An Azide-Functionalized Nitronyl Nitroxide Radical: Synthesis, Characterization and Staudinger-Bertozzi Ligation Reactivity

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Experimental - General considerations

Solvents were obtained from Caledon Laboratories, dried using an Innovative Technologies Inc. solvent purification system, collected under vacuum, and stored under a nitrogen atmosphere over 4 Å molecular sieves. 4-(3-azidopropoxy)-benzaldehyde,\textsuperscript{1} \( N,N' \)-dihydroxy-2,3-dimethyl-2,3-butanediamine,\textsuperscript{2} Staudinger-AuNP,\textsuperscript{3} TEG-AuNP\textsuperscript{3} and methyl-2-(diphenylphosphino)benzoate \textsuperscript{6} \textsuperscript{4} were prepared according to literature procedures. All other reagents were purchased from Sigma-Aldrich or Alfa Aesar and used as received. NMR Spectra were recorded on a 600 MHz (\textsuperscript{1}H: 599.5 MHz, \textsuperscript{13}C: 150.8 MHz) Varian INOVA instrument. \textsuperscript{1}H NMR spectra were referenced to residual CHCl\textsubscript{3} (7.27 ppm) and \textsuperscript{13}C NMR spectra were referenced to CDCl\textsubscript{3} (77.0 ppm). Mass spectrometry data were recorded in positive-ion mode using a high resolution Finnigan MAT 8200 spectrometer using electron impact ionization. UV-vis spectra were recorded using a Cary 300 Scan instrument. Four different concentrations were run for each sample and molar extinction coefficients were determined from the slope of a plot of absorbance against concentration. Infrared spectra were recorded on a KBr disk using a Bruker Vector 33 FT-IR spectrometer. Elemental analyses (C, H, N) were carried out by Laboratoire d’Analyse Élémentaire de l’Université de Montréal, Montréal, QC, Canada.

Transmission electron microscopy (TEM) images were recorded from a TEM Philips CM10 microscope. The TEM grids (Formvar carbon film on 400 mesh copper grids) were purchased from Electron Microscopy Sciences and samples were prepared by dropcasting a drop of nanoparticle solution directly onto the grid surface. The drop was then carefully removed after 30 s with a soft tissue. XPS analyses were carried out with a Kratos Axis Ultra spectrometer using a monochromatic Al K(\alpha) source (15 mA, 14 kV). The instrument work function was calibrated to give a binding energy (BE) of 83.96 eV for the Au 4f\textsubscript{7/2} line for metallic gold and
the spectrometer dispersion was adjusted to give a BE of 932.62 eV for the Cu 2p3/2 line of metallic copper. Specimens were mounted on a double sided adhesive tape and the Kratos charge neutralizer system was used on all specimens. Survey scan analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 160 eV. High resolution analyses were carried out with an analysis area of 300 x 700 μm and a pass energy of 20 eV. Spectra have been charge corrected to the main line of the carbon 1s spectrum set to 285.0 eV for aliphatic carbon (when needed). Spectra were analyzed using CasaXPS software (version 2.3.14).

**Electron Paramagnetic Resonance (EPR) Spectroscopy**

EPR measurements were made on ca. $10^{-6}$ M CH$_2$Cl$_2$ solutions of nitronyl nitroxide 5, functionalized and native Staudinger-AuNP and imino nitroxide 7 that had been subjected to three freeze-pump-thaw cycles in 0.4 mm quartz tubes using a JEOL JES-FA200 EPR. All measurements were made at 20 °C and g factors were referenced relative to a built-in manganese oxide marker within the resonance cavity of the instrument.

Quantification was done by comparing an EPR spectrum collected for a TEMPO solution of known concentration in toluene that was compared as outlined above. The integration of the TEMPO signal with respect to the manganese oxide marker was compared to that of the radical-functionalized Staudinger-AuNP sample. By assuming one molecule of TEMPO contributes one unpaired electron, the number of unpaired electrons present in the radical-functionalized Staudinger-AuNP sample could be determined.

**X-ray Crystallography Details**

A single crystal of nitronyl nitroxide 5 was mounted on a Mitegen polyimide micromount with a small amount of Paratone N oil. X-ray measurements were made on a Nonius diffractometer at a temperature of 150 K. The data collection strategy included a number of $\omega$ and $\phi$ scans which
collected data over a range of angles, $2\theta$. The frame integration was performed using SAINT.$^5$ The resulting raw data was scaled and absorption corrected using a multi-scan averaging of symmetry equivalent data using TWINABS.$^6$

The structures were solved by direct methods using the SIR 2011 program.$^7$ All non-hydrogen atoms were obtained from the initial solution. The hydrogen atoms were introduced at idealized positions and were allowed to ride on the parent atom. Twinning was present in the crystal and the twin domains were related by a $180^\circ$ rotation about [100]. The twin law used was:

\[
\begin{bmatrix}
0 & 0 & 1 \\
0 & -1 & 0 \\
0 & 0 & -1 \\
\end{bmatrix}
\]

and the twin fraction parameter refined to a value of 0.30670(172). The structural model was fit to the data using full matrix least-squares based on $F^2$. The calculated structure factors included corrections for anomalous dispersion from the usual tabulation. The structure was refined using the SHELXL-2014 program from SHELXL.$^8$ See Table S1 for additional crystallographic data.
Table S1. X-ray diffraction data collection and refinement details for 5.

<table>
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<tr>
<td>Chemical Formula</td>
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<tr>
<td>GOF</td>
<td>1.021</td>
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</table>

\[ R₁ = \frac{\sum (|F_o| - |F_c|)}{\sum F_o} , \quad wR₂ = \sqrt{\frac{\sum(w(F_o^2 - F_c^2))^2}{\sum(wF_o^4)}} \]

Experimental Procedures

Synthesis of 4-(3-azidoproxy)-substituted 1,3-dihydroxy-4,4,5,5,-tetramethyl-2-phenylimidazolidine 4

N,N'-dihydroxy-2,3-dimethyl-2,3-butanediamine (0.47 g, 3.2 mmol) and 4-(3-azidoproxy)-benzaldehyde (0.65 g, 3.2 mmol) were dissolved in 15 mL methanol and left to stir for 48 h. The product was filtered off as a white powder. Yield = 0.85 g, 80%. Mp = 139–140 °C. ¹H NMR (599.5 MHz, CDCl₃) δ 7.37 (d, 3J_HH = 8 Hz, 2H, aryl CH), 6.88 (d, 3J_HH = 8 Hz, 2H, aryl CH), 5.36 (s, 2H, OH), 4.64 (s, 1H, CH), 4.06 (t, 3J_HH = 5 Hz, 2H, CH₂), 3.52 (t, 3J_HH = 6 Hz, 2H, CH₂), 2.06–2.04 (m, 2H, CH₂), 1.06 (s, 6H, CH₃), 1.01 (s, 6H, CH₃). ¹³C{¹H} NMR (100.6 MHz,
CDCl₃): δ 158.9, 132.8, 130.0, 114.5, 90.3, 67.1, 64.7, 48.4, 29.0, 24.3, 17.1. FT-IR (KBr): 3203 (br, s), 2976 (s), 2903 (s), 2875 (m), 2101 (s), 1610 (m), 1511 (m), 1448 (m), 1308 (s), 1241 (s) cm⁻¹. UV-vis (CH₂Cl₂): λ_max = 292 nm (ε = 8,600 M⁻¹ cm⁻¹), 227 nm (ε = 9,100 M⁻¹ cm⁻¹). Mass Spec. (EI, +ve mode): exact mass calculated for [C₁₆H₂₅N₅O₃]⁺: 335.1957; exact mass found: 335.1949; difference: –2.54 ppm. Anal. Calcd. (%) for C₁₆H₂₅N₅O₃: C, 57.30; H, 7.51; N, 20.88. Found: C, 57.52; H, 7.62; N, 20.70.

Synthesis of radical functionalized Staudinger AuNP

Staudinger-AuNP (0.143 g) and azide-substituted nitronyl nitroxide 5 (4.095 g, 12.32 mmol) were dissolved in CH₂Cl₂ (25 mL). The mixture was stirred at room temperature for 48 h. Subsequently, the excess azide radical was removed by centrifugation (6000 rpm, 10 min), where the particles were allowed to settle and then could be removed using CH₂Cl₂:hexanes 1:6 as solvent. The last remaining traces of radical were removed by making a film of AuNP from CH₂Cl₂ inside a round bottom flask and rinsing it with hexanes followed by 95% ethanol. The cleaning procedure was monitored by IR spectroscopy, by following the disappearance of the azide peak at 2100 cm⁻¹.

Control Experiment

TEG-AuNP (0.037 g) and azide-substituted nitronyl nitroxide 5 (0.037 g, 0.110 mmol) were dissolved in 20 mL CH₂Cl₂. The mixture was stirred at room temperature for 48 h. Subsequently, the excess radical was removed by making a film of AuNP from CH₂Cl₂ inside a round bottom flask and rinsing with hexanes. Remaining traces of radical were removed by dissolving the TEG-AuNP in water and by filtering the insoluble radical through glass wool. The cleaning procedure was monitored by IR spectroscopy, by following the disappearance of the azide peak at ca. 2100 cm⁻¹.
Model Reaction – Synthesis of Imino Nitroxide 7

Methyl-2-(diphenylphosphino)benzoate 6 (0.500 g, 1.37 mmol) and azide-functionalized nitronyl nitroxide 5 (0.501 g, 1.51 mmol) were dissolved in 30 mL CH$_2$Cl$_2$ and stirred at 20 °C for 18 h. The solvent was removed in vacuo and the resulting red solid was purified by column chromatography (1:1 EtOAc:hexanes, silica) to yield imino nitroxide 7 as a microcrystalline orange solid. Yield = 0.400 g, 84%. Mp = 32–34 °C. FT-IR (KBr): 2975 (m), 2923 (m), 2869 (w), 2095 (s), 1607 (s), 1504 (s), 1468 (m), 1367 (m), 1302 (m), 1248 (s), 1176 (s) cm$^{-1}$. UV-vis (CH$_2$Cl$_2$): $\lambda_{\text{max}} = 461$ nm ($\varepsilon = 700$ M$^{-1}$ cm$^{-1}$), 252 nm ($\varepsilon = 25,500$ M$^{-1}$ cm$^{-1}$). Mass Spec. (EI, +ve mode): exact mass calculated for [C$_{16}$H$_{22}$N$_5$O$_2$·H$^+$]: 317.1852; exact mass found: 317.1845; difference: –2.24 ppm. Anal. Calcd. (%) for C$_{16}$H$_{22}$N$_5$O$_2$: C, 60.74; H, 7.01; N, 22.14. Found: C, 60.24; H, 7.08; N, 19.98.
Figure S1. $^1$H NMR spectrum of 4 in CDCl$_3$. The asterisks denote residual solvent/grease signals.

Figure S2. $^{13}$C$^{1}$H NMR spectrum of 4 in CDCl$_3$. The asterisk denotes solvent signal.
Figure S3. EPR spectrum (red) and simulated EPR spectrum (black) of azide substituted nitronyl nitroxide 5. Parameters used for simulation: linewidth = 0.09 mT, $a_{N1} = 0.760$ mT, and $g = 2.0075$.

Figure S4. EPR spectrum (red) and simulated EPR spectrum (black) of imino nitroxide 7. Parameters used for simulation: linewidth = 0.066 mT, $a_{N1} = 0.910$ mT, $a_{N2} = 0.495$ mT, and $g = 2.0472$. 
Figure S5. IR spectrum of radical functionalized AuNP after washing procedure recorded as a film on KBr disk.

Figure S6. EPR spectrum (red) and simulated EPR spectrum (black) of radical functionalized AuNP. Parameters used for simulation: linewidth = 0.16 mT, $a_{11} = 0.770$ mT, and $g = 2.0068$. 
Figure S7. XPS characterization of radical-functionalized Staudinger-AuNP.
Figure S8. IR spectrum of control TEG-AuNP after washing procedure recorded as a film on KBr disk.

Figure S9. EPR spectrum of radical-functionalized Staudinger-AuNP (black) and control TEG-AuNP (red) after washing procedure in CH₂Cl₂. Both samples are at the same concentration (3.3 mg mL⁻¹).
References


5. Bruker-Nonius, SAINT version 2012.12, 2012, Bruker-Nonius, Madison, WI 53711, USA

