Conformationally Strained *trans*-Cyclooctene (sTCO) Enables the Rapid Construction of ^{18}F-PET Probes via Tetrazine Ligation Supplementary Information

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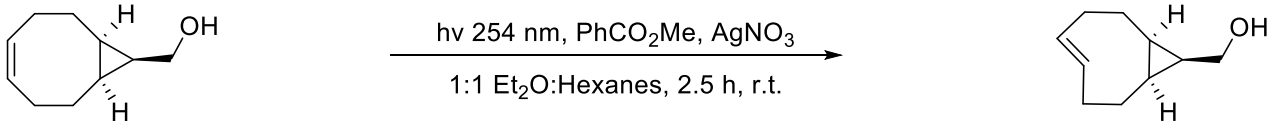
1. Synthetic Procedures

1.1. General Considerations: All reactions were carried out in glassware that was flame-dried under vacuum and cooled under nitrogen. All commercially available reagents and solvents were used as received. (rel-1R,8S,9S,4Z)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol and 4-nitrophenyl 4-(6-phenyl-1,2,4,5-tetrazin-3-yl)benzyl carbonate were prepared following known procedures.[1,2] Reactions were monitored by thin layer chromatography (TLC) performed on SiliCycle silica gel GF 250 μm plates and were visualized with ultraviolet (UV) light (254 nm) and/or $KMnO_4$ staining. Flash chromatography was performed using normal phase SiliCycle silica gel (40-63D, 60Å). Deactivated silica gel was prepared by treating silica gel with $EtSiCl_3$.[3] ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) chemical shifts are reported in ppm relative to $CHCl_3$, CH_2Cl_2 and MeOH (i.e. ^{1}H NMR δ = 7.26 and ^{13}C NMR = 77.0, ^{1}H NMR = 5.32 and ^{13}C NMR = 54.0, ^{1}H NMR = 3.31 and ^{13}C NMR = 49.1).

1.2. (rel-1R,8S,9S,4E)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol (5)

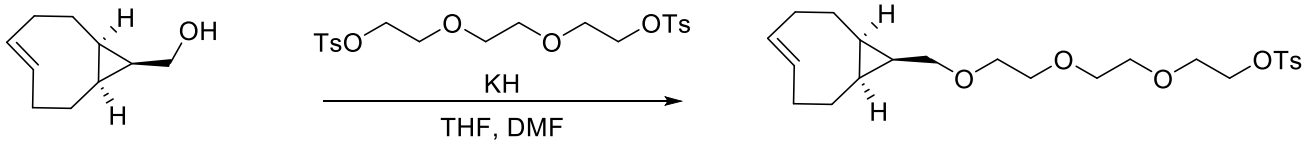


7 **5**

The flow photoisomerization procedure (ref 1) was followed using **7** (395 mg, 2.59 mmol) in 1:1 ether/hexanes (250 mL), methyl benzoate (705 mg, 5.18 mmol) and dodecane (491 mg, 2.88 mmol, standard for GC monitoring) in a 250 mL quartz tube. A 50 g Biotage® SNAP column was filled with normal silica gel (2.5 inches) and the remaining space was packed with 10% silver impregnated silica (5.70 g). The column was connected to a pump and flushed with 1:1 ether/hexanes (250 mL). Irradiation was carried out at 254 nm for 2.5 h at which GC monitoring

showed no more starting material. The column was flushed with 1:1 ether/hexanes (250 mL) and dried under air flow. The silica was placed into a flask and stirred in ammonium hydroxide (200 mL) and dichloromethane (200 mL) for 10 min. The silica was filtered and washed with additional ammonium hydroxide (100 mL) and dichloromethane (100 mL). The phases were separated and the aqueous layer was extracted an additional three times. The combined organic layers were washed twice with water, dried over Na_2SO_4, filtered and concentrated by rotary evaporation. Purification by column chromatography (25%, EtOAc : Hexanes) to yield 318 mg (2.09 mmol, 81%) of compound **5** as a colorless oil which was stored as a solution in MeOH at -15 °C. ^{1}H NMR (600 MHz, CD_3OD) δ: 5.88 (ddd, *J* = 16.2, 9.3, 6.2 Hz, 1H), 5.16 (dddd, *J* = 16.7, 10.6, 3.9, 1.1 Hz, 1H), 3.50 (d, *J* = 7.7 Hz, 2H), 2.31 (dtd, *J* = 11.4, 3.7, 2.4 Hz, 1H), 2.28 (ddd, *J* = 12.5, 8.4, 6.9 Hz, 1H), 2.21 – 2.15 (m, 1H), 2.13 – 2.09 (m, 1H), 1.96 – 1.86 (m, 2H), 1.20 (dt, *J* = 9.1, 7.7 Hz, 1H), 1.09 (tdd, *J* = 12.9, 11.2, 7.1 Hz, 1H), 0.85 – 0.71 (m, 2H), 0.60 (dtd, *J* = 13.0, 8.8, 4.6 Hz, 1H), (small peaks attributable to impurities were detected by ^{1}H NMR at 5.49, 4.09, 2.01, 1.29,1.24 and 0.90 ppm). ^{13}C NMR (151 MHz, CD_3OD) δ 139.4, 132.3, 59.5, 35.3, 34.8, 28.6, 28.3, 21.7, 20.2, 19.2; HRMS (EI) [M+H] *m/z*: calcd for $C_{10}H_{16}O$: 152.1201; found: 152.1181.

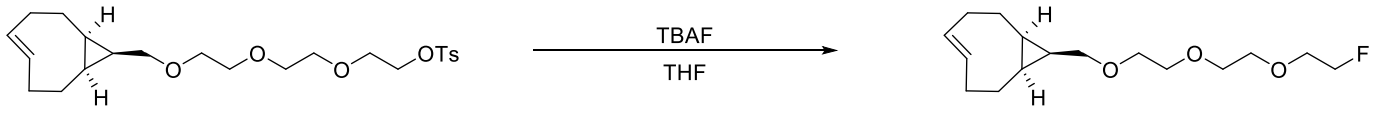
1.3. 2-(2-(2-((*syn*-(*E*)-bicyclo[6.1.0]non-4-en-9-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (8)



5 **8**

Triethylene glycol bis(*p*-toluenesulfonate) (972 mg, 2.12 mmol) was added into a flame-dried round bottom flask and dissolved in anhydrous THF (6.0 mL, 0.35M) and DMF (0.6 mL, 3.53M). **5** (100mg, 0.66 mmol) was added followed by potassium hydride (210 mg, 50% in paraffin, 2.63 mmol). The mixture was stirred at room temperature for 16 h after which saturated aqueous NH_4Cl solution was added followed by ether. The phases were separated and the aqueous layer was extracted an additional three times. The combined organic layers were dried over Na_2SO_4, filtered and concentrated by rotary evaporation. Purification by column chromatography (25 - 50%, EtOAc : Hexanes) yielded 85 mg (0.19 mmol, 30%) of desired compound **8** as a colorless oil which was stored as a solution in MeOH at -15 °C. ^{1}H NMR (600 MHz, CD_3OD) δ: 7.80 (d, *J* = 8.3 Hz, 2H) 7.45 (d, *J* = 8.4 Hz, 2H), 5.86 (ddd, *J* = 16.2, 9.3, 6.3 Hz, 1H), 5.16 (dddd, *J* = 16.8, 10.6, 3.9, 1.1 Hz, 1H), 4.18-4.12 (m, 2H), 3.68-3.34 (m, 2H), 3.60-3.52 (m, 8H), 3.46 – 3.41 (m, 2H), 2.46 (s, 3H), 2.34-2.27 (m, 1H), 2.26-2.19 (m, 1H), 2.19-2.11 (m, 1H), 2.11-2.04 (m, 1H), 1.98-1.84 (m, 2H), 1.30-1.19 (m, 1H), 1.14-1.01 (m, 1H), 0.87-0.69 (m, 2H) 0.65-0.55 (m, 1H), (small peaks attributable to impurities were detected by ^{1}H NMR at 4.63, 4.09, 2.01 and 1.24 ppm) ; ^{13}C APT NMR (100.6 MHz, CD_3OD) δ: 146.5, 139.4, 134.6, 132.4, 131.2, 129.2, 73.1, 71.7, 71.7, 71.6, 71.0, 69.9, 69.1, 35.5, 34.8, 28.8, 28.4, 21.7, 20.3, 19.3, 19.2, (a small peak attributable to dichloromethane was detected by ^{13}C at 54.9 ppm); HRMS (LIFDI-TOF) *m/z*: $[M]^+$ Calcd for $C_{23}H_{34}O_6S^+$ 438.2076; Found 438.2066.

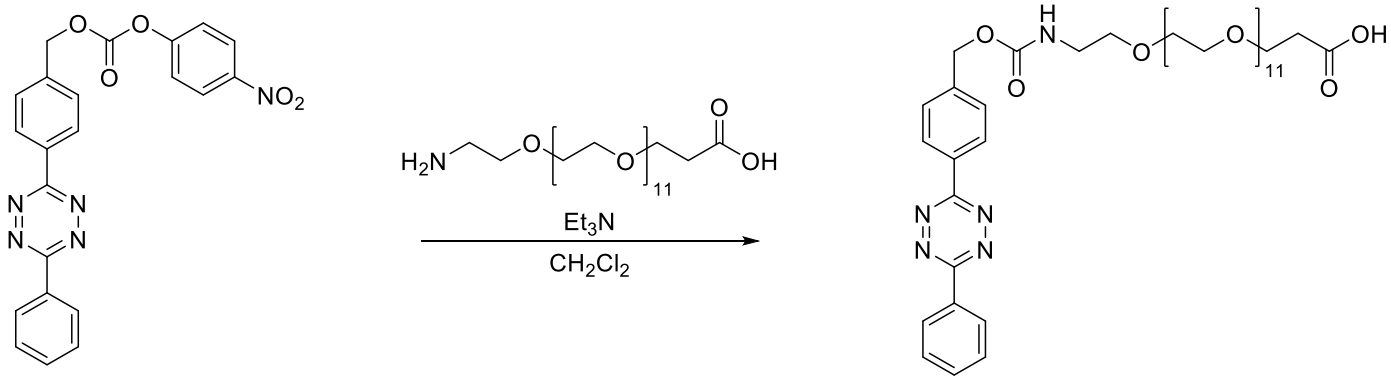
1.4. *syn*-(*E*)-9-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)bicycle[6.1.0]non-4-ene (9)



8 9

Tosylate **8** (10 mg, 0.02 mmol) was charged into a 4 dram vial and TBAF (0.5 mL, 1.0 M in THF) was added via syringe. The mixture was heated to 60 °C for 3.5 h and subsequently cooled to room temperature. The mixture was diluted with ethyl acetate, washed with saturated aqueous $NaHCO_3$ and dried over Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. Purification by column chromatography (25%, EtOAc : Hexanes) yielded 5 mg (0.02 mmol, 76%) of **9** as a colorless oil that was stored as a solution in MeOH at -15 °C. ^{1}H NMR (600 MHz, CD_3OD) δ: 5.87 (ddd, J = 16.2, 9.3, 6.2 Hz, 1H), 5.17 (ddd, J = 14.0, 10.6, 3.8 Hz, 1H), 4.52 (dt, J_{CF} = 48 Hz, J_{HH} = 4.1 Hz, 2H), 3.72 (dt, J_{CF} = 30.1 Hz, J_{HH} = 4.0 Hz, 2H), 3.68-3.59 (m, 6H), 3.59-3.54 (m, 2H), 3.44 (d, J = 7.5 Hz, 2H), 2.34-2.28 (m, 1H), 2.28-2.21 (m, 1H), 2.19-2.13 (m, 1H), 2.11-2.06 (m, 1H), 1.99-1/84 (m, 2H), 1.27-1.21 (m, 1H), 1.14-1.04 (m, 1H), 0.86-0.80 (m, 1H), 0.79-0.71 (m, 1H), 0.65-0.57 (m, 1H), (small peaks attributable to the cis isomer (5.61 ppm) and an impurity (1.29, 0.90 ppm) were also detected by ^{1}H NMR); ^{13}C APT NMR (100.6 MHz, MeOD) δ: 139.4, 132.4, 84.2 (d, JCF = 168 Hz), 71.82, 71.76, 71.74 (d, JCF = 20 Hz), 71.65, 71.0, 69.1, 35.5, 34.8, 28.8, 28.4, 20.3, 19.4, 19.3; ^{19}F NMR (376 MHz, CD_3OD) δ: -224.7 (tt, J = 48.1, 30.0 Hz); HRMS (Orbitrap) *m/z*: $[M + Na]^+$ Calcd for $C_{16}H_{27}FO_3Na$ 309.18364; Found 309.18453.

1.5. 3-oxo-1-(4-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)-2,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxa-4-azatritetracontan-43-oic acid (11)

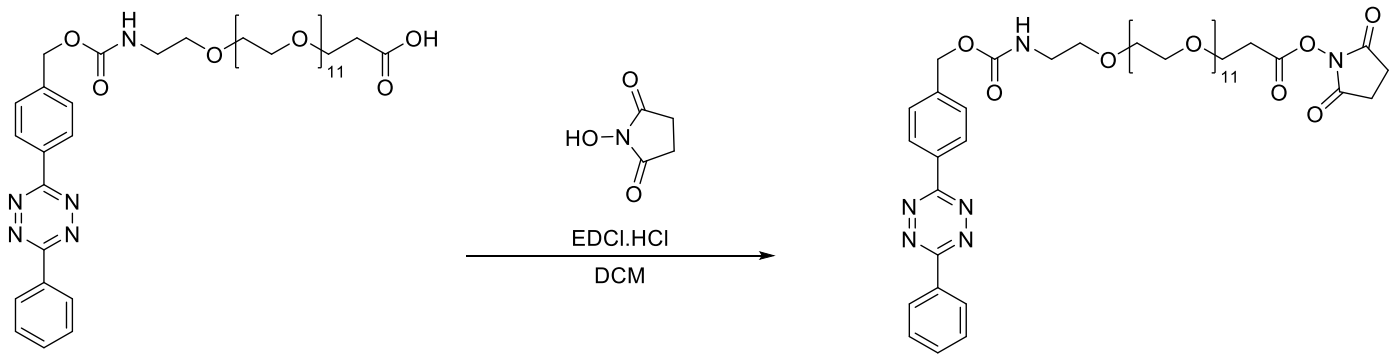


10 11

4-nitrophenyl 4-(6-phenyl-1,2,4,5-tetrazin-3-yl)benzyl carbonate (**10**) (43 mg, 0.10 mmol) and PEG12-Amino acid (31 mg, 0.05 mmol) were dissolved in anhydrous dichloromethane (4.0 mL, 0.01 M). Triethylamine (13.8 µL) was added and the reaction was stirred at room temperature for 30 h. 1N HCl (5 mL) was added and the aqueous phase was extracted with dichloromethane (3x). The combined organics were dried over Na_2SO_4, filtered and concentrated by rotary evaporation.

The crude was purified by column chromatography using deactivated silica gel (2.50 g, 0 - 5% MeOH : DCM) to yield 40 mg (0.04 mmol, 88%) of **11** as a purple solid. mp: 39 - 40 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.67-8.61 (m, 4H), 7.70-7.59 (m, 5H), 5.58 (t, J = 5.8 Hz, 1H), 5.22(m, 2H), 3.73 (t, J = 5.8 Hz, 2H), 3.66-3.54 (m, 48H), 3.39 (q, 5.4 Hz, 2H), 2.60 (bs, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3, 164.1, 163.8, 156.5, 141.8, 132.9, 131.8, 131.4, 129.5, 128.6, 128.2, 128.1, 70.8, 70.7 – 70.5 (19 C's), 70.4, 70.3, 70.1, 66.7, 66.0, 41.1, 35.1 (a peak attributed to CH_2Cl_2 was observed at 54 ppm); HRMS (LIFDI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{43}H_{65}N_5O_{16}Na$ 930.4324; Found 930.4336.

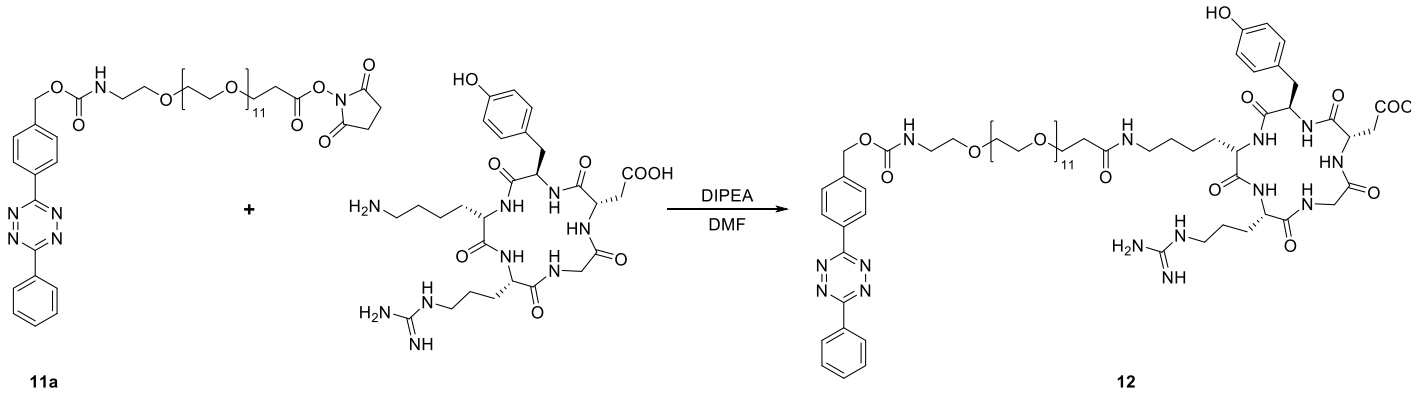
1.6. 2,5-dioxopyrrolidin-1-yl 3-oxo-1-(4-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)-2,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxa-4-azatritetracontan-43-oate (11a)



11 **11a**

Acid **11** (24 mg, 0.0264 mmol), N-hydroxysuccinimide (NHS) (5.0 mg, 0.0434 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCl) (8.0 mg, 0.0417 mmol) were added to a flame-dried round bottom flask. The mixture was dissolved in anhydrous dichloromethane (2.0 mL, 0.02 M) and stirred at room temperature for 16 h. The solution was directly applied to a column of deactivated silica gel (2.50 g) and washed with large amounts of dichloromethane after which product was eluted with 5% MeOH : DCM. Further purification using HILIC (2.50 g silica gel, 5% H_2O : MeOH) yielded 19 mg (0.02 mmol, 72%) of **11a** as a purple solid. mp: 37 - 39 °C; ^{1}H NMR (400 MHz, CD_2Cl_2) δ: 8.67-8.60 (m, 4H), 7.70-7.59 (m, 5H), 5.54 (bs, 1H, NH), 5.22 (s, 2H), 3.83 (t, J = 6.3 Hz, 2H), 3.67-3.52 (m, 48H), 3.39 (m, 2H), 2.88 (t, J = 6.3 Hz, 2H), 2.84-2.76 (bs, 4H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ: 169.5, 167.3, 164.4, 164.2, 156.5, 142.5, 133.0, 132.3, 131.8, 129.7, 128.7, 128.3, 128.2, 71.0, 70.9 – 70.7 (20 C's), 70.3, 66.0, 41.4, 32.5, 26.0; HRMS (LIFDI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{47}H_{68}N_6O_{18}Na$ 1027.4488; Found 1027.4487, : $[M + K]^+$ Calcd for $C_{47}H_{68}N_6O_{18}K$ 1043.4227, Found 1043.4200.

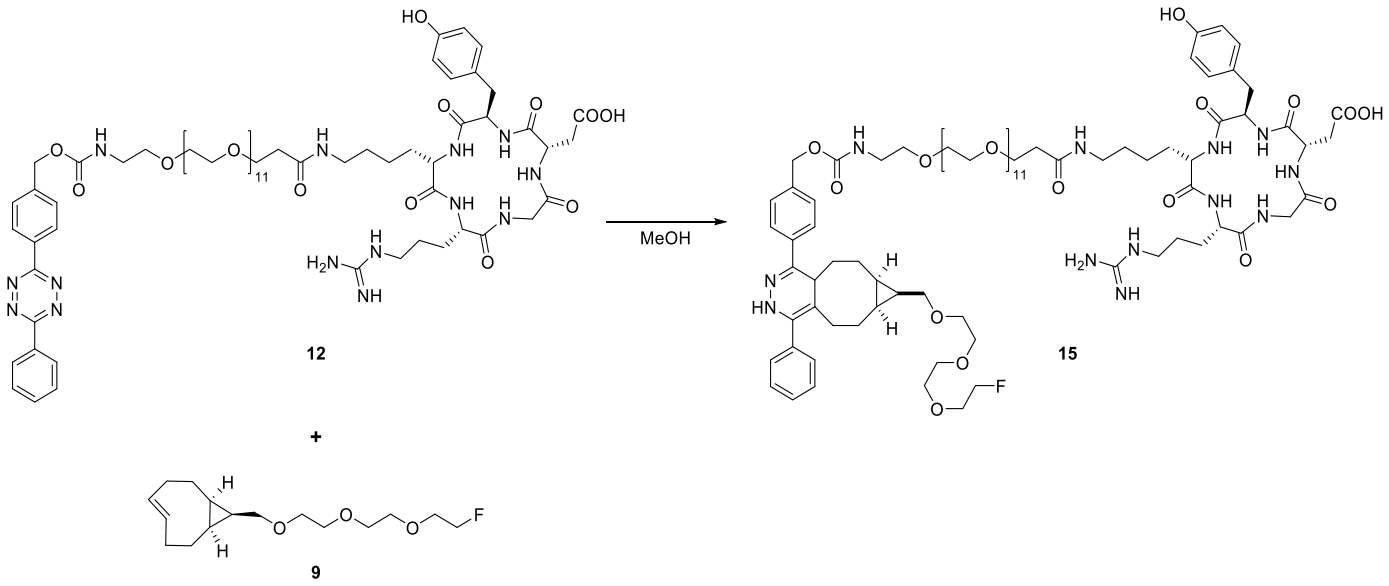
1.7 RGDyK-Tz (12)



11a 12

RGDyK (3.0 mg, 0.0048 mmol) was added to a 4 dram vial followed by TzPEG12NHS (**11a**) (10 mg, 0.0099 mmol) as a solution in anhydrous dimethylformamide (400 µL, 0.01 M). *N,N*-diisopropylethylamine (3.0 mg, 0.02 mmol) was added as a solution in anhydrous dimethylformamide (100 µL, 0.20 M) and the reaction was allowed to stir at room temperature for 18 h. H_2O was added and the solvents were removed via freeze drying. The residue was purified by RP HPLC to yield 7.2 mg (**12**) (0.005 mmol, 99 %) as a pink solid. HRMS (LIFDI-TOF) *m/z* $[M + Na]^+$ Calcd for $C_{70}H_{104}N_{14}O_{23}Na$ 1531.7296; Found 1531.7279.

1.8 RGDyK-Tz-sTCOPEGF (15)



12 15

9

Tetrazine-RGD conjugate (**12**) (0.3 mg, 0.0002 mmol) was dissolved in methanol (0.5 mL) and sTCOPEGF (**9**) (23 µL of a 2.5 mg/mL solution in MeOH, 0.06 mg, 0.0002 mmol) was added dropwise. The reaction was monitored by UV/Vis and was complete within 1 min. The product (**15**) was purified by reverse phase HPLC (C-18 column, 10% ACN + 0.1% formic acid to 100% ACN + 0.1% formic acid).

1.9 General procedure for stop-flow kinetic analysis of sTCO's and 11 at variable concentrations

The reaction between sTCOs (**4** & **5**) and the PEGylated tetrazine **11** was measured under pseudo-first order conditions in water : methanol 45:55 by following the exponential decay of the tetrazine at 298 nm over time using an SX 18MV-R stoppedflow spectrophotometer (Applied Photophysics Ltd.). Solutions were prepared for the sTCO concentrations see table below (water : methanol 45:55) and the tetrazine (0.1 mM in water : methanol 45:55) and thermostatted in the syringes of the spectrophotometer before measuring. An equal volume of each was mixed by the stopped flow device (resulting concentrations shown in the table below). 400 data points were recorded over a period of 1 second, and performed in sextuplicate at 298 K. The k_{obs} was determined by nonlinear regression analysis of the data points using Prism software (v. 6.00, GraphPad Software Inc.)

	Resulting concentration Tetrazine [mM]	Initial concentration sTCO [mM]	Resulting concentration sTCO [mM]	k_{obs}	k_2 [$M^{-1}s^{-1}$]	Mean k_2 [$M^{-1}s^{-1}$]
syn sTCO	0.05	0.49	0.245	8.415	34347	36,100 +/− 1,400
		0.98	0.49	18.41	37571	
		1.96	0.98	35.08	35796	
		2.94	1.47	54.18	36857	
anti sTCO		0.5	0.25	7.458	29832	31,700 +/− 1,300
		1.0	0.5	15.84	31680	
		2.0	1	32.51	32510	
		3.0	1.5	48.98	32653	

Table 1. Rate constants for the reaction of *trans*-cyclooctenes (sTCO's **4** & **5**) with PEGylated tetrazine **11** at 25 °C in water : methanol (45:55) measured under pseudo first order conditions using SX 18MV-R stoppedflow spectrophotometer. Values were determined from an average of four runs.

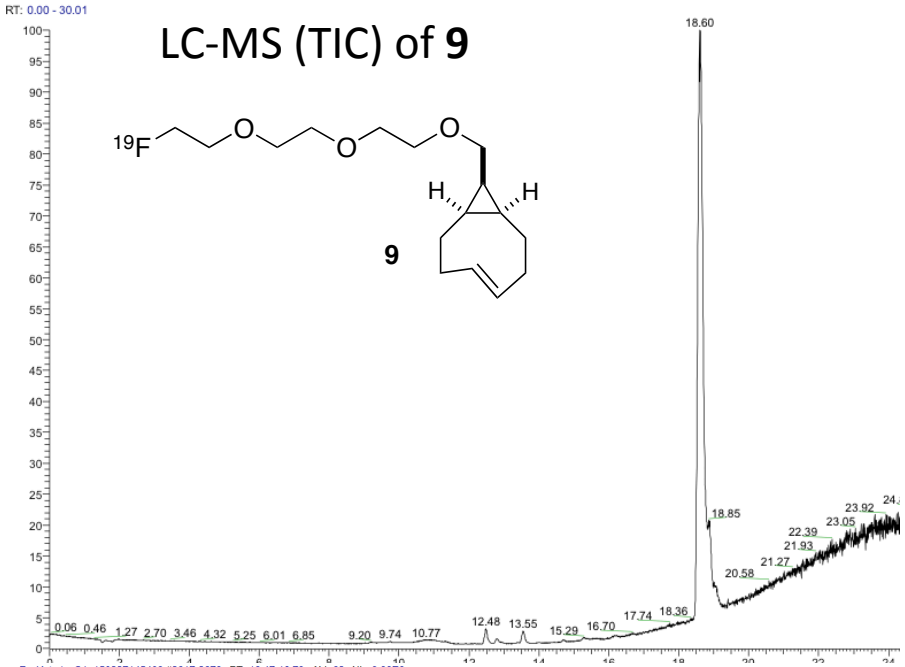
References:

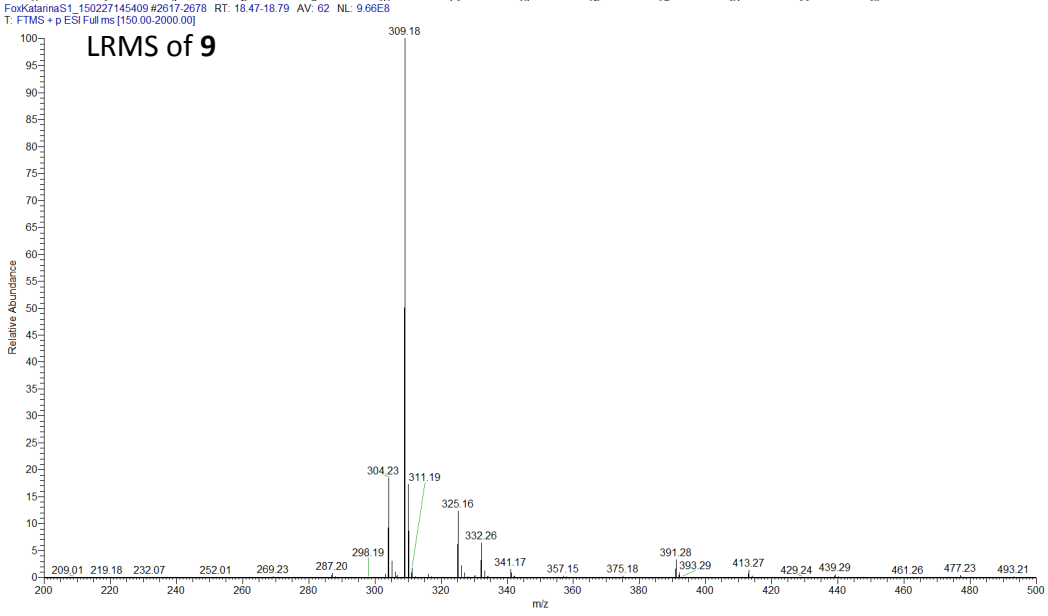
1. Taylor, M. T.; Blackman, M. L.; Dmitrenko, O.; Fox, J. M., *J. Am. Chem. Soc.* **2011**, *133*, 9646-9649.

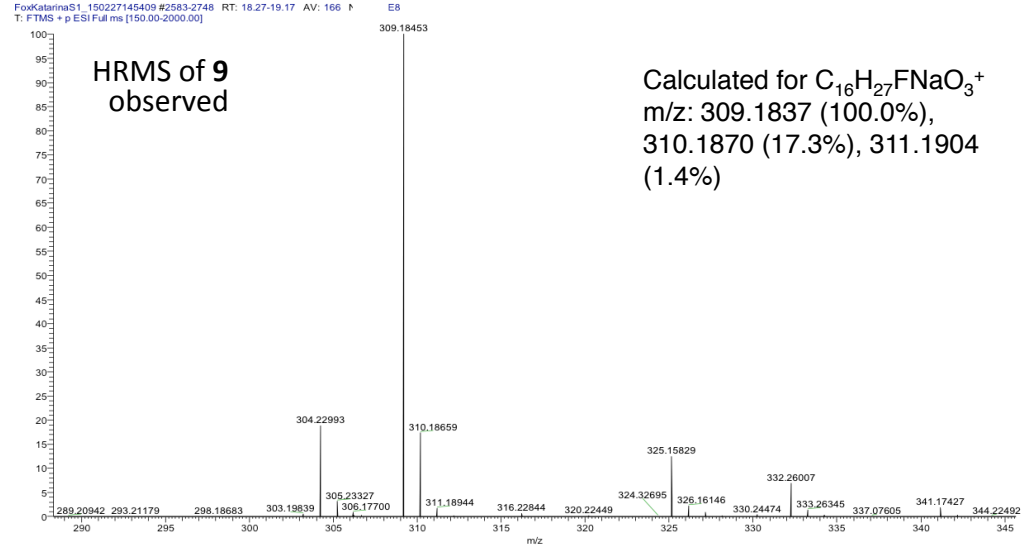
2. Zhang, H.; Dicker, K. T.; Xu, X.; Jia, X.; Fox, J. M. *ACS Macro Lett.* **2014**, *3*, 727-731.

3. Panne, P.; Fox, J. M., *J. Am. Chem. Soc.* **2007**, *129*, 22-23.

Figure S1. LC-MS trace and mass spectra for **9**

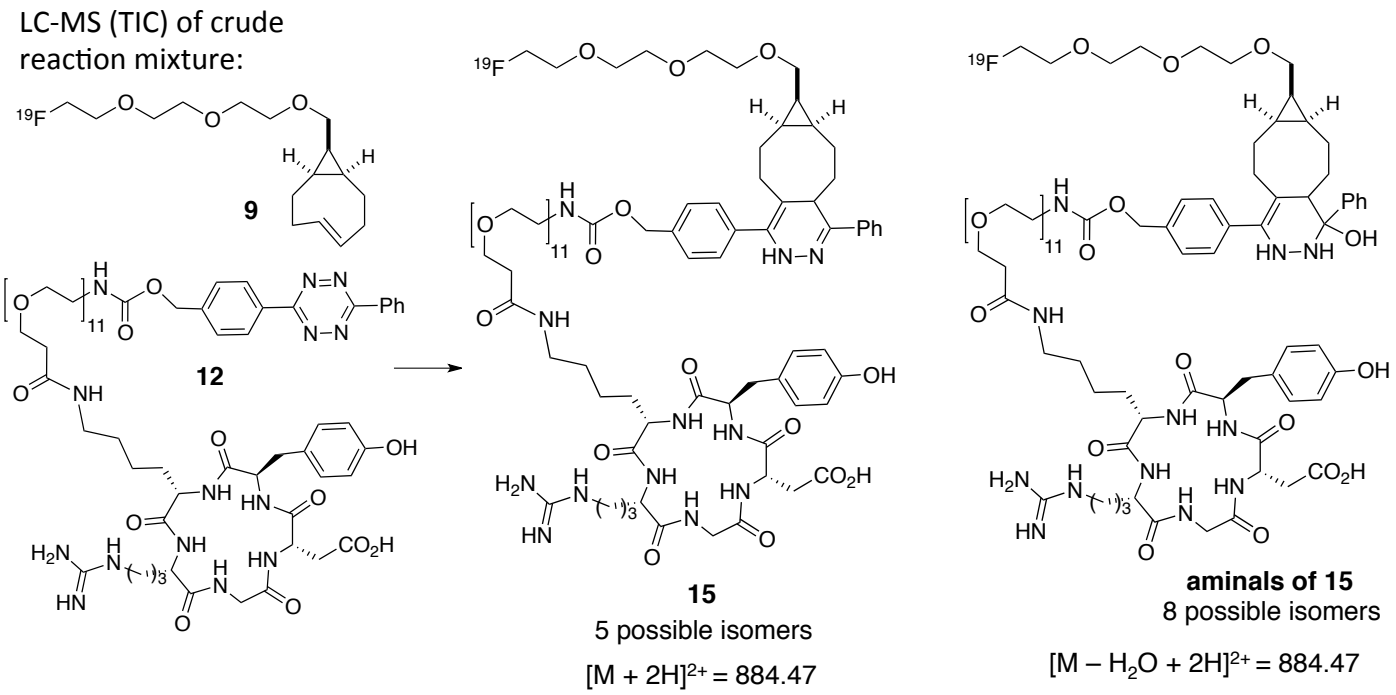






Calculated for $C_{16}H_{27}FNaO_3^+$
m/z: 309.1837 (100.0%), 310.1870 (17.3%), 311.1904 (1.4%)

Figure S2. LC-MS trace and mass spectra for crude **15**



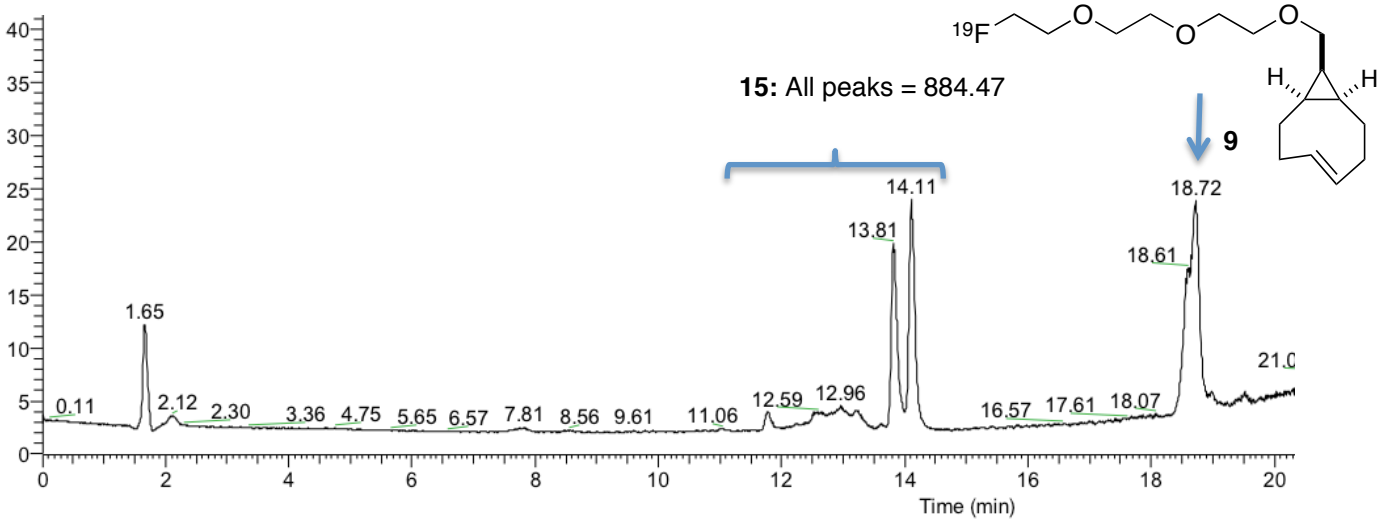
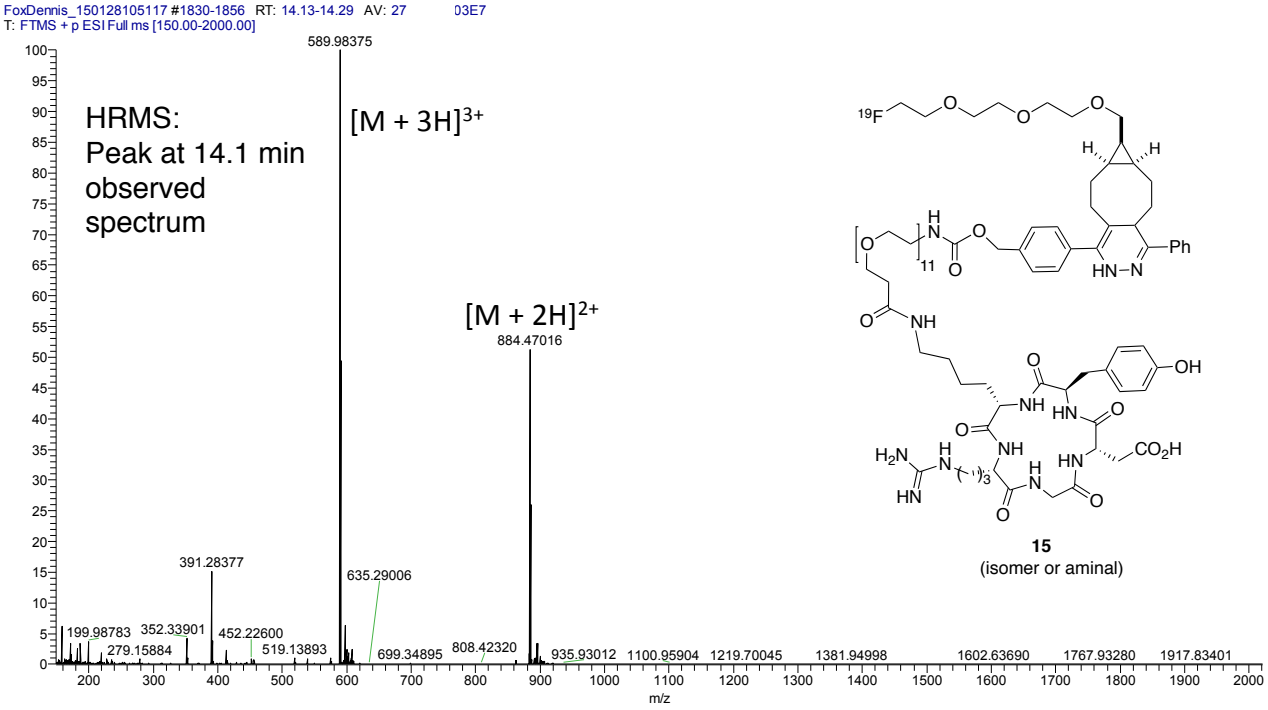
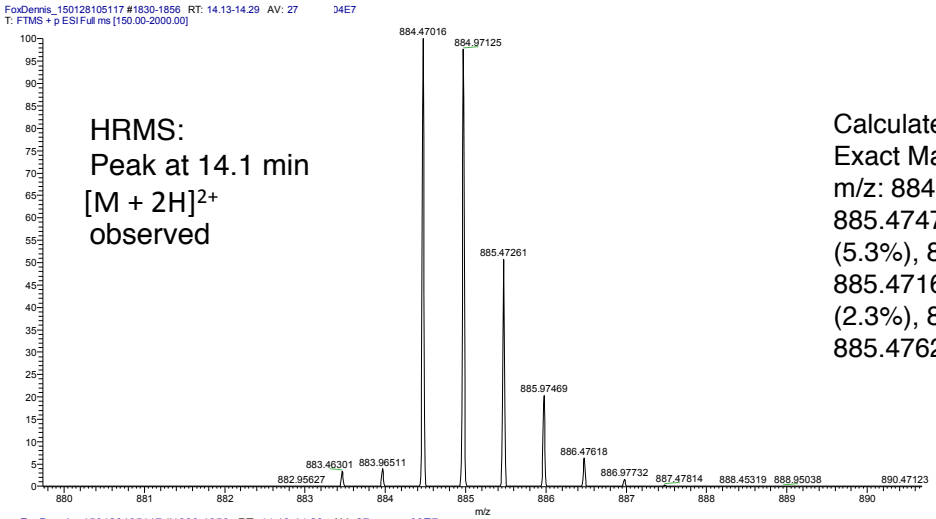
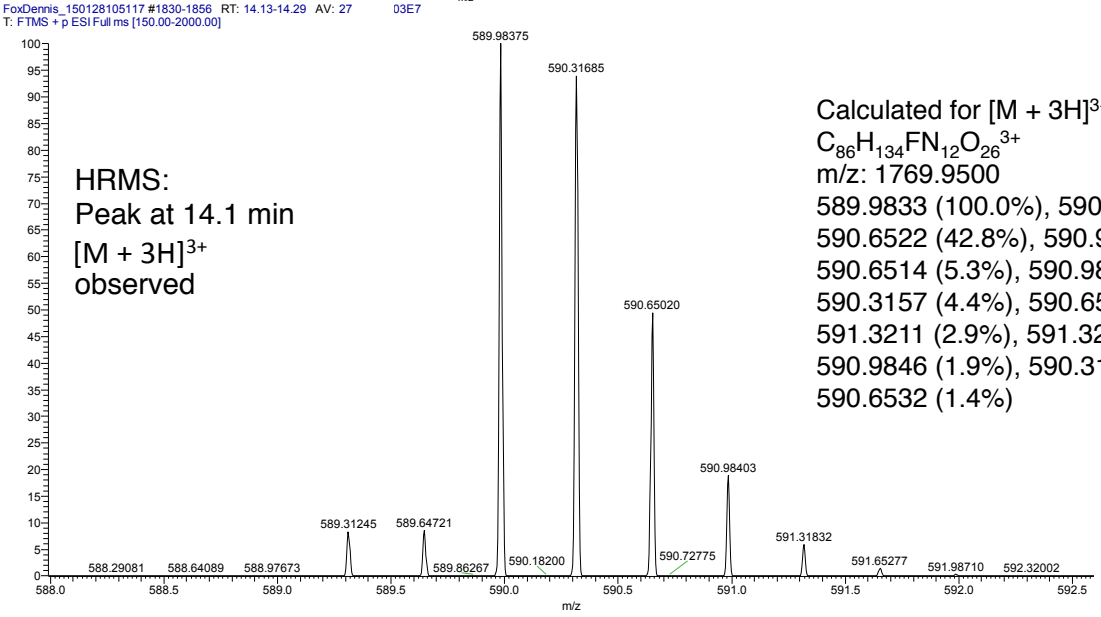


Figure S2 (continued). LC-MS trace and mass spectra for crude **15**



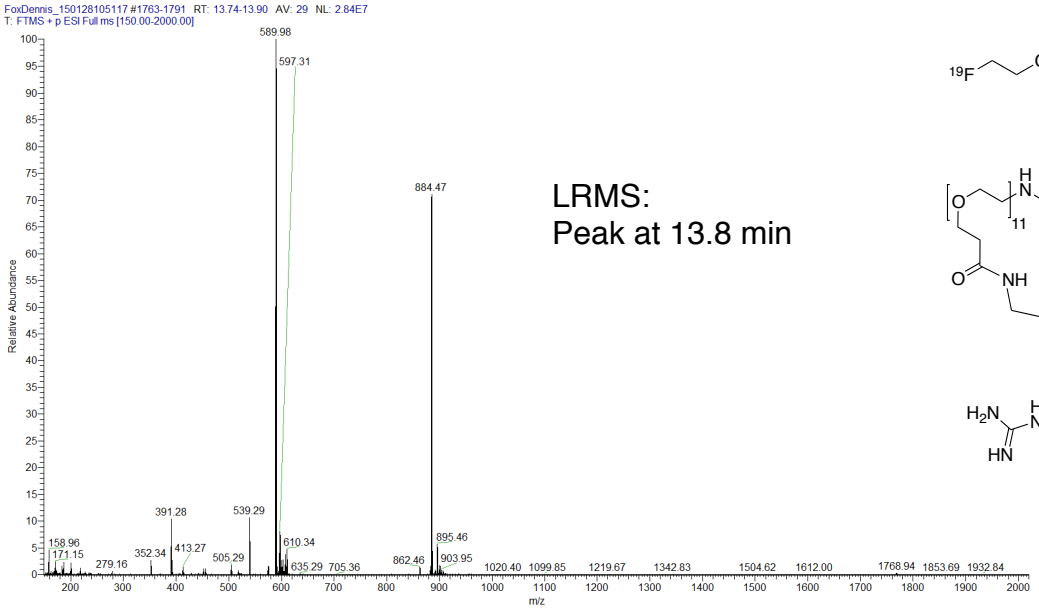


Calculated for $[M + 2H]^{2+}$, $C_{86}H_{133}FN_{12}O_{26}^{2+}$
Exact Mass: 1768.9427
m/z: 884.4714 (100.0%), 884.9731 (93.0%), 885.4747 (42.8%), 885.9764 (12.9%), 885.4735 (5.3%), 885.9752 (5.0%), 884.9699 (4.4%), 885.4716 (4.1%), 886.4781 (2.9%), 886.4769 (2.3%), 885.9732 (1.9%), 884.9745 (1.5%), 885.4762 (1.4%)

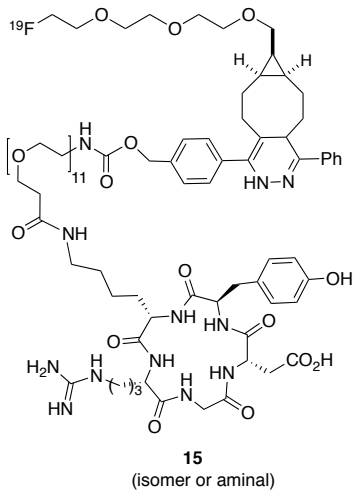


Calculated for $[M + 3H]^{3+}$,
$C_{86}H_{134}FN_{12}O_{26}^{3+}$
m/z: 1769.9500
589.9833 (100.0%), 590.3178 (93.0%), 590.6522 (42.8%), 590.9867 (12.9%), 590.6514 (5.3%), 590.9859 (5.0%), 590.3157 (4.4%), 590.6501 (4.1%), 591.3211 (2.9%), 591.3203 (2.3%), 590.9846 (1.9%), 590.3188 (1.5%), 590.6532 (1.4%)

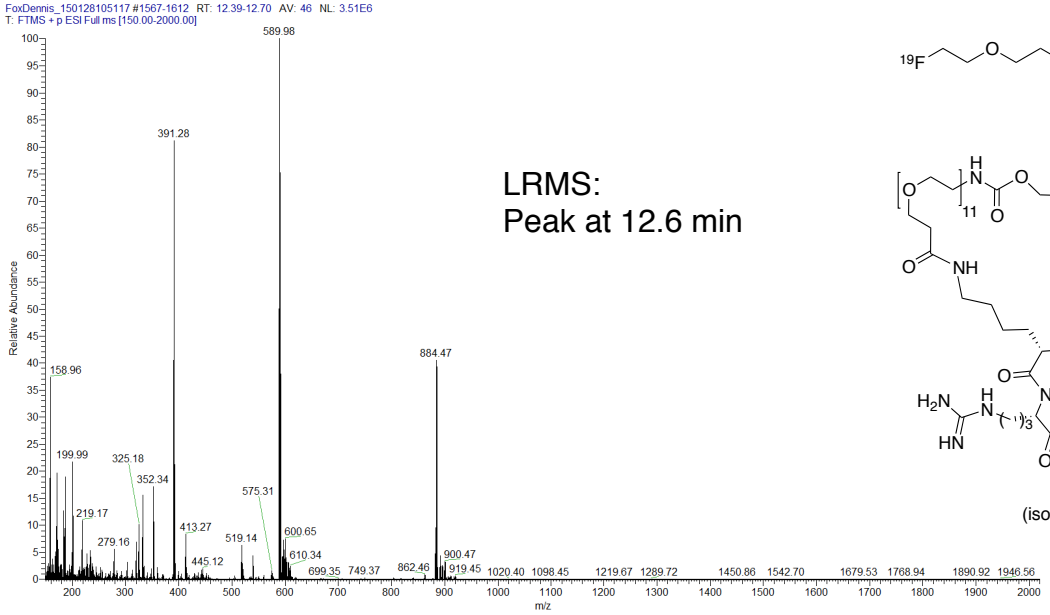
Figure S2 (continued). LC-MS trace and mass spectra for crude **15**



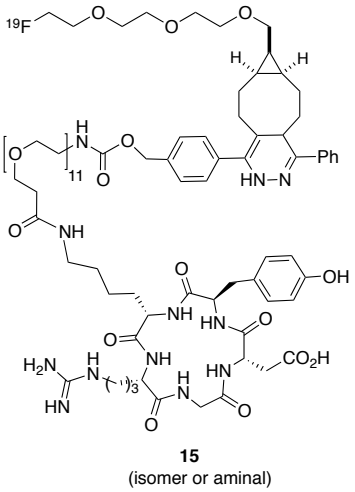
LRMS:
Peak at 13.8 min



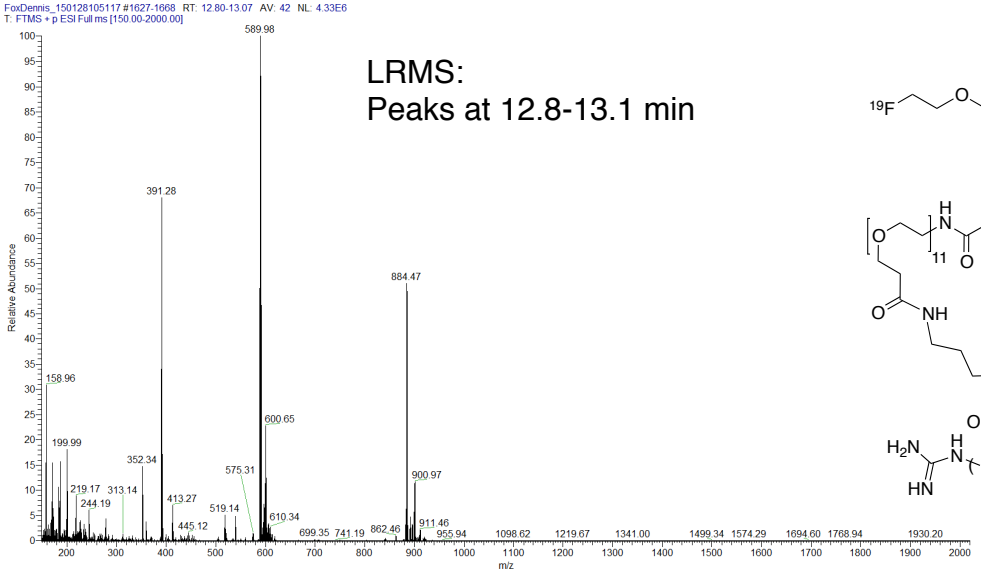
15
(isomer or aminal)



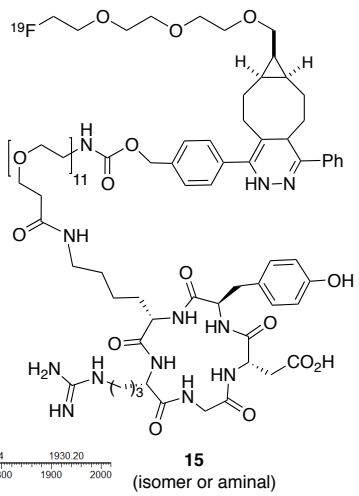
LRMS:
Peak at 12.6 min



15
(isomer or aminal)



LRMS:
Peaks at 12.8-13.1 min



15
(isomer or aminal)

Figure S3. HPLC profile of ^{18}F-**9**. a) Radio channel and b) UV channel of ^{18}F-**9** (co-injection with standard). Radio profile of ^{18}F-**9** after c) 1h incubation and d) 2h incubation in 1xPBS at 37 °C.

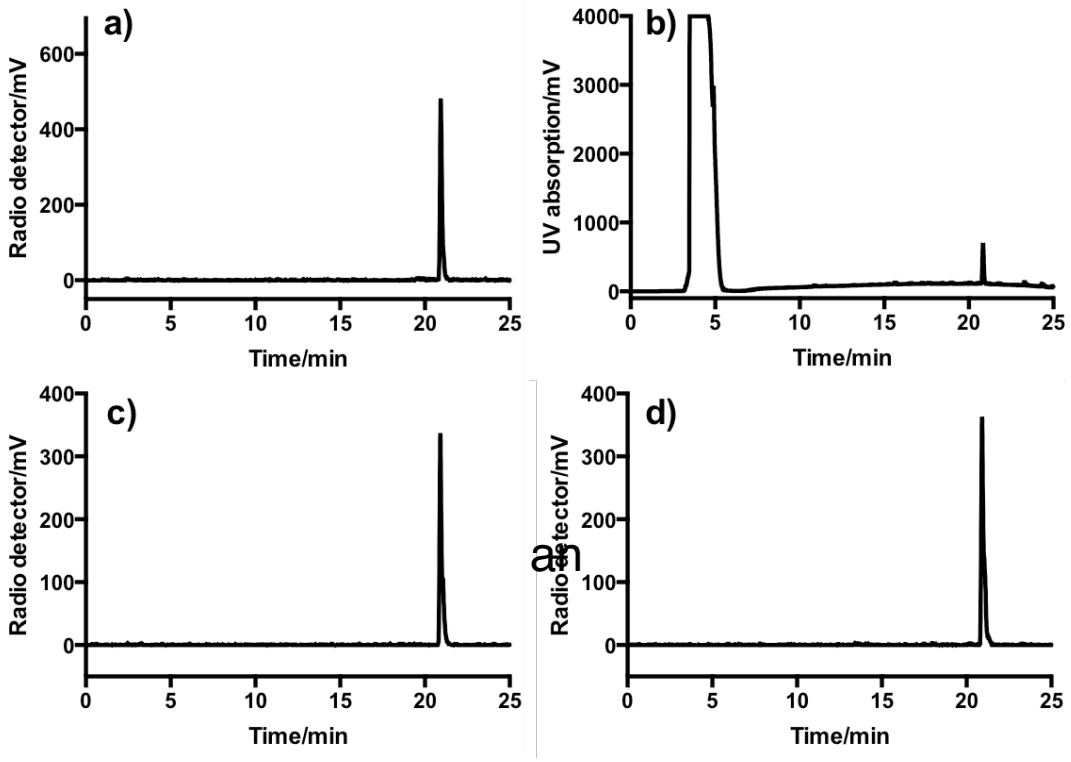


Figure S4. HPLC profile of crude reaction between ^{18}F-**9** and **12**. a) Radio channel and b) UV channel of the reaction when using excess amount of **12**. c) Radio channel and d) UV channel of the reaction when using excess amount of ^{18}F-**9** .

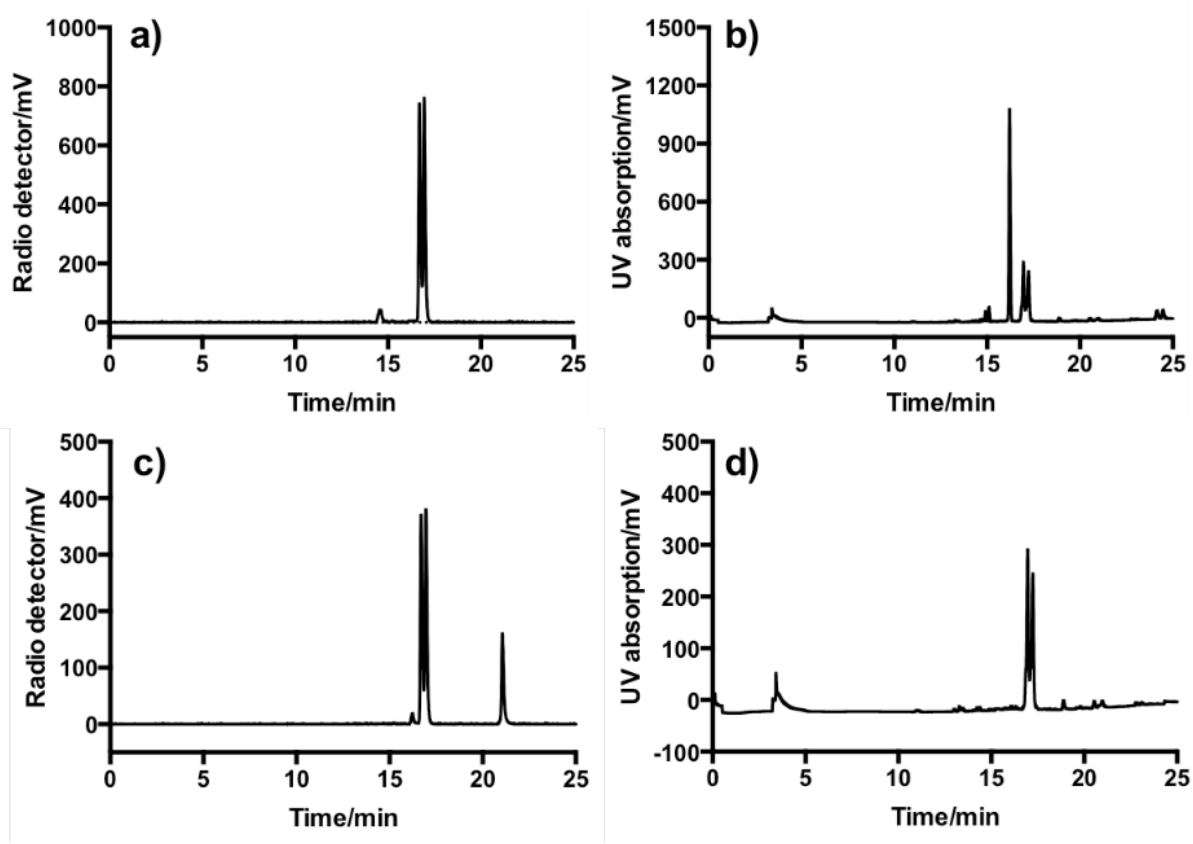
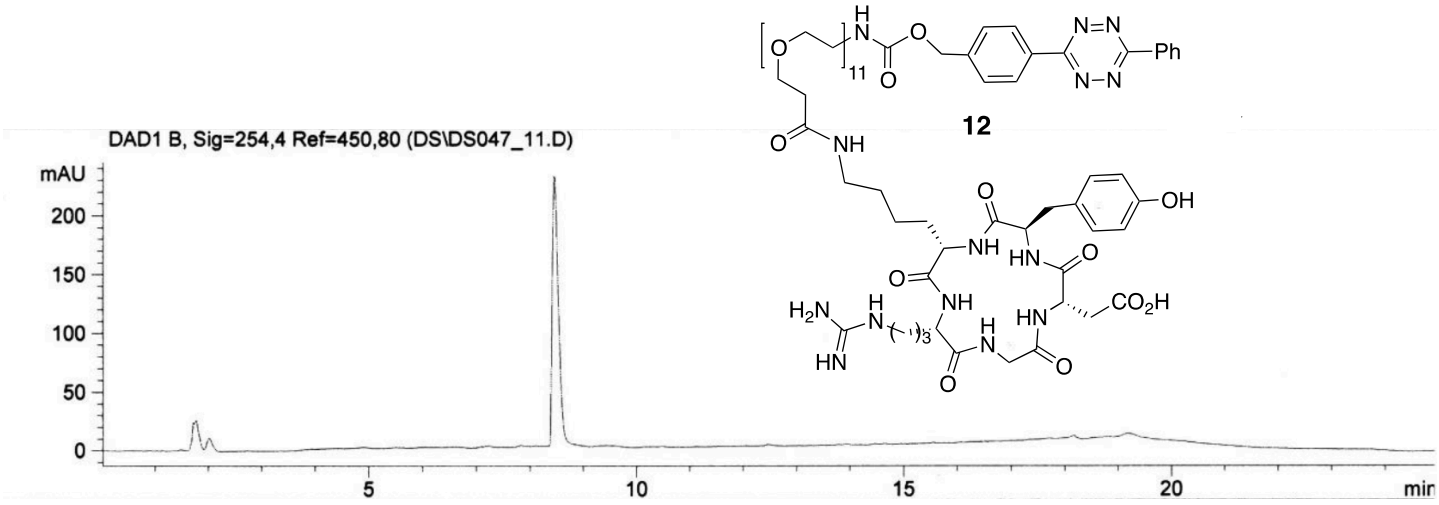


Figure S5. Reverse phase HPLC of purified **12.** Conditions: C18 Halo-column, 3.0 x 0.75 mm, 2.7μm. HPLC solvents were modified by 0.1% formic acid. The chromatogram was carried out with a gradient from 10% water in acetonitrile at t=0 min, to 100% acetonitrile at t=10 min, which was then held until the end of the run at t=20 min.



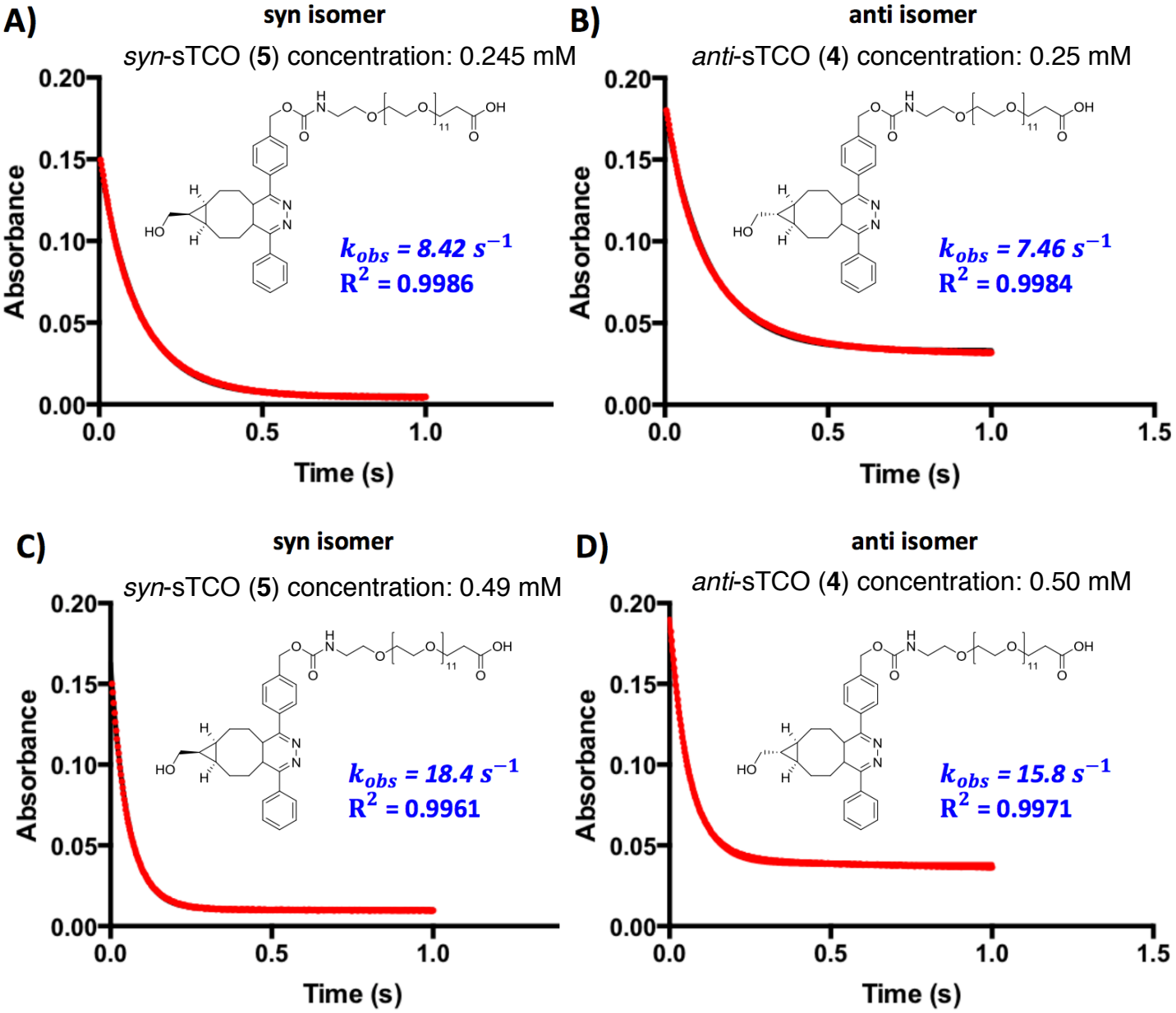


Figure S6. Stopped-flow monitored reaction of **11** (0.05 mM) with syn (**5**) and anti (**4**) diastereomers of sTCO. Each plot represents the average of 4 kinetic runs. The concentration of the sTCO used in each kinetic data set is given above each plot.

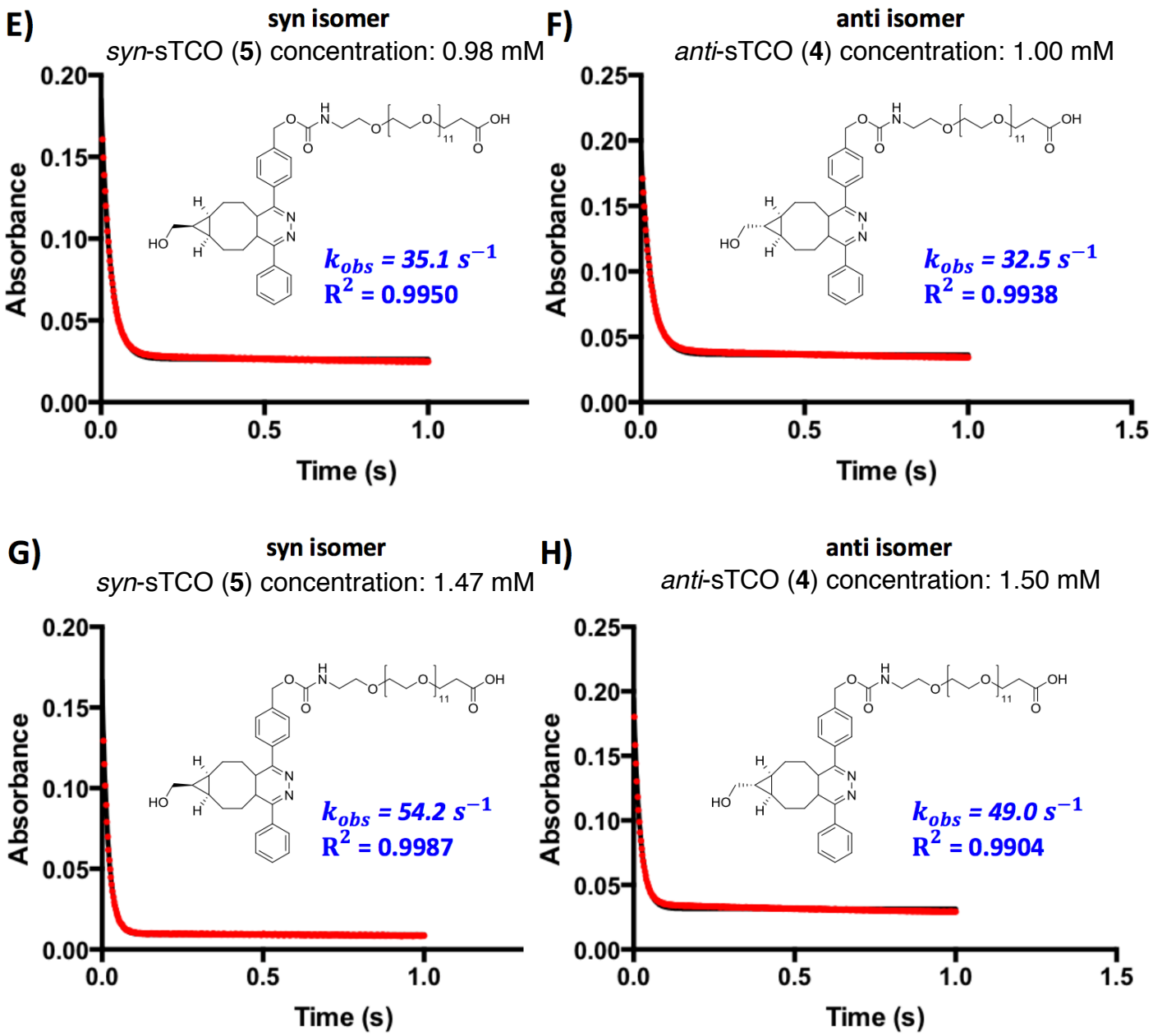


Figure S6 (continued)**.** Stopped-flow monitored reaction of **11** (0.05 mM) with syn (**5**) and anti (**4**) diastereomers of sTCO. Each plot represents the average of 4 kinetic runs. The concentration of the sTCO used in each kinetic data set is given above each plot.

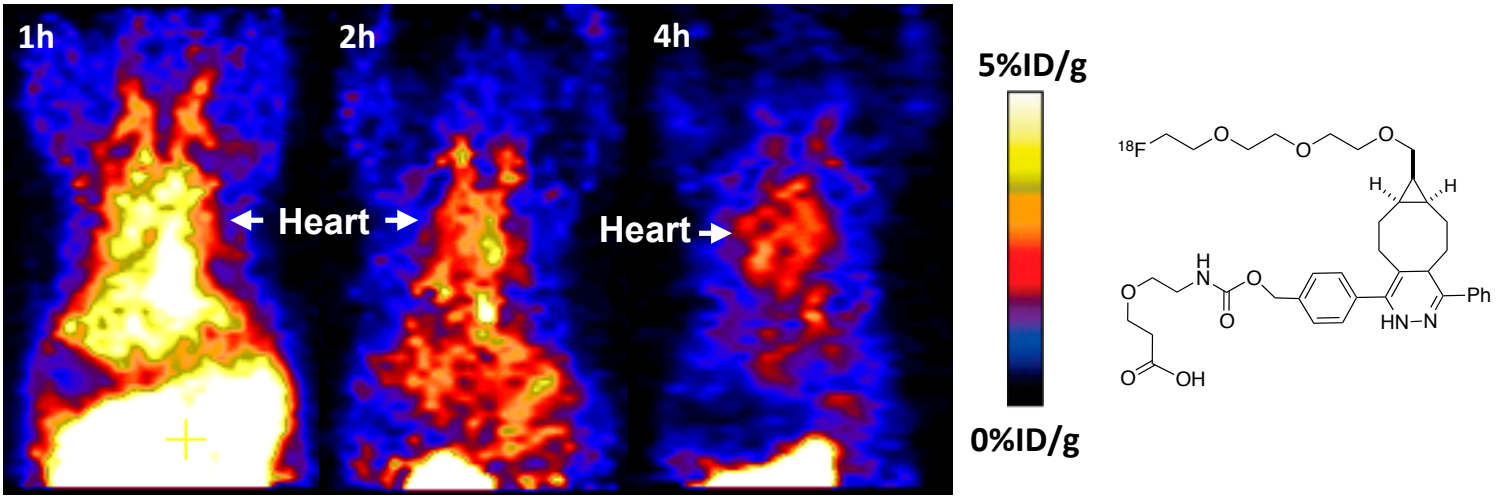


Figure S7. Small animal PET images for healthy mice injected with ^{18}F-sTCO-PEG-tetrazine conjugate (shown) and imaged at 1, 2, 4h post injection, respectively.

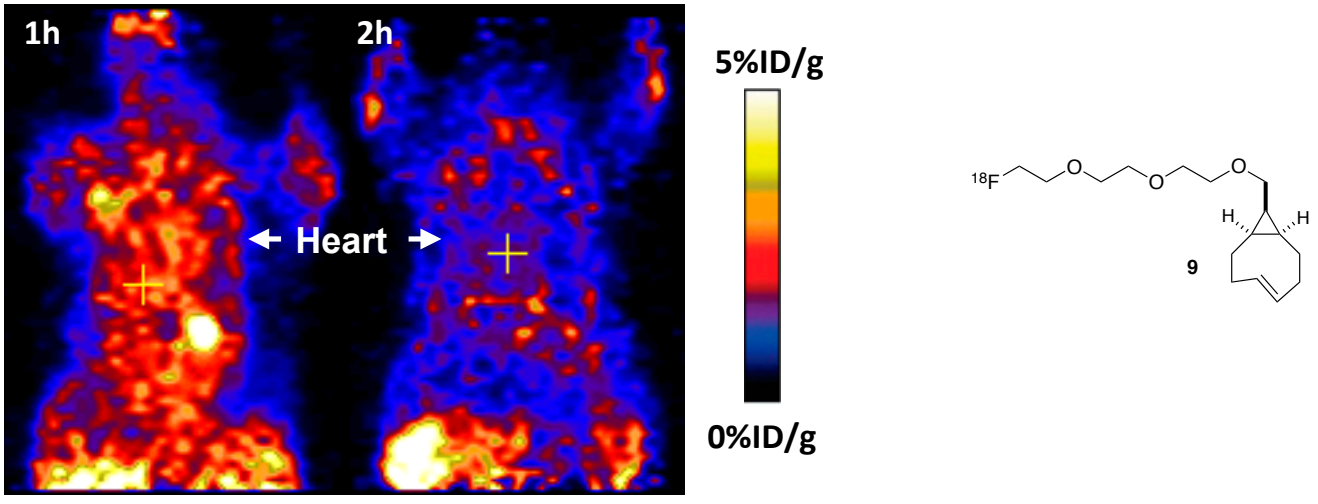


Figure S8. Small animal PET images for healthy mice injected with ^{18}F-sTCO **9** and imaged at 1 and 2 h post injection, respectively.

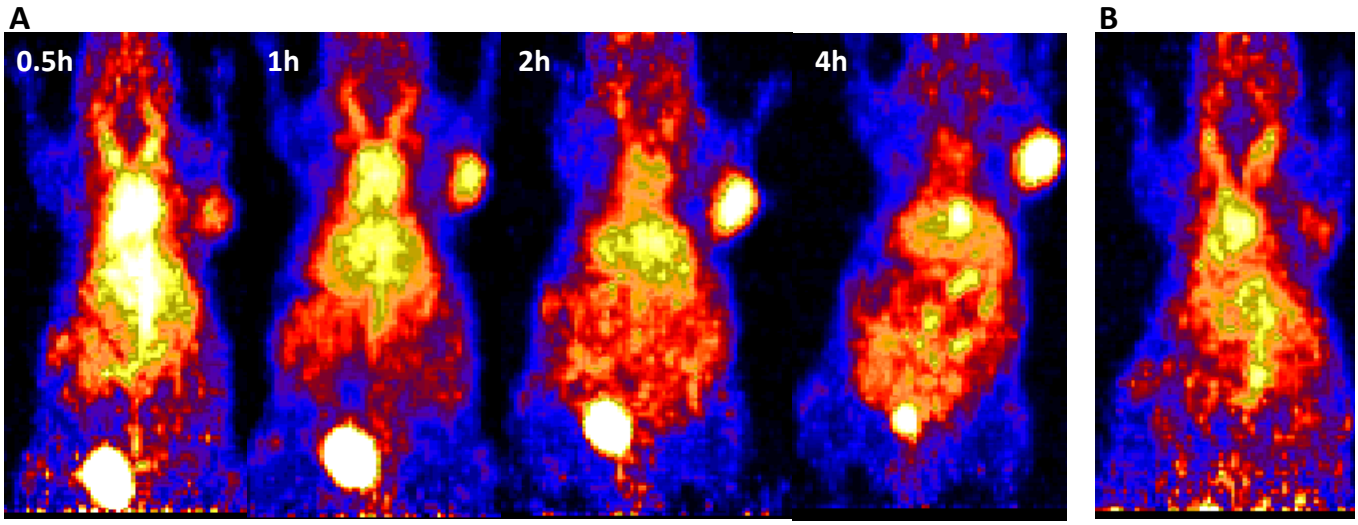


Figure S9. Two –dimensional maximum intensity projection of the mouse shown in Figure 2.

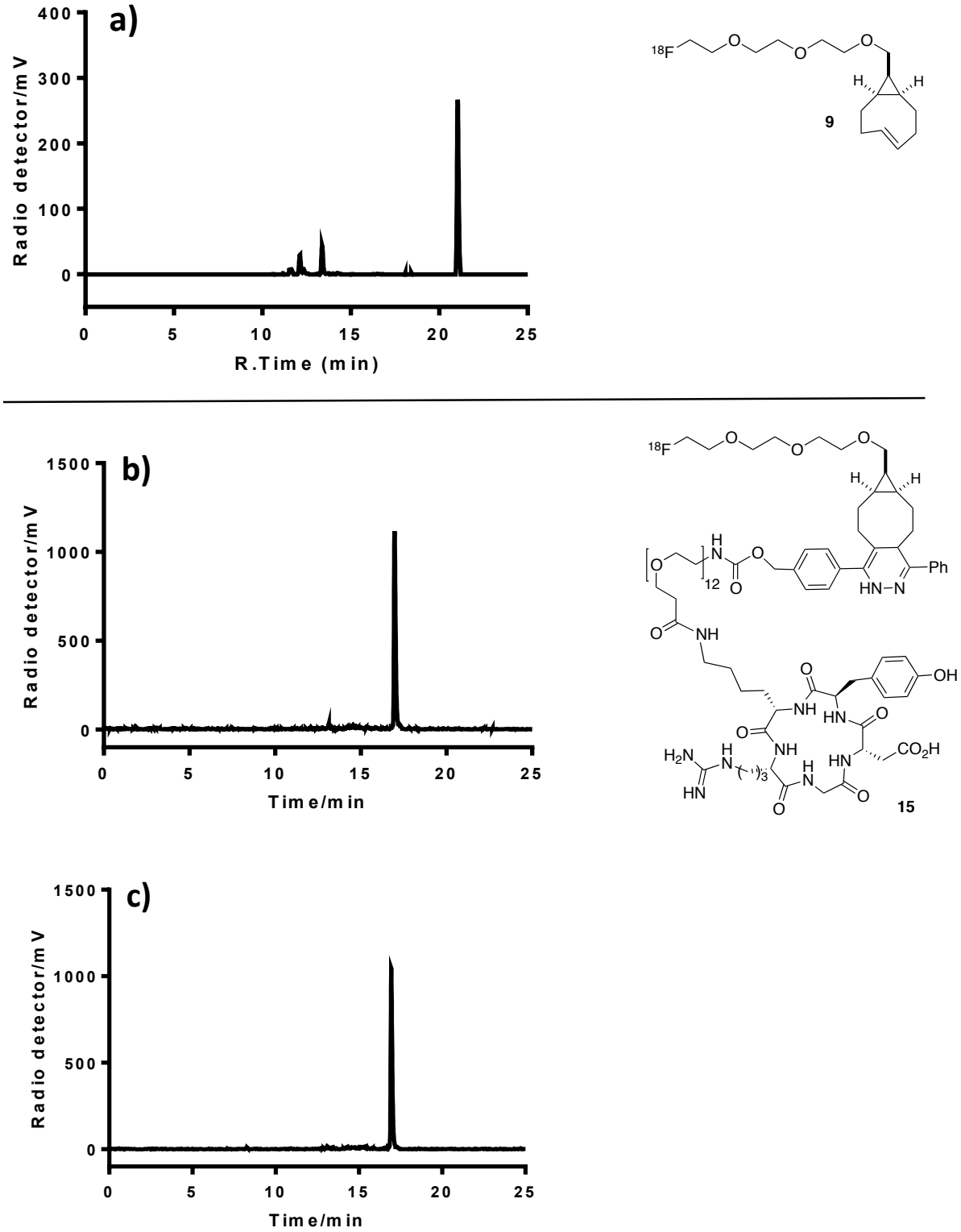
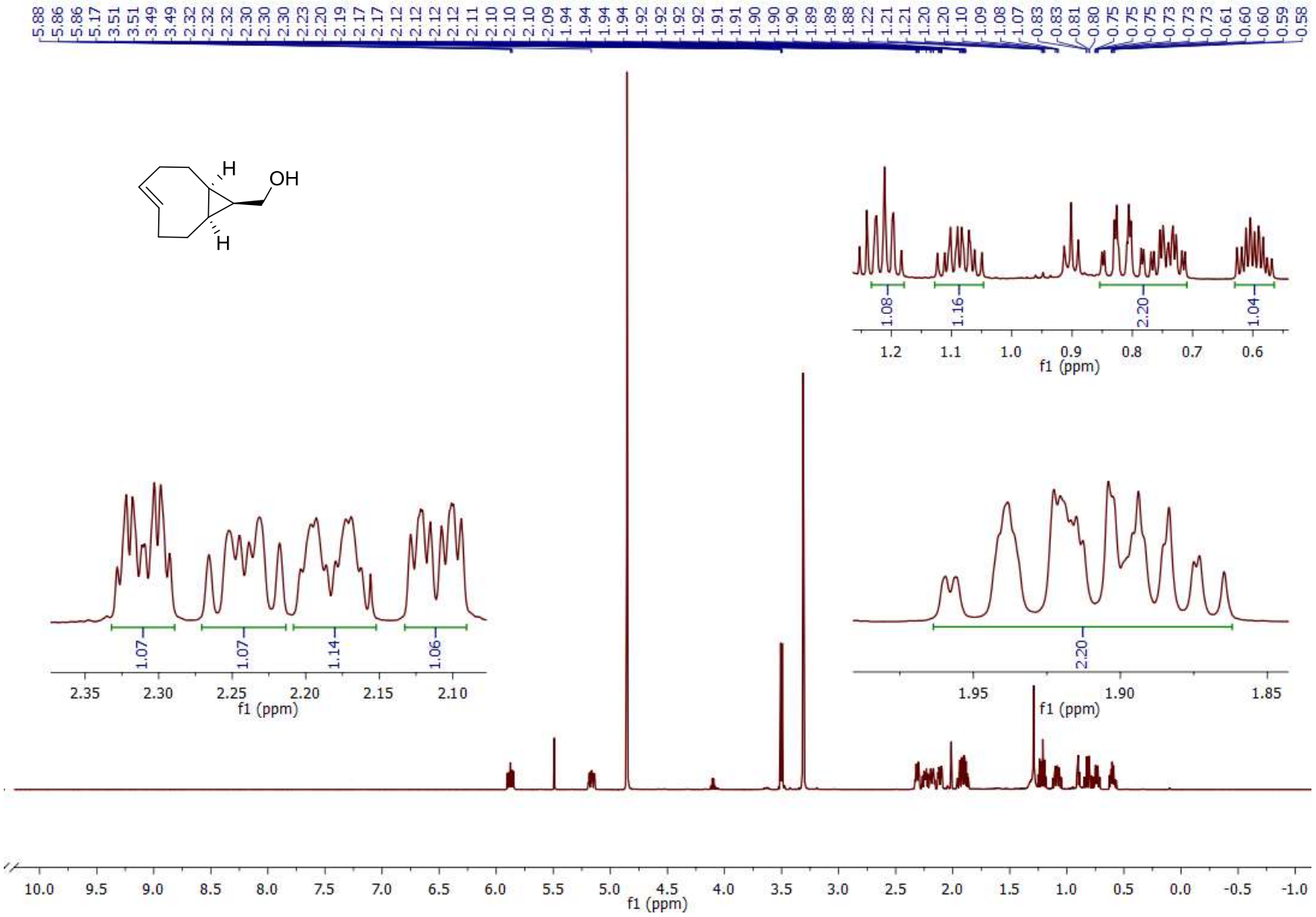


Figure S10. HPLC profile of serum stability test. a) 1h incubation of ^{18}F-**9** in FBS at 37°C. b) 2h and c) 4h incubation of ^{18}F-**15** in FBS at 37°C.

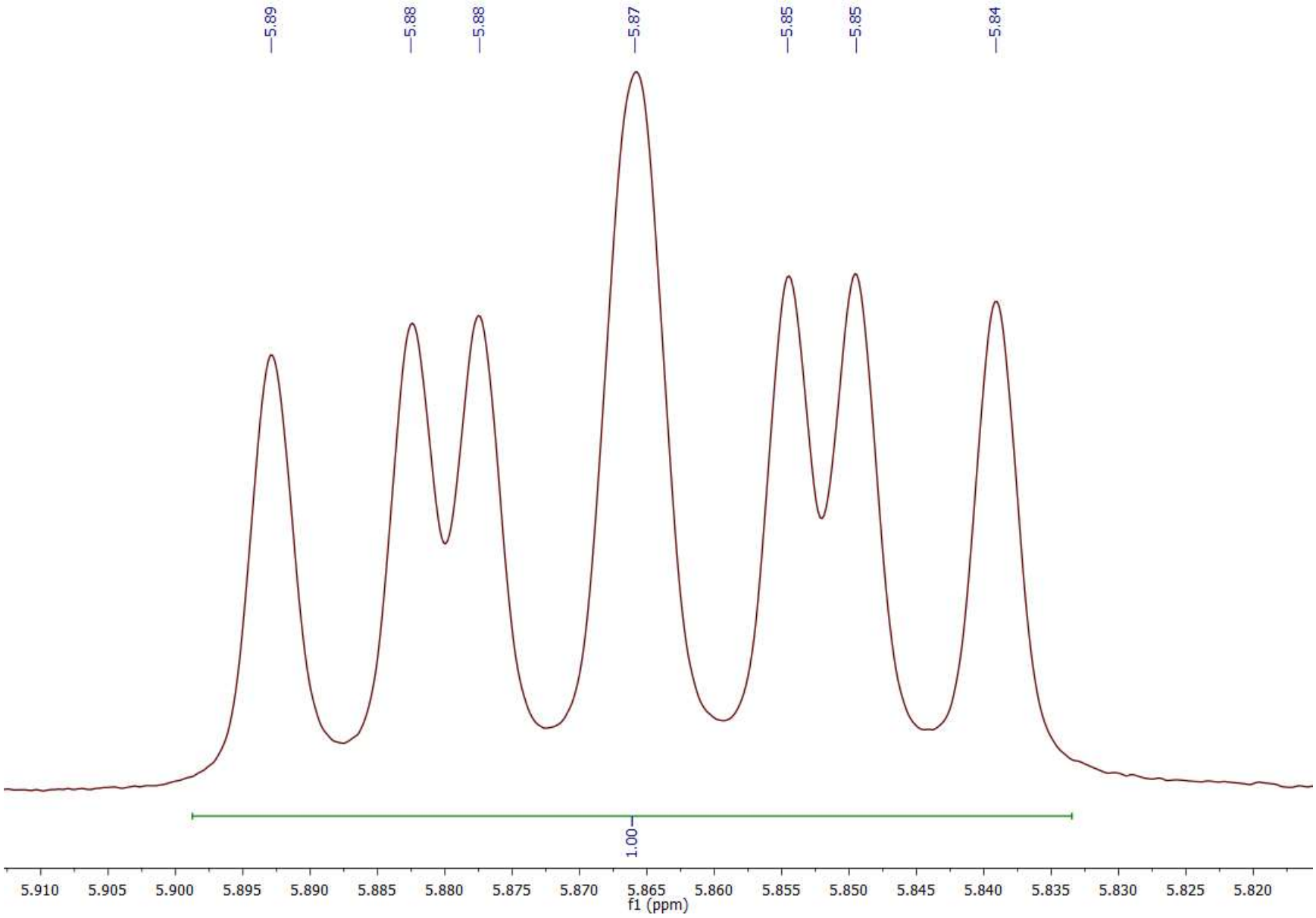
(rel-1R,8S,9S,4E)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol (**5**)

^{1}H NMR (600 MHz, CD_3OD)



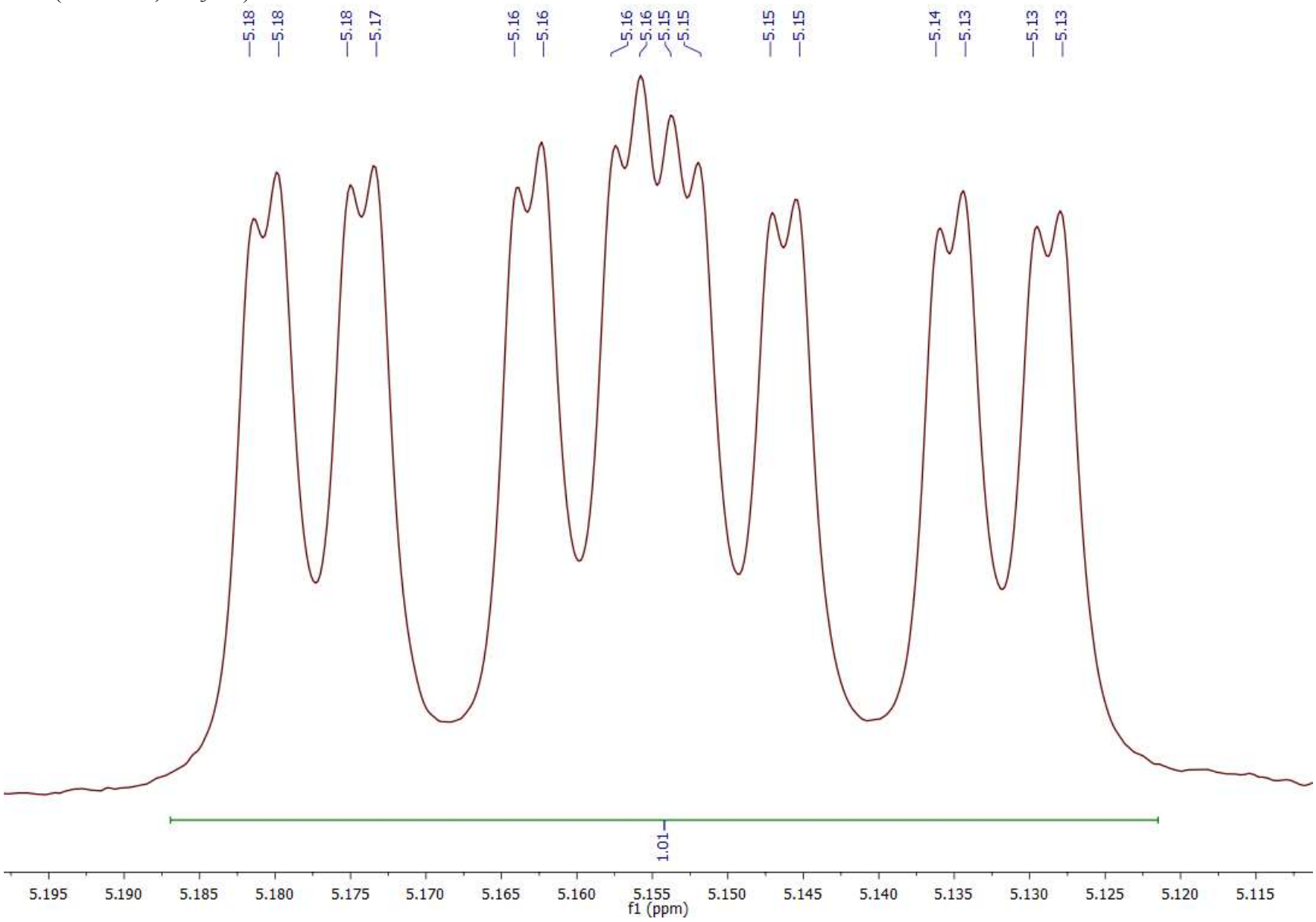
(rel-1R,8S,9S,4E)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol (**5**)

^{1}H NMR (600 MHz, CD_3OD)



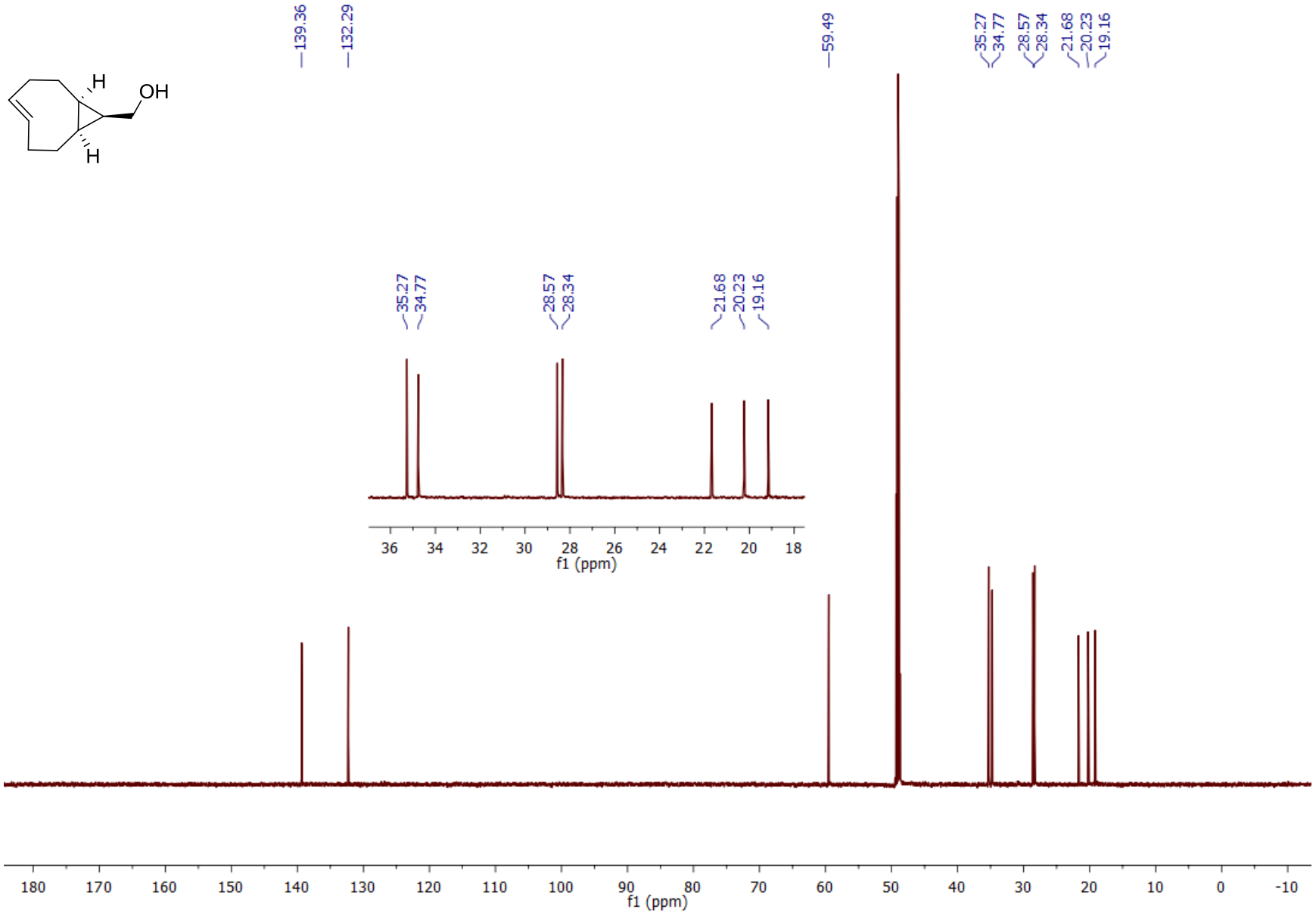
(rel-1R,8S,9S,4E)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol (**5**)

^{1}H NMR (600 MHz, CD_3OD)



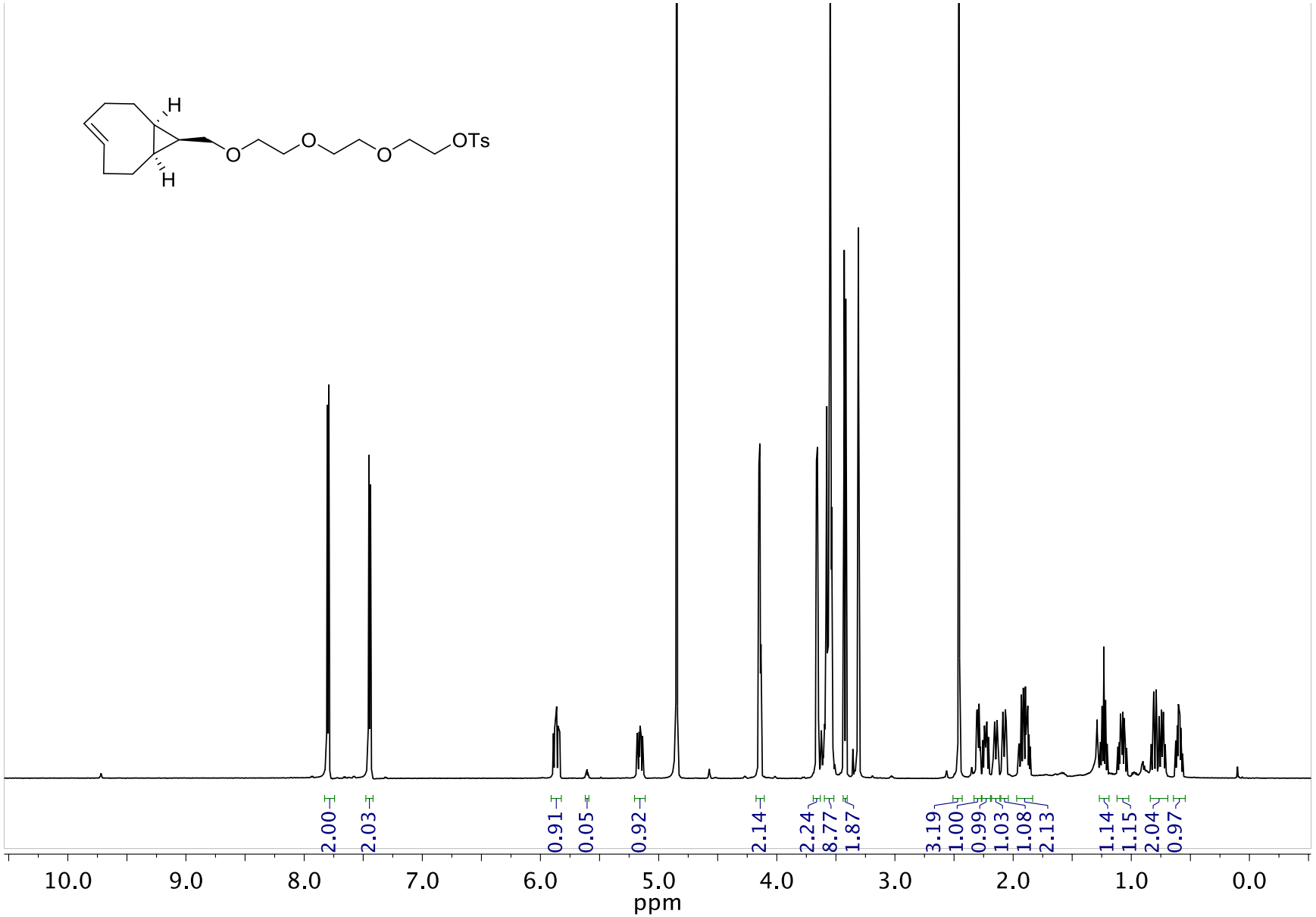
(rel-1R,8S,9S,4E)-Bicyclo[6.1.0]non-4-ene-9-ylmethanol (**5**)

^{1}H NMR (600 MHz, CD_3OD)



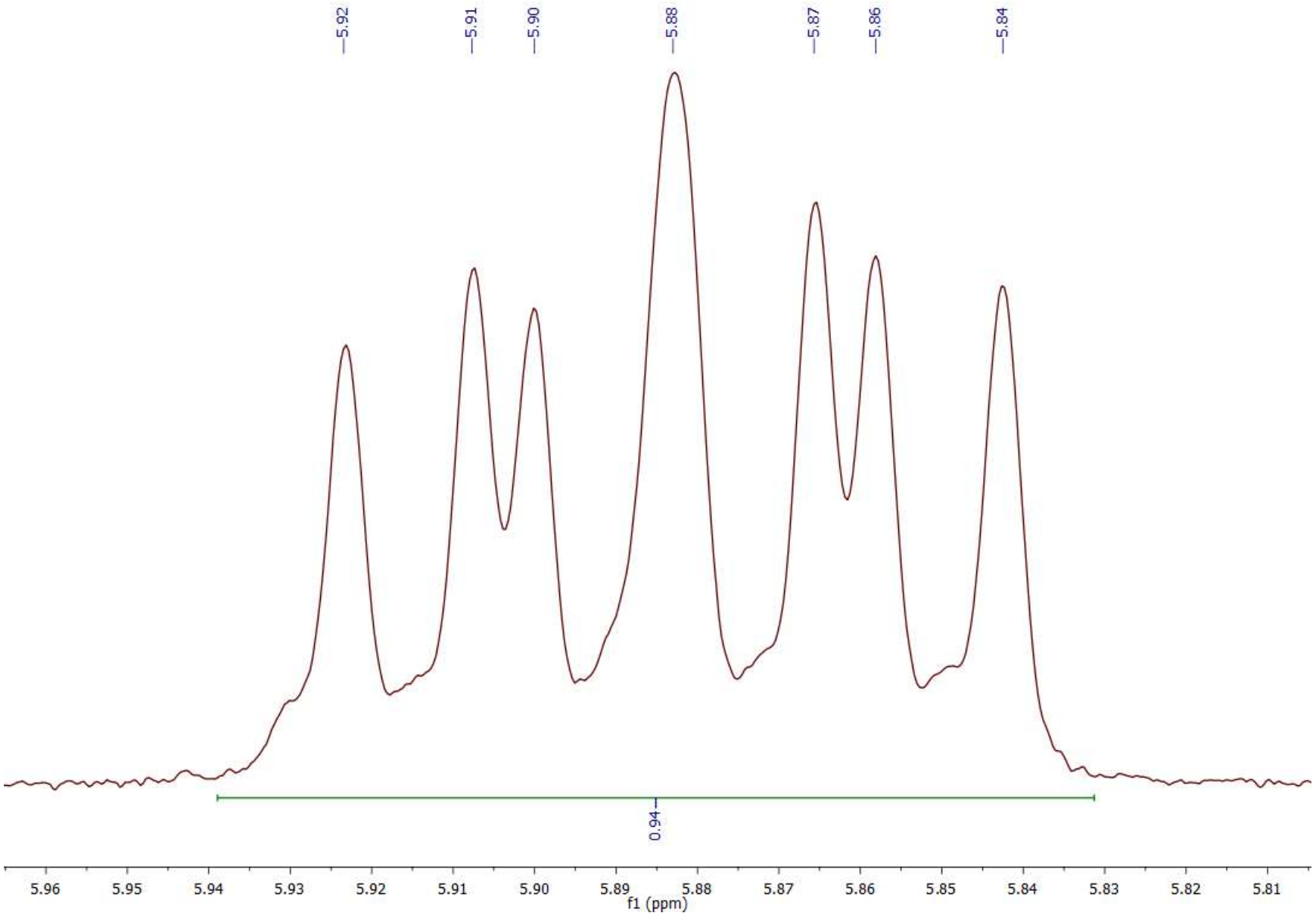
2-(2-(2-((*syn*-(*E*)-bicyclo[6.1.0]non-4-en-9-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**8**)

^{1}H NMR (600 MHz, CD_3OD)



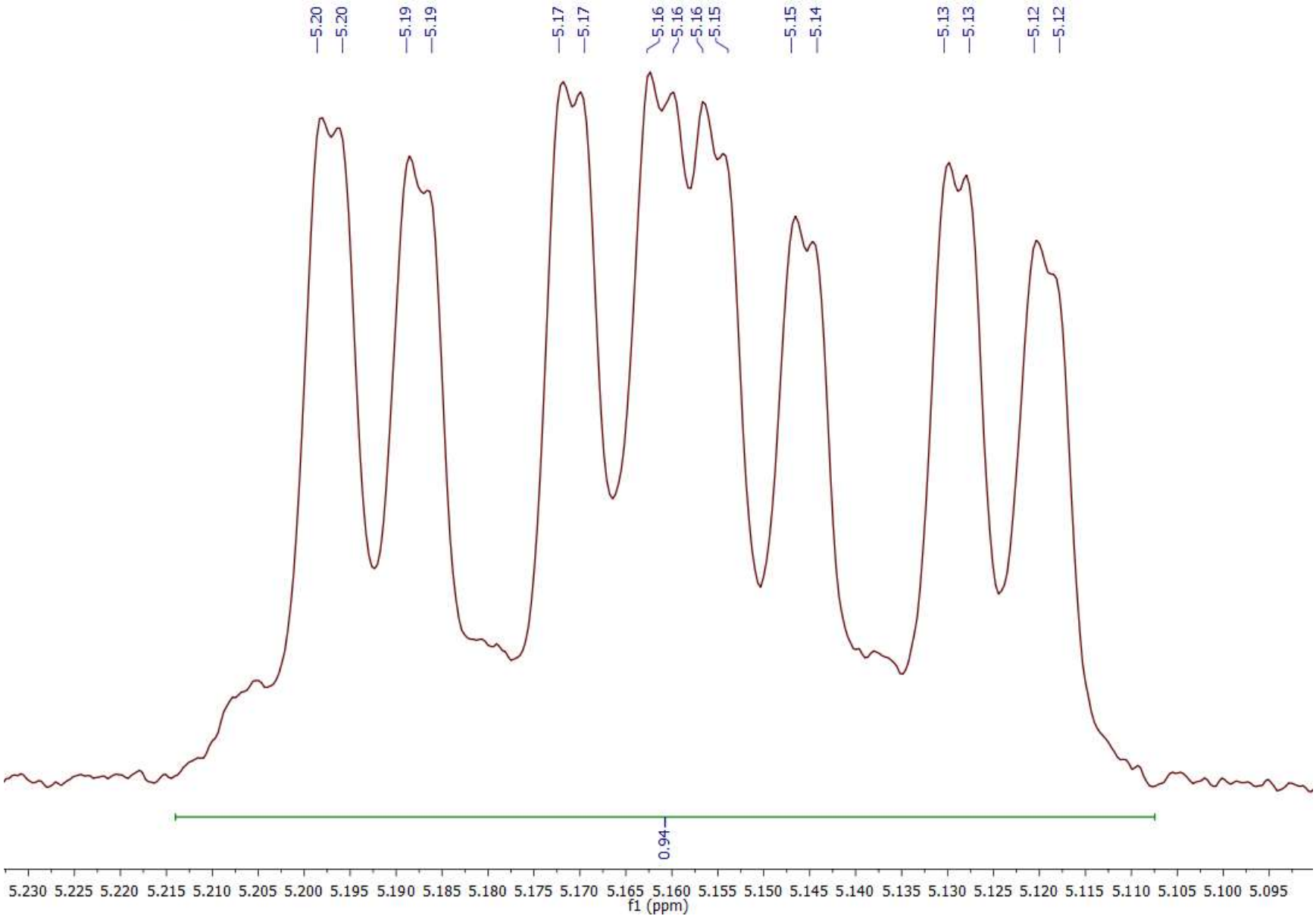
2-(2-(2-((*syn*-(*E*)-bicyclo[6.1.0]non-4-en-9-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (8)

^{1}H NMR (400 MHz, CD_3OD)



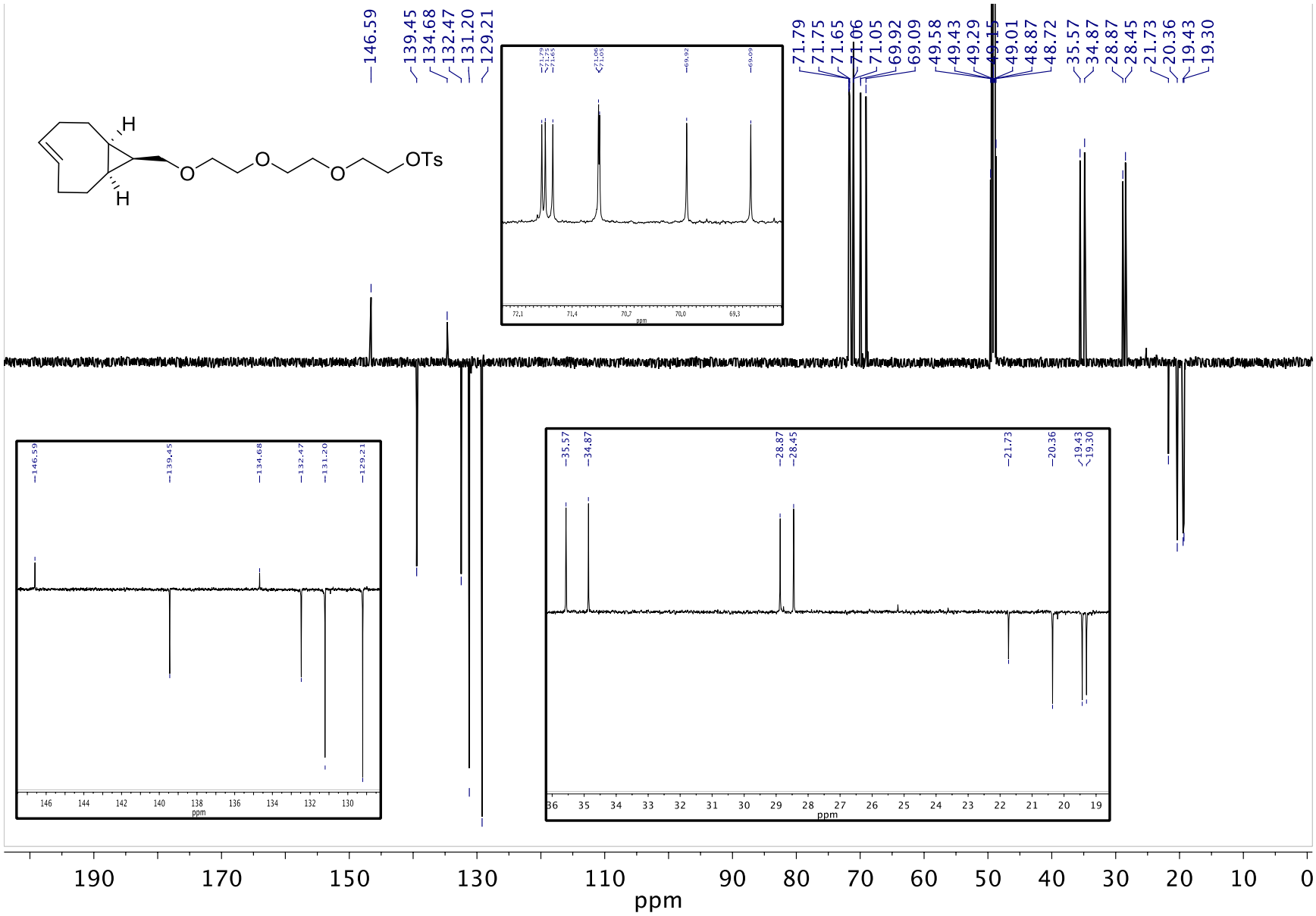
2-(2-(2-((*syn*-(*E*)-bicyclo[6.1.0]non-4-en-9-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (8)

^{1}H NMR (400 MHz, CD_3OD)



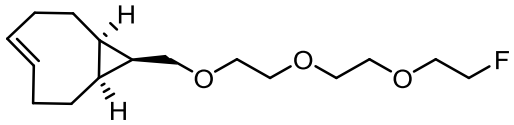
2-(2-(2-((*syn*-(*E*)-bicyclo[6.1.0]non-4-en-9-yl)methoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (8)

^{13}C APT NMR (100.6 MHz, CD_3OD)

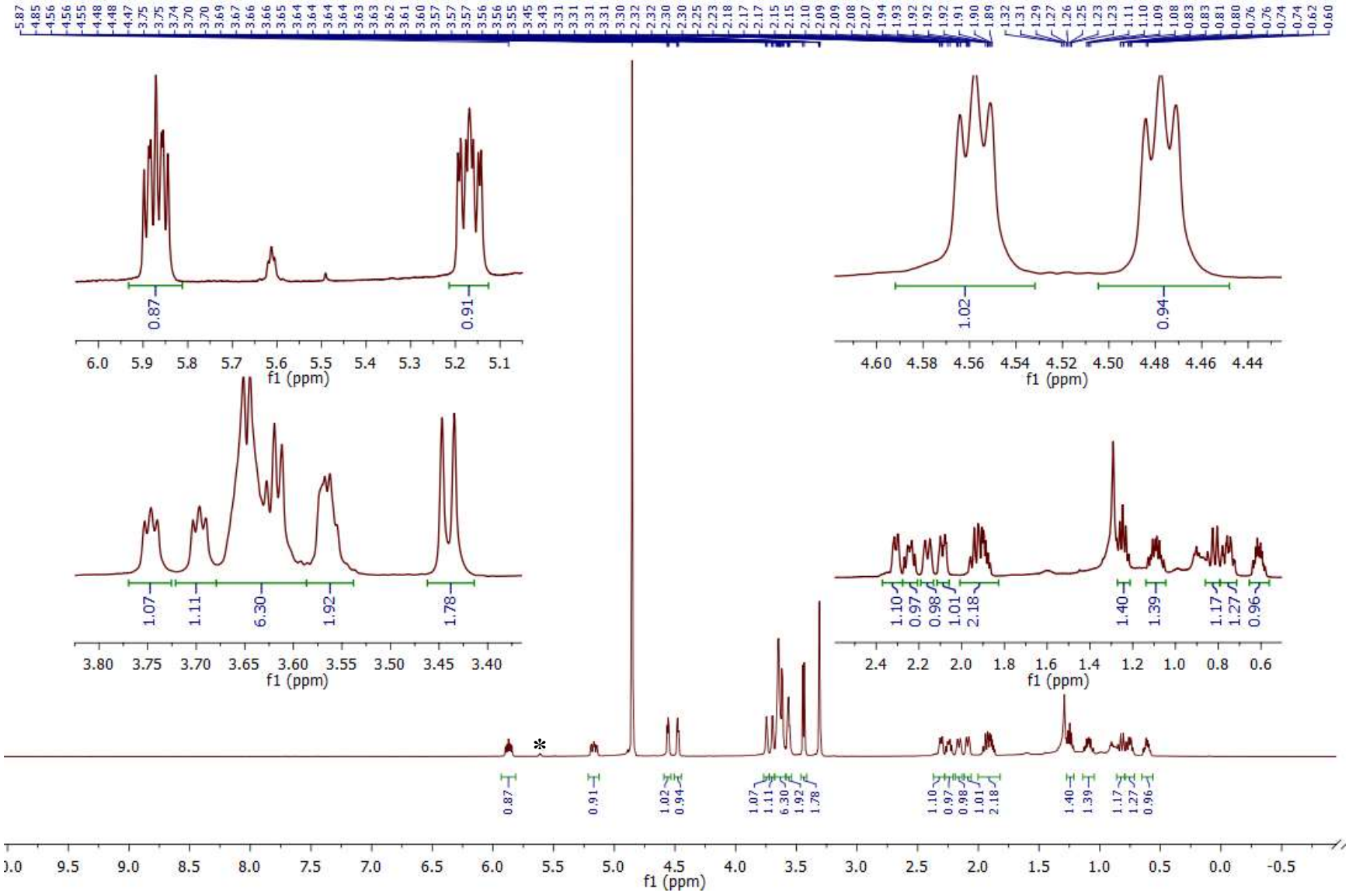


syn-(*E*)-9-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)bicyclo[6.1.0]non-4-ene (**9**)

^{1}H NMR (600 MHz, CD_3OD)

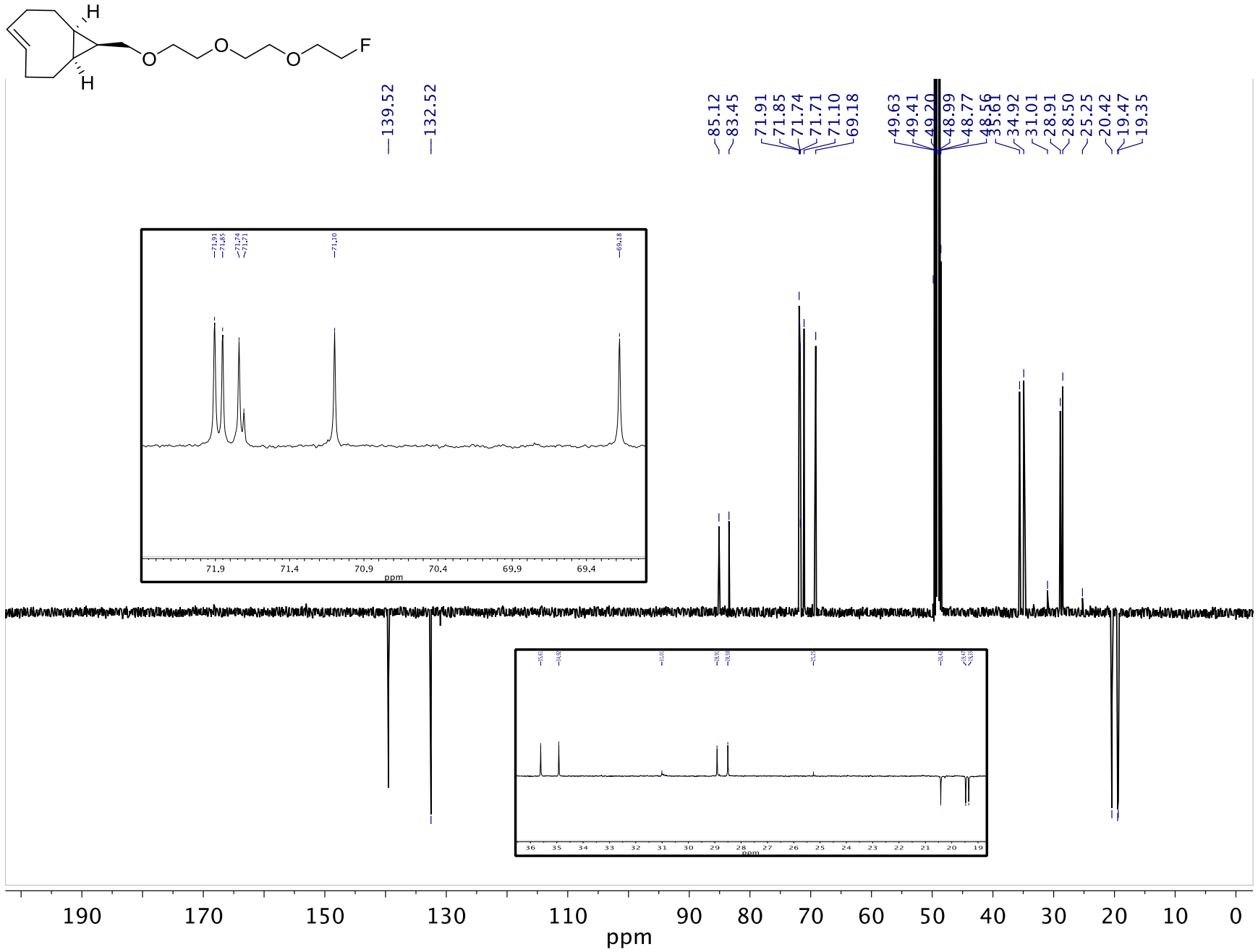


*Cis Isomer

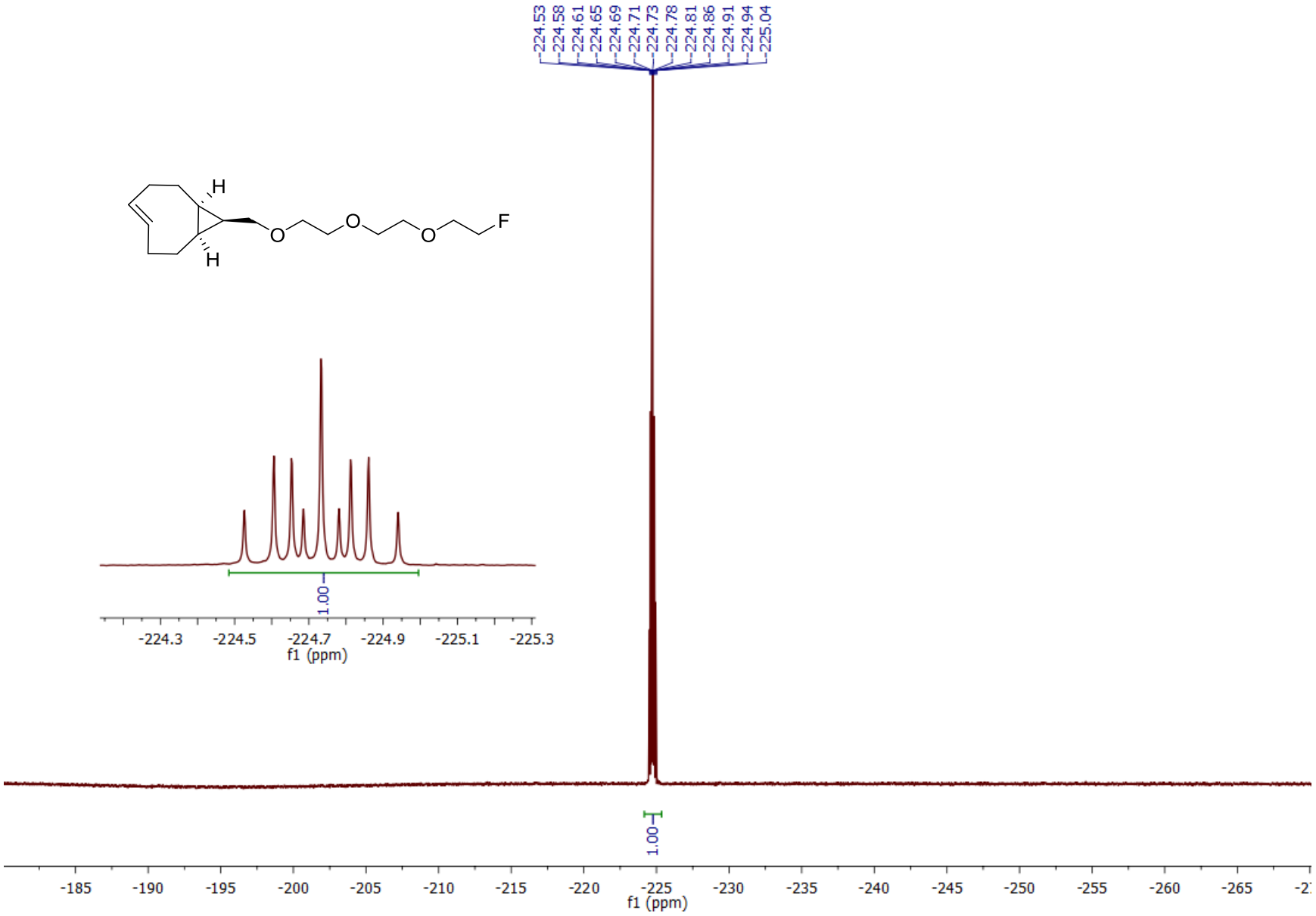


syn-(*E*)-9-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)bicyclo[6.1.0]non-4-ene (**9**)

^{13}C APT NMR (100.6 MHz, CD_3OD)

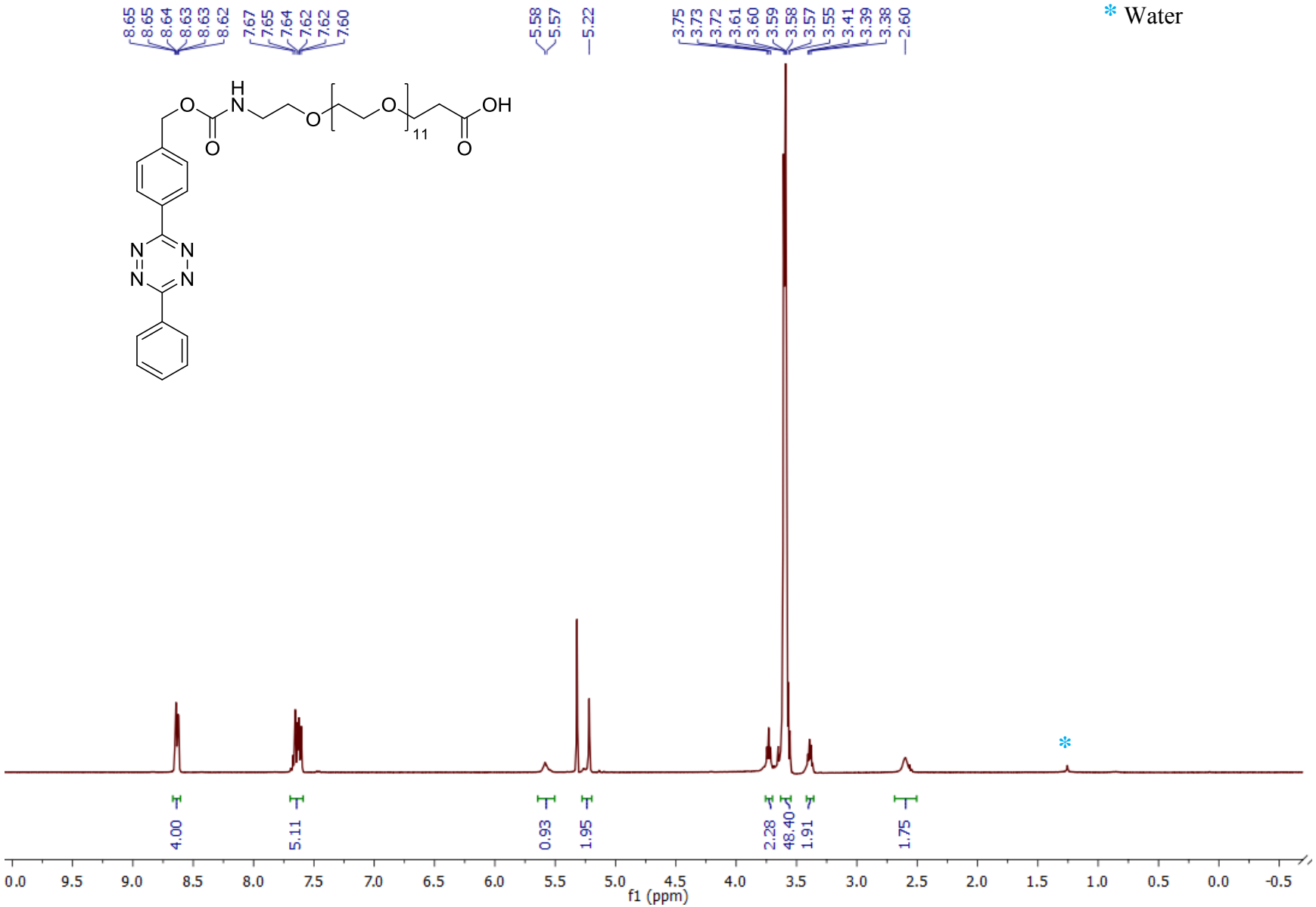


syn-(*E*)-9-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)bicyclo[6.1.0]non-4-ene (**9**)
^{19}F NMR



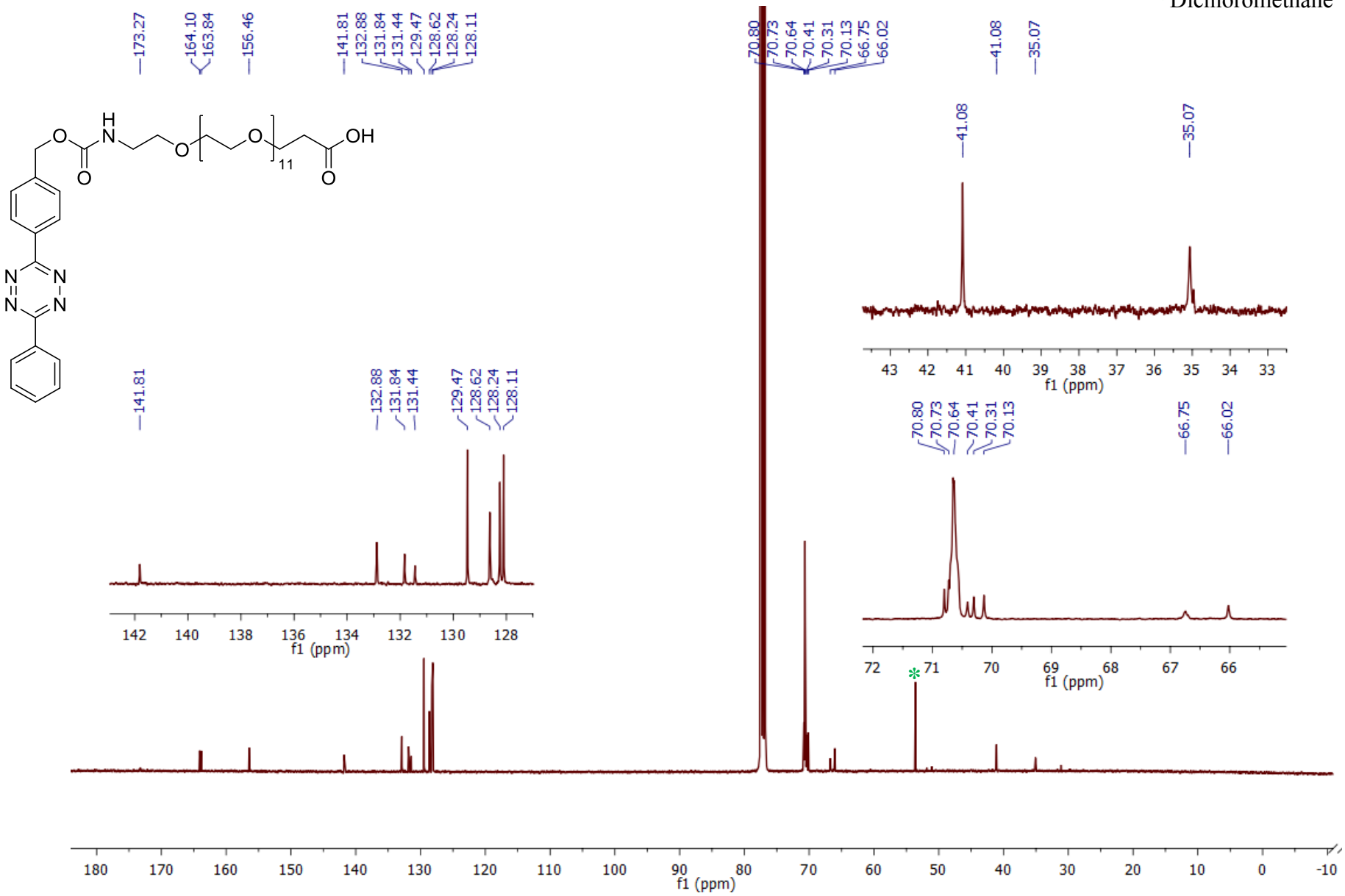
3-oxo-1-(4-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)-2,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxa-4-azatritetracontan-43-oic acid (**11**)

^{1}H NMR (400 MHz, CD_2Cl_2)



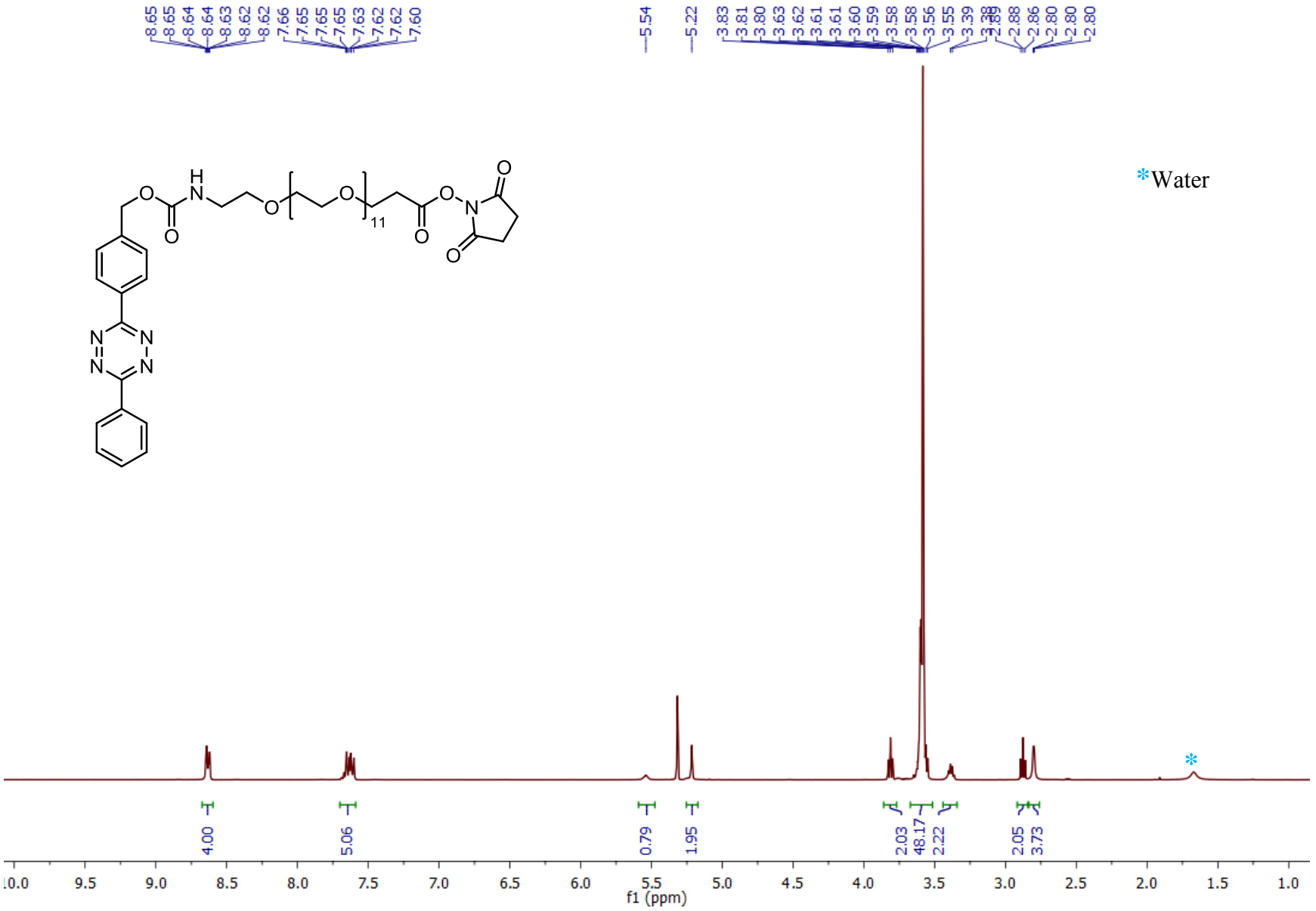
3-oxo-1-(4-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)-2,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxa-4-azatritetracontan-43-oic acid (**11**)

^{13}C NMR (101 MHz, $CDCl_3$)



2,5-dioxopyrrolidin-1-yl 3-oxo-1-(4-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)-2,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxa-4-azatritetracontan-43-oate

^{1}H NMR (400 MHz, CD_2Cl_2)



2,5-dioxopyrrolidin-1-yl 3-oxo-1-(4-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)-2,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxa-4-azatritetracontan-43-oate

^{13}C NMR (101 MHz, CD_2Cl_2)