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Stereoselective synthesis of 1-methoxyspiroindoline phytoalexins and their amino analogues

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ABSTRACT

The stereoselective synthesis of the spiroindoline phytoalexin (*R*)-(+)-1-methoxyspirobrassinin and its unnatural (*S*)-(−)-enantiomer were achieved by the bromine-induced spirocyclization of 1-methoxybrassinin using chiral auxiliaries (+)- and (−)-menthol, followed by oxidation of the obtained menthyl ethers. The TFA-catalyzed methanolysis of chiral 1-methoxyspirobrassinol menthyl ethers provided (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether and its other three unnatural stereoisomers. The enantiomers of the 2-amino analogues of the indole phytoalexin (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether were prepared by the TFA-catalyzed replacement of the chiral alkoxy group with an amine. The synthesized compounds were tested in vitro on cancer cell lines and examined with enantiopure 2-amino analogues of the indole phytoalexin (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether, which showed in most cases, lower activity than the corresponding racemates.

1. Introduction

Indole phytoalexins are low molecular weight secondary metabolites produced by plants of the family *Cruciferae*, after their exposure to physical, biological, or chemical stress.[1] Among them several spiroindoline[3,5]thiazolidine-type phytoalexins, such as (*S*)-(−)-spirobrassinin **1**, from Japanese radish (*Raphanus sativus*),[2,3] (*R*)-(+)-1-methoxyspirobrassinin **2**, from kohlrabi (*Brassica oleracea* var. *gongylodes*)[4,5], or (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether **3**, from Japanese radish (*Raphanus sativus*)[5,6] have been isolated (Fig. 1), their absolute configuration determined. The optically inactive spiroindoline phytoalexin 1-methoxyspirobrassinol **4** was isolated from Japanese radish and exists in solution as a mixture of diastereoisomers **4a** and **4b** in a 1:4 ratio due to its unstable hemiaminal structure.[6] (+)-Erucalexin **5**, with an as yet unknown absolute stereochemistry, have been isolated as a phytoalexin of dog mustard.[7]

In 2002, a spirocyclization strategy toward spiroindoline phytoalexins was developed based on the treatment of 1-methoxybrassinin **6** with dioxane dibromide in the presence of 5% of a nucleophile.[8] A mixture of racemic *trans*- and unnatural *cis*-diastereoisomer of 1-methoxyspirobrassinol methyl ether **3a** and **3b** in a 1:2 ratio was prepared using methanol as the nucleophile. Spirocyclization in the presence of water afforded a mixture of **4a** and **4b** in a 2:1 ratio. The *trans*-diastereoisomer is regarded as the one with the sulfur of the thiazoline ring and a group at the 2-position being located on the opposite sides of the indoline ring,

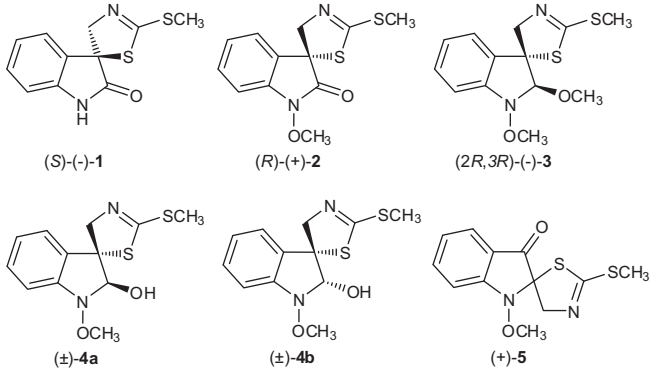

Figure 1. Selected indole phytoalexins.

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whereas the *cis*-diastereoisomer has the sulfur and the group at the 2-position on the same side of the indoline ring. Oxidation of the mixture of isomers **4a** and **4b** with CrO_3 afforded racemic (±)-**2**.[8] Direct biomimetic oxidation of 1-methoxybrassinin **6** to racemic (±)-1-methoxyspirobrassinin **2** was carried out by treatment with PCC in 40% yield.[7] (±)-1-Methoxyspirobrassinin **2** and *trans*-(±)-1-methoxyspirobrassinol methyl ether **3b** were resolved by chiral HPLC and the absolute configurations of natural (*R*)-(+)-**2** and (2*R*,3*R*)-(−)-**3** were determined by CD, VCD, and chemical correlation.[5] Analogues of the indole phytoalexin 1-methoxyspirobrassinol methyl ether can be prepared by replacing its 2-methoxy group with a 2-piperidyl,[9] 2-[*N*,*N*-bis(2-chloroethyl)amino][10] or 2-(substituted phenyl)amino group.[11]

Some indole phytoalexins also exhibit anticancer activity[12] and recently their antitrypanosomal effect was evaluated.[13] The natural isomers (*R*)-(+)-1-methoxyspirobrassinin **2** and (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether (−)-**3** were obtained in their individual enantiomeric forms and (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether (−)-**3** was found to be a more potent inhibitor of Jurkat cell proliferation than its (2*S*,3*S*)-(+)-enantiomer (+)-**3** and racemic (±)-**3**. On the other hand, in the case of 1-methoxyspirobrassinin **2**, there was no difference in potencies between the enantiomers, and both displayed weak overall activity against Jurkat cells.[5] Synthetic 2-amino derivatives of indole phytoalexins, such as 1-methoxyspirobrassinol methyl ether, were synthesized in order to achieve higher potency, as well as a better understanding of the structure–activity relationship. The introduction of a piperidyl moiety resulted in enhanced antiproliferative activity with the most potent anticancer effect exhibited by the *trans*-(±)-isomer against leukemic cell line CCRF-CEM with IC_{50} = 0.0263 - μmol × L^{-1}.[9] The *cis*-diastereoisomers of the 2-amino analogues of 1-methoxyspirobrassinol methyl ether with 4-methyl-, 3,4-dimethyl-, 3,4-dichloro-, and 4-nitrophenylamino have potencies higher than or comparable to cisplatin on Jurkat cells. The *trans*-diastereoisomers of 4-chloro- and 3,4-dichlorophenylamino analogues have higher potencies than the etoposide on MDA-MB-231 cells, and the 4-trifluoromethylphenylamino analogue was found to be the most potent among all of the compounds tested on A-549 cells and was approximately twice as potent than doxorubicin.[11]

2. Results and discussion

2.1. Chemistry

Herein we report the stereoselective synthesis of natural (+)-1-methoxyspirobrassinin (*R*)-(+)-**2**, unnatural (*S*)-(−)-**2** and diastereoisomers of 1-methoxyspirobrassinol methyl ether **3** as well as their 2-amino analogues. Some of these results have already been reported on.[14] We studied the spirocyclization of 1-methoxybrassinin in the presence of bulky chiral secondary alcohols as nucleophiles, which were reacted with methoxyiminium ion **A** (Scheme 1). It was supposed that the chiral secondary alcohol would approach the methoxyiminium ion **A** from the less hindered CH_2-side of the thiazoline ring in the direction of a Bürgi–Dunitz trajectory[15] with the alkoxy substituent being the most remote from the reaction center. The (*R*)-methoxyiminium intermediate (*R*)-**A** should be preferably attacked by the (*S*)-enantiomer of the alcohol from the less hindered CH_2-side of the thiazoline ring (Fig. 2). In the case of (*S*)-methoxyiminium intermediate (*S*)-**A**, the analogous attack of the (*S*)-alcohol should be unfavored. With the (*R*)-enantiomer of the alcohol, we expected the opposite. By analogy, the approach of the (*S*)-alcohol to the (*S*)-methoxyiminium ion, the (*R*)-alcohol to the (*R*)-methoxyiminium ion should be more favored from the sulfur side of the thiazoline ring.[15]

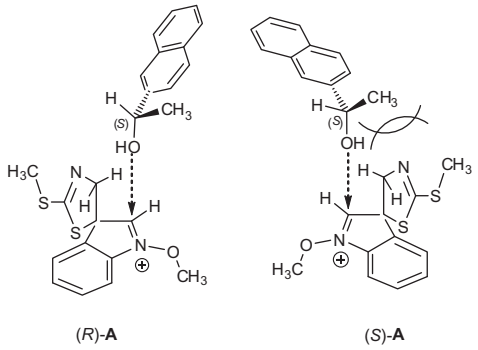
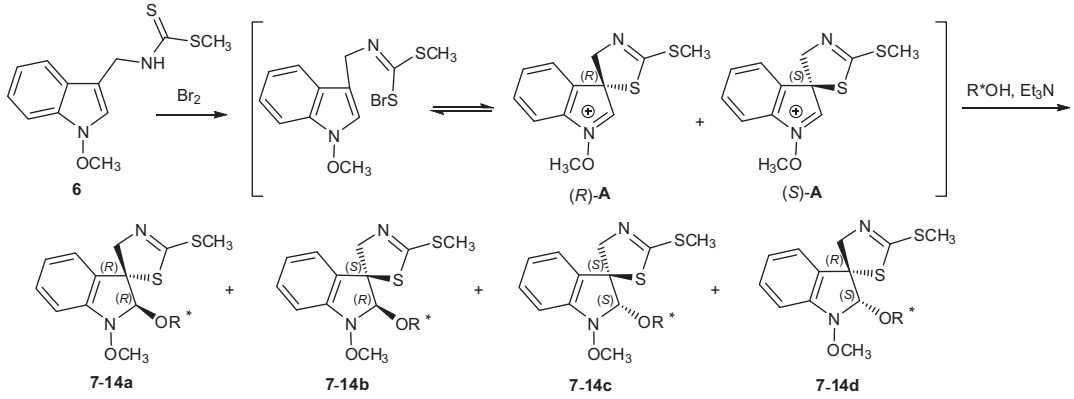


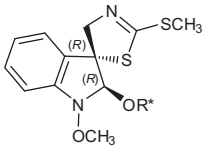
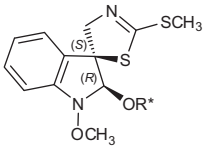
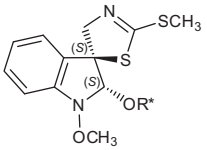
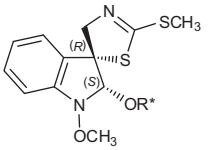
Figure 2.

We investigated the spirocyclization in the presence of (*S*)-(−)- and (*R*)-(+)-1-(2-naphthyl)ethanol, (1*S*,2*R*,5*S*)-(+)- and (1*R*,2*S*,5*R*)-(−)-menthol, (*R*)-(+)-1-phenylethanol, [(1*S*)-*endo*]-(−)-borneol, (*S*)-(+)-1-indanol, and (*S*)-*cis*-verbenol. In all cases, the formation of all four possible diastereoisomers was observed. From analysis of the reaction mixtures, it was found that the best diastereoselectivity was obtained in the presence of chiral 1-(2-naphthyl)ethanol (Table 1). Spirocyclization in the presence of (*S*)-(−)-1-(2-naphthyl)ethanol resulted in the formation of four diastereoisomers with the product of nucleophilic addition of this alcohol to the (*R*)-methoxyiminium ion from the *Re*-face as the major product **7a**, whereas (*R*)-(+)-1-(2-naphthyl)ethanol attacked the (*S*)-intermediate **A** mainly from the *Si*-face with the formation of diastereoisomer **8c** as the major product. The second most abundant isomers **7b** and **8d** correspond to the attack of the (*S*)-alcohol onto



Scheme 1. Spirocyclization of 1-methoxybrassinin **6** with bromine in the presence of a chiral alcohol.

Table 1
Stereochemistry and ratios of diastereoisomers **7-14** produced via Scheme 1

R*	Compound/ratio[a] (%)			
				
(*S*)-(−)-1-(2-Naphthyl)ethyl[b]	**7a**	**7b**	**7c**	**7d**
	69	22	2	7
(*R*)-(+)-1-(2-Naphthyl)ethyl[b]	**8a**	**8b**	**8c**	**8d**
	7	7	68	18
(1*S*,2*R*,5*S*)-(+)-Menthyl[b]	**9a**	**9b**	**9c**	**9d**
	57	18	20	5
(1*R*,2*S*,5*R*)-(−)-Menthyl[b]	**10a**	**10b**	**10c**	**10d**
	17	3	60	20
(*R*)-(+)-1-Phenylethyl[c]	**11a**	**11b**	**11c**	**11d**
	51	12	14	23
[(1*S*)-*endo*]-(−)-Borneyl[c]	**12a**	**12b**	**12c**	**12d**
	20	19	54	7
(*S*)-(+)-1-Indanyl[c]	**13a**	**13b**	**13c**	**13d**
	39	15	28	18
(*S*)-*cis*-Verbenyl[c]	**14a**	**14b**	**14c**	**14d**
	37	12	37	14

cis-Diastereoisomeric configuration was assigned to products that exhibited a cross peak in the NOESY spectra between the H-2 and Hb protons while a *trans*-diastereoisomeric configuration was assigned to products where this cross peak was absent.

[a] The ratios of diastereoisomers of compounds **7**–**14** were determined by integration of the non-overlapping singlets of the H-2 protons and the doublets of the H_a or H_b protons in the ^1H NMR spectra of the crude product mixtures.

[b] The absolute configurations of products **7**–**10** were determined in the literature.[14]

[c] The configurations of products **11**–**14** are relative, and were identified by analogy with compounds **7**–**10**.

the (*S*)-ion and the (*R*)-alcohol onto the (*R*)-ion from the sulfur side of the thiazolidine ring. Although the expected diastereoselectivity was achieved, the isolated yields of the major products of the 1-methoxyspirobrassinol 1-(2-naphthyl)ethyl ethers were only 23% for **7a** or 29% for **8c**. The second most abundant isomers were 11% for **7b** and 12% for **8d**. Using chiral alcohol (*R*)-(+)-1-phenylethanol resulted in lower stereoselectivity and yields than (*R*)-(+)-1-(2-naphthyl)ethanol. Reaction with the more common chiral auxiliary (1*S*,2*R*,5*S*)-(+)- and (1*R*,2*S*,5*R*)-(−)-menthol proceeded with similar diastereoselectivity, although the corresponding 1-methoxyspirobrassinol menthyl ethers **9a** and **10c** were obtained in higher isolated yields. The (±)-*trans*- and (±)-*cis*-diastereoisomers were isolated in 65% and 22% yield [for (+)-menthol] and 70% and 21% [for (−)-menthol] after chromatography on silica gel (eluent: *n*-hexane/diethyl ether 4:1). Additional chromatography with CH_2Cl_2 eluent provided the major diastereoisomers **9a** (38%) and **10c** (40%) with 99% ee. The bicyclic chiral alcohol [(1*S*)-*endo*]-(−)-borneol resulted in similar diastereoselectivity as in the case of (+)-menthol; after the first chromatography, the (±)-*trans*- and (±)-*cis*-diastereoisomers were isolated in 50% and 32% yields, but the major diastereoisomers could not be separated from the mixture of (±)-*trans*-diastereoisomers in all eluents tested. Using other chiral alcohols, such as (*S*)-(+)-1-indanol and (*S*)-*cis*-verbenol, did not improve the stereoselectivity, while the *trans*- and *cis*-diastereoisomers were formed in similar ratios and could not be separated.

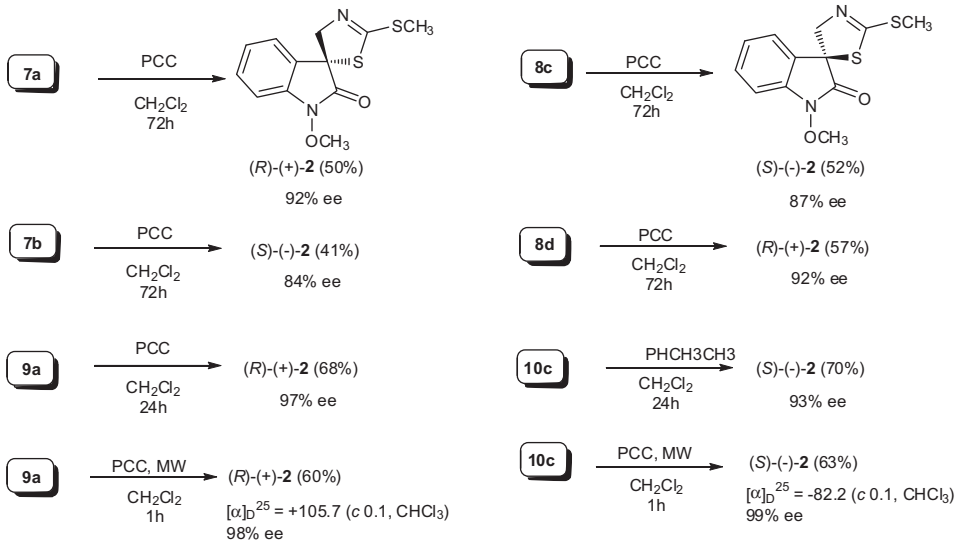
With the aim of improving the stereoselectivity of the spirocyclization with (+)-menthol, the temperature effect on the diastereoselectivity was studied. The diastereoselectivity was improved by raising the temperature, as can be seen in Table 2. It is interesting to note that the major diastereoisomer **9a** with a *trans*-configuration switched to **9b** with a *cis*-configuration at −70 °C. This could be due to a different mechanism at a low temperature. The reaction begins by the addition of a solution of bromine to a solution of 1-methoxybrassinin whereby an iminium ion is formed. A solution of chiral alcohol with 10 equiv of Et_3N is then added after 1 min to avoid substitution of the OH group of alcohol with bromine in the presence of HBr formed during the spirocyclization. The potential effect of Et_3N on the spirocyclization was confirmed with experiments with methanol (1.1 equiv) instead of menthol. The ratio of *trans*- and *cis*-diastereoisomer **3a** and **3b**, when the spirocyclization was carried out at 20 °C without Et_3N was 52:48, with Et_3N 39:61 and at −70 °C without Et_3N was 74:26, with Et_3N 17:83. It is possible that Et_3N attacks the intermediate methoxyiminium ion **A** from the less hindered thiazoline CH_2 side with the formation of an unstable triethylammonium ion. This further reacts with the methanol that approaches from the sulfur side, which results in the formation of the *cis*-diastereoisomer **3b**. A similar effect as with Et_3N was observed when using dioxane instead CH_2Cl_2 as the solvent, where the ratio of *trans*- and *cis*-diastereoisomer **3a** and **3b** was 36:64. This can be explained by the formation of an unstable oxonium ion after attacking the methoxyiminium ion **A** from the less hindered thiazoline CH_2 side with dioxane and further reaction of the ion with the methanol from the sulfur side.[5] The alcohol is presumably less reactive at lower temperatures and Et_3N reacts in preference. When using menthol as the secondary alcohol, it led to a remarkable decrease in the reactivity.

Table 2
Influence of temperature on the stereoselectivity of the spirocyclization of 1-methoxybrassinin **6** with (1*S*,2*R*,5*S*)-(+)-menthol

t (°C)	Compound/ratio (%)			
	9a	**9b**	**9c**	**9d**
60[a]	57	19	21	3
20[b]	57	18	20	5
−20[b]	55	25	16	4
−70[b]	36	45	8	11

[a] Solvent $CHCl_3$, 1.1 equiv of menthol, 10 equiv Et_3N.

[b] Solvent CH_2Cl_2, 1.1 equiv of menthol, 10 equiv Et_3N.

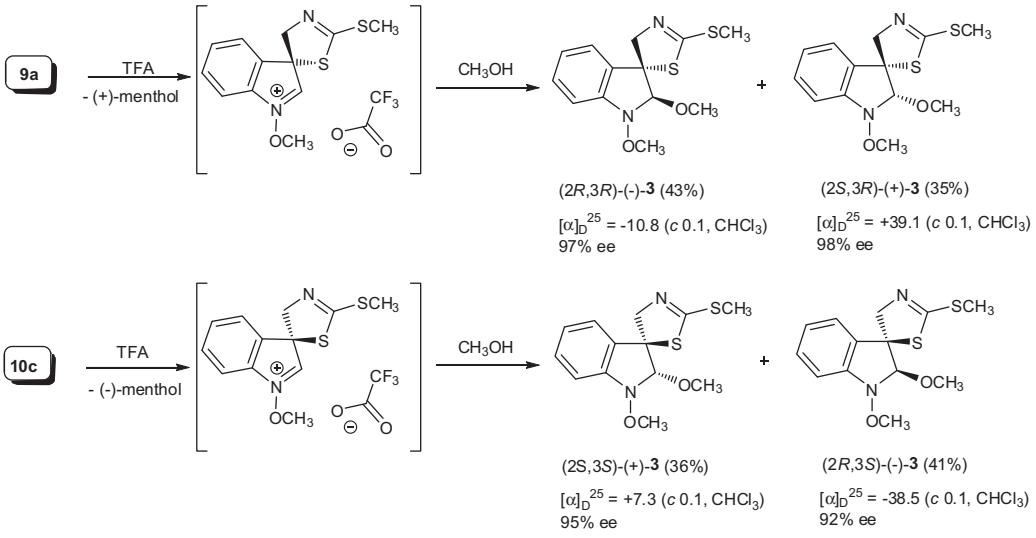


Scheme 2. Synthesis of enantiomerically pure 1-methoxyspirobrassinin (*R*)-(+)-**2** and (*S*)-(−)-**2**.

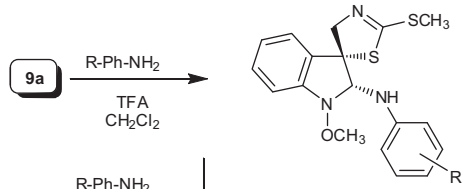
With the pure diastereoisomers in hand, the enantiomers of the indole phytoalexin 1-methoxyspirobrassinin (*R*)-(+)-**2** and (*S*)-(−)-**2** were synthesized (Scheme 2). Oxidation of the main diastereoisomer of naphthyl ether **7a** or **8c** with PCC gave natural (*R*)-(+)-1-methoxyspirobrassinin (*R*)-(+)-**2** in 50% yield (92% ee) or (*S*)-(−)-**2** in 52% yield (87% ee). Oxidation of diastereoisomer **7b** or **8d** resulted in (*S*)-(−)-**2** with 41% yield (84% ee) or (*R*)-(+)-**2** with 57% yield (92% ee). The reaction time was 72 h, but could be shortened to 3.5 or 3 h by the application of microwave irradiation. The same oxidation of the main and single isolated diastereoisomer of a menthyl ether proceeded smoothly over 24 h from **9a** to (*R*)-(+)-**2** with 68% yield (97% ee) and from **10c** to (*S*)-(−)-**2** with 70% yield (93% ee). By using microwave irradiation, the reaction time could be shortened to 1 h [(*R*)-(+)-**2** 98% and (*S*)-(−)-**2** 99%].

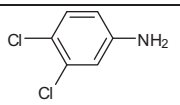
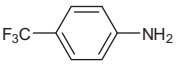
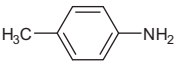
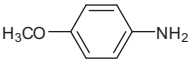
In order to prepare the natural (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether (2*R*,3*R*)-(−)-**3**, the methanolysis of **9a** was investigated. It was found that TFA-catalyzed methanolysis afforded a mixture of epimers (2*R*,3*R*)-(−)-**3** and (2*S*,3*R*)-(+)-**3** in a 1:1 ratio, and which were easily separable by column chromatography (Scheme 3). The analogous reaction of **10c** afforded the (2*S*,3*S*)-(+)-**3** and (2*R*,3*S*)-(−)-**3** isomers of 1-methoxyspirobrassinol methyl ether in a 1:1 ratio. The ee of the synthesized enantiomers were 92–98%. The reaction was carried out in dry CH_2Cl_2 to avoid a competitive reaction with water resulting in the formation of 1-methoxyspirobrassinol **5**.

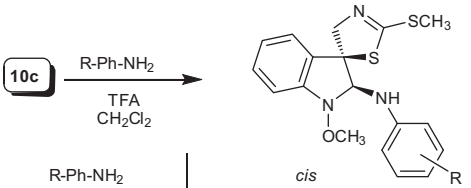
Recently a set of racemic 2-amino analogues of 1-methoxyspirobrassinol methyl ether **15**–**18** was synthesized and their anticancer activity determined.[11] The preparation of enantiomerically pure isomers of selected 2-amino analogues of 1-methoxyspirobrassinol methyl ethers **15**–**18** was accomplished with the aim of establishing and comparing anticancer activities of the racemates and its enantiomers. The synthesis is based on the acid-catalyzed nucleophilic substitution of the chiral alcohol of menthyl ether **9a** or **10c** with an amine (3,4-dichloroaniline, *p*-trifluoroaniline, *p*-toluidine and *p*-anisidine, Scheme 4). In all cases, 2 equiv of amine and 1.1 equiv of TFA were used. The reactions were performed in dry CH_2Cl_2. The reaction course was monitored by TLC and after consumption of the starting material, triethylamine was added. The reaction time depended on the nucleophilicity of the amine. Substitution of the menthyl group with 3,4-dichloroaniline required

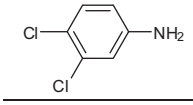
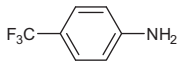
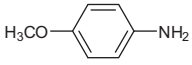


Scheme 3. Synthesis of the enantiomerically pure diastereoisomers of **3**.



R-Ph-NH_2	*cis*	*trans*	*dr* *cis*:*trans*
	(2*R*,3*R*)-(+)-**15** (62%) $[\alpha]_D^{25}$ = +116.2 (*c* 0.4, $CHCl_3$) 96% ee	(2*S*,3*R*)-(-)-**15** (26%) $[\alpha]_D^{25}$ = -75.9 (*c* 0.3, $CHCl_3$) 96% ee	70:30
	(2*R*,3*R*)-(+)-**16** (61%) $[\alpha]_D^{25}$ = +20.2 (*c* 0.15, $CHCl_3$) 99% ee	(2*S*,3*R*)-(-)-**16** (23%) $[\alpha]_D^{25}$ = -72.5 (*c* 0.07, $CHCl_3$) 98% ee	74:26
	(2*R*,3*R*)-(+)-**17** (60%) $[\alpha]_D^{25}$ = +211.7 (*c* 0.15, $CHCl_3$) 95% ee	(2*S*,3*R*)-(-)-**17** (15%) $[\alpha]_D^{25}$ = -74.7 (*c* 0.3, $CHCl_3$) 96% ee	84:16
	(2*R*,3*R*)-(+)-**18** (34%) $[\alpha]_D^{25}$ = +226.2 (*c* 0.1, $CHCl_3$) 96% ee	(2*S*,3*R*)-(-)-**18** (13%) $[\alpha]_D^{25}$ = -95.8 (*c* 0.15, $CHCl_3$) 90% ee	84:16



R-Ph-NH_2	*cis*	*trans*	*dr* *cis*:trans
	(2*S*,3*S*)-(-)-**15** (69%) $[\alpha]_D^{25}$ = -131.6 (*c* 0.4, $CHCl_3$) 99% ee	(2*R*,3*S*)-(+)-**15** (21%) $[\alpha]_D^{25}$ = +118.7 (*c* 0.3, $CHCl_3$) >99% ee	77:23
	(2*S*,3*S*)-(-)-**16** (69%) $[\alpha]_D^{25}$ = -43.4 (*c* 0.15, $CHCl_3$) 98% ee	(2*R*,3*S*)-(+)-**16** (18%) $[\alpha]_D^{25}$ = +42.7 (*c* 0.07, $CHCl_3$) 99% ee	70:30
	(2*S*,3*S*)-(-)-**17** (70%) $[\alpha]_D^{25}$ = -207.9 (*c* 0.15, $CHCl_3$) 99% ee	(2*R*,3*S*)-(+)-**17** (15%) $[\alpha]_D^{25}$ = +90.1 (*c* 0.3, $CHCl_3$) 98% ee	86:14
	(2*S*,3*S*)-(-)-**18** (31%) $[\alpha]_D^{25}$ = -135.2 (*c* 0.1, $CHCl_3$) 98% ee	(2*R*,3*S*)-(+)-**18** (9%) $[\alpha]_D^{25}$ = +116.3 (*c* 0.15, $CHCl_3$) 94% ee	74:26

Scheme 4. Synthesis of enantiomerically pure amino analogues of 1-methoxyspirobrassinol methyl ether **15**–**18**.

90 min while using *p*-trifluoroaniline required 150 min. The presence of an electron-donor substituent prolongs the reaction time; in the case of *p*-toluidine to 24 h and in the case of *p*-anisidine the reaction did not occur at room temperature and needed to be heated at reflux for 14.5 h. The products of the substitution were two epimers that differed at the C-2 carbon, in a ratio as indicated in Scheme 4. The ratio of the epimers was determined by integration of the differentiated protons for H_a, H_b, NH, H-2, OCH_3, and SCH_3. In all cases, the major products of the substitution of the menthyl group with an amine were the *cis*-diastereoisomers. Generally, the S_N1 reaction creates a racemic product (an equal amount of isomers). An excess of the *cis*-isomer is caused by subsequent epimerization of the products at the C-2 carbon under acidic condition, resulting in the formation of a thermodynamically more stable diastereoisomer.[11] The *cis*- and *trans*-epimers were isolated by column chromatography on silica gel. After the first chromatography, the *cis*-isomer had a menthol impurity and was separated from the *trans*-isomer, which was impure with amine. Further chromatography in CH_2Cl_2 provides the pure epimers with ees of 90–99%.

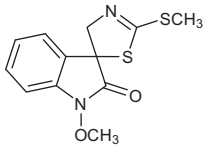
For all of the isomers prepared, **7a**, **8c**, **9a**, **10c**, **2**, **3**, and **15**–**18**, the CD spectra were examined and the enantiomeric structures of individual enantiomers were confirmed by absorption of the inverse circular polarized light.

2.2. Antiproliferative activity

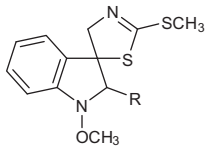
With the aim of comparing the antiproliferative activity of the racemates and enantiomers of indole phytoalexins and their 2-amino analogues selected human cancer cell lines were examined. The results of this investigation are in Table 3.

The antiproliferative activity of 1-methoxyspirobrassinin **2** was noted only on Jurkat cells. The highest effect was observed with (*S*)-(−)-**2** with IC_{50} = 18.6 μmol L^{-1}. Natural racemic (±)-*trans*- and unnatural racemic (±)-*cis*-1-methoxyspirobrassinol methyl ether (±)-*trans*-**3** and (±)-*cis*-**3** showed comparable antiproliferative activities on Jurkat and Hela cells with (2*S*,3*R*)-(+)- being the most effective with IC_{50} = 41 μmol L^{-1} on Jurkat and with IC_{50} = 33.6 μmol L^{-1} on A-549 cells. Our data indicated that the

Table 3
Antiproliferative activities of racemates and enantiomers of indole phytoalexins and their 2-amino analogues



2



3,15-18

Compound	R	Cell line, IC_{50} (μmol × L^{-1})					
		Jurkat	MCF-7	MDA-MB-231	HeLa	CCRF-CEM	A-549
(±)-**2**[a]	—	41.1	100	100	100	100	100
(*R*)-(+)-**2**		40.0	100	100	100	100	100
(*S*)-(−)-**2**		18.6	100	100	100	100	100
(±)-*trans*-**3**[b]	OCH_3	30.2	100	100	48.9	100	100
(2*R*,3*R*)-(−)-**3**		100	100	100	100	100	78
(2*S*,3*S*)-(+)-**3**		100	100	100	100	100	100
(±)-*cis*-**3**[b]		57.4	100	100	53.2	100	100
(2*S*,3*R*)-(+)-**3**		41	100	100	100	100	33.6
(2*R*,3*S*)-(−)-**3**		100	100	100	100	100	100
(±)-*cis*-**15**[11]	3,4-Di-Cl-C_6H_3NH	10.0	100	35.5	15.4	100	24.7
(2*R*,3*R*)-(+)-**15**		24.0	48.0	67.0	41.0	48.0	100
(2*S*,3*S*)-(−)-**15**		50.0	100	100	82.0	78.5	100
(±)-*trans*-**15**[11]		21.0	22.6	20.7	21.8	22.6	21.1
(2*S*,3*R*)-(−)-**15**		100	100	100	100	100	100
(2*R*,3*S*)-(+)-**15**		30.2	30.0	36.0	22.0	27.0	30.0
(±)-*cis*-**16**[11]	4-CF_3-C_6H_4NH	41.8	100	88	81.9	74.3	1.0
(2*R*,3*R*)-(+)-**16**		37.5	77.4	47.2	59.5	47.5	76.1
(2*S*,3*S*)-(−)-**16**		29	—	51.7	57.5	38.3	78.0
(±)-*trans*-**16**[11]		28.4	66.8	31.1	34.9	54.0	46.6
(2*S*,3*R*)-(−)-**16**		30.7	—	44.0	39.0	37.6	60.0
(2*R*,3*S*)-(+)-**16**		30.0	30.5	39.2	31.5	36.8	34.5
(±)-*cis*-**17**[11]	4-CH_3-C_6H_4NH	6.6	100	78.7	100	64.7	100
(2*R*,3*R*)-(+)-**17**		100	100	100	100	42.0	100
(2*S*,3*S*)-(−)-**17**		72.0	100	100	100	75.0	100
(±)-*trans*-**17**[11]		33.3	100	100	36.8	100	100
(2*S*,3*R*)-(−)-**17**		73.0	100	87.0	76.3	49.2	100
(2*R*,3*S*)-(+)-**17**		82.3	71.0	100	71.0	61.0	78.0
(±)-*cis*-**18**[11]	4-CH_3O-C_6H_4NH	34.8	100	100	81.5	10.0	73.9
(2*R*,3*R*)-(+)-**18**		38.6	>100	>100	60.2	54.7	>100
(2*S*,3*S*)-(−)-**18**		>100	>100	>100	>100	>100	>100
(±)-*trans*-**18**[11]		69.4	100	64.0	100	15.5	100
(2*S*,3*R*)-(−)-**18**		65.2	96.8	>100	63	>100	>100
(2*R*,3*S*)-(+)-**18**		70.5	>100	>100	46.7	90	>100

The potency of the 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 the amount of viable cells to 50% relative to untreated control cells, see Section 4.12.).

[a] Synthesized according the literature precedure.[8]

[b] Synthesized according the literature precedure.[8]

amino analogues of 1-methoxyspirobrassinol methyl ether showed enhanced antiproliferative activity. The binding of dichloro-, methoxy-, and methyl-groups to amino analogues of 1-methoxyspirobrassinol methyl ether significantly increased their activity with the (±)-*cis*-diastereoisomers being more effective on Jurkat cells. The addition of trifluoro substituents to methylaniline analogue resulted in decreased antiproliferative activity except for the A-549 cell line where the highest antiproliferative activity was noted with IC_{50} = 1 μmol L^{-1}. The (2*R*,3*S*)-(+)-diastereoisomer (2*R*,3*S*)-(+)-**16** showed the highest effect among the trifluoromethylaniline diastereoisomers on all cell lines.

3. Conclusion

In conclusion, we have developed a stereoselective approach to spiroindoline phytoalexins and their unnatural enantiomers involving the bromine-mediated spirocyclization of 1-methoxybrassinin in the presence of (+)- and (−)-menthol as the chiral auxiliary. Subsequent oxidation of the main and single isolated diastereoisomer of 1-methoxyspirobrassinol menthyl ether using PCC and microwave irradiation provided natural (*R*)-(+)-1-methoxyspirobrassinin (*R*)-(+)-**2** in 60% yield and its unnatural isomer (*S*)-(−)-**2** in 63% yield, with enantiomeric excesses of 98% and 99%, respectively. The synthesis of natural (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether (97% ee) as well its unnatural (2*S*,3*S*)-, (2*R*,3*S*)-, and (2*S*,3*R*)-isomers (92–98% ee) was achieved by TFA-catalyzed methanolysis of chiral 1-methoxyspirobrassinol menthyl ethers. The TFA-catalyzed replacement of the chiral menthoxy group with an amine afforded enantiomerically pure 2-amino analogues of indole phytoalexin (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether.

We also examined the cytostatic/cytotoxic activity of the synthesized compounds against selected human solid tumor and leukemia cell lines in vitro (Jurkat, MCF-7, MDA-MB-231, HeLa, CCRF-CEM and A-549). The tested enantiomerically pure 2-amino analogues of indole phytoalexin (2*R*,3*R*)-(−)-1-methoxyspirobrassinol methyl ether showed lower activity compared to the corresponding racemates in most cases.

4. Experimental

4.1. General

Melting points were determined on a Koffler micro melting point apparatus and are uncorrected. IR spectra were recorded on an IR-75 spectrometer (Zeiss Jena). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on a Varian Mercury Plus spectrometer. Chemical shifts (δ) are reported in ppm downfield from TMS as the internal standard and coupling constants (J) are given in Hertz. Microanalyses were performed with a Perkin–Elmer, Model 2400 analyzer. MALDI-TOF mass spectra were measured on a MALDI IV (Kratos Analytical). The samples were ionized with a N2-laser (k = 337 nm). The progress of the chemical reactions was monitored by thin layer chromatography, using Macherey-Nagel plates Alugram_Sil G/UV254. Preparative column chromatography was performed on Kieselgel 60 Merck Type 9385 (0.040–0.063 mm). The resolutions were performed with an Agilent 1120 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, an autosampler, a column oven, and a variable wavelength detector. The collection and evaluation of data were performed using EZChrom Elite Compact software. Analytical chiral HPLC was performed on a column CHIRALCEL® OD (ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm), CHIRALPAK® OD (ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm), CHIRALPAK® AD (ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm), CHIRALPAK IA [CARTRIDGE HOLDER ϕ 0.4 × 1 cm + ϕ 0.46 × 25 cm] and Larihc RN-CF6 (ϕ 0.46 × 25 cm). Optical rotations were measured at room temperature in a 10 cm cell on a polarimeter P3002 (Kruess) and a P-2000 Jasco polarimeter at the sodium D-line. CD spectra were obtained in a 1 mm quartz cell on a JASCO J-810 spectrometer. Microwave reactions were carried out on the focused microwave system (CEM Discover). The temperature content of the vessel was monitored using a calibrated infrared sensor mounted under the vessel. At the end of all reactions, the contents of vessel were cooled rapidly using a stream of compressed air.

4.2. Spirocyclization of 1-methoxybrassinin 6 with bromine in the presence of (*S*)-(−)-1-(2-naphthyl)ethanol

To a stirred mixture of 1-methoxybrassinin **6** (180 mg, 0.676 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (12.5 mL) at room temperature was added a freshly prepared solution of Br_2 (0.038 mL, 119 mg, 0.743 mmol) in dry CH_2Cl_2 (1.7 mL). After stirring for 1 min, a suspension of (*S*)-(−)-1-(2-naphthyl)ethanol (128 mg, 0.743 mmol), triethylamine (685 mg, 0.943 mL, 6.76 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (12.5 mL) was added. Stirring was continued for 15 min, after which the reaction mixture was diluted with CH_2Cl_2 (40 mL), and then washed with water (2 × 40 mL) and brine (40 mL). The organic layer was dried over anhydrous Na_2SO_4. The residue obtained after evaporation of solvent was subjected to chromatography on silica gel (35 g, petroleum ether/diethyl ether 4:1), affording 67 mg (23%) of diastereoisomer **7a** and 32 mg (11%) of diastereoisomer **7b** as colorless oils. Diastereoisomers **7c** and **7d** were not isolated.

4.2.1. (2*R*,3*R*)-1-Methoxyspirobrassinol[(*S*)-1-(2-naphthyl)ethyl] ether 7a

$[\alpha]_D^{25}$ = −179.3 (c 0.569, $CHCl_3$). IR ($CHCl_3$): ν 3053, 2973, 2933, 2893, 1573, 1460, 1367, 1300, 1127, 1073, 993, 947 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.87–7.81 (m, 4H, H-1′, H-4′, H-5′, H-8′), 7.58 (dd, 1H, J = 8.4, J = 1.6 Hz, H-3′), 7.48–7.44 (m, 2H, H-6′, H-7′), 7.30 (ddd, 1H, J = 7.6, J = 1.2, J = 0.6 Hz, H-4), 7.20 (td, 1H, J = 7.6, J = 1.2 Hz, H-6), 6.99 (td, 1H, J = 7.6, J = 1.1 Hz, H-5), 6.84 (ddd, 1H, J = 7.6, J = 1.1, J = 0.6 Hz, H-7), 5.17 (d, 1H, J = 15.2 Hz, H_b), 5.15 (s, 1H, H-2), 5.06 (quartet, 1H, J = 6.5 Hz, CH_3CH), 3.94 (d, 1H, J = 15.2 Hz, H_a), 3.67 (s, 3H, OCH_3), 2.65 (s, 3H, SCH_3), 1.62 (d, 3H, J = 6,5 Hz, CH_3CH). ^{13}C NMR (100 MHz): δ 164.0 (C=N), 140.2 (C-2′), 133.2 and 133.1 (C-8′a, C-5′a), 128.2, 128.0, 127.7, 126.1, 125.9, 125.7 and 124.5 ($C_{naphthyl}$), 148.2 (C-7a), 129.7 (C-6), 126.9 (C-3a), 123.9 (C-4), 123.6 (C-5), 112.7 (C-7), 105.6 (C-2), 78.9 (CH_3CH), 69.8 (C-3), 69.2 (CH_2), 63.9 (CH_3O), 23.9 (CH_3CH), 15.2 (SCH_3). NOESY: H_a/H-4, H_b; CH_3CH/H-2; H-7/H-6; H-5/H-6, H-4; H-3′/H-4′. MALDI-TOF MS, m/z (%): 477.4 $[M+K]^+$ (100), 458.4 $[M+Na]^+$ (17), 436.7 $[M+H]^+$ (42). Anal. Calcd for $C_{24}H_{24}N_2O_2S_2$: C, 66.02; H, 5.54; N, 6.42. Found: C, 65.82; H, 5.73; N, 6.25. CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 211 (−2.65), 217 (−2.07), 224 (−2.95), 241 (+0.07), 270 (−0.36), 294 (+0.41). >99% ee (CHIRALCEL® OD (ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 77:23 at a flow rate 1 mL/min); R_t = 5.69 min.

Enantiomeric product **8c** (85 mg, 29%) was prepared by the same procedure using (*R*)-(+)-1-(2-naphthyl)ethanol. Compound **8c**: $[\alpha]_D^{25}$ = +183.1 (c 0.633, $CHCl_3$). CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 211 (+5.60), 217 (+3.87), 224 (+4.94), 241 (−0.07), 270 (+0.58), 294 (−0.59). >99% ee (CHIRALCEL® OD (ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 77:23 at a flow rate 1 mL/min); R_t = 5.43 min.

4.2.2. (2*R*,3*S*)-1-Methoxyspirobrassinol[(*S*)-1-(2-naphthyl)ethyl] ether 7b

$[\alpha]_D^{25}$ = −166.4 (c 0.382, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88–7.82 (m, 4H, H-1′, H-4′, H-5′, H-8′), 7.60 (dd, 1H, J = 8.5, J = 1.7 Hz, H-3′), 7.50–7.44 (m, 2H, H–6′, H-7′), 7.27 (dd, 1H, J = 7.6, J = 1.2 Hz, H-4), 7.21 (td, 1H, J = 7.6, J = 1.2 Hz, H-6), 6.98 (dt, 1H, J = 7.6, J = 1.0 Hz, H-5), 6.84 (dd, 1H, J = 7.6, J = 1.0 Hz, H-7), 4.91 (quartet, 1H, J = 6.4 Hz, CH_3CH), 4.81 (s, 1H, H-2), 4.50 (d, J = 1H, 15.4 Hz, H_a), 4.44 (d, 1H, J = 15.4 Hz, H_b), 3.72 (s, 3H, OCH_3), 2.64 (s, 3H, SCH_3), 1.64 (d, 3H, J = 6.4 Hz, CH_3CH). ^{13}C NMR (100 MHz): δ 164.0 (C=N), 140.2 (C-2′), 133.2 and 133.1 (C-8′a, C-5′a), 129.9, 128.0, 127.7, 124.5, 126.1, 125.9 and 125.7 ($C_{naphthyl}$), 148.2 (C-7a), 129.7 (C-6), 126.9 (C-3a), 123.6 (C-4), 123.4 (C-5), 112.4 (C-7), 102.3 (C-2), 78.5 (CH_3CH), 73.3 (CH_2), 70.8 (C-3), 63.8 (CH_3O), 23.9 (CH_3CH), 15.2 (SCH_3). NOESY: H_b/H-2; CH_3CH/H-2; H-7/H-6; H-5/H-6, H-4. MALDI-TOF MS, m/z (%): 477.4 $[M+K]^+$ (52), 458.6 $[M+Na]^+$ (12), 436.5 $[M+H]^+$ (32). Anal. Calcd for $C_{24}H_{24}N_2O_2S_2$: C, 66.02; H, 5.54; N, 6.42. Found: C, 65.71; H, 5.82; N, 6.63.

4.3. Spirocyclization of 1-methoxybrassinin 6 with bromine in the presence of (1*S*,2*R*,5*S*)-(+)-menthol

To a stirred mixture of 1-methoxybrassinin **6** (133 mg, 0.5 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (7 mL) at room temperature was added a freshly prepared solution of Br_2 (0.028 mL, 88 mg, 0.55 mmol) in dry CH_2Cl_2 (1.25 mL). After stirring for 1 min, a suspension of (1*S*,2*R*,5*S*)-(+)-menthol (86 mg, 0.55 mmol), triethylamine (509 mg, 0.702 mL, 5 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (7 mL) was added. Stirring was continued for 20 min, after which the reaction mixture was diluted with CH_2Cl_2 (25 mL), and washed with 1 M HCl (13 mL) and water (20 mL). The organic layer was dried over Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (13 g, petroleum ether/diethyl ether 4:1), to afford 137 mg (65%) of a mixture of *trans*-diastereoisomers **9a**, **9c** (80:20) and 46 mg (22%) of the mixture of *cis*-diastereoisomers **9b**, **9d** (85:15). Subsequent chromatography of the mixture of *trans*-diastereoisomers **9a** and **9c** on silica gel (20 g, CH_2Cl_2) gave **9a** (79 mg, 38%) and **9c** (21 mg, 10%) as colorless oils.

4.3.1. (2*R*,3*R*)-1-Methoxyspirobrassinol-(1*S*,2*R*,5*S*)-menthyl ether 9a

$[\alpha]_D^{25}$ = −8.9 (*c* 0.74, $CHCl_3$). IR ($CHCl_3$): *ν* 2953, 2893, 2863, 1567, 1453, 1120, 1033, 987, 933 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): *δ* 7.29 (dd, 1H, *J* = 7.5, *J* = 1.2 Hz, H-4), 7.23 (td, 1H, *J* = 7.5, *J* = 1.2 Hz, H-6), 7.01 (td, 1H, *J* = 7.5, *J* = 1.0 Hz, H-5), 6.95 (dd, 1H, *J* = 7.5, *J* = 1.0 Hz, H-7), 5.16 (s, 1H, H-2), 5.02 (d, 1H, *J* = 15.3 Hz, H_b), 3.93 (s, 3H, OCH_3), 3.91 (d, 1H, *J* = 15.3 Hz, H_a), 3.59 (td, 1H, *J* = 10.5, *J* = 4.3 Hz, H-1′), 2.56 (s, 3H, SCH_3), 2.41 (quintet, 1H, *J* = 6.9, *J* = 2.0 Hz, H-8′), 2.24-2.19 (m, 1H, H-6′), 1.68-1.63 (m, 2H, H-3′, H-4′), 1.42-1.26 (m, 2H, H-2′, H-5′), 1.06-0.86 (m, 3H, H-3′, H-4′, H-6′), 0.94 (d, 3H, *J* = 6.9 Hz, H-7′), 0.92 (d, 3H, *J* = 6.9 Hz) and 0.83 (d, 3H, *J* = 6.9 Hz) [H-9′, H-10′]. ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 163.3 (C═N), 148.5 (C-7a), 129.4 (C-6), 128.7 (C-3a), 124.0 (C-4), 123.8 (C-5), 112.8 (C-7), 104.1 (C-2), 79.5 (C-1′), 70.1 (CH_2), 69.3 (C-3), 63.7 (OCH_3), 48.7 (C-5′), 41.6 (C-6′), 34.3 (C-4′), 31.5 (C-2′), 24.8 (C-8′), 23.1 (C-3′), 22.4 (C-7′), 21.4 and 16.3 (C-9′, C-10′), 15.0 (SCH_3). NOESY: H_a/H-4, H_b; OCH_3/H-7; H-2/H-1′, H-6′. MALDI-TOF MS, *m*/*z* (%): 420.9 $[M+H]^+$ (94). Anal. Calcd for $C_{22}H_{32}N_2O_2S_2$: C, 62.82; H, 7.67; N, 6.66. Found: C, 62.50; H, 7.90; N, 6.40. CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 214 (−39.2), 238 (+8.1), 264 (−2.8), 293 (+4.6). >99% ee (CHIRALCEL® OD *ϕ* 0.46 cm × 5 cm + *ϕ* 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 99.9:0.1 at a flow rate 1 mL/min); R_t = 13.71 min.

Enantiomeric product **10c** (85 mg, 40%) was prepared by the same procedure using (1*R*,2*S*,5*R*)-(−)-menthol. **10c**: $[\alpha]_D^{25}$ = +11.0 (*c* 0.88, $CHCl_3$). CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 214 (+38.3), 238 (−7.6), 264 (+2.8), 293 (−4.3). >99% ee (CHIRALCEL® OD *ϕ* 0.46 cm × 5 cm + *ϕ* 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 99.9:0.1 at a flow rate 1 mL/min); R_t = 15.52 min.

4.3.2. (2*S*,3*S*)-1-Methoxyspirobrassinol-(1*S*,2*R*,5*S*)-menthyl ether 9c

$[\alpha]_D^{25}$ = −50.7 (*c* 1.15, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): *δ* 7.29 (dd, 1H, *J* = 7.6, *J* = 1.2 Hz, H-4), 7.23 (td, 1H, *J* = 7.6, *J* = 1.2 Hz, H-6), 7.01 (td, 1H, *J* = 7.6, *J* = 1.1 Hz, H-5), 6.94 (dd, 1H, *J* = 7.6, *J* = 1.1 Hz, H-7), 5.19 (s, 1H, H-2), 5.15 (d, 1H, *J* = 15.5 Hz, H_b), 3.93 (s, 3H, OCH_3), 3.92 (d, 1H, *J* = 15.5 Hz, H_a), 3.58 (td, 1H, *J* = 10.5, *J* = 4.3 Hz, H-1′), 2.56 (s, 3H, SCH_3), 2.43 (dddd, 1H, *J* = 12.2, *J* = 4.3, *J* = 1.9, *J* = 1.6 Hz, H-6′), 2.35 (quintet, *J* = 6.9, 2.3 Hz, 1H, H-8′), 1.70-1.64 (m, 2H, H-3′, H-4′), 1.43-1.13 (m, 2H, H-2′, H-5′), 1.10-0.86 (m, 3H, H-3′, H-4′, H-6′), 0.94 (d, 3H, *J* = 6.9 Hz, H-7′), 0.92 (d, 3H, *J* = 6.9 Hz) and 0.79 (d, 3H, *J* = 6.9 Hz) [H-9′, H-10′]. ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 162.5 (C═N), 147.7 (C-7a), 129.9 (C-3a), 129.4 (C-6), 123.9 (C-4), 123.8 (C-5), 112.8 (C-7), 104.1 (C-2), 80.8 (C-1′), 70.7 (CH_2), 68.6 (C-3), 64.1 (OCH_3), 49.0 (C-5′), 41.3 (C-6′), 34.3 (C-4′), 31.5 (C-2′), 25.1 (C-8′), 22.8 (C-3′), 22.4 (C-7′), 21.5 and 15.8 (C-9′, C-10′), 15.0 (SCH_3). NOESY: H_a/H-4, H_b; OCH_3/H-7; H-2/H-1′, H-6′. Anal. Calcd for $C_{22}H_{32}N_2O_2S_2$: C, 62.82; H, 7.67; N, 6.66. Found: C, 62.62; H, 7.56; N, 6.44.

4.3.3. *cis*-1-Methoxyspirobrassinol-(1*S*,2*R*,5*S*)-menthyl ethers 9b and 9d

^1H NMR ($CDCl_3$, 400 MHz): *δ* 7.27–7.21 (m, 2H, H-4, H-6), 7.02 (dt, 0.85H, *J* = 0.7, *J* = 7.5 Hz, H-5 mj); 7.01 (dt, 0.15H, *J* = 1.1, *J* = 7.6 Hz, H-5 mn), 6.96 (dd, 0.85H, *J* = 7.7, *J* = 0.7 Hz, H-7 mj), 6.93 (dd, 0.15H, *J* = 7.8, *J* = 0.7 Hz, H-7 mn), 4.92 (s, 0.85H, H-2 mj), 4.82 (s, 0.15H, H-2 mn), 4.48 (d, 0.15H, *J* = 15.2 Hz, H_a mn), 4.41 (d, 0.85H, *J* = 15.1 Hz, H_a mj), 4.35 (d, 0.15H, *J* = 15.2 Hz, H_b mn), 4.31 (d, 0.85H, *J* = 15.1 Hz, H_b mj), 3.92 (s, 0.45H, OCH_3 mn), 3.91 (s, 2.55H, OCH_3 mj), 3.65 (dt, 0.85H, *J* = 4.2, *J* = 10.5 Hz, H-1′ mj), 3.48 (dt, 0.15H, *J* = 4.2, *J* = 10.5 Hz, H-1′ mn), 2.55 (s, 3H, SCH_3), 2.36 (quintet, 1H, *J* = 7.1, *J* = 2.5 Hz, H-8′), 2.17–2.12 (m, 1H, H-6′), 1.68–1.63 (m, 2H, H-3′, H-4′), 1.41–1.26 (m, 2H, H-2′, H-5′), 1.06–0.86 (m, 3H, H-3′, H-4′, H-6′), 0.93 (d, 3H, *J* = 6.6 Hz) and 0.91 (d, 3H, *J* = 7.1 Hz, H-9′, H-10′), 0.81 (d, 2.55H, *J* = 6.9 Hz, H-7′), 0.75 (d, 0.45H, *J* = 6.9 Hz, H-7′). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 166.8 mj, 166.2 mn (C═N), 148.1 mj, 147.4 mn (C-7a), 130.1 mj, 129.7 mn (C-3a), 129.6 (C-6), 124.1 mj, 123.9 mn (C-4), 123.0 mj, 122.9 mn (C-5), 113.1 mj, 112.6 mn (C-7), 102.6 mn, 99.3 mj (C-2), 81.4 mn, 78.8 mj (C-1′), 73.6 mn, 72.6 mj (CH_2), 71.3 mj, 70.8 mn (C-3), 64.0 mn, 63.5 mj (OCH_3), 48.8 mn, 48.6 mj (C-5′), 41.5 mn, 40.9 mj (C-6′), 34.3 (C-4′), 31.6 mj, 31.5 mn (C-2′), 24.9 mj, 24.8 mn (C-8′), 23.2 mj, 22.9 mn (C-3′), 22.4 and 21.4 (C-9′, C-10′), 16.4 mj, 15.9 mn (C-7′), 15.1 (SCH_3). NOESY: H_b/H-2; H_a/H-4; OCH_3/H-7.

4.4. Spirocyclization of 1-methoxybrassinin 6 with bromine in the presence of (*S*)-(−)-1-phenylethanol

To a stirred mixture of 1-methoxybrassinin **6** (118 mg, 0.442 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (6 mL) at room temperature was added a freshly prepared solution of Br_2 (1.12 mL, 0.478 mmol). The stock solution was obtained by dissolving bromine (0.040 mL) in dichloromethane (1.76 mL). After stirring for 1 min, a suspension of (*S*)-(−)-1-phenylethanol (59 mg, 0.487 mmol), triethylamine (447 mg, 0.615 mL, 4.42 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (6 mL) was added. Stirring was continued for 20 min, and then the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 1 M HCl (9 mL) and water (20 mL). The organic layer was dried over Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (20 g, *n*-hexane/ethyl acetate 8:1), to afford 29 mg (17%) of *trans*-diastereoisomer **11a**, 9 mg (5%) of *cis*-diastereoisomer **11b**, 12 mg (7%) of *trans*-diastereoisomer **11c,** and 13 mg (8%) of *cis*-diastereoisomer **11d**.

4.4.1. *trans*-1-Methoxyspirobrassinol[(*S*)-1-(phenyl)ethyl]ether 11a

$[\alpha]_D^{25}$ = −160.8 (*c* 0.25, $CHCl_3$). IR ($CHCl_3$): *ν* 2956, 2925, 2859, 1572, 1463, 1316, 1121 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): *δ* 7.42 (d, 2H, *J* = 7.1 Hz, H-2′, H-6′), 7.35 (dd, 2H, *J* = 7.6, *J* = 7.1 Hz, H-3′, H-5′), 7.29 (d, 1H, *J* = 7.6 Hz, H-4), 7.28 (d, 1H, *J* = 7.6 Hz, H-4′), 7.20 (dt, 1H, *J* = 1.2, *J* = 7.6 Hz, H-6), 6.99 (dt, 1H, *J* = 1.2, *J* = 7.6 Hz, H-5), 6.85 (d, 1H, *J* = 7.6 Hz, H-7), 5.12 (d, 1H, *J* = 15.3 Hz, H_b), 5.10 (s, 1H, H-2), 4.88 (quartet, 1H, *J* = 6.5 Hz, CH_3-C*H*), 3.90 (d, 1H, *J* = 15.3 Hz, H_a), 3.68 (s, 3H, OCH_3), 2.61 (s, 3H, SCH_3), 1.54 (d, 3H, *J* = 6.5 Hz, C*H*$_3$-CH). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 163.4 (C═N), 148.2 (C-7a), 142.9 (C-1′), 129.6 (C-6), 128.3 (C-3′, C-5′), 127.7 (C-4′), 127.1 (C-3a), 126.6 (C-2′, C-6′), 123.9 (C-4), 123.6 (C-5), 112.7 (C-7), 105.6 (C-2), 78.8 (CH_3-*C*H), 70.1 (CH_2), 69.3 (C-3), 63.8 (OCH_3), 23.9 (*C*H$_3$-CH), 15.0 (SCH_3). NOESY: H_a/H-4; OCH_3/H-7. Anal. Calcd for $C_{20}H_{22}N_2O_2S_2$: C, 62.15; H, 5.74; N, 7.25. Found: C, 62.22; H, 5.56; N, 7.44.

4.4.2. *cis*-1-Methoxyspirobrassinol[(*S*)-1-(phenyl)ethyl]ether 11b

$[\alpha]_D^{25}$ = −10.5 (*c* 0.20, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): *δ* 7.44–7.28 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 7.25 (dt, 1H, *J* = 1.2, *J* = 7.6 Hz, H-6), 7.22 (dd, 1H, *J* = 7.6, *J* = 1.2 Hz, H-4), 6.98 (dt, 1H, *J* = 1.0, *J* = 7.6 Hz, H-5), 6.86 (d, 1H, *J* = 7.6, Hz, H-7), 4.76 (s, 1H, H-2), 4.75 (quartet, 1H, *J* = 6.4, Hz, CH_3-C*H*), 4.49 (d, 1H, *J* = 15.4, Hz, H_a), 4.39 (d, 1H, *J* = 15.4 Hz, H_b), 3.72 (s, 3H, OCH_3), 2.61 (s, 3H, SCH_3), 1.56 (d, 3H, *J* = 6.4 Hz, C*H*$_3$-CH). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 167.0 (C═N), 148.1 (C-7a), 142.6 (C-1′), 129.9 (C-6), 128.5 (C-3a), 128.4 (C-3′, C-5′), 127.9 (C-4′), 127.1 (C-2′, C-6′), 123.6 (C-5), 123.3 (C-4), 112.5 (C-7), 102.2 (C-2), 78.4 (CH_3-CH), 73.4 (CH_2), 70.7 (C-3), 63.8 (OCH_3), 23.9 (*C*H$_3$-CH), 15.1 (SCH_3). NOESY: H_b/H-2; H_a/H-4; OCH_3/H-7. Anal. Calcd for $C_{20}H_{22}N_2O_2S_2$: C, 62.15; H, 5.74; N, 7.25. Found: C, 62.28; H, 5.76; N, 7.14.

4.4.3. *trans*-1-Methoxyspirobrassinol[(*S*)-1-(phenyl)ethyl]ether 11c

$[\alpha]_D^{25}$ = +173.0 (*c* 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): 7.37–7.21 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 7.22 (d, 1H, *J* = 7.6, H-6),

7.16 (d, 1H, J = 7.5, H-4), 6.99–6.92 (m, H-5, H-7), 5.06 (quartet, 1H, J = 6.5, Hz, CH_3-C*H*), 4.63 (s, 1H, H-2), 4.18 (d, 1H, J = 14.3, Hz, H_b), 4.02 (s, 3H, OCH_3), 3.69 (d, 1H, J = 14.3 Hz, H_a), 2.58 (s, 3H, SCH_3), 1.61 (d, 3H, J = 6.5 Hz, C*H*$_3$-CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.7 (C=N), 147.6 (C-7a), 142.6 (C-1′), 132.4 (C-3a), 130.8 (C-6), 128.6 (C-3′, C-5′), 128.1 (C-4′), 127.0 (C-2′, C-6′), 123.9 (C-5), 122.9 (C-4), 112.7 (C-7), 100.6 (C-2), 79.0 (CH_3-*C*H), 72.1 (CH_2), 68.1 (C-3), 63.8 (OCH_3), 23.9 (*C*H_3-CH), 15.1 (SCH_3). NOESY: H_a/H-4; OCH_3/H-7. Anal. Calcd for $C_{20}H_{22}N_2O_2S_2$: C, 62.15; H, 5.74; N, 7.25. Found: C, 62.18; H, 5.66; N, 7.15.

4.4.4. *cis*-1-Methoxyspirobrassinol[(*S*)-1-(phenyl)ethyl]ether 11d

$[\alpha]_D^{25}$ = −281.1 (*c* 0.09, $CHCl_3$). NMR ($CDCl_3$, 400 MHz): δ 7.37–7.28 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 7.23 (dt, 1H, J = 7.6, J = 1.1 Hz, H-6), 7.16 (dd, 1H, J = 7.6, J = 1.1 Hz, H-4), 6.98 (dt, 1H, J = 7.6, J = 0.5 Hz, H-5), 6.93 (dd, 1H, J = 7.6, J = 0.5 Hz, H-7), 5.06 (quartet, 1H, J = 6.5 Hz, CH_3-C*H*), 4.63 (s, 1H, H-2), 4.18 (d, 1H, J = 15.3 Hz, H_a), 4.02 (s, 3H, OCH_3), 3.70 (d, 1H, J = 15.3 Hz, H_b), 2.53 (s, 3H, SCH_3), 1.61 (d, 3H, J = 6.5 Hz, C*H*$_3$-CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7 (C=N), 147.6 (C-7a), 142.6 (C-1′), 129.7 (C-6), 129.4 (C-3a), 128.5 (C-3′, C-5′), 128.1 (C-4′), 127.0 (C-2′, C-6′), 123.9 (C-5), 122.9 (C-4), 112.7 (C-7), 100.6 (C-2), 79.0 (CH_3-CH), 72.1 (CH_2), 69.8 (C-3), 63.9 (OCH_3), 23.9 (*C*H_3-CH), 15.1 (SCH_3). NOESY: H_b/H-2; H_a/H-4; OCH_3/H-7. Anal. Calcd for $C_{20}H_{22}N_2O_2S_2$: C, 62.15; H, 5.74; N, 7.25. Found: C, 62.28; H, 5.76; N, 7.32.

4.5. Spirocyclization of 1-methoxybrassinin 6 with bromine in the presence of [(1*S*)-*endo*]-(−)-borneol

To a stirred mixture of 1-methoxybrassinin **6** (90 mg, 0.338 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (6 mL) at room temperature was added a freshly prepared solution of Br_2 (0.86 mL, 0.372 mmol). The stock solution was obtained dissolving of bromine (0.040 mL) in dichloromethane (1.76 mL). After stirring for 1 min, a suspension of [(1*S*)-*endo*]-(−)-borneol (57 mg, 0.372 mmol), triethylamine (342 mg, 0.472 mL, 3.38 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (6 mL) was added. Stirring was continued for 20 min, and then the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 1 M HCl (9 mL) and water (20 mL). The organic layer was dried over Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (20 g, *n*-hexane/diethyl ether 3:1), to afford 70 mg (50%) of the mixture of *trans*-diastereoisomers **12a**, **12c** (24:76) and 45 mg (32%) of the mixture of *cis*-diastereoisomers **12b**, **12d** (71:29).

4.5.1. *trans*-1-Methoxyspirobrassinol[(1*S*)-*endo*-borneyl]ether 12a and 12c

IR ($CHCl_3$): ν 2956, 2925, 2859, 1573, 1572, 1463, 1282, 1121, 1073, 943 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.32 (d, 0.8H, J = 7.5 Hz, H-4 mj), 7.30 (d, 0.2H, J = 7.5 Hz, H-4 mn), 7.24 (dd, 1H, J = 7.5, H-6), 7.01 (dd, 1H, J = 7.5 Hz, H-5), 6.92 (d, 1H, J = 7.5 Hz, H-7), 5.10 (d, 0.2H, J = 15.3 Hz, H_b mn), 5.08 (d, 0.8H, J = 15.0 Hz, H_b mj), 5.07 (s, 0.2H, H-2 mn), 5.01 (s, 0.8H, H-2 mj), 4.21 (d, 0.8H, J = 9.3 Hz, H-1′ mj), 4.10 (d, 0.2H, J = 10.3 Hz, H-1′ mn), 3.96 (s, 0.6H, OCH_3 mn), 3.94 (s, 2.4H, OCH_3 mj), 3.83 (d, 0.2H, J = 15.3 Hz, H_a mn), 3.79 (d, 0.8H, J = 15.0 Hz, H_a mj), 2.55 (s, 3H, SCH_3), 2.30–2.15 (m,1H), 2.12–2.04 (m, 1H), 1.74–1.66 (m, 2H), 1.33–1.20 (m, 3H), 0.99–0.95 (m, 1H), 0.87 (s, 3H, CH_3) and 0.86 (s, 3H, CH_3). NOESY: H_a/H-4; OCH_3/H-7.

4.5.2. *cis*-1-Methoxyspirobrassinol[(1*S*)-*endo*-borneyl]ether 12b and 12d

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28–7.24 (m, 2H, H-4, H-6), 7.01 (dd, 1H, J = 7.7, J = 6.8 Hz, H-5), 6.92 (d, 1H, J = 7.7 Hz, H-7), 4.74 (s, 0.3H, H-2 mn), 4.71 (s, 0.7H, H-2 mj), 4.51 (d, 0.7H, J = 15.3 Hz, H_a mj), 4.52 (d, 0.3H, J = 15.1 Hz, H_a mn), 4.38 (d, 0.3H, J = 15.1 Hz, H_b mn), 4.36 (d, 0.7H, J = 15.3 Hz, H_b mj), 4.18 (d, 1H, J = 8.7 Hz, H-1′), 3.96 (s, 0.9H, OCH_3 mn), 3.95 (s, 2.1H, OCH_3 mj), 2.56 (s, 2.1H, SCH_3 mj), 2.54 (s, 0.9H, SCH_3 mn), 2.27–2.10 (m, 2H), 1.74–1.67 (m, 2H), 1.37–1.22 (m, 3H), 0.99–0.94 (m, 1H), 0.87 (s, 3H, CH_3) and 0.85 (s, 3H, CH_3). NOESY: H_b/H-2; H_a/H-4.

4.6. Spirocyclization of 1-methoxybrassinin 6 with bromine in the presence of (*S*)-(+)-1-indanol

To a stirred mixture of 1-methoxybrassinin **6** (77 mg, 0.289 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (5.1 mL) at room temperature was added a freshly prepared solution of Br_2 (0.74 mL, 0.318 mmol). The stock solution was obtained by dissolving bromine (0.040 mL) in dichloromethane (1.76 mL). After stirring for 1 min, a suspension of (*S*)-(+)-1-indanol (43 mg, 0.318 mmol), triethylamine (292 mg, 0.40 mL, 2.89 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (5.1 mL) was added. Stirring was continued for 20 min, and then the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 1 M HCl (9 mL) and water (20 mL). The organic layer was dried over Na_2SO_4. The residue obtained after evaporation of the solvent was subjected to chromatography on silica gel (20 g, *n*-hexane/diethyl ether 3:1), affording 23 mg (20%) of the mixture of *trans*-diastereoisomers **13a**, **13c** (58:42) and 20 mg (17%) of diastereoisomer **13b** and 21 mg (18%) of diastereoisomer **13d**

4.6.1. *trans*-1-Methoxyspirobrassinol[(1*S*)-indanyl]ether 13a and 13c

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.56 (d, 0.6H, J = 7.2 Hz, H-7′ mj), 7.52 (d, 0.4H, J = 7.2 Hz, H-7′ mn), 7.31 (dd, 1H, J = 7.5, J = 7.1 Hz, H-6), 7.20–7.26 (m, 4H, H-4, H-4′, H-5′, H-6′), 7.01 (dd, 1H, J = 7.5 Hz, H-5), 6.94 (d, 1H, J = 7.1 Hz, H-7), 5.40 (dd, 0.6H, J = 5.1, J = 5.5 Hz, H-1′ mj), 5.36 (dd, 0.4H, J = 5.1, J = 5.5 Hz, H-1′ mn), 5.35 (s, 0.6H, H-2 mj), 5.31 (s, 0.4H, H-2 mn), 5.09 (d, 0.4H, J = 15.3 Hz, H_b mn), 5.01 (d, 0.6H, J = 15.3 Hz, H_b mj), 3.96 (s, 1.2H, OCH_3 mn), 3.89 (s, 1.8H, OCH_3 mj), 3.87 (d, 1H, J = 15.3 Hz, H_a), 3.10 (m, 1H, H-3′a), 2.83 (ddd, 1H, J = 14.9, J = 7.2, J = 6.5 Hz, H-3′b), 2.56 (s, 1.2H, SCH_3 mn), 2.55 (s, 1.8H, SCH_3 mj), 2.48 (ddd, 0.4H, J = 13.4, J = 5.5, J = 7.0 Hz, H-2′a mn), 2.41 (ddd, 0.4H, J = 13.4, J = 5.1, J = 6.9 Hz, H-2′b mn), 2.31 (ddd, 0.6 H, J = 5.5, J = 8.8, J = 12.9 Hz, H-2′a mj), 2.19 (ddd, 0.6 H, J = 5.1, J = 8.0, J = 12.9 Hz, H-2′b mj). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2 mn, 162.8 mj (C=N), 148.3 mn, 148.1 mj (C-7a), 144.0 mj, 143.9 mn (C-7′a), 142.5 mj, 142.2 mn (C-4′a), 129.6, 129.5 (C-6), 128.6, 128.5 (C-5′), 127.7, 127.1 (C-3a), 126.4, 126.3 (C-6′), 125.7, 125.2 (C-7′), 124.8, 124.7 (C-4′), 124.0 (C-5), 123.7, 123.6 (C-4), 112.7 (C-7), 106.9, 106.8 (C-2), 84.9 (C-1′), 70.1 (CH_2), 69.2 mn, 69.6 mj (C-3), 64.3 mn , 64.1 mj (OCH_3), 33.7 mj, 33.1 mn (C-2′), 30.0 mn, 29.9 mj (C-3′), 15.0. 14.9 (SCH_3). NOESY: H_a/H_b; H-1′/H-2′a,b; H-2′a/H-2′b; H-3′a/H-3′b.

4.6.2. *cis*-1-Methoxyspirobrassinol[(1*S*)-indanyl]ether 13b

$[\alpha]_D^{25}$ = +95.5 (*c* 0.29, $CHCl_3$). IR ($CHCl_3$): ν 2956, 2928, 1564, 1461, 1320, 1290, 1137, 1043 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.56 (d, 1H, J = 7.2 Hz, H-7′), 7.20–7.28 (m, 5H, H-4, H-4′, H-5′, H-6, H-6′), 7.01 (dd, 1H, J = 7.6 Hz, H-5), 6.95 (d, 1H, J = 7.9 Hz, H-7), 5.37 (dd, 1H, J = 5.4, J = 5.8 Hz, H-1′), 5.05 (s, 1H, H-2), 4.48 (d, 1H, J = 15.3, Hz, H_a), 4.38 (d, J = 15.3 Hz, H_b), 3.86 (s, 3H, OCH_3), 3.12 (ddd 1H, J = 15.9, J = 7.5, J = 5.6 Hz, H-3′a), 2.82 (ddd, 1H, J = 7.2, J = 7.6, J = 15.9 Hz, H-3′b), 2.55 (s, 3H, SCH_3), 2.43 (ddt, 1H, J = 5.4, J = 6.7, J = 13.3 Hz, H-2′a), 2.18 (ddt, 1H, J = 5.8, J = 7.6, J = 13.3 Hz, H-2′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.9 (C=N), 147.8 (C-7a), 143.8 (C-7′a), 142.4 (C-4′a), 129.8 (C-6), 128.6 (C-5′), 128.1 (C-3a), 126.4 (C-6′), 125.2 (C-7′), 124.8 (C-4′), 123.7

(C-5), 123.2 (C-4), 112.6 (C-7), 103.2 (C-2), 84.7 (C-1′), 72.9 (CH_2), 70.5 (C-3), 63.9 (OCH_3), 33.5 (C-2′), 30.0 (C-3′), 15.1 (SCH_3). NOESY: H_b/H-2; H_a/H-4; H-2/H-1′. Anal. Calcd for $C_{21}H_{22}N_2O_2S_2$: C, 63.29; H, 5.56; N, 7.03. Found: C, 63.28; H, 5.75; N, 7.22.

4.6.3. *cis*-1-Methoxyspirobrassinol[(1*S*)-indanyl]ether 13d

$[\alpha]_D^{25} = -292.5$ (*c* 0.08, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.52 (d, 1H, *J* = 7.8 Hz, H-7′), 7.28–7.26 (m, 3H, H-4′, H-5′, H-6), 7.25 (d, 1H, *J* = 7.5 Hz, H-4), 7.21(dd, 1H, *J* = 7.5, *J* = 7.8 Hz, H-6′), 7.01 (dd, 1H, *J* = 7.5 Hz, H-5), 6.96 (d, 1H, *J* = 7.8 Hz, H-7), 5.28 (dd, 1H, *J* = 4.1, *J* = 5.2 Hz, H-1′), 4.98 (s, 1H, H-2), 4.38 (d, 1H, *J* = 15.3 Hz, H_a), 4.09 (d, 1H, *J* = 15.3 Hz, H_b), 3.99 (s, 3H, OCH_3), 3.17 (dt, 1H, *J* = 7.2, *J* = 15.7 Hz, H-3′a), 2.83 (dt, 1H, *J* = 6.6, *J* = 15.7 Hz, H-3′b), 2.52 (s, 3H, SCH_3), 2.43-2.37 (m, 2H, H-2′a, H-2′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.4 (C=N), 148.1 (C-7a), 144.6 (C-7′a), 141.8 (C-4′a), 129.8 (C-6), 128.8 (C-5′), 127.8 (C-3a), 126.2 (C-6′), 125.7 (C-7′), 125.0 (C-4′), 123.7 (C-5), 123.2 (C-4), 112.6 (C-7), 102.6 (C-2), 84.6 (C-1′), 72.7 (CH_2), 70.3 (C-3), 64.1 (OCH_3), 33.6 (C-2′), 30.2 (C-3′), 15.1 (SCH_3). NOESY: H_b/H-2; H_a/H-4; H-2/H-1′. Anal. Calcd for $C_{21}H_{22}N_2O_2S_2$: C, 63.29; H, 5.56; N, 7.03. Found: C, 63.32; H, 5.59; N, 7.11.

4.7. Spirocyclization of 1-methoxybrassinin 6 with bromine in the presence of (*S*)-*cis*-verbenol

To a stirred mixture of 1-methoxybrassinin **6** (90 mg, 0.338 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (6 mL) at room temperature was added a freshly prepared solution of Br_2 (0.86 mL, 0.372 mmol). The stock solution was obtained by dissolving of bromine (0.040 mL) in dichloromethane (1.76 mL). After stirring for 1 min, a suspension of (*S*)-*cis*-verbenol (56 mg, 0.372 mmol), triethylamine (342 mg, 0.472 mL, 3.38 mmol) and powdered molecular sieves (3 Å) in dry CH_2Cl_2 (6 mL) was added. Stirring was continued for 20 min, and then the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 1 M HCl (9 mL) and water (20 mL). The organic layer was dried over Na_2SO_4. The residue obtained after evaporation of solvent was subjected to chromatography on silica gel (20 g, *n*-hexane/diethyl ether 4:1), affording 41 mg (29%) of the mixture of *trans*-diastereoisomers **14a**, **14c** (50:50) and 27 mg (19%) of the mixture of *cis*-diastereoisomers **14b**, **14d** (47:53).

4.7.1. *trans*-1-Methoxyspirobrassinol[(*S*)-verbenyl]ether 14a and 14c

IR ($CHCl_3$): *ν* 2987, 2923, 2869, 2845, 1566, 1463, 1149, 1130, 987 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.31 (d, 0.5H, *J* = 7.5 Hz, H-4), 7.28 (d, 0.5H, *J* = 7.5 Hz, H-4), 7.23 (dt,1H, *J* = 1.2, *J* = 7.5 Hz, H-6), 7.00 (dt, 1 H, *J* = 7.5, *J* = 0.9 Hz, H-5), 6.92 (d, 1H, *J* = 7.5 Hz, H-7), 5.54 (dd, 0.5H, *J* = 1.4, *J* = 2.7 Hz, H-3′), 5.45 (dd, 0.5H, *J* = 1.4, *J* = 2.5 Hz, H-3′), 5.09 (s, 1H, H-2), 5.06 (s, 1H, H-2), 5.04 (d, 0.5H, *J* = 15.3 H_b), 5.03 (d, 0.5H, *J* = 15.2 H_b), 4.60 (d, 0.5H, *J* = 1.4 Hz, H-2′), 4.57 (d, 0.5 H, *J* = 1.4 Hz, H-2′), 3.99 (s, 1.5H, OCH_3), 3,94 (s, 1.5H, OCH_3), 3.85 (d, 0.5H, *J* = 15.3 Hz, H_a), 3.84 (d, 0.5H. *J* = 15.2 Hz, H_a), 2.55 (s, 1,5H, SCH_3), 2.54 (s, 1.5H, SCH_3), 2.44-2.55 (m, 1.5H, H-1′, H-7′), 2.41 (ddd, 0.5H, *J* = 2.4, *J* = 4.1, *J* = 6.0 Hz, H-1′), 1.97 (dd, 1H, *J* = 4.2, *J* = 9.3 Hz, H-5′), 1.76 (t, 1.5H, *J* = 1.7 Hz, H-8′), 1.74 (t, 1.5H, *J* = 1.6 Hz, H-8′), 1.36 (s, 1.5H, H-9′), 1.35 (s, 1.5H, H-9′), 1.33 (dd, 1H, *J* = 5.9, *J* = 8.7 Hz, H-7′), 1.05 (s, 1.5H, H-10′), 1.03 (s, 1.5H, H-10′). ^{13}C NMR (100 MHz. $CDCl_3$): δ 162.7, 162.2 (C=N), 148.3, 148.0 (C-7a), 147.9, 147.6 (C-4′), 129.5, 129.4 (C-6), 128.6, 127.6 (C-3a), 124.1, 124.0 (C-4), 123.6, 123.5 (C-5), 117.4 (C-3′), 112.7, 112.6 (C-7), 108.0, 107.2 (C-2), 83.4, 82.8 (C-2′), 70.5, 70.2 (CH_2), 69.1, 68.7 (C-3), 64.1, 64.0 (OCH_3), 47.7 (C-5′), 46.7, 46.2 (C-1′), 39.6, 39.3 (C-6′), 35.6, 35.5 (C-7′), 26.9, 26.8 (C-9′), 23.0, 22.7 (C-8′), 22.7, 22.6 (C-10′), 14.9, 14.8 (SCH_3). NOESY: H_b/H-2; H_a/H-4.

4.7.2. *cis*-1-Methoxyspirobrassinol[(*S*)-verbenyl]ether 14b and 14d

^1H NMR ($CDCl_3$, 400 MHz): δ 7.25 (dt, 1H, *J* = 7.6, *J* = 1.1 Hz, H-6), 7.23 (d, 1H, *J* = 7.6 Hz, H-4), 7.03 (dt, 1H, *J* = 7.6, *J* = 0.8 Hz, H-5), 6.92 (dd, 1H, *J* = 7.6, *J* = 0.8 Hz, H-7), 5.55 (dt, 0.5H, *J* = 1.5, *J* = 4.3 Hz, H-3′), 5.42 (dt, 0.5H, *J* = 1.6, *J* = 4.2 Hz, H-3′), 4.82 (s, 0.5H, H-2), 4.77 (s, 0.5H, H-2), 4.64 (d, 0.5H, *J* = 1.6 Hz, H-2′), 4.59 (d, 0.5H, *J* = 1.5 Hz, H-2′), 4.53 (d, 0.5H, *J* = 15.4 Hz, H_a), 4.52 (d, 0.5H, *J* = 15.3 Hz, H_a), 4.37 (d, 0.5H, *J* = 15.3 Hz, H_b), 4.29 (d, 0.5H, *J* = 15.4 Hz, H_b), 3.98 (s, 1.5H, OCH_3), 3.93 (s,1.5H, OCH_3), 2.54 (s, 1.5H, SCH_3), (2.53 s, 1.5H, SCH_3), 2.44–2.57 (m, 1.5H, H-1′, H-7′), 2.33–2.37 (m, 0.5 H H-1′), 1.98 (t, 1H, *J* = 5.4 Hz, H-5′), 1.75 (t, 1.5H, *J* = 1.7 Hz, H-8′), 1.74 (t, 1.5H, *J* = 1.7 Hz, H-8′), 1.36 (s, 1.5H, H-9′), 1.35 (s, 1.5H, H-9′), 1.33 (d, 1H, *J* = 8.7 Hz, H-7′), 1.06 (s, 3H, H-10′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 166.5 (C=N), 148.6, 148.1 (C-7a), 148.0, 147.7 (C-4′), 129.7 (C-6), 128.9, 128.5 (C-3a), 123.71, 123.69 (C-5), 123.1, 123.0 (C-4), 117.2, 116.3 (C-3′), 112.5 (C-7), 103.6, 103.1 (C-2), 82.8, 81.8 (C-2′), 72.9 (CH_2), 70.3, 69.9 (C-3), 64.0, 63.9 (OCH_3), 47.9, 47.8 (C-5′), 46.4, 46.1 (C-1′), 39.6, 39.5 (C-6′), 35.6, 35.5 (C-7′), 26.9, 26.8 (C-9′), 23.0, 22.8 (C-8′), 22.7, 22.6 (C-10′), 15.1, 15.0 (SCH_3). NOESY: H_b/H-2; H_a/H-4.

4.8. Synthesis of (*R*)-(+)- and (*S*)-(−)-1-methoxyspirobrassinin (*R*)-(+)-2 and (*S*)-(−)-2 from 7a/8c, 7b/8d

(a) To a vigorously stirred slurry of PCC (75 mg, 0.345 mmol) and anhydrous $MgSO_4$ (62 mg, 0.515 mmol) in dry CH_2Cl_2 (0.3 mL) a solution of **7a** (50 mg, 0.115 mmol) in dry CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred for 72 h at room temperature, and then diluted with CH_2Cl_2 (2 mL). After adding a small amount of silica gel, the solvent was evaporated and the residue that was preabsorbed on silica gel was purified by silica gel column chromatography (6 g, petroleum ether/diethyl ether 4:1) to give 16 mg (50%) of (*R*)-(+)-1-metoxyspirobrassinin (*R*)-(+)-**2** as colorless needles, mp 129–131 °C (EtOAc/hexane), 92% ee. The spectroscopic data were fully identical with those of the natural product.[4] The absolute configuration was determined by direct comparison of the ECD spectra with published data.[5]

(b) Application of the same procedure on **8c** afforded 17 mg (52%) of (*S*)-(−)-**2**, 87% ee.

(c) Application of the same procedure on **7b** afforded 13 mg (41%) of (*R*)-(+)-**2**, 84% ee.

(d) Application of the same procedure on **8d** afforded 18 mg (57%) of (*S*)-(−)-**2**, 92% ee.

4.9. Synthesis of (*R*)-(+)- and (*S*)-(−)-1-methoxyspirobrassinin (*R*)-(+)-2 and (*S*)-(−)-2 from 9a/10c

(a) To a vigorously stirred slurry of PCC (138 mg, 0.642 mmol) and anhydrous $MgSO_4$ (116 mg, 0.936 mmol) in dry CH_2Cl_2 (0.45 mL), a solution of **9a** (90 mg, 0.214 mmol) in dry CH_2Cl_2 (1.8 mL) was added. The reaction mixture was stirred for 24 h at room temperature, and then diluted with CH_2Cl_2 (2 mL). After adding a small amount of silica gel, the solvent was evaporated and the residue that was preabsorbed on silica gel was purified by silica gel column chromatography (4 g, petroleum ether/diethyl ether 4:1) to give 43 mg (68%) of (*R*)-(+)-1-methoxyspirobrasinin (*R*)-(+)-**2** as colorless needles, mp 129–131 °C (EtOAc/hexane), 97% ee. The spectroscopic data were fully identical with those of the natural product.[4] The absolute configuration was determined by direct comparison of the ECD spectra with published data.[5]

(b) Application of the same procedure on **10c** afforded 42 mg (70%) of (*S*)-(−)-**2**, 93% ee.

(c) Microwave-assisted oxidation: PCC (131 mg, 0.6 mmol) and anhydrous $MgSO_4$ (108 mg, 0.9 mmol) was weighed in a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. A solution of **9a** (85 mg, 0.2 mmol) in dry CH_2Cl_2 (2.5 mL) was then added, the tube was closed with a silicon septum, and the reaction mixture was subjected to microwave irradiation for 1 h (power: 10 W, temperature: 60 °C). The reaction mixture was allowed to cool to room temperature and then transferred to a round bottom flask and worked-up as in the previous procedure to give 33 mg (60%) of (*R*)-(+)-**2**, $[\alpha]_D^{25}$ = +105.7 (*c* 0.1, $CHCl_3$); 98% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 85:15 at a flow rate 1 mL/min); R_t = 12.03 min.

(d) Application of the same procedure on **10c** afforded 35 mg (63%) of (*S*)-(−)-**2**, $[\alpha]_D^{25}$ = −82.2 (*c* 0.1, $CHCl_3$); 99% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 85:15 at a flow rate 1 mL/min); R_t = 13.86 min.

4.10. Synthesis of diastereoisomers of 1-methoxyspirobrassinol methyl ether (2*R*,3*R*)-3, (2*S*,3*S*)-3, (2*R*,3*S*)-3 and (2*S*,3*R*)-3

(a) To a solution of **9a** (49 mg, 0.117 mmol) in dry methanol was added TFA (15 mg, 0.01 mL, 0.13 mmol). The reaction mixture was stirred for 12 h, then the solvent was evaporated and the residue was subjected to chromatography on silica gel (10 g, cyclohexane/diethyl ether 2:1) to afford 15 mg (43%) of (2*R*,3*R*)-(−)-**3** and 12 mg (35%) of (2*S*,3*R*)-(+)-**3**. The spectroscopic data of (2*R*,3*R*)-(−)-**3** were fully identical with those of the natural product.[6] The spectroscopic data of (2*S*,3*R*)-(+)-**3** were fully identical with those of the corresponding racemic product.[5]

First eluted diastereoisomer (2*R*,3*R*)-(−)-**3**: colorless oil; R_f = 0.51 (cyclohexane/diethyl ether 2:1); $[\alpha]_D^{25}$ = −10.8 (*c* 0.1, $CHCl_3$); CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 214 (−26.5), 239 (+3.9), 263 (−0.7), 291 (+2.7). 97% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 75:25 at a flow rate 1 mL/min); R_t = 15.31 min.

Second eluted diastereoisomer (2*S*,3*R*)-(+)-**3**: colorless crystals; mp 77–79 °C (hexane); R_f = 0.31 (cyclohexane/diethyl ether 2:1); $[\alpha]_D^{25}$ = +39.1 (*c* 0.1, $CHCl_3$); CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 206 (−17.2), 233 (+22.8), 250 (−2.1), 291 (+7.7). 98% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 8.73 min.

(b) Application of the same procedure on **10c** afforded 13 mg (36%) of (2*S*,3*S*)-(+)-**3** and 15 mg (41%) of (2*R*,3*S*)-(−)-**3**. The spectroscopic data of (2*S*,3*S*)-(+)-**3** were fully identical with those of the natural product.[6] The spectroscopic data of (2*R*,3*S*)-(−)-**3** were fully identical with those of the corresponding racemic product.[5]

First eluted diastereoisomer (2*S*,3*S*)-(+)-**3**: colorless oil; R_f = 0.51 (cyclohexane/diethyl ether 2:1); $[\alpha]_D^{25}$ = +7.3 (*c* 0.1, $CHCl_3$); CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 214 (28.1), 239 (−3.9), 263 (1.0), 291 (−2.5). 95% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 75:25 at a flow rate 1 mL/min); R_t = 5.54 min.

Second eluted diastereoisomer (2*R*,3*S*)-(−)-**3**: colorless crystals; mp 77-79 °C (hexane); R_f = 0.31 (cyclohexane/diethyl ether 2:1); $[\alpha]_D^{25}$ = −38.5 (*c* 0.1, $CHCl_3$); CD (CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 206 (15.9), 233 (−21.3), 250 (2.2), 291 (−7.0). 92% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 10.26 min.

4.11. General procedure for the synthesis of the diastereoisomers of the 2-amino analogues of 1-methoxyspirobrassinol methyl ethers 15–18

To the suspension of **9a** (60 mg; 0.143 mmol) in dry CH_2Cl_2 (5 mL) with molecular sieves (3 Å) was added TFA (18 mg; 0.012 mL; 0.157 mmol) and (i) 3,4-dichloroaniline (46 mg, 0.286 mmol) (ii) *p*-trifluoromethylaniline (46 mg; 0.036 mL; 0.286 mmol), (iii) *p*-toluidine (31 mg; 0.286 mmol), (iv) *p*-anisidine (35 mg, 0.286 mmol). Reaction mixture was stirred for (i) 1.5 h, (ii) 2.5 h, (iii) 24 h, or (iv) refluxed 14.5 h, and then Et_3N (0.016 g; 0.022 mL; 0.157 mmol) was added, the solvent evaporated and the residue preabsorbed on silica gel was subjected to chromatography on silica gel.

4.11.1. Synthesis of the isomers of 1-methoxy-2-(3,4-dichlorophenylamino)-2′-(methylsulfanyl)spiro{indoline-3,5′-[4′,5′]dihydrotiazole} 15

Following the general procedure, products (2*R*,3*R*)-(+)-**15** and (2*S*,3*R*)-(−)-**15** were obtained using 3,4-dichloroaniline (46 mg, 0.286 mmol). Separation on silica gel (20 g, ethyl acetate/*n*-hexane 1:5) afforded product (2*R*,3*R*)-(+)-**15** impure with menthol and (2*S*,3*R*)-(−)-**15** impure with 3,4-dichloroaniline. Pure diastereoisomers were afforded after chromatography on silica gel (2*R*,3*R*)-(+)-**15** (15 g, CH_2Cl_2) and (2*S*,3*R*)-(−)-**15** (8 g, CH_2Cl_2). All spectroscopic data were fully identical with the described racemic products.[11]

(2*R*,3*R*)-(+)-**15**: 38 mg (62%); yellow oil; R_f = 0.41 (ethyl acetate/*n*-hexane 1:5); $[\alpha]_D^{25}$ = +116.2 (*c* 0.4, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (−21.8), 233 (16.6), 246 (6.3), 252 (7.9), 266 (−1.0), 292 (5.5). 96% ee (Larihc RN-CF6 ϕ 0,46 × 25 cm, *n*-heptane/ethanol = 95:5 at a flow rate 0.6 mL/min); R_t = 10.25 min.

(2*S*,3*R*)-(−)-**15**: 16 mg (26%); colorless crystals; mp 143–145 °C (acetone/hexane); R_f = 0.23 (ethyl acetate/*n*-hexane 1:5); $[\alpha]_D^{25}$ = −75.9 (*c* 0.3, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 216 (−38.5), 238 (−1.5), 249 (−6.6), 264 (4.9), 277 (0.2), 296 (2.8). 96% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 12.42 min.

Application of the same procedure on **10c** afforded:

(2*S*,3*S*)-(−)-**15**: 42 g (69%); yellow oil; R_f = 0.41 (ethyl acetate/*n*-hexane 1:5); $[\alpha]_D^{25}$ = −131.6 (*c* 0.4, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (21.9), 233 (−18.1), 246 (−7.2), 252 (−8.8), 266 (0.8), 292 (−6.0). 99% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 80:20 at a flow rate 1 mL/min); R_t = 6.84 min.

(2*R*,3*S*)-(+)-**15**: 13 g (21%); colorless crystals; mp 143–145 °C (acetone/hexane); R_f = 0.23 (ethyl acetate/*n*-hexane 1:5); $[\alpha]_D^{25}$ = +118.7 (*c* 0.3, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 216 (45.1), 238 (1.5), 249 (8.2), 264 (−6.0), 277 (0), 296 (−2.9). >99% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 10.75 min.

4.11.2. Synthesis of the isomers of 1-methoxy-2-(4-trifluoromethylphenylamino)-2′-(methylsulfanyl)spiro{indoline-3,5′-[4′,5′]dihydrotiazole} 16

Following the general procedure, products (2*R*,3*R*)-(+)-**16** and (2*S*,3*R*)-(−)-**16** were obtained using *p*-trifluoromethylaniline (46 mg; 0.036 mL; 0.286 mmol). Separation on silica gel (20 g, *n*-hexane/ethyl acetate 5:1) afforded product (2*R*,3*R*)-(+)-**16** impure with menthol and (2*S*,3*R*)-(−)-**16** impure with 4-trifluoromethylaniline. Pure diastereoisomers were afforded after chromatography on silica gel (2*R*,3*R*)-(+)-**16** (15 g, CH_2Cl_2) and (2*S*,3*R*)-(−)-**16** (7 g,

CH_2Cl_2). All spectroscopic data were fully identical with the described racemic products.[11]

(2*R*,3*R*)-(+)-**16**: 37 mg (61%); yellow oil; R_f = 0.28 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = +20.2 (*c* 0.15, $CHCl_3$); CD (*c* 0.023 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (−12.3), 233 (16.9), 245 (5.1), 253 (9.5), 267 (−0.1), 294 (6.6). 99% ee (CHIRALPAK® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/ethanol = 99:1 at a flow rate 1 mL/min); R_t = 10.67 min.

(2*S*,3*R*)-(−)-**16**: 14 mg (23%); white crystals, mp 114–116 °C (acetone/hexane), R_f = 0.13 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = −72.5 (*c* 0.07, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 213 (−50.4), 236 (0.4), 249 (−10.4), 264 (5.9), 276 (0.9), 292 (4.0). 98% ee (CHIRALPAK® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/ethanol = 99:1 at a flow rate 1 mL/min); R_t = 19.94 min.

Application of same procedure on **10c** afforded:

(2*S*,3*S*)-(−)-**16**: 42 mg (69%); yellow oil; R_f = 0.28 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = −43.4 (*c* 0.15, $CHCl_3$); CD (*c* 0.023 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (14.2); 233 (−18.5); 245 (−5.4); 253 (−10.1); 267 (0.9); 294 (−6.4). 98% ee (CHIRALPAK® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/ethanol = 99:1 at a flow rate 1 mL/min); R_t = 12.51 min.

(2*R*,3*S*)-(+)-**16**: 11 mg (18%); white crystals; mp 114–116 °C (acetone/hexane); R_f = 0.13 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = +42.7 (*c* 0.07, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 213 (38.1), 236 (−0.5), 249 (8.5), 264 (−4.3), 276 (−0.3), 292 (−2.6). 99% ee (CHIRALPAK® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/ethanol = 99:1 at a flow rate 1 mL/min); R_t = 17.42 min.

4.11.3. Synthesis of the isomers of 1-methoxy-2-(4-methylphenylamino)-2′-(methylsulfanyl)spiro{indoline-3,5′-[4′,5′]dihydrotiazole} 17

Following the general procedure, products (2*R*,3*R*)-(+)-**17** and (2*S*,3*R*)-(−)-**17** were obtained using *p*-toluidine (31 mg; 0.286 mmol). Separation on silica gel (20 g, *n*-hexane/ethyl acetate 8:1) afforded product (2*R*,3*R*)-(+)-**17** impure with menthol and (2*S*,3*R*)-(−)-**17** impure with 4-methylaniline. Pure diastereoisomers were afforded after chromatography on silica gel (2*R*,3*R*)-(+)-**17** (15 g, CH_2Cl_2) and (2*S*,3*R*)-(−)-**17** (4 g, CH_2Cl_2). All spectroscopic data were fully identical with the described racemic products.[11]

(2*R*,3*R*)-(+)-**17**: 32 mg (60%); colorless oil; R_f = 0.38 (*n*-hexane/ethyl acetate 8:1); $[\alpha]_D^{25}$ = +211.7 (*c* 0.15, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (−16.1), 233 (16.2), 262 (−0.4), 293 (6.7). 95% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 12.36 min.

(2*S*,3*R*)-(−)-**17**: 8 mg (15%); colorless crystals, mp 155–157 °C (acetone/hexane); R_f = 0.18 (*n*-hexane/ethyl acetate 8:1); $[\alpha]_D^{25}$ = −74.7 (*c* 0.3, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 213 (−14.5), 233 (−1.3), 244 (−3.8), 261 (3.1), 278 (1.2), 294 (1.8). 96% ee (CHIRALPAK IA [CARTRIDGE HOLDER Φ 0.4 × 1 cm + Φ 0.46 × 25 cm], *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 10.20 min.

Application of same procedure on **10c** afforded:

(2*S*,3*S*)-(−)-**17**: 37 mg (70%); colorless oil; R_f = 0.38 (*n*-hexane/ethyl acetate 8:1); $[\alpha]_D^{25}$ = −207.9 (*c* 0.15, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (15.9), 233 (−16.3), 262 (1.7), 293 (−5.3). 99% ee (CHIRALCEL® OD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 7.05 min.

(2*R*,3*S*)-(+)-**17**: 8 mg (15%); colorless crystals; mp 155–157 °C (acetone/hexane); R_f = 0.18 (*n*-hexane/ethyl acetate 8:1); $[\alpha]_D^{25}$ = +90.1 (*c* 0.3, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 213 (16.9), 233 (3.1), 244 (5.1), 261 (−1.9), 278 (0.6), 294 (0.1). 98% ee (CHIRALPAK IA [CARTRIDGE HOLDER ϕ 0.4 × 1 cm + ϕ 0.46 × 25 cm], *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 14.22 min.

4.11.4. Synthesis of the isomers of 1-methoxy-2-(4-methoxyphenylamino)-2′-(methylsulfanyl)spiro{indoline-3,5′-[4′,5′]dihydrotiazole} 18

Following the general procedure, products (2*R*,3*R*)-(+)-**18** and (2*S*,3*R*)-(−)-**18** were obtained using *p*-anisidine (35 mg, 0.286 mmol). Separation on silica gel (20 g, n-hexane/ethyl acetate 5:1) afforded product (2*R*,3*R*)-(+)-**18** impure with menthol and (2*S*,3*R*)-(−)-**18** impure with *p*-anisidine. Pure diastereoisomers were afforded after chromatography on silica gel (2*R*,3*R*)-(+)-**18** (10 g, CH_2Cl_2/MeOH 60:1) and (2*S*,3*R*)-(−)-**18** (4 g, CH_2Cl_2/ethyl acetate 30:1). All spectroscopic data were fully identical with the described racemic products.[11]

(2*R*,3*R*)-(+)-**18**: 19 mg (34%); white crystals; mp 178–180 °C (acetone/hexane); R_f = 0.24 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = +226.2 (*c* 0.1, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (−14.0), 233 (13.5), 263 (0.2), 292 (5.9). 96% ee (Larihc RN-CF6 ϕ 0,46 × 25 cm, *n*-heptane:ethanol = 95:5 at a flow rate 0.6 mL/min); R_t = 10.96 min.

(2*S*,3*R*)-(−)-**18**: 7 mg (13%); white crystals; mp 143–145 °C (acetone/hexane); R_f = 0.13 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = −95.8 (*c* 0.15, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 214 (−8.6), 232 (−1.3), 244 (−2.2), 261 (1.8), 280 (1.1), 297 (1.5). 90% ee (CHIRALPAK® AD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 26.00 min.

Application of the same procedure on **10c** afforded:

(2*S*,3*S*)-(−)-**18**: 17 mg (31%); white crystals; mp 178–180 °C (acetone/hexane); R_f = 0.24 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = −135.2 (*c* 0.1, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 210 (11.1), 233 (−11.0), 263 (1.0), 293 (−3.3). 98% ee (CHIRALPAK® AD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 15.48 min.

(2*R*,3*S*)-(+)-**18**: 5 mg (9%); white crystals; mp 143–145 °C (acetone/hexane); R_f = 0.13 (*n*-hexane/ethyl acetate 5:1); $[\alpha]_D^{25}$ = +116.3 (*c* 0.15, $CHCl_3$); CD (*c* 0.025 mM, CH_3OH, nm) λ_{ext} ($\Delta\varepsilon$): 214 (8.2), 232 (1.3), 244 (3.0), 261 (−0.9), 280 (0.4), 297 (0.1). 94% ee (CHIRALPAK® AD ϕ 0.46 cm × 5 cm + ϕ 0.46 cm × 25 cm, *n*-hexane/propan-2-ol = 95:5 at a flow rate 1 mL/min); R_t = 31.26 min.

4.12. Antiproliferative activity

4.12.1. Cell culture—Tumor cell lines

Jurkat (acute T-lymphoblastic leukemia), HeLa (cervical adenocarcinoma), MCF-7 (mammary gland adenocarcinoma), MDA-MB-231 (mammary gland adenocarcinoma), A-549 (non-small cell lung cancer), and CCRF-CEM cell line (acute T-lymphoblastic leukemia) cells were maintained in RPMI 1640 medium supplemented with Glutamax-I or in Dulbecco's modified Eagle's medium with Glutamax-I and glucose. Both of these media were supplemented with 10% (v/v) fetal calf serum, penicillin (100 IU × mL^{-1}), and streptomycin (100 μg × mL^{-1}) (all from Invitrogen, UK), in humidified air with 5% CO_2 at 37 °C. Before each cytotoxicity assay, cell viability was determined by the trypan blue (Invitrogen) exclusion method and found to be greater than 95%.

4.12.2. Cytotoxicity assay

The cytostatic/cytotoxic effects of the compounds were studied using the colorimetric microculture assay with the MTT endpoint.[16] 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 the formazan. The absorbance was measured at 540 nm and 630 nm–reference wavelength 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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