Designing Dendronic-Raman Markers for Sensitive Detection using Surface-Enhanced Raman Spectroscopy

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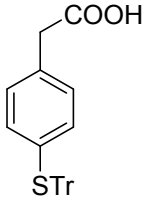
General Information

All glassware was dried in an oven overnight at 120 °C prior to use. Standard Schlenk techniques were used for carrying out reactions under argon atmosphere and thin layer chromatography (Merck TLC silica gel 60 F254) was used for monitoring. Silica gel of mesh size 230–400 was used for performing flash column chromatography. ^{1}H and ^{13}C NMRs was recorded on a Fourier transform NMR spectrometer (400 MHz for $^{1}HNMR$ and 125 Mhz for $^{13}CNMR$). Residual solvent peak: $CDCl_3$ (^{1}H NMR: 7.27 ppm, ^{13}C $\{^{1}H\}$ NMR: 77.16 ppm) were used as a reference for reporting chemical shifts in parts per million (ppm). IR was recorded using Frontier MIR/FTIR Spectrometer. Mass spectrometry was carried using Q-TOF instrument for HRMS and Autoflex speed instrument for MALDI. All Raman and SERS measurements were done on LabRam HR Evolution using a He-Ne laser of 633 nm excitation wavelength and 50 X objective lens. UV−visible extinction spectra of gold nanoparticle colloidal solution were recorded using a quartz cell in a lambda 900 spectrometer. TEM measurements were performed on a carbon-coated copper grid using a JEOL 3010 with an operating voltage of 300 KeV.

1. Compounds reported in literature



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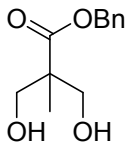

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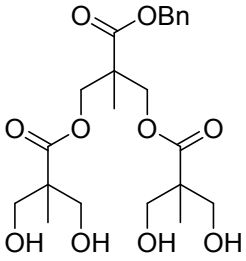
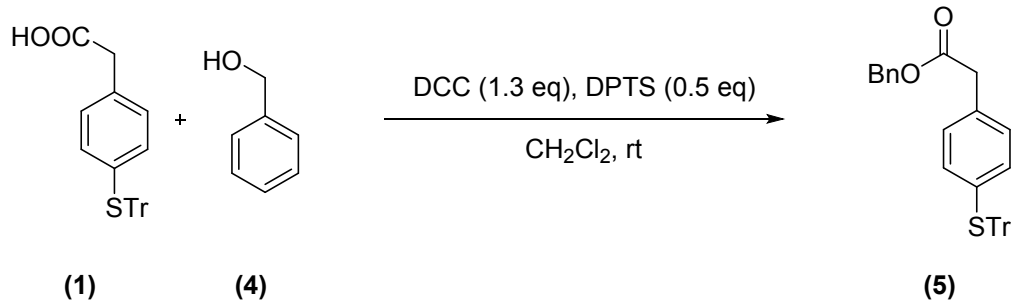


(3)[3]

2. Synthesis of trityl MT derivative (**5**)



(1) **(4)** **(5)**

Procedure:

In a 10 mL two-necked round bottom flask, compound **1** (100 mg, 0.22 mmol) and 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS) (30 mg, 0.12 mmol) were taken and the flask was purged with argon. Benzyl alcohol (100 µL, 0.9 mmol) was added to this flask followed by dichloromethane (DCM) (1.5 mL). A solution of N,N'-Dicyclohexyl carbodiimide (DCC) (65 mg, 0.317 mmol) in DCM (1 mL) was added dropwise at room temperature. Immediate precipitation of white residue occurred upon DCC addition. The reaction mixture was monitored for completion by TLC. After 2 hours, the reaction mixture was concentrated and further purified using flash column chromatography.

Column chromatography:

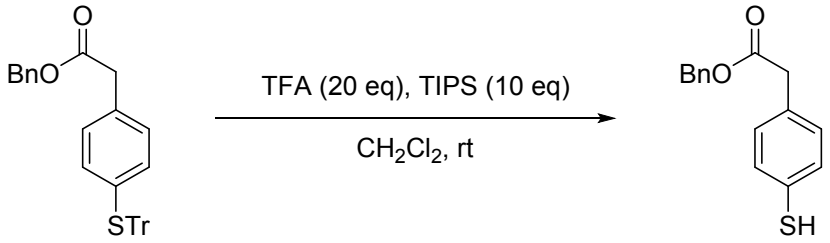
About 60 mL of silica was taken and packed into a column using hexanes. The crude compound obtained from the reaction was adsorbed over 2 mL of silica and loaded onto the column. The column was initially eluted with hexanes and 12 fractions of 25 mL each were collected. The compound started eluting in 5% EtOAc/hexanes (25 mL fractions). Fractions 16 through 19 were collected, concentrated and dried under high vacuum to obtain 100 mg of product (yield= 82%).

Characterization:

White solid; R_f: 0.33 in 10% EtOAc/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.16 (m, 20H), 6.94-6.89 (m, 4H), 5.10 (s, 2H), 3.54 (s, 2H). ^{13}C NMR{^{1}H} (125 MHz, $CDCl_3$): 40.9, 66.6,

70.8, 126.7, 127.7, 128.1, 128.25, 128.55, 129.0, 130.0, 133.3, 133.6, 134.8, 135.8, 144.5, 171.0. IR: 3056, 3035, 2924, 1732, 1445, 1329, 1151, 958, 737, 697 cm^{-1}. HRMS (ESI) m/z $(M-Na)^+$ Calculated for $C_{34}H_{28}O_2S$ 500.1810, Found 500.1683.

3. Synthesis of MT derivative



(5) **(MT)**

Procedure:

In a 5 mL Schlenk tube, compound **5** (111 mg, 0.22 mmol) was added and the tube was evacuated and then filled with argon. This process was repeated twice more. To the Schlenk tube, a mixture of triflouroacetic acid (TFA) (340 µL, 4.45 mmol) and DCM (1.4 mL) was injected under ice-cold conditions. The reaction mixture became deep yellow during addition. A mixture of triisopropylsilane (TIPS) (228 µL, 1.12 mmol) and DCM (1 mL) was added dropwise to the reaction. The reaction mixture turned pale slowly. The reaction was brought to room temperature and monitored via TLC. After completion (3 hours), the reaction was quenched using a few drops of water. The volatile components were removed by distillation under reduced pressure. The crude material was purified using flash column chromatography.

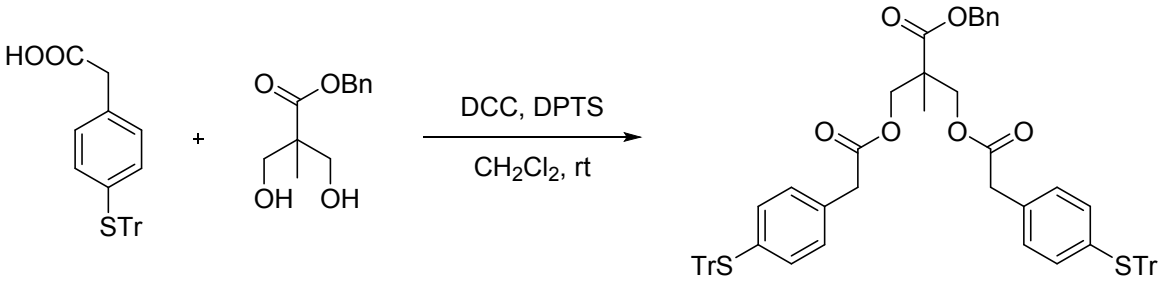
Column chromatography:

About 60 mL of silica was packed into a column using 2% EtOAc/hexanes. The crude compound obtained from the reaction was adsorbed on 2 mL of silica and loaded onto the column. The compound was eluted with 3% EtOAc/hexanes and 15 mL fractions were collected (20 fractions), concentrated and dried under high vacuum to obtain 40 mg of product (yield= 78%).

Characterization:

Yellowish-white solid; R_f: 0.23 in 10% EtOAc/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.33 (m, 5H), 7.25-7.15 (dd, J = 7.5Hz, 7.7Hz, 8H), 5.13 (s, 2H), 3.62 (s, 2H), 3.44 (s, 1H). ^{13}C NMR{^{1}H} (125 MHz, $CDCl_3$): 40.8, 66.7, 128.2, 128.3, 128.6, 129.5, 129.7, 130.0, 131.5, 136.0, 170.0. IR: 3065, 3036, 2957, 2561, 1724, 1600, 1494, 1016, 1002, 738, 701 cm^{-1}. HRMS (ESI) m/z (M-Na)$^+$ Calculated for $C_{15}H_{14}O_2S$ 258.0715 Found: 258.0607.

4. Synthesis of trityl DT derivative (**6**)



(1) **(2)** **(6)**

Procedure:

A 10 mL Schlenk tube was charged with compound **2** (65 mg, 0.29 mmol), compound **1** (300 mg, 0.73 mmol) and DPTS (73 mg, 0.29 mmol). The Schlenk tube was purged with argon. The compounds were dissolved in DCM (2 mL). A solution of DCC (72 mg, 0.35 mmol) in DCM (2 mL) was added dropwise at room temperature. Immediate precipitation of white residue occurred upon DCC addition. The reaction mixture was stirred till completion and monitored using TLC. After 3 hours, the reaction was removed from stirring, concentrated and further purified using flash column chromatography.

Column chromatography:

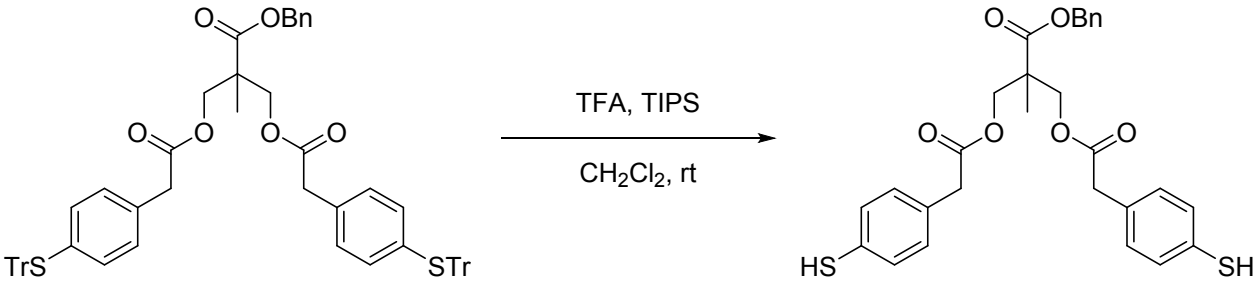
Approximately 70 mL of silica was taken in a column and packed with 10% EtOAc/hexanes. The crude product was dissolved in minimum amount of DCM and adsorbed on 3 mL of silica. The silica was dried under vacuum and loaded on the column. The column was then eluted with

10% EtOAc/hexanes and 25 mL fractions each were collected (10 fractions). The pure material eluted in 12% EtOAc/hexanes. Fractions 20 through 34 were collected. The fractions were concentrated and dried under high vacuum to obtain 230 mg of product. (Yield =79%)

Characterization:

White flaky solid, R_f: 0.37 in 12% EtOAc/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.16 (m, 35H), 6.89-6.81 (m, 8H), 5.00 (s, 2H), 4.17-4.10 (dd, J = 11Hz, 4H), 3.37 (s, 4H), 1.06 (s, 3H). ^{13}C NMR{^{1}H} (125 MHz, $CDCl_3$): 17.6, 40.8, 46.4, 65.6, 66.8, 70.7, 126.7, 127.9, 128.1, 128.4, 128.6, 129, 130.0, 133.0, 133.5, 134.5, 144.5, 165.5, 170.5. IR: 3084, 3054, 3029, 1738, 1733, 1594, 1444, 1129, 1016, 1002, 806, 739, 697 cm^{-1}. MALDI m/z $(M\text{-}Na)^+$ Calculated for $C_{66}H_{56}O_6S_2$ 1008.352 Found: 1031.693.

5. Synthesis of DT derivative



(6) **(DT)**

The procedure described in step 3 was used for the de-protection of compound **6**. Ten equivalents of TIPS were used in the reaction.

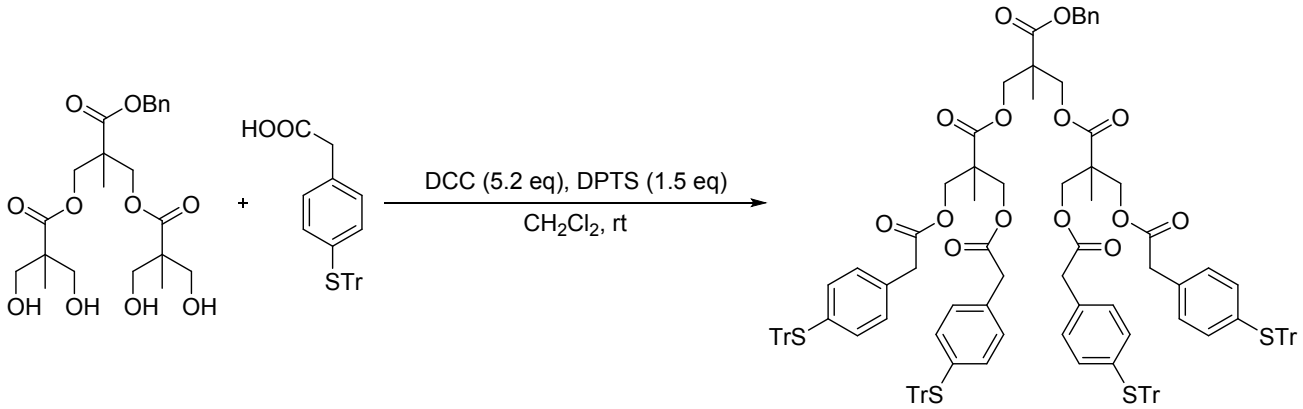
Column chromatography:

About 60 mL of silica was taken and packed using 5% EtOAc/hexanes. The crude compound obtained from the reaction was adsorbed on 2 mL of silica and loaded onto the column. The column was eluted with 5% EtOAc/hexanes and 15 mL fractions were collected (20 fractions). The pure compound was eluted in 15% EtOAc/hexanes. Fractions 42 through 52 were collected, concentrated and dried under high vacuum to obtain 35 mg of product (yield= 67%).

Characterization:

Colourless viscous liquid; R_f: 0.1 in 20% EtOAc/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.28 (m, 5H), 7.20-7.19 (d, J = 8Hz, 4H), 7.07-7.05 (d, J = 8Hz, 4H), 5.06 (s, 2H), 4.23-4.15 (dd, J = 11Hz, 4H), 3.47 (s, 4H), 3.42 (s, 2H), 1.15 (s, 3H). ^{13}C NMR {^{1}H} (125 MHz, $CDCl_3$): 17, 40, 46, 65, 66, 127.7, 128, 128.4, 128.6, 129.6, 130, 131.1, 135.5, 170, 172. IR: 3062, 3028, 2988, 2557, 1725, 1599, 1494, 1154, 1134, 1098, 1008, 803, 733, 695 cm^{-1}. HRMS (ESI) m/z (M-H_2O) Calculated for $C_{28}H_{28}O_6S_2$ 524.1327 Found: 524.1637

6. Synthesis of trityl TT derivative (**7**)



(3) **(1)** **(7)**

Procedure:

A 10 mL Schlenk tube was charged with compound **3** (182 mg, 0.40 mmol), compound **1** (816 mg, 1.99 mmol) and DPTS (150 mg, 0.60 mmol). The Schlenk tube was purged with argon. The compounds were dissolved in DCM (3 mL). A solution of DCC (427 mg, 2.06 mmol) and DCM (3 mL) was added dropwise at room temperature. The reaction mixture was monitored using TLC and stirred till completion. After 48 hours, the reaction mixture was concentrated and further purified using flash column chromatography.

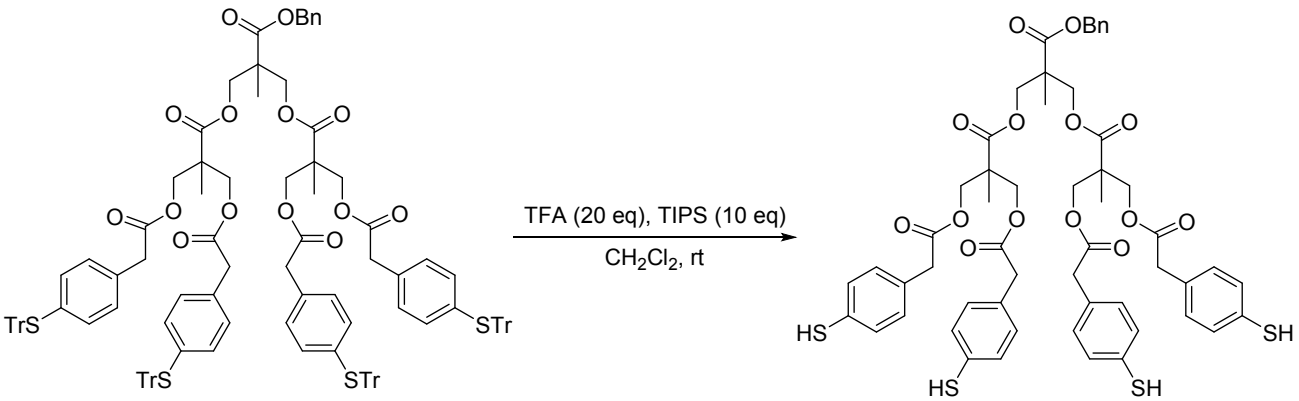
Column chromatography:

About 40 mL of silica was taken and packed using 10% EtOAc/hexanes. The crude compound obtained from the reaction was adsorbed on 1 mL of silica and loaded onto the column. The column was eluted with 10% EtOAc/hexanes and 15 mL fractions were collected (13 fractions). The pure compound was eluted in 20% EtOAc/hexanes. Fractions 26 through 32 were collected, concentrated and dried under high vacuum to obtain 500 mg of product (yield=62%).

Characterization:

White solid; R_f: 0.17 in 30% EtOAc/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.36 (d, *J* = 7.6Hz, 30H) 7.22-7.14 (m, 35H), 6.88-6.81 (dd, *J* = 8.2Hz, 7.9Hz, 16H), 5.08 (s, 2H), 4.15-3.98 (m, 12H), 3.40 (s, 8H), 1.11 (s, 3H), 0.94 (s, 6H). ^{13}C NMR {^{1}H} (125 MHz, $CDCl_3$): 17.6, 24.9, 25.8, 33.9, 40.5, 46.4, 46.6, 65.3, 65.7, 67.1, 70.7, 126.7, 127.7, 127.9, 128.4, 129.0, 130.0, 133.2, 133.5, 134.6, 144.5, 170.5, 171.8, 171.9. IR: 3084, 3030, 2930, 1738, 1626, 1594, 1580, 1444, 1239, 1218, 1125, 1016, 1003, 907, 732, 696 cm^{-1}. MALDI m/z $(M-Na)^+$ Calculated for $C_{130}H_{112}O_{14}S_4$ 2025.697 Found: 2025.612.

7. Synthesis of TT derivative



(7) **(TT)**

The procedure described in step 3 was used for the de-protection of compound **7**. Ten equivalents of TIPS were used in the reaction.

Column chromatography:

About 50 mL of silica was taken and packed using 5% EtOAc/hexanes. The crude compound obtained from the reaction was adsorbed on 2 mL of silica and loaded onto the column. The column was eluted with 5% EtOAc/hexanes followed by 30% EtOAc/hexanes and 40% EtOAc/hexanes. (15 mL fractions each) Fractions 58 through 70 were collected, concentrated and dried under high vacuum to obtain 35 mg of product (yield= 56%).

Characterization:

Colourless viscous liquid; R_f: 0.1 in 30% EtOAc/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 5H), 7.21-7.19 (d, J = 7.9Hz, 8H), 7.09-7.07 (d, J = 7.7Hz, 8H), 5.12 (s, 2H), 4.17-4.05 (m, 12H), 3.51 (s, 8H), 3.43 (s, 4H), 1.14 (s, 3H), 1.04 (s, 6H). ^{13}C NMR {^{1}H} (125 MHz, $CDCl_3$): 17.4, 17.6, 40.4, 46.4, 46.6, 65.3, 65.7, 67.1, 127.8, 128.4, 128.5, 128.7, 129.7, 130.0, 131.1, 135.4, 170.6, 171.8, 171.9. IR: 3028, 2980, 2566, 1731, 1600, 1494, 1237, 1218, 1126, 1099, 1015, 908, 802, 732, 698 cm^{-1}. HRMS (ESI) m/z $(M-Na)^+$ Calculated for $C_{54}H_{56}O_{14}S_4$ 1056.2553 Found 1056.2347.

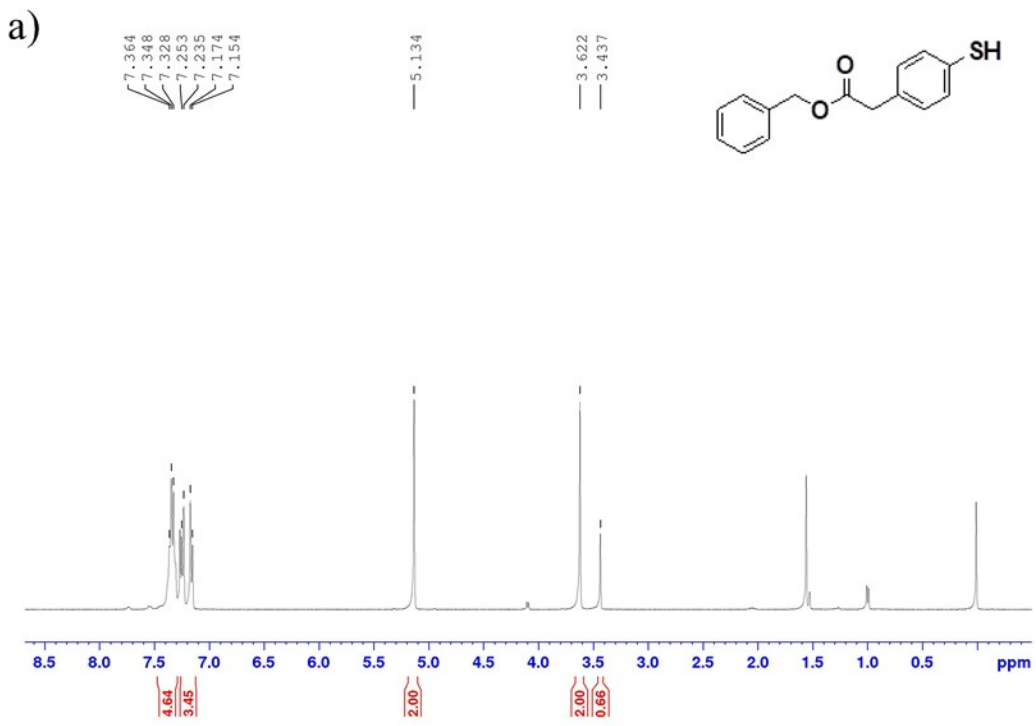
8. Synthesis of Gold nanoparticles (Aunp)

The colloidal gold nanoparticles were prepared by using the method given by Bastús et al.[4] Briefly, a 2.2 mM sodium citrate solution (150 mL) was heated in an oil-bath vigorous stirring. 1 mL of $HAuCl_4$ (25mM) was injected upon boiling. The solution was stirred for about 10 minutes. Light pink Au seeds were immediately prepared, and the solution was cooled to 90˚C for further growth of seeds. 1 mL of $HAuCl_4$ was injected twice. An interval of 30 minutes was maintained post every injection. 55 mL of the solution was pipetted out, followed by addition of 53 mL water and 2 mL sodium citrate solution (60 mM). This solution served as the seed for the next batch of Aunp and

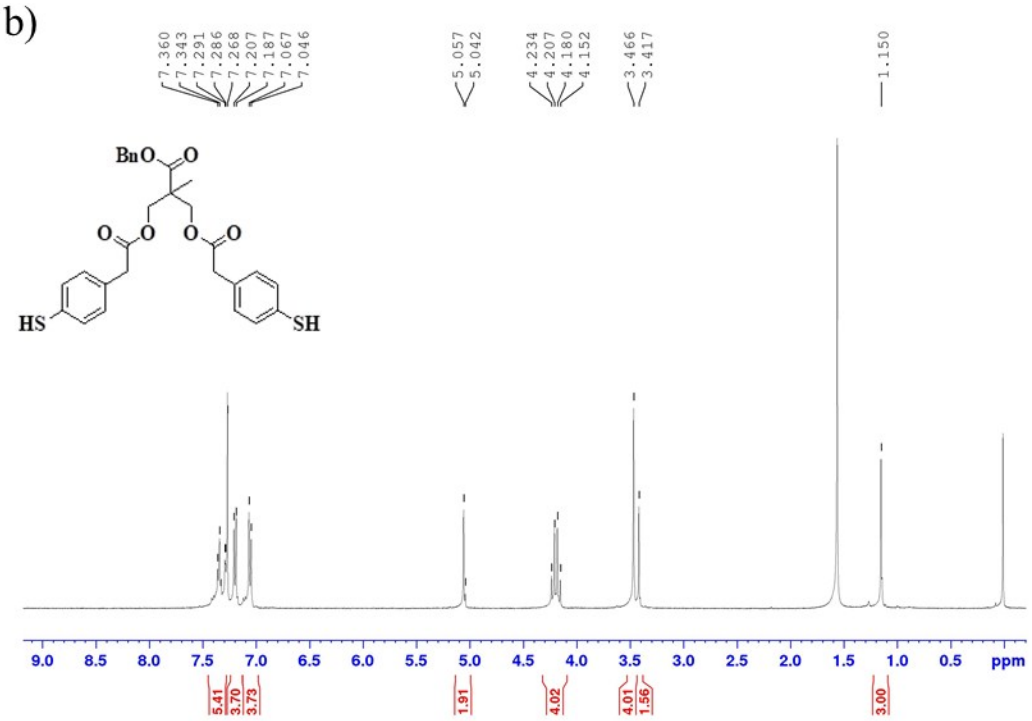
was repeated till batch 10. Aunp of size 60-70 nm gave the best SERS enhancement with the analyte molecules and were chosen for the studies. (Figure S2)

Experimental Method: For the SERS studies, 1 mL of Aunp were mixed and incubated with 10 µL of the analyte solution for 1 hour. The mixture was then centrifuged for 8 minutes at 7500 rpm. The supernatant was decanted and 10 µL of the residue was dropped on a siliconized glass substrate. The signal was recorded for the drop.

Figure S1: a) ^{1}H NMR spectra of MT derivative. b) ^{1}H NMR spectra of DT derivative (DT). c) ^{1}H NMR spectra of TT derivative.



b)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5.057
5.042
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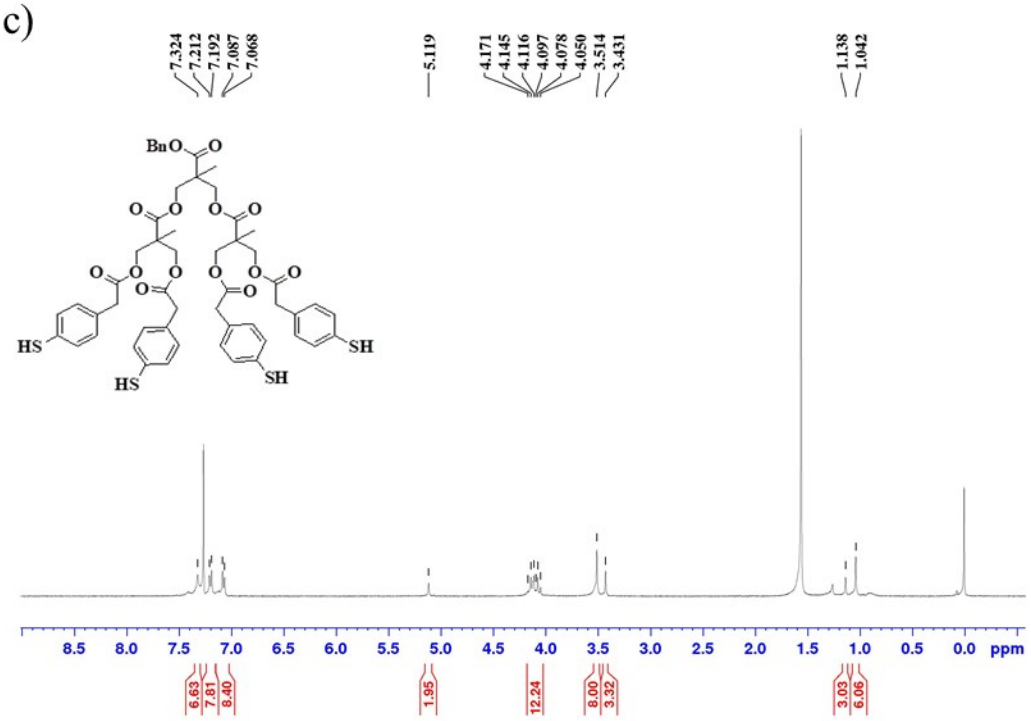
4.180
4.152
3.466
3.417
1.150
BnO
O
HS
SH
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
5.41
3.70
3.73
1.91
4.02
4.01
1.56
3.00

c)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7.087
7.068
5.119
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4.145
4.116
4.097
4.078
4.050
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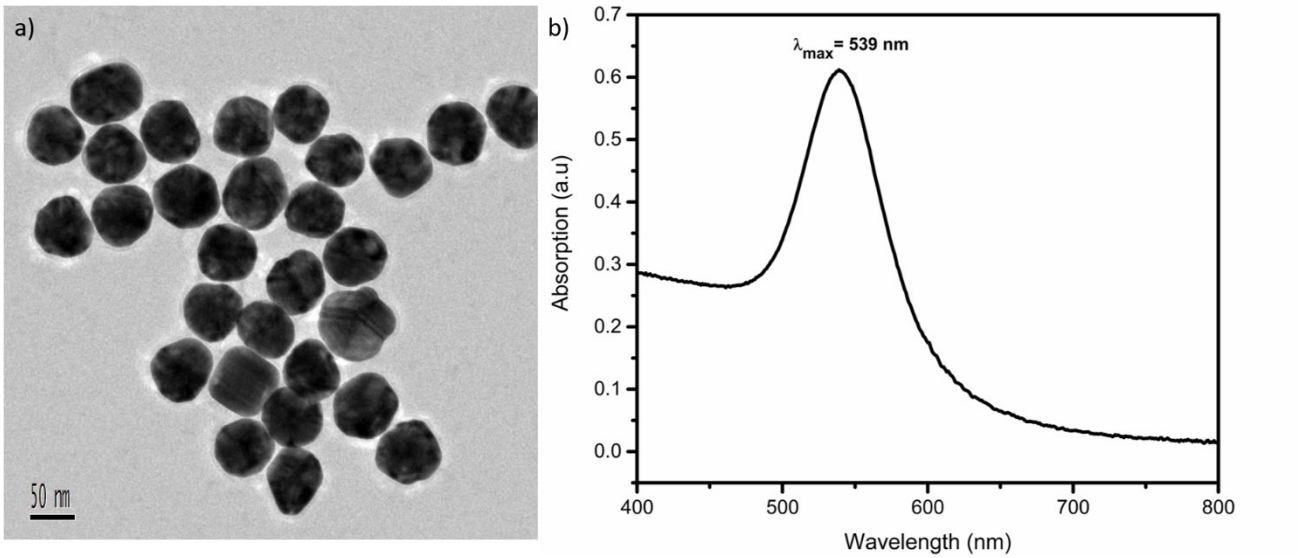
3.431
1.138
1.042
BnO
HS
SH
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
6.63
7.81
8.40
1.95
12.24
8.00
3.32
3.03
6.06

Figure S2: a) TEM image obtained for the gold nanoparticles synthesized of average size 60 nm.



b) Extinction spectra of the obtained gold colloidal nanoparticles.

Table S1: SERS intensities of 1004 cm^{-1}(ν_{12}) mode for synthesized thiophenol derivatives

	Concentration	I_{1004}/I_{1078}
MT	10^{-4}M	0.5
DT	10^{-4}M	0.2
TT	10^{-5}M	0.1

References

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[3] Feliu, N.; Walter, M. V.; Montañez, M. I.; Kunzmann, A.; Hult, A.; Nyström, A.; Malkoch, M.; Fadeel, B. *Biomaterials* **2012**, *33* (7), 1970–1981.

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