Electronic Supporting Information (ESI)

Photoactive chelates for radiolabelling proteins

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Experimental methods

General details

Unless otherwise stated, all chemicals were of reagent grade and purchased from SigmaAldrich (St. Louis, MO), Merck (Darmstadt, Germany), Tokyo Chemical Industry (Eschborn, Germany), abcr (Karlsruhe, Germany) or CheMatech (Dijon, France). Water (>18.2 MΩ·cm at 25 ºC, Puranity TU 3 UV/UF, VWR International, Leuven, Belgium) was used without further purification. Solvents for reactions were of reagent grade. Evaporation of the solvents was performed under reduced pressure by using a rotary evaporator (Rotavapor R-300, Büchi Labortechnik AG, Flawil, Switzerland) at the specified temperature and pressure or by using a high vacuum pump.

^{1}H and ^{13}C NMR spectra were measured in deuterated solvents on a Bruker AV-500 (^{1}H: 500 MHz, ^{13}C: 125.8 MHz) spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to the resonance of the residual solvent peaks, for example, with $CDCl_3$ δ_H = 7.26 ppm and δ_C = 77.2 ppm with respect tetramethylsilane (TMS, δ_H and δ_C = 0.00 ppm). Coupling constants (*J*) are reported in Hz. All resonances were assigned by using a combination of 1D and 2D NMR (HSQC, COSY) spectra. Peak multiplicities are abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), quint. (quintet), m (multiplet), and br (broad).

High-resolution electrospray ionisation mass spectra (HR-ESI-MS) were measured by the mass spectrometry service at the Department of Chemistry, University of Zurich.

Standard thin-layer chromatography (TLC) for synthesis employed Merck TLC plates silica gel *60* on an aluminium base with the indicated solvent system. The spots on TLC were visualised either by UV/visible light (254 nm) or by staining with $KMnO_4$.

Semi-preparative high-performance liquid chromatography (HPLC) purifications were performed using a Rigol HPLC system (Contrec AG, Dietikon, Switzerland) equipped with a C18 reverse-phase column (VP 250/21 Nucleodur C18 HTec, 21 mm ID x 250 mm, 5µm).

Analytical ultra-high-performance liquid chromatography (UHPLC) experiments were performed using a Hitachi Chromaster Ultra Rs system fitted with a reverse phase EC 250/4 Nucleodur C18 HTec (4 mm ID x 250 mm, 5µm) column. UHPLC used a flow rate of 1 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-2 min., 80% A; *t* = 12 min., 5% A; *t* = 14 min., 5% A; *t* = 16 min., 80% A; *t* = 18 min., 80% A). The system was also connected to a radioactivity detector (FlowStar^2 LB 514, Berthold Technologies, Zug, Switzerland) equipped with a 20 µL PET cell (MX-20-6, Berthold Technologies) for analysing radiochemical reactions. Proteins were analysed by using a Rigol HPLC system (Contrec AG, Dietikon, Switzerland) equipped with a size-exclusion column (Enrich SEC 70 column: 24 mL volume, 10 ± 2 µm particle size, 10 mm ID x 300 mm, Bio-Rad Laboratories, Basel, Switzerland).

Protein concentration was determined using a Nanodrop^{TM} One^{C} Microvolume UV-Vis Spectrophotometer (ThermoFisher Scientific, supplied by Witec AG, Sursee, Switzerland) in accordance with the manufacturer's protocol.

Photochemistry

Photochemical conjugation experiments were performed in transparent glass vials at the indicated concentrations. Stock solutions of trastuzumab and the different photoactivatable chelates (compounds **1**, **3** or **4**) were prepared in H_2O. Reaction mixtures were irradiated for the indicated reaction times at 23 °C without stirring using a portable, high-powered, light-emitting diode (LED, 365 nm). The LED intensity (set to 100% power, approximately 263 mW) was adjusted using a UV-LED controller (Opsytec Dr. Gröbel GmbH, Ettlingen, Germany). Note that calculation of exact power transferred to the reaction is non-trivial because it depends on the specific geometry of the experiment. The LED (365 nm) had a maximum emission intensity at 364.5 nm (FWHM of 9.1 nm).

Radiochemistry

All instruments for measuring radioactivity were calibrated and maintained in accordance with previously reported routine quality control procedures.[1] [^{68}Ga][Ga(H_2O)$_6$]Cl_3(aq.) was obtained from a ^{68}Ge/^{68}Ga-generator (Eckert&Ziegler, Model IGG100 Gallium-68 Generator), eluted with 0.1 M HCl(aq.). The eluted ^{68}Ga activity was trapped and purified by using a strong cation exchange column (Strata-XC, [SCX], Eckert&Ziegler). [^{68}Ga][Ga(H_2O)$_6$]Cl_3(aq.) was eluted from the SCX cartridge by using a solution containing 0.13 M HCl(aq.) and approx. 5 M NaCl(aq.) (SCX eluent). The generator yielded a ^{68}Ga stock solution with a measured molar activity of 201 ± 6.17 MBq/nmol. For radiolabelling experiments the ^{68}Ga stock solution was typically added as the limiting reagent to an aqueous reaction mixture buffered with NaOAc (≥0.2 M, pH4.4). Radioactive reactions were monitored by using instant thin-layer chromatography (iTLC). Glass-fibre iTLC plates impregnated with silica-gel (iTLC-SG, Agilent Technologies) were developed in citrate buffer (pH4.5, 0.2 M) and analysed on a radio-TLC detector (SCAN-RAM, LabLogic Systems Ltd, Sheffield, United Kingdom). Radiochemical conversion (RCC) was determined by integrating the data obtained by the radio-TLC plate reader and determining both the percentage of radiolabelled product (R_f = ca. 0.06 – 0.20) and 'free' ^{68}Ga (R_f = 1.0; present in the analyses as [^{68}Ga]Ga-citrate). Integration and data analysis were performed by using the software Laura version 5.0.4.29 (LabLogic).

Radiochemical purities (RCPs) of labelled protein samples were determined by size-exclusion chromatography (SEC) using two different columns and techniques. The first technique used an automated size-exclusion column (Bio-Rad Laboratories, ENrich SEC 70) connected to a Rigol HPLC system (Contrec AG, Dietikon, Switzerland) equipped with a UV/visible diode array detector, and a radioactivity detector (FlowStar^2 LB 514, Berthold Technologies, Zug, Switzerland). Isocratic elution with phosphate buffered saline (PBS, pH7.4) was used at a flow rate of 0.7 mL min^{-1}. The second method used a manual procedure involving size-exclusion column chromatography using a PD-10 desalting column (Sephadex G-25 resin, 85-260 µm, 14.5 mm ID x 50 mm, >30 kDa, GE Healthcare). For analytical procedures, PD-10 columns were eluted with PBS (pH7.4). A total of 40 x 200 µL fractions were collected up to a final elution volume of 8 mL. Note that the loading/dead-volume of the PD-10 columns is precisely 2.50 mL. For quantification of radioactivity, each fraction was measured on a gamma counter (HIDEX Automatic Gamma Counter, Hidex AMG, Turku, Finland) using an energy window between 480 – 558 keV for ^{68}Ga (511 keV emission) and a counting time of 30 s. Appropriate background and decay corrections were applied throughout. PD-10-SEC columns were also used for preparative purification and reformulation of radiolabelled products by collecting a fraction of the eluate corresponding to the high molecular weight protein (>30 kDa fraction eluted in the range between 0.0 – 1.6 mL).

Synthesis and characterisation data

Synthetic details and characterisation data for the intermediate *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-4-azidobenzamide, N_3-PEG_3-NH_2 were reported elsewhere.[2]

Synthesis of 2,2'-(7-(1-(4-azidophenyl)-1,17-dioxo-6,9,12-trioxa-2,16-diazaoctadecan-18-yl)-1,4,7-triazonane-1,4-diyl)diacetic acid, NOTA-PEG_3-ArN_3 (**1**)

To a stirred solution of NOTA-NHS ester (CheMatech, [used as the HPF_6 and TFA salt], 50 mg, 0.074 mmol) in dry DMF (2 mL) were added NEt_3 (36 mg, 0.360 mmol) and N_3-PEG_3-NH_2 (27 mg, 0.075 mmol). The clear, slightly yellow solution was stirred for 42 h at 23 °C under an N_2 atmosphere and protected from light. After evaporation of the solvent using a high vacuum pump the crude product was purified by semi-preparative HPLC (Nucleodur VP 250/21, 21 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 7 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-6 min., 80% A; *t* = 27 min., 5% A; *t* = 33 min., 5% A; *t* = 36 min., 80% A; *t* = 40 min., 80% A). Compound **1** was obtained as a white solid (18 mg, 0.078 mmol, 37%). ^{1}H NMR (D_2O, 500 MHz): δ (ppm) 7.76 (2H, arom., d, *J* = 8.4); 7.18 (2H, arom., d, *J* = 8.0); 3.92 (4H, br. s, 2 x NOTA-NC*H*$_2$COOH); 3.73 (2H, br. s, NOTA-NC*H*$_2$CONH); 3.67 – 3.58 (10H, m, 5 x CH_2, (OC*H*$_2$C*H*$_2$)$_2$OC*H*$_2$$CH_2$$CH_2$NHCOPh); 3.51 (2H, t, *J* = 6.6, CH_2CONHCH$_2$CH$_2$C*H*$_2$); 3.46 (2H, t, *J* = 6.8, PhCONHC*H*$_2$); 3.34 – 3.28 (8H, m, 4 x CH_2, NOTA moiety, NCH_2CH_2N); 3.25 (2H, t, *J* = 7.3, CH_2CONHC*H*$_2$$CH_2$); 3.16 (4H, m, 2 x CH_2, NOTA moiety, NCH_2CH_2N); 1.89 (2H, quint., *J* = 6.4, PhCONHCH$_2$C*H*$_2$); 1.75 (2H, quint., *J* = 6.7, CH_2CONHCH$_2$C*H*$_2$). ^{13}C{^{1}H} (D_2O; 125.8 MHz): δ (ppm) 171.9, 170.4, 169.6 (4 CO); 143.6, 130.0 (2C, arom.); 128.8, 119.1 (4C, arom., 4 x CH); 69.6, 69.5, 69.3, 69.3, 68.8 (5C, 5 x CH_2, (OC*H*$_2$C*H*$_2$)$_2$OC*H*$_2$$CH_2$$CH_2$NHCOPh); 68.3 (1C, CH_2CONHCH$_2$$CH_2$C*H*$_2$); 58.2 (1C, NOTA-NC*H*$_2$CONH); 56.1 (2C, 2 x NOTA-NC*H*$_2$COOH); 50.4, 49.9 (6C, 6 x CH_2, NOTA moiety, NC*H*$_2$C*H*$_2$N); 37.3 (1C, PhCONHC*H*$_2$); 36.5 (1C, CH_2CONHC*H*$_2$); 28.3 (2C, 2 x CH_2, CH_2CONHCH$_2$C*H*$_2$ + PhCONHCH$_2$C*H*$_2$). HR-ESI-MS (H_2O): *m/z* calc. for $[M+H]^+$ 651.34605, found 651.34630 (100%). RP-UHPLC (C18, 254 nm): t_R = 9.36 min. Analytical UHPLC method: Nucleodur EC 250/4, 4 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 1 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-2 min., 80% A; *t* = 12 min., 5% A; *t* = 14 min., 5% A; *t* = 16 min., 80% A; *t* = 18 min., 80% A.

Figure S1. Reverse-phase UHPLC chromatogram of compound **1** (λ = 254 nm).

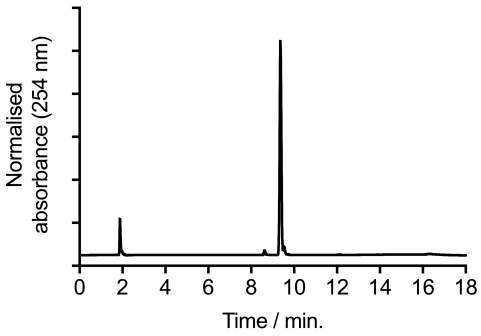


Figure S2. HR-ESI-MS data for compound **1**, *m*/*z* $[M+H]^+$ = 651.34630 (100%).

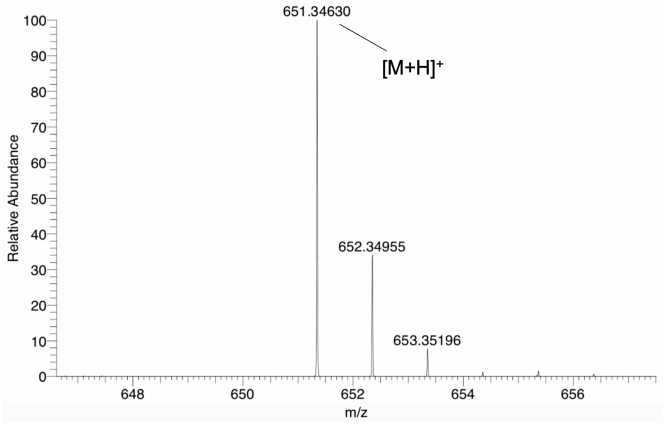


Figure S3. ^{1}H NMR spectrum (D_2O, 500 MHz) of compound **1**.

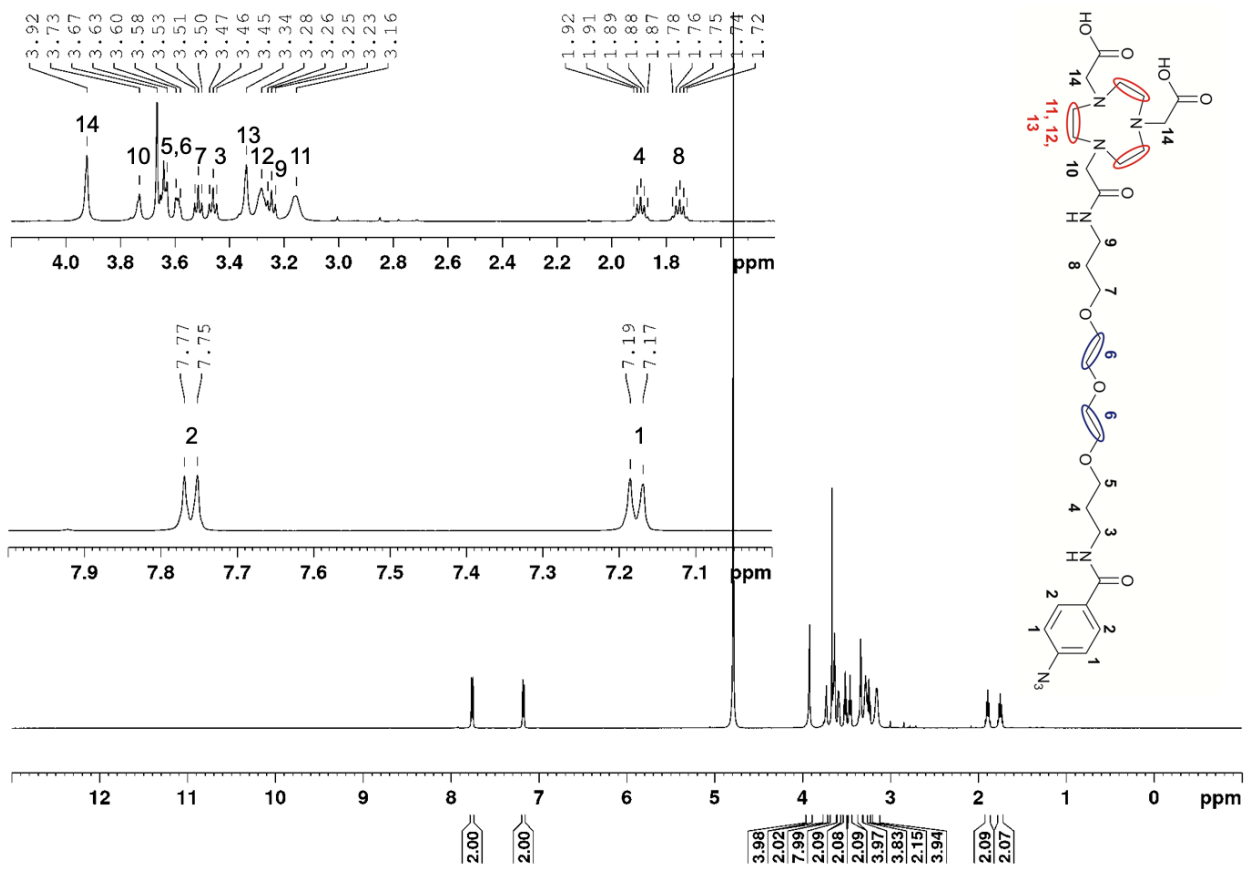


Figure S4. $^{13}C\{^{1}H\}$ NMR spectrum (D_2O, 125.8 MHz) of compound **1**.

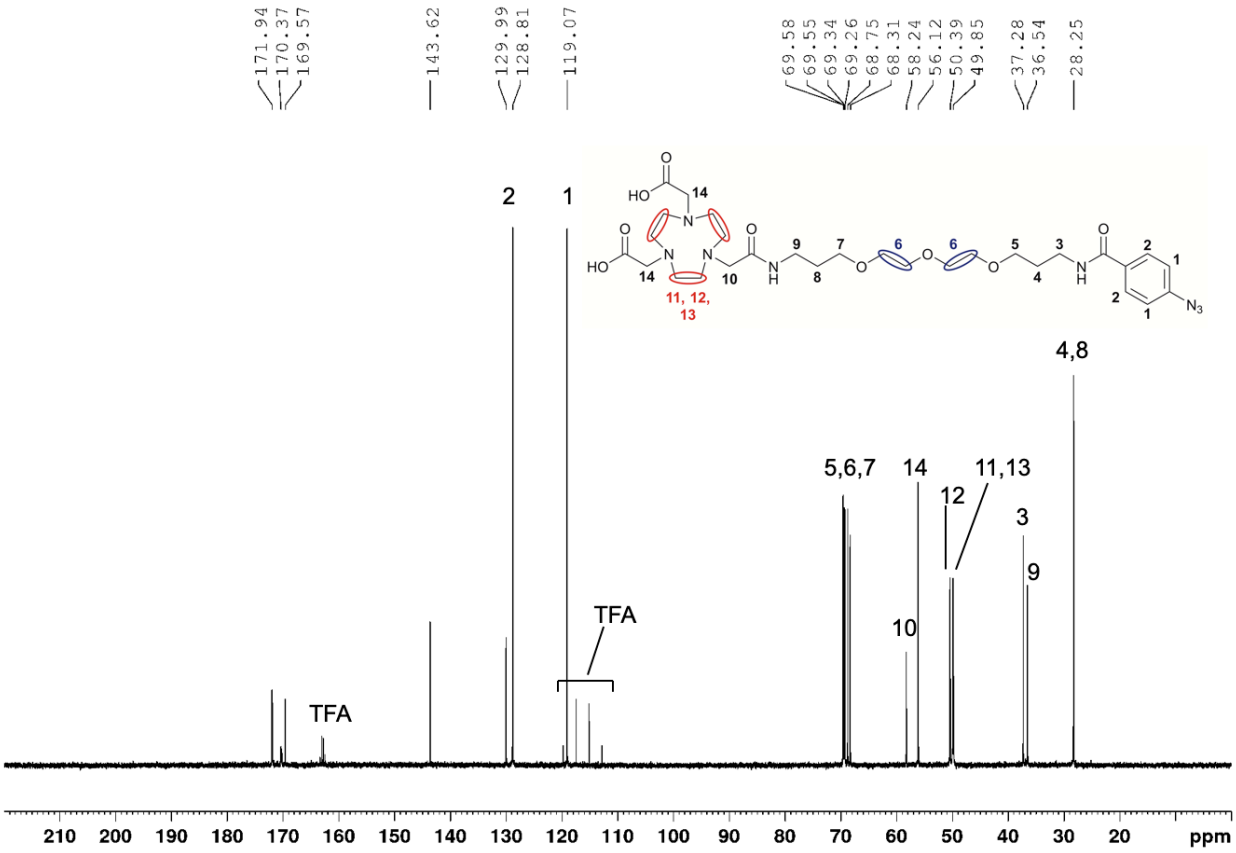


Figure S5. 2D-COSY spectrum (D_2O) of compound **1** (NOTA-PEG_3-ArN_3). (A) full spectrum, (B) zoom of the region between δ_H = 1.0 – 4.0 ppm.

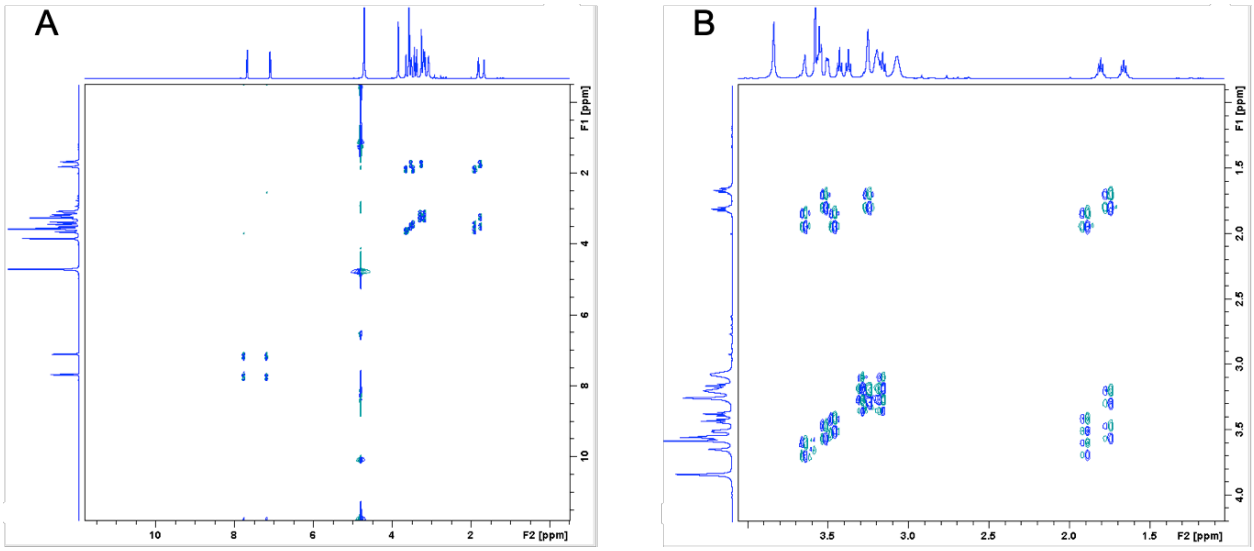
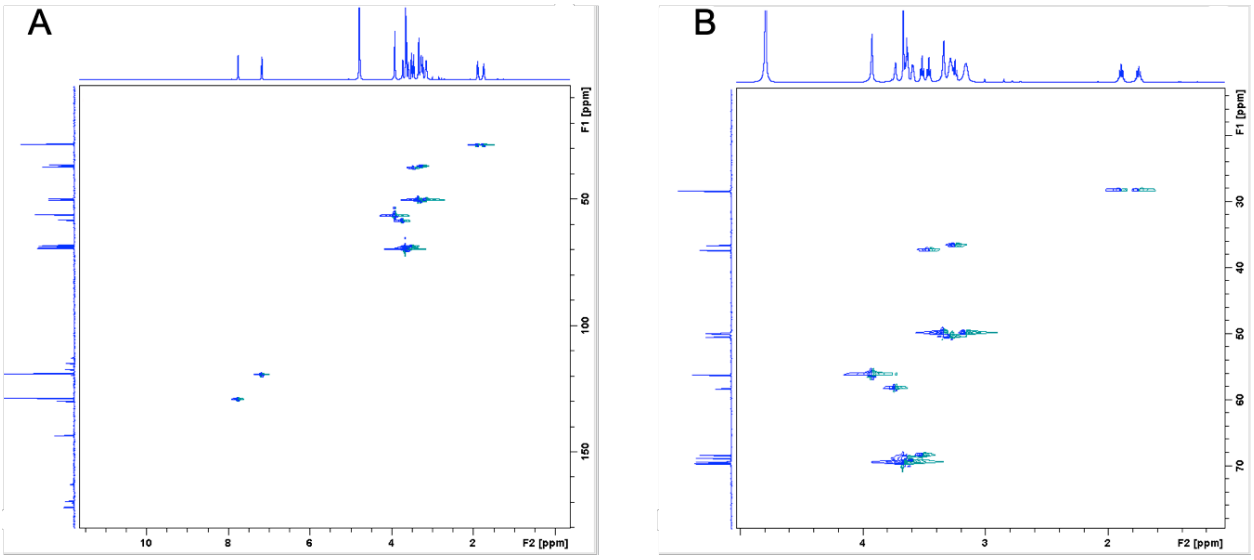


Figure S6. 2D-heteronuclear HSQC NMR spectrum (D_2O) of compound **1** (NOTA-PEG_3-ArN_3). (A) full spectrum, (B) zoom of the region between δ_H = 1.0 – 5.0 ppm and δ_C = 15 – 80 ppm.



Synthesis of 2,5-dioxopyrrolidin-1-yl 4-azidobenzoate (**2**)

To a stirred solution of 4-azidobenzoic acid (504 mg, 3.087 mmol) and *N*-hydroxysuccinimide (403 mg, 3.501 mmol) in CH_2Cl_2 (25 mL) was added EDC hydrochloride (667 mg, 3.479 mmol). The clear, slightly orange solution was stirred for 21 h at 23 ^oC under an N_2 atmosphere and protected from light. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed with H_2O (2 x 100 mL) and brine (1 x 75 mL). The organic layer was separated and dried over $MgSO_4$. Then the solvent was removed under reduced pressure to give compound **2** (quantitative, >90% purity by RP-UHPLC and ^1H NMR) as an off-white solid which was used without further purification. ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.12 (2H, arom., d, *J* = 8.6); 7.13 (2H, arom., d, *J* = 8.0); 2.90 (4H, s). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.8 MHz): δ (ppm) 169.4, 161.2 (3 CO), 147.1 (1C, arom.); 132.6 (2C, arom., 2 x CH); 121.4 (1C, arom.); 119.4 (2C, arom., 2 x CH); 25.8 (2C, 2 x CH_2). HR-ESI-MS (H_2O): *m/z* calc. for $[M+Na]^+$ 283.04378, found 283.04346 (100%). RP-UHPLC (C18, 254 nm): t_R = 11.07 min. Note: the minor peak at 12 min corresponds to 4-azidobenzoic acid which was potentially formed by hydrolysis under the RP-UHPLC conditions. Analytical RP-UHPLC method: Nucleodur EC 250/4, 4 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 1 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-2 min., 80% A; *t* = 12 min., 5% A; *t* = 14 min., 5% A; *t* = 16 min., 80% A; *t* = 18 min., 80% A.

Figure S7. Reverse-phase UHPLC chromatogram of compound **2** (λ = 254 nm).

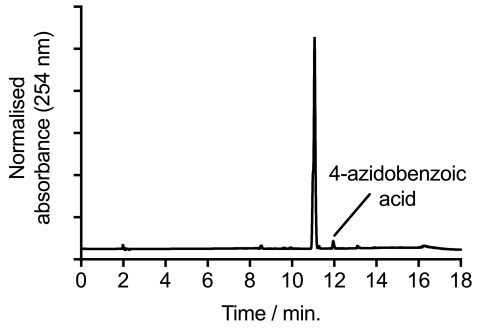


Figure S8. HR-ESI-MS data for compound **2**, *m/z* $[M+Na]^+$ = 283.04346 (100%).

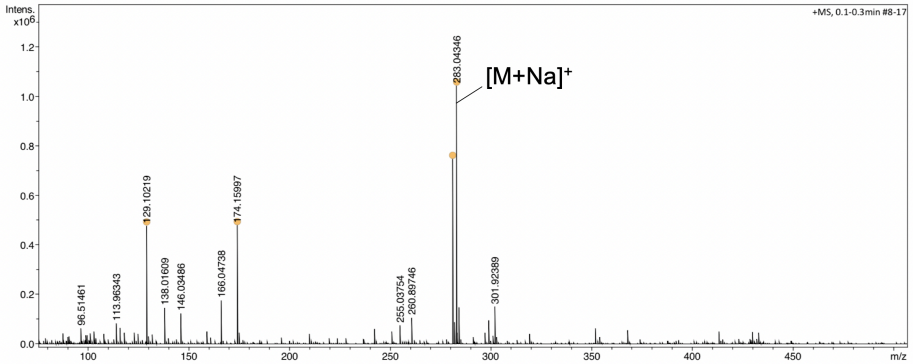


Figure S9. ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound **2**.

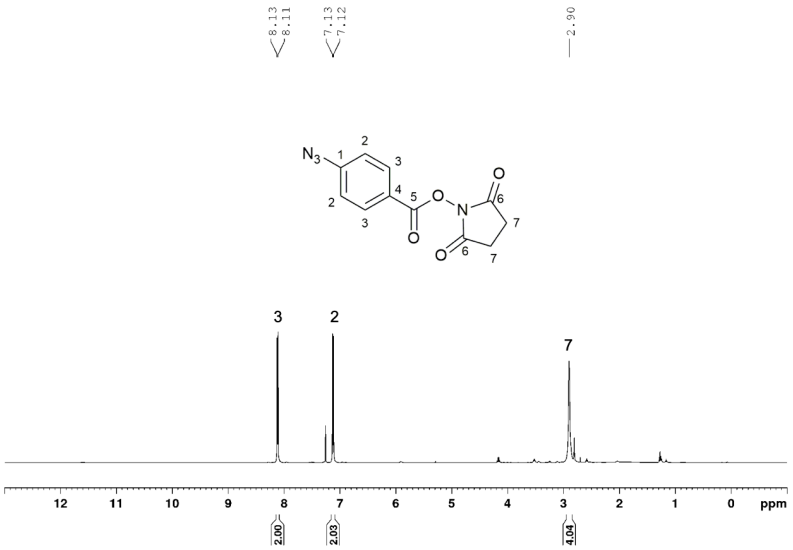
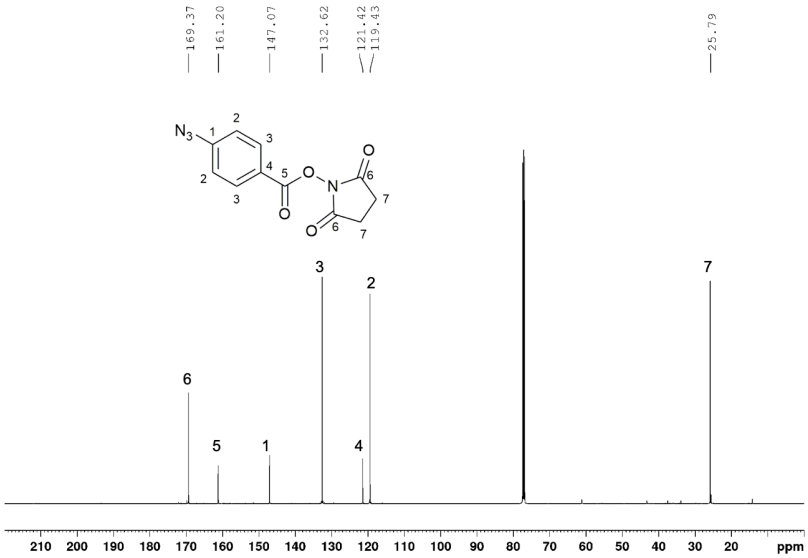


Figure S10. $^{13}C\{^{1}H\}$ NMR spectrum ($CDCl_3$, 125.8 MHz) of compound **2**.



Synthesis of 2,2',2''-(10-(1-(4-azidophenyl)-1,18-dioxo-5,8,11,14-tetraoxa-2,17-diazanonadecan-19-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid, DOTA-PEG_4-ArN_3 (**3**)

To a stirred solution of compound **2** (13 mg, 0.051 mmol) in dry DMF (3 mL) was added NEt_3 (18 µL, 13 mg, 0.130 mmol) and DOTA-PEG_4-NH_2 (CheMatech [used as the HCl salt with 3 equivalents of H_2O present], 31 mg, 0.043 mmol). The clear, colourless solution was stirred for 69 h at 23 °C under an N_2 atmosphere and protected from light. After evaporation of the solvent using a high vacuum pump, the crude product was purified by semi-preparative HPLC (Nucleodur VP 250/21, 21 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 7 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-6 min., 80% A; *t* = 27 min., 5% A; *t* = 33 min., 5% A; *t* = 36 min., 80% A; *t* = 40 min., 80% A). Compound **3** was obtained as a white solid (29 mg, 0.038 mmol, 89%). ^{1}H-NMR (D_2O, 313 K; 500 MHz): δ (ppm) 7.93 (2H, arom., d, *J* = 7.6), 7.31 (2H, arom., d, *J* = 8.1); 4.14 (4H, m), 3.87 – 3.73 (22H, m), 3.53 – 3.36 (18H, m). $^{13}C\{^1H\}$ NMR (D_2O, 313 K; 125.8 MHz): δ (ppm) 172.6 (br.), 169.8 (5 CO); 143.8, 130.1 (2C, arom.); 129.1, 119.3 (4C, arom. 4 x CH); 69.7, 69.6, 69.4, 69.0, 68.8, 55.0, 54.5 (br.), 53.6 (br.), 50.9 (br.), 49.1 (br.), 39.7, 39.1. HR-ESI-MS (MeCN): *m/z* calc. for $[M+2H]^{2+}$ 384.69796, found 384.69813 (100%); *m/z* calc. for $[M+H]^+$ 768.38864, found 768.38737; *m/z* calc. for $[M+Na]^+$ 790.37059, found 790.36972. RP-UHPLC (C18, 254 nm): t_R = 8.25 min. Analytical RP-UHPLC method: Nucleodur EC 250/4, 4 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 1 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-2 min., 80% A; *t* = 12 min., 5% A; *t* = 14 min., 5% A; *t* = 16 min., 80% A; *t* = 18 min., 80% A.

Figure S11. Reverse-phase UHPLC chromatogram of compound **3** (λ = 254 nm).

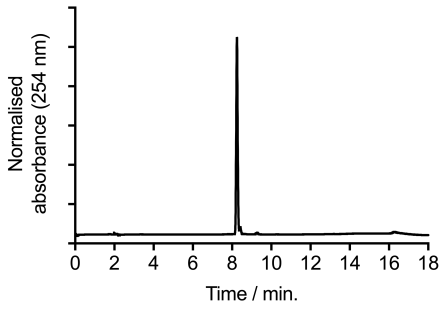


Figure S12. HR-ESI-MS data for compound **3**, *m*/*z* $[M+2H]^{2+}$ = 384.69813 (100%).

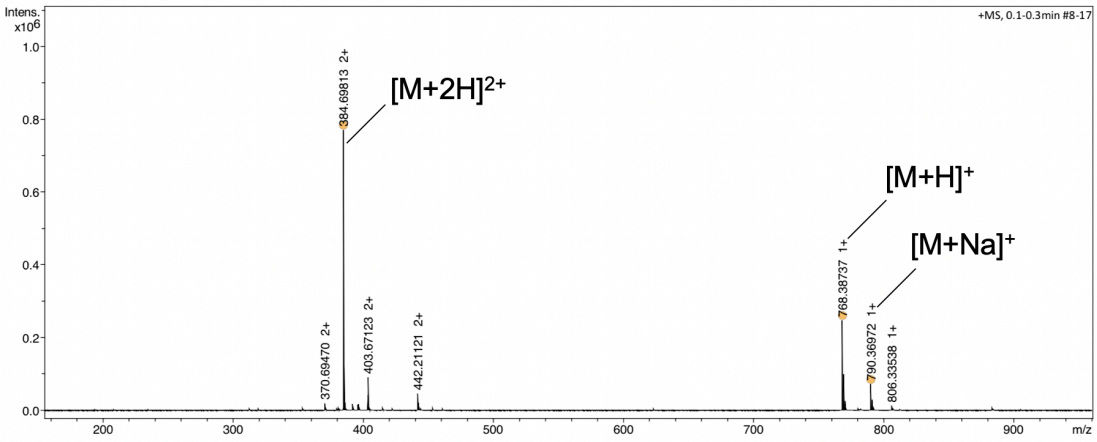


Figure S13. ^{1}H NMR spectrum (D_2O, 500 MHz) of compound **3**.

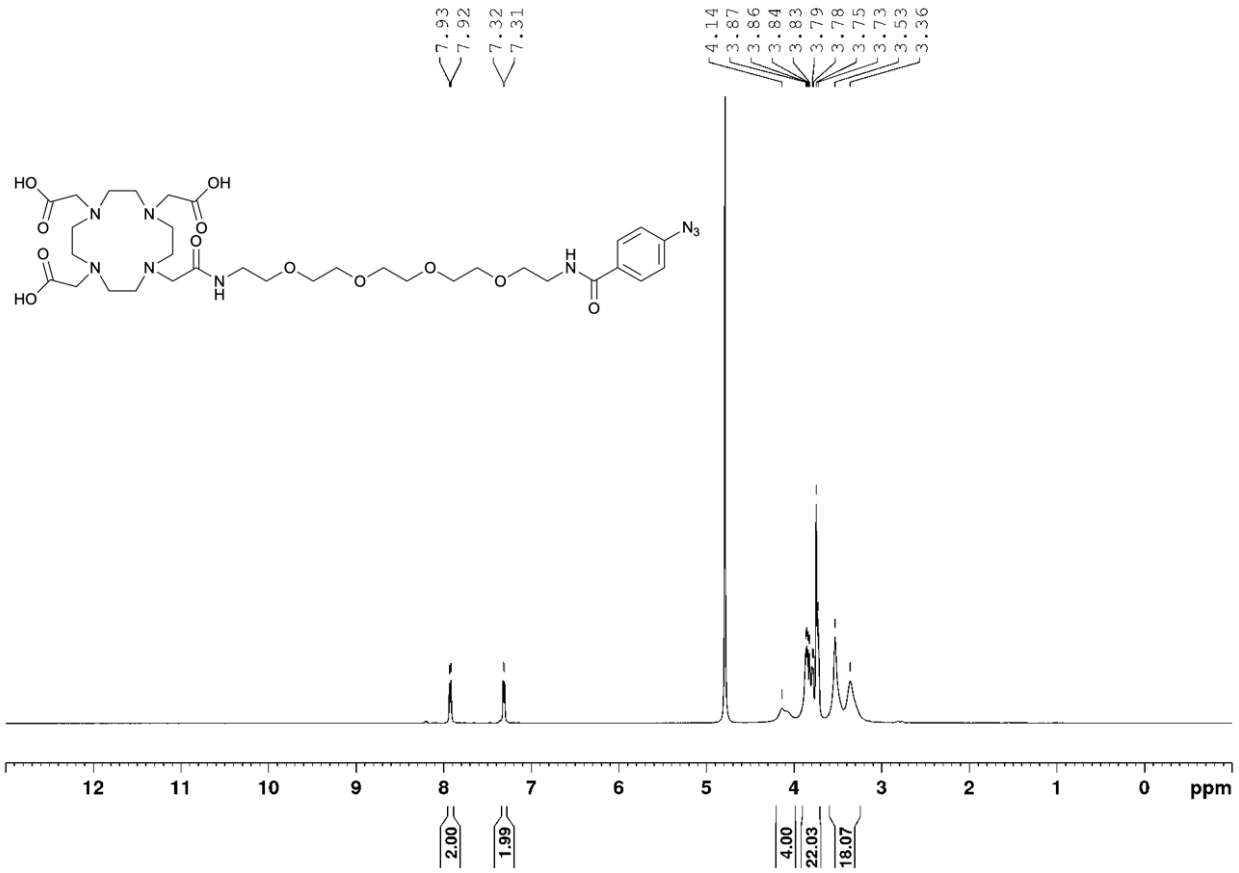
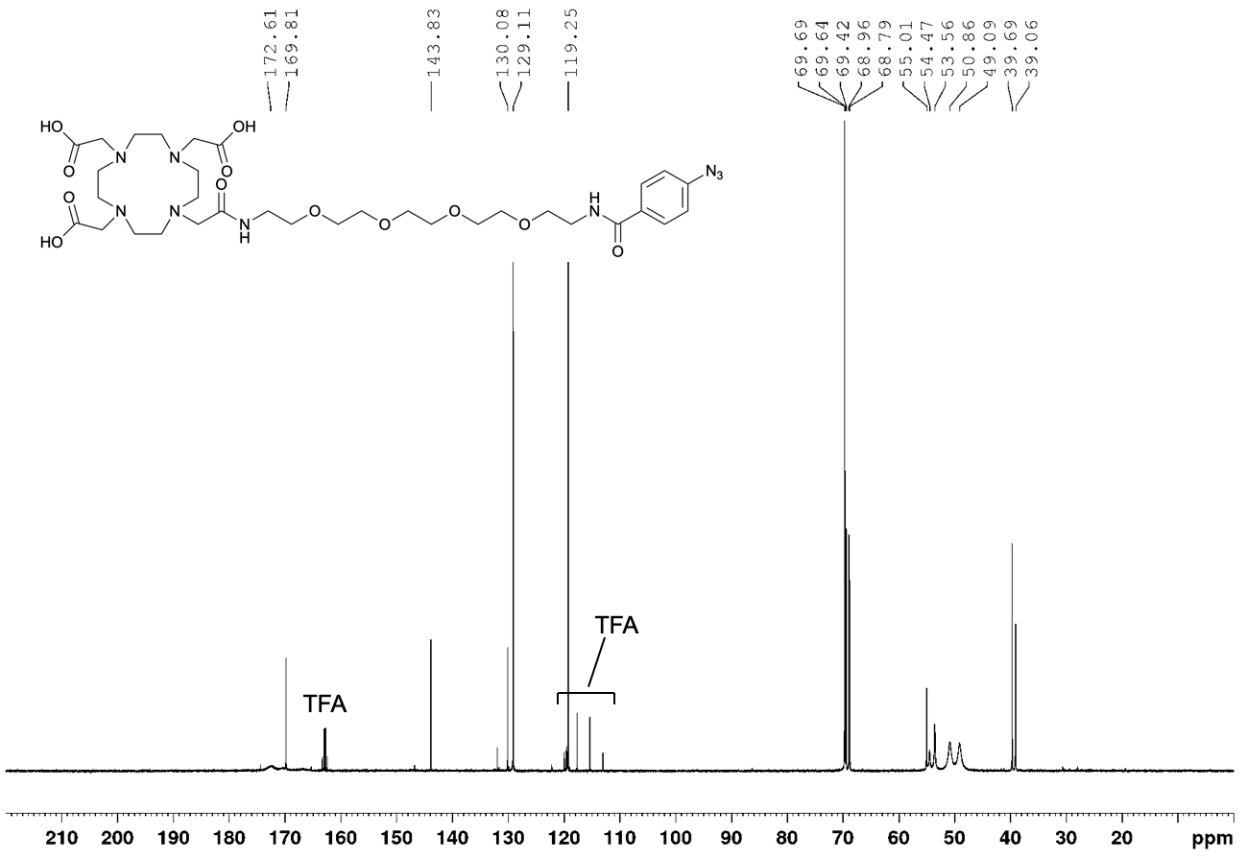


Figure S14. $^{13}C\{^{1}H\}$ NMR spectrum (D_2O, 125.8 MHz) of compound **3**.



Synthesis of 2,2',2''-(10-(1-(4-azidophenyl)-21-carboxy-1,18-dioxo-5,8,11,14-tetraoxa-2,17-diazahenicosan-21-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid, DOTAGA-PEG_4-ArN_3 (**4**)

To a stirred solution of 4-azidobenzoic acid (4 mg, 0.024 mmol) and HATU (13 mg, 0.034 mmol) in dry DMF (0.5 mL) was added DIPEA (7.5 µL, 6 mg, 0.043 mmol). After 1.5 h at 23 °C under N_2 atmosphere and protected from light, DOTAGA-PEG_4-NH_2 (CheMatech, [used as the 5xHCl salt and H_2O adduct], 21 mg, 0.023 mmol) was added with dry DMF (0.5 mL), followed by DIPEA (22.4 µL, 17 mg, 0.129 mmol). The slightly opaque, yellow reaction mixture was stirred at 23 °C for 44 h. Evaporation of the solvent using a high vacuum pump afforded an orange, oily residue. Upon addition of ice cold acetone (1 mL) followed by sonication, a white precipitate was formed, which was isolated by centrifugation. The crude product was purified by semi-preparative HPLC (Nucleodur VP 250/21, 21 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 7 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-4 min., 80% A; *t* = 24 min., 5% A; *t* = 28 min., 5% A; *t* = 32 min., 80% A; *t* = 36 min., 80% A). Compound **4** was obtained as a white solid (5 mg, 0.007 mmol, 29%). ^{1}H NMR (D_2O; 313 K; 500 MHz): δ (ppm) 7.89 (2H, arom., d, *J* = 8.6), 7.29 (2H, arom., d, *J* = 8.6); 4.08 – 4.01 (4H, m), 3.92 – 3.10 (39H, m), 2.59 – 2.57 (2H, m), 2.07 – 2.06 (2H, m). $^{13}C\{^1H\}$ NMR (D_2O; 313 K; 125.8 MHz): δ (ppm) 175.1, 174.4 (br.), 173.3 (br.), 170.0 (6 CO); 143.9, 130.2 (2C, arom.); 129.1, 119.3 (4C, arom. 4 x CH); 69.73, 69.72, 69.70, 69.67, 69.5, 69.0, 68.9, 59.8 (br.), 55.4 (br.), 53.7 (br.), 51.5 (br.), 51.1 (br.), 49.2 (br.), 48.0 (br.), 45.7 (br.), 44.6 (br.), 39.7, 39.1, 33.1, 22.2 (br.). HR-ESI-MS (H_2O): *m/z* calc. for $[M+H]^+$ 840.40977, found 840.40822 (100%). RP-UHPLC (C18, 254 nm): t_R = 8.41 min. Analytical RP-UHPLC method: Nucleodur EC 250/4, 4 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 1 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-2 min., 80% A; *t* = 12 min., 5% A; *t* = 14 min., 5% A; *t* = 16 min., 80% A; *t* = 18 min., 80% A.

Figure S15. Reverse-phase UHPLC chromatogram of compound **4** (λ = 254 nm).

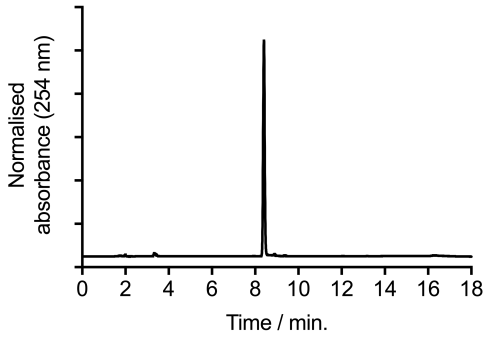


Figure S16. HR-ESI-MS data for compound **4**, *m*/*z* $[M+H]^+$ = 840.40822 (100%).

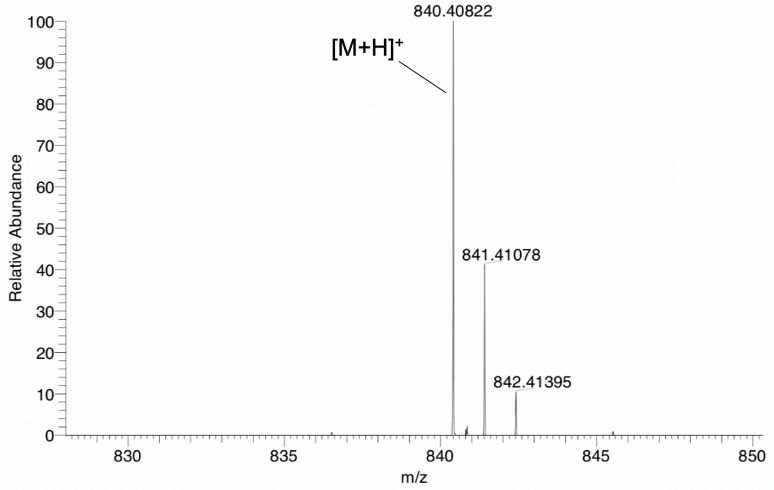


Figure S17. ^{1}H NMR spectrum (D_2O, 500 MHz) of compound **4**.

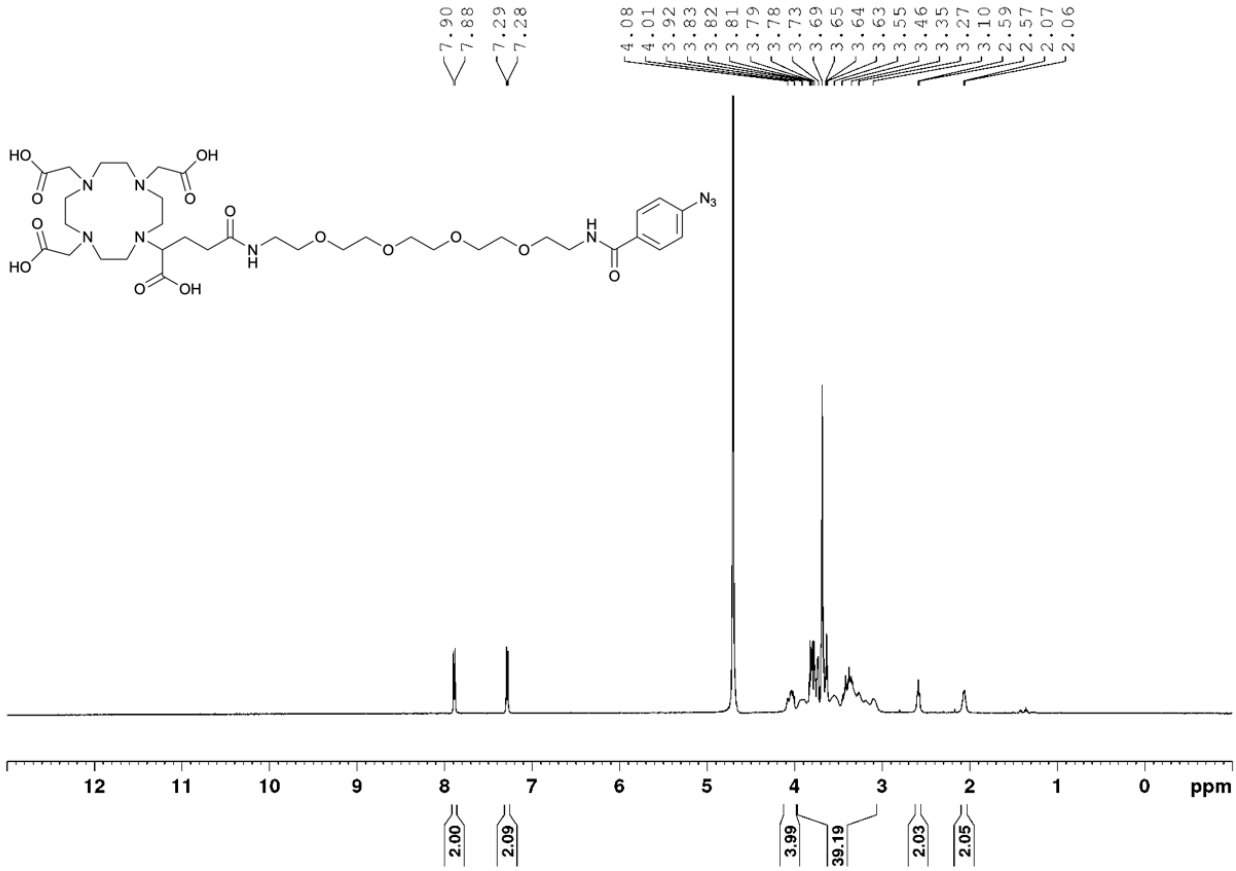
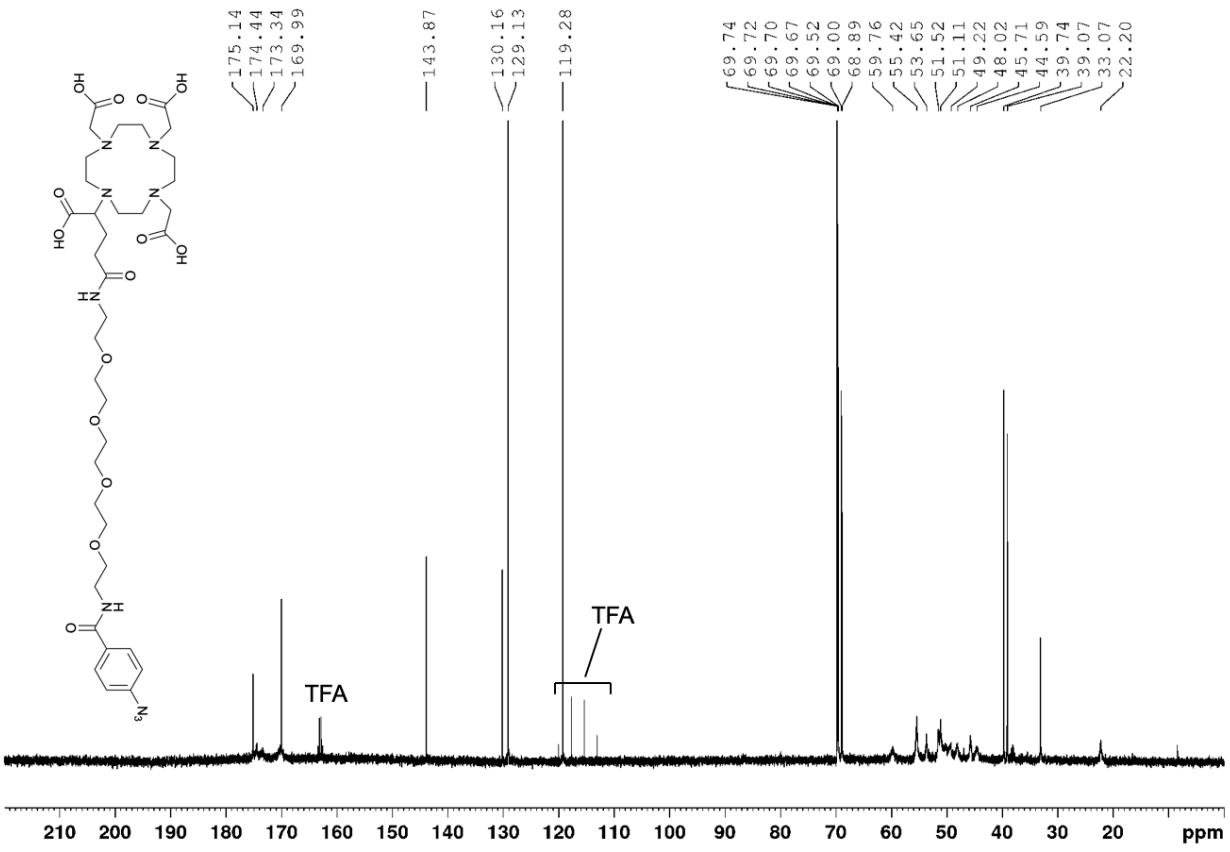


Figure S18. $^{13}C\{^{1}H\}$ NMR spectrum (D_2O, 125.8 MHz) of compound **4**.



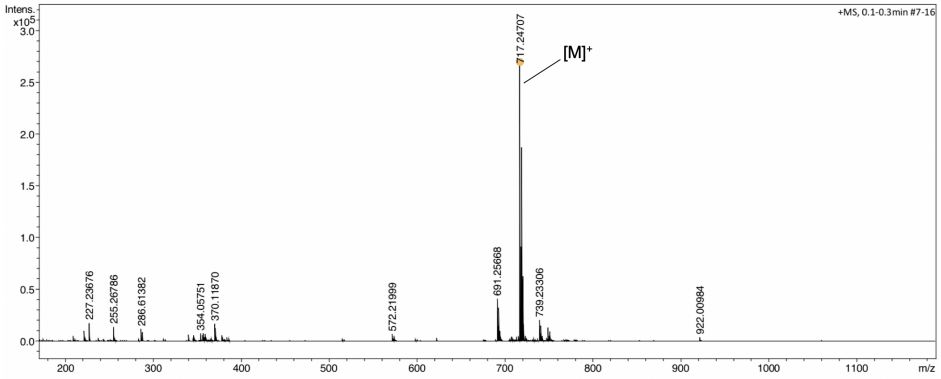
Synthesis of non-radioactive Ga^{3+} complexes

The gallium complexes of compounds **1**, **3** and **4** were synthesised by complexation using $Ga(NO_3)_3$. Briefly, to an aqueous solution of the ligand (10 mg mL^{-1}) in water was added an aliquot of a stock solution of $Ga(NO_3)_3$(aq.) (0.5 mg mL^{-1}). Complexation of Ga^{3+} ions by compound **1**, occurred immediately at room temperature without the need for stirring. Complexation of Ga^{3+} ions by compound **3** was complete after stirring at room temperature for ~18 h. Complexation of Ga^{3+} ions by compound **4** was found to be slower and required heating to 70 ^{o}C for 40 min. Gallium complexes were characterised by high-resolution electrospray ionisation mass spectrometry and by analytical RP-UHPLC (Nucleodur EC 250/4, 4 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 1 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-2 min., 80% A; *t* = 12 min., 5% A; *t* = 14 min., 5% A; *t* = 16 min., 80% A; *t* = 18 min., 80% A).

Characterisation of $[GaNOTA\text{-}PEG_3\text{-}ArN_3]^+$ (Ga-**1**$^+$)

HR-ESI-MS (H_2O): *m/z* calc. for M^+ 717.24815, found 717.24707 (100%). RP-UHPLC (C18, 254 nm): t_R = 8.62 min. Note: the complex has a +1 charge under physiological conditions (pH7.4).

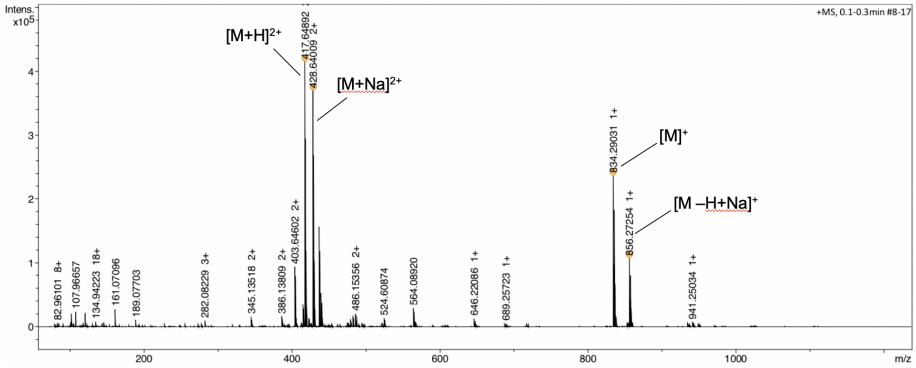
Figure S19. HR-ESI-MS data for complex Ga-**1**$^+$, *m/z* $[M+H]^+$ = 840.40822 (100%).



Characterisation of [GaDOTA-PEG$_4$-ArN$_3$] (Ga-**3**)

HR-ESI-MS (H_2O): *m/z* calc. for $[M+H]^{2+}$ 417.64901, found 417.64892 (100%); *m/z* calc. for $[M+Na]^{2+}$ 428.63998, found 428.64009; *m/z* calc. for M^+ 834.29074, found 834.29031; *m/z* calc. for $[M–H+Na]^+$ 856.27269, found 856.27254. RP-UHPLC (C18, 254 nm): t_R = 8.50 min. Note: the complex is neutral under physiological conditions (pH7.4) because the additional non-coordinated carboxylic acid group is deprotonated.

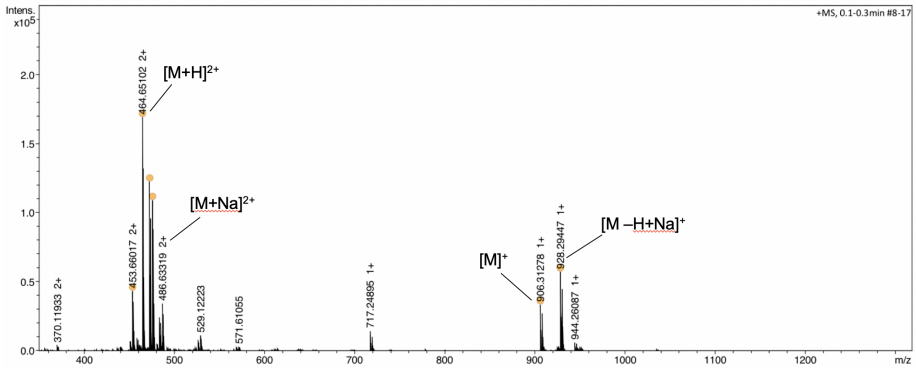
Figure S20. HR-ESI-MS data for complex Ga-**3**, *m/z* $[M+H]^+$ = 840.40822 (100%).



Characterisation of [GaDOTAGA-PEG$_4$-ArN$_3$]$^–$ (Ga-**4**$^–$)

HR-ESI-MS (H_2O): *m/z* calc. for $[M+H]^{2+}$ 453.65957, found 453.66017; *m/z* calc. for $[M+Na]^{2+}$ 464.65055, found 464.65102 (100%); *m/z* calc. for M^+ 906.31187, found 906.31278; *m/z* calc. for $[M–H+Na]^+$ 928.29382, found 928.29447. RP-UHPLC (C18, 254 nm): t_R = 8.71 min. Note: the complex has a –1 charge physiological conditions (pH7.4) because the two non-coordinated carboxylic acid groups are deprotonated.

Figure S21. HR-ESI-MS data for complex Ga-**4**$^–$, *m/z* $[M+H]^+$ = 840.40822 (100%).



Radiochemical synthesis

In all cases, the following analytical RP-UHPLC method was employed: Nucleodur EC 250/4, 4 mm ID x 250 mm (C18 HTec, 5 µm) at a flow rate of 1 mL min^{-1} with a linear gradient of A (distilled H_2O containing 0.1% TFA) and B (MeOH): *t* = 0-2 min., 80% A; t = 12 min., 5% A; *t* = 14 min., 5% A; *t* = 16 min., 80% A; *t* = 18 min., 80% A.

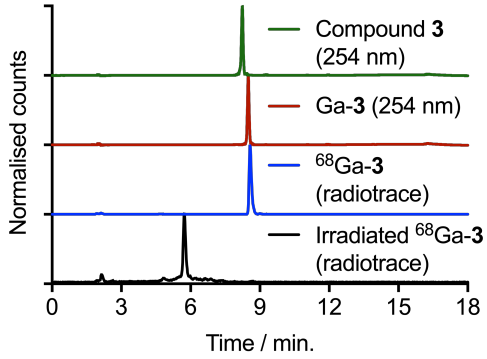
Radiosynthesis of $[^{68}Ga][GaNOTA\text{-}PEG_3\text{-}ArN_3]^+$ (^{68}Ga-**1**$^+$)

Radiolabelling reactions to prepare $[^{68}Ga][GaNOTA\text{-}PEG_3\text{-}ArN_3]^+$ (^{68}Ga-**1**$^+$) were accomplished by addition of an aliquot of $[^{68}Ga][Ga(H_2O)_6]Cl_3$(aq.) stock solution (29.2 MBq) to an aqueous solution of NOTA-PEG_3-ArN_3 (**1**, 100 µg, 1.54 x 10^{-7} mol) buffered with NaOAc (0.20 M, pH 4.4). The reaction was monitored by using radio-iTLC and was found to be complete after 5 min. at 23 °C giving a radiochemical conversion (RCC) >99% (R_f = 0.03 – 0.19). RP-UHPLC: t_R(radioactive trace) = 8.67 min. The identity of the radiolabelled compound ^{68}Ga-**1**$^+$ was confirmed by comparison of UHPLC retention times and by co-injection with an authenticated sample of ^{nat}Ga-**1**$^+$ (see Figure 2, main text).

Radiosynthesis of $[^{68}Ga]GaDOTA\text{-}PEG_4\text{-}ArN_3$ (^{68}Ga-**3**)

Radiolabelling reaction to prepare $[^{68}Ga]GaDOTA\text{-}PEG_4\text{-}ArN_3$ (^{68}Ga-**3**) was accomplished by addition of an aliquot of $[^{68}Ga][Ga(H_2O)_6]Cl_3$(aq.) stock solution (28.4 MBq) to an aqueous solution of DOTA-PEG_4-ArN_3 (**3**, 120 µg, 1.56 x 10^{-7} mol) buffered with NaOAc (0.29 M, pH 4.4). The reaction was monitored by using radio-iTLC and was found to be complete after 10 min. at 70 °C giving a radiochemical conversion (RCC) >99% (R_f = 0.06 – 0.21). RP-UHPLC: t_R(radioactive trace) = 8.57 min. The identity of the radiolabelled compound ^{68}Ga-**3** was confirmed by comparison of UHPLC retention times and by co-injection with an authenticated sample of ^{nat}Ga-**3**.

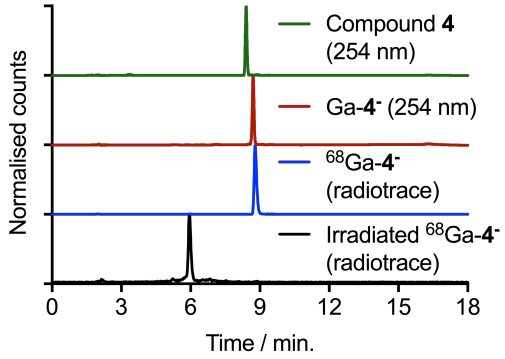
Figure S22. Normalised RP-UHPLC data showing, (green) a single peak for the elution of compound **3**, (red) a single peak observed for non-radioactive complex Ga-**3**, (blue) co-elution of ^{68}Ga-**3** confirming the identity of the radioactive complex, and (black) a single peak formed after irradiation of ^{68}Ga-**3** (365 nm, 15 min.).



Radiosynthesis of [^{68}Ga][GaDOTAGA-PEG$_4$-ArN$_3$]$^+$ (^{68}Ga-**4**$^-$)

Radiolabelling reaction to prepare [^{68}Ga][GaDOTAGA-PEG$_4$-ArN$_3$]$^+$ (^{68}Ga-**4**$^-$) was accomplished by addition of an aliquot of [^{68}Ga][Ga(H$_2$O)$_6$]Cl$_3$(aq.) stock solution (27.3 MBq) to an aqueous solution of DOTAGA-PEG$_4$-ArN$_3$ (**4**, 120 µg, 1.43 x 10^{-7} mol) buffered with NaOAc (0.29 M, pH 4.4). The reaction was monitored by using radio-iTLC and was found to be complete after 5 min. at 70 ^{o}C giving a radiochemical conversion (RCC) >99% (R_f = 0.06 – 0.22). RP-UHPLC: t_R(radioactive trace) = 8.80 min. The identity of the radiolabelled compound ^{68}Ga-**4**$^-$ was confirmed by comparison of UHPLC retention times and by co-injection with an authenticated sample of ^{nat}Ga-**4**$^-$.

Figure S23. Normalised RP-UHPLC data showing, (green) a single peak for the elution of compound **4**, (red) a single peak observed for non-radioactive complex Ga-**4**$^-$, (blue) co-elution of ^{68}Ga-**4**$^-$ confirming the identity of the radioactive complex, and (black) a single peak formed after irradiation of ^{68}Ga-**4**$^-$ (365 nm, 30 min.).



Photochemical reactivity tests

The photochemical reactivity of radiolabelled compounds ^{68}Ga-**1**$^+$, ^{68}Ga-**3**, and ^{68}Ga-**4**$^-$ was then evaluated by irradiating aliquots of the characterised compounds using the LED source (100% intensity, 365 nm) for between 10 – 30 min. at room temperature without stirring. The formation of photodegradation products was followed by UHPLC using the same method applied for the non-radioactive ^{nat}Ga and radioactive ^{68}Ga-complexes, as well as the free ligands (*vide supra*). For each complex, photochemical degradation was found to be complete within ~15 min. giving a one major degradation product that was observed by radioactive UHPLC.

One-pot photochemical conjugation and radiolabelling of trastuzumab

One-pot photoradiochemistry using NOTA-PEG_3-ArN_3 (**1**)

To a solution of NOTA-PEG_3-ArN_3 (**1**) (50 µg, 7.68 x 10^{-8} mol, 1.02 mM) buffered with NaOAc (0.53 M, pH4.4) was added [^{68}Ga][$Ga(H_2O)_6$]Cl_3(aq.) stock solution (30.8 ± 4.3 MBq, n = 3) resulting in a total reaction volume of 75 µL. Reactions were monitored by radio-iTLC. Formation of [^{68}Ga][GaNOTA-PEG_3-ArN_3]$^+$ (^{68}Ga-**1**$^+$) was complete after 5 min. incubation at 23 °C with radiochemical conversion (RCC) >99% (n = 3, R_f = 0.03 – 0.19 on iTLC). The pH of the reaction mixture was then adjusted to >7.5 by the addition of an aqueous solution of $NaHCO_3$ (1.0 M, 50 µL added). After adjusting the pH, an aliquot of pre-purified trastuzumab (1.015 mg, 7.00 x 10^{-9} mol, reaction concentration = 6.3 mg/mL) was added to give an initial chelate-to-trastuzumab ratio of 11.0 at the start of the photochemical conjugation step (total reaction volume ~160 µL). The reaction mixture was then irradiated using the LED (100% intensity, 365 nm) for 15 min. at room temperature without stirring. Aliquots of this crude reaction mixture were then analysed by using radio-iTLC, PD-10-SEC and SEC-UHPLC analysis.

Radio-iTLC analyses of the crude reactions after irradiation showed that ~30% (n = 3) of the radioactivity was bound to the antibody (R_f = 0.0). Note: integration of these radio-iTLC data is unreliable because the radiolabelled antibody fraction partially overlaps with the peak associated with ^{68}Ga-**1**$^+$ and the photodegraded ^{68}Ga-**1**$^+$ species (R_f = 0.03 – 0.19). Nevertheless, analytical PD-10-SEC measurements on the crude reaction mixtures confirmed this observation with an estimated RCP of 15.9 ± 1.8% (n = 3). Equivalent decay corrected SEC-UHPLC measurements indicated that the radiolabelled fraction of [^{68}Ga]GaNOTA-azepin-trastuzumab in the crude mixture was 15.5 ± 1.5% (n = 3).

Crude reaction mixtures were then purified by preparative PD-10-SEC eluting with PBS (collecting only the high purity 0.0 – 1.6 mL fraction). Prior to analysis, samples were concentrated using an Amicon Ultra-4 mL centrifugal filter (Millipore, 30 kDa MWCO, 4000 RPM, ~10 min.). The purified and formulated [^{68}Ga]GaNOTA-azepin-trastuzumab products (pH7.4) were obtained in <25 min. with decay corrected radiochemical yields (RCY) of 10.1 ± 0.7% (n = 3). The estimated lower limit on the molar activity (A_m / [MBq/nmol] of protein) of the formulated [^{68}Ga]GaNOTA-azepin-trastuzumab samples was 0.46 ± 0.09 MBq/nmol (n = 3, remeasured protein concentration). Purified products were then reanalysed by radio-iTLC, analytical PD-10-SEC and SEC-UHPLC. The RCP of purified [^{68}Ga]GaNOTA-azepin-trastuzumab was >99% (n = 3) by radio-iTLC, 91.3 ± 4.4% (n = 3) by analytical PD-10-SEC, and 95.2 ± 2.0% (n = 3) by SEC-UHPLC.

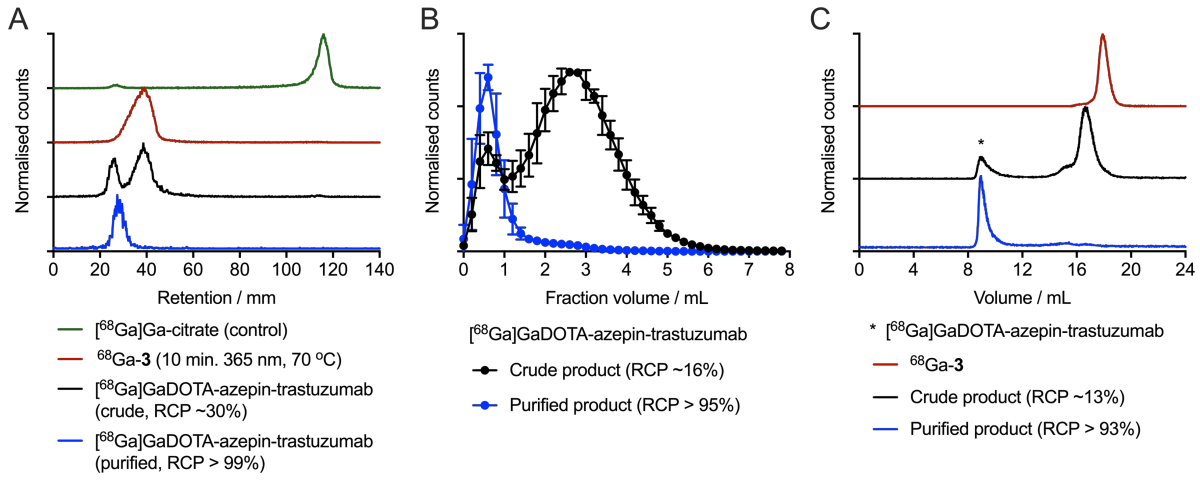
One-pot photoradiochemistry using DOTA-PEG_4-ArN_3 (**3**)

To a solution of DOTA-PEG_4-ArN_3 (**3**) (60 µg, 7.81 x 10^{-8} mol, 1.03 mM) buffered with NaOAc (0.53 M, pH4.4) was added [^{68}Ga][Ga(H_2O)$_6$]Cl_3(aq.) stock solution (31.6 ± 1.1 MBq, *n* = 3) resulting in a total reaction volume of 76 µL. Reactions were monitored by radio-iTLC. Formation of [^{68}Ga]GaDOTA-PEG_4-ArN_3 (^{68}Ga-**3**) was complete after 10 min. incubation at 70 °C with radiochemical conversion (RCC) >99% (*n* = 3, R_f = 0.06 – 0.21 on iTLC). The pH of the reaction mixture was then adjusted to >7.5 by the addition of an aqueous solution of $NaHCO_3$ (1.0 M, 50 µL added). After adjusting the pH, an aliquot of pre-purified trastuzumab (1.015 mg, 7.00 x 10^{-9} mol, reaction concentration = 6.3 mg/mL) was added to give an initial chelate-to-trastuzumab ratio of 11.2 at the start of the photochemical conjugation step (total reaction volume ~160 µL). The reaction mixture was then irradiated using the LED (100% intensity, 365 nm) for 15 min. at room temperature without stirring. Aliquots of this crude reaction mixture were then analysed by using radio-iTLC, PD-10-SEC and SEC-UHPLC analysis.

Radio-iTLC analyses of the crude reactions after irradiation showed that ~30% (*n* = 3) of the radioactivity was bound to the antibody (R_f = 0.0). Note: integration of these radio-iTLC data is unreliable because the radiolabelled antibody fraction partially overlaps with the peak associated with ^{68}Ga-**3** and the photodegraded ^{68}Ga-**3** species (R_f = 0.06 – 0.21). Nevertheless, analytical PD-10-SEC measurements on the crude reaction mixtures confirmed this observation with an estimated RCP of 16.2 ± 0.3% (*n* = 3). Equivalent decay corrected SEC-UHPLC measurements indicated that the radiolabelled fraction of [^{68}Ga]GaDOTA-azepin-trastuzumab in the crude mixture was 12.7 ± 3.2% (*n* = 3).

Crude reaction mixtures were then purified by preparative PD-10-SEC eluting with PBS (collecting only the high purity 0.0 – 1.6 mL fraction). Prior to analysis, samples were concentrated using an Amicon Ultra-4 mL centrifugal filter (Millipore, 30 kDa MWCO, 4000 RPM, ~10 min.). The purified and formulated [^{68}Ga]GaDOTA-azepin-trastuzumab products (pH7.4) were obtained in <30 min. with decay corrected radiochemical yields (RCY) of 8.3 ± 1.4% (*n* = 3). The estimated lower limit on the molar activity (A_m / [MBq/nmol] of protein) of the formulated [^{68}Ga]GaDOTA-azepin-trastuzumab samples was 0.37 ± 0.08 MBq/nmol (*n* = 3, remeasured protein concentration). Purified products were then reanalysed by radio-iTLC, analytical PD-10-SEC and SEC-UHPLC. The RCP of purified [^{68}Ga]GaDOTA-azepin-trastuzumab was >99% (*n* = 3) by radio-iTLC, 90.7 ± 1.1% (*n* = 3) by analytical PD-10-SEC, and 93.0 ± 3.0% (*n* = 3) by SEC-UHPLC.

Figure S24. Characterisation data for the one-pot photoradiochemical synthesis of [^{68}Ga]GaDOTA-azepin-trastuzumab from pre-purified mAb. (A) Radio-iTLC chromatograms, (B) analytical PD-10-SEC elution profiles, and (C) SEC-UHPLC chromatograms of the crude and purified product.



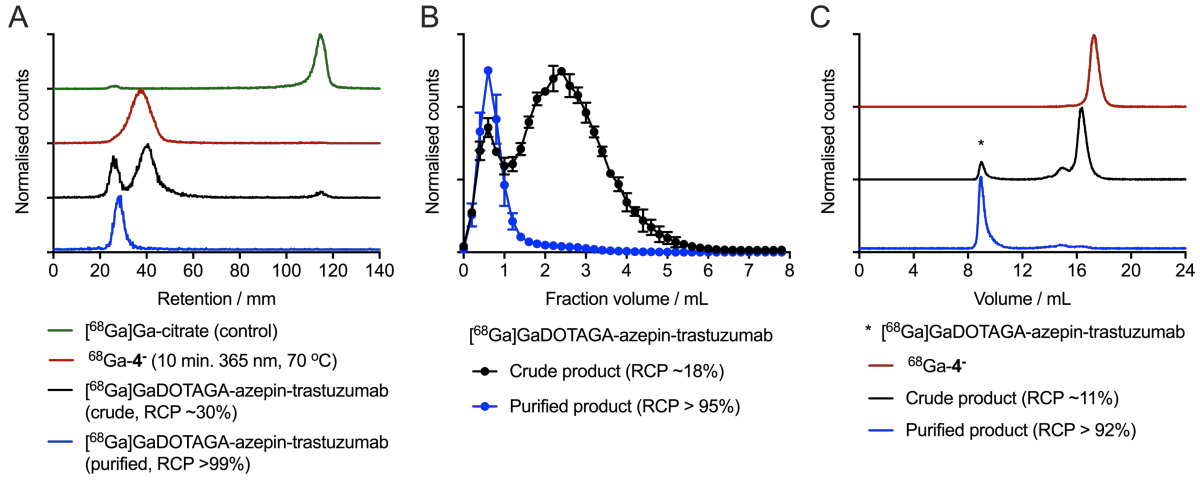
One-pot photoradiochemistry using DOTAGA-PEG_4-ArN_3 (**4**)

To a solution of DOTAGA-PEG_4-ArN_3 (**4**) (60 µg, 7.14 x 10^{-8} mol, 0.94 mM) buffered with NaOAc (0.53 M, pH4.4) was added [^{68}Ga][Ga(H_2O)$_6$]Cl_3(aq.) stock solution (31.6 ± 3.0 MBq, *n* = 3) resulting in a total reaction volume of 76 µL. Reactions were monitored by radio-iTLC. Formation of [^{68}Ga][GaDOTAGA-PEG_4-ArN_3]$^-$ (^{68}Ga-**4**$^-$) was complete after 10 min. incubation at 70 °C with radiochemical conversion (RCC) >99% (*n* = 3, R_f = 0.06 – 0.22 on iTLC). The pH of the reaction mixture was then adjusted to >7.5 by the addition of an aqueous solution of $NaHCO_3$ (1.0 M, 50 µL added). After adjusting the pH, an aliquot of pre-purified trastuzumab (1.015 mg, 7.00 x 10^{-9} mol, reaction concentration = 6.3 mg/mL) was added to give an initial chelate-to-trastuzumab ratio of 10.2 at the start of the photochemical conjugation step (total reaction volume ~160 µL). The reaction mixture was then irradiated using the LED (100% intensity, 365 nm) for 15 min. at room temperature without stirring. Aliquots of this crude reaction mixture were then analysed by using radio-iTLC, PD-10-SEC and SEC-UHPLC analysis.

Radio-iTLC analyses of the crude reactions after irradiation showed that ~30% (*n* = 3) of the radioactivity was bound to the antibody (R_f = 0.0). Note: integration of these radio-iTLC data is unreliable because the radiolabelled antibody fraction partially overlaps with the peak associated with ^{68}Ga-**4**$^-$ and the photodegraded ^{68}Ga-**4**$^-$ species (R_f = 0.06 – 0.22). Nevertheless, analytical PD-10-SEC measurements on the crude reaction mixtures confirmed this observation with an estimated RCP of 18.3 ± 0.7% (*n* = 3). Equivalent decay corrected SEC-UHPLC measurements indicated that the radiolabelled fraction of [^{68}Ga]GaDOTAGA-azepin-trastuzumab in the crude mixture was 11.1 ± 0.2% (*n* = 3).

Crude reaction mixtures were then purified by preparative PD-10-SEC eluting with PBS (collecting only the high purity 0.0 – 1.6 mL fraction). Prior to analysis, samples were concentrated using an Amicon Ultra-4 mL centrifugal filter (Millipore, 30 kDa MWCO, 4000 RPM, ~10 min.). The purified and formulated [^{68}Ga]GaDOTAGA-azepin-trastuzumab products (pH7.4) were obtained in <30 min. with decay corrected radiochemical yields (RCY) of 9.2 ± 0.6% (*n* = 3). The estimated lower limit on the molar activity (A_m / [MBq/nmol] of protein) of the formulated [^{68}Ga]GaDOTAGA-azepin-trastuzumab samples was 0.37 ± 0.07 MBq/nmol (*n* = 3, remeasured protein concentration). Purified products were then reanalysed by radio-iTLC, analytical PD-10-SEC and SEC-UHPLC. The RCP of purified [^{68}Ga]GaDOTAGA-azepin-trastuzumab was >99% (*n* = 3) by radio-iTLC, 92.2 ± 1.0% (*n* = 3) by analytical PD-10-SEC, and 92.2 ± 1.8% (*n* = 3) by SEC-UHPLC.

Figure S25. Characterisation data for the one-pot photoradiochemical synthesis of [^{68}Ga]GaDOTAGA-azepin-trastuzumab from pre-purified mAb. (A) Radio-iTLC chromatograms, (B) analytical PD-10-SEC elution profiles, and (C) SEC-UHPLC chromatograms of the crude and purified product.



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[2] M. Patra, L. S. Eichenberger, G. Fischer, J. P. Holland, *Angew. Chemie Int. Ed.* **2019**, *in press*.