Unprecedented chemical structure and biomimetic synthesis of erucalexin, a phytoalexin from the wild crucifer *Erucastrum gallicum*†

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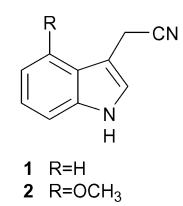
The isolation, structure determination, total synthesis and antifungal activity of erucalexin, a novel phytoalexin produced by the wild crucifer dog mustard are described. Erucalexin is a structurally unique plant alkaloid, representing the first example of a spiro[2*H*-indole-2,5′(4′*H*)-thiazol]-3-one, likely derived from a C-3–C-2 carbon migration in a 3-substituted indolyl nucleus.

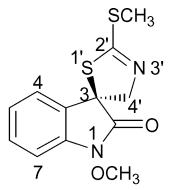
Introduction

The multifaceted defense systems of plants include an arsenal of metabolites known as phytoalexins, which are biosynthesized *de novo* in response to various sorts of stress such as microbial attack.[1] The phytoalexins produced by crucifer plants comprise an amazing class of tryptophan-derived alkaloids containing diverse functional groups that display selective activity against plant pathogens[2] as well as cancer chemopreventive activity.[3] Crucifers are economically valuable oilseeds (rapeseed and canola, *Brassica rapa* and *B. napus*), vegetables (cauliflower, *B. oleracea* var. *italica*, broccoli, *B. oleracea* var. *botrytis*, rutabaga, *B. napus* ssp. *rapifera*, and Brussels sprouts, *B. oleracea* var. *gemmifera*) and condiments (brown mustard, *B. juncea*, and wasabi, *Eutrema wasabi*).[2] Furthermore, the wild crucifer *Arabidopsis thaliana* is the first flowering plant to have its genome sequenced[4] and many wild crucifers[5] are sources of desirable agronomic traits of great interest in plant breeding. For example, dog mustard (*Erucastrum gallicum*) is a potential source of genetic resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary,[6] a fungal plant pathogen causing stem rot disease in crucifers and other plant families.[7,8]

Recently, analysis of the chemical response of dog mustard leaves to the pathogen *S. sclerotiorum* and to abiotic stress led to the isolation of three known phytoalexins[9] (**1**–**3**) and erucalexin (**4**), a new phytoalexin which was shown to have an unprecedented structure by spectroscopic analyses.[10] Subsequently, synthesis of model compounds and synthetic approaches to make this unique spiro[2*H*-indole-2,5′(4′*H*)-thiazol]-3-one ring system led to the discovery of a one-pot biomimetic spirocyclization applicable to both 2- and 3-indolinones. Here we report for the first time the chemical structure elucidation, synthesis and antifungal activity of erucalexin (**4**). The chemical structure of erucalexin (**4**) represents the first spiro[2*H*-indole-2,5′(4′*H*)-thiazol]-3-one described to date and suggests an intriguing rearrangement in its biosynthetic pathway.



1 R=H
2 R=OCH_3



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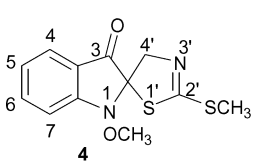

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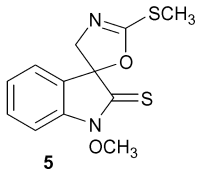

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Results and discussion

Erucalexin (**4**) was produced in leaves of dog mustard elicited with either *S. sclerotiorum* or $CuCl_2$, and was isolated as reported in the Experimental section ($[\alpha]_D$ +73, *c* 0.090, MeOH). The HRMS-EI data of erucalexin (**4**) indicated it to be an isomer of 1-methoxyspirobrassinin (**3**, $C_{12}H_{12}N_2S_2O_2$, obtained *m*/*z* 280.0342, calc. 280.0340), further corroborated by analysis of its NMR spectroscopic data. The NMR spectral data of **4** (Table 1) showed some similarities to that of 1-methoxyspirobrassinin (**3**), *i.e.* signals

Table 1 NMR data for erucalexin (**4**) dissolved in CD_3CN

Position	δ_H (multiplicity, *J* in Hz)[a]	δ_C[b,c]	HMBC correlations
2	—	95.4	
3	—	193.2	
3a	—	122.1	
4	7.68 (d, *J* = 8)	125.3	C-7a, 6, 3
5	7.21 (ddd, *J* = 8, 8, 1)	125.1	C-7, 3a, 6
6	7.73 (ddd, *J* = 8, 8, 1)	139.4	C-7a, 4, 5
7	7.32 (d, *J* = 8)	115.2	C-3a, 5, 6
7a	—	161.0	
2′	—	163.6	
4a′	4.75 (d, *J* = 16)	72.5	C-2, 2′
4b′	4.35 (br d, *J* = 16)	72.5	C-2′, 3
OMe	3.95 (s)	66.1	
SMe	2.61 (s)	16.3	C-2′, 4a′, 4b′

[a] Spectrum recorded at 500 MHz referenced to residual CD_2HCN, δ_H 1.94. Assigned from NOE and ^1H NMR decoupling experiments. [b] Spectra recorded at 125 MHz and referenced to CD_3CN, δ_C 118.69. [c] Assigned from HMQC and HMBC (500 MHz).

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† Electronic supplementary information (ESI) available: ^1H NMR spectra for compounds **3**, **4**, **6**, **9a**, **13**, **14**, **16**, **17**, **21**, **22**, **25**–**29**, **38**–**40**, **43**–**46**. See DOI: 10.1039/b515331j

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due to a methoxy group (δ_H 3.95, s; δ_C 66.1) attached to N-1, a thiomethyl group (δ_H 2.61, s; δ_C 16.3), a methylene group, and four aromatic protons on an *ortho*-disubstituted benzene ring. Irradiation of the methoxy protons (δ_H 3.95) caused an NOE enhancement of the aromatic proton signal at δ 7.32 (1H, d, $J = 8$ Hz) allowing its assignment as H-7 (Fig. 1); consecutive decoupling experiments allowed the assignment of the three remaining aromatic protons H-4, H-5 and H-6 (Table 1). The signal at δ_C 193.2 was suggestive of a 3-indolinone or a 2-indolethione ring rather than the 2-indolinone system present in 1-methoxyspirobrassinin (**3**). This hypothesis was supported by an HMBC correlation between H-4 and C-3/C-2 of the ring; additional HMBC correlations observed between the methylene protons and the carbon at δ_C 193.2, 95.4, and C-2′ (δ_C 163.6, S–C=N) led to two possible structures **4** or **5** (Fig. 1). Initially, structure **5** was chosen as a reasonable possibility based on ^{13}C NMR data and on the biogenetic pathway of this skeletal type of metabolite. Since the (H_3C)–S–C=N fragment was present in both **4** and **5**, the chemical shifts of C-3′ in **4** and in **5** were expected to be around 165 ppm, whereas the carbon chemical shifts of C-3 in **4** and C-2 in **5** were predicted to be *ca.* 195 ± 10 ppm. Because no structures similar to **4** or **5** were known, syntheses of model compounds **6** and **9** (**9a** R = CH_3) were carried out to determine the likely structure of erucalexin. Erucalexin was eventually proven to have structure **4** by total synthesis, as described below.

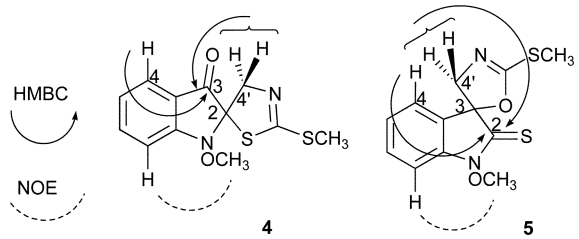
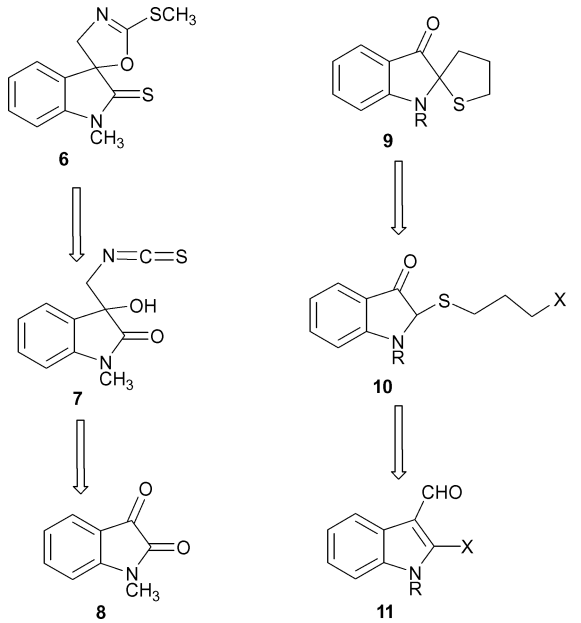
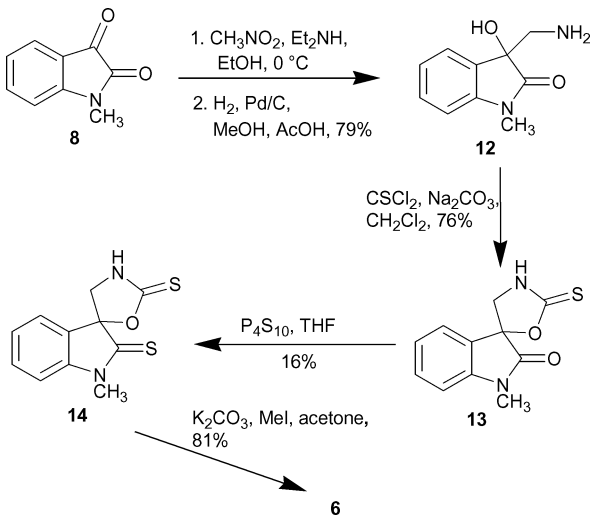


Fig. 1 Selected HMBC and NOE correlations.

To establish which of the proposed structures represented erucalexin, compound **6** (the *N*-methyl analogue of **5**) and compound **9** presented new but accessible targets using potentially known chemistry (Scheme 1). Compound **6** was synthesized in five steps from commercially available 1-methylisatin (**8**), as summarized in Scheme 2 and described in the Experimental section. Addition of nitromethane to **8** under basic conditions followed by catalytic hydrogenation[11] of the hydroxynitromethane product afforded (±)-**12** in 79% yield. Subsequent cyclization of (±)-**12** with thiophosgene[12] gave (±)-**13** with the desired spiro-ring system in 76% yield. Thiation of (±)-**13** using phosphorus pentasulfide in THF, a general procedure for conversion of carbonyl to thiocarbonyl, afforded (±)-**14** in 16% yield.[13] The use Lawesson's reagent[14] for thiation did not improve the yield of (±)-**14**. Subsequent methylation of (±)-**14** yielded the desired spirooxazoline **6** {(±)-2′-methylsulfanyl-spiro[1-methylindoline-3,5′-[4′,5′]-dihydrooxazole]-2-thione} in 81% yield. The spectroscopic data of **6** revealed significant differences from those of the isolated compound indicating that structure **5** was not erucalexin (**4**). Specifically, the UV spectrum of **6** [λ_{max} (log ε): 238 (4.1), 287 (3.6), 296 (3.7), 337 (3.8)] and that of **4** [λ_{max} (log ε): 234 (4.6), 262 (4.1), 368 (3.4)] were rather different.

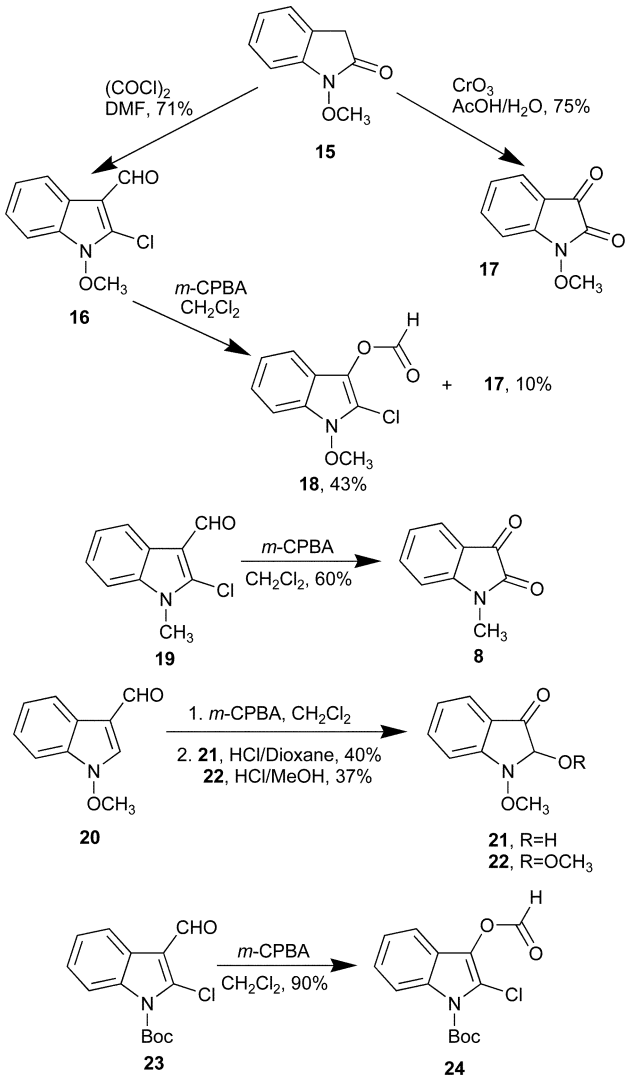


Scheme 1 Retrosynthetic analysis of model compounds **6** and **9**.



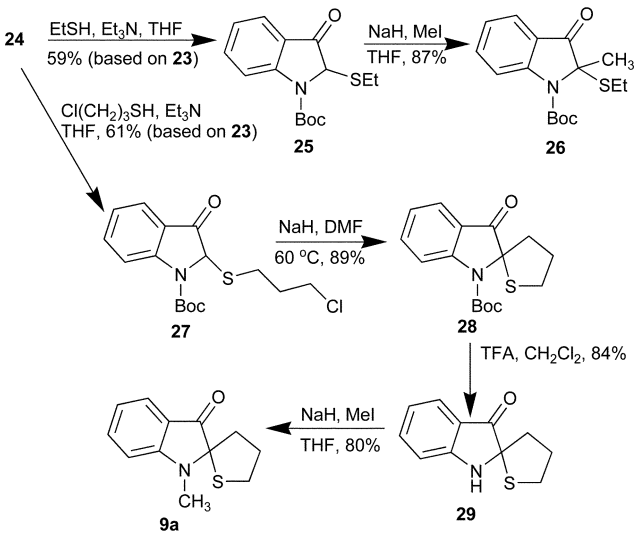
Scheme 2 Synthesis of compound **6**.

Baeyer–Villiger oxidation of indole-3-carboxaldehydes[15] **16**, **19** and **20** was thought to provide readily accessible starting materials for the syntheses of model compound **9** (**9a** R = CH_3 or **9b** R = OCH_3). Thus, 1-methoxy-2-chloroindole-3-carboxaldehyde [**16**, prepared by oxalyl chloride-mediated Vilsmeier formylation of 1-methoxyoxindole (**15**)] was treated with *m*-chloroperoxybenzoic acid (*m*-CPBA) in CH_2Cl_2 at room temperature to yield rather the unstable *O*-formate **18** in 43% yield, together with a small amount (10%) of 1-methoxyisatin (**17**) (Scheme 3).[16] Attempts to hydrolyze **18** or to carry out substitution at C-2 using sulfides or thiols as nucleophiles (EtSH, MeSNa) yielded only 1-methoxyisatin (**17**). Similarly, Baeyer–Villiger oxidation followed by HCl-mediated hydrolysis of 1-methyl-2-chloroindole-3-carboxaldehyde (**19**)[17] afforded only 1-methylisatin (**8**), whereas 1-methoxyindole-3-carboxaldehyde (**20**)[18] afforded (±)-1-methoxy-2-hydroxyindolin-3-one (**21**) (hydrolysis carried out in dioxane) or (±)-1,2-dimethoxyindolin-3-one (**22**) (hydrolysis carried out in



Scheme 3 Baeyer–Villiger oxidation of indole-3-carboxaldehydes **16**, **19**, **20** and **23**.

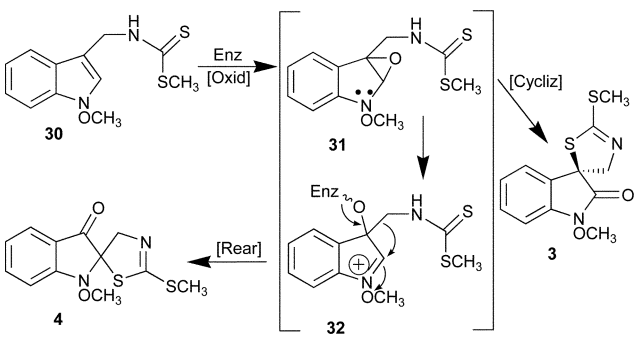
MeOH) (Scheme 3). Hence, since none of the aldehydes initially thought useful starting materials – **16**, **19** and **20** – yielded the desired product, oxidation of the *t*-Boc protected aldehyde **23**[19] was attempted. Baeyer–Villiger oxidation of **23** proceeded smoothly to yield *O*-formate **24** in 90% yield (Scheme 3). *O*-Formate **24** was isolated by flash column chromatography (FCC) and immediately treated with EtSH in the presence of Et_3N to afford (±)-1-Boc-2-ethylsulfanylindolin-3-one (**25**) in 59% yield (based on aldehyde **23**) (Scheme 4). Treatment of (±)-**25** with NaH, followed by CH_3I yielded (±)-1-Boc-2-ethylsulfanyl-2-methylindolin-3-one (**26**) in very good yield (87%), suggesting that the model compound **9a** would be accessible using similar methodology. Therefore, *O*-formate **24** was treated with 3-chloro-1-propanethiol to afford intermediate (±)-**27**, which upon intramolecular cyclization mediated by NaH yielded (±)-1-Boc-spiro(indoline-2,2′-tetrahydrothiophene)-3-one (**28**), possessing a hitherto unknown heterocyclic skeleton (Scheme 4). Finally, the protecting Boc group was removed using trifluoroacetic acid (TFA) in CH_2Cl_2 and the resulting (±)-spiro(indoline-2,2′-tetrahydrothiophene)-3-one (**29**) was methylated to yield model



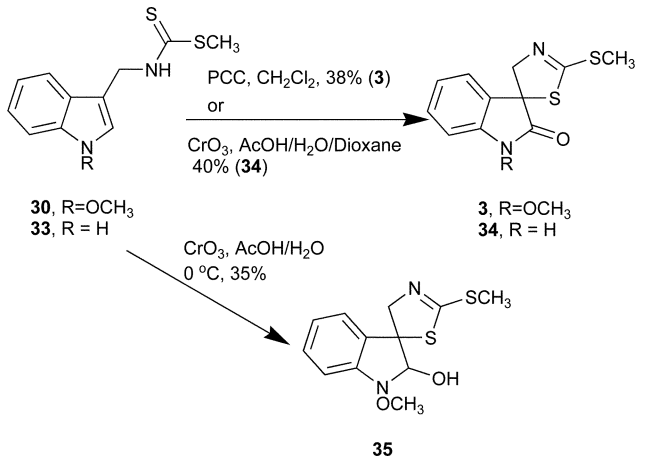
Scheme 4 Synthesis of model compound **9a**.

compound (±) **9a** (Scheme 4). As predicted, spectral data of (±) **9a** were similar to those of erucalexin (**4**): specifically, the UV spectrum of compound **9a** [λ_{max} (log ε): 235 (4.3), 264 (4.0), 353 (3.0)] and that of **4** [λ_{max} (log ε): 234 (4.6), 262 (4.1), 368 (3.4)] were similar, both containing the characteristic indolin-3-one absorption (>350 nm).

After obtaining a reasonable proof that the spiro structure **4** represented erucalexin, additional strategies to access the 3-indolinone-2-spirocyclic system of erucalexin (**4**) were considered. The most attractive of the various potential routes was designed based on possible biogenetic pathways to 1-methoxyspirobrassinin (**3**) and erucalexin (**4**) (Scheme 5). Our previous work[20] indicates that 1-methoxybrassinin (**30**) is a close biosynthetic precursor of 1-methoxyspirobrassinin (**3**). Furthermore, the structure of erucalexin (**4**), its co-occurrence with 1-methoxyspirobrassinin (**3**) in dog mustard leaves and an interesting study by Monde *et al.*[21] suggests that **3** could be a precursor of **4** as well. Nonetheless, despite the variety of oxidizing reagents and conditions used, our search for a reagent that transformed 1-methoxybrassinin (**30**) into **3** and **4** was unsuccessful, leading mostly to intractable mixtures of products of no synthetic utility. Unexpectedly, CrO_3 in acetic acid oxidized directly 1-methoxybrassinin (**30**) to (±)-1-methoxyspirobrassinin (**3**) (Scheme 6) in 21% yield; however, no traces of (±)-erucalexin (**4**) were detected. When the reaction was carried out at 0 °C, (±)-1-methoxyspirobrassinol (**35**)



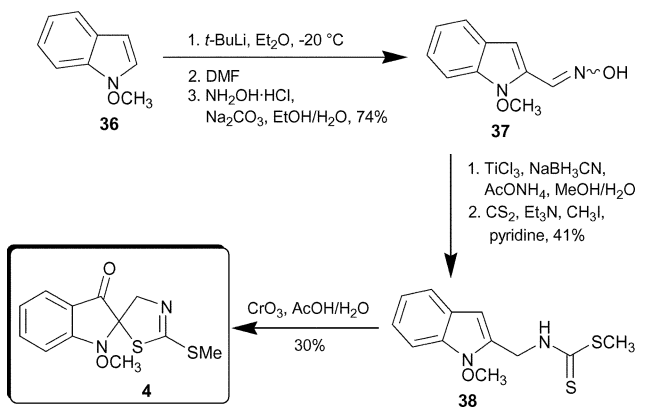
Scheme 5 Possible biogenetic pathways to erucalexin (**4**) and 1-methoxyspirobrassinin (**3**).



Scheme 6 Synthesis of (±)-1-methoxyspirobrassinin (**3**), (±)-spirobrassinin (**34**) and (±)-1-methoxyspirobrassinol (**35**).

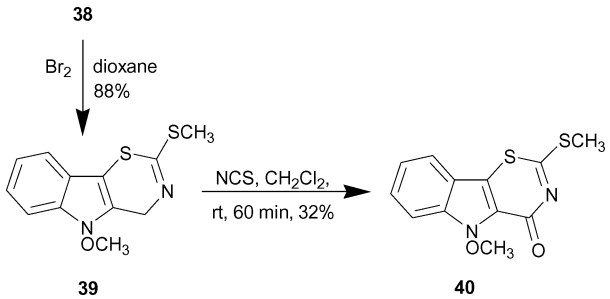
was obtained in 35% yield (Scheme 6). The yield of (±)-1-methoxyspirobrassinin (**3**) could be improved using pyridinium chlorochromate (PCC) as an oxidizing agent (38% yield). Importantly, oxidation of brassinin (**33**) by CrO_3 in acetic acid and dioxane afforded (±)-spirobrassinin (**34**) in 40% yield (Scheme 6). It is worthy of note that this is the first time that 'one-pot' oxidations of 1-methoxybrassinin (**30**) and brassinin (**33**) to (±)-1-methoxyspirobrassinin (**3**) and (±)-spirobrassinin (**34**), respectively, are reported. These transformations may be considered formal biomimetic syntheses of the phytoalexins **3** and **34**. Coincidentally, the absolute configuration of natural (+)-**3** was established recently to be *R* and (±)-**3** was synthesized from 1-methoxybrassinin (**30**) in a two-pot process.[22]

After observing that 1-methoxybrassinin (**30**) could be oxidized to (±)-1-methoxyspirobrassinin (**3**) with CrO_3 in acetic acid, it was conceivable that 1-methoxyisobrassinin (**38**) could likewise provide access to (±)-erucalexin (**4**) *via* oxidative cyclization. Toward this end, 1-methoxyindole (**36**)[23] appeared to be a suitable starting material for the synthesis of 1-methoxyisobrassinin (**38**). Thus, deprotonation of 1-methoxyindole (**36**) using *t*-BuLi,[24] followed by quenching with DMF afforded 1-methoxyindole-2-carboxaldehyde, which was directly converted to oxime **37** in 74% yield. Reduction of **37** with $TiCl_3$ and $NaBH_3CN$[25] afforded the corresponding amine, which was converted *in situ* to 1-methoxyisobrassinin (**38**) by standard treatment with carbon disulfide and iodomethane (Scheme 7).



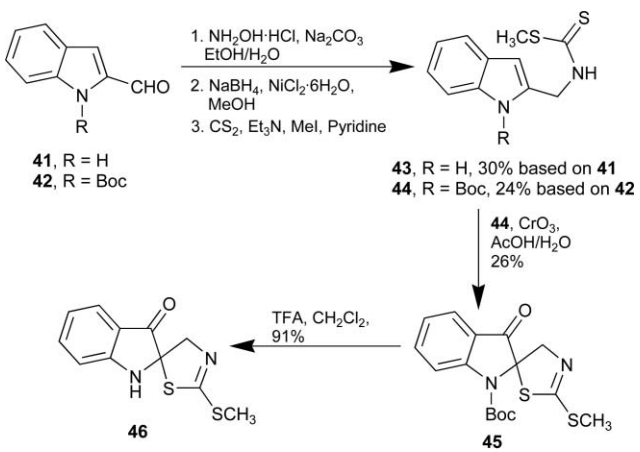
Scheme 7 Synthesis of (±)-erucalexin (**4**).

Gratifyingly, oxidative cyclization of 1-methoxyisobrassinin (**38**) with CrO_3 in acetic acid afforded (±)-erucalexin (**4**) in moderate yield (30%). Except for the optical rotation, the characterization data of the synthetic material and those of the natural product were identical in all respects. Additional attempts to synthesize erucalexin (**4**) from **38** using different oxidizing agents (Br_2 in dioxane, NBS in CH_2Cl_2, or pyridinium bromide perbromide in *t*-BuOH) and conditions led to formation of the tricyclic compound **39** (Scheme 8). With **39** in hand, we sought to carry out an oxidative rearrangement to form a 3-indolinone-2-spirocyclic compound. However, when **39** was treated with *m*-CPBA, an intractable mixture was obtained, whereas reaction of **39** with *N*-chlorosuccinimide (NCS) afforded **40** in 32% yield (Scheme 8). Other oxidizing reagents such as H_2O_2/Na_2WO_4 yielded **40** in <10% yield and NBS yielded **40** in 21%. Although a somewhat related rearrangement of a cyclic acetal into a lactone using *m*-CPBA/$BF_3 \cdot Et_2O$ has been observed, those conditions did not work in the case of compound **39**.[26]



Scheme 8 Oxidation of potential biosynthetic intermediate **38**.

To expand the scope of CrO_3-mediated oxidation we have also investigated the oxidative spirocyclization of isobrassinin (**43**) and 1-Boc-isobrassinin (**44**). To prepare isobrassinin (**43**), aldehyde **41**[27] was converted to the corresponding oxime, which upon treatment first with $NaBH_4/NiCl_2$,[28] followed by carbon disulfide and iodomethane afforded isobrassinin (**43**) in 30% yield, based on indole-2-carboxaldehyde (**41**) (Scheme 9). 1-Boc-Isobrassinin (**44**) was prepared similarly, using 1-Boc-indole-2-carboxaldehyde (**42**)[19] (Scheme 9) in 24% overall yield, based on 1-Boc-indole-2-carboxaldehyde (**42**). Oxidation of isobrassinin (**43**) under various conditions led to decomposition, as suggested by TLC monitoring



Scheme 9 Synthesis of (±)-demethoxyerucalexin (**46**).

Table 2 Antifungal activity[a] of natural 1-methoxyspirobrassinin (**3**) and erucalexin (**4**) against the plant pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum*

Compound	Concentration/M	% Inhibition[a]	
		R. solani	*S. sclerotiorum*
(+)-1-Methoxyspirobrassinin (**3**)	5.0×10^{-4}	68	53
	2.5×10^{-4}	14	34
	5.0×10^{-5}	No inhibition	10
(+)-Erucalexin (**4**)	5.0×10^{-4}	100	40
	2.5×10^{-4}	48	15
	5.0×10^{-5}	No inhibition	No inhibition

[a] Percent inhibition = 100 − [(growth on medium containing compound/growth on control medium) × 100]; results are the mean of three independent experiments conducted in triplicate (SD ± 1).

of the reaction mixture. On the other hand, 1-Boc-isobrassinin (**44**) afforded the oxidation product (±)-**45** in 26% yield (Scheme 9). Deprotection of (±)-**45** (TFA, CH_2Cl_2) gave *N*-demethoxy analog of erucalexin (±)-**46** (Scheme 9).

The antifungal activity of (+)-1-methoxyspirobrassinin (**3**) and (+)-erucalexin (**4**) was compared using a mycelia radial growth bioassay, as described in the Experimental section. These antifungal bioassays established that both (+)-1-methoxyspirobrassinin (**3**) and (+)-erucalexin (**4**) were active against *S. sclerotiorum* and *Rhizoctonia solani*, two of the most important pathogens of oilseed and vegetable crucifers. Erucalexin (**4**, 5.0×10^{-4} M) and 1-methoxyspirobrassinin (**3**, 5.0×10^{-4} M) were more inhibitory to *R. solani* (100 and 68% inhibition, respectively) than to *S. sclerotiorum* (40 and 53% inhibition, after 72 h of incubation, Table 2).

Conclusion

In conclusion, (+)-erucalexin (**4**) is produced in elicited leaves of *E. gallicum*, but is not detectable in non-elicited leaves, and shows antifungal activity against *S. sclerotiorum* and *R. solani*, two important pathogens of crucifer oilseeds. Therefore, erucalexin (**4**) is a novel phytoalexin having a 3-indoxyl system with a spiro ring at C-2. It is worthy of note that, although *E. gallicum* produces phytoalexins active against *S. sclerotiorum* and *R. solani*, it appears that to date only resistance to *S. sclerotiorum* has been reported.[6] Hence, it would be important to evaluate further the resistance of *E. gallicum* to other fungi, as it could be a valuable germplasm source of disease resistance for oilseed improvement.

The first one-pot biomimetic syntheses of both (±)-1-methoxyspirobrassinin (**3**) and (±)-spirobrassinin (**34**) from 1-methoxybrassinin (**30**) and brassinin (**33**), respectively, were accomplished. (±)-Erucalexin (**4**) was synthesized from 1-methoxyindole (**36**) in 9% overall yield. Further studies addressing the enantioselective synthesis, resolution and absolute configuration of erucalexin (**4**) are underway.

Experimental

General experimental procedures

All solvents were HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$ which were redistilled and THF and Et_2O which were dried over Na and benzophenone. Flash column chromatography: silica gel grade 60, 230–400 μm. Organic extracts were dried over Na_2SO_4 and the solvents were removed using a rotary evaporator. Analytical HPLC analysis was carried out with a high performance liquid chromatograph equipped with quaternary pump, automatic injector, and photodiode array detector (wavelength range 190–600 nm), degasser, and an ODS column (5 μm particle size silica, 4.6 id × 200 mm) equipped with an in-line filter. Mobile phase: H_2O–CH_3CN, 75 : 25 to 100% CH_3CN, for 35 min, linear gradient, and flow rate 1.0 ml min^{-1}. Specific rotations, $[\alpha]_D$ were determined at ambient temperature on a polarimeter using a 1 ml, 10 cm path length cell; the units are 10^{-1} deg cm^2 g^{-1} and the concentrations (*c*) are reported in g/100 ml. NMR spectra were recorded on 500 MHz spectrometers. For ^{1}H NMR (500 MHz) the chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. The δ values were referenced to $CDCl_3$ ($CHCl_3$ at 7.27 ppm), CD_3CN (CD_2HCN at 1.94 ppm). First-order behavior was assumed in the analysis of ^{1}H NMR spectra and multiplicities are as indicated by one or more of the following: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. Spin coupling constants (*J* values) are reported to the nearest 0.5 Hz. For ^{13}C NMR (125.8 MHz) the chemical shifts (δ values) were referenced to $CDCl_3$ (77.23 ppm), CD_3CN (118.69 ppm). The multiplicities of the ^{13}C signals refer to the number of attached protons: s = C, d = CH, t = CH_2, q = CH_3, and were determined based on HMQC correlations and magnitude of *J* values. Mass spectrometry (MS) [high resolution (HR), electron impact (EI)] data were obtained on a mass spectrometer using a solids probe. Fourier transform infrared (FTIR) spectra were recorded on a spectrometer and spectra were measured by the diffuse reflectance method on samples dispersed in KBr. Ultraviolet (UV) spectra were recorded on a spectrophotometer using a 1 cm path length quartz cell.

Plant material and growth

Seeds of dog mustard (*Erucastrum gallicum*, a wild crucifer) were obtained from Plant Gene Resources, Agriculture and Agric-Food Canada Research Station, Saskatoon, SK. The seeds were sown in commercial potting soil mixture, and plants were grown in a growth chamber under controlled environmental conditions (20/18 °C with 16/8 day/night cycle) for 2–5 weeks.

Fungal isolates

Fungal isolates were obtained from Agriculture and Agric-Food Canada Research Station, Saskatoon, SK. Cultures of *Sclerotinia sclerotiorum* clone #33 and *Rhizoctonia solani* AG-2 were maintained on potato dextrose agar (PDA) cultures.

Elicitation of phytoalexins with *Sclerotinia sclerotiorum*. Leaves from five-week-old plants (*E. gallicum)* were excised with a sharp blade, the petioles were wrapped with pre-moistened cotton wool and leaves placed in Petri plates (two leaves per plate). Each leaf was inoculated with five mycelium plugs placed upside down (4 mm cut from three-day-old PDA plates of *S. sclerotiorum* clone #33) and distributed evenly over the leaf surface. The Petri plates were sealed and incubated under constant fluorescent light for seven days. After every 24 h, leaves were frozen in liquid nitrogen, crushed with a glass rod and extracted with EtOAc by shaking at 120 rpm for 30 min. The EtOAc was filtered, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure and the extract was analyzed by HPLC. Two independent experiments were carried out. The HPLC analysis of the EtOAc extracts indicated the presence of the novel erucalexin (**4**, $t_R = 20.6$ min) and three other compounds with $t_R = 11.2$, 13.1, and 16.9 min, as previously reported,[9] not present in the control samples.

Elicitation of phytoalexins with $CuCl_2$. Five-week-old plants (*E. gallicum*) were sprayed to the point of run-off with $CuCl_2$ solutions at 24 h intervals for three days. Leaves were excised at 24 h intervals for seven days; control leaves were harvested from separate plants at the same time and treated in a similar manner throughout. After various incubation periods, leaves were worked up as described above and extracts were analyzed by HPLC. Three independent experiments were carried out.

Antifungal bioassays. The antifungal activity of 1-methoxy-spirobrassinin (**3**) and erucalexin (**4**) was determined using the following mycelia radial growth bioassay. Solutions of each compound in DMSO (5×10^{-2} M) were used to prepare assay solutions in minimal media (5×10^{-4} M, 2.5×10^{-4} M, 5×10^{-5} M) in serial dilution; control solutions contained 1% DMSO in minimal media. Sterile tissue culture plates (12-well, 24 mm diameter) containing test solutions and the control solution (1 ml per well) were inoculated with mycelia plugs placed upside down on the centre of each plate (5 mm cut from three-day-old and seven-day-old PDA plates of *S. sclerotiorum* clone #33 and *R. solani* AG 2-1, respectively) and incubated under constant light for seven days. The radial growth of mycelia the was measured with a ruler daily for one week. Three independent experiments were carried out, each one in triplicate.

Isolation and characterization of (+)-erucalexin (4). Plants (40, five-week-old) were sprayed with $CuCl_2$ (2×10^{-3} M) solution to the point of run-off, three times at 24 h intervals and allowed to stand for three days. Elicited leaves (180 g, fresh weight), were frozen in liquid nitrogen and crushed with a glass rod, and extracted with EtOAc in a manner similar to that followed for the time-course experiment. The EtOAc extract (1.2 g) was subjected to FCC (gradient elution, CH_2Cl_2, 100% to CH_2Cl_2–MeOH, 20 : 80), the fractions containing the HPLC peak at $t_R = 20.6$ min were combined (284 mg) and further fractionated by reverse phase FCC (gradient elution, CH_3CN–H_2O, 20 : 80) to CH_3CN, 100%) followed by reverse phase micro flash (2 cm plug of reverse phase C-18 silica gel in a Pasteur pipette, CH_3CN–H_2O (60 : 40) to yield 2.2 mg of (+)-erucalexin (**4**). This process was repeated four times to yield sufficient material for chemical characterization and bioassays (in total, from 200 plants and 6 g of extract *ca.* 12 mg of (+)-**4** were isolated). $[a]_D = 73$ (*c* 0.090, MeOH).

Synthesis

(±)-Erucalexin (4). A solution of CrO_3 (100 mg, 1.0 mmol) in water (0.3 ml) was added to a solution of 1-methoxyisobrassinin (**38**, 49 mg, 0.18 mmol) in acetic acid (1 ml) in one portion at room temperature. The mixture was stirred at rt for 5 min, was diluted with brine (20 ml) and was extracted (EtOAc). The combined organic extract was washed with 10% K_2CO_3 solution (20 ml) and dried. The solvent was concentrated and the residue was subjected to FCC (hexane–acetone, 5 : 1) to yield erucalexin (**4**, 16 mg, 30%) as an orange solid, mp 94–97 °C. HPLC: $t_R = 20.6$ min. δ_H (500 MHz, $CDCl_3$): 7.70 (d, $J = 8$ Hz, 1H), 7.66 (ddd, $J = 8$, 8, 1 Hz, 1H), 7.24 (d, $J = 8$ Hz, 1H), 7.14 (ddd, $J = 8$, 8, 1 Hz, 1H), 4.76 (d, $J = 16$ Hz, 1H), 4.49 (d, $J = 16$ Hz, 1H), 4.00 (s, 3H), 2.68 (s, 3H); δ_C (125 MHz, $CDCl_3$): 192.0 (s), 165.8 (s), 159.9 (s), 138.0 (d), 124.6 (d), 123.8 (d), 121.1 (s), 114.1 (d), 93.8 (s), 70.9 (t), 65.4 (q), 16.0 (q). HRMS (EI): calc. for $C_{12}H_{12}N_2O_2S_2$ (M^+) m/z 280.0340, found 280.0342; MS (EI) m/z (% relative int.): 280 [M^+] (56), 249 (74), 207 (30), 176 (100), 149 (15), 132 (64), 87 (38). ν_{max}(KBr)/cm^{-1}: 2923, 2850, 1725, 1608, 1576, 1461, 1301, 1106, 1064, 985, 759. λ_{max} (CH_3CN)/nm 234 (log ε, 4.6), 262 (4.1), 368 (3.4).

(±)-2′-Methylsulfanyl-spiro[1-methylindoline-3,5′-[4′,5′]-dihydro-oxazole]-2-thione (6). To a stirred suspension of powdered K_2CO_3 (2.5 mg, 0.018 mmol) in acetone (0.5 ml) were added spirooxazolidine **14** (4 mg, 0.016 mmol) and CH_3I (5 µl, 0.08 mmol). The mixture was stirred for 16 h at rt and was subjected to TLC (hexane–acetone, 5 : 1) to yield (±)-**6** (3.4 mg, 81%) as a slightly yellow solid, mp 130–132 °C. HPLC: $t_R = 16.8$ min. δ_H (500 MHz, CD_3CN): 7.50 (m, 2H), 7.27 (dd, $J = 7$, 7 Hz, 1H), 7.18 (d, $J = 7$ Hz, 1H), 4.27 (d, $J = 14$ Hz, 1H), 4.15 (d, $J = 14$ Hz, 1H), 3.56 (s, 3H), 2.55 (s, 3H); δ_C (125 MHz, CD_3CN): 204.5 (s), 165.4 (s), 146.3 (s), 133.1 (s), 132.4 (d), 126.4 (d), 125.8 (d), 111.9 (d), 92.8 (s), 69.5 (t), 32.4 (q), 15.4 (q). HRMS (EI): calc. for $C_{12}H_{12}N_2OS_2$ (M^+) m/z 264.0391, found 264.0391; MS (EI) m/z (% relative int.): 264 [M^+] (58), 217 (81), 190 (35), 175 (100), 89 (14), 71 (20). ν_{max}(KBr)/cm^{-1}: 2926, 1741, 1620, 1466, 1375, 1146. λ_{max} (CH_3CN)/nm 238 (log ε, 4.1), 287 (3.6), 296 (3.7), 337 (3.8).

(±)-1-Methyl-spiro(indoline-2,2′-tetrahydrothiophene)-3-one (9a). To a solution of (±)-spiro(indoline-2,2′-tetrahydrothiophene)-3-one (**29**) (14 mg, 0.068 mmol) in dry THF (0.5 ml) NaH (60% in mineral oil, 5 mg, 0.14 mmol) was added and the mixture was stirred for 5 min at rt, followed by the addition of CH_3I (17 µl, 0.27 mmol). The stirring continued for a further 3 h at rt, the solvent was evaporated and the residue was subjected to FCC (hexane–acetone, 5 : 1). Evaporation of the eluate afforded (±)-1-methyl-spiro(indoline-2,2′-tetrahydrothiophene)-3-one (**9a**) as a yellow oil. Yield: 12 mg (80%). HPLC: $t_R = 19.2$ min. δ_H (500 MHz, CD_3CN): 7.53 (m, 2H), 6.86 (d, $J = 8$ Hz, 1H), 6.78 (dd, $J = 8$, 8 Hz, 1H), 3.28 (m, 1H), 3.08 (m, 1H), 3.01 (s, 3H), 2.41 (m, 1H),

2.31 (m, 1H), 2.14 (m, 2H); δ_C (125 MHz, CD_3CN): 202.5 (s), 161.0 (s), 139.0 (d), 125.8 (d), 119.9 (s), 119.1 (d), 110.3 (d), 86.1 (s), 37.8 (t), 35.7 (t), 32.6 (t), 28.9 (q). HRMS (EI): calc. for $C_{12}H_{13}NOS$ (M^+) m/z 219.0718, found 219.0716; MS (EI) m/z (% relative int.): 219 [M^+] (100), 190 (58), 163 (80), 130 (15), 77 (17). ν_{max}(KBr)/cm^{-1}: 2927, 1707, 1615, 1483, 1369, 1318, 1146, 950, 745. λ_{max} (CH_3CN)/nm 235 (log ε, 4.3), 264 (4.0), 353 (3.0).

(±)-Spiro[1-methyl-3,5′-oxazolidin]-2-one-2′-thione (13). Et_2NH (21 µl, 0.20 mmol) was added to 1-methylisatin (**8**, 322 mg, 2.0 mmol) suspended in a solution of CH_3NO_2 [11] (325 µl, 5.8 mmol) and EtOH (1 ml) at 0 °C. The reaction mixture was kept at 0 °C for 10 min, and the solvent was removed under reduced pressure. The residue was dissolved in a solution of MeOH (6 ml) and acetic acid (0.5 ml), 10% Pd/C (50 mg) was added and mechanically agitated at 3 atm of H_2 for 16 h. The reaction mixture was filtered, the solvent evaporated and the residue subjected to FCC (CH_2Cl_2–MeOH–30% aqueous NH_3, 80 : 20 : 1) to yield (±)-**12** (304 mg, 79%) as a colorless oil. A solution of amine **12** (50 mg, 0.26 mmol, in CH_2Cl_2, 2 ml) was added dropwise (over a 10 min period) to a vigorously stirred mixture of 5% Na_2CO_3 and $CSCl_2$ (22 µl, 0.29 mmol in CH_2Cl_2, 4 ml) at rt. After 2 h, the aqueous layer was separated, extracted with CH_2Cl_2, the organic phases combined and, after removal of the solvent, the crude product was crystallized using acetone–hexane to yield (±)-spirooxazolidine **13** (46 mg, 76%) as colorless crystals, mp 180–182 °C. HPLC: t_R = 6.8 min. δ_H (500 MHz, CD_3CN): 8.04 (br s, D_2O exchange, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.49 (ddd, J = 7.5, 7.5, 1 Hz, 1H), 7.18 (ddd, J = 7.5, 7.5, 1 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H), 4.01 (d, J = 11 Hz, 1H), 3.96 (d, J = 11 Hz, 1H), 3.17 (s, 3H); δ_C (125 MHz, CD_3CN): 189.8 (s), 173.3 (s), 146.1 (s), 133.3 (d), 126.5 (s), 126.1 (d), 124.8 (d), 110.7 (d), 86.1 (s), 52.2 (t), 27.4 (q). HRMS (EI): calc. for $C_{11}H_{10}N_2O_2S$ (M^+) m/z 234.0463, found 234.0463; MS (EI) m/z (% relative int.): 234 [M^+] (44), 178 (58), 162 (100), 77 (12). ν_{max}(KBr)/cm^{-1}: 3300, 2925, 1730, 1616, 1524, 1471, 1376, 1241, 1165, 983, 755. λ_{max} (CH_3CN)/nm 211 (log ε, 4.0), 249 (3.5), 296 (2.9).

(±)-Spiro[1-methylindolin-3,5′-oxazolidine]-2,2′-dithione (14). To a solution of (±)-spirooxazoline **13** (23 mg, 0.1 mmol) in dry THF (4 ml) were added P_4S_{10} (222 mg, 0.50 mmol)[13] and $NaHCO_3$ (84 mg, 1.0 mmol) and the reaction mixture was stirred for 24 h under reflux (condenser equipped with a $CaCl_2$ trap). The solvent was evaporated, the residue was dissolved in water (20 ml) and extracted with CH_2Cl_2. The combined organic extract was dried, concentrated and the residue was subjected to FCC (hexane–EtOAc, 2 : 1) to yield starting material (5 mg, 22%) and dithione **14** (4 mg, 16%) as a slightly yellow solid, mp 155–157 °C. HPLC: t_R = 12.8 min. δ_H (500 MHz, CD_3CN): 7.98 (br s, D_2O exchange, 1H), 7.63 (d, J = 7 Hz, 1H), 7.54 (ddd, J = 7, 7, 1 Hz, 1H), 7.30 (ddd, J = 7, 7, 1 Hz, 1H), 7.20 (d, J = 7 Hz, 1H), 4.09 (d, J = 11 Hz, 1H), 4.01 (d, J = 11 Hz, 1H), 3.56 (s, 3H); δ_C (125 MHz, CD_3CN): 201.9 (s), 189.2 (s), 146.6 (s), 133.2 (d), 131.4 (s), 126.6 (d), 126.5 (d), 112.1 (d), 92.6 (s), 55.5 (t), 32.6 (q). HRMS (EI): calc. for $C_{11}H_{10}N_2OS_2$ (M^+) m/z 250.0235, found 250.0231; MS (EI) m/z (% relative int.): 250 [M^+] (10), 217 (50), 175 (40), 97 (50). ν_{max}(KBr)/cm^{-1}: 3267, 2925, 1701, 1614, 1527, 1467, 1376, 1164, 1098, 752. λ_{max} (CH_3CN)/nm 243 (log ε, 3.9), 287 (3.4), 337 (3.5).

1-Methoxy-2-chloroindole-3-carboxaldehyde (16). Dry DMF (4 ml) was cooled to 0 °C and oxalyl chloride (540 µl, 6.1 mmol) was added dropwise. 1-Methoxyoxindole (**15**)[29] (400 mg, 2.45 mmol) was added and the mixture was stirred for 1 h at 75 °C. After cooling to rt, the mixture was poured into 100 ml of water and extracted with Et_2O. The combined organic extract was dried, the solvent was evaporated and the residue subjected to FCC (hexane–acetone, 5 : 1). Crystallization from CH_2Cl_2–hexane afforded 1-methoxy-2-chloroindole-3-carboxaldehyde (**16**, 365 mg, 71%) as colorless crystals, mp 82–83 °C. HPLC: t_R = 17.8 min. δ_H (500 MHz, $CDCl_3$): 10.09 (s, 1H), 8.31 (d, J = 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 7.39 (dd, J = 8, 8 Hz, 1H), 7.35 (dd, J = 8, 8 Hz, 1H), 4.21 (s, 3H); δ_C (125 MHz, $CDCl_3$): 183.7 (s), 132.5 (s), 131.5 (s), 124.9 (d), 124.2 (d), 121.7 (d), 121.0 (s), 109.8 (s), 108.4 (d), 66.5 (q). HRMS (EI): calc. for $C_{10}H_8ClNO_2$ (M^+) m/z 209.0244, found 209.0243; MS (EI) m/z (% relative int.): 209 [M^+] (100), 166 (41), 114 (16). ν_{max}(KBr)/cm^{-1}: 2952, 1656, 1507, 1386, 1243, 1167, 1043, 952, 741.

Baeyer–Villiger oxidation of aldehydes 16 and 19. *m*-CPBA (43 mg, 0.25 mmol) was added to separate solutions of aldehydes **16** and **19** (0.125 mmol) in CH_2Cl_2 (1 ml), and the mixtures were stirred for 2 h at rt. Unstable *O*-formate **18** was separated by FCC (hexane–Et_2O, 10 : 1) to yield 12 mg (43%) of 1-methoxy-2-chloroindole-3-yl formate (**18**) containing a small amount (10%) of 1-methoxyisatin (**17**). When aldehyde **19** was used, 1-methylisatin (**8**) was isolated as the sole product (12 mg, 60%). HCl (one drop of 1 M) was added to a solution of *O*-formate **18** (17 mg, 0.08 mmol) in MeOH (1 ml) and the mixture was stirred for 1 h at rt. The solvent was evaporated and the residue was subjected to FCC (CH_2Cl_2). Evaporation of the eluate afforded 7 mg (52%) of 1-methoxyisatin (**17**) as a red solid, mp 105–107 °C (lit.[16] 110–113 °C).

To a solution of 1-methoxyoxindole (**15**, 41 mg, 0.25 mmol) in acetic acid (2 ml), a solution of CrO_3 (125 mg, 1.25 mmol) in water (0.5 ml) was added and the mixture was stirred at rt. After 1 h the reaction mixture was diluted with brine (20 ml), extracted with EtOAc, and the combined extract was washed with 10% K_2CO_3 solution (20 ml), dried and concentrated. The residue was subjected to FCC (CH_2Cl_2–MeOH, 98 : 2) to yield 1-methoxyisatin (**17**, 33 mg, 75%). HPLC: t_R = 8.2 min. δ_H (500 MHz, CD_3CN): 7.70 (dd, J = 7, 7 Hz, 1H), 7.58 (d, J = 7 Hz, 1H), 7.20 (dd, J = 7, 7 Hz, 1H), 7.15 (d, J = 7 Hz, 1H), 4.04 (s, 3H); δ_C (125 MHz, CD_3CN): 182.5 (s), 155.0 (s), 149.0 (s), 139.9 (d), 125.7 (d), 125.5 (d), 118.1 (s), 110.3 (d), 65.2 (q). HRMS (EI): calc. for $C_9H_7NO_3$ (M^+) m/z 177.0426, found 177.0428; MS (EI) m/z (% relative int.): 177 [M^+] (15), 149 (85), 104 (23), 91 (100), 78 (47), 63 (13). ν_{max}(KBr)/cm^{-1}: 2948, 1743, 1614, 1460, 1315, 1191, 1043, 935, 754.

Baeyer–Villiger oxidation of 1-methoxyindole-3-carboxaldehyde (20). *m*-CPBA (142 mg, 0.83 mmol) was added to a solution of 1-methoxyindole-3-carboxaldehyde (**20**,[18] 44 mg, 0.25 mmol) in CH_2Cl_2 (2 ml) and the mixture was stirred at rt for 16 h. The reaction mixture was diluted with CH_2Cl_2 (20 ml), washed with 10% K_2CO_3 solution (10 ml), the aqueous layer was re-extracted with CH_2Cl_2, and the combined organic extract was dried and concentrated. After chromatography of the residue (hexane–acetone, 5 : 1), the product was dissolved in dioxane (1.5 ml), HCl (1 M, 0.6 ml) was added, and the reaction mixture

was stirred at rt. After 16 h, the reaction mixture was diluted with brine (20 ml), extracted with EtOAc, and the combined organic extract was dried and concentrated. The residue was subjected to FCC (hexane–acetone, 2 : 1). Evaporation of the eluate afforded (±)-1-methoxy-2-hydroxyindolin-3-one (**21**) as a colorless oil (18 mg, 40%, based on aldehyde **20**). HPLC: t_R = 5.8 min. δ_H (500 MHz, $CDCl_3$): 7.67 (dd, J = 8, 8 Hz, 1H), 7.64 (d, J = 8 Hz, 1H), 7.31 (d, J = 8 Hz, 1H), 7.17 (dd, J = 8, 8 Hz, 1H), 5.15 (s, 1 H), 4.10 (s, 3H), 3.27 (br s, D_2O exchange, 1H); δ_C (125 MHz, $CDCl_3$): 194.5 (s), 159.0 (s), 137.9 (d), 124.2 (d), 124.0 (d), 120.9 (s), 115.4 (d), 93.4 (d), 64.9 (q). HRMS (EI): calc. for $C_9H_9NO_3$ (M^+) m/z 179.0582, found 179.0586; MS (EI) m/z (% relative int.): 179 [M^+] (27), 148 (100), 130 (10), 92 (24), 77 (21), 65 (37). ν_{max}(KBr)/cm^{-1}: 3355, 2935, 1726, 1612, 1461, 1208, 1150, 984, 811, 760.

When the acid-catalyzed (1 M HCl, 0.3 ml) hydrolysis was carried out in MeOH (2 ml) instead of dioxane (2 h at rt), followed by the work-up described above, (±)-1,2-dimethoxyindolin-3-one (**22**) was obtained (colorless oil, 18 mg, 37%, based on aldehyde **20**). HPLC: t_R = 13.6 min. δ_H (500 MHz, $CDCl_3$): 7.66 (m, 2H), 7.33 (d, J = 8 Hz, 1H), 7.15 (dd, J = 8, 8 Hz, 1H), 5.02 (s, 1 H), 4.08 (s, 3H), 3.52 (s, 3H); δ_C (125 MHz, $CDCl_3$): 193.2 (s), 159.5 (s), 137.8 (d), 124.1 (d), 123.8 (d), 122.0 (s), 115.4 (d), 98.0 (d), 64.5 (q), 54.2 (q). HRMS (EI): calc. for $C_{10}H_{11}NO_3$ (M^+) m/z 193.0739, found 193.0741; MS (EI) m/z (% relative int.): 193 [M^+] (20), 162 (100), 146 (43), 130 (98), 92 (15). ν_{max}(KBr)/cm^{-1}: 2941, 1729, 1610, 1463, 1216, 1146, 1032, 763.

Reaction of *O*-formate 24 with EtSH and MeSNa. To a solution of aldehyde **23**[19] (70 mg, 0.25 mmol) in CH_2Cl_2 (2 ml), *m*-CPBA (86 mg, 0.5 mmol) was added and the mixture was stirred for 2 h at rt. *O*-Formate **24** was separated by FCC (hexane–EtOAc, 8 : 1), affording 68 mg (90%) of 1-Boc-2-chloroindole-3-yl formate (**24**). *O*-Formate **24** (68 mg, 0.24 mmol) was dissolved in THF (2 ml), EtSH (72 µl, 0.96 mmol) or 3-chloro-1-propanethiol (45 µl, 0.46 mmol) were added, followed by addition of Et_3N (130 µl, 0.92 mmol). The mixtures were stirred for 2 h at rt, before being concentrated and the residues subjected to FCC (hexane–Et_2O, 10 : 1). Evaporation of the eluates afforded compounds (±)-**25** and (±)-**27**, respectively, as colorless oils. (±)-1-Boc-2-Ethylsulfanylindolin-3-one (**25**). Yield 40 mg, 59% (based on aldehyde **23**). HPLC: t_R = 29.7 min. δ_H (500 MHz, $CDCl_3$): 8.09 (br s, 1H), 7.75 (d, J = 8 Hz, 1H), 7.65 (dd, J = 8, 8 Hz, 1H), 7.17 (dd, J = 8, 8 Hz, 1H), 5.15 (s, 1H), 2.68 (m, 1H), 2.59 (m, 1H), 1.63 (s, 9H), 1.22 (t, J = 7 Hz, 3H); δ_C (125 MHz, $CDCl_3$): 195.4 (s), 152.6 (s), 150.5 (s), 137.6 (d), 124.5 (d), 123.6 (d), 123.5 (s), 116.7 (d), 83.4 (s), 65.3 (d), 28.5 (3 × q), 23.5 (t), 14.8 (q). HRMS (EI): calc. for $C_{15}H_{18}NO_3S$ ($M - H^+$) m/z 292.1007, found 292.1011; MS (EI) m/z (% relative int.): 292 [$M - H^+$] (6), 233 (88), 193 (26), 177 (100), 148 (16), 132 (94), 92 (26). ν_{max}(KBr)/cm^{-1}: 2978, 1714, 1604, 1467, 1681, 1365, 1280, 1153, 754.

(±)-1-Boc-2-(3-Chloropropyl)sulfanylindolin-3-one (27). Yield 52 mg, 61% (based on aldehyde **23**). HPLC: t_R = 32.2 min. δ_H (500 MHz, $CDCl_3$): 8.06 (br s, 1H), 7.75 (d, J = 8 Hz, 1H), 7.66 (dd, J = 8, 8 Hz, 1H), 7.18 (dd, J = 8, 8 Hz, 1H), 5.13 (s, 1H), 3.62 (m, 2H), 2.85 (m, 1H), 2.76 (m, 1H), 2.00 (m, 2H), 1.63 (s, 9H); δ_C (125 MHz, $CDCl_3$): 195.2 (s), 152.5 (s), 150.5 (s), 137.8 (d), 124.6 (d), 123.7 (d), 123.4 (s), 116.9 (d), 83.6 (s), 65.2 (d), 43.3 (t), 32.5 (t), 28.5 (3 × q), 26.7 (t). HRMS (EI): calc. for $C_{16}H_{20}ClNO_3S$ (M^+) m/z 341.0852, found 341.0843; MS (EI) m/z (% relative int.): 341 [M^+] (14), 286 (15), 268 (60), 233 (26), 177 (37), 132 (39). ν_{max}(KBr)/cm^{-1}: 2984, 1716, 1607, 1468, 1367, 1279, 1157, 1060, 757.

(±)-1-Boc-2-Ethylsulfanyl-2-methylindolin-3-one (26). To a solution of 1-Boc-2-ethylsulfanylindolin-3-one (**25**, 18 mg, 0.037 mmol) NaH, (60% in mineral oil, 6 mg, 0.15 mmol) in dry THF (0.5 ml) was added and the mixture was stirred for 10 min at rt followed by the addition of CH_3I (10 µl, 0.15 mmol). The stirring continued for 1 h at rt, the solvent was evaporated and the residue subjected to FCC (hexane–Et_2O, 10 : 1). Evaporation of the eluate afforded (±)-1-Boc-2-ethylsulfanyl-2-methylindolin-3-one (**26**) as a colorless oil. Yield: 10 mg (87%). HPLC: t_R = 32.8 min. δ_H (500 MHz, $CDCl_3$): 8.21 (d, J = 8 Hz, 1H), 7.79 (d, J = 8 Hz, 1H), 7.66 (dd, J = 8, 8 Hz, 1H), 7.17 (dd, J = 8, 8 Hz, 1H), 2.38 (m, 2H), 1.84 (s, 3H), 1.65 (s, 9H), 1.07 (t, J = 7 Hz, 3H); δ_C (125 MHz, $CDCl_3$): 197.9 (s), 152.3 (s), 151.0 (s), 137.9 (d), 124.7 (d), 123.7 (d), 122.3 (s), 117.0 (d), 83.5 (s), 72.9 (s), 28.7 (q), 23.7 (3 × q), 23.4 (t), 14.4 (q). HRMS (EI): calc. for $C_{16}H_{21}NO_3S$ (M^+) m/z 307.1242, found 307.1242; MS (EI) m/z (% relative int.): 307 [M^+] (100), 292 (55), 262 (29). ν_{max}(KBr)/cm^{-1}: 2935, 1712, 1608, 1465, 1350, 1259, 1162, 963, 754.

(±)-1-Boc-Spiro(indoline-2,2′-tetrahydrothiophene)-3-one (28). 1-Boc-2-(3-Chloropropyl)sulfanylindolin-3-one (**27**, 50 mg, 0.15 mmol) was dissolved in dry DMF (1 ml) and NaH (60% in mineral oil, 20 mg, 0.58 mmol) was added. The mixture was stirred for 15 min at 60 °C, before being cooled to 0 °C and diluted with brine (20 ml). The product was extracted with EtOAc, and the combined extract was washed with brine (2 × 25 ml), then dried and concentrated. The residue was subjected to FCC (hexane–Et_2O, 10 : 1). Evaporation of the eluate afforded (±)-1-Boc-spiro(indoline-2,2′-tetrahydrothiophene)-3-one (**28**) as a colorless solid. Yield: 40 mg (89%); mp 86–88 °C. HPLC: t_R = 31.3 min. δ_H (500 MHz, $CDCl_3$): 8.09 (br s, 1H), 7.77 (d, J = 8 Hz, 1H), 7.64 (dd, J = 8, 8 Hz, 1H), 7.16 (dd, J = 8, 8 Hz, 1H), 3.44 (m, 1H), 3.21 (m, 1H), 2.70 (m, 1H), 2.53 (m, 2H), 2.23 (m, 1H), 1.66 (s, 9H); δ_C (125 MHz, $CDCl_3$): 200.3 (s), 152.5 (s), 150.6 (s), 137.4 (d), 124.9 (d), 123.5 (d), 121.4 (s), 117.2 (d), 83.5 (s), 82.0 (s), 37.7 (t), 36.0 (t), 32.5 (t), 28.8 (3 × q). HRMS (EI): calc. for $C_{16}H_{19}NO_3S$ (M^+) m/z 305.1086, found 305.1084; MS (EI) m/z (% relative int.): 305 [M^+] (79), 249 (41), 232 (11), 205 (100), 177 (23), 149 (82). ν_{max}(KBr)/cm^{-1}: 2930, 1714, 1610, 1463, 1353, 1253, 1156, 979, 756.

(±)-Spiro(indoline-2,2′-tetrahydrothiophene)-3-one (29). 1-Boc-Spiro(indoline-2,2′-tetrahydrothiophene)-3-one (**28**, 28 mg, 0.092 mmol) was dissolved in CH_2Cl_2 (600 µl) and TFA (140 µl, 1.83 mmol) was added dropwise. The mixture was stirred for 1 h at rt, the solvent was evaporated and the residue subjected to FCC (hexane–acetone, 5 : 1). Evaporation of the eluate afforded (±)-spiro(indoline-2,2′-tetrahydrothiophene)-3-one (**29**) as a yellow oil. Yield: 15 mg (80%). HPLC: t_R = 12.3 min. δ_H (500 MHz, $CDCl_3$): 7.66 (d, J = 8 Hz, 1H), 7.48 (dd, J = 8, 8 Hz, 1H), 6.89 (dd, J = 8, 8 Hz, 1H), 6.83 (d, J = 8 Hz, 1H), 4.82 (br s, D_2O exchange, 1H), 3.26 (m, 1H), 3.19 (m, 1H), 2.51 (m, 1H), 2.25 (m, 1H), 2.15 (m, 2H); δ_C (125 MHz, $CDCl_3$): 201.3 (s), 159.3 (s), 137.7 (d), 125.4 (d), 120.2 (s), 120.0 (d), 112.6 (d), 80.3 (s), 42.0 (t), 35.0 (t), 31.2 (t). HRMS (EI): calc. for $C_{11}H_{11}NOS$ (M^+)

m/z 205.0561, found 205.0560; MS (EI) *m/z* (% relative int.): 205 [M$^+$] (75), 176 (25), 130 (10). ν_{max}(KBr)/cm^{-1}: 3333, 2930, 1690, 1617, 1467, 1321, 1061, 894, 752.

CrO_3-mediated oxidation of 1-methoxybrassinin (30) and brassinin (33). To a solution of 1-methoxybrassinin[25] (**30**, 16 mg, 0.06 mmol) in acetic acid (0.5 ml), a solution of CrO_3 (30 mg, 0.3 mmol) in water (0.2 ml) was added in one portion at rt. The mixture was stirred at rt for 20 min, was diluted with brine (10 ml) and then extracted (EtOAc). The combined organic extract was washed with 10% K_2CO_3 solution (10 ml) and was dried. The solvent was concentrated and the residue subjected to preparative TLC (multiple development, hexane–EtOAc, 4 : 1) to yield (±)-1-methoxyspirobrassinin (**3**), 4 mg (24%); when the reaction mixture was stirred at 0 °C, (±)-1-methoxyspirobrassinol (**35**)[30] was obtained, 6 mg (35%) instead of (±)-**3**.

To a solution of 1-methoxybrassinin (**30**, 19 mg, 0.07 mmol) in CH_2Cl_2 (1 ml), PCC (108 mg, 0.5 mmol) was added and the reaction mixture was stirred for 2 h at rt. The reaction mixture was diluted with CH_2Cl_2 (10 ml), silica gel was added and the solvent was evaporated. The residue was subjected to FCC (hexane–EtOAc, 4 : 1). Evaporation of the eluate afforded (±)-1-methoxyspirobrassinin (**3**),[9] 8 mg (38%).

To a solution of brassinin[2] (**33**, 19 mg, 0.07 mmol) in acetic acid (0.7 ml) and dioxane (0.3 ml), a solution of CrO_3 (50 mg, 0.5 mmol) in water (0.9 ml) was added in one portion at rt. The mixture was stirred at rt for 30 min, before being diluted with brine (20 ml) and extracted (EtOAc). The combined organic extract was washed with 10% K_2CO_3 solution (10 ml) and dried. The solvent was concentrated and the residue was subjected to FCC (hexane–acetone, 5 : 1) to yield (±)-spirobrassinin (**34**),[12] 10 mg (40%).

1-Methoxyisobrassinin (38). *t*-BuLi in pentane (3.7 ml, 4.8 mmol) was added dropwise to a solution of 1-methoxyindole[23] (**36**, 589 mg, 4 mmol) in dry Et_2O (10 ml, under an Ar atmosphere), cooled to −20 °C (ice–NaCl).[24] The mixture was stirred for 15 min at −20 °C, DMF (0.75 ml, 4.8 mmol) was added, the cooling bath was removed and the stirring continued at rt for 1 h. The reaction mixture was cooled to 0 °C, diluted with 1 M HCl (30 ml) and extracted with Et_2O. The combined organic extract was dried, the solvent evaporated and the residue was subjected to FCC (hexane–acetone, 5 : 1) to afford 1-methoxyindole-2-carboxaldehyde (568 mg, 81%) as a slightly yellow oil. A solution of $NH_2OH{\cdot}HCl$ (360 mg, 5.18 mmol) and Na_2CO_3 (247 mg, 2.33 mmol) in water (3 ml) was added to 1-methoxyindole-2-carboxaldehyde (568 mg, 3.24 mmol) in EtOH (12 ml). The mixture was refluxed for 20 min, before being concentrated, diluted with brine (30 ml) and extracted (EtOAc). The combined organic extract was dried and the solvent was evaporated to yield crude 1-methoxyindole-2-carboxaldehyde oxime (**37**, 567 mg, 92%) of sufficient purity to be used in the next step. $AcONH_4$ (424 mg, 5.5 mmol) and $NaBH_3CN$ (314 mg, 5 mmol) were added to a cooled solution of 1-methoxyindole-2-carboxaldehyde oxime (**37**, 95 mg, 0.5 mmol) in MeOH (3 ml) at 0 °C followed by the addition of a solution of $TiCl_3$ [prepared by mixing commercially available 30% $TiCl_3$ in 2 M HCl (1.55 ml), with 1 M NaOH solution (1.1 ml)] in one portion, and the mixture was stirred for 20 min at 0 °C. The reaction mixture was then diluted with aqueous NH_4OH (prepared by mixing 1.3 ml 30% NH_4OH with 33 ml water) and extracted with EtOAc. The combined organic extract was dried, the solvent was evaporated and the crude amine was used directly in the next step. To a solution of this amine dissolved in pyridine (1 ml) cooled to 0 °C, CS_2 (30 µl, 0.5 mmol) and Et_3N (70 µl, 0.5 mmol) were added. After 1 h at 0 °C, CH_3I (30 µl, 0.5 mmol) was added and the mixture was kept at 3 °C for 16 h. The reaction mixture was then diluted with 1.5 M H_2SO_4 (15 ml) and extracted with Et_2O. The combined organic extract was dried, the solvent was evaporated and the residue was subjected to FCC (hexane–EtOAc, 8 : 1) to yield 1-methoxyisobrassinin (**38**, 55 mg, 41%, slightly yellow oil). HPLC: t_R = 24.4 min. δ_H (500 MHz, $CDCl_3$): 7.57 (d, J = 8 Hz, 1H), 7.44 (d, J = 8 Hz, 1H), 7.28 (dd, J = 8, 8 Hz, 1H), 7.15 (m, D_2O exchange, 2H), 6.39 (s, 1 H), 5.15 (d, J = 4 Hz, 2H), 4.12 (s, 3H), 2.68 (s, 3H); δ_C (125 MHz, $CDCl_3$): 199.4 (s), 133.1 (d), 131.3 (s), 123.7 (s), 123.1 (s), 121.4 (d), 120.8 (d), 108.5 (d), 98.9 (d), 65.9 (q), 42.3 (t), 18.5 (q). HRMS (EI): calc. for $C_{12}H_{14}N_2OS_2$ (M$^+$) *m/z* 266.0548, found 266.0550; MS (EI) *m/z* (% relative int.): 266 [M$^+$] (10), 235 (100), 187 (35), 160 (62), 129 (83). ν_{max}(KBr)/cm^{-1}: 3326, 2935, 1502, 1306, 1084, 917, 745.

9-Methoxy-3-methylsulfanyl-1,3-thiazino[5,6-*b*]indole (39). A solution of dioxane dibromide (150 µl, 0.11 mmol, prepared by dissolving 40 µl of Br_2 in 1 ml dioxane) was added to 1-methoxyisobrassinin (**38**, 25 mg, 0.09 mmol) dissolved in dioxane (1 ml), and the mixture was stirred for 30 min at rt. The reaction mixture was diluted with brine (10 ml) and then extracted with EtOAc. The combined organic extract was dried, the solvent was evaporated and the residue was subjected to FCC (hexane–EtOAc, 8 : 1) to afford **39** (22 mg, 88%) as an orange solid, mp 45–47 °C. HPLC: t_R = 31.7 min. δ_H (500 MHz, $CDCl_3$): 7.45 (d, J = 8 Hz, 1H), 7.42 (d, J = 8 Hz, 1H), 7.28 (dd, J = 8, 8 Hz, 1H), 7.16 (dd, J = 8, 8 Hz, 1H), 5.08 (s, 2H), 4.08 (s, 3H), 2.57 (s, 3H); δ_C (125 MHz, $CDCl_3$): 165.0 (s), 133.5 (s), 124.1 (s), 123.3 (d), 121.0 (d + s), 118.3 (d), 109.1 (d), 95.8 (s), 66.1 (q), 46.6 (t), 15.8 (q). HRMS (EI): calc. for $C_{12}H_{12}N_2OS_2$ (M$^+$) *m/z* 264.0391, found 264.0392; MS (EI) *m/z* (% relative int.): 264 [M$^+$] (32), 191 (100), 176 (22), 160 (53), 120 (32), 89 (12). ν_{max}(KBr)/cm^{-1}: 2935, 1599, 1447, 1301, 1232, 1134, 948, 740.

9-Methoxy-3-methylsulfanyl-1,3-thiazino[5,6-*b*]indol-1-one (40). To a solution of **39** (9 mg, 0.034 mmol) in CH_2Cl_2 (0.5 ml) was added NBS (7 mg, 0.041 mmol) or NCS (6 mg, 0.041 mmol). The mixtures were stirred at rt as follows: NBS (30 min), NCS (60 min). The solvent was evaporated and the residues were subjected to preparative TLC (hexane–acetone, 5 : 1) to afford **40** in the following amounts: NBS (2 mg, 21%), NCS (3 mg, 32%), brownish solid, mp 143–146 °C. HPLC: t_R = 22.0 min. δ_H (500 MHz, $CDCl_3$): 7.65 (m, 2H), 7.57 (dd, J = 8, 8 Hz, 1H), 7.29 (dd, J = 8, 8 Hz, 1H), 4.31 (s, 3H), 2.80 (s, 3H); δ_C (125 MHz, $CDCl_3$): 173.4 (s), 158.9 (s), 135.3 (s), 128.7 (d), 122.2 (d), 120.5 (d), 119.2 (s), 118.1 (s), 113.9 (s), 110.4 (d) 67.1 (q), 15.4 (q). HRMS (EI): calc. for $C_{12}H_{12}N_2O_2S_2$ (M$^+$) *m/z* 278.0184, found 278.0187; MS (EI) *m/z* (% relative int.): 278 [M$^+$] (46), 205 (24), 174 (100), 120 (10), 102 (15). ν_{max}(KBr)/cm^{-1}: 2942, 1662, 1615, 1519, 1340, 1216, 941, 744.

Synthesis of isobrassinin (43) and 1-Boc-isobrassinin (44). To a solution of indole-2-carboxaldehyde (**41**)[27] or 1-Boc-indole-2-carboxaldehyde (**42**, 3.4 mmol) in EtOH (10 ml), a solution of $NH_2OH{\cdot}HCl$ (720 mg, 10.4 mmol) and Na_2CO_3 (660 mg, 6.2 mmol) in water (5 ml) was added. The mixtures were refluxed with stirring for 30 min, before benig concentrated and

extracted (EtOAc). The combined organic extracts were dried and concentrated to afford corresponding oximes in 99% yield. Oximes (3.3 mmol) and $NiCl_2 \cdot 6H_2O$ (778 mg, 3.3 mmol) were dissolved in MeOH (50 ml). $NaBH_4$ (804 mg, 21.3 mmol) was added and the mixtures were stirred for 5 min at rt. The black precipitates were filtered off, the solutions were concentrated (*ca.* 25%) and then poured into water (100 ml) containing 30% NH_4OH (4 ml). The mixtures were extracted with EtOAc, the combined extracts were dried and then concentrated. Obtained unstable indolyl-2-methyl amines (orange oils) were dissolved in pyridine (2 ml) and the solutions were cooled to 0 °C. Et_3N (1 ml, 7.13 mmol) and CS_2 (0.97 ml, 16.12 mmol) were added and the mixtures were set aside for 1 h at 3 °C, followed by addition of CH_3I (0.69 ml, 11.16 mmol). After 16 h at 3 °C the mixtures were diluted with 1.5 M H_2SO_4 (30 ml) and then extracted with Et_2O. The combined organic extracts were dried and then concentrated. The residues were subjected to FCC as follows: **43** (hexane–EtOAc, 2 : 1); **44** (hexane–Et_2O, 5 : 1). Evaporation of the eluates afforded residues that were crystallized from CH_2Cl_2–hexane solution. Isobrassinin (**43**, 234 mg, 30%, based on the aldehyde **41**) was obtained as slightly orange crystals, mp 82–84 °C. 1-Boc-Isobrassinin (**44**, 248 mg, 24%, based on the aldehyde **42**) was obtained as slightly orange crystals, mp 88–90 °C.

Isobrassinin (**43**), HPLC: $t_R = 21.3$ min. δ_H (500 MHz, $CDCl_3$): 8.96 (br s, D_2O exchange, 1H), 7.58 (d, $J = 8$ Hz, 1H), 7.37 (m, D_2O exchange, 2H), 7.21 (dd, $J = 8$, 8 Hz, 1H), 7.12 (dd, $J = 8$, 8 Hz, 1H), 6.43 (s, 1H), 5.10 (d, $J = 5.5$ Hz, 1H), 2.69 (s, 3H); δ_C (125 MHz, $CDCl_3$): 201.4 (s), 136.5 (d), 134.6 (s), 127.7 (s), 122.7 (d), 120.7 (d), 120.2 (d), 111.4 (d), 102.3 (s), 44.0 (t), 18.7 (q). HRMS (EI): calc. for $C_{11}H_{12}N_2S_2$ (M^+) m/z 236.0442, found 236.0445; MS (EI) m/z (% relative int.): 236 [M^+] (20), 188 (21), 163 (13), 130 (100). ν_{max}(KBr)/cm^{-1}: 3386, 3310, 2917, 1498, 1294, 1068, 919, 749, 634.

1-Boc-Isobrassinin (**44**), HPLC: $t_R = 35.4$ min. δ_H (500 MHz, $CDCl_3$): 8.11 (br s, D_2O exchange, 1H), 8.00 (d, $J = 8.5$ Hz, 1H), 7.53 (d, $J = 8.5$ Hz, 1H), 7.31 (dd, $J = 8.5$, 8.5 Hz, 1H), 7.24 (dd, $J = 8.5$, 8.5 Hz, 1H), 6.78 (s, 1H), 5.28 (d, $J = 6$ Hz, 1H), 2.62 (s, 3H), 1.75 (s, 9H); δ_C (125 MHz, $CDCl_3$): 198.6 (s), 151.5 (s), 136.1 (s), 134.8 (s), 129.0 (s), 124.8 (d), 123.4 (d), 121.3 (d), 115.8 (d), 112.2 (d), 85.4 (s), 44.6 (t), 28.5 (3 × q), 18.2 (q). HRMS (EI): calc. for $C_{16}H_{20}N_2O_2S_2$ (M^+) m/z 336.0966, found 336.0975; MS (EI) m/z (% relative int.): 336 [M^+] (20), 280 (16), 189 (12), 163 (23), 130 (100). ν_{max}(KBr)/cm^{-1}: 3369, 2974, 1725, 1481, 1452, 1371, 1331, 1156, 1119, 1078, 923, 747.

CrO_3-mediated oxidation of 1-Boc-isobrassinin (44). To a stirred suspension of 1-Boc-brassinin (**44**, 67 mg, 0.2 mmol) in acetic acid (1 ml), a solution of CrO_3 in water (0.5 ml) was added at rt. The mixture was stirred at rt for 18 h, before being diluted with brine (20 ml) and then extracted with EtOAc. The combined organic extract was dried, concentrated, and the residue was subjected to FCC (hexane–EtOAc, 4 : 1). Evaporation of the eluate afforded (±)-1-Boc-erucalexin (**45**, 18 mg, 26%) as a yellow oil. HPLC: $t_R = 30.1$ min. δ_H (500 MHz, $CDCl_3$): 8.27 (d, $J = 8$ Hz, 1H), 7.77 (d, $J = 8$ Hz, 1H), 7.69 (ddd, $J = 8$, 8, 1 Hz, 1H), 7.20 (ddd, $J = 8$, 8, 1 Hz, 1H), 4.71 (d, $J = 16$ Hz, 1H), 4.62 (d, $J = 16$ Hz, 1H), 2.58 (s, 3H), 1.59 (s, 9H); δ_C (125 MHz, $CDCl_3$): 196.2 (s), 171.4 (s), 152.8 (s), 150.4 (s), 138.4 (d), 124.9 (d), 124.2 (d), 121.2 (s), 117.1 (d), 85.7 (s), 84.4 (s), 74.7 (t), 28.2 (3 × q), 15.7 (q). HRMS (EI): calc. for $C_{16}H_{18}N_2O_3S_2$ (M^+) m/z 350.0759, found 350.0756; MS (EI) m/z (% relative int.): 350 [M^+] (16), 294 (14), 250 (14), 177 (41), 57 (100). ν_{max}(KBr)/cm^{-1}: 2927, 1719, 1600, 1464, 1352, 1303, 1253, 1156, 1089, 947, 755.

(±)-Demethoxyerucalexin (46). 1-Boc-Erucalexin (**45**, 17 mg, 0.049 mmol) was dissolved in CH_2Cl_2 (0.5 ml) and TFA (110 µl, 1.46 mmol) was added. The mixture was stirred for 2 h at rt, before being concentrated and subjected to FCC (hexane–acetone, 5 : 1). Evaporation of the eluate afforded (±)-demethoxyerucalexin (**46**, 12 mg, 91%) as an orange solid, mp 128–130 °C. HPLC: $t_R = 14.6$ min. δ_H (500 MHz, CD_3CN): 7.56 (m, 2H), 6.90 (m, 2H), 6.60 (br s, D_2O exchange, 1H), 4.42 (d, $J = 16$ Hz, 1H), 4.33 (d, $J = 16$ Hz, 1H), 2.60 (s, 3H); δ_C (125 MHz, CD_3CN): 198.7 (s), 163.2 (s), 160.6 (s), 139.7 (d), 125.9 (d), 121.0 (d), 120.0 (s), 113.6 (d), 86.6 (s), 75.3 (t), 16.2 (q). HRMS (EI): calc. for $C_{11}H_{10}N_2OS_2$ (M^+) m/z 250.0235, found 250.0236; MS (EI) m/z (% relative int.): 250 [M^+] (84), 235 (13), 203 (38), 177 (100), 145 (32), 117 (17), 87 (44). ν_{max}(KBr)/cm^{-1}: 3310, 2917, 1699, 1614, 1484, 1319, 1078, 938, 888, 748.

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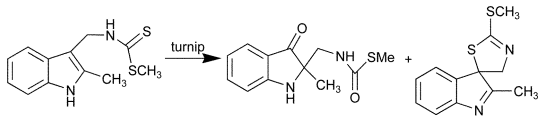

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