Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Hydrophilic ^{18}F-labeled *trans*-5-oxocene (oxoTCO) for efficient construction of PET agents with improved tumor-to-background ratios in neurotensin receptor (NTR) imaging

Mengzhe Wang[a], Raghu Vannam[b], William Lambert[b], Yixin Xie[b], Hui Wang[a], Ben Giglio[a], Xiaofen Ma[a], Zhanhong Wu[a], Joseph Fox[b]*, and Zibo Li[a]*

[a] Department of Radiology and Biomedical Research Imaging Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA

[b] Brown Laboratories, Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716, USA

*Correspondence to: *jmfox@udel.edu*, *ziboli@med.unc.edu*

S1. General Considerations

All commercially available solvents and chemicals were used without further purification. Anhydrous tetrahydrofuran was freshly distilled from sodium and benzophenone. Anhydrous dichloromethane was prepared by flowing through a nitrogen pressurized alumina column. All reactions (excluding TCO/tetrazine ligations) were carried out under nitrogen in dried glassware.

NMR data was collected on Bruker 400 MHz or 600 MHz instruments (100MHz and 150MHz respectively for ^{13}C NMR). Chemical shifts (δ) are reported in ppm and are referenced to the residual non-deuterated solvent peak: $CDCl_3$ (7.26 ppm), CD_3CN (1.94 ppm), MeOD (3.31 ppm) for ^{1}H-NMR spectra; $CDCl_3$ (77.2 ppm), CD_3CN (1.3 and 118.3 ppm), MeOD (49.0 ppm) for ^{13}C NMR spectra. Coupling constants (*J*) are measured to the nearest 0.1 Hz with splitting patterns designated accordingly: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; sext, sextet; m, multiplet. ^{13}C NMR is proton decoupled, and when an APT experiment was used, quaternary and methylene carbons appear 'up' (C or CH_2) and methane and methyl carbons appear 'down' (CH or CH_3).

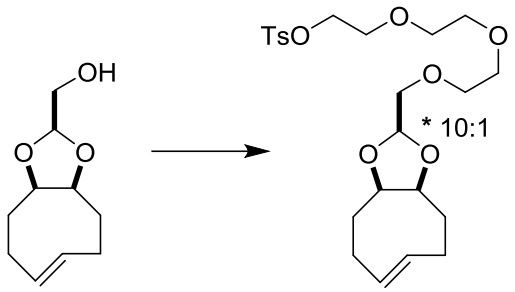
Thin layer chromatography was carried out on Merck/Millipore Silica Gel 60, F_{254}. Purifications by flash chromatography were accomplished on Silicycle 40-63D, 60Å silica gel. Reverse phase C18 chromatography was carried out on Yamazen Universal columns (Pore Size 120 Å, Particle Size 40-60 μm). Analytical reversed-phase HPLC using a kinetex 5μ C18 column (250 x 4.6mm) was performed on a SPD-M30A photodiode array detector (Shimadzu) and model 105S single-channel radiation detector (Carroll & Ramsey Associates). The flow was set to 1mL/min. Solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in acetonitrile. The mobile phase was 95% solvent A and 5% solvent B from 0–2min and ramped from 95% solvent A and 5% solvent B to 5% solvent A and 95% solvent B from 2 min to 22min.

Low resolution LCMS experiments were performed using a Waters SQD2 detector (ESI) that was coupled to a Waters Acquity H-Class UPLC. High resolution mass spectra were taken on a Waters GCT Premier instrument and Q extractive HF-X instrument.

Silver nitrate silica gel used for cyclooctene photoisomerizations was prepared using a procedure previously described [1]. Deactivated silica gel was also prepared using a procedure previously described [2]. *trans*-Cyclooctene photoisomerizations were carried out using a procedure previously described [1].

The *trans*-cyclooctenes sTCO, dTCO, and oxoTCO were synthesized using methods previously published. [3],[4],[5]. sTCO-Ts, sTCO-^{18}F and sTCO-^{19}F **(2)** were made by a published procedure [6].

S2. Synthesis

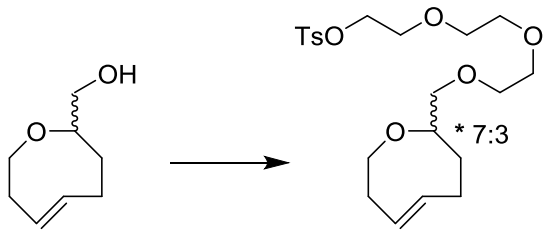


S1 **S2**

2-(2-(2-(((2*s/r*,3*aR*,9*aS,E*)-3a,4,5,8,9,9a-hexahydrocycloocta[d][1,3]dioxol-2-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate

To a suspension of potassium hydride (200 mg, 5.00 mmol, washed 3x with pentane from a mineral oil suspension) in dry THF/DMF (25 mL/ 2.5 mL) was added triethylene glycol di(p-toluenesulfonate) (2.14 g, 4.67 mmol) and ((2*s/r*,3*aR*,9*aS,E*)-3a,4,5,8,9,9a-hexahydrocycloocta[d][1,3]dioxol-2-yl)methanol **S1** (200 mg, 1.31 mmol) at rt. After 14 h, the resulting mixture was quenched with sat. NH_4Cl (20 mL) at 0 °C, extracted with diethyl ether (3x150 mL), washed with water (2×150 mL), dried over Na_2SO_4 and concentrated. The crude material was purified by silica gel chromatography using 0-70% acetone in hexane as an eluent to give the title compound **S2** (192 mg, 31%, d.r 10:1) as clear oil. This compound was stored at –20 °C as a solution in acetonitrile

^{1}H NMR (400 MHz, Acetonitrile-*d*3) δ 7.79 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 5.71 – 5.50 (m, 2H), 4.78 (t, *J* = 4.2 Hz, 1H), 4.16 – 4.07 (m, 2H), 3.99 – 3.83 (m, 2H), 3.62 – 3.58 (m, 2H), 3.57 – 3.54 (m, 2H), 3.53 – 3.46 (m, 6H), 3.43 (t, *J* = 3.9 Hz, 2H), 2.44 (s, 3H), 2.40 – 2.27 (m, 1H), 2.27 – 2.05 (m, 3H), 1.92 – 1.80 (m, 1H), 1.76 – 1.44 (m, 3H) ppm. ^{13}C NMR (101 MHz, Acetonitrile-*d*3) δ 146.3, 137.1, 133.9, 132.3, 131.0, 128.8, 101.0, 83.1, 81.2, 73.2, 71.7, 71.2, 71.1, 71.03, 69.96, 69.1, 39.4, 34.6, 31.9, 26.3, 21.7 ppm. HRMS (ESI) *m/z* : [M+H]+, Calcd for $C_{23}H_{35}O_8S$ is 471.2047; found 471.2045



(4) **(5)**

(E)-2-(2-(2-((3,4,7,8-tetrahydro-2H-oxocin-2-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5)

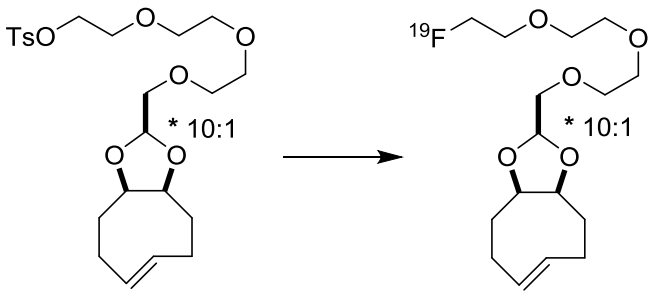
To a suspension of potassium hydride (50 mg, 1.2 mmol, washed 3x with pentane from a mineral oil suspension) in dry THF/DMF (8 mL/ 1 mL) was added triethylene glycol di(p-toluenesulfonate) (481 mg, 1.05 mmol) and (E)-(3,4,7,8-tetrahydro-2H-oxocin-2-yl)methanol **(4)** (50 mg, 0.35 mmol, 2.2:1 d.r.) at rt. After 14 h, the resulting mixture was quenched with sat. NH_4Cl (10 ml) at 0 °C, extracted with diethyl ether (3x50 mL), washed with water (2×50 mL), dried over Na_2SO_4 and concentrated. The crude material was purified by silica gel chromatography using 0 to 70% acetone in hexane as an eluent to give the title compound **(5)** (60 mg, 40%, d.r. 7:3) as a clear oil. This compound was stored at -20 °C as a solution in acetonitrile.

Peaks attributed to major diastereomer: ^{1}H NMR (600 MHz, Acetonitrile-*d*3) δ 7.79 (d, 8.3 Hz, 2H), 7.44 (d, 8.3 Hz, 2H), 5.66 (ddd, 15.5, 11.4, 3.3 Hz, 1H), 5.39 (ddd, 15.4, 10.9, 3.8 Hz, 1H), 4.12-4.09 (m, 2H), 3.92 (dd, J = 11.8, 6.1 H, 1H), 3.62-3.58 (m, 2H), 3.52-3.46 (m, 8H), 3.32-3.16 (m, 3H), 3.05 (dt, J=10.0, 6.2, 1H), 2.44 (s, 3H), 2.41-2.36 (m, 1H), 2.30-2.09 (m, 4H), 1.89-1.79 (m, 1H) ppm; ^{13}C NMR (151 MHz, Acetonitrile-*d*3) δ 146.7, 142.1, 134.3, 131.4, 129.2, 128.1, 85.0, 75.7, 74.6, 71.8, 71.61, 71.57, 71.5, 71.4, 69.61, 39.1, 39.0. 35.1, 22.09 ppm.

Peaks attributed to minor diastereomer: ^{1}H NMR (600 MHz, Acetonitrile-*d*3) δ 7.77 (d, overlapping with major isomer, 2H), 7.42 (d, overlapping, 2H), 5.73-5.66 (m, 1H), 5.48 (ddd, 16.0, 10.3, 5.0 Hz, 1H), 4.09-4.07 (m, 2H), 3.84-3.65 (m, 3H), 3.62-3.58 (m, 2H), 3.57-3.52 (m, 2H), 3.52-3.46 (m, 6H), 3.32-3.16 (m, 2H), 2.43 (s, 3H), 2.30-2.09 (m, 4H), 2.01-1.94 (m, 1H), 1.89-1.79 (m, 1H) ppm; ^{13}C NMR (151 MHz, Acetonitrile-*d*3, not all peaks accounted for due to overlap with major isomer) δ 146.7, 142.1, 139.6, 132.2, 131.4, 129.2, 80.1, 73.3, 71.46, 71.45, 71.32, 71.30, 69.63, 41.4, 36.4, 30.2, 22.07 ppm.

HRMS (ESI) *m/z* : [M+H]+, Calcd for $C_{21}H_{33}O_7S$ is 429.1942; found 429.1931

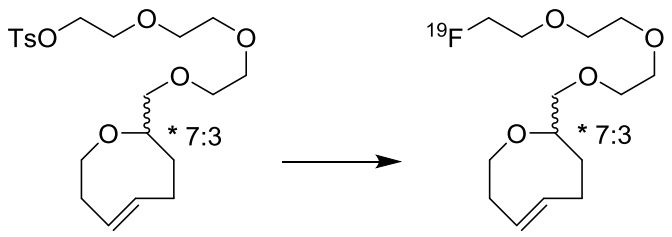
(2*s/r*,3*aR*,9*aS,E*)-2-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)-3a,4,5,8,9,9a-hexahydrocycloocta[d][1,3]dioxole (3)



S2 **(3)**

Tetrabutylammonium fluoride (TBAF, 1M in THF) was added to a sample vial containing **S2** (15 mg, 0.032 mmol) at rt. After 3 h, the reaction mixture was diluted with ethyl acetate and all the solvents were evaporated. To the resulting residue was added ethyl acetate and sat. NH_4Cl. The organic layer was separated and washed with water, dried with Na_2SO_4, filtered and purified by silica gel chromatography by using 0-100% ethyl acetate in hexane as an eluent to give the title compound **(3)** (9.0 mg, 89%) as clear oil. This compound was stored as a solution in acetonitrile at –20 °C.

^{1}H NMR (600 MHz, Acetonitrile-*d*3) δ 5.68-5.60 (m, 1H), 5.60-5.51 (m, 1H), 4.79 (t, J= 4.2 Hz, 1H), 4.51 (dm, J_{HF} = 48.0 Hz, 2H), 3.96-3.92 (m, 1H), 3.92-3.87 (m, 1H), 3.66 (dm, J_{HF} = 31.0 Hz, 2H), 3.61-3.53 (m, 8H), 3.48-3.40 (m, 2H), 2.38-2.31 (m, 1H), 2.24-2.18 (m, 1H), 2.18-2.08 (m, 2H), 1.91-1.82 (m, 1H), 1.75-1.60 (m, 2H), 1.56-1.46 (m, 1H) ppm. ^{13}C NMR (151 MHz, Acetonitrile-*d*3) δ 137.5, 132.8, 101.5, 84.71 (d, J_{CF} = 165.8 Hz), 83.6, 81.7, 73.7, 72.2, 71.7, 71.57, 71.55, 71.4 (d, J_{CF} = 19.1 Hz), 39.9, 35.1, 32.3, 26.7 ppm. HRMS (ESI) *m/z* : [M+H]+, Calcd for $C_{16}H_{28}O_5F$ 319.1915; found 319.1915

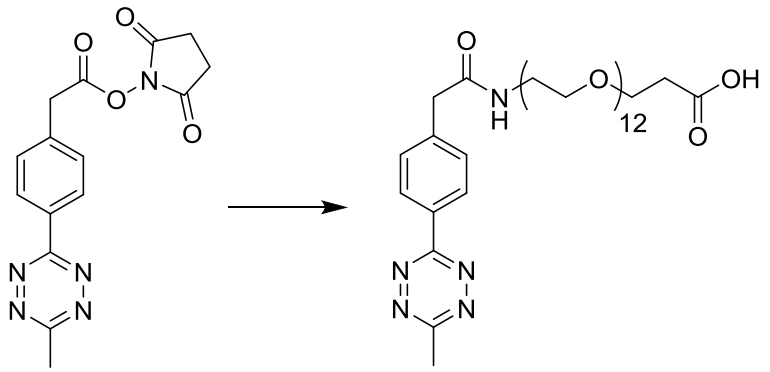


(5) **(6)**

(E)-2-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)-3,4,7,8-tetrahydro-2H-oxocine (6)

Tetrabutylammonium fluoride (TBAF, 1M in THF) was added to a sample vial containing **(5)** (15 mg, 0.032 mmol) at rt. After 3 h, the reaction mixture was diluted with ethyl acetate and the solvents were evaporated. To the resulting residue was added ethyl acetate and sat. NH_4Cl. The organic layer was separated and washed with water, dried with Na_2SO_4, filtered and purified by silica gel chromatography by using 0-100% ethyl acetate in hexane as an eluent to give the title compound **(6)** (8.0 mg, 91%) as clear oil. This compound was stored as a solution in acetonitrile at –20 °C.

Major diastereomer: ^{1}H NMR (600 MHz, Acetonitrile-*d*3) δ 5.67 (ddd, J = 15.3, 11.4, 3.3 Hz, 1H), 5.41 (ddd, J = 15.5, 10.9, 3.9 Hz, 1H), 4.51 (dm, J_{HF} = 48.0 Hz, 2H), 3.93 (dd, J = 11.8, 6.1 Hz, 1H), 3.70-3.68 (m, 1H), 3.65-3.63 (m, 1H), 3.60-3.50 (m, 8H), 3.33-3.18 (m, 3H), 3.07 (dt, J=10.0, 6.2, Hz, 1H) 2.44-2.17 (m, 3H), 1.91-1.85 (m, 1H), 1.67-1.57 (m, 1H), 1.41-1.28 (m, 1H) ppm. Alkene peaks attributable to the minor diastereomer were observed at δ 5.75-5.70 (m, 1H) and 5.50 (ddd, J = 15.7, 10.1, 5.0 Hz, 1H) ppm. ^{13}C NMR (151 MHz, Acetonitrile-*d*3) δ 142.1, 128.1, 85.2, 84.7 (d, J_{CF} = 165.9 Hz), 75.9, 74.6, 71.8, 71.7, 71.59, 71.57, 71.4 (d, J_{CF} = 19.2 Hz), 39.1, 39.0, 35.1 ppm. HRMS (ESI) *m/z* : [M+H]+, Calcd for $C_{14}H_{26}O_4F$ is 277.1810; found 277.1805

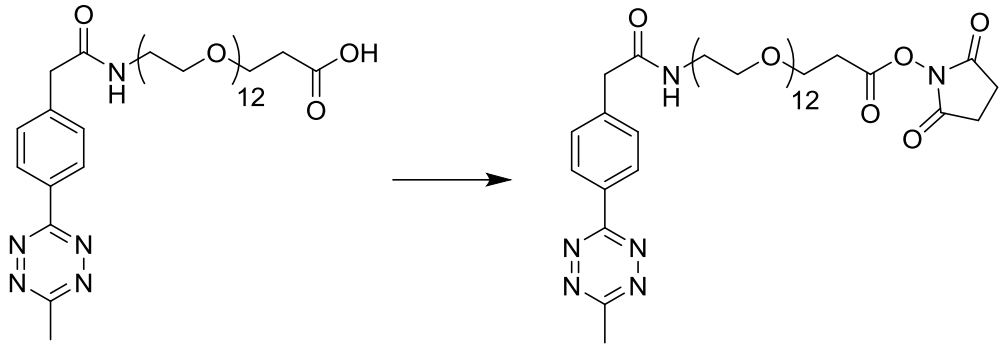


S3 **S4**

1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-2-oxo-6,9,12,15,18,21,24,27,30,33,36,39-dodecaoxa-3-azadotetracontan-42-oic acid (S4)

To a solution of 2,5-dioxopyrrolidin-1-yl 2-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)acetate **S3** (32 mg, 0.096 mmol) and 1-amino-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid (NH_2-PEG_{12}-COOH, 60 mg, 0.096) in DMF/DCM (4 mL/2 mL) was added diisopropylethylamine (34 µL, 0.18 mmol). After 14 h, the solvents were evaporated and the residue was re-dissolved in CH_2Cl_2 and 1N HCl. The organics were separated, dried over $MgSO_4$, and concentrated. The crude material was purified on deactivated silica with 0-5% methanol in dichloromethane to afford the title compound **S4** (72 mg, 90%) as pink solid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.55 – 8.50 (m, 2H), 7.54 – 7.49 (m, 2H), 6.72-6.60 (m, 1H), 3.75 (t, *J* = 6.3 Hz, 2H), 3.69 – 3.50 (m, 49H), 3.48 – 3.41 (m, 2H), 3.08 (s, 3H), 2.58 (t, *J* = 6.3 Hz, 2H) ppm. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 173.9, 170.6, 167.4, 164.1, 140.4, 130.7, 130.4, 128.4, 70.8, 70.74, 70.73, 70.7-70.6 (18 carbons), 70.5, 70.3, 69.9, 43.6, 39.7, 35.1, 21.3 ppm. HRMS (ESI) *m/z*: [M+H]+, Calcd for $C_{38}H_{64}O_{15}N_5$ is 830.4393; found 830.4376.

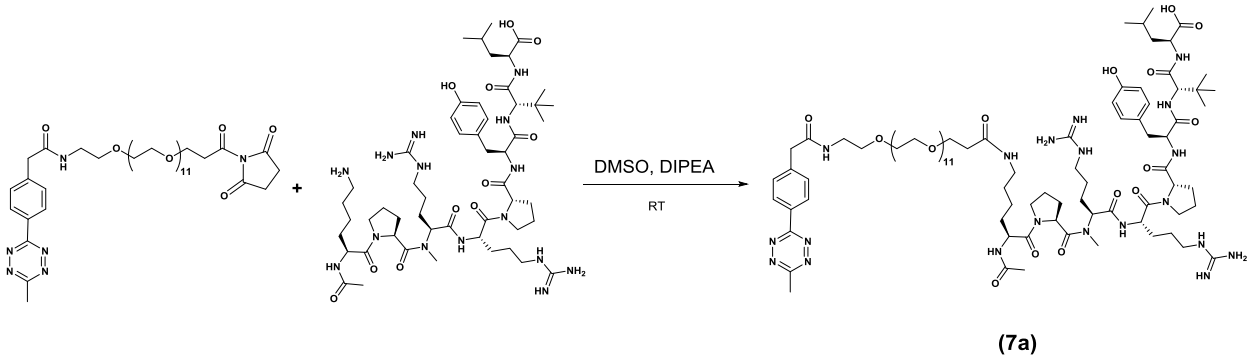


S4 **S5**

2,5-dioxopyrrolidin-1-yl 1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-2-oxo-6,9,12,15,18,21,24,27,30,33,36,39-dodecaoxa-3-azadotetracontan-42-oate

N-Hydroxysuccinimide (9.3 mg, 0.082 mmol) and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (15 mg, 0.078 mmol) were added to a flask containing a solution of **S4** (36 mg, 0.043 mmol) in CH_2Cl_2 (2 mL). After stirring for 24 h, the resulting solution was directly purified using deactivated silica with 0-5% methanol in dichloromethane to afford the title compound **S5** (35 mg, 87%) as a pink oil.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.54 (d, *J* = 7.9 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H), 6.59 (s, 1H), 3.84 (t, *J* = 6.4 Hz, 2H), 3.75 – 3.40 (m, 50H), 3.09 (s, 3H), 2.90 (t, *J* = 6.4 Hz, 2H), 2.87-2.76 (m, 4H) ppm. ^{13}C NMR APT (101 MHz, Chloroform-*d*) δ 170.4, 169.2, 167.4, 166.9, 164.1, 130.7, 130.4, 128.4, 70.9, 70.8, 70.7-70.6 (19 carbons), 70.4, 70.3, 43.6, 39.7, 32.3, 25.8, 21.4 ppm. HRMS (ESI) *m/z* : [M+H]+, Calcd for $C_{42}H_{67}O_{17}N_6$ is 927.4557; found 927.4540



Methylphenyl tetrazine-neurotensin (7a)

Neurotensin peptide Lys-NT20.3 (2.0 mg, 1.8 µmol) was dissolved in anhydrous DMSO (20 µL) followed by the addition of **S5** (2.5 mg, 2.7 µmol) dissolved in 20 µL DMSO. Diisopropylethylamine (5 µL) was added and the reaction was stirred at rt for 2 h. The crude mixture was purified by RP HPLC to afford methylphenyl tetrazine-neurotensin **(7a)** (3.4mg)

HRMS (ESI) *m/z* : [M+2H]2+, Calcd for $C_{90}H_{150}O_{25}N_{20}$ is 955.5535; found 955.5523

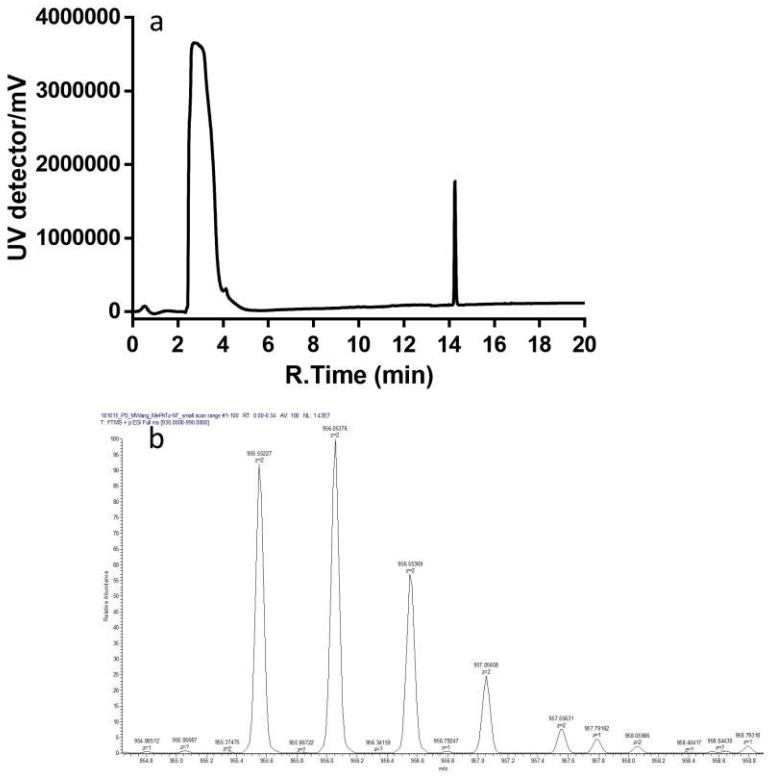
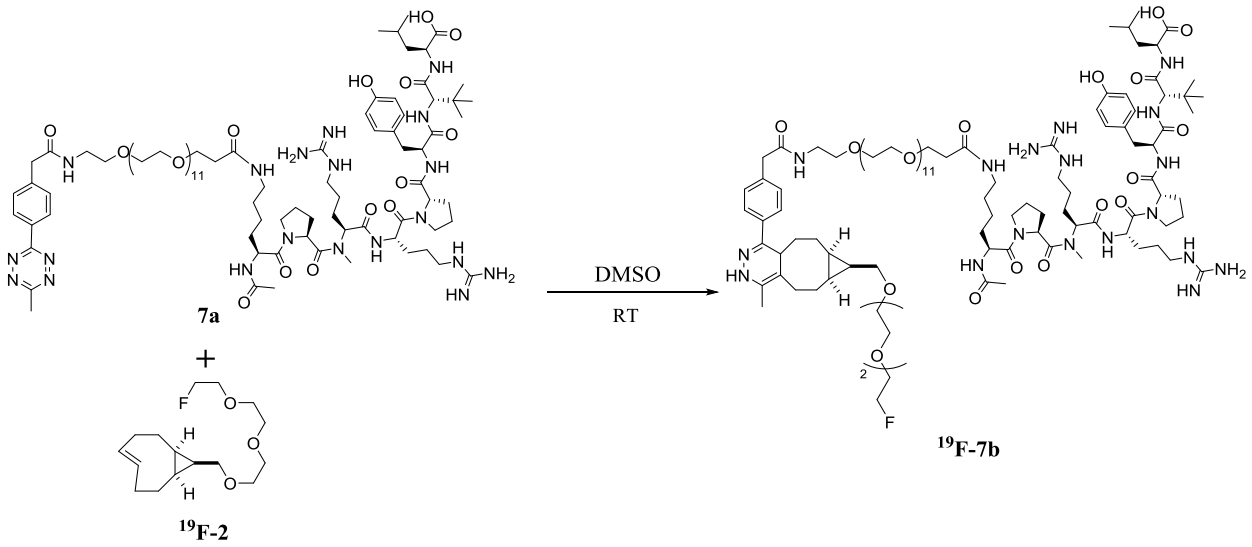


Fig S1 (a) HPLC profile and (b) HRMS of purified **7a**



sTCO-^{19}F labeled-neurotensin (7b)

Tetrazine-neurotensin conjugate **7a** (1 µL of a 10 mM solution in DMSO) was mixed with sTCO-^{19}F **(2)** (1 µL of a 24.5 mM solution in acetonitrile). The mixture was incubated at room temperature for 1 min and the crude residue was purified by reverse phase HPLC to provide the title compound.

HRMS (ESI) *m/z* : [M+3H]3+, Calcd for $C_{106}H_{178}FO_{28}N_{18}$ is 723.4342; found 723.4322

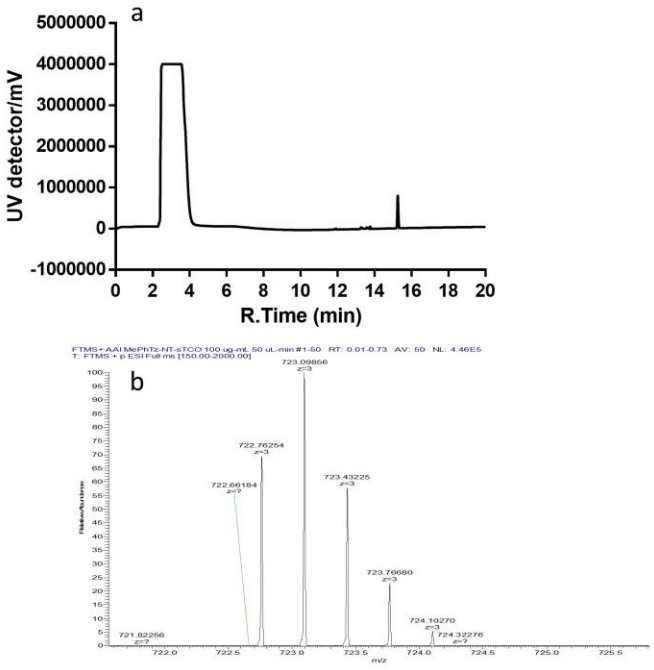
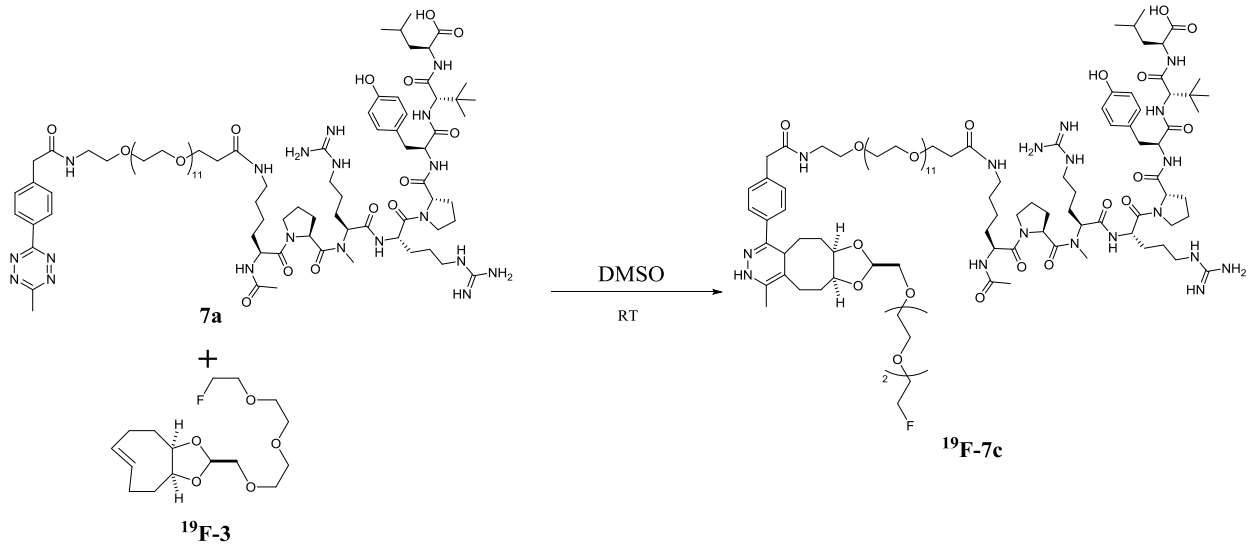


Fig S2 (a) HPLC profile and (b) HRMS of purified **^{19}F-7b**



dTCO-^{19}F labeled-neurotensin (7c)

Tetrazine-neurotensin conjugate **7a** (1 µL of a 10 mM solution in DMSO) was mixed with dTCO-^{19}F **(3)** (1 µL of a 22 mM solution in acetonitrile). The mixture was incubated at room temperature for 1 min and the crude residue was purified by reverse phase HPLC to provide the title compound.

HRMS (ESI) *m/z* : [M+3H]3+, Calcd for $C_{106}H_{178}FO_{30}N_{18}$ is 734.0975; found 734.0941

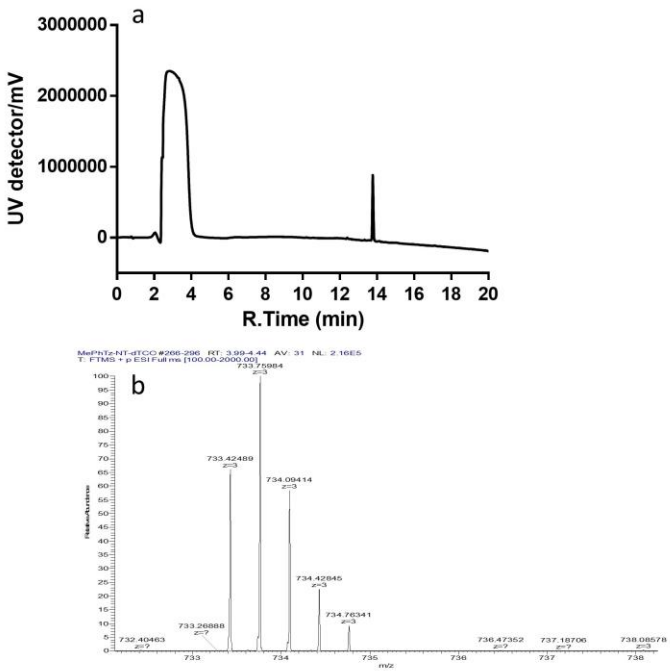
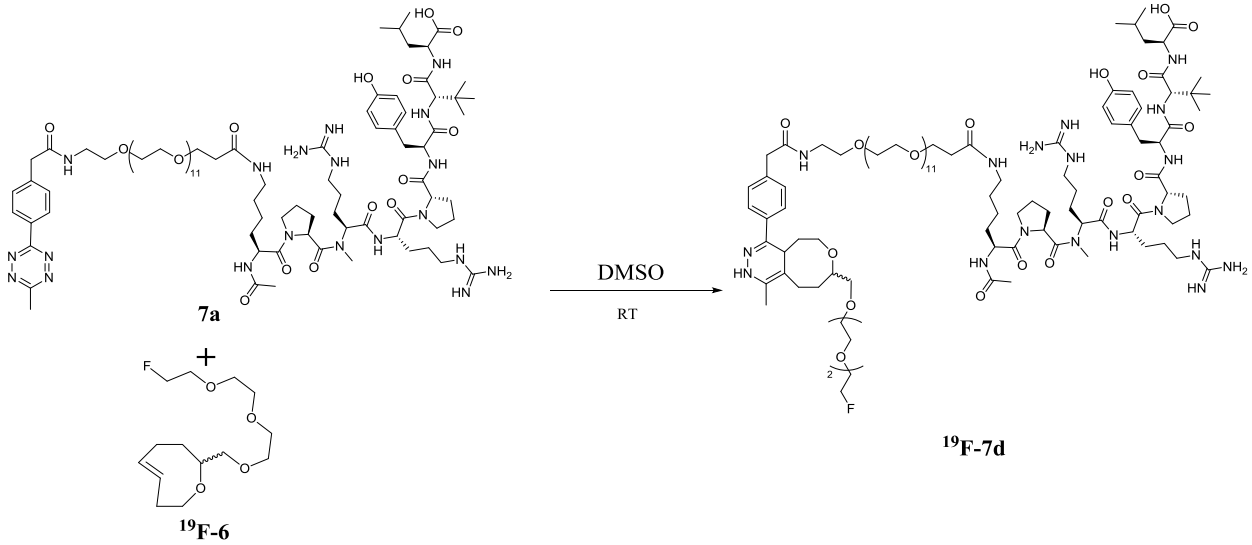


Fig S3 (a) HPLC profile and (b) HRMS of purified **^{19}F-7c**



oxoTCO-^{19}F labeled-neurotensin (7d)

Tetrazine-neurotensin conjugate **7a** (1 µL of a 10 mM solution in DMSO) was mixed with 1 µL oxoTCO-^{19}F **(6)** (25.3 mM solution in acetonitrile). The mixture was incubated at room temperature for 1 min and the crude residue was purified by reverse phase HPLC to provide the title compound.

HRMS (ESI) *m/z* : [M+3H]3+, Calcd for $C_{104}H_{176}FO_{29}N_{18}$ is 720.0939; found 720.0934

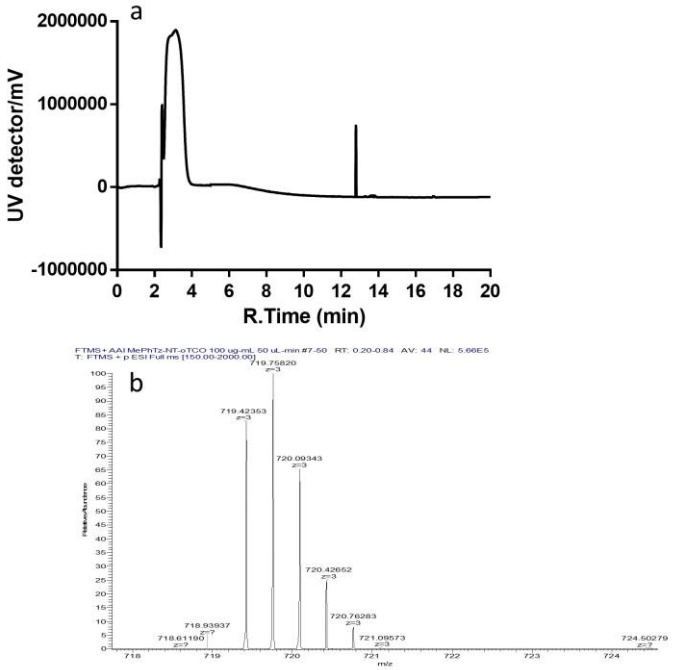


Fig S3 (a) HPLC profile and (b) HRMS of purified **^{19}F-7d**

S3. Radiochemistry

The radiolabeling reactions were performed based on the following protocol. sTCO-tosylate [6], dTCO-tosylate **S2** or oxoTCO-tosylate **(5)** (2.0 mg) was dissolved in anhydrous acetonitrile (20 µL) followed by the addition of ^{18}F-TBAF (200mCi). The mixture was heated at 85 ^{o}C for 10 min then quenched with 800 µL of water. The crude material was passed through a Sep-Pak light alumina cartridge before HPLC purification to yield ^{18}F-labeled sTCO **(**^{18}F-**2)**, dTCO **(**^{18}F-**3)** or oxoTCO **(**^{18}F-**6)**, which was then was mixed with 10 nmol of **(7a)**. After shaking for 10 seconds at room temperature, the crude residue was purified by reverse phase HPLC. The HPLC eluent containing ^{18}F-**7b**, ^{18}F-**7c** or ^{18}F-**7d** was collected and the organic solvent was removed by rotary evaporation. After adjusting the pH to 7, ^{18}F-**7b**, ^{18}F-**7c** or ^{18}F-**7d** was subjected to partition coefficient measurement and small animal studies.

In vitro stability

^{18}F-**6** was incubated in 1 x PBS buffer at 37^{o}C. After 1h, an aliquot of the solution (~20 µCi) was loaded on reverse phase HPLC for analysis.

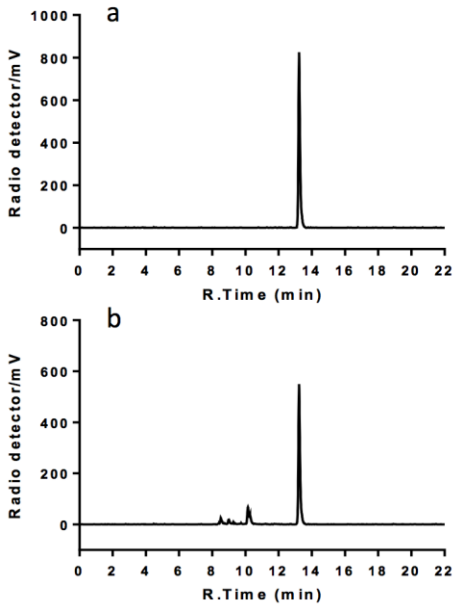


Fig S4 Radio-HPLC profile of (a) freshly prepared ^{18}F-**6**; (b) ^{18}F-**6** in PBS for 1h

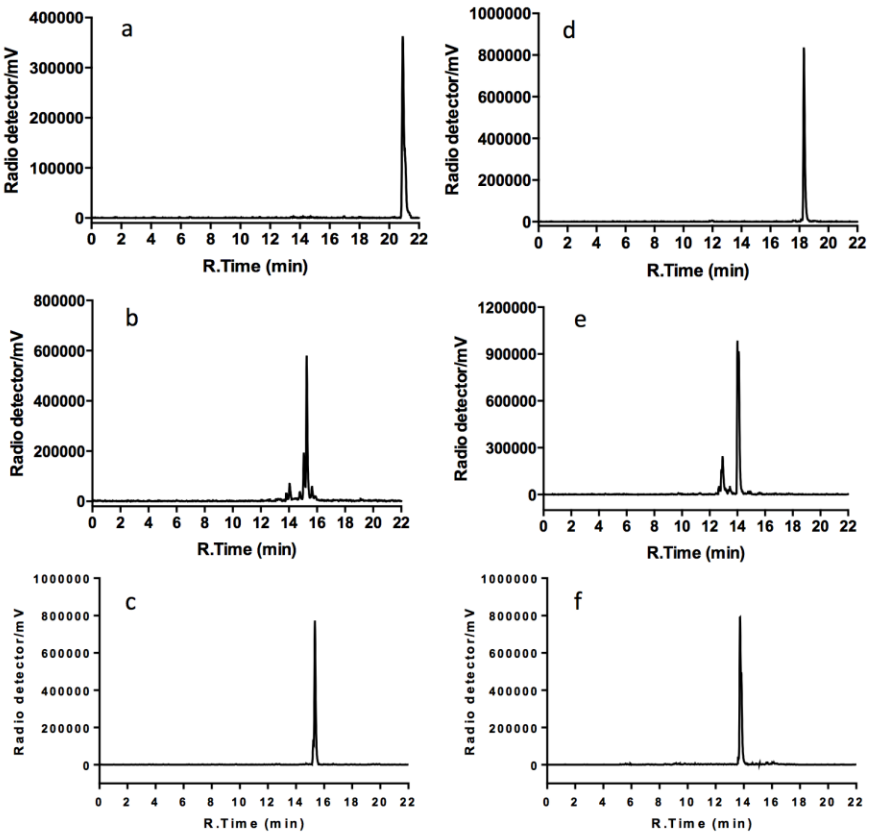


Fig S5 Radio-HPLC profile of (a) freshly prepared ^{18}F-**2**; (b) crude reactions of ^{18}F-**2** and **7a**; (c) freshly prepared ^{18}F-**7b**; (d) freshly prepared ^{18}F-**3**; (e) crude reactions of ^{18}F-**3** and **7a**; (f) freshly prepared ^{18}F-**7c**

Octanol-Water partition coefficient

All the ^{18}F labeled TCO tracers and their derived NT-probes were diluted in 500 µL PBS and 500 µL of octanol. After vigorous mixing for 10 min, the mixture was centrifuged (5min, 5000 rpm) to separate the aqueous and organic phases. The γ counts in aliquots of the organic and aqueous phases were measured by γ-counter (Perkin Elmer).

In vitro cell binding assay

HT29 cells were harvested and resuspended in chemokine binding buffer consisting of DMEM, 0.2% BSA, and 0.8 mM 1,10-phenanthroline. Cells were then placed in a 24-well plate (Corning, Tewksbury, MA) to have 2×10^5 cells per well. ^{125}I-NT (0.036 µCi/well, Perkin Elmer) and different concentrations of ^{19}F-**7b**, ^{19}F-**7c**, ^{19}F-**7d** and NT peptide, ranging from 1 pM to 10 µM were added to the wells. After 2 h of

incubation with gentle shaking at room temperature, the plate was washed three times with washing buffer (DMEM, 0.2% BSA). Cells in each well were lysed with 0.1 N NaOH and collected to measure with γ-counter (Perkin Elmer). IC_{50} values were calculated by GraphPad Prism software (GraphPad Software, CA).

Small animal PET imaging

Animals procedures were performed according to protocol approved by the UNC Institutional Animal Care and Use Committee. PC-3 tumor bearing mice were injected with 3.7 MBq (~100 μCi) of ^{18}F-**7b**, ^{18}F-**7c** or ^{18}F-**7d** via the tail vein. At 0.5 and 3.5 h post injection, static emission scans were acquired for 10min using a small animal PET scanner (GE eXplore Vista). For the blocking study, an excess amount, 100 μg, of NT peptide was co-injected with the ^{18}F-**7d** and PET images were acquired at 0.5 h post injection. The region of interests (ROIs) were converted to %ID/g based on the assumption of 1g/mL tissue density.

S4. NMR Data

See appended file

S5. References

[1] M. Royzen, G. P. A. Yap, J. M. Fox; J. Am. Chem. Soc. 2008, 130, 3760–3761 DOI: 10.1021/ja8001919

[2] P. Panne, J. M. Fox; J. Am. Chem. Soc. 2007, 129 (1), 22-23 DOI: 10.1021/ja0660195

[3] M. T. Taylor, B. L. Blackman, O. Dmitrenko, J. M. Fox; J. Am. Chem. Soc. 2011, 133 (25), 9646-9649 DOI: 10.1021/ja201844c

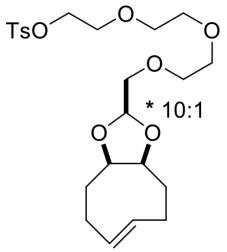
[4] A. Darko, S. Wallace, O. Dmitrenko, M. M. Machovina, R. A. Mehl, J. W. Chin, J. M. Fox; Chem. Sci. 2014, 5, 3770-3776 DOI: 10.1039/C4SC01348D

[5] W. D. Lambert, S. L. Scinto, O. Dmitrenko, S. J. Boyd, R. Magboo, R. A. Mehl, J. W. Chin, J. M. Fox, S. Wallace; Org. Biomol. Chem. 2017, 15, 6640-6644 DOI: 10.1039/C7OB01707C

[6] M. Wang, D. Svatunek, K. Rohlfing, Y. Liu, H. Wang, B. Giglio, H. Yuan, Z. Wu, Z. Li, J. Fox; Theranostics 2016, 6 (6), 887-895 DOI: 10.7150/thno.14742

2-(2-(2-(((2*s/r*,3*aR*,9*aS,E*)-3a,4,5,8,9,9a-hexahydrocycloocta[d][1,3]dioxol-2-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (S2)

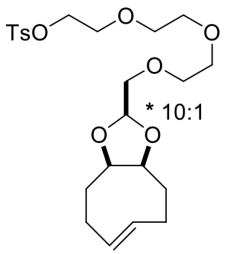
^{1}H NMR (400 MHz, Acetonitrile-*d*3)



S2

2-(2-(2-(((2*s/r*,3*aR*,9*aS,E*)-3a,4,5,8,9,9a-hexahydrocycloocta[d][1,3]dioxol-2-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (S2)

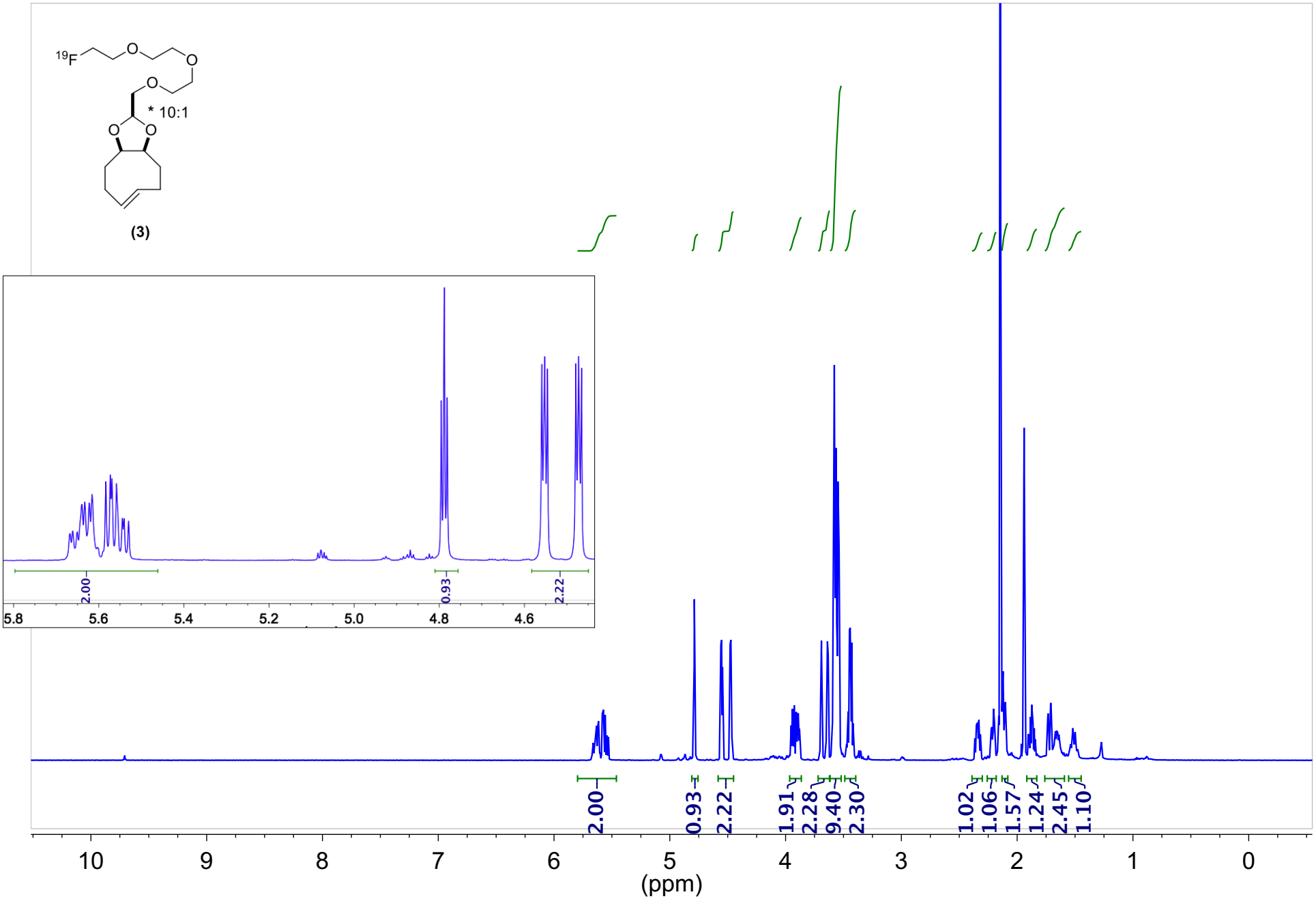
^{13}C NMR (101 MHz, Acetonitrile-*d*3)



S2

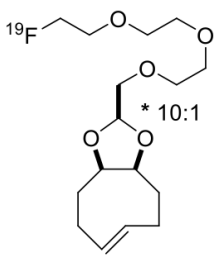
(2*s/r*,3*aR*,9*aS,E*)-2-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)-3a,4,5,8,9,9a-hexahydrocycloocta[d][1,3]dioxole (3)

^{1}H NMR (600 MHz, Acetonitrile-*d*3)



(2*s/r*,3*aR*,9*aS,E*)-2-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)-3a,4,5,8,9,9a-hexahydrocycloocta[d][1,3]dioxole (3)

^{13}C NMR (151 MHz, Acetonitrile-*d*3)



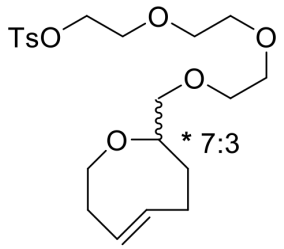
(3)

137.53
132.77
118.70
101.51
85.26
84.16
83.59
81.66
73.68
72.18
71.70
71.57
71.55
71.48
71.36
39.90
35.07
32.31
26.71
2.14
2.00
1.88
1.86 CD3CN
1.73 CD3CN
1.60
1.59 CD3CN
1.45
1.31

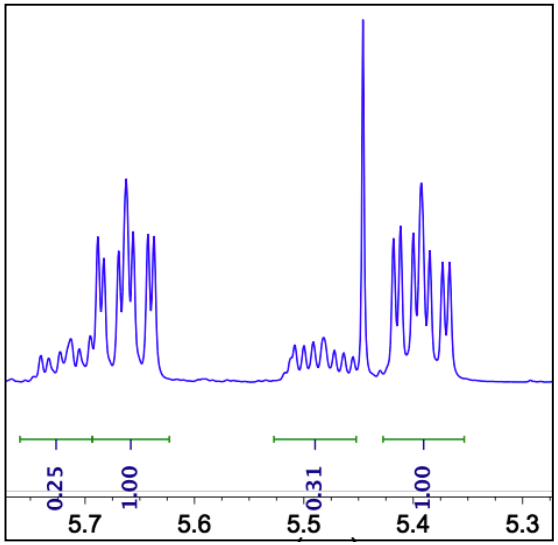
200 180 160 140 120 100 80 60 40 20 0
(ppm)

(E)-2-(2-(2-((3,4,7,8-tetrahydro-2H-oxocin-2-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5)

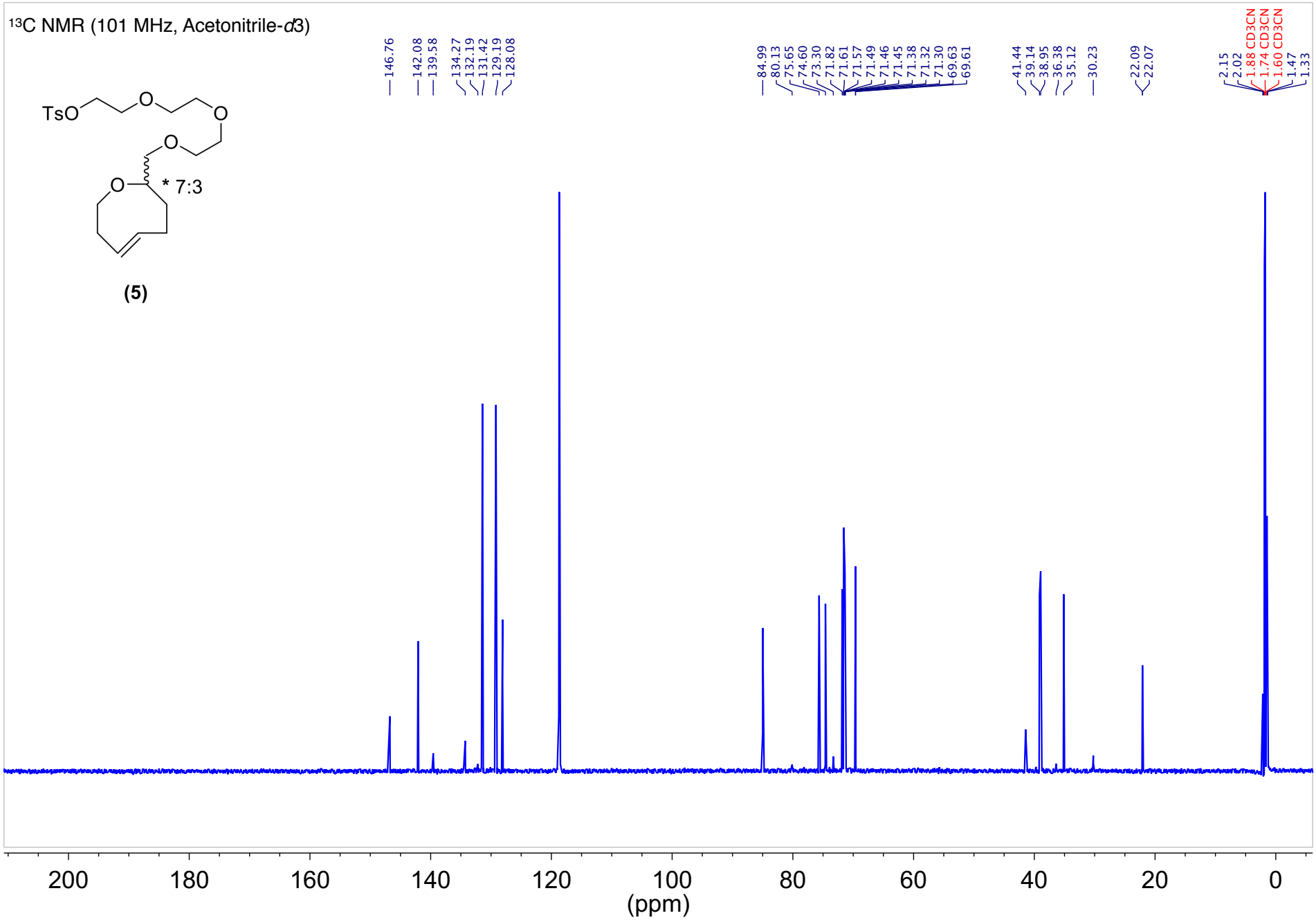
^{1}H NMR (400 MHz, Acetonitrile-*d*3)



(5)

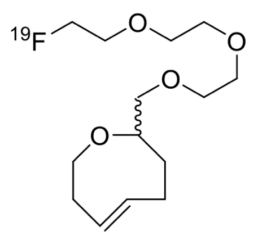


(E)-2-(2-(2-((3,4,7,8-tetrahydro-2H-oxocin-2-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5)



(E)-2-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)-3,4,7,8-tetrahydro-2H-oxocine (6)

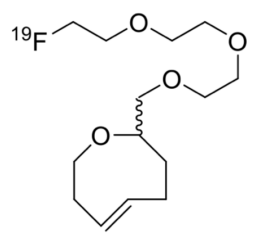
^{1}H NMR (600 MHz, Acetonitrile-*d*3)



(6)

(E)-2-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)-3,4,7,8-tetrahydro-2H-oxocine (6)

^{13}C NMR (101 MHz, Acetonitrile-*d*3)



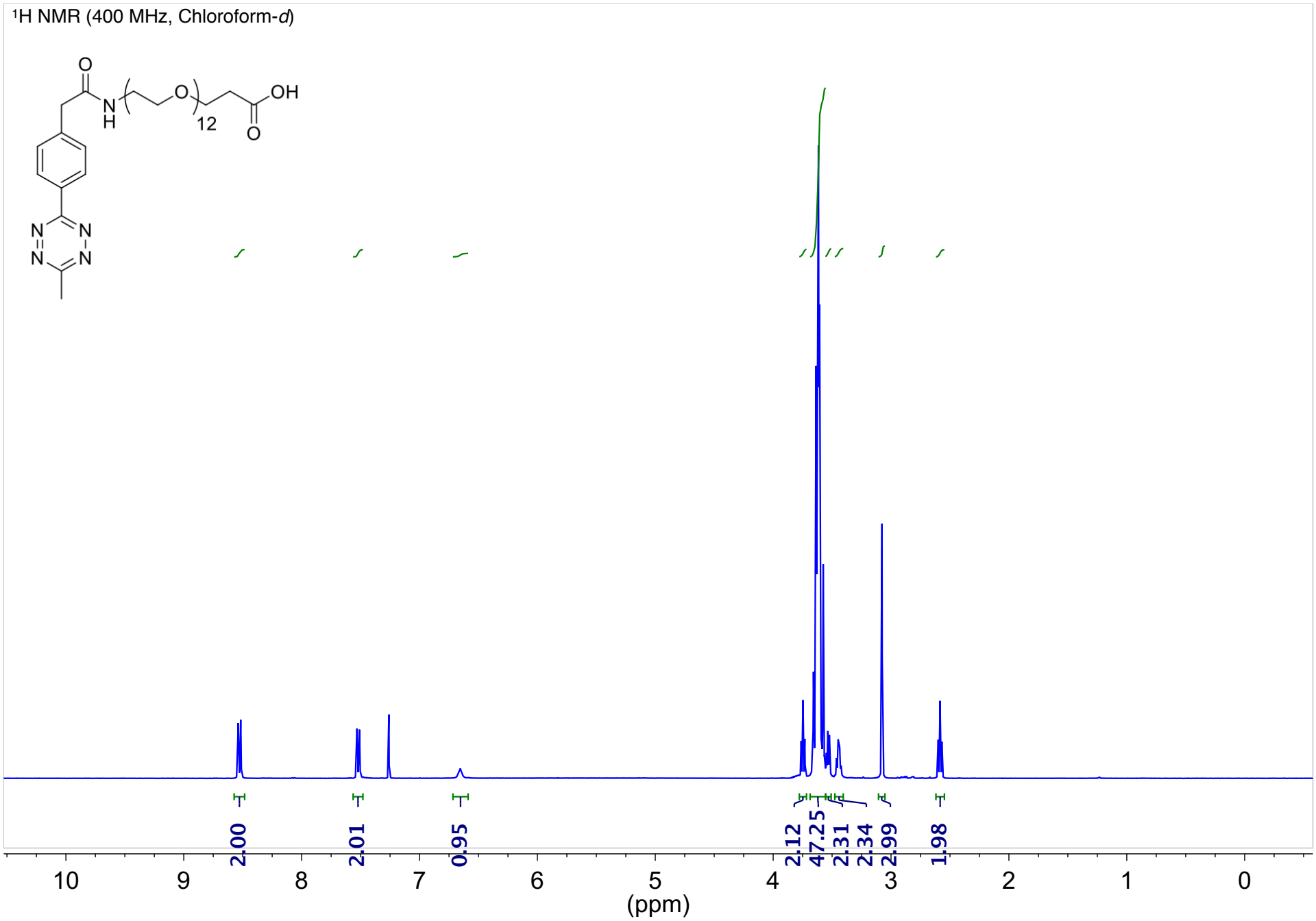
(6)

142.09
128.09
85.24
85.00
84.14
75.65
74.61
71.83
71.70
71.59
71.57
71.46
71.34
71.31
55.73
55.01
41.44
39.13
38.95
35.11
30.70
21.76
14.78
2.14
2.00 CD3CN
1.86 CD3CN
1.73 CD3CN
1.59
1.45
1.31

200 180 160 140 120 100 80 60 40 20 0
(ppm)

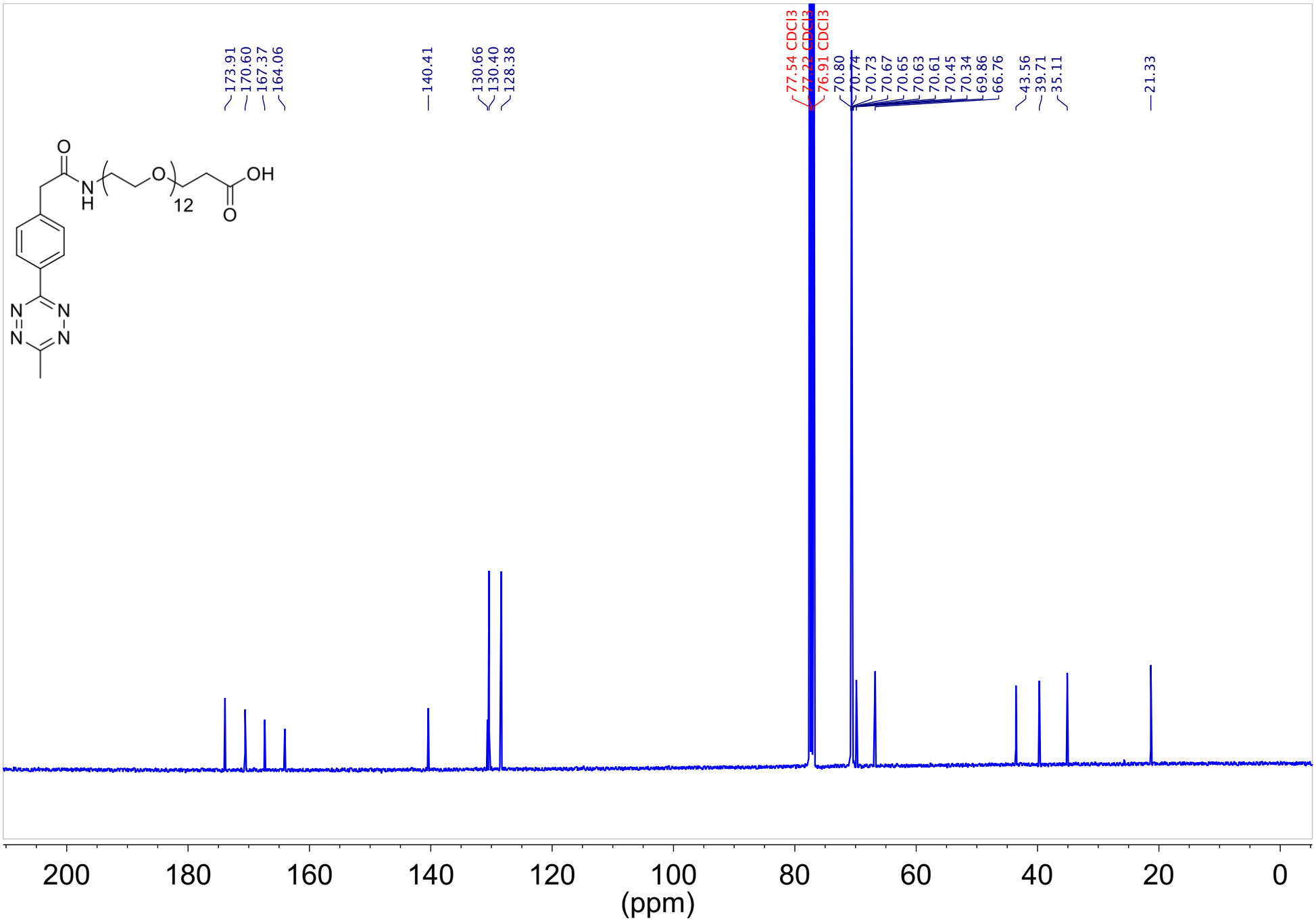
1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-2-oxo-6,9,12,15,18,21,24,27,30,33,36,39-dodecaoxa-3-azadotetracontan-42-oic acid

^{1}H NMR (400 MHz, Chloroform-*d*)

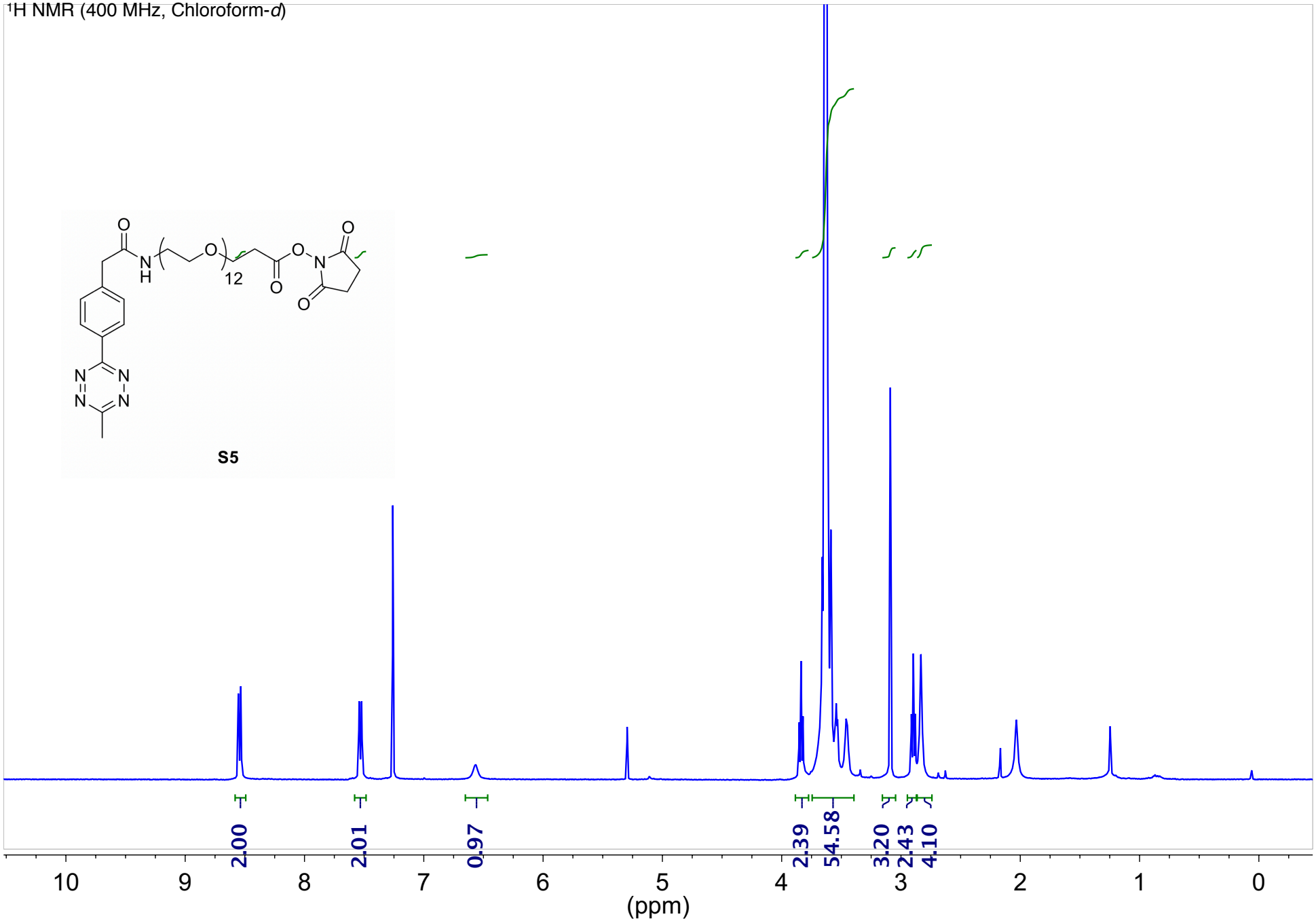


1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-2-oxo-6,9,12,15,18,21,24,27,30,33,36,39-dodecaoxa-3-azadotetracontan-42-oic acid

^{13}C NMR (101 MHz, Acetonitrile-*d*3)



2,5-dioxopyrrolidin-1-yl 1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-2-oxo-6,9,12,15,18,21,24,27,30,33,36,39-dodecaoxa-3-azadotetracontan-42-oate



2,5-dioxopyrrolidin-1-yl 1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-2-oxo-6,9,12,15,18,21,24,27,30,33,36,39-dodecaoxa-3-azadotetracontan-42-oate

^{13}C APT NMR (101 MHz, Acetonitrile-*d*3)