Appendix

Initial Synthetic Schemes for the Synthesis of 5-(*o*-nitrobenzyl)selenyl-2-hydroxypentanoic acid (NBSeOH)

A.1 Introduction:

This section describes several of the initial synthetic schemes that were tried to synthesize 5-(*o*-nitrobenzyl)selenyl-2-hydroxypentanoic acid (NBSeOH). All of the pathways described here did not lead to the finished product. A full analytical analysis of products and description of the reaction will be given only for the synthetic steps that yielded the desired product.

Mixed selenides are usually synthesized by the reduction of a diselenide that is then reacted with a organic halide (1-7). Figure A.1 illustrates the three different synthetic approaches that were utilized in the synthesis of NBSeOH. Routes 1 and 2 will be described here. Route 3, the only route that yielded the final desired product NBSeOH, is described in Chapter 2.

Route 1 is based on the reduction of di-*o*-nitrobenzyl diselenide (D) to produce a selenium ion that is reacted with (S) 5-chloro-2-hydroxypentanoic acid (C) (4, 5). The (S) 5-chloro-2-hydroxypentanoic acid is derived from the reduction of (S) 5-oxotetrahydrofuran-2-carboxylic acid, which is then halogenated. The scheme begins with the carboxylic acid on (S)-5-oxotetrahydrofuran 2-carboxylic acid protected as a *tert*-butyl ester (A) using ditertbutyldicarbonate in the presence of dimethylaminopyridine (8). The lactone was then reduced using $LiBH_4$ to yield product B (9, 10). Many different reaction conditions were tried to halogenate the primary alcohol over the secondary (11-17). However, product C was never isolated. I believe this is due to the recyclization of the diol during the halogenation reaction. Halogenation of the primary alcohol might be occurring, but is followed immediately by the secondary alcohol attacking and cyclizing the product.

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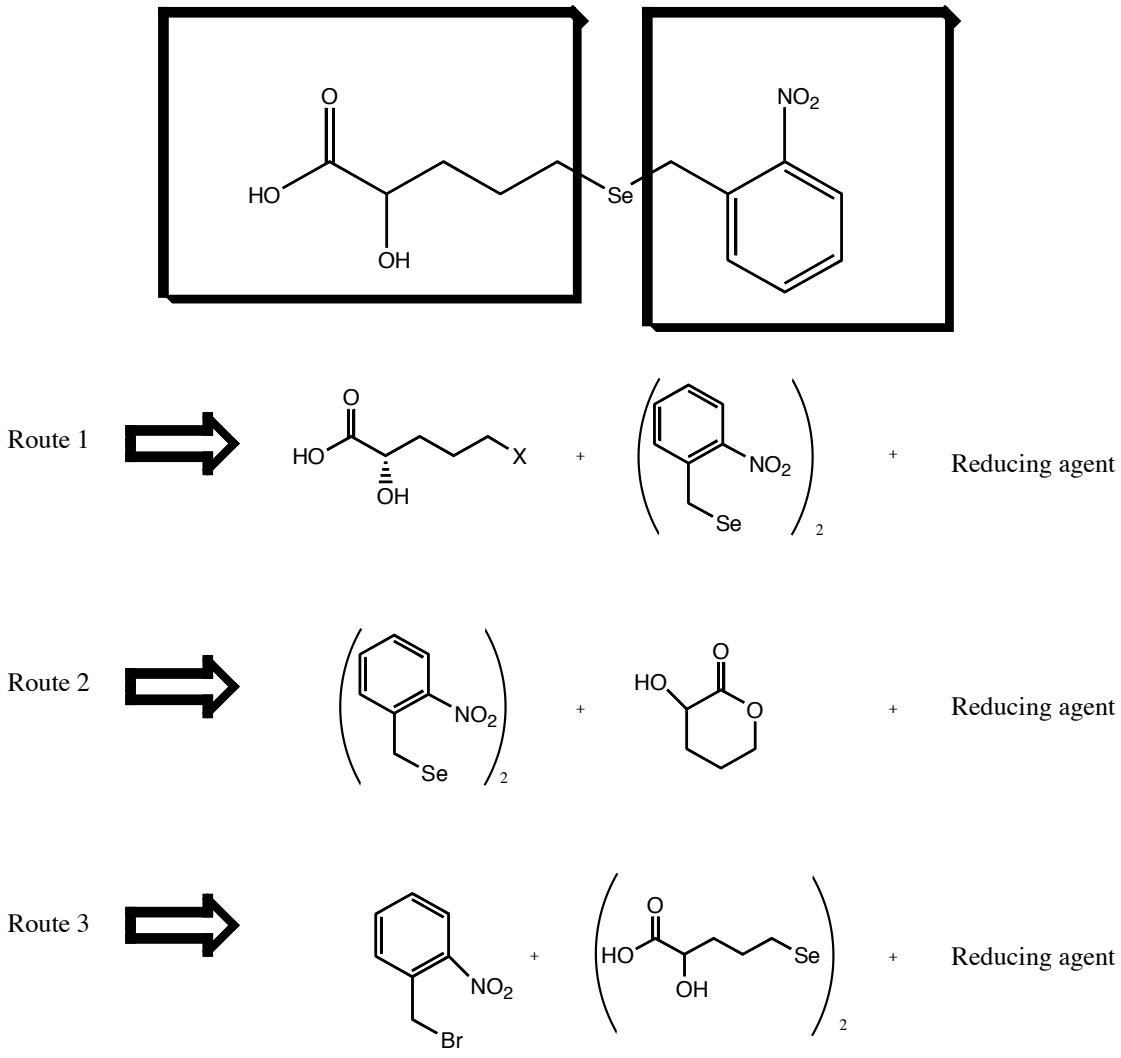
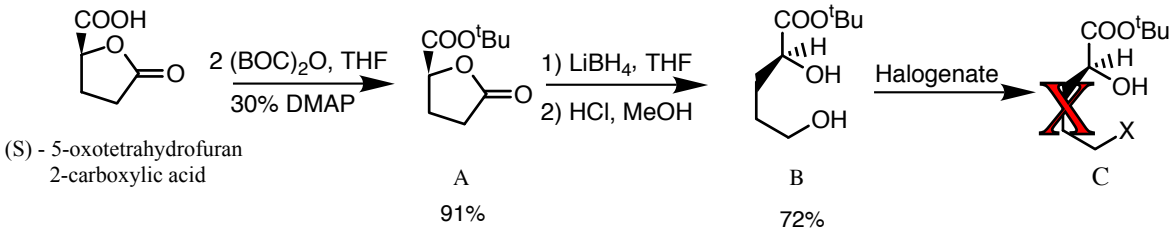

Route 1
Route 2
Route 3
Reducing agent
Reducing agent
Reducing agent

Figure A.1

Scheme 1



(S) - 5-oxotetrahydrofuran 2-carboxylic acid

A

91%

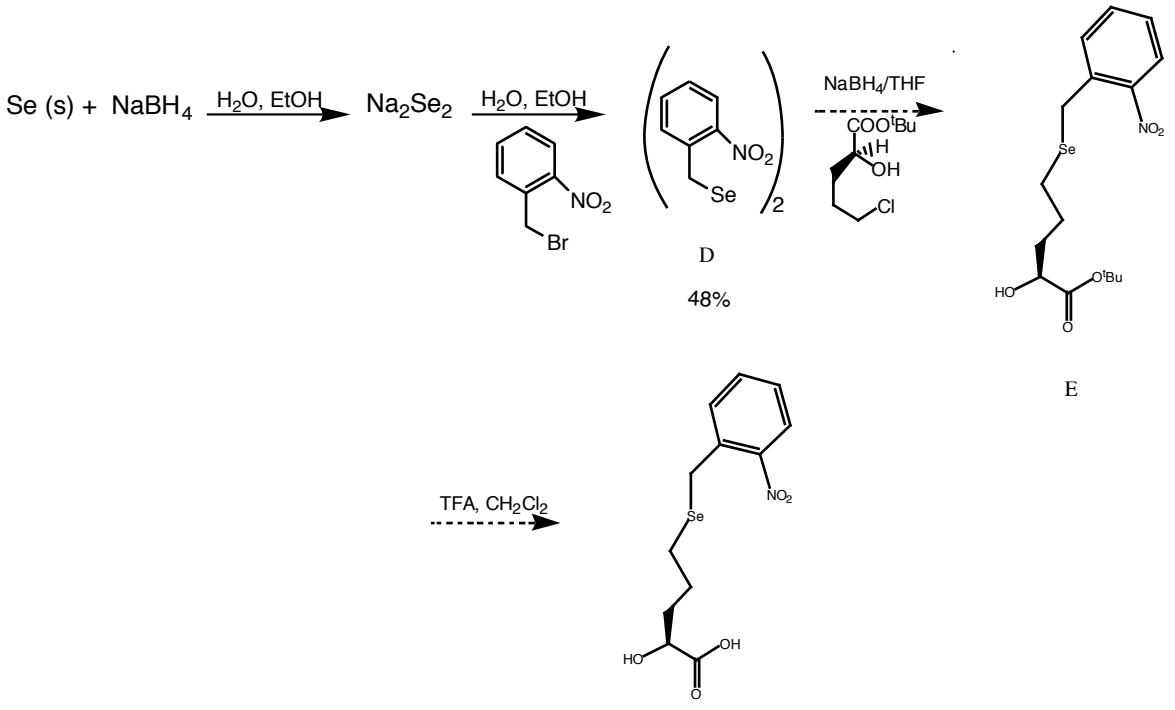
B

72%

C

Halogenation of 1' OH over 2' OH

PPh_3	CCl_4	Pyridine	Different solvents and reagent equivalences used.
PPh_3	CCl_4	Imidazole	Different solvents and reagent equivalences used.
PPh_3	CBr_4		1 equivalence of reagents, solvent THF.



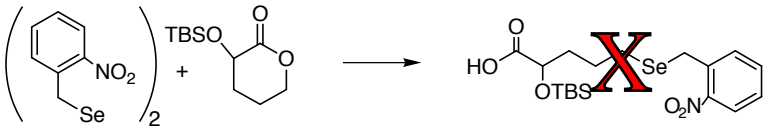
D

48%

E

Figure A.2

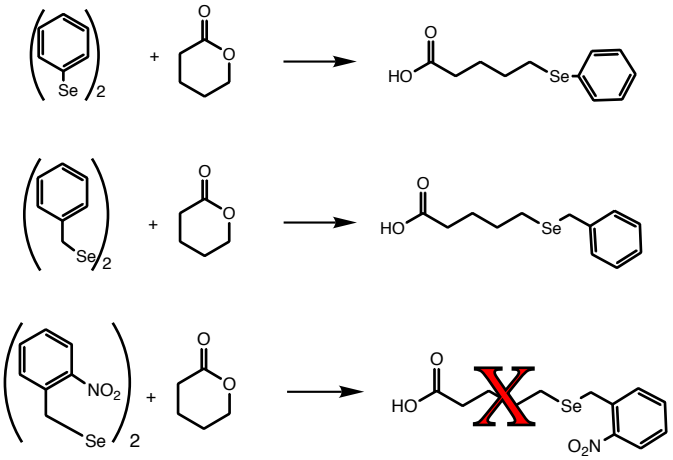
Scheme 2



Reducing Agents	Conditions
1.1 $NaBH_4$	DMF, 100°C 1 hr, RT 7 hours
2 $NaB(OCH_3)_3H$	DMF, heat
2 Na	THF, 2.4 DMPU

None of the above conditions worked !!

Model Compounds



Reducing Agents	Conditions
1.1 $NaBH_4$	DMF, 100°C 1 hr, RT 7 hours
2 Na	THF, 2.4 DMPU

Figure A.3

The di-*o*-nitrobenzyl diselenide was synthesized by the Klayman and Griffin method using disodium diselenide and *o*-nitrobenzyl bromide (18). The reaction went in a good yield and yellow crystals were isolated from the reaction. Due to the problems with the halogenation of product B, a new scheme using di-*o*-nitrobenzyl diselenide was designed (see figure A.3).

Selenium anions have been shown to cleave bulky esters and open lactone rings (1-3, 6, 7, 19). Route 2 was designed with this knowledge in mind. Route 2 involves the reduction of di-o-nitrobenzyl diselenide to generate a selenium anion that attacks the carbinol carbon of the TBS (*tert*-butyl-dimethylsilyl) protected 2-hydroxy-tetrahydro-pyran-2-one (See Scheme 2) (20). Sodium borohydride, the more congested tri-methoxy sodium borohydride, and sodium metal were all used to reduce di-*o*-nitrobenzyl diselenide. All three of these reducing agents have been used in the literature to reduce diselenides (1, 2, 6, 7). It is interesting to note that diselenides are yellow, and when they are reduced to their selenium anion they become colorless. In most of the literature procedures, the production of the selenium anion is monitored by the color change of the solution (4, 5). After the reaction mixture turns from yellow to colorless, the organic halide is added to the reaction. In all of my reduction reactions with di-*o*-nitrobenzyl diselenide, the reaction mixture starts out yellow, but when the reducing agent is added, the solution turns a dark black. I believe that the nitro group on the diselenide is getting reduced upon addition of a reducing agent and that this leads to complete havoc in the reaction (21). The starting diselenide has never been recovered from any of the reduction experiments. Based on these results several model reactions

were done using dibenzyl diselenide and diphenyl diselenide with □-valerlactone (Figure A.3). Both the dibenzyl diselenide and diphenyl diselenide could be reduced using sodium borohydride or sodium metal. The model reactions reveal that it is indeed the nitro moiety on the di-*o*-nitrobenzyl diselenide that is leading to the failure of the reaction.

Because the reduction of the di-*o*-nitrobenzyl diselenide could not be achieved, route 3 was created. This route synthesizes the hydroxy acid diselenide and reacts it with *o*-nitrobenzyl bromide (4, 5). This route leads to the final desired product NBSeOH. The route is discussed in detail in Chapter 2. In addition, many other reactions were tried to synthesize NBSeOH that are not described here but can be found in my lab notebooks.

A.2 Methods:

Reagents were purchased from Aldrich, Sigma, or other commercial sources. All bought chemicals were used without purification except □-valerlactone, which was purified by distillation under vacuum at (bp 58-60ºC/0.5 mm Hg). Anhydrous solvents were purchased from Mallinkrot Baker. Flash chromatography was done on 230-400 mesh silica gel with the solvent indicated. All NMR shifts are reported as □ ppm downfield from TMS. ^{1}H NMR and ^{13}C spectra were recorded using a GE QE-300 MHz spectrometer. Electrospray (ESI) ionization, quadrupole mass spectrometry was performed at the Caltech Protein/Peptide Micro Analytical Laboratory.

5-oxotetrahydrofuran 2-carboxylic acid *tert*-butyl ester (A) – An oven dried 250 ml round bottom was charged with (S) 5-oxotetrahydrofuran 2-carboxylic acid (2 g, 15.4 mmol),

BOC_2O (6.73 g, 30.8 mmol), and DMAP (564.4 mg, 4.62 mmol). The reagents were dissolved in 100 ml of anhydrous THF. The reaction was stirred overnight under argon. Solvent was removed by rotary-evaporation, and a thick yellow oil remained. The crude product was purified using flash chromatography run with a 60:40 ethyl acetate and petroleum ether solvent system. The Rf value for the product was 0.67. Yield: 92% ^1H ($CDCl_3$) □ 4.8 (m, 1 H), 2.51 (m, 4H), 2.3 (m, 2H), 1.49 (s, 9H); ^{13}C ($CDCl_3$) □ 26.274, 27.159, 28.287, 83.420, 176.347; ESI-MS: for $C_9H_{14}O_4$ [M] calculated is 186.1, found $[M+H]^+$ 187.2, and $[M+Na]^+$ 209.2

2,5-dihydroxy pentanoic acid *tert*-butyl ester (B) – Protected lactone (A) was dried under high vacuum for one day before performing the reaction. Drying the protected lactone increases the yield of the reduction reaction. To an oven dried 25 ml round bottom, $LiBH_4$ (122.5 mg, 5.38 mmol) and 5 ml anhydrous ether were added. In a separate round bottom, product A (500 mg, 2.69 mmol) was dissolved in 7 ml anhydrous ether. The dissolved lactone was then added via syringe to the $LiBH_4$. The reaction was heated to 40ºC for 4 hours and the reaction was then allowed to stir overnight at room temperature. The reaction mixture was diluted and quenched with 2 ml of saturated NH_4Cl solution and 5 ml of water at 0ºC. The product was extracted from the aqueous layer with 4 X 15 ml of ether. The organic layers were combined and dried over Na_2SO_4, gravity filtered, and rotary-evaporated. Yield: 55 % ^1H (CD_2Cl_2) □ 1.47 (s, 9H), 1.68 (m, 2H0, 1.72 (m, 2H), 3.62 (m, 2H), 4.1 (m, 1H); ^{13}C (CD_2Cl_2) □ 28.069, 28.620, 31.525, 62.554, 70.745, 82.471, 174; ESI-MS: for $C_9H_{18}O_4$ [M] is 190.1, found $[M+H]^+$ 191.2 and $[M+Na]^+$ 213.2

di-*o*-nitrobenzyl diselenide (D) – In a ventilated chemical fume hood, elemental selenium (1 g, 12.7 mmol) was weighed and added to a 150 ml 3-neck round bottom. Elemental selenium is highly toxic and is easily taken up by the lungs. The round bottom was also charged with sodium borohydride (0.32 g, 8.47 mmol). The round bottom was set up with an air bubbler that bubbled argon through the reaction. It should be noted that the reaction does not have to be kept dry; the argon atmosphere is used to remove any hydrogen selenide gas from the reaction. The hydrogen selenide gas is then trapped in lead (II) acetate saturated solution traps. The lead (II) acetate solutions convert hydrogen selenide gas back into elemental selenium. The round bottom was placed in an ice bath and 50 ml of absolute ethanol was added. The reaction is rather vigorous, care must be taken that argon pressure is strong enough to dissipate the hydrogen selenide gas. After the reaction began to slow down, the round bottom was removed from the ice bath and the reaction was heated. The reaction was allowed to reflux for 1.5 hours. During this time, another 15 ml of ethanol was added to the reaction. The reaction was allowed to cool to room temperature. When the solution finally reached room temperature, *o*-nitrobenzyl bromide (1.83 g, 8.46 mmol) was dissolved in 5 ml of ethanol and then added to the reaction via syringe. The reaction was allowed to stir at room temperature for several minutes. The reaction was then heated to 60°C using an oil bath for 4.5 hours. The reaction was then allowed to cool to room temperature and nitrogen was purged into the solution for another two hours. The reaction was then quenched using 4 ml of glacial acetic acid and stirred for 15 minutes. The reaction mixture was then transferred to a 300 ml round bottom and the solvent removed using rotary-evaporation. The product was

dissolved in hot chloroform, filtered, and the solvent was removed using rotary-evaporation. The crude product was then re-dissolved in hot chloroform, filtered, and the solvent removed using rotary-evaporation. A dark yellow solid was isolated. The product was re-crystallized using a 95:5 hexane to chloroform solution. Yellow crystals were isolated. Yield: 48 %, ^{1}H ($CDCl_3$) 4.2 (s, 4H), 7.27 (m, 2H), 7.43 (2H), 7.56 (2H), 8.07 (2H) ^{13}C ($CDCl_3$) 32.8, 125.9, 128.5, 132.25, 133.7, 136.8. Elemental analysis: theoretical 39.09 %C, 2.81 %H, 6.51 %N, 36.71 %Se; found 38.62 %C, 2.78 %H, 6.19 %N, 43.70 Se%. ESI-MS (High resolution): for $C_{14}H_{12}N_2O_4{}^{76}Se^{78}Se$ 427.9154, $C_{14}H_{12}N_2O_4{}^{78}Se^{86}Se$ 429.9135, $C_{14}H_{12}N_2O_4{}^{86}Se_2$ 431.9127; found 427.9178, 429.9117, 431.9125.

3-(*tert*-butyl-dimethylsilyl)hydroxytetrahydropyran-2-one – 3-hydroxy-tetrahydro-pyran-2-one (606 mg, 5.22 mmol), whose synthesis is described in Chapter 2 Methods section, was dissolved in 5.22 ml of anhydrous methylene chloride. 2,6-lutidiene (1.22 ml, 10.44 mmol) was added to the reaction via syringe. The reaction was then cooled to 0ºC and TBS-triflate (1.8 ml, 7.83 mmol) was added via syringe. The reaction was stirred for 48 hours at room temperature. The reaction was then diluted with 30 ml of CH_2Cl_2 and 15 ml of water. The product was extracted out of the aqueous layer with 3 X 20 ml of CH_2Cl_2. The organic fractions were combined, dried over $NaSO_4$, and decanted into a round bottom flask. The solvent was removed using rotary-evaporation. The crude product was then purified using a Kugelrohr. Yield: 80% ^{1}H (CD_2Cl_2) 4.38 (m, 1H), 3.89 (m, 2H), 2.21 (m, 1H), 1.89 (m, 3H), 0.93 (s, 9H), 0.26 (s, 6 H) ^{13}C (CD_2Cl_2) 173.82, 77.87, 69.57, 30.83, 25.83, 25.73, 18.13, -4.47

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