Supplementary Material (ESI) for Molecular BioSystems
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Supporting Information for

Prenyltransferase substrate binding pocket flexibility and its application in isoprenoid profiling

Youli Xiao,[a] Miranda Machacek,[a] Kent Lee,[a] Tomohisha Kuzuyama,[b] and Pinghua Liu*[a]

[a] *Department of Chemistry, Boston University, MA 02215, USA. Fax: (617) 353-6466; Tel: 671-353-2481*
[b] *Biotechnology Research Center, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, JAPAN*

E-mail: pinghua@bu.edu

Table of Contents

1. General information.

HPLC kinetic analysis was carried out on SHIMADZU CBM-20A system using a phenomenex® Luna 5U 100A column (4.6 mm × 250 mm) with the following method (60% A, 1-5 min; 60% A - 10% A, 5-15 min; 10% A, 15-20 min; 10% A – 60% A, 20-25 min; 60% A, 25-30 min with a flow rate of 1 mL/min and the elution process was monitored at 228 nm. Solvent A: water; Solvent B: acetonitrile). NMR spectra were recorded on a Varian UNITY PLUS 400 (400 MHz for ^{1}H-NMR). High-resolution mass spectra were obtained at the Boston University Mass Spectrometry Laboratory using a Waters Q-TOF mass spectrometer. All reagents and solvents were used as supplied by Sigma-Aldrich and Pharmco.

2. NphB purification.

Frozen cells (5 g) of *E. coli* BL21(DE3) harboring NphB/pASK-IBA5$^+$ construct were thawed in 25 ml of buffer (100 mM Tris-HCl, 150 mM NaCl, pH 7.5). Lysozyme (1.0 mg/mL) and DNase I (100 U/g cell) were then added into the cell suspension and the mixture was incubated on ice for 40 min with gentle agitation. The cells were disrupted by sonication (10 cycles of 30 s bursts). The supernatant and the cell debris were separated by centrifugation at 4 ^{o}C for 10 min at 20,000 g. To the supernatant (25 mL), streptomycin sulfate was added to a final concentration of 1% (w/v%) and the mixture was mixed on ice for 30 min with gentle agitation. The white DNA precipitate was then separated by centrifugation at 20,000 g for 30 min. The resulting supernatant was mixed with the Strep-Tactin resin (2 mL) and incubated on ice for 30 minutes. After the cell lysate was drained by gravity, the column was washed with washing buffer (100 mM Tris-HCl, 150 mM NaCl, pH 7.5) until the OD_{280} is smaller than 0.01. The recombinant protein was eluted with the elution buffer (2.5 mM desthiobiotin in 5 mM Tris-HCl buffer, pH 7.5) and dialyzed with dialysis buffer (100 mM Tris-HCl, pH 7.5). After the protein was concentrated by ultrafiltration, it was frozen by liquid nitrogen and stored at -80 ^{o}C.

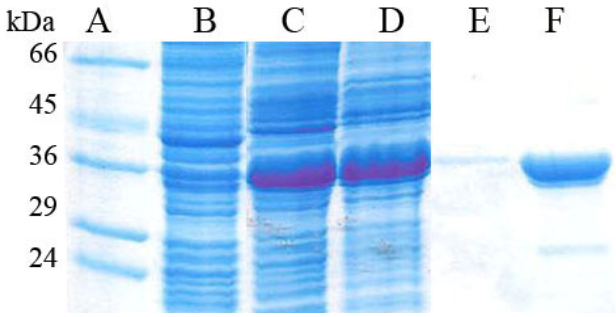
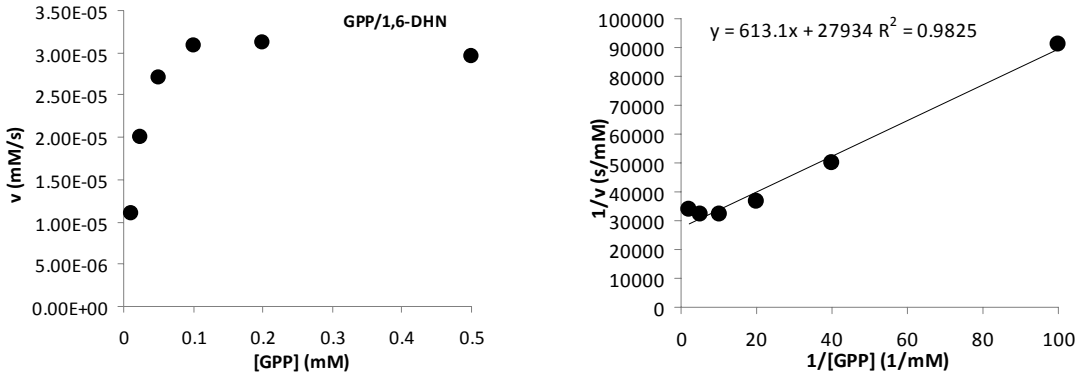


Figure 1S. SDS-PAGE analysis of purified NphB. Lane A: molecular weight makers from Sigma; Lane B: before induction with anhydrotetracycline; Lane C: after inducing; Lane D: Cell lysate; Lane E: washing fractions; Lane F: NphB eluted by desthiobiotin solution.

3. Kinetic analysis of GPP and azido-GPP.

1)



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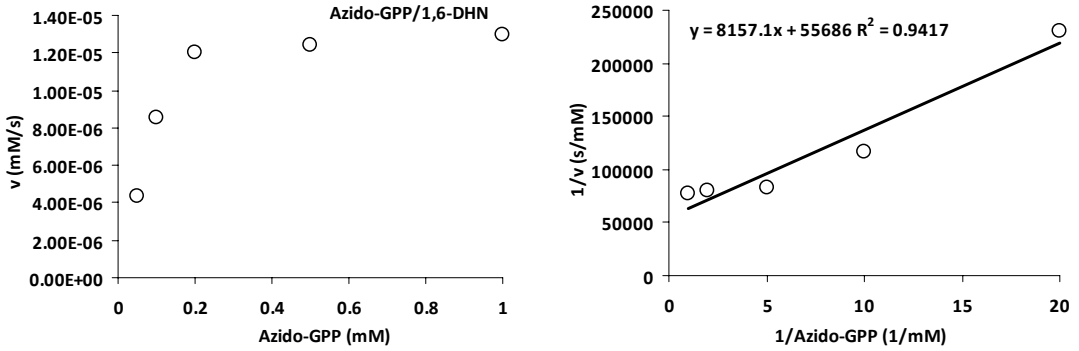
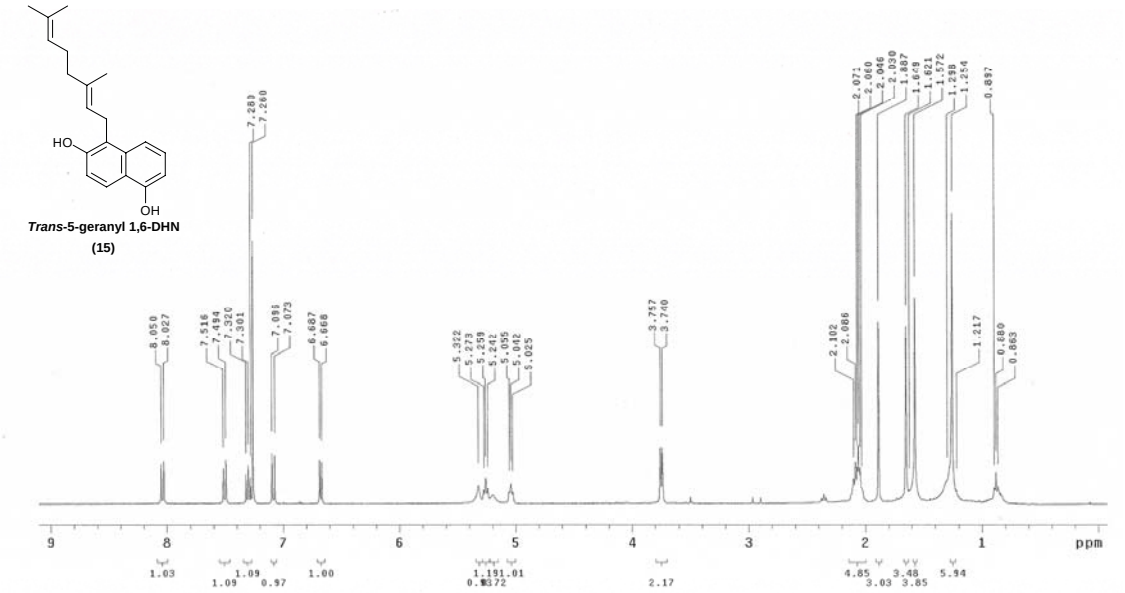


Figure 3S. Kinetic analysis of NphB using GPP and azido-GPP as substrates. Conditions: 5.0 mM 1,6-DHN, 5.0 mM $MgCl_2$, 100 mM Tris-Cl, pH = 9.0, and **1)** 0.01 mM – 0.5 mM GPP and 0.5 mg/mL NphB, **2)** 0.05 mM – 1.0 mM azido-GPP and 2.0 mg/mL NphB, respectively.

4. The ^{1}H-NMR and MS spectra of *trans*-5-geranyl 1,6-DHN.

1)



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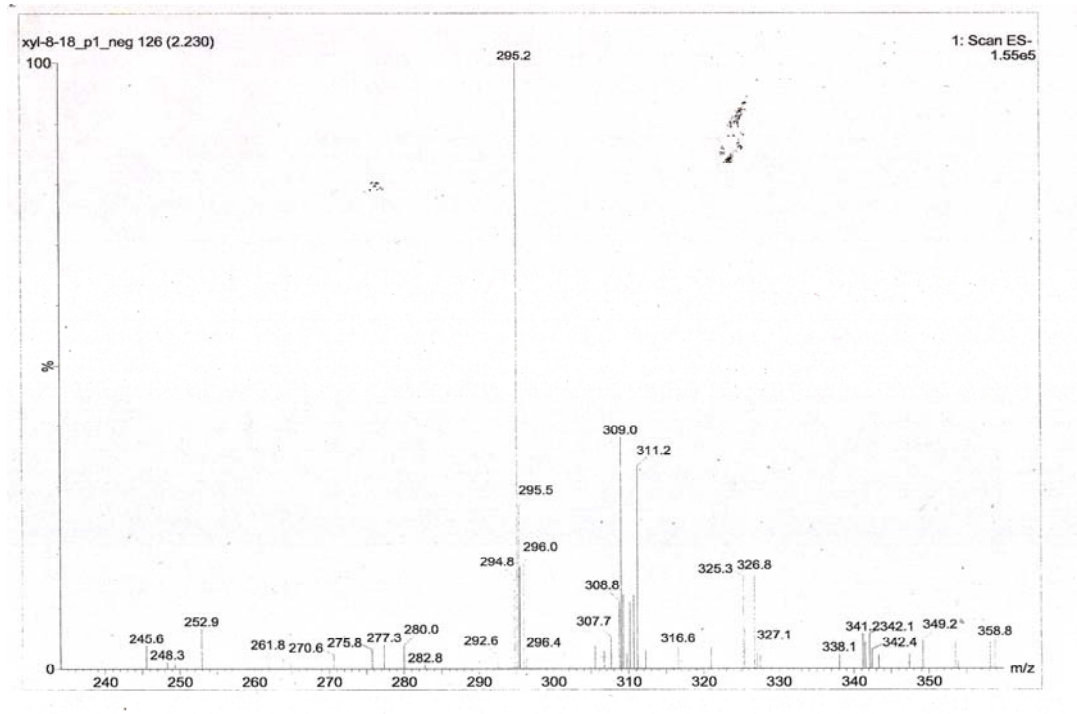
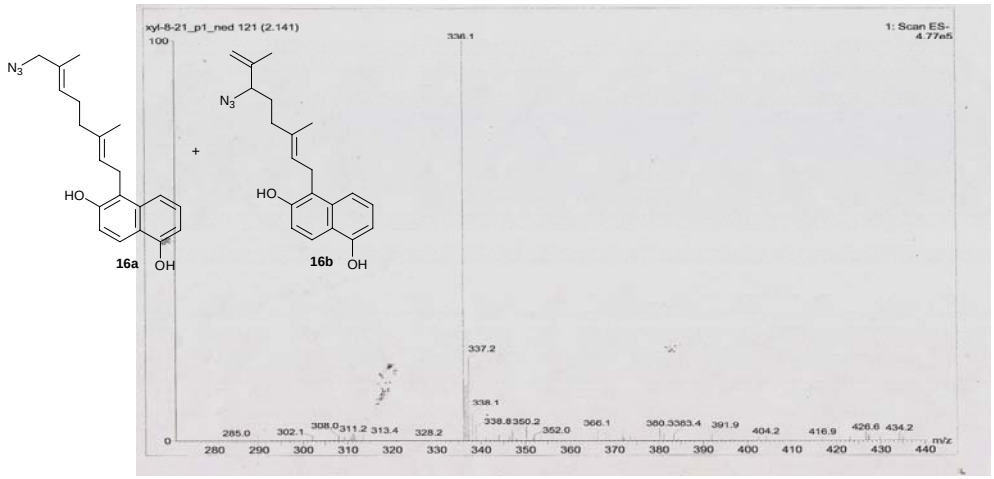


Figure 4S. 1) ^{1}H-NMR ($CDCl_3$): δ 8.04 (d, *J* = 9.2 Hz, 1 H), 7.50 (d, *J* = 8.4 Hz, 1 H), 7.30 (dd, *J* = 7.2 Hz 1 H), 7.08 (d, *J* = 9.2 Hz, 1 H), 6.68 (d, *J* = 7.2 Hz, 1 H), 5.26 (m, 1 H), 5.04 (m, 1 H), 3.75 (d, *J* = 6.4 Hz, 2 H), 2.05-2.10 (m, 4 H), 1.89 (s, 3 H), 1.65 (s, 3 H), 1.57 (s, 3 H). 2) LRMS (negative mode) calculated for $C_{20}H_{23}O_2$ $[M\text{-}H]^-$, 295.2; found at 295.2.

5. ^{1}H-NMR and MS spectra of *trans*-azido-5-geranyl 1,6-DHN.

1)



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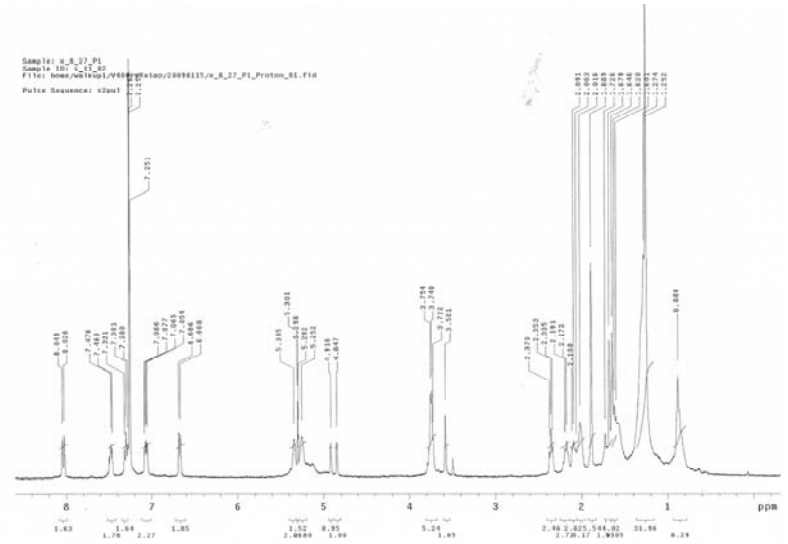


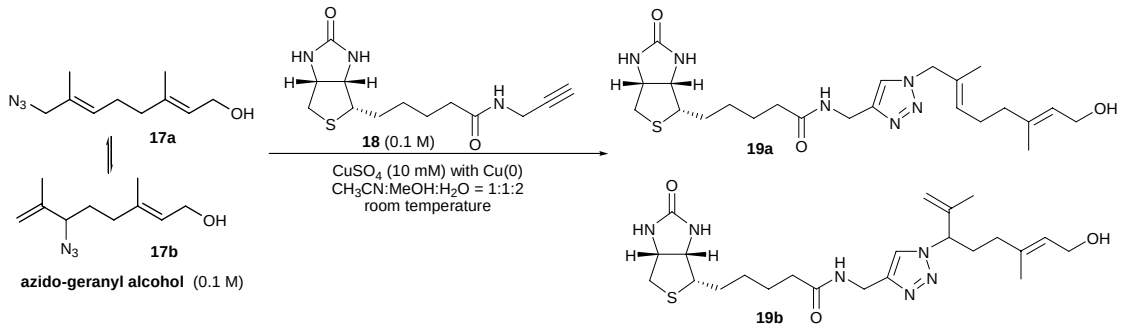
Figure 5S.

1) Low resolution MS (negative mode) calculated for $C_{20}H_{22}N_3O_2$ (**16a** & **16b**) $[M-H]^-$, 336.2; found at 336.1.

^{1}H NMR ($CDCl_3$) of **16a**: δ 8.04 (d, *J* = 8.8 Hz, 1 H), 7.32-7.48 (m, 1 H), 7.25-7.32 (m, 1 H), 7.05-7.09 (m, 1 H), 6.68 (d, *J* = 7.2 Hz, 1 H), 5.25-5.34 (m, 2 H), 3.72-3.75 (m, 2 H), 2.35 (m, 2 H), 2.02-2.19 (m, 4 H), 1.89 (s, 3 H), 1.64 (s, 3 H).

^{1}H NMR ($CDCl_3$) of the isomeric product **16b**: δ 8.04 (d, *J* = 8.8 Hz, 1 H), 7.32-7.48 (m, 1 H), 7.25-7.32 (m, 1 H), 7.05-7.09 (m, 1 H), 6.68 (d, *J* = 7.2 Hz, 1 H), 5.25-5.34 (m, 1 H), 4.92 (s, 1 H), 4.85 (s, 1 H), 3.72-3.75 (m, 2 H), 3.59 (s, 1 H), 2.02-2.19 (m, 4 H), 1.89 (s, 3 H), 1.68 (s, 3 H).

6. The MS spectrum of the product from "click chemistry" model reaction of 17 and 18.



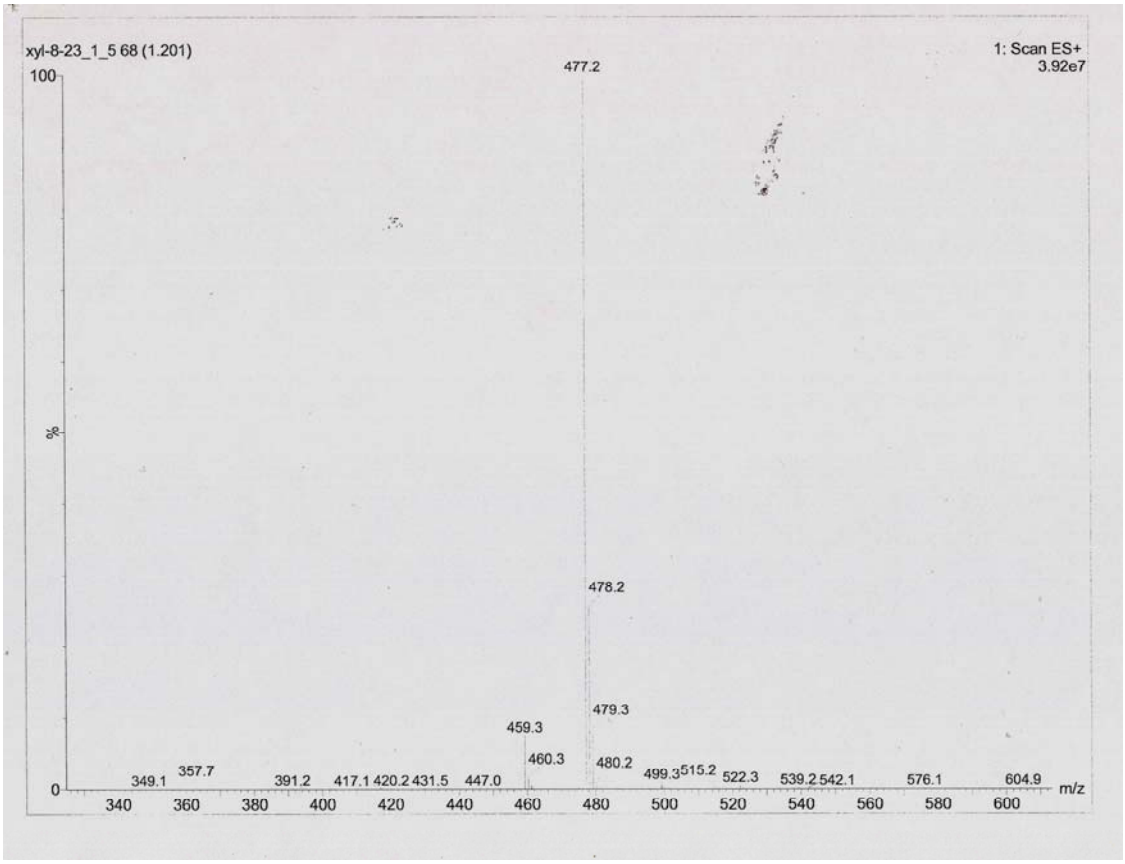
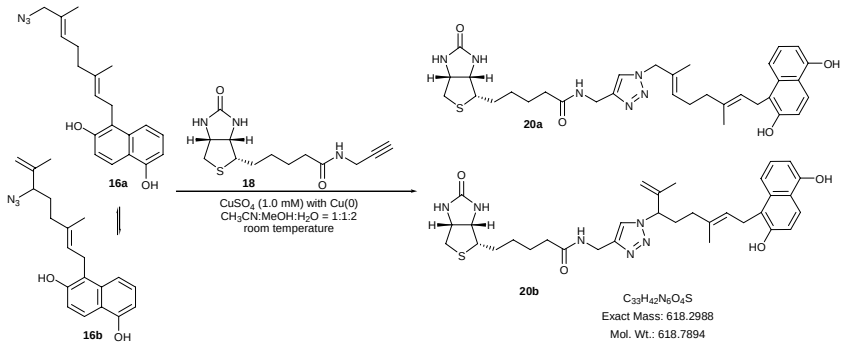


Figure 6S. MS Spectrum (positive mode) of the purified products (**19a** & **19b**). Calculated for $C_{23}H_{37}N_6O_3S$ $[M+H]^+$, 477.2; found at 477.2.

7. High resolution MS analysis of the reaction mixture of azido-tagged enzymatic products and alkyne-linked biotin molecule (18).

1)



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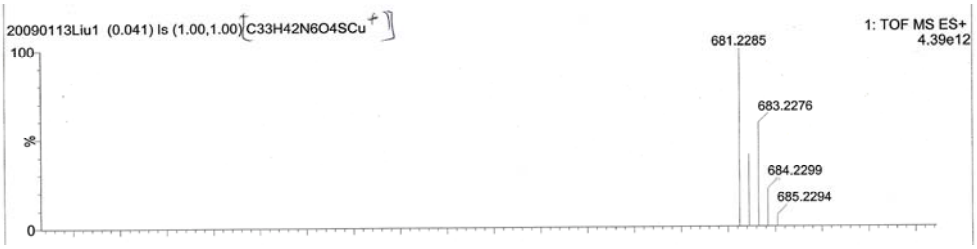

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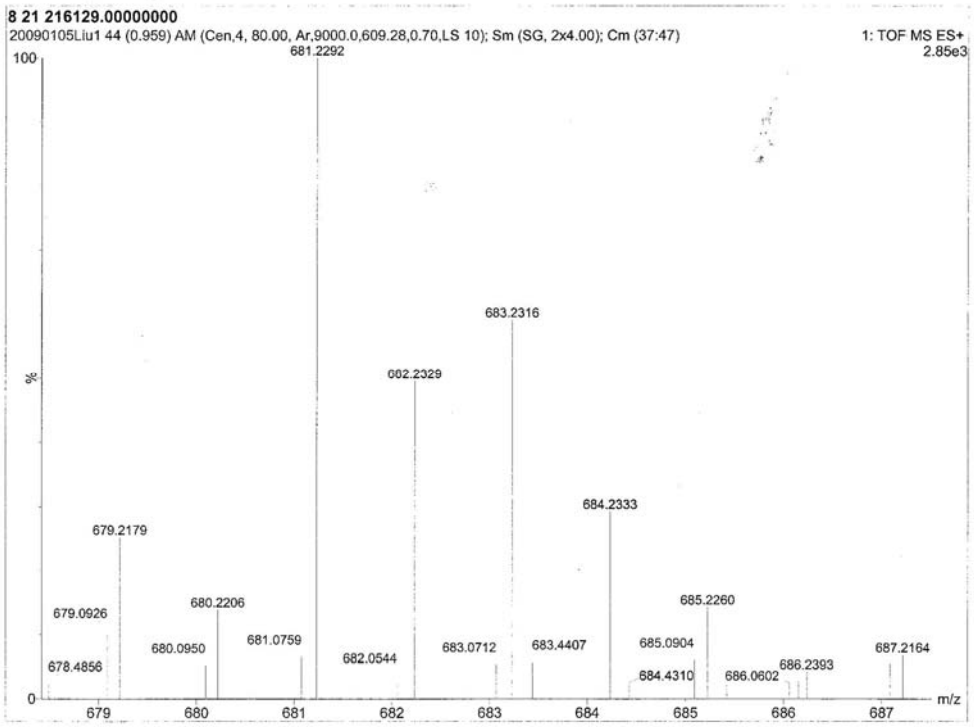

Figure 6S. 1) Reaction scheme. 2) Simulation analysis of the Cu(I) complex of the expected products. 3) High resolution mass spectrum of the reaction mixture (positive mode). Target products (**20a** & **20b**) calculated for $C_{33}H_{42}N_6O_4SCu$ $[M+Cu]^+$, 681.2285; found 681.2292.