Electronic Supplementary Information

In Situ Generation of Gold Nanoparticle on Protein Surface: Fischer Carbene Complex as Reducing Agent

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A. Synthesis of carbene complex 1

Scheme S1. Synthesis of Fischer carbene complex 1

2-[2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}-ethoxy]-ethanol ii. To a solution of hexa(ethylene glycol) i (5.64g, 5.04 mL, 20 mmol) in dry dichloromethane (60 mL), silver oxide (5.1 g, 22
mmol) was added portionwise for 1h at room temperature while stirring. Methyl iodide (3.4 g, 1.5mL, 24 mmol) was then added dropwise at 0 °C. Stirring was continued for 24 h. The reaction mixture was filtered through a column, washed with dichloromethane (3 x 10 mL). Removal of the solvent and flash column chromatography of the residual mass using dichloromethane/methanol (95:5) afforded the product (2.9 g, 50%) as colorless liquid; IR: \( \text{max} 3365 \text{ cm}^{-1} \); \(^1\)H NMR (300 MHz, CDCl\(_3\)):

- 3.33 (3H, s), 3.58 (24H, m);
- \(^{13}\)C NMR (75 MHz, CDCl\(_3\)):

58.1, 60.7, 69.7, 71.1, 71.9; Anal. Calcd. for C\(_{13}\)H\(_{28}\)O\(_7\): C, 52.69; H, 9.52; Found: C, 52.74; H, 9.48.

1-Bromo-2-[2-(2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy)-ethoxy]-ethane iii. To a stirred solution of hexa(ethylene glycol) monomethyl ether, ii (593 mg, 2 mmol) in dry dichloromethane (10 mL) under argon atmosphere carbon tetrabromide (0.8 g, 2.4 mmol) was added. Reaction mixture was cooled to 0 °C and triphenyl phosphine (0.8 g, 3 mmole) taken in dry dichloromethane (2 mL) was added dropwise to it. After stirring for 3 h the solvent was evaporated out. Ether (5 mL) was added, kept in refrigerator for 15 min and filtered. The same process (addition of ether, filtration and evaporation of solvent) was repeated thrice. The residue was subjected to flash column chromatography using dichloromethane/methanol (96:4) which afforded the colorless liquid product (502 mg, 70%). \(^1\)H NMR (300 MHz, CDCl\(_3\)):

- 3.37 (3H, s), 3.47 (2H, t, J = 6Hz), 3.62 (20H, m), 3.80 (2H, t, J = 6Hz); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)):

30.1, 58.8, 70.3, 70.4, 70.6, 71.7; Anal. Calcd. for C\(_{13}\)H\(_{27}\)BrO\(_6\): C, 43.46; H, 7.58; Found: C, 43.59; H, 7.66.

4-{2-[2-(2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy)-ethoxy]-ethoxy}-but-2-en-1-ol iv. Sodium metal (35 mg, 1.5 mmol) was added in small pieces to cis-2-butene-1,4-diol (616 mg, 7 mmol) for 4 h with stirring and was heated at 88 °C for 1 h. To it, the bromo compound iii (359 mg, 1 mmol) was added dropwise. Heating was continued at 88 °C for 5 h. Reaction mixture was allowed to come at room temperature and purified by flash column chromatography using dichloromethane/methanol as eluting solvent (95:5) provided the product (183 mg, 50%) as colorless liquid. R: \( \text{max} 3390 \text{ cm}^{-1} \); \(^1\)H NMR (300 MHz, CDCl\(_3\)):

- 3.37 (3H, s), 3.62 (24H, m), 4.11 (2H, d, J = 6.2Hz), 4.19 (2H, d, J = 6.2Hz), 5.70 (1H, m), 5.82 (1H, m); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)):

δ 58.3, 58.9, 61.5, 66.5, 69.2, 70.1, 70.3, 70.4, 71.8, 72.4, 127.9, 132.5; Anal. Calcd. for C\(_{17}\)H\(_{34}\)O\(_8\): C, 55.72; H, 9.35; Found: C, 55.78; H, 9.37.

1-Bromo-4-{2-[2-(2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy)-ethoxy]-ethoxy}-but-2-ene v. Phosphorous tribromide (100 mg, 0.375 mmol) was added drop wise to an ice-cold solution of alcohol iv
(137 mg, 0.375 mmol) in dry dichloromethane (1 mL). Stirring was continued at 0 °C for 30 min. Then methanol (1 mL) was added drop wise at 0 °C. Solvent was evaporated and the residue was subjected to flash column chromatography using acetone/pet ether (30:70) as eluting solvent to obtain pure product v (112 mg, 70%) as colorless liquid. 

\[ ^1H \text{ NMR } (300 \text{ MHz, CDCl}_3): \] 3.31 (3H, s), 3.56 (24H, m), 3.94 (2H, d, J = 6Hz), 4.10 (2H, d, J = 6Hz), 5.72 (1H, m), 5.87 (1H, m); 

\[ ^{13}C \text{ NMR } (75 \text{ MHz, CDCl}_3): \] 26.4, 58.9, 65.9, 69.6, 70.5, 70.4, 71.8, 128.1, 131.1; Anal. Calcd. for C_{17}H_{33}BrO_{7}: C, 47.56; H, 7.75; Found: C, 47.65; H, 7.80.

(B) Preparation and characterization of BSA-carbene conjugate 2

Protin-carbene conjugate was prepared and characterised by the method described by Jaouen and others. 

Reaction of the protein BSA (bovine serum albumin) with carbene complex 1 was performed in borate buffer (at pH 9). Thus, a solution of BSA (100 M, 1 ml) and a solution of carbene complex 1 (6000 M, 1 ml) was incubated for 6 h in borate buffer at 25 °C. The reaction medium was applied to a gel filtration column packed with Sephadex™ G-50 to separate protein carbene conjugate from unreacted carbene complex. Two clearly separated yellow bands were thus observed in the column; the upper band corresponds to the unreacted carbene complex and the lower band corresponds to the protein-carbene conjugate. A characteristic shift of the wavenumber of the 2 prominent CO bands was observed by IR upon conversion of the methoxycarbene into the protein carbene conjugate (Figure
S1. The UV-vis spectrum of protein carbene conjugate was also very similar to that of the aminocarbene complex generated by reaction of methoxycarbene complex with n-butylamine. Overlapping of two curves in the CD spectra (Fig. S2) reveals that conformation of protein remains unaltered after conjugation.

Quantification of the number of amino carbene adducts per protein molecule was done by the method described by Jaouen and others.² First, the concentration of protein was measured by the colorimetric assay described by Bradford.³ The concentration of aminocarbene complex was then determined spectroscopically, taking the adduct generated in situ by the reaction of carbene complex 7 with an excess of n-butylamine [ . (332 nm) = 5900 l mol⁻¹ cm⁻¹] as standard. It was thus estimated that 15 out of 60 amino groups of BSA were converted to aminocarbenes.

Figure S1. IR spectra of (A) carbene complex 1 (B) Carbene complex after reaction with BuNH₂ (C) after reaction with BSA
Figure S2. CD spectra of BSA (i) before reaction with carbene (red curve) (ii) after reaction with carbene (black curve)

C. Synthesis of gold nanoparticle from BSA-carbene conjugate

2 ml solution of BSA-carbene conjugate prepared according to the above procedure was added dropwise for 1 minute to 2 ml of 1 mM aqueous hydrogen tetrachloroaurate solution. Incubated at 25 °C for 2 days. The solution turned red slowly with a very characteristic surface plasmon centred at 530 nm in the UV-vis spectrum.

D. UV-vis absorption spectroscopy

UV-vis spectra of the gold nanoparticle prepared from the carbene complex 1 were recorded by diluting prepared gold colloidal solutions four times with water on a Varian Cary 50 Bio spectrophotometer. The spectral background absorption was subtracted by using the UV-vis spectra of the same solvent mixture.
**Figure S3.** UV-vis spectra of gold nanoparticles in aqueous medium synthesized from aq. HAuCl₄ (1mM) with Fischer carbene 1(1mM) aq. Solution.

**Figure S4.** UV-vis spectrums of gold nanoparticles in water using carbene salt 2 (1mM) with 0.01mM aq.HAuCl₄ in presence of oligoethyleneglycol dimethyl ethers with different chain lengths; (dme (n=1), tri (n=3), hexa (n=6), poly (avg n=11)).
E. Copies of NMR spectra

*Figure S5.* Proton NMR spectra of carbene complex 1
**Figure S6.** $^{13}$C NMR spectra of carbene complex 1
Figure S7. DEPT NMR of carbene complex 1
Figure S8. Proton NMR spectra of compound ii
Figure S9. C\textsuperscript{13} NMR spectra of compound ii
Figure S10. DEPT NMR spectra of compound ii
Figure S11. Proton NMR spectra of compound iii
Figure S12. C\textsuperscript{13} NMR spectra of compound iii
Figure S13. Proton NMR spectra of compound iv
Figure S14. C$^{13}$ NMR spectra of compound iv
Figure S15. DEPT NMR spectrum of compound iv
Figure S16. Proton NMR spectra of compound v
Figure S17. C13 NMR spectra of compound \( \text{v} \)
Figure S18. DEPT NMR spectra of compound v
F. TEM measurements

TEM measurements on the gold nanoparticle films cast onto carbon-coated TEM grids were carried out on a JEOL model 1200EX instrument at an accelerating voltage of 120 kV.

Figure S19. TEM micrograph of AuNPs generated with Fischer Carbene salt 2 and dimethoxyethane

![TEM micrograph of AuNPs](image_url)
**Figure S20.** TEM micrograph of AuNPs generated with Fischer carbene salt 2 and triethylene glycol dimethyl ether

![TEM micrograph of AuNPs generated with Fischer carbene salt 2 and triethylene glycol dimethyl ether](image)

**Figure S21.** TEM micrograph of AuNPs generated with Fischer carbene salt 2 and hexaethylene glycol diallylether

![TEM micrograph of AuNPs generated with Fischer carbene salt 2 and hexaethylene glycol diallylether](image)
Figure S22. TEM micrograph of AuNPs generated with Fischer carbene salt 2 and Polyethylene glycol demethyl ether
REFERENCES:

