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Synthesis, analysis and rearrangement of novel unnatural glucosinolates

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Abstract

As part of a structure activity study to examine the interaction of glucosinolates with leaf surfaces, a number of glucosinolates were synthesised bearing novel side chain functionalities. These included 7-carboxyheptyl, heptyl, and naphthyl side chains. For the carboxyheptyl glucosinolate, a novel intramolecular rearrangement reaction was observed during the final deprotection step, which generated an ester attached to the C-3 of glucose. Studies by ^{1}H NMR spectroscopy showed that the hydrophobic side chain associated with one face of the glucose ring and it was proposed that this was the driving force for the rearrangement. Similar hydrophobic interactions were also observed between the heptyl and naphthyl side chains and the glucose.

Keywords: Glucosinolates; *Cruciferae*; brassicas

1. Introduction

Glucosinolates are a group of thioglycosides that occur in all members of the *Cruciferae*, including the brassica crops such as cabbage, Brussels sprouts and oilseed rape.[1] Glucosinolates contain a common structure **5** and over 100 different examples have been isolated and characterised,[1] with a variety of substituents in the side chain R, including allyl (sinigrin), benzyl, indolyl and 4-hydroxybenzyl. Glucosinolates possess a range of important biological activities. Anti-nutritional and potentially toxic effects have been observed following consumption of high concentrations by mammals,[2] whereas reduced risk of cancer has been linked with consumption of brassicas by humans.[3] The anti-carcinogenic effects are thought to arise from isothiocyanates which are glucosinolate breakdown products.[4] In plants, the breakdown products show anti-fungal and anti-bacterial effects and deter attack by non-adapted herbivores.[5]

However, in some cases glucosinolates play a role in host–plant recognition[5] and leaf surface glucosinolates can act as ovi-position (egg-laying) stimulants for brassica adapted insects, e.g., cabbage and turnip root flies (*Delia radicum* and *Delia floralis*).[6,7] A combination of behavioural bioassays and electrophysiology have been used to test 7–11 naturally occurring glucosinolates, essentially the complete set of available compounds, in comparative studies on the closely related cabbage[8] and turnip[9] root flies. These studies demonstrated that there were significant correlations between overall length of the glucosinolate side chain and the biological activity,

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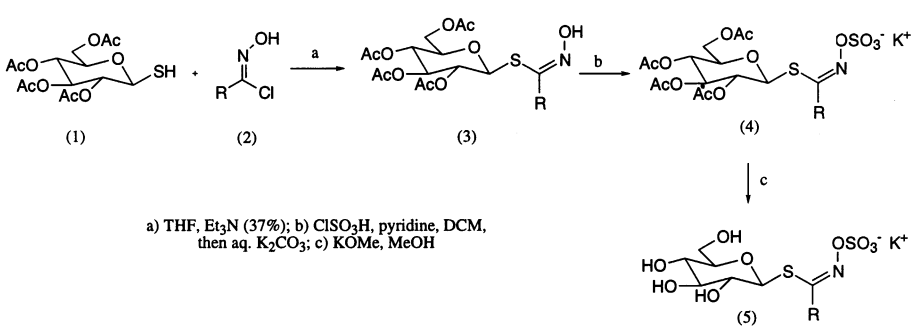
measured by behavioural or electrophysiological assays. It was also found that in model systems, the wax coating of the leaf is vital if any stimulatory activity is to be obtained.[10] Interaction of the glucosinolate side chain with the epicuticular waxes of the leaf surface may thus be essential in presenting the glucosinolate in the correct orientation for recognition by the insect. Similar observations have been reported for ovi-position by the diamondback moth (*Plutella xyostella*) in response to cabbage and sinigrin homogenates,[11] where waxes were shown to significantly increase the stimulatory effect of one glucosinolate, sinigrin, but were ineffective on their own. However, the structure activity study employed a diverse range of side chains including some aromatic and some indolyl, and it was thus decided to prepare a series of unnatural glucosinolates with simple alkyl side chains of varying lengths to re-examine this phenomenon in a more rational manner. As part of the series, a side chain with a polar end group was also required to determine whether this would disrupt interactions with the leaf surface and a glucosinolate with a 7-carboxyheptyl side chain was chosen as a suitable synthetic target. The final target was the naphthylmethyl glucosinolate which would act as a mimic for the natural indolyl glucosinolates but lack the nitrogen atom and thus any possible recognition due to this functionality.

2. Results and discussion

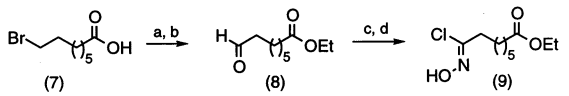
The most convenient synthetic route to glucosinolates **5** involves the coupling of an oximyl chloride derivative **2** to tetraacetyl thioglucose **1** to give the protected thiohydroximate **3**. This is followed by sulfation with chlorosulfonic acid in pyridine and deprotection using sodium methoxide in methanol (Scheme 1).[12] The oximyl chloride can be prepared from either an aldehyde[12] or a nitroalkane.[13] A large number of naturally occurring glucosinolates have been prepared using this methodology, in particular by Rollin and co-workers.[14]

For the alkyl glucosinolates the commercially available aldehydes were converted to the oximes, chlorinated using *N*-chlorosuccinimide in pyridine and the oximyl chlorides used without further purification (Scheme 1). Glucosinolates were prepared with propyl, butyl, pentyl, heptyl and nonyl side chains.

The naphthylmethyl glucosinolate **6** required 1-naphthylacetaldehyde as starting material. This proved to be difficult to synthesise. Reduction of 1-naphthylacetonitrile using Raney nickel failed as did the oxidation of 1-naphthaleneethanol using chromic acid, lead dioxide–sulfuric acid and lead tetraacetate. The latter instead gave high yields of 1-naphthaldehyde, the over oxidised product.[15] Oxidation using both milder Swern conditions and pyridinium chlorochromate (PCC) was attempted, but in all cases, a mixture was obtained of the desired acetaldehyde, 1-naphthaldehyde and starting material. Optimisation failed to give higher than 69% yield of the 1-naphthylacetaldehyde, which was difficult to separate from the by-products. The problem was finally solved by using an alternative reductive route. 1-Naphthylacetic acid was converted to its ethyl ester and then reduced to 1-naphthylacetaldehyde, in 94% yield, using di-*iso*-butyl aluminium hydride (DIBAL-H). Although this route required two steps, both were efficient and gave no undesirable side



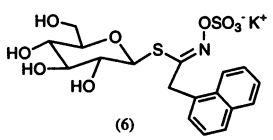
Scheme 1.



a) EtOH, $SOBr_2$, 81%; b) DMSO, KI, Na_2CO_3, 61%; c) $NH_2OH.HCl$, EtOH, pyridine, 85%; d) NCS, $CHCl_3$, Pyridine, 86%

Scheme 2.

products. The aldehyde was then used without purification for the preparation of 1-naphthylacetaldoxime in 52% yield. Chlorination of the oxime did not appear to go to completion by ^{1}H NMR, but the mixture of oxime and oximyl chloride was used for the coupling step without purification. This gave the thiohydroximate in 87% yield after recrystallisation. Sulfation and deprotection were then carried out as before to give the final glucosinolate **6**, but in this case in fairly poor 33% overall yield.

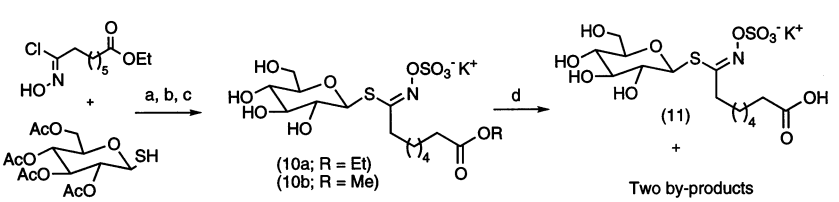


Synthesis of (7-carboxyheptyl)glucosinolate began with 8-bromooctanoic acid **7**. This was converted to the ethyl ester using thionyl bromide and ethanol. It was necessary to use thionyl bromide as the normal thionyl chloride method which gave a mixture of the 8-bromo and 8-chloro derivatives. Oxidation to the aldehyde, ethyl 8-oxooctanoate **8**, was accomplished in 61% yield by the iodide modified Kornblum oxidation procedure reported by Bauer and Macomber.[16] Reaction with hydroxylamine hydrochloride gave the oxime, which was treated with *N*-chlorosuccinimide to give ethyl 8-chloro-8-hydroxyiminooctanoate **9** in 86% yield (Scheme 2).

The coupling and sulfation were carried out as before to give **10a** in 72% yield (Scheme 3). However, during deprotection of **10a** transesterification took place to give the methyl ester **10b**. Analysis by ^{1}H and ^{13}C NMR spectroscopy (in D_2O) showed no signals for *O*-acetyl groups ($\sim$1.9–2.1 ppm) or the ethyl ester ($\sim$4.0–4.3 and 1.25 ppm), but a methyl signal was observed at 3.61 ppm, with a corresponding ^{13}C resonance at 55.1 ppm. Negative ion ESMS confirmed that the ethyl ester had undergone transesterification, and had been completely converted to the methyl ester **10b**. Further hydrolysis was thus required and so the methyl ester **10b** was treated with methanolic potassium hydroxide at room temperature for 24 h and the product purified by C18 reverse phase column chromatography. ^{1}H NMR at 500 MHz confirmed the presence of three glucose containing species in the aq fractions, one of which was shown to be a free glucoside. The peak for the anomeric proton resonance at 4.67 ppm could not be integrated due to overlap with the HOD signal, but by recording the spectra at 55 °C, the HOD signal was moved to 4.47 ppm, and the proportion of the two remaining species (2.5:1.0) was estimated from the H-1 peak integrals ratios. Resonance assignments for the major product were consistent with the desired acidic glucosinolate **11** (Table 1).

^{1}H–^{1}H spin-coupling constants for each of the three glycoside moieties in the product mixture are shown in Table 2. Large (9–10 Hz) $^3J_{HH}$ values indicated a diaxial relationship between vicinal ring protons, consistent with the β-glucose configuration, and $^4C_1$ chair conformation. $J_{H\text{-}5,H\text{-}6}$ and $J_{H\text{-}5,H\text{-}6}$ spin-coupling constants indicated that the favoured orientation of the hydroxymethyl group was the gauche–gauche rotamer.[17]

Further NMR studies were undertaken to characterise the rearrangement product. Haverkamp et al.[18] previously investigated changes in ^{1}H chemical shifts for glycosides



a) THF, Et_3N, 74%; b) $ClSO_3H$, pyridine, DCM, 72%; c) KOMe, MeOH, 98%; d) KOH, MeOH

Scheme 3.

Table 1
^{1}H and ^{13}C chemical shift assignments (ppm) for the glucose moiety of glucosinolates and related compounds in D_2O

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H6′	C-1	C-2	C-3	C-4	C-5	C-6
1	5.00	3.44	3.56	3.45	3.54	3.88	3.71	84.5	74.6	79.7	71.8	82.8	63.3
16	5.03	3.49	3.59	3.51	3.57	3.92	3.76						
6	4.60	3.31	3.11	3.32	2.71	3.48	3.41	84.6	74.5	79.6	71.3	82.5	62.8
14 or **15**	4.67	3.82	5.11	3.62	3.59	3.92	3.76	91.6	72.9	83.3	70.9	82.8	63.3
Glc-1-SH [a]	4.53	3.04	3.4	3.4	3.4	3.87	3.68	86.9					64.0

[a] β-1-thioglucose (sodium salt) from Ref. 23.

Table 2
^{1}H–^{1}H spin-coupling constants (Hz) for the glucose moiety of glucosinolates in D_2O

Compound	$J_{H\text{-}1,H\text{-}2}$	$J_{H\text{-}2,H\text{-}3}$	$J_{H\text{-}3,H\text{-}4}$	$J_{H\text{-}4,H\text{-}5}$	$J_{H\text{-}5,H\text{-}6}$	$J_{H\text{-}5,H\text{-}6'}$	$J_{H\text{-}6,H\text{-}6'}$
1	9.9	8.8	9.0	9.8	2.3	5.6	12.6
16	9.8	[a]	[a]	[a]	1.6	5.3	12.6
6	9.9	8.9	9.2	10.0	4.7	2.4	12.7

[a] Unresolved due to strong coupling.

upon acetylation, and reported characteristic α-effects (resonance frequency changes for the proton attached to the carbon which bears the *O*-acyl group) of 1–1.5 ppm downfield, and β-effects (for protons attached to adjacent carbon atoms) of approximately 0.2 ppm downfield. Chemical shifts for the glucose portion of the unknown product showed downfield shift changes of 1.55 ppm on H-3 (relative to the major product), and 0.38 and 0.17 ppm on H-2 and H-4, respectively, consistent with O-acylation on C-3. The transesterification was specific to the 3-position of the glucose ring, indicative of an intramolecular process.

In an HMBC experiment, H-3 showed a long range correlation (through two or three bonds) to a quaternary ^{13}C signal at 175.9 ppm. The same carbon was also correlated with proton resonances at one end of the aliphatic chain. The chemical shift of this quaternary carbon was upfield of the carbonyl carbon resonance of the free carboxylic acid (186.7 ppm). From these data we could infer that the side chain was connected to the glucose C-3 via an ester linkage. There were no correlations in the HMBC experiment between the opposite end of the aliphatic chain and the anomeric resonances of the glycoside. However, protons a and aí showed correlations to a quaternary ^{13}C resonance at 125.0 ppm, although the functionality of this carbon was not identified. A signal in the negative ion electrospray mass spectrum at m/z 348, and a small IR absorbance at 2252 cm^{-1} were taken as tentative evidence of isocyanate formation.

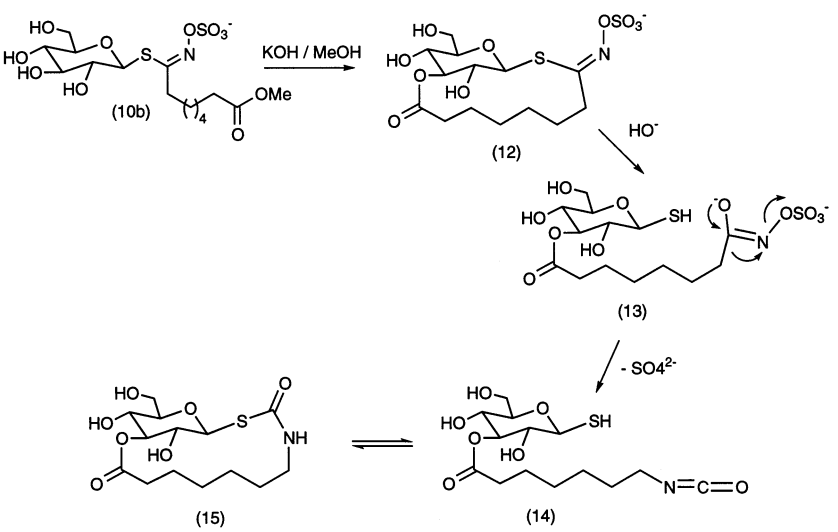
NOESY spectra (with 500 ms mixing time) showed strong NOEs between H-1 and protons attached to the first two carbons proximal to the thiooxime in the major product **11**. Weak NOEs were observed from the H-5 and the high-field H-6 protons to protons of the alkyl chain. No significant NOEs were observed connecting the aliphatic protons with either H-2 or H-4, indicating that the side chain associates exclusively with the B-face of the glucose ring. Based on NOE data for glucobrassicin (a natural glucosinolate bearing an indole side chain) Prestera et al. also inferred an association between a hydrophobic side chain and the B face of the glycoside.[19] The association, ascribed to an aromatic stacking interaction, is clearly also observed here with non-aromatic hydrophobic moieties.

A mechanism can be proposed to explain the observations as shown in Scheme 4. Firstly transesterification of **10b** takes place first, which is assisted by the conformation of the glucosinolate in solution which positions the alkyl side chain underneath the glucose ring. This will be in competition with the straightforward hydrolysis of the ester **10b** to give the

glucosinolate **11**. The bicyclic system **12** produced will be fairly strained and so is hydrolysed under the basic conditions employed to give a O-sulfated hydroxamic acid intermediate **13**. A Lossen-type rearrangement can then take place, analogous to that catalysed by myrosinase in the metabolism of glucosinolates, which would give the isocyanate **14**. The isocyanate should be very susceptible to nucleophilic attack and thus very unstable. However, there is some evidence for its presence in the IR spectrum of the product mixture and a molecule of this molecular weight is observed in the ESMS. As there is a strongly nucleophilic thiol group in close proximity to the isocyanate, this could result in a rapid intramolecular cyclisation to give **15** and the product could exist as an equilibrium mixture of **14** and **15**.

NMR studies were also carried out to investigate the solution conformations of the heptylglucosinolate **5** ($R = (CH_2)_6CH_3$) and naphthylmethylglucosinolate **6**. The heptylglucosinolate gave a similar set of NOEs connecting the H-1, H-3 and H-5 with the protons attached to the two carbon atoms proximal to the thiooximoyl functionality, implying that the side chain was also positioned under the glucose ring. For the naphthylmethylglucosinolate aromatic ring currents reduced the proton chemical shifts of the glucose moiety, as previously observed for aromatic glucosinolates.[19] The chemical shift differences were more pronounced for protons on the B-face of the glucose ring (H-1, H-3 and H-5) than for those on the A-face. NOEs involving the anomeric proton were unresolved from those involving the C-8 methylene protons using conventional 2D NOESY experiments. It was not clear whether the strong NOEs from the C-8 protons were masking significant weaker NOEs from H-1. A one-dimensional NOE spectrum, selective for H-1 (Fig. 1(b)), was obtained using the double-selective 1D TOCSY-NOESY pulse sequence described by Uhrin and Barlow.[20] The first pulse was selective for band of resonance frequencies encompassing protons H-2 through H-5, and magnetisation was transferred to H-1 by means of a 50 ms DIPSI-2 spin–lock sequence. The second selective pulse, beginning the NOESY portion of the pulse sequence, gave clean excitation of H-1 without excitation of the C-8 methylene protons. Similarly, 1D NOE spectra were obtained selectively for the combined H-2 and H-4 signals, H-3 and H-5. The resulting spectra (Fig. 1) indicate that the naphthyl side chain has a loose association with exclusively the B-face of the glucose ring.

The accessible conformational space for the glucosinolates spans a wide range, as there are several bonds with low energy barriers of rotation. The experimental evidence indicates that the hydrophobic moiety is associated with one face of the glucose ring for a significant proportion of the time, but few of the alternative conformations could be excluded since the



Scheme 4.

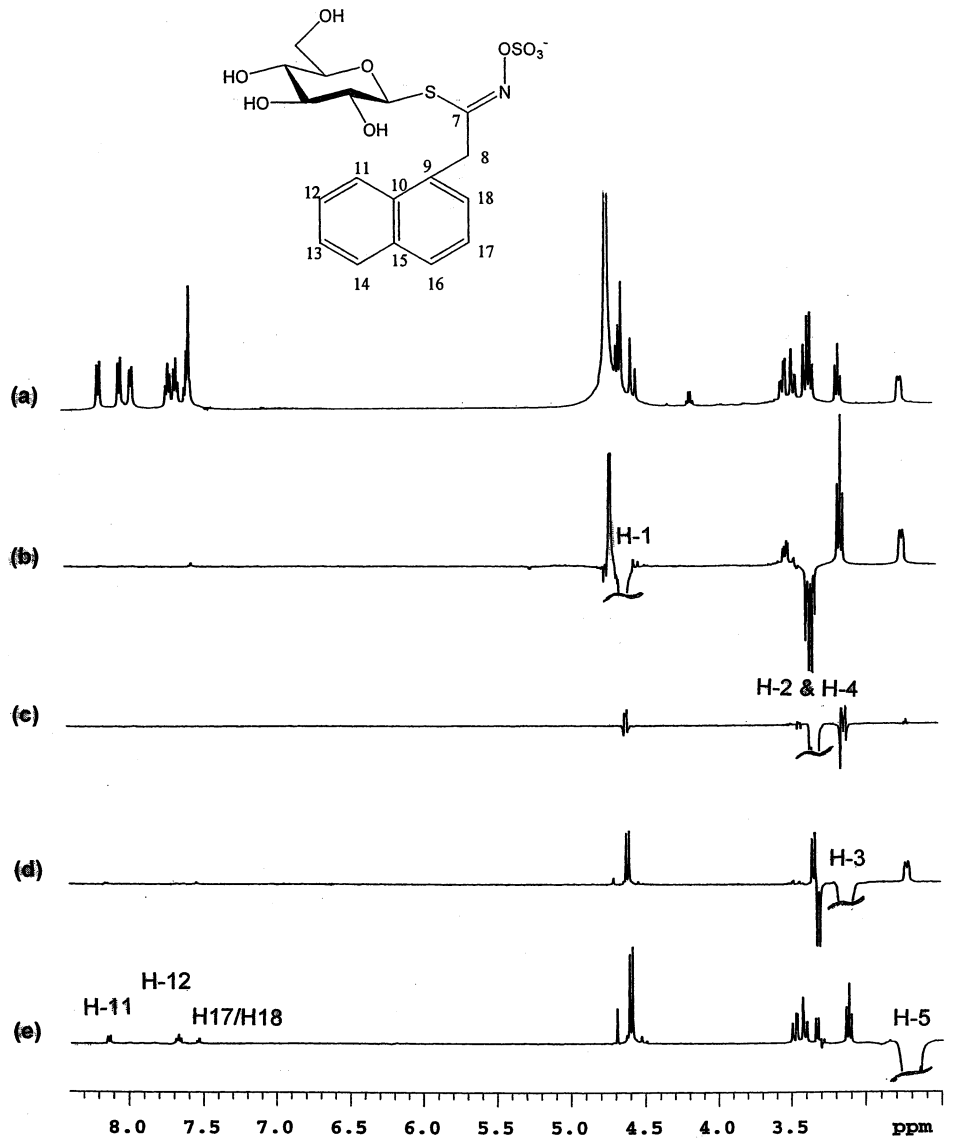


Fig. 1.

solution state structure is under-defined by NMR data.

An approximate model was constructed by a combination of molecular mechanics (AMBER) and semi-empirical (AM1) energy calculations, although a full conformational search was not conducted due to the low accuracy available for modelling the energy of the hydrophobic interactions. The model depicted in Fig. 2 shows the carboxylate in proximity with the C-3 hydroxyl of glucose. It is not clear on the basis of this model why intramolecular acylation should occur exclusively at the C-3 position, as the C-2 and C-4 hydroxyl groups appear to be equally accessible.

3. Conclusions

In conclusion it can be seen that during the synthesis of a novel 7-carboxyheptylglucosinolate an intramolecular rearrangement reaction has been observed. The first step was transesterification, resulting in attachment of the side chain via an ester linkage to the 3-hydroxy group of the glucose. This reaction is facilitated by the solution conformation of the glucosinolate where there is an interaction of the alkyl side chain one face of the glucose ring. It then appears that further reaction takes place, which cleaves the thiohydroximate moiety and probably produces an isocyanate after Lossen-type rearrangement. ^{1}H

NMR spectroscopy and molecular modelling calculations (AM1 and AMBER) on the 7-carboxyheptylglucosinolate, along with the novel heptyl and naphthylmethyl derivatives also synthesised, indicate that in all three cases the hydrophobic side chains associate with one face of the glucose ring.

4. Experimental

General methods.—Routine ^{1}H and ^{13}C NMR spectra were obtained using a Varian 2000 f.t spectrometer (^{1}H, 300 MHz;^{13}C, 75.42 MHz) and a Varian Gemini f.t spectrometer (^{1}H 200 MHz; ^{13}C 50.31 MHz). Mass spectra were recorded on an A.E.I MS-902 spectrometer. Optical rotations were measured at rt using an Optical Activity Ltd. AA 1000 polarimeter with 20 cm path-length cells. IR spectra were obtained on a Perkin–Elmer 1420 instrument or a Perkin–Elmer 1710 FT-IR spectrometer.

Heptyl glucosinolate.—2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (3 g, 8.23 mmol) was dissolved in dry THF (100 mL) before 1-chorooctaldoxime (1.46 g, 8.23 mmol), dissolved in dry THF (100 mL), was added. To this, dry triethylamine (7.81 g, 77.2 mmol) was added and the resulting solution was stirred, under a N_2 atmosphere, at rt for 22 h. Diethyl ether (65 mL) was then added and the solution washed with 1 M H_2SO_4 (65 mL). After removal of approximately 60% of the organic layer, the remaining organics and aq layer were extracted with EtOAc (65 mL). The organic layers were combined, dried ($MgSO_4$) and concentrated under reduced pressure to afford a brown solid. The product was extracted as before and recrystallised from EtOAc–petroleum ether (bp 40–60 °C) to afford the title compound as a white solid (2.86 g, 69%): mp 127 °C; $[\alpha]_D^{25}$ −17.1° (*c* 0.14, $CHCl_3$); IR (Nujol); *ν* 3300 (OH), 1730 (CO), 1600 cm^{-1} (C=N); ^{1}H NMR (200 MHz, CD_3OD) 5.40 (1 H, t, $J_{3,4}$ 9 Hz, H-3), 5.28 (1 H, d, $J_{1,2}$ 10 Hz, H-1), 5.05 (2 H, m, H-2,4), 4.92 (1 H, br s, OH), 4.25 (1 H, dd, $J_{5,6b}$ 7, $J_{6a,6b}$ 12 Hz, H-6_b), 4.13 (1 H, dd, $J_{5,6a}$ 2, $J_{6a,6b}$ 12 Hz, H-6a), 3.99 (1 H, m, H-5), 2.55 (2 H, t, *J* 6 Hz, $CH_2(CH_2)_5CH_3$), 2.07, 2.04, 2.05, 2.00 (12 H, 4 × s, CH_3COO), 1.66 (2 H, t, *J* 6 Hz, $CH_2(CH_2)_4CH_3$), 1.35 (8 H, br s, $(CH_2)_4CH_3$), 0.93 (3 H, t, *J* 7 Hz, CH_3); ^{13}C NMR (50.31 MHz, CD_3COCD_3) 171.00, 170.67, 170.39, 170.08 (CO), 151.66 (C=N), 80.34 (C-1), 76.58 (C-5), 74.61 (C-3), 71.35 (C-2), 69.61 (C-4), 63.51 (C-6), 32.96, 30.63, 29.85, 28.36, 21.03, 20.97 (CH_2), 20.92 (CH_3COO), 14.73 (CH_3); *m/z* (CI) 506 (MH^+, 7%), 331 (100, $C_6H_7O(OAc)_4^+$) and 271 (40, $C_6H_7O(OAc)_3^+$); Anal. Calcd for $C_{22}H_{35}NO_{10}S$: C, 52.26; H, 6.98; N, 2.77. Found: C, 52.39; H, 7.12; N, 2.68; (HRMS) $C_{22}H_{36}NO_{10}S$ requires MH^+ 506.6871. Found MH^+ 506.2060.)

A solution of chlorosulfonic acid (3.03 g, 26.0 mmol) in dry CH_2Cl_2 (30 mL) was added to a stirred mixture of dry pyridine (38.6 g, 488.3 mmol) and dry CH_2Cl_2 (30 mL) maintained at 0 °C and under a N_2 atmosphere. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylheptyl thiohydroximate (1.3 g, 2.6 mmol) dissolved in dry CH_2Cl_2 (20 mL) was then added and the resulting mixture stirred as given previously. Potassium hydrogen carbonate (1.67 g, 16.7 mmol) was added in water (100 mL) and this mixture allowed to stir for 30 min before partial concentration under reduced pressure followed by co-evaporation with toluene. Further aq potassium hydrogen carbonate (5.0 g, 50.1 mmol) was added and the two-phase system partially concentrated under reduced pressure to afford a brown solid. Purification by chromatography, on silica gel, eluting with petroleum ether (bp 40–60 °C):

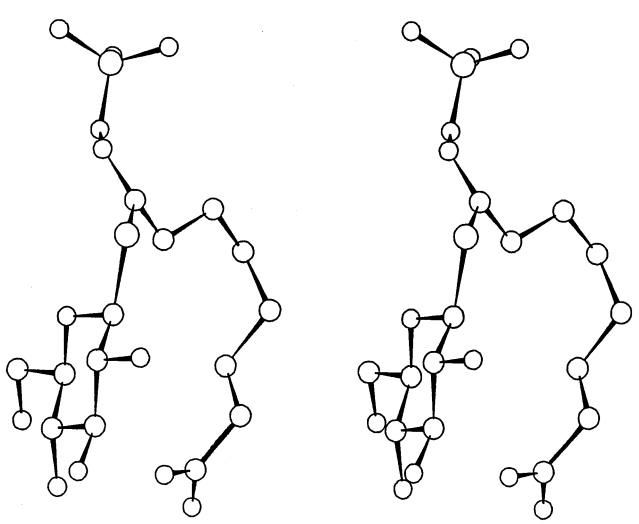


Fig. 2.

EtOAc (1:1) with 10% (v/v) MeOH, allowed recovery of the title compound as a white solid (697 mg, 43%): mp 107–109 °C; $[\alpha]_D^{25}$ −16.4° (*c* 0.14, MeOH); IR (Nujol); *v* 1750 (CO), 1580 (C=N), 1250 cm^{-1} (COOC); ^1H NMR (200 MHz, CD_3OD) 5.40 (1 H, t, $J_{3,4}$ 9 Hz, H-3), 5.36 (1 H, d, $J_{1,2}$ 10 Hz, H-1), 5.03 (2 H, 2 × t, *J* 10, 9 Hz, H-2,4), 4.24 (2 H, dd, $J_{5,6b}$ 4, $J_{6a,6b}$ 12 Hz, H-6_b), 4.13 (2 H, dd, $J_{5,6a}$ 2, $J_{6a,6b}$ 12 Hz, H-6_a), 4.03 (1 H, m, H-5), 2.66 (2 H, t, *J* 8 Hz, $CH_2(CH_2)_5CH_3$), 2.07, 2.04, 1.99 (12 H, 3 × s, CH_3COO), 1.85 (2 H, br m, $CH_2(CH_2)_4CH_3$), 1.34 (8 H, br s, $(CH_2)_4$), 0.91 (3 H, t, *J* 6 Hz, CH_3); ^{13}C NMR (50.31 MHz, CD_3OD) 172.51, 171.82, 171.55, 171.25 (CO), 159.94 (C=N), 81.18 (C-1), 77.03 (C-5), 75.31 (C-3), 71.72 (C-2), 69.80 (C-4), 63.68 (C-6), 33.98, 33.25, 30.54, 30.49, 28.78, 24.02 (CH_2), 21.02, 20.88 (CH_3COO) 14.78 (CH_3); *m*/*z* (ES^-) 584 ($[M-K]^-$, 100%); Anal. Calcd for $C_{22}H_{34}KNO_{13}S_2$: C, 42.36; H, 5.49; N, 2.25. Found: C, 42.08; H, 5.52; N, 1.98.

Finally a solution of potassium methoxide was prepared by adding a catalytic amount of potassium (up to 100 mg) to dry MeOH (1 mL) at rt and under a N_2 atmosphere. A small volume of this solution was added to 2,3,4,6-tetra-*O*-acetyl-heptyl glucosinolate (460 mg, 0.74 mmol), dissolved in dry MeOH (10 mL), until the pH reached 8–9. The resulting solution was stirred overnight under a N_2 atmosphere before being concentrated under reduced pressure to afford a gold coloured solid (337, 100%): $[\alpha]_D^{25}$ −14.0° (*c* 0.2, H_2O); IR (Nujol) *v* 3400 cm^{-1} (OH); ^1H NMR (200 MHz, CD_3OD) 4.83 (1 H, d, $J_{1,2}$ 10, H-1), 3.85 (1 H, d, $J_{6a,6b}$ 10 Hz, H-6_b), 3.65 (1 H, dd, $J_{5,6a}$ 4, $J_{6a,6b}$ 10 Hz, H-6_a), 3.34 (4 H, m, H-2,3,4,5), 2.69 (2 H, t, *J* 7, Hz $CH_2(CH_2)_5CH_3$), 1.71 (2 H, br m, CH_2-$(CH_2)_4CH_3$), 1.33 (8 H, br m, $(CH_2)_4$), 0.91 (3 H, t, *J* 7 Hz, CH_3); ^{13}C NMR (50.31 MHz, CD_3OD) 162.66 (C=N), 84.01 (C-1), 82.59 (C-5), 79.83 (C-3), 74.44 (C-2), 71.39 (C-4), 62.90 (C-6), 33.99, 33.25, 30.65, 30.47, 29.08, 24.02 (CH_2), 14.77 (CH_3); *m*/*z* (ES^-) 416 ($[M-K]^-$, 100%); Anal. Calcd for $C_{14}H_{26}KNO_9S_2$: C, 36.91; H, 5.75; N, 3.07. Found: C, 37.12; H, 5.89; N, 2.84.

1-Naphthylacetic acid ethyl ester.—1-Naphthylacetic acid (20 g, 107.4 mmol) was dissolved in dry EtOH (500 mL) before thionyl chloride (14.06 g, 118.2 mmol) was added. The resulting solution was heated under reflux for 3.5 h before concentration under reduced pressure to afford the title compound as a yellow oil (22.95 g, 100%): IR (neat); *v* 3050 (aromatic) 2900–3000 (alkyl), 1730 (CO), 1520, 1600 (aromatic), 1150–1275 cm^{-1} (COOC); ^1H NMR (200 MHz, $CDCl_3$) 8.06 (1 H, d, *J* 8 Hz, H-2), 7.88 (2 H, m, H-4,8), 7.54 (4 H, m, H-3,5,6,7), 4.20 (2 H, q, *J* 7 Hz, OCH_2CH_3), 4.11 (2 H, s, $CH_2COCH_2CH_3$), 1.27 (3 H, t, *J* 7 Hz, CH_3); ^{13}C NMR (50.31 MHz, $CDCl_3$) 172.19 (CO), 134.36 (ArC-10), 132.67 (ArC-9), 131.25 (ArC-1), 129.29, 128.61, 126.89, 126.34, 126.09, 126.01, 124.41 (ArC), 61.54 (OCH_2CH_3), 39.85 (CH_2CO-CH_2CH_3), 14.79, 14.73 (OCH_2CH_3).

1-Naphthylacetaldehyde.—1-Naphthylacetic acid ethyl ester (10 g, 46.7 mmol) was dissolved in dry toluene (200 mL) and the solution cooled to −78 °C before DIBAL-H (1.5 M in toluene; 12.74 g, 89.6 mmol) was added. The resulting solution was stirred at −78 °C for 2 h under a N_2 atmosphere before dry MeOH (100 mL) was added and the solution allowed to warm slowly to rt. A solution of potassium sodium tartrate (60 g in 100 mL H_2O) was added and the biphasic system stirred overnight. The organic layer was removed and the aq layer washed with Et_2O (2 × 150 mL). The organic layers were combined, dried ($MgSO_4$) and concentrated under reduced pressure to yield a yellow oil (7.43 g, 94%): ^1H NMR (200 MHz, $CDCl_3$) 9.79 (1 H, t, *J* 2 Hz, CHO), 7.90 (3 H, m, ArH), 7.46 (4 H, m, ArH), 4.11 (2 H, d, *J* 2 Hz, CH_2); ^{13}C NMR (50.31 MHz, $CDCl_3$) 200.28 (CHO), 134.43 (ArC-10), 132.80 (ArC-9), 48.89 (CH_2), 129.47, 129.03, 128.89, 127.26, 126.63, 126.22, 126.15, 124.10(ArC).

1-Naphthylacetaldoxime.—1-Naphthylacetaldehyde (6.65 g, 39.1 mmol) was dissolved in EtOH (70 mL) and pyridine (7 mL) before hydroxylamine hydrochloride (5 g, 71.9 mmol) was added. The resulting solution was heated under reflux, with stirring, for 2.5 h before being concentrated under reduced pressure. Water was then added resulting in

the appearance of a yellow solid which was recrystallised from EtOH to afford the title compound (3.78 g, 52%) as a mixture of isomers (40% anti:60% syn): mp 127–128 °C (lit.,[15] 123–124 °C); ^{1}H NMR (200 MHz, $CDCl_3$) 8.80 (1 H, br s, NOH), 8.08–7.38 (7 H, m, ArH), 6.84 (1 H, t, *J* 5 Hz, CH=N), 4.20 (2 H, d, *J* 5 Hz, CH_2 (syn)), 4.02 (2 H, d, *J* 5 Hz, CH_2 (anti)); ^{13}C NMR (50.31 MHz, $CDCl_3$) 151.49 (CH=N (syn) 151.15 (CH=N (anti)), 134.38, 133.31, 129.52, 129.34, 128.41, 128.29, 127.61, 127.43, 127.36, 127.29, 127.08, 126.94, 126.72, 126.63, 126.44, 126.11, 126.06, 124.22, 124.11, 124.02 (ArC (syn, anti)), 33.95, 30.19 (CH_2); *m*/*z* (CI) 186 (MH^+, 100%), 141 (52, $[M - CH_2NOH]^+$).

Chloro-1-naphthylacetaldoxime.—1-Naphthylacetaldoxime (6.5 g, 35.1 mmol) was added to a stirred solution of $CHCl_3$ (50 mL) and pyridine (1.39 g, 17.6 mmol) before *N*-chlorosuccinimide (4.69 g, 35.1 mmol) was added slowly. The resulting solution was stirred at rt for 4 h then poured onto ice-water. The organic layer was separated and washed with water (50 mL) and satd NaCl solution (50 mL) while the aq layer was extracted with Et_2O (75 mL). The organics were combined, dried ($MgSO_4$) and concentrated under reduced pressure. This afforded a mixture of the (1:2) oxime and oximyl chloride (5.53 g, 48% oximyl chloride): ^{1}H NMR (200 MHz, $CDCl_3$) 8.86 (1 H, br s, NOH), 8.09–7.69 (3 H, m, H-2,4,8), 7.32–7.61 (4 H, m, H-3,5,6,7), 4.24 (2 H, s, CH_2C=N); ^{13}C NMR (50.31 MHz, $CDCl_3$) 140.07 (C=N), 122.87-133.83 (ArC), 40.20 (CH_2).

2,3,4,6-Tetra-O-*acetyl*-β-D-*glucopyranosyl*-*1-naphthylmethyl thiohydroximate*.—This was prepared as for the heptyl derivative using 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (4.32 g, 11.9 mmol) and crude chloro-1-naphthylacetaldoxime (3.65 g, representing ca. 11 mmol of the oxime). Recrystallisation from EtOH afforded the title compound as a white solid (5.62 g, 87%): mp 208–210 °C; $[\alpha]_D^{25}$ + 4.64° (*c* 0.14, MeOH); IR (Nujol); *v* 3300 (OH), 1720, 1750 (CO), 1620 (C=N), 1225, 1250 cm^{-1} (COOC); ^{1}H NMR (200 MHz, $CDCl_3$) 7.80 (3 H, m, H-2′,4′,8′), 7.40 (4 H, m, H-3′,5′,6′,7′), 4.84 (3 H, m, H-2,3,4), 4.64 (1 H, d, $J_{1,2}$ 9 Hz, H-1), 4.26 (2 H, 2 × d, *J* 18 Hz, CH_2), 3.94 (1 H, dd, $J_{5,6b}$ 5, $J_{6a,6b}$ 12 Hz, H-6b), 3.79 (1 H, d, $J_{6a,6b}$ 12 Hz, H-6a), 3.05 (1 H, m, H-5), 1.91, 1.90, 1.85 (12 H, 3 × s, CH_3); ^{13}C NMR (50.31 MHz, $CDCl_3$) 171.34, 170.76, 169.98, 169.88(COO), 150.17 (C=N), 134.18, 132.33, 131.67, 129.47, 128.33, 126.89, 126.37, 126.02, 125.90, 123.12, (ArC), 80.05 (C-1), 75.92 (C-5), 74.06 (C-3), 70.34 (C-2), 68.13 (C-4), 62.36 (C-6), 35.57 (CH_2), 20.89, 20.78(CH_3); *m*/*z* (CI) 548 (MH^+, 8%), 331 (100, $C_6H_7O(OAc)_4^+$) and 271 (33, C_6H_7O-$(OAc)_3^+$); Anal. Calcd for $C_{26}H_{29}NO_{10}S{\cdot}0.5$ H_2O: C, 56.11; H, 5.43; N, 2.53. Found: C, 56.10; H, 5.16; N, 2.48; HRMS $C_{26}H_{30}NO_{10}S$ requires M*H*$^+$ 548.5880. Found M*H*$^+$ 548.1590.

2,3,4,6-Tetra-O-*acetyl*-*1-naphthylmethyl glucosinolate*.—Sulfonation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-1-naphthylmethyl thiohydroximate (3.50 g, 6.4 mmol) was carried out as for the heptyl derivative. Purification by flash chromatography on silica gel, eluting with (1:1) petroleum ether (bp 40–60 °C)–EtOAc then (9:1) EtOAc–MeOH afforded the protected glucosinolate as a brown solid (2.54 g, 60%): mp (dec.) 110–112 °C; $[\alpha]_D^{25}$ −27.5° (*c* 0.14, H_2O); IR (Nujol); *v* 3000 (aromatic), 1750 (CO), 1225 cm^{-1} (COOC); ^{1}H NMR (200 MHz, CD_3OD) 8.19 (1 H, d, *J* 8 Hz, H-2′), 7.96 (1 H, d, *J* 8 Hz, H-8′), 7.86 (1 H, d, *J* 8 Hz, H-4′), 7.56 (4 H, m, H-3′,5′,6′,7′), 4.89 (4 H, m, H-1,2,3,4), 4.53 (2 H, s, CH_2), 4.01 (1 H, dd, $J_{5,6a}$ 5, $J_{6a,6b}$ 12 Hz, H-6b), 3.75 (1 H, d, $J_{6a,6b}$ 12 Hz, H-6_a), 3.30 (1 H, m, H-5), 1.95 (12 H, m, CH_3COO); ^{13}C NMR (50.31 MHz, CD_3OD) 172.42, 171.64, 171.32, 171.07 (COO), 158.87 (C=N), 124.49–135.66 (ArC), 81.36 (C-1), 77.12 (C-5), 75.13 (C-3), 71.30 (C-2), 69.31 (C-4), 63.27 (C-6), 36.51 (CH_2), 20.91, 20.76 (CH_3); *m*/*z* (ES^-) 626 ($[M - K]^-$, 100%); Anal. Calcd for $C_{26}H_{28}KNO_{13}S_2$: C, 46.91; H, 4.24; N, 2.10. Found: C, 47.12; H, 4.54; N, 2.07.

1-Naphthylmethyl glucosinolate (**6**).—Deprotection of 2,3,4,6-tetra-*O*-acetyl-1-naphthylmethyl glucosinolate (500 mg, 0.75 mmol) was carried out in dry MeOH (10 mL) with a catalytic amount of potassium metal as for the heptyl derivative. Purification by flash chromatography, on silica gel, eluting initially with EtOAc then (4:1) EtOAc–MeOH afforded the

product as a gold-coloured foam (198 mg, 53%): $[\alpha]_D^{25}$ −3.8° (*c* 0.2, H_2O); IR (Nujol); *v* 3390 cm^{-1} (OH); ^1H NMR (200 MHz, CD_3OD) 8.22 (1 H, d, *J* 8 Hz, H-2′), 7.91 (1 H, d, *J* 8 Hz, H-8′), 7.81 (1 H, d, *J* 8 Hz, H-4′), 7.41–7.65 (4 H, m, H-3′,5′,6′,7′), 4.82 (1 H, d, $J_{1,2}$ 6 Hz, H-1), 4.37 (2 H, m, CH_2), 3.74 (1 H, dd, $J_{5,6b}$ 2, $J_{6a,6b}$ 12 Hz, H-6b), 3.56 (1 H, dd, $J_{5,6a}$ 6, $J_{6a,6b}$ 12 Hz, H-6_a), 3.17 (2 H, t, *J* 9 Hz, H-2,4), 2.93 (1 H, t, $J_{3,4}$ 9 Hz, H-3), 2.82 (1 H, m, H-5); ^{13}C NMR (50.31 MHz, CD_3OD) 162.03 (C=N), 135.59, 133.54, 132.98, 130.20, 129.12, 128.03, 127.40, 127.10, 126.85, 124.65 (ArC), 83.81, 83.78 (C-1), 82.47 (C-5), 79.40 (C-3), 74.26 (C-2), 71.10 (C-4), 62.76, 62.67 (C-6), 36.35 (CH_2); *m*/*z* (ES^-) 458 ($[M-K]^-$, 100%); Anal. Calcd for $C_{18}H_{20}KNO_9S_2 \cdot 0.5\ H_2O$: C, 42.68; H, 4.18; N, 2.76. Found: C, 42.48; H, 4.73; N, 2.40.

Ethyl 8-bromooctanoate.—8-Bromooctanoic acid (5.78 g, 25.9 mmol) was dissolved in dry EtOH (125 mL) before thionyl bromide (5.92 g, 28.5 mmol) was added and the resulting solution heated under reflux with stirring for 2 h under a N_2 atmosphere. Concentration under reduced pressure afforded a brown oil which was purified by flash chromatography, on silica gel, eluting initially with petroleum ether (bp 40–60 °C) then (9:1) petroleum ether–EtOAc. This yielded the title compound as a pale yellow oil (5.26 g, 81%); IR (neat) 2858, 2935 (alkyl), 1736 (CO), 1373–1465 (alkyl), 1183 cm^{-1} (COOC); ^1H NMR (200 MHz, $CDCl_3$) 4.12 (2 H, q, *J* 7 Hz, OCH_2), 3.40 (2 H, t, *J* 7 Hz, $BrCH_2$), 2.29 (2 H, t, *J* 7 Hz, CH_2COO), 1.85 (2 H, quintet, *J* 7 Hz, $BrCH_2CH_2$), 1.62 (2 H, quintet, *J* 7 Hz, CH_2CH_2COO), 1.39 (6 H, m, $(CH_2)_3$), 1.25 (3 H, t, *J* 7 Hz, CH_3); ^{13}C NMR (50.31 MHz, $CDCl_3$) 174.26 (COO), 60.70 (OCH_2CH_3), 34.76, 34.41, 33.18, 29.39, 28.89, 28.45, 25.31 (CH_2), 14.72 (CH_3); *m*/*z* (CI) 252, 254 (MH^+, 6%), 251, 253 (55, 56, M^+), 205, 207 (5, $[M-C_2H_5OH]^+$).

Ethyl 8-oxooctanoate (**8**).—Following the procedure of Bauer and Macomber,[21] KI (3.33 g, 20.1 mmol) and Na_2CO_3 (2.11 g, 20.1 mmol) were suspended in Me_2SO (150 mL) at 80–85 °C before ethyl 8-bromoooctanoate (5 g, 20.1 mmol) was added with stirring. This mixture was stirred at the above temperature for 11 h before being rapidly cooled and poured onto ice-cold brine. The product was extracted with Et_2O (2 × 300 mL) and the organic layer washed with water (150 mL), brine (150 mL), satd $NaHCO_3$ (150 mL) and more brine (150 mL) before being dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash chromatography, on silica gel, eluting with (9:1) petroleum ether (bp 40–60 °C)–EtOAc. This afforded the title compound as a colourless oil (2.27 g, 61%): IR (neat); *v* 2722, 2860, 2937 (alkyl), 1735 (CO), 1349–1466 (alkyl), 1182 cm^{-1} (COOC); ^1H NMR (200 MHz, $CDCl_3$) 9.71 (1 H, t, *J* 2 Hz, CHO), 4.07 (2 H, q, *J* 7 Hz, $COOCH_2$), 2.39, 2.38 (2 H, 2 × t, *J* 7 Hz, CH_2CO), 2.24 (2 H, t, *J* 7 Hz, CH_2COO), 1.46 (4 H, m, $(CH_2)_2$), 1.30–1.15 (7 H, m, $(CH_2)_2$, CH_3); ^{13}C NMR (50.31 MHz, $CDCl_3$) 203.12 (CHO), 174.13 (COO), 60.65 (OCH_2CH_3), 44.21 (CH_2CHO), 34.64 (CH_2COO), 29.25, 29.20, 25.14, 22.28 (CH_2), 14.68 (CH_3); *m*/*z* (CI) 187 (MH^+, 86%), 171 (5, $[M-CH_3]^+$), 141 (100, $[M-OCH_2CH_3]^+$).

Ethyl 8-hydroxyiminooctanoate.—Ethyl 8-oxooctanoate (2.26 g, 12.11 mmol) was dissolved in EtOH (45 mL) and pyridine (4.5 mL) before hydroxylamine hydrochloride (1.55 g, 22.3 mmol) was added and the resulting solution heated under reflux for 2.5 h. Concentration under reduced pressure and addition of water afforded a white solid which was removed by filtration under reduced pressure. This was identified as the oxime existing as a mixture of (1:1) syn and anti isomers, (2.06 g, 85%): mp 28 °C; IR (Nujol); *v* 3050–2200 (OH), 2820, 2900 (alkyl), 1700 cm^{-1} (CO); ^1H NMR (200 MHz, $CDCl_3$) 7.92 (1 H, br s, NOH), 7.40 (1 H, t, *J* 6 Hz, CH=N (anti)), 6.72 (1 H, t, *J* 5 Hz, CH=N (syn)), 4.11 (2 H, q, *J* 8 Hz, OCH_2), 2.28 (2 H, t, *J* 8 Hz, CH_2COO), 2.18 (2 H, q, *J* 7 Hz, CH_2CH), 1.61 (2 H, m, CH_2), 1.48 (2 H, m, CH_2), 1.34 (4 H, m, $(CH_2)_2$), 1.24 (3 H, t, *J* 7 Hz, CH_3); ^{13}C NMR (50.31 MHz, $CDCl_3$) 174.39 (CO), 152.51 (CH=N (syn)), 152.45 (CH=N (anti)), 60.76 (OCH_2), 34.75 29.87, 29.47, 29.25, 29.17, 26.80, 26.29, 25.40, 25.25 (CH_2), 14.73 (CH_3); *m*/*z* (CI) 202 (MH^+, 100%), 184 (11, $[M-OH]^+$), 138 (18, $[M-C_2H_7O_2]^+$); Anal. Calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.51; N, 6.96. Found: C, 59.62; H, 9.59; N, 6.87.

Ethyl 8-chloro-8-hydroxyiminooctanoate (**9**).—The oxime (2 g, 10.1 mmol) was dissolved in $CHCl_3$ (32 mL) and pyridine (397 mg, 5 mmol) before *N*-chlorosuccinimide (1.34 g, 10.1 mmol) was added slowly at 0 °C. The resulting solution was stirred at rt for 3.5 h before being poured onto ice-water. The organic layer was separated and washed with water (30 mL) then brine (30 mL) and the aq layer was extracted with Et_2O (2×30 mL). The organic layers were combined, dried ($MgSO_4$) and concentrated under reduced pressure to afford a pale yellow oil (2.05 g, 86%): ^{1}H NMR (200 MHz, $CDCl_3$) 4.10 (2 H, q, *J* 7 Hz, OCH_2), 2.46 (2 H, t, *J* 7 Hz, CH_2COO), 2.27 (2 H, t, *J* 7 Hz, $CH_2C{=}N$), 1.61 (4 H, m, $(CH_2)_2$), 1.47–1.19 (7 H, m, $(CH_2)_2$, CH_3); ^{13}C NMR (50.31 MHz, $CDCl_3$) 174.55 (CO), 144.34 (C=N), 60.85 ($COOCH_2$), 25.20, 26.51, 28.57, 29.11, 34.72, 36.92 (CH_2), 14.70 (CH_3).

*2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucopyranosyl-(7-ethoxycarbonylheptyl) thiohydroximate.*—The synthesis was carried out as for the heptyl derivative using 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (2.24 g, 6.14 mmol) and the oximyl chloride (2.03 g, 8.59 mmol) in dry THF (65 mL). Recrystallisation from a mixture of hexane and EtOAc afforded the title compound as a white solid (2.57 g, 74%): mp 90 °C; $[\alpha]_D^{25}$ −12.1° (*c* 0.14, $CHCl_3$); IR (Nujol): *v* 3240 (OH), 1685, 1715 (CO), 1225 cm^{-1} (COOC); ^{1}H NMR (200 MHz, CD_3OD) 5.39 (1 H, t, $J_{3,4}$ 10 Hz, H-3), 5.28 (1 H, d, $J_{1,2}$ 10 Hz, H-1), 5.05, 4.98 (2 H, $2 \times$ t, *J* 10, 10 Hz, H-2,4), 4.83 (1 H, br s, NOH), 4.22–4.07 (4 H, m, OCH_2 and *H*-6^a,6^b), 4.00 (1 H, m, H-5), 2.55 (2 H, t, *J* 7 Hz, $CH_2C{=}N$), 2.33 (2 H, t, *J* 7 Hz, CH_2COO), 2.06, 2.03, 1.99, 1.98 (12 H, $4 \times$ s, CH_3COO), 1.65 (4 H, m, $(CH_2)_2$), 1.40 (4 H, m, $(CH_2)_2$), 1.25 (3 H, t, *J* 7 Hz, CH_3); ^{13}C NMR (50.31 MHz, CD_3OD) 174.37 ($COOCH_2CH_3$), 171.00, 170.37, 170.03, 169.78 (CH_3COO), 151.06 (C=N), 79.50 (C-1), 75.48 (C-5), 73.95 (C-3), 70.54 (C-2), 68.49 (C-4), 62.27 (C-6), 60.25 (OCH_2), 33.81 31.86, 28.64, 28.50, 27.11, 24.69, 19.54, 19.45, 19.39 (CH_2), 13.41 (OCH_2CH_3); *m/z*(CI) 564 (MH^+, 2%), 331 (100, $C_6H_7O(OAc)_4^+$), 271 (33, $C_6H_7O(OAc)_3^+$); Anal. Calcd for $C_{24}H_{37}NO_{12}S$: C, 51.15; H, 6.62; N, 2.49. Found: C, 51.19; H, 6.72; N, 2.49.

*2,3,4,6-Tetra-*O*-acetyl-(7-ethoxycarbonylheptyl) glucosinolate.*—Chlorosulfonic acid (9.1 g, 78.1 mmol) was added to dry CH_2Cl_2 (65 mL) and the resulting solution was added slowly to an ice-cold solution of dry CH_2Cl_2 (65 mL) and dry pyridine (58.6 g, 741 mmol) maintained under a N_2 atmosphere. The thiohydroximate (2.2 g, 3.9 mmol) was added as a solution in dry CH_2Cl_2 (45 mL) at rt and the resulting solution was stirred under a N_2 atmosphere for 24 h. Potassium hydrogen carbonate (11.7 g, 117 mmol) was added in water (100 mL) and the resulting two-phase system was concentrated under reduced pressure to allow removal of the organics. This yielded a substantial quantity of salts which were removed by filtration before the aq layer was extracted with EtOAc. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure to afford a cream-coloured foam. This was purified by flash chromatography, on silica gel, eluting with (1:1) petroleum ether (bp 40–60 °C)–EtOAc then (4:1) EtOAc–MeOH. This afforded the title compound as a cream-coloured solid (1.91 g, 72%): mp (dec.) 109–111 °C; $[\alpha]_D^{25}$ −11.4° (*c* 0.14, H_2O); IR (Nujol); *v* 1700 (CO), 1560 (C=N), 1200 cm^{-1} (COOC); ^{1}H NMR (200 MHz, CD_3OD) 5.41 (1 H, t, $J_{3,4}$ 10 Hz, H-3), 5.37 (1 H, d, $J_{1,2}$ 10 Hz, H-1), 5.06, 4.99 (2 H, $2 \times$ t, *J* 10, 10 Hz, H-2,4), 4.30-3.88 (5 H, m, OCH_2, H-5,6^a,6^b), 2.67 (2 H, t, *J* 7 Hz, $CH_2C{=}N$), 2.34 (2 H, t, *J* 7 Hz, CH_2COO), 2.07, 2.04, 2.02, 1.99 (12 H, $4 \times$ s, CH_3COO), 1.67 (4 H, m, $(CH_2)_2$), 1.41 (4 H, m, $(CH_2)_2$), 1.25 (3 H, t, *J* 7 Hz, CH_3); ^{13}C NMR (50.31 MHz, CD_3OD) 175.89 ($COOCH_2CH_3$), 172.50, 171.80, 171.52, 171.23 (COO), 159.77 (C=N), 81.19 (C-1), 77.03 (C-5), 75.33 (C-3), 71.75 (C-2), 69.86 (C-4), 63.69 (C-6), 61.72 (OCH_2), 35.30, 33.83, 30.09, 29.97, 28.45, 26.13 (CH_2), 21.03, 20.87 (CH_3COO), 14.89 (CH_3); *m/z* (ES^-) 642 ($[M-K]^-$, 100%); Anal. Calcd for $C_{24}H_{36}KNO_{15}S_2$: C, 42.28; H, 5.32; N, 2.05. Found: C, 42.53; H, 5.41; N, 2.29.

(7-Methoxycarbonylheptyl) glucosinolate (**10b**).—2,3,4,6 - Tetra - *O* - acetyl - (7 - ethoxycarbonylheptyl) glucosinolate (750 mg, 681.8 mmol) was dissolved in dry MeOH (10 mL) before a catalytic quantity of potassium

methoxide was added with stirring until the pH reached 8–9. The resulting solution was stirred at rt, under a N_2 atmosphere, for 24 h. Concentration under reduced pressure afforded a cream-coloured foam (540 mg, 98%): $[\alpha]_D^{25}$ −12° (*c* 0.2, H_2O); IR (Nujol); *v* 3400 (OH), 2850, 2930 (alkyl), 1720 (CO), 1250 cm^{-1} (COOC); ^1H NMR (200 MHz, D_2O) 4.93 (1 H, d, $J_{1,2}$ 9 Hz, H-1), 3.81 (1 H, dd, $J_{5,6b}$ 2, $J_{6a,6b}$ 11 Hz, H-6_b), 3.63 (1 H, m, H-6_a), 3.61 (3H, s, OCH_3), 3.42 (4 H, m, H-2,3,4,5), 2.63 (2 H, t, *J* 7 Hz, CH_2C=N), 2.32 (2 H, t, *J* 7 Hz, CH_2COO), 1.57 (4 H, m, $(CH_2)_2$), 1.30 (4 H, m, $(CH_2)_2$); ^{13}C NMR (50.31 MHz, D_2O) 180.60 (COO), 167.53 (C=N), 84.73 (C-1), 83.02 (C-5),7 9.99 (C-3), 74.87 (C-2), 72.01 (C-4), 63.52 (C-6), 55.05 (OCH_3), 36.57, 34.99, 30.77, 30.67, 29.63, 27.10 (CH_2); *m*/*z* (ES^-) 460 ($[M-K]^-$, 100%); Anal. Calcd for $C_{15}H_{26}KNO_{11}S_2 \cdot 0.5\ H_20$ requires C, 35.42; H, 5.35; N, 2.75. Found: C, 35.26; H, 5.15; N, 2.65.

Attempted synthesis of (7-carboxyheptyl) glucosinolate (**11**).—(7-Methoxycarbonylheptyl) glucosinolate (100 mg, 0.20 mmol) was dissolved in MeOH (600 μL) before 1 M potassium hydroxide (300 μL, 0.30 mmol) was added and the resulting solution was stirred overnight at rt. Analysis by TLC ((4:1) EtOAc–MeOH) showed the reaction to be incomplete and more potassium hydroxide (100 μL, 0.10 mmol) was added. The resulting solution was stirred as before for several hours prior to concentration under reduced pressure. Methanol was then added and the remaining solid was removed by filtration before purification, on C_{18} reverse-phase silica, eluting with water then MeOH. NMR analysis of the aq fractions showed (7-carboxyheptyl) glucosinolate as a mixture with two side-products; ^1H NMR (500 MHz, D_2O) 5.00 (H-1), 3.88 (H-6^b), 3.71 (H-6^a), 3.56 (H-3), 3.54 (H-5), 3.45 (H-4), 3.44 (H-2), 2.69 (H-1′), 2.17 (H-6′), 1.71 (H-2′), 1.55 (H-5′), 1.40 (H-3′), 1.33 (H-4′); ^{13}C NMR (125.8 MHz, D_2O) 186.68 (COOH), 167.63 (C=N), 84.50 (C-1), 82.80 (C-5), 79.74 (C-3), 74.63 (C-2), 71.81 (C-4), 63.26 (C-6), 40.23 (C-6′), 34.80 (C-1′), 31.00, 30.67, 29.59, 28.43 (C-2′,3′,4′,5′); *m*/*z* (ES^-) 222 ($[M-2K]^{2-}$, 100%).

NMR studies.—NMR spectra were acquired using a Varian Unity+ spectrometer, operating at 500.3 MHz (^1H frequency), with a nominal probe temperature of 30 °C. One-dimensional ^1H spectra were recorded with 20 s relaxation delay, to ensure full relaxation between pulses and accurate quantification from peak integrals. Prior to transformation, all data were apodised with a cosine-bell function followed by zero-filling.

To suppress zero-quantum correlations in NOESY experiments, the mixing time was incremented on each pulse of the 16-step phase cycle.[20] The total variation of the mixing time was ±5%. 1D TOCSY-NOESY spectra were acquired using the implementation described by Uhrin and Barlow.[20] All selective pulses used the quiet-sneeze pulse shape, spanning a single bandwidth. Spectra were acquired with 400 ms NOE mixing time, a recycle time of 5.4 s per transient, and a digital resolution after transformation of 0.2 Hz/point. After 1000 scans the limit of detection of a signal above the noise was estimated to be an NOE of 0.2% (equivalent to 4.9 Å time-averaged $^1H-^1H$ distance).

J-coupling constants were measured from homonuclear *J*-resolved spectra, with a digital resolution of 0.25 Hz/point in f_1 after transformation.

Molecular modelling.—Minimum energy models of the acidic glucosinolate **11** were computed using either the AM1 semi-empirical parameter set implemented within the MOPAC software package, or the AMBER molecular mechanics force field, implemented within the DISCOVER package (MSI, San Diego). All calculations were run on a Silicon Graphics O2 computer, with R5000 processor. Initial models were constructed from β-D-glucose (optimised using AMBER), with the hydrophobic acyl chain extended away from the saccharide. Following an initial AM1 optimisation to refine the geometry of the thiooxime functionality the structure was modified by restrained molecular mechanics minimisation to place the alkyl chain into an orientation consistent with experimental NOE data. Potentials of 10 kcal/mol/Å^2 (up to a maximum of 10 kcal/mol) were applied to restrain the protons of the two methylene groups closest

to the thiooxime to within 5 Å of Glc H-1 and to within 5 Å of Glc H-5. As the conformation was under-defined by experimental data at the distal end of the carboxyheptyl chain an additional weak restraint (up to 2 kcal/mol maximum) was applied between Glc C-3 and the carbonyl carbon of the carboxylate. Since there are no reliable molecular mechanics parameters available for a thiooxime, crude parameters for bond lengths and bond angles were added to the AMBER force field library, based upon the AM1 optimised geometry. Bond lengths for the thiooxime were consistent with the corresponding values in the crystal structure of sinigrin,[22] with the exception of *b*(N–O) which was 1.27 Å by AM1 and 1.44 Å in sinigrin. Most bond angles were also similar in the AM1 and crystal structure models, but significant differences were observed for *t*(S–C-7–C-8) (109.1° by AM1 vs. 125.3° in sinigrin), *t*(N–O–S) (119.0° by AM1 vs. 109.4° in sinigrin) and *t*(C-7–N–O) (120.1° by AM1 vs. 110.7° in sinigrin). Models are thus presented as a qualitative description only. The values used for the sulfate group parameters were those reported by Huige and Altona.[24] Torsional potentials for the thiooxime were set to zero. The resulting structure was subjected to a final AM1 minimisation to compensate for the unrefined AMBER parameters in the thiooxime.

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