Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Evaluation of DFO-HOPO as an octadentate chelator for zirconium-89

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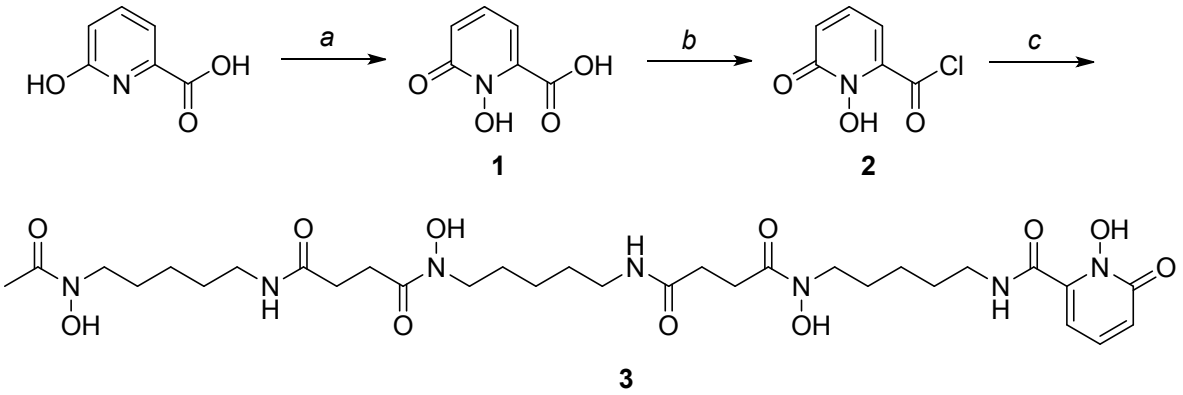
Table of contents

MATERIALS AND METHODS

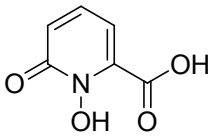
All the reagents and solvents were purchased from commercial sources and used without further purification unless otherwise stated. HPLC grade acetonitrile, water and trifluoroacetic acid (TFA), anhydrous dimethyl formamide (DMF), anhydrous tetrahydrofuran (THF), glacial acetic acid, trimethylamine (TEA), thionyl chloride ($SOCl_2$) sodium carbonate (Na_2CO_3) and methanol (MeOH) were purchased from Fisher Scientific (Loughborough, UK). Ethylenediaminetetraacetic acid (EDTA), mouse serum, 6-hydoxypicolinic acid, zirconium (IV) chloride ($ZrCl_4$, anhydrous 99.99%), deferoxamine mesylate salt (DFO) and peracetic acid were purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Phosphate buffered saline (PBS) was purchased from Gibco (Life Technologies, Paisley, UK). Silica-glass (SG) ITLC chromatography paper was purchased from Agilent Technologies. Salicylic acid (SA) ITLC chromatography paper used for serum stability analysis was generously gifted by Agilent Technologies. ^{89}Zr in 1M oxalic acid was purchased from Perkin Elmer, USA. Initial stock solutions (50mM) of DFO and **3** were prepared by dissolving the chelators in DMSO. Stock solutions were prepared by diluting aliquots of the 50mM solution in 0.5M HEPES pH 7.
Lyophilisation was performed on a Concentrator Plus (Eppendorf, Stevenage, UK). Sample incubation was performed using a Thermomixer (Eppendorf, Stevenage, UK).

SYNTHESIS OF DFO-HOPO (3) AND ^{nat}Zr-3



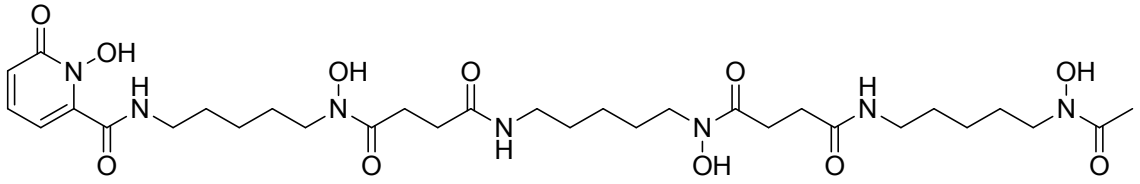
Scheme S1. Synthesis of N1-hydroxy-N1-(5-(4-(hydroxy(5-(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamido)pentyl)amino)-4-oxobutanamido)pentyl)-N4-(5-(N-hydroxyacetamido)pentyl)succinamide (DFO-HOPO, **3**). Reagents and conditions: *a*) CH_3COOOH (1.2 eq), AcOH, TFA, 80 °C, 12 h; *b*) $SOCl_2$ (1 mL), dry THF, 75 °C. *c*) DFO mesylate, TEA, dry DMF, rt, 16 h.

1-Hydroxy-6-oxo-1,6-dihydropyridine-2-carboxylic acid (1)[1]



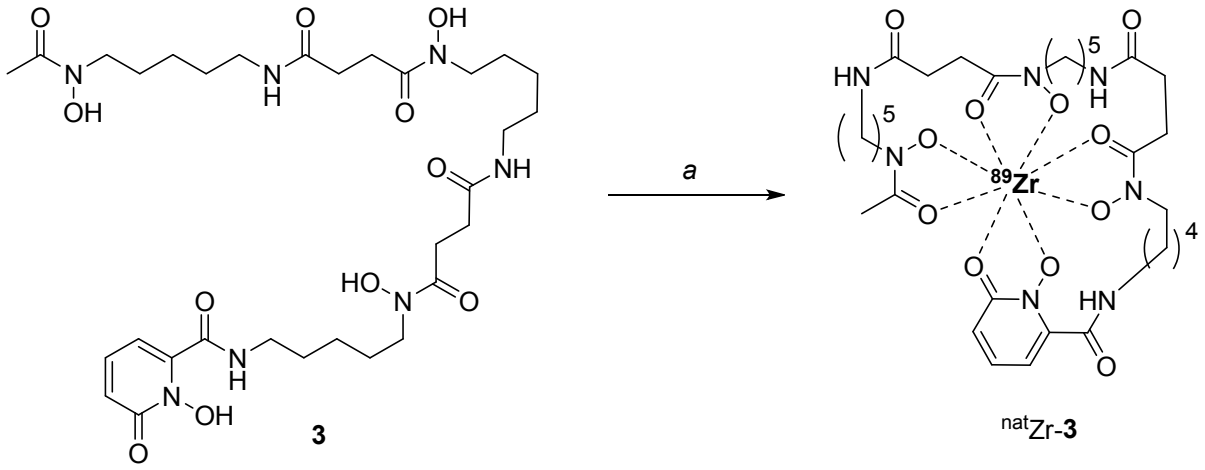
The preparation of **1** was adapted from a literature procedure.[1] 6-Hydoxypicolinic acid (2.00 g, 14.36 mmol) was added to a solution of glacial acetic acid (12 mL), trifluoroacetic acid (20 mL) and peracetic acid (3.5 mL, 17.24 mmol) and the mixture was stirred at ambient temperature for 1 h under N_2. The reaction was then heated to 80 °C for 12 h during which time a precipitate formed. The reaction was allowed to cool and the precipitate was filtered and washed with ice-cold MeOH (15 mL) to give product (**1**) as an off-white solid (1.84 g, 11.86 mmol, 83%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.45 (dd, J = 9.0, 7.0 Hz, 1H), 6.72 (dd, J = 9.0, 1.7 Hz, 1H), 6.64 (dd, J = 7.0, 1.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 106.7, 120.8, 137.1, 139.3, 157.6, 162.4.

N1-Hydroxy-N1-(5-(4-(hydroxy(5-(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamido)pentyl)amino)-4-oxobutanamido)pentyl)-N4-(5-(N-hydroxyacetamido)pentyl)succinamide (3).[2]



The preparation of **3** was adapted from a literature procedure.[2] Thionyl chloride (1 mL) was added to a suspension of compound **1** (50 mg, 0.32 mmol) in dry THF (1 mL) and the reaction mixture was heated to 75 °C overnight. After the reaction was allowed to cool to ambient temperature, the solvent was removed *in vacuo* to yield 1-hydroxy-6-oxo-1,6-dihydropyridine-2-carbonyl chloride (**2**) as an oily residue which was used without further purification. Deferoxamine mesylate (200 mg, 0.32 mmol) was added to a solution of compound **2** (55.4 mg, 0.32 mmol) in dry DMF (2 mL) followed by trimethylamine (45 μL, 0.32 mmol). The reaction was stirred at ambient temperature for 16 h. The bulk solvent was removed *in vacuo*. Acetone (1 mL) was added to the residue and the formed precipitate was collected and washed with acetone (3 mL). Since a highly pure compound was required for radiochemistry applications, the product was further purified by semi-preparative HPLC (Gradient 2) to give a solid yellow powder after lyophilisation (10 mg, 3%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.62 (s, 3H), 8.73 (t, J = 5.6 Hz, 1H), 7.78 (t, J = 5.6 Hz, 2H), 7.39 (dd, J = 9.1, 6.9 Hz, 1H), 6.56 (dd, J = 9.1, 1.7 Hz, 1H), 6.26 (dd, J = 6.9, 1.7 Hz, 1H), 3.46 (q, J = 7.3 Hz, 6H), 3.18 (d, J = 6.4 Hz, 1H), 3.11 – 2.91 (m, 4H), 2.62 – 2.52 (m, 4H), 2.27 (m, 4H), 1.96 (s, 3H), 1.61 – 1.43 (m, 9H), 1.44 – 1.32 (m, 4H), 1.32 – 1.11 (m, 7H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 20.8, 24.0, 26.5, 28.0, 28.8, 29.3, 30.4, 38.9, 47.2, 47.5, 104.0, 119.6, 137.7, 142.9, 137.7, 142.9, 157.9, 160.6, 170.6, 171.7, 172.4. ESI-HRMS (*m/z*): $[M + H]^+$ calcd for $C_{31}H_{52}N_7O_{11}$: 698.3725; found: 698.3722 $[M + H]^+$ (100), 349.700 $[M + H]^{2+}$ (19), 720.370 $[M + Na]^+$ (19)

^{nat}Zr-DFO-HOPO (^{nat}Zr-3)



Scheme S2. Synthesis of ^{nat}Zr-DFO-HOPO (^{nat}Zr-**3**). Reaction conditions: *a*) $ZrCl_4$, MeOH, 15 min, rt.

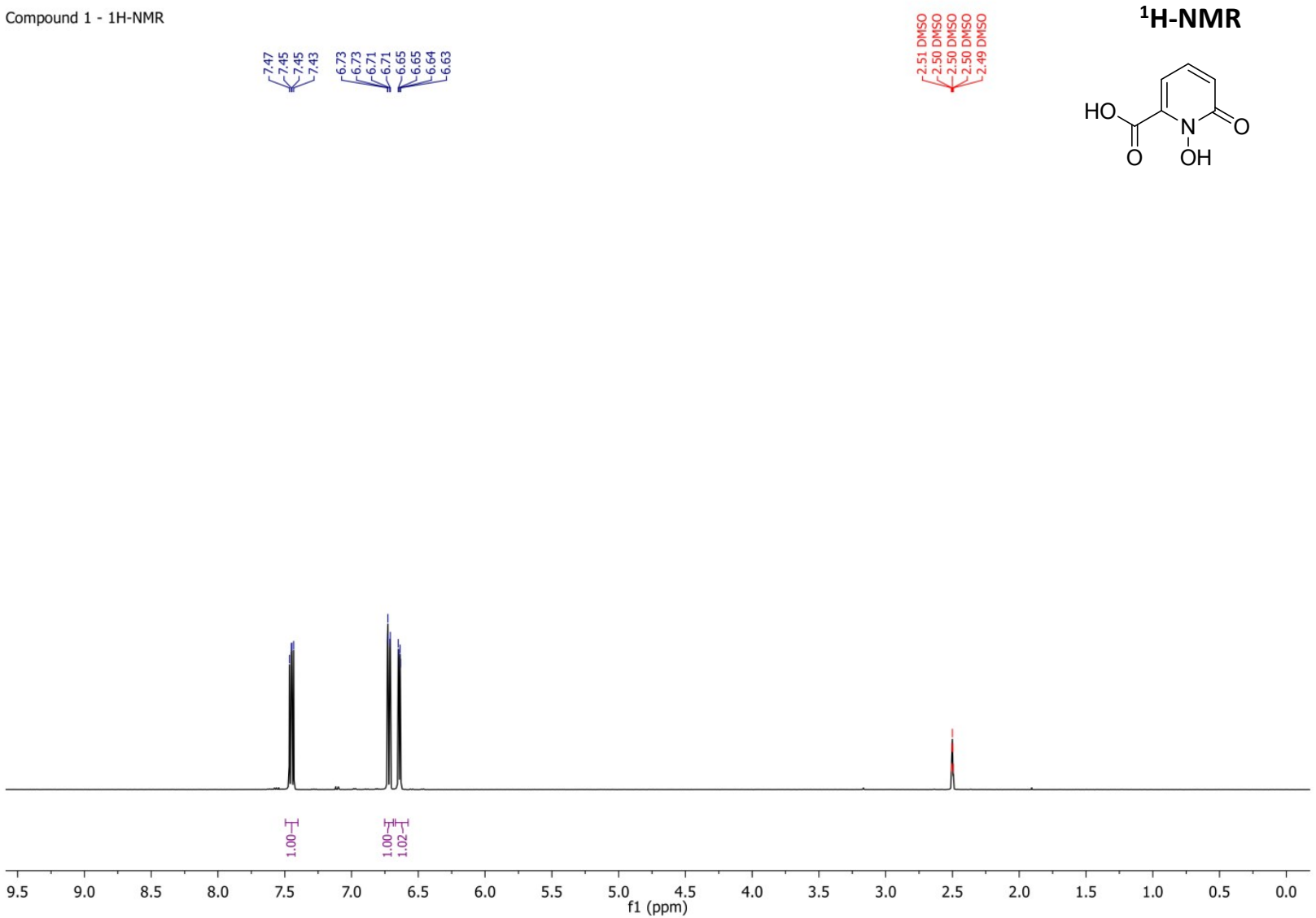
In a 1.5 mL centrifuge tube, a $ZrCl_4$ solution (1.5 mg, 6.6 µmol, in 500 µL MeOH) was added to **3** (3.1 mg, 4.4 µmol) in MeOH (500 µL). The mixture was vortexed for 15 min at room temperature. The cloudy sample was centrifuged to pellet the precipitate. Lyophilisation of the collected supernatant yielded the product as a white powder. ESI-HRMS: (*m/z*): $[M + H]^+$ calcd for $C_{31}H_{48}N_7O_{11}Zr$: 784.2459; found: 784.2470 $[M + H]^+$ (100); 806.226 $[M + Na]^+$ (53)

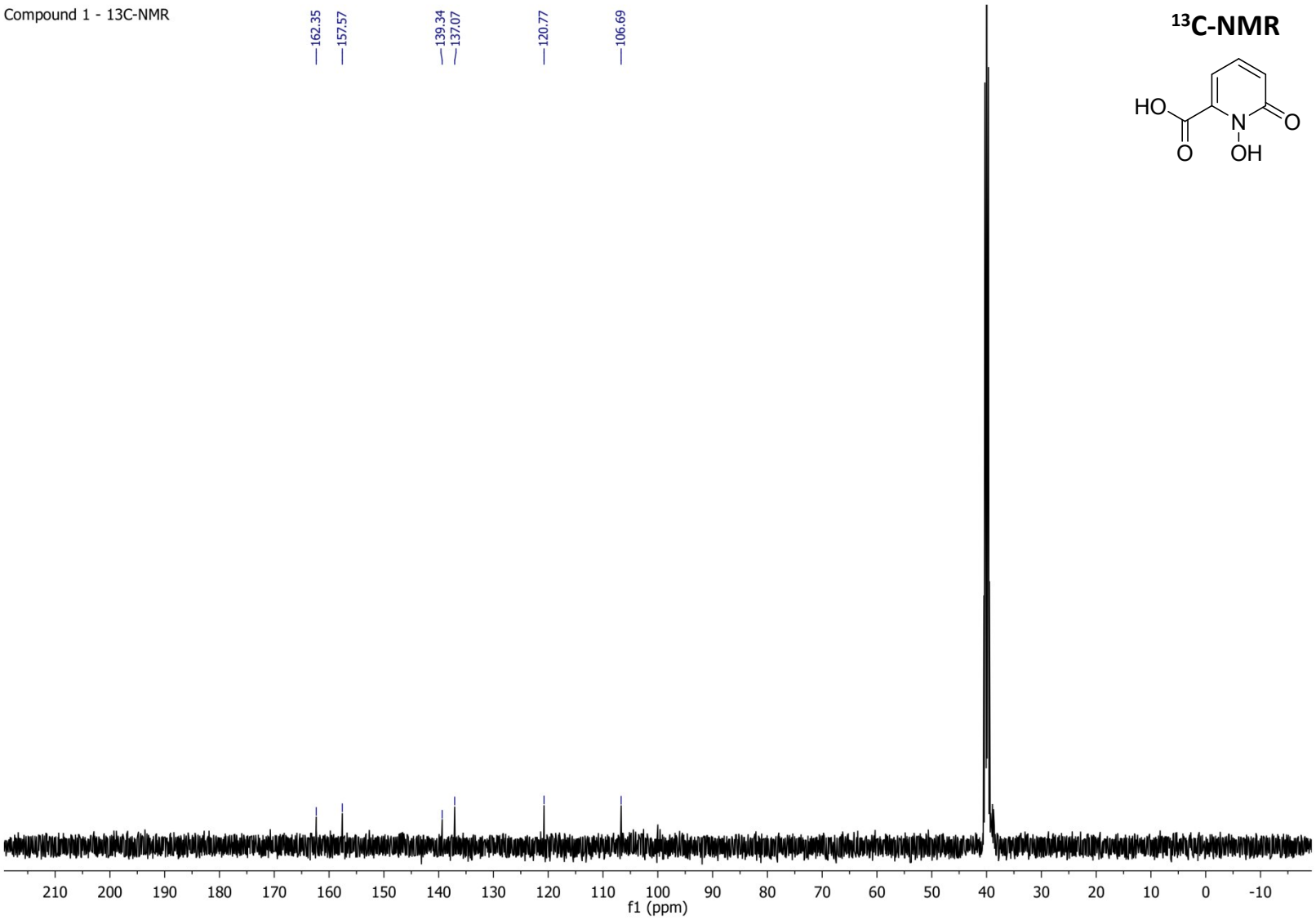
NMR AND MS DATA

^{1}H and ^{13}C NMR spectra were obtained using a Bruker 500 MHz spectrometer operating at room temperature. Chemical shifts (δ) are reported in parts per million (ppm) and residual solvent peaks have been used as an internal reference. Peak multiplicities have been abbreviated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet).

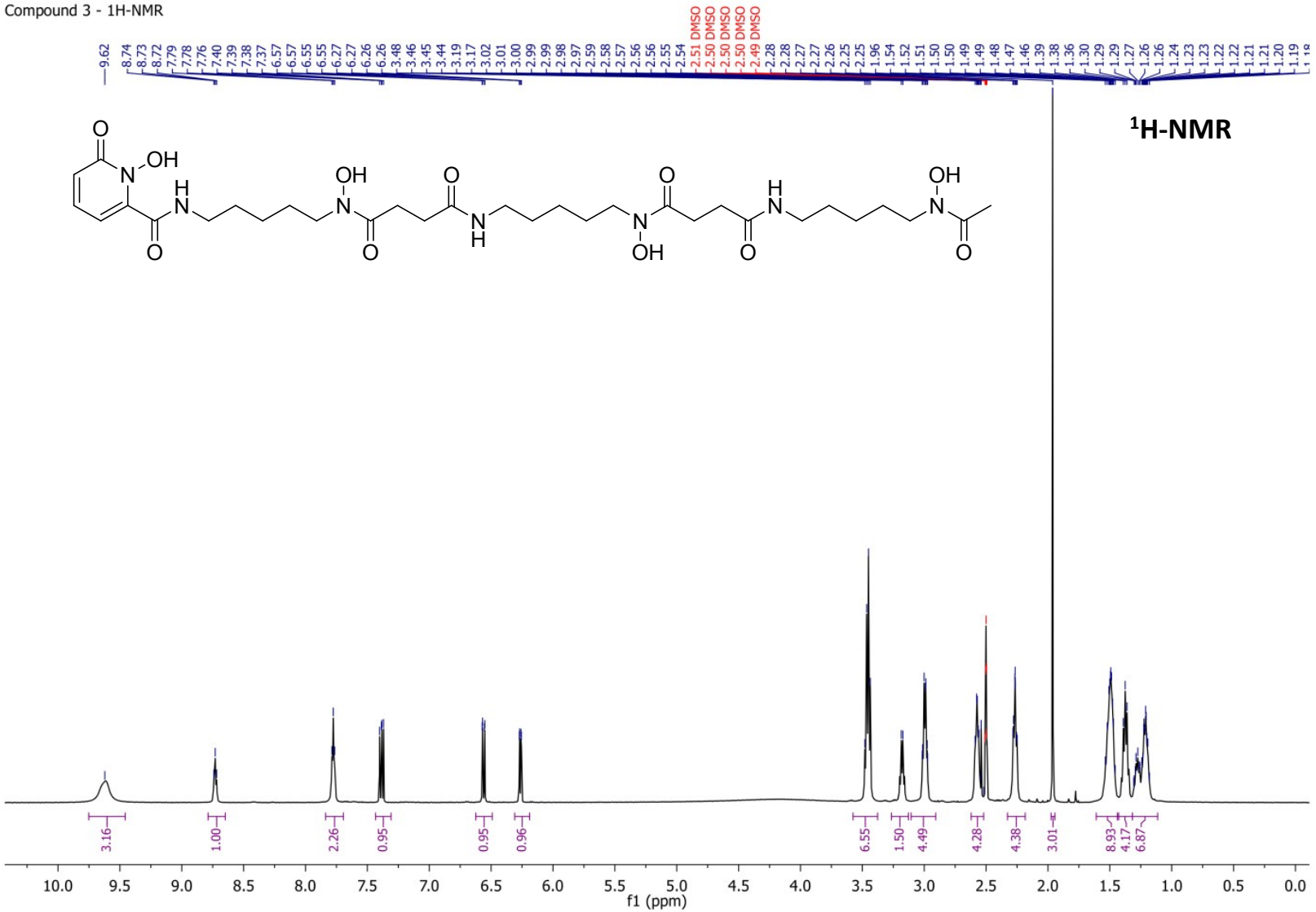
Liquid chromatography (LC) coupled to electrospray ionization high resolution mass spectrometry (ESI-HRMS) was performed using an Agilent 1200 series LC pump with a 6210 time-of-flight (TOF) mass analyser.

1-Hydroxy-6-oxo-1,6-dihydropyridine-2-carboxylic acid (1)

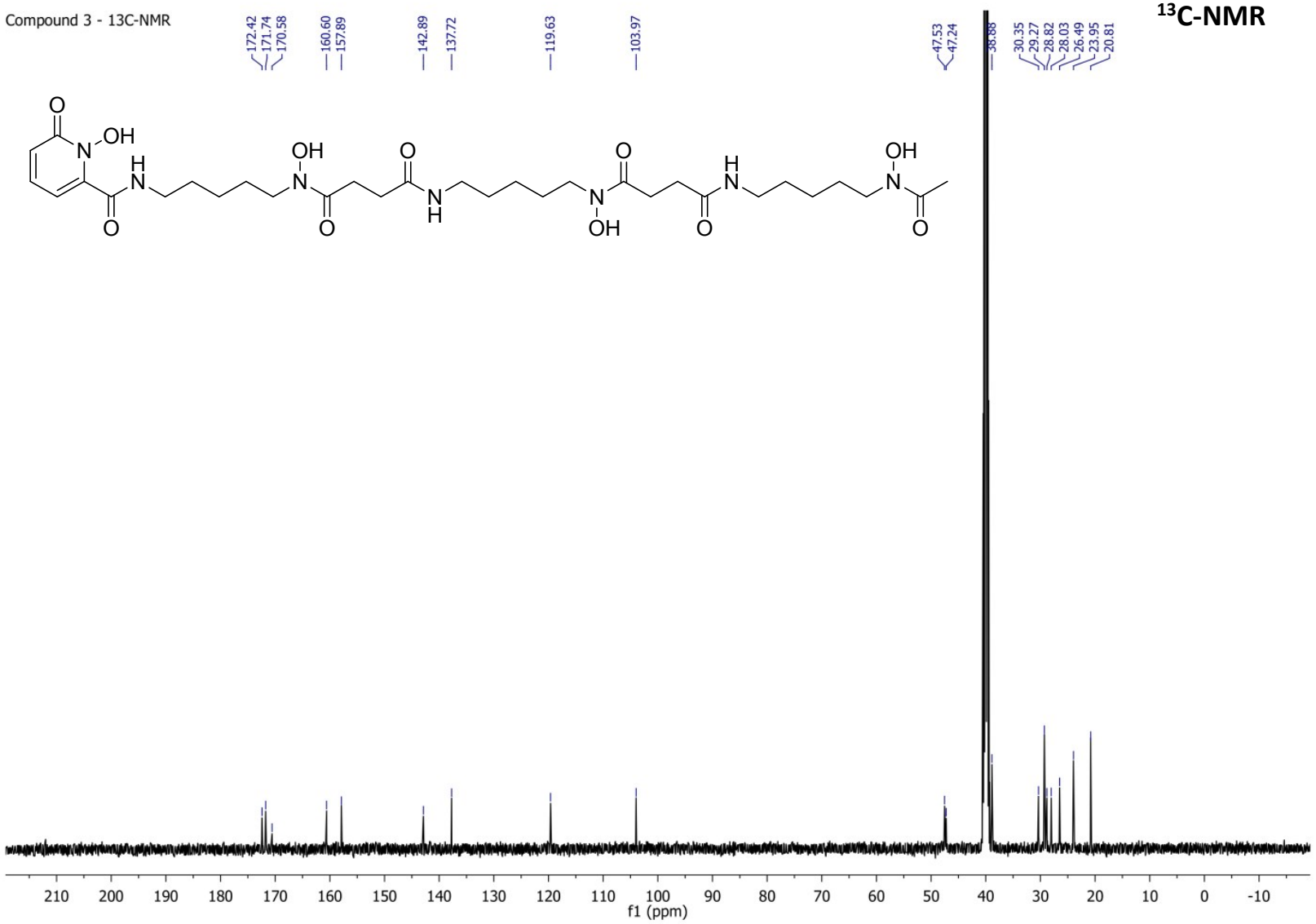



Compound 1 - 13C-NMR
162.35
157.57
139.34
137.07
120.77
106.69
13C-NMR
HO
O
N
OH
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

***N*1-Hydroxy-*N*1-(5-(4-(hydroxy(5-(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamido)pentyl)amino)-4-oxobutanamido)pentyl)-*N*4-(5-(N-hydroxyacetamido)pentyl)succinamide. (DFO-HOPO, 3)**



^{13}C-NMR


Compound 3 - 13C-NMR
172.42
171.74
170.58
160.60
157.89
142.89
137.72
119.63
103.97
47.53
47.24
38.88
30.35
29.27
28.82
28.03
26.49
23.95
20.81
f1 (ppm)

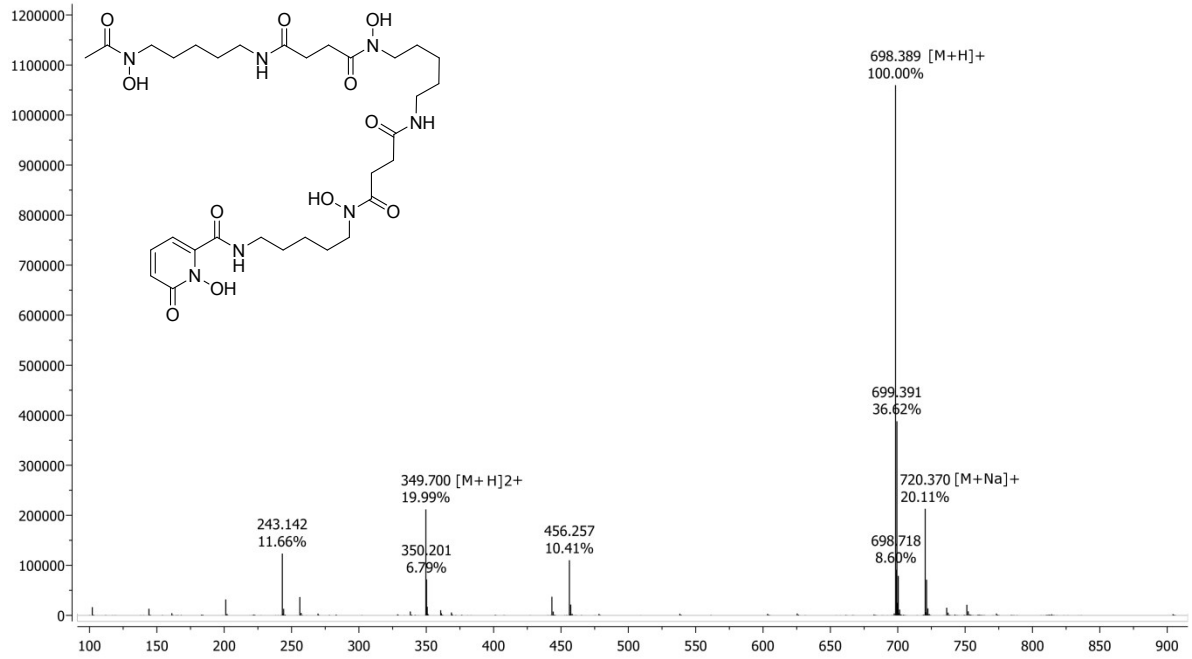


Figure S1. ESI-HRMS analysis of chelator **3**.

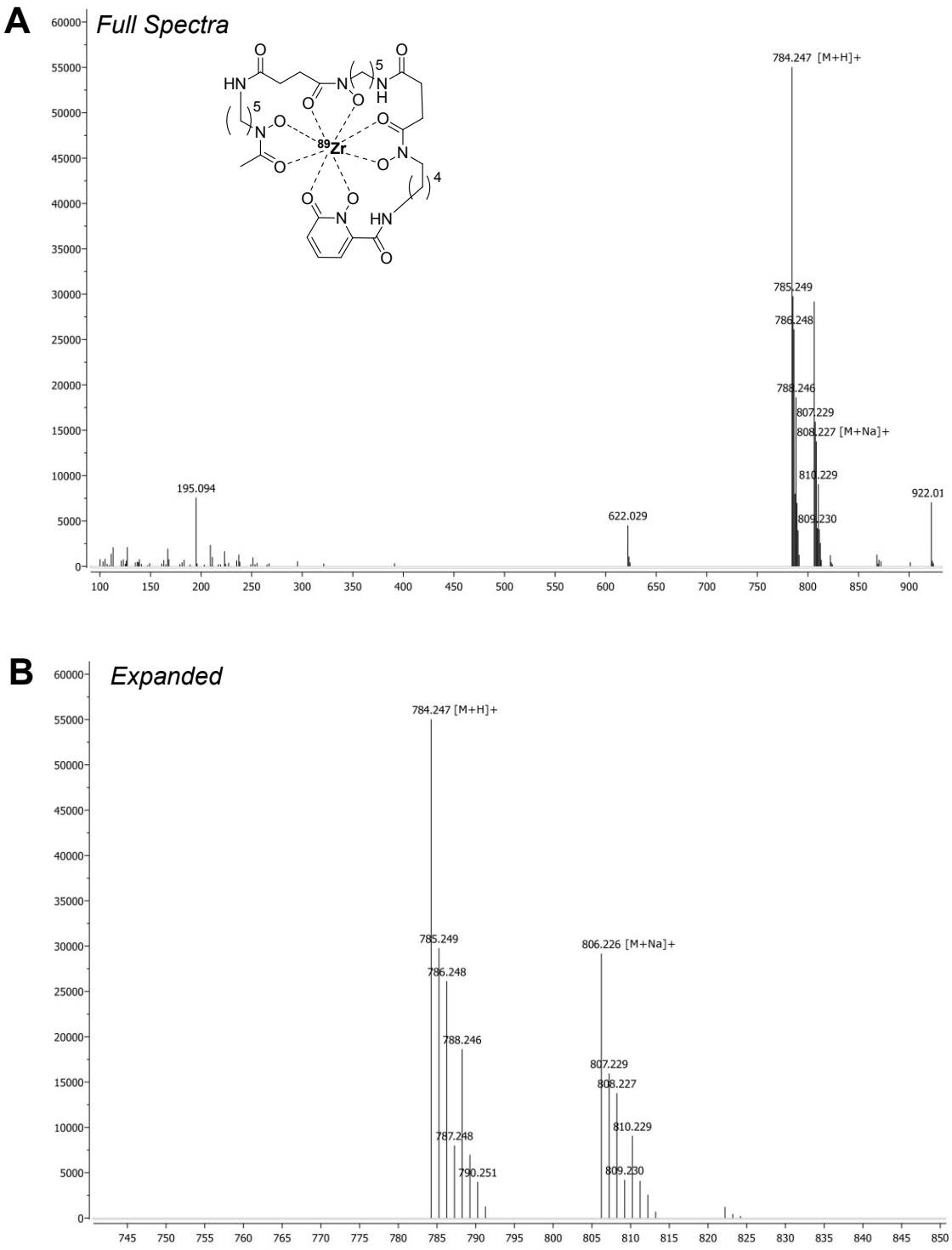


Figure S2. ESI-HRMS analysis of ^{nat}Zr-**3**. The full (**A**) and expanded (**B**) spectra show a 1:1 binding ration of Zr^{4+} to the DFO-HOPO chelator (**3**).

RP-HPLC, UV-VIS AND IR DATA

Analytical and semi-preparative HPLC analyses were carried out on an Agilent Infinity 1260 quaternary pump system equipped with a 1260 Diode array (Agilent Technologies, UK). Elution profiles were analysed using Laura software (Lablogic, Sheffield, UK). The radioactivity of the eluate was monitored using an IN/US Systems Gamma-ram Model 4 NaI radiodetector (Lablogic, Sheffield, UK). Retention times (RT) are expressed as minutes:seconds (min:sec).

Samples were analysed on a Zorbax Eclipse XDB C18 column, 4.6 × 150 mm, 5 µm (Agilent Technologies) using **Gradient 1**: 0-20 min 3%-90% B, 20.1-21 min 90%-3% B, 21.1-35 min 3% B at a flow rate of 1 mL/min with 0.1% TFA in water as eluent A and 0.1% TFA in acetonitrile as eluent B.

Compound **3** was purified by semi-preparative RP-HPLC on a Gemini C18, 10 × 250 mm, 10 µm (Phenomenex) using **Gradient 2:** 0-30 min 3%-90% B, 30.1-31 min 90%-3% B, 40 min 3% B at a flow rate of 3 mL/min with 0.1% TFA in water as eluent A and 0.1% TFA in acetonitrile as eluent B.

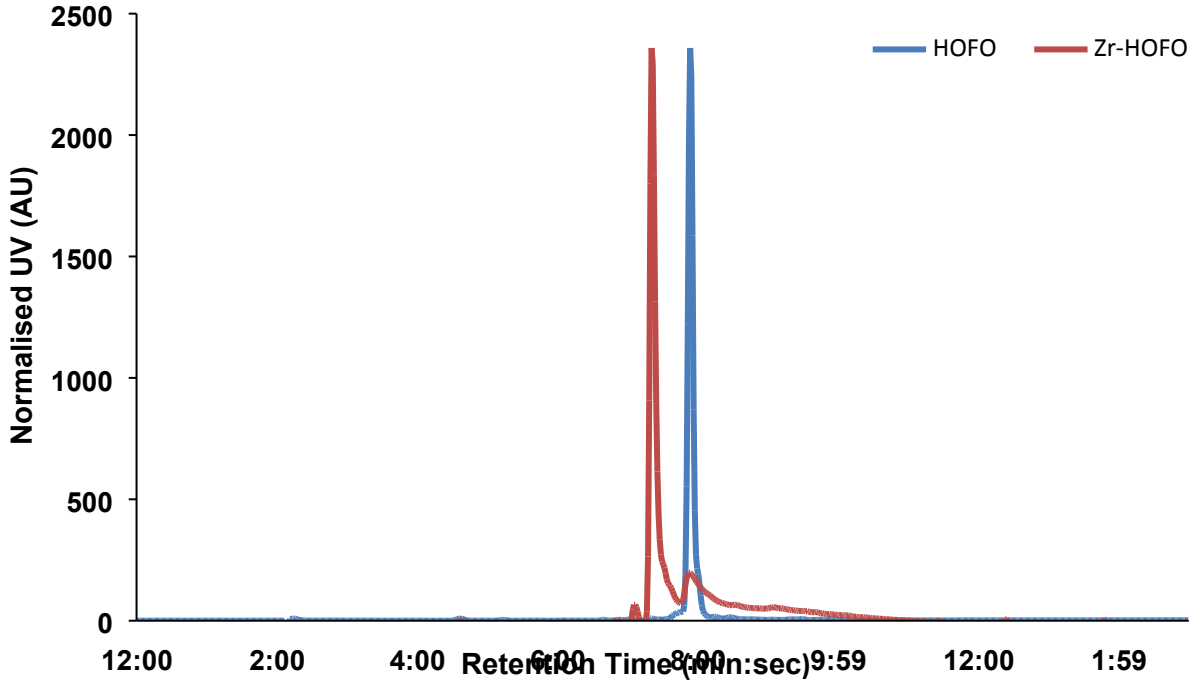


Figure S3. Overlaid HPLC chromatograms (Gradient 1) of **3** (RT = 7:53) and ^{nat}Zr-**3** (RT = 7:20). The absorbance was recorded at the wavelength of 345 nm.

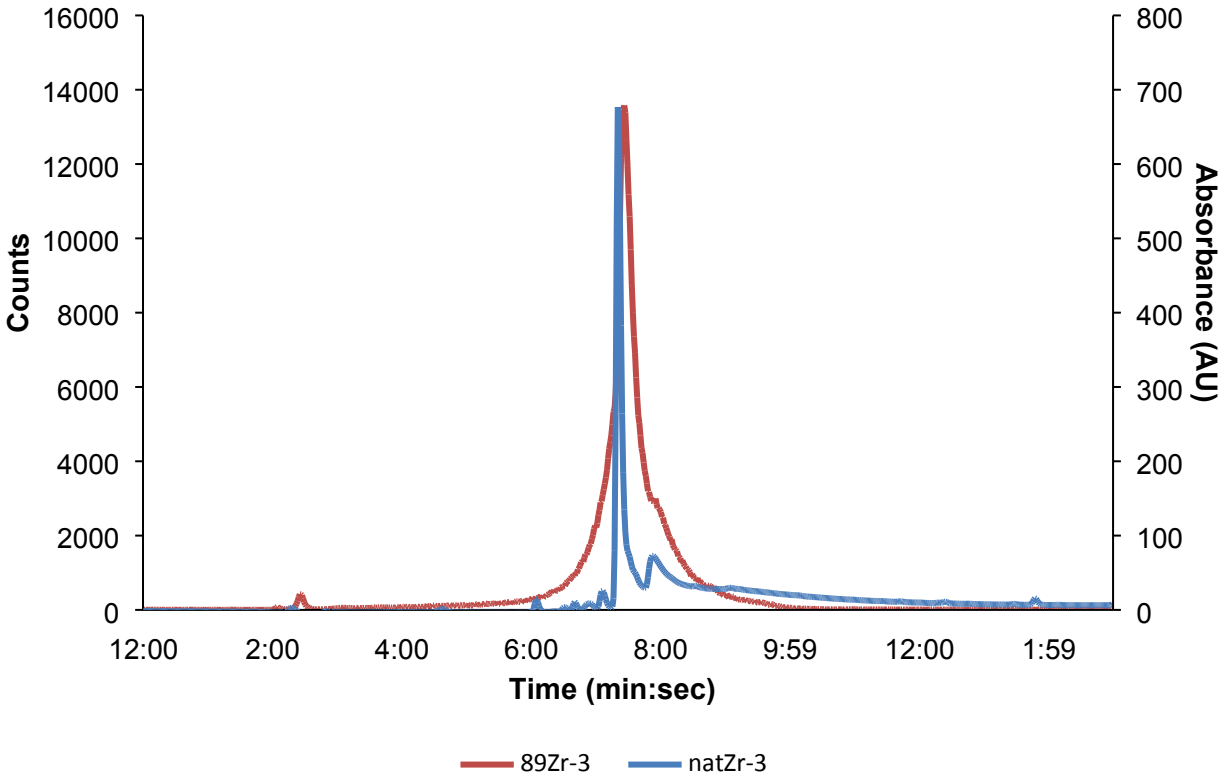


Figure S4. HPLC chromatograms (Gradient 1) of ^{nat}Zr-**3** (RT = 7:20. The absorbance was recorded at the wavelength of 345 nm) and 24 hours old ^{89}Zr-**3** (RT = 7:26. The radioactivity was measured in γ-counts). The sequential position of the UV and radioactive detectors caused the few seconds of separation between the two peaks.

UV-Vis analysis

UV-vis measurements were performed on a NanoDrop 2000 spectrophotometer (Thermo Scientific, UK).

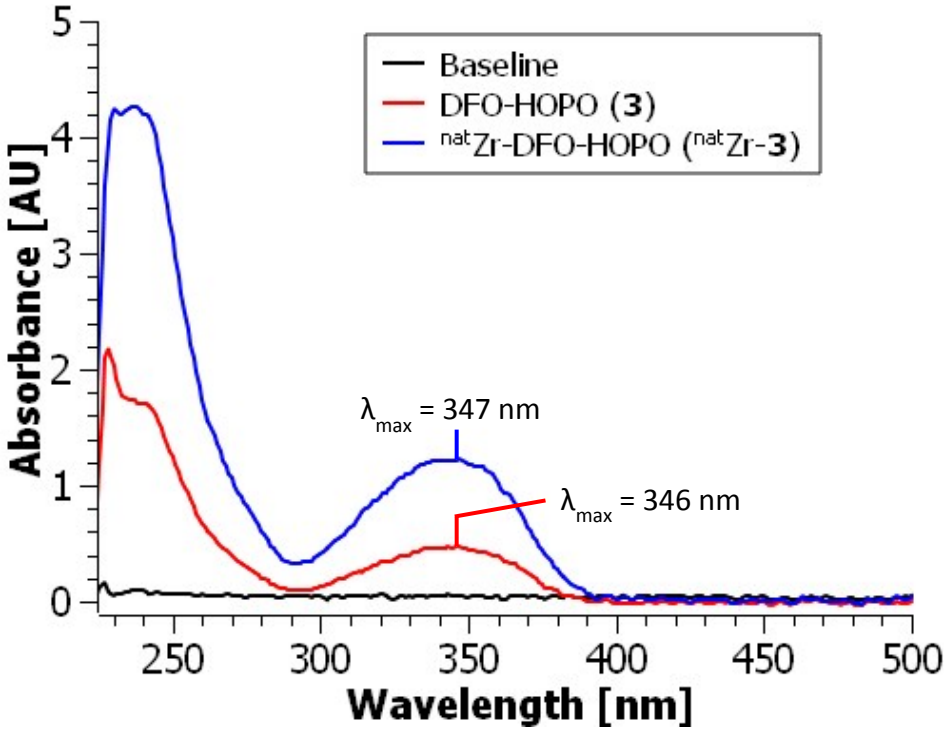


Figure S5. The UV-vis spectra of chelator **3** and the complex ^{nat}Zr-**3** show no detectable changes in the λ_{max} after the incorporation of the metal.

IR analysis

IR measurements were performed on a Bruker Alpha P FT-IR spectrometer (Bruker, UK).

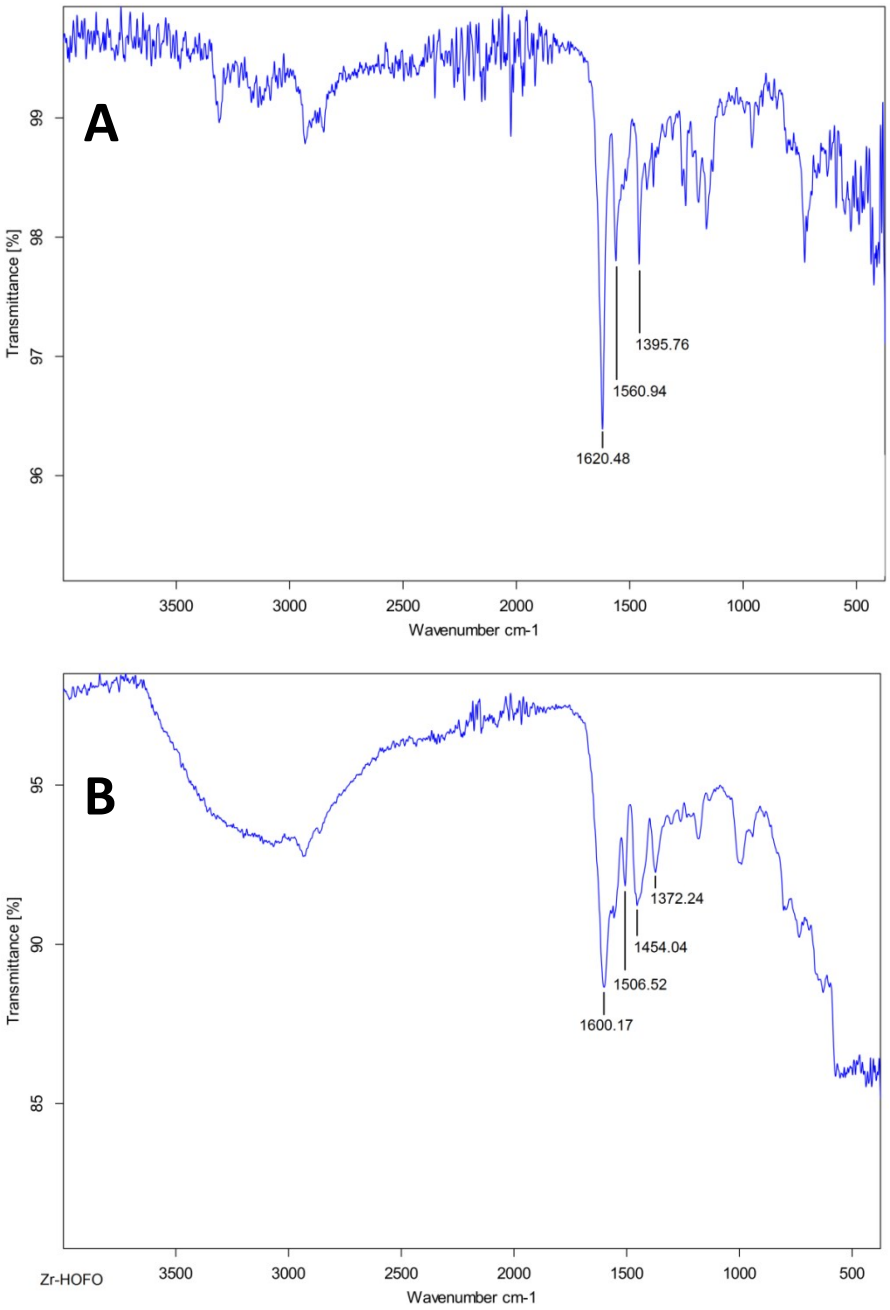


Figure S6. IR spectra of chelator **3** (**A**) and ^{nat}Zr-**3** (**B**). The free chelator shows a strong stretching band at ca 1620 cm^{-1} and a ring stretching band at ca 1560 cm^{-1}. With bands at ca 1660 cm^{-1} and 1506 cm^{-1}, a red-shift is observed in the complex ^{nat}Zr-**3**.

DFT CALCULATIONS

Density functional theory (DFT) calculations were performed for the Zr-**3** complex to investigate the geometry and coordination of **3** around the Zr^{4+} ion. Gas phase DFT calculations were performed using the Jaguar software package (Schrödinger Release 2016-4: Jaguar, Schrödinger LLC, New York, NY, 2016) utilising the standard hybrid B3LYP functional in conjunction with the double zeta form of the Los Alamos effective core potential (ECP) basis set, LACVP.[3, 4] The neutral, gas phase optimised geometry of DFO was retrieved from Deri *et al.* and transformed to incorporate the HOPO moiety using the Maestro build tool (Schrödinger Release 2016-4: Jaguar, Schrödinger LLC, New York, NY, 2016).[1] The energy optimised Zr-**3** structure was prepared for publication using PyMOL (PyMOL: The Molecular Graphics System, Version 1.8, Schrödinger, LLC.) Detailed data regarding DFT calculation and the coordinates of the optimised geometry can be found in DFT_calculation.log and DFT_structure.mol2 files.

	NO-Zr (Å)	**CO-Zr (Å)**
(Terminal) CONO 1	2.15	2.28
CONO 2	2.14	2.36
CONO 3	2.14	2.31
1,2-HOPO CONO	2.27	2.22

Table S1. DFT calculated Zr-O bond lengths. DFT calculations were performed using the Jaguar software package using the B3LYP functional with the LACVP basis set.

RADIOLABELLING WITH ^{89}Zr(IV) AND STABILITY STUDIES OF ^{89}Zr-3

General procedure for the radiolabelling of chelator 3 and DFO with $^{89}Zr^{4+}$

Depending on the number and kind of radiolabelling experiments, a neutralised stock solution of $^{89}Zr^{4+}$ was prepared and then used for the various radiolabelling reactions. Briefly, the ^{89}Zr neutralised stock solution was prepared as follows:
Aqueous 2 M Na_2CO_3 (6-12.6 µL) was added to a solution of ^{89}Zr in 1M oxalic acid (12.5-28 µL, 20-45 MBq) followed by 0.5 M HEPES pH 7 (32-70 µL) to reach pH 7.
Both **3** and DFO chelators were radiolabelled at the required concentrations and specific activities with the neutralised ^{89}Zr solution at room temperature for 1 h. The reactions were analysed by radio-TLC using either ITLC-SG or ITLC-SA strips depending on the type of experiment.

Selection of the mobile phase for the radio-ITLC of ^{89}Zr-3 and ^{89}Zr-DFO

Using SG-ITLC strips, the following mobile phases (of different composition and pH) were investigated to analyse ^{89}Zr-**3** and ^{89}Zr-DFO:

1) 50 mM EDTA pH 5
2) 0.1 M ammonium acetate + 25 mM EDTA pH 7
3) 0.1 M sodium citrate pH 2

The migration of the products was indicated as retention factor (R_f).
Because of a clearer migration of the products and the separation between the complexes, 0.1 M ammonium acetate pH 7 (with or without EDTA, depending on the nature of the experiments) was chosen as the mobile phase for radio-ITLC analysis.

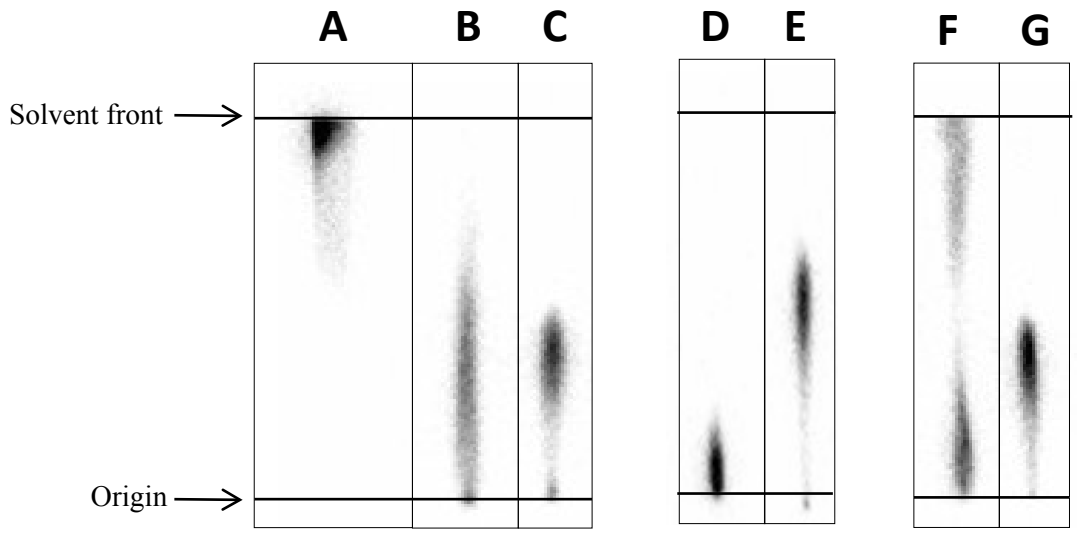


Figure S7. The reaction mixtures containing either ^{89}Zr-DFO or ^{89}Zr-**3** (ca 3 MBq/nmol) were analysed on SG-ITLC strips using different mobile phases. When 50 mM EDTA pH 5 was utilised as mobile phase (left), un-chelated ^{89}Zr migrates with the solvent front (R_f = 1.0) (**A**); ^{89}Zr-DFO complex migrates as a smeared band with an R_f = 0.57 (**B**); ^{89}Zr-**3** migrates with an R_f = 0.48 (**C**). Using 0.1 M ammonium acetate + EDTA pH 7 as mobile phase (centre): ^{89}Zr-DFO complex migrates with an R_f = 0.11 (**D**); ^{89}Zr-**3** migrates with an R_f = 0.66 (**E**). Using 0.1 M sodium citrate pH 2 as mobile phase (right): in the case of ^{89}Zr-DFO, a main band (R_f = 0.28) together with a band which migrates with the solvent front (R_f = 1) were detected (**F**); ^{89}Zr-**3** migrates with an R_f = 0.48 (**G**). Either the low pH or the high content of citrate (a chelating buffer) of the mobile phase could have caused the demetallation (14.4 ± 4.65%) of the ^{89}Zr-DFO complex, while ^{89}Zr-**3** showed to be stable in the same conditions.

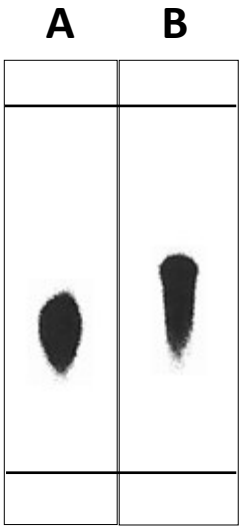


Figure S8. Radio-ITLC analysis of ^{89}Zr-**3** (**A**) and ^{89}Zr-DFO (**B**) (ca 15 MBq/nmol) using SG-ITLC strips and 0.1 M sodium citrate pH 5 as mobile phase. ^{89}Zr-DFO shows just one band (R_f = ca 0.58) suggesting that the demetallation observed in 0.1 M sodium citrate pH 2 was due to the low pH and not to a transchelation process promoted by the high concentration of citrate in the mobile phase.

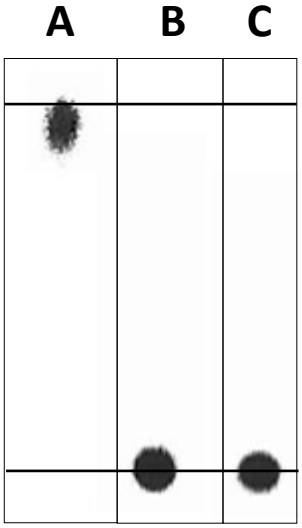


Figure S9. Radio-ITLC analysis of ^{89}Zr-DFO and ^{89}Zr-**3** using SA-ITLC strips and 0.1 M ammonium acetate + 25 mM EDTA pH 7 as mobile phase. ^{89}Zr that is not associated to the chelators migrates with the mobile phase front (**A**), while both ^{89}Zr-DFO (**B**) and ^{89}Zr-**3** (**C**) remain at the origin. Because of the clear separation between the non-chelator-associated and

chelator-associated radioactivity, this stationary and mobile phase system was used for serum stability analysis.

Radiolabelling of DFO-HOPO (3) and analysis of the effects of the radiolabelling conditions on the product composition

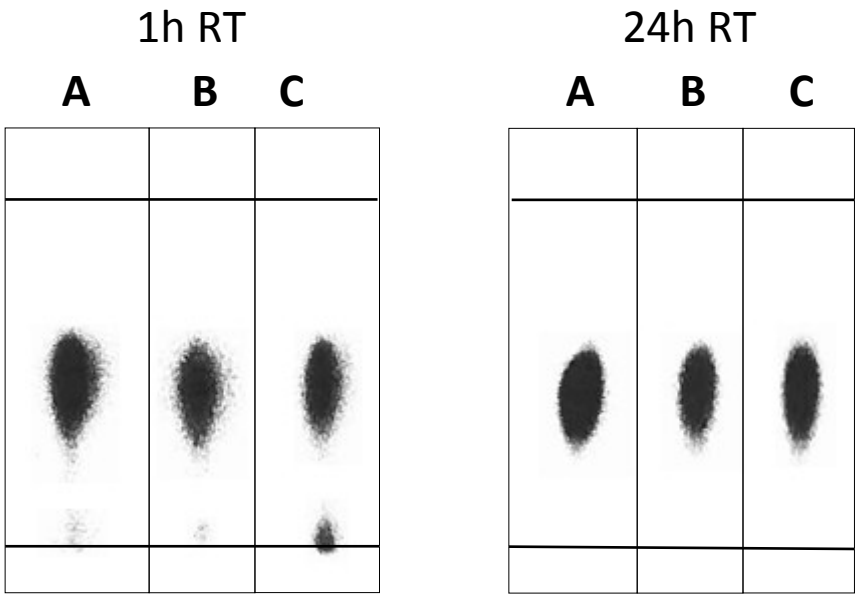


Figure S10. Representative radio-ITLC analysis of ^{89}Zr-**3** at different specific activities: 5 MBq/nmol (final concentration of **3** = 24 μM) (**A**), 15 MBq/nmol (final concentration of **3** = 8 μM) (**B**) and 20 MBq/nmol (final concentration of **3** = 6.5 μM) (**C**) after incubation for 1 h (left) and 24 h (right) at ambient temperature. The analysis was performed using SG-ITLC strips and 0.1 M ammonium acetate + 25 mM EDTA pH 7 as mobile phase. Quantitative (>98%) radiolabelling could be achieved up to 20 MBq/nmol even at low concentration of the chelator (6.5 μM).

The analysis of the 1 h mixtures show the presence of two bands having different R_f. The quantity of product having an R_f = ca 0.1 increases with the increase of the specific activity (i.e. the decrease of the quantity of chelator **3** in solution). After 24 h at ambient temperature, only the product having R_f = ca 0.6 is detected.

Specific activity (MBq/nmol)	^{89}Zr Incorporation (1h, rt)	Product R_f = 0.11 (%)	Product R_f = 0.6 (%)
5	>99 %	6.5	93.5
15	>99 %	13.4	86.6
20	>99 %	20.0	80.0

Table S2. Data from the radio-ITLC analysis of ^{89}Zr-**3** at different specific activities shown in Figure S10 (5, 15, and 20 MBq/nmol with a chelator concentration of 24, 8, and 6.5 μM respectively). The results show that the quantity of product having an R_f = ca 0.1 increases with the increase of the specific activity (i.e. the decrease of the quantity of chelator **3** in solution).

	Reaction temperature	^{89}Zr Incorporation (%)	Product R_f = 0.11 (%)
15 MBq/nmol	rt	>99	6.3
	0°C	95.5	12.3
20 MBq/nmol	rt	>99	8.5
	80°C	>99	0.24
60 MBq/nmol	rt	70	13.1
	80°C	98	3.9

Table S3. ^{89}Zr-**3** was synthesised at different specific activities (15, 20 and 60 MB/nmol with chelator concentrations of 8.5, 3 and 1 μM respectively) either at 0°C, rt, or 80°C (1 h reaction time). Radio-ITLC analysis (SG-ITLC strips using 0.1 M ammonium acetate + 25 mM EDTA pH 7 as mobile phase) shows the correlation between the reaction temperature and both the ^{89}Zr incorporation and the quantity of the product having an R_f = ca 0.1. The results show that a higher ^{89}Zr incorporation and a lower quantity of product having R_f = ca 0.1 were achieved with the increase of the temperature.

Determination of $LogD_{7.4}$ for ^{89}Zr-3

24 Hours old ^{89}Zr-**3** (ca 10 KBq, only the product with Rf = 0.6 present) was added to 0.5 mL of PBS (pH 7.4) followed by an equal volume of *n*-octanol. The mixture was vortexed for 10 min and then centrifuged at 100 × g for 10 min. The experiments were performed in triplicate. Three 100 µL samples were taken from each layer and the amount of activity was measured in a 2480 WIZARD^2 Automatic Gamma Counter (Perkin Elmer, UK) as counts per minute (cpm). The distribution coefficient at pH 7.4 ($LogD_{7.4}$) was expressed as the mean ± standard deviation (SD) and calculated using the formula: $LogD = \log[(counts_{octanol})/(counts_{PBS})]$.

	$LogD_{7.4}$ (mean ± SD)
^{89}Zr-**3**	-0.87 ± 0.03

EDTA challenge study at pH 7 (1:100 ratio chelator/EDTA)

A 0.1M EDTA solution (3.7 µL, 0.37 µmol) was added to solutions containing either ^{89}Zr-DFO or ^{89}Zr-**3** (3.7 MBq, 1MBq/nmol) followed by 0.5 M HEPES pH 7 to have a final volume of 0.5 mL and pH 7. Each sample was immediately analysed by radio-TLC to obtain the initial percent of ^{89}Zr incorporation (T = 0 min). Samples containing the radiolabelled chelators and no EDTA were used as controls. All reaction mixtures were incubated at 37°C with agitation (400 rpm) in a Thermomixer. The experiments were performed in triplicate. Samples were monitored at 1 h, 3 h, 1 d, 3 d and 7 d post incubation on SG-ITLC using 0.1 M ammonium acetate pH 7 as mobile phase. The control solutions were analysed using 0.1 M ammonium acetate pH 7 + 25 mM EDTA as mobile phase. The stability of the complexes was determined by calculating the ratio between the radioactivity associated with the complex (R_f = 0.6 for ^{89}Zr-**3** and R_f = 0.11 for ^{89}Zr-DFO) and what migrated with the solvent front (^{89}Zr-EDTA, R_f = 1).

^{89}Zr-3 and ^{89}Zr-DFO transchelation experiments

^{89}Zr-**3** and ^{89}Zr-DFO (3.7 MBq, 3 MBq/nmol) were mixed with a ca 3200-fold molar excess of either DFO or **3** respectively (32.4 µL of a 125 µM solution of the chelator, 4 µmol) followed by

0.5 M HEPES pH 7 to a final volume of 1 mL. Each sample was immediately analysed by radio-TLC to obtain the initial percent of ^{89}Zr incorporation (T = 0 min). Samples containing only the radiolabelled chelators were used as controls. All reaction mixtures were incubated at 37°C with agitation (400 rpm) in a Thermomixer. The experiments were performed in triplicate. Samples were monitored at 1 h, 3 h, 1 d, 3 d and 7 d post incubation on SG-ITLC using 0.1 M ammonium acetate pH 7 as mobile phase. The transchelation was determined by the ratio between ^{89}Zr-**3** (R_f = 0.66) and ^{89}Zr-DFO (R_f = 0.11).

***In vitro* serum stability**

^{89}Zr-**3** and ^{89}Zr-DFO (3.8 MBq, 1 MBq/nmol) were added to mouse serum (0.5 mL). Each sample was immediately analysed by radio-TLC to obtain the initial percent of ^{89}Zr incorporation (T = 0 min). A sample containing ^{89}Zr-oxalate was used as control. The mixtures were incubated at 37°C with agitation (400 rpm) in a Thermomixer. The experiments were performed in triplicate. Samples were monitored at 3 h, 1 d, 3 d and 7 d post incubation on SA-ITLC using 0.1 M ammonium acetate + 25 mM EDTA pH 7 as mobile phase. The SA-ITLC paper was preferred to the SG-ITLC one because of the clearer separation between the non-chelator-associated and chelator-associated radioactivity.
The stability towards demetallation of the radiolabelled chelators was determined by calculating the ratio between the radioactivity at the origin of the strip (associated to the intact ^{89}Zr-DFO and ^{89}Zr-**3**) and what migrated towards the solvent front.

***IN VIVO* STUDIES**

All experiments were performed in compliance with licences issued under the UK Animals (Scientific Procedures) Act 1986 and following local ethical review. Studies were compliant with the United Kingdom National Cancer Research Institute Guidelines for Animal Welfare in Cancer Research. PET/CT imaging studies were conducted using an Albira PET/SPECT/CT imaging system. Healthy female NCr athymic mice (6-8 week-old) were administered ^{89}Zr-**3** in 0.9% sterile saline (7-8 MBq/mouse, 15 MBq/nmol. Prepared the day before and checked by radio-TLC to confirm both the ^{89}Zr incorporation and the presence of only the product with R_f = 0.6 before injection) by intravenous tail vein injection and approximately 5 minutes prior to

imaging were anesthetised using isoflurane/O_2 mixture (1.5-2.0 % v/v) and placed prone in the centre of the scanner's field of view. The whole body PET images were acquired at different time points (1, 4 and 24 h) for the duration of 15 min with a 358 to 664 keV energy window. Images were reconstructed using a MLEM algorithm (12 iterations) with a voxel size of 0.5 × 0.5 × 0.5 mm^3. No attenuation or partial-volume averaging corrections were applied. Whole body standard high resolution CT scans were performed with the X-ray tube set-up at a voltage of 45 kV, current of 400 μA and 250 projections (1s per projection) and a voxel size of 0.5 × 0.5 × 0.5 mm3. The CT images were reconstructed using a FBP algorithm. The image analysis was performed using the PMOD software package (PMOD Technologies Ltd, CH).

For biodistribution studies, either ^{89}Zr-DFO or ^{89}Zr-**3** in 0.9% sterile saline (0.9-1 MBq, 15 MBq/nmol) was administered to healthy female NCr athymic mice (6-8 week-old). The mice were euthanised by cervical dislocation at 1, 4 and 24 h after injection. The major organs/tissues were dissected, weighed, and the radioactivity was measured in a 2480 WIZARD^2 Automatic Gamma Counter (PerkinElmer, UK). The percentage of the injected dose per gram of tissue (%ID/g) was determined for each organ/tissue. The data are expressed as the average of n = 3 mice ± SD. Significant differences were assessed by unpaired *t*-test using GraphPad Prism (GraphPad Software for Windows 7.01 version, California, US).

Organs	^{89}Zr-DFO (%ID/g ± SD)			^{89}Zr-3 (%ID/g ± SD)		
	1h	4h	24h	1h	4h	24h
Blood	0.046±0.022	0.007±0.002	0.002±0.000	0.205±0.072	0.012±0.003	0.003±0.000
Heart	0.023±0.009	0.012±0.007	0.011±0.001	0.069±0.016	0.007±0.002	0.003±0.000
Lung	0.100±0.032	0.030±0.007	0.041±0.014	0.271±0.069	0.074±0.051	0.004±0.001
Kidneys	1.721±0.327	1.441±0.248	0.926±0.112	1.397±0.099	0.303±0.048	0.075±0.007
Spleen	0.033±0.007	0.026±0.004	0.033±0.001	0.080±0.012	0.010±0.004	0.004±0.001
Liver	0.217±0.035	0.117±0.018	0.059±0.006	0.245±0.031	0.073±0.004	0.019±0.001
Pancreas	0.015±0.006	0.007±0.003	0.008±0.001	0.035±0.007	0.003±0.001	0.005±0.003
Bone	0.028±0.005	0.022±0.006	0.037±0.002	0.083±0.011	0.015±0.004	0.004±0.001
Stomach	0.088±0.084	0.024±0.009	0.015±0.001	0.220±0.144	0.048±0.020	0.002±0.000
SI	0.039±0.005	0.032±0.009	0.016±0.000	1.346±1.442	0.216±0.227	0.003±0.000
LI	0.080±0.038	0.060±0.009	0.017±0.002	0.241±0.154	0.898±0.252	0.002±0.001
Muscle	0.021±0.010	0.016±0.015	0.011±0.008	0.051±0.001	0.007±0.001	0.004±0.003

Table S4. Biodistribution results (%ID/g) for ^{89}Zr-DFO and ^{89}Zr-**3** at 1, 4 and 24 h p.i. SI = small intestine; LI = large intestine. The clearance of the two radiocomplexes is prevalently renal for ^{89}Zr-DFO and a combination of renal and hepatobiliary for ^{89}Zr-**3**.

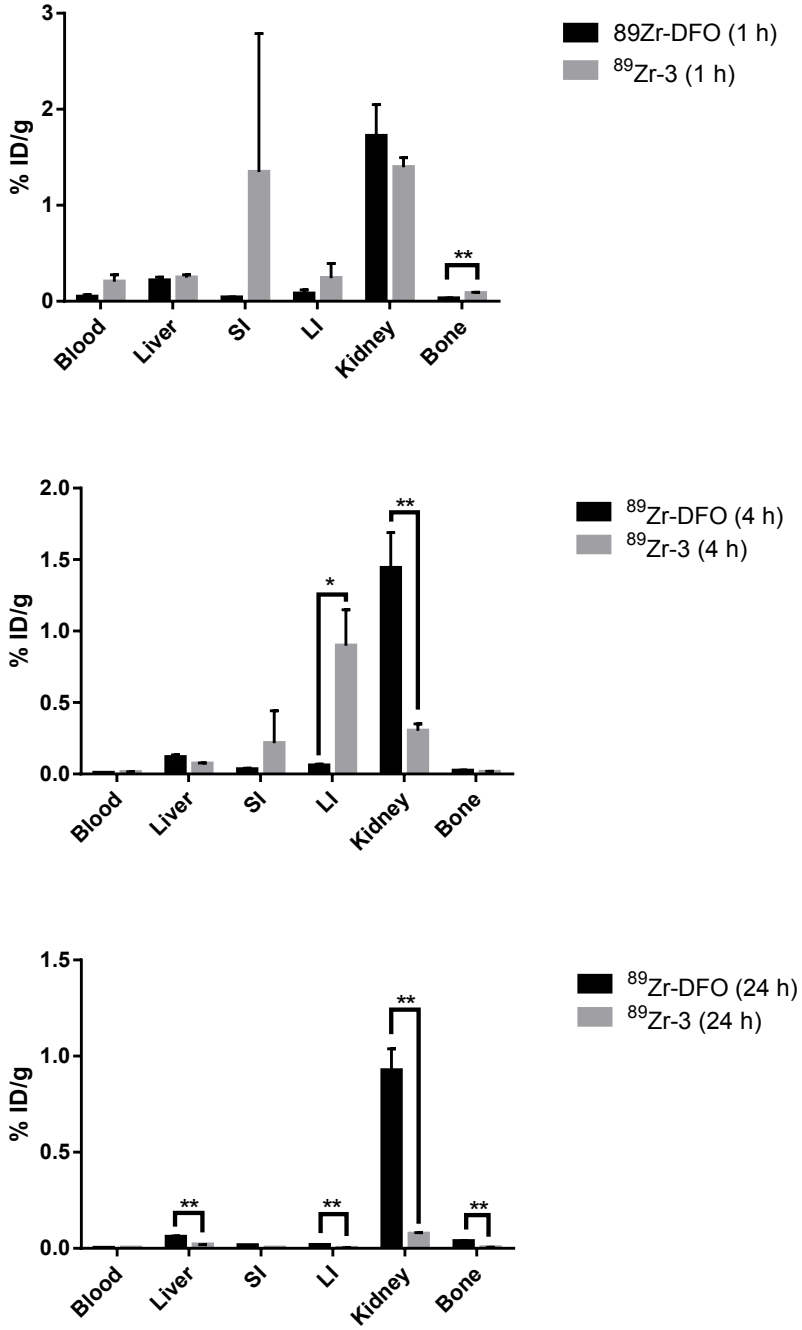


Figure S11. Biodistribution data for ^{89}Zr-**3** and ^{89}Zr-DFO at 1, 4 and 24 h p.i. in selected organs. SI = small intestine; LI = large intestine. Significance was calculated using unpaired *t*-test (* = $P \leq 0.05$and ** = $P \leq 0.001$). The levels of ^{89}Zr in the bone could be correlated to either the quantity of ^{89}Zr present in the animals (1 and 24 h p.i.) or to an improved *in vivo* stability of ^{89}Zr-**3** compared to ^{89}Zr-DFO (24 h p.i).

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