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A total synthesis of $(R,S)_S$-glucoraphanin

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ABSTRACT

A total synthesis of $(R,S)_S$-glucoraphanin (GRP) has been completed by a novel, simple and convenient method in high overall yield (17% over seven steps). The study describes a method for the synthesis of natural and unnatural (methylsulfinyl)alkyl glucosinolates (GLs) and also opens useful pathways to synthesize GRP as well as other sulfinyl GLs.

1. Introduction

GLs are β-thioglucoside *N*-hydroxysulfates with a side chain (R) and a sulfur-linked β-D-glucopyranose moiety. These are natural compounds, which are found in a large number of *Brassica* species such as cabbage, broccoli, and canola. 4-Methylsulfinylbutyl glucosinolate (GRP) is the most abundant glucosinolate in broccoli[1] or *Cardaria draba*[2] and is hydrolyzed by the enzyme myrosinase to sulforaphane that imparts numerous health benefits. Studies have shown that sulforaphane eliminates carcinogens in living tissue[3,4] and it is an inducer of phase 2 enzymes (glutathione *S*-transferase and quinone reductase),[5] which is thought to result in cancer protection.[6,7] A series of synthetic analogs of sulforaphane were developed both as a mixture of $(R,S)_S$-isomers and as R_S-sulforaphane.[8–11] Although sulforaphane has potential applications, it is volatile compared to GRP, which is a stable non-volatile compound. It is practical therefore to create a GRP based nutraceutical, which retains the health advantages of sulforaphane. Some studies in extraction of GRP from plants have been developed.[2,12–15] However, up to now, there have been only a few studies in synthesis of labeled GRP,[16,17] or oxidation of natural glucoercin.[5] The total chemical synthesis of GRP and the applications of the synthetic compound have not yet been studied. Here, we report a versatile synthetic approach for the synthesis of GRP.

2. Results and discussion

2.1. Synthesis of 5-(methylthio)pentanal

From previous studies, 5-(methylthio)pentanal **1** could be synthesized by either reduction of the corresponding ester or carboxylic acid.[18–20] Dawson's group used 5-bromopentanenitrile as starting material to synthesize aldehyde **1** in two steps in 18% overall yield.[18] However, using this pathway to synthesize the aldehyde **1** has some disadvantages such as low overall yield and the toxicity of methanethiol.

To avoid the disadvantages and improve the yield, the aldehyde **1** was synthesized by an oxidation pathway (Scheme 1). The chloride **2** was treated with sodium methanethiolate in MeOH/H_2O (3:1) for 4 h at rt to form **3** in 85% yield. The alcohol **3** was then oxidized by Swern reaction[21] to create the aldehyde **1** in 80% yield. Thus, the overall yield was improved to 68% yield over two steps, compared with 18% in Dawson's method. Furthermore, the final aldehyde **1** could be used directly in the next step without purification.

2.2. Synthesis of 5-(methylsulfinyl)pentanal oxime

Firstly, the 5-(methylsulfinyl)pentanal oxime **8** was synthesized following Morrison's method (Scheme 1).[16,17] To avoid oxidation of the aldehyde, in the first step, the aldehyde **1** was protected by reaction with ethane-1,2-diol in toluene in the presence of

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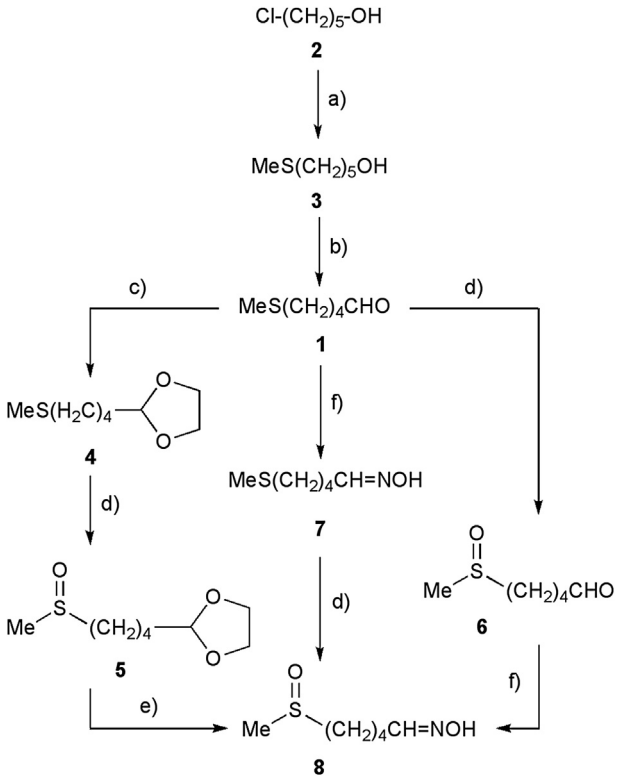


Scheme 1. Synthesis of 5-(methylsulfinyl)pentanal oxime **8**. Reagents and conditions: (a) CH_3SNa, MeOH/H_2O (3:1), 4 h; (b) $(COCl)_2$, DMSO, Et_3N, DCM, −78 °C; (c) $HOCH_2CH_2OH$, *p*-$MePhSO_3H$, toluene, reflux, 3 h; (d) $NaIO_4$, MeOH/H_2O (2:1), 3 h; (e) $H_2NOH{\cdot}HCl$ 5.0 equiv, 1 M H_2SO_4, CH_3CN/H_2O (3:1); (f) $H_2NOH{\cdot}HCl$, NaOAc, MeOH/H_2O (1:3).

p-toluenesulfonic acid as a catalyst for 3 h at reflux temperature to yield the 1,3-dioxolane **4** in 88% yield.[22]

From previous studies,[23–27] the oxidation reaction of sulfides to sulfoxides can be performed using a wide range of oxidation reagents. So, to find the best conditions for oxidation of **4** to **5**, combinations of reagents and solvents were investigated at different temperatures; the results are summarized in Table 1.

Table 1
Oxidation of **4** in a variety of conditions

Entry	Oxidation reagents	Solvent(s)	Temp (°C)	Reaction time (min)	Yield (%)
1	H_2O_2	H_2O	60	60	78
2	H_2O_2	MeOH/H_2O	rt	180	47
3	*m*-CPBA	DCM	rt	120	85[a]
4	NCS	MeOH	0	60	73[a]
5	NCS	Dioxane/H_2O	rt	60	64
6	HNO_3/wet SiO_2	DCM	rt	30	84[a]
7	SiO_2–HNO_3/KBr	DCM	rt	30	60
8	PVP–HNO_3/KBr	DCM	rt	30	76
9	$NaIO_4$	MeCN	−10	60	80
10	$NaIO_4$	MeOH/H_2O	rt	120	65
11	$NaIO_4$	MeOH/H_2O	0	60	82

[a] The converted yield of sulfide; note the product was a mixture of sulfoxide and sulfone.

Initially, the oxidation reaction employed hydrogen peroxide in H_2O at 60 °C (Table 1, entry 1) and hydrogen peroxide in MeOH/H_2O at rt (Table 1, entry 2). The yield of these reactions was 78 and 47%, respectively. The reaction was also carried out using *m*-CPBA/DCM, *N*-chlorosuccinimide (NCS)/MeOH, NCS/dioxane–H_2O, HNO_3/wet-SiO_2, SiO_2–HNO_3/KBr, and polyvinyl pyrrolidone (PVP) PVP–HNO_3/KBr (Table 1, entries 3–8) but the yield was not much improved and the products of reaction contained both the sulfoxide and the sulfone. Thus, it was difficult to control the amount of oxidant and reaction conditions to get only compound **5**. Moreover, the purification of the products was not an easy task.

From the studies of Afzali-Ardakani's group and Leonard's group,[28,29] sodium metaperiodate was used as oxidant for the reaction in three different sets of conditions (Table 1, entries 9–11). As can be seen from Table 1, the best result is with $NaIO_4$ (Table 1, entry 11). The reaction occurred within a 1 h reaction time in an excellent isolated yield (82%). Compound **5** was obtained as a mixture of $(R,S)_S$-isomers and could be used directly in the next step without purification. Sodium metaperiodate was the most efficient and convenient reagent for the oxidation of the sulfide to the sulfoxide and though the yield was similar to that in entry 1, it was used in the following reactions.

The deprotection of the dioxolane **5** could not be carried out under normal conditions involving aqueous acid, because of the formation of condensation products.[17] This problem was solved by carrying out the protection in the presence of excess hydroxylamine in order to trap the aldehyde in situ, before competing condensation reactions could occur, and so form the desired oxime **8** in one step. Following the research on the oximation,[17] the reaction was carried out with excess hydroxylamine hydrochloride in H_2O, but the yield was only about 18% (Table 2, entry 1). To study the effect of concentration of hydroxylamine hydrochloride on the reaction, the amount of the reagent was increased from 2 to 10 equiv. It was found when the concentration of hydroxylamine hydrochloride was more than 5 equiv, the yield of the reaction reached a maximum value. Thus 5 equiv of hydroxylamine hydrochloride was chosen for the next phase of the study.

Table 2
Deprotection of the dioxolane and oximation of **5** in a variety of conditions

Entry[a]	Acid	Solvent	Temp[b] (°C)	Yield of **8**[c] (%)
1	—	H_2O	rt	18
2	—	H_2O	100	17
3	—	MeOH/H_2O	rt	50
4	—	MeOH/H_2O	60	46
5	—	CH_3CN/H_2O	rt	48
6	—	CH_3CN/H_2O	60	45
7	1 M H_2SO_4	H_2O	rt	37
8	1 M H_2SO_4	H_2O	100	32
9	1 M H_2SO_4	MeOH/H_2O	rt	53
10	1 M H_2SO_4	MeOH/H_2O	60	41
11	1 M H_2SO_4	CH_3CN/H_2O	rt	54
12	1 M H_2SO_4	CH_3CN/H_2O	60	34

[a] Amount of $H_2NOH{\cdot}HCl$ 5 equiv, H_3O^+ 1.2 equiv.
[b] Reaction was conducted for 24 h and monitored by TLC.
[c] Isolated yield of **8**.

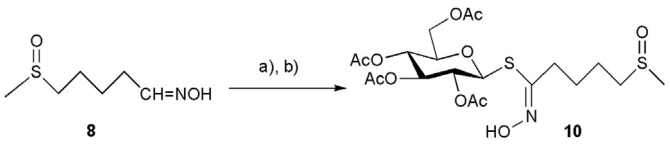
To find the best conditions for the reaction, different reaction conditions were applied. The results are shown in Table 2. It is shown that the reaction should be conducted at rt rather than with warming. However, the low yield of reaction was still a limitation (around 54% yield). This is because the acid conditions reduce the nucleophilicity of the hydroxylamine. Consequently, after finishing the reaction, the reaction mixture always had the aldehyde **6** present, although hydroxylamine was added in an excess amount. It is difficult to purify the oxime from reaction residue because of the presence of the aldehyde **6** and ethylene glycol. The oxime **8** could be lost in the aqueous phases because of its high polarity and hence water solubility. An attempt was made to improve the reaction yield by using MeOH for acetalation of the aldehyde, but the results were not satisfactory. As a result, the overall yield of **8** was only 39% for the 1,3-dioxolane (34% for the dimethyl acetal) over three steps.

Fortunately, from these studies, it was found that the aldehyde **6** could be isolated, so a process to synthesize **8** without carbonyl protection was planned (Scheme 1). The aldehyde **1** was oxidized with sodium metaperiodate in MeOH/H_2O (2:1) for 3 h at rt. After work-up and purification by flash column chromatography eluting with DCM/MeOH (9:1), compound **6** was obtained as a colorless oil (75% yield). The oximation of aldehydes normally employs hydroxylamine hydrochloride in MeOH or EtOH in the presence of pyridine,[9,30] but it was shown that the reaction of **6** with hydroxylamine hydrochloride in MeOH formed the acetal 1,1-dimethoxy-5-(methylsulfinyl)pentane as a side product. Consequently, the isolated yield of **8** was low (49%). To improve the yield of the reaction, the oximation of **6** was carried out following Wang's method.[31] The aldehyde was treated with hydroxylamine hydrochloride (3 equiv) and CH_3COONa (3 equiv) in MeOH/H_2O (1:3). After work-up, the oxime **8**, as a mixture isomers, was obtained as a colorless oil (76% yield) by flash column chromatography.

By this method, the overall yield of **8** was enhanced to 57%. However, it was also found that the aldehyde **6** was not very stable, which could be a reason for the low yield of the oximation reaction. This problem was solved by doing the oximation reaction first and then oxidation of the sulfur atom (Scheme 1). It was pleasing that the yields of the oximation and oxidation reactions were successfully improved to 92 and 91%, respectively, by this strategy. As a result, compound **8** was obtained in 84% overall yield over two steps. The study has shown that the pathway to synthesize oxime **8** following the oximation of aldehyde **3** and then oxidation of the resulting oxime **7** was the simplest and most convenient method. The results can be widely applied to synthesize other alkylsulfinyl alkyl oximes.

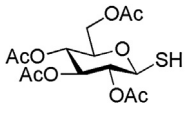
2.3. Coupling of thiol glucose 9 with 5-(methylsulfinyl)pentanal oxime

In most cases of glucosinolate synthesis, the oxime **8** is then converted to the chlorooxime using NCS, before coupling to 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl thiol **9** (compound **9** can be synthesized according to Floyd's method[32]). However, in the literature,[16,17] the chlorooxime was noted as an unstable compound and in our hands could not be isolated. Therefore, the chlorination and subsequent coupling reactions were carried out in one pot (Scheme 2). The oxime **8** was reacted with NCS in the presence of pyridine to



Scheme 2. The synthesis of *S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(5-(methylsulfinyl)pentyl)thiohydroximate **10**. Reagents and conditions: (a) NCS (1.05 equiv), DCM, pyridine; (b) **9**, Et_3N, DCM.

form the chlorooxime and then it was immediately treated with a mixture of thioglucose **9** and triethylamine in DCM. Consequently, the thiohydroximate **10** was obtained in 47% yield over two steps. The NMR, MS, IR data confirm that compound **10** is *S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(5-(methylsulfinyl)pentyl)thiohydroximate.



It was found that when the amount of NCS was larger than 1.1 equiv, side products appeared, which were related to the oxidation of the sulfur atom of the thiol **9** or the oxime **8**. This may be because the excess NCS caused the oxidation reaction over and above the chlorination reaction. However, when the amount of NCS was 1 equiv or less, there was insufficient for the chlorination to reach completion (monitored by TLC). Consequently, there was a limitation in the yield of **10**. It was found that the amount of NCS should be controlled in the range 1.05–1.10 equiv. This study also found that the amount of pyridine should be the same as the amount of NCS to avoid the adverse effect of pyridine in the second step, which may reduce the yield of the coupling reaction.

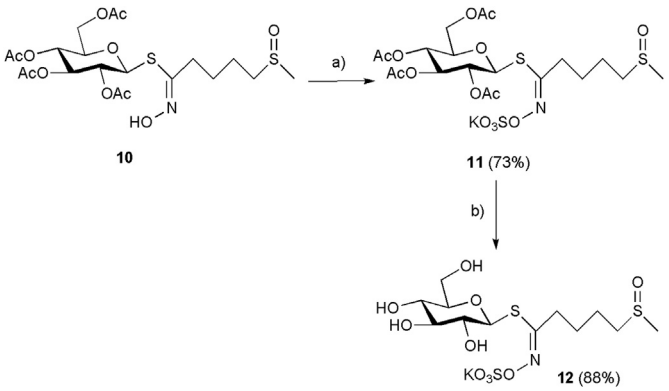
Investigation of the solvent effects on the reaction (Table 3) has found that DCM is the best solvent for the reaction because of the highest yield and ease of work-up and purification.

Table 3
The coupling of **8** and **9** in a variety of solvents

Entry	Solvent(s)	Yield (%) of **10**
1	Et_2O	37
2	DCM	47
3	DCM/Et_2O	40
4	THF	43
5	DCM/THF	38
6	$CHCl_3$	22

2.4. Synthesis of GRP

Sulfation of **10** was accomplished with pyridine sulfur trioxide complex in pyridine (Scheme 3).[33] The resulting potassium salt **11** (73% yield) was isolated by flash column chromatography on silica gel. The sulfating reaction was first conducted in DCM but the yield was not high because of the limited dissolution of **10** in the reaction mixture. Thus, pyridine was used as a convenient solvent for the reaction. However, it was found in the work-up that removing the pyridine solvent in the presence of aqueous potassium bicarbonate resulted in deterioration of the product by deacetylation and/or desulfation.[34] The problem was solved by quick extraction of organic phases by chloroform and then $CHCl_3$/MeOH (20%) before work-up and purification.



Scheme 3. Synthesis of GRP **12**. Reagents and conditions: (a) (1) Py·SO_3, pyridine, 24 h, rt, (2) $KHCO_3$, H_2O; (b) MeOK, MeOH, 2 h.

De-O-acetylations were performed by dissolving **11** in MeOH in the presence of MeOK as a catalyst. The final GRP **12** (88% yield) was successfully purified by flash column chromatography on silica gel.[35] Compound **12** was obtained as a mixture of $(R,S)_S$-isomers in overall 17% yield over seven steps. This is much higher than the literature result (9% yield over nine steps).[16,17] The optical rotation

data {$[\alpha]_D^{20}$ −14.8 (*c* 1.0, H_2O) (lit. $[\alpha]_D$ −15 (*c* 1.0, H_2O))} compared favorably with the literature.[5] However, it was not possible to differentiate between the R_S-isomer and the S_S-isomer using the ^1H and ^{13}C NMR data, which matched the literature data.[5,36] An attempt to separate the R_S-isomer from the S_S-isomer by HPLC was unsuccessful.

3. Conclusion

A total synthesis of $(R,S)_S$-GRP has been completed. This compound is now being used for bioassay and other applications. This study has found a novel method for synthesis of sulfinylalkyl glucosinolates.

4. Experimental

4.1. General methods

Melting points (mp) were recorded on a hot stage apparatus and are uncorrected. Optical rotations were measured at the stated temperatures in the stated solvent on a polarimeter at the sodium D-line (589 nm); $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra (ν_{max}) were recorded on an FT-IR spectrometer. Samples were analyzed as KBr drift (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 300 MHz spectrometer operating at 300 MHz for proton and 75 MHz for carbon nuclei. Chemical shifts were 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 recorded in $CDCl_3$, 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 mass spectrometer at 300 °C and scan rate of 5500 *m*/*z*/s using either water/methanol/acetic acid in a ratio of 0:99:1 or 50:50:1 as a mobile phase. Accurate mass measurement was by mass spectrometry with a heated electrospray ionization (HESI) 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 320 °C with a source heater temperature of 350 °C 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 F_{254} 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).

4.2. 5-(Methylthio)pentan-1-ol (3)

5-Chloropentanol (2.50 g, 20.5 mmol) was added to a stirred solution of sodium methylmercaptide (1.83 g, 23.5 mmol) in MeOH/H_2O (3:1 v/v) (20 mL). The solution was further stirred at rt overnight and it was then extracted with chloroform (3×25 mL). The organic phase was collected and dried over anhydrous Na_2SO_4. Following filtration, the filtrate was evaporated under reduced pressure and the crude product was purified by flash column chromatography on silica gel eluting with 95% DCM/MeOH. The pure alcohol **3** was isolated as a colorless oil (2.33 g, 85%). R_f=0.64 in 90% DCM/MeOH; IR (NaCl) ν_{max} 3369 (OH), 2933, 2858, 1456, 1436, 1068, 1049 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 3.59 (t, $J_{1,2}$=6.3 Hz, 2H, CH_2OH), 2.45 (t, $J_{4,5}$=7.2 Hz, 2H, CH_2S), 2.04 (s, 3H, CH_3S), 1.89 (s, 1H, OH), 1.60–1.46 (m, 6H, CH_2CH_2CH_2CH_2OH); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 62.2 (C-1), 33.8 (C-5), 31.9 (C-2), 28.5 (C-4), 24.5 (C-3), 15.1 (CH_3S); HRMS (ESI) *m*/*z* for $C_6H_{15}OS$ $[M+H]^+$, calcd 135.0838, found 135.0837.

4.3. 5-(Methylthio)pentanal (1)

To a stirred solution of oxalyl chloride (1.67 mL, 19.0 mmol) in DCM (40 mL) at −78 °C under Ar was added DMSO (2.66 mL, 38.1 mmol). After 15 min, a solution of alcohol **2** (1.70 g, 12.7 mmol) in DCM (10 mL) was added dropwise and the resulting reaction mixture was stirred for 15 min. TEA (7.00 mL, 50 mmol) was added and the reaction mixture was stirred for 15 min and then allowed to warm to rt. Water (50 mL) was then added and the aqueous layer was re-extracted with DCM (3×30 mL). The combined organic layers were washed with saturated solutions of NH_4Cl, $NaHCO_3$, and NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. Compound **3** was obtained as a colorless oil (1.34 g, 80%) by flash column chromatography eluting with 80% hexane/ethyl acetate. R_f=0.38 in 70% hexane/EtOAc; IR (NaCl) ν_{max} 2916, 2858, 2719, 1722, 1427, 1390, 1355, 1126, 1072 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 9.72 (t, 1H, *J*=1.5 Hz, 1H, C*H*O), 3.49–3.40 (m, 4H, H2 and H5), 2.05 (s, 3H, CH_3S), 1.77–1.56 (m, 4H, H3 and H4); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 200.5 (C-1), 43.0 (C-5), 33.4 (C-2), 28.1 (C-4), 20.7 (C-3), 15.1 (CH_3S); HRMS (ESI) *m*/*z* for $C_6H_{13}OS$ $[M+H]^+$, calcd 133.0682, found 133.0681.

4.4. 2-(4-(Methylthio)butyl)-1,3-dioxolane (4)[22]

A mixture of 5-(methylthio)pentanal **3** (280 mg, 2.1 mmol), ethylene glycol (0.50 mL, 6.6 mmol), and *p*-toluenesulfonic acid (44.0 mg, 0.2 mmol) in toluene (20 mL) was heated at reflux for 3 h using a Dean–Stark trap. The mixture was cooled to rt and K_2CO_3 (1 g) was added. After stirring for 10 min, the contents were washed with 10% K_2CO_3 solution. The aqueous layer was extracted with DCM (3×20 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Compound **4** was obtained as colorless oil (330 mg, 88%). R_f=0.44 in 70% hexane/EtOAc; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 4.85 (t, 1H, *J*=4.5 Hz, $(CH_2O)_2CH$), 3.98–3.86 (m, 4H, $(CH_2O)_2CH$), 2.51–2.44 (m, 2H, CH_3SCH_2), 2.08 (s, 3H, CH_3S), 1.71–1.48 (m, 6H, $CH_3S(CH_2)_3$); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 104.4 ($CH(OCH_2)_2$), 64.7 ($CH(OCH_2)_2$), 34.1, 33.5, 29.0, 23.2, 15.3 (CH_3S); HRMS (ESI) *m*/*z* for $C_8H_{17}O_2S$ $[M+H]^+$, calcd 177.0949, found 177.0940.

4.5. 2-(4-(Methylsulfinyl)butyl)-1,3-dioxolane (5)

A solution of sodium metaperiodate (652 mg, 3.0 mmol) in water (10 mL) was added dropwise to a vigorously stirred ice-cold solution of **4** (511 mg, 2.9 mmol) in MeOH (20 mL). The reaction mixture was stirred for 3 h, then filtered at the pump and the filter cake was washed with MeOH. The combined filtrates were concentrated to ca. 10 mL and then extracted with $CHCl_3$ (3×20 mL). The combined $CHCl_3$ extracts were washed with water, dried, and concentrated under reduced pressure. Compound **5** was obtained as a yellow oil (460 mg, 82%) by flash column chromatography eluting with 90% DCM/MeOH. R_f=0.28 in 10% MeOH/DCM; IR (NaCl) ν_{max} 3408, 2949, 2916, 2887, 1720, 1409, 1130, 1018 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 4.84 (t, 1H, *J*=4.5 Hz, $(CH_2O)_2CH$), 3.95–3.78 (m, 4H, $(CH_2O)_2CH$), 2.51–2.44 (m, 2H, CH_3SOCH_2), 2.08 (s, 3H, CH_3SO), 1.83–1.54 (m, 6H, $CH_3SO(CH_2)_3$); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 103.6 ($CH(OCH_2)_2$), 64.5 ($CH(OCH_2)_2$), 54.1 (CH_3SOCH_2), 38.1 (CH_3SO), 32.9 (CH_2CH_2CH), 22.8 ($CH_2CH_2CH_2CH$),

22.1 ($CH_2CH_2CH_2CH$); HRMS (ESI) m/z for $C_8H_{17}O_3S$ $[M+H]^+$, calcd 193.0898, found 193.0892.

4.6. 5-(Methylsulfinyl)pentanal (6)

Compound **6** was prepared by the method of Leonard et al.[29] A solution of sodium metaperiodate (692 mg, 3.2 mmol) in water (10 mL) was added dropwise to a vigorously stirred ice-cold solution of **3** (400 mg, 3.0 mmol) in MeOH (20 mL). The reaction mixture was stirred for 3 h, filtered off, and washed with MeOH. The combined filtrates were concentrated to ca. 10 mL and then extracted with $CHCl_3$ (3×20 mL). The combined $CHCl_3$ extracts were washed with water, dried, and concentrated under reduced pressure. Flash column chromatography eluting with 90% DCM/MeOH gave compound **6** as a colorless oil (340 mg, 75%). R_f=0.33 in 10% MeOH/DCM; IR (NaCl) ν_{max} 3400, 2924, 2852, 2729, 1720, 1664, 1633, 1409, 1024 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 9.68 (t, 1H, J=1.5 Hz, 1H, CHO), 2.66–2.58 (m, 2H, H5), 2.48–2.42 (m, 5H, CH_3S and H2), 1.74–1.64 (m, 4H, H3 and H4); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 201.1 (CHO), 53.8 (C-5), 42.9 (C2), 38.2 (CH_3SO), 21.7, 20.7 (C3 and C4); HRMS (ESI) m/z for $C_6H_{13}O_2S$ $[M+H]^+$, calcd 149.0636, found 149.0630.

4.7. 5-(Methylthio)pentanal oxime (7)

A solution of hydroxylamine hydrochloride (360 mg, 5.5 mmol) and CH_3COONa (760 mg, 5.5 mmol) in water (15 mL) was stirred in an ice-bath. To this solution was added a solution of aldehyde **3** (230 mg, 1.7 mmol) in MeOH (5 mL). The mixture was stirred at 0 °C for 2 h and then it was stirred at rt for 1 h. The reaction mixture was concentrated in vacuo; the residue was dissolved in water and extracted with DCM (3×20 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with 90% hexane/ethyl acetate to give oxime **7** as a colorless oil (240 mg, 92%). R_f=0.2 in 80% hexane/EtOAc; IR (NaCl) ν_{max} 3369 (OH), 2915, 2856, 2720, 1720, 1600 (CH=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 8.92 (s, 1H, OH), 7.34 (t, 1H, J=6.3 Hz, CH=N), 2.46–2.09 (m, 4H, H2 and H5), 2.01 (s, 3H, CH_3S), 1.58–1.51 (m, 4H, H3 and H4); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 151.3 (CH=N), 55.4 (C-5), 28.6 (C-2), 28.0 (C-4), 25.2 (C-3), 15.1 (CH_3S); HRMS (ESI) m/z for $C_6H_{14}NOS$ $[M+H]^+$, calcd 148.0791, found 148.0789.

4.8. 5-(Methylsulfinyl)pentanal oxime (8)

*4.8.1. Synthesis of **8** from **5**.* To a stirred solution of **5** (683 mg, 3.6 mmol) and 2 N H_2SO_4 (5 mL) in CH_3CN/H_2O (3:1, 20 mL) was added hydroxylamine hydrochloride (1.24 g, 17.8 mmol). The mixture was stirred at rt for 48 h. The reaction mixture was concentrated in vacuo and then the residue was dissolved in water and extracted with $CHCl_3$ (3×20 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The oxime **8**, as a mixture (*R*,*S*)-sulfoxide isomers, was obtained as a colorless oil (310 mg, 54%) by flash column chromatography eluting with 90% DCM/MeOH.

*4.8.2. Synthesis of **8** from **6**.* A solution of hydroxylamine hydrochloride (430 mg, 6.5 mmol) and CH_3COONa (890 mg, 6.5 mmol) in water (15 mL) was stirred in an ice-bath. To this mixture was added a solution of aldehyde **6** (320 mg, 2.2 mmol) in MeOH (5 mL). The mixture was stirred at 0 °C for 2 h and then it was stirred at rt for 1 h. The reaction mixture was concentrated at reduced pressure and the residue was dissolved in water and extracted with $CHCl_3$ (3×20 mL). The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The oxime **8**, as a mixture (*R*,*S*)-sulfoxide isomers, was obtained as a colorless oil (270 mg, 76%) by flash column chromatography eluting with 90% DCM/MeOH.

*4.8.3. Synthesis of **8** from **7**.* A solution of sodium metaperiodate (370 mg, 1.7 mmol) in water (5 mL) was added dropwise to a vigorously stirred ice-cold solution of **7** (240 mg, 1.6 mmol) in MeOH (15 mL). The reaction mixture was stirred for 3 h, then filtered at the pump and the filter cake was washed with MeOH. The combined filtrates were concentrated to ca. 10 mL and then extracted with $CHCl_3$ (3×20 mL). The combined $CHCl_3$ extracts were washed with water, dried, and concentrated under reduced pressure. Compound **8**, as a mixture $(R,S)_S$-sulfoxide isomers, was obtained as a colorless oil (240 mg, 91%) by flash column chromatography eluting with 90% DCM/MeOH. R_f=0.17 in 10% MeOH/DCM; IR (NaCl) ν_{max} 3419, 2943, 2866, 2833, 1716, 1647, 1124, 1045, 1018 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 7.31 (t, 1H, J=6.3 Hz, 1H, CH=N), 6.61 (s, 1H, NO*H*), 2.71–2.54 (m, 2H, H5), 2.51 (s, 3H, CH_3SO), 2.35–2.13 (m, 2H, H2), 1.78–1.49 (m, 4H, H3 and H4); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 150.3 (CH=N), 53.4, 53.3 (C-5 isomers), 38.8 (CH_3SO), 28.6 (C-2), 25.2, 24.8 (C-4 isomers), 21.9, 21.6 (C-3 isomers); HRMS (ESI) m/z for $C_6H_{14}NO_2S$ $[M+H]^+$, calcd 164.0740, found 164.0740.

4.9. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl thiol (9)

To a solution of D-glucose (3.00 g, 16.6 mmol) in dry pyridine (33 mL) at 0 °C under a nitrogen atmosphere was slowly added acetic anhydride (31.5 mL, 333 mmol). The reaction mixture was stirred at 0 °C for 1 h before a catalytic amount of DMAP (200 mg, 1.67 mmol) was added. As the reaction mixture was allowed to reach rt, it becomes slightly exothermic. After 6 h, the clear yellow mixture was slowly poured into rapidly stirred ice-water (125 mL), giving a sticky solid. After EtOAc extraction (3×45 mL), evaporation of the solvent and co-evaporation with dry toluene (3×20 mL), peracetylated glucose was obtained as a yellow solid (5.84 g, 90%). A solution of pentaacetyl-D-glucopyranose (2.00 g, 5.1 mmol) in DCM (20 mL) was stirred in an ice bath while HBr/HOAc (6 mL, 45 wt %) was added drop-wise. After an hour, the solution was washed with ice-water and cold saturated $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated to leave the glucosyl bromide as a pale yellow oil (1.83 g). The oil was dissolved in dry acetone (20 mL), and the solution was added to freshly activated 4 Å molecular sieves (2 g) and thiourea (500 mg, 6.6 mmol). The mixture was maintained at reflux temperature (60 °C) under a nitrogen atmosphere for 2.5 h, cooled, and filtered through Celite. Solvent removal and trituration of the syrupy residue with hexane (3×20 mL) gave the isothiouronium bromide **7** as a colorless amorphous powder. The crude product was dissolved in DCM (20 mL), a solution of $Na_2S_2O_5$ (2.00 g) in water (20 mL) was added, and the mixture was maintained at reflux under a nitrogen atmosphere for 1 h. After cooling, the organic layer was separated and washed with water, saturated NaCl solution, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. Pure **9** was obtained by flash column chromatography on silica gel eluting with 0–3% MeOH/DCM as a solid (1.60 g, 86%). R_f=0.3 in 50% hexane/EtOAc; mp=114–115 °C (lit. 113–114 °C);[24] $[\alpha]_D^{20}$ +10.5 (c 1.0, $CHCl_3$) (lit.+11);[24] ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 5.18–5.02 (m, 2H, H3 and H4), 4.93 (dd, $J_{1,2}$=9.6 Hz, $J_{2,3}$=9.3 Hz, 1H, H2), 4.51 (dd, $J_{1,2}$=9.6 Hz, $J_{1,SH}$=9.9 Hz, 1H, H1), 4.23 (dd, $J_{5,6b}$=4.8 Hz, $J_{6a,6b}$=12.3 Hz, 1H, H6b), 4.11 (dd, $J_{5,6a}$=2.4 Hz, $J_{6a,6b}$=12.3 Hz, 1H, H6b), 3.66–3.17 (m, 1H, H5), 2.29 (d, $J_{1,SH}$=9.9 Hz, 1H, SH), 2.08–1.96 (m, 12H, CH**3**COO); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 170.2, 169.7, 169.2, 168.9 (4×CH_3COO), 87.3 (C-1), 75.9 (C-3), 73.2 (C-2, C-3), 67.7 (C-4), 61.6 (C-6), 20.6, 20.3(2), 20.2 (4×CH_3COO); HRMS (ESI) m/z for $C_{14}H_{19}O_9S$ $[M-H]^-$, calcd 363.0755, found 363.0746.

4.10. *S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(5-(methylsulfinyl)pentyl)thiohydroximate (10)

To a suspension of **8** (100 mg, 0.6 mmol) in DCM (5 mL) was added pyridine (0.06 mL, 0.63 mmol), then *N*-chlorosuccinimide (80.0 mg, 0.63 mmol). The mixture was stirred for 2.5 h at rt under N_2, then thiol **9** (230 mg, 0.6 mmol) in DCM (5 mL) was added. The resulting mixture was treated with TEA (0.35 mL, 2.5 mmol). The reaction mixture was stirred for 2 h at rt under N_2 then acidified with aqueous 1 M H_2SO_4 (7 mL/mmol of sugar). The mixture was left to stand for about 10 min and then separated. The aqueous phase was extracted with DCM (3×20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. Compound **10**, as a foam (150 mg, 47%), was obtained by flash column chromatography eluting with 90% DCM/MeOH. R_f=0.43 in 90% DCM/MeOH; mp=108–109 °C; IR (NaCl) ν_{max} 3288 (OH), 2922, 2850, 1751 (C=O), 1600 (C=N), 1375, 1226, 1039 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 5.26–4.99 (m, 4H, H1, H2, H3, H4), 4.12–4.07 (m, 2H, H6a and H6b), 3.76–3.71 (m, 1H, H5), 2.81–2.67 (m, 2H, $SOCH_2$), 2.58–2.53 (m, 5H, CH_3SO and CH_2C=N), 2.04, 2.01, 1.99, 1.97 (4×s, 12H, CH_3COO), 1.82–1.80 (m, 4H, $SOCH_2CH_2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 170.2, 169.8, 169.0, 168.8 (4×CH_3COO), 149.4 (C=N), 79.6 (C-1), 75.4 (C-5), 73.4 (C-3), 69.9 (C-2), 67.8 (C-4), 61.8 (C-6), 53.4 (C-2′), 37.8 (CH_3SO), 31.8 (C-5′), 25.5 (C-4′), 21.6 (C-3′), 20.4, 20.3, 20.2, 20.17 (4×CH_3COO); HRMS (ESI) *m*/*z* for $C_{20}H_{31}O_{11}NNaS_2$ $[M+Na]^+$, calcd 548.1236, found 548.1219.

4.11. Potassium 2,3,4,6-tetra-*O*-acetylglucoraphanin (11)

To a stirred solution of the thiohydroximate **10** (120 mg, 0.2 mmol) in dry pyridine (5 mL) was added pyridine–sulfur trioxide complex (95.0 mg, 0.6 mmol). After stirring at rt under N_2 for 24 h, an additional portion of the pyridine–sulfur trioxide complex (19.0 mg, 0.1 mmol) was added and stirring was continued for 2 h. After that, a solution of $KHCO_3$ (850 mg, 8.4 mmol) in water (10 ml) was added and the mixture was stirred for 30 min and then concentrated under reduced pressure. The residue was dissolved in water and extracted with chloroform (3×40 mL) and then 20% MeOH/$CHCl_3$ (2×30 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. Compound **11** was obtained by flash chromatography eluting with 80% DCM/MeOH as a white solid (103 mg, 73%). R_f=0.16 in 80% DCM/MeOH; mp=155–156 °C (dec); IR (KBr drift) ν_{max} 2971, 2873, 1741 (C=O), 1654 (C=N), 1433, 1368, 1250, 1224, 1061 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (300 K) δ 5.38–5.35 (m, 2H, H1 and H3), 5.07–4.93 (m, 2H, H2 and H4), 4.19–4.02 (m, 3H, H5, H6a, and H6b), 2.89–2.63 (m, 2H, $SOCH_2$), 2.63–2.61 (m, 5H, CH_3SO and CH_2C=N), 2.04, 2.01, 1.99, 1.95 (4×s, 12H, CH_3COO), 1.91–1.89 (m, 4H, $SOCH_2CH_2CH_2$); ^{13}C NMR (75 MHz, CD_3OD) (300 K) δ 170.4, 169.7, 169.4, 169.1 (4×CH_3COO), 156.6 (C=N), 79.0 (C-1), 75.0 (C-5), 73.3 (C-3), 69.6 (C-2), 67.8 (C-4), 61.5 (C-6), 52.6 (C-5′), 36.3 (CH_3SO), 31.3 (C-2′), 25.0 (C-4′), 21.0 (C-3′), 18.9(2), 18.7(2) (4×CH_3COO); HRMS (ESI) *m*/*z* for $C_{20}H_{30}O_{14}NS_3$ $[M-K]^-$, calcd 604.0834, found 604.0812.

4.12. (*R*,*S*)S-Glucoraphanin (12)

To a solution of **11** (50.0 mg, 0.08 mmol) in anhydrous MeOH (10 ml) under N_2 atmosphere was added dry MeOK (5.00 mg, 0.1 mmol) until pH=8–9. After stirring for 2 h at rt, the solution was made neutral by the addition of glacial acetic acid then it was concentrated under reduced pressure. GRP **12** as a white solid (32.4 mg, 88%) was obtained by flash column chromatography eluting with EtOAc/MeOH/H_2O (16:4:1). R_f=0.08 in EtOAc/MeOH/H_2O (16:4:1); mp>115 °C (dec); $[\alpha]_D^{20}$ −14.8 (*c* 1.0, H_2O) (lit.[5] $[\alpha]_D$ −15 (*c* 1.0, H_2O)); IR (KBr drift) ν_{max} 3316 (OH), 2976, 2868, 1651 (C=N), 1495, 1265, 1063 cm^{-1}; ^1H NMR (300 MHz, D_2O) (300 K) δ 4.85 (d, $J_{1,2}$=9.9 Hz, 1H, H1), 3.75–3.18 (m, 6H, H2, H3, H4, H5, H6a, and H6b); 2.77–2.75 (m, 2H, $SOCH_2$), 2.55–2.53 (m, 5H, CH_3SO and CH_2C=N), 1.66–1.62 (m, 4H, $SOCH_2CH_2CH_2$); ^{13}C NMR (75 MHz, D_2O) (300 K) δ 155.4 (C=N), 81.1 (C-1), 79.7 (C-5), 76.7 (C-3), 71.8 (C-2), 68.8 (C-4), 60.3 (C-6), 51.8 (C-5′), 36.0 (CH_3SO), 30.9 (C-2′), 25.4 (C-4′), 20.8 (C-3′); HRMS (ESI) *m*/*z* for $C_{12}H_{22}O_{10}NS_3$ $[M-K]^-$, calcd 436.0411, found 436.0433.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2013.07.097.

References and notes

1. West, L. G.; Meyer, K. A.; Balch, B. A.; RossiI, F. J.; Schultz, M. R.; Haas, G. W. *J. Agric. Food Chem.* **2004**, *52*, 916–926.
2. Powell, E. E.; Hill, G. A.; Juurlink, B. H.; Carrier, D. J. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 985–991.
3. Fahey, J. W.; Zalcmann, A. T.; Talalay, P. *Phytochemistry* **2001**, *56*, 5–51.
4. Zhang, Y.; Talalay, P.; Cho, C. G.; Posner, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399–2403.
5. Iori, R.; Bernardi, R.; Gueyrard, D.; Rollin, P.; Palmieri, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1047–1048.
6. Kiddle, G.; Bennett, R. N.; Botting, N. P.; Davidson, N. E.; Robertson, A. A. B.; Wallsgrove, R. M. *Phytochem. Anal.* **2001**, *12*, 226–242.
7. Pereira, F. M. V.; Rosa, E.; Fahey, J. W.; Stephenson, K. K.; Carvalho, R.; Aires, A. *J. Agric. Food Chem.* **2002**, *50*, 6239–6244.
8. Vermeulen, M.; Zwanenburg, B.; Chittenden, G. J. F.; Verhagen, H. *Eur. J. Med. Chem.* **2003**, *38*, 729–737.
9. Mays, J. R.; Roska, R. W.; Sarfaraz, S.; Mukhtar, H.; Rajski, S. R. *ChemBioChem* **2008**, *9*, 729–747.
10. Posner, G. H.; Cho, C. G.; Green, J. V.; Zhang, Y.; Talalay, P. *J. Med. Chem.* **1994**, *37*, 170–176.
11. Khiar, N.; Werner, S.; Mallouk, S.; Lieder, F.; Alcudia, A.; Ferna'ndez, I. *J. Org. Chem.* **2009**, *74*, 6002–6009.
12. Rochfort, S.; Caridi, D.; Stinton, M.; Trenerry, V. C.; Jones, R. *J. Chromatogr., A* **2006**, *1120*, 205–210.
13. Rangkadilok, N.; Nicolas, M. E.; Bennett, R. N.; Premier, R. R.; Eagling, D. R.; Taylor, P. W. J. *Sci. Hortic.* **2002**, *96*, 11–26.
14. Fahey, J. W.; Wade, K. L.; Stephenson, K. K.; Chou, F. E. *J. Chromatogr., A* **2003**, *996*, 85–93.
15. Trenerry, V. C.; Caridi, D.; Elkins, A.; Donkor, O.; Jones, R. *Food Chem.* **2006**, *98*, 179–187.
16. Botting, P. N.; Robertson, A. A. B.; Morrison, J. *J. Labelled Compd. Radiopharm.* **2007**, *50*, 260–263.
17. Morrison, J. J.; Botting, N. P. *Tetrahedron Lett.* **2007**, *48*, 1891–1894.
18. Dawson, G. W.; Hick, A.; Bennett, R.; Donald, A.; Pickett, J.; Wallsgrove, R. *J. Biol. Chem.* **1993**, *268*, 27154–27159.
19. Buckingham, D. A.; Rauchfuss, T. B. *J. Chem. Soc., Chem. Commun.* **1978**, 705–707.
20. Šmejkal, T.; Breit, B. *Angew. Chem.* **2008**, *120*, 4010–4013.
21. Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480–2482.
22. Bates, R. W.; Sridhar, S. *Synlett* **2009**, 1979–1981.
23. Madesclaire, M. *Tetrahedron* **1986**, *42*, 5459–5495.
24. Lakouraj, M. M.; Tajbakhsh, M.; Shirini, F.; Asady Tamami, M. V. *Synth. Commun.* **2005**, *35*, 775–784.
25. Ladziata, U.; Koposov, A. Y.; Lo, K. Y.; Willging, J.; Nemykin, V. N.; Zhdankin, V. V. *Angew. Chem., Int. Ed.* **2005**, *44*, 7127–7131.
26. Zolfigol, M. A.; Amani, K.; Choghamarani, A. G.; Hajjami, M.; Nasrabadi, R. A.; Jafari, S. *Catal. Commun.* **2008**, *9*, 1739–1744.
27. Zolfigol, M. A.; Amani, K.; Hajjami, M.; Choghamarani, A. G. *Monatsh. Chem.* **2008**, *139*, 895–899.
28. Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817–4820.
29. Leonard, N. J.; Johnson, C. R. *J. Org. Chem.* **1962**, *27*, 282–284.
30. Davidson, N. E.; Rutherford, T. J.; Botting, N. P. *Carbohydr. Res.* **2000**, *330*, 295–307.

31. Wanga, E. C.; Huanga, K. S.; Chena, H. M.; Wua, C. C.; Jiun Lina, G. J. *J. Chin. Chem. Soc.* **2004**, *51*, 619–627.
32. Floyd, N.; Vijayakrishnan, B.; Koeppe, J. R.; Davis, B. G. *Angew. Chem., Int. Ed.* **2009**, *48*, 7798–7802.
33. Benn, M. H. *Can. J. Chem.* **1963**, *41*, 2836–2838.
34. Zhang, Q.; Lebl, T.; Kulczynska, A.; Botting, N. P. *Tetrahedron* **2009**, *65*, 4871–4876.
35. Vo, Q. V.; Trenerry, C.; Rochfort, S.; Wadeson, J.; Leyton, C.; Hughes, A. B. *Bioorg Med. Chem* **2013**, in press.
36. Vergara, F.; Wenzler, M.; Hansen, B. G.; Kliebenstein, D. J.; Halkier, B. A.; Gershenzon, J.; Schneider, B. *Phytochemistry* **2008**, *69*, 2737–2742.