SYNTHESIS OF INDOLE GLYCOSINOLATES, SUGAR VARIANTS OF NATURALLY OCCURRING GLUCOBRASSICIN[1]

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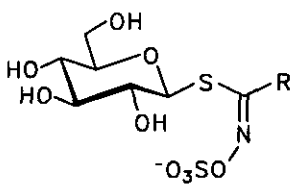
Abstract - *Eight sugar-variants (3c-10c) of naturally occurring glucobrassicin (2c) were synthesized via nucleophilic addition of glycosyl mercaptans on a common nitrile-oxide intermediate.*

INTRODUCTION

Glucosinolates (**1**) are an important structurally homogeneous class of compounds widely distributed in *Cruciferae*.[2-4] Among the more than 100 glucosinolates which have been listed after identification, tryptophan-lineaged indole structures[5] - the glucobrassicin group (**2**) - are largely encountered in vegetables belonging to the *Raphanus* and *Brassica* genera and consumed by humans, *i.a.* Chinese cabbages like pe-tsai and pak-choi.[6]



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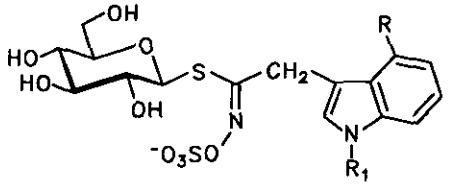
R= alkenyl, hydroxyalkenyl, alkylsulfinyl, aromatic, indol-3-ylmethyl

(the counter ion is generally K^+)

* Dedicated to Prof. Edward C. Taylor on the occasion of his 70th birthday



R_1= H	R= H	glucobrassicin (cpd **2c**)
R_1= OCH_3	R= H	neoglucobrassicin
R_1= H	R= OH	4-hydroxyglucobrassicin
R_1= H	R= OCH_3	4-methoxyglucobrassicin
R_1= OSO_3^-	R= H	sulfoglucobrassicin
R_1= $COCH_3$	R= H	N-acetylglucobrassicin

2

These indole glucosinolates are known to induce marked physiological activities, showing for example strong effects on the drug metabolizing enzyme system or on the chemically induced carcinogenesis.[7,8]

The degradative cascade of glucosinolates is mainly under the control of a thioglucoside glucohydrolase[9] called **myrosinase** (EC 3. 2. 3. 1.) the study of which has therefore important applications for both the biological and technological aspects of the food and feed stuff industries.[10]

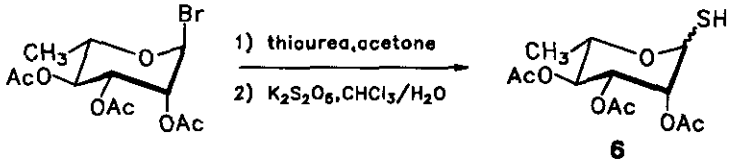
In order to provide a deeper understanding of this enzyme, it is important that its structure and main physico-chemical properties be characterized. We now describe the synthesis of several sugar-variants of glucobrassicin (**2c**), which can be used as model substrates in the structural study of the active site of myrosinase.

RESULTS AND DISCUSSION

The nitronate-pathway strategy recently devised by us[11] for the synthesis of glucobrassicin (**2c**) was further extended to the case of eight sugar-variants (**3c-10c**), starting from a previously described[12] common indole precursor (**11**).

Miscellaneous protected glycosyl mercaptans (**3-10**) were prepared according to literature procedures[13] with the exception of the unprecedented 2,3,4-tri-*O*-acetyl-1-thio-L-rhamnopyranose (**6**), which was obtained in the form of an anomeric mixture through a sequence adapted from Cerny[13] (Scheme 1).

Scheme 1



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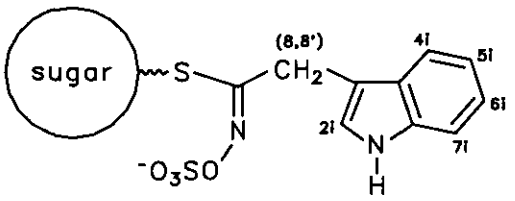
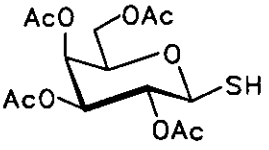
The key step of the glycobrassicin syntheses consisted in the coupling reaction between the thiosugar (**3-10**) and indol-3-ylacethydroximoyl chloride (**12**), which was readily obtained[11]

Miscellaneous glycobrassicins synthesized



	sugar
2c	D-Glc
3c	D-Gal
4c	D-Man
5c	D-GlcNAc
6c	L-Rha
7c	D-Xyl
8c	D-Ara
9c	Malto
10c	Cellobio



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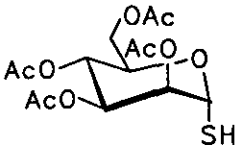

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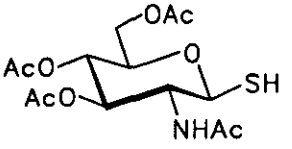

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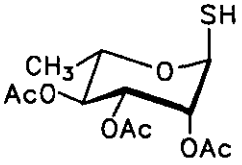

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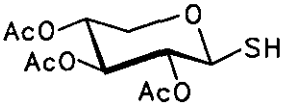

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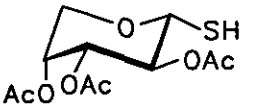

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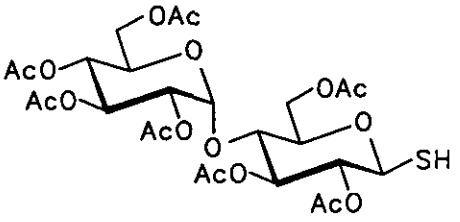

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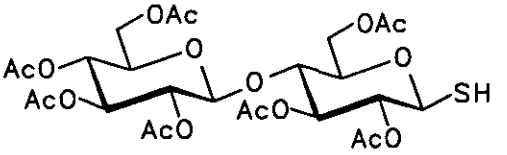

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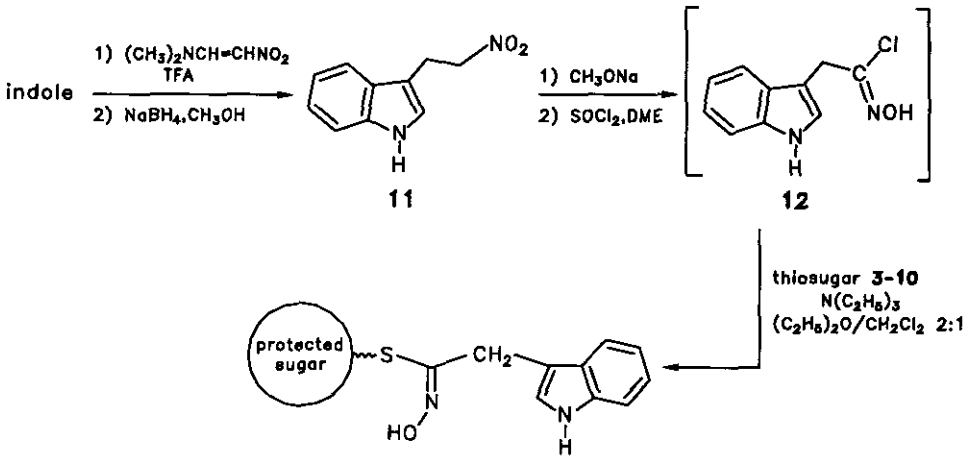
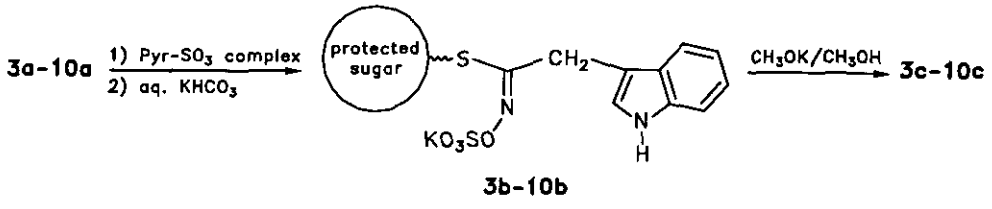
from **11**, to afford the glycosyl thiohydroximates (**3a-10a**) with yields ranging from 60 to 80% (Scheme 2).

Scheme 2



O-Sulfation at the hydroximino site of **3a-10a** was best effected with SO_3-pyridine complex, freshly prepared from chlorosulfonic acid[14] and used *in situ* to produce the proglycosinolates (**3b-10b**) as potassium salts in 60-85% yield (Scheme 3).

Scheme 3



Final deprotection by base-catalyzed methanolysis of the acetate groups yielded (70-90%) the target molecules (**3c-10c**), the purity of which was carefully checked by reverse phase ion-pairing hplc according to the usual method for intact glucosinolate determination[15] completed by fast scanning uv detection. Additional checking was provided by reverse phase hplc of the enzymically desulfated glycobrassicins.[16]

Characterization data for all the glycobrassicin derivatives synthesized are reported in Table 1; ^1H nmr data are listed in Tables 2-6. The potential inhibitory effects of **3c-10c** on myrosinase are currently under investigation.[17]

Table 1. Characterization data for compounds (**3a-10a, 3b-10b, 3c-10c**).

Compd	Yield (%)	$[\alpha]_D$ (c 1.0, solvent)	Mol. formula	LRms m/z	HRms calcd/found
3a	67	+17° ($CHCl_3$)	$C_{24}H_{28}N_2O_{10}S$	537 $(M+1)^+$	536.1465/536.1458
4a	75	+46° ($CHCl_3$)	$C_{24}H_{28}N_2O_{10}S$	537 $(M+1)^+$	536.1465/563.1449
5a	60	-8° (CH_3CN)	$C_{24}H_{29}N_3O_9S$	536 $(M+1)^+$	535.1624/535.1624
6a	65	-40° ($CHCl_3$)	$C_{22}H_{26}N_2O_8S$	480 $(M+1)^+$	478.1410/478.1412
7a	60	-22° ($CHCl_3$)	$C_{21}H_{24}N_2O_8S$	465 $(M+1)^+$	464.1253/464.1247
8a	70	-30° ($CHCl_3$)	$C_{21}H_{24}N_2O_8S$	465 $(M+1)^+$	464.1253/464.1229
9a	73	+51° ($CHCl_3$)	$C_{36}H_{44}N_2O_{18}S$	825 $(M+1)^+$	824.2310/824.2290
10a	80	-18° ($CHCl_3$)	$C_{36}H_{44}N_2O_{18}S$	825 $(M+1)^+$	824.2310/824.2287
3b	65	+7° (MeOH)	$C_{24}H_{27}N_2O_{13}KS_2$	615 $(M-K)^-$	654.0591/654.0578
4b	70	-4° (MeOH)	$C_{24}H_{27}N_2O_{13}KS_2$	615 $(M-K)^-$	654.0591/654.0589
5b	70	-16° (MeOH)	$C_{24}H_{28}N_3O_{12}KS_2$	614 $(M-K)^-$	653.0751/653.0730
6b	61	-13° (MeOH)	$C_{22}H_{25}N_2O_{11}KS_2$	557 $(M-K)^-$	596.0537/596.0528
7b	73	+4° (MeOH)	$C_{21}H_{23}N_2O_{11}KS_2$	543 $(M-K)^-$	582.0380/582.0364
8b	60	-43° (MeOH)	$C_{21}H_{23}N_2O_{11}KS_2$	543 $(M-K)^-$	582.0380/582.0384
9b	66	+34° (MeOH)	$C_{36}H_{43}N_2O_{21}KS_2$	903 $(M-K)^-$	942.1437/942.1418
10b	70	-30° (MeOH)	$C_{36}H_{43}N_2O_{21}KS_2$	903 $(M-K)^-$	942.1437/942.1425
3c	76	+11° (H_2O)	$C_{16}H_{19}N_2O_9KS_2$	509 $(M+Na)^+$	486.0169/486.0149
4c	85	-24° (H_2O)	$C_{16}H_{19}N_2O_9KS_2$	509 $(M+Na)^+$	486.0169/486.0157
5c	90	+19° (H_2O)	$C_{18}H_{22}N_3O_9KS_2$	528 $(M+Na)^+$	527.0434/527.0422
6c	68	-40° (H_2O)	$C_{16}H_{19}N_2O_8KS_2$	493 $(M+Na)^+$	470.0220/470.0221
7c	90	-27° (H_2O)	$C_{15}H_{17}N_2O_8KS_2$	479 $(M+Na)^+$	456.0063/456.0053
8c	90	-28° (H_2O)	$C_{15}H_{17}N_2O_8KS_2$	479 $(M+Na)^+$	456.0063/456.0047
9c	80	+26° (H_2O)	$C_{22}H_{29}N_2O_{14}KS_2$	671 $(M+Na)^+$	648.0697/648.0680
10c	70	-20° (H_2O)	$C_{22}H_{29}N_2O_{14}KS_2$	671 $(M+Na)^+$	648.0697/648.0674

Table 2. ^{1}H Nmr data of **3a-8a** in $CDCl_3$. Apparent coupling constant (Hz) in parentheses.

	3a	**4a**	**5a**	**6a**	**7a**	**8a**
H-1 $J_{1,2}$	4.95d (10.0)	5.76s	5.19d (10.3)	5.65s	5.15d (7.5)	4.59d (8.1)
H-2 $J_{2,3}$	5.17dd (10.0)	5.22-5.28m	3.96m	5.17-5.23m	4.85dd (7.5)	5.26dd (8.3)
H-3 $J_{3,4}$	4.79dd (3.2)	5.22-5.28m	4.93-5.05m	5.17-5.23m	4.96dd (7.5)	4.92m
H-4 $J_{4,5}$ (or $J_{4,5a}$)	5.30d	5.22-5.28m	4.93-5.05m	5.04dd (10.0)	4.84ddd (4.5)	5.16m (5.1)
H-5 (or H-5a) $J_{5,6a}$ (or $J_{5a,5b}$)	3.56dd (6.7)	4.26m (5.1)	3.36m (5.0)	4.14dq	4.16dd (12.2)	4.12dd (12.2)
H-5b $J_{4,5b}$					3.19dd (7.8)	3.37dd (2.4)
H-6a $J_{6a,6b}$	4.05dd (11.8)	4.22dd (12.2)	4.09dd (12.2)	CH_3 1.16d		
H-6b $J_{5,6b}$	3.98dd (6.3)	3.98d	3.97dd (2.1)	(6.2)		
Ac	1.93s,1.94s 2.04s,2.12s	1.96s,1.99s 2.03s,2.08s	1.71s,1.94s 1.96s,1.97s	1.91s,1,95s 2.03s	1.88s,2.02s	1.86s,2.03s 2.08s
H-8,H-8'	4.02d,4.12d (16.2)	4.00d,4.09d (16.2)	4.04s	3.99d,4.09d (16.2)	3.98d,4.05d (16.6)	3.98d,4.09d (16.2)
H-2i	7.12d (2.8)	7.10d (1.4)	7.00s	7.01d (2.2)	7.05d (2.4)	7.08d (2.1)
H-4i	7.62d (7.9)	7.61d (7.9)	7.56d (7.9)	7.57d (7.9)	7.50d (7.7)	7.59d (7.9)
H-5i	7.15dd (7.5)	7.12dd (7.5)	7.07dd (7.1)	7.09dd (7.5)	7.12dd (7.5)	7.13dd (7.5)
H-6i	7.23dd (7.5)	7.20dd (7.5)	7.16dd (7.1)	7.16dd (7,6)	7.20dd (7.5)	7.27dd (7.5)
H-7i	7.39d (7.9)	7.35d (7.9)	7.33d (7.9)	7.29d (7.9)	7.35d (7.7)	7.36d (7.9)

Table 3.^{1}H Nmr data of **3b-8b** in DMSO-d_6. Apparent coupling constant (Hz) in parentheses.

	3b	**4b**	**5b**	**6b**	**7b**	**8b**
H-1 $J_{1,2}$	5.35d (10.0)	5.82s	5.21d (10.5)	5.78s	5.30d (9.1)	5.27d (9.5)
H-2 $J_{2,3}$	4.94dd (10.0)	5.01-5.16m	3.80q (9.9)	5.05m (3.5)	4.77dd (9.1)	4.91dd (9.5)
H-3 $J_{3,4}$	5.14dd (3.5)	5.01-5.16m	5.01dd (9.9)	4.97d (9.9)	5.08dd (9.1)	5.09dd (3.6)
H-4 $J_{4,5}$ (or $J_{4,5a}$)	5.29d	5.01-5.16m	4.79dd (9.9)	4.86dd (9.9)	4.84ddd (5.3)	5.15m
H-5 (or H-5a) $J_{5,6a}$ (or $J_{5a,5b}$)	4.27dd (5.1)	4.17m (5.2)	3.56m (5.2)	3.96dq	3.95dd (13.0)	3.89d (13.0)
H-5b $J_{4,5b}$					3.49dd (9.7)	3.83dd (2.4)
H-6a $J_{6a,6b}$	3.95-4.08m (12.2)	4.14dd (12.2)	4.06dd (12.3)			
H-6b $J_{5,6b}$	3.98dd (7.4)	3.94d	3.77dd (2.2)	CH_3 0.96d (6.2)		
OAc	1.85s,1.89s 1.97s,2.11s	1.92s,1.99s 2.01s,2.02s	1,71s,1.88s 1.94s,1.966s	1.91s,1,99s 2.03s	1.81s,1.95s 1.97s	1.82s,1.92s 2.07s
H-8,H-8'	3.95-4.08m	3.97d,4.08d (16.2)	3.98d,4.05d (16.4)	3.96d,4.04d (16.4)	3.98s	3.98s
H-2i	7.34s	7.18s	7.25d (2.3)	7.15d (2.2)	7.33d (2.8)	7.33d (2.4)
H-4i	7.66d (7.9)	7.59d (7.9)	7.70d (7.9)	7.58d (7.9)	7.65d (7.9)	7.65d (7.9)
H-5i	6.96dd (7.5)	6.96dd (7.1)	6.97dd (7.5)	6.95dd (7.5)	6.95dd (7.5)	6.96dd (7.5)
H-6i	7.08dd (7.5)	7.07dd (7.1)	7.08dd (7.5)	7.06dd (7.5)	7.07dd (7.5)	7.07dd (7.5)
H-7i	7.35d (7.9)	7.33d (7.9)	7.36d (7.9)	7.33d (7.9)	7.34d (7.9)	7.35d (7.9)

Table 4. ^{1}H Nmr data of 3c-8c in D_2O. Apparent coupling constant (Hz) in parentheses.

	3c	4c	5c	6c	7c	8c
H-1 $J_{1,2}$	4.78d (9.5)	5.762s	4.95d (10.4)	5.70s	4.77d (9.5)	4.74d (9.5)
H-2 $J_{2,3}$	3.46-3.62m	3.84s	3.68dd (10.2)	3.81-3.91m	3.29dd (9.1)	3.55dd (9.5)
H-3 $J_{3,4}$	3.36dd (3.3)	3.59-3.73m	3.31dd (9.5)	3.67dd (9.8)	3.17dd (8.9)	3.38dd (3.6)
H-4 $J_{4,5}$ (or $J_{4,5a}$)	3.85d	3.59-3.73m	3.41dd (9.5)	3.41dd (9.8)	3.53ddd (5.4)	3.85m (2.0)
H-5 (or H-5a) $J_{5,6a}$ (or $J_{5a,5b}$)	3.31dd (5.1)	3.81m	3.07m	3.81-3.91m (12.6)	3.79dd (13.0)	3.72dd (12.8)
H-5b $J_{4,5b}$					2.90t	3.28d
H-6a $J_{6a,6b}$	3.46-3.62m	3.59-3.73m	3.65m	CH_3 1.14d		
H-6b $J_{5,6b}$	3.46-3.62m	3.59-3.73m	3.65m	(6.3)		
Ac			1.86s			
H-8,H-8'	4.19d,4.33d (16.2)	4.12d,4.35d (16.2)	4.15d,4.27d (16.3)	4.12d,4.31d (16.3)	4.17d,4.26d (16.2)	4.16d,4.27d (16.2)
H-2i	7.37s	7.35s	7.33s	7.34s	7.36s	7.33s
H-4i	7.79d (7.9)	7.76d (7.9)	7.76d (7.9)	7.77d (7.9)	7.78d (7.9)	7.77d (7.9)
H-5i	7.22dd (7.5)	7.17dd (7.1)	7.21dd (7.5)	7.19dd (7.5)	7.23dd (7.5)	7.21dd (7.5)
H-6i	7.30dd (7.5)	7.26dd (7.1)	7.29dd (7.5)	7.27dd (7.5)	7.30dd (7.5)	7.29dd (7.5)
H-7i	7.56d (7.9)	7.51d (7.9)	7.56d (7.9)	7.53d (7.9)	7.56d (7.9)	7.55d (7.9)

Table 5.^{1}H Nmr data of sugar moiety of **9a-10a** in $CDCl_3$, **9b-10b** in DMSO-d_6 and **9c-10c** in D_2O. Apparent coupling constant (Hz) in parentheses.

	9a	**10a**	**9b**	**10b**	**9c**	**10c**
H-1 $J_{1,2}$	5.06d (9.9)	4.97d (9.5)	5.36d (10.1)	5.32d (10.1)	4.84d (9.9)	4.86d (9.5)
H-2 $J_{2,3}$	4.80dd (9.1)	4.87dd (9.5)	4.68dd (9.6)	4.71dd (9.7)	3.31d (9.1)	3.34-3.48m
H-3 $J_{3,4}$	5.09dd (8.7)	4.99dd (9.5)	5.26dd (8.9)	5.11dd (9.1)	3.42dd (9.5)	3.34-3.48m
H-4 $J_{4,5}$	3.84dd (9.5)	3.67dd (9.5)	3.83-4.09m	3.78dd (9.5)	3.47-3.66m	3.34-3.48m
H-5 $J_{5,6a}$	3.31m (2.4)	3.17m (1.9)	3.83-4.09m (2.5)	3.82-4.01m	2.94m (2.4)	3.08m (4.0)
H-6a $J_{6a,6b}$	4.28dd (12.2)	4.28dd (12.2)	4.33dd (12.2)	4.18-4.26m	3.82dd (12.2)	3.63dd (12.2)
H-6b $J_{5,6b}$	4.01-4.10m	3.91dd (5.5)	3.83-4.09m	3.82-4.01m	3.47-3.66m	3.59dd (2.4)
H-1' $J_{1',2'}$	5.33d (4.0)	4.43d (7.9)	5.19d (3.9)	4.78d (8.9)	5.26d (4.0)	4.47d (7.9)
H-2' $J_{2',3'}$	4.82dd (10.3)	4.86dd (8.7)	4.81dd (10.4)	4.62dd (8.9)	3.47-3.66m	3.27dd (8.3)
H-3' $J_{3',4'}$	5.31dd (10.1)	5.11dd (9.5)	5.16dd (10.0)	5.22dd (9.7)	3.47-3.66m	3.48dd (9.5)
H-4' $J_{4',5'}$	5.02dd (10.1)	5.03dd (9.5)	4.93dd (9.7)	4.86dd (9.7)	3.47-3.66m	3.34-3.48m
H-5' $J_{5',6a'}$	3.91m (4.3)	3.61m (4.3)	3.83-4.09m (5.7)	3.82-4.01m	3.47-3.66m (4.7)	3.34-3.48m
H-6a' $J_{6a',6b'}$	4.23dd (12.2)	4.32dd (12.2)	4.15dd (16.3)	3.82-4.01m	3.74dd (12.2)	3.89d (12.2)
H-6b' $J_{5',6b'}$	4.01-4.10m	4.01dd (2.0)	3.83-4.09m	3.82-4.01m	3.47-3.66m	3.72dd (4.7)
OAc	1.91s,1.97s 1.99s,2.01s 2.02s,2.09s 2.10s	1.93s,1.97s 2.00s,2.01s 2.06s,2.08s	1.80s,1,88s 1.90s,1.93s 1.94s,1.97s	1.80s,1.86s 1.89s,1.91s 1.94s,1.95s 1.97s		

Table 6. ^{1}H Nmr data of the indole moiety of **9a** & **10a** in $CDCl_3$, **9b** & **10b** in DMSO-d_6 and **9c** & **10c** in D_2O. Apparent coupling constant (Hz) in parentheses.

	9a	**10a**	**9b**	**10b**	**9c**	**10c**
H-8,H-8'	4.01d,4.10d (16.2)	4.01d,4.08d (16.2)	3.83-4.09m	4.01s	4.18d,4.27d (16.4)	4.30d,4.44d (16.6)
H-2i	7.11s	7.11d (2.4)	7.31d (2.4)	7.36s	7.34s	7.38s
H-4i	7.63d (7.9)	7.60d (7.9)	7.64d (7.9)	7.65d (7.9)	7.76d (7.9)	7.79d (7.9)
H-5i	7.17dd (7.5)	7.12dd (7.5)	6.92dd (7.5)	6.93dd (7.5)	7.20dd (7.5)	7.22dd (7.5)
H-6i	7.25dd (7.5)	7.21dd (7.5)	7.04dd (7.5)	7.07dd (7.5)	7.28dd (7.5)	7.31dd (7.5)
H-7i	7.41d (7.9)	7.39d (7.9)	7.37d (7.9)	7.34d (7.9)	7.53d (7.9)	7.56d (7.9)

EXPERIMENTAL

General methods. Optical rotations were measured with a Jobin-Yvon Digital type 71 polarimeter at 22°C. ^{1}H Nmr spectra were recorded at 300°K on a Bruker AM 300 (300.13 MHz for ^{1}H) spectrometer; chemical shifts are expressed in parts per million downfield from TMS. Mass spectra for **3a-10a** were recorded (CI mode) on a Nermag-R-10-10C spectrometer; those for **3b-10b** were recorded (FAB$^-$ mode) on a Nermag-R-10-10H; those for **3c-10c** (LSIms mode) on a VG-Autospec-Q apparatus. Thin layer chromatography was run on aluminium plates precoated with silica gel $60F_{254}$ (E. Merck, Darmstadt, Germany); detection was effected by observation under short wavelength uv light (254 nm), then dipping the chromatograms into a solution of ceric ammonium nitrate $[Ce(NH_4)_2(NO_3)_6]$ in 20% sulfuric acid and charring them with a heat gun. Column chromatography was performed using silica gel 60 (0.063-0.200 mm, E.Merck) and flash chromatography was conducted with silica gel (0.040-0.063 mm, E.Merck). 3-(2-Nitroethyl)indole (**11**) was prepared by the method of Büchi and Mak.[18] Glycosyl mercaptans (**3-10**) were synthesized according to reference 13.

2,3,4-Tri-*O*-acetyl-1-thio-α,β-L-rhamnopyranose (6). 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[19] (3 g, 8.5 mmol) and thiourea (0.78 g, 10 mmol) were dissolved in anhydrous acetone (20 ml) and the mixture was refluxed 30 min. After evaporation, the raw anomeric mixture of intermediate isothiouronium salts was obtained in the form of a white foam

which was used in the second step without further purification.
Potassium metabisulfite ($K_2S_2O_5$, 443 mg, 2.3 mmol) was dissolved in 10 ml of water at 85°C. Chloroform (8 ml) was added, immediately followed by the isothiouronium salt (1 g, 2.3 mmol) and the mixture was refluxed during 15 min. After cooling and extraction with chloroform (2 x 10 ml), the organic phase was dried on $MgSO_4$ and evaporated. The residue was purified by flash chromatography (3:2 petroleum ether/ethyl acetate) to give **6** (α/β mixture 85:15) as a colorless syrup (423 mg, 60 % yield) which slowly crystallized (mp *ca.* 68-70°C) after a few hours standing at 4°C. $[\alpha]_D$ -85° (*c* 1.0, $CHCl_3$). ir ν (film): 2570 (SH), 1750 (COO) cm^{-1}. HRms Calcd for $C_{12}H_{18}O_7S$: 306.0773; found: 306.0755. ^1H Nmr ($CDCl_3$) **α-anomer** : δ 1.24 (3H, d, $J_{5,6}$ = 6.3, CH_3); 1.99, 2.06, 2.17 (9H, 3s, OAc); 2,24 (1H, d, $J_{SH,1}$ = 6.0, SH); 4.22 (1H, dq, H_5); 5.10 (1H, ft, $J_{3,4} = J_{4,5}$ = 10.0, H_4); 5.29-5.35 (2H, m, H_2, H_3); 5.48 (1H, bd, H_1).
β-anomer : δ 1,27 (d, 3H, $J_{5,6}$ = 6,3, CH_3); 1,97, 2,04, 2,22 (3s, 9H, OAc); 2,49 (d, 1H, $J_{SH,1}$ = 9,9, SH); 3,58 (dq, 1H, H_5); 4,86 (bd, 1H, H_1); 5,03 (m, 1H, H_4); 5,42 (bs, 1H, H_2).

General procedure for the synthesis of thiohydroximates (3a-10a). To a stirred solution of freshly prepared indol-3-ylacethydroximoyl chloride[11] (**12**) (250 mg, 1.2 mmol) in 10 ml of an anhydrous ether/dichloromethane (2:1 v/v) mixture under argon were successively added the glycosyl mercaptan (**3-10**) (1 mmol) dissolved in dry dichloromethane (2 ml) and freshly distilled triethylamine (0.43 ml, 3 mmol). Triethylammonium hydrochloride immediately precipitated from the solution. After stirring for 1 h, the mixture was acidified with 0.5 M H_2SO_4, extracted with dichloromethane and the organic layer was dried ($MgSO_4$) and evaporated to dryness. The remaining solid was purified by column chromatography using ethyl acetate/petroleum ether (1:1 v/v) or methanol/dichloromethane (3:97 v/v) to afford amorphous **3a-10a** in 60-80% yield.

O-Sulfation of 3a-10a to the proglycosinolates (3b-10b). To a cooled (0°C) and stirred solution of pyridine (2 ml) in dry dichloromethane (2 ml) under argon, a solution of chlorosulphonic acid (0.3 ml, 4.5 mmol) in dry dichloromethane (2 ml) was added over a period of 15 min, followed by a solution of **3a-10a** (0.2 mmol) in dry dichloromethane (5 ml). After stirring for 24 h at room temperature, the medium was treated with a solution of potassium hydrogen carbonate (120 mg, 1.4 mmol) in water (2ml) and stirred for another 30 min; the solvents were evaporated and traces of pyridine removed by coevaporation with toluene. The residue was purified by column chromatography (eluent methanol/dichloromethane 1:9 v/v) to give an amorphous powder **3b-10b** (60-85%).

Deprotection of 3b-10b to the glycosinolates (3c-10c). To a stirred solution of **3b-10b** (0.1 mmol) in anhydrous methanol (10 ml) a few drops of a 1M solution of potassium methoxide were added in order to reach pH 8-9. When the reaction had finished (tlc), the solvent was

evaporated. The remaining solid was dissolved in water then submitted to freeze-drying, whereby compounds (**3c-10c**) were obtained (70-90%) in the form of hygroscopic white amorphous solids.

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