Note added after first publication

This Supplementary information replaces the version previously published on 20 June 2018, which contained errors in the 1H NMR data description (some proton integrals were incorrect).

Supplementary Information

N,1,4-Tri(4-alkoxy-2-hydroxybenzyl)-DAZA: Efficient one-pot synthesis and labelling with ^{68}Ga for PET liver imaging *in ovo*

Julia Greiser,[a] Christian Kühnel,[a] Helmar Görls,[b] Wolfgang Weigand*[b] and Martin Freesmeyer*[a]

Synthetic procedures

Synthesis of bicyclic aminals

Synthesis of 1

1,4-Diazepan-6-amine (65 mg, 0.57 mmol) and 4-methoxy-2-hydroxybenzaldehyde (172 mg, 1.13 mmol) were added to methanol (10 mL) and the mixture was stirred at rt for 1 h. The yellow residue was removed by filtration, washed with methanol, and dried *in vacuo* (205 mg, 0.54 mmol, 94%).

^{1}H NMR (400.1 MHz, $CDCl_3$): δ 13.17 (s, broad), 11.85 (s, broad), 8.28 (s, 1H), 7.26–7.23 (m, 1H), 7.12 (d, $^3J_{H,H}$ = 8.4 Hz, 1H), 6.45–6.36 (m, 4H), 5.21 (s, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.71–3.64 (m, 1H), 3.37–2.90 (m, 8H).

^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 165.0, 163.7, 163.5, 161.2, 158.5, 132.7, 128.0, 112.5, 106.9, 105.5, 101.8, 101.2, 87.4, 60.6, 59.1, 55.6, 55.3, 50.6.

MS (DEI): *m/z* 383.2 ([M]$^+$, 100%).

EA (%) (for $C_{21}H_{25}N_3O_4$): C 65.46 (65.78), H 6.72 (6.57), N 11.07 (10.96).

Synthesis of 2

The synthesis was performed as described for **1** but with 1,4-diazepan-6-amine (30 mg, 0.26 mmol) and 4-ethoxy-2-hydroxybenzaldehyde (86 mg, 0.52 mmol). Yield: 100 mg, 0.24 mmol, 94%.

^{1}H NMR (400.1 MHz, $CDCl_3$): δ 13.16 (s, broad), 11.84 (s, broad), 8.27 (s, 1H), 7.24–7.22 (m, 1H), 7.12–7.09 (m, 1H), 6.45–6.41 (m, 2H), 6.38–6.35 (m, 2H), 5.21 (s, 1H), 4.07–3.96 (m, 4H), 3.72–3.64 (m, 1H), 3.49–2.90 (m, 8H), 1.43–1.36 (m, 6H).

^{13}C{^{1}H} NMR (62.9 MHz, $CDCl_3$): δ 165.0, 163.4, 163.0, 160.5, 158.5, 132.7, 128.0, 112.3, 107.4, 106.1, 102.3, 101.6, 87.4, 63.8, 63.5, 60.6, 59.1, 50.6, 15.0, 14.8.

MS (ESI positive, methanol): *m/z* 434.2 ([M + Na]$^+$, 100%), 412.2 ([M + H]$^+$, 45%).

EA (%) (for $C_{23}H_{29}N_3O_4$): C 66.90 (67.13), H 7.13 (7.10), N 10.26 (10.21).

Synthesis of DAZA ligands

Synthesis of TMeOHB-DAZA

$NaBH_4$ (71 mg, 1.89 mmol) was added to a suspension of **1** (200 mg, 0.52 mmol) in a chloroform/methanol mixture (1/1, 15 mL). The mixture was stirred for 1 h at rt. Solvents were removed under reduced pressure and the residue was suspended in methanol. The suspension was filtered and the colourless residue was washed with methanol and dried *in vacuo* (163 mg, 0.31 mmol, 60%).

^{1}H NMR (400.1 MHz, $CDCl_3$): δ 6.88 (d, $^3J_{H,H}$ = 8.1 Hz, 2H), 6.52 (d, $^3J_{H,H}$ = 8.4 Hz, 1H), 6.41–6.35 (m, 5H), 6.27 (dd, $^3J_{H,H}$ = 8.3 Hz, $^2J_{H,H}$ = 2.5 Hz, 1H), 3.82 (d, $^2J_{H,H}$ = 13.4 Hz, 2H), 3.75 (s, 6H), 3.74 (s, 3H), 3.67 (d, $^2J_{H,H}$ = 13.4 Hz, 2H), 3.37 (s, 2H), 2.98–2.72 (m, 9H).

$^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 161.1, 160.6, 159.2, 158.7, 129.7, 129.2, 114.4, 114.0, 105.9, 105.2, 102.1, 102.0, 62.5, 58.3, 57.9, 55.4, 54.7, 51.0, 49.4.

MS (ESI positive, methanol): *m/z* 546.2 ($[M + Na]^+$, 45%), 524.3 ($[M + H]^+$, 100%).

EA (%) (for $C_{29}H_{37}N_3O_6 \cdot 0.5$ MeOH): C 65.58 (65.66), H 7.07 (7.28), N 7.89 (7.79).

Synthesis of TEtOHB-DAZA

$NaBH_4$ (22 mg, 0.58 mmol) was added to a stirred suspension of **2** (120 mg, 0.29 mmol) in methanol. The yellow suspension turned to a colourless solution within 10 min. The solution was stirred for 1 h at rt and then left to stand overnight. A colourless residue was precipitated. The residue was removed by filtration, washed with methanol, and dried *in vacuo* (85 mg, 0.15 mmol, 52%).

^1H NMR (400.1 MHz, $CDCl_3$): δ 6.86 (d, $^3J_{H,H}$ = 8.1 Hz, 2H), 6.51 (d, $^3J_{H,H}$ = 8.3 Hz, 1H), 6.40–6.33 (m, 5H), 6.25 (dd, $^3J_{H,H}$ = 8.3 Hz, $^2J_{H,H}$ = 2.5 Hz, 1H), 3.96 (q, $^3J_{H,H}$ = 7.0 Hz, 6H), 3.82 (d, $^2J_{H,H}$ = 13.4 Hz, 2H), 3.66 (d, $^2J_{H,H}$ = 13.4 Hz, 2H), 3.35 (s, 2H), 2.98–2.71 (m, 9H), 1.41–1.36 (m, 9H).

$^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 160.4, 159.9, 159.2, 158.6, 129.7, 129.2, 114.3, 113.9, 106.4, 105.6, 102.6, 102.6, 63.5, 63.4, 62.5, 57.9, 54.6, 49.4, 15.0.

MS (ESI positive, methanol): *m/z* 588.4 ($[M + Na]^+$, 100%), 566.4 ($[M + H]^+$, 62%), 438.3 ($[M - (CH_2C_6H_4OOC_2H_5) + Na]^+$, 25%), 416.3 ($[M - (CH_2C_6H_4OOC_2H_5) + H]^+$, 46%).

EA (%) (for $C_{32}H_{43}N_3O_6 \cdot H_2O$): C 65.49 (65.84), H 7.43 (7.77), N 7.27 (7.20).

Synthesis of 3a and 4a for mechanistic studies

Synthesis of 3a

Cycloheptylamine (41 mg, 0.36 mmol) and 4-ethoxy-2-hydroxybenzaldehyde (60 mg, 0.36 mmol) were combined in methanol and stirred at rt overnight. Removal of the solvent *in vacuo* gave **3a** as a yellow solid in quantitative yield.

^1H NMR (400.1 MHz, $CDCl_3$): δ 14.26 (s, 1H), 8.09 (s, 1H), 7.04 ($^3J_{H,H}$ = 8.5 Hz, 1H), 6.36–6.31 (m, 2H), 4.03 (q, $^3J_{H,H}$ = 7.0 Hz, 2H), 3.44–3.39 (m, 1H), 1.91–1.53 (m, 12H), 1.40 (t, $^3J_{H,H}$ = 7.0 Hz, 3H).

$^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 167.8, 163.4, 160.7, 132.6, 112.1, 106.8, 102.1, 67.9, 63.6, 36.4, 28.4, 24.2, 14.8.

MS (ESI positive, methanol): *m/z* 284.4 ($[M + Na]^+$, 100%), 262.3 ($[M + H]^+$, 85%).

Synthesis of 4a

Homopiperazine (30 mg, 0.31 mmol) and 4-ethoxy-2-hydroxybenzaldehyde (51 mg, 0.31 mmol) were combined in dichloromethane. Å3 molecular sieves were added and the mixture was stirred at rt overnight. The mixture was filtered and removal of the solvent from the filtrate gave **4a** as a colourless solid (60 mg, 0.24 mmol, 78%).

^1H NMR (400.1 MHz, $CDCl_3$): δ 12.42 (s, 1H), 7.20–7.17 (m, 1H), 6.34–6.32 (m, 2H), 5.12 (s, 1H), 3.97 (q, $^3J_{H,H}$ = 7.0 Hz, 2H), 3.35–3.27 (m, 2H), 3.05–2.82 (m, 7H), 2.06–1.92 (m, 1H), 1.37 (t, $^3J_{H,H}$ = 7.0 Hz, 3H).

$^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 167.3, 158.8, 127.7, 113.3, 105.8, 102.2, 88.1, 63.4, 54.4, 50.0, 18.7, 15.0.

MS (DEI): *m/z* 248.2 ($[M]^+$, 100%).

Synthesis of Ga(III) complexes

Synthesis of Ga-TMeOHB-DAZA

An aqueous solution of Ga(III) chloride (0.11 M; 0.1 mmol, 0.87 mL) was added to a solution of TMeOHB-DAZA (50 mg, 0.10 mmol) in methanol/chloroform (1/1, 10 mL). Aqueous ammonia (20%) was added dropwise to raise the pH of the solution to 3.8. The solution was stirred for 2 h at rt, and then the solvent was removed under reduced pressure. The residue was redissolved in ethanol and purified by reversed-phase chromatography (RP-C18- silica, water → ethanol) to yield the Ga(III) complex as a colourless solid (20 mg, 0.03 mmol, 35%).

^{1}H NMR (400.1 MHz, 25°C, MeOH-d_4): δ 7.51-7.26 (m, 1H), 6.95-6.88 (m, 2H, three doublets visible with $^{3}J_{H,H}$ = 8.3 Hz, 8.3 Hz, 8.0 Hz), 6.62-6.22 (m, 6H), 4.38-4.17 (m, 3H), 3.80-3.44 (m, 13H, underlying doublet with $^{2}J_{H,H}$ = 12.8 Hz), 3.22-2.78 (m, 8H).

^{1}H NMR (300.1 MHz, 25°C, MeOH-d_4): δ 6.86 (s, 0.4H), 6.85 (s, 0.1H), 6.83 (s, 0.5H), 6.79 (dd, $^{3}J_{H,H}$ = 8.2 Hz, $^{4}J_{H,H}$ = 3.3 Hz, 1.8H), 6.54 (m, 1.0H), 6.44 (d, $^{4}J_{H,H}$ = 2.4Hz, 1.0H), 6.30 (d, $^{4}J_{H,H}$ = 2.5 Hz, 2.5Hz, 0.5H), 6.27 (d, $^{4}J_{H,H}$ = 2.5 Hz, 2.5Hz, 0.9H), 6.25 (d, $^{4}J_{H,H}$ = 2.5 Hz, 2.5Hz, 0.4H), 6.3 (d, $^{4}J_{H,H}$ = 2.5 Hz, 2.5Hz, 0.5H), 6.20 (d, $^{4}J_{H,H}$ = 2.5 Hz, 2.5Hz, 0.4H), 4.61 (d, $^{2}J_{H,H}$ = 14.5 Hz, 1.0H), 4.21 (d, $^{2}J_{H,H}$ = 12.5 Hz, 1.0H), 4.06 (d, $^{2}J_{H,H}$ = 12.1 Hz, 1.0H), 3.96 (d, $^{2}J_{H,H}$ = 13.1 Hz, 1.0H), 3.77 (s, 2.8H), 3.74 (s, 3.0H), 3.70-3.56 (m, 3.2H), 3.54 (s, 2.7H), 3.38-3.35 (m, 1.2H), 3.06-2.75 (m, 6.1H).

^{13}C{^{1}H} NMR (100.6 MHz, 25°C, MeOH-d_4): δ 165.8, 164.3, 163.6, 163.1, 162.7, 132.6, 132.5, 131.8, 116.4, 115.1, 106.9, 106.6, 106.4, 105.6, 104.7, 103.4, 62.5, 60.8, 59.9, 55.8, 55.6, 55.5, 55.4, 47.0.

MS (ESI positive, methanol]): *m/z* 614.1 ([M(^{71}Ga) + Na]$^{+}$, 75%), 612.1 ([M(^{69}Ga) + Na]$^{+}$, 100%), 592.2 ([M(^{71}Ga) + H]$^{+}$, 55%), 590.2 ([M(^{69}Ga) + H]$^{+}$, 75%).

EA (%) (for $C_{29}H_{34}GaN_3O_6$·MeOH): C 58.09 (57.90), H 6.08 (6.15), N 6.42 (6.75).

Synthesis of Ga-TEtOHB-DAZA

An aqueous solution of Ga(III) chloride (0.11 M, 0.09 mmol, 0.8 mL) was added to a solution of TEtOHB-DAZA (50 mg. 0.09 mmol) in hot methanol (35 mL). Aqueous ammonia (20%) was added dropwise to raise the pH of the solution to 3.8. The solution was stirred for 2 h at rt and then the solvent was removed under reduced pressure. The residue was washed with water to yield the Ga(III) complex as a colourless solid (37 mg, 0.06 mmol, 67%).

^{1}H NMR (500.3 MHz, 25°C, DMSO-d_6): δ 10.05 (s, 0.2H), 9.58 (s, 0.6H), 7.34-7.09 (m, 0.5), 6.92-6.90 (d, $^{3}J_{H,H}$ = 7.8 Hz, 1H), 6.87-6.82 (m, 0.9H), 6.78 (d, $^{3}J_{H,H}$ = 8.3 Hz, 0.3H), 6.47 (d, $^{4}J_{H,H}$ = 2.1 Hz, 0.2H), 6.42 (dd, $^{3}J_{H,H}$ = 8.4 Hz, $^{4}J_{H,H}$ = 2.5 Hz, 0.2H), 6.36 (s, 0.4H), 6.35 (d, $^{4}J_{H,H}$ = 2.3 Hz, 0.5H), 6.25 (s, 0.8), 6.22 (d, $^{4}J_{H,H}$ = 2.5 Hz, 0.2H), 6.20-6.17 (m, 2.7H), 6.09 (dd, $^{3}J_{H,H}$ = 8.3 Hz, $^{4}J_{H,H}$ = 2.5 Hz, 0.4H), 4.36 (d, $^{2}J_{H,H}$ = 14.2 Hz, 0.2H), 4.20 (d, $^{2}J_{H,H}$ = 13.0 Hz, 0.8H), 4.04-3.74 (m, 9.8H), 3.33-3.16 (m, 2.7H), 3-08-2.97 (m, 1.7H, underlying doublet visible with $^{2}J_{H,H}$ = 14.1 Hz), 2.94-2.59 (m, 5.4H), 1.32-1.15 (m, 9.1H).

^{13}C{^{1}H} NMR (100.6 MHz, 25°C, DMSO-d_6): δ 165.1, 165.0, 163.6, 160.6, 160.4, 159.9, 159.4, 157.4, 156.3, 133.7, 131.4, 130.7, 128.8, 116.6, 114.8, 114.1, 113.7, 111.6, 106.7, 106.0, 105.4, 105.1, 104.8, 104.5, 103.4, 101.9, 101.5, 62.8, 62.6, 62.3, 60.8, 59.5, 58.9, 55.7, 55.1, 54.2, 49.3, 14.8, 14.6.

MS (ESI positive, methanol]): *m/z* 656.3 ([M(^{71}Ga) + Na]$^{+}$, 75%), 654.3 ([M(^{69}Ga) + Na]$^{+}$, 100%), 634.3 ([M(^{71}Ga) + H]$^{+}$, 50%), 632.3 ([M(^{69}Ga) + H]$^{+}$, 65%).

EA (%) (for $C_{32}H_{40}GaN_3O_6$·2.75H_2O): C 56.12 (56.43), H 6.19 (6.73), N 5.78 (6.17).

Additional Figures

Fig 4 Exemplary dynamic 90 min PET scan of [^{68}Ga]Ga-TEtOHB-DAZA administered to an incubated ostrich egg at breeding day 35. The dynamic scan visualizes tracer injection and influx via the vitelline vein (1min) followed by blood circulation (app. 1-5 min) and rapid liver enhancement starting at ca. 5 min post injection. Hepatobiliary excretion into the bowel began about 30 min post injection. For anatomical reference CT images of the skeletal and vessel structure as well as the egg shell are shown.

Fig 4 is provided as a separate mp4 file.

NMR spectra of Ga-TMeOHB-DAZA

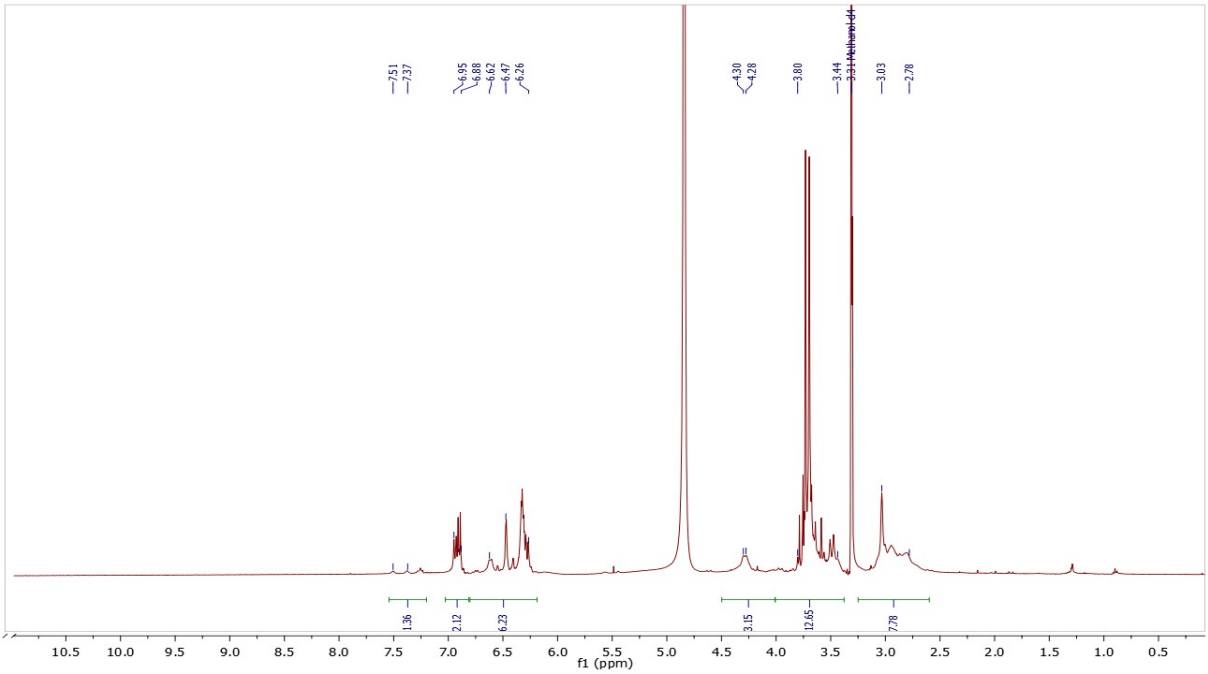

Fig. S1 ^{1}H NMR spectrum of Ga-TMeOHB-DAZA in MeOH-d_4 at 25°C (400 MHz).

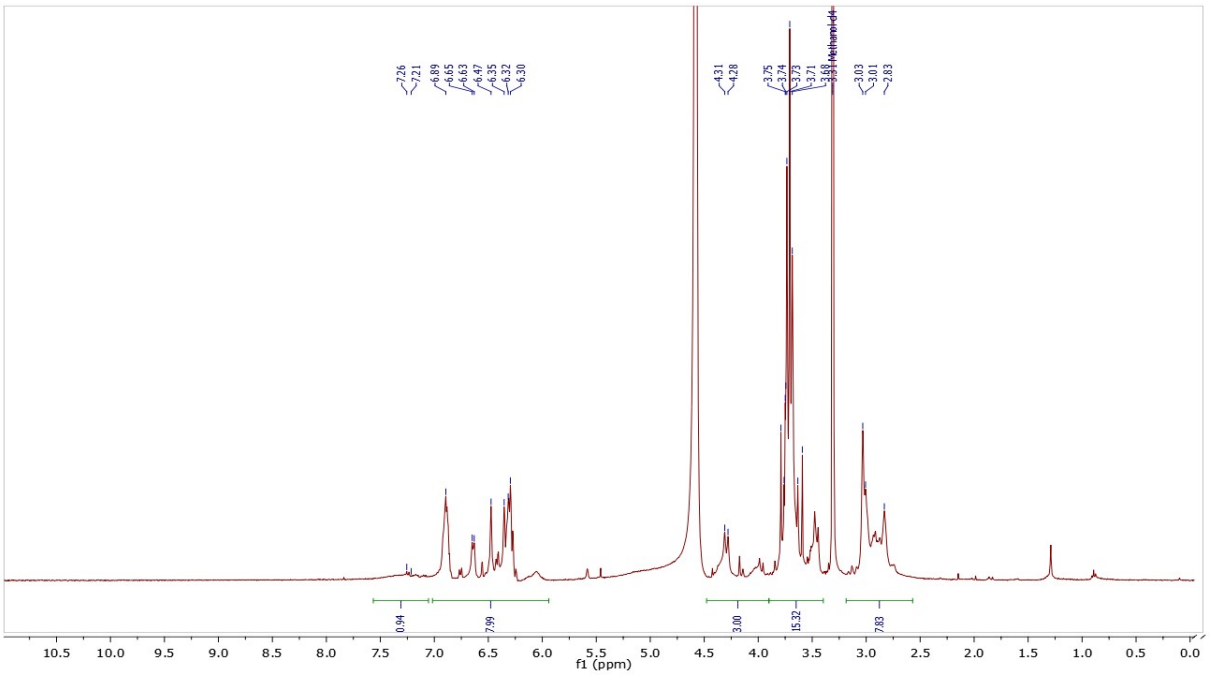

Fig. S2 ^{1}H NMR spectrum of Ga-TMeOHB-DAZA in MeOH-d_4 at 50°C (400 MHz).

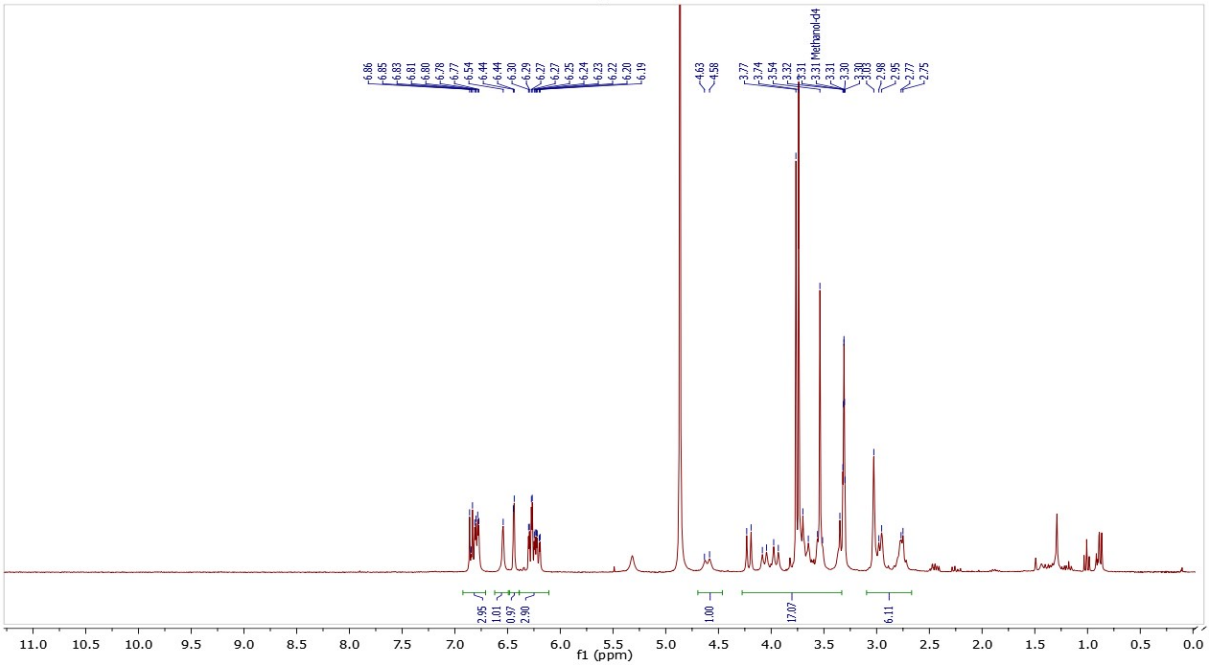

Fig. S3 Particularly well resolved ^{1}H NMR spectrum of Ga-TMeOHB-DAZA in MeOH-d_4 at 25°C (300 MHz). Signals at δ1.29-0.86 likely stem from grease.

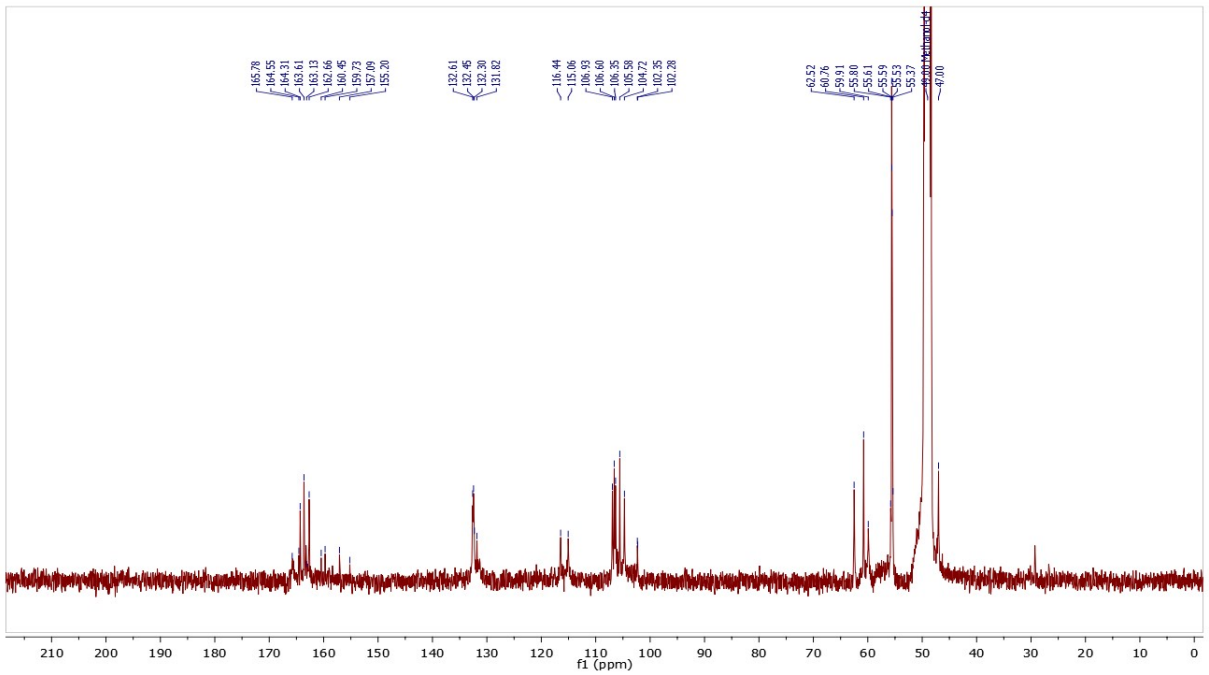

Fig. S4 ^{13}C{^{1}H} NMR spectrum of Ga-TMeOHB-DAZA in MeOH-d_4 at 25°C (100 MHz).

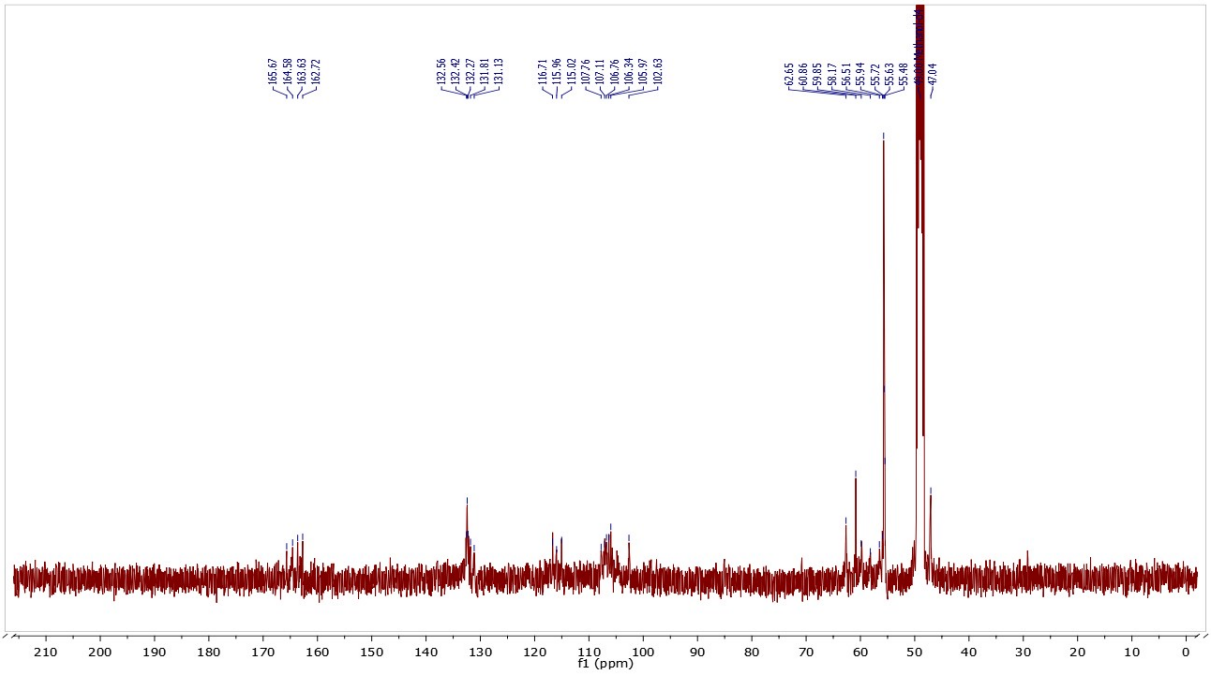

Fig. S4 $^{13}C\{^{1}H\}$ NMR spectrum of Ga-TMeOHB-DAZA in MeOH-d_4 at 50°C (100 MHz).

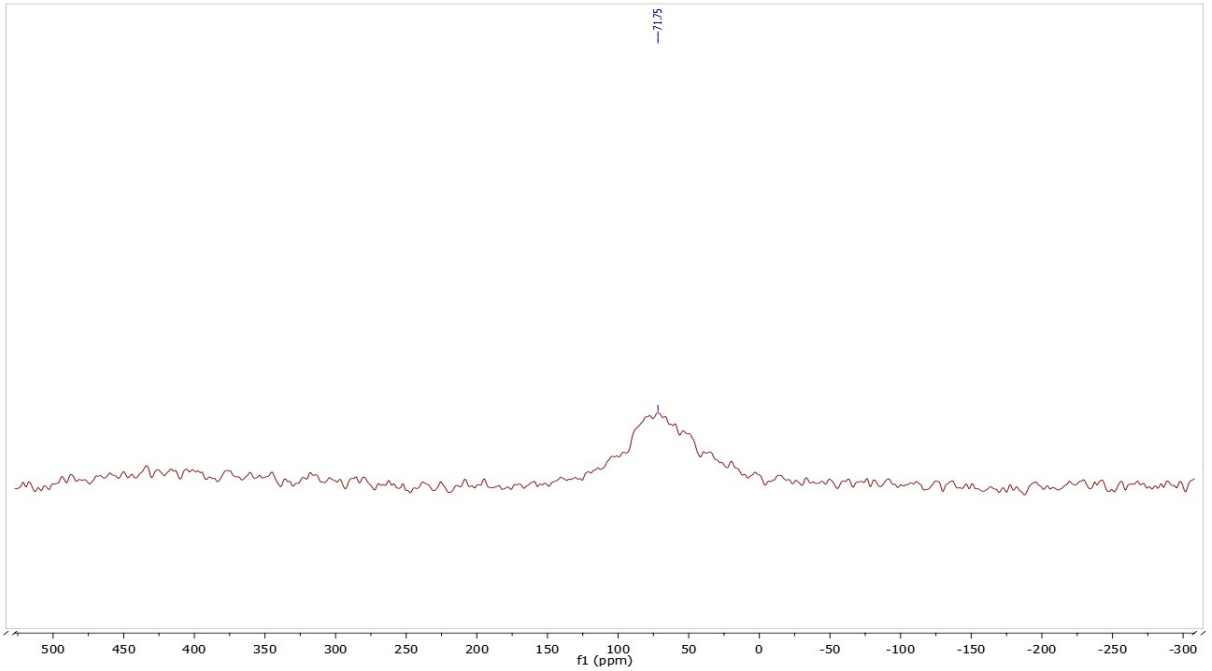

Fig. S5 71Ga NMR spectrum of Ga-TMeOHB-DAZA in MeOH-d4 at 25°C (122 MHz). Number of Scans: 20480.

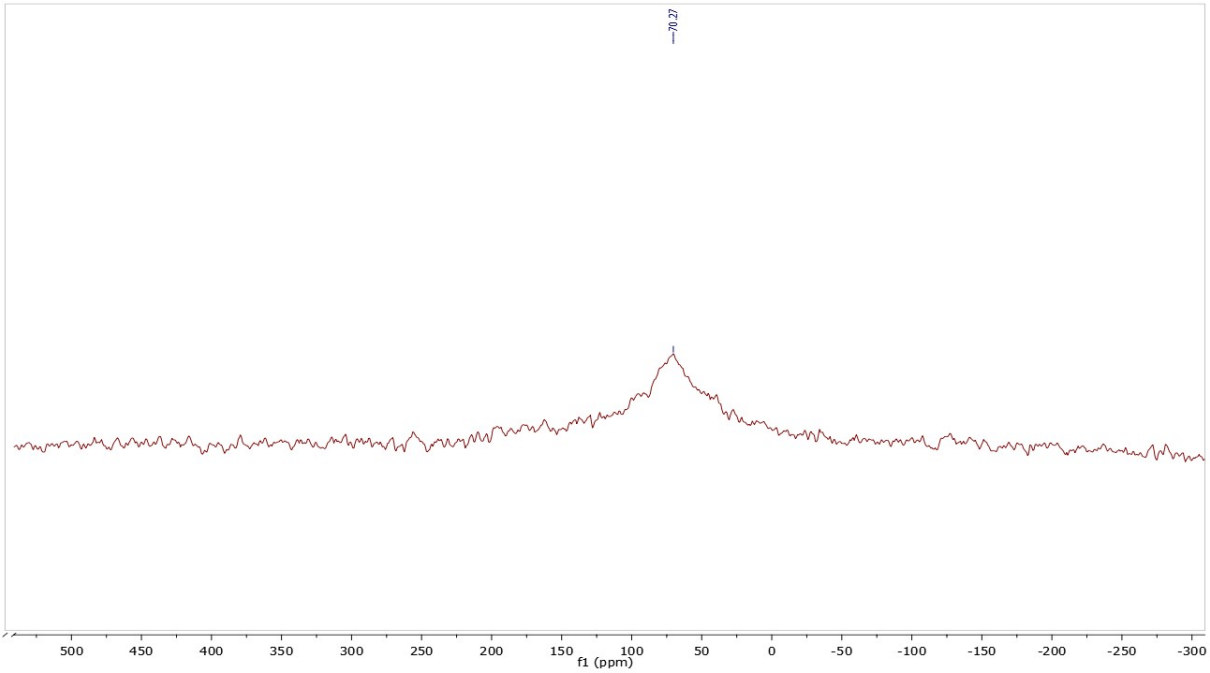

Fig. S6 ^{71}Ga NMR spectrum of Ga-TMeOHB-DAZA in MeOH-d_4 at 50°C (122 MHz). Number of Scans: 17242.

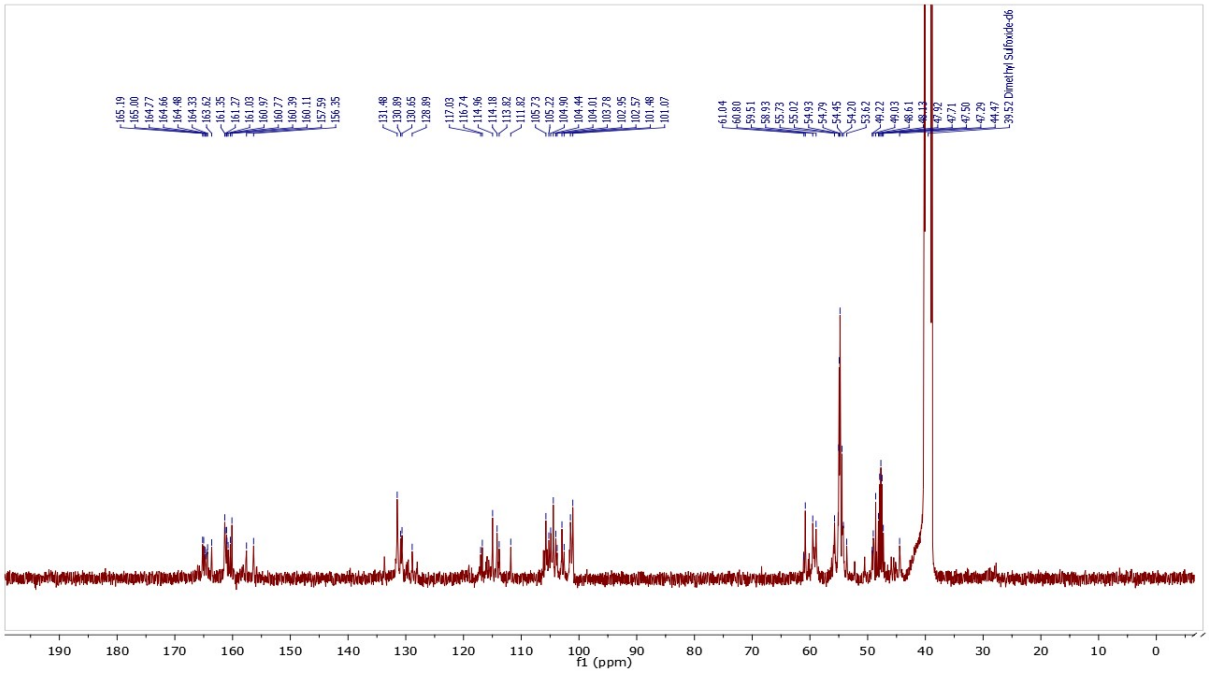

Fig. S7 ^{13}C{^{1}H} NMR spectrum of Ga-TMeOHB-DAZA in DMSO-d_6 at 25°C (100 MHz).

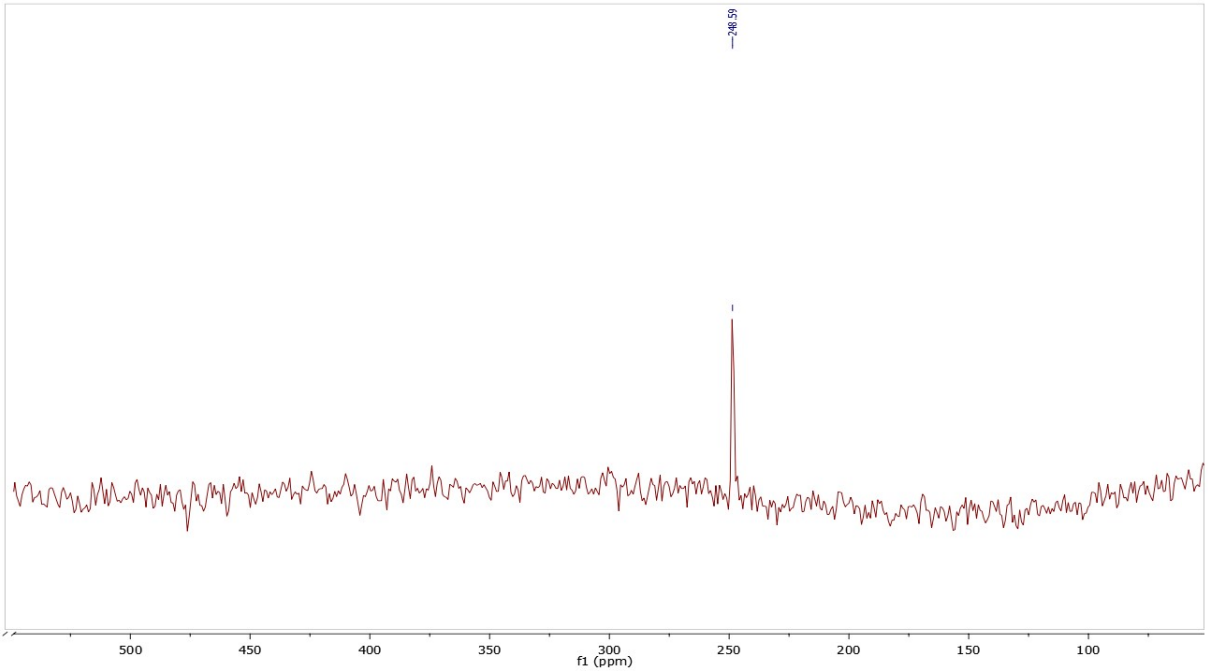

Fig. S8 ^{71}Ga NMR spectrum of Ga-TMeOHB-DAZA in DMSO-d_6 at 25°C (122 MHz). Number of Scans: 20480.

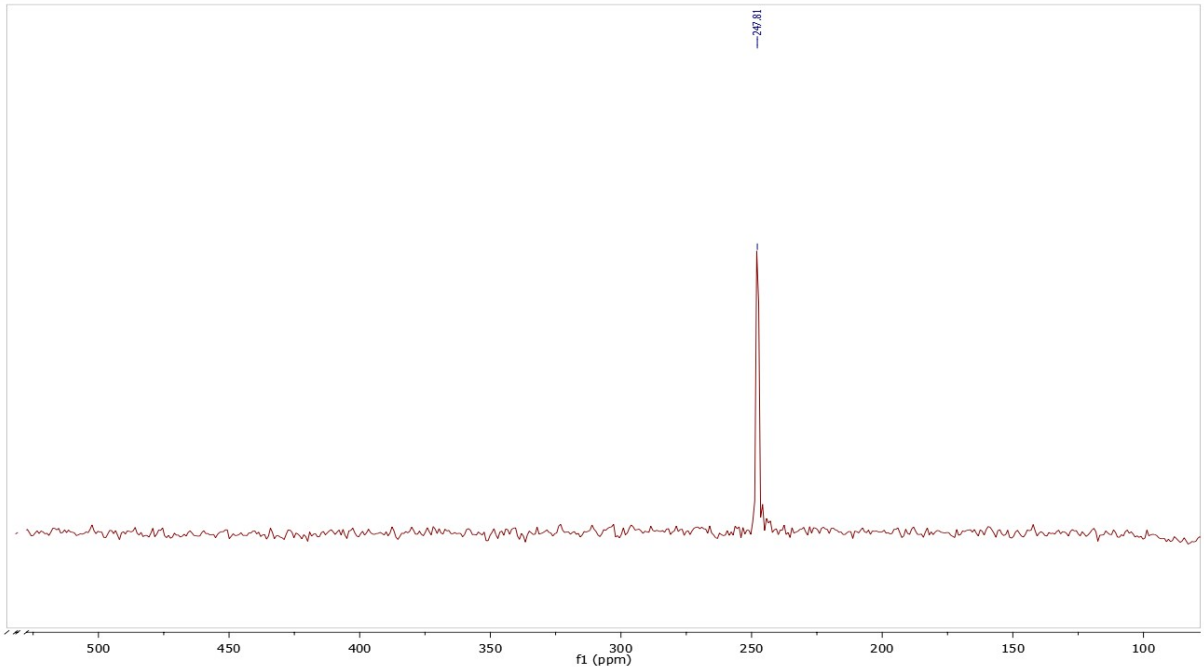

Fig. S9 ^{71}Ga NMR spectrum of Ga-TMeOHB-DAZA in DMSO-d_6 at 50°C (122 MHz). Number of Scans: 20480.

NMR spectra of Ga-TEtOHB-DAZA

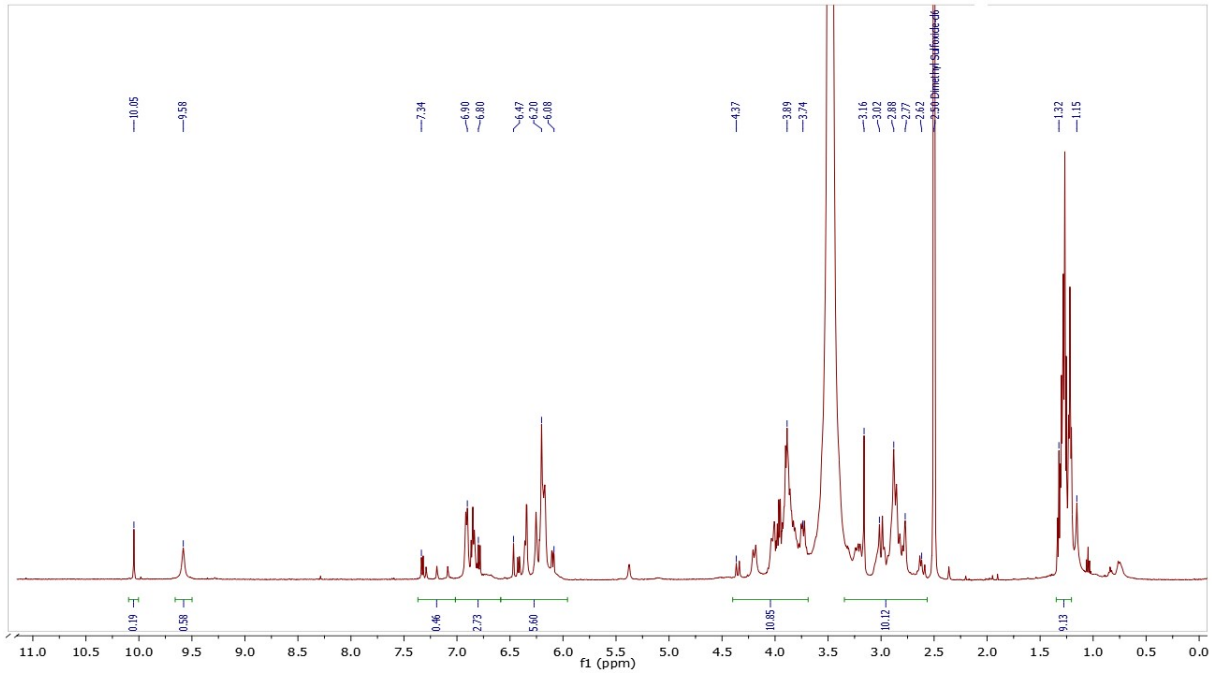

Fig. S10 ^{1}H NMR spectrum of Ga-TEtOHB-DAZA in DMSO-d_6 at 25°C (500 MHz).

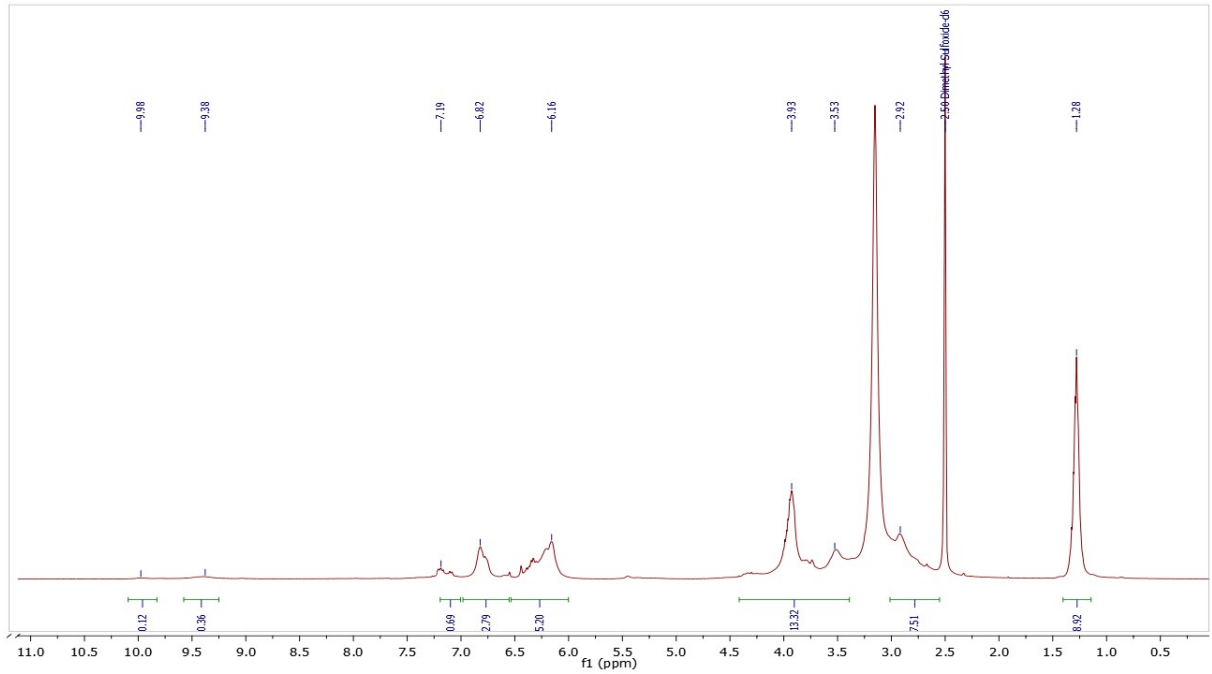

Fig. S11 ^{1}H NMR spectrum of Ga-TEtOHB-DAZA in DMSO-d_6 at 70°C (400 MHz).

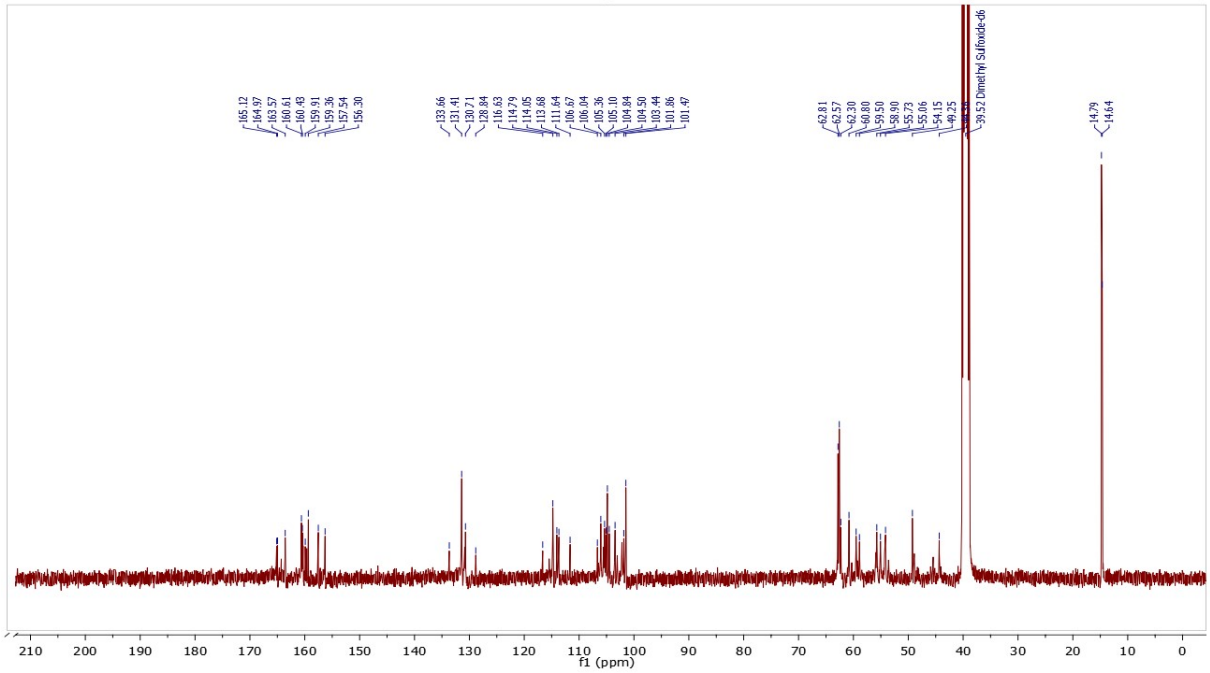

Fig. S12 $^{13}C\{^{1}H\}$ NMR spectrum of Ga-TEtOHB-DAZA in DMSO-d_6 at 25°C (100 MHz).

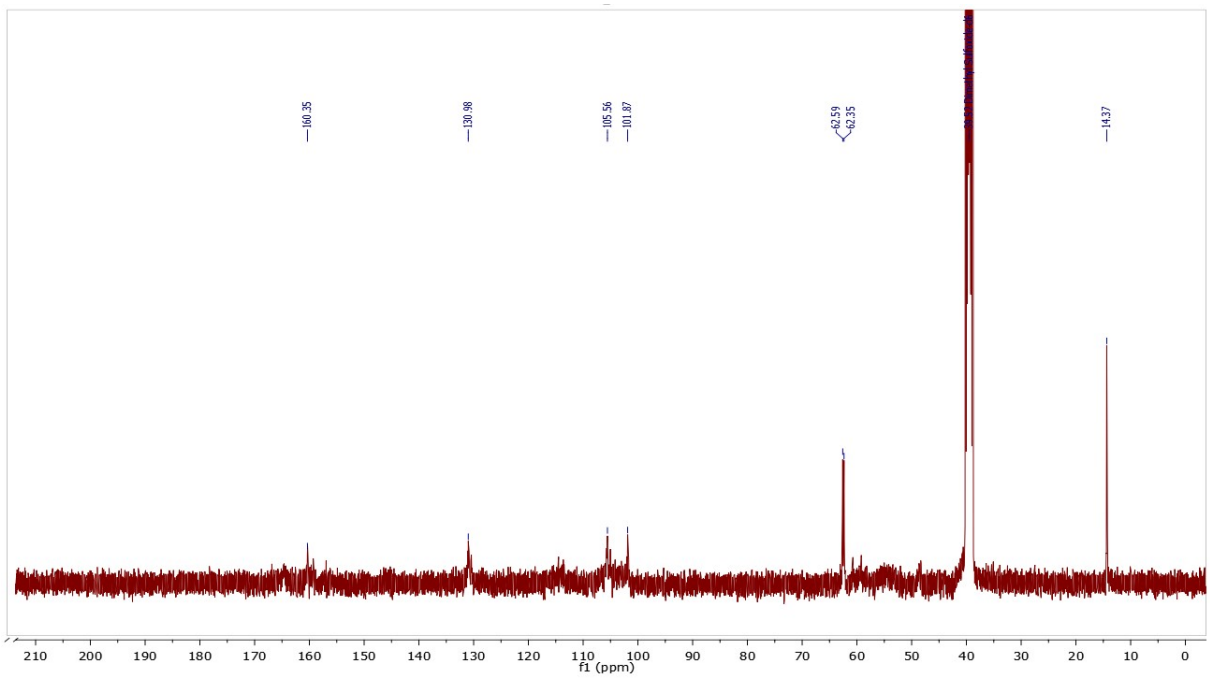

Fig. S13 $^{13}C\{^{1}H\}$ NMR spectrum of Ga-TEtOHB-DAZA in DMSO-d_6 at 70°C (100 MHz).

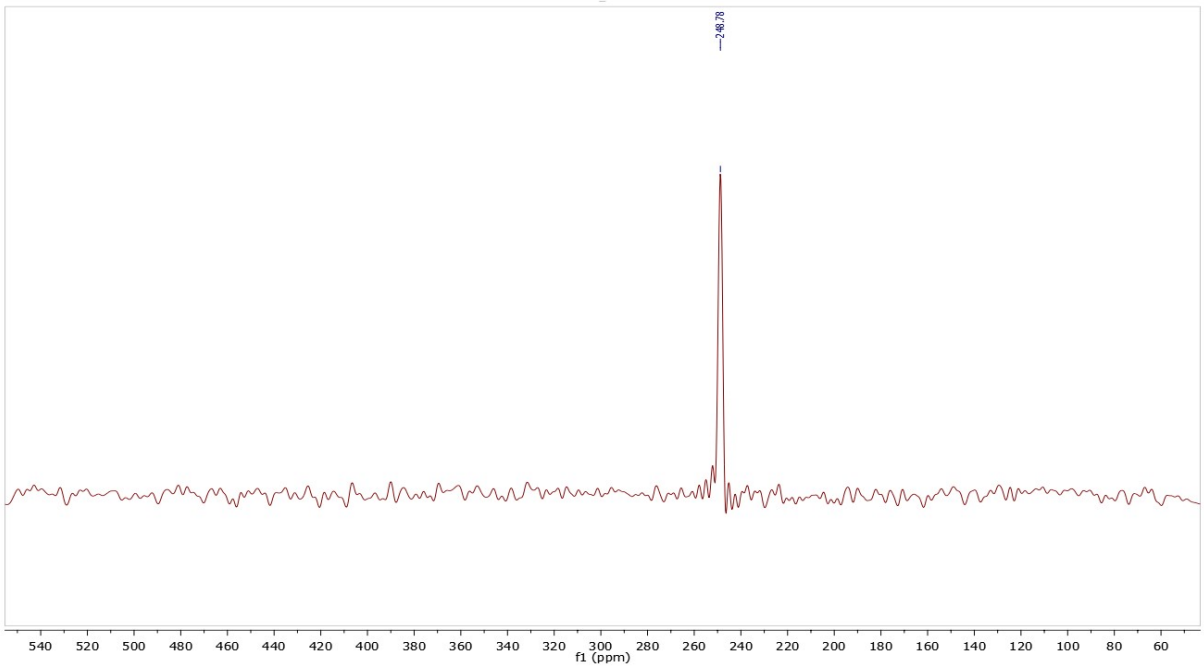

Fig. S14 ^{71}Ga NMR spectrum of Ga-TEtOHB-DAZA in DMSO-d_6 at 25°C (122 MHz). Number of Scans: 20480.

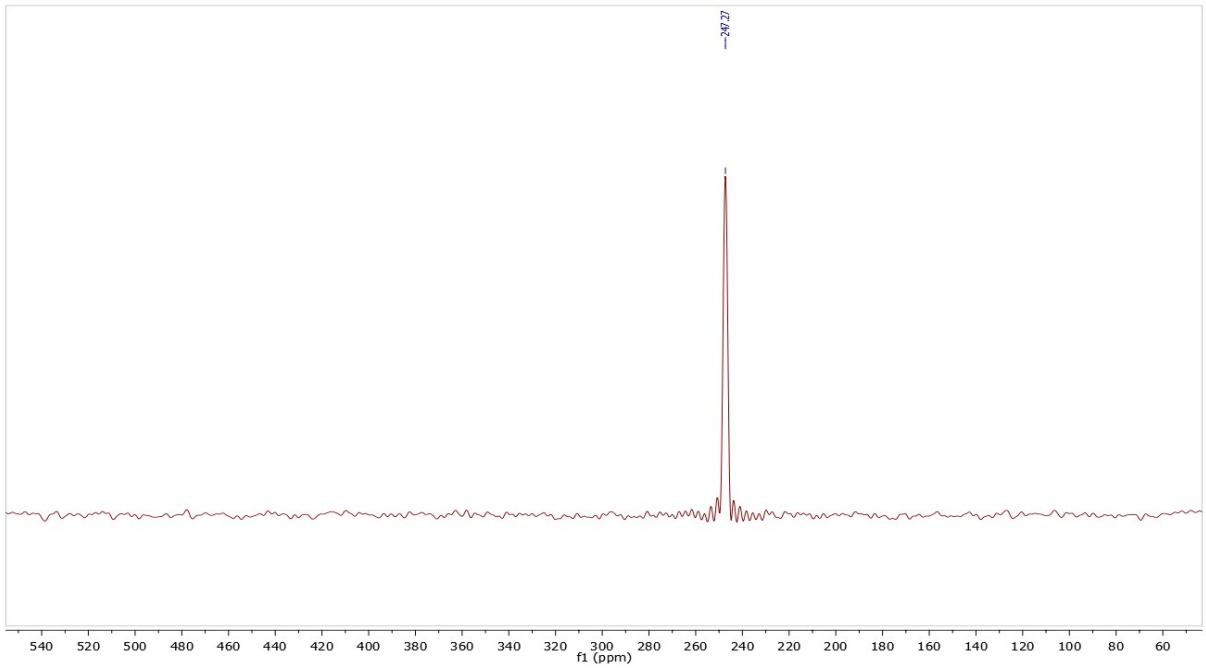

Fig. S15 ^{71}Ga NMR spectrum of Ga-TEtOHB-DAZA in DMSO-d_6 at 70°C (122 MHz). Number of Scans: 18461.

Examples of mass spectra (ESI positive, methanol/chloroform) from mechanistic studies (Table 1)

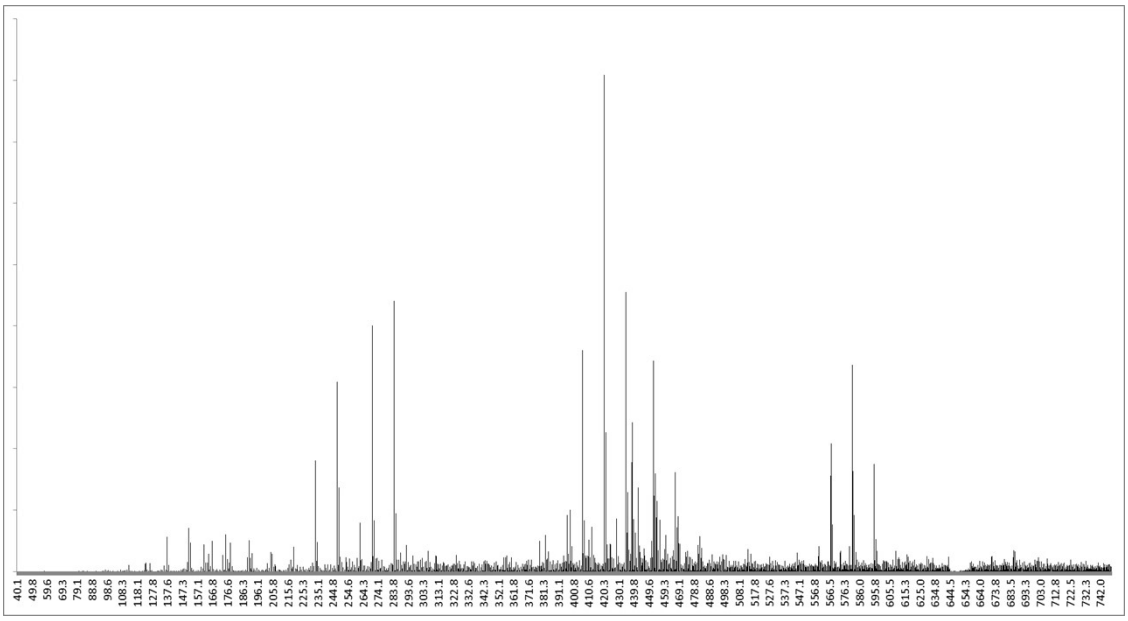

Fig. S16 Mass spectrum of Aii. Reaction Conditions: **1** (1 eq.) and 4-ethoxybenzaldehyde (1 eq.) in methanol/chloroform (1/1), rt, two weeks.

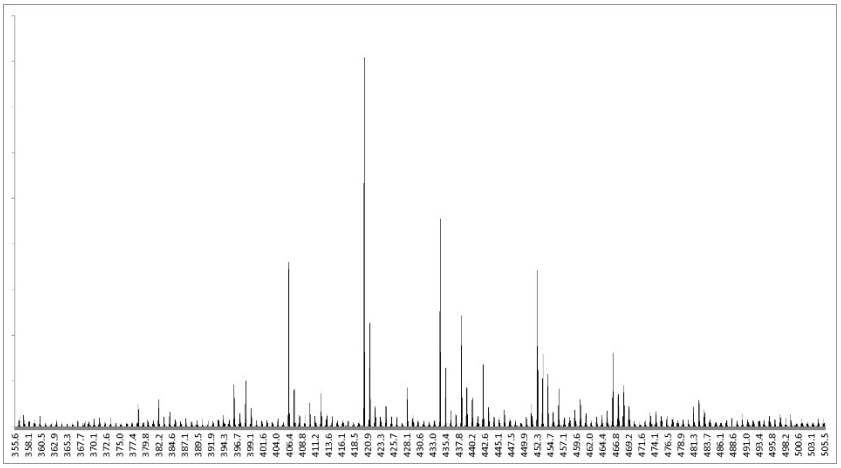

Fig. S17 Signal set for bicyclic structures (**I**) in mass spectrum of Aii: *m/z* 406.2 ([M + Na]$^+$, two R = Me, **1**), 420.2 ([M + Na]$^+$, one R = Me, one R = Et), 434.2 ([M + Na]$^+$, two R = Et, **2**), 438.3 ([M + Na + MeOH]$^+$, two R = Me, **1**), 452.3 ([M + Na + MeOH]$^+$, one R = Me, one R = Et), 466.3 ([M + Na + MeOH]$^+$, two R = Et, **2**).

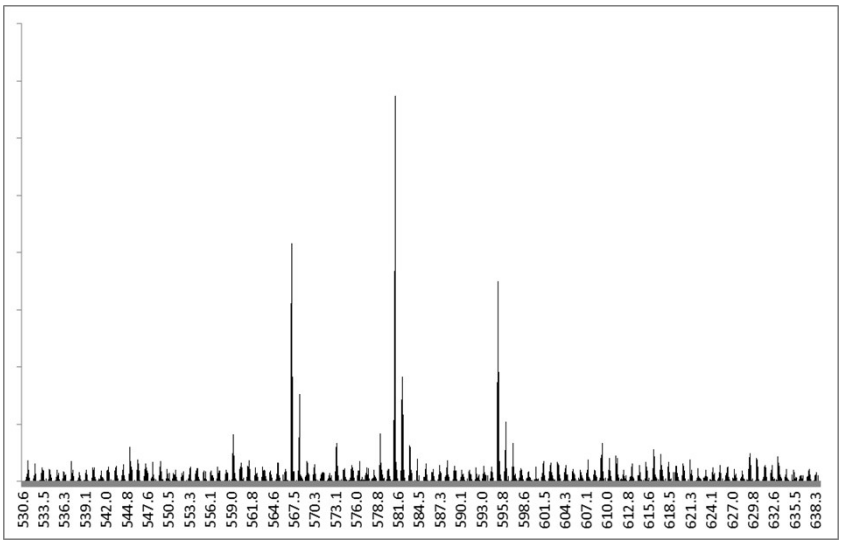

Fig. S18 Signal set for hemiaminals (**III**) in mass spectrum of Aii: *m/z* 567.2 ([M^+], two R = Me, one R = Et), 581.1 ([M^+], one R = Me, two R = Et), 595.2 ([M^+], all R = Et).

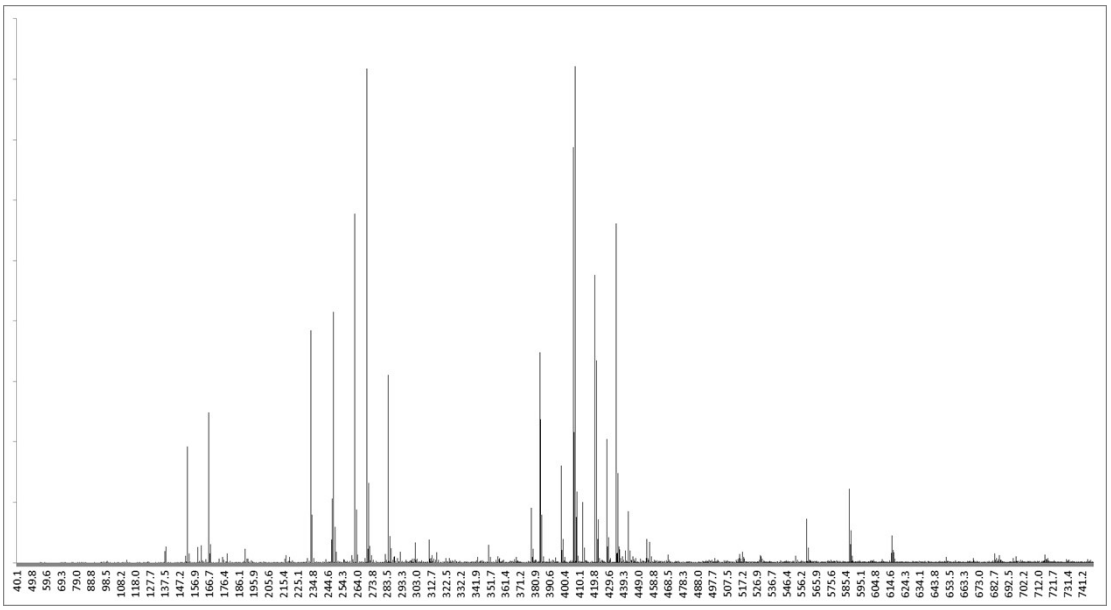

Fig. S19 Mass spectrum of Bi. Reaction Conditions: **1** (1 eq.) and **3a** (1 eq.) in methanol/chloroform (1/1), rt, 24h.

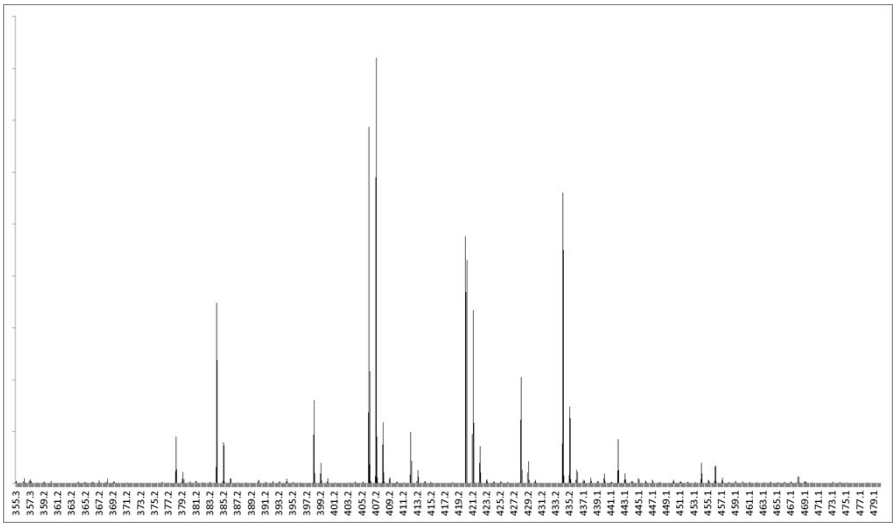

Fig. S20 Signal set for bicyclic structures (**I**) in mass spectrum of Bi: *m/z* 384.2 ($[M + H]^+$, two R = Me, **1**), 398.2 ($[M + H]^+$, one R = Me, one R = Et), 406.2 ($[M + Na]^+$, two R = Me, **1**), 412.3 ($[M + H]^+$, two R = Et, **2**), 420.2 ($[M + Na]^+$, one R = Me, one R = Et), 434.2 ($[M + Na]^+$, two R = Et, **2**).

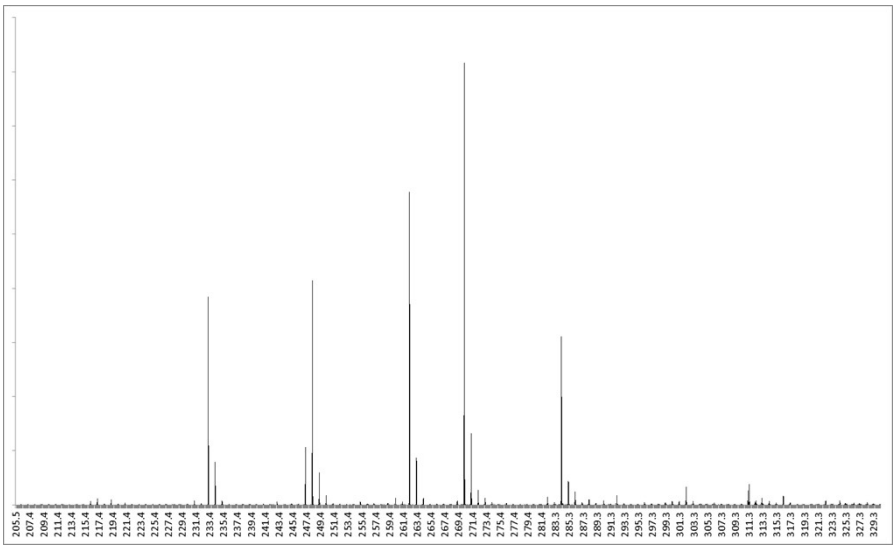

Fig. S21 Signal set for starting material (**3a**) and **3b** in mass spectrum of Bi: *m/z* 248.2 ([M + H]$^+$, **3b**), 262.2 ([M + H]$^+$, **3a**), 270.2 ([M + Na]$^+$, **3b**), 284.2 ([M + Na]$^+$, **3a**).

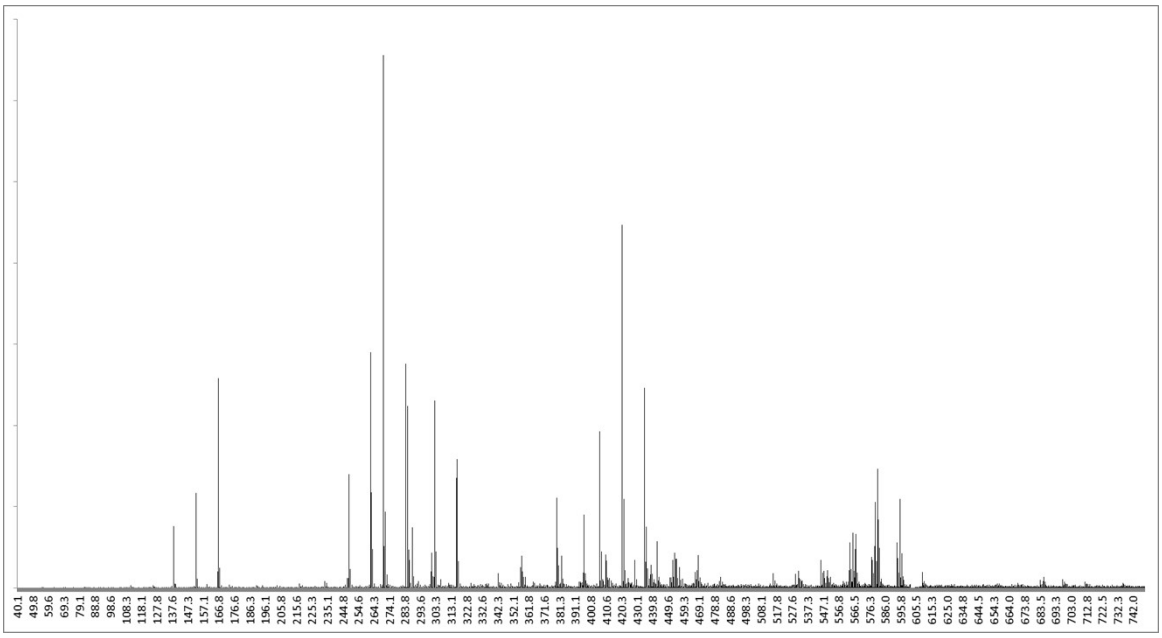

Fig. S22 Mass spectrum of Bii. Reaction Conditions: **1** (1 eq.) and **3a** (1 eq.) in methanol/chloroform (1/1), rt, two weeks.

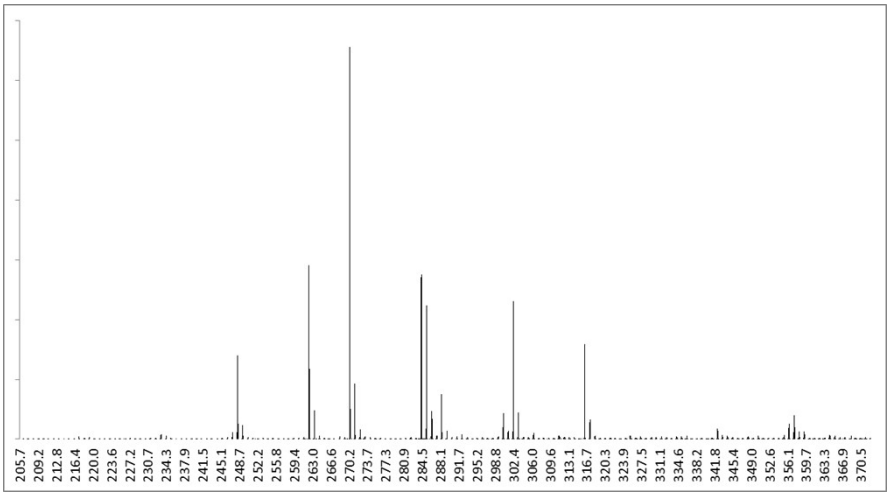

Fig. S23 Signal set for starting material (**3a**) and **3b** in mass spectrum of Bii: *m/z* 248.2 ([M + H]$^+$, **3b**), 262.2 ([M + H]$^+$, **3a**), **270.2** ([M + Na]$^+$, **3b**), 284.2 ([M + Na]$^+$, **3a**).

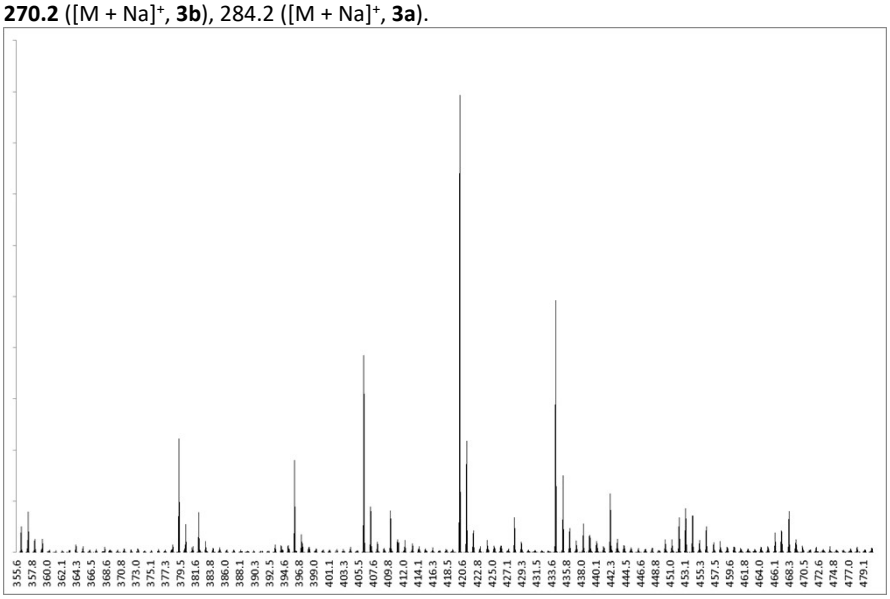

Fig. S24 Signal set for bicyclic structures (**I**) in mass spectrum of Bii: *m/z* 406.2 ([M + Na]$^+$, two R = Me, **1**), 420.2 ([M + Na]$^+$, one R = Me, one R = Et), 434.2 ([M + Na]$^+$, two R = Et, **2**).

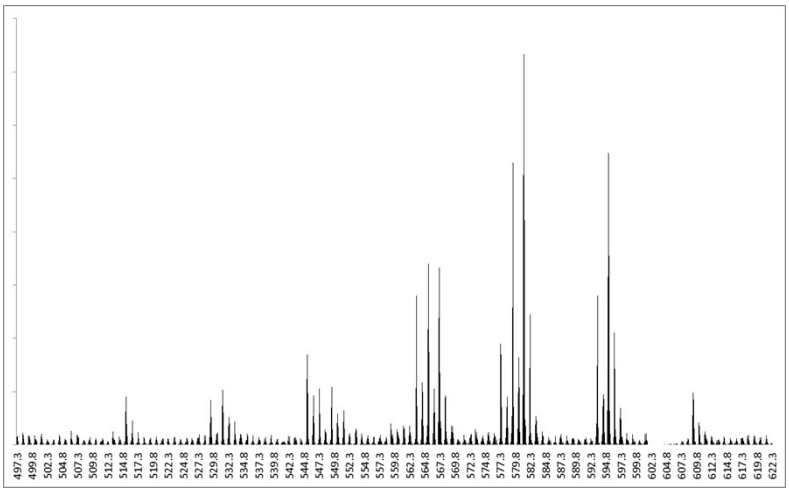

Fig. S25 Signal set for hemiaminals (**III**) in mass spectrum of Bii: *m/z* 567.2 ([M^+], two R = Me, one R = Et), 581.2 ([M^+], one R = Me, two R = Et), 595.2 ([M^+], all R = Et).

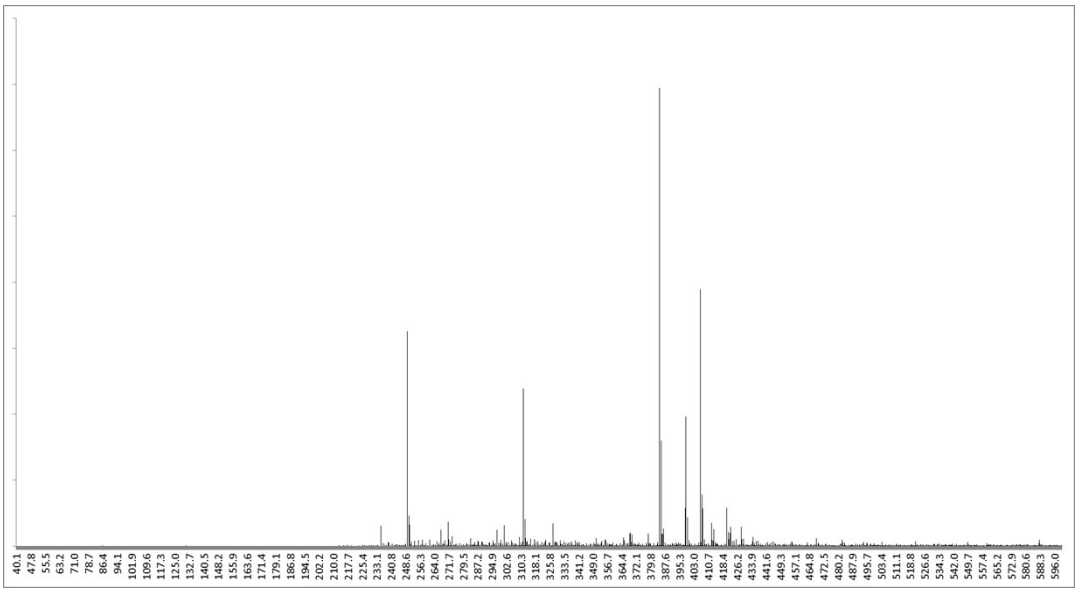

Fig. S26 Full mass spectrum of Cii. Reaction Conditions: **1** (1 eq.) and **4a** (1 eq.) in methanol/chloroform (1/1), rt, two weeks.

Signal set for starting material (**4a**) and **4b** in mass spectrum of Cii: *m/z* 235.2 ($[M + H]^+$, **4b**), 249.2 ($[M + H]^+$, **4a**), 271.2 ($[M + Na]^+$, **4a**).
Signal set for bicyclic structures (**I**) in mass spectrum of Cii: m/z = 384.2 ($[M + H]^+$, two R = Me, **1**), 398.2 ($[M + H]^+$, one R = Me, one R = Et), 406.2 ($[M + Na]^+$, two R = Me, **1**), 420.2 ($[M + Na]^+$, one R = Me, one R = Et).

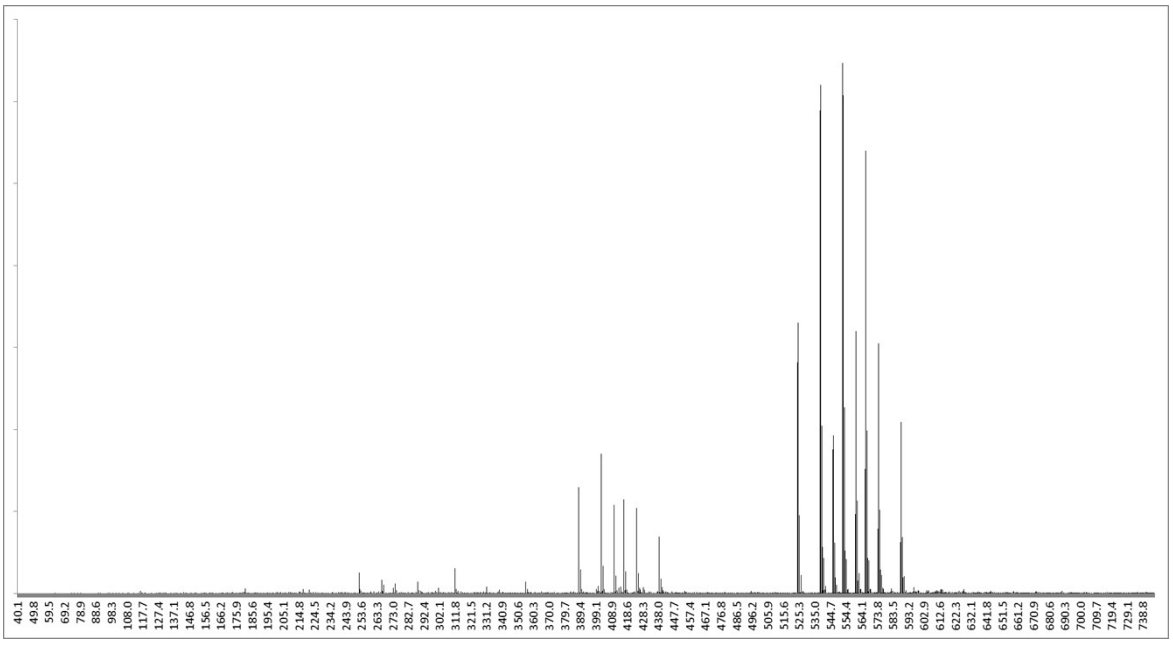

Fig. S27 Mass spectrum of Diii. Reaction Conditions: **1** (1 eq.) and **2** (1 eq.) with $NaBH_4$ (2 eq.) in methanol/chloroform (1/1), rt, 24h.

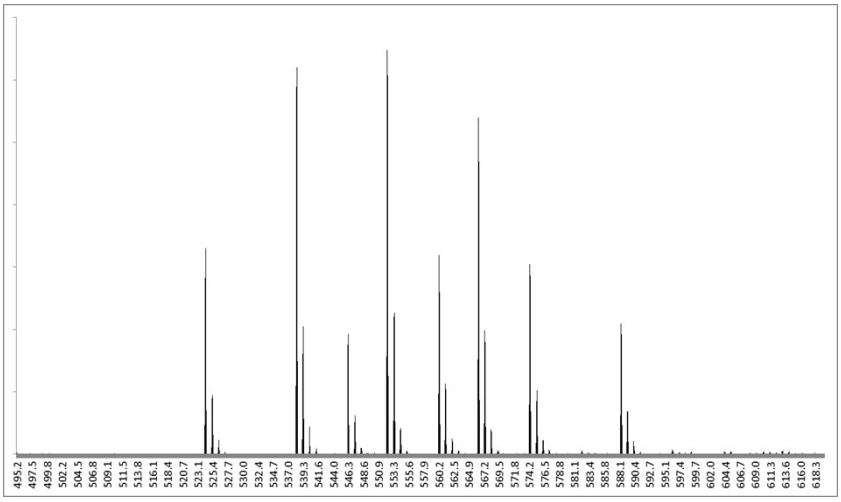

Fig. S28 Signal set for trialkylated DAZA (**IV**) in mass spectrum of Diii: *m/z* 524.2 ($[M + H]^+$, three R = Me, **TMeOHB-DAZA),** 538.2, ($[M + H]^+$, two R = Me, one R = Et), 546.2 ($[M + Na]^+$, three R = Me, **TMeOHB-DAZA),** 552.3 ($[M + H]^+$, one R = Me, two R = Et), 560.2 ($[M + Na]^+$, two R = Me, one R = Et), 566.3 ($[M + H]^+$, three R = Et, **TEtOHB-DAZA**), 574.2 ($[M + Na]^+$, one R = Me, two R=Et), 588.3 ($[M + Na]^+$, three R = Et, **TEtOHB-DAZA**).

Radiolabelling and stability determination of [^{68}Ga]Ga-species

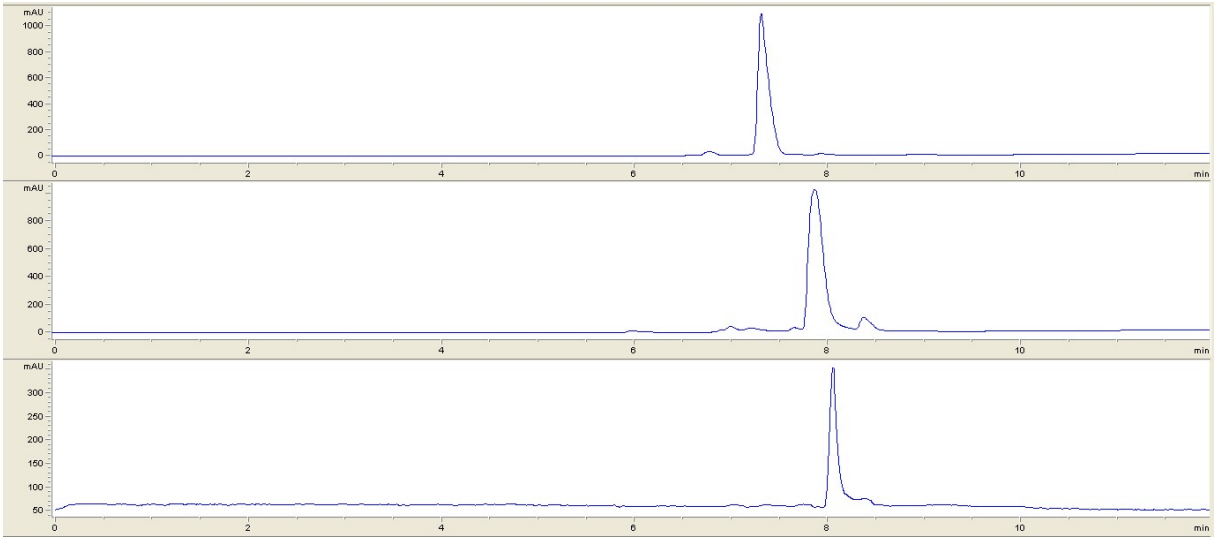

Fig. S29 HPLC chromatograms of ligand TMeOHB-DAZA (top, UV-vis channel 254 nm), cold Ga-TMeOHB-DAZA (middle, UV-vis channel 254 nm) and [^{68}Ga]Ga-TMeOHB-DAZA (bottom, radioactive channel). The slight shift between the retention time of Ga-TMeOHB-DAZA and [^{68}Ga]Ga-TMeOHB-DAZA is due to the UV-vis detector and the radioactive detector being connected in series.

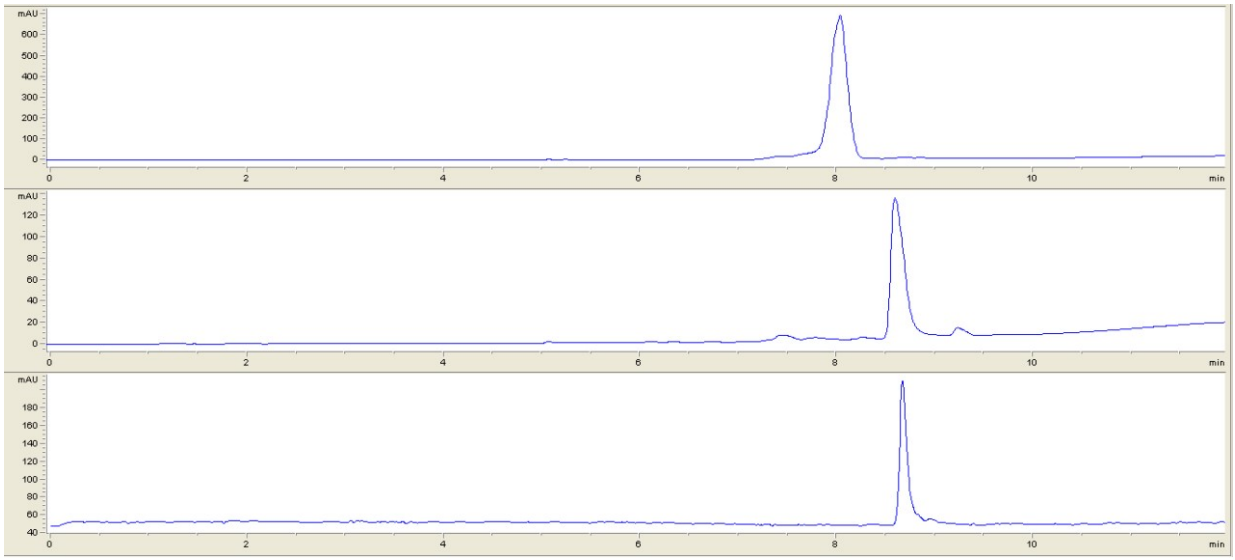

Fig. S30 HPLC chromatograms of ligand TEtOHB-DAZA (top, UV-vis channel 254 nm), cold Ga-TEtOHB-DAZA (middle, UV-vis channel 254 nm) and [^{68}Ga]Ga-TEtOHB-DAZA (bottom, radioactive channel). The slight shift between the retention time of Ga-TEtOHB-DAZA and [^{68}Ga]Ga-TEtOHB-DAZA is due to the UV-vis detector and the radioactive detector being connected in series.

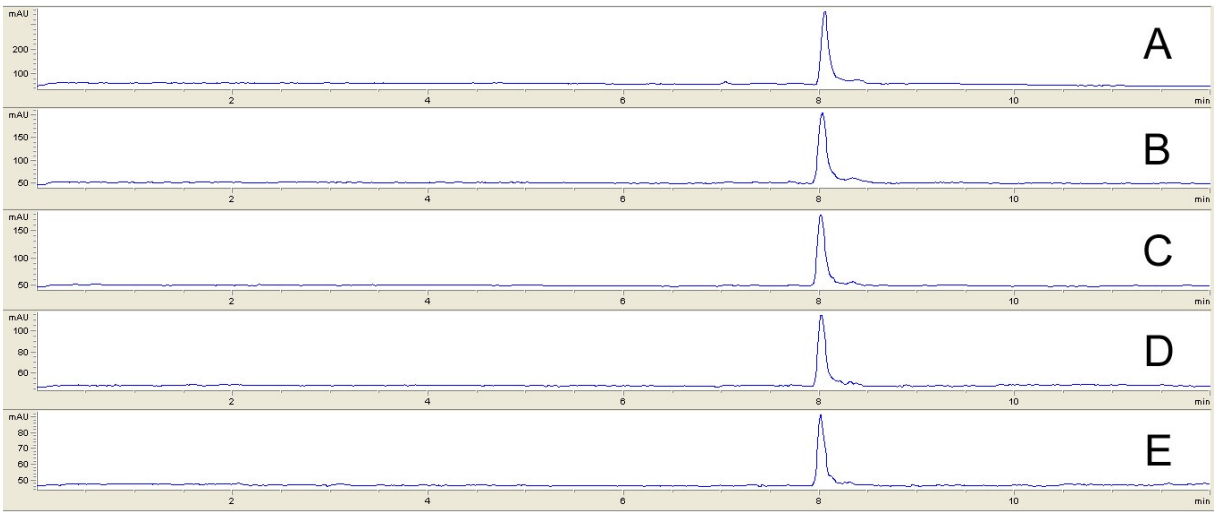

Fig. S31 Radio HPLC chromatograms of aliquots of [^{68}Ga]Ga-TMeOHB-DAZA in PBS buffered human serum (pH 7.4) incubated at 37°C. Samples were taken before incubating (A) and following incubation for 1h (B), 2h (C), 3h (D) and 4h (E), respectively. Prior to HPLC analysis aliquots were passed through a C18 cartridge to remove colloids.

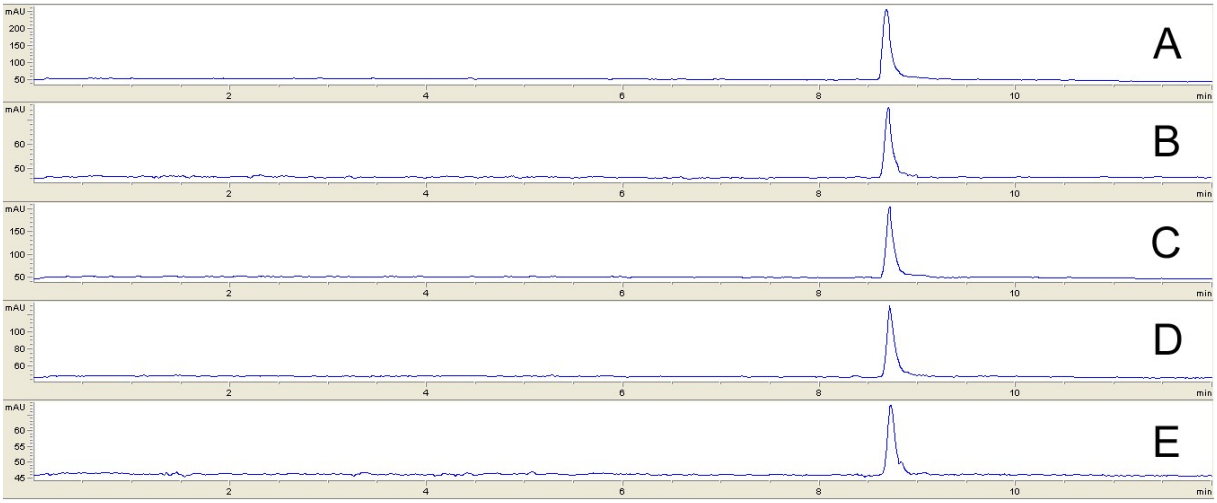

Fig. S32 Radio HPLC chromatograms of aliquots of [^{68}Ga]Ga-TEtOHB-DAZA in PBS buffered human serum (pH 7.4) incubated at 37°C. Samples were taken before incubating (A) and following incubation for 1h (B), 2h (C), 3h (D) and 4h (E), respectively. Prior to HPLC analysis aliquots were passed through a C18 cartridge to remove colloids.

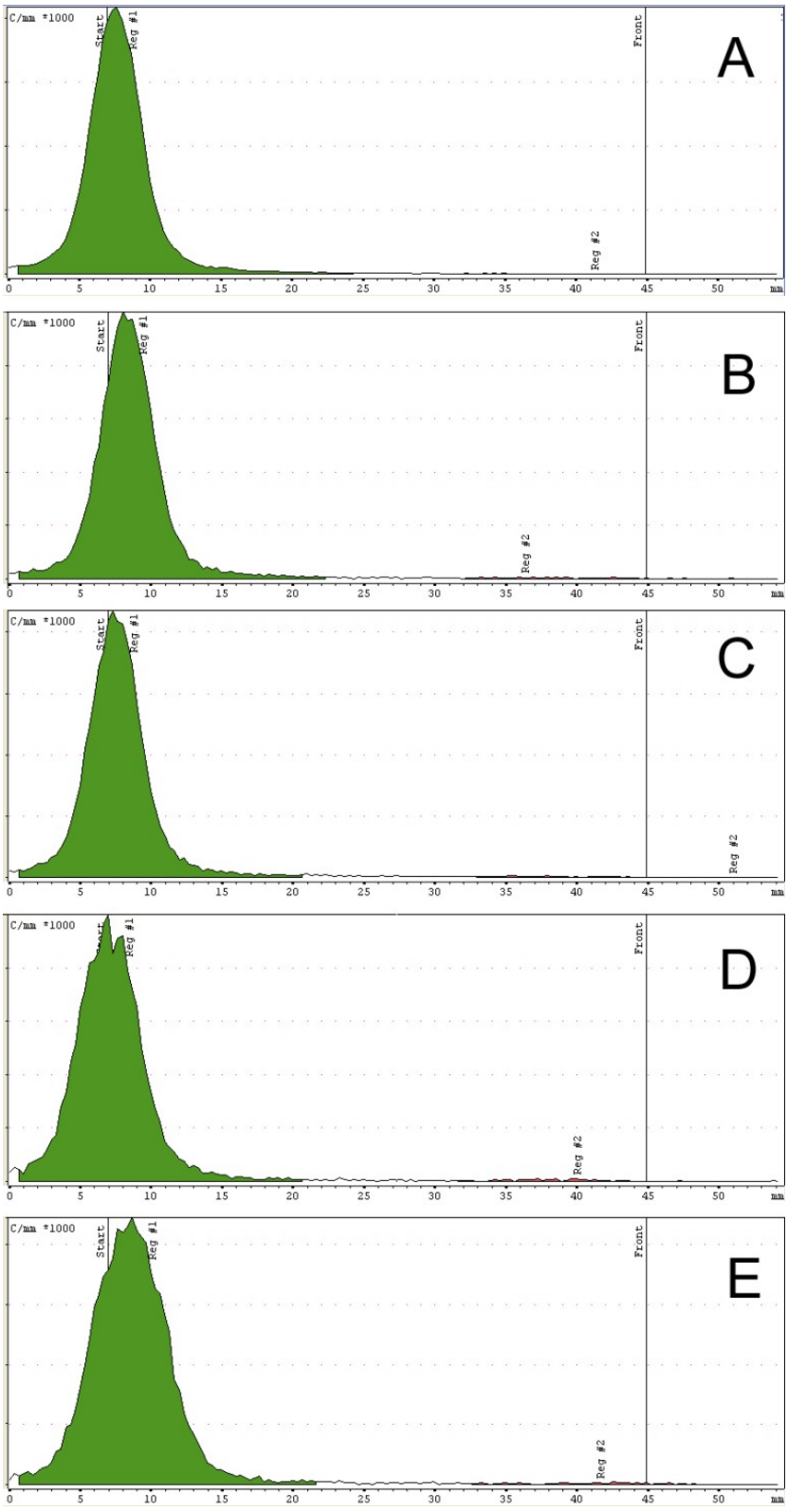

Fig. S33 Radio TLC chromatograms (silica coated alumina plates, 0.1 M sodium citrate) of samples of [^{68}Ga]Ga-TMeOHB-DAZA in PBS buffered human serum (pH 7.4) incubated at 37°C. Aliquots were taken before incubating (A) and following incubation after 1h (B), 2h (C), 3h (D) and 4h (E).

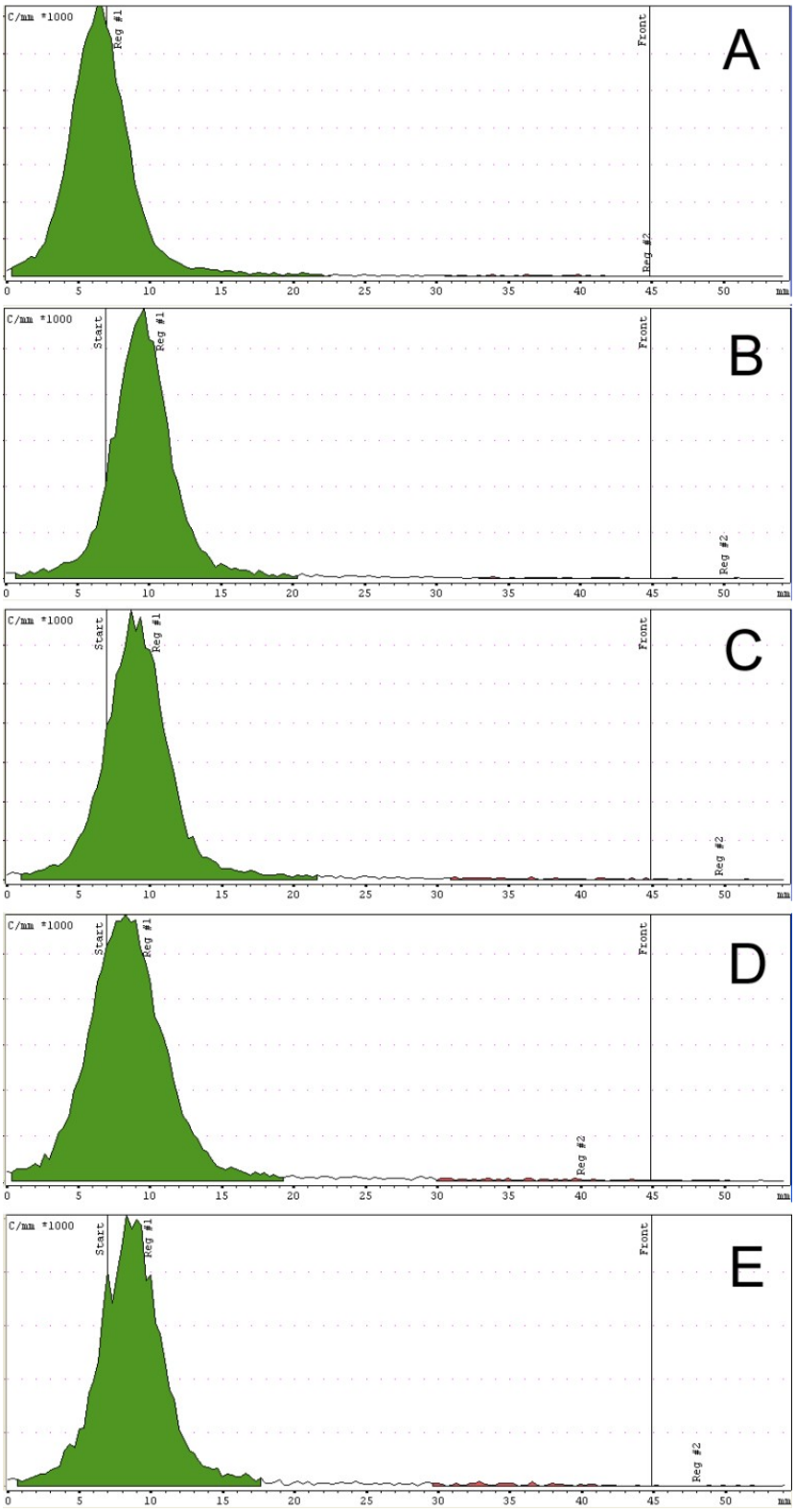

Fig. S34 Radio TLC chromatograms (silica coated alumina plates, 0.1 M sodium citrate) of samples of [^{68}Ga]Ga-TEtOHB-DAZA in PBS buffered human serum (pH 7.4) incubated at 37°C. Aliquots were taken before incubating (A) and following incubation after 1h (B), 2h (C), 3h (D) and 4h (E).

Table S1 Crystallographic data and refinement details for X-ray structure determination of **1,** TMeOHB-DAZA, and TEtOHB-DAZA

Compound	**1**	TMeOHB-DAZA	TEtOHB-DAZA
Formula	$C_{21}H_{25}N_3O_4$	$C_{29}H_{37}N_3O_6$	$C_{32}H_{43}N_3O_6$
Fw (g mol^{-1})	383.44	523.62	565.69
T (°C)	−140(2)	−140(2)	−140(2)
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/n$	$P2_1/c$	$P\bar{1}$
a (Å)	11.6451(6)	14.0088(5)	7.2228(2)
b (Å)	15.7066(7)	5.7639(2)	11.9597(3)
c (Å)	11.7436(6)	33.2059(14)	17.8857(4)
α (°)	90	90	77.175(2)
β (°)	117.744(2)	98.854(2)	87.679(2)
γ (°)	90	90	79.603(1)
V (Å^3)	1901.02(16)	2649.27(17)	1481.73(6)
Z	4	4	2
ρ (g cm^{-3})	1.340	1.313	1.268
μ (cm^{-1})	0.94	0.92	0.88
Measured data	12 803	24 731	9072
Data with $I > 2\sigma(I)$	2805	4444	5403
Unique data (R_{int})	4248/0.1051	6023/0.0509	6573/0.0308
wR_2 (all data, on F^2)[a]	0.1607	0.1836	0.1338
R_1 ($I > 2\sigma(I)$)[a]	0.0846	0.0805	0.0660
S[b]	1.186	1.056	1.130
Res. dens. (e Å^{-3})	0.333/−0.259	0.732/−0.373	0.303/−0.251
Absorption method	Multiscan	Multiscan	Multiscan
Absorpt. corr. $T_{min}/_{max}$	0.6223/0.7456	0.6511/0.7456	0.4405/0.7456
CCDC No.	1820196	1820197	1820198

[a]Definition of *R* indices: $R_1 = (\Sigma \,||F_o| - |F_c||)/\Sigma|F_o|$; $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$ with $w^{-1} = \sigma^2(F_o^2) + (aP)^2 + bP$; $P = [2F_c^2 + \mathrm{Max}(F_o^2]/3$.
[b]$S = \{\Sigma[w(F_o^2 - F_c^2)^2]/(N_o - N_p)\}^{1/2}$.