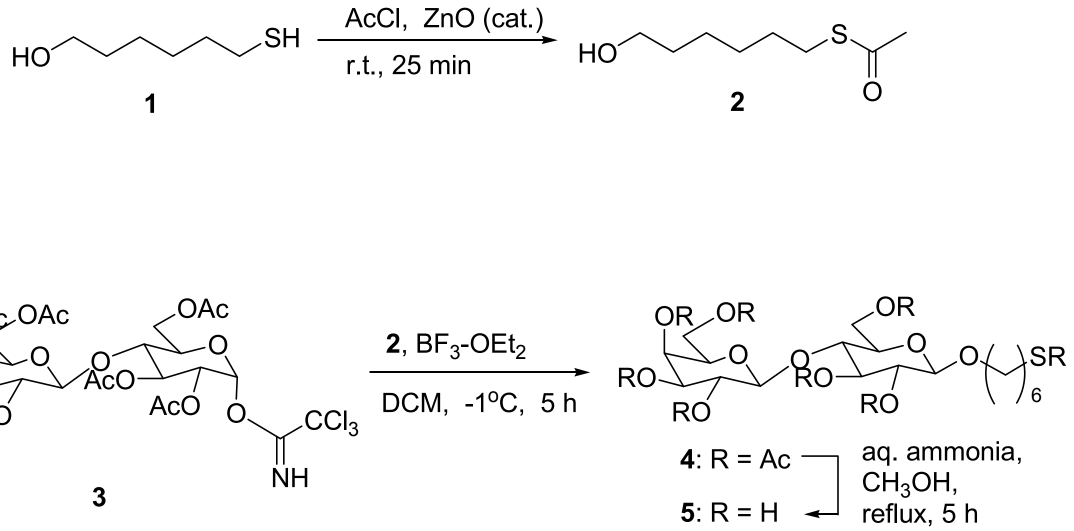
Bi-ligand functionalized gold nanoparticles-based calcium ion probe and its application to the detection of calcium ions in serum

Min Sik Eom[a], Woojeong Jang[b], Yoon Seo Lee[a], Gildon Choi[c], Yong-Uk Kwon[b]* and Min Su Han[a]*

Synthesis of 1-thiohexyl β-D-lactopyranoside



Scheme 1S. Synthetic routes for 1-thiohexyl β-D-lactopyranoside

Experimental Section

General methods. Chemical reagents were purchased from commercial sources and used without further purification unless otherwise noted. Moisture sensitive reactions were performed under argon atmosphere. NMR spectra were recorded on a Bruker AVANCE III 3000 spectrometer with tetramethylsilane as the internal standard. IR spectra were recorded on a Varian Scimitar Series spectrometer. MALDI-TOF MS was performed on a Voyager-DE STR biospectrometry workstation (Applied Biosystems) with α-hydroxy cinnamic acid as a matrix.

6-Acetylthio-1-hexanol (2).

Acetyl chloride (0.35 mL, 5.0 mmol) and zinc oxide powder (26 mg, 0.3 mmol) were mixed by stirring and 6-mercapto-1-hexanol (0.68 mL, 5.0 mmol) was then added.[1] A yellow-brown color developed immediately and became dark as the reaction progressed. After 30 min, the mixture was subjected to flash silica gel column chromatography (n-hexane/EtOAc = 20/1) to afford the pure oily product, 6-acetylthio-1-hexanol (0.56 g, 64% yield). R_f = 0.47 (n-hexane/EtOAc = 10/1); ^{1}H NMR (300 M Hz, $CDCl_3$) δ 1.31-1.47 (m, 5H, *H*O, H_2C(3) and H_2C(4)), 1.58-1.68 (m, 4H, H_2C(2) and H_2C(5)), 2.05 (s, 3H, CH_3COS), 2,53 (q, *J* = 7.4 Hz, 2H, H_2COH), 4.06 (t, *J* = 6.6 Hz, 2H, H_2CSCOCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0 , 24.5, 25.4, 28.0, 28.5, 33.8, 64.4, 171.2; IR (film) ν (cm^{-1}) 3448.0, 2964.6, 2935.7, 2017.5, 1640.9.

1-Thioacetyl-hexyl 2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-D-lactopyranoside (4).

To a solution of hepta-*O*-acetyl-α-D-lactopyranosyl trichloroacetimidate[2] (1.56 g, 2.0 mmol) in CH_2Cl_2 (15 ml) were added *S*-acetyl-6-mercapto-1-hexanol (350 mg, 2.0 mmol) and BF_3–OEt_2 (25 µL, 0.2 mmol) at −15 °C. After the mixture was stirred for 5 h, the reaction was quenched by the addition of TEA (0.1 ml). The mixture was evaporated and chromatographed on silica gel to provide the desired product (1.30 g, 82%) as a glassy white solid. R_f = 0.27 (n-hexane/EtOAc = 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33-1.45 (4H, m), 1.55-1.67 (4H, m), 1.97 (s, 3H), 2.05-2.07 (m, 15H), 2.11 (s, 3H), 2.15 (s, 3H), 2.57-2.72 (m, 2H), 3.58-3.64 (m, 1H), 3.79 (t, *J* = 9.5 Hz, 1H), 3.87 (t, *J* = 6.0 Hz, 1H), 4.02-4.17 (m, 5H), 4.44-4.50 (m, 3H), 4.90-4.98 (m, 2H), 5.11 (dd, *J* = 7.5, 10.2 Hz, 1H), 5.21 (t, *J* = 9.1 Hz, 1H), 5.35 (d, *J* = 3.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.5, 20.6, 20.7, 20.8, 20.9 21.0, 25.5, 28.3, 28.4, 29.5, 30.0, 60.8, 62.3, 64.4, 66.6, 69.1, 70.3, 70.7, 71.0, 73.8, 76.2, 83.5, 101.1, 169.1, 169.6, 169.7, 170.0, 170.1, 170.3, 170.4, 171.2; MS (MALDI-TOF) *m/z* calcd for $C_{34}H_{50}O_{19}SNa$ 817.3, found 817.5 $[M + Na]^+$.

1-Thiohexyl β-D-lactopyranoside (5).

Aqueous ammonia (30 ml) was added to a solution of 1-thioacetyl-hexyl 2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-D-lactopyranoside (250 mg, 3.1 mmol) in methanol (50 ml). The mixture was refluxed at 80 °C for 5h. The resulting mixture was concentrated in vacuo, diluted with water, and lyophilized to give 1-thiohexyl β-D-lactopyranoside (144 mg). ^{1}H NMR (300 MHz, D_2O) δ 1.21-1.63 (m, 8H), 2.57-2.73 (m, 2H), 3.26 (t, J = 9.3 Hz, 1H), 3.41-3.88 (m, 13H), 4.35 (d, J = 8.1 Hz, 1H) 4.44 (d, J = 9.3 Hz, 1H);^{13}C NMR (75MHz, D_2O) δ 24.5, 27.7, 29.2, 29.8, 31.1, 60.1, 61.0, 61.7, 68.5, 70.9, 72.0, 72.5, 75.3, 75.7, 78.1, 78.6, 85.2, 102.8; MS (MALDI-TOF) *m/z* calcd for $C_{18}H_{34}O_{11}SNa$ 481.2, found 481.3 $[M + Na]^+$.

References

1. Bandgar, B. P.; More, P. E.; Kamble, V. T.; Sawant, S. S. *Aust. J. Chem.* **2008**, *61*, 1006.
2. Chang, C. W. T.; Hui, Y.; Elchert, B.; Wang, J.; Li, J.; Rai, R. *Org. Lett.* **2002**, *4*, 4603.

Synthesis of bi-ligand functionalized gold nanoparticles

All glassware was washed with freshly prepared aqua regia (3:1=HCl:HNO_3) followed by extensive rinsing with doubly distilled H_2O. Citric acid stabilized Au particles with 13 nm diameters were prepared by adding 50 mL of a citrate solution (38.8 mM) to 500 mL of boiling 1.0 mM $HAuCl_4{\cdot}3H_2O$ with vigorous stirring. After the appearance of a deep red color, boiling and stirring continued for 15 min. The solution was then allowed to cool to room temperature with continued stirring.

Various molar ratio mixtures of 1-thiohexyl carboxylic acid and 1-thiohexyl β-D-lactopyranoside in ethanol:TDW = 1:1 were prepared for a total concentration of 0.01M. Then, 10mL of the citric acid stabilized AuNPs (11.2nM) was added to 0.9 mL of the mixture drop by drop with vigorous stirring. The mixture was then incubated for 24 hours.

Determination of the detection limit of A_2S_1-AuNPs for Ca^{2+}

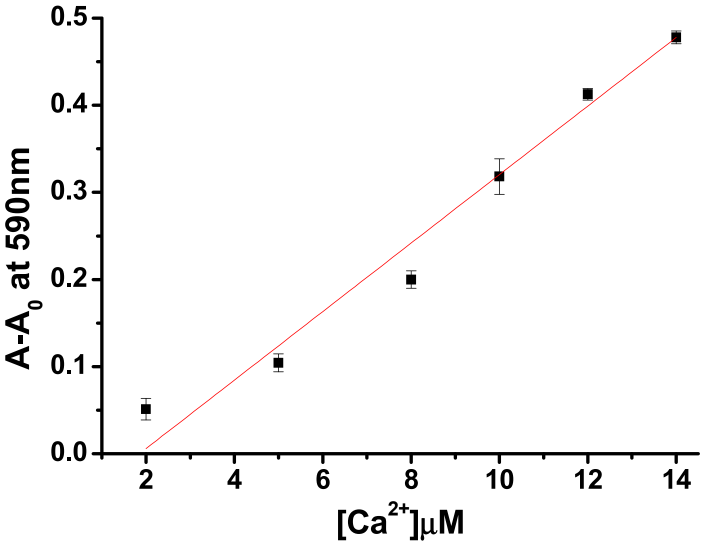


Figure S1

Intercept = -0.07283

Slope = 0.03933

$R^2 = 0.97191$

LOD = 1.85 x 10^{-6} M

1. M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland *Anal. Chem.* **1996,** *68*, 1414.

Titration curve of A_2S_1 for Ca^{2+} and Mg^{2+} in buffer solution

A stock solution of metal ions in pH 7.4 buffer solution was added to the assay solution AuNPs (3 nM) in a pH 7.4 buffer solution such that the final concentration was between 0 µM and 200 µM. Changes in the absorbance of the assay mixtures were measured 3 minutes after adding the metal ions.

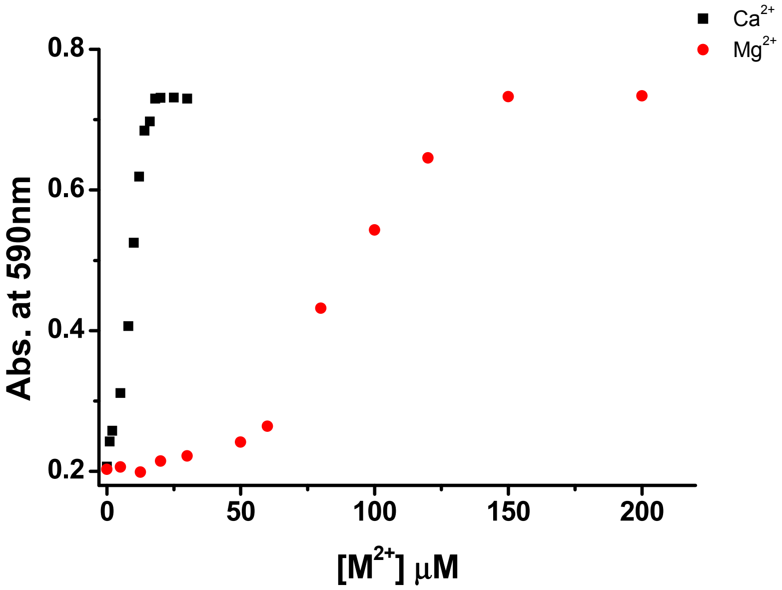


Figure S2

Comparison of titration curve of A_2S_1 for Ca^{2+} in FBS with that for Ca^{2+} in buffer solution

FBS serums were acidified to pH 2 with HCl and incubated for 3 hours at 80 ºC. After treatment, the solution was filtrated with a spin filter of 3 kDa pore size to remove serum protein. The Ca^{2+} concentration of the filtrate was determined with an inductively coupled plasma atomic emission spectrometer (ICP-AES). Based on the result from ICP-AES, the filtrate was added to the assay solution AuNPs (3 nM) in a pH 7.4 buffer solution such that the final concentration of Ca^{2+} was between 0 μM and 30 μM. Changes in the absorbance of the assay mixtures were measured 3 minutes after adding the filtrate.

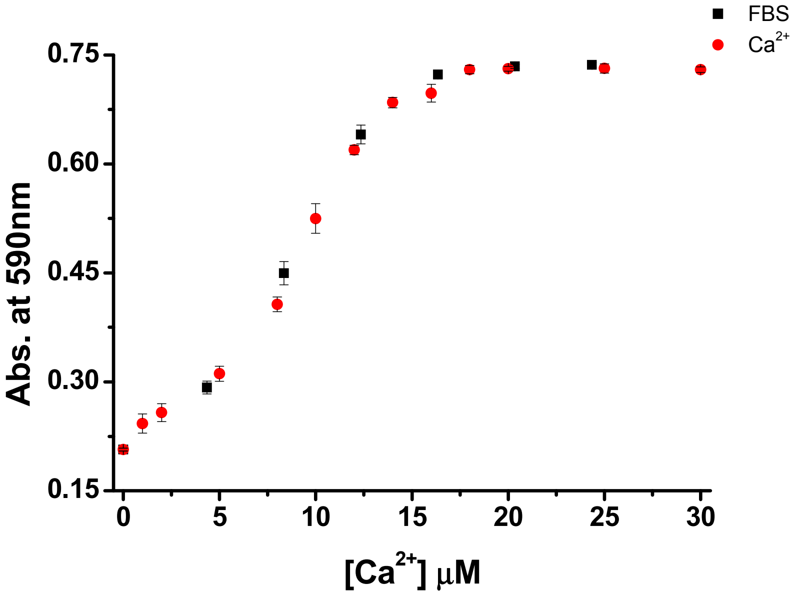


Figure S3

UV/Vis spectra of A_2S_1-AuNPs in the presence of alkaline earth metal ions

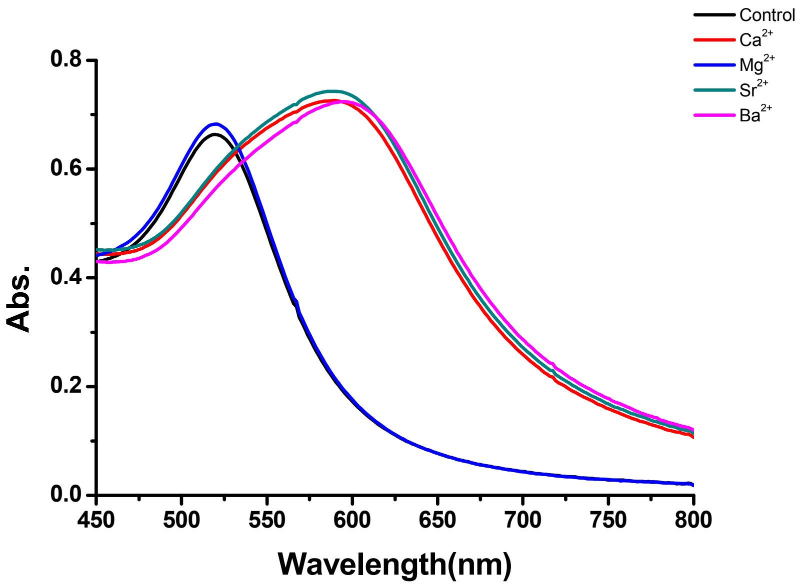


Figure S4. The UV/Vis spectra obtained 3 min after the addition of various cations (20 μM) to pH 7.4 buffer solution (Tris-HCl + 50 mM NaCl) containing A_2S_1-AuNPs (3 nM).