Protection against Peroxynitrite-Mediated Nitration Reaction by Intramolecularly Coordinated Diorganoselenides

Sangit Kumar,[†] Harkesh B. Singh,*[,†] and Gotthelf Wolmershäuser[‡]

Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai 400 076, India, and Fachbereich Chemie, Universität Kaiserslautern, Postfach 3049, Kaiserslautern 67653, Germany

Received May 5, 2005

A series of intramolecularly Se···X (X = N, O) coordinated diorganoselenides and -sulfides are synthesized by using the heteroatom-directed lithiation route and characterized by multinuclear (^{1}H, ^{13}C, ^{77}Se) NMR, IR spectroscopy, and electrospray mass spectrometry (ES-MS). The intramolecular Se···O interactions in diorganoselenides **26**−**31** have been studied by multinuclear NMR studies in solution and in the solid state by single-crystal X-ray crystallography. The protection against peroxynitrite-mediated nitration reaction (PN assay) by diorganoselenides/-sulfides (with and without intramolecular coordination) has been evaluated. The PN assay data of diorganoselenides reveal that the selenides **20**−**22**, having a basic amino group (sp^3-N donor) in close proximity of selenium, are more active compared to the diorganoselenides **16**−**19**, having an imino group (sp^2-N donor), and also show much higher protective action than the unsubstituted diorganoselenides **14** and **15**. The diorganoselenides **16**−**31** were oxidized to corresponding selenoxides **44**−**59**. The redox properties of the selenoxides **13** and **44**−**59** have been investigated by cyclic voltammetry and potentiometric titration experiments. Two redox potentials for in situ-generated ferrocenyl selenoxides (**50, 54**−**59**) were observed. The reduction of selenoxides **13** and **48** to ebselen (**2**) and selenide **20** with benzenethiol (PhSH) was monitored by ES-MS.

Introduction

Peroxynitrite ($ONOO^-$/ONOOH), formed by the combination of nitric oxide ($^\bullet$NO) and superoxide ($O_2^{-\bullet}$) anion radicals, is a potent oxidizing agent. The radicals are generated by the endothelial cells.[1] The reactivity of peroxynitrite (PN) can be considered beneficial at the level of the whole organism because of its cytotoxicity to bacteria and other invading organisms.[2] However, excessive production of PN can damage normal tissue and induce DNA damage as well as initiate lipid peroxidation in biomembranes or low-density lipoproteins.[3] PN also causes tyrosine nitration of proteins and is known to deactivate a variety of enzymes.[4] Thus, its high reactivity with biological molecules and its high mobility even in the presence of biological membranes implicate its many disease states.[1,3]

Low molecular mass biologically occurring compounds such as CO_2, ascorbate, cysteine, and methionine or tryptophan have been shown to react with PN and protect biomolecules against PN-mediated damage.[5] In addition to these small molecules, some water-soluble manganese and iron porphyrins as well as other macrocyclic metal complexes and heme-containing proteins have been shown to act against PN-mediated reactions.[6] Selenoproteins such as glutathione peroxidase (GPx) and selenoprotein P and synthetic organoselenium compounds such as **1**−**12** have also been shown to reduce PN species (Chart 1).[7,8] It has also been demonstrated that some diorganotellurides might provide a defense system against PN.[7k,o]

Sies et al. have reported that selenomethionine (**1**) protects against PN more effectively than its sulfur analogue, methionine, because the selenium atom in **1** can be easily oxidized to the corresponding selenoxide and the oxidized species can be effectively and rapidly reduced to **1** by glutathione (GSH).[7b,p] They have also shown that the synthetic organoselenium compound, ebselen (**2**), which has been the subject of clinical trials to evaluate its anti-inflammatory properties,[9] is oxidized to the corresponding selenoxide **13** by the reaction with PN. The selenoxide is reduced back to ebselen at the expense of GSH, thus permitting a catalytic cycle as shown in Scheme 1.[7d]

* To whom correspondence should be addressed. E-mail: chhbsia@chem.iitb.ac.in.

[†] Indian Institute of Technology Bombay.

[‡] Universität Kaiserslautern.

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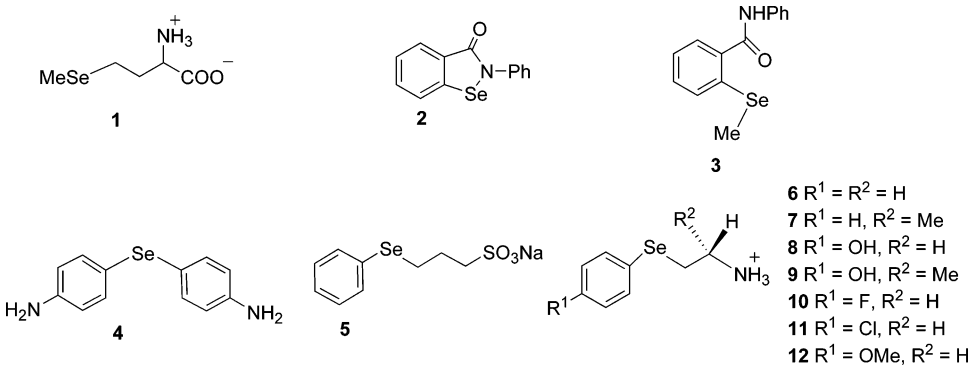
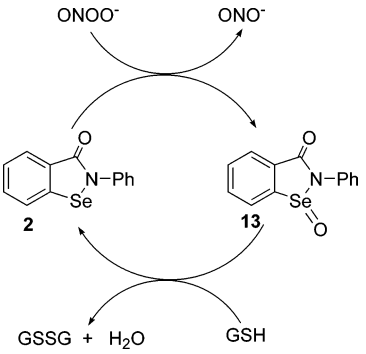
10.1021/om050353c CCC: $33.50
Publication on Web 12/15/2005

Chart 1. Organoselenium Compounds 1−12 with Protective Action against PN



Scheme 1. Redox Cycle of Ebselen (2) with PN



Furthermore, it has been reported that **1** and **2** can protect DNA from single-strand break formation caused by PN and the rate of the reaction is about 3 orders of magnitude faster than that of biologically occurring small molecules, such as ascorbate, cysteine, and methionine.[7d,e] Musaev et al.[10] have reported theoretical studies on the reaction of **2** and related derivatives with PN and found that the ebselen derivatives with weak Se−N bonds are active toward coordination with PN and peroxide (O−O) bond cleavage. Recent theoretical studies on the mechanism of the reaction of PN with dimethyl selenide have shown that the reaction proceeds via an O atom transfer mechanism and produces the corresponding selenoxide and NO_2^- anion. More recently May et al.[8b] have reported that the synthetic diorganoselenides (**6**−**12**) can protect against PN-induced DNA damage effectively in a catalytic fashion at the expense of GSH or ascorbate via a thiolselenurane intermediate.[8b] These studies have provided evidence that the selenium redox cycling can enhance the protective effects of organoselenium compounds against oxidant-induced DNA damage.

Intramolecularly coordinated organoselenium compounds have attracted considerable interest as glutathione peroxidase (GPx) enzyme mimetics.[11,12] Our group has recently reported the antioxidant property (thiol peroxidase-like activity) of a series of intramolecularly coordinated diorganodiselenides and ditellurides and found that diselenides having a ferrocenylamine redox active group show excellent thiol peroxidase activity.[12b−f] These observations prompted us to evaluate the protective action of intramolecularly coordinated organoselenium compounds against PN-mediated nitration reaction, because the intramolecular interaction in diorganoselenium compounds can (a) tune the electronic environment of selenium; (b) stabilize the selenoxide which is the intermediate in the PN assay; and (c) play a crucial role in the reduction of selenoxide to selenide. In this paper we report, for the first time, a systematic investigation on the use of intramolecularly coordinated diorganoselenides in PN-mediated nitration reactions (Chart 2).

In view of the excellent protective action against PN of ebselen (**2**) and selenides **3** and **6**−**12**, having amide and amino functional groups against PN, we contemplated incorporating intramolecularly coordinating amine and amide functional groups and the redox active ferrocenyl group in the desired selenides for potent protective action against PN-mediated nitration reaction. The protective effects of the intramolecularly coordinated ferrocenyl selenides/sulfides **22** and **26**−**34** incorporating *tert*-amine and amide functional groups have been studied to delineate the role of a ferrocene-like redox active

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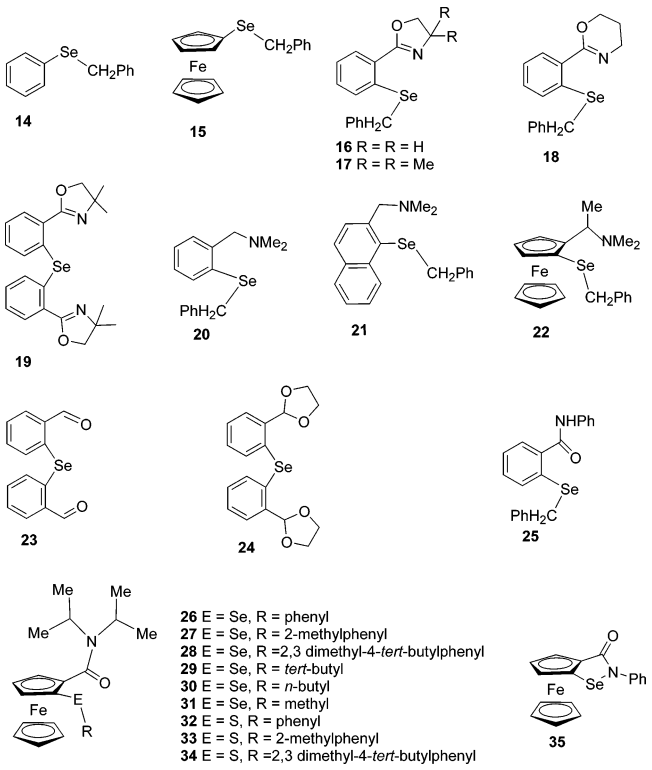
Chart 2. Diorganoselenides/Sulfides 14−34 with Protective Action against PN-Mediated Nitration Reaction



group in the PN assay. To incorporate the ebselen moiety in ferrocene, the synthesis of ferrocene-ebselen (**35**) was also attempted.

The reduction of selenoxides to selenides has been investigated by potentiometric titrations, and the reaction intermediates involved in the reduction of ebselen oxide (**13**) and selenoxide **48** by PhSH have been characterized by ES-MS. The redox properties of selenides/sulfides **22** and **26**−**34** and selenoxides/sulfoxides **13** and **44**−**62** were also evaluated by cyclic voltammetry in addition to the detailed synthesis and spectroscopic studies of the diorganoselenides. To gain insight into the intramolecular interactions, the crystal structures of **26**−**28** and **33**−**34** are determined by single-crystal X-ray crystallography.

Results and Discussion

Synthesis. Compounds **2**,[13] **15**,[14] **16**,[15] **18**,[12d] **17** and **20**,[16] **19**,[17] **23**,[18] and **24**[19] were synthesized by literature methods, and **25**[20] and **26**, **32**, and **39**[21] were synthesized by literature methods with minor modification. *N*,*N*-Dimethylaminomethyl-2-(benzylseleno)naphthalene (**21**) was prepared by the standard protocol shown in Scheme 2. The reaction of **36** with *n*-BuLi gave a lithiated intermediate (**37**). The reaction of **37** with selenium powder, followed by quenching of the resulting lithium selenolate **38** with benzyl chloride yielded the desired selenide **21**.

It is worth noting that the lithium halogen exchange reaction proceeded smoothly only at −78 °C and not at room temperature. On the other hand the synthesis of (*R*,*S*)-[1-(*N*,*N*-dimethylamino)ethyl]-2-(benzylseleno)ferrocene (**22**) by direct lithiation of (*R*)-(*N*,*N*-dimethylamino)ethylferrocene followed by selenium insertion[22] and then quenching with benzyl chloride was unsuccessful. Alternatively, the novel selenide **22** was prepared by the reduction of (*RR*,*SS*)-bis-2-[1-(*N*,*N*-dimethylamino)ethyl]ferrocenyl diselenide[22] followed by quenching of the resulting selenolate with benzyl chloride. Synthesis of *N*-phenyl-2-(benzylseleno)benzamide (**25**) was achieved by the direct metalation of benzanilide, followed by the addition of selenium powder and then quenching with benzyl chloride in one pot. The synthesis of **25** has been reported earlier by a multistep route. Schiesser et al.[20] have reported the synthesis of **25** from 2-benzylselenobenzoic acid (which itself requires several steps)[20c,23] by the addition of phosgene and subsequent quenching with aniline.

Ferrocenyl selenides/sulfides **26**−**34** were prepared by deprotonation of *N*,*N*-diisopropyl ferrocenecarboxamide (**39**) followed by quenching of the resulting arenelithium (**40**) with the corresponding diselenides/disulfides (Scheme 3). Reaction of **39** with the diselenides/disulfides (in 1:1 ratio) afforded the ferrocenyl selenides/sulfides **26**−**34** in 35−45% yield. Interestingly, deprotonation of **39** using 3.2 equiv of *n*-BuLi and TMEDA, followed by quenching with 4 equiv of corresponding diselenides, gave the ferrocenyl selenides in excellent yield (80−96%), and no deprotonation of the second Cp ring of **39** was noticed. All attempts to synthesize the benzylic compound ($RSeCH_2Ph$, R = *N*,*N*-diisopropyl ferrocenylcarboxamide) were unsuccessful.

The synthesis of ferrocene-ebselen (**35**) was first approached by the lithiation of **41** and cyclization of lithium selenolate dianion **42**, as shown in Scheme 4, and the reaction gave the corresponding diselenide **43**.[24] Also, the reaction of the dilithiated intermediate with $SeCl_2$ did not afford **35**, but led to decomposition. The synthesis of **35** from diselenide **43** following the procedures reported for the synthesis of **2** (radical cyclization,[20b] bromination, and then cyclization by NEt_3[20c]) was unsuccessful.

Selenoxides/sulfoxides **13** and **44**−**62** were generated in situ by the oxidation of selenides and sulfides by using H_2O_2 as an alternate to PN (Scheme 5)[10a] and were used directly for further investigations.

Spectroscopic Studies. The ^{1}H NMR chemical shifts for $-\underline{CH_2}NMe_2$ and $-CH_2N(\underline{CH_3})_2$ protons of **21** exhibit considerable downfield shifts (∼1 ppm) as compared to naphthylamine ligand (**36**), which indicates the presence of an intramolecular Se···N interaction in **21**. The ^{77}Se NMR chemical shifts of organoselenium compounds are quite informative about the electronic environment on the selenium nucleus. The ^{77}Se NMR chemical shifts of diorganyl selenides are listed in Table 1. The

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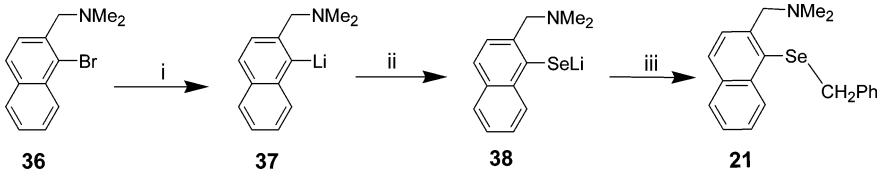
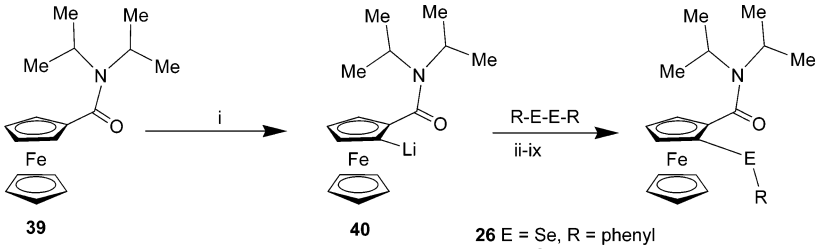
(24) The details of the synthesis of compound **43** will be reported elsewhere. Here we present only the reaction scheme for the attempted synthesis of ferrocene-ebselen (**36**).

Scheme 2. Synthesis of Selenide 21[a]



[a] Reagents and conditions: (i) *n*-BuLi, THF, −78 °C; (ii) Se powder, 5 h, 0 °C; (iii) $C_6H_5CH_2Cl$.

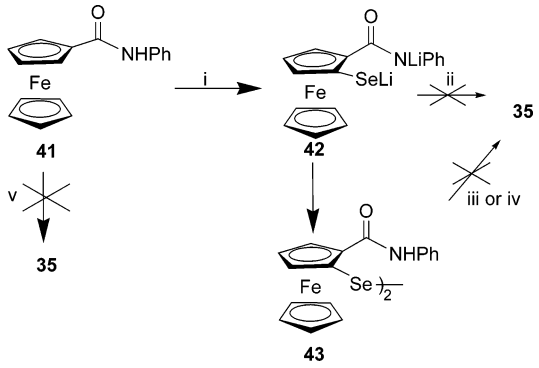
Scheme 3. Synthesis of Selenides/Sulfides (26−34)[a]



26 E = Se, R = phenyl
27 E = Se, R = 2-methylphenyl
28 E = Se, R = 2,3-dimethyl-4-*tert*-butylphenyl
29 E = Se, R = *tert*-butyl
30 E = Se, R = *n*-butyl
31 E = Se, R = methyl
32 E = S, R = phenyl
33 E = S, R = 2-methylphenyl
34 E = S, R = 2,3-dimethyl-4-*tert*-butylphenyl

[a] Reagents and conditions: (i) *n*-BuLi, TMEDA, −78 °C; (ii) diphenyl diselenide, THF, −78 to 0 °C, (iii) dimethyl diselenide, −78 °C; (iv) di(*n*-butyl) diselenide, −78 °C; (v) di(*tert*-butyl) diselenide, −78 °C; (vi) di(2,6-dimethyl-4-*tert*-butylphenyl) diselenide, THF, −78 °C; (vii) di(2-methylphenyl) diselenide, THF, −78 to 0 °C; (viii) diphenyl disulfide; (iv) di(2,6-dimethyl-4-*tert*-butylphenyl) disulfide; (ix) di(2-methylphenyl) disulfide.

Scheme 4. Attempted Synthesis of Ferrocene-Ebselen (35)[a]



[a] Reagent and conditions: (i) *n*-BuLi, Se; (ii) $CuBr_2$, (iii) Br_2, CuI, NEt_3, DMF; (iv) *tert*-butyl peroxide; (v) *n*-BuLi, $SeCl_2$.

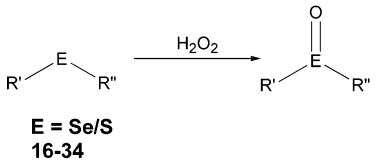
^{77}Se NMR chemical shifts for naphthylamine selenide **21** and ferrocenylamine selenide **22** are observed at 262 and 260 ppm, respectively, and are upfield shifted as compared to the benzylamine selenide **20** (321 ppm).[16] The ^{77}Se NMR chemical shift of **25** (376 ppm) is comparable with that of related selenides [(2-benzylseleno)benzamides (>N-*R* = hexyl, Bu, benzyl)] (361−371 ppm)[20b] and indicates a stronger Se···O interaction than ferrocenecarboxamide-based selenides (vide infra). The ^{77}Se NMR chemical shifts of **26**−**31** are in the order (ppm) phenyl > 2-methylphenyl > 2,6-dimethyl-4-*tert*-butylphenyl > *tert*-butyl > *n*-butyl ≫ methyl and can be correlated by the electron-donating nature of the −Se-*R* group (except methyl). The significant upfield ^{77}Se NMR chemical shift for selenide **28** (184 ppm) compared with **26** and **27** (294 and 242 ppm) indicates the strong electron donation of the 2,6-dimethyl-4-*tert*-butylphenyl group. Selenide **31** exhibits a signal at 85 ppm, which is significantly upfield shifted as compared to other related selenides. Ferrocenyl aryl selenides/sulfides **26**−**28** and **32**−**34** did not show any optical rotation, whereas ferrocenyl alkyl selenides **29**−**31** showed optical rotation (vide infra, Experimental Section).[21,25]

Protection against PN-Mediated Nitration of 4-Hydroxyphenylacetate (4-HPA). Protection by diorganoselenides and sulfides against nitration of 4-HPA was studied by following the method reported by Sies et al.[7b] Figure 1 shows the protection by selected diorganoselenides against PN-mediated nitration of 4-HPA.

The half-maximal inhibitory concentrations (IC_{50} values) obtained for the selenides/sulfides **14**−**34** are summarized in Table 1 along with their protective action against PN-mediated nitration reaction at 20 μM of selenides/sulfides **14**−**34** [% concentration of 4-hydroxy-3-nitrophenylacetate (NO_2−HPA) formed in the presence of 20 μM of testing compounds]. Most of the selenides exhibited some protective action in the PN assay. The result obtained for **2** is also included for a comparison.[7b] The concentration of NO_2−HPA in the absence of any test compound was ∼50 μM, and this value was set as 100%. The IC_{50} value for **2**, which has been previously used, was 63 μM (entry a, Table 1) and is in good agreement with the value (IC_{50} = 60 μM) reported in the literature.[7b] The IC_{50} value of simple selenide **14** is 134 μM (entry b, Table 1), and a slight enhancement in the inhibition was observed when the phenyl group in **14** was replaced by a redox active group, ferrocene (compound **15**; IC_{50} = 120 μM; entry b, Table 1). As expected, ferrocene alone did not have any effect on the formation of NO_2−HPA in the PN assay. Intramolecularly Se···N coordinated diorganoselenides **16** and **18**, having a sp^2-N donor atom in close proximity of selenium, exhibited lower protective action (IC_{50} = 157, 149 μM; entries d, f; Table 1) as compared with selenide **14**. This is probably due to the presence of an imino-nitrogen in **16**−**19**, which is not basic enough to enhance the nucleophilicity of selenium.[12e] On the other hand, the intramolecularly

(25) Slocum, D. W.; Tucker, S. P.; Engelmann, T. R. *Tetrahedron Lett.* **1970**, 621. Roman, E.; Astruc, D.; des Abbayes, H. *J. Organomet. Chem.* **1981**, *219*, 211.

Scheme 5. In Situ Preparation of Selenoxides and Sulfoxides (44−62)



E = Se/S
16-34

44-59 E = Se
44 R' = 2-phenyl-2-oxazolinyl, R" = benzyl
45 R' = 4,4-dimethyl-2-phenyl-2-oxazolinyl, R" = benzyl
46 R' = 2-phenyl-2-oxazinyl, R" = benzyl
47 R' = R" = 4,4-dimethyl-2-phenyl-2-oxazolinyl
48 R' = *N,N*-dimethylaminobenzyl, R" = benzyl
49 R' = *N,N*-dimethylaminonaphthyl, R" = benzyl
50 R' = (*R*)-1-[*N,N*-dimethylaminoethylferrocenyl], R" = benzyl
51 R' = R" = 2-benzaldehyde
52 R' = R" = 2-phenylacetal
53 R' = benzamide, R" = benzyl
54-62 R' = *N,N*-diisopropylferrocenecarboxamide,
54 R" = phenyl
55 R" = 2-methylphenyl
56 R" = 2,5-dimethyl-4-*tert*-butylphenyl
57 R" = *tert*-butyl
58 R" = *n*-butyl
59 R" = methyl
60-63 E = S
60 R" = phenyl
61 R" = 2-methylphenyl
62 R" = 2,5-dimethyl-4-*tert*butylphenyl

Table 1. IC_{50} Values of Testing Compounds; Nitration in 4-HPA at 20 μM of Testing Compounds, $E_{1/2}$; ^{77}Se NMR Chemical Shifts of Diorganoselenides

entry	compd	IC_{50} (μM)[a]	nitration of 4-HPA (20 μM)[b]	$E_{1/2}$ (mV vs Fc in CH_3CN)[c]	^{77}Se NMR (ppm)[d]
a	**2**	63	78 ± 12		942
b	**14**	134	89 ± 5		
c	**15**	120	87 ± 8	65	287
d	**16**	157	61 ± 7		376
e	**17**	ND	ND	ND	420
f	**18**	149	85 ± 13		362
g	**19**	ND	ND		420
h	**20**	43	83 ± 7		321
i	**21**	34	79 ± 6		262
j	**22**	28	65 ± 6	+52 ± 3	260
k	**23**	130	95 ± 11		393
l	**24**	54	87 ± 13		321
m	**25**	176	81 ± 9		377
n	**26**	80	85 ± 14	+193 ± 4	294
o	**27**	72	86 ± 7	+189 ± 2	242
p	**28**	52	77 ± 8	+139 ± 6	184
q	**29**	84	86 ± 5	+124 ± 7	172
r	**30**	90	88 ± 9	+126 ± 4	162
s	**31**	102	96 ± 12	+128 ± 5	86
t	**32**	>250	97 ± 14	+191 ± 2	
u	**33**	>250	96 ± 19	+190 ± 3	
v	**34**	>250	95 ± 15	+128 ± 6	
w	**39**		98 ± 17	+125 ± 1	

[a] Concentration of compound causing 50% inhibition of PN-mediated reaction (see also Figure 1). [b] Concentration of NO_2−HPA (%) when 20 μM of testing compound was used in PN assay. [c] $E_{1/2}$ oxidation potentials values for ferrocenyl selenides/sulfides and Fc = ferrocene. [d] Chemical shifts measured in $CDCl_3$ at RT and chemical shifts δ relative to Me_2Se; ND, not determined.

Se···N coordinated diorganoselenide **20**, having a sp^3-N donor atom, shows much better protective action (IC_{50} = 43 μM; entry h, Table 1) than the unsubstituted diorganoselenides **14** and **15**. Interestingly, very effective protective action was observed when the phenyl group in selenide **20** was replaced by a naphthyl or a ferrocenyl with the same functionality (**21** and **22**). The IC_{50} values (34, 28 μM, entries i, j, Table 1) observed for these compounds are much lower than that of all other compounds in the present study. This is probably due to the presence of a *tert*-amino group, because the presence of a *tert*-amino group in close proximity of selenium may enhance the nucleophilicity of selenium. The IC_{50} values of the intramolecularly Se···O coordinated selenides **23** and **24** are observed to be 130 and 54 μM, respectively (entries k, l, Table 1). The lower activity of these compounds may be ascribed to the electron-withdrawing

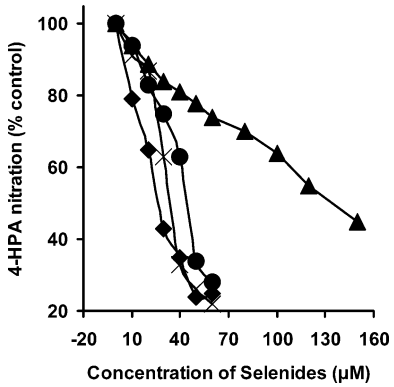


Figure 1. Effect of selenides [**14** (▲), **20** (●), **21** (×), and **22** (■)] on the nitration of 4-HPA caused by PN. Results are given as means ± SD (n = 5−7).

effect of the aldehyde group. This is in agreement with the reports of Sies et al.[7k] and May et al.[8] that aryl selenides with electron-withdrawing substituents show very poor activity. It is worth noting that selenide **25**, incorporating an amide functionality of ebselen, led to a decrease in the protective action (176 μM; entry m, Table 1) as compared to **2**, which is similar to the observation[7c] that the methyl derivative of **2** essentially reduces the protective effect. Further studies show that the aryl selenides **26**−**28** (80, 72, 52 μM; entries n, o, p) are considerably more active than the corresponding alkyl selenides **29**−**31** (84, 90, 102 μM; entries q, r, s, Table 1). The aryl selenide **28**, which contains an electron-donating substituent in the *para* position and two methyl substituents in the *ortho* positions, was found to be an efficient scavenger of PN (IC_{50} = 52 μM).[7k,8] In contrast to the selenium compounds, the sulfides **32**−**34** are found to be essentially inactive under identical experimental conditions. The very high IC_{50} values observed for these compounds indicate that there is no significant inhibition on the control activities (entries t, u, v, Table 1). The high reactivity of ferrocenyl selenides **26**−**28** as compared with their sulfur analogues may be ascribed to the higher nucleophilic character of selenium compared with sulfur.[26] Interestingly, diphenyl diselenide and bis[2-phenyl-2-oxazolinyl]diselenide are devoid of any protective action in the PN assay. Ebselen oxide (**13**) and selenoxide **48**, which are

(26) Wada, M.; Nobuki, S.; Tenkyuu, Y.; Natsume, S.; Asahara, M.; Erabi, T. *J. Organomet. Chem.* **1999**, 580, 282.

Table 2. Redox Potentials Obtained from Potentiometric Titrations and Reduction Potentials (E_{pc}) Obtained from Cyclic Voltammetry for Selenoxides and Sulfoxides

entry	compd	$E_{1/2}$, E_{pc}(mV)[a] vs NHE	E(mV)[b] vs NHE
a	**13**	−852 ± 7	*e*
b	**44**	−949 ± 10	*e*
c	**45**	−968 ± 12	*e*
d	**46**	−924 ± 6	*e*
e	**47**	ND	
f	**48**	−1176 ± 5	+423
g	**49**	−1200 ± 4	+385
h	**50**	+150, −1295 ± 6	+380
i	**51**	−896 ± 3	+490
j	**52**	−1168 ± 6	+415
k	**53**	−907 ± 11	+435
l	**54**	+680, −1205	+420
m	**55**	+638, −1270	+405
n	**56**	+618, −1350	+398
o	**57**	+622, −1361	+385
p	**58**	+654, −1362	+384
q	**59**	+646, −1359	+395
r	**60**	+656	*e*
s	**61**	+658	*e*
t	**62**	+645	*e*

[a] $E_{1/2}$ values correspond to ferrocene in ferrocenyl selenoxide/sulfoxides; E_{pc} values correspond to selenoxides/sulfoxides. [b] Redox potentials obtained from potentiometric titration. [e] A well-defined titration curve could not be obtained using sodium dithionite.

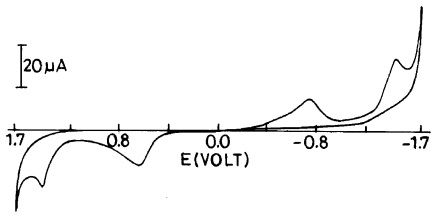


Figure 2. Representative cyclic voltammogram of selenoxide (**54**).

already in the oxidized form, also do not show any protective action in the PN assay.

Cyclic Voltammetry Studies. Peak potentials (E_{pc}) for selenoxides were for the first time reported by May et al.[8b] For comparison purposes, the E_{pc} values for in situ-generated selenoxides **13** and **44**−**62** were obtained under similar conditions by cyclic voltammetry. Cyclic voltammetry experiments were carried out in acetonitrile for selenides/sulfides **22** and **26**−**34** and in aqueous solutions for selenoxides/sulfoxides **13** and **44**−**62** at pH 5.5. Half-wave potentials ($E_{1/2}$) and peak potentials (E_{pc}) for reductive waves are given in Tables 1 and 2. In the case of selenides **22** and **26**−**31** the wave (which is not well defined) due to the selenium atom was observed at 1.60−1.90 V. These species exhibit electrochemically irreversible voltammograms, as evidenced by the absence of reduction waves. The additional feature may be due to the selenium acting as a redox center.[14] The selenoxides **13**, **44**−**49**, and **51**−**53** show a single redox wave, whereas ferrocene selenoxides **50** and **54**−**59** show two redox waves. A representative cyclic voltammogram for **54** is given in Figure 2.

In ferrocene selenoxides **54**−**59**, the redox potentials ($E_{1/2}$) due to the ferrocenyl moiety are observed in the range from +622 to +680 mV. In contrast, a significantly low $E_{1/2}$ value was observed for the ferrocenylamine selenoxide **50** (+150 mV; entry h, Table 2), which may be due to electron donation of the *tert*-aminoethyl group to the −Cp ring. The redox waves due to the selenoxide moiety are irreversible for **44**−**46** and **48**−**53** and quasi-reversible for **13** and **54**−**59** as evident from a large separation of the cathodic and anodic peak potentials. The peak potentials for the reductive waves (E_{pc}) are observed

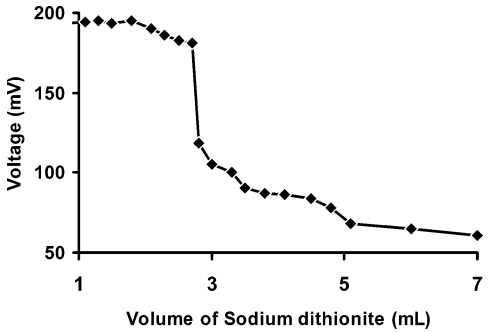


Figure 3. Potentiometric titration of selenoxide **54** (1 mM) by using 10 mM sodium dithionite as the titrant in 100 mM sodium acetate, pH 5.5.

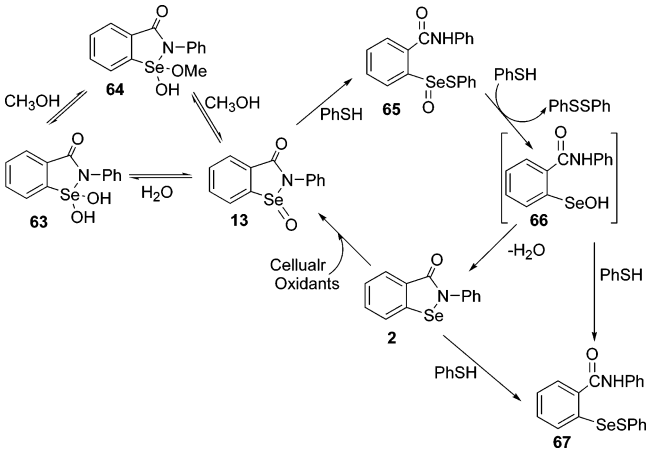
in the range from −852 to −1362 mV (Table 2). The E_{pc} values are in good agreement with the related compounds reported in the literature, which generally range from −892 to −1160 mV.[8b] Ferrocenyl alkyl selenoxides **57**−**59** exhibit E_{pc} values (−1361, −1362, and −1359 mV; entries o, p, q, Table 2) that are close to or slightly lower than those observed for the ferrocenyl aryl selenoxides **54**−**56** (E_{pc} = −1205, −1270, −1350 mV; entries m, n, o, Table 2). Selenoxide **56** with an electron-donating *tert*-butyl group at the *para* position shows a considerably lower E_{pc} value (−1350 mV) than the selenoxides **54** and **55**, and this is in accordance with the report of May et al.[8b] that electron-donating *para* substituents in benzene result in a decrease in E_{pc}. It is also evident from Table 1 that the E_{pc} values of selenoxides **51** and **52** are influenced by the presence of electron-withdrawing and -donating *ortho* groups in the aromatic rings (entries i, j, Table 2).

Potentiometric Titrations. To further understand the redox properties of the selenoxides, potentiometric titrations have been carried out by following the method reported by May et al.[8b] The results from the potentiometric titrations of selenoxides **13** and **44**−**59** were compared with their redox potentials (E) and related potentiometric values for some reported selenoxides (Table 2). A representative plot for potentiometric titration of **54** is shown in Figure 3.

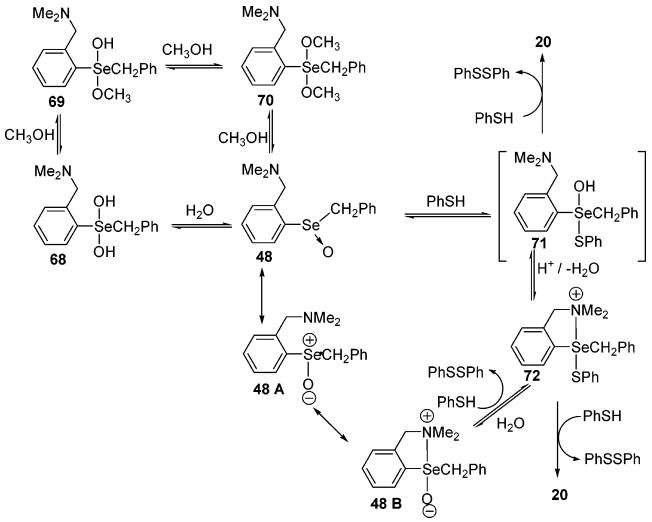
The salient features of the experimental results are (a) electron-withdrawing groups in the aromatic ring in **51** and **53** result in the increase in E (+490, +435 mV; entries i, k, Table 2); (b) naphthyl and ferrocenyl selenoxides **49** and **50** show lower E values (+385, +380 mV, entries g, h, Table 2) than the phenyl analogues, **48** (+423 mV; entry f, Table 2); and (c) ferrocenyl aryl selenoxides **54**−**56** exhibit higher E (+418, +405, +398 mV; entries m, n, o, Table 2) than the ferrocenyl alkyl selenoxides, **57**−**59** (E = +385, +384, +395 mV). It is worth mentioning that the selenoxides that exhibited lower reduction potentials in the cyclic voltammetry experiments show lower redox potentials (E) also in the potentiometric titrations (Table 2).

Electrospray Mass Spectrometry (ES-MS) Study. It has been well documented that diorganoselenides can be oxidized to selenoxides by PN and reduced back to diorganoselenides by reducing agents such as GSH or ascorbate, via the formation of a thiolselenurane intermediate.[8b] To understand the nature of the intermediates in the reduction of selenoxides to selenides in detail, the reduction of selenoxides **13** and **48** was monitored by ES-MS by using benzenethiol (PhSH) as an alternate to GSH. The electrospray mass spectra of organoselenium compounds gave distinct molecular ion peaks with characteristic isotopic patterns (illustrated for **13**, **29**−**31**, **48**, **54**, **65**, **67**−**69**, and **72** in Figures S50, S54, and S58−71 in the Supporting Informa-

Scheme 6. Proposed Mechanism for the Reduction of Ebselen Oxide (13) to Ebselen (2)



Scheme 7. Proposed Mechanism for the Reduction of Selenoxide 48 to Selenide 20



tion). The ES-MS of ebselen oxide (**13**) shows the molecular ion peak at 292 (*m*/*z*), and this compound may be in equilibrium with dihydroxy- and hydroxymethoxy-selenuranes; **63** and **64** (Scheme 6).

Although the ES-MS of **13** was somewhat complicated, it produced thiolseleninate (**65**, *m*/*z*, 400), when treated with 1 equiv of PhSH. When the 1:1 reaction mixture of **13** and PhSH was treated with an additional equivalent of PhSH, the molecular ion peaks for **2** and selenenyl sulfide **67** (*m*/*z*, 382) were detected in the ES-MS. This suggests that ebselen is regenerated from thiolseleninate (**65**) via the formation of intermediate **66**, although the formation of **66** has not been observed in the ES-MS, presumably due to its high reactivity. The conversion of selenenic acid **66** into selenenyl sulfide **67** also takes place as shown in Scheme 6.[27] In the case of intramolecularly Se···N coordinated selenoxide **48**, the ES-MS shows the molecular ion peaks for dihydroxy- (**68**, *m*/*z* 336), hydroxymethoxy- (**69**, *m*/*z*, 352), and dimethoxyselenuranes (**70**, *m*/*z*, 365) in addition to the selenoxide **48** (*m*/*z*, 322) (Scheme 7).

It seems that the solvation of **48** is more favorable than ebselen oxide (**13**), and this may be due the more basic nature of the selenoxide oxygen in **48**, which may in turn be due to

(27) (a) Glass, R. S.; Farooqui, F.; Sabahi, M.; Ehler, K. W.*J. Org. Chem.* **1989**, *54*, 1092. (b) Haenen, G. R. M.; De Rooij, B. M.; Vermeulen, N. P. E.; Bast, A. *Mol. Pharmacol.* **1990**, *37*, 412.

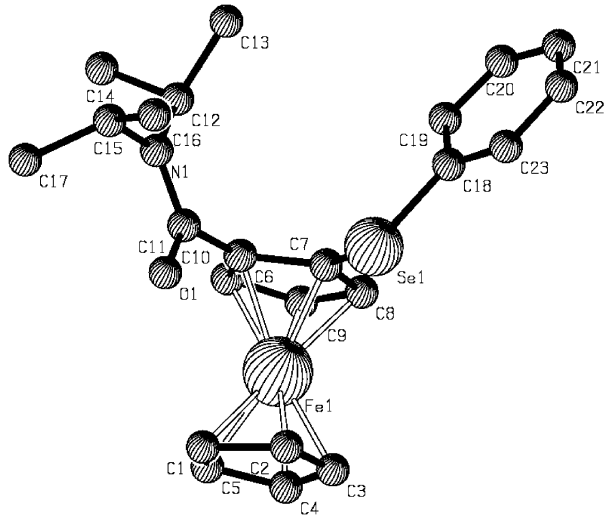


Figure 4. Molecular structure of **26**.

Table 3. Bond Lengths [Å] and Angles [deg] for 26−28, 33, and 34

	26	**27**	**28**
Se(1)−C(7)	1.885(3)	1.891(4)	1.898(3)
Se(1)−C(18)	1.914(4)	1.925(5)	1.927(3)
N(1)−C(12)	1.473(4)	1.472(6)	1.488(3)
C(7 or 6)−Se(1)−C(18)	101.1(15)	100.5(2)	99.9(10)
N(1)···Se(1)−C(18)	104.1(9)	78.2(3)	151.5(2)
O(1)···Se(1)−C(18)	100.9(4)	109.2(5)	164.6(7)

	33	**34**
S(1)−C(7)	1.744(3)	1.776(7)
S(1)−C(18)	1.780(3)	1.780(8)
N(1)−C(12)	1.470(4)	1.49(9)
C(7)−S(1)−C(18)	102.4(14)	105.3(4)
N(1)···S(1)−C(18)	78.7(11)	69.4(3)

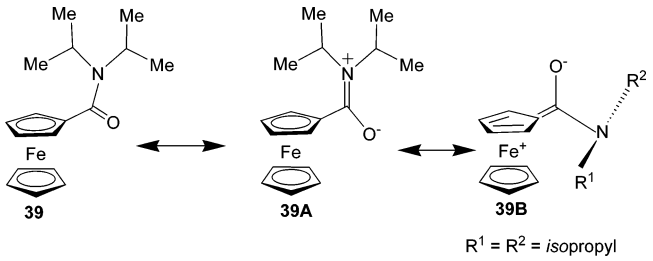
the presence of an *ortho* N-donor atom near selenium.[28] It appears that nitrogen donates its lone pair of electrons to selenium and increases the basic nature of selenoxide oxygen in **48** via resonance (**48A** and **48B**). The 1:1 molar reaction of **48** with PhSH shows the formation of **72** (*m*/*z*, 412), but does not show the formation of the thioseleneurane intermediate (**71**). This suggests that the reduction of selenoxide **48** takes place via **71**. The formation of **72** may also be possible directly by the reaction of selenoxide **48** with thiol. A similar mechanism has been proposed for the tellurium analogue of **48** by Detty et al.[29] It is worth mentioning that the oxidized selenomethionine residue in proteins can be reduced back to **1** rapidly by GSH through the formation of a three-electron Se···N species.[7p] Thus this Se···N interaction may also play a crucial role in the overall reduction of selenoxide **48** as shown in Scheme 7. The ES-MS experiments on the reaction of selenoxides **13** and **48** with PhSH suggest that the selenoxide **48** is reduced back to selenide **20** via a different reaction intermediate.

X-ray Crystallographic Study. The selenides **26**, **27**, and **28** are isostructural. The molecular structure of a representative selenide (**26**) is shown in Figure 4. Significant bond angles and bond lengths are given in Table 3. The distance between carbon and selenium (−Se−C−) and angle (−C−Se−C−) are well within the range of reported values for other diorganoselenides.[16,30]

(28) (a) Goodman, M. A.; Detty, M. R. *Organometallics* **2004**, *23*, 3016. (b) Shimizu, T.; Enomoto, M.; Takai, H.; Kamigata, N. *J. Org. Chem.* **1999**, *64*, 8242.

(29) You, Y.; Ahsan, K.; Detty, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 4918.

Chart 3. Resonance in 39 Based on the Related *N,N*-Dimethylferrocenecarboxamide[35]



The intramolecular Se···N distance (4.0741 Å) in selenide **26** is longer than the sum of van der Waals radii (3.45 Å)[31] of nitrogen and selenium, whereas the Se···O distance (3.5069 Å) is close to the van der Waals radius (3.40 Å). This crucial observation can be rationalized by resonance (vide infra, Chart 3). Compound **27** is isostructural to **26**. In this structure the Se···N distance is 4.102 Å, which is slightly larger than that for compound **26**. The Se···O distance is 4.1012 Å. The Se···N distance (4.4728 Å) in selenide **28** is longer than that observed for **26** and **27**. Surprisingly the Se···O distance (2.9425 Å) in **28** is shorter than the sum of van der Waals radii (Figure 5). Compared to the selenides **26** and **27**, the shorter distance between the selenium and oxygen in selenide **28** indicates that steric effects may play an obvious role.

The diorganosulfur molecules **33** and **34** are isostructural to **27** and **28**, respectively. The molecular structure of a representative sulfide (**33**) is shown in Figure 6. The carbon−sulfur bond distances and bond angles are comparable with the reported diorganosulfides.[15,32,33] The intramolecular S(1)···O(1) and S(1)···N(1) distances in **33** and **34** are longer than their van der Waals radii (O; S; 3.30 Å; N; S; 3.35 Å) and indicate that there is no significant intramolecular interaction between oxygen and sulfur.

The C−O bond lengths for compounds **26** and **27** (1.212−1.220 Å) are longer than C═O bond lengths (1.17−1.20 Å), while the C(11)−N bond distances (1.347−1.363 Å) for **26**−**28** are significantly shorter than the C(22)−N bond (1.488−1.463 Å) (Table 4). These indicate the double-bond character in C(11)−N as reflected in the resonance structures (**39A** and **39B**) shown in Chart 3.[34] The Se···O interactions in selenides **26**−**28** may also be correlated with the C(11)−N distances. The Se···O, Se···N, C(11)−O, and C(11)−N bond distances are shown in Table 4 for comparison. For selenide **28**, where the Se···O interaction is strong, the C(11)−N bond length [1.351(4) Å] is significantly shorter than the C−N [C(12)−N 1.488(3); C(15)−N 1.465(4) Å] single bond. The same is true for **26** and **27**. These distances suggest the existence of resonating structures in selenides **26** and **27**. Owing to the charged nature of the second resonance structures, **39A** and **39B**, one may expect O to be an electron donor. This diminishes the possibility of any intramolecular interaction of the nitrogen atom with selenium.

Steric crowding by two isopropyl groups, which are bonded to nitrogen, may be an additional factor to diminish the possibility of a selenium and nitrogen intramolecular interaction. The Se···O intramolecular distances in selenides based on **39** are in contrast to oxazoline-based ferrocenyl selenide[30] and oxazoline-based phenyl selenides,[16] in which selenium atoms are in contact with nitrogen. However, in both the ligands (oxazoline and carboxamide) both heteroatoms (O and N) are capable of forming a five-membered ring. The Se···O distances in **26**−**28** and Se···N distances in oxazoline-based selenides indicate that resonance plays a crucial role in Se···X (X = O, N) interamolecular interactions. Similarly the nature of S···O and S···N distances can be explained in **33** and **34**. However, there is no significant S···O interaction between sulfur and oxygen in **33** and **34**.

Conclusion

This study reveals that the selenides having basic amino groups with weak intramolecular Se···N interaction (**20**−**22**) are more active than the selenides having imino groups with strong Se···N interaction (**16**−**19**) against PN-mediated nitration reactions. The selenoxides of diorganoselenides, which show better protective action in the PN assay, can also be reduced back to selenides. Intramolecularly coordinated diaryl selenoxides (**47**, **51**, **52**, and **54**−**56**), aryl benzyl selenoxides (**44**−**46**, **48**−**50**, and **53**), and aryl alkyl selenoxide (**59**), lacking a β-hydrogen, do not undergo any selenoxide elimination reaction. The absence of selenoxide elimination reactions[28,35] may help in recycling the selenoxides back to selenides without loss of any activity. The present study will enhance our understanding of the mechanism and protective action of the model compounds and may ultimately yield insight that results in improved protective action of selenides against PN-mediated nitration reactions.

Experimental Section

General Procedures. All reactions were carried out under nitrogen or argon using standard vacuum-line techniques. Solvents were purified by standard procedures[36] and were freshly distilled prior to use. Melting points were recorded in capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained at 300 MHz in $CDCl_3$ on a Varian VXR 300S spectrometer. ^{1}H NMR chemical shifts are cited with respect to $SiMe_4$ as internal standard. IR spectra were recorded as KBr pellets on a Nicolet Impact 400 FTIR spectrometer. The ^{77}Se NMR spectra were obtained at 95.35 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 ppm. Elemental analyses were performed on a Carlo-Erba model 1106 elemental analyzer. Optical rotations were measured by a JASCO Model DIP 370 digital polarimeter.

***N,N*-Dimethylaminomethyl-2-(benzylseleno)naphthalene (21).** To a THF (35 mL) solution of 1-bromo-2-(*N,N*-dimethylaminomethyl)naphthalene (**36**)[37] (0.66 g, 2.5 mmol) was added dropwise *n*-BuLi (1.7 mL, 3 mmol, 1.6 M solution in pentane) at −78 °C. The solution was stirred for 20 min, and then Se powder (0.198 g, 2.5 mmol) was added in portions to the solution at 0 °C. After the disappearance of selenium powder, benzyl chloride (0.33 mL, 2.8 mmol) was added to the solution and the stirring was continued for 1 h at 0 °C and then at room temperature for 8 h. The solution was washed with a saturated NH_4Cl solution, and the

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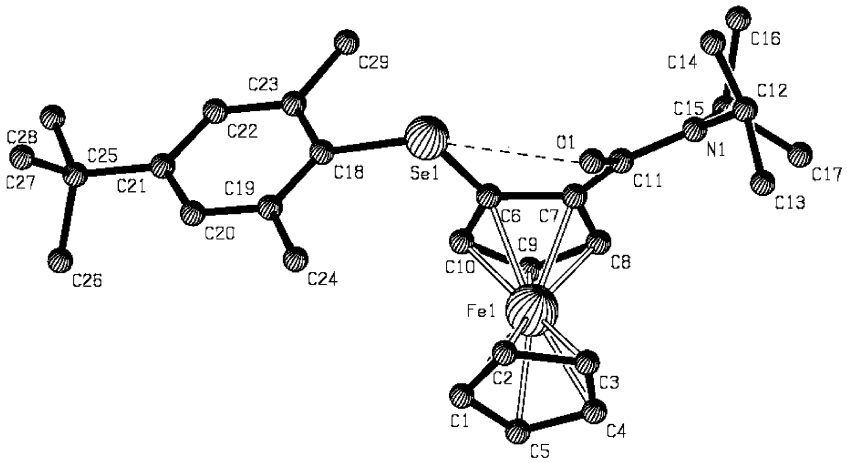

Figure 5. Molecular structure of **28**.

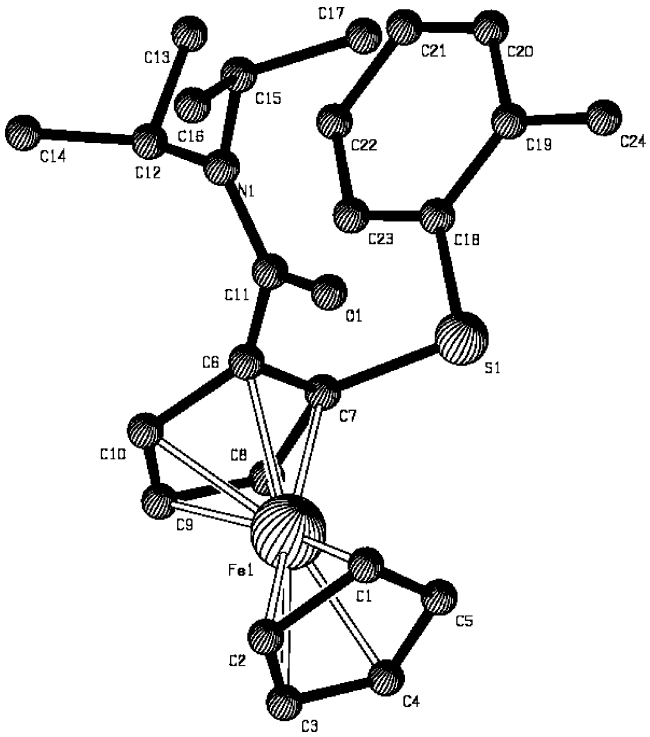

Figure 6. Molecular structure of **33**.

Table 4. Structural Comparison of Selenides 26−28 and Sulfides 33 and 34

compound	Se/S···O (Å)	Se/S···N (Å)	O−C(11) (Å)	N−C(11) (Å)
26	3.506(9)	4.074(1)	1.212(4)	1.363(4)S
27	3.760(2)	4.101(2)	1.220(6)	1.347(5)
28	2.950(2)	4.493(4)	1.216(3)	1.353(3)
33	3.703(3)	4.023(2)	1.222(4)	1.351(4)
34[a]	3.543(7)	4.254(7)	1.242(9)	1.246(9)

[a] Average distances from two asymmetric molecules.

product was extracted with ether and dried over Na_2SO_4. The solvent was removed in vacuo. To the residue was added hexane/CH_2Cl_2 (2:3), and it was kept for crystallization to obtain a white-colored compound. Yield: 0.75 g (84%); mp 186−189 °C; ^{1}H NMR ($CDCl_3$) δ 3.18 (s, 6H), 5.18 (s, 2H), 5.34 (s, 2H), 7.45−7.55 (m, 6H), 7.65−7.73 (m, 2H), 7.84−7.89 (m, 2H), 8.17−8.20(d, 1H); ^{13}C NMR ($CDCl_3$) δ 48.08, 48.86, 67.50, 69.00, 124.91, 126.62, 127.38, 127.44, 127.54, 128.30, 128.62, 128.86, 129.43, 130.25, 132.66, 133.28, 133.54, 133.75; ^{77}Se NMR ($CDCl_3$) δ 262. Anal. Calcd (%) for $C_{20}H_{21}NSe$ (354.2): C, 67.81; H, 5.97; N, 3.95. Found: C, 67.62; H, 5.76; N, 3.46. ES-MS: *m*/*z* 355.

***N*-Phenyl-2-(benzylseleno)benzamide (25).** To a stirred solution of benzanilide (2.0 g, 10.0 mmol) in dry THF (50 mL) was added *n*-BuLi (12.8 mL, 20 mmol, 1.6 M solution in hexane) at 0 °C under N_2. To the dark red-colored solution of the dilithiated product formed after 30 min, Se powder (0.8 g, 10 mmol) was added under a brisk flow of N_2 and the stirring was continued further for 30 min. Benzyl chloride (1.2 mL, 10 mmol) was added to the homogeneous dark orange solution via syringe. After 1 h, the reaction mixture was poured in water and extracted with $CHCl_3$. The organic layer was washed with 2 × 50 mL of water, dried over Na_2SO_4, and concentrated in vacuo to give a white-colored solid. Recrystallization from EtOH afforded a white crystalline powder. Yield: 2.04 g (56%); mp 192−194 °C; ^{1}H NMR ($CDCl_3$) δ 4.12 (s, 2H), 7.19−7.89 (many signals, 15H); ^{77}Se NMR ($CDCl_3$) δ 377. Anal. Calcd (%) for $C_{20}H_{17}NOSe$ (366.2): C, 65.59; H, 4.67; N, 3.82. Found: C, 65.38; H, 5.13; N, 4.64. All other data are in accordance with the literature values.[20a]

Preparation of (*R*,*S*)-1-[1-(*N*,*N*-Dimethylamino)ethyl]-2-(benzylseleno)ferrocene (22). In a two-necked 50 mL round-bottomed flask containing a magnetic stirring bar was placed (*RR*,*SS*)-bis-2-[1-(*N*,*N*-dimethylamino)ethyl]ferrocenyl diselenide[22] (0.1 g, 0.32 mmol) in THF (30 mL) under N_2. Lithium superhydride (0.7 mL, 0.7 mmol, 1 M solution in THF) was added to the flask at 0 °C, and the reaction mixture was stirred for an additional 30 min. Benzyl chloride (0.1 mL, 0.7 mmol) in 10 mL of THF was added to the resulting solution, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was treated with brine (100 mL) and then extracted with CH_2Cl_2 (3 × 25 mL). The extract was dried over Na_2SO_4 and evaporated to leave a red oil of **22**, which was purified by column chromatography on alumina with hexane/ethyl acetate (9:3) as an eluent to get pure **22** as an orange oil. Yield: 0.16 g, (57%); $[\alpha]^{25}$ 58.0 (*c* 0.034, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.32 (d, 3H), 2.18 (s, 6H), 3.63−3.78 (m, 1H) 4.14−4.49 (m, 3H), 4.17 (s, 5H), 4.42 (s, 2H), 6.84−7.29 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 15.73, 19.44, 23.85, 29.36, 31.86, 53.90, 60.56, 68.51, 69.72, 70.18, 70.52, 71.10, 85.38, 115.73, 119.88, 129.54, 156.83, 211.09; IR (neat) ν 2897, 2961, 2845, 1468, 1228, 1118, 82 cm^{-1}; ES-MS *m*/*z* 413.

Preparation of *N*,*N*-Diisopropylferrocenecarboxamide (39). A stirred solution of ferrocenecarboxylic acid (2.30 g, 10 mmol) in dry CH_2Cl_2 (15 mL) was treated dropwise with oxalyl chloride (1.74 mL, 20 mmol) under N_2 at room temperature, and the stirring was continued for 30 min at this temperature to obtain a red-colored solution. Excess oxalyl chloride was removed under vacuo, CH_2Cl_2 was added, and the solution was cooled to 0 °C. To this were added dry diisoprylamine (1.01 g, 10 mmol) in CH_2Cl_2 (25 mL) and triethylamine (1.4 mL, 10 mmol), and the stirring was continued overnight. The reaction mixture was treated with a saturated NH_4Cl solution and extracted with CH_2Cl_2 (2 × 50 mL).

The combined organic layer was washed with water (100 mL), dried over Na_2SO_4, filtered, and concentrated under vacuo to give a dark red-colored solid. Purification was accomplished by column chromatography using SiO_2 (60−120 mass) and petroleum ether (60−80 °C)/ethyl acetate (10:2). Crystallization from pentane and hexane (3:2) provided orange-colored needles. Yield: 2.89 g, (92%); mp 86−88 (lit. 88−89 °C);[21] ^{1}H NMR ($CDCl_3$) δ 1.23−1.48 (b, 12H), 3.18−3.69 (b, 2H), 4.27 (s, 5H) 4.26 (m, 2H), 4.56 (m, 2H); IR (KBr) ν 2980, 2945, 1628, 1580, 1462, 1373, 1206, 1034, 818 cm^{-1}. Anal. Calcd (%) for $C_{17}H_{23}FeNO$ (313.2): C, 65.21; H, 7.40; N, 4.47. Found: C, 64.97; H, 7.52; N, 4.51. All other data are consistent with the values reported in the literature.[21]

Synthesis of *N,N*-Diisopropyl-2-(phenylseleno)ferrocenecarboxamide (26). Under a N_2 atmosphere *n*-BuLi (2.07 mL, 3.7 mmol, 1.6 M solution in hexane) was added to a stirred solution of TMEDA (0.48 mL, 3.6 mmol) in dry Et_2O (10 mL) at −78 °C, and the stirring was continued further for 10 min. A solution of **39** (0.313 g, 1.0 mmol) in dry Et_2O (15 mL) was added dropwise via cannula, and the resulting red-colored reaction mixture was stirred for 45 min at −78 °C. Diphenyl diselenide (1.18 g, 3.8 mmol) in dry THF (8 mL) was added by syringe, and the stirring was continued for an additional 30 min at −78 °C and 1 h at room temperature. The reaction mixture was quenched with a saturated NH_4Cl solution and the organic layer separated. The aqueous layer was extracted with 2 × 50 mL of Et_2O, dried over Na_2SO_4, and concentrated in vacuo. Purification by column chromatography using petroleum ether (60−80 °C)/ethyl acetate (10:2) gave **26** as an orange solid. Recrystallization from pentane/hexane (3:1) provided orange crystals. Yield: 0.44 g (94%), mp 136−138 (lit. 133−135 °C);[21] ^{1}H NMR ($CDCl_3$) δ 0.52 (b, 3H), 1.03 (b, 3H), 1.32−163 (b, 6H); 3.22−3.42 (b, 1H); 3.62−3.85 (b, 1H); 4.29 (m, 1H); 4.38 (m, 1H); 4.41 (s, 5H); 4.51 (m, 1H); 7.12−7.24 (m, 3H); 7.32−7.40 (m, 2H); ^{77}Se NMR ($CDCl_3$) δ 294; IR (KBr) ν 2943, 2924, 1632, 1580, 1460, 1206, 818 cm^{-1}. Anal. Calcd (%) for $C_{23}H_{27}FeNOSe$ (468.2): C, 58.99; H, 5.81; N, 2.99. Found: C, 58.89; H, 5.68; N, 2.82. All other data are consistent with the values reported in the literature.[21]

Synthesis of *N,N*-Diisopropyl-2-{(2-methyphenyl)seleno}-ferrocenecarboxamide (27). A solution of **39** (0.626 g, 2.0 mmol) in Et_2O (10 mL) was added to a mixture of *n*-BuLi (3.7 mL, 6.7 mmol) and TMEDA (1.05 mL 6.6 mmol) in Et_2O (15 mL). Bis-(2-methylphenyl) diselenide[38] (2.26 g, 7 mmol) was added to the reaction mixture. Standard workup and purification by column chromatography using petroleum ether (60−80 °C) and ethyl acetate (18:2) afforded **27** as an orange solid. Recrystallization from CH_2Cl_2/hexane (1:5) provided orange crystals. Yield: 0.93 g (96%); mp 126−129 °C; ^{1}H NMR ($CDCl_3$) δ 0.47 (b, 3H), 0.97 (b, 3H), 1.25−1.47 (b, 6H); 2.36 (s, 3H), 3.25 (b, 1H); 3.70 (b, 1H); 4.32 (m, 1H); 4.39 (s, 5H); 4.47 (m, 2H); 6.93−7.00 (m, 2H); 7.06−7.26 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.19, 20.86, 21.67, 29.78, 45.88, 50.35, 68.32, 68.53, 71.53, 75.72, 92.40, 125.79, 126.36, 128.92, 129.81, 135.31, 137.05, 166.42; ^{77}Se NMR ($CDCl_3$) δ 242; IR (KBr) ν 2973, 2927, 1637, 1466, 1374, 1314, 1209, 828, 744 cm^{-1}. Anal. Calcd (%) for $C_{24}H_{29}FeNOSe$ (482.2): C, 59.78; H, 6.06; N, 2.90. Found: C, 59.16; H, 6.84; N, 2.65.

Preparation of *N,N*-Diisopropyl-2-{(2,5-dimethyl-4-*tert*-butylphenyl)seleno}ferrocenecaboxamide (28). To a stirred solution of the lithiated derivative **40** was added bis[2,5-dimethyl-4-*tert*-butylphenyl] diselenide[12c] (4.82 g, 10 mmol) in 8 mL of dry THF by syringe over 30 min at −78 °C. The reaction mixture was further stirred for 45 min at this temperature and 2 h at 60 °C. The reaction mixture on usual workup and purification by column chromatography using petroleum ether (60−80 °C) and ethyl acetate (19:2) afforded the desired compound as an orange solid. Recrystallization from toluene at −30 °C provided red pellets. Yield: 1.3 g (98%), mp 110−113 °C; ^{1}H NMR ($CDCl_3$) δ 0.73 (b, 3H), 1.10 (b, 3H), 1.26 (s, 9H), 1.35−1.57 (b, 6H), 2.52 (s, 6H), 3.28−3.62 (m, 1H), 3.42−3.68 (b, 1H), 4.09(t, 1H), 4.14 (m, 1H), 4.26 (m, 1H), 4.41 (s, 5H), 7.02 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 21.02, 24.98, 31.29, 34.315, 46.05, 50.24, 66.30, 66.87, 71.73, 73.05, 76.78, 77.10, 77.42, 80.56, 86.18, 124.74, 129.88, 142.29, 151.11, 167.66; ^{77}Se NMR ($CDCl_3$) δ 184; IR (KBr) ν 2973, 2927, 2868, 1643, 1446, 1321, 1032, 828, cm^{-1}. Anal. Calcd (%) for $C_{29}H_{39}FeNOSe$ (552.3): C, 63.07; H, 7.11; N, 2.53. Found: C, 62.83; H, 7.10; N, 2.45.

Preparation of *N,N*-Diisopropyl-2-(*tert*-butylseleno)ferrocenecaboxamide (29). To a stirred lithiated product **40** (prepared as described for compound **26**) was added bis(*tert*-butyl) diselenide (2.2 g, 8.0 mmol) via syringe over 15 min at −78 °C. The reaction mixture was further stirred for 45 min at this temperature and 2 h at room temperature. The reaction mixture was worked up and purified by chromatography using petroleum ether (60−80 °C) and ethyl acetate (30:2) to afford **29** as an orange colored-liquid. Yield: 0.78 g (87%); $[\alpha]^{25}$ −0.89 (*c* 0.025, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.08−1.68 (many signal, 12H), 2.68 (m, 9H), 4.20 (t, 1H), 4.30 (m, 1H), 4.32 (s, 5H), 4.40 (t, 1H); ^{13}C NMR ($CDCl_3$) δ 13.76, 21.09, 23.15, 24.19, 28.53, 29.21, 31.64, 32.73, 44.81, 46.03, 50.43, 67.39, 67.55, 71.37, 72.17, 71.38, 72.71, 73.03, 75.29, 76.88, 77.20, 77.52, 90.04, 167.31; ^{77}Se NMR ($CDCl_3$) δ 172; IR (neat) ν 3098, 2960, 2921, 1644, 1460, 1374, 1321, 1038, 821 cm^{-1}; ES-MS *m*/*z* 450.

Preparation of *N,N*-Diisoprpyl-2-(*n*-butylseleno)ferrocenecaboxamide (30). Compound **30** was synthesized in a similar manner as described for **26**, from **39** (0.876 g, 2.8 mmol) and di-(*n*-butyl) diselenide (2.75 g, 10 mmol). Purification by column chromatography using petroleum ether (60−80 °C) and ethyl acetate (32:2) afforded **30** as an orange-colored liquid. Yield: 0.1 g (86%); $[\alpha]^{25}$ 1.78 (*c* 0.025, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.80 (t, 3H), 1.08 (b, 6H), 1.21−1.82 (many signal, 10H), 3.22 (b, 1H), 3.82 (b, 1H), 2.71 (m, 2H), 4.20 (t, 1H), 4.26 (b, 1H), 4.32 (s, 5H), 4.37 (b, 1H); ^{13}C NMR ($CDCl_3$) δ 13.77, 21.09, 23.16, 28.53, 29.84, 32.73, 46.08, 50.43, 67.39, 67.53, 71.37, 73.05, 75.10, 76.92, 90.06, 167.3; ^{77}Se NMR ($CDCl_3$) δ 162; IR (neat) ν 3098, 2960, 2868, 1644, 1460, 1374, 1321, 834 cm^{-1}; ES-MS *m*/*z* 450.

Preparation of *N,N*-Diisopropyl-2-(methylseleno)ferrocenecaboxamide (31). Selenide **31** was synthesized by a similar method as that described for **30** except the addition of dimethyl diselenide in place of di(*n*-butyl) diselenide. The crude was purified by column chromatography using petroleum ether/Et_2O (20:5) to afford **31** as an orange-colored liquid. Yield: 0.8 g (87%); $[\alpha]^{25}$ −16.66 (*c* 0.025, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89−1.25 (b, 6H), 1.38−1.66 (b, 6H), 2.21 (s, 3H), 3.51 (b, 1H), 4.07 (b, 1H), 4.20 (t, 1H), 4.29 (b, 1H), 4.31 (b, 1H), 4.32 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 8.48, 21.10, 29.85, 46.11, 50.47, 67.48, 67.69, 71.29, 71.59, 89.18, 167.54; ^{77}Se NMR ($CDCl_3$) δ 85; IR (neat) ν 3105, 2973, 2920, 1644, 1460, 1374, 1321, 821 cm^{-1}; ES-MS *m*/*z* 406.

Synthesis of *N,N*-Diisopropyl-2-(phenylthio)ferrocenecarboxamide (32). Sulfide **32** was prepared by a similar method as that described for **26** except the addition of diphenyl disulfide. Standard workup and purification by column chromatography using petroleum ether (60−80 °C)/ethyl acetate afforded **32** as an orange solid. Recrystallization from pentane/hexane (3:1) provided orange crystals. Yield: 0.39 g (92%), mp 150−153 (154−155 °C);[21] ^{1}H NMR ($CDCl_3$) δ 0.44 (b, 3H), 0.97 (b, 3H), 1.37−1.59 (b, 6H); 3.25 (b, 1H); 3.66 (b, 1H); 4.31 (m, 1H); 4.38 (m, 2H); 4.40 (s, 5H); 4.53 (m, 2H); 7.03−7.13 (m, 3H); 7.14−7.26 (m, 2H); IR (KBr) ν 2941, 2919, 1637, 1586, 1465, 1216, 821 cm^{-1}. Anal. Calcd (%) for $C_{23}H_{27}FeNOS$ (421.4): C, 65.56; H, 6.46; N, 3.32; S, 7.61. Found: C, 65.37; H, 6.25; N, 3.54; S, 7.43. All other data are consistent with the values reported in the literature.[21]

Synthesis of *N,N*-Diisopropyl-2-[(2-methyl-phenyl)thio]ferrocenecarboxamide (33). Synthesis of **33** was achevied by a similar method as that reported for **27**, except the addition of bis(2-

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methylphenyl) disulfide[39] in place of bis(2-methylphenyl)diselenide. Standard workup and purification by column chromatography using petroleum ether (60−80 °C) and ethyl acetate (18:2) afforded **33** as an orange solid. Recrystallizion from CH_2Cl_2/hexane (1:5) provided orange crystals. Yield: 0.70 g (80%), mp 135−137 °C; ^{1}H NMR ($CDCl_3$) δ 0.44 (b, 3H), 0.97 (b, 3H), 1.26−1.59 (b, 6H); 2.37 (s, 3H), 3.25 (b, 1H); 3.70 (b, 1H); 4.33 (m, 1H); 4.43 (s, 5H); 4.52 (m, 2H); 6.94−6.95 (m, 2H); 7.05−7.26 (m, 2H); IR (KBr) ν 2978, 2934, 1643, 1469, 1371, 1308, 1204, 821, 743 cm^{-1}. Anal. Calcd (%) for $C_{24}H_{29}FeNOS$ (435.4): C, 66.20; H, 6.71; N, 3.21; S, 7.36. Found: C, 65.87; H, 6.51; N, 3.57; S, 7.31.

Preparation of *N,N*-Diisopropyl-2-[(2,5-dimethyl-4-*tert*-butylphenyl)thio]ferrocenecaboxamide (34). To a stirred solution of arenelithium (**39**) was added bis(2,5-dimethyl-4-*tert*-butylphenyl) disulfide[40] (3.86 g, 10 mmol in 8 mL of dry THF) via syringe over 30 min at −78 °C. The reaction mixture was worked up and purified by column chromatography using petroleum ether (60−80 °C) and ethyl acetate (19:2) to afford the desired compound as an orange solid. Recrystallization from toluene at −30 °C provided red pellets. Yield: 1.1 g (87%), mp 127−129 °C; ^{1}H NMR ($CDCl_3$) δ 0.0.68 (b, 3H), 1.05 (b, 3H), 1.25 (s, 9H), 1.28−1.58 (b, 6H), 2.45 (s, 6H), 3.21−3.53 (m, 1H), 3.33−3.57 (b, 1H), 4.01 (t, 1H), 4.16 (m, 1H), 4.21 (m, 1H), 4.30 (s, 5H), 7.02 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 20.90, 22.61, 29.72, 31.26, 34.31, 65.62, 66.05, 71.62, 71.75, 86.47, 87.73, 125.26, 126.80, 131.64, 141.83, 150.96, 166.9; IR (KBr) ν 2977, 2931, 2873, 1631, 1453, 1327, 1027, 825 cm^{-1}. Anal. Calcd (%) for $C_{29}H_{39}FeNOS$ (505.5): C, 68.90; H, 7.78; N, 2.77; S, 6.34. Found: C, 68.78; H, 7.23; N, 2.81; S, 6.27.

Peroxynitrite Synthesis. PN synthesis was carried out by modifying the method described by Beckman et al.[41] Sodium nitrite (0.6 M), hydrogen peroxide (0.6 M in 0.7 M HCl), and sodium hydroxide (1.2 M) were mixed by quenched flow reactor at 0 °C. Excess hydrogen peroxide was removed by MnO_2 treatment. The concentration of PN was conveniently assayed by diluting the PN stock solution to 100-fold in 1.2 M NaOH and measuring the absorbance at 302 nm (ϵ = 1670 M^{-1} cm^{-1}).

General Procedure for the Determination of IC_{50} Values of Diorganselenides in PN Assay. Experiments were conducted at room temperature in a 0.1 M sodium phosphate buffer (pH 7.4) containing Fe(III)/EDTA (0.5 mM) [Fe(III)/EDTA complex was prepared by mixing equimolar solutions of Fe(III) chloride and sodium EDTA], 4-HPA (1 mM), and various concentrations of the testing compounds in methanol. PN (50 μM) was added to the above reaction mixture under constant stirring. Samples were mixed for 30 min at room temperature. The pH was adjusted to 10−11 with 1 M NaOH before absorbance was measured at 430 nm. The yield of the nitrated product (4-hydroxy-3-nitrophenyl acetate) was calculated by using ϵ = 4400 M^{-1} cm^{-1}. Alternatively, as a control, the experiment was carried out without PN. The result of the reaction of 4-HPA with PN and methanol alone was set equal to 100%.

Preparation of Selenoxides and Sulfoxides 44−62. The selenoxides were prepared by the reaction of corresponding diorganoselenides/sulfides **16**−**34** with 1.5 equiv of H_2O_2 in methanol at 0 °C. The progress of the reaction was monitored by TLC. Once the reaction was complete, MnO_2 powder was added to the reaction mixture to remove the excess of H_2O_2, and the reaction mixture was further stirred for 1 h. Then MnO_2 powder was removed by filtration and the filtrate concentrated in vacuo to give the selenoxides/sulfoxides **44**−**62**, which were directly used for cyclic voltammetry, potentiometric titrations, and ES-MS study. Selenoxide **48** was also prepared by the reported method by using *m*-chloroperbenzoic acid oxidation of selenide **20**.[18] Selenoxide **48** obtained from this method and the above method (H_2O_2) exhibited the same cyclic voltammetry and potentiometric titration data.

Table 5. Crystal Data and Structure Refinement for 26−28

	26	**27**	**28**
empirical formula	$C_{23}H_{27}FeNOSe$	$C_{24}H_{29}FeNOSe$	$C_{29}H_{39}FeNOSe$
fw	468.27	482.29	552.42
cryst syst	triclinic	triclinic	triclinic
space group	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$
a (Å)	7.3377(7)	7.4339(7)	7.6049(6)
b (Å)	11.6860(13)	10.4051(12)	12.2227(10)
c (Å)	13.4351(16)	14.8043(16)	15.1327(12)
α (deg)	66.420(12)	83.841(13)	90.729(10)
β (deg)	85.705(13)	81.238(12)	93.247(10)
γ (deg)	86.313(12)	78.814(12)	104.268(10)
V (Å^3)	1052.1(2)	1106.7(2)	1360.53(19)
Z	2	2	2
D(calcd) (Mg/m^3)	1.478	1.447	1.348
abs coeff (mm^{-1})	2.459	2.340	1.912
no. of obsd reflns [$I > 2\sigma(I)$]	14 830	15 535	21 303
final $R(F)$ [$I > 2\sigma(I)$][a]	0.0343	0.0412	0.0339
$wR(F^2)$ [$I > 2\sigma(I)$]	0.0709	0.0876	0.0758
no. of data/restraints/params	3754/0/248	3923/0/258	5386/0/307
goodness fit on F^2	0.772	0.824	0.912

[a] Definitions: $R(F_0) = \sum||F_o| - |F_c||/\sum|F_o|$ and $wR(F_o^2) = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_c^2)^2]\}^{1/2}$.

Table 6. Crystal Data and Structure Refinement for 33 and 34

	33	**34**
empirical formula	$C_{24}H_{29}FeNOS$	$C_{29}H_{39}FeNOS$
fw	435.39	505.52
cryst syst	triclinic	triclinic
space group	$P\bar{1}$	$P\bar{1}$
a (Å)	7.4118(8)	8.0669(7)
b (Å)	10.4149(12)	17.1460(18)
c (Å)	14.6040(17)	19.849(2)
α (deg)	83.639(14)	91.560(13)
β (deg)	81.266(13)	90.312(12)
γ (deg)	79.564(12)	98.494(12)
V (Å^3)	1091.8(2)	2714.2(5)
Z	2	4
D(calcd) (Mg/m^3)	1.324	1.237
abs coeff (mm^{-1})	0.800	0.653
no. of obsd reflns [$I > 2\sigma(I)$]	16 825	17 041
final $R(F)$ [$I > 2\sigma(I)$][a]	0.0509	0.0577
$wR(F^2)$ [$I > 2\sigma(I)$]	0.1327	0.1095
no. of data/restraints/params	4298/0/258	6512/0/613
goodness fit on F^2	1.052	0.752

[a] Definitions: $R(F_o) = \sum||F_o| - |F_c||/\sum|F_o|$ and $wR(F_o^2) = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_c^2)^2]\}^{1/2}$.

Cyclic Voltammetry Study. Cyclic voltammetry was performed with a CH-1660 scanning potentiostat/galvanostat interfaced to a base PC using the EG&G model 270 software package. Cyclic voltammograms of selenoxides/sulfoxides **13** and **44**−**62** were obtained by using a glassy carbon working electrode, a platinum coil counter electrode, and a calomel reference electrode in 100 mM sodium acetate buffer (pH = 5.5) under argon. The scan rate was 100 mV/s, and full IR compensation was employed in all measurements. The fresh solutions of in situ-generated selenoxides/sulfoxides **13** and **44**−**62** in CH_3OH were used for cyclic voltammetry experiments. A blank experiment was carried out in sodium acetate aqueous buffer under argon (for more information, see Supporting Information, pp 19−29, Figures S13−S23).

Potentiometric Titrations. The cell reduction potentials (E) for the selenoxides were obtained by potentiometric titration with the reducing agent sodium dithionite as a titrant. Measurements were performed in a two-electrode-cell system with a single compartment containing a platinum spiral wire indicator electrode and an aqueous

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saturated calomel electrode (SCE) connected to a millivoltmeter. Measurements were carried out at 25 °C under argon in 100 mM sodium acetate, pH 5.5, with an initial concentration of selenoxide of 1 mM. Sodium dithionite (10 mM) was titrated into the cell with a 30 min equilibration period between each additional aliquot. All reported potentials were corrected to potentials versus the normal hydrogen electrode (NHE). A stock solution of selenoxides **13** and **44**−**62** was prepared in methanol. In the case of selenoxides and sulfoxides **44**−**47** and **60**−**62** no significant changes in voltage were observed.

ES-MS Experiment. ES-MS were performed at room temperature on a Q-Tof micro (YA-105) mass spectrometer. Mass spectra were obtained with a Platform II single quadrupole mass spectrometer (Micromass, Altrincham, UK) using a CH_3OH mobile phase. A solution of PhSH (11 mg, 0.10 mmol) in CH_3OH/H_2O (1:1) was added to a solution of ebselen oxide (**13**) (29 mg, 0.10 mmol) in MeOH. The resulting solution was stirred for 15 min, and then ES-MS of the reaction mixture was obtained. Similarly ES-MS of the reaction of PhSH and selenoxide **48** was obtained (for more information, see Supporting Information, pp 68−80, Figures S59−S69).

X-ray Structure Determination of Compounds 26−**28 and 33**−**34.** The diffraction measurements were performed on a STOE (Darmstadt, Germany) IPDS imaging plate single-crystal diffractometer at room temperature with graphite-monochromated Mo Kα radiation ($\lambda = 0.7107$ Å). The structures were determined by routine heavy-atom using SHELXS-90[42] and Fourier methods and refined by full-matrix least squares with the non-hydrogen atoms anisotropic and hydrogen with fixed isotropic thermal parameters of 0.07 Å^2 by means of the SHELEXL-97 program.[43] Hydrogens were partially located from difference electron-density maps, and the rest were fixed at predetermined positions. Scattering factors were from common sources. Some details of the structural refinement are given in Tables 5 and 6.

Acknowledgment. We heartily thank Dr. G. Mugesh (Indian Institute of Science, Bangalore, India) for helpful discussions. This work was supported by the Department of Science and Technology, New Delhi (DST grant to H.B.S.), and Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi (Senior Research Fellowship to S.K.).

Supporting Information Available: The synthesis of **41** and **43** and details of the PN assay (for compounds **2**, **14**, **20**−**22**, **24**, and **26**−**31**), ^{77}Se NMR (for compounds **21**, **22**, and **25**−**31**), ES-MS spectra (for compounds **13**, **29**−**31**, **48**, **54**, **65**, **67**−**69**, and **72**), colored molecular structures of **26**−**28**, **33**, and **34**, and CIF data for **26**−**28**, **33**, and **34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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