Supporting Information

Detection and identification of designer drugs by nanoparticle-based NMR chemosensing

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1. Experimental Procedures

**General:** Solvents were purified by standard methods. All commercially available reagents and substrates were used as received. Compounds S1-4, were prepared as detailed in Section 7 of this SI.

TLC analyses were performed using Merck 60 F254 precoated silica gel glass plates. Column chromatography was carried out on Macherey-Nagel silica gel 60 (70-230 mesh).

NMR spectra were recorded using a Bruker AV III 500 spectrometer operating at 500 MHz for $^1$H, 125.8 MHz for $^{13}$C. Chemical shifts are reported relative to internal Me$_4$Si. Multiplicity is given as follow: s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, br = broad peak.

ESI-MS mass spectra were obtained with an Agilent Technologies LC/MSD Trap SL mass spectrometer.

HRMS mass spectra were obtained with a Mariner Applied Biosystem (API-TOF) mass spectrometer (MeOH, 0.5% formic acid).

TEM images were recorded on a Jeol 300 PX electron microscope. One drop of sample was placed on the sample grid and the solvent was allowed to evaporate. TEM images were analysed with ImageJ software to measure the diameters distribution and average values.

TGA were run on 1 mg nanoparticle samples using a Q5000 IR model TA instrument from 30 to 1000 °C under a continuous air flow.

Fluorescence spectra were recorded on a Perkin Elmer LS50B fluorimeter.
2. Synthesis of Sulfonate thiols (S1, S2, S3, S4)

Thiol S1 was prepared as reported in the following scheme:

\[
\begin{align*}
\text{Na}_2\text{SO}_3 & \quad \text{MeOH:H}_2\text{O}, \quad 95 \, ^\circ\text{C}, \quad 97\% \\
\text{SO}_3\text{Na} & \quad \text{MeONa} \\
\text{S1} & \quad \text{MeOH}, \quad \text{hv}, \quad 85\% \\
& \quad \text{AcSH}, \quad \text{DMPA} \\
\text{SO}_3\text{Na} & \quad \text{SO}_3\text{Na}
\end{align*}
\]

**Sodium undec-10-ene-1-sulfonate (1).**

11-bromoundec-1-ene (510 mg, 2.190 mmol, 1 equiv) was dissolved in 9 mL of H\textsubscript{2}O and 4 ml of MeOH. Na\textsubscript{2}SO\textsubscript{3} (554 mg, 4.400 mmol, 2 equiv) was added and the mixture was stirred under reflux overnight. Then the solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (DCM:MeOH from 9:1 to 1:1), affording the desired sulfonate compound 1 (533 mg, 97%).

\[\text{1H-NMR (D}_2\text{O, 200 MHz)}: \delta \quad 1.26 \text{ (br s, 12H)}, 1.68 \text{ (m, 2H)}, 1.99 \text{ (m, 2H)}, 2.82 \text{ (m, 2H)}, 4.92 \text{ (m, 2H)}, 5.80 \text{ (m, 1H)}.\]

\[\text{13C-NMR (D}_2\text{O, 500 MHz): } \delta \quad 23.95, 27.68, 27.70, 28.12, 28.24, 28.40, 28.46, 33.11, 51.08, 113.89, 140.46.\]

\[\text{MS (ESI) m/z): } 233.1 ([M]-).\]

**Sodium 11-(acetylthio)undecane-1-sulfonate (2).**

Sulfonate 1 (200 mg, 0.78 mmol, 1 equiv) was dissolved in 2 mL of MeOH and the solution was degased under N\textsubscript{2} bubbling for 30 min. Then thiaoctic acid (220 \textmu l, 3.120 mmol, 4 equiv) and 2,2-dimethoxy-2-phenylacetophenone (DMPA, 20 mg, 0.078 mmol, 0.1 equiv) were added and the mixture was stirred under UV irradiation (365 nm) for 2.5 h. The solvent was evaporated under vacuum and the crude was purified by flash column chromatography (DCM:MeOH from 9:1 to 1:1), affording the desired sulfonate compound 2 (220 mg, 85%).

\[\text{1H-NMR (D}_2\text{O, 200 MHz)}: \delta \quad 1.25 \text{ (br s, 14H)}, 1.39 \text{ (m, 2H)}, 1.64 \text{ (m, 2H)}, 2.31 \text{ (s, 3H, CH3-)}, 2.69 \text{ (m, 4H)}.\]

\[\text{MS (ESI) m/z): } 309.1 ([M]+).\]
Sodium 11-mercaptoundecane-1-sulfonate (S1).

The acetylated sulfonate 2 (84 mg, 0.250 mmol, 1 equiv) was dissolved in 2 mL of dry MeOH and then sodium methoxide was added (32 mg, 0.590 mmol, 2.4 equiv). After stirring for 2.5 hours under N₂ atmosphere, the reaction was quenched adding IR 120H⁺ resin until pH neutralization, then the resin was filtered off, the solvent was evaporated, giving the deprotected Sodium 11-mercaptoundecane-1-sulfonate S1 (70 mg, yield 95%), that was freshly used for the nanoparticles’ synthesis.

¹H-NMR (MeOD, 300 MHz): δ 1.32 (br s, 14H), 1.57 (m, 2H), 1.78 (m, 2H), 2.49 (m, 2H), 2.79 (m, 2H).

¹³C-NMR (MeOD, 500 MHz): δ 23.78, 23.96, 27.71, 28.10, 28.24, 28.40, 28.46, 28.55, 33.01, 51.08.

TOF ES- HRMS: [M] calcd. for C₁₁H₂₃O₃S₂=267.109. Found =267.113

Spectroscopic data are in agreement with those reported in literature.¹
Thiol S2 was prepared as reported in the following scheme:

8-(Acetylthio)octanoic acid (3).

8-bromo octanoic acid (4 g, 17.928 mmol, 1 eq) was dissolved in 100 mL of aceton, then KSAc (2.46 g, 21.514 mmol, 1.2 eq) was added and the mixture was stirred at room temperature overnight. Then the solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (PE:EtOAc from 8:2 to 7:3), affording the desired compound 3 (3.76 g, 96%).

\[ ^1H-NMR\ (CDCl_3, 500 MHz): \delta 1.35 (m, 6H), 1.58 (m, 2H), 1.64 (m, 2H), 2.30 (s, 3H), 2.36 (t, J=7.5Hz, 2H), 2.89 (t, J=7.3Hz, 2H). \]

\[ ^13C-NMR\ (CDCl_3, 300 MHz): \delta 24.54, 28.54, 28.70, 28.85, 29.07, 29.41, 30.64, 33.98, 179.98, 196.12. \]

4-(8-(acetylthio)octanamido)benzenesulfonate · DIPEA (4).

Compound 3 (1 g, 4.581 mmol, 1 equiv) was dissolved in 10 mL of dry DCM and then pentafluoro phenol (pFp 626 µl, 5.955 mmol, 1.3 equiv) and EDC*HCl (1.14 g, 5.955 mmol, 1.3 equiv) were added. The mixture was stirred for 20 h, then the solvent was evaporated under reduced pressure. The crude was dissolved in dry DMF (15 mL), then sulfanilic acid (937 mg, 5.406 mmol, 1.18 equiv) and DIPEA (3 mL, 17.408 mmol, 3.8 equiv) were added; the reaction was stirred at 68°C and monitored by TLC (7:3 Tol:MeOH). After completion the solvent was evaporated and the crude was purified by flash column chromatography (Tol:MeOH 7:3), affording the desired sulfonate compound 4 (1.54 g, 85%).

\[ ^1H-NMR\ (MeOD, 500): \delta 1.39 (m br, 27H), 1.57 (m br, 2H), 1.70 (m br, 2H), 2.32 (s, 3H), 2.42 (t, J=7.5 Hz, 2H), 2.88 (t, J=7.3 Hz, 2H), 3.23 (q, J=7.4 Hz, 2H), 3.72 (hept, J=6.6 Hz, 2H), 7.38, 7.76 (m, 4H) \]
S6

C- NMR (MeOD, 500): 11.93, 16.03, 17.47, 25.36, 28.27, 28.47, 28.55, 28.77, 29.35, 36.63, 42.51, 54.47, 118.82, 126.31, 140.27, 140.57, 173.33, 196.06

TOF ES HRMS: [M-] calcd. for C₁₆H₂₂O₅NS₂=372.094  Found =372.102.

Sodium 4-(8-mercaptooctanamido)benzenesulfonate (S2).

The acetylated sulfonate 4 (223 mg, 0.445 mmol, 1 equiv) was dissolved in 5 mL of dry MeOH and then sodium methoxide was added (72 mg, 1.334 mmol, 3 equiv); after stirring at for 3 hours under N₂ atmosphere, the reaction was quenched adding IR 120H⁺ resin until pH neutralization, then the resin was filtered off, the solvent was evaporated, giving the deprotected Sodium 4-(8-mercaptooctanamido)benzenesulfonate S₂ (145 mg, yield 90%), that was freshly used for the nanoparticles’ synthesis.

H-NMR (MeOD, 300): δ 1.36 (m, 8H), 1.56 (m br, 1H), 1.69 (m br, 2H), 2.41 (t, J=7.5 Hz, 2H), 2.88 (t, J=7.3 Hz, 2H), 7.38, 7.76 (m, 4H)

C-NMR (MeOD, 500): δ 25.29, 28.46, 28.58, 28.63, 28.97, 29.39, 33.22, 136.17, 137.76, 139.54, 141.07.

TOF ES HRMS: [M-] calcd. for C₁₄H₂₀O₄NS₂=330.083  Found =330.085
Thiol S3 was prepared as reported in the following scheme:

\[
\begin{align*}
\text{Br} & \xrightleftharpoons[5,23 \text{ mmol}]{\text{MeOH, 75°C, 98%}} \text{NaN}_3 \xrightarrow{\text{MeOH, 75°C, 98%}} \text{N}_3 \\
\text{SOCl}_2, \text{DMF} & \xrightarrow{70°C, 83\%} \text{CH}_2\text{Cl}_2, \text{H}_2\text{O} \\
\text{AcSO}_3\text{H} & \xrightarrow{\text{DMPA, h}, 85\%} \text{S}_3
\end{align*}
\]

8-azidoct-1-ene (5).

8-bromoct-1-ene (1 g, 5.23 mmol, 1 equiv) and NaN₃ (605 mg, 9.29 mmol, 1.77 equiv) were refluxed overnight in MeOH (7 mL). Then the solvent was removed under reduced pressure and the mixture was carefully extracted in DCM/H₂O. The organic layers were collected, dried over Na₂SO₄ anhydrous concentrated, obtaining 791 mg (98% yield) of a colorless liquid.

\(^{1}\text{H-NMR} (\text{CDCl}_3, 500 \text{ MHz}): \delta 1.41 \text{ (br s, 6H)}, 1.62 \text{ (m, 2H)}, 2.08 \text{ (m, 2H)}, 3.28 \text{ (t, J=7.0 Hz, 2H)}, 5.00 \text{ (m, 2H)}, 5.83 \text{ (m, 1H)}.

\(^{13}\text{C-NMR} (\text{CDCl}_3, 500 \text{ MHz}): \delta 26.57, 28.60, 28.72, 28.80, 33.64, 51.46, 114.38, 138.88

Oct-7-en-1-amine (6).

In a rbf, under N₂ atmosphere, 1M THF solution of LiAlH₄ (12 mL, 12 mmol, 2.3 equiv) was diluted with dry THF (30 mL) and the obtained solution was cooled at 0°C. Compound 5 (0.791 g, 5.13 mmol, 1 equiv) was dissolved in dry THF (20 mL) and this solution was slowly added (over 20
min) to the stirring LiAlH₄ solution. The ice bath was removed and the mixture was stirred under rt for 2 hours. After reaction completion, NaOH 1% solution (40 mL) was added and the mixture was extracted three times with Et₂O (40 mL). The organic layers were collected and dried over Na₂SO₄ anhydrous. No further purifications were required and due to highly volatility of compound 6, the solution was concentrated and used for the next step as a 0,3 M Et₂O solution (17 ml, 99%).

¹H-NMR (CDCl₃, 500 MHz): δ 1.35 (m, 6H) 1.42 (m, 2H), 2.09 (m, 2H) 2.66 (t, J=6.9 Hz, 2H), 4.97 (m, 2H), 5.85 (m, 1H).

¹³C-NMR (CDCl₃, 500 MHz): δ 26.72, 28.94, 29.00, 33.74, 33.96, 42.25, 113.82, 139.24.

MS (ESI) m/z: 128.2 ([M+H]+)

4-(chlorosulfonyl)benzoylchloride (7).

Benzensulphonic acid (1,5 g, 6,25 mmol, 1 equiv), thionyl chloride (15 mL, 76,88 mmol, 12 equiv) and dry DMF (0,1 mL) were stirred at 70°C for 4 hours. Then the mixture was concentrated under vacuum and Toluene was added. The solution was filtered and the solvent was evaporated under vacuum, obtaining 1,31 g (87%) of compound 7.

¹H-NMR (CDCl₃, 500 MHz): δ 8.23 (m, 2H) 8.39 (m, 2H)

¹³C-NMR (CDCl₃, 500 MHz): δ 127.75, 132.42, 138.54, 148.99, 167.03

Sodium 4-(oct-7-en-1-ylcarbamoyl)benzenesulfonate (8).

Compound 7 (165 mg, 0,69 mmol, 1 equiv) and DIPEA (300 µl, 1,72 mmol, 2,5 equiv) were dissolved in dry THF and the resulting solution was stirred for 10 minutes under N₂ atmosphere at -78°C. Then compound 6 (1,3 mL of Et₂O 0,3 M solution, 0,39 mmol, 0,58 equiv) was added in two portions over 30 minutes and the mixture was stirred for 2 hours at -78°C. Then the solution was brought to rt and filtered. The solvent was removed under reduced pressure and the obtained crude was dissolved in 40 mL of CHCl₃ and subsequently extracted with NaHCO₃ saturated solution, HCl 1M and finally brine. The organic layer was dried with Na₂SO₄, the solvent was evaporated under reduced pressure and the mixture was purified via flash chromatography (PE:EtOAc from 9:1 to 7:3). The obtained product was dissolved in H₂O and refluxed for 3 hours. Then water was evaporated under reduced pressure giving, without need of further purifications, the desired sulfonate 8 (57 mg, 43%).
1H-NMR (MeOD, 200 MHz): δ 1.44 (br s, 6H, -CH2-) 1.67 (m, 2H), 2.11 (m, 2H), 3.40 (t, 2H), 5.06 (m, 2H), 5.83 (m, 1H) 7.91 (m, 4H).

MS (ESI) m/z: 333.2 ([M+Na])

Sodium 4-((7-(acetylthio)heptyl)carbamoyl)benzenesulfonate (9).

Compound 8 (127 mg, 0.38 mmol, 1 equiv) was dissolved in 2 mL of MeOH and the solution was degased under N2 bubbling for 30 min. Then thioacetic acid (115 µl, 1.63 mmol, 4 equiv) and 2,2-dimethoxy-2-phenylacetophenone (DMPA, 6 mg, 0.02 mmol, 0.05 equiv) were added and the mixture was transferred in UV cuvettes and stirred under UV irradiation (365 nm) for 2.5 h. The solvent was evaporated under vacuum and the crude was purified by flash column chromatography (DCM:MeOH from 9:1 to 8:2), affording the desired sulfonate compound 9 (125 mg, 85%).

1H-NMR (MeOD, 200 MHz): δ 1.37 (br s), 1.59 (m, 4H), 2.29 (s, 3H), 3.38 (m, 4H), 7.89 (m, 4H).

13C-NMR (MeOD, 200 MHz): δ 26.8 29.5 29.7 30.0, 30.6, 31.9, 40.2, 128.1, 129.9, 138.8, 157.9, 167.5, 195.2.

Sodium 4-((7-mercaptoheptyl)carbamoyl)benzenesulfonate (S3).

The protected thiol 9 (45 mg, 0.12 mmol, 1 equiv) was dissolved in 2.5 mL of dry MeOH and then sodium methoxide was added (20 mg, 0.35 mmol, 3 equiv); after stirring at for 3 hours under N2 atmosphere, the reaction was quenched adding IR 120H+ resin until pH neutralization, then the resin was filtered off and the solvent was evaporated. Flash chromatography purification (8:2 DCM:MeOH) gave the deprotected S3 (38 mg, yield 90%), that was freshly used for the nanoparticles’ synthesis.

1H-NMR (MeOD, 200 MHz): δ 1.37 (br s, 6H), 1.59 (m, 4H), 3.38 (m, 4H), 7.89 (m, 4H).

1H-NMR (MeOD, 500 MHz): δ 23.55, 26.61, 27.93, 28.28, 28.29, 28.99, 33.80, 39.70, 125.73, 126.89, 136.02, 147.73, 167.94

TOF ES+ HRMS: [M+Na]+ calcd. for C15H22NO4S2=M=344.099. Found =344.106
Thiol S4 was prepared as reported in the following scheme:

\[
\text{Br} \quad \text{Cl} \quad \text{Si} \quad \text{Cl} \quad \text{Br}
\]

1) I₂, Mg, dry THF
2) Et₂O, 0°C, 97%

\[
\text{Cl} \quad \text{Si} \quad \text{Cl}
\]

10

\[
\text{LiBr, TOABr}
\]

76%

\[
\text{Br} \quad \text{Si} \quad \text{Br}
\]

11

\[
\text{Na}_2\text{SO}_3, \quad \text{H}_2\text{O}, \text{EtOH}
\]

80%

\[
\text{Br} \quad \text{Si} \quad \text{SO}_3\text{Na}
\]

S4

\[
\text{MeONa, dry MeOH}
\]

90%

\[
\text{AcS} \quad \text{Si} \quad \text{SO}_3\text{Na}
\]

13

\[
\text{AcSH, DMPA, h}_ν, \quad 81%
\]

\[
\text{Br} \quad \text{Si} \quad \text{SO}_3\text{Na}
\]

12

(Chloromethyl)dimethyl(pent-4-enyl)silane (10).

Mg turnings (693 mg, 29.713 mmol, 1.1 equiv) were vigorously stirred overnight in a two neck flask under N₂. Then 3 mL of dry THF and a catalytic amount of I₂ are added and the solution is heated in order to activate the Mg. Then 5-bromopent-1-ene (3.2 mL, 27.012 mmol, 1 equiv) and dry THF (57 mL) were added drop by drop. The solution was stirred under N₂ at 40°C for 6 hours.

Then the solution was cooled to 0°C and slowly added via canula to a 0°C solution of chloro(chloromethyl)dimethylsilane (4.3 mL, 32.414 mmol, 1.2 equiv) in dry Et₂O. The obtained solution was stirred overnight at room temperature and then it was quenched by adding a saturated solution of NH₄Cl. Then the mixture was extracted with Et₂O for five times, the organic layers were collected, dried over Na₂SO₄ and then concentrated under reduced pressure. The obtained crude was purified by flash column chromatography (100% PE), affording the desired compound 10 (4.7 g, 97%).

\(^{1}\text{H NMR}\) (500 MHz, CDCl₃) δ 6.03 – 5.75 (m, 1H), 5.21 – 4.98 (m, 2H), 2.89 (s, 2H), 2.25 – 2.13 (m, 2H), 1.59 – 1.49 (m, 2H), 0.81 – 0.72 (m, 2H), 0.22 (s, 6H).

\(^{13}\text{C NMR}\) (126 MHz, CDCl₃) δ 138.60, 114.74, 37.44, 30.34, 23.03, 13.23, 4.62.

\(^{29}\text{Si NMR}\) (99 MHz, CDCl₃) δ 3.71.

(Bromomethyl)dimethyl(pent-4-enyl)silane (11).

In a Schlenk tube compound 10 (2.5 g, 14.143 mmol, 1 equiv), LiBr (previously dried at 150°C under N₂, 3.69 g, 42.430 mmol, 3 equiv) and TOABr (1.50 g, 2.829 mmol, 0.2 equiv) were added and the mixture was stirred for 24 h at 60°C. The obtained crude was purified by flash column chromatography (100% PE), affording the desired compound 11 (2.37 g, 76%).
1H NMR (500 MHz, CDCl₃) δ 6.02 – 5.79 (m, 1H), 5.21 – 4.98 (m, 2H), 2.58 (s, 2H), 2.24 – 2.11 (m, 2H), 1.59 – 1.49 (m, 2H), 0.82 – 0.72 (m, 2H), 0.23 (s, 6H).

13C NMR (126 MHz, CDCl₃) δ 138.61, 114.74, 37.43, 23.07, 17.09, 13.69, -4.04.

29Si NMR (99 MHz, CDCl₃) δ 3.69.

Sodium (dimethyl(pent-4-enyl)silyl)methanesulfonate (12).

In a Schlenk tube compound 11 (400 mg, 1.808 mmol, 1 equiv) and Na₂SO₃ (456 mg, 3.616 mmol, 2 equiv) were dissolved in 13 mL of H₂O:EtOH 1:1 and the reaction was stirred for 24 h at 60°C. The obtained crude was purified by reverse phase chromatography (C18 resin, from 100% H₂O to 100% MeOH), affording the desired compound 12 (352 mg, 80%).

1H NMR (500 MHz, MeOD) δ 5.96 – 5.70 (m, 1H), 5.09 – 4.90 (m, 2H), 2.58 (s, 2H), 2.16 – 2.01 (m, 2H), 1.54 – 1.37 (m, 2H), 0.81 – 0.63 (m, 2H), 0.18 (s, 6H).

13C NMR (126 MHz, MeOD) δ 138.5, 113.6, 41.9, 37.3, 23.0, 14.4, -4.2.

29Si NMR (99 MHz, CDCl₃) δ 0.85.

MS (ESI) m/z: 221.1 ([M]⁺)

Sodium ((5-(acetylthio)pentyl)dimethylsilyl)methanesulfonate (13).

Sulfonate 12 (221.3 mg, 1.471 mmol, 1 equiv) was dissolved in 3 mL of MeOH and the solution was degased by N₂ bubbling for 30 min. Then thioacetic acid (415 μl, 5.890 mmol, 4 equiv) and 2,2-dimethoxy-2-phenylacetophenone (DMPA, 39 mg, 0.147 mmol, 0.1 equiv) were added and the mixture was stirred under UV irradiation (365 nm) for 2.5 h. The solvent was evaported under vacuum and the crude was purified by flash column chromatography (DCM:MeOH from 9:1 to 8:2), affording the desired sulfonate compound 13 (381 mg, 81%).

1H NMR (500 MHz, MeOD) δ 2.88 (t, J = 7.3 Hz, 2H), 2.59 (s, 2H), 2.32 (s, 2H), 1.64 – 1.53 (m, 2H), 1.46 – 1.35 (m, 2H), 0.78 – 0.65 (m, 2H), 0.18 (s, 6H).

13C NMR (126 MHz, MeOD) δ 196.23, 41.87, 32.24, 29.11, 28.99, 28.41, 22.85, 14.67, -4.16.

29Si NMR (99 MHz, MeOD) δ -0.84.

MS (ESI) m/z: 297.0 ([M]⁺)

Sodium ((5-mercaptopentyl)dimethylsilyl)methanesulfonate (S4).

The acetylated sulfonate 13 (30 mg, 0.0987 mmol, 1 equiv) was dissolved in 1 mL of dry MeOH and then sodium methoxide was added (16 mg, 0.296 mmol, 3 equiv). After stirring for 2.5 hours under N₂ atmosphere, the reaction was quenched adding IR 120H⁺ resin until pH neutralization,
then the resin was filtered off, the solvent was evaporated, giving the deprotected Sodium 11-
mercaptoundecane-1-sulfonate S4 (25 mg, yield 90%), that was freshly used for the nanoparticles’
synthesis.

\[ 1^1H \text{ NMR (500 MHz, MeOD)} \delta 2.57 (s, 2H), 2.52 – 2.45 (m, 2H), 1.66 – 1.56 (m, 2H),
1.49 – 1.32 (m, 4H), 0.76 – 0.66 (m, 2H), 0.18 (s, 6H). \]

\[ 1^3C \text{ NMR (126 MHz, MeOD)} \delta 41.90, 37.31, 33.56, 24.70, 23.30, 15.06, -4.16. \]

\[ 2^9Si \text{ NMR (99 MHz, MeOD)} \delta -0.87. \]

**TOF ES HRMS**: [M-] calcd. for \( C_{8}H_{19}O_{3}S_{2}Si \)=255.055. Found = 255.049.
3. Synthesis and characterization of monolayer protected gold nanoparticles

Monolayer protected gold nanoparticles (S1, S2, S3, S4-AuNPs) were prepared following a previously reported two-step procedure.\(^2\) A solution of HAuCl\(_4\)·3H\(_2\)O (50 mg, 0.127 mmol, 1 equiv) in water (2 mL) was extracted with a solution of tetraoctylammonium bromide (0.175 g, 0.318 mmol, 2.5 equiv) in N\(_2\) purged toluene (125 mL). To the resulting reddish-orange organic solution dioctylamine (0.613 g, 2.539 mmol, 20 equiv) was added (the amount of dioctylamine was calculated in order to obtain 2 nm nanoparticles). The mixture is vigorously stirred under N\(_2\) for 1.5 hours. During this period of time the color of the mixture fades. Then the solution is cooled at 0°C and a NaBH\(_4\) solution (48.0 mg, 1.269 mmol, 10 equiv) in H\(_2\)O (1 mL) is then rapidly added. The color of the solution turns rapidly to black and after 1.5 hours of stirring at 0°C, the aqueous layer is removed. To the obtained nanoparticle solution, the desired thiol S1-4 (0.254 mmol, 2 equiv) dissolved in 3 mL of MeOH was rapidly added. The reaction mixture was stirred for 3 hours at 0°C. All the formed AuNPs were unsoluble in toluene, hence the mixtures were centrifuged and the collected AuNPs were washed under sonication 7 times, with EtOAc and MeOH. The resulting NPs were finally purified by gel permeation chromatography with Sephadex G-25.

3.1. Characterization of S1-, S2-, S3-, S4-AuNPs

TEM analysis of the different samples of small nanoparticles (Figures S1, S2, S3, S4) yields an average diameter for S1-AuNPs of 1.8 ± 0.2 nm, for S2-, S3-, S4-AuNPs of 1.6 ± 0.4 nm. This data, together with the loss of organic weigh obtained by TGA analysis (Figures S5,S6, S7, S8), indicate that the formula for AuNP is \(\text{Au}_{127}\text{SR}_{39}\) for S1-AuNPs, \(\text{Au}_{180}\text{SR}_{54}\) for S2-AuNPs, \(\text{Au}_{127}\text{SR}_{55}\) for S3-AuNPs, \(\text{Au}_{127}\text{SR}_{44}\) for S4-AuNPs. The gold core of AuNP was approximated as a sphere, and the weight loss considered is related to the thiol minus NaHSO3 that stays as inorganic residue. NMR analysis (Figure S9, S10, S11, S12) indicates monolayer formation (broadening of all signals and missing of the \(\text{SCH}_2\text{CH}_2\) protons’ signals). UV-vis spectra (Figure S13, S14, S15, S16) were recorded.
Figure S1. Sample TEM images S1-AuNP and size distribution: average diameter = 1.6 nm (σ= 0.4 nm).
Figure S2. Sample TEM images S2-AuNP and size distribution: average diameter = 1.8 nm (σ= 0.2 nm).
Figure S3. Sample TEM images of S3-AuNP and size distribution: average diameter = 1.6 nm (σ = 0.4 nm).
**Figure S4.** Sample TEM images S4-AuNP and size distribution: average diameter = 1.6 nm ($\sigma$ = 0.3 nm).
Figure S5. TGA analysis of S1-AuNPs sample, under air atmosphere.

Figure S6. TGA analysis of S2-AuNPs sample, under air atmosphere.
Figure S7. TGA analysis of S3-AuNPs sample, under air atmosphere.

Figure S8. TGA analysis of S4-AuNPs sample, under air atmosphere.
Figure S9. $^1$H-NMR (500 MHz) spectrum in D$_2$O of: a) deprotected thiol S1; b) S1-AuNPs; c) diffusion filtered spectrum of S1-AuNPs. (* indicates the residual solvents).

Figure S10. $^1$H-NMR (500 MHz) spectrum of: a) deprotected thiol S2 in MeOD; b) S2-AuNPs in D$_2$O; c) diffusion filtered spectrum of S2-AuNPs in D$_2$O. (* indicates the residual solvents).
Figure S11. $^1$H-NMR (500 MHz) spectrum of: a) protected thiol 9; b) Diffusion filter of S3-AuNPs and c) $^1$HNMR of the S3-AuNPs (* indicates the residual solvents).

Figure S12. $^1$H-NMR (500 MHz) spectra of: a) Protected thiol (MeOD) 13; b) $^1$H-NMR of the S4-AuNPs and c) Diffusion filter of S4-AuNPs (D$_2$O) (* indicates the residual solvents).
Figure S13. UV-Vis spectrum of S1-AuNPs (0.1 mg/mL) at 25°C in water.

Figure S14. UV-Vis spectrum of S2-AuNPs (0.1 mg/mL) at 25°C in water. Absorbance peak of thiol aromatic moiety at 270 nm.
Figure S15. UV-Vis spectrum of S3-AuNPs (0.1 mg/mL) at 25°C in water. Absorbance peak of thiol aromatic moiety at 270nm.

Figure S16. UV-Vis spectrum of S4-AuNPs (0.1 mg/mL) at 25°C in water.
4. Fluorescence experiments

4.1 Direct titrations

The fluorescence titrations were performed using a Perkin Elmer LS50B instrument. Intensities generated upon subsequent additions of a 3mM (2µl) solution of the analytes to a 1.4µM solution of the AuNP (100µM in thiol) in buffered H₂O (HEPES 2 mM, pH=7) were recorded after the signal had stabilized (2-3 min). Each point is the average of three measurements. The fluorescence intensities were plotted vs the concentration of analyte added. The titration were fitted to the following 1:1 binding model using DynaFit for Windows.

```
[task]
data = equilibria
task = fit

[mechanism]
P + L ⇌ P.L : Kd dissoc

[constants]
Kd = 0.00000 ?

[concentrations]
P = 0.00000 ?

[data]
variable L
directory NAME
sheet NAME.csv
column 2 | response L = 2e4 ? | label I, au

[output]
directory NAME

[end]
```
**Figure S17.** Plot of the fluorescence intensities vs the concentration of serotonine (13) added. In red the fitting curve obtained from DynaFit. Conditions S1-AuNPs 0.1 mM, HEPES 2 mM.

**Figure S18.** Fluorescence spectra obtained from the titration of S1-AuNPs (0.1mM) with serotonine (solution 3mM, 2µL additions) at 25°C in water. Every spectrum is recorded three times.
Figure S19. Simulated titration experiments for different binding constants ([binding sites] = 3×10^{-5} M). From the bottom: $K = 1\times10^6 \text{ M}^{-1}$, $5\times10^5 \text{ M}^{-1}$, $2\times10^5 \text{ M}^{-1}$, $1\times10^5 \text{ M}^{-1}$, $5\times10^4 \text{ M}^{-1}$, $2\times10^4 \text{ M}^{-1}$, $1\times10^4 \text{ M}^{-1}$. Inspection of the plot clearly indicates that binding constants smaller than $5\times10^4 \text{ M}^{-1}$ (red) are difficult to be measured in these experimental conditions.
4.2 Displacement titrations

The fluorescence displacement titrations were performed using the same procedure reported in the previous section 4.1. Conditions: additions of a 3mM (2µl) solution of the analytes to a 1.4 µM solution of the AuNP (100 µM in thiol) in buffered H₂O (HEPES 2 mM, pH=7) containing dopamine 72 µM. Fitting model as follows.

```
[task]
  data = equilibria
  task = fit

[mechanism]
  P + L ⇌ P.L    :    Kd    dissoc
  P + D ⇌ P.D    :    Kc    dissoc

[constants]
  Kd = 0.000000
  Kc = 0.000000 ?

[concentrations]
  P = 0.000040 ?
  L = 0.000029

[data]
  variable D
  directory NAME
  sheet NAME.csv
  column 2 | response L = 2e7 ? | label I, au

[output]
  directory NAME/fit_tit

[end]
```
Figure S20. Plot of the fluorescence intensities vs the concentration of phenethylamine (9) added. In red the fitting curve obtained from DynaFit. Conditions S1-AuNPs 1.4 µM, Dopamine 72 µM, HEPES 2mM K(dopamine)=1.2×10^5

4.3 Comparison of S1, S2, S3, S4-AuNPs

Table S1. Data of Figure 7 of the paper. Binding parameters of analytes 10, 11, 18, 19 to S1, S2, S3, S4-AuNPs in water. The errors reported are derived from fitting errors from estimation of K.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>AuNp</th>
<th>(K, M^{-1})</th>
<th>[binding sites], M</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>S1</td>
<td>(4.1±0.4)×10^5</td>
<td>(4.2±0.1)×10^5</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>(9.3±1.2)×10^4</td>
<td>(4.0±0.3)×10^5</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>-</td>
<td>-b</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>(1.1±0.2)×10^5</td>
<td>(2.4±0.2)×10^5</td>
</tr>
<tr>
<td>11</td>
<td>S1</td>
<td>(4.8±0.5)×10^5</td>
<td>(3.9±0.1)×10^5</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>(8.5±1.5)×10^4</td>
<td>(4.7±0.4)×10^5</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>-b</td>
<td>-b</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>(1.0±0.2)×10^5</td>
<td>(2.3±0.3)×10^5</td>
</tr>
<tr>
<td>18</td>
<td>S1</td>
<td>(6.1±1.4)×10^5</td>
<td>(3.3±0.1)×10^5</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>(5.1±0.3)×10^6</td>
<td>(4.1±0.1)×10^5</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>-b</td>
<td>-b</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>(4.3±0.2)×10^6</td>
<td>(3.1±0.1)×10^5</td>
</tr>
<tr>
<td>19</td>
<td>S1</td>
<td>(2.2±0.1)×10^6</td>
<td>(5.5±0.1)×10^5</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>(1.3±0.1)×10^6</td>
<td>(5.7±0.1)×10^5</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>(3.6±0.1)×10^6</td>
<td>(4.7±0.1)×10^5</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>(8.5±0.1)×10^6</td>
<td>(5.4±0.1)×10^5</td>
</tr>
</tbody>
</table>

a) [AuNp] = 10×10^−5 M, pH 7.0 (HEPES buffer 10 mM); b) no binding observed.
5. Affinity tuning and NMR sensitivity

**Figure S21.** Plot of the log K vs log D (pH=7.4) values relative to the binding of the luminescent analytes 10, 11, 18 and 19 to S1/S4-AuNp (orange: S1-AuNp, blue: S2-AuNp, green: S3-AuNp, grey: S4-AuNp). The lines represent the linear fit of the data for S1-AuNp, S2-AuNp and S4-AuNp, in the case of S3-AuNp the green dotted line is not the result of a fit and it has been drawn with the same slope of the one relative to S2-AuNp. The blue area represents the binding constant values which cannot be measured by fluorescence titrations.

**Figure S22.** Simulated plots of the NOE pumping signal intensities for different binding constants ([binding sites] = 5×10⁻⁵ M). From the top: K = 5×10⁵ M⁻¹, 1×10⁵ M⁻¹, 5×10⁴ M⁻¹, 1×10⁴ M⁻¹, 5×10³ M⁻¹, 1×10³ M⁻¹.

The grey area evidences the typical analyte concentration used in this work (2 mM): signal intensity is not significantly affected by the binding constant when above 5×10⁴ M⁻¹.

The grey dotted line represents the limit of detection with the typical signal intensities found in this work: expected limit of detection for binding constant larger than 5×10⁴ M⁻¹ is about 0.25 mM. Larger values are expected in experiments because of the partition of the analytes in the monolayer, which increases the relaxation times.
6. Additional NMR experiments

NOE-pumping spectra were acquired as reported in previous works\textsuperscript{3,4}. Main parameters used: spectral width = 6 KHz, acquired points = 16 k, number of scans = 3072, recycle delay = 2 s, mixing time = 1.2 s, diffusion delay $\Delta = 50$ ms.

STD spectra were performed using the Bruker \textit{stddiff.2} sequence with a saturation time $D_{20}=2$ s saturating at 10000 Hz for the off resonance experiment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figureS23.png}
\caption{\textsuperscript{1}H-NMR NOE pumping-CPMGz full spectra (3072 scan, 4 h) of AuNP-S1 (14 $\mu$M in D2O), HEPES buffer (10.0 mM) and different analytes (2 mM): (a) – (k). For 4-nitrophenethylamine (e) the NOE pumping spectrum is shown (same acquisition parameters).}
\end{figure}
Figure S24. (a) $^1$H-NMR; (b) diffusion filter spectrum (640 scan, 40 min); (c) NOE-Pumping experiment (3072 scan, 4h); (d) NOE-Pumping CPMGz experiment (3072 scan, 4h). Conditions: AuNP-S2 (15 µM), phloretic acid (2 mM), HEPES (10 mM, pD 7.0), D$_2$O. Phloretic acid is not detected in these conditions.
Figure S25. Comparison between NOE pumping experiment and STD experiment (a) $^1$H-NMR of the mixture; (b) diffusion filter spectrum; (c) NOE-Pumping experiment; (d) STD-NMR experiment (on res. 40 Hz). Conditions: (15 μM), Phenylalanine (1 mM), N-Methylphenetylamine (1 mM), HEPES (10 mM, pD 7.0), D$_2$O. In the inset is highlighted the aromatic region.
**Figure S26.** N-Methylphenethylamine detection: S4-AuNP (15 μM), Phenylalanine (1 mM), N-Methylphenethylamine (from bottom to the top: 10, 30, 50, 100, 200, 500, 1000, 2000 μM), HEPES (10 mM, pD 7.0), D$_2$O: (a) $^1$H-NMR and (b) STD-NMR experiment (on res. 40 Hz) of the same mixtures.
7. $^1$H, $^{13}$C and $^{29}$Si NMR spectra of the synthesized compounds

Figure S27. $^1$H-NMR of compound 3 in CDCl$_3$.

Figure S28. $^{13}$C-NMR of compound 3 in CDCl$_3$. 

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Figure S29. $^1$H-NMR of compound 4 in MeOD.

Figure S30. $^{13}$C-NMR of compound 4 in MeOD.
Figure S31. $^1$H-NMR of compound S2 in MeOD.

Figure S32. $^1$H-NMR of compound 5 in CDCl$_3$. 
Figure S33. $^{13}$C-NMR of compound 5 in CDCl$_3$.

Figure S34. $^1$H-NMR of compound 6 in CDCl$_3$. 
Figure S35. $^{13}$C-NMR of compound 6 in CDCl$_3$.

Figure S36. $^1$H-NMR of compound 7 in CDCl$_3$. 
Figure S37. $^{13}$C-NMR of compound 7 in CDCl$_3$.

Figure S38. $^1$H-NMR of compound 8 in MeOD.
Figure S39. $^1$H-NMR of compound 9 in MeOD$_3$.

Figure S40. $^{13}$C-NMR of compound 9 in MeOD.
Figure S41. $^1$H-NMR of compound S3 in MeOD$_3$.

Figure S42. $^{13}$C-NMR of compound S3 in MeOD.
Figure S43. $^1$H-NMR of compound 10 in CDCl$_3$.

Figure S44. $^{13}$C-NMR of compound 10 in CDCl$_3$. 
Figure S45. $^{29}$Si-NMR of compound 10 in CDCl$_3$.

Figure S46. $^1$H-NMR of compound 11 in CDCl$_3$. 
Figure S47. $^{13}$C-NMR of compound 11 in CDCl$_3$.

Figure S48. $^{29}$Si-NMR of compound 11 in CDCl$_3$.
Figure S49. $^1$H-NMR of compound 12 in MeOD.

Figure S50. $^{13}$C-NMR of compound 12 in MeOD.
Figure S51. $^{29}$Si-NMR of compound 12 in MeOD.

Figure S52. $^1$H-NMR of compound 13 in MeOD.
Figure S53. $^{13}$C-NMR of compound 13 in MeOD.

Figure S54. $^{29}$Si-NMR of compound 13 in MeOD.
Figure S55. $^1$H-NMR of compound S4 in MeOD.

Figure S56. $^{13}$C-NMR of compound S4 in MeOD.
Figure S57. $^{29}$Si-NMR of compound S4 in MeOD.
References