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The synthesis of novel hexa-^{13}C-labelled glucosinolates from [$^{13}C_6$]-D-glucose

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ABSTRACT

An isotopically labelled building block, 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-[$^{13}C_6$]glucopyranose (**4**), is obtained from the commercially available [$^{13}C_6$]-D-glucose. This hexa-^{13}C-labelled thioglucose can be employed to make any glucosinolate (**8**) for use as an internal standard for isotopic dilution LCMS analysis. Herein three typical glucosinolates in their hexa-^{13}C-labelled form: [*glucose*-$^{13}C_6$]gluconasturtiin, [*glucose*-$^{13}C_6$]sinigrin and [*glucose*-$^{13}C_6$]glucoerucin are synthesised by coupling the isotopically labelled thioglucose (**4**) with the corresponding hydroximoyl chlorides followed by sulfation with pyridine sulfur trioxide and deacetylation with a catalytic amount of potassium methoxide, respectively.

1. Introduction

Glucosinolates are a family of naturally occurring sulfur-containing compounds present in all cruciferous plants including broccoli, cabbage, Brussels sprouts and oilseed rape.[1] Glucosinolates share a common structure that comprises a D-glucopyranosyl unit, an anomeric thiohydroximoyl-*O*-sulfate function and a variable side chain. Over 120 different glucosinolates have been isolated and characterised. In terms of the side chain R, glucosinolates can be divided into several major groups: aliphatic, arylaliphatic, ω-methylthioalkyl and heterocyclic (e.g., indole).[1,2] There is increasing evidence from both epidemiological and animal studies that consumption of broccoli and other cruciferous vegetables is associated with a lowered risk of cancers especially those of the gastrointestinal and respiratory tracts.[3–5] The anti-cancer effects have been attributed to the high content of glucosinolates in these vegetables. Hydrolysis of glucosinolate during food preparation, cooking, chewing and digestion, mediated by the compartmentalised plant enzyme myrosinase (thioglucoside glucohydrolase EC 3.2.3.1),[1,6] which appears also in mammalian intestinal bacteria,[7] gives an unstable thiohydroximate-*O*-sulfate aglycon intermediate. This unstable intermediate reacts further to give a wide range of degradation products (isothiocyanates, nitriles, epithiocyanates, oxazolidine-2-thiones, thiocyanates) with diverse biological activities including anti-cancer effects.[8,9] This has generated increasing interest in the accurate analysis of glucosinolates using stable isotopic labelled analogues as internal standards for isotopic dilution methods in LC/MS and/or LC/MS/MS analysis.[10–13] Isotopically labelled internal standards allow for correction of losses of the analytes during sample preparation and consequently provide more accurate quantitative analysis results. To reduce interference due to the natural isotopic distribution in the mass spectrum, a suitable internal standard should have at least a three mass unit difference from the analyte. Most of the existing methods for isotopic labelling of glucosinolates have incorporated the isotopes into the side chain, which is more appropriate for metabolism studies.[11,14–18] The drawback of this approach is that a new synthesis needs to be developed for each glucosinolate. Our group has recently demonstrated a general strategy for glucosinolates deuterated in the glucose fragment, using 2,3,4,6-tetra-*O*-acetylthio-β-D-[1-^2H, 6-$^2H_2$]glucopyranose to synthesise 1′-^2H,6′-$^2H_2$]desulfogluconasturtiin.[19] The triply deuterated building block itself needs 9–14 synthetic steps, depending upon the starting material. In this project, a greatly simplified multiply ^{13}C-labelled building block 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-[$^{13}C_6$]glucopyranose is prepared in excellent yield in three steps from the commercially available [$^{13}C_6$]-D-glucose. Coupling of this ^{13}C-labelled thioglucopyranose with various hydroximoyl chlorides would afford any glucosinolate in another three-step sequence. Herein three typical hexa-^{13}C-labelled glucosinolates were synthesised according to our standard methodology for glucosinolates,[14,19,20] after optimisation using unlabelled materials.

2. Results and discussion

2.1. Synthesis of [*glucose*-$^{13}C_6$]glucosinolates

Scheme 1 shows the general procedure for the synthesis of the title compounds. Firstly [$^{13}C_6$]-D-glucose (**1**) was converted into 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**) with acetic anhydride and hydrobromic acid in 88% yield after recrystallisation. The reaction is governed by the anomeric effect, giving the α-anomer as the only product, which is confirmed by the small size of coupling constant, $^3J_{H1,H2}$=3.8 Hz. Heating the bromide (**2**) and

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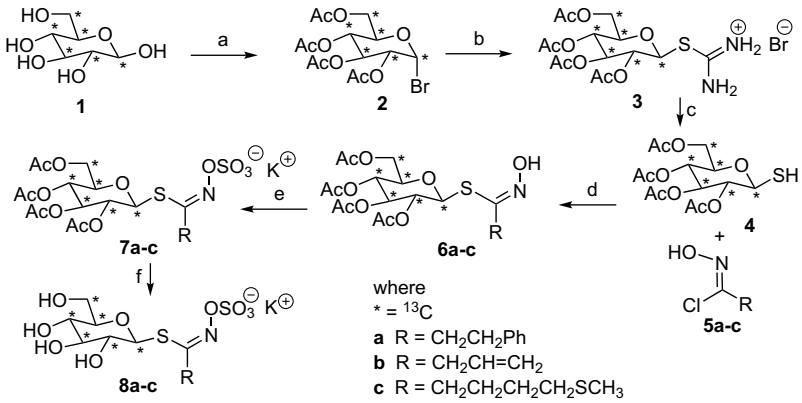
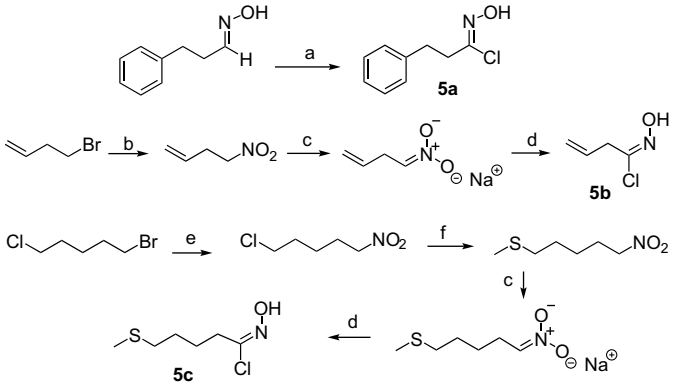


Scheme 1. Hexa-^{13}C-labelled glucosinolates and desulfoglucosinolates from [$^{13}C_6$]-D-glucose. Reagents and conditions: (a) HBr, AcOH, Ac_2O (88%); (b) thiourea, acetone (71–80%); (c) $K_2S_2O_5$, H_2O, CH_2Cl_2 (67–93%); (d) Et_3N, THF (50–93%); (e) (i) Py·SO_3 complex, CH_2Cl_2, (ii) aq 2 M $KHCO_3$ (47–78%); (f) KOMe, MeOH (83–88%).

thiourea in acetone under reflux led to the precipitation of the isothiouronium bromide (**3**) in good yield in the β-configuration due to the neighbouring group effect of the acetate group adjacent to the anomeric carbon atom. Simple hydrolysis of the isothiouronium bromide (**3**) in the presence of potassium metabisulfite in a biphasic system of water and DCM gave thioglucose (**4**), the key isotopically labelled building block, in good to excellent yield. The large size of $^3J_{H1,H2}$ of 10 Hz in **3** and **4** confirms the β-configuration.

With the hexa-^{13}C-labelled thioglucose in hand, subsequent work concentrated on its coupling with the hydroximoyl chloride (Scheme 2), a protocol initially established by Benn et al.[20] Phenethylhydroximoyl chloride (**5a**) derived from phenylpropionaldehyde oxime in DCM in the presence of half an equivalent of pyridine or triethylamine was known to be unstable and has to be prepared immediately before the coupling. We reasoned that the instability of this compound lies in the presence of organic base, which catalyses the premature removal of HCl and formation of the nitrile oxide. Therefore the generation of **5a** was improved by reacting phenylpropionaldehyde oxime with *N*-chlorosuccinimide in DMF without base by modifying a literature procedure.[21] A more stable, colourless oil of **5a**, which gradually solidified upon storage at room temperature, was thus obtained by simple work-up with water and extraction with diethyl ether.[22] To guarantee that no labelled material was wasted, the coupling reaction was carried out with an excess of the hydroximoyl chloride to maximise the conversion of the [$^{13}C_6$]thioglucose. The required 2,3,4,6-tetra-*O*-acetyl-β-D-gluconasturtiin (**6a**) was thus obtained in 88% yield.



Scheme 2. Synthesis of hydroximoyl chloride. Reagents and conditions: (a) *N*-chlorosuccinimide, DMF, rt, overnight; (b) $AgNO_2$, CH_2Cl_2 (45%); (c) NaOEt, EtOH (55% and 88%); (d) HCl(g), Et_2O (100%); (e) $NaNO_2$, DMSO, 47%; (f) NaI, CH_3SNa, MeOH, reflux, 42%.

For sinigrin, the required allylhydroximoyl chloride (**5b**, Scheme 2) was generated immediately before the coupling from the sodium salt of (*E*)-4-nitrobut-1-ene using HCl(g) in dioxane and diethyl ether.[16] The (*E*)-4-nitrobut-1-ene was synthesised using (*E*)-4-bromobut-1-ene and silver nitrite in water followed by deprotonation with sodium ethoxide.[23] For glucoerucin, 4-methylthiobutylhydroximoyl chloride (**5c**, Scheme 2) was prepared in the same way as (**5b**).[16] The requisite nitroalkane was synthesised from 1-bromo-5-chloropentane, reacting first with $NaNO_2$ in DMSO and then sodium thiomethoxide in the presence of sodium iodide in refluxing methanol.[24] Coupling of **5b** and **5c** with [$^{13}C_6$]thioglucose **4** afforded **6b** and **6c** in 80% and 50% yields, respectively (Scheme 1). In all cases the *Z*-isomer is obtained exclusively due to stereoelectronic effects.[25]

The O-sulfation proved to be a tricky step as evidenced by our previous work,[4,14,15] therefore further optimisation with unlabelled material was carried out using excess Py·SO_3 in pyridine. Monitoring the reaction closely by TLC has shown that this appears to be a clean reaction but work-up by removing the solvent pyridine in the presence of aqueous $KHCO_3$ resulted in deterioration of the product by deacylation and/or desulfation. This problem was solved by using DCM as the solvent while keeping the pyridine to as small amount as possible. The crude product was further purified by column chromatography, however, recrystallisation from boiling EtOH (85%)[26,18] required extra care as desulfation occurred in our hands to some extent. Once the *O*-sulfates (**7a–c**) were purified, the final products (**8a–c**) could be obtained in good yield by straightforward deacetylation with a catalytic amount of potassium methoxide in MeOH.

2.2. Characterisation of [*glucose*-$^{13}C_6$]derivatives

Extensive ^{1}H,^{13}C and ^{13}C,^{13}C coupling leads to severe overlaps and second order coupling effects in the conventional ^{1}H and ^{13}C NMR spectra, which substantially hampers characterisation of the isotopically labelled glucose derivatives. However, by employing 2D NMR techniques it was possible to assign all the ^{1}H and ^{13}C resonances, derive significant J_{HH}, J_{HC} and J_{CC} coupling constants and thus characterise all synthesised ^{13}C-labelled compounds unambiguously. Alongside standard ^{1}H,^{1}H-COSY spectra, two less common techniques, 2D ^{1}H *J*-resolved spectrum with ^{13}C decoupling and 2D ^{1}H,^{13}C HSQC without ^{13}C decoupling have been used. Examples of the application of these techniques on compounds **2** and **3** are shown in Figures 1 and 2, respectively. Figure 1 shows the ^{1}H *J*-resolved spectrum with ^{13}C decoupling of compound **2**, which has only chemical shift information present on the F_2-axis. Homonuclear spin–spin coupling patterns are displayed along the F_1-axis and heteronuclear coupling was removed by ^{13}C decoupling in order to simplify the

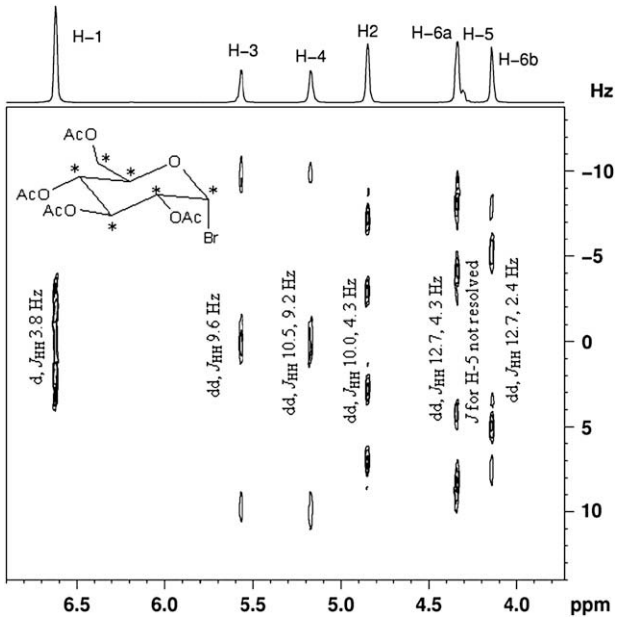


Figure 1. Homonuclear *J*-resolved spectrum of **2**.

spectrum. Thus, one can get rid of overlaps and easily obtain the ^{1}H chemical shifts and J_{HH} coupling constants. 2D ^{1}H,^{13}C HSQC without ^{13}C decoupling (Fig. 2) provides standard correlation of ^{1}H and ^{13}C chemical shifts. In addition, homonuclear ^{13}C,^{13}C coupling patterns are apparent along the F_1-axis and splitting of crosspeaks along the F_2-axis allows derivation of the $^{1}J_{HC}$ coupling constants.

3. Conclusion

In conclusion, we have demonstrated a general synthesis of stable isotopically labelled derivatives of glucosinolates from the commercially available [$^{13}C_6$]-D-glucose. Theoretically, any [*glucose*-$^{13}C_6$]glucosinolate can be achieved by this methodology by varying the hydroximoyl chloride required for the side chain, which would make accurate analysis of individual glucosinolates by LCMS practical. Further work on other [*glucose*-$^{13}C_6$]glucosinolates and [*glucose*-$^{13}C_6$]desulfoglucosinolates is underway.

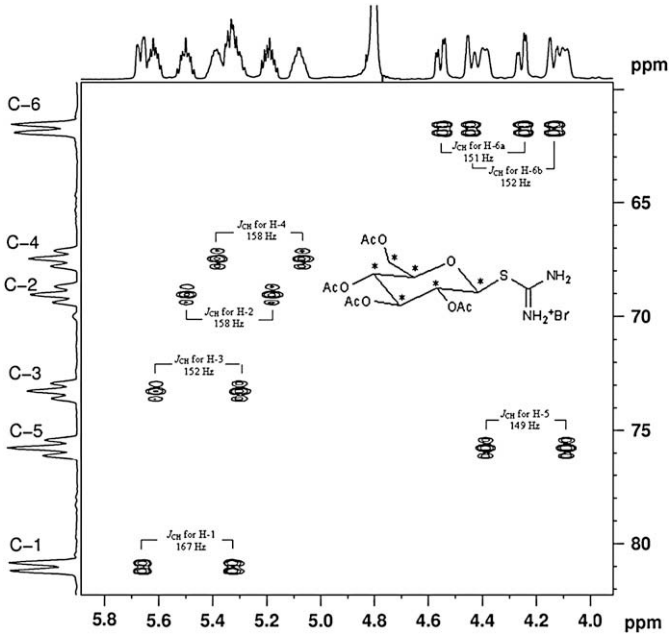


Figure 2. 2D ^{1}H,^{13}C HSQC without ^{13}C decoupling of **3**.

4. Experimental

4.1. General

[$^{13}C_6$]-D-glucose was purchased from ISOTEC of Sigma–Aldrich. Other reagents were used as supplied without further purification unless stated otherwise. Anhydrous tetrahydrofuran, diethyl ether and dichloromethane were obtained from a solvent purification system (MBraun, SPS-800). Pyridine, triethylamine and methanol were distilled over calcium hydride under an argon atmosphere. Acetone was dried over 4 Å molecular sieve for 48 h. Petrol is defined as petroleum ether 40–60 °C. All reactions were performed in overnight oven-dried glassware with magnetic stirring under an argon atmosphere. Air- and moisture-sensitive liquids and solutions were transferred by syringe or cannula and introduced into reaction vessels through rubber septa. Thin layer chromatography was performed on Kieselgel 60 F_{254} plastic plates pre-coated with a 0.25 mm thickness of silica gel. Visualisation was achieved by inspection under UV light (254 nm) followed by staining with either potassium permanganate or sulfuric acid/methanol dips. Column chromatography was performed on Kieselgel 60 (230–400 mesh) silica gel.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were acquired on a Bruker AV-400 spectrometer. Chemical shifts are reported as parts per million downfield from tetramethylsilane using residues of deuterated solvents as references. Connectivities were assigned using two-dimensional (COSY, HSQC) experiments. Coupling constants J_{CH}, J_{CC} and J_{HH} were denoted $^{1}J_{CH}$, $^{1}J_{CC}$ and $^{3}J_{HH}$, respectively, and were measured in hertz using 1D or 2D spectra and where necessary, the *J*-resolving experiments. Mass spectra were acquired by electrospray ionisation (ESI) on a Micromass LCT spectrometer. Infrared spectra were recorded as KBr discs on a Perkin Elmer 1420 instrument.

4.2. Synthetic procedure for [*glucose*-$^{13}C_6$]derivatives

*4.2.1. 2,3,4,6-Tetra-O-acetyl-α-D-[$^{13}C_6$]glucopyranosyl bromide (**2**)*

Hydrogen bromide in acetic acid (45% w/v 40 ml, 222 mmol) was added dropwise to [$^{13}C_6$]-D-glucose (25.0 g, 134 mmol) in acetic anhydride (100 ml, 1.06 mol) at −10 to 5 °C under an argon atmosphere. After 4 h, further 45% w/v hydrogen bromide in acetic acid (92 ml, 623 mmol) was added and the solution stirred at room temperature overnight. The reaction mixture was taken up in dichloromethane and poured onto ice/water. The organic phase was carefully neutralised with an ice/saturated sodium hydrogen carbonate solution. The organic layer was then washed with brine and dried over magnesium sulfate. The solvent was evaporated at reduced pressure to give the pale yellow oil, which solidified upon cooling to 0 °C. The product was recrystallised from diethyl ether to give **2** as a white crystalline solid (49.3 g, 88%), which is heat labile and should be stored in the freezer. Mp 89–90 °C (lit.[27] (unlabelled) 88–89 °C). [Found: C, 40.47%; H, 4.71. $^{13}C_6C_8H_{19}BrO_9$ requires C, 40.31; H, 4.59%.] $[\alpha]_D^{20}$ +194.8 (*c* 2.42, $CHCl_3$) (lit.[28] (unlabelled) +197. 84 (*c* 2.42, $CHCl_3$)); ν_{max} (KBr disc) 1744 (C=O), 1246, 1288, 1035 (COO) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 6.59 (1H, dd, J_{CH} 185 Hz, J_{HH} 3.8 Hz, H-1), 5.54 (1H, ddd, J_{CH} 149 Hz, 2×J_{HH} 9.6 Hz, H-3), 5.14 (1H, ddd, J_{CH} 147 Hz, J_{HH} 10.5 and 9.2 Hz, H-4), 4.82 (1H, ddd, J_{CH} 151 Hz, J_{HH} 10.0 and 4.3 Hz, H-2), 4.31 (1H, ddd, J_{CH} 147 Hz, $^{2}J_{HH}$ 12.7 Hz, J_{HH} 4.3 Hz, H-6a), 4.21 (1H, m, J_{CH} 146 Hz, J_{HH} not resolved, H-5), 4.12 (1H, ddd, J_{CH} 149 Hz, $^{2}J_{HH}$ 12.7 Hz, J_{HH} 2.4 Hz, H-6b), 2.23, 2.17, 1.97, 1.91 (12H, 4×s, 4×CH_3); δ_C (100.10 Hz, $CDCl_3$) 170.5, 169.9, 169.8, 169.5 (4×C=O), 86.5 (enhanced d, J_{CC} 38 Hz, C-1), 72.3 (enhanced dd, 2×J_{CC} 43 Hz, C-5), 71.1 (enhanced m, J_{CC} not resolved, C-3), 70.6

(enhanced m, J_{CC} not resolved, C-2), 67.1 (enhanced dd, 2×J_{CC} 39 Hz, C-4), 60.9 (enhanced d, J_{CC} 45 Hz, C-6), 20.7, 20.64, 20.56 (3×s, 4×CH_3); *m*/*z* (ES^+): 439 and 441 $(M+Na)^+$.

4.2.2. 2,3,4,6-Tetra-O-acetyl-β-D-[$^{13}C_6$]glucopyranosyl isothiouronium bromide (**3**)

Thiourea (3.01 g, 39.55 mmol) was added to **2** (16.5 g, 39.55 mmol) in dry acetone (40 ml) under argon. The solution was heated at reflux for 30 min and then cooled to 0 °C to give a white precipitate. The precipitate was removed by filtration followed by washing with acetone to give **3** as a white crystalline solid (13.55 g). The filtrate was concentrated and triturated with ethyl acetate. The precipitate was collected by filtration and washed with EtOAc and ice-cooled acetone successively to give another crop of product (2.0 g), which was not used for analysis but directly for next step of reaction. Total yield: 15.55 g, 80%. Mp 203–205 (lit.[29] (unlabelled) 205 °C). [Found: C, 36.68; H, 4.43; N, 5.48. $^{13}C_6C_9H_{23}BrN_2O_9S$ requires C, 36.51; H, 4.70; N, 5.68%.] $[\alpha]_D^{20}$ −17.0 (*c* 1.0, MeOH) (lit.[30] (unlabelled) −17.3 (*c* 1.0, MeOH)); ν_{max} (KBr disc) 3312, 3273, 3059, 3021 (NH), 1752 (C=O), 1657 (C=N), 1226, 1039 (COO) cm^{-1}; δ_H (400 MHz, D_2O) 5.49 (1H, dd, J_{CH} 167 Hz, J_{HH} 10.0 Hz, H-1), 5.45 (1H, ddd, J_{CH} 152 Hz, J_{HH} 9.5 Hz, H-3), 5.34 (1H, ddd, J_{CH} 158 Hz, J_{HH} 10.8, 8.9 Hz, H-2), 5.23 (2×0.5H, ddd, J_{CH} 158 Hz, J_{HH} 10.5 Hz, H-4), 4.41 (1H, ddd, J_{CH} 151 Hz, $^2J_{HH}$ 12.1 Hz, J_{HH} 4.3 Hz, H-6a), 4.29 (1H, m, J_{CH} 152 Hz, $^2J_{HH}$ 12.7 Hz, H-6b), 4.24 (1H, m, J_{CH} 149 Hz, H-5), 2.15, 2.13, 2.11, 2.08 (12H, 4×s, 4×CH_3); δ_C (100.10 Hz, D_2O) 173.5, 172.9, 172.5, 172.3 (4×C=O), 167.4 (C=N), 81.25 (enhanced d, J_{CC} 42 Hz, C-1), 75.6 (enhanced dd, 2×J_{CC} 42 Hz, C-5), 73.3 (enhanced dd, 2×J_{CC} 41 Hz, C-3), 69.0 (enhanced ddd, 2×J_{CC} 41 Hz, $^2J_{CC}$ 2.9 Hz, C-2), 67.5 (enhanced ddd, 2×J_{CC} 41 Hz, $^2J_{CC}$ 2.7 Hz, C-4), 61.8 (enhanced d, J_{CC} 44 Hz, C-6), 20.1, 20.0, 19.9 (3×s, 4×Ac CH_3); *m*/*z* (ES^+): 413 $(M-Br)^+$, 337 $(M-Br-NH_2C(S)NH_2)^+$; HRMS (ES^+): $(M-Br)^+$ found 413.1331, $^{13}C_6C_9H_{23}N_2O_9S$ requires 413.1326.

4.2.3. 2,3,4,6-Tetra-O-acetyl-1-thio-β-D-[$^{13}C_6$]glucopyranose (**4**)

Potassium metabisulfite (3.154 g, 14.19 mmol) and **3** (7.0 g, 14.19 mmol) were suspended in degassed water and dichloromethane (1:1, 30 ml). The biphasic solution was heated at reflux under an argon atmosphere for 30 min and then cooled to room temperature. The organic phase was washed with water (three times) and the aqueous layer washed with dichloromethane. The combined organic layers were dried ($MgSO_4$) and the solvent evaporated at reduced pressure to give a white gum. The product was recrystallised from methanol to give **3** as a white crystalline solid (4.88 g, 93%). Mp 113–115 °C (lit.[31] (unlabelled) 112–114 °C). [Found: C, 45.55; H, 5.58. $^{13}C_6C_8H_{20}O_9S$ requires C, 45.41; H, 5.44%.] $[\alpha]_D^{20}$ +5.7 (*c* 1.0, $CHCl_3$) (lit.[31] (unlabelled) +6.3 (*c* 1.2, $CHCl_3$)); ν_{max} (KBr disc) 2584 (S–H), 1744 (C=O), 1239, 1031 (COO) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.17 (1H, m, J_{CH} 148 Hz, J_{HH} 9.0 Hz, H-3), 5.08 (1H, m, J_{CH} 159 Hz, J_{HH} 9.9 Hz, H-4), 4.96 (1H, m, J_{CH} 150 Hz, J_{HH} 9.6 Hz, H-2), 4.54 (1H, m, J_{CH} 157 Hz, J_{HH} 9.8 Hz, H-1), 4.23 (1H, m, J_{CH} 149 Hz, $^2J_{HH}$ 12.4 Hz, J_{HH} 4.8 Hz, H-6a), 4.12 (1H, m, J_{CH} 150 Hz, $^2J_{HH}$ 12.4 Hz, J_{HH} 2.4 Hz, H-6b), 3.71 (1H, m, J_{CH} 143 Hz, H-5), 2.31 (1H, dt, J_{HH} 9.9 Hz, $^2J_{CH}=^3J_{CH}$ 3.9 Hz, SH), 2.10, 2.08, 2.03, 2.01 (12H, 4×s, 4×CH_3); δ_C (100.10 Hz, DMSO-d_6) 170.9, 170.3, 169.9, 169.5 (4×C=O), 78.7 (enhanced m, J_{CC} 46 Hz, $^2J_{CC}$ not resolved, C-1), 76.3 (enhanced dd, 2×J_{CC} 46 Hz, C-5), 73.8 (enhanced m, C-3), 73.4 (enhanced m, C-2), 68.0 (enhanced m, J_{CC} 47 Hz, C-4), 62.0 (enhanced d, J_{CC} 45 Hz, C-6), 21.0, 20.9 and 20.8 (3×s, partial overlaps, 4×CH_3); *m*/*z* (ES^+) 393 $(M+Na)^+$; HRMS (ES^+); $(M+Na)^+$ found 393.0925, $^{13}C_6C_8H_{20}O_9SNa$ requires 393.0927.

4.2.4. 2,3,4,6-Tetra-O-acetyl-β-D-[glucose-$^{13}C_6$]glucopyranosyl phenethylthiohydroximate (**6a**)

To a solution of 3-phenylpropionaldehyde oxime (1.2 g, 8.0 mmol) in DMF at 0 °C was added *N*-chlorosuccinimide (1.09 g, 8.0 mmol).[22,23] The mixture was stirred at rt overnight before diluting with water and extracting with diethyl ether. The combined extracts were dried over $MgSO_4$ and the solvent was evaporated under reduced pressure to give a pale yellow oil of phenethylhydroximate chloride **5a** (1.5 g, 100%). The oil (1.2 g, 6.5 mmol) was taken up in anhydrous THF (30 ml) and **4** (1.7 g, 4.6 mmol) was added. To this solution was added triethylamine (4.5 ml, 32 mmol). The mixture was stirred overnight before being poured onto ice-water (200 ml). The precipitate was collected by filtration and washed with copious amounts of water before being air dried. The crude product was purified by column chromatography (SiO_2, EtOAc/petrol=1:2) to give **6a** as a white solid (2.21 g, 93%). Mp 205–207 °C (lit.[26] (unlabelled) 198 °C). [Found: C, 53.51; H, 5.76; N, 2.76. $^{13}C_6C_{17}H_{29}NO_{10}S$ requires C, 53.38; H, 5.65; N, 2.71%.] $[\alpha]_D^{20}$ −10.6 (*c* 1.0, $CHCl_3$) (lit.[32] (unlabelled) −11 (*c* 2.0, CCl_4)); ν_{max} (KBr disc) 3316 (OH), 1746, 1713 (C=O, C=N), 1253, 1226, 1034 (COO) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 11.31 (1H, s, OH), 7.30–7.17 (5H, m, Ph H), 5.61 (1H, m, J_{CH} 164 Hz, J_{HH} 10.2 Hz, H-1), 5.47 (1H, dm, J_{CH} 152 Hz, 2×J_{HH} 9.9 Hz, H-2), 4.89 (2×1H, m, J_{CH} 152 Hz, 4×J_{HH} 9.8 Hz, H-2 and H-4), 4.14 (1H, m, J_{CH} 147 Hz, H-5), 4.01 (2×1H, m, J_{CH} 147 Hz, H-6), 2.95–2.76 (4H, m, 2×CH_2), 2.02, 1.99, 1.96, 1.76 (12H, 4×s, 4×CH_3); δ_C (100.10 Hz, DMSO-d_6) 169.9, 169.5, 169.3, 169.1 (4×C=O), 149.1 (C=N), 140.9, 128.3, 128.2, 125.9 (4×s, Ph-C), 77.8 (enhanced d, J_{CC} 42 Hz, C-1), 74.3 (enhanced dd, 2×J_{CC} 43 Hz, C-5,), 72.7 (enhanced dd, 2×J_{CC} 41 Hz, C-3), 69.6 (enhanced dd, 2×J_{CC} 41 Hz, C-2), 68.1 (enhanced dd, 2×J_{CC} 41 Hz, C-4), 62.2 (enhanced d, *J* 45 Hz, C-6), 32.3 (s, 2×CH_2), 20.3, 20.2, 20.1 (3×s, overlapped, 4×CH_3); *m*/*z* (ES^+) 540 $(M+Na)^+$, HRMS (ES^+): $(M+Na)^+$ found 540.1592, $^{13}C_6C_{17}H_{29}NO_{10}SNa$ requires 540.1611.

4.2.5. 2,3,4,6-Tetra-O-acetyl-β-D-[glucose-$^{13}C_6$]glucopyranosyl gluconasturtiin (**7a**)

To a solution of **6a** (2.18 g, 4.21 mmol) in DCM (10 ml) were added pyridine sulfur trioxide (3.35 g, 21.1 mmol) and pyridine (3.4 ml, 42.1 mmol). The mixture was heated under reflux for 4 h and cooled to room temperature. Potassium hydrogen carbonate (2 M, 35 ml, 70 mmol) was added and the mixture was stirred for another half an hour and then the two-phase mixture was concentrated under reduced pressure. The solid residue was washed with aqueous $KHCO_3$ (2 M), cold water, cold EtOH and diethyl ether successively and the residue was purified by chromatography (SiO_2, DCM/MeOH=100:20) to give the product as a white solid (2.23 g, 83%). Mp 200–202 °C dec (lit.[32] (unlabelled) 198–200 °C dec). [Found: C, 42.48; H, 4.41; N, 2.10. $^{13}C_6C_{17}H_{28}NO_{13}S_2K\cdot H_2O$ requires C, 42.26; H, 4.63; N 2.14%.] $[\alpha]_D^{20}$ −12.0 (*c* 0.5, MeOH) (lit.[32] (unlabelled) −11 (*c* 1.0, 50% aq EtOH)); ν_{max} (KBr disc) 1751 (C=O), 1227, 1063 ($ROSO_3^-$), 1035 (COO) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 7.36–7.28 (5H, m, PhH), 5.68 (1H, dd, J_{CH} 163 Hz, J_{HH} 8.6 Hz, H-1), 5.48 (1H, m, J_{CH} 152 Hz, J_{HH} 9.7 Hz, H-3), 4.91 (2×1H, m, J_{CH} 152 Hz, J_{HH} 10.1 Hz, H-2+H-4), 4.13 (1H, m, J_{CH} 146 Hz, H-5), 4.00 (2×1H, m, J_{CH} 147 Hz, H-6), 2.95–2.80 (4H, m, 2×CH_2), 2.04, 2.00, 1.96, 1.73 (12H, 4×s, 4×CH_3); δ_C (100.10 Hz, DMSO-d_6) 169.6, 169.4 (C=O), (C=N not observed due to the strong solvent peak), 140.8, 128.4, 128.3, 126.1 (4×Ph C), 77.9 (enhanced d, J_{CC} 42 Hz, C-1), 74.4 (enhanced dd, 2×J_{CC} 43 Hz, C-5), 72.7 (enhanced dd, 2×J_{CC} 41 Hz, C-3), 69.5 (enhanced dd, 2×J_{CC} 41 Hz, C-2), 68.1 (enhanced dd, 2×J_{CC} 46 Hz, C-4), 62.2 (enhanced d, J_{CC} 44 Hz, C-6), 33.0 (CH_2), 32.4 (CH_2), 20.4, 20.3, 20.1 (3×s, overlapped at δ 20.4, 4×CH_3); *m*/*z* (ES^-) 596 $(M-K)^-$; HRMS (ES^-): $(M-K)^-$, found 596.1214. $^{13}C_6C_{17}H_{28}NO_{13}S_2$ requires 596.1203.

4.2.6. [glucose-$^{13}C_6$]Gluconasturtiin (**8a**)

To a suspension of **7a** (0.66 g, 1.0 mmol) in methanol (30 ml) was added a catalytic amount of potassium metal under argon. The reaction mixture was stirred overnight before Dowex-50 was added to neutralise the solution. Stirring was continued for

a further 30 min before the resin was removed by filtration and the solvent evaporated at reduced pressure to give the product as a gum. The gum was taken up in water and the solution was freeze dried to give a white amorphous powder (0.402 g, 83%). Mp 164 °C dec (lit.[32] (unlabelled) 170–172 °C dec). [Found: C, 36.16; H, 3.72; N, 2.78. $^{13}C_6C_9H_{20}NO_9S_2K\cdot 0.5KHCO_3$ requires C, 35.97; H, 3.99; N, 2.71%.] $[\alpha]_D^{20}$ −22 (*c* 1.0, H_2O) (lit.[32] (unlabelled) −23 (*c* 1.0, H_2O)); IR (KBr disc) 3424 (OH), 1237, 1039 ($ROSO_3^-$) cm^{-1}; δ_H (400 MHz, D_2O) 7.41–7.28 (5H, m, Ph), 4.99 (1H, dd, J_{CH} 161 Hz, J_{HH} 9.1 Hz, H-1), 3.94 (1H, m, J_{CH} 147 Hz, $^2J_{HH}$ 12.0 Hz, H-6a), 3.77 (1H, m, J_{CH} 147 Hz, $^2J_{HH}$ 12.0 Hz, H-6b), 3.59 (2×0. 5H, m, J_{CH} 141 Hz, H-3), 3.56 (1H, m, J_{CH} 144 Hz, H-5), 3.54 (2×1H, m, J_{CH} 148 Hz, H-2+H-4), 3.12–2.97 (4H, m, 2×CH_2); δ_C (100.10 Hz, D_2O) 163.3 (C═N), 140.5, 128.71, 128.68, 126.6 (4×Ph C), 81.6 (enhanced d, J_{CC} 40 Hz, C-1), 80.0 (enhanced dd, 2×J_{CC} 42 Hz, C-5), 77.0 (enhanced dd, 2×J_{CC} 39 Hz, C-3), 71.8 (enhanced dd, 2×J_{CC} 39 Hz, C-2), 68.1 (enhanced dd, 2×J_{CC} 40 Hz, C-4), 62.2 (enhanced d, J_{CC} 43 Hz, C-6), 33.9, 32.5 (2×CH_2); *m*/*z* (ES^-) 428 (M−K^+); HRMS (ES^-): [M−K]$^-$, found 428.0785. $^{13}C_6C_9H_{20}NO_9S_2$ requires: 428.0781.

4.2.7. 2,3,4,6-Tetra-O-acetyl-β-D-[glucose-$^{13}C_6$]glucopyranosyl allylthiohydroximate (**6b**)

To a suspension of sodium 4-nitrobut-1-ene[16] (1.11 g, 9 mmol) in Et_2O (100 ml) cooled to −78 °C was added dropwise 4 M HCl in dioxane (16 ml, 72 mmol). The suspension gradually changed from yellow to bright blue. The suspension was further stirred for 30 min before the temperature was raised to −40 °C and triethylamine was added dropwise. The blue colour of the suspension changed back to pale brown to which **4** (1.6 g, 4.32 mmol) in THF (30 ml) was added. The mixture was left stirring at room temperature overnight and then taken up in water (200 ml). The layers were separated and the aqueous layer was extracted with EtOAc (two times). The combined extracts were rinsed with water and brine successively and the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, EtOAc/petrol) to give the product as a white powder (1.56 g, 80%). Mp 168–170 °C (lit.[20] (unlabelled) 164–165 °C). [Found: C, 47.75; H, 5.23; N, 2.92. $^{13}C_6C_{12}H_{25}NO_{10}S$ requires C, 47.68; H, 5.56; N, 3.09%.] $[\alpha]_D^{20}$ −12.2 (*c* 0.14, $CHCl_3$) (lit.[20] (unlabelled) −13 (*c* 0.14, $CHCl_3$)); ν_{max} (KBr disc) 3310 (OH), 1750, 1707 (C═O, C═N), 1605 (C═C), 1253, 1220 (COO^-) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 11.37 (1H, s, OH), 5.95 (1H, ddd, J_{HH} 17.1, 10.2, 6.2 Hz, H-2′), 5.44 (1H, d, J_{CH} 167 Hz, J_{HH} 8.4 Hz, H-1), 5.37 (1H, m, 1H, J_{CH} 151 Hz, H-3), 5.18 (1H, ddt, *J* 17.2, 1.6, 1.2 Hz, H-3′), 5.11 (1H, ddt, J_{HH} 10.08, 1.6, 1.2 Hz, H-3′), 4.91 (1H, m, J_{CH} 154 Hz, H-4), 4.85 (1H, m, J_{CH} 155 Hz, H-2), 4.10 (1H, m, J_{CH} 149 Hz, H-5), 4.06 (2×1H, m, J_{CH} 149 Hz, H-6), 3.30 (2H, m, H-1′), 2.01, 2.00, 1.99, 1.96 (12H, 4×s, 4×CH_3); δ_C (100.10 Hz, DMSO-d_6) 169.9, 169.6, 169.3, 169.1 (4×C═O), C═N not observed due to the strong signal of the solvent and the enhanced signal of ^{13}C labelling atoms, 133.8 (C-2′), 116.9 (C-3′), 78.0 (enhanced d, J_{CC} 42 Hz, C-1), 74.3 (enhanced dd, 2×J_{CC} 42 Hz, C-5), 72.8 (enhanced dd, 2×J_{CC} 41 Hz, C-3), 69.7 (enhanced dd, 2×J_{CC} 40 Hz, C-2), 68.0 (enhanced dd, 2×J_{CC} 41 Hz, C-4), 62.1 (enhanced d, J_{CC} 45 Hz, C-6), 35.7 (C-1′), 20.47, 20.3 (2×s, overlapped, 4×CH_3); *m*/*z* (ES^+) 476 (M+Na)$^+$; HRESIMS$^+$ (ES^+): 476.1299, $^{13}C_6C_{12}H_{25}NO_{10}SNa$ requires: 476.1298.

4.2.8. 2,3,4,6-Tetra-O-acetyl-β-D-[glucose-$^{13}C_6$]glucopyranosyl sinigrin (**7b**)

To a solution of **6a** (1.427 g, 3.15 mmol) in DCM (60 ml) were added pyridine sulfur trioxide (2.51 g, 15.77 mmol) and pyridine (1.5 ml, 18.6 mmol). The mixture was heated under reflux for 6 h and cooled to room temperature and stirred overnight. Aqueous potassium hydrogen carbonate solution (2 M, 20 ml, 40 mmol) was added and the mixture was stirred for 30 min and then the two-phase mixture was concentrated under reduced pressure. The solid residue was washed with aqueous $KHCO_3$ (2 M), cold water, cold EtOH and diethyl ether successively to get the crude product. The residue was purified by chromatography (SiO_2, DCM/MeOH=100:20) to get the product as a white solid: (0.875 g, 47%). Mp 185–195 °C dec (lit.[20] (unlabelled) 193–195 °C). [Found: C, 37.77; H, 3.93; N, 2.20. $^{13}C_6C_{12}H_{24}NO_{13}S_2K$ requires C, 37.83; H, 4.23; N, 2.45%.] $[\alpha]_D^{20}$ −14.4 (*c* 0.14, H_2O) (lit.[20] (unlabelled) −16 (*c* 0.14, H_2O)); ν_{max} (KBr disc) 1751 (C═O), 1283, 1244, 1061 ($ROSO_3^-$) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 5.96 (1H, ddd, J_{HH} 17.0, 10.4, 6.2 Hz, H-2′), 5.47 (1H, dd, J_{CH} 163 Hz, J_{HH} 9.5 Hz, H-1), 5.36 (1H, m, J_{CH} 150 Hz, H-3), 5.23 (1H, dd, J_{HH} 17.2 and 1.5 Hz, H-3′), 5.16 (1H, dd, J_{HH} 10.2 and 1.3 Hz, H-3′), 4.91 (1H, m, J_{CH} 151 Hz, H-4), 4.85 (1H, m, J_{CH} 151 Hz, H-2), 4.09 (1H, m, J_{CH} 147 Hz, H-5), 4.05 (2×1H, m, J_{CH} 147 Hz, H-6), 3.37 (1H, m, H-1′), 2.02, 2.01, 2.00, 1.96 (12H, 4s, 4×CH_3); δ_C (100.10 Hz, DMSO-d_6) 170.0, 169.6, 169.3, 169.1 (4×C═O), 152.3 (C═N), 133.1 (C-2′), 117.52 (C-3′), 78.1 (enhanced d, J_{CC} 42 Hz, C-1), 74.3 (enhanced dd, 2×J_{CC} 43 Hz, C-5), 72.8 (enhanced dd, 2×J_{CC} 41 Hz, C-3), 69.5 (enhanced dd, 2×J_{CC} 41 Hz, C-2), 67.9 (enhanced dd, 2×J_{CC} 40 Hz, C-4), 62.0 (enhanced d, J_{CC} 44 Hz, C-6), 35.7 (C-1′), 20.5, 20.4, 20.3 (partially overlapped, 4×CH_3); *m*/*z* (ES^+) 594 (M+Na)$^+$; 610 (M+K)$^+$; *m*/*z* (ES^-) 532 (M−K)$^-$; HRMS (ES^-): [M−K]$^-$ found 532.0895, $^{13}C_6C_{12}H_{24}NO_{13}S_2$ requires: 532.0890.

4.2.9. [glucose-$^{13}C_6$]Sinigrin (**8b**)

To a suspension of **7b** (0.818 g, 1.43 mmol) in MeOH (30 ml) was added a catalytic amount of MeOK in MeOH. The mixture was stirred at room temperature for overnight. The solution was carefully neutralised with Dowex-50 to pH 7.0 and then filtered through a pad of Celite and concentrated under reduced pressure at room temperature. The residue was dissolved in the smallest amount of H_2O. To this aqueous solution was added ethanol until cloudy. The mixture was stored at 4 °C overnight. The product precipitated out as colourless needle crystals (0.475 g, 82%). Mp 128–130 °C (lit.[20] (unlabelled) 125–127 °C). [Found, C, 28.91; H, 4.19; N, 3.15. $^{13}C_6C_4H_{16}NO_9S_2K\cdot H_2O$ requires C, 29.12; H, 4.15; N, 3.39%.] $[\alpha]_D^{20}$ −15.9 (*c* 0.2, H_2O) (lit.[20] (unlabelled) −17.0 (*c* 0.2, H_2O)); ν_{max} (KBr disc) 3492, 3406, 3186 (OH), 1650 (C═C), 1574 (C═N), 1274, 1240, 1087, 1055, 1010 ($ROSO_3^-$) cm^{-1}; δ_H (400 MHz, D_2O) 6.03 (1H, ddd, J_{HH} 17.2, 10.4 and 6.2 Hz, H-2′), 5.32 (1H, ddt, J_{HH} 17.2, 1.7 and 1.2 Hz, H-3′), 5.28 (1H, ddt, J_{HH} 10.4, 1.7 and 1.3 Hz, H-3′), 5.05 (1H, dd, J_{CH} 160.3 Hz, J_{HH} 9.8 Hz, H-1), 4.13 (1H, m, J_{CH} 143 Hz, $^2J_{HH}$ 12.5 Hz, H-6a), 3.94 (1H, ddd, J_{CH} 143 Hz, $^2J_{HH}$ 12.5 Hz, J_{HH} 5.6 Hz, H-6b), 3.54 (1H, m, J_{CH} 142 Hz, H-5), 3.53 (1H, m, J_{CH} 143 Hz, H-3), 3.45 (1H, H m, J_{CH} 148 Hz, H-4), 3.44 (2×0.5H, m, J_{CH} 146 Hz, H-2), 3.56–3.50 (4H, m, H-3+H-5+2× H-1′), 3.47–3.42 (2H, m, H-2+H-4); δ_C (100.10 Hz, D_2O) 163.0 (C═N), 132.0 (C-2′), 118.3 (C-3′), 81.5 (enhanced d, J_{CC} 40 Hz, C-1), 79.9 (enhanced dd, 2×J_{CC} 42 Hz, C-5), 77.0 (enhanced dd, 2×J_{CC} 39 Hz, C-3), 71.8 (enhanced dd, 2×J_{CC} 39 Hz, C-2), 69.0 (enhanced dd, 2×J_{CC} 40 Hz, C-4), 60.5 (enhanced d, J_{CC} 43 Hz, C-6), 36.4 (C-1′); *m*/*z* (ES^-) 364 (M−K^+); HRMS (ES^-): (M−K)$^-$ found 364.0470, $^{13}C_6C_4H_{16}NO_9S_2$ requires: 364.0468.

4.2.10. 2,3,4,6-Tetra-O-acetyl-β-D-[glucose-$^{13}C_6$]glucopyranosyl-4-methythiobutylthiohydroximate (**6c**)

To a suspension of sodium 1-methylthio-5-nitropentane[16,25] (0.58 g, 3.13 mmol) in Et_2O (100 ml) cooled to −78 °C was added dropwise 4 M HCl in dioxane (1.57 ml, 6.3 mmol). The suspension gradually changed from yellow to bright blue. The suspension was further stirred for 30 min before the temperature was raised to −40 °C and triethylamine (2.78 ml, 12.2 mmol) was added dropwise. The blue suspension changed back to pale brown and **4** (0.65 g, 1.76 mmol) was added. The mixture was allowed to warm up to room temperature and left stirring overnight and then taken up in water. The layers were separated and the aqueous layer was further extracted with EtOAc (two times). The combined extracts were rinsed with water and brine successively, dried over $MgSO_4$

and the solvent was removed under reduced pressure. The residue was recrystallised from Et_2O to give the first crop of product as a white solid (0.20 g). The mother liquor was concentrated under reduced pressure and the residue was purified by column chromatography [SiO_2, EtOAc/petrol=1:1] to give the second crop of product total yield: 0.456 g, 50%. Mp 132–134 °C. [Found: C, 46.89; H, 5.87; N, 2.74. $^{13}C_6C_{14}H_{31}NO_{10}S_2$ requires C, 46.59; H, 6.06; N, 2.72%.] $[\alpha]_D^{20}$ −16.7 (*c* 1.0, $CHCl_3$) (lit.[24] (unlabelled) −17 (*c* 1.0, $CHCl_3$)); IR (KBr disc) 3320 (OH), 1747, 1712 (C=O), 1612 (C=N), 1253, 1227 (COO) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.02 (1H, s, OH), 5.27 (2×0.5H, m, J_{CH} 151 Hz, H-3), 5.02 (1H, m, J_{CH} 156 Hz, H-4), 5.01 (1H, m, J_{CH} 161 Hz, H-1), 4.99 (1H, m, J_{CH} 155 Hz, H-2), 4.12 (1H, m, J_{CH} 150 Hz, H-6a), 4.09 (1H, m, J_{CH} 149 Hz, H-6b), 3.707 (2×0.5H, m, J_{CH} 145 Hz, H-5), 2.55 (4H, m, H-1′+H-4′), 2.11, 2.09, 2.06, 2.04, 2.02 (15H, 5×s, SCH_3+4×CH_3), 1.82–1.65 (4H, m, H-2′+H-3′); δ_C (100.10 Hz, DMSO-d_6) 170.8, 170.4, 169.6 and 169.4 (4×C=O), 152.2 (C=N), 80.1 (enhanced d, J_{CC} 42 Hz, C-1), 76.2 (enhanced dd, 2×J_{CC} 43 Hz, C-5), 74.0 (enhanced dd, 2×J_{CC} 41 Hz, C-3), 70.3 (enhanced dd, 2×J_{CC} 42 Hz, C-4), 68.3 (enhanced dd, 2×J_{CC} 42 Hz, C-2), 62.4 (enhanced d, J_{CC} 45 Hz, C-6), 33.9, 32.3, 28.4, 26.1 (4×s, 4×CH_2), 20.93, 20.86, 20.78 (3×s, overlapped, 4×CH_3), 15.7 (SCH_3); *m*/*z* (ES^+) 538 $(M+Na)^+$; HRMS (ES^+): $(M+Na)^+$ found 538.1473, $^{13}C_6C_{14}H_{31}NO_{10}S_2Na$ requires 538.1488.

4.2.11. 2,3,4,6-Tetra-O-acetyl-β-D-[glucose-$^{13}C_6$]glucopyranosyl-4-methythiobutyl-O-sulfate thiohydroximate (**7c**)

To a solution of **6c** (0.200 g, 0.388 mmol) in DCM (10 ml) were added pyridine sulfur trioxide (0.3 g, 1.88 mmol) and pyridine (0.1 ml, 1.2 mmol). The mixture was heated under reflux for 4 h and cooled to room temperature. Potassium hydrogen carbonate (2 M, 10 ml, 20 mmol) was added and the mixture was stirred for another half an hour and then the two-phase mixture was concentrated under reduced pressure. The residue was filtered and then washed with ice cold aqueous $KHCO_3$ (2 M), cold water, cold EtOH and diethyl ether successively to get the product as a white solid (0.189 g, 77%). Mp 174–176 °C. [Found: C, 35.90; H, 4.31; N, 2.10. $^{13}C_6C_{14}H_{30}NO_{13}S_3K\cdot 0.5KHCO_3$ requires C, 36.00; H, 4.49; N, 2.05%.] $[\alpha]_D^{20}$ −18.8 (*c* 1.0, H_2O); ν_{max} (KBr disc) 1751 (C=O), 1629, 1560 (C=N), 1279, 1236, 1064, 1035 ($ROSO_3^-$ and COO) cm^{-1}; δ_H (300 MHz, DMSO-d_6) 5.51 (2×0.5H, dd, J_{CH} 165 Hz, J_{HH} 10.0 Hz, H-1), 5.45 (2×0.5H, m, J_{CH} 153 Hz, H-3), 4.93 (2×0.5H, m, J_{CH} 154 Hz, H-4), 4.87 (2×0.5H, m, J_{CH} 157 Hz, H-2), 4.13 (1H, m, J_{CH} 152 Hz, H-5), 4.05 (2×1H, m, J_{CH} 148 Hz, H-6), 2.57 (4H, 2×CH_2 m, H-1′+H-4′), 2.05, 2.03, 2.02, 2.00, 1.96 (15H, 5×s, 4×CH_3+SCH_3), 1.64 (4H, m, 2×CH_2); δ_C (100.10 Hz, DMSO-d_6) 170.0, 169.6, 169.3, 169.1 (4×C=O), 153.5 (C=N), 78.1 (enhanced d, J_{CC} 42 Hz, C-1), 74.3 (enhanced dd, 2×J_{CC} 43 Hz, C-5), 72.7 (enhanced dd, 2×J_{CC} 41 Hz, C-3), 69.5 (enhanced dd, 2×J_{CC} 41 Hz, C-2), 68.0 (enhanced dd, 2×J_{CC} 41 Hz, C-4), 62.2 (enhanced d, J_{CC} 44.3 Hz, C-6), 32.7, 31.0, 27.9, 25.7 (4×CH_2), 20.5, 20.4, 20.3 (acetyl CH_3), 14.5 (SCH_3); *m*/*z* (ES^+) 656 (M+Na), 672 (M+K); *m*/*z* (ES^-) 594 $[M-K]^-$; HRMS (ES^-): $[M-K]^-$ found 594.1060, $^{13}C_6C_{14}H_{30}NO_{13}S_3$ requires 594.1081.

4.2.12. [glucose-$^{13}C_6$]Glucoerucin (**8c**)

To a solution of **7c** (0.148 g, 0.2 mmol) in MeOH (30 ml) was added a catalytic amount of MeOK in MeOH. The mixture was stirred at room temperature overnight. The solution was neutralised carefully with Dowex-50 ion-exchange resin to pH 7.0 and filtered through a pad of Celite and concentrated at room temperature. The residue was dissolved in H_2O (10 ml) and the solution was freeze dried to give the product as a fluffy white solid (108 mg, 100%). Mp 158–162 °C dec. [Found: C, 30.65; H, 4.54; N, 2.91. $^{13}C_6C_6H_{22}NO_9S_3K$ requires C, 30.95; H, 4.76; N, 3.00%.] $[\alpha]_D^{20}$ −19.6 (*c* 1.0, H_2O) (lit.[24] (unlabelled) −20 (*c* 1.0, H_2O)); ν_{max} (KBr disc) 3423 (br s, OH), 1579 (C=N), 1274, 1240, 1087, 1055, 1010 ($ROSO_3^-$) cm^{-1}; δ_H (300 MHz, D_2O) 5.05 (1H, dd, J_{CH} 160 Hz, J_{HH} 9.5 Hz, H-1), 3.90 (1H, dd, J_{CH} 144 Hz, J_{HH} 12.3 Hz, H-6a), 3.64 (1H, ddd, J_{CH} 142 Hz, J_{HH} 12.5 and 5.6 Hz, H-6b), 3.51 (1H, m, J_{CH} 142.8 Hz, H-5), 3.50 (1H, m, J_{CH} 144 Hz, H-3), 3.40 (1H, m, J_{CH} 145 Hz, H-4), 3.39 (1H, m, J_{CH} 148 Hz, H-2), 2.74 (2H, t, J_{HH} 7.4 Hz, CH_2), 2.59 (2H, t, J_{HH} 7.1 Hz, CH_2), 2.10 (3H, s, SCH_3), 1.82 (2H, quintet, J_{HH} 7.4 Hz, CH_2), 1.72 (2H, quintet, J_{HH} 7.0 Hz, CH_2); δ_C (100.10 Hz, D_2O) 164.3 (C=N), 81.7 (enhanced d, J_{CC} 40 Hz, C-1), 80.1 (enhanced dd, 2×J_{CC} 41 Hz, C-5), 77.0 (enhanced dd, 2×J_{CC} 39 Hz, C-3), 71.8 (enhanced dd, 2×J_{CC} 39 Hz, C-2), 69.0 (enhanced dd, 2×J_{CC} 39 Hz, C-4), 60.5 (enhanced d, J_{CC} 43 Hz, C-6), 32.5, 31.6, 27.2, 25.6 (4×CH_2), 13.9 (SCH_3); *m*/*z* (ES^-) 426 $(M-K)^-$; HRMS (ES^-): $[M-K]^-$ found 426.0676, $^{13}C_6C_6H_{22}NO_9S_3$ requires: 426.0658.

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