Multifaceted ligand design facilitates chemical- or peptide-mediated linking of hollow gold nanoshells with tuned interparticle distance, interference and cytotoxicities

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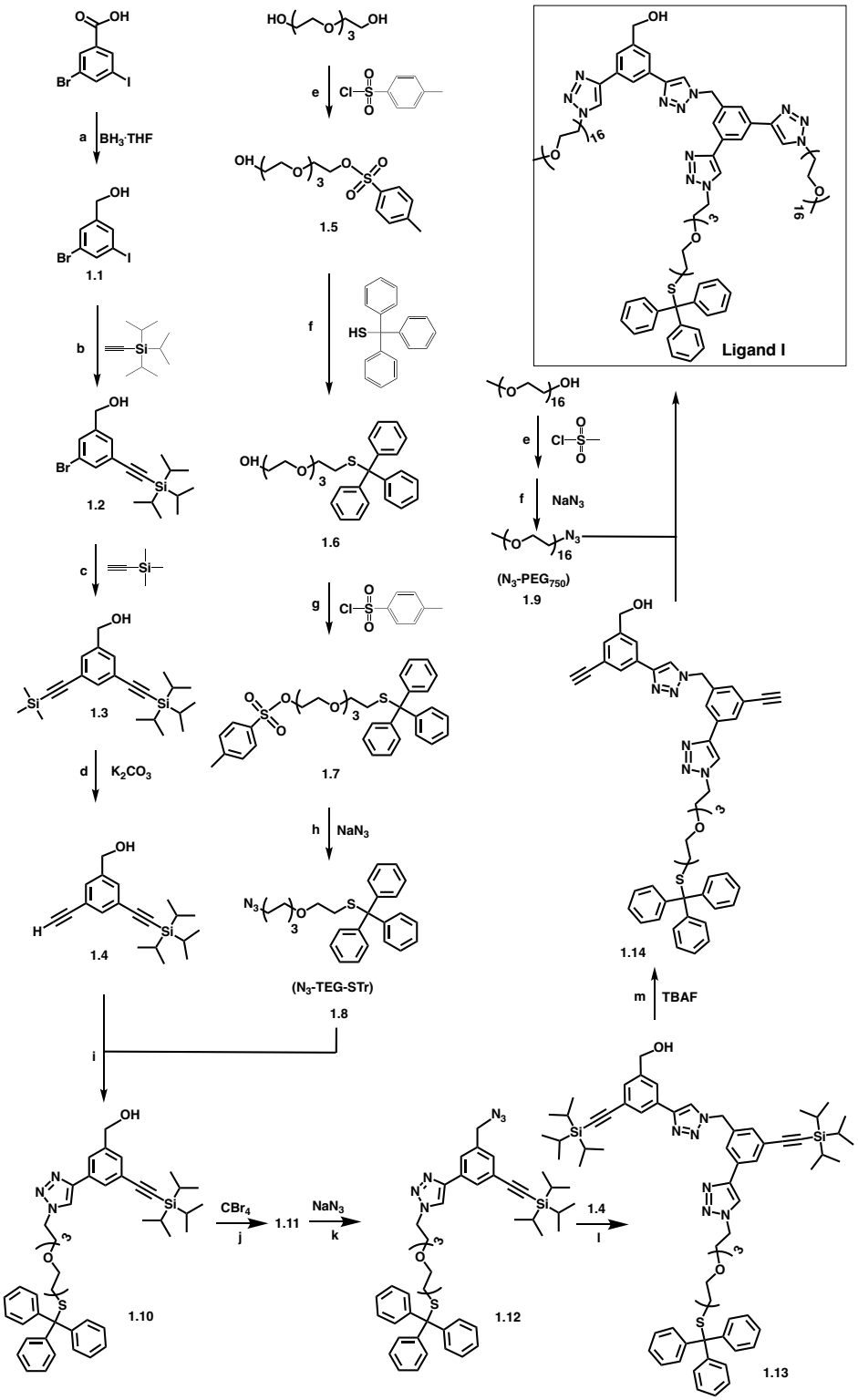


Scheme S1 Synthesis of **OH-CH_2-Core-(PEG_{750})$_2$-(TEGSR) Ligand I**.

Synthetic details:

Compounds **1.1** to **1.7** were prepared by adaptation and modification of the literature procedures reported in references [1-6].

Compound **1.8** In a 25 mL round bottom flask, **1.7** (4 g, 6.59 mmol) was dissolved along with NaN_3 (2.57 g, 39.55 mmol) in 10 mL of THF, and stirred at 50°C for 5 hours. The reaction mixture was transferred to a separatory funnel and washed with water against ethyl acetate. Organic phase was washed with brine, dried over Na_2SO_4 and concentrated in vacuo to afford a white solid in a quantitative yield. ^{1}H NMR ($CDCl_3$, 300MHz): δ 2.43, (t, 2H, -C$\underline{H_2}$-S-, $J_{H\text{-}H}$ = 6.7Hz), 3.33-3.68 (m, 12H, -O-C$\underline{H_2}$- C$\underline{H_2}$-), 7.24-7.48 (m, 15H, H_{Ar}). ^{13}C {^{1}H} NMR ($CDCl_3$, 75MHz): δ 31.7, 50.7, 66.6, 69.6, 70.0, 70.2, 70.6, 70.7, 126.7, 127.9, 129.6, 144.9. Measured HR-MS (ESI +ve Mode) m/z = 500.19667, calculated m/z = 500.19783 for $C_{27}H_{31}O_3N_3NaS$ ($[M+Na]^+$).

Compound **1.9** was using an adaption and modification a literature procedure [7]. Poly(ethylene glycol) methyl ether (12.85 g, 17.14 mmol) was dissolved in 30 mL DCM in a round bottom flask, before cooling to 0 °C. While stirring, triethylamine (3.12 g, 30.85 mmol) was added. The reaction solution turned cloudy after adding mesyl chloride (2.94 g, 25.71 mmol) dropwise at 0 °C. It was left to stir at room temperature overnight. The reaction mixture was washed with 1M $HCl_{(aq)}$ and 1M $NaOH_{(aq)}$, and the organic layer dried over anhydrous $MgSO_4$ and filtered. DCM was removed under reduced pressure to afford a white paste with 90% yield.
Characterization: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.09 (s, 3H, -S-C$\underline{H_3}$), 3.38 (s, 3H, -O-C$\underline{H_3}$), 3.54-3.78 (m, 64H, -O-C$\underline{H_2}$-), 4.38-4.40 (m, 2H, -CH_2-C$\underline{H_2}$-S-).

In a round bottom flask, the above product (12.77 g, 15.41 mmol), NaN_3 (2 g, 30.82 mmol) and tetrabutylammonium iodide (81 mg, 0.4 mmol) were dissolved in 5 mL DMF. The reaction was left to stir at 35 °C overnight. DCM was added to the reaction mixture and washed with minimal water multiple times until excess reagent and DMF were removed to afford a white paste with quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.30 (s, 3H, -O-C$\underline{H_3}$), 3.40 (t, 2H, -C$\underline{H_2}$-O-CH_3, $J_{H\text{-}H}$ = 4.7Hz), 3.48 (t, 2H, -C$\underline{H_2}$-CH_2-O-CH_3, $J_{H\text{-}H}$ = 4.9Hz), 3.59-3.63 (m, 60H, -O-C$\underline{H_2}$-), 3.70 (t, 2H, -CH_2-C$\underline{H_2}$-N_3, $J_{H\text{-}H}$ = 4.9Hz).

Compound **1.10**: A mixture of **1.8** (2.83 g, 5.92 mmol), **1.4** (1.85 g, 5.92 mmol) and sodium ascorbate (0.47 g, 2.37 mmol) were placed in a 25 mL Schlenk flask and dissolved in 8 mL of THF. The mixture was stirred at 45°C for two minutes under nitrogen. An aqueous solution (2 mL) of $CuSO_4 \cdot 5H_2O$ (0.30 g 1.18 mmol) was added dropwise to the reaction flask, followed by stirring for 12 hours at 45°C under nitrogen. THF was removed under reduced pressure to afford the crude product. DCM (10 mL) was added and the mixture stirred for an hour in the presence of Na-EDTA to remove copper. The mixture was filtered and concentrated in vacuo. Purification was done using column chromatography on silica gel first with pure DCM, and then increasing polarity to 50 % ethyl acetate in DCM to afford a white oil. ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.14 (s, 21H, Si-C$\underline{H_2}$-(C$\underline{H_3}$)$_2$), 2.37 (t, 2H, -S-C$\underline{H_2}$-CH_2, $J_{H\text{-}H}$ = 6.7), 3.27 (t, 2H, -S-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 6.7), 3.41-3.57 (m, 8H, -O-C$\underline{H_2}$-CH_2), 3.84 (t, 2H, -triazole-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 4.8Hz), 4.49 (t, 2H, -triazole-C$\underline{H_2}$-CH_2-, $J_{H\text{-}H}$ = 4.8Hz), 4.67 (s, 2H, -Bz-C$\underline{H_2}$-OH), 7.15-7.37 (m, 15H, H_{Ar}), 7.41 (s, H, H_{Ar}), 7.78 (s, H, H_{Ar}), 7.87 (s, H, H_{Ar}), 8.02 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 11.2, 18.2, 31.8, 50.0, 63.2, 66.3, 69.1, 69.1, 70.1, 70.1, 70.2, 70.3, 89.6, 107.4, 121.7, 123.5, 123.8, 126.6, 126.9, 127.8, 129.0, 129.5, 131.9, 143.7, 145.0, 146.0. Measured HR-MS (ESI +ve Mode) m/z = 812.3890, calculated m/z = 812.3888 for $C_{47}H_{59}O_4N_3NaSSi$ ($[M+Na]^+$).

Compound **1.11**: In a 25 mL Schlenk flask, **1.10** (1.86 g, 2.36 mmol) and CBr_4 (1.02 g, 3.06 mmol) were dissolved in 10 mL of dry THF and stirred at 0°C while adding triphenylphosphine (TPP, 0.80 g, 3.06 mmol) under nitrogen. The reaction was stirred at 0°C under nitrogen for an hour, and it turned cloudy over time. The mixture was allowed to warm up to room temperature and left to stir under nitrogen for 36 hours. THF was removed in vacuo, and purification was carried out using column chromatography with silica gel, in which the crude product was washed with pure DCM and then the polarity was increased to 20 % ethyl acetate in DCM to afford a white oil in 70 % yield. ^{1}H NMR (D_6-acetone, 500MHz): δ 1.19 (s, 21H, -Si-C<u>H</u>-($C\underline{H_3}$)$_2$), 2.35 (t, 2H, -S-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 6.6Hz), 3.31 (t, 2H, -S-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 6.7Hz), 3.44-3.62 (m, 8H, -O-C$\underline{H_2}$-), 3.94 (t, 2H, -triazole-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 5.1Hz), 4.58 (t, 2H, -triazole-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 5.1Hz), 4.69 (s, 2H, -Bz-C$\underline{H_2}$-Br), 7.23-7.40 (m, 15H, H_{Ar}), 7.56 (s, H, H_{Ar}), 7.98 (s, H, H_{Ar}), 8.08 (s, H, H_{Ar}), 8.49 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 75MHz): δ 11.2, 18.2, 31.8, 32.5, 50.0, 66.3, 69.0, 69.2, 70.1, 70.2, 70.3, 90.6, 106.58, 122.08, 124.2, 126.4, 126.6, 126.7, 127.5, ,127.8, 129.5, 131.5, 132.6, 139.5, 145.0, 145.5, 147.9. HR-MS (ESI +ve Mode) m/z = 874.3064, calculated m/z = 874.3044 for $C_{47}H_{58}BrO_3N_3NaSSi$ ($[M+Na]^+$).

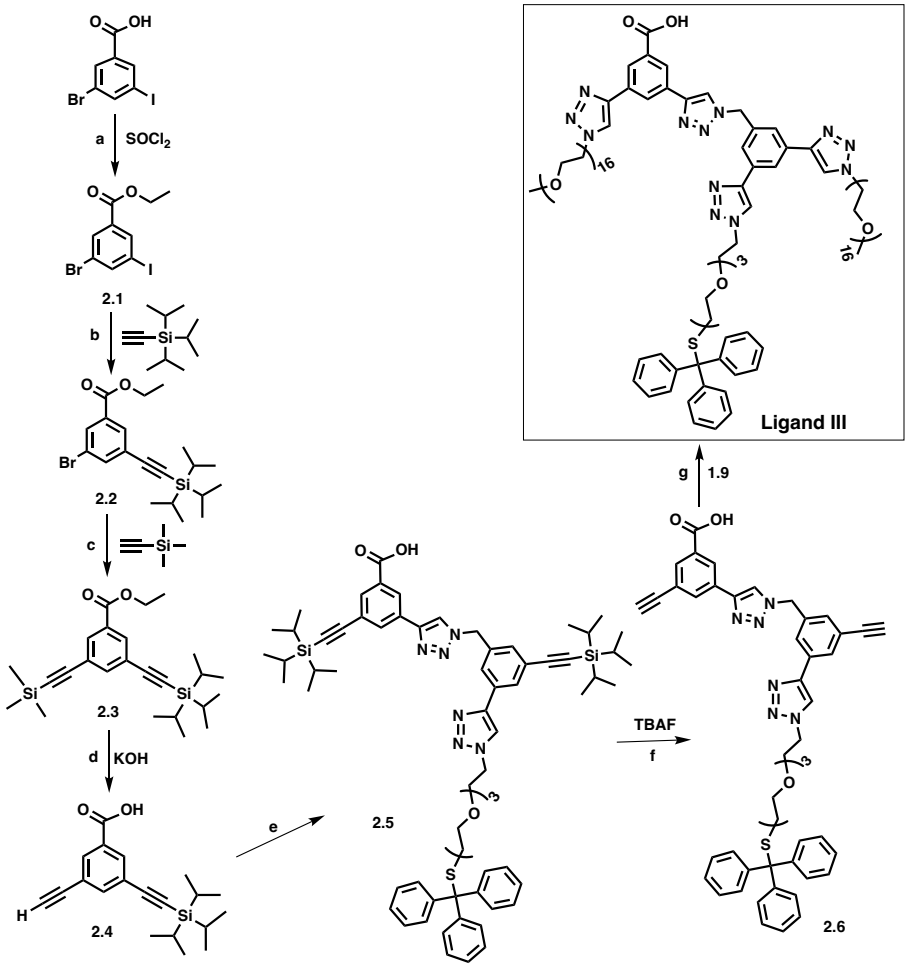
Compound **1.12**: **1.11** (1.03 g, 1.20 mmol) was reacted with NaN_3 (469 mg, 7.20 mmol) in 10 mL of THF in a 25 mL round bottom flask, and the mixture was stirred for 5h at 50°C. It was then transferred to a separatory funnel and washed with ethyl acetate against water. Then the organic phase was washed with brine, dried over Na_2SO_4 and concentrated via vacuum to afford yellow oil in quantitative yield. ^{1}H NMR (D_6-acetone, 500MHz): δ 1.19 (s, 21H, -Si-C<u>H</u>-($C\underline{H_3}$)$_2$), 2.35 (t, 2H, -S-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 6.6Hz), 3.31 (t, 2H, -S-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 6.6Hz), 3.43-3.63 (m, 8H, -O-C$\underline{H_2}$-), 3.95 (t, 2H, -triazole-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 5.1Hz), 4.55 (s, 2H, -Bz-C$\underline{H_2}$-N_3), 4.59 (t, 2H, -triazole-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 5.1Hz), 7.22-7.40 (m, 15H, H_{Ar}), 7.49 (s, H, H_{Ar}), 7.97 (s, H, H_{Ar}), 8.00 (s, H, H_{Ar}), 8.50 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 11.2, 18.2, 31.8, 50.1, 53.6, 66.3, 69.1, 69.1, 70.0, 70.1, 70.3, 90.6, 106.7, 122.1, 124.1, 125.4, 126.6, 127.8, 128.0, 129.5, 130.6, 132.5, 137.3, 145.0, 145.5. Measured HR-MS (ESI +ve Mode) m/z = 837.3975, calculated m/z = 837.3953 for $C_{47}H_{58}O_3N_6NaSSi$ ($[M+Na]^+$).

Compound **1.13**: In a 5 mL Schlenk flask, **1.12** (400 mg, 0.49 mmol), **1.4** (199 mg, 0.64 mmol) and sodium ascorbate (39 mg, 0.20 mmol) were dissolved in 1.5 mL of degassed THF and stirred at 45°C for 2 minutes before adding $CuSO_4{\cdot}5H_2O$ (25 mg, 0.10 mmol) in 0.5 mL water dropwise under nitrogen. The reaction was stirred at 45°C under nitrogen for 12 hours. THF was removed under reduced pressure, and the crude mixture was dissolved in DCM and stirred for an additional hour in the presence of Na-EDTA. It was filtered and concentrated under reduced pressure to obtain the crude product, which was purified using column chromatography on silica gel, first a clean wash with DCM followed by increasing polarity up to 50% ethyl acetate in DCM to afford a pale yellow solid in 77 % yield. ^{1}H NMR (D_6-acetone, 500MHz): δ 1.17 (s, 42H, -Si-C<u>H</u>-($C\underline{H_3}$)$_2$), 2.32 (t, 2H, -S-C$\underline{H_2}$-CH_2, $J_{H\text{-}H}$ = 6.6Hz), 3.30 (t, 2H, -S-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = =6.6Hz), 3.42-3.60 (m, 8H, -O-C$\underline{H_2}$-C$\underline{H_2}$-), 3.92 (t, 2H, -triazo-CH_2-C$\underline{H_2}$-, $J_{H\text{-}H}$ = 5.0Hz), 4.56 (t, 2H, -triazo-C$\underline{H_2}$-CH_2, -, $J_{H\text{-}H}$ = 5.0Hz), 4.69 (s, 2H, HO-C$\underline{H_2}$-), 5.75 (s, 2H, -triazole-C$\underline{H_2}$-Bz), 7.21-7.38 (m, 15H, H_{Ar}), 7.47 (s, H, H_{Ar}), 7.54 (s, H, H_{Ar}), 7.91 (s, H, H_{Ar}), 7.92 (s, H, H_{Ar}), 7.97 (s, H, H_{Ar}), 8.02 (s, H, H_{Ar}), 8.46 (s, H, $H_{triazole}$), 8.52 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 11.2, 18.2, 31.8, 50.1, 53.1, 63.0, 63.2, 66.3, 69.0, 69.1, 70.0, 70.1, 70.1, 70.3, 89.7, 90.9, 106.6, 107.3, 121.2, 122.1, 123.5,

123.8, 124.3, 125.4, 126.6, 127.0, 127.8, 128.3, 129.1, 129.5, 130.6, 131.5, 132.7, 137.1, 143.8, 145.0, 145.3, 146.6. Measured HR-MS (ESI +ve Mode) m/z =1149.5893, calculated m/z = 1149.5862 for $C_{67}H_{86}O_4N_6NaSSi$ ($[M+Na]^+$).

Compound **1.14**: **1.13** (200 mg, 0.18 mmol) was dissolved in 1 mL of dry THF in a 25 mL round bottom flask under nitrogen. 0.44 mL (1M TBAF, 0.44 mmol in THF) was added dropwise at 0 °C and reaction was left stirring for 1 hour. The reaction was warmed to room temperature and stirred for 12 hours under nitrogen. THF was removed under reduced pressure followed by DCM extraction against water. Organic phase was dried over Na_2SO_4, filtered and concentrated. Purification was performed using column chromatography on silica, using firstly neat DCM for washing the crude product and then increasing the polarity to 50 % ethyl acetate in DCM. Crystalline light yellow product was obtained in a quantitative yield. ^{1}H NMR (D_6-acetone, 500MHz): δ 2.33 (t, 2H, -S-C$\underline{H_2}$-CH_2-, J_{H-H} = 6.6Hz), 3.30 (t, 2H, -S-CH_2-C$\underline{H_2}$-, J_{H-H} =6.6Hz), 3.42-3.61 (m, 8H, -O-C$\underline{H_2}$-), 3.66 (s, H, -C≡C-$\underline{H}$), 3.74 (s, H, -C≡C-$\underline{H}$), 3.93 (t, 2H, -triazole-CH_2-C$\underline{H_2}$-, J_{H-H} = 5.1Hz), 4.58, (t, 2H, -triazo-C$\underline{H_2}$-CH_2, J_{H-H} = 5.1Hz), 4.68 (s, 2H, HO-C$\underline{H_2}$-), 5.76 (s, 2H, -triazole-C$\underline{H_2}$-Bz), 7.21-7.39 (m, 15H, H_{Ar}), 7.45 (s, H, H_{Ar}), 7.49 (s, H, H_{Ar}), 7.90 (s, H, H_{Ar}), 7.94 (s, H, H_{Ar}), 8.00 (s, H, H_{Ar}), 8.01 (s, H, H_{Ar}), 8.47 (s, H, $H_{triazole}$), 8.55 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 31.8, 50.1, 53.0, 63.0, 63.1, 66.3, 69.0, 69.1, 70.0, 70.1, 70.1, 70.3, 78.2, 79.2, 82.6, 83.2, 121.2, 122.1, 122.6, 123.4, 123.9, 125.4, 126.6, 127.1, 127.5, 127.8, 128.0, 128.5, 129.1, 129.5, 130.5, 131.5, 132.7, 137.2, 143.8, 145.0, 145.3, 146.6. Measured HR-MS (ESI +ve Mode) m/z =1149.5893, calculated m/z = 1149.5862 for $C_{67}H_{86}O_4N_6NaSSi$ ($[M+Na]^{2+}$). Measured HR-MS (ESI +ve Mode) m/z = 837.3191, calculated m/z = 837.3193 for $C_{49}H_{46}O_4N_6NaS$ ($[M+Na]^{3+}$).

Ligand I: A mixture of **1.14** (150 mg, 0.18 mmol), N_3-PEG_{750} (304 mg, 0.40 mmol) and Na-Ascorbate (15 mg, 0.07 mmol) were placed in a 5 mL Schlenk flask, and dissolved in 2 mL of degassed THF, which was stirred at 45 °C for 2 minutes before adding $CuSO_4 \cdot 5H_2O$ (9 mg, 0.04 mmol) in 0.5 mL water dropwise under nitrogen. The reaction was continued to stir for 36 hours at 45 °C under nitrogen. THF was removed in vacuo and the mixture was dissolved in DCM and stirred for an hour in the presence of Na-EDTA. The crude product was filtered and concentrated under reduced pressure and precipitated by adding ether dropwise to obtain **1.15**. Supernatant containing excess of N_3-PEG_{750} was pipetted out. This procedure was repeated several times to obtain the product as an amber oil in 87 % yield.
Characterization: ^{1}H NMR (D_6-acetone, 500MHz): δ 2.33 (t, 2H, -S-C$\underline{H_2}$-CH_2-, J_{H-H} = 6.6Hz), 3.29 (s, 6H, -O-C$\underline{H_3}$), 3.41-3.64 (m, 130H, -O-C$\underline{H_2}$-), 3.95-3.97 (m, 6H, -triazole-CH_2-C$\underline{H_2}$-), 4.59-4.65 (m, 6H, -triazole-C$\underline{H_2}$-CH_2), 4.74 (s, 2H, HO-C$\underline{H_2}$-Bz), 5.82 (s, 2H, -triazole-C$\underline{H_2}$-Bz), 7.22-7.39 (m, 15H, H_{Ar}), 7.90 (s, 2H, H_{Ar}), 7.95 (s, 2H, H_{Ar}), 8.31 (s, H, $H_{triazole}$), 8.45 (s, 2H, $H_{triazole}$), 8.51 (s, H, H_{Ar}), 8.51 (s, H, H_{Ar}), 8.57 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 31.8, 50.0, 50.1, 50.1, 53.6, 57.9, 63.7, 66.3, 69.1, 69.2, 70.0, 70.2, 70.2, 70.3, 70.4, 71.8, 121.0, 121.1, 121.6, 122.0, 122.2, 122.9, 124.4, 126.7, 127.9, 129.5, 131.7, 132.0, 132.9, 137.3, 143.8, 145.0, 146.1, 146.7, 147.4. GPC: Mn=2910, PD=1.13.



Scheme S2 Synthesis of **COOH-Core-$(PEG_{750})_2$-(TEGSR) Ligand III**.

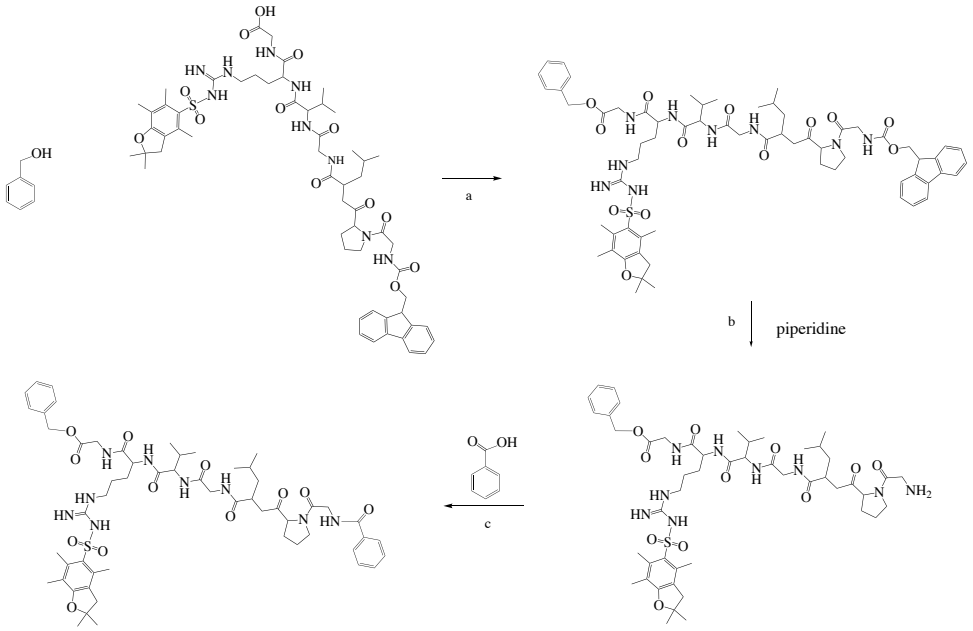
Compounds **2.1** to **2.4** were prepared by adaptation and modification of the literature procedure reported in reference [8].

Compound **2.5**: In a 5 mL Schlenk flask, **2.4** (140 mg, 0.43 mmol), **1.12** (350 mg, 0.43 mmol) and Na-Ascorbate (34 mg, 0.17 mmol) were dissolved in 2 mL of degassed THF and stirred at 45°C. After 2 minutes of stirring, $CuSO_4 \cdot 5H_2O$ (21 mg, 0.09 mmol) was added in 0.5 mL water dropwise under nitrogen. The reaction was stirred at 45°C under nitrogen for 12 hours. THF was removed under reduced pressure, and the reaction mixture was dissolved in DCM containing Na-EDTA and stirred for additional hour. After filtering and concentrating the crude product under reduced pressure, the product was purified using column chromatography on silica gel. A DCM eluent was used as a first wash, and then polarity was increased by adding a mixture of 5 % MeOH in DCM to afford a light yellow powder in 89 % yield. ^{1}H NMR (D_6-acetone, 500MHz): δ 1.17 (s, 21H, -Si-C<u>H</u>-C<u>H$_3$</u>)$_2$), 1.18 (s, 21H, -Si-C<u>H</u>-C<u>H$_3$</u>)$_2$), 2.31 (t, 2H, -S-C<u>H$_2$</u>- CH$_2$-, $J_{H\text{-}H}$ = 6.6Hz), 3.29 (t, 2H, -S-CH$_2$-C<u>H$_2$</u>-, $J_{H\text{-}H}$ = 6.6Hz), 3.43-3.60 (m, 8H, -O-C<u>H$_2$</u>-), 3.93 (t, 2H, -triazole-CH$_2$-C<u>H$_2$</u>-, $J_{H\text{-}H}$ = 5.1Hz), 4.57 (t, 2H, -triazole-C<u>H$_2$</u>-CH$_2$, $J_{H\text{-}H}$ = 5.0Hz), 5.78 (s, 2H, -triazole-C<u>H$_2$</u>-Bz), 7.20-7.37 (m, 15H, H_{Ar}), 7.55 (s, H, H_{Ar}), 7.99 (s, H, H_{Ar}), 8.02 (s, H, H_{Ar}), 8.05 (s, H, H_{Ar}), 8.24 (s, H, H_{Ar}), 8.47 (s, H, $H_{triazole}$), 8.56 (s, H, H_{Ar}), 8.67 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 11.1, 18.1, 31.7, 50.1, 53.2, 66.3, 69.0, 69.1, 70.0, 70.1, 70.1, 70.3, 90.9, 91.4, 105.9, 106.6, 121.9, 122.2, 124.2, 124.3, 125.4,

126.4, 126.6, 127.8, 128.4, 129.5, 130.7, 131.7, 131.8, 132.2, 132.3, 132.7,136.8, 145.0, 145.3, 145.7, 165.7. HR-MS (ESI +ve Mode) m/z =1163.56717, calculated m/z = 1163.56546 for $C_{67}H_{84}O_5N_6NaSSi_2$ ($[M+Na]^+$), m/z =1141.58517, calculated m/z = 1141.58352 for $C_{67}H_{85}O_5N_6SSi_2$ ($[M+H]^+$).

Compound **2.6**: **2.5** (400 mg, 0.35 mmol) was dissolved in 1 mL dry THF under nitrogen in a 25 mL round bottom flask, followed by the addition of 0.84 mL Bu_4NF (0.84 mmol) dropwise, while the reaction was stirred at 0°C. The reaction was left to stir under nitrogen overnight. THF was removed under reduced pressure and then the crude mixture was extracted using DCM against water. $MgSO_4$ was added to the organic phase to dry it for an hour, filtered and concentrated before loading on a silica gel. Purification of the sample was done by washing it on the column with pure DCM followed by increasing polarity with 5 % MeOH in DCM to afford a light yellow powder with 96 % yield. ^{1}H NMR (D_6-acetone, 500MHz): δ 2.33 (t, 2H, -S-C$\underline{H_2}$- CH_2-, J_{H-H} = 6.6Hz), 3.30 (t, 2H, -S-CH_2-C$\underline{H_2}$-, J_{H-H} = 6.6Hz), 3.42-3.61 (m, 8H, -O-C$\underline{H_2}$-), 3.74 (s, H, -C≡C-$\underline{H}$), 3.81 (s, H, -C≡C-$\underline{H}$), 3.93 (t, 2H, -triazole-CH_2-C$\underline{H_2}$-, J_{H-H} = 5.1Hz), 4.58 (t, 2H, -triazole-C$\underline{H_2}$-CH_2, J_{H-H} = 5.1Hz), 5.79 (s, 2H, -triazole-C$\underline{H_2}$-Bz), 7.21-7.39 (m, 15H, H_{Ar}), 7.52 (s, H, H_{Ar}), 8.00 (s, H, H_{Ar}), 8.03 (s, H, H_{Ar}), 8.05 (s, H, H_{Ar}), 8.24 (s, H, H_{Ar}), 8.48 (s, H, $H_{triazole}$), 8.58 (s, H, H_{Ar}), 8.71 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 31.8, 50.1, 53.2, 66.3, 69.0, 69.1, 70.0, 70.1, 70.1, 70.3, 79.2, 79.6, 82.1, 82.64 121.9, 122.2, 123.4, 123.5, 125.6, 126.6, 127.7, 127.8, 128.5, 129.5, 130.6, 132.3, 132.4, 132.7, 137.0, 145.0, 145.3, 145.7, 165.37. Measured HR-MS (ESI +ve Mode) m/z = 829.31477, calculated m/z = 829.31667 for $C_{49}H_{45}O_5N_6S$ ($[M+H]^+$), 851.29654, calculated m/z = 851.29861 for $C_{49}H_{44}O_5N_6NaS$($[M+Na]^+$).

Ligand III: In a 5 mL Schlenk flask, **2.6** (200 mg, 0.24 mmol) and N_3-PEG_{750} (483 mg, 0.48 mmol) and Na-Ascorbate (19 mg, 0.10 mmol) were dissolved in 2 mL of degassed THF and stirred at 45°C for 2 minutes before dropwise addition of $CuSO_4{\cdot}5H_2O$ (12 mg, 0.05 mmol) in 0.5 mL degassed water under nitrogen. The reaction was further stirred at 45°C under nitrogen for 36 hours. THF was removed under reduced pressure, and the reaction mixture was dissolved in DCM containing Na-EDTA, and stirred for an hour. The mixture was filtered and concentrated under reduced pressure to obtain a crude product, which was re-dissolved in DCM and crashed out with dropwise addition of ether. Pipetting out the supernatant containing excess N_3-PEG_{750}, and repeating this process gave compound **2.7**. After removing the solvent under vacuum, an amber oil was afforded in 84 % yield. ^{1}H NMR (D_6-acetone, 500MHz): δ 2.33 (t, 2H, -S-C$\underline{H_2}$-CH_2, J_{H-H} = 6.6Hz), 3.29-3.30 (m, 8H, C$\underline{H_3}$-O-, -O-C$\underline{H_2}$-), 3.43-3.64 (m, 147H, -O-C$\underline{H_2}$-C$\underline{H_2}$- and -S-CH_2-C$\underline{H_2}$-), 3.95-3.99(m, 6H, -triazole-CH_2-C$\underline{H_2}$-), 4.61 (t, 2H, triazole-C$\underline{H_2}$-CH_2-, J_{H-H} = 5.0Hz), 4.65 (q, 4H, triazole-C$\underline{H_2}$-CH_2-, J_{H-H} = 5.0Hz), 5.84 (s, 2H, -triazole-C$\underline{H_2}$-Bz), 7.21-7.38 (m, 15H, H_{Ar}), 7.97 (s, 2H, H_{Ar}), 8.46 (s, H, $H_{triazole}$), 8.51 (s, 2H, H_{Ar}), 8.55 (s, H, H_{Ar}), 8.56 (s, H, H_{Ar}), 8.60 (s, H, $H_{triazole}$), 8.67 (s, H, $H_{triazole}$), 8.74 (s, H, $H_{triazole}$). ^{13}C {^{1}H} NMR (D_6-acetone, 125MHz): δ 31.8, 50.1, 50.1, 50.2, 53.7, 57.9, 66.32, 69.1, 69.1, 69.2, 70.0, 70.2, 70.2, 70.3, 71.8, 121.7, 122.0, 122.2, 124.5, 125.5, 125.5, 126.4, 126.6, 127.9, 129.5, 131.8, 132.4, 132.6, 132.9, 137.1, 145.0, 145.8, 146.1, 146.5, 166.44. Measured HR-MS (ESI +ve Mode) m/z =770.04528, calculated m/z = 770.04898 for $C_{111}H_{171}O_{35}N_{12}Na_2S$ ($[M+Na_2+H]^{3+}$). GPC: Mn = 2832, PDI = 1.29.



Reaction conditions: a) DPTS, DIC, dry DCM, rt, 24 hr, b) 20% DMF, c) DPTS, DIC, dry DCM, rt, 24 hr.

Scheme S3 Model reactions for the synthesis of linked HAuNS.

1,4-dimethylpyridin-1-ium,4-methylbenzenesulfonate (DPTS) required for the reaction below was prepared by adapting a reported procedure [9].

Synthesis of **Fmoc-P-Ester**: In a Schlenk flask, benzyl alcohol (45.06 mg, 0.4167 mmol), Fmoc-P (564.625 mg, 0.5 mmol), DPTS (52 mg, 0.4167 mmol) and stir bar were added under N_2 stream. 2 mL dry DCM was then added, followed by 8.87 mg N,N'-diisopropylcarbodiimide (DIC) at 0°C while stirring. The reaction stirred at room temperature under N_2 overnight. DCM was removed in vacuo and purified on silica gel with 10% MeOH in DCM to afford a light yellow solid in 71% yield. HR-MS (ESI +ve Mode) 1241.5619, calculated m/z = 1241.5676 for $C_{63}H_{82}N_{10}NaO_{12}S$ ($[M+Na]^+$).

Synthesis of **P-Ester**: In a Schlenk flask, **Fmoc-P-Ester** (100 mg, 0.08 mmol), 1mL 10% piperidine (piperidine:DMF:THF = 1:1:8) and a stir bar were placed under N_2 stream. 1 mL dry THF was then added, and the reaction stirred at room temperature under N_2 for 8 hours. The solvent was then removed in vacuo and the product purified on silica gel column with 10% MeOH in DCM to afford a white solid in quantitative yield. HR-MS (ESI +ve Mode) 997.5209, calculated m/z = 997.5176 for $C_{48}H_{73}N_{10}O_{11}S$ ($[M+H]^+$).

Synthesis of **Amide-P-Ester**: In a Schlenk flask, benzoic acid (5.29 mg, 0.433 mmol), **P-ester** (36 mg, 0.036 mmol), DPTS (9.7 mg, 0.036 mmol) and stir bar were placed under N_2 stream. 1 mL dry DCM was added, followed by the addition of 6.8 mg DIC while stirring. The reaction was stirred at room temperature under N_2 for 24 hours. DCM was reduced under reduced pressure and the product purified using prep-silica gel chromatography with 10% MeOH in DCM to afford a white solid with 75% yield. HR-MS (ESI +ve Mode) 1123.5249, calculated m/z = 1123.5257 for $C_{55}H_{76}N_{10}NaO_{12}S$ ($[M+Na]^+$).

Synthesis of citrate capped HAuNS was carried out using a procedure developed by Schwartzberg *et al* [10]. Briefly, trisodium citrate (1.24g, 4.20 mmol) was added to a three necks flask while it was under positive pressure of constant argon flow. Cobalt (II) chloride hexahydrate (150 mg, 0.63 mmol) and 142.5 mg of sodium borohydride solution (3.77 mmol in 2 mL MilliQ H_2O) were added under positive pressure of Argon to form cobalt nanoparticles. Cobalt nanoparticle solution was then added to $HAuCl_4$ solution (12 mg, 0.035 mmol in 900 mL MilliQ H_2O). The mixture was left to stir under argon for ten minutes before opening to air for four hours to complete oxidation. Gold nanoshells were then collected by centrifuging at 4700 rpm for two hours at 4°C. Separate pallets were collected into 15 mL centrifuge tube after supernatant removal. Then second centrifugation was performed at 4700 rpm for three hours at 4 °C. Pallets were stored at 4 °C before use.

***In situ* ligand detritylation and HAuNS functionalization**: In a typical procedure, in a Schlenk flask, the desired ligand (0.05 mmol) was dissolved in dry and degassed DCM under nitrogen. Upon addition of 0.1 mL TFA to the reaction mixture, the color for the solution changed from yellow to bright orange. The mixture was stirred for 30 seconds before the addition of 0.2 mL triethylsilane, which turned the reaction mixture to a colorless solution. The reaction was stirred at room temperature under a nitrogen stream for 8h. Hollow gold nanoshells solution was purged with nitrogen overnight before it was added directly to the mixture, then the reaction was left stirring at room temperature overnight. The functionalized hollow gold nanoshells were collected by centrifuging at 4600 rpm for 2h at 4 °C. Supernatant was removed carefully, followed by addition of DCM before another cycle of centrifuging. The purification process was repeated twice to ensure all the reactants and reagents were removed. The solution containing the ligand attached hollow gold nanoshells was concentrated to a final volume of about 4 mL.

Synthesis of ChemLinked-HAuNS. A typical procedure for linking HAuNS chemically is summarized here. In a Schlenk flask, solutions of OH-CH_2-Core-$(PEG_{750})_2$-TEGS-HAuNS and COOH-Core-$(PEG_{750})_2$-TEGS-HAuNS were placed with a stir bar, and dried over reduced pressure while stirring. HAuNS settled at the bottom of the reaction flask. The reduced pressure was maintained for 5 minutes, when the HAuNS visually appeared to be dry. DPTS and degassed DCM were added into the reaction flask under nitrogen stream. The reaction mixture was cooled with an ice bath before adding DIC. The reaction mixture was stirred at room temperature under N_2 for 24h. HAuNS settled at the bottom. The supernatant was pipetted out slowly to remove unreacted L-HAuNS and excess regents, and the residue washed with DCM. The procedure was repeated three times to afford ChemLinked-HAuNS which were then transfer into DMSO via centrifugation.

Synthesis of BioLinked-HAuNS: A general synthetic procedure is outlined below.
Synthesis of Fmoc-P-O-CH_2-Core-$(PEG)_2$-TEGSR: Fmoc-P, OH-CH_2-Core-$(PEG)_2$-TEGSR and DPTS and 5 mL dry DCM were added to a Schlenk flask under a constant nitrogen stream. DIC was then added while stirring, and the reaction was left to stir at room temperature for 24h. MeOH was then added to the reaction mixture to precipitate excess Fmoc-P. The supernatant that contained *Fmoc-P-O-CH_2-Core-$(PEG)_2$-TEGSR* was washed with MeOH, and the procedure was repeated to afford a light yellow powder.

Synthesis of Fmoc-P-O-CH_2-Core-$(PEG)_2$-TEGS-HAuNS: Fmoc-P-O-CH_2-Core-$(PEG)_2$-TEGSR from above (0.05 mmol) was placed in a Schlenk flask with a stir bar under nitrogen stream, together with 2 mL of DCM. 0.1 mL TFA was added while stirring and the solution turned bright yellow. 0.2 mL TES was added while stirring and the color of solution turned colorless. The reaction was left stirred at room temperature under nitrogen for 8 hours. HAuNS was degassed with nitrogen stream overnight before added to the reaction mixture. The reaction was left stirred at under nitrogen overnight. Collected Fmoc-P-O-CH_2-Core-$(PEG)_2$-TEGS-HAuNS *via* centrifuge at 4600 rpm for two hours at 4 °C. Supernatant was removed carefully, followed by addition of DCM before another cycle of centrifuging. The purification process was repeated twice to ensure all the reactants and reagents were removed. The solution containing the final ligand attached to hollow gold nanoshells was concentrated to a final volume of about 4 mL.

Synthesis of P-OH-PEG-L-HAuNS: Fmoc-P-O-CH_2-Core-$(PEG)_2$-TEGS-HAuNS solution and stirred bar were added to a Schlenk flask. Solvent was removed under reduced pressure slowly to preserve most of Fmoc-P-O-CH_2-Core-$(PEG)_2$-TEGS-HAuNS before 1 mL dry THF was added to the reaction flask. 1mL 10% piperidine solution (piperidine: DMF: THF=1:1:8) was added to the reaction flask under N_2 stream. Left the reaction stirred at room temperature under nitrogen for 8h. Removed THF under decreased pressure. Ethyl acetate as added to reaction mixture and washed against water. Ethyl acetate was removed *via* vacuum and yield P-O-CH_2-Core-$(PEG)_2$-TEGS-HAuNS powder which was then resuspend in DCM quickly.

BioLinking reaction: Functionalized HAuNS (COOH-Core-$(PEG)_2$-TEGS-HAuNS) were transferred to DCM and added to a Schlenk flask together with P-O-CH_2-Core-$(PEG)_2$-TEGS-HAuNS solution. DCM was removed under reduced pressure while stirring and continued for about 5 minutes. DPTS and dry DCM were added to the reaction flask under a nitrogen stream. DIC was added dropwise while stirring. The reaction was left to stir at room temperature under N_2 for 24h. The reaction mixture was then transferred to a centrifuge tube and DCM was added to the tube to wash it. HAuNS were left to settle to the bottom, and DCM was pipetted out carefully. Cycles of washing and removing DCM were repeated two times. BioLinked-HAuNS were transferred to DMSO *via* centrifugation.

Table S1 Thermogravimetric analysis (TGA) of functionalized HAuNS.

Ligand	Avg. Mol. Wt.	TGA Residue (%)	Ligand (%)
OH-CH_2-Core-$(PEG_{750})_2$-TEGS-HAuNS	2338.1	37.1	62.9
COOH-Core-$(PEG_{750})_2$-TEGS-HAuNS	2352.8	42.2	57.8
OH-CH_2-Core-$(PEG_{2000})_2$-TEGS-HAuNS	4893.9	65.3	34.7
COOH-Core-$(PEG_{2000})_2$-TEGS-HAuNS	4907.9	50.5	49.5

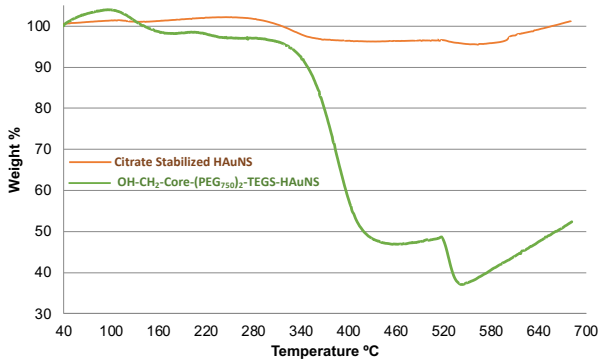


Fig. S1 Comparative TGA analysis of citrate stabilized- and OH-CH_2-Core-$(PEG_{750})_2$-TEGS-HAuNS.

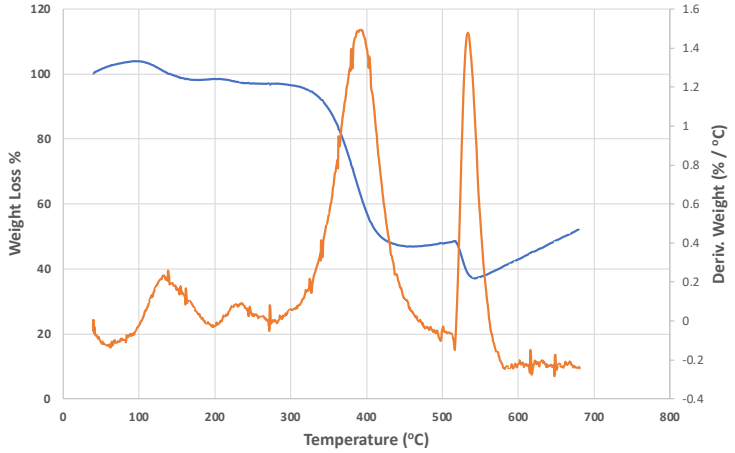


Fig. S2: TGA analysis of OH-CH_2-Core-$(PEG_{750})_2$-TEGS-HAuNS.

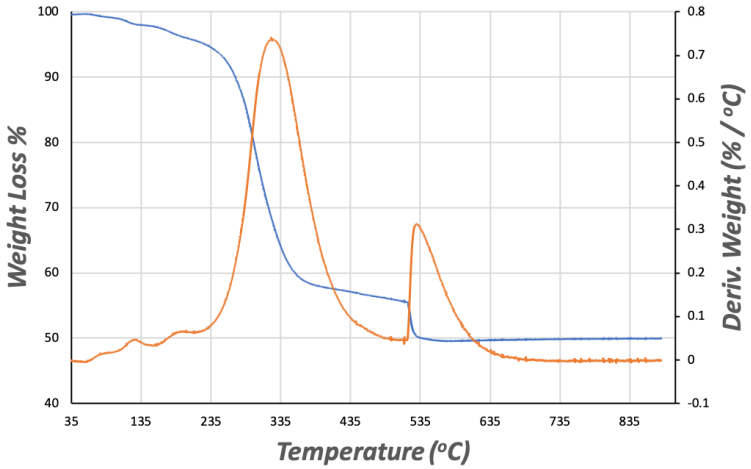


Fig. S3: TGA analysis of COOH-Core-$(PEG_{2000})_2$-TEGS-HAuNS.

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Fig. S4 ^{1}H NMR of **1.8**

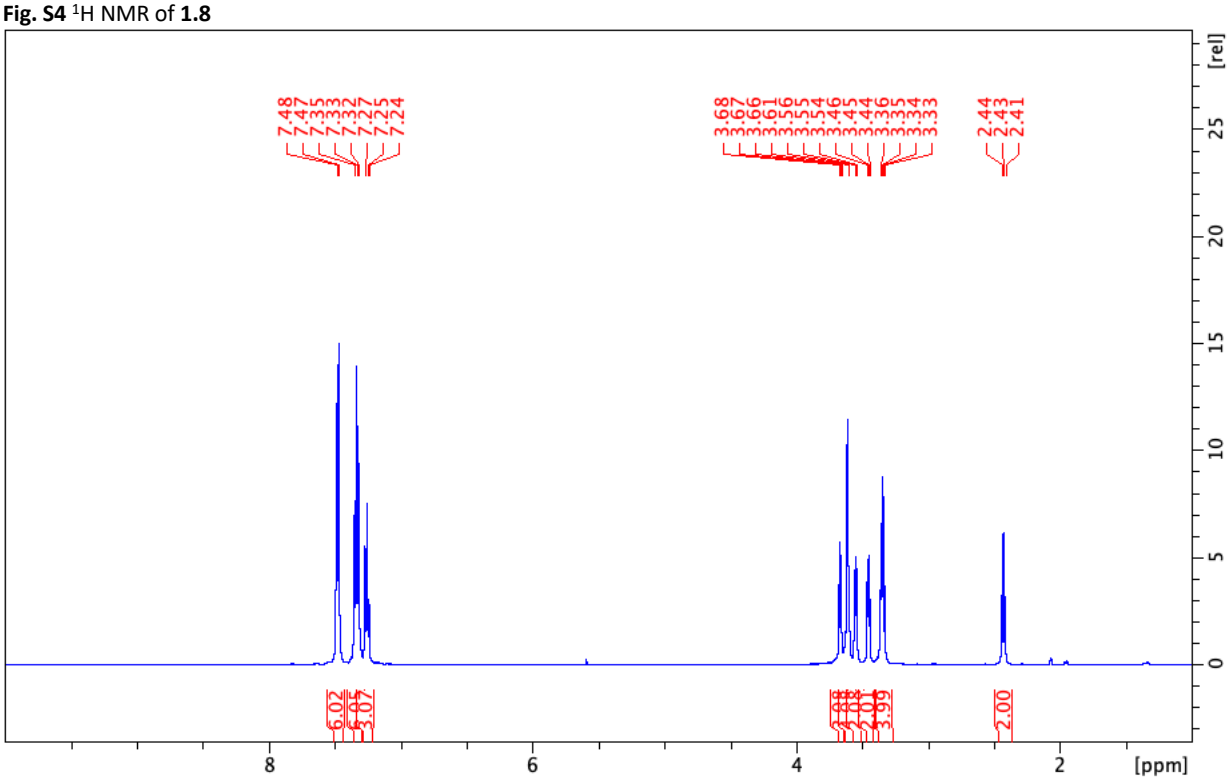


Fig. S4.1 ^{13}C NMR of **1.8**

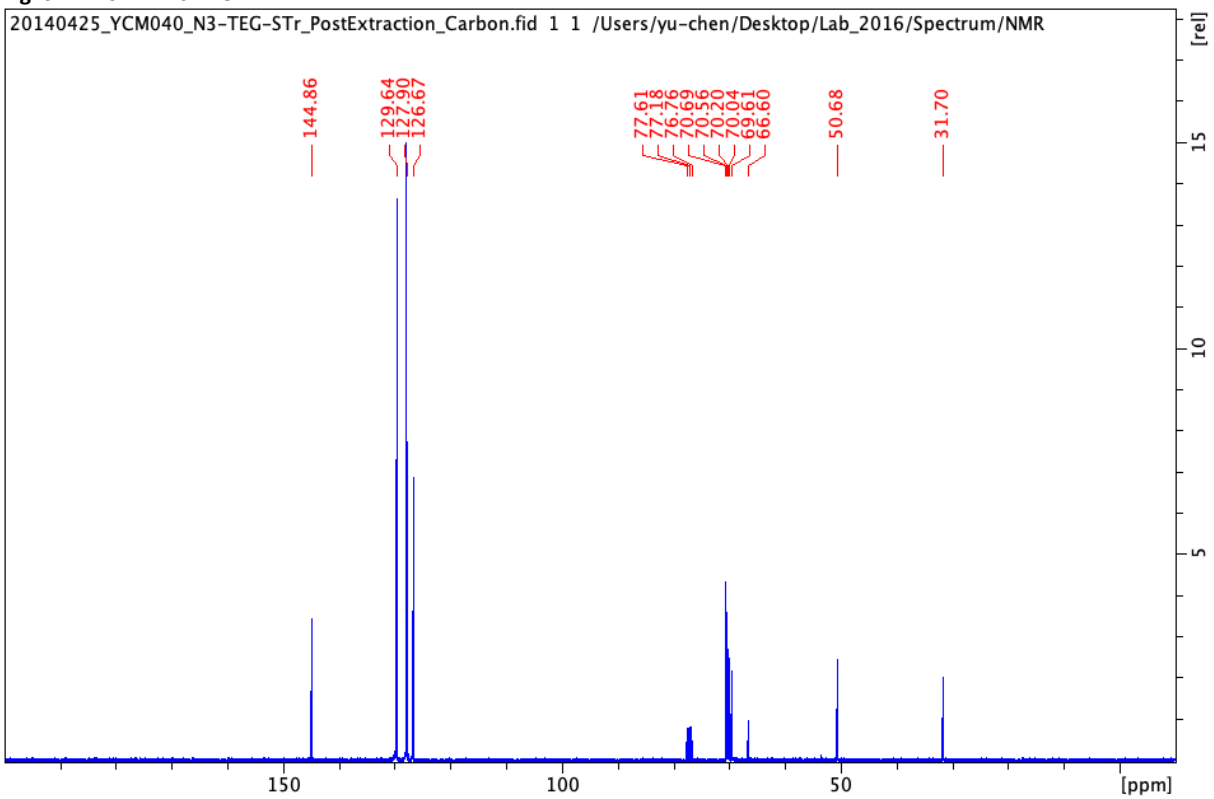


Fig. S5 ^{1}H NMR of **1.12**

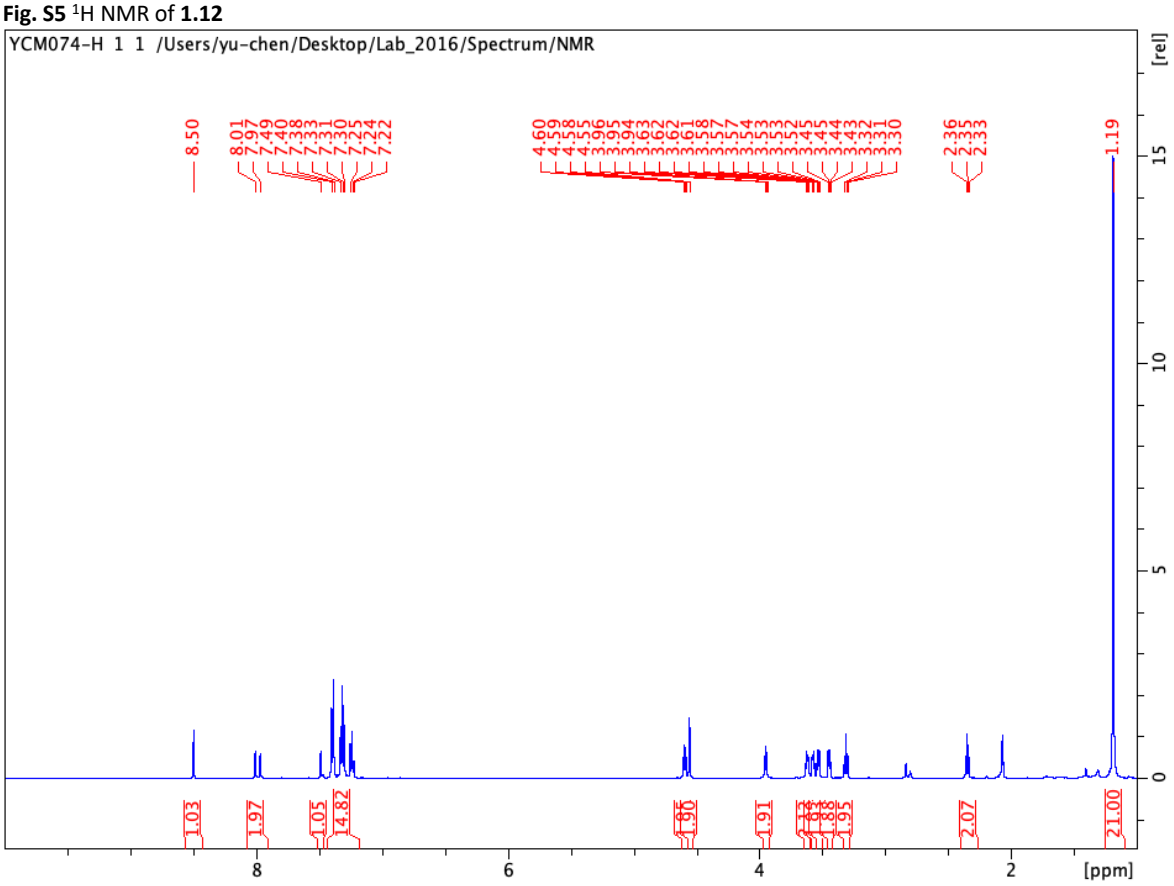


Fig. S5.1 13C NMR of **1.12**

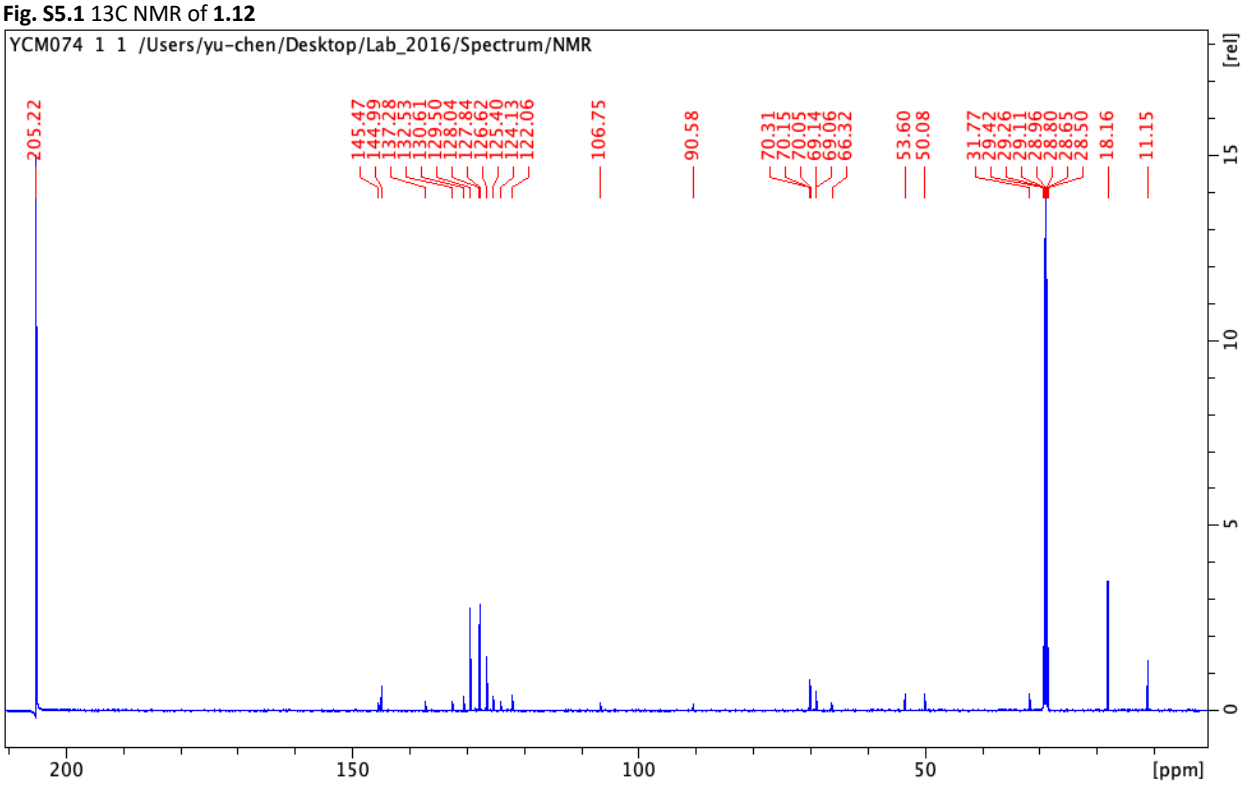


Fig. S6 ^{1}H NMR of **1.13**

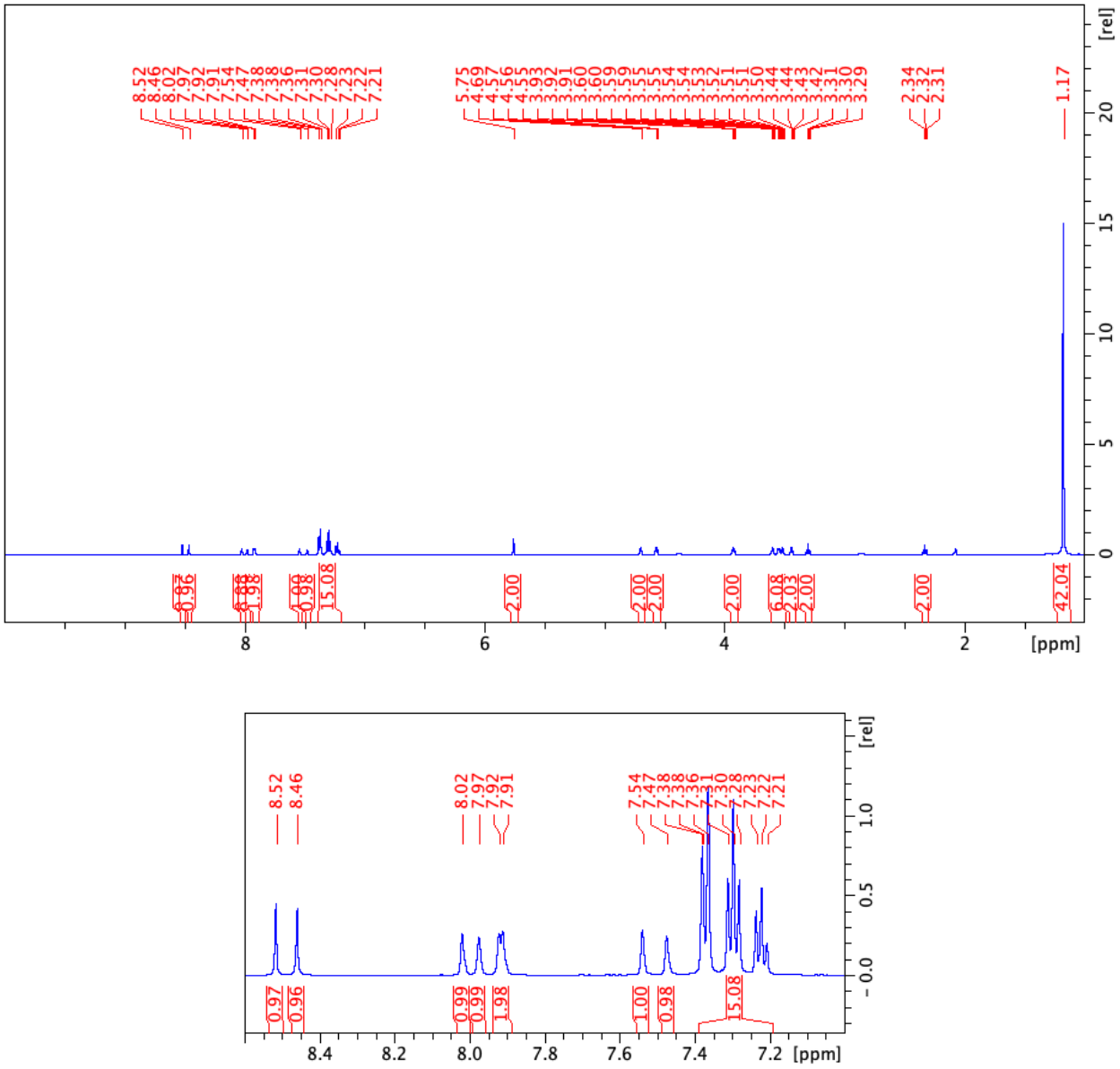


Fig S7 ^{1}H NMR of **1.14**

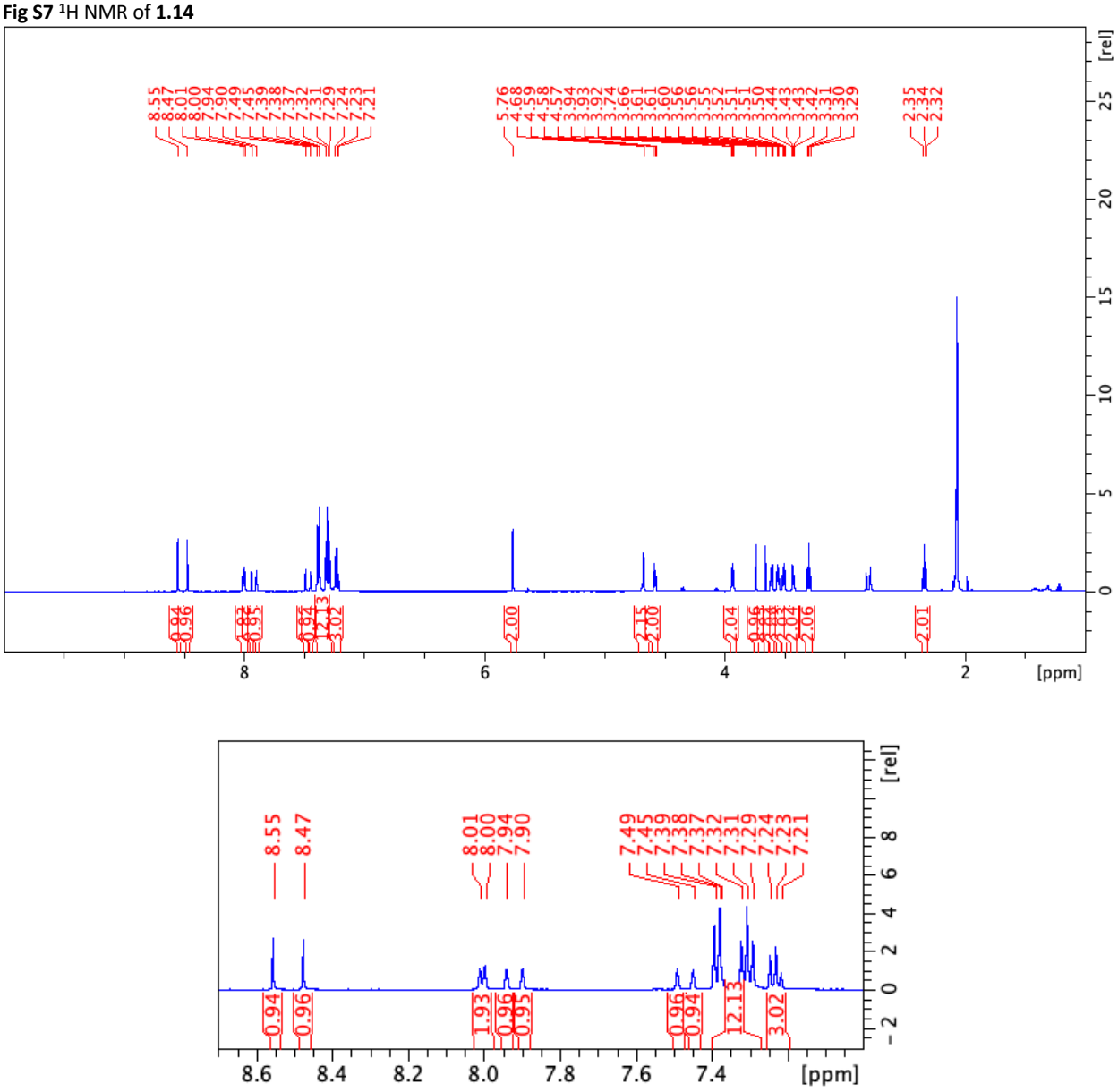


Fig. S8 ^{1}H NMR of **1.15**

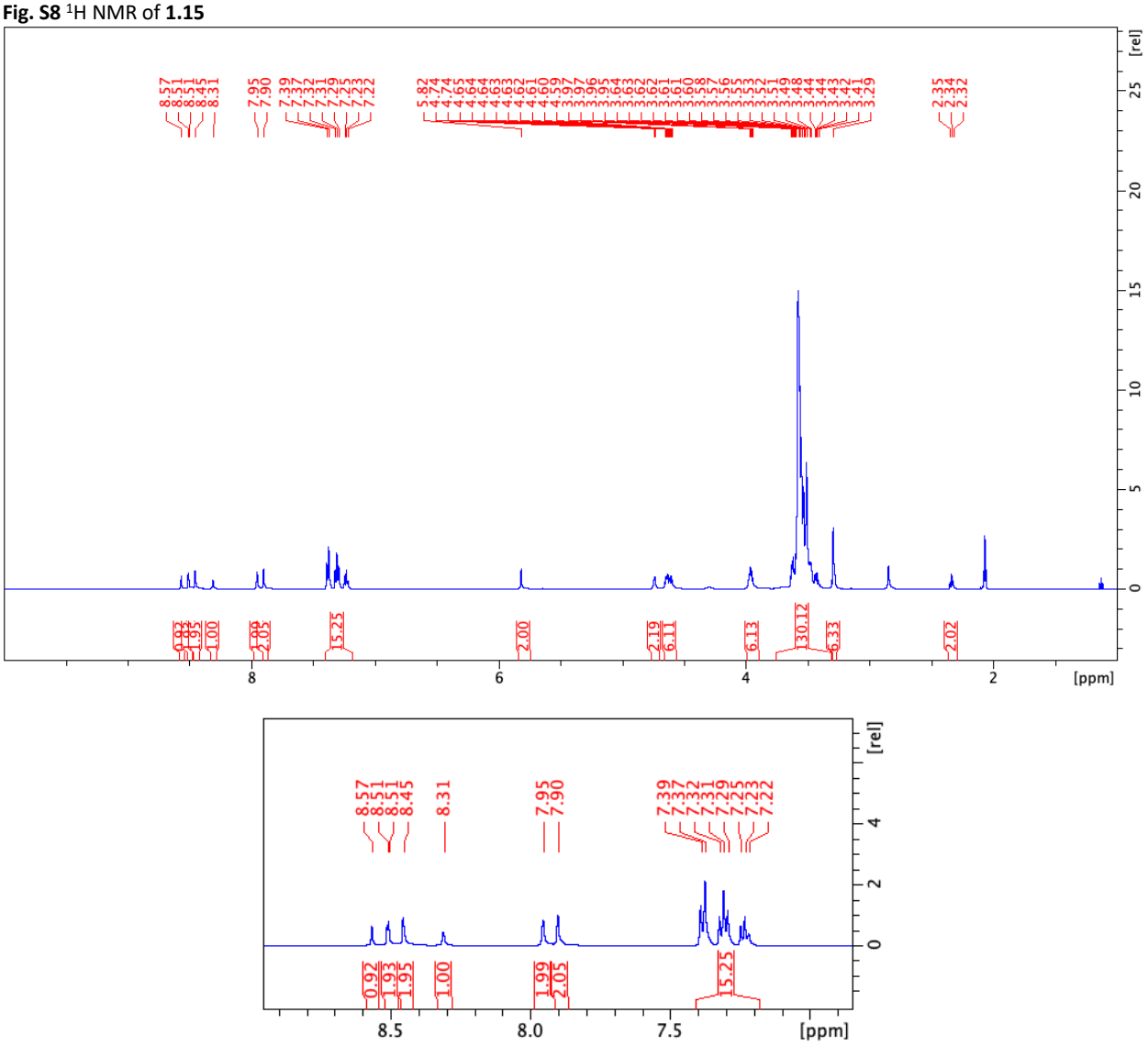


Fig. S8.1 ^{13}C NMR of **1.15**

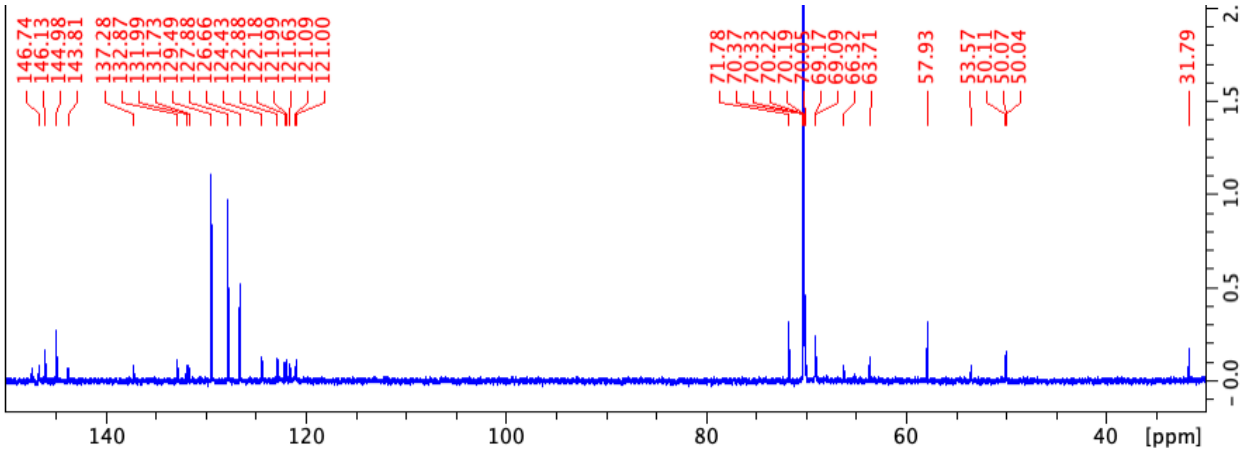


Fig. S9 ^{1}H NMR of **2.5**

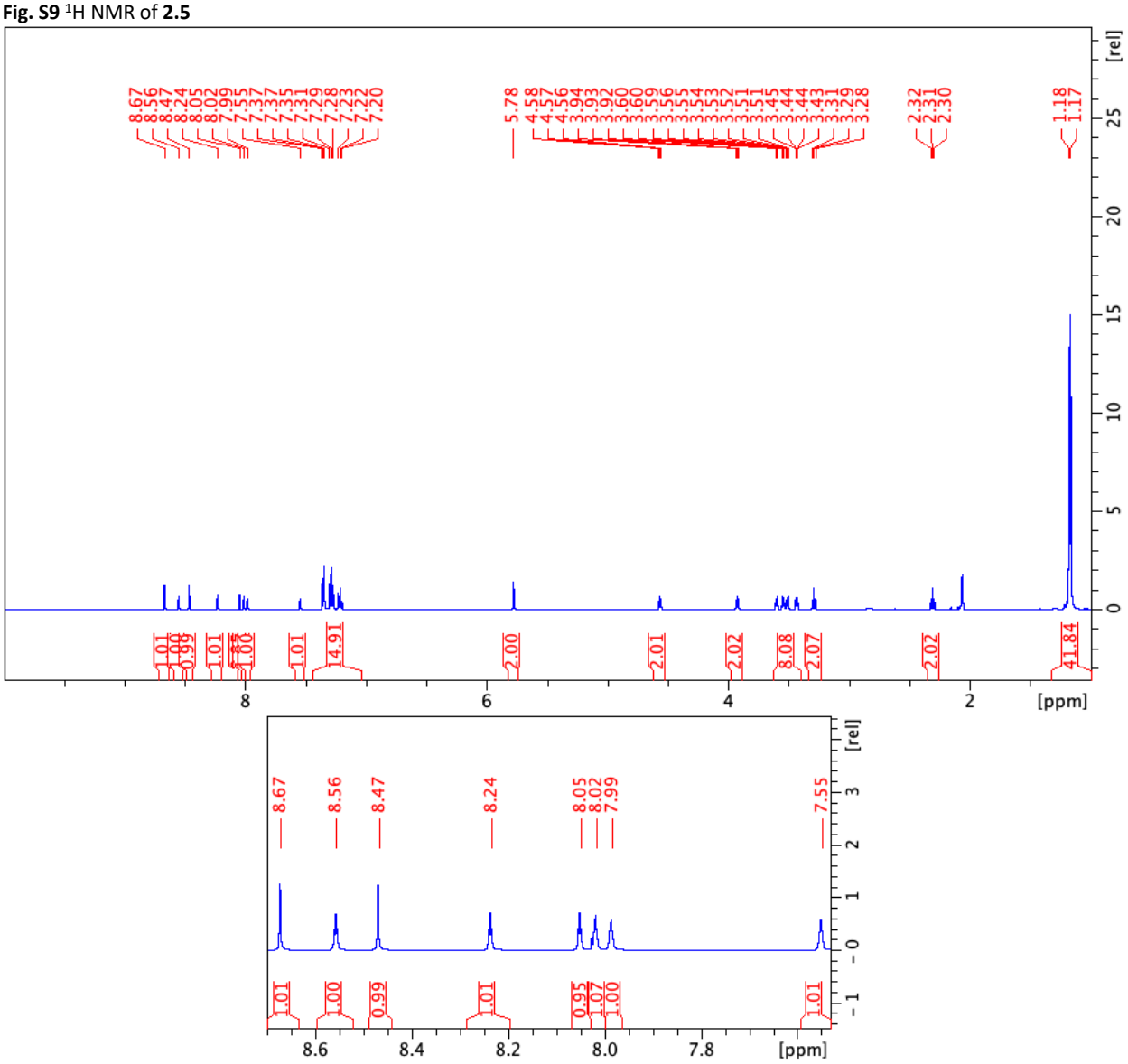


Fig. S9.1 ^{13}C NMR of **2.5**

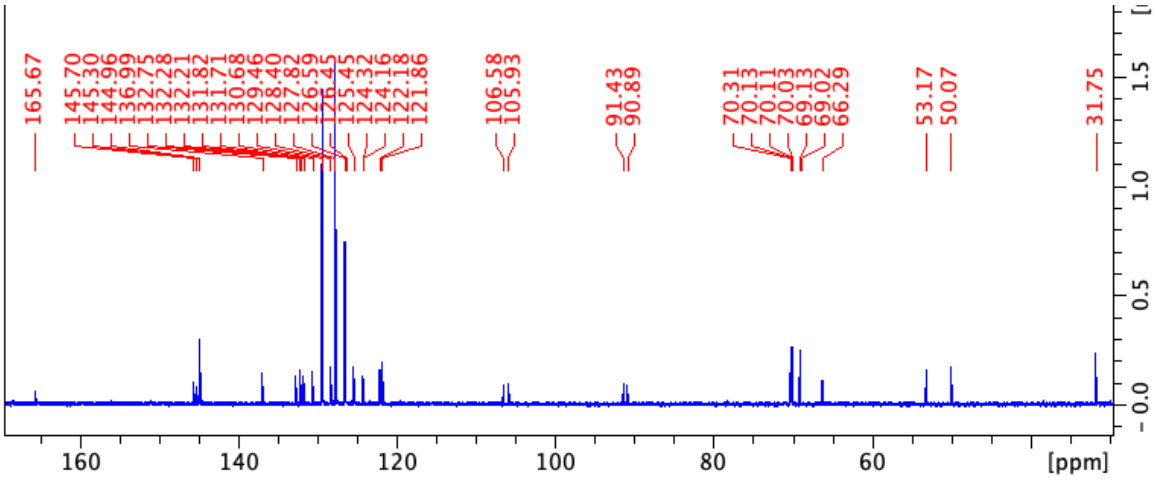


Fig. S10 ^{1}H NMR of **2.6**

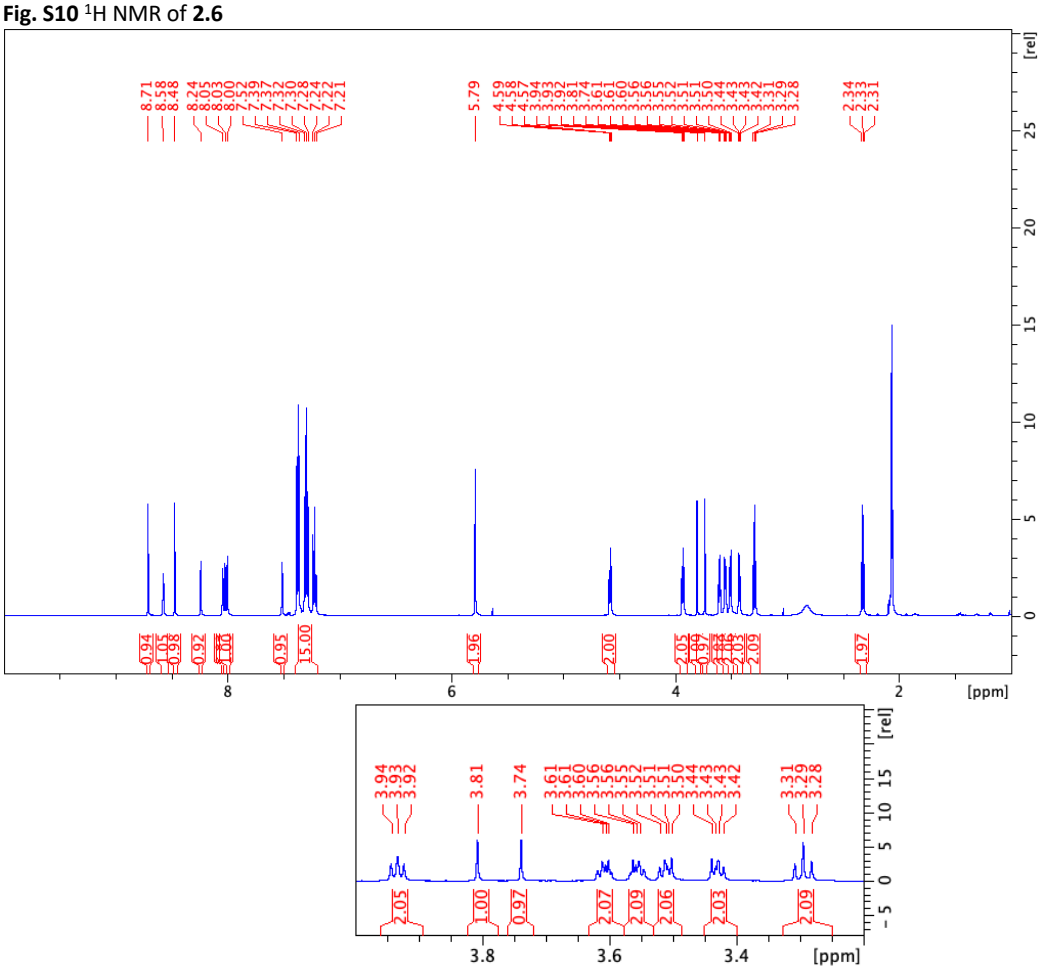


Fig. S10.1 ^{13}C NMR of **2.6**

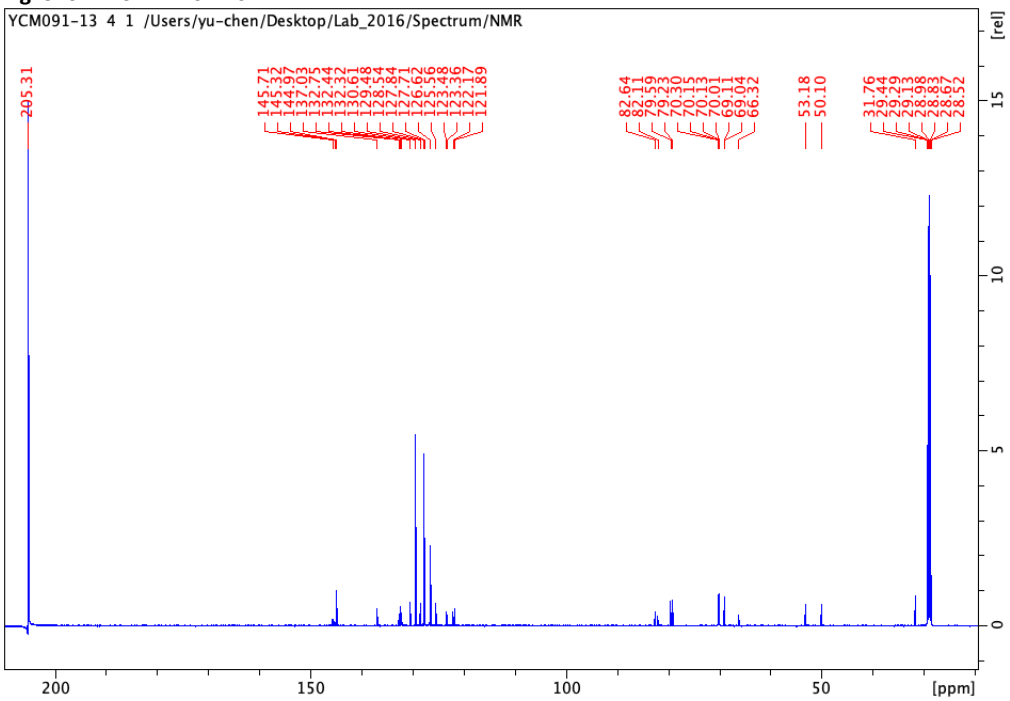


Fig. S11 ^{1}H NMR of **2.7**

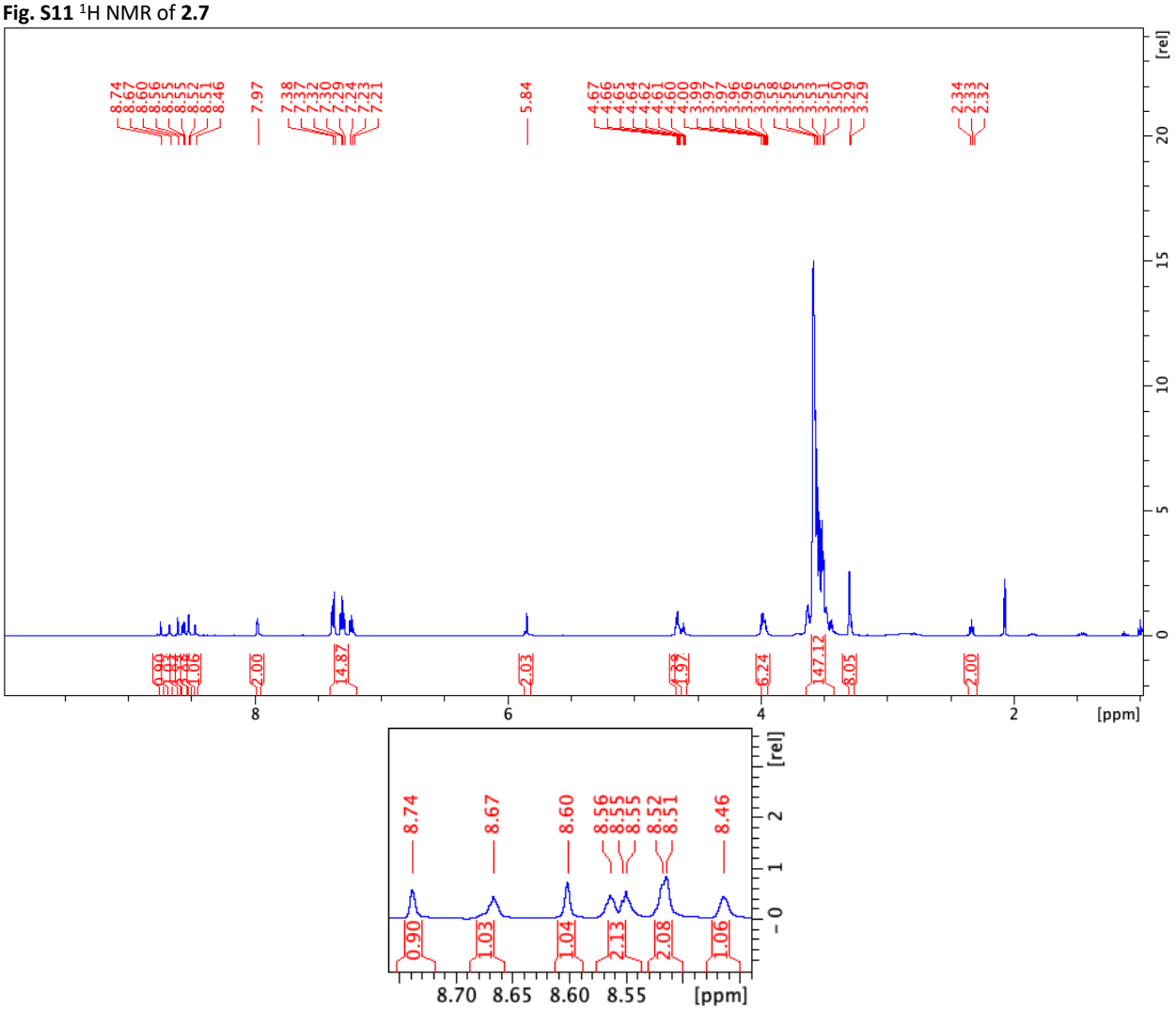


Fig. S11.1 13C NMR of **2.7**

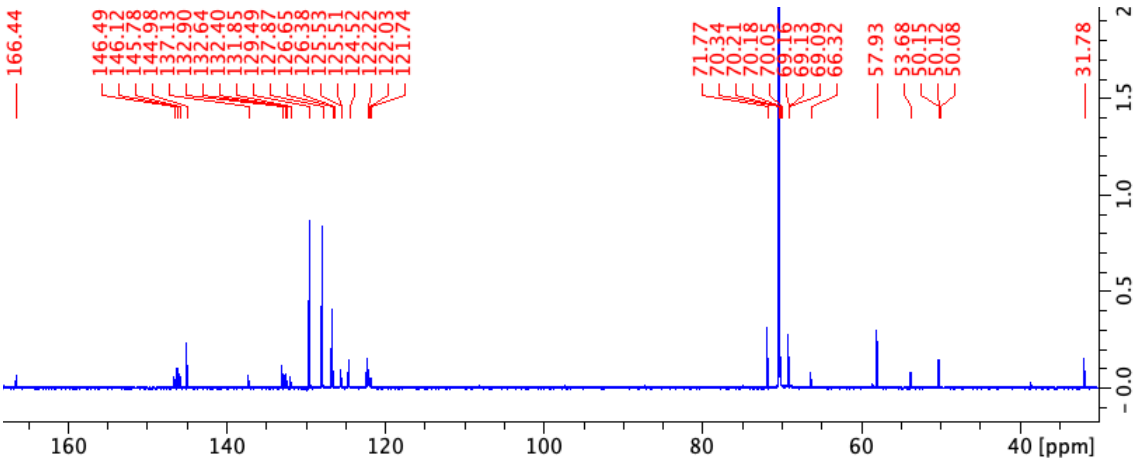


Fig. S12 ^{1}H NMR/2D DOSY spectra of Fmoc-P-Ester

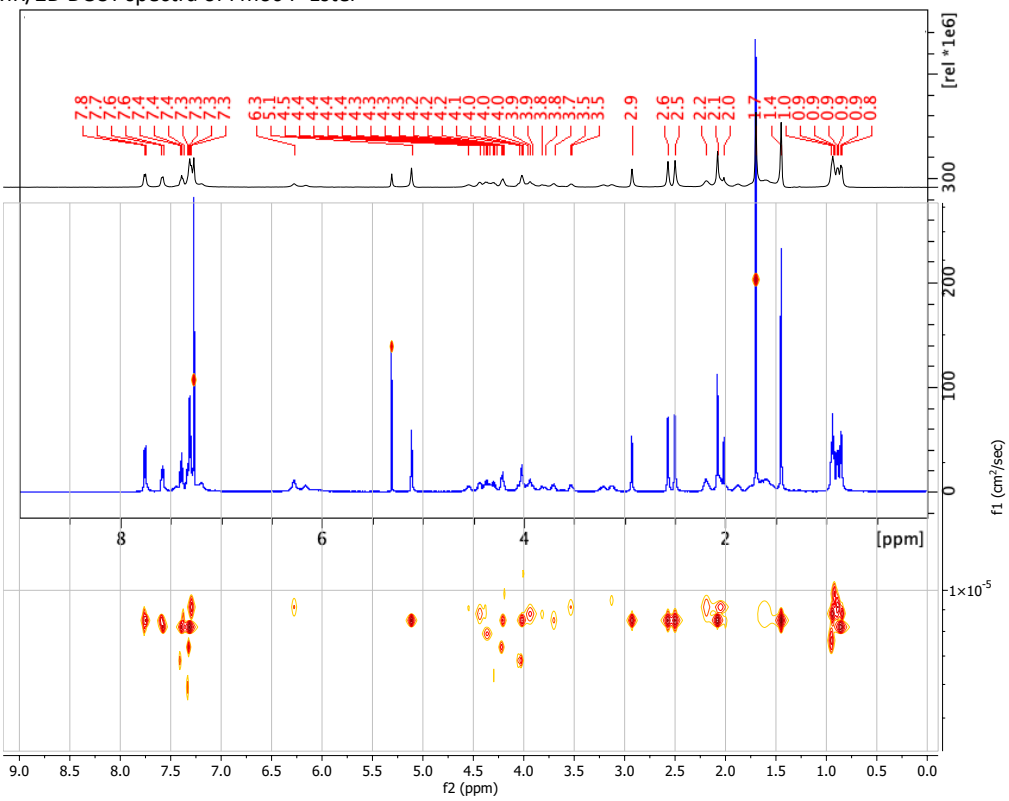


Fig. S13 ^{1}H NMR spectra of P-Ester

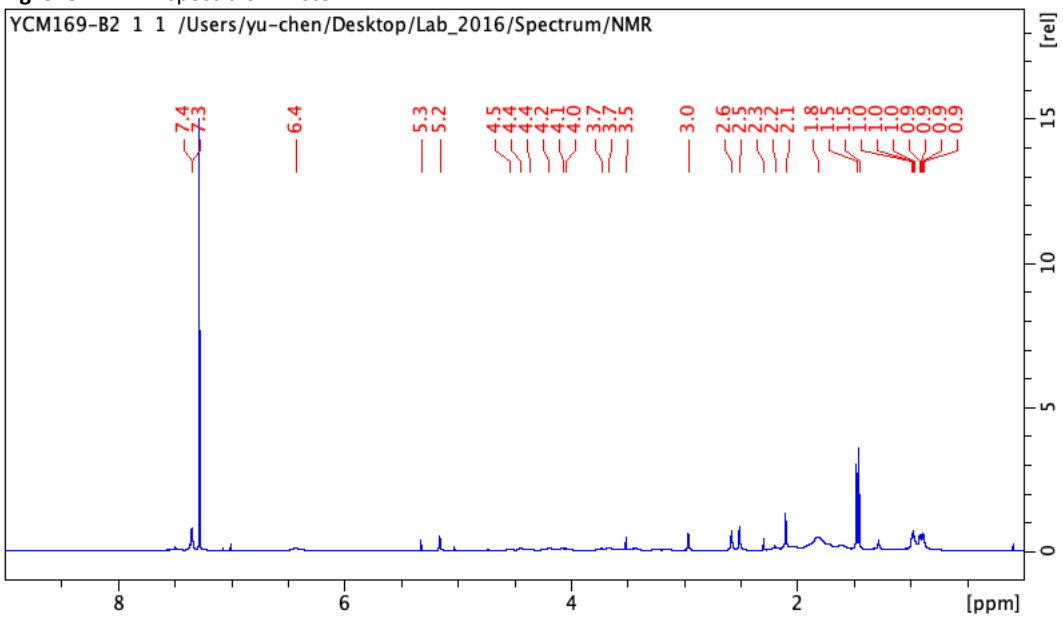
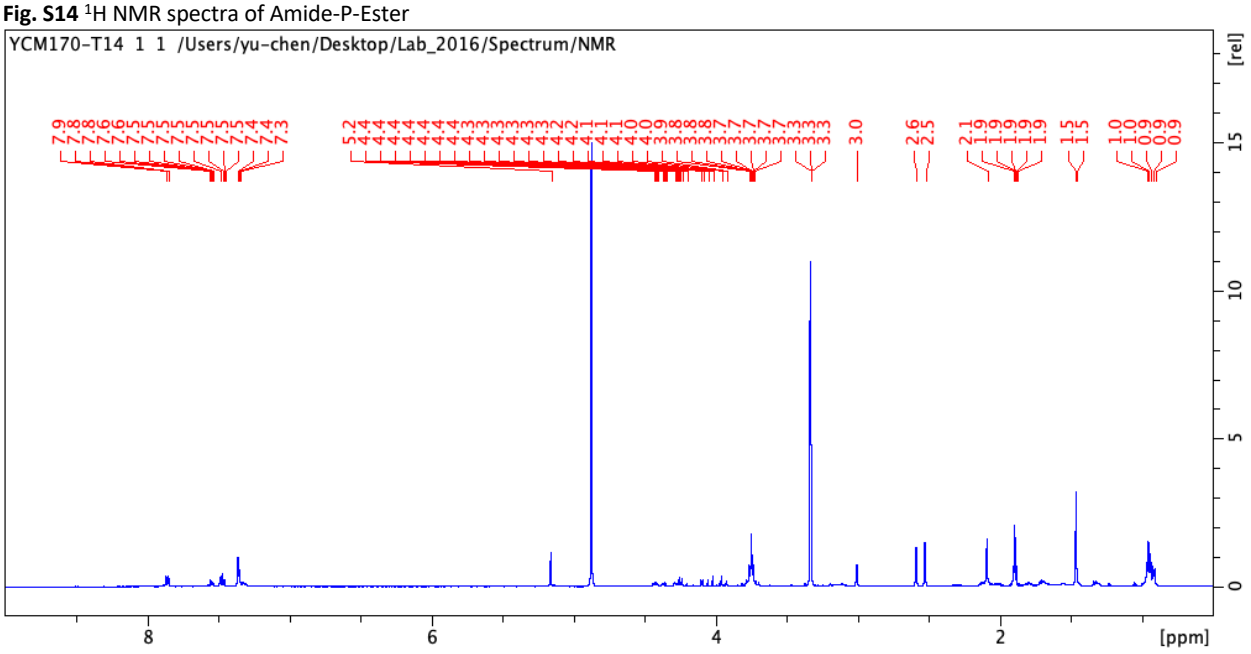


Fig. S14 ^{1}H NMR spectra of Amide-P-Ester



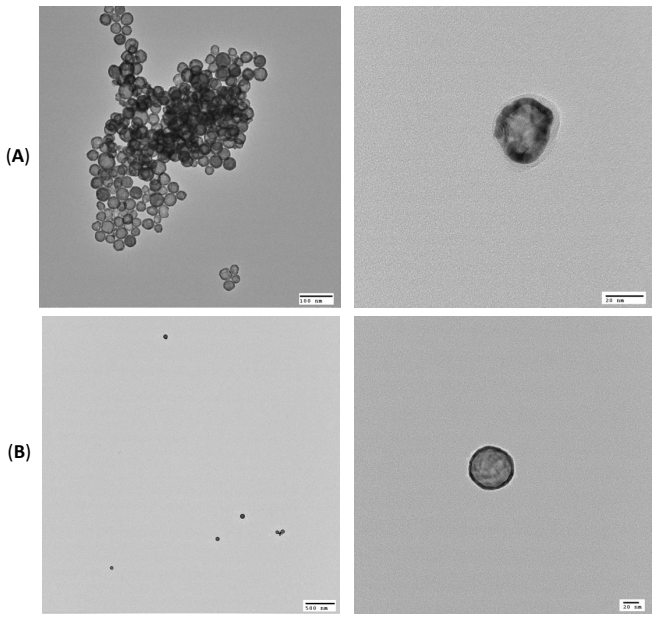


Fig. S15 TEM micrographs of (**A**) OH-CH_2-Core-$(PEG_{750})_2$-TEGS-HAuNS ; (**B**) COOH-Core-$(PEG_{750})_2$-TEGS-HAuNS.

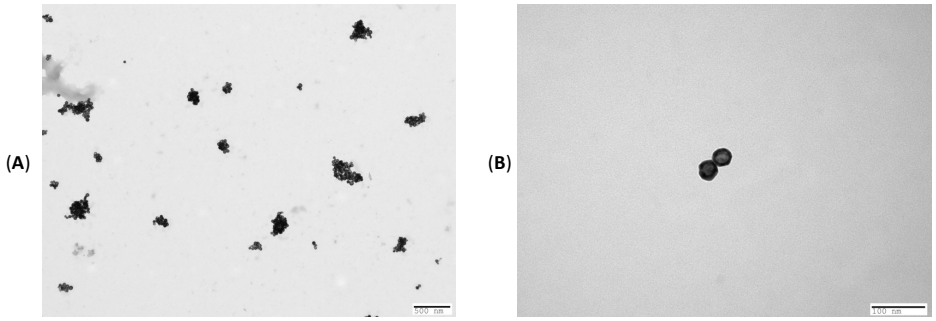


Fig. S16 TEM micrographs of TEM ChemLinked-PEG_{750} HAuNS: (A) Concentrated; (B) Diluted solution.

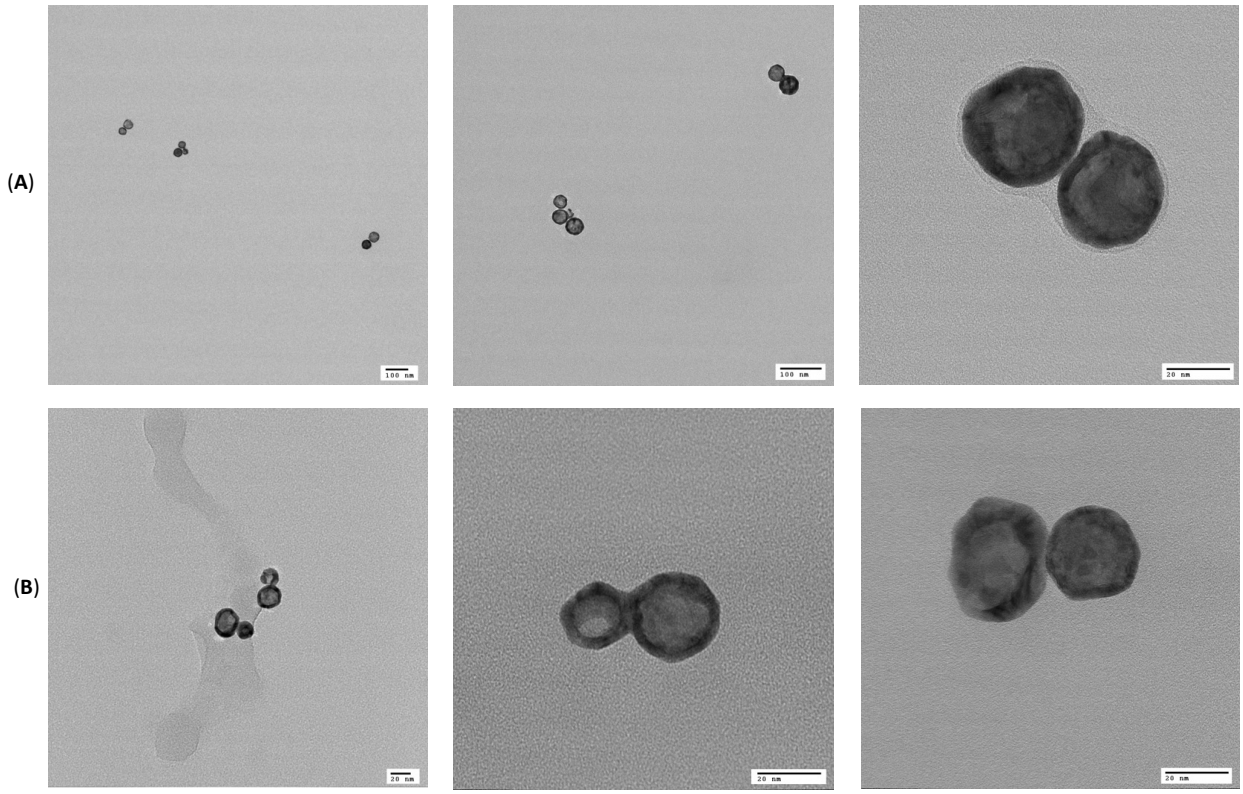


Fig. S17 TEM micrographs of ChemLinked-PEG_{750} (**A**) and ChemLinked-PEG_{2000} (**B**) HAuNS from different batches.

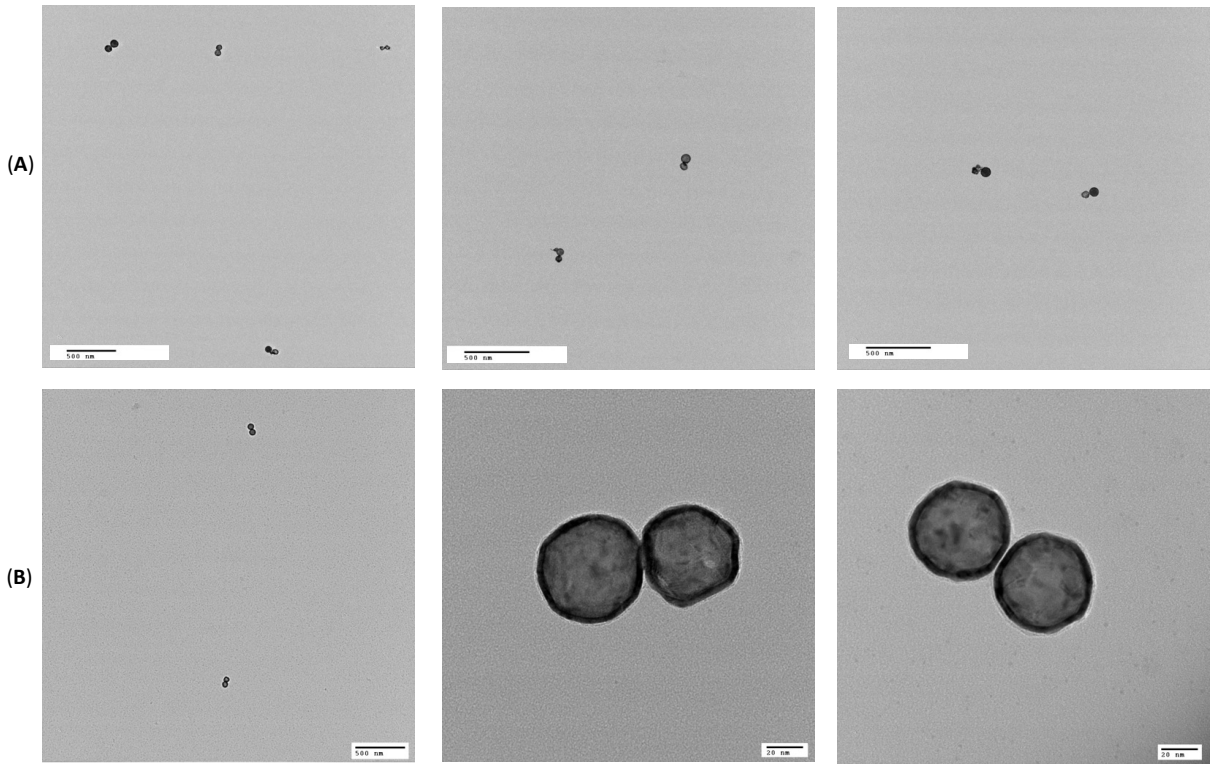


Fig. S18 TEM micrographs of BioLinked-PEG$_{750}$ (**A**) and BioLinked-PEG$_{2000}$ (**B**) HAuNS from different batches.

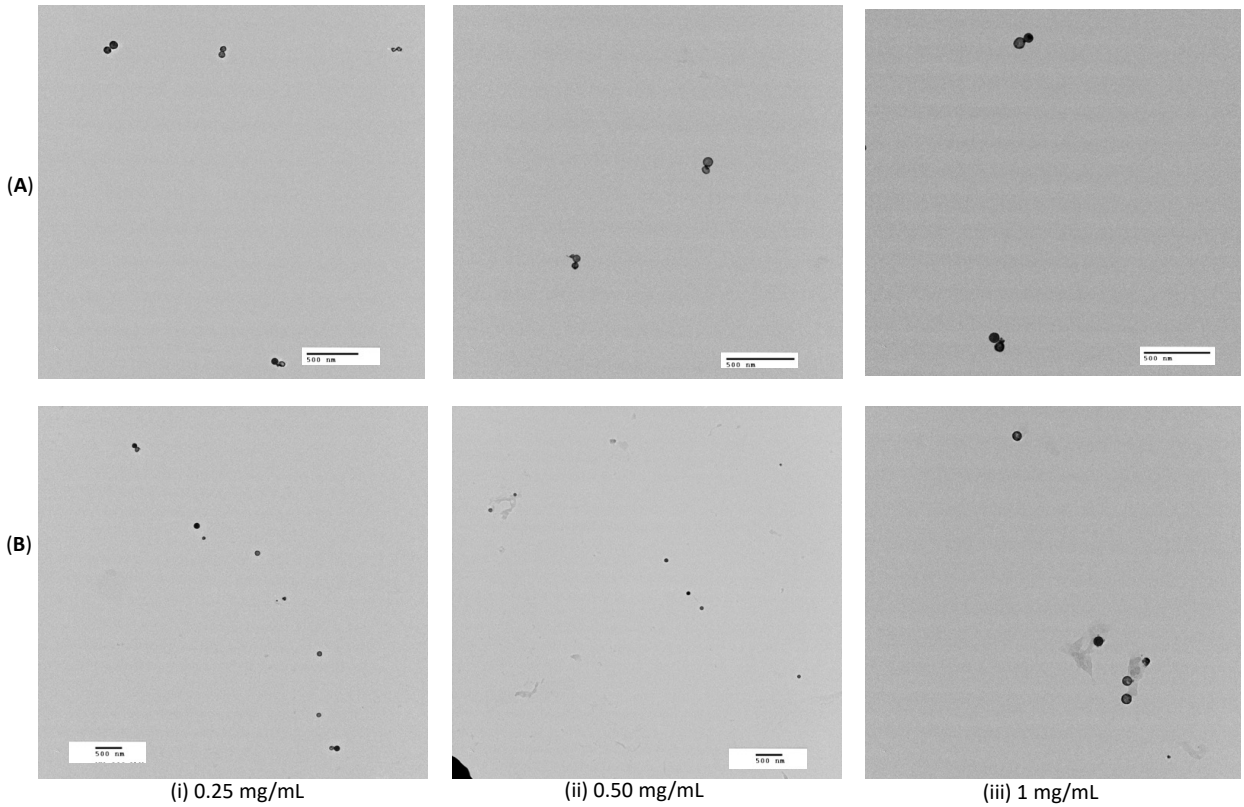


Fig. S19 TEM Micrographs of BioLinked-PEG_{750} HAuNS: (**A**) Before treatment with enzyme; (**B**) After treatment with enzyme, (i) 0.25 mg/mL; (ii) 0.50 mg/mL; (iii) 1 mg/mL.