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Toward Pyridine-Fused Selenium-Containing Antioxidants

Tahli Fenner and Carl H. Schiesser*

School of Chemistry, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria, Australia, 3010. Tel. (+61)-3-8344-4180, Fax (+61)-3-9347-5180.

* Author to whom correspondence should be addressed; e-mail: carlhs@unimelb.edu.au.

Abstract: Photolysis of the thiohydroximate ester derivative **21** of 2-carboethoxy-2-(2-(benzylseleno)pyridin-3-yl)tridecylcarboxylic acid (**20**) affords 2-dodecyl-2-carboethoxy-2,3-dihydroselenolo[2,3-b]pyridine (**22**) in 89% yield in a process presumably involving intramolecular homolytic substitution by a tertiary alkyl radical at selenium with loss of a benzyl radical. Work toward extending this methodology to the preparation of pyridine-fused selenium analogues of antioxidants is described.

Keywords: Free radical, homolytic substitution, selenium, antioxidants.

Introduction

Interest in selenium containing therapeutics has grown over the last thirty years [1]. Simple organoselenium compounds have been prepared, such as selenazolopyrimidone (**1**), that show antitumor activity against mouse leukemia [2]. (Aminoethyl)phenylselenide (**2**) shows excellent antihypertensive activity and selenazine derivatives such as **3** show both antibacterial and antitumor activity [3]. Despite this, the major therapeutic benefit that selenium currently offers appears to be in the form of dietary supplements [4]. Selenium is an essential trace element and dietary deficiency can lead to ailments including gum disease, as well as debilitating conditions such as Keshan's disease [4e]. The biochemistry and pharmacology of selenium is of intense current interest. Selenium is now know to be intimately involved in the activity of enzymes such as glutathione peroxidase and thioredoxin reductase, that catalyse chemistry essential to the protection of biomolecules against oxidative stress and free radical damage [5].

1   **2**   **3**
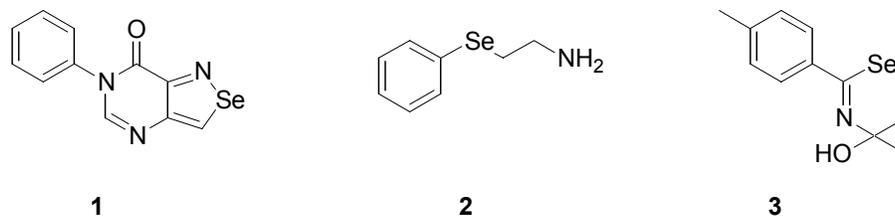
Reactive oxygen species (ROS) are a byproduct of normal aerobic metabolism [6]. Superoxide is formed when electrons leaked from the electron transport chain react with molecular oxygen and is also the product of some enzymatic processes [6b,7]. Superoxide dismutase converts superoxide to hydrogen peroxide, which can, in turn, lead to the formation of hydroxyl and lipid peroxyl radicals [6b,7]. Hydroxyl radicals are extremely reactive and can cause damage to important biomolecules that include DNA, while lipid peroxidation has been implicated in diseases such as atherosclerosis [6a,8]. Reactive oxygen species do have some positive roles in biology, such as providing defense against infection [6a,9], however, excessive concentrations of ROS can lead oxidative stress [6a,9]. Nature has evolved clever methods for controlling ROS. Preventative antioxidant enzymes such as glutathione peroxidase remove ROS through redox cycling [5], while chain breaking antioxidants such as *Vitamin E*, interrupt the chain reactions responsible for the formation of radical species [10]. It should be noted that *Vitamin E* refers to a family of related compounds known as *tocopherols* of which α-tocopherol (**4**) is most potent [10b].

4
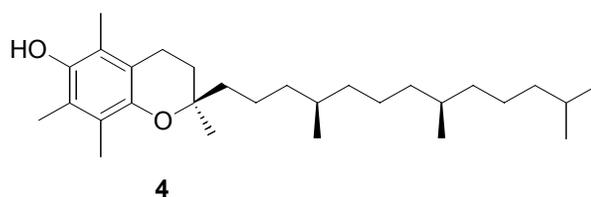
In recent years there has been much interest in developing new, more potent antioxidants such as Ebselen (**5**), that also acts as a glutathione peroxidase mimic and is due to be released for human use as a non-steroidal anti-inflammatory [11]. Water-soluble Vitamin E analogues that have been prepared include Trolox (**6**) [12], while our group has used intramolecular homolytic substitution chemistry to prepare a selenium containing analogue **7** of Vitamin E that possesses dual mode of action [13].

5   **6**   **7**
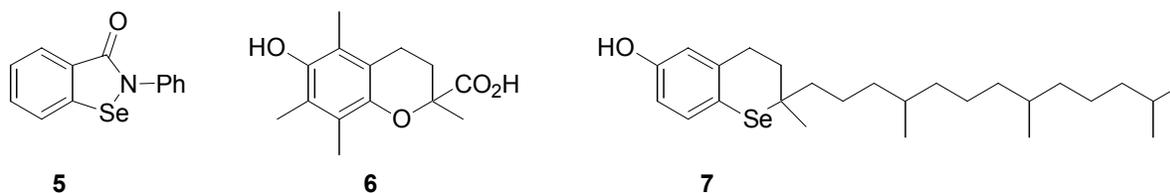
Work in our laboratories has also been directed at the development of water-soluble, dual acting, tocopherol/ebselen hybrids such as **8** and to pyridine-fused tocopherol systems such as **9**. Our recent efforts towards the synthesis of the latter system are described below.

8   **9**
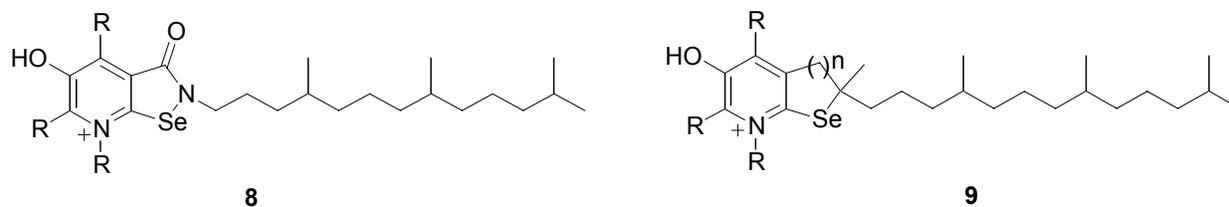
Results and Discussion

Preliminary investigations began with the attempted preparation of model compound **10** that contained many of the salient feature of structures **8** and **9**. To that end ethyl 2-chloronicotinate (**11**) was reacted with sodium benzylselenoate, generated *in situ* by reduction of dibenzyl diselenide with sodium borohydride, to give ethyl 2-(benzylseleno)nicotinate (**12**) in 64% yield (Scheme 1).

Scheme 1.
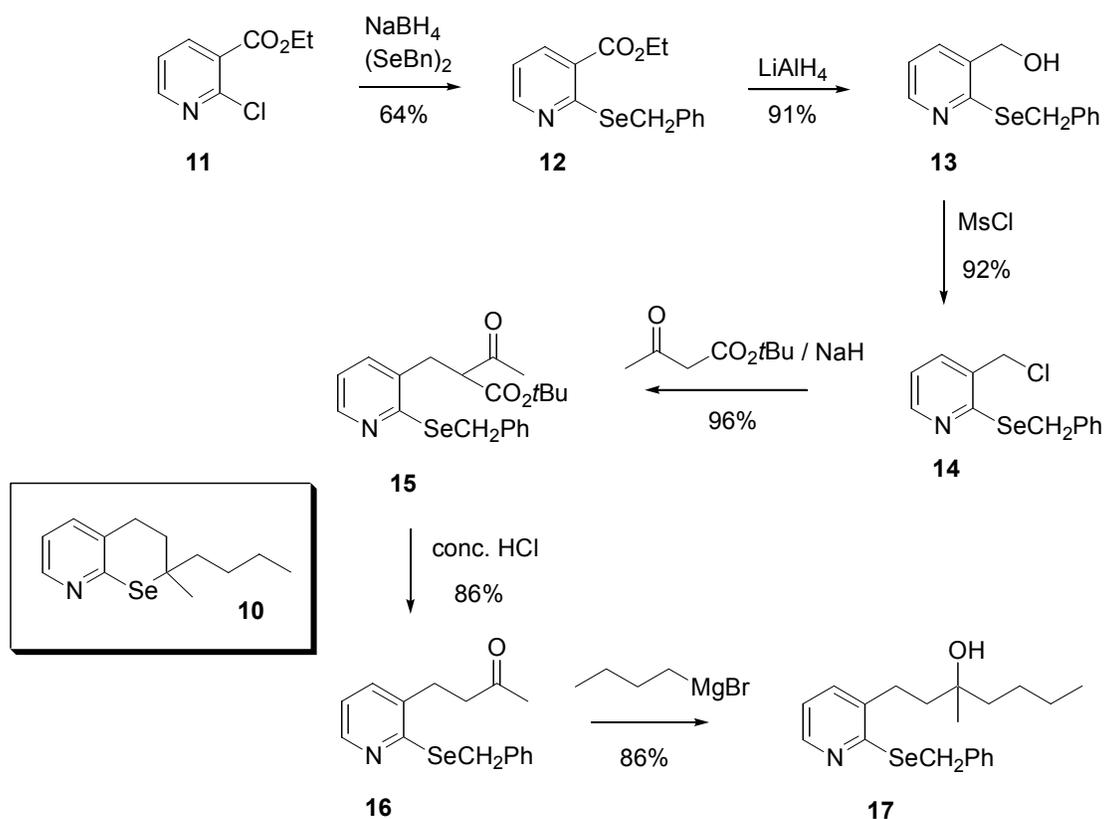
Further reduction with lithium aluminium hydride afforded alcohol **13** in 91% yield. It is interesting to note that the alternative sequence of reactions, namely reduction of the ester **11** followed by treatment with sodium benzylselenoate proceeded only very poorly. We attribute this observation to the electrophilicity of the pyridine ring in the various substrates, specifically that the electron-withdrawing ester substituent is able to increase ring reactivity in a manner that the alcohol can not. The alcohol **13** was further reacted with methanesulfonyl chloride to give the chloride **14** in 92% yield, which was subsequently reacted with *t*-butyl acetoacetate and base to

give the ketoester **15** following the procedure developed by Malmström [13]. Treatment of **15** with concentrated hydrochloric afforded the required ketone **16** in excellent overall yield. Finally, reaction of **16** with n-butylmagnesium bromide gave the desired tertiary alcohol **17** in 86% yield (Scheme 1).

Following our previously published work, we expected that alcohol **17** could be converted into the PTOC oxalate ester, originally described by Barton and Crich as a suitable precursor for the generation of carbon-centred radicals from tertiary alcohols. To our surprise, reaction of **17** with oxalyl chloride , followed by sodium omadine®, afforded none of the desired cyclized product **10**, despite the reaction being carried out under a number of different conditions. ^{1}H-NMR spectroscopy of the only identified product suggested strongly that elimination has occurred to afford a mixture of isomeric alkenes (Scheme 2).

Scheme 2.

Given the problems associated with the methodology described above, it became apparent that an alternative method for the generation of the required radical was needed. Following a modification of the procedure described in Scheme 1, chloride **14** was reacted with *t*-butyl ethyl malonate to afford selenide **18** in moderate (54%) yield (Scheme 3).

Scheme 3.

Further treatment with sodium hydride and 1-bromododecane provided diester **19** in 72% yield, which was selectively deprotected by the action of trifluoroacetic acid to afford 2-carboethoxy-2-(2-(benzylseleno)pyridin-3-yl)tridecylcarboxylic acid (**20**), suitable for conversion into a radical precursor.

Kim recently described a new method for generating carbon-centred radicals from thiohydroximate esters of carboxylic acids and we have generally found this precursor to be superior to those described by Barton and coworkers [14] for reasons including stability, especially in tertiary systems [15]. Therefore, reaction of N-methyl-hydroxydithiocarbamate and dicyclohexylcarbodiimide (DCC) with **20** afforded precursor **21** which was isolated as a yellow oil after column chromatography. Photolysis of **21** in heptane afforded the target 2-dodecyl-2-carboethoxy-2,3-dihydroselenolo[2,3-b]pyridine (**22**) in 89% yield. The dihydroselenolo[2,3-b]-pyridine **22** displayed a ^{77}Se-NMR signal at δ 547.6, characteristic of the structurally related selenium heterocycles [16]. Having successfully demonstrated that tertiary carbon-centred radicals, such as **23** are capable of undergoing intramolecular homolytic substitution to afford dihydroselenolo[2,3-b]pyridines such as **22**, we next turned our attention to the preparation of a precursor for the synthesis of a six-membered ring such as that found in the initial target compound **10**.

23
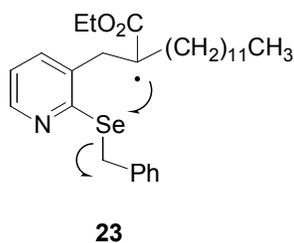
To that end, 2-chloronicotinaldehyde (**25**) was added to a solution of ethyl propiolate and lithium hexamethyldisilazine in THF at –78°C (Scheme 4).

Scheme 4.
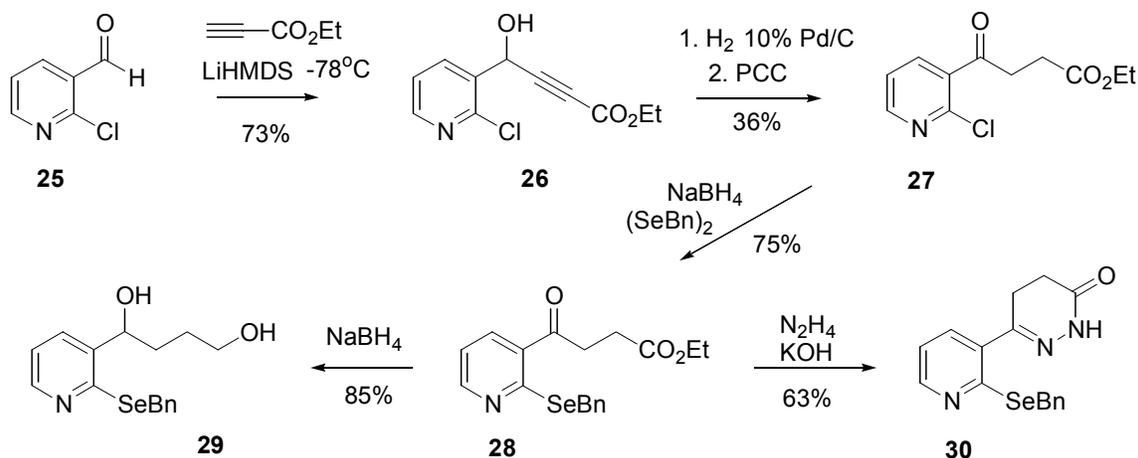
After stirring at –78°C for 4 hours, the reaction was quenched using saturated ammonium chloride solution to give the acetylinic alcohol **26** in 73% yield after purification. It is important that the reaction mixture is quenched at low temperature, as significantly lower yields were obtained when the mixture was allowed to warm addition of the ammonium chloride solution. Hydrogenolysis of alcohol **26** followed by PCC oxidation afforded ketone **27** in poor (36%) yield. Significant amounts of by-product appeared to accompany this transformation. Despite this, ketone (**27** was further reacted with sodium benzylselenoate, generated as described above, to afford ethyl 3-oxo-3-(2-(benzylseleno)pyridin-3-yl)butyrate (**28**) in acceptable yield.

With **28** in hand, the next task was the removal of the ketone functional group. We envisaged that Wolff-Kischner or Clemenson techniques would result in the required compound. Unfortunately, the action of zinc-mercury amalgam (Clemenson reduction) returned only **28**, while reaction with alkaline hydrazine (Wolff-Kischner reduction) afforded a single product that was isolated in 63% yield and that was eventually assigned to be pyradizinone **30**. The formation of **30** can be rationalized by the nucleophilic capture of the initial ketone/hydrazine adduct by the proximate ester moiety, with subsequent dehydration.

28   **31**
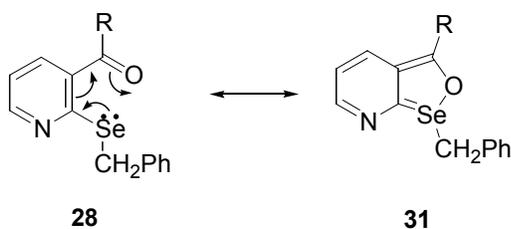
Ketone **28** proved to surprisingly difficult to reduce using standard techniques. Treatment with a large excess of sodium borohydride at room temperature resulted in no reduction, however in refluxing ethanol, clean conversion to the diol **29** was observed after several hours. Single crystal x-ray analysis of **28** provided interesting structural information that may help in our understanding of the relative inertness of the ketone moiety in **28** to reduction (Figure 1).

Figure 1 Perspective diagram of **28**
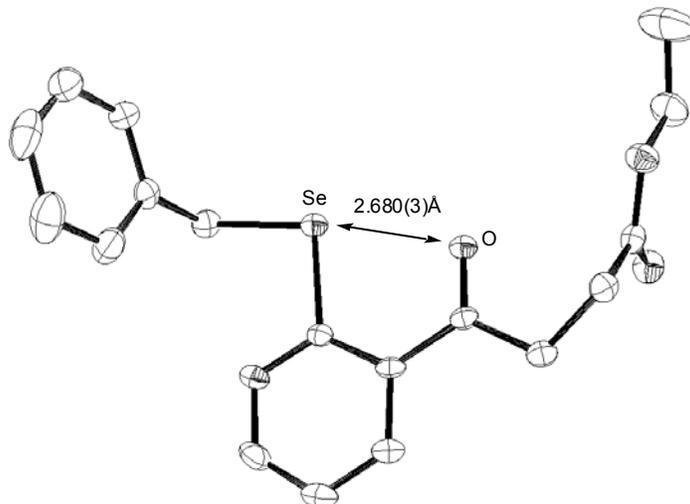
There appears to be a remarkably short oxygen-selenium separation in 28, which at 2.68Å is suggestive of significant contribution of resonance structure **31** to the overall bonding in **28**. Indeed, the O–Se separation in **28** is, to the best of our knowledge, the shortest such interaction reported, being some 0.04Å shorter than the previous "record" of 2.72Å reported by us recently [17].

Conclusions

We have presented work toward the preparation of novel pyridine-fused, selenium-containing antioxidants and anti-inflammatory agents and have demonstrated that, in a model compound, intramolecular homolytic substitution is an effective method for the preparation of these ring systems. The unusual reactivity of ethyl 3-oxo-3-(2-(benzylseleno)pyridin-3-yl)butyrate (**28**) is attributed to strong non-bonded O–Se interactions that are supported by x-ray analysis

Acknowledgements

References and Notes

1. (a) May, S. W. *Exp. Opin. Invest. Drugs* **1999**, *8*, 1017; (b) May, S.W. *Exp. Opin. Invest. Drugs* **2002**, *11*, 1261; (c) Mugesh, G.; du Mont, W.W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125.
2. Ito, H.; Wang, J.Z.; Shimura, K.; Sakakihara, J.; Ueda, T.; *Anticancer Res*. **1990**, *10*, 891.
3. (a) Pollock, S.H.; Herman, H.H.; Fowler, L.C., Edwards, A.S.; Evans, C.O.; May, S.W. *J. Pharm. Exp. Ther*. **1988**, *246*, 227; (b) Koketsu, M.; Ishihara, H.; Hatsu, M. *Res. Commun. Mol. Path. Pharmacol.* **1998**, *101*, 179; (c) Koketsu, M.; Ishihara, H.; Wu, W.; Koji, M.; Saiki, I. *Eur. J. Pharm. Sci.* **1999**, *9*, 157.
4. (a) Arthur, J. R.; McKenzie, R. C.; Beckett, G. J. *J. Nutr.* **2003**, *133*, 1457S; (b) Beck, M. A.; Levander, O. A.; Handy, J. *J. Nutr.* **2003**, *133*, 1463S; (c) Jackson, M. J.; Broome, C. S.; Francis, M. *J. Nutr.* **2003**, *133*, 1557S; (d) Neve, J. *Curr. Opin. Clin. Nutr.* **2002**, *5*, 659. (e) Rayman, M. P. *The Lancet* **2000**, *356*, 233; (f) Rayman, M. P. *Proc. Nutr. Soc.* **2002**, *61*, 203.
5. (a) Arteel, G. E.; Sies, H. *Environ. Toxicol. Pharmacol.* **2001**, *10*, 153; (b) Birringer, M.; Pilawa, S.; Flohe, L. *Nat. Prod. Rep.* **2002**, *19*, 693; (c) Nordberg, J.; Arner, E. S. J. *Free Rad. Biol. Med.* **2001**, *31*, 1287; (d) Gamble, S.C.; Wiseman, A. Goldfarb, P.S. *J. Chem. Tech. Biotechnol.* **1997**, *68*, 123; (e) Ursini, F.; Bindoli, A. *Chem. Phys. Lipids.* **1987**, *44*, 255.
6. (a) Droge, W. *Physiol. Rev.* **2002**, *82*, 47-95; (b) Fridovich, I. *Science*. **1978**, *201*, 875.
7. Deby, C.; Goultier, R. *Biochem. Biopharm.* **1990**, *39*, 3, 399.
8. (a) Ames, B.N.; Shigenaga, M.K.; Hagen, T.M. *Proc. Natl. Acad. Sci. USA*. **1993**, *90*, 7915; (b) Alexander, R. W. *Hypertension.* **1995**, *25*, 155; (c) Niki, E. *Chem. Phys. Lipids.* **1987**, *44*, 227.
9. Nordberg, J.; Arner, E. S. J. *Free Rad. Biol. Med.* **2001**, *31*, 1287.

10. (a) Niki, E.; *Chem. Phys. Lipids.* **1987**, *44*, 227; (b) Burton, G.W.; Ingold, K.U. *Acc. Chem. Res.* **1986**, *19*, 194; (c) Burton, G.W.; Doba, T.; Gabe, E.J.; Hughes, L.; Lee, F.L.; Prasad, L.; Ingold, K.U. *J. Am. Chem. Soc.* **1985**, *107*, 7053.

11. (a) Mugesh, G.; Singh, H. B. *Chem. Soc. Rev.* **2000**, *29*, 347; (b) Schewe, T. *Gen. Pharmac.* **1995**, *26*, 1153; (c) Sies, H.; Masumoto, H. *Adv. Pharmacol.* **1997**, *38*, 229; (d) Nakamura, Y.; Feng, Q.; Kumagai, T.; Ohigashi, H.; Osawa, T.; Noguchi, N.; Niki, E.; Uchida, K. *J. Biol. Chem.* **2002**, *277*, 2687.

12. Sagach, V.F.; Scrosati, M.; Fielding, J.; Rossoni, G.; Galli, C.; Visioli, F. *Pharmacol. Res.* **2002**, *45*, 435; (b) Forrest, V.; Kang, Y.; McCain, D.E.; Robinson, D.H.; Ramakrisgnan, N. *Free Rad. Biol. Med.* **1994**, 675.

13. Al-Maharik, N.; Engman, L.; Malmström, J.; Schiesser, C.H. *J. Org. Chem.* **2001**, *66*, 6286.

14. Barton, D. H. R.; Crich, D.; Motherwell, W. B., *Tetrahedron*, **1985**, *41*, 3901.

15. Kim, S.; Lim, C.; Song, S.; Kang, H. *Chem. Commun.* **2001**, 1410.

16. Duddeck, H., *Prog. NMR. Spec.*, **1995**, *27*, 1.

17. Carland, M. W.; Schiesser, C. H.; White, J. M, *Aust. J. Chem.*, **2004**. *57*, 97; See also: Gable, R. W.; Laws, M. J.; Schiesser, C. H., *Acta Cryst.* **1997**, *53*, 641.