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Research Article

The synthesis of glucosinolates deuterium labelled in the glucose fragment

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Summary

A facile synthesis is described for 2,3,4,6-tetra-*O*-acetyl-thio-β-D-[1-^{2}H,6-^{2}H$_2$]glucopyranose, which has been used to make an isotopically labelled desulphoglucosinolate, [1′-^{2}H,6′-^{2}H$_2$]desulfogluconasturtiin. This isotopically labelled building block can be employed to make any glucosinolate or desulfoglucosinolate for use as internal standards for MS based analytical methods. Copyright © 2006 John Wiley & Sons, Ltd.

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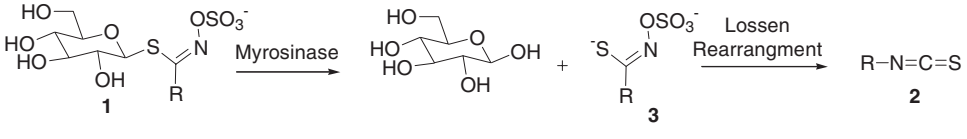
Introduction

Glucosinolates (**1**) are a class of naturally occurring thioglucosides present in all members of the *Cruciferae*, including the brassica crops such as cabbage, Brussels sprouts and oilseed rape.[1] Glucosinolates contain a common structure and over 100 different examples have been isolated and characterized,[1] with a variety of substituents in the side chain R, including allyl, benzyl, indolyl and 4-hydroxybenzyl. They are metabolized by the plant enzyme myrosinase[2,3] during food preparation, cooking and chewing (Scheme 1) to give the



Scheme 1.

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corresponding isothiocyanate (**2**) as the major product. The isothiocyanate is the formed via a Lossen-type rearrangement of the unstable thiohydroximate-*O*-sulfonate aglycone (**3**) initially produced.

There is considerable interest in glucosinolates for two major reasons. Firstly, they play an important role in plants.[4] The glucosinolates and myrosinase are compartmentalized and only interact to release the noxious isothiocyanates following tissue damage, usually caused by pests. The isothiocyanates then deter further attack by non-adapted herbivores. However, specialist pests have evolved to employ the volatile isothiocyanates as host–plant recognition cues. Cabbage and turnip root flies (*Delia radicum* and *Delia floralis*), use leaf surface glucosinolates as ovi-position (egg-laying) stimulants.[5] Secondly, there is convincing epidemiological evidence that consumption of broccoli and other cruciferous vegetables is associated with a decreased risk of cancer and this association is strongest for cancers of the gastrointestinal and respiratory tracts.[6] The anti-cancer effects have been attributed to the presence of glucosinolates in these vegetables, although the anti-cancer activity appears to reside with the isothiocyanate.[7] The mechanism of the anti-cancer activity is still controversial and could be a result of either inhibition of the Phase 1 detoxification enzymes,[8] upregulation of the Phase 2 detoxification enzymes[9] or even induction of apoptosis of tumour cells.[10]

The interest in glucosinolates has generated a need for accurate, and reproducible, glucosinolate analysis and a number of methods have been developed using LC-MS[11–13] and LC-MS/MS[14] techniques. However, there are currently few isotopically labelled internal standards available to aid quantitation. Most of the existing methods for isotopic labelling of glucosinolates have incorporated the isotopes into the side chain.[12] The drawback of this method is that a new synthesis needs to be developed for each glucosinolate. An answer to this problem is to label the sugar half of the glucosinolate, and this can then be used whatever the structure of the side chain. Therefore we herein report the synthesis of [1′-^{2}H,6′-^{2}H$_2$] desulfogluconasturtiin, which demonstrates a strategy for the synthesis of sugar labelled glucosinolates as internal standards for analysis.

Results and discussion

In previous studies in our laboratory a deuterated desulfogluconasturtiin was prepared as an internal standard for glucosinolate analysis,[15] using [^{2}H$_5$]bromobenzene as the source of the isotopic labels. This synthesis was then modified to make [*phenyl*-^{2}H$_5$]gluconasturtiin which was used in metabolic studies in rats.[16] As a result of this experience gluconasturtiin was used as our model glucosinolate in these further studies.

For an optimum internal standard a mass increase of three units is required and it was thought that three deuterium atoms could be incorporated into the

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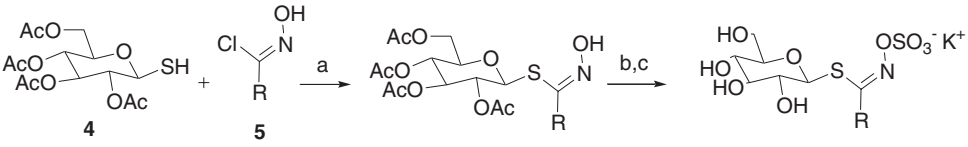

Scheme 2. General glucosinolate synthesis. Reagents and conditions: (a) Et_3N, THF; (b) pyridine.SO_3, CH_2Cl_2; (c) KOMe, MeOH

glucose at C-1 and C-6 via reduction of suitable derivatives with deuterated reducing agents. As the key step in glucosinolate synthesis, as originally developed by Benn,[17] is the coupling of a protected thioglucose (**4**) with a suitable oximyl chloride (**5**) (Scheme 2), the initial synthetic target was 2,3,4,6-tetra-*O*-acetyl-thio-β-D-[1-$^2H_1$,6-$^2H_2$]glucopyranose (**6**), which can be then be incorporated directly into any established glucosinolate synthesis.

Although *D*-[6-$^2H_2$]glucose is commercially available it is also readily made via reduction of a suitable methyl glucuronide with lithium aluminium deuteride. The stating material for the labelling was methyl (methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid)uronate (**7**). The method of Bernet and Vasella[18] was used to make methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside which was oxidized to **7** in two steps using Swern conditions[19] followed by pyridinium dichromate (PDC) in methanol/dichloromethane, to afford the methyl ester (**7**) in 58% yield. This oxidation is also reported in the literature in one step using either chromium trioxide in sulfuric acid/acetone[20,21] or PDC in DMF,[22] followed by methylation with diazomethane in each case.

The first two deuterium atoms where then introduced at C-6 by reduction with lithium aluminium deuteride in 91% yield, followed by benzylation to give the methyl 2,3,4-tribenzyl-α-*D*-[6-$^2H_2$]glucopyranoside (**8**) (Scheme 3). Comparison of the spectral data with that of the unlabelled compound clearly demonstrated the presence of the two deuterium atoms, from the expected increase in molecular weight and absence of the 6-CH_2 signals in the ^{1}H NMR spectrum. Hydrolysis of the methyl glycoside using glacial acetic acid and 1.0 M sulfuric acid under reflux was followed by oxidation with pyridinium chlorochromate (PCC) in dry dichloromethane to give the protected [6-$^2H_2$]glucono-γ-lactone (**9**) in 79% yield. Introduction of the third deuterium atom at C-1 was achieved by reduction with sodium borodeuteride in quantitative yield. Removal of the three benzyl groups and acetylation were carried out in a one pot reaction using acetic anhydride and BF_3.etherate[23] to give 1,2,3,4,6-pentaacetyl-β-*D*-[1-^{2}H,6-$^2H_2$]glucose (**10**) as a 5:1 mixture of α and β anomers.

From this point onwards the synthesis employed our standard glucosinolate methodology.[16,24] The 1,2,3,4,6-pentaacetyl-*D*-[1-^{2}H,6-$^2H_2$]glucose was converted to the glucopyranosyl bromide using HBr in acetic acid, reacted with

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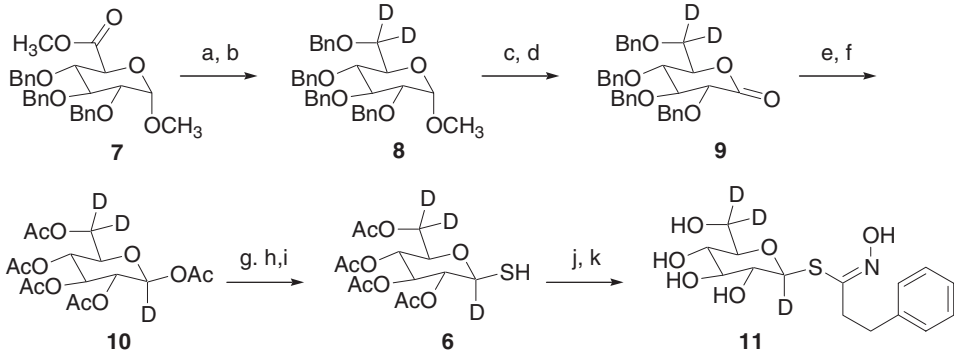

Scheme 3. Synthesis of [1′-^{2}H, 6′-^{2}H$_2$]desulfogluconasturtiin (11). Reagents and conditions: (a) $LiAlD_4$, Et_2O (91%); (b) NaH, BnBr, DMF (95%); (c) AcOH, aq. H_2SO_4 (64%); (d) PCC, CH_2Cl_2 (79%); (e) $NaBD_4$, THF/H_2O (100%); (f) Ac_2O, $BF_3.Et_2O$, O^{o}C (82%); (g) HBr, ACOH (83%); (h) thiourea, acetone (98%); (i) $K_2S_2O_5$, H_2O, CH_2Cl_2 (71%); (j) phenethyl oximyl chloride, Et_3N, Et_2O (100%); (k) KOMe, MeOH (64%)

thiourea in acetone and the resulting thiouronium salt was then hydrolysed using aqueous potassium metabisulfite to give the target molecule, 2,3,4,6-tetra-*O*-acetyl-thio-β-D-[1-^{2}H,6-^{2}H$_2$]glucopyranose (**6**), in 58% yield over the three steps. To demonstrate incorporation of the labelled sugar into a glucosinolate framework, as discussed above, [1′-^{2}H,6′-^{2}H$_2$]desulfogluconasturtiin was the preferred example. Generally the coupling reaction between the oximyl chloride and protected thioglucose gives reasonable yields but it was decided to optimize the reaction further to ensure no waste of labelled material. Examination of test reactions using unlabelled starting materials showed that there was some disulfide by-product. Therefore, it was decided to carry out the reaction with an excess of the oximyl chloride to maximize the conversion of the labelled thioglucose. Using 2.3 equivalents of the oximyl chloride the coupled product could thus be obtained in quantitative yield. Final deprotection using catalytic potassium methoxide gave the [1′-^{2}H,6′-^{2}H$_2$]desulfogluconasturtiin (**11**) in 64% yield. The spectral data confirmed the labelling pattern. In the ^{1}H NMR spectrum the signals for 1′-H and 6′-CH_2 were absent and a 3 mass unit increase was observed in the mass spectrum, compared to the unlabelled material.

A facile synthesis has thus been developed for 2,3,4,6-tetra-*O*-acetyl-thio-β-D-[1-^{2}H,6-^{2}H$_2$]glucopyranose and it has shown that this can be used to make an isotopically labelled desulfoglucosinolate, [1′-^{2}H, 6′-^{2}H$_2$]desulphogluconasturtiin. In principle this methodology can now be employed to make any glucosinolate or desulfoglucosinolate that may be required and

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allow a whole family of compounds to be prepared as internal standards for MS based analytical methods, using the same isotopically labelled building block.

Experimental

General

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.46 MHz, respectively, using Bruker Avance 500 and Varian Gemini 2000 spectrometers. The chloroform peaks (7.27 ppm for ^{1}H, 77.00 ppm for ^{13}C) were used as references. The EI and CI mass spectra were obtained on a VG Autospec mass spectrometer, and the ESI and APCI mass spectra on a Micromass LCT mass spectrometer. Diethyl ether and tetrahydrofuran were distilled over sodium and dichloromethane over calcium hydride. DMSO was freshly distilled from calcium hydride.

Methyl 2,3,4-tri-O-benzyl-α-D-[6-$^{2}H_2$]glucopyranoside

Methyl (methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid)uronate (8.0 g, 16.2 mmol) was dissolved in dry diethyl ether (100 ml) at 0°C and treated with lithium aluminium deuteride (1.0 M solution in diethyl ether) (8.12 ml, 8.12 mmol). The reaction was stirred at room temperature overnight, quenched using 1.0 M sulphuric acid and extracted using ethyl acetate (2 × 100 ml). The organic extracts were washed with brine (100 ml), dried ($MgSO_4$) and the solvent evaporated at reduced pressure. The product was purified by column chromatography on silica gel using ethyl acetate–hexane (1:2) as the eluant to give methyl 2,3,4-tri-*O*-benzyl-α-D-[6-$^{2}H_2$]glucopyranoside as a white crystalline solid (6.87 g, 91%); m.p. 50–51°C; $[\alpha]_D$ +21.7° (c 1.0 in $CHCl_3$); (Found: C, 71.58; H, 6.71. Calculated for $C_{28}H_{30}{}^{2}H_2O_6$: C, 72.08; H, 6.91%); ν_{max} (nujol)/cm^{-1} 3479 (OH); δ_H (200 MHz; $C^{2}HCl_3$) 3.37 (3H, s, OCH_3), 3.51 (1H, dd, $J_{1,2}$ 3, $J_{2,3}$ 10, H-2), 3.53 (1H, t, $J_{3,4} = J_{4,5}$ 10, H-4), 3.63 (1H, d, $J_{4,5}$ 10, H-5), 4.02 (1H, t, $J_{2,3} = J_{3,4}$ 10, H-3), 4.57 (1H, d, *J* 3, H-1), 4.60–5.10 (6H, m, 3 × $PhCH_2$), 7.20–7.40 (15H, m, 3 × $PhCH_2$); δ_C (50.3 MHz; $C^{2}HCl_3$) 55.7 (OCH_3), 71.0 (C-5), 74.0 ($PhCH_2$), 75.6 ($PhCH_2$), 76.3 ($PhCH_2$), 77.8 (C-4), 80.4 (C-2), 82.5 (C-3), 98.7 (C-1), 128.2–129.0 (15CH, 3 × $PhCH_2$), 138.5, 138.6, 139.2 (3 × quaternary C, 3 × Ph); *m/z* (CI) 467 ($[MH]^+$, 1%), 435 (37), 375 (29), 343 (29), 271 (60), 181 (94) and 91 (100, $[PhCH_2]^+$).

*Methyl 2,3,4,6-tetra-O-benzyl-α-D-[6-$^{2}H_2$]glucopyranoside (**8**)*

Sodium hydride (60% dispersion in mineral oil) (1.6 g, 61 mmol) was washed with dry diethyl ether (2 × 5 ml) then treated with benzyl bromide (4.80 g, 3.4 ml, 27.5 mmol). Methyl 2,3,4-*O*-benzyl-α-D-[6-$^{2}H_2$]glucopyranoside (6.3 g,

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13.5 mmol) in dry *N*,*N*-dimethylformamide (65 ml) was added to the reaction and after 2 h at room temperature the reaction was quenched by dropwise addition of methanol (10 ml). The solvent was evaporated at reduced pressure then the residue was taken up in diethyl ether (250 ml) and washed with water (200 ml). The aqueous phase was extracted with diethyl ether (2×100 ml) then the combined organic extracts were washed with brine (200 ml), dried ($MgSO_4$) and the solvent evaporated at reduced pressure to give the crude product. The resulting oil was purified by column chromatography using ethyl acetate-hexane (1:2) as the eluant to give methyl 2,3,4,6-tetra-*O*-benzyl-α-D-[6-$^2H_2$]glucopyranoside was given as a clear viscous oil (7.14 g, 95%); $[\alpha]_D$ +37.4° (c 1.0 in $CHCl_3$); (Found: C, 75.53; H, 6.86. Calculated for $C_{35}H_{36}{}^2H_2O_6$: C, 75.51; H, 6.88%); δ_H (200 MHz; $CDCl_3$) 3.40 (3H, s, OCH_3), 3.60 (1H, dd, $J_{1,2} = 3$, $J_{2,3} = 10$, H-2), 3.70 (1H, t, $J_{2,3} = J_{3,4} = 10$, H-4), 3.77 (1H, d, $J_{4,5} = 10$, H-5), 4.00 (1H, t, $J_{3,4} = J_{4,5}$ 10, H-3), 4.40–5.10 (9H, m, $4 \times PhCH_2$, H-1), 7.10–7.40 (20H, m, $4 \times PhCH_2$); δ_C (50.3 MHz; $CDCl_3$) 55.7 (OCH_3), 70.4 (C-5), 73.9 ($PhCH_2$), 74.0 ($PhCH_2$), 75.6 ($PhCH_2$), 76.3 ($PhCH_2$), 78.1 (C-4), 80.2 (C-2), 82.6 (C-3), 98.7 (C-1), 128.2–129.0 (20CH, $4 \times PhCH_2$), 138.4, 138.6, 138.7, 139.2 ($4 \times$ quaternary C, $4 \times$ Ph); *m*/*z* (CI) 557 ($[MH]^+$, 1%), 271 (60), 219 (33), 181 (94) and 91 (100, $[PhCH_2]^+$).

*2,3,4,6-Tetra-O-benzyl-*D*-[6-$^2H_2$]glucopyranoside*

Glacial acetic acid (55 ml) and 1.0 M sulfuric acid (14 ml) were heated at 105°C and a solution of methyl 2,3,4,6-tetra-*O*-benzyl-D-[6-$^2H_2$]glucopyranoside (6.87 g, 12.3 mmol) in glacial acetic acid (8 ml) was added. The reaction was kept at 105°C for 1 h then cooled on ice. The crystalline product was removed by filtration, washed with ice water and dried at reduced pressure. The white crystals were then washed with hexane and dried in a desiccator to give 2,3,4,6-tetra-*O*-benzyl-D-[6-$^2H_2$]glucopyranoside as a white crystalline solid (4.28 g, 64%), which was a mixture of α and β anomers; m.p. 132–133°C; $[\alpha]_D$ +18.4° (c 1.0 in $CHCl_3$); (Found: C, 74.60; H, 6.75. Calculated for $C_{34}H_{34}{}^2H_2O_6$: C, 75.25; H, 6.69%); ν_{max} (nujol)/cm^{-1} 3370 (OH); δ_H (300 MHz; $CDCl_3$) 2.70 (1H, s, OH), 3.42–3.46 (1H, m, H-2b), 3.55 (1H, d, $J_{4,5} = 10$, H-5), 3.60–3.70 (4H, m, H-2α, 3β, 4α and β), 3.98 (1H, t, $J_{2,3} = J_{3,4}$ 10, H-3α), 4.05 (1H, d, $J_{4,5} = 10$, H-5α), 4.40–5.00 (17H, m, $4 \times PhCH_2$ α and β, H-1b), 5.20 (1H, d, $J_{1,2}$ 3.6, H-1α), 7.10–7.40 (40H, m, $4 \times PhCH_2$ α and β); δ_C (50.3 MHz; $CDCl_3$) 70.4 (C-5α), 73.8, 74.0 ($3 \times PhCH_2$), 75.0 ($3 \times PhCH_2$, 5b), 76.3 ($2 \times PhCH_2$), 78.2 (C-4α, 4β), 80.4 (C-2α), 82.2 (C-3α), 83.5 (C-2β), 85.0 (C-3β), 91.8 (C-1α), 98.0 (C-1β), 128.1–130.0 (20CH, $4 \times PhCH_2$), 138.4, 138.6, 138.7, 139.2 ($4 \times$ quaternary C, $4 \times$ Ph); *m*/*z* (FAB) 565 ($[M + Na]^+$, 34%), 543 (2, $[MH]^+$), 417 (15) and 181 (100, $[glucoseH]^+$).

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*2,3,4,6-Tetra-O-benzyl-D-[6-$^2H_2$]glucono-γ-lactone (**9**)*

Pyridinium chlorochromate (7.00 g, 40 mmol) was added in one portion to 2,3,4,6-tetra-*O*-benzyl-α-D-[6-$^2H_2$]glucopyranoside (3.62 g, 6.7 mmol) and powdered 4 Å molecular sieves (7.0 g) in dry dichloromethane (90 ml). The reaction was stirred at room temperature for 1 h then hexane (100 ml) and diethyl ether (200 ml) were added. The solution was filtered through silica gel and the solvent evaporated at reduced pressure. The crude product was purified using column chromatography on silica using hexane-diethyl ether (7:1) as the eluant. 2,3,4,6-Tetra-*O*-benzyl-D-[6-$^2H_2$]glucono-γ-lactone was obtained as a clear oil (2.83 g, 79%); $[\alpha]_D$ +33.2° (c 2.0 in $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1765 (C=O); δ_H (200 MHz; $CDCl_3$) 3.95 (1H, t, *J* 7, H-4), 4.00 (1H, t, *J*7, H-3), 4.15 (1H, d, *J*7, H-2), 4.45–5.10 (9H, m, H-5 and 4 × $PhCH_2$), 7.10–7.50 (20H, m, 4 × $PhCH_2$); δ_C (50.3 MHz; $CDCl_3$) 74.0 ($PhCH_2$), 74.3 (2 × $PhCH_2$), 74.5 ($PhCH_2$), 76.5 (C-5), 77.9, 78.5 (C-2, 4), 81.4 (C-3), 125.5–126.3 (20CH, 4 × $PhCH_2$), 134.7, 135.3, 135.4 (4 × quaternary C, 4 × Ph), 169.9 (C=O); *m/z* (CI) 541 ($[M+H]^+$, 40%), 540 (5), 271 (64), 181 (64), 107 (65) and 91 (100, $[PhCH_2]^+$).

2,3,4,6-Tetra-O-benzyl-D-[1-^2H,6-$^2H_2$]glucopyranoside

2,3,4,6-Tetra-*O*-benzyl-D-[6-$^2H_2$]glucono-γ-lactone (2.7 g, 3.9 mmol) was dissolved in tetrahydrofuran (13 ml) and cooled to 0°C under nitrogen. Sodium borohydride (0.84 g, 2.3 mmol) was dissolved in water and added to the lactone solution dropwise. The reaction was stirred for 24 h, quenched with 1 M sulfuric acid then extracted with ethyl acetate (2 × 75 ml) and washed with brine (80 ml). The organic extracts were dried ($MgSO_4$) and the solvent evaporated at reduced pressure to give 2,3,4,6-tetra-*O*-benzyl-D-[1-^2H,6-$^2H_2$]glucopyranoside as a white crystalline solid (2.7 g, 100%); m.p. 120.5–122°C; $[\alpha]_D$ +37.2° (c 1.0 in $CHCl_3$); ν_{max} (nujol)/cm^{-1} 3370 (OH); δ_H (200 MHz; $CDCl_3$) 3.1 (1H, s, OH), 3.40–3.70 (6H, m, H-2α, 3*β*, 4α and *β*, 5*β*), 3.98 (1H, t, $J_{2,3} = J_{3,4}$ 10, H-3α), 4.05 (1H, d, $J_{4,5}$ 10, H-5α), 4.40–5.00 (16H, m, 4 × $PhCH_2$ α and *β*), 7.10–7.40 (40H, m, 4 × $PhCH_2$ α and *β*); δ_C (50.3 MHz; $CDCl_3$) 70.4 (C-5α), 73.8, 74.0 (3 × $PhCH_2$), 75.0 (3 × $PhCH_2$, 5*β*), 76.3 (2 × $PhCH_2$), 78.2 (C-4α, 4*β*), 80.4 (C-2α), 82.2 (C-3α), 83.5 (C-2*β*), 85.0 (C-3*β*), 128.1–130.0 (20CH, 4 × $PhCH_2$), 138.4, 138.6, 138.7, 139.2 (4 × quaternary C, 4 × Ph); *m/z* (FAB) 566 ($[M+Na]^+$), 12%), 544 (2, $[MH]^+$), 543 (5, $[M]^+$), 418 (14) and 181 (100, $[glucoseH]^+$).

*1,2,3,4,6-Penta-O-acetyl-D-[1-^2H,6-$^2H_2$]glucopyranose (**10**)*

2,3,4,6-Tetra-*O*-benzyl-D-[1-^2H,6-$^2H_2$]glucopyranose (1.50 g, 0.276 mmol) in acetic anhydride (200 ml) was treated with boron trifluoride diethyl etherate (2.88 ml, 22 mmol) at 0°C and the reaction stirred overnight. Sodium hydrogen

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carbonate was used to neutralize the reaction mixture which was then extracted using ethyl acetate (2×200 ml) and washed with water (4×200 ml). The organic extracts were dried ($MgSO_4$) and the solvent evaporated at reduced pressure. The residue was purified by column chromatography on silica gel using 40–60 petroleum ether-ethyl acetate (3:2) as the eluant to give 1,2,3,4,6-penta-*O*-acetyl-D-[1-^{2}H,6-$^2H_2$]glucopyranose as a white crystalline solid (0.89 g, 82%); m.p. 111–112°C; $[\alpha]_D$ +98.1° (c 0.6 in $CHCl_3$); (Found: C, 48.34; H, 5.46. Calculated for $C_{16}H_{19}{}^2H_3O_{11}$: C, 48.85; H, 5.64%); ν_{max} (nujol)/cm^{-1} 1735 (CO); δ_H (200 MHz; $CDCl_3$) 2.00–2.20 (30H, m, $5 \times$ OC(O)CH_3 α and β), 3.80 (1H, d, $J_{4,5}$ 10, H-5β), 4.10 (1H, d, $J_{4,5}$ 10, H-5α), 5.10 (1H, d, $J_{2,3}$ 10, H-2α), 5.14 (1H, t, $J_{3,4} = J_{4,5}$ 10, H-4α), 5.46 (1H, t, $J_{2,3} = J_{3,4}$ 10, H-3α), 5.00–5.50 (3H, m, H-2β, 3β, 4β); δ_C (50.3 MHz; $CDCl_3$) 20.9–21.4 ($5 \times$ OC(O)CH_3 α and β), 68.1 (C-4β), 68.3 (C-4α), 69.5 (C-2α), 70.1, 70.3 (C-3α, 5α), 70.6 (C-2β), 73.0, 73.2 (C-3β, 5β), 169.3, 169.9, 170.2, 170.7, 171.0 ($5 \times$ OC(O)CH_3 α and β); *m/z* (CI) 394 ([MH]$^+$, 1%), 334 (100, [M-OAc]$^+$), 274 (28), 214 (6) and 172 (35).

2,3,4,6-Tetra-O-acetyl-α-D-[1-^{2}H,6-$^2H_2$]glucopyranosyl bromide

1,2,3,4,6-Penta-*O*-acetyl-D-[1-$^2H_1$,6-$^2H_2$]glucopyranose (0.7 g, 1.78 mmol) was dissolved in acetic anhydride (0.6 ml) and 45% w/v hydrogen bromide in acetic acid (1 ml) was added. The reaction mixture was stirred for 3 h then further 45% w/v hydrogen bromide in acetic acid (3 ml) was added and stirring continued overnight. The residue was dissolved in dichloromethane (10 ml) and poured into ice/water (20 ml) with stirring. The organic layer was then added carefully to an ice/saturated sodium hydrogen carbonate solution (20 ml) with stirring. Once the gas evolution became less vigorous the organic phase was added to saturated sodium hydrogen carbonate solution (20 ml). The organic layer was dried ($MgSO_4$) and the solvent evaporated at reduced pressure to give a golden oil which solidified upon cooling to 0°C. The product was recrystallized from 40–60 petroleum ether–diethyl ether to give 2,3,4,6-tetra-*O*-acetyl-α-D-[1-$^2H_1$,6-$^2H_2$]glucopyranosyl bromide as a white crystalline solid (0.614 g, 83%); m.p. 84–87°C; $[\alpha]_D$ +186° (c 2.42 in $CHCl_3$); ν_{max} (nujol)/cm^{-1} 1730 (CO); δ_H (200 MHz; $CDCl_3$) 2.00 (3H, s, OC(O)CH_3), 2.05 (3H, s, OC(O)CH_3), 2.10 (6H, 2 s, $2 \times$ OC(O)CH_3), 4.20 (1H, d, $J_{4,5}$ 10, H-5), 4.80 (1H, d, $J_{2,3}$ 10, H-2), 5.15 (1H, t, $J_{3,4} = J_{4,5}$ 10, H-4), 5.55 (1H, t, $J_{2,3} = J_{3,4}$ 10, H-3); δ_C (50.3 MHz; $CDCl_3$) 21.0–21.1 ($4 \times$ OC(O)CH_3), 64.6 (C-4), 70.6 (C-2), 71.0 (C-3), 72.4 (C-5), 169.9, 170.2, 170.3, 171.0 ($4 \times$ OC(O)CH_3); *m/z* (CI) 433, 431 ([M + NH_4]$^+$, 5%), 334 (30, [M-Br]$^+$), 292 (44), 216 (7) and 172 (58).

2,3,4,6-Tetra-O-acetyl-β-D-[1-^{2}H,6-$^2H_2$]-glucopyranosylisothiouronium bromide

Thiourea (0.33 g, 4.35 mmol) was added to 2,3,4,6-tetra-*O*-acetyl-β-D-[1-^{2}H,6-$^2H_2$]glucopyranosyl bromide (0.6 g, 1.45 mmol) in dry acetone (5 ml)

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under nitrogen. The solution was heated at reflux for 15 min then the reaction mixture allowed to cool. The solvent was removed at reduced pressure and the crude product purified by column chromatography on silica gel with 40–60 petroleum ether–ethyl acetate (2:3) followed by methanol as eluant, to give 2,3,4,6-tetra-*O*-acetyl-*β*-D-[1-$^2H_1$,6-$^2H_2$]glucopyranosylisothiouronium bromide as a white crystalline solid (0.7 g, 98%); m.p. 182.5–183.5°C; $[\alpha]_D$ −15.6° (c 1.0 in CH_3OH); ν_{max} (nujol)/cm^{-1} 3360–3310 (NH), 1730 (CO); δ_H (200 MHz; D_2O) 2.10 (3H, s, $COCH_3$), 2.15 (3H, s, $COCH_3$), 2.20 (3H, s, $COCH_3$), 2.22 (3H, s, $COCH_3$), 4.20 (1H, d, $J_{4,5}$ 10, H-5), 5.15 (1H, t, $J_{3,4} = J_{4,5}$ 10, H-4), 5.25 (1H, d, $J_{2,3}$ 10, H-2), 5.40 (1H, t, $J_{2,3} = J_{3,4}$ 10, H-3); δ_C (50.3 MHz; D_2O) 23.3–23.5 (4 × OC(O)CH_3), 68.6 (C-4), 70.1 (C-2), 74.1 (C-3), 76.7 (C-5), 170.5 (C=N), 173.2, 173.3, 173.6, 174.4 (4 × $COCH_3$); *m/z* (CI) 490 ([M]$^+$, 7%), 444 (32), 410 (17), 334 (67), 292 (26), 248 (30), 216 (24) and 172 (25).

*2,3,4,6-Tetra-O-acetyl-thio-β-D-[1-^2H,6-$^2H_2$]glucopyranose (**6**)*

Potassium metabisulfite (0.24 g, 1.26 mmol) was dissolved in water (4 ml) and heated to 75°C. Dichloromethane (5 ml) was added carefully followed by 2,3,4,6-tetra-*O*-acetyl-*β*-D-[1-$^2H_1$,6-$^2H_2$]glucopyranosylisothiouronium bromide (0.24 g, 1.22 mmol). The biphasic solution was heated under reflux for 15 min then cooled to room temperature. The organic phase was washed with water (3 × 5 ml) then the aqueous layer washed with dichloromethane (2 × 5 ml). The combined organic layers were dried ($MgSO_4$) and the solvent evaporated at reduced pressure to give a white solid. After purification using column chromatography on silica gel with 40–60 petroleum ether–ethyl acetate (1:1) as eluant, 2,3,4,6-tetra-*O*-acetyl-thio-*β*-D-[1-^2H,6-$^2H_2$]glucopyranose was obtained as a white crystalline solid (0.127 g, 71%); m.p. 117–119°C; $[\alpha]_D$ −8.7° (c 1.0 in CH_3OH); (Found: C, 45.66; H, 5.45. Calculated for $C_{14}H_{17}{}^2H_3O_9S$: C, 45.77; H, 5.49%); ν_{max} (nujol)/cm^{-1} 3460 (SH), 1735 (CO); δ_H (200 MHz; $CDCl_3$) 2.00 (3H, s, OC(O)CH_3), 2.02 (3H, s, OC(O)CH_3), 2.10 (3H, s, OC(O)CH_3), 2.12 (3H, s, OC(O)CH_3), 3.70 (1H, d, $J_{4,5}$ 10, H-5), 4.98 (1H, d, $J_{2,3}$ 10, H-2), 5.09 (1H, t, $J_{3,4} = J_{4,5}$ 10, H-4), 5.18 (1H, t, $J_{2,3} = J_{3,4}$ 10, H-3); δ_C (50.3 MHz; $CDCl_3$) 21.1–21.3 (4 × OC(O)CH_3), 68.5 (C-4), 73.9 (C-2), 74.0 (C-3), 76.6 (C-5), 169.9, 170.2, 170.6, 171.2 (4 × OC(O)CH_3); *m/z* (CI) 368 ([MH]$^+$, 2%), 334 (100, [M-SH]$^+$), 274 (29) and 172 (62).

2,3,4,6-Tetra-O-acetyl-β-D-[1-^2H,6-$^2H_2$]desulphogluconasturtiin

Hydrocinnamaldehyde oximyl chloride (0.06 g, 0.32 mmol) was suspended in dry diethyl ether (3.0 ml) and a solution of 2,3,4,6-tetra-*O*-acetyl-thio-*β*-D-[1-$^2H_1$,6-$^2H_2$]glucopyranose (0.052 g, 0.14 mmol) in dry diethyl ether (1.5 ml) was added. The reaction was treated with dry triethylamine (0.3 ml) and stirred

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overnight. 1 M sulfuric acid (5 ml) was added to the reaction and the solution extracted using ethyl acetate (3×15 ml). The organic extracts were dried ($MgSO_4$) and the solvent evaporated at reduced pressure to give a white solid. The product was purified by column chromatography on silica gel using 40–60 petroleum ether–ethyl acetate (3:2) as the eluant. 2,3,4,6-Tetra-*O*-acetyl-*β*-D-[1-$^2H_1$,6-$^2H_2$]desulfogluconasturtiin was obtained as a white solid (0.075 g, 100%); m.p. 152–154°C; $[\alpha]_D$ −19.6° (c 1.0 in $CHCl_3$); (Found: C, 52.66; H, 6.08; N, 2.74. Calculated for $C_{23}H_{26}{}^2H_3NO_{10}S.0.5H_2O$: C, 52.76; H, 5.78; N, 2.68%); ν_{max} (nujol)/cm^{-1} 3300 (OH), 1750 (C=O); δ_H (300 MHz; $CDCl_3$) 1.90–2.10 ($4 \times 3H$, 4 s, $4 \times OC(O)CH_3$), 2.70–3.00 (4H, m, H-8, 9), 3.65 (1H, d, $J_{4,5}$ 10, H-5), 4.96–5.00 (2H, 2t, *J* 10, H-2, 4), 5.20 (1H, t, *J* 10, H-3), 7.10–7.30 (5H, m, phenyl); δ_C (75.45 MHz; $CDCl_3$) 20.5–20.6 ($4 \times OC(O)CH_3$), 33.2 (CH_2), 34.3 (CH_2), 68.1 (C-4), 70.1 (C-2), 73.7 (C-3), 76.0 (C-5), 126.7 (C-4′), 128.4 (C-3′, 5′), 128.8 (C-2′, 6′), 140.6 (C-1′), 152.0 (C-7), 169.4, 169.5, 170.4, 170.8 ($4 \times OC(O)CH_3$); *m*/*z* (EI) 515 ($[MH]^+$, 5%), 334 (47), 243 (61), 172 (30), 150 (64), 132 (100), 105 (15) and 91 (27, $[PhCH_2]^+$).

β-D-[1′-^2H, 6′-$^2H_2$]desulfogluconasturtiin (**11**)

To 2,3,4,6-tetra-*O*-acetyl-*β*-D-[1′-^{2}H,6′-$^2H_2$]desulfogluconasturtiin (0.5 g, 0.98 mmol) in methanol was added a catalytic amount of potassium metal under nitrogen. The reaction was stirred for 18 h then Amberlite IR-120 resin was added. Stirring was continued for a further 15 min before the Amberlite was removed by filtration and the solvent removed by evaporation at reduced pressure to give an extremely hygroscopic white amorphous solid (0.057 g, 64%); m.p. 78–80°C; $[\alpha]_D$ −50.3° (c 1.0 in CH_3OH); (Found: C, 49.62; H, 6.00; N, 3.58. Calculated for $C_{15}H_{18}{}^2H_3NO_6S.0.8H_2O$: C, 49.87; H, 6.32; N, 3.88%); ν_{max} (nujol)/cm^{-1} 3300 (OH); δ_H (300 MHz; CD_3OD) 3.00–3.20 (4H, m, CH_2CH_2), 3.60–3.80 (4H, m, H-2, 3, 4, 5), 7.25 (5H, s, phenyl); δ_C (75.45 MHz; CD_3OD) 34.8 (CH_2), 35.3 (CH_2), 71.3 (C-4), 74.5 (C-2), 79.8 (C-5), 82.2 (C-3), 127.4 (C-4′), 129.6, 129.7 (C-2′, 3′, 5′, 6′), 142.7 (C-1'), 154.0 (C=N); *m*/*z* (CI) 347 ($[MH]^+$, 1%), 150 (11), 131 (46) and 91 (100, $[PhCH_2]^+$).

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