Supporting Information to Accompany:

Copper-free Click for PET: Rapid 1,3-Dipolar Cycloadditions with a Fluorine-18 Cyclooctyne

Richard D. Carpenter,[†] Sven H. Hausner,[†] Julie L. Sutcliffe[†,‡,£,]*

[†]Department of Biomedical Engineering, [‡]Division of Hematology/Oncology, Department of Internal Medicine, and [£]Center for Molecular and Genomic Imaging, University of California, Davis, California 95616

jlsutcliffe@ucdavis.edu

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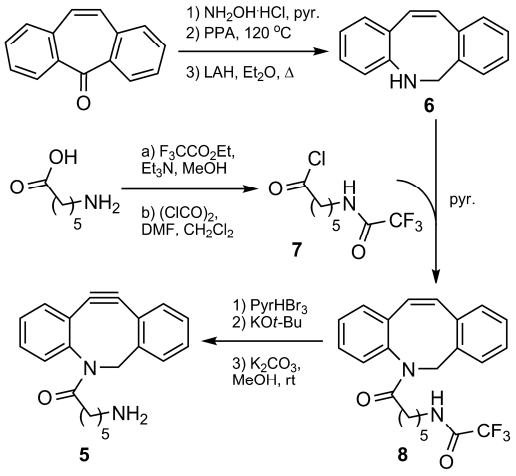
Supporting Information Scheme 1. Synthesis of the ADIBO amine (**5**) precursor.



Supporting Information Table 1. Stability of [^{18}F]-**4** and [^{18}F]-**12** in rat serum at various time points.[a]

Compound	Fraction of intact radiolabeled compound		
	30 min	60 min	120 min
[^{18}F]-**4**	>99%	98.3%	98.2%
[^{18}F]-**12**	>99%	>99%	96.8%

[a]The fraction of intact radiolabeled compound was determined by analytical radio reversed-phase HPLC.

General Procedures

Reagents and Solvents: Reagents and solvents were purchased from Aldrich, Acros, TCI America, Novabiochem, Alfa Aeser, and VWR and used without further purification with the exception of the following. Tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were passed through a column of alumina and stored over activated 3 Å molecular sieves. Triethylamine and *N*,*N*-diisopropylethylamine (DIEA) were distilled from sodium hydride. Methanol and pyridine were dried over 3 Å molecular sieves. All dry organic solvents used for radiochemical syntheses were used as purchased (AcroSeal; Acros) and stored over activated 3 Å molecular sieves. [^{18}F]Fluoride was produced from the $^{18}O(p,n)^{18}F$ nuclear reaction on [^{18}O]H_2O purchased from Medical Isotopes, Inc. using a Siemens CTI RDS 111 negative ion cyclotron.

Chromatography and Purification: Thin layer chromatography (TLC) used glass plates coated with silica gel 60 F254 (UV indicator), developed in a chamber, and visualized under ultraviolet light (λ_{ex} = 254 nm) as well as by treatment with cerium molybdate solution prepared from 40 g of ammonium pentamolybdate + 1.6 g of cerium(IV) sulfate + 800 mL diluted sulfuric acid (1:9, with water, v/v). Silica gel was purchased from Silicycle (SiliaFlash® P60, 40-63 μm, 60Å). Radiochemical solid-phase extraction (SPE) was done with C_{18} SepPak Plus cartridges (Waters) that were pre-conditioned with denatured ethanol (10 mL), water (10 mL), and air (3 x 10 mL). Reversed-phase HPLC was used to purify and analyze the products; solvent A: 0.05% trifluoroacetic acid (TFA) in water (v/v); solvent B: acetonitrile. HPLC systems were equipped with both UV absorbance detector (UV, 220 nm) and a radioactivity detector

(photomultiplier tube; PMT) connected in series, which accounts for the slight difference between detected retention times for corresponding ^{18}F- and ^{19}F-compounds. Analytical HPLC: Phenomenex Jupiter 4 µm Proteo 90 Å column (250 × 4.6 mm, 4 µm), solvent B isocratic 9% for 2 min, then linear gradient to 81% over 30 min, flow rate 1.5 mL/min. Semi-preparative HPLC: Phenomenex Jupiter 10 µm Proteo 90 Å (250 × 10 mm, 10 µm), solvent B isocratic 9% for 2 min, then linear gradient to 81% over 30 min, flow rate 3 mL/min. ^{1}H NMR spectra were obtained using a Bruker Instrument (500 MHz) and recorded in chloroform-*d* with the solvent signal used as a reference (7.26 ppm). LC-MS was done using a Waters Alliance instrument equipped with a Waters Nova-Pak C_{18} column (3.9 x 150 mm) using 0.1% TFA in water (v/v; solvent A) and 0.1% TFA in acetonitrile (v/v; solvent B) with a linear gradient over 25 min from 5% solvent B to 80%.

Animal Handling: All animals were handled in accordance with a protocol approved by the University of California, Davis, Animal Use and Care Committee.

Experimental Protocols

Synthesis of ADIBO-[^{19}F]Fluorobenzamide ([^{19}F]-4). ADIBO-amine **5** (4 mg, 12.3 µmol) was dissolved in anhydrous DMF (500 µL) and *N,N*-diisopropylethylamine (DIEA; 1 drop) was added. [^{19}F]SFB (5 mg, 21.1 µmol) was then added, and the reaction was allowed to proceed for 75 min at room temperature. The reaction mixture was diluted with 1:1 acetonitrile:0.1% TFA in water (v/v; 2 mL) and purified *via* reversed-phase semi-preparative HPLC. Pooled fractions were freeze dried to afford ADIBO-[^{19}F]fluorobenzamide [^{19}F]-**4** as a colorless fluffy solid (2.1 mg, 39% yield). ^{1}H NMR ($CDCl_3$, 500 MHz, 25 ˚C) δ 7.83-6.44 (m, 12H), 5.16 (d, *J* = 12 Hz, 1H), 3.69-0.9 (m, 11H); ESI-MS (*m/z*) 441.11 $(M + H)^+$ (Supporting Information Figure 1). The chemical purity was determined to be 99% by HPLC (Supporting Information Figure 2), retention times of [^{19}F]-**4** were 28.7, 29.2, and 29.5 min (Supporting Information Figure 4).

Synthesis of *N*-Succinimidyl 4-[^{18}F]Fluorobenzoate ([^{18}F]SFB) ([^{18}F]-10).[1] 4-[^{18}F]Fluorobenzoic acid ([^{18}F]FBA), was prepared according to our previously described protocol using the aryltrimethylammonium precursor **9**.[1] [^{18}F]SFB ([^{18}F]-**10**) was prepared with slight modifications to Zalutsky's protocol.[2] Briefly, [^{18}F]FBA (686.0 MBq – 37.11 GBq) in anhydrous acetonitrile (500 µL) was added to a 5-mL screw-cap conical vial containing trimethylammonium hydroxide in water (45% wt/v; 10 µL). The solution was azeotropically dried (3 x 1 mL of acetonitrile) at 100 °C using a gentle stream of helium. After the final evaporation, *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyluronium

[1] Hausner, S. H.; Marik, J.; Gagnon, M. K.; Sutcliffe, J. L. *In Vivo* Positron Emission Tomography (PET) Imaging with an $\alpha_v\beta_6$ Specific Peptide Radiolabeled Using ^{18}F-"click" Chemistry: Evaluation and Comparison with the Corresponding 4-[^{18}F]fluorobenzoyl- and 2-[^{18}F]fluoropropionyl Peptides. *J. Med. Chem.* **2008**, *51*, 5901-5904.

[2] Vaidyanathan, G.; Zalutsky, M. R. Synthesis of *N*-succinimidyl 4-[^{18}F]fluorobenzoate, an Agent for Labeling Proteins and Peptides with ^{18}F. *Nat. Protoc.* **2006**, *1*, 1655-1661.

tetrafluoroborate (TSTU; 12 mg) in anhydrous acetonitrile (1 mL) was added *via* syringe, and the reaction was heated at 100 ^{o}C for 5 min in the sealed vial. The reaction mixture was then aspirated into a syringe containing water (9 mL) and acetic acid (150 µL) and shaken. Crude [^{18}F]-**10** was then trapped onto a C_{18} SepPak Plus, washed (10 mL of 10% aqueous acetonitrile), dried (3 x 10 mL of air), and eluted with anhydrous acetonitrile (3 mL) and air (5 mL) into a clean 5-mL screw-cap conical vial. The solution was dried azeotropically at 100 ^{o}C using a gentle stream of helium, followed by evaporation of fresh acetonitrile twice (2 x 1 mL) to afford dry [^{18}F]SFB ([^{18}F]-**10**; 284.2 MBq – 6.586 GBq; 43 + 22% d.c. RCY; synthesis time of 73 + 27 min; *n* = 4). The radiochemical purity was 80.4 + 20.3% as determined by analytical radio HPLC. The radioactive reversed-phase HPLC trace of [^{18}F]-**10** correlated with the UV trace of [^{19}F]-**10**, retention time was 20.3 min.

Synthesis of ADIBO-[^{18}F]Fluorobenzamide ([^{18}F]-4). ADIBO-amine **5** (2.5 mg) in DIEA (10 µL) and anhydrous DMF (300 µL) was added to dried [^{18}F]SFB ([^{18}F]-**10**) (284.6 MBq – 6.586 GBq). The reaction was heated to 37 ^{o}C for 30 min with stirring. The reaction mixture was aspirated into a 10-mL syringe containing 0.2 M NaOH (5 mL), trapped onto a C_{18} SepPak Plus cartridge, washed with water (10 mL), dried with air (3 x 10 mL) and eluted with acetonitrile (3 mL) and air (5 mL). The solution was heated at 60 ^{o}C and the acetonitrile was evaporated using a gentle stream of helium to afford crude ADIBO-[^{18}F]fluorobenzamide ([^{18}F]-**4**; 74.0 MBq – 3.615 GBq; 64 + 15% d.c. RCY with a synthesis time of 89 + 24 min). The crude radiochemical purity was 80.4 + 3.3% as determined by analytical radio reversed-phase HPLC. HPLC trace of

[^{18}F]-**4** correlated with the UV trace of [^{19}F]-**4**, retention times of [^{18}F]-**4** were 28.9, 29.3, and 29.7 min (Supporting Information Figures 3 and 4).

General Procedure for Fluorine-19 Copper-free Click Reactions: *N*-(6-(1-Benzyl-1*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-*d*]azocin-8(9*H*)-yl)-6-oxohexyl)-4-[^{19}F]fluorobenzamide ([^{19}F]-11). ADIBO-[^{19}F]fluorobenzamide ([^{19}F]-**4**; 1 mg, 23 µmol) was dissolved in DMF (150 µL) and treated with an alkyl azide (benzyl azide; 10 mg, 75 µmol). The reaction was warmed to 40 ^{o}C for 1 h, diluted with 1:1 acetonitrile:0.1% TFA in water (v/v; 2 mL) and purified *via* semi-preparative reversed-phase HPLC. Pooled fractions were freeze dried to afford [^{19}F]-**11** as a light brown solid (0.5 mg; 39% yield): ESI-MS (*m/z*) 574.26 $(M + H)^+$ (Supporting Information Figure 5). The chemical purity was determined to be >95% by reversed-phase HPLC for the mixture of triazole regioisomers (Supporting Information Figure 6), retention times of [^{19}F]-**11** were 27.5 and 27.7 min (Supporting Information Figure 10).

Synthesis of 32-(8-(6-(4-[^{19}F]Fluorobenzamido)hexanoyl)-8,9-dihydro-1*H*-dibenzo[*b,f*][1,2,3]triazolo-[4,5-*d*]azocin-1-yl)-5-oxo-3,9,12,15,18,21,24,27,30-nonaoxa-6-azadotriacontan-1-oic acid ([^{19}F]-12). Following the General Procedure for Fluorine-19 Copper-free Click Reactions using 32-azido-5-oxo-3,9,12,15,18,21,24,27,30-nonaoxa-6-azadotriacontan-1-oic acid (15 mg, 27 µmol), afforded [^{19}F]-**12** as a reddish brown solid (1.4 mg; 61% yield): ESI-MS (*m/z*) 995.24 $(M + H)^+$, 1012.81 $(M + H_3O)^+$, 498.38 $[(M + 2H)^{2+}] / 2$ (Supporting Information Figure 7). The chemical purity was determined to be >99% by reversed-phase HPLC for the mixture of triazole regioisomers (Supporting Information Figure 8), retention times of [^{19}F]-**12** were 20.7 and 21.0 min (Supporting Information Figure 12).

General Procedure for Fluorine-18 Copper-free Click Reactions: *N*-(6-(1-Benzyl-1*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-*d*]azocin-8(9*H*)-yl)-6-oxohexyl)-4-[^{18}F]fluorobenzamide ([^{18}F]-11). Dried crude ADIBO-[^{18}F]fluorobenzamide [^{18}F]-**4** (54.4 – 232.7 MBq), in a 5-mL screw-cap conical vial was reacted with an azide (benzyl azide; 8-25 mg, 60-188 µmol) in anhydrous DMF (150 µL). The reaction proceeded at 37 °C and an aliquot was taken at 30 min and immediately analyzed by analytical radio HPLC (74 + 4.8% radiochemical conversion; n = 3). Additional samples were measured at 1 h (75 + 1.8% radiochemical conversion) and 4 h (78 + 2.4% radiochemical conversion). The crude radiochemical purity was 50.6%, while purified [^{18}F]-**11** was >99% radiochemically pure as determined by analytical radio reversed-phase HPLC for the mixture of triazole regioisomers (Supporting Information Figure 9). The radioactive reversed-phase HPLC trace of [^{18}F]-**11** correlated with the UV trace of [^{19}F]-**11**, retention times of [^{18}F]-**11** were 27.6 and 27.8 min (Supporting Information Figures 9 and 10).

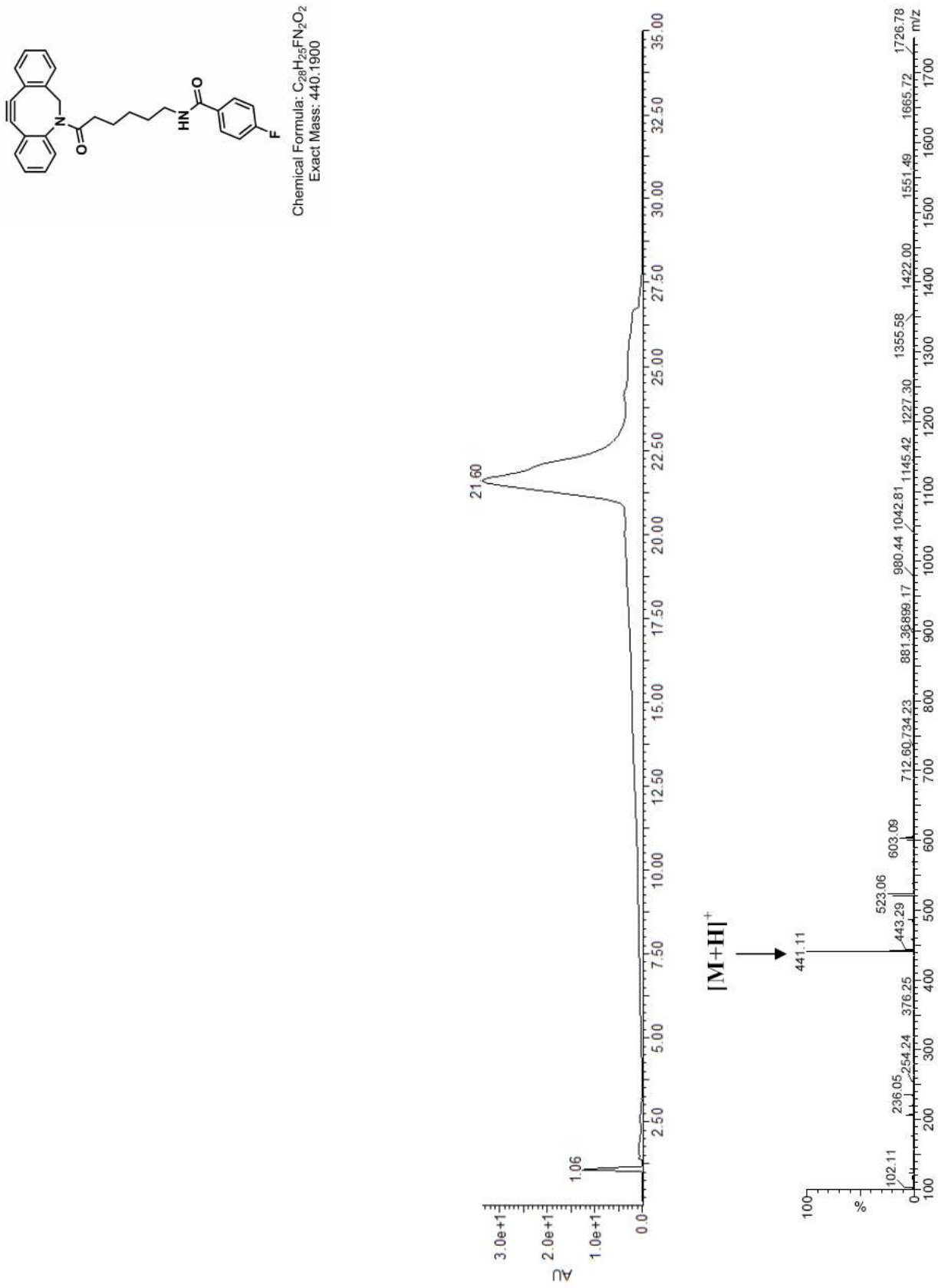
Synthesis of 32-(8-(6-(4-[^{18}F]Fluorobenzamido)hexanoyl)-8,9-dihydro-1*H*-dibenzo[*b,f*][1,2,3]triazolo-[4,5-*d*]azocin-1-yl)-5-oxo-3,9,12,15,18,21,24,27,30-nonaoxa-6-azadotriacontan-1-oic acid ([^{18}F]-12). Following the General Procedure for Fluorine-18 Copper-free Click Reactions, [^{18}F]-**4** (54.4 – 232.7 MBq) was reacted with 32-azido-5-oxo-3,9,12,15,18,21,24,27,30-nonaoxa-6-azadotriacontan-1-oic acid (3-15 mg, 5-27 µmol) as the azide source, and aliquots were taken at the 2 h and 6 h time points. Radiochemical conversion was 64 + 8.5% and crude radiochemical purity was 48 + 7.4% for the mixture of triazole regioisomers (n = 3 at each time point) as determined by analytical radio reversed-phase HPLC. Purified [^{18}F]-**12** was >99% radiochemically pure as determined by analytical radio reversed-phase HPLC for the

mixture of triazole regioisomers (Supporting Information Figure 11). The radioactive trace of [^{18}F]-**12** correlated with the UV trace of [^{19}F]-**12**, retention times of [^{18}F]-**12** were 20.9 and 21.2 min (Supporting Information Figures 11 and 12).

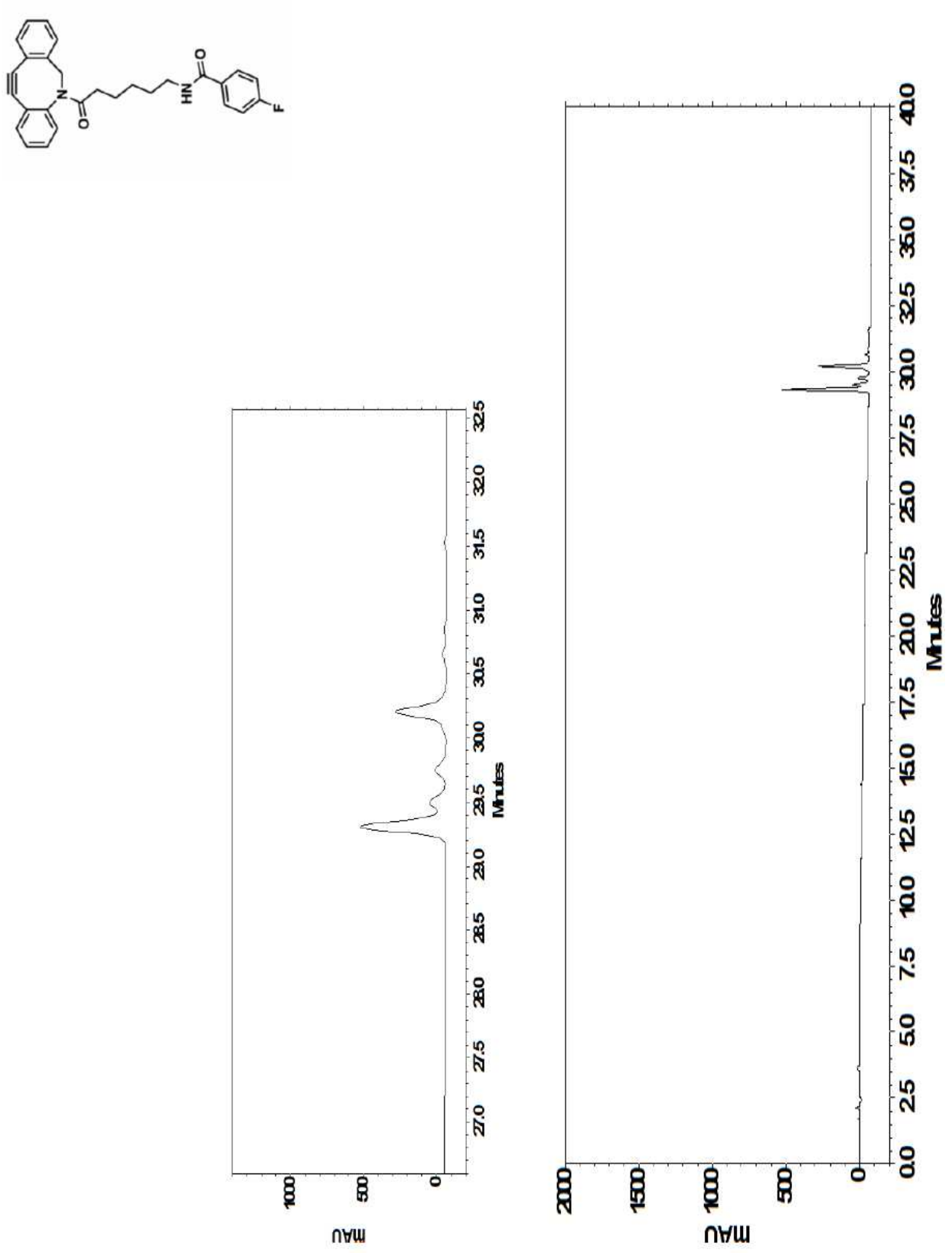
Serum Stability Studies. The radiolabeled compound ([^{18}F]-**4** or [^{18}F]-**12**) was purified by reversed-phase HPLC. Fractions containing pure product were pooled, diluted with water (15 mL), trapped onto a C_{18} SepPak Plus cartridge, washed with water (5 mL), dried with air (15 mL) and eluted with absolute ethanol (1.5 mL) containing 5 μL glacial acetic acid. The solvent was evaporated at 40-50 ^{o}C using a gentle stream of nitrogen gas and the radiolabeled compound was formulated in PBS/saline (containing 10% v/v absolute ethanol for [^{18}F]-**4**) at pH 7.0-7.4. Radiochemical purities of purified [^{18}F]-**4** and [^{18}F]-**12** were confirmed by analytical radio reversed-phase HPLC. Serum was obtained from male Sprague Dawley rats (Charles River Laboratories). Following euthanasia blood was collected, allowed to clot for 1 h at room temperature, and centrifuged (2300×g, 10 min). Serum (1 mL) was collected and combined with an aliquot of the formulated radiolabeled compound ([^{18}F]-**4** or [^{18}F]-**12**, 25 μL; approx. 2 MBq) in an Eppendorf tube.[3] The tube was gently shaken and kept at 37 ^{o}C. Aliquots (100 μL) were withdrawn at selected time points (30, 60 and 120 min), mixed with absolute ethanol (4 ^{o}C, 500 μL) and centrifuged (2300×g, 2.5 min) to precipitate serum proteins. The percentage of intact radiolabeled compound at each time point was determined by analytical radio reversed-phase HPLC of supernatant samples.

[3] Gottumukkala, V.; Heinrich, T. K.; Baker, A.; Dunning, P.; Fahey, F. H.; Treves, S. T.; Packard, A. B. Biodistribution and stability studies of [^{18}F]fluoroethylrhodamine B, a potential PET myocardial perfusion agent. *Nucl. Med. Biol.* **2010**, *37*, 365-370.

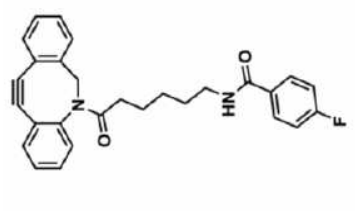
Supporting Information Figure 1. LC/MS traces of [^{19}F]-**4**.

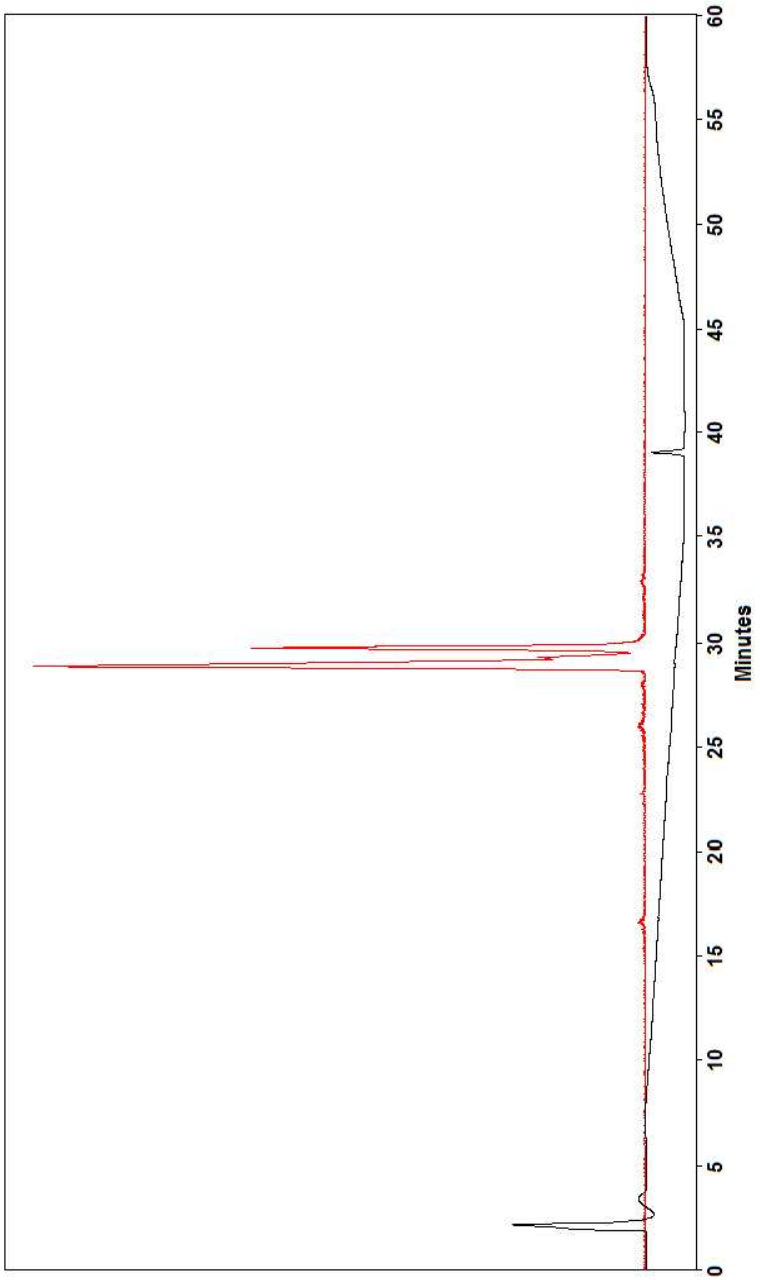


Supporting Information Figure 2. Analytical HPLC trace of [^{19}F]-**4** (λ = 220 nm).

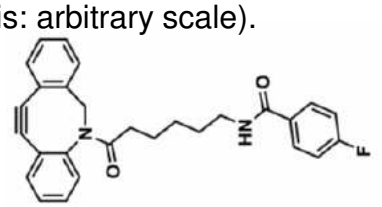


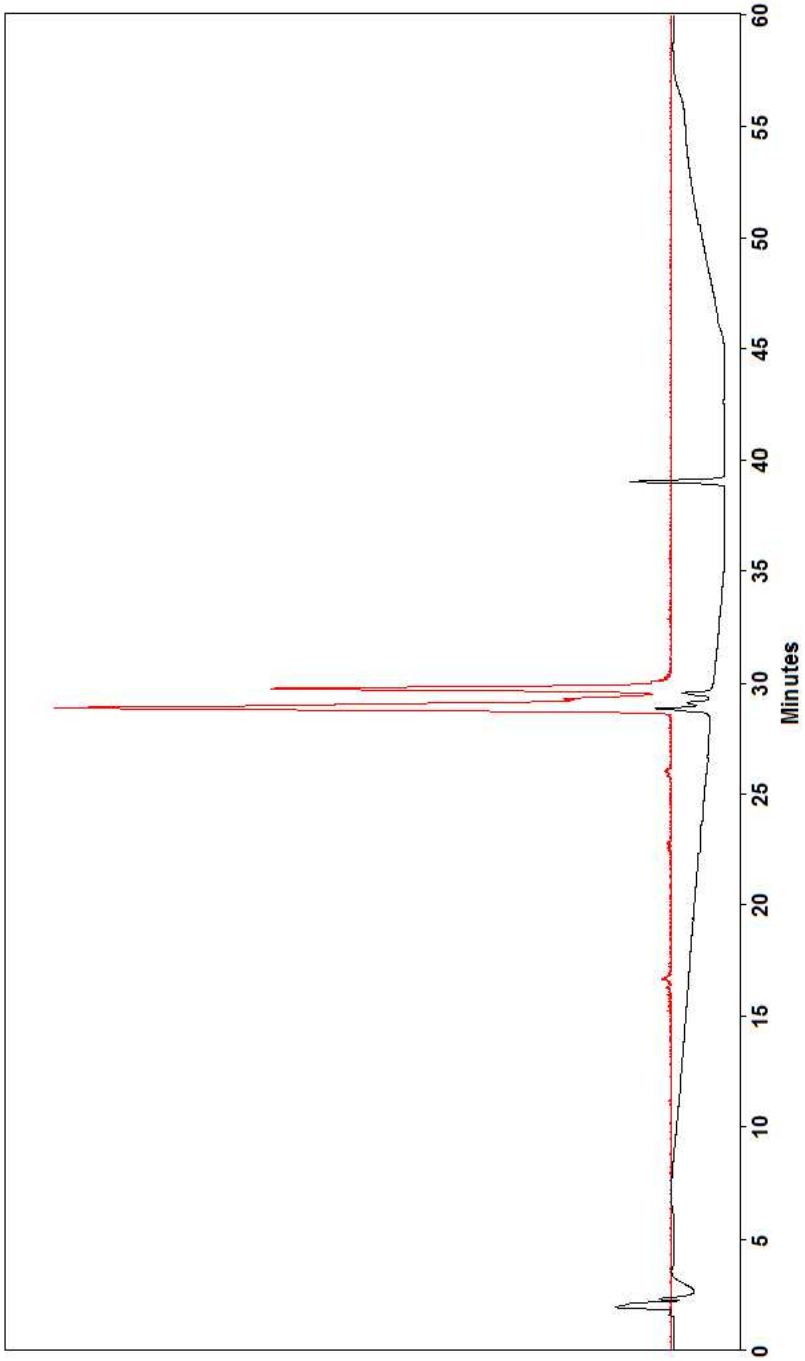
Supporting Information Figure 3. Analytical HPLC UV (λ = 220 nm) trace (black) overlaid with the radio HPLC trace (red) of purified [^{18}F]-**4** (y-axis: arbitrary scale).



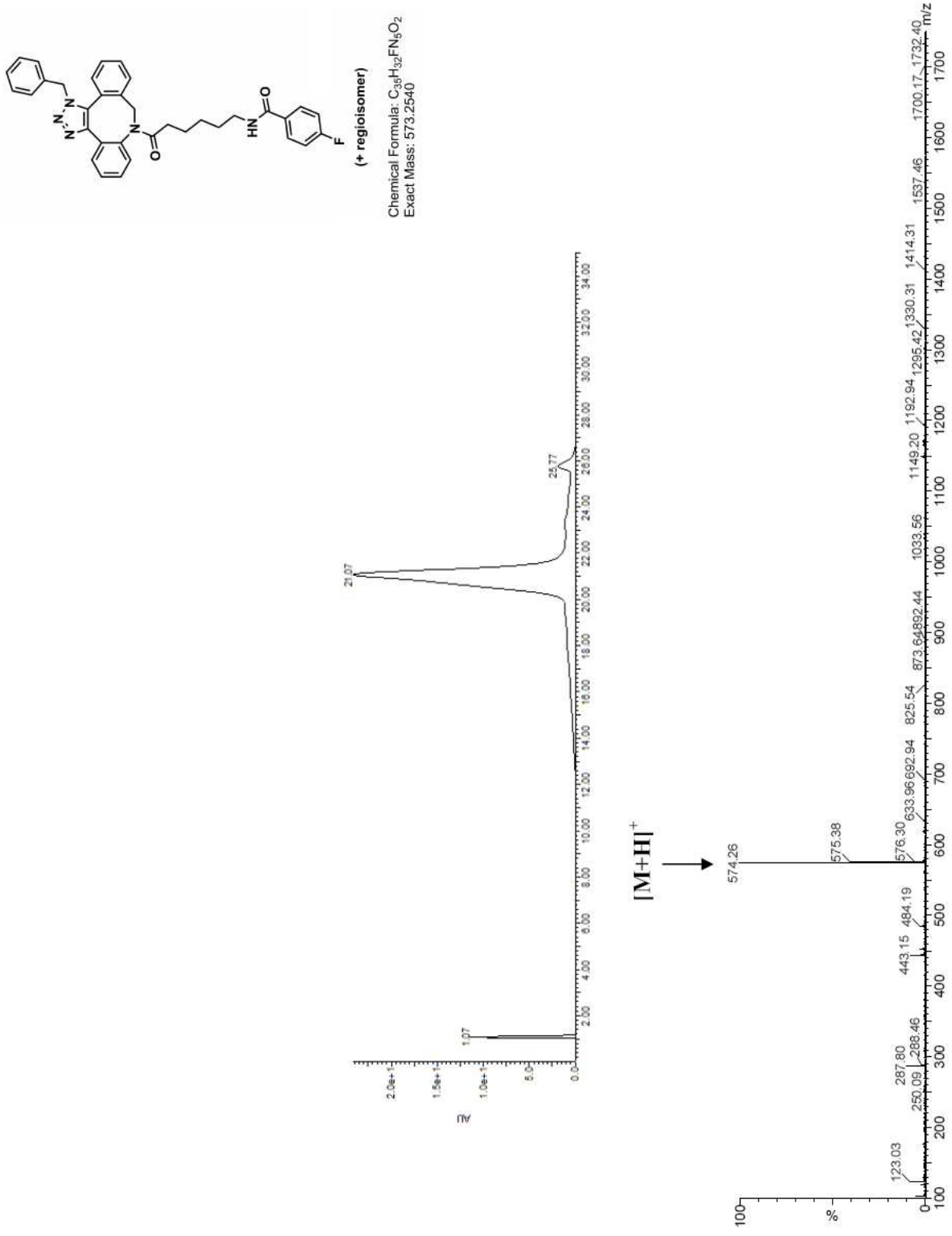


Supporting Information Figure 4. Analytical HPLC UV (λ = 220 nm) trace (black) overlaid with the radio HPLC trace (red) of purified [^{18}F]-**4** spiked with purified [^{19}F]-**4** (y-axis: arbitrary scale).

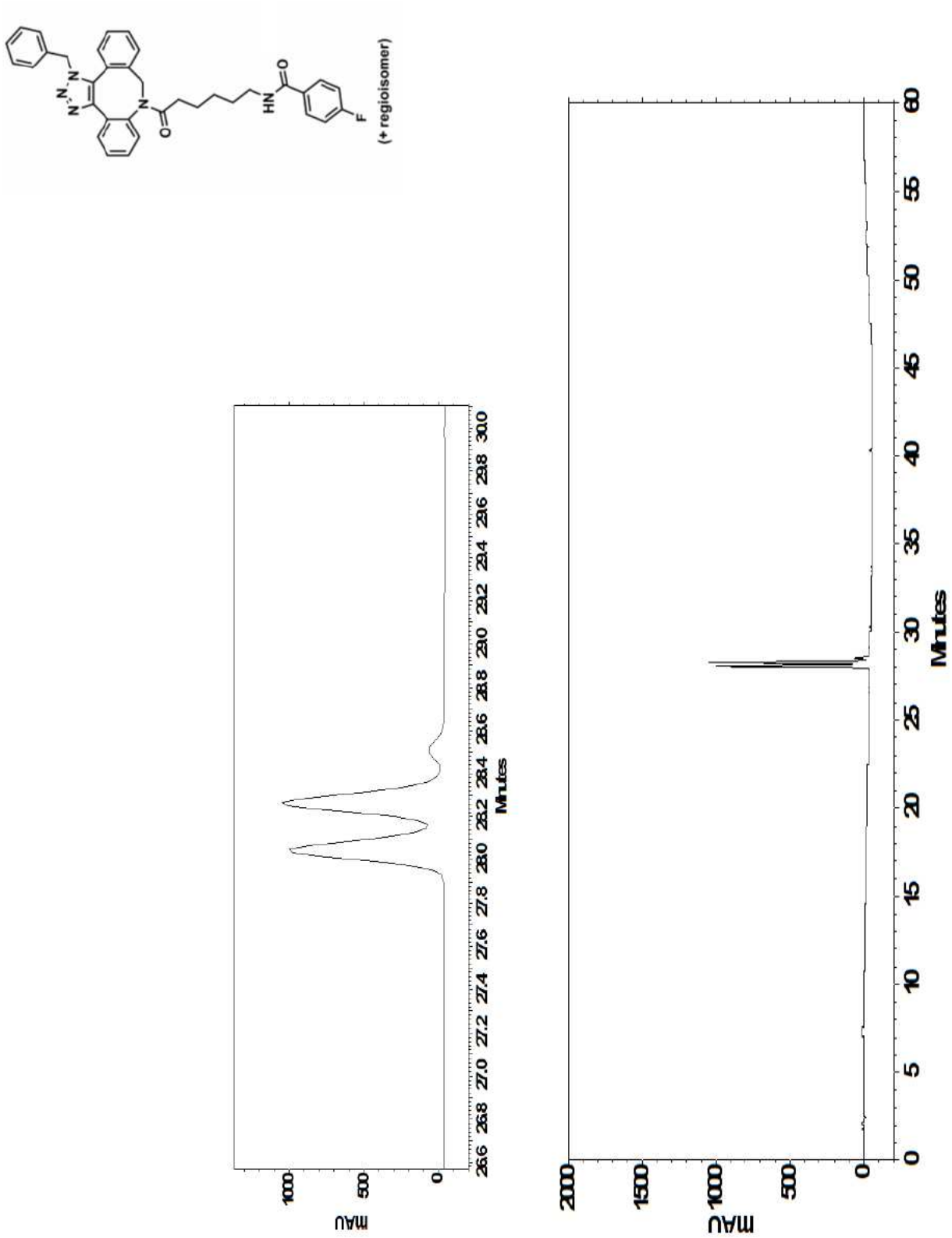




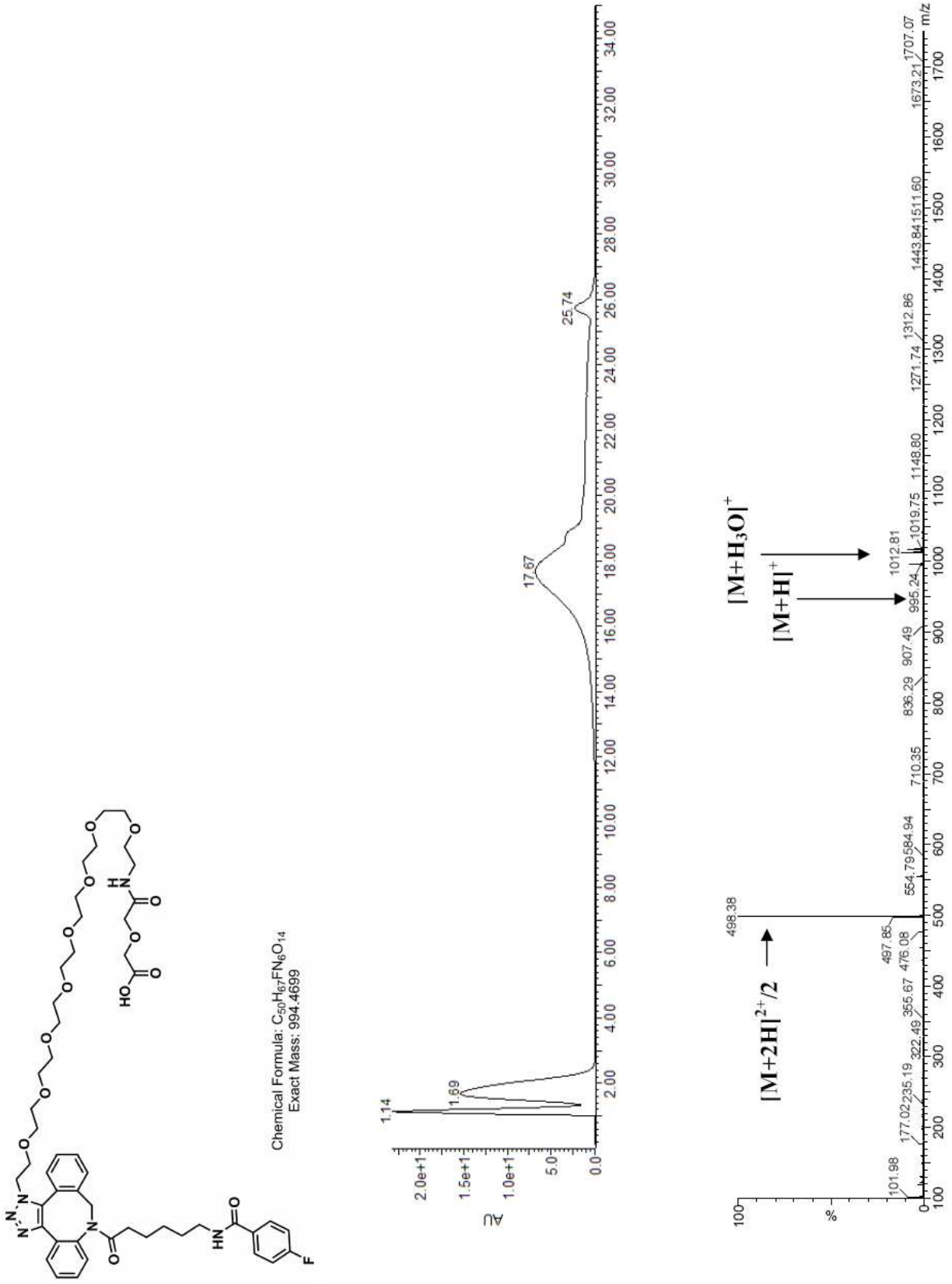
Supporting Information Figure 5. LC/MS traces of [^{19}F]-**11**.



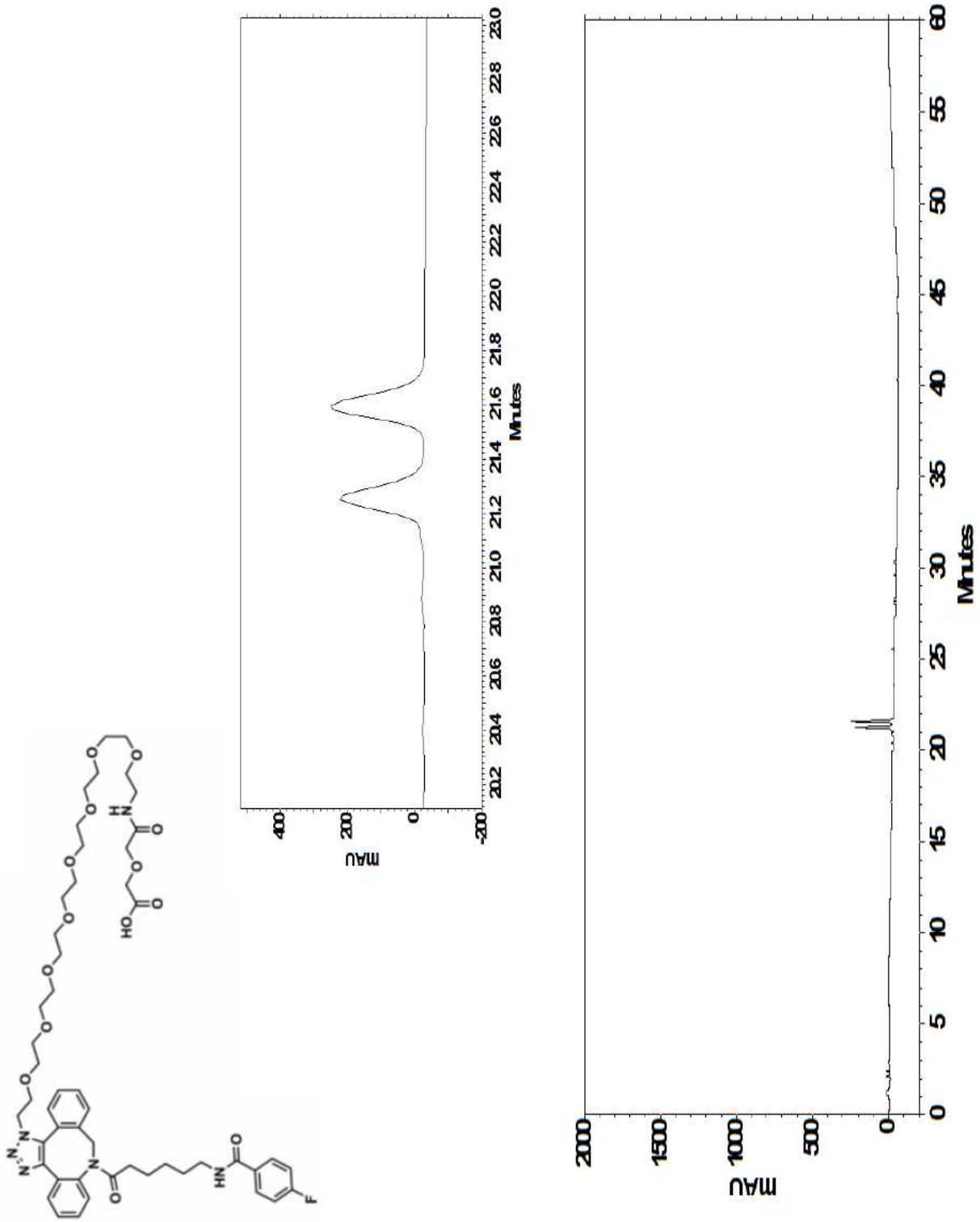
Supporting Information Figure 6. Analytical HPLC trace of [^{19}F]-**11** (λ = 220 nm).



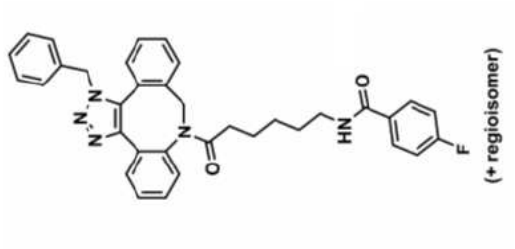
Supporting Information Figure 7. LC/MS traces of [^{19}F]-**12**.

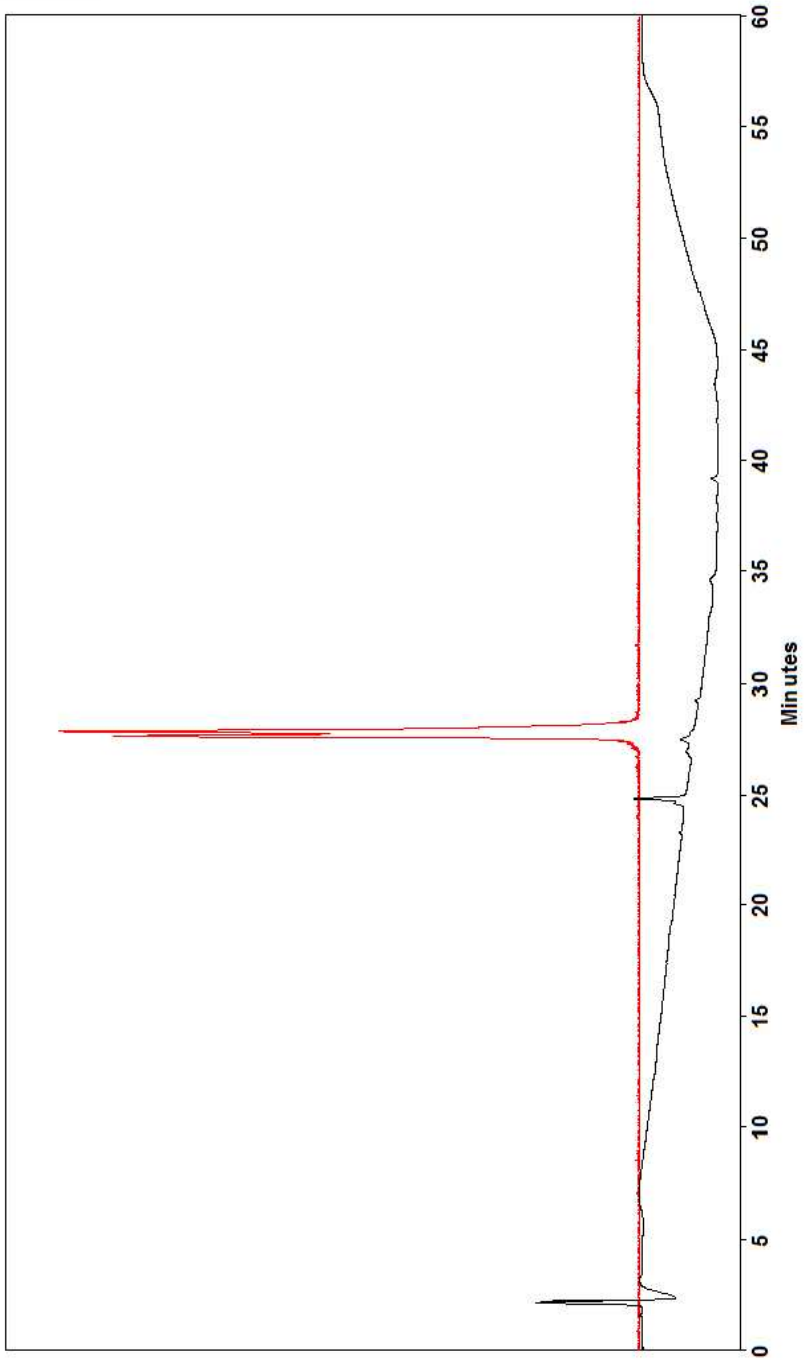


Supporting Information Figure 8. Analytical HPLC trace of [^{19}F]-**12** (λ = 220 nm).

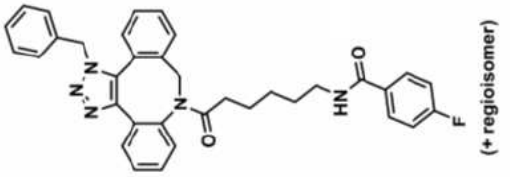


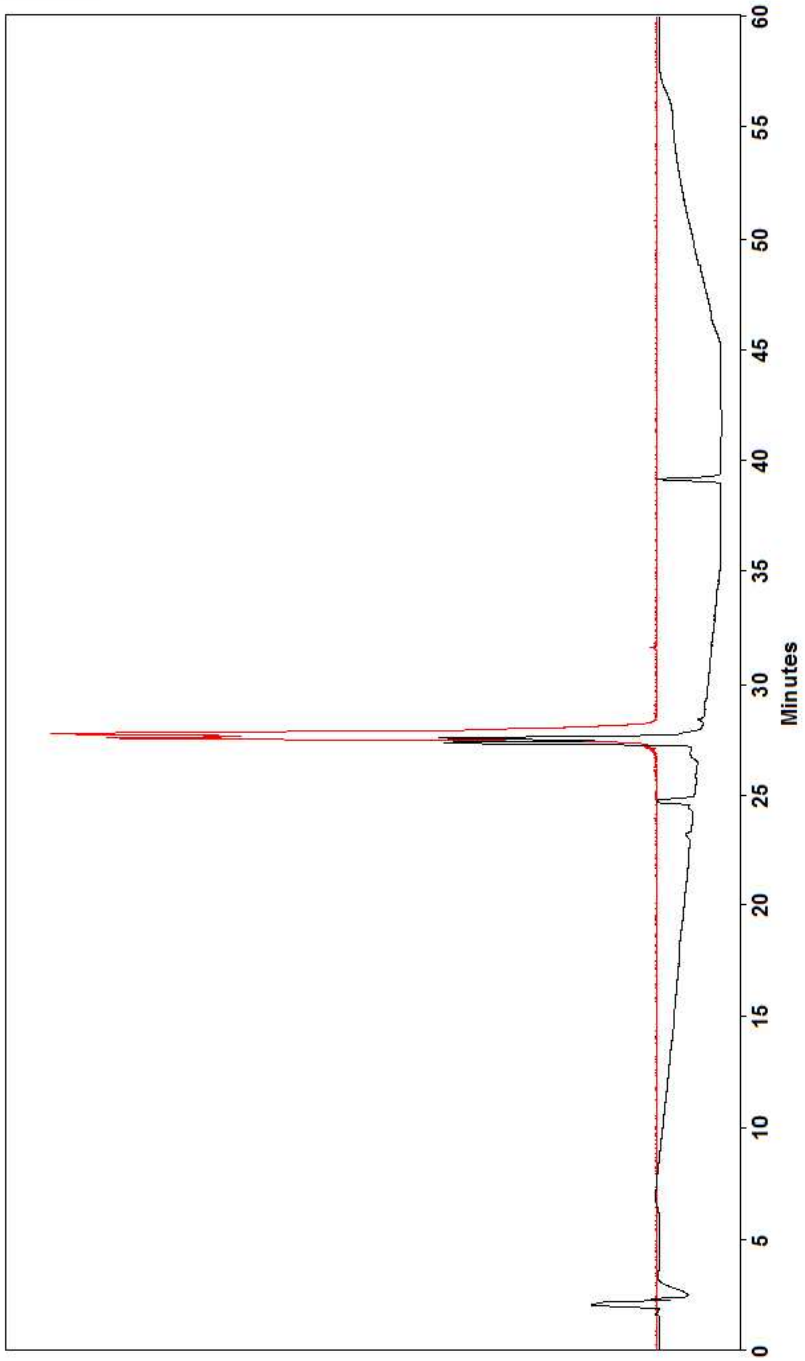
Supporting Information Figure 9. Analytical HPLC UV (λ = 220 nm) trace (black) overlaid with the radio HPLC trace (red) of purified [^{18}F]-**11** (y-axis: arbitrary scale).



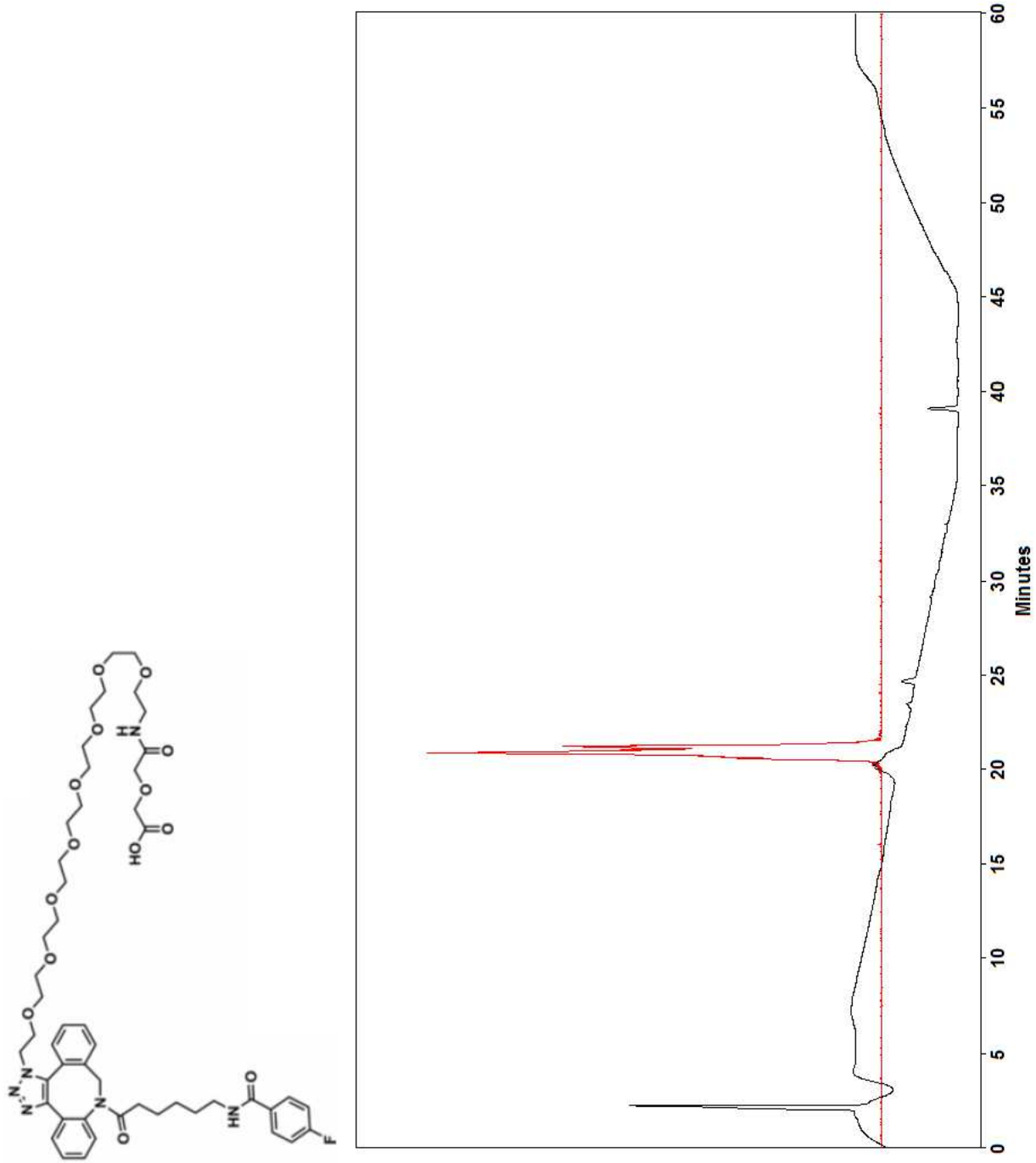


Supporting Information Figure 10. Analytical HPLC UV (λ = 220 nm) trace (black) overlaid with the radio HPLC trace (red) of purified [^{18}F]-**11** spiked with purified [^{19}F]-**11** (y-axis: arbitrary scale).





Supporting Information Figure 11. Analytical HPLC UV (λ = 220 nm) trace (black) overlaid with the radio HPLC trace (red) of purified [^{18}F]-**12** (y-axis: arbitrary scale).



Supporting Information Figure 12. Analytical HPLC UV (λ = 220 nm) trace (black) overlaid with the radio HPLC trace (red) of purified [^{18}F]-**12** spiked with purified [^{19}F]-**12** (y-axis: arbitrary scale).