

Short Research Article

The synthesis of isotopically labelled glucosinolates for analysis and metabolic studies[†]

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Abstract: Glucosinolates are dietary natural products with important cancer chemoprevention properties. The syntheses of a number of stable isotopically labelled (²H, ¹³C) glucosinolates, and their desulfo-analogues, are described. These compounds are used as internal standards for analysis and for metabolic studies. Copyright © 2007 John Wiley & Sons, Ltd.

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Introduction

Epidemiological and animal studies have provided convincing evidence that the consumption of broccoli and other cruciferous vegetables is strongly associated with a decreased risk of cancer.¹ The association is strongest for cancers of the gastrointestinal and respiratory tracts. Glucosinolates **1**, a class of naturally occurring thioglucosides, are thought to be responsible for the observed anti-cancer effects, as they are found in high levels in these vegetables. Glucosinolates are metabolized 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 **2**, formed *via* a Lössén-type rearrangement of the unstable thiohydroximate-*O*-sulfonate aglycone which is the initial product. In plant cells the glucosinolates and myrosinase are compartmentalized, so that they can 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 aglycone 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 in fact thought to be 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 The most active isothiocyanate appears to be sulforaphane found in broccoli because of its ability to mono-induce Phase-II enzymes.⁷ However, when broccoli is consumed, humans are not directly exposed to sulforaphane, but instead to its glucosinolate precursor glucoraphanin. Enzymatic hydrolysis to release sulforaphane takes place following tissue disruption by chewing of vegetables, although cooking may inactivate myrosinase and then hydrolysis must occur catalysed by the bacterial enzymes in the intestinal tract. So the issue of human exposure is complex and as yet poorly understood. Stable isotopically labelled derivatives of glucoraphanin, and other glucosinolates, are thus required for studies to establish the metabolism and bioavailability of glucosinolates and isothiocyanates and to search for possible new biomarkers of exposure.

Another important use for isotopically labelled glucosinolates is as internal standards for analysis by LC-MS techniques.⁸ Some methods have been developed for the measurement of intact glucosinolate, while others employ a desulfonation step to produce the uncharged desulfo-glucosinolates as analytes, and so both are required in isotopically labelled form.

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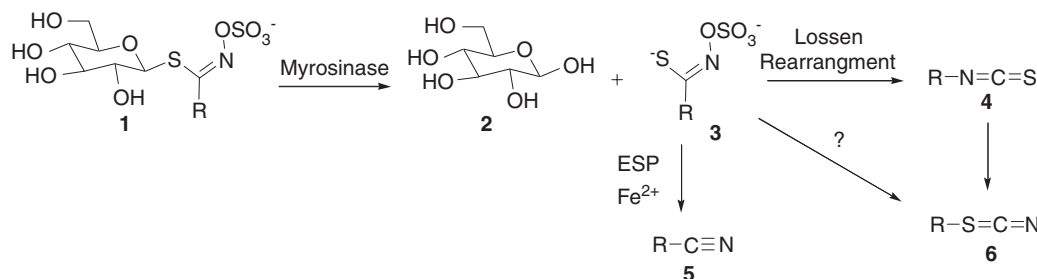
Results and discussion

Synthesis of isotopically labelled gluconasturtiin

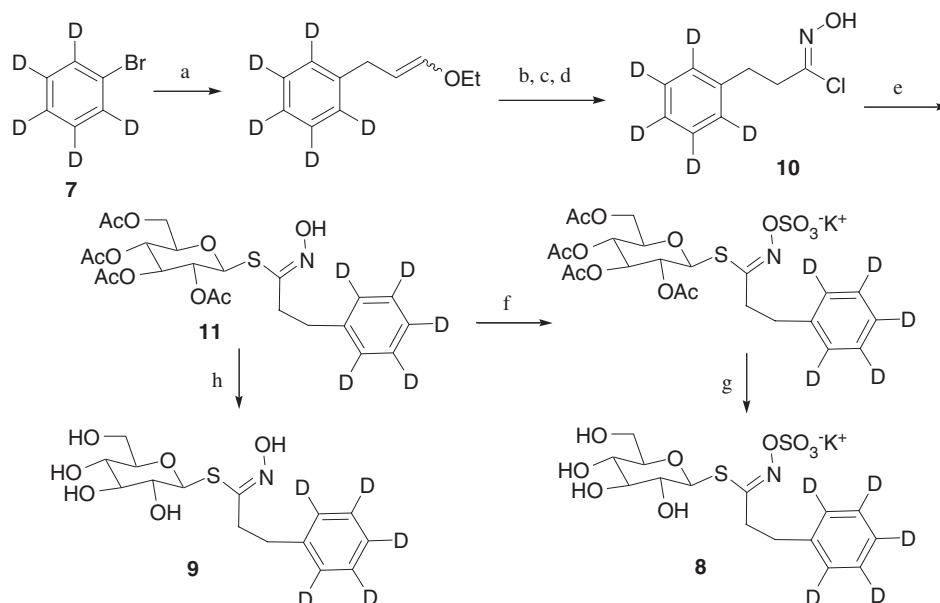
Our initial studies centred on the glucosinolate, gluconasturtiin, which has a phenethyl side chain. It was decided to make a derivative with deuterium substitution in the benzene ring and [$^2\text{H}_5$]bromobenzene **7** was used as the starting material. The full syntheses of [$phenyl\text{-}^2\text{H}_5$]gluconasturtiin⁹ **8** and [$phenyl\text{-}^2\text{H}_5$]desulfoglucanasturtiin¹⁰ **9** are given in Scheme 2. The required side chain was prepared *via* conjugate addition of the Grignard reagent derived from [$^2\text{H}_5$]bromobenzene with acrolein diethyl acetal. The key step in constructing the glucosinolate was the coupling of the oximyl chloride **10** with the thiolate derived from tetraacetyl thioglucose,¹¹ which gave the protected

desulfoglucosinolate **11** in 74% yield. The *Z*-isomer is obtained exclusively due to stereoelectronic effects.¹² The ^1H and ^{13}C NMR data for the final products were identical to those of the unlabelled compounds except for the lack of any signal for the aromatic protons, while electrospray mass spectrometry confirmed the molecular mass. Currently, the [$phenyl\text{-}^2\text{H}_5$]gluconasturtiin, and also similarly labelled versions of its metabolites, are being used to investigate their metabolism in rats.

Studies using LC-APCI-MS showed that using [$phenyl\text{-}^2\text{H}_5$]desulfoglucanasturtiin as an internal standard combined with single ion monitoring, increased the levels of detection a hundred fold as compared with traditional HPLC methods.¹³ Also the [$phenyl\text{-}^2\text{H}_5$]gluconasturtiin has been employed in new LC-MS/MS method for the analysis of glucosinolates and their



Scheme 1



Scheme 2 Reagents and conditions: (a) Mg, THF, then 5% CuBr, THF then acrolein diethyl acetal (100%); (b) acetone/ H_2O (1:4), HCl (93%); (c) $\text{NH}_2\text{OH}\cdot\text{HCl}$, NaOAc, EtOH, reflux (88%); (d) *N*-Chlorosuccinimide, pyridine, CHCl_3 (82%); (e) Et_3N , THF, tetraacetyl thioglucose (74%); (f) ClSO_3H , pyridine, DCM (20%); (g) KOMe, MeOH (99%); (h) NaOMe, MeOH (99%).

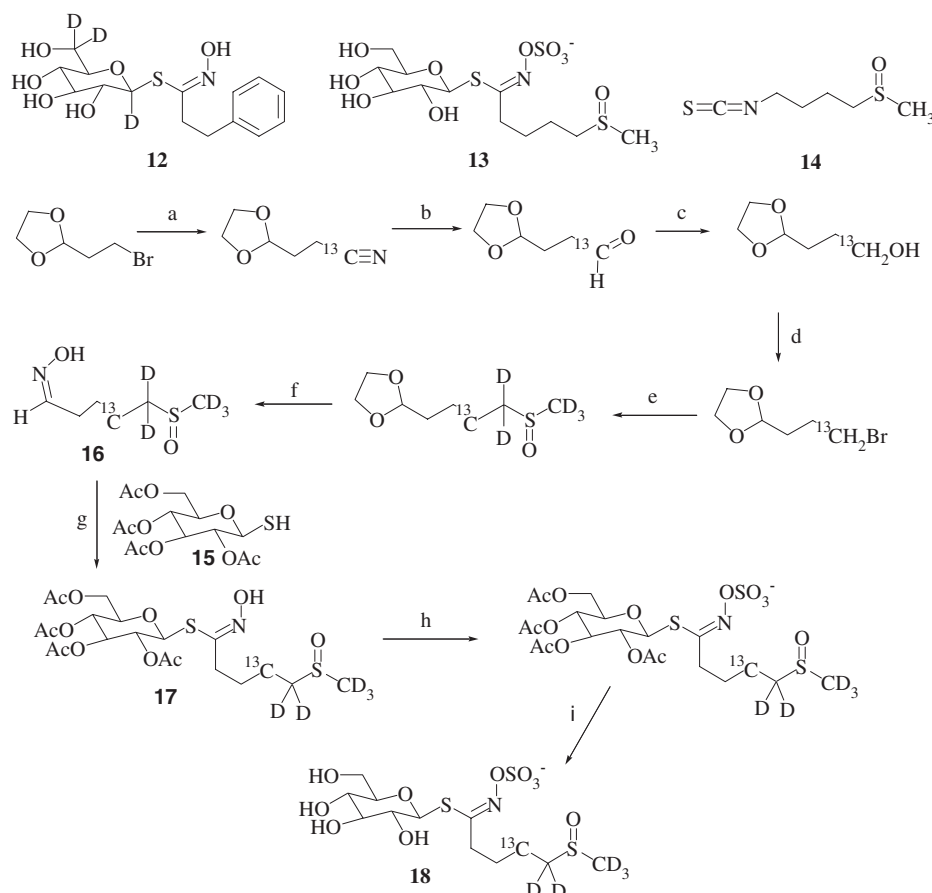
metabolites.¹⁴ However, a drawback with this strategy for the design and synthesis of internal standards is that the deuterium atoms are incorporated into the side chain of the desulfoglucosinolate and so a different synthetic procedure will be required for each glucosinolate. Therefore a synthesis has now been developed whereby three deuterium atoms were incorporated into the glucose to give [1'-²H, 6'-²H₂]desulfoglucosinasturtiin **12**. This means that the same sugar fragment can be used for the preparation of any desulfoglucosinolate, regardless of the nature of the side chain, thus making the procedure much more flexible.

Synthesis of isotopically labelled glucoraphanin

The next target was an isotopically labelled version of glucoraphanin **13**, the precursor of the isothiocyanate sulforaphane **14**, which was to be employed in metabolic studies. In this case an isotopically labelled 5-carbon chain aldehyde with terminal methyl sulfoxide functionality was required for coupling to the

thioglucose fragment. It was decided to use dioxolane protection for the aldehyde, following some preliminary studies with other protecting groups. For the isotopic labelling the end of the side chain was provided by [¹³H₆]dimethyl sulfoxide, with one mole of [¹³C]cyanide ion used for a chain extension reaction, to provide an overall 6 mass unit increase over the unlabelled glucoraphanin. The synthesis is shown in Scheme 3.

A key feature of the synthesis was the *in situ* coupling of the oximyl chloride with the tetraacetylthioglucose **15**. It was found that the oximyl chloride was too unstable to be isolated, presumably due to competing cyclisation reactions with the sulfoxide, however when *N*-chlorosuccinimide was used to chlorinate the oxime precursor **16** in the presence of base and **15**, the coupled product **17** was obtained in a reasonable yield in one step. The spectroscopic data for the final product, [10-¹³C, 11, 12-²H₅]glucoraphanin **18**, were in accord with previous literature data.^{15,16} The presence of the isotopic labels was confirmed by the increase of 6 mass units from that of the unlabelled material. The ¹³C NMR



Scheme 3 (a) K¹³CN, Bu₄N⁺Br⁻, H₂O (75%); (b) DIBAL, Et₂O (83%); (c) NaBH₄, MeOH (80%); (d) Ph₃P, CBr₄ (61%); (e) CD₃SOCD₂Na⁺, CD₃SOCD₃, 10 eq. NaH (96%); (f) NH₂OH.HCl, H₃O⁺ (100%); (g) *N*-Chlorosuccinimide, pyridine, then tetraacetylthioglucose **15** and Et₃N (45%); (h) Py.SO₃, pyridine (74%); (i) MeOK, MeOH (89%).

spectrum showed an enhanced signal for the ^{13}C atom at 21.0 ppm.

Conclusion

A range of isotopically labelled glucosinolates and their desulfonated derivatives have been synthesised for use as internal standards in analysis or for metabolic studies.

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