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Synthesis of spiroindoline phytoalexin (*S*)-(−)-spirobrassinin and its unnatural (*R*)-(+)-enantiomer

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ABSTRACT

The stereoselective syntheses of the cruciferous indole phytoalexin (*S*)-(−)-spirobrassinin and its unnatural (*R*)-(+)-enantiomer were achieved by bromine-induced spirocyclization of (−)- and (+)-1-(8-phenylmenthoxycarbonyl)brassinin in the presence of water to give the corresponding spirobrasinol derivatives, followed by oxidation to the derivatives of spirobrassinin and finally removal of the chiral auxiliary.

1. Introduction

Phytoalexins include a chemically diverse group of low molecular weight secondary metabolites biosynthesized de novo by plants as a defence response to several forms of stress (microbes, UV light, heavy metal salt). They are not present in naturally healthy plant tissues.[1,2] Cruciferous phytoalexins represent a particular group of these stress metabolites specifically produced by economically and dietary important plants of the family Cruciferae (syn. Brassicaceae), cultivated worldwide (e.g., cabbage, turnip, Chinese cabbage, Japanese radish, wasabi, broccoli, rapeseed and arabidopsis). The phytoalexins of crucifers are indole alkaloids derived from (*S*)-tryptophan, most of which contain a sulfur atom derived from cysteine.[2,3] Amongst the cruciferous phytoalexins, several spiroindoline[3,5′] thiazolidine-type compounds have been described including (*S*)-(−)-spirobrassinin [(−)-**1**, from Japanese radish (*Raphanus sativus*)],[4] (*R*)-(+)-1-methoxyspirobrassinin [(+)-**2**, from kohlrabi (*Brassica oleracea* var. *gongylodes*)],[5] 1-methoxyspirobrassinol (**3**) and *trans*-(2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether [(−)-**4**, from Japanese radish (*R. sativus*), Fig. 1].[6]

Racemic (±)-spirobrassinin [(±)-**1**] was first synthesized by treatment of (±)-dioxibrassinin (**5**) with either thionyl chloride or methanesulfonyl chloride. Synthetic (±)-spirobrassinin [(±)-**1**] was enantioresolved by (*S*)-(−)-phenylethyl isocyanate giving natural (*S*)-(−)-**1** and (*R*)-(+)-**1**. The absolute configuration of (*S*)-(−)-spirobrassinin [(−)-**1**] was determined by calculated electronic CD methods and X-ray crystallographic analysis after derivatization with (−)-camphanoyl chloride.[7a,b] A one-pot synthesis of (±)-spirobrassinin [(±)-**1**] was achieved by direct oxidative cyclization of brassinin (**6**) using CrO_3.[7c] The first enantioselective synthesis of (*S*)-(−)-spirobrassinin [(−)-**1**] is based on a MsCl-mediated cyclization of unnatural (*R*)-(+)-dioxibrassinin [(+)-**5**] prepared by a catalytic asymmetric Henry reaction of isatin with nitromethane,

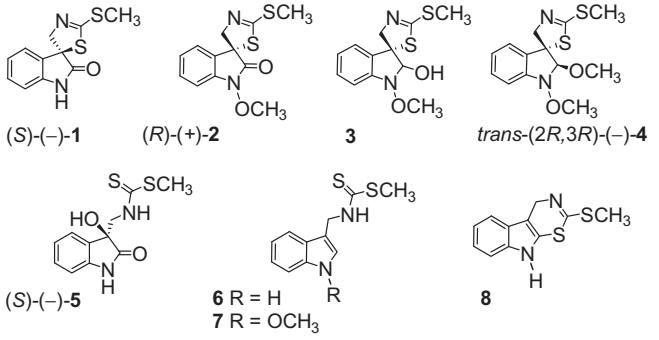

Fig. 1. Indole phytoalexins.

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followed by hydrogenation and reaction with carbon disulfide and methyl iodide.[7d,e] (±)-1-Methoxyspirobrassinol [(±)-**3**] and (±)-1-methoxyspirobrassinol methyl ether [(±)-**4**] were synthesized by a dioxane dibromide-mediated cyclization of 1-methoxybrassinin (**7**) in dioxane in the presence of water or methanol as a nucleophile.[8a] The racemate of (±)-1-methoxyspirobrassinin [(±)-**2**] was obtained by oxidation of (±)-**3** and (±)-**4**.[8b] The stereoselective syntheses of naturally occurring (*R*)-(+)-1-methoxyspirobrassinin [(+)-**2**] and its unnatural (*S*)-(−)-enantiomer (−)-**2** were achieved by spirocyclization of 1-methoxybrassinin (**7**) using (−)- and (+)-menthol as a nucleophile and subsequent oxidation of the obtained menthyl ethers. TFA-catalyzed methanolysis of chiral 1-methoxyspirobrassinol menthyl ethers afforded *trans*-(2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether [(2*R*,3*R*)-(−)-**4**] and its (2*S*,3*S*)-, (2*S*,3*R*)-, (2*R*,3*S*)-isomers.[8c] The phytoalexin spirobrassinin (**1**) is known to exhibit the unusual phenomenon of enantiomeric self-disproportionation on achiral-phase chromatography (ESDAC) whereby the enantiomeric composition of a non-racemic mixture varies across the eluting chromatographic peak. This behaviour is rationalized by the formation of two different complexes, both of which are in dynamic equilibrium with the free molecules.[7a,b] Complexation was also evident by NMR spectroscopy including the observation of distinct NMR signals for each enantiomer in the non-racemic mixture—the phenomenon of self-induced diastereomeric anisochromism (SIDA).[9] The ^{1}H, ^{13}C and ^{15}N spectroscopic enantioresolution of chiral phytoalexins spirobrassinin (**1**), 1-methoxyspirobrassinin (**2**) and 1-methoxyspirobrassinol methyl ether (**4**) was induced by application of the chiral solvating agents [2,2,2-trifluoro-1-(9-anthryl)ethanol, (*R*)-(+)-1,1′-bi-2-naphthol, (*R*)-(−)-1-phenyl-2,2,2-trifluoroethanol] in benzene.[10,11]

In addition to their moderate antimicrobial activities,[3] indole phytoalexins from cruciferous plants display significant anticancer activity. Brassinin (**6**), cyclobrassinin (**8**) and (±)-spirobrassinin [(±)-**1**] have been shown to exhibit cancer chemopreventive activity in 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced precancerous lesions in mouse mammary gland organ culture.[12] Various indole phytoalexins demonstrate in vitro cytostatic/cytotoxic effects against cultured human solid tumour and leukaemia cell lines.[13,14] Indole phytoalexins produced by cruciferous plants show significant antiproliferative effects on intracellular amastigotes of *Trypanosoma cruzi* and may be prospective candidates for antichagasic drug design.[15]

2. Results and discussion

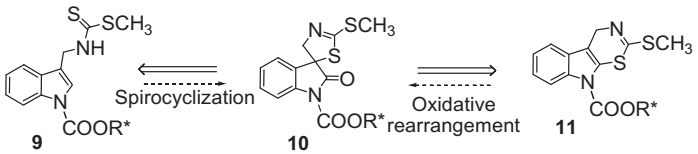
In the present paper we report a new stereoselective approach to the spirooxindole phytoalexin (*S*)-(−)-spirobrassinin [(−)-**1**] as well as its unnatural enantiomer (*R*)-(+)-spirobrassinin [(+)-**1**]. For this purpose we investigated the stereoselectivity of spirocyclization of brassinin derivatives possessing chiral groups on the indole nitrogen (**9**, Scheme 1). Our further aim was the study of the oxidative rearrangement of chiral 9-substituted derivatives of cyclobrassinin **11** in the stereoselective synthesis of spirobrassinin (**1**). The spirocyclization was performed with bromine in dichloromethane in the presence of water as a nucleophile. It was assumed that one of the four possible diastereoisomers of chiral derivatives of spirobrassinol would be the major product. Oxidation with pyridinium chlorochromate (PCC) afforded diastereoisomers of 1-substituted spirobrassinin **10** and removal of the chiral group by treatment with sodium methoxide provided the enantiomers of (−)-**1** and (+)-**1**. Oxidative rearrangement of 9-substituted derivatives of cyclobrassinin **11** to 1-substituted spirobrassinin **10** was achieved by treatment with oxone and sodium chloride (Scheme 1).

For the experiments we selected the (1*R*,2*S*,5*R*)-menthoxycarbonyl, (1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl, (1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl, (1*S*)-phenylethoxycarbonyl and (1*S*)-*endo*-borneoxycarbonyl group as a chiral auxiliary.

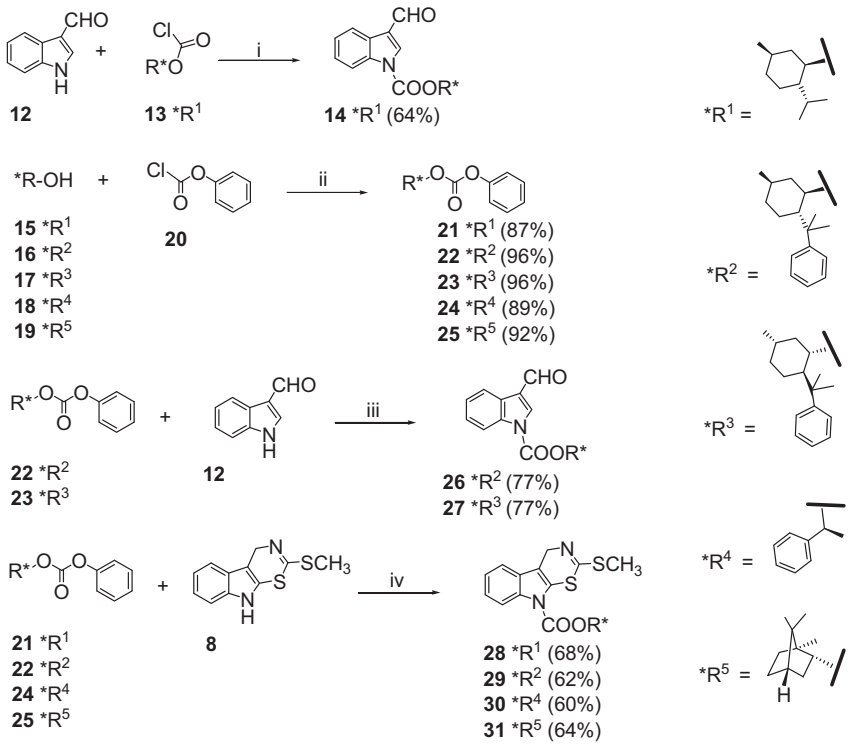
Indole-3-carboxaldehyde (**12**), which was used as a starting compound, was transformed by reaction with (1*R*,2*S*,5*R*)-(−)-menthyl chloroformate (**13**) in tetrahydrofuran in the presence of triethylamine to give 1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl] indole-3-carboxaldehyde (**14**) (Scheme 2). The starting point in the synthesis of 1-[(1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl]indole-3-carboxaldehyde (**26**) was to develop a method for attaching a (1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl group onto the indole nitrogen without working with toxic phosgene or triphosgene. An effective and simple solution was to acylate the indole-3-carboxaldehyde (**12**) with chiral carbonate **22**, prepared by the reaction of (1*R*,2*S*,5*R*)-(−)-8-phenylmenthol (**16**) with phenyl chloroformate (**20**) in pyridine according to the procedure published for (1*R*,2*S*,5*R*)-(−)-menthol (**15**).[16] [(1*S*,2*R*,5*S*)-8-Phenylmenthyl]phenyl carbonate (**23**) was synthesized from (1*S*,2*R*,5*S*)-8-(+)-phenylmenthol (**17**) (Scheme 2) in an analogous way. Reaction of chiral alcohol (1*S*)-(−)-1-phenylethanol (**18**) and [(1*S*)-*endo*]-(−)-borneol (**19**) with phenyl chloroformate (**20**) in pyridine afforded carbonates **24** and **25**. Aldehyde **26** and its enantiomer **27** were prepared in good yields by the reaction of indole-3-carboxaldehyde (**12**) with carbonate **22** or **23** using sodium hydride. The required starting chiral compounds **28**–**31** were obtained by acylation of cyclobrassinin (**8**) with carbonates **21**–**25** using sodium hydride in acetonitrile.

The key intermediates, chiral derivatives of brassinins **38**–**40**, were prepared by the modified procedure used previously for 1-Boc-brassinin.[17] Treatment of aldehydes **14**, **26** and **27** with hydroxylamine hydrochloride provided the corresponding oximes **32**–**34** as mixtures of *Z*- and *E*-isomers in high yields. The reduction of oximes **32**–**34** with sodium borohydride in the presence of catalytic nickel boride afforded the unstable amines **35**–**37**, which decomposed within a minute to unidentified material. Therefore no useful spectra of these compounds could be recorded and they were used as crude products immediately after isolation. Treatment of amines **35**–**37** with CS_2 and CH_3I in methanol in the presence of triethylamine afforded 1-substituted derivatives of brassinin **38**–**40** in 51% yield (Scheme 3).

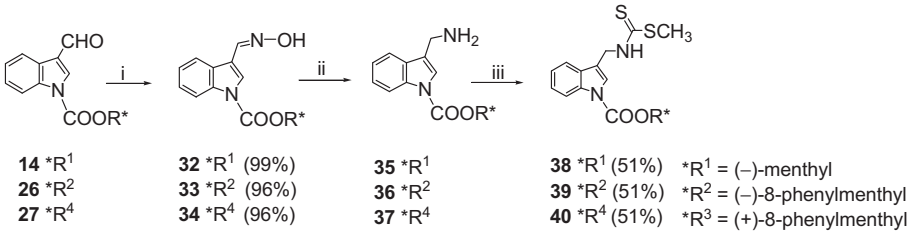
With **38**–**40** in hand, the stereoselectivity of the key bromospirocyclization was investigated. 1-[(1*R*,2*S*,5*R*)-Menthoxycarbonyl] brassinin (**38**) was subjected to the conditions of the spirocyclization in dichloromethane using bromine in the presence of water as the nucleophile (Scheme 4). The result of this reaction was the formation of trans-diastereoisomers **41a** and **41b** in 51% yield in a 50:50 ratio. cis-Diastereoisomers **41c** and **41d** were isolated as minor products in 3% yield (Table 1).



Scheme 1.



Scheme 2.



Scheme 3.

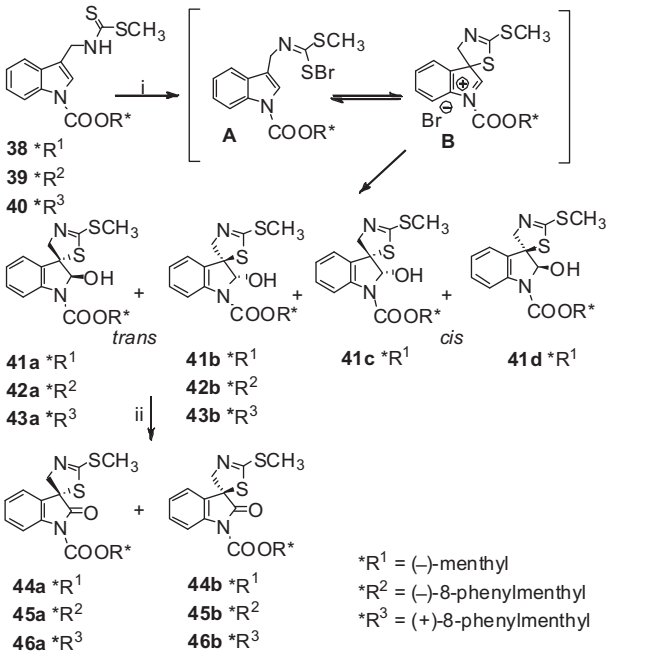
Above-mentioned findings are in accordance with the previous spirocyclization reactions of 1-Boc-, 1-acetyl-, 1-benzoyl- and 1-methoxycarbonylbrassinin using bromine in the presence of water that always afforded trans-diastereoisomers as the major products.[8a,18]

The trans-diastereoisomer is regarded as the one in which the sulfur of the thiazoline ring and the hydroxy group in position-2 are located on the opposite sides of indoline ring, while the cis-diastereoisomer has the sulfur and 2-hydroxy group on the same side of indoline ring.

trans-Diastereoisomers **41a** and **41b** were not separable therefore their NMR spectrum was recorded as a mixture. The ^{1}H NMR spectra of **41a** and **41b** were almost identical except for a 0.01 ppm difference in the shifts of the H_a protons of the thiazoline ring. The structures of the diastereoisomers were determined by NMR spectroscopic methods, including 2D HSQC, HMBC and NOESY experiments (Fig. 2). Products **41c** and **41d** exhibited cross peaks between the H-2 and H_b protons in the NOESY spectra providing evidence to suggest they were the cis-diastereoisomers. In the case of **41a** and **41b** the NOESY spectrum did not show the interaction between the H_b and OH protons required to confirming their trans-configuration. The interaction between the H_b and H-2 protons was also not observed. Thus, the structure of trans-diastereoisomers was assigned to compounds **41a** and **41b**.

Examination of the ^{1}H NMR spectra of spiroindoline[3,5′]thiazolidine-type compounds revealed a significant difference in the chemical shifts between the H-2 protons of trans- and cis-diastereoisomers. In all cases the $\delta(\text{H-2})_{\text{trans}}$ appeared at lower field compared to $\delta(\text{H-2})_{\text{cis}}$.[8a,19] This relation is also observed for trans-diastereoisomers **41a** and **41b** and cis-diastereoisomers **41c** and **41d**. Diastereoisomers **41a** and **41b** show a chemical shift for the H-2 proton at 5.97 ppm and so the trans-configuration was assigned, whilst the cis-diastereoisomers **41c** and **41d** show a signal for the H-2 proton at 5.65 ppm.

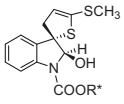
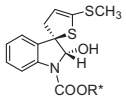
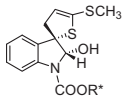
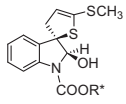
For the synthesis of the target spirobrassinin (**1**), trans-diastereoisomers **41a** and **41b**, as major products, were used.



Scheme 4.

Their oxidation using Jones reagent afforded the mixture of diastereoisomers **44a** and **44b** in a moderate 47% yield. With the aim of improving the yield of oxidation, Jones reagent was substituted with PCC, which increased the yield to 71%. The ratio of

Table 1
Stereochemistry, ratio and yields of diastereoisomers 1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl] (**41**), 1-[(1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl] (**42**) and 1-[(1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl]spirobrassinol (**43**)

*R	Compound/ratio[a] (%)/yield (%)			
				
	trans		cis	
*R1	**41a**	**41b**	**41c**	**41d**
	40[b]	40[b]	10[b]	10[b]
	30[c]	58[c]	4[c]	8[c]
	51[d]		3[d]	
*R2	**42a**	**42b**	nd	
	50[e]	50[e]		
	63[f]			
*R3	**43a**	**43b**	nd	
	50[e]	50[e]		
	63[f]			

*R1=(−)-menthyl.
*R2=(−)-8-phenylmenthyl.
*R3=(+)-8-phenylmenthyl.
[a] The ratio of diastereoisomers **41a**–**41d** was determined by deconvolution of the partially overlapping signals of the H_a protons for **41a** and **41b** and the H_b protons for **41c** and **41d** in the ^{1}H NMR spectrum of the crude mixture. The ratio of products **42a**,**42b** and **43a**,**43b** was determined by integration of the non-overlapping signals of the H-2 protons in the ^{1}H NMR spectrum of the crude product mixture.
[b] Ratio of diastereoisomers **41a**–**41d** obtained by spirocyclization of **38** at room temperature.
[c] Ratio of diastereoisomers **41a**–**41d** obtained by spirocyclization of **38** at −70 °C.
[d] Yields of the mixture of trans-isomers **41a**, **41b** and cis-isomers **41c**, **41d**.
[e] Ratio of diastereoisomers **42a**, **42b** and **43a**, **43b** obtained by spirocyclization of **39** and **40** at room temperature.
[f] Yields of the mixture of trans-isomers **42a**, **42b** and trans-isomers **43a**, **43b**.

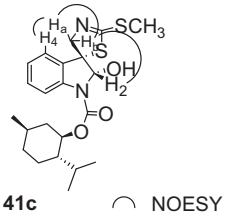
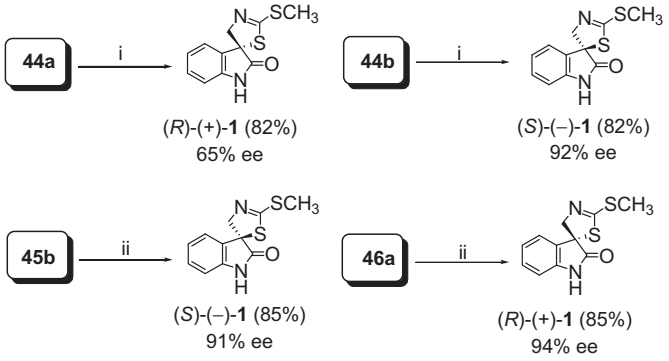


Fig. 2. NOESY correlations of compound **41c**.

diastereoisomers **44a** and **44b** was 50:50, which was determined by deconvolution of the partially overlapping signals of the H_b protons in the ^{1}H NMR spectrum of the crude mixture using MestReNova software.[20] The ^{1}H NMR spectra of **44a** and **44b** were almost identical except for a 0.01 ppm difference in the shifts of the H_b protons of the thiazoline ring. Diastereoisomers **44a** and **44b** showed very close R_f values in various eluents, which prevented their separation by column chromatography. Diastereoisomer **44b** was separated by crystallization from a solution of dichloromethane/*n*-hexane. The pure diastereoisomer **44b** was isolated as white crystals in 24% yield. Removal of the (1*R*,2*S*,5*R*)-menthoxycarbonyl group from pure diastereoisomer **44b** was achieved by treatment with sodium methoxide in methanol giving (*S*)-(−)-spirobrassinin [(*S*)-(−)-**1**] in 82% yield (Scheme 5). Its absolute



Scheme 5.

configuration was determined by direct comparison of the ECD spectrum with the published data of the natural product.[7a] The enantiomeric excess of the synthesized enantiomer (*S*)-(−)-**1**, determined by chiral HPLC, was 92%. Diastereoisomer **44a** was obtained by evaporation of the solvent from the filtrate after filtration of crystals of diastereoisomer **44b**. Deprotection resulted in formation of (*R*)-(+)-spirobrassinin [(*R*)-(+)-**1**]. Most major ECD signals showed opposing signs in comparison to ECD signals of (*S*)-(−)-**1** obtained from **44b**. The optical purity of *R*-enantiomer (*R*)-(+)-**1** was 65% ee (Scheme 5).

With the aim to improve the stereoselectivity, spirocyclization of compound **38** was performed at lower temperatures from −110 °C up to −70 °C leading to a moderate increase in the stereoselectivity. The ratio of diastereoisomers **44a** and **44b** obtained after subsequent oxidation of crude product **41** with PCC is listed in Table 2.

Low diastereoselectivity in synthesis of derivatives **41a** and **41b** prompted us to examine the impact of the (1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl group on the cyclization of derivate **39**. We expected the π stacking effect to lock reactant **39** preferentially in one conformation.[21]

Table 2
Influence of temperature on the stereoselectivity of spirocyclization of brassinin derivatives **38**–**40**

Solvent	Temperature of spirocyclization	Ratio		
		44a/**44b**[a]	**45a**/**45b**[b]	**46a**/**46b**[b]
CH_2Cl_2	rt	50:50	50:50	50:50
THF	rt	48:52	50:50	—
Et_2O	rt	48:52	50:50	—
CH_2Cl_2	−20 °C	—	40:60	60:40
CH_2Cl_2	−70 °C	36:64	27:73	—
CH_2Cl_2	−90 °C	36:64	18:82	82:18
THF	−100 °C	42:58	25:75	—
Et_2O	−110 °C	42:58	12:88	86:14

[a] The ratio of diastereoisomers **44a** and **44b** was determined by deconvolution of the partially overlapping signals of the H_b protons in the ^{1}H NMR spectrum of the crude mixture.
[b] The ratio of products **45a**/**45b** and **46a**/**46b** was determined by integration of the non-overlapping signals of the H-7 protons in the ^{1}H NMR spectrum of the crude product mixture.

Spirocyclization of 1-[(1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl] brassinin (**39**) using bromine in the presence of water at room temperature resulted in the formation of trans-diastereoisomers **42a** and **42b** in 63% yield that were not separable (Table 1). Oxidation of their mixture with PCC provided (1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl derivatives of spirobrassinin **45a** and **45b** in a 50:50 ratio isolated in 34% and 32% yields after chromatography on silica gel (Scheme 4). Performing spirocyclization at lower temperatures led to preferential formation of diastereoisomer **45b** (Table 2). Thus spirocyclization in dichloromethane at −90 °C and subsequent oxidation gave diastereoisomer **45b** in 54% yield. The ratio of products **45a** and **45b** was determined by integration of the non-overlapping signals of the H-7 protons in the ^{1}H NMR spectrum of the crude product mixture. With the pure diastereoisomer **45b** in hand, the *S*-enantiomer of indole phytoalexin spirobrassinin [(*S*)-(−)-**1**] was synthesized by removing the chiral group with sodium methoxide (Scheme 5). The absolute configuration of the enantiomer was identified by comparison of its ECD spectrum with the published data.[7a] The enantiomeric excess of the prepared (*S*)-enantiomer was 91%.

Cyclization of 1-[(1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl]brassinin (**40**) in dichloromethane using bromine in the presence of water at room temperature afforded trans-diastereoisomers **43a** and **43b** in 63% yield also as a non-separable mixture (Scheme 4). Subsequent oxidation of this mixture with PCC led to epimers **46a** and **46b** in a 50:50 ratio. As can be seen from Table 2, the spirocyclization of **40** at the lower reaction temperatures led to the preferential formation of diastereoisomer **46a**. Spirocyclization performed at −90 °C in dichloromethane and subsequent oxidation afforded diastereoisomer **46a** as the major product in 54% yield. (*R*)-(+)-Spirobrassinin [(*R*)-(+)-**1**] was obtained after removal of the (1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl group from diastereoisomer **46a** with sodium methoxide in 85% yield (Scheme 5). The ECD spectrum of prepared (*R*)-(+)-spirobrassinin [(*R*)-(+)-**1**] was the mirror image of the ECD spectrum of (*S*)-(−)-spirobrassinin [(−)-**1**] obtained from **45b**.

For the explanation of the stereoselectivity of the spirocyclization, the conformational analysis of brassinin **40** within (1*S*,2*R*,5*S*)-8-phenylmenthoxy group was done using DFT methods at the B3LYP 6-31** level in the gas-phase using the JAGUAR program.[22] In Fig. 3 there are depicted structures of the five most stable conformations represented by their relative energies (kJ/mol) and the percentage of their abundance (Table 3). Conformers C1–C5 differ in the orientation of the (1*S*,2*R*,5*S*)-8-phenylmenthoxy group and indole core. The energy of conformer C5 is lower by more than 4 kJ/mol relative to the energy of other unentered conformers. In the most stable conformer C1, as well as in the conformer C3, the phenyl nucleus of the (1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl group spatially restricts the access to the C-3 atom of indole moiety from the *Re* face. In the case of conformers C2 and C4, neither the *Re* nor the *Si* face is shielded. Conformation C5 has more restraints *Si* face. The spatial arrangement of the energetically favourable conformers C1 and C3, in comparison with conformer C5, probably allows effective interaction of the O=C–O group with the phenyl core (π-stacking).[21] Assuming equal reactivity of individual conformers in the spirocyclization reaction, our experimentally found stereoselectivity supports the prediction that the dominant product will be (3*R*)-diastereoisomer of 1-[(1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl]spirobrassinol (**43a**) as a result of sulfur atom attacking from the *Si* face.

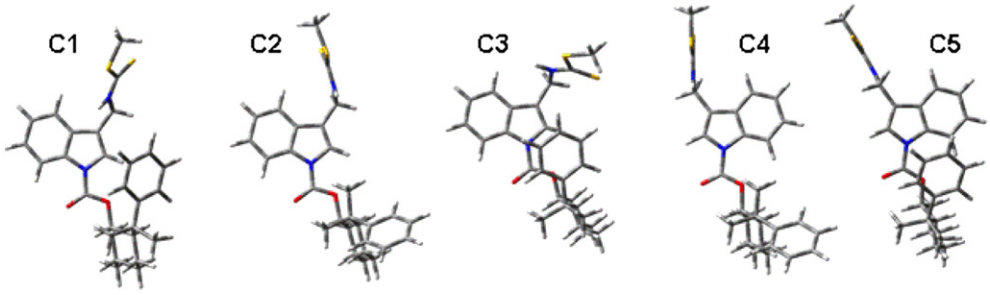


Fig. 3. Molecular geometries of the most stable conformers C1–C5 of derivate **40** resulting from DFT B3LYP 6-31** minimalization.

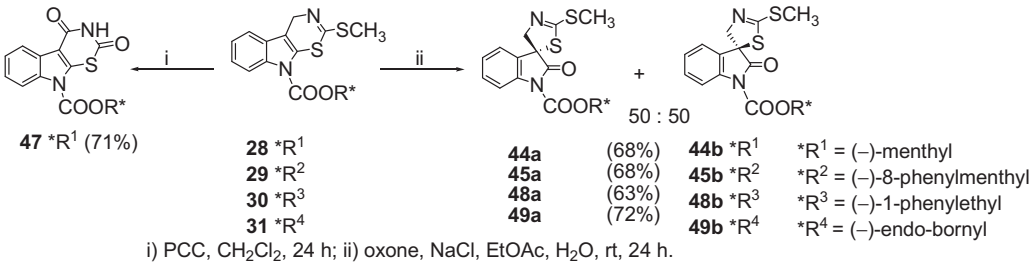
Table 3
Relative energies and the percentage abundance of the five most stable conformers C1–C5 of 1-[(1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl]brassinin (**40**)

Conformer	C1	C2	C3	C4	C5
Δ*E* (kJ/mol)	0.00	5.19	6.82	7.95	8.37
Percentage of abundance (%)[a]	79.5	9.7	5.1	3.1	2.6

[a] The variety of conformations was accounted for by population-weight averaged assessment of energies.[23]

Oxidative rearrangement using halogenated electrophilic agents (*N*-bromosuccinimide, *tert*-butyl hypochlorite) or epoxidation with dimethyldioxirane allows the conversion of condensed indole derivatives in to spirooxindoles.[24a–c] This fact led us to study the epoxidation and oxidative rearrangement of 9-substituted derivatives of cyclobrasinin **28**–**31** in the stereoselective synthesis of spirobrassinin (**1**). Chiral derivatives of cruciferous phytoalexins cyclobrassinin **28**–**31** were selected as starting compounds with the aim of studying the stereoselectivity of the oxidative rearrangement. The oxidative rearrangement was explored with chiral cyclobrassinin

28. The use of several oxidants (*N*-bromosuccinimide, *N*-chlorosuccinimide, dioxane dibromide, H_2O_2, *tert*-butyl hydroperoxide, *m*-chloroperoxybenzoic acid, dimethyldioxirane) did not afford 1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]spirobrassinin (**44**). The use of PCC, CrO_3, 2-iodoxybenzoic acid or Davis oxaziridine resulted in the oxidation of the CH_2 and SCH_3 groups leading to the formation of 9-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]-2*H*-3,4-dihydro-[1,3]thiazino[6,5-*b*]indole-2,4-dione (**47**) (Scheme 6). Conversion of chiral cyclobrassinins **28**–**31** into the desired spirobrassinin derivatives **44**, **46**, **48** and **49** was achieved only by oxidative chlorination using oxone and sodium chloride, however, the reaction proceeded with no stereoselectivity. The diastereoselectivity of the rearrangement was not influenced probably due to the big distance between the reaction centre and the chiral auxiliary on the indole nitrogen.



Scheme 6.

3. Conclusion

In summary, we have developed a stereoselective approach to the cruciferous spirooxindole phytoalexin (*S*)-(−)-spirobrassinin [(*S*)-(−)-**1**] and its unnatural (*R*)-(+)-enantiomer [(*R*)-(+)-**1**]. The key step of *the* synthesis involves a bromine-mediated cyclization of chiral (−)-8-phenylmenthoxycarbonyl or (+)-8-phenylmenthoxycarbonyl derivatives of brassinin in the presence of water with the formation of non-separable diastereoisomers of chiral derivatives of spirobrassinol. Spirocyclization of **39** or **40** performed in dichloromethane at −90 °C °C followed by oxidation with PCC provided diastereoisomers **45a**,**45b** or **46a**,**46b** in 66% yield and 82:18 diastereomeric ratio (dr). Removal of the chiral auxiliary afforded (*S*)-(−)-spirobrassinin [(*S*)-(−)-**1**] and its unnatural (*R*)-(+)-enantiomer [(*R*)-(+)-**1**] in 10% overall yield, with enantiomeric excesses of 91% and 94%, respectively.

4. Experimental

4.1. Chemistry

Melting points were determined on a Koffler micro melting point apparatus and are uncorrected. IR spectra were recorded on an IR-75 spectrometer (Zess Jena). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on a Varian Mercury Plus spectrometer. Chemical shifts (δ) are reported in parts per million downfield from TMS as internal standard and coupling constants (J) are given in hertz. Microanalyses were performed with a Perkin–Elmer, Model 2400 analyzer. The EI mass spectra were recorded on a GS-MS Trio 1000 (Fisons Instruments) spectrometer at an ionization energy of 70 eV, whereas MALDI-TOF mass spectra of **14**, **32**, **41** and **44** were measured on a MALDI IV (Shimazu, Kratos Analytical). The samples were ionized with a N_2-laser (λ=337 nm). The progress of chemical reactions was monitoring by thin layer chromatography, using Macherey-Nagel plates Alugram® Sil G/UV254. Preparative column chromatography was performed on Kieselgel 60 Merck Type 9385 (0.040–0.063). Optical rotations were measured at room temperature in a 10 cm cell with a polarimeter JASCO P-2000 and P3002 (Kreuss) at the sodium D-line. CD spectra were obtained in a 1 mm quartz cell on a JASCO J-810 spectrometer. The enantiomeric excess of synthesized enantiomers was determined by analytical chiral HPLC, using a CHIROBIOTIC T (Astec, 25×0.46 cm ID) column, using the eluent *n*-hexane/ethanol (8:2) at flow rate 0.6 mL/min. (1*R*,2*S*,5*R*)-(−)-Menthyl chloroformate (ee>95.0%, Fluka), (1*R*,2*S*,5*R*)-(−)-menthol (ee 96%, Aldrich), (1*R*,2*S*,5*R*)-(−)-8-phenylmenthol (ee>98%, Fluka), (1*S*,2*R*,5*S*)-(+)-8-phenylmenthol (ee>98%, Fluka), (1*S*)-(−)-1-phenylethanol (ee>98,5%, Fluka) and [(1*S*)-*endo*]-(−)-borneol (ee 98%, Aldrich) were used as received.

*4.1.1. 1-[(1R,2S,5R)-Menthoxycarbonyl]indole-3-carboxaldehyde (**14**).* To a solution of indole-3-carboxaldehyde (**12**) (1.45 g, 10.0 mmol) in THF (40 mL) at 0 °C was added triethylamine (1.06 g, 1.46 mL, 10.5 mmol). The reaction mixture was stirred at 0 °C for 10 min. After that (1*R*,2*S*,5*R*)-(−)-menthyl chloroformate (**13**) (2.30 g, 2.23 mL, 10.5 mmol) was added and the reaction mixture was stirred at 0 °C for 20 min and for 30 min at room temperature. After the reaction was finished, THF was evaporated. The residue obtained after evaporation of the solvent was subjected to column chromatography (15 g silica gel, cyclohexane/ethyl acetate 8:1). The obtained compound was further crystallized from hot ethanol to afford aldehyde **14**. Yield: 2.11 g (64%), white crystals, mp 62–65 °C (ethanol), $[\alpha]_D^{20}$ −10.0 (*c* 0.70, $CHCl_3$), R_f 0.56 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{20}H_{25}NO_3$ requires: C, 73.37; H, 7.70; N, 4.28. Found: C, 73.01; H, 7.98; N, 4.56. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.12 (s, 1H, CHO), 8.30 (dd, 1H, *J* 7.7, 0.6, H-4), 8.27 (s, 1H, H-2), 8.18 (d, 1H, *J* 8.1, H-7), 7.46–7.36 (m, 2H, H-6, H-5), 5.01 (dt, 1H, *J* 10.9, 4.2, H-1′), 2.26–2.22 (m, 1H, H-8′), 2.04–1.97 (m, 1H, H-6′), 1.83–1.75 (m, 2H, H-3′, H-4′), 1.69–1.54 (m, 2H, H-2′, H-5′), 1.28–0.94 (m, 3H, H-3′, H-4′, H-6′), 0.97 (d, 3H, *J* 6.6), 0.96 (d, 3H, *J* 6.9) [H-9′, H-10′], 0.84 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.8 (CHO), 150.0 (C=O), 136.1 (C-2), 136.0 (C-3a), 126.3 (C-5), 126.1 (C-7a), 124.8 (C-6), 122.2 (C-4), 122.0 (C-3), 115.2 (C-7), 79.3 (C-1′), 47.3 (C-2′), 40.9 (C-6′), 34.0 (C-4′), 31.5 (C-5′), 26.5 (C-8′), 23.4 (C-3′), 22.0, 20.8 (C-9′, C-10′), 16.4 (C-7′). IR ($CHCl_3$) ν_{max} 2960, 2927, 2880, 1740 and 1667 (C=O), 1540, 1447, 1347, 1213, 1093, 946 cm^{1}. MS (MALDI-TOF), *m*/*z* (%): 350.72 $[M+Na]^+$ (17), 328.02 $[M+H]^+$ (41).

*4.1.2. General procedure for the synthesis of carbonates **21**–**25**.* To a solution of chiral alcohol **15**–**19** (4.30 mmol) in dry pyridine (1 mL) cooled in an ice-bath, phenyl chloroformate (**20**) (0.741 g, 0.600 mL, 4.73 mmol) was added slowly with vigorous stirring. A white precipitate was formed. The mixture was stirred at room temperature for 12 h and then was poured into water (50 mL). The

product was extracted with diethyl ether (1×50 mL and 2×25 mL). The extract was washed with 10% aqueous citric acid (2×10 mL), aqueous sodium hydrogen carbonate (1×10 mL) and finally water (1×10 mL). The extract was dried over Na_2SO_4 and the solvent was evaporated.

*4.1.2.1. [(1R,2S,5R)-Menthyl]phenyl carbonate (**21**).*[16] Following the general procedure, product **21** was obtained using (1*R*,2*S*,5*R*)-(−)-menthol (**15**) (0.675 g, 4.30 mmol). Yield: 1.03 g (87%), lit.[16] 95%, colourless oil, R_f 0.88 (*n*-hexane/ethyl acetate 5:1). Spectroscopic data were consistent with those reported in the literature.[16]

*4.1.2.2. [(1R,2S,5R)-8-Phenylmenthyl]phenyl carbonate (**22**).* Following the general procedure, product **22** was obtained using (1*R*,2*S*,5*R*)-(−)-8-phenylmenthol (**16**) (1.00 g, 4.30 mmol). Yield: 1.46 g (96%), colourless oil, $[\alpha]_D^{20}$ −63.4 (*c* 0.81, $CHCl_3$), R_f 0.81 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{23}H_{28}O_3$ requires: C, 78.38; H, 8.01. Found: C, 78.61; H, 7.83. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.09 (m, 10H, H-arom), 4.72 (dt, 1H, *J* 10.7, 4.5, H-1′), 2.06–2.00 (m, 2H, H-2′, H-6′), 1.65–1.61 (m, 2H, H-3′, H-4′), 1.50–1.46 (m, 1H, H-5′), 1.41 (s, 3H), 1.32 (s, 3H) [H-9′, H-10′], 1.25–0.81 (m, 3H, H-3′, H-4′, H-6′), 0.89 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9 (C=O), 151.4, 150.9, 129.5, 128.3, 125.9, 125.7, 125.6, 121.3 (C-arom), 79.8 (C-1′), 51.0 (C-2′), 41.8 (C-6′), 40.2 (C-8′), 34.6 (C-4′), 31.6 (C-5′), 27.0, 26.9 (C-9′, C-10′), 26.8 (C-3′), 21.9 (C-7′). IR ($CHCl_3$) ν_{max} 2953, 1733 (C=O), 1227 cm^1. MS (EI), *m*/*z* (%): 216 (38), 214 (64), 119 (100), 105 (40), 91 (46), 77 (29).

*4.1.2.3. [(1S,2R,5S)-8-Phenylmenthyl]phenyl carbonate (**23**).* Following the general procedure, product **23** was obtained using (1*S*,2*R*,5*S*)-(+)-8-phenylmenthol (**17**) (1.00 g, 4.30 mmol). Yield: 1.46 g (96%), colourless oil, $[\alpha]_D^{20}$ +59.8 (*c* 0.56, $CHCl_3$), R_f 0.81 (*n*-hexane/ethyl acetate 5:1). The spectral data were fully identical with those of enantiomeric product **22**.

*4.1.2.4. [(1S)-Phenylethyl]phenyl carbonate (**24**).* Following the general procedure, product **24** was obtained using (1*S*)-(−)-1-phenylethanol (**18**) (0.525 g. 0.460 mL, 4.30 mmol). Yield: 0.927 g (89%), colourless oil, $[\alpha]_D^{20}$ −78.6 (*c* 0.31, $CHCl_3$), R_f 0.64 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{15}H_{14}O_3$ requires: C, 74.36; H, 5.82. Found: C, 74.02; H, 5.46. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.14 (m, 10H, H-arom), 5.82 (quartet, 1H, *J* 6.6, CH), 1.67 (d, 3H, *J* 6.6, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.3 (C=O), 151.2, 140.8, 129.8, 129.6, 128.8, 128.6, 126.8, 126.4, 126.2, 121.3, 121.2 (C-arom), 77.6 (CH), 22.5 (CH_3). IR ($CHCl_3$) ν_{max} 2967, 1760 (C=O), 1587, 1233 cm^1. MS (EI), *m*/*z* (%): 247 $[M]^+$ (1), 214 (45), 105 (100), 94 (16), 77 (97).

*4.1.2.5. [(1S)-endo-Bornyl]phenyl carbonate (**25**).* Following the general procedure, product **25** was obtained using [(1*S*)-*endo*]-(−)-borneol (**19**) (0.622 g, 4.30 mmol). Yield: 1.09 g (92%), colourless oil, $[\alpha]_D^{20}$ −14.4 (*c* 0.32, $CHCl_3$), R_f 0.8 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{17}H_{22}O_3$ requires: C, 74.42; H, 8.08. Found: C, 74.73; H, 7.74. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.35 (m, 2H, H-3″, H-5″), 7.29–7.17 (m, 3H, H-2″, H-6″, H-4″), 4.49 (dd, 1H, *J* 10.0, 5.4, H-2′), 2.46–2.38 (m, 1H, H-3′), 2.01–1.95 (m, 1H, H-6′), 1.83–1.71 (m, 2H, H-5′, H-4′), 1.39–1.28 (m, 2H, H-6′, H-5′), 1.21–1.17 (m, 1H, H-3′), 0.93 (s, 3H), 0.92 (s, 3H) [H-8′, H-9′], 0.89 (s, 3H, H-10′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.2 (C=O), 151.4 (C-1″), 129.8, 129.6 (C-3″, C-5″), 126.1 (C-4″), 121.3 (C-2″, C-6″), 85.1 (C-2′), 49.2 (C-7′), 48.2 (C-1′), 44.9 (C-4′), 36.6 (C-3′), 28.2 (C-5′), 27.1 (C-6′), 19.9, 19.1 (C-8′, C-9′), 13.7 (C-10′). IR ($CHCl_3$) ν_{max} 2958, 2885, 1731 (C=O), 1591, 1265 cm^1. MS (EI), *m*/*z* (%): 274 $[M]^+$ (1), 137 (100), 95 (84).

*4.1.3. 1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]indole-3-carboxaldehyde (**26**) and 1-[(1S,2R,5S)-8-phenylmenthoxycarbonyl]indole-3-carboxaldehyde (**27**).* To a suspension of NaH (0.384 g, 9.60 mmol, 60% suspension in mineral oil) in dry acetonitrile (5 mL) was added indole-3-carboxaldehyde (**12**) (0.383 g, 2.64 mmol). After stirring for 10 min at room temperature, the mixture was cooled in an ice-bath and a solution of carbonate **22** or **23** (0.847 g, 2.40 mmol) in dry acetonitrile (5 mL) was added. The reaction mixture was stirred in an ice cooling bath for 1.5 h, then poured into cold water (200 mL) and the product was extracted with ethyl acetate (1×100 mL and 1×50 mL). The extract was dried over Na_2SO_4 and the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (30 g, cyclohexane/ethyl acetate 8:1). Data for **26**: yield: 0.742 g (77%), white crystals, mp 53–55 °C (ethanol), $[\alpha]_D^{20}$ −56.3 (*c* 0.46, $CHCl_3$), R_f 0.51 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{26}H_{29}NO_3$ requires: C, 77.39; H, 7.24; N, 3.47. Found: C, 77.53; H, 7.01; N, 3.64. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (s, 1H, CHO), 8.21–8.19 (m, 1H, H-7), 8.11 (d, 1H, *J* 7.2, H-4), 7.39–7.31 (m, 2H, H-5, H-6), 7.28–7.25 (m, 2H, H-2″, H-6″), 7.08–7.04 (m, 2H, H-3″, H-5″), 6.95–6.91 (m, 1H, H-4″), 6.73 (s, 1H, H-2), 5.08 (dt, 1H, *J* 10.7, 4.3, H-1′), 2.37–2.31 (m, 1H, H-2′), 2.09–2.06 (m, 2H, H-3′, H-6′), 1.84–1.80 (m, 1H, H-4′), 1.71–1.54 (m, 1H, H-5′), 1.36 (s, 3H), 1.21 (s, 3H) [H-9′, H-10′], 1.33–0.94 (m, 3H, H-3′, H-4′, H-6′), 0.94 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.1 (CHO), 152.5 (C-1″), 149.1 (C=O), 136.8 (C-2), 136.1 (C-3a), 128.4 (C-3″, C-5″), 126.1 (C-5), 125.9 (C-7a), 125.4 (C-4″), 125.1 (C-2″, C-6″), 124.8 (C-6), 122.1 (C-7), 121.3 (C-3), 115.2 (C-4), 78.8 (C-1′), 50.8 (C-2′), 41.8 (C-6′), 39.5 (C-8′), 34.7 (C-4′), 31.6 (C-5′), 31.1 (C-9′), 26.5 (C-3′), 21.9 (C-7′), 21.8 (C-10′). IR ($CHCl_3$) ν_{max} 2927, 1723 (C=O), 1673 (C=O), 1393, 1093 cm^1. MS (EI), *m*/*z* (%): 404 $[M+H]^+$ (11), 403 $[M]^+$ (28), 119 (94), 105 (100), 91 (62). Data for **27**: yield: 0.742 g (77%), white crystals, mp 53–55 °C (ethanol), $[\alpha]_D^{20}$ +54.3 (*c* 0.50, $CHCl_3$), R_f 0.66 (cyclohexane/ethyl acetate 8:1). The spectral data were fully identical with those of enantiomeric product **26**.

*4.1.4. General procedure for the preparation of 9-substituted derivatives of cyclobrassinin **28**–**31**.* To a suspension of NaH (0.176 g, 4.40 mmol, 60% suspension in mineral oil) in dry acetonitrile (12 mL) was added cyclobrassinin (**8**)[25] (0.264 g, 1.10 mmol). After stirring for 10 min at room temperature, the mixture was cooled in an ice-bath and a solution of carbonate **21**, **22**, **24** or **25** (1.00 mmol) in dry acetonitrile (12 mL) was added. The reaction mixture was stirred with cooling for 1.5 h, then poured into cold water (30 mL) and the product was extracted with ethyl acetate (1×30 mL and 1×20 mL). The extract was dried over Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on silica gel (50 g, cyclohexane/ethyl acetate 8:1).

*4.1.4.1. 9-[(1R,2S,5R)-Menthoxycarbonyl]cyclobrassinin (**28**).* Following the general procedure, product **28** was obtained using carbonate **21** (0.276 g, 1.00 mmol). Yield: 0.283 g (68%), light yellow oil, $[\alpha]_D^{20}$ −20.1 (*c* 0.24, $CHCl_3$), R_f 0.66 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{22}H_{28}N_2O_2S_2$ requires: C, 63.43; H, 6.77; N, 6.72. Found: C, 63.18; H, 7.10; N, 6.65. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 1H, *J* 7.6, H-8), 7.42–7.40 (m, 1H, H-5), 7.31–7.24 (m, 2H, H-7, H-6), 5.02 (s, 2H, CH_2), 4.91 (dt, 1H, *J* 10.9, 4.4, H-1′), 2.53 (s, 3H, SCH_3), 2.25–2.22 (m, 1H, H-8′), 2.11–2.00 (m, 1H, H-6′), 1.80–1.67 (m, 2H, H-3′, H-4′), 1.61–1.53 (m, 2H, H-2′, H-5′), 1.29–0.82 (m, 3H, H-3′, H-4′, H-6′), 0.97 (d, 3H, *J* 6.4), 0.96 (d, 3H, *J* 6.9) [H-9′, H-10′], 0.82 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5 (C=N), 150.9 (C=O), 136.3 (C-8a), 127.7 (C-4b), 124.3 (C-9a), 124.2 (C-7), 123.3 (C-6), 117.1 (C-5), 115.1 (C-8), 108.8 (C-4a), 78.8 (C-1′), 47.9 (CH_2), 47.1 (C-2′), 40.9 (C-6′), 34.0 (C-4′), 31.5 (C-5′), 26.2 (C-8′), 23.2 (C-3′), 21.9, 20.9 (C-9′, C-10′), 16.2 (C-7′), 15.1 (SCH_3). IR ($CHCl_3$) ν_{max} 2958, 2872, 1732 (C=O), 1664, 1541, 1317 cm^1. MS (EI), *m*/*z* (%): 416 $[M]^+$ (22), 161 (100).

*4.1.4.2. 9-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]cyclobrassinin (**29**).* Following the general procedure, product **29** was obtained

using carbonate **22** (0.352 g, 1.00 mmol). Yield: 0.305 g (62%), light yellow oil, $[\alpha]_D^{20}$ −21.2 (*c* 0.26, $CHCl_3$), R_f 0.73 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{28}H_{32}N_2O_2S_2$ requires: C, 68.26; H, 6.55; N, 5.69. Found: C, 68.01; H, 6.37; N, 5.90. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (br s, 1H, H-8), 7.36–7.34 (m, 1H, H-5), 7.22–7.20 (m, 4H, H-2″, H-6″, H-7, H-6), 6.90–6.86 (m, 2H, H-3″, H-5″), 6.69–6.61 (m, 1H, H-4″), 5.17 (dt, 1H, *J* 10.8, 4.7, H-1′), 5.15 (d, 1H, *J* 17.7, H_b), 4.77 (d, 1H, *J* 17.7, H_a), 2.54 (s, 3H, SCH_3), 2.25–2.22 (m, 1H, H-2′), 2.04–1.81 (m, 2H, H-3′, H-6′), 1.73–1.69 (m, 1H, H-4′), 1.56–1.47 (m, 1H, H-5′), 1.38 (s, 3H), 1.27 (s, 3H) [H-9′, H-10′], 1.33–0.84 (m, 3H, H-3′, H-6′, H-4′), 0.93 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3 (C=N), 150.7 (C=O), 150.3 (C-1″), 136.4 (C-8a), 127.9 (C-3″, C-5″), 127.7 (C-4b), 127.6 (C-9a), 125.3 (C-2″, C-6″), 124.9 (C-4″), 124.0 (C-7), 123.2 (C-6), 117.0 (C-5), 115.5 (C-8), 108.8 (C-4a), 78.3 (C-1′), 51.5 (C-2′), 48.0 (CH_2), 42.4 (C-6′), 39.9 (C-8′), 34.5 (C-4′), 31.7 (C-5′), 28.4 (C-9′), 26.8 (C-3′), 24.8 (C-10′), 21.9 (C-7′), 15.4 (SCH_3). IR ($CHCl_3$) ν_{max} 2960, 2856, 1720 (C=O), 1662, 1450, 1321 cm^{-1}. MS (EI), *m*/*z* (%): 492 $[M]^+$ (25), 161 (100).

*4.1.4.3. 9-[(1S)-Phenylethoxycarbonyl]cyclobrassinin (**30**).* Following the general procedure, product **30** was obtained using carbonate **24** (0.242 g, 1.00 mmol). Yield: 0.229 g (60%), light yellow oil, $[\alpha]_D^{20}$ −53.7 (*c* 0.17, $CHCl_3$), R_f 0.55 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{20}H_{18}N_2O_2S_2$ requires: C, 62.80; H, 4.74; N, 7.32. Found: C, 62.48; H, 4.98; N. 7.47. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, 1H, *J* 7.1, H-8), 7.50–7.48 (m, 2H, H-2′, H-6′), 7.43–7.29 (m, 4H, H-5, H-3′, H-5′, H-4′), 7.28–7.25 (m, 2H, H-7, H-6), 6.13 (quartet, 1H, *J* 6.6, CH), 5.00 (s, 2H, CH_2), 2.52 (s, 3H, SCH_3), 1.81 (d, 3H, *J* 6.6, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6 (C=N), 150.7 (C=O), 140.4 (C-1′), 136.5 (C-8a), 129.0 (C-3′, C-5′), 128.3 (C-4b), 127.9 (C-9a), 126.6 (C-2′, C-6′), 126.3 (C-4′), 124.5 (C-7), 123.6 (C-6), 117.4 (C-5), 115.4 (C-8), 109.3 (C-4a), 77.2 (CH), 48.1 (CH_2), 22.5 (CH_3), 15.4 (SCH_3). IR ($CHCl_3$) ν_{max} 2986, 1720 (C=O), 1587, 1373 cm^{-1}. MS (EI), *m*/*z* (%): 382 $[M]^+$ (19), 161 (100), 105 (82).

*4.1.4.4. 9-[(1S)-endo-Borneoxycarbonyl]cyclobrassinin (**31**).* Following the general procedure product **31** was obtained using carbonate **25** (0.274 g, 1.00 mmol). Yield: 0.265 g (64%), light yellow oil, $[\alpha]_D^{20}$ −35.4 (*c* 0.24, $CHCl_3$), R_f 0.63 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{22}H_{26}N_2O_2S_2$ requires: C, 63.74; H, 6.32; N, 6.76. Found: C, 63.52; H, 6.45; N, 6.49. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, 1H, *J* 7.9, H-8), 7.42–7.40 (m, 1H, H-5), 7.32–7.25 (m, 2H, H-7, H-6), 5.23 (dd, 1H, *J* 10.0, 5.4, H-2′), 5.00 (s, 2H, CH_2), 2.58–2.48 (m, 1H, H-3′), 2.53 (s, 3H, SCH_3), 2.21–2.14 (m, 1H, H-6′), 1.90–1.78 (m, 2H, H-5′, H-4′), 1.54–1.36 (m, 2H, H-6′, H-5′), 1.35–1.31 (m, 1H, H-3′), 1.08 (s, 3H), 0.98 (s, 3H) [H-8′, H-9′], 0.94 (s, 3H, H-10′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7 (C=N), 151.8 (C=O), 136.6 (C-8a), 127.9 (C-4b), 124.6 (C-9a), 124.4 (C-7), 123.5 (C-6), 117.4 (C-5), 115.3 (C-8), 109.2 (C-4a), 85.6 (C-2′), 49.4 (C-7′), 48.4 (C-1′), 48.1 (CH_2), 44.9 (C-4′), 36.8 (C-3′), 28.3 (C-5′), 28.0 (C-6′), 20.0, 19.1 (C-8′, C-9′), 15.4 (SCH_3), 14.7 (C-10′). IR ($CHCl_3$) ν_{max} 2956, 2881, 1734 (C=O), 1614, 1450, 1352 cm^{-1}. MS (EI), *m*/*z* (%): 414 $[M]^+$ (24), 161 (75), 81 (100).

*4.1.5. 1-[(1R,2S,5R)-Menthoxycarbonyl]indole-3-carboxaldehyde oxime (**32**).* To a stirred solution of aldehyde **14** (1.60 g, 4.50 mmol) in ethanol (28 mL) was added a solution of hydroxylammonium chloride (0.533 g, 7.70 mmol) and sodium carbonate (0.370 g, 3.50 mmol) in water (2.4 mL) and the mixture was stirred for 30 min at room temperature. After evaporation of ethanol and addition of water (12 mL), the oxime was extracted with diethyl ether (1×40 mL, 1×30 mL and 1×20 mL) and after drying with Na_2SO_4 the solvent evaporated. Yield: 1.65 g (99%) of a mixture of *E*- and *Z*-isomer in a 70:30 ratio, white crystals, mp 56–59 °C (diethyl ether), $[\alpha]_D^{20}$ −40.1 (*c* 0.52, $CHCl_3$), R_f 0.49 and 0.53 (cyclohexane/acetone 2:1). Anal. Calcd for $C_{20}H_{26}N_2O_3$ requires: C, 70.15; H, 7.65; N, 8.18. Found: C, 69.82; H, 7.79; N, 8.02. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 0.3H, CH=N min.), 8.32 (s, 0.7H, CH=N maj.), 8.24 (d, 0.3H, *J* 8.1, H-4 min.), 8.19 (d, 0.7H, *J* 8.1, H-4 maj.), 8.11 (d, 0.7H, *J* 7.9, H-7 maj.), 7.86 (br s, 1H, OH), 7.81(s, 0.7H, H-2 maj.), 7.79 (s, 0.3H, H-2 min.), 7.73 (d, 0.3H, *J* 7.8, H-7 min.), 7.40 (dt, 1H, *J* 7.3, 1.4, H-6), 7.34 (dt, 0.3H, *J* 8.2, 0.9, H-5 min.), 7.32 (dt, 0.7H, *J* 7.9, 0.8, H-5 maj.), 4.99 (dt, 0.3H, *J* 10.8, 4.4, H-1′ min.), 4.96 (dt, 0.7H, *J* 10.8, 4.4, H-1′ maj.), 2.23–2.20 (m, 1H, H-8′), 2.05–1.95 (m, 1H, H-6′), 1.80–1.74 (m, 2H, H-3′, H-4′), 1.68–1.53 (m, 2H, H-2′, H-5′), 1.27–0.93 (m, 3H, H-3′, H-4′, H-6′), 0.96 (d, 3H, *J* 6.4), 0.95 (d, 3H, *J* 7.1) [H-9′, H-10′], 0.84 (d, 0.9H, *J* 7.0, H-7′ min.), 0.83 (d, 2.1H, *J* 7.0, H-7′ maj.). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.6 (C=O min.), 150.3 (C=O maj.), 145.0 (CH=N maj.), 138.8 (CH=N min.), 136.0 (C-7a maj.), 134.5 (C-7a min.), 131.1 (C-3a maj.), 128.8 (C-3a min.), 127.6 (C-2 maj.), 127.1 (C-2 min.), 125.6 (C-6 maj.), 125.2 (C-6 min.), 123.8 (C-5 maj.), 123.5 (C-5 min.), 122.4 (C-4 maj.), 118.3 (C-4 min.), 115.5 (C-7 min.), 115.2 (C-7 maj.), 114.7 (C-3 maj.), 110.0 (C-3 min.), 78.5 (C-1′ min.), 78.4 (C-1′ maj.), 47.3 (C-2′ maj.), 47.2 (C-2′ min.), 41.0 (C-6′ maj.), 40.9 (C-6′ min.), 34.2 (C-4′ min.), 34.1 (C-4′ maj.), 31.6 (C-5′ min.), 31.5 (C-5′ maj.), 26.5 (C-8′ maj.), 25.9 (C-8′ min.), 23.6 (C-3′ min.), 23.5 (C-3′ maj.), 22.2 (min.), 22.0 (maj.), 21.0 (min.), 20.8 (maj.) (C-9′, C-10′), 16.5 (C-7′ min.), 16.4 (C-7′ maj.). IR ($CHCl_3$) ν_{max} 3580 (OH), 2960, 2873, 1707 (C=O), 1440, 1333, 1226, 1080, 1020, 940 cm^{-1}. MS (MALDI-TOF), *m*/*z* (%): 365.91 $[M+Na]^+$ (5), 343.45 $[M+H]^+$ (35), 327.3 (100).

*4.1.6. 1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]indole-3-carboxaldehyde oxime (**33**) and 1-[(1S,2R,5S)-8-phenylmenthoxycarbonyl]indole-3-carboxaldehyde oxime (**34**).* To a stirred solution of aldehyde **26** or **27** (0.706 g, 1.75 mmol) in ethanol (14 mL) was added a solution of hydroxylammonium chloride (0.190 g, 2.73 mmol) and sodium carbonate (0.135 g, 1.28 mmol) in water (1.7 mL) and the mixture was stirred for 30 min at room temperature. After evaporation of ethanol and addition of water (8 mL), the oxime was extracted with diethyl ether (2×30 mL) and after drying with Na_2SO_4 the solvent evaporated. Data for **33**: yield: 0.703 g (96%) of a mixture of *E*- and *Z*-isomer in a 70:30 ratio, white crystals, mp 56–58 °C (diethyl ether), $[\alpha]_D^{20}$ −62.9 (*c* 0.56, $CHCl_3$), R_f 0.50 and 0.41 (*n*-hexane/acetone 3:1). Anal. Calcd for $C_{26}H_{30}N_2O_3$ requires: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.90; H, 6.93; N, 6.45. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (br s, 0.3H, OH min.), 9.86 (br s, 0.7H, OH maj.), 8.20–8.08 (m, 0.7H, H-7 maj.), 8.12 (s, 0.7H, CH=N maj.), 8.03 (ddd, 0.7H, *J* 7.7, 0.8, 0.6, H-4 maj.), 8.01–7.92 (m, 0.3H, H-7 min.), 7.72 (s, 0.3H, CH=N min.), 7.69–7.67 (m, 0.3H, H-4 min.), 7.37–7.28 (m, 2H, H-5, H-6), 7.26–7.24 (m, 2.4H, H-2, H-2″, H-6″ maj.), 7.22–7.20 (m, 0.6H, H-2″, H-6″ min.), 7.10–7.06 (m, 1.4H, H-3″, H-5″ maj.), 6.99–6.96 (m, 1.3H, H-3″, H-5″ min., H-4″ maj.), 6.82–6.79 (m, 0.3H, H-4″ min.), 5.15 (dt, 0.3H, *J* 10.7, 4.5, H-1′ min.), 5.05 (dt, 0.7H, *J* 10.7, 4.3, H-1′ maj.), 2.29 (dt, 0.7H, *J* 12.6, 3.5, H-2′ maj.), 2.21 (dt, 0.3H, *J* 12.1, 3.5, H-2′ min.), 2.08–1.97 (m, 2H, H-3′, H-6′), 1.88–1.69 (m, 1H, H-4′), 1.65–1.51 (m, 1H, H-5′), 1.39 (s, 0.9H, H-9′ min.), 1.36 (s, 2.1H, H-9′ maj.), 1.22 (s, 0.9H, H-10′ min.), 1.21 (s, 2.1H, H-10′ maj.), 1.33–0.83 (m, 3H, H-3′, H-4′, H-6′), 0.92 (d, 2.1H, *J* 6.5, H-7′ maj.), 0.91 (d, 0.9H, *J* 5.4, H-7′ min.). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2 (C-1″ maj.), 151.1 (C-1″ min.), 150.0 (C=O min.), 149.5 (C=O maj.), 145.4 (CH=N maj.), 139.1 (CH=N min.), 136.2 (C-7a maj.), 134.6 (C-7a min.), 131.4 (C-3a min.), 128.7 (C-3″, C-5″ min.), 128.4 (C-3″, C-5″ maj.), 128.3 (C-2 min.), 128.2 (C-2 maj.), 126.9 (C-3a maj.), 125.5 (C-6), 125.4 (C-4″ min.), 125.3 (C-4″ maj.), 125.2 (C-2″, C-6″ maj.), 125.1 (C-2″, C-6″ min.), 123.9 (C-5 maj.), 123.5 (C-5 min.), 122.4 (C-4 maj.), 118.4 (C-4 min.), 115.7 (C-7 min.), 115.3 (C-7 maj.), 113.9 (C-3 maj.), 109.8 (C-3 min.), 78.5 (C-1′), 51.3 (C-2′ min.), 50.9 (C-2′ maj.), 42.2 (C-6′ min.), 41.9 (C-6′ maj.), 39.8 (C-8′ min.), 39.5 (C-8′ maj.), 34.7 (C-4′ maj.), 34.6 (C-4′ min.), 31.7 (C-5′ min.), 31.0 (C-5′ maj.), 30.4 (C-9′ min.), 29.8 (C-9′ maj.), 26.8 (C-3′ min.), 26.6 (C-3′ maj.), 24.2 (C-10′ maj.), 22.7 (C-10′ min.), 22.6 (C-7′ min.), 21.9 (C-7′ maj.). IR ($CHCl_3$) ν_{max} 3560 (OH), 2953, 1713 (C=O), 1367, 1220,

1087 cm^{-1}. MS (EI), *m*/*z* (%): 420 $[M+H]^+$ (4), 419 $[M]^+$ (14), 204 (34), 160 (61), 119 (95), 105 (100). Data for **34**: yield: 0.703 g (96%) of a mixture of *E*- and *Z*-isomer in a 70:30 ratio, white crystals, mp 56–58 °C (diethyl ether), $[\alpha]_D^{20}$ +66.2 (*c* 0.54, $CHCl_3$), R_f 0.50 and 0.41 (*n*-hexane/acetone 3:1). The spectral data were fully identical with those of enantiomeric product **33**.

*4.1.7. 1-[(1R,2S,5R)-Menthoxycarbonyl]indol-3-ylmethyl amine (**35**).* To a solution of $NiCl_2 \cdot 6H_2O$ (0.784 g, 3.30 mmol) in methanol (30 mL) was added oxime **32** (1.03 g, 3.00 mmol) in methanol (42 mL) followed by $NaBH_4$ (0.908 g, 24.0 mmol) in one portion with stirring and cooling with flowing cold water. After 5 min, methanol in the mixture was evaporated to ¼ of its original volume and mixture was poured into a saturated solution of NH_4Cl (150 mL). After extraction with ethyl acetate (1×100 mL and 1×60 mL), drying the extract with Na_2SO_4 and evaporation of the solvent, the crude amine **35** (0.985 g) was obtained. The crude amine **35** was employed in the next reaction without purification.

*4.1.8. 1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]indol-3-ylmethyl amine (**36**) and 1-[(1S,2R,5S)-8-phenylmenthoxycarbonyl]indol-3-ylmethyl amine (**37**).* To a solution of $NiCl_2 \cdot 6H_2O$ (0.238 g, 1.00 mmol) in methanol (20 mL) was added oxime **33** or **34** (0.419 g, 1.00 mmol) in methanol (20 mL) followed by $NaBH_4$ (0.378 g, 10.0 mmol) in one portion with stirring and cooling with flowing cold water. After 5 min, methanol in the mixture was evaporated to ¼ of its original volume and mixture was poured into a saturated solution of NH_4Cl (50 mL). After extraction with ethyl acetate (2×40 mL), drying the extract with Na_2SO_4 and evaporation of the solvent, the crude amine **36** or **37** (0.404 g) was obtained. The crude amine **36** or **37** was employed in the next reaction without purification.

*4.1.9. 1-[(1R,2S,5R)-Menthoxycarbonyl]brassinin (**38**).* To a stirred solution of crude freshly prepared amine **35** (0.985, 3.00 mmol) in methanol (21 mL) were added triethylamine (0.910 g, 1.25 mL, 9.00 mmol) and carbon disulphide (0.685 g, 0.541 mL, 9.00 mmol). After stirring for 5 min at room temperature, methyl iodide (1.28 g, 0.560 mL, 9.00 mmol) was added and stirring was continued for 1 h. The solvent was evaporated and the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (90 g, cyclohexane/ethyl acetate 8:1). Yield: 0.64 g (51%), colourless oil, $[\alpha]_D^{20}$ −61.9 (*c* 0.73, $CHCl_3$), R_f 0.53 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{22}H_{30}N_2O_2S_2$ requires: C, 63.12; H, 7.22; N, 6.69. Found: C, 63.27; H, 7.51; N, 6.42. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, 1H, *J* 8.0, H-7), 7.66 (s, 1H, H-2), 7.58 (d, 1H, *J* 7.6, H-4), 7.38–7.34 (m, 1H, H-6), 7.29–7.23 (m, 1H, H-5), 7.03 (br s, 1H, NH), 5.05 (d, 2H, *J* 4.7, CH_2), 4.95 (dt, 1H, *J* 10.9, 4.4, H-1′), 2.66 (s, 3H, SCH_3), 2.27–2.20 (m, 1H, H-8′), 2.05–1.95 (m, 1H, H-6′), 1.83–1.74 (m, 2H, H-3′, H-4′), 1.69–1.53 (m, 2H, H-2′, H-5′), 1.28–0.94 (m, 3H, H-3′, H-4′, H-6′), 0.96 (d, 3H, *J* 6.5), 0.95 (d, 3H, *J* 6.7) [H-9′, H-10′], 0.83 (d, 3H, *J* 6.8, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.0 (C=S), 150.5 (C=O), 135.6 (C-7a), 129.1 (C-3a), 125.2 (C-6), 124.8 (C-2), 123.2 (C-5), 119.1 (C-4), 115.9 (C-3), 115.5 (C-7), 78.4 (C-1′), 47.2 (C-2′), 42.4 (CH_2), 40.9 (C-6′), 34.1 (C-4′), 31.5 (C-5′), 26.5 (C-8′), 23.5 (C-3′), 22.0, 20.8 (C-9′, C-10′), 18.2 (SCH_3), 16.4 (C-7′). IR ($CHCl_3$) ν_{max} 3366 (NH), 2960, 2926, 1700 (C=O), 1440, 1373, 1286, 1073 cm^{-1}. MS (EI), *m*/*z* (%): 418 $[M+H]^+$ (6), 130 (100).

*4.1.10. 1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]brassinin (**39**) and 1-[(1S,2R,5S)-8-phenylmenthoxycarbonyl]brassinin (**40**).* To a stirred solution of crude freshly prepared amine **36** or **37** (0.404 g, 1.00 mmol) in methanol (10 mL) were added triethylamine (0.304 g, 0.418 mL, 3.00 mmol) and carbon disulphide (0.229 g, 0.181 mL, 3.00 mmol). After stirring for 5 min at room temperature, methyl iodide (0.424 g, 0.187 mL, 3.00 mmol) was added and stirring was continued for 1 h. The solvent was evaporated and the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (50 g, *n*-hexane/ethyl acetate 5:1). Data for **39**: yield: 0.252 g (51%), colourless oil, $[\alpha]_D^{20}$ −63.5 (*c* 0.79, $CHCl_3$), R_f 0.60 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{28}H_{34}N_2O_2S_2$ requires: C, 67.98; H, 6.93; N, 5.66. Found: C, 68.25; H, 6.74; N, 5.41. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H, H-7), 7.49 (d, 1H, *J* 7.6, H-4), 7.35–7.23 (m, 4H, H-6, H-5, H-2″, H-6″), 7.12 (t, 2H, *J* 7.3, H-3″, H5″), 7.02–7.00 (m, 1H, H-4″), 6.87 (s, 1H, H-2), 6.22 (br s, 1H, NH), 5.03 (dt, 1H, *J* 10.6, 4.2, H-1′), 4.88 (s, 2H, CH_2), 2.69 (s, 3H, SCH_3), 2.29 (dt, 1H, *J* 12.1, 3.5, H-2′), 2.08–2.04 (m, 1H, H-6′), 1.92–1.96 (m, 1H, H-3′), 1.80–1.76 (m, 1H, H-4′), 1.63–1.59 (m, 1H, H-5′), 1.36 (s, 3H), 1.20 (s, 3H) [H-9′, H-10′], 1.32–0.87 (m, 3H, H-3′, H-4′, H-6′), 0.92 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.9 (C=S), 152.5 (C-1″), 149.7 (C=O), 135.8 (C-7a), 129.0 (C-3a), 128.4 (C-3″, C-5″), 125.3 (C-2″, C-6″), 125.1 (C-6), 125.0 (C-2), 124.9 (C-4″), 123.3 (C-5), 118.9 (C-4), 115.6 (C-7), 114.9 (C-3), 77.9 (C-1′), 50.8 (C-2′), 42.7 (CH_2), 41.9 (C-6′), 39.6 (C-8′), 34.7 (C-4′), 31.6 (C-5′), 30.3 (C-9′), 26.6 (C-3′), 22.9 (C-10′), 21.9 (C-7′), 18.5 (SCH_3). IR ($CHCl_3$) ν_{max} 3233 (NH), 2947, 1773 (C=O), 1387, 1087 cm^{-1}. MS (EI), *m*/*z* (%): 495 $[M+H]^+$ (2), 447 (10), 119 (100), 105 (96). Data for **40**: yield: 0.252 g (51%), colourless oil, $[\alpha]_D^{20}$ +61.5 (*c* 0.62, $CHCl_3$), R_f 0.60 (*n*-hexane/ethyl acetate 5:1). The spectral data were fully identical with those of enantiomeric product **39**.

*4.1.11. Spirocyclization of 1-[(1R,2S,5R)-menthoxycarbonyl]brassinin (**38**).* To a stirred solution of 1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl] brassinin (**38**) (0.418 g, 1.00 mmol) in a mixture of dichloromethane/water (9 mL/1 mL) at room temperature was added a freshly prepared solution of Br_2 (2.57 mL, 1.10 mmol). The stock solution was obtained by dissolving of bromine (0.060 mL) in dichloromethane (2.64 mL). The reaction mixture was stirred for 20 min at room temperature, then triethylamine (0.223 g, 0.305 mL, 2.20 mmol) was added. Stirring was continued for 10 min, and the reaction mixture was diluted with dichloromethane (50 mL), washed with brine (2×50 mL). The organic layer was dried over Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on silica gel (35 g, cyclohexane/ethyl acetate 8:1), affording a mixture of trans-diastereoisomers **41a** and **41b** and mixture of cis-diastereoisomers **41c** and **41d**.

*4.1.11.1. trans-1-[(1R,2S,5R)-Menthoxycarbonyl]spirobrassinol (**41a**, **41b**).* Yield: 0.224 g (51%) of a mixture of diastereoisomers **41a** and **41b** in a 50:50 ratio, colourless oil, R_f 0.18 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{22}H_{30}N_2O_3S_2$ requires: C, 60.80; H, 6.96; N, 6.45. Found: C, 61.09; H, 7.18; N, 6.22. IR ($CHCl_3$) ν_{max} 3437 (OH), 2958, 2872, 1713 (C=O), 1568 (C=N), 1483, 1281, 955 cm^{-1}. MS (MALDI-TOF), *m*/*z* (%): 460.17 $[M+Na]^+$ (26), 435.26 $[M+H]^+$ (90).

*Compound **41a***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (br s, 1H, H-7), 7.39 (d, 1H, *J* 7.5, H-4), 7.29 (dd, 1H, *J* 7.5, 7.6, H-6), 7.07 (dd, 1H, *J* 7.5, 7.5, H-5), 5.97 (s, 1H, H-2), 5.29 (s, 1H, OH), 5.07 (d, 1H, *J* 15.5, H_b), 4.84 (ddd, 1H, *J* 10.5, 10.5, 4.3, H-1′), 4.30 (d, 1H, *J* 15.5, H_a), 2.60 (s, 3H, SCH_3), 2.17–2.16 (m, 1H, H-8′), 1.97–1.94 (m, 1H, H-6′), 1.76–1.72 (m, 2H, H-3′, H-4′), 1.57–1.51 (m, 2H, H-2′, H-5′), 1.17–0.95 (m, 3H, H-3′, H-4′, H-6′), 0.94 (d, 3H, *J* 7.0), 0.92 (d, 3H, *J* 7.0) [H-9′, H-10′], 0.82 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7 (C=N), 150.5 (C=O), 140.9 (C-7a), 129.9 (C-6), 129.5 (C-3a), 124.1 (C-4), 123.8 (C-5), 114.9 (C-7), 91.7 (C-2), 77.2 (C-1′), 70.5 (C-3), 67.7 (CH_2), 47.3 (C-2′), 40.9 (C-6′), 34.1 (C-4′), 31.5 (C-5′), 26.4 (C-8′), 23.4 (C-3′), 21.9, 20.8 (C-9′, C-10′), 16.3 (C-7′), 15.4 (SCH_3). NOESY correlation (400 MHz, $CDCl_3$): H_a/H-4, H_a/H_b, H-7/H-6, H-4/H-5.

*Compound **41b***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H, H-7), 7.39 (d, 1H, *J* 7.5, H-4), 7.29 (dd, 1H, *J* 7.5, 7.6, H-6), 7.07 (dd, 1H, *J* 7.5, 7.5, H-5), 5.97 (s, 1H, H-2), 5.29 (s, 1H, OH), 5.07 (d, 1H, *J* 15.5, H_b),

4.84 (ddd, 1H, *J* 10.5, 10.5, 4.3, H-1′), 4.29 (d, 1H, *J* 15.5, H_a), 2.60 (s, 3H, SCH_3), 2.17–2.16 (m, 1H, H-8′), 1.97–1.94 (m, 1H, H-6′), 1.76–1.72 (m, 2H, H-3′, H-4′), 1.57–1.51 (m, 2H, H-2′, H-5′), 1.17–0.95 (m, 3H, H-3′, H-4′, H-6′), 0.94 (d, 3H, *J* 7.0), 0.92 (d, 3H, *J* 7.0) [H-9′, H-10′], 0.82 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7 (C=N), 150.5 (C=O), 140.9 (C-7a), 129.9 (C-6), 129.5 (C-3a), 124.1 (C-4), 123.8 (C-5), 114.9 (C-7), 91.7 (C-2), 77.2 (C-1′), 70.5 (C-3), 67.7 (CH_2), 47.3 (C-2′), 40.9 (C-6′), 34.1 (C-4′), 31.5 (C-5′), 26.4 (C-8′), 23.4 (C-3′), 21.9, 20.8 (C-9′, C-10′), 16.3 (C-7′), 15.4 (SCH_3). NOESY correlation (400 MHz, $CDCl_3$): H_a/H-4, H_a/H_b, H-7/H-6, H-4/H-5.

*4.1.11.2. cis-1-[(1R,2S,5R)-Menthoxycarbonyl]spirobrassinol (**41c**, **41d**).* Yield: 0.014 g (3%) of a mixture of diastereoisomers **41c** and **41d** in a 50:50 ratio, colourless oil, R_f 0.59 (cyclohexane/ethyl acetate 8:1). Anal. Calcd for $C_{22}H_{30}N_2O_3S_2$ requires: C, 60.80; H, 6.96; N, 6.45. Found: C, 61.05; H, 6.68; N, 6.22. MS (MALDI-TOF), *m*/*z* (%): identical with spectrum of **41a** and **41b**.

*Compound **41c***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (br s, 1H, H-7), 7.39 (d, 1H, *J* 7.5, H-4), 7.30–7.26 (m, 1H, H-6), 7.15–6.99 (m, 1H, H-5), 5.65 (s, 1H, H-2), 4.97 (s, 1H, OH), 4.83 (ddd, 1H, *J* 11.1, 11.1, 4.3, H-1′), 4.38 (d, 1H, *J* 15.1, H_b), 4.02 (d, 1H, *J* 15.1, H_a), 2.57 (s, 3H, SCH_3), 2.19–2.14 (m, 1H, H-8′), 2.02–1.95 (m, 1H, H-6′), 1.74–1.72 (m, 2H, H-3′, H-4′), 1.64–1.50 (m, 2H, H-2′, H-5′), 1.27–0.86 (m, 3H, H-3′, H-4′, H-6′), 0.93 (d, 3H, *J* 7.1), 0.92 (d, 3H, *J* 7.1) [H-9′, H-10′], 0.81 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7 (C=N), 153.5 (C=O), 139.9 (C-7a), 130.0 (C-6), 129.7 (C-3a), 124.3 (C-4), 124.0 (C-5), 115.1 (C-7), 88.4 (C-2), 75.7 (C-1′), 75.6 (C-3), 64.5 (CH_2), 47.5 (C-2′), 41.6 (C-6′), 34.4 (C-4′), 31.5 (C-5′), 26.5 (C-8′), 23.5 (C-3′), 22.2, 21.1 (C-9′, C-10′), 16.4 (C-7′), 15.4 (SCH_3). NOESY correlation (400 MHz, $CDCl_3$): H_a/H-4, H_b/H-2, H_a/H_b.

*Compound **41d***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (br s, 1H, H-7), 7.39 (d, 1H, *J* 7.5, H-4), 7.30–7.26 (m, 1H, H-6), 7.15–6.99 (m, 1H, H-5), 5.65 (s, 1H, H-2), 4.97 (s, 1H, OH), 4.83 (ddd, 1H, *J* 11.1, 11.1, 4.3, H-1′), 4.37 (d, 1H, *J* 15.1, H_b), 4.03 (d, 1H, *J* 15.1, H_a), 2.57 (s, 3H, SCH_3), 2.19–2.14 (m, 1H, H-8′), 2.02–1.95 (m, 1H, H-6′), 1.74–1.72 (m, 2H, H-3′, H-4′), 1.64–1.50 (m, 2H, H-2′, H-5′), 1.27–0.86 (m, 3H, H-3′, H-4′, H-6′), 0.93 (d, 3H, *J* 7.1), 0.92 (d, 3H, *J* 7.1) [H-9′, H-10′], 0.82 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7 (C=N), 154.2 (C=O), 139.9 (C-7a), 130.0 (C-6), 129.7 (C-3a), 124.3 (C-4), 124.0 (C-5), 115.1 (C-7), 88.4 (C-2), 75.7 (C-1′), 75.6 (C-3), 60.6 (CH_2), 47.5 (C-2′), 41.5 (C-6′), 34.3 (C-4′), 31.5 (C-5′), 26.7 (C-8′), 23.6 (C-3′), 22.2, 21.1 (C-9′, C-10′), 16.6 (C-7′), 15.4 (SCH_3). NOESY correlation (400 MHz, $CDCl_3$): H_a/H-4, H_b/H-2, H_a/H_b.

*4.1.12. Spirocyclization of 1-[(1R,2S,5R)-8-phenylmenthoxycarbonyl] brassinin (**39**) and 1-[(1S,2R,5S)-8-phenylmenthoxycarbonyl]brassinin (**40**).* To a stirred solution of 1-[(1*R*,2*S*,5*R*)-8-phenylmenthoxycarbonyl]brassinin (**39**) or-1-[(1*S*,2*R*,5*S*)-8-phenylmenthoxycarbonyl]brassinin (**40**) (0.198 g, 0.400 mmol) in a mixture of dichloromethane/water (3.6 mL/0.4 mL) cooled to −90 °C was added a freshly prepared solution of Br_2 (1.10 mL, 0.440 mmol). The stock solution was obtained by dissolving of bromine (0.040 mL) in dichloromethane (1.76 mL). The reaction mixture was stirred for 20 min at −90 °C, then triethylamine (0.089 g, 0.124 mL, 0.880 mmol) was added. Stirring was continued for 10 min and the reaction mixture was diluted with dichloromethane (20 mL), then washed with brine (2×20 mL). The organic layer was dried over Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on silica gel (20 g, *n*-hexane/ethyl acetate 5:1), affording a mixture of trans-diastereoisomers **42a** and **42b** in an 18: 82 ratio or **43a** and **43b** in an 82:18 ratio.

*4.1.12.1. trans-1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]spirobrassinol (**42a**, **42b**).* Yield: 0.129 g (63%) of a mixture of diastereoisomers **42a** and **42b** in an 18:82 ratio, colourless oil, R_f 0.21 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{28}H_{34}N_2O_3S_2$ requires: C, 65.85; H, 6.71; N, 5.49. Found: C, 65.93; H, 6.49; N, 5.70. MS (EI), *m*/*z* (%): 511 $[M]^+$ (2), 251 (47), 119 (100), 105 (99), 91 (64).

*Compound **42a***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, 1H, *J* 8.1, H-7), 7.38–7.18 (m, 5H, H-4, H-6, H-2″, H-6″, H-5), 7.04–7.01 (m, 2H, H-3″, H-5″), 6.91–6.86 (m, 1H, H-4″), 5.95 (s, 1H, H-2), 5.03 (dt, 1H, *J* 10.8, 4.3, H-1′), 4.89 (d, 1H, *J* 15.0, H_b), 4.15 (d, 1H, *J* 15.0, H_a), 2.60 (s, 3H, SCH_3), 2.19–2.12 (m, 1H, H-2′), 2.05–1.82 (m, 2H, H-3′, H-6′), 1.73–1.71 (m, 1H, H-4′), 1.53–1.47 (m, 1H, H-5′), 1.38 (s, 3H), 1.21 (s, 3H) [H-9′, H-10′], 1.31–0.88 (m, 3H, H-3′, H-6′, H-4′), 0.89 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4 (C=N), 152.3 (C-1″), 148.9 (C=O), 140.8 (C-7a), 130.1 (C-6), 128.6 (C-3a), 128.1 (C-3″, C-5″), 126.1 (C-5), 125.5 (C-2″, C-6″), 125.1 (C-4″), 123.8 (C-4), 114.8 (C-7), 90.1 (C-2), 77.4 (C-1′), 70.8 (C-3), 68.5 (CH_2), 50.8 (C-2′), 42.5 (C-6′), 39.7 (C-8′), 34.7 (C-4′), 31.7 (C-5′), 29.9 (C-9′), 26.7 (C-3′), 23.5 (C-10′), 21.9 (C-7′), 15.4 (SCH_3). NOESY correlation (400 MHz, $CDCl_3$): H_a/H-4, H_a/H_b.

*Compound **42b***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H, H-7), 7.32–7.16 (m, 5H, H-4, H-6, H-2″, H-6″, H-5), 7.09–6.80 (m, 2H, H-3″, H-5″), 6.74–6.61 (m, 1H, H-4″), 5.84 (s, 1H, H-2), 5.03 (dt, 1H, *J* 10.8, 4.3, H-1′), 4.23 (d, 1H, *J* 15.6, H_a), 4.23 (d, 2H, *J* 15.6, H_b), 2.58 (s, 3H, SCH_3), 2.18–2.14 (m, 1H, H-2′), 1.97–1.85 (m, 2H, H-3′, H-6′), 1.73–1.70 (m, 1H, H-4′), 1.58–1.45 (m, 1H, H-5′), 1.39 (s, 3H), 1.23 (s, 3H) [H-9′, H-10′], 1.26–0.88 (m, 3H, H-3′, H-6′, H-4′), 0.90 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4 (C=N), 153.0 (C-1″), 151.3 (C=O), 139.4 (C-7a), 130.3 (C-6), 129.7 (C-3a), 128.2 (C-3″, C-5″), 125.2 (C-2″, C-6″, C-5), 123.9 (C-4″), 123.6 (C-4), 115.3 (C-7), 92.0 (C-2), 77.4 (C-1′), 70.2 (C-3), 68.1 (CH_2), 51.6 (C-2′), 42.7 (C-6′), 39.8 (C-8′), 34.7 (C-4′), 31.7 (C-5′), 29.9 (C-9′), 26.8 (C-3′), 24.0 (C-10′), 21.9 (C-7′), 15.4 (SCH_3). NOESY correlation (400 MHz, $CDCl_3$): H_a/H-4, H_a/H_b.

*4.1.12.2. trans-1-[(1S,2R,5S)-8-Phenylmenthoxycarbonyl]spirobrassinol (**43a**, **43b**).* Yield: 0.129 g (63%) of a mixture of diastereoisomers **43a** and **43b** in an 82:18 ratio, colourless oil, R_f 0.21 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{28}H_{34}N_2O_3S_2$ requires: C, 65.85; H, 6.71; N, 5.49. Found: C, 65.99; H, 6.86; N, 5.76. Properties and spectral data of compounds **43a** and **43b** were fully identical with those of enantiomers **42a** and **42b**.

*4.1.13. 1-[(1R,2S,5R)-Menthoxycarbonyl]spirobrassinin (**44a**, **44b**).* A solution of a mixture of **41a** and **41b** (0.172 g, 0.400 mmol) in dry dichloromethane (0.2 mL) was added to a vigorously stirred slurry of pyridinium chlorochromate (0.256 g, 1.20 mmol) and anhydrous magnesium sulfate (0.216 g, 1.80 mmol) in dry dichloromethane (0.2 mL). The reaction mixture was stirred for 24 h at room temperature and then diluted with dichloromethane (5 mL). After adding a small amount of silica gel, the solvent was evaporated and residue preabsorbed on silica was subjected to silica gel column chromatography (12 g, *n*-hexane/ethyl acetate 5:1) affording a mixture of **44a** and **44b** as a colourless oil. The obtained mixture of **44a** and **44b** was further crystallized from dichloromethane/*n*-hexane to afford pure diastereoisomer **44b**. Diastereoisomer **44a** was obtained after filtration of crystals of diastereoisomer **44b** and evaporation of the solvent.

Data for **44a**: yield: 0.082 g (47%), colourless oil, $[\alpha]_D^{20}$ −51.3 (*c* 0.16, $CHCl_3$), R_f 0.32 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{22}H_{28}N_2O_3S_2$ requires: C, 61.08; H, 6.52; N, 6.48. Found: C, 61.39; H, 6.40; N, 6.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, 1H, *J* 8.2, H-7), 7.42–7.34 (m, 2H, H-4, H-6), 7.23 (dd, 1H, *J* 7.6, 7.6, H-5), 4.91 (ddd, 1H, *J* 10.9, 10.9, 4.3, H-1′), 4.73 (d, 1H, *J* 15.2, H_b), 4.51 (d, 1H, *J* 15.2, H_a), 2.62 (s, 3H, SCH_3), 2.21–2.18 (m, 1H, H-8′), 2.15–2.09 (m, 1H, H-6′), 1.77–1.71 (m, 2H, H-3′, H-4′), 1.65–1.51 (m, 2H, H-2′, H-5′), 1.26–0.88 (m, 3H, H-3′, H-4′, H-6′), 0.94 (d, 3H, *J* 6.4), 0.93 (d, 3H, *J* 6.9) [H-9′, H-10′], 0.81 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4 (C=O), 163.7 (C=N), 150.3 (C=O), 138.3 (C-7a), 130.1 (C-6),

129.9 (C-3a), 125.5 (C-5), 124.1 (C-4), 115.2 (C-7), 78.7 (C-1′), 75.9 (CH_2), 65.0 (C-3), 46.8 (C-2′), 40.7 (C-6′), 34.0 (C-4′), 31.5 (C-5′), 25.9 (C-8′), 23.1 (C-3′), 21.9, 20.9 (C-9′, C-10′), 16.0 (C-7′), 15.6 (SCH_3). IR ($CHCl_3$) ν_{max} 2958, 2872, 1774 (C=O), 1734 (C=O), 1589 (C=N), 1290 cm^{-1}. MS (MALDI-TOF), *m*/*z* (%): 455.66 $[M+Na]^+$ (34), 433.61 $[M+H]^+$ (18), 177.83 (100).

Data for **44b**: yield: 0.041 g (24%), colourless crystals, mp 143–145 °C (dichloromethane/*n*-hexane), $[\alpha]_D^{20}$ −10.6 (*c* 0.60, $CHCl_3$), R_f 0.4 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{22}H_{28}N_2O_3S_2$ requires: C, 61.08; H, 6.52; N, 6.48. Found: C, 61.29; H, 6.40; N, 6.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, 1H, *J* 8.2, H-7), 7.42–7.39 (m, 2H, H-4, H-6), 7.23 (dd, 1H, *J* 7.5, 9.1, H-5), 4.91 (ddd, 1H, *J* 10.9, 10.9, 4.3, H-1′), 4.74 (d, 1H, *J* 15.3, H_b), 4.51 (d, 1H, *J* 15.3, H_a), 2.62 (s, 3H, SCH_3), 2.21–2.18 (m, 1H, H-8′), 2.15–2.09 (m, 1H, H-6′), 1.77–1,71 (m, 2H, H-3′, H-4′), 1.65–1.51 (m, 2H, H-2′, H-5′), 1.26–0.88 (m, 3H, H-3′, H-4′, H-6′), 0.94 (d, 3H, *J* 6.4), 0.93 (d, 3H, *J* 6.9) [H-9′, H-10′], 0.81 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4 (C=O), 163.7 (C=N), 150.3 (C=O), 138.3 (C-7a), 130.1 (C-6), 129.2 (C-3a), 125.4 (C-5), 124.1 (C-4), 115.2 (C-7), 78.7 (C-1′), 75.9 (CH_2), 65.0 (C-3), 46.8 (C-2′), 40.7 (C-6′), 34.0 (C-4′), 31.5 (C-5′), 25.1 (C-8′), 23.1 (C-3′), 21.9, 20.9 (C-9′, C-10′), 16.0 (C-7′), 15.6 (SCH_3). IR (KBr) ν_{max} 2947, 2922, 1774 (C=O), 1734 (C=O), 1597 (C=N), 1288 cm^{-1}. MS (MALDI-TOF), *m*/*z* (%): 455.66 $[M+Na]^+$ (34), 433.61 $[M+H]^+$ (18), 177.83 (100).

*4.1.14. 1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]spirobrassinin (**45a**, **45b**) and 1-[(1S,2R,5S)-8-phenylmenthoxycarbonyl]spirobrassinin (**46a**, **46b**).* A solution of a mixture of **42a** and **42b** or **43a** and **43b** (0.129 g, 0.250 mmol) in dry dichloromethane (0.5 mL) was added to a vigorously stirred slurry of pyridinium chlorochromate (0.377 g, 1.75 mmol) and anhydrous magnesium sulfate (0.258 g, 1.25 mmol) in dry dichloromethane (0.5 mL). The reaction mixture was stirred for 24 h at room temperature and then diluted with dichloromethane (2 mL). After adding a small amount of silica gel, the solvent was evaporated and residue preabsorbed on silica was subjected to silica gel column chromatography (20 g, dichloromethane/ethyl acetate 30:1) and diastereoisomers **45a** and **45b** or **46a** and **46b** were separated.

*4.1.14.1. 1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]spirobrassinin (**45a**, **45b**).* Data for **45a**: yield: 0.015 g (12%), colourless oil, $[\alpha]_D^{20}$ −17.5 (*c* 0.16, $CHCl_3$), R_f 0.78 (dichloromethane/ethyl acetate 30:1). Anal. Calcd for $C_{28}H_{32}N_2O_3S_2$ requires: C, 66.11; H, 6.34; N, 5.51. Found: C, 66.43; H, 6.07; N, 5.36. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66–7.64 (m, 1H, H-7), 7.33–7.29 (m, 2H, H-4, H-6), 7.25–7.23 (m, 2H, H-2″, H-6″), 7.20–7.16 (m, 1H, H-5), 6.99–6.95 (m, 2H, H-3″, H-5″), 6.80–6.77 (m, 1H, H-4″), 5.10 (dt, 1H, *J* 10.8, 4.7, H-1′), 4.59 (d, 1H, *J* 15.1, H_b), 4.42 (d, 1H, *J* 15.1, H_a), 2.62 (s, 3H, SCH_3), 2.24–2.16 (m, 1H, H-2′), 2.01–1.86 (m, 2H, H-3′, H-6′), 1.74–1.69 (m, 1H, H-4′), 1.60–1.45 (m, 1H, H-5′), 1.38 (s, 3H), 1.20 (s, 3H) [H-9′, H-10′], 1.32–0.84 (m, 3H, H-3′, H-6′, H-4′), 0.92 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8 (C=O), 164.0 (C=N), 151.2 (C-1″), 149.5 (C=O), 138.2 (C-7a), 129.9 (C-6), 129.6 (C-3a), 127.9 (C-3″, C-5″), 125.5 (C-2″, C-6″, C-5), 125.1 (C-4″), 124.0 (C-4), 115.4 (C-7), 77.2 (C-1′), 76.4 (CH_2), 64.9 (C-3), 50.8 (C-2′), 41.8 (C-6′), 39.8 (C-8′), 34.5 (C-4′), 31.7 (C-5′), 29.4 (C-9′), 26.6 (C-3′), 23.5 (C-10′), 21.9 (C-7′), 15.9 (SCH_3). MS (EI), *m*/*z* (%): 509 $[M]^+$ (4), 251 (54), 119 (100), 105 (68), 91 (55).

Data for **45b**: yield: 0.068 g (54%), colourless oil, $[\alpha]_D^{20}$ −89.3 (*c* 0.47, $CHCl_3$), R_f 0.64 (dichloromethane/ethyl acetate 30:1). Anal. Calcd for $C_{28}H_{32}N_2O_3S_2$ requires: C, 66.11; H, 6.34; N, 5.51. Found: C, 66.27; H, 6.15; N, 5.79. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60–7.58 (m, 1H, H-7), 7.35–7.23 (m, 4H, H-4, H-6, H-2″, H-6″), 7.19–7.16 (m, 1H, H-5), 6.99–6.95 (m, 2H, H-3″, H-5″), 6.78–6.69 (m, 1H, H-4″), 5.12 (dt, 1H, *J* 10.8, 4.7, H-1′), 4.60 (d, 1H, *J* 15.2, H_b), 4.40 (d, 1H, *J* 15.2, H_a), 2.62 (s, 3H, SCH_3), 2.24–2.17 (m, 1H, H-2′), 1.98–1.88 (m, 2H, H-3′, H-6′), 1.74–1.68 (m, 1H, H-4′), 1.59–1.45 (m, 1H, H-5′), 1.39 (s, 3H), 1.24 (s, 3H) [H-9′, H-10′], 1.29–0.84 (m, 3H, H-3′, H-6′, H-4′), 0.92 (d, 3H, *J* 6.5, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9 (C=O), 164.1 (C=N), 151.4 (C-1″), 149.4 (C=O), 138.3 (C-7a), 129.9 (C-6), 129.1 (C-3a), 127.9 (C-3″, C-5″), 125.5 (C-2″, C-6″), 125.4 (C-5), 124.9 (C-4″), 124.0 (C-4), 115.4 (C-7), 77.6 (C-1′), 76.2 (CH_2), 65.1 (C-3), 50.9 (C-2′), 42.0 (C-6′), 39.8 (C-8′), 34.5 (C-4′), 31.7 (C-5′), 29.5 (C-9′), 26.6 (C-3′), 23.5 (C-10′), 21.9 (C-7′), 15.9 (SCH_3). MS (EI), *m*/*z* (%): 509 $[M]^+$ (4), 251 (49), 119 (100), 105 (76), 91 (57).

*4.1.14.2. 1-[(1S,2R,5S)-8-Phenylmenthoxycarbonyl]spirobrassinin (**46a**, **46b**).* Data for **46a**: yield: 0.068 g (54%), colourless oil, $[\alpha]_D^{20}$ +100.0 (*c* 0.49, $CHCl_3$), R_f 0.64 (dichloromethane/ethyl acetate 30:1). Anal. Calcd for $C_{28}H_{32}N_2O_3S_2$ requires: C, 66.11; H, 6.34; N, 5.51. Found: C, 65.91; H, 6.09; N, 5.20. Properties and spectral data of compound **46a** were fully identical with those of enantiomer **45b**.

Data for **46b**: yield: 0.015 g (12%), colourless oil, $[\alpha]_D^{20}$ +31.7 (*c* 0.26, $CHCl_3$), R_f 0.78 (dichloromethane/ethyl acetate 30:1). Anal. Calcd for $C_{28}H_{32}N_2O_3S_2$ requires: C, 66.11; H, 6.34; N, 5.51. Found: C, 66.31; H, 6.07; N, 5.28. Properties and spectral data of compound **46b** were fully identical with those of enantiomer **45a**.

*4.1.15. Enantiomers of spirobrassinin [(R)-(+)-**1**, (S)-(−)-**1**] from **44a** and **44b**.* To a stirred solution of **44a** or **44b** (0.030 g, 0.070 mmol) in dry methanol (1.0 mL) was added CH_3ONa (0.006 g, 0.104 mmol). The mixture was stirred at room temperature for 20 min, then the solvent was evaporated and the residue was purified by silica gel column flash chromatography (2.5 g, *n*-hexane/ethyl acetate 2:1) affording (*R*)-(+)-**1** [0.014 g, 82% from **44a**] and (*S*)-(−)-**1** [0.014 g, 82% from **44b**]. *(R)-(+)-Spirobrassinin [(R)-(+)-**1**]*: colourless needles, mp 156–158 °C (*n*-hexane/acetone), $[\alpha]_D^{20}$ +29.2 (*c* 0.13, $CHCl_3$), 65% ee, ECD (CH_3OH, *c* ~ 0.1 mM) λ_{ext} ($\Delta\varepsilon$): 200 (+5.7), 210 (+0.80), 218 (−10.9), 237 (+1.35), 260 (+2.37), 278 (+0.74), 305 (+2.2) nm. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H, NH), 7.36 (d, 1H, *J* 7.1, H-4), 7.26 (ddd, 1H, *J* 7.8, *J* 7.7, *J* 1.2, H-6), 7.09 (ddd, 1H, *J* 7.7, *J* 7.1, *J* 1.0, H-5), 6.94 (d, 1H, *J* 7.8, H-7), 4.68 (d, 1H, *J* 15.1, H_b), 4.51 (d, 1H, *J* 15.1, H_a), 2.62 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5 (C=O), 164.1 (C=N), 139.5 (C-7a), 131.2 (C-3a), 129.7 (C-6), 124.5 (C-4), 123.7 (C-5), 110.5 (C-7), 75.0 (CH_2), 64.7 (C-3), 15.7 (SCH_3). Spectral data of (*R*)-(+)-**1** were fully identical with those of natural product.[4] *(S)-(−)-Spirobrassinin [(S)-(−)-**1**]*: colourless needles, mp 153–154 °C (*n*-hexane/acetone), $[\alpha]_D^{20}$ −55.4 (*c* 0.13, $CHCl_3$), 92% ee, ECD (CH_3OH, *c* ~ 0.1 mM) λ_{ext} ($\Delta\varepsilon$): 200 (−11.7), 210 (−1.04), 218 (+21.26), 237 (−1.62), 260 (−5.22), 278 (−1.40), 305 (−4.42) nm. Spectral data of (*S*)-(−)-**1** were fully identical with those of natural product.[4]

*4.1.16. Enantiomers of spirobrassinin [(S)-(−)-**1**, (R)-(+)-**1**] from **45b** and **46a**.* To a stirred solution of **45b** or **46a** (0.028 g, 0.055 mmol) in dry methanol (0.5 mL) was added CH_3ONa (0.009 g, 0.165 mmol). The mixture was stirred at room temperature for 1.5 h, then the solvent was evaporated and the residue was purified by silica gel column flash chromatography (2.5 g, *n*-hexane/ethyl acetate 2:1) affording (*S*)-(−)-**1** [0.012 g, 85% from **45b**] and (*R*)-(+)-**1** [0.012 g, 85% from **46a**]. *(S)-(−)-Spirobrassinin [(S)-(−)-**1**]*: colourless needles, mp 153–154 °C (*n*-hexane/acetone), $[\alpha]_D^{20}$ −55.1 (*c* 0.12, $CHCl_3$), 91% ee, ECD (CH_3OH, *c* ~ 0.1 mM) λ_{ext} ($\Delta\varepsilon$): 200 (−15.57), 210 (−5.24), 218 (+24.22), 237 (−0.38), 260 (−5.59), 278 (−1.63), 305 (−5.32) nm. Spectral data of (*S*)-(−)-**1** were fully identical with those of natural product.[4] *(R)-(+)-Spirobrassinin [(R)-(+)-**1**]*: colourless needles, mp 152–153 °C (*n*-hexane/acetone), $[\alpha]_D^{20}$ +56.0 (*c* 0.12, $CHCl_3$), 94% ee, ECD (CH_3OH, *c* ~ 0.1 mM) λ_{ext} ($\Delta\varepsilon$): 200 (+15.57), 210 (+5.24), 218 (−24.22), 237 (+0.38), 260 (+5.59), 278 (+1.63), 305 (+5.32) nm. Spectral data of (*R*)-(+)-**1** were fully identical with those of natural product.[4]

*4.1.17. General procedure of preparation of 1-substituted derivatives of spirobrassinin **44**, **45**, **48**, **49** from 9-substituted derivatives of*

cyclobrassinin ***28***–***31***. To a stirred solution of cyclobrassinin derivatives **28**–**31** (0.075 mmol) in ethyl acetate (0.2 mL) was added solution of NaCl (0.160 mL, 0.083 mmol; the stock solution was obtained by dissolving of 0.030 g of NaCl in 1.00 mL of water) and oxone (0.051 g, 0.083 mmol). The reaction mixture was stirred for 24 h. The reaction mixture was diluted with ethyl acetate (5 mL) and water (5 mL), extracted with ethyl acetate (2×5 mL). The organic layer was washed with a saturated solution of $Na_2S_2O_3$ (1×5 mL) and dried over Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on silica gel (2.5 g, *n*-hexane/ethyl acetate 5:1 or *n*-hexane/ethyl acetate 3:1), affording mixture of diastereoisomers **44a** and **44b**, **45a** and **45b**, **48a** and **48b**, **49a** and **49b** in a 50:50 ratio.

*4.1.17.1. 1-[(1R,2S,5R)-Menthoxycarbonyl]spirobrassinin (**44a**, **44b**).* Following the general procedure, product **44** was obtained using cyclobrassinin derivative **28** (0.031 g, 0.075 mmol). Yield: 0.021 g (68%) of a mixture of diastereoisomers **44a** and **44b** in a 50:50 ratio.

*4.1.17.2. 1-[(1R,2S,5R)-8-Phenylmenthoxycarbonyl]spirobrassinin (**45a**, **45b**).* Following the general procedure, product **45** was obtained using cyclobrassinin derivative **29** (0.037 g, 0.075 mmol). Yield: 0.026 g (68%) of a mixture of diastereoisomers **45a** and **45b** in a 50:50 ratio.

*4.1.17.3. 1-[(1S)-Phenylethoxycarbonyl]spirobrassinin (**48a**, **48b**).* Following the general procedure, product **48** was obtained using cyclobrassinin derivative **30** (0.029 g, 0.075 mmol). Yield: 0.019 g (63%) of a mixture of diastereoisomers **48a** and **48b** in a 50:50 ratio, light yellow oil, R_f 0.42 (*n*-hexane/ethyl acetate 3:1). Anal. Calcd for $C_{20}H_{18}N_2O_3S_2$ requires: C, 60.28; H, 4.55; N, 7.03. Found: C, 60.52; H, 4.68; N, 7.19. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.88 (m, 2H, 2×H-7), 7.53–7.49 (m, 4H, 2×H-2″, 2×H-6″), 7.43–7.29 (m, 10H, 2×H-4, 2×H-3″, 2×H-5″, 2×H-4″, 2×H-6), 7.24–7.18 (m, 2H, 2×H-5), 6.09 (quartet, 2H, *J* 6.6, 2×CH), 4.76 (d, 1H, *J* 15.3, H_b), 4.73 (d, 1H, *J* 15.3, H_b), 4.51 (d, 1H, *J* 15.3, H_a), 4.49 (d, 1H, *J* 15.3, H_a), 2.63 (s, 3H, SCH_3), 2.62 (s, 3H, SCH_3), 1.72 (d, 3H, *J* 6.6, CH_3), 1.71 (d, 3H, *J* 6.6, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 173.4 (C=O), 163.1, 163.0 (C=N), 148.9, 148.8 (C=O), 139.5, 139.4 (C-1′), 137.1, 137.0 (C-7a), 129.0 (C-6), 127.7 (C-3′, C-5′), 128.6 (C-3a), 127.2 (C-4′), 125.2 (C-2′, C-6′), 124.7 (C-5), 123.1 (C-4), 114.3 (C-7), 75.6 (CH), 75.0, 74.9 (CH_2), 64.0, 63.9 (C-3), 21.7, 21.6 (CH_3), 14.6 (SCH_3). IR ($CHCl_3$) ν_{max} 3020, 1767 (C=O), 1727 (C=O), 1593 (C=N), 1207 cm^1. MS (EI), *m*/*z* (%): 398 $[M]^+$ (2), 249 (17), 105 (100).

*4.1.17.4. 1-[(1S)-endo-Borneoxycarbonyl]spirobrassinin (**49a**, **49b**).* Following the general procedure, product **49** was obtained using cyclobrassinin derivative **31** (0.031 g, 0.075 mmol). Yield: 0.023 g (72%) of a mixture of diastereoisomers **49a** and **49b** in a 50:50 ratio, light yellow oil, R_f 0.64 (*n*-hexane/ethyl acetate 3:1). Anal. Calcd for $C_{22}H_{26}N_2O_3S_2$ requires: C, 61.37; H, 6.09; N, 6.51. Found: C, 61.63; H, 5.83; N, 6.75. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, 2H, *J* 8.1, 2×H-7), 7.41 (dd, 2H, *J* 7.5, 1.0, 2×H-4), 7.36–7.34 (m, 2H, 2×H-6), 7.24–7.20 (m, 2H, 2×H-5), 5.10 (dd, 2H, *J* 10.0, 5.4, 2×H-2′), 4.76 (d, 1H, *J* 15.3, H_b), 4.75 (d, 1H, *J* 15.3, H_b), 4.52 (d, 1H, *J* 15.3, H_a), 4.51 (d, 1H, *J* 15.3, H_a), 2.62 (s, 6H, 2×SCH_3), 2.51–2.44 (m, 2H, 2×H-3′), 2.31–2.21 (m, 2H, 2×H-6′), 1.79–1.73 (m, 4H, 2×H-5′, 2×H-4′), 1.42–1.39 (m, 4H, 2×H-6′, 2×H-5′), 1.26–1.20 (m, 2H, 2×H-3′), 0.95 (s, 12H) [2×H-8′, 2×H-9′], 0.91 (s, 6H, 2×H-10′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6 (C=O), 163.9 (C=N), 151.2 (C=O), 138.5 (C-7a), 130.2 (C-6), 129.5 (C-3a), 125.8 (C-5), 124.3 (C-4), 115.4 (C-7), 84.8 (C-2′), 76.4 (CH_2), 65.2 (C-3), 49.3 (C-7′), 48.1 (C-1′), 45.0 (C-4′), 36.9 (C-3′), 28.2 (C-5′), 27.5 (C-6′), 19.9, 19.1 (C-8′, C-9′), 15.9 (SCH_3), 13.7 (C-10′). IR ($CHCl_3$) ν_{max} 2958, 2879, 1772 (C=O), 1734 (C=O), 1589 (C=N), 1290 cm^1. MS (EI), *m*/*z* (%): 430 $[M]^+$ (17), 250 (38), 137 (53), 81 (100).

*4.1.18. 9-[(1R,2S,5R)-Menthoxycarbonyl]-2H-3,4-dihydro-[1,3]thiazino[6,5-b]indol-2,4-dione (**47**).* A solution of compound **28** (0.021 g, 0.050 mmol) in dry dichloromethane (0.2 mL) was added to a vigorously stirred slurry of pyridinium chlorochromate (0.028 g, 0.130 mmol) in dry dichloromethane (0.2 mL). The reaction mixture was stirred for 24 h at room temperature and then diluted with dichloromethane (5 mL). After adding a small amount of silica gel, the solvent was evaporated and the residue preabsorbed on silica was subjected to silica gel column chromatography (2 g, *n*-hexane/ethyl acetate 5:1). Yield: 0.014 g (71%), light yellow oil, $[\alpha]_D^{20}$ −19.4 (*c* 0.20, $CHCl_3$), R_f 0.39 (*n*-hexane/ethyl acetate 5:1). Anal. Calcd for $C_{21}H_{24}N_2O_4S$ requires: C, 62.98; H, 6.04; N, 6.99. Found: C, 62.72; H, 6.04; N, 6.71. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H, NH), 8.35–8.33 (m, 1H, H-5), 8.11–8.08 (m, 1H, H-8), 7.44–7.42 (m, 2H, H-7, H-6), 5.01 (dt, 1H, *J* 10.9, 4.5, H-1′), 2.28–2.22 (m, 1H, H-8′), 2.07–1.99 (m, 1H, H-6′), 1.86–1.71 (m, 2H, H-3′, H-4′), 1.61–1.53 (m, 2H, H-2′, H-5′), 1.37–0.88 (m, 3H, H-3′, H-4′, H-6′), 0.99 (d, 3H, *J* 6.5), 0.98 (d, 3H, *J* 6.8) [H-9′, H-10′], 0.84 (d, 3H, *J* 6.9, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5 (C=O), 160.4 (C=O), 150.7 (C=O), 139.8 (C-8a), 136.4 (C-4b), 126.5 (C-9a), 126.2 (C-7), 125.5 (C-6), 121.5 (C-5), 115.2 (C-8), 107.9 (C-4a), 81.3 (C-1′), 47.4 (C-2′), 41.1 (C-6′), 34.1 (C-4′), 31.8 (C-5′), 26.6 (C-8′), 23.5 (C-3′), 22.1, 21.0 (C-9′, C-10′), 16.4 (C-7′). IR ($CHCl_3$) ν_{max} 3156, 3040, 2954, 2926, 2868, 1728, 1682, 1664, 1522, 1448, 1312 cm^1. MS (EI), *m*/*z* (%): 400 $[M]^+$ (16), 262 (28), 175 (44), 83 (100).

4.2. Molecular modelling computational protocols

The 2D molecular structures drawn and saved in MDL mol format were imported into Accelrys Discovery Studio[26] in order to convert them into 3D form and add hydrogens. The 'clean geometry' option of the program was used to remove the unwanted short contacts. The 3D structures thus obtained were saved in MDL mol format, consecutively. The geometries obtained in this way were imported into MAESTRO (Schrodinger, LLC) and submitted from there for ab initio minimization runs using the JAGUAR program[22] on DFT level with 6-31** basis sets. The geometries of conformers were optimized in the gas-phase. The variety of conformations was accounted for by population-weight averaged assessment of energies.[23]

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