Electronic Supplementary Material (ESI) for Chemical Communications.

Trastuzumab gold-conjugates: synthetic approach and *in vitro* evaluation of anticancer activities in breast cancer cell lines.

Natalia Curado,[a] Guillaume Dewaele-Le Roi,[a,b] Sophie Poty,[d] Jason S. Lewis,[d-f,*] Maria Contel.[a-c,*]

[a]Department of Chemistry, Brooklyn College, The City University of New York, Brooklyn, NY, 11210, USA. [b]Chemistry, [c]Biochemistry and [d]Biology PhD Programs, The Graduate Center, The City University of New York, 365 Fifth Avenue, New York, NY, 10016, USA. [e]Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [f]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [g]Departments of Radiology and Pharmacology, Weill Cornell Medical College, New York, NY, USA. [h]Radiochemistry and Molecular Imaging Probes Core, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

Electronic Supporting Information

1. Experimental section

1.1 General information and instrumentation for characterization and stability studies of the new compounds.

NMR spectra were recorded in a Bruker AV400 (^{1}H-NMR at 400 MHz, ^{13}C{^{1}H} NMR at 100.6 MHz and ^{31}P{^{1}H} NMR at 161.9 MHz). Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz (Hz), using $CDCl_3$, d^6-DMSO or PBS-D_2O as solvent, unless otherwise stated. ^{1}H and ^{13}C NMR resonances were measured relative to solvent peaks considering tetramethylsilane = 0 ppm, and ^{31}P{^{1}H} NMR was externally referenced to H_3PO_4 (85%). IR spectra (4000-500 cm^{-1}) were recorded on a Nicolet 6700 Fourier transform infrared spectrophotometer on solid state (ATR accessory). Elemental analyses were performed on a Perkin-Elmer 2400 CHNS/O series II analyzer by Atlantic Microlab Inc. (US). Mass spectra electrospray ionization high resolution (MS-ESI-HR) were performed on a Waters Q-Tof Ultima. The theoretical isotopic distributions have been calculated using enviPat Web 2.0. Abbreviations were used along the Supporting Information for Trastuzumab (Tz) and Auranofin (AF).

1.2 Synthesis

[AuCl(tht)],[1] [$AuN_3(PPh_3)$],[2] [$AuCl(PPh_3)$],[3] [Au(mba)(PPh_3)],[4] were prepared as previously reported. Chemicals were purchased as indicated: H[$AuCl_4$] (STREM Chemicals), $Si(CH_3)_4$, PTAD-alkyne (**b**), PPh_3 and 4-mercaptobenzoic acid (Sigma Aldrich), Tl(acac), N-hydroxysuccinimide and N-N'-diisopropylcarbodiimide (Alfa Aesar) and 2,5-Dioxopyrrolidin-1-yl pent-4-ynoate (**a**), 1-(2-(2-(Prop-2-ynyloxy)ethoxy)ethyl)-1H-pyrrole-2,5-dione (**c**) and 1-(2-aminoethyl)-1H-pyrrole-2,5-dione 2,2,2-trifluoroacetate (**d**) (Abosyn Chemical Inc.). Trastuzumab (Tz) was obtained from Genentech in its usual clinical formulation (440 mg, lyophilized).
Reaction solvents were purchased anhydrous from Fisher Scientific (BDH, ACS Grade) and Sigma-Aldrich, used without further purification, and dried in a SPS machine and kept over molecular sieves (3 Å, beads, 4-8 mesh), otherwise over sodium if necessary. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. and were kept over molecular sieves (3 Å, beads, 4-8 mesh). Celite (Celite 545, Diatomaceous Earth) was purchased from VWR International and used as received.

Synthesis of compound 1 [Au(PPh_3)(a)]. [$AuN_3(PPh_3)$] (0.065 g, 0.13 mmol) and **a** (0.028 g, 0.14 mmol) were dissolved in dry toluene and stirred for 2 days at room temperature under nitrogen. The white precipitate was filtered, washed with cold dry toluene and *n*-pentane, and dried under vacuum to afford a white product (0.050 g, 0.07 mmol) in 55% yield. ^{1}H NMR (400 MHz, d^6-DMSO, 25ºC): δ(ppm) 14.06 (s, 1H, N*H*), 7.62-7.34 (m, 15H, H_{Ar}), 3.11 (bt, 4H, CH_2), 2.80 (bt, 2H, CH_2), 2.60 (bt, 2H, CH_2), 2.30 (bs, 2H, CH_2,). ^{31}P NMR (161 MHz, d^6-DMSO, 25ºC): δ(ppm) 43.24. ^{13}C{^{1}H}

NMR (128 MHz, d^6-DMSO, 25ºC): δ(ppm) 173.31 (*C*=O), 170.64 (2xC_{Suc}=O), 168.90 (N-C_{triaz}=C) 149.78 (C=C_{triaz}-NH), 134.38 ($C_{Ar\text{-}PPh_3}$), 132.42 ($C_{p\text{-}Ar\text{-}PPh_3}$), 130.04 ($C_{Ar\text{-}PPh_3}$), 125.87 ($C_{ipso\text{-}Ar\text{-}PPh_3}$), 32.13 ($CH_2$), 25.91 ($CH_2$), 25.69 (2x$CH_2$). MS (ESI): *m/z* Calcd 696.12. Found: 697.13 $[M+H]^+$. Anal. Calcd. for $C_{27}H_{24}AuN_4O_4P{\cdot}H_2O$: C, 45.45; H, 3.53; N, 7.85. Found: C, 45.61; H, 3.63; N, 7.63.

Synthesis of compound 2 [Au(PPh$_3$)(b)]. Under inert atmosphere, crystalline [$AuN_3(PPh_3)$] (0.065 g, 0.13 mmol) and PTAD-alkyne ligand **b** (0.042 g, 0.18 mmol) were stirred in dry tetrahydrofuran (5 mL) at 45°C for 4 days. The white solid obtained was filtered, washed with dry diethyl ether and *n*-hexane, and dried under vacuum to afford the desired product (0.065 g, 0.089 mmol) as a white solid in 68% yield. ^{1}H NMR (400 MHz, d^6-DMSO, 25ºC): δ(ppm) 14.26 (s, 1H, N-H_{Tr}), 10.35 (bs, 2H, NH_{PTAD}), 7.57-7.54 (m, 15H, H_{Ar}), 7.24 (d, 2H, $H_{Ar\text{-}PTAD}$), 7.13 (d, 2H, $H_{Ar\text{-}PTAD}$) 5.23 (s, 2H, CH_2). ^{31}P{^{1}H} NMR (161 MHz, d^6-DMSO, 25ºC): δ(ppm) 43.57. ^{13}C{^{1}H} NMR (100 MHz, d^6-DMSO, 25ºC): δ(ppm) 157.86 ($C_{ipso\text{-}PTAD}$), 153.81 (*C*=O), 153.67 ($C_{ipso\text{-}triazole}$) 147.58 ($C_{ipso\text{-}triazole}$), 133.91 ($C_{Ar\text{-}PPh_3}$), 131.96 ($p\text{-}C_{Ar\text{-}PPh_3}$), 129.62 ($C_{Ar\text{-}PPh_3}$), 129.10 ($C_{ipso\text{-}Ar\text{-}PPh_3}$), 127.60 ($C_{Ar\text{-}PTAD}$), 124.23 ($C_{ipso\text{-}PTAD}$), 115.03 ($C_{Ar\text{-}PTAD}$), 63.84 ($CH_2$). MS (ESI): *m/z* Calcd. 732.14. Found: 733.14 $[M+H]^+$. Anal. Calcd. for $C_{29}H_{25}AuN_6O_3P{\cdot}H_2O$: C, 46.41; H, 3.49; N, 11.20. Found: C, 46.36; H, 3.49; N, 10.81.

Synthesis of compound 3 [Au(PPh$_3$)(c)]. [$AuN_3(PPh_3)$] (0.090 g, 0.18 mmol) and PEG linker **c** (0.046 g, 0.20 mmol) were suspended in dry toluene and stirred for 2 days at room temperature under nitrogen. The white precipitate was filtered, washed with cold dry toluene and diethyl ether, and dried *in vacuo* to afford a pale salmon solid (0.070 g, 0.1 mmol) in 54% yield. ^{1}H NMR (400 MHz, d^6-DMSO, 25ºC): δ(ppm) 14.15 (s, 1H, N*H*), 7.62-7.40 (m, 15H, H_{Ar}), 5.44 (d, 2H, $^3J_{HH}$ = 10.8, C*H*), 4.56 (t, 4H, $^3J_{HH}$ = 9.2, CH_2), 3.57-3.38 (m, 6H, CH_2). ^{31}P{^{1}H} NMR (161 MHz, d^6-DMSO, 25ºC): δ(ppm) 43.36. Anal. Calcd. for $C_{29}H_{28}AuN_4O_4P$: C, 48.08; H, 3.90; N, 7.73. Found: C, 47.66; H, 4.05; N, 7.61.

Synthesis of compound 4a [Au(PPh$_3$)(NHS)]: Compound [Au(mba)(PPh_3)] (0.3 g, 0.49 mmol) was dissolved in dichloromethane (9 mL) under nitrogen. N-hydroxysuccinimide (0.056 g, 0.49 mmol) and N-N'-diisopropylcarbodiimide (77 μL, 0.49 mmol) were added to the previous solution and the resulting suspension was stirred for 4 hours at room temperature. The desired compound was extracted from the crude with water (5x30 mL) and dried over $MgSO_4$. The solution was dried under vacuum and a light yellow solid was recovered in 80% yield (0.278 g). ^{1}H NMR (400 MHz, $CDCl_3$, 25ºC): δ(ppm) 7.81 (d, 2H, $^3J_{HH}$ = 7.81, H_{mba}) 7.69 (d, 2H, $^3J_{HH}$ = 7.69, H_{mba}), 7.48-7.58 (m, 15H, H_{Ar}), 2.88 (bs, 4H, C$H_{2\text{-}Suc}$). ^{31}P{^{1}H} NMR (161 MHz, $CDCl_3$, 25ºC): δ(ppm) 38.76.

Synthesis of compound 4 [Au(PPh$_3$)(d)]: The active ester **4a** (0.25 g, 0.35 mmol) was dissolved in a mixture of acetonitrile/dichloromethane (2:1), and **d** (0.2 g, 0.77 mmol) was added to the solution. Then, Et_3N (245 μL, 5 eq.) was added dropwise to the previous solution. The suspension was stirred at r.t. for 5 hours, and dried under vacuum afterwards. The crude was dissolved in dichloromethane, and the organic phase collected after extraction with brine solution (4x20mL). The solution was dried over $MgSO_4$ and the volume of solvent reduced *in vacuo* to the minimum amount. Column chromatography on silica gel (ethyl acetate) followed by precipitation with diethyl

ether/hexane (5:1) yielded the final compound as a pale yellow solid (29% yield). ^{1}H NMR (400MHz, $CDCl_3$, 25ºC): δ(ppm) 7.66 (d, 2H, $^{3}J_{HH}$ = 7.66, H_{mba}),7.64-7.50 (m, 17H, H_{mba} and H_{ar}), 6.74 (bd, 2H, C*H*-C*H* maleimide), 6.52 (t, 1H, $^{3}J_{HH}$ = 4.93, N*H*(CO)), 3.85-3.82 (m, 2H, CH_2), 3.67 (dt, 2H, $^{1}J_{HH}$ = 3.40, $^{3}J_{HH}$ = 8.57, CH_2) ppm. ^{31}P{^{1}H} NMR (400MHz, $CDCl_3$, 25ºC) δ(ppm) 38.23 ppm. ^{13}C{^{1}H}NMR (100MHz, $CDCl_3$, 25ºC) δ(ppm) 171.32 (C=O maleimide), 167.66 (C=O amide), 148.27 (4-mba), 134.48 ($C_{Ar\text{-}PPh_3}$), 134.3 (*C*H maleimide), 132.59 (1-mba), 132.4 (mba), 131.99 (mba), 129.57 ($C_{Ar\text{-}PPh_3}$), 129.42 ($C_{Ar\text{-}PPh_3}$), 126.8 ($C_{Ar\text{-}PPh_3}$), 39.68 (*C*H_2 close to the amide), 37.65 (*C*H_2 close to maleimide). IR (ATR): υ = 3328 (w, amine N-H stretch), 1703 (s, amide C=O) cm^{-1}. MS (ESI): *m/z* Calcd 734.11. Found: 750.31 $[C_{30}H_{28}AuN_2O_2PS{\cdot}CH_3CN]^{+}$. Anal. Calcd. for $C_{30}H_{26}AuN_2O_3PS$: C, 50.69; H, 3.57; N, 3.81; S, 4.36. Found: C, 50.36; H, 3.67; N, 3.46: S: 4.27.

Synthesis of Gold Antibody Drug Conjugates.

Synthesis of Tras-1: The pH of a solution of Tz in PBS buffer (95 μM, ~210 μL) was adjusted to 9 (with a basic solution of Na_2CO_3 in distilled water). A solution of **1** in dry dimethylformamide (*ca.* 19 mM) was prepared and added in aliquots of 2 μL to avoid the precipitation of the compound in solution achieving a final concentration of the solvent in solution below 10%. The reaction was incubated at 37°C for 1.5 h under mild agitation, and purified by size exclusion using PD-10 Columns (GE Healthcare).

Synthesis of Tras-4: To a Tz solution (22.9 μM, ~300 μL) in borate buffer (25 mM NaCl, 1 mM EDTA, 25 mM sodium borate, pH 8.0) were added 10 equiv. of a stock solution of compound **4** in dry DMSO in aliquots of 2 μL to avoid high concentration of DMSO in solution. Next, 5 equiv. of freshly prepared TCEP in PBS (22.9 mM) were added and the reaction incubated at 37°C for 2 h under mild agitation. Purification of the product was achieved by exclusion using PD-10 Columns (GE Healthcare).

2. NMR spectra of compounds 1-4.

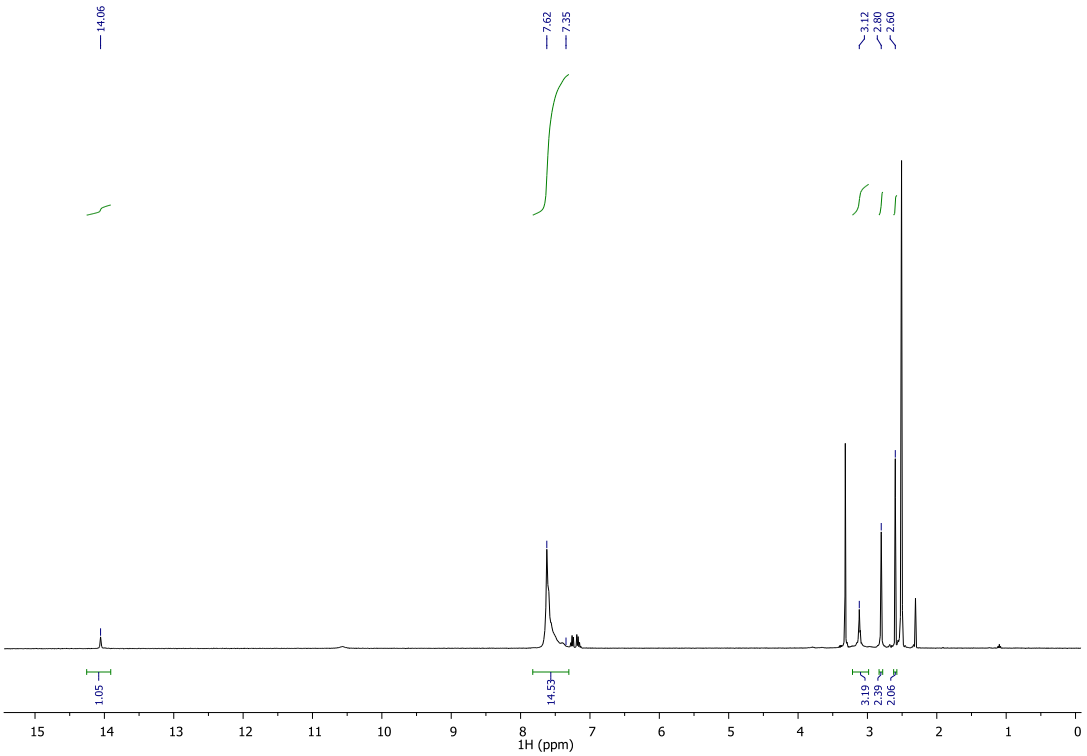
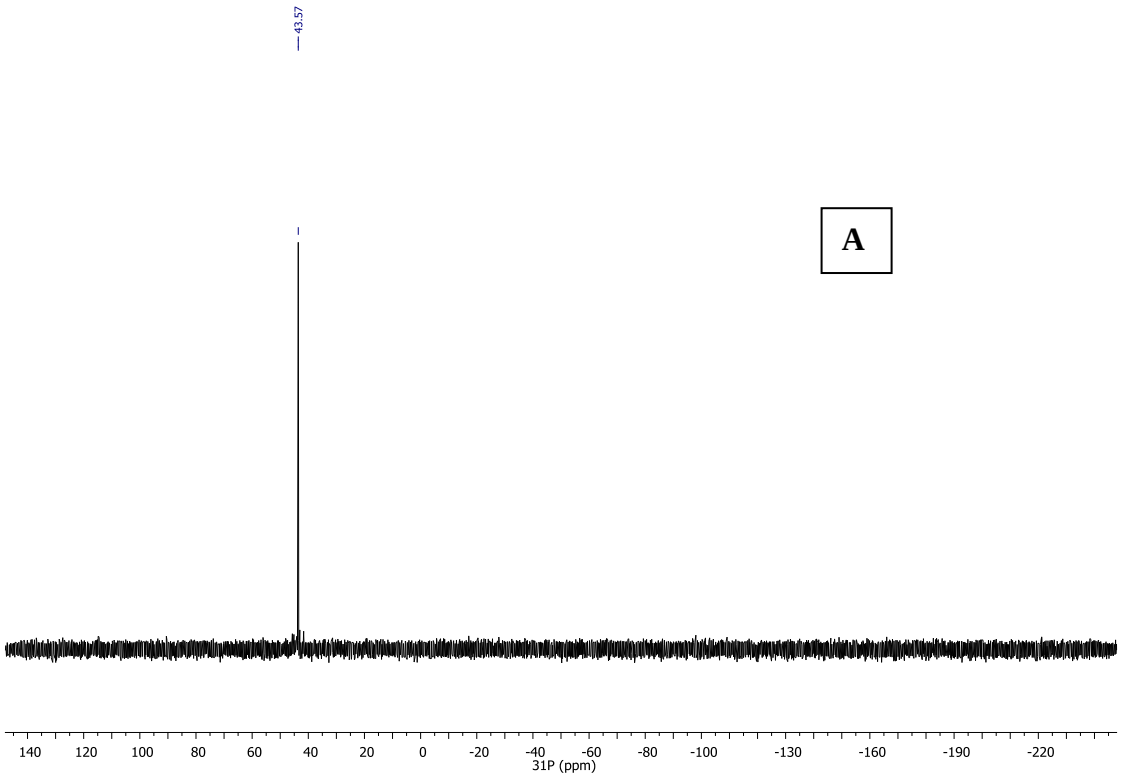


Figure S1. ^{1}H NMR spectrum of compound **1** in d^{6}-DMSO.



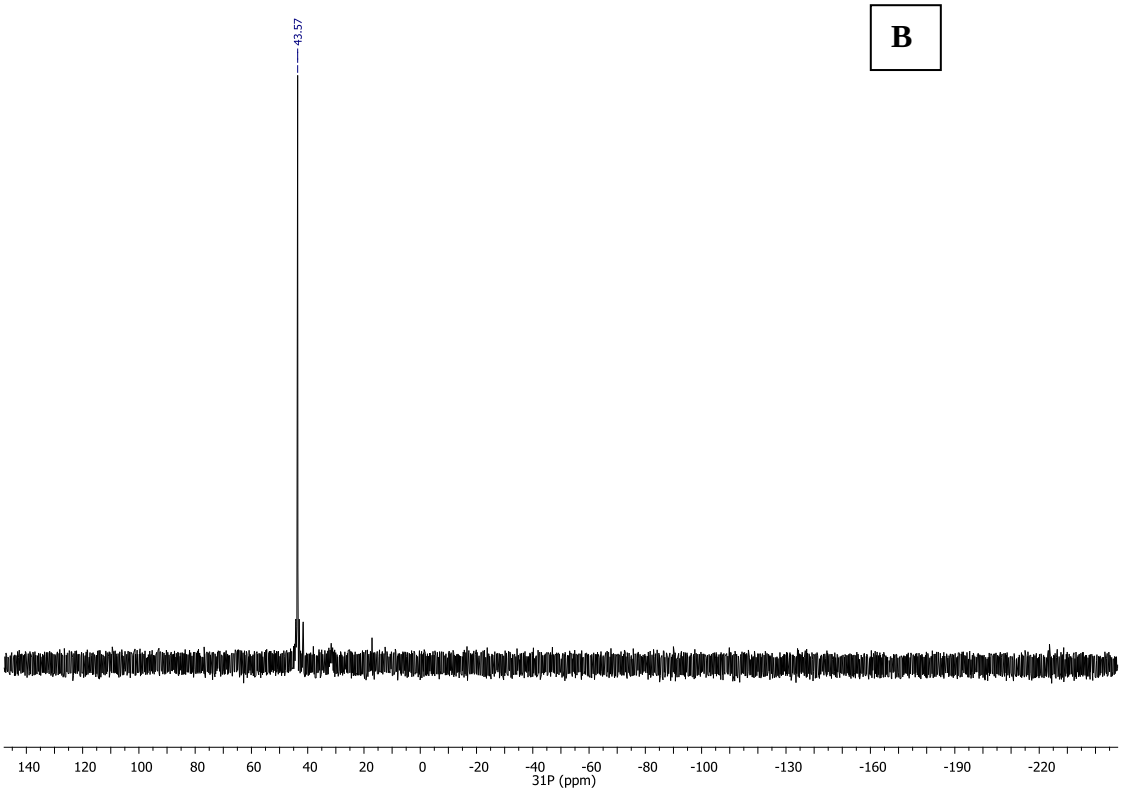


Figure S2. $^{31}P\{^{1}H\}$ NMR spectrum of compound **1** in d^{6}-DMSO. Figure A (t = 15 min). Figure B (t=30 min).

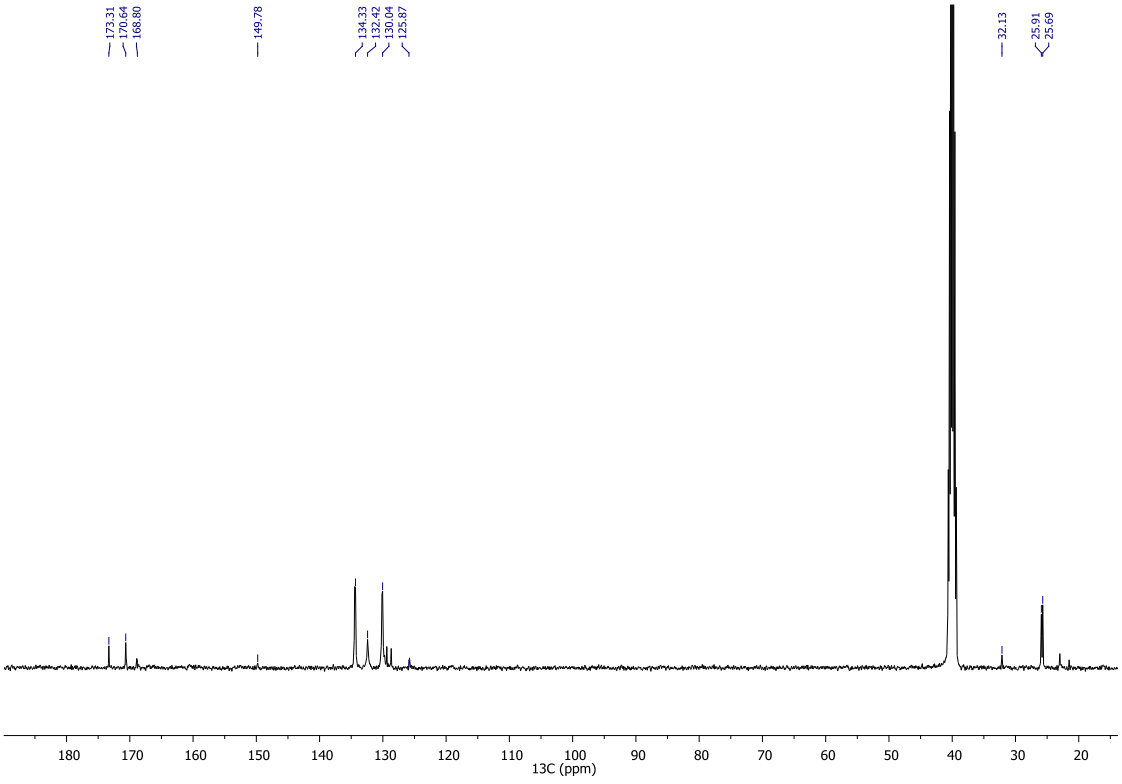


Figure S3. $^{13}C\{^{1}H\}$ NMR spectrum of compound **1** in d^{6}-DMSO.

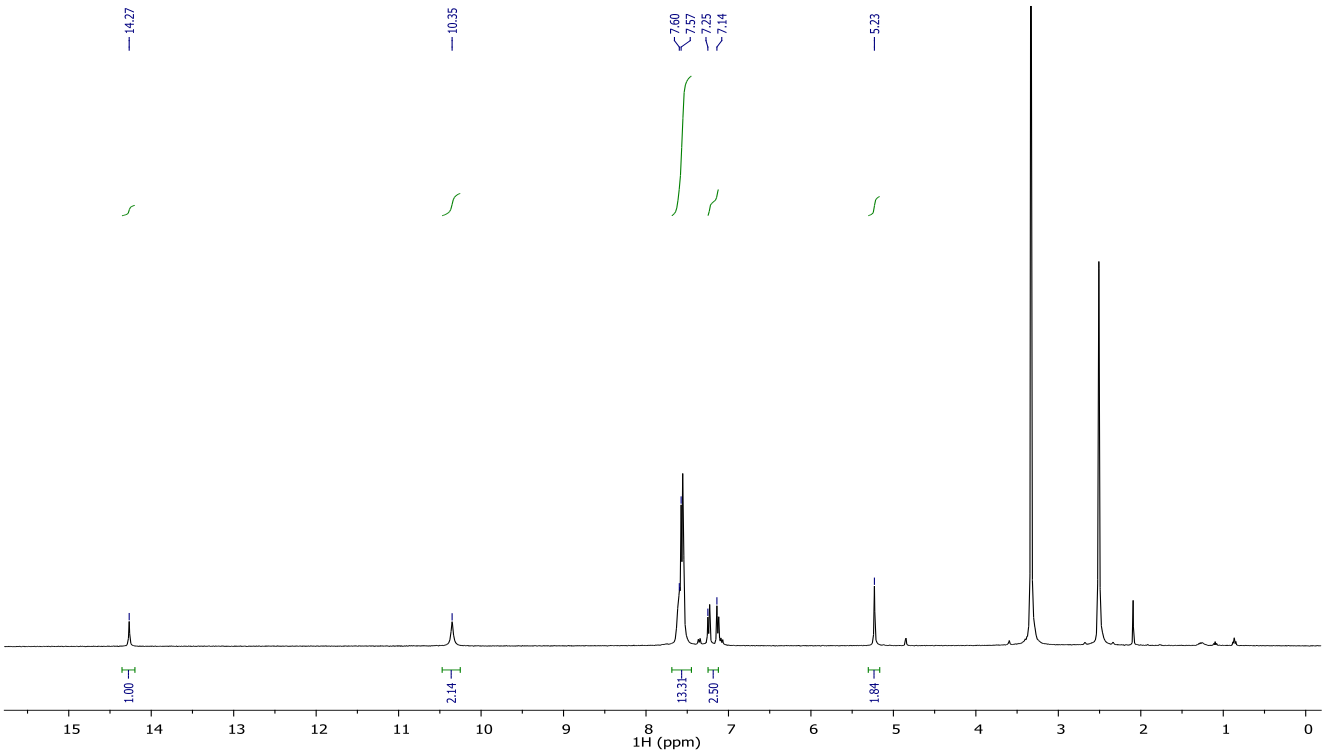


Figure S4. ^{1}H NMR spectrum of compound **2** in d^{6}-DMSO.

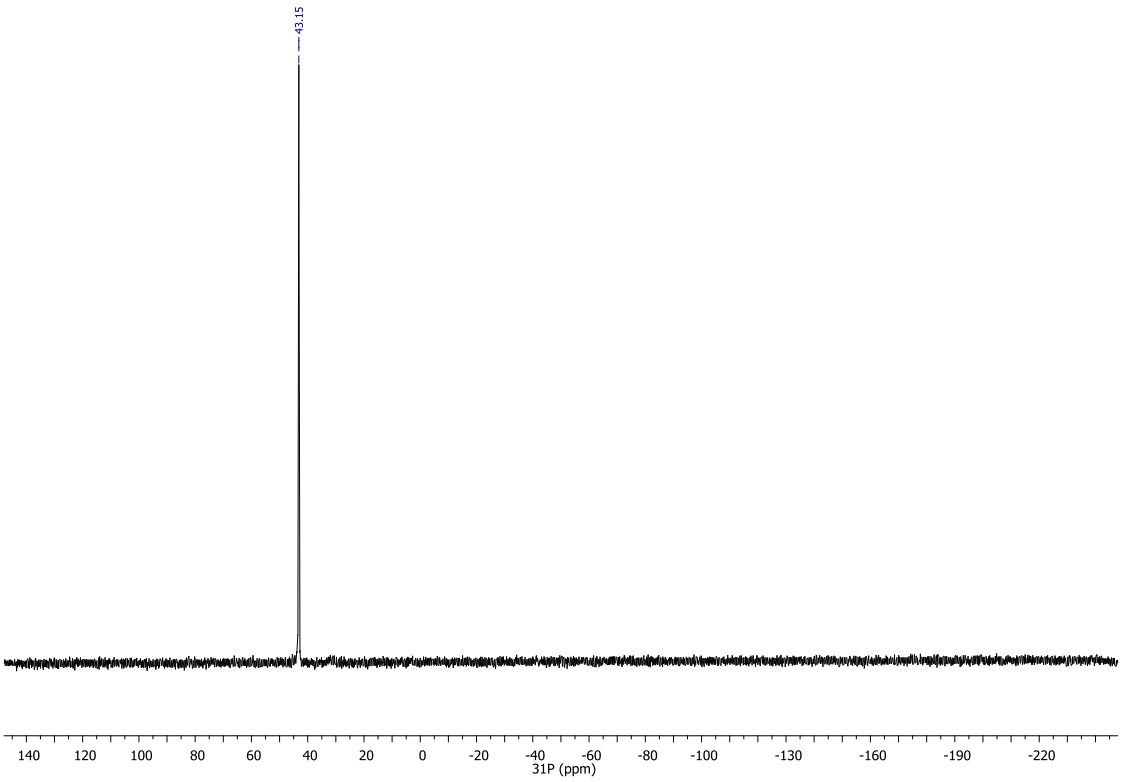


Figure S5. ^{31}P{^{1}H} NMR spectrum of compound **2** in d^{6}-DMSO.

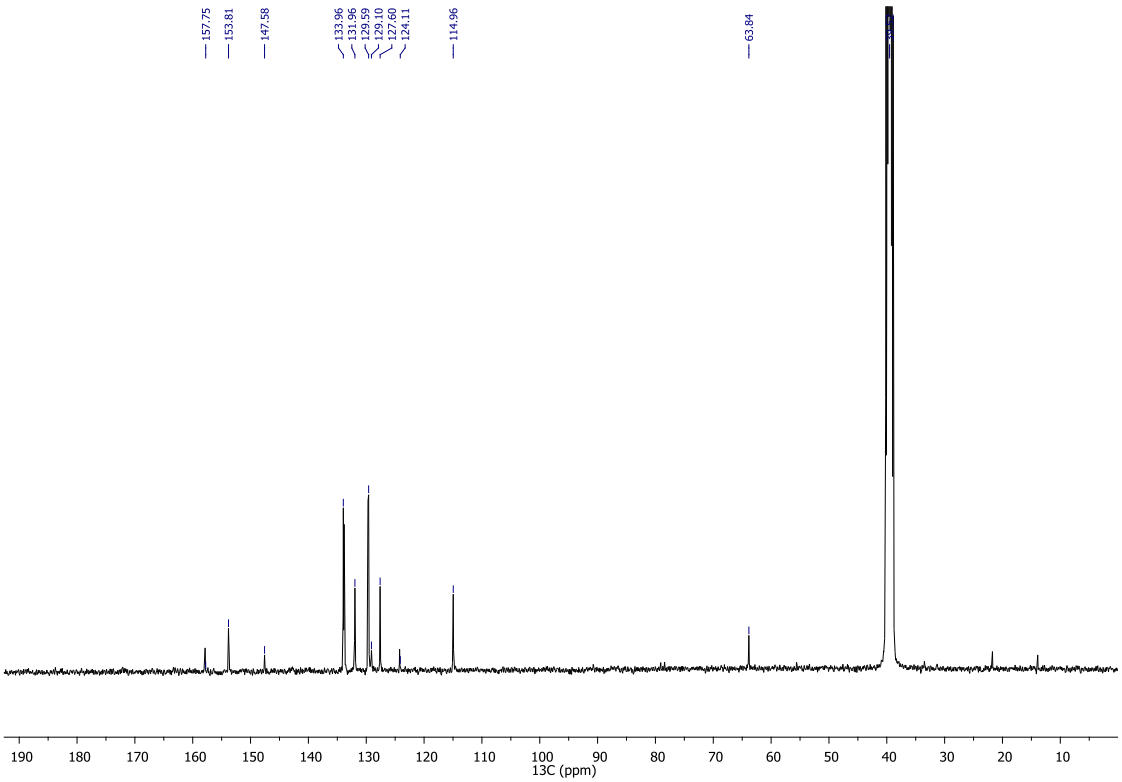


Figure S6. $^{13}C\{^{1}H\}$ NMR spectrum of compound **2** in d^{6}-DMSO.

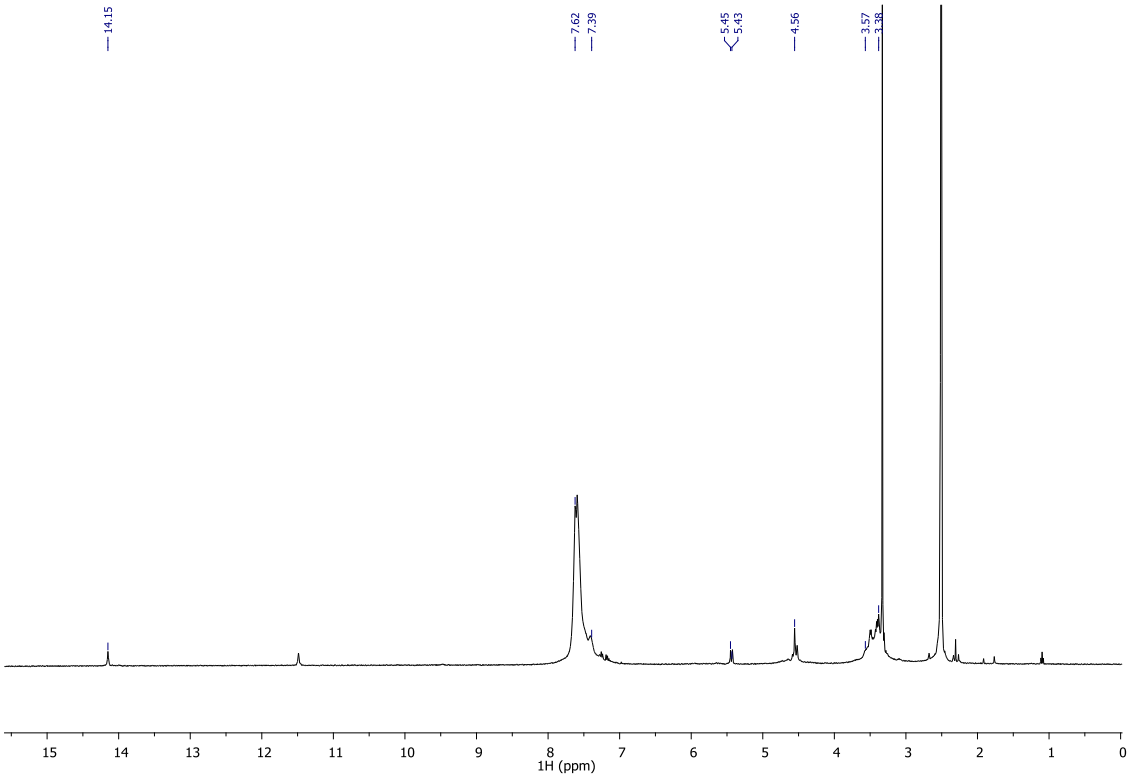


Figure S7. ^{1}H NMR spectrum of compound **3** in d^{6}-DMSO. Solutions of compound **3** in d^{6}-DMSO rapidly turned orange and unknown peaks appear.

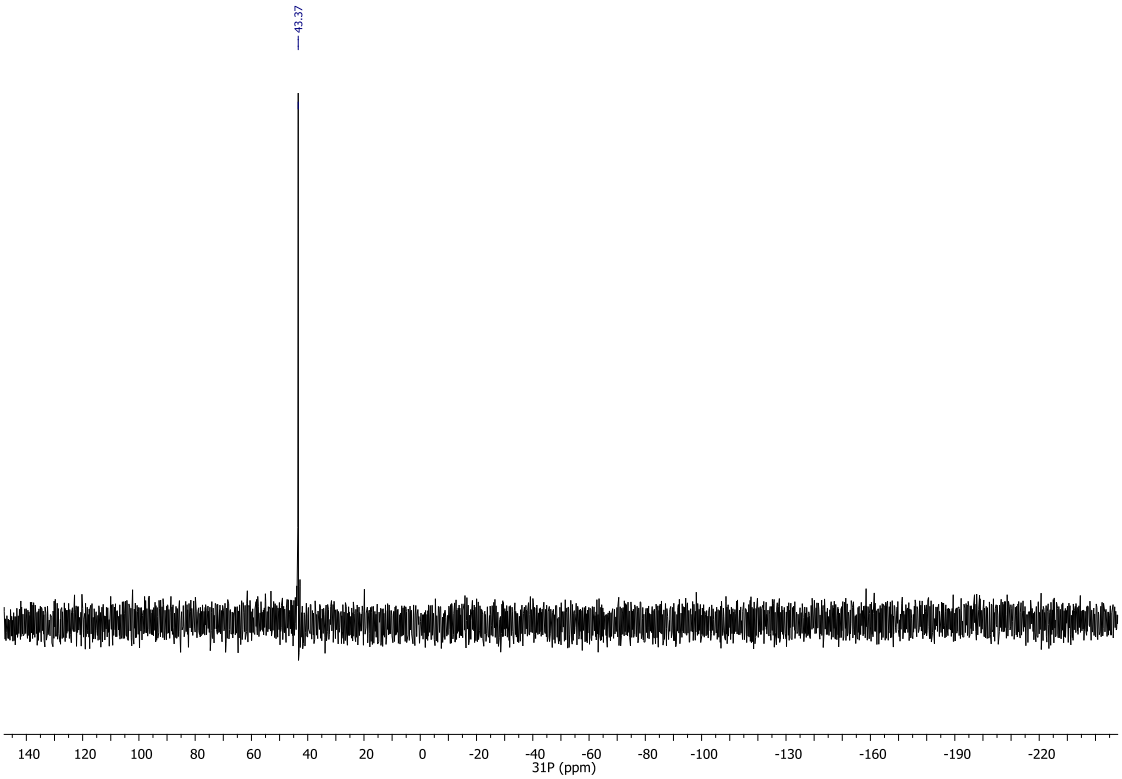


Figure S8. $^{31}P\{^{1}H\}$ NMR spectrum of compound **3** in d^{6}-DMSO. Solutions of compound **3** in d^{6}-DMSO rapidly turned orange and unknown peaks appear.

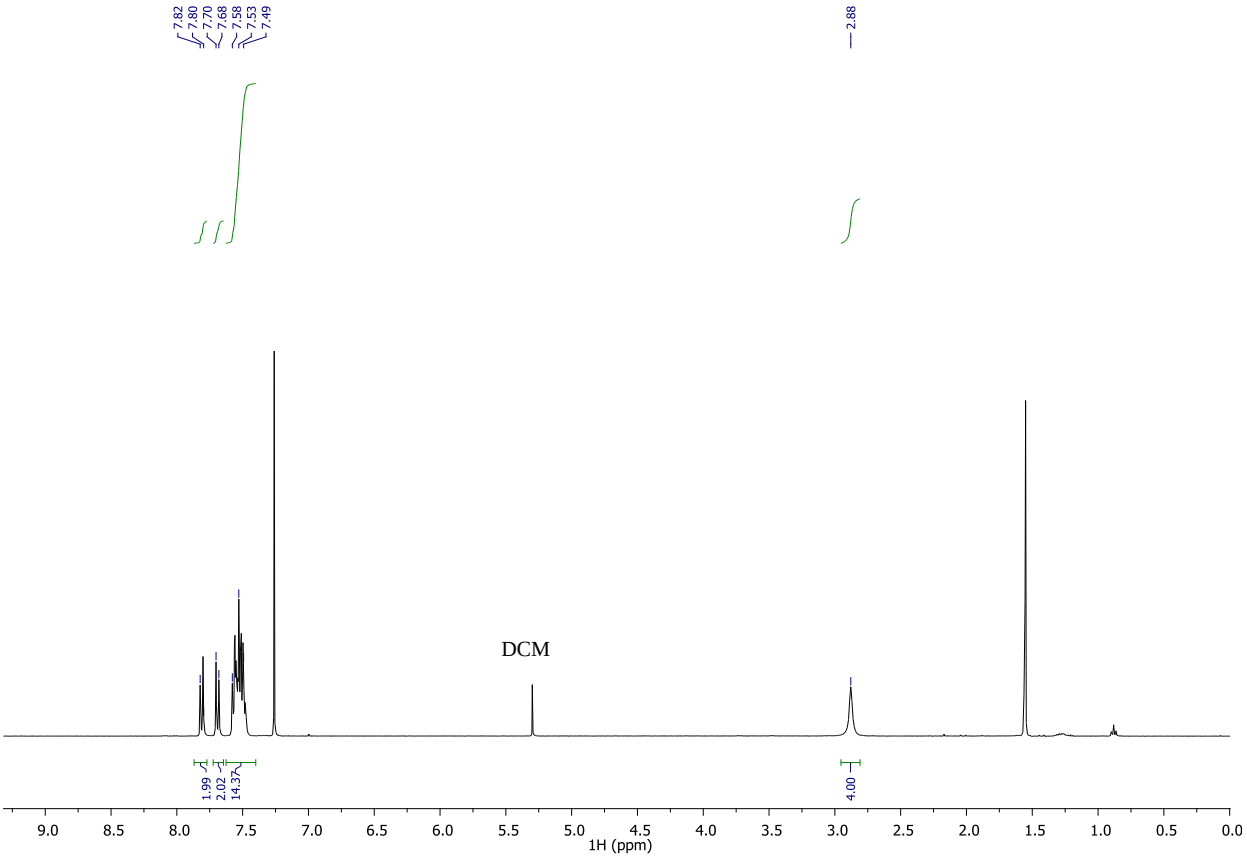


Figure S9. ^{1}H NMR spectrum of compound **4a** in $CDCl_3$.

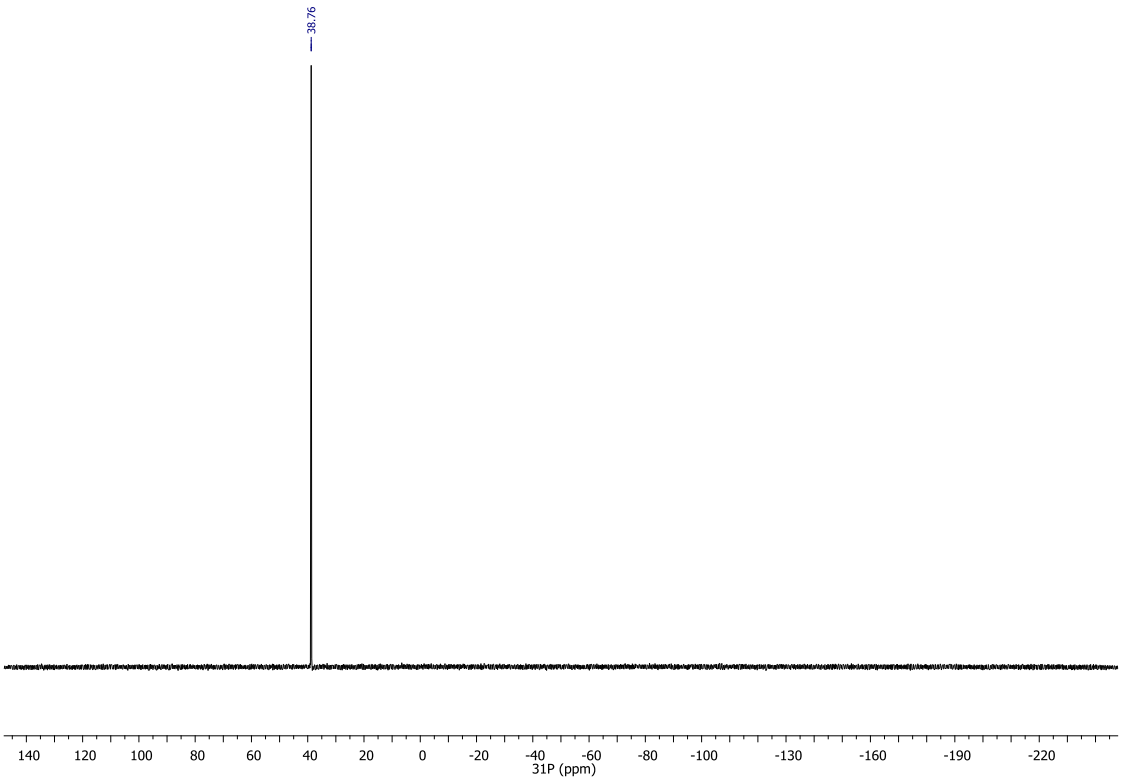


Figure S10. $^{31}P\{^{1}H\}$ NMR spectrum of compound **4a** in $CDCl_3$.

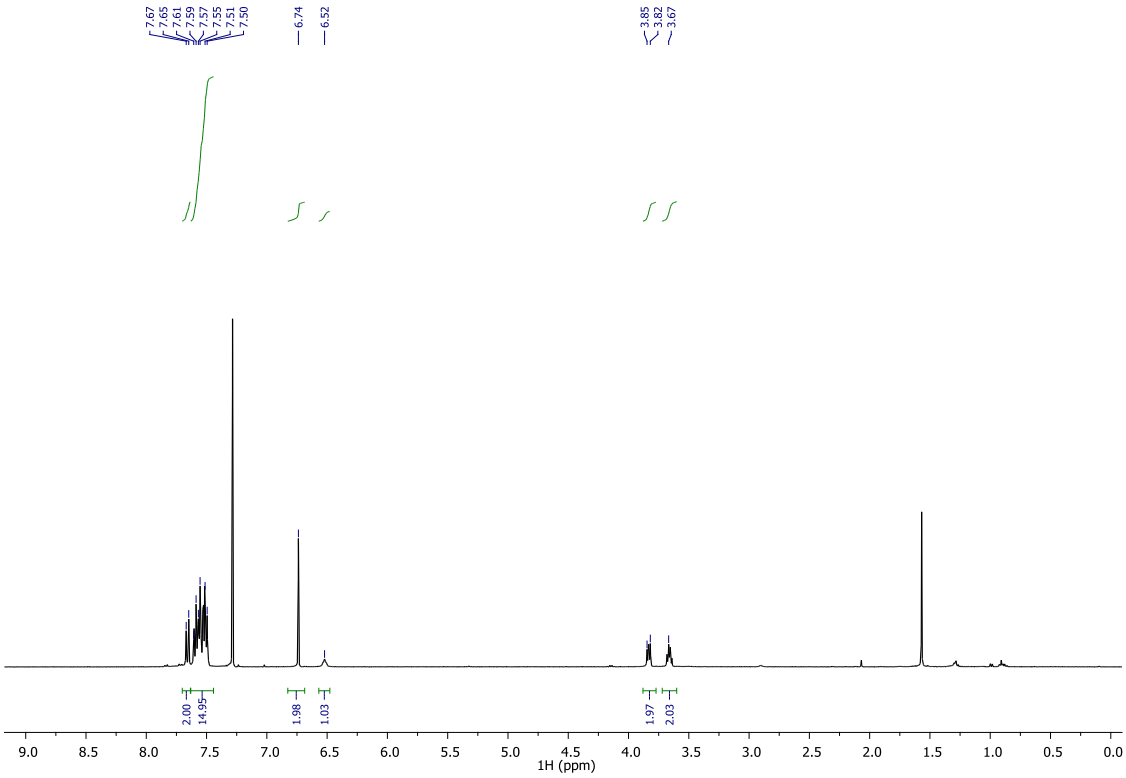


Figure S11. ^{1}H NMR spectrum of compound **4** in $CDCl_3$.

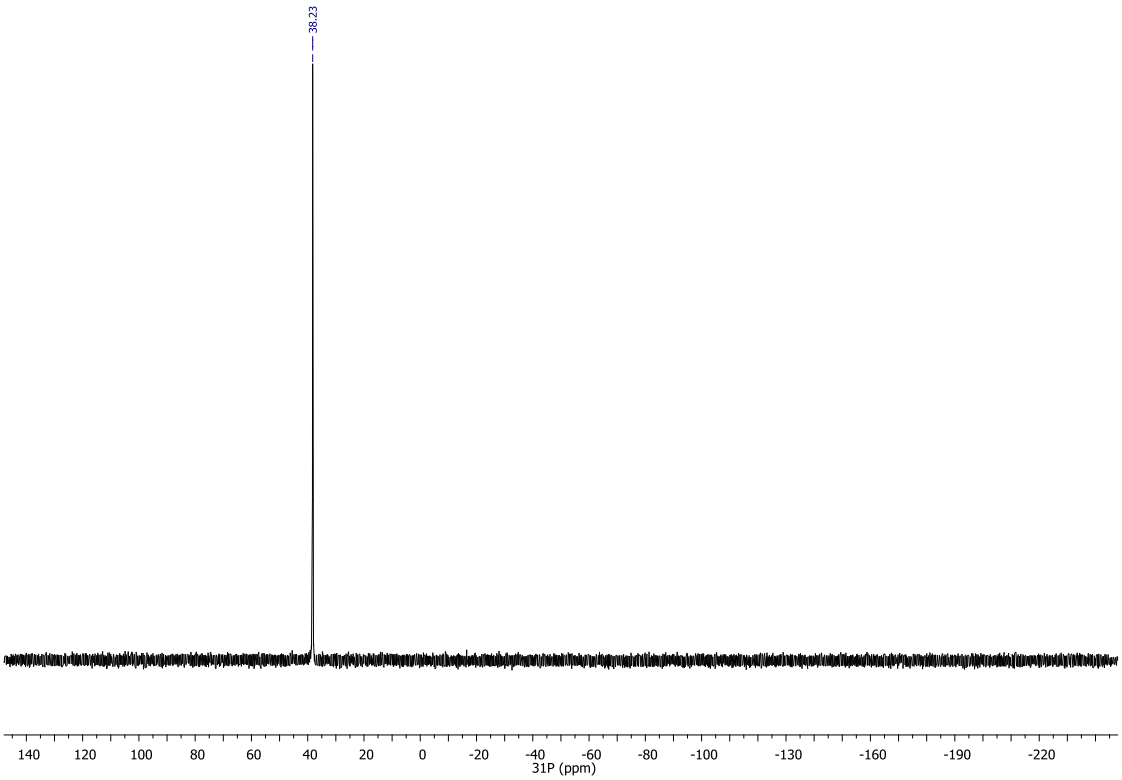


Figure S12. $^{31}P\{^{1}H\}$ NMR spectrum of compound **4** in $CDCl_3$.

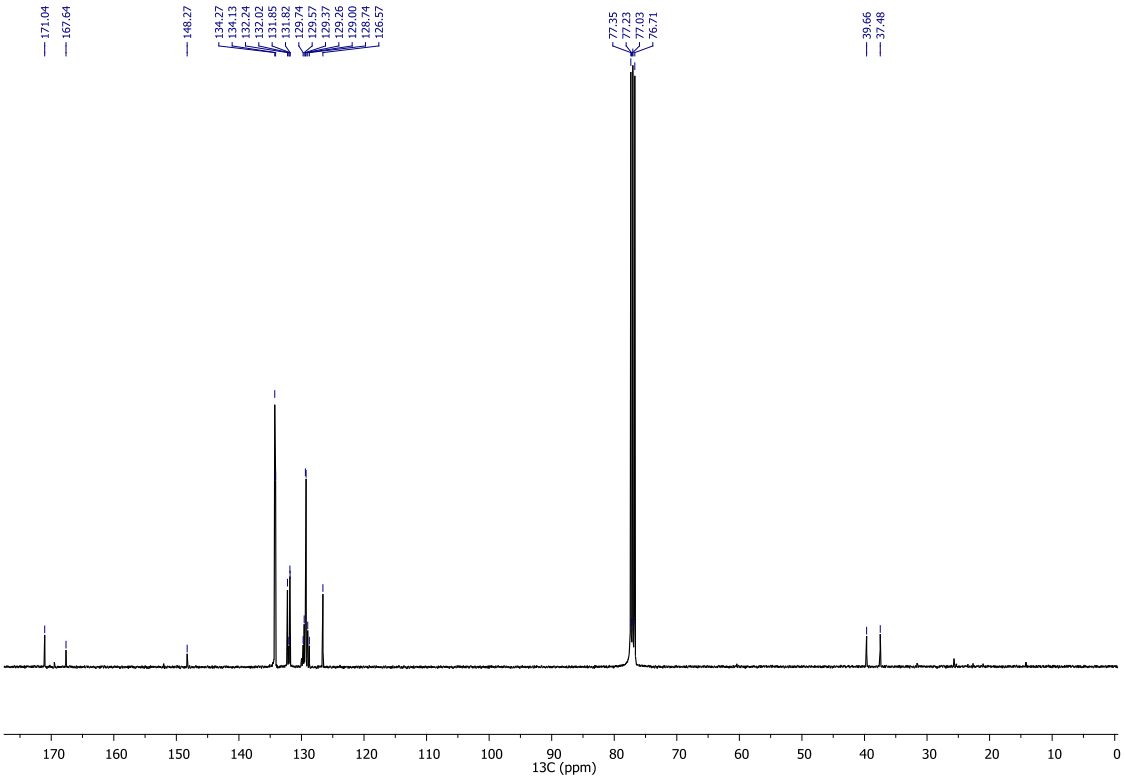


Figure S13. $^{13}C\{^{1}H\}$ NMR spectrum of compound **4** in $CDCl_3$.

3. NMR spectra of decomposition of compounds 1, 2 and 4 in d^6-DMSO and d^6-DMSO/PBS-D_2O

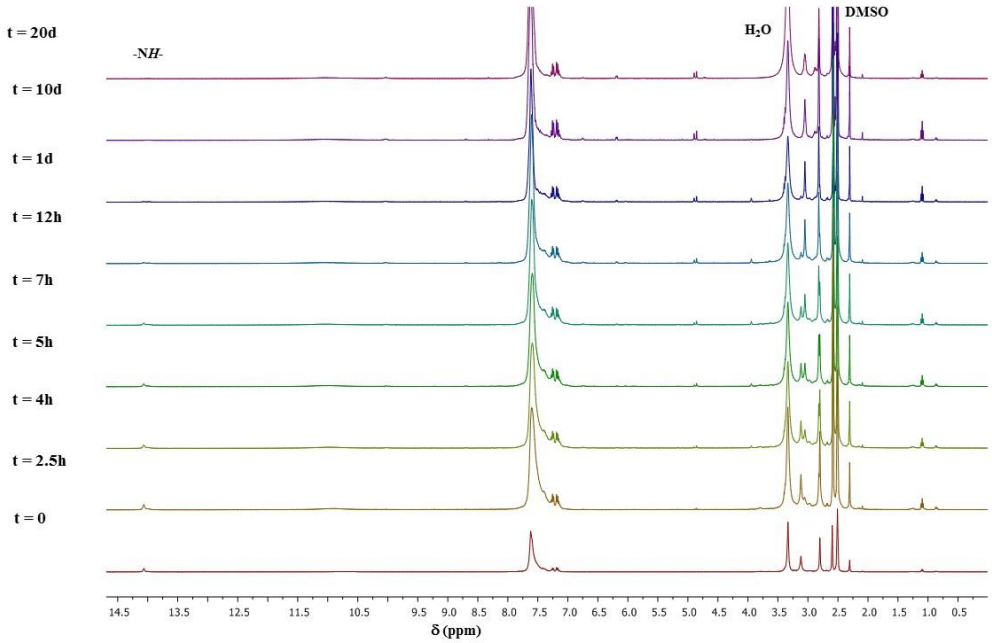


Figure S14. Time course ^{1}H NMR spectrum in d^6-DMSO of compound **1**.

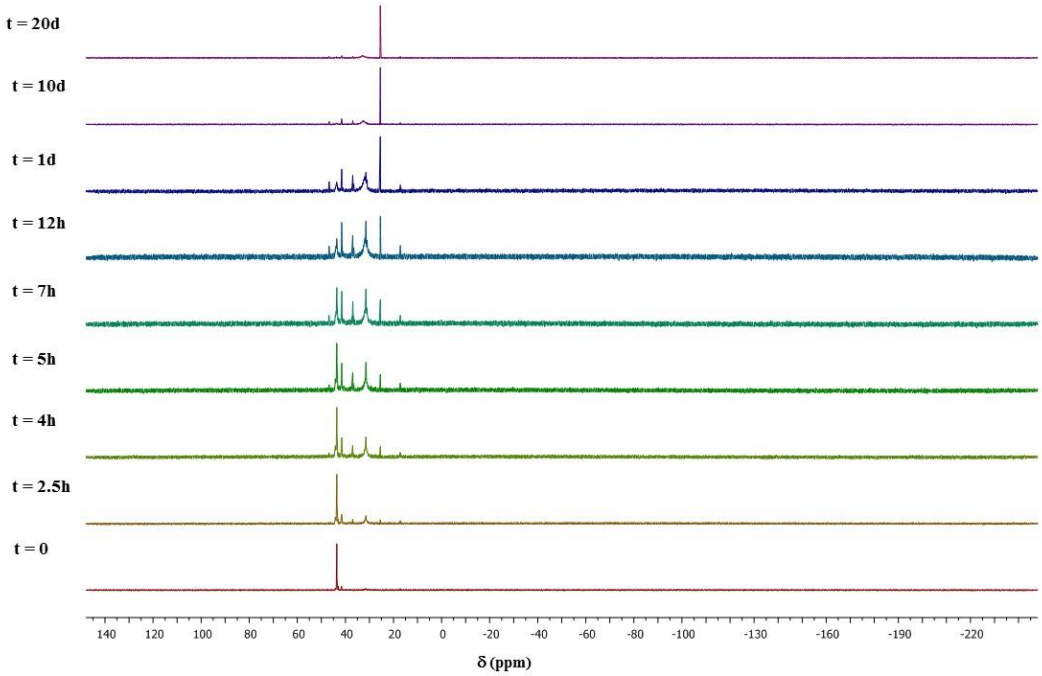


Figure S15. Time course $^{31}P\{^{1}H\}$ NMR spectrum in d^6-DMSO of compound **1**.

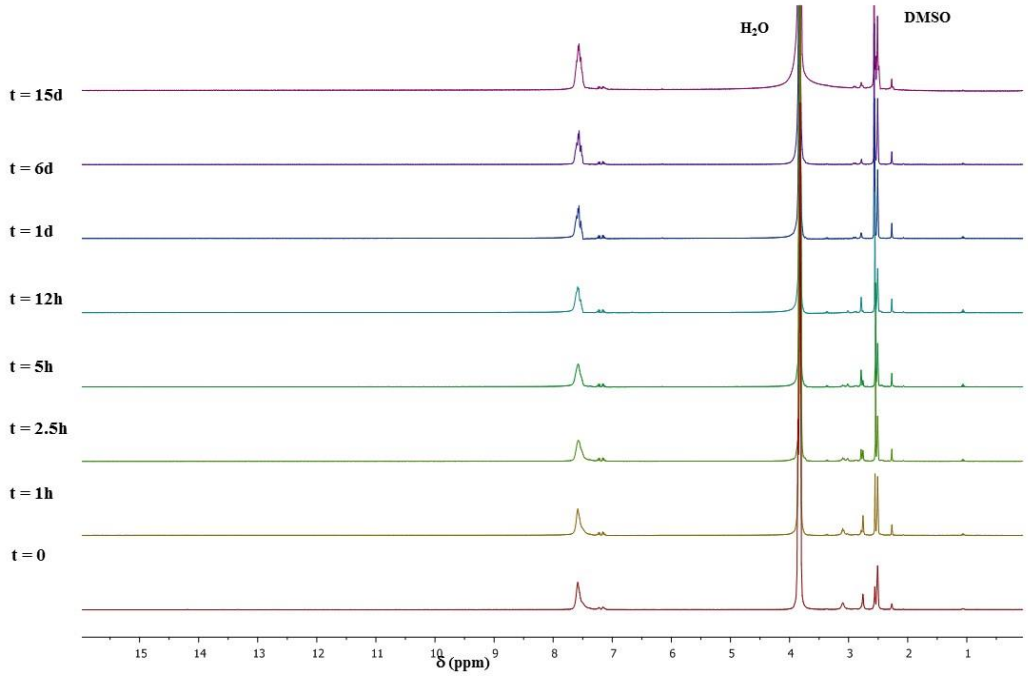


Figure S16. Time course ^{1}H NMR spectrum in 3:1 d^6-DMSO/PBS-D_2O of compound **1**.

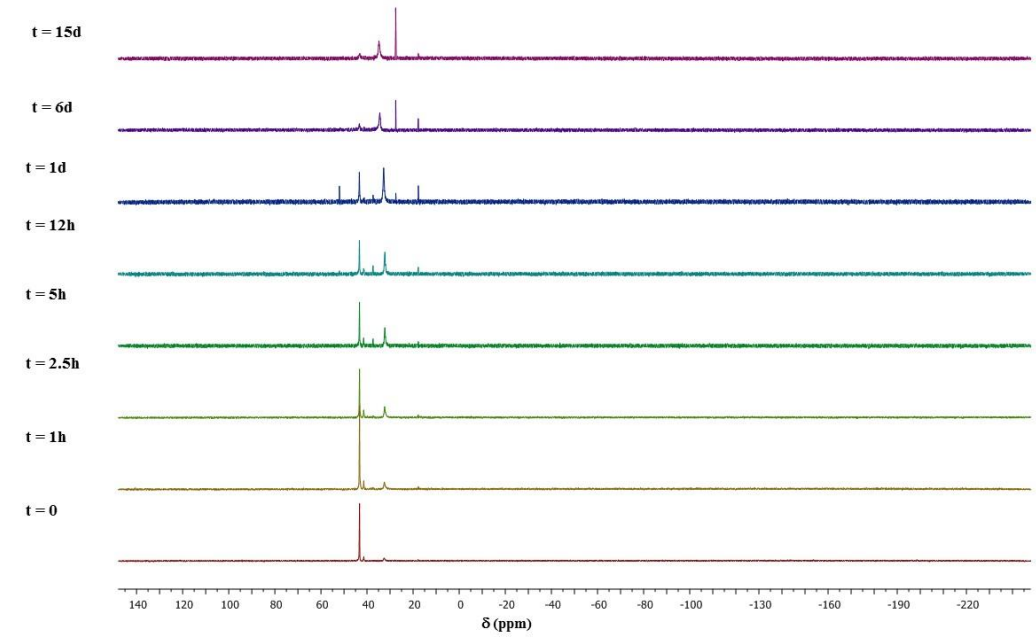


Figure S17. Time course $^{31}P\{^{1}H\}$ NMR spectrum in 3:1 d^6-DMSO/PBS-D_2O of compound **1**

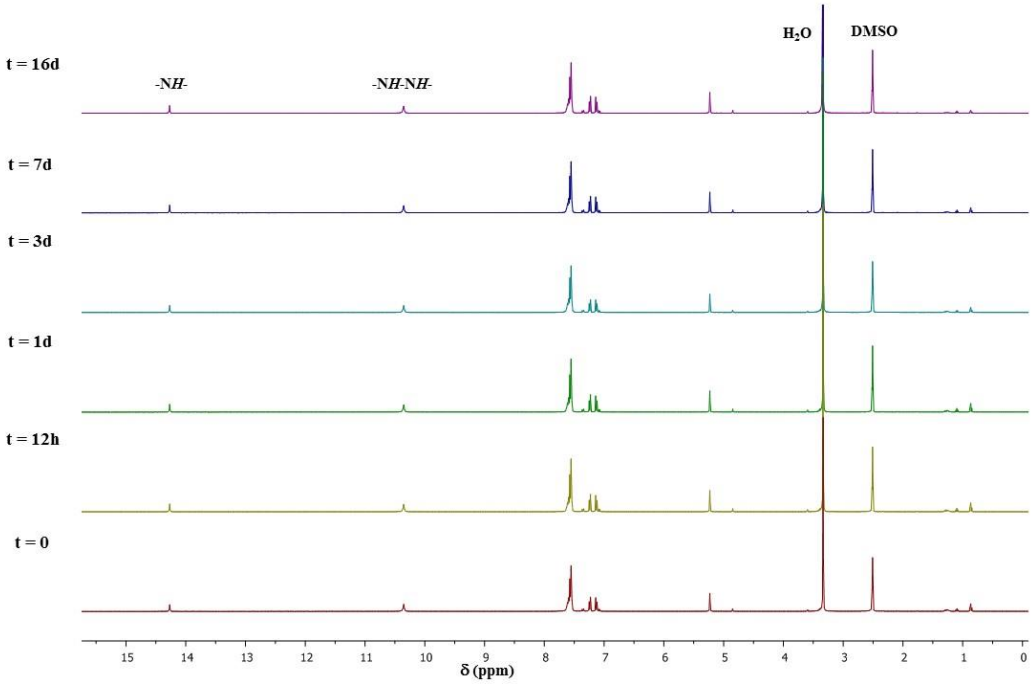


Figure S18. Time course ^{1}H NMR spectrum in d^6-DMSO of compound **2**.

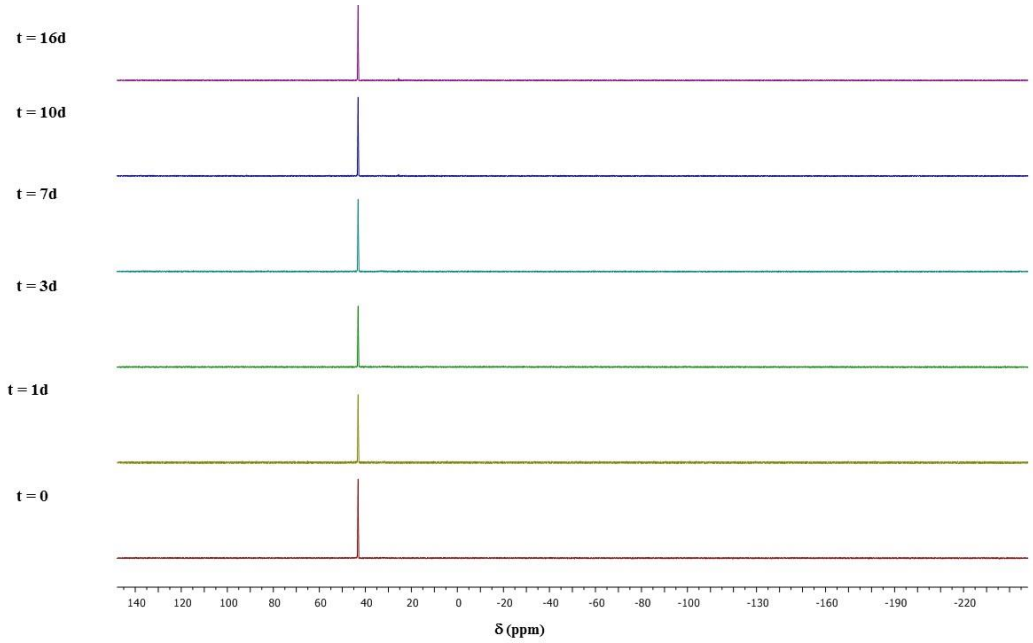


Figure S19. Time course $^{31}P\{^{1}H\}$ NMR spectrum in d^6-DMSO of compound **2**.

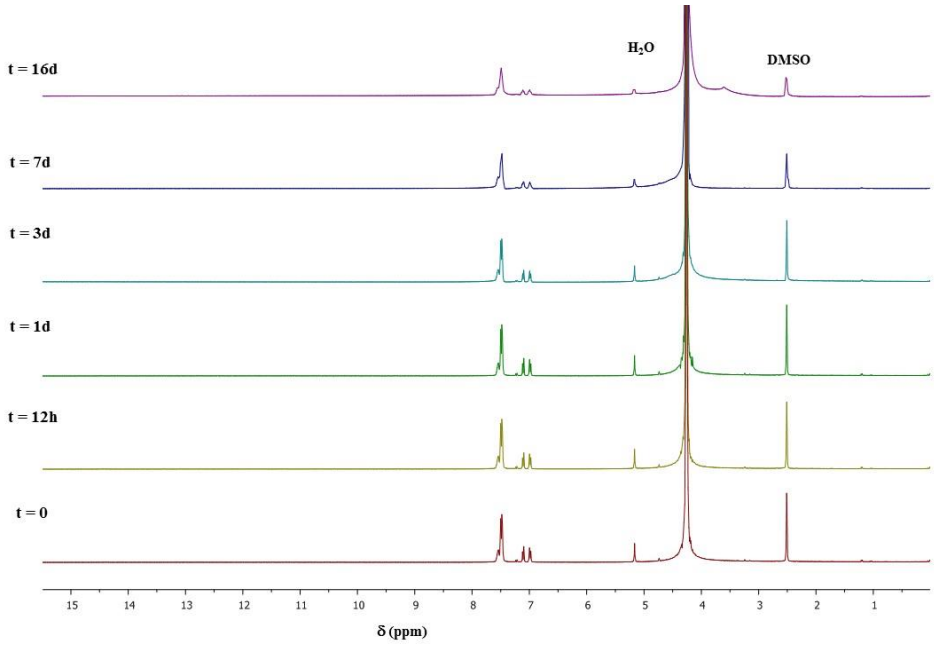


Figure S20. Time course ^{1}H NMR spectrum in 3:1 d^6-DMSO/PBS-D_2O of compound **2**.

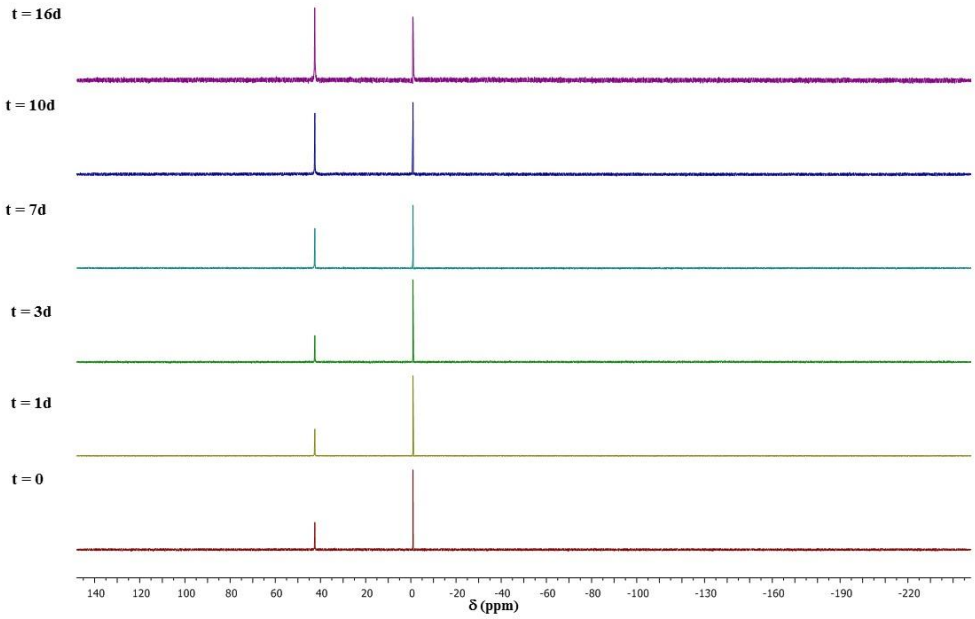


Figure S21. Time course $^{31}P\{^{1}H\}$ NMR spectrum in 3:1 d^6-DMSO/PBS-D_2O of compound **2**.

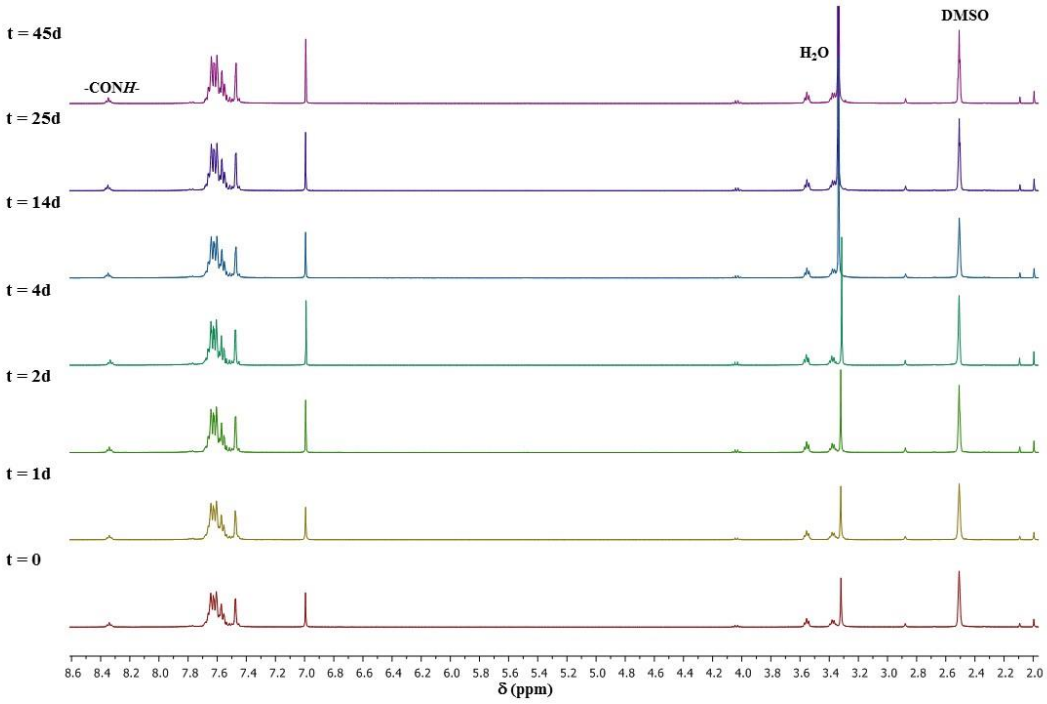


Figure S22. Time course ^{1}H NMR spectrum in d^6-DMSO of compound **4**.

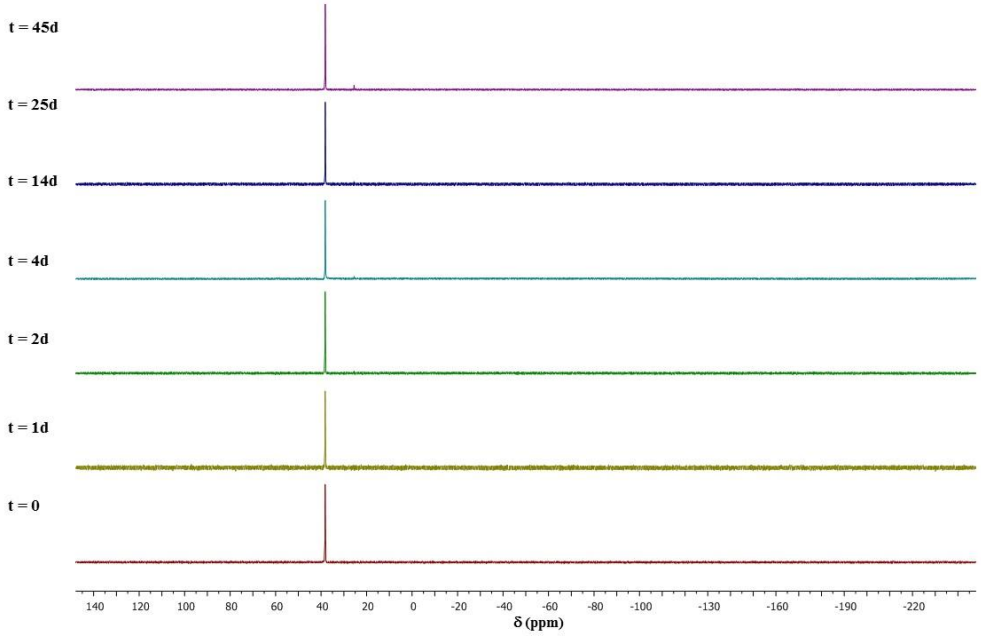


Figure S23. Time course $^{31}P\{^{1}H\}$ NMR spectrum in d^6-DMSO of compound **4**.

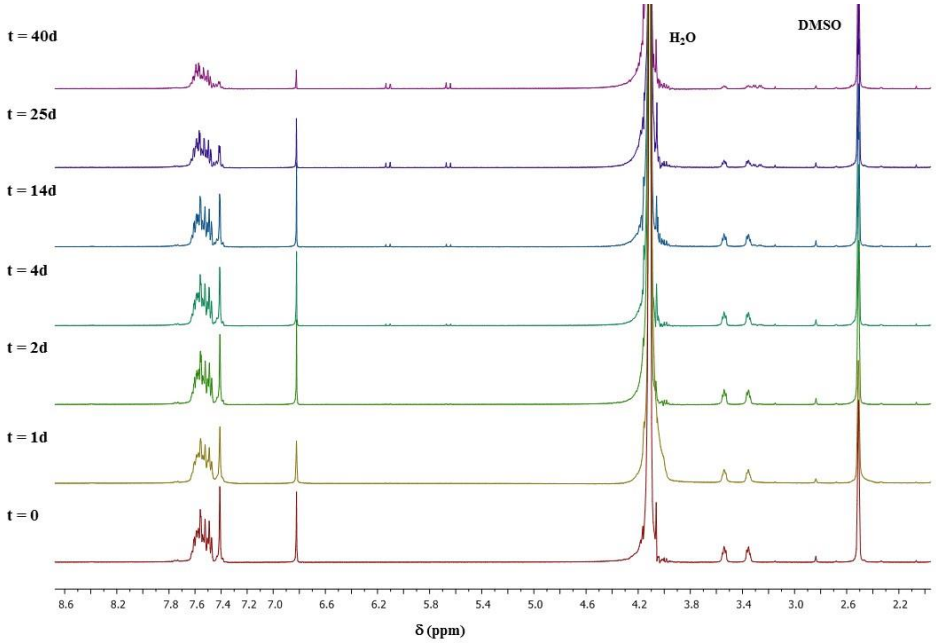


Figure S24. Time course ^{1}H NMR spectrum in 3:1 d^6-DMSO/PBS-D_2O of compound **4**.

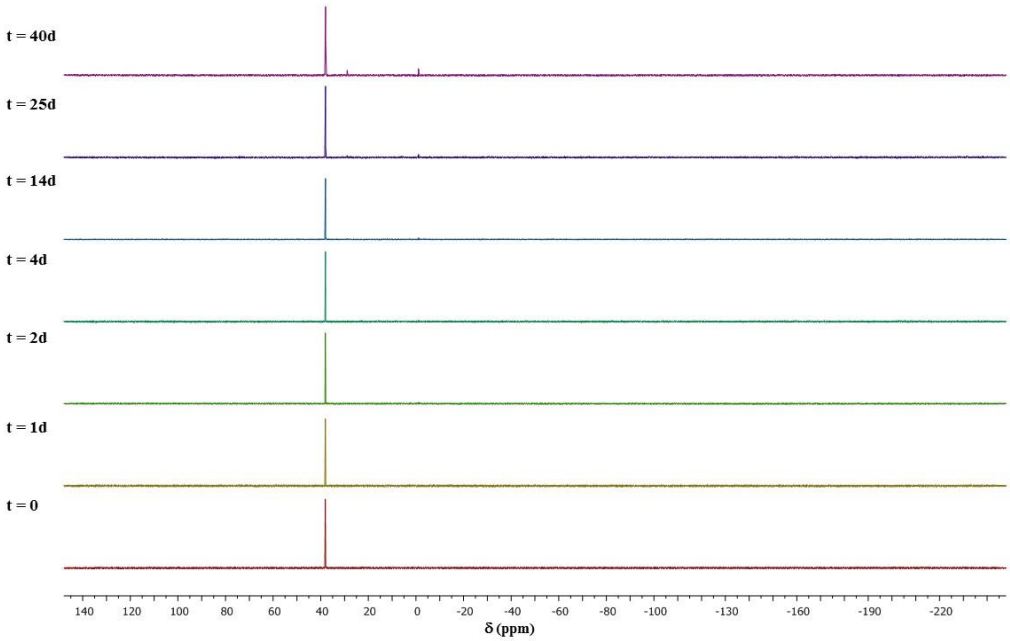
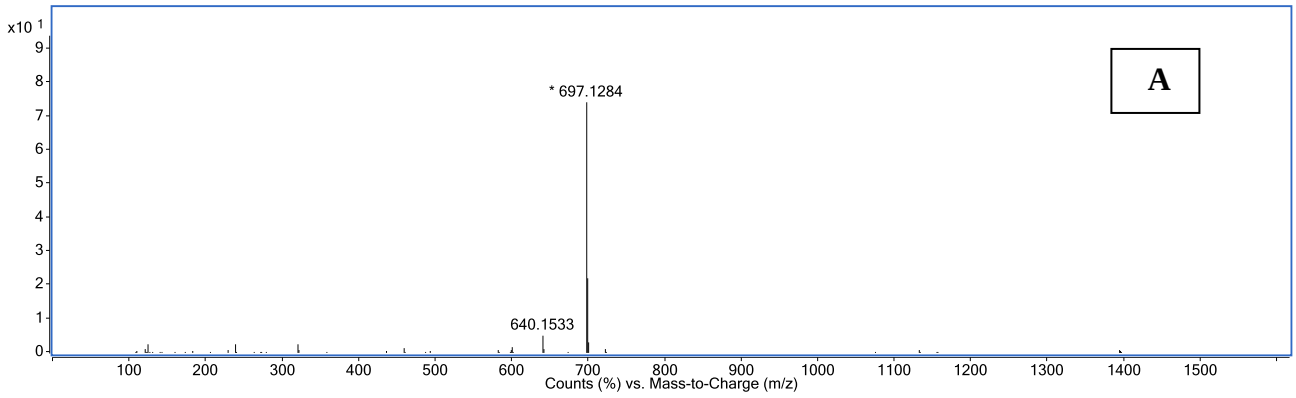


Figure S25. Time course $^{31}P\{^{1}H\}$ NMR spectrum in 3:1 d^6-DMSO/PBS-D_2O of compound **4**.

4. Mass spectra (ESI+) of compounds 1, 2 and 4



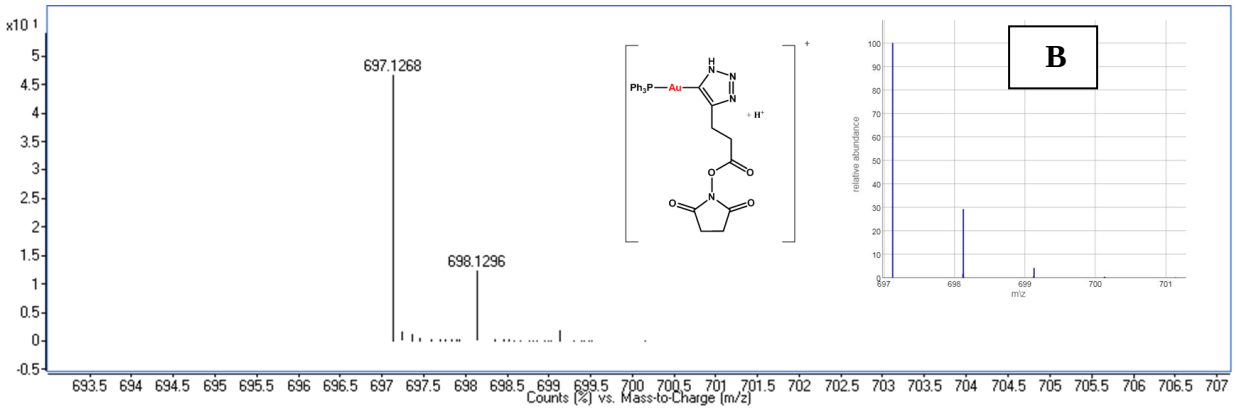
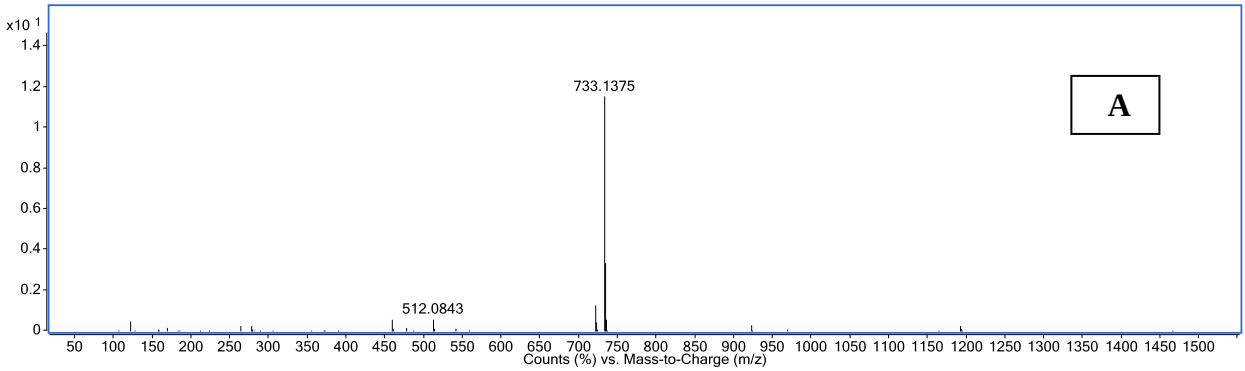


Figure S26. MS ESI+ of compound **1** in 1% DMSO-Acetonitrile (full experimental spectrum (A); selected experimental peak + theoretical isotopic pattern (B)).



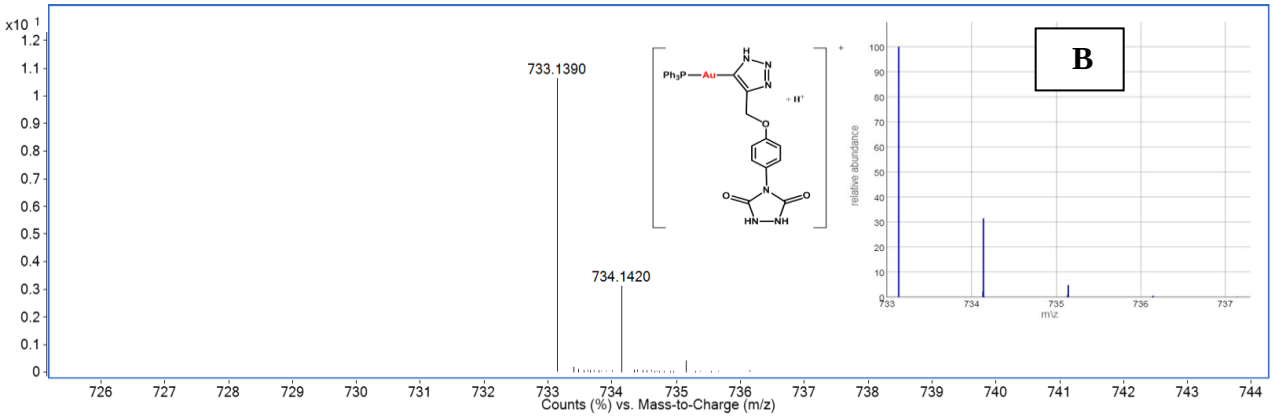


Figure S27. MS ESI+ of compound **2** in 1% DMSO-Acetonitrile (full experimental spectrum (A); selected experimental peak + theoretical isotopic pattern (B)).

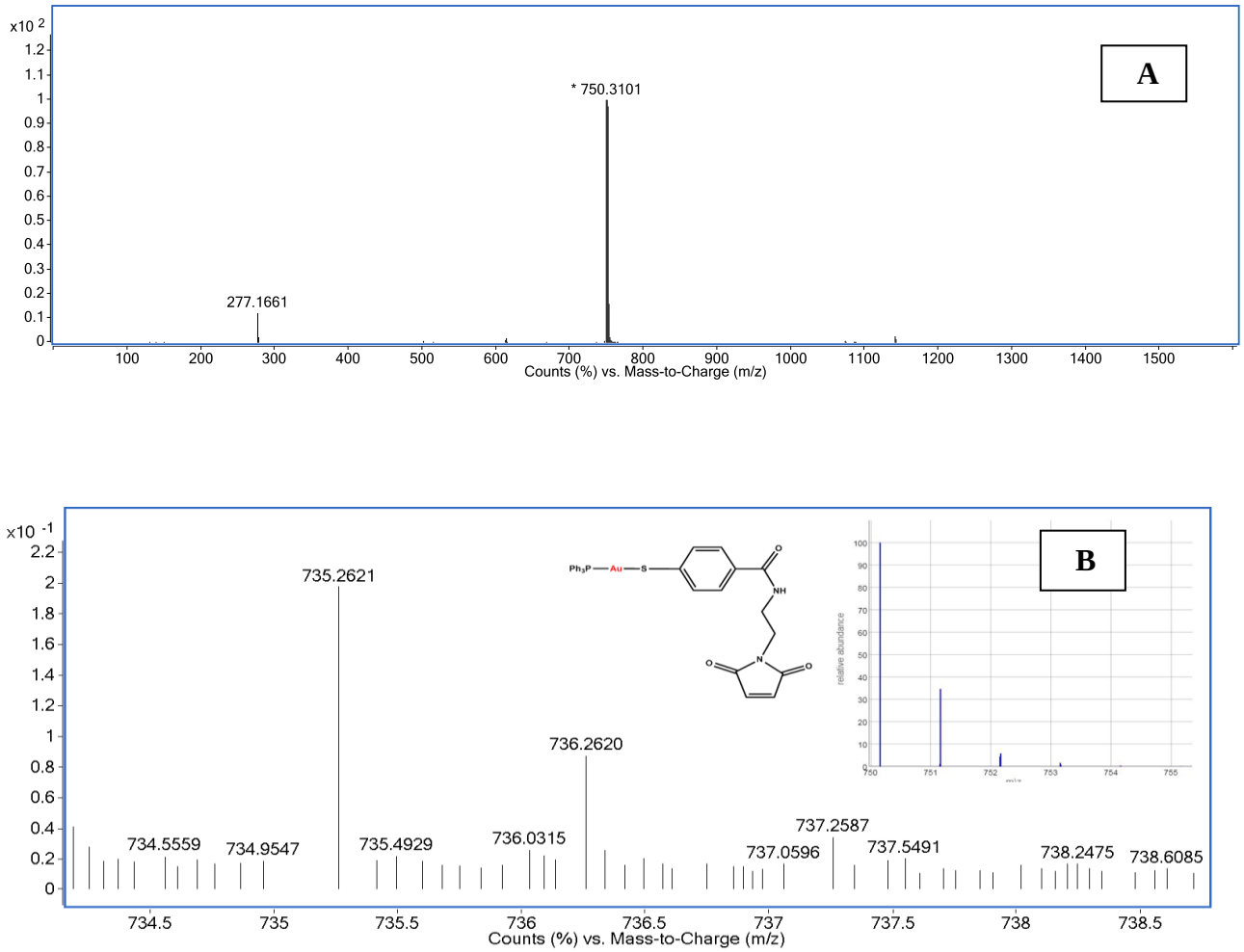


Figure S28. MS ESI+ of compound **4** in 1% DMSO-Acetonitrile (full experimental spectrum (A); selected experimental peak + theoretical isotopic pattern (B)).

5. Size Exclusion HPLC spectra of AGCs Tras1 and Tras4.

To determine the purity of the synthesized antibody gold-conjugates, size exclusion HPLC analysis were performed on a Shimadzu system equipped with a DGU-20A degasser, a SPD-M20A UV detector, a LC-6AD pump system and a CBM-20A communication BUS module, using a Superdex^{TM} 200 Increase 10/300 GL column with an isocratic 0.01 M phosphate buffer, 0.14 M NaCl (pH 7.4).

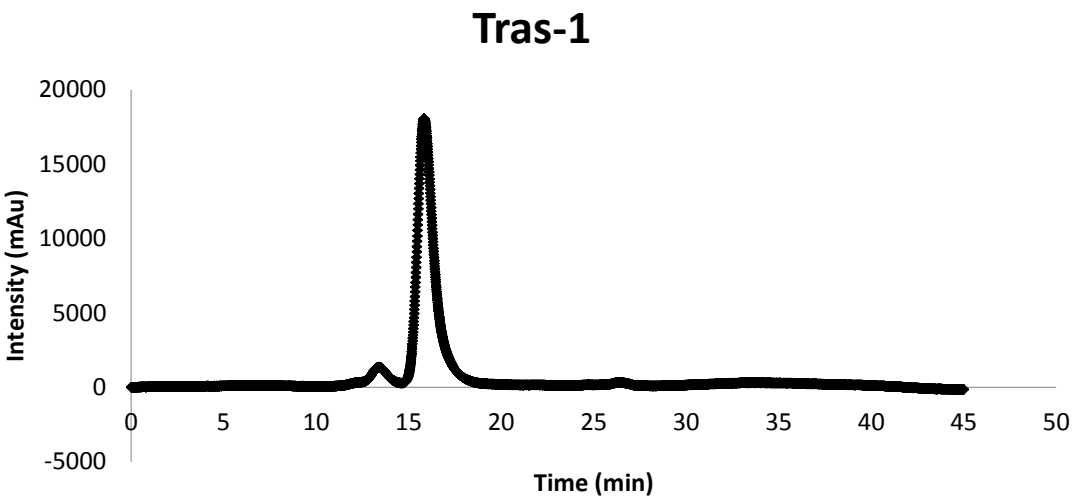


Figure S29. Chromatogram of **Tras-1**.

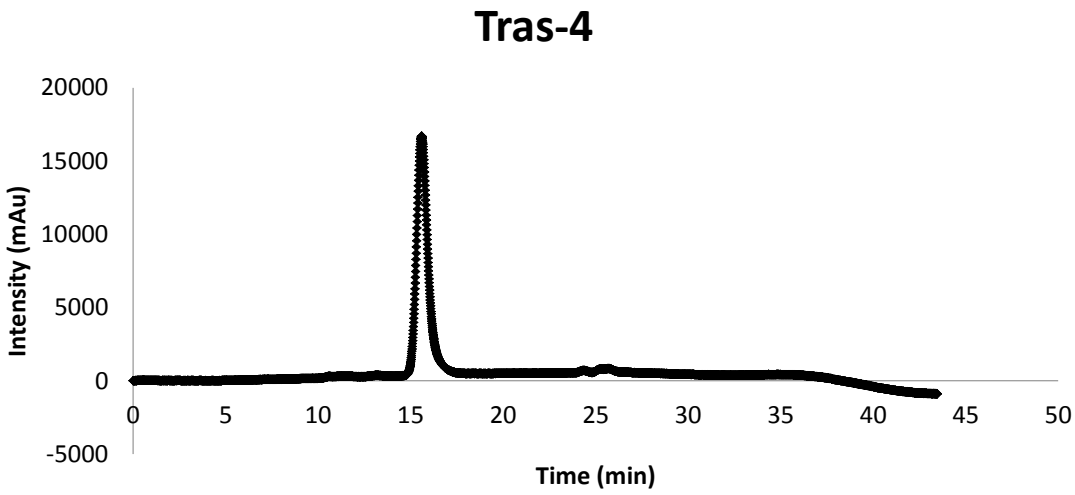


Figure S30. Chromatogram of **Tras-4**.

6. MALDI-TOF spectra of AGCs Tras1 and Tras4 and DAR calculations.

To determine the number of gold-complexes conjugated per antibody, the immunoconjugates were analyzed by MALDI-TOF MS/MS at the Alberta Proteomics and Mass Spectrometry Facility, University of Alberta, Canada. 1 μL of each sample (1

mg/mL) was mixed with 1 μL of sinapic acid (10 mg/ml in 50% acetonitrile:water and 0.1% trifluoroacetic acid). 1 μL of the sample/matrix solution was then spotted onto a stainless steel target plate and allowed to air dry. All mass spectra were obtained using a Bruker Ultraflex MALDI-TOF/TOF (Bruker Daltonic GmbH). Ions were analyzed in positive mode and external calibration was performed by use of a standard protein mixture (Bovine Serum Albumin). It has to be realized that antibodies have up to 20 lysine residues, however, not all of them are available for bioconjugation.[5] In the case of trastuzumab, antibody drug conjugates with DARs higher than 3.5 are not commonly reported.[6,7] DAR between 2 and 4 are known to have a better pharmacokinetic profile and we therefore aimed for this ratio. Bioconjugation reactions were performed for MALDI-TOF as indicated below:

Tras-1:

a) 1 equiv. Tz + 24 equiv. **1**.

b) 1 equiv. Tz + 12 equiv. **1**.

Tras-4:

a) 1 equiv. Tz + 10 equiv. **4** + 5 equiv. TCEP.

b) 1 equiv. Tz + 10 equiv. **4** + 10 equiv. TCEP.

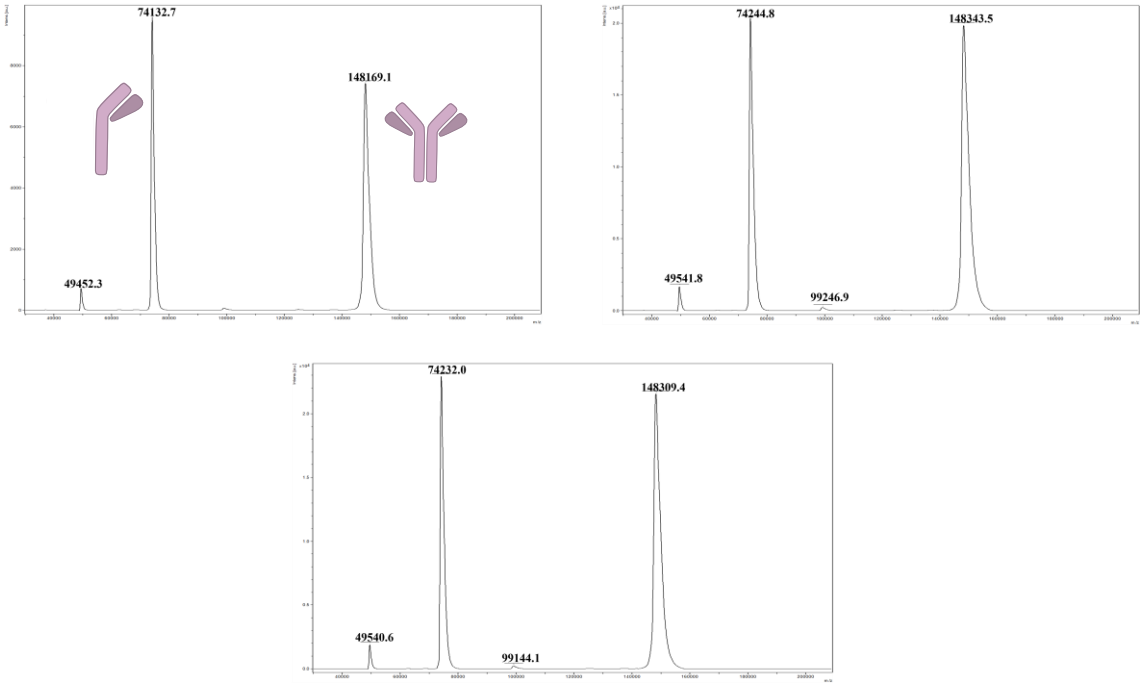


Figure S31. MALDI-TOF of Tz (run in triplicate).

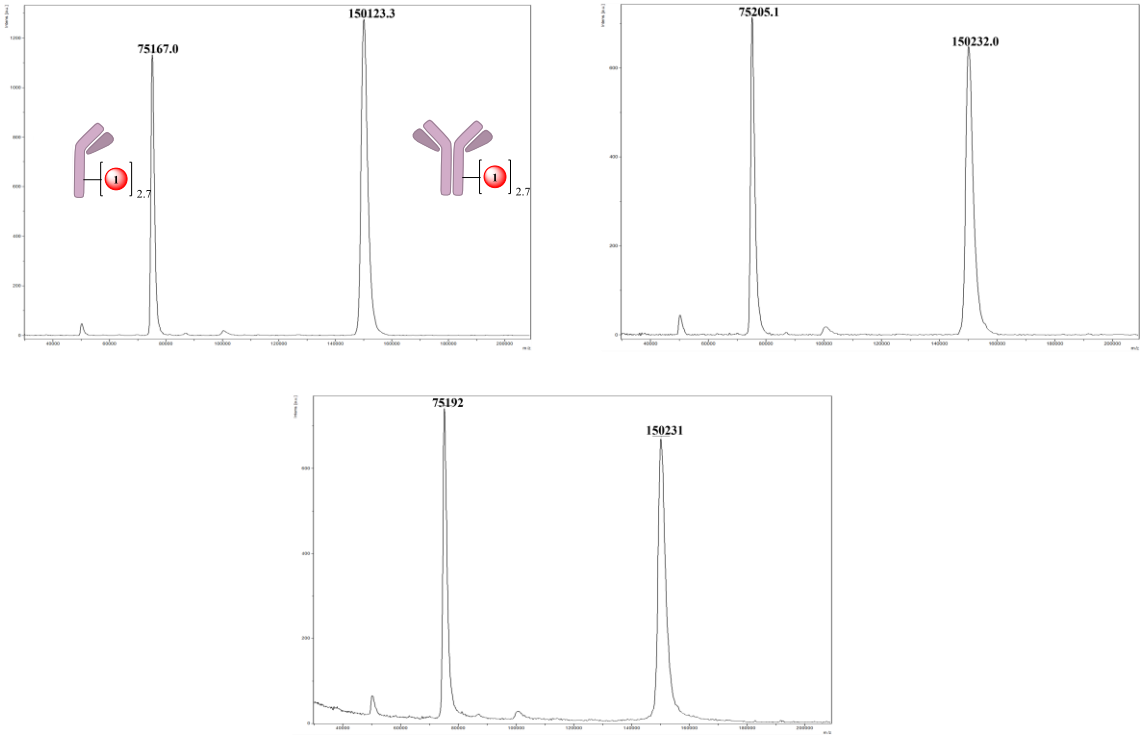


Figure S32. MALDI-TOF of **Tras-1**: 1 equiv. Tz + 24 equiv. **1** (run in triplicate).

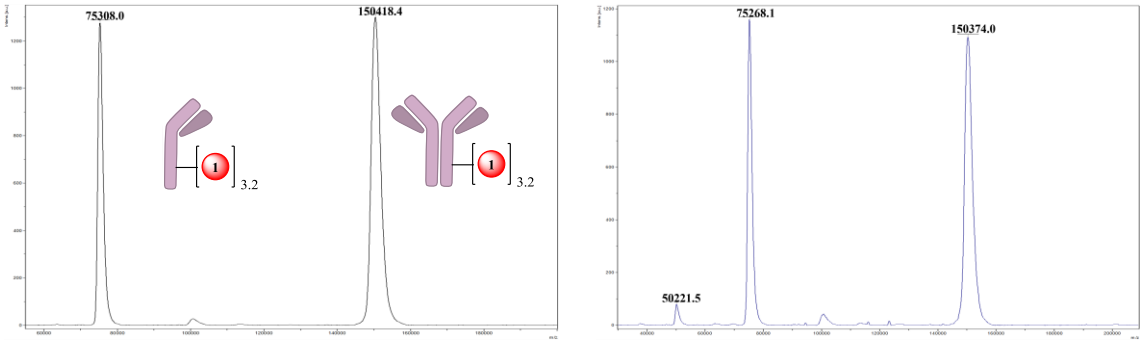


Figure S33. MALDI-TOF of **Tras-1**: 1 equiv. Tz + 12 equiv. **1** (run in duplicate).

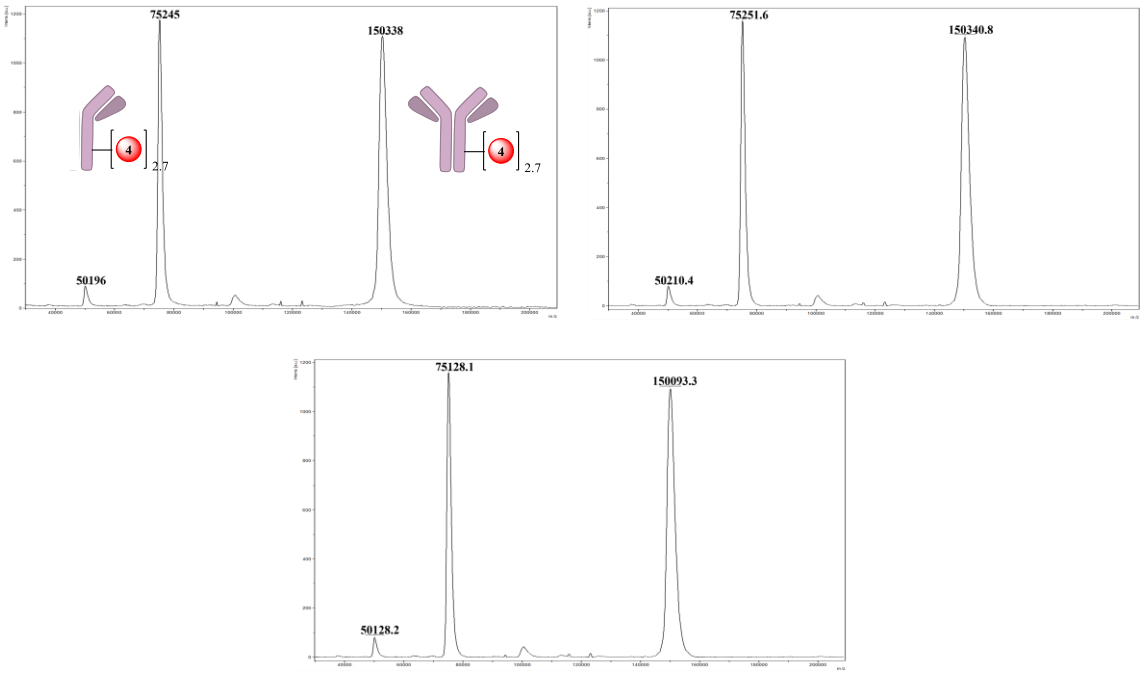


Figure S34. MALDI-TOF of **Tras-4**: 1 equiv. Tz + 10 equiv. **4** + 5 equiv. TCEP (run in triplicate).

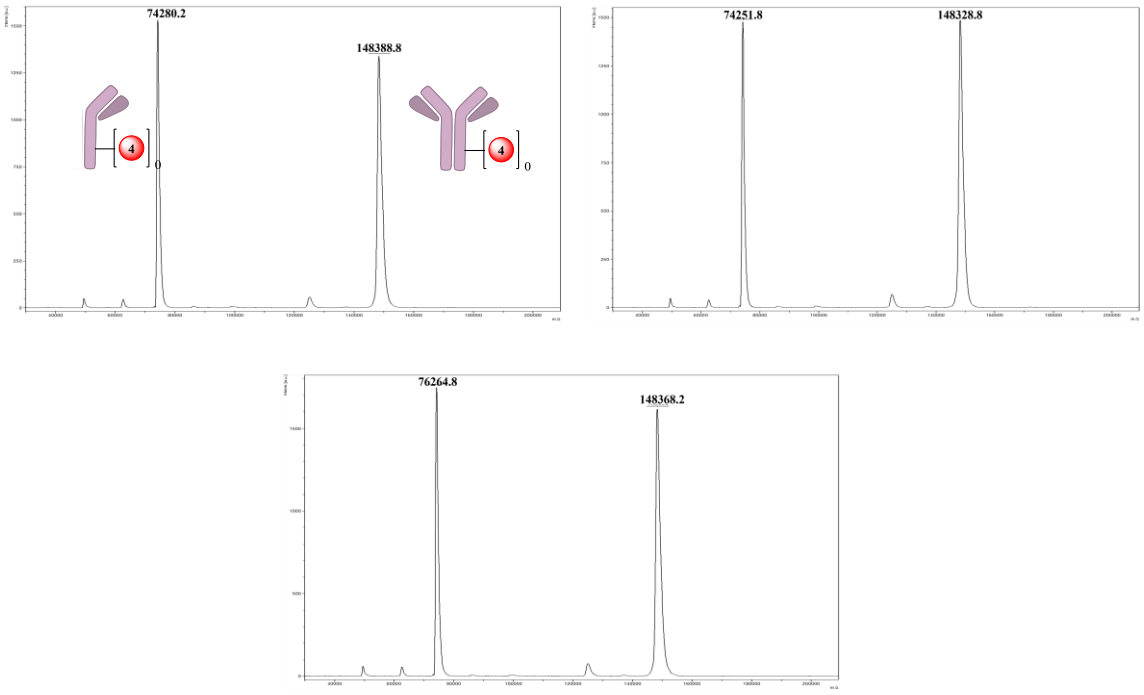


Figure S35. MALDI-TOF of **Tras-4**: 1 equiv. Tz + 10 equiv. **4** + 10 equiv. TCEP (run in triplicate).

	Experimental (average)	Calculated	DAR
Tz	148,273.7	-	-
Tras-1 (a)	150,195	149,665 (DAR = 2) 150,361 (DAR = 3)	2.7
Tras-1 (b)	150,396	149,665 (DAR = 2) 150,361 (DAR = 3)	3.2
Tras-4 (a)	150,257	149,742 (DAR = 2) 150,476 (DAR = 3)	2.7
Tras-4 (b)	148,361	-	0

$$DAR = \frac{P_{ADC} - P_{Tz}}{M_w}$$

P_{ADC} = Average of the triplicate measurement of the peak in the ADC

P_{Tz} = Average of the triplicate measurement of the peak in Tz

Mw = Molecular weight of the gold compound

Table S1. Experimental average m/z MALDI-TOF, calculated values for selected DAR and DAR values. Bottom: Formula to calculate DAR values.

7. Enzyme-linked immunosorbent assay (ELISA) for binding assays of AGCs Tras1 and Tras4 and Trastuzumab to HER2.

Binding affinity to HER2 receptor was determined by ELISA. A 96-well plate was coated overnight at 4 °C with HER2 (100 µL of a 0.25 µg/mL solution in PBS). One row of wells was coated with PBS only as a negative control. Wells were washed with PBS (x3) and blocked with a 1% BSA solution in PBS (100 µL) at 20 °C for 1 h. Next, the wells were washed with PBS (100 µL x 3). Tz and respective AGCs were diluted in PBS yielding the following concentrations: 300 nM, 100 nM, 30 nM, 10 nM, 3.0 nM, 1.0 nM, 0.3 nM, 0.1 nM, 0.03 nM and 0.01 nM (quadruplicates). The dilution series was

added, including PBS only and AGC at 30 nM in the absence of HER2 as negative controls. The plate was incubated for 2 h at 20 °C. Wells were washed with PBS (100 μL x 2) and the detection antibody (100 μL of anti-human IgG, Fab-specific-HRP solution, 1:5000 in PBS) was added followed by incubation for 1 h at 20 °C. After the washing step (100 μL x 2), freshly prepared TMB solution (50:50 Peroxide Solution (H_2O_2)/Peroxide Substrate (TMB)) was added to each well (100 μL) and the reaction was stopped after 20 min by addition of 1 M H_2SO_4 (100 μL). The colorimetric reaction was measured at 450 nm and the absorption was corrected by subtracting the average of negative controls. Each measurement was done in quadruplicates.

8. Stability Studies of AGCs Tras-1 and Tras-4

Procedure

100 μL of gold antibody conjugates (1 mg/mL) in PBS (prepared as described in previous sections) were incubated in 900 μL of human serum at 37 °C (triplicate for each gold antibody conjugate). Aliquots of 100 μL were taken at different time points (2h, 24h, 48h, 72h, 7d) and dissolved up to 500 μL in MilliQ water (triplicates). The resulting solutions were centrifuged and filtered using 30kDa Amicon filters for 12min at 4000 rpm. Two solutions were separated (solution at the upper part of the filter and filtrate at the bottom). Samples were digested with an aqua regia solution of HNO_3 (65%): HCl (35%). For those samples showing incomplete digestion by means of the regular procedure stated before, the concentration of nitric acid was adjusted to 5%. Then, sulfuric acid, hydrogen peroxide and heat were used and applied for the later samples. A different attempt of digestion using protease (37 °C overnight) following by aqua regia was also performed. None of the alternative procedures to aqua regia were successful with samples forming aggregates (upper solution in the filter of **Tras-1** and **Tras-4** in serum at any time point).

A gold elemental analysis of the samples was carried out using a Perkin-Elmer Optima 7000 DV spectrometer. The concentration of Au (mg/L) in the sample and the percentage of recovery (% recovery) were reported. The percentage recovery (%) was calculated by using the following equation:

$$\% \text{ recovery} = [(Ci-Ca)/Ca]*100$$

where C_i and C_a define Au concentration in the measured aqueous phases before and after passing through the filter, respectively. The ICP-OES results revealed the total recovery of Au-based particles in the samples.

Table S2. % of Au found on the samples, before and after filtration.

Name of the sample	Description	Concentration of Au in the sample (mg/L)	% Au
1	Control **Tras-1** (digestion of the 1 mg/mL crude solution of gold antibody conjugate without filtration)	0.73	93%
2	Control **Tras-4** (digestion of the 1 mg/mL crude solution of gold antibody conjugate without filtration)	0.83	106%
3	Control **Tras-1**filtration (digestion of the upper solution of 1 mg/mL crude solution of gold antibody conjugate after filtration)	0.68	88%
4	Control **Tras-1** filtration (digestion of the bottom filtrate of 1 mg/mL crude solution of gold antibody conjugate after filtration)	0	0%
5	Control **Tras-4** filtration (digestion of the upper solution of 1 mg/mL crude solution of gold antibody conjugate after filtration)	0.80	103%
6	Control **Tras-4** filtration (digestion of the bottom filtrate of 1 mg/mL crude solution of gold antibody conjugate after filtration)	0	0%
7	Digestion of the upper solution after filtration of gold antibody conjugate (**Tras-1** or **Tras-4**) in serum at any time point	Aggregates were formed after all digestion attempts (described in procedure)	N/A
8	Digestion of the bottom filtrate after filtration of gold antibody conjugate (**Tras-1** or **Tras-4**) in serum at any time point	0	0%

Method

The recovery of the gold-based ADCs particles by filtration was evaluated by means of inductively coupled plasma optical emission spectroscopy (ICP-OES). All the experimental points were repeated in triplicates to confirm reproducibility. The average of the three measurements was reported as the final result.

The percentage of recovery (% recovery) was determined by using the equation on the previous section. The elemental analysis for Au distribution was carried out using a Perkin-Elmer Optima 7000 DV spectrometer. Samples were first digested with an aqua regia solution of HNO_3 (65%): HCl (35%), and then the concentration of nitric acid was adjusted to 5% to be within the calibration curve range (from 5ppm to 10ppb). Calibration solutions were prepared from a certified stock of a gold single element solution (Sigma-Aldrich, TraceCERT$^{@}$, 999 ± 2 mg/L). The instrument was calibrated using a six-point calibration curve between 0.01 and 5 ppm and checked by three QC samples at the low, middle and high points on the curve. The operating conditions employed for ICP-OES determination were: 1,300 W RF power, 15 $L.min^{-1}$ plasma flow, 0.2 $L.min^{-1}$ auxiliary flow, 0.8 $L.min^{-1}$ nebulizer flow, and 0.8 $mL.min^{-1}$ sample uptake rate. Signals at a wavelength of 267.595 nm were monitored. The low limit of quantification was determined to be 0.01ppm.

9. Cell viability analysis for all compounds and AGCs described.

MCF7, MCF10A and BT-474 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium (DMEM) (Mediatech) supplemented with 10% FBS, 1% NEAA and 1% penicillin-streptomycin (MCF7 AND MCF10A) or DME HG/F12K, NEAA, 2 mM glutamine, 10% FBS/PS and incubated at 37°C and 5% CO_2 in a humidified incubator.

Cytotoxic profiles (EC_{50}) of compounds **[AuCl(PPh$_3$)]**, **[Au(mba)(PPh$_3$)]**, **1**, **2**, **4**, **Tras-1**, **Tras-2**, **Tz**, and **AF** were obtained by assessing the viability of human breast tumor cells MCF7 and BT-474 and nontumorigenic human breast cell line MCF10A treated with the appropriate culture medium containing the specific compounds. Concentrations ranged from 100 μM, to 0.1 μM (**[AuCl(PPh$_3$)]**, **[Au(mba)(PPh$_3$)]**, **1**, **2**, **4** and **AF**), and 60-20 μM, to 0.001 μM (**Tras-1**, **Tras-2**, **Tz**). After 72h, cell viability

was determined by means of the colorimetric cell viability assay PrestoBlue (Invitrogen, Carlsbad, CA) according to the manufacturer's intructions. EC_{50} were fit using GraphPad Prism 7 to best match the experimental data. All compounds were dissolved in DMSO, DMF and 50:50 DMSO/Tryethylene glycol (**[AuCl(PPh$_3$)]**, **[Au(mba)(PPh$_3$)], 1**, **2**, **4** and **AF**) and PBS (phosphate buffered saline solution) for **Tz**, **Tras-1**, **Tras-4**, with a final concentration of the organic solvent of 0.1% maximum. AF has been described to be stable for longer than 72 hours in DMSO solution at room temperature.[8]

Tables S32 (top) and S4 (bottom). EC_{50} Values (μM) in MCF7, MCF10A and BT474 cell lines determined with **[AuCl(PPh$_3$)]** (DMF), **[Au(mba)(PPh$_3$)]** (0.5:0.5 DMSO/TEG), **1** (DMSO), **2** (DMSO), **4** (0.5:0.5 DMSO/TEG), **Tras-1** (PBS), **Tras-4** (PBS), **Tz** (PBS), and **AF** (DMSO) as control.

	[AuCl(PPh$_3$)]	**[Au(mba)(PPh$_3$)]**	**1**	**2**	**4**	**AF**
MCF-7	3.57 ± 0.33	2.36 ± 0.32	38.76 ± 4.85	11.63 ± 1.62	2.73 ± 0.86	4.09 ± 0.07
BT-474	3.32 ± 0.10	3.51 ± 0.16	23.28 ± 0.31	57.83 ± 3.58	0.81 ± 0.01	3.94 ± 0.33
MCF-10A	18.94 ± 1.30	5.52 ± 0.36	44.57 ± 5.15	-	6.77 ± 0.67	3.19 ± 0.45

	Tz	**Tras-1**	**Tras-4**
MCF-7	> 60	2.67 ± 0.70	0.63 ± 0.05
BT-474	> 60	1.73 ± 0.17	0.32 ± 0.01
MCF-10A	> 50	5.69 ± 0.45	4.04 ± 0.20

Statistical analysis was performed using GraphPad Prism 7. Unpaired two-tailed t-tests were performed to compare EC_{50} values as described below. We compared EC_{50} values of the AGCs **Tras-1** with respect to Tz and compound **1**, of AGCs **Tras-4** with respect to Tz and compound **4**. We also compared the value of **Tras-4** with respect to the gold(I) starting material [Au(mba)(PPh$_3$)].

***P* values**

MCF7

Tras1 vs Tz: unpaired t test, two-tailed P<0.001

Tras4 vs Tz: unpaired t test, two-tailed P<0.001

Tras 1 vs 1: unpaired t test, two-tailed P<0.001

Tras 4 vs 4: unpaired t test, two-tailed P=0.013

Tras 4 vs {Au(mba)(PPh$_3$)]: unpaired t test, two-tailed P<0.001

BT474

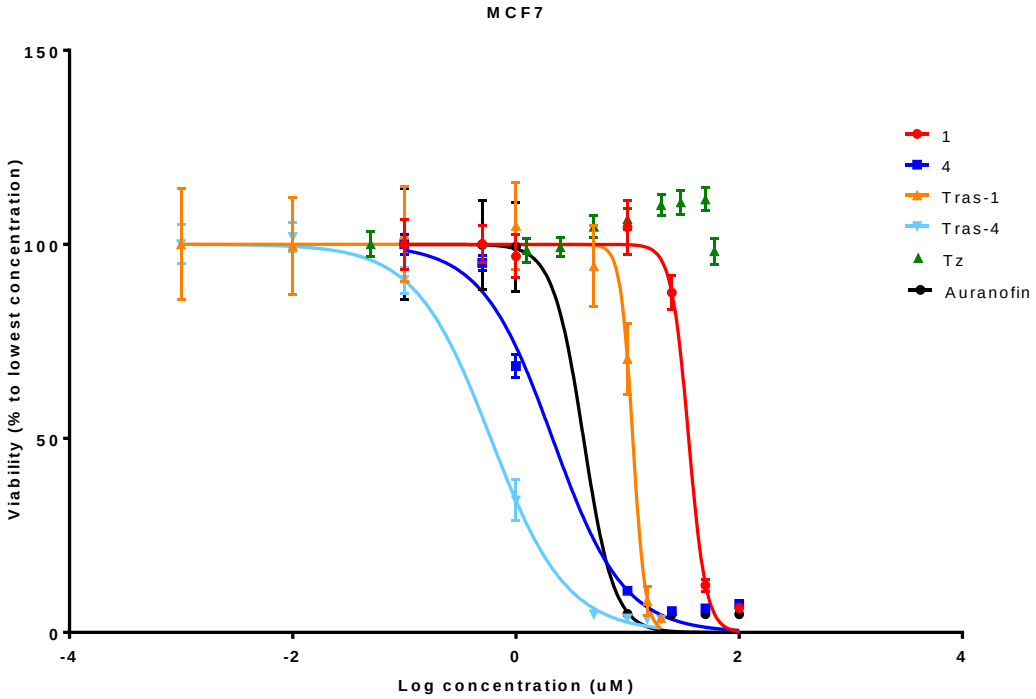
Tras1 vs Tz: unpaired t test, two-tailed P<0.001

Tras4 vs Tz: unpaired t test, two-tailed P<0.001

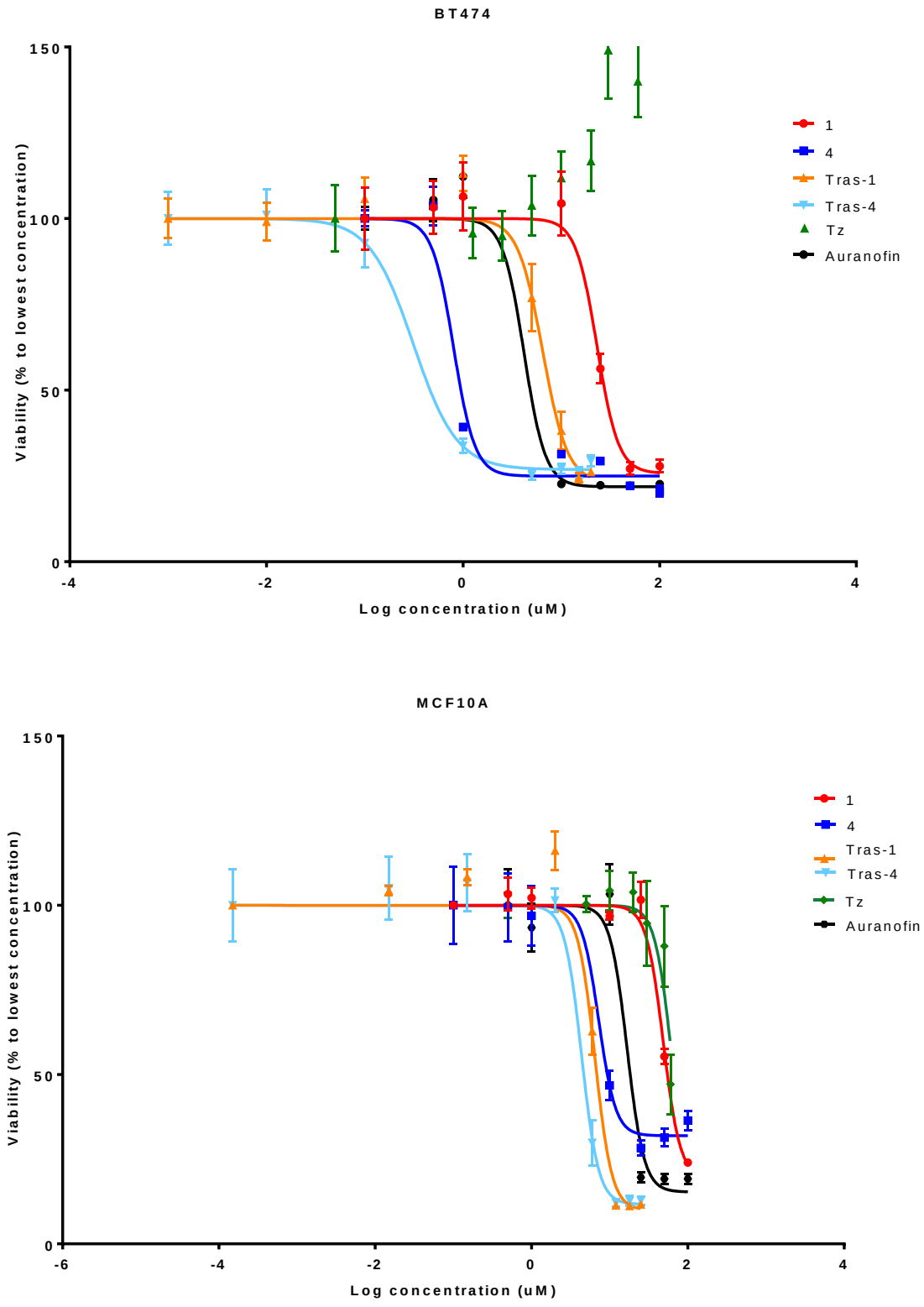
Tras 1 vs 1: unpaired t test, two-tailed P<0.001

Tras 4 vs 4: unpaired t test, two-tailed P<0.001

Tras 4 vs {Au(mba)(PPh3)]: unpaired t test, two-tailed P<0.001



Figures S36. Viability of selected compounds and conjugates in MCF7 cell lines. Results are shown as a percentage and correspond to 2 biological replicates (mean±s.d.).



Figures S37-38. Viability of selected compounds and conjugates in BT474 and MCF10A (from top to bottom) cell lines. Results are shown as a percentage and correspond to 2 biological replicates (mean±s.d.).

References:

(1) R. Usón, A. Laguna and M. Laguna, *Inorg. Synth.*, 1989, **26**, 85.
(2) D.V. Partyka, J.B. Updegraff III, M. Zeller, A. D. Hunter and T. G. Gray, *Organometallics*, 2007, **26**, 183.
(3) A. C. J. Heinrich, *Synlett*, 2015, **26**, 1135.
(4) J. Fernandez-Gallardo, B. T. Elie, T. Sadhuka, S. Prabha, M. Sanau, S.A. Rotenberg, J.W. Ramos and M. Contel, *Chem. Sci.* 2015, **6**, 5269.
(5) L. Chen, L. Wang, H. Shion, C. Yu, Y.Q. Yu, L. Zhei, M. Li, W. Chen and K. Gao. *MABS*, **2016,** *8*, 1210.
(6) M. Barok, H. Joensuu, and J. Isola. *Breast Cancer Res*. **2014**, 16:09.
(7) M.T. Kim, Y. Chen, J. Marhoul and F. Jacobson. *Bioconjugate Chem*. **2014**, *25*, 1223.
(8) J.P. Owings, N.N. McNair, Y.F. Mui, T.N. Gustafsoon, A.Holmgren, M. Contel, J.B. Goldberg and J.R. Mead. *FEMS Microbiology Letters*, **2016**, *363*, fnw148