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Authors

Pailloux, Sylvie L
Nguyen, Sean
Zhou, Stephanie
et al.

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Synthesis and chemical reactivity of a 6-Me-3,2-hydroxypyridinone dithiazolide with primary amines: a route to new hexadentate chelators for hard metal(III) ions

Sylvie L. Pailloux[a], **Sean Nguyen**[a], **Stephanie Zhou**[a], **Marisa E. Hom**[a], **Michelle N. Keyser**[a], **Danil Smiles**[a], and **Kenneth N. Raymond**[a]

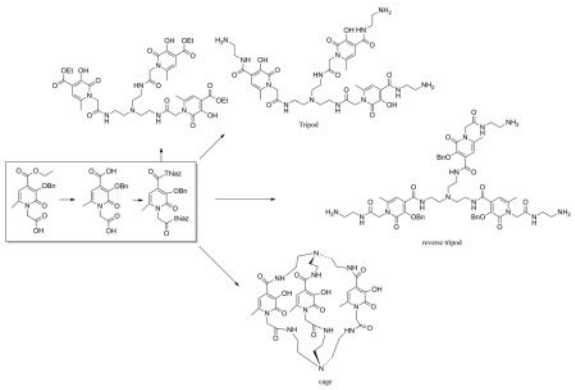
Kenneth N. Raymond: raymond@socrates.berkeley.edu

[a]Department of Chemistry, University of California, Berkeley, California 94720-1460, Fax: 510-642-5324

Abstract

A hydroxypyridinone building block, bifunctionalized with thiazoline, has been prepared from orthogonally protected 2-(3-(benzyloxy)-4-(ethoxycarbonyl)-6-methyl-2-oxopyridin-1(2H)-yl) acetic acid. The reactivity of the dithiazolide has been explored with two primary amines, leading to the synthesis and characterization of four new hexadentate ligands. Their complexes with selected hard trivalent ions pertinent to potential molecular imaging applications have been surveyed.

Graphical Abstract



INTRODUCTION

Hydroxypyridinones (HOPOs) have been developed for the last 20 years as chelators for hard metal ions [1]. The HOPO 6 membered ring has three major isomers [1]: 3,4-HOPO, 3,2-HOPO, and 1,2-HOPO, as depicted in Figure 1. Some HOPOs have been used in pharmaceutical applications, since their affinity for iron makes them good candidates for treating metal overload, protozoic and bacterial infections, as well as neurodegeneration [2]. HOPOs also have been employed as MMP inhibitors as well as for antidiabetic and anticancer treatments when coordinated to d-block elements such as Zn, Mo, V, Ru and Os [3]. Additionally, HOPOs with Gd and Ga have been utilized as imaging probes and as

decorporation agents of actinides [4]. Lastly, several studies of luminescence performance of 1,2-HOPO complexes with europium have been published and they display good quantum yields [5,6].

We have developed powerful MRI contrast agents involving 1,2-HOPO and 1-Me-3,2-HOPO which have been connected with a tripodal primary amine (TREN, TACN, Mesyl) to form hexadentate ligands that strongly complex Gd(III) [7,8,9]. These complexes have two or three sites available for water coordination (q), improving the relaxivity of the complex by a factor of 2 or 3 in comparison to all other contrast agents currently on the market, which have a q=1. To allow the incorporation of solubilizing moieties (dendrimers) or the conjugation to nano-supports (MS2, Ester Amine (EA) dendrimer), one HOPO moiety has been replaced by a terephthalamide (TAM) that has two amides built into the motif [10,11,12]. However, the thermodynamic stability of the Gd complexes is lowered when more than one HOPO unit is substituted by a TAM moiety [13, 14].

To mitigate the thermodynamic stability problem, a new hydroxypyridinone (6-Me-3,2-HOPO) has been developed and is described herein. This scaffold enables functionalization of the 1-*N* position, allowing for structural adjustment and the formation of neutral complexes with M(III) metal ions. This chemistry also supports the formation of a cage environment, which increases the kinetic stability of the complex [1,4]. With the exception of work by Gopalan [15], Rapoport [16] and Van der Eycken [17] very little work has been reported on 3,2-HOPOs that enables reactivity at the 1-*N* position or leads to the development of new HOPO systems. We describe herein a scalable synthesis for a new HOPO derivative bi-functionalized at positions 1 and 4 with thiazolide rings. Facile reaction of this reagent with primary amines enables for the synthesis of tripodal and bi-macrocyclic cage ligands that are capable of hexadentate coordination with hard metal ions such as Gd(III) and Ga(III).

RESULTS AND DISCUSSION

The formation of the HOPO ring **3** (Scheme 1) followed a procedure described by Feist [18]. However yields obtained were typically lower by 20 – 30%. The ^{1}H NMR spectrum was consistent with previously described results [13, 18], and the ^{13}C NMR spectrum showed a characteristic peak at δ (ppm) 102.18 for the only carbon in the ring carrying a proton [13]. A single X-ray diffraction determination of **3** has been previously described [13].

The reaction of HOPO **3** with tert-butyl iodoacetate presents a possible competition between N-alkylation and O-alkylation. This was previously addressed by us through the use of cetylpyridinium for phase transfer that favors the O-alkylation [13]. In a second approach, we studied the use of strong bases such as 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) which favors O- over N-alkylation in a homogeneous mixture [19, 20]. By using this approach we succeeded in synthesizing 6-Me-3,2-HOPO(Bn) **4** with better yield and reproducibility as well as a simpler purification. ^{1}H and ^{13}C NMR spectra for **4** were consistent with previously described spectra [13]. Specifically, the ^{13}C NMR spectrum contains a diagnostic methylene carbon δ (ppm) 74.04. The molecular structure of **4** was unequivocally confirmed by single crystal X-ray diffraction structure determination. Compound **5** results from N-

alkylation of the enolate of an amide (Scheme 1). We first envisaged the synthesis of the diester **5** using potassium on alumina [13]. In this case the tert-butyl ester **5** was obtained with moderate yield. Alternatively, by using potassium carbonate in DMF, the O-alkylated **5a** was also obtained (Scheme 2).

In comparison with **5, 5a** is considered to be more thermodynamically stable due to higher aromaticity. The ^{1}H NMR spectra of thermodynamic **5a** and kinetic **5** compounds are presented in Figure 2. The methylene resonance for the O-alkylated compound **5a** shows a more aromatic shift (δ = 6.96 ppm) than the N-alkylated compound **5** (δ = 6.19 ppm). In addition the molecular structure **5** was confirmed by single X-ray crystallography diffraction methods.

Functional similarity between hydroxypyridinone and quinolinone systems suggested the development of a one-step selective N-alkylation over O-alkylation of compound **4** through the use of magnesium tert-butoxide [21]. In the present system, it is expected that the Mg(II) ion coordinates with the carbonyl O-atom of the cyclic amide to form a five-membered ring intermediate (Scheme 3) that favors N-alkylation, producing compound **6** with 80% yield [21]. It is noted that the acid **6** was first obtained by acid hydrolysis of the tert-butyl ester **5** with high yield (Scheme 1).

The amide bond formation was first attempted by generating the N-hydroxysuccinimide (NHS) active ester intermediate**, 6a** (Scheme 4). Compound **10c**, was obtained with moderate yield. Saponification of the ester function of **10c** was obtained, but the activation of position 4 in active ester NHS or thiazolide failed. Benzyl group of compound **10c** was removed in acidic condition and permitted the synthesis of the Gd complex **12c** in the following step.

To access a cage structure or more soluble structures, the di-thiazolide intermediate **8** was developed (Scheme 4).

Following saponification of **6,** the di-acid **7** was combined with TBTU and 2-mercaptothiazolide to give **8** with a good overall yield. Compound **8** displays four characteristic triplets in its ^{1}H NMR spectrum at 4.62, 4.31, 3.42 and 2.88 ppm. These peaks correspond to the four methylene groups present in the two thiazolides fragments.

Primary amines tert-butyl-(2-aminoethyl)carbamate and TREN regioselectively reacted with the thiazolide (on the N side) of **8** with formation of **9** and **9a** respectively (Scheme 5). The ^{1}H NMR spectra of **9** and **9a** show two methylene signals δ (ppm) 4.32 and 2.89 **(9)** and 4.30 and 2.98 **(9a)** corresponding to the resonance of the thiazolide left in position 4 of the ring. The mono-thiazolide of **9** and **9a** reacts further with the primary aliphatic amines of TREN and tert-butyl (2-aminoethyl)carbamate respectively to form **10** and **10a**. The methylene protons of TREN were observed at δ (ppm) 3.01 and 2.24 for **10** and δ (ppm) 3.19 and 2.53 for **10a** in their respective ^{1}H NMR spectra. The macrocyclic cage **10b** was obtained by reaction of one equivalent of TREN with one equivalent of **9a** using high dilution conditions. In all cases the deprotections of BOC and benzyl groups were achieved by treatment with acid.

The coordination chemistry of the new hexadentate ligand was surveyed with hard, trivalent cations. In particular, one equivalent of each of the deprotected ligands **11-11b** (Scheme 5) and **11c** (Scheme 4) was combined with one equivalent of gadolinium chloride hexahydrate ($GdCl_3{\cdot}6H_2O$) at reflux in methanol (MeOH) and in the presence of pyridine. The resulting Gd complex **12-12c** was characterized by microanalysis and by mass spectrometry, and the latter showed the expected isotopic distributions and mass/charge values. In general, the coordination of the deprotected ligand **11-11c** to the M(III) ions results in broadening of the peaks in the IR spectra compared to the free ligands. In the cases of the Gd complexes with the tripodal ligand (**11, 11a, 11c**), a shift ($\Delta\nu_{CO}$~ 30cm) of the band assigned to the carbonyl of the ring toward lower frequency upon coordination was observed from 1660–1650 cm^{-1} for the ligand to 1630–1620 cm^{-1} for the complexes **12, 12a, 12c**. The bi-macrocycle cage **11b** was also complexed to $Ga(NO_3)_3$ and $GdCl_3{\cdot}6H_2O$. In these complexes the shift for the carbonyl of the ring stretching frequencies in the IR spectra were not as significant as observed with complex Gd(III) **12b** and complex Ga(III) **12d**. However, the mass spectra and microanalyses confirmed the formation of these complexes. The Gd(III) complexes **12, 12a** and **12c** appear to be monomeric with the respective ligands **11-11a** and **11c** bonded in hexadentate coordination modes. This is in contrast to the Gd(III) complex **12b** where it is thought that only two HOPO units coordinate the Gd(III) ion, and that the third HOPO moiety assisted with the formation of polymers by coordinating to a second Gd(III) ion. The Ga(III) ion in complex **12d** is assumed to be inside the cage of **11b.** This conclusion was reached based upon the size of a similar cage (e.g. BC-TREN-TAM) that has been shown to be more appropriate for the incorporation of Ga(III) ion (radius = 76 pm for 6 coordinate) than Gd(III) ion (radius = 119 pm for 8 coordinate) [22, 23].

In conclusion, an optimal synthesis for a key intermediate dithiazolide HOPO **8** has been developed, that supports formation of four new ligands and their formation of Gd(III) and Ga(III) complexes is described. This work is intended to support MRI and PET molecular imaging.

EXPERIMENTAL

General information

Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. All organic extracts were dried over anhydrous $MgSO_4$ and solvents removed *in vacuo*. Infrared spectra were recorded with the Nicolet 580 spectrophotometer using KBr tablets. ^1H spectra were recorded on a Bruker AVB 300 at 300 MHz, a Bruker AVB 400 at 400 MHz, or a Bruker AVB 600 at 600 MHz. ^{13}C spectra were collected using a Bruker AVB 400 at 100 MHz or a Bruker AVB 600 at 150 MHz. The residual solvent peak or TMS was used as an internal reference. Elemental analysis, mass spectrometry (HR = high resolution; ESI-MS = electrospray ionization mass spectrometry), and X-ray diffraction data were obtained at the analytical facilities at the University of California, Berkeley.

Ethyl 3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylate (3)—In a round bottom flask diethyl oxalacetate (100.0 g, 0.476 mol, 1 *eq.*) was dissolved in dry

tetrahydrofuran (THF) (600 mL). Chloroacetone (47.0 g, 0.50 mol, 1.05 *eq.*) was added to the reaction mixture and then ammonia gas was bubbled through the mixture (40 min.), resulting in a red mixture. A catalytic quantity of aluminum chloride ($AlCl_3$, 6.44 g, 0.05 mol, 0.09 *eq.*) was added slowly, and the mixture was stirred (4 days, 23°C). The yellow heterogeneous mixture was centrifuged (6000 rpm, 30 min.), and the resulting yellow solid was quenched using aqueous 6M HCl solution. The solid was collected by filtration and dried in a desiccator to give **3** as a beige solid (18.1 g, 19%). IR (KBr, ν cm^{-1}): 1678 (C=O, ester), 1644 (C=O, ring).^{1}H NMR (300 MHz, $CDCl_3$ + TMS): δ (ppm): 12.77 (s, 1H), 10.77 (s, 1H), 6.53 (s, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 2.34 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H); ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$ + TMS): (δ, ppm): 169.21, 160.18, 150.74, 133.27, 115.40, 102.20, 62.28, 18.63, 14.09. $[M+H]^+$ calcd for $C_9H_{12}NO_4$ 198.0766, Found 198.0762 *m/z*.

Ethyl 3-(benzyloxy)-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylate (4)—1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 13.6 g, 89.0 mmol, 1.5 *eq.*) was added to a solution containing **3** (11.5 g, 58.6 mmol, 1 *eq.*) in isopropanol (200 mL). The reaction mixture was refluxed at 83 °C under N_2 before adding benzyl bromide (15.2 g, 88.9 mmol, 1.5 *eq.*) dropwise. Refluxing was maintained for four hours and then the solvent was evaporated. The resulting dark brown oil was dissolved in dichloromethane (DCM, 30 mL), washed with aqueous 3M HCl solution (2×30 mL), and then with Millipore water (3×30 mL). The organic layers were combined and dried with Na_2SO_4, and the solvent was evaporated. Diisopropyl ether (250 mL) was added to the oily residue which precipitated after a day and **4** was recovered as a light brown solid (9.43 g, 56%). IR (KBr, ν cm^{-1}):1712 (C=O, ester), 1646(C=O, ring). ^{1}H NMR (300 MHz, $CDCl_3$ + TMS): (δ, ppm): 12.31 (s, 1H), 7.31–7.53 (m, 5H), 6.17 (s, 1H), 5.27 (s, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 2.33 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H); ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$): (δ, ppm): 165.32, 162.20, 144.54, 139.59, 137.26, 133.24, 128.55, 128.27, 128.03, 104.35, 74.01, 61.69, 18.54, 14.11.$[M+H]^+$ calcd for $C_{16}H_{18}NO_4$ 288.1236, Found 288.1232 *m/z*.

Ethyl 3-(benzyloxy)-1-(2-(tert-butoxy)-2-oxoethyl)-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylate (5)—Dimethoxyethane (5mL) was added under inert conditions to a sample of **4** (0.3 g, 1.04 mmol, 1 *eq.*) and KF alumina (182 mg, 3.13 mmol, 3 *eq.*) previously purged with N_2. Tert-butyl iodoacetate (182 mg, 3.13 mmol, 3 eq.) was added dropwise under N_2. The reaction mixture was heated (2 h, 45 °C) then left to stir (12 h, 23 °C). The alumina was removed by filtration and the filtrate was concentrated to dryness. The residue was purified by flash chromatography using a gradient of MeOH in DCM. The compound (recovered at 5% of MeOH) with the shortest retention time in HPLC C18 was characterized as **5** (146 mg, 35%) and the last eluted was found to be the starting material **4**. IR (KBr, ν cm^{-1}):1740 (C=O, ester), 1661 (C=O, ring). ^{1}H NMR (500 MHz, $CDCl_3$): (δ, ppm): 7.49 (d, *J* = 5.0 Hz, 2H), 7.35 (dd, *J* = 5.0 Hz, 2H), 7.30 (d, *J* = 5.0 Hz, 1H), 6.19 (s, 1H), 5.24 (s, 2H), 4.73 (s, 2H), 4.27 (q, *J* = 7.0 Hz, 2H), 2.38 (s, 3H), 1.48 (s, 9H), 1.27 (t, *J* = 7.0 Hz, 3H,).^{13}C{^{1}H} NMR (500 MHz, $CDCl_3$) (δ, ppm): 166.5, 165.2, 160.2, 144.9, 139.7, 137.1, 130.3, 128.5, 128.2, 127.9, 104.0, 82.8, 73.8, 61.5, 46.5, 27.9, 20.0, 14.0. $[M+H]^+$ calcd for $C_{22}H_{28}NO_6$: 402.1838, Found 402.1916 *m/z*. *Anal.* Calcd (found) for $C_{22}H_{27}NO_6$: C, 65.82 (65.18); H, 6.78 (6.63); N, 3.49 (3.02).

2-(3-(benzyloxy)-4-(ethoxycarbonyl)-6-methyl-2-oxopyridin-1(2H)-yl) acetic acid (6)—Sample of **4** (4.10 g, 14.3 mmol, 1 *eq.*), magnesium di-*tert*-butoxide ($Mg(OtBu)_2$, 5.28 g, 31.0 mmol, 2.2 *eq.*), and potassium *tert*-butoxide (KOtBu, 1.79 g, 16.0 mmol, 1.1 *eq.*) were combined in a round bottom flask, purged three times with nitrogen and then THF (200ml) was added. A solution of bromoacetic acid (5.29 g, 38.0 mmol, 2.7 *eq.*) in dry THF (20 mL) was added dropwise and the reaction mixture was stirred (12 h, 23 °C). The solvent was evaporated, and the dark brown oil was dissolved in dichloromethane (DCM, 30 mL). The mixture was quenched with aqueous 3M HCl solution (2×30 mL) and washed with Millipore water (2×30 mL). The organic layers were collected and dried with Na_2SO_4, and the solvent was evaporated. Diisopropyl ether was added and the mixture was stirred (12 h, 23 °C) to cause precipitation. The brown solid **6** was collected by filtration and dried to give compound **6** as a beige solid (3.9 g, 80%). IR (KBr, ν cm^{-1}): 1733 (C=O, acid), 1716 (C=O, ester), 1645(C=O, ring). ^1H NMR (300 MHz, $CDCl_3$ + TMS): (δ, ppm): 7.28–7.46 (m, 5H), 6.27 (s, 1H), 5.19 (s, 2H), 4.82 (s, 2H), 4.26 (q, J = 7.1 Hz, 2H), 2.26 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H); $^{13}C\{^1H\}$ NMR (400 MHz, $CDCl_3$ + TMS): (δ, ppm): 170.09, 164.85, 160.94, 144.89, 140.18, 136.92, 131.42, 128.60, 128.27, 128.04, 105.43, 74.24, 61.80, 46.54, 20.15, 14.07. $[M\text{-}H]^-$ calcd for $C_{18}H_{18}NO_6$ 344.1134, Found 344.1147 *m/z. Anal.* Calcd (found) for $C_{18}H_{19}NO_6$: C, 62.60 (61.45); H, 5.55 (5.69); N, 4.06 (4.16).

Ethyl 3-(benzyloxy)-1-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-6-methyl-2-oxo-1,2 dihydropyridine-4-carboxylate (6a)—A solution of N-hydroxysuccinimide (NHS, 0.66 g, 5.79 mmol, 2 *eq.*) and N,N′-dicyclohexylcarbodiimide (DCC, 0.89 g, 4.35 mmol, 1.5 *eq.*) in dimethylformamide (DMF, 3 mL) was added dropwise to a solution containing **6** (1 g, 2.89 mmol, 1 *eq.*) in DMF (10 mL) and the mixture was stirred (24 h, 23 °C). The volatile components were removed under vacuum and the residue was dissolved in DCM (20 mL) and water (10 mL). The organic phase was collected and dried over $MgSO_4$ and concentrated until dryness. The residue was crystallized from isopropyl alcohol (10 mL) to give **6a** as a white solid (0.78 g, 71%). IR (KBr, ν cm^{-1}): 1707 (C=O, ester), 1648 (C=O, ring). ^1H NMR (300MHz, $CDCl_3$): (δ, ppm): 7.31–7.44 (m, 5H), 6.21 (s, 1H), 5.26 (s, 2H,), 5.18 (s, 2H), 4.26 (q, J = 7.1 Hz, 2H), 2.86 (s, 4H), 2.34 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H). A*nal.* Calcd (found) for $C_{22}H_{22}N_2O_8$: C, 59.73 (57.53); H, 5.01 (6.03); N, 6.33 (6.78).

3-(benzyloxy)-1-(carboxymethyl)-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylic acid (7)—A solution of NaOH (1.33 g, 33.3 mmol, 2.9 *eq.*) in water (40 mL) was added to a solution containing **6** (3.98 g, 11.5 mmol, 1 *eq.*) in ethanol (140 mL). The reaction mixture was stirred (1.5 h, 23 °C) and reaction progress was monitored via TLC (DCM:MeOH 9:1). The solvent was evaporated, the resulting dark oil was suspended in ethyl acetate and the solution was concentrated twice to ensure that all the ethanol was removed. The oil was dissolved in water (20 ml) and the flask was placed in an ice bath. Compound **7** was recovered by acidification of the aqueous solution with aqueous 3M HCl solution (50 mL). A light brown precipitate was recovered after stirring (12h, 23°C) then dried to give **7** as a white solid (3.4 g, 98%). IR (KBr, ν (cm^{-1}): 1720 (C=O, acid), 1647 (C=O, ring). ^1H NMR (300 MHz, DMSO-d6): (δ, ppm): 7.35–7.49 (m, 5H), 6.25 (s, 1H), 5.09 (s, 2H), 4.79 (s, 2H), 2.31 (s, 3H); $^{13}C\{^1H\}$ NMR (600 MHz, DMSO-d6): (δ, ppm):

170.30, 167.45, 160.47, 143.48, 142.79, 138.21, 133.18, 129.23, 129.20, 128.95, 103.80, 73.96, 47.10, 20.51. $[M+H]^+$ calcd for $C_{16}H_{16}NO_6$ 318.0978 Found 318.0977m/z, $[M+Na]^+$ calcd for $C_{16}H_{15}NO_6Na$ 340.0797, Found 340.0804 *m/z* $[M-H]^-$ calcd for $C_{16}H_{14}NO_6$ 316.0821, Found 316.0835 m/z. *Anal.* Calcd (found) for $C_{16}H_{21}NO_9$ (L+3H_2O): C, 51.75 (56.46); H, 5.70 (4.90); N, 3.77 (4.12).

3-(benzyloxy)-6-methyl-1-(2-oxo-2-(2-thioxothiazolidin-3-yl)ethyl)-4-(2-thioxothiazolidine-3-carbonyl)pyridin-2(1*H*)-one (8)—*N,N*-diisopropylethylamine (DIPEA, 5.00 g, 38.7 mmol, 4.6 *eq.*) was added dropwise to a suspension of **7** (2.68 g, 8.44 mmol, 1 *eq.*), *O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TBTU, 6.26 g, 19.5 mmol, 2.3 *eq.*), 4-(dimethylamino)pyridine (DMAP, 0.11 g, 0.925 mmol, 0.11 *eq.*), and 2-mercaptothiazoline (2.26 g, 19.0 mmol, 2.2 *eq.*) in DCM (80 mL). After addition, the reaction mixture turned dark brown and it was stirred (40 min., 23°C). The solvent was evaporated and immediately purified using CombiFlash® (gradient of EtOAc in DCM). The fractions were collected at 40% EtOAc, concentrated, and washed with diethyl ether to give **8** as a yellow solid (2.9 g, 66%). IR (KBr, ν cm^{-1}):1699 (C=O amide), 1653 (C=O, ring), 1597 (C=S, thiaz). ^1H NMR (300 MHz, $CDCl_3$ + TMS): (δ, ppm): 7.31–7.43 (m, 5H), 6.02 (s, 1H), 5.63 (s, 2H), 5.26 (s, 2H), 4.62 (t, *J* = 7.5 Hz, 2H), 4.31 (t, *J* = 7.3 Hz, 2H), 3.42 (t, *J* = 7.5 Hz, 2H), 2.88 (t, *J* = 7.3 Hz, 2H), 1.58 (s, 3H). $^{13}C\{^1H\}$ NMR (400 MHz, $CDCl_3$ + TMS): (δ, ppm): 202.06, 200.79, 168.13, 165.95, 159.22, 141.39, 140.49, 137.57, 132.96, 128.39, 128.33, 128.10, 104.17, 73.87, 55.84, 55.07, 51.29, 29.33, 29.14, 20.47. $[M+H]^+$ calcd for $C_{22}H_{22}N_3O_4S_4$: 520.0493, Found: 520.0484 *m/z*. A*nal.* Calcd (found) for $C_{22}H_{21}N_3O_4S_4$: C, 50.85 (50.40); H, 4.07 (4.10); N, 8.09 (8.32).

***Tert*-butyl (2-(2-(3-(benzyloxy)-6-methyl-2-oxo-4-(2-thioxothiazolidine-3-carbonyl)pyridin- 1(2*H*)-yl)acetamido)ethyl)carbamate (9)—**A solution of tert-butyl (2-aminoethyl)carbamate (703 mg, 4.39 mmol, 1.1 *eq.*) in DCM (40 mL) was added at 1.2 mL/hour using an automated syringe to a solution of **8** (2.00 g, 3.84 mmol, 1 *eq.*) in DCM (100 mL). The reaction mixture was stirred for four hours after the end of the addition before being concentrated and purified using CombiFlash® (gradient of MeOH in DCM). The fractions were collected (10% MeOH) and evaporated to dryness to give **9** as a light yellow solid (1.87 g, 86%). IR (KBr, ν cm^{-1}): 1697(C=O, ester), 1685 (C=O, amides), 1654 (C=O, ring), 1598 (C=S, thiaz). ^1H NMR (300 MHz, $CDCl_3$ + TMS): (δ, ppm): 7.32–7.43 (m, 5H), 6.90 (s, 1H), 6.03 (s, 1H), 5.27 (s, 2H), 4.91 (s, 1H), 4.70 (*J* = 7.3 Hz, 2H), 2.43 (s, 3H), 1.43 (s, 9H); $^{13}C\{^1H\}$ NMR (400 MHz, $CDCl_3$ + TMS): (δ, ppm): 200.98, 167.49, 165.76, 159.88, 141.38, 141.28, 137.45, 133.40, 128.44, 128.37, 128.22, 104.75, 79.55, 74.09, 55.07, 50.78, 48.81, 40.30, 29.14, 28.39, 20.52. $[M+H]^+$ calcd for $C_{26}H_{33}N_4O_6S_2$ 561.1842, Found 561.1835 *m/z. Anal.* Calcd (found) for $C_{26}H_{32}N_4O_6S_2$: C, 55.70 (55.32); H, 5.75 (4.99); N, 9.99 (9.50).

***N,N′,N″*-(nitrilotris(ethane-2,1-diyl))tris(2-(3-hydroxy-6-methyl-2-oxo-4-(2-thioxothiazolidine-3-carbonyl)pyridin-1(2*H*)-yl)acetamide) (9a)—**A solution containing TREN (43 mg, 0.29 mmol, 0.53 *eq.*) and DIPEA (78 mg, 0.60 mmol, 1.1 *eq.*) in DCM (37 mL) was added at a rate of 1.5 mL/hour using an automated syringe to a solution

containing **8** (290 mg, 0.56 mmol, 1 *eq.*) in DCM (50 mL). The reaction mixture was stirred (12 h, 23 °C), the solvent was evaporated and the mixture purified through CombiFlash® (gradient of MeOH in DCM). The fractions of the column were collected (10% MeOH) and evaporated. The yellow residue was solubilized in a minimum of DCM, followed by a slow precipitation with addition of diethyl ether to give **9a** as a yellow solid (143 mg, 57%). IR (KBr, ν cm^{-1}):1700 (C=O, amides), 1655 (C=O, ring), 1600 (C=S, thiaz). ^{1}H NMR (300 MHz, $CDCl_3$ + TMS): (δ, ppm): 8.09 (t, *J* = 6.2 Hz, 3H), 7.29–7.31 (m, 15H), 5.94 (s, 3H), 5.13 (s, 6H), 4.65 (s, 6H), 4.30 (t, *J* = 7.3 Hz, 6H), 3.19 (s, 6H), 2.98 (t, *J* = 7.3 Hz, 6H), 2.53 (s, 6H), 2.17 (s, 9H); $^{13}C\{^{1}H\}$ NMR (400 MHz, $CDCl_3$ + TMS): (δ, ppm): δ (ppm): 200.92, 167.01, 175.75, 159.67, 142.12, 141.37, 137.43, 133.46, 128.52, 128.18, 127.79, 104.25, 75.65, 55.24, 50.83, 48.47, 38.54, 29.22, 20.43. $[M+H]^+$ calcd for $C_{63}H_{67}N_{10}O_{12}S_6$ 1347.3264, Found 1347.3253 *m/z. Anal.* Calcd (found) for $C_{63}H_{66}N_{10}O_{12}S_6$: C, 56.15 (55.9); H, 4.94 (5.02); N, 10.39 (10.50).

Tri-*tert*-butyl (((2,2′,2″-((((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(carbonyl))tris(3-(benzyloxy)-6-methyl-2-oxopyridine-4,1(2*H*)-diyl))tris(acetyl))tris(azanediyl))tris(ethane-2,1-diyl))tricarbamate (10)—A solution of TREN (0.506 g, 3.45 mmol, 0.36 *eq.*) and DIPEA (1.52 g, 11.2 mmol, 1.2 *eq.*) in DCM (50 mL) was added at a rate of 1.5 mL/hour via automated syringe to a solution of **9** (5.31 g, 9.47 mmol, 1 *eq.*) in DCM (120 mL). After complete addition the mixture was stirred (4 h, 23 °C) before being evaporated and purified using CombiFlash® (gradient of MeOH in DCM). The fractions were collected (5% MeOH) and evaporated to give **10** as a white solid (2.28 g, 49%). IR (KBr, ν cm^{-1}): 1691 (C=O, amide), 1677 (C=O, ester), 1658 (C=O, ring). ^{1}H NMR (300 MHz, $CDCl_3$ + TMS): (δ, ppm): 7.77 (s, 3H), 7.43 (s, 3H), 7.28 (s, 15H), 6.60 (s, 3H), 5.13 (s, 6H), 4.72 (s, 6H), 3.38 (s, 6H), 3.27 (s, 6H), 3.01 (s, 3H), 2.39 (s, 9H), 2.24 (s, 6H), 1.41 (s, 27H); $^{13}C\{^{1}H\}$ NMR (400 MHz, $CDCl_3$ + TMS): (δ, ppm): 167.44, 163.23, 160.28, 156.65, 143.81, 140.44, 136.25, 130.81, 128.79, 128.73, 128.63, 105.39, 79.53, 74.70, 51.86, 48.40, 40.37, 40.22, 37.24, 28.38, 20.33. $[M+Na]^+$ calcd for $C_{75}H_{99}N_{13}O_{18}Na$ 1492.7129, Found 1492.7120 *m/z*, $[M+H]^+$ calcd for $C_{75}H_{100}N_{13}O_{18}$ 1470.7311 Found 1470.7312 *m/z. Anal.* Calcd (found) for $C_{75}H_{99}N_{13}O_{18} + 3H_2O$: C, 59.08 (58.92); H, 6.94 (6.72); N, 11.94 (11.98).

Tri-*tert*-butyl (((1,1′,1″-(((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(2-oxoethane-2,1-diyl))tris(3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-1,4-diyl-4-carbonyl))tris(azanediyl))tris(ethane-2,1-diyl))tricarbamate (10a)—A solution containing **9a** (0.143 g, 0.106 mmol, 1 *eq.*) in DCM (50 mL) was added dropwise at a rate of 1.5 mL/hour via automated syringe to a solution of DIPEA (69 mg, 0.53 mmol, 5 *eq.*) and tert-butyl (2-aminoethyl)carbamate (51 mg, 0.32 mmol, 3 *eq.*) in DCM (20 mL). The solvent was evaporated and the mixture was purified via CombiFlash® (gradient of MeOH in DCM). The fractions were collected (5% MeOH) and evaporated. The recovered clear oil was re-dissolved in a minimal amount of DCM and precipitated by slow addition of diethyl ether to give **10a** as a white solid (63 mg, 41%). IR (KBr, ν cm^{-1}):1710 (C=O, amides), 1690 (C=O, ester), 1650 (C=O, ring), ^{1}H NMR (300 MHz, $CDCl_3$ + TMS): (δ, ppm): 8.16 (s, 3H), 7.93 (s, 3H), 7.35 (s, 15H), 6.59 (s, 3H), 5.17 (s, 6H), 5.06 (s, 3H), 4.80 (s, 6H), 3.28 (s, 12H), 3.14 (s, 6H), 2.64 (s, 6H), 2.27 (s, 9H), 1.41 (s, 27H); $^{13}C\{^{1}H\}$ NMR

(400 MHz, $CDCl_3$ + TMS) (δ, ppm): 166.91, 163.79, 160.30, 156.07, 143.27, 141.01, 136.01, 130.2, 128.94, 128.85, 128.44, 105.59, 79.30, 74.39, 53.77, 48.38, 40.37, 39.82, 38.79, 28.41, 20.35. $[M+H]^+$ calcd for $C_{75}H_{100}N_{13}O_{18}$ 1470.7311, Found 1470.7306 *m/z. Anal.* Calcd (found) for $C_{75}H_{99}N_{13}O_{18}$: C, 61.25 (60.62); H, 6.79 (6.81); N, 12.38 (12.04).

Macrocycle BC-TREN-tris-6-Me-3,2-HOPO(Bn) (10b)—A first solution containing **9a** (0.34 g, 0.23mmol, 1 *eq.*) in DCM (25 mL) was prepared. A second solution of TREN (0.03 g, 0.23 mmol, 1 *eq.*) and DIPEA (0.12 g, 0.93 mmol, 4 *eq.*) in DCM (25 mL) was also prepared. Both solutions were simultaneously added to DCM (110 mL) at a rate of 1 mL/hour using automated syringes. The reaction was stirred for 1 day after the end of the addition. TLC showed the formation of a single major spot. The reaction mixture was evaporated and purified using CombiFlash® (gradient of MeOH 0.1% $N(Et)_3$ in DCM). The fractions were collected (5% MeOH) and evaporated to give compound **10b** as a light beige solid (142 mg, 53%). IR (KBr, ν cm^{-1}):1720 (C=O, amide), 1654(C=O, ring). ^1H NMR (300MHz, CD_3OD): (δ, ppm): 7.21 (br s, 15H), 6.27 (br s, 3H), 5.20 (br s, 6H), 4.80 (br s, 6H), 3.45 (br s, 6H), 3.05 (br s, 6H), 2.21 (br s, 21H). $^{13}C\{^1H\}$ NMR (600Mhz, $CDCl_3$ with a drop of MeOH 600MHz): (δ, ppm): 169.0, 163.53, 159.76, 143.23, 140.71, 135.93, 131.10, 128.09 104.0, 74.17, 50.41, 48.22, 38.43, 36.93, 19.74. $[M+H]^+$ calcd for $C_{60}H_{70}N_{11}O_{12}$ 1136.5205, Found 1136.5227 *m/z. Anal.* Calcd (found) for $C_{60}H_{69}N_{11}O_{12}$: C, 59.6 (53.25); H, 5.92 (6.75); N, 12.74 (13.20).

Diethyl 1,1′-(((((2-(2-(3-(benzyloxy)-4-(ethoxycarbonyl)-6-methyl-2-oxopyridin-1(2H)-yl)-N-methylacetamido)ethyl)azanediyl)bis(ethane-2,1-diyl))bis(azanediyl))bis(2-oxoethane-2,1-diyl))bis(3-(benzyloxy)-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylate) (10c)—A solution containing DIPEA (90mg, 0.69 mmol, 5 *eq.*) and tris(2-aminoethyl)amine (TREN, 20 mg, 0.14 mmol, 1 *eq.*) in DCM (5 mL) was added to a solution of **6a** (0.2 g, 0.45 mmol, 3.3 *eq.*) in dry DCM (5 mL). The reaction mixture was stirred (12h, 23°C) then the organic phase was washed with water (2×5 mL). The organic phase was dried using $MgSO_4$ and concentrated to dryness to give compound **10c** as a light beige solid (85 mg, 50%). IR (KBr, ν cm^{-1}): 1731 C=O, ester), 1690 (C=O, amide), 1655 (C=O, ring). ^1H NMR (300MHz, $CDCl_3$): (δ, ppm): 7.36–7.38 (m, 2H), 7.23–7.29 (m, 3H), 5.04 (s, 2H), 4.67 (br s, 2H), 4.24 (q, J=7.2 Hz, 2H), 3.21 (br s, 2H), 2.55 (br s, 2H), 2.04 (s, 3H), 1.25 (t, J=7.2 Hz, 3H,). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 600 MHz): (δ, ppm): 166.89, 164.82, 160.65, 144.70, 141.73, 137.23, 131.20, 128.38, 127.98, 127.73, 104.73, 73.80, 61.66, 52.87, 38.38, 33.96, 20.22, 14.09. $[M+H]^+$ calcd for $C_{60}H_{70}N_7O_{15}$ 1128.493, Found: 1128.4914 *m/z. Anal.* Calcd. (found) for $C_{60}C_{69}N_7O_{15}$: C, 63.87 (64.20); H, 6.16 (5.09); N, 8.69 (8.53).

***N*,*N*′,*N*″-(nitrilotris(ethane-2,1-diyl))tris(1-(2-((2-aminoethyl)amino)-2-oxoethyl)-3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide) (11)—**A solution containing **10** (231 mg, 0.16 mmol, 1 *eq.*) in glacial acetic acid (5 mL) and concentrated HCl (5 mL) was added to a DTPA-washed round-bottom flask. The resulting yellow mixture was stirred (12 h, 23 °C) then the solvent was evaporated. The yellow solid was dissolved in a minimal amount of Millipore water and transferred to a centrifuge tube containing diethyl ether. MeOH was added to the biphasic mixture until a white precipitate

appeared. The precipitate was centrifuged (6000 rpm, 30 min.), and the process was repeated three times to give **11** as a light yellow crystalline solid (169 mg, 98%). IR (KBr, ν cm^{-1}): 1717 (C=O, amide), 1658 (C=O, ring).^{1}H NMR (300 MHz, CD_3OD): δ (ppm): 6.44 (s, 2H), 4.83 (br s, 6H), 3.92 (br s, 6H), 3.57 (t, *J* = 5.7 Hz, 6H), 3.14 (t, *J* = 5.7 Hz, 6H), 2.27 (s, 9H). $^{13}C\{^{1}H\}$ NMR (400 MHz, $CDCl_3$ + TMS): δ (ppm): 167.71, 166.66, 159.17, 145.46, 135.15, 117.24, 102.76, 52.14, 47.54, 38.58, 36.82, 34.52, 19.91. $[M+H]^+$ calcd for $C_{39}H_{58}N_{13}O_{12}$ 900.4328, Found 900.4330 *m/z. Anal* Calcd for (found) $C_{39}H_{61}N_{13}O_{12}Cl_4$: C, 44.79 (44.32); H, 5.88 (6.51); N, 17.41 (17.02).

1,1′,1″-(((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(2-oxoethane-2,1-diyl))tris(N-(2-aminoethyl)-3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide) (11a)—A solution containing **10a** (148 mg, 0.10 mmol) in glacial acetic acid (2 mL) and concentrated HCl (2 mL) was added to a 25 mL DTPA-washed round-bottom flask. The resulting yellow mixture was stirred (12 h, 23 °C), and then the solvent was evaporated. The yellow solid was dissolved in a minimal amount of Millipore water, and transferred to a centrifuge tube containing diethyl ether. MeOH was added to the biphasic mixture until a white precipitate appeared. The precipitate was centrifuged (6000 rpm, 30 min.), and the process was repeated three times, to give **11a** as a light yellow crystalline solid (51 mg, 56%). IR (KBr, ν cm^{-1}): 1691(C=O, amides), 1658 (C=O, ring). ^{1}H NMR (300 MHz, DMSO-d6): (δ, ppm): 8.67 (s, 6H), 8.04 (s, 9H), 6.44 (s, 3H), 4.65 (s, 6H), 3.66, (s, 6H), 3.47 (s, 6H), 3.28 (s, 6H), 2.91(s, 6H), 2.12 (s, 9H).$^{13}C\{^{1}H\}$ NMR (400 MHz, $CDCl_3$ + TMS): (δ, ppm): 170.24, 167.51, 159.72, 144.29, 136.03, 116.0, 104.58, 54.30, 48.03, 39.12, 37.13, 34.82, 19.07.$[M-H]^-$ calcd for $C_{39}H_{56}N_{13}O_{12}$ 898.4171 Found 898.4158 *m/z. Anal.* Calcd (found) for $C_{39}H_{73}Cl_4N_{13}O_{18}$ (L.4HCl, $6H_2O$): C, 40.59 (39.05); H, 6.38 (7.10); N, 15.78 (9.60), (very hydroscopic).

Macrocycle BC-TREN-tris-6-Me3,2-HOPO (11b)—A solution containing **10b** (142 mg, 0.12 mmol) in concentrated HCl (2 mL) and glacial acetic acid (2 mL) was added to a DTPA-washed round-bottom flask. The reaction was stirred (48 h, 23°C). The solvent was removed and the residue was precipitated using a mixture of water, MeOH and diethyl ether to give compound **11b** as a light beige solid (86 mg, 80%). IR (KBr, ν cm^{-1}):1680 (C=O, amides), 1658 (C=O, ring). ^{1}H NMR (300MHz, DMSO-d6): (δ, ppm): 8.65 (OH, br s), 8.45 (NH, br s), 7.87 (NH, br s), 6.35 (3H, s), 4.72 (6H, s), 3.34 (6H,m), 3.10 (6H, m), 2.47(12H, m), 2.09 (9H,s). $^{13}C\{^{1}H\}$ NMR (600MHz, DMSO-d6): (δ, ppm): 185.0, 169.7, 159.2, 145.0, 135.6, 117.5, 103.1, 56.5, 46.5, 46.4, 34.8, 34.5, 18.8; $[M+H^+]$ calcd for $C_{39}H_{52}N_{11}O_{12}$ 866.3797, Found 866.3792 *m/z. Anal* Calcd (found) for $C_{39}H_{53}N_{11}O_{12}Cl_2$: C, 49.89 (50.40); H, 5.69 (6.43); N, 16.41 (16.21).

Triethyl-1,1′,1″-(((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(2-oxoethane-2,1-diyl))tris(3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylate) (11c)—A solution containing **10c** (140 mg, 0.12 mmol) in glacial acetic acid (1.5 mL) and concentrated HCl (1.5 mL) was added to a DTPA-washed round-bottom flask. The mixture was stirred (12h, 23°C). The solvent was removed, then the residue was solubilized in a minimum of MeOH and a solid was precipitated with diethyl ether. The solid was recovered after centrifugation of the suspension (6000 rpm, 30 min) to give

compound **11c** as a light beige solid (100 mg, 95%). IR (KBr, ν cm^{-1}): 1700 (C=O, ester), 1685 (C=O amide), 1655(C=O, ring). ^{1}H NMR (300MHz, $CDCl_3$): (δ, ppm): 10.41 (s, 1H), 8.23 (s, 1H), 6.33 (s, 1H), 4.91 (s, 2H), 4.36 (q, 2H), 3.25 (s, 2H), 2.57 (s, 2H), 2.33 (s, 3H), 1.37 (t, 7.2 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (600MHz, $CDCl_3$): (δ, ppm): 167.45, 166.03, 159.38, 147.03, 134.67, 114.25, 102.37, 64.84, 61.00, 48.69, 19.71, 14.03, please note that one methylene is under the MeOH peak. $[M+H]^+$ calcd for $C_{39}H_{52}H_7O_{15}$: 858.3521, Found: 858.3528 *m/z. Anal* Calcd (found) for $C_{39}H_{52}N_7O_{15}Cl$: C, 52.37 (53.01); H, 5.86 (6.06); N, 10.97 (11.62).

Gd(III)-*N*,*N*′,*N*″-(nitrilotris(ethane-2,1-diyl))tris(1-(2-((2-aminoethyl)amino)-2-oxoethyl)-3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide) (12)— A solution of gadolinium (III) chloride hexahydrate ($GdCl_3{\cdot}6\ H_2O$, 72 mg, 0.19 mmol, 1.5 *eq.*) in water (2 mL) was added to a solution containing **11** (139 mg, 0.13 mmol, 1 *eq.*) in Millipore water (5 mL) and placed in a 25 mL DTPA-washed round-bottom flask. Pyridine (497 mg, 6.28 mmol, 47 *eq.*) was added to the reaction mixture and was refluxed (12h, 95°C). The solvent was evaporated. The solid was dissolved in a minimal amount of MeOH and added to a centrifuge tube containing diethyl ether. The precipitate was recovered after centrifugation (6000 rpm, 30 min.), and the process was repeated three times, to give complex **12** as a light grey solid (160 mg, 98%). IR (KBr, ν (cm^{-1}):1680 (C=O, amides), 1624(C=O, ring). $[M+H]^+$ calcd for $C_{39}H_{55}N_{13}O_{12}Gd$ 1055.3344, Found 1055.3348 *m/z Anal*. Calcd (found) for $C_{42}H_{73}Gd2N_{10}O_{20}Cl_3$ ($GdL(H_2O)_2$ + $Gd{\cdot}Cl_3{\cdot}6H_2O$): C, 34.58 (31.19); H, 5.04 (5.63); N, 9.60 (10.54).

Gd-1,1′,1″-(((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(2-oxoethane-2,1-diyl))tris(N-(2-aminoethyl)-3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide) (12a)—A solution of $GdCl_3{\cdot}6\ H_2O$ (2.31 mg, 6.22 mmol, 1 *eq.*) in Millipore water (1 mL) was added to **11a** (6.51 mg, 6.22 mmol, 1 *eq.*) in MeOH (5ml). Pyridine (20 μL) was then added and the reaction mixture was stirred (24 h, 95°C). After cooling, the complex was recovered by precipitation using a minimum of MeOH and addition of diethyl ether until a white precipitate appeared. The precipitate was washed three times with diethyl ether and recovered after centrifugation (6000 rpm, 30 min), to give complex **12a** as a light grey solid (6 mg 91%). IR (KBr, ν (cm^{-1}): 1690 (C=O, amide), 1624 (C=O, ring). $[M+H]^+$ calcd for $C_{39}H_{55}N_{13}O_{12}Gd$ 1055.3256, Found 1055.3338 *m/z. Anal.* Calcd (found) for $C_{39}H_{58}N_{13}O_{17}Gd_3$ ($LGd(H_2O)$ + Gd_2O_3): C, 32.24 (32.04); H, 4.02 (5.33); N, 12.53 (11.06).

Gd-BC-TREN-tris-6-Me3,2-HOPO (12b)—A solution of $GdCl_3{\cdot}6\ H_2O$ (4.29 mg, 0.01 mmol, 1*eq.*) in MeOH (2 mL) was added to a solution containing **11b** (10 mg, 0.01 mmol, 1 *eq.*) in MeOH (5 mL), followed by the addition of pyridine (4.25 mg, 0.03 mmol, 3 eq.). The reaction was stirred (24 h, 95°C), then the solvent was removed and the residue was precipitated in a minimum of MeOH with a slow addition of diethyl ether to give complex **12b** as a white grey solid (6 mg, 48%). IR (KBr, ν (cm^{-1}):1682 (C=O, amides), 1650 (C=O, ring). $[M-H]^-$ calcd for $C_{39}H_{48}ClN_{11}O_{12}Gd$ ($L^{-3}Gd^{+3}Cl$) 1055.2413, Found 1055.2395 *m/z*; $[M-H]^-$ calcd for $C_{39}H_{50}ClN_{11}O_{13}Gd$ ($L^{-3}Gd^{+3}Cl.H_2O$) 1073.2519, Found 1073.2487 *m/z.*

Anal. Calcd (found) for $C_{40}H_{66}N_{10}O_{20}GdCl_5$ ($L^{-2}Gd^{-3}2Cl\ 2H_2O + GdCl_3{\cdot}6H_2O$): C, 32.05 (31.85); H, 4.44 (5.27); N, 9.35 (8.74).

Gd-triethyl-1,1′,1″-(((nitrilotris(ethane-2,1-diyl))tris(azanediyl))tris(2-oxoethane-2,1-diyl))tris(3-hydroxy-6-methyl-2-oxo-1,2-dihydropyridine-4-carboxylate) (12c)—A solution of $GdCl_3{\cdot}6H_2O$ (40 mg, 0.011 mmol, 1 *eq.*) in MeOH (5 mL) was added to a solution containing **11c** (94 mg, 0.11 mmol, 1 *eq.*) in MeOH (1 mL), followed by the addition of pyridine (40 mg, 0.326 mmol, 3 *eq.*). The reaction mixture was stirred (24 h, 95°C). After cooling, the complex was recovered in a minimum of MeOH by slow addition of diethyl ether. The precipitate was washed three times with diethyl ether and recovered after centrifugation (6000 rpm, 30 min) to give complex **12c** as a white solid (85 mg, 60 %). IR (KBr, ν (cm^{-1}): 1700 (C=O, ester), 1650 (C=O, amide) 1567 (C=O, ring). $[M+H]^+$ calcd for $C_{39}H_{51}Gd_2N_7O_{16}Cl_3$ 1294.0928, Found 1294.0776 *m/z*, *Anal.* Calcd (found) for $C_{39}H_{50}Gd_2N_7O_{16}Cl_3$: C, 36.20 (32.78); H, 3.90 (5.01); N, 7.72 (7.67).

Ga-BC-TREN-tris-6-Me3,2-HOPO (12d)—A solution of Ga(acetyl acetate) (Ga-Acac 8.48 mg, 0.02 mmol,1 eq.) in MeOH (2 mL) was added to a solution of **11b** (20 mg, 0.02 mmol, 1 eq) in MeOH (5 mL) and water (2 mL) followed by the addition of pyridine (131 mg, 1.66 mmol, 72 *eq.*). The reaction mixture was stirred (24 h, 95 °C). The solvent was removed and the residue was precipitated using a minimum of MeOH and slow addition of diethyl ether. The solid was recovered after centrifugation (6000 rpm, 30 min) to a give **12d** as a white powder (8 mg, 36%). IR (KBr, ν (cm^{-1}): 1654 (C=O, amide), 1648 (C=O, ring). $[M+H]^+$ calcd. for $C_{39}H_{49}N_{11}O_{12}Ga$ 932.2812, Found 932.2822 *m/z*, $[M+H+H_2O]^+$ calcd. For $C_{39}H_{50}N_{11}O_{13}Ga$ 950.2924, Found 950.2931 *m/z*, $[M+H+2H_2O]^+$ calcd. for $C_{39}H_{52}N_{11}O_{14}Ga$ 968.3029, Found 968.3042 *m/z*. *Anal.* Calcd (found) for $C_{39}H_{64}GaN_{11}O_{26.}$ [LGa+ $Ga(OH)_4$. $2H_2O$]: C, 33.90 (33.08); H, 4.67 (5.14); N, 11.15 (10.11).

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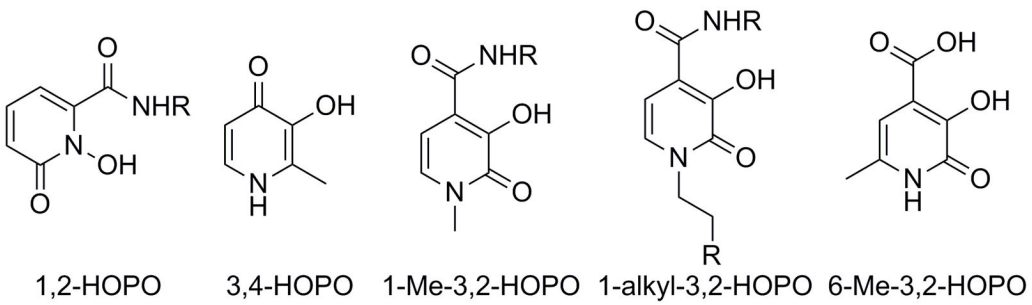


Figure 1.
Examples of the hydroxypyridinone family.

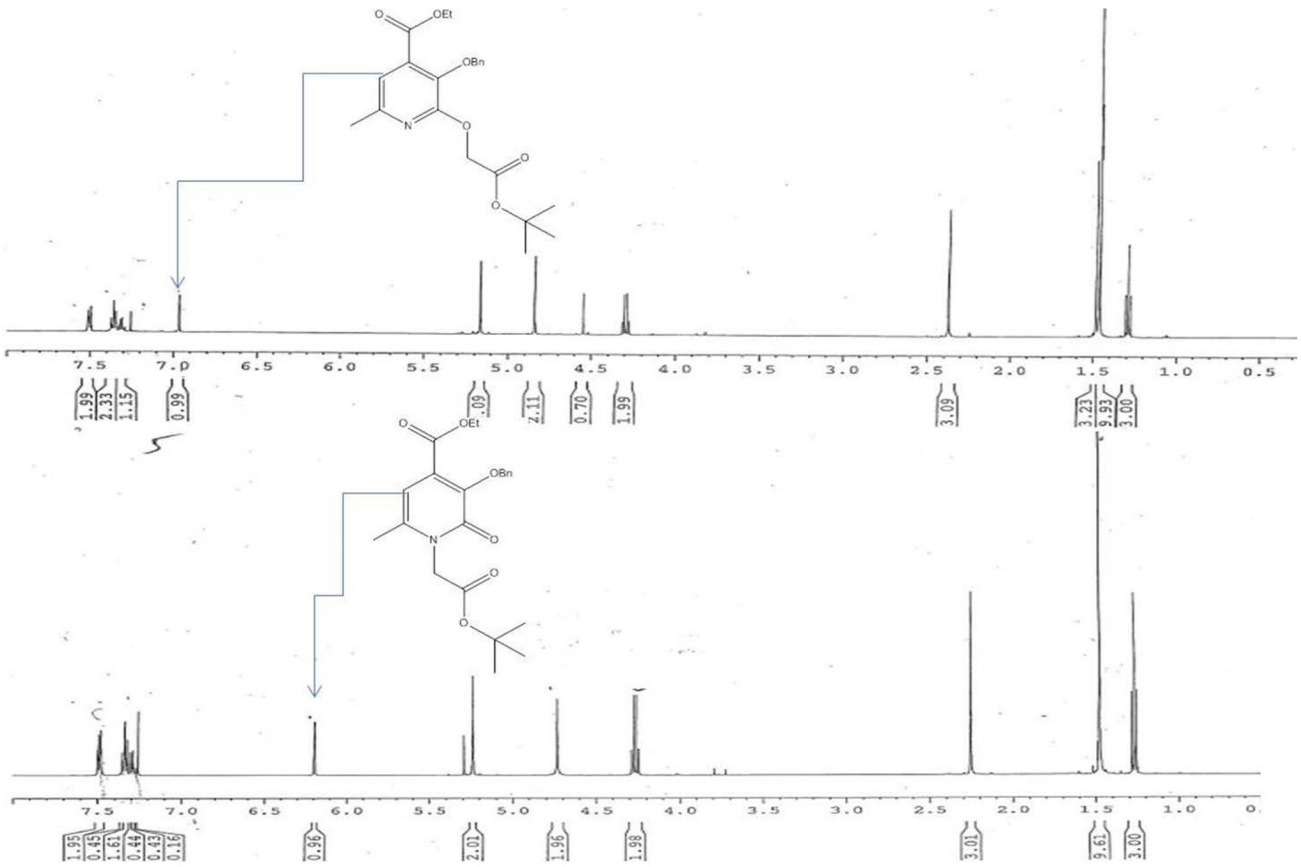
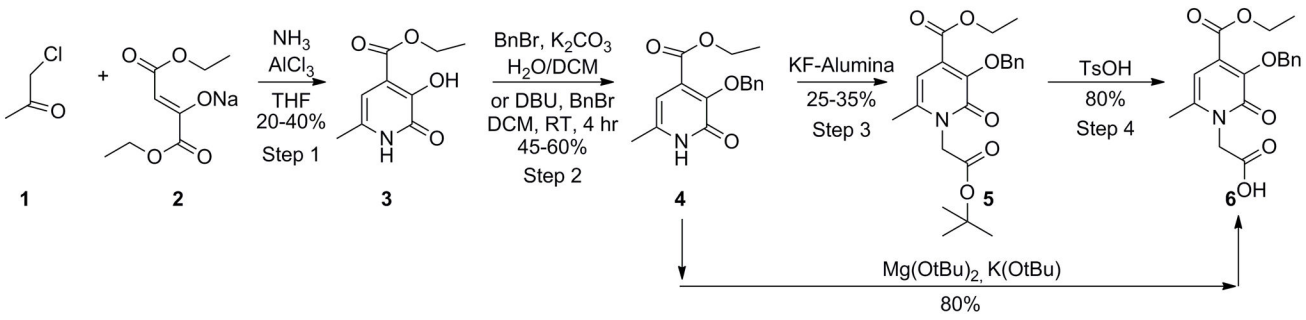


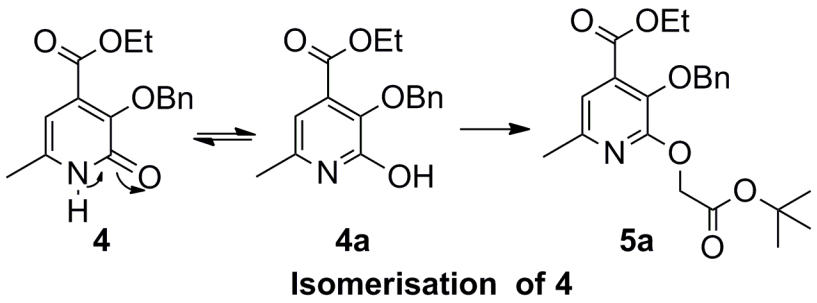
Figure 2.

^{1}H NMR comparison between resulting products of competitive N- **5**, (bottom) vs O-alkylation **5a** (top)

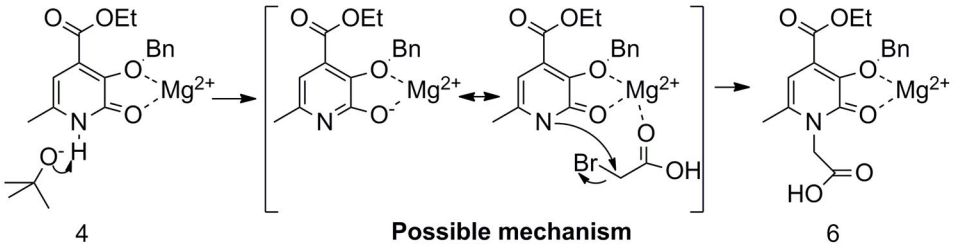


Scheme 1.

Synthesis of bifunctionalized 6-methyl-3,2-HOPOs, **5** and **6**.

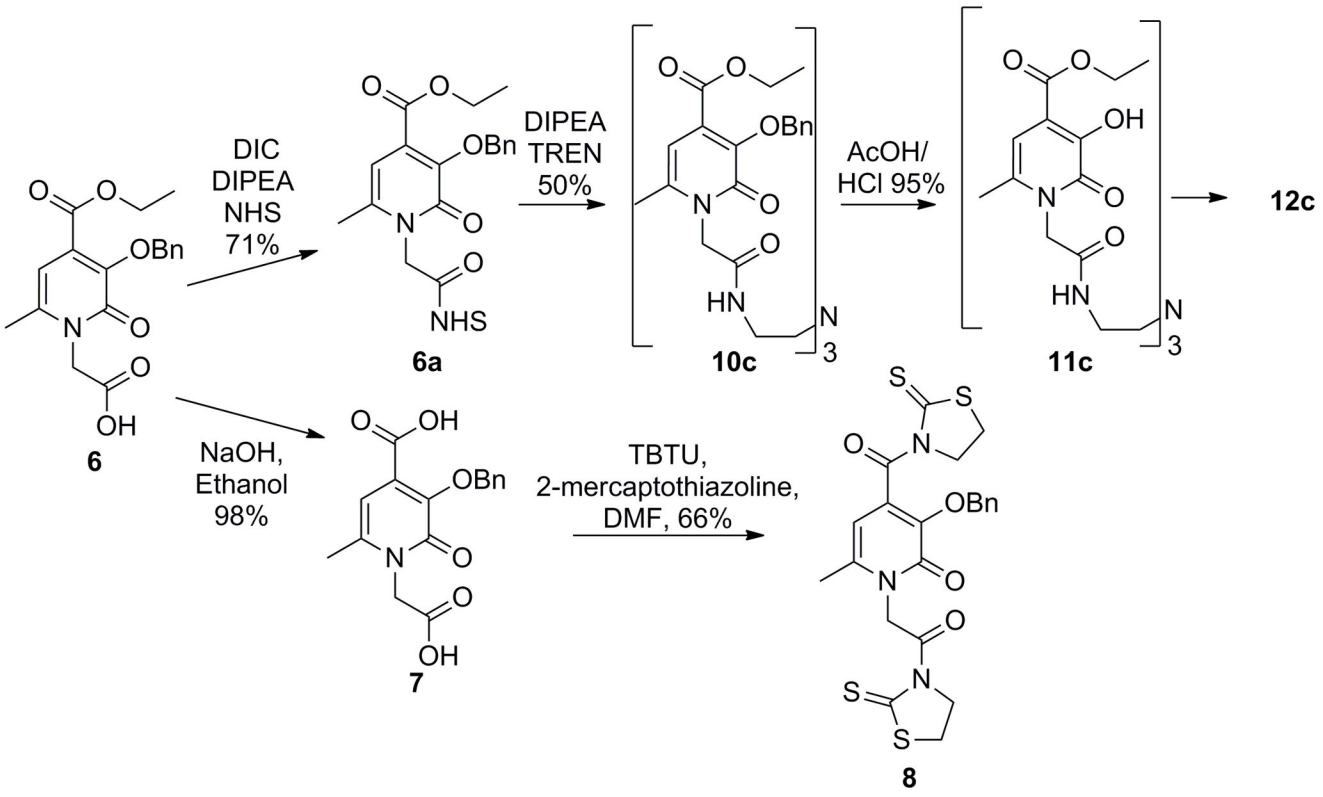


Scheme 2.
O-alkylation of the enolate form of 6-Me-3,2-HOPO **4**.

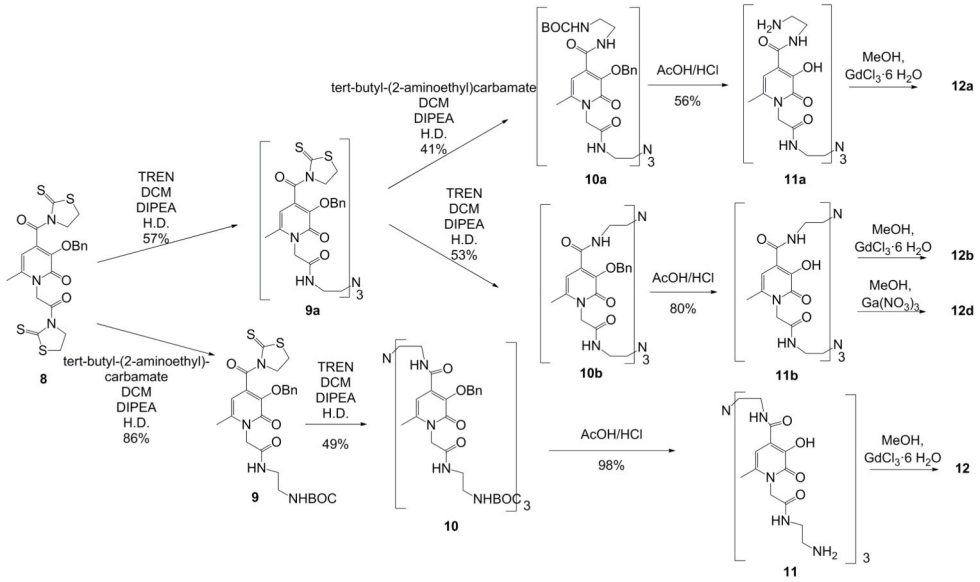


Scheme 3.

Possible mechanism of N-Alkylation of 6-Me-3,2-HOPO **4**.



Scheme 4.
Reactivity of functionalized 6-Me-3,2-HOPO.



Scheme 5.

Synthesis of hexadentate HOPO based ligands 11-11b.