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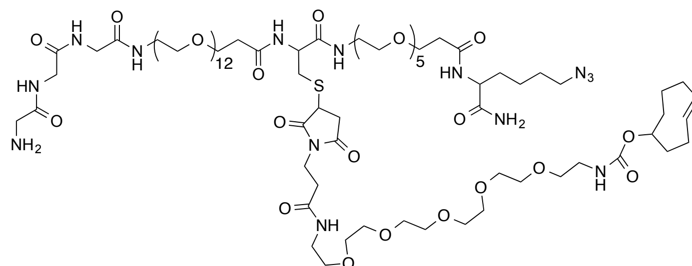
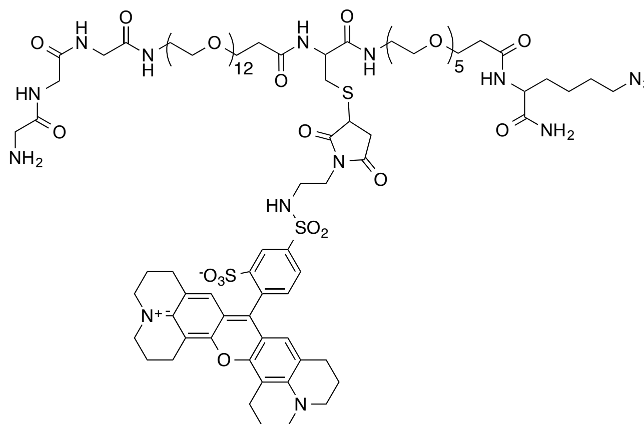
Synthesis of $(Gly)_3$-PEG_{12}-Cys(TCO)-PEG_5-Lys(azide).



The peptide $(Gly)_3$-PEG_{12}-Cys-PEG_5-Lys(azide) was synthesized by standard solid phase peptide synthesis. Maleimide-TCO (from Conju-bio) was dissolved in 0.05 M $NaHCO_3$ buffer pH 8.3. The peptide was added and left to stir at room temperature for 1 h until LC-MS indicated near-complete conversion to the product. The solution was filtered and purified by reverse phase-HPLC with a semi-preparative column (Phenomenex, C_{18} column , Gemini, 5 μm, 10x250 mm) at a flow rate of 5.0 mL/min.; solvent A: 0.1% formic acid in H_2O, solvent B: 0.1% formic acid in CH_3CN. Product eluted at 35–40% solvent B. Fractions containing pure product were collected and lyophilized. LC-MS calculated for $C_{83}H_{151}N_{14}O_{34}S$ $[M+H]^+$ 1920.0, found 1919.0.

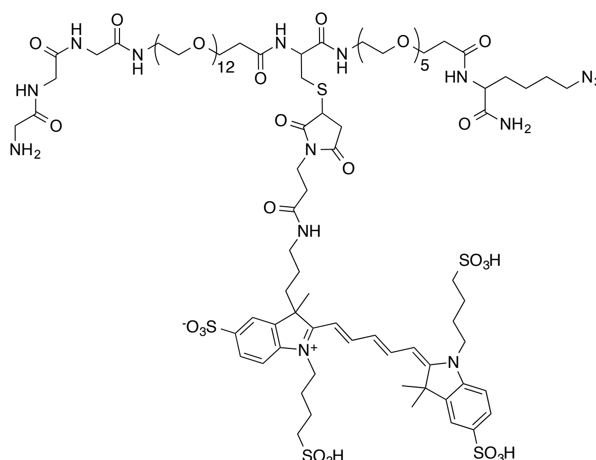
Synthesis of $(Gly)_3$-PEG_{12}-Cys(Texas Red)-PEG_5-Lys(azide).



The peptide $(Gly)_3$-PEG_{12}-Cys-PEG_5-Lys(azide) was synthesized by standard solid phase peptide synthesis and was dissolved in 0.05 M $NaHCO_3$ buffer pH 8.3. Maleimide-Texas Red (from

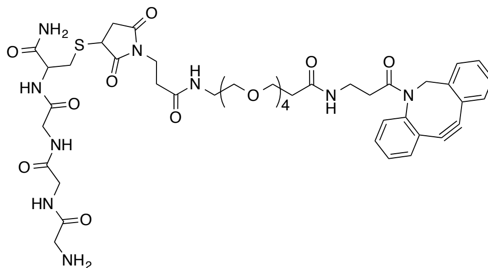
Vector Labs) was dissolved in DMSO and then was added to the solution and left to stir at room temperature for 1 h until LC-MS indicated near-complete conversion to the product. The solution was filtered and purified by reverse phase-HPLC with a semi-preparative column (Phenomenex, C_{18} column, Gemini, 5 μm, 10x250 mm) at a flow rate of 5.0 mL/min.; solvent A: 0.1% TFA in H_2O, solvent B: 0.1% TFA in CH_3CN. Product eluted at 40–45% solvent B. Fractions containing pure product were collected and lyophilized. LC-MS calculated for $C_{92}H_{142}N_{15}O_{32}S_3$ $[M+H]^+$ 2064.9, found 2063.9.

Synthesis of (Gly)$_3$-PEG$_{12}$-Cys(Alexa647)-PEG$_5$-Lys(azide).



The peptide (Gly)$_3$-PEG$_{12}$-Cys-PEG$_5$-Lys(azide) was synthesized by standard solid phase peptide synthesis. Maleimide-Alexa647 (from Life Technology) was dissolved in 0.05 M $NaHCO_3$ buffer pH 8.3. The peptide was added and left to stir at room temperature for 1 h until LC-MS indicated near-complete conversion to the product. The solution was filtered and purified by reverse phase-HPLC with a semi-preparative column (Phenomenex, C_{18} column, Gemini, 5 μm, 10x250 mm) at a flow rate of 5.0 mL/min.; solvent A: 0.1% TFA in H_2O, solvent B: 0.1% TFA in CH_3CN. Product eluted at 30–35% solvent B. Fractions containing pure product were collected and lyophilized. LC-MS calculated for $C_{97}H_{158}N_{15}O_{39}S_5$ $[M+H]^+$ 2317.9, found 2318.4.

Synthesis of $(Gly)_3$-DBCO.



The tetrapeptide $(Gly)_3$-Cys was synthesized by standard solid phase peptide synthesis and was dissolved in 0.05 M $NaHCO_3$ buffer pH 8.3. Maleimide-DBCO (from Click Chemistry Tools) was dissolved in DMSO and then was added to the solution and left to stir at room temperature for 1 h until LC-MS indicated near-complete conversion to the product. The solution was filtered and purified by reverse phase-HPLC with a semi-preparative column (Phenomenex, C_{18} column, Gemini, 5 μm, 10x250 mm) at a flow rate of 5.0 mL/min.; solvent A: 0.1% TFA in H_2O, solvent B: 0.1% TFA in CH_3CN. Product eluted at 35–40% solvent B. Fractions containing pure product were collected and lyophilized. LC-MS calculated for $C_{45}H_{60}N_9O_{13}S$ $[M+H]^+$ 966.4, found 966.4.

Enzymatic incorporation of substrates into proteins using sortase.

The penta-mutant sortase A, with an improved k_{cat}, was used (1). Reaction mixtures (1 mL) contained Tris·HCl (50 mM, pH 7.5), $CaCl_2$ (10 mM), NaCl (150 mM), triglycine-containing probe (500 µM), LPETG-containing substrate (100 µM), and sortase (5 µM) (2, 3). After incubation at 4 °C with agitation for 2 h, reaction products were analyzed by LC-MS. Yields were generally >90%. When the yield was below 90%, the reaction was allowed to proceed for an additional two hours, with addition of sortase to 10 µM and triglycine-containing probe to 1 mM. Ni-NTA beads were added to the reaction mixture with agitation for 5 min at 25 °C followed by centrifugation to remove sortase and any remaining unreacted His-tagged substrate. The final product was purified by size exclusion chromatography in PBS or Tris·HCl (50 mM, pH 7.5). The labeled protein was stored at -80 °C with 5% glycerol for up to six months.

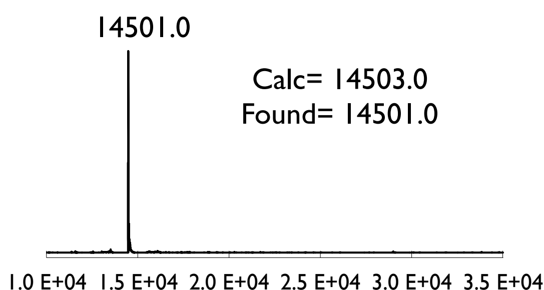
Dimerization of VHHs.

The general procedure was as follows: the DBCO-VHH (1.3 eq, in PBS) was added to the azide-X-VHH (where X is either TCO, Texas Red or Alexa647) and the reaction was left to proceed at room temperature for ~1-3 hours with constant agitation, where LC-MS analysis revealed (generally) above 80% conversion to the corresponding dimer. The dimer was then purified via size exclusion chromatography (FPLC) using PBS as the eluting solvent. The labeled dimer was stored at -80 °C with 5% glycerol for up to six months.

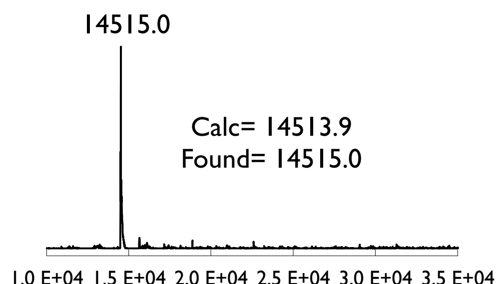
LC-MS analysis of VHHs, their corresponding sortagged products and dimers.

DC8 and its derivatives.

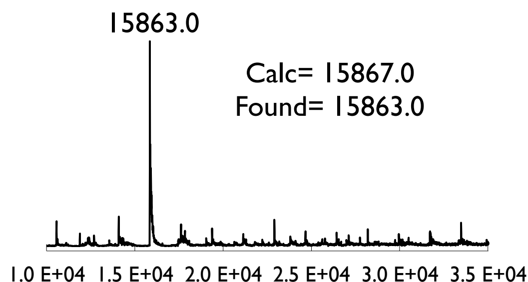
DC8



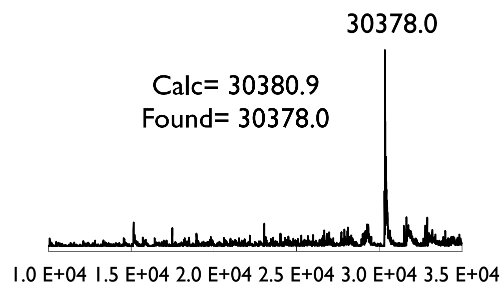
DC8-DBCO



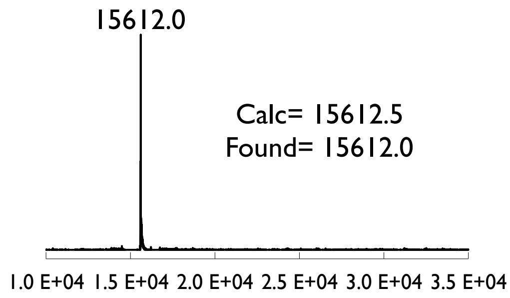
DC8-azide-Alexa647



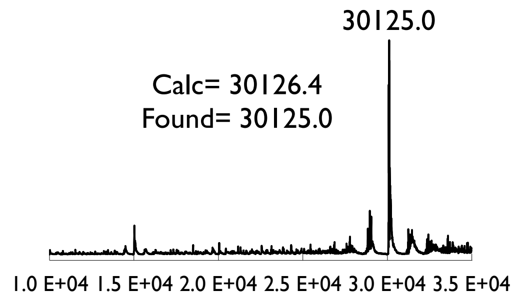
DC8-DC8-Alexa647 (homo dimer)



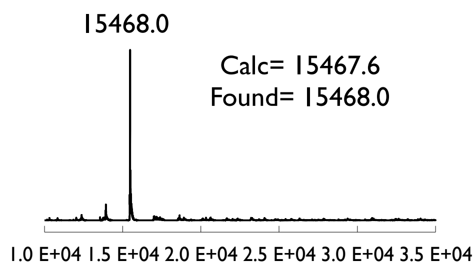
DC8-azide-Texas Red



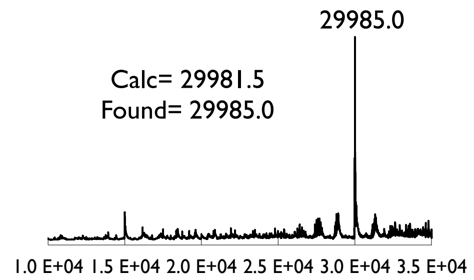
DC8-DC8-Texas Red (homo dimer)



DC8-azide-TCO

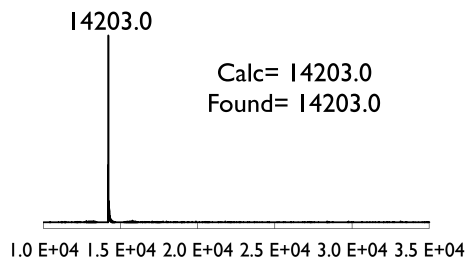


DC8-DC8-TCO (homo dimer)

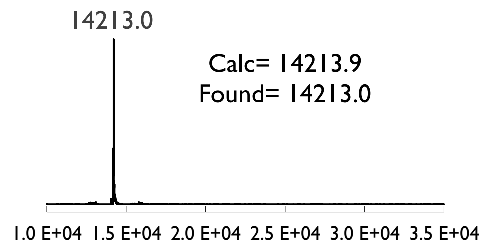


DC13 and its derivatives.

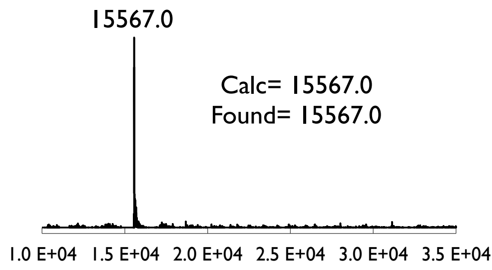
DC13



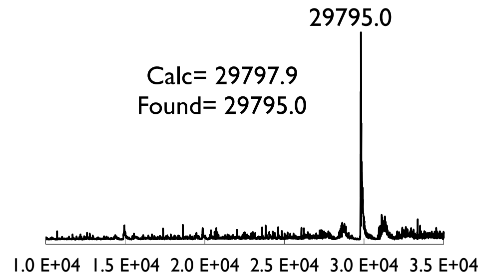
DC13-DBCO



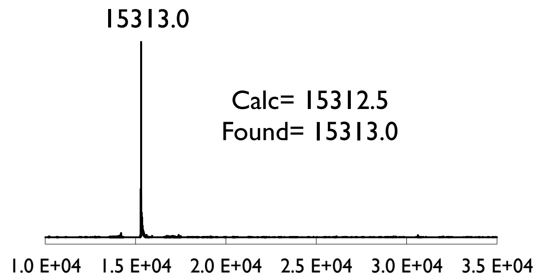
DC13-azide-Alexa647



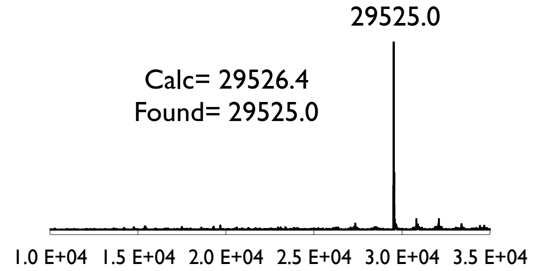
DC13-DC13-Alexa647 (homo dimer)



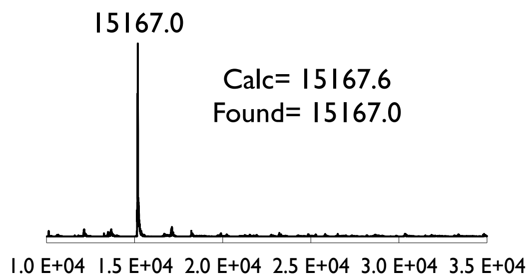
DC13-azide-Texas Red



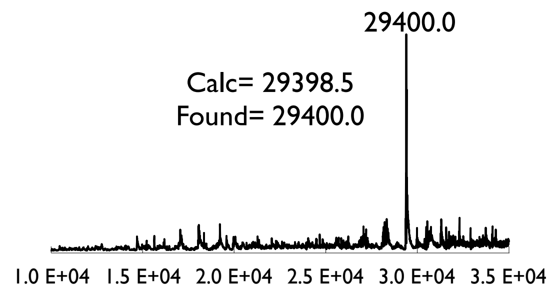
DC13-DC13-Texas Red (homo dimer)



DC13-azide-TCO



DC13-DC13-TCO (homo dimer)



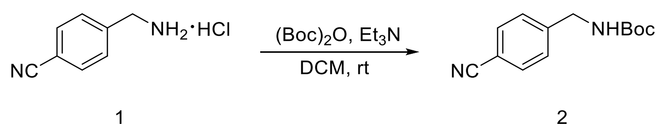
PEGylation of VHHs.

The general procedure was as follows: the DBCO-PEG (4 eq, in PBS) was added to the azide-X-VHH (where X is either TCO, Texas Red or Alexa647) and the reaction was left to proceed at room temperature for ~1-2 hours with constant agitation, where SDS-PAGE analysis revealed (generally) above 80% conversion to the corresponding PEGylated product. The final PEGylated product was purified via size exclusion chromatography (FPLC) using PBS as the eluting solvent. The labeled PEGylated protein was stored at -80 °C with 5% glycerol for up to six months.

Two-photon imaging.

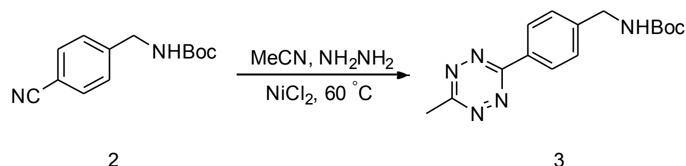
Two-photon imaging was performed with Olympus BX61 upright microscope (Olympus 25X 1.05 NA Plan Objective), fitted with a SpectraPysics MaiTai DeepSee laser. Images were acquired using 910 nm excitation and following filters; CFP (460-510), GFP (495-540) and a third filter (575-630) for the Texas Red signal. Second harmonics (collagen) were also detected in the CFP filter. Images were acquired with 5 µm Z-resolution with Olympus FluoView FC1000 software. Tile images were saved as JPEG files. Images were processed to obtain a scale bar in Imaris v 7.4.0; no intensity or contrast adjustments were made.

Synthesis and characterization of [^{19}F]SFB-Tetrazine.

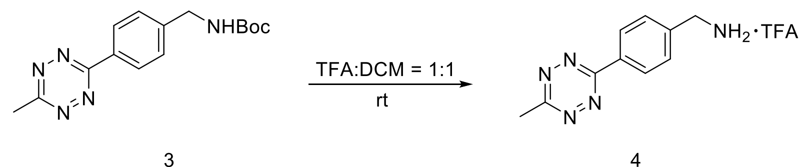


***tert*-butyl (4-cyanobenzyl)carbamate (2)**: 4-(Aminomethyl)benzonitrile hydrochloride **1** (2.82 g, 16.7 mmol) and triethylamine (4.7 mL, 33.7 mmol) were dissolved in anhydrous CH_2Cl_2 (50 mL) at 0 °C. To this stirred solution was added di-*tert*-butyl dicarbonate (4.38 g, 20.1 mmol), and the reaction allowed to warm to room temperature and stirred for 16 hours. The reaction mixture was evaporated in vacuo, and the residue was re-dissolved in diethyl ether (50 mL), which was

washed successively with 0.5 M aq. HCl (2 x 25 mL), saturated $NaHCO_3$ (2 x 25 mL) and brine (25 mL). The organic layer was dried with $MgSO_4$, filtered and evaporated in vacuo to give an off-white solid. The residue was purified by flash column chromatography (Hexanes/EtOAc = 10/1) to afford *tert*-butyl (4-cyanobenzyl) carbamate **2** (3.69 g, yield 95 %) as a colorless solid. It was further characterized according to the literature procedure (4).

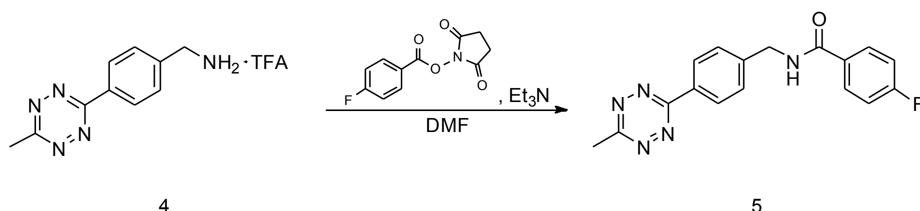


***tert*-butyl (4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzyl) carbamate (3)**: A stirred mixture of carbamate **2** (1.5 g, 6.46 mmol), MeCN (3.4 mL, 64.6 mmol) and anhydrous $NiCl_2$ (418 mg, 3.23 mmol) was treated dropwise with hydrazine (5 mL, 161.5 mmol). The purple reaction mixture was stirred at 60 °C for 24 hours. Afterwards a solution of $NaNO_2$ (8.8 g, 127 mmol) in H_2O (65 mL) was carefully added. HCl (2 N solution) was added until the evolution of nitrous oxides ceased. The dark red solution was extracted with ethyl acetate (3 x 60 mL). The extract was combined and dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (Hexanes/EtOAc = 8/1) to afford *tert*-butyl (4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzyl) carbamate **3** (1.22 g, yield 63 %) as a red solid. It was further characterized according to the literature (5).



(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)methanamine (4): In a 100 mL reaction vessel was charged the solution of tetrazine **3** (301 mg, 1.0 mmol) in DCM (12 mL). Trifluoroacetic acid (12 mL) was added dropwise. The mixture was stirred at room temperature for 2 h. Afterwards the mixture was evaporated and suspended into Et_2O (20 mL) for recrystallization at -20 °C. The

supernatant was decanted and the residue was dried under vacuum for 2 hours to afford (4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)methanamine **4** (200 mg, yield 99%) as red solid. The product was further characterized according to the literature (6).



4-fluoro-*N*-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzyl)benzamide (5): A solution of the tetrazine amine TFA salt **4** (50 mg, 0.25 mmol) in anhydrous DMF (3.5 mL) was added 2,5-dioxopyrrolidin-1-yl 4-fluorobenzoate (50 mg, 0.223 mmol) and Et_3N (0.35 mL, 2.5 mmol). The resulting solution was then stirred at room temperature overnight under argon gas. The reaction mixture was quenched with water (15 mL), and then extracted with ethyl ether (10 mL × 3). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexanes/EtOAc=7/1) to afford 4-fluoro-*N*-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzyl)benzamide **5** (29 mg, 36%) as a red solid powder.

^{1}H NMR (300 MHz, DMSO) δ 9.18 (t, J = 6.1 Hz, 1H), 8.43 (d, J = 8.4 Hz, 2H), 7.99 (dd, J = 5.5, 3.3 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 7.31 (t, J = 7.8 Hz, 2H), 4.60 (d, J = 6.1 Hz, 2H), 2.97 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 167.5, 165.9 (d, J = 23.3 Hz), 163.6, 162.8, 144.8, 131.0 (d, J = 3.1 Hz), 130.8, 130.3 (d, J = 8.9 Hz), 128.5, 127.9, 115.7 (d, J = 21.7 Hz), 43.0, 21.3.; HRMS calc'd for $C_{17}H_{15}FN_5O^+$ $[M+H]^+$, 324.1261; found 324.1265.

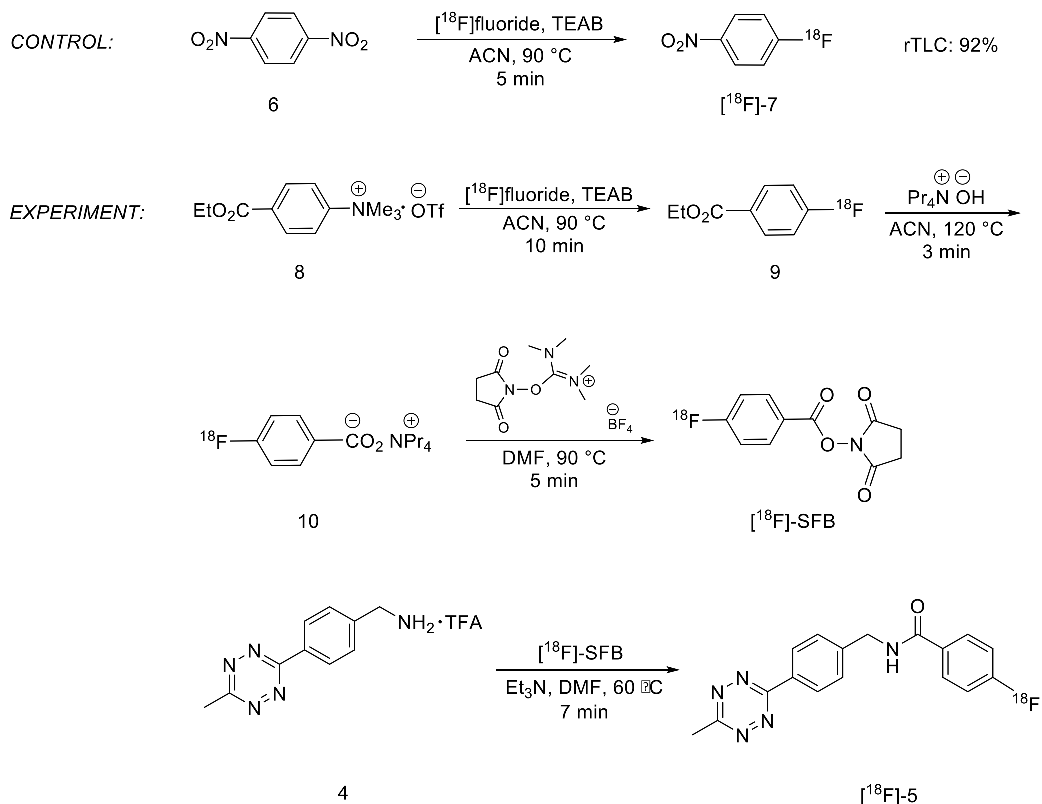
Radiochemical synthesis of [^{18}F]-Tetrazine ([^{18}F]-5).

General methods for radioisotope production: a GE PETtrace 16.5 MeV cyclotron was used for [^{18}F]fluoride production by the $^{18}O(p,n)^{18}F$ nuclear reaction to irradiate ^{18}O-enriched water.

[^{18}F]fluoride was delivered to a lead-shielded hot cell in ^{18}O-enriched water by nitrogen gas pressure.

General methods for analysis of radiofluorination reactions: radioactivity was quantified using a Capintec Radioisotope Calibrator (CRC-712M) ion chamber. Radiochemical incorporation yields were determined by radioTLC. EMD TLC Silica gel 60 plates (10 x 2 cm) were spotted with an aliquot (1-5 μL) of crude reaction mixture approximately 1.5 cm from the bottom of the plate (baseline). TLC plates were developed in a chamber containing ethyl acetate until within 2 cm of the top of the plate (front). Analysis was performed using a Bioscan AR-2000 radio-TLC imaging scanner and WinScan software. Radiochemical identity and purity were determined by radioHPLC. A Phenomenex Luna C18, 250 x 4.6 mm, 5 μm HPLC column was used with a Waters 1515 Isocratic HPLC Pump equipped with a Waters 2487 Dual λ Absorbance Detector, a Bioscan Flow-Count equipped with a NaI crystal, and Breeze software.

Manual radiolabeling.



[^{18}F]Fluoride was prepared for radiofluorination by the following method: a solution of base (tetraethylammonium bicarbonate (TEAB), 6 mg) in acetonitrile and water (1 mL, v/v 7:3) was added to an aliquot of target water (≤ 1 mL) containing the appropriate amount of [^{18}F]fluoride in a V-shaped vial sealed with a teflon-lined septum. The vial was heated to 110 °C while nitrogen gas was passed through a P_2O_5-Drierite™ column followed by the vented vial. When no liquid was visible in the vial, it was removed from heat, anhydrous acetonitrile (1 mL) was added, and the heating was resumed until dryness. This step was repeated an additional three times. The vial was then cooled at room temperature under nitrogen pressure. The contents were resolubilized in CH_3CN (0.6 mL). A solution of TEA[^{18}F] (0.2 mL) was added into another V-shaped vial charged with 1,4-dinitrobenzene **6** (2 mg) and CH_3CN (0.2 mL). The mixture was heated at 90 °C for 5 min, and then quenched with HPLC mobile phase (40% CH_3CN, 60% 0.1 M $NH_4{\cdot}HCO_2$(aq), 0.2 mL). TLC plate was spotted with crude mixture (2 μL) and developed with 100% EtOAc to determine the radiochemical conversion. The RCC value (92%) revealed that the TEA[^{18}F] solution was ready for radiolabeling. (total time: 8 min)

<u>*Note:*</u> The quality of TEA[^{18}F] is crucial for the following radiolabeling, thus the control is necessary to evaluate the quality.

Ethyl 4-(trimethylammonium triflate)benzoate **8** (4.8 mg) in anhydrous MeCN (0.6 mL) was added to the above dried TEA[^{18}F] solution (0.4 mL) and the mixture was heated at 90 °C for 10 min to produce ethyl 4-[^{18}F]fluorobenzoate **9**. The ethyl ester was subsequently hydrolyzed to form **10** using tetrapropylammonium hydroxide (20 μL, 1.0 M in water) at 120 °C for 3 min, and then the mixture azeotropically dried using MeCN (1 mL). Subsequently, a solution of N,N,N',N'-Tetramethyl-O-(N-succinimidyl) uronium tetrafluoroborate (10 mg) in DMF (0.3 mL) was added and the solution heated at 90 °C for 5 min. The mixture was cooled down to ambient temperature. Afterwards a solution of tetrazine amine TFA salt **4** (1.7 mg) in DMF (0.3 mL) was added into the mixture, followed by addition of Et_3N (20 μL). Then the reaction was heated at 60 °C for 7 min, quenched with HPLC mobile phase (60% CH_3CN, 40% 0.1 M $NH_4{\cdot}HCO_2$(aq), 2 mL). The solution was diluted with water (15 mL), passed through C18 cartridge, washed with water (10 mL), and eluted with acetonitrile (1.5 mL) to determine the radiochemical yield (RCY) and identity via co-injection with standard **[^{19}F]-5**.

- <u>Radiochemical yield (non-decay corrected)</u>

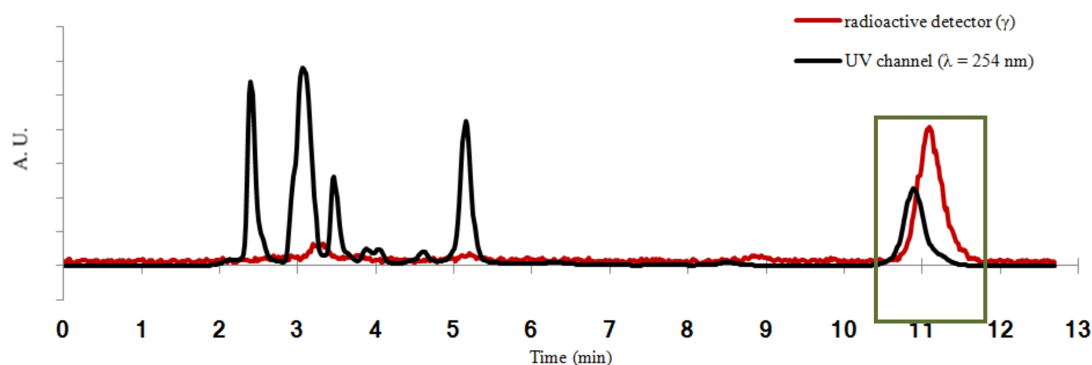
	1	2	3	4	5	mean	standard deviation
RCY (%)	27	35	21	19	34	27	7

- <u>RadioHPLC chromatogram:</u>

Column: luna 5u C18 100 Å 250 × 4.6 mm

Mobile phase: 60% CH_3CN, 40% 0.1 M $NH_4 \cdot HCO_2$(aq)

Flow rate: 1mL/min



Automated synthesis by GE TracerLab FX_{FN} method.

Following completion of bombardment, the [^{18}F]fluoride was transferred to the GE TRACERlab™ FX_{FN} radiosynthesis module via helium gas overpressure. A schematic diagram of the GE medical systems commercial TRACERlab™ FX_{FN} radiosynthesis module used for the synthesis of **[^{18}F]-5** is shown in Figure S-01.

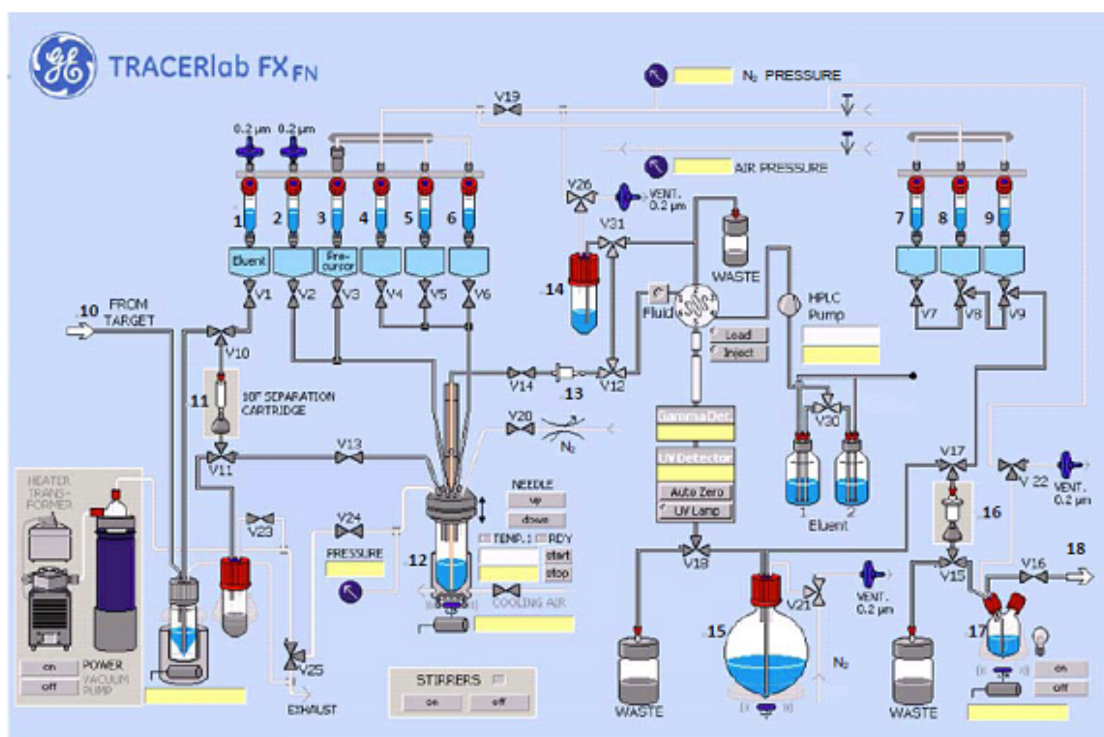


Figure S-01: Schematic of the GE TRACERlab™ FX_{FN} radiosynthesis module automated synthesis manifold for **[^{18}F]-5**.

Automated synthesis involves the following: (1) azeotropic drying of [^{18}F]fluoride; (2) [^{18}F]fluorination; and (3) HPLC purification, followed by solid-phase formulation of the final product. The synthesis module was operated in the following sequences with numerical references to Figure S-01.

- [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction using a GE cyclotron and delivered to the radiosynthesis module via 10. The [^{18}F]fluoride was quantitatively trapped on a QMA carbonate ion exchange solid phase extraction (SPE) light cartridge (Waters; activated with 6 mL of trace grade H_2O).
- Automated synthesis began with the elution of resin-bound [^{18}F]fluoride using a solution of tetraethylammonium bicarbonate (6 mg in 500 µL H_2O and 500 µL CH_3CN), pre-loaded into 1 and delivered to the reactor (12).
- The reaction mixture (12) was dried azeotropically at 85 °C under N_2 flow and vacuum over

5 min, then at 110 °C under N_2 flow and vacuum for 2 min, then cooled down to 90 °C.

- Ethyl 4-(trimethylammonium triflate)benzoate **8** (5 mg in 1.0 mL CH_3CN) pre-loaded into 3 was added to 12. The reactor was sealed via the closure of valve V13, V20 and V24 and the reaction mixture was maintained at 90 ºC for 10 min.
- The reaction mixture was then cooled to 40 °C, vented via valve V24, and tetrapropylammonium hydroxide (1.0 M in water, 20 µL in 0.5 mL CH_3CN) pre-loaded into 4 was added to 12. The reactor was sealed via the closure of valve V24 and the reaction mixture was heated to 120 ºC and this temperature was maintained for 3 min, then cooled down to 70 °C.
- The reaction mixture (12) was dried azeotropically by addition of 1 mL anhydrous CH_3CN, preloaded into 5, at 70 °C under N_2 flow and vacuum over 6 min.
- N,N,N',N'-Tetramethyl-O-(N-succinimidyl) uronium tetrafluoroborate (TSTU, 10 mg) in DMF (0.5 mL) pre-loaded into 6 was added to 12. The reactor was sealed via the closure of valve V24 and the reaction mixture was heated to 90 ºC and this temperature was maintained for 5 min, then cooled down to 60 °C.
- A mixture of tetrazine amine TFA salt **4** (6.0 mg) and Et_3N (40 µL) in DMF (0.5 mL) pre-loaded into 2 was added to 12. The reaction mixture was maintained at 60 ºC for 7 min.
- The crude reaction mixture was eluted into 14, which was preloaded with 20:80 CH_3CN/ 0.1 M ammonium formate solution (3 mL). The contents of 14 were transferred to the HPLC loop via N_2 pressure using a fluid detector, injected onto a semi-preparative column (Luna C18 semi-preparative, 250 × 10.00 mm, 5µ), and eluted with 40:60 CH_3CN/ 0.1 M ammonium formate by volume at a flow rate of 5 mL/min. The eluent was monitored by UV (λ = 254 nm) and radiochemical detectors connected in series.
- A typical semi-preparative HPLC chromatogram is shown in Figure S-02. The fraction containing the major radiochemical product (t_R = 20.1 min) was collected, via valve 18, into a large dilution vessel (15), which was preloaded with 23 mL of sterile water for injection (United States Pharmacopeia (USP); Hospira).

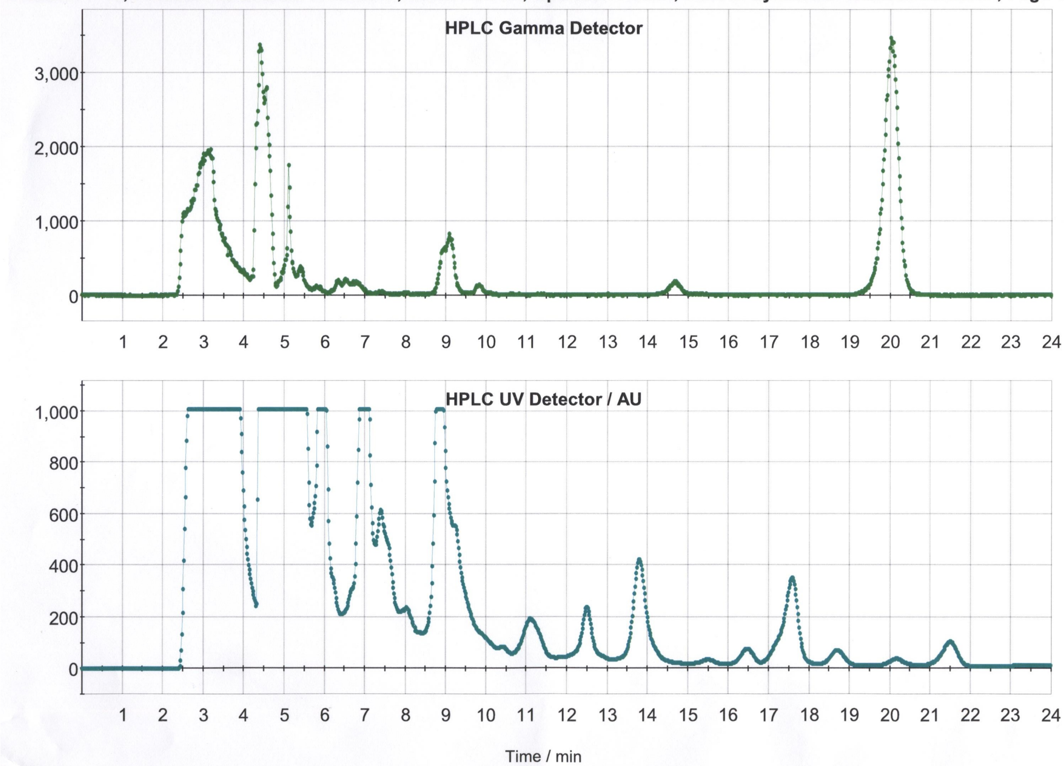


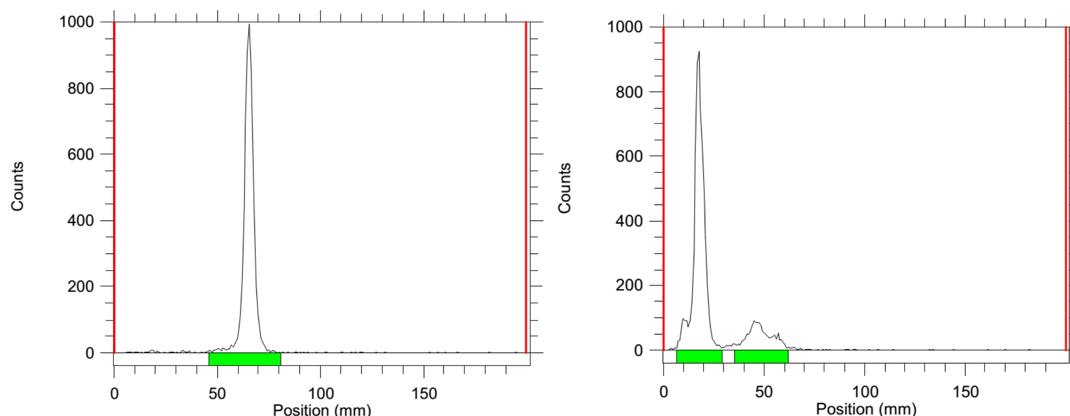
Figure S-02: Semi-preparative HPLC trace of a typical radiosynthesis of **[^{18}F]-5**.

- The diluted HPLC fraction was then loaded onto a C18 SPE cartridge (16) (Waters; preactivated with 5 mL EtOH followed by 10 mL H_2O).
- Cartridge 16 was washed with 10 mL sterile water for injection, USP, preloaded into 7, to remove traces of salts, HPLC mobile phase, and [^{18}F]fluoride. Then 16 was eluted with 1.5 mL CH_3CN, preloaded in 8, into collection vial 17.

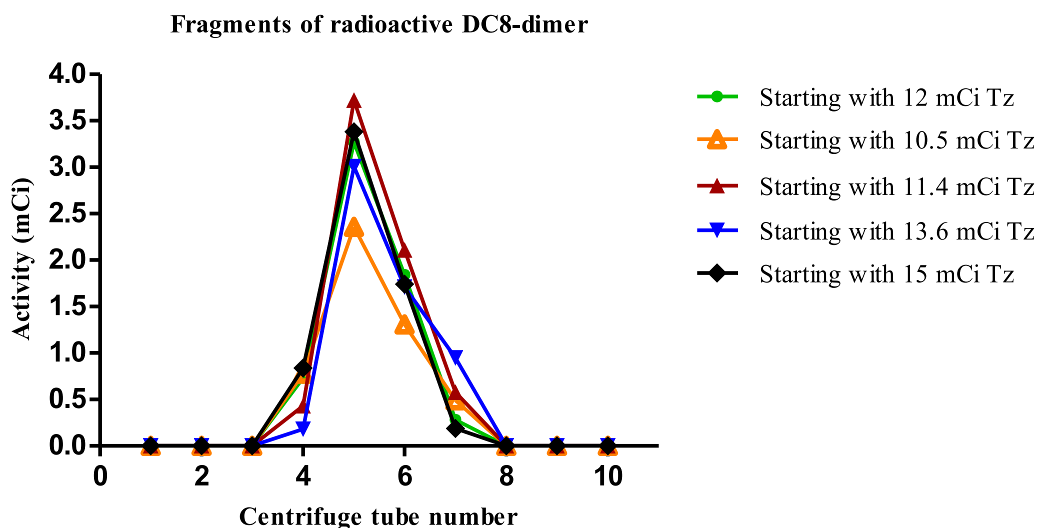
Analyses of radioactive mixtures were performed by HPLC with an in-line UV (λ = 254 nm) detector in series with a CsI PIN diode radioactivity detector. Uncorrected radiochemical yields of **[^{18}F]-5** were 10 ± 3% (n = 8) relative to starting [^{18}F]fluoride.

Synthesis and characterization of [^{18}F]-VHHs.

General procedure: the solution of [^{18}F]-Tetrazine **5** obtained from FX_{FN} was concentrated at 70 °C under N_2 flow for 10 min, then cooled down to room temperature. A centrifuge tube (1.5 mL) was loaded with PBS (150 μL) and a solution of [^{18}F]-Tetrazine **5** in CH_3CN (50 μL), then the radioactivity was measured by a dose calibrator (10~15 mCi). VHH-TCO (either monomer or dimer) in PBS (100 μL) was added into the centrifuge tube at the last step. The reaction was allowed to proceed with constant agitation at room temperature for ~20 min. The mixture was analyzed by radio-TLC (100% EtOAc, R_f [^{18}F]-Tz **5** = 0.6, R_f [^{18}F]-VHHs = 0.0) showing more than 80% radiochemical conversion. The reaction mixture was loaded onto a PD-10 size-exclusion cartridge (GE Healthcare), and PBS (2 × 500 μL) was used to assist transfer. Afterwards the activity of the reaction centrifuge tube was measured by the dose calibrator (< 50 μCi), confirming a complete transfer. The PD-10 cartridge was eluted with PBS (10 × 500 μL), and each fragment was collected into a new 1.5 mL tube. The desired product [^{18}F]-VHHs usually eluted at tubes #4-7. Characterization (using [^{18}F]-DC8-dimer as an example): rTLC chromatography (left [^{18}F]-Tz **5**; right [^{18}F]-DC8-dimer; at 20 min)



Fragments collection through PD-10 cartridge



After size-exclusion chromatography, a 47 ± 9% (n = 5, non-decay corrected) radiochemical yield was obtained.

MicroPET Imaging Studies.

All procedures and animal protocols were approved by the Massachusetts General Hospital subcommittee on research animal care. [^{18}F]VHHs (20-40 μCi) was injected into the tail-vein of each animal. Mice were serially imaged using a microPET (Sofie, G4-PET). For all imaging experiments, mice were anesthetized using 2% isoflurane in O_2 at a flow rate of ~1.5 L/min, positioned in a prone position along the long axis of the microPET scanner and imaged. Images were reconstructed using a filtered back projection reconstruction algorithm. For image analysis, cylindrical regions of interest (ROIs) were manually drawn from three dimensional filtered back projection (FBP) reconstructed PET images using AMIDE software. Regional radioactivity was expressed as the percentage standardized uptake value [% SUV = % ID/mL × body weight (g)]. Two- and three-dimensional visualizations were produced using the DICOM viewer OsiriX (© Pixmeo SARL, 2003-2014).

Sequence of DC8, and DC13

DC8:
Nucleic Acid:

CAGGTGCAGCTGCAGGAGTCAGGGGGAGGATTGGTGCAGCCTGGGGGGTCTCTGAG
ACTCTCCTGTACAGCCTCTGGATTCACATTCAGTACTTACTACATGAGCTGGGTCCG
CAAGGCTCCAGGGAAGGGGCCCGAGTGGGTCTCAGTTATGAATAGTAGTGGTGGTG
ACACAAGGTATGCAGACTTCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCC
AAGAACACACTGTATCTCCAAATGAACAGCCTGAAACCTGAGGATACGGCCCTGTA
TTACTGTGCGCAAGGTAGATCAGATATATACCCAACCTTCACGCGGGGCCAGGGGA
CCCAGGTCACCGTCTCCTCAGGAGGACTGCCGGAAACCGGC

Peptide:
QVQLQESGGGLVQPGGSLRLSCTASGFTFSTYYMSWVRKAPGKGPEWVSVMNSSGGD
TRYADFVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCAQGRSDIYPTFTRGQGTQVT
VSSGSLPETGGHHHHHH

DC13:
Nucleic Acid:
CAGGTTCAACTGCAAGAGAGTGGCGGGGGCCTGGTTCAGACCGGTGGTTCTCTCCG
GCTCTCGTGTGCCGCAAGTGGAGTAGATTTTAACTGGTATAGCATGGGTTGGTTCAG
GCAAGCCCCTGGCAAAGAGCGGGAGTATGTGGCTTCGATTGACCAGGGAGGCGAGT
TGGATTACGCAATATCAGTAAAGGGCAGATTCACGATCTCCCGAGACAACGCGAAG
AATATGGTGTATCTCCAGATGAATTCGTTAAAGCCCGAAGACACCGCTGTATACTAC
TGTGCCGCAGATTTTTCCGGCCGGGGTGCGTCAAACCCTGACAAGTATAAATATTGG
GGACAGGGAACCCAAGTGACCGTCAGCAGCGGTGGGTTGCCCGAAACTGGAGGAC
ACCATCACCATCACCAT

Peptide:
QVQLQESGGGLVQTGGSLRLSCAASGVDFNWYSMGWFR
QAPGKEREYVASIDQGGELDYAISVKGRFTISRDNAKNMVYLQMNSLKPEDTAVYYCA
ADFSGRGASNPDKYKYWGQGTQVTVSSGGLPETGGHHHHHH

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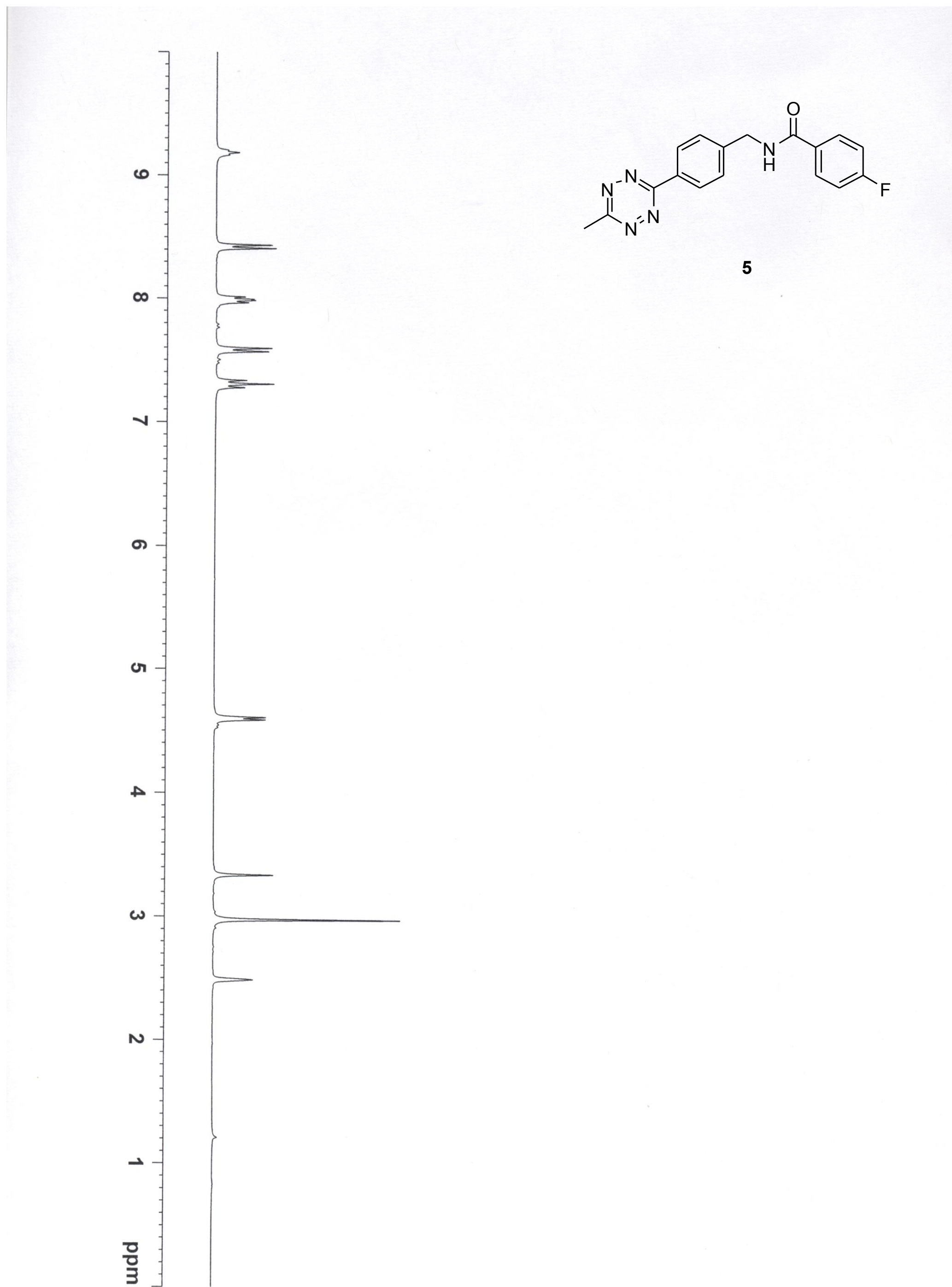
NMR spectra of [^{19}F]tetrazine 5

^{1}H-NMR



^{13}C-NMR