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The synthesis of isotopically labelled glucoraphanin for metabolic studies

John J. Morrison and Nigel P. Botting*

School of Chemistry, University of St. Andrews, St. Andrews, Fife KY16 9ST, United Kingdom

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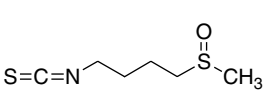
Abstract—The first synthesis of a stable isotopically labelled derivative of the glucosinolate glucoraphanin, namely [10-^{13}C,11,12-$^{2}H_5$]glucoraphanin, is described. This also represents the first total chemical synthesis of glucoraphanin itself.

There is convincing evidence from both epidemiological and animal studies that consumption of broccoli and other cruciferous vegetables is associated with a decreased risk of cancer and this association is the strongest for cancers of the gastrointestinal and respiratory tracts.[1] The anti-cancer effects have been attributed to the high content of specific phytochemicals known as glucosinolates in these vegetables. Glucosinolates **1**, are a class of naturally occurring thioglucosides, which are metabolised by the plant enzyme myrosinase during food preparation, cooking and chewing (Scheme 1).[2,3] The major product of this metabolism is the corresponding isothiocyanate **4**, formed via a Lossen-type rearrangement of the unstable thiohydroximate-*O*-sulfonate aglycon initially produced. In plants, glucosinolates and myrosinase are compartmentalised and only interact following tissue damage. In mammals, there also appears to be myrosinase activity in intestinal bacteria, which may contribute to glucosinolate degradation in vivo.[4] The aglycon can also break down to give nitriles **5** and thiocyanates **6**, depending on the conditions and presence of other factors such as ESP (epithiospecifier protein) and ferrous ions.[5]

The anti-cancer activity is, allegedly, due to the up-regulation of the xenobiotic-detoxifying Phase-II enzymes and/or the down regulation of the xenobiotic-activating Phase-I enzymes by the isothiocyanates derived from the glucosinolates.[6] Particular attention has been devoted to isothiocyanate sulforaphane **7** as being the major anti-carcinogenic compound in broccoli because of its ability to mono-induce Phase-II enzymes.[7] However, it is important to note that when broccoli is consumed, humans are not directly exposed to sulforaphane, but instead, to its glucosinolate precursor glucoraphanin **8**. The necessary enzymatic hydrolysis to release sulforaphane can take place following tissue disruption by chewing of vegetables, although cooking may inactivate myrosinase and then hydrolysis must be catalysed by the bacterial enzymes in the intestinal tract. The issue of human exposure is therefore complex and as yet poorly understood. In order to begin to answer some of the questions regarding the metabolism and bioavailability of glucosinolates and isothiocyanates a stable isotopically labelled derivative of glucoraphanin has been prepared for use in metabolic studies, to identify possible new biomarkers of exposure to glucosinolates and their metabolites. Prior to this study no total synthesis of glucoraphanin had been previously published, although the methylsulfanyl analogue, glucoerucin, had been prepared.[8] However, a semi-synthetic method for glucoraphanin has also been developed, employing chemoselective oxidation of glucoerucin, isolated from *Eruca sativa*.[9]



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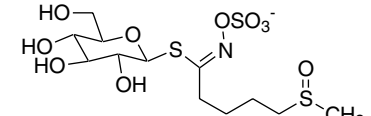

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The key-step in glucosinolate synthesis is the stereospecific coupling of 2,3,4,6-tetra-*O*-acetyl-l-thio-β-D-gluco-

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* Corresponding author. Tel.: +44 1334 463856; e-mail: npb@st-andrews.ac.uk

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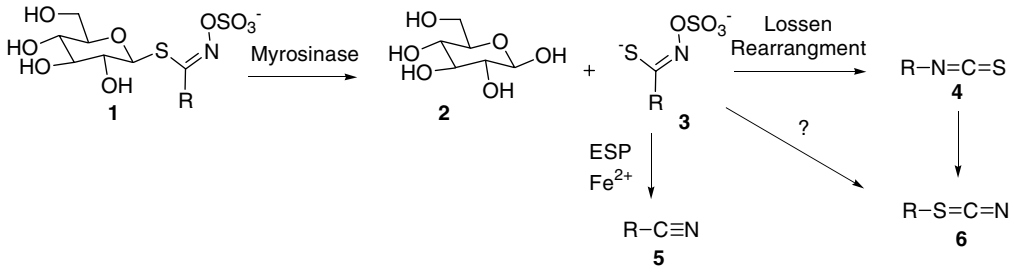
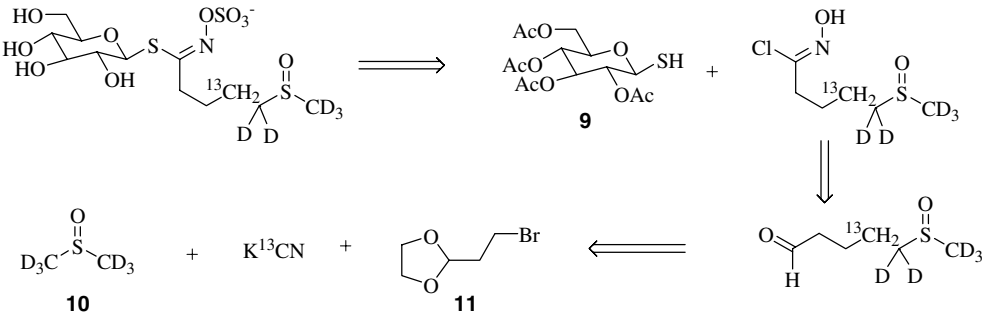


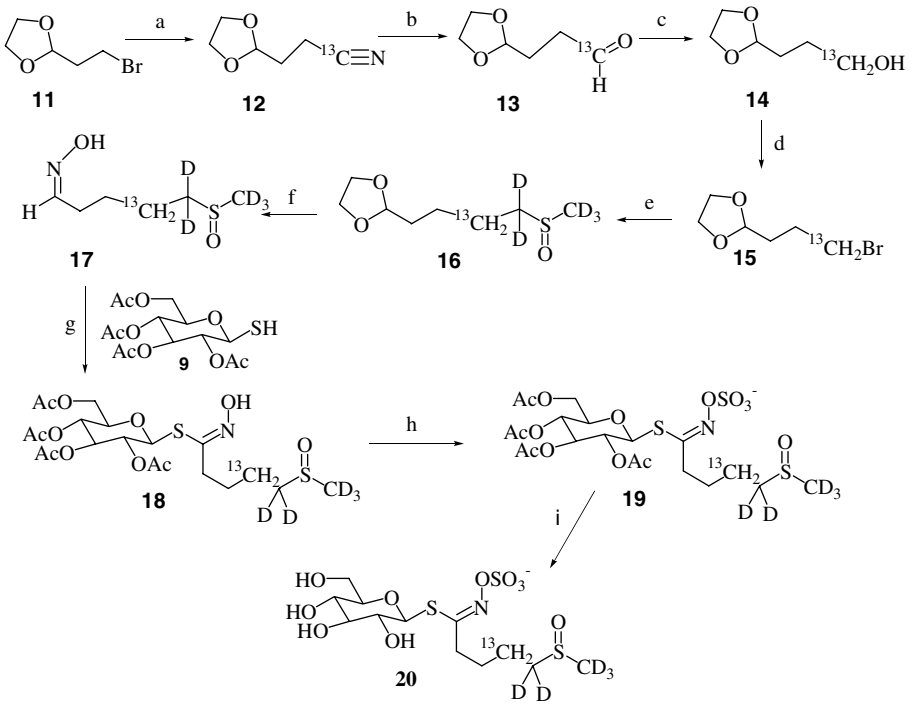
Scheme 1. General metabolism for glucosinolates.

pyranose **9** with the appropriate hydroximoyl chloride,[10] and so the target was to synthesise an isotopically labelled 5-carbon chain aldehyde with terminal methyl sulfoxide functionality. After some preliminary studies it was decided to use dioxolane protection for the aldehyde during the early steps of the synthesis. For the isotopic labelling, [$^2H_6$]dimethyl sulfoxide **10** was used to provide the end of the side chain and one mole of [^{13}C]cyanide ion was used for a chain extension reaction, to provide an overall six mass unit increase over the unlabelled glucoraphanin. The retrosynthesis is shown in Scheme 2.

The commercially available bromoalkyldioxolane **11** was thus reacted with potassium [^{13}C]cyanide under aqueous conditions to give nitrile **12** in 75% yield (Scheme 3). The next step was reduction of the nitrile to aldehyde **13** using DIBAL-H, however, careful isolation of the product was required otherwise hydrolysis of the dioxolane took place to give an unwanted dialdehyde. The first attempts only gave a 17% yield but then a modified, milder, work-up using silica gel and tartaric acid was discovered in the literature,[11] which gave good yields on a small scale and with some modification gave a respectable 83% yield on scale-up. Sodium borohydride smoothly reduced the aldehyde to alcohol **14**, however, the subsequent bromination to give **15** proved to be challenging. The triphenylphosphine/carbon tetrabromide system, commonly used in our laboratory as a mild non-acidic bromination method, was employed for this step as there were concerns that acid would be likely to open the dioxolane. This worked well on a small scale but for preparative purposes the removal of the by-products, that is Ph_3PO and CBr_4, made purification very difficult. Eventually, following optimisation of the purification procedure, the bromide was obtained in 61% yield. Alkylation of bromide **15** with the anion of [$^2H_6$]DMSO proceeded in excellent yield as this had been thoroughly investigated in the model studies. Normally simple deprotection of the dioxolane under aqueous acidic conditions would afford the isolable aldehyde, but in this case problems were encountered due to the formation of condensation products and despite several attempts, the aldehyde could not be isolated. This problem was overcome by carrying out the deprotection in the presence of excess hydroxylamine in order to trap out the aldehyde in situ, before competing condensation reactions could take place, and thus form the desired oxime **17** in one step. This worked well and in fact gave **17** in quantitative yield. Normally the oxime is then converted to the oximyl chloride, using *N*-chlorosuccinimide (NCS), before coupling to acetylthioglucose **9**. However in this case the chlorination failed and the oximyl chloride, known to be an unstable species, could not be isolated. Chlorination was also attempted with chlorine gas under a variety of conditions but again the desired product was not obtained and the starting material was also consumed in a side reaction, which apparently modified the sulfoxide. This problem was solved by carrying out the chlorination and coupling reaction in one pot. Thus, chlorination of **17** with NCS in the presence of pyridine should give the oximyl chloride followed by immediate elimination of HCl to provide the unstable nitrile *N*-oxide. The *N*-oxide is then trapped out by the thiolate produced from the protected thioglucose **9** and triethylamine, which were also present in the reaction mixture. This strategy was surprisingly successful and gave the coupled product **18** in 45% yield over the



Scheme 2. Retrosynthesis of [^{13}C,$^2H_5$]glucoraphanin.



Scheme 3. Synthesis of isotopically labelled glucoraphanin. Reagents and conditions: (a) $K^{13}CN$, $Bu_4N^+Br^-$, H_2O (75%); (b) DIBAL-H, Et_2O (83%); (c) $NaBH_4$, MeOH (80%); (d) Ph_3P, CBr_4 (61%); (e) $CD_3SOCD_2^-Na^+$, CD_3SOCD_3, 10 equiv NaH (96%); (f) $NH_2OH{\cdot}HCl$, H_3O^+ (100%); (g) *N*-chlorosuccinimide, pyridine, then tetraacetylthioglucose and Et_3N (45%); (h) $Py{\cdot}SO_3$, pyridine (74%); (i) MeOK, MeOH (89%).

two steps,[12] which was comparable to the yields obtained for the two step process in previous glucosinolate syntheses.

In our previous work, the O-sulfonation step has been achieved using chlorosulfonic acid in pyridine,[13–15] however, trial reactions showed that the major product in this case had a molecular mass of 16 Da less than that expected. This appeared to be due to a concomitant reduction of the sulfoxide to sulfide. Attempts to separate the sulfide and sulfoxide were unsuccessful. However, $Py{\cdot}SO_3$ complex[16] in pyridine gave complete sulfonation to give **19** and mass spectrometry gave no indication of the sulfide by-product. Deacetylation using one equivalent of potassium methoxide in methanol then gave the desired final product[17] [10-^{13}C,11,12-$^{2}H_5$]glucoraphanin **20**. The presence of the isotopic labels was confirmed by the increase of 6 mass units from that of the unlabelled material. The ^{13}C NMR spectrum clearly showed an enhanced signal for the ^{13}C atom at 20.8 ppm and ^{1}H–^{13}C couplings to the protons at C-9 and C-8 were observed in the ^{1}H NMR spectrum. The loss of deuterium at carbons 11 and 12 through exchange with protons from solvent was found to be negligible through the synthesis, resulting in >95% deuterium incorporation at each position.

In conclusion, the first synthesis of a stable isotopically labelled derivative of the glucosinolate glucoraphanin, namely, [10-^{13}C,11,12-$^{2}H_5$]glucoraphanin, has been developed. This compound is now being used for metabolic studies to search for novel biomarkers for exposure to the glucosinolate. This also represents the first total chemical synthesis of glucoraphanin itself.

Acknowledgement

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References and notes

1. van Poppel, G.; Verhoeven, D. T. H.; Verhagen, H.; Goldbohm, R. A. *Adv. Exp. Med. Biol.* **1999**, *472*, 159–168.
2. Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 123–201.
3. Mithen, R. F.; Dekker, M.; Verkerk, R.; Rabot, S.; Johnson, I. T. *J. Sci. Food Agric.* **2000**, *80*, 967–984.
4. Rouzaud, G.; Rabot, S.; Ratcliffe, B.; Duncan, A. J. *Brit. J. Nutr.* **2003**, *90*, 395–404.
5. Bones, A. M.; Rossiter, J. T. *Phytochemistry* **2006**, *67*, 1053–1067.
6. Thornalley, P. J. *Anti-Cancer Drugs* **2002**, *13*, 331–338.
7. Zhang, Y.; Talalay, P.; Cho, C.-G.; Posner, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399–2403.
8. Mavratzotis, M.; Dourtoglou, V.; Lorin, C.; Rollin, P. *Tetrahedron Lett.* **1996**, *37*, 5699–5700.
9. Iori, R.; Bernardi, R.; Gueyrard, D.; Rollin, P.; Palmieri, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1047–1048.
10. Brochard, L.; Joseph, B.; Viaud, M. C.; Rollin, P. *Synth. Commun.* **1994**, *24*, 1403–1414.
11. Wadsworth, A. H.; Newman, J. J.; Wipperman, M. D.; Fellows, I.; Sutherland, D. R. *J. Labelled Compd. Radiopharm.* **2000**, *43*, 11–28.
12. All new compounds gave satisfactory spectroscopic and microanalytical data. Selected spectroscopic data for tetracetyl desulfo-[10-^{13}C,11,12-$^{2}H_5$]glucoraphanin **18**; δ_H

(300 MHz, $CDCl_3$): 1.75–1.90 (2H, m, CH_2-9), 1.84 (2H, dt, J_{10}, $^{13}C = 120.6$ Hz, $J_{9,10} = 7.6$ Hz, CH_2-10), 2.01, 2.04, 2.05, 2.08 (12H, 4 × s, Ac), 2.50–2.63 (2H, m, CH_2-8), 3.72–3.82 (1H, m, H-5), 4.11–4.26 (2H, m, CH_2-6), 5.00–5.15 (3H, m, H-l,2,4), 5.24–5.31 (1H, m, H-3); δ_C (75.47, $CDCl_3$): 20.5, 20.6, 20.7 (CH_3CO), 21.8 (enhanced, C-10), 25.8 (d, $J_{9,10} = 36.7$, C-9), 32.1 (d, $J_{8,10} = 6.0$, C-8), 38.8 (br, m, C-12), 52.6 (br, m, C-11), 62.1 (C-6), 68.1 (C-4), 70.2 (C-2), 73.7 (C-3), 75.8 (C-5), 79.9 (C-1), 149.7 (C-7), 169.2, 169.3, 170.1, 170.5 (C=O); m/z (+ve ion electrospray) $C_{19}{}^{13}CH_{26}{}^{2}H_5NO_{11}S_2Na$ req. 554.1581, found 554.1584.

13. Blanc-Muesser, M.; Driguez, H.; Joseph, B.; Viaud, M. C.; Rollin, P. *Tetrahedron Lett.* **1990**, *31*, 3867–3868.
14. Viaud, M. C.; Rollin, P.; Latxague, L.; Gardrat, C. *J. Chem. Res. (S)* **1992**, *7*, 207.
15. Davidson, N. E.; Rutherford, T. J.; Botting, N. P. *Carbohydr. Res.* **2001**, *330*, 295–307.
16. Keller, T. H.; Yelland, L. J.; Benn, M. H. *Can. J. Chem.* **1984**, *62*, 437–440.
17. Spectroscopic data for [10-^{13}C,11,12-$^{2}H_5$]glucoraphanin **20**; δ_H (300 MHz, D_2O): 1.76–1.82 (2H, m, H-9), 1.92 (2H, dt, $J_{H,C} = 122.7$ Hz, $J_{9,10} = 7.2$ Hz, CH_2-10), 2.64–2.87 (2H, m, H-8), 3.41–3.61 (4H, m, H-2,3,4 and H-5), 3.71 (1H, dd, $J_{5,6a} = 5.6$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6a), 3.90 (1H, dd, $J_{5,6b} = 1.8$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6b), 5.16 (1H, d, $J_{1,2} = 9.9$ Hz, H-l); δ_C (75.47, D_2O): 20.8 (enhanced, C-10), 25.5 (d, $J_{9,10} = 34.7$, C-9), 31.59 (d, $J_{8,10} = 6.0$, C-8), 36.0 (br, m, C-12), 51.1 (br, m, C-11), 60.7 (C-6), 69.1 (C-4), 71.9 (C-2), 77.1 (C-3) 80.2 (C-5), 81.7 (C-1), 163.6 (C-7); m/z (−ve ion electrospray) $C_{11}{}^{13}CH_{17}{}^{2}H_5NO_{10}S_3$ req. 442.0753, found 442.0749.