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Novel selenium containing non-detergent sulphobetaines

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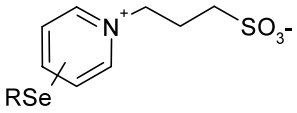
Abstract—The first selenium containing non-detergent sulphobetaines are described. They have been prepared in multi-gram quantities from readily available pyridyl precursors. Incorporation of selenium was achieved using nucleophilic organoselenide anions. The resulting products were shown by ^{77}Se NMR spectroscopy to be homogeneous with respect to selenium oxidation state.

Non-detergent sulphobetaines (NDSB's) are small bipolar compounds containing two oppositely charged polar head groups linked by a short (usually 3 carbon) hydrophobic chain. They are readily prepared from tertiary amines (both aliphatic and aromatic) and have found use as additives in protein X-ray crystallographic experiments. As well as being effective in the extraction,[1] solubilization[2] and renaturation[3] of proteins, NDSB's have also been shown to enhance both the size and the rate of growth[4] of protein crystals for use in structural studies.

As part of a research program to develop strategies towards high-throughput X-ray crystallography we were interested in preparing novel NDSB's containing functionalities that might facilitate rapid structure determination; in particular we sought to introduce selenium into NDSB's. Selenium, as an anomalous scatterer of X-rays, is very commonly utilized in protein X-ray crystallography in the form of selenomethionine (SeMet), where it is used as an isosteric replacement for the naturally occuring sulphur containing amino acid methionine (Met). Incorporation of SeMet into proteins allows for their structures to be determined using the multi-wavelength anomalous dispersion[5] (MAD) technique. Multi-wavelength anomalous solvent-contrast (MASC) has also been described[6] and uses anomalous scatterers in the solvent envelope of the protein to allow structure determination.

Herein we report the first synthesis of selenium containing non-detergent sulphobetaines **1**–**3** (Se-NDSB's) containing either one or two selenium atoms (Fig. 1). Short syntheses from cheap and readily available commercial precursors have been developed and allow for the rapid synthesis of multi-gram quantities of these new materials. ^{77}Se NMR spectroscopic analysis has been used to demonstrate that introduction of selenium can be effected essentially homogenously with respect to selenium oxidation state (selenide level).

Due to their commercial availability with a wide ranging pattern of substitution we chose to prepare pyridyl-derived NDSB's. Selenium,[7,8] in common with its fellow chalcogens, has a very rich and varied chemistry and can be readily incorporated into organic molecules in an electrophilic, nucleophilic or radical sense. We envisaged that addition of selenium via the use of common electrophilic reagents (e.g. PhSeBr) would be unlikely to succeed due to the inherent nucleophilic nature of pyridines whereas radical approaches (using tin reagents) were felt to be undesirable for large scale synthesis.



1 4-SePh
2 3,5-di-SePh
3 4-SeMe

Figure 1. Selenium containing non-detergent sulphobetaines.

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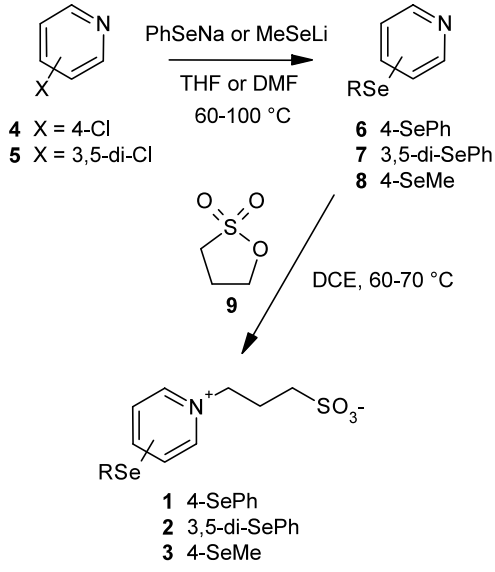
In contrast, nucleophilic organoselenide anions are easily prepared and handled on a large scale by in situ metal hydride reduction (e.g. NaH)[9] of organic diselenides or by the action of organometallics (e.g. alkyl lithiums)[10] on elemental selenium. In our hands, the synthesis of seleno-NDSB's proved to be possible using either of these methods (Scheme 1) but for large scale work the organometallic route proved to be the method of choice.

A bright yellow solution of diphenyl diselenide in dimethylformamide was reduced with an excess of sodium hydride under an argon atmosphere. After 1 h at 60°C the solution had decolourized, indicative of the formation of phenylselenide anion. 4-Chloropyridine **4**[11] was added and the mixture held at 80°C for a further 4 h. Workup and chromatographic purification on silica afforded the desired pyridyl selenide **6**[12] (50–60%) which reacted smoothly with one equivalent of 1,3-propane sultone **9** in hot 1,2-dichloroethane (DCE) to afford the desired 4-phenylselenyl NDSB **1**[13] in high yield (88%). Purification of **1** was simple due to its poor solubility in DCE; upon cooling of the reaction mixture the pure product was collected by suction filtration.

The same procedure was also used in an attempt to effect the incorporation of two selenium atoms into the pyridine nucleus. Thus 3,5-dichloropyridine **5** reacted with phenylselenide under identical conditions to afford the bis-selenyl pyridine **7**[14] after chromatographic purification albeit in low yield (35%). The poor yield in this case is thought to reflect both the reduced probability of two sequential S_NAr processes and also the fact that such substitutions in pyridyl systems are more favorable when the halide leaving group is located either *ortho*- or *para*- to the ring nitrogen. Nonetheless, bis-selenyl pyridine **7** could be prepared in gram quantities and reacted smoothly with sultone **9** to afford high yields (80–85%) of 3,5-di-phenylselenyl NDSB **2**.[15]



Scheme 1.

Our initial observations regarding the aqueous solubilites of Se-NDSB's **1** and **2** were disappointing. Unfortunately neither **1** nor **2** proved soluble enough to prepare solutions concentrated enough[16] for them to be applicable for protein solubilization or renaturation studies; poor solubility was particularly notable in the case of the bis-selenide **2** which was only very sparingly water soluble. These findings were in stark contrast with the analogous 4-benzyl NDSB **10**[17,18] (4-benzylpyridine, 1,3-propane sultone, DCE, 60°C; 95%), the 4-*tert*-butyl NDSB **11**[19] (4-*tert*-butylpyridine, 1,3-propane sultone, DCE, 60°C; 98%) and the commercially available[20] benzyldimethylamine derivative **12** (NDSB256) all of which have high aqueous solubility (Fig. 2).

Given that the introduction of a lipophilic selenium atom in conjunction with a phenyl moiety appeared to result in poor aqueous solubility we turned our attention to the introduction of smaller, less lipophilic substituents. We envisaged that the methylselenide anion would have much less of a detrimental effect and were also attracted by the higher level of nucleophilicity that alkyl selenide anions appeared to possess when compared with their aryl equivalents (where the anion is extensively resonance stabilized).

We thus treated a suspension of elemental selenium in anhydrous tetrahydrofuran under argon with an ethereal solution of methyl lithium at room temperature. The selenium was rapidly consumed to afford a white suspension of MeSeLi. Upon reaction with **4** at reflux, workup and chromatographic purification, the desired pyridine **8** was isolated in good yield (65–75%). Moreover, the process was amenable to large scale synthesis; multi-gram quantities of **8** could be readily prepared by this method. Subsequent treatment of **8** with sultone **9** afforded Se-NDSB **3**[21] in excellent yield (9–10 g scale, 85–90%).

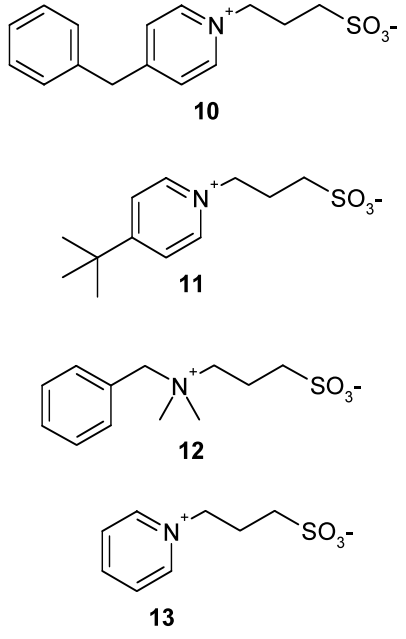


Figure 2.

Gratifyingly, Se-NDSB **3** was appreciably more soluble in water than either **1** or **2**; we were able to prepare aqueous solutions of **3** that were approximately 800 mM, a significant increase in concentration compared to either of the phenylselenyl derivatives. The corresponding pyridyl derived NDSB **13** (NDSB201) has previously been shown[22] to increase protein yields approximately 5-fold upon renaturation when used at similar concentrations.

Se-NDSB's **1**–**3** were characterized extensively by multinuclear NMR spectroscopy (^{1}H, ^{13}C and ^{77}Se) and were shown to be essentially pure by reference to their ^{1}H and ^{13}C NMR spectra. We also paid particular attention to the homogeneity of **1**–**3** with respect to selenium oxidation state and used ^{77}Se NMR spectroscopy to show that no detectable expeditious oxidation had occurred during synthesis or isolation.

^{77}Se NMR shifts for **1**–**3** are shown in Table 1; in all cases only a single selenium signal was observed, indicative of the desired selenium homogeneity. The chemical shifts were in good agreement with literature values[23] for Ar_2Se and ArSeMe species and confirmed selenium to be present at the selenide oxidation level. Notably, the strong mesomeric electronic withdrawing effect felt by substituents either *ortho*- or *para*- to pyridinium nitrogen was evident in the observed selenium chemical shifts for **1** and **3**; both were deshielded by around 100 ppm relative to their phenyl analogues (Ph_2Se δ ~400, PhSeMe δ ~200). This effect was far less pronounced (~35 ppm downfield shift) in the *meta*- substituted derivative **2**; shifts of this level are similar to those induced by the strongly electron withdrawing but electronically neutral *p*-nitrophenyl moiety.

Whilst the majority of MAD studies using SeMet substituted proteins are performed under mildly reducing conditions, recent studies[24] have shown the anomalous signal from oxidized selenium to be much stronger. Though the precise selenium oxidation level appears to be unimportant in MAD experiments, the homogeneity of selenium appears to be crucial. In samples where heterogeneity is present structure determination proves impossible as the selenium signal is 'smeared' out over several wavelengths and its intensity is thus greatly reduced. The structure of the SeMet substituted bacterial membrane protein TolC has been reported recently[25] but its structure could only be solved after very brief oxidative treatment with hydrogen peroxide which ensured the necessary selenium homogeneity.

Table 1. Selenium NMR data for Se-NDSBs

Se-NDSB	δ ^{77}Se[a]	Solvent
1	492[b]	D_2O+$(CD_3)_2CO$
2	446	CD_3CN
3	327[b]	D_2O+$(CD_3)_2CO$

[a] Chemical shifts are quoted relative to Me_2Se (δ 0.0).

[b] $(CD_3)_2CO$ added as co-solvent due to immiscibility of Me_2Se and D_2O.

In summary, we have prepared the first selenium containing non-detergent sulphobetaines in multi-gram quantities from readily available pyridyl precursors. Incorporation of selenium was readily achieved using nucleophilic aryl or alkylselenide anions prepared in situ from the corresponding aryl diselenides or elemental selenium respectively. The resulting Se-NDSB's were shown by ^{77}Se NMR spectroscopy to be homogeneous with respect to selenium oxidation state making them potentially useful as additives in protein structure determination experiments using the MAD or MASC techniques.

Acknowledgements

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13. NMR data for **1**: δ_H (250 MHz, D_2O) 8.29 (2H, d, *J* 7 Hz, H-2 and H-6), 7.74–7.60 (4H, m, H-3, H-5 and *m*-Ph), 7.60–7.48 (3H, m, *o*-Ph and *p*-Ph), 4.50 (2H, t, *J* 7.5 Hz, NCH_2), 2.88 (2H, t, *J* 7.5 Hz, SCH_2), 2.31 (2H, quintet, *J* 7.5 Hz, $NCH_2CH_2CH_2S$); δ_C (100 MHz, D_2O) 163.3, 141.7, 136.6, 131.0, 130.8, 126.2, 123.7, 58.5, 47.0 and 25.9; δ_{Se} (76.3 MHz, D_2O+d_6-Me_2CO) 492.
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15. NMR data for **2**: δ_H (250 MHz, CD_3CN) 8.60 (2H, s, H-2 and H-6), 7.54–7.39 (7H, H-4, *m*-Ph and *p*-Ph), 7.39–7.28 (4H, m, *o*-Ph), 4.55 (2H, t, *J* 7.5 Hz, NCH_2), 2.74 (2H, t, *J* 7.5 Hz, SCH_2) and 2.30 (2H, quintet, *J* 7.5 Hz, $NCH_2CH_2CH_2S$); δ_C (100 MHz, CD_3CN) 145.3, 142.1, 138.0, 136.6, 131.5, 131.2, 125.9, 61.3, 47.8 and 27.7; δ_{Se} (76.3 MHz, CD_3CN) 446.
16. NDSB's are most effective for protein solubilization and renaturation when used at high concentrations, typically 2 M or higher.
17. NMR data for **10**: δ_H (250 MHz, D_2O) 8.60 (2H, d, *J* 7.5 Hz, H-2 and H-6), 7.77 (2H, d, *J* 7.5 Hz, H-3 and H-5), 7.40–7.20 (5H, m, Ph), 4.58 (2H, t, *J* 7 Hz, CH_2N), 4.20 (2H, s, CH_2Ph), 2.87 (2H, t, *J* 7.5 Hz, SCH_2) and 2.32 (2H, quintet, *J* 7.5 Hz, $NCH_2CH_2CH_2S$).
18. NDSB **10** is now commercially available from Raschig GmbH, Mundenheimer Str. 100, D-67061 Ludwigshafen, Germany.
19. NMR data for **11**: δ_H (250 MHz, D_2O) 8.63 (2H, d, *J* 7.5 Hz, H-2 and H-6), 8.00 (2H, d, *J* 7.5 Hz, H-3 and H-5), 4.61 (2H, t, *J* 7 Hz, NCH_2), 2.87 (2H, t, *J* 7 Hz, SCH_2), 2.28 (2H, quintet, *J* 7 Hz, $NCH_2CH_2CH_2S$) and 1.30 (9H, s, ^{t}Bu).
20. NDSB256 is available from Calbiochem®, 10394 Pacific Center Court, San Diego, California, 92039-2087, USA.
21. Synthesis of Se-NDSB **3**. A stirred suspension of selenium powder (4.74 g, 60.0 mmol) in anhydrous tetrahydrofuran (80 ml) under an argon atmosphere was treated dropwise with a solution of methyl lithium in diethyl ether (1.6 M, 43 ml, 68.8 mmol) and the resulting suspension stirred at room temperature under argon for 1 h. 4-Chloropyridine (6.80 g, 59.9 mmol) in anhydrous tetrahydrofuran (10 ml) was added and the mixture stirred and held at reflux for 48 h. Upon cooling the solvent was removed in vacuo and the residual oil partitioned between water (200 ml) and diethyl ether (200 ml). The aqueous layer was extracted with further portions of diethyl ether (2×100 ml), the combined ethereal extracts were dried (Na_2SO_4), filtered and the solvent removed in vacuo. The resulting yellow oil was subjected to flash chromatography on silica. Elution with diethyl ether afforded 4-methylselenylpyridine **8** (7.31 g, 71%) as a pale yellow oil. δ_H (250 MHz, $CDCl_3$) 8.33 (2H, d, *J* 7.5 Hz, H-2 and H-6), 7.22 (2H, d, *J* 7.5 Hz, H-3 and H-5) and 2.45 (3H, s, SMe. 1,3-Propane sultone (4.90 g, 40.16 mmol) in 1,2-dichloroethane (15 ml) was added to a solution of **8** (6.50 g, 37.79 mmol) in 1,2-dichloroethane (35 ml) and the mixture was stirred and held at reflux for 6 h. Upon cooling the precipitate was collected by suction filtration and washed with dichloromethane to afford Se-NDSB **3** (9.54 g, 86%) as a colourless solid. δ_H (250 MHz, D_2O) 8.29 (2H, d, *J* 7.5 Hz, H-2 and H-6), 7.84 (2H, d, *J* 7.5 Hz, H-3 and H-5), 4.46 (2H, t, *J* 7 Hz, NCH_2), 2.84 (2H, t, *J* 7 Hz, SCH_2), 2.46 (3H, s, SMe) and 2.28 (2H, quintet, *J* 7 Hz, $NCH_2CH_2CH_2S$); δ_C (100 MHz, D_2O) 162.1, 140.7, 125.6, 57.9, 46.5, 25.3 and 6.1; δ_{Se} (76.3 MHz, D_2O+d_6-Me_2CO) 327.
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