J. Chem. Sci., Vol. 117, No. 6, November 2005, pp. 621–628.

Thiol peroxidase-like activity of some intramolecularly coordinated diorganyl diselenides[#]

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MS received 19 April 2005; revised 11 November 2005

Abstract. Several new diaryl diselenides having intramolecular coordinating groups have been synthesized by ortho-lithiation/Na_2Se_2 routes in good yield. Bis[2-(N-phenylferrocenecarboxamide)] diselenide (**10**), bis[2-(N-*tert*-butylferrocenecarboxamide)] diselenide (**11**), (*S*)(*S*)-bis[2(-*N*-phenethylferrocenecarboxamide)] diselenide (**12**) were synthesized by the ortho-lithiation route. Bis[2-(N,N-dimethylaminomethylnaphthyl)] diselenide (**13**) was synthesized by lithium/bromide exchange reaction whereas bis(2,4-dinitrophenyl) diselenide (**14**) was prepared by the reaction of disodium diselenide with 2,4-dinitro-1-chlorobenzene. Thiol peroxidase-like activities of the diorganodiselenides have been evaluated by using H_2O_2 as substrate and PhSH as cosubstrate. Diselenides (**13**) and (**14**) with dimethylaminomethyl- or nitro-donor groups in close proximity to selenium, show much better thiol peroxidase-like activities compared to diselenides **10–12** with amide donor groups. Cyclic voltammetry study of diselenides **10–12** derived from redox-active ferrocenamide has been carried out.

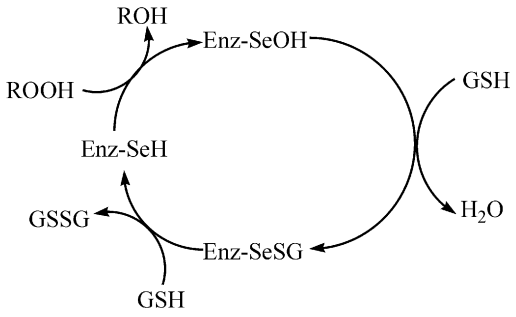
Keywords. Selenium; diorganodiselenide; ferrocenamide; thiol peroxidase; antioxidant.

1. Introduction

Glutathione peroxidase is a selenoenzyme, which is well known for its antioxidant function. This selenoenzyme catalyses the reduction of various harmful peroxides produced in biological systems and protects the cell from oxidative stress.[1] The enzyme (Enz-SeH) reduces the peroxides to the corresponding alcohol or water and in the process gets converted to the corresponding selenenic acid (Enz-SeOH). The cofactor glutathione (GSH) is next introduced into the enzyme forming the Enz-SeSG complex, which then reacts with a second molecule of GSH to regenerate the reduced enzyme (Enz-SeH) and the oxidized disulphide product, GSSG (Scheme 1).[2]

A variety of small organoselenium compounds have been reported as mimics of glutathione peroxidase enzyme (chart 1).[3] These include N–Se heterocycles,[4,5] recently reported Se–O heterocycles,[6,7] artificial selenoenzyme selenosubtilisin,[8] selenopeptides,[9] variously substituted diselenides,[10] and their tellurium analogues.[11,12] We have previously reported the thiol peroxidase-like activities of a range of intramolecularly coordinated diorganodiselenides (**1–9**) (chart 1).[10c,10d,10e] It was observed that the diselenides (**1–2**) with basic *tert*-aminoferrocenyl coordinating groups, showed much better thiol peroxidase-like activity compared to other related diselenides.

In this context, we contemplated investigating the thiol peroxidase-like activity of other related diselenides based on ferrocene.[13] In this paper, we report the synthesis, characterization and thiol peroxidase-like activity of ferrocenamide-based diselenides (**10–12**). In our earlier study, it was further observed that diselenide **3** with a non-conjugated amine



Scheme 1. Catalytic mechanism of glutathione peroxidase enzyme.

*For correspondence
[#]Dedicated to the memory of the late Professor Bhaskar G Maiya

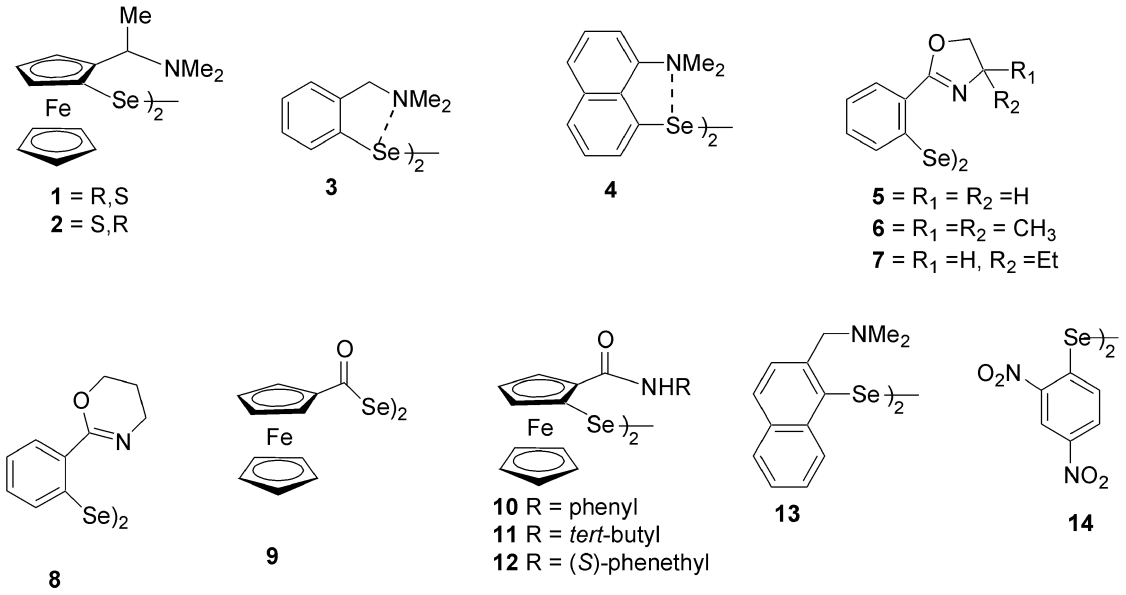


Chart 1. Diorganodiselenides as thiol peroxidase mimics.

group, exhibited significant thiol peroxidase-like activity whereas deselenide **4** with a conjugated amine donor group was inactive under identical conditions. To delineate the effects of conjugated and non-conjugated N,N′-dimethylamino groups, we decided to prepare and study the thiol peroxidase-like activity of **13** which has both the naphthyl group and the non-conjugated amino group. Thiol peroxidase-like activity of diselenide **14** with an ortho-coordinating nitro group is also compared.

2. Experimental section

2.1 *Materials*

Ferrocene carboxylic acid was prepared from ferrocene by following the literature method.[14]

2.1a *General procedures:* All reactions were carried out under nitrogen or argon using standard vacuum-line techniques. Solvents were purified by standard procedures and were freshly distilled prior to use. Melting points were recorded in capillary tubes and are uncorrected. IR spectra were recorded as KBr pellets on a Nicolet Impact 400 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ on a Varian VXR 300S spectrometer. ^{1}H chemical shifts are cited with respect to $SiMe_4$ as internal standard. The ^{77}Se spectra were obtained at 95·35 MHz in $CDCl_3$ on a Bruker AMX500 spectrometer using diphenyl diselenide as external standard. Chemical shifts are reported relative to dimethyl selenide (^{77}Se) (0 ppm) by assuming that the resonance of the standard is at 461 respectively. Elemental analyses were performed on a Carlo–Erba model 1106 elemental analyser. Optical rotations were measured using a Jasco Model DIP 370 digital polarometer. Electrospray mass spectra (ES-MS) were performed at room temperature on a Q-Tof micro (YA-105) mass spectrometer. FAB mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kv, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature. *m*-Nitrobenzyl alcohol NBA was used as the matrix unless specified otherwise. The matrix peak may appear at *m*/*z* 136, 137, 154, 289, 307 in the absence of any metal ions. If metal ions such as Na^+ are present these peaks may be shifted accordingly. For isotopes the value given is for the most intense peak. Cyclic voltammetry: CH I660 scanning potentiostat; Pt working and auxiliary electrodes; Ag/Ag^+ (0·1 M $AgNO_3$ in CH_3CN) as reference electrode; 0·1 M $[NBu_4][ClO_4]$ in CH_3CN as a supporting electrolyte; scan rate 200 mV s^{-1}; under these condition $[Fe(C_5H_5)_2]/[Fe(C_5H_5)_2]^+$ has $E_{1/2}$ of + 0·060 V.

2.1b *Synthesis of N-phenylferrocenecarboxamide* **(15):**[15] To a stirred solution of ferrocene carboxylic acid (2·30 g, 10 mmol) in CH_2Cl_2 (10 ml) was added

oxalyl chloride (1·32 ml, 15 mmol) via syringe at room temperature under nitrogen. Gas evolution was accompanied by the formation of a dark red coloured homogeneous solution. After 30 min, solvent was removed under *vacuo*. The resultant crude ferrocenoyl chloride was isolated as a red oil that crystallized on standing. It was taken up in CH_2Cl_2 (20 ml) and added via syringe to a solution of phenyl amine (0·93 g, 10 mmol) and triethyl amine (2·1 ml, 15 mmol) under nitrogen at 0°C. The reactiom mixture was stirred overnight. The dark red coloured reaction mixture was washed with 2×50 ml of water extracted with 2×50 ml of CH_2Cl_2, dried over Na_2SO_4 and concentrated under *vacuo*. The crude product was purified by column chromatography using SiO_2 (60–120 mesh) and CH_2Cl_2/CH_3OH (9·8 : 0·2) to give compound **15** as a yellow crystalline powder. Yield: 2·85 g (93·4%), m.p. 200–202°C; Anal. Calcd. for $C_{17}H_{15}FeNO$: C, 66·91; H, 4·95; N, 4·58%; Found: C, 66·59, H, 4·95; N, 4·81%; IR (KBr): 3294, 3085, 2921, 1642, 1313, 696 (cm^{-1}); ^1H NMR ($CDCl_3$) *d* 4·26 (*s*, 5H), 4·44 (*t*, 2H), 4·78 (*t*, 2H), 7·08–7·16 (*m*, 1H), 7·32–7·41 (*m*, 2H), 7·56–7·64 (*m*, 2H).

2.1c *Synthesis of N-tert-butylferrocenecarboxamide* **(16):**[15] Ferrocenecarboxamide (**16**) was synthesized by a similar method to that described for **15** at 15 mmol scale. The standard workup gave the crude product, which was purified by column chromatography using SiO_2 (60–120 mesh) and CH_2Cl_2/CH_3OH (9·8 : 0·2) to give compound **16** as a yellow crystalline powder. Yield: 3·93 g (93%), m.p. 205–208°C; Anal. Calcd. for $C_{15}H_{19}FeNO$: C, 63·17; H, 6·72; N, 4·91%; Found: C, 62·87, H, 6·58; N, 5·17%; IR (KBr): 3315, 3079, 2973, 1630, 1551, 1453, 1308, 1222, 821 (cm^{-1}); ^1H NMR ($CDCl_3$) *d* 1·44 (*s*, 9H), 4·32 (*t*, 3H), 4·65 (*t*, 2H); 5·4 (broad *d*, 1H), GC retention time = 12·97 min, GC–MS (% abundance): 285 (85%), 229 (100%), 211 (50%), 137 (60%).

2.1d *Synthesis of (S)-N-phenethylferrocenecarboxamide* **(17):**[16] Ferrocenecarboxamide (**17**) was prepared by a similar method to that described for **15** at 2 mmol scale. The crude product was purified by column chromatography using SiO_2 (60–120 mesh) and CH_2Cl_2/CH_3OH (9·8 : 0·2) to gave compound **17** as a yellow crystalline powder. Yield: 0·6 g (90%), m.p. 198–202°C; [*a*] = + 34·99 (0·1c, $CHCl_3$); Anal. Calcd. for $C_{17}H_{15}FeNO$: C, 68·48; H, 5·74; N, 4·20%. Found: C, 68·37, H, 4·95; N, 4·81%. IR (KBr): 3310, 3085, 2921, 1642, 1313, 696 (cm^{-1}). ^1H NMR ($CDCl_3$) *d* 1·56 (*d*, 3H), 4·11 (*s*, 5H), 4·31 (*t*, 2H), 4·61 (*d*, 1H), 4·67 (*d*, 1H), 5·28 (*m*, 1H), 5·82 (*d*, 1H), 7·27 (*m*, 1H,), 7·37 (*m*, 4H); ^{13}C NMR ($CDCl_3$): *d* 21·7, 48·5, 67·83, 68·41, 69·28, 70·43, 70·44, 76·07, 126·42, 127·4, 128·7, 143·6, 169·3. ES–MS: *m*/*z* = 333 (100) [M^+].

2.1e *Synthesis of bis[2-(N-phenyl ferrocenylcarboxamide)] diselenide* (**10**): To a stirred solution of ferrocenecarboxamide **15** (0·61 g, 2 mmol) in dry THF (35 ml) under N_2 at 0°C was added *n*-BuLi (2·25 ml, 4·2 mmol, 1·6 M solution in hexane) dropwise (≈ 1 drop/10 s). A dark red coloured, homogenous solution of dianion (**18**) was formed after 40 min. Elemental selenium (0·16 g, 2 mmol) was added to the resulting reaction mixture under a brisk flow of N_2 to exclude the air. The selenium powder was consumed rapidly to give a homogeneous solution of blackish red coloured lithium selonolate (**19**). The reaction mixture was poured into saturated aqueous solution of $K_3Fe(CN)_6$ (0·85 g, 2·5 mmol), and then placed in a separating funnel and extracted with CH_2Cl_2 (2×25 ml). Standard work up, followed by column chromatography using SiO_2 (60–120 mesh), CH_2Cl_2/CH_3OH (18 : 2) gave a red colour solid. Recrystallisation from CH_2Cl_2/CH_3OH afforded dark red coloured crystals. Yield: 0·52 g (68%), m.p. 138–140°C; Anal. Calcd. for $C_{34}H_{28}Fe_2N_2O_2Se_2$: C, 53·32; H, 3·68; N, 3·65%. Found: C, 52·48, H, 3·71; N, 3·28%; IR (KBr): 3302, 3093, 2927, 2855, 1657, 1604, 1532, 1440, 1249, 762 (cm^{-1}). ^1H NMR ($CDCl_3$) *d* 4·21 (*s*, 10H), 4·34–4·56 (*m*, 4H), 5·04–5·10 (*m*, 2H), 7·06–7·14 (*m*, 4H), 7·26–7·36 (*m*, 4H), 7·48 (*d*, 2H), 9·07 (*d*, 2H); ^{13}C NMR ($CDCl_3$): *d* 71·3, 71·49, 72·03, 72·65, 72·85, 73·65, 80·39, 119·6, 123·8, 128·8, 167·8. ^{77}Se NMR ($CDCl_3$) *d* 484, 489; MS (FAB): *m*/*z* 768 (M^+), 701, 613, 460, 307, 289, 165, 154, 138, 120, 107.

2.1f *Synthesis of bis[2-(N-tert-butylferrocene carboxamide)] diselenide* **(11)**: Compound **11** was synthesized as described for **10**, from N-tert-butylferrocenylcarboxamide at 2 mmol scale and purified by column chromatography by using SiO_2 (60–120 mesh), CH_2Cl_2/CH_3OH (15 : 2) to give red coloured solid. Recrystallization from CH_2Cl_2 and hexane afforded dark red coloured crystals. Yield: 0·51 g, (71%), m.p. 142–145°C; Anal. Calcd. for $C_{30}H_{36}Fe_2N_2O_2Se_2$: C, 49·64; H, 4·99; N, 3·85%. Found: C, 50·87, H, 5·79; N, 3·42%. IR (KBr): 3342, 2967, 2987, 2855, 1670, 1644, 1525, 1466, 1229,

815 (cm^{-1}). ^{77}Se NMR ($CDCl_3$) *d* 450, 457; MS (FAB): *m*/*z* 726 (M^+).

2.1g *Synthesis of (S, S) bis[2-(N-phenethylferrocene carboxamide)]diselenide* (**12**): Diselenide (**12**) was prepared similarly as described for related diselenide (**10**) at 2 mmol scale. The oxidized product was purified by column chromatography using SiO_2 (60–120 mesh), CH_2Cl_2/CH_3OH (18 : 2) to give a red coloured solid. Recrystallization from CH_2Cl_2/CH_3OH afforded dark red coloured crystals. Yield: 0·56 g (68%), m.p. 140–145°C; [*a*] = + 96·82 (0·1c, $CHCl_3$); Anal. Calcd. for $C_{38}H_{38}Fe_2N_2O_2Se_2$: C, 55·38; H, 4·64; N, 3·40%. Found: C, 55·12, H, 3·98; N, 3·28%; IR (KBr): 3326, 3084, 3027, 2967, 2924, 1682, 1527, 1449, 1374, 1263, 821, 699 cm^{-1}; ^1H NMR ($CDCl_3$) *d* 1·60 (*d*, 6H), 4·09 (*q*, 3H), 4·24 (*s*, 10H), 4·32–5·28 (*m*, 6H), 5·29 (*d*, 2H), 7·26–7·35 (*m*, 10H); ^{13}C NMR ($CDCl_3$): *d* 21·7 48·5, 67·83, 68·41, 69·28, 70·43, 70·44, 76·07, 126·42, 127·4, 128·7, 143·6, 169·3; ^{77}Se NMR ($CDCl_3$) *d* 455, 460; ES–MS: *m*/*z* 823 (M^+).

2.1h *Synthesis of 1-bromo-2-bromomethylnaphtalene* **(20)**:[17] A CCl_4 solution (150 ml) of 2-methylbromonaphthalene (9·95 g, 7 ml, 45 mmol) and N-bromosuccinamide (8·9 g, 50 mmol) was refluxed in the presence of benzoyl peroxide (0·1 g) for 16 h under N_2. The solid formed by the reaction was filtered off and the solvent removed in *vacuo*. Recrystallization from hexane at 0°C afforded a white crystalline solid, **20**. Yield: 12·96 g (96%), m.p. 106–107 (lit. 107–109°C),[17] Anal. Calcd. for $C_{11}H_8Br_2$: C, 44·06; H, 2·69%; Found: C, 43·99; H, 2·38%; GC retention time = 12·26 min; IR (KBr) 3058, 2954, 2822, 1690, 1512, 1466, 1287, 1045, 821 cm^{-1}, ^1H NMR ($CDCl_3$) *d* 4·78 (*s*, 2H), 7·26–7·78 (*m*, 4H), 8·12–8·28 (*m*, 2H); GC–MS (% abundance), 301 (50), 221 (100), 222 (70).

2.1i *Synthesis of 1-bromo-2-(N,N-dimethylaminomethyl)naphthalene* **(21)**:[18] To a THF solution (100 ml) of 1-bromo-2-bromomethylnaphthalene; **20** (3 g, 10 mmol) and dimethyl amine (0·9 g, 20 mmol, 4·0 ml (40% aqueous solution) were added dropwise at 0°C under N_2 and stirring was continued for a further 1 h at 0°C. Then the reaction mixture was refluxed for 19 h. The reaction mixture was poured into cold water and extracted with 3×25 ml of Et_2O, dried over Na_2SO_4, concentrated in *vacuo* at 45°C and purified by column chromatography using SiO_2 (60–120 mesh) and petroleum ether (60–80°C)/ethyl acetate (6 : 4) to give light yellow coloured oil of **21**. Yield: 2·27 g (86%); GC retention time = 13·17 min; ^1H NMR ($CDCl_3$) *d* 2·25 (*s*, 6H), 3·68 (*s*, 2H), 7·42 (*t*, 2H), 7·56 (*t*, 2H) 8·38 (*d*, 1H); 8·17 (*d*, 1H), IR n_{max} (neat): 3058, 2954, 2822, 1690, 1512, 1466, 1287, 1045, 821 cm^{-1}; GC–MS (% abundance), 263, 221 (100); 219 (70).

2.1j *Synthesis of bis[2-(N,N-dimethylaminomethylnaphthyl]diselenide* **(13):** To a solution of **21** (1·84 g, 10 mmol) in dry Et_2O (75 ml) was added dropwise *n*-BuLi (7·7 ml, 12·3 mmol, 1·6 M solution in hexane) under N_2 at –78°C over a period of 10 min. The mixture was stirred for 30 min at this temperature and 30 min at 0°C. Selenium powder (0·79 g, 10 mmol) was added portion wise at 0°C. The reaction mixture was stirred further for 5 h from 0°C to room temperature. The reaction mixture was poured into cold aqueous saturated $NaHCO_3$ and O_2 was passed at a moderate rate for 30 min. The organic phase was separated, dried over Na_2SO_4 and filtered. The filtrate was concentrated to 15 ml. A light yellow solid, which precipitated out from the resulting solution on cooling, was filtered out. The compound was recrystallized from ethanol to give yellow needles. Yield: 1·2 g (46%), m.p. 165–167°C; Anal. Calcd. for $C_{26}H_{28}N_2Se_2$: C, 59·34; H, 5·36; N, 5·32%; Found: C, 58·76, H, 5·25, N, 5·67%; IR (KBr): 3072, 2967, 2927, 2769, 1506, 1460, 1262, 1032, 821 cm^{-1}; ^1H NMR ($CDCl_3$) *d* 2·45 (*s*, 12H), 3·98 (*s*, 2H), 7·42 (*t*, 2H), 7·58–7·71 (*m*, 4H), 7·98 (*t*, 4H), 8·92 (*d*, 2H); ^{13}C NMR ($CDCl_3$) *d* 24·59, 125·05, 126·39, 127·88, 127·93, 128·88, 129·42, 129·89, 132·38, 135·87, 142·55. ^{77}Se NMR ($CDCl_3$) *d* 356; ES–MS (*m*/*z*) 527 (M^+).

2.1k *Synthesis of bis(2,4-dinitrophenyl) diselenide* **(14):**[19] Sodium (0·23 g, 10 mmol), selenium powder (0·8 g, 10 mmol) and naphthalene (0·12 g, 1 mmol) were refluxed in dry THF (15 ml) under N_2 for overnight. The colour of the reaction mixture turned to brown. To this reaction mixture, 2,4-dinitro-chloro-benzene (2·02 g, 10 mmol) in dry THF (6 ml) was added at 0°C. The stirring was continued for an additional 1 h, the product was filtered off, and washed with ether 4×20 ml and then with hexane. It was recrystallized from CH_3OH and dried under *vacuum* to afford a yellow crystalline solid. Yield: 1·87 g (76%), m.p. 262–264 (lit. 268°C); Anal. Calcd. for $C_{12}H_6N_4O_8Se_2$: C, 29·30; H, 1·23; N, 11·38%; Found: C, 28·86, H,

1·16, N, 11·98%; IR (KBr): *n*, 3067, 2936, 2857, 1618, 1622, 1542,1448, 758 cm^{-1}; ^1H NMR ($CDCl_3$) *d* 7·87 (*d*, 2H), 8·76 (*d*, 2H), 8·98 (*s*, 2H); ^{13}C NMR ($CDCl_3$) *d* 122·14, 128·51, 136·37, 136·88, 148·29, 147·64. ^{77}Se NMR ($CDCl_3$ + DMSO) *d* 525. ES–MS: *m*/*z* 348 (M^+), 3413, 258, 239, 213, 183, 155, 129.

2.2 *Kinetic analysis*

The reactions of model compounds with benzenethiol (PhSH) and H_2O_2 were studied in methanol by following the appearance of the disulphide absorption at 305 nm, at 25°C.[10b] Each initial velocity was measured at least six times and calculated from the first 5–10% of the reaction. For the peroxidase activity, the rates were corrected for the background reaction between H_2O_2 and PhSH. The actual concentration of PhSH in the kinetic apparatus was measured from the 305 nm absorbance, and rates were corrected for any variation in the concentration of PhSH. The molar extinction coefficient of PhSSPh ($e = 1{\cdot}24 \times 10^{-3}\ M^{-1}\ cm^{-1}$) at the wavelength was much larger than that of PhSH ($e = 9\ M^{-1}\ cm^{-1}$). The concentration of PhSH was, therefore, calculated from the absorbance (*a*) according to the following equation: $C = (e_1C_0 - 2a)/(e_2 - 2e_2) \approx C_0 - 2a/e_1$. The initial reduction rate of H_2O_2 (n_0) was then determined by $1/n_0$ vs 1/[PhSH] plots using the Grapher program. Concentration of the H_2O_2 stock was determined by permanganate titration. To investigate the dependence of rate on substrate concentrations, the reaction rates were determined at several concentrations of one substrate while keeping the concentration of the other constant. The Lineweaver–Burk plots were obtained using the Grapher 1·09 version, 2D-Graphing System for Windows program.[20] For each set of experiments a straight line was drawn by choosing the best fit method.

3. Results and discussion

3.1 *Synthesis*

N-Phenylferrocenecarboxamide (**15**), N-*tert*-butylferrocenecarboxamide (**16**) and (*S*)-N-phenethylferrocenecarboxamide (**17**) were synthesized from the ferrocenoyl chloride and corresponding primary amines in the presence of catalytic amount of triethyl amine. Ferrocenoyl chloride was generated *in situ* by the reaction of ferrocene carboxylic acid and oxalyl chloride (scheme 2). Initially the lithiation of ferrocenamides (**15–17**) with *n*-BuLi was attempted in diethyl ether. However, the reaction was unsuccessful.

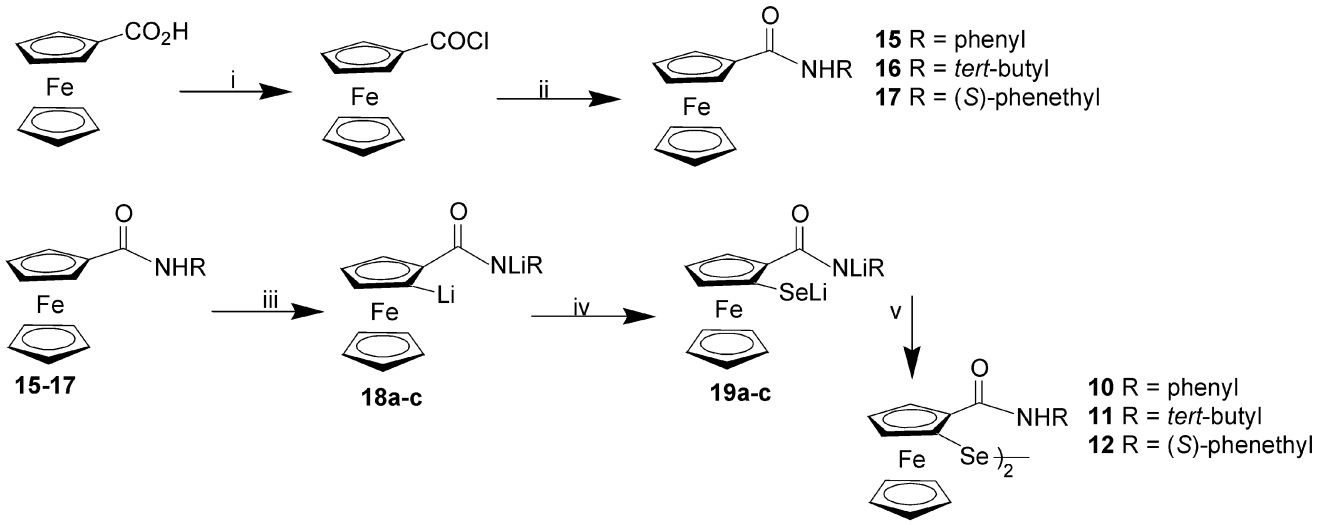
Lithiation of ferrocenecarboxamides was successful in THF. Addition of selenium to the dilithiated species (**18a–c**) provided a dark red coloured solution of dilithium areneselenolates (**19a–c**) at low temperature. Oxidation of **19a** using aqueous $K_3Fe(CN)_6$ gave the corresponding diselenide (**10**) in 68% yields. Similarly oxidative workup of **19b** and **19c** provided corresponding diselenides (**11** and **12**) in good yields (71–79%). These diselenides were purified by column chromatography.

Synthesis of diselenide (**13**) was accomplished by the well-established organolithium route (scheme 3). Bromine/lithium exchange with *n*-BuLi in Et_2O proceeded smoothly to give the desired aryllithium (**22**). This reagent slowly reacts with elemental selenium to give aryllithium selenolate (**23**). Oxidation of **23** provided the desired diselenide. Diselenide (**14**) was synthesized by the reaction of disodium diselenide (Na_2Se_2) and 2,4-dinitro-1-chlorobezene.[19]

3.2 *Spectroscopic study*

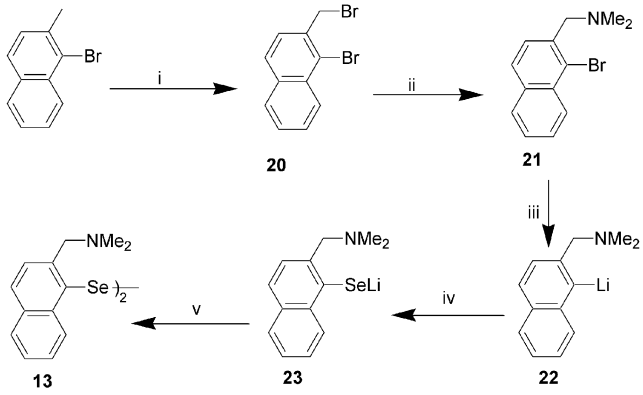
Ferrocenecarboxamide-based diselenides (**10–12**) show two closely spaced signals in the ^{77}Se NMR spectra (*vide supra*, see experimental section) and the mean values are reported here for comparison. The ^{77}Se NMR signal for compound **13** was observed at 356 ppm and this is shifted upfield compared to related diselenide **4** (484 ppm), phenyl analogue, **2** (430 ppm), and bis[2-(N,N-dimethylaminomethyl)ferrocenyl] diselenide (576 ppm).[10d,21,22] The downfield shift of the ^{77}Se NMR peaks is due to strong S…N nonbonding interaction as widely accepted, although the values for the shift do not correspond exactly to the strength of the Se…N interaction. Thus, upfield shift in **13** shows the presence of weak intramolecular Se…N intearaction compared to diselenides **3** and **4**. The ^{77}Se NMR chemical downfield shift for diselenide **14** with two electron-withdrawing nitro groups is 525 ppm.

In the IR spectra, n_{NH} vibrations for diselenides **10–12** are 3302, 3342 and 3326 cm^{-1} respectively. These values are observed at frequencies slightly higher than their precursors **15–17** (3294 and 3315, 3310 cm^{-1}). Similarly $n_{C=O}$ vibrations for diselenides (**10–12**) are shifted slightly upper frequency range 12–40 cm^{-1} compared to **15–17** and indicating the presence of interaction between selenium and oxygen of the amide group.



Reagents and conditions: (i) *n*-BuLi, THF, –15°C, 30 min; (ii) Se powder, 0°C, 2 h; (iii) aqueous $K_3Fe(CN)_6$; (iv) *t*-BuLi, THF, 0°C, 30 min; (v) Se powder, 0°C, 1 h; (vi) aqueous $K_3Fe(CN)_6$.

Scheme 2. Synthesis of diselenides (**10–12**) and their precursors (**15–17**).



Reagents and conditions: (i) NBS, CCl_4, reflux, 24 h; (ii) aqueous $HNMe_2$, THF, reflux; (iii) *n*-BuLi, Et_2O, –78°C, 30 min; (iv) Se powder, 0°C, 5 h; (v) O_2, aqueous $NaHCO_3$

Scheme 3. Synthesis of diselenide (**13**) and bromonaphthylamine (**21**).

3.3 *Thiol peroxidase-like activity*

Catalytic activity was studied according to the thiol assay method reported by Tomoda *et al*[10b] using benzenethiol (PhSH) as a glutathione alternative. The thiol assay has an advantage over the coupled assay due to use of a solvent system which is more compatible with diorganodiselenides.[23] The initial rates (n_0) for the reduction of H_2O_2 (3·75 mM) by thiol (1 mM) in the presence of various catalysts (0·01 mM) were determined in methanol medium by monitoring the UV absorption at 305 nm due to the formation of diphenyl disulfide (PhSSPh). The catalytic activities of the compounds are summarised in table 1.

Activities of some known catalysts have been carried out for comparison with those of the new diselenides prepared in this study. The uncatalysed reduction rate is very slow ($n_0 = 0{\cdot}15 \pm 0{\cdot}04$ *m*M min^{-1}), but a considerable enhancement in the rate is observed when the simple diphenyl diselenide was added ($n_0 = 0{\cdot}58 \pm 0{\cdot}17$ *m*M min^{-1}). Acitivites of diselenides **1**, **3** and **4** (entries a, b, c) are in close agreement with the reported values (table 1).[10d] Diselenides **10–12** show low reaction rates ($n_0 = 13{\cdot}38 \pm 0{\cdot}2$, $15{\cdot}97 \pm 0{\cdot}3$, $18{\cdot}98 \pm 0{\cdot}1$ *m*M min^{-1}, entries d, e, f) for H_2O_2 oxidation of benzenethiol. These diselenides show 40-fold lower activity than the ferrocene-based diselenides **1–2** ($n_0 = 574{\cdot}01 \pm 24$ *m*M min^{-1}). However, these diselenides are better catalysts than simple diferrocenyldiselenide ($n_0 = 3{\cdot}39 \pm 0{\cdot}3$ *m*M min^{-1}) and benzamide based diselenide, which is inactive.[10d] Diselenide (**13**), with a basic amino group, is found to be an efficient catalyst ($279{\cdot}8 \pm 10$ *m*M min^{-1}, entry g, table 1), which shows ~ 10-fold higher activity than the corresponding phenyl based diselenide **3** ($28{\cdot}38 \pm 4$ *m*M min^{-1}entry b, table 1). There was no noticeable effect on the reduction rate when only bromo naphthylamine (**21**) [$n_0 = 0{\cdot}78 \pm 0{\cdot}2$ *m*M min^{-1} entry i) is used as catalyst. It is interesting to note that the diselenide **4** has the basic amino group in close proximity to selenium and is inactive. In diselenide **4** basic amino groups are in conjugation with

selenium whereas in **13**, there is no such conjugation present. It is worth mentioning here that the simple dinaphthyl diselenide (5·4 *m*M min^{-1}) shows nearly 10-fold higher activity than diphenyl diselenide (0·55 *m*M min^{-1}).[10d] Diselenide **14** with two nitro groups exhibits 170·66 ± 9 *m*M min^{-1} (entry h) activity. The introduction of nitro group in ortho position to selenium in ebselen (2-phenyl-1,2-benzisoselenazol-3(2H-one)) has been shown to strongly enhance GP*x* activity and it has been suggested that influence of the elctronic effects on selenium by the interaction of nitro group may be responsible for the high GP*x* activity of nitrated ebselen.[24]

3.4 *Cyclic voltammetric study*

The redox properties of diselenides **10–12** and their precursors **15–17** were investigated by cyclic voltammetry in CH_3CN solution. A representative cyclic voltammogram for diselenide **10** shown in figure 1, reveals a reversible redox wave of equal intensity with half-wave potential ($E_{1/2}$) value of + 311 mV. Half-wave potentials values for diselenide **10–12** are 311, 292 and 287 mV and for their ligands **15–17** are 228, 206, 204 mV respectively.

$E_{1/2}$ values for these compounds **10–12** are considerably more positive than the corresponding value for ferrocene. This may be due to the strong electron-withdrawing nature of the amide groups, which are bonded directly to the Cp ring, making the oxidation more difficult than that of unsubstituted ferrocene.[25] Diselenides (**10–12**) have higher oxidation potentials than their precursors.

Table 1. The initial reduction rate (n_0) for diselenides.

Entry	Compounds	(n_0) *m*M min^{-1}	Reference
a	**1**	574·01 ± 24	10d
b	**3**	28·38 ± 4	10d
c	**4**	Inactive	10d
d	**10**	13·38 ± 0·2	This work
e	**11**	15·97 ± 0·3	This work
f	**12**	18·98 ± 0·1	This work
g	**13**	279·8 ± 10	This work
h	**14**	170·66 ± 9	This work
i	**21**	0·78 ± 0·2	This work

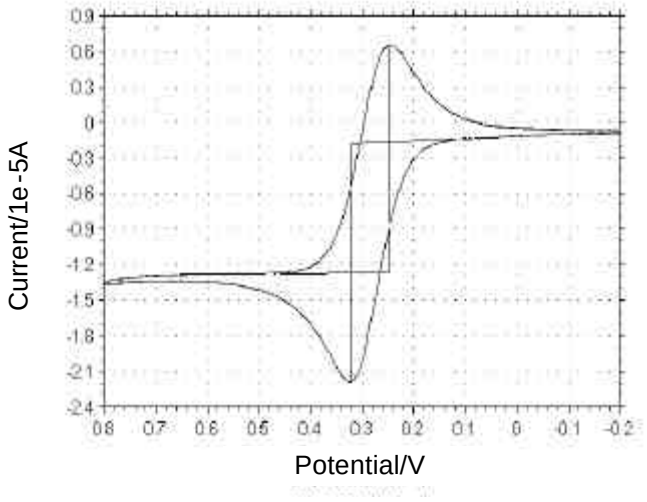


Figure 1. Cyclic voltammogram of compounds **10**.

4. Conclusion

In summary, a comparison of thiol peroxidase-like activities of various diorganoselenides has been made. The noticeable observations are as follows: (i) ferrocenecarboxamides based diselenide (**10–12**) show better activity compared to the benzamide based diselenides; (ii) diselenide (**13**) with an amino group without conjugation with the aromatic substrate shows better activity than diselenide **4**, which has an amino group in conjugation with the aromatic substrate.

Acknowledgments

This work was supported by the Department of Science and Technology, and the Council of Scientific and Industrial Research, Government of India, New Delhi.

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