Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information
A Novel Clickable MSAP Agent for Dual Fluorescence/Nuclear Labeling of Biovectors

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General information

All chemicals were obtained from commercial suppliers and used without further purification. $^{68}GaCl_3$ was obtained from a $^{68}Ga/^{68}Ge$ generator (Eckert and Ziegler Europe®, Berlin, Germany). $^{111}InCl_3$ (370.0 MBq/ml in HCl, pH 1.5–1.9) was purchased from Tyco Healthcare® (Petten, the Netherlands). Reaction vessels for solid phase synthesis were purchased from Chemglass® (Vineland, NJ, USA). Reactions were magnetically stirred and monitored by thin-layer chromatography on aluminum-backed pre-coated plates (Silica gel 60 F254) (Merck®, Darmstadt, Germany), and visualized with ultraviolet light or by staining with 10% phosphomolybdic acid in neat ethanol. Flash chromatography was performed on silica gel of 40–63 μm particle size (Sigma-Aldrich®, Zwijndrecht, the Netherlands). Concentration refers to rotary evaporation. Fmoc-based solid-phase peptide synthesis (SPPS) was conducted on an C.S. Bio® CS136 automated peptide synthesizer (Menlo Park, CA, USA). Reversed-phase high-performance liquid chromatography (HPLC) was carried out on a Waters® 2659 series system (Etten-Leur, the Netherlands) equipped with a diode array detector and a radio-detector. Low-resolution electrospray ionization (ESI) mass spectra were recorded on a TSQ Quantum Ultra™ triple quadrupole mass spectrometer from Thermo Fisher Scientific® (Lansingerland, the Netherlands). Fluorescence spectroscopy were performed on Hitachi F-4500 form Hitachi High-Technologies Corporation® (Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were recorded in D_2O and $CDCl_3$ on a Bruker® AVANCE 400 (Leiden, the Netherlands) at ambient temperature. Chemical shifts are given as δ values in ppm and coupling constants J are given in Hz. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets) and br (broad signal). The analysis of the products was performed by HPLC on an analytical column (Gemini®, Phenomenex C-18, 5 μm, 250.0 × 4.6 mm) with a gradient elution of MeCN (5% to 95% in H_2O, containing 0.1% TFA) at a flow rate of 1 mL/min over 30 min. The purification of the peptides was established using a semi-preparative column (Luna®, Phenomenex C-18, 5 μm, 250.0 × 10.0 mm) with a gradient elution of MeCN (10% to 90% in H_2O) at a flow rate of 3 mL/min over 30 min. The CAIX ligand, acetazolamide **6**, was prepared as described in our previous report.[22]

Experimental information

Chemistry and Radiolabeling

6-(2-oxo-2-(2,3,5,6-tetrafluorophenyl)ethoxy)benzo[d]thiazole-2-carbonitrile (5)

The 2-((2-cyanobenzo[*d*]thiazol-6-yl)oxy)acetic acid (CBT-COOH) was prepared as described in our previous report.[25] The mixture of CBT-COOH (480.0 mg, 2.1 mmol), 2,3,5,6-tetrafluorophenol (450.0 mg, 2.7 mmol), DIC (491.0 μL, 3.2 mmol) and Et_3N (280.0 μL, 2.1 mmol) in DCM (20.0 mL) was stirred at rt for 4 h. The reaction mixture was diluted by addition of DCM (40.0 mL), and then washed with 1.0 N $HCl_{(aq)}$ (50.0 mL) and water (50.0 mL). The organic layers were collected, dried with $MgSO_4$, and concentrated to give crude product as a white solid. The crude product was purified by flash chromatography (DCM/Hexanes = 1:1 to 100% DCM, silica gel) to give the tetrafluorophenyl ester **5** as a white solid (580.0 mg, 74%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 1H, J = 8.8 Hz), 7.42 (d, 1H, J =

2.8 Hz), 7.37 (dd, 1H, *J* = 2.8 and 8.8 Hz), 6.99–7.08 (m, 1H), 5.10 (s, 2H). ESI-MS: m/z 380.9 $[M - H]^-$.

CBT-Lys(DOTA)-Lys-C(O)NH_2 (3)

Compound **3** was prepared by solid phase synthesis in a 10 mL reaction vessel. All the reactions were performed at rt with agitation at 150 rpm. The Fmoc-deprotection step was achieved by treatment of the resin with 20% piperidine in DMF for 15 min. The washing steps were performed after each coupling and Fmoc-deprotection steps with DMF (2 x 5.0 mL) and DCM (2 x 5.0 mL). The Rink amide MBHA resin (100.0 mg, loading capacity: 0.65 mmol/g) was swollen with DMF (5.0 mL) for 1 h, and then treated with 20% piperidine in DMF (5.0 mL) for 1 h to remove the Fmoc-protecting group before the first coupling step. A mixture of Fmoc-Lys(Boc)-OH (91.0 mg, 0.19 mmol), HBTU (76.0 mg, 0.32 mmol), OxymaPure (46.0 mg, 0.32 mmol) and DIPEA (83.0 µL, 0.65 mmol) was added and agitated for 2 h, followed by the treatment of the resin with Ac_2O (94.5 µL, 1.0 mmol) in DMF (5.0 mL) to cap the unreacted amino groups. Subsequent couplings with Fmoc-Lys(ivDde)OH (113.0 mg, 0.19 mmol) and tri-*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA-tris(*tert*-butyl ester)) (93.0 mg, 0.16 mmol) were accomplished by a standard Fmoc-deprotection/coupling sequence. The protected peptide was treated with 5% hydrazine in DMF (5.0 mL) for 2 h to remove the ivDde protecting group. A mixture of **5** (48.0 mg, 0.19 mmol) and DIPEA (83.0 µL, 0.65 mmol) in DMF (5.0 mL) was added to the resin and the mixture was agitated at rt for 2 h. Simultaneous removal of side-chain protecting groups and cleavage of the product from solid support was accomplished by stirring the resin in a mixture of trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/thioanisole (5 mL, v/v/v = 75:5:20) at rt for 16 h. The cleavage cocktail was concentrated, the residue was washed with ice-cold ether (3 x 12.0 mL) and purified by semi-preparative HPLC to give **3** as a white solid after lyophilization (26.2 mg, 46%). ^{1}H NMR (400 MHz, D_2O): δ 8.15 (d, 1H, *J* = 9.2 Hz), 7.61 (d, 1H, *J* = 2.4 Hz), 7.43 (dd, 1H, *J* = 2.4, 9.2 Hz), 4.85 (d, 2H, *J* = 4.8 Hz), 4.39 (dd, 1H, *J* = 6.0, 8.8 Hz), 4.29 (dd, 1H, *J* = 5.2, 8.8 Hz), 3.79 (br, 8H), 3.31 (br, 12 H), 3.03-3.06 (m, 4H), 2.94 (t, 2H, *J* = 7.6 Hz), 2.87 (m, 2H), 1.70–1.88 (m, 4H), 1.60–1.68 (m, 2H), 1.29–1.48 (m, 6 H). ESI-MS: *m/z* 898.69 $[M + Na]^+$.

CBT-Lys(DOTA)-Lys(sCy5)-C(O)NH_2 (4)

A mixture of **3** (3.5 mg, 4.0 µmol), sulfo-Cy5-NHS (3.0 mg, 3.8 µmol) and DIPEA (10 µL, 77 µmol) in DMF (4 mL) was stirred at rt for 16 h. Solvent was then removed under vacuum. The residue was dissolved in H_2O/MeCN (v/v = 1:1) and purified by semi-preparative HPLC to yield **4** as a blue solid upon lyophilization (5.5 mg, 95%). The compound purity was determined to be 95% by HPLC (Figure S1A). ESI-MS: m/z 751.62 $[M + 2H]^{2+}$.

Cys-D-Phe-Gln-Trp-Ala-Val-Gly-His-NHCH[CH_2-$CH(CH_3)_2$]$_2$ (7)

Linear peptide **7** was synthesized on an automated peptide synthesizer using a standard Fmoc-based solid phase peptide synthesis (SPPS) protocol. All the coupling reactions were carried out in DMF (5 mL) with HBTU (3.9 equiv.), Oxyma Pure (4.0 equiv.) and DIPEA (8.0 equiv.) for 1 h. Fmoc deprotection

was accomplished by treatment of the resin with a 20% solution of piperidine in DMF (15.0 mL) for 15 min. Amide formation and Fmoc deprotection were monitored by Kaiser test. Double couplings were performed when the reaction was not complete. The synthesis was initiated by coupling Fmoc-His(Trt)-OH (0.99 g, 1.6 mmol, 2.0 equiv.) on a 2-chlorotrityl resin (0.5 g, loading capacity: 1.6 mmol/g) in DCM (5 mL) and followed by a capping procedure using DCM/MeOH/DIPEA (20 mL, v:v:v = 80:15:5) for 15 min at room temperature. After capping, subsequent conjugations with 4 equivalents of the following amino acids was carried out: Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Trp(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-D-Phe-OH and Fmoc-Cys(Trt). Selective cleavage of the linear peptide sequence from the resin was achieved by treatment with HFIP/DCM (4 mL, v/v = 20: 80) for 1 h at rt. After filtration, the solution was collected and concentrated by using rotary evaporator to give the peptide as white solid (1.59 g, 0.8 mmol). To a mixture of the *C*-terminal acid-free peptide (1.59 g, 0.8 mmol), PyBop (1.04 g, 2.0 mmol), DIPEA (0.69 µL, 4.0 mmol) in DMF (3.0 mL) was added 4-amino-2,6-dimethylheptane (0.36 g, 2.0 mmol) in DMF (3 mL). After stirring at rt for 1 h, the reaction mixture was concentrated and triturated with cold diethyl ether to give the crude protected peptide intermediate (1.60 g). A solution of diethylamine (1 mL, 9.6 mmol) in THF (2.0 mL) was added to a solution of peptide intermediate in DMF (3.0 mL) to remove the Fmoc-protecting group on the cysteine. The reaction mixture was stirred at rt for 2 h and followed by precipitation of the product in cold diethyl ether. The global deprotection of the peptide was performed by treatment of the residue with trifluoroacetic acid, triisopropylsilane and water (v/v/v = 95:2.5:2.5). The reaction mixture was stirred at rt for 2 h, followed by the evaporation of the solvent and trituration of the crude peptide in cold diethyl ether (light yellow solid, 371.5 mg). A fraction of the crude product (11.8 mg) was purified by semi-preparative HPLC to give pure **7** as a white solid (6.1 mg, 23%). The compound purity was estimated to 96% by HPLC (Figure S1B). ESI-MS: *m/z* 1073.75 $[M+H]^+$.

DOTA-sCy5-Luc-Acetazoamide (8)

6 (0.5 mg, 0.63 µmol), **4** (0.5 mg, 0.33 µmol) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl) (0.2 mg, 0.69 µmol) were dissolved in PBS (0.01 M, pH 7.4, 150 µL) and incubated under agitation at 37 °C for 2 h. The reaction was monitored by RP-C18 HPLC. **8** was purified by semi-preparative RP-C18 HPLC (t_R = 13.2 min) to yield a blue solid (0.7 mg, 93%). The purity was determined by HPLC (98%) (Figure S1C). ESI-MS: m/z 1139.47 $[M + 2H]^{2+}$, 760.03 $[M + 3H]^{3+}$.

DOTA-sCy5-Luc-D-Phe-Gln-Trp-Ala-Val-Gly-His-NHCH[CH_2-$CH(CH_3)_2$]$_2$ (9)

A mixture of **4** (3.0 mg, 2.0 µmol), peptide **7** (1.1 mg, 1.0 µmol) and TCEP·HCl (0.6 mg, 2.2 µmol) in PBS (0.01 M, pH 7.4, 200.0 µL) and DMF (70 µL) was stirred at rt for 2 h. The reaction was monitored by HPLC and the dual-labeled product **9** was obtained by semi-preparative HPLC purification as a blue solid (1.76 mg, 69%). The compound purity was determined by HPLC (99%) (Figure S1D). ESI-MS: m/z 1279.32 $[M + 2H]^{2+}$, 853.69 $[M + 3H]^{3+}$.

Magnetic CBT-bearing scavenger resin (10)

Magnetic amine-decorated beads (0.5 mL, ~ 6.0 µmol, loading capacity: 12.0 µmol/mL) were separated from the storage buffer and washed trice with a mixture of PBS (0.01 M, pH 7.4) and DMF (0.5 mL, v/v = 1:1) and were resuspended in PBS/DMF (300.0 µL, v/v: 1:1). CBT-TFP (**5**) (20.0 mg, 52.0 µmol) in DMF (300.0 µL) was added and the mixture was agitated at rt at 250 rpm for 2.5 h. Subsequently, the beads were washed with DMF (3 × 0.5 mL), H_2O (3 × 0.5 mL), a mixture of PBS and DMF (3 × 0.5 mL, v/v = 1:1), resuspended in PBS/DMF (0.5 mL, v/v = 1:1) and stored at 4 °C.

^{111}In-Radiolabeling - direct labeling approach

$^{111}InCl_3$ (9.7 MBq, 10.0 µL) was added to a solution of **8** (2.3 µg, 1.0 nmol) or **9** (2.6 µg, 1.0 nmol) in sodium acetate buffer (0.2 M, pH 5.5, 200.0 µL) at 90 °C for 15 min. The reaction was monitored by radio-iTLC (eluant: 0.1 M sodium citrate, pH 5.5; $R_{f\ \text{product}}$ = 0) and radio-HPLC. [^{111}In]**8** and [^{111}In]**9** were obtained with a radiochemical yield of 98% and 95% (d. c.), respectively. The molar activity of [^{111}In]**8** and [^{111}In]**9** were 9.1 and 8.8 MBq/nmol. The radiochemical purity of [^{111}In]**8** (t_R = 13.2 min) and [^{111}In]**9** (t_R = 15.8 min) were determined by radio-HPLC, showing greater than 95% (Figure S2 and S3). The nonradioactive standards, [^{nat}In]**8** and [^{nat}In]**9**, were prepared by mixing $In(NO_3)_3$ (1 g/mL, 10 µL, 33.3 nmol) and **8** (50 µg, 22.7 nmol) or **9** (51 µg, 20.0 nmol) in 200 µL of NaOAc buffer pH 5.5 and heating at 90 °C for 15 min. The products were analyzed by UV-HPLC and ESI-MS. [^{nat}In]**8**: t_R = 13.2 min, ESI-MS: m/z 1194.50 $[M + 2H]^{2+}$; m/z 796.84 $[M + 3H]^{3+}$. [^{nat}In]**9**: t_R = 15.8 min, ESI-MS: m/z 1334.84 $[M + 2H]^{2+}$; m/z 889.87 $[M + 3H]^{3+}$.

^{68}Ga-Radiolabeling - direct labeling approach

$^{68}GaCl_3$ was eluted from the ^{68}Ge/^{68}Ga-Generator as an aqueous solution containing 0.05 M HCl and 3.0 M NaCl. $^{68}GaCl_3$ (22.4 MBq, 25.0 µL) was added to a solution of **8** (2.3 µg, 1.0 nmol) or **9** (2.6 µg, 1.0 nmol) in sodium acetate buffer (0.2 M, pH 5.5, 200.0 µL) at 90 °C for 15 min. The reaction conversion was monitored by radio-iTLC (eluant: 0.1 M sodium citrate, pH 5.5; $R_{f\,\text{product}}$ = 0) and radio-HPLC. [^{68}Ga]**8** and [^{68}Ga]**9** were obtained with a radiochemical yield of 99 and 98% (d. c.) and a molar activity of 17.2 and 17.1 MBq/nmol, respectively. The radiochemical purity of [^{68}Ga]**8** (t_R = 13.2 min) and [^{68}Ga]**9** (t_R = 15.4 min) as determined by radio-HPLC were greater than 98% (Figure S4 and S5). The nonradioactive standards, [^{nat}Ga]**8** and [^{nat}Ga]**9**, were prepared by mixing $Ga(NO_3)_3$ (1 g/mL, 20 µL, 78.7 nmol) and **8** (55.3 µg, 70 nmol) or **9** (122.6 µg, 47.9 nmol) in 200 µL of NaOAc buffer pH 5.5 and heating at 90 °C for 15 min. The products were analyzed by UV-HPLC and ESI-MS. [^{nat}Ga]**8**: t_R = 13.2 min, ESI-MS: m/z 1181.20 $[M + 2H]^{2+}$; m/z 787.74 $[M + 3H]^{3+}$. [^{nat}Ga]**9**: t_R = 15.4 min, ESI-MS: m/z 1311.25 $[M + 2H]^{2+}$; m/z 874.75 $[M + 3H]^{3+}$.

Two-step radiolabeling

$^{111}InCl_3$ (20.0 µL, 40.7 MBq) or $^{68}GaCl_3$ (50.0 µL, 57.8 MBq) was added to a solution of **4** (5.0 µg, 3.3 nmol) in sodium acetate buffer (0.2 M, pH 5.5, 200 µL) at 90 °C for 15 min. The reaction was monitored

by radio-iTLC (eluant: 0.1 M sodium citrate, pH 5.5; $R_{f\,\text{product}}$ = 0) and radio-HPLC. The reaction mixture was cooled for 5 min, and then passed through a C-18 light cartridge to remove the acidic buffer and non-reacted radiometals. The cartridge was washed with water (10.0 mL) and eluted with ethanol (1 mL) to give [^{111}In]**4** or [^{68}Ga]**4** with a radiochemical yield of 99% and 93% (d. c.), respectively. The molar activity of [^{111}In]**4** and [^{68}Ga]**4** were 12.3 and 13.6 MBq/nmol, respectively. The radiochemical purity of [^{111}In]**4** (t_R = 13.5 min) and [^{68}Ga]**4** (t_R = 13.5 min) were determined by radio-HPLC, showing greater than 99% (Figure S6 and S7). The non-radioactivity [^{nat}In]**4** and [^{nat}Ga]**4** were prepared by mixing **4** (30 µg, 20.0 nmol) and $In(NO_3)_3$ (1 g/mL, 10 µL, 33.3 nmol) and by mixing **4** (98.9 µg, 65.9 nmol) and $Ga(NO_3)_3$ (1 g/mL, 20 µL, 78.7 nmol), respectively, in 200 µL of NaOAc buffer pH 5.5 and heating at 90 ˚C for 15 min. the products were analyzed by UV-HPLC and ESI-MS. [^{nat}In]**4**: t_R = 13.5 min**,** ESI-MS: m/z 807.13 $[M + 2H]^{2+}$. [^{nat}Ga]**4**: t_R = 13.5 min, ESI-MS: m/z 1567.16 $[M + H]^{+}$; m/z 784.66 $[M + 2H]^{2+}$. For the click reaction, compound **6** (15.8 µg, 20.0 nmol) or **7** (51.9 µg, 30.0 nmol), TCEP·HCl (6.2 µg, 22.0 nmol or 9.3 µg, 33.0 nmol) in PBS (0.01 M, pH 7.4, 500 µL) were mixed in a 1.5 mL microtube at rt for 10 min. To the mixture, [^{111}In]**4** (1.0 nmol, 12.3 MBq) or [^{68}Ga]**4** (1.0 nmol, 13.6 MBq) was added, and the reaction was agitated (800 rpm) at 37 ˚C. After 20 min, the CBT-scavenger beads (50 µL, ~600 nmol) were added into the reaction mixture and agitated at 37 ˚C for 5 min. Efficiency of the purification was monitored by HPLC. [^{111}In]**8**, [^{111}In]**9**, [^{68}Ga]**8** and [^{68}Ga]**9** were obtained with RCYs of 96, 95, 99 and 98% (d. c.), respectively, and molar activity of 12.1, 12.0, 9.5 and 9.4 MBq/nmol, respectively. The radiochemical purity of the products was determined by radio-HPLC, and they all showed a purity over 95% (Figure 4C and S9).

Fluorescence Spectroscopy

Excitation and emission spectra were recorded using a Hitachi F-4500 fluorescence spectrophotometer at λ_{em} = 662 and λ_{ex} = 646 nm. To record excitation and emission spectra, an aqueous solution of the samples (1 µM) in PBS buffered at pH 7.4 were prepared.

Determination of $LogD_{7.4}$

Distribution coefficients ($LogD_{7.4}$ values) were determined by a shake-flask method. Sample containing the radioligand was dissolved in 600 µL solution of PBS (0.01 M, pH 7.4) and n-octanol (v/v = 1:1). The mixture was vortexed vigorously and then centrifuged for 10 min for phase separation. Samples (100 µL) of the two phases were taken out and analyzed by γ-counter. $LogD_{7.4}$ value was calculated by using the following equation: $LogD_{7.4}$ = log [(counts in octanol phase)/(counts in aqueous phase)]. All the experiments were performed in triplicates.

Stability and transchelation challenge studies

Stability and transchelation challenge tests were performed as described in previous reports.[22] The radiolabeled samples (1~2 MBq) were mixed with 300 µL of PBS (0.01 M, pH 7.4), human serum or PBS/ethylenediaminetetraacetic acid (EDTA) solution (34 mM EDTA in PBS) at 37 °C for 1 to 3 h. After

incubation, acetonitrile (300 μL) was added to the samples in human serum and centrifuged (8000 × g) for 5 min. The supernatant solution was loaded onto a radio-HPLC system for analysis. The other samples were directly analyzed by radio-HPLC without any pretreatment.

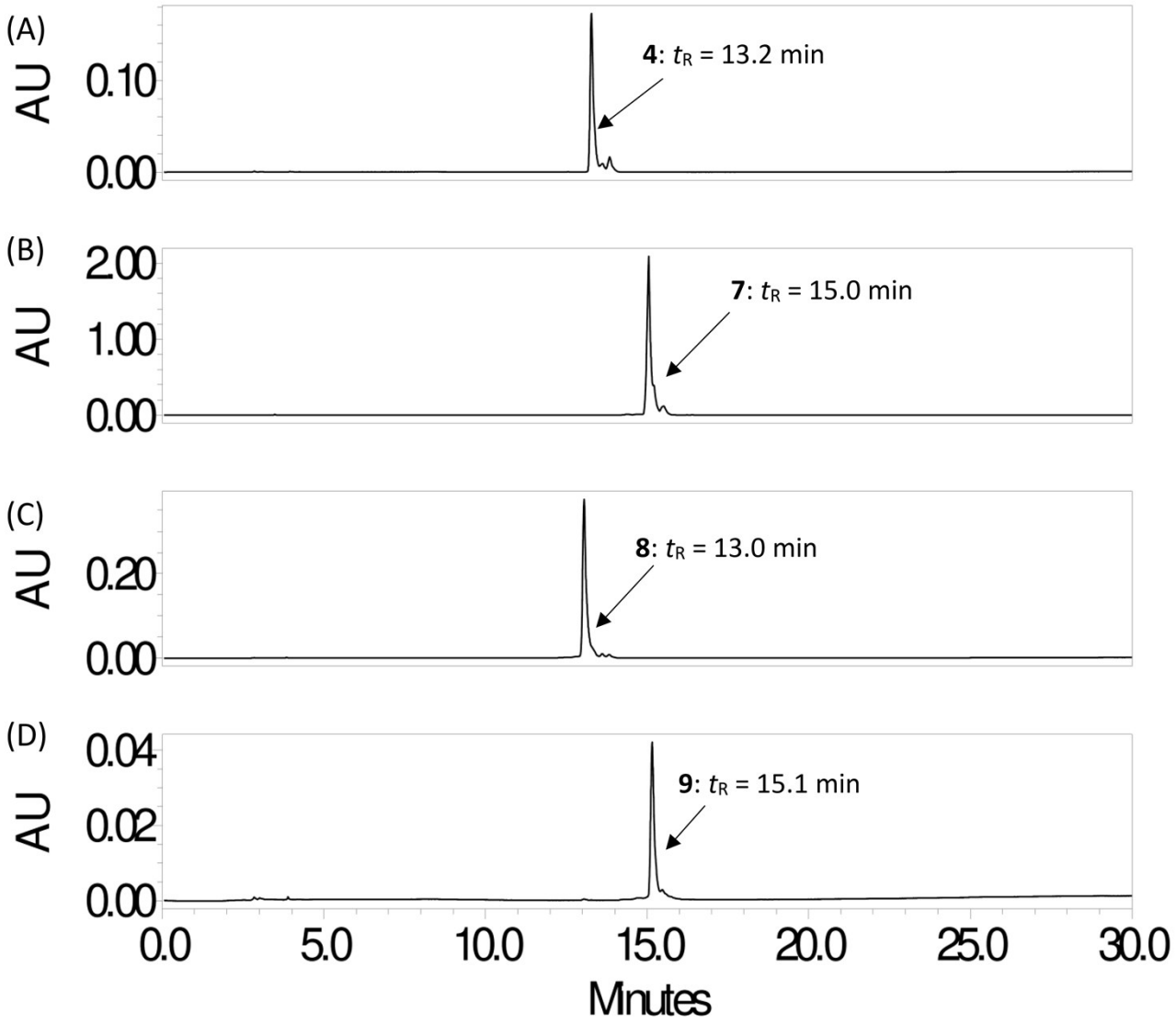
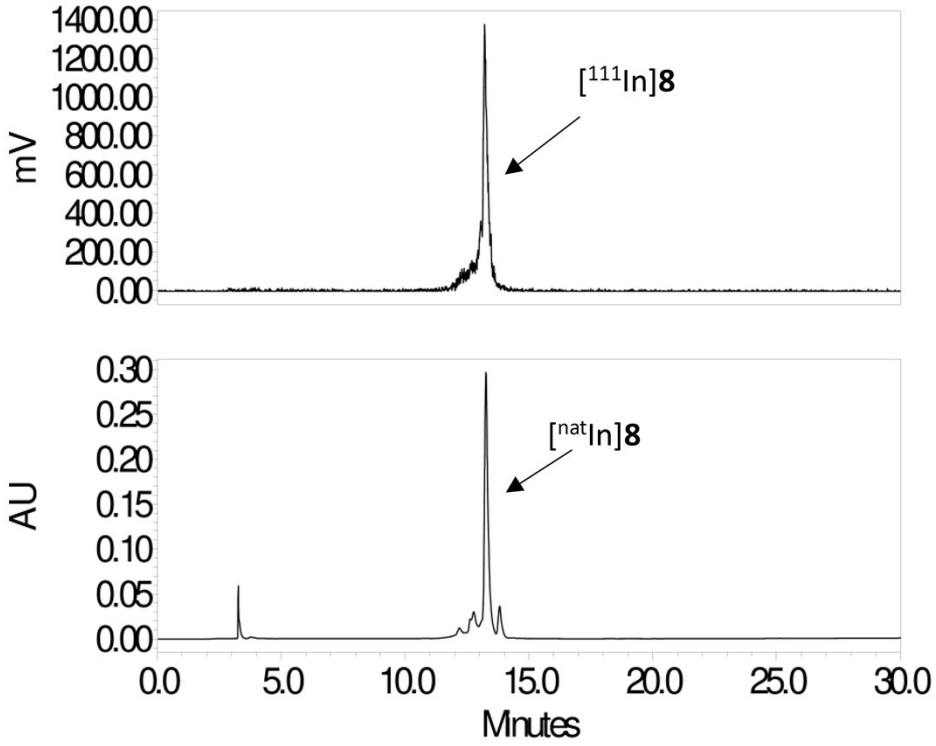


Figure S1. Determination of the purity of **4** (A), **7** (B), **8** (C) and **9** (C) by analytical HPLC. The retention time of **4**, **7**, **8** and **9** are 13.2, 15.0, 13.0 and 15.1 min, respectively. The purity of **4**, **7**, **8** and **9** were all found to be higher than 95%.

(A)



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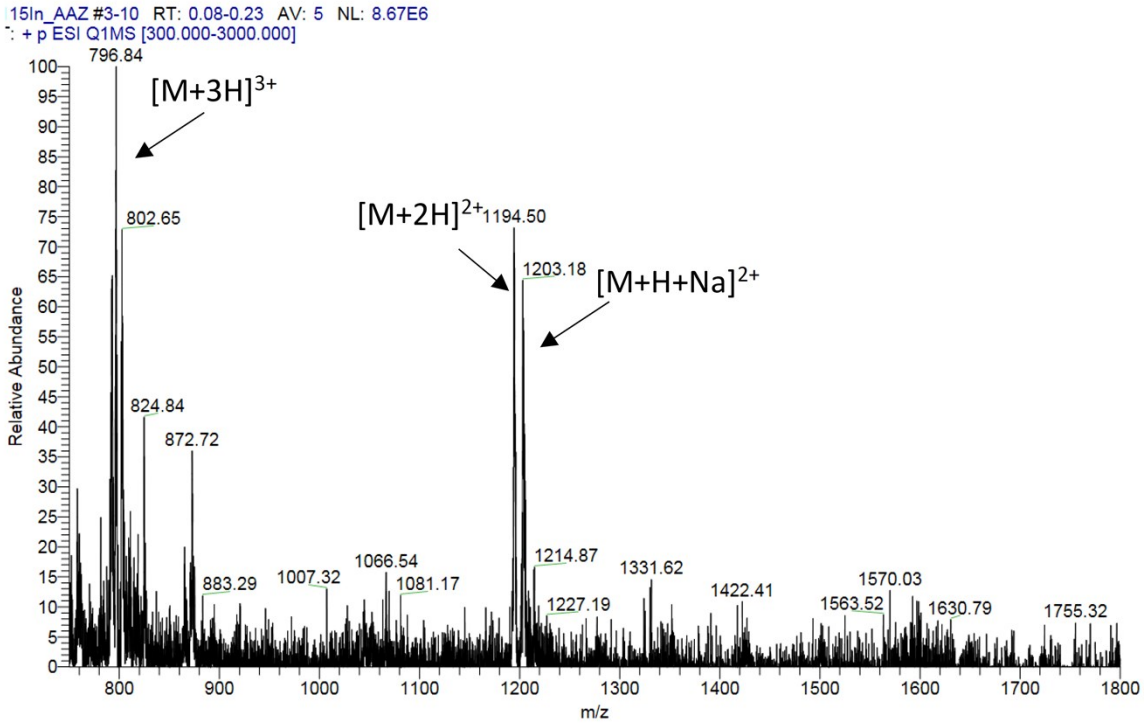
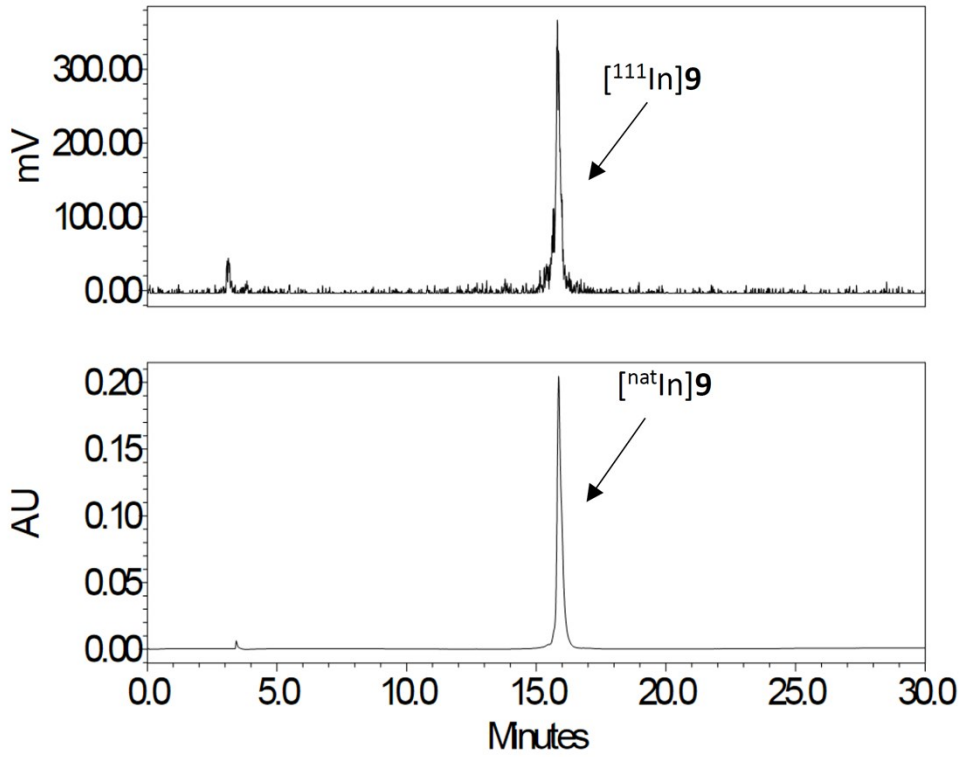


Figure S2. Identification of [^{111}In]**8**. (A) Radio-chromatogram of [^{111}In]**8** and UV absorbance at 320 nm of its corresponding non-radioactive analog [^{nat}In]**8**. The retention time of [^{111}In]**8** and [^{nat}In]**8** were 13.2 and 13.2 min, respectively. (B) Mass spectrum of [^{nat}In]**8**.

(A)



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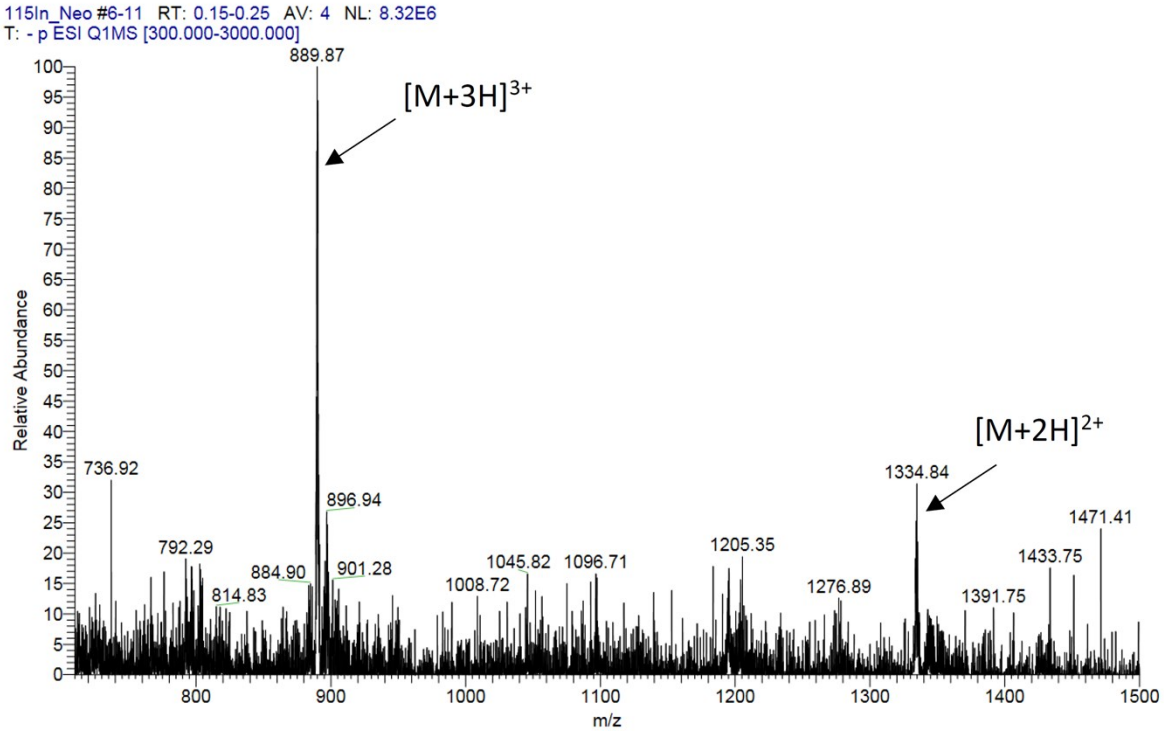
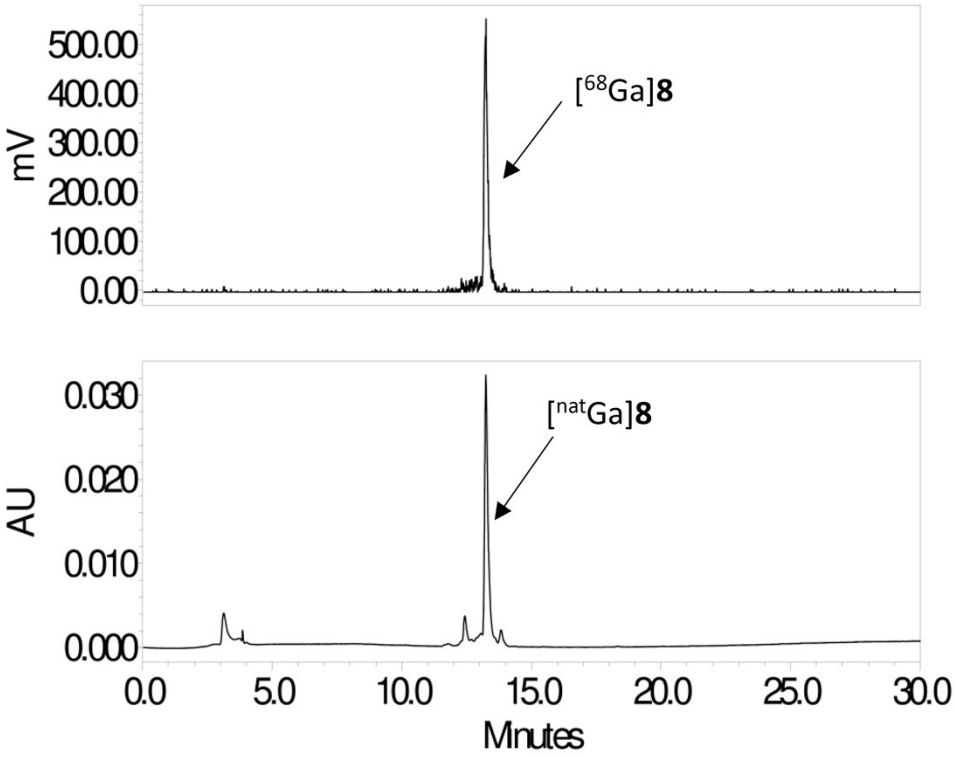


Figure S3. Identification of [^{111}In]**9**. (A) Radio-chromatogram of [^{111}In]**9** and UV absorbance at 320 nm of its corresponding non-radioactive analog [^{nat}In]**9**. The retention time of [^{111}In]**9** and [^{nat}In]**9** were 15.8 and 15.8 min, respectively. (B) Mass spectrum of [^{nat}In]**9**.

(A)



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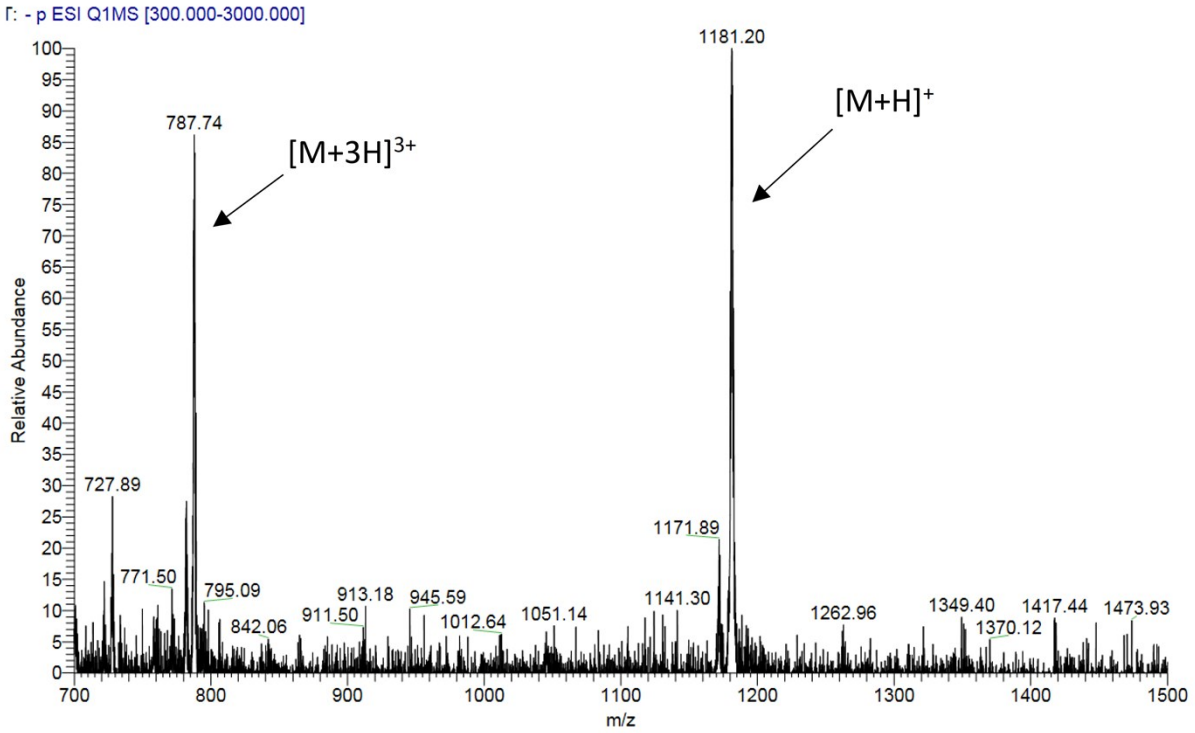
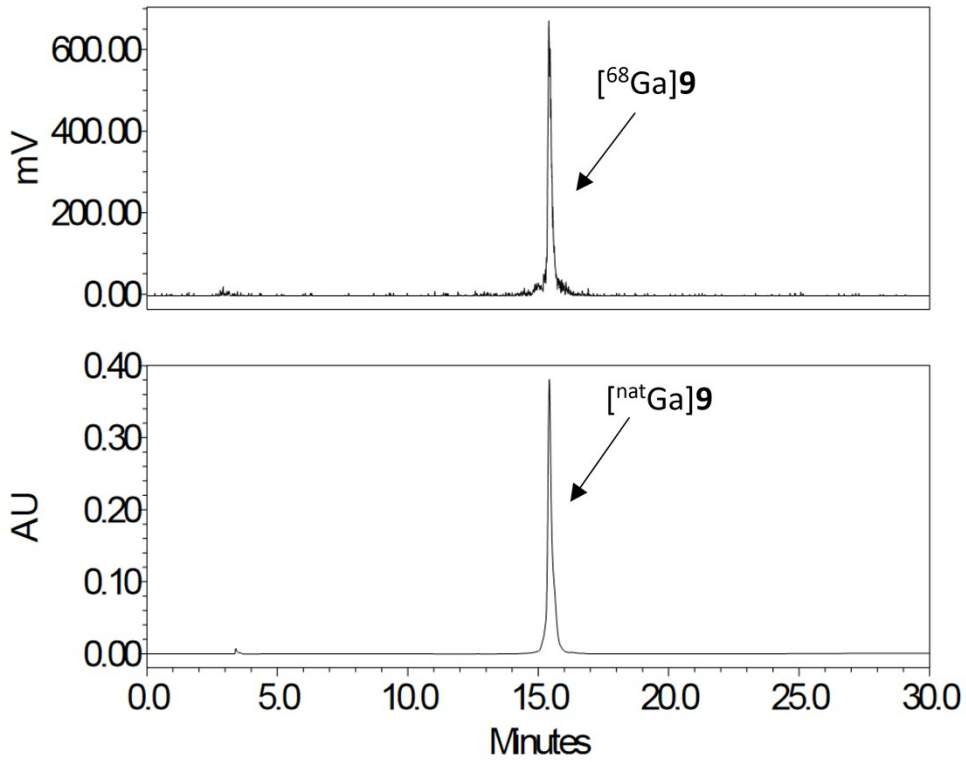


Figure S4. Identification of [^{68}Ga]**8**. (A) Radio-chromatogram of [^{68}Ga]**8** and UV absorbance at 320 nm of its corresponding non-radioactive analog [^{nat}Ga]**8**. The retention time of [^{68}Ga]**8** and [^{nat}Ga]**8** were 13.2 and 13.2 min, respectively. (B) Mass spectrum of [^{nat}Ga]**8**.

(A)



(B)

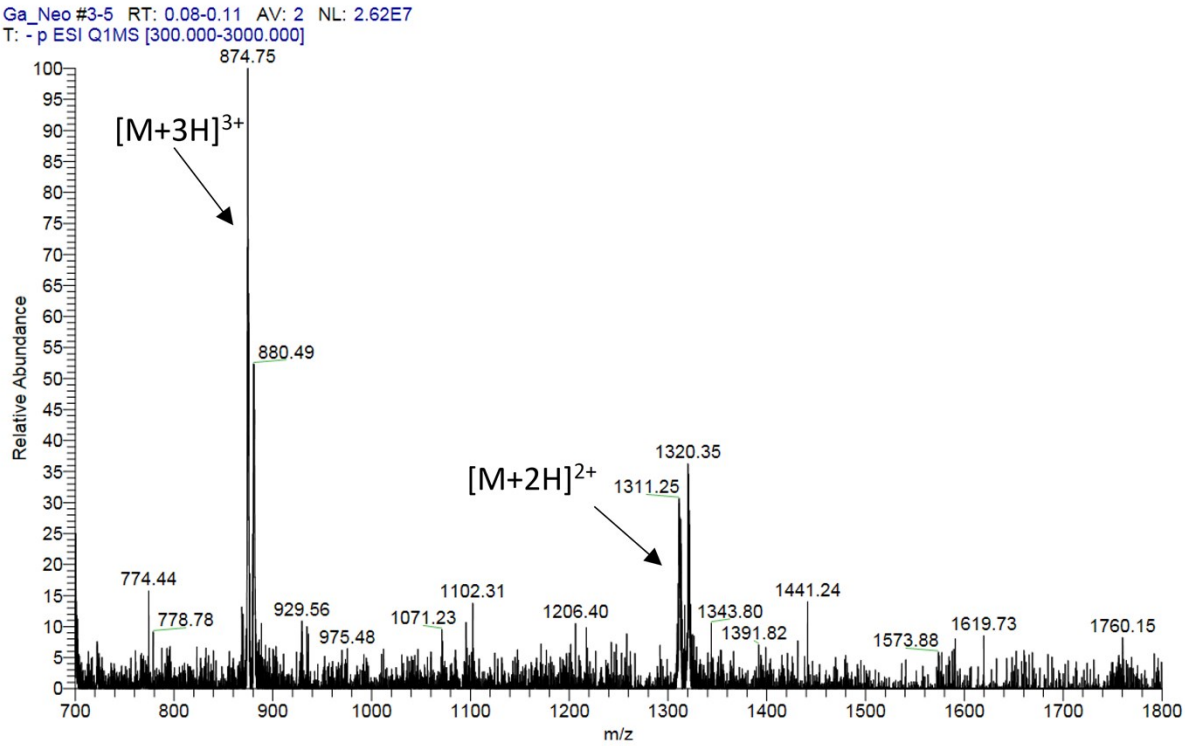
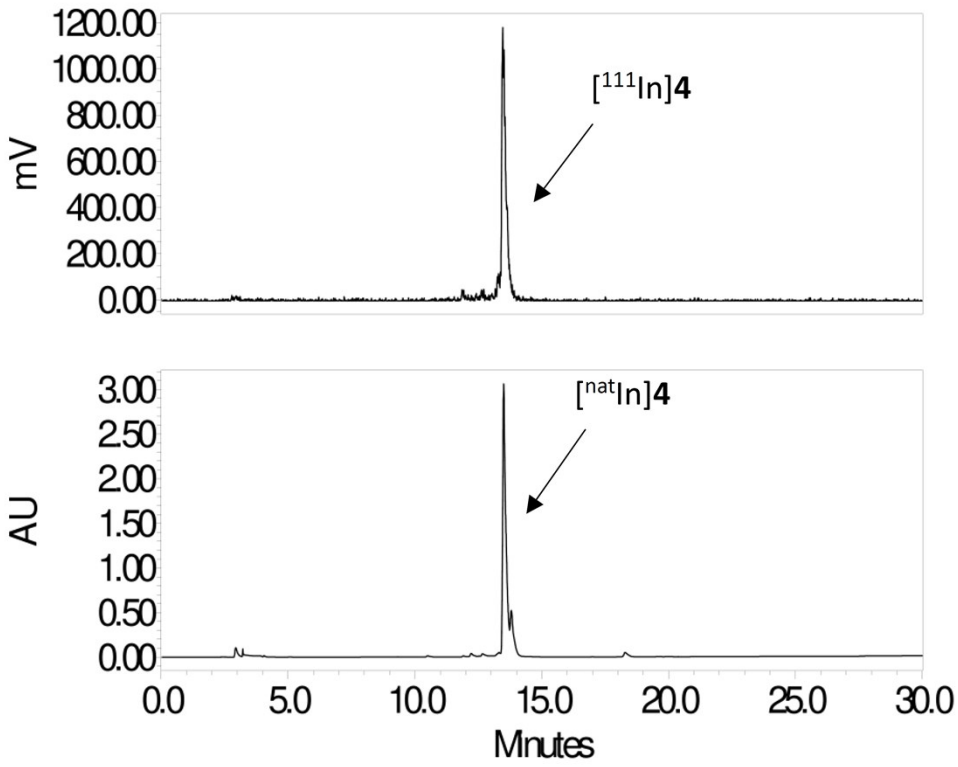


Figure S5. Identification of [^{68}Ga]**9**. (A) Radio-chromatogram of [^{68}Ga]**9** and UV absorbance at 320 nm of its corresponding non-radioactive analog [^{nat}Ga]**9**. The retention time of[^{68}Ga]**9** and [^{nat}Ga]**9** were 15.4 and 15.4 min, respectively. (B) Mass spectrum of [^{nat}Ga]**9**.

(A)



(B)

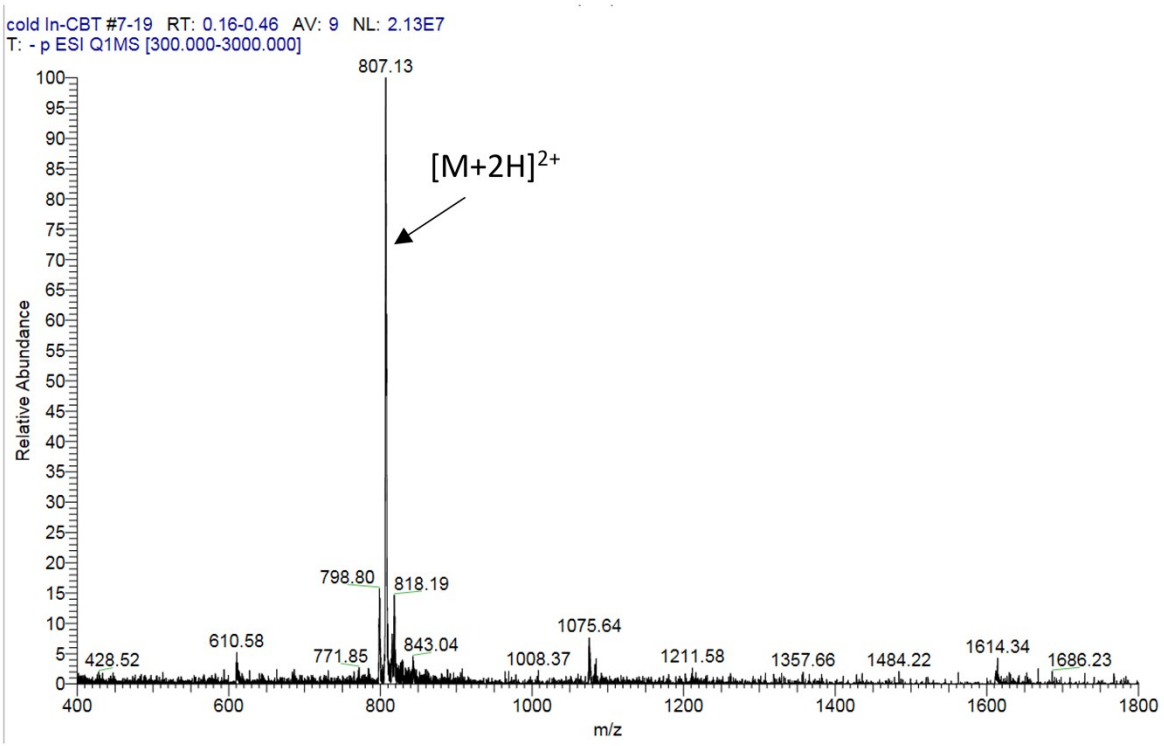
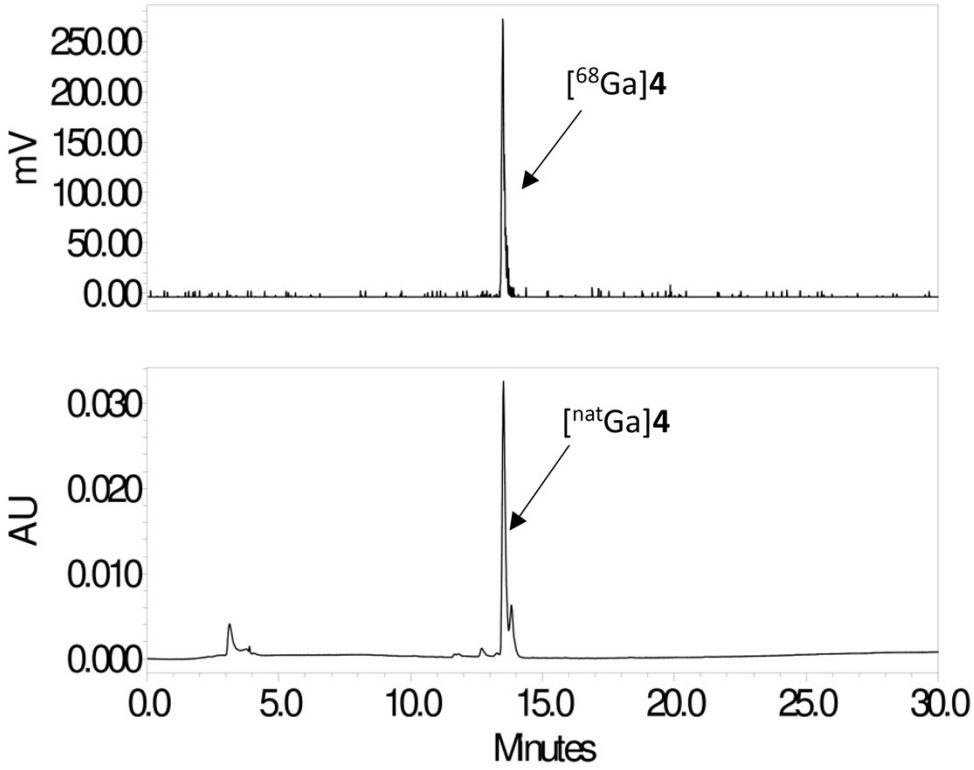


Figure S6. Identification of [^{111}In]**4**. (A) Radio-chromatogram of [^{111}In]**4** and UV absorbance at 320 nm of its corresponding non-radioactive analog [^{nat}In]**4**. The retention time of [^{111}In]**4** and [^{nat}In]**4** were 13.5 and 13.5 min, respectively. (B) Mass spectrum of [^{nat}In]**4**.

(A)



(B)

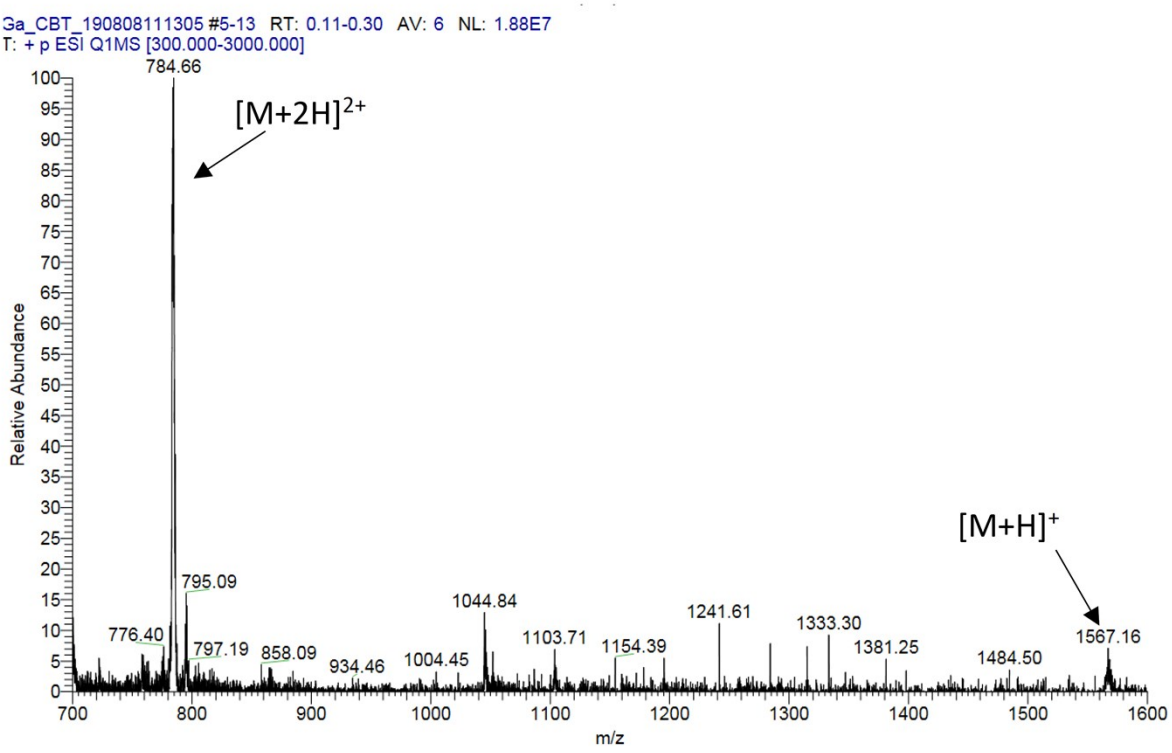


Figure S7. Identification of [^{68}Ga]**4**. (A) Radio-chromatogram of [^{68}Ga]**4** and UV absorbance at 320 nm of its corresponding non-radioactive analog [^{nat}Ga]**4**. The retention time of[^{68}Ga]**4** and [^{nat}Ga]**4** were 13.5 and 13.5 min, respectively. (B) Mass spectrum of [^{nat}Ga]**4**.

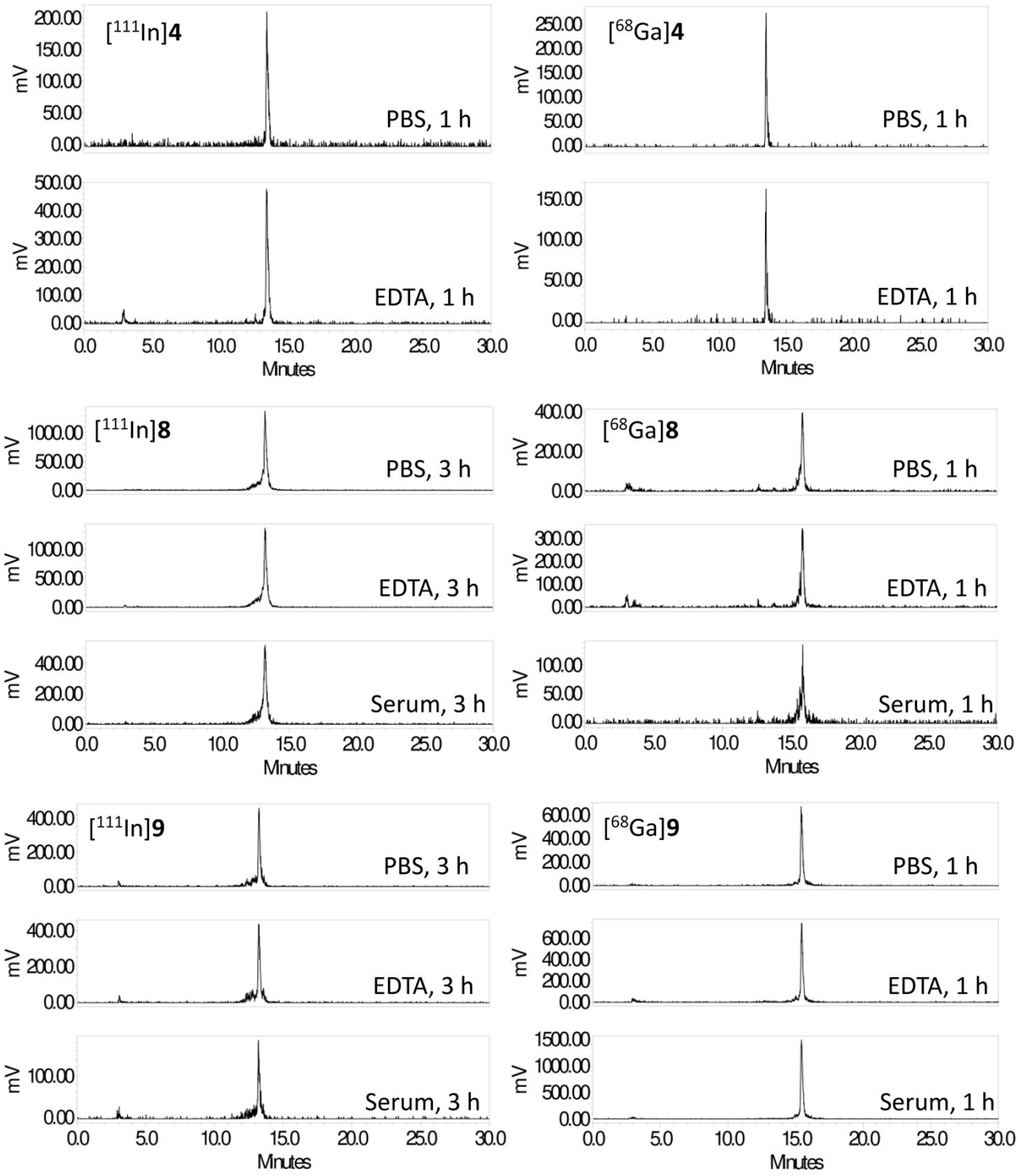


Figure S8. Determination of the stability of ^{111}In- or ^{68}Ga-labeled **4**, **8** and **9** in PBS (0.01 M, pH 7.4), EDTA solution (34 mM in PBS) and human serum by radio-HPLC. All the compounds were stable under these conditions.

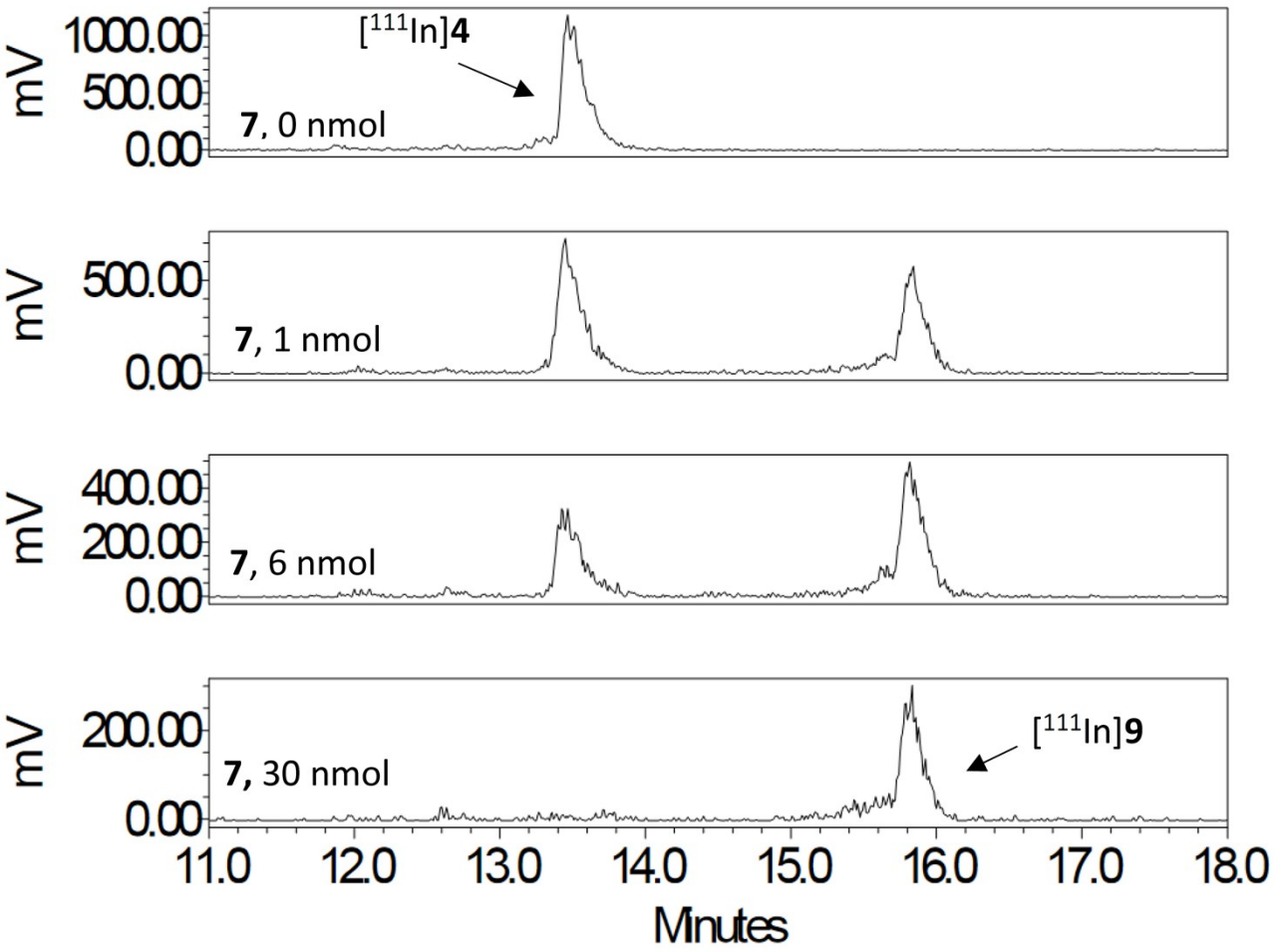


Figure S9. Radio-high performance liquid chromatography (HPLC) monitoring of the click reaction between [^{111}In]**4** and various concentration of **7**. The reaction was performed in PBS buffer (0.01 M, pH 7.4) at 37 ^{o}C for 20 min. The retention time of [^{111}In]**4** and [^{111}In]**9** product are 13.5 and 15.8 min, respectively.

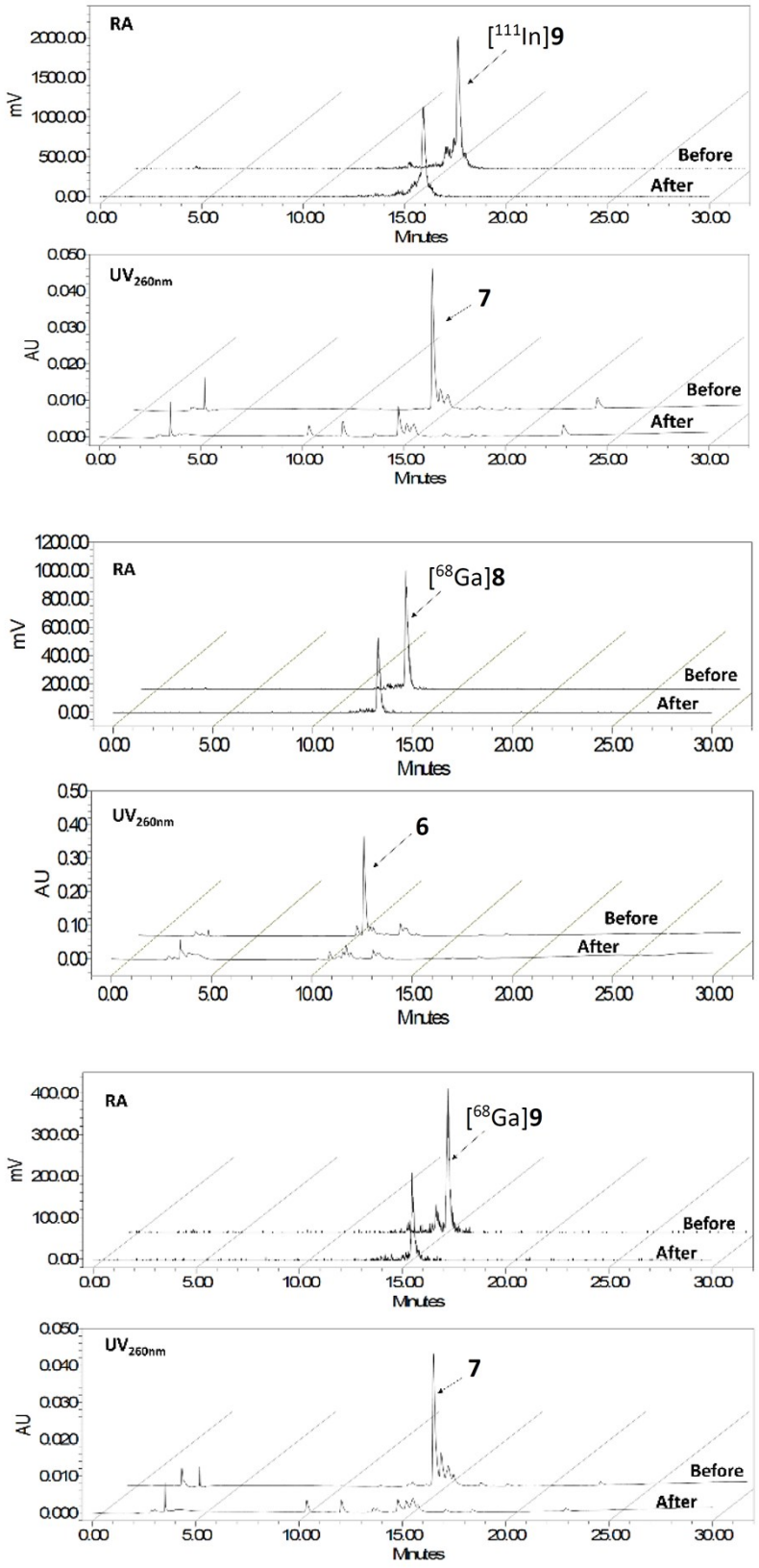


Figure S10. HPLC monitoring of substrate (**6** and **7**) scavenging after click reaction of CBT-beads. Excess of substrate is quantitatively removed from the luciferin product upon treatment with CBT-beads (37 ˚C, 5 min).