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Selective sensing of sulfate anions in water with cyclopeptide-decorated gold nanoparticles

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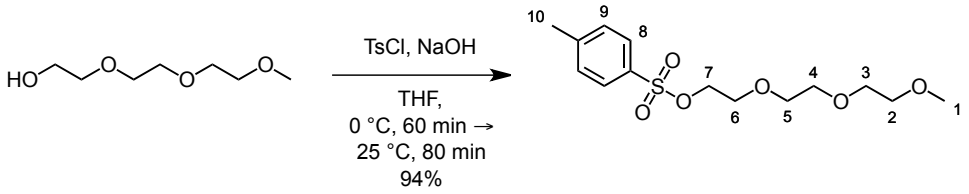
CONTENT

Syntheses of Compounds 1-5

General details. Solvents were dried according to standard procedures prior to use if necessary. Starting materials and reagents were commercially available and were used without further purification. Analyses were carried out as follows: ATR-IR, Perkin Elmer Spectrum 100 FT-IR-Spectrometer with Universal ATR Sampling Accessory Unit; Centrifuge, Eppendorf Centrifuge 5702 R; Centrifugal Concentrators, Vivaspin® 15R (MWCO 5000 Da) Sartorius; elemental analysis, Elementar vario Micro cube; ESI-MS, Bruker Esquire 3000 Plus and Esquire 6000; NMR, Bruker AVANCE™ III 400 and 600 (peak assignments were confirmed by using H,H-COSY, HSQC and HMQC spectra, ^{1}H and ^{13}C NMR spectra were referenced to the residual solvent signals ($CDCl_3$: δ^H = 7.26 ppm, δ^C = 77.2 ppm; DMSO-d_6: δ^H = 2.50 ppm, δ^C = 39.52 ppm, MeOD-d_4: δ^H = 3.31 ppm, δ^C = 49.00 ppm); MALDI-TOF-MS, Bruker Ultraflex TOF/TOF; melting points, Müller SPM-X 300; precision balance, Kern ABT 100-5M; preparative chromatography, silica gel 60 A (0.06-0.20 mm) Acros Organics; preparative HPLC, Dionex UltiMate 3000; column, ThermoFisher BetaBasic-18, 250 × 21.2 mm, 5 µm particle size; temperature, 25 °C; flow, 10 mL/min; eluent, water/acetonitrile with the following gradient: 0-5 min, 10% acetonitrile; 5-31 min, linear increase of organic to 90%, 31-40 min, 90% acetonitrile, 40-41 min, linear decrease to 10% organic; 41-42 min, 10% acetonitrile; size exclusion chromatography, Sephadex® LH-20 and G-10 GE Healthcare; reversed-phase chromatography, RP-8 POLYGOPREP® 60-50 C8 (40−63 µm) Macherey Nagel; TEM, JEOL JEM-2100 LaB6 Transmission Electron Microscope equipped with a Gatan Orius SC1000 CCD camera; UV-vis, Varian Cary 100.

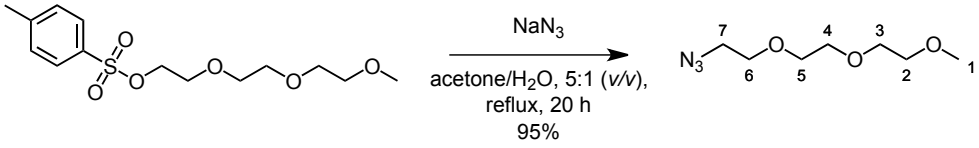
The following abbreviations are used: Ala, β-alanine; Apa, 6-aminopicolinic acid; Pro, L-proline; Apro (4*S*,2*S*)-4-aminoproline; AuNP, gold nanoparticle; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate; EDC, *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide hydrochloride; DIPEA, ethyldiisopropylamine; sulfo-NHS, *N*-hydroxysulfosuccinimide sodium salt.

2-(2-(2-Methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate[1]



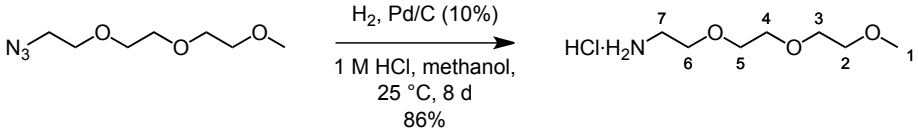
Triethylene glycol monomethylether (8.21 g, 50.0 mmol) was dissolved in THF (15 mL) and the resulting solution cooled to 0 °C. A solution of NaOH (3.86 g, 96.5 mmol) in water (16 mL) was added dropwise under stirring followed by a solution of *p*-tosyl chloride (12.4 g, 65.0 mmol) in THF (18 mL). The reaction mixture was stirred for 60 min at 0 °C and further 80 min at 25 °C. Afterwards, the solution was diluted with diethyl ether (125 mL) and 1 M NaOH (40 mL) and the aqueous phase was separated. The organic phase was washed with water (2 × 50 mL) dried over $MgSO_4$ and concentrated in vacuo. Yield: 15.0 g (47.1 mmol, 94%) colourless oil; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$): δ = 7.78 (m, 2H, H^8), 7.33 (m, 2H, H^9), 4.15 (t, 2H, ^3J = 4.0 Hz, H^7), 3.67 (t, 2H, ^3J = 4.0 Hz, H^6), 3.51-3.61 (m, 8H, H^2, H^3, H^4, H^5), 3.36 (s, 3H, H^1), 2.44 (s, 3H, H^{10}) ppm.

1-Azido-2-(2-(2-methoxyethoxy)ethoxy)ethane[1]



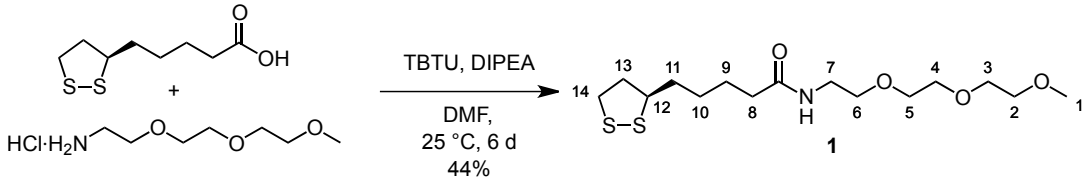
2-(2-(2-Methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (7.96 g, 25.0 mmol) and sodium azide (2.44 g, 37.5 mmol) were dissolved in acetone (30 mL) and water (6 mL). The mixture was heated to reflux for 20 h. Afterwards, the acetone was removed *in vacuo* and the remaining solution was diluted with water (20 mL). The reaction mixture was extracted with diethyl ether (4 × 20 mL), the combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. Yield: 4.51 g (23.8 mmol, 95%) colourless oil; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$): δ = 3.64-3.68 (m, 8H, H^2, H^3, H^4, H^5), 3.53-3.57 (m, 2H, H^6), 3.37-3.39 (m, 5H, H^1, H^7) ppm.

2-(2-(2-Methoxyethoxy)ethoxy)ethanamine Hydrochloride



1-Azido-2-(2-(2-methoxyethoxy)ethoxy)ethane (5.67 g, 30.0 mmol) was dissolved in methanol (100 mL). A suspension of Pd/C (570 mg, 10 weight%) in water (5 mL) and 1 M aqueous HCl (33.0 mL, 33.0 mmol) were added. The reaction mixture was stirred for 8 d at 25 °C under an atmosphere of hydrogen. Afterwards, the catalyst was removed by filtering the reaction mixture through celite, and the filtrate was evaporated to dryness. Yield: 5.17 g (25.9 mmol, 86%) pale yellow oil; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$): δ = 6.31 (s, 2H, NH), 3.77 (t, 2H, ^{3}J = 4.0 Hz, H^2), 3.61-3.68 (m, 6H, H^3, H^4, H^5), 3.35-3.39 (m, 5H, H^1, H^6), 3.05-3.15 (m, 2H, H^7) ppm.

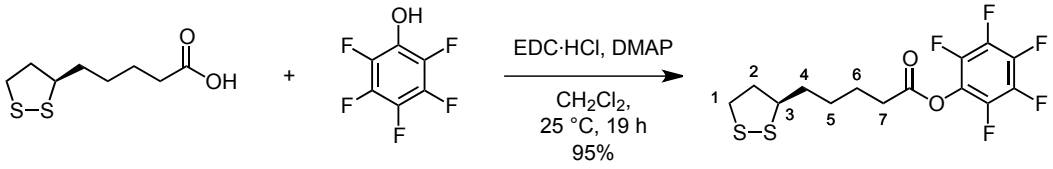
Ligand 1



(*R*)-Lipoic acid (4.12 g, 20.0 mmol) and 1-amino-2-(2-(2-methoxyethoxy)ethoxy)ethane hydrochloride (5.17 g, 25.9 mmol) were dissolved in DMF (50 mL). TBTU (8.31 g, 25.9 mmol) and DIPEA (13.2 mL, 77.7 mmol) were added and the reaction mixture was stirred for 6 d at 25 °C. Afterwards, the solvent was removed *in vacuo* and the residue was purified chromatographically over silica (EtOAc/petroleum ether, 2:1 (*v/v*) → acetone). The thus obtained crude product was further purified by preparative HPLC. Pure fractions were collected and evaporated to dryness. Yield: 3.11 g (8.84 mmol, 44%) pale yellow oil; specific rotation, $[\alpha]_D^{25}$ = 57.2 (*c* = 1, methanol), ^{1}H NMR (400 MHz, 25 °C, MeOD-d_4): δ = 3.52-3.65 (m, 11H, H^2, H^3, H^4, H^5, H^6, H^{12}), 3.34-3.36 (m, 5H, H^1, H^7), 3.07-3.21 (m, 2H, H^{14}), 2.43-2.50 (m, 1H, H^{13}), 2.21 (t, 2H, ^{3}J = 8.0 Hz, H^8), 1.85-1.93 (m, 1H, H^{13}), 1.60-1.72 (m, 4H, H^9, H^{11}), 1.39-1.53 (m, 2H, H^{10}) ppm; ^{13}C NMR (101 MHz, 25 °C, MeOD-d_4): δ = 176.1 (CO), 72.9 (C^2), 71.6 + 71.4 + 71.2 + 70.6 (C^3 + C^4 + C^5 + C^6), 59.1 (C^1), 57.6 (C^7), 41.3 (C^{12}), 40.4 (C^{14}), 39.4 (C^{13}), 36.8 (C^8), 35.8 (C^{11}), 29.9 (C^9), 26.7 (C^{10}) ppm; MS (MALDI-TOF) *m/z* (%): 352.3 $[M+H]^+$ (100%), 374.3 $[M+Na]^+$ (7%); IR (ATR): 311 (w), 2923 (m), 2863 (m), 1646 (s),

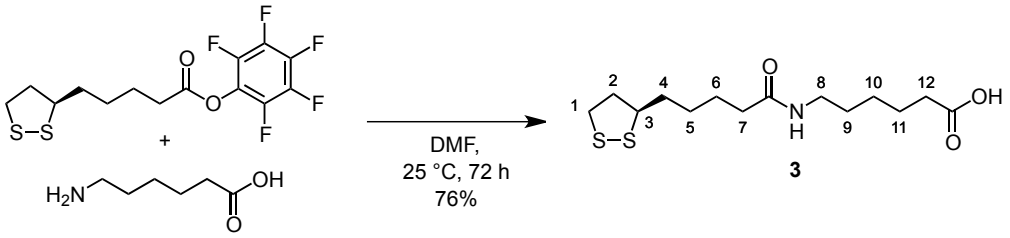
1095 (s), 850 (w) cm^{-1}; CHN calculated for $C_{15}H_{29}NO_4S_2 \cdot 0.75H_2O$: C, 49.35%, H, 8.42%, N, 3.84%, S, 17.57%, found: C, 49.24%, H, 8.09%, N, 3.68%, S, 17.39 %.

(*R*)-Perfluorophenyl 5-(1,2-dithiolan-3-yl)pentanoate



(*R*)-Lipoic acid (2.06 g, 10.0 mmol) and pentafluorophenol (2.02 g, 11.0 mmol) were dissolved in dichloromethane (40 mL). A solution of EDC·HCl (2.11 g, 11.0 mmol) and DMAP (122 mg, 1.00 mmol) in dichloromethane (20 mL) was added dropwise under stirring. The reaction mixture was stirred for 19 h at 25 °C. The solution was washed with water (3 × 20 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness. Yield: 3.55 g (9.53 mmol, 95%) yellow oil; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$): δ = 3.56-3.62 (m, 1H, H^3), 3.10-3.23 (m, 2H, H^1), 2.69 (t, 2H, ^3J = 7.4 Hz, H^7), 2.44-2.51 (m, 1H, H^2), 1.88-1.97 (m, 1H, H^2), 1.69-1.86 (m, 4H, H^4, H^6), 1.51-1.65 (m, 2H, H^5) ppm.

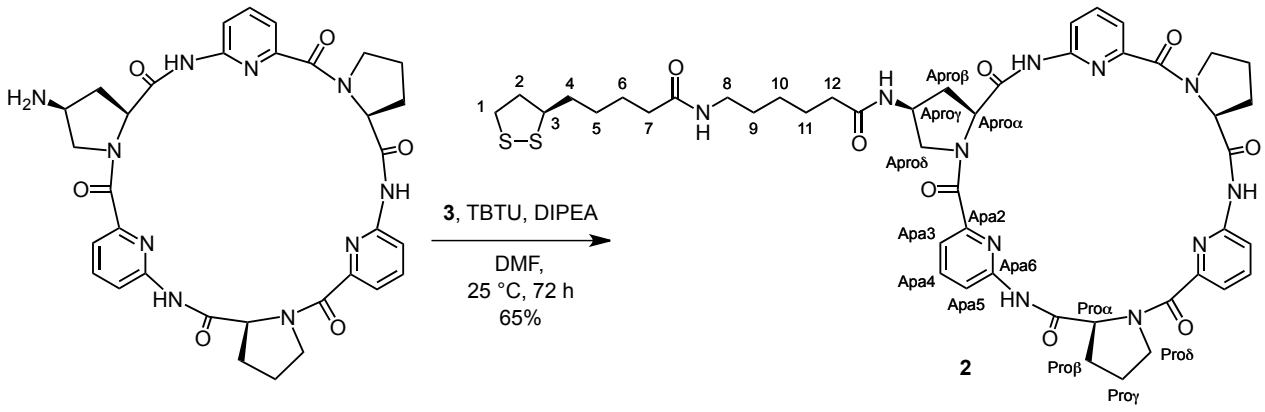
Ligand 3



6-Aminohexanoic acid (1.19 g, 9.07 mmol) and (*R*)-perfluorophenyl 5-(1,2-dithiolan-3-yl)pentanoate (3.71 g, 9.98 mmol) were dissolved in DMF (40 mL). The mixture was stirred for 72 h at 25 °C. The solvent was removed *in vacuo*, the residue was dissolved in dichloromethane (50 mL) and washed with 1 M aqueous HCl (2 × 30 mL) and water (30 mL). The organic phase was dried over $MgSO_4$, the solvent was removed *in vacuo* and the residue was triturated with diethyl ether. The resulting solid was filtered, washed with diethyl ether, and dried. Yield: 2.21 g (6.92 mmol, 76%) pale yellow solid; mp: 79 °C; specific rotation: $[\alpha]_D^{24}$ = 66.0 (*c* = 0.5, methanol); ^{1}H NMR (400 MHz, 25 °C, DMSO-d_6): δ = 7.76 (t, 1H, ^3J = 5.3 Hz, NH), 3.56-3.61 (m, 1H, H^3), 3.09-3.19 (m, 2H, H^1), 2.97-

3.02 (m, 2H, H^8), 2.36-2.44 (m, 1H, H^2), 2.18 (t, 2H, ^3J = 7.4 Hz, H^{12}), 2.03 (t, 2H, ^3J = 7.3 Hz, H^7), 1.83-1.89 (m, 1H, H^2), 1.21-1.68 (m, 12H, H^4, H^5, H^6, H^9, H^{10}, H^{11}) ppm; ^{13}C NMR (101 MHz, 25 °C, DMSO-d_6): δ = 174.6 (COOH), 171.9 (CONH), 56.2 (C^3), 38.3 (C^1), 38.2 (C^2), 35.3 (C^{12}), 34.2 (C^7), 33.7 (C^4), 29.0 (C^9), 28.4 (C^5), 26.0 (C^{10}), 25.2 (C^6), 24.3 (C^{11}) ppm; MS (ESI-TOF, negative mode) *m/z* (%): 318.1 $[M–H]^–$ (100%), 354.0 $[M+Cl]^–$ (14%), 637.1 $[M_2–H]^–$ (31%); IR (ATR): 3299 (w), 2919 (w), 2869 (w), 2852 (w), 1694 (m), 1632 (s), 1534 (s), 1269 (w), 941 (w) $cm^{–1}$; CHN calculated for $C_{14}H_{25}NO_3S_2$: C, 52.63%, H, 7.89%, N, 4.38%, S, 20.07%, found: C, 52.46%, H, 7.87%, N, 4.66%, S, 19.69 %.

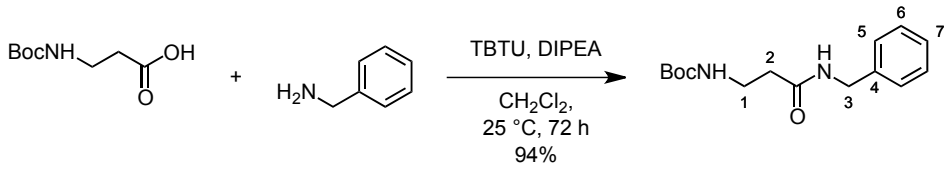
Ligand 2



The synthesis started from the Cbz-protected version of a known cyclopeptide containing one (4*S*,2*S*)-4-aminoproline residue.[2] The unprotected cyclopeptide was obtained by dissolving the protected form (196 mg, 245 µmol) in dichloromethane/methanol, 1:1 (*v/v*) (50 mL). 10% Pd/C (25.0 mg) and $Pd(OH)_2$/C (25.0 mg) were added followed by 1 M aqueous HCl (270 µL, 270 µmol). The reaction mixture was stirred for 4 d at 25 °C under an atmosphere of hydrogen, the catalysts were removed by filtering the mixture through celite, and the filtrate was evaporated to dryness. The thus obtained colourless solid was used in the next step without further purification. This monoamine and **3** (118 mg, 368 µmol) were dissolved in DMF (15 mL). TBTU (118 mg, 368 µmol) and DIPEA (125 µL, 735 µmol) were added and the reaction mixture was stirred for 72 h at 25 °C. Afterwards, the solvent was removed *in vacuo*. The residue was purified by RP-8 column chromatography. Initially, methanol/water, 1:5 (*v/v*) was used as the eluent. The solvent composition was gradually changed to methanol/water, 1:2 (*v/v*) and finally to methanol/water, 2:1 (*v/v*) with which the product eluted. The

fractions containing pure **2** were collected and evaporated to dryness. The residue was dissolved in methanol (5 mL) and the product was precipitated by adding diethyl ether. The solid was centrifuged and dried. Yield: 154 mg (159 µmol, 65%) pale yellow solid; mp: 202 °C; specific rotation: $[\alpha]_D^{26}$ = −241.7 (*c* = 0.25, methanol); ^{1}H NMR (600 MHz, 25 °C, MeOD-*d*$_4$): δ = 7.75-7.82 (m, 3H, ApaH^4), 7.54-7.59 (m, 3H, ApaH^3), 7.32-7.43 (m, 3H, ApaH^5), 5.79-5.83 (m, 1H, AproH$^\alpha$), 5.61-5.69 (m, 2H, ProH$^\alpha$), 4.47-4.49 (m, 1H, AproH$^\gamma$), 4.02-4.05 (m, 1H, AproH$^\delta$), 3.80-3.84 (m, 2H, ProH$^\delta$), 3.74-3.79 (m, 2H, ProH$^\delta$), 3.69-3.72 (m, 1H, AproH$^\delta$), 3.55-3.59 (m, 1H, H^3), 3.14-3.18 (m, 1H, H^1), 3.08-3.11 (m, 1H, H^1), 3.04-3.07 (m, 2H, H^8), 2.89-2.93 (m, 1H, AproH$^\beta$), 2.66-2.69 (m, 2H, ProH$^\beta$), 2.42-2.48 (m, 1H, H^2), 2.19 (t, 2H, ^3J = 6.0 Hz, H^{12}), 2.06-2.17 (m, 5H, AproH$^\beta$ + ProH$^\beta$ + H^7), 1.99-2.04 (m, 2H, ProH$^\gamma$), 1.91-1.97 (m, 2H, ProH$^\gamma$), 1.86-1.90 (m, 1H, H^2), 1.69-1.74 (m, 1H, H^4), 1.57-1.66 (m, 3H, H^4, H^5), 1.36-1.48 (m, 4H, H^6, H^{11}), 1.30-1.33 (m, 2H, H^9), 1.16-1.20 (m, 2H, H^{10}) ppm; ^{13}C NMR (151 MHz, 25 °C, MeOD-*d*$_4$): δ = 176.7 (C^{12}CO), 176.3 (C^{7}CO), 173.0 (ProCO), 168.8 (ApaCO), 153.2 + 153.1 + 152.5 (ApaC^6), 150.1 + 150.0 (ApaC^2), 140.6 (ApaC^4), 121.8 (ApaC^3), 117.6 + 117.5 (ApaC^5), 63.5 + 63.4 (ProC$^\alpha$), 62.2 (AproC$^\alpha$), 57.7 (C^3), 53.5 (AproC$^\delta$), 50.0 + 49.9 (ProC$^\delta$), 48.0 (AproC$^\gamma$), 41.3 (C^2), 40.1 (C^8), 39.3 (C^1), 38.8 (AproC$^\beta$), 36.9 (C^{12}), 36.8 (C^7), 35.5 (C^4), 33.9 (ProC$^\beta$), 29.8 (C^{11}), 29.7 (C^9), 27.2 (C^{10}), 26.7 (C^6), 26.4 (C^5), 23.7 + 23.6 (ProC$^\gamma$) ppm; IR (ATR): 3270 (w), 2933 (w), 2869 (w), 1704 (w), 1625 (m), 1572 (s), 1532 (s), 1464 (s), 1418 (s), 1398 (s), 1298 (m), 1155 (m), 759 (m) cm^{-1}; MS (ESI-TOF, negative mode) *m/z* (%): 966.4 [M–H]$^-$ (27%), 1002.4 [M+Cl]$^-$ (100%); CHN calculated for $C_{47}H_{57}N_{11}O_8S_2 \cdot 2H_2O$: C, 56.22%, H, 6.12%, N, 15.34%, S, 6.39%, found: C, 56.14%, H, 6.08%, N, 15.29%, S, 6.61%.

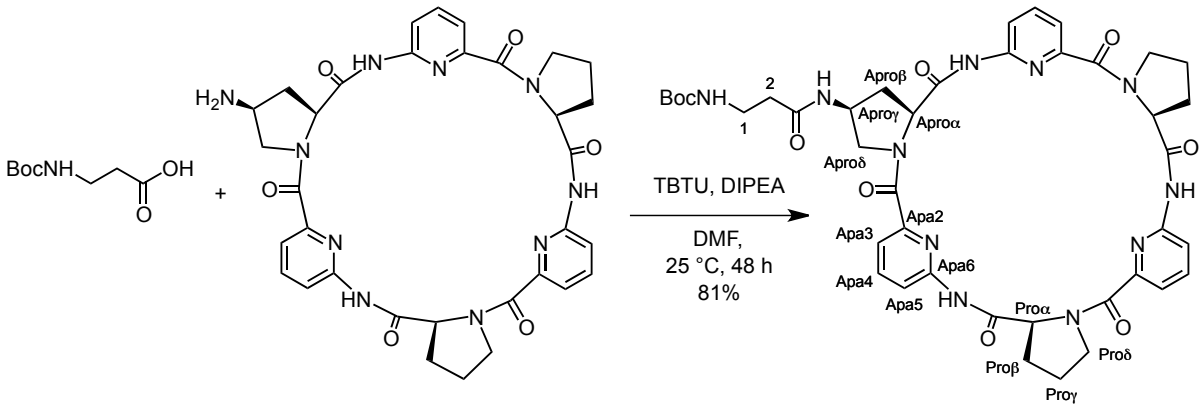
Boc-Protected Precursor of 4



Boc-β-Alanine (568 mg, 3.00 mmol) and benzylamine (328 µL, 3.00 mmol) were dissolved in dichloromethane (10 mL). TBTU (1.16 g, 3.60 mmol) and DIPEA (1.53 mL, 9.00 mmol) were added and the reaction mixture was stirred for 72 h at 25 °C. The solvent was removed *in vacuo* and the residue was purified by column chromatography (SiO_2, ethyl acetate/hexane, 4:1 (*v/v*)). Yield: 787

mg (2.83 mmol, 94%) colourless solid; ^{1}H NMR (600 MHz, 25 °C, $CDCl_3$): δ = 7.32-7.24 (m, 5H, PhH), 6.27 (s, b, 1H, NH), 5.22 (s, b, 1H, NH), 4.41 (d, 2H, ^{3}J = 5.5 Hz, H^3), 3.39 (t, 2H, ^{3}J = 6.0 Hz, H^1), 2.42 (t, 2H, ^{3}J = 5.9 Hz, H^2), 1.40 (s, 9H, BocH) ppm; ^{13}C NMR (151 MHz, 25 °C, $CDCl_3$): δ = 171.5 (BnNHCO), 156.3 (BocCO), 138.2 (C^4), 128.8 (C^7), 127.9 (C^6), 127.7 (C^5), 79.5 (BocC), 43.7 (C^3), 36.9 (C^1), 36.4 (C^2), 28.5 ($BocCH_3$) ppm; MS (ESI-TOF, positive mode) *m/z* (%): 301.1 $[M+Na]^+$ (16%), 579.3 $[M_2+Na]^+$ (100%); CHN calculated for $C_{15}H_{22}N_2O_3$: C, 64.73%, H, 7.97%, N, 10.06%, found: C, 64.68%, H, 7.98%, N, 10.06%.

Boc-Protected Precursor of 5



The synthesis started from the Cbz-protected version of a known cyclopeptide containing one (4*S*,2*S*)-4-aminoproline residue.[2] The unprotected cyclopeptide was obtained by dissolving the protected form (81 mg, 101 µmol) in dichloromethane/methanol, 1:1 (*v/v*) (50 mL). 10% Pd/C (15.0 mg) and $Pd(OH)_2$/C (15.0 mg) were added followed by 1 M aqueous HCl (110 µL, 110 µmol). The reaction mixture was stirred for 4 d at 25 °C under an atmosphere of hydrogen, the catalysts were removed by filtering the mixture through celite, and the filtrate was evaporated to dryness. The thus obtained colourless solid was used in the next step without further purification. It was dissolved together with Boc-β-alanine (21.0 mg, 101 µmol) in DMF (10 mL). TBTU (38.9 mg, 121 µmol) and DIPEA (51.5 µL, 303 µmol) were added and the reaction mixture was stirred for 48 h at 25 °C. Afterwards, the solvent was removed *in vacuo*. The residue was purified by column chromatography (SiO_2, dichloromethane/acetone, 10:1 (*v/v*) → dichloromethane/methanol, 10:1 (*v/v*) → 8:1 (*v/v*) → 5:1 (*v/v*)). The fractions containing pure product were collected and evaporated to dryness. The residue was dissolved in methanol (5 mL), the product was precipitated by adding diethyl ether, separated by

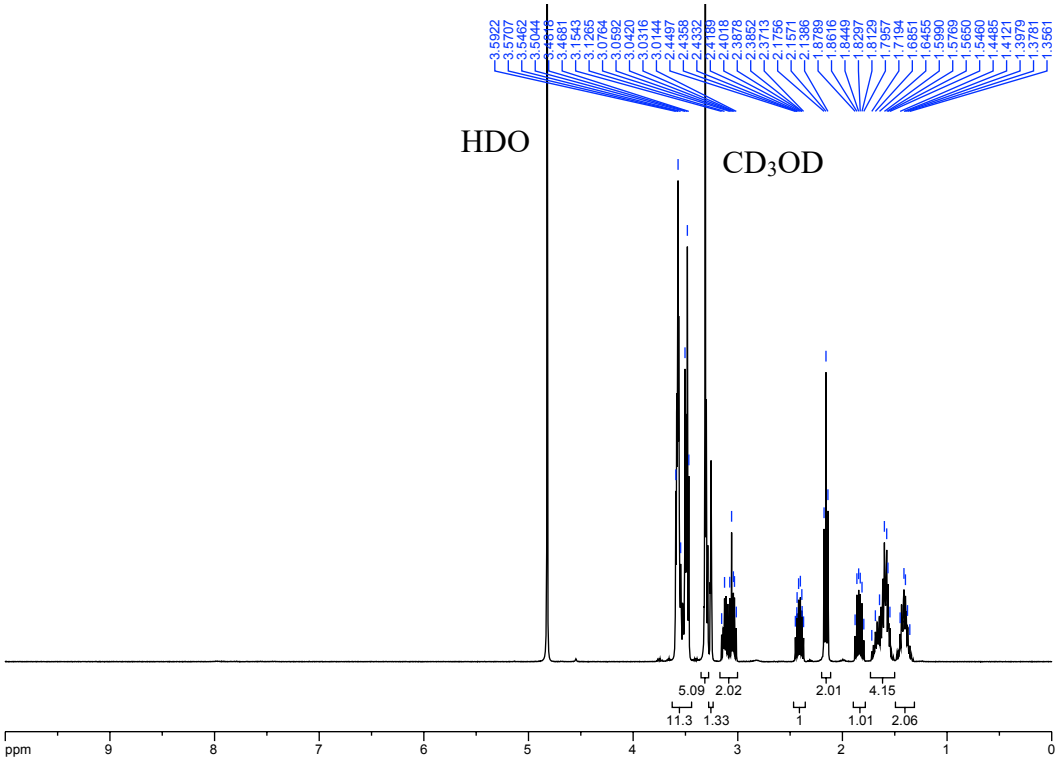
centrifugation and dried. Yield: 69 mg (82 µmol, 81%) colourless solid; mp: 241 °C; specific rotation, $[\alpha]_D^{25}$ = −359.3 (*c* = 0.5, methanol); ^{1}H NMR (400 MHz, 25 °C, MeOD-d_4): δ = 7.72-7.78 (m, 3H, ApaH^4), 7.57-7.59 (m, 3H, ApaH^3), 7.41 (t, 3H, ^3J = 8.0 Hz, ApaH^5), 5.90-6.00 (m, 3H, AproH$^\alpha$ + ProH$^\alpha$), 4.39-4.42 (m, 1H, AproH$^\gamma$), 4.04-4.08 (m, 1H, AproH$^\delta$), 3.71-3.84 (m, 4H, ProH$^\delta$), 3.59-3.64 (m, 1H, AproH$^\delta$), 3.19 (t, 2H, ^3J = 6.0 Hz, H^1), 2.95-3.02 (m, 1H, AproH$^\beta$), 2.65-2.69 (m, 2H, ProH$^\beta$), 2.22-2.27 (m, 2H, H^2), 2.00-2.11 (m, 3H, ProH$^\beta$ + AproH$^\beta$), 1.87-1.96 (m, 4H, ProH$^\gamma$), 1.37 (s, 9H, BocH) ppm; ^{13}C NMR (151 MHz, 25 °C, MeOD-d_4): δ = 174.1 (AlaCO), 172.8 + 172.7 (ProCO), 172.2 + 168.7 + 168.5 (ApaCO), 158.2 (BocCO), 153.2 + 153.1 + 152.8 (ApaC^6), 150.4 + 150.3 + 150.2 (ApaC^2), 140.4 + 140.4 + 140.3 (ApaC^4), 121.8 + 121.7 (ApaC^3), 117.6 + 117.4 + 117.4 (ApaC^5), 80.1 (BocC), 63.7 + 63.6 (ProH$^\alpha$), 62.5 (AproH$^\alpha$), 53.6 (AproH$^\delta$), 49.6 (ProH$^\delta$), 48.1 (AproH$^\gamma$), 38.5 (AproH$^\beta$), 37.9 (C^2), 37.3 (C^1), 33.8 (ProH$^\beta$), 28.7 (BocCH$_3$), 23.7 + 23.6 (ProH$^\gamma$) ppm; IR (ATR): 3256 (w), 3030 (w), 2979 (w), 2880 (w), 1700 (m), 1627 (m), 1572 (s), 1527 (s), 1465 (m), 1424 (w), 1399 (m), 1296 (w), 1158 (w), 760 (w) cm^{-1}; MS (MALDI-TOF) *m/z* (%): 738.2 [M–Boc+H]$^+$ (9%), 860.3 [M+Na]$^+$ (100%), 876.4 [M+K]$^+$ (9%); CHN calculated for $C_{41}H_{47}N_{11}O_9 \cdot 2.5H_2O$: C, 55.77%, H, 5.94%, N, 17.45%, found: C, 55.88%, H, 5.74%, N, 17.35%.

Boc-Deprotection of the Precursors of 4 and 5

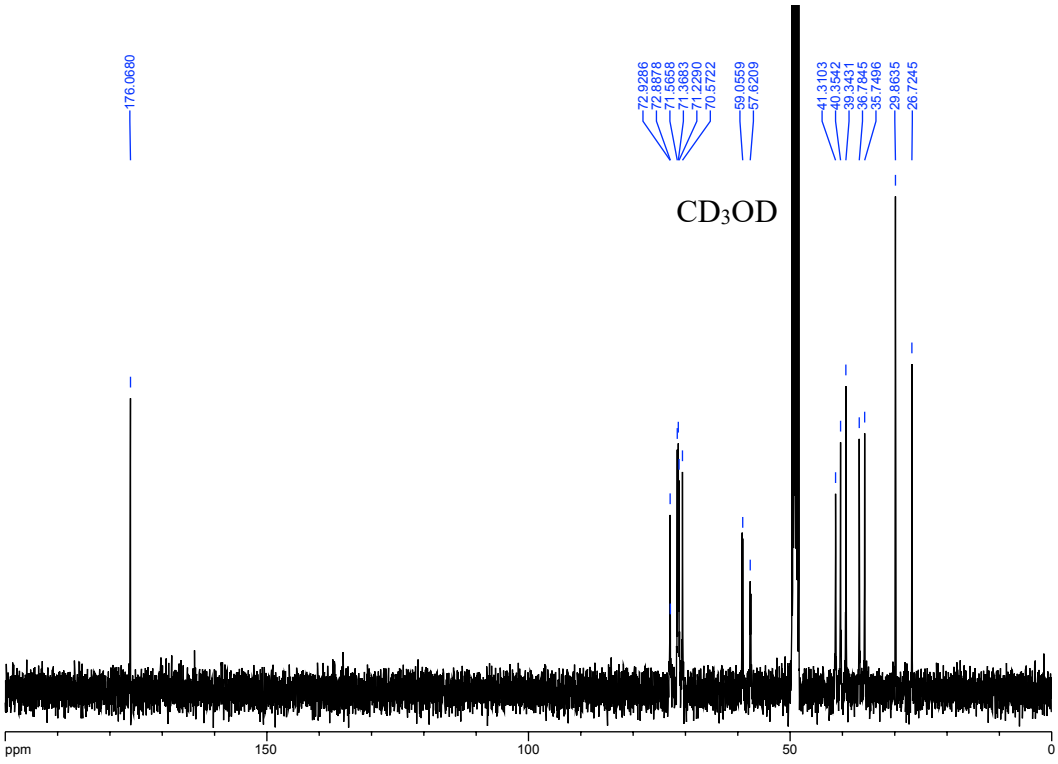
A solution of the Boc-protected amine in 1,4-dioxane (20 mL/mmol) is cooled to 0 °C and a 6 N solution of HCl in 1,4-dioxane (10 mL/mmol) is added dropwise. The reaction mixture is stirred for 20 min at 0 °C and for 2 h at 25 °C. Afterwards, the solvent is removed *in vacuo* and the remaining residue directly used for the nanoparticle functionalisation without further purification.

NMR and Mass Spectra of 1

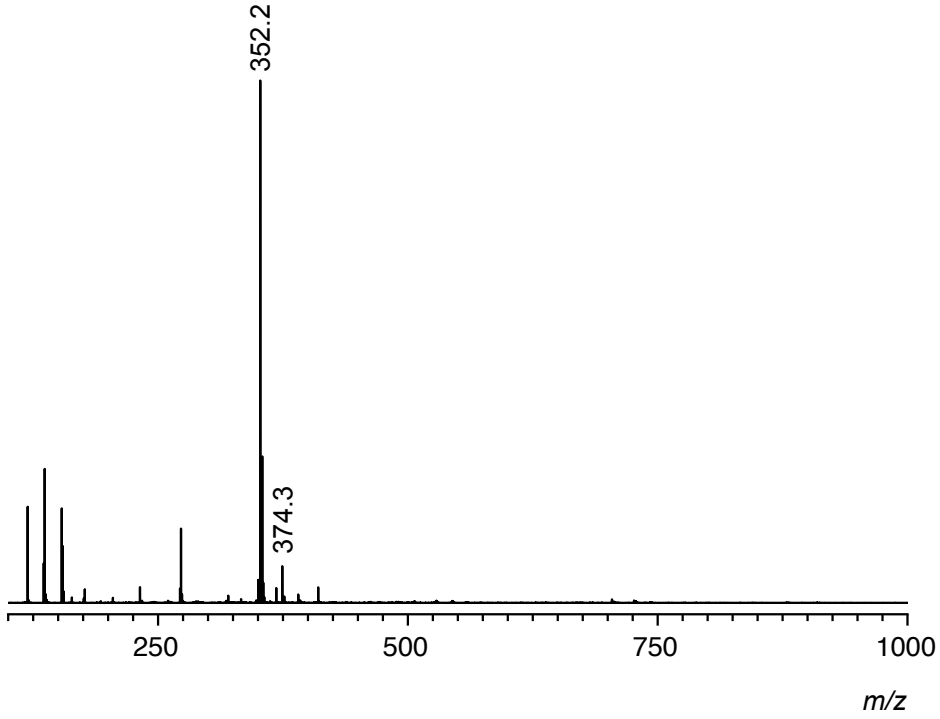
^{1}H NMR (400 MHz, methanol-d_4)



^{13}C NMR (101 MHz, methanol-d_4)



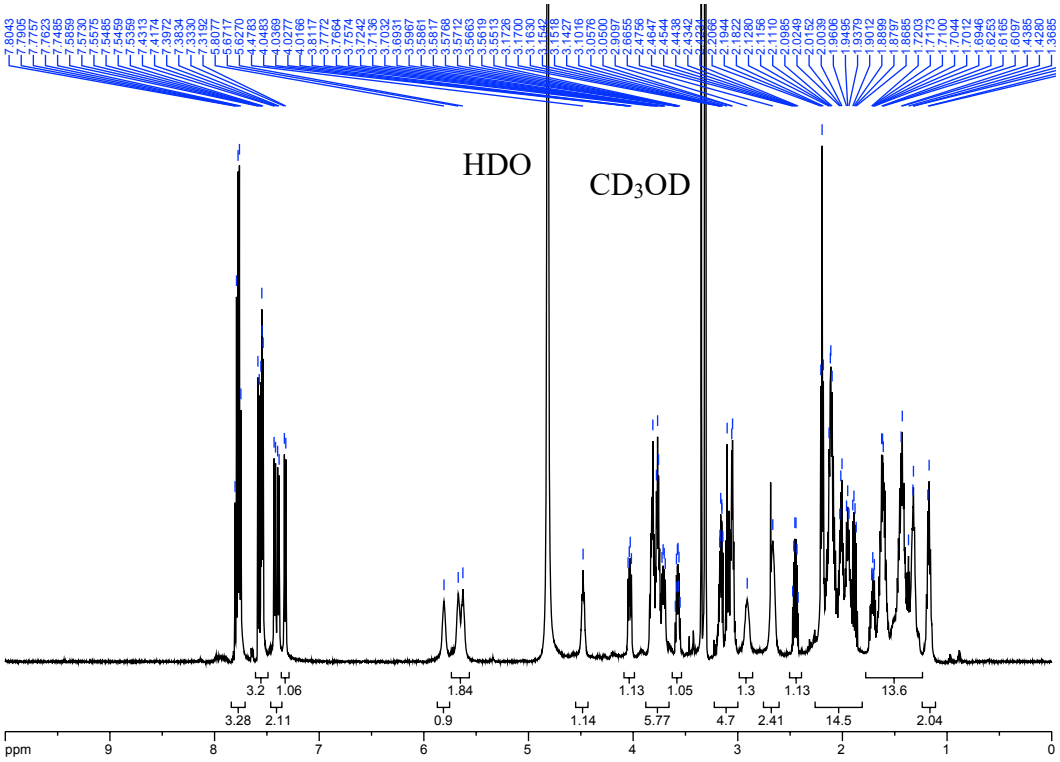
MALDI-TOF MS (positive mode)



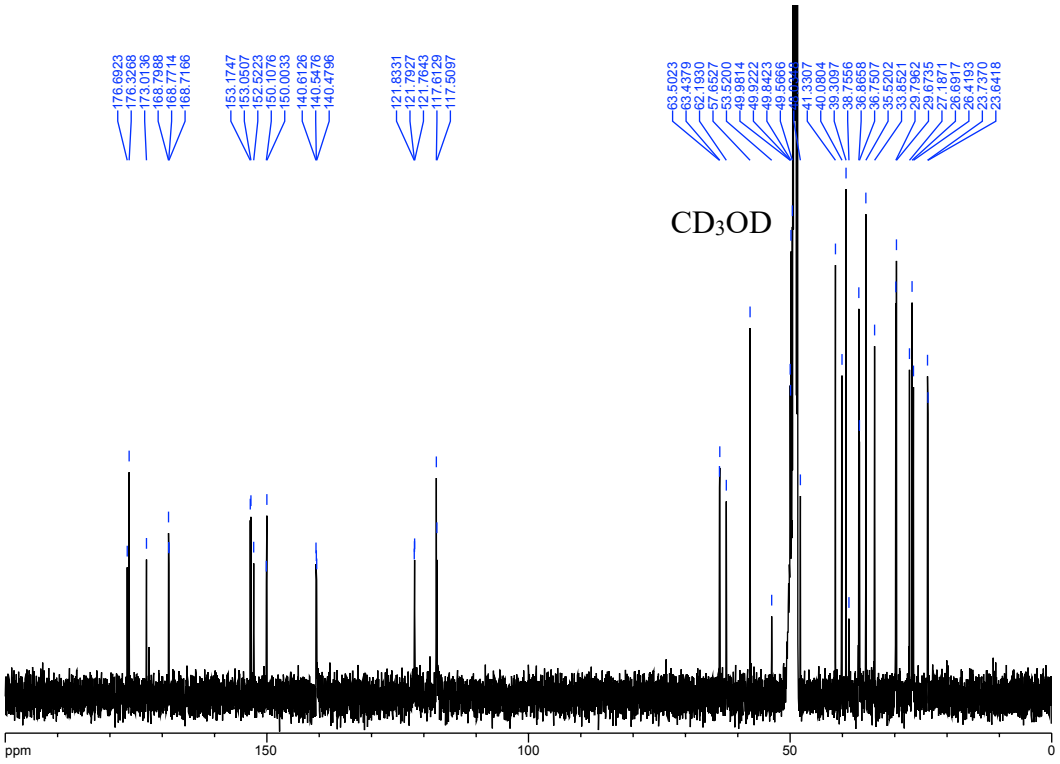
		m/z calcd.	*m/z exp.*
$[M+H]^+$	$C_{15}H_{29}NO_4S_2 + H^+$	352.2	352.3
$[M+Na]^+$	$C_{15}H_{29}NO_4S_2 + Na^+$	374.1	374.3

NMR and Mass Spectra of 2

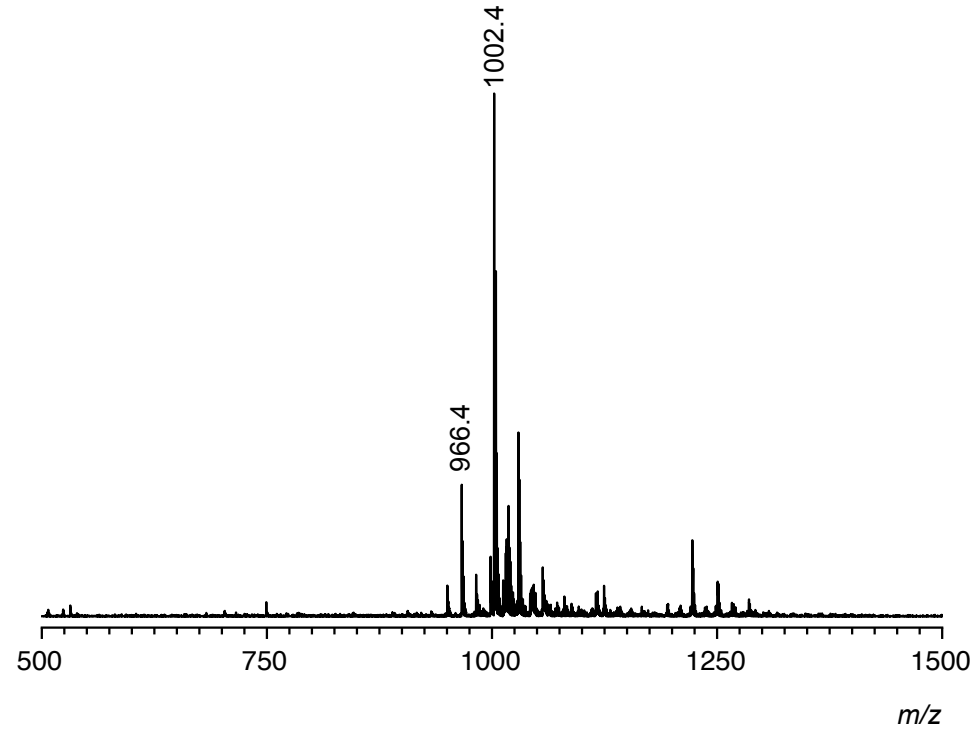
^{1}H NMR (400 MHz, methanol-d_4)



^{13}C NMR (101 MHz, methanol-d_4)



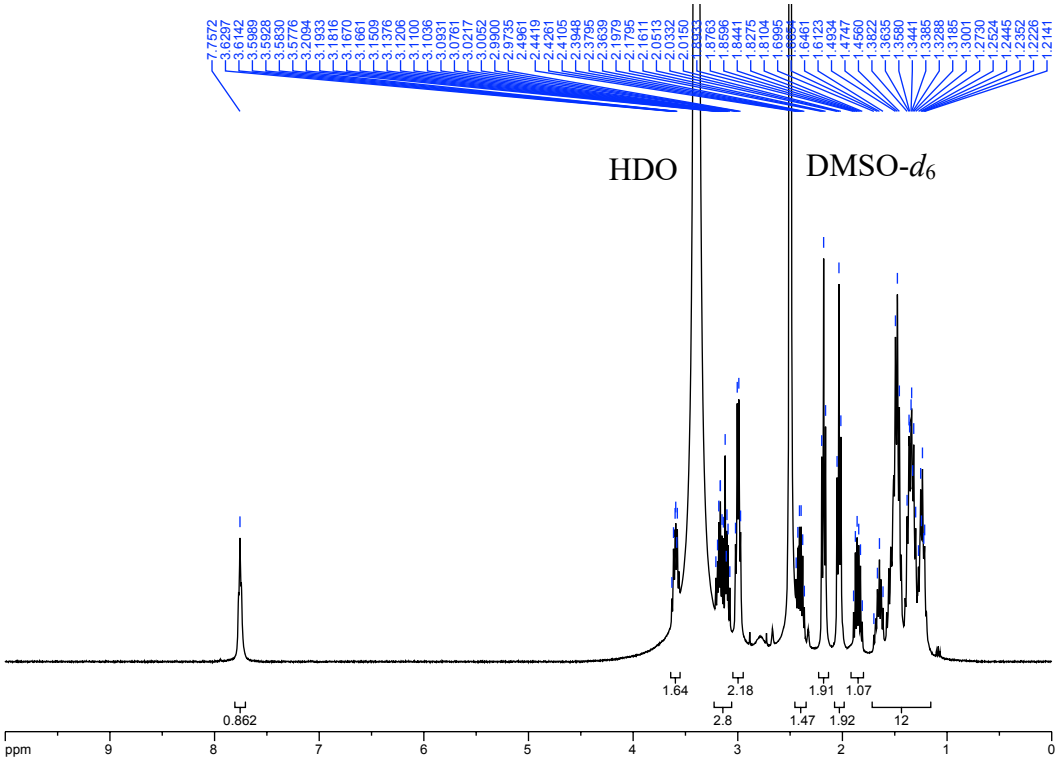
ESI-TOF MS (negative mode)



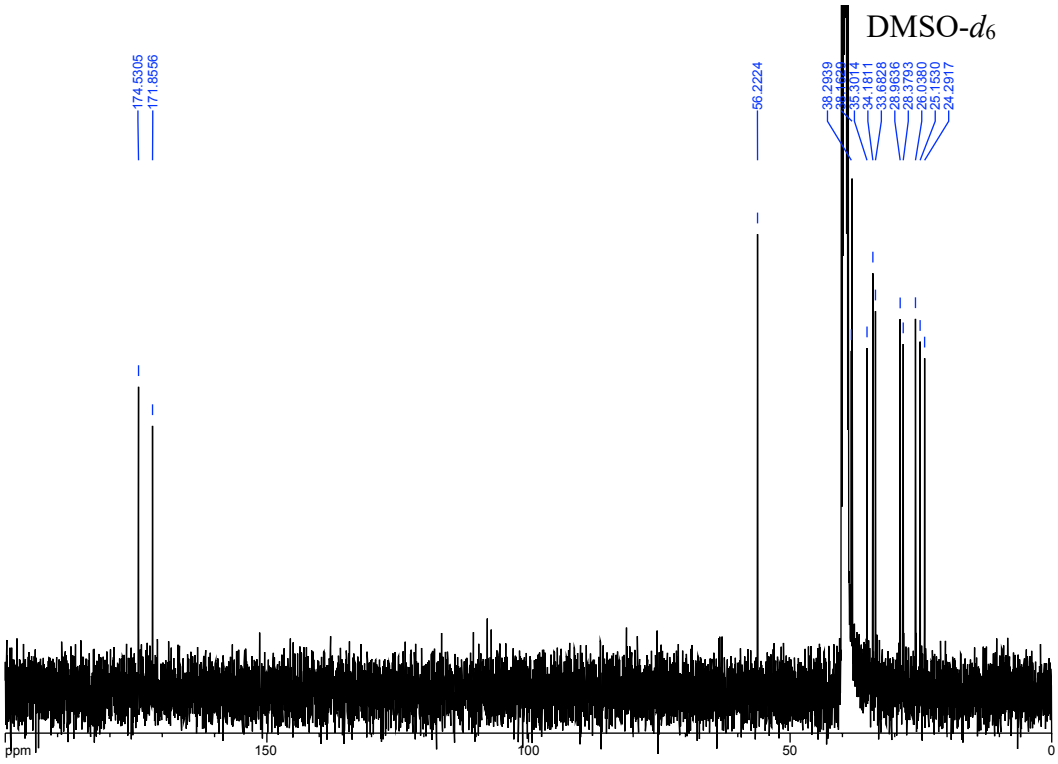
		m/z calcd.	*m/z exp.*
$[M–H]^-$	$C_{47}H_{57}N_{11}O_8S_2 – H^+$	966.4	966.4
$[M+Cl]^-$	$C_{47}H_{57}N_{11}O_8S_2 + Cl^-$	1002.4	1002.4

NMR and Mass Spectra of 3

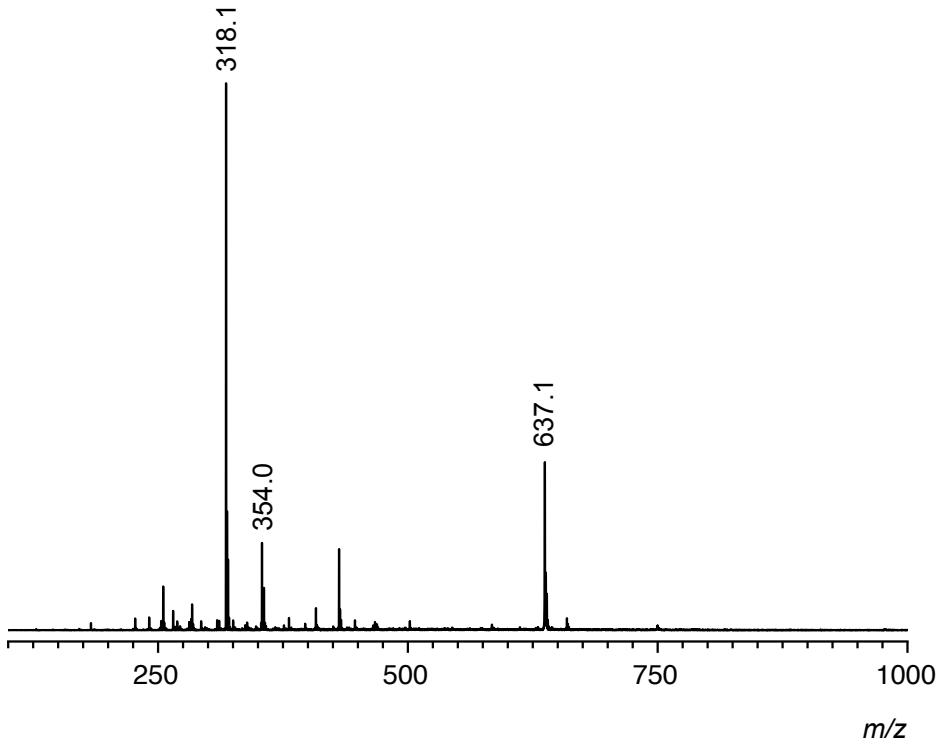
^{1}H NMR (400 MHz, DMSO-d_6)



^{13}C NMR (101 MHz, DMSO-d_6)



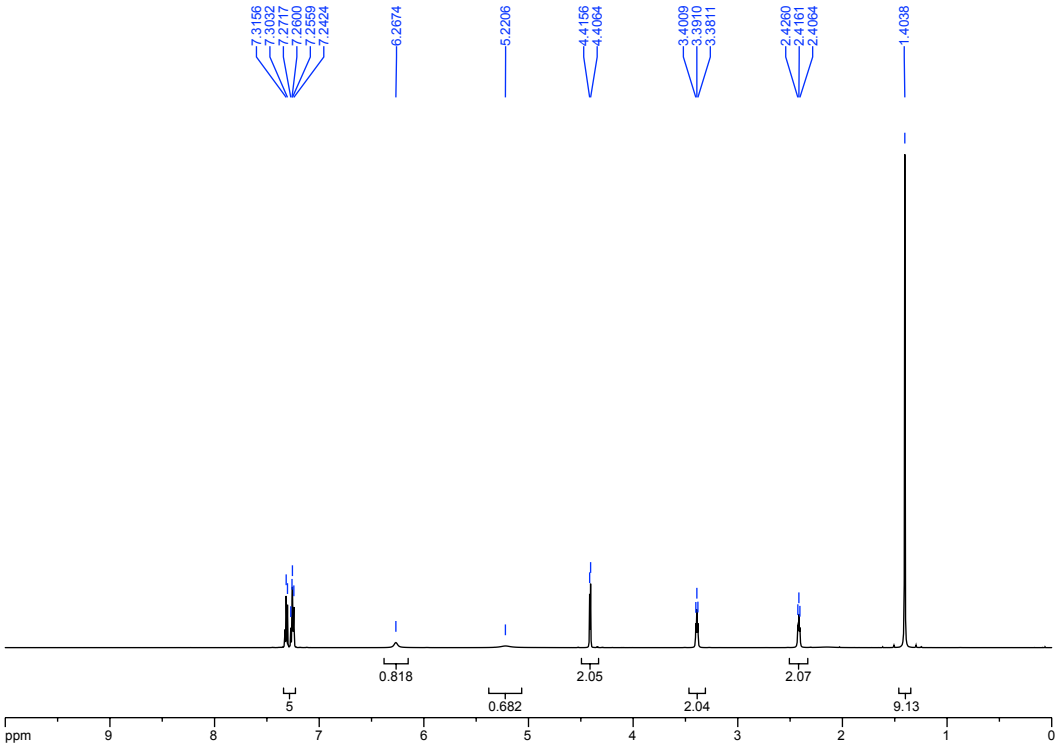
ESI-TOF MS (negative mode)



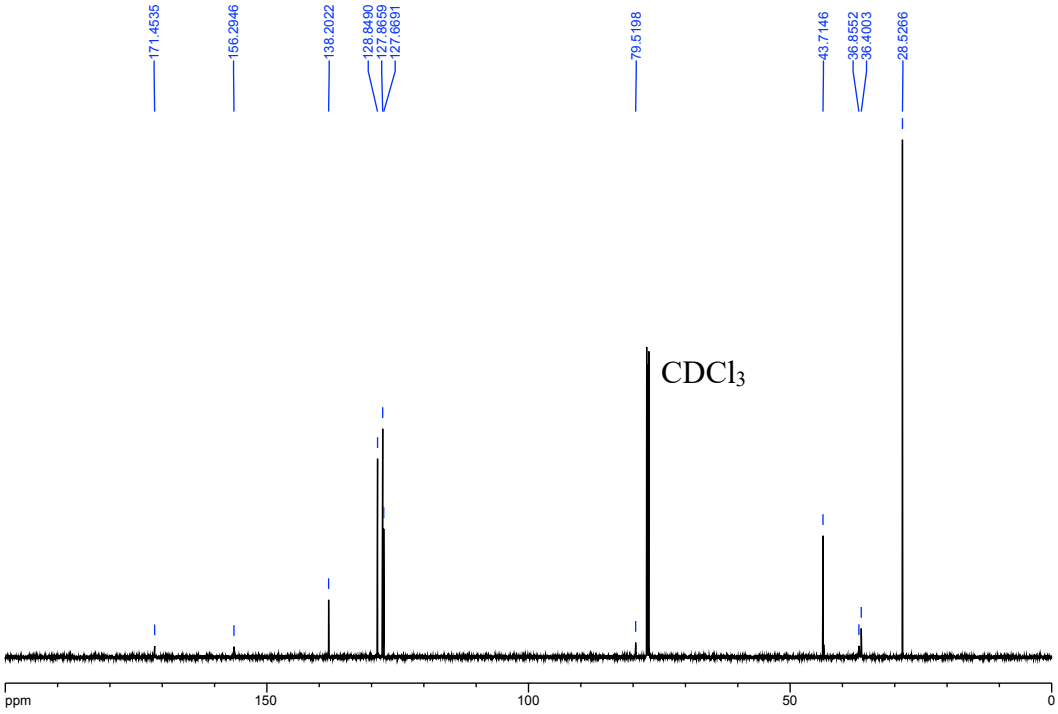
		m/z calcd.	*m/z exp.*
$[M–H]^–$	$C_{14}H_{25}NO_3S_2 – H^+$	318.1	318.1
$[M+Cl]^–$	$C_{14}H_{25}NO_3S_2 + Cl^–$	354.1	354.0
$[M_2+Cl]^–$	$(C_{14}H_{25}NO_3S_2)_2 – H^+$	637.2	637.1

NMR and Mass Spectra of the Boc-Protected Precursor of 4

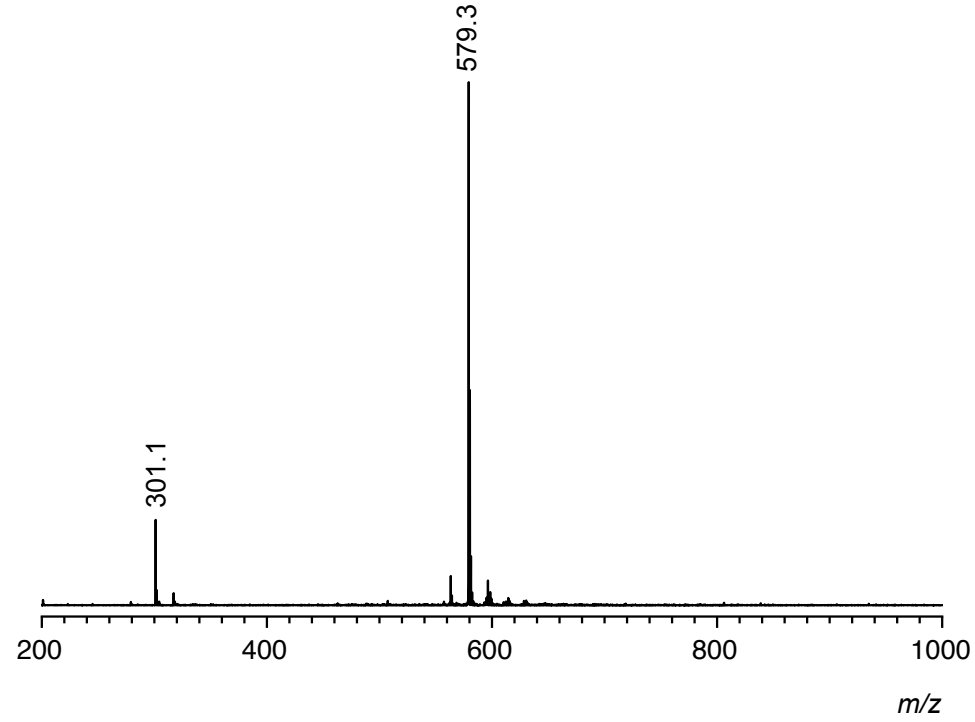
^{1}H NMR (600 MHz, $CDCl_3$)



^{13}C NMR (101 MHz, MeOD-d_4)



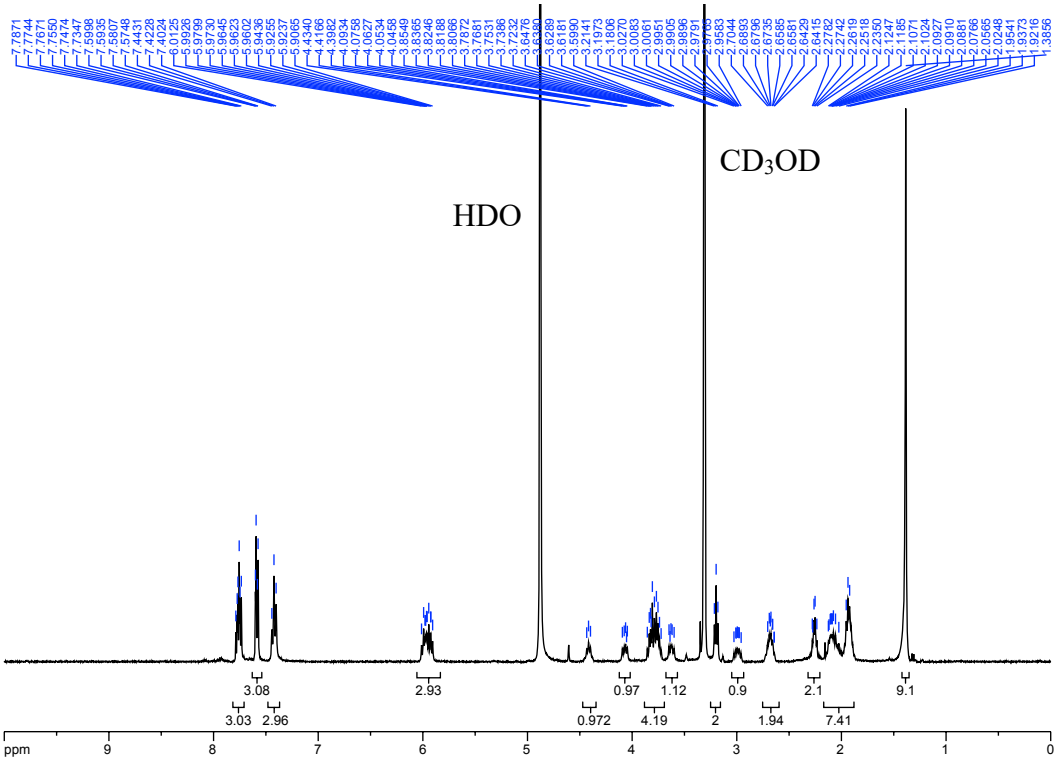
ESI-TOF MS (positive mode)



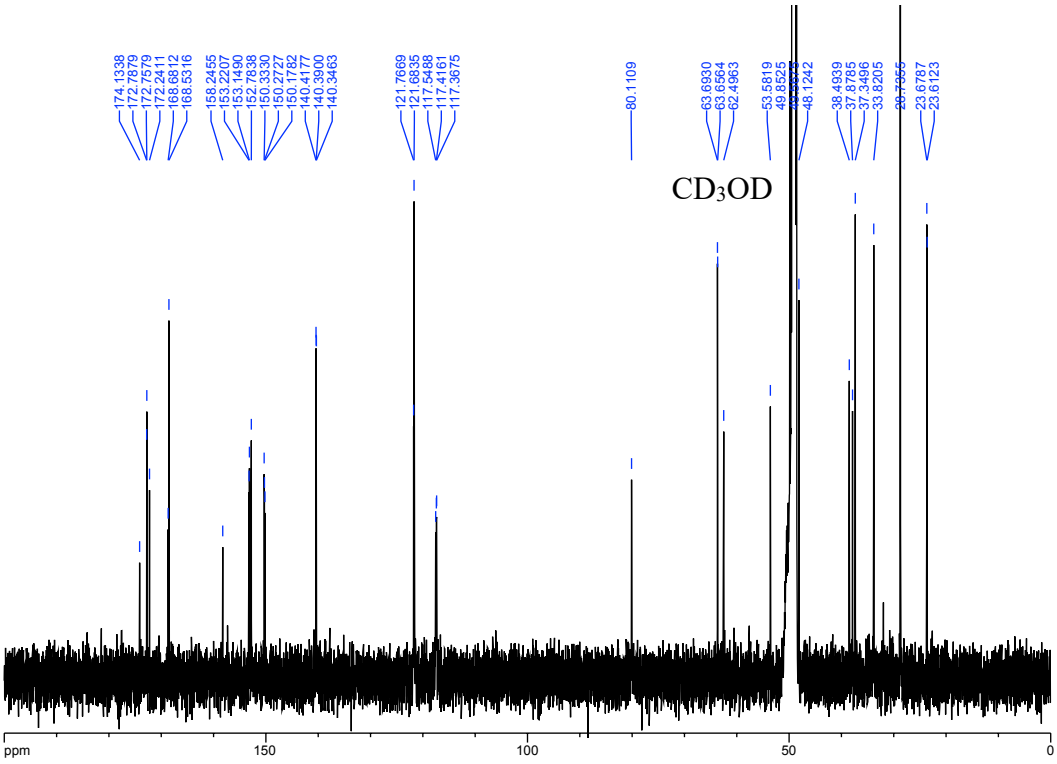
		m/z calcd.	*m/z exp.*
$[M+Na]^+$	$C_{15}H_{22}N_2O_3 + Na^+$	301.2	301.1
$[M_2+Na]^+$	$(C_{15}H_{22}N_2O_3)_2 + Na^+$	579.3	579.3

NMR and Mass Spectra of the Boc-Protected Precursor of 5

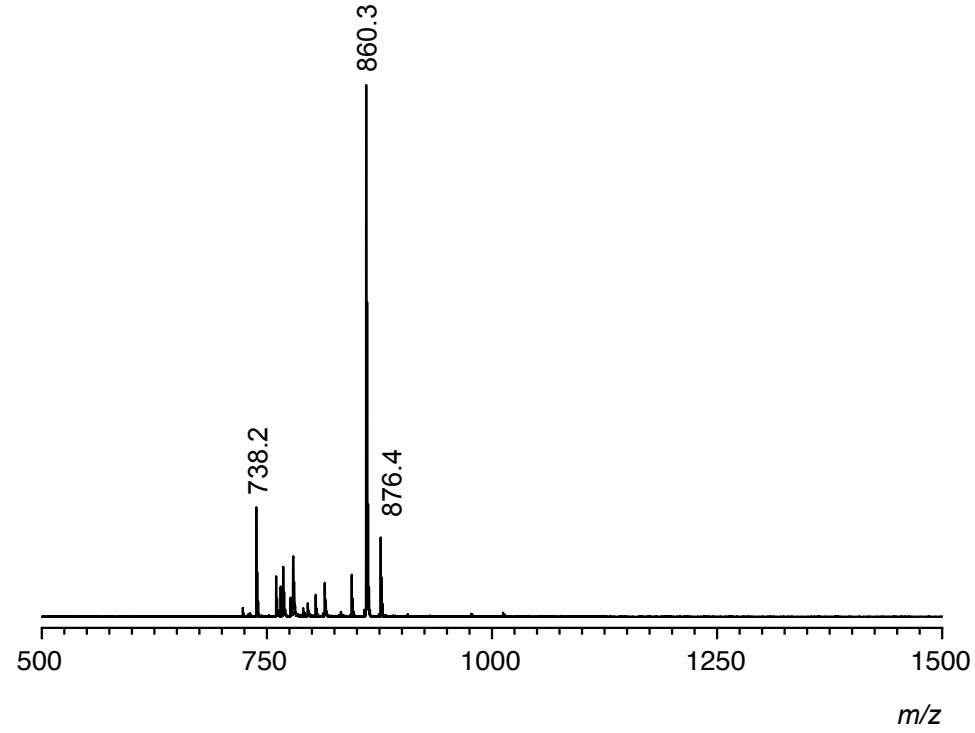
^{1}H NMR (400 MHz, MeOD-d_4)



^{13}C NMR (101 MHz, MeOD-d_4)



MALDI-TOF MS (positive mode)



		m/z calcd.	*m/z exp.*
$[M–C_4H_8–CO_2+H]^+$	$C_{41}H_{47}N_{11}O_9–C_5H_9O_2+H^+$	738.3	738.2
$[M+Na]^+$	$C_{41}H_{47}N_{11}O_9+Na^+$	860.3	860.3
$[M+K]^+$	$C_{41}H_{47}N_{11}O_9+K^+$	876.3	876.4

Nanoparticle Synthesis and Characterisation

Analytical Methods

^{1}H NMR Spectroscopy. The ^{1}H NMR spectroscopic measurements were performed on a Bruker Avance™ III 400 spectrometer at 400 MHz and 22 °C. MeOD-d_4 and D_2O were used as purchased.

UV-vis Spectroscopy. The UV-vis measurements were performed by using a Varian Cary 100 spectrometer in semi-micro PMMA disposable cuvettes. All measurements were performed at 22 °C by using HPLC grade water as the solvent. The spectra were recorded between 300 and 800 nm. The AuNPs and the salts were weighed by using an analytical precision balance. Blank measurements were performed with water.

Transmission Electron Microscopy. A droplet of an aqueous AuNP solution was placed on a holey carbon grid (Plano S147-4) and dried under ambient conditions. A JEOL JEM-2100 LaB6 Transmission Electron Microscope (TEM) equipped with a Gatan Orius SC1000 CCD camera was used for bright-field imaging at 200 kV accelerating voltage. The images have a size of 1024×1024 pixels (acquisition time 0.5 s). The average diameters of the AuNPs were determined by processing the images with ImageJ followed by statistical analysis with MS Excel.

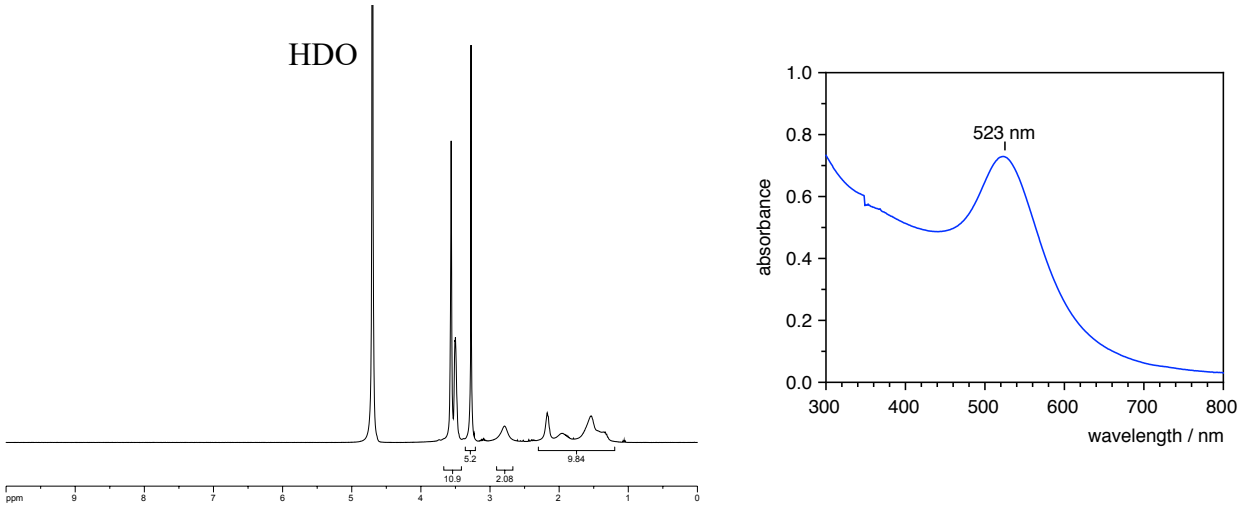
Syntheses

Citrate-stabilised AuNPs (NP^{Cit}).[3] Trisodium citrate dihydrate (484 mg, 1.65 mmol) was dissolved in water (250 mL) and the resulting solution was refluxed for 15 min. Meanwhile, a solution of $HAuCl_4$ (44.8 mg, 132 μmol) in water (1 mL) was also heated to 100 °C and added quickly to the refluxing citrate solution. The reaction mixture was further refluxed for 20 min and then allowed to cool to 25 °C. Prior to functionalisation, an aliquot of the resulting nanoparticles solution was dialysed against water for 24 h at 25 °C.

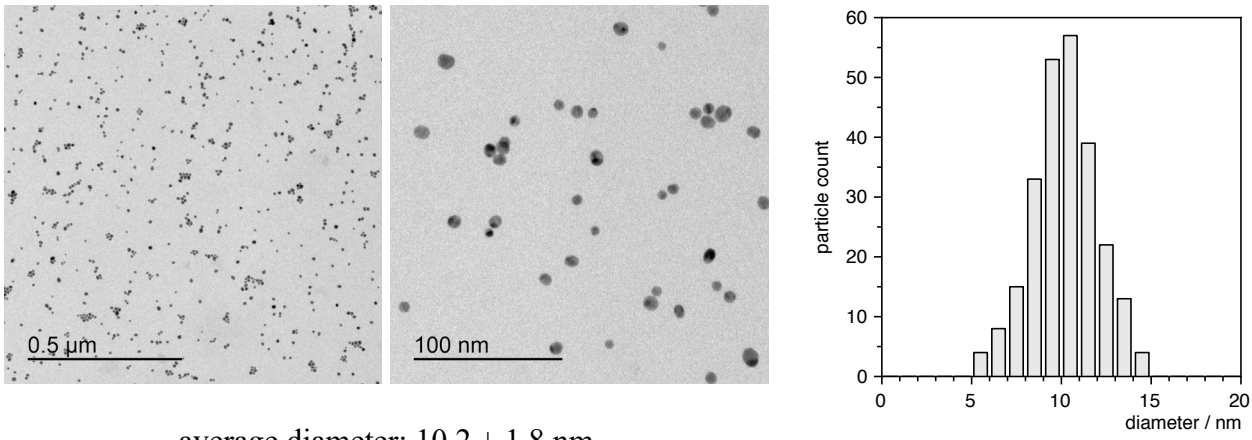
TEG-stabilised AuNPs (NP^{TEG}). To a dialysed **NP^{Cit}** stock solution (20 mL), a solution of **1** (1.00 mL, 100 μmol, 0.1 M in methanol) was added and the reaction mixture was stirred for 24 h at 25 °C. The solvent was removed *in vacuo*, the residue was re-dissolved in water (1.0 mL) and the AuNPs

purified first by size exclusion chromatography (Sephadex® G-10, water/methanol, 1:1 (*v/v*)) and then by membrane filtration. For this, the AuNPs were dissolved in water/methanol, 1:1 (*v/v*), the solution subjected to centrifugal concentrators with a MWCO membrane (5000 Da) and filtered off by centrifugation (3000 rpm, 12 °C). Membrane filtration was repeated three times. The resulting AuNPs were collected and dried. Yield: 3.5 mg.

(a) (b)



(c) (d)



average diameter: 10.2 ± 1.8 nm

Figure S1: ^{1}H NMR spectrum in D_2O (a) and UV-vis spectrum (b) of **NP^{TEG}** in water, and TEM image (c) together with the histogram illustrating the size distribution of the nanoparticles derived from the TEM image (d).

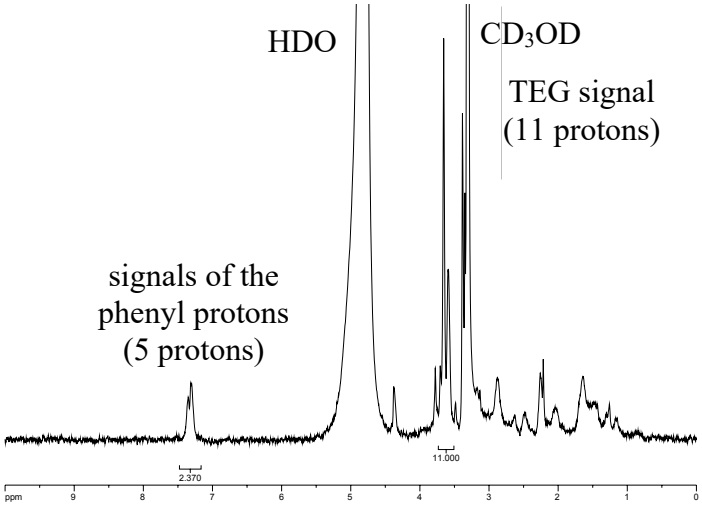
Attempted Synthesis of NP^{CP} by simultaneous immobilisation of 1 and 2. Dialysed NP^{Cit} stock solution (20 mL) was diluted with an organic solvent (methanol or DMF, 20-40 mL) and treated with solutions of, first, **2** (in methanol or DMF) and subsequently **1** (0.1 M in methanol or DMF). The times between the addition of the two solutions were varied. After the addition of both solutions was complete, the reaction mixture was stirred for 48 h at 25 °C. Afterwards, the solvent was removed *in vacuo*, the residue was re-dissolved in water/methanol, 1:2 (*v/v*) (1.2 mL) and the AuNPs purified first by size exclusion chromatography (Sephadex® LH-20, water/methanol, 1:2 (*v/v*)) and then by membrane filtration. For this, the AuNPs were dissolved in water/methanol, 1:1 (*v/v*), the solution subjected to centrifugal concentrators with a MWCO membrane (5000 Da) and filtered off by centrifugation (3000 rpm, 12 °C) with. Membrane filtration was repeated three times. The resulting AuNPs were collected and dried. The ^{1}H NMR spectroscopic characterisation of the products showed that none of the AuNPs thus obtained contained substantial amounts of immobilised cyclopeptide in spite of the systematic variation of the reaction conditions.

Mixed Monolayer-Protected NP^{COOH}. Dialysed NP^{Cit} stock solution (20 mL) was diluted with methanol (20 mL). Afterwards, solutions of **3** (100 µL, 10.0 µmol, 0.1 M in methanol) and of **1** (900 µL, 90.0 µmol, 0.1 M in methanol) were added simultaneously and the reaction mixture was stirred for 24 h at 25 °C. The solvent was removed *in vacuo*, the residue was re-dissolved in water/methanol, 1:2 (*v/v*) (1.2 mL) and the AuNPs purified first by size exclusion chromatography (Sephadex® LH-20, water/methanol, 1:2 (*v/v*)) and then by membrane filtration. For this, the AuNPs were dissolved in water/methanol, 1:1 (*v/v*), the solution subjected to centrifugal concentrators with a MWCO membrane (5000 Da) and filtered off by centrifugation (3000 rpm, 12 °C) with. Membrane filtration was repeated three times. The resulting AuNPs were collected and dried.

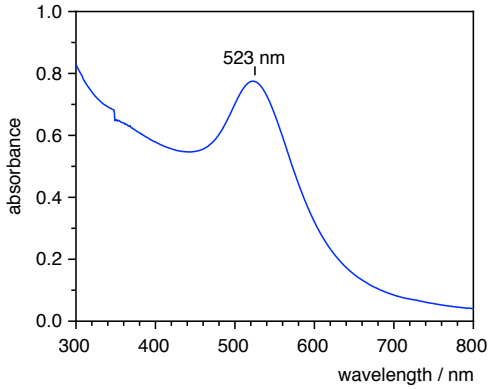
Mixed Monolayer-Protected NP^{Bn}. NP^{COOH} was dissolved in water/acetonitrile, 1:1 (*v/v*) (800 µL). A solution of sulfo-NHS (3.26 mg, 15.0 µmol) and EDC·HCl (2.88 mg, 15.0 µmol) in water (200 µL) was added. Compound **4** (1.3 mg, 5.00 µmol) was dissolved in water/acetonitrile, 1:1 (*v/v*) (400 µL) and the pH of the solution was adjusted to 8 with 0.1 M aqueous NaOH. This solution was added to the reaction mixture, whose pH was then again adjusted to 8 with 0.1 M aqueous NaOH. The

resulting mixture was stirred for 19 h at 25 °C. Afterwards, the nanoparticles were purified by size exclusion chromatography (Sephadex® LH-20, water/methanol, 1:2 (*v*/*v*). The solvent was evaporated and the product dried. Yield: 2.9 mg. According to the ^{1}H NMR spectroscopic analysis (Figure S2), 32 ± 1% of the ligands immobilised on the nanoparticle contained terminal benzylamine groups.

(a)



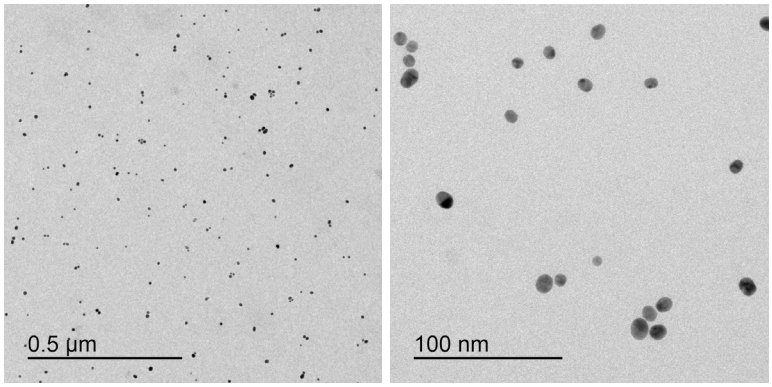
(b)



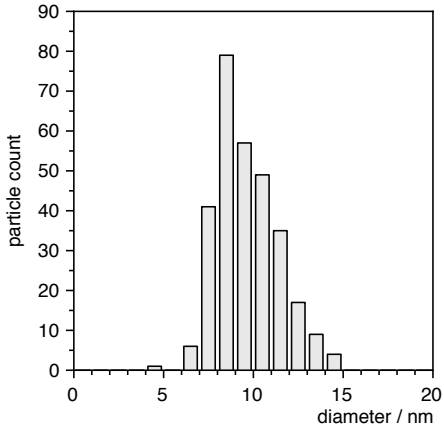
relative content of the functionalised ligand:

$$Content\ (\%) = \frac{2.399/5}{(2.399/5 + 11/11)} \times 100 = 32\%$$

(c)



(d)

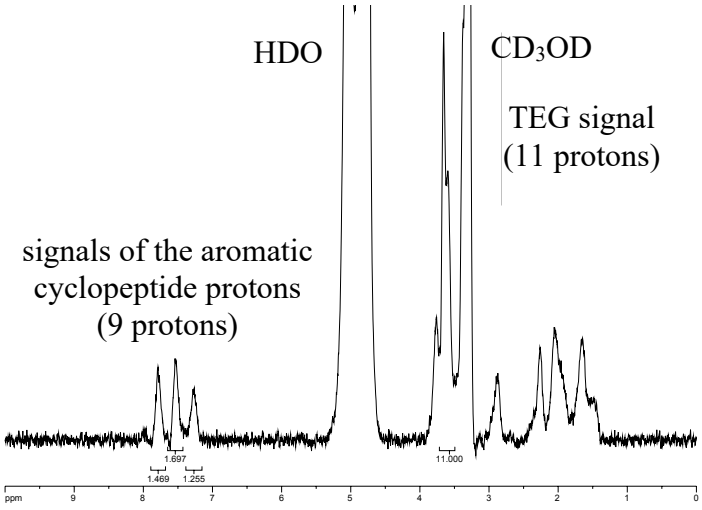


average diameter: 9.7 ± 1.7 nm

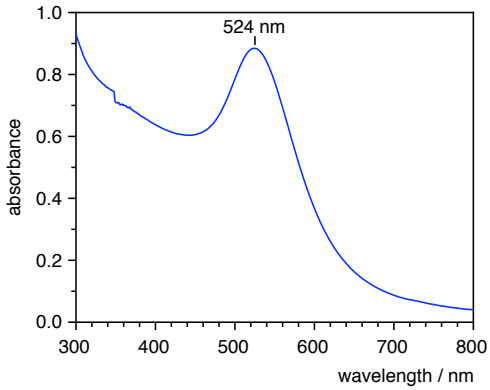
Figure S2: ^{1}H NMR spectrum in D_2O/MeOD-d_4, 1:1 (*v*/*v*) (a) and UV-vis spectrum (b) of **NP^{Bn}** in water, and TEM image (c) together with the histogram illustrating the size distribution of the nanoparticles derived from the TEM image (d).

Mixed Monolayer-Protected NP^{CP}. **NP^{COOH}** was dissolved in water/acetonitrile, 1:1 (*v/v*) (800 µL). A solution of sulfo-NHS (3.3 mg, 15.0 µmol) and EDC·HCl (2.9 mg, 15.0 µmol) in water (200 µL) was added. Compound **5** (4.1 mg, 5.00 µmol) was dissolved in water/acetonitrile, 1:1 (*v/v*) (400 µL) and the pH of the solution was adjusted to 8 with 0.1 M aqueous NaOH. This solution was added to the reaction mixture, whose pH was then again adjusted to 8 with 0.1 M aqueous NaOH. The resulting mixture was stirred for 19 h at 25 °C. Afterwards, the nanoparticles were purified by size exclusion chromatography (Sephadex® LH-20, water/methanol, 1:2 (*v/v*). The solvent was evaporated and the product dried. Yield: 2.7 mg. According to the ^{1}H NMR spectroscopic analysis (Figure S3), 32 ± 1% of the ligands immobilised on the nanoparticle contained terminal cyclopeptide moieties.

(a)



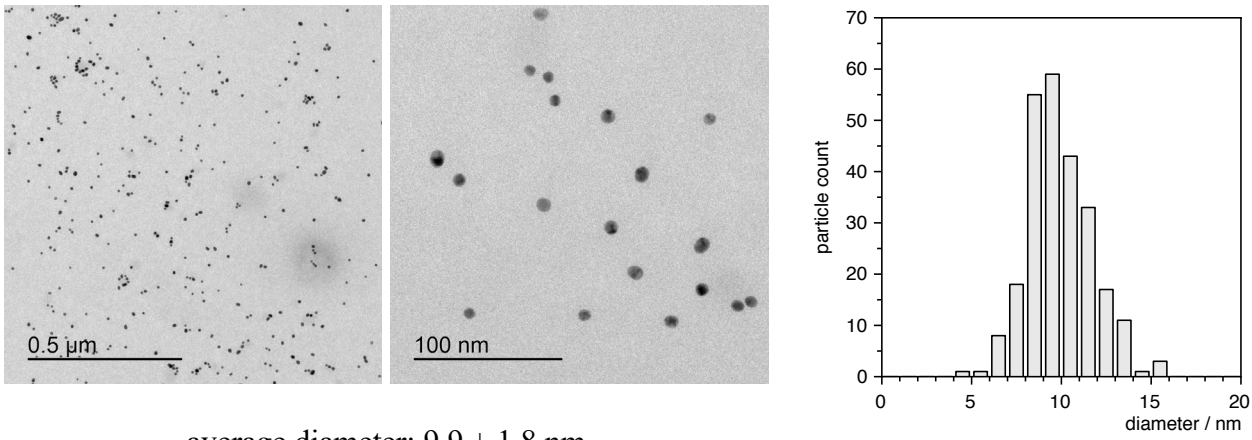
(b)



relative content of the functionalised ligand:

$$Content\ (\%) = \frac{(1.469 + 1.697 + 1.255)/9}{[(1.469 + 1.697 + 1.255)/9 + 11/11]} \times 100 = 33\%$$

(c) (d)



average diameter: 9.9 ± 1.8 nm

Figure S3: ^{1}H NMR spectrum in D_2O/MeOD-d_4, 1:1 (*v/v*) (a) and UV-vis spectrum (b) of **NP^{CP}** in water, and TEM image (c) together with the histogram illustrating the size distribution of the nanoparticles derived from the TEM image (d).

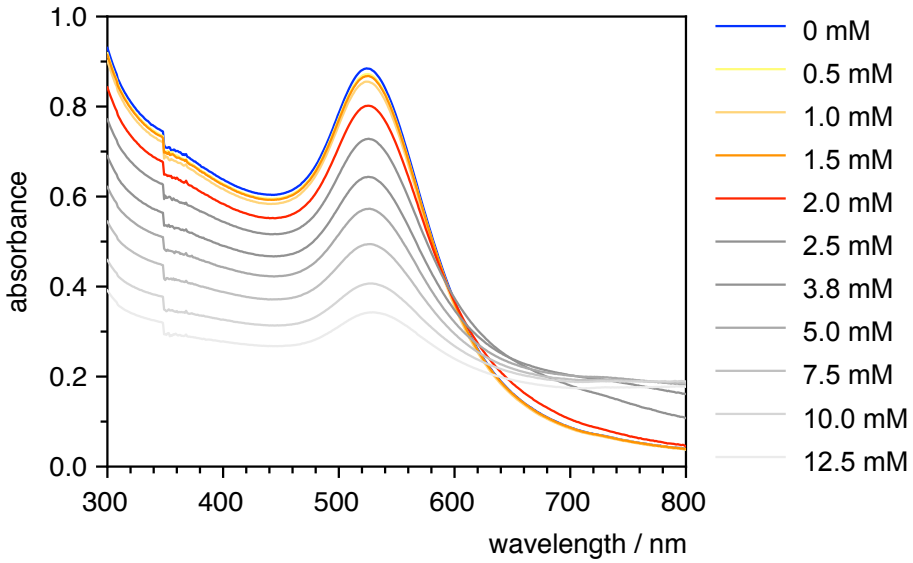
Binding Studies

Visual Binding Study. All experiments were performed by using HPLC grade water. The nanoparticles and salts were weighed by using an analytical precision balance and dissolved in water to obtain solutions containing 0.5 mg/mL of nanoparticles and salt solutions with a concentration of 0.1 M. As salts, NaCl, NaBr, NaI, Na_2SO_4, Na_2CO_3, Na_2HPO_4, Na_2HAsO_4, $Na_4P_2O_7$, $NaNO_3$ were used. Ten vials were prepared, each containing 100 µL of the nanoparticle stock solution. The solutions were diluted with water (100 µL). One vial was used as blank while each of the other vials was treated with a salt solution. Initially 1 µL of the salt stock solution was added and the amounts were then progressively increased in steps of 1 µL until the vials contained overall 5 µL of the corresponding salt solution. Once 5 µL were present, effects of the Na_2SO_4 solutions on the nanoparticles were observed. Photographs of the vials were taken after 5 and 30 minutes. This experiment was repeated at least three times.

UV-vis Spectroscopic Binding Study. The UV-vis spectroscopic measurements were performed on a Varian Cary 100 at 22 °C by using semi-micro PMMA disposable cuvettes with a 1 cm path length and HPLC grade water as the solvent. The nanoparticles and Na_2SO_4 were weighed by using an analytical precision balance. A stock solution of $\mathbf{NP^{CP}}$ was prepared containing 0.5 mg/mL of the nanoparticle in water and a 0.1 M aqueous solution of Na_2SO_4. Blank measurements were performed with water.

A series of samples were prepared, each containing the $\mathbf{NP^{CP}}$ stock solution (500 µL) and varying amounts of the Na_2SO_4 stock solution (0, 5, 10, 15, 20, 25, 37.5, 50, 75, 100, 125 µL). Each sample was made up with water to a total volume of 1 mL, and the respective UV-vis spectra were recorded between 300 and 800 nm exactly 5 minutes after sample preparation. The resulting spectra are shown in Figure S4a. Figure S4b shows a comparison of the UV-vis spectrum of $\mathbf{NP^{CP}}$ with the spectra obtained in the presence of 2.5 mM of Na_2SO_4 after 5 and 30 min.

(a)



(b)

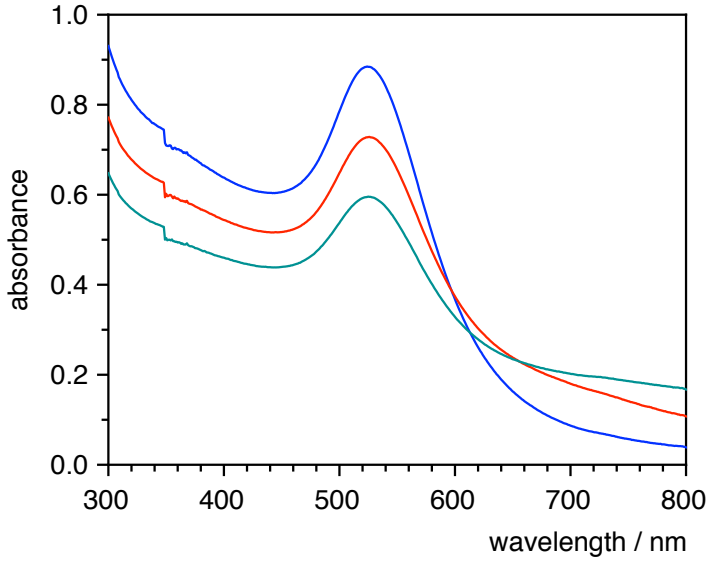


Figure S4: UV/VIS spectra of **NP^{CP}** (0.25 mg/mL) in water containing between 0 and 12.5 mM of Na_2SO_4 measured after 5 min (a). In (b), the UV-vis spectra of the solution containing 2.5 mM of Na_2SO_4 measured after 5 (red) and 30 min (green) are compared with the UV-vis spectrum of **NP^{CP}** (blue).

An analogous measurement was performed by adding a 0.1 M aqueous NaI solution (25 µL) to the **NP^{CP}** stock solution (500 µL) and adding water to obtain a total volume of 1 mL. The corresponding UV-vis spectrum is depicted in Figure S5 together with the spectrum of **NP^{CP}** in water.

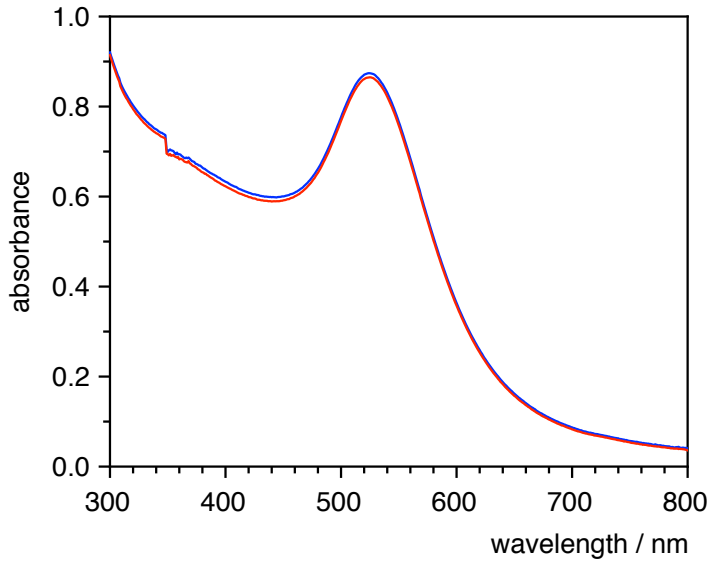
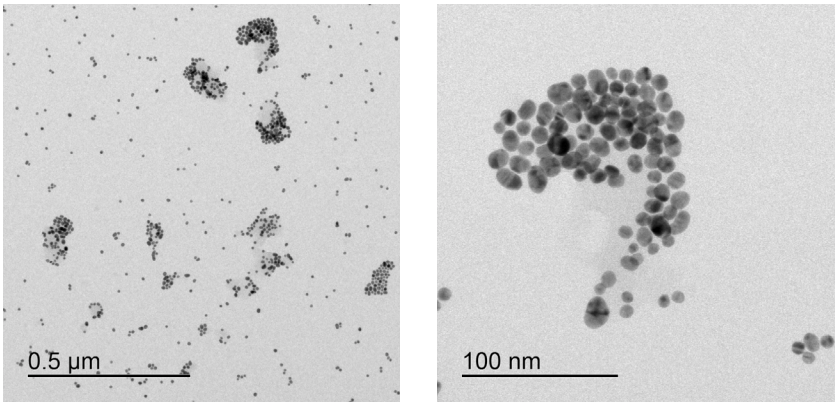


Figure S5: UV/VIS spectra of **NP^{CP}** (0.25 mg/mL) in water (blue) and spectrum of a solution containing additional 2.5 mM of NaI (red).

Transmission Electron Microscopy. Stock solutions in water were prepared containing of **NP^{CP}** (0.25 mg/mL) and of Na_2SO_4 (0.1 M). The Na_2SO_4 stock solution (5 µL) was added to the nanoparticle stock solution (200 µL) and TEM images of this mixture were taken after 5 and 30 minutes. For the measurement, a droplet of an aqueous AuNP solution was placed on a holey carbon grid (Plano S147-4) and dried under ambient conditions.

(a)



(b)

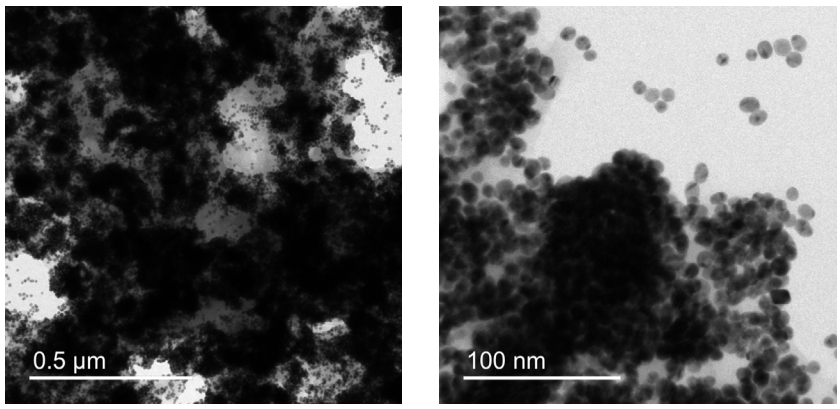


Figure S6: TEM images of **NP^{CP}** 5 min (a) and 30 min (b) after the addition of Na_2SO_4.

Competition Experiment. Stock solutions of **NP^{CP}** (0.5 mg/mL) and NaCl (1 M), Na_2HPO_4 (1 M), and Na_2SO_4 (0.1 M) were prepared in HPLC grade water. Three vials were prepared, each containing 100 µL of the **NP^{CP}** stock solution and additional 100 µL of water. No salt solutions were added to the first vial. The NaCl and the Na_2HPO_4 stock solution (5 µL each) were added to the two other vials. To the third vial, the Na_2SO_4 stock solution (5 µL) was additionally added. All vials were made up to total volumes of 215 µL with water. A photograph of the vials, taken after 5 min, is shown in Fig. 5 of the main manuscript.

References

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3. I. Ojea-Jiménez, N. G. Bastús and Victor Puntes, *J. Phys. Chem. C*, 2011, **115**, 15752–15757.