Supporting Information for

Novel Chemoselective ^{18}F-Radiolabeling of Thiol-Containing Biomolecules under Mild Aqueous Conditions

Aristeidis Chiotellis,[a] Filippo Sladojevich,[a] Linjing Mu,[a] Adrienne Müller-Herde,[a] Ibai E. Valverde,[b] Vladimir Tolmachev,[c] Roger Schibli,[a] Simon M. Ametamey,[a] Thomas L. Mindt [a,b,d,]*

a. Center for Radiopharmaceutical Sciences ETH-PSI-USZ, Institute of Pharmaceutical Sciences ETH, Vladimir-Prelog Weg 4, CH-8093 Zurich, Switzerland; e-mail: t.mindt@gmx.ch

b. Division of Radiopharmaceutical Chemistry, University of Basel Hospital, Petersgraben 4, CH-4031 Basel, Switzerland

c. Department of Immunology, Genetics and Pathology, Medical Radiation Science Program,, Rudbeck Laboratory, Uppsala University, SE-75181 Uppsala, Sweden

d. Ludwig Boltzmann Institute for Applied Diagnostics, General Hospital of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

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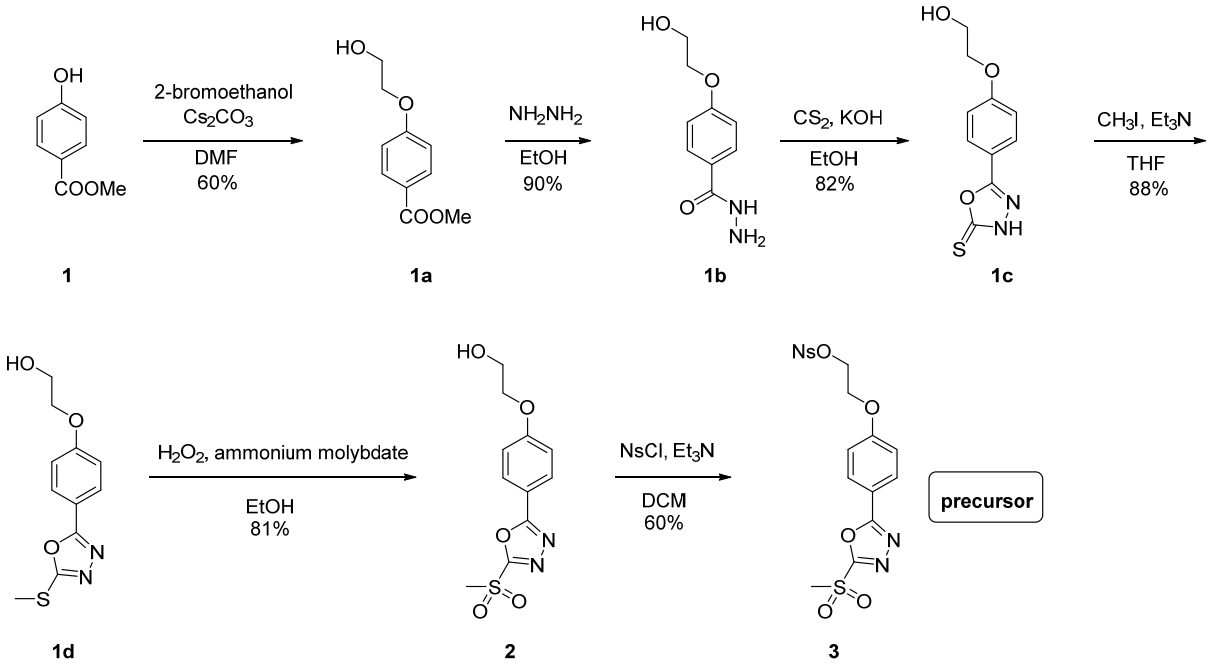
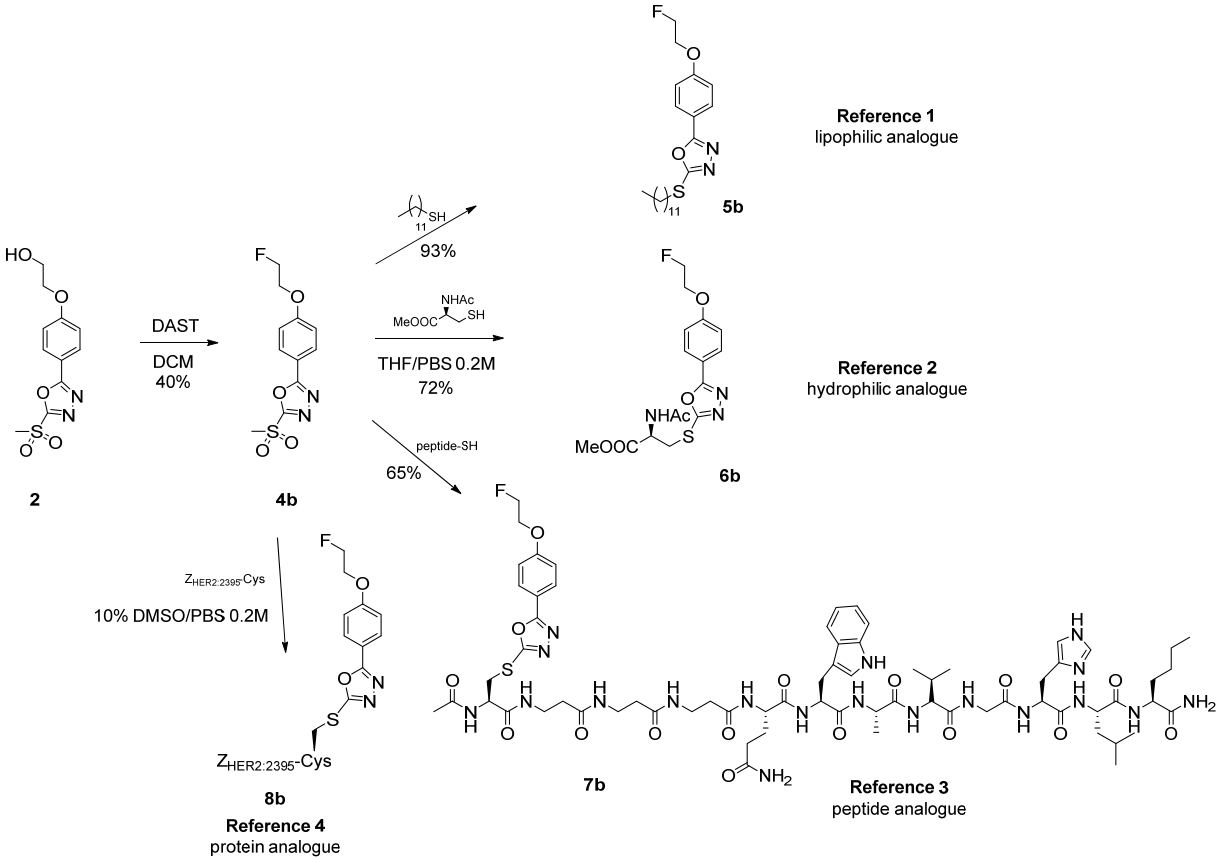
2. Schemes



Scheme S1. Synthesis of radiolabeling precursor **3**.



Scheme S2. Synthesis of non-radioactive ^{19}F-reference compounds.

3. Material and Methods

All reagents and starting materials were purchased from commercial suppliers and used without further purification. All solvents used for reactions were purchased as anhydrous grade from Acros Organics (puriss., dried over molecular sieves, H_2O < 0.005%) and were used without further purification unless otherwise stated. Solvents for extractions, column chromatography and thin layer chromatography (TLC) were purchased as commercial grade. All non aqueous reactions were performed under an argon atmosphere using flame-dried glassware and standard syringe/septa techniques. In general, reactions were magnetically stirred and monitored by TLC performed on Merck TLC glass sheets (silica gel 60 F_{254}). Spots were visualized with UV light (λ = 254 nm) or through staining with anisaldehyde solution or basic aq. $KMnO_4$ solution and subsequent heating. Chromatographic purification of products was performed using Fluka silica gel 60 for preparative column chromatography (particle size 40 – 63 µm). Reactions at 0 °C were carried out in an ice/water bath. Reactions at -78 °C were carried out in a dry ice/acetone bath. For the synthesis of the phenyloxadiazole compounds, the general procedures described by Toda et al.[1] were accordingly adopted.

Nuclear magnetic resonance (NMR) spectra were recorded in chloroform-*d*, acetonitrile-d_3 or methanol-d_4 on a Bruker Av-400 spectrometer at room temperature. The measured chemical shifts are reported in δ (ppm) and the residual signal of the solvents was used as the internal calibration standard (chloroform-*d*: ^{1}H = 7.24 ppm, ^{13}C = 77.23 ppm; acetone-d_6: ^{1}H = 2.05 ppm, ^{13}C = 29.9 ppm; methanol-d_4: ^{1}H = 3.31 ppm, ^{13}C = 49.15 ppm, acetonitrile-d_3 : ^{1}H = 1.94 ppm, ^{13}C = 13.9 ppm). All ^{13}C-NMR spectra were measured with complete proton decoupling. Data of NMR spectra were recorded as follows: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, dm = doublet of multiplets, br = broad signal. The coupling constant *J* is reported in Hertz (Hz). Electrospray (ES) mass spectra (HRMS) were obtained with a Bruker FTMS 4.7 T BioAPEXII spectrometer.

High-performance liquid chromatography (HPLC) was performed on a Merck-Hitachi L-7000 system equipped with an L-7400 tunable absorption detector. Analytical HPLC was performed with a reverse phase column (Sunfire© C18 column 4.6 x 150 mm, 5 µm) with the following solvent system: water/0.1% TFA (solvent A), acetonitrile (solvent B); 0–3 min: 5% B, 3–19 min: 5–90% B, 19 - 24 min: 95% B, 24 – 25 min: 90 – 5% B, 25 – 30 min 5% B. The flow rate was 1 mL/min and UV detection at 280 nm. Semi-preparative HPLC was performed with a reverse phase semi-preparative column (Sunfire© C18 column 10 x 150 mm, 5 µm) using the same system as mentioned before but with a flow rate of 4 mL/min and UV detection at 280 nm.

Analytical radio-HPLC was performed on an Agilent 1100 system equipped with multi-UV-wavelength and Raytest Gabi Stardetectors and Gina Star software. For the low molecular compounds, a reverse phase column was used (LiChrospher 100RP-18 5 µm LiChroCART 4 x 250 mm) with the following solvent systems: System A: water (solvent A), acetonitrile (solvent B); flow 1 mL/min; 0–3 min: 50% B, 3–15 min: 50–95% B, 15 - 23 min: 95% B, 23 – 25 min: 95 – 50% B, 25 – 30 min 50% B; UV = 280 nm. System B: water (solvent A), acetonitrile (solvent B); flow 1 mL/min; 0–3 min: 10% B, 3–15 min: 10–90% B, 15 - 23 min: 90% B, 23 – 25 min: 90 – 10% B, 25 – 30 min 10% B; UV = 280 nm. System C: water/0.1% TFA (solvent A), acetonitrile (solvent B): flow 1 mL/min; 0–3 min: 5% B, 3–20 min: 5–90% B, 20 - 25 min: 90% B, 25 – 27 min: 90 – 5% B, 27 – 30 min 5% B: UV = 280 nm. For affibody labeling, an Agilent Zorbax 300SB-C18 (4.6 x 150 mm, 3.5 µm) column was used with the following system: System D: water/0.1% TFA (solvent A), acetonitrile/0.1% TFA (solvent B); flow 1 mL/min; 0–2 min: 15% B, 2–22 min: 15–65% B, 22 - 25 min: 65% B, 25 – 26 min: 65 – 15% B, 26 – 30 min 15% B; UV = 280 nm. Semipreparative purification of radiolabeled material was performed on a Merck-Hitachi L6200A system equipped with Knauer variable wavelength detector and an Emberline radiation detector using a reverse phase column (Sunfire© C18 column 10 x 150 mm, 5 µm) with the following solvent systems: System E: NH_4HCO_3 10mM (solvent A), acetonitrile (solvent B); flow 4 mL/min; 0–5 min: 5% B, 5–10 min: 5–40% B, 10 - 25 min: 40% B, UV = 280 nm. System F: H_2O (solvent A), acetonitrile (solvent B); flow 4 mL/min; 0–7 min: 50% B, 7–10 min: 50–95% B, 10 - 20 min: 95% B, UV = 280 nm; System G: H_2O (solvent A), acetonitrile (solvent B); flow 4 mL/min; 0–7 min: 10% B, 7–10 min: 10–40% B, 10 - 20 min: 40% B, UV = 280 nm; System H: H_2O 0.1% TFA (solvent A), acetonitrile (solvent B); flow 4 mL/min; 0–5 min: 5% B, 5–10 min: 5–30% B, 10 - 20 min: 30% B, 20-25 min: 30-40% B, 25-35 min: 40% B, UV = 280 nm. For reaction monitoring during radiosynthesis a Waters Ultra-performance liquid chromatography (UPLC) system was used with an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm, Waters) and an attached coincidence detector (FlowStar LBS13, Berthold). The mobile phase consisted of the following system: water/0.1% TFA (solvent A), acetonitrile/0.1% TFA (solvent B); flow 0.6 mL/min; 0–0.3 min: 0% B, 0.3–2.2 min: 0–70% B, 2.2 – 2.6 min: 70% B, 2.6 – 3.0 min: 70 – 0% B; UV = 280 nm. Specific activity for peptide [^{18}F]**7a** and affibody [^{18}F]**8a** was calculated by comparing ultraviolet peak intensity of final formulated products with calibration curves of corresponding non-radioactive standards of known concentrations.

Materials for peptide synthesis

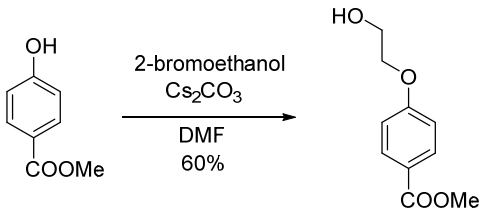
Chemicals and solvents were used without further purification. Fmoc-amino acids, Rink Amide MBHA LL resin (100-200 mesh), HATU was purchased from Merck Biosciences (Nottingham,

UK). Solvents were purchased from Acros Organics (Geel, Belgium), Merck (Darmstadt, Germany), and Sigma Aldrich (Buchs, Switzerland). All other chemicals were from Sigma Aldrich (Buchs, Switzerland). Polypropylene syringes fitted with polypropylene frits and a polypropylene plunger were obtained from MultiSyntech (Witten, Germany) and teflon taps from Biotage (Uppsala, Sweden).

HPLC analysis and purification were performed on Bischoff HPLC systems. The analytical HPLC system was composed of a Bischoff LC-CaDi 22-14 interface, a UV-vis Lambda 1010 detector, and two HPLC compact pumps 2250. HPLC preparative purification was carried out on a Bischoff HPLC system composed of a Bischoff LC-CaDi 22-14 interface, a UV-vis Lambda 1010 detector and two HPLC compact pumps 2250. A Phenomenex Jupiter 4 μm Proteo 90 Å 250 × 4.6 mm was used for analytical separations. A Nucleodur C18 ISIS, 5 μm, 250 × 16 mm column (Macherey Nagel) was used for preparative purification. HPLC solvents A and B were a 0.1% solution of TFA in H_2O and 0.1% solution of TFA in acetonitrile respectively.

4. Syntheses

Synthesis and characterization of compound 1a

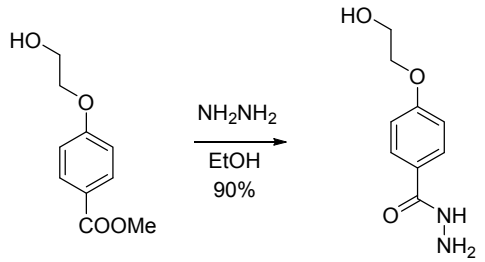


1 **1a**

methyl 4-(2-hydroxyethoxy)benzoate (1a):

Methyl 4-hydroxybenzoate **1** (3.00 g, 19.72 mmol) was dissolved in DMF (65 mL). Finely powdered Cs_2CO_3 (16.06 g, 49.3 mmol) was added at room temperature, followed by 2-bromoethanol (3.70 g, 29.6 mmol). The reaction mixture was heated at 75 ºC and stirred under a N_2 atmosphere for 10 hours. An additional amount of 2-bromoethanol (1.77 g, 14.16 mmol) was added and heating was resumed overnight. Afterwards, the reaction mixture was cooled to room temperature, poured to ice water and extracted with ether (x3). The combined organic extracts were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (EtOAc/Hexane 2:1) to obtain product **1a** as a white solid (2.34 g, 60%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 8.01 - 7.97 (m, 2 H), 6.96 - 6.91 (m, 2 H), 4.16 - 4.12 (m, 2 H), 4.00 – 3.97 (m, 2 H), 3.88 (s, 3 H), 1.99 (br.s, 1 H). ^{13}C NMR (101 MHz, chloroform-*d*) δ ppm 166.8, 162.3, 131.6, 123.0, 114.1, 69.3, 61.3, 51.9. HRMS (ESI-TOF) (m/z): Calculated for $[C_{10}H_{13}O_4+H]^+$, 197.0808. Found, 198.0809.

Synthesis and characterization of compound 1b



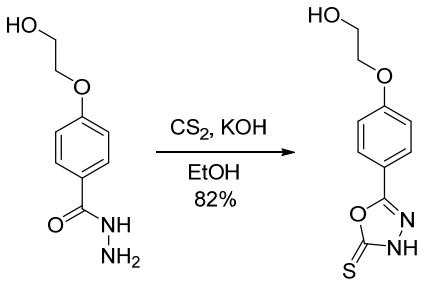
1a **1b**

4-(2-hydroxyethoxy)benzohydrazide (1b)

Compound **1a** (0.498 g, 2.54 mmol) was dissolved in absolute EtOH (12 mL) and hydrazine monohydrate (1.48 mL, 30.5 mmol) was added at room temperature. The reaction mixture was refluxed for 16 hours. Volatiles were then removed under reduced pressure to obtain a solid that was triturated with EtOAc/hexane (1:1) mixture to obtain product **1b** as an off-white solid (0.447 g, 90%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.79 - 7.72(m, 2 H), 7.04 - 6.97 (m,

2 H), 4.11 - 4.08 (m, 2 H), 3.90 - 3.86 (m, 2 H). ^{13}C NMR (101 MHz, methanol-d_4) δ ppm 169.6, 163.4, 130.2, 126.6, 115.5, 70.9, 61.7. HRMS (ESI-TOF) (m/z): Calculated for $[C_9H_{13}N_2O_2+H]^+$, 197.0921. Found, 197.0921.

Synthesis and characterization of compound 1c

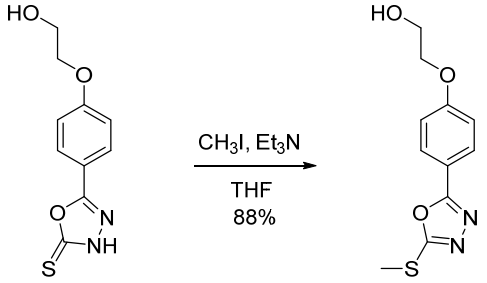


1b **1c**

5-(4-(2-hydroxyethoxy)phenyl)-1,3,4-oxadiazole-2(3H)-thione (1c)

4-(2-Hydroxyethoxy)benzohydrazide **1b** (0.432 g, 2.20 mmol) was dissolved in absolute EtOH (10 mL). Finely powdered KOH (0.124 g, 2.20 mmol) was added at room temperature. followed by CS_2 (0.450 mL, 7.49 mmol). The reaction mixture was stirred for 20 minutes at room temperature and then heated to reflux for 12 hours. Two thirds of the EtOH were removed under reduced pressure and ~ 5 mL of cold water were added. The resultant mixture was then acidified to pH 1-2 (pH paper) with 1M HCl. The white precipitate that formed was collected by filtration, washed with cold water and dried until constant weight to yield product **1c** as a white solid (430 mg, 82%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm: 7.90 - 7.83 (m, 2 H), 7.15 - 7.08 (m, 2 H), 4.16 - 4.11 (m, 2 H), 3.92 - 3.88 (m, 2 H). ^{13}C NMR (101 MHz, methanol-d_4) δ ppm 180.0, 163.8, 162.8, 129.3, 116.8, 116.5, 71.1, 61.6. HRMS (ESI-TOF) (m/z): Calculated for $[C_{10}H_{11}N_2O_3S+H]^+$, 239.0485. Found, 239.0484.

Synthesis and characterization of compound 1d



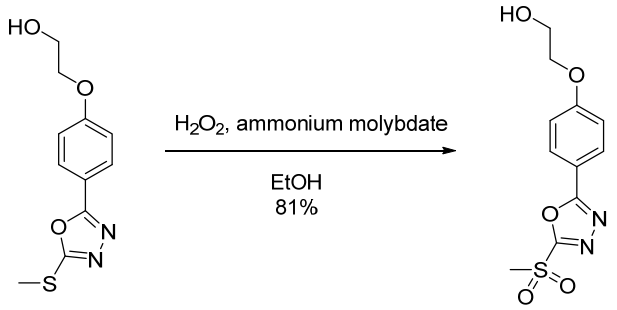
1c **1d**

2-(4-(5-(methylthio)-1,3,4-oxadiazol-2-yl)phenoxy)ethan-1-ol (1d)

Compound **1c** (0.514 g, 2.16 mmol) was dissolved in anhydrous THF (25 mL). NEt_3 (376 µL, 2.70 mmol) was added and the reaction mixture was cooled to 0 °C. Methyl iodide (148 µL, 2.37

mmol) was added, the cooling bath was removed and the reaction mixture was stirred for 2 hours. The reaction mixture was diluted with H_2O and extracted three times with EtOAc. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to obtain the title compound as a white solid (480 mg, 88%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.97 - 7.90 (m, 2 H), 7.03 - 6.97 (m, 2 H), 4.17 - 4.13 (m, 2 H), 4.02 - 3.98 (m, 2 H), 2.76 (s, 3 H), 2.19 (br.s, 1 H). ^{13}C NMR (101 MHz, chloroform-*d*) δ ppm 165.6, 164.4, 161.3, 128.4, 116.5, 115.0, 69.4, 61.2, 14.6. HRMS (ESI-TOF) (m/z): Calculated for $[C_{11}H_{13}N_2O_3S+H]^+$, 253.0641. Found, 253.0640.

Synthesis and characterization of compound 2

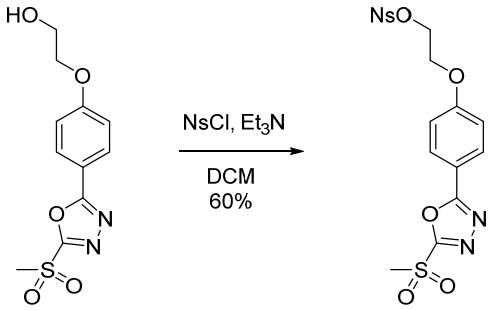


1d **2**

2-(4-(5-(methylsulfonyl)-1,3,4-oxadiazol-2-yl)phenoxy)ethan-1-ol (2)

Compound **1d** (200 mg, 0.793 mmol) was dissolved in absolute EtOH (5 mL) and the solution was cooled to 0 °C. Ammonium molybdate tetrahydrate (196 mg, 0.159 mmol) was added followed by H_2O_2 30% wt solution in water (239 µL, 2.38 mmol). The cooling bath was removed and the heterogeneous mixture was vigorously stirred for 45 minutes. At this point a second aliquot of H_2O_2 30% wt solution in water was added at room temperature (480 µL, 4.76 mmol). A thick slurry soon formed and 6 mL of absolute EtOH were added. After 30 minutes a third aliquot of H_2O_2 30% wt solution in water (239 µL, 2.38 mmol) was added at room temperature. After 45 minutes all starting material was consumed as indicated by TLC. The reaction mixture was cooled to 0 ºC, excess H_2O_2 was quenched with acqueous sodium thiosulfate sat. solution. The reaction mixture was further diluted with water and extracted three times with EtOAc. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was purified by flash column chromatography (solid deposition, 100% EtOAc) to obtain product **2** as an off-white solid (183 mg, 81%). ^{1}H NMR (400 MHz, acetone-d_6) δ ppm 8.10 - 8.02 (m, 2 H), 7.22 - 7.15 (m, 2 H), 4.21 (t, J = 4.6 Hz, 2 H), 4.09 (t, J = 5.8 Hz, 1 H), 3.95 - 3.90 (m, 2 H), 3.63 (s, 3 H). ^{13}C NMR (101 MHz, acetone-d_6) δ ppm 167.5, 164.3, 163.4, 130.7, 161.7, 116.0, 71.5, 61.5, 43.9. Calculated for $[C_{11}H_{13}N_2O_5S+H]^+$, 285.0540. Found, 285.0540.

Synthesis and characterization of compound 3

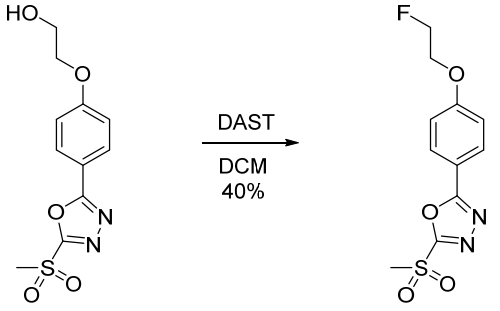


2 **3**

2-(4-(5-(methylsulfonyl)-1,3,4-oxadiazol-2-yl)phenoxy)ethyl 4-nitrobenzenesulfonate (3)

Alcohol **2** (130 mg, 0.46 mmol) was dissolved in anhydrous CH_2Cl_2 (4.5 mL). NEt_3 (94 µL, 0.67 mmol) and DMAP (16.8 mg, 0.14 mmol) were sequentially added. The reaction mixture was cooled to 0 ºC and 4-nitrobenzenesulfonyl chloride (132 mg, 0.59 mmol) was added. The cooling bath was removed and the reaction mixture was stirred at room temperature for 3 hours. Water was added and the reaction mixture was extracted three times with CH_2Cl_2. The combined organic extracts were dried (MgSO4) and concentrated under reduced pressure. The crude material was purified by flash column chromatograpy (Hexane/EtOAc 2:1) to obtain 129 mg of the title compound as a white solid (60%). Precursor was stable at -25 °C for a period over 6 months. ^{1}H NMR (400 MHz, acetonitrile-d_3) δ ppm 8.40 - 8.35 (m, 2 H), 8.17 - 8.13 (m, 2 H), 8.04 - 8.00 (m, 2 H), 7.03 - 6.98 (m, 2 H), 4.55 - 4.51 (m, 2 H), 4.34 - 4.29 (m, 2 H), 3.48 (s, 3 H). ^{13}C NMR (101 MHz, acetonitrile-d_3) δ ppm 167.7, 163.3, 163.0, 142.6, 130.8, 130.7, 126.1, 116.8, 71.4, 67.1, 44.3. HRMS (ESI-TOF) (m/z): Calculated for $[C_{17}H_{16}N_3O_9S_2+H]^+$, 470.0322. Found, 470.0326.

Synthesis and characterization of compound 4b



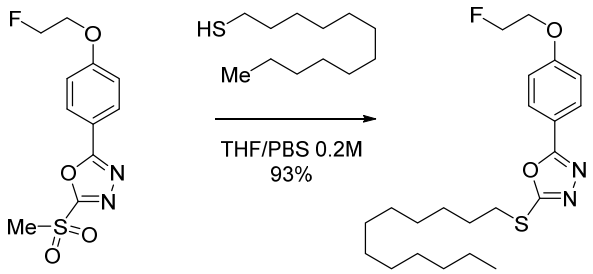
2 **4b**

2-(4-(2-fluoroethoxy)phenyl)-5-(methylsulfonyl)-1,3,4-oxadiazole (4b)

Alcohol **2** (15 mg, 0.053 mmol) was dissolved in anhydrous CH_2Cl_2 (1.0 mL). The reaction mixture was cooled to 0 °C with an ice bath and DAST (9 µL, 0.07 mmol) was added. The cooling bath was removed and the mixture was stirred until complete disappearence of the starting

material (TLC monitoring, approximately 1.5 h). The reaction mixture was quenched with water and extracted with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), concentrated under reduced pressure and purified by gravity column chromatography using hexane/ethyl acetate 5:5 to yield compound **4b** as a white solid (6 mg, 40%). ^{1}H NMR (400 MHz, acetone-d_6) δ ppm 8.14 - 8.06 (m, 2 H), 7.28 - 7.20 (m, 2 H), 4.83 (dm, J_{HF}=47.8 Hz, 2 H), 4.44 (dm, J_{HF}=29.0 Hz, 2 H), 3.63 (s, 3 H). ^{13}C NMR (101 MHz, acetone-d_6) δ ppm 167.6, 163.9, 163.6, 130.9, 116.9, 116.7, 83.2 (d, $^1J_{C\text{-}F}$ = 168.4 Hz), 69.2 (d, $^2J_{C\text{-}F}$ = 19.3 Hz), 44.0. HRMS (ESI-TOF) (m/z): Calculated for $[C_{11}H_{12}FN_2O_4S+H]^+$, 287.0496 Found, 287.0498.

Synthesis and characterization of compound 5b

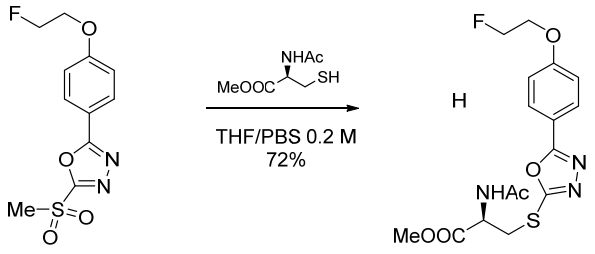


4b **5b**

2-(dodecylthio)-5-(4-(2-fluoroethoxy)phenyl)-1,3,4-oxadiazole (5b)

Compound **4b** (7.2 mg, 0.025 mmol) was dissolved in THF (1 mL) and Phosphate Buffer Solution 200 mM, pH=7.4 (1 mL) was added. Dodecanethiol (4.2 mg, 0.021 mmol) dissolved in THF (0.2 mL) was then added and the reaction mixture was stirred at 23 °C for 2 hours. The reaction mixture was diluted with water, extracted with EtOAc. The organic pahses were dried ($MgSO_4$), concentrated under reduced pressure and purified by column chromatography (Hexane/EtOAc 4:1) to yield coompound **5b** as a white solid (8 mg, 93%). ^{1}H NMR (400 MHz, chloroform-d) δ ppm 7.97 - 7.92 (m, 2 H), 7.05 - 6.99 (m, 2 H), 4.79 (dm, $^2J_{H\text{-}F}$=47.4 Hz, 2 H), 4.28 (dm, $^3J_{H\text{-}F}$=27.4 Hz, 2 H), 3.28 (t, J = 7.4 Hz, 2 H), 1.83 (quintet, $J_1 = J_2$ = 7.4 Hz, 2 H), 1.50 - 1.41 (m, 2 H), 1.26 (br, 16 H), 0.90 – 0.85 (m, 3 H). ^{13}C NMR (101 MHz, chloroform-d) δ ppm 165.4, 164.0, 161.0, 128.5, 116.9, 115.0, 81.5 (d, $^1J_{C\text{-}F}$ = 171.5 Hz), 67.2 (d, $^2J_{C\text{-}F}$ = 20.7 Hz), 32.7, 31.9, 29.6, 29.5, 29.4, 29.32, 29.26, 29.0, 28.6, 22.7, 14.1. HRMS (ESI-TOF) (m/z): Calculated for $[C_{22}H_{34}FN_2O_2S+H]^+$, 409.2320. Found, 409.2316.

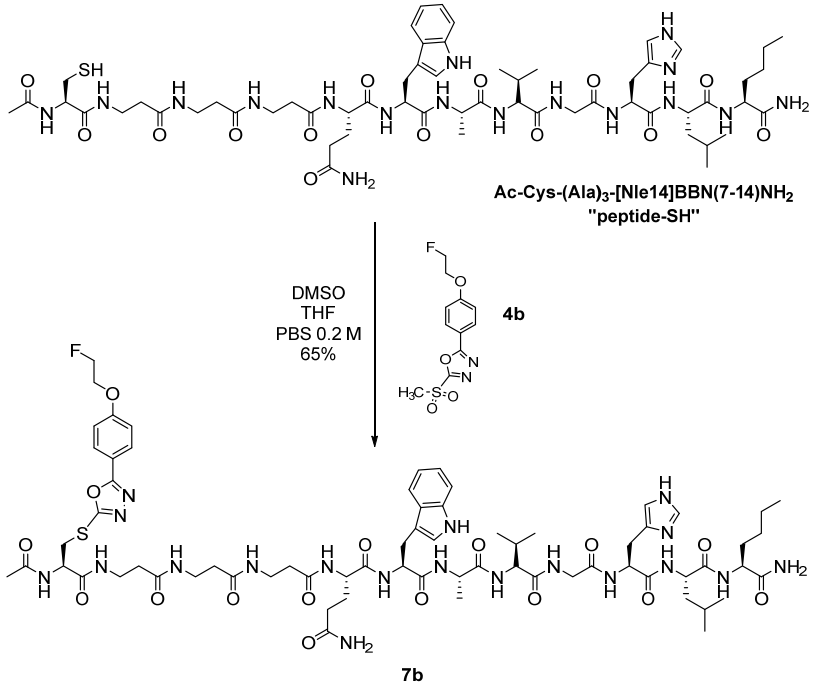
Synthesis and characterization of compound 6b



methyl N-acetyl-S-(5-(4-(2-fluoroethoxy)phenyl)-1,3,4-oxadiazol-2-yl)-L-cysteinate (6b)

Methyl acetyl-L-cysteinate (5 mg, 0.025 mmol) in THF (0.3 mL) was added to a solution of **4b** (8.7 mg, 0.03 mmol) in THF (0.9 mL) and Phosphate Buffer Solution 200 mM, pH=7.4 (1.2 mL) and the reaction was stirred at 23 °C for 2 hours. The reaction mixture was diluted with water and extracted with EtOAc. The organic pahses were dried over sodium sulfate, concentrated under reduced pressure and purified by column chromatography on silica using ethyl acetate to yield compound **6b** as a white solid (7 mg, 72%). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.97 – 7.91 (m, 2 H), 7.06 – 6.99 (m, 2 H), 6.87 (d, J = 7.1 Hz, 1 H), 5.03 – 4.96 (m, 1 H), 4.78 (dm, $^{2}J_{H\text{-}F}$ = 47.3 Hz, 2 H), 4.28 (dm, $^{3}J_{H\text{-}F}$ = 27.6 Hz, 2 H), 3.81 (dd, J_1 = 14.6 Hz, J_2 = 4.4 Hz, 1 H), 3.77 (s, 3 H), 3.72 (dd, J_1 = 14.6 Hz, J_2 = 6.1 Hz, 1 H), 2.00 (s, 3 H). ^{13}C NMR (101 MHz, chloroform-*d*) δ ppm 170.2, 170.1, 166.0, 163.1, 161.2, 128.6, 116.3, 115.1, 81.6 (d, $^{1}J_{C\text{-}F}$ = 171.4 Hz), 67.2 (d, $^{2}J_{C\text{-}F}$ = 20.7 Hz), 53.0, 52.6, 34.3, 23.0. HRMS (ESI-TOF) (m/z): Calculated for $[C_{16}H_{18}FN_3O_5S+H]^+$, 384.1024. Found, 384.1024.

Synthesis and characterization of compound 7b



A. Synthesis of the bombesin peptide derivative AcCys(βAla)$_3$[Nle^{14}]BBN(7-14)NH$_2$ (peptide-SH)

The peptide was synthesized by automated and manual synthesis. 0.1 mmol of a Rink MBHA LL resin was placed in a reactor for automated peptide synthesizer and residues Fmoc-Nle-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Trp(Boc)-OH, Fmoc-Gln(Trt)-OH, and Fmoc-βAla-OH (3 x) were coupled on the resin using the general procedure for automated peptide synthesis.

The resin was then placed in a syringe fitted with a polypropylene frit and a teflon tap. To the resin swollen in DMF were successively added Fmoc-Cys(Trt)-OH (2 equiv., 0.2 mmol), HATU (2 equiv.) and *i*-Pr$_2$NEt (5 equiv.) and the suspension was shaken at RT for 1 h. The solvent and excess reagents were then removed by filtration and the resin was thoroughly washed by DMF and CH_2Cl_2. The completion of the reaction was checked by the Kaiser test. The coupling was followed by Fmoc-deprotection with 20% piperidine/DMF solution (3 x 3 minutes). Acetylation of the N-terminal amine function was achieved manually by treatment with an acetylation solution (Ac$_2$O/*i*-Pr$_2$NEt/DMF (5:3:92)) for 1 h.

The peptide resin was then cleaved and deprotected by 2 h treatment with a mixture of TFA/H$_2$O/*i*-Pr$_3$SiH/PhOH (87.5:5:2.5:5), and the peptide was precipitated with ice-cold diethyl ether, centrifuged and washed twice with cold diethyl ether. The precipitate was purified by preparative HPLC to obtain the desired peptide in 40% yield after lyophilization. HRMS (ESI-TOF) (m/z): Calcd for $[C_{58}H_{90}N_{17}O_{14}S+H]^+$, 1280.6568 Found, 1280.6551

B. Synthesis of compound 7b

To a solution of Ac-Cys-(βAla)$_3$-[Nle^{14}]BBN(7-14)NH$_2$-(peptide-SH; 6 mg, 4.69 μmol) in DMSO (0.2 mL) and PBS 0.2 M, pH=7.4 (1.8 mL) was added a solution of **4b** (1.6 mg, 5.63 μmol) in THF (0.2 mL) and the reaction was stirred at room temperature for 2 h at which point HPLC confirmed the complete consumption of the starting peptide (product t_R = 13.4 min). The reaction mixture was quenched with saline (1 mL) and it was purified by semi-preparative HPLC with the system specified in the Materials and Methods section (t_R = 11.5 min). The relevant fractions were combined and lyophilized to provide the product as a white powder (4.5 mg, 65%). HRMS (ESI-TOF) (m/z): Calculated for $[C_{68}H_{98}FN_{19}O_{16}S+2H]^{2+}$, 743.8566. Found, 743.8576.

Synthesis and characterization of compound 8b

A. Synthesis of affibody $Z_{HER2:2395}$-CYS.

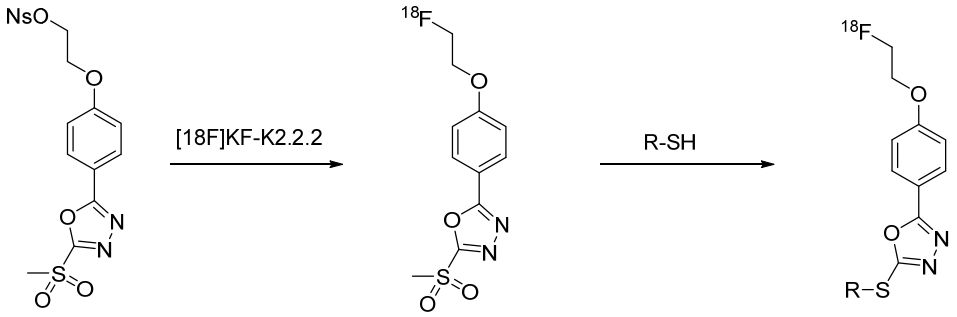
Anti-HER2 $Z_{HER2:2395}$ affibody molecule bearing a C-terminal cysteine was produced by Affibody AB (Solna, Sweden) as described earlier and provided in freeze-dried form. (Ahlgren S, Orlova A, Rosik D, Sandström M, Sjöberg A, Baastrup B, Widmark O, Fant G, Feldwisch J, Tolmachev V. Evaluation of maleimide derivative of DOTA for site-specific labeling of recombinant affibody molecules. *Bioconjug Chem.* **2008**, *19*, 235-43).

B. **Synthesis of compound 8b**

Affibody $Z_{HER2:2395}$-Cys (1 mg, 0.14 µmol) was dissolved in 1 ml PBS 0.6 M (pH=7.4) and 15 µL of DTT (1 M solution in water, freshly prepared) was subsequently added. The mixture was incubated for 2 h at 37 °C. The reduced affibody was purified by passing the solution through a NAP-5 size exclusion column (GE Healthcare Life Sciences) preconditioned with well-degassed PBS 0.2 M/Na ascorbate 0.02 M) and eluting with the same afore-mentioned solution. The first 900 µL of the high molecular weight (HMW) fraction were collected and then a solution of **4b** (0.2 mg, 0.7µmol) in 100 µL DMSO was added. The reaction was stirred at 37 °C for 2 h and then at 55 °C for 1 h. After cooling down, the mixture was passed through a NAP-5 column preconditioned with PBS 0.2 M (pH = 7.4) and the first 900 µL were collected. An aliquot of 400 µL was purified via a single injection on an analytical column (Agilent Zorbax 300SB-C18, 4.6 x 150 mm, 3.5 µm) using system D, t_R = 9.70 min). The structure of the product was confirmed by mass spectrometry. Calculated for $[M+7H]^{7+}$, 1029.86. Found, 1029.84.

5. Radiosyntheses

General Scheme:



3 **4a**, [^{18}F]**FPOS** **5a - 8a**

[^{18}F]-fluoride was obtained via the ^{18}O(p,n)^{18}F reaction using 98% enriched ^{18}O-water. $^{18}F^-$ was trapped on a light QMA carbonate cartridge (Waters). Kryptofix $K_{2.2.2.}$ solution (Kryptofix $K_{2.2.2.}$ 2.5 mg, K_2CO_3 1 mg in MeCN/water (2.3:1)) was used for the elution of $^{18}F^-$ from the cartridge. The solvents were evaporated at 110 °C under vacuum with a slight inflow of nitrogen gas. After addition of acetonitrile (1 mL), azeotropic drying was carried out. This procedure was repeated twice to afford dry [^{18}F]KF-$K_{2.2.2.}$ complex. A solution of nosylate **3** (3 mg, 6.4×10^{-3} mmol) in a mixture of dry MeCN (300 µl) and dry t-BuOH (300 µl) was added to the dried [^{18}F]KF-$K_{2.2.2}$ complex. The reaction mixture was heated at 85 °C for 10 min to afford crude **4a**. For radiosyntheses of **5a** and **6a,** [^{18}F]FPOS **4a** was used directly while for **7a** and **8a**, it was purified by semi-prep HPLC using system E. Average radiochemical yield of **4a** was 27±6% (n=7; 6.4 - 16 GBq), decay corrected from End of Bombardment (EOB). Average synthesis time was 60 min (without purification) and 90 min (with purification).

*Radiosynthesis of model compounds **5a, 6a***

After cooling down, PBS 0.2M, pH=7.4 (0.5 mL) was added to the crude **4a**, followed by dodecacyl thiol (3.5 mg, 0.017 mmol) in THF (0.3 mL) or methyl acetyl-L-cysteinate (5 mg, 0.028 mmol) in THF (0.3 mL). The reaction mixture was stirred at 37 °C for 15 min after which time complete conversion of prosthetic group 4a was observed. The products were then purified by semi-preparative HPLC using system F for **5a** (t_R = 16.2 min) and system G for **6a** (t_R = 10 min) respectively. The identity of the labelled compounds was confirmed by co-injection with the corresponding non-radioactive standards; system A for **5a** (t_R = 21.2 min) and system B for **6a** (t_R = 12.5 min).

*Radiosynthesis of model peptide **7a***

After cooling down, the crude reaction of **4a** was diluted with aqueous NH_4HCO_3 10 mM (2.4 mL) and purified by semi-preparative HPLC using system E, with **4a** eluting at about 19 min which was collected in a vial containing 30 mL of water. The product was trapped on a C18 light SepPak cartridge (Waters), which was preconditioned with ethanol (5 mL) and water (10 mL). The product was eluted with 1 mL of acetonitrile. The solvent was removed under reduced pressure at 80 °C to a volume of approximately 0.2 mL and then peptide-SH in DMSO (50 µl) and PBS 0.2 M (0.2 mL) was added. For peptide-SH (2 mg, 1.6×10^{-3} mmol), the reaction was

stirred at 37 °C for 15 min which led to quantitative consumption of labelled intermediate **4a** providing cleanly **7a**. For peptide-SH (0.5 mg) the reaction was stirred at 37 °C for 15 min after which time some **4a** remained unreacted (conversion approximately 33%). The reaction was diluted with water 0.1% TFA (2.5 mL) and was purified by semi-preparative HPLC using system H with **7a** eluting at 23.2 min. Specific activity was 53 GBq/µmol and radiochemical yield was 2%. In another experiment, the reaction was repeated with 0.5 mg of peptide-SH, stirring at 55 °C for 15 min. These conditions led to quantitative conversion of **4a** to **7a**. In all cases, the identity of **7a** was confirmed by co-injection with the corresponding non radioactive standard; HPLC system C (t_R = 14.7 min).

Radiosynthesis of affibody [^{18}F]$Z_{HER2:2395}$-Cys **(8a)**

Before radiolabelling, a stock solution of affibody (2 mg/mL in PBS 0.2 M, pH=7.4) was treated with dithiothreitol (DTT) in order to reduce spontaneously formed intermolecular disulfide bonds as previously described[2]. Briefly, 0.5 mL of affibody solution was mixed with 15 µl of DTT (1 M solution in water, freshly prepared) and incubated for 2 h at 37 °C. The mixture was purified by passing the solution through a NAP-5 size exclusion column (GE Healthcare Life Sciences) preconditioned with well-degassed PBS 0.2 M/Na ascorbate 0.02 M) and eluting with the same afore-mentioned solution. The first 900 µl of the high molecular weight (HMW) fraction were used for coupling with **4a**. For this experiment, the HPLC-purified intermediate **4a** was eluted with 1 mL of ethanol after trapping it on a C18 light SepPak cartridge and the volume was reduced to approximately 0.1 mL at 80 °C under reduced pressure. Purified reduced affibody was then added and the reaction was heated at 55 °C for 15 min. The conversion to the labelled affibody was approximately 40% as estimated by radio-TLC (80% aqueous acetone, R_f (**8a**) is at the baseline, R_f (**4a**) is at the front of the solvent. After cooling, the reaction was purified via a NAP-5 column and as before, the product eluted with the first 900 µl of PBS 0.2M/Na ascorbate 0.02M. Quality control and specific activity determination were performed with analytical HPLC using system D (t_R = 10.6 min). Average radiochemical yield was 3% (d.c.) with specific activity in the range of 8-17 GBq/µmol (n=3).

6. Small animal PET/CT imaging

Animals

Animal experiments were approved by the local veterinarian department of the Canton Aargau and conducted in accordance with the Swiss law of animal protection and welfare. Five-week old female CD1 nude mice were purchased from Charles River Laboratories (Sulzfeld, Germany) and inoculated subcutaneously in the left and right shoulder region each with 5x10^6 HER2-positive SKOV3 cells (n=3) or with 1x10^7 HER2-negative RAMOS cells (n=2).

Cell Culture

SKOV3ip cells (a kind gift from Prof. P. Altevogt, German Cancer Center, Heidelberg, Germany) were cultured in DMEM medium and Ramos cells (Leibniz Institute DSMZ, Braunschweig Germany) in RPMI 1640 medium at 37 ^{0}C in a humidified atmosphere containing 5% CO_2. The medium were supplemented with 10% fetal calf serum (FCS), 2 mM of glutamine, 100 units/mL

of penicillin, 0.1 µg/mL of streptomycin, 0.25 µg/mL of fungizone, and additionally 1 mM sodium pyruvate for RPMI. All media and additives were obtained from BioConcept (Allschwil, Switzerland).

In vivo PET/CT Imaging

After 4 to 5 weeks of tumor growth mice were injected intravenously via the tail vein with 7-20 MBq of ^{18}F-Affibody (15 ug peptide per mouse, 8-17 GBq/µmol) and scanned in a 2-bed whole body configuration from 2-3 or 4-5 hours post injection (30 minutes of acquisition time per bed position) in the small-animal PET/CT scanner Super Argus (Sedecal, Madrid, Spain). Ten minutes before scan start, mice were anesthetized in an incubation chamber containing 5% isoflurane (Rothacher, Switzerland) in a mixture (1/1) of air and oxygen. During the scans, the mice were kept anesthetized with 2%–3% isoflurane in air/oxygen (200 mL/min each). The respiration rate was monitored (Model 1025 L, SA Instruments) and maintained at 90 bpm by adjusting the isoflurane dose if required. Body temperature was kept at 37 °C by a fan controlled by a rectal temperature probe. Each PET scan was followed by a CT acquisition. PET data were reconstructed to a voxel size of 0.3875 × 0.3875 × 0.775 mm with a 3-dimensional FORE/2-dimensional OSEM algorithm in one 60 minutes time frame. Scatter and random correction were applied. PET data were analyzed with the PMOD software package (PMOD Technologies Ltd., Zurich, Switzerland). For direct comparison of accumulated radioactivity in each mouse, radioactivity was normalized to the injected dose and body weight of the animal to reveal the standardized uptake values (SUV).

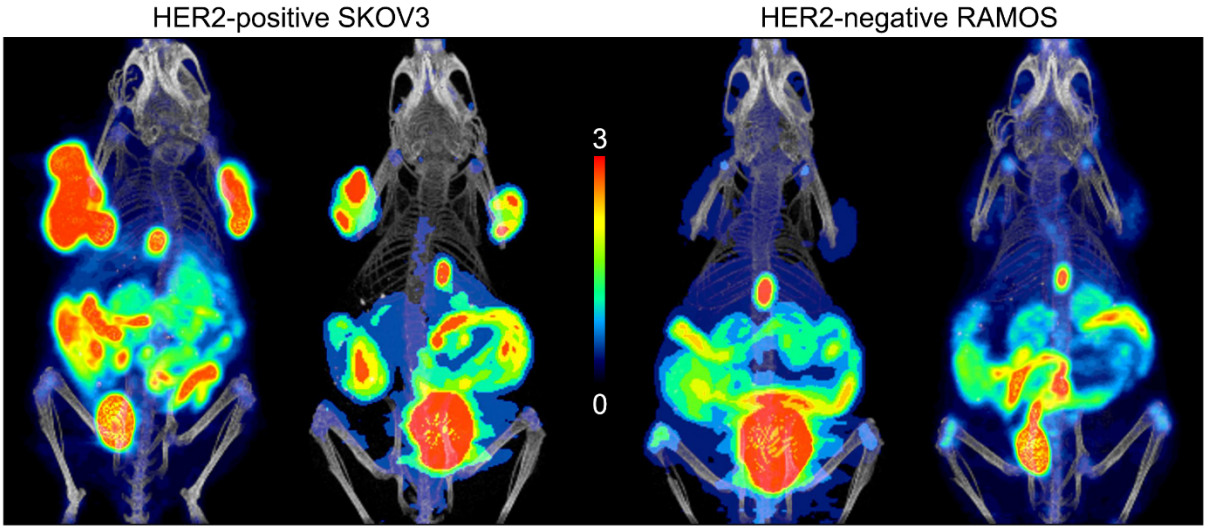


Figure S1: Representative 3-dimensional maximal intensity projections of PET/CT scans performed with two mice bearing HER2-positive SKOV3 tumors (left) and two mice bearing HER2-negative RAMOS tumors (right). Image acquisition was performed from 2-3 hours after injection of [^{18}F]$Z_{HER2:2395}$-Cys **8a** (7-20 MBq, 15 µg). Images were averaged over the whole scan period. The maximal SUV (color bar) is scaled to 3 for all animals.

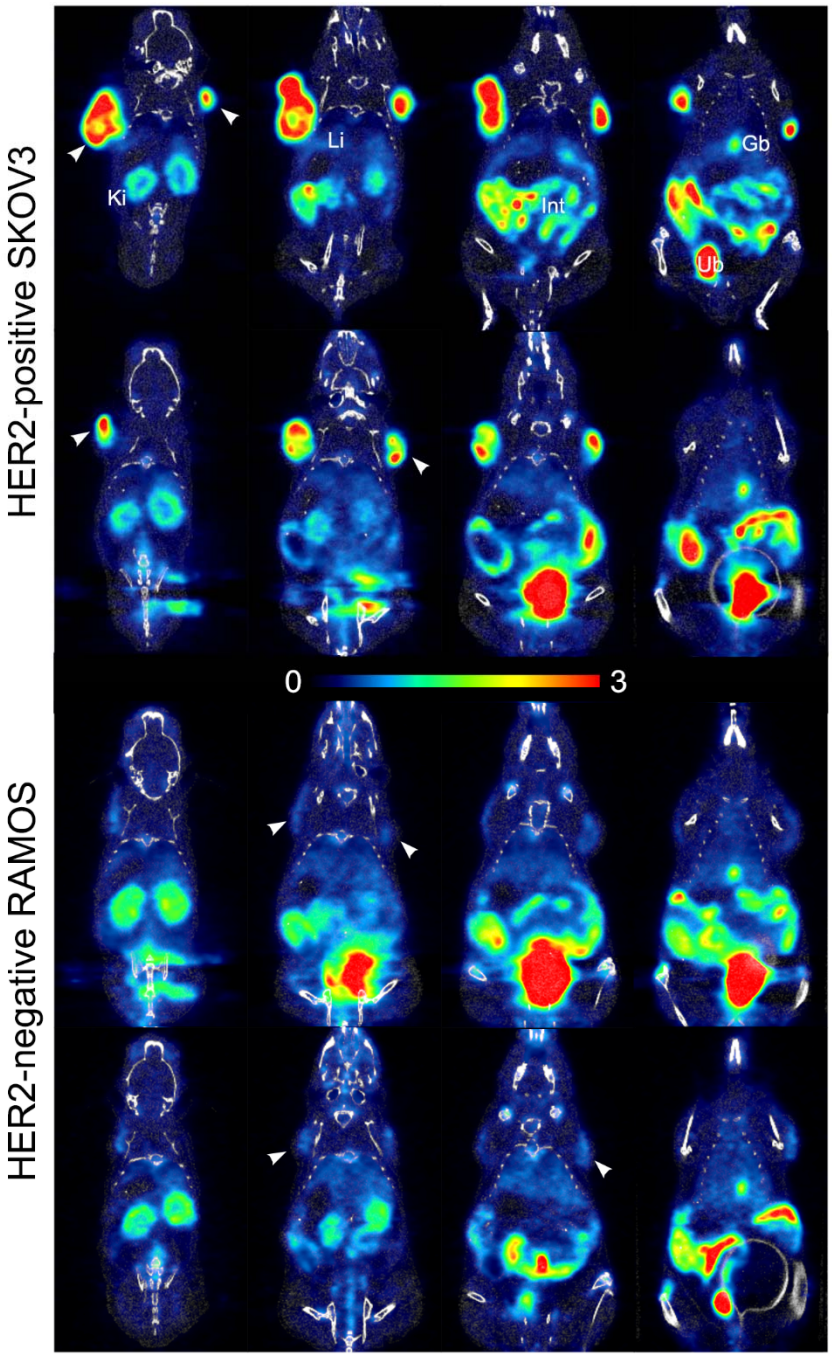
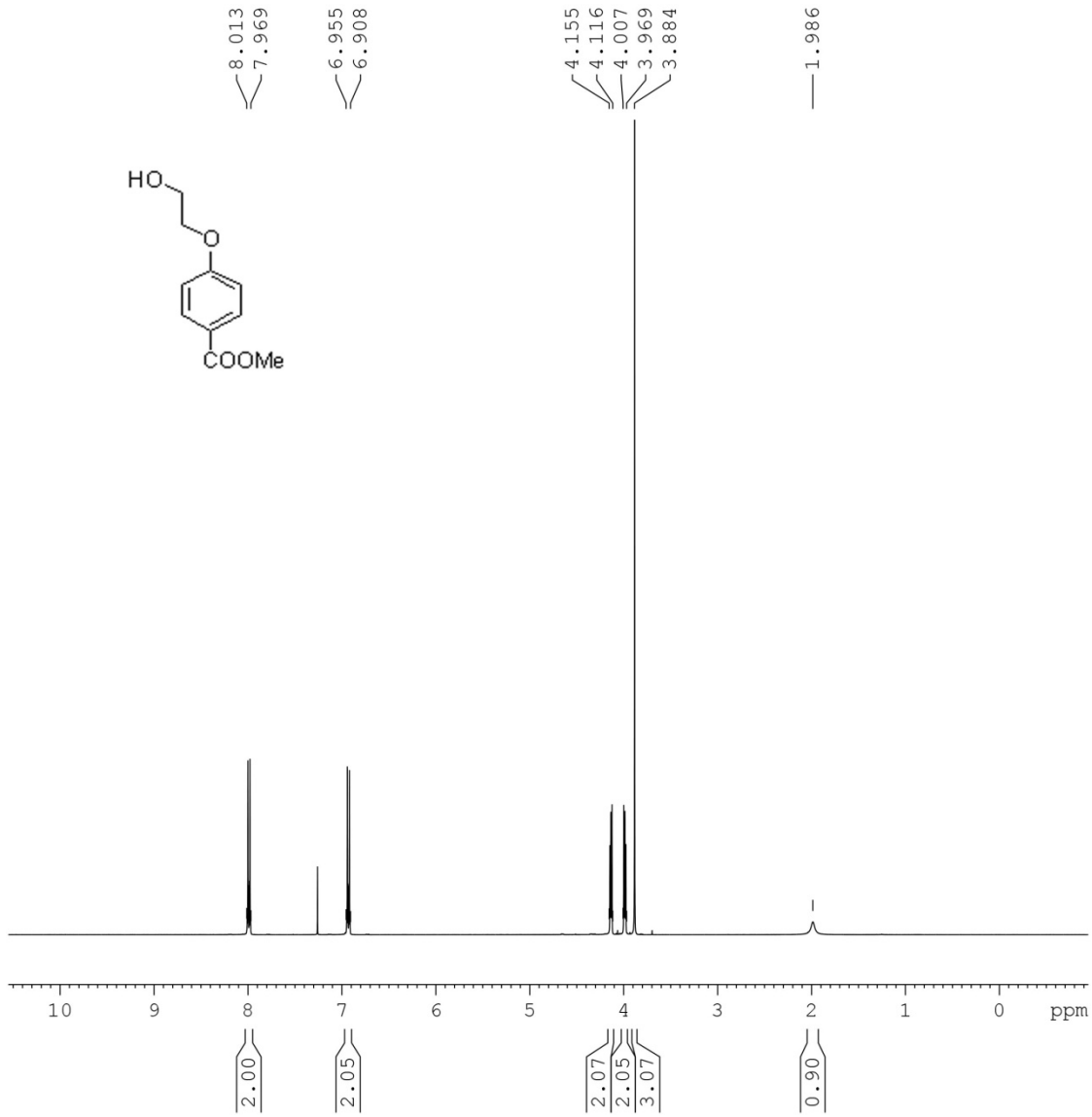


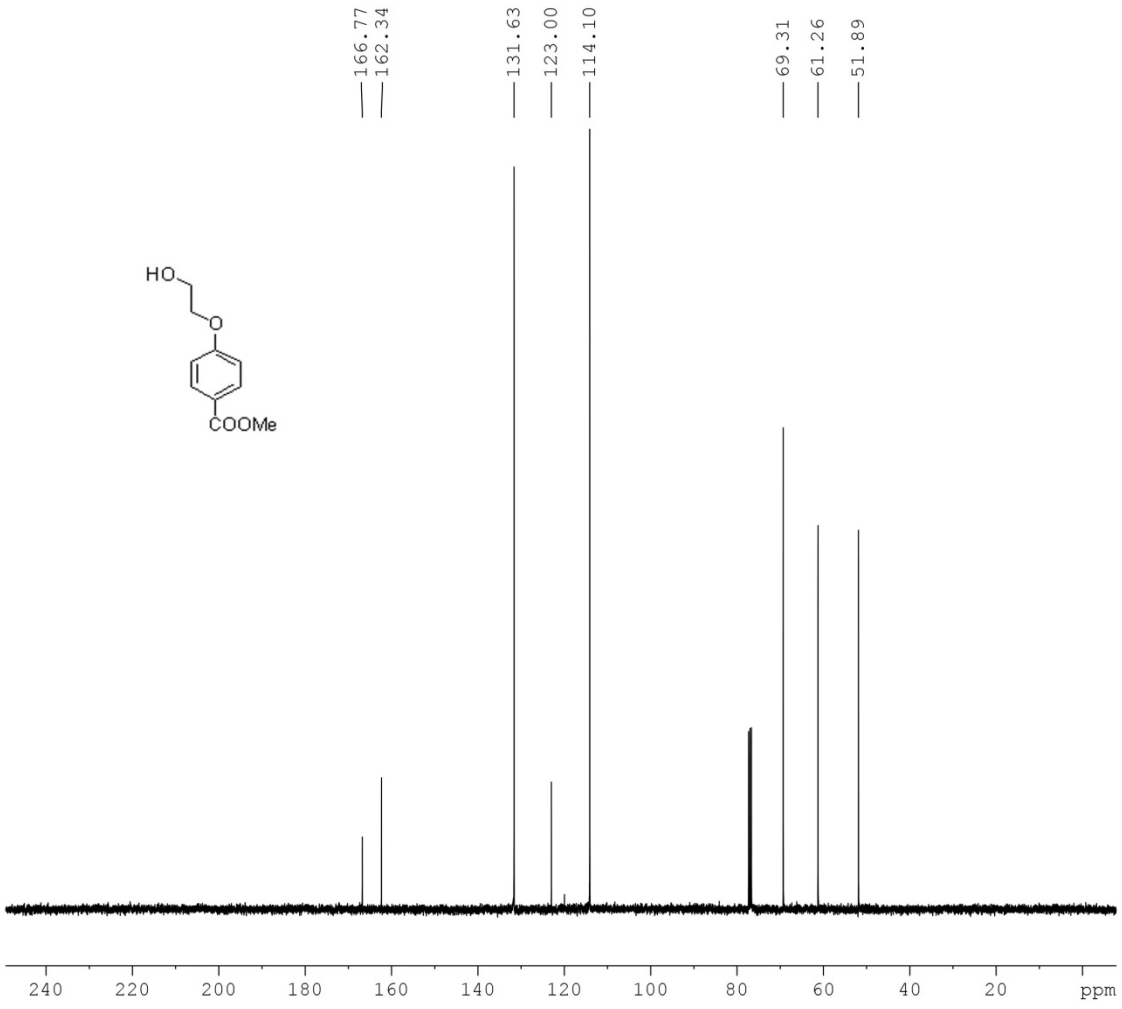
Figure S2: Series of 4 horizontal whole body PET/CT slices (ventral to dorsal) of two mice bearing HER2-positive SKOV3 tumors (top) and two mice bearing HER2-negative RAMOS tumors (bottom) (see arrow heads) which were injected with dy [^{18}F]FPOS-$Z_{HER2:2395}$-Cys **8a** and scanned from 2 to 3 hours post injection. The maximal SUV (color bar) is scaled to 3 for all animals. Ki, kidneys; Li, liver; Int, intestine; Gb, gall bladder; Ub urinary bladder.

Uptake in kidneys and the urinary bladder is the result of renal elimination of the radiotracer, a common feature reported for the protein. Low levels of radioactivity were also found in the intestines and gall bladder indicating hepatobiliary excretion to some extent. This may be the result of the more lipophilic character of radiohalogenated affibody formats in comparison to reported radiometallated analogues.[3]

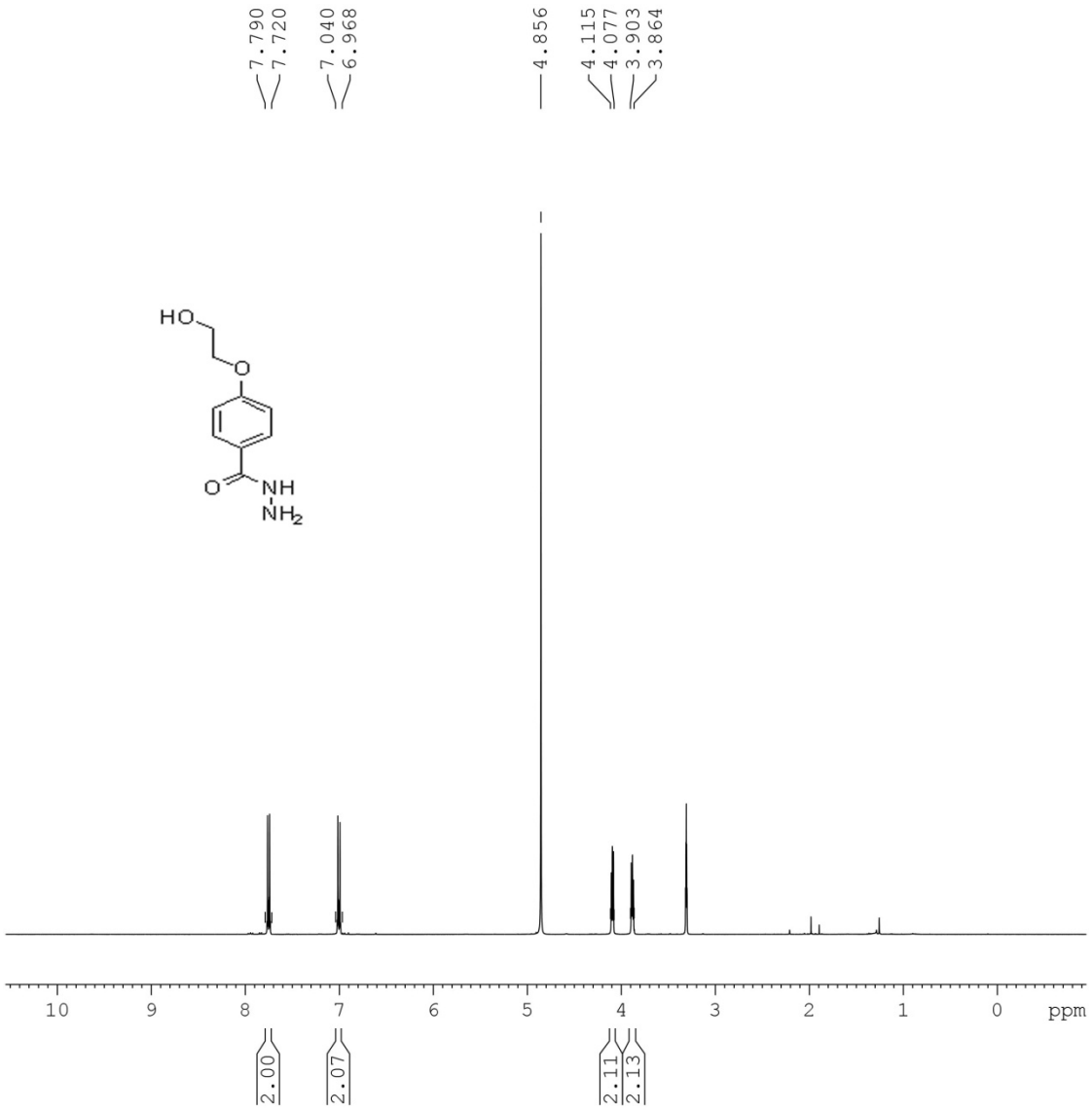
7. NMR spectra

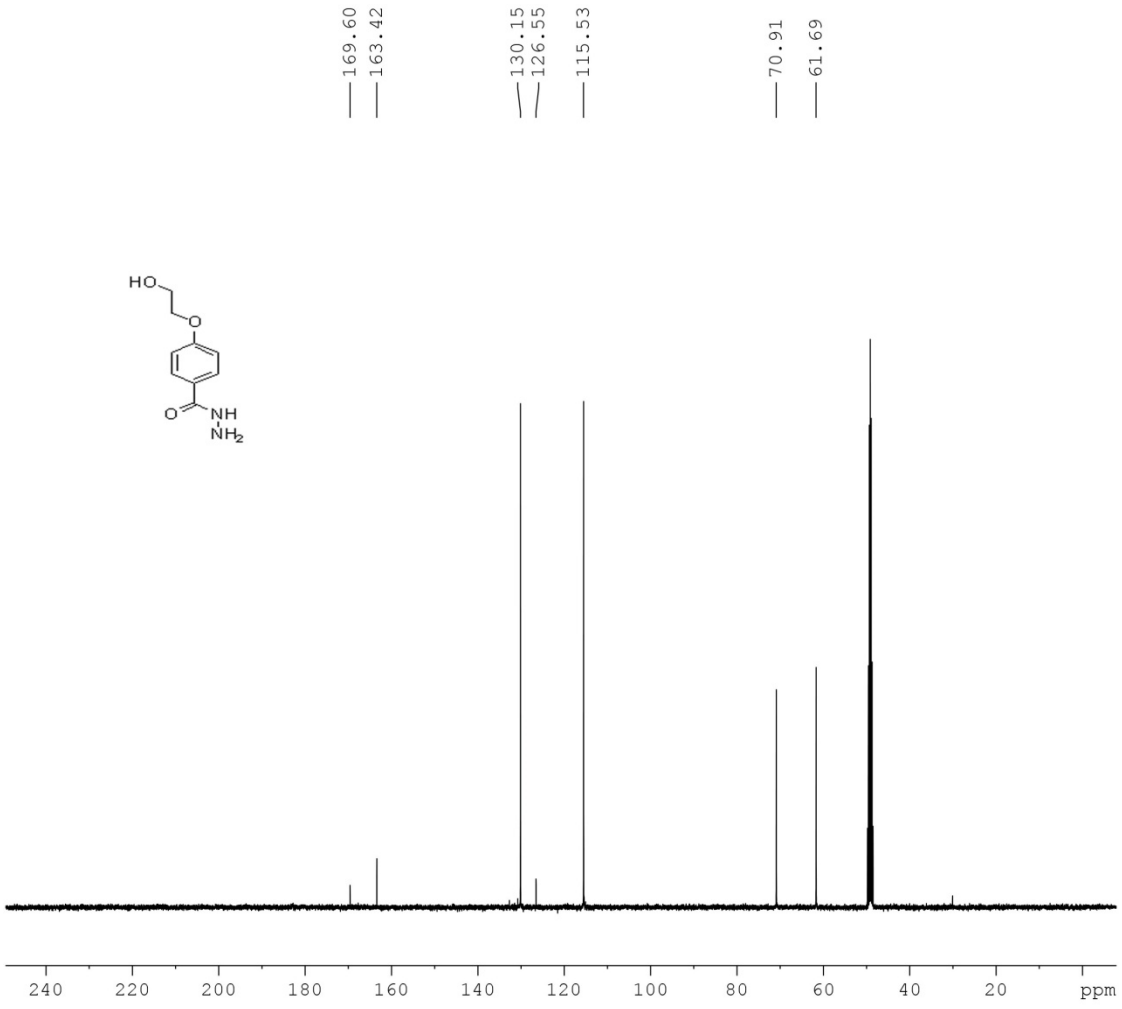
Compound **1a**



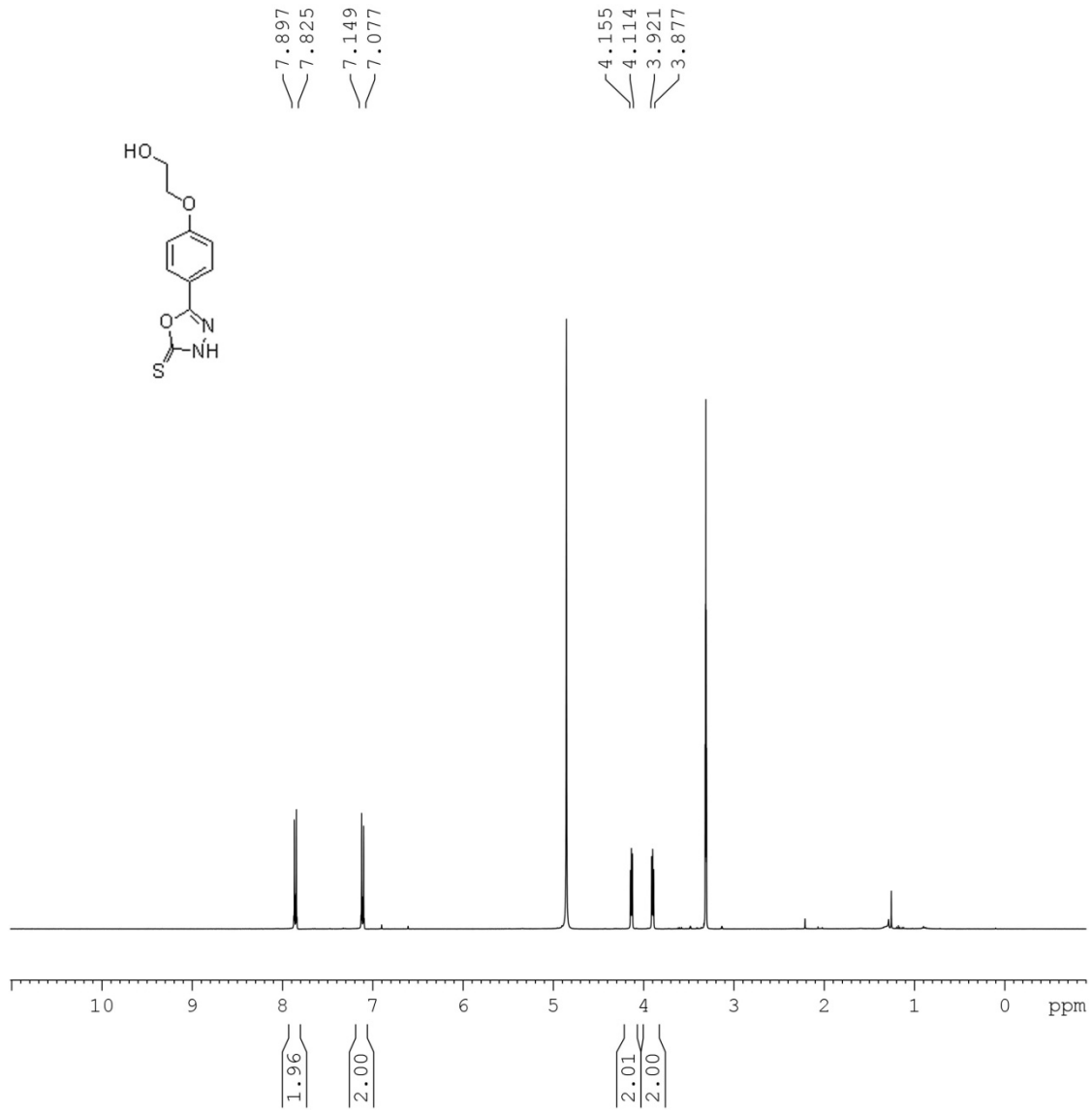

166.77
162.34
131.63
123.00
114.10
69.31
61.26
51.89
HO
O
COOMe
240
220
200
180
160
140
120
100
80
60
40
20
ppm

Compound **1b**


7.790
7.720
7.040
6.968
4.856
4.115
4.077
3.903
3.864
HO
O
O
NH
NH2
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
2.07
2.11
2.13


169.60
163.42
130.15
126.55
115.53
70.91
61.69
HO
O
O
NH
NH2
240
220
200
180
160
140
120
100
80
60
40
20
ppm

Compound **1c**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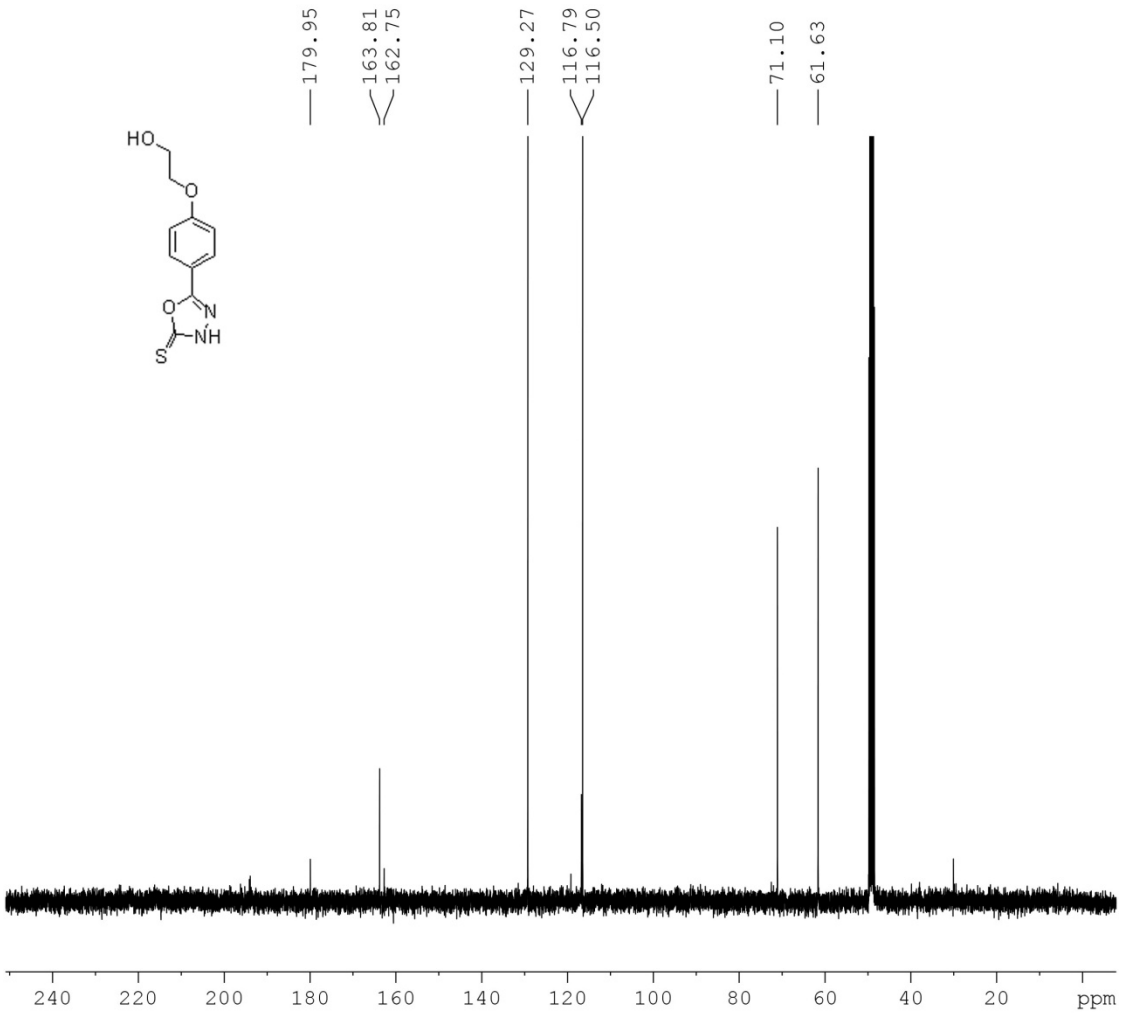
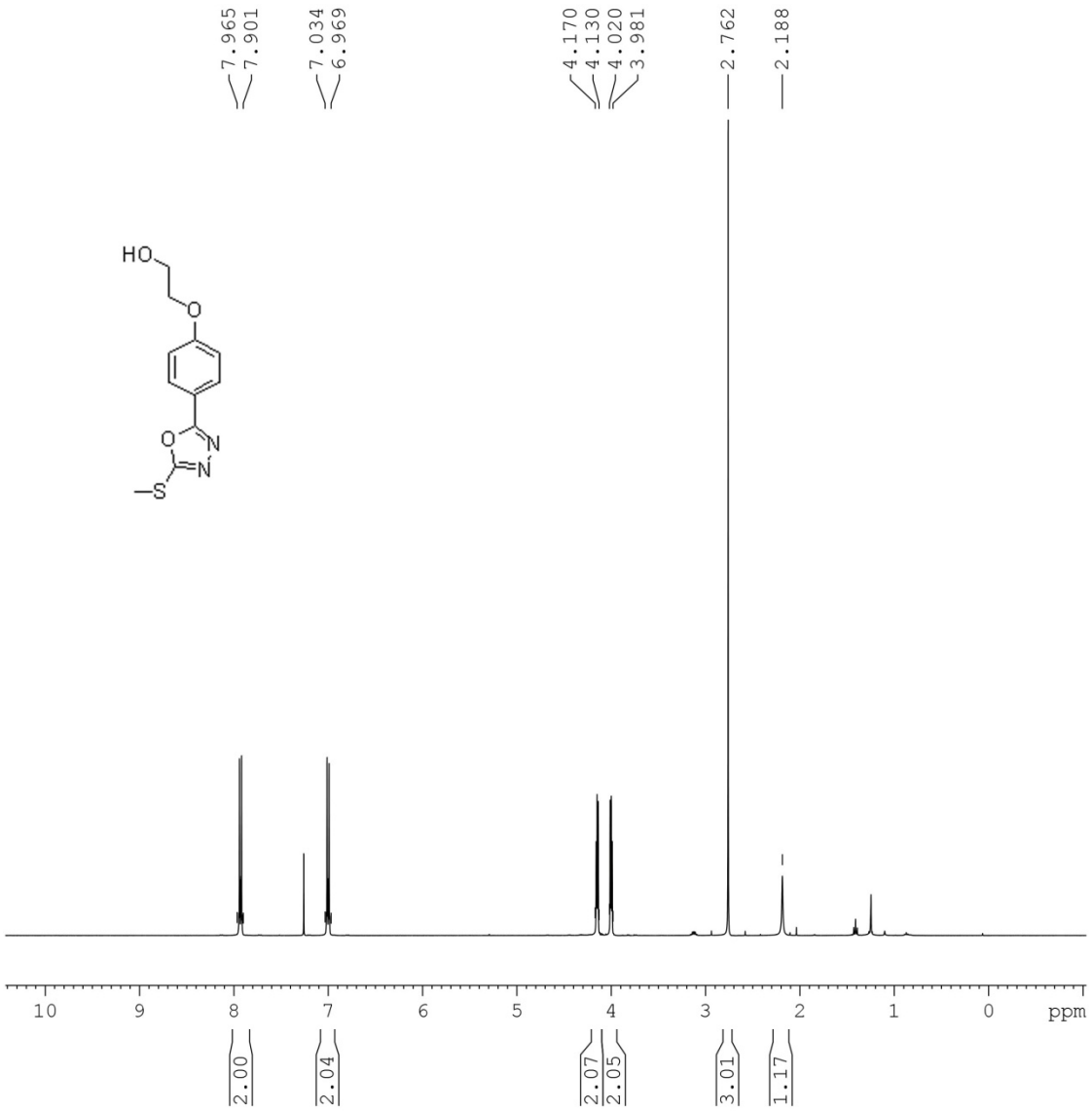
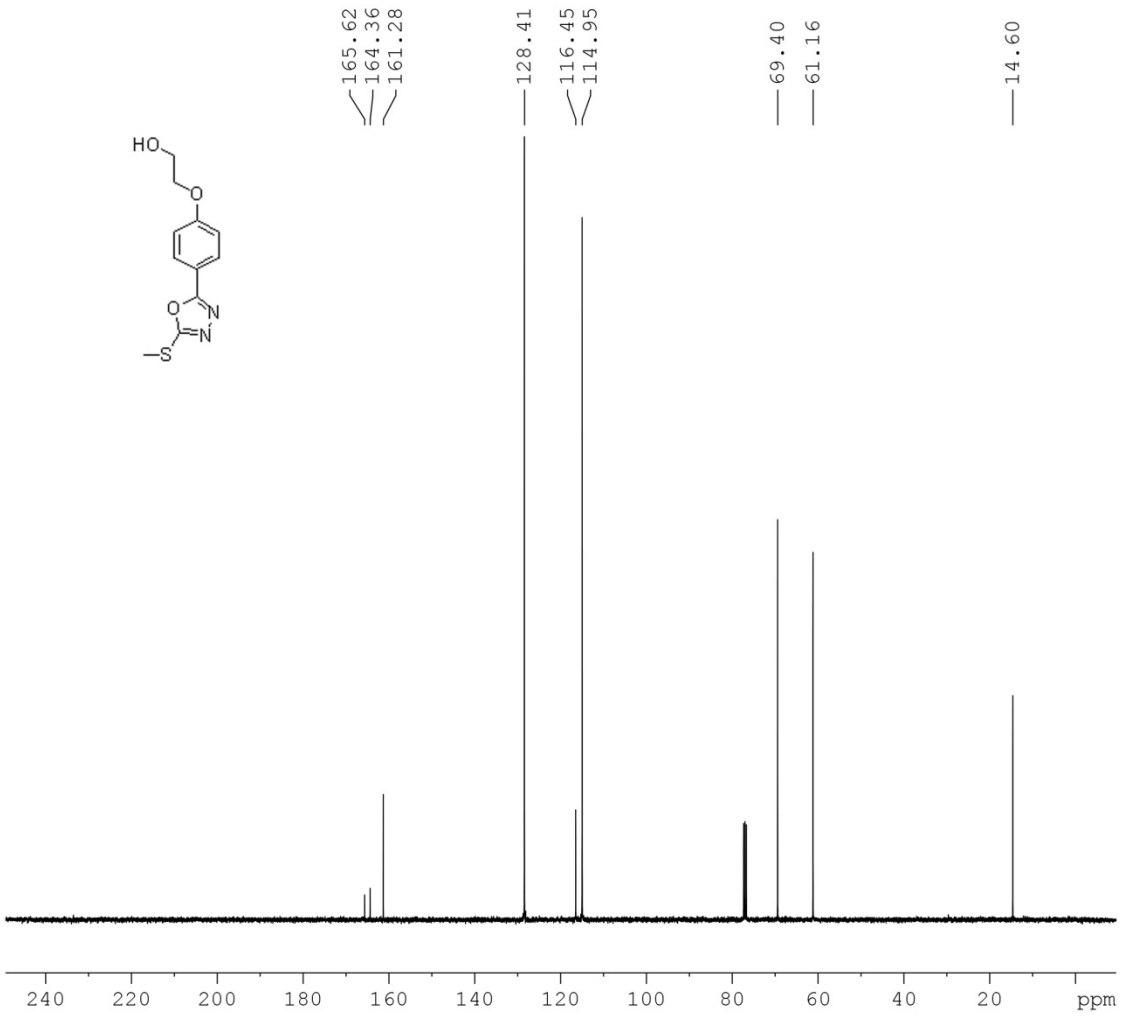
7.825
7.149
7.077
4.155
4.114
3.921
3.877
HO
O
O
N
NH
S
10
9
8
7
6
5
4
3
2
1
0
ppm
1.96
2.00
2.01
2.00

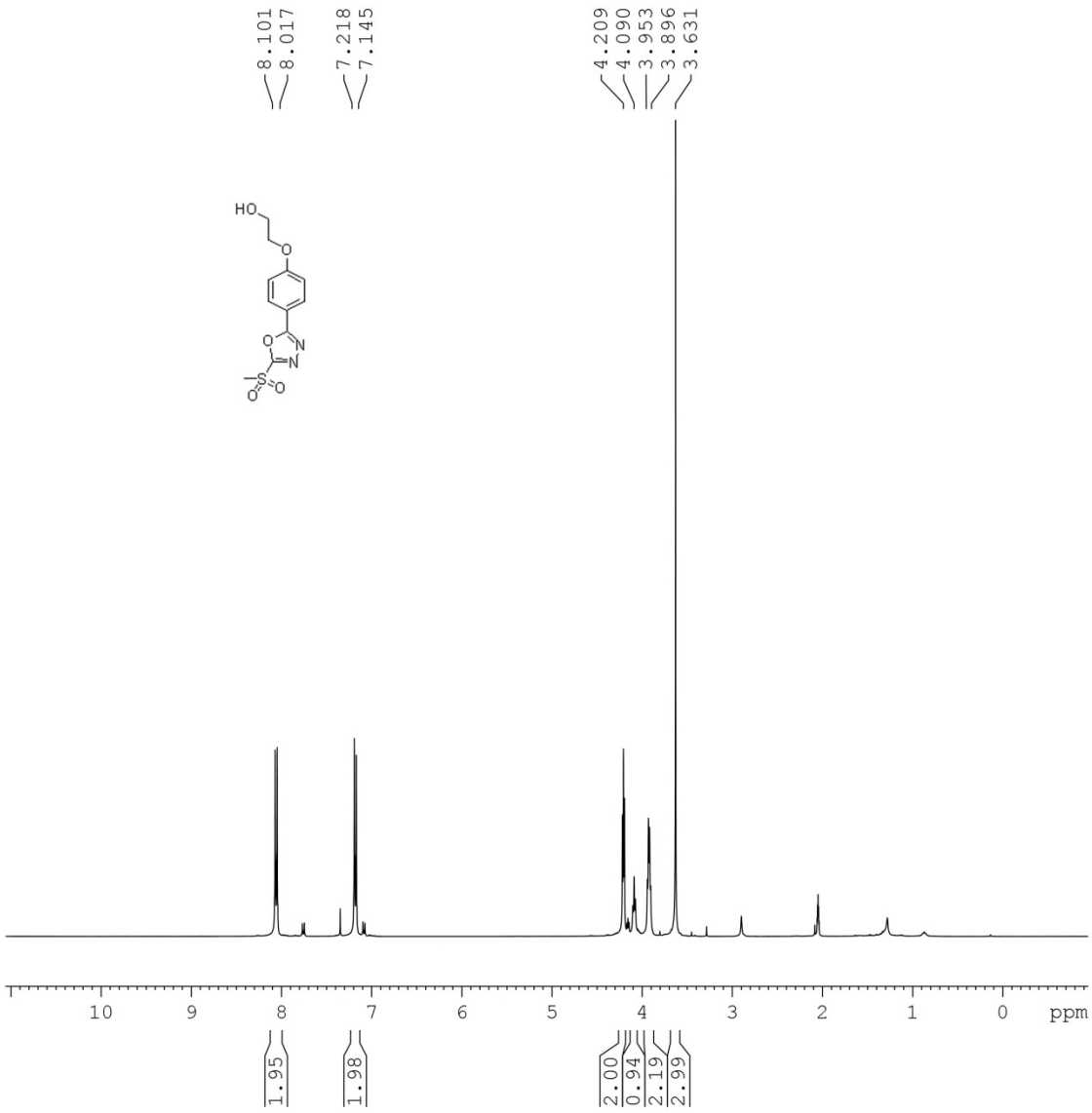

179.95
163.81
162.75
129.27
116.79
116.50
71.10
61.63
HO
O
O
N
NH
S
240
220
200
180
160
140
120
100
80
60
40
20
ppm

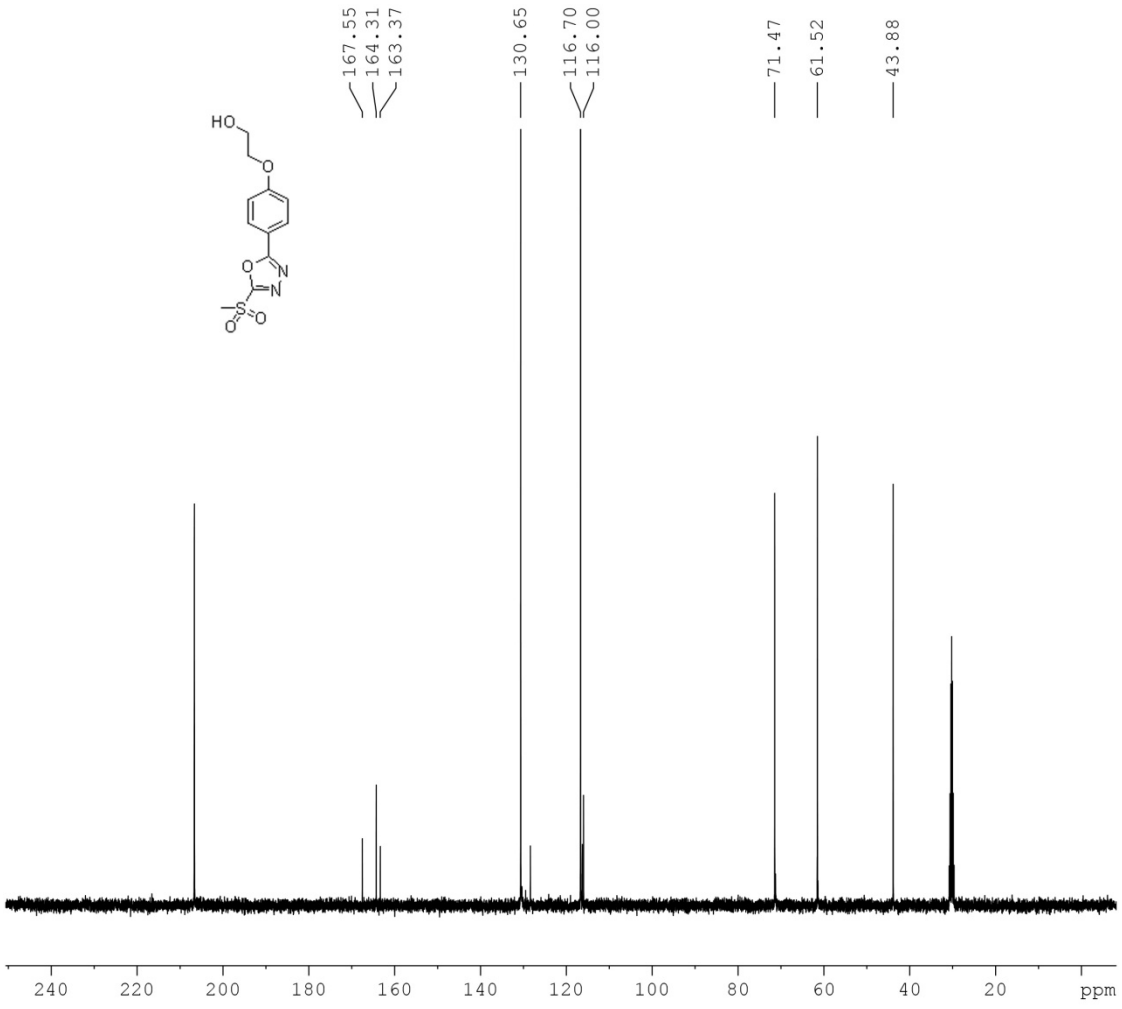
Compound **1d**


7.965
7.901
7.034
6.969
4.170
4.130
4.020
3.981
2.762
2.188
HO
O
O
N
N
S
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
2.04
2.07
2.05
3.01
1.17

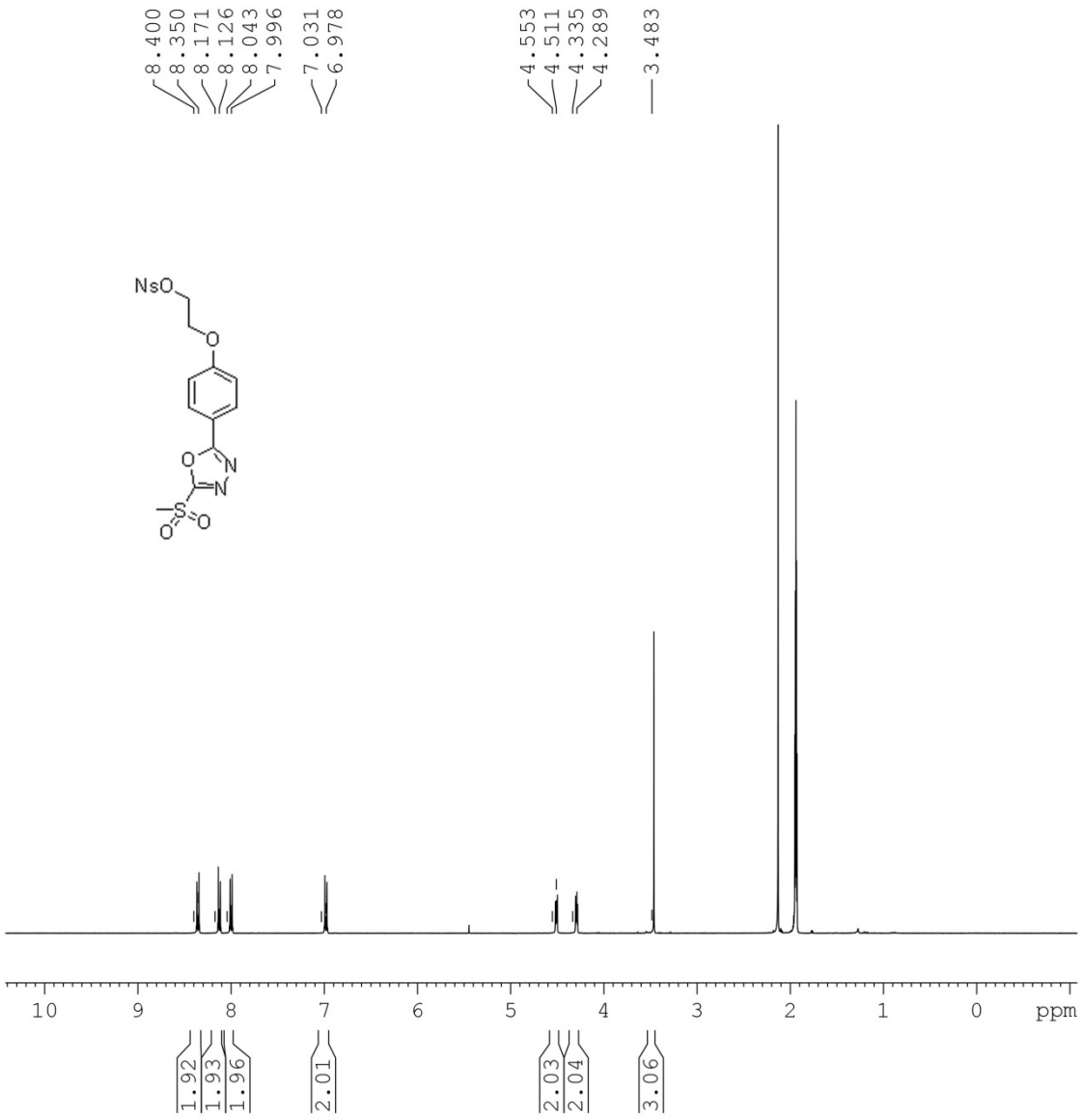

165.62
164.36
161.28
128.41
116.45
114.95
69.40
61.16
14.60
HO
O
O
N
N
S
240
220
200
180
160
140
120
100
80
60
40
20
ppm

Compound **2**


8.101
8.017
7.218
7.145
4.209
4.090
3.953
3.896
3.631
HO
O
O
N
O
S
O
O
10
9
8
7
6
5
4
3
2
1
0
ppm
1.95
1.98
2.00
0.94
2.19
2.99


167.55
164.31
163.37
130.65
116.70
116.00
71.47
61.52
43.88
HO
O
O
N
O
S
O
O
240
220
200
180
160
140
120
100
80
60
40
20
ppm

Compound **3**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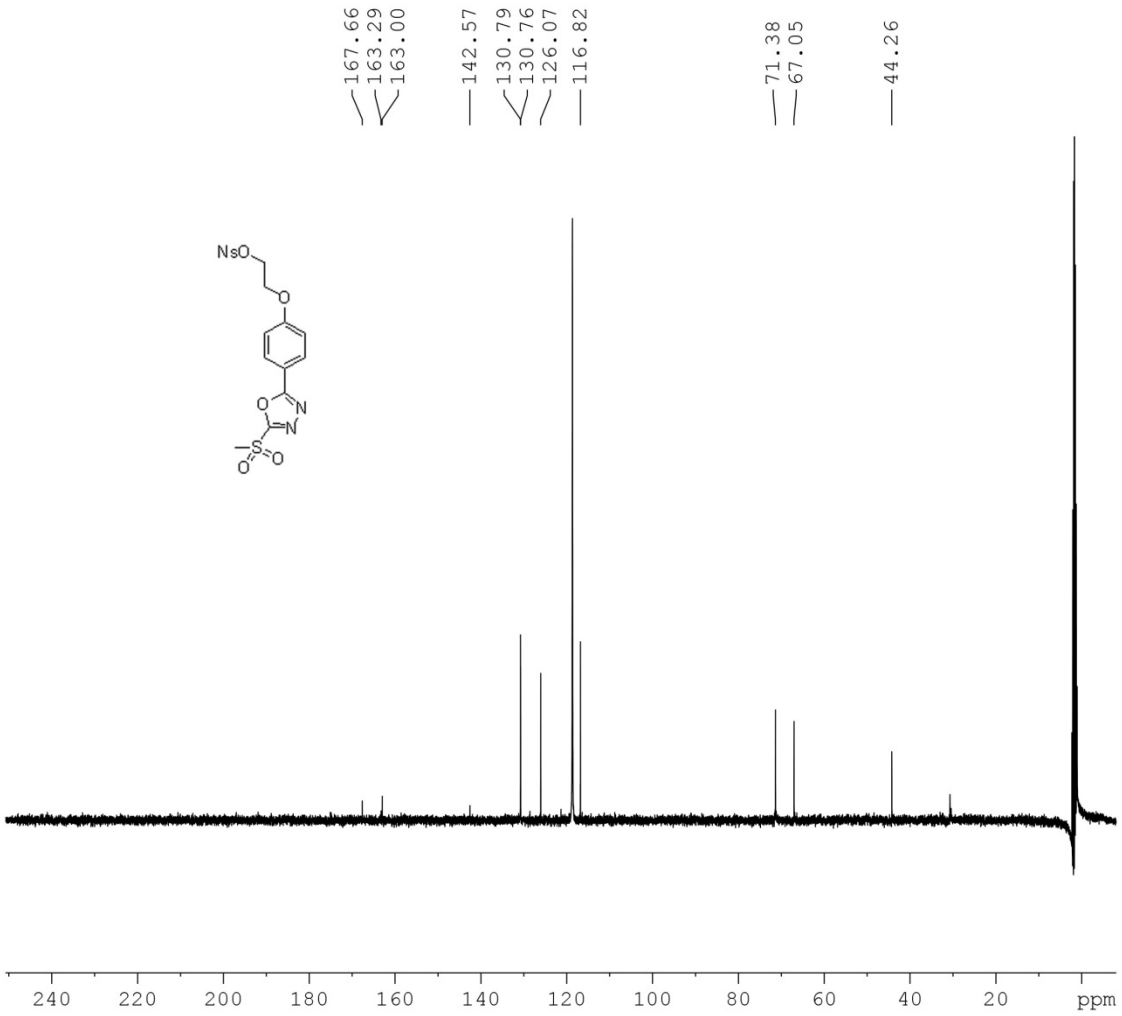
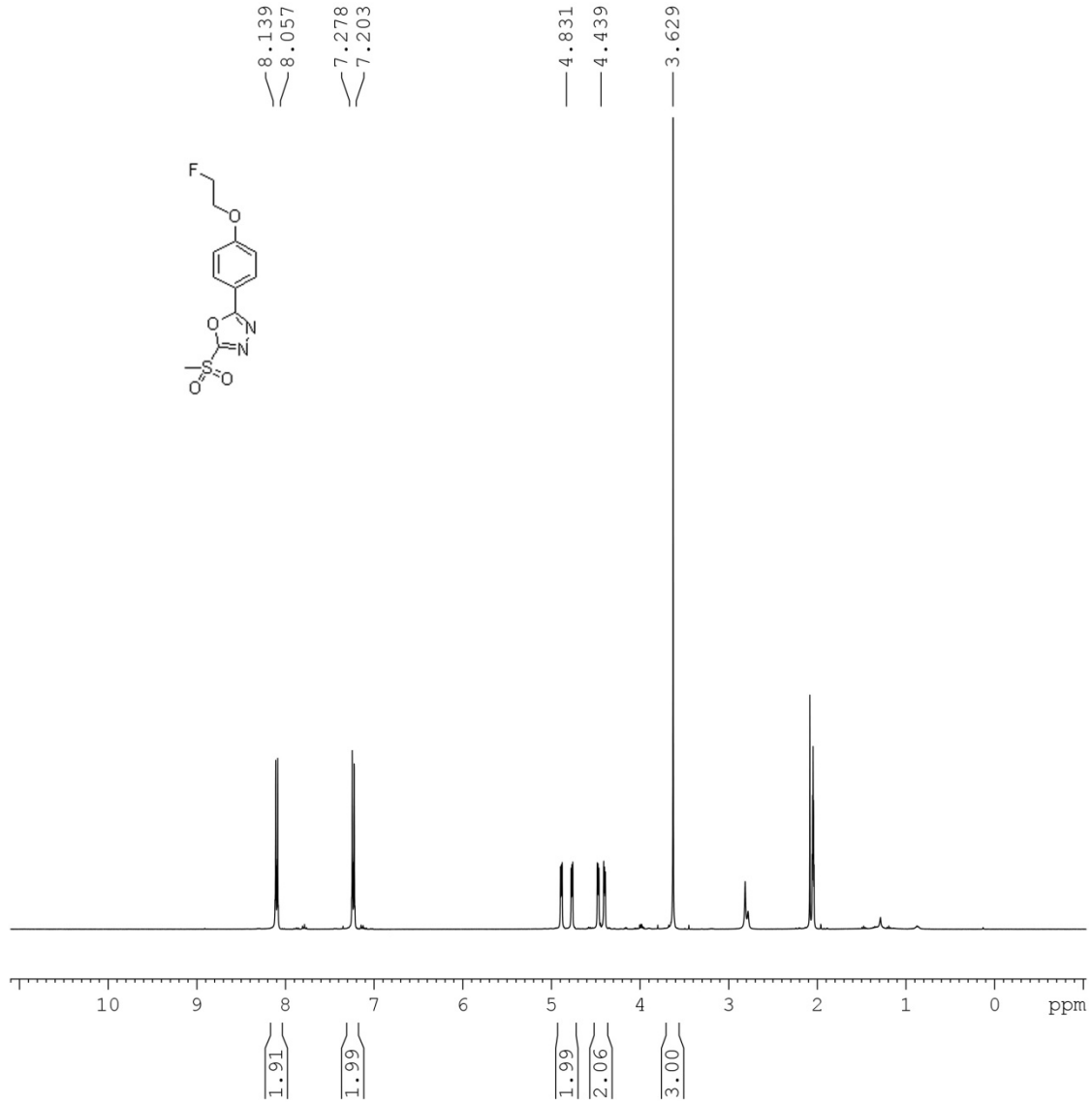
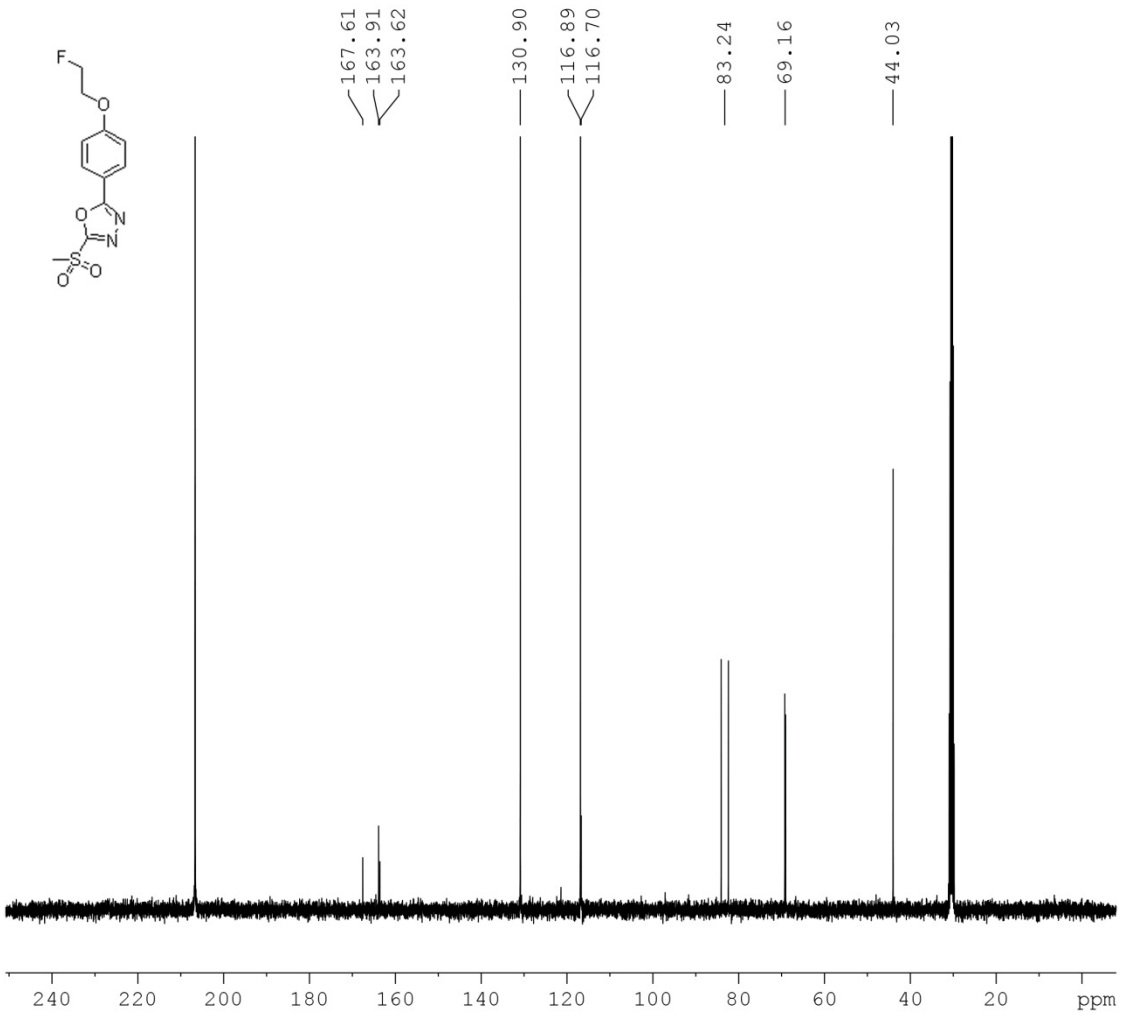
8.350
8.171
8.126
8.043
7.996
7.031
6.978
4.553
4.511
4.335
4.289
3.483
NsO
O
O
N
N
S
O
O
10
9
8
7
6
5
4
3
2
1
0
ppm
1.92
1.93
1.96
2.01
2.03
2.04
3.06

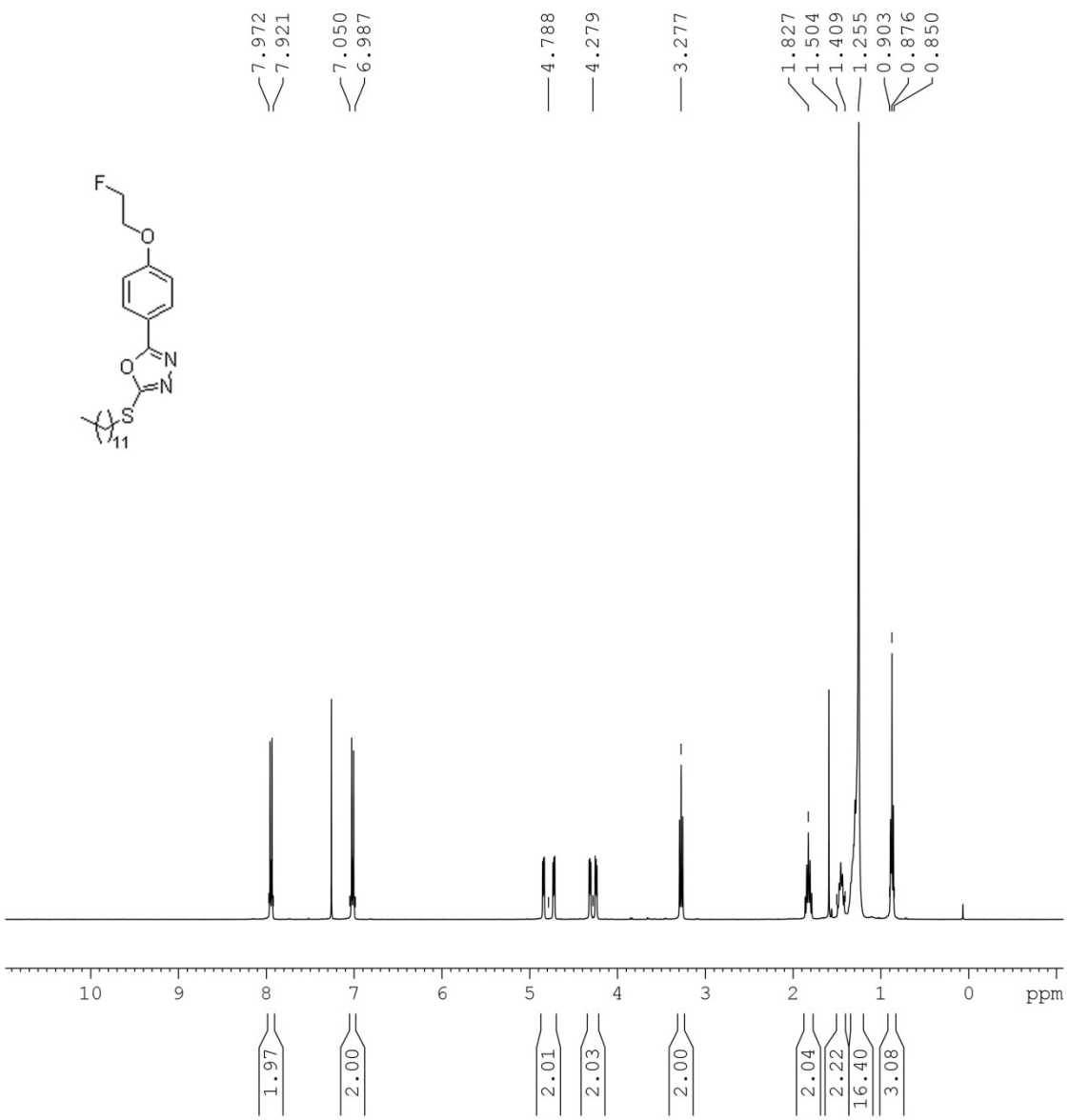

167.66
163.29
163.00
142.57
130.79
130.76
126.07
116.82
71.38
67.05
44.26
NsO
O
O
N
S
O
O
240
220
200
180
160
140
120
100
80
60
40
20
ppm

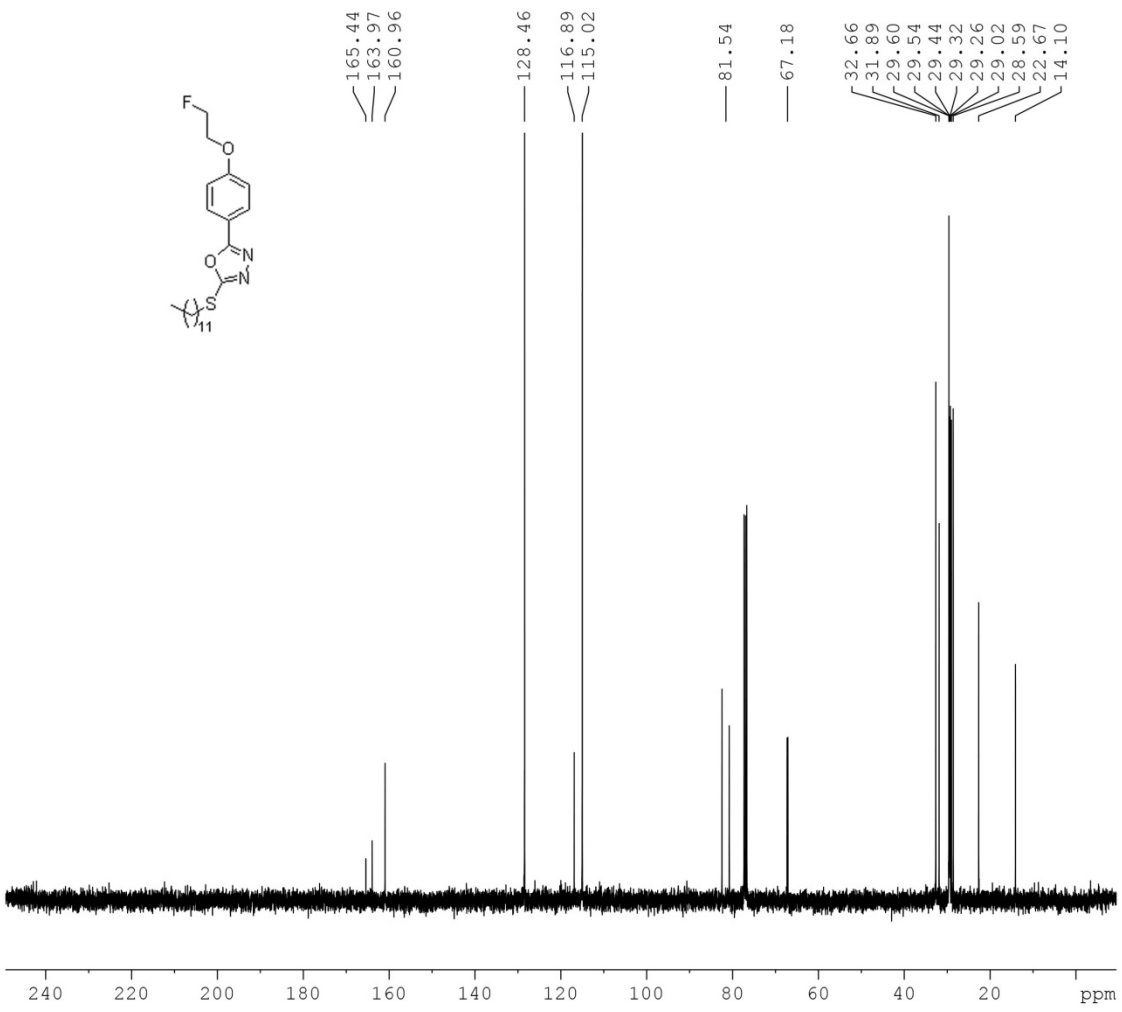
Compound **4b**: [^{19}F]FPOS



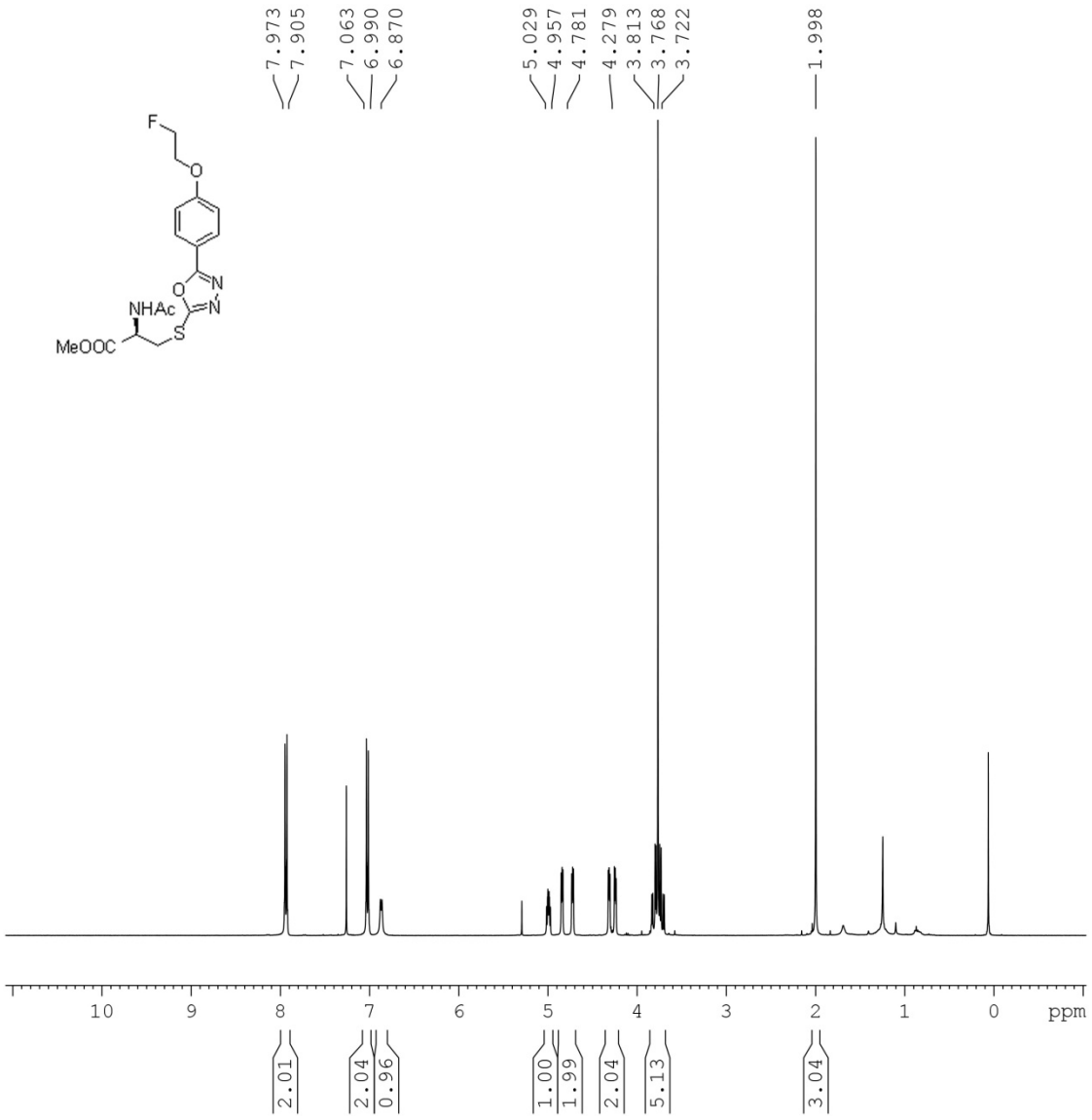

F
O
O
N
N
S
O
O
167.61
163.91
163.62
130.90
116.89
116.70
83.24
69.16
44.03
240
220
200
180
160
140
120
100
80
60
40
20
ppm

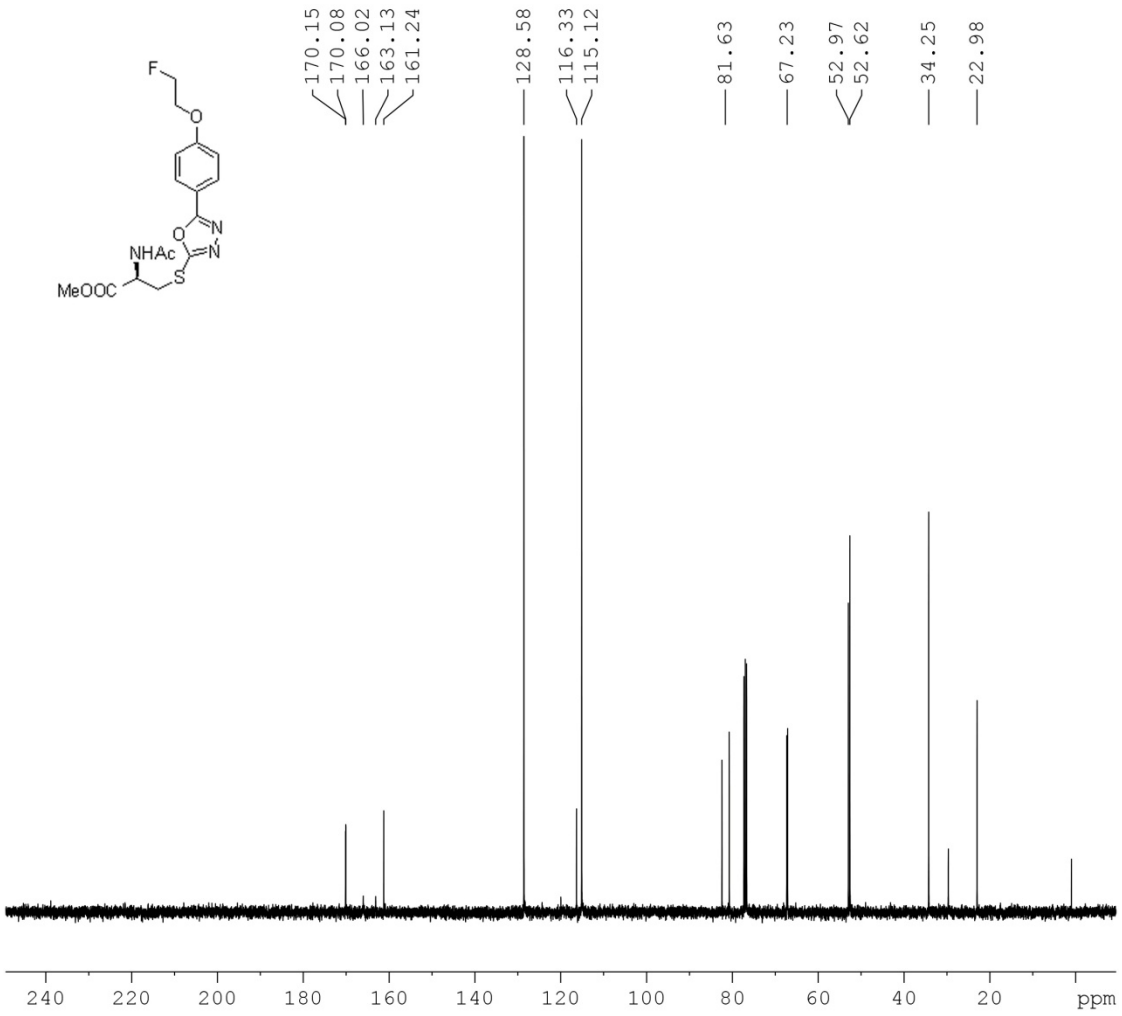
Compound **5b**


7.972
7.921
7.050
6.987
4.788
4.279
3.277
1.827
1.504
1.409
1.255
0.903
0.876
0.850
F
O
O
N
N
S
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.97
2.00
2.01
2.03
2.00
2.04
2.22
16.40
3.08


165.44
163.97
160.96
128.46
116.89
115.02
81.54
67.18
32.66
31.89
29.60
29.54
29.44
29.32
29.26
29.02
28.59
22.67
14.10
F
O
O
N
N
S
11
240
220
200
180
160
140
120
100
80
60
40
20
ppm

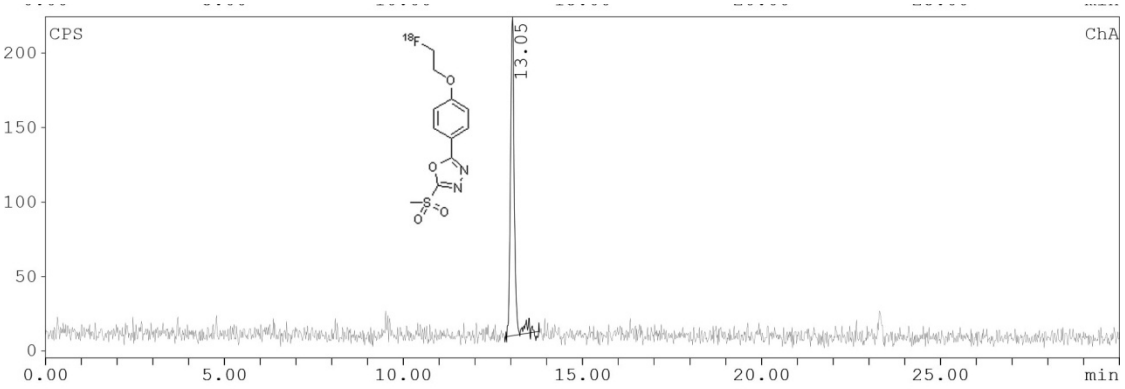
Compound **6b**


7.973
7.905
7.063
6.990
6.870
5.029
4.957
4.781
4.279
3.813
3.768
3.722
1.998
F
O
O
N
N
NHAc
S
MeOOC
10
9
8
7
6
5
4
3
2
1
0
ppm
2.01
2.04
0.96
1.00
1.99
2.04
5.13
3.04


170.15
170.08
166.02
163.13
161.24
128.58
116.33
115.12
81.63
67.23
52.97
52.62
34.25
22.98
F
O
N
O
N
NHAc
S
MeOOC
240
220
200
180
160
140
120
100
80
60
40
20
ppm

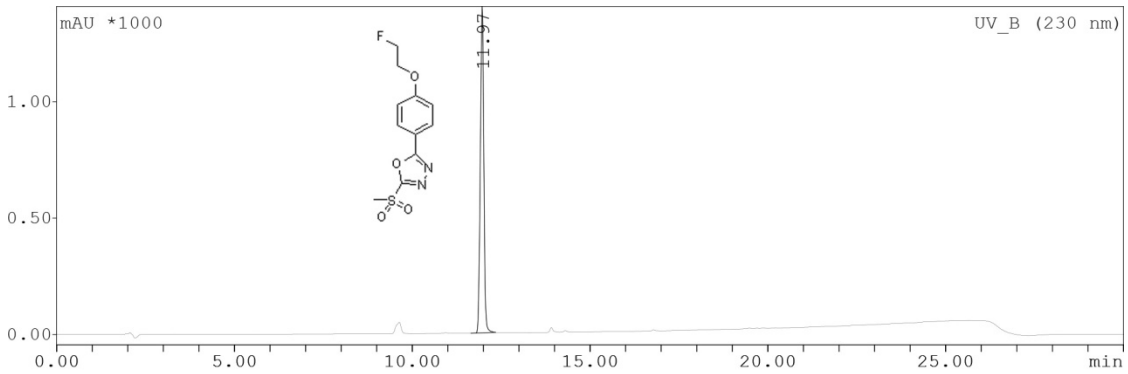
8. γ- and UV-HPLC chromatograms

γ-HPLC of compound **4a** and UV-HPLC of compound **4b** (no co-injection; HPLC chromatograms were obtained with different HPLC systems which resulted in different retention times of the hot and cold compounds)



Integration ChA

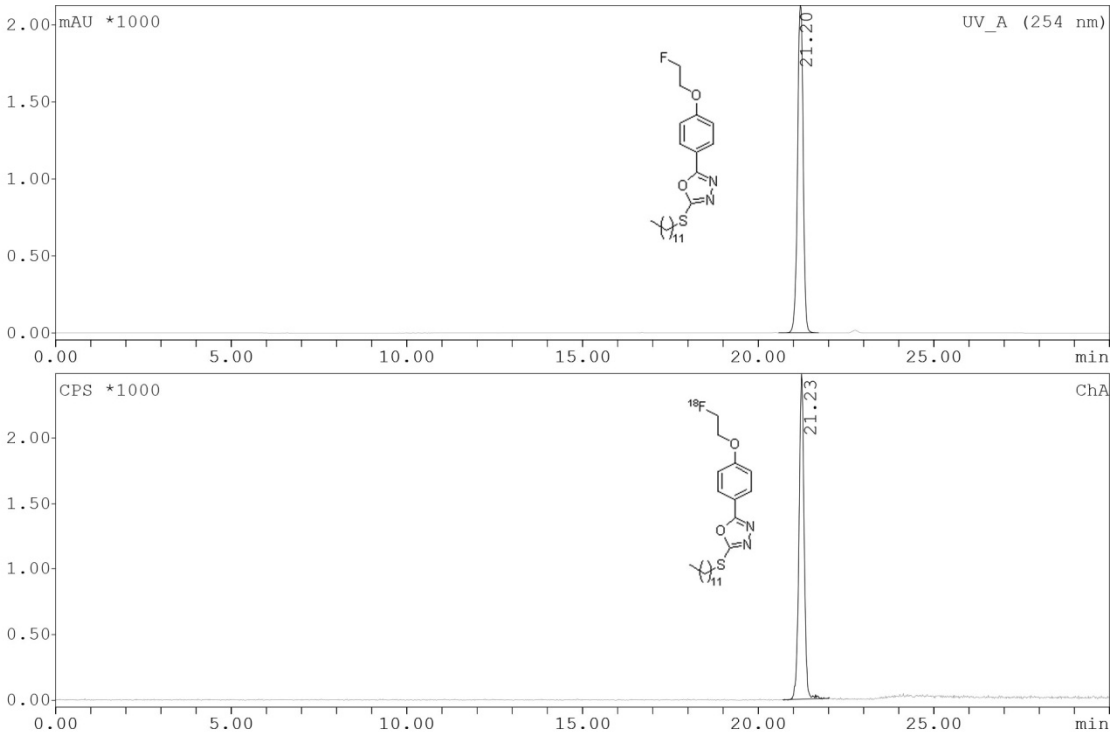
Substance	R/T min	Type	Area Counts	%Area %
Reg #1	13.05	BB	1589.581	100.00
Sum in ROI			1589.581	100.00
Area (total)			20742.764	
Ext. BKG.			0.00 CPS	



Integration UV_B (230 nm)

Substance	R/T min	Type	Area mAU*min	%Area %
Reg #1	11.97	BB	9525.045	100.00
Sum in ROI			9525.045	100.00

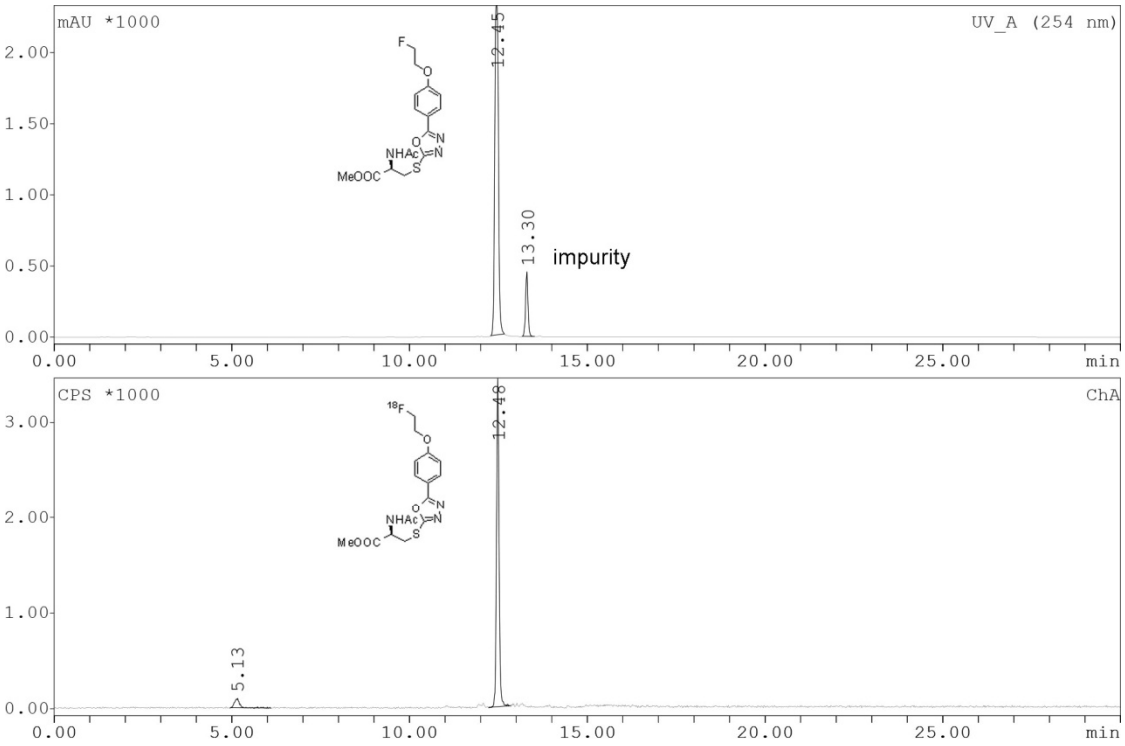
γ-HPLC of compound **5a** and UV-HPLC of compound **5b** (co-injection)



Integration ChA

Substance	R/T min	Type	Area Counts	%Area %
Reg #1	21.23	BB	25083.62	100.00
Sum in ROI			25083.62	100.00
Area (total)			41567.34	
Ext. BKG.			0.00 CPS	

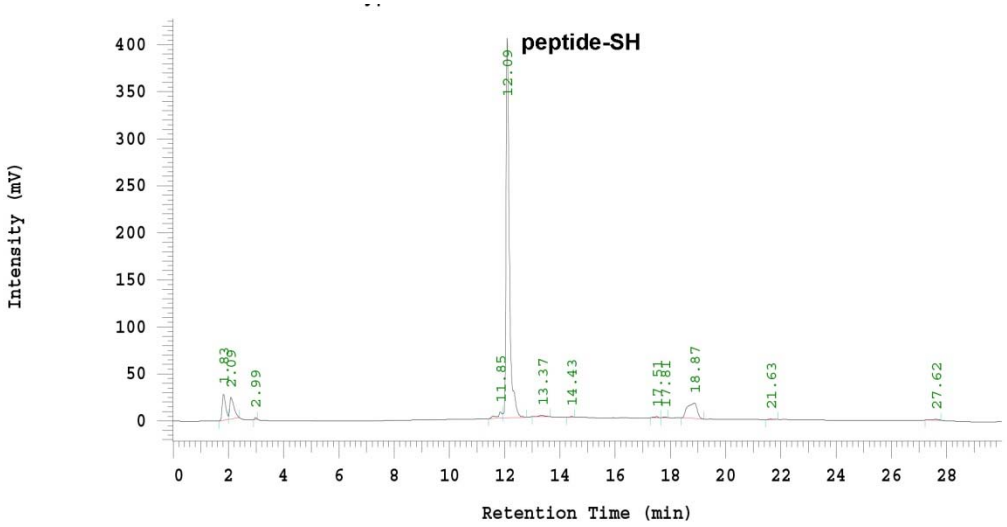
γ-HPLC of compound **6a** and UV-HPLC of compound **6b** (co-injection)



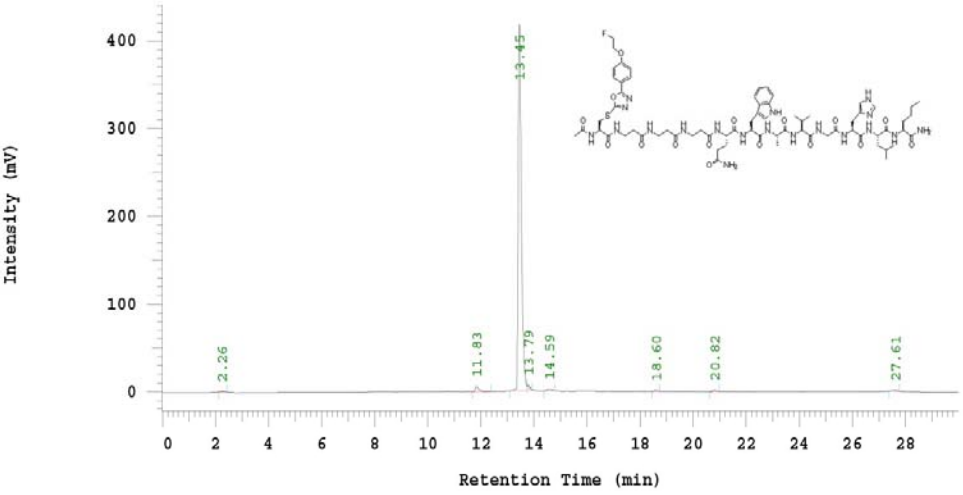
Integration ChA

Substance	R/T min	Type	Area Counts	%Area %
Reg #1	5.13	BB	925.30	5.19
Reg #2	12.48	BB	16894.50	94.81
Sum in ROI			17819.80	100.00
Area (total)			52765.32	
Ext. BKG.			0.00 CPS	

UV-HPLC of AcCys(βAla)$_3$[Nle^{14}]BBN(7-14)NH$_2$ (peptide-SH)

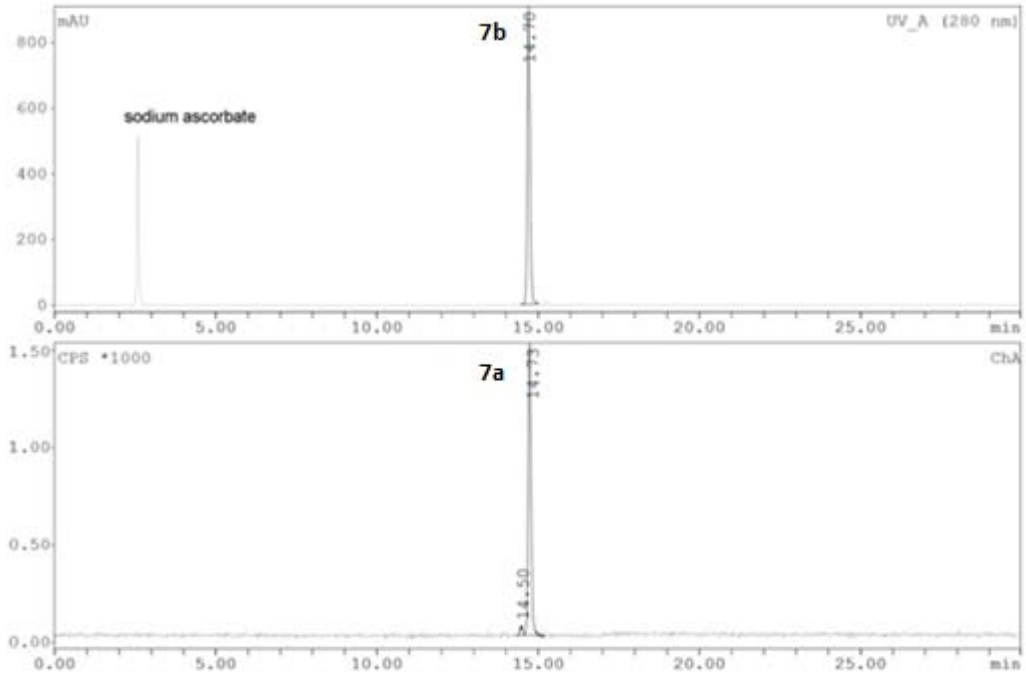


UV-HPLC of compound **7b**



No.	RT	Area	Area %
1	2.26	7751	0.227
2	11.83	58740	1.719
3	13.45	3277287	95.912
4	13.79	12538	0.367
5	14.59	25677	0.751
6	18.60	10183	0.298
7	20.82	14570	0.426
8	27.61	10231	0.299
		3416977	100.000

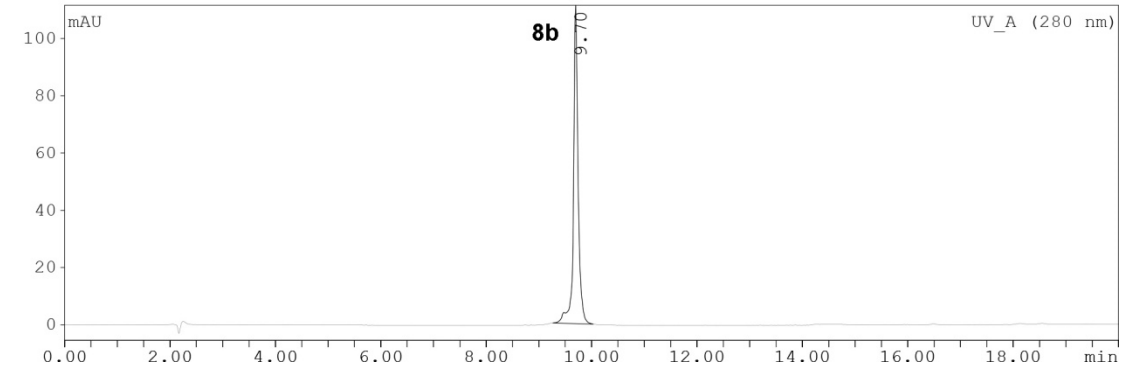
γ-HPLC of compound **7a** and UV-HPLC of compound **7b** (co-injection)

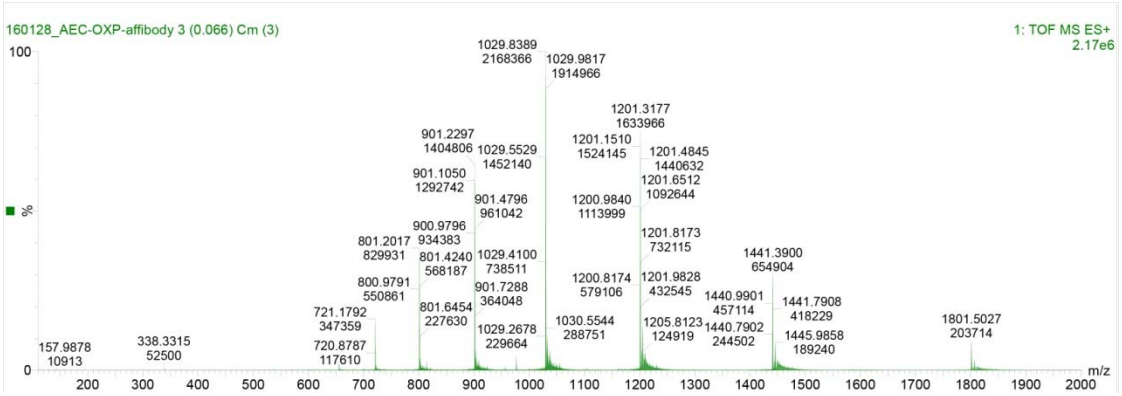


Integration ChA

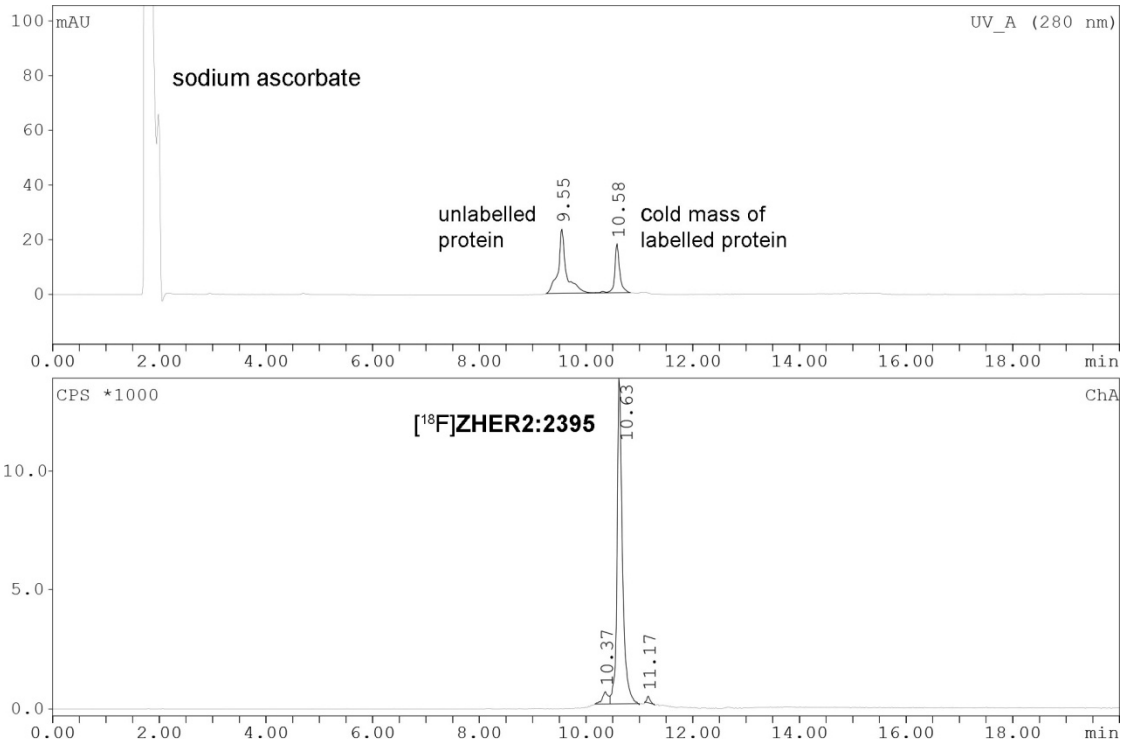
Substance	R/T min	Type	Area Counts	%Area %
Reg #1	14.50	BD	259.277	2.69
Reg #2	14.73	DB	9385.216	97.31
Sum in ROI			9644.493	100.00
Area (total)			79449.135	
Ext. BKG.			0.00 CPS	

UV-HPLC and MS analysis of compound **8b** (no co-injection with hot **8a**)





γ-HPLC and UV trace of purified compound **8a**



Integration ChA

Substance	R/T min	Type	Area Counts	%Area %
Reg #1	10.37	BD	3822.40	3.83
Reg #2	10.63	DB	95019.31	95.09
Reg #3	11.17	BB	1079.06	1.08
Sum in ROI			99920.77	100.00
Area (total)			152794.26	
Ext. BKG.			0.00 CPS	

9. References

1. Toda, N.; Asano, S.; Barbas, C. F., Rapid, Stable, Chemoselective Labeling of Thiols with Julia-Kocienski-like Reagents: A Serum-Stable Alternative to Maleimide-Based Protein Conjugation. *Angew Chem Int Edit* **2013,** *52* (48), 12592-12596.
2. Ahlgren, S.; Orlova, A.; Rosik, D.; Sandstrom, M.; Sjoberg, A.; Basstrup, B.; Widmark, O.; Fant, G.; Feldwisch, J.; Tolmachev, V., Evaluation of maleimide derivative of DOTA for site-specific labeling of recombinant affibody molecules. *Bioconjugate Chem* **2008,** *19* (1), 235-243.
3. M. Glaser, P. Iveson, S. Hoppmann, B. Indrevoll, A. Wilson, J. Arukwe, A. Danikas, R. Bhalla, D. Hiscock, J. Nucl. Med. 2013, 54, 1981-1988.