

**“Click” chemistry toward *bis*(DOTA-derived) heterometallic complexes: potential bimodal MRI/PET(SPECT) molecular probes.**

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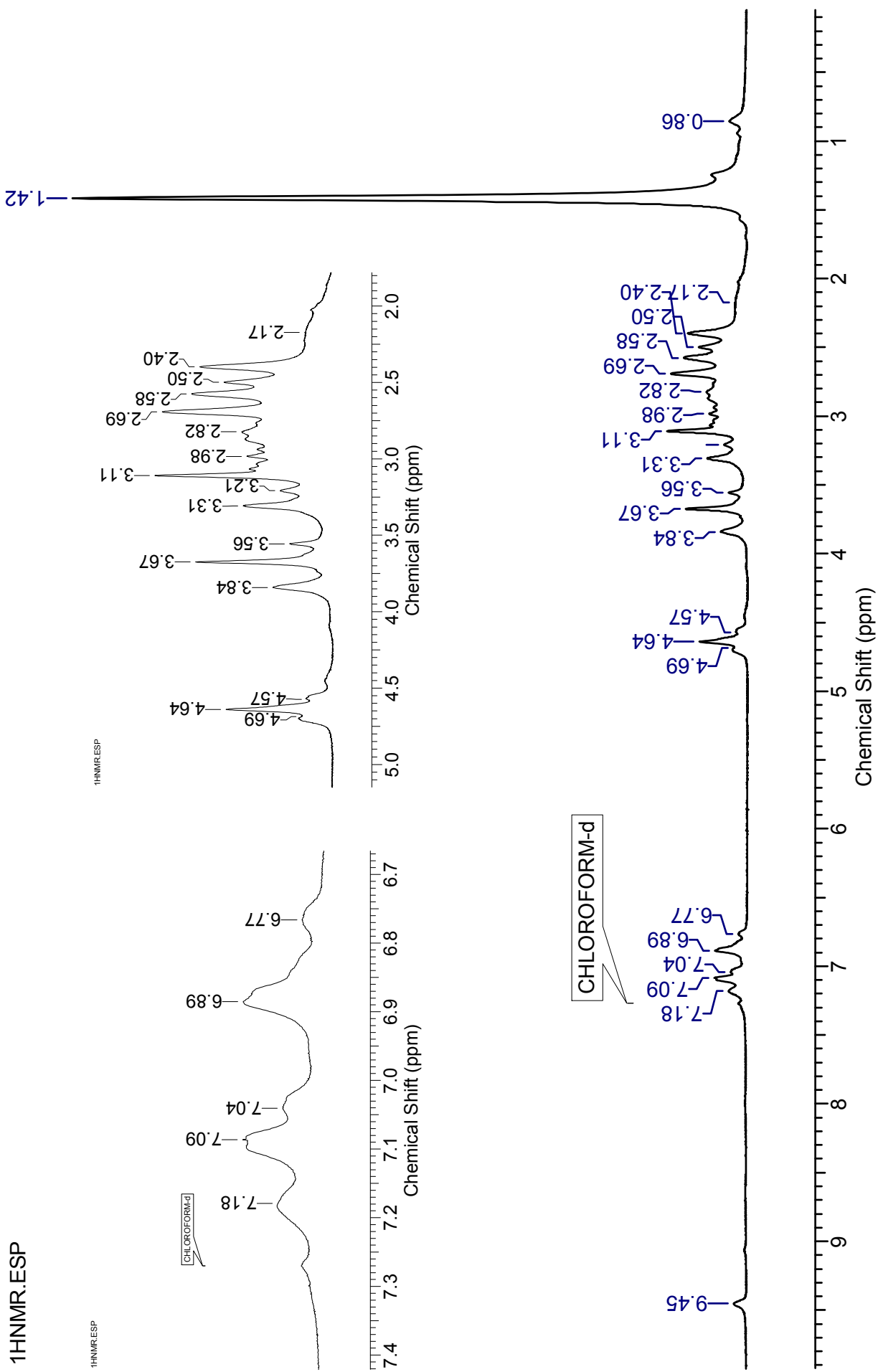
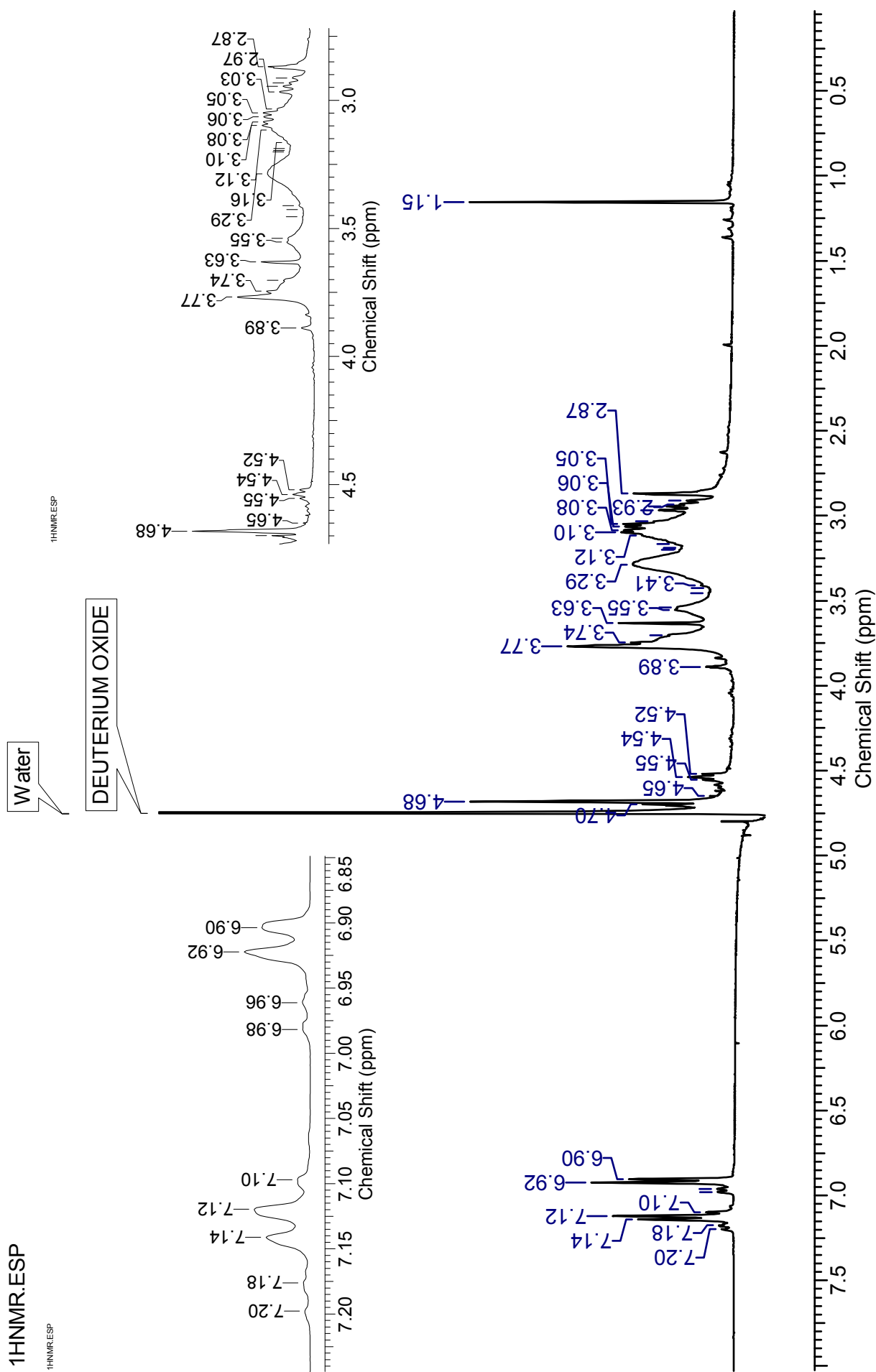


Figure S1:  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3\text{-d}$ ) of compound 6a.



**Figure S2:**  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ ) of an unmetallated version of complex **6b**.

1HNMR.ESP

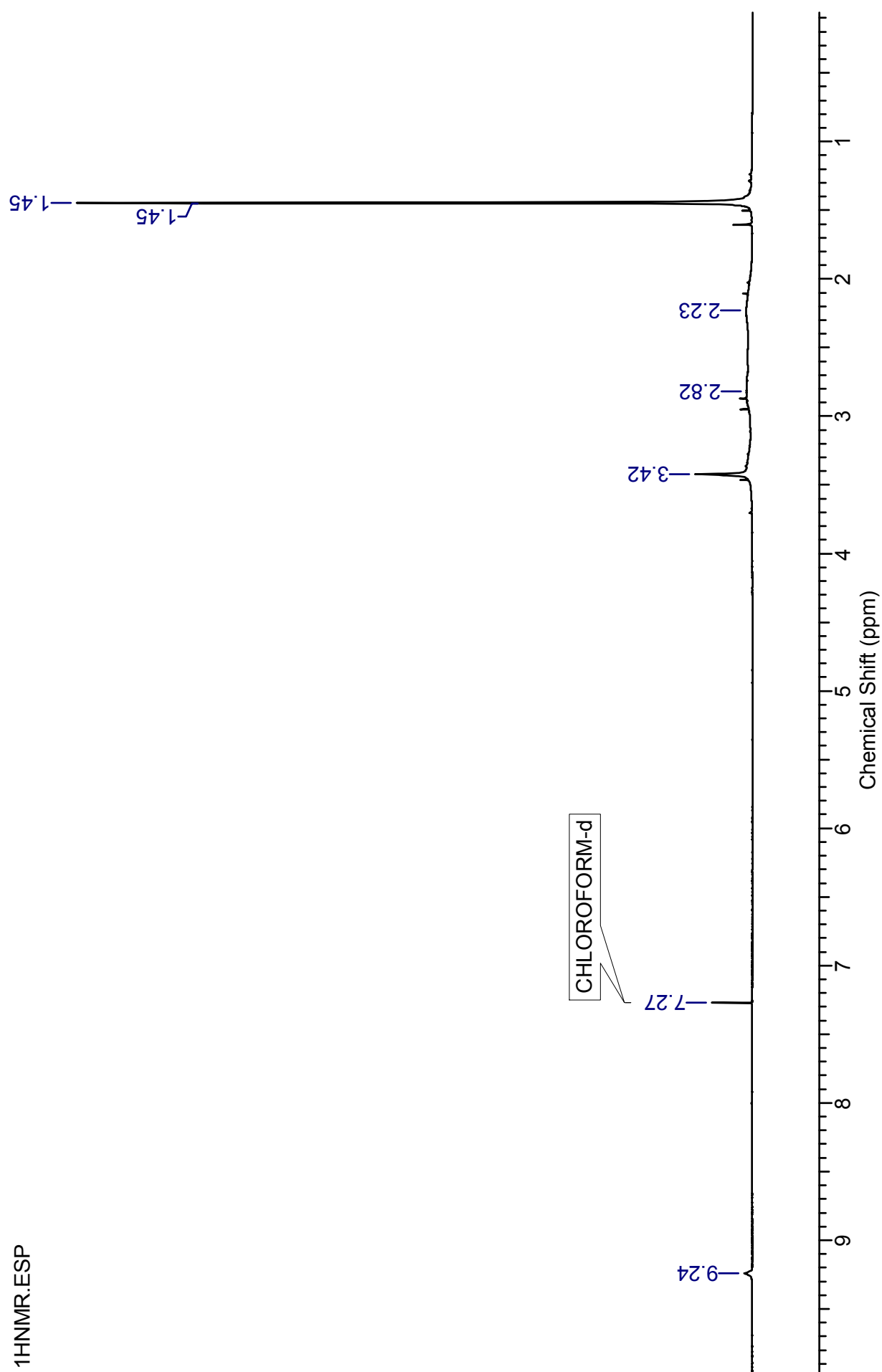
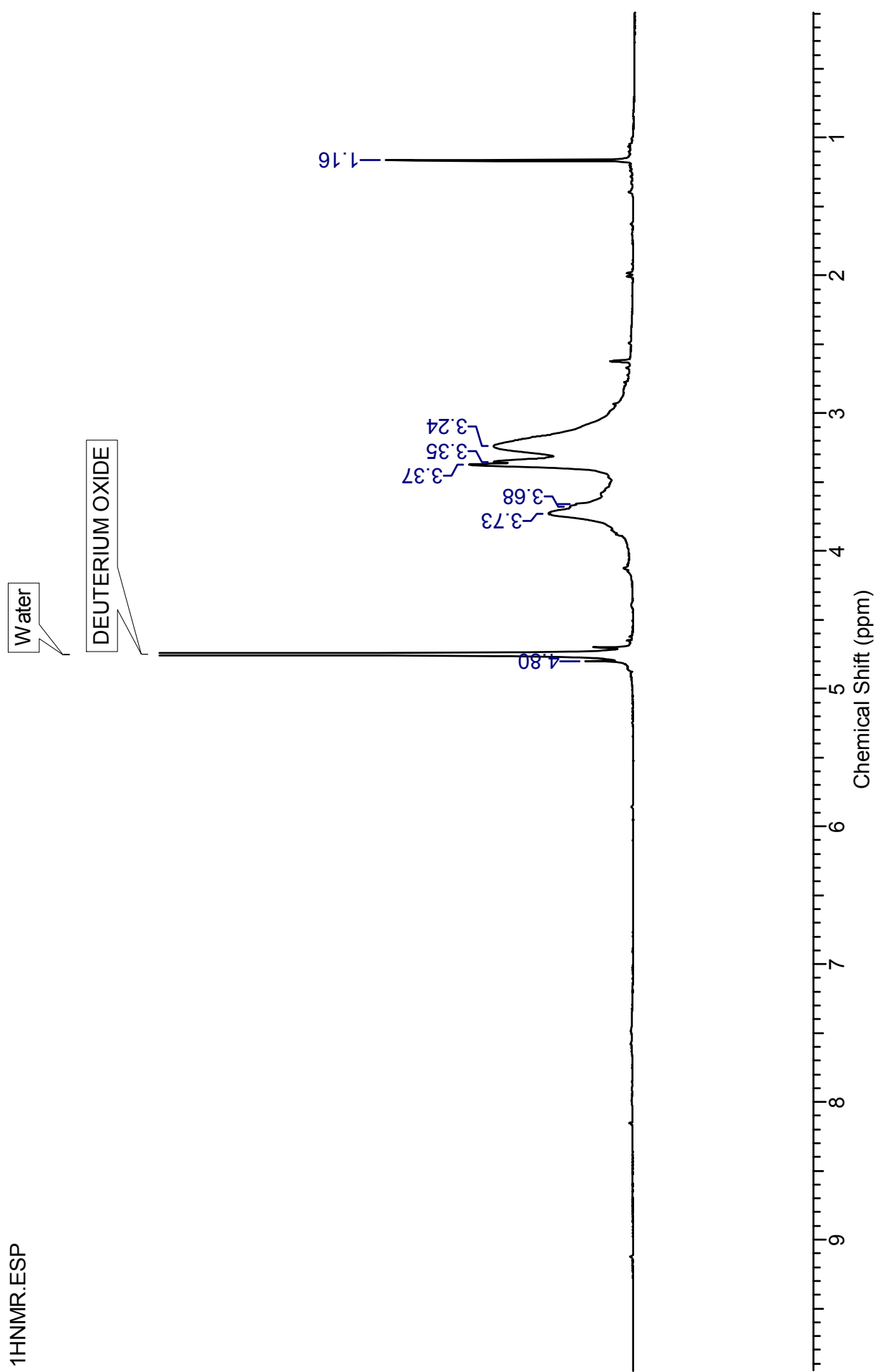
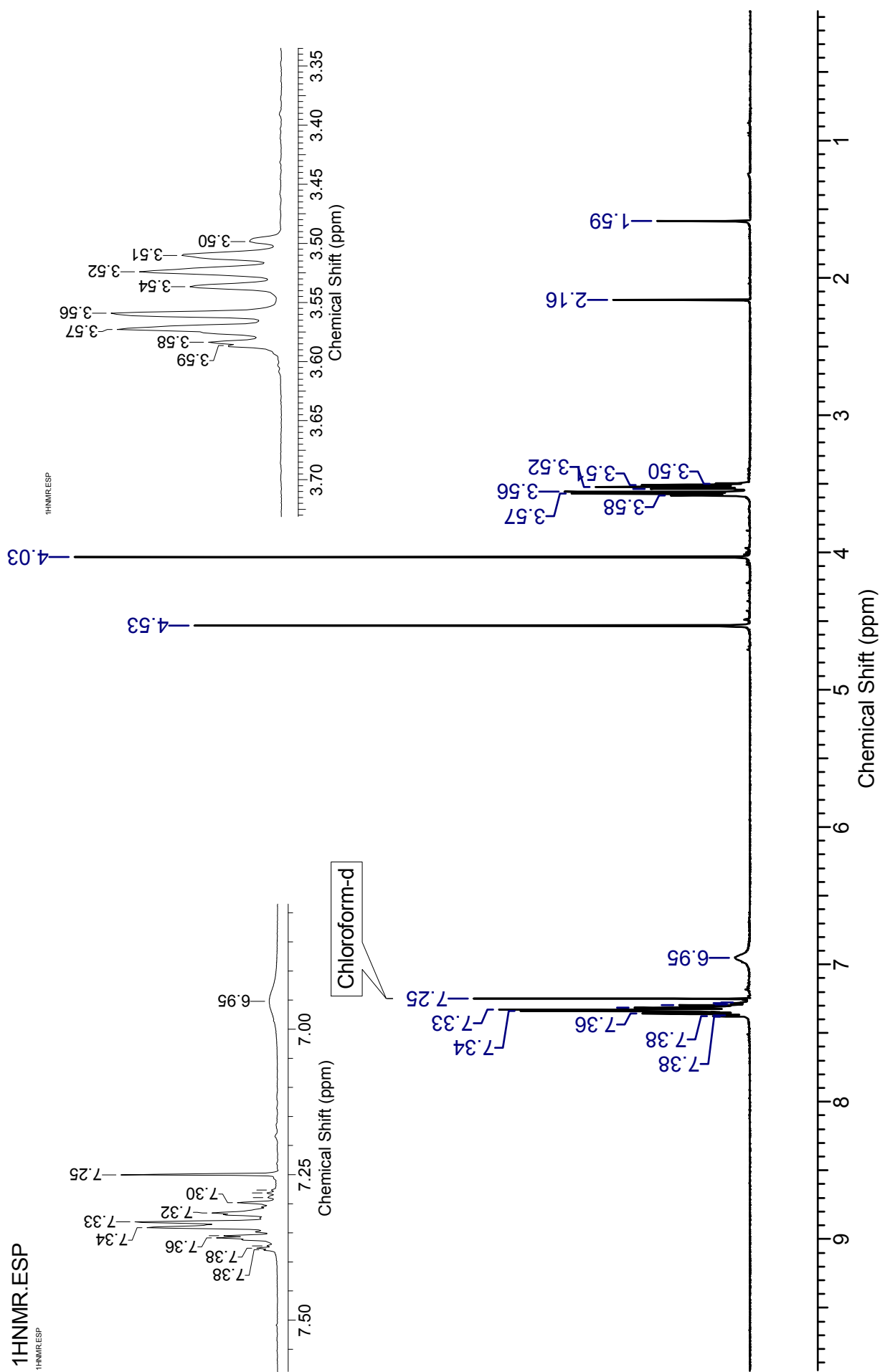


Figure S3: <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) of compound 7a

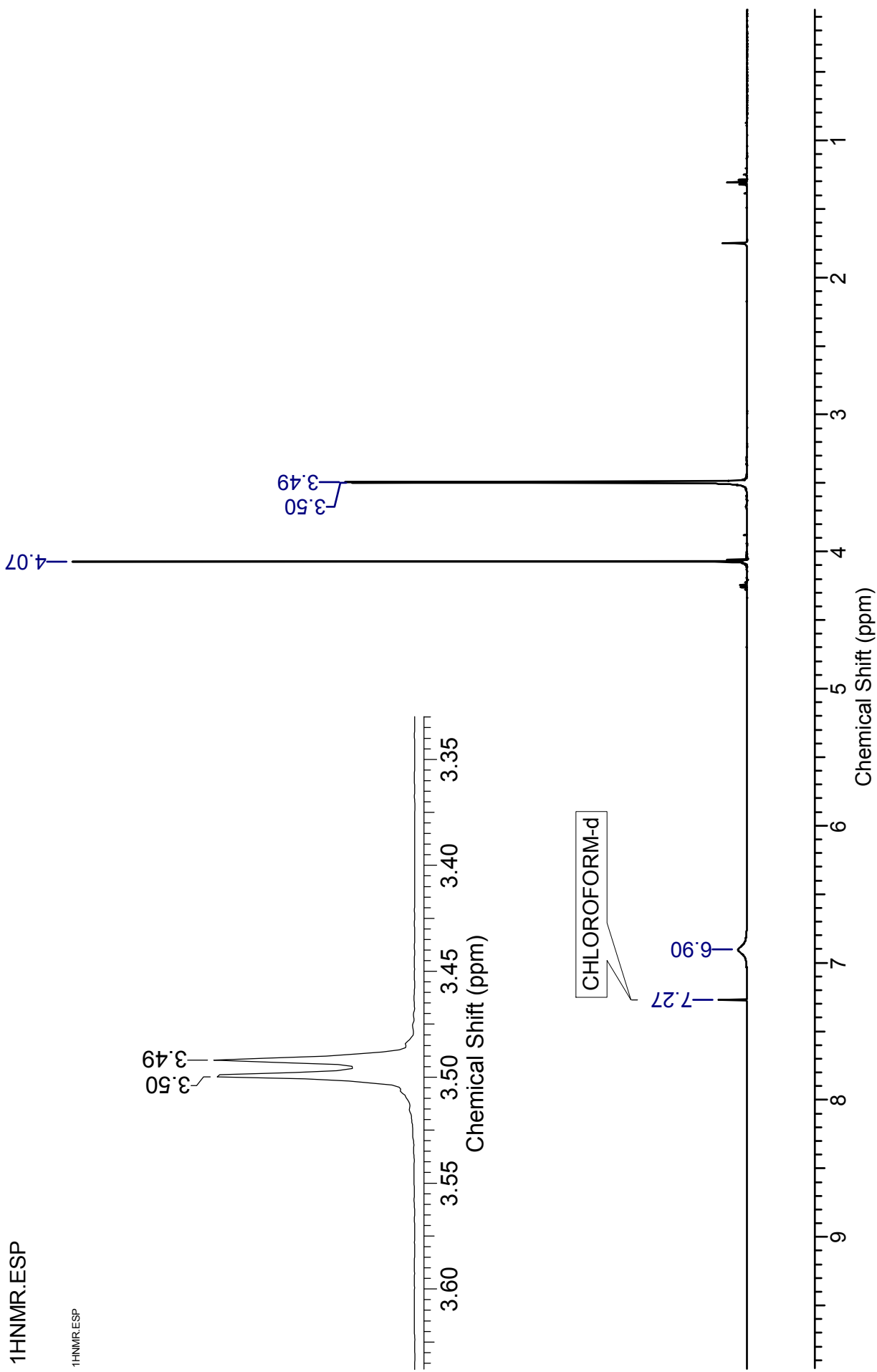




**Figure S4:**  $^1\text{H}$  NMR spectrum ( $\text{D}_2\text{O}$ ) of an ester deprotected version of ligand 7a.

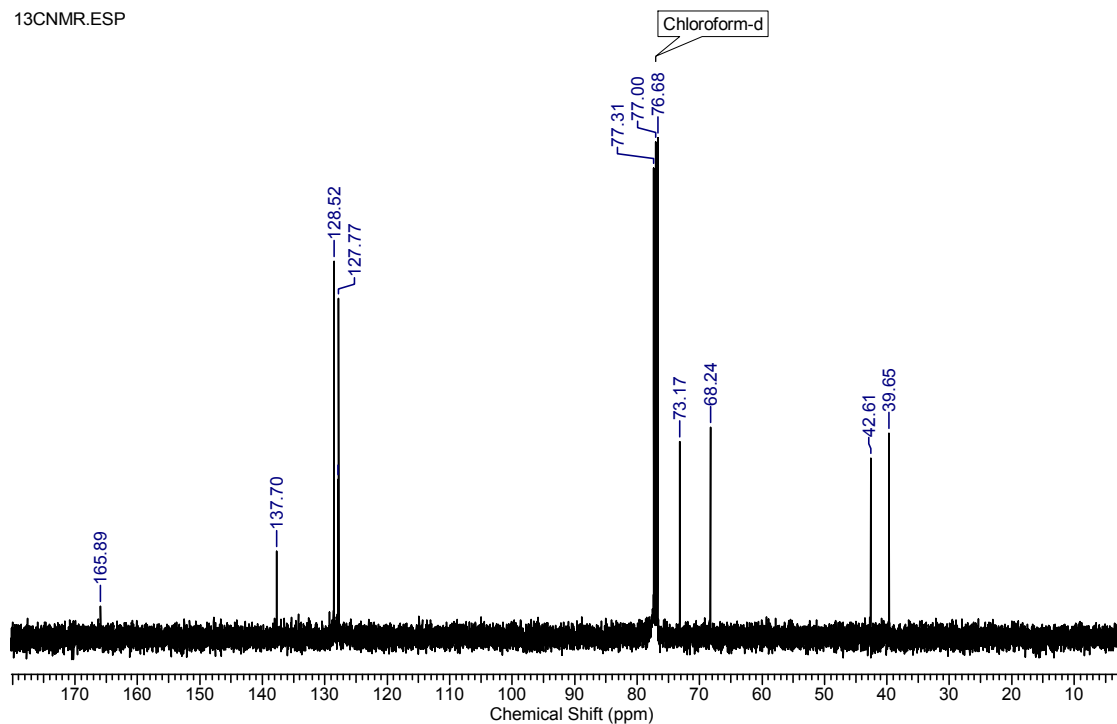


**Figure S5:** <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) of compound 12.



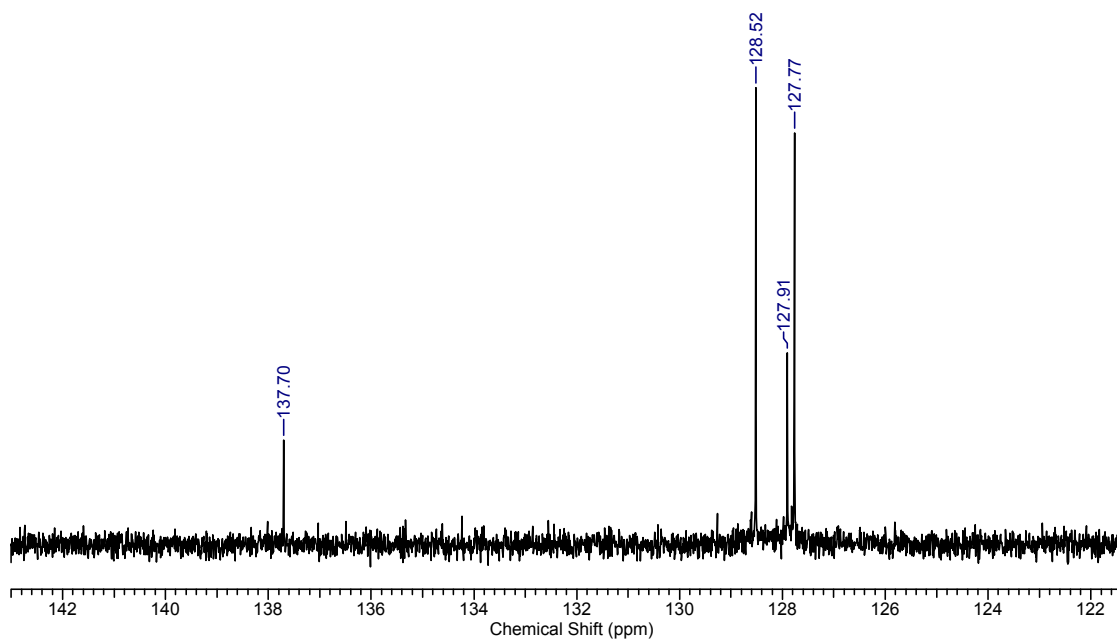
**Figure S6:**  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ ) of compound **15**.

13CNMR.ESP



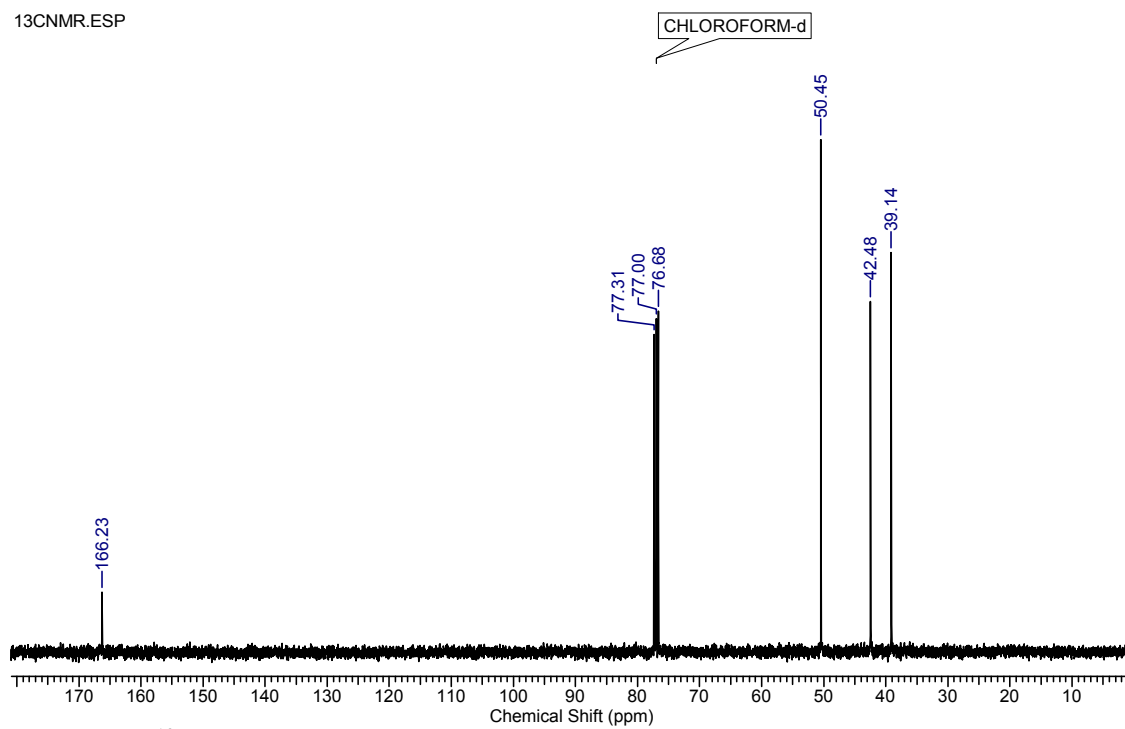
**Figure S7:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>) of compound **12**.

13CNMR.ESP



**Figure S8:** <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>) of compound **12**, an expansion of the aromatic carbon region.

13CNMR.ESP

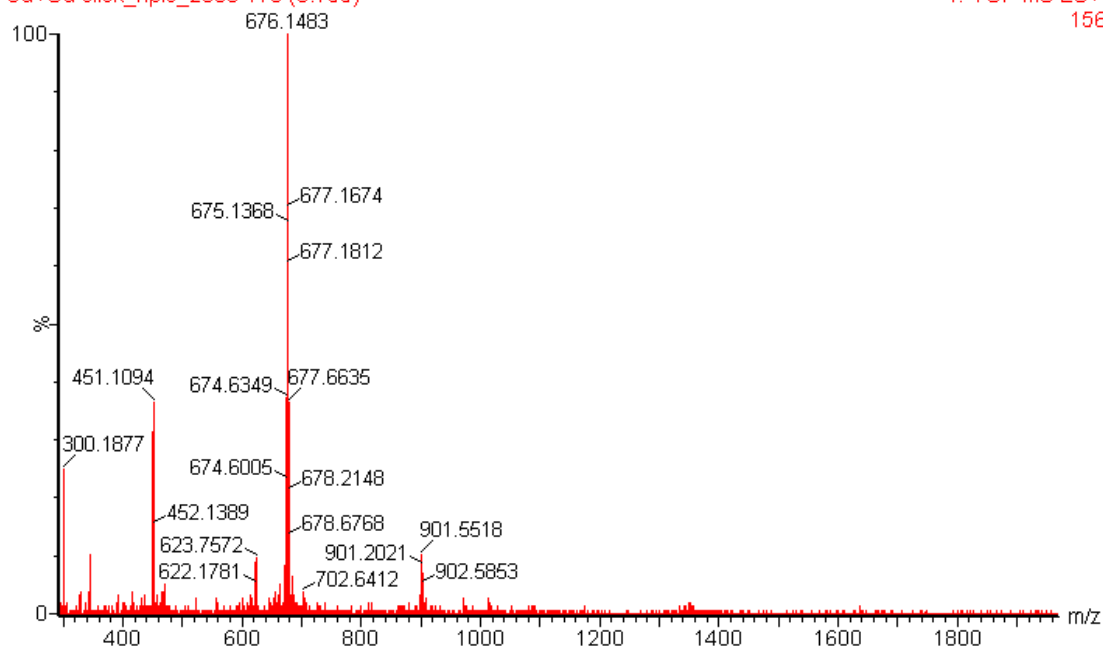


**Figure S9:**  $^{13}\text{C}$  NMR spectrum ( $\text{CDCl}_3$ ) of compound **15**.

**Mojmir38**

Cu+Gd click\_hplc\_2603 170 (3.755)

1: TOF MS ES+  
156

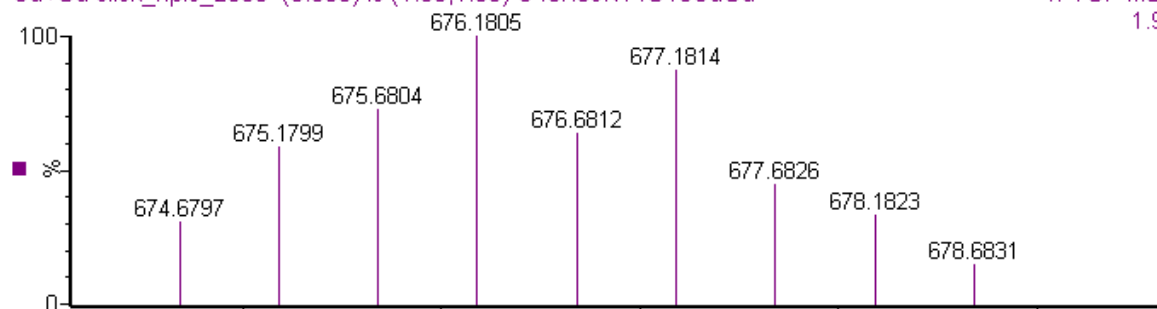


**Figure S10:** HR-ESI-MS spectrum of compound **5a** showing a proper charge state envelope.

**Mojmir38**

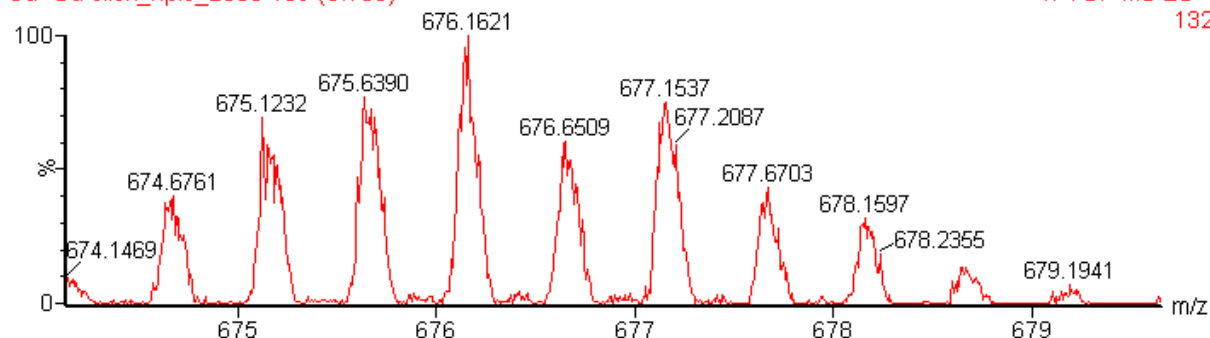
Cu+Gd click\_hplc\_2603 (0.036) Is (1.00,1.00) C48H69N14O18CuGd

1: TOF MS ES+  
1.94e12



Cu+Gd click\_hplc\_2603 169 (3.738)

1: TOF MS ES+  
132

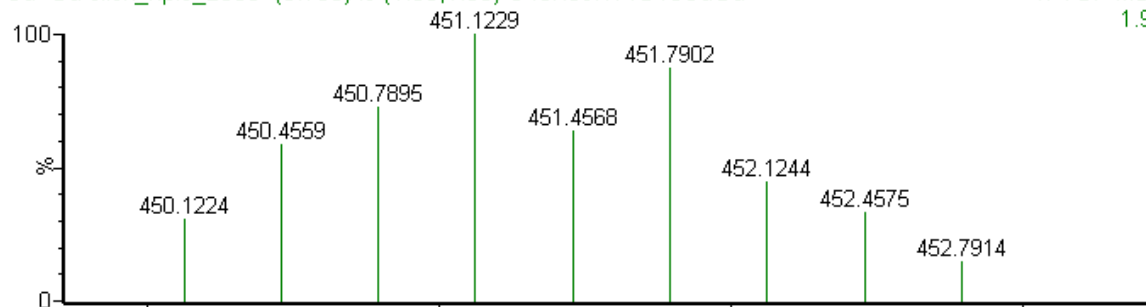


**Figure S11:** HR-ESI-MS spectrum of compound **5a**,  $M^{2+}$  charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of heavy metals.

### Mojmir38

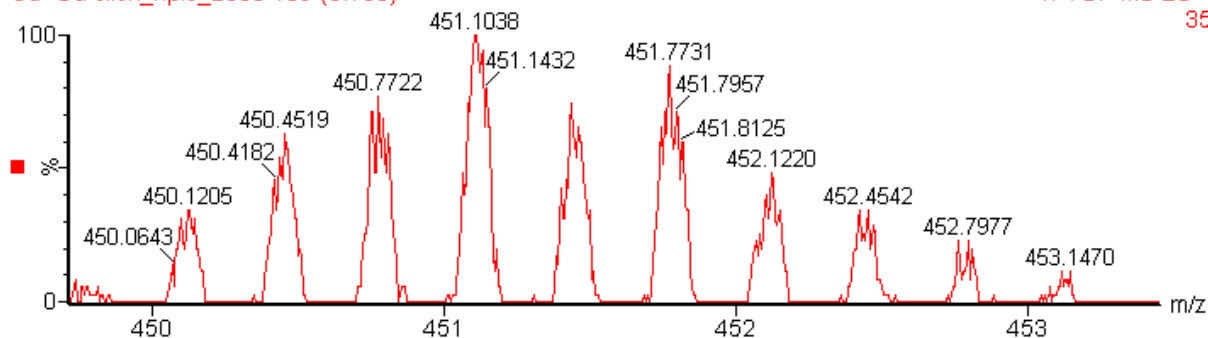
Cu+Gd click\_hplc\_2603 (3.738) Is (1.00,1.00) C<sub>48</sub>H<sub>69</sub>N<sub>14</sub>O<sub>18</sub>CuGd

1: TOF MS ES+  
1.94e12



Cu+Gd click\_hplc\_2603 169 (3.738)

1: TOF MS ES+  
35



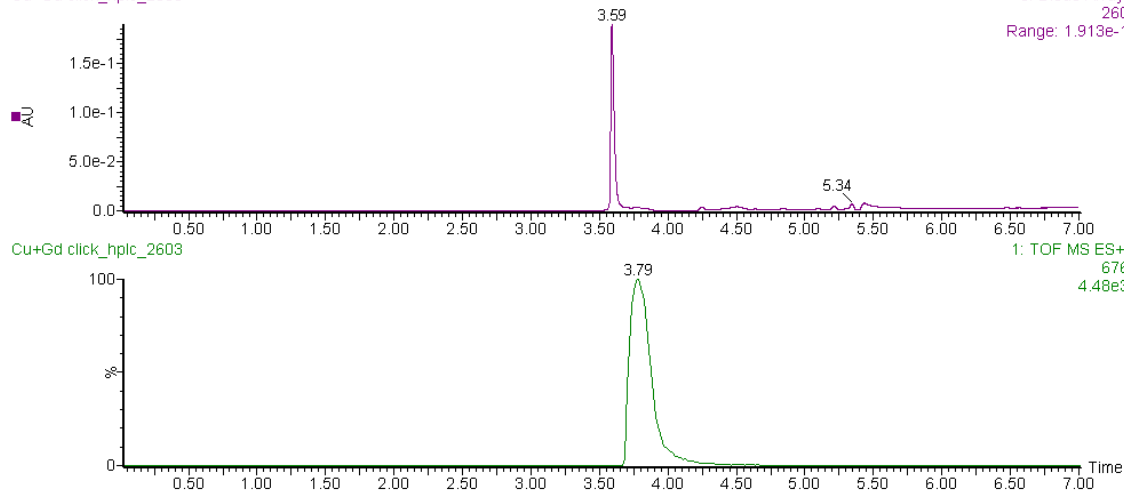
**Figure S12:** HR-ESI-MS spectrum of compound **5a**, M<sup>3+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of heavy metals.

### Mojmir38

Cu+Gd click\_hplc\_2603

26-Mar-2012

3: Diode Array  
260  
Range: 1.913e-1



**Figure S13:** UPLC chromatograms (Method B) of compound **5a**, MS detector (bottom), UV detector (top).

Mojmir16

Ga+Gd click\_130312 fr2 167 (3.684)

1: TOF MS ES+  
203

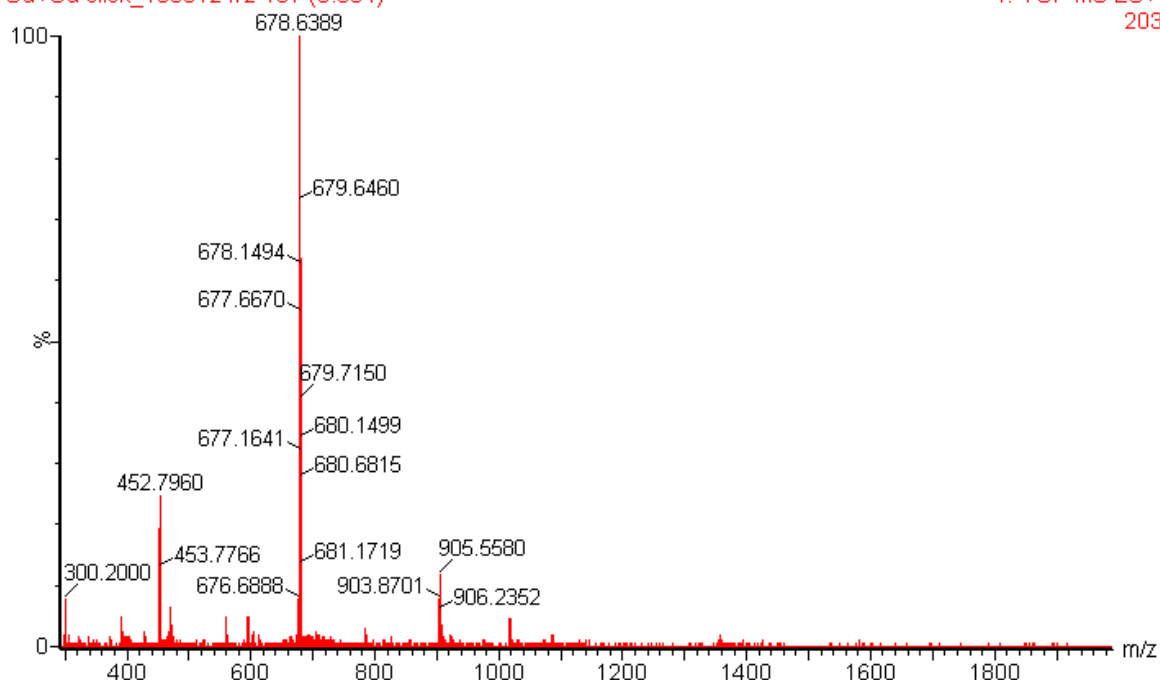
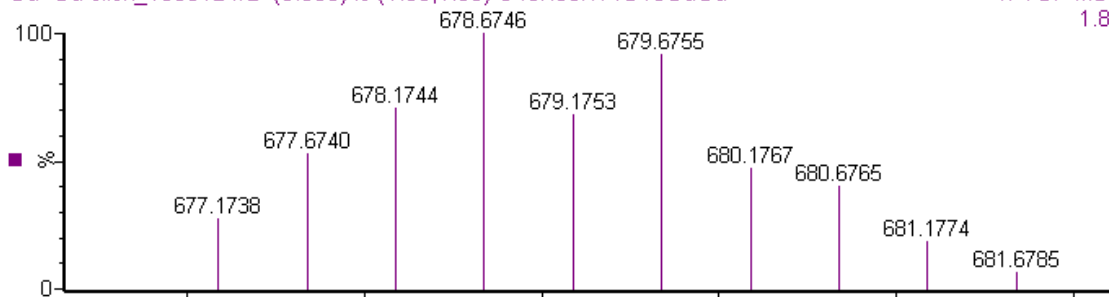


Figure S14: HR-ESI-MS spectrum of compound **5b** showing a proper charge state envelope.

Mojmir16

Ga+Gd click\_130312 fr2 (0.035) Is (1.00,1.00) C<sub>48</sub>H<sub>68</sub>N<sub>14</sub>O<sub>18</sub>GaGd

1: TOF MS ES+  
1.89e12



Ga+Gd click\_130312 fr2 167 (3.684)

1: TOF MS ES+  
203

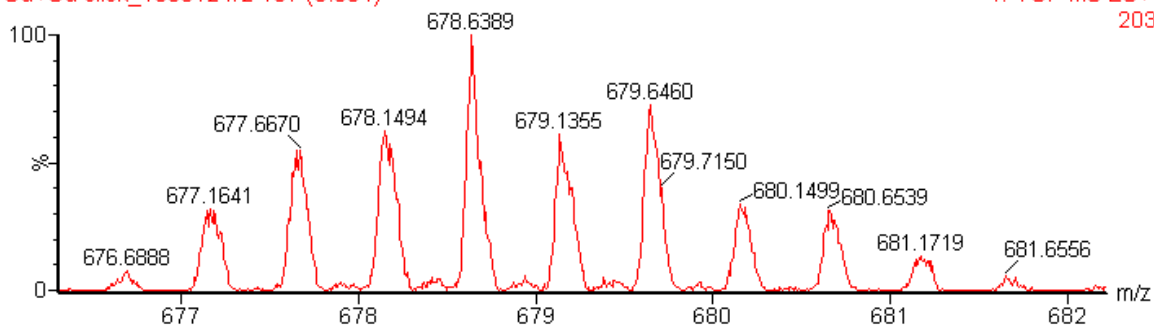


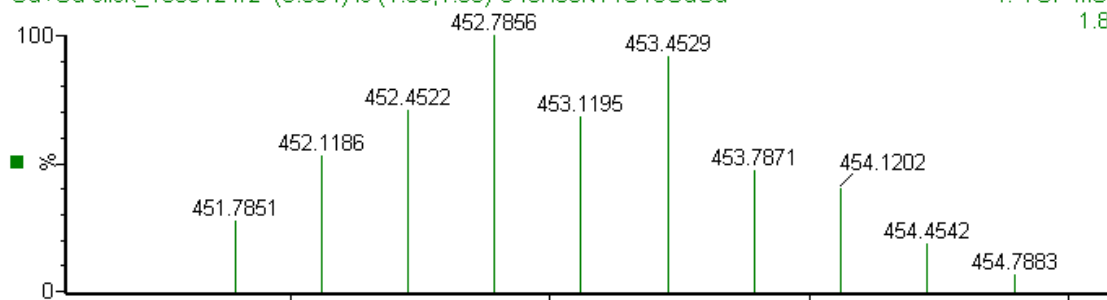
Figure S15: HR-ESI-MS spectrum of compound **5b**, M<sup>2+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of heavy metals.



Mojmir16

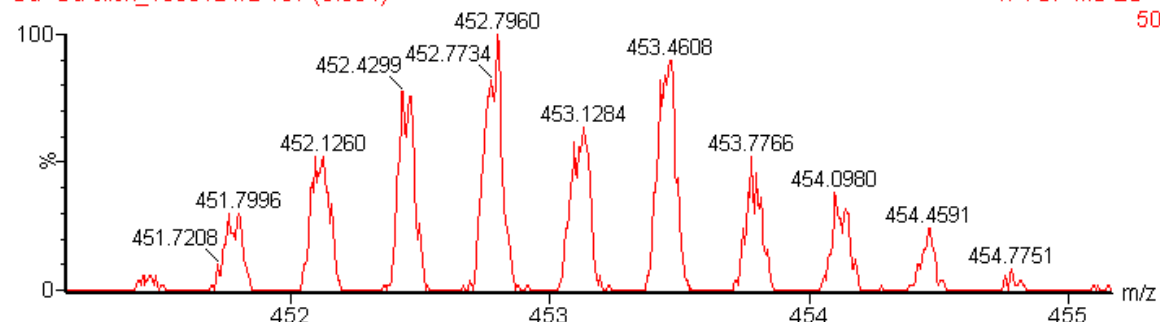
Ga+Gd click\_130312 fr2 (3.684) Is (1.00,1.00) C<sub>48</sub>H<sub>68</sub>N<sub>14</sub>O<sub>18</sub>GaGd

1: TOF MS ES+  
1.89e12



Ga+Gd click\_130312 fr2 167 (3.684)

1: TOF MS ES+  
50



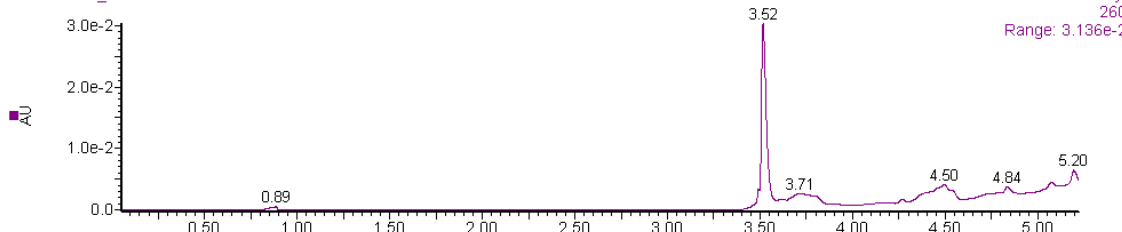
**Figure S16:** HR-ESI-MS spectrum of compound **5b**, M<sup>3+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of heavy metals.

Mojmir16

Ga+Gd click\_130312 fr2

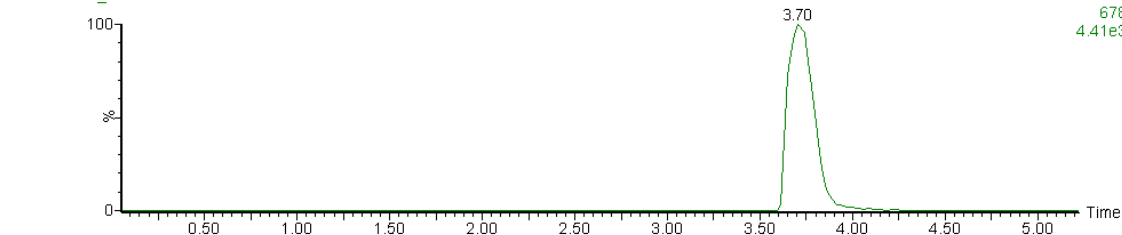
13-Mar-2012

3: Diode Array  
260  
Range: 3.136e-2

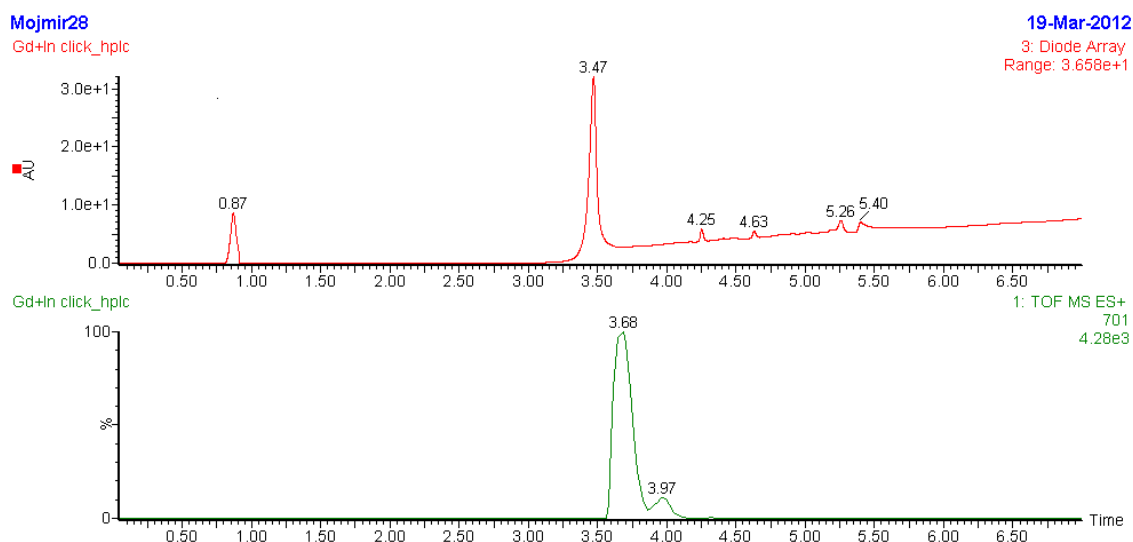


Ga+Gd click\_130312 fr2

1: TOF MS ES+  
678  
4.41e3

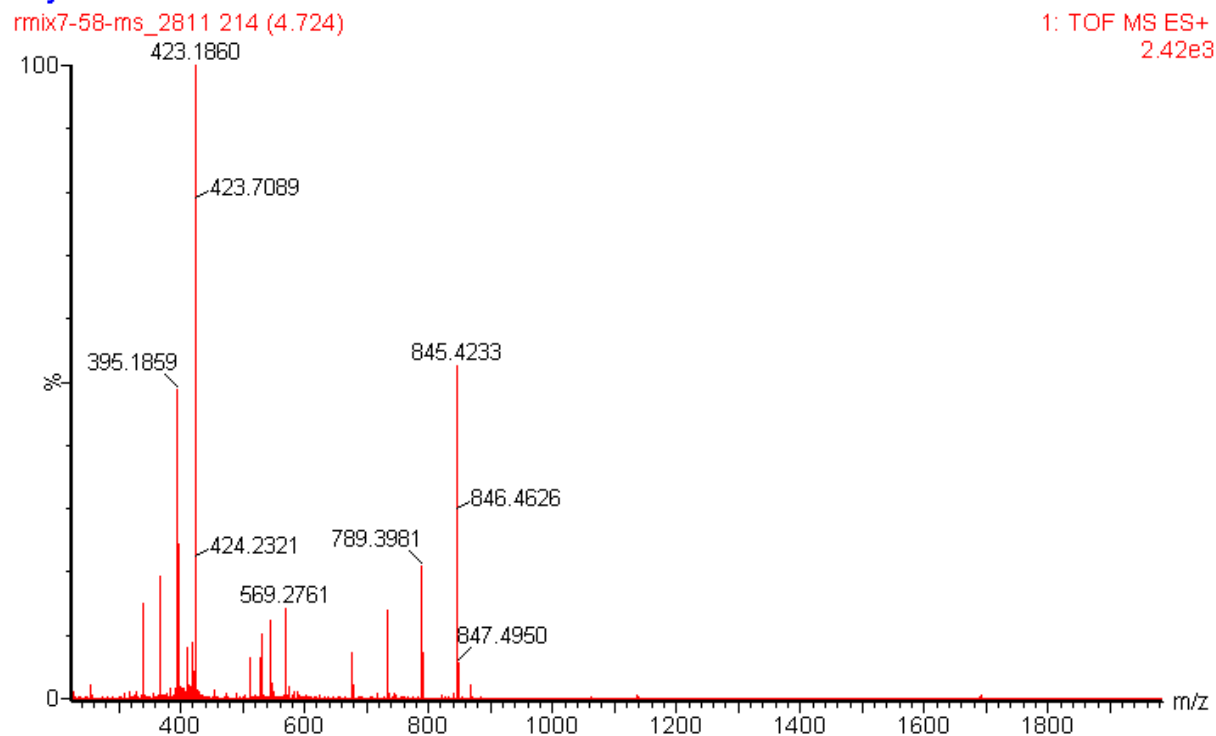


**Figure S17:** UPLC chromatograms (Method B) of compound **5b**, MS detector (bottom), UV detector (top).



**Figure S18:** UPLC chromatograms (Method B) of compound **5d**, MS detector (bottom), UV detector (top). The remaining MS data for compound **5d** are shown in the body of the paper (Figure 2).

**Mojmir 67**



**Figure S19:** HR-ESI-MS spectrum of compound **6a** showing a proper charge state envelope. The *t*-Bu ester functionalities present in **6a** are not stable under the conditions of the analysis therefore  $M^+ - 56$ ,  $M^+ - 112$  and  $M^+ - 168$  ions are observed for the singly charged species and  $M^+ - 28$ ,  $M^+ - 56$  and  $M^+ - 84$  ions are observed for the doubly charged species.

**Mojmir 67**

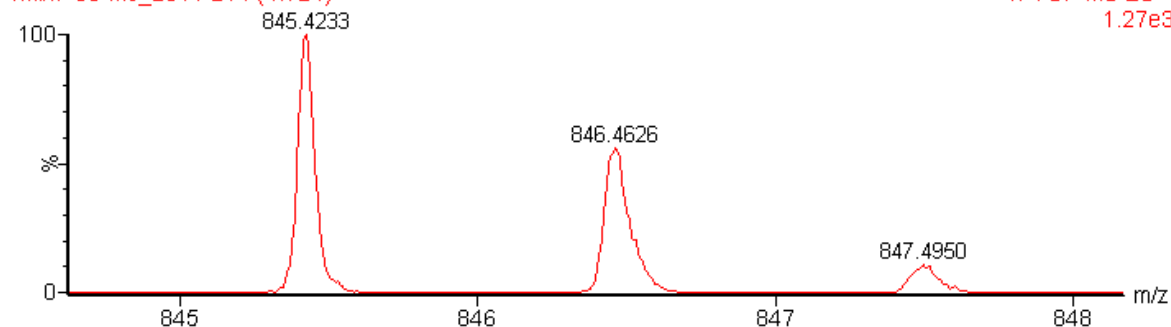
rmix7-58-ms\_2811 (4.724) Is (1.00,1.00) C<sub>43</sub>H<sub>68</sub>N<sub>6</sub>O<sub>11</sub>

1: TOF MS ES+  
5.86e12



rmix7-58-ms\_2811 214 (4.724)

1: TOF MS ES+  
1.27e3

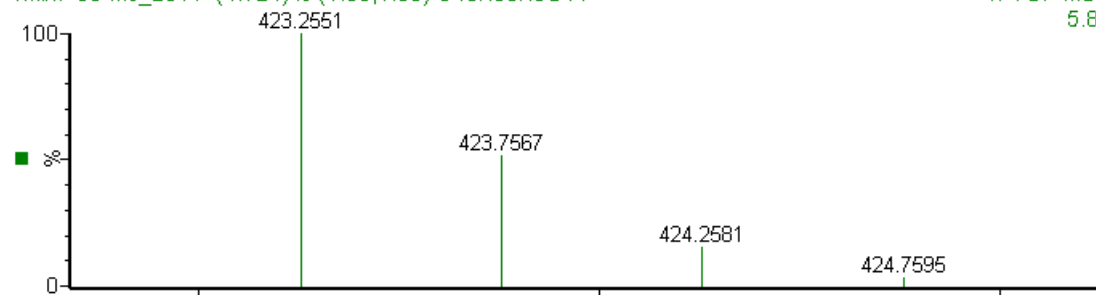


**Figure S20:** HR-ESI-MS spectrum of compound **6a**, M<sup>+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum.

**Mojmir 67**

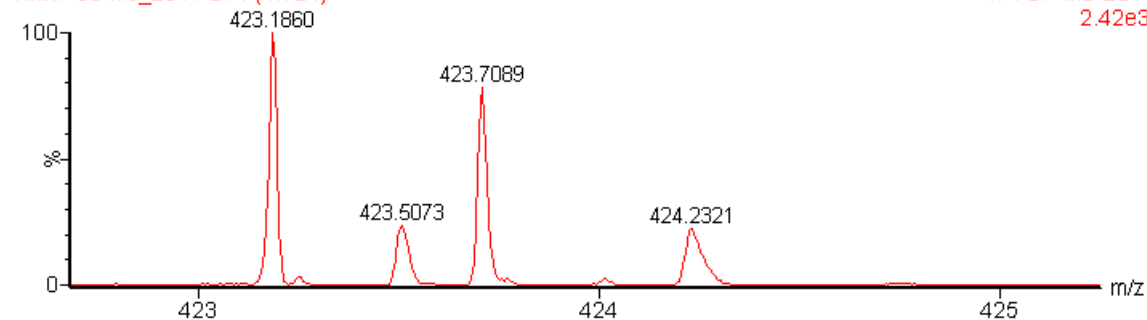
rmix7-58-ms\_2811 (4.724) Is (1.00,1.00) C<sub>43</sub>H<sub>68</sub>N<sub>6</sub>O<sub>11</sub>

1: TOF MS ES+  
5.86e12

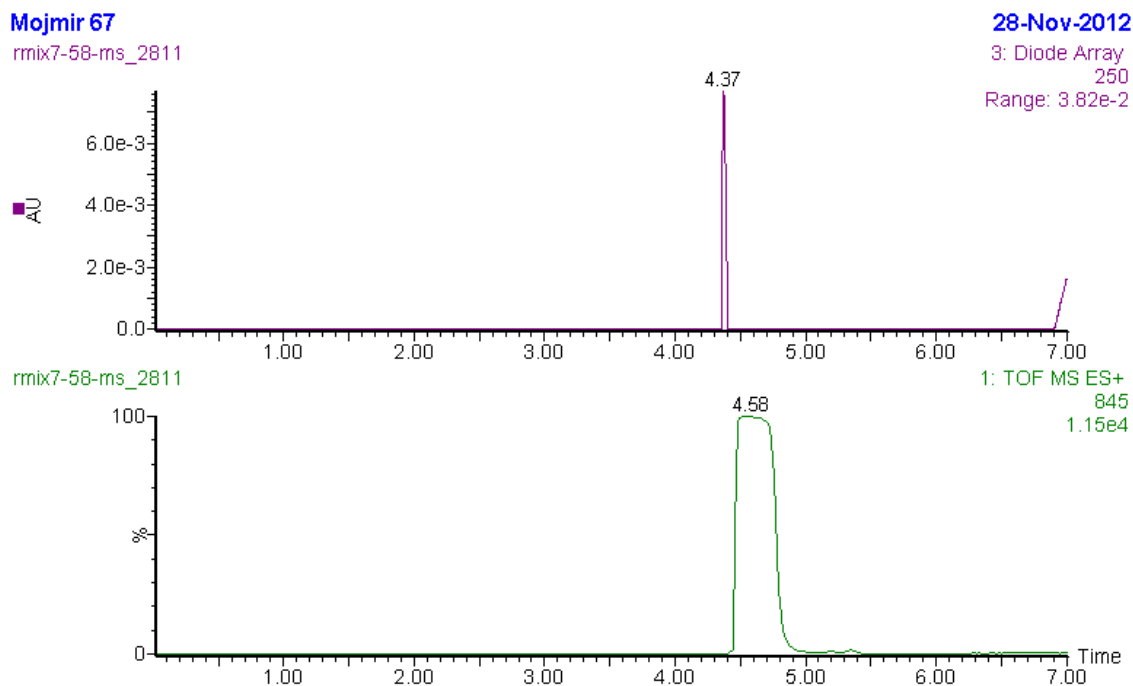


rmix7-58-ms\_2811 214 (4.724)

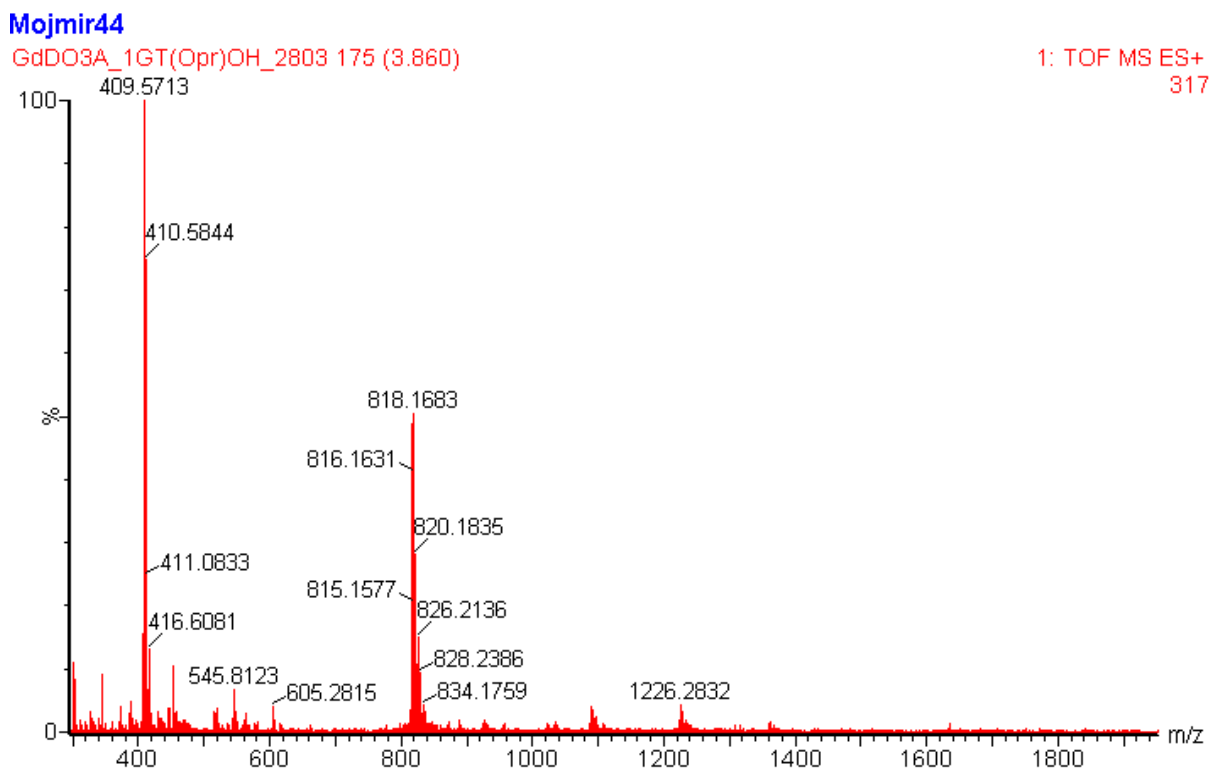
1: TOF MS ES+  
2.42e3



**Figure S21:** HR-ESI-MS spectrum of compound **6a**, M<sup>2+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum.



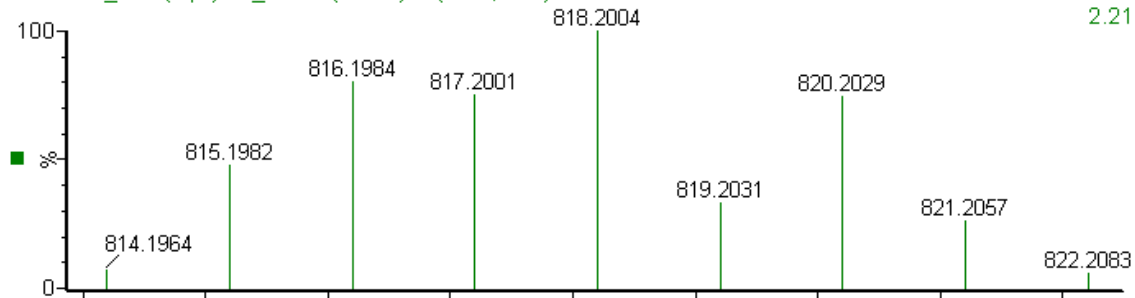
**Figure S22:** UPLC chromatograms (Method B) of compound **6a**, MS detector (bottom), UV detector (top).



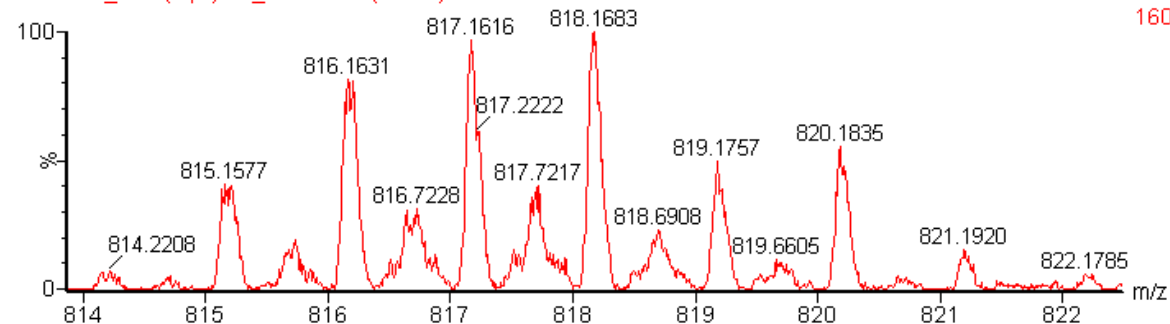
**Figure S23:** HR-ESI-MS spectrum of compound **6b** showing a proper charge state envelope.

Mojmir44

GdDO3A\_1GT(Opr)OH\_2803 (3.860) Is (1.00,1.00) C<sub>30</sub>H<sub>39</sub>N<sub>6</sub>O<sub>11</sub>Gd 1: TOF MS ES+ 2.21e12



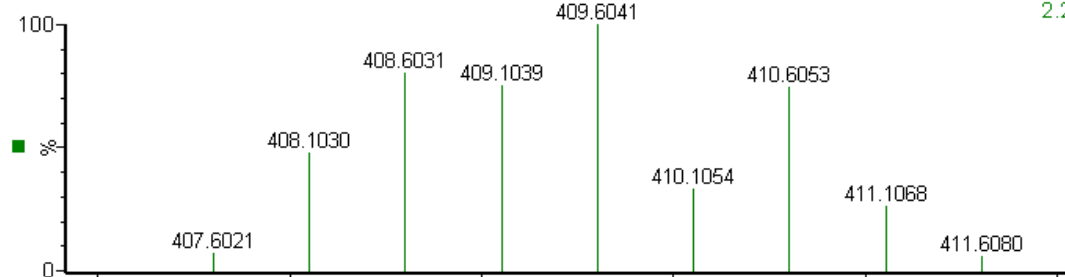
GdDO3A\_1GT(Opr)OH\_2803 175 (3.860) 1: TOF MS ES+ 160



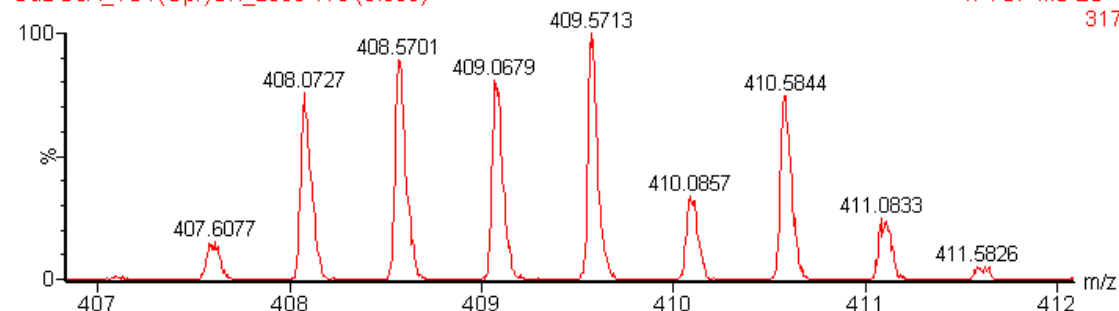
**Figure S24:** HR-ESI-MS spectrum of compound **6b**,  $M^+$  charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of Gd.

Mojmir44

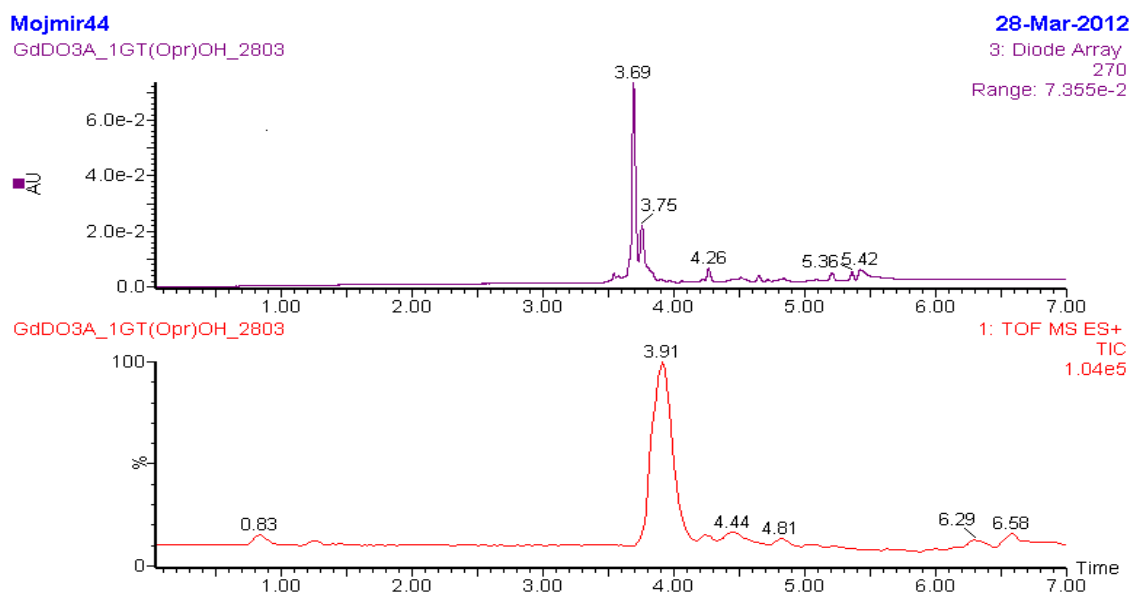
GdDO3A\_1GT(Opr)OH\_2803 (3.860) Is (1.00,1.00) C<sub>30</sub>H<sub>39</sub>N<sub>6</sub>O<sub>11</sub>Gd 1: TOF MS ES+ 2.21e12



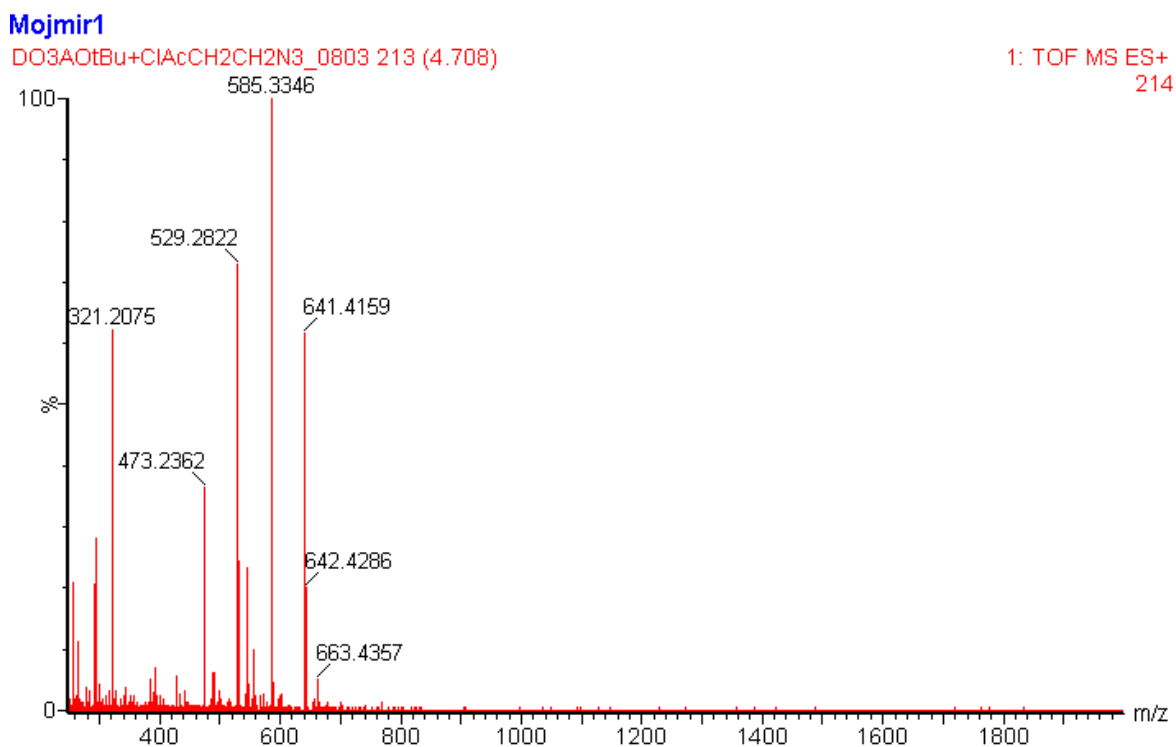
GdDO3A\_1GT(Opr)OH\_2803 175 (3.860) 1: TOF MS ES+ 317



**Figure S25:** HR-ESI-MS spectrum of compound **6b**,  $M^{2+}$  charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of Gd.



**Figure S26:** UPLC chromatograms (Method B) of compound **6b**, MS detector (bottom), UV detector (top).

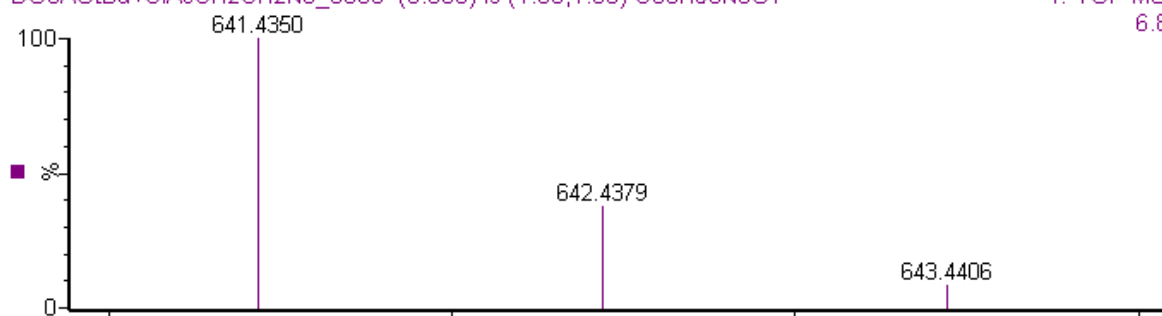


**Figure S27:** HR-ESI-MS spectrum of compound **7a** showing a proper charge state envelope. The *t*-Bu ester functionalities present in **7a** are not stable under the conditions of the analysis therefore  $M^+-56$ ,  $M^+-112$  and  $M^+-168$  ions are observed for the singly charged species and  $M^+-28$ ,  $M^+-56$  and  $M^+-84$  ions are observed for the doubly charged species.

**Mojmir1**

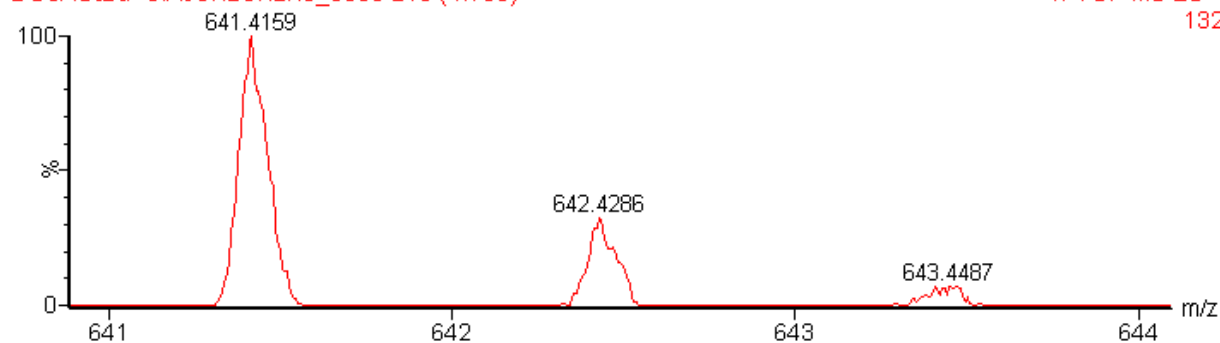
DO3AOTBu+ClAcCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>\_0803 (0.036) Is (1.00,1.00) C<sub>30</sub>H<sub>56</sub>N<sub>8</sub>O<sub>7</sub>

1: TOF MS ES+  
6.80e12



DO3AOTBu+ClAcCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>\_0803 213 (4.708)

1: TOF MS ES+  
132



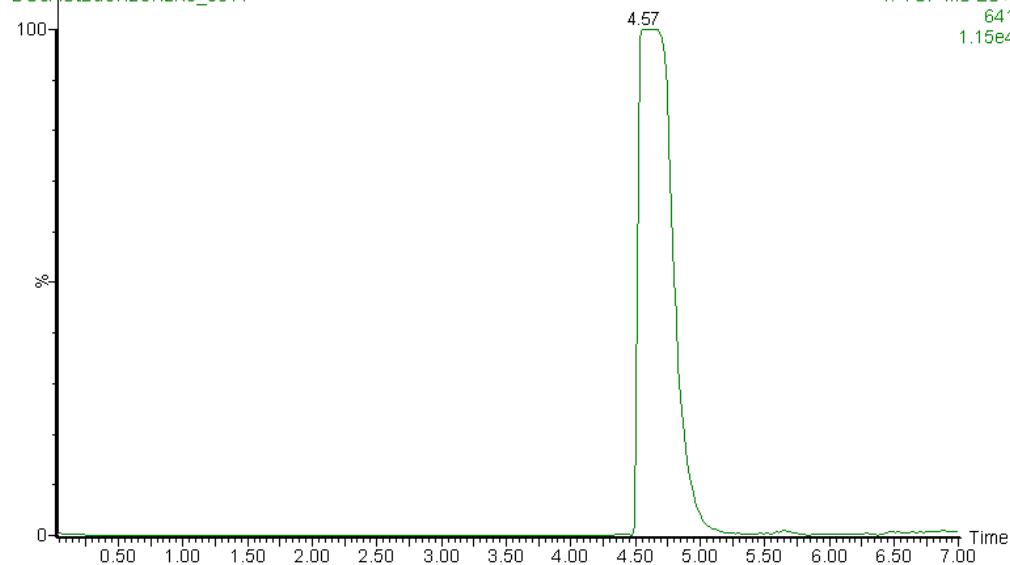
**Figure S28:** HR-ESI-MS spectrum of compound **7a**,  $M^+$  charge state observed (bottom) spectrum and calculated (top) spectrum.

**Mojmir 7**

DO3AOTBuCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>\_0611

06-Nov-2012

1: TOF MS ES+  
641  
1.15e4

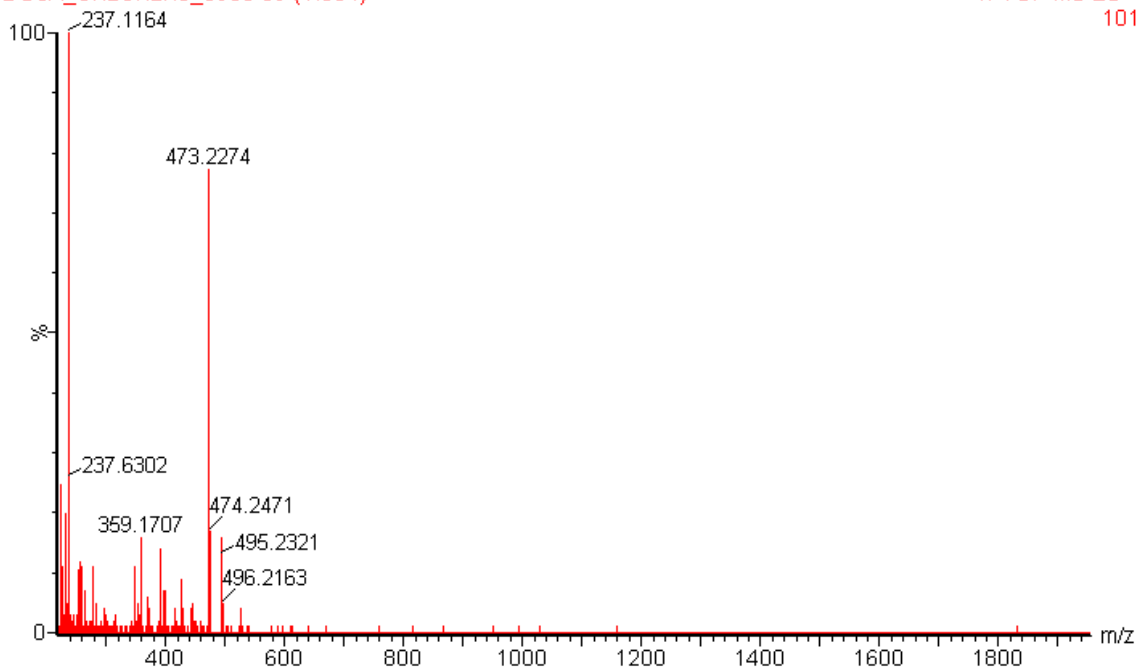


**Figure S29:** UPLC chromatogram (Method B) of compound **7a**, MS detector. Compound **7a** does not contain a suitable chromophore to allow for the UV detection.

Mojmir6

DO3A\_CH2CH2N3\_0903 59 (1.304)

1: TOF MS ES+  
101

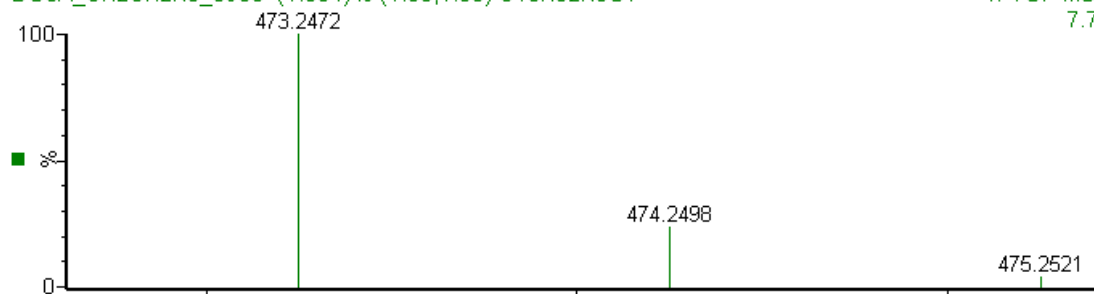


**Figure S30:** HR-ESI-MS spectrum of the ester deprotected version of compound **7a** showing a proper charge state envelope.

Mojmir6

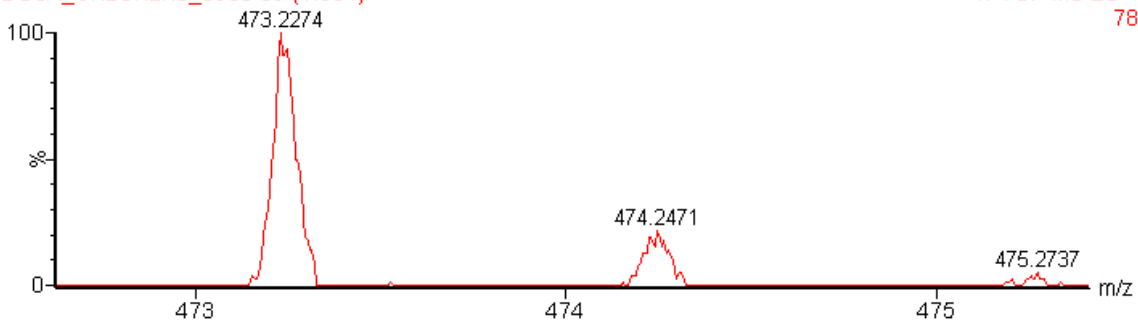
DO3A\_CH2CH2N3\_0903 (1.304) Is (1.00,1.00) C18H32N8O7

1: TOF MS ES+  
7.79e12



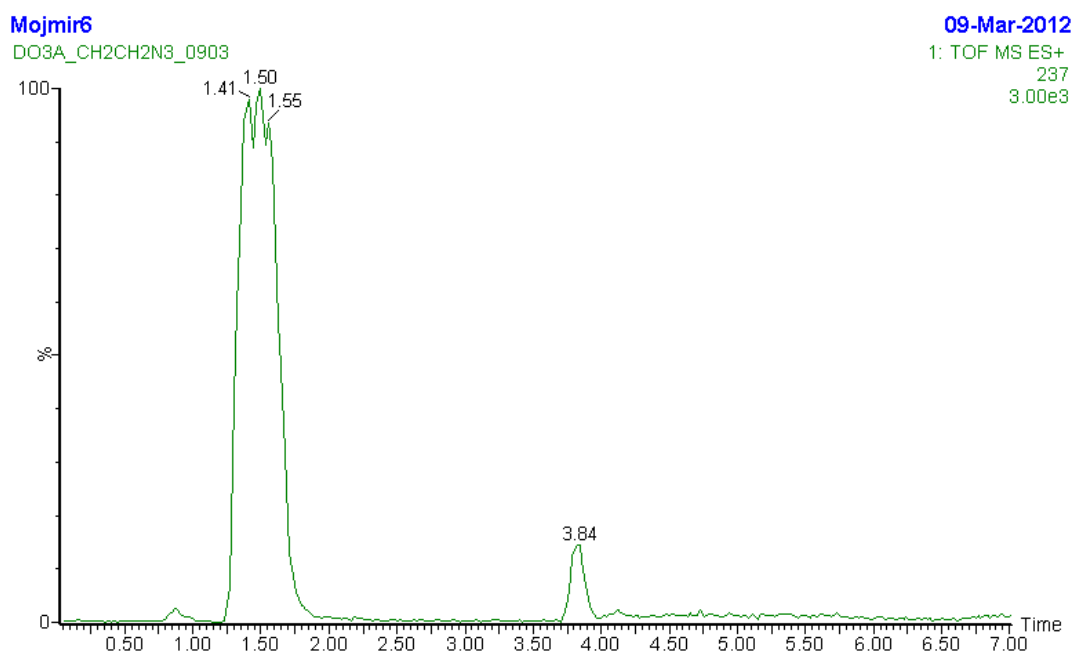
DO3A\_CH2CH2N3\_0903 59 (1.304)

1: TOF MS ES+  
78



**Figure S31:** HR-ESI-MS spectrum of the ester deprotected version of compound **7a**,  $M^+$  charge state observed (bottom) spectrum and calculated (top) spectrum.





**Figure S32:** UPLC chromatogram (Method B) of the ester deprotected version of compound **7a**, MS detector. Compound does not contain a suitable chromophore to allow for the UV detection.

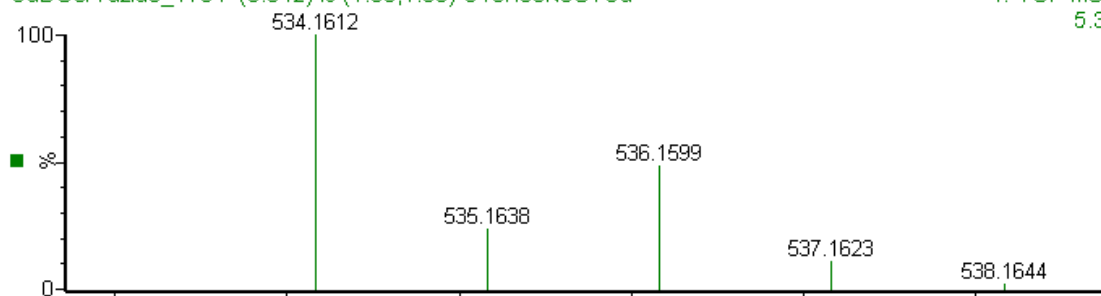


**Figure S33:** HR-ESI-MS spectrum of compound **7b** showing a proper charge state envelope.

**Mojmir 18**

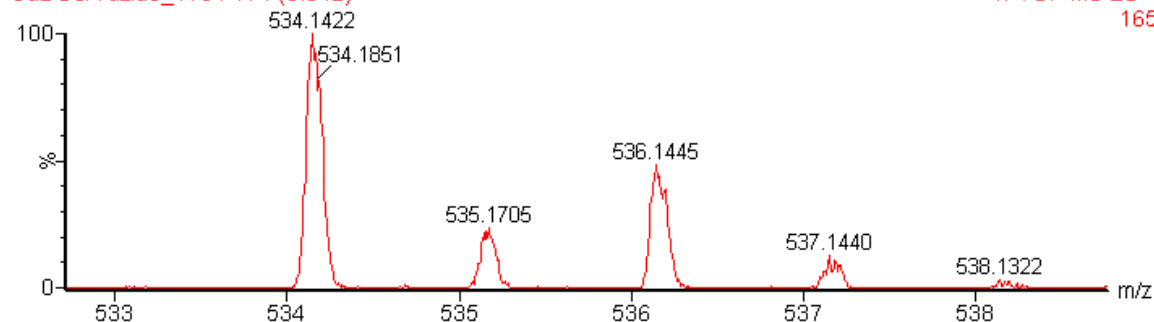
CuDO3A azide\_1704 (3.842) Is (1.00,1.00) C<sub>18</sub>H<sub>30</sub>N<sub>8</sub>O<sub>7</sub>Cu

1: TOF MS ES+  
5.39e12



CuDO3A azide\_1704 174 (3.842)

1: TOF MS ES+  
165

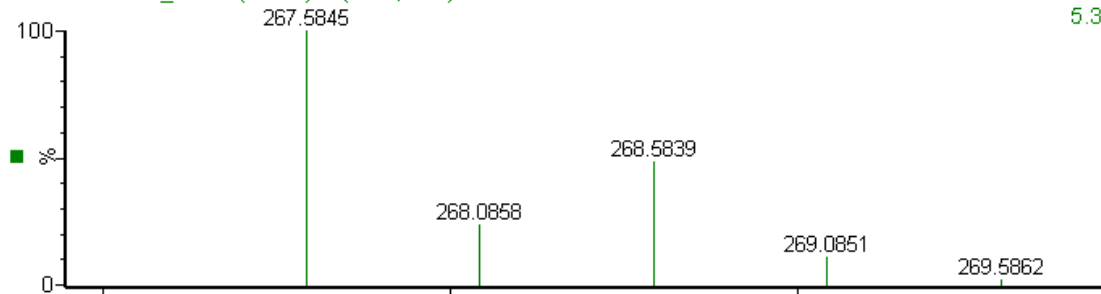


**Figure S34:** HR-ESI-MS spectrum of compound **7b**, M<sup>+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of Cu.

**Mojmir 18**

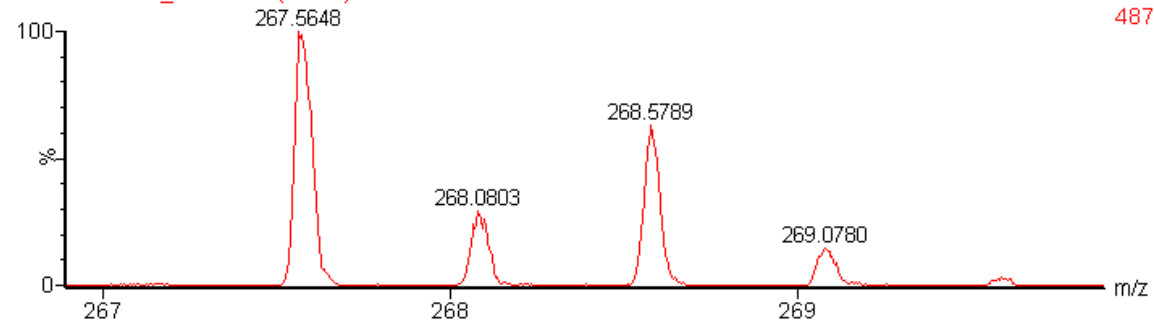
CuDO3A azide\_1704 (3.842) Is (1.00,1.00) C<sub>18</sub>H<sub>30</sub>N<sub>8</sub>O<sub>7</sub>Cu

1: TOF MS ES+  
5.39e12

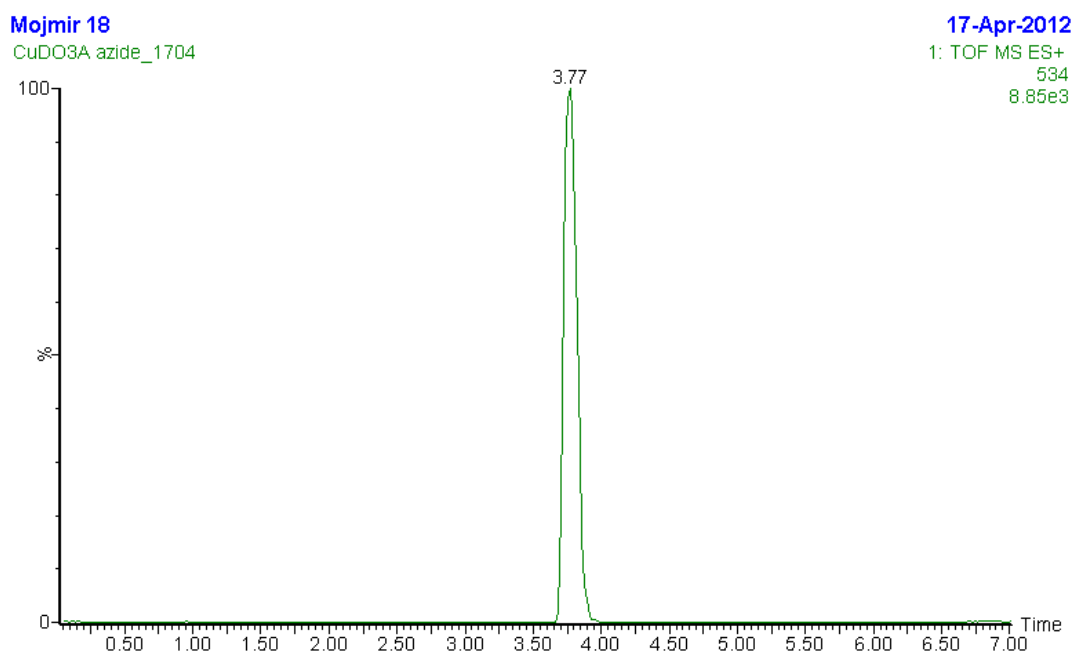


CuDO3A azide\_1704 174 (3.842)

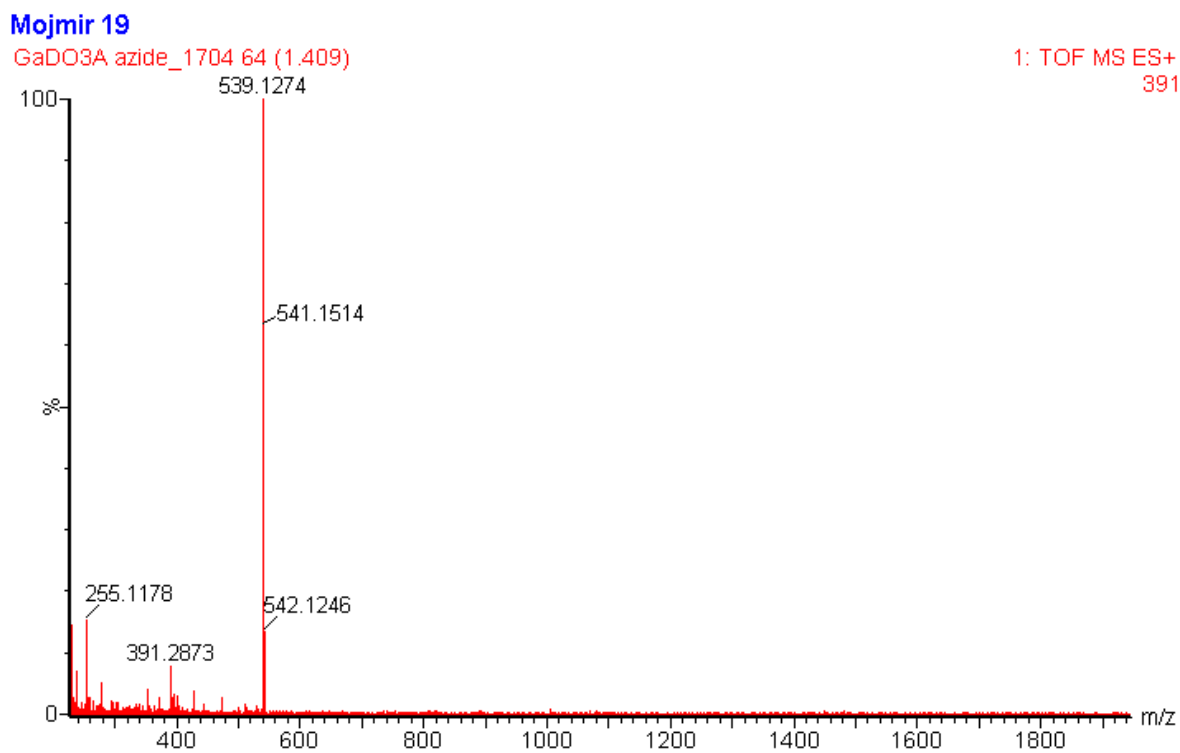
1: TOF MS ES+  
487



**Figure S35:** HR-ESI-MS spectrum of compound **7b**, M<sup>2+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of Cu.



**Figure S36:** UPLC chromatogram (Method B) of compound **7b**, MS detector. Compound **7b** does not contain a suitable chromophore to allow for the UV detection.

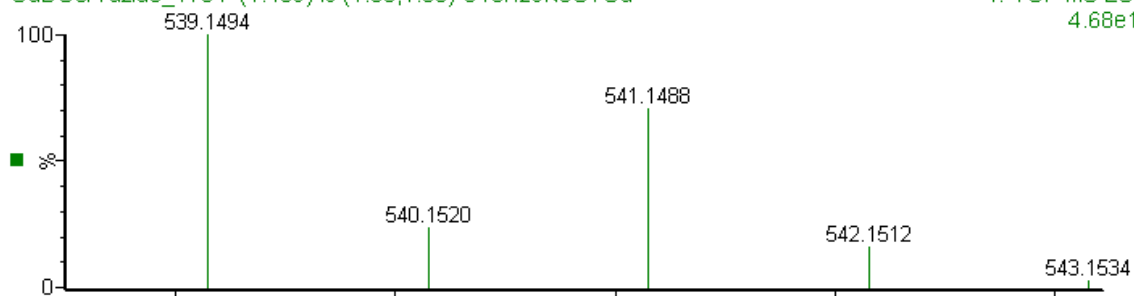


**Figure S37:** HR-ESI-MS spectrum of compound **7c**.

Mojmir 19

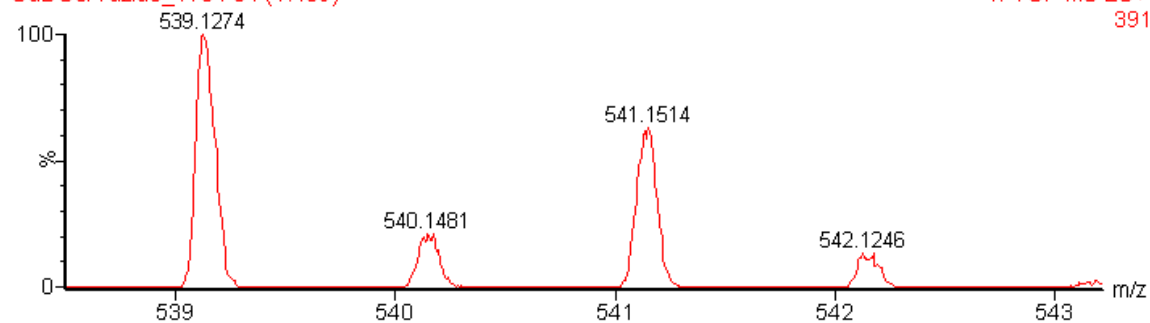
GaDO3A azide\_1704 (1.409) Is (1.00,1.00) C<sub>18</sub>H<sub>29</sub>N<sub>8</sub>O<sub>7</sub>Ga

1: TOF MS ES+  
4.68e12



GaDO3A azide\_1704 64 (1.409)

1: TOF MS ES+  
391



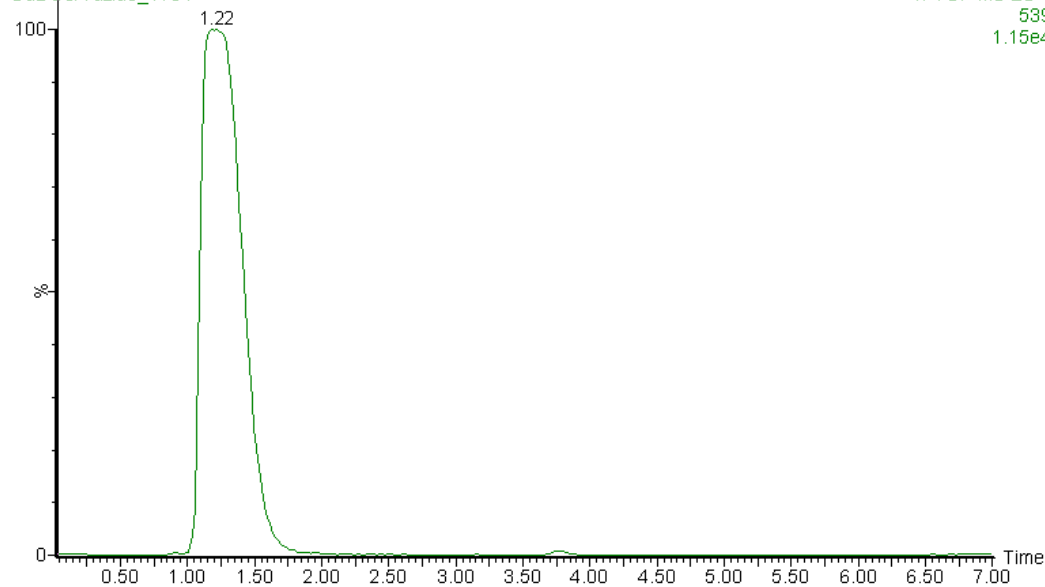
**Figure S38:** HR-ESI-MS spectrum of compound **7c**,  $M^+$  charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of Ga.

Mojmir 19

GaDO3A azide\_1704

17-Apr-2012

1: TOF MS ES+  
539  
1.15e4



**Figure S39:** UPLC chromatogram (Method B) of compound **7c**, MS detector. Compound **7c** does not contain a suitable chromophore to allow for the UV detection.

**Mojmir 20**

InDO3A azide\_1704 48 (1.056)

1: TOF MS ES+  
658

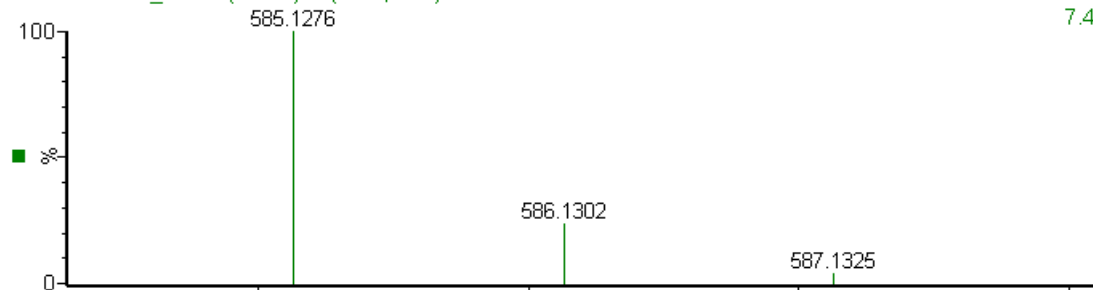


**Figure S40:** HR-ESI-MS spectrum of compound 7d showing a proper charge state envelope.

**Mojmir 20**

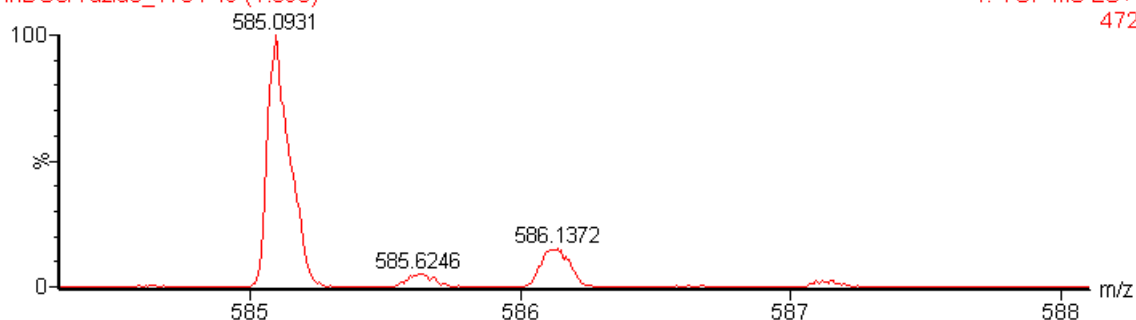
InDO3A azide\_1704 (1.093) Is (1.00,1.00) C18H29N8O7In

1: TOF MS ES+  
7.47e12



InDO3A azide\_1704 49 (1.093)

1: TOF MS ES+  
472



**Figure S41:** HR-ESI-MS spectrum of compound 7d,  $M^+$  charge state observed (bottom) spectrum and calculated (top) spectrum, note that In only possesses one stable isotope.

Mojmir 20

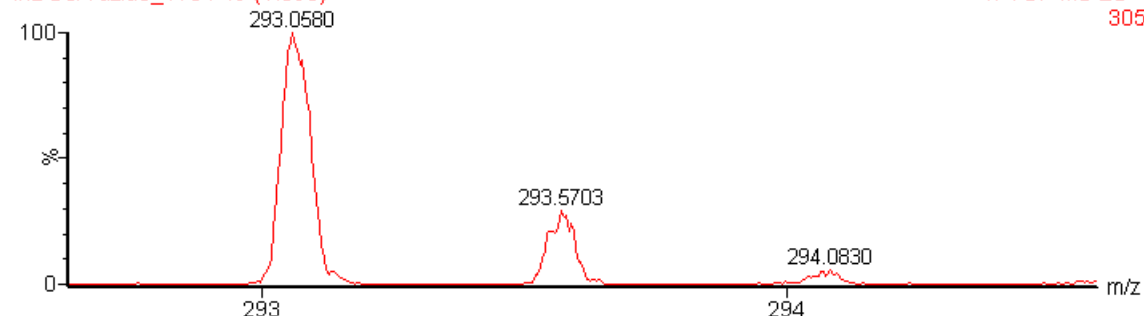
InDO3A azide\_1704 (1.093) Is (1.00,1.00) C<sub>18</sub>H<sub>29</sub>N<sub>8</sub>O<sub>7</sub>In

1: TOF MS ES+  
7.47e12



InDO3A azide\_1704 49 (1.093)

1: TOF MS ES+  
305



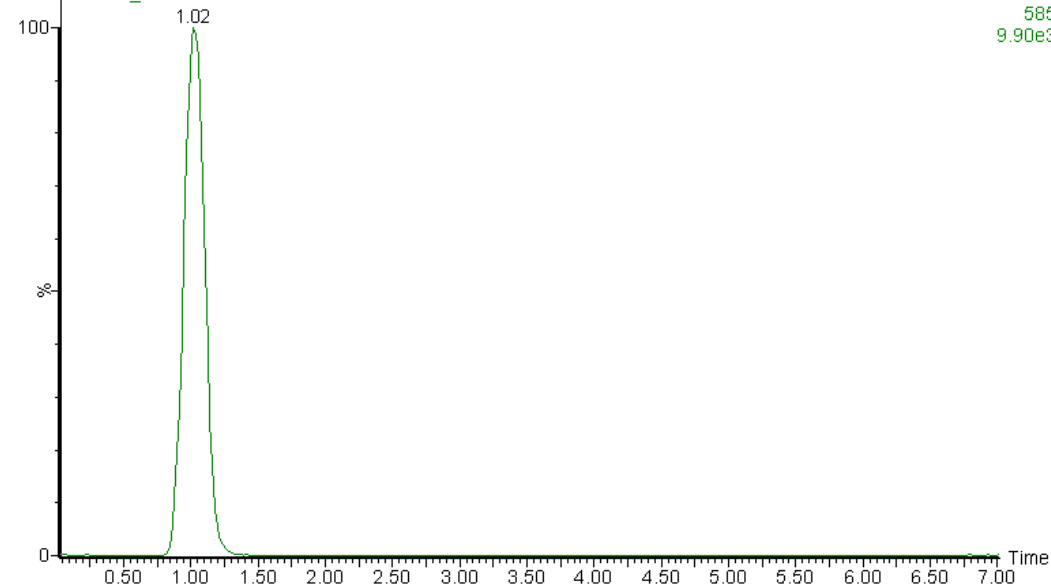
**Figure S42:** HR-ESI-MS spectrum of compound **7d**, M<sup>2+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum, note that In only possesses one stable isotope.

Mojmir 20

InDO3A azide\_1704

17-Apr-2012

1: TOF MS ES+  
585  
9.90e3

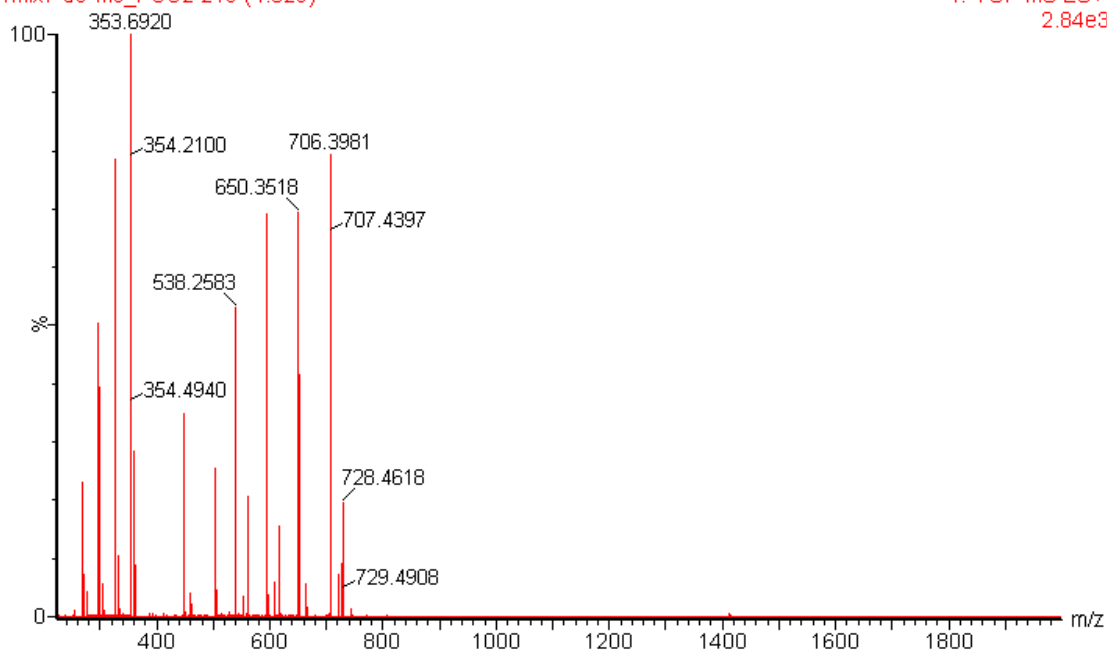


**Figure S43:** UPLC chromatogram (Method B) of compound **7d**, MS detector. Compound **7d** does not contain a suitable chromophore to allow for the UV detection.

Mojmir 71

rmix7-59-ms\_FCC2 219 (4.829)

1: TOF MS ES+  
2.84e3

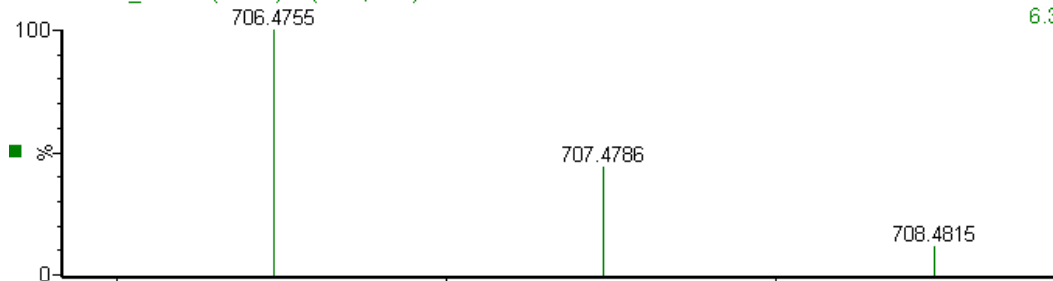


**Figure S44:** HR-ESI-MS spectrum of compound **13** showing a proper charge state envelope. The *t*-Bu ester functionalities present in **13** are not stable under the conditions of the analysis therefore  $M^+-56$ ,  $M^+-112$  and  $M^+-168$  ions are observed for the singly charged species and  $M^+-28$ ,  $M^+-56$  and  $M^+-84$  ions are observed for the doubly charged species.

Mojmir 71

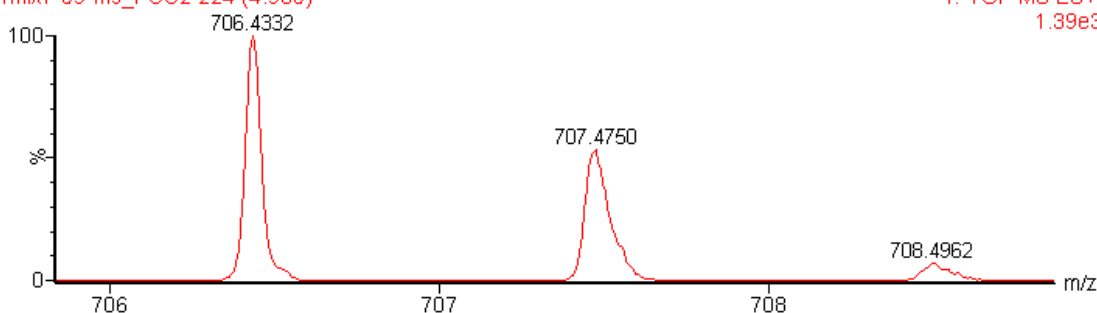
rmix7-59-ms\_FCC2 (4.935) Is (1.00,1.00) C37H63N5O8

1: TOF MS ES+  
6.34e12



rmix7-59-ms\_FCC2 224 (4.935)

1: TOF MS ES+  
1.39e3

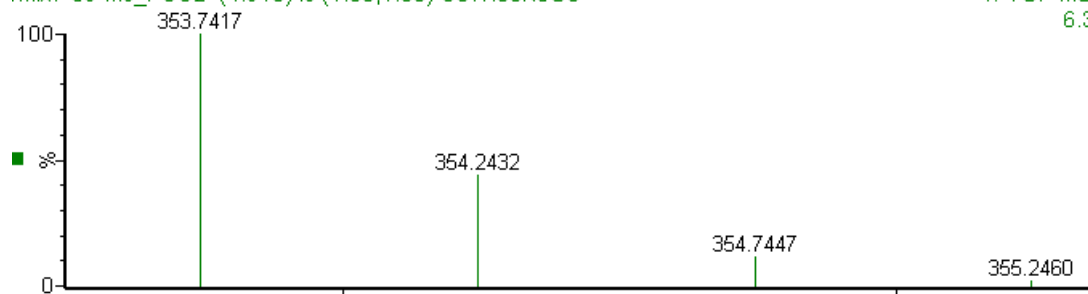


**Figure S45:** HR-ESI-MS spectrum of compound **13**,  $M^+$  charge state observed (bottom) spectrum and calculated (top) spectrum.

Mojmir 71

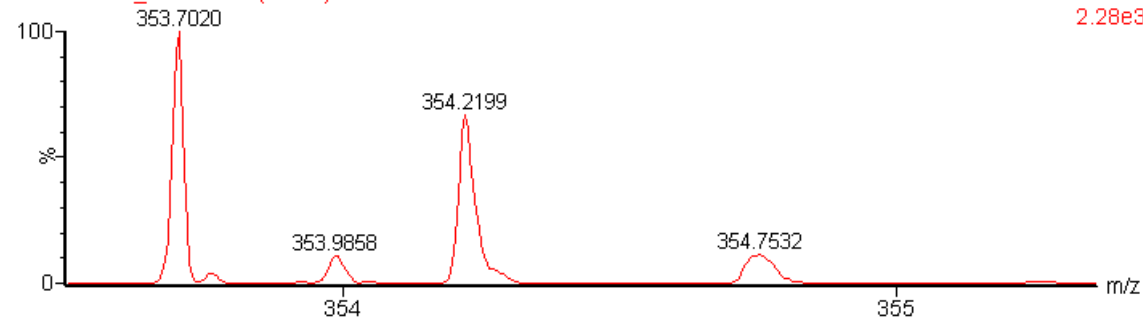
rmix7-59-ms\_FCC2 (4.918) Is (1.00,1.00) C37H63N5O8

1: TOF MS ES+  
6.34e12



rmix7-59-ms\_FCC2 223 (4.918)

1: TOF MS ES+  
2.28e3



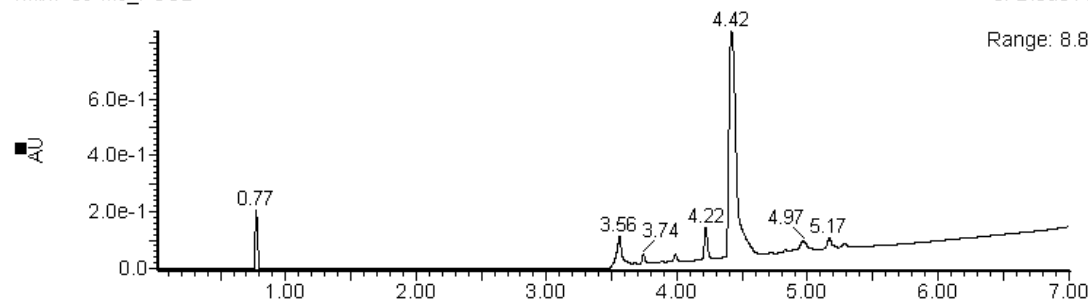
**Figure S46:** HR-ESI-MS spectrum of compound **13**,  $M^{2+}$  charge state observed (bottom) spectrum and calculated (top) spectrum.

Mojmir 71

rmix7-59-ms\_FCC2

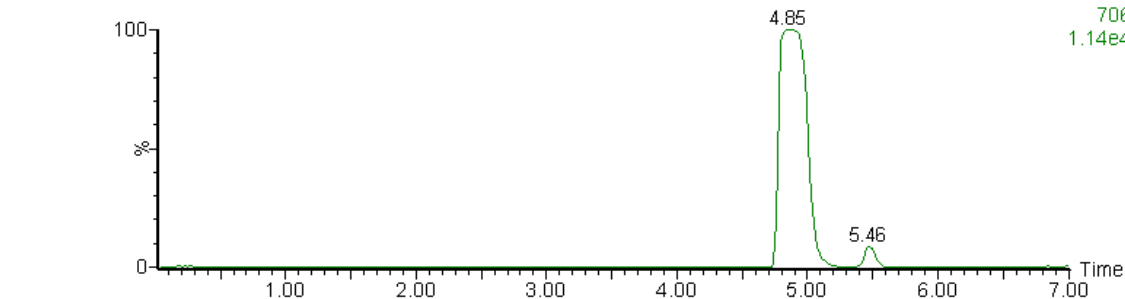
28-Nov-2012

3: Diode Array  
240  
Range: 8.84e-1



rmix7-59-ms2

1: TOF MS ES+  
706  
1.14e4



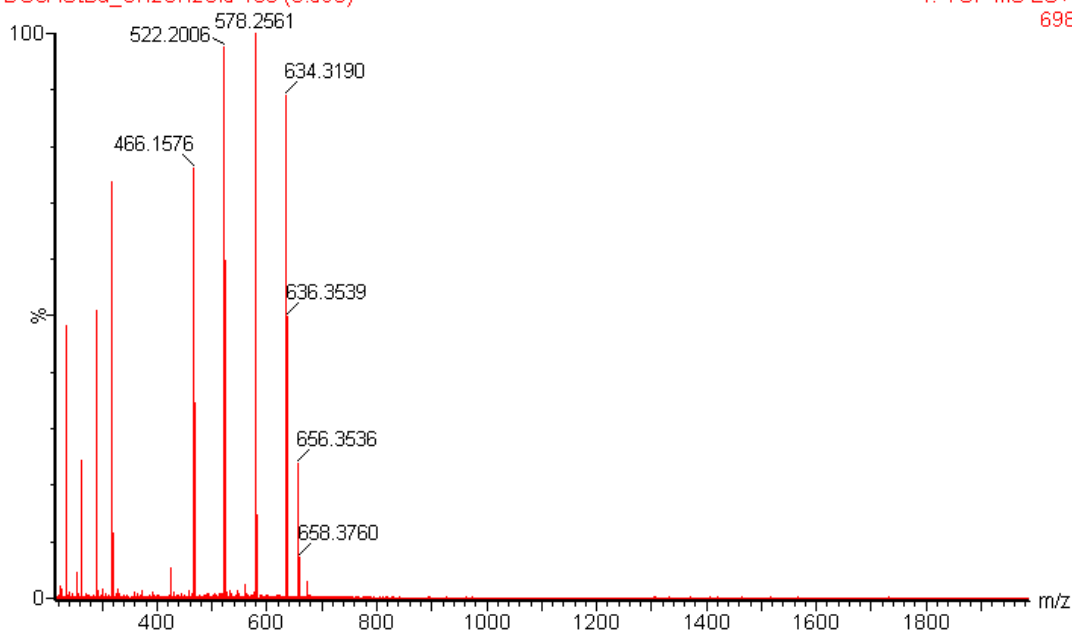
**Figure S47:** UPLC chromatograms (Method B) of compound **13**, MS detector (bottom), UV detector (top).



**Mojmir 6**

DO3A*O*tBu\_CH2CH2Cl5 163 (3.596)

1: TOF MS ES+  
698

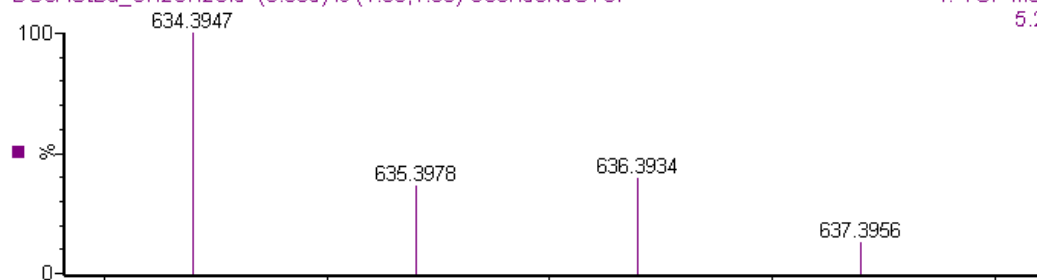


**Figure S48:** HR-ESI-MS spectrum of DO3A-*O*tBu-Ac 2-chloroethylamine showing a proper charge state envelope. The *t*-Bu ester functionalities present in this intermediate are not stable under the conditions of the analysis therefore  $M^+ - 56$ ,  $M^+ - 112$  and  $M^+ - 168$  ions are observed for the singly charged species and  $M^+ - 28$ ,  $M^+ - 56$  and  $M^+ - 84$  ions are observed for the doubly charged species.

**Mojmir 6**

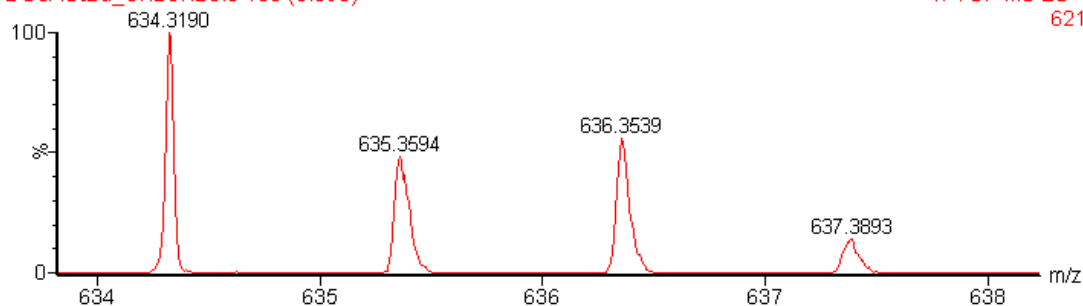
DO3A*O*tBu\_CH2CH2Cl5 (0.035) Is (1.00,1.00) C30H56N5O7Cl

1: TOF MS ES+  
5.21e12



DO3A*O*tBu\_CH2CH2Cl5 163 (3.596)

1: TOF MS ES+  
621

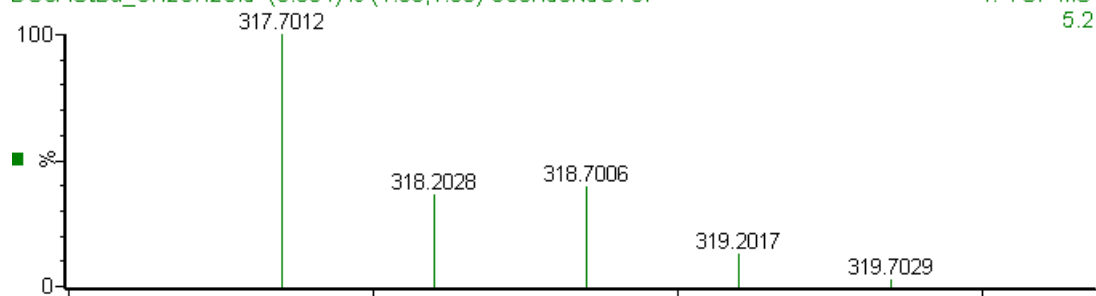


**Figure S49:** HR-ESI-MS spectrum of DO3A-*O*tBu-Ac 2-chloroethylamine,  $M^+$  charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of Cl.

**Vojmir 6**

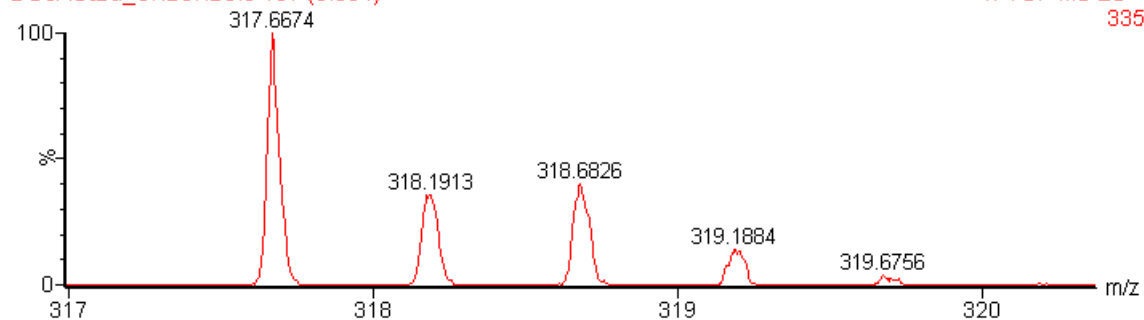
DO3A*o*tBu\_CH2CH2Cl5 (3.684) Is (1.00,1.00) C30H56N5O7Cl

1: TOF MS ES+  
5.21e12

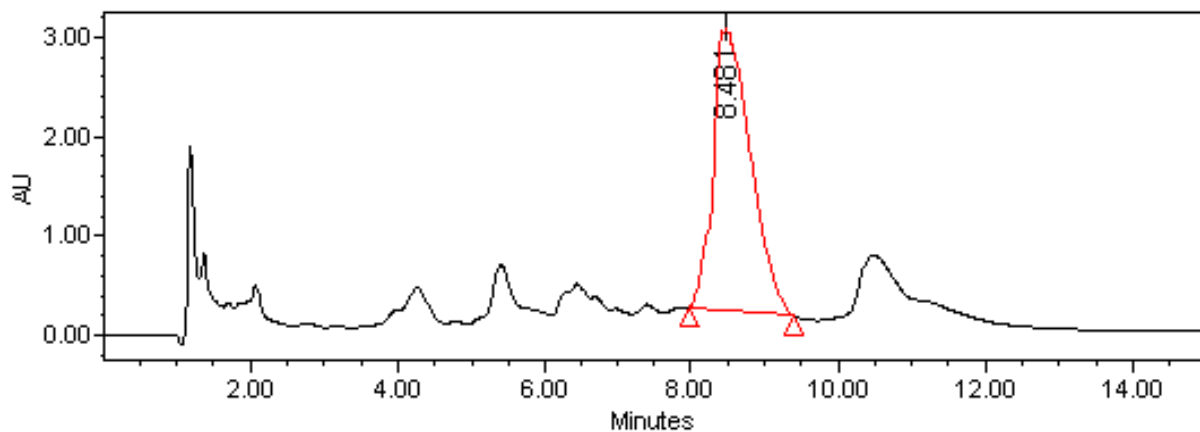


DO3A*o*tBu\_CH2CH2Cl5 167 (3.684)

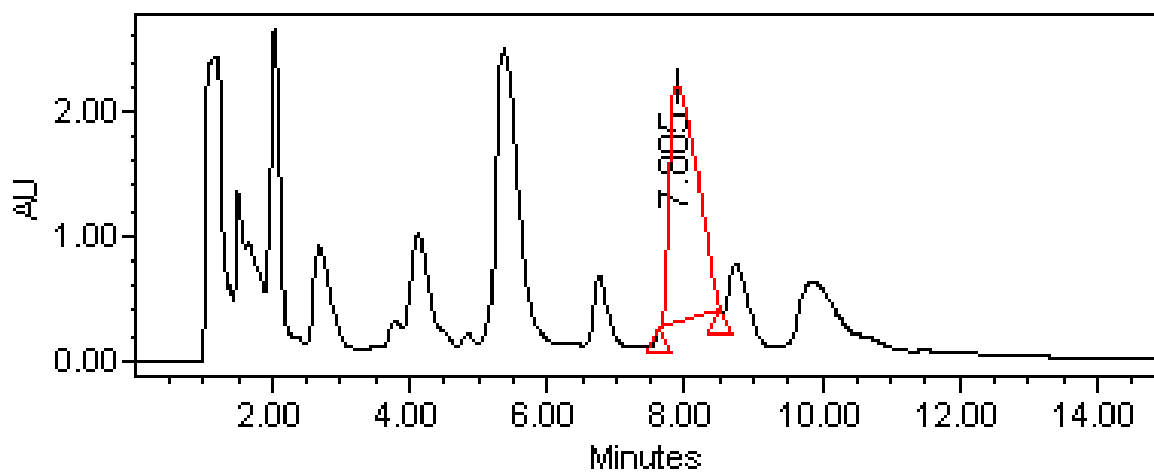
1: TOF MS ES+  
335



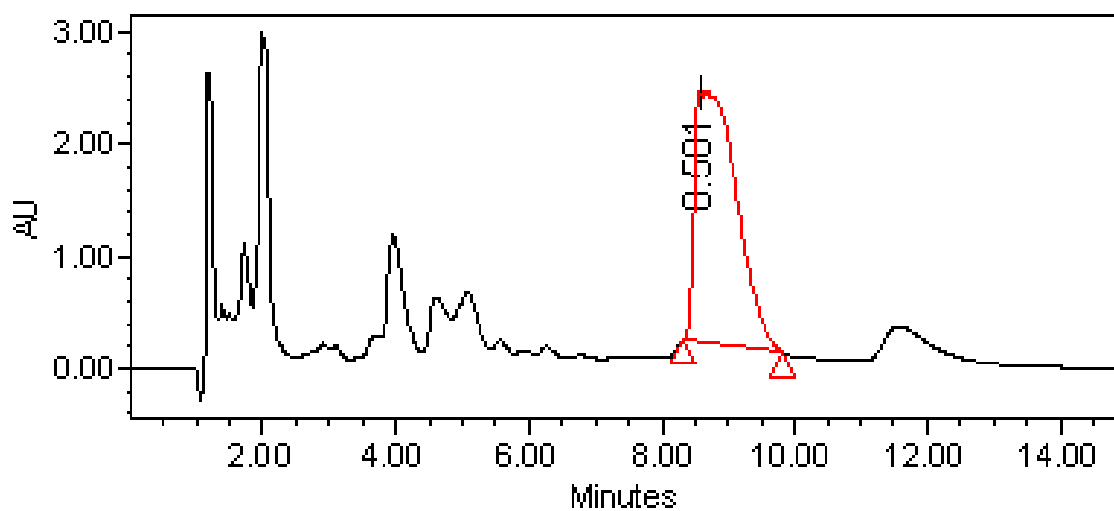
**Figure S50:** HR-ESI-MS spectrum of DO3A-*O*tBu-Ac 2-chloroethylamine, M<sup>2+</sup> charge state observed (bottom) spectrum and calculated (top) spectrum, note the isotope pattern due to the presence of Cl.



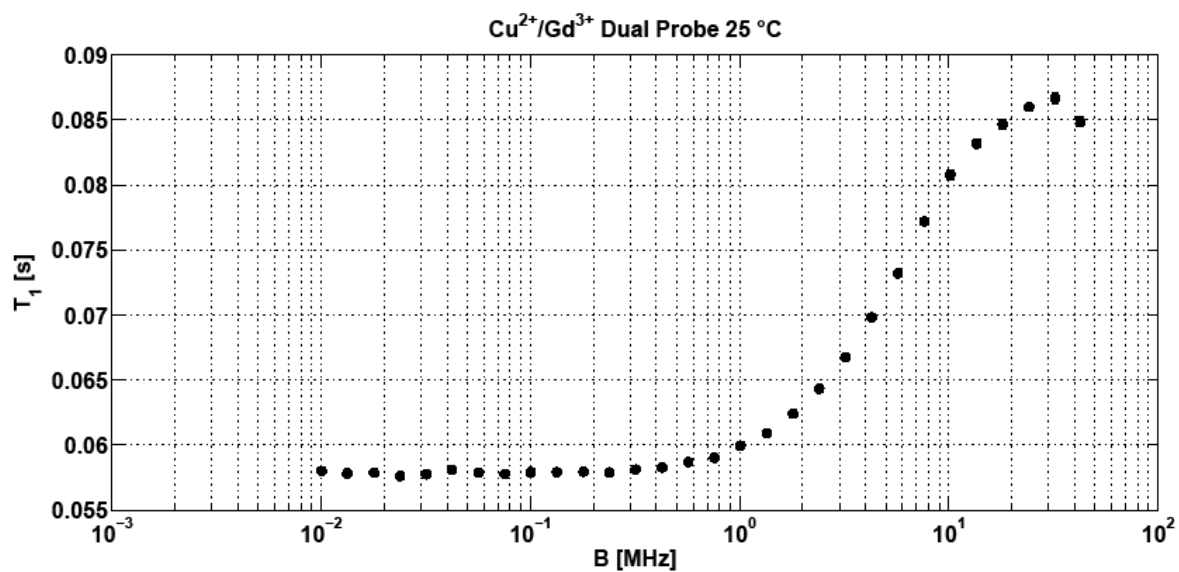
**Figure S51:** HPLC chromatogram (Method A) obtained during the semi-preparative purification of crude reaction mixture containing the  $\text{Gd}^{3+}/\text{Cu}^{2+}$  heterometallic complex **5a** ( $t_R$  8.5 min).



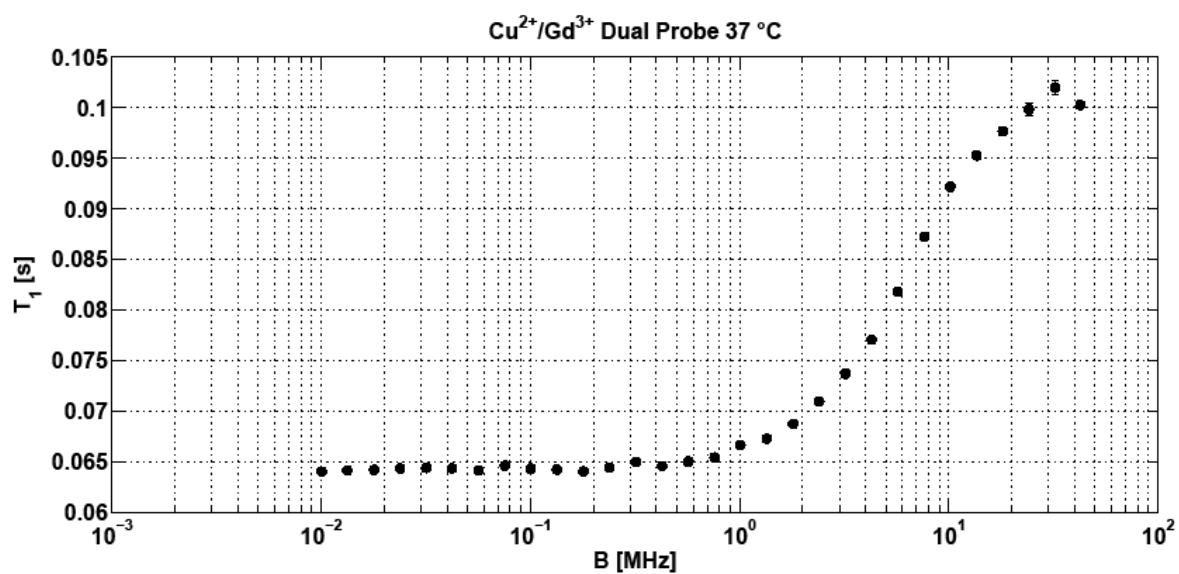
**Figure S52:** HPLC chromatogram (Method A) obtained during the semi-preparative purification of crude reaction mixture containing the  $\text{Gd}^{3+}/\text{Ga}^{3+}$  heterometallic complex **5b** ( $t_R$  7.8 min).



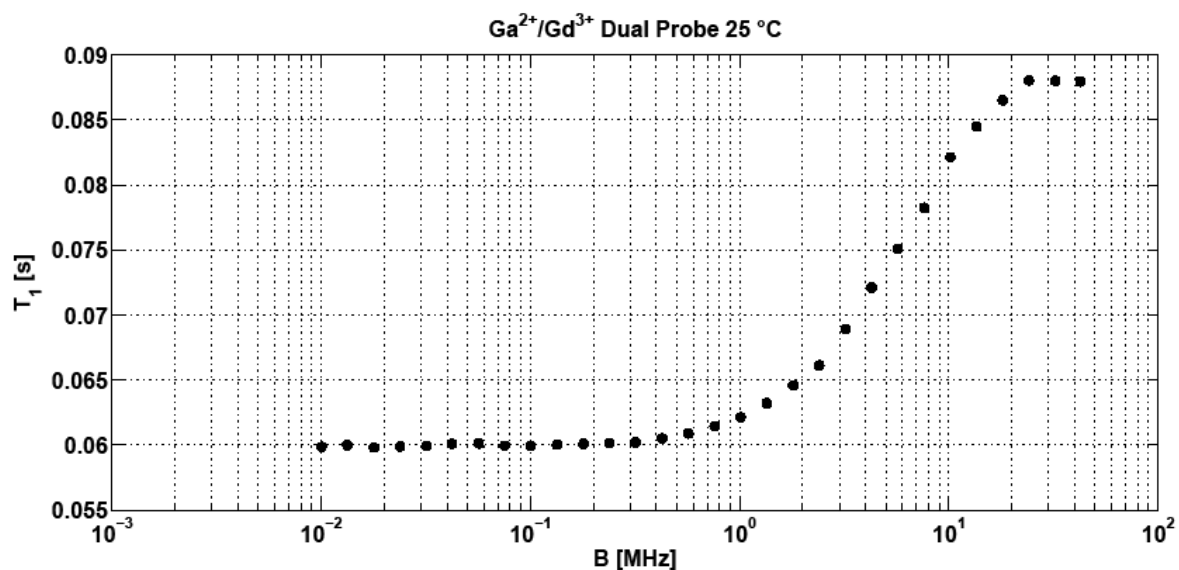
**Figure S53:** HPLC chromatogram (Method A) obtained during the semi-preparative purification of crude reaction mixture containing the  $\text{Gd}^{3+}/\text{In}^{3+}$  heterometallic complex **5c** ( $t_{\text{R}}$  8.5 min).



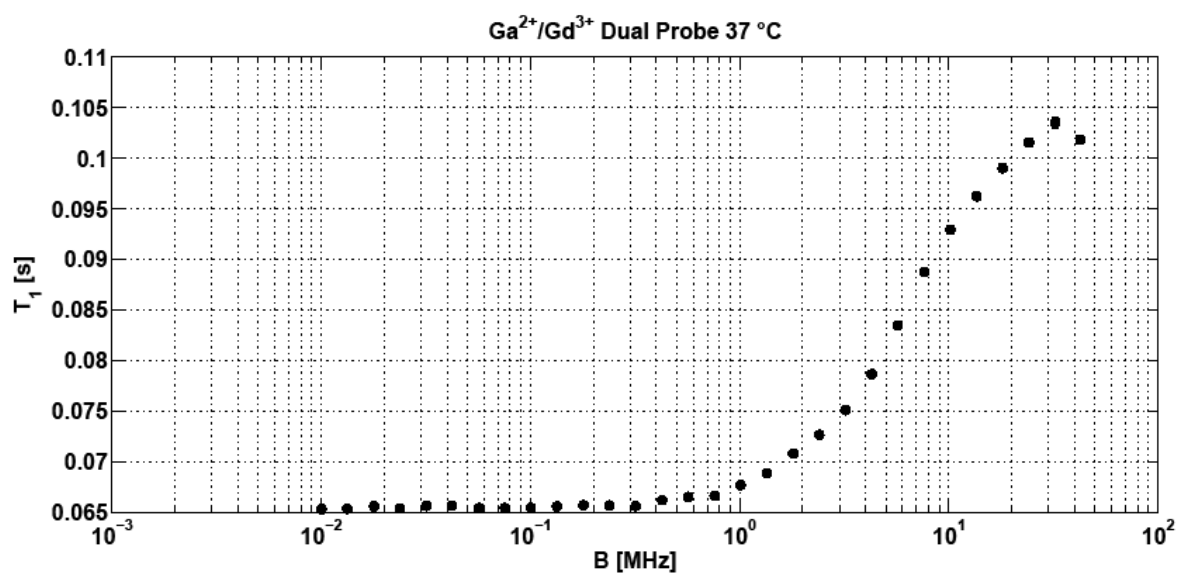
**Figure S54:** Dependence of T<sub>1</sub> relaxation time on the magnetic field strength for the Gd<sup>3+</sup>/Cu<sup>2+</sup> heterometallic complex **5a**, acquired at 25 °C.



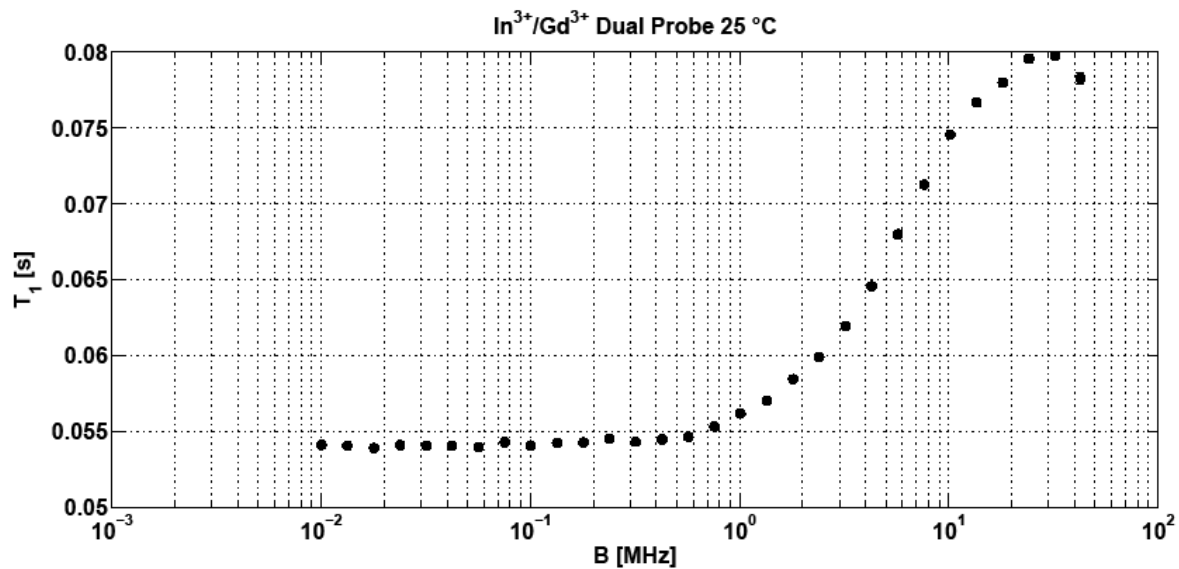
**Figure S55:** Dependence of T<sub>1</sub> relaxation time on the magnetic field strength for the Gd<sup>3+</sup>/Cu<sup>2+</sup> heterometallic complex **5a**, acquired at 37 °C.



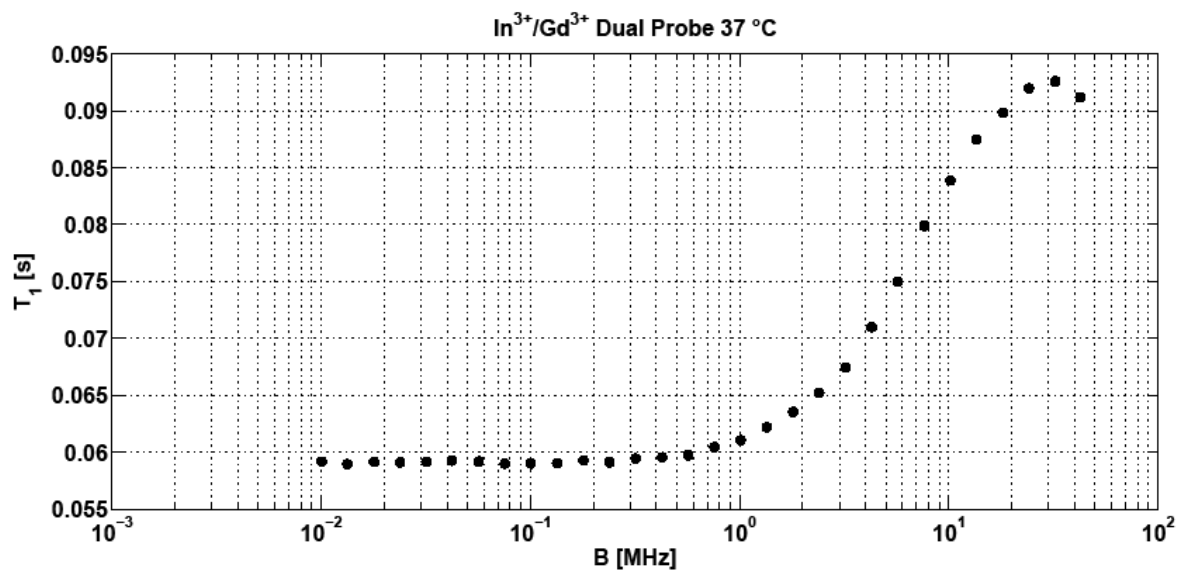
**Figure S56:** Dependence of T<sub>1</sub> relaxation time on the magnetic field strength for the Gd<sup>3+</sup>/Ga<sup>3+</sup> heterometallic complex **5b**, acquired at 25 °C.



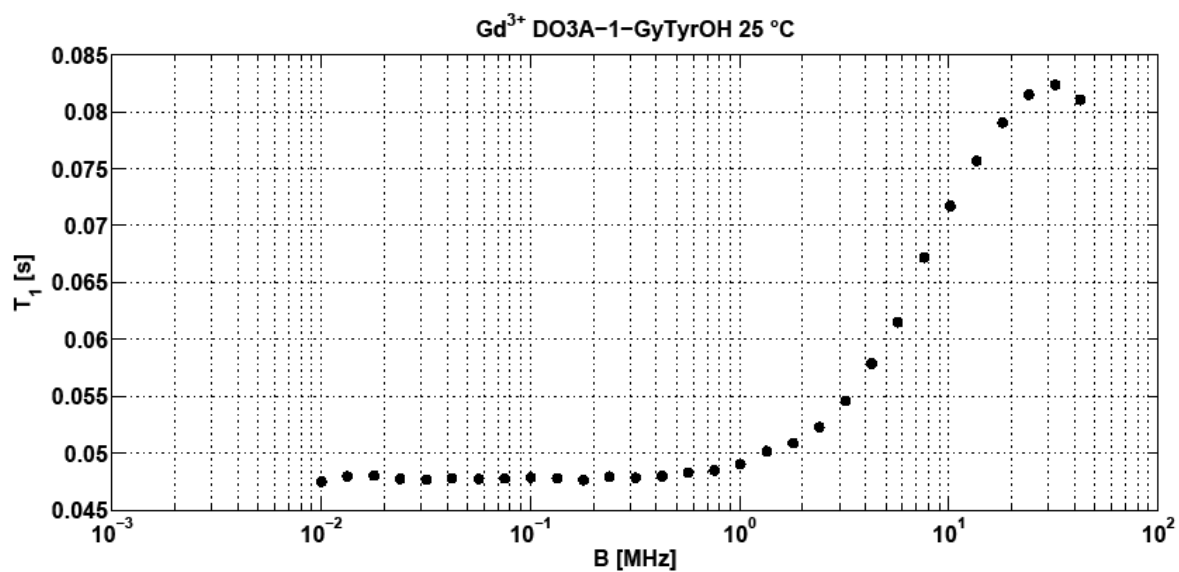
**Figure S57:** Dependence of T<sub>1</sub> relaxation time on the magnetic field strength for the Gd<sup>3+</sup>/Ga<sup>3+</sup> heterometallic complex **5b**, acquired at 37 °C.



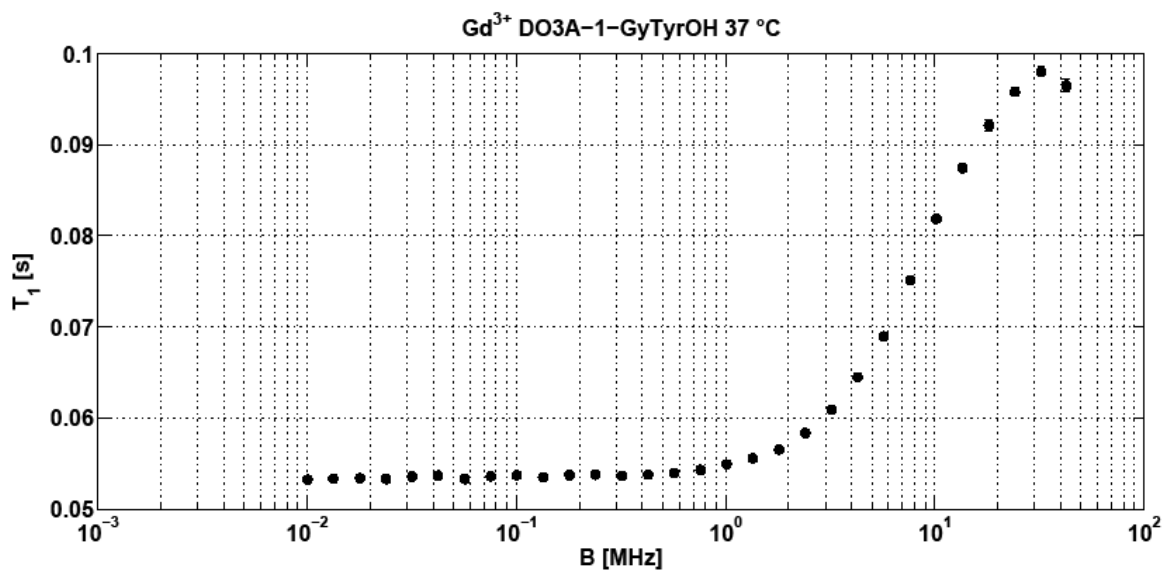
**Figure S58:** Dependence of  $T_1$  relaxation time on the magnetic field strength for the  $\text{Gd}^{3+}/\text{In}^{3+}$  heterometallic complex **5c**, acquired at 25 °C.



**Figure S59:** Dependence of  $T_1$  relaxation time on the magnetic field strength for the  $\text{Gd}^{3+}/\text{In}^{3+}$  heterometallic complex **5c**, acquired at 37 °C.

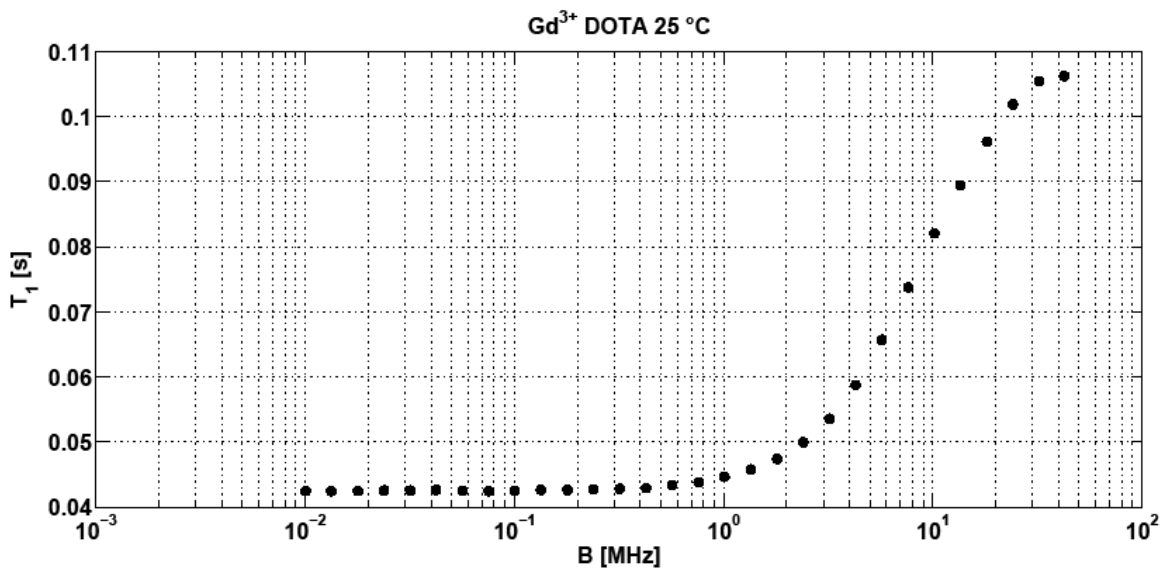


**Figure S60:** Dependence of  $T_1$  relaxation time on the magnetic field strength for the alkyne building block **6b**, acquired at 25 °C.

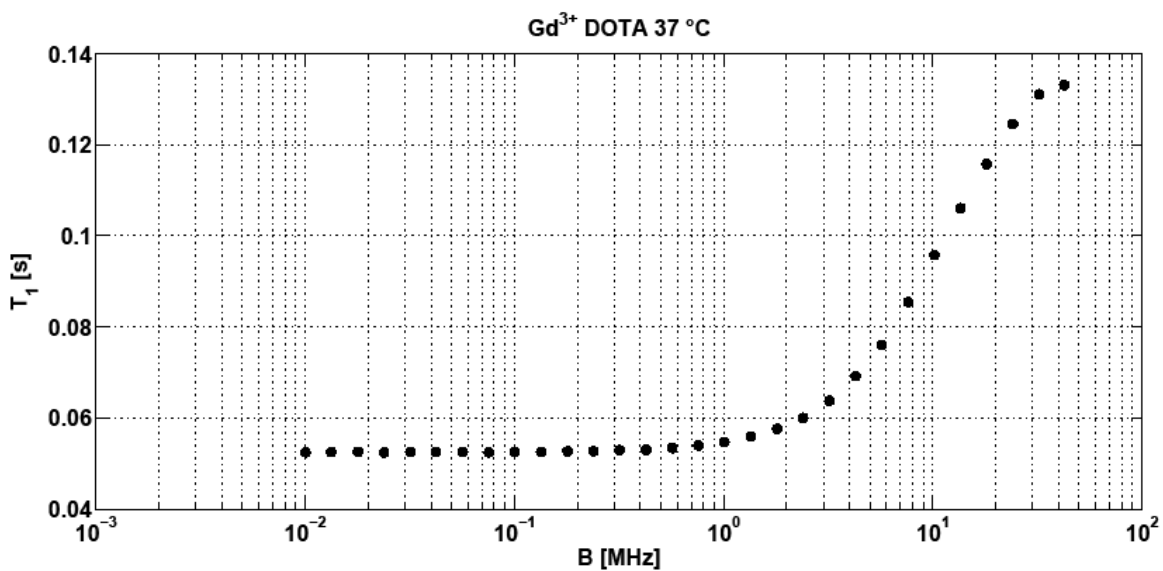


**Figure S61:** Dependence of  $T_1$  relaxation time on the magnetic field strength for the alkyne building block **6b**, acquired at 37 °C.

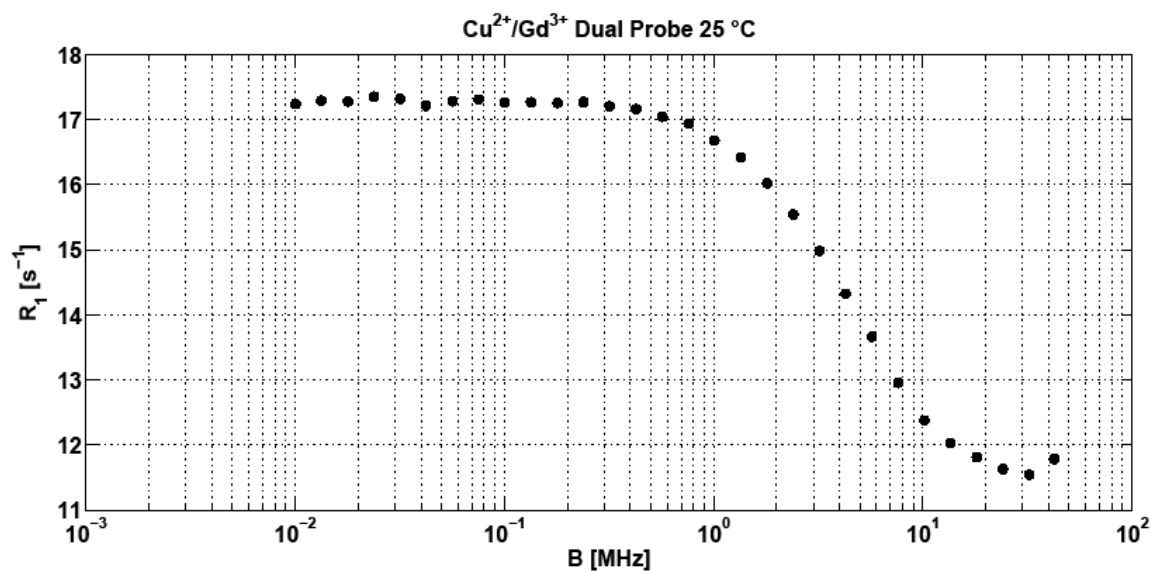




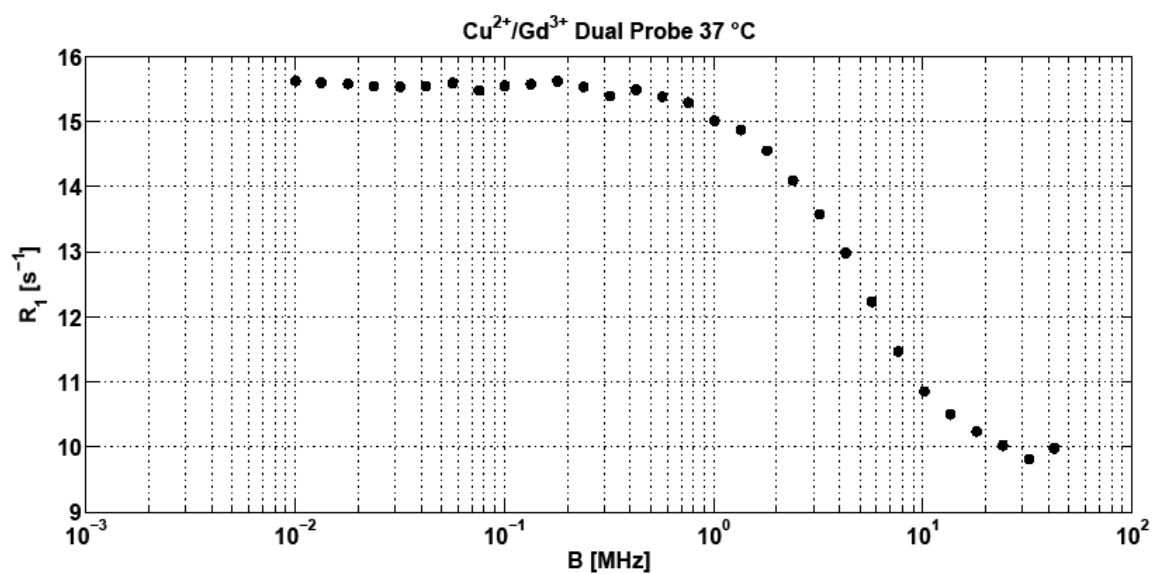
**Figure S62:** Dependence of  $T_1$  relaxation time on the magnetic field strength for the commercial MRI contrast agent Dotarem (**16**), acquired at 25 °C.



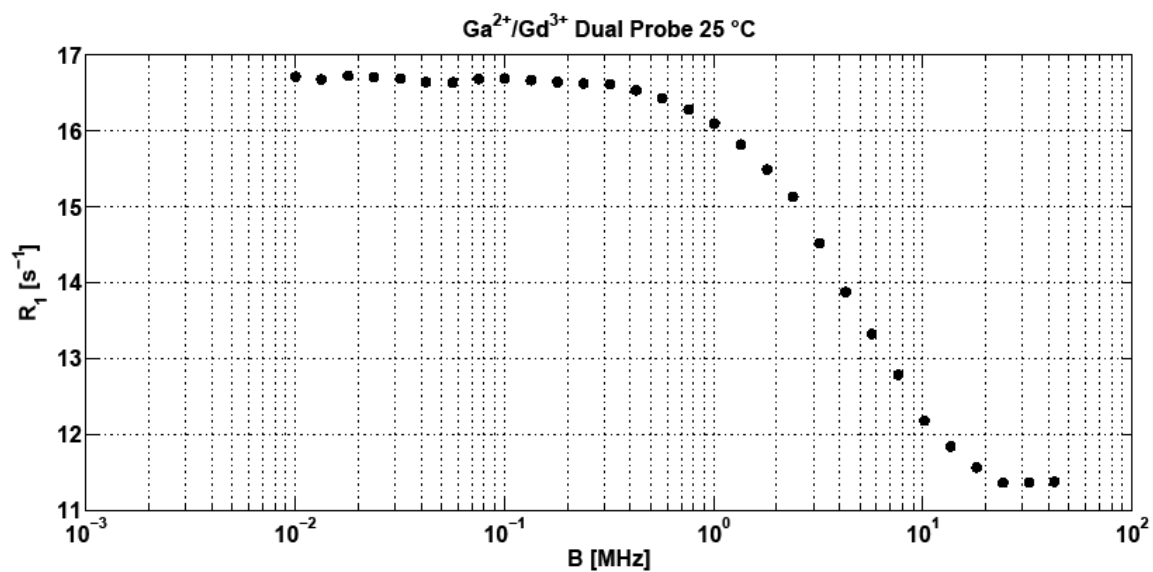
**Figure S63:** Dependence of  $T_1$  relaxation time on the magnetic field strength for the commercial MRI contrast agent Dotarem (**16**), acquired at 37 °C.



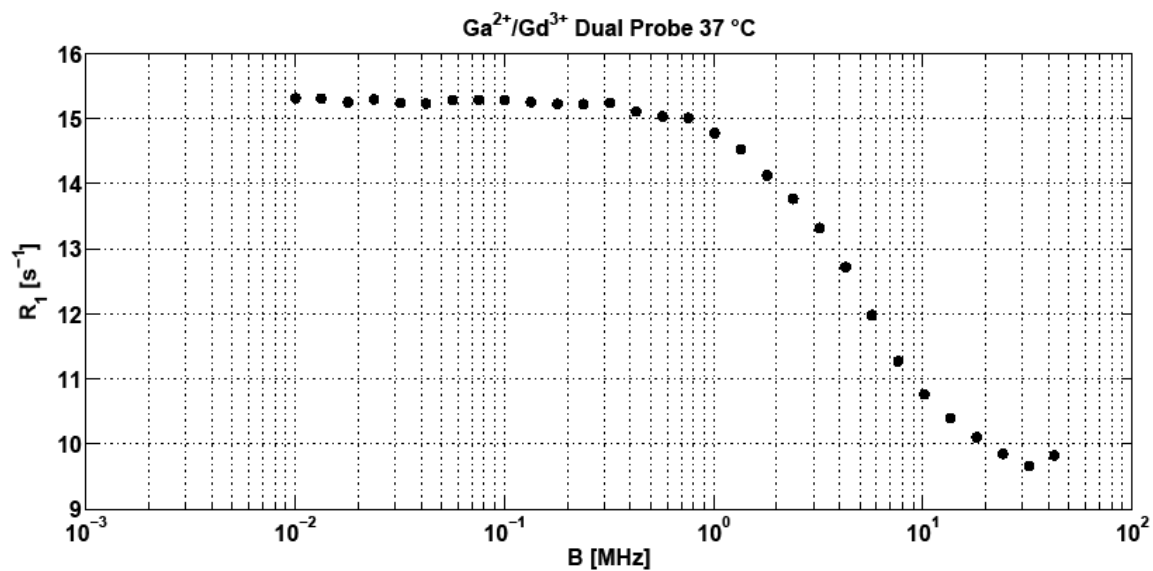
**Figure S64:** Dependence of  $R_1$  relaxivity on the magnetic field strength for the  $Gd^{3+}/Cu^{2+}$  heterometallic complex **5a**, acquired at 25 °C.



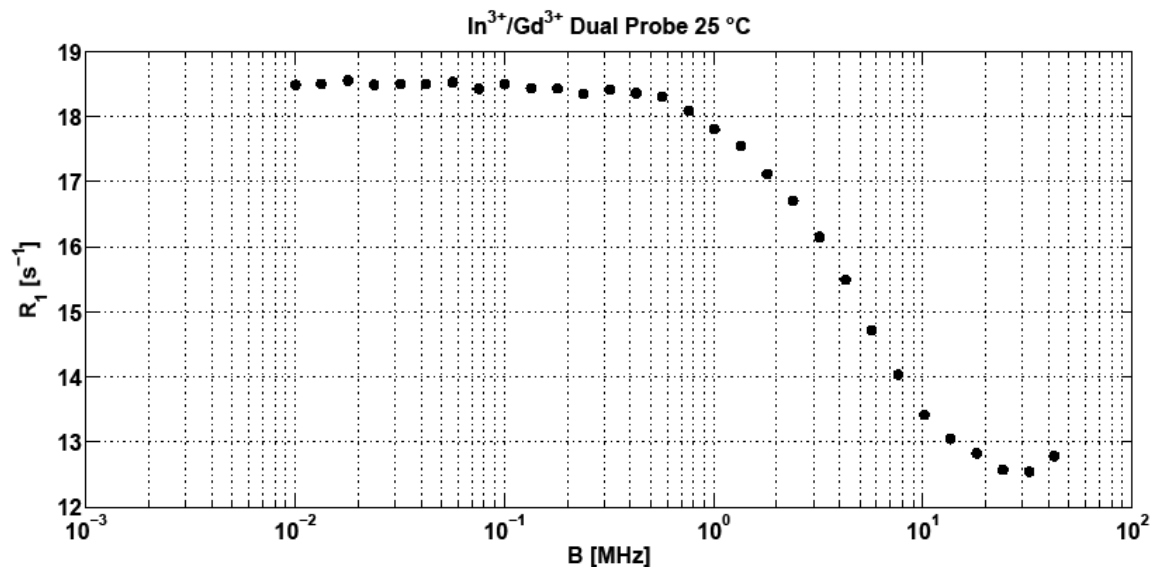
**Figure S65:** Dependence of  $R_1$  relaxivity on the magnetic field strength for the  $Gd^{3+}/Cu^{2+}$  heterometallic complex **5a**, acquired at 37 °C.



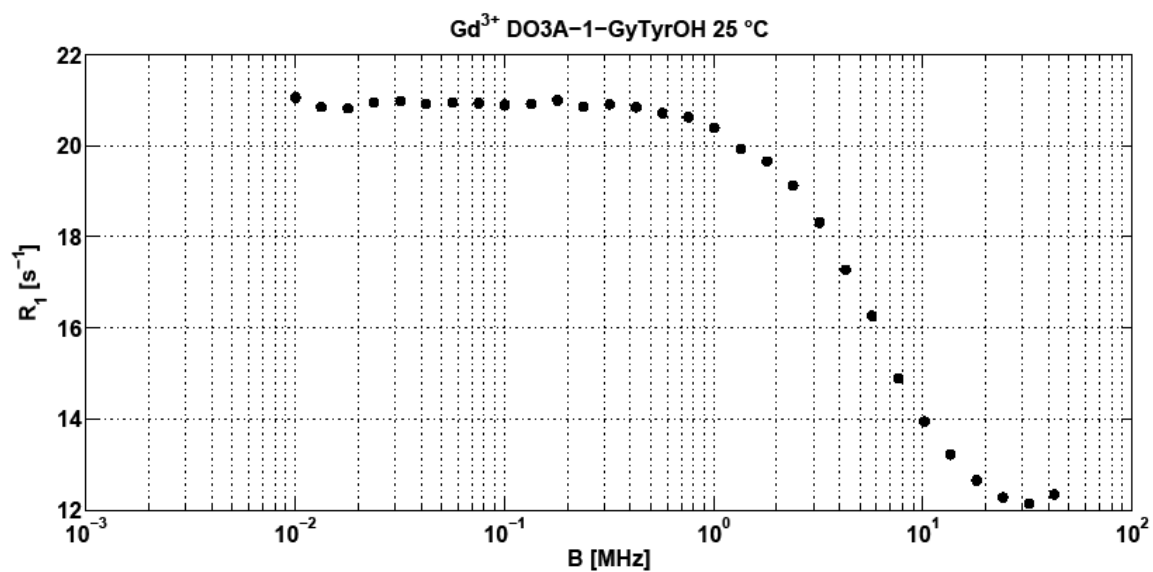
**Figure S66:** Dependence of R<sub>1</sub> relaxivity on the magnetic field strength for the Gd<sup>3+</sup>/Ga<sup>3+</sup> heterometallic complex **5b**, acquired at 25 °C.



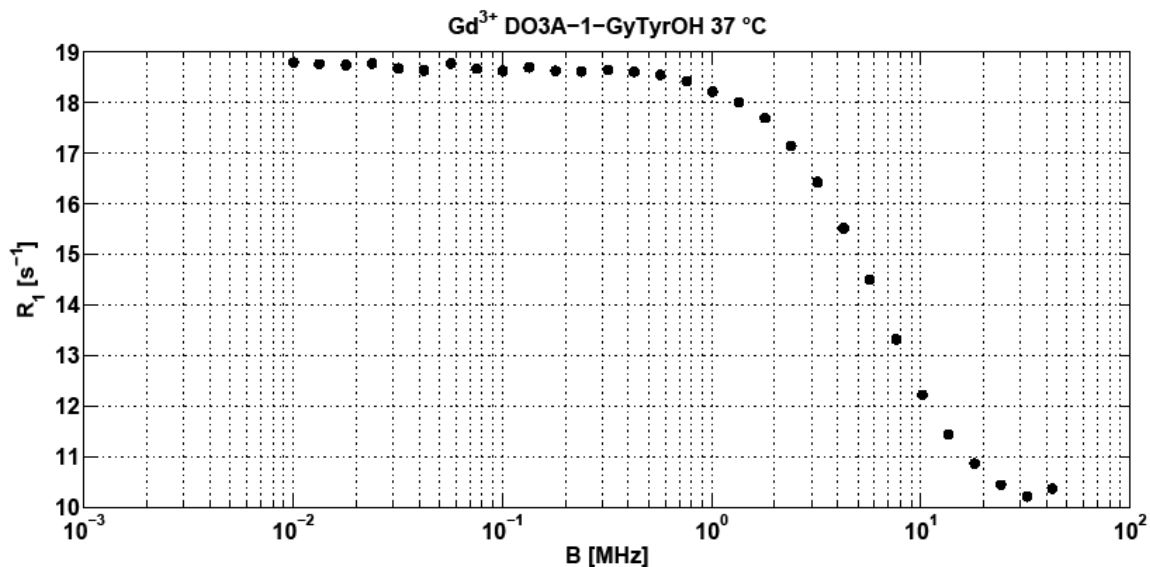
**Figure S67:** Dependence of R<sub>1</sub> relaxivity on the magnetic field strength for the Gd<sup>3+</sup>/Ga<sup>3+</sup> heterometallic complex **5b**, acquired at 37 °C.



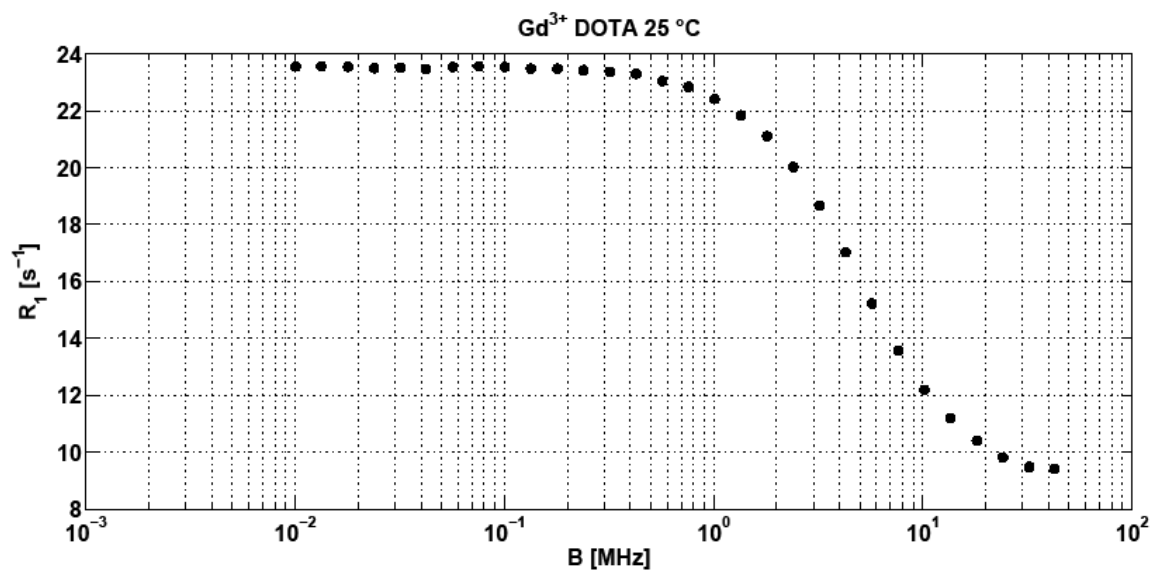
**Figure S68:** Dependence of  $R_1$  relaxivity on the magnetic field strength for the  $Gd^{3+}/In^{3+}$  heterometallic complex **5c**, acquired at 25 °C. Related NMRD profile acquired at 37 °C is shown in the body of the paper (Figure 5).



**Figure S69:** Dependence of  $R_1$  relaxivity on the magnetic field strength for the alkyne building block **6b**, acquired at 25 °C.



**Figure S70:** Dependence of  $R_1$  relaxivity on the magnetic field strength for the alkyne building block **6b**, acquired at 37 °C.



**Figure S71:** Dependence of  $R_1$  relaxivity on the magnetic field strength for the commercial MRI contrast agent Dotarem (**16**), acquired at 25 °C. Related NMRD profile acquired at 37 °C is shown in the body of the paper (Figure 5)