JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Nitro-, Azo-, and Amino Derivatives of Ebselen: Synthesis, Structure, and Cytoprotective Effects

Vijay P. Singh,*[,†] Jia-fei Poon,[†] Jiajie Yan,[†] Xi Lu,[‡] Marjam Karlsson Ott,[‡] Ray J. Butcher,[§] Paul J. Gates,[∥] and Lars Engman*[,†]

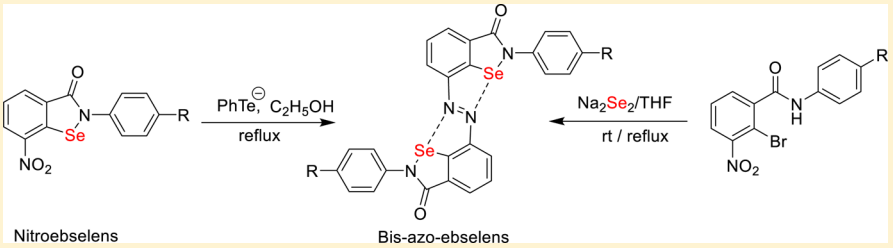
[†]Department of Chemistry−BMC, Uppsala University, Box 576, SE-751 23 Uppsala, Sweden

[‡]Division of Applied Materials Science, Department of Engineering Sciences, Uppsala University, Uppsala, Sweden

[§]Department of Chemistry, Howard University, Washington, D.C. 20059, United States

[∥]University of Bristol, School of Chemistry, Bristol, BS8 1TS, United Kingdom

Supporting Information

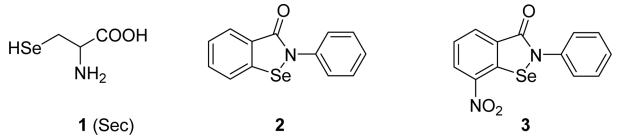


ABSTRACT: Novel azo-bis-ebselen compounds **7** were prepared by reduction of 7-nitro-2-aryl-1,2-benzisoselenazol-3(2*H*)-ones **3** and **6** with sodium benzenetellurolate, $NaTeC_6H_5$, and by reaction of 2-bromo-3-nitrobenzamides with Na_2Se_2. The X-ray structure of **7b** showed that the molecule, due to strong intramolecular secondary Se···N interactions, is completely planar. Azo-compounds **7** upon further reaction with $NaTeC_6H_5$ were reductively cleaved to provide 2 equiv of the corresponding aromatic amine. The weak Se−N bond was not stable enough to survive the reaction conditions, and diselenides **8** were isolated after workup. Whereas azo-bis-ebselens **7** were poor mimics of the glutathione peroxidase (GPx)-enzymes, nitroebselens **3**, **6**, and **11b** and diselenides **8** were 3−6-fold more active than ebselen. Based on ^{77}Se NMR spectroscopy, a catalytic cycle for diselenide **8b**, involving aminoebselen **14**, was proposed. As assessed by chemiluminescence measurements, the good GPx-mimics could reduce production of reactive oxygen species (ROS) in stimulated human mononuclear cells more efficiently than Trolox. No toxic effects of the compounds were seen in MC3T3-cells at 25 μM.

INTRODUCTION

Selenocysteine (Sec, **1**) is recognized as the 21st proteinogenic amino acid. It is an essential part of the active site of selenoproteins in humans and animals. To date, 23 selenoprotein families have been annotated.[1] The human selenoproteome consists of 25 selenoproteins.[2] Whereas some of these have a known structure and function (GPx, iodothyronine deiodinases, thioredoxin reductases),[3,4] others are as yet insufficiently characterized. The substitution of selenium for sulfur in cysteine (Cys) has many implications.[5] At physiological pH, the more acidic selenol (pK_a = 5.43)[6] is essentially deprotonated. Sec is therefore a more reactive nucleophile than Cys. Also, redox-cycling (oxidation/reduction) occurs more readily in Sec than in Cys. In addition to catalase, the GPx-enzymes are the most important hydroperoxide-decomposing enzymes in humans.[7] Gluthathione (GSH) is used as the stoichiometric reducing agent (eq 1). In the proposed catalytic cycle for the action of the GPx-enzymes selenium is present in the form of selenol, selenenic acid, and selenosulfide.[8]

$$\mathrm{HOOH/ROOH + 2GSH \xrightarrow{GPx} H_2O/ROH + GSSG + H_2O} \quad (1)$$



1 (Sec) **2** **3**

The reports on the existence of GPx triggered a search for small-molecule compounds that could mimic the action of the large enzyme. The benzisoselenazolone ebselen (**2**) was the first compound of this kind.[9] Since the mid-1980s, the suitability of ebselen as a pharmaceutical agent has been extensively probed. Due to its GPx-activity, it has been found to reduce oxidative stress.[10] It is/has been used in clinical trials for the prevention or treatment of cardiovascular diseases, arthritis, stroke, and cancer.[11] Considered as a safe drug-like compound with a history of use in human clinical trials, ebselen has also been included in the National Institutes of Health Clinical

Received: October 5, 2016
Published: December 2, 2016

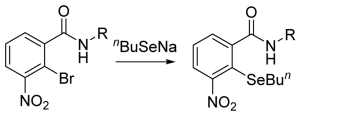
DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313−321

Collection.[12] Ebselen has been subjected to numerous structural modifications in order to improve its GPx-activity.[13] An early study by Parnham and co-workers showed that the nitro-derivative **3** of ebselen was 9-fold better than the parent as a catalyst for the glutathione-induced reduction of *t*-BuOOH.[14] However, this finding has not been much explored and the reason for the rate enhancement has been attributed to both electronic[15] and steric[16] effects.

We thought it would be interesting to prepare benzisoselenazolones carrying a variety of *N*-substituents (nitro-, azo-, and amino groups) in position 7. In the following we describe their synthesis and structure as well as their GPx-like activities and cytoprotective effects.

RESULTS AND DISCUSSION

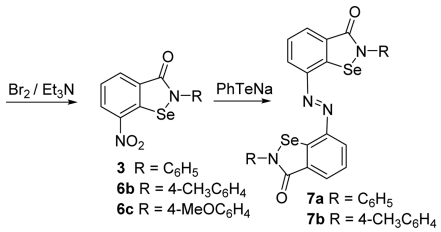
Synthesis. We envisaged obtaining azo- and amino derivatives of ebselen by reduction of the corresponding nitro compounds. Nitro-substituted ebselens were prepared using a slightly modified[17] version of the procedure developed by Christians and co-workers.[18] The required benzamides **4a–c** (eq 2) for this reaction were obtained in high yields by addition



4a R = C_6H_5
4b R = 4-$CH_3C_6H_4$
4c R = 4-$MeOC_6H_4$

5a R = C_6H_5
5b R = 4-$CH_3C_6H_4$
5c R = 4-$MeOC_6H_4$

(2)

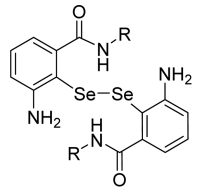


3 R = C_6H_5
6b R = 4-$CH_3C_6H_4$
6c R = 4-$MeOC_6H_4$

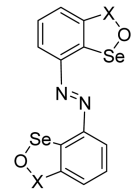
7a R = C_6H_5
7b R = 4-$CH_3C_6H_4$

of aniline, *para*-toluidine, and *para*-anisidine, respectively, to 2-bromo-3-nitrobensoyl chloride. Their conversion to benzisoselenazolones **3**/**6b–c** involved aromatic nucleophilic substitution with ^{n}BuSeNa, generated *in situ* from Bu_2Se_2 and $NaBH_4$ in ethanol, followed by bromine-induced cyclization. Reduction of the nitro group in the presence of the weak Se–N bond turned out to be difficult. Sodium benzenetellurolate, NaTePh,[19] generated in ethanol by sodium borohydride reduction of diphenyl ditelluride (Ph_2Te_2), was found to cleanly reduce compound **3** to azo-bis-ebselen derivative **7a** in 51% yield. However, in the case of **6b**, the corresponding azo-derivative **7b** was isolated as the minor product (9%) along with diselenide **8b** (16%). Obviously, in **8b**, the nitro group has been reduced all the way to an amine and the Se–N bond cleaved reductively. Oxidation of the resulting selenol then provided the diselenide product. Reduction of azo-compound **7b** with NaTePh produced diselenide **8b** as the only product (87%).

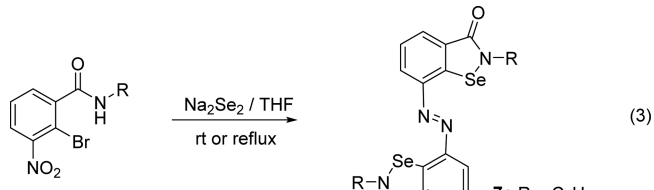
An alternative method for the preparation of azo-derivatives of ebselen was also tried. This is based on the finding that 2-bromo-3-nitrobenzylic alcohols, when reacted with disodium diselenide (Na_2Se_2), produced azo-derivatives **9** of 2-oxaselenaindane.[20] Reaction of *in situ* prepared Na_2Se_2 with compounds **4a–d** at ambient temperature produced the corresponding azo-bis-ebselens **7a** (29%), **7b** (39%), **7c** (21% when heated at reflux), and **7d** (68%) (eq 3). Due to poor



8b R = 4-$CH_3C_6H_4$
8d R = 1-naphthyl



9 X = CH_2 or $CHCH_3$

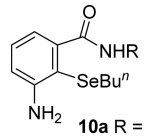


(3)

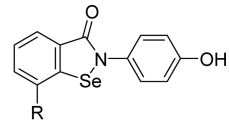
4a R = C_6H_5
4b R = 4-$CH_3C_6H_4$
4c R = 4-$MeOC_6H_4$
4d R = 1-naphthyl

7a R = C_6H_5
7b R = 4-$CH_3C_6H_4$
7c R = 4-$MeOC_6H_4$
7d R = 1-naphthyl

solubility, we were unable to characterize the methoxy derivative **7c**. Further reaction of compound **7d** with NaTePh afforded diselenide **8d** in 52% yield. Under similar reaction conditions, benzamides **5a–c**, carrying a butylseleno group in position 2, were cleanly reduced to the corresponding amines **10a–c** in high yields. We recently reported on the cytoprotective effects of the radical-trapping and hydroperoxide-decomposing ebselenol **11a**.[21] Curious about the effects of an ortho-coordinating nitro group, compound **6c** was *O*-demethylated with 3 equiv of BBr_3 in CH_2Cl_2 to afford the nitroebselenol **11b** in 88% yield.



10a R = C_6H_5
10b R = 4-Me-C_6H_5
10c R = 4-MeO-C_6H_4



11a R = H
11b R = NO_2

Structure. The structures of azo-bis-ebselen derivative **7b** and diselenide **8b** were determined by X-ray crystallography. Dark black crystals of **7b** suitable for X-ray crystallographic analysis were obtained by slow evaporation of a $CHCl_3$ solution at −20 °C. The structure (Figure 1) shows strong intramolecular secondary Se···N interactions [Se1···N1 2.442(5) Å] notably shorter than the sum of the van der Waals radii of Se and N (3.45 Å).[22] Normally, the bond length of a Se–N covalent bond is 1.87 Å. The strong secondary Se···N interactions cause a slight elongation of this bond (Se1–N2 = 1.940(3) Å). The coordination geometry is strongly distorted from a linear arrangement with the bond angle N2–Se1–N1 = 156.56(13)°, thus, indicating a T-shaped geometry around the Se atom. Because of these strong interactions, compound **7b** adopts a completely planar conformation. The crystal structure of **7b** showed π–π stacking interactions between the benzene ring with a centroid–centroid distance of 3.711 Å (Figure S3 in the Supporting Information).

Suitable orange crystals of diselenide **8b**, suitable for X-ray crystallographic analysis, were obtained by slow evaporation of an ethyl acetate/pentane solution at room temperature. The structure (Figure 2) indicates a V-shaped geometry around the selenium atom with bond angles C1a–Se1–Se2 and C1b–Se2–Se1 of 104.26(5)° and 103.65(5)°, respectively. The

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313–321

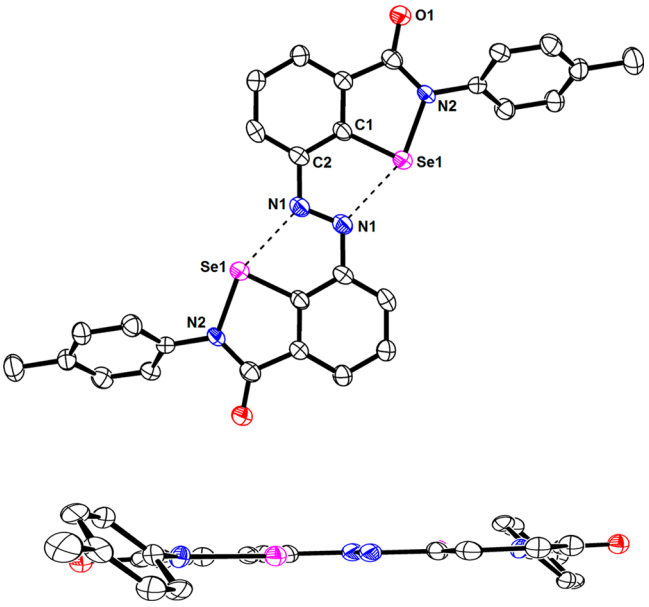


Figure 1. ORTEP diagram (up) top view and (down) side view of **7b**. Hydrogen atoms and solvated-$CHCl_3$ molecules are omitted for clarity. Thermal ellipsoids are set at 50% probability. Significant bond lengths [Å] Se1···N1 2.442(5); Se1−C1 1.851(4); Se1−N2 1.940(3), and angles [deg] N2−Se1−C1 84.04(16); N2−Se1···N1 156.56(13); C1−Se1−N1 72.55(15).

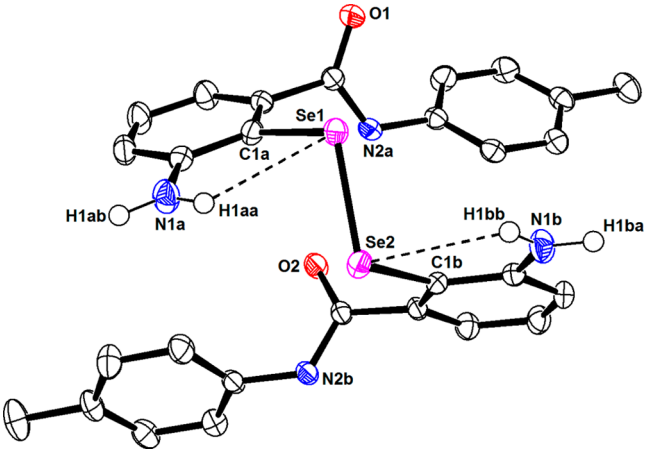


Figure 2. ORTEP diagram of **8b**. Hydrogen atoms are omitted for clarity. Thermal ellipsoids are set at 50% probability. Significant bond lengths [Å] Se1−C1a 1.9171(15); Se1−Se2 2.3602(2); Se2−C1b 1.9156(15), and angles [deg] C1a−Se1−Se2 104.26(5); C1b−Se2−Se1 103.65(5); C1a−Se1−Se2−C1b 98.47(7).

C1a−Se1−Se2−C1b dihedral angle of 98.47(7)° indicates a "transoid" conformation. As indicated in Figure 2, selenium is hydrogen bonded to the $-NH_2$ group in the ortho-position. The Se···H distance of 2.734 Å is significantly shorter than the sum of the van der Waals radii of selenium and hydrogen (3.10 Å).[22]

Bonding. To find out more about the effect of intramolecular secondary Se···N/O interactions on bonding and ^{77}Se NMR chemical shifts, density functional theory (DFT) calculations were carried out. The geometries of **6b**, **6c**, **7a**, **7b**, **7d**, and **11b** were fully optimized in the gas phase at the B3LYP/6-311+G(d) level of theory (for optimized geometries and coordinates, see the Supporting Information). The optimized geometry of **7b** showed good agreement with the X-ray crystal structure. The structures of **7a** and **7d** were also found to be completely planar with strong Se···N interactions. The second perturbation energies $E_{Se\cdots N}$ and $E_{Se\cdots O}$ shown in Table 1 were obtained by NBO analysis. The values reflect the effectiveness of orbital interactions between the low-laying σ^*_{Se-N} and n_N/n_O. The magnitude of the $n_N \rightarrow \sigma^*_{Se-N}$ and $n_O \rightarrow \sigma^*_{Se-N}$ orbital interactions suggests a significant covalent interaction between the nitrogen lone pair and the low-lying antibonding orbital of the Se atom. The NBO results indicate that the Se···N interactions were much stronger than the Se···O interactions. The calculated ^{77}Se NMR chemical shifts were in good agreement with the experimental values.

The topology of the electron density at the Se···N/O bond critical point (BCP) was evaluated according to Bader's theory of atoms in molecules (AIM) using the AIM2000 software package (for molecular graphs, see Figure S6 in the Supporting Information). Parameters were calculated in order to support the NBO calculations in the form of electron density ($\rho_{Se\cdots N/O}$), Laplacian ($\nabla^2\rho_{Se\cdots N/O}$), and the total electron energy density ($H_{Se\cdots N/O}$) for the Se···N/O interactions (Tables S19 and S20 in the Supporting Information). Whereas compounds **7a−b** and **7d** showed negative values for $H_{Se\cdots N}$, $H_{Se\cdots O}$ for compounds **6b−c** and **11b** were positive, suggesting weaker electrostatic interactions between Se and O atoms.

Glutathione Peroxidase-like Activity. The GPx-like activity of antioxidants **2−3**, **6b−c**, **7a−b**, **7d**, **8b**, **8d**, and **11b** was assessed by the coupled reductase assay, using H_2O_2 as a substrate and GSH as a thiol cofactor in the presence of glutathione reductase (GR). GR serves to reduce oxidized glutathione (GSSG) formed by the action of the GPx-catalyst on H_2O_2/GSH, using β-nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. GPx-activities were assessed as the initial rates (v_0) for the consumption of NADPH by UV-spectroscopy at 340 nm during the initial 10 s of reaction. The values shown in Table 2 were corrected for the spontaneous oxidation of GSH by H_2O_2 (24.3 ± 1.2 μM·min^{-1}). Ebselen (46.5 ± 0.8 μM·min^{-1}) and **3** (111.4 ± 4.6 μM·min^{-1}) were included as benchmarks and references in the study. Nitroebselens **6b**, **6c**, and **11b** were found to be nearly three times more active than ebselen (**2**) and slightly better than **3**. This enhanced activity of the nitro compounds can probably be ascribed to intramolecular secondary Se···O interactions. Such or similar effects are well described in the literature.[14,17,20b,24] Azoebselens **7a**, **7b**, and **7d** turned out to be considerably poorer GPx-mimics than **2** and **3**. Interestingly, diselenide **8b** showed an activity (271.6 ± 5.7 μM·min^{-1}) almost 6-fold larger than recorded for ebselen. The other diselenide **8d**, carrying a bulky naphthyl group, also showed good GPx-activity (133.8 ± 2.7 μM·min^{-1}).

Consumption of Hydrogen Peroxide. The performance of some of the best GPx-mimics **8b**, **8d**, and **11b** (10 mol %) together with GSH, NADPH, GR, and H_2O_2 in phosphate buffer was also followed for a much longer time (250 min). As shown in Figure 3, a significant decrease in the absorbance of NADPH was seen during ca. 80 min. Thereafter, the consumption of NADPH corresponds more or less to the background reaction. Ebselen (**2**), included as a reference in Figure 3, could not match the activity of **8b**, **8d**, and **11b**.

Mechanistic Studies. In order to obtain some mechanistic insight, ^{77}Se NMR experiments in DMSO-d_6 were carried out with diselenide **8b** (δ = 369 ppm). Addition of 1 equiv of H_2O_2 (Scheme 1) did not cause much of a change in the spectrum

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313−321

Table 1. Data for 3, 6b–c, 7a–b, 7d, and 11b Obtained by DFT Calculations at the B3LYP/6-311+G(d,p) Level in the Gas Phase[a]

	second-order perturbation energy				
compds	$r_{Se\cdots O/N}$ [Å]	$E_{Se\cdots O/N}$ (kcal/mol)	r_{Se-N} [Å]	NPA charges q_{Se}	^{77}Se NMR (in ppm)[b]
3[17,23]	2.593 (2.573)	12.63	1.924 (1.896)	+0.753	910 (924)
6b	2.598	12.35	1.922	+0.753	905 (929)
6c	2.604	12.00	1.921	+0.750	910 (932)
7a	2.532	17.32	1.946	+0.734	904 (931)
7b	2.537 (2.442)	16.89	1.944 (1.941)	+0.733	903 (927)
7d	2.539	16.57	1.943	+0.732	925 (946)
11b	2.601	12.22	1.922	+0.751	912 (923)

[a]The NBO analysis was calculated at the B3LYP/6-311+G(d,p) level using the B3LYP/6-311+G(d)-level-optimized geometries. [b]The ^{77}Se NMR values are referenced to Me_2Se (δ = 0). The experimental values are given in parentheses.

Table 2. GPx-like Activities of Ebselen, 3, 6b–c, 7a–b, 7d, 8b, 8d, and 11b As Determined by the Initial Rate of NADPH Consumption (ν_0) in the Presence of H_2O_2, GSH, and Glutathione Reductase

antioxidants	GPx-like activity, ν_0 (μM·min^{-1})	activity relative to ebselen
2, ebselen	46.5 ± 0.8	1
3	111.4 ± 4.6	2.4
6b	135.2 ± 4.1	2.9
6c	137.4 ± 2.3	3.0
7a	29.5 ± 0.6	0.6
7b	2.3 ± 0.1	0.05
7d	9.7 ± 0.2	0.2
8b	271.6 ± 5.7	5.8
8d	133.8 ± 2.7	2.9
11b	123.9 ± 0.7	2.7

[a]Assay conditions: Phosphate buffer (100 mM), pH 7.5 with ethylenediaminetetraacetic acid (EDTA; 1 mM), GSH (1 mM), NADPH (0.2 mM), GR (1.3 unit·mL^{-1}), H_2O_2 (0.80 mM), and catalysts (20 μM). Stock solutions (2 mM) of catalysts **2**, **3**, and **6a–b** were prepared in MeOH and stock solutions of catalysts **7**, **8**, and **11b** were prepared in DMSO. Initial rates (ν_o) were corrected for the spontaneous oxidation of GSH (24.3 ± 1.2 μM·min^{-1}). Errors correspond to ± SD for triplicates.

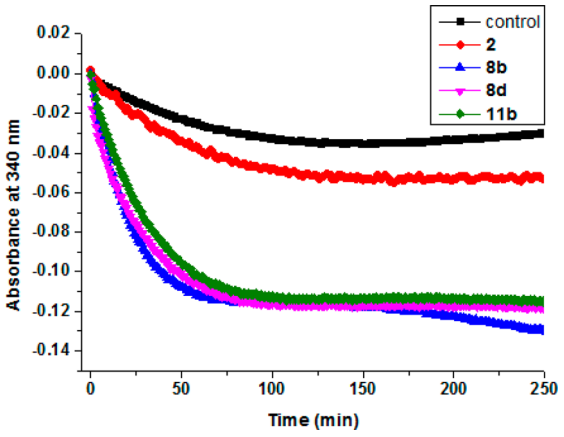
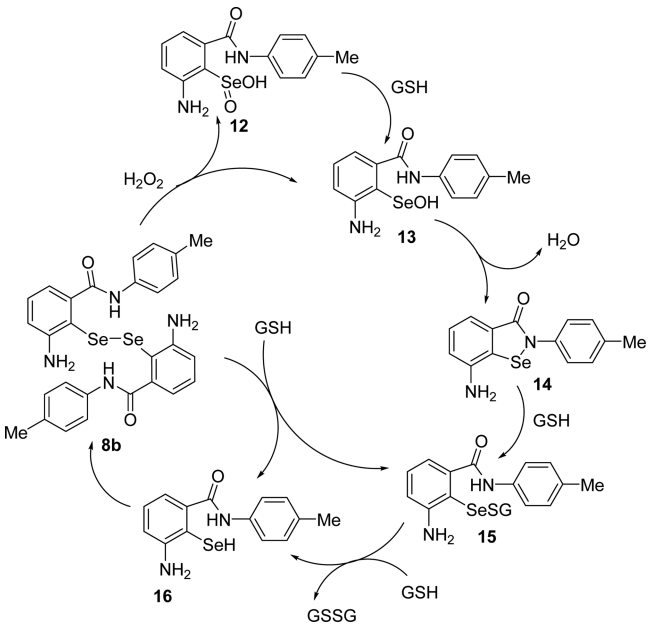


Figure 3. NADPH-consumption with time in the absence (control) and presence of catalysts **2** (ebselen), **8b**, **8d**, and **11b**. A stock solution of catalyst **2** was prepared in MeOH, and stock solutions of catalysts **8b**, **8d**, and **11b** were prepared in DMSO. Assay conditions: reactions were carried out with phosphate buffer (100 mM), pH 7.5, with EDTA (1 mM), GSH (0.10 mM), NADPH (0.20 mM), GR (1.3 unit·mL^{-1}), H_2O_2 (20 μM), and catalyst (2 μM).

Scheme 1. Proposed Mechanism for the Reduction of H_2O_2 in the Presence of GSH and Diselenide 8b



(see the ^{77}Se NMR spectrum in the Supporting Information). Upon addition of another equivalent of H_2O_2 to the above mixture, one new signal appeared at δ = 1156 ppm, corresponding to seleninic acid **12** (or a dehydrated product thereof, a *Se*-oxide of aminoebselen). Now, 1 equiv of GSH was added to the mixture produced containing unreacted **8b** and **12**. This caused the disappearance of the peak at δ = 1156 and the appearance of two new peaks at δ = 935 ppm and δ = 344 ppm, corresponding to amino derivative **14** of ebselen and selenol **16**, respectively. Presumably, compound **14** is formed by cyclization of an intermediate selenenic acid **13**.[17,24,25] Further addition of 2 equiv of GSH to the mixture caused the disappearance of the peaks corresponding to **12**, **14**, and **16**, and only the peak at 369 ppm, corresponding to diselenide **8b**, remained in the ^{77}Se NMR spectrum. When GSH (1 equiv) was added to a solution of **8b** in DMSO-d_6, the peak for **16** at δ = 346 ppm appeared.

It is not surprising that the peak from selenol **16** is shifted only 25 ppm upfield from that of diselenide **8b**. This is due to strong intramolecular secondary Se···O interactions with the carbonyl group and additional substituent effects from the aromatic rings. A similar downfield chemical shift of 232 ppm for the corresponding selenol derived from ebselen was also

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313–321

accounted for in terms of strong Se···O interactions.[24b] In none of the above experiments did we see a peak corresponding to selenosulfide **15**. It is probably a highly reactive species. We speculate that hydrogen bonding of Se to the ortho-amino group facilitates attack by thiol.

Cytoprotective Effects. An overproduction of ROS/reactive nitrogen species (RNS) in biological systems has many deleterious effects. For example, oxidative damage of DNA is directly linked to cardiovascular and neurodegenerative diseases.[26,27] Since many of the novel antioxidants showed excellent hydroperoxide-decomposing activities, we decided to test their protective effects in cellular systems. Freshly isolated human mononuclear cells (MNC) were stimulated with phorbol myristate acetate (PMA) to produce ROS/RNS in the presence of antioxidants **3**, **6b–c**, **7a–b**, **7d**, **8b**, **8d**, **11b**, ebselen (**2**), and Trolox at 25 μM. The total chemiluminescence (CL; extra- and intracellular) was then recorded in a luminol amplified assay. As shown in Figure 4, CL was

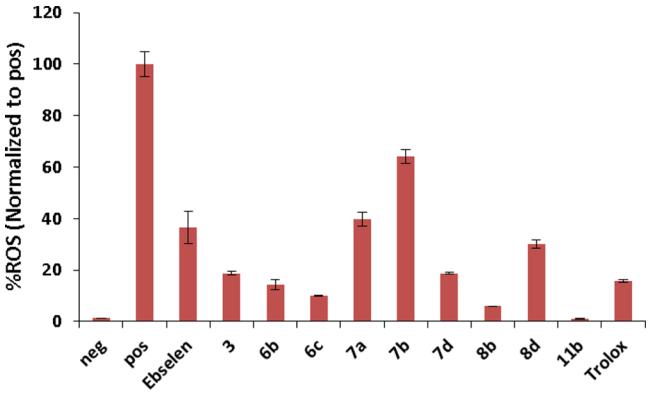


Figure 4. Chemiluminescence (normalized to positive control) monitored at λ = 425 nm in PMA-stimulated MNC-cells exposed to 25 μM of antioxidants Ebselen (**2**), **3**, **6b–c**, **7a–b**, **7d**, **8b**, **8d**, **11b**, and Trolox. N = 3 to 6 for each group from four independent experiments.

significantly reduced for all compounds tested. Good GPx-mimics such as nitro compounds **6b**, **6c**, and **11b** and diselenide **8b** all afforded better cytoprotection than Trolox. Nitroebselenol **11b** was by far the most potent protective agent.

Cell Viability. In order to reveal any toxicity of antioxidants **3**, **6b–c**, **7a–b**, **7d**, **8b**, **8d**, and **11b**, MC3T3-cells (a preosteoblast cell line) were exposed to 25 μM of compound and the cell viability was checked after 1 and 3 days by using the Alamar Blue assay (Figure 5). No toxic effects of the compounds were seen after 3 days.

CONCLUSION

In the search for novel ebselen derivatives with improved antioxidative properties we have described straightforward syntheses of azo-bis-ebselens from nitroebselens and 2-bromo-3-nitrobenzamides. Reduction of the azo-group to the corresponding amine was effected by NaTePh, but this transformation was accompanied by cleavage of the Se–N bond, resulting in formation of diaryl diselenides carrying both amino- and benzamide groups in the ortho positions. As revealed by X-ray crystallography and DFT-calculations, the intramolecular Se···N-coordination in azo-bis-ebselens was much stronger than the corresponding Se···O interaction in nitroebselens. This may be the reason for the poor GPx-activity

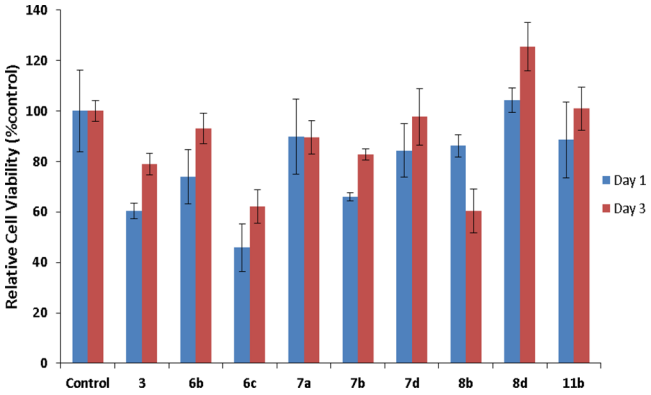


Figure 5. Relative cell viability of MC3T3 cells in the presence of 25 μM of antioxidants **3**, **6b–c**, **7a–b**, **7d**, **8b**, **8d**, and **11b** as determined by Alamar Blue measurements after 1 and 3 days at λ_{em} = 590 nm.

of the azo-bis-ebselens. Both nitroebselens **6** and diselenides **8** outperformed ebselen when it comes to catalysis of GSH-induced reduction of hydrogen peroxide. Considering their low toxicity and ability to inhibit ROS/RNS in stimulated human MNC-cells, we feel that compounds of this sort would be useful as models and tools in the development of novel treatments for diseases with a component of oxidative stress (cardiovascular diseases, stroke, Alzheimer's and Parkinson's diseases).

EXPERIMENTAL SECTION

2-Bromo-3-nitrobenzoic acid (98% purity) was purchased and used as such. ^{1}H and ^{13}C NMR spectra for all compounds prepared were recorded on 300 MHz (^{1}H: 300 MHz; ^{13}C: 75 MHz) and 400 MHz (^{1}H: 399.97 MHz; ^{13}C: 100 MHz) spectrometers, using the residual solvent peaks of $CDCl_3$ (^{1}H: δ 7.26; ^{13}C: δ 77.2), CD_3OD (^{1}H: δ 3.31; ^{13}C: δ 49.0), and DMSO-d_6 (^{1}H: δ 2.50; ^{13}C: δ 39.5), as indirect references to TMS (δ = 0 ppm). ^{77}Se NMR spectra were recorded on 300 MHz (^{77}Se: 57 MHz) and 400 MHz (^{77}Se: 76 MHz) spectrometers with Ph_2Se_2 (δ = 460 ppm) as an indirect reference to Me_2Se (δ = 0 ppm). Flash column chromatography was performed using silica gel (0.04–0.06 mm). Melting points are uncorrected. The high resolution mass spectra (HRMS) were obtained using a time-of-flight (TOF) instrument equipped with electrospray ionization (ESI) and electron impact (EI^+) operating in the positive ion mode. Tetrahydrofuran (THF) and CH_2Cl_2 were dried in a solvent purification system by passing them through an activated alumina column before use.

Typical Procedure for Benzamide Formation: 2-Bromo-3-nitro-*N*-phenylbenzamide (4a). A mixture of 2-bromo-3-nitrobenzoic acid (1.0 g, 4.06 mmol) and thionyl chloride (5 mL) was refluxed for 3 h in the presence of a catalytic amount of *N,N*-dimethylformamide (DMF). Excess thionyl chloride was then removed under reduced pressure. The residue was dissolved in dichloromethane (10 mL). Aniline (0.74 g, 8.13 mmol) in CH_2Cl_2 (15 mL) was added dropwise at room temperature. The resulting solution was then stirred overnight. The reaction mixture was poured in water (10 mL) and extracted with dichloromethane. The separated organic layers were combined and dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using ethyl acetate/*n*-pentane (1:1) as an eluent to give the title product (1.13 g, 87%) as a solid. Physical and spectroscopic data were in good agreement with the literature.[17] The ^{1}H and ^{13}C NMR spectra of **4a** have been included in the Supporting Information.

2-Bromo-3-nitro-*N*-*p*-tolylbenzamide (4b). White solid. Yield: 1.30 g (96%); mp 166–168 °C; ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H), 7.18 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.53 (t, J = 7.8 Hz, 1H), 7.62 (br s, 1H), 7.71 (dd, J = 1.7, 7.7 Hz, 1H), 7.76 (dd, J = 1.6, 7.9 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 21.1, 111.6, 120.5, 126.2, 128.9,

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313–321

129.9, 131.9, 134.6, 135.4, 141.5, 151.3, 164.2; HRMS (TOF MS ES$^+$) m/z calcd for $C_{14}H_{11}N_2O_3Br$ $[M + H]^+$: 335.0031; found: 335.0035.

2-Bromo-*N*-(4-methoxyphenyl)-3-nitrobenzamide (4c). The crude residue was purified by chromatography on silica gel with 60% ethyl acetate/pentane as an eluent to afford the title compound as a white solid. Yield: 1.34 g (94%); mp 180−182 °C; ^{1}H NMR (CD_3OD): δ 3.80 (s, 3H), 6.93 (dd, J = 2.2, 6.9 Hz, 2H), 7.57 (dd, J = 2.2, 6.9 Hz, 2H), 7.66 (t, J = 7.8 Hz, 1H), 7.74 (dd, J = 1.8, 7.8 Hz, 1H), 7.89 (dd, J = 1.7, 7.9 Hz, 1H); ^{13}C NMR (CD_3OD): δ 55.9, 112.0, 115.1, 123.2, 126.6, 130.2, 132.3, 143.2, 152.8, 158.5, 166.9; HRMS (TOF MS ES$^+$) m/z calcd for $C_{14}H_{11}N_2O_4Br$ $[M + H]^+$: 350.9980; found: 350.9975.

2-Bromo-*N*-(1-naphthyl)-3-nitrobenzamide (4d). The crude residue was washed with pentane to obtain the title compound as a brown solid of sufficient purity. Yield: 0.89 g (59%); mp 243−246 °C; ^{1}H NMR (DMSO-d_6): δ 7.56−7.59 (several peaks, 3H), 7.80 (m, 3H), 7.89 (d, J = 8.0 Hz, 1H), 7.97−8.00 (several peaks, 2H), 8.10 (dd, J = 1.2, 6.8 Hz, 1H), 8.20 (t, J = 7.2 Hz, 1H), 10.75 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 110.5, 122.6, 122.9, 125.3, 125.6, 126.2, 126.3, 126.4, 128.2 (2C), 129.5, 131.8, 132.7, 133.8, 141.9, 150.9, 165.6; HRMS (TOF MS EI$^+$) m/z calcd for $C_{17}H_{11}N_2O_3Br$ $[M]^+$: 369.9953; found: 369.9959.

Typical Procedure for the Introduction of a Butylseleno Group by Nucleophilic Aromatic Substitution: 2-Butylseleno-3-nitro-*N*-phenylbenzamide (5a). Benzamide **4a** (0.75 g, 2.33 mmol) was added to an *in situ* prepared solution of BuSeNa (4 equiv) in EtOH (30 mL) at 0 °C under an inert atmosphere. The mixture was stirred at room temperature for 2 h and then warmed at 50 °C for 20 h. The solvent was removed under reduced pressure, and the residue dissolved in $CHCl_3$ and washed with water. After drying over anhydrous Na_2SO_4, evaporation of the solvent, and column chromatography using 20% ethyl acetate/pentane as eluent, the pure title compound (0.51 g, 55%) was isolated. Physical and spectroscopic data were in good agreement with the literature.[17] The ^{1}H, ^{13}C, and ^{77}Se NMR spectra of **5a** have been included in the Supporting Information.

2-Butylseleno-3-nitro-*N*-*p*-tolylbenzamide (5b). Orange liquid (semisolid). Solidified in the freezer. Yield: 0.59 g (67%); mp 69−73 °C; ^{1}H NMR ($CDCl_3$): δ 0.78 (t, J = 7.3 Hz, 3H), 1.22−1.29 (several peaks, 2H), 1.45−1.55 (several peaks, 2H), 2.36 (s, 3H), 2.87 (t, J = 7.5 Hz, 2H), 7.20 (d, J = 8.2 Hz, 2H), 7.49−7.55 (several peaks, 3H), 7.83 (dd, J = 1.3, 7.9 Hz, 1H), 7.91 (dd, J = 1.4, 7.6 Hz, 1H), 8.24 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 13.5, 21.1, 22.7, 31.1, 31.9, 120.0, 122.4, 125.5, 128.9, 129.9, 133.4, 135.0, 135.1, 142.9, 155.1, 165.2; ^{77}Se NMR ($CDCl_3$): δ 283; HRMS (TOF MS ES$^+$) m/z calcd for $C_{18}H_{20}N_2O_3Se$ $[M + H]^+$: 393.0717; found: 393.0721.

2-Butylseleno-*N*-(4-methoxyphenyl)-3-nitrobenzamide (5c). Yellow solid. Yield: 0.50 g (56%); mp 94−97 °C; ^{1}H NMR ($CDCl_3$): δ 0.78 (t, J = 7.3 Hz, 3H), 1.26 (m, 2H), 1.51 (m, 2H), 2.88 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 6.93 (dd, J = 2.2, 7.7 Hz, 2H), 7.51 (t, J = 7.8 Hz, 1H), 7.57 (dd, J = 2.2, 5.4 Hz, 2H), 7.82 (dd, J = 1.5, 8.0 Hz, 1H), 7.90 (dd, J = 1.5, 7.6 Hz, 1H), 8.22 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 13.5, 22.7, 31.0, 31.9, 55.6, 114.5, 121.7, 122.3, 125.3, 128.8, 130.7, 133.1, 143.0, 155.0, 157.0, 165.2; ^{77}Se NMR ($CDCl_3$): δ 283; HRMS (TOF MS ES$^+$) m/z calcd for $C_{18}H_{20}N_2O_4Se$ $[M + H]^+$: 409.0667; found: 409.0663.

Typical Procedure for the Synthesis of Nitroebselen Analogues: 7-Nitro-2-phenyl-1,2-benzisoselenazol-3(2*H*)-one (3). To a stirred solution of selenide **5a** (0.72 g, 1.84 mmol) in dry $CHCl_3$ (10 mL) was added Br_2 (0.10 mL, 1.84 mmol) in dry $CHCl_3$ (5 mL) containing Et_3N (0.257 mL, 1.84 mmol) at 0 °C under an inert atmosphere. The reaction mixture was then stirred for 3 h at room temperature, and water (10 mL) was added. The separated organic layer was dried over anhydrous Na_2SO_4. Removal of the solvent and purification of the residue by silica gel column chromatography (elution with 25% ethyl acetate/*n*-pentane) afforded the title compound (0.58 g, 95%) as a red solid. Physical and spectroscopic data were in good agreement with the literature.[17] The ^{1}H, ^{13}C, and ^{77}Se NMR spectra of **3** have been included in the Supporting Information.

7-Nitro-2-*p*-tolyl-1,2-benzisoselenazol-3(2*H*)-one (6b). Red solid. Yield: 0.50 g (82%); mp 184 °C; ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H), 7.27 (d, J = 7.9 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.72 (t, J = 7.8 Hz, 1H), 8.46 (dd, J = 0.9, 7.5 Hz, 1H), 8.57 (dd, J = 0.9, 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 21.3, 125.2, 127.6, 127.9, 130.3, 131.6, 135.3, 135.8, 136.6, 137.4, 142.2, 164.0; ^{77}Se NMR ($CDCl_3$): δ 929; HRMS (TOF MS ES$^+$) m/z calcd for $C_{14}H_{10}N_2O_3Se$ $[M + H]^+$: 334.9935; found: 334.9943.

2-(*p*-Methoxyphenyl)-7-nitro-1,2-benzisoselenazol-3(2*H*)-one (6c). Purification with 40% ethyl acetate/*n*-pentane afforded the pure title compound as a red solid. Yield: 67%; mp 206−209 °C; ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 3H), 6.99 (d, J = 9.0 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.73 (t, J = 7.6 Hz, 1H), 8.47 (d, J = 7.6 Hz, 1H), 8.58 (d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 55.8, 114.9, 127.1, 127.6, 127.9, 131.0, 131.4, 135.3, 136.6, 142.2, 158.9, 164.2; ^{77}Se NMR ($CDCl_3$): δ 932; HRMS (TOF MS ES$^+$) m/z calcd for $C_{14}H_{10}N_2O_4Se$ $[M + H]^+$: 350.9884; found: 350.9876.

Typical Procedure A for the Preparation of Azo-bis-ebselen Compounds: Bis-[2-phenyl-1,2-benzisoselenzol-3-(2*H*)-one-7-yl]diazene (7a). Nitroebselen **3** (275 mg, 0.86 mmol) was added to an *in situ* prepared colorless solution of PhTeNa (1.72 mmol) from the reaction of Ph_2Te_2 (352 mg, 0.86 mmol) and $NaBH_4$ (65 mg, 1.73 mmol) in EtOH (10 mL) at room temperature under an inert atmosphere. The mixture was then heated at reflux for 20 h, allowed to cool, poured into water, and extracted with $CHCl_3$. The separated $CHCl_3$ extract was dried over anhydrous Na_2SO_4, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel. The column was first eluted with pentane/ethyl acetate (1:1) then with $CHCl_3$ and finally with $CHCl_3$/MeOH (98:2) to give the title compound as a dark brown solid. Yield: 0.125 g (51%); mp > 320 °C; ^{1}H NMR ($CDCl_3$): δ 7.32 (t, J = 7.2 Hz, 2H), 7.49 (t, J = 7.2 Hz, 4H), 7.66 (dd, J = 8.0 Hz, 4H), 7.80 (t, J = 7.6 Hz, 2H), 8.40 (d, J = 7.2 Hz, 2H), 8.61 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 125.3, 126.7, 128.3, 129.6, 130.2, 131.7, 131.8, 132.1, 139.4, 144.5, 164.0; ^{77}Se NMR ($CDCl_3$): δ 931; HRMS (MALDI) m/z calcd for $C_{26}H_{16}N_4O_2Se_2$ $[M + H]^+$: 576.9682; found: 576.9689.

Bis-[2-*p*-tolyl-1,2-benzisoselenzol-3-(2*H*)-one-7-yl]diazene (7b). Heating at reflux for 40 h. Purification by column chromatography using 4% MeOH/$CHCl_3$ afforded the title compound as a dark brown solid. Yield: 30 mg (9%); mp > 320 °C; ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 6H), 7.29 (d, J = 7.6 Hz, 4H), 7.52 (d, J = 8.0 Hz, 4H), 7.78 (t, J = 7.2 Hz, 2H), 8.38 (d, J = 7.6 Hz, 2H), 8.57 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 21.3, 125.4, 128.2, 130.2, 131.2, 131.8, 132.1, 136.7 (2C), 144.6, 164.1; ^{77}Se NMR ($CDCl_3$): δ 927; HRMS (TOF MS ES$^+$) m/z calcd for $C_{28}H_{20}N_4O_2Se_2$ $[M + H]^+$: 604.9995; found: 604.9970.

Typical Procedure B for the Preparation of Azo-bis-ebselen Compounds: Bis-[2-phenyl-1,2-benzisoselenzol-3-(2*H*)-one-7-yl]diazene (7a). To a brown suspension of *in situ* prepared Na_2Se_2 (1.24 mmol) in dry THF (20 mL) under an inert atmosphere was slowly added a solution of benzamide **4a** (0.20 g, 0.62 mmol) in THF (10 mL) at room temperature. After heating at reflux for 5 h and cooling to room temperature, water (20 mL) was added and stirring was continued for 10 min. Following extraction with $CHCl_3$, the combined organic layers were dried over anhydrous Na_2SO_4. Removal of the solvent and purification of the residue by silica gel column chromatography, eluting first with pentane/ethyl acetate (1:1), then with $CHCl_3$, and finally with $CHCl_3$/MeOH (98:2), afforded compound **7a** (62 mg, 35%). Similarly, compound **7b** (340 mg, 36%) was prepared from **4b** (1.00 g, 2.98 mmol).

Bis-[1-naphthyl-1,2-benzisoselenzol-3-(2*H*)-one-7-yl]-diazene (7d). The solution of **4d** and Na_2Se_2 was stirred for 8 h at room temperature before workup. Purification by column chromatography using 5% MeOH/$CHCl_3$ as an eluent afforded the title compound (68%) as a dark brown solid; mp > 320 °C; ^{1}H NMR ($CDCl_3$): δ 7.49−7.61 (several peaks, 8H), 7.77 (t, J = 8.0 Hz, 2H), 7.85 (d, J = 8.0 Hz, 2H), 7.95 (t, J = 6.0 Hz, 4H), 8.42 (d, J = 7.6 Hz, 2H), 8.53 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 123.5, 125.8, 126.8 (2C), 127.1, 128.3, 128.7, 129.1, 130.8, 130.9, 131.4, 131.7, 132.4, 134.8, 135.0, 144.5, 165.3; ^{77}Se NMR ($CDCl_3$): δ 946; HRMS

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313−321

(TOF MS EI$^+$) m/z calcd for $C_{34}H_{20}N_4O_2Se_2$ $[M]^+$: 675.9917; found: 675.9913.

Typical Procedure for the Synthesis of Diselenides: Bis[3-amino-*N*-(1-naphthyl)benzamide-2-yl] Diselenide (8d). The azo-bis-ebselen **7d** (50 mg, 0.074 mmol) was added to an *in situ* prepared colorless solution of NaTePh (0.592 mmol) prepared from Ph_2Te_2 (61 mg, 0.148 mmol) and $NaBH_4$ (11 mg, 0.296 mmol) in EtOH (10 mL) at room temperature under an inert atmosphere. The mixture was then heated at 80 °C for 1 h and allowed to cool to room temperature. After addition of water, the mixture was extracted with ethyl acetate and dried over anhydrous $MgSO_4$. Evaporation of the solvent and purification by column chromatography, using ethyl acetate as an eluent afforded the title compound (25 mg, 52%) as a yellow solid; mp 236−240 °C; ^{1}H NMR (DMSO-d_6): δ 5.63 (s, 4H), 6.45 (d, J = 7.1 Hz, 2H), 6.80 (d, J = 7.8 Hz, 2H), 6.90 (t, J = 7.5 Hz, 2H), 7.25 (d, J = 7.0 Hz, 2H), 7.25−7.52 (several peaks, 6H), 7.87 (d, J = 8.2 Hz, 2H), 7.91 (d, J = 8.2 Hz, 2H), 7.97 (d, J = 7.9 Hz, 2H), 10.27 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 110.1, 114.3, 114.8, 122.6, 123.0, 125.3, 125.5, 125.7, 125.8, 127.8, 128.4, 130.3, 133.1, 133.5, 144.5, 150.9, 169.2; ^{77}Se NMR (DMSO-d_6): δ 364; HRMS (TOF MS ES$^+$) m/z calcd for $C_{34}H_{26}N_4O_2Se_2$ $[M + Na]^+$: 705.0286; found: 705.0304.

Bis[3-Amino-*N*-(*p*-tolyl)benzamide-2-yl] Diselenide (8b). Orange solid. Yield: 44 mg (87%); mp 219−221 °C; ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 6H), 5.46 (s, 4H), 6.60 (d, J = 7.0 Hz, 2H), 6.79 (d, J = 8.2 Hz, 2H), 7.05 (d, J = 8.2 Hz, 4H), 7.10 (t, J = 7.8 Hz, 2H), 7.33 (d, J = 8.2 Hz, 4H), 10.08 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 20.5, 110.2, 114.6, 115.0, 120.0, 128.8, 130.4, 132.4, 136.3, 144.7, 150.8, 167.3; ^{77}Se NMR (DMSO-d_6): δ 368; HRMS (TOF MS ES$^+$) m/z calcd for $C_{28}H_{26}N_4O_2Se_2$ $[M + H]^+$: 611.0464; found: 611.0475.

Diselenide **8b** was also isolated in 16% yield when **6b** was allowed to react with NaTePh.

Typical Procedure for Reduction of 2-Butylseleno-3-nitrobenzamides: 3-Amino-2-butylseleno-*N*-phenyl Benzamide (10a). To *in situ* prepared NaTePh (0.96 mmol) from the reaction of Ph_2Te_2 (192 mg, 0.48 mmol) and $NaBH_4$ (35 mg, 0.94 mmol) in EtOH (10 mL) was added **5a** (96 mg, 0.24 mmol) at room temperature under nitrogen. The mixture was then heated at reflux overnight. After cooling to room temperature the solvent was evaporated and the residue was dissolved in dichloromethane. After treatment with water the organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. Chromatographic purification, eluting with 40% ethyl acetate/pentane, afforded the title compound (70 mg, 96%) as a white solid; mp 76−78 °C; ^{1}H NMR ($CDCl_3$): δ 0.83 (t, J = 7.5 Hz, 3H), 1.33 (m, 2H), 1.58 (m, 2H), 2.81 (t, J = 7.5 Hz, 2H), 4.62 (s, 2H), 6.83 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 6.9 Hz, 1H), 7.12−7.22 (several peaks, 2H), 7.37 (t, J = 7.8 Hz, 2H), 7.63 (d, J = 7.8 Hz, 2H), 7.79 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 13.6, 23.0, 29.3, 32.6, 110.4, 115.8, 117.6, 120.1, 124.5, 129.2, 130.1, 138.2, 144.4, 149.6, 168.0; ^{77}Se NMR ($CDCl_3$): δ 145; HRMS (TOF MS EI$^+$) m/z calcd for $C_{17}H_{20}N_2OSe$ $[M]^+$: 348.0741; found: 348.0742.

3-Amino-2-butylseleno-*N*-(*p*-tolyl)benzamide (10b). Yield: 82%; mp 88−90 °C; ^{1}H NMR (CD_3OD): δ 0.84 (t, J = 7.0 Hz, 3H), 1.35 (m, 2H), 1.58 (m, 2H), 2.32 (s, 3H), 2.77 (t, J = 7.4 Hz, 2H), 6.74 (dd, J = 1.2, 7.5 Hz, 1H), 6.86 (dd, J = 1.2, 8.2 Hz, 1H), 7.32−7.18 (several peaks, 3H), 7.52 (d, J = 8.3 Hz, 2H); ^{13}C NMR (CD_3OD): δ 13.9, 21.0, 23.9, 28.7, 33.6, 111.6, 116.2, 116.7, 121.7, 130.2, 130.9, 135.1, 137.3, 146.7, 151.6, 171.5; ^{77}Se NMR (CD_3OD): δ 137; HRMS (TOF MS EI$^+$) m/z calcd for $C_{18}H_{22}N_2OSe$ $[M]^+$: 362.0897; found: 362.0901.

3-Amino-2-(butylseleno)-*N*-(4-methoxyphenyl)benzamide (10c). Yield: 79%; mp 100−104 °C; ^{1}H NMR (DMSO-d_6): δ 0.79 (t, J = 7.4 Hz, 3H), 1.25−1.31 (several peaks, 2H), 1.45−1.51 (several peaks, 2H), 2.71 (t, J = 7.4 Hz, 2H), 3.73 (s, 3H), 5.52 (br s, 2H), 6.58 (d, J = 7.0 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 7.12 (t, J = 7.8 Hz, 1H), 7.61 (d, J = 8.6 Hz, 2H), 9.99 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 13.4, 22.3, 26.9, 31.9, 55.2, 108.6, 113.7, 114.1, 114.4, 120.9, 129.4, 132.7, 145.9, 150.3, 155.2, 167.7; ^{77}Se NMR (DMSO-d_6): δ 144; HRMS (TOF MS EI$^+$) m/z calcd for $C_{18}H_{22}N_2O_2Se$ $[M]^+$: 378.0846; found: 378.0844.

2-(4-Hydroxyphenyl)-7-nitrobenzisoselenazol-3(2*H*)-one (11b). Solution of BBr_3 (0.86 mmol, 0.86 mL) was added into a stirred solution of **6c** (0.27 mmol, 0.10 g) in dry CH_2Cl_2 (10 mL) at −78 °C under an inert atmosphere. Stirring was then continued overnight at room temperature. The reaction mixture was diluted with dichloromethane and poured into a solution of brine. The separated organic layer was dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to afford a red solid which was washed with diethyl ether and dried under vacuum to give the pure title compound (85 mg, 88%); mp 263−265 °C; ^{1}H NMR (DMSO-d_6): δ 6.86 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 9.0 Hz, 2H), 7.86 (t, J = 7.9 Hz, 1H), 8.38 (d, J = 7.4 Hz, 1H), 8.71 (d, J = 8.2 Hz, 1H), 9.77 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 115.9, 127.1, 128.3, 128.8, 129.3, 130.3, 134.8, 135.8, 142.0, 156.6, 163.6; ^{77}Se NMR (DMSO-d_6): δ 923; HRMS (TOF MS EI$^+$) m/z calcd for $C_{13}H_8N_2O_4Se$ $[M]^+$: 335.9649; found: 335.9642.

X-ray Crystallographic Analysis. X-ray crystallographic studies for obtaining the structures of compounds **7b** and **8b** were carried out using graphite-monochromatized Mo Kα radiation (λ = 0.7107 Å). The structures were solved by direct methods (SHELXS-2013) and refined by a full-matrix least-squares procedure on F^2 for all reflections using SHELXL-2013 software.[28] Hydrogen atoms were localized by geometrical means. A riding model was chosen for refinement. The isotropic thermal parameters of hydrogen atoms were fixed at 1.5 times and 1.2 times U(eq) of the corresponding carbon atoms for sp^3 C−H and sp^2 C−H bonds, respectively. Crystallographic data for the structures reported in this paper, containing supplementary crystallographic data for this paper, have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publications: CCDC 1047473 (for compound **7b**) and CCDC 1047474 (for compound **8b**). These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

*Crystal Data for **7b**.* $C_{30}H_{22}Cl_6N_4O_2Se_2$, M_r = 841.14, monoclinic, space group $C2/c$, a = 22.883(7) Å, b = 12.0629(12) Å, c = 15.901(5) Å, $\alpha = \gamma$ = 90°, β = 132.43(5)°, V = 3239.5(15) Å^3, λ = 0.71073 Å, Z = 4, T = 123(2) K, ρ_{calcd} = 1.725 Mg/m^3, GOF = 1.049, R1 = 0.0707, wR2 = 0.1422 [$I > 2\sigma(I)$]; R1 = 0.1291, wR2 = 0.1754 (all data). Of the 18 727 reflections that were collected, 6174 were unique (R_{int} = 0.1199).

*Crystal Data for **8b**.* $C_{28}H_{26}N_4O_2Se_2$, M_r = 608.45, monoclinic, space group $P12/c1$, a = 8.9772(2) Å, b = 26.5501(5) Å, c = 10.9101(3) Å, $\alpha = \gamma$ = 90°, β = 96.200(2)°, V = 2585.16(10) Å^3, λ = 0.71073 Å, Z = 4, T = 123(2) K, ρ_{calcd} = 1.563 Mg/m^3, GOF = 1.069, R1 = 0.0543, wR2 = 0.0813 [$I > 2\sigma(I)$]; R1 = 0.0981, wR2 = 0.0921 (all data). Of the 59 472 reflections that were collected, 16 750 were unique (R_{int} = 0.0599).

Computational Details. Computational calculations for compounds **6b−c**, **7a−b**, **7d**, **11b** were executed by using the Gaussian 09 suite of quantum chemical programs.[29] The hybrid B3LYP exchange correlation functional was implemented for density functional theory (DFT) calculations.[30] The geometry optimizations were carried out at the B3LYP level of DFT by using the 6-311+G(d) basis sets. The quantifications of orbital interaction were done by natural bond orbital (NBO) analysis at the B3LYP/6-311+G(d, p) level.[31] The ^{77}Se NMR calculations were performed at the B3LYP/6-311+G (d, p) level on B3LYP/6-311+G(d)-level-optimized geometries by using the gauge-including atomic orbital (GIAO) method (referenced with respect to the peak of Me_2Se).[32] Atoms in molecules (AIM)[33] calculations have also been used to confirm a distinct bond critical point between the two interacting atoms.

Coupled Reductase Assay. The glutathione peroxidase-like activity of compounds prepared was determined by UV-spectroscopy following the protocol by Wilson[34] with slight modifications. The test mixture contained GSH (1 mM), ethylene diamine tetraacetate (EDTA, 1 mM), glutathione reductase (GR) (1.3 unit·mL^{-1}), and β-nicotinamide adenine dinucleotide phosphate (NADPH, 0.2 mM) in potassium phosphate buffer (100 mM), pH 7.5. Catalysts (20 μM) were added to the test mixture at 21 °C, and the reaction was initiated

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313−321

by addition of H_2O_2 (0.8 mM). Initial reaction rates were based on the consumption of NADPH as assessed by UV-spectroscopy at 340 nm. The initial reduction rates were triplicated and calculated from the first 10 s of reaction by using 6.22 mM^{-1} cm^{-1} as the extinction coefficient for NADPH. GPx-data reported in Table 2 are means ± SD.

Biological Studies. Blood buffy coats were diluted in a 1:1 ratio with 1 × phosphate buffered saline (PBS), and mononuclear cells were segregated using Ficoll-Paque plus density gradient centrifugation. Briefly, the diluted blood was gently placed on top of Ficoll-Paque and then centrifuged at 400 g for 30 min. After removing the plasma layer, the mononuclear (MNC) layer was collected, washed with PBS, and then spun at 100 g for 15 min. This washing procedure was repeated for a total of 3 times. Total cell number was counted using a hemocytometer and trypan blue exclusion method. The blood pellet was then suspended in 3% dextran/0.9% saline for 20 min. The supernatant was then collected, and the cell pellet was collected after centrifugation at 250 g for 10 min. To remove erythrocytes, the pellet was subjected to 0.2% saline solution for 20 s and then an equal volume of 1.6% saline was added. Then the cells were spun at 250 g for 10 min, and the pellet was suspended in PBS.

Chemiluminescence Assay. To measure the release of ROS from monocytes, a luminol amplified chemiluminescence assay was conducted. All measurements were performed in white 96 well plates at 37 °C in 1 × PBS with 50 mM of luminol, 0.1 M NaOH, 2 μg/mL of horse radish peroxidase, different concentrations of ebselen, **3**, **6b–c**, **7a–b**, **7d**, **8b**, **8d**, and **11b**, and Trolox initially diluted in DMSO. Approximately 2 × 10^5 cells were plated in each well and then stimulated to generate ROS using 500 nM of 1 μM phorbol myristate acetate (PMA). Luminescence readings were taken using a multiplate reader every 2 min for 2 h. Total ROS was quantified by determining the area under the chemiluminescence kinetic curve. Experiments were repeated 4–5 times with n = 3 per group per experiment. For statistical analysis, ANOVA was performed with Scheffe posthoc tests. P values below 0.05 were considered significant.

Cell Proliferation and Toxicity Assay. MC3T3 (plating density: 5 × 10^3 and 20 × 10^3 per well respectively in a 96 well plate) was allowed to adhere after plating for 24 h in α-MEM supplemented with 10% FBS and 1% pen/strep. Then the cells were exposed to antioxidants **3**, **6b–c**, **7a–b**, **7d**, **8b**, **8d**, and **11b** (25 μM) for 24 h, washed, and then fed with fresh α-MEM every other day. To assess cell viability, the Alamar Blue assay was conducted on days 1 and 3 after treatment. Briefly, cells were washed with 1 × PBS and then incubated with Alamar Blue (1:20 dilution in phenol red free α-MEM) for 1.5 h, and then fluorescence was read with a plate reader at 560 nm excitation and 590 nm emissions. Experiments were repeated twice with n = 6 for each group.

Mechanistic Studies. A solution of H_2O_2 (3.7 μL, 0.033 mmol) was added to an NMR tube containing diselenide **8b** (20 mg, 0.033 mml) in 400 μL of DMSO-d_6, and the ^{77}Se NMR spectrum was recorded after 30–60 min. Then another equivalent of H_2O_2 was added, and the ^{77}Se NMR spectrum was recorded again. Several portions (1 + 2 equiv) of GSH (10 mg, 0.033 mmol) in 100 μL of H_2O were then syringed into the NMR tube. After every new addition of thiol, the ^{77}Se NMR spectrum was recorded.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02418.

X-ray crystallographic data (CIF)

^{1}H, ^{13}C, and ^{77}Se NMR data for all new compounds prepared; results from kinetic studies of compounds prepared and coordinates of optimized geometries (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: vijay.singh@kemi.uu.se.

*E-mail: lars.engman@kemi.uu.se.

ORCID

Lars Engman: 0000-0001-7751-629X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Stiftelsen Å-Forsk (16-364) and Stiftelsen Olle Engkvist Byggmästare (2016/159) and Carl Tryggers Stiftelse för Vetenskaplig Forskning (CTS 13:346) are gratefully acknowledged for financial support.

■ REFERENCES

(1) Romagné, F.; Santesmasses, D.; White, L.; Sarangi, G. K.; Mariotti, M.; Hübler, R.; Weihmann, A.; Parra, G.; Gladyshev, V. N.; Guigó, R.; Castellano, S. *Nucleic Acids Res.* **2014**, *42*, D437–D443.

(2) Kryukov, G. V.; Castellano, S.; Novoselov, S. V.; Lobanov, A. V.; Zehtab, O.; Guigó, R.; Gladyshev, V. N. *Science* **2003**, *300*, 1439–1443.

(3) Lu, J.; Holmgren, A. *J. Biol. Chem.* **2009**, *284*, 723–727.

(4) Hatfield, D. L., Berry, M. J., Gladyshev, V. N., Eds. *Selenium: Its Molecular Biology and Role in Human Health*, 3rd ed.; Springer-Verlag: New York, 2012.

(5) Jacob, C.; Giles, G. I.; Giles, N. M.; Sies, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4742–4758.

(6) Arnold, A. P.; Tan, K.; Rabenstein, D. L. *Inorg. Chem.* **1986**, *25*, 2433–2437.

(7) Halliwell, B., Gutteridge, J. M. C., Eds. *Free Radicals in Biology and Medicine*, 4th ed.; Oxford University Press: Oxford, 2007; p 110.

(8) Epp, O.; Ladenstein, R.; Wendel, A. *Eur. J. Biochem.* **1983**, *133*, 51–69.

(9) (a) Müller, A.; Cadenas, E.; Graf, P.; Sies, H. *Biochem. Pharmacol.* **1984**, *33*, 3235–3239. (b) Wendel, A.; Fausel, M.; Safayhi, H.; Tiegs, G.; Otter, R. *Biochem. Pharmacol.* **1984**, *33*, 3241–3245. (c) Sies, H. *Free Radical Biol. Med.* **1993**, *14*, 313–323.

(10) (a) Yamaguchi, T.; Sano, K.; Takakura, K.; Saito, I.; Shinohara, Y.; Asano, T.; Yasuhara, H. *Stroke* **1998**, *29*, 12–17. (b) Morisseau, C.; Sahdeo, S.; Cortopassi, G.; Hammock, B. D. *Anal. Biochem.* **2013**, *434*, 105–111. (c) Soares, J. C. M.; Folmer, V.; Da Rocha, J. B. T.; Nogueira, C. W. *Cell. Biol. Int.* **2014**, *38*, 625–630. (d) Park, S.; Kang, S.; Kim, D. S.; Shin, B. K.; Moon, N. R.; Daily, J. W., III *Free Radical Res.* **2014**, *48*, 864–874. (e) Wang, X.; Yun, J.-W.; Lei, X. G. *Antioxid. Redox Signaling* **2014**, *20*, 191–203. (f) Saxena, N.; Rao, P. V. L; Bhaskar, A. S. B.; Bhutia, Y. D. *Int. Immunopharmacol.* **2014**, *21*, 412–425. (g) Tan, S. M.; Deliyanti, D.; Figgett, W. A.; Talia, D. M.; de Haan, J. B.; Wilkinson-Berka, J. L. *Exp. Eye Res.* **2015**, *136*, 1–8. (h) Martini, F.; Bruning, C. A.; Soares, S. M.; Nogueira, C. W.; Zeni, G. *Curr. Pharm. Des.* **2014**, *21*, 920–924.

(11) (a) Parnham, M. J.; Sies, H. *Biochem. Pharmacol.* **2013**, *86*, 1248–1253. (b) Azad, G. K.; Tomar, R. S. *Mol. Biol. Rep.* **2014**, *41*, 4865–4879.

(12) NIH Clinical Collections (NCC), http://nihsmr.evotec.com/evotec/sets/ncc. Screening of this database for inhibitors of inositol monophosphate recently identified ebselen as a possible lithium mimetic for bipolar disorder. Singh, N.; Halliday, A. C.; Thomas, J. M.; Kuznetsova, O. V.; Baldwin, R.; Woon, E. C. Y.; Aley, P. K.; Antoniadou, I.; Sharp, T.; Vasudevan, S. R.; Churchill, G. C. *Nat. Commun.* **2013**, *4*, 1332.

(13) (a) Bhabak, K. P.; Mugesh, G. *Chem. - Eur. J.* **2007**, *13*, 4594–4601. (b) Satheeshkumar, K.; Mugesh, G. *Chem. - Eur. J.* **2011**, *17*, 4849–4857. (c) Selvakumar, K.; Shah, P.; Singh, H. B.; Butcher, R. J. *Chem. - Eur. J.* **2011**, *17*, 12741–12755. (d) Balkrishna, S. J.; Kumar, S.; Azad, G. K.; Bhakuni, B. S.; Panini, P.; Ahalawat, N.; Tomar, R. S.;

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313–321

Detty, M. R.; Kumar, S. *Org. Biomol. Chem.* **2014**, *12*, 1215−1219. (e) Pacuła, A. J.; Ścianowski, J.; Aleksandrzak, K. B. *RSC Adv.* **2014**, *4*, 48959−48962. (f) Elsherbini, M.; Hamama, W. S.; Zoorob, H. H.; Bhowmick, D.; Mugesh, G.; Wirth, T. *Heteroat. Chem.* **2014**, *25*, 320−325. (g) Wang, Z.; Wang, Y.; Li, W.; Luo, Z.; Sun, Y.; Wu, R.; Huang, L.; Li, X. *Dalton Trans.* **2015**, *44*, 20913−20925.
(14) Parnham, M. J.; Biedermann, J.; Bittner, Ch.; Dereu, N.; Leyck, S.; Wetzig, H. *Agents Actions* **1989**, *27*, 306−308.
(15) Mugesh, G.; du Mont, W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125−2179.
(16) Pearson, J. K.; Boyd, R. J. *J. Phys. Chem. A* **2008**, *112*, 1013−1017.
(17) Singh, V. P.; Singh, H. B.; Butcher, R. J. *Eur. J. Org. Chem.* **2011**, *2011*, 5485−5497.
(18) Lambert, C.; Christiaens, L. *Tetrahedron* **1991**, *47*, 9053−9060.
(19) Ohe, K.; Uemura, S.; Sugita, N.; Masuda, H.; Taga, T. *J. Org. Chem.* **1989**, *54*, 4169−4174.
(20) (a) Singh, V. P.; Singh, H. B.; Butcher, R. J. *Chem. - Asian J.* **2011**, *6*, 1431−1442. (b) Wirth, T.; Fragale, G. *Chem. - Eur. J.* **1997**, *3*, 1894−1902.
(21) Kumar, S.; Yan, J.; Poon, J.; Singh, V. P.; Lu, X.; Ott, M. K.; Engman, L.; Kumar, S. *Angew. Chem., Int. Ed.* **2016**, *55*, 3729−3733.
(22) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441−445.
(23) Dupont, L.; Dideberg, O.; Sbit, M.; Dereu, N. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1988**, *44*, 2159−2161.
(24) (a) Sarma, B. K.; Mugesh, G. *Chem. - Eur. J.* **2008**, *14*, 10603−10614. (b) Sarma, B. K.; Mugesh, G. *J. Am. Chem. Soc.* **2005**, *127*, 11477−11485.
(25) Back, T. G.; Dyck, B. P. *J. Am. Chem. Soc.* **1997**, *119*, 2079−2083.
(26) Loft, S.; Deng, X. S.; Tuo, J.; Wellejus, A.; Sorenson, M.; Poulsen, H. E. *Free Radical Res.* **1998**, *29*, 525−539.
(27) Brewer, G. J. *Exp. Biol. Med.* **2007**, *232*, 323−335.
(28) Sheldrick, G. M. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **2008**, *64*, 112−122.
(29) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E., Jr.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision c.01 and d.01; Gaussian, Inc.: Wallingford, CT, 2010 and 2013.
(30) (a) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1988**, *37*, 785−789. (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.
(31) (a) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899−926. (b) Weinhold, F. Natural Bond Orbital (NBO), version 5.0.
(32) Bayse, C. A. *Inorg. Chem.* **2004**, *43*, 1208−1210.
(33) (a) Bader, R. W. F. *Atoms in Molecules: A Quantum Theory*; Oxford University Press: New York, 1990. (b) Popelier, P. *Atoms In Molecules: An Introduction*: Pearson, Harlow, U.K., 2000. (c) Beigler-Konig, F.; Schonbohm, J.; Bayles, D. *J. Comput. Chem.* **2001**, *22*, 545−559.
(34) (a) Wendel, A. *Methods Enzymol.* **1981**, *77*, 325−333. (b) Wilson, S. R.; Zucker, P. A.; Huang, R. C.; Spector, A. *J. Am. Chem. Soc.* **1989**, *111*, 5936−5939.

DOI: 10.1021/acs.joc.6b02418
J. Org. Chem. 2017, 82, 313−321