Spirocyclization reactions and antiproliferative activity of indole phytoalexins 1-methoxybrassinin and its 1-substituted derivatives

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Abstract

The effect of the reaction temperature and the solvent on the diastereoselectivity of the spirocyclization of 1-methoxybrassinin leading to 1-methoxyspirobrassinol methyl ether was studied. 1-Acyl derivatives of 1-methoxyspirobrassinol and 1-methoxyspirobrassinol methyl ether were prepared by the bromine-mediated spirocyclization reactions of derivatives of brassinin bearing an acyl group on the indole nitrogen with water or methanol as nucleophilic agents. The cyclization of 1-acyl derivatives of brassinin afforded the *trans*-diastereoisomer as the major product, whereas using 1-methoxybrassinin afforded the *cis*- and *trans*-isomers in a ratio near to 1:1. Bromospirocyclization of brassinin and 1-methylbrassinin in the presence of methanol resulted in the formation of spirobrassinin and 1-methylspirobrassinin. The newly synthesized analogues of indole phytoalexins exhibited more significant antiproliferative activity against human leukemia cell lines than the natural phytoalexins.

Keywords: Indole phytoalexins, spirocyclization, diastereoselectivity, 1-methoxyspirobrassinol methyl ether, antiproliferative activity

Introduction

In 1940 Müller first proposed the phytoalexin concept.[1] Phytoalexins play a significant role in the defence response of plants. These secondary metabolities, which are synthesized *de novo* in response to biotic or abiotic stress, are part of the plant chemical defense mechanism.[2] Indole phytoalexins produced by crucifers were first reported in 1986 by Takasugi.[3] To date, 44 indole phytoalexins have been isolated from economically and dietary important plants of the family *Cruciferae* (syn *Brassicaceae*), which are cultivated worldwide (*e.g.* cabbage, turnip, Chinese cabbage, Japanese radish, wasabi, broccoli, rapeseed and arabidopsis).[4] The 44 cruciferous phytoalexins have been divided into six groups according to simple structural features.[4] A unique group of these natural products are spiroindoline structures containing a spirocyclic ring in the C-3 position [(*S*)-(–)-spirobrassinin [(–)-**1**],[5] (*R*)-(+)-1-methoxyspirobrassinin [(+)-**2**],[6] 1-

methoxyspirobrassinol (**3**)[7] and *trans*-(2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether [(2*R*,3*R*)-(−)-**4a**, (Figure 1)].[7] In 1987, the first spiroindoline phytoalexin (*S*)-(−)-spirobrassinin [(−)-**1**] was isolated from *Pseudomonas cichorii*-inoculated Japanese radish (*Raphanus sativus*).[5] Natural (−)-spirobrassinin [(−)-**1**] was assigned the absolute configuration (*S*) on the base of X-ray crystallographic analysis and CD studies.[8,9] (±)-Spirobrassinin [(±)-**1**] was synthesized by thionyl chloride- or methanesulfonyl chloride-mediated cyclization of (±)-dioxibrassinin.[8,9] A stereoselective synthesis of (*S*)-(−)-spirobrassinin [(−)-**1**] was achieved by bromine-induced spirocyclization of (−)-1-(8-phenylmenthoxycarbonyl)brassinin with water, followed by oxidation and removal of the chiral auxiliary.[10] (*R*)-(+)-1-Methoxyspirobrassinin [(+)-**2**] was isolated in 1994 from kohlrabi after UV irradiation[6] while *trans*-(2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether [(2*R*,3*R*)-(−)-**4a**] and optically inactive 1-methoxyspirobrassinol (**3**) were isolated in 1995 from Japanese radish after inoculation with *Pseudomonas cichorii*.[7] Compound **3** exists in solution as a mixture of two diastereoisomers *trans*-**3a** and *cis*-**3b** in a 1:4 ratio, owing to its unstable hemiaminal structure.[7] The enantiomers of (±)-1-methoxyspirobrassinin [(±)-**2**] and *trans*-(±)-1-methoxyspirobrassinol methyl ether [*trans*-(±)-**4a**] were resolved by chiral HPLC and the absolute configurations of natural (*R*)-(+)-**2** and (2*R*,3*R*)-(−)-**4a** were determined by electronic circular dichroism (ECD), vibrational circular dichroism (VCD) and chemical correlation.[11]

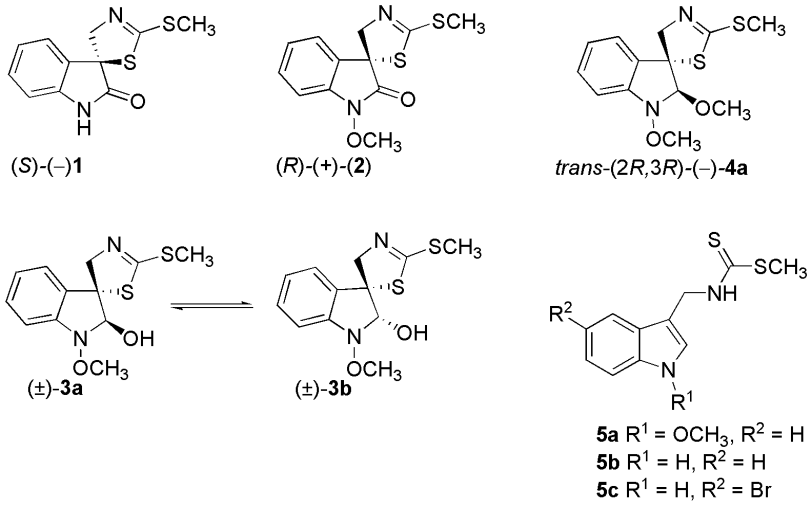


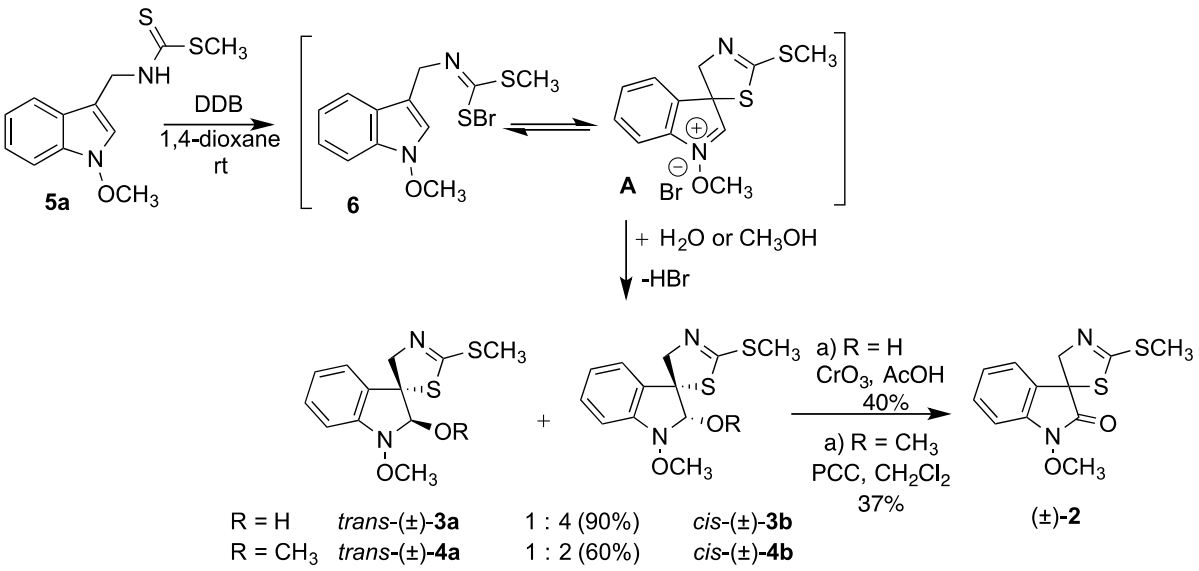
Figure 1. Selected indolic phytoalexins.

The first syntheses of 1-methoxyspirobrassinol (**3**) and 1-methoxyspirobrassinol methyl ether (**4**) were achieved by the 1,4-dioxane-dibromide (DDB)-mediated spirocyclization of 1-methoxybrassinin (**5a**) in 1,4-dioxane in the presence of water or methanol. The reaction probably proceeds via sulfenyl bromide **6**, which cyclizes to spiroindoleninium ion **A**. When 1-methoxybrassinin (**5a**) was cyclized in the presence of water, 1-methoxyspirobrassinol [*trans*-(±)-**3a**] and [*cis*-(±)-**3b**] was produced. In the presence of methanol as a nucleophile, a mixture of diastereoisomers, natural *trans*-(±)-**4a** and unnatural *cis*-diastereoisomer *cis*-(±)-**4b** in a ratio 1:2, was obtained (Scheme 1).[12] Oxidation of a mixture of diastereoisomers *trans*-

(±)-**3a** and *cis*-(±)-**3b** with CrO_3[12] or a mixture of diastereoisomers *trans*-(±)-**4a** and *cis*-(±)-**4b** with PCC afforded racemic 1-methoxyspirobrassinin [(±)-**2**].[11]

Indole phytoalexins have been previously shown to exert antibacterial, antifungal[5-7,13,14] and anticancer properties[11,15-18] and can serve as lead compounds for anticancer drug design. Brassinin (**5b**) and spirobrassinin [(±)-**1**] are effective in inhibiting the formation of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced preneoplastic lesions in a mouse mammary gland.[17] In addition, brassinin (**5b**) has been reported to exhibit dose-dependent inhibition of DMBA-induced and TPA-promoted skin carcinogenesis.[19] Izutani et al. demostrated that brassinin (**5b**) inhibits cell growth in human colon cancer cells by arresting the G_1 phase via inceased expression of p21 and p27.[20] Spiroindoline phytoalexins and their derivatives exhibit an antiproliferative effect against human cancer cell lines.[15,21-26] Brassinin (**5b**) and its synthetic derivative 5-bromobrassinin (**5c**) act as bioavailable competitive inhibitors of indoleamine 2,3-dioxygenase (IDO), a tryptophan-catabolizing enzyme that promotes tumor escape via a mechanism of immune tolerance.[27,28] 1-Methoxybrassinin (**5a**) displayed significant antiproliferative effect on intramolecular amastigotes of *Trypanosoma cruzi* and demonstrated a higher potency than shown by the currently used antichagasic agents (nifurtimox, benznidazol).[29] Kristofikova et al. documented the in vitro effect of anti-aggregation of spirobrassinin [(±)-**1**] in the cerebrospinal fluid of patients with multiple sclerosis.[30]

In this paper we describe our investigations into the diastereoselectivity of spirocyclization of 1-methoxybrassinin (**5a**) in the presence of various alcohols as nucleophiles, in various solvents and at various temperatures. We have also examined the bromine-initiated spirocyclization of 1-acyl derivatives of brassinin in the presence of water and methanol and studied the influence of acyl groups on the diastereoselectivity of bromospirocyclizations in comparison with 1-methoxybrassinin (**5a**). To our knowledge, no spiroindoline derivatives had been prepared by bromocyclization of brassinin (**5b**).

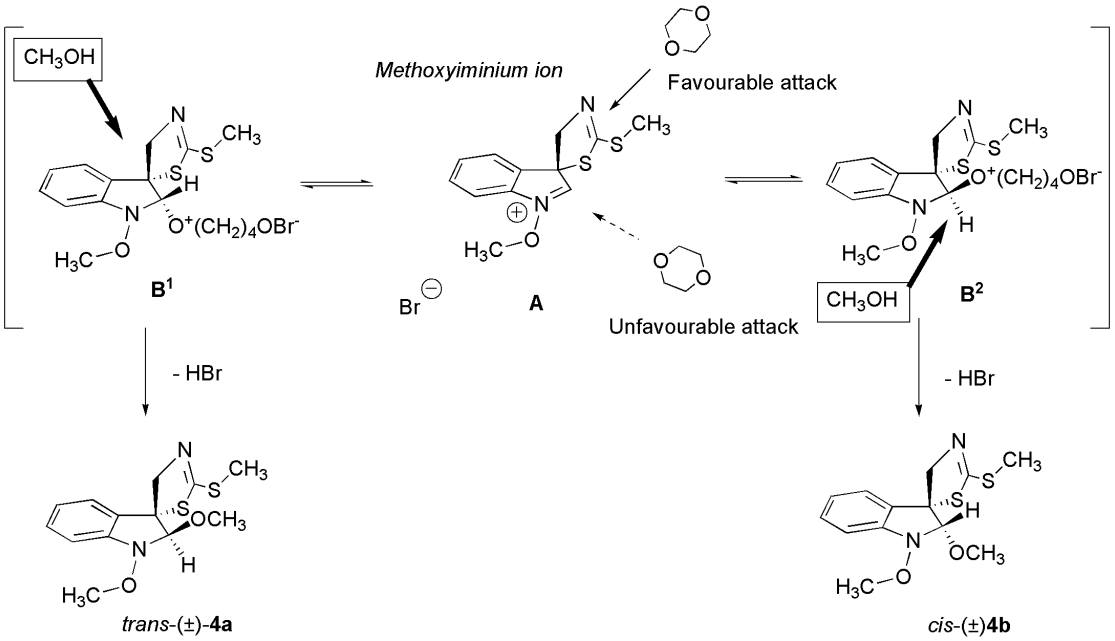


Scheme 1. Spirocyclization of 1-methoxybrassinin (**5a**) in the presence of water or methanol.

Results and Discussion

First the effect of the solvent (dichloromethane, diethyl ether, diisopropyl ether, tetrahydrofuran, 1,4-dioxane) was studied on the diastereoselectivity of the bromine-induced spirocyclization reaction of 1-methoxybrassinin (**5a**) at rt. A distinct change in diastereoselectivity was observed upon replacing 1,4-dioxane as a solvent with dichloromethane. Bromine was used instead of 1,4-dioxane dibromide as a convenient cyclization agent. The reaction mixture was stirred for 15 min at rt and then triethylamine was added to trap the hydrogen bromide liberated during the reaction. Under these conditions a mixture of isomers containing a small excess of the *trans*-diastereoisomer *trans*-(±)-**4a** was obtained (Table 1, entry 1). This result highlights the influence of the solvent on the diastereoselectivity of spirocyclization. When using 1,4-dioxane it is postulated that rather than direct addition of methanol on the methoxyiminium ion **A**, which is favourable from both sides, a solvent molecule attacks methoxyiminium ion **A** from the less hindered thiazoline CH_2 side with the formation of an unstable oxonium ion B.[2] Subsequently methanol attacks the oxonium ion **B**[2] from the sulfur side, which results in the formation of the *cis*-diastereoisomer *cis*-(±)-**4b** (Scheme 2).

The designations *trans*- and *cis*-diastereoisomers are used for differentiation of diastereoisomers. The *trans*-diastereoisomer is regarded as the one with the sulfur of thiazoline ring and methoxy group at C-2 located on the opposite sides of indoline ring, whereas the *cis*-diastereoisomer has the sulfur and 2-methoxy group on the same side of indoline ring.



Scheme 2. The mechanism of the spirocyclization of 1-methoxybrassinin (**5a**) in 1,4-dioxane.

This mechanism was also supported by cyclizations with other ethers used as solvent (diethyl ether, diisopropyl ether and tetrahydrofuran). The *trans*-diastereoisomer *trans*-(±)-**4a** was obtained as the minor product (Table 1, entries 3 and 4) using diisopropyl ether and

tetrahydrofuran, whereas the use of diethyl ether provided a 1:1 mixture of diastereoisomers *trans*-(±)-**4a** and *cis*-(±)-**4b** (Table 1, entry 2). Cyclization reactions carried out in methanol and *n*-heptane (Table 1, entries 6 and 7), in which methanol directly attacks the methoxyiminium ion **A**, provided a 1:1 mixture of *trans*-(±)-**4a** and *cis*-(±)-**4b** diastereoisomers as expected.

The effect of triethylamine on the diastereoselectivity of spirocyclization of 1-methoxybrassinin (**5a**) was also determined. Performing the spirocyclization with bromine in anhydrous dichloromethane and subsequent addition of a solution of Et_3N in methanol, 1-methoxyspirobrassinol methyl ethers were isolated in a 39:61 ratio in favor of *cis*-diastereoisomer *cis*-(±)-**4b** (Table 1, entry 8). It is postulated that in this case the Et_3N preferably approaches the intermediate methoxyiminium ion **A** from the less hindered CH_2 side of thiazoline ring with the formation of an unstable triethylammonium ion analogous to that produced from 1,4-dioxane.

Table 1. The effect of solvent on the diastereoselectivity of the spirocyclization of the 1-methoxybrassinin (**5a**) at room temperature

Entry	Conditions	Ratio[a] *trans*-(±)-**4a** : *cis*-(±)-**4b**	Yield[b] (%)
1	Br_2, CH_2Cl_2/MeOH (v:v 9:1)	54:46	65
2	Br_2, Et_2O/MeOH (v:v 9:1)	50:50	67
3	Br_2, diisopropyl ether/MeOH (v:v 9:1)	40:60	73
4	Br_2, THF/MeOH (v:v 9:1)	43:57	67
5	DDB, 1,4-dioxane/MeOH (v:v 9:1)	36:64[12]	60
6	Br_2, MeOH (v:v 9:1)	50:50	76
7	Br_2, *n*-heptane/MeOH (v:v 9:1)	50:50	67
8	Br_2, CH_2Cl_2, after 1 min. 1.1 eq. MeOH, 10 eq. Et_3N	39:61	89

[a]The ratios of diastereoisomers *trans*-(±)-**4a** : *cis*-(±)-**4b** were determined by integration of separate signals corresponding to H-2, H_a and H_b protons in the ^1H NMR spectrum of the crude product mixture.
[b]Crude product.

With the aim of influencing the diastereoselectivity of the spirocyclization of **5a**, the effect of temperature on the reaction was examined. Performing experiments above and below rt confirmed that temperature has a distinct effect on the diastereoselectivity of the spirocyclization of 1-methoxybrasssinin (**5a**). Reactions performed at low temperature led predominantly to the *trans*-diastereoisomer *trans*-(±)-**4a** (Table 2), whereas at rt or at 40-60 °C in 1,4-dioxane a preference for the *cis*-diastereoisomer *cis*-(±)-**4b** was observed. The best ratio was achieved at -70 °C in THF as solvent (Table 2, entry 16).

Table 2. The effect of temperature on the diastereoselectivity of the spirocyclization of 1-methoxybrassinin (**5a**)

Entry	Conditions	Temperature	Ratio[a] *trans*-(±)-**4a** : *cis*-(±)-**4b**	Yield[b] (%)
1	Br_2, CH_2Cl_2/MeOH (v:v 9:1)	rt	54:46	65
2		0 °C	62:38	73
3		-20 °C	65:35	73
4		-60 °C	74:26	70
5	Br_2, Et_2O/MeOH (v:v 9:1)	rt	50:50	67
6		0 °C	61:39	73
7		-20 °C	68:32	73
8		-60 °C	75:25	70
9	Br_2, diisopropyl ether/MeOH	rt	40:60	73
10	(v:v 9:1)	0 °C	61:39	70
11		-20 °C	69:31	73
12		-60 °C	72:28	73
13	Br_2, THF/MeOH (v:v 9:1)	rt	43:57	67
14		0 °C	68:32	70
15		-20 °C	70:30	70
16		-70 °C	85:15	67
17	DDB, 1,4-dioxane/MeOH (v:v	60 °C	27:73	70
18	9:1)	40 °C	28:72	70
19		rt	36:64[12]	60
20		0 °C	63:37	73
21		-20 °C	75:25	73
22	Br_2, MeOH	rt	50:50	76
23		-20 °C	61:39	73
24		-60 °C	70:30	73
25	Br_2, *n*-heptane/MeOH (v:v 9:1)	rt	50:50	67
26		-60 °C	63:37	71
27	Br_2, *n*-heptane, after 1 min. 1.1 eq. MeOH	-60 °C	70:30	63
28	Br_2, CH_2Cl_2, after 1 min.	rt	39:61	89
29	1.1 eq. MeOH, 10 eq. Et_3N	-75 °C	17:83	88

[a]The ratios of diastereoisomers *trans*-(±)-**4a** : *cis*-(±)-**4b** were determined by integration of separate signals corresponding to H-2, H_a and H_b protons in the ^{1}H NMR spectrum of the crude product mixture.
[b]Crude product.

Under these conditions a mixture of *trans*-diastereoisomer *trans*-(±)-**4a** and *cis*-diastereoisomer *cis*-(±)-**4b** was obtained in an 85:15 ratio. It is postulated that at low temperature, molecules of methanol form intermolecular hydrogen bonds with the solvent used

as well as with each other to create bulky associates. Such associates reacts with the methoxyiminium ion **A** from the less hindered thiazoline CH_2 side with the preferential formation of *trans*-diastereoisomer *trans*-(±)-**4a**.

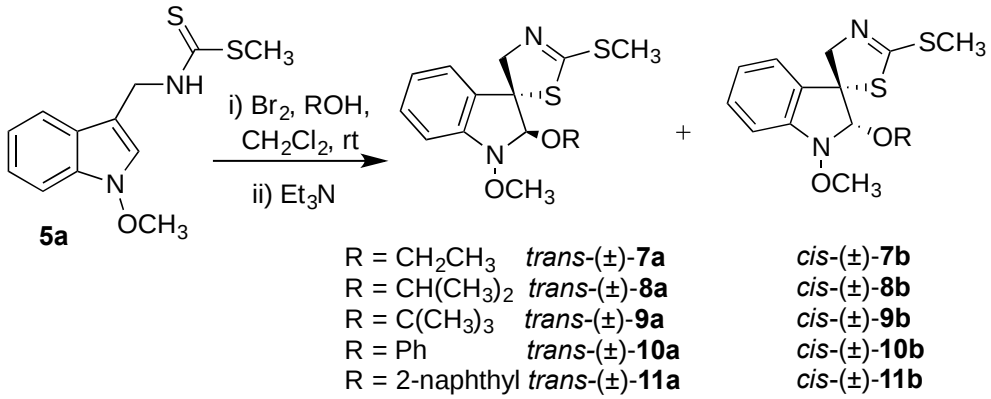
Finding the effect of solvent on the diastereoselectivity of the spirocyclization of **5a** led us to use methanol as a nucleophile in the form of a large complex. Therefore we performed the bromospirocyclization of 1-methoxybrassinin (**5a**) in anhydrous dichloromethane with sodium methoxide in the presence of 15-crown-5 ether. To a stirred mixture of 1-methoxybrassinin (**5a**) in anhydrous dichloromethane was added bromine. After stirring for one minute, a freshly prepared solution of the complex CH_3ONa/15-crown-5 in anhydrous dichloromethane was added. In the product misture, *cis*-diastereoisomer *cis*-(±)-**4b** predominated (Table 3, entry 1). Probably, the complex CH_3ONa/15-crown-5-ether was decomposed by the influence of hydrogen bromide liberated during reaction and 15-crown-5-ether liberated from the complex had the same effect on the diastereoselectivity as did 1,4-dioxane. To prevent decomposition of this complex, triethylamine (2 eq.) was added to the reaction mixture to trap the hydrogen bromide and then a solution of complex CH_3ONa/15-crown-5-ether in anhydrous dichloromethane was added. As can be seen from Table 3 (entry 2), this spirocyclization of **5a** led to formation of the *trans*-diastereoisomer *trans*-(±)-**4a** preferentially. Performing the spirocyclization with anhydrous K_2CO_3 (2 eq., Table 3, entry 3), the natural diastereoisomer of 1-methoxyspirobrassinol methyl ether *trans*-(±)-**4a** was prepared with improved diastereoselectivity in a 69:31 ratio. Probably, the complex of sodium methoxide with 15-crown-5-ether as nucleophile approaches the intermediate methoxyiminium ion **A** preferemtially from the less hindered CH_2 side of thiazoline ring and leads to formation *trans*-diastereoisomer *trans*-(±)-**4a**.

Table 3. Spirocyclization of the 1-methoxybrassinin (**5a**) in the presence of the complex MeONa/15-crown-5 ether at room temperature

Entry	Conditions	Ratio[a] *trans*-(±)-**4a** : *cis*-(±)-**4b**	Yield[b] (%)
1	Br_2, CH_2Cl_2, MeONa/15-crown-5	39:61	68
2	Br_2, CH_2Cl_2, Et_3N, MeONa/15-crown-5	64:36	71
3	Br_2, CH_2Cl_2, K_2CO_3, MeONa/15-crown-5	69:31	71[11]

[a]The ratios of diastereoisomers *trans*-(±)-**4a** : *cis*-(±)-**4b** were determined by integration of separate signals corresponding to H-2, H_a and H_b protons in the ^{1}H NMR spectrum of the crude product mixture. [b]Crude product.

We also investigated the effect of the bulkiness of alcohols (ethanol, isopropyl alcohol, *tert*-butanol, phenol and naphth-2-ol) on the diastereoselectivity of the spirocyclization of 1-methoxybrassinin (**5a**, Scheme 3). Diastereoisomers *trans*-(±)-**7a**, *cis*-(±)-**7b** and *trans*-(±)-**9a**, *cis*-(±)-**9b** were obtained in a 57:43 ratio (Table 4, entries 2 and 4). The use of isopropyl alcohol provided a 62:38 mixture of isomers *trans*-(±)-**8a**, *cis*-(±)-**8b** (Table 4, entry 3). The bromocyclization reaction of 1-methoxybrassinin (**5a**) in the presence of phenol and naphth-2-ol as nucleophiles afforded mixtures of diastereoisomers (±)-**10a**-(±)-**10b** and (±)-**11a**-(±)-**11b** with a slight excess of the *cis*-isomer (Table 4, entries 5 and 6).



Scheme 3. Cyclization reactions of 1-methoxybrassinin (**5a**) with alcohols.

Table 4. The effect of the bulkiness of alcohols (ethanol, isopropyl alcohol, *tert*-butanol, phenol and naphth-2-ol) on the diastereoselectivity of spirocyclization of 1-methoxybrassinin (**5a**) at room temperature

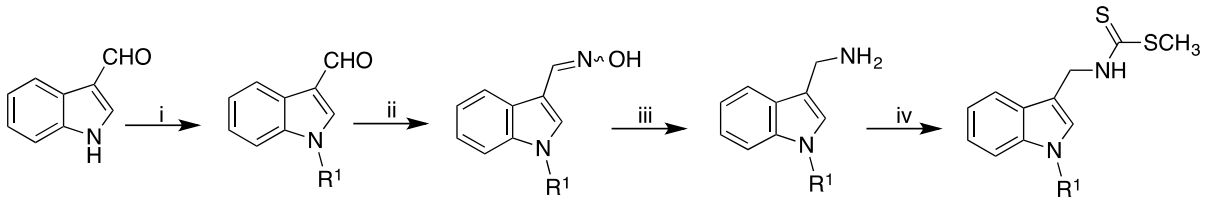
Entry	Compound	R	Ratio[a] *trans*-(±)- : *cis*-(±)-	Yield[b] (%)
1	(±)-**4a**, (±)-**4b**	Me	54:46	65
2	(±)-**7a**, (±)-**7b**	Et	57:43	68
3	(±)-**8a**, (±)-**8b**	*i*-Pr	62:38	77
4	(±)-**9a**, (±)-**9b**	*t*-Bu	57:43	71
5	(±)-**10a**, (±)-**10b**	Ph	32:68	88[23]
6	(±)-**11a**, (±)-**11b**	2-naphthyl	32:68	65

[a]The ratios of diastereoisomers (±)-**7a**:(±)-**7b**-(±)-**11a**:(±)-**11b** were determined by integration of separate signals corresponding to H-2, H_a and H_b protons in the ^{1}H NMR spectrum of the crude product mixture. [b]Crude product.

We also decided to study the influence of an acyl group on the diastereoselectivity of the bromospirocyclization reactions of 1-acylderivatives, **12**, and **23**-**25**, of brassinin. For the experiments we selected *tert*-butoxycarbonyl, acetyl, benzoyl and methoxycarbonyl groups. The key intermediate 1-Boc-brassinin (**12**) was prepared by the previously reported procedure.[31] Commercially available indole-3-carboxaldehyde (**13**) was used as a starting compound for the preparation 1-acetyl (**23**), 1-benzoyl (**24**) and 1-(methoxycarbonyl)brassinin (**25**, Scheme 4). N-Acyl aldehydes **14**-**16** were synthesized by various methods, using acetic anhydride/DMAP in THF (**14**, 91%), benzoyl chloride/Et_3N in THF (**15**, 98%) or methyl chloroformate/NaH in acetonitrile (**16**, 85%). Treatment of aldehydes **14** and **15** with hydroxylamine hydrochloride in THF in the presence of sodium acetate as the base provided oximes **17** and **18** as mixtures of *Z*- and *E*-isomers. Oxime **19** was obtained from aldehyde **16** using $NH_2OH.HCl$, Na_2CO_3, EtOH, H_2O in 80% yield. Nickel boride-catalyzed reduction of **17** using sodium borohydride afforded the unstable amine **20** which was employed as a crude product immediately after isolation. Subsequent reaction of amine **20** with CS_2 and CH_3I in

methanol in the presence of Et_3N resulted in the formation of 1-acetylbrassinin (**23**) in 22% yield. A better result was obtained, when dichloromethane was used for the extraction of amine **20** and also as a solvent in the reaction with CS_2 and CH_3I. Under these conditions 1-acetylbrassinin (**23**) was isolated in 78% yield. The reduction of oxime **18** by sodium borohydride and subsequent treatment of amine **21** with CS_2 and CH_3I in methanol afforded 1-benzoylbrassinin (**24**) in 20% yield. Replacement of the methanol as solvent by dichloromethane again improved the yield to 36% (Scheme 4). If we performed the reduction of **18** with sodium cyanoborohydride, the yield of 1-benzoylbrassinin (**24**) was 32%.

1-(Methoxycarbonyl)brassinin (**25**) was prepared from the unstable 1-(methoxycarbonyl)indole-3-ylmethyl amine (**22**, obtained by nickel boride-catalyzed reduction of **19** with sodium borohydride) by the reaction with CS_2 and CH_3I in methanol in 58% yield after two reaction steps .



13

14 $R^1 = COCH_3$ **15** $R^1 = COC_6H_5$ **16** $R^1 = COOCH_3$

17 $R^1 = COCH_3$ **18** $R^1 = COC_6H_5$ **19** $R^1 = COOCH_3$

20 $R^1 = COCH_3$ **21** $R^1 = COC_6H_5$ **22** $R^1 = COOCH_3$

23 $R^1 = COCH_3$ **24** $R^1 = COC_6H_5$ **25** $R^1 = COOCH_3$

i) (a) $R^1 = COCH_3$; $(CH_3CO)_2O$, DMAP, THF, 0 °C, 1 h, 91%; (b) $R^1 = COC_6H_5$; PhCOCl, Et_3N, THF, 0 °C, 45 min.,98%; (c) $R^1 = COOCH_3$; CH_3OCOCl, NaH, CH_3CN, rt, 10 min, 85%;

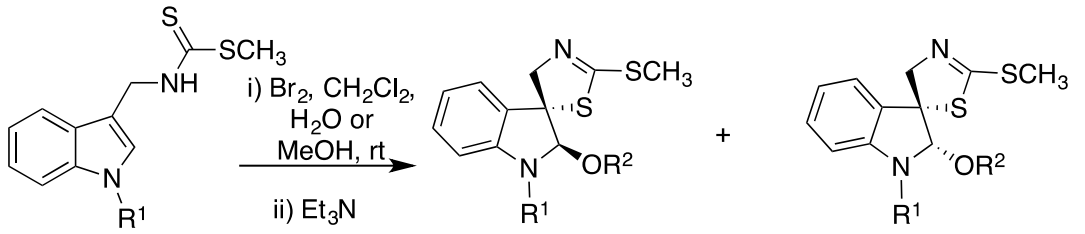
ii) (a) $R^1 = COCH_3$; $NH_2OH.HCl$, CH_3COONa, THF/H_2O, rt, 4 h, 88%; (b) $R^1 = COC_6H_5$; $NH_2OH.HCl$, CH_3COONa, THF/H_2O, rt, 4 h, 88%; (c) $R^1 = COOCH_3$; $NH_2OH.HCl$, Na_2CO_3, $EtOH/H_2O$, rt, 10 min, 80%;

iii) $NiCl_2.6H_2O$, $NaBH_4$, MeOH;

iv) (a) $R^1 = COCH_3$; Et_3N, CS_2, MeI, CH_2Cl_2, rt, 1 h, 78%; (b) $R^1 = COC_6H_5$; Et_3N, CS_2, MeI, CH_2Cl_2, rt, 30 min, 36%; (c) $R^1 = COOCH_3$; Et_3N, CS_2, MeI, MeOH, rt, 15 min, 58%.

Scheme 4. Synthesis of 1-acyl derivatives **12**, **23-25** of brassinin.

Derivatives of brassinin **12**, **23-25** were dissolved in a mixture dichloromethane/water or methanol (v/v 9:1) and then 1.1 equivalents of bromine were added (Scheme 5). After 15 minutes of stirring triethylamine was added for the neutralization of hydrogen bromide liberated by the spirocyclization. In such a way were prepared 1-acetyl- (**26**), 1-benzoyl- (**27**), 1-methoxycarbonyl- (**28**) and 1-Boc-spirobrassinol (**29**) as well as 1-acetyl- (**30**), 1-benzoyl- (**31**), 1-methoxycarbonyl- (**32**) and 1-Boc-spirobrassinol methyl ether (**33**). The ratios of diastereoisomers (±)-**26a**-(±)-**33b** and yields are summarized in Table 5. In all cases the *trans*-diastereoisomer *trans*-(±)-**26a**-(±)-**33a** was obtained in preference. *trans*-Diastereoisomer *trans*-(±)-**33a** was also the major product using 1,4-dioxane as the solvent. Cooling the reaction mixture did not change the stereoselectivity. The cyclization reaction of **12** accomplished at –60 °C also led predominantly to the *trans*-diastereoisomer *trans*-(±)-**33a** (Table 5). For comparison, Table 5 includes the ratios of diastereoisomers of 1-methoxyspirobrassinol [(±)-**3a**-(±)-**3b**] and 1-methoxyspirobrassinol methyl ether [(±)-**4a**-(±)-**4b**].



23	$R^1 = COCH_3$	*trans*-(±)-**26a**	*cis*-(±)-**26b**	$R^1 = COCH_3$	$R^2 = H$
24	$R^1 = COC_6H_5$	*trans*-(±)-**27a**	*cis*-(±)-**27b**	$R^1 = COC_6H_5$	$R^2 = H$
25	$R^1 = COOCH_3$	*trans*-(±)-**28a**	*cis*-(±)-**28b**	$R^1 = COOCH_3$	$R^2 = H$
12	$R^1 = COOC(CH_3)_3$	*trans*-(±)-**29a**	*cis*-(±)-**29b**	$R^1 = COOC(CH_3)_3$	$R^2 = H$
		trans-(±)-**30a**	*cis*-(±)-**30b**	$R^1 = COCH_3$	$R^2 = OCH_3$
		trans-(±)-**31a**	*cis*-(±)-**31b**	$R^1 = COC_6H_5$	$R^2 = OCH_3$
		trans-(±)-**32a**	*cis*-(±)-**32b**	$R^1 = COOCH_3$	$R^2 = OCH_3$
		trans-(±)-**33a**	*cis*-(±)-**33b**	$R^1 = COOC(CH_3)_3$	$R^2 = OCH_3$

Scheme 5. Bromospirocyclization of 1-acyl derivatives **12**, **23-25**

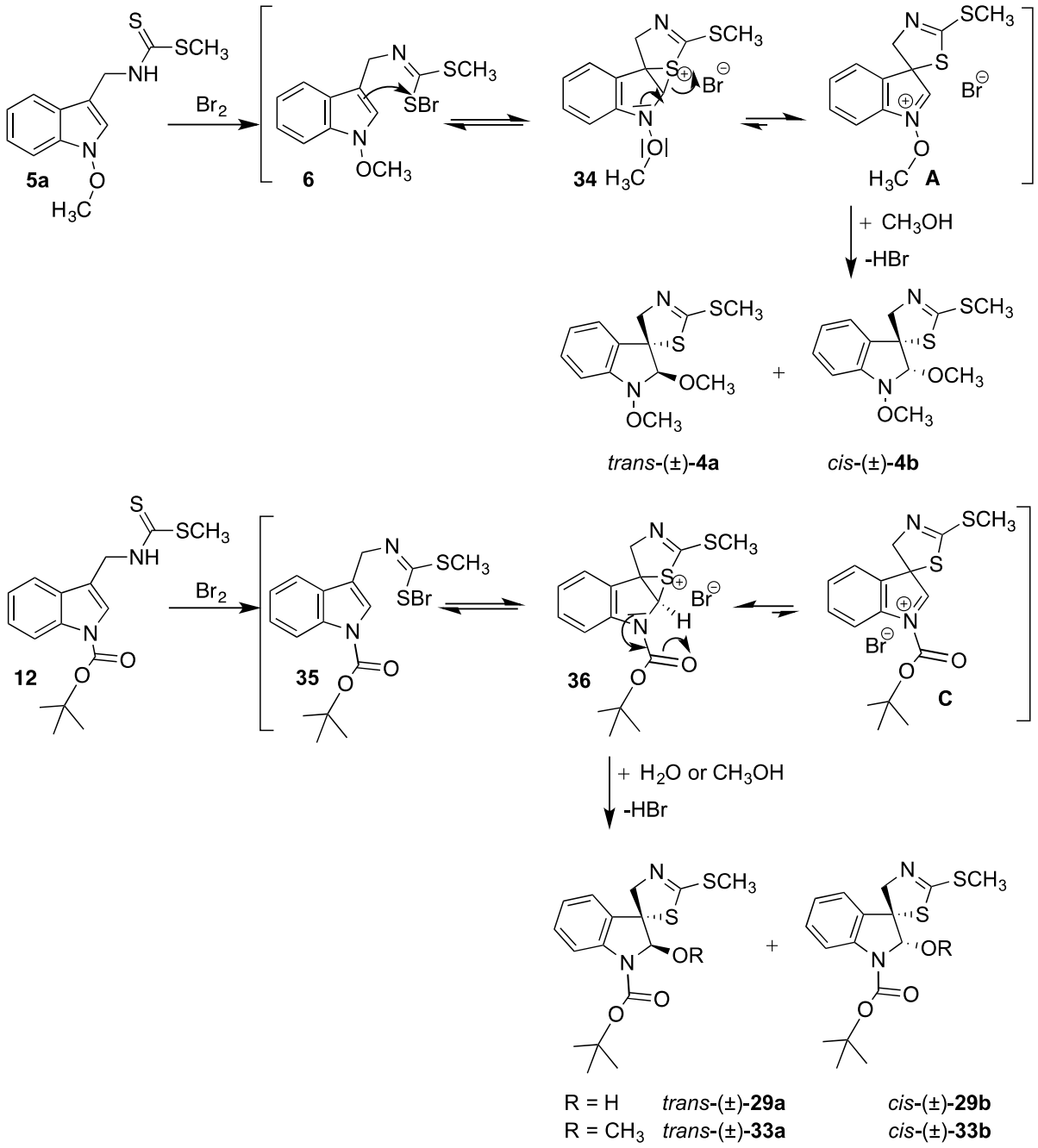
Table 5. Ratios and yields of diastereoisomers of 1-acyl derivatives of 1-methoxyspirobrassinol methyl ether (±)-**26a**-(±)-**33b**

Compounds	R^1	R^2	Conditions	Ratio[a]	Yield[b]
				trans-(±)- : *cis*-(±)-	(%)
(±)-**26a,** (±)-**26b**	$COCH_3$	H	CH_2Cl_2, rt	71:29	79
(±)-**27a,** (±)-**27b**	COC_6H_5		CH_2Cl_2, rt	64:36	77
(±)-**28a,** (±)-**28b**	$COOCH_3$		CH_2Cl_2, rt	83:17	38
(±)-**29a,** (±)-**29b**	$COOC(CH_3)_3$		CH_2Cl_2, rt	77:23	53
(±)-**29a,** (±)-**29b**	$COOC(CH_3)_3$		1,4-dioxane, rt	80:20[12]	47
(±)-**3a,** (±)-**3b**	OCH_3		CH_2Cl_2, rt	21:79	90
(±)-**3a,** (±)-**3b**	OCH_3		1,4-dioxane, rt	20:80[12]	90
(±)-**30a,** (±)-**30b**	$COCH_3$	OCH_3	CH_2Cl_2, rt	67:33	67
(±)-**31a,** (±)-**31b**	COC_6H_5		CH_2Cl_2, rt	74:26	66
(±)-**32a,** (±)-**32b**	$COOCH_3$		CH_2Cl_2, rt	71:29	49
(±)-**33a,** (±)-**33b**	$COOC(CH_3)_3$		CH_2Cl_2, rt	71:29	65
(±)-**33a,** (±)-**33b**	$COOC(CH_3)_3$		CH_2Cl_2, -60 °C	78:22	65
(±)-**33a,** (±)-**33b**	$COOC(CH_3)_3$		1,4-dioxane, rt	71:29	51
(±)-**4a,** (±)-**4b**	OCH_3		CH_2Cl_2, rt	54:46	65
(±)-**4a,** (±)-**4b**	OCH_3		CH_2Cl_2, -60 °C	74:26	70
(±)-**4a,** (±)-**4b**	OCH_3		1,4-dioxane, rt	36:64[12]	60

[a]The ratios of diastereoisomers (±)-**26a**-(±)-**33b** were determined by integration of separate signals corresponding to H-2, H_a and H_b protons in the ^1H NMR spectrum of the crude product mixture.
[b]Crude product.

Study of the bromine-mediated spirocyclization reaction of 1-methoxybrassinin (**5a**) and 1-acyl derivatives **12**, **23-25** in the presence of water or methanol revealed different

diastereoselectivity. Under the same conditions, *trans*-diastereoisomers predominated from 1-acyl derivatives, whereas with 1-methoxybrassinin (**5a**) the *cis*- and *trans*-isomers were obtained in a near 1: 1 ratio (Tables 1, 4 and 5). The diastereoselectivity can be explained by a different mechanism. In both cases reactions probably start at the thiocarbamoyl group creating a sulfenyl bromide **6**, **35**. In the case of the methoxy derivative, the sulfenyl bromide **6** undergoes electrophilic attack on the sulfur with the formation of 1-methoxyspiroindoleninium intermediate **A**. Subsequent nucleophilic addition of methanol gives spiroindoline structures *trans*-(±)-**4a**, *cis*-(±)-**4b** (Scheme 6). In the case of the 1-acyl derivatives, delocalization of the lone electron pair on nitrogen to the carbonyl group stabilizes sulfonium intermediate **36** and the nucleophile approaches from the side opposite to sulfur with formation predominantly of *trans*-diastereoisomers, *trans*-(±)-**29a**, side *trans*-(±)-**33a** (Scheme 6).



Scheme 6. Different mechanisms of the bromine-mediated spirocyclization reactions of 1-methoxybrassinin (**5a**) and 1-acyl derivatives.

The ratios of diastereoisomers (±)-**26a**-(±)-**33b** were determined by the ^{1}H NMR spectra of the crude products after dilution with dichloromethane, washing with brine, drying and evaporation of the solvent. The ratios of diastereoisomers (±)-**26a**-(±)-**33b** were determined by integration of well separated signals corresponding to the H-2, H_a and H_b protons. Chromatographic separation of the mixture of diastereoisomers of 1-methoxycarbonylspirobrassinol afforded pure *trans*-(±)-**28a** and pure *cis*-diastereoisomer *cis*-(±)-**28b** as crystalline substances. *trans*- and *cis*-Diastereoisomers **30a**,**30b**-**33a**,**33b** were separated by column chromatography. In the case of 1-acetylspirobrassinol (**26**) and 1-benzoylspirobrassinol (**27**), the *trans*- and *cis*-diastereoisomers were not separable owing to isomerization during the attempted separation on silica gel. This fact was confirmed by a simple experiment. Prepared products **26** or **27** were applied to a TLC plate and the plate was developed. After waiting for one hour, the plate was turned by 90° and developed again. Detection using UV showed that from each original spot there were now two spots for the two diastereoisomers (Figure 2). The products **26** and **27** were isolated as a mixture of *trans*- and *cis*-diastereoisomers by column chromatography. It is supposed that diastereoisomers (±)-**26a**-(±)-**26b** and (±)-**27a**-(±)-**27b** isomerize at C-2 atom like the diastereoisomers of 1-methoxyspirobrassinol [*trans*-(±)-**3a**, *cis*-(±)-**3b**]. In the case of 1-methoxyspirobrassinol (**3**), isomerization was explained by facile interconversion of hemi-aminal and aminoaldehyde.[7]

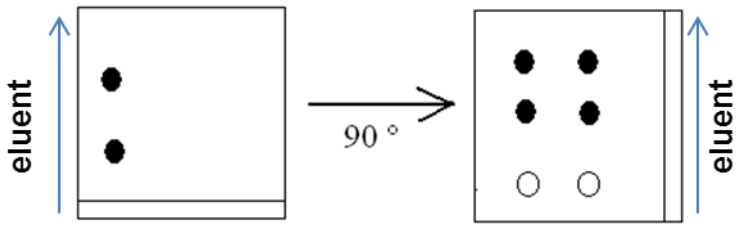


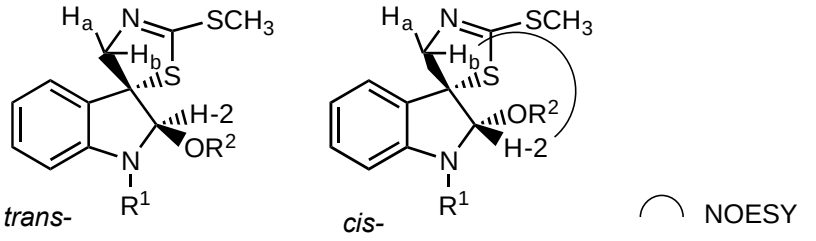
Figure 2. Evidence of isomerization of *trans*- and *cis*-diastereoisomers of 1-acetyl-(±)-**26a**-(±)-**26b** and 1-benzoylspirobrassinol (±)-**27a**-(±)-**27b**.

The structures of individual diastereoisomers were confirmed by NMR studies, including COSY, HSQC, HMBC and NOESY experiments. The *cis*-diastereoisomers **7b**-**11b** and **26b**-**33b** exhibited in their NOESY spectra a cross peak between H-2 and H_b protons confirming their *cis*-configuration. The NOESY specta of structures **7a**-**11a** and **26a**-**33a** did not show the interactions between H_b and OH or alkoxy group, which would have confirmed their *trans*-diastereoisomeric structure. However, interactions between H-2 and H_b were also not observed thus the structures of *trans*-diastereoisomer was assigned to these products.

Inspection of the ^{1}H NMR spectra of **7**-**11** and **26**-**33** revealed a significant difference in the chemical shifts between the H-2 protons of the *trans*- and *cis*-diastereoisomers. In all cases the $\delta(\text{H-2})_{trans}$ appeared at lower field compared to $\delta(\text{H-2})_{cis}$ (Table 6). The higher shielding of H-2 in the *cis*-diastereoisomers is probably caused by anisotropic shielding by the C=N double

bond of the thiazoline ring. This correlation is valid for *trans*- and *cis*-diastereoisomers of 1-methoxyspirobrassinol (**3**),[7] 1-methoxyspirobrassinol methyl ether (**4**)[7,11] and 1-Boc-spirobrassinol (**29**),[12] in which the diastereoisomeric structures were confirmed by NOE experiments. This consistent chemical shift difference was observed in $CDCl_3$ or DMSO-d_6.

Table 6. Chemical shifts of H-2 proton in *trans*- and *cis*-diastereoisomers **7a-11b** and **26a-33b**



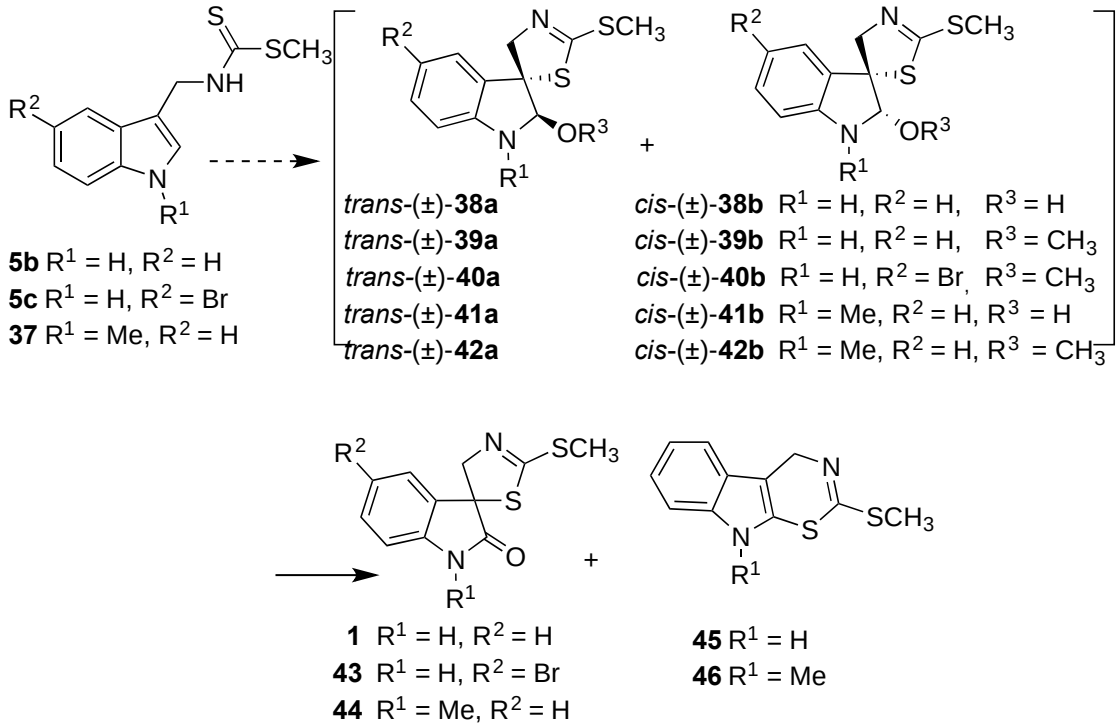
Compound	R^1	R^2	Isomer	^{1}H NMR δ(H-2) ppm
(±)-**3a**	OCH_3	H	*trans*[a]	5.30[7]
(±)-**3b**			*cis*[a]	4.80[7]
(±)-**4a**		CH_3	*trans*[a]	4.94[7]
(±)-**4b**			*cis*[a]	4.62[11]
(±)-**7a**		CH_2CH_3	*trans*[a]	5.02
(±)-**7b**			*cis*[a]	4.70
(±)-**8a**		$CH(CH_3)_2$	*trans*[a]	5.07
(±)-**8b**			*cis*[a]	4.75
(±)-**9a**		$C(CH_3)_3$	*trans*[a]	5.26
(±)-**9b**			*cis*[a]	4.93
(±)-**10a**		Ph	*trans*[a]	5.78[23]
(±)-**10b**			*cis*[a]	5.49[23]
(±)-**11a**		2-naphthyl	*trans*[a]	5.96
(±)-**11b**			*cis*[a]	5.67
(±)-**26a**	$COCH_3$	H	*trans*[a]	5.73
(±)-**26b**			*cis*[a]	5.42
(±)-**30a**		CH_3	*trans*[a]	5.41
(±)-**30b**			*cis*[a]	5.20
(±)-**27a**	COC_6H_5	H	*trans*[a]	5.95
(±)-**27b**			*cis*[a]	5.49
(±)-**31a**		CH_3	*trans*[a]	5.52
(±)-**31a**			*cis*[a]	5.22
(±)-**28a**	$COOCH_3$	H	*trans*[a]	5.95
(±)-**28b**			*cis*[a]	5.64
(±)-**32a**		CH_3	*trans*[a]	5.56
(±)-**32b**			*cis*[a]	5.29
(±)-**29a**	$COOC(CH_3)_3$	H	*trans*[b]	5.63[12]
(±)-**29b**			*cis*[b]	5.49[12]
(±)-**33a**		CH_3	*trans*[b]	5.42[31]
(±)-**33b**			*cis*[b]	5.31[31]

[a]$CDCl_3$.[b]DMSO-d_6.

Huggershoff´s oxidative bromocyclization of brassinin (**5b**) and 1-methylbrassinin (**37**) provided cyclobrassinin (**45**) or 9-methylcyclobrassinin (**46**). The formation of cyclobrassinin (**45**) or 9-methylcyclobrassinin (**46**) was achieved using various brominating agents (pyridinum tribromide **45** 34%,[3,32] NBS **45** 35%,[19] **46** 61%,[33] 1,4-dioxane dibromide **45** 45%,[12] **46** 40%,[12] phenyltrimethylammonium tribromide **45** 59%[34]). No comment was made on whether or not these cyclizations afforded spirocyclic structures as minor products.

Therefore we decided to examine the cyclization of brassinin (**5b**) and 1-methylbrassinin (**37**) using several cyclization agents (Br_2, DDB, I_2, NBS, NCS, Me_3PhNBr_3) and solvents (dichloromethane, 1,4-dioxane, methanol). Bromocyclizations of brassinin (**5b**) and 1-methylbrassinin (**37**) in dichloromethane and 1,4-dioxane with water as a nucleophile did not provide the desired spiroindoline[3,5']thiazoline derivatives (±)-**38a**-(±)-**38b** and (±)-**41a**-(±)-**41b** but only unidentified products (Table 7, entries 1,2).

Bromine-mediated cyclization of brassinin (**5b**) and 1-methylbrassinin (**37**) in the presence of methanol as a nucleophile led to the the formation of spirobrassinin [(±)-**1**] and 1-methylspirobrassinin [(±)-**44**], respectively (Table 7, entry 11). It is postulated that the initially formed unstable and nonisolable spirobrassinol methyl ether [(±)-**39a**,(±)-**39b**] and 1-methylspirobrassinol methyl ether [(±)-**42a**,(±)-**42b**] undergo oxidation with bromine to provide spirobrassinin [(±)-**1**] and 1-methylspirobrassinin [(±)-**44**] (Scheme 7). Transformation of brassinin (**5b**) into spirobrassinin [(±)-**1**] was studied with an excess of bromine. The use of 2.2 equivalents of bromine afforded 5-bromospirobrassinin [(±)-**43**] in 18% yield (Table 7, entry 15). On the basis of the low yield it is assumed that firstly, bromation takes place on the indole core of compounds (±)-**39a**-(±)-**39b** at C-5 and subsequently oxidation resulted in the formation of 5-bromospirobrassinin [(±)-**43**]. Application of four equivalents of bromine led to an increased yield (Table 7, entry 16). To prevent competitive bromination of the aromatic core, 5-bromobrassin (**5c**) was used in a cyclization with four equivalents of bromine. 5-Bromospirobrassinin [(±)-**43**] was obtained in 64% yield (Table 7, entry 19). The proposed mechanism of oxidation of spirobrassinol methyl ether [(±)-**39a**,(±)-**39b**] is depicted in Scheme 8.



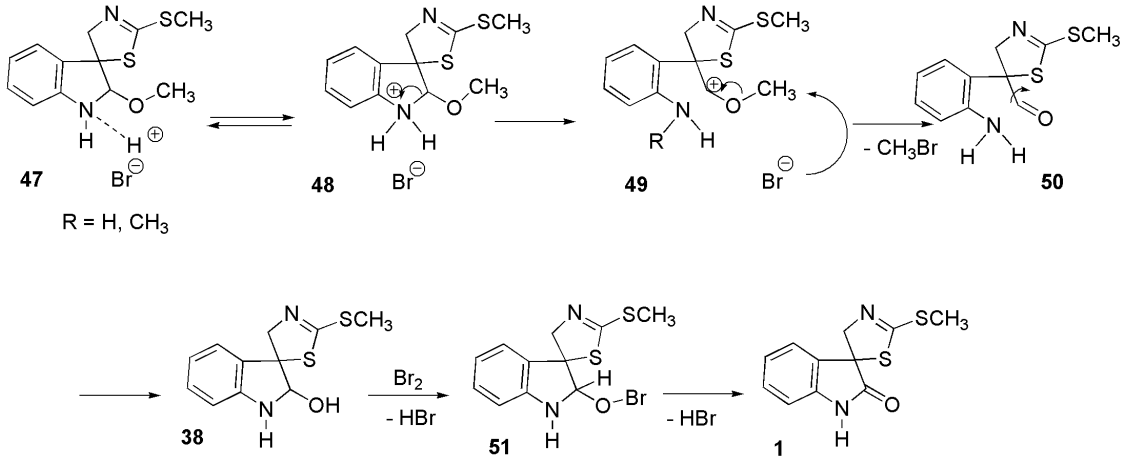
Scheme 7. Bromine-mediated cyclization of brassinin (**5b**) and 1-methylbrassinin (**37**).

Table 7. Spirocyclization of brassinin (**5b**) and 1-methylbrassinin (**37**): reaction conditions and yields

Entry	Conditions	$R^1 = H$		$R^1 = CH_3$	
		Yield (%)		Yield (%)	
		1	**45**	**44**	**46**
1	1.1eq. Br_2, CH_2Cl_2/H_2O, Et_3N, rt	decomposition	-	decomposition	-
2	1.1eq. DDB, 1,4-dioxane/H_2O, Et_3N, rt	decomposition	-	decomposition	-
3	1.1eq. Br_2, MeOH, Et_3N, rt	24	-	27	-
4	1 eq. I_2, MeOH, Et_3N, rt	decomposition	-	30	-
5	1 eq. Me_3PhNBr_3, MeOH, Et_3N, rt	18	-	13	-
6	1.1eq. Br_2, CH_2Cl_2/MeOH, Et_3N	32	-	33	-
7	1.1eq. $SOCl_2$, 1,4-dioxane/MeOH, Et_3N, rt	16	-	25	-
8	1.1eq. NBS, CH_2Cl_2/MeOH, Et_3N, rt	21	-	33	-
9	1.1eq. NCS, CH_2Cl_2/MeOH, Et_3N, rt	40	-	40	-
	1.1eq. NBS, 1,4-dioxane/MeOH, Et_3N, rt	32	-	45	-
10	1.1eq. NCS, 1,4-dioxane/MeOH, Et_3N, rt	42	-	35	-
11	1.1eq. DDB, 1,4-dioxane/MeOH, Et_3N, rt	47	-	55	-

Table 7 (continued)

12	1.1eq. DDB, 1,4-dioxane/EtOH, Et_3N, rt	39	-	68	-
13	1.1eq. DDB, 1,4-dioxane/*i*-PrOH, Et_3N, rt	31	11	65	8
14	1.1eq. DDB, 1,4-dioxane/*t*-BuOH, Et_3N, rt	-	42	60	13
15	2.2 eq. DDB, 1,4-dioxane/MeOH, Et_3N, rt	18 (**43**)	-	-	-
16	4 eq. DDB, 1,4-dioxane/MeOH, Et_3N, rt	49 (**43**)	-	-	-
17	**5c**, 1.1eq. DDB, 1,4-dioxane/MeOH, Et_3N, rt	48 (**43**)	-	-	-
18	**5c**, 2.2eq. DDB, 1,4-dioxane/MeOH, Et_3N, rt	64 (**43**)	-	-	-
19	**5c**, 4 eq. DDB, 1,4-dioxane/MeOH, Et_3N, rt	64 (**43**)	-	-	-



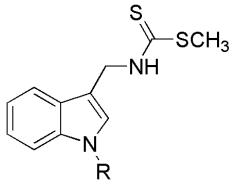
Scheme 8. A plausible reaction mechanism.

The antiproliferative effect (using the colorimetric MTT assay) of the newly synthesized substances was evaluated on six human cancer cell lines; Jurkat (acute T-lymphoblastic leukemia), MCF-7 and MDA-MB-231 (mammary gland adenocarcinomas), HeLa (cervical adenocarcinoma), CEM (acute T-lymphoblastic leukemia) and A-549 (non-small cell lung cancer). IC_{50} values for the synthesized compounds are presented in Tables 8 and 9. For comparison, Table 8 also includes IC_{50} values for conventional chemotherapeutic agents (cisplatin and etoposide) and 1-methoxybrassinin (**5a**), brassinin (**5b**), 1-Boc-brassinin (**12**) synthesized previously.

1-(Methoxycarbonyl)brassinin (**25**) displayed the highest antiproliferative activity with IC_{50} from <10 to 32.5 μmol × L^{-1} with the greatest activity in CEM cells (Table 8). 1-Benzoylbrassinin (**24**) reduced the proliferation capacity of CEM cells with IC_{50} 25.8 μmol × L^{-1}. 1-Acetylbrassinin (**23**) did not demonstrate any activity in all the cancer cell lines examined. 1-(Methoxycarbonyl)brassinin (**25**) and 1-benzoylbrassinin (**24**) exhibited more significant inhibitory effects than natural phytoalexins 1-methoxybrassinin (**5a**) and brassinin (**5b**) against all of the tested cancer lines.

2-Alkoxy analogues of 1-methoxyspirobrassinol methyl ether **7a-11b** possess relatively weak antiproliferative activity with IC_{50} values ranging from 50 to >100 μmol × L^{-1} (Table 9). Similar results were obtained with the 1-acyl analogues of 1-methoxyspirobrassinol methyl ether **26a-33b**. The highest antiproliferative effects were noted with 1-Boc-spirobrassinol [(±)-**29a**,(±)-**29b**] and 1-Boc-spirobrassinol methyl ether [(±)-**33a**,(±)-**33b**], where measured IC_{50} values 29.8–43.4 μmol × L^{-1} were obtained with leukemic cells (Jurkat and CEM).

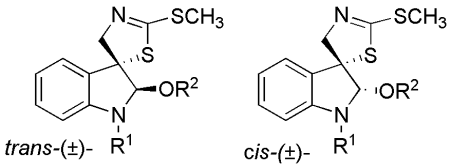
Table 8. Antiproliferative activity of 1-methoxybrassinin (**5a**) and its derivatives



Compound	R	Cancer Cell line, IC_{50} (μmol × L^{-1})					
		Jurkat	MCF-7	MDA	HeLa	CEM	A-549
5b[24]	H	>100	>100	>100	>100	90.2	>100
5a[24]	OCH_3	37.5	100	100	100	63.5	100
23	$COCH_3$	>100	>100	>100	>100	>100	>100
24	COC_6H_5	32.4	56.1	35.2	29.0	25.8	55.2
25	$COOCH_3$	32.5	32.5	32	28.5	<10	31.8
12[24]	Boc	17.8	23.0	21.4	16.9	19.6	21.4
Cisplatin		12	11.4	14.7	7.7	4.4	12.2
Etoposide		1.2	10.9	21.2	3.9	1.1	14.3

The potency of compounds was determined using the MTT (Thiazolyl Blue Tetrazolium Bromide) assay after 72 h incubation of cells and presented as IC_{50} (concentration of a given compound that decreased amount of viable cells to 50% relative to untreated control cells).

Table 9. Antiproliferative activity of 1-methoxyspirobrassinol methyl ether (**4**) and its analogues



Compound	R^1	R^2	Cancer Cell line, IC_{50} (μmol × L^{-1})					
			Jurkat	MCF-7	MDA	HeLa	CEM	A-549
trans-(±)-**4a**[24]	OCH_3	CH_3	30.2	100	100	48.9	100	100
cis-(±)-**4b**[24]			57.4	100	100	53.2	100	100
trans-(±)-**7a**		CH_2CH_3	70.4	85.6	>100	>100	83.7	85.4
cis-(±)-**7b**			>100	>100	>100	>100	>100	>100
trans-(±)-**8a**		$CH(CH_3)_2$	73.8	NT	>100	>100	>100	>100
cis-(±)-**8b**			>100	NT	>100	>100	>100	>100
trans-(±)-**9a**		$C(CH_3)_3$	50.0	NT	>100	84.8	>100	72.8
cis-(±)-**9b**			59.6	NT	>100	>100	>100	68.8
trans-(±)-**10a**[23]		Ph	100	100	100	100	100	100
cis-(±)-**10b**[23]			100	100	100	100	100	100
trans-(±)-**11a**		2-naphthyl	>100	>100	>100	>100	>100	>100
cis-(±)-**11b**			>100	>100	>100	>100	>100	>100
(±)-**26a**,**b**	$COCH_3$	H	>100	>100	>100	>100	>100	>100
trans-(±)-**30a**		CH_3	49.4	>100	>100	>100	>100	>100
cis-(±)-**30b**			>100	>100	>100	>100	>100	>100
(±)-**27a**,**b**	COC_6H_5	H	50.0	>100	>100	>100	>100	>100
trans-(±)-**31a**		CH_3	42.0	>100	>100	74.0	38.0	>100
cis-(±)-**31b**			53.0	78.0	>100	67.0	31.0	68.0
trans-(±)-**28a**	$COOCH_3$	H	>100	>100	>100	>100	>100	>100
cis-(±)-**28b**			>100	>100	>100	>100	>100	>100
trans-(±)-**32a**		CH_3	>100	>100	>100	96.0	>100	>100
cis-(±)-**32b**			>100	>100	>100	>100	>100	85.0
trans-(±)-**29a**	Boc	H	34.0	100	82.8	78.0	30.6	100
cis-(±)-**29b**			29.8	100	95.0	93.6	27.1	100
trans-(±)-**33a**[24]		CH_3	37.3	70.2	87.0	74.3	37.9	70.5
cis-(±)-**33b**[24]			43.4	100	97.7	77.6	41.9	96.3

The potency of compounds was determined using the MTT (Thiazolyl Blue Tetrazolium Bromide) assay after 72 h incubation of cells and presented as IC_{50} (concentration of a given compound that decreased amount of viable cells to 50% relative to untreated control cells).
NT not tested

Conclusions

The effect of the solvent and temperature was investigated with the aim of influencing the diastereoselectivity of the bromine-initiated spirocyclization of 1-methoxybrassinin (**5a**) with methanol. It was found that the use of ether solvents gives rise to a preference for the *cis*-diastereoisomer *cis*-(±)-**4b**, whereas at low temperature the *trans*-diastereoisomer *trans*-(±)-**4a** is preferred. The bromospirocyclization of brassinin bearing an acyl group (acetyl, benzoyl, methoxycarbonyl and *tert*-butoxycarbonyl) **12**, **23-25** on the indole nitrogen afforded predominantly the *trans*-diastereoisomer. Bromine-induced spirocyclization reactions of brassinin (**5b**) and 1-methylbrassinin (**37**) in the presence of methanol produced spirobrassinin [(±)-**1**] and 1-methylspirobrassinin [(±)-**44**]. The antiproliferative activity of the newly synthesized compounds against selected human cancer cell lines was examined. Substances **25**, (±)-**29a**, (±)-**29b**, (±)-**33a**, (±)-**33b** exhibited the highest inhibitory effects on the growth of CEM cells.

Experimental Section

General. Melting points were determined on a Koffler hot-stage apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were measured on a Varian Mercury Plus spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts (δ) are reported in ppm downfield from TMS as the internal standard and the 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. IR spectra were recorded on an IR-75 spectrometer (Zeiss Jena). Flash column chromatography was performed on the Kieselgel Merck Type 9385 at 230-400 mesh. The progress of chemical reactions was monitored by thin layer chromatography (TLC), using Macherey–Nagel plates Alugram® Sil G/UV254. Preparative column chromatography was performed on Kieselgel 60 Merck Type 9385 (0.040–0.063).

Spirocyclization of 1-methoxybrassinin (5a) in the presence of methanol *trans*-(±)- and *cis*-(±)-1-Methoxyspirobrassinol methyl ether [*trans*-(±)-4a and *cis*-(±)-4b]. **Method A** (Table 1 and Table 2): To a stirred solution of 1-methoxybrassinin (**5a**; 0.027 g, 0.1 mmol) in a mixture of anhydrous solvent/methanol (0.9 mL/0.1 mL) at rt (or 0 °C, -20 °C, -60 °C) was added a freshly prepared solution of Br_2 (0.25 mL, 0.11 mmol). The stock solution was obtained by dissolving bromine (0.04 mL) in 1.76 mL of the used solvent. The reaction mixture was stirred for 15 min, then Et_3N (0.022 g, 0.031 mL, 0.22 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with brine (2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and the crude product, obtained after evaporation of the solvent, was subjected to ^{1}H NMR spectroscopy to determine the ratio of diastereoisomers *trans*-(±)-**4a** and c*is*-(±)-**4b**.
Method B (Table 2, entries 28 and 29): To a stirred solution of 1-methoxybrassinin (**5a**; 0.027 g, 0.1 mmol) in dichloromethane (0.9 mL) at rt (or -75 °C) was added a freshly prepared

solution of Br_2 (0.25 mL, 0.11 mmol). The stock solution was obtained by dissolving bromine (0.04 mL) in anhydrous CH_2Cl_2 (1.76 mL). The reaction mixture was stirred for 1 min, then methanol (0.004 g, 0.005 mL, 0.11 mmol) and Et_3N (0.101 g, 0.139 mL, 1.00 mmol) were added. Stirring was continued for 15 min and the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with brine (2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and the crude product, obtained after evaporation of the solvent, was subjected to ^{1}H NMR spectroscopy to determine the ratio of diastereoisomers *trans*-(±)-**4a** and c*is*-(±)-**4b**.

Method C (Table 3, entry 3): To a stirred mixture of 1-methoxybrassinin (**5a**; 0.210 g, 0.79 mmol) and powdered molecular sieves (3 Å) in anhydrous CH_2Cl_2 (4.2 mL) were added powdered anhydrous K_2CO_3 (0.220 g, 1.6 mmol) and a freshly prepared solution of bromine [2.1 mL, 0.9 mmol; the stock solution was obtained by dissolving bromine (0.05 mL) in anhydrous CH_2Cl_2 (2.25 mL)]. After stirring for 1 min, a freshly prepared solution of complex CH_3ONa-15-crown-5-ether in anhydrous CH_2Cl_2 (1.9 mL, 0.90 mmol) was added. The stock solution was prepared by dissolving of CH_3ONa (0.054 g 1.0 mmol) in anhydrous MeOH (2 mL) with a subsequent addition of 15-crown-5-ether (0.220 g, 0.20 mL, 1 mmol). MeOH was thoroughly evaporated and the residue was dissolved in anhydrous CH_2Cl_2 (2 mL). Stirring was continued for 10 min, and the reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with brine (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on 25 g silica gel (*n*-hexane/Et_2O 3:1), affording natural diastereoisomer *trans*-(±)-**4a** (0.086 g, 37%) and unnatural *cis*-(±)-**4b** (0.026 g, 19%).

The spectral data were identical with those of the natural product *trans*-(±)-**4a**[7] and unnatural product *cis*-(±)-**4b**.[11]

Spirocyclization of 1-methoxybrassinin (5a) in the presence of ethanol

***trans*-(±)- and *cis*-(±)-1-Methoxyspirobrassinol ethyl ether [*trans*-(±)-7a and *cis*-(±)-7b].** To a stirred solution of 1-methoxybrassinin (**5a**; 0.081 g, 0.3 mmol) in a mixture of anhydrous CH_2Cl_2/EtOH (2.7 mL/0.3 mL) at rt was added a freshly prepared solution of Br_2 (0.77 mL, 0.33 mmol). The stock solution was obtained by dissolving of bromine (0.04 mL) in 1.76 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 15 min, then Et_3N (0.067 g, 0.09 mL, 0.66 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with brine (2 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (10 g, *n*-hexane/Me_2CO 5:1) and diastereoisomers *trans*-(±)-**7a**, *cis*-(±)-**7b** were separated.

***trans*-(±)-1-Methoxyspirobrassinol ethyl ether [*trans*-(±)-7a].** Yield: 0.036 g (39%), bright yellow oil, R_f 0.62 (*n*-hexane/Me_2CO 5:1). Anal. Calcd for $C_{14}H_{18}N_2O_2S_2$ requires: C, 54.17; H, 5.84; N, 9.02. Found: C, 54.39; H, 6.01; N, 9.23. MS (EI), *m/z* (%): 310 $[M]^+$ (8), 279 (83), 251 (30), 117 (100). IR ($CHCl_3$) ν_{max}: 3007, 1567 (C=N), 1460, 1380, 1193 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.22 (m, 2H, H-4, H-6), 7.01 (ddd, *J* 7.5, *J* 7.5, *J* 1.0, 1H, H-5), 6.93 (d, *J* 7.8, 1H, H-7), 5.02 (s, 1H, H-2), 4.97 (d, *J* 15.3, 1H, H_b), 3.98 (dq, *J* 9.7, *J* 7.0, 1H, $\underline{CH_2}CH_3$), 3.95 (s, 3H, N-OCH_3), 3.89 (d, *J* 15.3, 1H, H_a), 3.82 (dq, *J* 9.7, *J* 7.0, 1H, $\underline{CH_2}CH_3$), 2.57 (s, 3H, SCH_3), 1.30 (t, *J* 7.0, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4 (C=N),

148.3 (C-7a), 129.8 (C-6), 127.9 (C-3a), 124.1 (C-4), 123.9 (C-5), 113.1 (C-7), 107.8 (C-2), 70.1 (CH_2), 69.1 (C-3), 67.9 ($\underline{CH_2}CH_3$), 64.1 (N-OCH_3), 15.8 ($CH_2\underline{CH_3}$), 15.2 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_a/H-4, H-6/H-7, H-4/H-5.

***cis*-(±)-1-Methoxyspirobrassinol ethyl ether [*cis*-(±)-7b].** Yield: 0.027 g (29%), bright yellow oil, R_f 0.43 (*n*-hexane/Me_2CO 5:1). Anal. Calcd for $C_{14}H_{18}N_2O_2S_2$ requires: C, 54.17; H, 5.84; N, 9.02. Found: C, 53.86; H, 5.67; N, 9.18. MS of compound *cis*-(±)-**7b** was fully identical with MS of *trans*-(±)-**7a** diastereoisomer. IR ($CHCl_3$) ν_{max}: 3013, 1560 (C=N), 1447, 1380, 1193 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.28-7.24 (m, 2H, H-6, H-4), 7.01 (ddd, *J* 7.5, *J* 7.5, *J* 0.7, 1H, H-5), 6.93 (d, *J* 7.7, 1H, H-7), 4.70 (s, 1H, H-2), 4.49 (d, *J* 15.2, 1H, H_a), 4.31 (d, *J* 15.2, 1H, H_b), 3.98 (dq, *J* 7.1, *J* 9.5, 1H, $\underline{CH_2}CH_3$), 3.95 (s, 3H, N-OCH_3), 3.81 (dq, *J* 7.1, *J* 9.5, 1H, $\underline{CH_2}CH_3$), 2.56 (s, 3H, SCH_3), 1.32 (t, *J* 7.1, 3H, $CH_2\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9 (C=N), 147.9 (C-7a), 130.1 (C-6), 128.6 (C-3a), 124.1 (C-5), 123.3 (C-4), 112.9 (C-7), 104.5 (C-2), 73.1 (CH_2), 70.4 (C-3), 67.9 ($\underline{CH_2}CH_3$), 64.1 (N-OCH_3), 15.8 ($CH_2\underline{CH_3}$), 15.3 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H-2/H_b, H-6/H-7, H-4/H-5.

Spirocyclization of 1-methoxybrassinin (5a) in the presence of isopropyl alcohol

***trans*-(±)- and *cis*-(±)-1-Methoxyspirobrassinol isopropyl ether [*trans*-(±)-8a and *cis*-(±)-8b].** To a stirred solution of 1-methoxybrassinin (**5a**; 0.081 g, 0.3 mmol) in a mixture of anhydrous CH_2Cl_2/*i*-PrOH (2.7 mL/0.3 mL) at rt was added a freshly prepared solution of Br_2 (0.77 mL, 0.33 mmol). The stock solution was obtained by dissolving of bromine (0.04 mL) in 1.76 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 15 min, then Et_3N (0.067 g, 0.09 mL, 0.66 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with brine (2 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (10 g, *n*-hexane/Me_2CO 5:1) and diastereoisomers *trans*-(±)-**8a**, *cis*-(±)-**8b** were separated.

***trans*-(±)-1-Methoxyspirobrassinol isopropyl ether [*trans*-(±)-8a].** Yield: 0.046 g (47%), bright yellow oil, R_f 0.59 (*n*-hexane/Me_2CO 5:1). Anal. Calcd for $C_{15}H_{20}N_2O_2S_2$ requires: C, 55.53; H, 6.21; N, 8.63. Found: C, 55.81; H, 6.47; N, 8.35. MS (EI), *m/z* (%): 324 $[M]^+$ (7), 293 (30), 251 (93), 117 (60), 43 (100). IR ($CHCl_3$) ν_{max}: 2980, 1547, 1373, 1187 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* 7.7, 1H, H-4), 7.23 (ddd, *J* 7.7, *J* 7.7, *J* =1.2, 1H, H-6), 7.00 (ddd, *J* 7.7, *J* 7.7, *J* =1.0, 1H, H-5), 6.92 (d, *J* 7.7, 1H, H-7), 5.07 (s, 1H, H-2), 4.99 (d, *J* 15.2, 1H, H_b), 4.06 [sep, *J* 6.1, 1H, $\underline{CH}(CH_3)_2$], 3.95 (s, 3H, N-OCH_3), 3.85 (d, *J* 15.2, 1H, H_a), 2.57 (s, 3H, SCH_3), 1.31 [d, *J* 6.1, 3H, $CH(\underline{CH_3})_2$], 1.25 [d, *J* 6.1, 3H, $CH(\underline{CH_3})_2$]. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3 (C=N), 148.6 (C-7a), 129.6 (C-6), 127.9 (C-3a), 124.3 (C-4), 123.9 (C-5), 112.5 (C-7), 106.3 (C-2), 76.3 [$\underline{CH}(CH_3)_2$], 70.3 (CH_2), 69.7 (C-3), 64.4 (N-OCH_3), 24.1 and 24.0 [$CH(\underline{CH_3})_2$], 15.2 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_a/H-4, $OCH_3/CH(\underline{CH_3})_2$, H-6/H-7, H-4/H-5.

***cis*-(±)-1-Methoxyspirobrassinol isopropyl ether [*cis*-(±)-8b].**Yield: 0.029 g (30%), bright yellow oil, R_f 0.40 (*n*-hexane/Me_2CO 5:1). Anal. Calcd for $C_{15}H_{20}N_2O_2S_2$ requires: C, 55.53; H, 6.21; N, 8.63. Found: C, 55.72; H, 5.96; N, 8.85. MS of compound *cis*-(±)-**8b** was fully identical with MS of *trans*-(±)-**8a** diastereoisomer. IR ($CHCl_3$) ν_{max}: 2973, 1563, 1367, 1187,

1106 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.28-7.24 (m, 2H, H-6, H-4), 7.00 (ddd, *J* 7.6, *J* 7.6, *J* 1.1, 1H, H-5), 6.93 (dd, *J* 8.2, *J* 1.0, 1H, H-7), 4.75 (s, 1H, H-2), 4.48 (d, *J* 15.2, 1H, H_a), 4.32 (d, *J* 15.2, 1H, H_b), 3.98 [sep, *J* 6.1, 1H, $\underline{CH}(CH_3)_2$], 3.94 (s, 3H, N-OCH_3), 2.55 (s, 3H, SCH_3), 1.34 [d, *J* 6.1, 3H, $CH(\underline{CH_3})_2$], 1.26 [d, *J* 6.1, 3H, $CH(\underline{CH_3})_2$]. ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0 (C=N), 148.2 (C-7a), 130.6 (C-6), 127.7 (C-3a), 123.8 (C-5), 123.4 (C-4), 112.7 (C-7), 103.2 (C-2), 74.1 [$\underline{CH}(CH_3)_2$], 72.8 (CH_2), 70.5 (C-3), 64.2 (N-OCH_3), 22.8 and 22.7 [$CH(\underline{CH_3})_2$], 15.3 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H-2/H_b, $\underline{CH}(CH_3)_2/CH(\underline{CH_3})_2$, H-6/H-7, H-4/H-5.

Spirocyclization of 1-methoxybrassinin (5a) in the presence of *tert*-butanol

***trans*-(±)- and *cis*-(±)-1-Methoxyspirobrassinol *tert*-butyl ether [*trans*-(±)-9a and *cis*-(±)-9b].** To a stirred solution of 1-methoxybrassinin (**5a**; 0.081 g, 0.3 mmol) in a mixture of anhydrous CH_2Cl_2/*t*-BuOH (2.7 mL/0.3 mL) at rt was added a freshly prepared solution of Br_2 (0.77 mL, 0.33 mmol). The stock solution was obtained by dissolving of bromine (0.04 mL) in 1.76 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 15 min, then Et_3N (0.067 g, 0.09 mL, 0.66 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with brine (2 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (10 g, *n*-hexane/Me_2CO 5:1) and diastereoisomers *trans*-(±)-**9a**, *cis*-(±)-**9b** were separated.

***trans*-(±)-1-Methoxyspirobrassinol *tert*-butyl ether [*trans*-(±)-9a].** Yield: 0.042 g (41%), bright yellow oil, R_f 0.64 (*n*-hexane/Me_2CO 5:1). Anal. Calcd for $C_{16}H_{22}N_2O_2S_2$ requires: C, 56.77; H, 6.55; N, 8.28. Found: C, 56.52; H, 6.74; N, 8.06. MS (EI), *m/z* (%): 338 $[M]^+$ (2), 251 (100), 57 (77). IR ($CHCl_3$) ν_{max}: 3000, 1560, 1387, 1186, 1120 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.30-7.28 (m, 1H, H-4), 7.22 (ddd, *J* 7.5, *J* 7.5, *J* 1.2, 1H, H-6), 6.99 (ddd, *J* 7.5, *J* 7.5, *J* 1.0, 1H, H-5), 6.98 (m, 1H, H-7), 5.26 (s, 1H, H-2), 5.07 (d, *J* 15.2, 1H, H_b), 3.92 (s, 3H, N-OCH_3), 3.86 (d, *J* 15.2, 1H, H_a), 2.53 (s, 3H, SCH_3), 1.35 [s, 9H, $C(CH_3)_3$]. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3 (C=N), 148.6 (C-7a), 129.6 (C-6), 127.9 (C-3a), 124.3 (C-4), 123.6 (C-5), 112.5 (C-7), 101.0 (C-2), 76.3 [$\underline{C}(CH_3)_3$], 70.3 (CH_2), 69.7 (C-3), 64.4 (N-OCH_3), 29.3 [$C(\underline{CH_3})_3$], 15.2 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_a/H-4, H-2/$C(\underline{CH_3})_3$, H-6/H-7, H-4/H-5.

***cis*-(±)-1-Methoxyspirobrassinol *tert*-butyl ether [*cis*-(±)-9b].** Yield: 0.031 g (30%), bright yellow oil, R_f 0.51 (*n*-hexane/Me_2CO 5:1). Anal. Calcd for $C_{16}H_{22}N_2O_2S_2$ requires: C, 56.77; H, 6.55; N, 8.28. Found: C, 56.94; H, 6.37; N, 8.12. MS of compound *cis*-(±)-**9b** was fully identical with MS of *trans*-(±)-**9a** diastereoisomer. IR ($CHCl_3$) ν_{max}: 3020, 1500, 1400, 1200, 913, 720, 660 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.28-7.23 (m, 2H, H-6, H-4), 7.01-6.97 (m, 1H, H-5), 6.93-6.91 (m, 1H, H-7), 4.93 (s, 1H, H-2), 4.43 (d, *J* 15.4, 1H, H_a), 4.37 (d, *J* 15.4, 1H, H_b), 3.91 (s, 3H, N-OCH_3), 2.54 (s, 3H, SCH_3), 1.35 [s, 9H, $C(CH_3)_3$]. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8 (C=N), 148.8 (C-7a), 129.9 (C-6), 126.9 (C-3a), 123.5 (C-5, C-4), 112.5 (C-7), 98.7 (C-2), 76.3 [$\underline{C}(CH_3)_3$], 72.4 (CH_2), 71.3 (C-3), 64.3 (N-OCH_3), 28.9 [$C(\underline{CH_3})_3$], 15.3 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H-2/H_b, H-2/$C(\underline{CH_3})_3$, H-6/H-7, H-4/H-5.

Spirocyclization of 1-methoxybrassinin (5a) in the presence of naphth-2-ol

***trans*-(±)- and *cis*-(±)-1-Methoxyspirobrassinol naphth-2-yl ether [*trans*-(±)-11a and *cis*-(±)-11b].** To a stirred solution of 1-methoxybrassinin (**5a**; 0.054 g, 0.2 mmol) in anhydrous CH_2Cl_2 (3 mL) at rt was added a freshly prepared solution of Br_2 (0.52 mL, 0.22 mmol). The stock solution was obtained by dissolving of bromine (0.04 mL) in 1.76 mL of anhydrous CH_2Cl_2. After stirring for 1 min, the solution of naphth-2-ol (0.032 g, 0.22 mmol) and triethylamine (0.202 g, 0.279 mL, 2.0 mmol) in anhydrous CH_2Cl_2 (3 mL) was added. Stirring was continued for 15 min, then the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 1M HCl (5 mL) and brine (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (10 g, *n*-hexane/EtOAc 3:1) and diastereoisomers *trans*-(±)-**11a**, *cis*-(±)-**11b** were separated. *trans*-Diastereoisomer *trans*-(±)-**11a** contained small amount of naphth-2-ol as an impurity which was removed by repeated chromatography on silica gel (20 g, *n*-hexane/Me_2CO 1:1).

***trans*-(±)-1-Methoxyspirobrassinol naphth-2-yl ether [*trans*-(±)-11a].** Yield: 0.016 g (20%), bright yellow oil, R_f 0.68 (*n*-hexane/EtOAc 3:1). Anal. Calcd for $C_{22}H_{20}N_2O_2S_2$ requires: C, 64.68; H, 4.93; N, 6.86. Found: C, 64.49; H, 4.61; N, 6.61. IR ($CHCl_3$) ν_{max}: 3054, 2929, 2847, 1585, 1462, 1212, 941, 746 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.82-7.75 (m, 3H, H-arom), 7.65-7.58 (m, 1H, H-arom), 7.48 -7.29 (m, 5H, H-arom), 7.14-7.02 (m, 2H, H-arom), 5.96 (s, 1H, H-2), 5.25 (d, *J* 15.4, 1H, H_b), 4.06 (d, *J* 15.4, 1H, H_a), 3.91 (s, 3H, N-OCH_3), 2.50 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8 (C=N), 155.9 (C-arom), 147.9 (C-arom), 134.3 (C-arom), 129.9 (CH-arom), 129.6 (CH-arom), 127.8 (C-arom), 127.6 (CH-arom), 127.2 (CH-arom), 126.9 (C-arom), 126.4 (CH-arom), 124.5 (CH-arom), 124.0 (CH-arom), 123.9 (CH-arom), 119.5 (CH-arom), 113.0 (CH-arom), 112.4 (CH-arom), 107.4 (C-2), 70.3 (CH_2), 69.4 (C-3), 64.1 (N-OCH_3), 15.0 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b.

***cis*-(±)-1-Methoxyspirobrassinol naphth-2-yl ether [*cis*-(±)-11b].** Yield: 0.037 g (45%), bright yellow oil, R_f 0.55 (*n*-hexane/EtOAc 3:1). Anal. Calcd for $C_{22}H_{20}N_2O_2S_2$ requires: C, 64.68; H, 4.93; N, 6.86. Found: C, 64.45; H, 4.72; N, 6.58. IR ($CHCl_3$) ν_{max}: 3054, 2929, 2847, 1585, 1462, 1212, 941, 746 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.81-7.68 (m, 3H, H-arom), 7.63-7.56 (m, 1H, H-arom), 7.48 -7.24 (m, 5H, H-arom), 7.11-7.01 (m, 2H, H-arom), 5.67 (s, 1H, H-2), 4.46 (d, *J* 15.3, 1H, H_a), 4.35 (d, *J* 15.3, 1H, H_b), 3.88 (s, 3H, N-OCH_3), 2.53 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9 (C=N), 155.9 (C-arom), 147.3 (C-arom), 134.2 (C-arom), 130.1 (CH-arom), 130.0 (CH-arom), 128.8 (C-arom), 127.7 (CH-arom), 127.2 (CH-arom), 126.5 (CH-arom), 126.3 (C-arom), 124.6 (CH-arom), 124.0 (CH-arom), 123.3 (CH-arom), 119.6 (CH-arom), 112.8 (CH-arom), 112.4 (CH-arom), 103.1 (C-2), 72.6 (CH_2), 70.7 (C-3), 63.9 (N-OCH_3), 15.1 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H-2/H_b,

1-Acetylindole-3-carboxaldehyde (14). To a solution of indole-3-carboxaldehyde (**13**; 2.90 g, 20.0 mmol) in THF (66 mL) at 0 ºC was added Ac_2O (6.12 g, 5.6 mL, 60.0 mmol) and catalytic amount of DMAP. The reaction mixture was stirred for 1 h at rt. After the reaction was finished, THF was evaporated. The residue was dissolved in CH_2Cl_2 (120 ml) and the solution washed with 5% solution of KOH (100 mL), 1M HCl (100 ml) and H_2O (80 ml). After drying over anhydrous Na_2SO_4 and evaporation of solvent, aldehyde **14** was obtained by

crystallization from the hot EtOH. Yield: 3.42 g (91%), bright yellow crystals, R_f 0.47 (*n*-hexane/Me_2CO 2:1), m.p. 165-166 ºC (hot ethanol), lit.[35] 167-169 ºC (*n*-hexane/EtOAc). Spectral and analytical data are consistent with literature values.[35]

1-Benzoylindole-3-carboxaldehyde (15). To a solution of indole-3-carboxaldehyde (**13**; 3.0 g, 20.0 mmol) in THF (70 mL) at 0 ºC was added Et_3N (10.12 g, 14.0 mL, 100 mmol). The reaction mixture was stirred at 0 ºC for 10 min. After that, PhCOCl (3.93 g, 3.25 mL, 28.0 mmol) was added and the reaction mixture was stirred at 0 ºC for 45 min. After the reaction was finished, THF was evaporated. The residue obtained after evaporation of the solvent was subjected to column chromatography (30 g silica gel, *n*-hexane/EtOAc 4:1). The obtained compound was further crystallized from CH_2Cl_2/*n*-hexane to afford aldehyde **15**. Yield: 4.88 g (98%), white crystals, R_f 0.56 (*n*-hexane/Me_2CO 2:1), m.p. 68-71 ºC (CH_2Cl_2/*n*-hexane). Anal. Calcd for $C_{16}H_{11}NO_2$ requires: C, 77.10; H, 4.45; N, 5.62. Found: C, 76.77; H, 4.69; N, 5.41. MS (EI), *m/z* (%): 249 $[M]^+$ (43), 105 $[C_6H_5C{=}O]^+$ (100), 77 $[C_6H_5]^+$ (79). IR ($CHCl_3$) ν_{max}: 3026, 1686 (C=O), 1673 (C=O), 1440, 706 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H, CHO), 8.32-8.30 (m, 1H, H-7), 8.12-8.10 (m, 1H, H-4), 7.94 (s, 1H, H-2) , 7.78-7.76 (m, 2H, H-2´, H-6´), 7.70-7.66 (m, 1H, H-4´), 7.62-7.56 (m, 2H, H-3´, H-5´), 7.49-7.42 (m, 2H, H-5, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.8 (CHO), 168.5 (C=O), 137.6 (C-2), 136.8 (C-1´), 133.0 (C-4´), 129.4 (C-2´, C-6´), 129.3 (C-7a), 129.0 (C-3´, C-5´), 126.6 (C-6), 126.2 (C-3a), 125.6 (C-5), 122.2 (C-3), 122.0 (C-4), 116.1 (C-7).

1-Methoxycarbonylindole-3-carboxaldehyde (16). To a suspension of NaH (2.4 g, 60.0 mmol, 60% suspension in mineral oil) in anhydrous MeCN (60 mL) was added indole-3-carboxaldehyde (**13**; 2.17 g, 15.0 mmol). After stirring for 5 min at rt, methyl chloroformate (2.83 g, 2.3 mL, 30.0 mmol) was added. The reaction mixture was stirred for 10 min, then poured into cold water (200 mL) and the product was extracted with EtOAc (1 × 150 mL and 1 × 100 mL). The extract was dried over Na_2SO_4. The residue obtained after evaporation of the solvent was crystallized from CH_2Cl_2/*n*-hexane to afford aldehyde **16**. Yield: 2.59 g (85%), bright yellow crystals, R_f 0.54 (*n*-hexane/Me_2CO 2:1), m.p. 94-96 ºC (CH_2Cl_2/*n*-hexane). Anal. Calcd for $C_{11}H_9NO_3$ requires: C, 65.02; H, 4.46; N, 6.89. Found: C, 64.79; H, 4.61; N, 6.73. MS (EI), *m/z* (%): 203 $[M]^+$ (100), 158 (78), 130 (47), 116 (81), 89 (35), 59 $[CH_3OCO]^+$ (37). IR ($CHCl_3$) ν_{max}: 3016, 1755 (C=O), 1673 (C=O), 1440, 1345, 1226, 1096 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 10.08 (s, 1H, CHO), 8.28 (dd, *J* 7.3, *J* 1.4, 1H, H-4), 8.22 (s, 1H, H-2), 8.16 (d, *J* 7.3, 1H, H-7), 7.43 (ddd, *J* 7.3, *J* 7.3, *J* 1.4, 1H, H-6), 7.38 (ddd, *J* 7.3, *J* 7.3, *J* 1.1, 1H, H-5), 4.11 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9 (CHO), 145.9 (C=O), 131.2 (C-2), 131.1 (C-7a), 121.5 (C-6), 121.1 (C-3a), 120.1 (C-5), 117.5 (C-3), 117.4 (C-4), 110.3 (C-7), 49.8 (CH_3).

1-Acetylindole-3-carboxaldehyde oxime (17). To a stirred solution of aldehyde (**14**; 3.42 g, 18.3 mmol) in THF (80 mL) was added a solution of hydroxylammonium chloride (1.98 g, 28.5 mmol) and NaOAc (1.72 g, 12.6 mmol) in water (14 mL) and the mixture was stirred for 4 h at rt. After evaporation of THF and addition of water (80 mL), the oxime **17** was extracted with EtOAc (1 × 350 mL, 1 × 250 mL). The extract was dried over Na_2SO_4 and the residue obtained after evaporation of the solvent was further crystallized from Me_2CO/*n*-hexane to afford oxime **17** as a mixture of *E*- and *Z*-isomer.

Yield: 3.26 g (88%), white crystals, R_f 0.44 (*n*-hexane/Me_2CO 2:1), m.p. 145-148 ºC (Me_2CO/*n*-hexane). Anal. Calcd for $C_{11}H_{10}N_2O_2$ requires: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.27; H, 4.80; N, 13.51. MS (EI), *m/z* (%): 203 $[M+H]^+$ (7), 202 $[M]^+$ (66), 160 (100), 43 $[CH_3CO]^+$ (78). IR (KBr) ν_{max}: 3229 (OH); 1706 (C=O); 1620 (C=N); 1539; 1433; 1365; 1200; 1119; 932; 745 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.64 (bs, 0.3H, OH min.), 10.73 (bs, 0.7H, OH maj.), 8.60 (s, 0.3H, CH= min.), 8.40 (d, *J* 8.2, 1H, H-7), 8.26 (s, 0.7H, CH= maj.), 8.14 (d, *J* 7.6, 1H, H-4), 7.72 (s, 0.3H, H-2 min.), 7.63 (s, 0.7H, H-2 maj.), 7.39-7.34 (m, 1H, H-6), 7.31-7.28 (m, 1H, H-5), 2.67 (s, 0.9H, CH_3 min.), 2.64 (s, 2.1H, CH_3 maj.). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3 (C=O min.), 168.7 (C=O maj.), 143.5 (CH= maj.), 137.3 (C-7a min.), 136.4 (C-7a maj.), 134.8 (CH= min.), 130.3 (C-3a min.), 129.0 (C-2 min.), 127.4 (C-3a maj.), 126.8 (C-2 maj.), 126.1 (C-6 maj.), 125.6 (C-6 min.), 124.3 (C-5), 122.6 (C-4), 118.3 (C-7 min.), 116.7 (C-3 maj.), 116.5 (C-7 maj.), 111.7 (C-3 min.), 24.1 (CH_3).

1-Benzoylindole-3-carboxaldehyde oxime (18). To a stirred solution of aldehyde (**15**; 1.0 g, 4.0 mmol) in THF (26 mL) was added a solution of hydroxylammonium chloride (0.43 g, 6.3 mmol) and NaOAc (0.38 g, 2.8 mmol) in water (5 mL) and the mixture was stirred for 4 h at rt. After evaporation of THF and addition of water (26 mL), the oxime **18** was extracted with EtOAc (2 × 80 mL). The extract was dried over Na_2SO_4 and the residue obtained after evaporation of the solvent was further crystallized from EtOAc/*n*-hexane to afford oxime **18** as a mixture of *E*- and *Z*-isomer. Yield: 0.93 g (88%), bright yellow crystals, R_f 0.46 (*n*-hexane/Me_2CO 2:1), m.p. 115-117 ºC (EtOAc/*n*-hexane). Anal. Calcd for $C_{16}H_{12}N_2O_2$ requires: C, 72.72; H, 4.58; N, 10.60. Found: C, 72.48; H, 4.90; N, 10.77. MS (EI), *m/z* (%): 264 $[M]^+$ (27), 105 $[C_6H_5C=O]^+$ (100), 77 $[C_6H_5]^+$ (73). IR ($CHCl_3$) ν_{max}: 3579 (OH), 3020, 1680 (C=O), 1446, 1339, 1165, 1125 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 11.35 (bs, 0.3H, OH min), 10.34 (bs, 0.7H, OH maj.), 8.46 (s, 0.3H, CH=N min.), 8.42-8.40 (m, 0.3H, H-7 min), 8.37-8.35 (m, 0.7H, H-7 maj.), 8.21 (s, 0.7H, CH=N maj.), 8.20-8.18 (m, 0.7H, H-4 maj.), 7.78-7.76 (m, 0.3H, H-4 min.), 7.74-7.72 (m, 2H, H-2´, H-6´), 7.65-7.61 (m, 1H, H-4´), 7.56-7.51 (m, 2H, H-3´, H-5´), 7.44 (s, 1H, H-2), 7.44-7.39 (m, 1H, H-6), 7.37-7.33 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9 (C=O min.), 168.3 (C=O maj.), 143.4 (CH=N maj.), 137.3 (C-4´ min.), 136.4 (C-7a maj.), 135.1 (C-7a min.), 134.0 (CH=N min.), 133.8 (C-1´ min.), 132.3 (C-1´ maj.), 132.2 (C-4´ maj.), 129.3 (C-2´, C-6´ min.), 129.1 (C-2´, C-6´ maj.), 128.7 (C-3´, C-5´ maj.), 128.6 (C-3´, C-5´ min.), 128.3 (C-2), 127.6 (C-3a), 125.7 (C-6 maj.), 125.3 (C-6 min.), 124.4 (C-5 maj.), 124.2 (C-5 min.), 122.5 (C-4 maj.), 118.2 (C-4 min.), 116.3 (C-3 maj.), 116.2 (C-7 min.), 116.1 (C-7 maj.), 111.0 (C-3 min.).

1-Methoxycarbonylindole-3-carboxaldehyde oxime (19). To a stirred solution of aldehyde (**16**; 2.03 g, 10.0 mmol) in EtOH (40 mL) was added a solution of hydroxylammonium chloride (1.04 g, 15.0 mmol) and Na_2CO_3 (0.73 g, 7.0 mmol) in water (5 mL) and the mixture was stirred for 10 min at rt. After evaporation of EtOH and addition of water (10 mL), the oxime **19** was extracted with EtOAc (1 × 80 mL and 1 × 50 mL). The extract was dried over Na_2SO_4 and the residue obtained after evaporation of the solvent was further crystallized from CH_2Cl_2/*n*-hexane to afford oxime **19** as a mixture of *E*- and *Z*-isomer. Yield: 1.74 g (80%), white crystals, R_f 0.47 (*n*-hexane/Me2CO 2:1), m.p. 122-124 ºC (CH_2Cl_2/*n*-hexane). Anal. Calcd for $C_{11}H_{10}N_2O_3$ requires: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.83; H, 4.37; N, 12.58. MS (EI), *m/z* (%): 219 $[M+H]^+$ (9), 218 $[M]^+$ (90), 175 (47), 159 (56), 142 (76), 132 (57), 131

(79), 130 (87), 115 (76), 114 (76), 77 (52), 59 $[CH_3OCO]^+$ (100). IR ($CHCl_3$) ν_{max}: 3578 (OH), 1732 (C=O), 1433, 1345, 1246, 1082, 932 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 0.3H, CH= min.), 8.29 (s, 0.7H, CH= maj.), 8.24 (d, *J* 8.1, 0.3H, H-7 min.), 8.19 (d, *J* 8.0, 0.7H, H-7 maj.), 8.11 (d, *J* 7.8, 0.7H, H-4 maj.), 7.79 (s, 0.7 H, H-2 maj.), 7.77 (s, 0.3H, H-2 min.), 7.72 (d, *J* 7.8, 0.3H, H-4 min.), 7.42-7.31 (m, 2H, H-6, H-5), 4.08 (s, 0.9H, OCH_3), 4.06 (s, 2.1H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.0 (C=O), 144.9 (CH=N maj.), 138.8 (C-2 min.), 135.9 (C-7a maj.), 134.4 (C-7a min.), 130.9 (CH=N min.), 128.6 (C-3a min.), 127.5 (C-2 maj.), 126.9 (C-3a maj.), 125.7 (C-6 maj.), 125.2 (C-6 min.), 123.9 (C-5 maj.), 123.6 (C-5 min.), 122.5 (C-4 maj.), 118.3 (C-4 min.), 115.3 (C-7 min.), 115.0 (C-7 maj., C-3 maj.), 109.9 (C-3 min.), 55.2 (CH_3O min.), 55.1 (CH_3O maj.).

General procedure for the preparation of 1-acyl derivatives of indole-3-ylmethyl amine 20-22.

To a solution of $NiCl_2 \cdot 6H_2O$ (1.05 g, 4.4 mmol) in MeOH (40 mL) was added oxime (**17-19**; 4.0 mmol) in MeOH (30 mL) followed by $NaBH_4$ (1.51 g, 40.0 mmol) in one portion with stirring and cooling with flowing cold water. After 5 min, MeOH in the mixture was evaporated to ¼ of its original volume and mixture was poured into a saturated solution of NH_4Cl (250 mL). After extraction with CH_2Cl_2 for compounds **20** and **21** or EtOAc for compound **22** (1 × 150 mL, 1 × 100 mL, 2 × 50 mL), drying the extract over Na_2SO_4 and evaporation of the solvent, the crude amine **20-22** was obtained. The crude amine **20-22** was employed in the next reaction without purification.

1-(Acetyl)indole-3-ylmethyl amine (20). Following the general procedure, amine **20** was obtained using oxime (**17**; 0.6 g, 3.0 mmol).

1-(Benzoyl)indole-3-ylmethyl amine (21). Following the general procedure, amine **21** was obtained using oxime (**18**; 1.06 g, 4.0 mmol).

1-(Methoxycarbonyl)indole-3-ylmethyl amine (22). Following the general procedure, amine **22** was obtained using oxime (**19**; 0.87 g, 4.0 mmol).

General procedure for the preparation of 1-acetylbrassinin (23) and 1-benzoylbrassinin (24).

To a stirred solution of crude freshly prepared amine (**20**; 0.565 g, 3.0 mmol or **21**; 1.00 g, 4.0 mmol) in CH_2Cl_2 (25 mL or 40 mL) was added Et_3N (0.91 g, 1.25 mL, 9.0 mmol or 1.21 g, 1.67 mL, 12.0 mmol) and CS_2 (0.685 g, 0.54 mL, 9.0 mmol or 0.91 g, 0.72 mL, 12.0 mmol). After stirring for 5 min at rt, MeI (1.28 g, 0.57 mL, 9.0 mmol or 1.70 g, 0.75 mL, 12.0 mmol) was added and stirring was continued for 1 h or 30 min. The solvent was evaporated and the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel **1-Acetylbrassinin (23).** Following the general procedure, product **23** was obtained using of amine (**20**; 0.565 g, 3.0 mmol) and isolated on silica gel (25 g, *n*-hexane/Me_2CO 2:1). The obtained compound was crystallized from Me_2CO/*n*-hexane to afford 1-acetylbrassinin (**23**). Yield: 0.651 g (78%), bright yellow crystals, R_f 0.59 (*n*-hexane/Me_2CO 2:1), m.p. 155-156 ºC (Me_2CO/*n*-hexane). Anal. Calcd for $C_{13}H_{14}N_2OS_2$ requires: C, 56.09; H, 5.07; N, 10.06. Found: C, 55.72; H, 4.89; N, 10.30. MS (EI), *m/z* (%): 279 $[M+H]^+$ (2), 278 $[M]^+$ (10), 130 (100), 43 $[CH_3CO]^+$ (27). IR ($CHCl_3$) ν_{max}: 3366 (NH), 1687 (C=O), 1440, 1373, 1120, 1080 cm^{-1}. ^1H

NMR (400 MHz, DMSO-d_6) δ 9.83 (bs, 1H, NH), 8.38 (d, *J* 8.0, 1H, H-7), 7.65 (d, *J* 7.7, 1H, H-4), 7.58 (s, 1H, H-2), 7.36-7.26 (m, 2H, H-6, H-5), 5.01 (d, *J* 5.0, 1.8H, CH_2), 4.74 (d, *J* 5.4, 0.2H, CH_2), 2.62 (s, 3H, CH_3), 2.61 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 198.9 (C=S), 168.8 (C=O), 135.9 (C-7a), 129.8 (C-3a), 125.5 (C-2), 125.0 (C-6), 123.8 (C-5), 119.6 (C-4), 118.0 (C-3), 116.7 (C-7), 42.1 (CH_2), 24.2 (CH_3), 18.1 (SCH_3).

1-Benzoylbrassinin (24). Following the general procedure, product **24** was obtained using of amine (**21**; 1.00 g, 4.0 mmol) and isolated on silica gel (60 g, *n*-hexane/EtOAc 2:1). The obtained compound was further crystallized from dichloromethane/*n*-hexane to afford 1-benzoylbrassinin (**24**). Yield: 0.490 g (36%), bright yellow crystals, R_f 0.66 (*n*-hexane/EtOAc 2:1), m.p. 109-111 ºC (CH_2Cl_2/*n*-hexane). Anal. Calcd for $C_{18}H_{16}N_2OS_2$ requires: C, 63.50; H, 4.74; N, 8.23. Found: C, 63.21; H, 4.99; N, 8.01. MS (EI), *m/z* (%): 340 $[M]^+$ (35), 234 (80), 105 $[C_6H_5C{=}O]^+$ (100), 77 $[C_6H_5]^+$ (81). IR ($CHCl_3$) ν_{max}: 3365 (NH), 1679 (C=O), 1446, 1352, 1172, 1086 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* 8.2, 1H, H-7), 7.71-7.69 (m, 2H, H-2´, H-6´), 7.64-7.59 (m, 2H, H-4, H-4´), 7.55-7.51 (m, 2H, H-3´, H-5´), 7.43-7.39 (m, 1H, H-6), 7.36-7.32 (m, 2H, H-5, H-2), 7.11 (bs, 1H, NH), 5.01 (d, *J* 4.3, 1.5H, CH_2), 4.71 (s, 0.5H, CH_2), 2.71 (s, 0.75H, SCH_3), 2.63 (s, 2.25H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.1 (C=S), 168.4 (C=O), 136.4 (C-7a), 134.1 (C-1´), 132.1 (C-4´), 129.3 (C-3a), 129.1 (C-2´, C-6´), 128.7 (C-3´, C-5´), 126.7 (C-2), 125.6 (C-6), 124.2 (C-5), 119.0 (C-4), 116.6 (C-3, C-7), 42.3 (CH_2), 18.2 (SCH_3).

1-(Methoxycarbonyl)brassinin (25). To a stirred solution of crude freshly prepared amine (**22**; 0.817 g, 4.0 mmol) in MeOH (25 mL) was added Et_3N (1.21 g, 1.67 mL, 12.0 mmol) and CS_2 (0.91 g, 0.72 mL, 12.0 mmol). After stirring for 5 min at rt, MeI (1.70 g, 0.75 mL, 12.0 mmol) was added and stirring was continued for 15 min. The solvent was evaporated and the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (25 g, *n*-hexane/EtOAc 2:1). The obtained compound was further crystallized from CH_2Cl_2/*n*-hexane to afford 1-(methoxycarbonyl)brassinin (**25**). Yield: 0.683 g (58%), bright yellow crystals, R_f 0.46 (*n*-hexane/EtOAc 2:1), m.p. 128-131 ºC (CH_2Cl_2/*n*-hexane). Anal. Calcd for $C_{13}H_{14}N_2O_2S_2$ requires: C, 53.04; H, 4.79; N, 9.52. Found: C, 52.81; H, 5.08; N, 9.74. MS (EI), *m/z* (%): 294 $[M]^+$ (13), 188 (78), 59 $[CH_3OCO]^+$ (100). IR ($CHCl_3$) ν_{max}: 3367 (NH), 3020, 1725 (C=O), 1439, 1371, 1276, 732 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* 7.5, 1H, H-7), 7.59 (s, 1H, H-2), 7.56 (d, *J* 7.7, 1H, H-4), 7.39-7.35 (m, 1H, H-6), 7.30-7.27 (m, 1H, H-5), 7.12 (s, 1H, NH), 5.03 (d, *J* 4.7, 1.7H, CH_2), 4.74 (s, 0,3H, CH_2), 4.01 (s, 3H, OCH_3), 2.73 (s, 0.45H, SCH_3), 2.66 (s, 2.55H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.1 (C=S), 151.1 (C=O), 135.5 (C-7a), 128.9 (C-3a), 125.2 (C-6), 124.5 (C-2), 123.3 (C-5), 119.0 (C-4), 116.3 (C-3), 115.3 (C-7), 53.9 (OCH_3), 42.3 (CH_2), 18.2 (SCH_3).

General procedure for the spirocyclization of 1-acyl derivatives of brassinin 23-25 with bromine in the presence of water. To a stirred solution of 1-acyl derivatives of brassinin **23-25** (0.5 mmol) in a mixture of CH_2Cl_2/water (3.6 mL/0.4 mL) at rt was added freshly prepared solution of Br_2 (1.26 mL, 0.55 mmol). The stock solution was obtained by dissolving of 0.04 mL of bromine in 1.76 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 15 min, then Et_3N (0.111 g, 0.15 mL, 1.1 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with brine (2 × 25 mL). The

organic layer was dried over anhydrous Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography.

***trans*-(±)- and *cis*-(±)-1-Acetylspirobrassinol [*trans*-(±)-26a and *cis*-(±)-26b].** Following the general procedure, products *trans*-(±)-**26a** and *cis*-(±)-**26b** were obtained using 0.139 g (0.5 mmol) of 1-acetylbrassinin (**23**) and isolated on silica gel (15 g, *n*-hexane/EtOAc 1:3) as mixture of products *trans*-(±)-**26a** : *cis*-(±-)-**26b** in a 71:29 ratio. Yield: 0.116 g (79%), sallow oil, R_f (*trans*) 0.52 (*n*-hexane/EtOAc 1:3), R_f (*cis*) 0.37 (*n*-hexane/EtOAc 1:3). Anal. Calcd for $C_{13}H_{14}N_2O_2S_2$ requires: C, 53.04; H, 4.79; N, 9.52. Found: C, 53.31; H, 4.50; N, 9.83. MS (EI), *m/z* (%): 295 $[M+H]^+$(9), 294 $[M]^+$ (47), 251 (50), 43 $[CH_3CO]^+$ (100). IR ($CHCl_3$) ν_{max}: 3279 (OH), 3013, 1649 (C=O), 1547 (C=N), 1466, 1378, 1099 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* 7.6, 0.7H, H-7 *trans*), 7.82 (d, *J* 7.6, 0.3H, H-7 *cis*), 7.43 (dd, *J* 7.5, *J* 0.6, 0.7H, H-4 *trans*), 7.36 (d, *J* 7.4, 0.3H, H-4 *cis*), 7.32-7.27 (m, 1H H-6), 7.11 (ddd, *J* 0.9, *J* 7.5, *J* 7.5, 1H, H-5), 6.11 (s, 0.3H, OH *cis*), 5.73 (s, 0.7H, H-2 *trans*), 5.42 (s, 0.3H, H-2 *cis*), 5.17 (s, 0.7H, OH *trans*), 4.95 (d, *J* 15.6, 0.7H, H_b *trans*), 4.35 (d, *J* 15.3, 0.3H, H_b *cis*), 4.31 (d, *J* 15.6, 0.7H, H_a *trans*), 3.93 (d, *J* 15.3, 0.3H, H_a *cis*), 2.57 (s, 2.1H, SCH_3 *trans*), 2.54 (s, 0.9H, SCH_3 *cis*), 2.39 (s, 3H, CH_3 *cis*, *trans*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1 (C=O), 166.2 (C=N), 141.4 (C-7a), 130.0 (C-6), 128.5 (C-3a), 124.7 (C-5), 123.9 (C-4), 117.2 (C-7 *trans*), 114.1 (C-7 *cis*), 93.0 (C-2 *cis*), 88.5 (C-2 *trans*), 75.1 (CH_2 *cis*), 71.5 (C-3 *trans*), 67.6 (C-3 *cis*), 66.3 (CH_2 *trans*), 23.3 (CH_3), 15.2 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H-4 (*trans*), H-2/H_b (*cis*), H-5/H-4, H-6/H-7.

***trans*-(±)- and *cis*-(±)-1-Benzoylspirobrassinol [*trans*-(±)-27a and *cis*-(±)-27b].** Following the general procedure, products *trans*-(±)-**27a** and *cis*-(±)-**27b** were obtained using 0.170 g (0.5 mmol) of 1-benzoylbrassinin (**24**) and isolated on silica gel (15 g, CH_2Cl_2/Me_2CO 8:1) as a mixture of products *trans*-(±)-**27** : *cis*-(±-)-**27b** in a 64:36 ratio. Yield: 0.137 g (77%), sallow oil, R_f (*trans*) 0.67 (CH_2Cl_2/Me_2CO 8:1), R_f (*cis*) 0.43 (CH_2Cl_2/Me_2CO 8:1). Anal. Calcd for $C_{18}H_{16}N_2O_2S_2$ requires: C, 60.65; H, 4.52; N, 7.86. Found: C, 60.31; H, 4.79; N, 7.53. MS (EI), *m/z* (%): 357 $[M+H]^+$ (11), 356 $[M]^+$ (35), 251 (71), 105 $[C_6H_5C=O]^+$ (100), 77 $[C_6H_5]^+$ (92). IR ($CHCl_3$) ν_{max}: 3365 (OH), 3006, 1666 (C=O), 1560 (C=N), 1466, 1365, 1086, 939 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* 7.1, 0.6 H, H-2´, H-6´ *cis*), 7.62 (d, *J* 7.5, 1.4 H, H-2´, H-6´ *trans*), 7.56-7.53 (m, 1H, H-4´), 7.48-7.38 (m, 4H, H-7, H-4, H-3´, H-5´), 7.22-7.05 (m, 2H, H-6, H-5), 5.95 (s, 0.7H, H-2 *trans*), 5.49 (s, 0.3H, H-2 *cis*), 4.97 (d, *J* 15.6, 0.7H, H_b *trans*), 4.63 (s, 1H, OH *cis*, *trans*), 4.37 (d, *J* 15.2, 0.3H, H_b *cis*), 4.34 (d, *J* 15.6, 0.7H, H_a *trans*), 3.98 (d, *J* 15.2, 0.3H, H_a *cis*), 2.55 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (C=O *trans*), 170.1 (C=O *cis*), 165.9 (C=N *cis*), 164.7 (C=N *trans*), 140.5 (C-7a *trans*), 140.0 (C-7a *cis*), 135.1 (C-1´ *cis*), 134.8 (C-1´ *trans*), 131.6 (C-4´ *trans*), 131.4 (C-4´ *cis*), 131.2 (C-3a), 129.5 (C-6 *trans*), 129.4 (C-6 *cis*), 128.7 (C-3´, C-5´ *trans*), 128.6 (C-3´, C-5´ *cis*), 127.9 (C-2´, C-6´ *trans*), 127.8 (C-2´, C-6´ *cis*), 125.0 (C-5 *cis*), 124.4 (C-5 *trans*), 124.3 (C-4 *cis*), 124.1 (C-4 *trans*), 117.0 (C-7 *cis*), 116.1 (C-7 *trans*), 92.7 (C-2 *trans*), 89.2 (C-2 *cis*), 74.1 (CH_2 *cis*), 70.4 (C-3 *trans*), 66.7 (CH_2 *trans*), 64.3 (C-3 *cis*), 15.2 (SCH_3 *trans*), 15.1 (SCH_3 *cis*). NOESY correlations (400 MHz, $CDCl_3$): H_a/H-4 (*trans*), H_a/H_b (*trans*), H-2/H_b(*cis*), H_a/H_b (*cis*).

***trans*-(±)- and *cis*-(±)-1-Methoxycarbonylspirobrassinol [*trans*-(±)-28a and *cis*-(±)-28b].** Following the general procedure, products *trans*-(±)-**28a** and *cis*-(±)-**28b** were obtained using

0.147 g (0.5 mmol) of 1-(methoxycarbonyl)brassinin (**25**) and separated on silica gel (30 g, CH_2Cl_2/Me_2CO 9:1). Both diastereoisomers *trans*-(±)-**28a** and *cis*-(±)-**28b** were crystallized from CH_2Cl_2/*n*-hexane.

***trans*-(±)-1-Methoxycarbonylspirobrassinol [*trans*-(±)-28a].** Yield: 0.048 g (31%), white crystals, R_f 0.48 (CH_2Cl_2/Me_2CO 9:1), mp 135-138 °C (CH_2Cl_2/*n*-hexane). Anal. Calcd for $C_{13}H_{14}N_2O_3S_2$ requires: C, 50.30; H, 4.55; N, 9.03. Found: C, 50.49; H, 4.37; N, 8.85. MS (EI), *m/z* (%): 311 $[M+H]^+$ (16), 310 $[M]^+$ (65), 203 (100), 159 (87), 117 (47), 87 (87), 72 (63), 59 $[CH_3OCO]^+$ (58). IR ($CHCl_3$) ν_{max}: 3567 (OH), 3099, 1699 (C=O), 1547 (C=N), 1433, 1073 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (bs, 1H, H-7), 7.38 (d, *J* 7.5, 1H, H-4), 7.31-7.26 (m, 1H, H-6), 7.10-7.06 (m, 1H, H-5), 5.95 (s, 1H, H-2), 5.04 (d, *J* 15.5, 1H, H_b), 4.64 (bs, 1H, OH), 4.31 (d, *J* 15.5, 1H, H_a), 3.91 (s, 3H, OCH_3), 2.55 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8 (C=N), 153.8 (C=O), 140.6 (C-7a), 129.9 (C-6), 129.8 (C-3a), 123.9 (C-4, C-5), 114.9 (C-7), 91.6 (C-2), 70.6 (C-3), 67.2 (CH_2), 53.2 (OCH_3), 15.2 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_a/H-4, H-4/H-5, H-5/H-6, H-6/H-7.

***cis*-(±)-1-Methoxycarbonylspirobrassinol [*cis*-(±)-28b].** Yield: 0.011 g (7%), white crystals, R_f 0.57 (CH_2Cl_2/Me_2CO 9:1), mp 129-132 °C (CH_2Cl_2/*n*-hexane). Anal. Calcd for $C_{13}H_{14}N_2O_3S_2$ requires: C, 50.30; H, 4.55; N, 9.03. Found: C, 50.58; H, 4.39; N, 9.31. MS of compound *cis*-(±)-**28b** was fully identical with MS of *trans*-(±)-**28a** diastereoisomer. IR ($CHCl_3$) ν_{max}: 3526 (OH), 3132, 1706 (C=O), 1567 (C=N), 1476, 1378, 1083 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.68 (bs, 1H, H-7), 7.39 (d, *J* 7.5, 1H, H-4), 7.31-7.26 (m, 1H, H-6), 7.10-7.06 (m, 1H, H-5), 6.11 (bs, 1H, OH), 5.64 (s, 1H, H-2), 4.37 (d, *J* 15.1, 1H, H_b); 3.99 (d, *J* 15.1, 1H, H_a), 3.91 (s, 3H, OCH_3), 2.58 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3 (C=N), 153.7 (C=O), 139.1 (C-7a), 130.1 (C-3a), 129.8 (C-6), 124.1 (C-4), 124.0 (C-5), 114.9 (C-7), 88.0 (C-2), 75.2 (CH_2), 73.4 (C-3), 53.2 (OCH_3), 15.1 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_b/H-2, H-4/H-5, H-5/H-6.

***trans*-(±)- and *cis*-(±)-1-Boc-spirobrassinol [*trans*-(±)-29a and *cis*-(±)-29b].** To a stirred solution of 1-Boc-brassinin (**12**; 0.027 g, 0.08 mmol) in a mixture of CH_2Cl_2/water (0.9 mL/0.1 mL) at rt was added freshly prepared solution of Br_2 (0.20 mL, 0.088 mmol). The stock solution was obtained by dissolving of 0.04 mL of bromine in 1.76 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 15 min, then Et_3N (0.017 g, 0.024 mL, 0.18 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with brine (2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on 8 g silica gel (*n*-hexane/Me_2CO 3:1) and diastereoisomers *trans*-(±)-**29a** and *cis*-(±)-**29b** were separated.

***trans*-(±)-1-Boc-spirobrassinol [*trans*-(±)-29a].** Yield: 0.012 g (42%), white solid, R_f 0.35 (*n*-hexane/Me_2CO 3:1), mp 73-75 °C ($CHCl_3$/light petroleum). The spectral data were fully identical with those of previously described product *trans*-(±)-**29a**.[12]

***cis*-(±)-1-Boc-spirobrassinol [*cis*-(±)-29b].** Yield: 0.003 g (11%), colourless plates, R_f 0.67 (*n*-hexane/Me_2CO 3:1), mp 126-128 °C (CH_2Cl_2/light petroleum). The spectral data were fully identical with those of previously described product *cis*-(±)-**29b**.[12]

General procedure for the spirocyclization of 1-acyl derivatives of brassinin 23-25 with bromine in the presence of methanol. To a stirred solution of 1-acyl derivatives of brassinin **23-25** (0.5 mmol) in a mixture of anhydrous CH_2Cl_2/MeOH (3.6 mL/0.4 mL) at rt was added freshly prepared solution of Br_2 (1.26 mL, 0.55 mmol). The stock solution was obtained by dissolving of 0.04 mL of bromine in 1.76 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 15 min, then Et3N (0.111 g, 0.15 mL, 1.1 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with brine (2 × 25 mL). The organic layer was dried over anhydrous Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography.

***trans*-(±)- and *cis*-(±)-1-Acetylspirobrassinol methyl ether [*trans*-(±)-30a and *cis*-(±)-30b].** Following the general procedure, products *trans*-(±)-**30a** and *cis*-(±)-**30b** were obtained using 0.139 g (0.5 mmol) of 1-acetylbrassinin (**23**) and separated on silica gel (25 g, *n*-hexane/EtOAc 1:1). Both diastereoisomers *trans*-(±)-**30a** and *cis*-(±)-**30b** were crystallized from Et_2O/*n*-hexane.

***trans*-(±)-1-Acetylspirobrassinol methyl ether [*trans*-(±)-30a].** Yield: 0.064 g (42%), white crystals, R_f 0.63 (*n*-hexane/EtOAc 1:1), mp 89-91 ºC (Et_2O/*n*-hexane). Anal. Calcd for $C_{14}H_{16}N_2O_2S_2$ requires: C, 54.52; H, 5.23; N, 9.08. Found: C, 54.76; H, 5.08; N, 9.32. MS (EI), *m/z* (%): 309 $[M+H]^+$ (68), 265 (56), 43 $[CH_3CO]^+$ (100). IR ($CHCl_3$) ν_{max}: 2993, 1653 (C=O), 1553 (C=N), 1467, 1373, 1080 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* 6.3, 1H, H-7), 7.38 (d, *J* 7.3, 1H, H-4), 7.33-7.29 (m, 1H, H-6), 7.14-7.11 (m, 1H, H-5), 5.41 (s, 1H, H-2), 4.83 (d, *J* 15.7, 1H, H_b), 4.35 (d, *J* 15.7, 1H, H_a), 3.34 (s, 3H, CH_3O), 2.58 (s, 3H, SCH_3), 2.37 (s, 3H, CH_3C=O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5 (C=O), 164.8 (C=N), 142.1 (C-7a), 130.1 (C-6), 128.9 (C-3a), 124.6 (C-5), 123.4 (C-4), 117.1 (C-7), 100.2 (C-2), 71.1 (C-3), 66.6 (CH_2), 55.6 (OCH_3), 23.4 (CH_3C=O), 15.2 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_a/H-4, H-6/H-7, H-4/H-5.

***cis*-(±)-1-Acetylspirobrasinol methyl ether [(±)-30b].** Yield: 0.038 g (25%), white crystals, R_f 0.46 (*n*-hexane/EtOAc 1:1), mp 91-93 ºC (Et_2O/*n*-hexane). Anal. Calcd for $C_{14}H_{16}N_2O_2S_2$ requires: C, 54.52; H, 5.23; N, 9.08. Found: C, 54.86; H, 4.97; N, 9.31. MS of compound *cis*-(±)-**30b** was fully identical with MS of *trans*-(±)-**30a** diastereoisomer. IR ($CHCl_3$) ν_{max}: 3007, 1653 (C=O), 1546 (C=N), 1467, 1373, 1087 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* 7.1, 1H, H-7), 7.42 (d, *J* 7.5, 1H, H-4), 7.32-7.27 (m, 1H, H-6), 7.14-7.10 (m, 1H, H-5), 5.20 (s, 1H, H-2), 4.34 (d, *J* 15.2, 1H, H_b), 3.93 (d, *J* 15.2, 1H, H_a), 3.36 (s, 3H, CH_3O), 2.59 (s, 3H, SCH_3), 2.37 (s, 3H, CH_3C=O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7 (C=O), 167.1 (C=N), 140.9 (C-7a), 130.2 (C-3a), 129.8 (C-6), 124.7 (C-5), 123.3 (C-4), 116.7 (C-7), 95.3 (C-2), 75.7 (CH_2), 72.9 (C-3), 55.3 (OCH_3), 23.5 (CH_3C=O), 15.1 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_b/H-2, H-6/H-7, H-4/H-5.

***trans*-(±)- and *cis*-(±)-1-Benzoylspirobrassinol methyl ether [*trans*-(±)-31a and *cis*-(±)-31b].** Following the general procedure, products *trans*-(±)-**31a** and *cis*-(±)-**31b** were obtained using 0.170 g (0.5 mmol) of 1-benzoylbrassinin (**24**) and separated on silica gel (40 g, *n*-hexane/Et_2O 1:1). Both diastereoisomers *trans*-(±)-**31a** and *cis*-(±)-**31b** were crystallized from Me_2CO/*n*-hexane.

***trans*-(±)-1-Benzoylspirobrasinol methyl ether [(±)-31a].** Yield: 0.091 g (49%), white crystals, R_f 0.38 (*n*-hexane/Et_2O 1:1), mp 112-115 ºC (Me_2CO/*n*-hexane). Anal. Calcd for

$C_{19}H_{18}N_2O_2S_2$ requires: C, 61.60; H, 4.90; N, 7.56. Found: C, 61.91; H, 4.64; N, 7.82. MS (EI), *m/z* (%): 371 $[M+H]^+$ (5), 370 $[M]^+$ (39), 265 (72), 105 $[C_6H_5C=O]^+$ (98), 77 $[C_6H_5]^+$ (100). IR ($CHCl_3$) ν_{max}: 3006, 1675 (C=O), 1560 (C=N), 1469, 1372, 1092 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* 6.6, 2H, H-2´, H-6´), 7.52-7.44 (m, 4H, H-4´, H-3´, H-5´, H-7), 7.39 (d, *J* 7.4, 1H, H-4), 7.26-7.10 (m, 2H, H-6, H-5), 5.52 (s, 1H, H-2), 4.79 (d, *J* 15.7, 1H, H_b), 4.36 (d, *J* 15.7, 1H, H_a), 3.21 (s, 3H, OCH_3), 2.56 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7 (C=O), 164.5 (C=N), 141.6 (C-7a), 135.5 (C-1´), 130.8 (C-3a, C-4´), 129.6 (C-6), 128.6 (C-3´, C-5´), 127.6 (C-2´, C-6´), 124.8 (C-5), 123.5 (C-4), 117.4 (C-7), 99.9 (C-2), 70.9 (C-3), 66.2 (CH_2), 57.2 (OCH_3), 15.2 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H-4, H_a/H_b.

***cis*-(±)-1-Benzoylspirobrasinol methyl ether [(±)-31b].** Yield: 0.031 g (17%), white crystals, R_f 0.27 (*n*-hexane/Et_2O 1:1), mp 113-116 °C (Me_2CO/*n*-hexane). Anal. Calcd for $C_{19}H_{18}N_2O_2S_2$ requires: C, 61.60; H, 4.90; N, 7.56. Found: C, 61.33; H, 5.19; N, 7.30. MS of compound *cis*-(±)-**31b** was fully identical with MS of *trans*-(±)-**31a** diastereoisomer. IR ($CHCl_3$) ν_{max}: 3006, 1668 (C=O), 1560 (C=N), 1461, 1370, 1098 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.57 -7.38 (m, 7H, H-2´, H-6´, H-4´, H-3´, H-5´, H-4, H-7), 7.22-7.18 (m, 1H, H-6), 7.14-7.10 (m, 1H, H-5), 5.22 (s, 1H, H-2), 4.42 (d, *J* 15.1, 1H, H_b), 3.98 (d, *J* 15.1, 1H, H_a), 3.25 (s, 3H, OCH_3), 2.57 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (C=O), 167.2 (C=N), 140.6 (C-7a), 135.9 (C-1´), 132.4 (C-3a), 131.1 (C-4´), 129.4 (C-6), 128.9 (C-3´, C-5´), 127.7 (C-2´, C-6´), 125.2 (C-5), 124.0 (C-4), 117.4 (C-7), 96.9 (C-2), 74.8 (CH_2), 73.6 (C-3), 57.9 (OCH_3), 15.3 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_b/H-2.

***trans*-(±)- and *cis*-(±)-1-Methoxycarbonylspirobrassinol methyl ether [*trans*-(±)-32a and *cis*-(±)-32b].** Following the general procedure, products *trans*-(±)-**32a** and *cis*-(±)-**32b** were obtained using 0.147 g (0.5 mmol) of 1-(methoxycarbonyl)brassinin (**25**) and separated on silica gel (30 g, *n*-hexane/EtOAc 2:1). Diastereoisomer *trans*-(±)-**32a** was crystallized from Et_2O/*n*-hexane. Diastereoisomer *cis*-(±)-**32b** was isolated as a colourless oil.

***trans*-(±)-1-Methoxycarbonylspirobrasinol methyl ether [(±)-32a].** Yield: 0.053 g (33%), white crystals, R_f 0.48 (*n*-hexane/EtOAc 2:1), mp 125-128 °C (Et_2O/*n*-hexane). Anal. Calcd for $C_{14}H_{16}N_2O_3S_2$ requires: C, 51.83; H, 4.97; N, 8.63. Found: C, 52.09; H, 4.73; N, 8.82. MS (EI), *m/z* (%): 325 $[M+H]^+$ (15), 324 $[M]^+$ (96), 245 (100), 87 (71), 72 (57), 59 $[CH_3OCO]^+$ (98). IR ($CHCl_3$) ν_{max}: 1702 (C=O), 1553 (C=N), 1476, 1436, 1372, 1066 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (bs, 1H, H-7), 7.35 (d, *J* 7.5, 1H, H-4), 7.31-7.26 (m, 1H, H-6), 7.09-7.05 (m, 1H, H-5), 5.56 (s, 1H, H-2), 4.86 (d, *J* 15.6, 1H, H_b), 4.33 (d, *J* 15.6, 1H, H_a), 3.90 (s, 3H, $COOCH_3$), 3.50 (s, 3H, OCH_3), 2.57 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6 (C=N), 153.8 (C=O), 140.8 (C-7a), 129.8 (C-6, C-3a), 124.0 (C-5), 123.5 (C-4), 115.9 (C-7), 98.8 (C-2), 70.7 (C-3), 66.3 (CH_2), 57.8 (OCH_3), 53.1 ($COO\underline{C}H_3$), 15.1 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H-4, H-2/OCH_3, H-4/H-5, H-6/H-7.

***cis*-(±)-1-Methoxycarbonylspirobrasinol methyl ether [(±)-32b].** Yield: 0.026 g (16%), colourless oil, R_f 0.38 (*n*-hexane/EtOAc 2:1). Anal. Calcd for $C_{14}H_{16}N_2O_3S_2$ requires: C, 51.83; H, 4.97; N, 8.63. Found: C, 52.11; H, 4.69; N, 8.47. MS of compound *cis*-(±)-**32b** was fully identical with MS of *trans*-(±)-**32a** diastereoisomer. IR ($CHCl_3$) ν_{max}: 1699 (C=O), 1560 (C=N), 1460, 1433, 1368, 1266, 1085 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (bs, 1H, H-7), 7.36 (d, *J* 7.5, 1H, H-4), 7.30-7.26 (m, 1H, H-6), 7.08-7.05 (m, 1H, H-5), 5.29 (s, 1H, H-2), 4.34 (d, *J* 15.1, 1H, H_b), 3.91 (s, 3H, $COOCH_3$), 3.90 (d, *J* 15.1, 1H, H_a), 3.53 (s, 3H, OCH_3),

2.58 (s, 3H, SCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8 (C=N), 153.3 (C=O), 139.4 (C-7a), 131.7 (C-3a), 129.5 (C-6), 124.0 (C-5), 123.7 (C-4), 115.5 (C-7), 95.6 (C-2), 74.8 (CH_2), 73.2 (C-3), 58.2 (OCH_3), 53.1 ($COO\underline{C}H_3$), 15.1 (SCH_3). NOESY correlations (400 MHz, $CDCl_3$): H_a/H_b, H_b/H-2, H-2/OCH_3, H-4/H-5, H-5/H-6, H-6/H-7.

***trans*-(±)- and *cis*-(±)-1-Boc-spirobrassinol methyl ether [*trans*-(±)-33a and *cis*-(±)-33b].** *Method A*: To a stirred solution of 1-Boc-brassinin (**12**; 0.027 g, 0.08 mmol) in a mixture of CH_2Cl_2/MeOH (0.9 mL/0.1 mL) at rt was added freshly prepared solution of Br_2 (0.20 mL, 0.088 mmol). The stock solution was obtained by dissolving of 0.04 mL of bromine in 1.76 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred for 15 min, then Et_3N (0.017 g, 0.024 mL, 0.18 mmol) was added. Stirring was continued for 5 min and the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with brine (2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on 5 g silica gel (petroleum ether/EtOAc 5:1), affording mixture of products *trans*-(±)-**33a** : *cis*-(±)-**33b** in a 71:29 ratio. Subsequent chromatography of the mixture of diastereoisomers (±)-**33a** and (±)-**33b** on 5 g of silica gel (CH_2Cl_2) gave (±)-**33a** (0.013 g, 45%) and (±)-**33b** (0.006 g, 20%).
Method B: To a stirred solution of 1-Boc-brassinin (**12**; 0.150 g, 0.446 mmol) in a mixture of 1,4-dioxane/MeOH (5.4 mL/0.6 mL) at rt was added freshly prepared solution of DDB (2.96 mL, 0.491 mmol). The stock solution was obtained by dissolving of 0.05 mL of bromine in 6.0 mL of 1,4-dioxane. The reaction mixture was stirred for 15 min, then Et_3N (0.99 g, 0.137 mL, 0.971 mmol) was added. Stirring was continued for 5 min and the mixture poured mixture into water (90 mL), the product extracted with EtOAc (2 × 30 mL), the extract washed with brine (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on 15 g of silica gel (petroleum ether/EtOAc 5:1), affording mixture of products *trans*-(±)-**33a** : *cis*-(±)-**33b** in a 71:29 ratio. Subsequent chromatography of the mixture of diastereoisomers (±)-**33a** and (±)-**33b** on 15 g of silica gel (CH_2Cl_2) gave (±)-**33a** (0.068 g, 42%) and (±)-**33b** (0.014 g, 9%).
***trans*-(±)-1-Boc-spirobrassinol methyl ether [*trans*-(±)-33a].** Yield: 0.068 g (42%), colourless solid, R_f 0.12 (CH_2Cl_2), mp 68-70 °C. The spectral data were fully identical with those of previously described product *trans*-(±)-**33a**.[31]
***cis*-(±)-1-Boc-spirobrassinol methyl ether [*cis*-(±)-33b].** Yield: 0.014 g (9%), colourless oil, R_f 0.19 (CH_2Cl_2). The spectral data were fully identical with those of previously described product c*is*-(±)-**33b**.[31]

Spirocyclization of brassinin (5b) or 1-methylbrassinin (37) with DDB (1.1 eq.) in the presence of methanol. To a stirred solution of brassinin (**5b**; 0.035 g, 0.15 mmol) or 1-methylbrassinin (**37**; 0.038 g, 0.15 mmol) in a mixture of 1,4-dioxane/MeOH (1.8 mL/0.2 mL) at rt was added freshly prepared solution of DDB (0.38 mL, 0.165 mmol). The stock solution was obtained by dissolving of 0.04 mL of bromine in 1.76 mL of anhydrous of 1,4-dioxane. The reaction mixture was stirred for 15 min, then Et_3N (0.033 g, 0.046 mL, 0.33 mmol) was added. Stirring was continued for 5 min and the mixture poured mixture into water (10 mL), the product extracted with CH_2Cl_2 (2 × 10 mL), the extract washed with brine (2 × 10 mL).

The organic layer was dried over anhydrous Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on 5 g of silica gel (*n*-hexane/EtOc 2:1).
Spirobrassinin [(±)-1]. Yield: 0.018 g (47%), colourless crystals, R_f 0.24 (*n*-hexane/`EtOAc 2:1), mp 159-160 ºC (Me_2CO/*n*-hexane). The spectral data were fully identical with those of natural product [(-)-**1**].[5]
1-Methylspirobrassinin [(±)-44]. Yield: 0.022 g (55%), white solid, R_f 0.25 (*n*-hexane/EtOAc 2:1). The spectral data were fully identical with those of previously described product (±)-**44**.[36]

Spirocyclization of brassinin (5b) with DDB (4 eq.) in the presence of methanol. To a stirred solution of brassinin (**5b**; 0.035 g, 0.15 mmol) in a mixture of 1,4-dioxane/MeOH (1.8 mL/0.2 mL) at rt was added freshly prepared solution of DDB (1.4 mL, 0.6 mmol). The stock solution was obtained by dissolving of 0.04 mL of bromine in 1.76 mL of anhydrous of 1,4-dioxane. The reaction mixture was stirred for 15 min, then Et_3N (0.121 g, 0.167 mL, 1.2 mmol) was added. Stirring was continued for 5 min and the mixture poured mixture into water (10 mL), the product extracted with CH_2Cl_2 (2 × 10 mL), the extract washed with brine (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on 5 g of silica gel (*n*-hexane/EtOAc 2:1).
5-Bromospirobrassinin [(±)-43]. Yield: 0.024 g (49%), pale yellow oil, R_f 0.22 (*n*-hexane/EtOAc 2:1). The spectral data were fully identical with those of previously described product (±)-**43**.[37]

Spirocyclization of 5-bromobrassinin (5c) with DDB (4 eq.) in the presence of methanol. To a stirred solution of brassinin (**5c**; 0.047 g, 0.15 mmol) in a mixture of 1,4-dioxane/MeOH (1.8 mL/0.2 mL) at rt was added freshly prepared solution of DDB (1.4 mL, 0.6 mmol). The stock solution was obtained by dissolving of 0.04 mL of bromine in 1.76 mL of anhydrous of 1,4-dioxane. The reaction mixture was stirred for 15 min, then Et_3N (0.121 g, 0.167 mL, 1.2 mmol) was added. Stirring was continued for 5 min and the mixture poured mixture into water (10 mL), the product extracted with CH_2Cl_2 (2 × 10 mL), the extract washed with brine (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to chromatography on 5 g of silica gel (*n*-hexane/EtOAc 2:1).
5-Bromospirobrassinin [(±)-43]. Yield: 0.031 g (64%), pale yellow oil, R_f 0.22 (*n*-hexane/EtOAc 2:1). The spectral data were fully identical with those of previously described product (±)-**43**.[37]

Biological effects

Cell lines. Jurkat (human T-cell acute lymphoblastic leukemia), HeLa (human cervical adenocarcinoma) and MCF-7 (human breast adenocarcinoma, estrogen receptor-positive) were obtained from the European Collection of Cell Cultures (United Kingdom), CCRF-CEM cell line (human T-cell acute lymphoblastic leukemia) from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). MDA-MB-231 (human breast

adenocarcinoma, estrogen receptor-negative) and A-549 cell lines (human lung adenocarcinoma) were kindly provided by Dr. M. Hajdúch (Olomouc, Czech Republic).
The cells were routinely maintained in RPMI 1640 medium with L-glutamine and HEPES (Jurkat, HeLa and CCR-CEM) or Dulbecco's modified Eagle's medium with Glutamax- I (MCF-7, MDA-MB-231 and A-549) supplemented with 10% fetal calf serum, penicillin (100 IU x mL^{-1}) and streptomycin (100 lg x mL^{-1}) (all from Invitrogen, USA), in humidified air with 5% CO_2 at 37 °C. Before each cytotoxicity assay, cell viability was determined by the trypan blue exclusion method and found to be greater than 95%.
Cytotoxicity assay. The antiproliferative effects of compounds were studied using the colorimetric microculture assay with the MTT endpoint.[38] Briefly, 5×10^3 cells were plated per well in 96-well polystyrene microplates (Sarstedt, Germany) in 100 μL of the culture medium containing tested chemicals at final concentrations of 10^{-6}-10^{-4} mol × L^{-1}. After 72 h incubation, 10 μL of MTT (5 mg × mL^{-1}, Sigma-Aldrich) was added into each well. After an additional 4 h at 37 °C, during which insoluble formazan was produced, 100 μL of 10% (m/m) sodium dodecylsulfate (SDS, Sigma-Aldrich) was added into each well and another 12 h were allowed for the dissolution of formazan. The absorbance was measured at 540 nm and 630 nm – reference wavelenght by the automated uQuant™ Universal Microplate Spectrophotometer (Biotek Instruments Inc., Winooski, VT USA). The blank corrected absorbance of the control wells was taken as 100% and the results were expressed as a percentage of the control.

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