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Synthesis and Mass Spectrometric Analysis of Aromatic and Indole Glucosinolates

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The synthesis and mass spectrometric analysis of aromatic and indole glucosinolates were investigated in this study. It was found that besides the peaks at *m/z* 75, 80, 96, 97, 195, 259, 275, the mass spectra also contained peaks that served unambiguously to identify a particular glucosinolate based on the loss of SO_3 from its unambiguousness to identify a specific ion $[M-K]^-$, and other ions such as $[R\text{-}C(=NOH)S]^-$ and $[R\text{-}C(S)OSO_2]^-$ generated from the fragmentation of the backbone skeleton of GLs.

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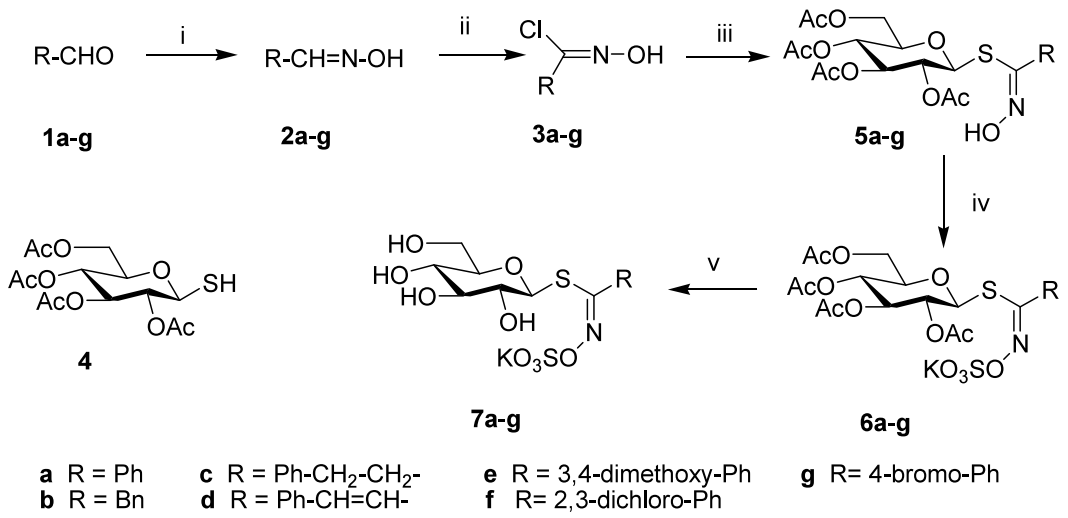
Glucosinolates (GLs) are *β*-thioglucoside *N*-hydroxysulphates with a side chain (R) and a sulphur-linked *β*-D-glucopyranose moiety. These are natural compounds which are found in a significant number of *Brassica* species such as cabbage, broccoli, and canola [1]. Owing to the fact that the potential biological activity and medicinal uses [2], the synthesis and the analysis of structure and bio-activities of GLs have been paid much attention [3]. Several mass spectrometric techniques have been used for qualitative and quantitative determination of GLs [4−9]. Electrospray ionization in the negative ion mode is one of the most convenient methods to use, as GLs readily produce negative ions which fragment under collision-induced dissociation conditions to give several diagnostic product ions [4,10,11]. The studies indicated that the peaks at *m/z* 75, 96, 97 are useful to identify glucosinolates in a complex mixture as well as are widely used for qualitative and quantitative investigations of glucosinolates [4−10]. However, up to now, the mass spectrometric analysis of indole GLs (Glucobrassicin, neo-glucobrassicin and 4-methoxyglucobrassicin) has not been adequately studied yet. Here we report the synthesis and mass spectrometric analysis of two important families of GLs, including aromatic and indole GLs.

RESULT AND DISCUSSION

Synthesis of Glucosinolates

Aromatic glucosinolates. Aromatic GLs were synthesized following the literature (Scheme 1) [12,13]. The chlorooxime **3a-g** was synthesized following the aldoxime pathway [14–16]. The aldehyde **1a-g** was reacted with hydroxylamine hydrochloride in MeOH in the presence of pyridine to yield oxime **2a-g**, which was treated with *N*-chlorosuccinimide (NCS) in *N,N*-dimethylformamide (DMF) to create, hydroxymoyl chloride **3a-g** in an excellent yield (74–97% yield over two steps). The coupling of 2,3,4,6-tetra-*O*-acetyl-l-thio-*β*-D-glucopyranose **4** [17,18] with hydroxymoyl chlorides **3a-g** was conducted by a general method [14,19,20]. To guarantee that no thiol material was wasted, the coupling was carried out with an excess of the hydroxymoyl chloride **3a-g** to maximize the conversion of **5a-g**. The thiohydroximates were purified by flash chromatography eluting with dichloromethane (DCM)/MeOH in excellent yield (81%–96%). In all cases, the coupling was stereospecific in that only the (*Z*)-isomers were formed [21,13]. The sulphation was conducted in the normal way by reaction of thiohydroximates **5a-g** with pyridine-



Conditions: (i) $NH_2OH.HCl$, MeOH, pyridine, rt, 2.5 h; (ii) NCS, DMF, 0 °C - rt, 3 h; (iii) **4**, Et_3N, DCM:Et_2O (2:1)
(iv) (1) $Pyr.SO_3$, DCM, reflux, 18 h; (2) $KHCO_3$, H_2O, 2 h; (v) MeOK, MeOH, 3 h

Scheme 1. Synthesis of aromatic GLs.

Table 1. Overall yield of synthetic aromatic GLs **7**.

No.	R	Yield (%)					Overall
		2	**3**	**5**	**6**	**7**	
a	Ph	94	89	96	64	90	46
b	Bn	94	84	83	85	82	46
c	$PhCH_2CH_2$	97	87	81	72	96	47
d	PhCH=CH	–	83	86	83	89	47
e	3,4-dimethoxyphenyl	95	84	96	68	93	48
f	2,3-dichlorophenyl	99	87	90	81	99	62
g	4-bromophenyl	98	99	83	85	99	68

sulphur trioxide reagent in DCM at refluxing temperature (around 40ºC) to form the potassium salts **6a-g**. The GLs **7a-h** were obtained in a good yield (46%–68% overall yield) by straightforward de-*O*-acetylation of **6a-g** with a catalytic amount of potassium methoxide in MeOH [22,12].

*Indole Glucosinolates***.** The indole GLs were synthesized following the nitronate method (Scheme 2) [23]. The conventional Henry reaction of the aldehydes **8a-c** with nitromethane in the presence of ammonium acetate at reflux for 2 h yielded the nitroalkenes **9a-c** in 78%–99% yields. The nitrovinyl group was then reduced by $NaBH_4$ in tetrahydrofuran (THF) and MeOH to form the nitroalkanes **10a-c** in 75%–79% yield [24–26, 21]. The nitroalkanes **10a-c** were reacted with sodium methoxide in MeOH to make sodium nitronate derivatives which were treated with thionyl chloride in 1,2-dimethoxyethane (DME) at –40ºC to convert to (indol-3-yl)acetohydroxymoyl chlorides. The coupling of the hydroxymoyl chlorides and thiol **4** was carried out by a general method in DCM/Et_2O in the presence of Et_3N to make indole thiohydroxymates **11a-c** (42%–46% overall yield from the nitroalkanes) [23].

Sulphation and de-*O*-acetylation of **11a-c** were carried out by Vo's method using pyridine-sulphur trioxide complex in DCM and then MeOK in MeOH to create final indole GLs **13a-c** in 21%–29% overall yield (Scheme 2, Table 2) [23].

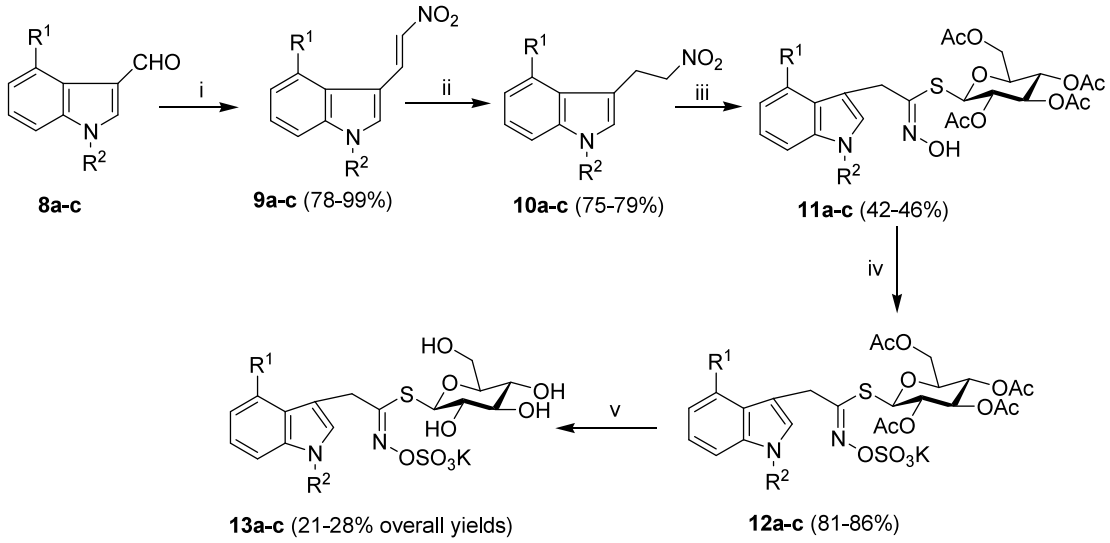
Mass Spectrometric Analysis of the Synthetic GLs

It is claimed that GLs are ideal candidates for negative-ion electro-spray ionization mass spectrometry because of the sulphate moiety in their molecular structure [4]. The product ion spectra of all GLs anions are known to show intense signals at *m/z* 75 (HO-CH=CH-S^-), *m/z* 96 ($SO_4^{-\bullet}$), *m/z* 97 (HSO_4^-), *m/z* 119, *m/z* 195 [4,9, 27–29].

The CID spectra of GLs also show two peaks at *m/z* 259 and 275 as prominent peaks to identify the presence of GLs (Figure 1 and Figure 3) [10, 30, 31]. Two other significant fragment ion peaks observed in GL product ion spectra are those for $[R\text{-}C(=NOH)S]^-$ and $[R\text{-}C(S)OSO_2]^-$.

Investigation of MS on synthetic aromatic GLs has indicated that the $[M\text{-}K]^-$ ion was the significant ion (100%) in the spectra. An accurate mass determination has also confirmed the ions $[M\text{-}K]^-$ as molecular ions of synthetic aromatic GLs for negative-ion electro-spray ionization mass spectrometry. Studies in MS/MS of molecular ions ($[M\text{-}K]^-$) of synthetic aromatic GLs has shown that the peaks *m/z* 259 and 275 were prominent peaks. Besides, the peaks $[R\text{-}C(=NOH)S]^-$ and $[R\text{-}C(S)OSO_2]^-$ were also noticeable observed ions in most of synthetic GLs. The fragmentation to form the ions $[R\text{-}C(=NOH)S]^-$ and $[R\text{-}C(S)OSO_2]^-$ was confirmed and a proposed pathway based on available experimental details is illustrated in Figures 2 and 3.

Another interesting observation is that the product ion spectra of all investigated GLs also show a low but specific peak fragment ion $[M\text{-}K\text{-}SO_3]^-$(Figure 3). This ion was formed by loss of a sulphur oxide from the molecular anion $[M\text{-}K]^-$, and its formation is illustrated by a proposed pathway in Figure 2.



a $R^1 = R^2 = H$; **b** $R^1 = H$, $R^2 = OMe$; **c** $R^1 = OMe$, $R^2 = H$

Conditions: (i) $MeNO_2$, $AcONH_4$, reflux, 2 h; (ii) $NaBH_4$, THF, MeOH, rt, 6 h; (iii) (1) MeONa, MeOH; (2) $SOCl_2$, DME, - 40 °C; (3) **4,** DCM/Et_2O, Et_3N; (iv) (1) $Pyr.SO_3$, pyridine, rt, 18 h; (2) $KHCO_3$, H_2O; (v) MeOK, MeOH, 3 h

Scheme 2. Synthesis of indole GLs.

Table 2. Yields of indole GLs.

R_1, R_2	Yield (%) Sulphates **12**	Indole GLs **13**	Overall
$R^1 = R^2 = H$	**a** 86%	**a** 96%	21%
$R^1 = H$, $R^2 = OMe$	**b** 81%	**b** 99%	22%
$R^1 = OMe$, $R^2 = H$	**c** 82%	**c** 97%	29%

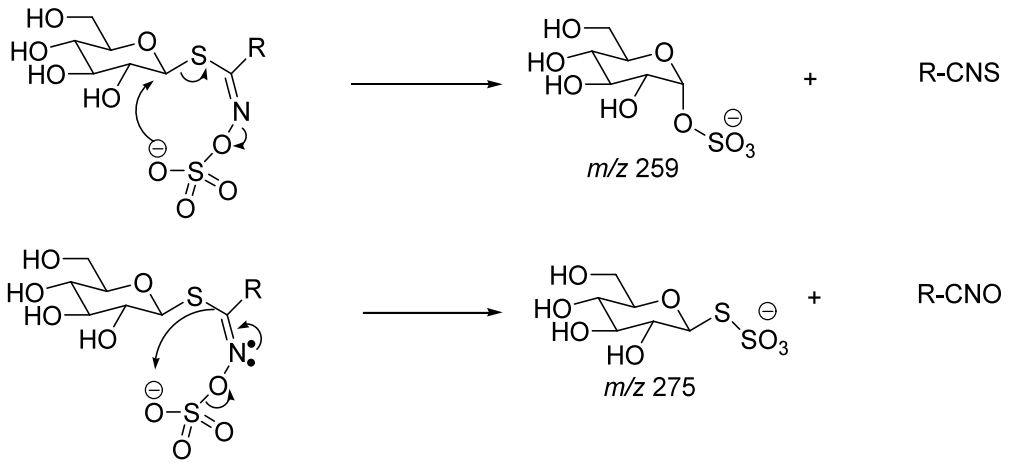


Figure 1. Formation of m/z 259 and 275 ions from synthetic GLs.

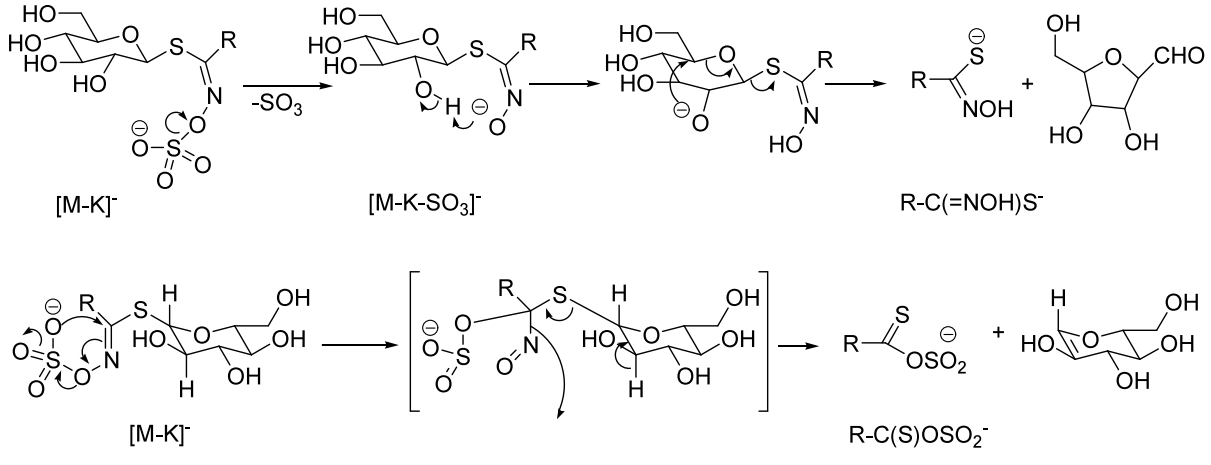


Figure 2. Formation of *m/z* of $[R\text{-}C(=NOH)S]^-$ and $[R\text{-}C(S)OSO2]^-$ ions from GLs.

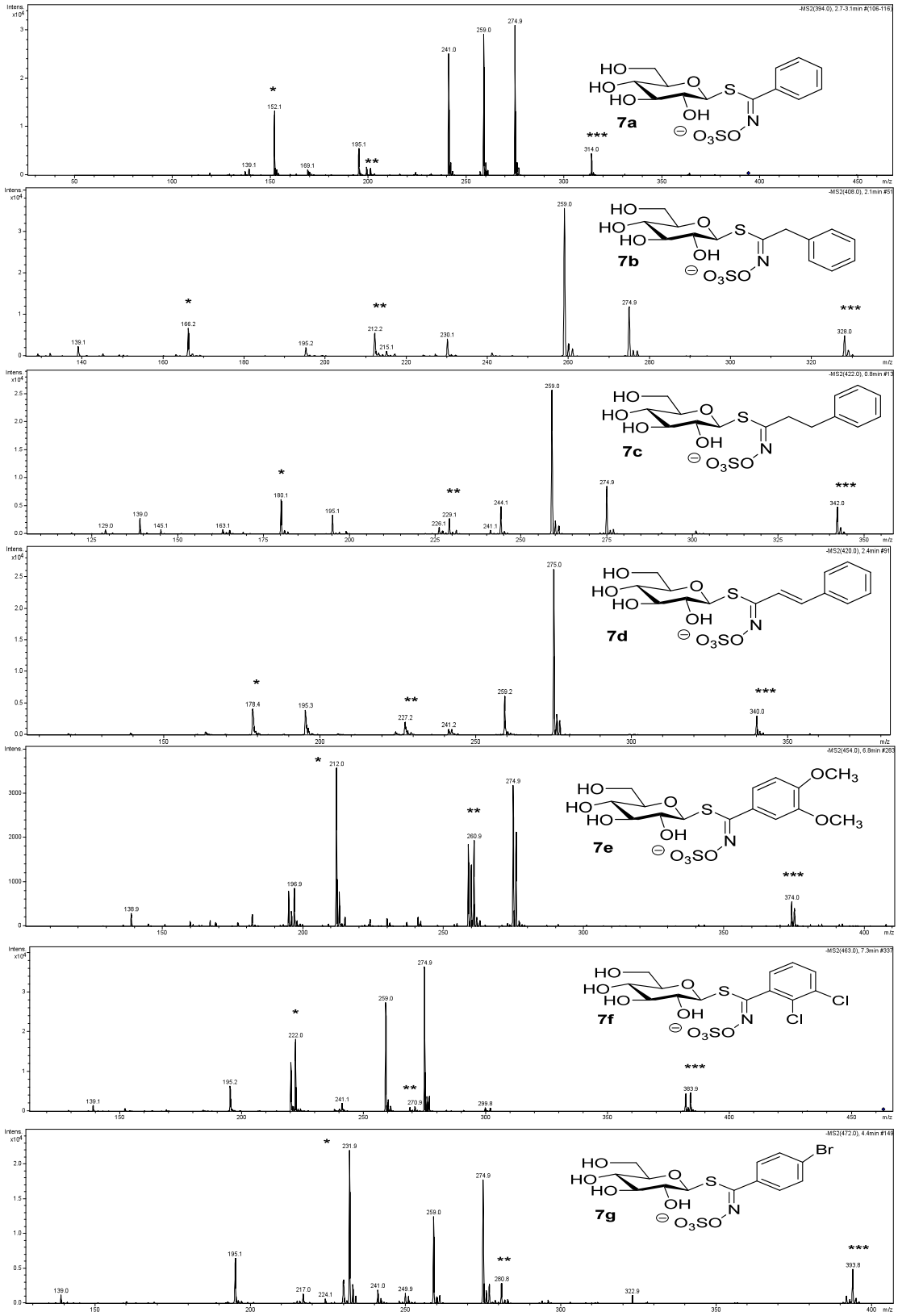


Figure 3. MS/MS of molecular ions [M-K]; *: $[R\text{-}C(=NOH)S]^-$; ** : $[R\text{-}C(S)OSO_2]^-$; *** : $[M\text{-}K\text{-}SO_3]^-$.

Therefore, it is claimed that, beside the product ion spectra of all investigated GLs such as 75, 80, 96, 97, 195, 259, 275, other ions such as $[M\text{-}K\text{-}SO_3]^-$, $[R\text{-}C(=NOH)S]^-$ and $[R\text{-}C(S)OSO_2]^-$ were specific ions to prove the presence of particular R groups in GLs (Table 3).

MS of synthetic indole GLs showed that the $[M\text{-}K]^-$ ion was the base peak (100%) in the spectra. An accurate mass determination had also confirmed the ions $[M\text{-}K]^-$ as molecular ions of synthetic GLs for negative-ion electro-spray ionization mass spectrometry. Studies in MS/MS of the molecular ions ($[M\text{-}K]^-$) of the synthetic indole GLs showed that while the peaks *m/z* 259 and 275 were prominent peaks for GB (**13a**) and MGB (**13c**), the peak *m/z* 446 was the most significant observed fragment ion of NGB (**13b**) (Figure 4). This ion was formed by loss of a methoxy of the N-indole from the molecular anion $[M\text{-}K]^-$ (477 $[M\text{-}K]^-$ – 31 $[CH_3O]$ = 446 [M-K-MeO]). The study of MS^3 of $[M\text{-}K]^-$ from NGB (MS of ion *m/z* 446) indicated that the peaks *m/z* 259 and 275 were considerable ions (Figure 5). This could be confirmed by the presence of GL structure of ion *m/z* 446 and the ion *m/z* 446 formed by the loss of a methoxy group.

The peak $[R\text{-}C(=NOH)S]^-$ was also a noticeably observed ion in GB (*m/z* 205) and MGB (*m/z* 235) spectra. The fragmentation to form the ion $[R\text{-}C(=NOH)S]^-$ was confirmed and a pathway is proposed based on the experimental details, as illustrated in Figures 4 and 6.

EXPERIMENTAL

General Procedures

Melting points (mp) were recorded on a Reichert 'thermopan' hot stage apparatus and were uncorrected. Optical rotations were measured at the stated temperatures in the stated solvent on a Perkin Elmer 141 polarimeter at the sodium d-line (589 nm); [α]D values are given in 10^{-1} $degcm^2g^{-1}$. Infrared spectra (ν_{max}) were recorded on a Bruker Vector 22 Fourier-Transform Spectrometer or a Perkin Elmer 1720-X FT-IR Spectrometer. Samples were analyzed using KBr Diffuse Reflectance Fourier Transform spectra (for solids) or as thin films on NaCl plates (for liquids/oils). Unless otherwise specified, proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer operating at 300 MHz for proton and 75 MHz for carbon nuclei. Chemical shifts are recorded as δ values in parts per million (ppm). Spectra were acquired in deuterated chloroform ($CDCl_3$) at 300 K unless otherwise stated. For ^1H NMR spectra were recorded in $CDCl_3$ and the peak due to residual $CHCl_3$ (δ_H 7.24) was used as the internal reference, while the central peak (δ_C 77.0) of the $CDCl_3$ triplet was used as the reference for proton-decoupled ^{13}C NMR spectra. Low-resolution mass spectra were measured on a Brüker Daltonics Esquire 6000 mass spectrometer at 300ºC and a scan rate of 5500 *m/z*/second using either water/methanol/acetic acid in a ratio of 0/99/1 or 50/50/1 as a mobile phase. The accurate mass measurement was by mass spectrometry

Table 3. A list of specific peaks observed in negative–mode CID fragmentation of $[M\text{-}K]^-$ ions of the synthetic GLs.

Comp.	R (group)	$[M\text{-}K]^-$	$[M\text{-}K\text{-}SO_3]^-$	Fragment ions specific to each R	
				$R\text{-}C(=NOH)S^-$	$R\text{-}C(S)OSO_2^-$
7a	Ph	394	314	152	201
7b	Bn	408	328	166	215
7c	$Ph\text{-}CH_2\text{-}CH_2$	422	342	180	229
7d	Ph-CH=CH	420	340	178	227
7e	3,4-Dimethoxyphenyl	454	374	212	261
7f	2,3-Dichlorophenyl	463	383	220, 222 (2:3)	269, 271 (2:3)
7g	4-Bromophenyl	473	393	232	281

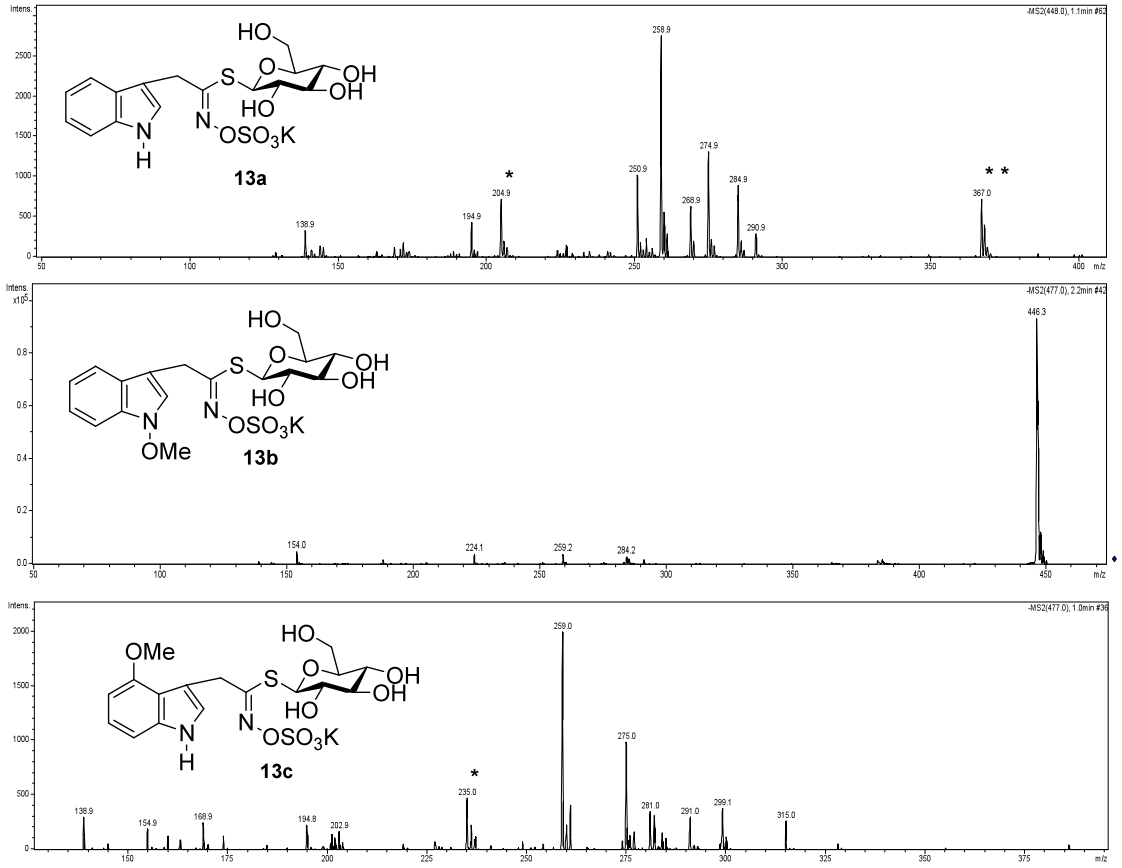


Figure 4. MS/MS of the synthetic indole GLs; * : $[R\text{-}C(=NOH)S]^-$; ** : $[M\text{-}K\text{-}SO_3]^-$.

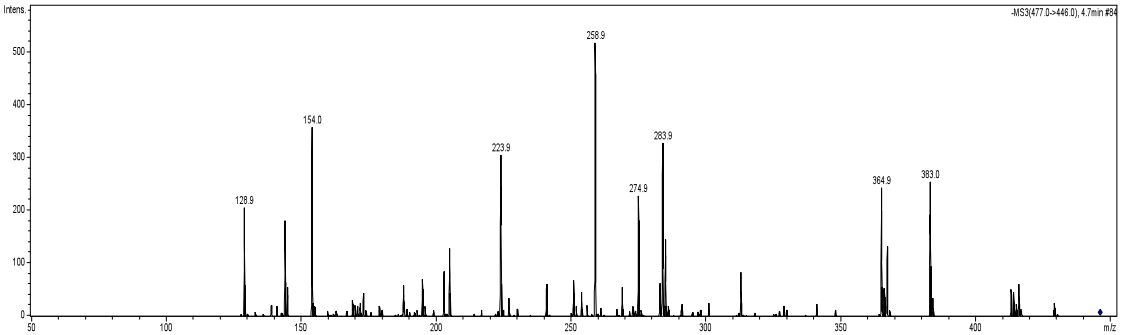


Figure 5. MS/MS/MS of the $[M\text{-}K]^-$ of NGB (MS of ion *m/z* 446).

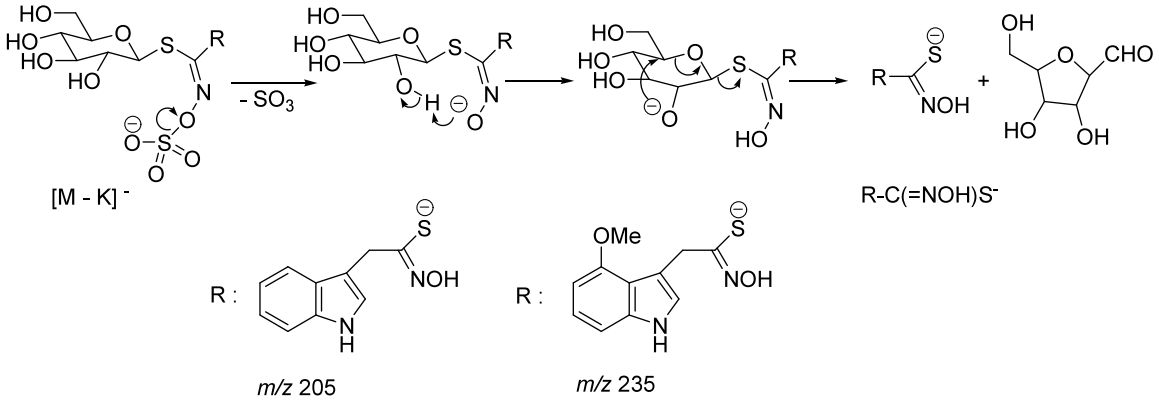


Figure 6. Formation of *m/z* of R-C(=NOH)S⁻ ion from GB and MGB.

utilising an LTQ Orbitrap Velos instrument (Thermo Scientific, Waltham, MA, USA; Bremen, Germany) with a heated electrospray ionisation source. The mass spectrometer was operated with full scan (50–1000 amu) in positive or negative FT mode (at a resolution of 100 000). The analyte was dissolved in water/methanol/acetic acid in a ratio of 0/99/1 or 50/50/1 and infused via syringe pump at a rate of 5 µl/min. The heated capillary was maintained at 320ºC with a source heater temperature of 350ºC and the sheath, auxiliary and sweep gases were at 40, 15 and 8 units, respectively. Source voltage was set to 4.2 kV. Solvents were dried over standard drying agents and freshly distilled before use. Ethyl acetate and hexane used for chromatography were distilled prior to use. All solvents were purified by distillation. Reactions were monitored by TLC on silica gel 60 F254 plates with detection by UV fluorescence or charring with a basic potassium permanganate stain. Flash column chromatography was performed on silica gel 60 particle size 0.040–0.063 µm (230–400 mesh).

General Procedure for the Preparation of Aromatic GLs[12]

Stirred solution of hydroxymoyl chloride **3a-g** (**3a-g** were synthesized following the literature) [12], (1.5 eq.) in dry Et_2O:DCM (2:1, 45 ml) was added a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylthiol **4** (**4** were synthesized following the literature)[22], (1 eq.) in dry DCM (6 ml). The resulting mixture was treated with Et_3N (6 eq.) in Et_2O (12 ml). The reaction mixture was stirred for 2 h at rt under N_2 then acidified with 1 M H_2SO_4 (7 ml/mmol of sugar). The mixture was left to stand for 10 min and then separated. The aqueous phase was extracted with DCM (3 × 30 ml). The combined organic layers were dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The thiohydroxymates **5a-g** were obtained by flash chromatography eluting with 0–3% MeOH/DCM. To a stirred solution of the thiohydroxymate **5a-g** (1 eq.) in dry DCM (40 ml) was added pyridine sulphur trioxide complex (2.5 eq.). After stirring and refluxing under Ar for 18 h, an additional portion of the complex (0.3 eq.) was added and stirred continously for 2 h. After that, a solution of $KHCO_3$ (4 eq.) in water (40 ml) was added and the mixture was stirred for 30 min and then it was concentrated under reduced pressure. The residue was dissolved in water and extracted with chloroform (2 × 30 ml) and then with 80% $CHCl_3$/MeOH (3 × 30 ml). The organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. To remove excess pyridine, the mixture was co-distilled several times with toluene. The compounds **6a-h** were obtained by flash chromatography eluting with DCM/MeOH.

To a solution of *O*-acetylglucosinolate **6a-g** in anhydrous MeOH (20 ml) under an N_2 atmosphere was added dry MeOK (0.4 eq.) until pH = 8–9 was reached. After stirring for 3 h at rt, the solution was made neutral by the addition of glacial acetic acid then the solution was concentrated under reduced pressure. The GLs **7a-h** were obtained by flash chromatography eluting with EtOAc:MeOH:H_2O (16:4:1).

*Phenylglucosinolate **7a**.*

Pure **7a** was obtained as a white solid (99 mg, 46% overall yield). R_f = 0.26 in EtOAc:MeOH:H_2O 16:4:1; mp = 119–121ºC (dec.); $[\alpha]_D^{21}$ = –12.5 (c = 1.8, H_2O); IR (KBr DRIFT) ν_{max} = 3322 (OH), 1632 (C=N), 1555, 1444, 1252, 1062 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.55–7.43 (m, 5H, ArH), 4.19 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 3.60 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.52 (dd, $J_{5,6b}$ = 4.2 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.39 (dd, $J_{2,3}$ = 8.7 Hz, $J_{1,2}$ = 10.2 Hz, 1H, H2), 3.29 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 6.6 Hz, 1H, H4), 3.17 (dd, $J_{2,3}$ = 8.7 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H3), 2.64–2.59 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 163.6 (C=N), 130.6 (C-1 of Ph), 129.9 (p-Ph-C), 128.6 (m-Ph-C), 128.4 (o-Ph-C), 83.1 (C-1), 79.5 (C-5), 76.6 (C-3), 71.2 (C-2), 68.4 (C-4), 59.8 (C-6); HRMS (ESI) *m/z* for $C_{13}H_{16}O_9NS_2K$ $[M-K]^-$, calcd 394.0266, found 394.0256.

*Glucotropaeolin **7b**.*

Pure **7b** was obtained as a white solid (101 mg, 46% overall yield). R_f = 0.14 in 15% EtOAc/MeOH; mp = 132–134ºC (dec.); $[\alpha]_D^{18}$ = +5 (c = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3317 (OH), 1650 (C=N), 1567, 1495, 1265, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.37–7.26 (m, 5H, ArH), 4.61–4.59 (m, ^{1}H, H1), 4.05 (s, 2H, CH_2Ph), 3.60–3.46 (m, 2H, H6a and H6b), 3.33–3.20 (m, 4H, H2, H3, H4 and H5); ^{13}C-NMR (75 MHz, D2O) (300 K): δ 162.3 (C=N), 134.7 (C-1 of Ph), 128.8, 127.7 (m- + o-Ph-C), 127.2 (p-Ph-C), 80.9 (C-1), 79.5 (C-5), 76.6 (C-3), 71.4 (C-2), 68.4 (C-4), 59.9 (C-6), 37.8 (CH_2Ph); HRMS (ESI) *m/z* for $C_{14}H_{18}O_9NS_2$ $[M-K]^-$, calcd 408.0423, found 408.0414.

*Gluconasturtiin **7c**.*

Pure **7c** was obtained as a white solid (651 mg, 47% overall yield). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 169–171ºC (dec.); $[\alpha]_D^{20}$ = –22.5 (c = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3335 (OH), 1568 (C=N), 1484, 1412, 1257, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.32–7.18 (m, 5H, ArH), 4.80 (d, $J_{1,2}$ = 9.3 Hz, 1H, H1), 3.76 (dd, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6a), 3.44–3.01 (m, 4H, H2, H3, H4 and H5), 3.01–2.86 (m, 4H, $PhCH_2CH_2$); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 163.0 (C=N), 140.2 (C-1 of Ph), 128.4 (C-3 and C-5 of Ph), 128.4 (C-2 and C-6 of Ph), 126.3 (C-4 of Ph), 81.3 (C-1), 79.7 (C-5), 76.7 (C-3), 71.5 (C-2), 68.7 (C-4), 60.2 (C-6), 33.5 ($PhCH_2CH_2$), 32.2 ($PhCH_2CH_2$); HRMS (ESI) *m/z* for $C_{15}H_{20}O_9NS_2$ $[M-K]^-$, calcd 422.0585 found 422.0570.

*2-Phenylethenylglucosinolate **7d**.*

Pure **7d** was obtained as a yellow solid (206 mg, 47% overall yield). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 117–119ºC (dec.); $[\alpha]_D^{20}$ = –40.2 (c = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3374 (OH), 2887, 1628 (C=N), 1575, 1537, 1494, 1448, 1271, 1060 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.54–7.51(m, 2H, H2' and H6'-Ph-H), 7.38–7.34 (m, 3H, H3', H4' and H5'-Ph-H), 7.26 (d, *J* = 15.9 Hz, 1H, PhCH=CH), 6.85 (d, *J* = 15.9 Hz, 1H, Ph*CH*=CH), 4.92 (d, *J* = 9.6 Hz, 1H, H1), 3.78-3.55 (m, 2H, H6a and H6b), 3.42–3.33 (m, 4H, H2, H3, H4 and H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 160.7 (C=N), 138.9 (PhCH=CH), 134.5 (C-1 of Ph), 129.6 (C-4 of Ph), 128.8 (C-3, C-5 of Ph), 127.3 (C-2, C-6 of Ph), 117.5 (Ph*CH*=CH), 82.4 (C-1), 80.1 (C-5), 76.7 (C-3), 71.7 (C-2), 68.8 (C-4), 60.3 (C-6) HRMS (ESI) *m/z* for $C_{15}H_{18}O_9NS_2$ $[M-K]^-$, calcd 420.0428 found 420.0415.

*3,4-Dimethoxyphenylglucosinolate **7e**.*

Pure **7e** was obtained as a white solid (110 mg, 48% overall yield). R_f = 0.11 in EtOAc/MeOH/H_2O (16:4:1); mp = 104–106ºC (dec.); $[\alpha]_D^{20}$ = +12.3 (c = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3317

(OH), 1650 (C=N), 1567, 1495, 1265, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.13–7.11 (m, 2H, H2' and H6'-Ph-H), 7.07–7.04 (m, 1H, H5'-Ph-H), 4.27 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 3.81 (s, 6H, 2 × CH_3O), 3.58–3.46 (m, 2H, H6a and H6b), 3.38–3.14 (m, 3H, H2, H3 and H4), 2.67–2.63 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 162.9 (C=N), 149.9 (C-4 of Ph), 147.8 (C-3 of Ph), 122.8 (C-1 of Ph), 122.4 (C-2 of Ph), 112.0 (C-5 of Ph), 111.2 (C-6 of Ph), 83.5 (C-1), 79.6 (C-5), 76.7 (C-3), 71.3 (C-2), 68.4 (C-4), 59.8 (C-6), 35.6, 55.5 (CH_3OPh); HRMS (ESI) *m/z* for $C_{15}H_{20}O_{11}NS_2$ $[M-K]^-$, calcd 454.0483 found 454.0470.

*2,3-Dichlorophenylglucosinolate **7f**.*

Pure **7f** was obtained as a white solid (145 mg, 62% overall yield). R_f = 0.14 in EtOAc/MeOH/H_2O (16:4:1); mp = 117–119ºC (dec.); $[\alpha]_D^{18}$ = +60 (c = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3354 (OH), 2882, 1566 (C=N), 1412, 1276, 1265, 1064 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.62–7.57 (m, 1H, H4'-Ph-H), 7.38–7.29 (m, 2H, H5'and H6'-Ph-H), 4.01 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 3.47–3.41 (m, 2H, H6a and H6b), 3.31–3.18 (m, 2H, H2 and H4), 3.10 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H3), 2.43–2.38 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 160.5 (C=N), 132.7 (C-3 of Ph), 132.4 (C-4 of Ph), 131.1 (C-1 of Ph), 130.3 (C-2 of Ph), 129.3 (C-6 of Ph), 127.7 (C-5 of Ph), 82.7 (C-1), 79.4 (C-5), 76.5 (C-3), 70.9 (C-2), 68.0 (C-4), 59.1 (C-6); HRMS (ESI) *m/z* for $C_{13}H_{14}O_9Cl_2NS_2$ $[M-K]^-$, calcd 461.9493 found 461.9470.

*4-Bromophenylglucosinolate **7g**.*

Pure **7g** was obtained as a white solid (223 mg, 68% overall yield). R_f = 0.2 in EtOAc/MeOH/H_2O (16:4:1); mp = 140–142ºC (dec.); $[\alpha]_D^{24}$ = +10.2 (c = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3337 (OH), 2875, 1568 (C=N), 1485, 1410, 1257, 1066 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.66 (d, *J* = 8.4 Hz, 2H, H3' and H5'-Ph-H), 7.44 (d, *J* = 8.4 Hz, H2' and H6'-Ph-H), 4.22 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 3.62 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.53 (dd, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.36 (dd, $J_{2,3}$ = 9.0 Hz, $J_{1,2}$ = 9.9 Hz, 1H, H2), 3.28 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.3 Hz, 1H, H4), 3.18 (dd, $J_{2,3}$ = 9.0 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H3), 2.69–2.66 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 162.5 (C=N), 131.6 (C-3 and C-5 of Ph), 130.4 (C-2 and C-6 of Ph), 129.1 (C-4 of Ph), 124.7 (C-1 of Ph), 83.1 (C-1), 79.6 (C-5), 76.7 (C-3), 71.3 (C-2), 68.4 (C-4), 59.8 (C-6); HRMS (ESI) *m/z* for $C_{13}H_{15}O_9NS_2$ $[M-K]^-$, calcd 471.9377 found 471.9365.

General procedure for the preparation of indole GLs[23]

To a stirred solution of the indole nitroalkane **10a-c** (**10a-c** were synthesized following the literature)[23], (1.0 eq. mol) in dry MeOH (15 ml) under a nitrogen atmosphere was added sodium methoxide (2.0 eq. mol). After 20 min the reaction was concentrated under reduced pressure giving the nitronate as a white solid which was dried under a high vacuum for 15 min. The nitronate was cooled to –40ºC under nitrogen and then it was treated with dry DME (20 ml) at –40ºC. A solution of thionyl chloride (2.6 eq. mol) in dry DME (5 ml) at –40ºC was added to the nitronate dropwise to give a burgundy coloured solution. After 30 min at –40ºC, water (30 ml) was added and the solution was extracted with DCM (3 × 50 ml). The organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give an indol-3-yl acetohydroxymoyl chloride which was left under a high vacuum for 15 min and then reacted directly in the next step. Indol-3-yl acethydroxymoyl chloride in dry Et2O:DCM (2:1, 30 ml) was treated with a solution of 2,3,4,6-tetra-*O*-acetyl-l-thio-*β*-D-glucopyranose **4** (1.0 eq. mol) and dry triethylamine (6.0 eq. mol) in dry DCM (10 ml). The reaction was stirred for 2 h giving an orange solution. The reaction mixture was acidified with 1 M H_2SO_4 (7 ml/mmol sugar) then extracted using DCM (3 × 30 ml). The organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The indole thiohydroxymates **11a-c** were obtained by flash column chromatography on silica gel eluting with 2–5% MeOH/DCM. To a stirred solution of the thiohydroxymate **11a–c** (1 eq.) in dry pyridine (40 ml) was added pyridine sulphur trioxide complex (2.5 eq.). After stirring under N_2 for 18 h, an additional portion of the complex (0.3

eq.) was added and stirring was continued for 2 h. After that, a solution of $KHCO_3$ (4 eq.) in water (40 ml) was added and the mixture was stirred for 30 min and then it was concentrated under reduced pressure. The residue was dissolved in water and extracted with chloroform (3 × 40 ml). The organic layers were dried ($MgSO_4$), filtered and concentrated under reduce pressure. To remove excess pyridine, the mixture was co-distilled several times with toluene. The compounds **12a–c** were obtained by flash chromatography eluting with 80%–85% DCM/MeOH. To a solution of *O*-acetylglucosinolate **12a–c** in anhydrous MeOH (20 ml) under an N_2 atmosphere was added dry MeOK (0.8 eq.) until pH = 8–9 was reached. After stirring for 3 h at rt, the solution was made neutral by the addition of glacial acetic acid and then the solution was concentrated under reduced pressure. The GLs **13a–c** were obtained by flash chromatography eluting with EtOAc:MeOH:H_2O (16:4:1)[12].

*Glucobrassicin **13a**.*

Pure **13a** was obtained as a purple solid (390 mg, 21% overall yield). R_f = 0.14 in EtOAc/MeOH/H_2O (16:4:1); mp = 124–126ºC (dec.); $[\alpha]_D^{20} = -12$ (*c* = 1.0, H_2O); IR (KBr DRIFT) ν_{max} 3378, 1600, 1236, 1041 cm^{-1}; ^1H NMR (300 MHz, D_2O) (300 K) δ 7.66 (d, $J_{4i,5i}$ = 7.8 Hz, 1H, H4i), 7.44 (d, J6i,7i = 8.1 Hz, 1H, H7i), 7.22 (s, 1H, H2i), 7.19−7.06 (m, 2H, H5i and H6i), 4.72 (d, $J_{1,2}$ = 9.6 Hz, 1H, H1), 4.21 (d, J_{AB} = 16.2 Hz, 1H, C*H*HC=N), 4.13 (d, J_{AB} = 16.2 Hz, 1H, C*H*HC=N), 3.46−3.45 (m, 2H, H6a and H6b), 3.30−3.06 (m, 3H, H2, H3 and H4), 2.85−2.81 (m, 1H, H5); ^{13}C NMR (75 MHz, D_2O) (300 K) δ 162.7 (C=N), 135.8 (C-8i), 125.7 (C-9i), 123.6 (C-2i), 121.8 (C-6i), 119.2 (C-5i), 118.0 (C-4i), 111.6 (C-7i), 107.7 (C-3i), 81.0 (C-1), 79.4 (C-5), 76.5 (C-3), 71.3 (C-2), 68.2 (C-4), 59.8 (C-6), 28.9 (CH_2C=N); HRMS (ESI) *m/z* for $C_{16}H_{19}O_9N_2S_2$ [M-K]$^-$, calcd 447.0537, found 447.0537.

*Neoglucobrassicin **13b**.*

Pure **13b** was obtained as a purple solid (77.0 mg, 22% overall yield). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 109–111ºC (dec.); $[\alpha]_D^{20} = -2.5$ (c = 1, H_2O); IR (KBr DRIFT) ν_{max} 3126, 2936, 1713, 1573, 1453, 1242, 1060 cm^{-1}; ^1H NMR (300 MHz, D_2O) (300 K) δ 7.64 (d, *J* = 7.5 Hz, 1H, H4i), 7.45 (d, *J* = 7.5 Hz, 1H, H7i), 7.39 (s, 1H, H2i), 7.22 (t, *J* = 7.5 Hz, 1H, H6i), 7.09 (t, *J* = 7.5 Hz, 1H, H5i), 4.65 (d, *J* = 10.2 Hz, H1), 4.09 (d, J_{AB} = 16.7 Hz, 1H, *CH*HC=N), 4.02 (d, J_{AB} = 16.7 Hz, 1H, *CH*HC=N), 3.96 (s, 3H, CH_3O), 3.47−3.41 (m, 2H, H6a and H6b), 3.25 (t, *J* = 9.0 Hz, 1H, H4), 3.22 (t, *J* = 9.0 Hz, 1H, H2), 3.09 (t, *J* = 9.0 Hz, 1H, H3), 2.87−2.83 (m, 1H, H5); ^{13}C NMR (75 MHz, D_2O) (300 K) δ 162.3 (C=N), 131.9 (C-8i), 122.8 (C-9i), 122.4 (C-6i), 122.3 (C-2i), 120.1 (C-5i), 118.6 (C-4i), 108.4(C-7i), 105.0 (C-3i), 81.2 (C-1), 79.6 (C-5), 76.5 (C-3), 71.4 (C-2), 68.3 (C-4), 65.7 (CH_3O), 59.8 (C-6), 28.7 (CH_2C=N); HRMS (ESI) *m/z* for $C_{17}H_{21}O_{10}N_2S_2$ [M-K]$^-$, calcd 477.0643, found 477.0643.

*4-Methoxyglucobrassicin **13c**.*

Pure **13c** was obtained as a dark-green solid (145 mg, 29% overall yield). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 104–106ºC (dec.); $[\alpha]_D^{20} = -51$ (*c* = 1, H_2O); IR (KBr DRIFT) ν_{max} 3452, 3265, 2908, 2841, 1259, 1060 cm^{-1}; ^1H NMR (300 MHz, D_2O) (300 K) δ 7.09–7.01 (m, 3H, H2i, H6i and H7i), 6.57 (d, $J_{5i,6i}$ = 7.2.Hz, 1H, H5i), 4.77 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 4.32 (d, J_{AB} = 16.8 Hz, 1H, C*H*HC=N), 4.28 (d, J_{AB} = 16.8 Hz, 1H, *CH*HC=N), 3.85 (s, 3H, CH_3O), 3.47 (dd, $J_{5,6a}$ = 3.9 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.35 (dd,$J_{5,6b}$ = 2.3 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.27−3.16 (m, 2H, H2 and H4), 3.05 (t, $J_{2,3}$ = J3,4 = 9.0 Hz, 1H, H3), 2.67−2.64 (m, 1H, H5); ^{13}C NMR (75 MHz, D_2O) (300 K) δ 163.6 (C=N), 153.4 (C-4i), 137.4 (C-8i), 122.7 (C-6i), 122.5 (C-2i), 115.4 (C-9i), 108.0 (C-3i), 105.1 (C-7i), 99.7 (C-5i), 81.5 (C-1), 79.2 (C-5), 76.6 (C-3), 71.2 (C-2), 67.9 (C-4), 59.5 (C-6), 54.8 (CH_3O), 29.7 (CH_2C=N); HRMS (ESI) *m/z* for $C_{17}H_{21}O_{10}N_2S_2$ [M-K]$^-$, calcd 477.0643, found 477.0624.

CONCLUSION

A series of important aromatic and indole GLs were successfully synthesized. The structure and purity of the synthetic compounds have been confirmed

by NMR and HRMS data. The fragmentation pathways of the aromatic and indole glucosinolate anions under CID conditions were investigated. The study showed that, in the low-mass region, besides the peaks at *m/z* 75, 80, 96, 97, 119, 195, 259, 275 other ions $[M\text{-}K\text{-}SO_3]^-$, $[R\text{-}C(=NOH)S]^-$ and $[R\text{-}C(S)OSO_2]^-$ were specific ions to prove the presence of particular R groups in GLs. This could be used to identify glucosinolates in a complex mixture or used for qualitative and quantitative investigations of GLs.

ABBREVIATIONS

DCM, dichloromethane; DME, 1,2-dimethoxyethane; DMF, *N,N*-dimethylformamide; ESI, electrospray ionization; FTMS, Fourier transform mass spectrometry; GB, glucobrassicin; GLs, Glucosinolates; HESI, heated electrospray ionization; HRMS, high resolution mass spectrometry; IR, infra-red; MGB, 4-methoxyglucobrassicin; MS, mass spectrometry; NCS, *N*-chlorosuccinimide; NGB, neoglucobrassicin; NMR, nuclear magnetic resonance; rt, room temperature; THF, tetrahydrofuran; TLC, thin-layer chromatography; UV, ultra-violet.

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