Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Small Gold Nanoparticles for Interfacial Staudinger-Bertozzi Ligation: from Synthesis to Bioconjugation

Pierangelo Gobbo*[a], Wilson Luo[a], Sung Ju Cho[a], Xiaoxiao Wang[a], Mark C. Biesinger[b], Robert H. E. Hudson[a] and Mark S. Workentin*[a]

[a] *The University of Western Ontario and the Centre for Materials and Biomaterials Research, Richmond Street, London, Ontario, Canada. E-mail: mworkent@uwo.ca; Tel: +1 519-661-2111 extn 86319*
[b] *Surface Science Western, The University of Western Ontario, 999 Collip Circle, London (ON), N6G 0J3 (Canada).*

# Supporting Information

# Table of contents

## Materials and Methods

The following reagents were used as received. Triethylene glycol monomethylether, tetraethylene glycol, 1-methyl-2-aminoterephthalate, sodium azide, triphenylmethanethiol, triphenylphosphine, anhydrous deuterated chloroform ($CDCl_3$), anhydrous deuterated dimethyl sulfoxide (DMSO-$d_6$), tetrachloroauric acid trihydrate, sodium borohydride, *p*-Toluenesulfonyl chloride, triisopropylsilane (TIPS), *N,N*-Diisopropylethylamine (DIPEA), O-Benzotriazole- N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) were purchased from Sigma-Aldrich. All common solvents, triethylamine (TEA), magnesium sulfate, sodium sulfate anhydrous, potassium iodide, sodium sulphite, sodium bicarbonate, dry methanol, hydrochloric acid, trifluoroacetic acid, sodium hydroxide, sodium chloride were purchased from Caledon. Palladium(II) diacetate trimer and diphenylphosphine were purchased from Alfa Aesar. Deuterated water ($D_2O$) was purchased from Cambridge Isotope Laboratories. Ethanol was purchased from Commercial Alcohols. Glacial acetic acid (99.7%) and sodium nitrite were purchased from BDH. Dialysis membranes (MWCO 6000-8000 Da) were purchased from Spectra/Por. All solvents for peptide synthesis were peptide grade, except water (18.2 MΩ cm). N-Fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids (Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Gly-OH, Fmoc-L-Asp(OtBu)-OH), Fmoc-L-Lys(Boc)-OH, Fmoc-L-Cys(Trt)-OH), [2-(2-(Fmoc-amino)ethoxy)ethoxy]acetic acid (AEEA linker), 2-chlorotrityl chloride resin (100-200 mesh, loading: 1.5 meq/g), trifluoroacetic acid (TFA), triethylsilane (TES) and piperidine were commercially available. All the materials for preparation of the azide monomer were purchased and used as previously reported.[1]

$^1H$, $^{13}C$ and $^{31}P$ NMR spectra were recorded on an Inova 400 MHz using $CDCl_3$, DMSO-$d_6$ or $D_2O$ as solvent and were calibrated against the residual protonated solvent or using $H_3PO_4$ as standard.

UV-Visible spectra have been recorded using a Varian Cary 100 bio spectrometer and 7 mm quartz cuvettes. The nanoparticles sample was dissolved in spectroscopic grade dichloromethane. The background was automatically subtracted from each spectrum.

Thermogravimetric analyses (TGA) were recorded by loading the sample in a 70 μL ceramic crucible and heating from 25 ºC to 750 ºC at rate of 10 ºC $min^{-1}$. The experiment was run under a nitrogen flow of 70 mL $min^{-1}$ in a Mettler Toledo TGA/SDTA 851 instrument.

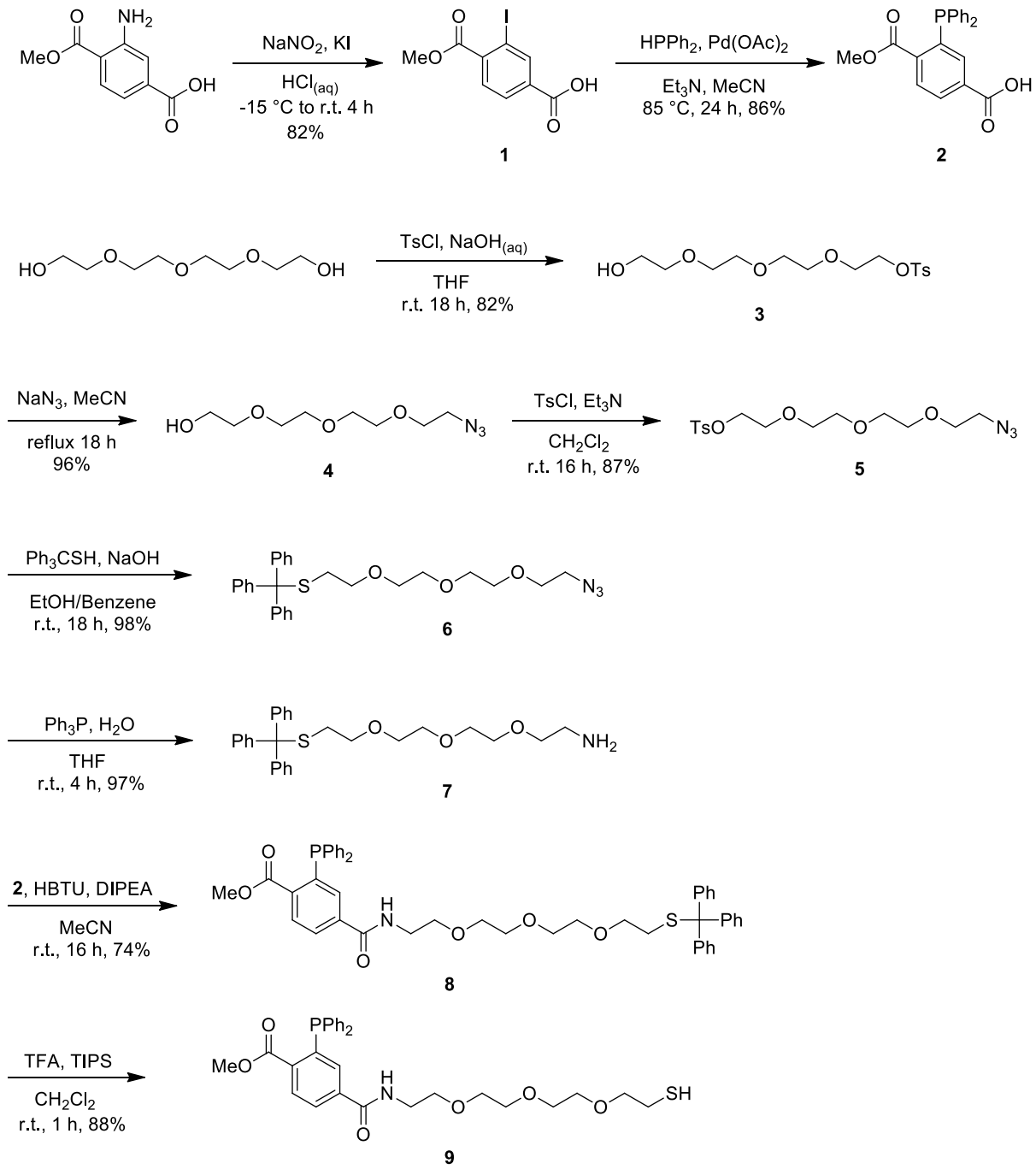
Transmission electron microscopy (TEM) images were recorded from a TEM Philips CM10 microscope. The TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and prepared by dropcasting a drop of nanoparticles solution directly onto the grid surface. The drop was then carefully removed after 30 seconds with a soft tissue.

XPS analyses were carried out with a Kratos Axis Ultra spectrometer using a monochromatic Al K(alpha) source (15mA, 14kV). XPS can detect all elements except hydrogen and helium, probes the surface of the sample to a depth of 5-7 nanometers, and has detection limits ranging from 0.1 to 0.5 atomic percent depending on the element. The instrument work function was calibrated to give a binding energy (BE) of 83.96 eV for the Au 4f7/2 line for metallic gold and the spectrometer dispersion was adjusted to give a BE of 932.62 eV for the Cu 2p3/2 line of metallic copper. Specimens were mounted on a double sided adhesive tape and the Kratos charge neutralizer system was used on all specimens. Survey scan analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 160 eV. High resolution analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 20 eV. Spectra have been charge corrected when needed to the main line of the carbon 1s spectrum set to 285.0 eV for aliphatic carbon. Spectra were analyzed using CasaXPS software (version 2.3.14).

Kinetic measurements using $^{31}P$ NMR spectroscopy were performed at 25ºC as follows. Concentrated stock solutions of Staudinger-AuNP, phosphine-thiol **(9)** and benzyl azide were prepared in 1 mL of anhydrous deuterated solvent (either $CDCl_3$ or DMSO-$d_6$) and sealed and stored in the

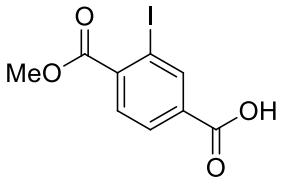
freezer. For the model reaction in solution and at 25ºC phosphine-thiol **(9)** was dissolved in deuterated solvent to obtain a 0.043 M or a 0.073 M solution. To this solution 1 equivalent of $Ph_3P{=}O$ was dissolved and used as the internal standard. Benzyl azide was dissolved in 1 mL of deuterated solvent to obtain a 1.727 M or 2.767 M solution. For the experiments at the nanoparticles interface 100 mg of Staudinger-AuNP were dissolved in 1 mL of anhydrous $CDCl_3$ to obtain a 0.043 M solution in active interfacial methyl 2-(diphenylphosphino)benzoate. In a typical kinetic experiment 300 μL of phosphine thiol or Staudinger-AuNP solution were insert into a dry NMR tube and a spectrum was recorded. Subsequently 50 μL of nanopure water and 150 μL of benzyl azide stock solution were injected and the NMR tube was shaken vigorously. The reaction was monitored by $^{31}P$ NMR at 25ºC until at least 60% of the reaction was complete. Typically the first spectrum was acquired after 2-3 min from the addition of water and benzyl azide. Peak areas were integrated and normalized towards the $Ph_3P{=}O$ peak for the experiments in solution and towards the oxidized methyl 2-(diphenylphosphino)benzoate at the nanoparticle's interface.

## Synthesis of Methyl 2-(Diphenylphosphino)Benzoate Ligand




**Scheme SI1:** Synthetic strategy for the synthesis of the Methyl 2-(Diphenylphosphino)Benzoate Ligand.

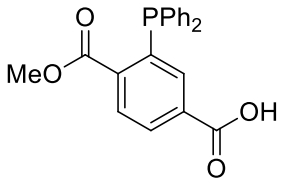
**Synthesis of 3-iodo-4-(methoxycarbonyl)benzoic acid (1)[2]**




**1**

The solution of 1-methyl-2-aminoterephthalate (3.0 g, 15.4 mmol) in hydrochloric acid (4 M, 80 mL) was cooled in an ice-salt bath, to which was slowly added an aqueous solution (25 mL) of sodium nitrite (1.2 g, 17.4 mmol) over a period of 0.5 h. The mixture was stirred at this temperature for another 0.5 h. An aqueous solution (100 mL) of potassium iodide (13.0 g, 77.0 mmol) was cooled to -15 °C and then added into the reaction mixture. The mixture was stirred at room temperature for 4 h. After addition of a saturated sodium sulphite aqueous solution (50 mL), the precipitation was collected by filtration and washed with a large amount of water. Recrystallization of this crude product from a methanol/water solution gave **(1)** as yellow crystals (3.85 g, 82%). $^1$H NMR (400 MHz, $CDCl_3$) δ 3.97 (s, 3H), 7.84 (d, $J$ = 8.0 Hz, 1H), 8.11 (dd, $J$ = 8.1, 1.6 Hz, 1H), 8.68 (d, $J$ = 1.7 Hz, 1H).

**Synthesis of 3-(diphenylphosphino)-4-(methoxycarbonyl)benzoic acid (2)[2]**

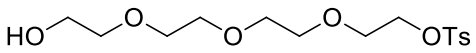



**2**

To an MeCN (15 mL) solution of **(1)** (2.0 g, 6.5 mmol) were added anhydrous triethylamine (2.7 mL, 19.5 mmol) and $Pd(OAc)_2$ (146 mg, 0.65 mmol) under an argon atmosphere. Diphenylphosphine (1.4 mL, 7.8 mmol) was slowly added at room temperature with stirring. The resultant solution was heated at reflux for 24 h. After removal of the solvent, the residue was dissolved in $CH_2Cl_2/H_2O$ (300 mL, 1:1 in v/v). The organic layer was separated and washed with HCl (1 M, 20 mL x 3), dried

over $MgSO_4$, and evaporated to give a crude product. Recrystallization from cold MeOH (5 mL) afforded **(2)** as a yellow solid (1.6 g, 67%). $^1$H NMR (400 MHz, $CDCl_3$) δ 3.75 (s, 3H), 7.29-7.34 (m, 10H), 7.66 (br, 1H), 8.07 (br, 2H). $^{31}$P NMR (162 MHz, CDCl3) δ -3.32 (s).

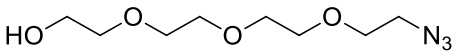
**Synthesis of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (3)**




**3**

To a solution of tetraethylene glycol (112.5 g, 579 mmol) in THF (150 mL) was added aqueous NaOH (2.2 M, 50 mL). The resultant mixture was stirred vigorously for 20 min. A solution of *p*-Toluenesulfonyl chloride (10.7 g, 56.2 mmol) in THF (80 mL) was added dropwise slowly via a dropping funnel and the mixture was stirred at ambient temperature for 18 h. After addition of $H_2O$ (200 mL) and $CH_2Cl_2$ (150 mL), the organic layer separated, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was then purified by flash column chromatography on silica gel (EtOAc:Hexanes = 2:1 to EtOAc 100%) to afford **(3)** as a yellow oil (17.3 g, 88%). $^1$H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 2.44 (s, 1H), 3.57 – 3.74 (m, 16H), 4.13 – 4.16 (m, 2H), 7.33 (d, $J$ = 8.0 Hz, 2H), 7.79 (d, $J$ = 8.4 Hz, 2H).

**Synthesis of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol (4)[2]**

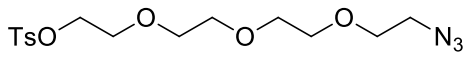



**4**

Sodium azide (1.8 g, 27.1 mmol) was added to a solution of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate **(3)** (6.3 g, 18 mmol) in MeCN (50 mL). The mixture was heated at reflux for 18 h. The solution was allowed to cool and $H_2O$ (50 mL) was added. The aqueous phase was extracted with $CH_2Cl_2$ (3 x 50 mL) and the combined organic portions

were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **(4)** as a yellow oil (3.78 g, 96%) which was used directly in the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$) δ 2.53 (s, 1H), 3.36 – 3.42 (m, 2H), 3.58 – 3.61 (m, 2H), 3.64 – 3.68 (m, 10H), 3.70 – 3.73 (m, 2H).

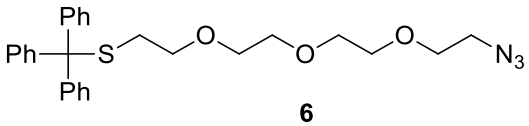
**Synthesis of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5)[3]**




**5**

To a solution of azide **(4)** (2.3 g, 10.5 mmol) and triethylamine (2.2 mL, 15.8 mmol) in $CH_2Cl_2$ (30 mL) was added p-Toluenesulfonyl chloride (3.0 g, 15.7 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h. Water (20 mL) was then added to the reaction mixture. The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$ (30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel (EtOAc:Hexanes = 3:1 to EtOAc 100%) to afford **(5)** as a pale yellow oil (3.6 g, 92%). $^1$H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 3.36 (t, $J$ = 5.0 Hz, 2H), 3.58 (s, 4H), 3.62 – 3.68 (m, 8H), 4.13 – 4.15 (m, 2H), 7.33 (dd, $J$ = 8.0, 0.6 Hz, 2H), 7.78 (d, $J$ = 8.3 Hz, 2H).

**Synthesis of 13-azido-1,1,1-triphenyl-5,8,11-trioxa-2-thiatridecane (6)**

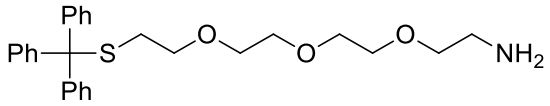



**6**

Triphenylmethanethiol (2.0 g, 7.2 mmol) was dissolved in a solution of EtOH/benzene (1:1, 16 mL) and NaOH (0.36 g, 9.0 mmol) in $H_2O$ (4 mL) was added. To this mixture was added a solution of **(5)** (2.2 g, 5.9 mmol) in EtOH/benzene (1:1, 16 mL). The reaction mixture was stirred at room temperature

for 18 h. Once the reaction was completed (checked by TLC), the mixture was poured into a $NaHCO_3$ saturated solution. The organic layer was washed with $NaHCO_3$ (3x) and brine (3x). The organic portion was then dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using hexanes/EtOAc 3:1 to 1:1 (v/v) as the eluent (2.8 g, 98%). $^1$H NMR (400 MHz, $CDCl_3$) δ 2.44 (t, *J* = 6.9 Hz, 2H), 3.31 (t, *J* = 6.9 Hz, 2H), 3.34 – 3.38 (m, 2H), 3.46 (dd, *J* = 5.7, 3.8 Hz, 2H), 3.57 – 3.60 (m, 2H), 3.63 – 3.68 (m, 6H), 7.18 – 7.31 (m, 9H), 7.40 – 7.45 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 31.6, 50.6, 66.5, 69.55, 69.97, 70.12, 70.50, 70.62, 126.6, 127.8, 129.6, 144.8.

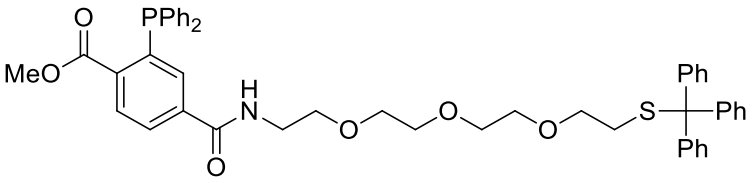
**Synthesis of 1,1,1-triphenyl-5,8,11-trioxa-2-thiatridecan-13-amine (7)[4]**




**7**

Triphenylphosphine (3.3 g, 12.6 mmol) and $H_2O$ (0.6 mL) were added to a solution of **(6)** (5.4 g, 11.3 mmol) in THF (20 mL) under an argon atmosphere. After stirring at room temperature for 4 h, the mixture was concentrated under reduced pressure. Purification of the crude product by flash column chromatography, eluting with $CHCl_3$/MeOH/TEA (3:3:1), provided **(7)** as a yellow oil (4.9 g, 96%). $^1$H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 2H), 2.43 (t, *J* = 6.9 Hz, 2H), 2.85 (t, *J* = 5.1 Hz, 2H), 3.30 (t, *J* = 6.9 Hz, 2H), 3.45 (dd, *J* = 5.7, 3.9 Hz, 2H), 3.49 (t, *J* = 5.2 Hz, 2H), 3.55 – 3.63 (m, 6H), 7.18 – 7.30 (m, 9H), 7.39 – 7.43 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 31.6, 41.6, 66.6, 69.6, 70.12, 70.22, 70.42, 70.54, 73.0, 126.6, 127.8, 129.6, 144.8. ESI-MS calcd for $C_{27}H_{34}NO_3S^+$ [M+H$^+$] 452.2259, found 452.2240.

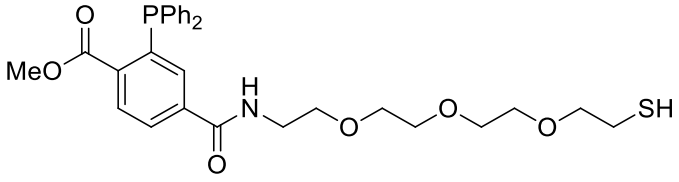
**Synthesis of methyl 2-(diphenylphosphanyl)-4-((1,1,1-triphenyl-5,8,11-trioxa-2-thiatridecan-13-yl)carbamoyl)benzoate (8)**



**8**

Compound **(2)** (1.0 g, 2.7 mmol) and HBTU (1.4 g, 3.7 mmol) were combined into a flask under argon. MeCN (20 mL), primary amine **(7)** (1.4 g, 3.1 mmol) and DIPEA (1.3 mL, 7.4 mmol) were injected. The resultant solution was stirred at room temperature under Ar for 16 h. The crude product was purified by column chromatography over silica gel using hexanes/EtOAc (1:2) as the eluent (1.6 g, 74%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 2.39 (t, *J* = 6.9 Hz, 2H), 3.26 (t, *J* = 6.9 Hz, 2H), 3.42 (dd, *J* = 5.6, 3.8 Hz, 2H), 3.50 – 3.61 (m, 10H), 3.73 (s, 3H), 6.56 (s, 1H), 7.15 – 7.43 (m, 26H), 7.77 (dd, *J* = 8.0, 1.7 Hz, 1H), 8.07 (dd, *J* = 8.0, 3.6 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 31.6, 39.8, 52.2, 69.52, 69.58, 70.10, 70.23, 70.39, 70.51, 126.6, 127.8, 128.60, 128.67, 129.08, 129.56, 130.8, 132.8, 133.76, 133.96, 137.4, 144.7, 166.28, 166.62. $^{31}$P NMR (162 MHz, $CDCl_3$) δ -3.51 (s). ESI-MS calcd for $C_{48}H_{49}NO_6PS^+$ [M+H$^+$] 798.3018, found 798.3022.

**Synthesis of methyl 2-(diphenylphosphanyl)-4-((2-(2-(2-(2-mercaptoethoxy)ethoxy)ethoxy)ethyl)carbamoyl)benzoate (9)**



**9**

To a solution of **(8)** (0.8 g, 1.0 mmol) in 5% TFA/$CH_2Cl_2$ (v/v, 40 mL) was added TIPS (0.25 mL, 1.2 mmol) as a carbocation scavenger. The reaction mixture was stirred under Ar for 1 h. The solution was washed with 0.2 M $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated. The crude product was

purified by flash column chromatography on silica gel (EtOAc:Hexanes = 3:1 to EtOAc 100%) to afford **(9)** as a yellow oil (0.5 g, 90%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 1.56 (t, *J* = 8.2 Hz, 1H), 2.64 (dd, *J* = 14.5, 6.6 Hz, 2H), 3.53 – 3.63 (m, 14H), 3.74 (s, 3H), 6.54 (t, *J* = 4.4 Hz, 1H), 7.25 – 7.37 (m, 11H), 7.79 (d, *J* = 8.1 Hz, 1H), 8.08 (dd, *J* = 8.0, 3.6 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 24.1, 39.7, 52.1, 69.5, 70.03, 70.12, 70.34, 70.43, 72.7, 126.6, 128.47, 128.54, 128.83, 130.65, 130.67, 132.6, 133.66, 133.86, 136.38, 136.57, 137.01, 137.12, 137.26, 141.19, 141.48, 166.29, 166.55. $^{31}$P NMR (162 MHz, $CDCl_3$) δ -3.82 (s). ESI-MS calcd for $C_{29}H_{35}NO_6PS^+$ [$M+H^+$] 556.1923, found 556.1902.

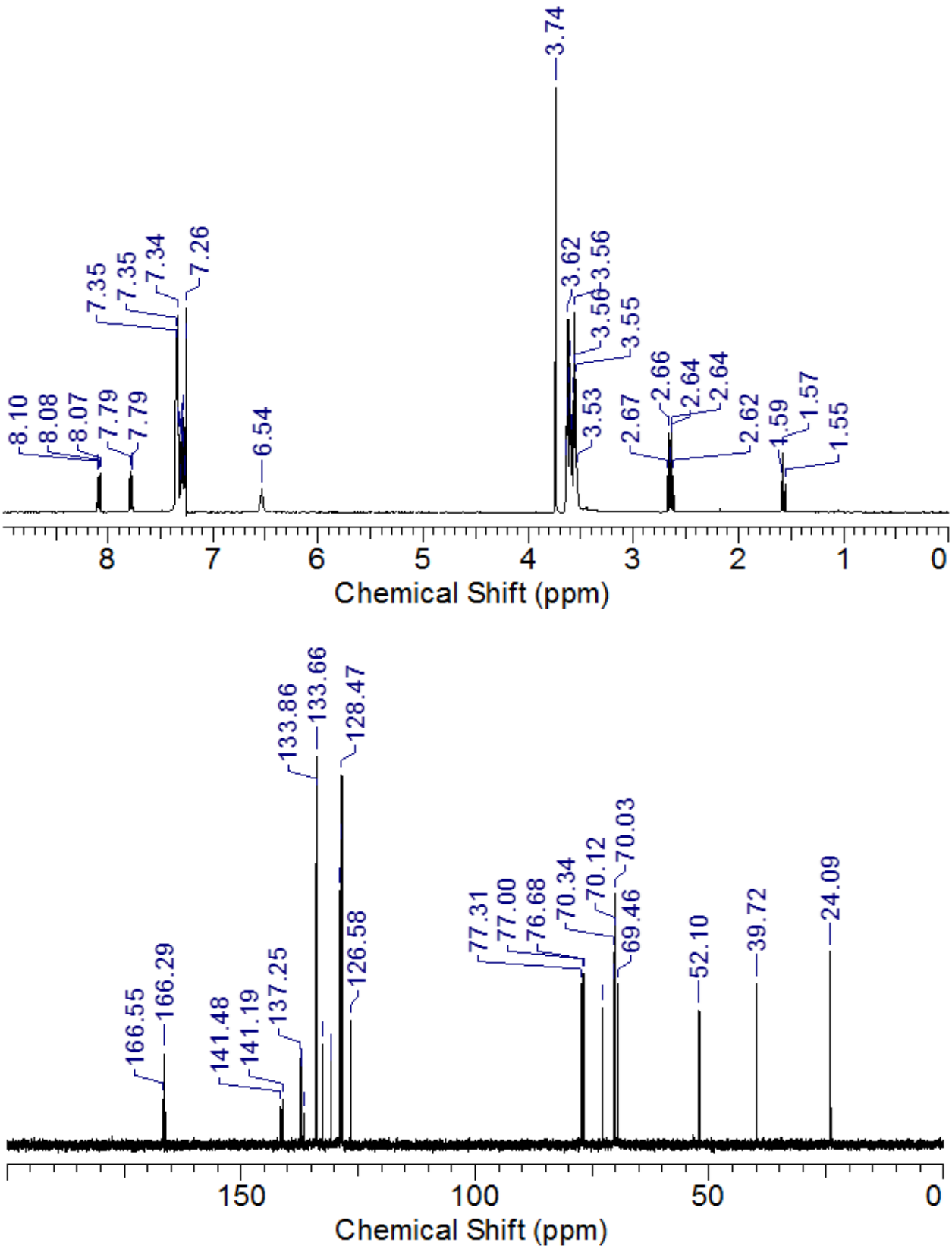
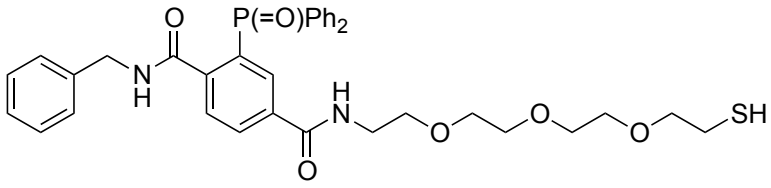



**Fig. SI1:** Top: $^{1}H$ NMR spectrum of compound **(9)**. Spectrum referenced against residual $CHCl_3$. Bottom: $^{13}C$ NMR spectrum of compound **(9)**. Spectrum referenced against residual $CHCl_3$.

**Staudinger Ligation Product Using Model Compound (9)**




**10**

Thiol **(9)** (50 mg, 0.1 mmol), benzy lazide (13.3 mg, 0.1 mmol), 2 drops of $H_2O$ in MeCN (5mL) stirred at room temperature for overnight. The resulting solution was concentrated in vacuo to give the Staudinger ligation product. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 1.58 (t, *J* = 8.2 Hz, 1H), 2.59 – 2.65 (m, 2H), 3.51 – 3.64 (m, 14H), 4.07 (d, *J* = 5.48 Hz, 2H), 6.90 (t, *J* = 4.3 Hz, 1H), 7.17 – 7.29 (m, 5H), 7.42 – 7.52 (m, 4H), 7.54 – 7.71 (m, 7H), 7.95 (dd, *J* = 8.2 Hz, *J* = 1.6 Hz, 1H), 7.99 – 8.04 (m, 1H), 9.07 (t, *J* = 5.1 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 24.2, 29.6, 39.9, 44.1, 69.4, 70.07, 70.20, 70.38, 70.51, 72.7, 127.17, 127.92, 128.49, 128.72, 128.84, 129.80, 129.88, 130.17, 130.19, 130.77, 130.94, 131.62, 131.72, 131.84, 132.52, 132.55, 132.72, 132.85, 135.56, 135.66, 137.5, 143.20, 143.28, 165.5, 166.45, 166.49. $^{31}$P NMR (162 MHz, $CDCl_3$) δ 34.87 (s). ESI-MS calcd for $C_{35}H_{40}N_2O_6PS^+$ $[M+H^+]$ 647.2345, found 647.2333.

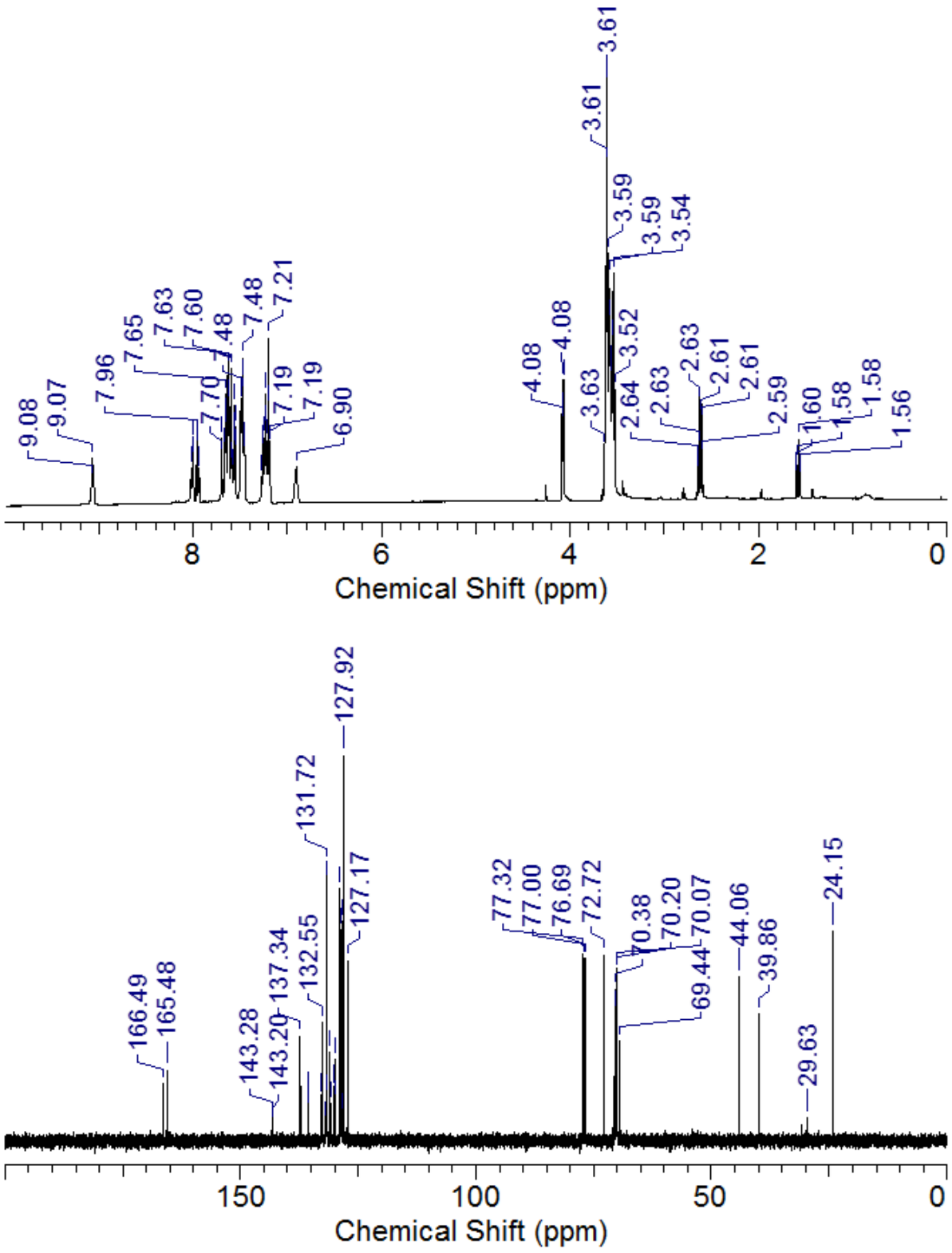



**Fig. SI2:** Top: $^{1}$H NMR spectrum of Staudinger ligation product **(10)**. Spectrum referenced against residual $CHCl_3$. Bottom: $^{13}$C NMR spectrum of Staudinger ligation product **(10)**. Spectrum referenced against residual $CHCl_3$.

## Synthesis of Staudinger-AuNP

Me-$EG_3$-AuNPs were synthesized according to our previously established procedure.[5] $HAuCl_4 \cdot 3H_2O$ (1.4564 g, 3.7 mmol, 1.0 eq.) was dissolved in a mixture of dry methanol (503 mL) and glacial acetic acid (83 mL). To this yellow solution was added Me-$EG_3$-SH (2.0 g, 11 mmol, 3.0 eq.). The slightly darkened solution was stirred vigorously for 2 h and the solution color slightly faded. A solution of $NaBH_4$ (1.3997 g, 37 mmol, 10.0 eq.) in nanopure $H_2O$ (96 mL) was added dropwise to the reaction mixture under vigorous stirring. The mixture turned dark brown immediately. After overnight stirring at ambient temperature, the solution was concentrated and rediluted with brine. The Me-$EG_3$-AuNPs were extracted with toluene while adding sodium chloride to the aqueous phase after each extraction to maintain the saturation. The aqueous phase was eventually colorless. The combined organic phases were then concentrated in vacuo. Evaporation of the solvent left a thin film of nanoparticles which was then rinsed with hexanes to remove the excess free thiol. The crude Me-$EG_3$-AuNPs were dissolved in nanopure $H_2O$ and further purified by overnight dialysis. $^1$H NMR (400 MHz, $CDCl_3$) δ 3.34 (-$CH_3$), 3.58, 3.66 (-$CH_2$-).

To a solution of Me-$EG_3$-AuNP (200 mg) in $CH_2Cl_2$ (45 mL) was added a solution of thiol **(9)** (100 mg, 0.18 mmol) in $CH_2Cl_2$ (5 mL) under an argon atmosphere while stirring vigorously. After 0.25 h of stirring, the solution was concentrated under reduced pressure. The residue was dissolved in $CH_2Cl_2$ (6 mL) and hexanes (33 mL) was added to precipitate the Staudinger AuNPs. The resulting suspension was placed under centrifugation at 5000 rpm for 5 min and the supernatant containing free thiols was removed. This washing procedure was performed four more times and the resulting Staudinger AuNPs were dried in vacuo.

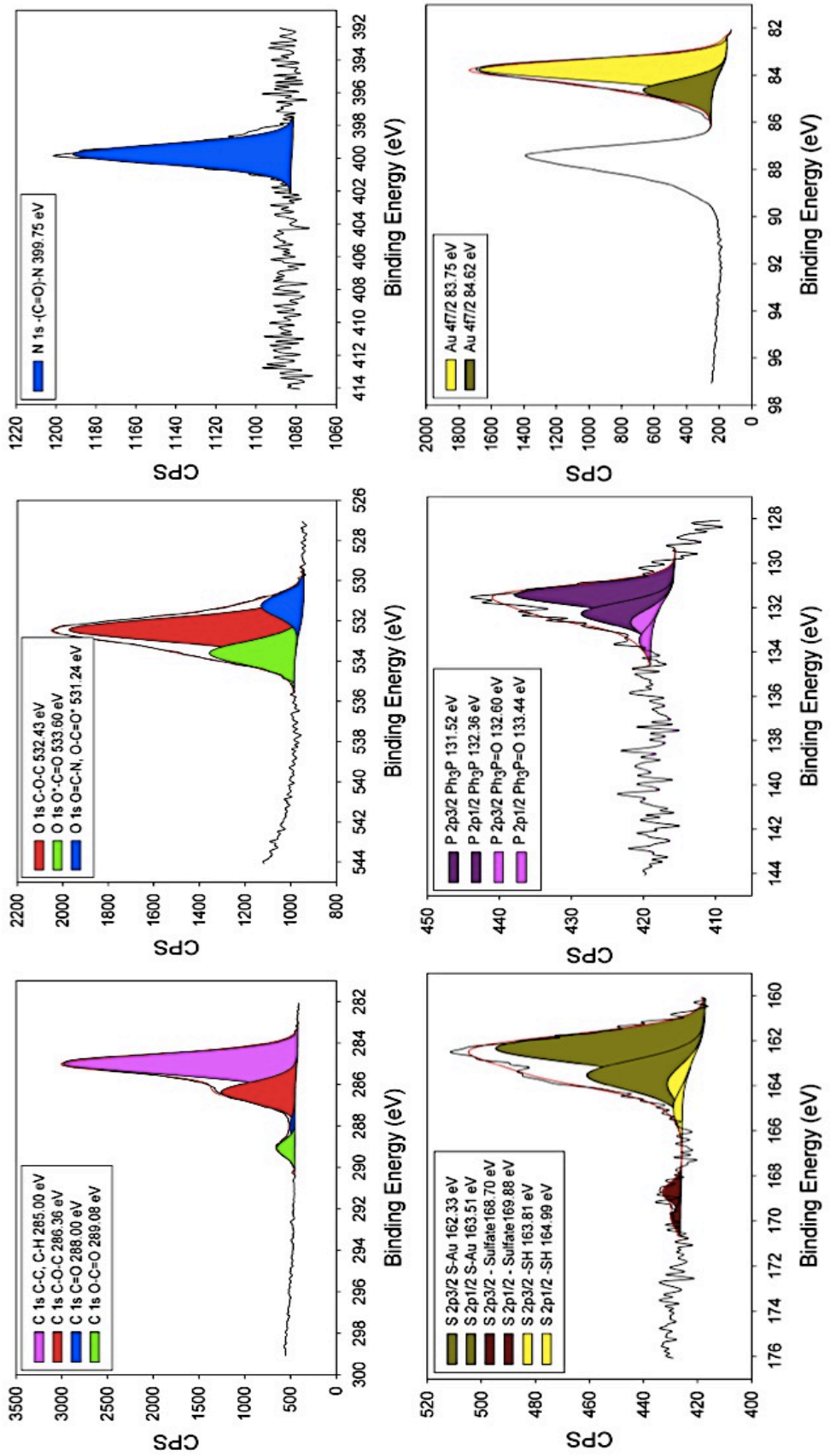



**Fig. SI3:** XPS characterization of Staudinger-AuNP.

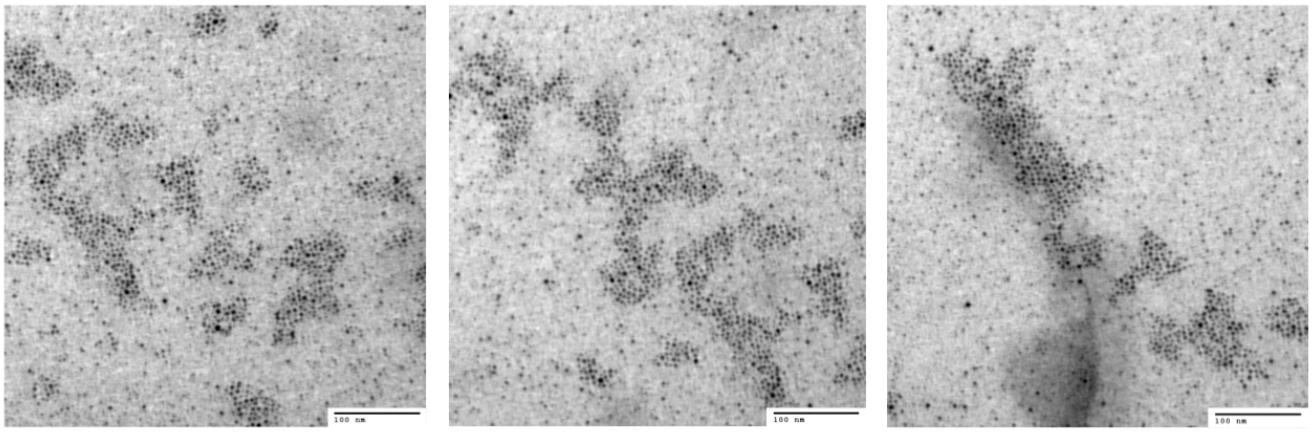


**Fig. SI4:** TEM images of Staudinger-AuNP.

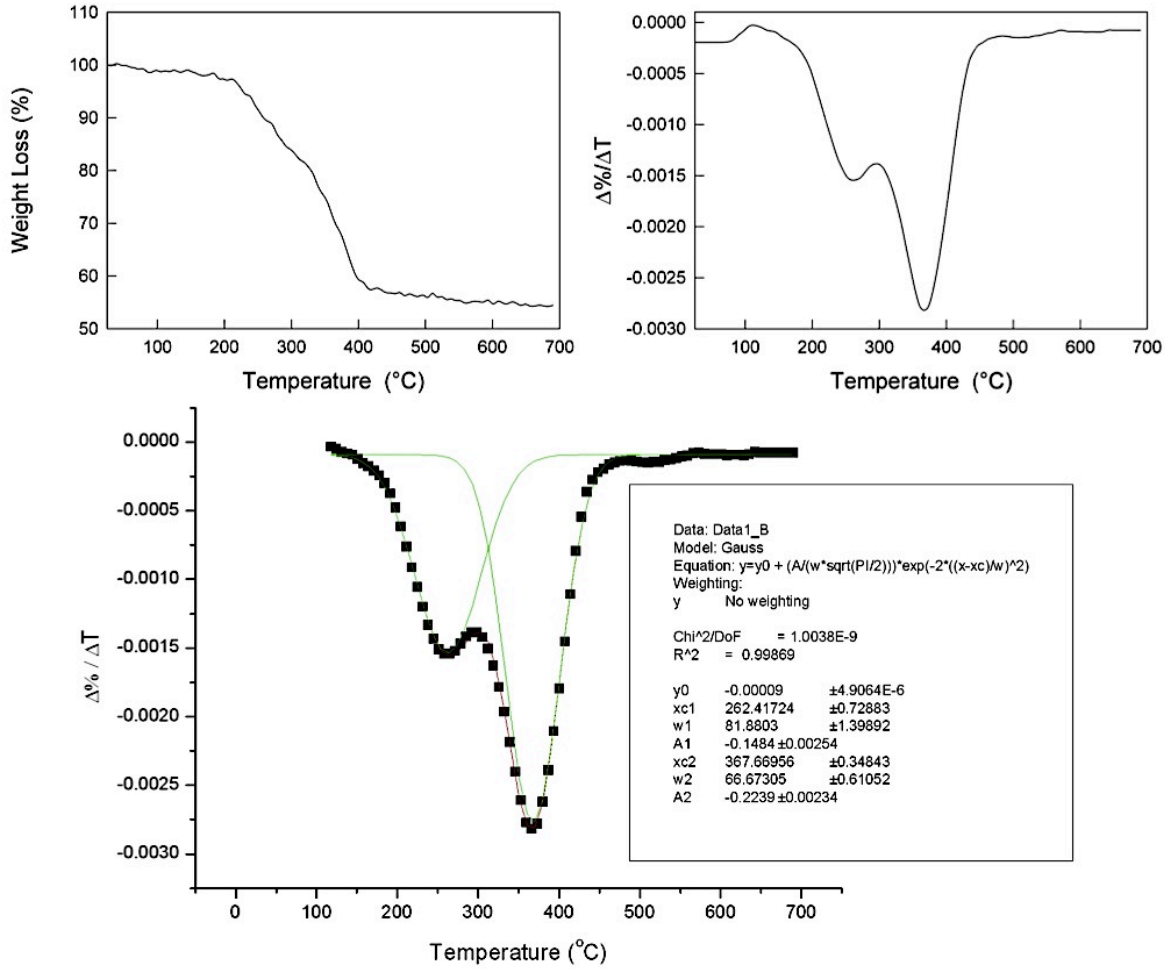



**Fig. SI5:** TGA characterization of Staudinger-AuNP.

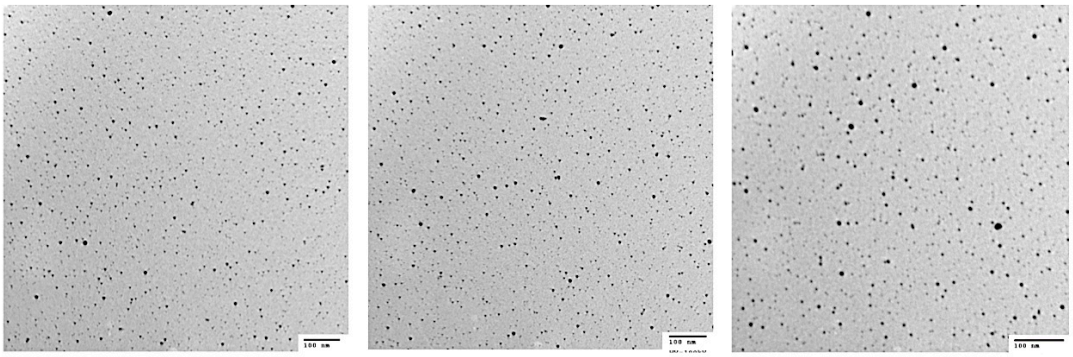


**Fig. SI6:** TEM images of Staudinger-AuNP after reaction with benzyl azide.

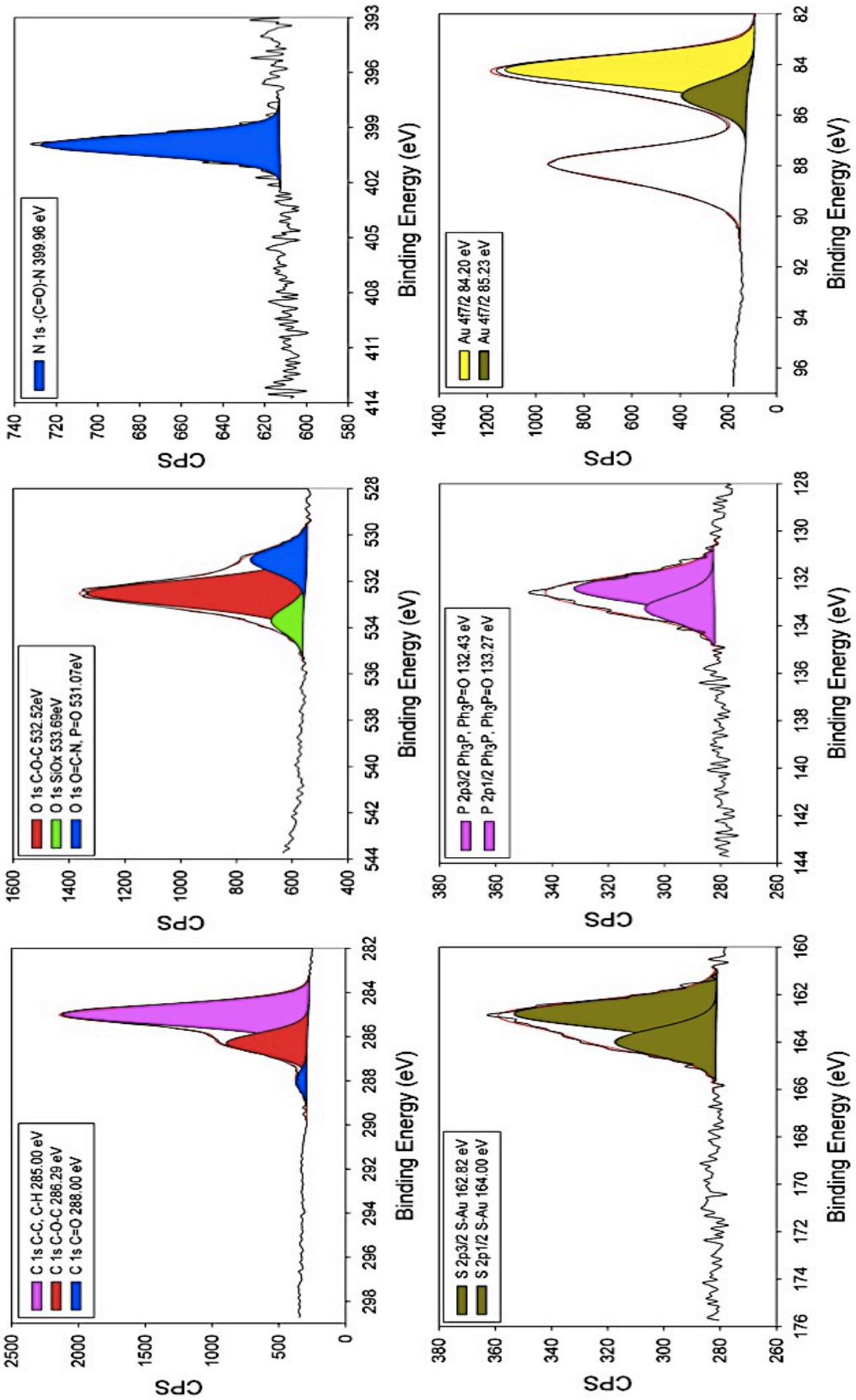



**Fig. SI7:** XPS characterization of Staudinger-AuNP after reaction with benzyl azide.

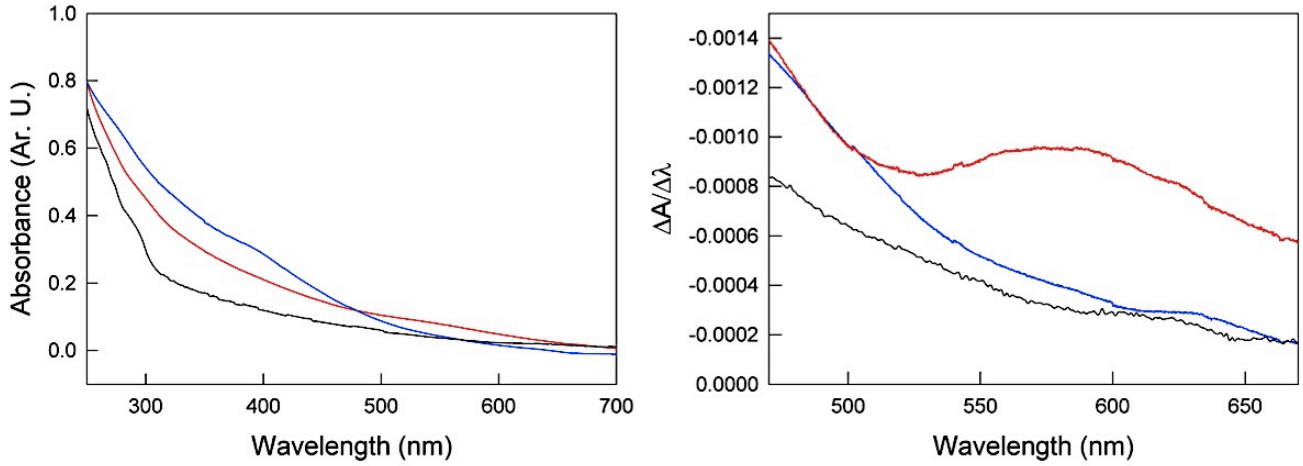



**Fig. SI8:** Left plot: UV-Vis spectra of AuNP samples recorded in dichloromethane. Right plot: UV-Vis derivative. Blue curve: Me-$EG_3$-AuNP; red curve: Staudinger-AuNP; black curve Staudinger-AuNP after reaction with benzyl azide.

## Calculation of the nanoparticles raw formula

From the deconvolution of the TGA derivative (see **figure SI4**, bottom) it is possible to calculate the weight amount of template ligand (Me-$EG_3$-SH) and phosphine ligand per milligram of Staudinger-AuNP. A fraction of the phosphine ligand is present as triphenylphosphine-oxide (8% from the $^{31}P$ NMR spectrum). It is possible to calculate the amount, in grams, of oxidized phosphine ligand ($mg_{P=O}$) and active phosphine ($mg_{P:}$) per milligram of AuNP from the following equations:

$$mg_{P:} = \frac{M_{TGA(P:+P=O)} \cdot MW_{P:} \cdot n_{\%P:}}{MW_{P:} \cdot n_{\%P:} + MW_{P=O} \cdot n_{\%P=O}}$$

$$mg_{P=O} = M_{TGA} - mg_{P:}$$

Where:

$M_{TGA(P:+P=O)}$ = mass of phosphine ligand (P: + P=O) per milligram of AuNP from TGA

$MW_{P:}$ = molecular weight of phosphine ligand

$MW_{P=O}$ = molecular weight of phosphine oxide ligand

$n_{\%P:}$ = molar percentage of phosphine ligand from $^{31}P$ NMR

$n_{\%P=O}$ = molar percentage of phosphine oxide ligand from $^{31}P$ NMR (normalized to 1)

Knowing the molecular weights of the three different ligands (MeO-$EG_3$-$S^-$; thiolate **(9)**; oxidized thiolate **(9)**) it is possible to know the molar percentage of each ligand per milligram of nanoparticles.

The number of gold atoms ($N_{Au}$) can be calculated from the following formula[6]:

$$N_{Au} = \frac{\pi \rho d^3 N_A}{6 M_{Au}}$$

Where:

$\rho$ = density of face centered cubic(fcc) gold lattice (19.3 g $cm^{-1}$)

$d_3$ = average diameter of nanoparticles in centimeters (from TEM images)

$N_A$ = Avogadro constant

$M_{Au}$ = mole atomic weight of gold (196.9665 g mol-1)

This assuming that the AuNPs are spherical and that their size is monodispersed.

The total number of ligands ($N_L$) can be calculated using the following formula:

$$N_L = \frac{N_{Au}\, M_{Au} M_{TGA}}{(1 - M_{TGAorg})(MW_{P:} n_{\%P:} + MW_{MeO} n_{\%MeO} + MW_{P=O} n_{\%P=O})}$$

Where:

$M_{TGA}$ = organic percentage from TGA

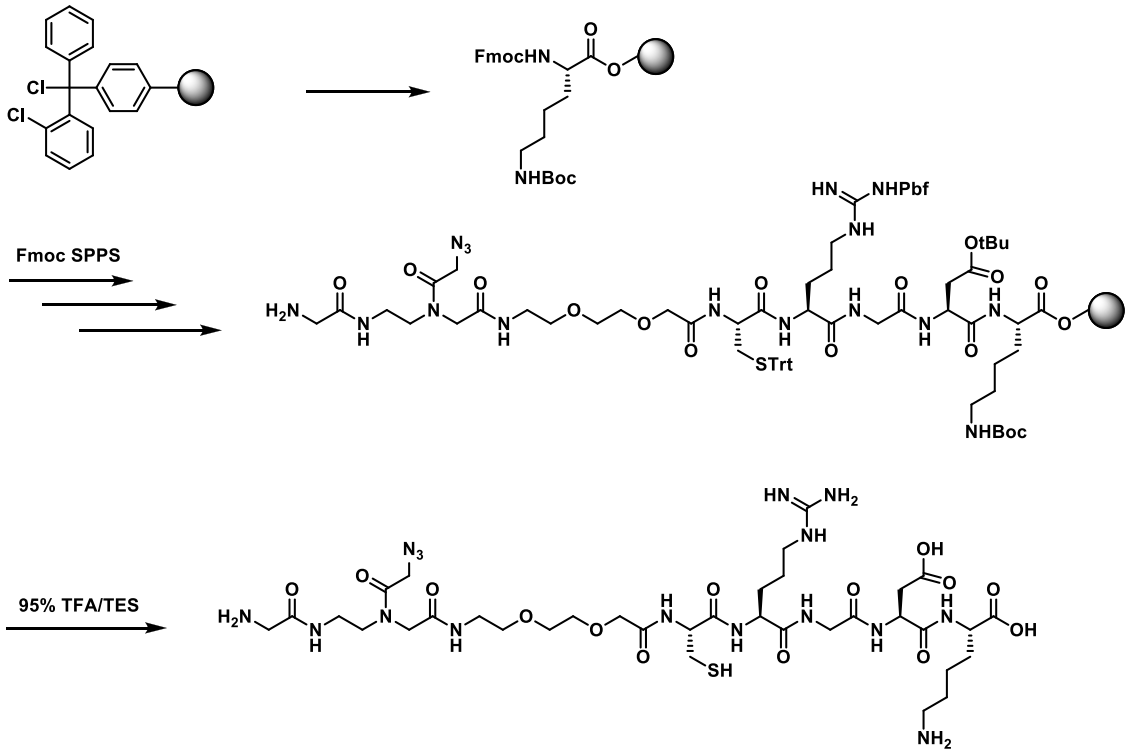
$MW_{MeO}$ = molecular weight of MeO-$EG_3$-$S^-$ ligand

$n_{\%MeO}$ = molar percentage of MeO-$EG_3$-$S^-$ ligand

From the number of ligands per particle, the number of gold atoms per particles, and the molar percentage of the three different ligands it is possible to obtain the nanoparticles raw formula.

## Synthesis of Azide-CRGDK peptide

The peptide was prepared at 0.1 mmol scale automatically (CEM Liberty Blue Automated Microwave Peptide Synthesizer) via standard Fmoc SPPS procedure by using 2-chlorotrityl chloride resin as solid support. The coupling of the first residue used 1.5 eq. Fmoc-protected amino acid and 4 eq. of DIEA in a DCM solution for 2 hours. The coupling of each residue used 5 eq. (relative to the loading of resin) Fmoc-protected amino acids, 5 eq. of HBTU and 10 eq. of DIEA in a DMF. Cleavage of the peptide from the dried resin was performed by suspending the resin in cleavage cocktail containing TFA (95%), TES (5%) for 1 hour. The filtrate was concentrated to a viscous solution by flushing it with $N_2$. The crude product was dissolved in water and purified by HPLC. The characterization of the peptide was performed by ESI-MS: calculated mass [M] 962.4352; found [M+1] 963.4155. Purity: 92.6% determined by ultra-performance liquid chromatography (UPLC) with a C18 column and using a linear gradient of acetonitrile and MQ water containing 0.1% HCOOH.




**Scheme SI2:** Solid-phase synthesis of $N_3$-CRGDK.

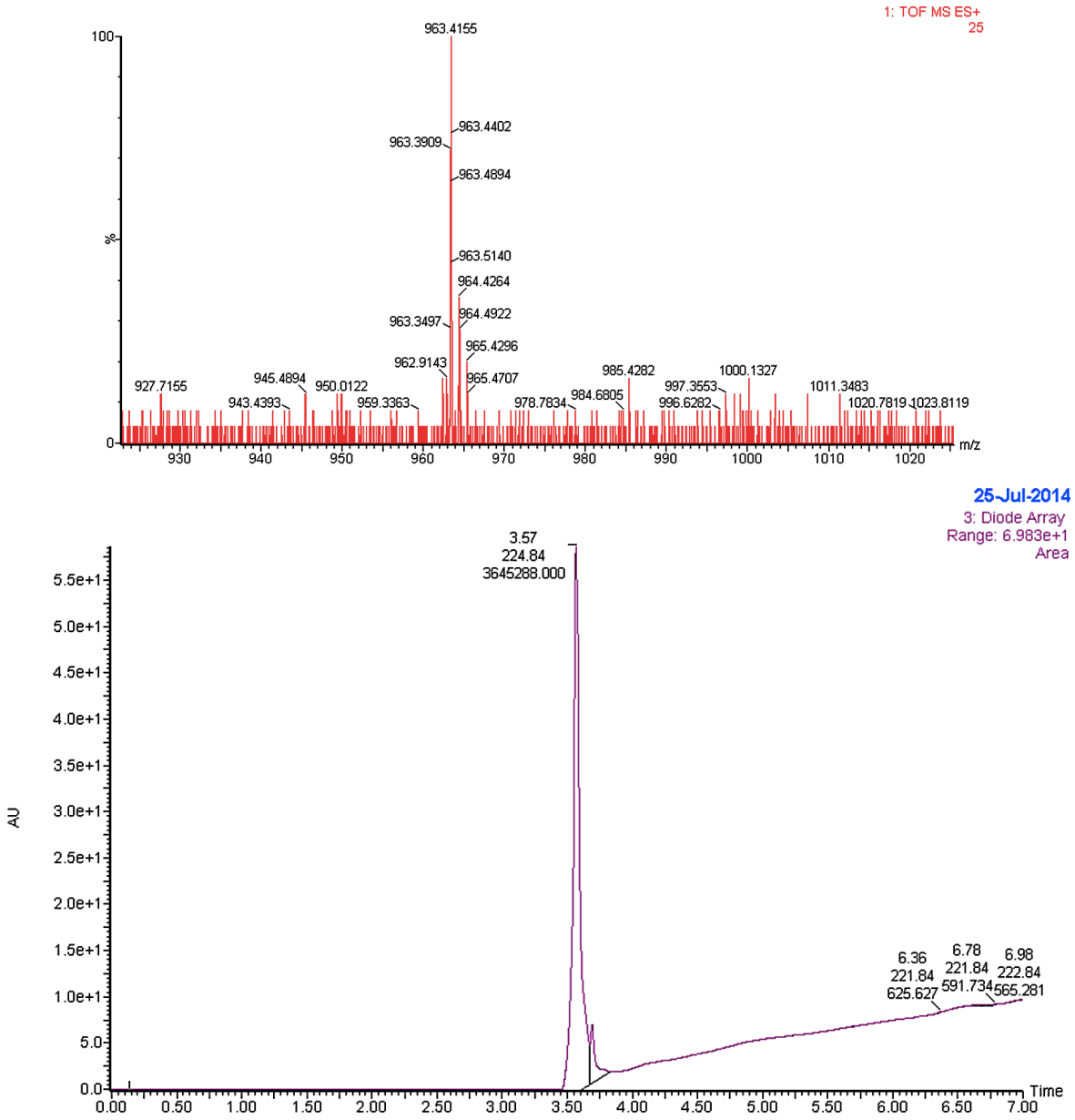



**Fig. SI9:** ESI-MS and UPLC spectra of $N_3$-CRGDK.

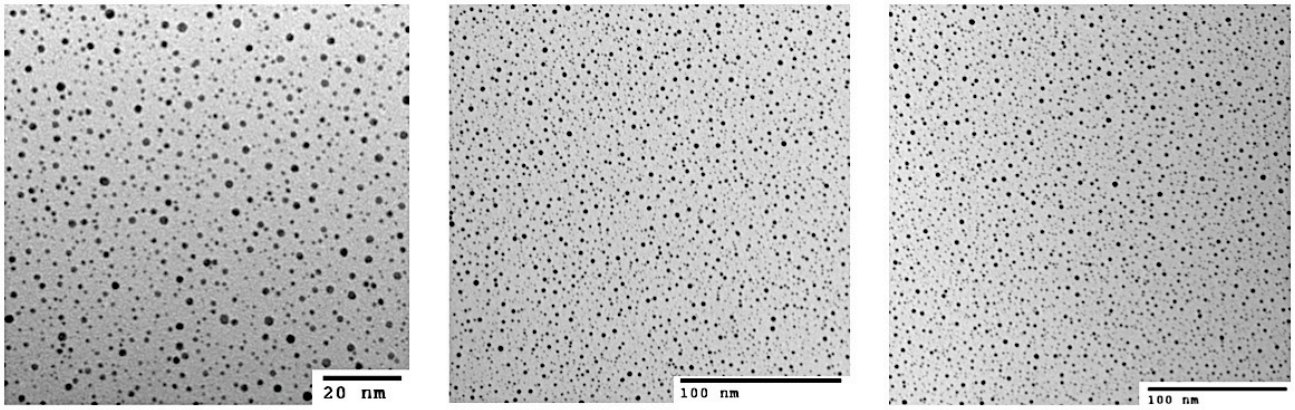



**Fig. SI10:** TEM images of Staudinger-AuNP after reaction with azide-CRGDK peptide.

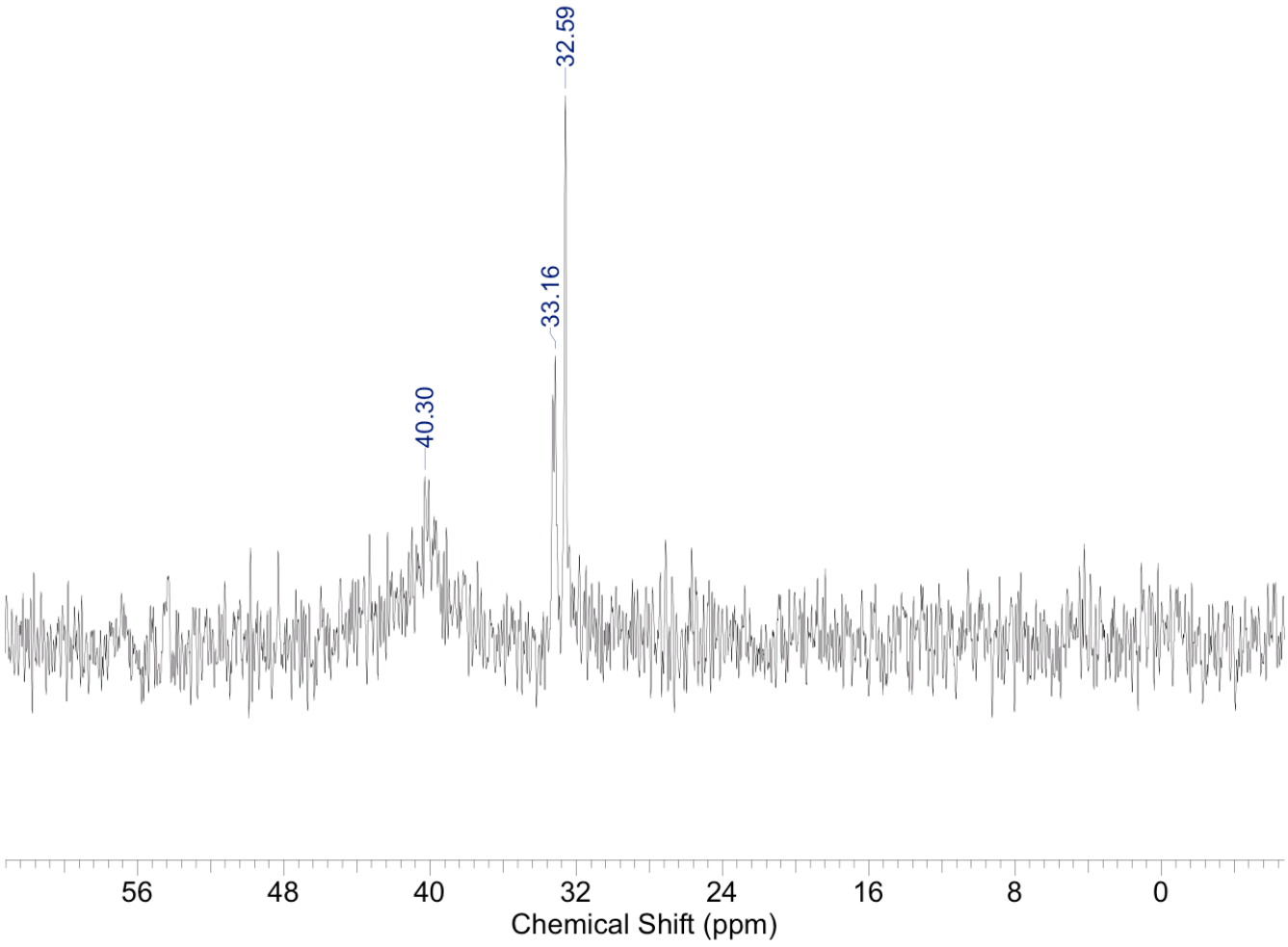



**Fig. SI11:** $^{31}$P NMR spectrum of Staudinger-AuNPs reacted with azide-functionalized CRGDK peptide.

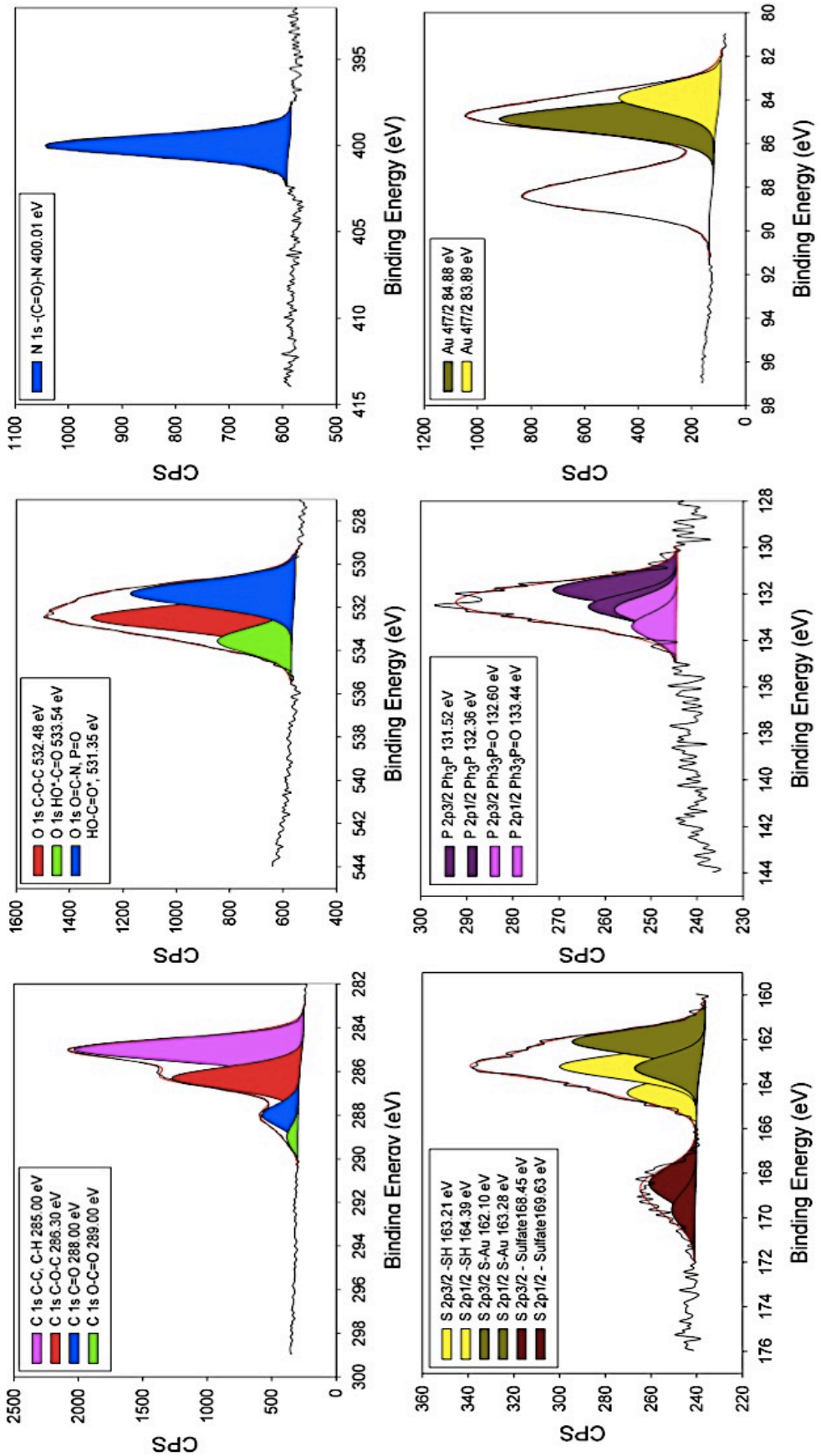



**Fig. SI12:** XPS characterization of Staudinger-AuNP after reaction with azide-CRGDK peptide.

## References


1. A. H. St Amant, C. Engbers and R. H. Hudson, *Artificial DNA, PNA & XNA*, 2013, **4**, 4-10.
2. K. J. Zhou, J. F. Li, Y. J. Lu, G. Z. Zhang, Z. W. Xie and C. Wu, *Macromolecules*, 2009, **42**, 7146-7154.
3. B. C. Sanders, F. Friscourt, P. A. Ledin, N. E. Mbua, S. Arumugam, J. Guo, T. J. Boltje, V. V. Popik and G. J. Boons, *J Am Chem Soc*, 2011, **133**, 949-957.
4. M. S. Cubberley and B. L. Iverson, *J Am Chem Soc*, 2001, **123**, 7560-7563.
5. P. Gobbo and M. S. Workentin, *Langmuir*, 2012, **28**, 12357-12363.
6. M. Milne, P. Gobbo, N. McVicar, R. Bartha, M. S. Workentin and R. H. E. Hudson, *J Mater Chem B*, 2013, **1**, 5628-5635.