Synthesis of 2-Phenyl-1,2-benziso[^{77}Se]selenazol-3(2H)-one: "Ebselen"

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SUMMARY

Summary: Synthesis of 2-Phenyl-1,2-benziso[^{77}Se]selenazol-3(2H)-one has been accomplished in one step from 94% ^{77}Se and commercially available starting materials in 76% yield based on the enriched elemental selenium.

Keywords: 2-Phenyl-1,2-benziso[^{77}Se]selenazol-3(2H)-one, [^{77}Se]Ebselen, ^{77}Selenium.

INTRODUCTION

2-Phenyl-1,2-benzisoselenazol-3(2H)-one, Ebselen, is one of the first examples of a selenium containing heterocycle which displays significant pharmacological activities. In general it is accepted that it is effective for the treatment of diseases caused by oxidative cell damage, and is described as exhibiting glutathione peroxidase-like activity *in vitro*[3]. Its toxicity in mice is >6g/kg[4]. More recent reports indicate that Ebselen have a broad spectrum of positive pharmacological effects. For example, Ebeselen has shown promise in the protection against cisplatin-induced neurotoxicity[5], is in clinical trials as an anti-inflammatory drug[5], and provides some benefits for stroke victims[6].

It has been reported that Ebselen reacts rapidly with sulfhydryl compounds, giving rise to cleavage of the Se-N bond[7]. Our interest in Ebselen arises from the potential to study its interaction with cellular targets by ^{77}Se NMR spectroscopy. Recently, the selenium atom has been exploited as a novel spectroscopic reporter group in the study of various inorganic, organic and biochemical systems[8]. The sensitivity of the ^{77}Se nucleus (6.93×10^{-3} with respect to ^{1}H and 2.98 compared to ^{13}C), its natural abundance (7.5%) and spin (I=1/2) make it an excellent candidate for an NMR reporter nucleus[9]. Selenium has the special feature of possessing a large chemical shift range (~3400 ppm), and the selenium nucleus is extremely sensitive to its electronic

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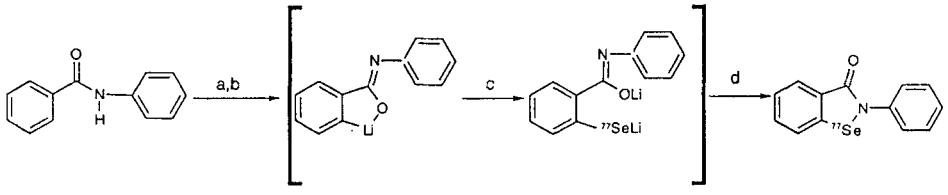
environment[10]. For example, comparison of ^{77}Se and ^{31}P chemical shifts reveals that the sensitivity of the selenium shielding to changes in electronic structure is several times greater than that of phosphorus[11]. These properties make the selenium atom an ideal candidate for *in vitro* NMR spectroscopy studies. Stable isotope labeling is required because many of these studies are performed using low concentrations of biomolecules.

RESULTS AND DISCUSSION

Previous syntheses of Ebselen have been reported[12]. We chose to investigate a recently published one-step synthesis by Engman and co-workers for the synthesis of ^{77}Se enriched Ebselen[13]. This synthetic route was appealing because it used commercially available benzanilide and elemental selenium. Engman and co-workers used an ortho-directed metallation using *n*-butyllithium to generate the dianion **2** (Scheme 1). The red-orange dianion was treated with elemental selenium (natural abundance) to give the insertion product **3**. Oxidation with $CuBr_2$ gave **4** in 63% yield.



1 2 3 4

Scheme 1 *Reagents:* (a) 1.15 equiv, LDA; (b) 1.15 equiv, *n*-butyllithium; (c) 1.00 equiv of Se°; (d) $CuBr_2$

We were able to construct **4** consistently by employing a slightly different approach. We generated the lithium amide with lithium diisopropylamide at 0 °C during a period of 15 min. The solvents were then removed *in vacuo* to a constant pressure of 0.1 mmHg. The pale yellow solid was then heated to 40-50 °C to ensure complete removal of diisopropyl amine. The solids were then taken up in THF and cooled to -78 °C and 1.15 equiv of *n*-butyllithium were added. The reaction was allowed to warm to ambient temperature. Upon warming the yellow solution became orange-red indicative of dianion formation. Addition of Se° followed by stirring for 1.5 h affords the insertion product. Cooling to -78 °C and adding finely ground $CuBr_2$ followed by isolation according to the literature procedure gave consistent yields (50-68%) of Ebselen. With these positive results we next turned our attention to the synthesis of ^{77}Se enriched Ebselen. Enriched elemental ^{77}Se can be commercially obtained. However, from our experience[14], consistent and reliable results can only be obtained if this enriched material is sublimed first. Slowly heating the selenium in a long necked round bottom flask at 0.1 mmHg (in a closed system) provides for a mixture of red and gray selenium which deposits above the heating level on the round bottom flask. The dianion is then transferred to the sublimation flask to generate the insertion product **3**. Again, quenching with finely ground $CuBr_2$ afforded the labeled **4**. Purification by flash chromatography gave **4** in 68% yield.

CONCLUSION

^{77}Se labeled Ebselen has synthesized in one step from commercially available benzanilide and enriched elemental ^{77}Se in good yield. Experiments using ^{77}Se labeled Ebselen may provide insight into the mechanisms of inhibition in enzymes such as pyruvate dehydrogenase which is a key enzyme in carbohydrate metabolism.

Experimental--Analytical thin-layer chromatography (TLC) was carried out on glass plates (silica gel 60 Å, 250 mm thickness) obtained from EM Scientific. TLC visualization was accomplished with a UV lamp, I_2 staining, and an ethanolic solution of phosphomolybdic acid (PMA). Flash chromatography was performed using the Still protocol[15]. Microanalyses were performed on a Perkin Elmer instrument at Los Alamos National Laboratory, CST-12.

NMR Methods--Proton, The ^{1}H, ^{13}C, and ^{77}Se NMR spectra were recorded as DMSO-d6 solutions on a Bruker AM-200 or WM-300 NMR spectrometers. ^{1}H chemical shifts are expressed in parts per million with respect to tetramethylsilane at 0.0 ppm; ^{13}C chemical shifts are referenced with respect to internal DMSO-d6 (δ = 39.5.0 ppm with respect to tetramethylsilane at 0.0 ppm); ^{77}Se chemical shifts are reference with respect to diphenyldiselenide in $CDCl_3$ (δ = 471 ppm with respect to dimethylselenide in a 60% solution[16] in $CDCl_3$).

Chemicals--^{77}Se° (94%) was obtained from Isotec Inc. Se°, benzanalide, *n*-butyllithium, lithium diisopropylamine, $CuBr_2$, was obtained from Aldrich Chemical Co. Diisopropylamine was distilled over calcium hydride and stored over KOH prior to use. Tetrahydrofuran was distilled over potassium benzophenone ketal prior to use.

2-Phenyl-1,2-benzisoselenazol-3(2H)-one (4)--mp 179-180 °C (lit.[13] mp 180-181°C); ^{1}H (DMSO-d6) δ 7.26 (t, J = 7 Hz, 1H), 7.46 (m, 3H), 7.66 (m, 3H) 7.91 (d, J = 8 Hz, 1H), 8.09 (d, J = 8 Hz, 1H); ^{13}C (DMSO-d6) δ 165.1, 139.7, 138.9, 132.3, 129.2, 128.5, 128.0, 126.3, 125.9, 125.8, 124.7; ^{77}Se (DMSO-d6) δ 928; Anal. Calcd. for $C_{13}H_9NOSe$: C, 56.95; H, 3.19; N, 5.05. Found: C, 56.95; H, 3.31; N, 5.11.

2-Phenyl-1,2-benziso[^{77}Se]selenazol-3(2H)-one (4)--In a long single necked flask was placed 0.128 g (0.649 mmol) of **1**. This was pumped to a constant 0.1 mmHg and then placed over argon. To this was added 3 mL of freshly distilled THF followed by 0.497 mL (1.15 equiv; 1.5 M) of LDA. After 15 min the temperature of the yellow solution was reduced to -78°C and 0.298 mL (1.15 equiv, 2.5 M) of *n*-butyllithium was added. The reaction was stirred for 0.5 h and then slowly brought to ambient temperature. The resulting red-orange solution was stirred for 0.5 h and then transferred to freshly sublimed selenium (0.050 g, 94% ^{77}Se, 0.649 mmol). The solid selenium reacted within minutes. The mixture was then stirred for 1.5 h and then chilled to -78°C and finely ground $CuBr_2$ was added. This suspension was stirred for 0.5 h and then warmed to ambient temperature. Stirring was continued for 1 h. The solution was then poured onto 100 mL of H_2O/glacial acetic acid (99:1 v/v). The mixture was then extracted 4x with 50 mL portions of methylene chloride. The extracts were then dried over sodium sulfate, filtered and then reduced to give 0.170 g of a yellow solid. Purification by flash chromatography (4:2 v/v hexane: ethyl acetate) to gave 0.120 g of **4**. Further purification by recrystallization from ethanol affords a pale yellow solid. mp 178-180 °C; ^{1}H (DMSO-d6) δ 7.26 (t, J = 7 Hz, 1H), 7.46 (m, 3H), 7.66 (m, 3H), 7.91 (d, J = 8 Hz, 1H), 8.09 (d, J = 8 Hz, 1H); ^{13}C (DMSO-d6) δ 165.1 (d, $^2J_{C\text{-}Se}$ = 10

Hz), 139.6, 138.1, 132.3 (d, $J_{C\text{-}Se}$ = 5.6 Hz), 129.2, 128.4, 128.0 (d, $J_{C\text{-}Se}$ = 3 Hz), 126.4, 126.0, 125.7 (d, $J_{C\text{-}Se}$ = 10 Hz), 124.7 (d, $J_{C\text{-}Se}$ = 3 Hz); ^{77}Se (DMSO-d6) δ 928, 973 ($CDCl_3$)[17].

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