Electronic Supplementary Material (ESI) for Analytical Methods
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Electronic Supplementary information

Fischer carbene complex with hydrophilic OEG-tentacles decorates antibody surface with *in situ* generated gold nanoparticles for rapid, sensitive visual detection of proteins

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1. Materials and Methods

All reactions were carried out under an atmosphere of argon. Column chromatography was typically performed using 230-400 mesh silica gel. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance 300 or 500 MHz spectrometers at room temperature with $CDCl_3$ as solvent. IR of compounds **1**, **2**, **3** were measured on a FTIR-8300 spectrophotometer using $CHCl_3$ as solvent. The aminolysis reaction was monitored on a Nicolet 380 FT-IR spectrophotometer. UV-vis spectra were recorded on a CARY 100 Bio spectrophotometer. TEM pictures were taken on a JEM-2011 (JEOL) microscope. Synthetic reagents were bought from Sigma-Aldrich and Merck and used as received. Solvents like DCM, ether, THF were dried using standard protocols. IgG from rabbit serum (Reagent grade, > 95%, MW 150 KDa), Anti-Rabbit IgG (whole molecule, raised in goat), IgG from goat serum (Reagent grade, ≥ 95%, SDS-PAGE or HPLC), IgG from porcine serum (Reagent grade, ≥ 95%, SDS-PAGE), IgG from bovine serum (Reagent grade, ≥ 95%, SDS-PAGE or HPLC) and Nitrocellulose membrane (Protran BA 85, 0.45μm, 15 cm x 15cm) were purchased from Sigma-Aldrich.

2. Synthesis of hydrophillic Fischer carbene complex

2.1 Synthesis of 1-bromoethoxy poly(ethylene glycol) methyl ether (1)

To a stirred solution of methoxypolyethylene glycol 350 (avg. Mol. Wt ~350), (7 g, 20 mmol) in dry dichloromethane (20 ml) under argon atmosphere, carbon tetrabromide (8 g, 24 mmol) was added. Reaction mixture was cooled to 0˚C and triphenylphosphine (7.8 g, 30 mmol) dissolved in dry dichloromethane(10 ml) was added to it drop wise. After stirring for 3 hours the solvent was evaporated under reduced pressure and the residue was extracted with cold ether (3 x 50 ml). The combined ether layers were concentrated to one-third of its volume in rotavap and stored in refrigerator overnight followed by filtering off the precipitate formed. Then the ether was completely removed under vaccum. The residue was subjected to flash column chromatography using methanol/ dichloromethane (2:98) to afford the product **1** (R_f ~ 0.26 in 2 % methanol/DCM), 3.85 g (55%).

Compound **1**:	Colourless liquid
IR ($CHCl_3$, in cm^{-1})	2871.8, 1462.9, 1350.1, 1252.7, 1109, 970.1
^{1}H NMR ($CDCl_3$, 500MHz)	δ 3.23 (s, 3H), 3.32 (t, 2H, J = 6.25 Hz), 3.40 (t, 2H, J = 2.5 Hz), 3.46-3.55 (m, 22H), 3.66 (t, 2H, J = 6.25 Hz)
^{13}C NMR ($CDCl_3$, 125 MHz)	δ 14.16, 22.72, 29.38, 30.37, 31.55, 31.95, 49.56, 59.06, 70.55, 70.63, 70.71, 71.26, 71.98, 78.45
HRMS (Molecular formula $C_{15}H_{31}O_7Br$)	Calc $[M+Na]^+$ 425.1151
	Found $[M+Na]^+$ 425.1151

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2.2 Synthesis of 3, 4-bis(methyl polyethylene glycoxy)benzaldehyde (2)

In a 25 ml two necked round bottomed flask, K_2CO_3 (1.4 g, 10 mmol) was taken and activated under reduced pressure for 15 minutes. Then it was cooled and dry THF was added. 3, 4-dihydroxybenzldehyede (415 mg, 3 mmol) was dissolved in dry THF and the solution was added drop wise to K_2CO_3 soluiton and refluxed for 30 minutes. Then the bromo compound (**1**) was added with a syringe and refluxed for 72 hours. Then the THF was removed under vacuum. Excess K_2CO_3 was filtered through celite using ethyl acetate. The ethyl acetate layer was then washed with 2 M NaOH (4 ml) and then washed with distilled water thrice using a separating funnel. The ethyl acetate solution was dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography using methanol/ dichloromethane (4:96) to afford the product **2** (R_f 0.2 in 3% MeOH / DCM), 1.3g (65%).

Compound **2**:	Colourless liquid
IR ($CHCl_3$, in cm^{-1})	2873.7, 1681.8 (ν_{-CHO}), 1595, 1585.4, 1510, 1454.2, 1436.9, 1350.1, 1269.07, 1107.1, 950.8, 848.6
^1H NMR ($CDCl_3$, 500 MHz)	δ 3.36 (s, 6H), 3.53 (t, 4H, J = 4.5 Hz), 3.62-3.65 (m, 40H), 3.72 (t, 4H, J = 4.75 Hz), 3.86-3.90 (m, 4H), 4.19-4.24(m, 4H), 6.99(d, 1H, J = 8 Hz), 7.42 (d, 2H, J = 6 Hz), 9.82 (s, 1H)
^{13}C NMR ($CDCl_3$, 125 MHz)	δ 59.04, 68.71, 68.78, 69.46, 69.59, 70.53, 70.60, 70.63, 70.69, 70.92, 70.98, 71.97, 112.14, 112.69, 126.63, 130.35, 149.24, 154.40, 190.86
HRMS (Molecular formula $C_{37}H_{66}O_{17}$)	Calc $[M+Na]^+$ 805.4198 Found $[M+Na]^+$ 805.4197

2.3 Synthesis of Fischer carbene complex (3)

The methyl carbene complex (**a**)[1] $(CO)_5W{=}C(Me)OMe$ (382 mg, 1mmol) was taken in a r. b. flask under argon atmosphere and the aldehyde (**2**) (790 mg, 1 mmol) dissolved in dry ether was added to it. Then dry Et_3N (0.6 ml, 4 mmol) and dry TMSCl (0.5 ml, 3 mmol) was added successively and stirred at room temperature for 20 hours. Solvent was evaporated and extracted with ether. Flash chromatography with acetone/ petroleum ether (45:55) afforded the product as an inseparable mixture of *cis* and *trans* isomers 3a and 3b (R_f 0.3 in 50% acetone/ pet ether), 440 mg (56% yield).

Compound **3**:	Dark red sticky liquid
IR ($CHCl_3$, in cm^{-1})	2921.1, 2872.5, 2061.9, 1929.6, 1267.7, 1108.5
^1H NMR ($CDCl_3$, 300 MHz)	δ 3.37 (s, 12H), 3.54 (t, 8H, J = 4.05 Hz), 3.64-3.79 (m, 80H), 3.84-3.90 (m, 8H), 4.14-4.21 (m, 8H), 4.50 (s, 3H), 4.62 (s, 3H), 6.68-6.73 (m, 3H), 6.85 (d, 3H, J = 6.7 Hz), 6.92 (2H, J = 8.2 Hz), 7.16-7.25 (m, 8H), 7.73 (d, 2H, J = 15.3Hz)

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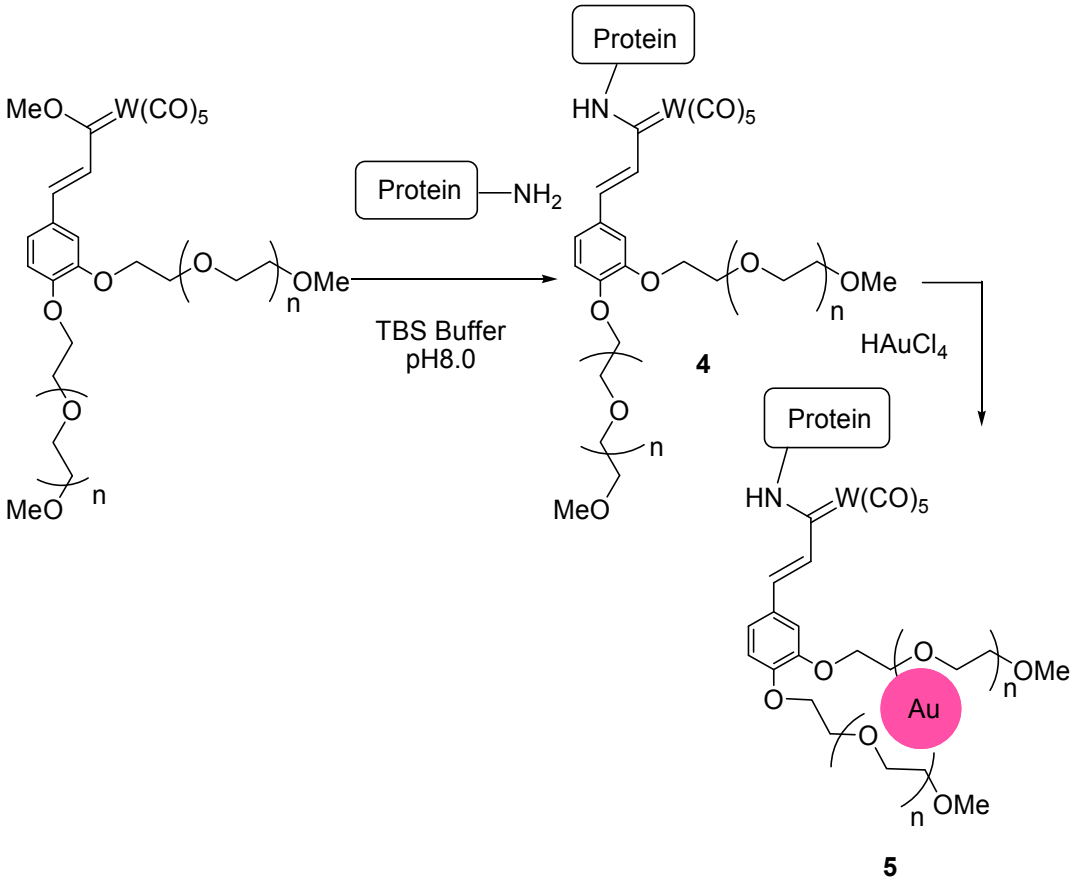
^{13}C NMR ($CDCl_3$, 75 MHz) δ 33.00, 37.73, 59.00, 68.62, 68.74, 69.00, 69.12, 69.50, 69.70, 69.77, 70.53, 70.67, 70.76, 70.85, 70.89, 71.91, 113.96, 114.67, 115.05, 115.22, 124.85, 127.67, 136.05, 141.76, 149.18, 152.24, 197.71, 198.37, 203.76, 305.29

HRMS (Molecular formula $C_{45}H_{70}O_{22}W$) Calc $[M+K]^+$ 1185.3505
Found $[M+K]^+$ 1185.3510

3. Protocol for protein detection‡

A solution of Anti-rabbit IgG (3 μM, 5 ml), raised in goat, that binds specifically to Rabbit-IgG, in Tris- buffered saline (TBS, pH 8.0) was initially treated with the PEG-tethered, hydrophilic Fischer carbene complex **3** (0.1 mM, 0.5 ml). To a solution (1 ml) of the Fischer carbene–antibody conjugate, 100 μl of 0.1 mM aqueous solution of $HAuCl_4$ was added and the solution was allowed to stand for 5 minutes (Scheme S-1). The colloidal suspension was centrifuged at 1350 rpm for 5 minutes to separate the AuNp-tagged antibody which was re-dispersed in 5 ml of fresh buffer.



Scheme S-1. Aminolysis of Fischer carbene and generation of AuNp-tagged antibody

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10 μl of 1 μM Rabbit-IgG (10^{-11} moles of protein) from rabbit serum in TBS (pH 8.0) was immobilized by spotting the same on the nitrocellulose membrane (9.2 mm x 1.3 mm) with a micropipette.

Control experiments were conducted to reveal the selectivity and specificity of the recognition reaction by spotting 10 μl of 1 μM IgG from bovine serum, porcine serum and goat serum in TBS buffer on the same nitrocellulose membrane (as positive control). The membrane was blocked with 5% casein for 30 minutes and washed with TBS-T (TBS pH 8.0 containing 0.05% Tween 20) to avoid any non-specific adsorption of protein. The immobilized antigens were then incubated for a period of 15 minutes at room temperature by immersing in 5 ml of the pink solution (3 μM) containing the AuNp-tagged antibody **5**. The experiments were performed in duplicate to eliminate errors that could arise due to washing related change of concentration of the microspotted target antigens.

To study the sensitivity of this method, Rabbit IgG solutions of lower concentrations (10^{-7}M, 10^{-8} M, 10^{-9} M corresponding to 1 pmol, 10^{-13} mol, 10^{-14} mol and 1 fmol of protein respectively) were prepared and spotted on the nitrocellulose membrane. Similarly, Anti-rabbit IgG solutions of different concentrations (3, 2, 1 μM) were prepared in TBS and used for detection once the minimum detectable limit of the bound antigen was known.

10 μl of Rabbit serum was spotted on a piece of nitrocellulose membrane. After blocking the rest of the membrane with 5% casein, the membrane was thoroughly washed with TBS-T and immersed in the AuNp-tagged antibody solution. Unlike the previous experiments the membrane had to be kept for a few hours to show a faint rose pink spot.

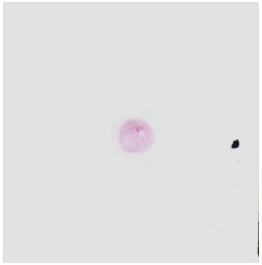

Fig S-1. Detection of Rabit IgG in serum

‡ Note: The general procedure for ELISA on membranes was followed.[2,3,4]

4. UV-vis absorption spectroscopy

UV-vis spectrum of Fischer carbene in aqueous solution was recorded. A peak was observed at 406 nm. Upon treatment with Anti-Rabbit IgG, aminolysis started corresponding to which a decrease in the peak intensity of the UV absorption band was noted. The spectral background absorption was corrected by using the UV-vis spectrum of water.

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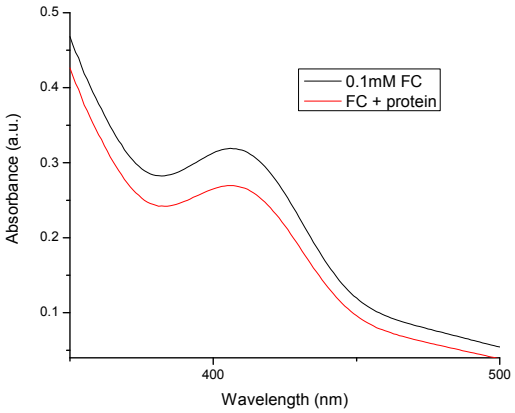

Fig S-2. UV-vis spectrum showing decrease in peak intensity at 406 nm due to aminolysis at carbene centre

5. IR spectroscopy

On treating the Fischer carbene complex with Anti-rabbit IgG, there was a shift in peak position from ~1930 cm^{-1} to ~ 1917 cm^{-1} due to aminolysis at the carbene centre.

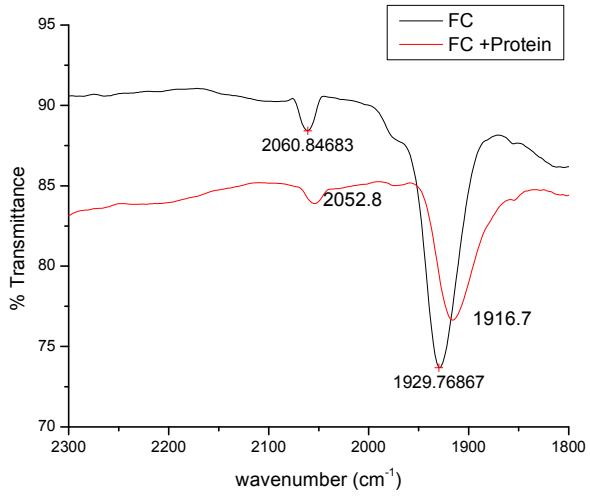

Fig S-3i. IR spectrum showing shift in peak position due to aminolysis

The shifted peak of amino-carbene complex was present even after treating the Fischer carbene-antibody conjugate with $HAuCl_4$ indicating some of the residual $W(CO)_5$ fragment.

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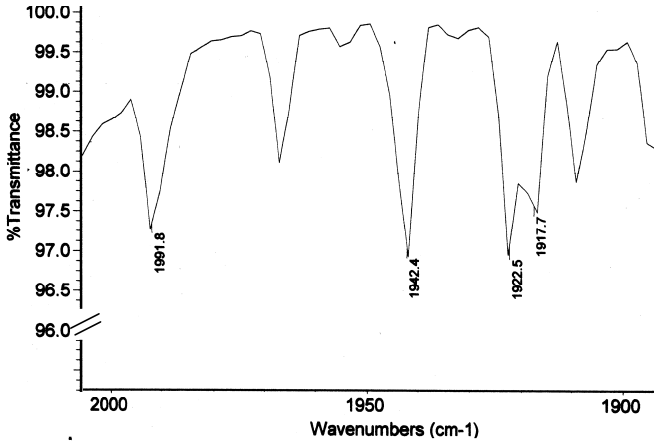

Fig S-3ii. IR spectrum showing presence of amino-carbene peak after treatment with $HAuCl_4$.

6. UV-vis spectrum of gold nanoparticles

UV–vis spectrum of the gold nanoparticles prepared from the Fischer carbene-antibody conjugate **4** were recorded by diluting the prepared gold colloidal solutions four times with water. A blank set in which $HAuCl_4$ was added to the antibody solution, was also recorded. The former showed a distinct peak at 529 nm. The spectral background absorption was corrected by using the UV-vis spectra of water.

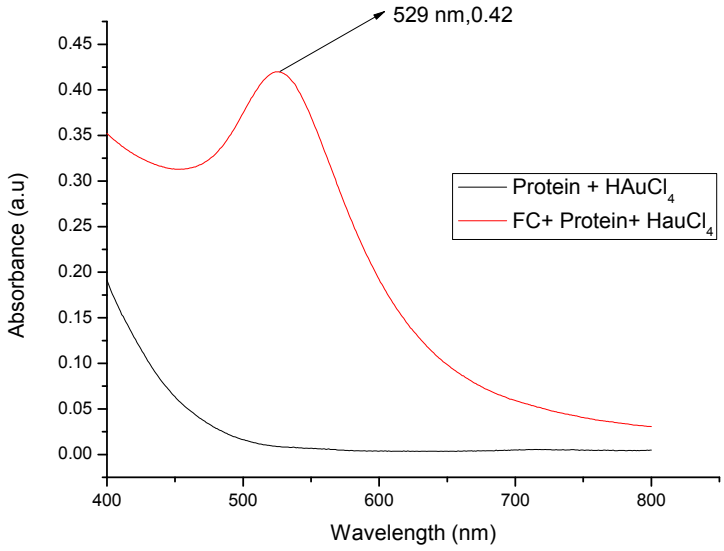

Fig S-4. UV-vis spectrum showing distinct peak at 529 nm characteristic of AuNp

7. Characterization of Gold nanoparticles by TEM

AuNps were generated by treating $HAuCl_4$ (0.01 mM, 0.5 ml) with aqueous solution of an acyl metal complex[1] $(CO)_5W{=}C(CH_3)ONEt_4$ (0.1 mM, 0.5 ml), the Fischer carbene complex **3** (0.1 mM, 0.5 ml) and the Fischer carbene-antibody conjugate. TEM images of AuNps are listed below.

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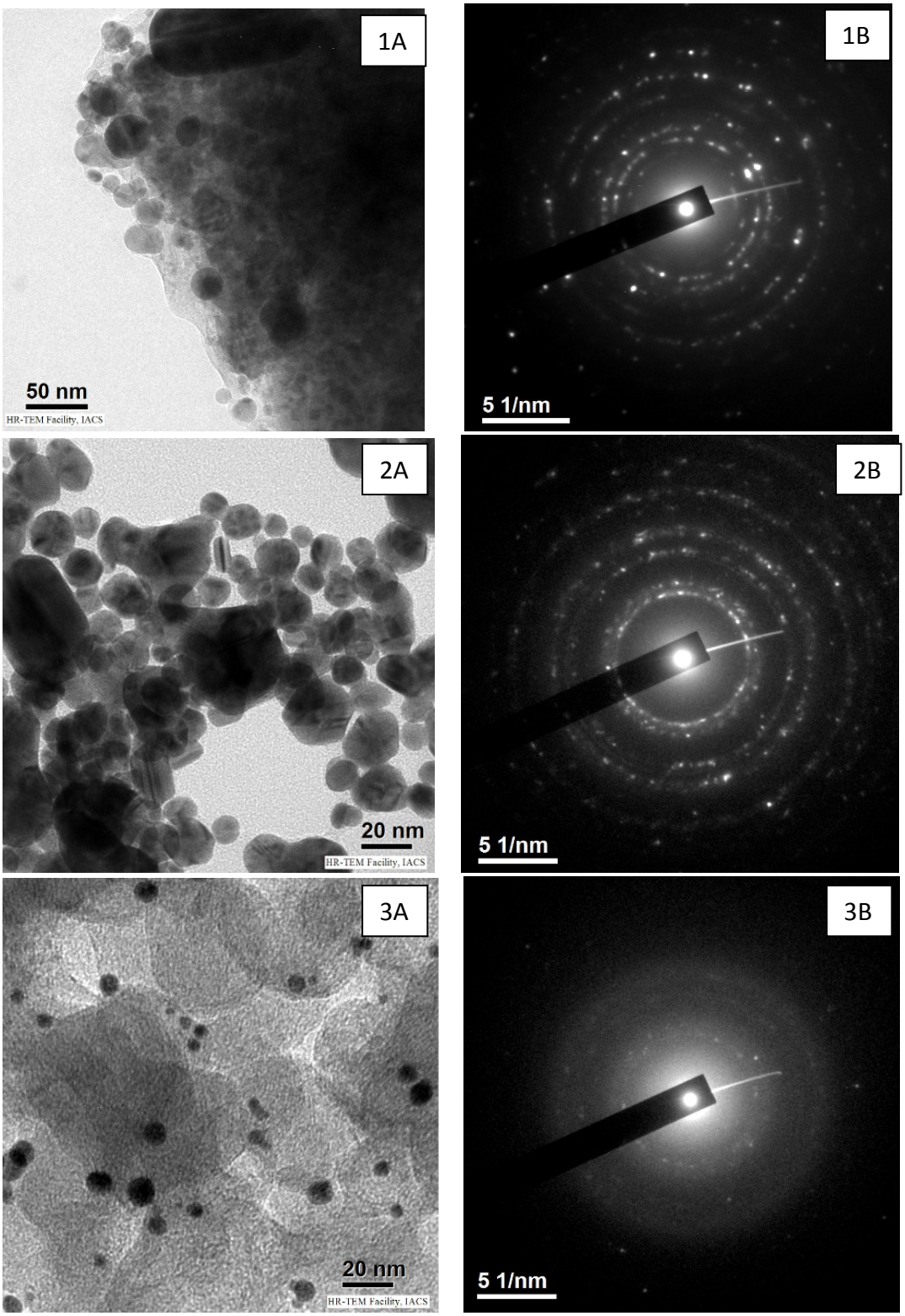

Figure S-5. TEM image of the AuNps and corresponding Electron diffraction patterns of AuNps generated by acyl metal complex and $HAuCl_4$ (1A & 1B); generated by Fischer carbene complex **3** and $HAuCl_4$ (2A & 2B); generated by Fischer carbene-antibody conjugate and $HAuCl_4$ (3A & 3B).

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8. ^{1}H and ^{13}C spectra of compounds:

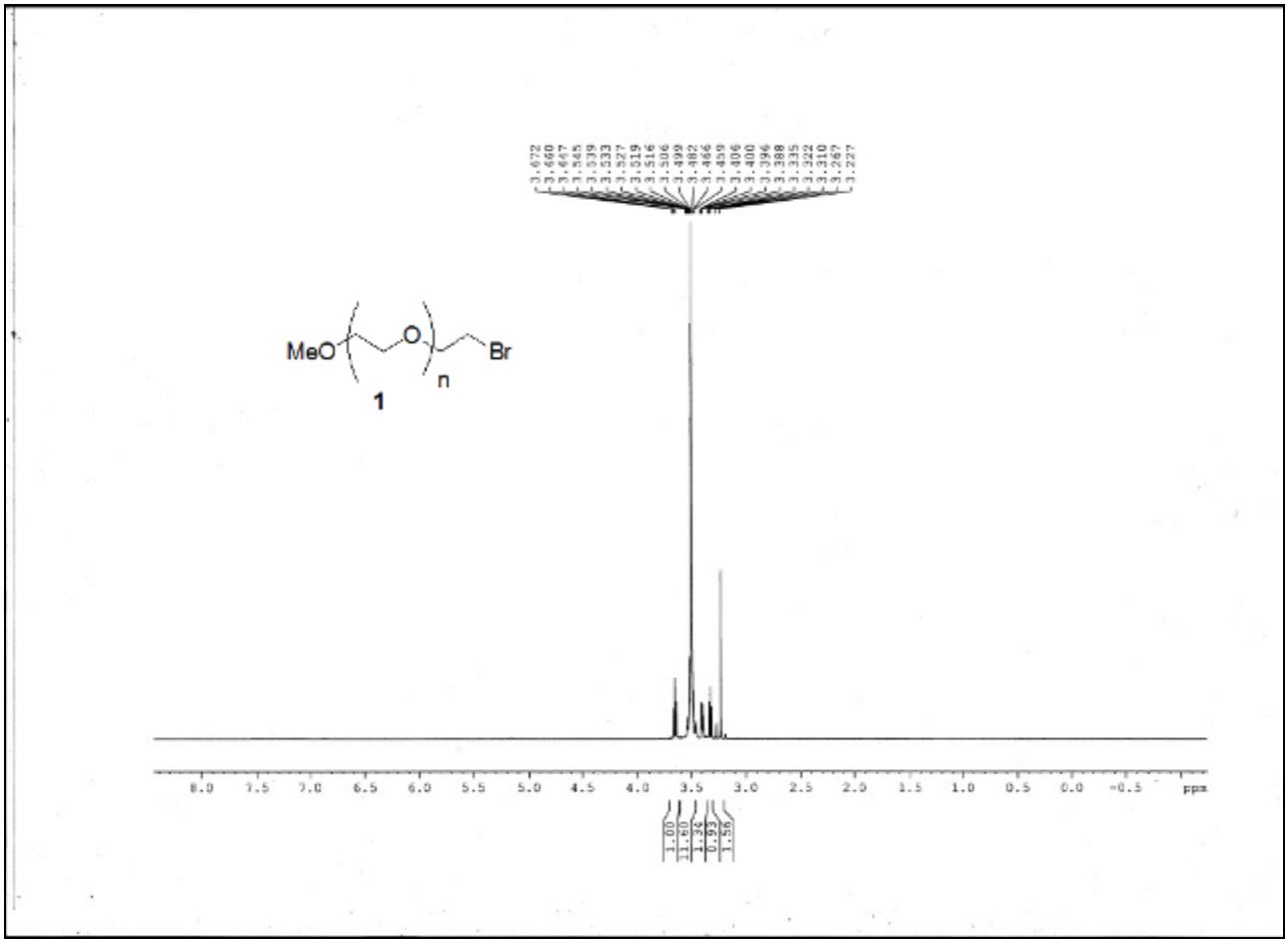

Fig S-6. ^{1}H spectrum of compound **1**

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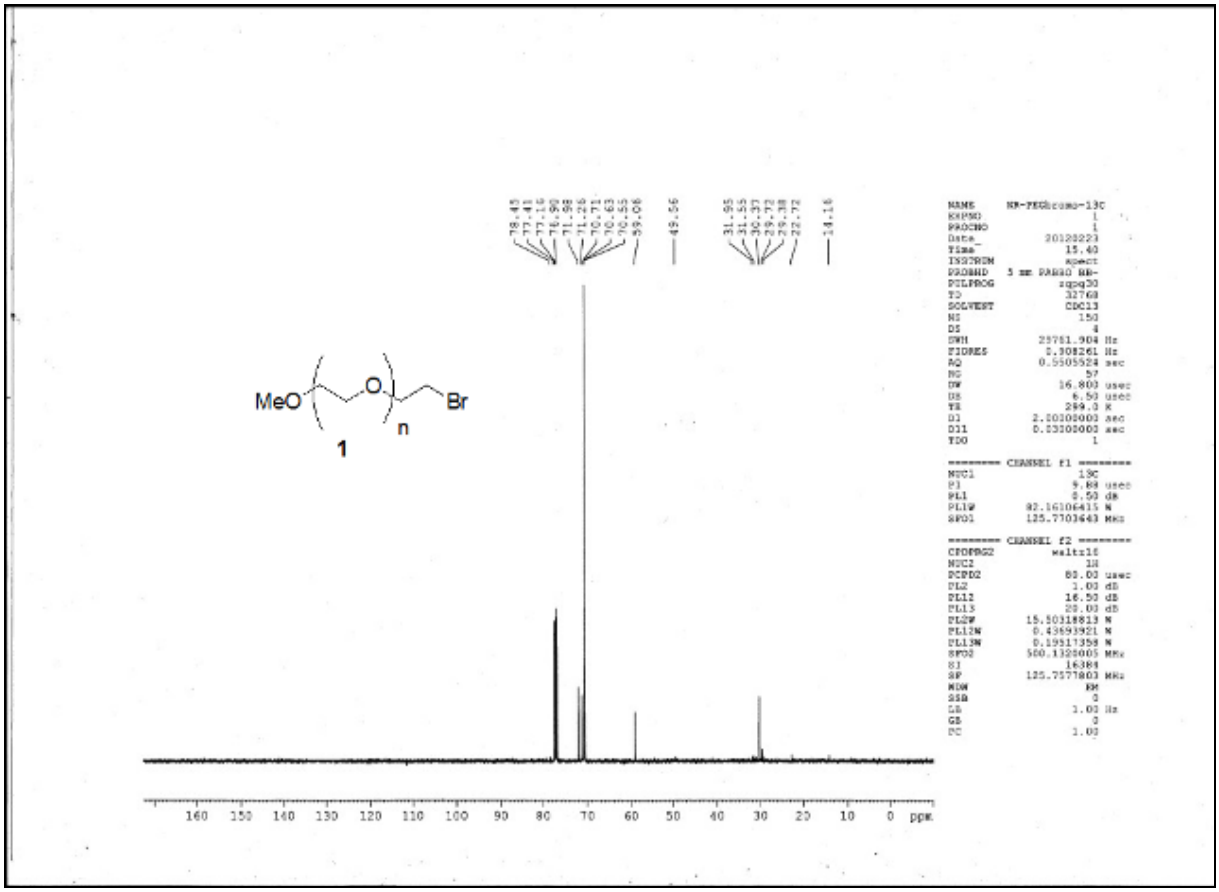

Fig S-7. ^{13}C spectrum of compound **1**

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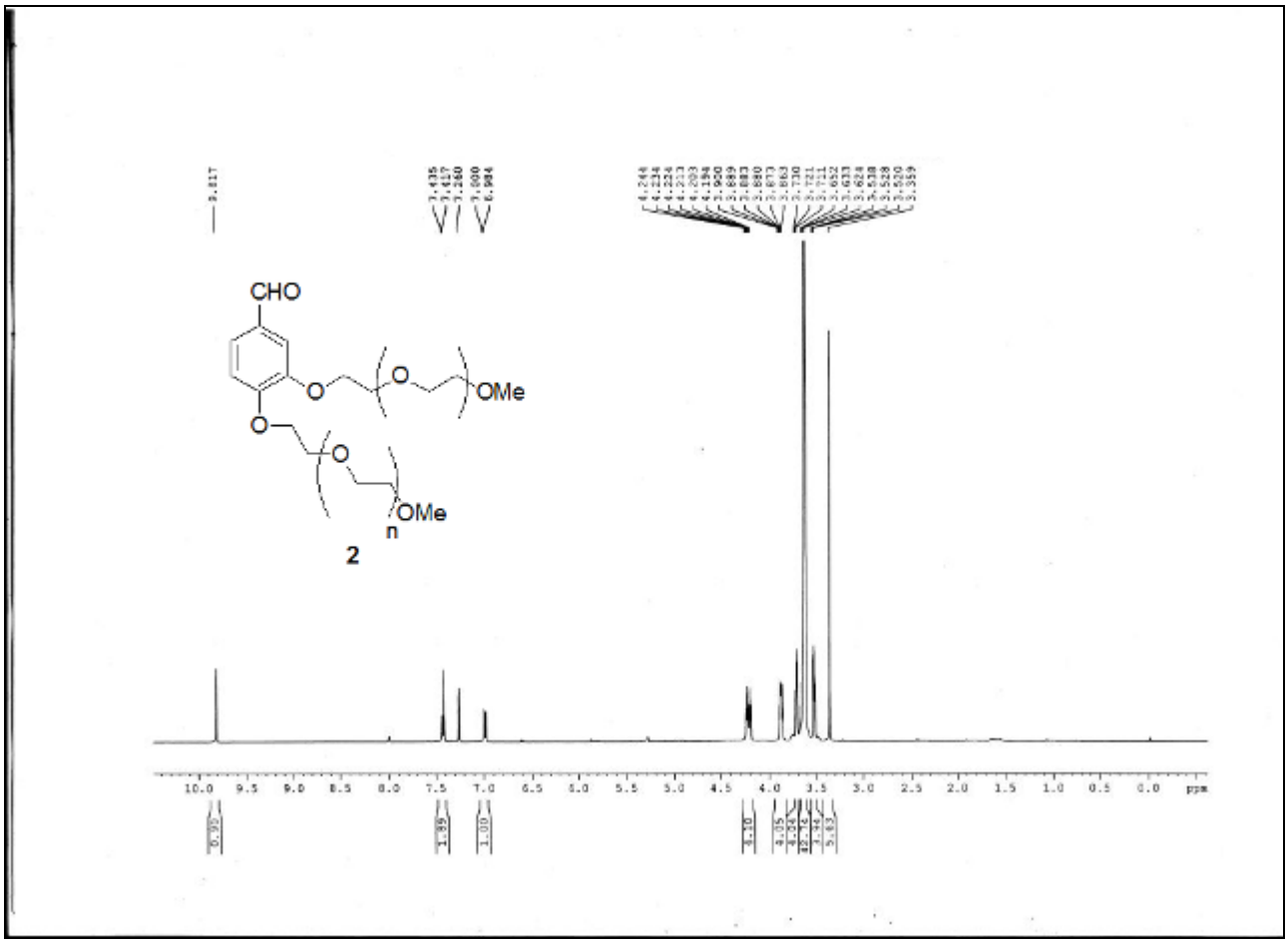

Fig S-8. ^{1}H spectrum of compound 2

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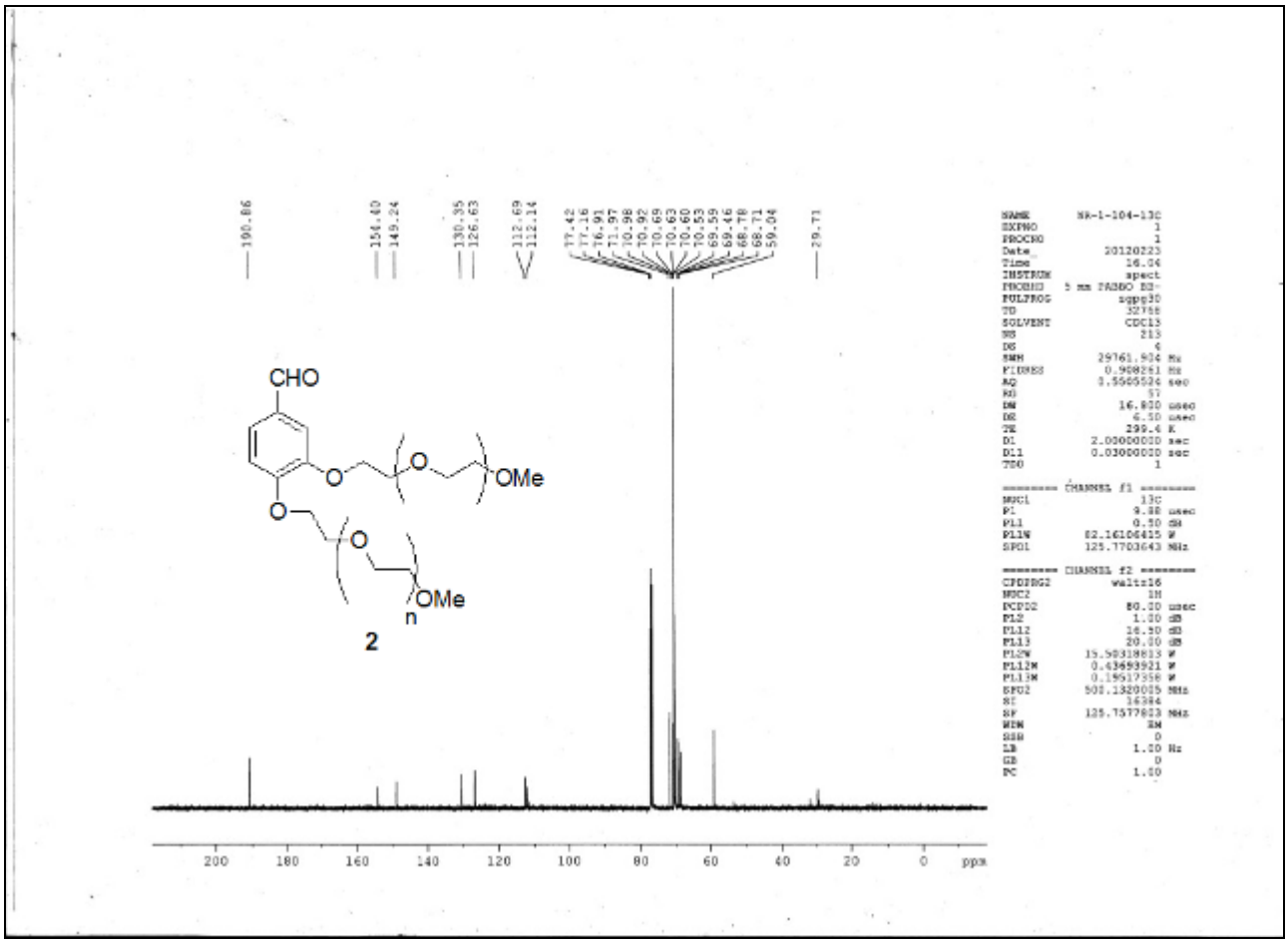

Fig S-9. ^{13}C spectrum of compound **2**

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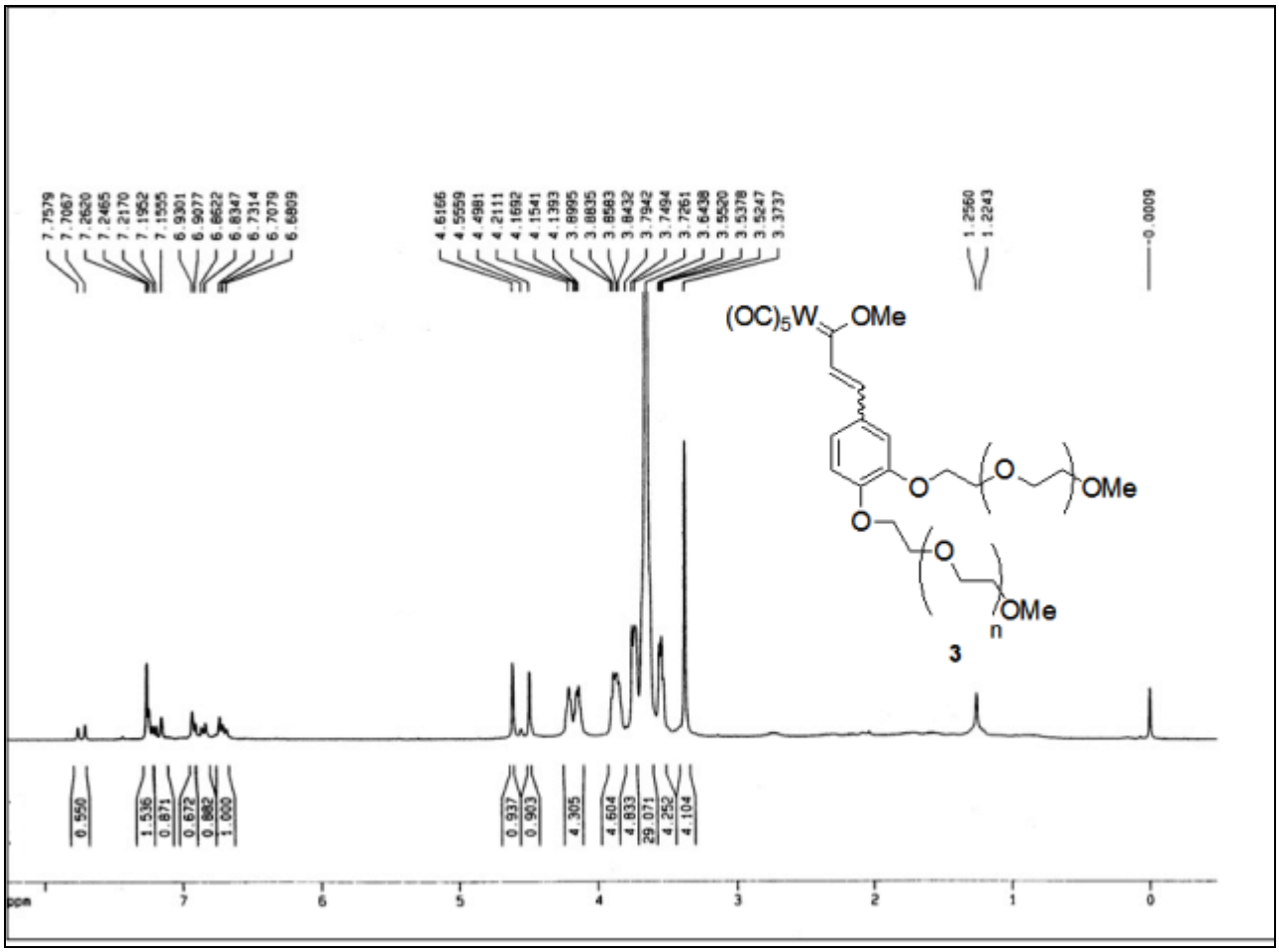

Fig S-10. ^{1}H spectrum of compound **3**

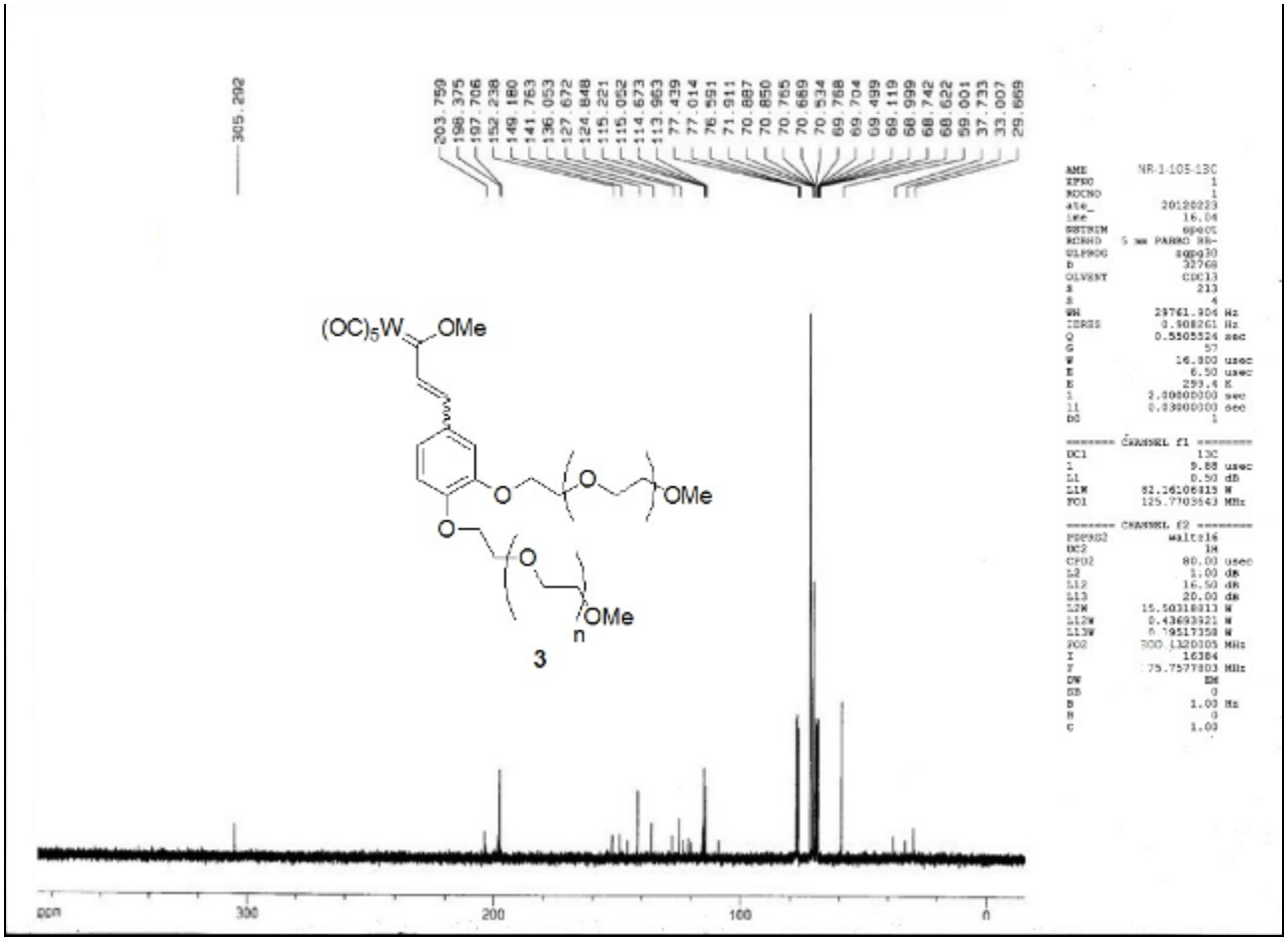

Fig S-11. ^{13}C spectrum of compound **3**

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