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Synthesis of Methyl 2-Acetamido-2-deoxy-1-seleno-β-D-gluco- and galacto-pyranoside: Selenium Metabolites in Human Urine

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An efficient synthesis of two methyl 2-acetamido-2-deoxy-1-seleno-β-D-hexopyranosides is reported. The synthesized compounds, which have recently been identified in human urine, will be used in further studies on the metabolism and toxicology of selenium.

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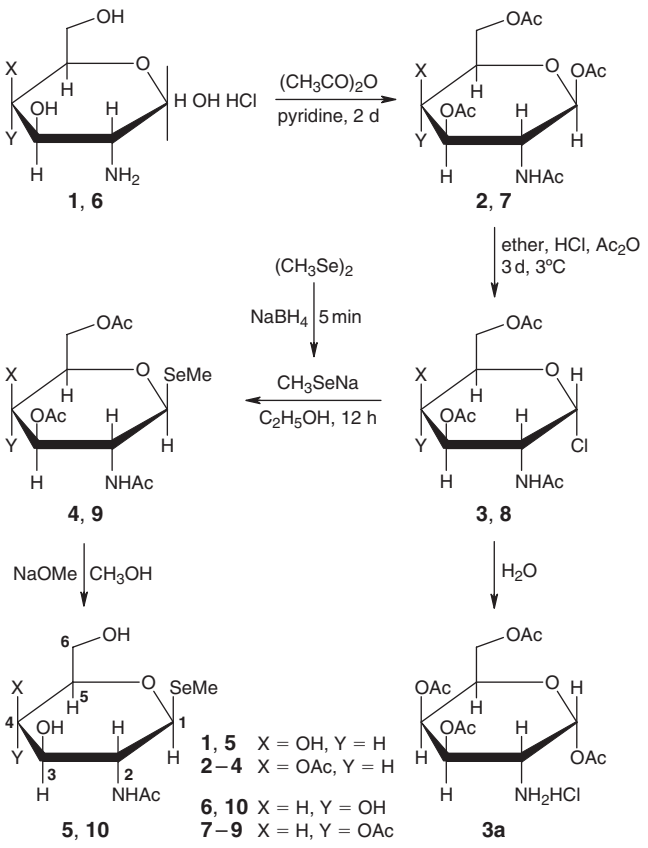
The metalloid selenium is of interest in human health because not only is it an essential trace element but also it can elicit toxic effects at modest intake levels. The threshold for selenium toxicity in man may be as low as 700 μg Se day^{-1}, which is just 10–20-fold of the estimated physiological requirements.[1,2] Despite this narrow window of beneficiality, the public perception of selenium is that of a health promoting agent, and selenium supplements in the form of tablets are widely taken with an apparent disregard to potential problems resulting from overindulgence.



Scheme 1. Reaction scheme for the synthesis of methyl 2-acetamido-2-deoxy-1-seleno-β-D-gluco- and galacto-pyranoside.

Our understanding of the biotransformation processes delineating beneficial and detrimental effects of selenium requires knowledge of the metabolic products, and methods to identify, quantify, and toxicologically assess the selenium metabolites in humans are currently important areas of research. The identification of selenium metabolites in urine has proved difficult and several incorrect structures have been assigned over the last ten years.[3] A breakthrough in this field, however, came in 2002 with the report by Japanese researchers,[4] of a selenium-containing hexosamine (**5**, Scheme 1, termed selenosugar) in rat urine, and the subsequent report by Danish researchers of **5** and its diastereomer **10** as human urinary metabolites.[5] The Japanese group reported the synthesis of both **5** and **10** in a single paragraph, in separate publications,[4,6] but scant experimental details were provided and the compounds were not adequately characterized. A full toxicological assessment of these metabolites awaits the synthesis of pure, well characterized compounds in reasonable quantities. We report details of a simple and efficient synthesis of two selenium metabolites found in human urine. In addition, a crystal structure analysis (Fig. 1) and a ^{77}Se NMR spectrum of the synthetically prepared selenium metabolite **5** are presented.

Although the key intermediates **3** and **8** are commercially available, they are rather expensive and we chose to synthesis them in-house. Thus the hexosamine hydrochlorides **1** and **6** (Scheme 1) were easily converted into the fully protected β-pentaacetates **2** and **7** following the generally accepted acylation method using acetic anhydride in pyridine.[7] The pentaacetate **2** (82% yield) was precipitated by pouring the reaction mixture into ice-cold water, whereas **7** (77%)

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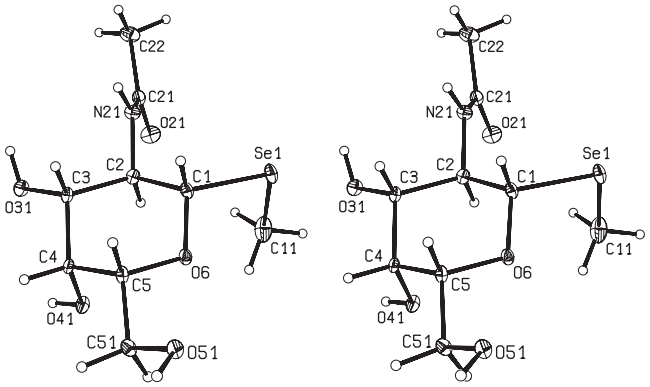

Fig. 1. Stereoscopic ORTEP[13] diagram of **5** showing the atomic numbering scheme. The probability ellipsoids are drawn at the 50% probability level. Selected bond lengths and angles: Se1–C1 1.957(2), Se1–C11 1.951(3), C2–N21 1.448(3), N21–C21 1.351(3), C21–O21 1.240(3) Å; C11–Se1–C1 98.42(11), C21–N21–C2 123.0(2)°.

crystallized directly from the clear reaction solution. To obtain the chlorides **3** and **8** the use of hydrogen chloride in ether was favoured.[8] On our first attempt (beginning with **2**), however, evaporation of the organic layer lead to an oil which on work-up produced the hydrochloride **3a**. This rearrangement has been described previously.[9] Subsequently, we performed the reaction under argon, and the organic layer was evaporated followed by coevaporation several times with chloroform (to remove all traces of acetic acid), giving the chlorides as solids (**3**, 87%; **8**, 89%). These products, pure by NMR spectroscopy, were used without further purification. For the introduction of the selenium at the anomeric centre, we used an alkylselenide ion,[10] generated from dimethyl diselenide by reduction with sodium borohydride, which was then reacted directly in solution to give the methyl tri-*O*-acetyl-2-acetamido-2-deoxy-1-seleno-β-D-hexopyranosides **4** and **9**. Deprotection of residual hydroxyl groups[7] according to Zemplén in basic medium lead to the desired methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside **5** and methyl 2-acetamido-2-deoxy-1-seleno-β-D-glucopyranoside **10**. No purification by column chromatography was needed during the synthesis, and the overall yield was 56% for **5** and 52% for **10**.

The described synthesis has provided quantities of two important selenium metabolites that will be used in our future studies on the biotransformation of selenium in humans.

Experimental

D-Galactosamine hydrochloride was purchased from Lactan/Roth (Graz), D-glucosamine hydrochloride from Lancaster (Frankfurt), dimethyl diselenide from Acros-Organics (Geel), and sodium borohydride (p.a.) was obtained from Merck (Darmstadt). Organic solvents were freshly distilled.

Thin-layer chromatography was performed on Merck TLC plastic sheets of silica gel 60 F_{254} using ethyl acetate as eluent. For visualization of the compounds, a dip solution of vanillin (1.5 g)/H_2SO_4 (20 mL)/water (150 mL)/ethanol (125 mL) was used.

^{1}H, ^{13}C, and ^{77}Se NMR spectra were recorded with Varian (400 and 600 MHz) and Bruker (360 and 500 MHz) instruments. Chemical shifts are given in ppm. Calibration ^{1}H: $CHCl_3$ 7.24 ppm, $(CH_3)_2SO$ 2.49 ppm, SeO_2 1300 ppm; ^{13}C: $CDCl_3$ 77 ppm, $(CH_3)_2SO$ 39.7 ppm.

Electrospray ionization mass spectra (ESIMS) were recorded on an Agilent G1946D single quadrupole mass spectrometer with flow injection using a mixture of water/acetonitrile (4 : 1 v/v) and MeOH as solvents.

Melting points (uncorrected) were recorded on a Gallenkamp melting point apparatus, rotation values on a Perkin Elmer Polarimeter 341, and elemental analysis on an Elemental Analyzer 1108, Fisons Instruments.

X-Ray crystal structure analysis was performed with a modified STOE four-circle diffractometer.

The structures for the well known intermediates **2**, **7**, **3**, and **8**, in addition to the by-product **3a**, were confirmed by NMR spectroscopy (see Accessory Materials).

*Methyl Tri-*O*-acetyl-2-acetamido-2-deoxy-1-seleno-β-D-hexopyranosides **4**, **9***

For the introduction of the methylseleno group we followed in general a known procedure used for glucopyranosides.[10] Thus, a mixture of dimethyl diselenide (470 mg, 2.5 mmol) and sodium borohydride (189 mg, 5 mmol) in dry ethanol (15 mL) under argon was stirred at room temperature until the yellow-orange colour disappeared (5–10 min). The solution was then cooled to 0°C (ice bath) and the chloro sugar **3** or **8** (600 mg, 1.64 mmol) was added. The mixture was allowed to warm to room temperature and stirred for 12 h. Acetic acid (0.5 mL) was added and the mixture was stirred for an additional hour before the solvent was removed by evaporation. The residue was taken up in ethyl acetate (50 mL), the solution washed with water and saturated aqueous sodium bicarbonate solution, and then quickly dried ($MgSO_4$). Evaporation of the organic layer gave the protected methylseleno sugar as a white solid. Recrystallization from chloroform gave fine needles (**4**, 612 mg, 88%; **9**, 584 mg, 84%).

*Compound **4***, mp 205–208°C (dec.; lit.[4] 218–220°C), $[\alpha]_D^{20}$ −10 [*c* 0.7 in $(CH_3)_2SO$] (Found: C 42.6, H 5.7, N 3.1. $C_{15}H_{23}NO_8Se$ requires C 42.5, H 5.4, N 3.3%). δ_H (400 MHz, $CDCl_3$) 5.53 (1H, d, *J* 9.8, NH), 5.36 (1H, d, *J* 2.8, H4), 5.04 (1H, dd, *J* 10.2, 2.8, H3), 4.69 (1H, d, *J* 10, H1), 4.35 (1H, q, *J* 10, H2), 4.14–4.06 (2H, m, H6), 3.94 (1H, t, *J* 6, H5), 2.13 (3H, s, Me of OAc), 2.11 (3H, s, Me of SeMe), 2.01, 1.98 (6H, s, Me of OAc), 1.94 (3H, s, Me of NHAc). δ_C (100 MHz, $CDCl_3$) 170.8–170.3 (CO), 79.0 (C1), 75.5 (C5), 71.2 (C3), 66.9 (C4), 61.6 (C6), 49.7 (C2), 23.3 (Me of NHAc), 20.7 (Me of OAc), 2.7 (Me of SeMe). *m/z* (ESI^+, 10 V) 464 $[M(^{80}Se) + K]^+$, 448 $[M(^{80}Se) + Na]^+$, 426 $[M(^{80}Se) + H]^+$, 330 $[M + H - CH_3SeH]^+$. *m/z* (ESI^-, 10 V) 460 $[M(^{80}Se) + Cl]^-$, 424 $[M(^{80}Se) - H]^-$.

*Compound **9***, mp 192–195°C (dec.), $[\alpha]_D^{20}$ −31 [*c* 1.2 in $(CH_3)_2SO$] (Found: C 42.7, H 5.6, N 3.2. $C_{15}H_{23}NO_8Se$ requires C 42.5, H 5.4, N 3.3%). δ_H (500 MHz, $CDCl_3$) 5.62 (1H, d, *J* 9.8, NH), 5.09–5.05 (2H, m, H3, H4), 4.65 (1H, d, *J* 10, H1), 4.23–4.19 (2H, m, H2, H6), 4.11 (1H, AB part of an ABX dd, *J* 12.3, 2, H6), 3.68–3.64 (1H, m, H5), 2.10 (3H, s, Me of SeMe), 2.07, 2.03 (9H, s, Me of OAc), 1.93 (3H, s, Me of NHAc). δ_C (125 MHz, $CDCl_3$) 171.2–169.3 (CO), 78.4 (C1), 77.3 (C5), 73.8, 68.3 (C3, C4), 62.3 (C6), 53.4 (C2), 23.2 (Me of NHAc), 20.7–20.6 (Me of OAc), 2.7 (Me of SeMe). *m/z* (ESI^+, 50 V) 448 $[M(^{80}Se) + Na]^+$. *m/z* (ESI^-, 50 V) 460 $[M(^{80}Se) + Cl]^-$.

*Methyl 2-Acetamido-2-deoxy-1-seleno-β-D-hexopyranosides **5**, **10***

For removal of the acetyl groups the general deacylation procedure according to Zemplén was chosen.[7] To the protected methylseleno sugar **4** or **9** (500 mg, 1.18 mmol) in dry methanol (10 mL), 1 M NaOMe solution (60 μL) was added. The mixture was kept at room temperature and stirred for approx. 15 min (monitored by TLC) and then neutralized with AMBERLITE IR 120 H^+. The mixture was filtered and concentrated under vacuum. Recrystallization from water gave the selenosugar as white crystals (**5**, 310 mg, 88%; **10**, 316 mg, 90%).

*Compound **5***, mp 247–250°C [dec.; lit.[4] 255–257°C (dec.)], $[\alpha]_D^{20}$ +23 (*c* 0.8 in H_2O) lit.[4] $[\alpha]_D^{24}$ +16.4 (*c* 0.51 in H_2O) (Found: C 35.9, H 5.6, N 4.6. $C_9H_{17}NO_5Se$ requires C 36.2, H 5.7, N 4.7%). δ_H [400 MHz, $(CD_3)_2SO$] 7.59 (1H, d, *J* 9.8, NH), 4.49 (1H, d, *J* 10, H1), 3.90 (1H, q, *J* 10, H2), 3.71 (1H, d, *J* 2.8, H4), 3.49 (2H, dd, *J* 5.6, 2, H6), 3.39

(1H, dd, *J* 10, 2.8, H3), 3.32 (1H, t, *J* 5.6, H5), 1.95 (3H, s, Me of SeMe), 1.79 (3H, s, Me of NHAc). δ_C [100 MHz, $(CD_3)_2SO$] 169.4 (CO), 80.5 (C1), 79.8 (C5), 72.4 (C3), 67.8 (C4), 60.7 (C6), 51.4 (C2), 23.1 (Me of NHAc), 2.5 (Me of SeMe). δ_{Se} [68.6 MHz, $(CD_3)_2SO$] 201 (s, Se). *m/z* (ESI^+, 100 V) 322 $[M(^{80}Se) + Na]^+$. *m/z* (ESI^-, 100 V) 334 $[M(^{80}Se) + Cl]^-$, 298 $[M(^{80}Se) - H]^-$, 202 $[M - H - CH_3SeH]^-$.

Compound ***10***, mp 188–190°C (dec.), $[\alpha]_D^{20}$ −26° (*c* 0.8 in H_2O) (Found: C 34.3, H 5.7, N 4.2. $C_9H_{17}NO_5Se \cdot H_2O$ requires C 34.2, H 6.0, N 4.4%). δ_H [400 MHz, $(CD_3)_2SO$] 7.71 (1H, d, *J* 10, NH), 4.53 (1H, d, *J* 10, H1), 3.67 (1H, AB part of an ABX d, *J* 11.2, H6), 3.59 (1H, q, *J* 10, H2), 3.43 (1H, AB part of an ABX dd, *J* 11.2, 5.2, H6), 3.26 (1H, t, *J* 10, H3), 3.12–3.06 (2H, m, H5, H4), 1.96 (3H, s, Me of SeMe), 1.79 (3H, s, Me of NHAc). δ_C [100 MHz, $(CD_3)_2SO$] 169.1 (CO), 82.4 (C5), 79.6 (C1), 75.4 (C3), 70.6 (C4), 61.3 (C6), 55.1 (C2), 23.0 (Me of NHAc), 2.1 (Me of SeMe). *m/z* (ESI^+, 10 V) 322 $[M(^{80}Se) + Na]^+$, 300 $[M(^{80}Se) + H]^+$, 204 $[M + H - CH_3SeH]^+$. *m/z* (ESI^-, 100 V) 334 $[M(^{80}Se) + Cl]^-$, 298 $[M(^{80}Se) - H]^-$, 202 $[M - H - CH_3SeH]^-$.

X-Ray Crystal Structure Analysis of ***5***

All the measurements were performed using graphite-monochromatized $Mo_{K\alpha}$ radiation at 95 K. $C_9H_{17}NO_5Se$, M_r 298.20, orthorhombic, space group $P2_12_12_1$, *a* 5.7611(15), *b* 10.3368(17), *c* 19.260(3) Å, *V* 1147.0(4) $Å^3$, *Z* 4, D_{calc} 1.727 g cm^{-3}, μ 3.280 m^{-1}. A total of 2221 reflections were collected (θ_{max} 30.0°), from which 2176 were unique (R_{int} 0.0171), with 2019 having $I > 2\sigma(I)$. The structure was solved by direct methods (*SHELXS-97*)[11] and refined by full-matrix least-squares techniques against F^2 (*SHELXL-97*).[12] The non-hydrogen atoms were refined with anisotropic displacement parameters. The H-atoms were refined with idealized geometries. Due to the Se atom, the absolute structure of the chiral molecule could be reliably confirmed. For 160 parameters final *R* indices of *R* 0.0244 and wR^2 0.0575 (GOF 1.050) were obtained. The largest peak in a difference Fourier map was 0.333 e $Å^{-3}$. The final atomic parameters, as well as bond lengths and angles are deposited at the Cambridge Crystallographic Data Centre (CCDC 245179).

The crystal structure analysis of **5** confirmed the compound as methyl 2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside (Fig. 1).

Accessory Materials

NMR assignments for compounds **2**, **3**, **3a**, **7**, and **8** are available from the author or, until November 2009, the *Australian Journal of Chemistry*.

Acknowledgments

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