

## Electronic Supporting Information (ESI)

### Microfluidic preparation of $^{89}\text{Zr}$ -radiolabeled proteins by flow photochemistry

Daniel F. Earley,<sup>1</sup> Amaury Guillou,<sup>1</sup> Dion van der Born,<sup>2</sup> Alex J. Poot,<sup>3</sup> and Jason P. Holland<sup>1\*</sup>

1. Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland
2. FutureChemistry Agro Business Park 10, 6708 PW, Wageningen, The Netherlands
3. Department of Radiology and Nuclear Medicine, UMC Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

**\* Corresponding Author:**

Prof. Dr Jason P. Holland

Tel: +41.44.63.53.990

E-mail: [jason.holland@chem.uzh.ch](mailto:jason.holland@chem.uzh.ch)

Website: [www.hollandlab.org](http://www.hollandlab.org)

**First Author:**

Daniel F. Earley

Email: [daniel.earley@chem.uzh.ch](mailto:daniel.earley@chem.uzh.ch)

## Table of Contents

<b>Figure S1.</b> $^1\text{H}$ (400 MHz, $\text{CDCl}_3$ , 298 K) NMR spectrum of compound <b>2</b> .....	3
<b>Figure S2.</b> $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, $\text{CDCl}_3$ , 298 K) NMR spectrum of compound <b>2</b> .....	3
<b>Figure S3.</b> HRMS (negative mode) spectrum of compound <b>2</b> .....	3
<b>Figure S4.</b> $^1\text{H}$ (400 MHz, $\text{CDCl}_3$ , 298 K) NMR spectrum of compound <b>4</b> .....	4
<b>Figure S5.</b> $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, $\text{CDCl}_3$ , 298 K) NMR spectrum of compound <b>4</b> .....	4
<b>Figure S6.</b> HRMS spectrum of compound <b>4</b> .....	4
<b>Figure S7.</b> $^1\text{H}$ (400 MHz, $\text{CDCl}_3$ , 298 K) NMR spectrum of compound <b>5</b> .....	5
<b>Figure S8.</b> $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, $\text{CDCl}_3$ , 298 K) NMR spectrum of compound <b>5</b> .....	5
<b>Figure S9.</b> HRMS spectrum of compound <b>5</b> .....	5
<b>Figure S10.</b> $^1\text{H}$ (400 MHz, MeOD, 298 K) NMR spectrum of compound <b>6</b> .....	6
<b>Figure S11.</b> $^{13}\text{C}\{^1\text{H}\}$ (101 MHz, MeOD, 298 K) NMR spectrum of compound <b>6</b> .....	6
<b>Figure S12.</b> HRMS (negative mode) spectrum of compound <b>6</b> .....	6
<b>Figure S13.</b> $^1\text{H}$ (500 MHz, $\text{DMSO-d}^6$ , 298 K) NMR spectrum of compound <b>1</b> .....	7
<b>Figure S14.</b> $^{13}\text{C}\{^1\text{H}\}$ (125 MHz, $\text{DMSO-d}^6$ , 298 K) NMR spectrum of compound <b>1</b> .....	7
<b>Figure S15.</b> 2D-COSY ( $\text{DMSO-d}^6$ ) NMR spectrum of compound <b>1</b> .....	8
<b>Figure S16.</b> 2D-heteronuclear HSQC ( $\text{DMSO-d}^6$ ) NMR spectrum of compound <b>1</b> .....	8
<b>Figure S17.</b> HRMS spectrum of compound <b>1</b> .....	9
<b>Supporting Results</b> .....	9
$^{89}\text{Zr}$ -Radiolabelling of DFO-PEG <sub>3</sub> -Et-ArN <sub>3</sub> ( <b>1</b> ).....	9
<b>Figure S18.</b> Characterisation data for the radiochemical synthesis of [ $^{89}\text{Zr}$ ]ZrDFO-PEG <sub>3</sub> -Et-ArN <sub>3</sub> . A) Radio-iTLC chromatograms of ‘free’ $^{89}\text{Zr}$ complexed as [ $^{89}\text{Zr}$ ]Zr-DTPA (blue) and [ $^{89}\text{Zr}$ ]ZrDFO-PEG <sub>3</sub> -Et-ArN <sub>3</sub> (black); B) HPLC chromatograms of the ligand (compound <b>1</b> ; blue), [ $^{\text{nat}}\text{Zr}$ ]ZrDFO-PEG <sub>3</sub> -Et-ArN <sub>3</sub> (green) and [ $^{89}\text{Zr}$ ]ZrDFO-PEG <sub>3</sub> -Et-ArN <sub>3</sub> (black). .....	9
<b>References</b> .....	<b>Error! Bookmark not defined.</b>

Figure S1.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ , 298 K) NMR spectrum of compound 2

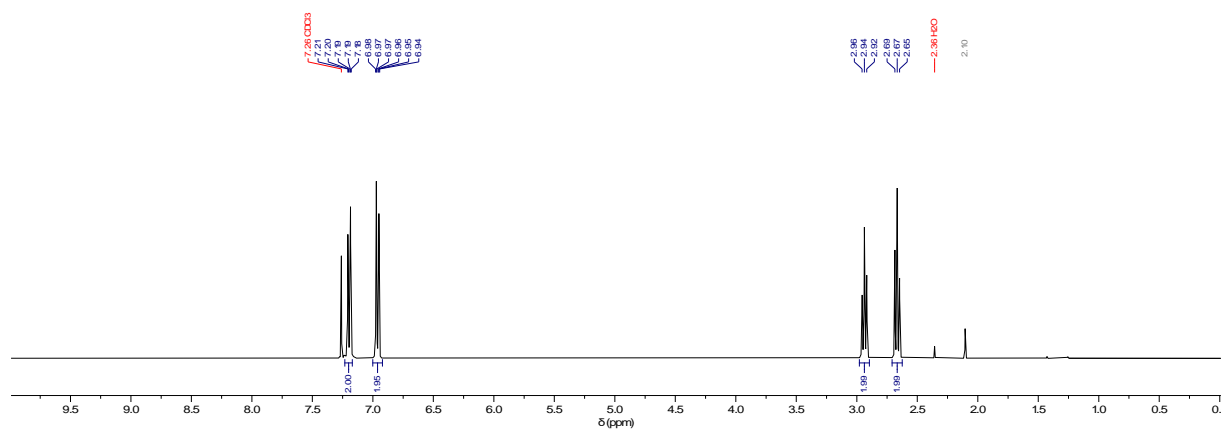


Figure S2.  $^{13}\text{C}\{^1\text{H}\}$  (101 MHz,  $\text{CDCl}_3$ , 298 K) NMR spectrum of compound 2

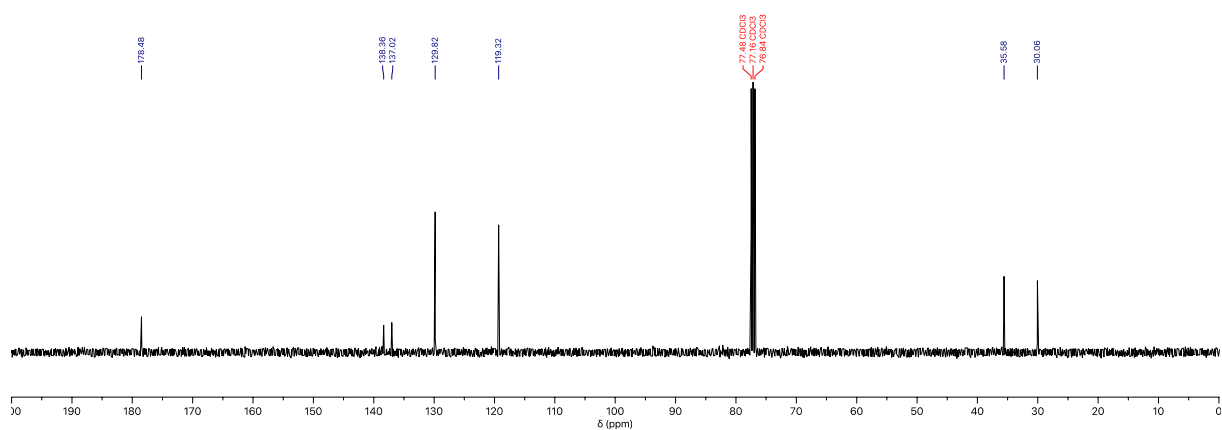


Figure S3. HRMS (negative mode) spectrum of compound 2

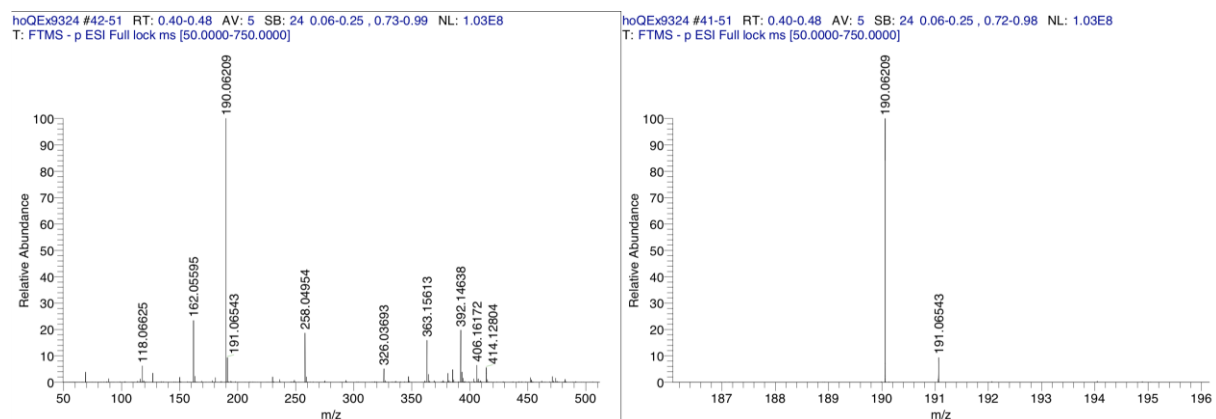


Figure S4.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ , 298 K) NMR spectrum of compound 4

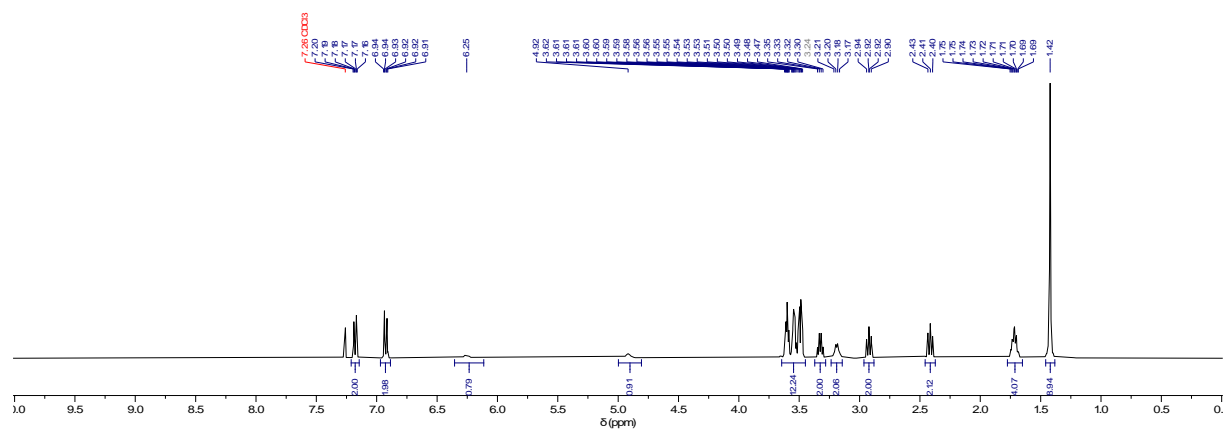


Figure S5.  $^{13}\text{C}\{^1\text{H}\}$  (101 MHz,  $\text{CDCl}_3$ , 298 K) NMR spectrum of compound 4

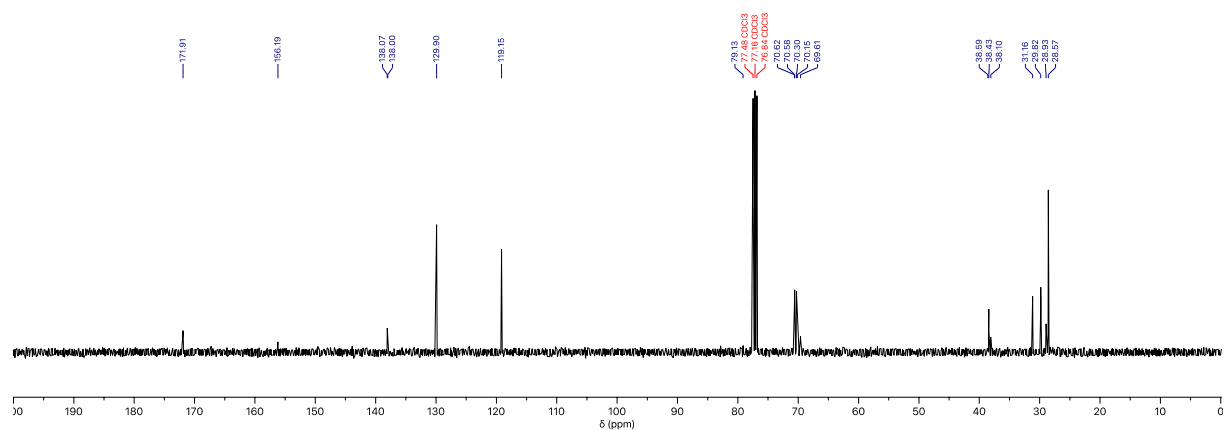


Figure S6. HRMS spectrum of compound 4

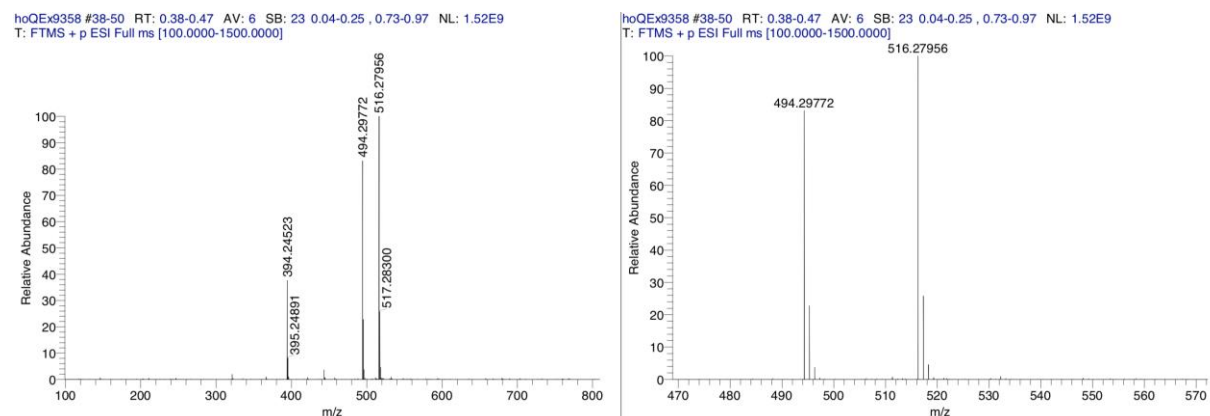


Figure S7.  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ , 298 K) NMR spectrum of compound 5

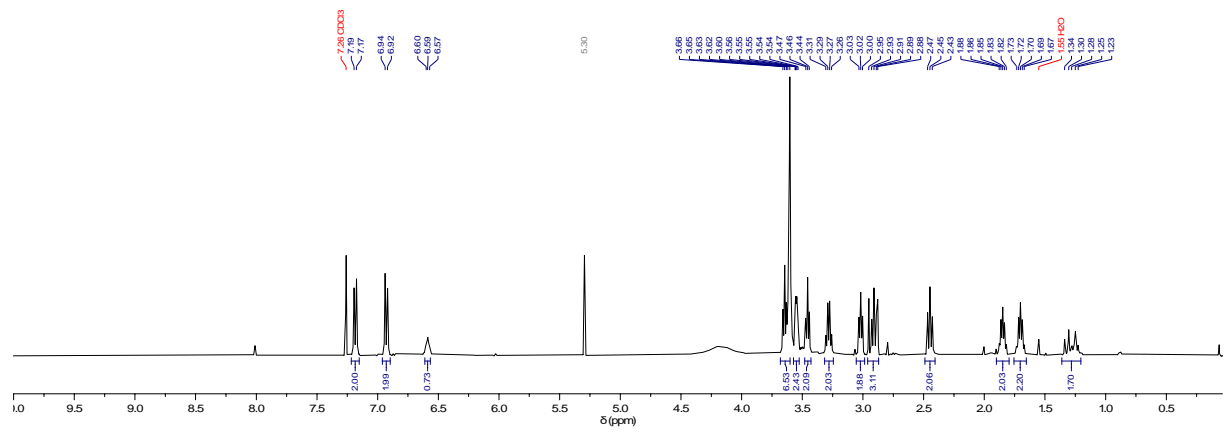


Figure S8.  $^{13}\text{C}\{^1\text{H}\}$  (101 MHz,  $\text{CDCl}_3$ , 298 K) NMR spectrum of compound 5

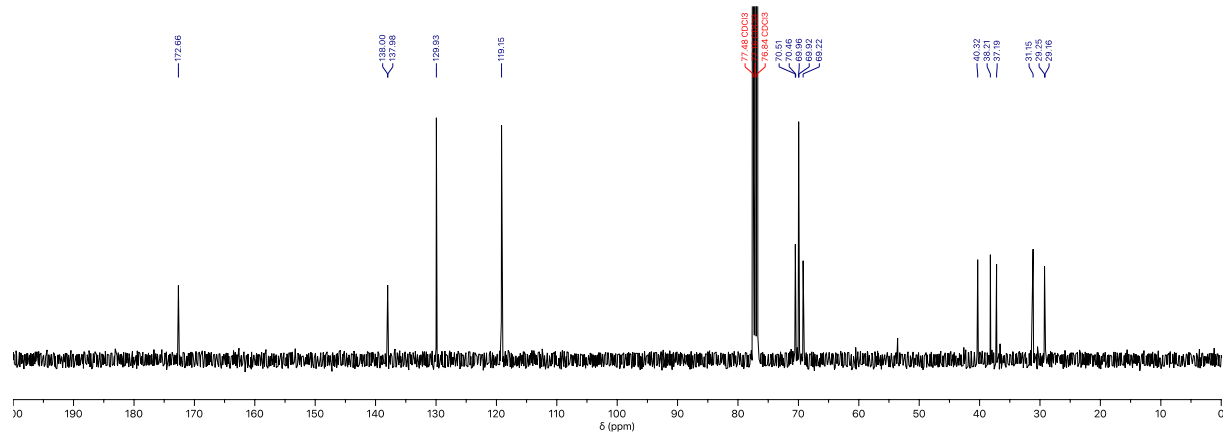
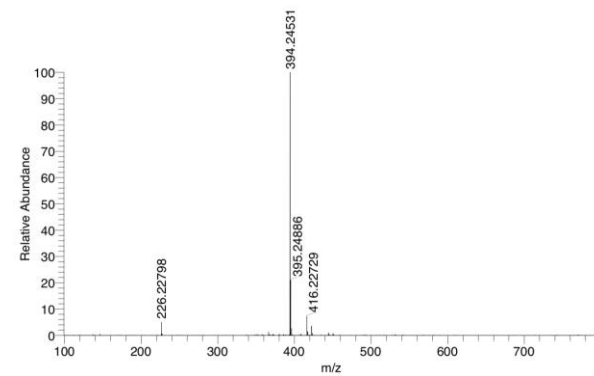
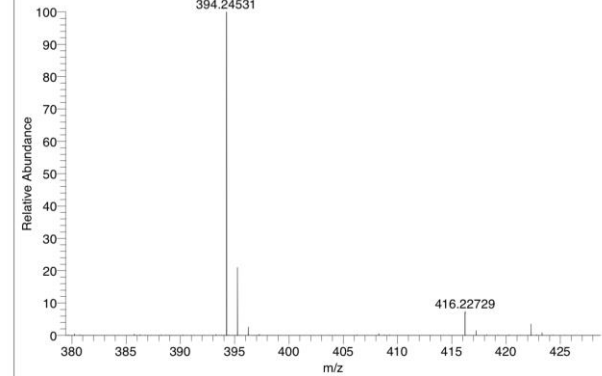


Figure S9. HRMS spectrum of compound 5

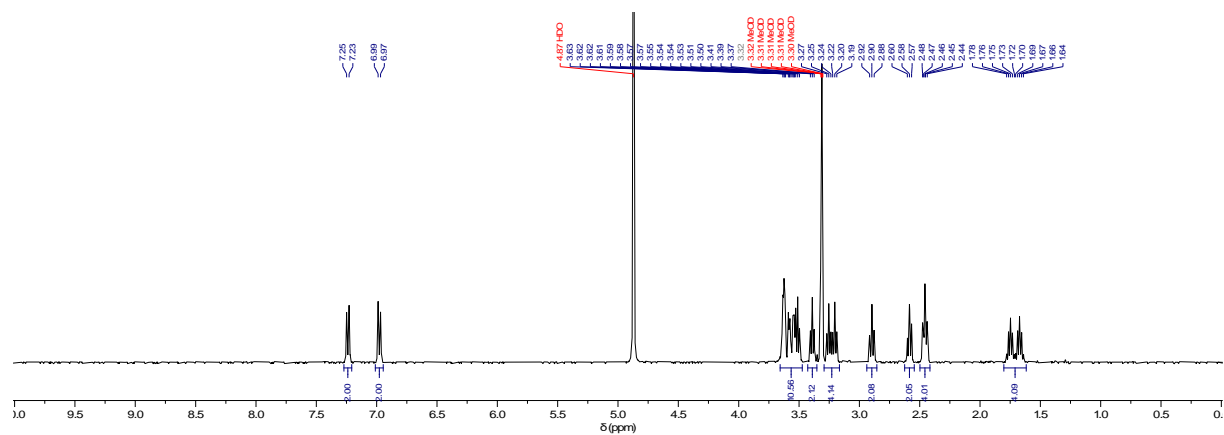
hoQEx9353 #38-50 RT: 0.38-0.47 AV: 6 SB: 24 0.04-0.25 , 0.73-0.97 NL: 3.33E9  
T: FTMS + p ESI Full ms [100.0000-1500.0000]



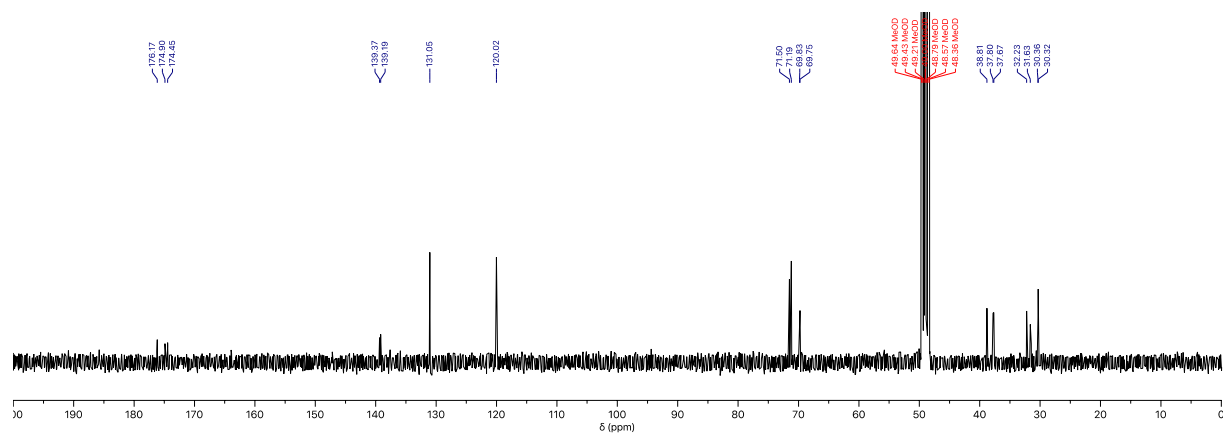
hoQEx9353 #38-50 RT: 0.38-0.47 AV: 6 SB: 24 0.04-0.25 , 0.73-0.97 NL: 3.33E9  
T: FTMS + p ESI Full ms [100.0000-1500.0000]



**Figure S10.**  $^1\text{H}$  (400 MHz, MeOD, 298 K) NMR spectrum of compound **6**

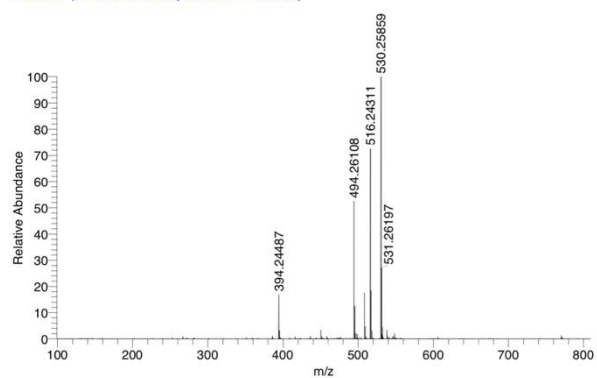


**Figure S11.**  $^{13}\text{C}\{^1\text{H}\}$  (101 MHz, MeOD, 298 K) NMR spectrum of compound **6**



**Figure S12.** HRMS (negative mode) spectrum of compound **6**

hoQEx9357 #38-50 RT: 0.38-0.47 AV: 6 SB: 23 0.04-0.25 , 0.73-0.97 NL: 4.06E8  
T: FTMS + p ESI Full lock ms [100.0000-1500.0000]



hoQEx9357 #38-50 RT: 0.38-0.47 AV: 6 SB: 23 0.04-0.25 , 0.73-0.97 NL: 4.06E8  
T: FTMS + p ESI Full lock ms [100.0000-1500.0000]

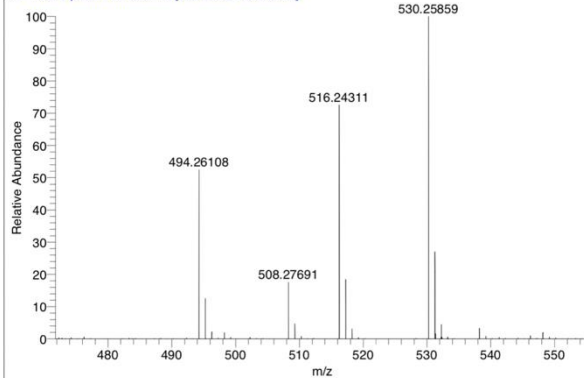


Figure S13.  $^1\text{H}$  (500 MHz,  $\text{dms}\text{-}d^6$ , 298 K) NMR spectrum of compound **1**

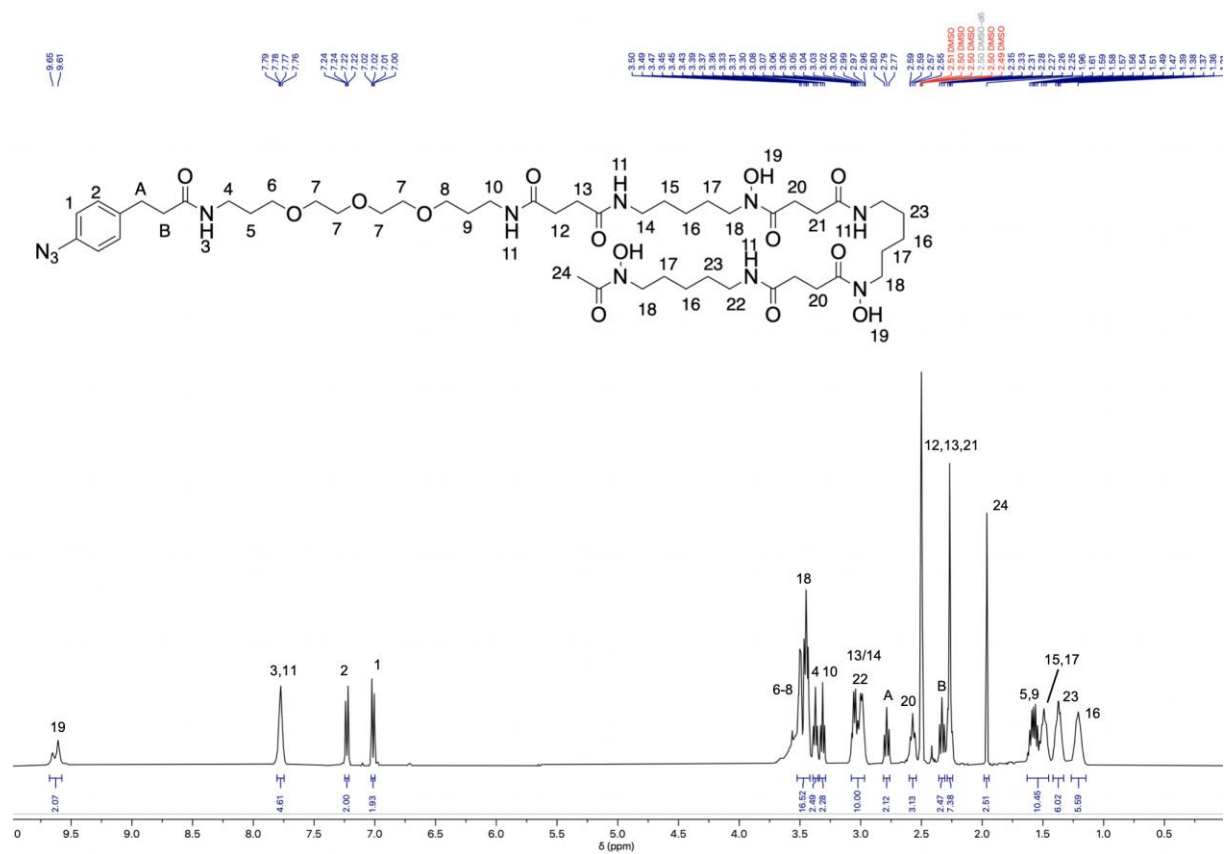
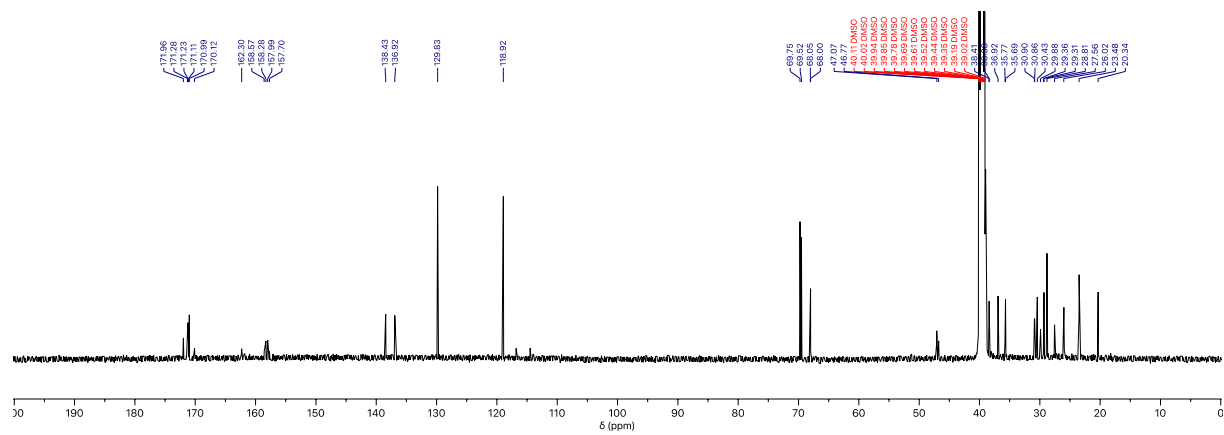
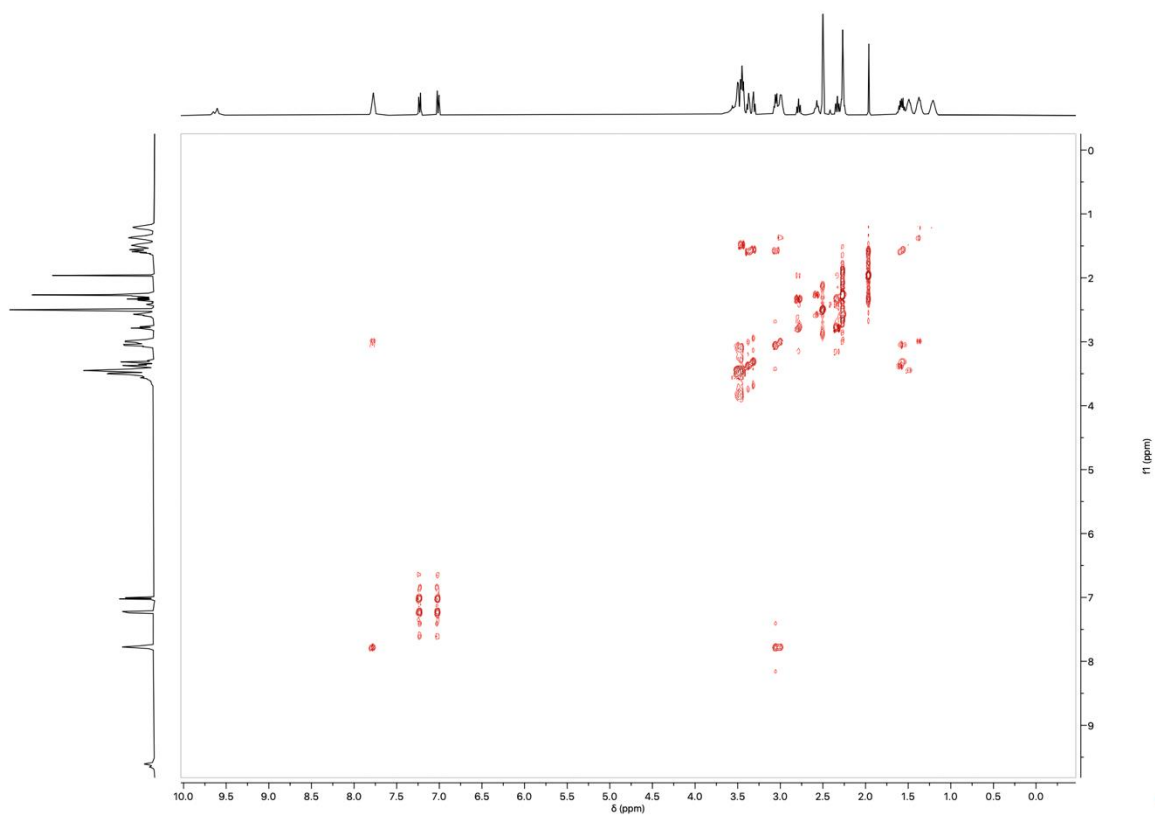


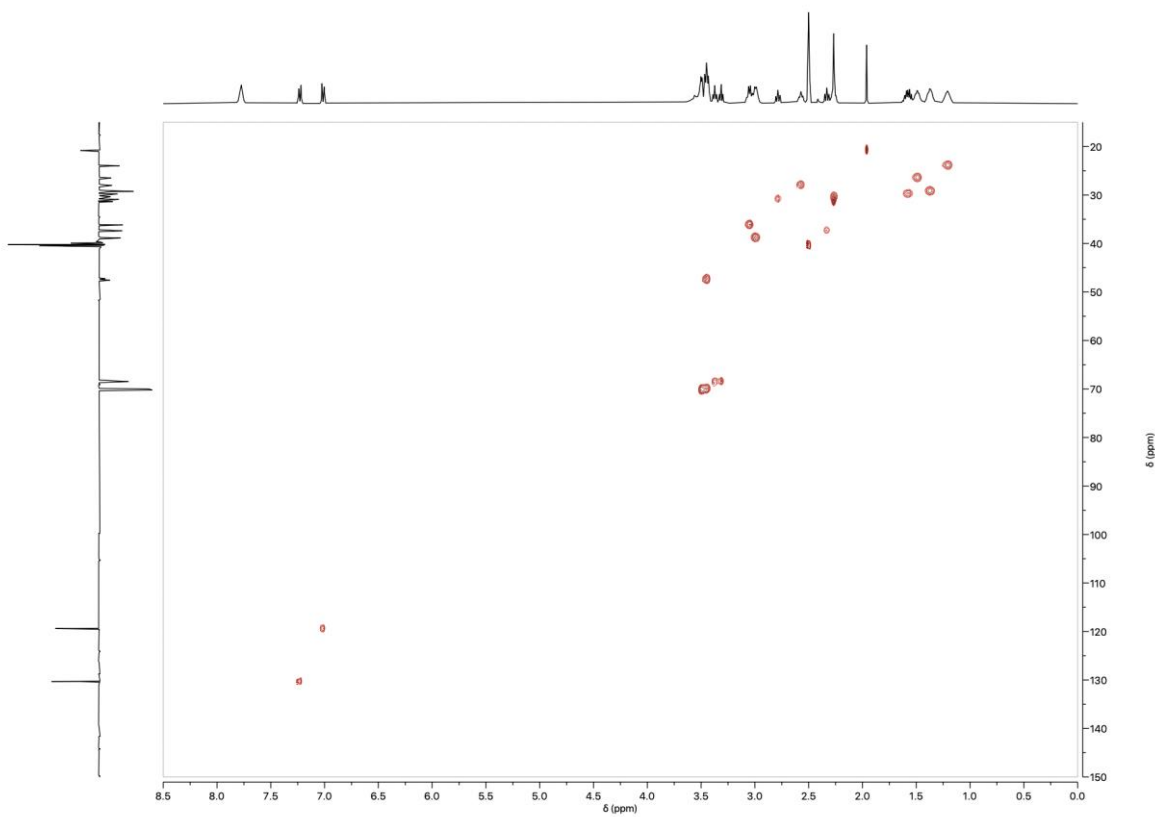
Figure S14.  $^{13}\text{C}\{^1\text{H}\}$  (125 MHz,  $\text{dms}\text{-}d^6$ , 298 K) NMR spectrum of compound **1**



**Figure S15.** 2D-COSY ( $dms\text{-}d^6$ ) NMR spectrum of compound **1**

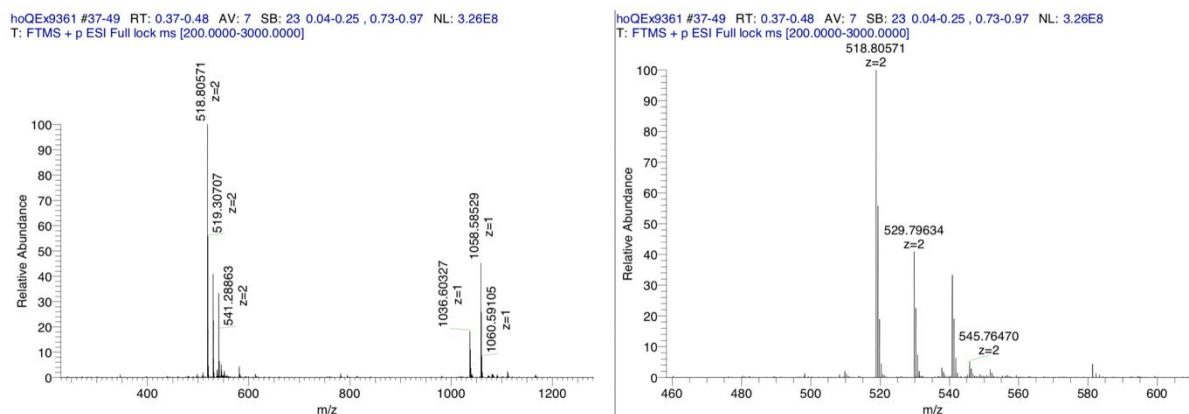


**Figure S16.** 2D-heteronuclear HSQC ( $dms\text{-}d^6$ ) NMR spectrum of compound **1**





**Figure S17.** HRMS spectrum of compound **1**



## Supporting Results

### <sup>89</sup>Zr-Radiolabelling of DFO-PEG<sub>3</sub>-Et-ArN<sub>3</sub> (**1**)

The [<sup>89</sup>Zr]Zr-radiolabelling of DFO-PEG<sub>3</sub>-Et-ArN<sub>3</sub>, compound **1** to give the [<sup>89</sup>Zr]Zr-radiolabelled complex [<sup>89</sup>Zr]ZrDFO-PEG<sub>3</sub>-Et-ArN<sub>3</sub> (<sup>89</sup>Zr-**1**<sup>+</sup>) was performed in triplicate. As an example, incubation of ligand **1** (20 μL; 2 mM stock solution; MW = 1036.22) with [<sup>89</sup>Zr]Zr-oxalate (40 μL; 5.532 MBq) in Chelex<sup>®</sup> H<sub>2</sub>O (40 μL) was performed in the dark for 10 mins at pH 8.0. The formation of <sup>89</sup>Zr-**1**<sup>+</sup> was confirmed by radio iTLC and radio HPLC analysis (Figure S18).

**Figure S18.** Characterisation data for the radiochemical synthesis of [<sup>89</sup>Zr]ZrDFO-PEG<sub>3</sub>-Et-ArN<sub>3</sub>. A) Radio-iTLC chromatograms of 'free' <sup>89</sup>Zr complexed as [<sup>89</sup>Zr]Zr-DTPA (blue) and [<sup>89</sup>Zr]ZrDFO-PEG<sub>3</sub>-Et-ArN<sub>3</sub> (black); B) HPLC chromatograms of the ligand (compound **1**; blue), [<sup>nat</sup>Zr]ZrDFO-PEG<sub>3</sub>-Et-ArN<sub>3</sub> (green) and [<sup>89</sup>Zr]ZrDFO-PEG<sub>3</sub>-Et-ArN<sub>3</sub> (black).

