Electronic Supplementary Information

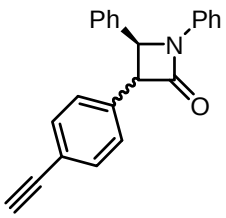
Carbon-bonded silver nanoparticles: alkyne-functionalized ligands for SERS imaging of mammalian cells

David C. Kennedy,[a] Craig McKay,[b] Li-lin Tay,[c] Yanouchka Rouleau[a] and John P. Pezacki*[a,b]

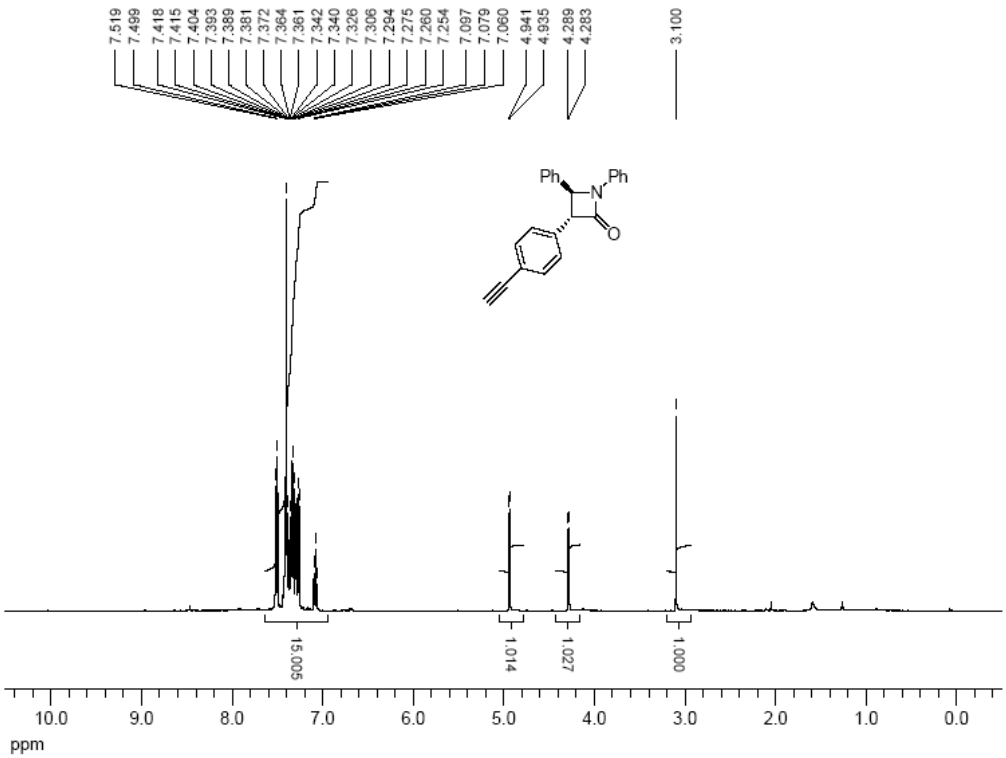
[a] *Steacie Institute for Molecular Sciences, National Research Council Canada, 100 Sussex Drive, Ottawa, K1A 0R6;*
[b] *Chemistry Department, University of Ottawa, 10 Marie Curie, Ottawa, K1N 6N5.*
[c] *Institute for Microstructural Sciences, National Research Council Canada, 1200 Montreal Road, M-50, Ottawa, K1A 0R6.*

Materials: All chemical reagents were purchased from Sigma-Aldrich and were used without further purification. EG_3SH[1] were prepared according literature procedures. Deuterated solvents were purchased from Cambridge Isotope laboratories. Thin layer chromatography (TLC) was carried out on Analtech Uniplate® silica gel plates (60 Å F254, layer thickness 250μm) using UV light to visualize the course of the reaction. Flash column chromatography was performed using silica gel (60 Å, particle size 40–63 μm). ^{1}H NMR and ^{13}C NMR spectra were obtained using a 400 MHz Bruker NMR spectrometer. Chemical shifts are reported as δ referenced to solvent and coupling constants (*J*) are reported in Hz. *N*-phenyl-α-phenylnitrone was prepared by micelle catalysis.[2] 2 mM solutions of DTSP in DMF and of EG_3SH in MeOH were prepared. Silver NPs (~25 nm in diameter) were synthesized with modified Lee and Meisel protocol.[3] Briefly, a solution of $AgNO_3$ (50.0 ml, 1.0 mM) in 18.2 MΩ deionized water was heated to boil under reflex. A 1.0 ml of 51.0 mM sodium citrate solution was added to the boiling $AgNO_3$ solution. The colour of the solution slowly turned into grayish-yellow. The solution was refluxed at boiling condition for another 60 minutes. The Ag sol was cooled to room temperature before storage at 4°C.

Procedure:



3-(4-Ethynyl-phenyl)-1,4-diphenyl-azetidin-2-one: A mechanically stirred solution of *N*-phenyl-α-phenylnitrone (297.2 mg, 1.5 mmoles) in SDS/H_2O (15 mL, 0.1 M) was cooled to 0˚C and was added (+)-Na-ascorbate (476 mg, 2.4 mmoles), $CuSO_4{\cdot}5H_2O$ (299.6 mg, 1.2 mmoles), pyridine (971 μL, 12 mmoles), ethanolamine buffer pH = 10 (1.5 mL) and 1,4-diethynyl benzene (151.4mg, 1.2 mmoles). The reaction mixture was warmed to r.t. over 30 minutes and was stirred for an additional 8 hrs. The reaction progress was monitored by TLC (9:1/Hx:EtOAc). Upon disappearance of the starting nitrone, the reaction was diluted with brine (10 mL) and extracted into EtOAC (3 x 10 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated by rotary evaporation. The resulting brown residue was purified by flash chromatography (95:5 to 90:10/Hx:EtOAc) yielded the trans (42mg, 0.13 mmoles) and cis (38 mg, 0.12 mmoles) β-lactams (21%). *trans* isomer: ^{1}H NMR (400MHz, $CDCl_3$) δ 7.52-7.06 (m, 14H), 4.94 (d, J = 2.6 Hz, 1H), 4.29 (d, J = 2.5 Hz, 1H), 3.10 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 137.3, 137.2, 135.4, 132.8, 129.4, 129.2, 128.8, 127.5, 125.9, 124.2, 121.8, 117.2, 83.2, 77.8, 64.9, 63.6. *cis* isomer: ^{1}H NMR (400MHz, $CDCl_3$) δ 7.41-7.01 (m, 14H), 4.97 (d, J = 6.1 Hz, 1H), 5.00 (d, J = 6.1 Hz, 1H), 3.00 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 137.5, 134.0, 133.0, 132.7, 131.9, 129.4, 129.1, 128.8, 128.4, 128.1, 127.5, 127.0, 125.9, 124.2, 121.8, 120.8, 117.2, 83.3, 60.2, 60.0. ESI/MS calculated for $C_{23}H_{17}NO$ M+1= 324.2, found 324.2.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1.027
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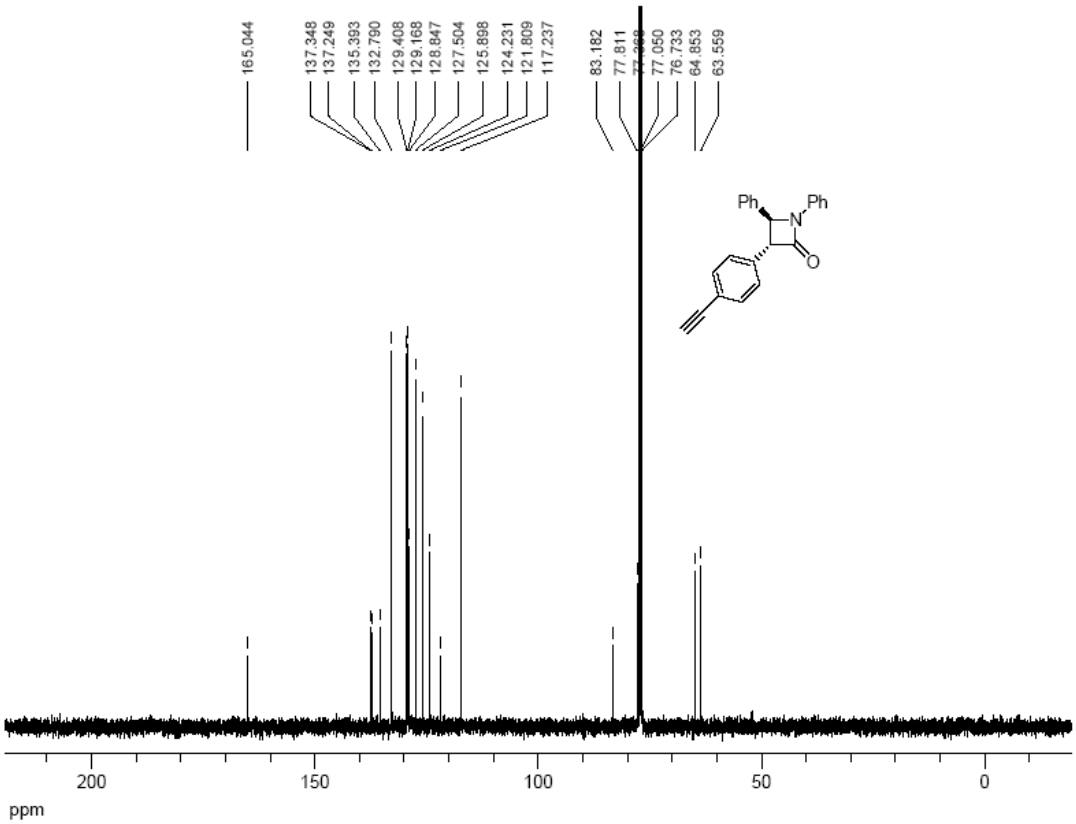
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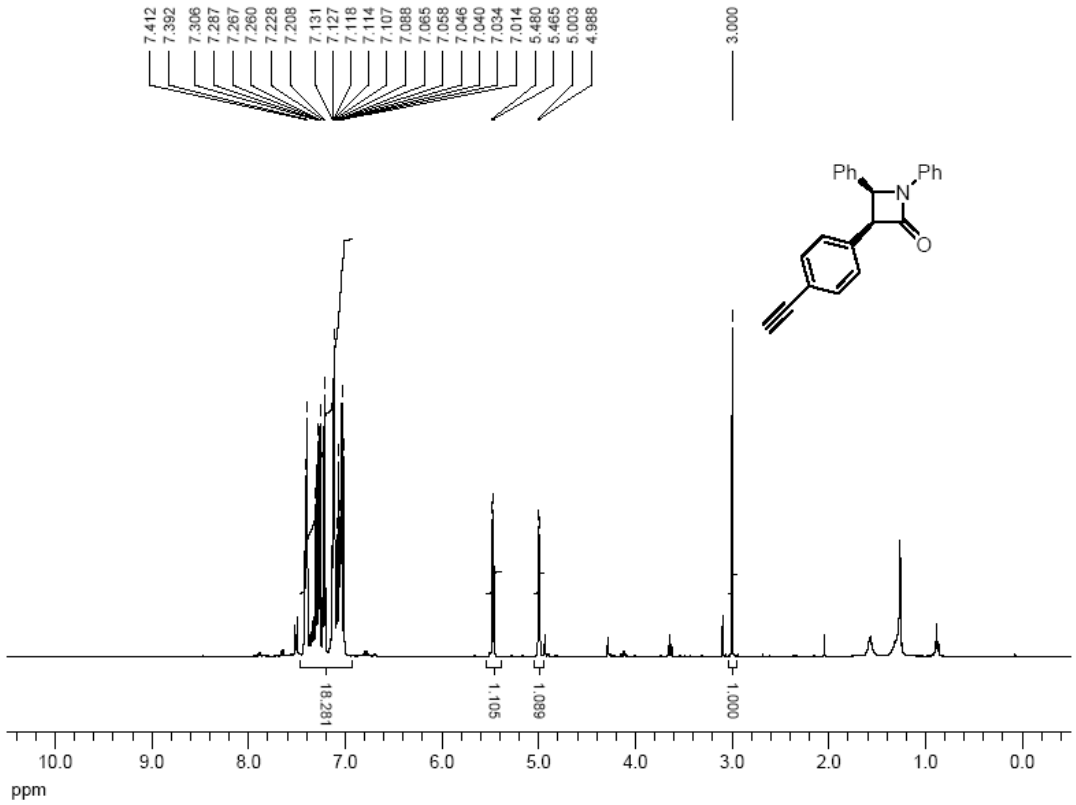
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7.014
5.480
5.465
5.003
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3.000
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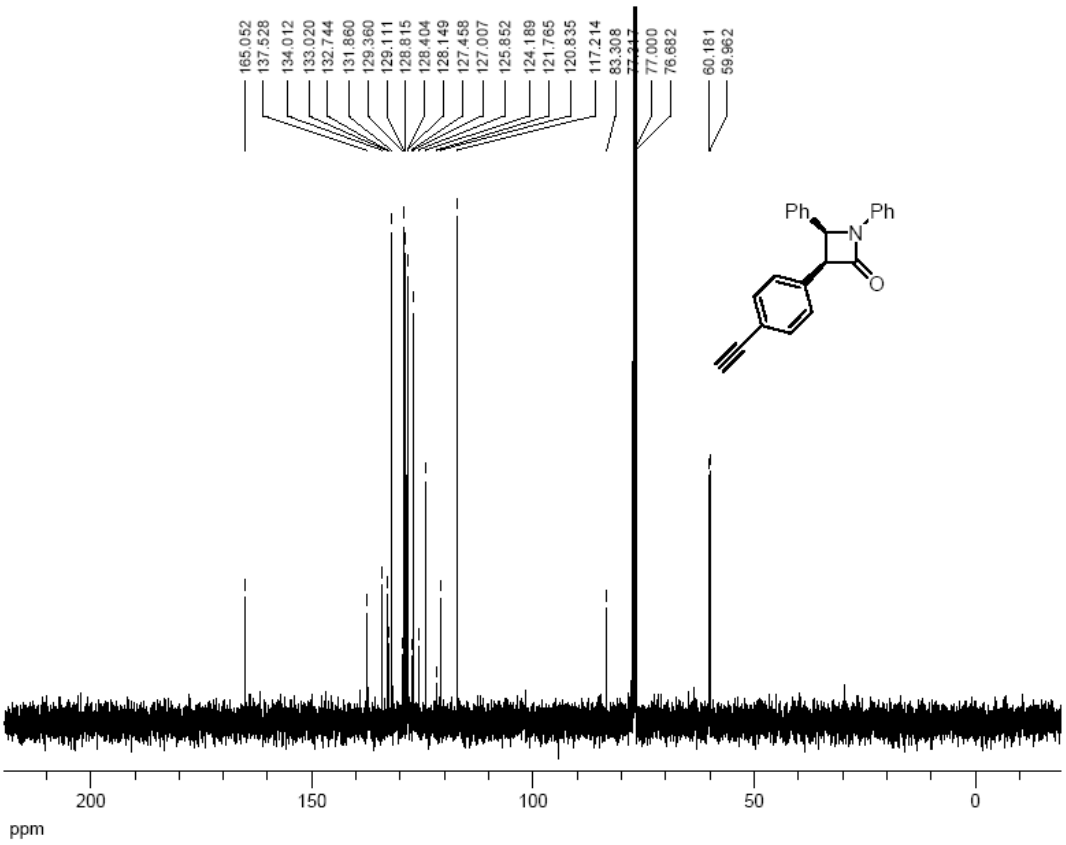
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83.308
77.317
77.000
76.682
60.181
59.962
Ph
Ph
N
O
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100
50
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ppm

Functionalization of Nanoparticles: To 1 mL of Ag particles (~10^{13}/mL) in a glass vial was added either (a) 1 μL of 2mM DTSP, 6 μL of 2mM phenylacetylene and 6 μL of 2mM EG_3SH or (b) 15 μL of 2 mM alkynyl-β-lactam. The resulting solutions were left stirring for 4 hours. Solution (b) was transferred to a 1.5 mL Eppendorf micro-centrifuge tube and spun at 13.4 k rpm for 20 min to pellet the particles. The supernatant was then removed and the particles resuspended in 500 μL of water. To (a), 50 μL of the secondary antibody solution (Affinipure bovine anti-goat IgG (H+L) 2.4 mg/mL (Jackson Immunoresearch)) was added and the resulting solution mixed by pipetting it slowly several times. This solution was left in the fridge at 4°C overnight (16 hrs). After 16 hrs, the solution was warmed to rt and 30 μL of 30% bovine serum albumin (BSA) (Sigma) was added. This solution was mixed with a pipette and left to stand for 30 min. at rt. After 30 min., the solution was transferred to a 1.5 mL Eppendorf micro-centrifuge tube and spun at 13.4 k rpm for 20 min to pellet the particles. The supernatant was then removed and the particles resuspended in 500 μL of PBS. The resulting solutions were determined to be approximately 1 nM by UV-Vis and were stored at 4°C.

Cell Culture and Sample Preparation: Huh7.5 cells (ATCC, Manassas, VA) were grown in Dulbecco's modified Eagle's medium (Invitrogen, Burlington, ON) supplemented with 10% fetal bovine serum (FBS) (NorthBio, Toronto, ON) under standard culture conditions (37 °C, 5% CO_2) and were plated onto silica wafers in a 12-well plate. After 24 hrs, cells were fixed and rinsed with PBS. To this, anti-occludin antibody (AB) (Occludin (Y-12)) goat polyclonal IgG (Santa Cruz Biotechnology 0.2 mg/mL, 4 uL used per mL PBS) was added, and the cells stored in the fridge with the AB for 24 hrs at 4 °C. Cells were then rinsed 3 times with PBS and then the 1mL NP solution in PBS was used to cover the cells after the final wash. This cells were then stored again for 24 hrs at 4 °C with the NPs. After 24 hrs, the NP solution was recovered, and cells were again rinsed with 3 times with PBS. The cells were then stored at 4 °C in PBS until imaged.

Raman Imaging and Spectroscopy: Raman spectroscopy and microscopy were acquired with a commercial microRaman system (LabRAM HR, Horiba Jobin Yvon) equipped with a software controlled XYZ stage and a thermal-electric cooled CCD detector. In typical SERS experiments, samples were excited with 632.8 nm radiation at a power density of ~ 10^4 W/cm^2. Incident radiation was coupled into an Olympus BX51 optical microscope and focused to ~ 1 μm diameter spot through a 100X objective. The same objective also collects the retro-reflected radiation and guides it to a notch filter which removes the Rayleigh radiation. In the Raman mapping experiments, a fine set of grid points within an area of interest is defined in the software and imaged by raster the sample under the tightly focused laser beam. At each of the grid point, a full Raman spectrum was acquired. The SERS images were generated with 1 second acquisition time (2 accumulations) with a power density of 10^4 W/cm^2. The multiple accumulations are necessary for the spike removal algorithm to function. Upon completion of the mapping, Raman intensity map of the C≡C vibrational mode is regenerated by fitting and removing the associated background for each spectrum in the predefined spatial grid. The C≡C intensity is displayed as a thermal map. This is achieved by the Labspec 5.25 software (Horiba Jobin Yvon). The solid Raman spectrum (Fig. 2, top) was acquired by 632.8 nm irradiation at a power density of ~ 10^5 W/cm^2.

Additional Imaging Data:

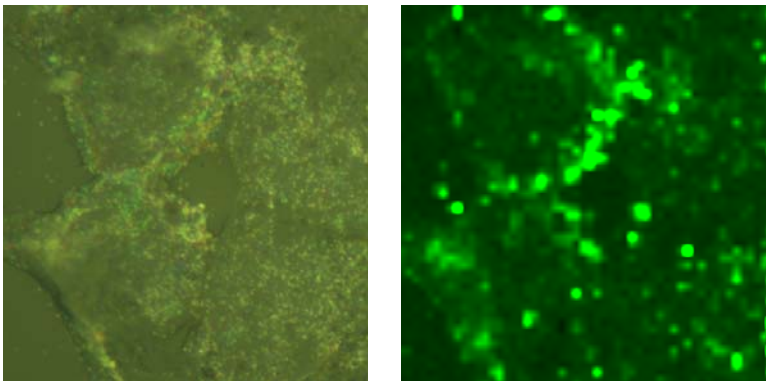

Figure S1. Additional brightfield and SERS image of Huh7.5 cells showing a high abundance of occludin localized away form tight junctions.

a) b)

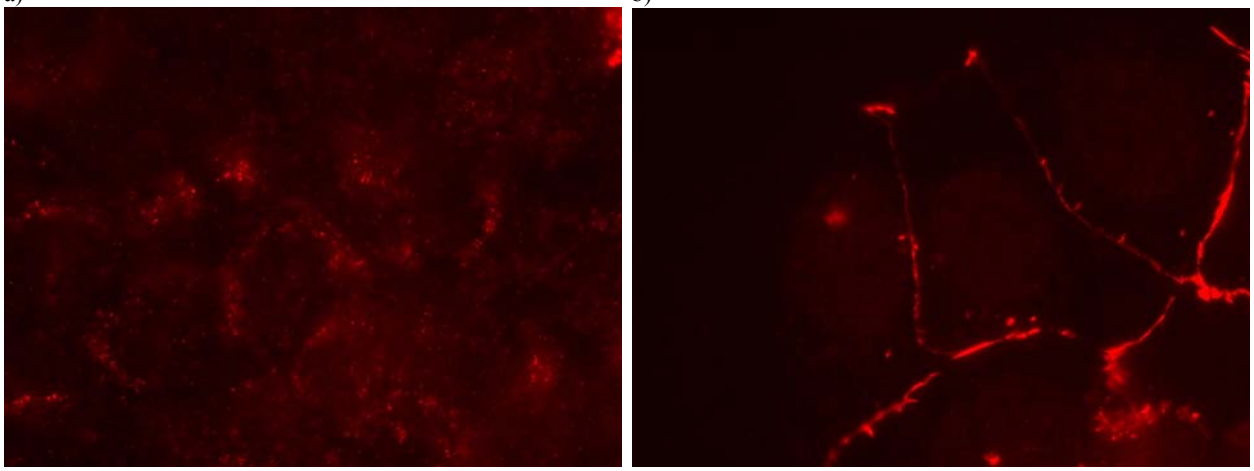

Figure S2. Fluorescence microscopy images of Huh7.5 cells showing extracellular antibody targeting (a) and intracellular antibody targeting (b).

References:

1 M. Zheng, Z. Li, and X. Huang, *Langmuir*, 2004, **20**, 4226.
2 A. Chatterjee, D.K. Maiti, P. K. Bhattacharya *Org. Lett.* 2003, **5**, 3967.
3 P.C. Lee and J. D. Meisel, *J. Phys. Chem.* 1982, **86**, 3391.