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Amino Acid Based Gallium-68 Chelators Capable of Radiolabeling at Neutral pH

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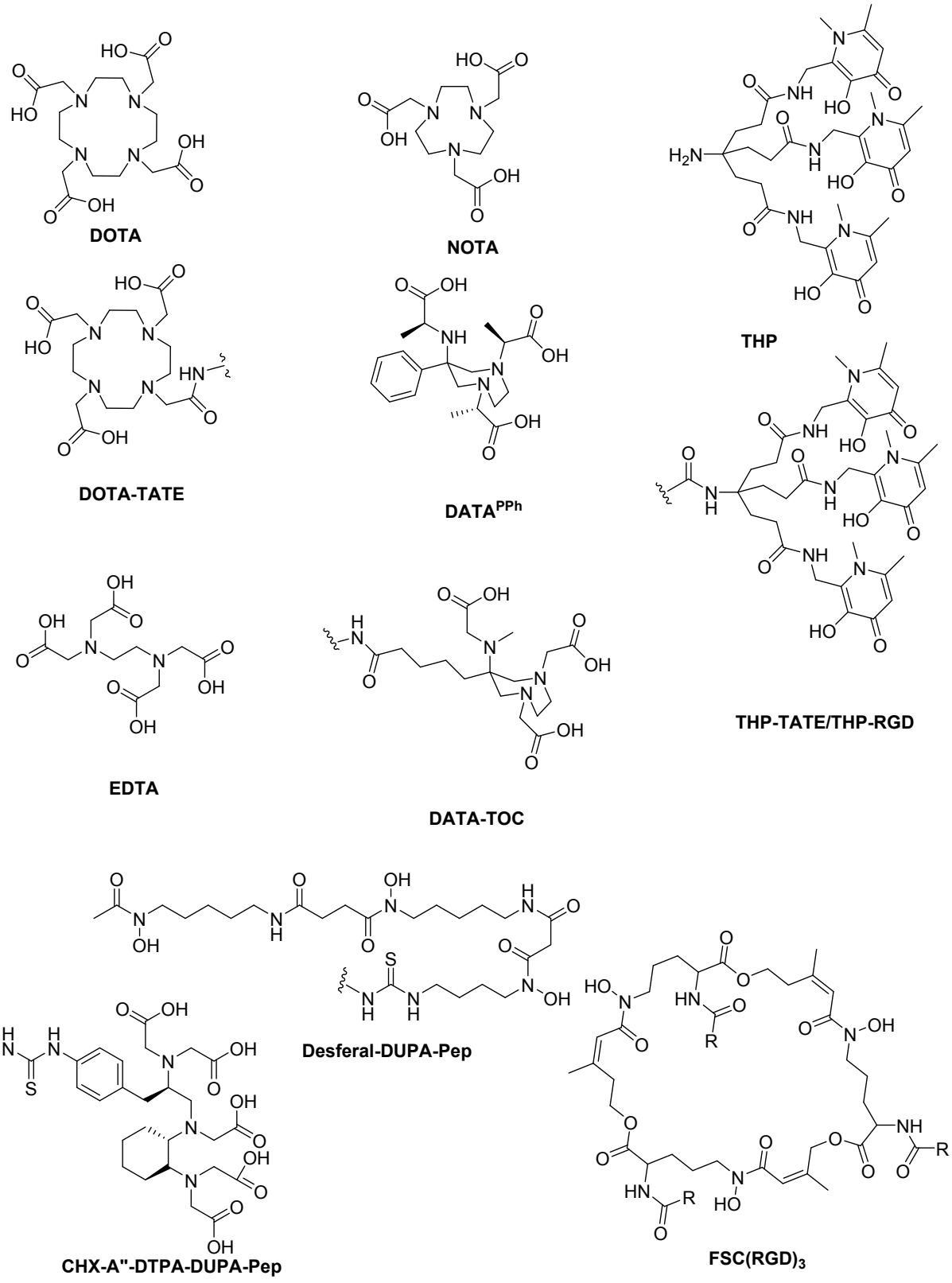


Figure S 1: Structure of literature Ga(III) chelators discussed in this report

1.1 Ligands

Table S 1: Overall protonation constants (β_{hlm}) for ligands (T = 25 °C, I = 0.1 M (NMe_4)Cl)

	H_3Dpaa	H_3Dpaa.dab	H_4Dpaa.ga

HL	7.38(2)	11.35(1)	7.17(1)
H_2L	11.11(2)	16.74(2)	11.84(2)
H_3L	13.93(2)	20.51(3)	15.76(2)
H_4L	-	23.20(3)	18.51(2)

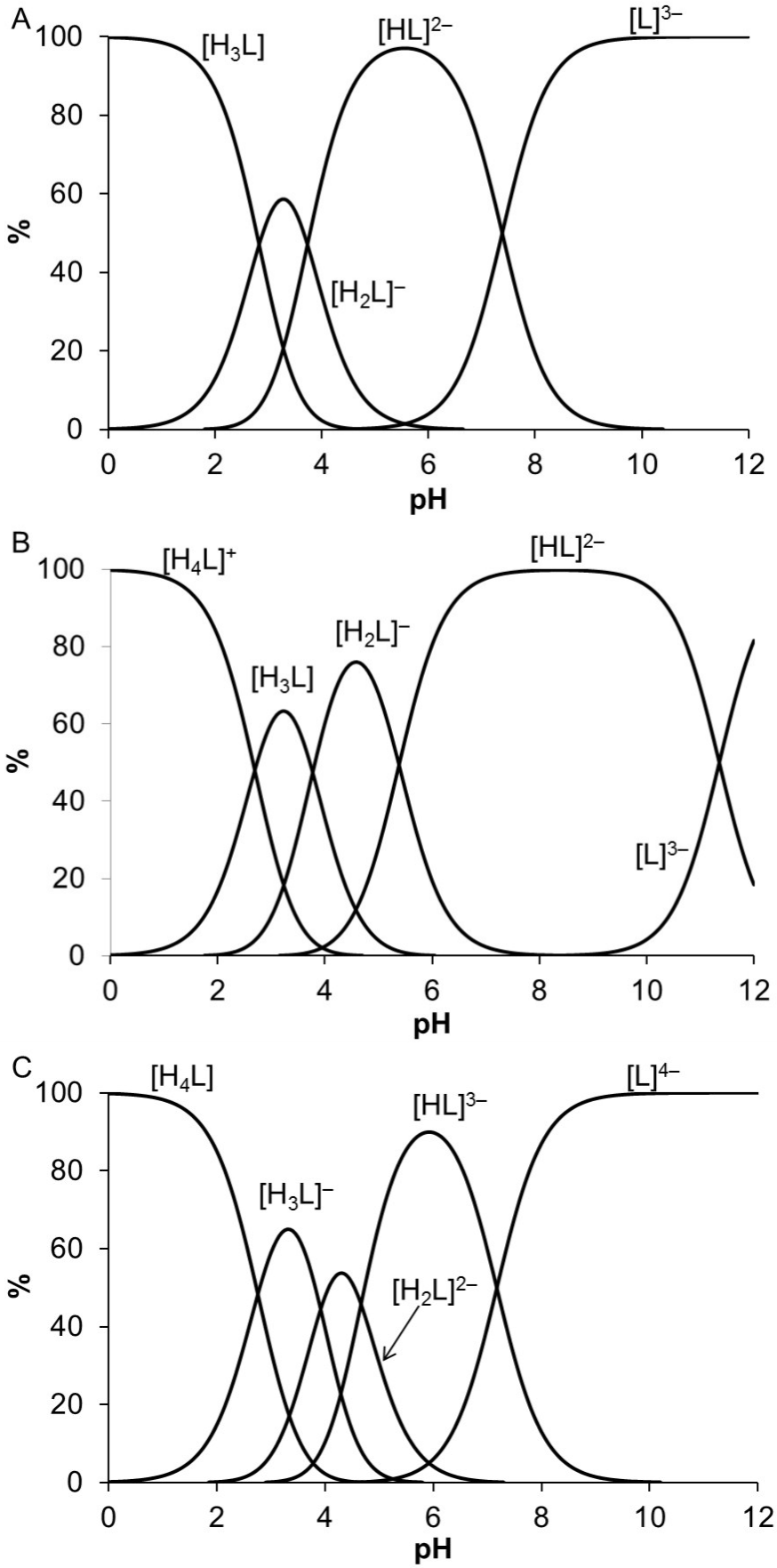


Figure S 2: Speciation diagrams of ligands A) H_3Dpaa B) H_3Dpaa.dab C) H_4Dpaa.ga. (*T* = 25 ºC, *I* = 0.1 M (NMe_4)Cl, [L] = 0.004 M).

1.2 Complexes

Table S 2: Equilibrium constants (*logK*) obtained for complexes

Equilibrium (Charges are omitted)	**H_3Dpaa**			**H_3Dpaa.dab**			**H_4Dpaa.ga**		
	Ga(III)[a,b]	Cu(II)[a]	Zn(II)[a]	Ga(III)[a]	Cu(II)[a, c]	Zn(II)[a,c]	Ga(III)[a]	Cu(II)[a,c]	Zn(II)[a,c]
M + L ↔[M(L)]	18.53	10.85	11.93	22.08	19.1	15.8	18.36	14.52	13.38
[M(HL)] ↔ [M(L)] + H	1.0	3.38	2.33	5.40	5.0	6.8	4.04	4.54	4.65
[M(H_2L)] ↔ [M(HL)] + H	-	-	-		2.8	1.8	-	3.10	1.77
[M(L)] + H_2O ↔ [M(L)(OH)] + H	4.41	9.86	11.27	[c]	12.5	12.2	5.27	10.52	12.24
[M(L)(OH)] + H_2O ↔ [M(L)(OH)$_2$] + H	9.63	12.00	-	[c]	-	-	-	-	-

[a] Determined by potentiometric titration ([L] = [M] = 0.004 M, *T* = 25 °C, *I* = 0.1 M (NMe_4)Cl), [b] Determined by UV-VIS titration ([L] = [M] = 0.1 mM, *T* = 25 °C, pH = 2-7)[c] Determined by UV-VIS titration [L] = [M] = 0.01 mM, *T* = 25 °C, pH = 0-2)

Table S 3: Overall stability constants, $log\beta_{hlm,}$ of the complexes. (*T* = 25 ºC, *I* = 0.1 M (NMe_4)Cl). Charges are omitted.

Ligand	**H_3Dpaa**			**H_3Dpaa.dab**			**H_4Dpaa.ga**		
Metal	Ga(III)[a,b]	Cu(II)[a]	Zn(II)[a]	Ga(III)[a]	Cu(II)[a,c]	Zn(II)[a,c]	Ga(III)	Cu(II)[a, c]	Zn(II)[a, c]
[M(L)]	18.53(5)	10.85(1)	11.93(3)	22.08(1)	19.1(1)	15.8(1)	18.36(3)	14.52(7)	13.38(7)
[M(HL)]	19.5(2)	14.23(1)	14.26(4)	27.48(2)	24.1(1)	22.6(1)	22.40(3)	19.06(7)	18.03(7)
[M(H_2L)]	-	-	-		26.9(1)	24.3(1)	-	22.16(7)	19.80(8)
[M(L)(OH)]	14.12(2)	0.99(2)	0.66(3)		6.6(1)	3.6(1)	13.09(1)	4.00(7)	1.14(7)
[M(L)(OH)$_2$]	4.49(8)	-11.01(2)	-	a	-	-			

[a] Determined by potentiometric titration ([L] = [M] = 0.004 M, *T* = 25 °C, *I* = 0.1 M (NMe_4)Cl), [b] Determined by UV-VIS titration ([L] = [M] = 0.1 mM, *T* = 25 °C, pH = 2-7) [c] Determined by UV-VIS titration ([L] = [M] = 0.01 mM, *T* = 25 °C, pH = 0-2)

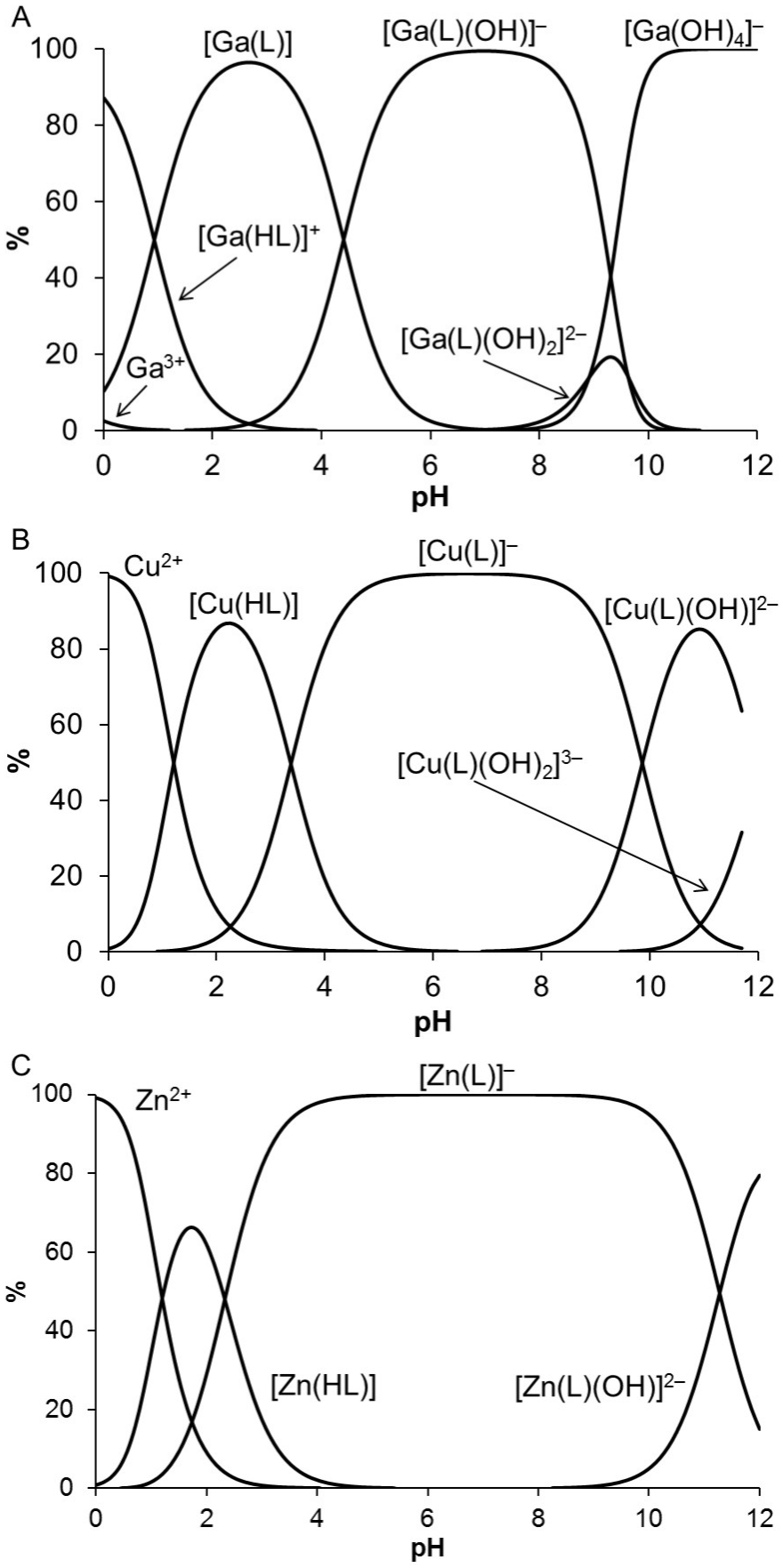


Figure S 3: Speciation diagram for Metal – H_3Dpaa systems. A) Ga(III), B) Cu(II), C) Zn(II) (*T* = 25 ºC, *I* = 0.1 M $(NMe_4)Cl$, [L] = [M] =0.004 M)

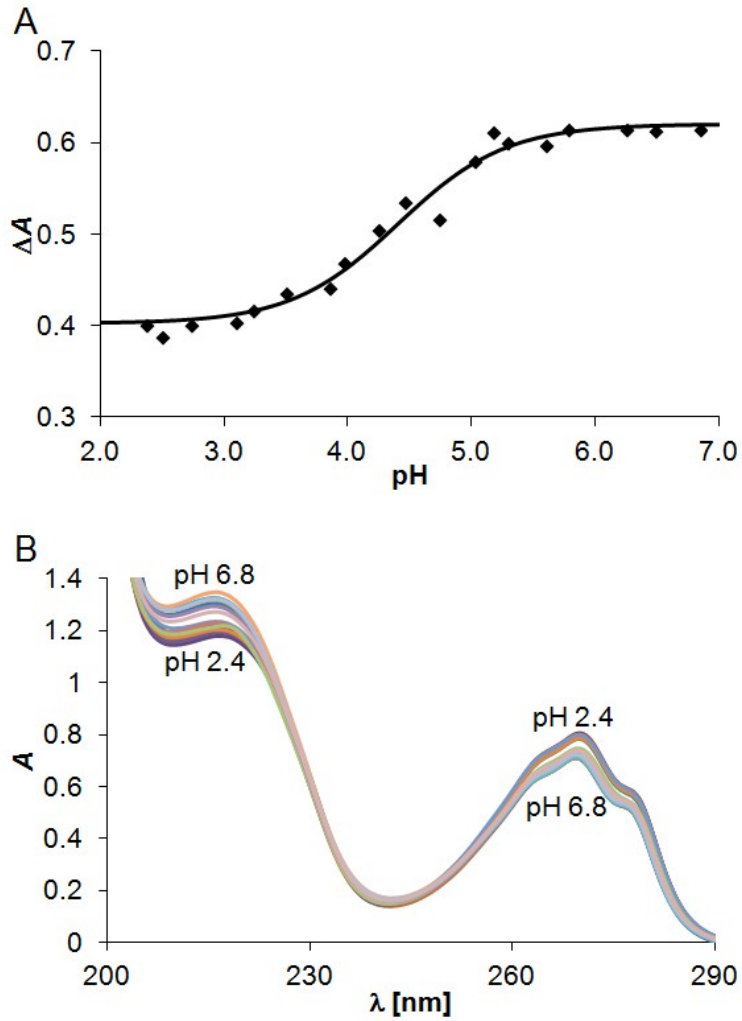


Figure S 4 : UV-Vis titration of GaDpaa: UV-VIS titration (A) of Ga(III)-H_3Dpaa, difference of absorbance $\Delta A = A_{216\ nm} - A_{270\ nm}$ was used to evaluate results, the line corresponds to the best fits. The corresponding UV-VIS spectra (B) of Ga(III)-H_3Dpaa system.(T = 25 ºC, [L] = [M] =0.1 mM.)

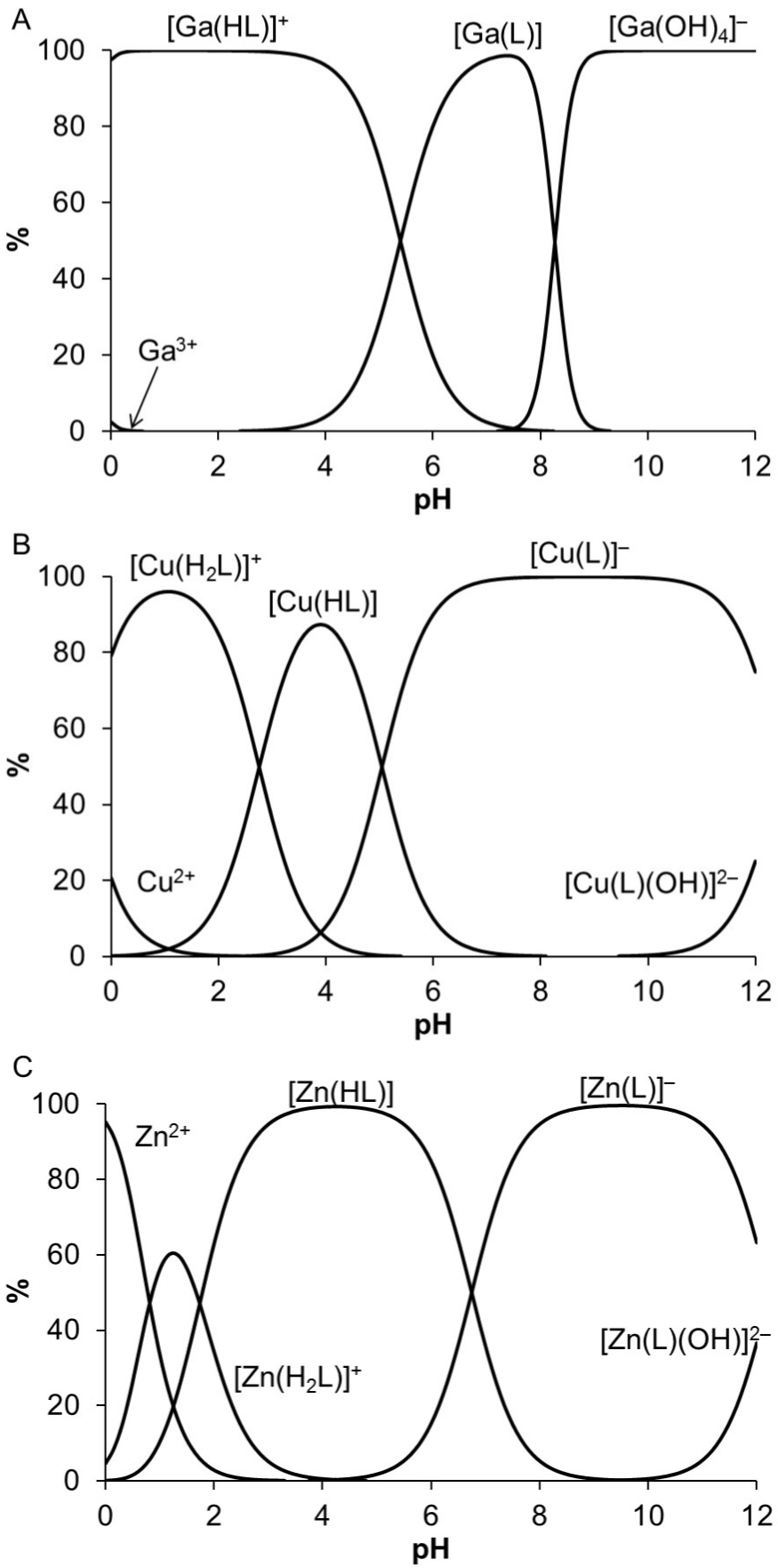


Figure S 5: Speciation diagram for Metal – H_3Dpaa.dab systems. C) Ga(III) A) Cu(II), B) Zn(II) (T = 25 ºC, I = 0.1 M (NMe_4)Cl, [L] = [M] =0.1 mM.)

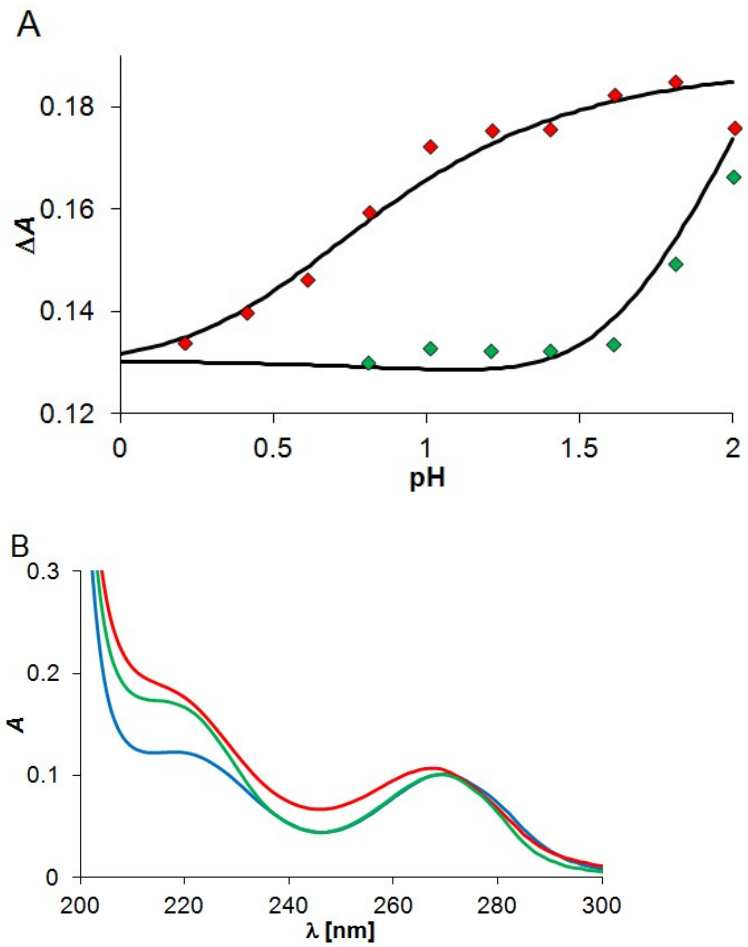


Figure S 6: UV-VIS titration at 220 nm (A) of Cu(II)-H_3Dpaa.dab (red) and Zn(II)- H_3Dpaa.dab (green), the lines correspond to the best fits. The corresponding UV-VIS spectra (B) of H_3Dpaa.dab (blue), Cu(II)- H_3Dpaa.dab (red) and Zn(II)- H_3Dpaa.dab (green) at pH = 2.

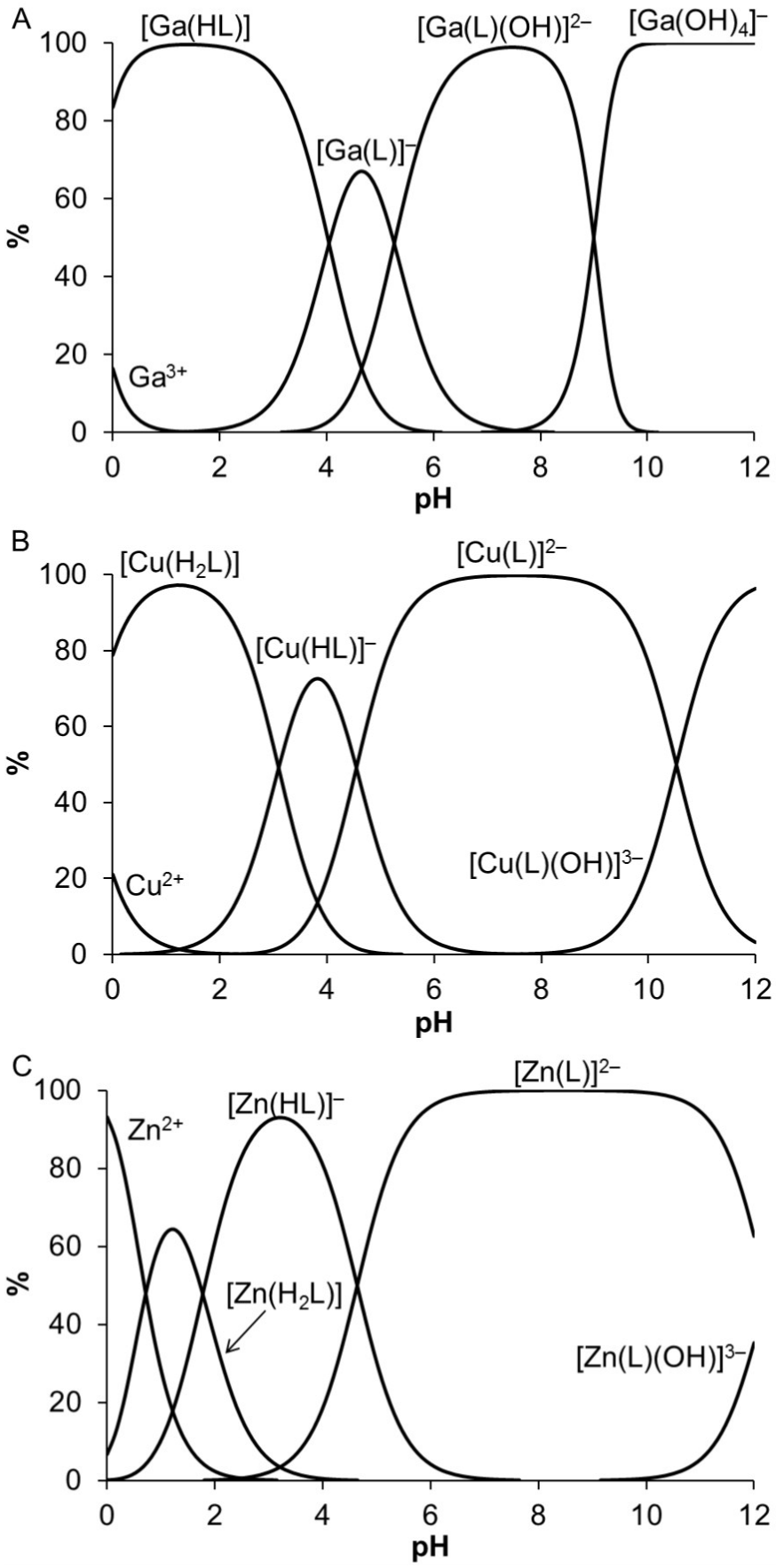


Figure S 7: Speciation diagram for Metal – H_4Dpaa.ga systems. A) Ga(III), B) Cu(II), C) Zn(II) (T = 25 ºC, I = 0.1 M $(NMe_4)Cl$, [L] = [M] =0.004 M).

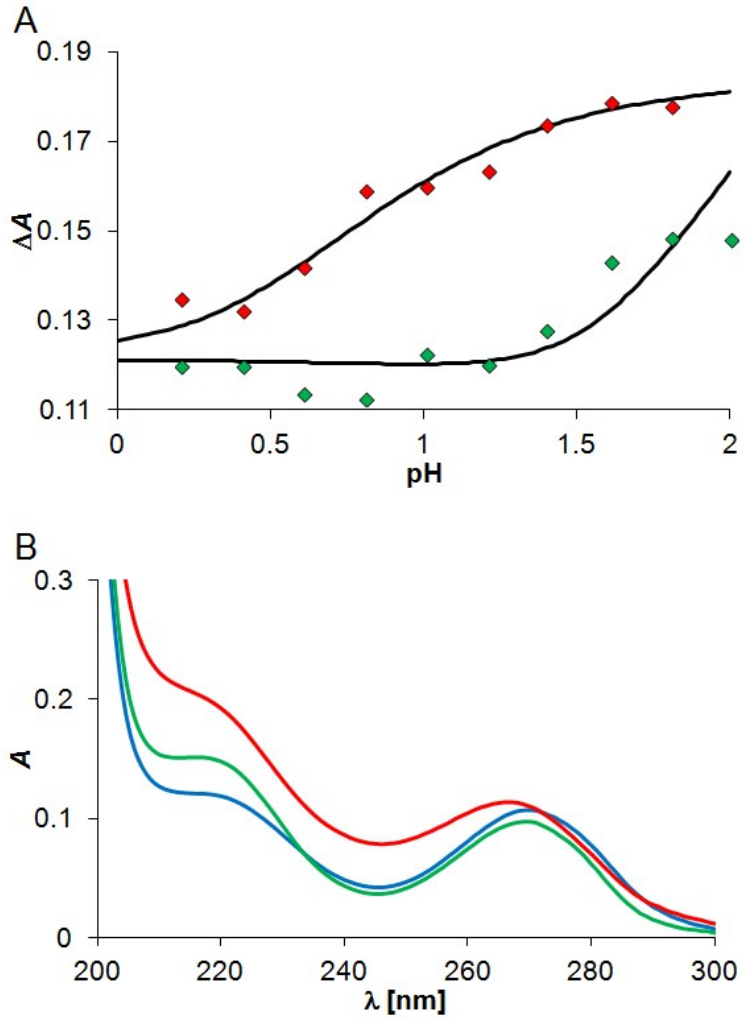


Figure S 8: UV-VIS titration at 220 nm (A) of Cu(II)-H_4Dpaa.ga (red) and Zn(II)- H_4Dpaa.ga (green), the lines correspond to the best fits. The corresponding UV-VIS spectra (B) of H_4Dpaa.ga (blue), Cu(II)- H_4Dpaa.ga (red) and Zn(II)- H_4Dpaa.ga (green) at pH = 2. (*T* = 25 ºC, [L] = [M] =0.01 mM

1.3 Radio-HPLC

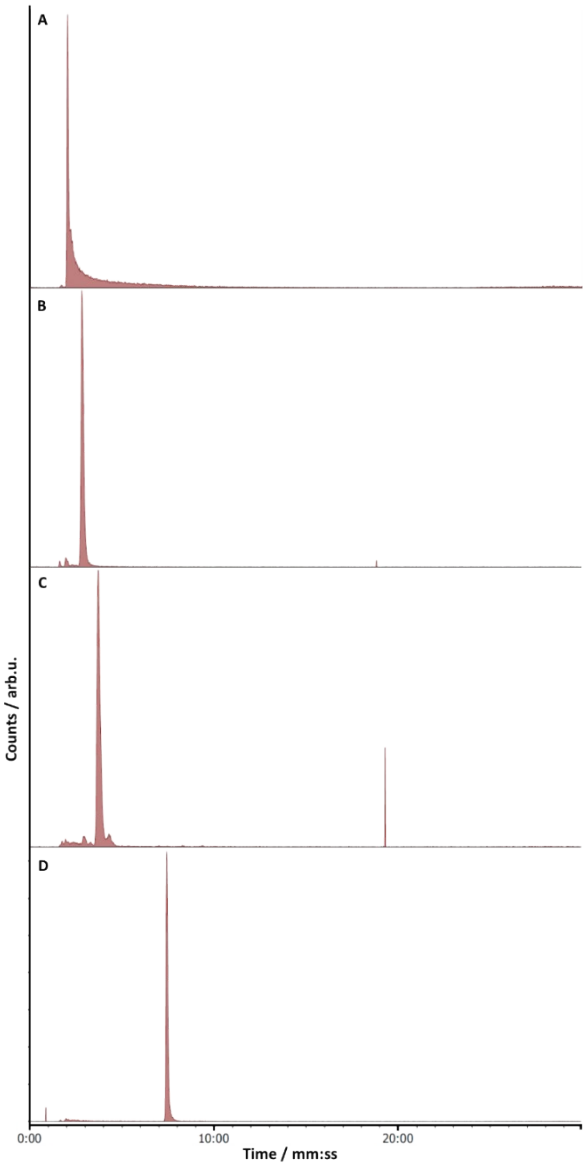


Figure S 9: HPLC traces of A) ^{68}Ga, B,C,D) radiolabeling mixtures containing ^{68}Ga and B) H_3Dpaa C) H_3Dpaa.dab, D) H_4Dpaa.ga. ([L] = 100 µM, pH = 7.4, *I* = PBS, *T* = 37.7 °C, *t* = 5 minutes)

1.4 Radio-TLC

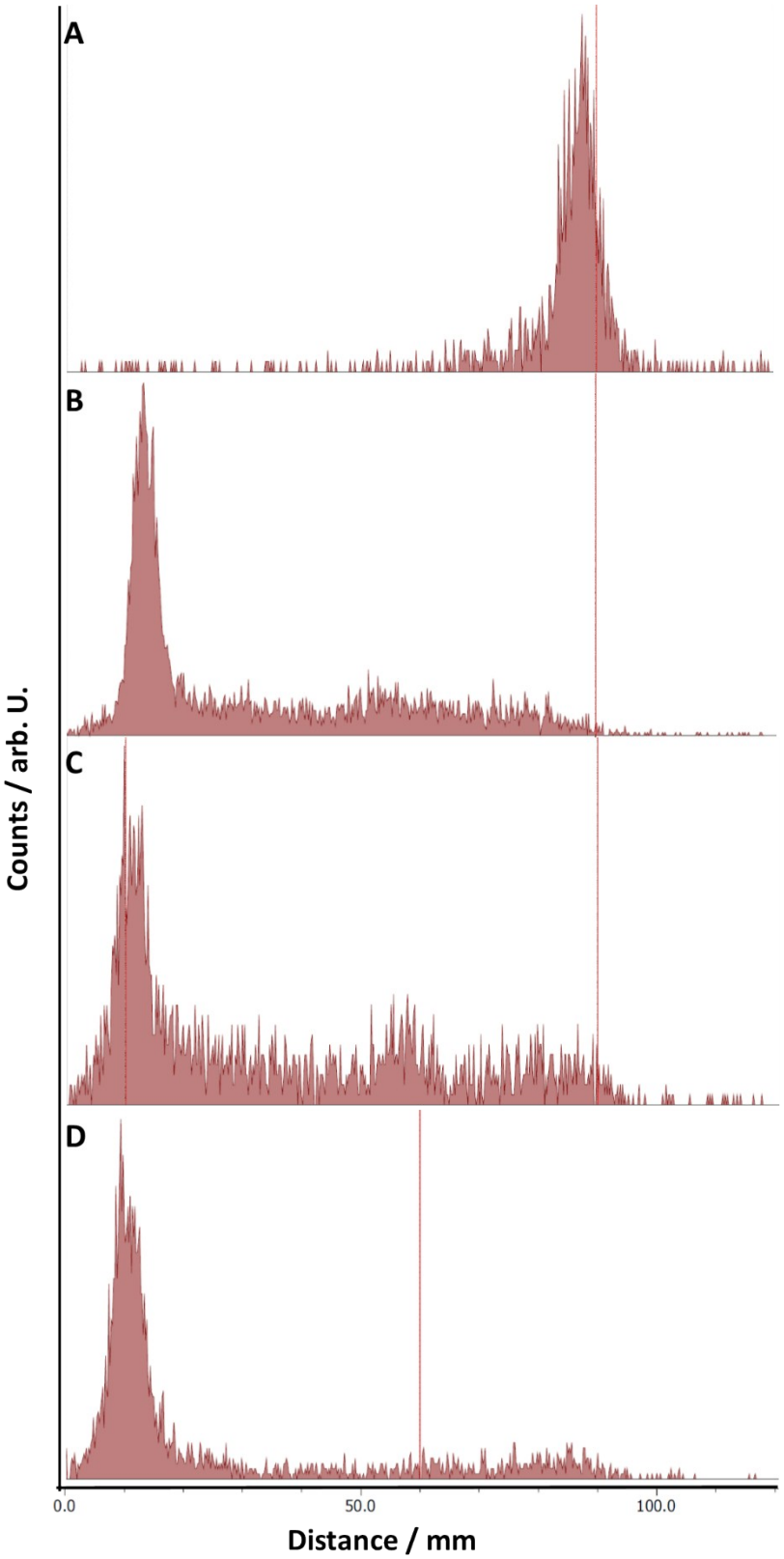


Figure S 10: Radio-TLC of a) $^{68}GaCl_3$, b) $^{68}GaCl_3$ + H_3Dpaa, b) $^{68}GaCl_3$ + $H_3Dpaa.dab$, D) $^{68}GaCl_3$ + $H_4Dpaa.ga$. [L] = 100 µM, *I* = PBS, *T* = 37.5 °C, *t* = 15 mins.

1.5 Specific Activity

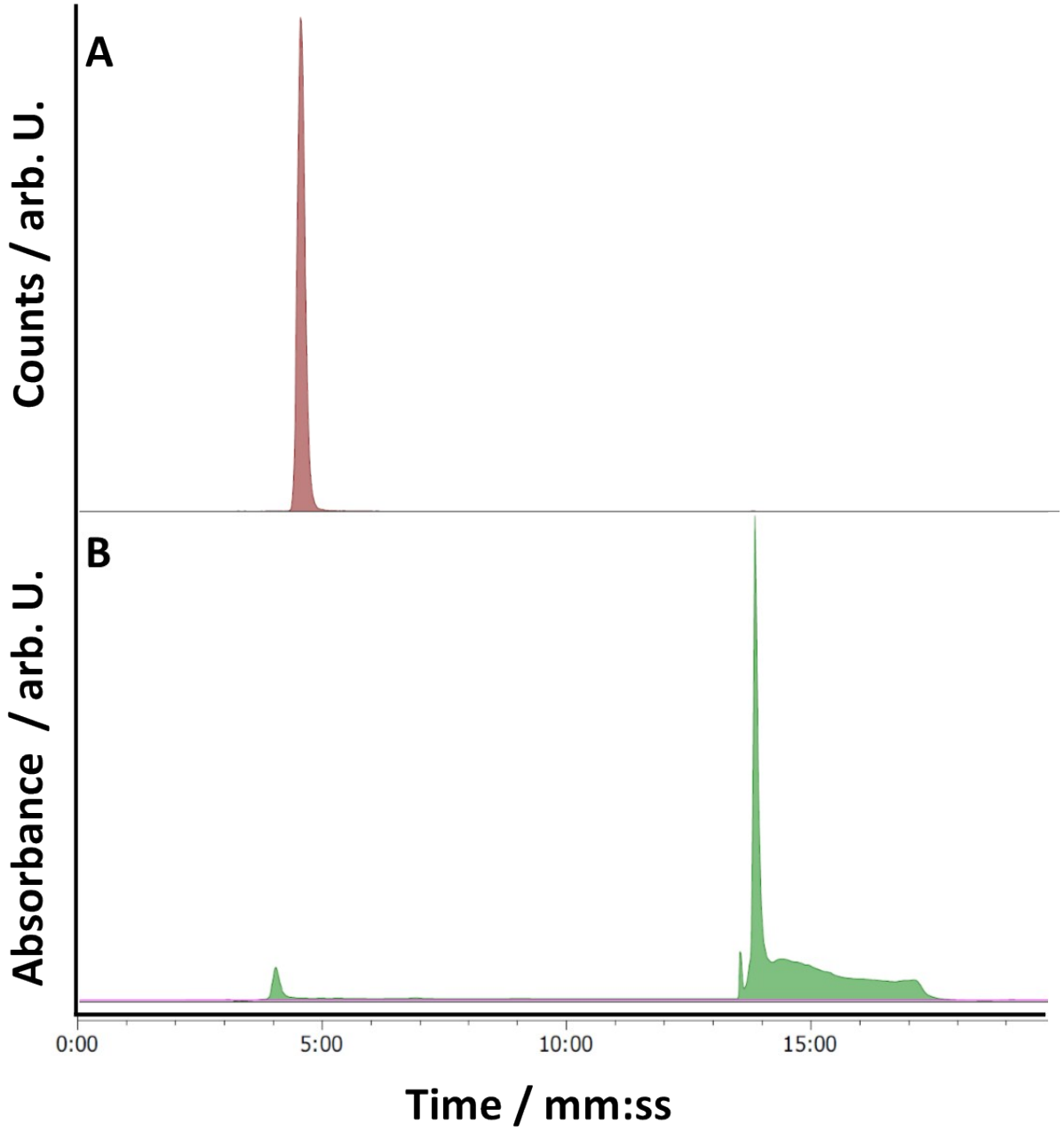


Figure S 11: Semi-preparative HPLC chromatogram for the purification of ^{68}GaDpaa. A) Radio-HPLC trace. B) UV-HPLC trace

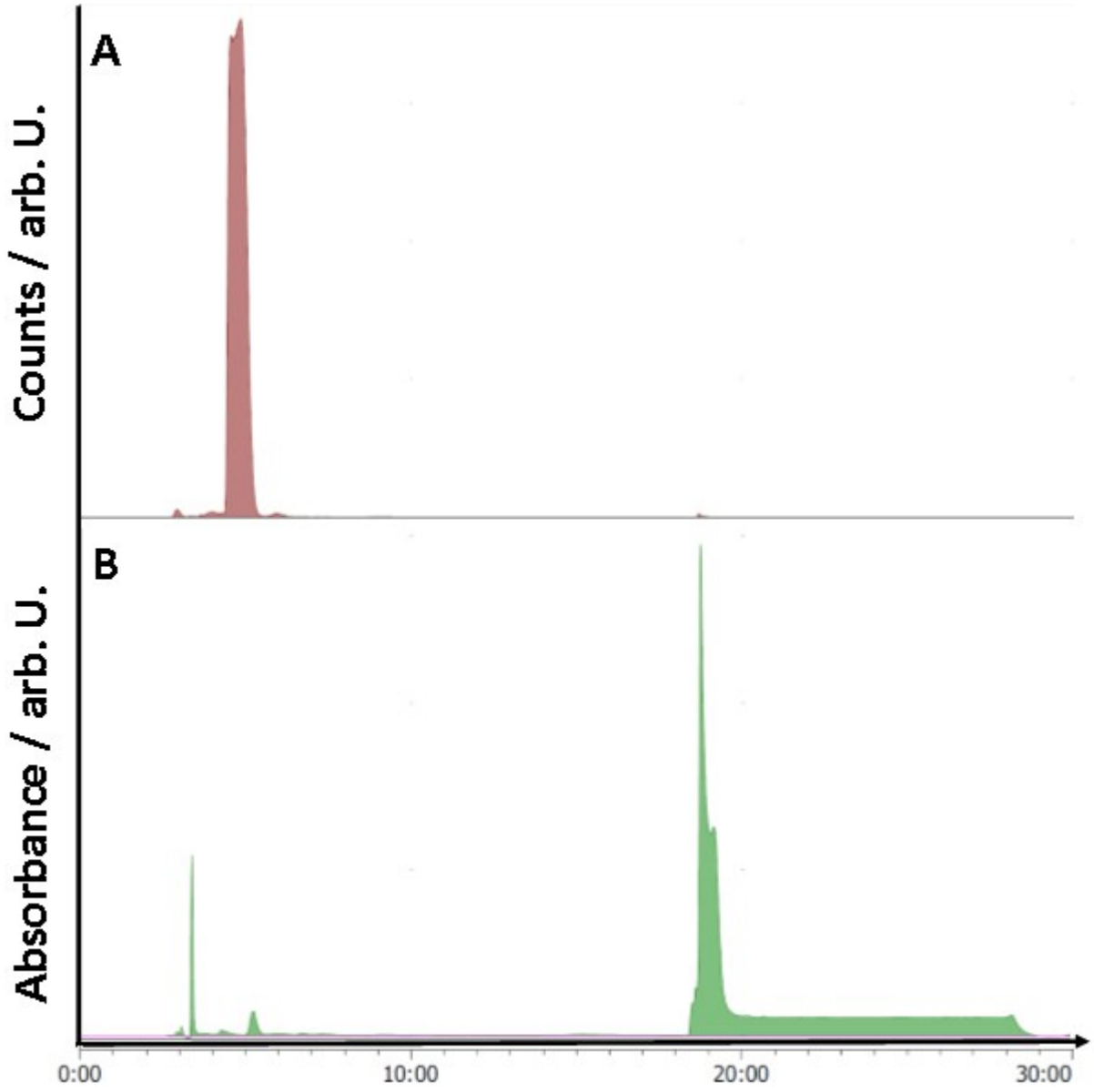


Figure S 12: Semi-preparative HPLC chromatogram for the purification of ^{68}GaDpaa.dab. A) Radio-HPLC trace. B) UV-HPLC trace

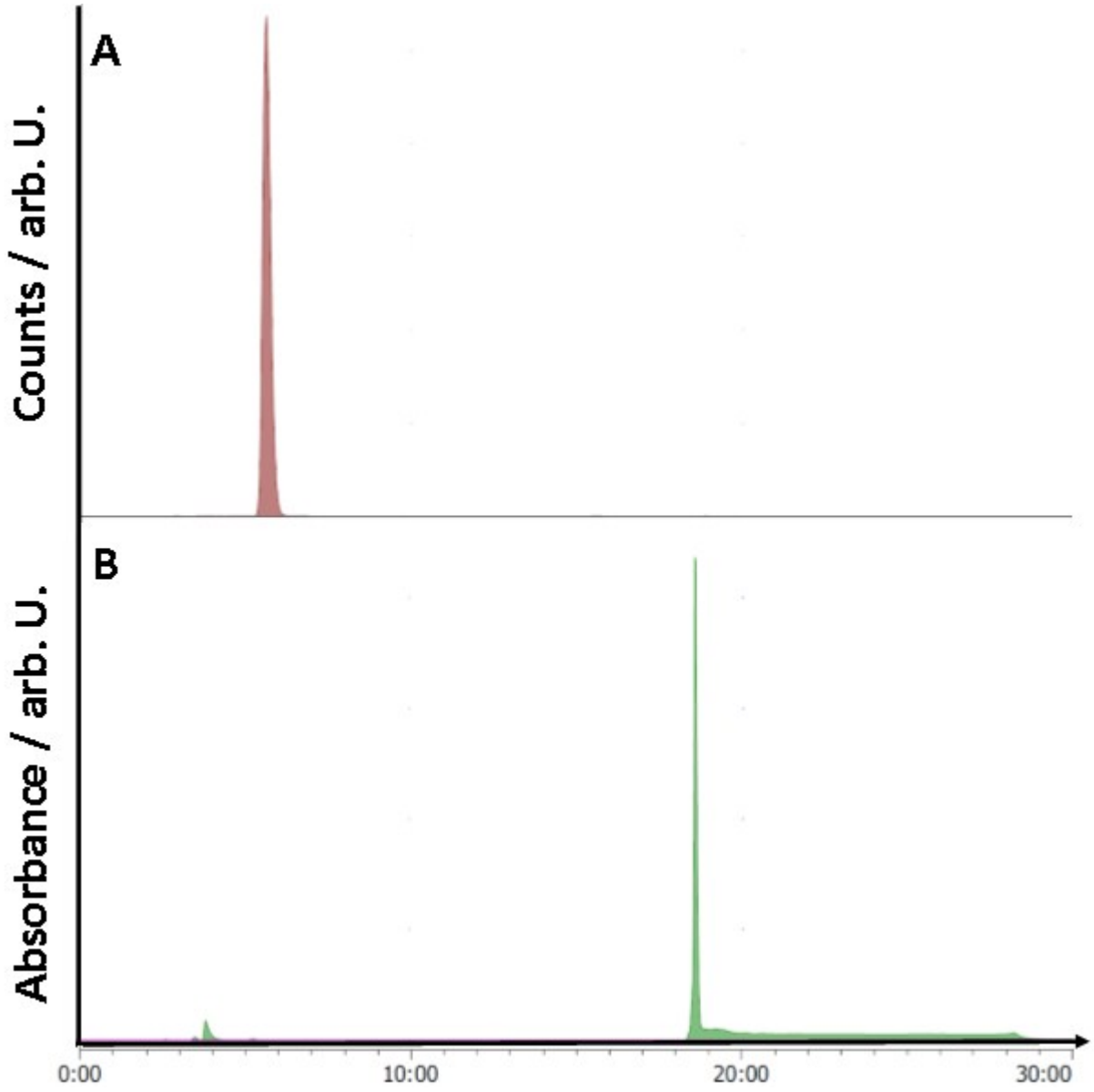


Figure S 13: Semi-preparative HPLC chromatogram for the purification of ^{68}GaDpaa.ga. A) Radio-HPLC trace. B) UV-HPLC trace

1.6 Radiolabelling of standard chelates

Table S 4: Radiochemical yields (RCY) of standard chelators with ^{68}Ga. (pH = 7.5, *T* = 25 °C, [L] = 100 uM, *I* = 0.1 M phosphate buffer)

Ligand	5 minutes	15 minutes
DOTA	0	0
NOTA	33	48
EDTA	95	95
THP	95	95

1.7 Stability to transferrin

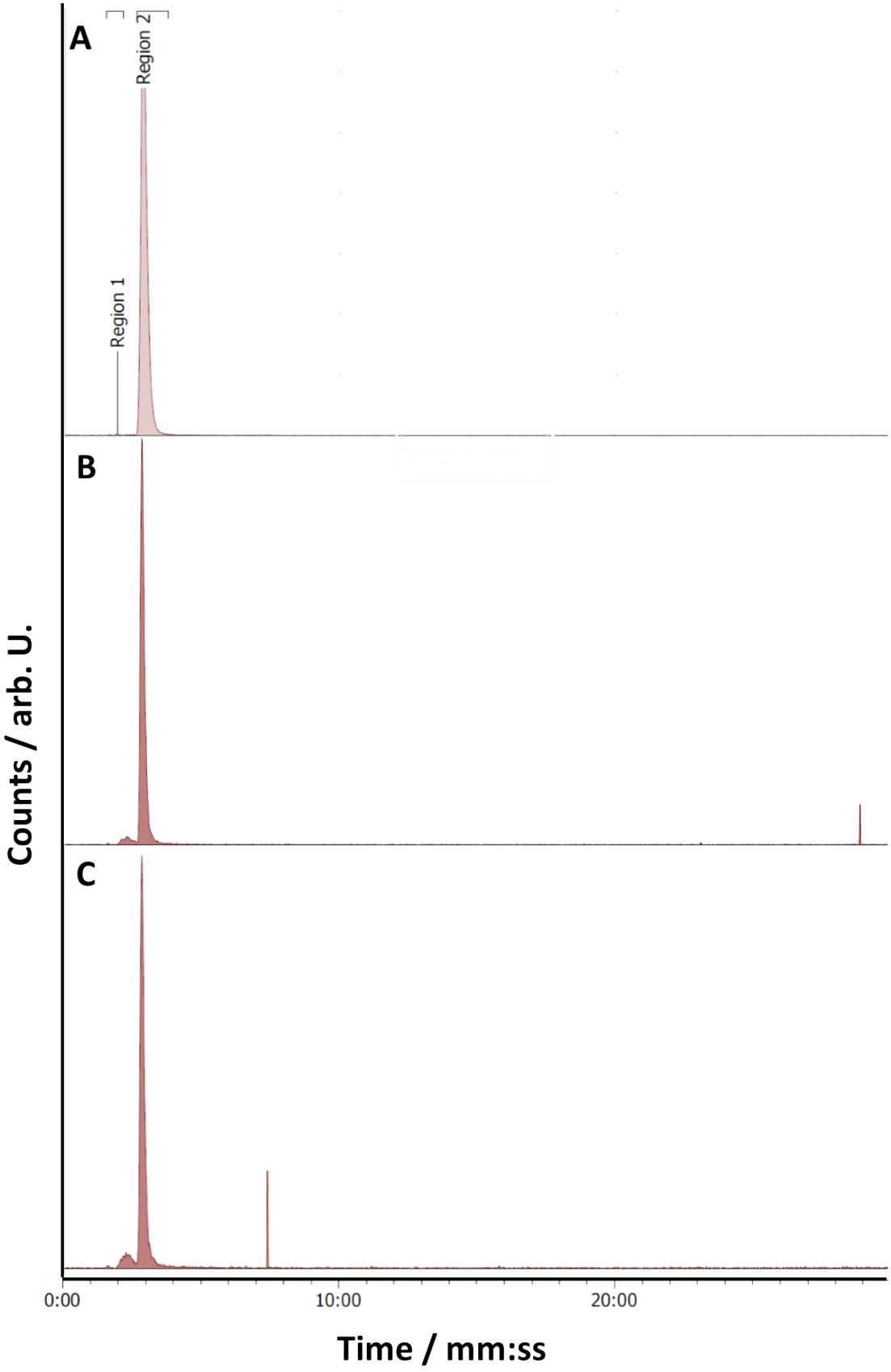
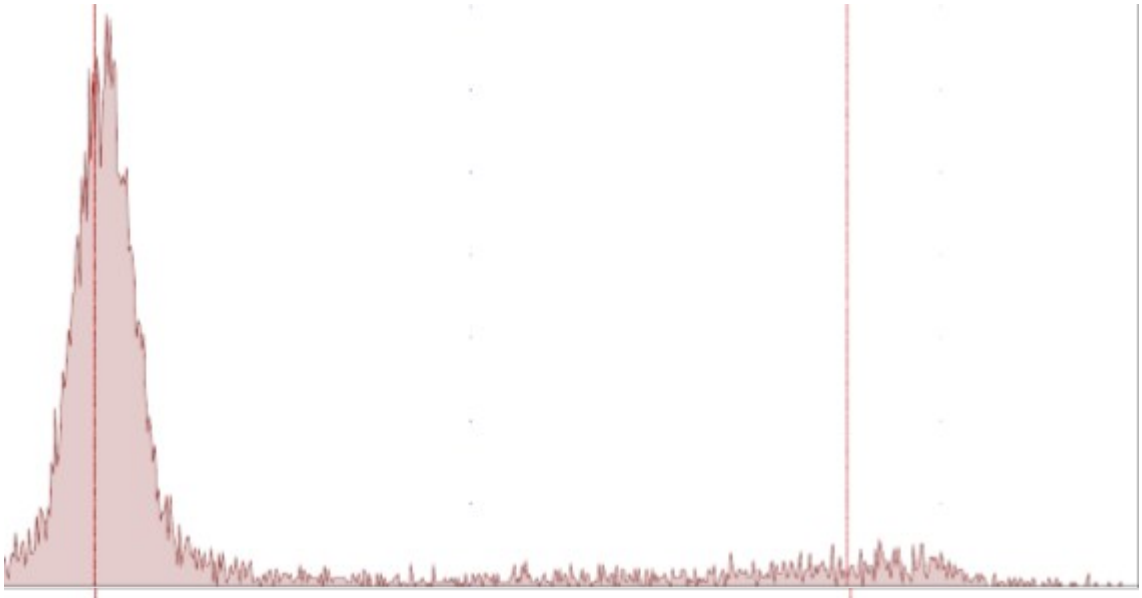
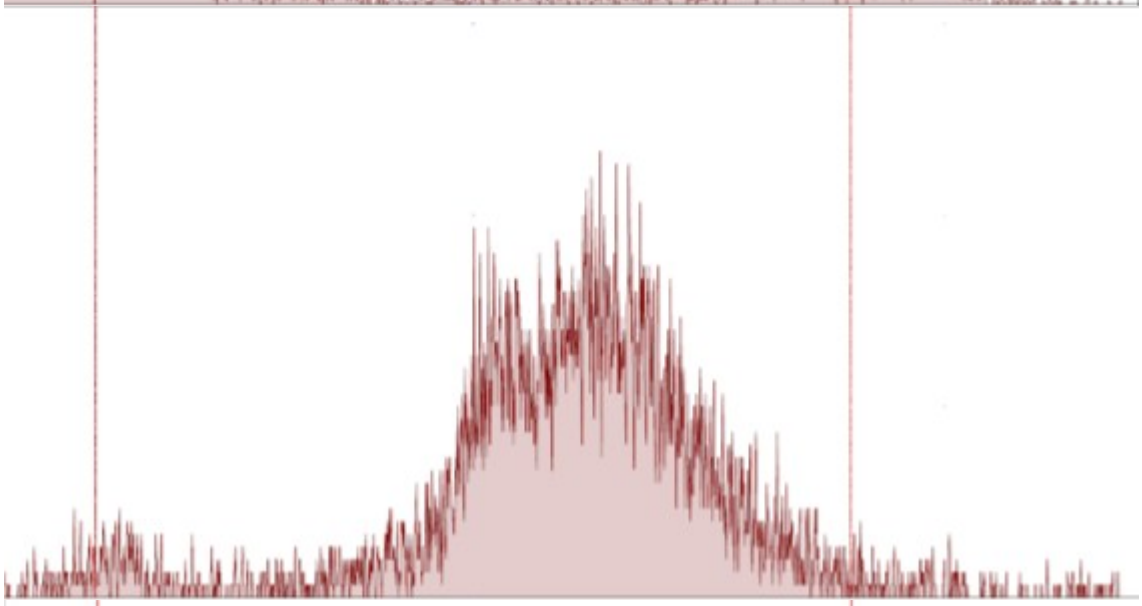


Figure S 14: Stability of GaDpaa to transferrin. A) $^{68}GaCl_3$ + H_3Dpaa (*I* = 0.1 M aq. NaOAc, pH = 4.5, *T* = RT). B) A after incubation with transferrin for 1 hour, C) A after incubation with transferrin for 2 hours.

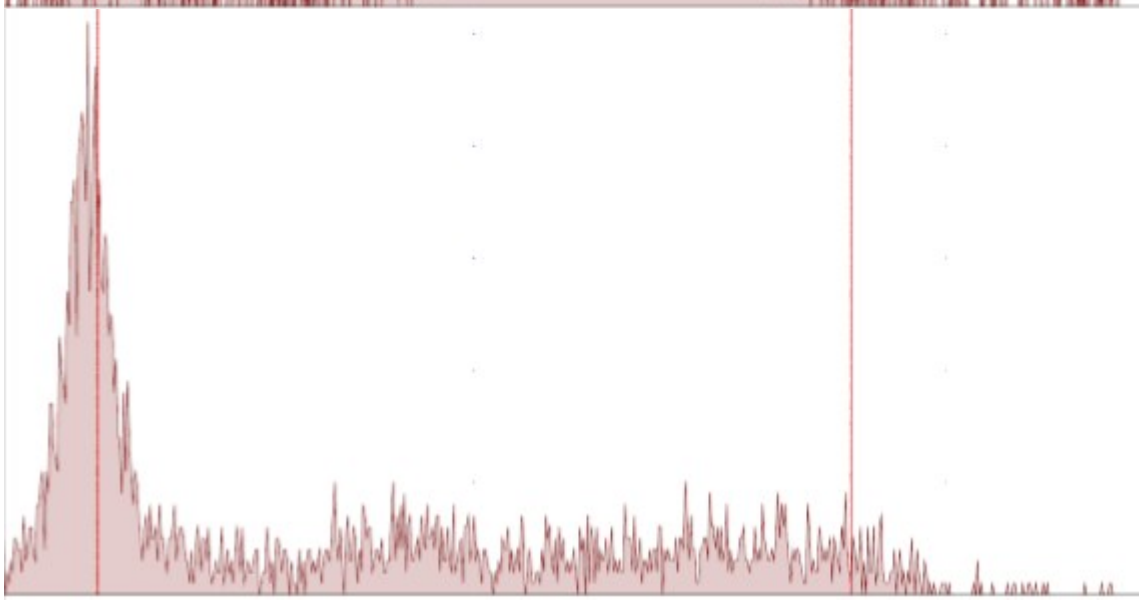
A



B



C



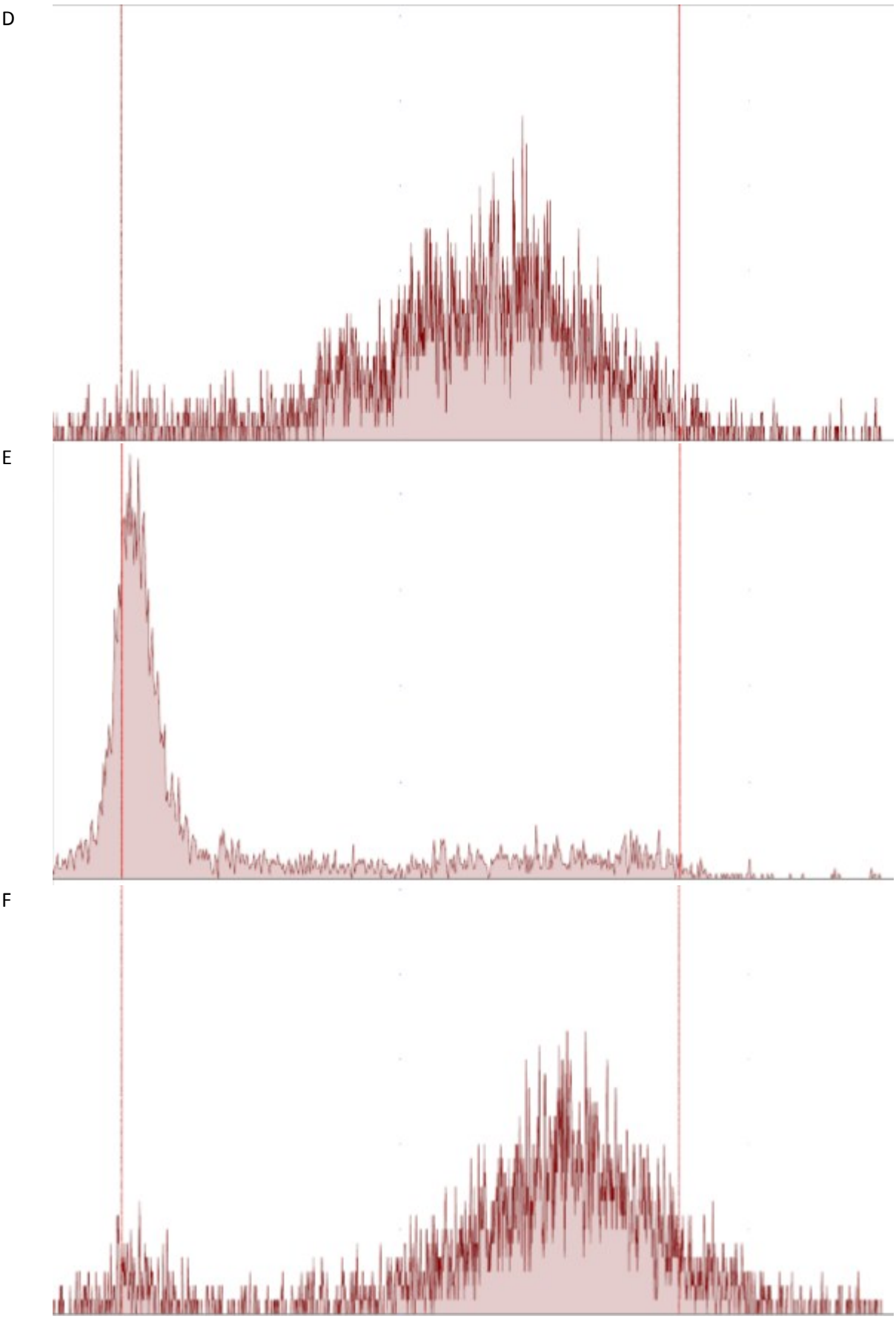


Figure S 15: Stability of radiolabelled complexes assessed by radio-TLC. A) Radiolabelling mixture containing 100 µM H_3Dpaa in PBS after 15 minutes incubation at 37 °C. B) Solution containing 100 µL of radiolabelling mixture A and 1.5 mL FBS after incubation at 37 °C for 30 minutes. C) Radiolabelling mixture containing 100 µM H_3Dpaa.dab in PBS after 15

minutes incubation at 37 °C. D) Solution containing 100 µL of radiolabelling mixture C and 1.5 mL FBS after incubation at 37 °C for 30 minutes. E) Radiolabelling mixture containing 100 µM H_4Dpaa.ga in PBS after 15 minutes incubation at 37 °C. F) Solution containing 100 µL of radiolabelling mixture E and 1.5 mL FBS after incubation at 37 °C for 30 minutes.

2. NMR Data

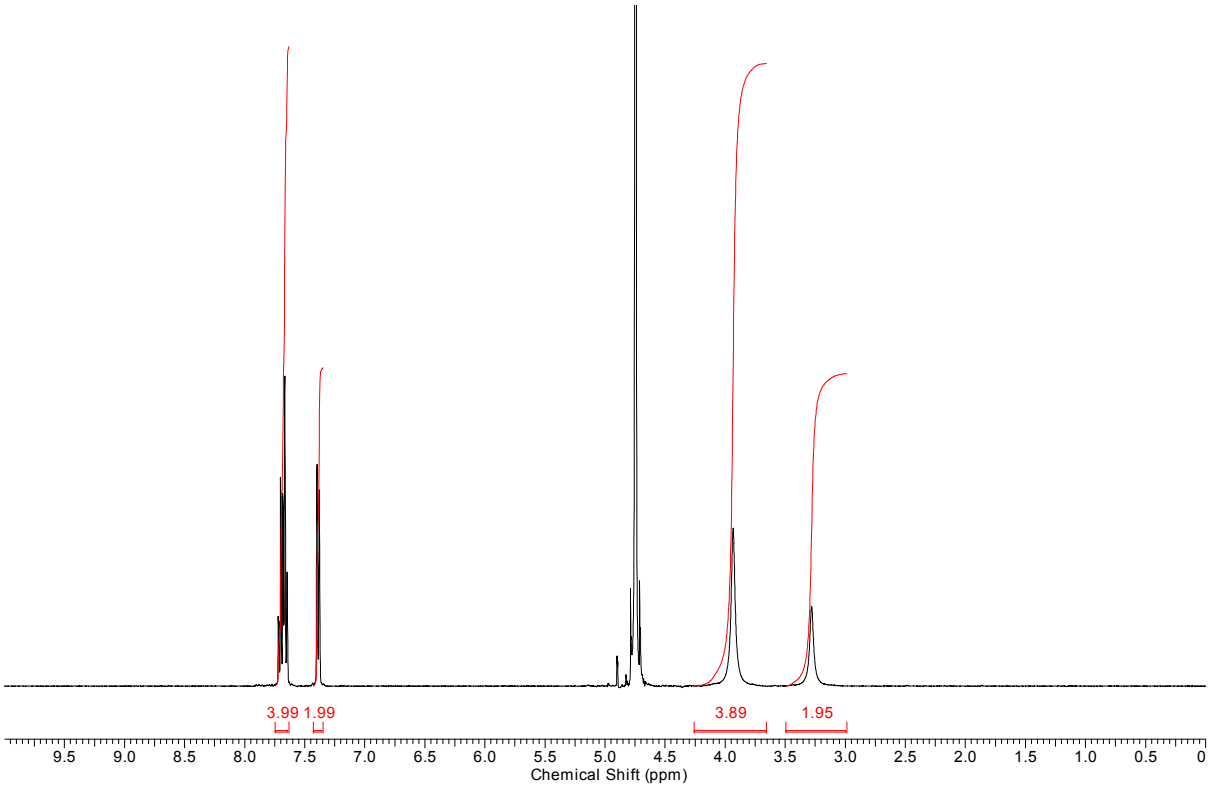


Figure S 16: ^{1}H NMR of H_3Dpaa in D_2O (pD = 8.8)

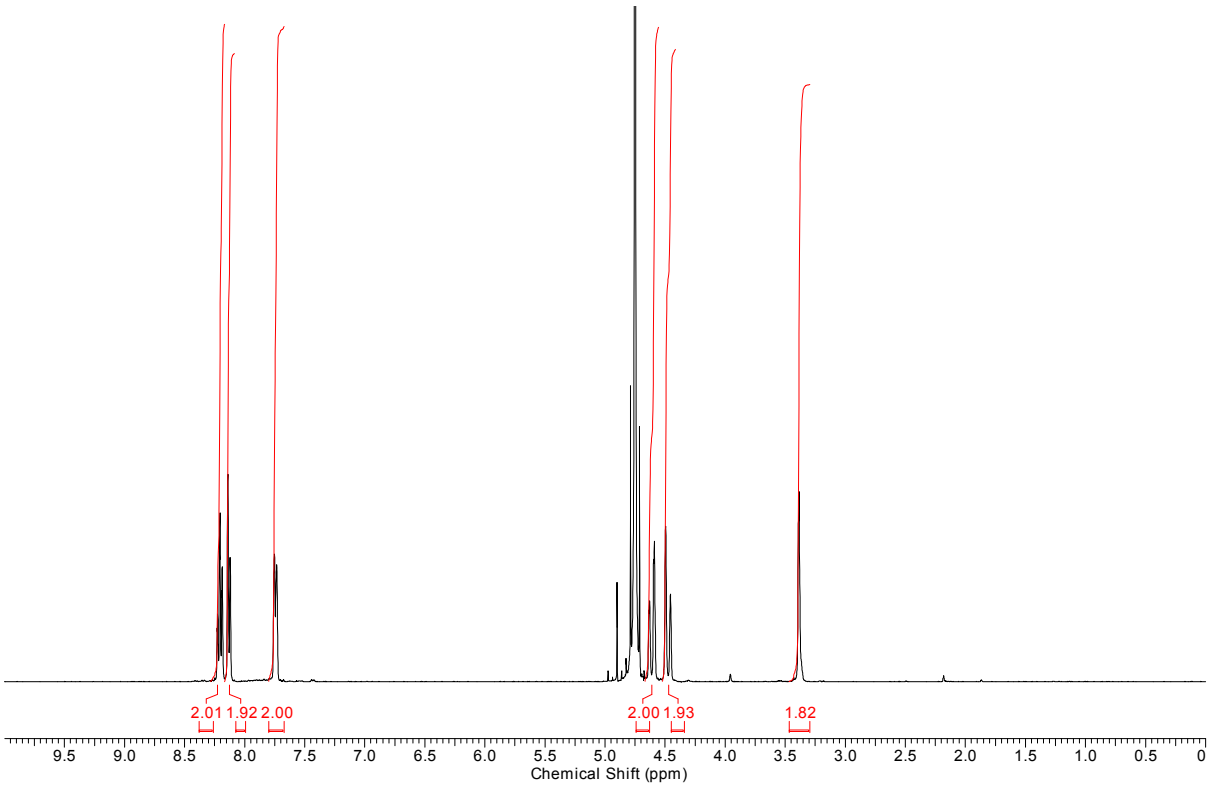


Figure S 17: ^{1}H NMR of [Ga(Dpaa)] in D_2O (pD = 8.8)

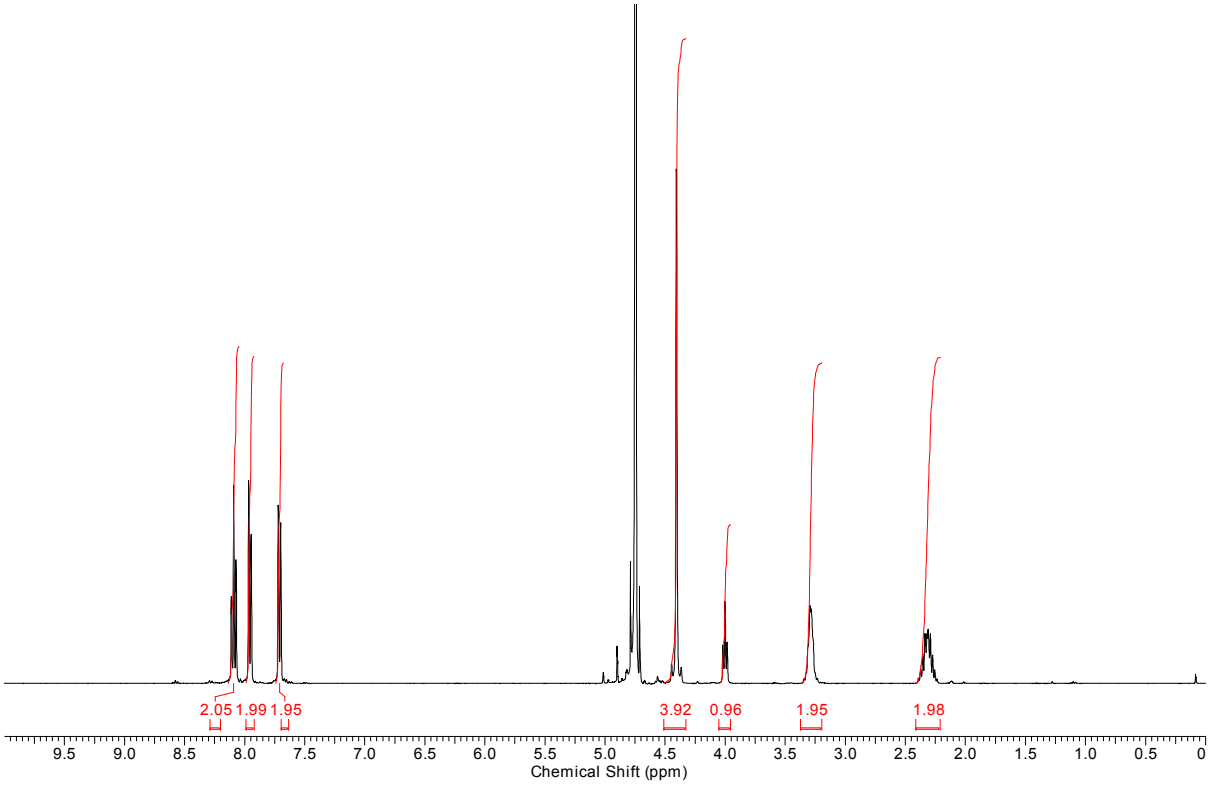


Figure S 18: ^{1}H NMR of H_3Dpaa.dab in D_2O (pD = 1.6)

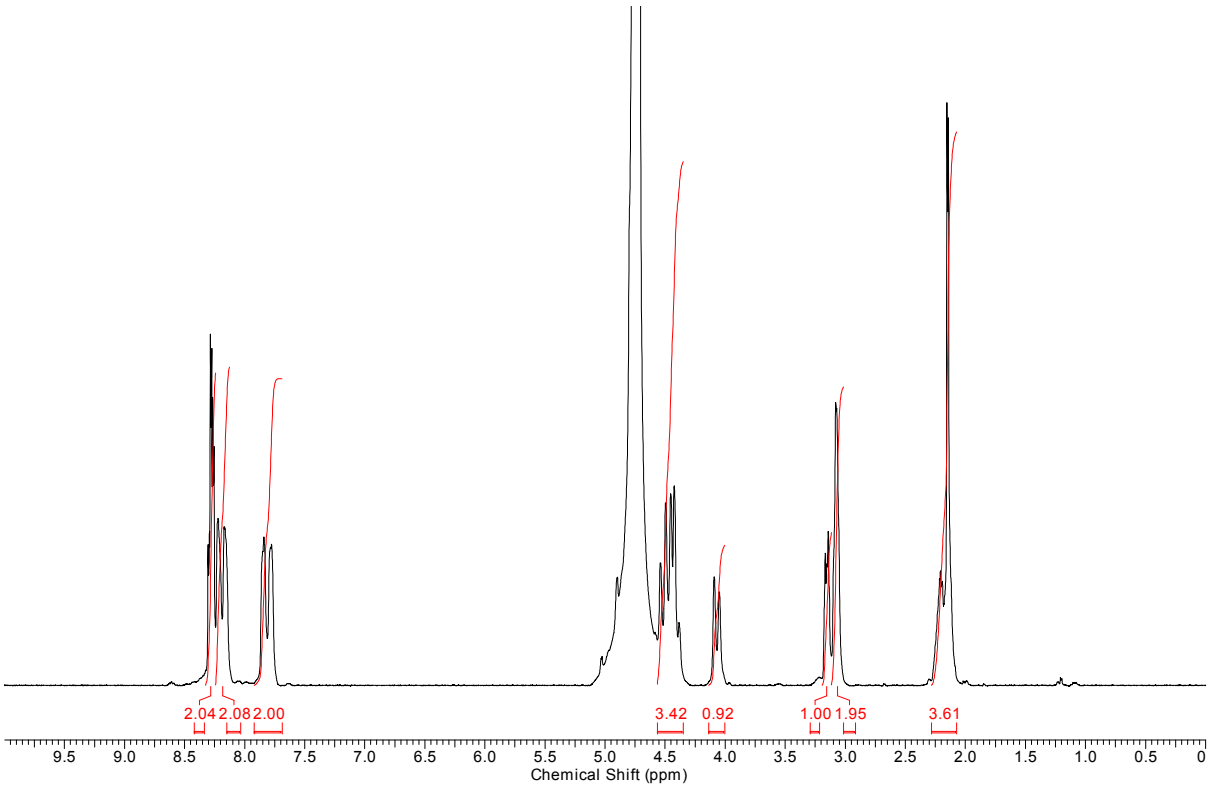


Figure S 19: ^{1}H NMR of [Ga(Dpaa.dab)] in D_2O (pD = 1.1)

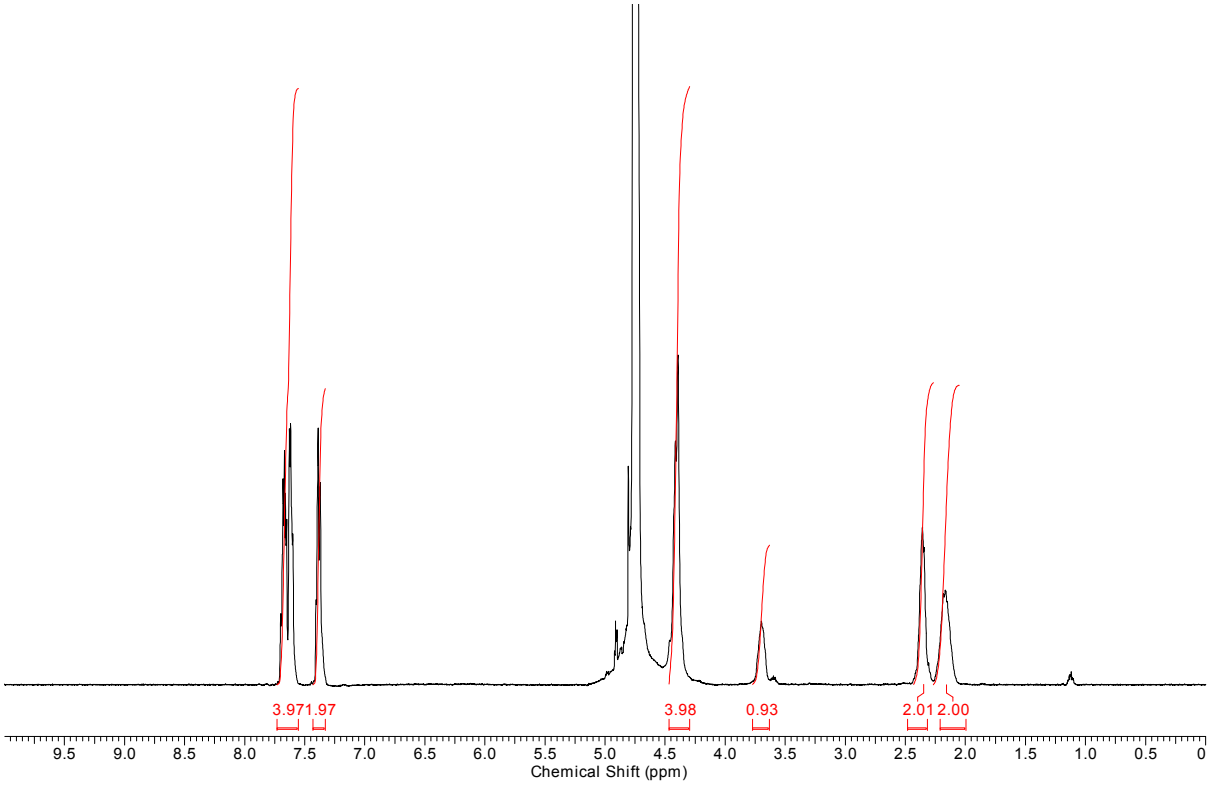


Figure S 20: ^{1}H NMR of H_4Dpaa.ga in D_2O (pD = 7.1)

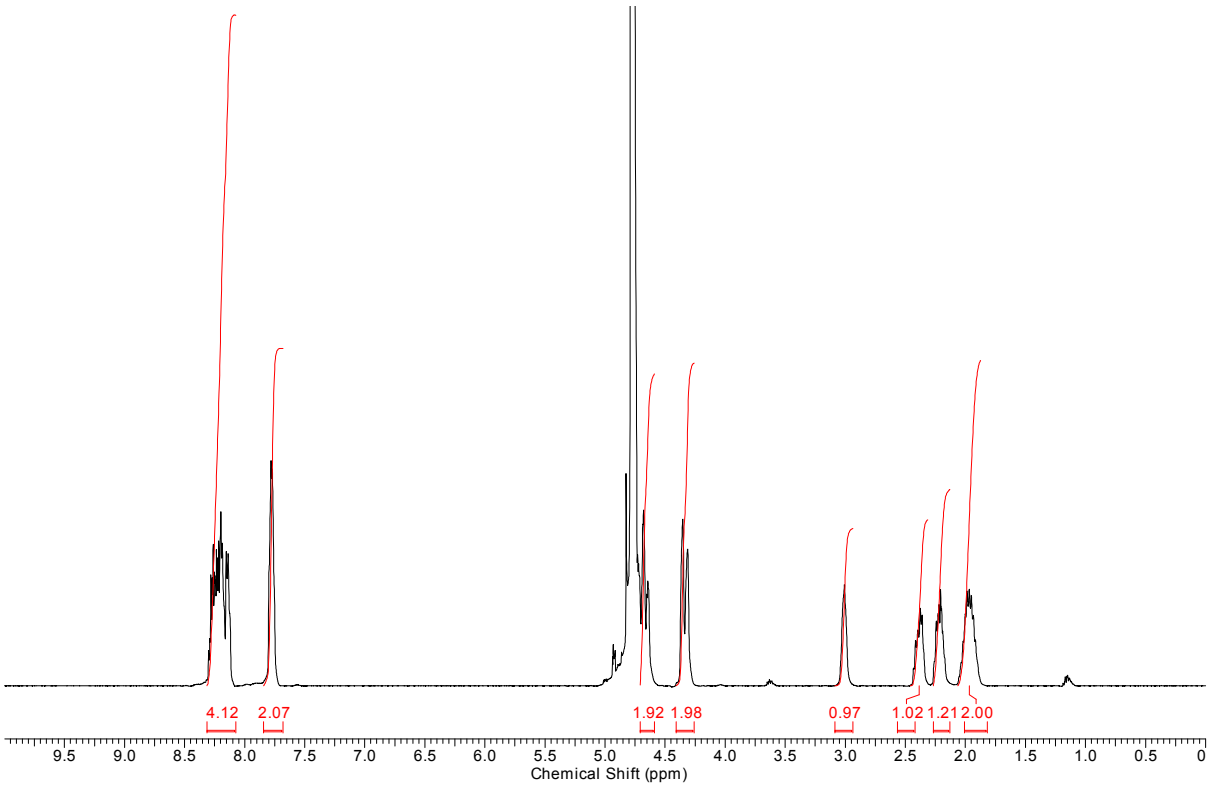


Figure S 21: ^{1}H NMR of [Ga(Dpaa.ga)] in D_2O (pD = 6.0)

3. Crystal Data

3.1 H_4Dpaa.ga crystals structure

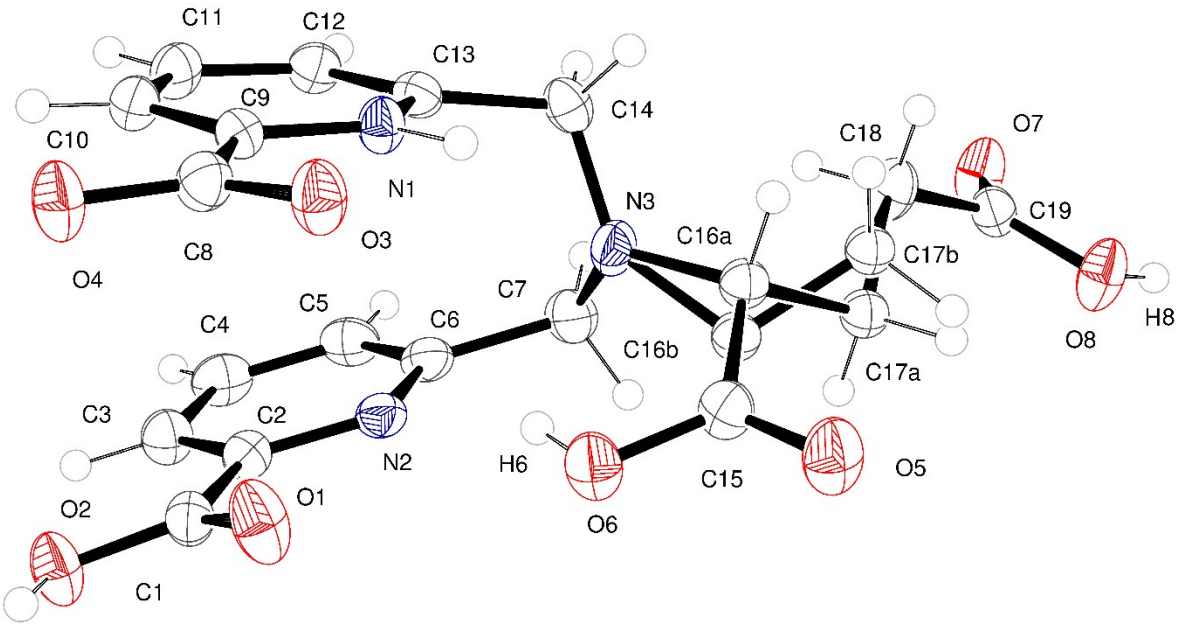


Figure S 22: ORTEP representation of H4Dpaa with atoms drawn as 50% probability ellipsoids. Small-scale disorder (C16a/C17a & C16b/C17b) was modelled using standard techniques.

Table S 5: Crystal data and structure refinement for H_4Dpaa.ga .

Identification code	shelx	
Empirical formula	C19 H19 N3 O8	
Formula weight	417.37	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 2_1/n	
Unit cell dimensions	a = 7.0852(6) Å	a= 90°
	b = 32.290(4) Å	b= 94.108(7)°
	c = 7.6786(6) Å	g = 90°
Volume	1752.2(3) Å^3	
Z	4	
Density (calculated)	1.582 Mg/m^3	
Absorption coefficient	0.125 mm^{-1}	
F(000)	872	
Crystal size	0.275 x 0.130 x 0.056 mm^3	
Theta range for data collection	1.261 to 25.550°.	
Index ranges	-8<=h<=8, -38<=k<=38, -9<=l<=9	
Reflections collected	9845	
Independent reflections	3229 [R(int) = 0.0571]	
Completeness to theta = 25.242°	99.90%	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	3229 / 0 / 275	
Goodness-of-fit on F2	0.792	
Final R indices [I>2sigma(I)]	R1 = 0.0440, wR2 = 0.0727	
R indices (all data)	R1 = 0.1003, wR2 = 0.0804	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.400 and -0.220 e.Å^{-3}	

3.2 GaDpaa crystal structure

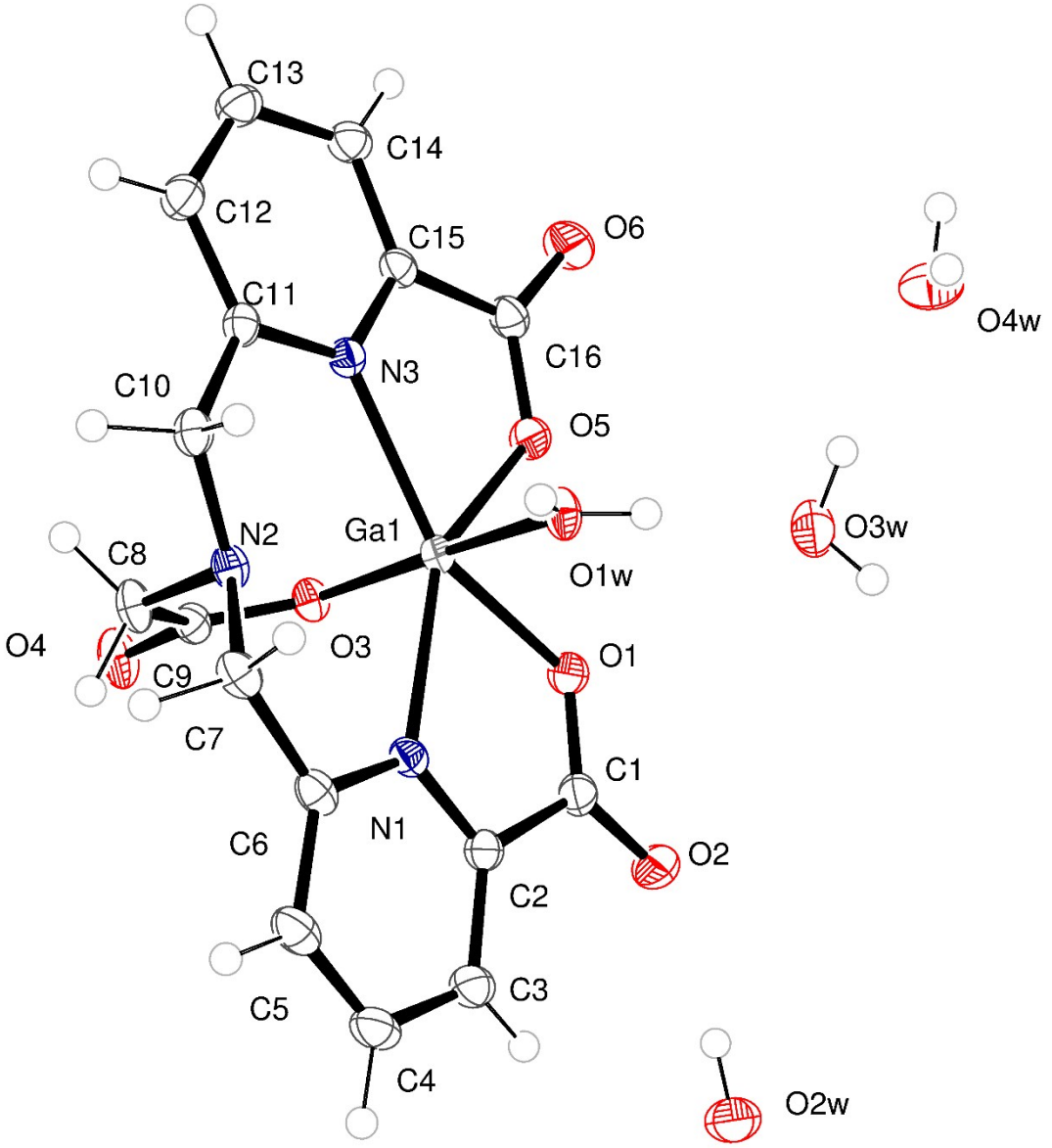


Figure S 23: ORTEP diagram of Ga.Dpaa with atoms drawn as 50 % thermal ellipsoids.

Table S 6: Crystal data and structure refinement for Ga.Dpaa

Identification code	Ga.Dpaa	
Empirical formula	C32 H40 Ga2 N6 O20	
Formula weight	968.14	
Temperature	100(2) K	
Wavelength	0.6889 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 7.06810(10) Å	a= 93.6180(10)°
	b = 8.21920(10) Å	b= 93.7920(10)°
	c = 15.9952(2) Å	g = 91.9130(10)°
Volume	924.69(2) $Å^3$	
Z	1	
Density (calculated)	1.739 Mg/m^3	
Absorption coefficient	1.425 mm^{-1}	
F(000)	496	
Crystal size	0.040 × 0.005 × 0.005 mm^3	
Theta range for data collection	1.239 to 36.179°.	
Index ranges	-11 ≤ h ≤ 12, -13 ≤ k ≤ 13, -27 ≤ l ≤ 27	
Reflections collected	20701	
Independent reflections	8614 [R(int) = 0.0570]	
Completeness to theta = 24.415°	99.40%	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	8614 / 18 / 304	
Goodness-of-fit on F2	1.01	
Final R indices [I>2sigma(I)]	R1 = 0.0425, wR2 = 0.1063	
R indices (all data)	R1 = 0.0491, wR2 = 0.1099	
Extinction coefficient	none	
Largest diff. peak and hole	1.606 and -0.543 $e.Å^{-3}$	

3.3 GaDpaa.ga crystal structure

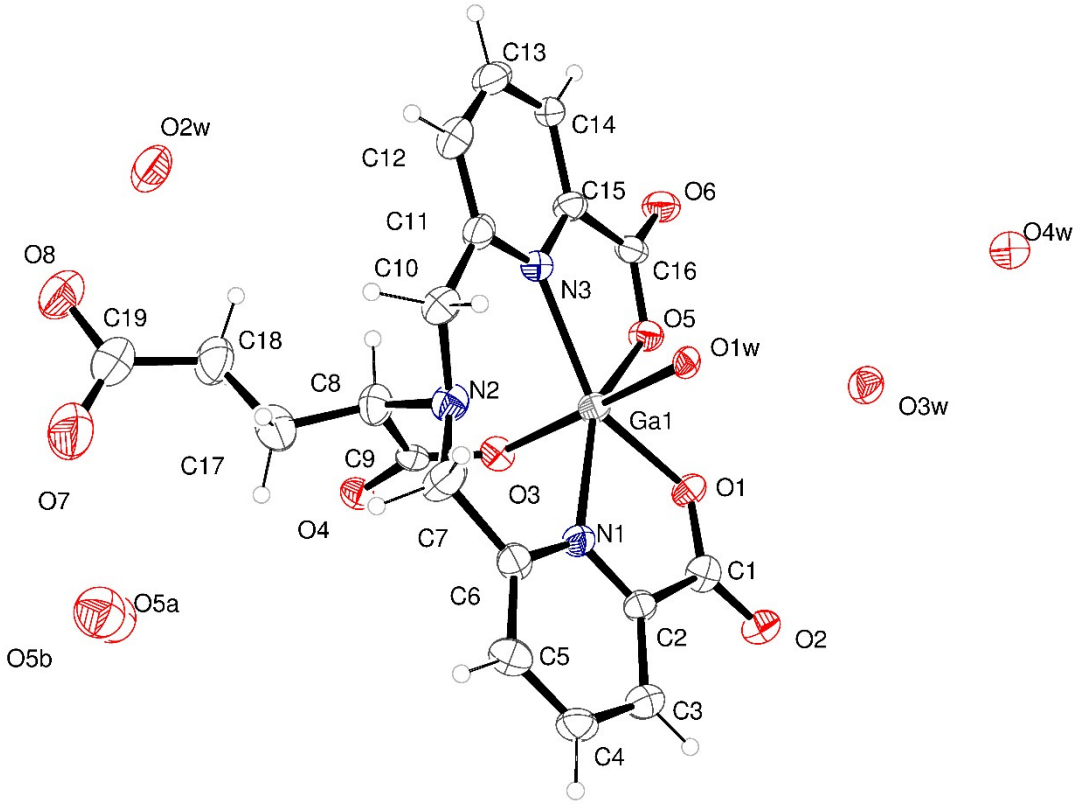


***Figure S 24**:* ORTEP diagram of GaDpaa.ga with atoms drawn as 50 % thermal ellipoids.

The compound crystallises in the centric space group Pccn (Z = 8) with discrete complexes and unbound water molecules. Between the complexes there are C–H···O interactions and there is evidence for π···π interactions between face-on pyridine rings. The complexes are arranged such that there are broad channels running parallel to the crystallographic ¬c-axis. Within these channels are located the unbound water molecules.

The structure presented above was determined using synchrotron radiation (λ = 0.6889 Å) and a data collection that lasted 12 minutes in total. The structure determination confirms the chemical connectivity and the binding of gallium, although the fit parameters are only moderately good (R1 = 0.1133 for data with I > 2σI). The crystal examined was found to suffer from damage in the X-ray beam; this causes a loss of intensity in the X-ray scattering and always leads to poor quality of fit of the model to data. Interestingly in this case, a total of 70 minutes X-ray scattering data were collected from one crystal and it is possible to refine the structure using all of the data (70 minutes). This gives a hint as to the mode of decomposition of the compound in the X-ray beam. The structure refined from all data is shown below.

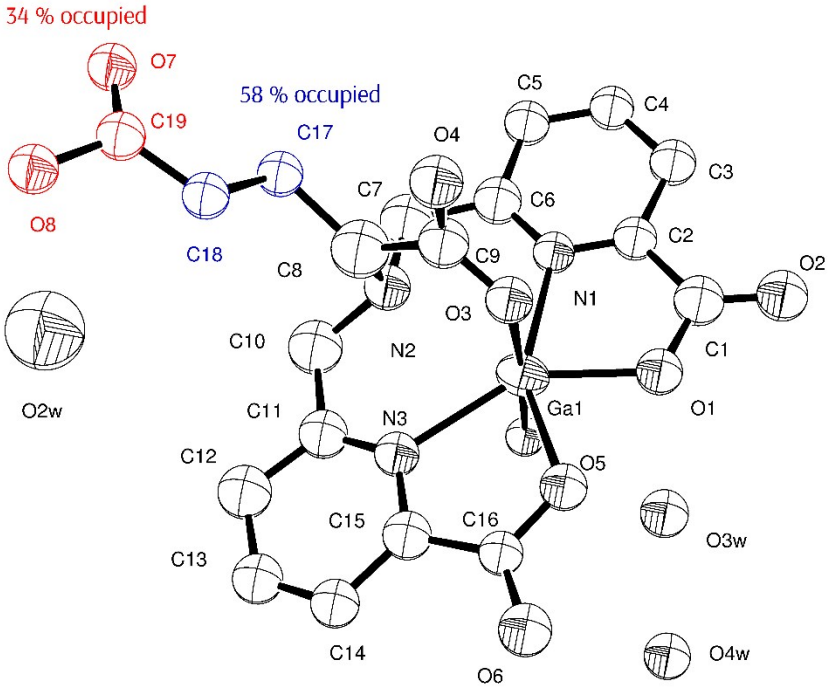


Figure S 25: ORTEP representation of decomposed form of GaDpaa.ga. Hydrogen atoms have been omitted for clarity. Atoms are drawn at the 30% probability level.

The major portion of the ligand is unchanged, but two key changes are observed. There is loss of water (O5a/O5b) and the ligand starts to decompose. There is evidence that this decomposition involves loss of the arm containing unbound carboxylate and in particular with loss of CO_2. In the structure refinement the carboxylate O7-C19-O8 is 34 % occupied and C17&C18 are 58% occupied.

The decomposition of the ligand and loss of water is accompanied by fracture of the crystal and loss of scattered intensity.

Table S 7: Crystal data and structure refinement for GaDpaa.ga. (12 minutes – limited decomposition)

Identification code	GaDpaa.ga	
Empirical formula	C19 H15 Ga N3 O12.87	
Formula weight	561.02	
Temperature	100(2) K	
Wavelength	0.6889 Å	
Crystal system	Orthorhombic	
Space group	P c c n	
Unit cell dimensions	a = 20.7850(12) Å	a= 90°
	b = 30.2224(18) Å	b= 90°
	c = 7.2085(6) Å	g = 90°
Volume	4528.2(5) $Å^3$	
Z	8	
Density (calculated)	1.646 Mg/m^3	
Absorption coefficient	1.186 mm^{-1}	
F(000)	2272	
Crystal size	0.060 × 0.005 × 0.005 mm^3	
Theta range for data collection	1.152 to 24.835°.	
Index ranges	-18 ≤ h ≤ 25, -34 ≤ k ≤ 36, -5 ≤ l ≤ 8	
Reflections collected	12211	
Independent reflections	4251 [R(int) = 0.2109]	
Completeness to theta = 24.415°	98.70%	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	4251 / 0 / 324	
Goodness-of-fit on F2	1.009	
Final R indices [I>2sigma(I)]	R1 = 0.1133, wR2 = 0.2750	
R indices (all data)	R1 = 0.1749, wR2 = 0.3333	
Extinction coefficient	none	
Largest diff. peak and hole	2.429 and -1.265 $e.Å^{-3}$	