Versatile "click" synthesis of 1-hydroxy-1,1-methylenebisphosphonic acids with thioalkoxy substituents for the preparation of stable gold nanoparticles

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Experimental

All water was distilled and subsequently purified to Millipore Milli-Q quality. For Au NP synthesis, all glassware used was cleaned with concentrated nitric acid (60%), then rinsed thoroughly with water before use. UV-Visible Absorption spectroscopy of Au NPs dipersions was recorded on a Jasco V630 spectrophotometer at neutral pH. All experiments under microwave (MW) irradiations were run in a 30 mL MW tube, using an Anton Paar monowave 300 microwave synthesis reactor.^{1}H NMR spectra (400 MHz), proton-decoupled ^{13}C NMRspectra (100.6 MHz) and proton decoupled ^{31}P spectra (162.0 MHz) were recorded on a Bruker Avance 400 spectrometer andchemical shifts are reported in parts per million (ppm) on the δ scale. High Resolution Mass Spectrometry (HR-MS) experiments were realized on a LTQ Orbitrap Velos (Thermo Scientific) in positive mode using an ESI source or by MALDITOF/MS (Ultraflex TOF/TOF, Bruker Daltonique) in a DHB matrix in positive mode. MS spectra recorded in the Orbitrap mass analyzer allowing a mass accuracy around1–2 ppm. DLS and ζ potential analyses were performed on a nano ZS (red badge) ZEN3600 Zetasizer at neutral pH. TEM images were taken on a FEI CM10 electron microscope. Samples were prepared by dropping of the colloidal solution onto holey carbon-coated Cu grid 10 times. FTIR absorption spectra were recorded on a Nicolet 380Thermo Scientfic FTIR spectrophotometer. Concentrated Au NPs colloidal solution (neutral pH, pre-washed by ultrafiltration) was added to analytical grade KBr and left dried at 80°C overnight. The pellet was prepared with this crude powder. The fluorescence of free Rhodamine 123 and coupled Au NPs was determined by using a spectrofluorimeter (Jobin-Yvon FluoroMax+) with excitation at 500 nm and emission at 522 nm.

1 and **2** were synthesized as previously described.[24, 39]

General procedure for obtaining of compound 1a-c and 2a-c (all solvents are freshly degassed prior use)**:** 1.1 mmole (for **1a-c**) or 0.55 mmole (for **2a-c**) of mercapto propionic acid (116 mg for **1a** 0.58 mg for **2a**) or ω-HS-PEG-carboxylic acids (488 mg for **1b**, 244mg for **2b**, 2.35 g for **1c** and 1.18 g for **2c**) were dissolved in 3 mL of DMF and added in a 20 mL tube to 0.5 mmole of the compound **1** (147.5 mg) or **2** (134 mg) in 3 mL of distilled water. A small amount of 1-hydroxycyclohexylphenyl ketone is then added and the tube is sealed and stirred under a UV lamp at 360 nm during 3 hours. 8 mL of water are then added to the mixture and the resulting solution is washed with 3 times 10 mL of dichloromethane and 3 times 10 mL of diethylic ether. The aqueous solution is then lyophilized and a white powder is obtained. This protocol yield from 80 to 90 % of pure product (products are obtained as monosodium salts) except for compound 1c were a mixture of the mono and difunctionalized compound was obtained. **1a**: ^{31}P NMR (162 MHz, D_2O) δ 18.46. ^{1}H NMR (400 MHz, D_2O) δ 2.86 (m, 1H, CH-S), 2.76 (d, J = 6.3 Hz, 2H, CH_2-S), 2.72 (m, 4H, 2xCH_2-S), 2.54 (m, 4H, 2xCH_2CO_2H), 1.82 (m, 2H), 1.63 (m, 1H), 1.46 (m, 3H), 1.33 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.3 (s, *C*OOH), 74.1 (t, J = 134.8 Hz, P-*C*-P), 45.3 (s, *C*H-S-), 37.0 (s, *C*H_2-COOH), 34.8 (s, *C*H_2-S-), 34.4, 33.5, 33.0 (s, *C*H_2-S-), 27.3, 26.9, 25.1, 23.3 (t, J = 6.6 Hz, *C*H_2-C$(PO_3H_2)_2$). HR-MS (ESI$^+$ Q Tof) $C_{13}H_{26}NaO_{11}P_2S_2$: m/z $(M+H)^+$: 507.02918 ; calc: 507.0289. **1b**: ^{31}P NMR (162 MHz, D_2O) δ 18.53. ^{1}H NMR (400 MHz, D_2O) δ 3.68 (m, 4H, 2xCH_2CO_2H), 3.52 (m, 60H, CH_2O), 2.78 (m, 1H, CH-S), 2.70 (d, J = 6.3 Hz, 2H, CH_2-S), 2.52 (m, 4H, 2xCH_2-S), 1.80 (m, 2H), 1.64 (m, 1H), 1.48 (m, 3H), 1.33 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 176.5 (s, *C*OOH), 74.1 (t, J = 135.0 Hz, P-*C*-P), 69.9 (*C*H_2O), 69.6 (*C*H_2O), 69.5 (*C*H_2O), 69.3 (*C*H_2O), 66.3 (*C*H_2O), 45.6 (s, *C*H-S-), 37.2 (s, *C*H_2-COOH), 34.6 (s, *C*H_2-S-), 33.5, 33.3, 31.4 (s, *C*H_2-S-), 29.2, 27.0, 23.5 (t, J = 6.3 Hz, *C*H_2-C$(PO_3H_2)_2$). HR-MS (ESI$^+$ Q Tof) $C_{43}H_{84}Na_2O_{27}P_2S_2$: m/z $(M+H)^+$: 603.7501 ; calc: 603.5919.

1c (compound 1c was obtained in mixture with the monofunctionalized product as visible on the MS MALDI spectra): ^{31}P NMR (162 MHz, D_2O) δ 18.78. ^{1}H NMR (400 MHz, D_2O) δ 3.75 (m, 4H, CH_2CO_2H) 3.60 (m, 356H) 3.39 (m,2H), 2.83 (m, 1H, CH-S), 2.78 (m, 2H, CH_2-S), 2.66 (m, 4H, 2xCH_2-S), 1.83 (m, 2H), 1.66 (m, 1H), 1.48 (m, 3H), 1.34 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 175.4 (s, *C*OOH), 129.1*, 127.1*, 74.0 (t, J = 135.3 Hz, P-*C*-P), 69.9 (*C*H_2O), 69.8 (*C*H_2O), 69.6 (*C*H_2O), 69.4 (*C*H_2O), 68.4 (*C*H_2O), 68.3 (*C*H_2O), 48.8, 45.6 (s, *C*H-S-), 37.2 (s, *C*H_2-COOH), 33.5 (s, *C*H_2-S-), 33.3, 31.4 (s, *C*H_2-S-), 30.7, 29.2, 27.0, 23.43 (t, J = 6.1 Hz, *C*H_2-C(PO_3H_2)$_2$). *vinylic carbon corresponding to mono addition. **2a**: ^{31}P NMR (162 MHz, D_2O) δ = 18.46. ^{1}H NMR (400 MHz, D_2O) δ = 2.79 (t, J = 6.8 Hz, 2H), 2.66 (t, J = 7.1 Hz, 2H), 2.60 (t, = 7.1 Hz, 2H), 1.93 (m, 2H), 1.60 (m, 4H). ^{13}C NMR (126 MHz, D_2O) δ = 177.1 (s, *C*OOH), 74.0 (t, J = 135.3 Hz, P-*C*-P), 34.4 (s, *C*H_2-S-), 33.2, 30.9 (s, *C*H_2-S-), 29.7, 26.1, 23.0 (t, J = 6.4 Hz, *C*H_2-C(PO_3H_2)$_2$). HR-MS (ESI$^+$ Q Tof) $C_8H_{18}NaO_9P_2S$: m/z $(M+H)^+$: 375.00414 ; calc: 375.0044. **2b**: ^{31}P NMR (162 MHz, D_2O) δ = 18.73. ^{1}H NMR (400 MHz, D_2O) δ 3.65 (m, 2H), 3.62 – 3.47 (m, 30H), 2.64 (t, J = 6.4 Hz, 2H), 2.57 – 2.45 (m, 4H), 1.81 (m, 2H), 1.50 (m, 4H). ^{13}C NMR (101 MHz, D_2O) δ 176.2 (s, *C*OOH), 73.9 (t, J = 135.8 Hz, P-*C*-P), 69.5 (*C*H_2O), 69.2 (*C*H_2O), 66.1 (*C*H_2O), 34.3 (s, *C*H_2-S-), 33.1, 31.0 (s, *C*H_2-S-), 30.3, 29.8, 23.02 (t, J = 6.4 Hz, *C*H_2-C(PO_3H_2)$_2$). **2c**: ^{31}P NMR (162 MHz, D_2O) δ = 18.39. ^{1}H NMR (400 MHz, D_2O) δ = 3.70 (m, 16H), 2.76 (t, J = 6.4 Hz, 2H), 2.60 (t, J = 7.1 Hz, 2H), 2.54 (t, J = 6.4 Hz, 2H), 1.93 (m, 2H), 1.61 (m, 2H). ^{13}C NMR (126 MHz, D_2O) δ =178.0 (s, *C*OOH), 74.1 (t, J = 135.0 Hz, P-*C*-P), 69.52 (m), 69.4 (*C*H_2O), 69.4 (*C*H_2O), 69.3 (*C*H_2O), 66.9 (*C*H_2O), 35.8 (s, *C*H_2-S-), 33.2, 31.1 (s, *C*H_2-S-), 30.4, 29.1, 23.11 (t, J = 6.3 Hz, *C*H_2-C(PO_3H_2)$_2$).

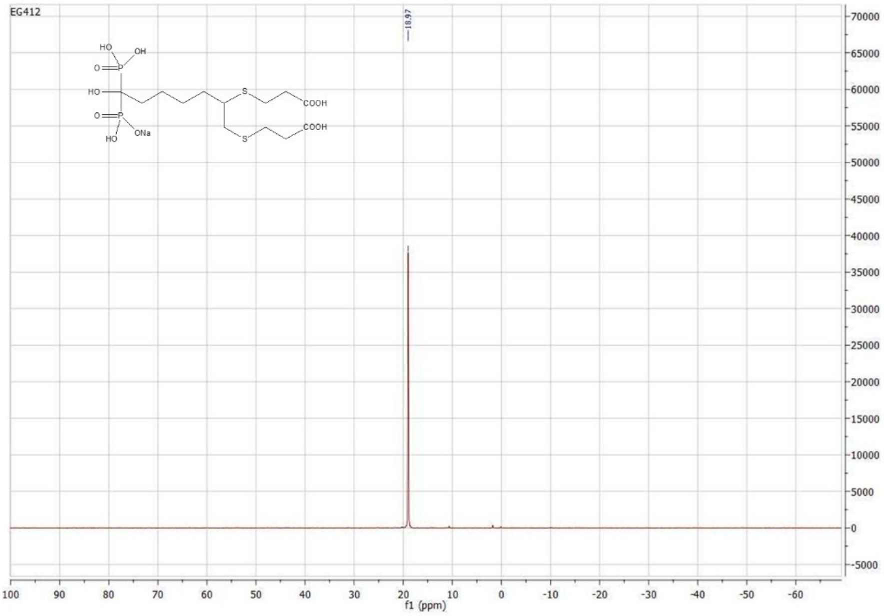
General procedure for oxidation of compound 2a and 2c. To a solution of compound **2a** or **2c** (0.5 mmole, 176mg for **2a**, 1.18 g for **2c**) in 4 mL of water, 282 mg of m-CPBA (1.65 mmol, 284 mg) were added in 4 mL of DMF under vigorous stirring. The solution is stirred for 24h at rroom temperature and then washed with 3 times 10 mL of diethylic ether. The aqueous solution is then lyophilized and a white powder is obtained. This protocol yield from 70% of pure product (obtained as monosodium salts) **3a**: ^{31}P NMR (162 MHz, D_2O) δ 18.40. ^{1}H NMR (400 MHz, D_2O) δ 3.84 (t, J = 7.1 Hz, 2H), 3.70 – 3.52 (m, 2H), 3.19 (t, J = 7.1 Hz, 2H), 2.26 (m, 2H), 2.08 (m, 2H), 1.99 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 174.8 (s, *C*OOH), 73.7 (t, J = 145.4 Hz, P-*C*-P), 52.4 (*C*H_2SO_2), 47.9 (*C*H_2SO_2), 33.4, 27.2, 22.6, 22.2. HR-MS (ESI$^+$ Q Tof) $C_8H_{18}NaO_{11}P_2S$: m/z $(M+H)^+$: 407.127 ; calc: 406.994. **3c**: ^{31}P NMR (162 MHz, D_2O) δ =18.14. ^{1}H NMR (400 MHz, D_2O) δ = 3.89 (t, J = 5.6 Hz, 2H), 3.83 (s, 2H), 3.77 (d, J = 4.2 Hz, 2H), 3.59 (s, 178H), 3.40 (t, J = 5.6 Hz, 2H), 3.27 – 3.13 (m, 2H), 1.83 (m, 2H), 1.72 (m, 2H), 1.64 (m, 2H). ^{13}C NMR (126 MHz, D_2O) δ = 177.2 (s, *C*OOH), 73.9 (t, J = 137.9 Hz, P-*C*-P), 69.6 (*C*H_2O), 69.4 (*C*H_2O), 63.4 (*C*H_2O), 53.5 (*C*H_2SO_2), 51.8 (*C*H_2SO_2), 33.1, 22.7 (t, J = 6.2 Hz, *C*H_2-C(PO_3H_2)$_2$), 21.8.

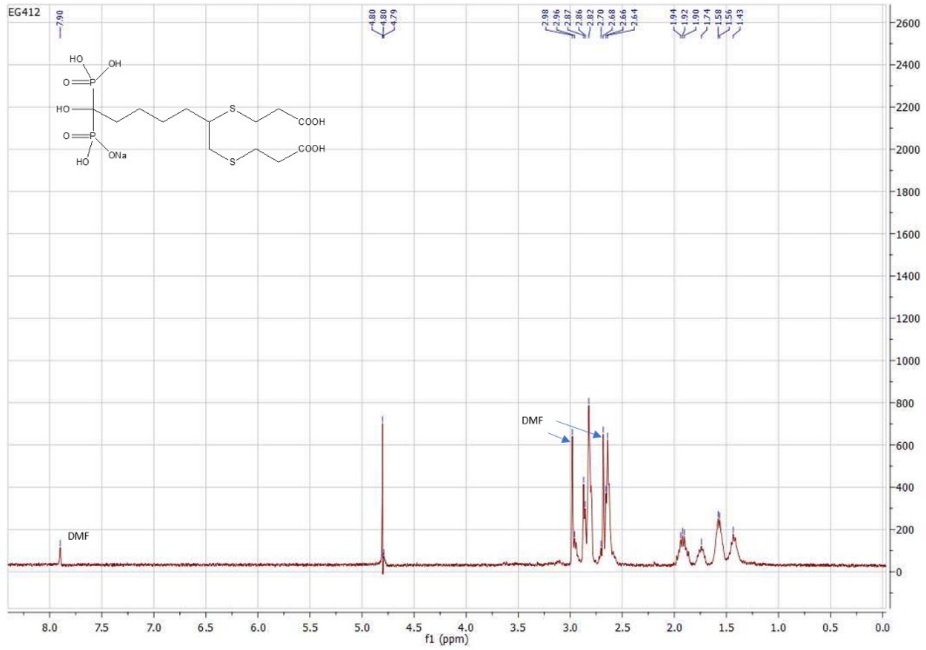
General procedure for the Au NP **synthesis under MW irradiation:** In a 4 mL vial, 900 µL of water, 125 µL of a 20 mM gold solution ($HAuCl_4 \cdot 3H_2O$, ≥ 99,9 %, Sigma-Aldrich, St Louis, MO) and 100 µL of a 10 mM solution of compound **2c, 3c** or the commercial phosphonate-PEG were mixed. The resulting solution was heated under MW (stirring at 1200 rpm) following three stages (1) heat as fast as possible to 100°C (2) hold at 100°C for 10 min (3) cool down.

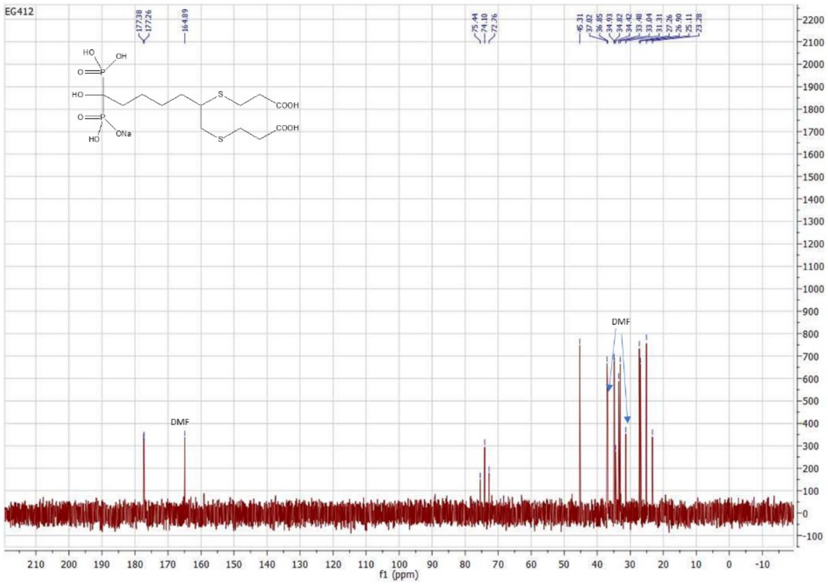
General procedure for the PEGylated Au NP **synthesis with sodium ascorbate:** in a 20 mL vial, 125 µL of a 20 mM gold solution ($HAuCl_4 \cdot 3H_2O$, ≥ 99,9 %, Sigma-Aldrich, St Louis, MO), 82 µL of a 10 mM solution of compound **3c** and 164 µL of a 31 mM solution of phosphonate-PEG (PEG PHOSPHONIC CARBOXYLIC ACID, ref. SP-1P-10-002 specific polymers) were mixed in 9.5 mL of water. The pH of the solution was then fixed at 10.5 with a 100 mM NaOH solution. Then, 100 µL of 50 mM solution of sodium ascorbate (C6H7NaO6, ≥ 98 %, Sigma-Aldrich, St Louis, MO) were added rapidly at room temperature. The mixture was stirred for 10 min at room temperature and afforded a pink Au NP solution. The Au NP solution is then washed several times with water and purified using ultrafiltration over a 30kDa membrane (Amicon®, Millipore).

Rhodamine coupling onto the PEGylated Au NP**:** To 400 µL of PEGylated Au NP solution ([Au] = 2,5 mM,), 30 µL of a 650 µM EDC solution ($C_8H_{17}N_3$, ≥ 97 %, Sigma-Aldrich, St Louis, MO) and 30 µL of a 650 µM NHS solution were added. Then, the pH of the solution was fixed at pH=3. The resulting mixture was stirred for 2.5 h before addition of 30 µL of a 130 µM rhodamine 123 solution ($C_{21}H_{17}ClN_2O_3$, ≥ 85 %, Sigma-Aldrich, St Louis, MO). A triethylamine solution (25 g/L) was added to reach a pH = 10 and the solution was stirred for 16 h. The Au NP solution was washed several times with water and treated with HBSS 30 min to remove unbounded rhodamine before a last filtration.

Compound 1a (monosodium salt) RMN ^{31}P, ^{1}H, ^{13}C and MS spectra



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33.48
33.04
31.31
27.26
26.90
25.11
23.28
HO
OH
O=P
HO
S
COOH
O=P
HO
ONa
S
COOH
DMF
DMF
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
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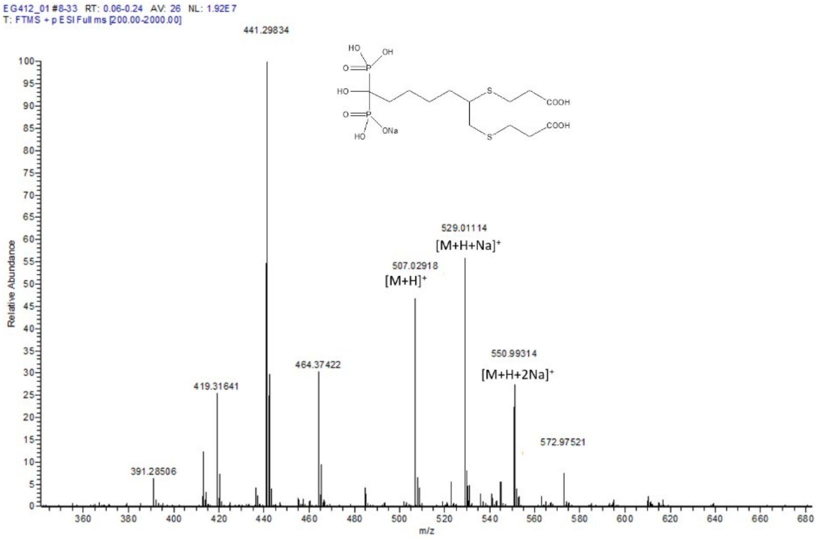
f1 (ppm)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HO
S
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O=P
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S
COOH
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Relative Abundance
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90
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80
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420
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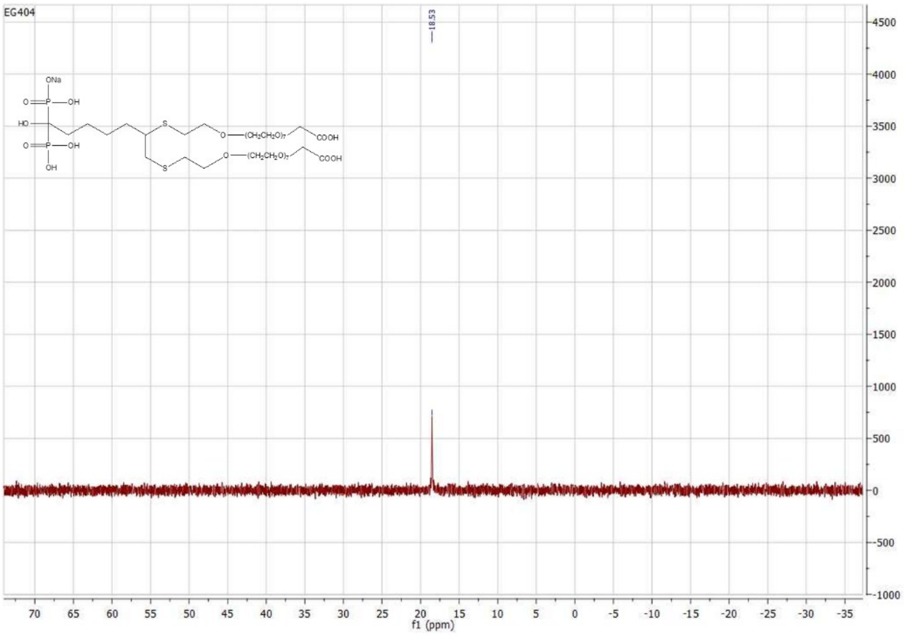
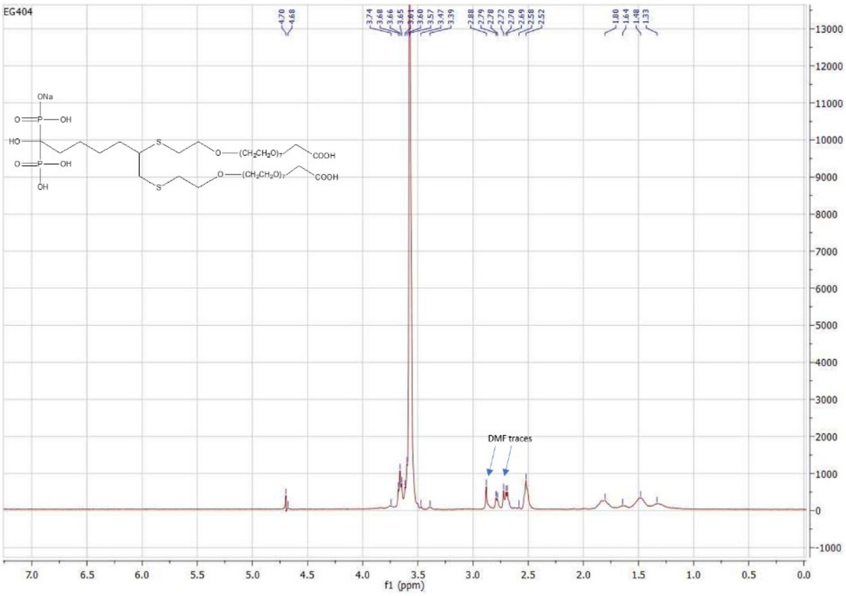
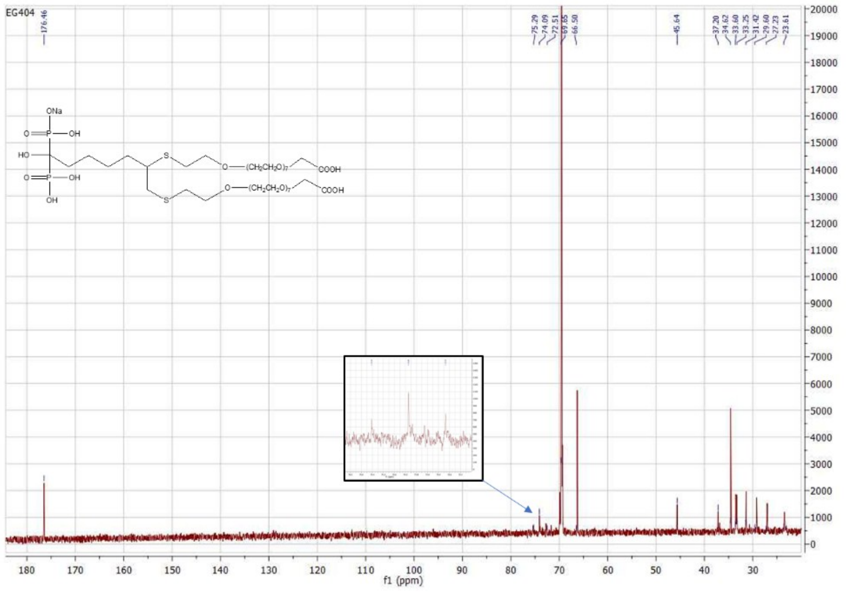
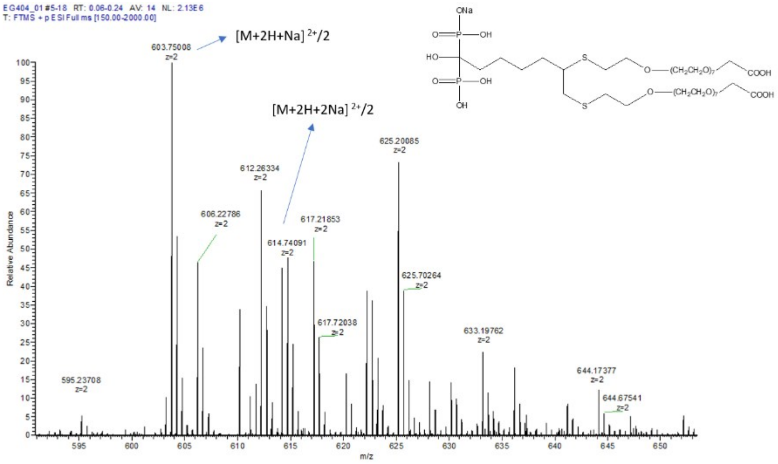
m/z

Compound 1b RMN 31P, 1H, 13C and MS spectra

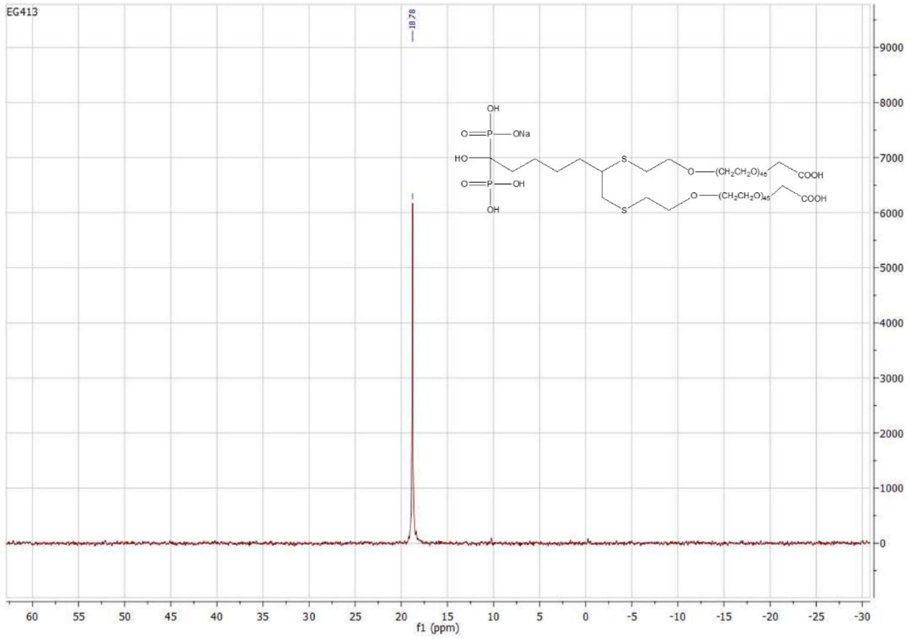


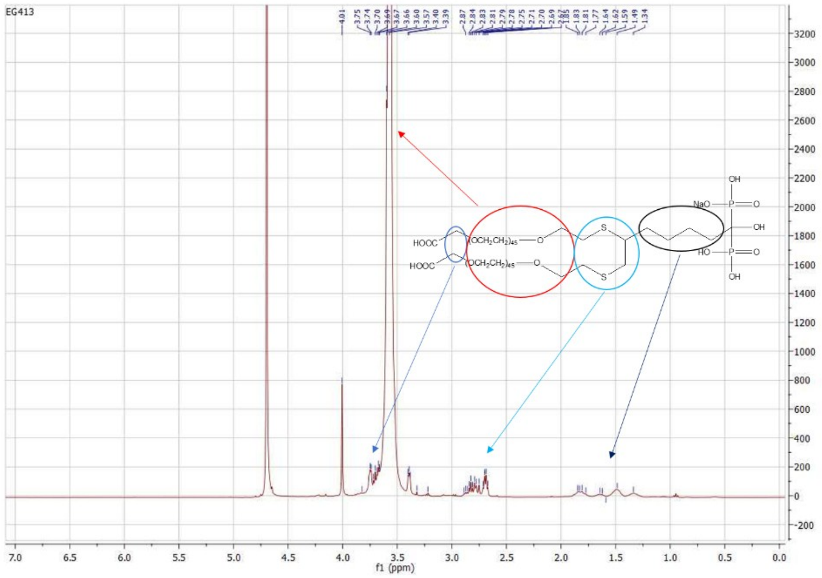


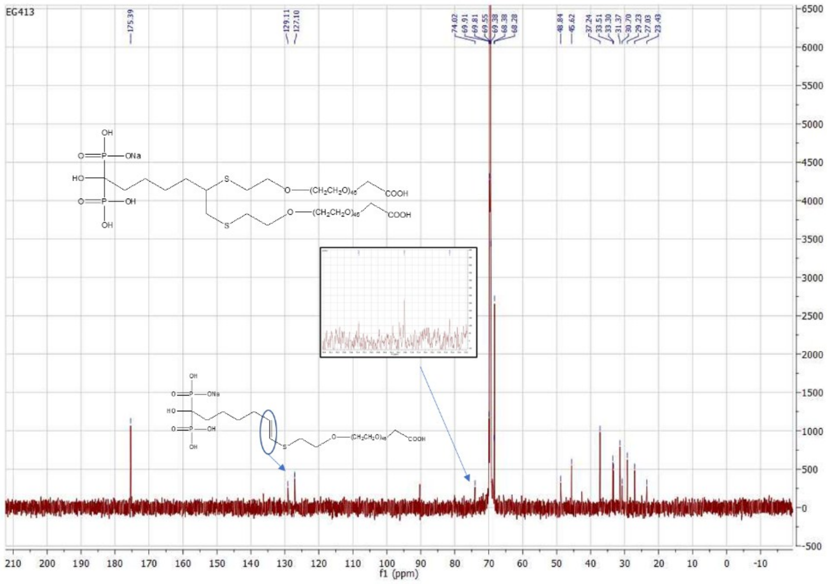


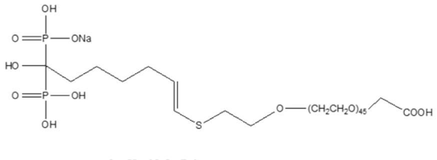


Compound 1c RMN 31P, 1H, 13C and MS spectra









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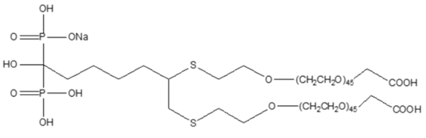

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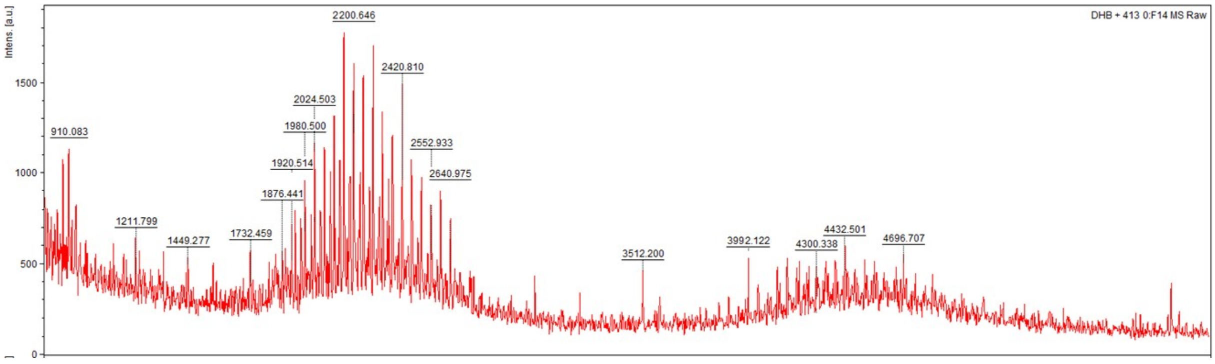
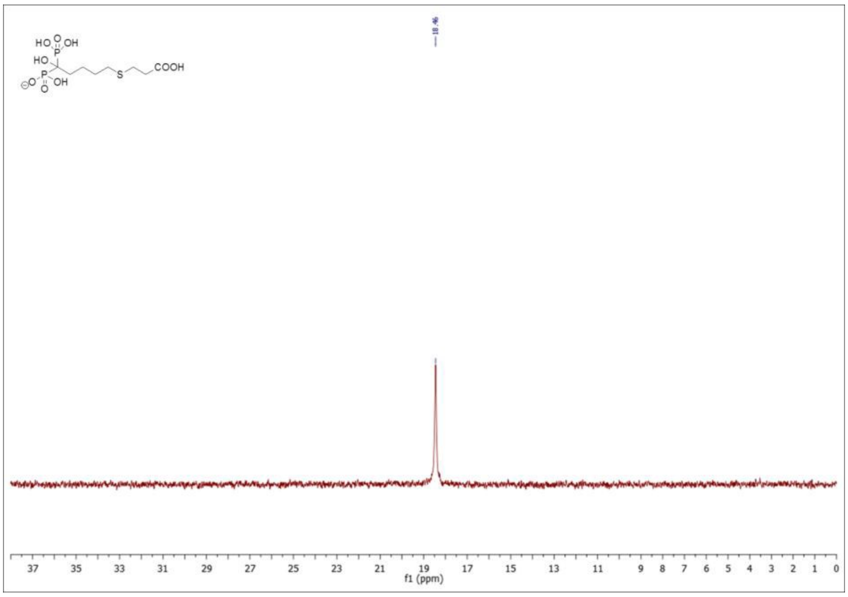
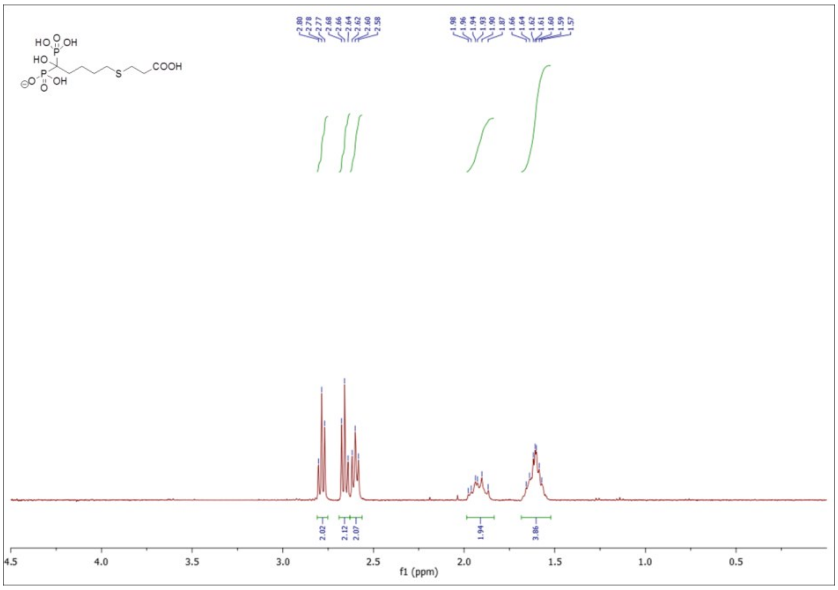
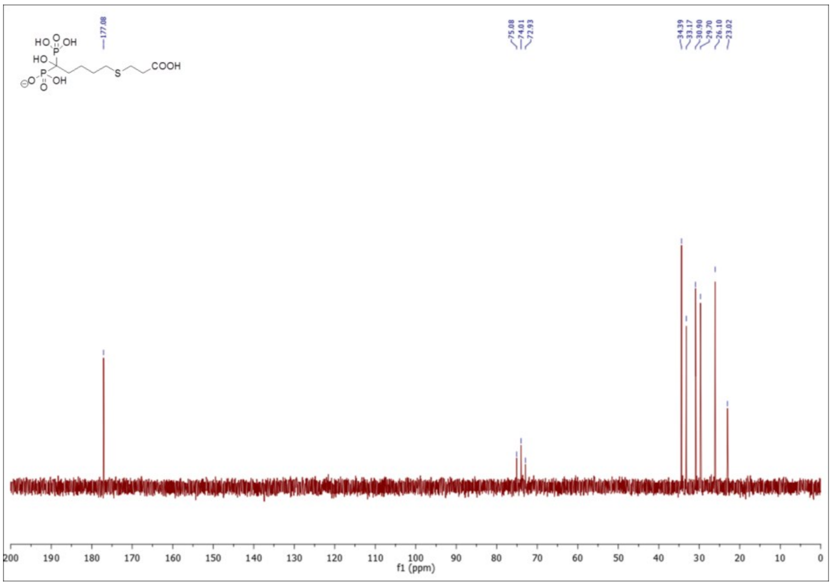
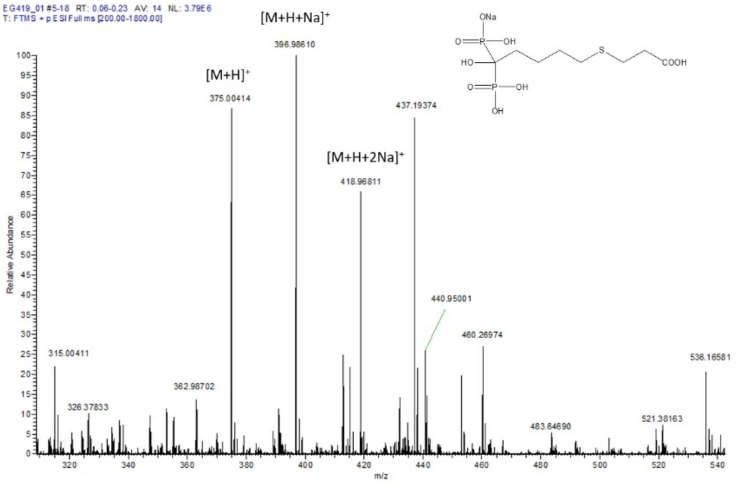


Compound 2a RMN 31P, 1H, 13C and MS spectra

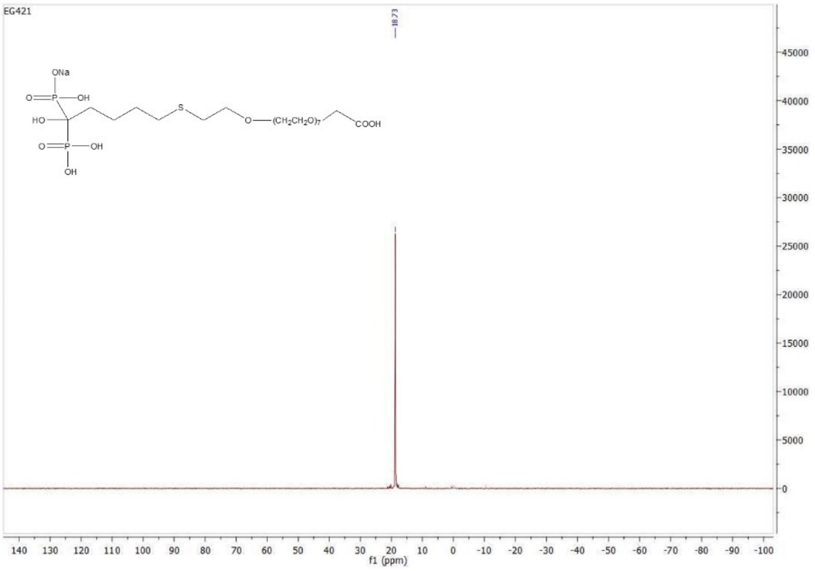


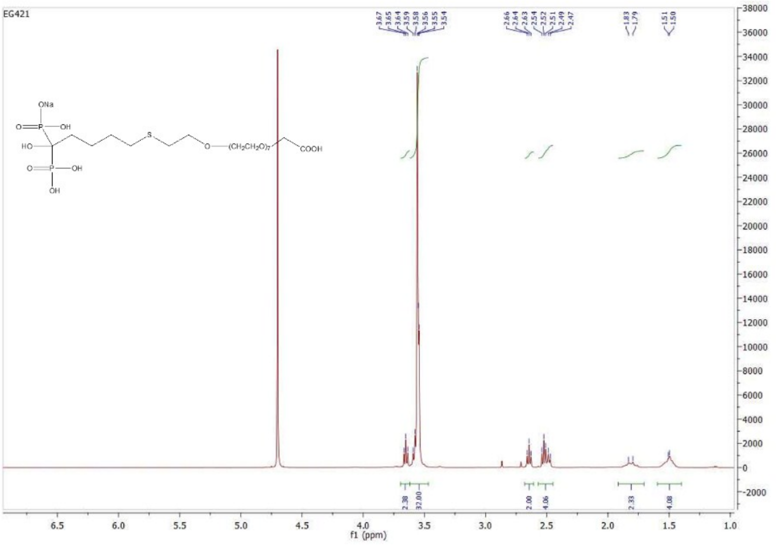


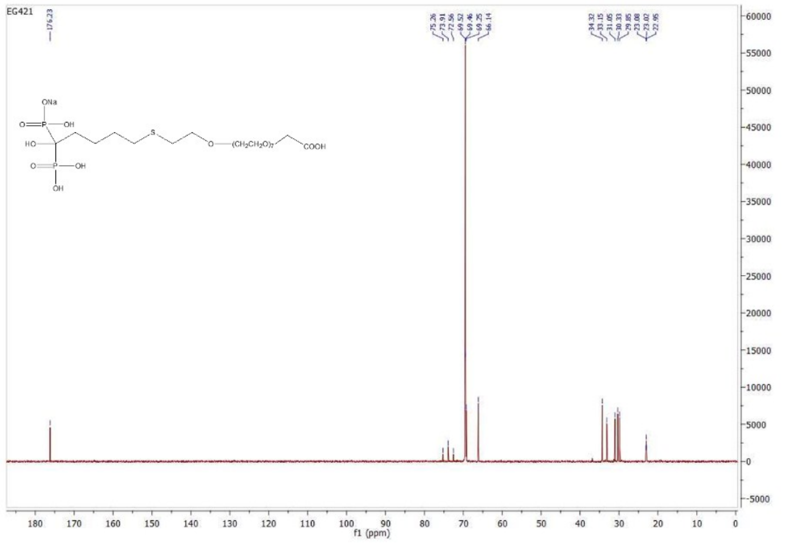




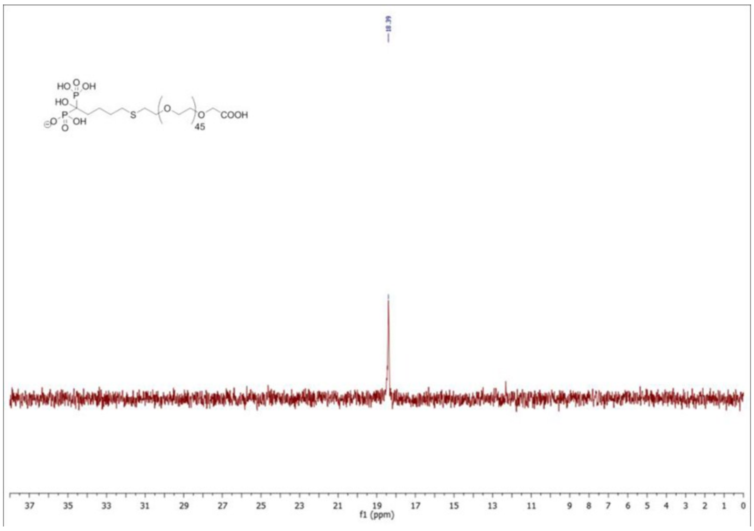
Compound 2b RMN 31P, 1H and 13C

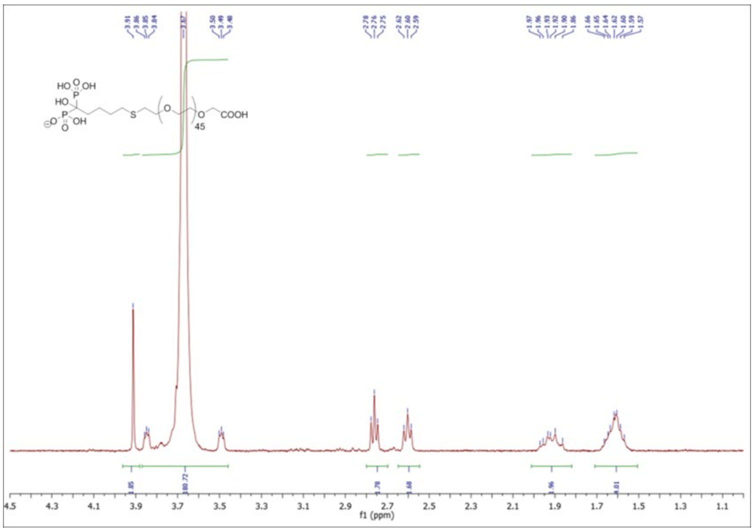


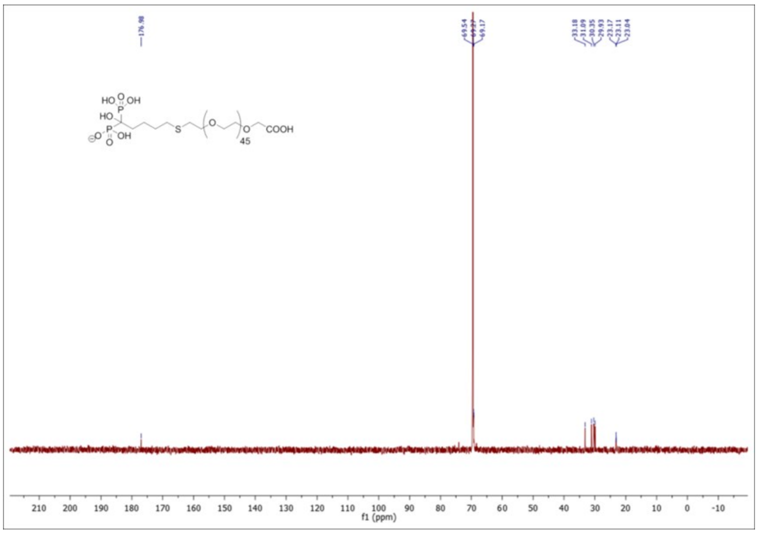


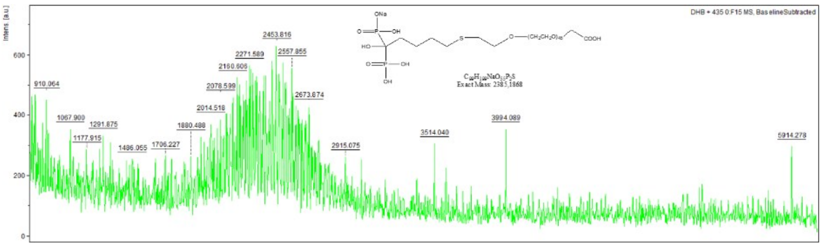


Compound 2c RMN 31P, 1H, 13C and MS spectra

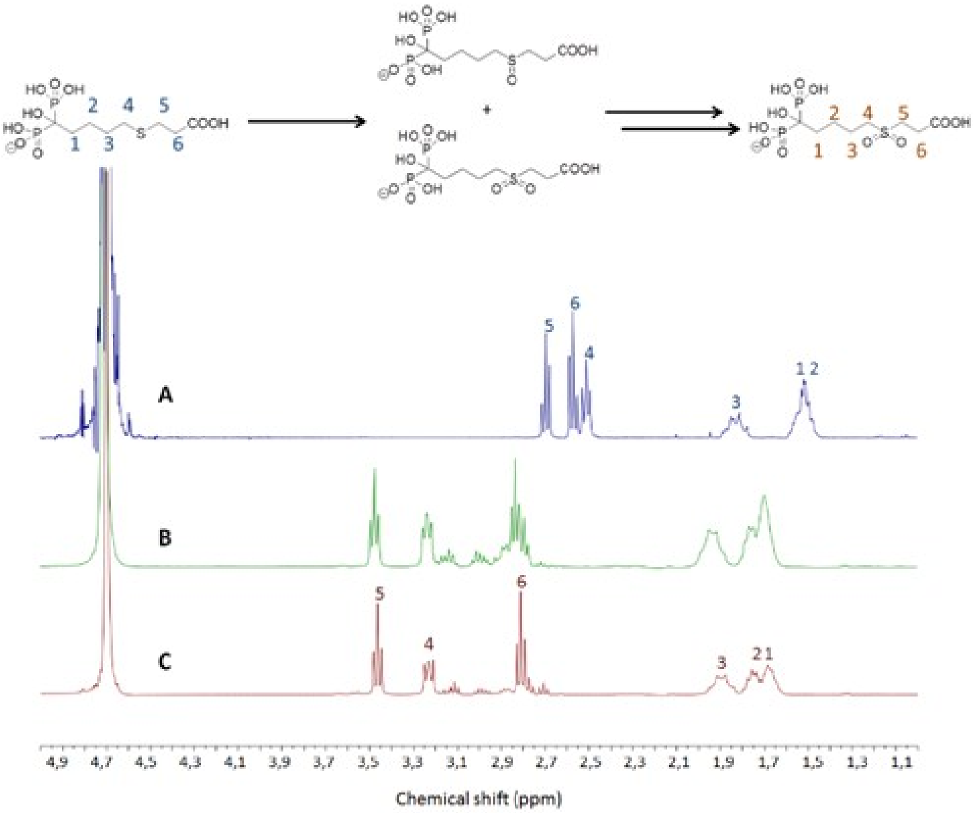




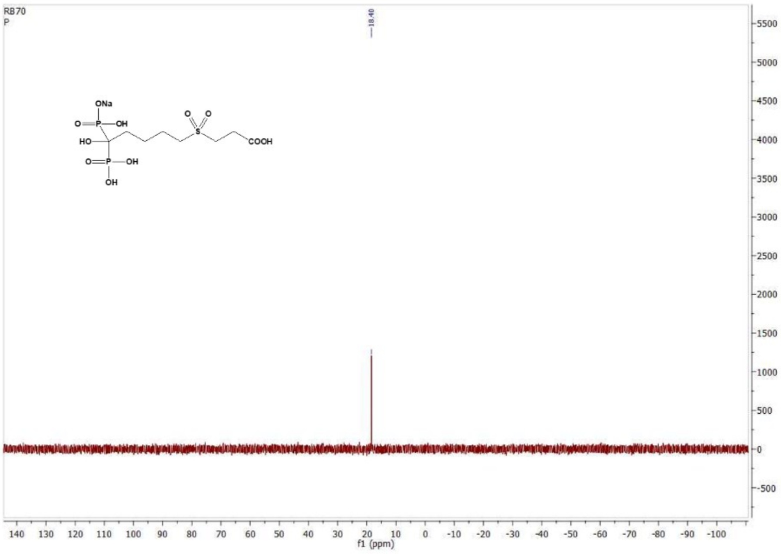


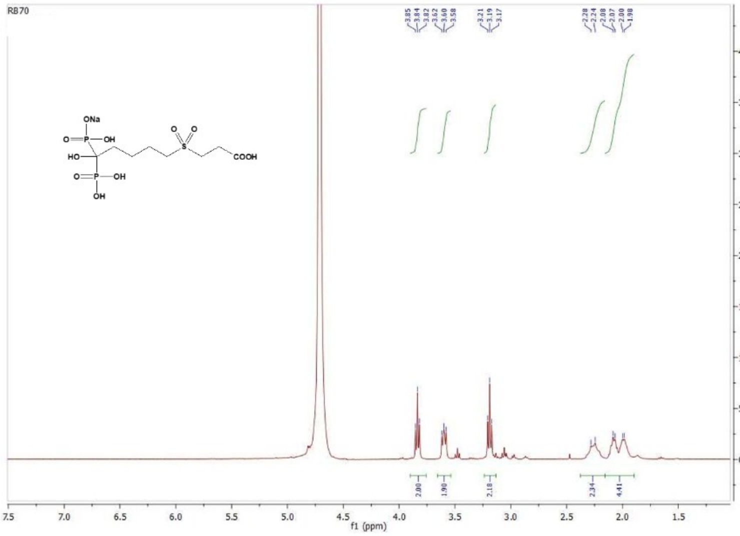


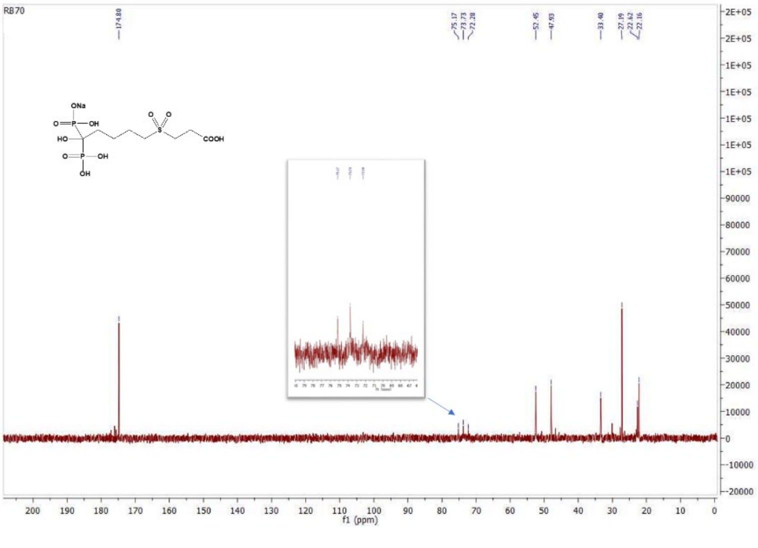
1H NMR study of 2a oxidation. The product of the thiol-ene reaction in deoxygenated condition (A) after several days in water (un degassed) (B) and after oxidation with m-CPBA (C).

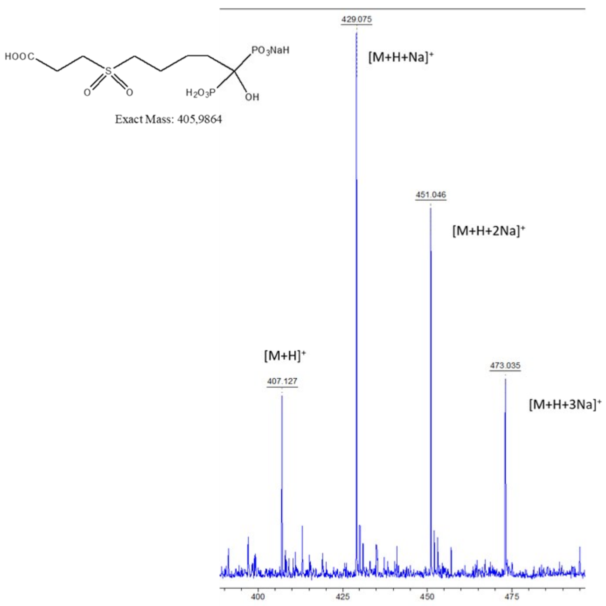


Compound 3a RMN 31P, 1H, 13C and MS spectra

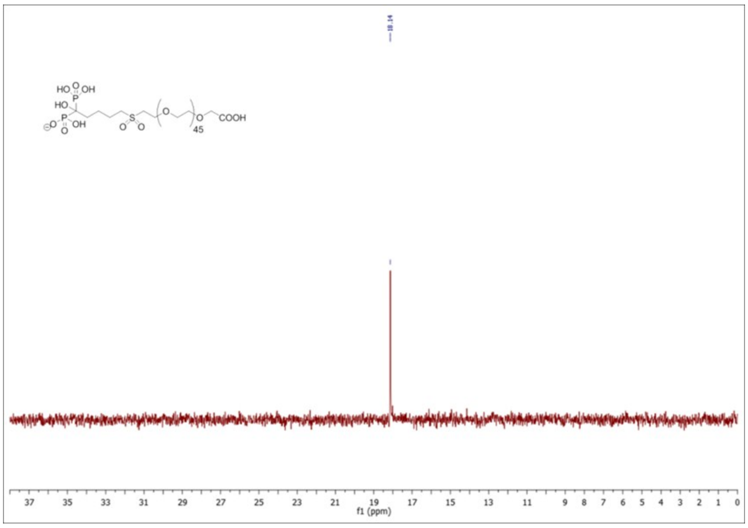


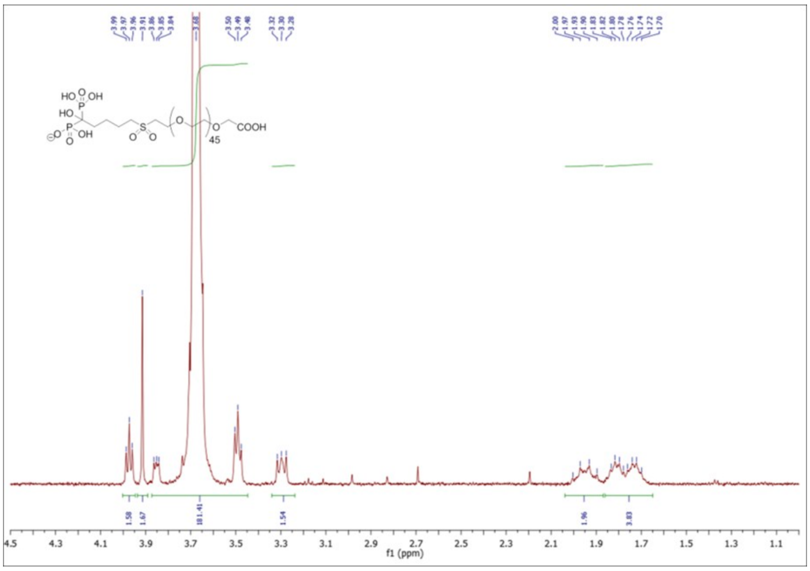


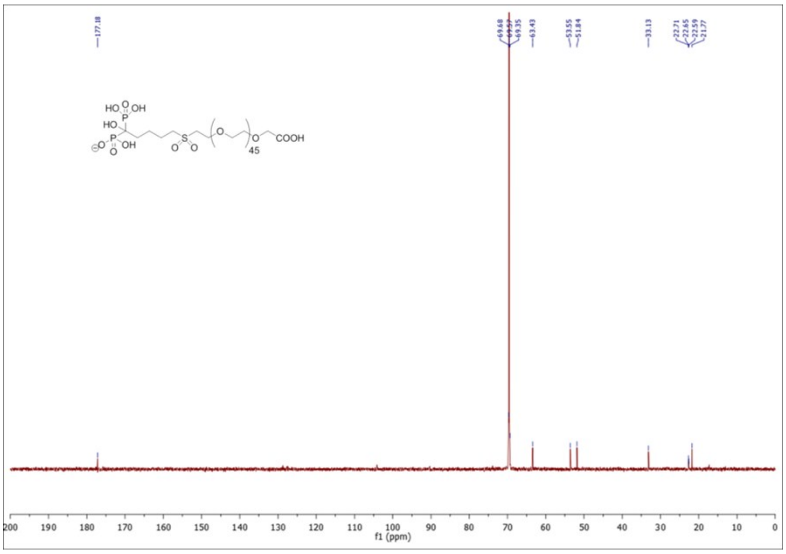




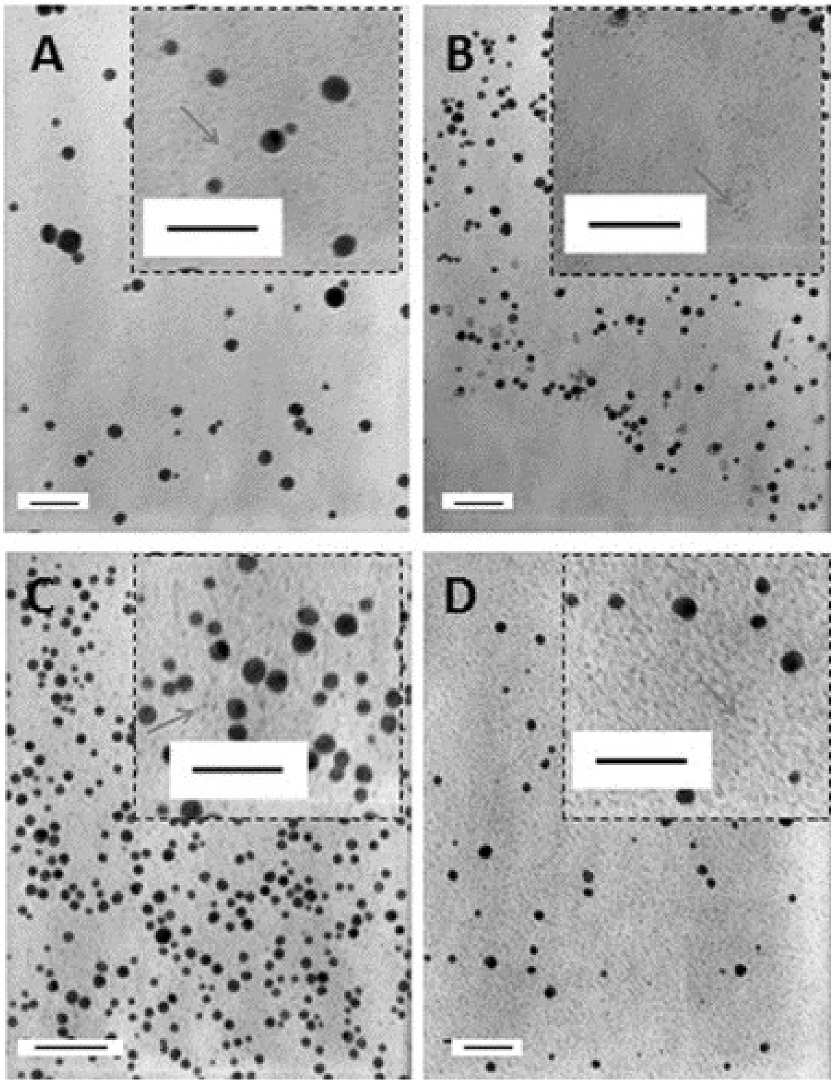
Compound 3c RMN 31P, 1H and 13C




3.99
3.97
3.96
3.91
3.86
3.85
3.84
3.68
3.50
3.49
3.48
3.32
3.30
3.28
2.00
1.97
1.93
1.90
1.83
1.82
1.80
1.78
1.76
1.74
1.72
1.70
HO
OH
O
P
HO
O
P
OH
O
S
O
O
O
45
COOH
1.58
1.67
181.41
1.54
1.96
3.83
4.5
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
2.3
2.1
1.9
1.7
1.5
1.3
1.1
f1 (ppm)


177.18
69.68
69.57
69.35
63.43
53.55
51.84
33.13
22.71
22.65
22.59
21.77
HO
OH
O
P
HO
O
P
OH
O
S
O
O
O
45
COOH
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Au NP obtained with compound 2c at: pH = 4.1 (A); pH = 8 (B); pH = 9.6 (C); pH = 11.8 (D) (scale bare is 50 nm). On insert arrows are indicating the small Au seeds.



Au NPs obtained with compound 3c at: pH = 4.6 (A); pH = 6.5 (B); pH = 8.5 (C); pH = 10.4 (D) (scale bare is 500 nm).