Novel fluorinated ligands for gold nanoparticle labelling with application in ^{19}F-MRI.

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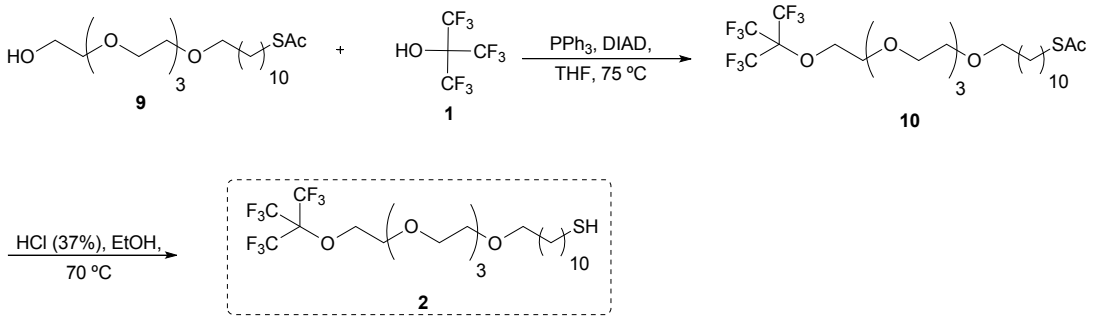
Note added after first publication: This Supplementary Information file replaces the one originally published on 31st January 2017 due to an error in the ligand percentage used to prepare **NP4**.

1. Ligand synthesis

General remarks: Starting materials **1**, **9**, **11** and all chemical reagents for ligand synthesis were purchased from Sigma Aldrich and used without further purification. PEG derivatives **7** and **14** were purchased from Rapp Polymere. Compounds **5**,[1] **6**,[2] **8**[3] and **16**[4] were prepared as reported elsewhere. Unless otherwise stated, reactions were run under air and non-anhydrous conditions. ^{1}H, ^{13}C and ^{19}F-NMR spectra were recorded in $CDCl_3$ (purchased from Cortecnet) solution in a Bruker 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the residual signal of the solvent used, except in the case of ^{19}F-NMR spectra, which are referred to TFA (δ = -76.55 ppm). Coupling constants (J) are expressed in Hertz (Hz). TLC was carried out on SiO_2 (silica gel 60 F254, Merck), and the spots were located with UV light or by staining with anysaldehyde staining solution, obtained by mixing anylsaldehyde (10 mL), EtOH (180 mL), H_2SO_4 (10 mL) and AcOH (0.4 mL). Purification of compounds by chromatography was performed on a Biotage SP4 automated flash chromatography system, Biotage AB, Uppsala, Sweden or by conventional flash chromatography on SiO_2 (silica gel 60, Merck, 230–400 mesh ASTM). Drying of organic extracts after work-up of reactions was performed over anhydrous Na_2SO_4. Evaporation of solvents was accomplished with a Buchi rotary evaporator. MALDI-TOF mass analyses of new compounds were performed on an Ultraflextreme III time-of-flight mass spectrometer equipped with a pulsed Nd:YAG laser (355 nm) and controlled by FlexControl 3.3 and FlexAnalysis 3.3 softwares (Bruker Daltonics, Bremen , Germany). For other measurements, a time-of-flight mass spectrometer (ESI-TOF) LCT Premier XE from Waters (Milford, MA, USA) with an electrospray ionization source, working in positive / W mode was used.

Synthetic procedures.



*Scheme S1: Synthesis of ligand **2**.*

Compound 10. *Typical procedure*. Perfluoro *tert*-butanol **1** was added to a stirred solution of commercially available alcohol **9** (123 µL, 280 µmol) and triphenylphosphine (149 mg, 560 µmol) in anhydrous THF (3 mL) at room temperature and under argon atmosphere. The so-

[1] M.C. Martos-Maldonado, M.B. Thygesen, K.J. Jensen, A. Vargas-Berenguel, *Eur. J. Org. Chem*., 2013, **14**, 2793.

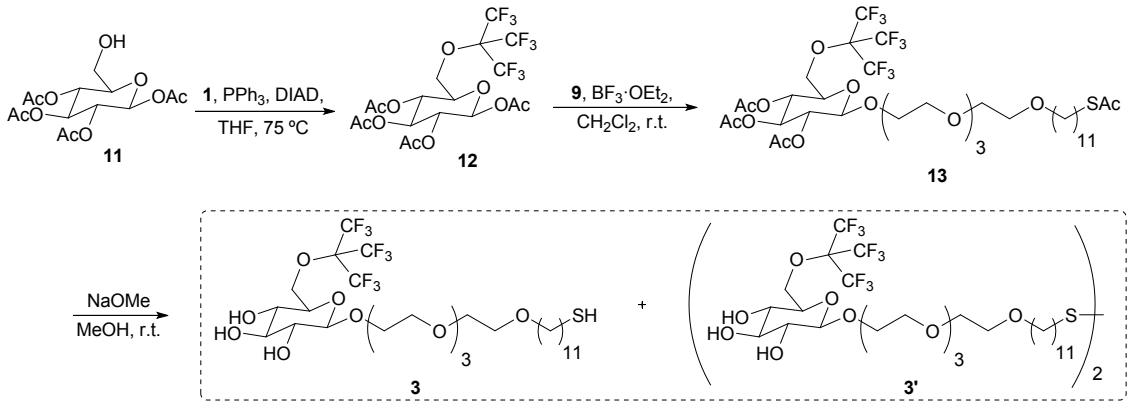
[2] R. Iida, H. Kawamura, K. Niikura, T. Kimura, S. Sekiguchi, Y. Joti, Y. Bessho, H. Mitomo, Y. Nishino, K. Ijiro, *Langmuir* 2015, **31**, 4054.

[3] (a) A.J. Lin, R.A. Sperling, J.K. Li, T.Y. Yang, P.Y. Li, M. Zanella, W.H. Chang, W.J. Parak, *Small*, 2008, **4**, 334; (b) M.T. Fernandez-Arguelles, A. Yakovlev, R.A. Sperling, C. Luccardini, S. Gaillard, A. Sanz-Medel, J.M. Mallet, J.C. Brochon, A. Feltz, M. Oheim, W.J. Parak, *Nano Lett.,* 2007, **7**, 2613.

[4] D. Szabó, J. Mohl, A.-M. Bálint, A. Bodor, J. Rábai, *J. Fluorine Chem*., 2006, **127**, 1496.

obtained mixture was refluxed for at least 5 minutes. Then, a solution of DIAD (116 µL, 560 µmol) in anhydrous THF (250 µL) was added dropwise over 30 minutes while refluxing. After the addition was finished, the reaction mixture was allowed to reflux for another 3 hours. Subsequently, the solvent was evaporated and the crude mixture purified by flash chromatography (5 % Et_2O/CH_2Cl_2) to give compound **10** (157 mg, 87 %) as a colourless oil. ^{1}H NMR (500MHz, $CDCl_3$) δ 4.12 (t, *J* = 5.0 Hz, 2H), 3.70 (t, *J* = 4.9 Hz, 2H), 3.68 – 3.58 (m, 10H), 3.59 – 3.50 (m, 2H), 3.41 (t, *J* = 6.8 Hz, 2H), 2.83 (t, *J* = 7.4 Hz, 2H), 2.28 (s, 3H), 1.53 (p, *J* = 7.1 Hz, 4H), 1.38 – 1.12 (m, 14H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.01, 120.28 (q, $J_{C\text{-}F}$ = 293.4 Hz), 79.71 (q, $J_{C\text{-}F}$ = 30.0 Hz), 71.50, 71.04, 70.64, 70.58, 70.01, 69.38, 69.23, 30.57, 29.58, 29.50, 29.45, 29.42, 29.11, 29.06, 28.77, 26.04; ^{19}F NMR (470 MHz, $CDCl_3$) δ -71.48 (s). HRMS (MALDI): *m/z* calculated for $C_{25}H_{41}F_9O_6SNa$: 663.2372 $[M+Na]^+$; found, 663.2396.

Compound 2. *Typical procedure.* Protected thiol **10** (270 mg, 420 µmol) was dissolved in a mixture of HCl (37% v/v, 920 µL) and EtOH (7.6 mL), and stirred for 14 hours at 70 °C. Afterwards, the reaction mixture was allowed to reach room temperature, the pH neutralised with NH_4OH and the product extracted 3 times with CH_2Cl_2. The organic layer was dried and concentrated in vacuo. Compound **2** was obtained as a colourless oil (241 mg, 96 %) as a thiol:disulfide mixture (24:1), and was used without further purification. To keep the product as much as possible as a thiol and avoid disulfide formation, compound **2** was stored at 4 °C and under argon atmosphere. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.11 (t, *J* = 4.9 Hz, 2H), 3.69 (t, *J* = 4.9 Hz, 2H), 3.64 – 3.57 (m, 10H), 3.55 – 3.50 (m, 2H), 3.40 (t, *J* = 6.8 Hz, 2H), 2.47 (q, *J* = 7.4 Hz, 2H), 1.54 (dp, *J* = 14.0, 7.1 Hz, 4H), 1.33 (t, *J* = 7.5 Hz, 2H), 1.30 – 1.18 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.29 (q, $J_{C\text{-}F}$ = 293.7 Hz), 79.71 (q, $J_{C\text{-}F}$ = 29.6 Hz), 123.78, 121.45, 119.12, 116.82, 116.79, 80.07, 79.83, 79.59, 79.36, 77.00, 71.50, 71.05, 70.63, 70.58, 70.02, 69.38, 69.23, 34.02, 29.59, 29.52, 29.47, 29.44, 29.03, 28.34, 26.04, 24.61; ^{19}F NMR (470 MHz, $CDCl_3$) δ -71.48 (s). HRMS (MALDI): *m/z* calculated for $C_{46}H_{76}F_{18}O_{10}S_2Na$: 1217.4485 $[M+Na]^+$; found, 1217.4409.



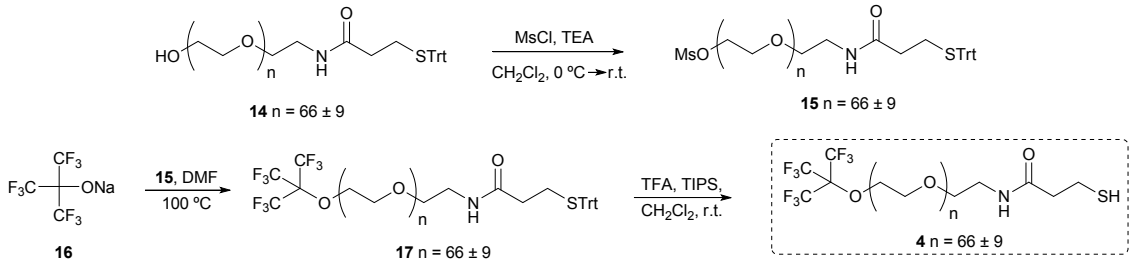
*Scheme S2: Synthesis of ligand **3**.*

Compound 12. *Typical procedure*. Perfluoro *tert*-butanol **1** was added to a stirred solution of commercially available glucose derivative **11** (100 mg, 280 µmol) and triphenylphosphine (149 mg, 560 µmol) in anhydrous THF (3 mL) at room temperature and under argon atmosphere. The so-obtained mixture was refluxed for at least 5 minutes. Then, a solution of DIAD (116 µL, 560 µmol) in anhydrous THF (250 µL) was added dropwise over 20 minutes while refluxing.

After the addition was finished, the reaction mixture was allowed to reflux for another 3 hours. Subsequently, the solvent was evaporated and the crude mixture purified by flash chromatography (5 % Et_2O/CH_2Cl_2) to give compound **12** (159 mg, 99.9 %) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.71 (d, *J* = 8.1 Hz, 1H), 5.24 (t, *J* = 9.3 Hz, 1H), 5.16 – 5.09 (m, 2H), 4.12 (m, 2H), 3.86 (ddd, *J* = 10.1, 4.1, 2.9 Hz, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.15, 169.15, 169.07, 168.85, 120.06 (q, J_{C-F} = 293.5 Hz), 91.53, 79.56 (q, J_{C-F} = 29.3 Hz), 72.87, 72.77, 70.01, 67.87, 67.55, 20.68, 20.51, 20.27. ^{19}F NMR (470 MHz, $CDCl_3$) δ -71.48 (s). HRMS (MALDI): *m/z* calculated for $C_{18}H_{19}F_9O_{10}Na$: 589.0727 $[M+Na]^+$; found, 589.0750.

Compound 13. *Typical procedure*. $BF_3{\cdot}OEt_2$ (75 µL, 610 µmol) was added to a mixture of **12** (150 mg, 260 µmol) and **9** (128 µL, 290 µmol) in dry CH_2Cl_2 (2.1 mL) at room temperature and under argon atmosphere. After 6 hours, unreacted $BF_3{\cdot}OEt_2$ was quenched by slow addition of triethylamine, until pH ≥ 7. The reaction mixture was then washed 3 times with CH_2Cl_2, the organic extracts were dried and the solvent removed in vacuo. The residue was purified by consecutive flash chromatography (firstly with 50 % Hexanes/AcOEt; secondly with 30 % $AcOEt/CH_2Cl_2$) to give compound **13** (93 mg, 38 %) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.20 (t, *J* = 9.4 Hz, 1H), 5.02 – 4.93 (m, 2H), 4.61 (d, *J* = 7.9 Hz, 1H), 4.15 – 4.02 (m, 2H), 3.90 (dt, *J* = 11.1, 4.3 Hz, 1H), 3.73 (ddd, *J* = 11.3, 6.4, 3.3 Hz, 2H), 3.68 – 3.59 (m, 10H), 3.57 (dd, *J* = 5.9, 3.8 Hz, 2H), 3.44 (t, *J* = 6.8 Hz, 2H), 2.85 (t, *J* = 7.4 Hz, 2H), 2.31 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.56 (m, 4H), 1.39 – 1.14 (m, 14H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.99, 170.19, 169.28, 120.10 (q, J_{C-F} = 293.4 Hz), 100.63, 79.61 (q, J_{C-F} = 30.0 Hz) 72.70, 72.36, 71.49, 71.10, 70.59, 70.54, 70.19, 70.00, 68.84, 68.65, 29.66, 29.59, 29.50, 29.45, 29.40, 29.10, 29.06, 28.76, 26.04, 20.62, 20.55, 20.33. ^{19}F NMR (470 MHz, $CDCl_3$) δ -71.48 (s). HRMS (MALDI): *m/z* calculated for $C_{37}H_{57}F_9O_{14}SNa$: 951.3217 $[M+Na]^+$; found, 951.3221.

Compound 3 and 3'. *Typical procedure*: Protected compound **13** (50 mg, 54 µmol) was dissolved in CH_2Cl_2 (400 µL) and MeOH (1.3 mL). Sodium methoxide (3 mg, 54 µmol) was added and the reaction stirred at room temperature for 4.5 hours. Afterwards, the reaction mixture was neutralised with Amberlist IR-120 H^+, filtered and evaporated to dryness. Compounds **3** and **3'** were obtained as a pale yellow oil (40 mg, 98 %, **3**:**3'** = 35:65) and were used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.46 – 4.30 (m, 2H), 4.15 (t, *J* = 8.2 Hz, 1H), 3.96 (d, *J* = 10.3 Hz, 1H), 3.79 – 3.67 (m, 2H), 3.68 – 3.61 (m, 11H), 3.61 – 3.55 (m, 2H), 3.55 – 3.46 (m, 2H), 3.44 (t, *J* = 6.9 Hz, 2H), 3.42 – 3.32 (m, 2H), 2.68 (t, *J* = 7.5 Hz, 2H, -C*H*$_2$-S-S), 2.52 (q, *J* = 7.4 Hz, 2H, -C*H*$_2$-SH), 1.67 (p, *J* = 7.5 Hz, 2H), 1.64 – 1.48 (m, 2H), 1.45 – 1.10 (m, 14H).; ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.28 (q, J_{C-F} = 293.5 Hz), 102.72, 79.69 (q, J_{C-F} = 29.5 Hz), 74.42, 73.25, 71.51, 70.45, 70.40, 70.32, 70.25, 70.19, 69.91, 69.80, 69.23, 68.06, 39.24, 34.02, 29.69, 29.54, 29.50, 29.44, 29.19, 26.01, 24.63, 22.67. ^{19}F NMR (470 MHz, $CDCl_3$) δ -71.48 (s). HRMS (MALDI): *m/z* calculated for $C_{58}H_{96}F_{18}O_{20}S_2Na$: 1541.5541 $[M+Na]^+$; found, 1541.5613.



*Scheme S3: Synthesis of ligand **4**.*

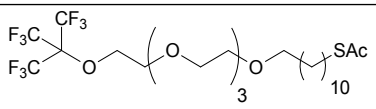
Compound 15. *Typical procedure*. Commercially available PEG derivative **14** (400 mg, 120 µmol) was dissolved in CH_2Cl_2 (1.2 mL) and TEA (98 µL, 710 µmol) was added. Subsequently, MsCl (42 µL, 540 µmol) was added dropwise at 0 °C. The reaction was allowed to reach room temperature slowly and stirred for 6 hours in total since mesyl chloride addition. The crude mixture was washed 4 times with a saturated solution of NH_4Cl, and then twice with brine. The organic layer was dried and the solvent removed in vacuo. Mesylated compound **15** was obtained as a pale yellow solid (409 mg, 99.9%) and was used without further purification in the next step. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, *J* = 7.7 Hz, 6H), 7.19 (t, *J* = 7.6 Hz, 6H), 7.11 (t, *J* = 7.3 Hz, 3H), 6.01 (t, *J* = 5.3 Hz, 1H), 4.32 – 4.24 (m, 2H), 3.55 (m, PEG), 3.28 (q, *J* = 5.3 Hz, 2H), 3.00 (s, 2H), 2.40 (t, *J* = 7.4 Hz, 2H), 1.97 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.71, 144.50, 129.36, 127.69, 126.44, 70.41, 70.34, 70.24, 70.02, 69.55, 69.11, 68.79, 39.01, 37.51, 35.16, 27.50. HRMS (ESI): *m/z* calculated for $C_{157}H_{291}NO_{70}S_2Na_3$: 1147.9455 $[M+3Na]^{3+}$; found 1147.9548.

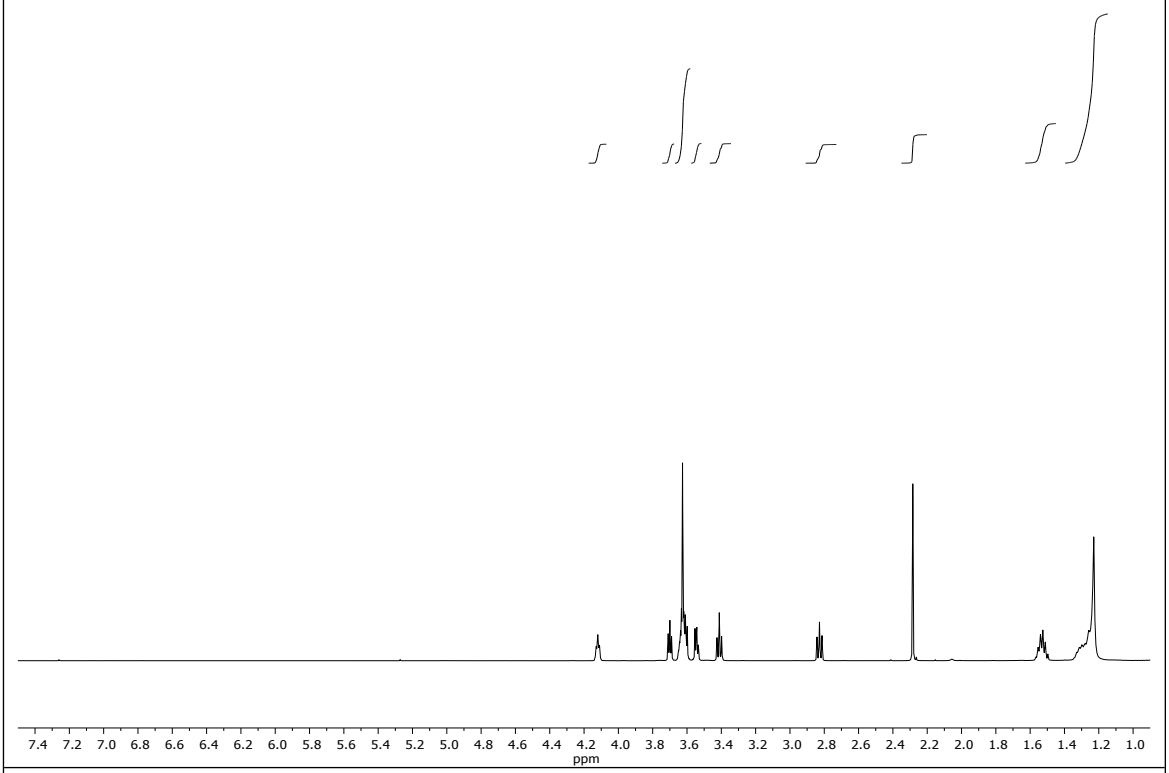
Compound 17. *Typical procedure*. Mesylated compound **15** (296 mg, 87 µmol) was dissolved in DMF (1.8 mL) and **16**4 was added (85 mg, 330 µmol). The mixture was stirred at 100 °C for 14 hours. The crude mixture was allowed to reach room temperature and washed 5 times with a saturated solution of NH_4Cl. The organic layer was dried and the solvent removed in vacuo. Compound **17** was obtained as a pale orange solid (281 mg, 91 %) and was used without further purification in the next step. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, *J* = 7.7 Hz, 6H), 7.24 (d, *J* = 7.8 Hz, 6H), 7.17 (t, *J* = 7.0 Hz, 2H), 6.06 (br s, 1H), 4.21 – 4.04 (m, 2H), 3.93 – 3.17 (m, PEG), 2.46 (t, *J* = 7.5 Hz, 2H), 2.02 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.85, 144.62, 129.49, 127.81, 126.56, 120.21 (q, $J_{C\text{-}F}$ = 293.8 Hz), 83.71, 70.97, 70.46, 70.14, 69.72, 69.38, 69.30, 39.14, 35.30, 27.63. ^{19}F NMR (470 MHz, $CDCl_3$) δ -71.48 (s). HRMS (ESI): *m/z* calculated for $C_{160}H_{292}F_9N_4O_{68}S$: 1189.6567 $[M+3NH_4]^{3+}$; found 1189.6462.

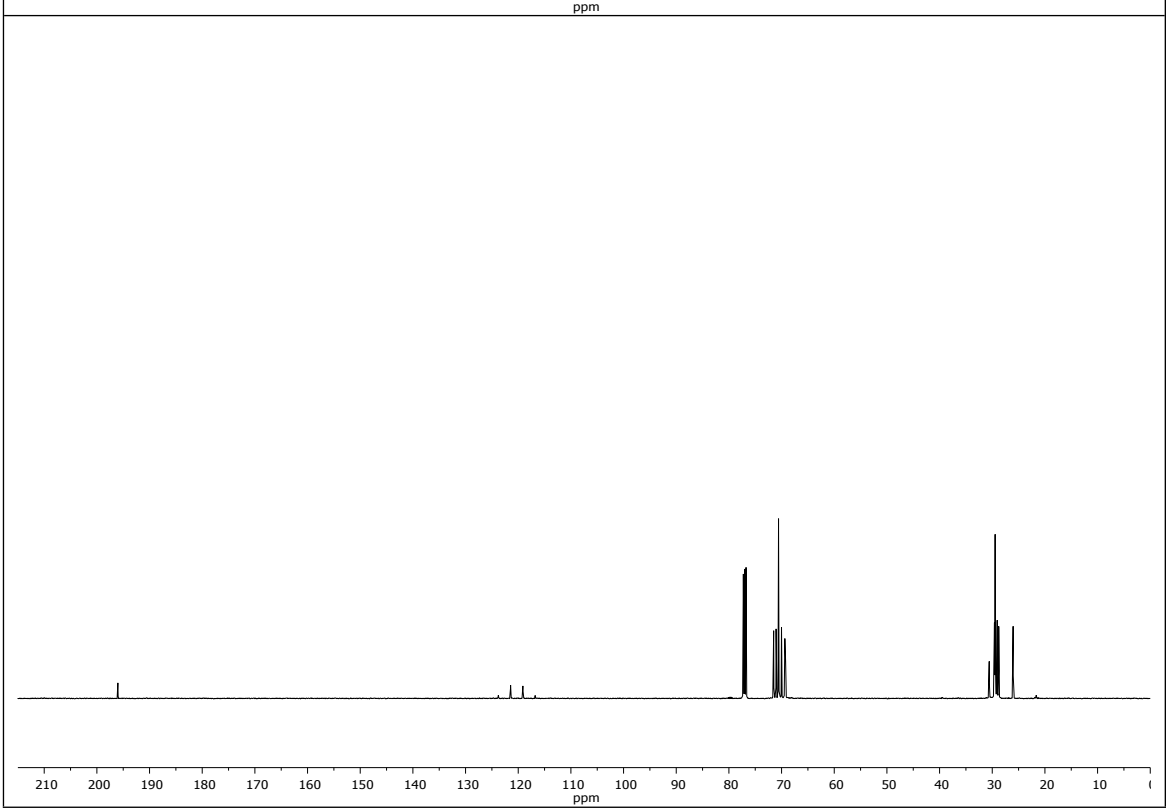
Compound 4. *Typical procedure*. Compound **17** (218 mg, 62 µmol) was dissolved in CH_2Cl_2 (7.4 mL) and TFA (454 µL, 620 µmol) was added, followed by TIPS (624 µL, 310 µmol). Subsequently, TFA (454 µL, 620 µmol) and TIPS (624 µL, 310 µmol) were added again to the reaction mixture and it was then allowed to stir for 15 minutes at room temperature. All liquids were completely removed under high vacuum and the so-obtained residue was triturated with Et_2O and filtered washing with Et_2O. Compound **4** was obtained as a white solid (189 mg, 90%). To keep the product as a thiol and avoid disulfide formation, compound **4** was stored at 4 °C and under argon atmosphere. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (bs, 1H), 4.12 (t, *J* = 4.9 Hz, 2H), 3.75 (d, *J* = 4.3 Hz, 2H), 3.70 (t, *J* = 4.9 Hz, 2H), 3.61 – 3.53 (m, PEG), 3.46 (t, *J* = 4.9 Hz, 2H), 3.42 (q, *J* = 5.2 Hz, 2H), 2.77 (q, *J* = 7.2 Hz, 2H), 2.50 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.12, 119.28 (q, $J_{C\text{-}F}$ = 298.7 Hz), 83.46, 70.90, 70.43, 70.39, 70.32, 70.22,

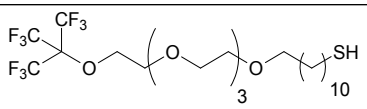
69.94, 69.75, 69.27, 40.03, 39.09, 20.44; ^{19}F NMR (470 MHz, $CDCl_3$) δ -71.48 (s). HRMS (MALDI): *m/z* calculated for $C_{141}H_{274}F_9NO_{68}SNa$: 3295.7483 $[M+Na]^+$; found, 3295.7485.

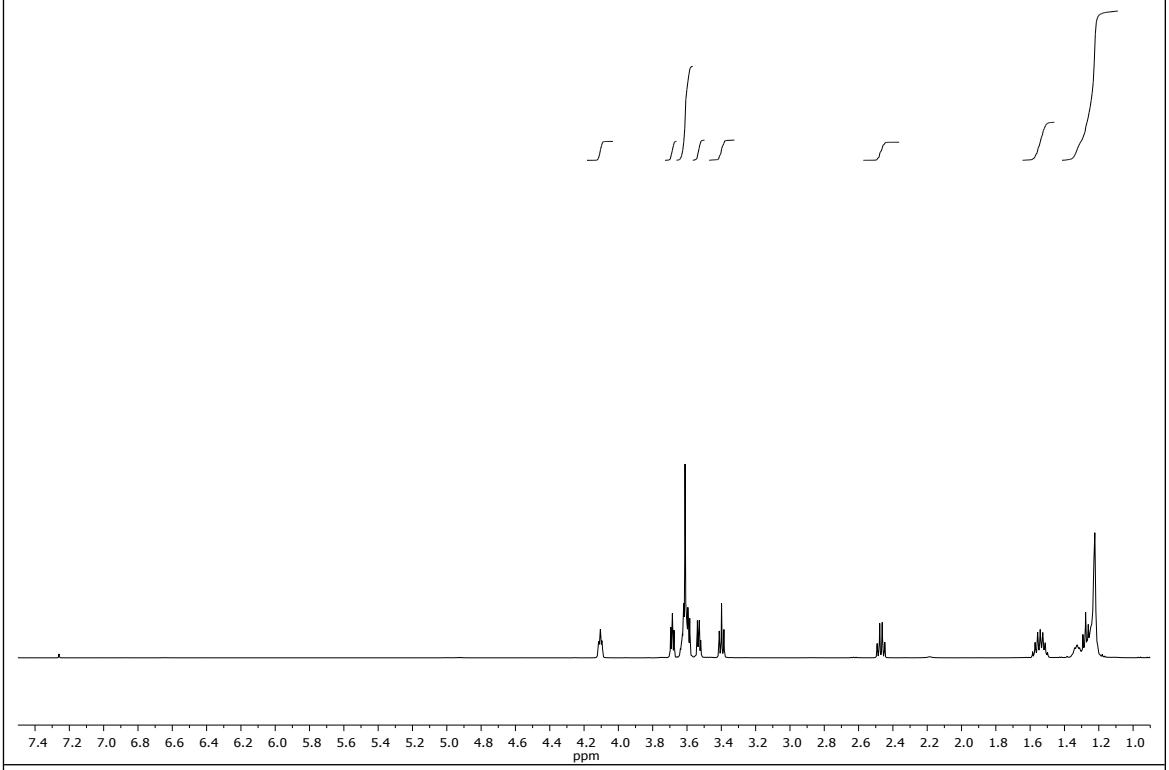
2. ^{1}H and ^{13}C NMR spectra of new compounds.

Compound **10**	





Compound **2**	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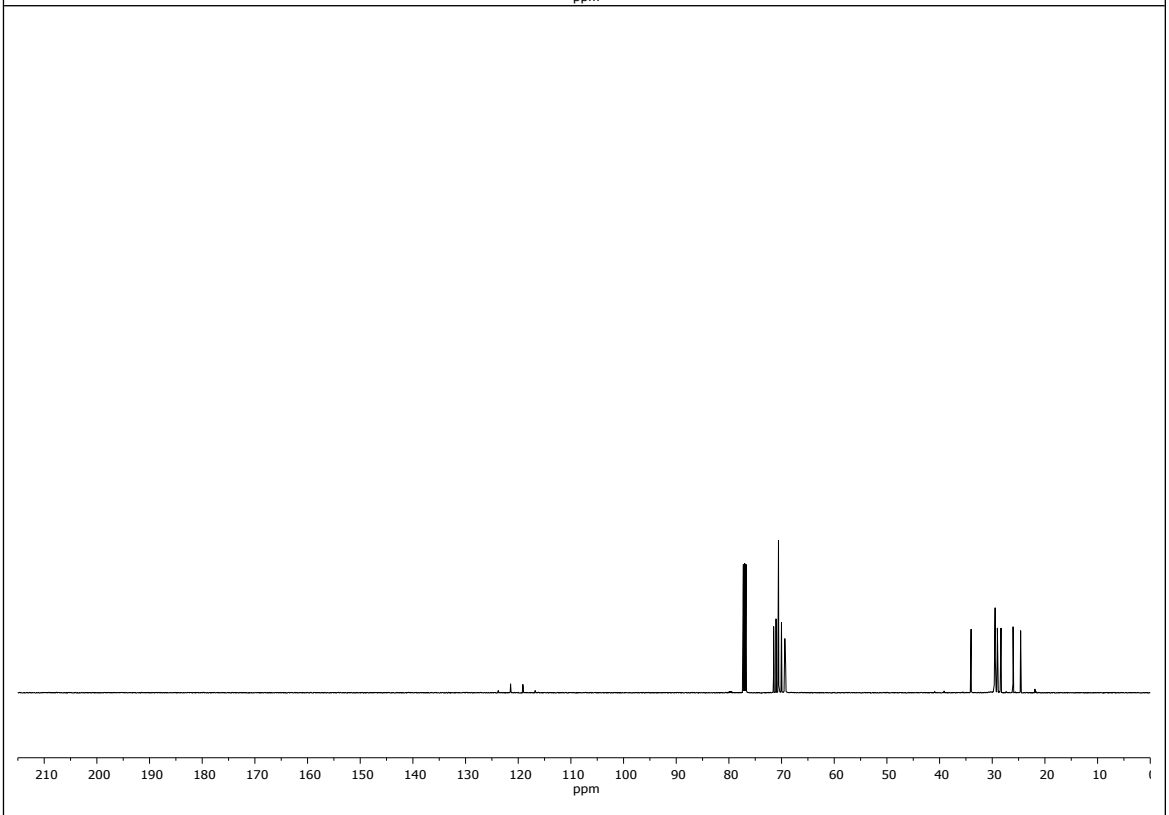
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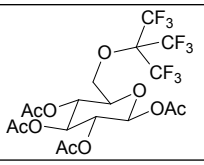
Compound **12**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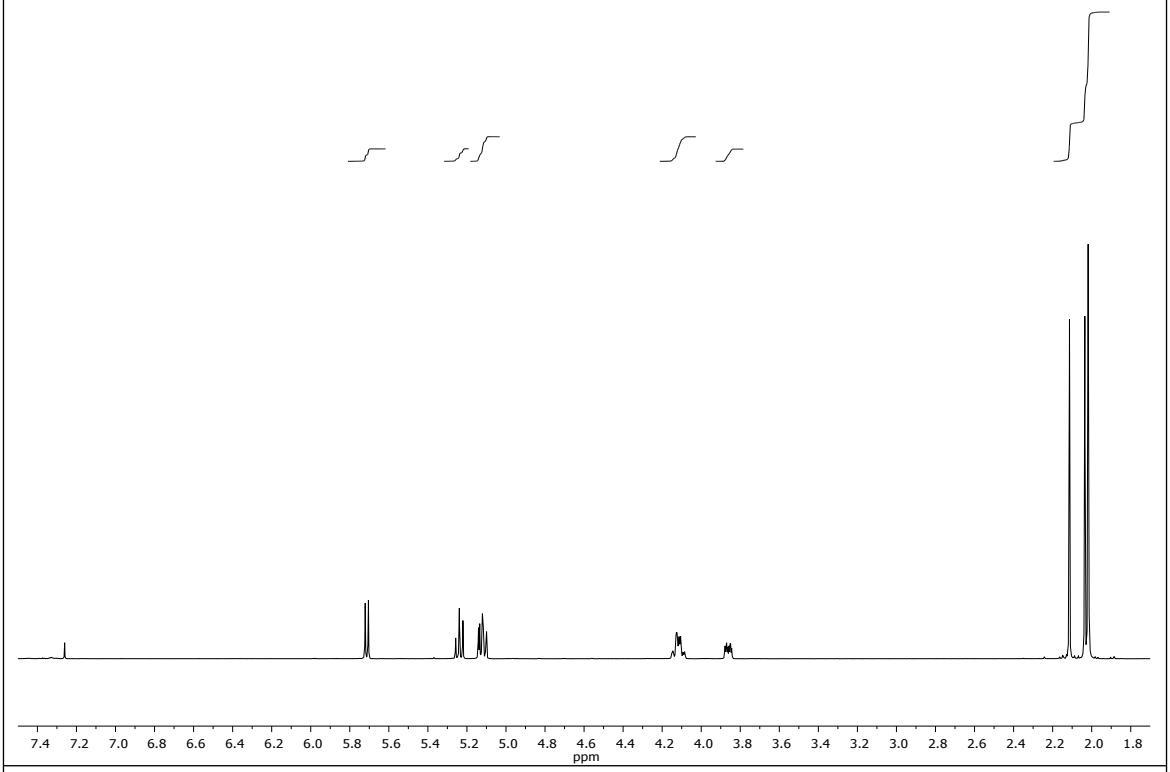
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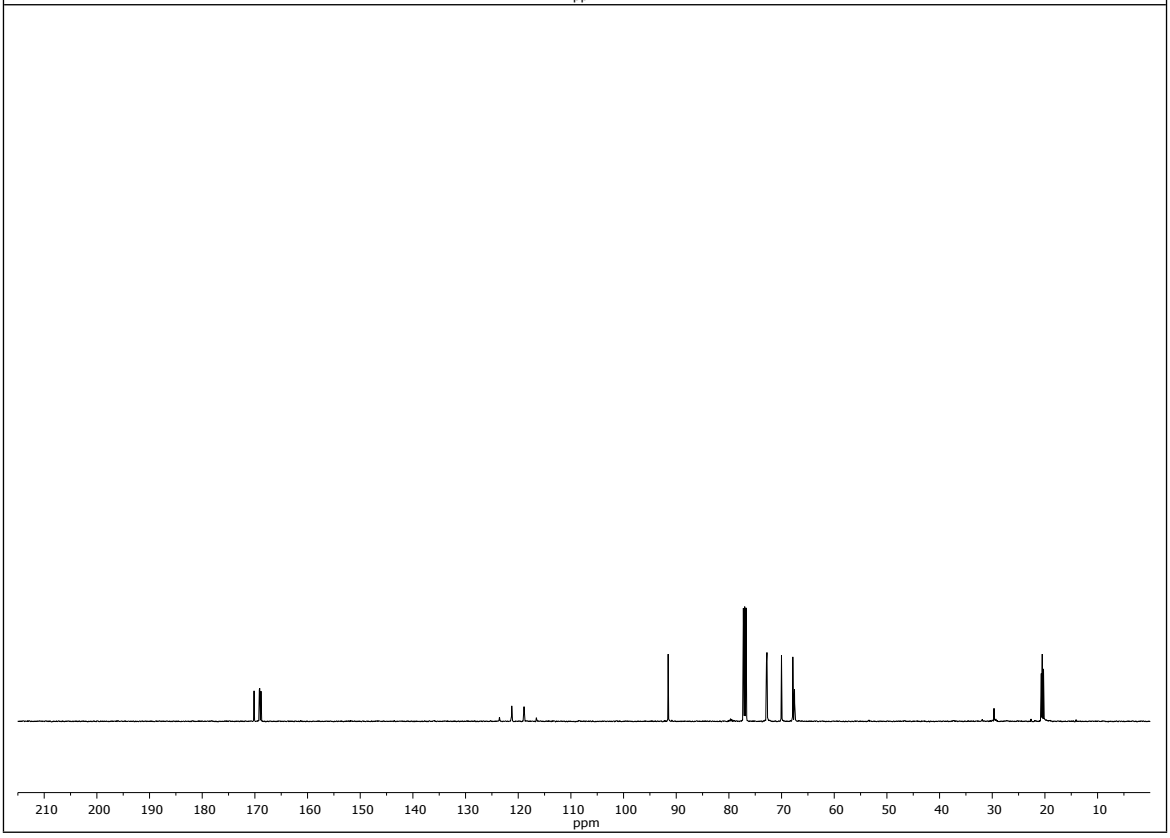
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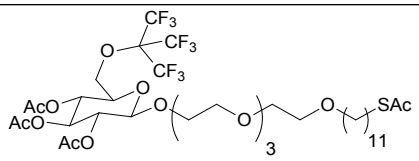
Compound **13**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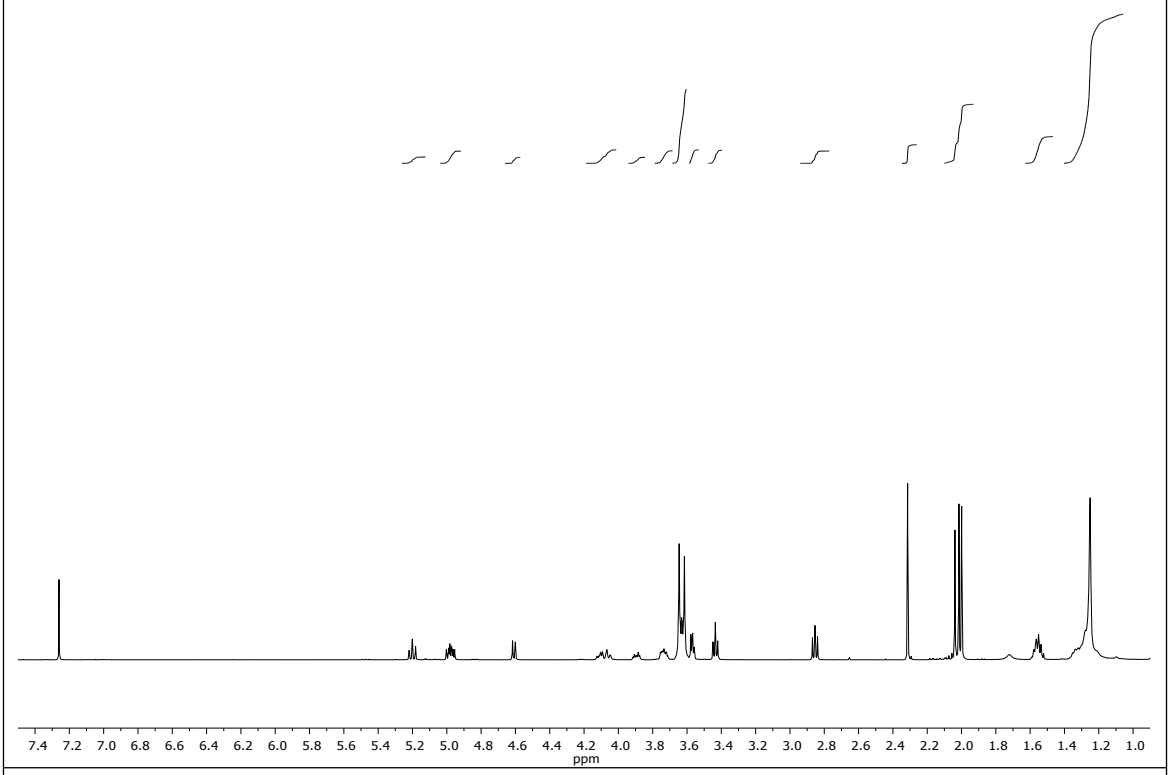
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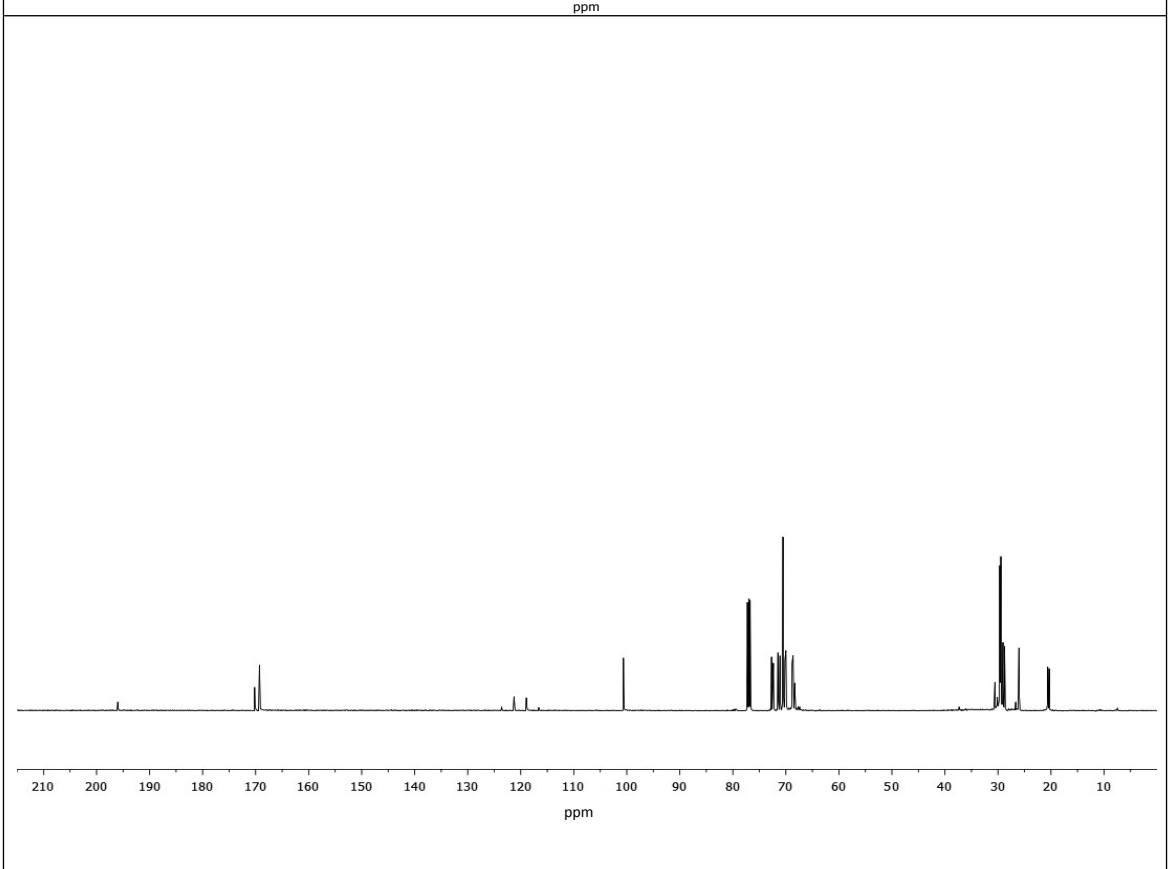
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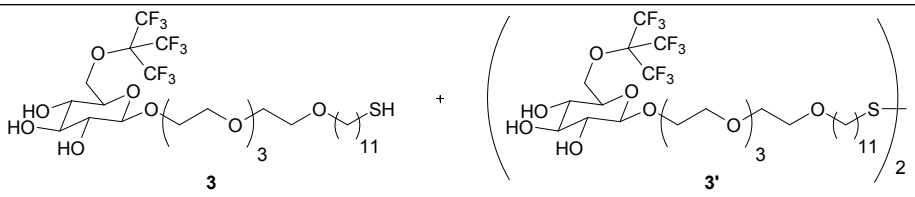
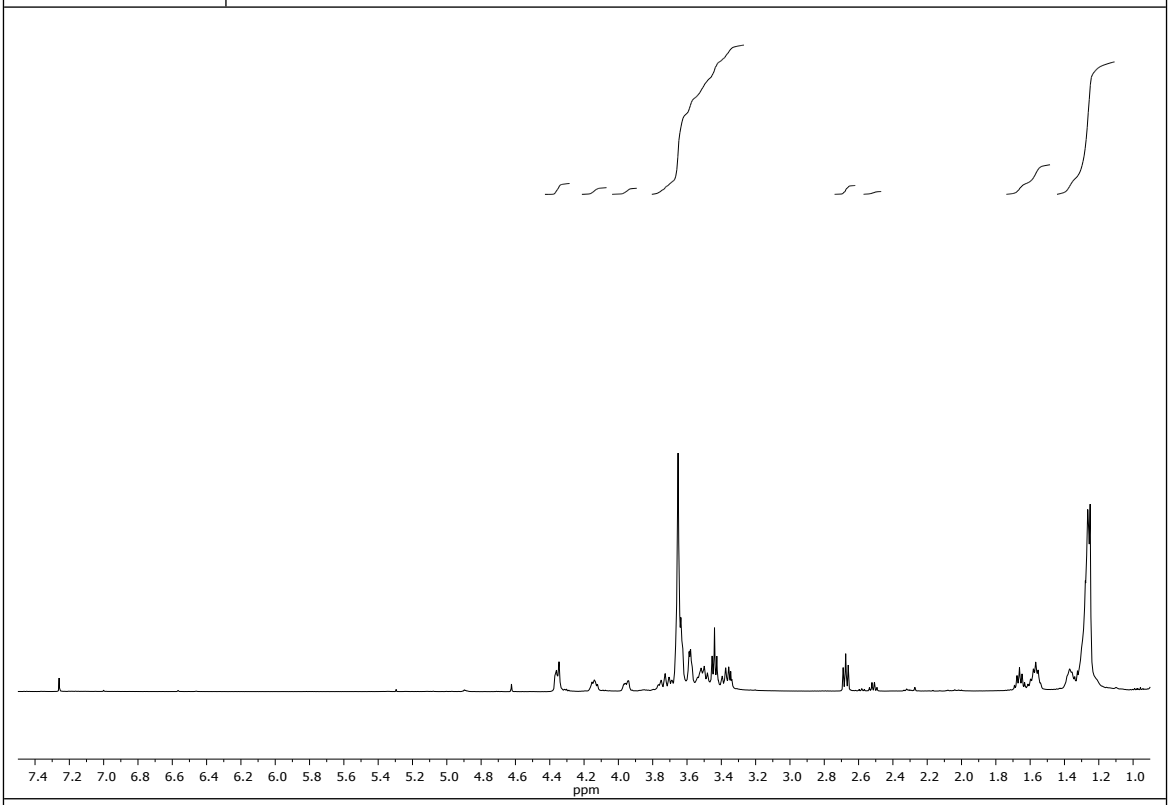
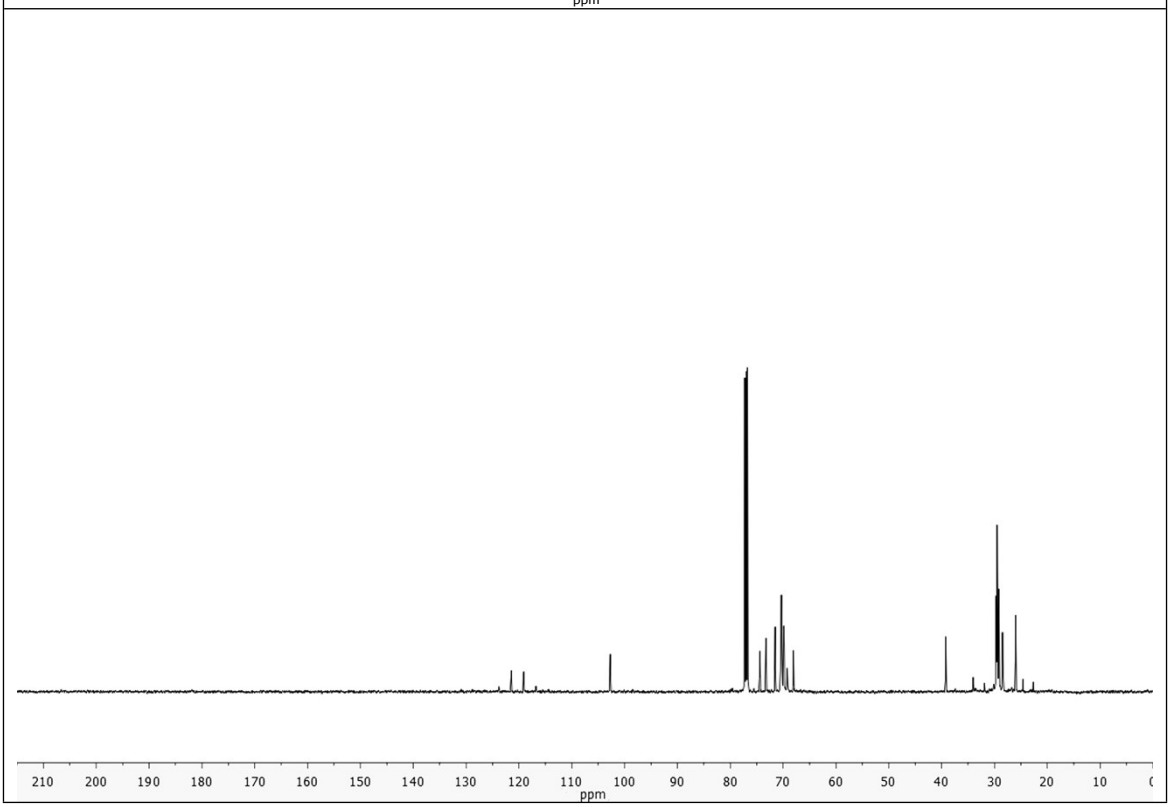
Compound 3 and 3’	





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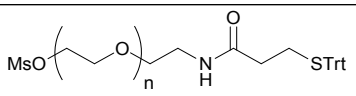
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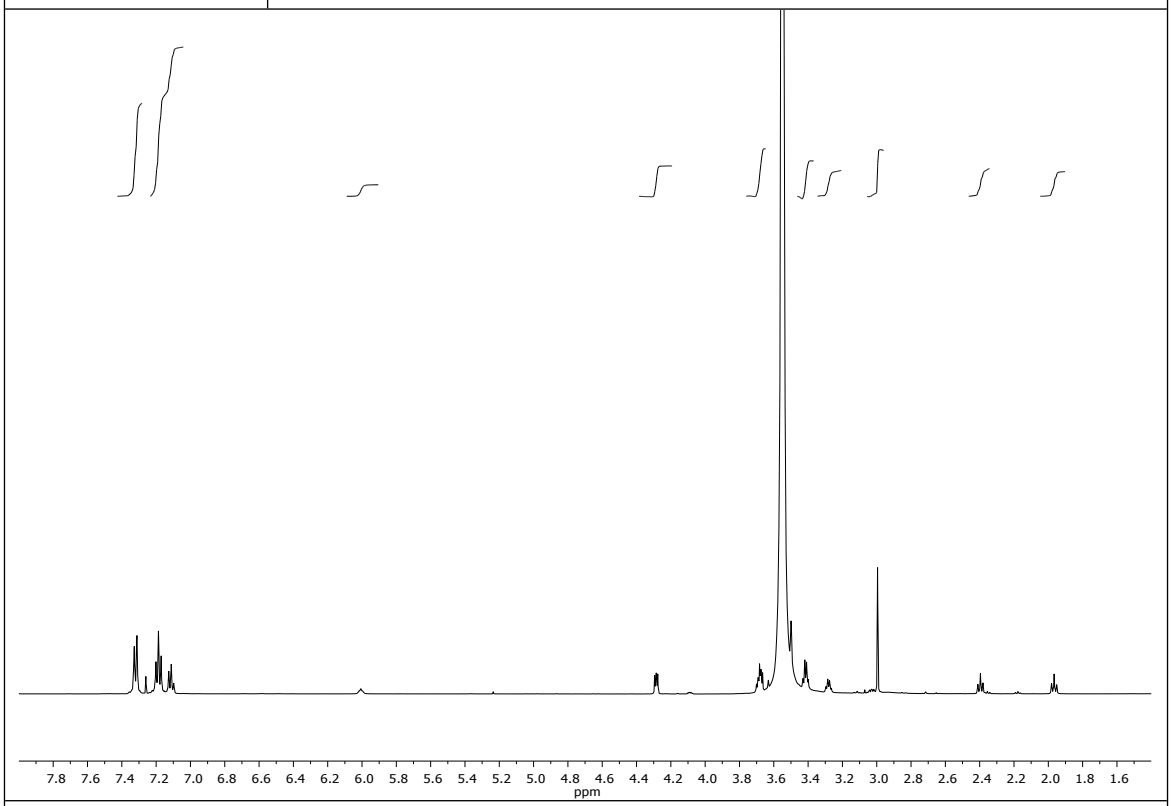
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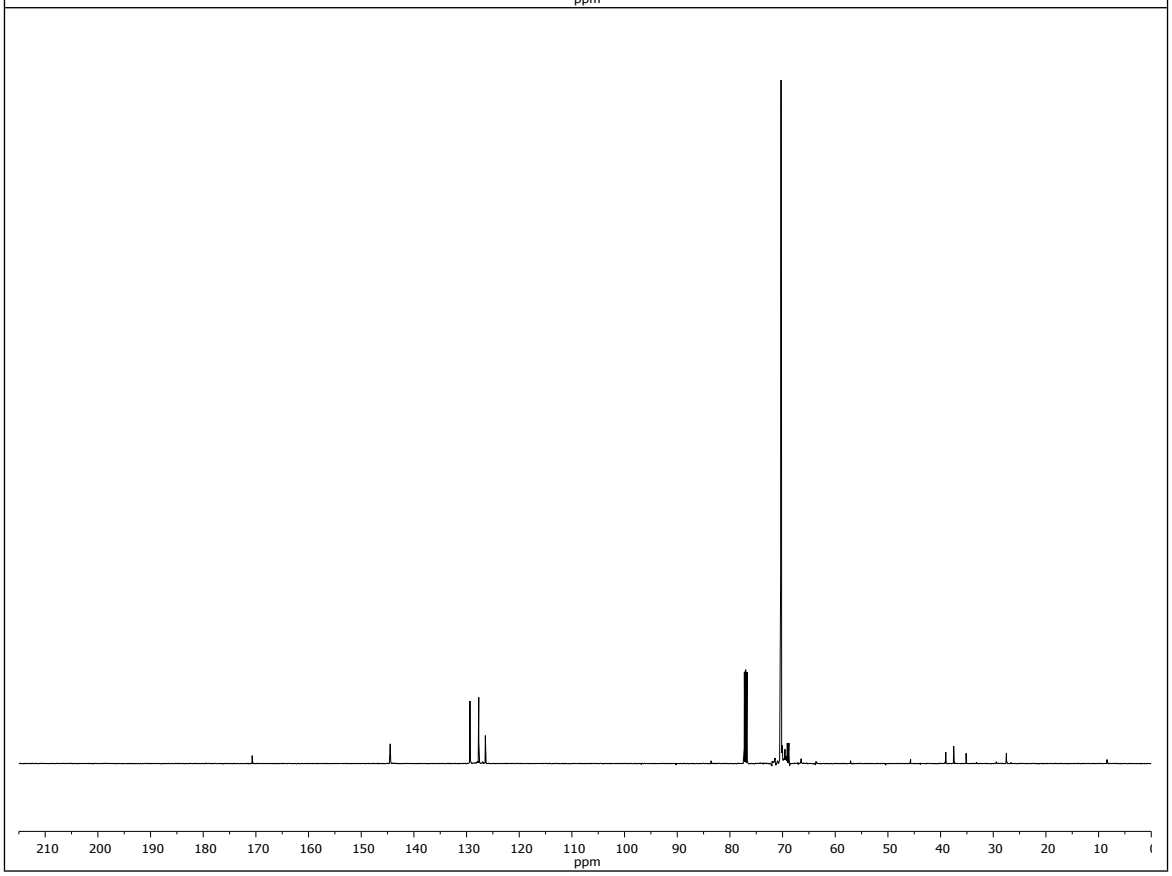
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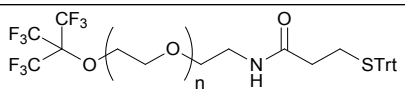
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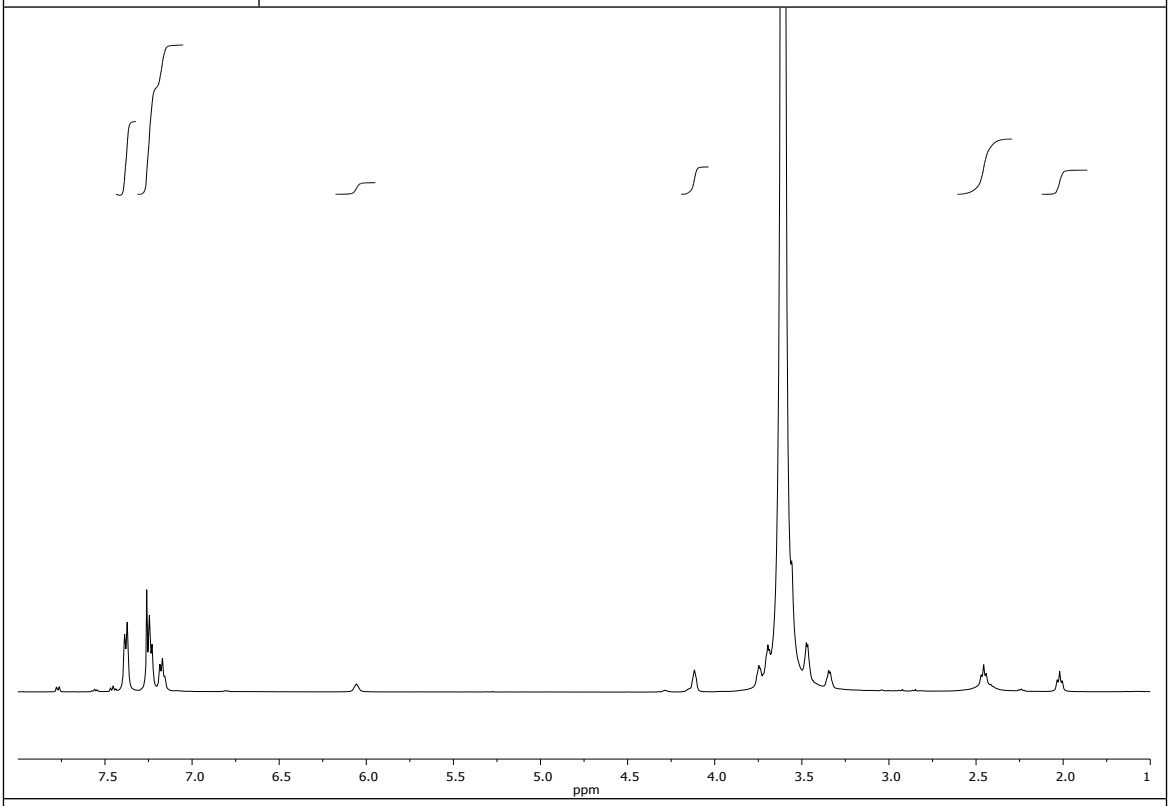
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2.0
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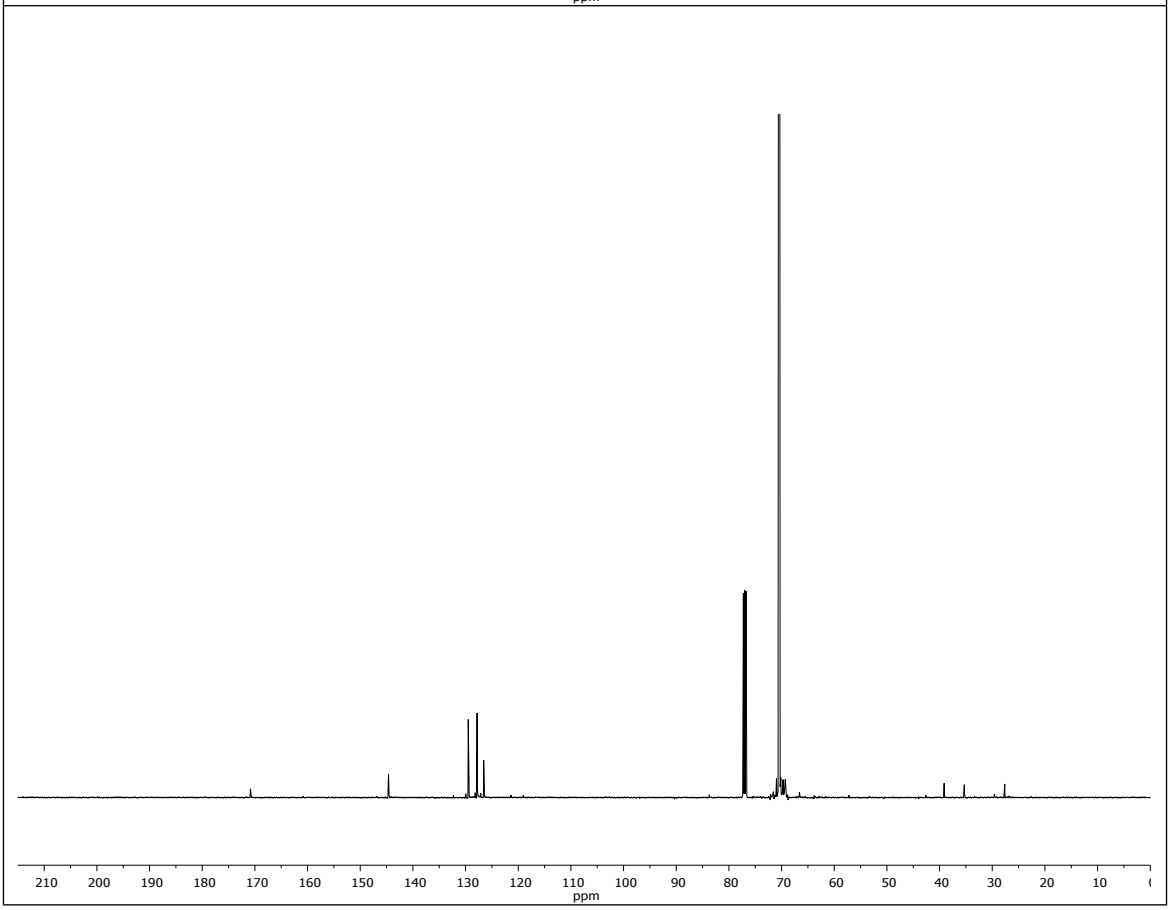
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200
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160
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110
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90
80
70
60
50
40
30
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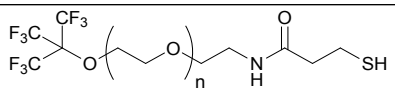
ppm

Compound 4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CF3
F3C
O
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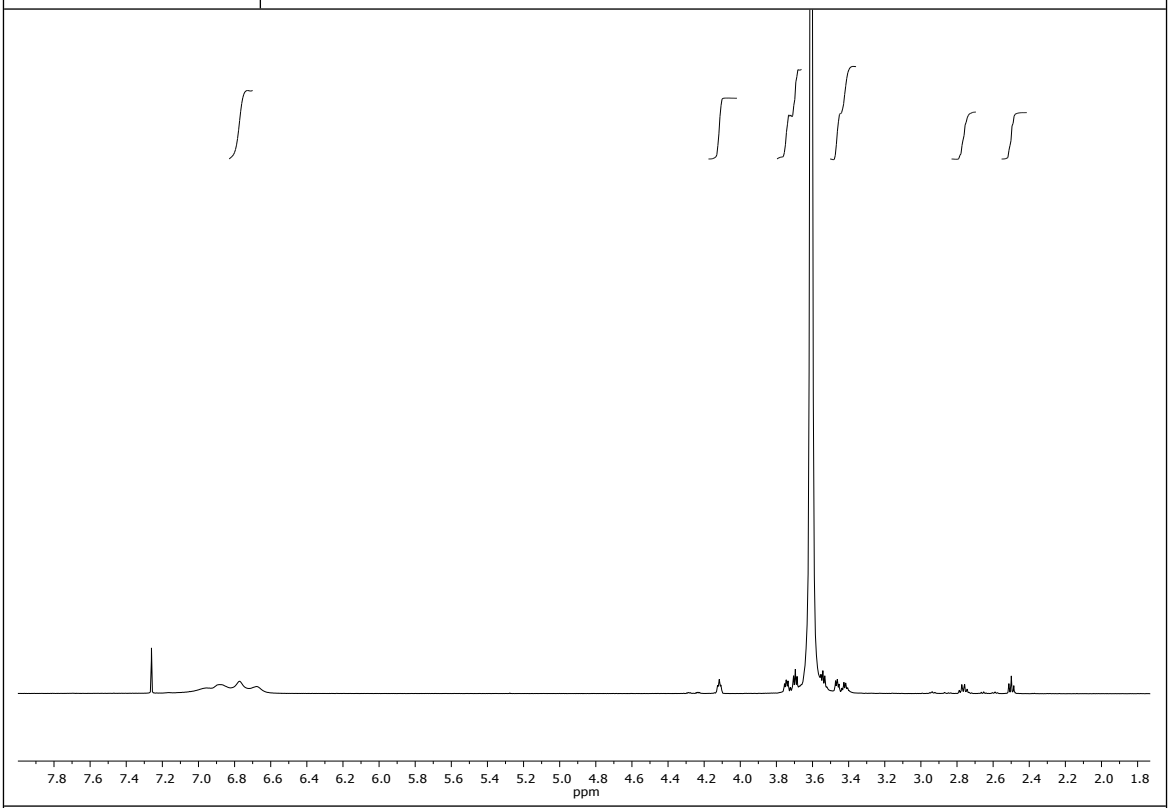
n
N
H
O
SH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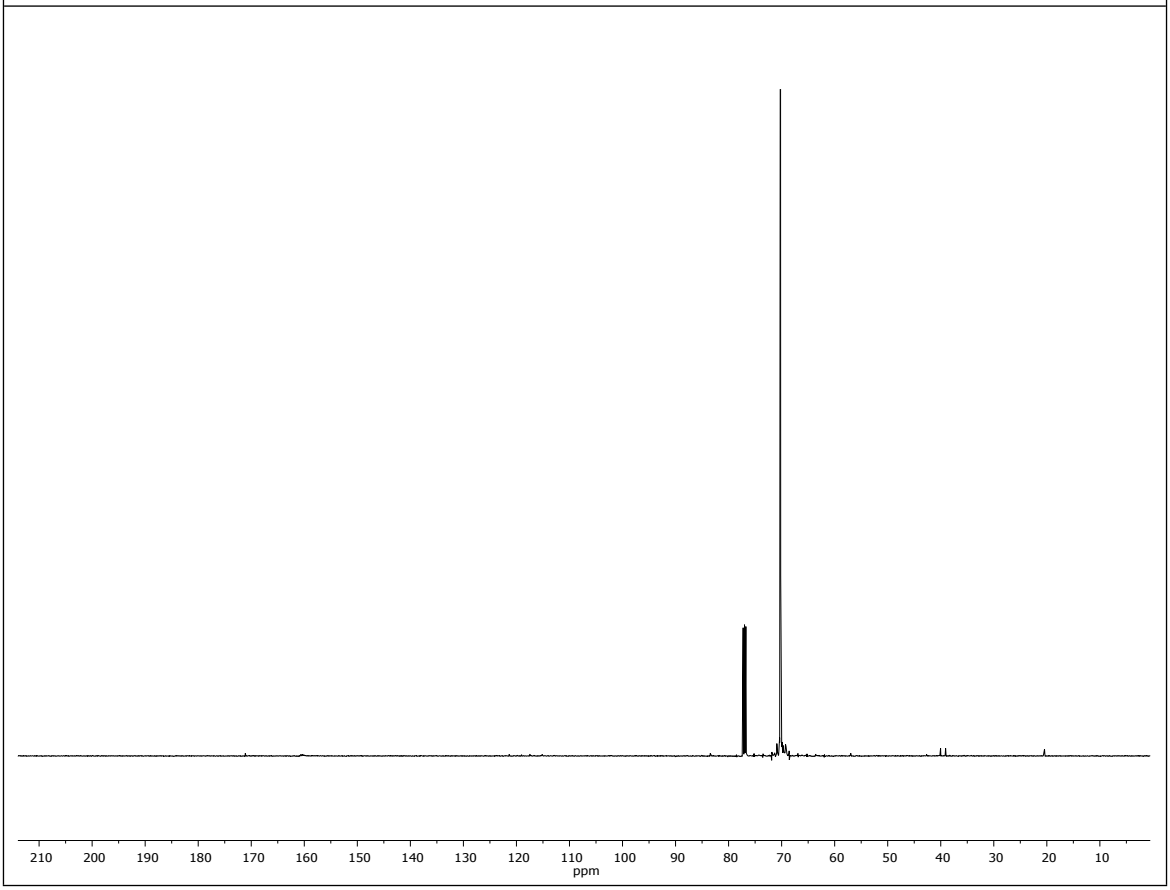
ppm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ppm

3. NP synthesis and characterization.

General considerations. $HAuCl_4$ was purchased from Strem Chemicals and $NaBH_4$ from Sigma Aldrich. Solvents (analysis quality) were purchased from Panreac and used as received. Water is MilliQ quality. A Sigma bench centrifuge was used for centrifugations at speed smaller than 1.4 x 10^4 RCF. A Beckmann Coulter floor ultracentrifuge was used for centrifugations at speed greater than 5 x 10^4 RCF. UV-Vis absorption spectra were measured in a Varian spectrophotometer. Transmission electron microscopy (TEM) images were acquired in both JEOL JEM 2100F and JEOL JEM-1400PLUS microscopes by deposition of the sample on top of a copper grid coated with a layer of carbon. Size measurement was performed with free ImageJ software and size distribution analysis and histogram production was done with Origin software.

Synthesis of NP2-4, NP2A-C and NP3A-B. *General procedure*: The corresponding ligands **2**-**7** dissolved in CH_2Cl_2 (0.45-4 equiv., see Table S1-S3) were added under stirring onto CH_2Cl_2 (8 mL/µmol of $HAuCl_4$). Subsequently, a solution of $NaBH_4$ (0.1 M, 5-7.5 equiv., see Table S2) was freshly prepared with ice-cold MeOH and allowed to stand for 1 minute. During that minute, a freshly prepared solution of $HAuCl_4$ (25 mM, 1 equiv.) in ice-cold methanol was added to the reaction mixture. One minute after the preparation of $NaBH_4$ solution, the latter was added dropwise to the ligand/gold mixture. The reaction was stirred for 30 seconds and then allowed to stand for 14 hours without stirring and capped to avoid solvent evaporation. On next day, the solvent was evaporated in the rotary evaporator and the so-obtained NPs were purified by centrifugation until no free ligand was detected by ^{1}H-NMR. See Table S4 for centrifugation details for each NP. The synthesis workflow is depicted in Figure S1. The so-obtained NPs were characterized by UV-Vis, TEM and ^{19}F-NMR.

Synthesis of NP2D. *Typical procedure*. Starting **NP2** was prepared following the general procedure described above starting from 31250 nmol of $HAuCl_4$. The so-obtained NPs were purified by centrifugation as described in Table S4 and then resuspended in approximately 125 mL of CH_2Cl_2. Subsequently a solution of **8** in CH_2Cl_2 (5 mL, 0.5 M) was added to **NP2** and the mixture sonicated for 2 minutes in a sonication bath. Then, the solvent was slowly removed in the rotary evaporator (350-450 mm Hg). NaOH (0.1 M) was added to the resulting dry mixture until NPs were completely solubilised with the help of sonication. The latter solution was centrifuged twice at 1.4 x 10^4 RCF for 5 minutes to remove the bigger aggregates and then centrifuged again 3 times at 5.15 x 10^4 RCF for 30 minutes to remove excess of **8**. The so-obtained pellet was reconstituted in water and loaded into a 1 % agarose gel for purification by gel electrophoresis (125 V, 30 min), as described before.3

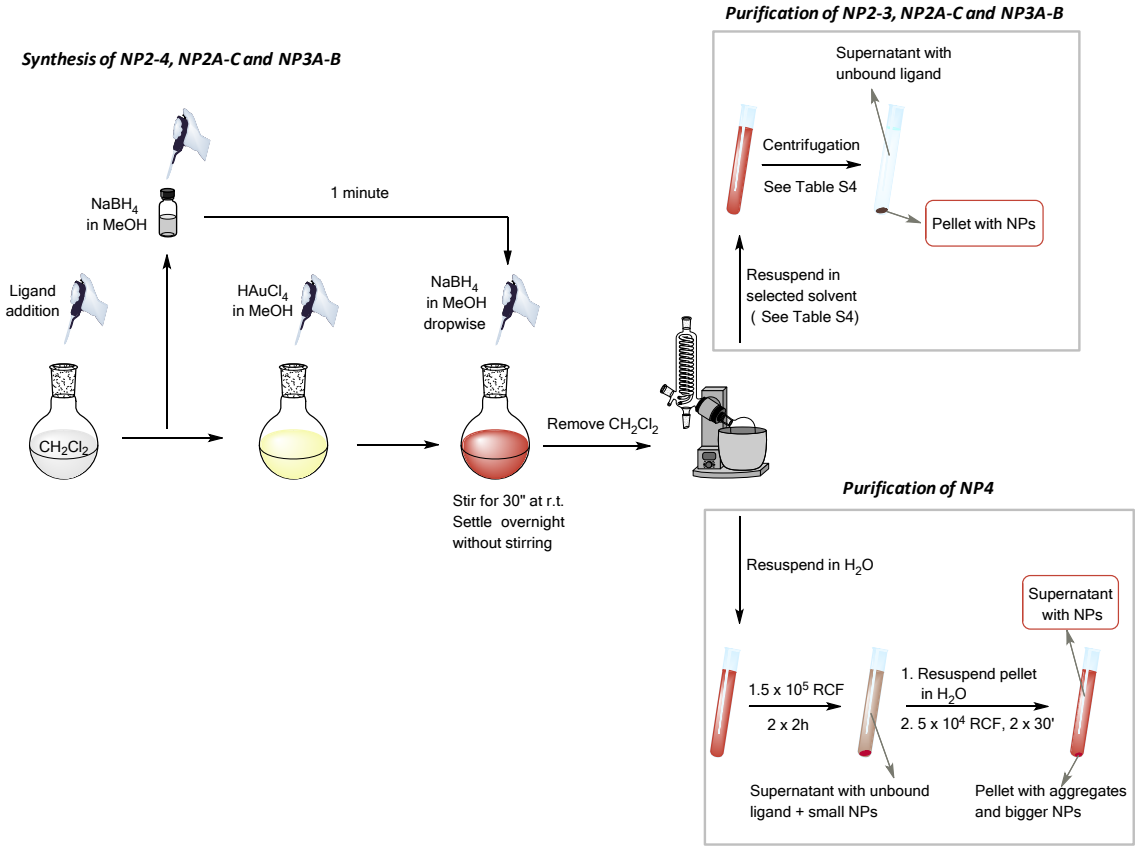

Figure S1: Schematic representation of the workflow used to prepare NP2-4, NP2A-C and NP3A-B.

*Table S1. Percentage of each ligand used to prepare **NP2-4**, **NP2A-C** and **NP3A-B**.*

Ligand / NP	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)
NP2	100	-	-	-	-	-
NP2A	50	-	-	50	-	-
NP2B	50	-	-	-	50	-
NP2C	85	-	-	-	-	15
NP3	-	100	-	-	-	-
NP3A	-	50	-	50	-	-
NP3B	-	75	-	25	-	-
NP4	-	-	100	-	-	-

*Table S2. Ligand and $NaBH_4$ equivalents with respect to $HAuCl_4$ used to prepare **NP2-4**, **NP2A**-**C** and **NP3A**-**B**.*

	NP2	**NP2A**	**NP2B**	**NP2C**	**NP3**	**NP3A**	**NP3B**	**NP4**
2-7 (equiv.)*	**2** (3)	**2**+**5** (4)	**2**+**6** (4)	**2**+**7** (0.45)	**3** (3)	**2**+**5** (3)	**2**+**6** (3)	**4** (0.45)
$NaBH_4$ (equiv.)	5	5	5	5	7.5	7.5	7.5	5

* Values between brackets refer to the optimized equivalents used for the preparation of each NP.

*Table S3. Reagent concentrations used to prepare **NP2-4**, **NP2A**-**C** and **NP3A**-**B**.*

	2	**3**	**4**	**5**	**6**	**7**	**$HAuCl_4$**	**$NaBH_4$**
mM (CH_2Cl_2)	42.7	10.5	2.5	15.3	46.5	2.5		
mM (MeOH)							25	100

*Table S4. Centrifugation conditions for the purification of **NP2-4**, **NP2A**-**C** and **NP3A**-**B**.*

NP	**Solvent for centrifugation**	**Speed (RCF x 10^3)**	**Time (min)**	**Number of washes**	**Pellet recovery**
NP2	MeOH	14	3	3	CH_2Cl_2
NP2A	MeOH	14	4	3	CH_2Cl_2
NP2B	MeOH/Acetone (1/1)	16	20	4	CH_2Cl_2
NP2C	MeOH	14	4	4	CH_2Cl_2
NP3	CH_2Cl_2	14	4	4	MeOH
NP3A	MeOH/CH_2Cl_2 (1/1)	12	15	4	MeOH
NP3B	H_2O	175	120	2	H_2O
NP4	H_2O	150	120	2	H_2O

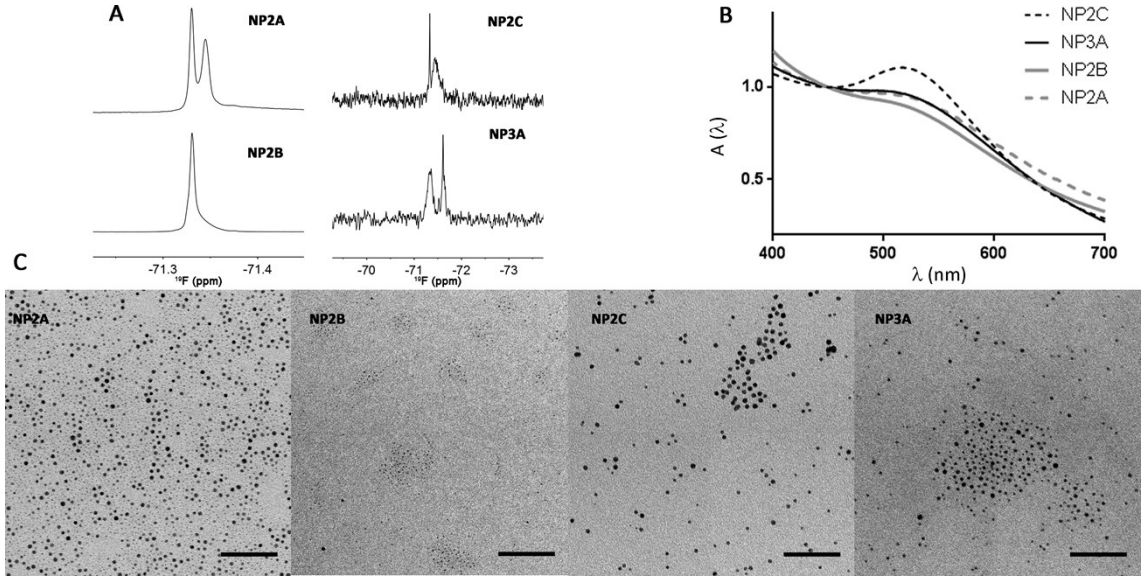

*Figure S2. A) ^{19}F-NMR spectra of **NP2A**-**C** (in CD_2Cl_2) and **NP3A** (in CD_3OD). B) UV-Vis absorption spectra of **NP2A**-**C** (in CH_2Cl_2) and **NP3A** (in CH_3OH) normalized at λ = 450 nm. C) TEM micrographs of **NP2A**-**C** and **NP3A**. Scale bars represent 50 nm.*

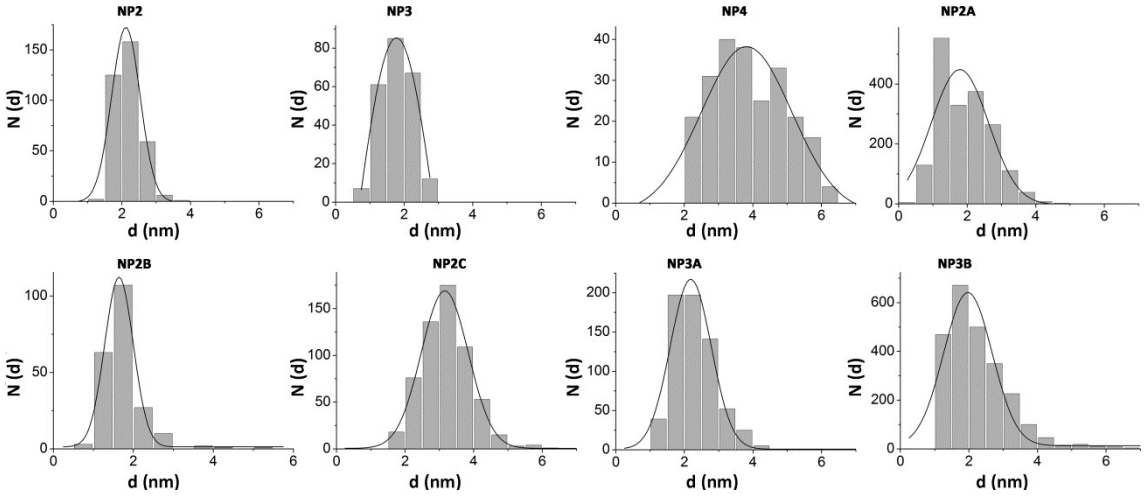

*Figure S3. Histograms for size distribution N (d) and Gaussian fit for each NP. Diameters (d) of the Au cores as visualised by TEM for each NP are the following: **NP2**: (2.1 ± 0.4) nm; **NP3**: (1.8 ± 1.2) nm; **NP4**: (3.8 ± 1.3) nm; **NP2A**: (1.8 ± 0.8) nm; **NP2B**: (1.6 ± 0.4) nm; **NP2C**: (3.2 ± 0.7) nm; **NP3A**: (2.2 ± 0.6) nm; **NP3B**: (2.0 ± 0.7) nm.*

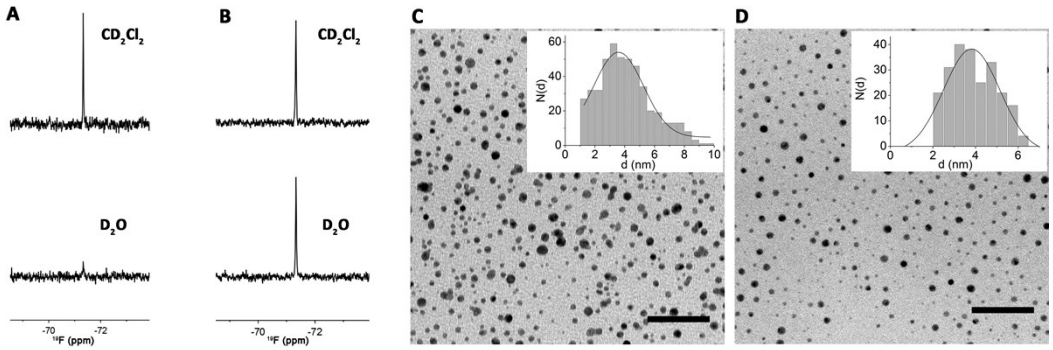

*Figure S4. ^{19}F-NMR spectra comparison in CD_2Cl_2 and in D_2O for **NP4**. A) 0.2 equivalents of ligand **4** used and B) 0.45 equivalents of ligand **4** used. TEM micrographs and size distribution for **NP4** prepared with C) 0.2 equivalents of **4**, d = 3.6 ± 1.7 nm and D) 0.45 equivalents of **4**, d = 3.8 ± 1.3 nm.*

Gold content measurement by ICP-MS. The content of elemental Au in selected NP samples was obtained by ICP-MS (Agilent 7700 Series). The samples were dried in glass vials prior to measurement and weighted 1.32 mg (**NP4**) and 4.77 mg (**NP2**) per vial prior to digestion. Following these weightings, 1.2 mL of aqua regia consisting of 1 part of HNO_3 and 3 parts of HCl were added directly to the NP samples in their glass vials. The samples were then digested under constant agitation for 4 hours at room temperature. 200 µL of the digested gold solution was then transferred to a 6 mL perfluoroalkoxy alkane tube (PFA) and diluted using 1.8 mL of a 2 wt% HCl solution to prevent digestion of the machinery, as well as to provide an ion-stable environment with constant background conditions for all samples. Measurements were done using 5 repetitions per sample, 100 sweeps, and a peak pattern of 3 peaks. All data given were calculated as the mean of all 5 measurements taken for each sample. According to these measurements, the gold content (as determined in terms of elemental Au by ICP-MS) was found to be 40.7% and 10.4% of the total mass (of the weighted NP powder) for **NP4** and **NP2**, respectively. The rest of the mass corresponded to organic ligands **4** and **2** in each case (59.3% and 89.6% for **NP4** and **NP2**, respectively) and knowing the molecular weight of each ligand type, the calculation of fluorinated ligand moles in any sample is straightforward. The molar concentration values of the **NP4** aqueous solutions used for cells assays were calculated by using the inorganic core diameters obtained by TEM (d), the density of bulk gold (ρ =19.3 g/cm^3), and the ICP-MS data. From the core diameter and the density of bulk gold, the mass of gold in one NP was calculated. The molar mass of one NP was assumed to be the molar mass of the Au core, neglecting the mass contribution of the organic surface coating.

4. ^{19}F-MRI imaging.

General remarks. MRI experiments were performed in a magnetic resonance scanner at Bruker BioSpec 11.7 T with 16 cm bore for small rodents (Bruker Biospin) along a 9 cm shielded gradient insert capable of 750 mT/m. The acquisition of ^{19}F-MRI images was done employing a 40 mm inner diameter ^{1}H/^{19}F coil, which also allowed the acquisition of a reference proton

image before the ^{19}F-MRI image recording. All ^{19}F images were acquired without proton decoupling.

Phantoms were prepared by dissolving each sample in 100-200 µL of solvent inside 3 mm wide NMR tubes. Those tubes were carefully inserted in 2 % agarose prepared inside 50 mL falcon tubes. The gel surface was sealed with liquid glue to avoid gel dryness over time (Figure S4). Experiments shown in Figure 5A were performed with samples dissolved in CD_2Cl_2 and those in Figure 5B were done using water as the sample solvent.

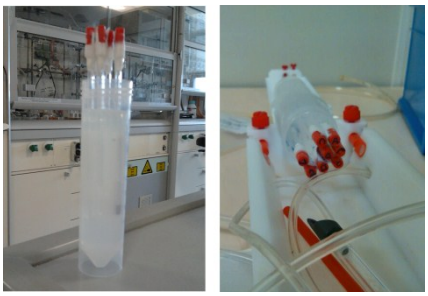

Figure S5. Phantom photos.

Imaging parameters for images shown in Figure 5A. ^{1}H MRI images were acquired with the following parameters: Bruker's implementation of FLASH experiment, TR/TE = 200/6 ms , Flip angle 30 deg, FOV 32 mm x 32 mm , Matrix 256 x 256, 8 slices, slice thickness 4 mm, 1 average, total acquisition time 1 minute.

^{19}F MRI images were acquired with the following parameters: Bruker's implementation of fast spin echo experiment (RAREVTR) acquiring 8 consecutive echoes, TR = 2600 ms, RARE acceleration factor = 8, Effective TE = 15, 55, 95, 135, 175, 215, 255, 295 ms, 1024 averages, total acquisition time 2.25 hours. Images were acquired with the same geometric parameters and position as the ^{1}H reference, except for the matrix that was 32 x 32, making the pixel 1 mm x 1 mm x 4 mm. The magnitude of the sum of the 8 echoes images yields the final ^{19}F image.

Imaging parameters for images shown in Figure 5B. ^{1}H MRI images were acquired with the following parameters: Bruker's implementation of FLASH experiment, TR/TE = 200/6 ms , Flip angle 30 deg, FOV 32 mm x 32 mm , Matrix 256 x 256, 2 slices, slice thickness 8 mm, 1 average, total acquisition time 1 minute.

^{19}F MRI images were acquired with the following parameters: Bruker's implementation of fast spin echo experiment (RAREVTR) acquiring 8 consecutive echoes, TR = 2600 ms, RARE acceleration factor = 8, Effective TE = 15, 55, 95, 135, 175, 215, 255, 295 ms, 1024 averages, total acquisition time 2.25 hours. Images were acquired with the same geometric parameters and position as the ^{1}H reference, except for the matrix that was 32 x 32, making the pixel 1 mm x 1 mm x 4 mm. The magnitude of the sum of the 8 echoes images yields the final ^{19}F image.

In all cases, regions of interest (ROIs) were manually segmented on the ^{1}H-MRI reference image and loaded on the ^{19}F-MRI image. The signal to noise ratio (SNR) was calculated by the following formula:

$$SNR = I_{ROI}/SD_{NOISE}$$

Where:

I_{ROI} = Intensity of each selected ROI

SD_{NOISE} = standard deviation outside the phantom.

5. Cell viability and apoptosis assays.

Reagents. All cell lines were purchased from ATCC-LGC and cultured in DMEM media (Sigma) completed with 2 nM L-glutamine (Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin (Gibco) and 10 % heat-inactivated fetal bovine serum (Gibco). The PBS buffer was purchased in tablets and prepared following manufacturer procedures (Sigma-Aldrich) corresponding to 10 mM phosphate buffer containing 137 mM NaCl and 2.7 mM KCl at pH 7.4.

Cell culture and counting. Human breast adenocarcinoma (MDA-MB-231), melanoma (MDA-MB-435S), and cervix carcinoma (C33-A) cells were cultured in complete media at 37 °C and 5 % CO_2 in tissue culture- treated 75 cm^2-flasks (Nunc). For cell passage, cells were lifted from the flasks by incubation at 37 °C with trypsin-EDTA solution 1x (2.5 g porcine trypsin and 0.2 g EDTA·4Na per liter of Hanks' Balanced Salt, Sigma), spun at 10^3 RCF for 5 min and the pellet re-suspended in 1 mL of media. For cell counting, the cell suspension was serially diluted 1:10 in PBS and 1:2 in the exclusion dye Trypan Blue solution (0.4 % in 0.81 % sodium chloride and 0.06 % potassium phosphate, dibasic, Sigma). 10 µL of the diluted cell suspension was counted in a haemocytometer chamber under transmitted light in an inverted microscope (DMIL, Leica).

MTS cytotoxicity assay.[5] 1.2 x 10^5 MDA-MB-231, 10^5 MDA-MB-435S and 10^5 C33-A cells were seeded in 200 µL per well of complete media at 37 °C and 5 % CO_2 in a 96-microwell plate and cultured for 3 days. Then, media was replaced with 50 µL of media containing **NP4** at the NP concentrations of 0.5, 1.5 and 3 µM in quadruplicate wells and incubated for 24 h (Figure 5E) at 37 °C and 5 % CO_2. Cells were washed by pipetting with 100 µL per well with complete DMEM media. Pipetting had to be carried out carefully to avoid lifting the cells from the bottom of the wells. Then, following manufacturer instructions, medium containing CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)), Promega) was added to all wells to a final dilution of 1:20. Following incubation at 37 °C and 5 % CO_2 for 30 min, absorbance measurements of the soluble formazan product generated by the metabolically active cells were taken at 492 nm in a micro plate spectrophotometer (GeniosPro, Tecan). Data are

[5] J. A. Barltrop, T. C. Owen, A. H. Cory, J. G. Cory, *Bioorg. Med. Chem. Lett.,* 1991, **1**, 611.

expressed as a percentage of absorbance of treated cells related to the untreated control cells and represented as means of quadruplicates ± SD.

Annexin V apoptosis assay.[6] 5 x 10^3 MDA-M-231, 4 x 10^3 C33-A and 10^4 MDA-MB-435S cells were seeded in 250 µL complete media per well in a 48 microwell plate and cultured for 48 h at 37 °C and 5 % CO_2. Then, they were incubated for 24 h with 150 µL media containing either 1 µM staurosporin (Sigma) or **NP4** at the concentrations of 0.5, 1.5 and 3 µM at 37 °C and 5 % CO_2. Both media and cells were transferred to a 96 well round bottom microwell plate (Costar) and centrifuged at 800 RCF for 10 min at 4 °C. For detachment from the 48 microwell plate, cells were incubated at 37 °C with 150 µL Cell Disassociation Solution (Sigma) for 5-10 min and pipetting. The pellets were re-suspended in 150 µL Annexin-binding buffer (0.01 M HEPES, pH 7.4; 140 mM NaCl; 2.5 mM $CaCl_2$) containing 0.09 % sodium azide (ABBA), spun again and re-suspended in 50 µL of ABBA containing 1.5 µL PE-conjugated Annexin V (BD Biosciences). Following incubation in ice for 15-20 min in the dark, they were centrifuged at 800 RCF for 10 min at 4 °C. Supernatans were discarded by aspiration and a volume of 200 µL of ABBA containing 1 µg/mL of 6-diamidino-2-phenylindole (DAPI, Molecular Probes) was added and samples were kept on ice prior to acquisition by flow cytometry (BD FACSCanto™II). Data analysis was performed using the FlowJo DIVA software (Version 7.6.1; Tree Star Inc). Cells stained with both annexin V-PE and DAPI were considered to be late apoptotic, cells stained only with annexin V-PE were considered to be early apoptotic cells, and cells only stained with DAPI were consider necrotic cells . Results of the mean fluorescence intensity were normalized to the untreated control cells. The experiment was repeated three times and the data is represented as the average of the three experiments (n=3) ± SD (Figure 5F).

6. Fluorine content calculation for **NP4**.

With the gold core radius measured in TEM (r = 1.9 nm), the density of bulk gold (ρ =19.3 g/cm^3) and assuming that gold NPs are perfect spheres, we obtained that the weight of one single **NP4** (only the gold core) was 5.6 x 10^{-19} g, as shown below:
Vol_{NP4} = (1.9 x 10^{-7})^3 4π/3 = 2.9 x 10^{-20} cm^3
$Mass_{NP4}$ = 19.3 x 2.9 x 10^{-20} = 5.6 x 10^{-19} g for one single **NP4**

According to ICP-MS data, from the 1320 µg of **NP4** analysed, 537 µg were of gold and 783 µg were of fluorinated ligand **4**, 40.7% and 59.3% of the total mass, respectively. From these data and the M_w of **4** (approx 3400 g/mol), we could calculate the number of gold NP in 537 µg of gold and the amount of ligands per NP:
(537 x 10^{-6})/ 5.6 x 10^{-19} = 9.5 x 10^{14} gold NPs in the analysed sample.
(783 x 10^{-6})/3400 = 2.3 x 10^{-7} mol of **4** in the analysed sample.
2.3 x 10^{-7}/9.5 x 10^{14} = 2.4 x 10^{-22} mol of **4** per **NP4**
(2.4 x 10^{-22}) x (6.023 x 10^{23}) = 145 ligands **4** per **NP4**

Since each ligand has 9 fluorine atoms:
145 x 9 = 1305 fluorine atoms per **NP4**

[6] I. Vermes, C. Haanen, H. Steffens-Nakken, C. Reutelingsperger, *J. Immunol. Methods*. 1995, **184**, 39.

7. List of abbreviations.

ABBA	Annexin-binding buffer
Ac	Acyl
DAPI	6-diamidino-2-phenylindole
DIAD	Diisopropyl azodicarboxylate
DMF	Dimethyl formamide
EDTA	Ethylenediaminetetraacetic acid
equiv	Equivalent
ESI-TOF	Electrospray ionization - Time of flight
Et	Ethyl
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HRMS	High Resolution Mass Spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
^{i}Pr	Iso-propyl
MALDI-TOF	Matrix-assisted laser desorption/ionization - Time of flight
Me	Methyl
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
Ms	Mesyl
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
m/z	Mass to charge ratio
NMR	Nuclear Magnetic Resonance
NP	nanoparticle
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
PFC	Perfluorocarbon
PMA	poly(isobutylene-alt-maleic anhydride)
ppm	Parts per million
RCF	Relative Centrifugal Force
SD	Standard Deviation
SNR	Signal to Noise Ratio
TEA	Triethylamine
TEG	Tetraethylene glycol
TEM	Transmission Electron Microscopy
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin Layer Chromatography
UV	Ultraviolet
Vis	Visible
vs.	versus