Supporting information

Hyperbranched Polyselenides as Glutathione Peroxidase Mimics

Huaping Xu[1], Jian Gao[1], Yapei Wang[1], Zhiqiang Wang[1], Mario Smet*[2], Wim Dehaen[2], Xi Zhang*[1]

[1] *Key Lab of Organic Optoelectronics & Molecular Engineering, Department of Chemistry, Tsinghua University, Beijing 100084 and Key Lab for Supramolecular Structure & Materials, College of Chemistry, Jilin University, Changchun 130023, P. R. China*
Email: xi@mail.tsinghua.edu.cn
[2] *Laboratory for Organic Synthesis, University of Leuven, Celestijnenlaan 200F, B-3001 Leuven, Belgium Email: Mario.Smet@chem.kuleuven.be*

Materials, Instruments and Measurements:

1,3,5-Tris-bromomethyl-2,4,6-trimethyl-benzene was prepared as previously published (see G. Van Wuytswinkel et al., *J. Chem. Soc., Perkin Trans 1* **2000**, 1337-1340) and used without further purification. 3,5-Dimethylbenzyl bromide was bought from Lancaster. Diethylene glycol monomethyl ether (2-(2-methoxyethoxy) ethanol) was bought from Fluka. Other chemicals were analytically pure. The solvents used were all freshly distilled.

^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM□ECA300 (300 MHz) spectrometer using TMS as the internal standard. ^{77}Se-NMR spectrum was recorded on a Bruker Avance 500 (500MHz) spectrometer with $(CH_3)_2Se$ as an external standard. UV-VIS spectra were obtained using a Hitachi U- 3010 spectrophotometer. Gel permeation chromatography (GPC) measurements were performed on a Waters chromatograph connected to a Waters 410 differential refractometer with CH_2Cl_2 or THF as an eluent. X-ray photoelectron spectroscopy data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300 W Al Kα radiation.

Synthesis of glutathione peroxides mimics

Preparation of NaHSe[1]: Selenium (79 mg, 1 mmol) was added to sodium borohydride (80 mg, 2.1 mmol) in water (1 ml). Vigorous foaming (H_2) immediately occurred and the selenium was consumed in less than 5 min. The virtually colorless solution of the resulting NaHSe was ready for use without further treatment.

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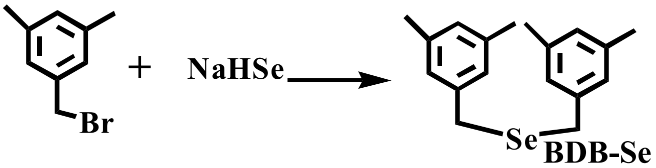
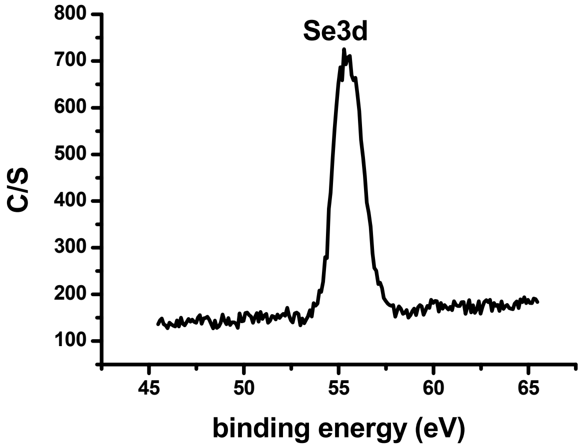


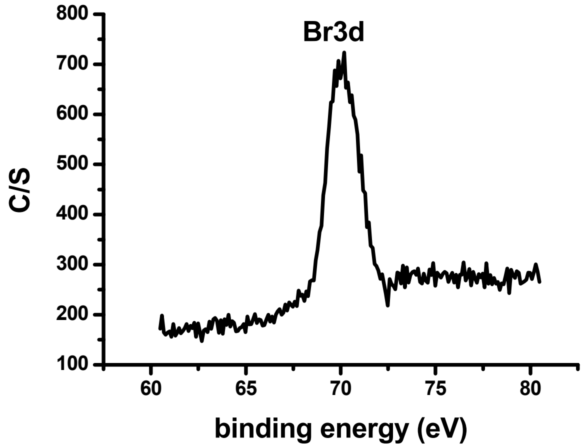
Synthesis and characterization of bis(3,5-dimethylbenzyl) selenide (BDB-Se): NaHSe (0.46 mmol) was added in solution under magnetic stirring to 3,5-dimethylbenzyl bromide(0.186 g, 0.93 mmol) dissolved in THF (20 ml) under argon atmosphere. The mixture was stirred for 6 h at 50 °C. Then the solvent was removed and the residue was purified by silica gel column chromatography eluting with 8:1 cyclohexane:chloroform . Evaporation of the solvent gave the yellow title compound

bis(3,5-dimethylbenzyl) selenide (yield around 50%).
^{1}H-NMR (300MHz, $CDCl_3$) ppm: δ 2.31 (s, 12H, Ar-*CH_3*,), 3.70 (s, 4H, -*CH_2*-Se-*CH_2*-), 6.87-6.89 (6H, Ar-*H*); ^{13}C-NMR (75MHz, $CDCl_3$) ppm: δ 21.35, 27.83, 126.92, 128.50, 138.03, 139.18; ESI-MS: Calcd. 318.09 found 335.17(plus NH_4^+)

Synthesis of hyperbranched polyselenide HP-Br: NaHSe (1 mmol) was added in solution under magnetic stirring to 1,3,5-tris-bromomethyl-2,4,6-trimethyl-benzene (0.396 g, 1 mmol) dissolved in THF (30 ml) under argon atmosphere. The mixture was stirred for 8h at 45 °C. Then the mixture was filtered and washed with THF several times. The filtrate was collected and evaporation of the solvent gave the title compound as a yellow powder (yield around 45%).
^{1}H-NMR (300MHz, $CDCl_3$) ppm: δ 2.33-2.39 (broad, 9H), 3.79-3.82 (broad, 4H), 4.56-4.58 (broad, 2H); ^{77}Se-NMR (500MHz, $CDCl_3$) ppm: δ 196.3; GPC: Mn = 1640 g/mol, Mw = 1780 g/mol, Polydispersity =1.08;

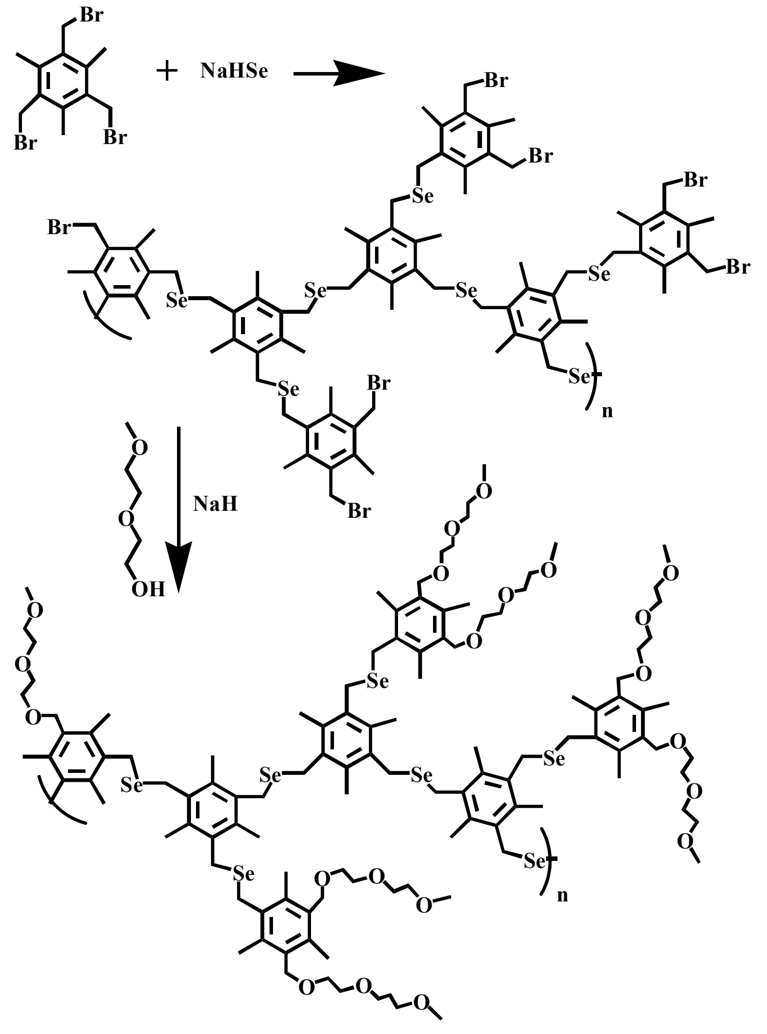
XPS: the molar ratio between Se : Br in HP-Br is 1.05:1.





Synthesis of hyperbranched polyselenide HP-EO: NaH (0.3 g, dispersion in mineral oil) was added to 2-(2-methoxyethoxy) ethanol (15 ml) under argon atmosphere and the solution was kept at 45 °C for 1 h. Then HP-Br (97 mg) was added. The mixture was stirred for 24 h during which the yellow powder dissolved completely. After washing with H_2O and extraction with CH_2Cl_2, the organic layer was dried over Na_2SO_4. Then the solvent was removed and the residue purified by silica gel column chromatography eluting with methanol. Evaporation of the solvent gave the title compound as a yellow powder (yield around 80%).

^{1}H-NMR (300MHz, $CDCl_3$) ppm: δ 2.30-2.45 (broad, 9H), 3.37-3.84 (broad, 15H), 4.56 (broad, 2H); GPC: Mn = 2165 g/mol, Mw = 2761 g/mol, Polydispersity = 1.28



Measurement of Catalytic Activity in organic solvent

Initial Reduction Rates (v_0)[2]

The catalytic GPx model reaction was initiated by adding an excess amount of H_2O_2 to a CH_3OH solution of PhSH (1 mM) containing a selenium catalyst. The optical density was measured at different time intervals for each experiment. The initial portion of the curve was a straight line with a constant slope and afterwards the rate became zero when all the substrate had been consumed. Therefore, only the initial portion of the straight line was considered for calculating the initial rate of the reaction. These initial reduction rates were determined by calculating the thiol concentration after a given time using the following equation.

$$\mathbf{C = (\varepsilon_1 C_0 - 2A)/(\varepsilon_1 - 2\varepsilon_2) \quad \ldots\ldots(a)}$$

Where C is the concentration of PhSH, C_0 is the initial concentration of PhSH, A is the absorbance, ε_1 is the molar extinction coefficient of PhSSPh and ε_2 is the molar extinction coefficient of PhSH. Since the molar extinction coefficient of PhSSPh ($\varepsilon_1 = 1.24 \times 10^3\ M^{-1}.cm^{-1}$) at this wavelength is much larger than that of PhSH ($\varepsilon_2 = 9\ M^{-1}.cm^{-1}$), the term $2\varepsilon_2$ in equation **a** can be neglected. Therefore, the equation **a** becomes

$C = C_0 - 2A/\varepsilon_1$ ……(b)

The initial reduction rate of H_2O_2 (**v_0**) was then determined by 1/v vs 1/C plots.

Measurement of Catalytic Activity in aqueous solution

Coupled Reductase Assay:[3]

$$2GSH + ROOH \xrightarrow{Enzyme} GSSG + ROH + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{Reductase} 2GSH + NADP^+$$

The GPx activities of these compounds were measured using Wilson's method with minor modification. The assay mixture contained 50 mM phosphate buffer, pH 7.0, 1 mM EDTA, 1 mM GSH, 0.5 mM H_2O_2, 0.25 mM NADPH, 1 unit of glutathione reductase, and a moderate amount of test compound at 25 °C. The reaction was initiated by the subsequent addition of H_2O_2 and the absorbance at 340 nm was recorded for a few minutes to calculate the rate of NADPH consumption.

Reference:

(1) Klayman, D. S.; Griffin, T. S. *J. Am. Chem. Soc.* 1973, **95(1)***,* 197

(2) Iwaoka, M.; Tomoda, S. *J. Am. Chem. Soc.*1994, **116**, 2557

(3) Wilson, S. R.; Zucker, P. A.; Huang, R.-R. C.; Spector, A. *J. Am. Chem. Soc.* 1989, **111**, 5936