Supporting Infomation

Gold nanoparticle-based colorimetric chiral discrimination of histidine: Application to determining the enantiomeric excess of histidine

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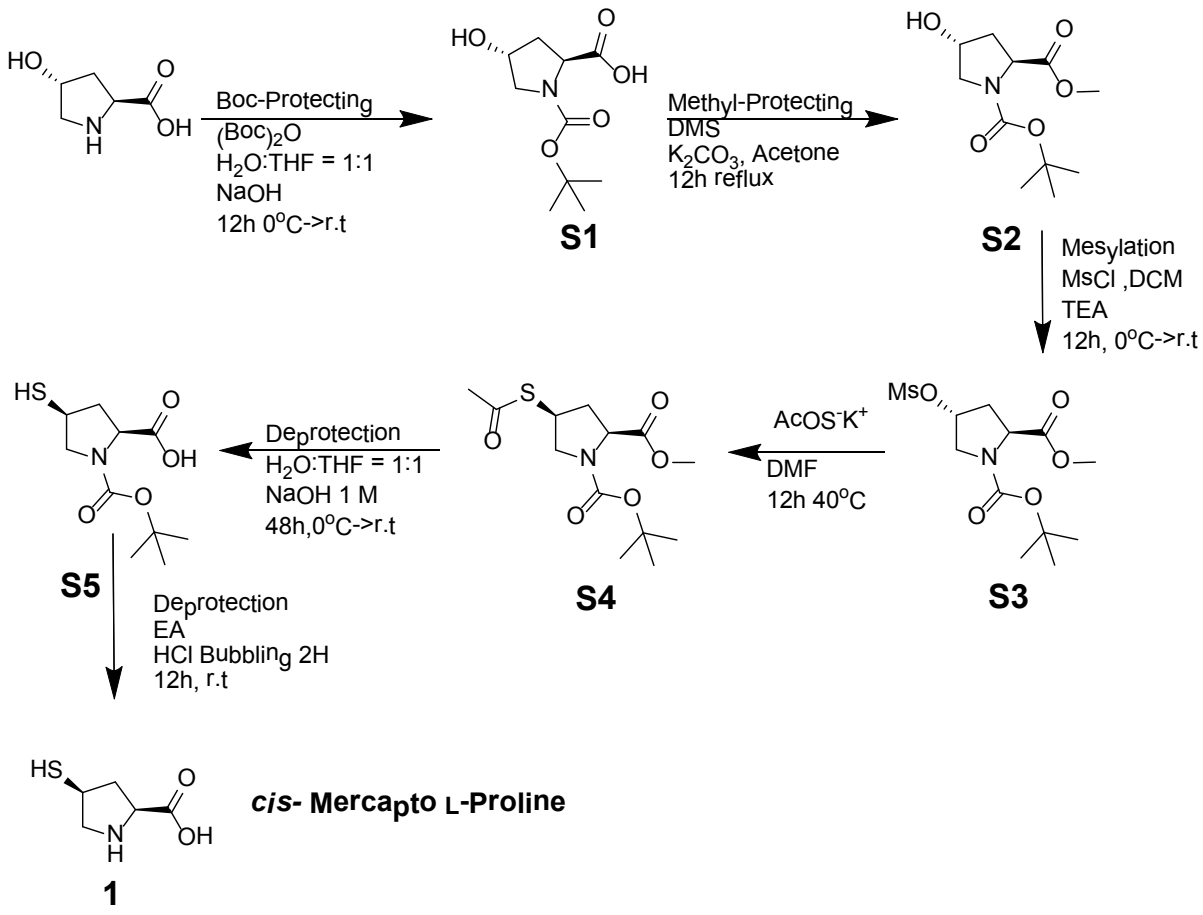
General methods

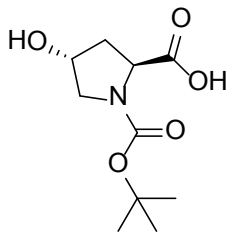
Chemical reagents were purchased from commercial sources and used without further purification unless otherwise noted. NMR spectra were recorded on a Varian Gemini 2000 (300MHz). UV-Vis spectrometric studies were carried out on an S-3100 purchased from SCINCO. Exact mass spectra were recorded on Tempo nano HPLC/QSTAR Elite from Applied Biosystems. FT-IR was recorded on Nicolet 6700 from Thermo Scientific.

Synthesis



Scheme S1. Synthetic procedure of *cis* –mercapto L-proline as ligand.

N-Boc-*trans*-4-hydroxy L-proline (S1)

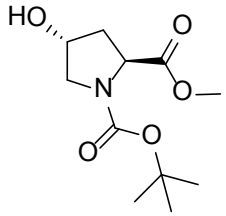


S1

trans-Hydroxy L-Proline (3.39 g, 30 mmol) was dissolved in 50 mL of 1:1 THF/H_2O. To this solution were added 50 mL of 10% (w/v) NaOH(*aq*) and Boc anhydride (7.85 g, 0.36 mol), and the resulting solution was stirred at room temperature during 12 h. The THF was removed by rotary evaporation under reduced pressure. The residue was dissolved in EtOAc, and the pH was adjusted to 2 by the addition of 10% (w/v) $KHSO_4$(*aq*).

The acidic solution was extracted several times with EtOAc. The combined organic extracts were washed with H_2O and brine, and then dried over anhydrous $MgSO_4$. The desiccant was removed by filtration, and the solvent was removed by rotary evaporation under reduced pressure to give **S1** (5.23 g, 82%) as a white solid. This compound was used next step without further purification.

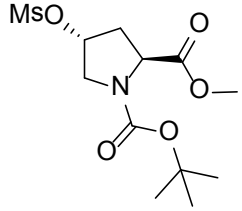
N-Boc-*trans*-4-hydroxy L-proline-methyl ester (S2)



S2

To a solution of **S1** (5.23 g, 24.5 mmol) in acetone (60 mL) was added a Dimethyl sulfide (DMS, 2 eq., 6.2 g 49 mmol) and K_2CO_3 (3 eq., 73.5 mmol) and the resulting solution was stirred at reflux condition during 12 h. The mixture was concentrated and the residue was dissolved in EtOAc. The solution washed with H_2O and brine, and then dried over anhydrous $MgSO_4$(*s*). The obtained residue was purified by silica gel chromatography [ether/EtOAc 1:1 (v/v)] to give compound **S2** as a white powder (4.81g, 19.6mmol, yield 80%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.42 (9H, 2 s, Boc), 2.05–2.31 (2H, m, Pro C3H_2), 3.48–3.67 (2H, m, Pro C5H_2), 3.75 (3H, s, $COOCH_3$), 4.39–4.51 (2H, m, Pro αCH and Pro C4H).

N-Boc-*trans*-4-mesyloxy-L-proline-methyl ester (S3)

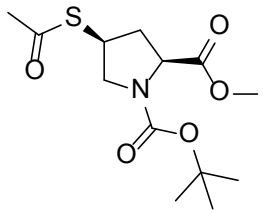


S3

A solution of **S2** (1.82 g, 5.67 mmol) and TEA (1.18 mL, 8.51 mmol) in DCM (23.0 mL) was added mesyl chloride (MsCl, 527 µL, 6.81 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then quenched with 1 M HCl(*aq*). The mixture was extracted with DCM, and the obtained organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered and evaporated. The obtained residue was purified by silica gel

chromatography [hexane/EtOAc 4:1 (v/v) *Rf* = 0.3] give compound **S3** as a white powder (5.06 g, 15.6 mmol, yield 80%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.42-1.57 (9H, 2s, Boc), 2.22–2.29, 2.60-2.70 (2H, m, Pro C3H_2), 3.06 (3H, s, CH_3SO_2), 3.70–3.88 (5H, m, Pro C5H_2 and $COOCH_3$), 4.38-4.50 (1H, m, Pro αCH), 5.24 (1H, m, Pro C4H).

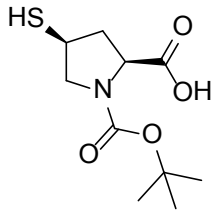
N-Boc-*cis*-4-acetylthio-L-proline-methyl ester (S4)



S4

Compound of **S3** (5.06 g, 15.6 mmol) was dissolved in DMF (80 mL) and the solution was treated with potassium thioacetate (1.94 g, 17.0 mmol). The reaction mixture was stirred at 50 ℃ for 12 h and evaporated. The concentrate was dissolved in EtOAc, and the solution was washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated. The obtained residue was purified by column chromatography [hexane/EtOAc = 4:1 (v/v) *Rf* = 0.5] to give yellowish white powder **S4** (1.48 g, 4.9 mmol, yield 31%). ^{1}H-NMR (300 MHz, CDCl3) δ 1.41, 1.46 (9H, 2s, Boc), 1.97 and 2.73 (2H, 2 m, Pro C3H_2), 2.33 (s, 3H, CH_3CO); 3.34 and 3.97 (3H, 2 m, Pro C5H_2 and Pro C4H), 3.74 (3H, s, $COOCH_3$), 4.27-4.40 (1H, m, Pro αCH).

N-Boc-*cis*-mercapto L- proline (S5)

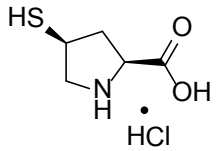


S5

Compound of **S4** (1.40 g, 4.6 mmol) was dissolved in THF (10 mL). And then the solution was slowly added 1 M NaOH(*aq*) (10 mL) at 0 ℃. The reaction mixture was stirred at room temperature for 20 h and evaporated to remove THF. The aqueous solution was diluted with sat. $NaHCO_3$(*aq*) and was washed with ether. After addition of EtOAc, the aqueous layer was acidified with 1 M HCl(*aq*) and was extracted with EtOAc. The

extract was washed with brine, dried over $MgSO_4$, filtered and evaporated. The obtained residue was purified by recristallization with ether and EtOAc as white powder. ^{1}H-NMR (300 MHz, CDCl3) δ = 1.42, 1.48 (9H, 2s, Boc), 180, 1.81 (1H, d, SH), 2.00-2.19 (1H, 2m, Pro C3H_2) 2.69-2.77 (1H, 2 m, Pro C3H_2), 3.25-3.32 (2H, 2m, Pro C5H_2 and Pro C4H), 3.93-4.01 (1H, 2m, Pro C5H_2), 4.27-4.37 (1H, 2m, Pro αCH), 9.09 (1H, br, COOH).

cis-mercapto L-proline (1)



1

Compound of **S5** (650 mg, 2.6mmol) was dissolved in EtOAc (10 mL). And then the solution was slowly added HCl(*g*) at room temperature during 2 h. The reaction mixture was stirred at room temperature during 10 h, yellowish precipitate was generated in the solution. The solution filtered to remove the solvent and remained the filtrate recrystallized with methanol and ether to give white solid (280mg, 1.9mmol 73%). ^{1}H-NMR (300 MHz, D_2O) δ = 165-1.66 (1H, d, SH), 2.14-2.20 (1H, 2m, Pro C3H_2), 2.84- 2.93 (1H, 2 m, Pro C3H_2), 3.29-3.40 (1H, 2m, Pro C5H_2), 3.67-3.87 (2H, 2m, Pro C5H_2 and Pro C4H), 4.44-4.53 (1H, 2m, Pro αCH)). FT-IR (KBr) 3436, 2970, 2842, 2750, 2561, 1965, 1738, 1633, 1404, 1372, 1263, 1017 cm^{-1}. HRMS (ESI-FTMS) *m/z* $C_5H_{10}O_2NS$ $[(M+H)^+]$ calcd for 148.0427 founded 148.0426.

Preparation of AuNPs

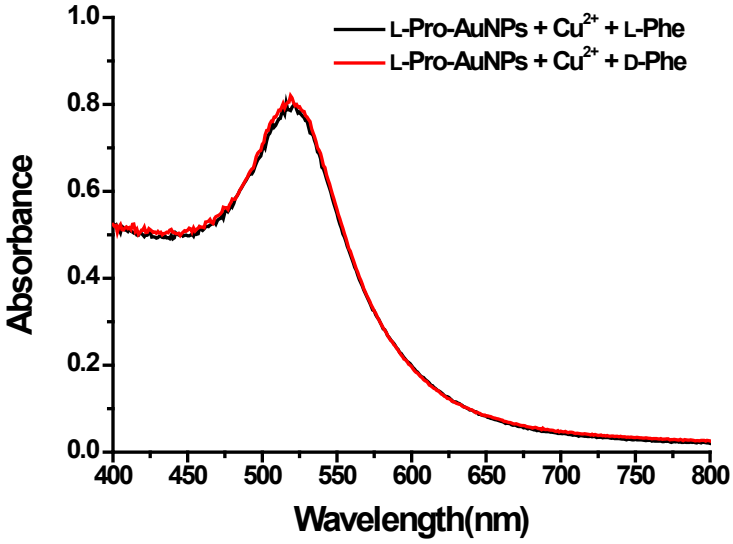
All glassware was washed with freshly prepared aqua regia (3:1 = HCl:HNO_3) followed by extensive rinsing with doubly distilled H_2O. Citric acid-stabilized Au particles with a diameter of 13 nm were prepared by adding 50 mL of a citrate solution (38.8 mM) to 500 mL of boiling 1.0 mM $HAuCl_4$ with vigorous stirring. After the appearance of a deep red color, boiling and stirring were continued for 15 min. The solution was then allowed to cool to room temperature with continued stirring to give 10.0 nM of 13 nm AuNPs.

Colorimetric assay for enantiomeric excess of histidine

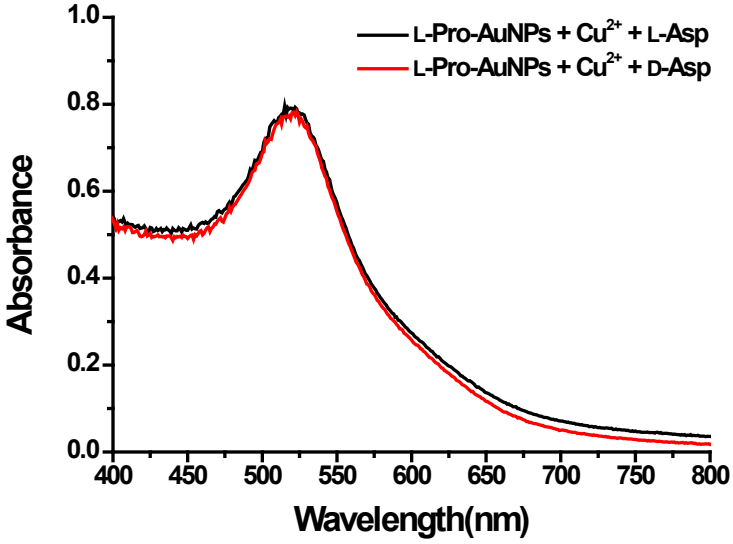
The 300 μL of 10 nM AuNPs solution was sequentially mixed with 100 μL of **1** (30 μM) solution and then, 1 min later, added to 600 μL of His (300 μM) combined with Cu^{2+} (150 μM). The mixtures were analyzed using UV/Vis spectroscopy at an extinction wavelength of 620 nm to determine the change of AuNPs solution during 15 min. The mixtures were continuously stirred at room temperature with a magnetic stir bar to ensure homogeneity.

Discrimination of various chiral amino acid

a)



b)



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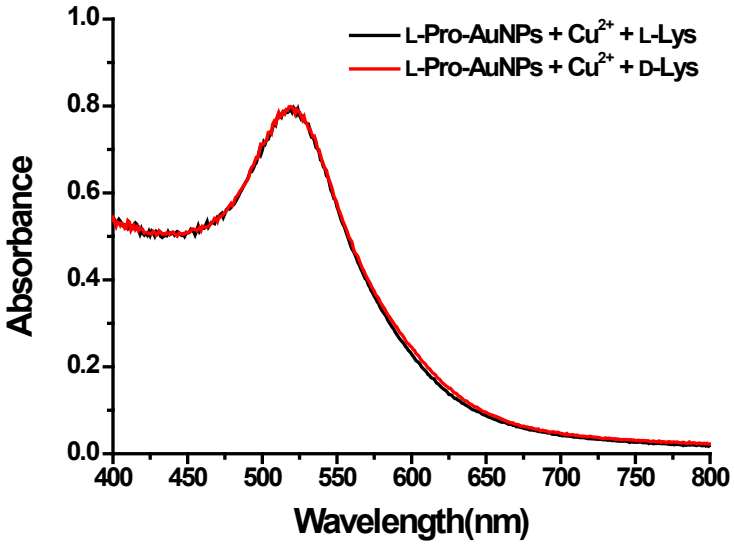
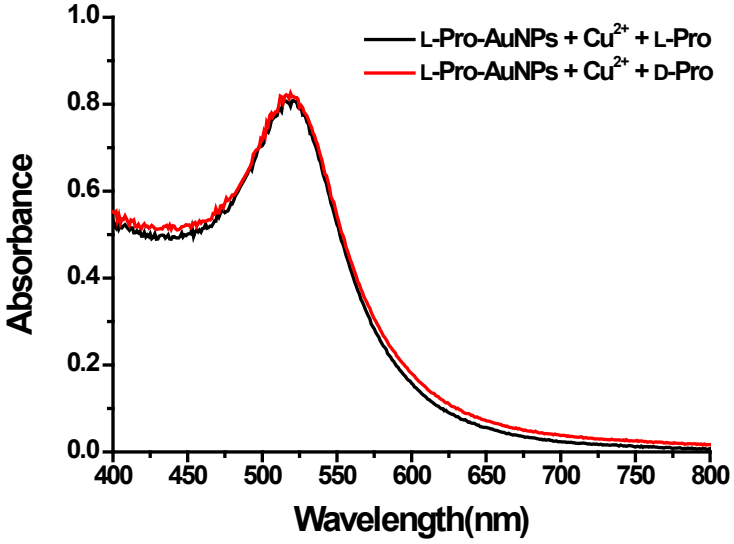


d)



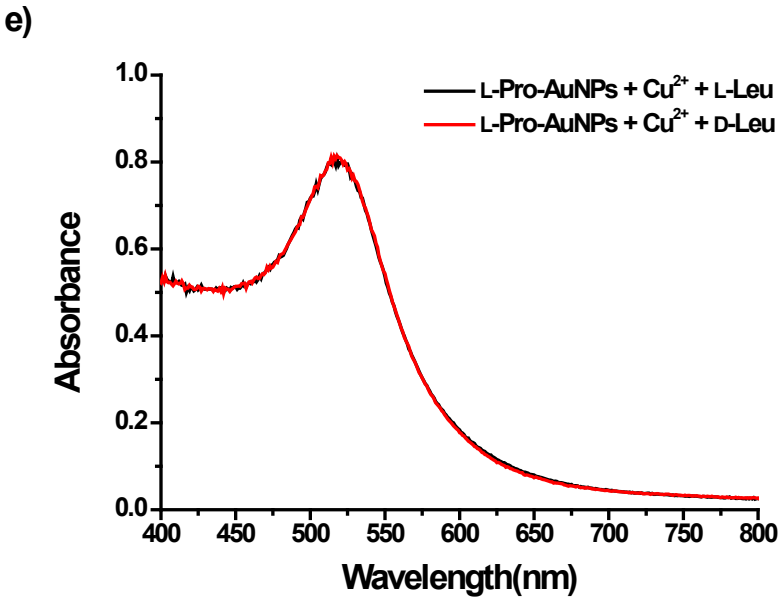
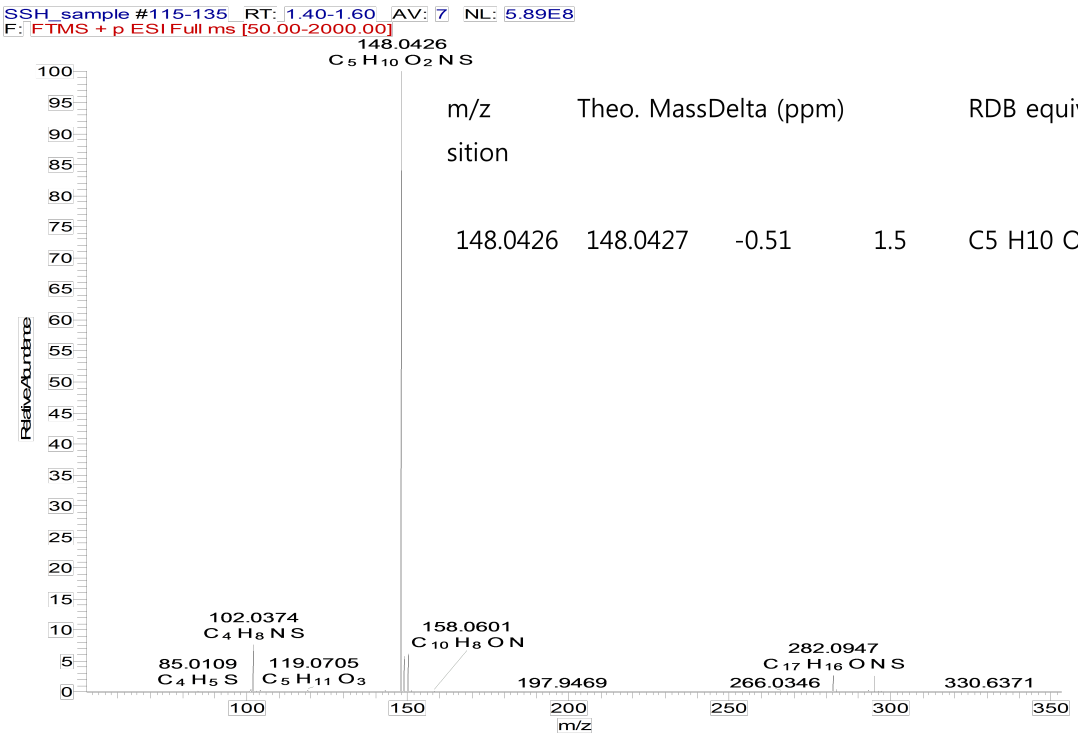


Figure S1. UV/Vis spectra obtained by the addition of enantiomerically pure L- and D-amino acid (300 μM) combined with Cu^{2+} (150 μM) after 15 min to L-Pro-AuNPs (3 nM): (a) Phe, (b) Asp, (c) Lys, (d) Pro (e) Leu**.**



m/z	Theo. Mass	Delta (ppm)		RDB equiv.	Compo sition
148.0426	148.0427	-0.51	1.5		C5 H10 O2 N S

Figure S2. HRMS spectrum of **1**

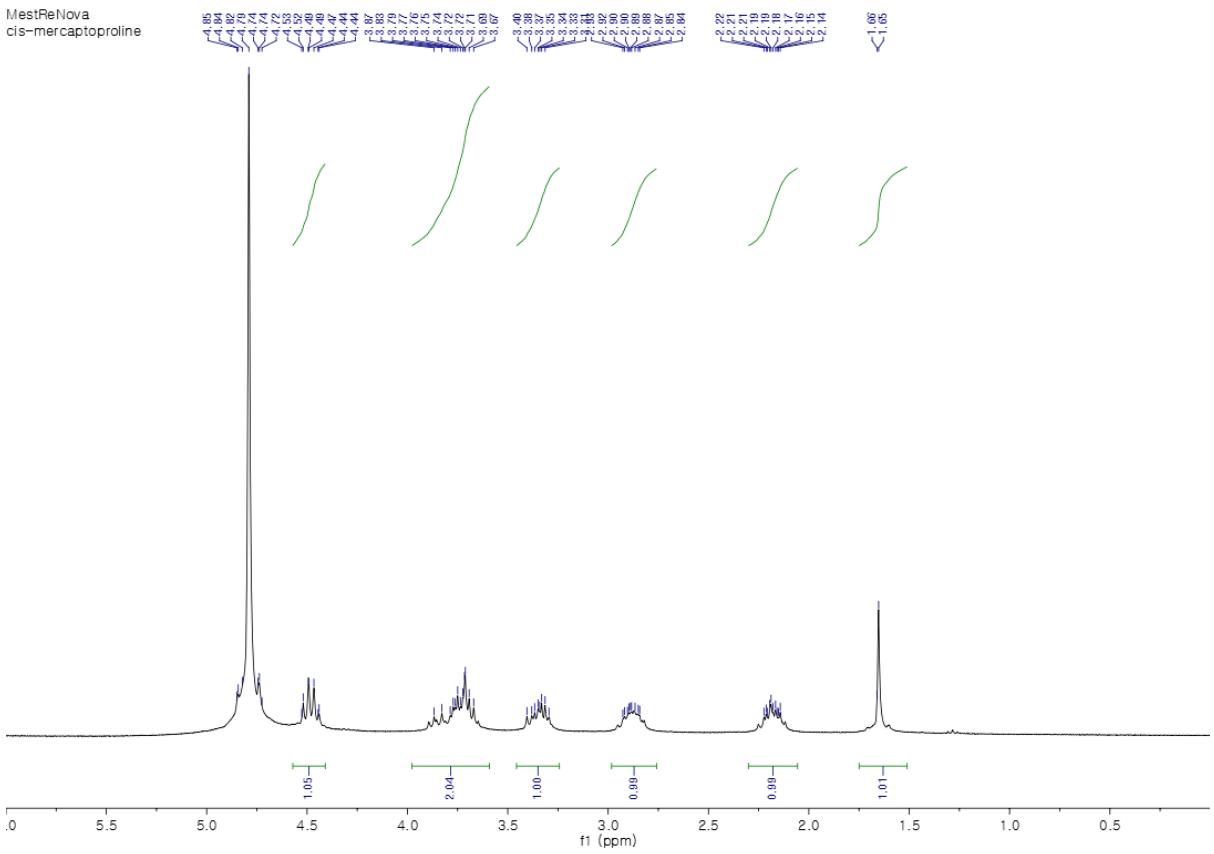


Figure S3. ^{1}H-NMR spectrum of **1**

References

1. A. Mollica, M. P. Paradisi, K. Varani, S. Spisani, G. Lucente, *Bioorg. Med. Chem.*, 2006, **14**, 2253.

2. V. Eswarakrishnan, L. Field, *J.Org. Chem.*, 1981, **46**, 4182.