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The first synthesis of natural alkaloid capparine A

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ABSTRACT

Substances containing a spirooxindole framework display important biological activities. Natural alkaloid capparine A [(*S*)-(–)-**1**] has an anti-inflammatory effect. In the present study, attention has been paid to the first total synthesis of natural capparine A [(*S*)-(-)-**1**]. Racemic capparine A [(±)-**1**] was synthesized by bromospirocyclization of 6-methoxy-1-Boc-brassinin with water, followed by oxidation of obtained spirobrassinol derivatives and removal of the Boc group. Synthesized racemic capparine A [(±)-**1**] was enantioresolved by derivatization with (1*R*,2*S*,5*R*)-menthyl chloroformate, chromatographic separation of diastereoisomers and the cleavage of the chiral auxiliary using sodium methoxide. Screening of anti-proliferative activity against human cancer cells revealed no anti-proliferative activity of the capparine A [(*S*)-(–)-**1**].

Keywords: natural alkaloids, capparine A, indole phytoalexins, spirobrassinin, spirocyclization

1. Introduction

In 2008, natural alkaloid capparine A {6-methoxyl-2´-(methylthio)spiro[3*H*-indole-3,5´(4´*H*)thiazol]-2(1*H*)-one, (*S*)-(–)-**1**, Fig 1.} was first isolated from the whole plant of *Capparis himalayensis* (family *Capparidacea*).[1] Two years later, capparine A [(*S*)-(–)-**1**] was identified from the caper fruit of *Capparis spinosa*.[2] Its structure was determined by spectral methods and confirmed on the basis of X-ray crystallographic analysis.[1] Pharmacological studies have shown that the capparine A [(–)-**1**] has an anti-inflammatory effect on the carrageenan-induced paw edema in mice.[2] Moreover, the immature flower buds, leaves, unripe fruits, seeds, shoots and bark of roots of the above mentioned plants are still used for the treatment of rheumatism, stomach problems, headache and toothache in traditional medicine. The mechanism of inhibition is not yet known.[2]

Due to its structure, capparine A [(*S*)-(–)-**1**] can also be defined as a 6-methoxy derivative of spirobrassinin. Spirobrassinin [(*S*)-(–)-**2**, Fig 1.] belongs to the group of spiroindoline phytoalexins biosynthesized by plants of the family *Brassicaceae* as a defense response to physical, biological or chemical stress.[3] Spirobrassinin [(–)-**2**] offers a broad range of biological activities, such as antimicrobial, chemopreventive, antitumor, antitrypanosomal, anti-aggregation properties.[4-14]

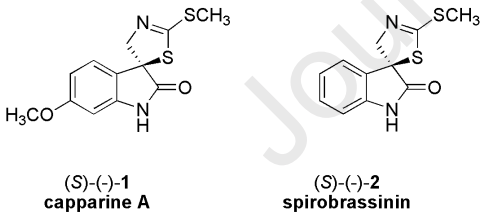


Fig 1. Structures of spirooxindole alkaloids capparine A [(*S*)-(–)-**1**] and spirobrassinin [(*S*)-(–)-**2**].

Unpublished total synthesis and significant biological activity of capparine A [(*S*)-(–)-**1**] prompted us to synthesize and test this alkaloid as a potential antitumor compound due to its structural similarity to the indole phytoalexin spirobrassinin (**2**).

2. Results and discussion

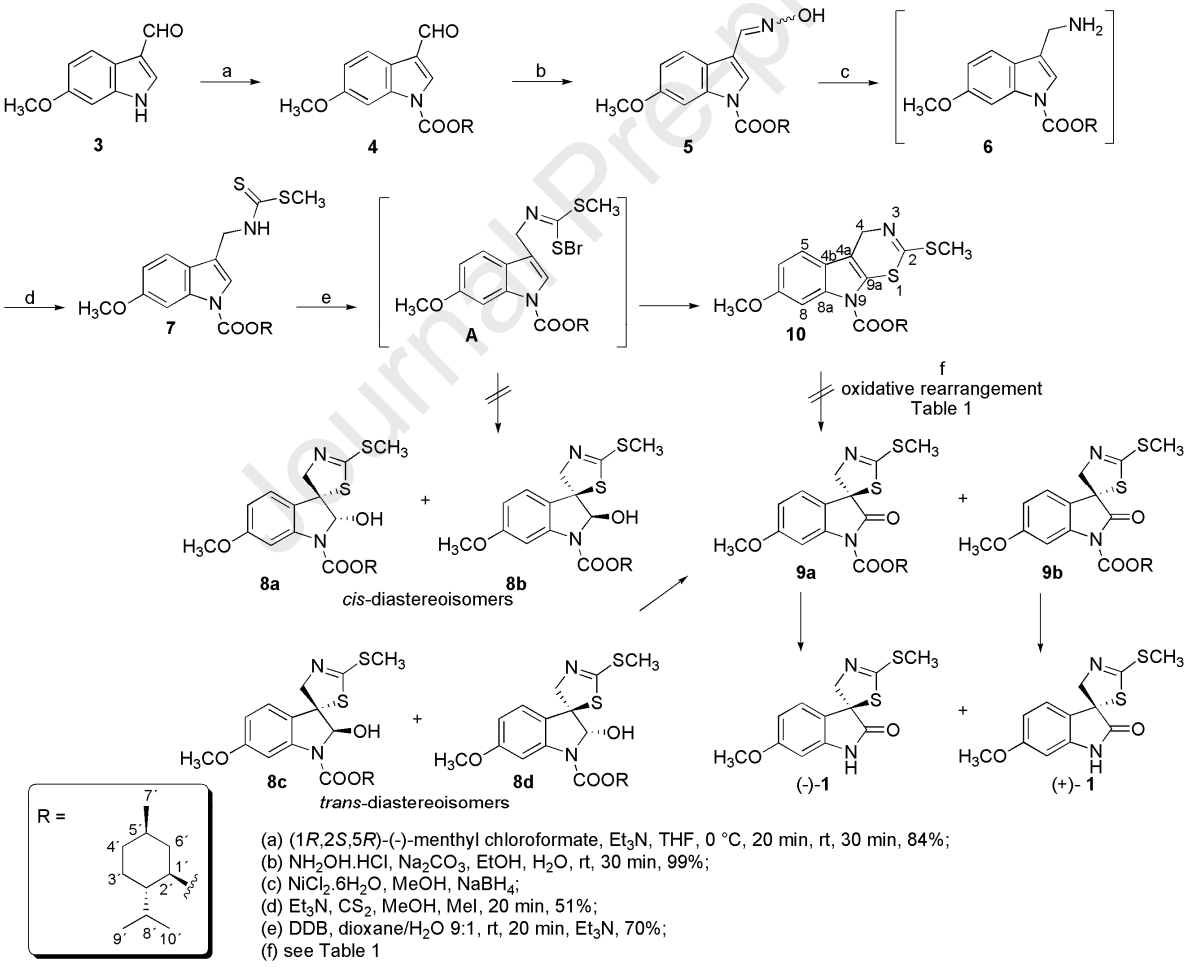
In the preparation of capparine A [(*S*)-(–)-**1**], the principle of stereoselective synthesis was primary proposed based on the use of a chiral acyl derivative of 6-methoxybrassinin **7**, which would undergo bromospirocyclization in the presence of water as a nucleophile to give four diastereoisomers of spirobrassinol **8** assuming that one diastereoisomer would be major. Subsequent oxidation of obtained spirobrassinol **8** to the oxindole structure **9** and removal of the auxiliary chiral group would give the (*S*)-(–)-enantiomer of capparine A [(*S*)-(–)-**1**, Scheme 1].

Following this principle, the starting 6-methoxyindole-3-carboxaldehyde (**3**) was first converted to the 6-methoxy-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]indole-3-carbaldehyde (**4**) by reaction with (1*R*,2*S*,5*R*)-menthyl chloroformate in the presence of triethylamine as base in 84% yield (Scheme 1). In the next step, the reaction of aldehyde **4** with hydroxylamine hydrochloride gave a quantitative mixture of *E*- and *Z*-oxime **5**. Reduction of mixture of *E*- and *Z*-oxime **5** with sodium borohydride catalyzed by nickel boride generated *in situ* from $NiCl_2.6H_2O$ and $NaBH_4$ provided an unstable amine **6**. The crude amine **6** was immediately reacted with carbon disulfide, methyl iodide and triethylamine as a base to give 6-methoxy-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]brassinin (**7**) in a 51% yield after two reaction steps starting from oxime **5** (Scheme 1).

In a study of the stereoselective synthesis of capparine A [(*S*)-(–)-**1**], the electrophilic spirocyclization reaction of chiral brassinin **7** was subsequently investigated in dichloromethane using bromine as a cyclizing agent and water as a nucleophile. This reaction was expected to result in the formation of *cis*-diastereoisomers **8a**,**8b** and *trans*-diastereoisomers **8c**,**8d** of 6-methoxy-1-[(1*R*,2S,5*R*)-menthoxycarbonyl]spirobrassinol. However, after the reaction, only one product was observed in the reaction mixture, namely 7-methoxy-9-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]cyclobrassinin (**10**). Its structure was confirmed by 1D and 2D NMR methods. Based on this result, we decided to implement spirocyclization under other reaction conditions. We hypothesized that dioxane dibromide in dioxane in the presence of water as a nucleophile is more suitable due to the better miscibility of water in dioxane. The dioxane dibromide-mediated spirocyclization of brassinin **7** in dioxane also afforded cyclobrassinin **10** as a sole product (Scheme 1). After sequential spirocyclization of brassinin **7** with bromine and subsequent oxidation by PCC, only the formation of cyclobrassinin **10** was also observed on the TLC plate.

The hope for us was known oxidative rearrangements of fused indole derivatives which is frequently used in the synthesis of spirooxindole.[15,16] We believed that the oxidative chlorination using oxone and sodium chloride or with NaOCl afford spiro products **9a** and **9b**. Unfortunately, by carrying out the reactions under these reaction conditions (Table 1), we did not achieve the rearrangement to spirooxindole derivative **9**, and decomposition products were observed in the reaction mixture in addition to the starting material.

From the above results, we hypothesize that the formation of the cyclostructure is due to the presence of a methoxy group attached at the 6-position on the benzene nucleus of indole, because the reaction of 1-[(1*R*,2S,5*R*)-menthoxycarbonyl]brassinin and 9-[(1*R*,2S,5*R*)-menthoxycarbonyl]cyclobrassinin carried out under the same reaction conditions gave spirocompounds.[16]



Scheme 1.

Table 1 Reaction conditions for oxidative rearrangement

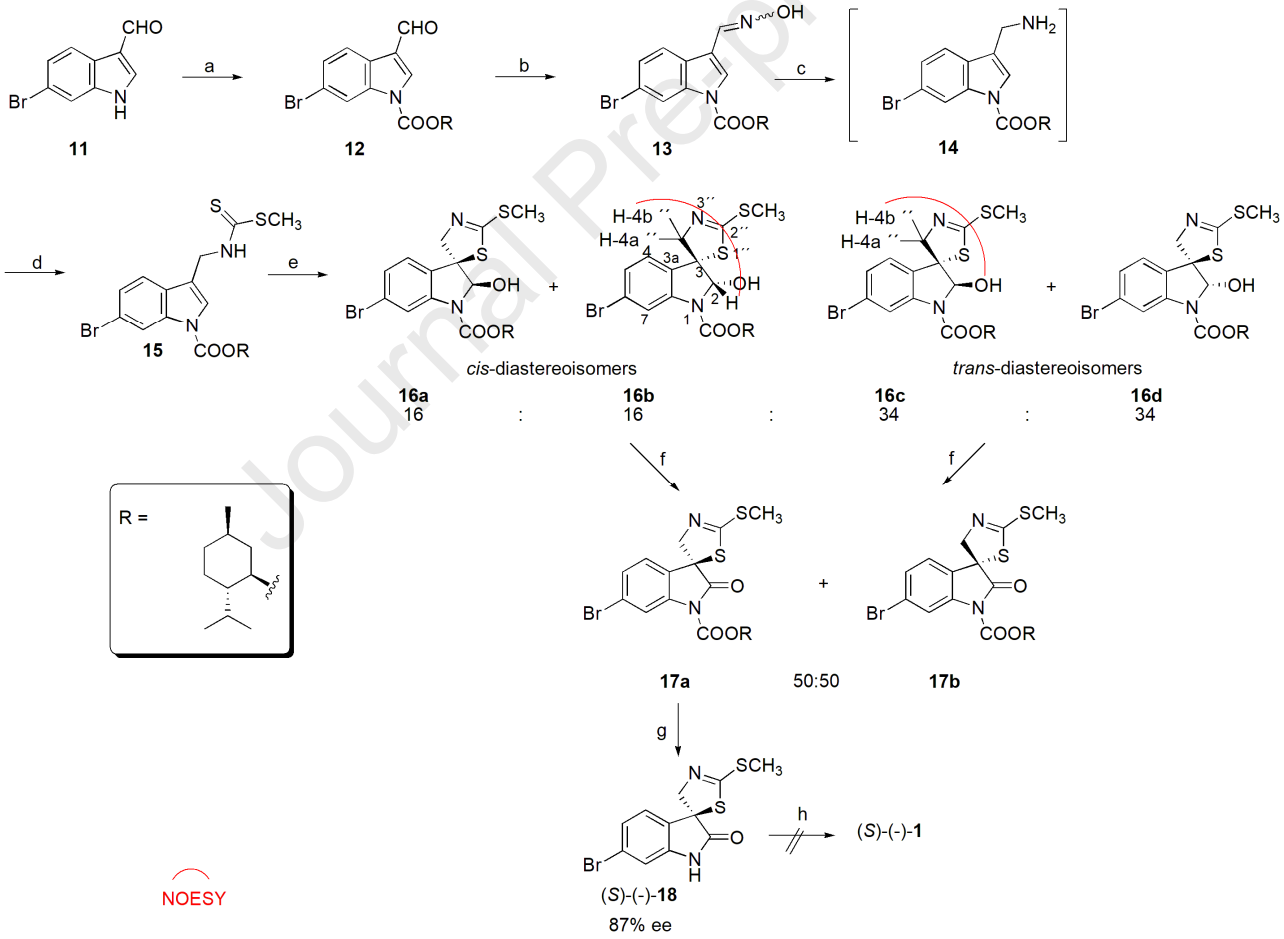
Entry	Reaction conditions		
	Reagent (equiv.)	**Temperature**	**Reaction time (h)**
1	oxone (1.1), aq NaCl (11)	r.t.	24
2	oxone (3), aq NaCl (11)	r.t.	24
3	oxone (3), aq NaCl (11)	60 °C	24
4	NaOCl, (1.5), AcOH	r.t.	1
5	NaOCl, (10), THF	0 °C	4

After an unsuccessful attempt at the synthesis of capparine A [(*S*)-(–)-**1**], we decided to try the relatively well-described method of nucleophilic substitution of bromine for methoxy group in position 6 for indoles and 2,3-indolines.[17] The synthesis plan was based on the preparation of a chiral 6-bromo-1-acyl derivative of brassinin **15**, which would give chiral spirobrassinins **17** by cyclization with bromine in the presence of water and subsequent oxidation of spirobrassinols **16**. Subsequent removal of the chiral group would give the (*S*)-(–)-enantiomer of 6-bromospirobrasinin [(*S*)-(–)-**18**], which in the last step would undergo nucleophilic substitution in the presence of sodium methoxide to give capparine A [(*S*)-(–)-**1**, Scheme 2].

6-Bromo-1*H*-indole-3-carboxaldehyde (**11**) was used as a starting material in this synthetic strategy for the synthesis of the key intermediate 6-bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]brassinin (**15**). Aldehyde **11** was transformed by reaction with (1*R*,2*S*,5*R*)-methyl chloroformate to the corresponding chiral aldehyde **12** in an 86% yield (Scheme 2). From aldehyde **12**, 6-bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]brassinin (**15**) was subsequently synthesized analogously to 6-methoxy-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]brassinin (**7**) in 60% yield relative to oxime **13** (Scheme 2).

Spirocyclization of 1-acylbrassinin **15** with DDB in the presence of water as a nucleophile resulted in the formation of four diastereoisomers **16a**,**16b, 16c**,**16d** in the ratio 16:16:34:34. The ratio of diastereoisomers **16a-16d** was determined by the integration of non-overlapping singlets of the H-2 protons in the ^{1}H NMR spectrum of crude product mixture. The yield of chromatographically isolated mixture of *cis*-diastereoisomers **16a**,**16b** was 30% and the mixture of *trans*-diastereoisomers **16c**,**16d** was 48% (Scheme 2). The mixture of *cis*-diastereoisomers **16a**,**16b** as well as the mixture of *trans*-diastereoisomers **16c**,**16d** were not chromatographically separable. Oxidation of individual mixtures of diastereoisomers **16a**,**16b** and **16c**,**16d** using PCC provided 6-bromo-1-[(1*R*,2*S*,5*R*)-

menthoxycarbonyl]spirobrassinin derivatives **17a** and **17b** in the ratio of 50:50. The ratio of diastereoisomers was determined by accurate quantification of partially overlapping signals of the H-4b´ protons in the ^{1}H NMR spectrum using the MestReNova software.[18] Diastereoisomers **17a** and **17b** showed very close R_f values in various eluents. By multiple chromatographic separation using hexane/ethyl acetate 6:1 as an eluent, only pure diastereoisomer **17a** was possible to separate. Removal of the chiral auxiliary from isomer **17a** by treatment with sodium methoxide afforded (*S*)-(–)-6-bromospirobrasinin [(*S*)-(–)-**18**] in 89% yield. Its absolute configuration at carbon C-3 was assigned by comparing the obtained ECD spectrum with the spectrum of natural (*S*)-(–)-spirobrassinin [(*S*)-(–)-**2**][19] The CD curve of (-)-**18** corresponds to the spectrum of (-)-**2** suggesting that (-)-**18** also has an *S* configuration. Enantiomer (–)-**18** showed moderate Cotton effects at 210 (Δε –13.9) nm and 228 (Δε +25.9) nm.



(a) (1*R*,2*S*,5*R*)-(-)-menthyl chloroformate, Et_3N, THF, 0 °C, 20 min, rt, 30 min, 86%;
(b) $NH_2OH.HCl$, Na_2CO_3, EtOH, H_2O, rt, 30 min, 95%;
(c) $NiCl_2.6H_2O$, MeOH, $NaBH_4$;
(d) Et_3N, CS_2, MeOH, MeI, 20 min, 60%;
(e) DDB, dioxane/H_2O 9:1, rt, 20 min, Et_3N, **16a,16b**-30%, **16c,16d**-48%;
(f) PCC, CH_2Cl_2, rt, 24 h, from **16a,16b**, 61%, from **16c,16d**, 76%;
(g) MeONa, MeOH, rt, 20 min, 89%;
(h) MeONa, CuI, DMF, reflux, Table 2.

Scheme 2.

Pedras[20] and Klika[21] described a simple and inexpensive method for determining enantiopurity of some spirooxindole phytoalexins using a chiral NMR shift agent. The enantiomeric excess of the obtained (*S*)-6-bromospirobrassinin [(*S*)-(−)-**18**] was 87% ee and was determined using the chiral shift agent (*R*)-(+)-1,1'-bi-2-naphthol (~ 3 equiv.) in C_6D_6. The enantiomeric excess of the resolved (*S*)-6-bromospirobrassinin [(*S*)-(−)-**18**] sample was accurately measured by integration of the areas of the ^{1}H NMR peaks corresponding to the SCH_3 group and the doublet of H-4b´ proton of each enantiomer. The optical rotation of prepared (*S*)-6-bromospirobrassinin [(*S*)-(−)-**18**] {$[\alpha]_D^{27}$ = –50.0 (*c* 0.16, $CHCl_3$)} had the same sign and a comparable magnitude as (*S*)-spirobrassinin [(*S*)-(–)-**2**] {$[\alpha]_D^{20}$ –53.0 (*c* 0.30 $CHCl_3$)}.[22]

Subsequently, nucleophilic substitution of (*S*)-(–)-6-bromospirobrassinin [(*S*)-(–)-**18**] was performed in the presence of sodium methoxide (10 equiv.) and copper iodide (2 equiv.) as a catalyst in DMF. After 6 hours of reflux, only the formation of decomposition products was observed on a TLC plate. By lowering the temperature to 80 °C, in addition to the decomposition products, an unidentifiable product was isolated. ^{1}H and ^{13}C NMR spectra of the unidentifiable product showed a deficit of the SCH_3 group. It was assumed that copper (CuI) probably showed an affinity for the sulfur of the SCH_3 group. Therefore, further substitution attempts were made with 0.5 equiv. of copper iodide (Table 2). However, neither by changing the amount of reagent, catalyst or by changing the temperature and time, was the desired substitution product (–)-**1** observed in the reaction mixture. In experiments 4-6, in addition to by-products, unreacted starting material was observed on the TLC plate.

Table 2: Nucleophilic substitution of (*S*)-(–)-6-bromospirobrassinin [(*S*)-(–)-**18**]

Entry	**Reaction conditions**		
	Reagent (equiv.)	**Temperature**	**Reaction time (h)**
1	CH_3ONa (10), CuI (2)	120 °C	6
2	CH_3ONa (10), CuI (2)	80 °C	6
3	CH_3ONa (10), CuI (0.5)	120 °C	6
4	CH_3ONa (10), CuI (0.5)	100 °C	6
5	CH_3ONa (10), CuI (0.5)	80 °C	6
6	CH_3ONa (20), CuI (0.5)	80 °C	12

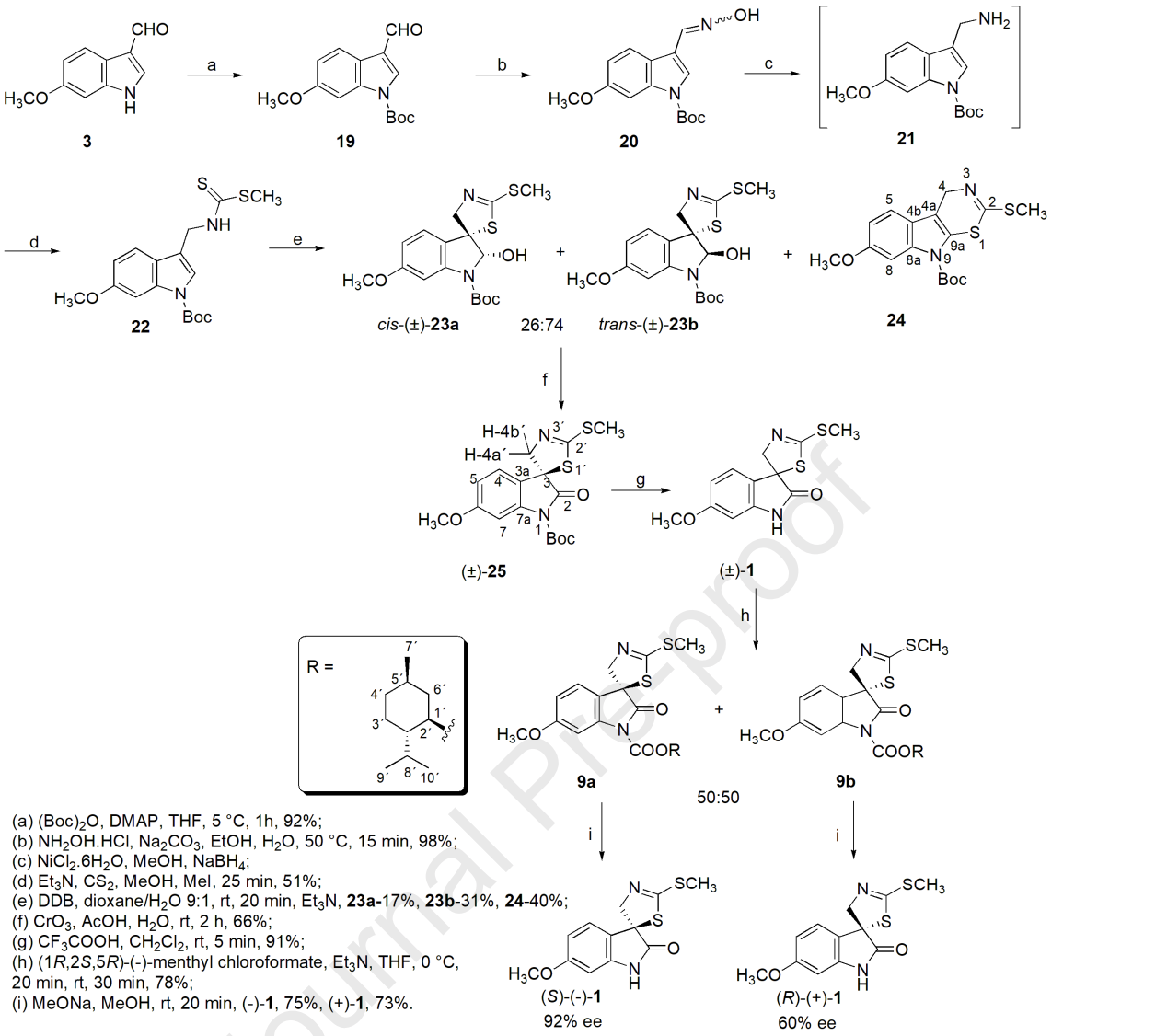
The repeated unsuccessful result of the preparation of capparine A [(*S*)-(–)-**1**] finally led us to consider the possibility of using a *tert*-butoxycarbonyl group in the preparation of achiral 6-

methoxy-1-Boc-brassinin (**22**), which would provide by bromospirocyclization with water easily oxidizing spirobrassinols **23** to oxindole (±)-**25**. After deprotection of the Boc group and attachment of the chiral auxiliary group to the racemate (±)-**1**, spirooxindole structures **9a** and **9b** can be obtained. After separation of diastereoisomers **9a** and **9b** and the subsequent removal of the chiral group, give (*S*)-capparine A [(*S*)-**1**, Scheme 3].

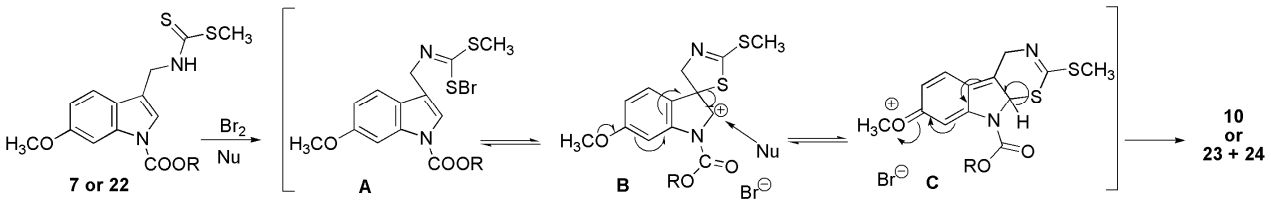
According to the above strategy, 6-methoxyindole-3-carboxaldehyde (**3**) was used as a starting material for the synthesis of brassinin **22**. Reaction of aldehyde **3** with di-t*ert*-butyl dicarbonate in the presence of DMAP as a catalyst provided 6-methoxy-1-(*tert*-butoxycarbonyl)indole-3-carboxaldehyde (**19**)[23] in 92% yield. 6-Methoxy-1-(*tert*-butoxycarbonyl)brassinin (**22**) was obtained in a yield of 51% by the sequence of known reactions (Scheme 3).

Subsequent cyclization of the prepared brassinin **22** by the action of DDB as a bromospirocyclizing agent in a mixture dioxane/water (9:1) provided *cis*- (±) - and *trans*-(±) - diastereoisomers of 6-methoxy-1-(*tert*-butoxycarbonyl)spirobrassinol [(±)-**23a** and (±)-**23b**] in 48% yield, which were chromatographically separable in the eluent hexane/ethyl acetate 2:1. Their ratio of 26:74 in favor of the isomer **23b** was determined based on ^{1}H NMR spectrum of the crude product by integrating the different hydrogen signals H-4b´ and H-2. The low yield of cyclization was caused by the formation of cyclobrassinin derivative **24** (40%, Scheme 3).

The presence of the cyclo product in the spirocyclization reactions of 6-methoxy-substituted brassinins **7** or **22** is probably conditioned by the influence of the methoxy group, which fundamentally affects the overall course of the reactions. Bromospirocyclizations performed with 1-substituted brassinins without OCH_3 in position 6 in the presence of methanol or water as nucleophiles proceeded to form spiro compounds without observation of the cyclo product.[24,16] In the case of cyclization of 6-methoxybrassinins **7** and **22**, the reaction begins at the thiocarbamoyl group, where the action of bromine produces sulfenyl bromide **A**. Subsequently, the electrophilic sulfur attacks position 3 of the indole nucleus to provide spirointermediate **B**, which may form spiro compound **23**, or it rearranges onto cyclobrassinin structure **C** by opening the spiro ring. The 6-methoxy group delocalizes the electrons of the oxygen lone pairs into the ring up to the carbon C-3 of the indole in intermediate **B**. This forces the C-S bond to migrate to the C-2 position with the formation an intermediate C (Scheme 4). The difference of the size of the carbamates (Boc, menthoxycarbonyl) can be responsible for the extent of the formation of the cyclo product.



Scheme 3.



Scheme 4.

Oxidation of a mixture of diastereoisomers (±)-**23a** and (±)-**23b** with chromium oxide in acetic acid gave racemic 6-methoxy-1-(*tert*-butoxycarbonyl)spirobrasdinin [(±)-**25**] in 66% yield, which by removing the *tert*-butoxycarbonyl group with acid trifluoroacetic acid gave racemic capparine A [(±)-**1**] in 91% yield. Synthetic capparine [(±)-**1**] was enantioresolved by chiral auxiliary method. Racemic capparine [(±)-**1**] reacted with (1*R*,2*S*,5*R*)-mentyl

chloroformate with the addition of triethylamine for reaction acceleration to produce two chiral derivatives **9a** and **9b** (Scheme 3). After multiple chromatographic separation of isomers **9a** and **9b** in hexane/ethyl acetate 6:1 as an eluent, pure isomer **9a** and isomer **9b** enriched with **9a** were obtained. In the last step, removal of chiral auxiliary with sodium methoxide afforded (*S*)-(–)-6-methoxyspirobrassinin, i.e. capparine A [(*S*)-(–)-**1**, 92% ee] from isomer **9a** in 75% yield and (*R*)-(+)-6-methoxyspirobrassinin [(*R*)-(+)-**1**, 60% ee] from the isomer **9b** in 73% yield (Scheme 3). The overall yield of synthesized capparine A [(*S*)-(–)-**1**] was 9% after 9 reaction steps. Spectroscopic data (NMR, IR) of (*S*)-(–)-**1** and (*R*)-(+)-**1** were fully identical with those of the natural capparine A [(*S*)-(–)-**1**].[1] The optical rotation of synthesized (*S*)-(–)-**1**{$[\alpha]_D^{27}$ −10.6 (*c* 0.16, MeOH)} had a comparable magnitude and the same sign as natural capparine A [(*S*)-(−)-**1**] {$[\alpha]_D^{25}$ −9.88 (*c* 0.16, MeOH)}.[1] The value of optical rotation of unnatural capparine A [(*R*)-(+)-**1**] was $[\alpha]_D^{27}$ = +3.7 (*c* 0.10, MeOH). Circular dichroism (CD) proved to be a powerful tool to verify the chirality of enantiomers. CD spectroscopy is extremely sensitive, fast, simple and relatively inexpensive method. We realized CD studies to confirm the absolute configuration of prepared capparine A [(*S*)-(–)-**1**] and [(*R*)-(+)-**1**]. Figure 2 displays CD spectra and UV-vis absorption spectra of (–)-**1** and (+)-**1**. Their CD curves are completely mirror images. CD spectrum of prepared (–)-**1** displays comparable curve shape like the spectrum of spirobrassinin [(*S*)-(–)-**2**][19] and spectrum of (+)-**1** like (*R*)-(+)-**2**,[19] suggesting that (–)-**1** has an (*S*)-configuration and (+)-**1** has (*R*)-configuration. Enantiomer (–)-**1** showed an exciton-type split at 228 (Δε +30.9) nm in the short-wavelength region and two negative Cotton effects at 271 (Δε −8.2) and 315 (Δε −11.1) in the long-wavelength region (Fig. 2). Enantiomer (+)-**1** showed an exciton-type split at 228 (Δε −10.2) nm in the short-wavelength region and two negative Cotton effects at 271 (Δε +2.8) and 315 (Δε +3.7) in the long-wavelength region (Fig. 4). UV spectra of capparine A [(*S*)-(-)-**1**] and its enantiomer (*R*)-(+)-**1** show absorption at 228 nm, (log ε +1.69 for (*S*)-(-)-**1**, log ε +0.65 for (*R*)-(+)-**1**, Fig. 2).

To determined accurate ee values of the target capparine A [(*S*)-(–)-**1**] and its enantiomer (*R*)-(+)-**1**, a chiral NMR shift agent (*R*)-(+)-1,1´-bi-2-naphthol (~ 3 equiv.) was used in non-polar C_6D_6 (Fig 3. and Fig 4.). Complete enantioresolution and distinct signals for each enantiomer were observed for the singlet of the SCH_3 group and the doublet of H-4b´ proton in the ^1H NMR spectrum (Fig 3. and Fig 4.). By the integration of the areas corresponding to the individual signals for both enantiomers was determined enantiomeric excess for (-)-**1** ee = 92%. The (+)-**1** enantiomer showed optical purity ee = 60%.

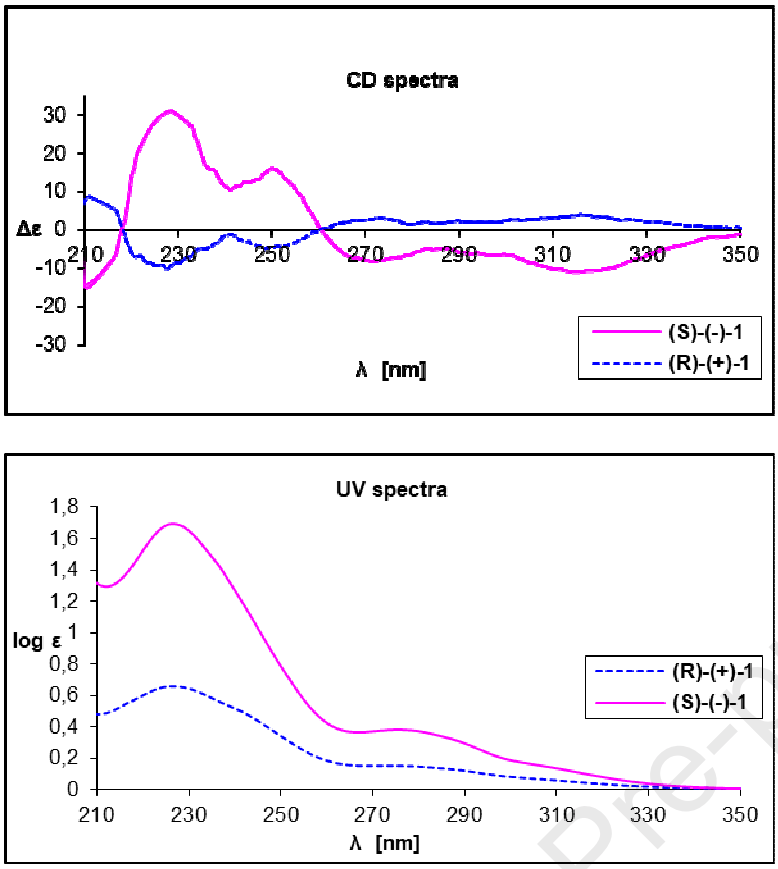


Fig. 2 CD and UV spectra of (*S*)-capparine A [(*S*)-(–)-**1**] and (*R*)-capparine A [(*R*)-(+)-**1**] (CH_3OH, c ~ 0.075 mM).

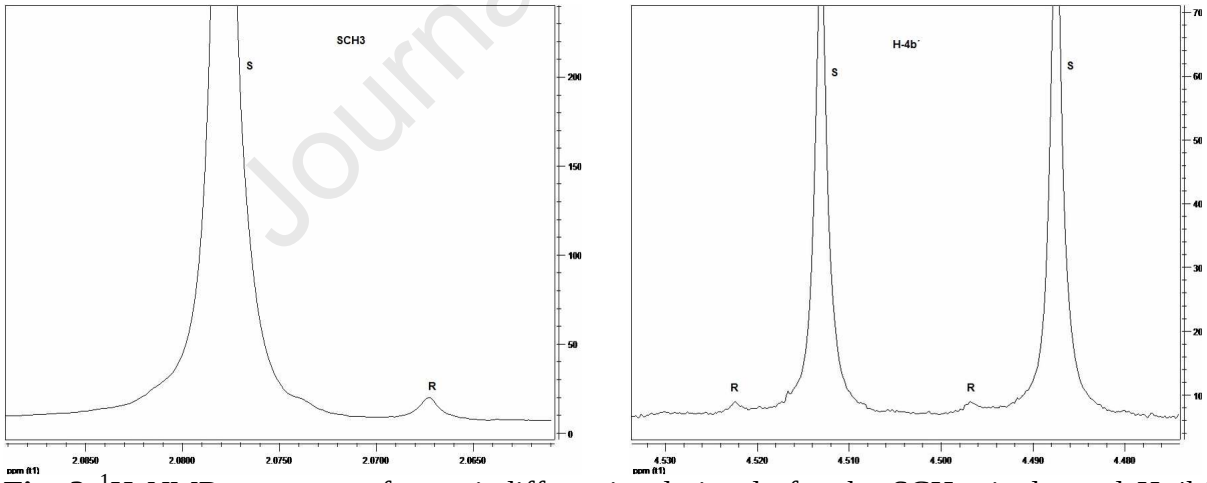


Fig. 3 ^{1}H NMR spectrum of enantiodifferentiated signals for the SCH_3 singlet and H-4b´ doublet of (*S*)-capparine A [(*S*)-(–)-**1**] with (*R*)-(+)-1,1´-bi-2-naphthol (~ 3 equiv.) in C_6D_6

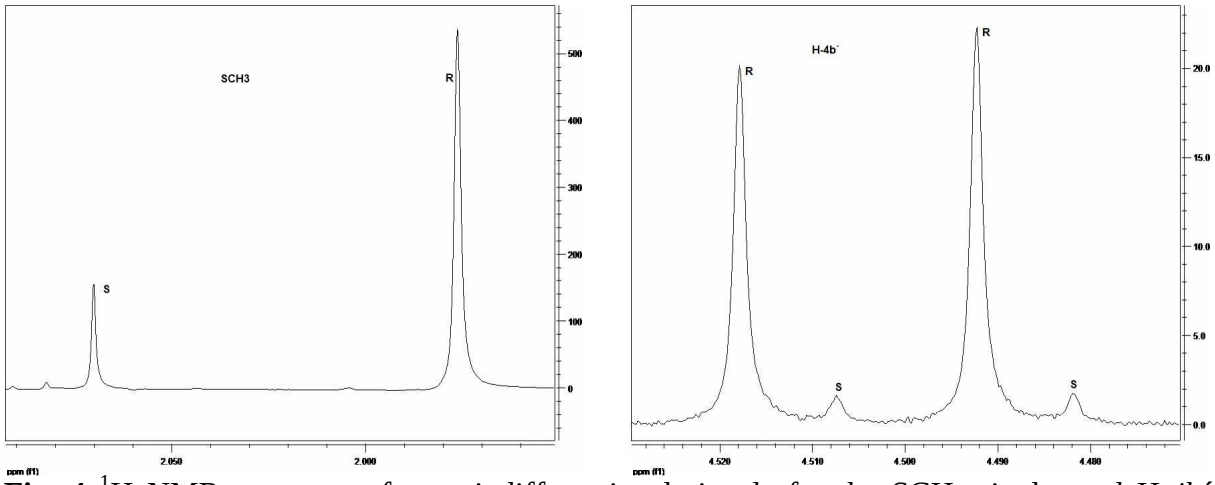


Fig. 4 ^{1}H NMR spectrum of enantiodifferentiated signals for the SCH_3 singlet and H-4b´ doublet of (*R*)-capparine A [(*R*)-(+)-**1**] with (*R*)-(+)-1,1´-bi-2-naphthol (~ 3 equiv.) in C_6D_6

Racemic (±)-**1** and natural enantiomer of the capparine A [(*S*)-(–)-**1**] were screened for anti-proliferative/cytotoxic activities on the panel of the following ten human cancer cell lines: Caco-2, HCT116 (colorectal carcinoma), CCRF-CEM (acute T-lymphoblastic leukemia), MCF-7, MDA-MB-231 (mammary gland adenocarcinoma), HeLa (cervical adenocarcinoma), A-549 (non-small cell lung cancer), U-87 MG (glioblastoma astrocytoma), SK-OV-3, A2780 (ovarian carcinoma) and a non-malignant cell line NIH 3T3 (murine fibroblasts). Cytotoxicity assay against cancer cell lines was performed by the MTT (Thiazolyl Blue Tetrazolium Bromide) method.[25] The potencies of racemic (±)-**1** and enantiopure capparine A [(*S*)-(–)-**1**] are presented in Table 3 as IC_{50} values. This table also include IC_{50} values for the conventional anticancer agent cisplatin for comparison. Racemic capparine A [(±)-**1**] exhibited moderate activity on the proliferation of the HeLa, MCF-7 and CEM cells. Natural enantiomer (*S*)-capparine A [(*S*)-(–)-**1**] showed no anti-proliferative activity against all human cancer cell lines. The unnatural (+)-enantiomer of capparine A [(*R*)-(+)-**1**] seems to be more promising than the natural alkaloid regarding this respect and its antiproliferative activity is currently examined.

Table 3: Antiproliferative activities of racemic (±)-**1** and enantiopure capparine A (*S*)-(-)-**1**

Comp.	Cell line IC_{50} (μmol × L^{-1})										
	HeLa	**MCF-7**	**MDA**	**A-549**	**CEM**	**HCT116**	**CaCo-2**	**U-87 MG**	**SK-OV-3**	**A2780**	**3T3**
(±)-**1**	52.4	57.6	>100	>100	44.95	nt	nt	nt	nt	nt	nt
(*S*)-(–)-**1**	>100	>100	>100	nt	nt	>100	>100	>100	>100	>100	>100
Cisplatin[26]	13.1	15.6	17.5	9.5	nt	15.3	15.2	nt	nt	nt	20.9

nt – not tested

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).

3. Conclusion

In this paper, we report the first concise total synthesis of the natural alkaloid capparine A [(*S*)-(–)-**1**]. Synthesis of racemic capparine A [(±)-**1**] was achieved by the bromine-mediated spirocyclization of 6-methoxy-1-Boc-brassinin in the presence of water as a nucleophile with the formation of spirobrassinols, subsequent oxidation of spirobrassinol derivatives and removal of the Boc group. To enantioresolve synthesized racemic capparine A [(±)-**1**], a chiral auxiliary method was applied. Enantioresolution of (±)-**1** was achieved by derivatization with (1*R*,2*S*,5*R*)-menthyl chloroformate, chromatographic separation of diastereoisomers and their cleavage with sodium methoxide. An examination of the anti-proliferative activity of the capparine A [(*S*)-(–)-**1**] against human cancer cells revealed no effect on cell proliferation.

4. Experimental

4.1. Chemistry

^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.61 MHz) spectra were measured at room temperature on a Varian Mercury Plus spectrometer. Spectra were recorded in $CDCl_3$ unless otherwise stated with an internal standard tetramethylsilane (TMS, 0.00 ppm). Coupling constants (*J*) were obtained by first-order analysis and measured in Hertz (Hz). Infrared spectra were taken on an Avatar FT-IR 6700 spectrometer (Thermo Scientific, UK) using an attenuated total reflectance (ATR) method in the range 4000 – 400 cm^{-1}. Microanalyses were performed on a Perkin-Elmer CHN 2400 elementar analyzer. Melting points were determined on a Kofler hot-stage apparatus and remained uncorrected. Optical rotations were measured at room temperature in a 10 cm cell on a polarimeter JASCO P-2000 at the sodium D-line. CD spectra were obtained in a 10 mm quartz cell on a JASCO J-810 spectrometer. The enantiomeric excess of synthesized enantiomers was determined by solution-state ^{1}H NMR spectroscopy using the chiral NMR shift agent (*R*)-(+)-1,1´-bi-2-naphthol in C_6D_6. The chemical reactions were monitored on TLC-sheets ALUGRAM® SIL G/UV_{254} (Macherey-Nagel, Germany). Detection was carried out with ultraviolet light (254 nm). Preparative column chromatography was performed on Kieselgel 60 Merck Type 9385 (0.040-0.063 mm).

4.1.1. General procedure for the synthesis of aldehydes **4** and **12**.

To a corresponding solution of aldehyde **3**[27], **11** (4.926 mmol) in THF (20 mL) at 0 °C was added triethylamine (0.523 g, 0.72 mL, 5.172 mmol). The reaction mixture was stirred at 0 °C

for 10 min. (1*R*,2*S*,5*R*)-(–)-Menthyl chloroformate (1.131 g, 1.1 mL, 5.172 mmol) was then added and the reaction mixture was stirred at 0 °C for 20 min and for 30 min at room temperature. After the reaction was finished, THF was evaporated. The residue obtained after evaporation of the solvent was subjected to column chromatography on silica gel. The obtained products were further crystallized from hot ethanol to afford aldehydes **4** and **12**.

4.1.1.1. 6-Methoxy-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]indole-3-carboxaldehyde (**4**). Following the general procedure, product **4** was obtained using aldehyde **3**[27] (0.863 g, 4.926 mmol) and separated on silica gel (30 g, *n*-hexane/acetone 3:1). Yield: 1.48 g (84%); white crystals; mp 121–123 °C (ethanol); $[\alpha]_D^{23}$ –77.1 (*c* 1.08, $CHCl_3$); R_f 0.55 (*n*-hexane/acetone 3:1); IR (neat) ν_{max} 2954, 2925, 2869, 1741 and 1675 (C=O), 1550, 1493, 1397, 1264 cm^{-1}; Anal. Calcd for $C_{21}H_{27}NO_4$ requires (357.44): C, 70.56; H, 7.61; N, 3.92. Found: C, 70.45; H, 7.50; N, 3.91; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (s, 1H, CHO), 8.15 (s, 1H, H-2), 8.13 (d, 1H, *J* = 8.7 Hz, H-4), 7.75 (d, 1H, *J* = 2.3 Hz, H-7), 7.00 (dd, 1H, *J* = 8.7 Hz, *J* = 2.3 Hz, H-5), 4.98 (dt, 1H, *J* = 10.9 Hz, *J* = 4.5 Hz, H-1´), 3.88 (s, 3H, OCH_3), 2.28–2.21 (m, 1H, H-6´), 2.07–1.97 (m, 1H, H-8´), 1.84–1.72 (m, 2H, H-3´, H-4´), 1.70–1.54 (m, 2H, H-2´, H-5´), 1.28–0.94 (m, 3H, H-3´, H-4´, H-6´), 0.97 (d, 3H, *J* = 7.0 Hz), 0.96 (d, 3H, *J* = 6.5 Hz) [H-9´, H-10´], 0.85 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.8 (CHO), 159.1 (C-6), 150.1 (C=O), 137.3 (C-7a), 135.0 (C-2), 122.8 (C-4), 122.2 (C-3), 119.7 (C-3a), 113.9 (C-5), 99.4 (C-7), 79.3 (C-1´), 55.7 (OCH_3), 47.4 (C-2´), 41.0 (C-6´), 34.1 (C-4´), 31.6 (C-5´), 26.6 (C-8´), 23.6 (C-3´), 22.0, 20.9 (C-9´, C-10´), 16.5 (C-7´).

4.1.1.2. 6-Bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]indole-3-carboxaldehyde (**12**). Following the general procedure, product **12** was obtained using aldehyde **11** (1.104 g, 4.926 mmol) and separated on silica gel (30 g, *n*-hexane/acetone 5:1). Yield: 1.72 g (86%); white crystals; mp 56–58 °C (ethanol); $[\alpha]_D^{23}$ –71.9 (*c* 0.80, $CHCl_3$); R_f 0.72 (*n*-hexane/acetone 5:1); IR (neat) ν_{max} 2955, 2926, 2870, 1748 and 1662 (C=O), 1546, 1426, 1226 cm^{-1}; Anal. Calcd for $C_{20}H_{24}BrNO_3$ requires (406.31): C, 59.12; H, 5.95; N, 3.45. Found: C, 59.03; H, 5.87; N, 3.39; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.09 (s, 1H, CHO), 8.39 (d, 1H, *J* = 1.7 Hz, H-7), 8.23 (s, 1H, H-2), 8.16 (d, 1H, *J* = 8.4 Hz, H-4), 7.51 (dd, 1H, *J* = 8.4 Hz, *J* = 1.7 Hz, H-5), 4.50 (dt, 1H, *J* = 11.0 Hz, *J* = 4.5 Hz, H-1´), 2.28–2.21 (m, 1H, H-6´), 2.10–1.90 (m, 1H, H-8´), 1.85–1.75 (m, 2H, H-3´, H-4´), 1.72–1.54 (m, 2H, H-2´, H-5´), 1.28–0.95 (m, 3H, H-3´, H-4´, H-6´), 0.98 (d, 6H, *J* = 6.5 Hz, H-9´, H-10´), 0.85 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.4 (CHO), 149.5 (C=O), 136.6 (C-7a), 136.0 (C-2), 128.1 (C-5), 124.9

(C-3a), 123.3 (C-4), 121.6 (C-3), 120.0 (C-6), 118.5 (C-7), 79.7 (C-1´), 47.2 (C-2´), 40.8 (C-6´), 33.9 (C-4´), 31.5 (C-5´), 26.5 (C-8´), 23.5 (C-3´), 21.9, 20.7 (C-9´, C-10´), 16.4 (C-7´).

4.1.2. General procedure for synthesis of oximes **5**, **13** and **20**.

To a stirred solution of corresponding aldehyde **4**, **12**, **19** (4.932 mmol) in ethanol (31 mL) was added a solution of hydroxylammonium chloride (0.582 g, 8.384 mmol) and sodium carbonate (0.41 g, 3.797 mmol) in water (2.6 mL). The mixture was stirred for 30 min at room temperature (**5**, **13**) or heated at 50 °C for 15 min (**20**). After evaporation of ethanol and addition of water (13 mL), the product was extracted with diethyl ether (**5**, **20**) or ethyl acetate (**13**) (1 × 40 mL, 1 × 30 mL, 1 × 20 mL) and after drying with anhydrous Na_2SO_4 the solvent evaporated. The obtained products were further crystallized from dichloromethane/*n*-hexane or ethyl acetate/*n*-hexane to afford oximes **5**, **13** and **20**.

4.1.2.1. 6-Methoxy-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]indole-3-carboxaldehyde oxime (**5**). Following the general procedure, product **5** was obtained using aldehyde **4** (1.763 g, 4.932 mmol). Yield: 1.82 g (99%) of a mixture of *E*- and *Z*-isomer in a 70:30 ratio; white crystals (dichloromethane/*n*-hexane); R_f 0.52 and 0.35 (*n*-hexane/ethyl acetate 3:1); IR (neat) ν_{max} 3265 (OH), 2955, 2931, 2852, 1728 (C=O), 1614, 1493, 1386 cm^{-1}; Anal. Calcd for $C_{21}H_{28}N_2O_4$ requires (372.46): C, 67.72; H, 7.58; N, 7.52. Found: C, 67.62; H, 7.50; N, 7.42; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 0.3H, CH=N min.), 8.26 (s, 0.7H, CH=N maj.), 7.97 (d, 0.7H, *J* = 8.7 Hz, H-4 maj.), 7.83 (s, 0.3H, H-7 min.), 7.78 (s, 0.7H, H-7 maj.), 7.71 (s, 0.3H, H-2 min.), 7.69 (s, 0.7H, H-2 maj.), 7.58 (d, 0.3H, *J* = 8.7 Hz, H-4 min.), 6.97 (dd, 0.3H, *J* = 8.7 Hz, *J* = 2.3 Hz, H-5 min.), 6.94 (dd, 0.7H, *J* = 8.7 Hz, *J* = 2.4 Hz, H-5 maj.), 4.95 (dt, 1H, *J* = 10.8 Hz, *J* = 4.3 Hz, H-1´), 3.89 (s, 0.9H, OCH_3 min.), 3.88 (s, 2.1H, OCH_3 maj.), 2.28–2.20 (m, 1H, H-6´), 2.12–1.99 (m, 1H, H-8´), 1.84–1.72 (m, 2H, H-3´, H-4´), 1.72–1.54 (m, 2H, H-2´, H-5´), 1.28–0.93 (m, 3H, H-3´, H-4´, H-6´), 0.96 (d, 3H, *J* = 7.1 Hz), 0.95 (d, 3H, *J* = 6.3 Hz) [H-9´, H-10´], 0.84 (d, 0.9H, *J* = 6.9 Hz, H-7´ min.), 0.83 (d, 2.1H, *J* = 6.9 Hz, H-7´ maj.); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.5 (C-6 maj.), 158.3 (C-6 min.), 150.6 (C=O min.), 150.4 (C=O maj.), 145.0 (CH=N maj.), 138.8 (C-2 min.), 137.1 (C-7a maj.), 135.6 (C-7a min.), 129.7 (CH=N min.), 126.3 (C-2 maj.), 123.0 (C-4 maj.), 122.4 (C-3a min.), 120.7 (C-3a maj.), 118.8 (C-4 min.), 114.8 (C-3 maj.), 112.9 (C-5 min.), 112.8 (C-5 maj.), 110.9 (C-3 min.), 99.4 (C-7 min.), 99.3 (C-7 maj.), 78.4 (C-1´ min.), 78.3 (C-1´ maj.), 55.6 (OCH_3 min.), 55.5 (OCH_3 maj.), 47.3 (C-2´ maj.), 47.2 (C-2´ min.), 41.0 (C-6´ maj.), 40.9 (C-6´ min.), 34.2 (C-4´ min.), 34.1 (C-4´ maj.), 31.5 (C-5´ min.), 31.4 (C-5´ maj.), 26.5

(C-8´ maj.), 25.9 (C-8´ min.), 23.6 (C-3´ min.), 23.5 (C-3´maj.), 22.2, 21.0 (C-9´, C-10´ min.), 21.9, 20.7 (C-9´, C-10´ maj.), 16.5 (C-7´ min.), 16.4 (C-7´ maj.).

4.1.2.2. 6-Bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]indole-3-carboxaldehyde oxime (**13**). Following the general procedure, product **13** was obtained using aldehyde **12** (2.0 g, 4.932 mmol). Yield: 1.97 g (95%) of a mixture of *E*- and *Z*-isomer in a 60:40 ratio; white crystals (dichloromethane/*n*-hexane); R_f 0.50 and 0.42 (*n*-hexane/acetone 3:1); IR (neat) ν_{max} 3304 (OH), 2954, 2923, 2869, 1736 (C=O), 1431, 1371, 1241 cm^{-1}; Anal. Calcd for $C_{20}H_{25}BrN_2O_3$ requires (421.33): C, 57.01; H, 5.98; N, 6.65. Found: C, 56.92; H, 5.86; N, 6.51; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 0.4H, CH=N min.), 8.44 (s, 0.4H, H-7 min.), 8.39 (s, 0.6H, H-7 maj.), 8.27 (s, 0.6H, CH=N maj.), 7.98 (d, 0.6H, *J* = 8.4 Hz, H-4 maj.), 7.75 (s, 1H, H-2), 7.58 (d, 0.4H, *J* = 8.4 Hz, H-4 min.), 7.45 (dd, 0.4H, *J* = 8.4 Hz, *J* = 1.5 Hz, H-5 min.), 7.42 (dd, 0.6H, *J* = 8.4 Hz, *J* = 1.7 Hz, H-5 maj.), 4.96 (dt, 1H, *J* = 10.8 Hz, *J* = 4.4 Hz, H-1´), 2.27–2.18 (m, 1H, H-6´), 2.12–1.90 (m, 1H, H-8´), 1.88–1.72 (m, 2H, H-3´, H-4´), 1.70–1.54 (m, 2H, H-2´, H-5´), 1.30–0.95 (m, 3H, H-3´, H-4´, H-6´), 0.96 (d, 3H, *J* = 6.6 Hz), 0.90 (d, 3H, *J* = 6.9 Hz) [H-9´, H-10´], 0.83 (d, 1.8H, *J* = 6.9 Hz, H-7´ maj.), 0.79 (d, 1.2H, *J* = 6.9 Hz, H-7´ min.); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.9 (C=O), 144.3 (CH=N maj.), 136.6 (C-7a maj.), 135.2 (C-7a min.), 131.6 (CH=N min.), 127.6 (C-2), 127.4 (C-3a min.), 127.0 (C-5 maj.), 126.8 (C-5 min.), 125.9 (C-3a maj.), 123.7 (C-4 maj.), 119.5 (C-6 maj.), 119.4 (C-4 min.), 118.9 (C-6 min.), 118.7 (C-7 min.), 118.4 (C-7 maj.), 114.7 (C-3 maj.), 110.0 (C-3 min.), 78.8 (C-1´), 47.5 (C-2´ min.), 47.2 (C-2´ maj.), 41.5 (C-6´ min.), 40.9 (C-6´ maj.), 34.4 (C-4´ min.), 34.0 (C-4´ maj.), 31.5 (C-5´ min.), 31.3 (C-5´ maj.), 26.3 (C-8´ min.), 25.5 (C-8´ maj.), 23.6 (C-3´ min.), 23.4 (C-3´maj.), 22.1, 20.9 (C-9´, C-10´ min.), 21.9, 20.7 (C-9´, C-10´ maj.), 16.4 (C-7´ maj.), 16.3 (C-7´ min.).

4.1.2.3. 6-Methoxy-1-(*tert*-butoxycarbonyl)indole-3-carboxaldehyde oxime (**20**). Following the general procedure, product **20** was obtained using aldehyde **19**[23] (1.357 g, 4.932 mmol). Yield: 1.40 g (98%) of a mixture of *E*- and *Z*-isomer in a 70:30 ratio; white crystals (ethyl acetate/*n*-hexane); R_f 0.68 and 0.50 (*n*-hexane/ethyl acetate 2:1); IR (neat) ν_{max} 3389 (OH), 2979, 2930, 1718 (C=O), 1430, 1381, 1213 cm^{-1}; Anal. Calcd for $C_{15}H_{18}N_2O_4$ requires (290.31): C, 62.06; H, 6.25; N, 9.65. Found: C, 61.91; H, 6.19; N, 9.46; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (s, 0.3H, CH=N min.), 8.27 (s, 0.7H, CH=N maj.), 7.93 (d, 0.7H, *J* = 8.7 Hz, H-4 maj.), 7.77 (s, 0.6H, H-2 min., H-7 min.), 7.74 (s, 0.7H, H-7 maj.), 7.65 (s, 0.7H, H-2 maj.), 7.55 (d, 0.3H, *J* = 8.7 Hz, H-4 min.), 6.94 (dd, 0.3H, *J* = 8.7 Hz, *J* = 2.2 Hz, H-5 min.),

6.91 (dd, 0.7H, *J* = 8.7 Hz, *J* = 2.2 Hz, H-5 maj.), 3.88 (s, 0.9H, OCH_3 min.), 3.87 (s, 2.1H, OCH_3 maj.), 1.68 [s, 2.7H, $C(CH_3)_3$ min.], 1.67 [s, 6.3H, $C(CH_3)_3$ maj.]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.3 (C-6 maj.), 158.1 (C-6 min.), 149.5 (C=O min.), 149.3 (C=O maj.), 144.9 (CH=N maj.), 138.9 (C-2 min.), 136.9 (C-7a maj.), 135.4 (C-7a min.), 130.2 (CH=N min.), 126.8 (C-2 maj.), 122.7 (C-4 maj.), 122.3 (C-3a min.), 120.6 (C-3a maj.), 118.7 (C-4 min.), 114.3 (C-3 maj.), 112.7 (C-5 min.), 112.6 (C-5 maj.), 109.6 (C-3 min.), 99.3 (C-7 min.), 99.2 (C-7 maj.), 84.4 [$\underline{C}(CH_3)_3$ min.], 84.3 [$\underline{C}(CH_3)_3$ maj.], 55.5 (OCH_3), 28.1 [$C(\underline{C}H_3)_3$].

4.1.3. General procedure for synthesis of brassinins **7**, **15** and **22**.

To a solution of $NiCl_2.6H_2O$ (0.938 g, 3.946 mmol) in methanol (36 mL) was added corresponding oxime **5**, **13** and **20** (3.587 mmol) in methanol (50 mL) followed by $NaBH_4$ (1.357 g, 35.87 mmol) in one portion with stirring and cooling with flowing cold water. After 5 min, methanol in the reaction mixture was evaporated to ¼ of its original volume and mixture was poured into 180 mL of water containing 10 mL of 26% NH_4OH. After extraction of products with ethyl acetate (1 × 120 mL, 1 × 70 mL and 1 × 50 mL), drying the extracts with Na_2SO_4 and evaporation of the solvent, the obtained crude amines **6**, **14** and **21** were immediately dissolved in methanol (25 mL) and triethylamine (1.089 g, 1.5 mL, 10.76 mmol) and carbon disulfide (0.819 g, 0.65 mL, 10.76 mmol) were added. After stirring for 5 min, methyl iodide (1.527 g, 0.67 mL, 10.76 mmol) was added and stirring was continued for 20 min at room temperature. The solvent was evaporated and the residue obtained after evaporation of the solvent was subjected to chromatography on silica gel to give brassinins **7**, **15** and **22**.

4.1.3.1. 6-Methoxy-1-[(1*R*,*2S*,5*R*)-menthoxycarbonyl]brassinin (**7**). Following the general procedure, product **7** was obtained using oxime **5** (1.336 g, 3.587 mmol) and isolated on silica gel (60 g, *n*-hexane/ethyl acetate 4:1). Yield: 0.82 g (51%); pale yellow oil; $[\alpha]_D^{23}$ –53.9 (*c* 1.18, $CHCl_3$); R_f 0.45 (*n*-hexane/ethyl acetate 4:1); IR (neat) ν_{max} 3285 (NH), 2953, 2920, 2867, 1728 (C=O), 1618, 1488, 1391 cm^{-1}; Anal. Calcd for $C_{23}H_{32}N_2O_3S_2$ requires (448.64): C, 61.57; H, 7.19; N, 6.24. Found: C, 61.39; H, 7.15; N, 6.12; ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (s, 1H, H-7), 7.53 (s, 1H, H-2), 7.44 (d, 1H, *J* = 8.6 Hz, H-4), 7.05 (br s, 1H, NH), 6.91 (dd, 1H, *J* = 8.6 Hz, *J* = 2.2 Hz, H-5), 5.01 (s, 1.6H, CH_2), 4.92 (dt, 1H, *J* = 10.9 Hz, *J* = 4.4 Hz, H-1´), 4.71 (s, 0.4H, CH_2), 3.88 (s, 3H, OCH_3), 2.74 (s, 0.5H, SCH_3), 2.66 (s, 2.5H, SCH_3), 2.26–2.18 (m, 1H, H-6´), 2.06–1.99 (m, 1H, H-8´), 1.80–1.70 (m, 2H, H-3´, H-4´), 1.67–1.55 (m, 2H, H-2´, H-5´), 1.28–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.96 (d, 3H, *J* = 7.1 Hz),

0.95 (d, 3H, *J* = 6.3 Hz) [H-9´, H-10´], 0.83 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 198.9 (C=S), 158.4 (C-6), 150.5 (C=O), 136.7 (C-7a), 123.3 (C-2), 122.8 (C-3a), 119.6 (C-4), 115.9 (C-3), 112.5 (C-5), 99.6 (C-7), 78.0 (C-1´), 55.6 (OCH_3), 47.3 (C-2´), 42.6 (CH_2), 41.0 (C-6´), 34.1 (C-4´), 31.5 (C-5´), 26.5 (C-8´), 23.5 (C-3´), 22.0, 20.8 (C-9´, C-10´), 18.2 (SCH_3), 16.4 (C-7´).

4.1.3.2. 6-Bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]brassinin (**15**). Following the general procedure, product **15** was obtained using oxime **13** (1.511 g, 3.587 mmol) and isolated on silica gel (40 g, *n*-hexane/ethyl acetate 6:1). Yield: 1.07 g (60%); pale yellow oil; $[\alpha]_D^{23}$ –35.7 (*c* 0.14, $CHCl_3$); R_f 0.52 (*n*-hexane/ethyl acetate 6:1); IR (neat) ν_{max} 3294 (NH), 2954, 2920, 2849, 1732 (C=O), 1454, 1386, 1246 cm^{-1}; Anal. Calcd for $C_{22}H_{29}BrN_2O_2S_2$ requires (497.51): C, 53.11; H, 5.88; N, 5.63. Found: C, 53.01; H, 5.69; N, 5.52; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 8.39 (s, 1H, H-7), 7.63 (s, 1H, H-2), 7.45 (d, 1H, *J* = 8.3 Hz, H-4), 7.40 (dd, 1H, *J* = 8.4 Hz, *J* = 1.4 Hz, H-5), 6.97 (br s, 1H, NH), 5.05 (s, 1.6H, CH_2), 4.93 (dt, 1H, *J* = 10.8 Hz, *J* = 4.4 Hz, H-1´), 4.76 (s, 0.4H, CH_2), 2.67 (s, 3H, SCH_3), 2.28–2.18 (m, 1H, H-6´), 2.10–1.90 (m, 1H, H-8´), 1.88–1.72 (m, 2H, H-3´, H-4´), 1.70–1.54 (m, 2H, H-2´, H-5´), 1.30–0.95 (m, 3H, H-3´, H-4´, H-6´), 0.96 (d, 6H, *J* = 6.5 Hz, H-9´, H-10´), 0.83 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 199.2 (C=S), 149.3 (C=O), 136.4 (C-7a), 128.0 (C-3a), 126.6 (C-5), 125.3 (C-2), 120.3 (C-4), 119.1 (C-6), 118.7 (C-7), 115.8 (C-3), 78.6 (C-1´), 47.2 (C-2´), 41.9 (CH_2), 40.9 (C-6´), 34.0 (C-4´), 31.5 (C-5´), 26.5 (C-8´), 23.5 (C-3´), 21.9, 20.7 (C-9´, C-10´), 18.2 (SCH_3), 16.4 (C-7´).

4.1.3.3. 6-Methoxy-1-(*tert*-butoxycarbonyl)brassinin (**22**). Following the general procedure, product **22** was obtained using oxime **20** (1.041 g, 3.587 mmol) and isolated on silica gel (30 g, *n*-hexane/acetone 5:1). Yield: 0.67 g (51%); pale yellow crystals; mp 118–120 °C (ethyl acetate/*n*-hexane); R_f 0.38 (*n*-hexane/acetone 5:1); IR (neat) ν_{max} 3261 (NH), 2976, 2930, 1716 (C=O), 1435, 1367 cm^{-1}; Anal. Calcd for $C_{17}H_{22}N_2O_3S_2$ requires (366.50): C, 55.71; H, 6.05; N, 7.64. Found: C, 55.60; H, 5.95; N, 7.53; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.73 (s, 1H, H-7), 7.49 (s, 1H, H-2), 7.42 (d, 1H, *J* = 8.6 Hz, H-4), 7.00 (br s, 1H, NH), 6.90 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, H-5), 4.99 (d, 2H, *J* = 4.3 Hz, CH_2), 3.87 (s, 3H, OCH_3), 2.65 (s, 3H, SCH_3), 1.67 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 198.9 (C=S), 158.3 (C-6), 149.5 (C=O), 136.7 (C-7a), 123.6 (C-2), 122.8 (C-3a), 119.5 (C-4), 115.4 (C-3), 112.4 (C-5), 99.6 (C-7), 84.0 [$\underline{C}(CH_3)_3$], 55.6 (OCH_3), 42.6 (CH_2), 28.2 [$C(\underline{C}H_3)_3$], 18.2 (SCH_3).

4.1.4. General procedure for cyclization of brassinins **7**, **15** and **22**.

To a solution of corresponding brassinin **7**, **15** or **22** (0.402 mmol) in a mixture of dioxane/water (3.6 mL/0.4 mL) at room temperature was added a freshly prepared solution of DDB (1.0 mL, 0.442 mmol). The stock solution was obtained by dissolving of bromine (0.04 mL) in dioxane (1.76 mL). The reaction mixture was stirred for 20 min at room temperature, and then triethylamine (0.089 g, 0.12 mL, 0.884 mmol) was added. Stirring was continued for 5 min, and the reaction mixture was poured into water (60 mL). After extraction with ethyl acetate (2 × 40 mL), the combined organic phase was washed with brine (40 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by chromatography on silica gel.

4.1.4.1. 7-Methoxy-9-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]cyclobrassinin (**10**). Following the general procedure, product **10** was obtained using brassinin **7** (0.180 g, 0.402 mmol) and isolated on silica gel (20 g, *n*-hexane/ethyl acetate 6:1). Yield: 0.125 g (70%); pale yellow oil; $[\alpha]_D^{23}$ –72.8 (*c* 0.36, $CHCl_3$); R_f 0.55 (*n*-hexane/ethyl acetate 6:1); IR (neat) ν_{max} 2954, 2926, 2868, 1723 (C=O), 1615, 1487, 1211 cm^{-1}; Anal. Calcd for $C_{23}H_{30}N_2O_3S_2$ requires (446.63): C, 61.85; H, 6.77; N, 6.27. Found: C, 61.72; H, 6.54; N, 6.14; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H, H-8), 7.28 (d, 1H, *J* = 8.6 Hz, H-5), 6.89 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, H-6), 4.98 (dt, 1H, *J* = 10.9 Hz, *J* = 4.5 Hz, H-1´), 4.97 (d, 2H, *J* = 3.0 Hz, H-4), 3.86 (s, 3H, OCH_3), 2.53 (s, 3H, SCH_3), 2.28–2.20 (m, 1H, H-6´), 2.17–2.03 (m, 1H, H-8´), 1.85–1.72 (m, 2H, H-3´, H-4´), 1.70–1.50 (m, 2H, H-2´, H-5´), 1.30–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.97 (d, 3H, *J* = 7.1 Hz), 0.96 (d, 3H, *J* = 6.6 Hz) [H-9´, H-10´], 0.82 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.7 (C-7), 155.9 (C-2), 150.8 (C=O), 137.5 (C-8a), 123.0 (C-9a), 121.6 (C-4b), 117.6 (C-5), 112.1 (C-6), 109.0 (C-4a), 100.0 (C-8), 78.8 (C-1´), 55.6 (OCH_3), 47.9 (C-4), 47.1 (C-2´), 41.0 (C-6´), 34.0 (C-4´), 31.5 (C-5´), 26.2 (C-8´), 23.2 (C-3´), 21.9, 20.9 (C-9´, C-10´), 16.2 (C-7´), 15.0 (SCH_3).

4.1.4.2. *cis*-6-Bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]spirobrasinol (**16a** and **16b**) and *trans*-6-bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]spirobrasinol (**16c** and **16d**).

Following the general procedure, a mixture of *cis*-diastereoisomers **16a**,**16b** (30%, in a ratio 50:50) and *trans*-diastereoisomers **16c**,**16d** (48%, in a ratio 50:50) was obtained using brassinin **15** (0.20 g, 0.402 mmol) and isolated on silica gel (40 g, *n*-hexane/ethyl acetate 6:1). Data for **16a**,**16b**: Yield: 0.062 g (30%); pale yellow oil; R_f 0.27 (*n*-hexane/ethyl acetate 6:1); IR (neat) ν_{max} 3412 (OH), 2954, 2924, 2868, 1709 (C=O), 1597, 1566 (C=N), 1476, 1385,

1276 cm^{-1}; Anal. Calcd for $C_{22}H_{29}BrN_2O_3S_2$ requires (513.51): C, 51.46; H, 5.69; N, 5.46. Found: C, 51.31; H, 5.54; N, 5.44; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (br s, 1H, H-7), 7.20 (dd, 1H, *J* = 8.1 Hz, *J* = 1.5 Hz, H-5), 7.24 (d, 1H, *J* = 8.1 Hz, H-4), 5.64 (s, 1H, H-2), 4.83 (dt, 1H, *J* = 10.6 Hz, *J* = 4.4 Hz, H-1´), 4.38 (d, 1H, *J* = 15.1 Hz, H-4b´´), 4.01 (d, 1H, *J* = 15.1 Hz, H-4a´´), 2.58 (s, 3H, SCH_3), 2.23–2.11 (m, 1H, H-6´), 2.04–1.90 (m, 1H, H-8´), 1.80–1.70 (m, 2H, H-3´, H-4´), 1.58–1.46 (m, 2H, H-2´, H-5´), 1.30–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.94 (d, 3H, *J* = 6.8 Hz), 0.93 (d, 3H, *J* = 7.3 Hz) [H-9´, H-10´], 0.82 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3 (C-2´´), 148.5 (C=O), 140.6 (C-7a), 129.8 (C-3a), 126.7 (C-5), 125.3 (C-4), 123.4 (C-6), 118.3 (C-7), 88.5 (C-2), 77.2 (C-1´), 75.4 (C-4´´), 72.6 (C-3), 47.3 (C-2´), 41.2 (C-6´), 34.0 (C-4´), 31.4 (C-5´), 26.5 (C-8´), 23.4 (C-3´), 21.9, 20.8 (C-9´, C-10´), 16.4 (C-7´), 15.2 (SCH_3).

Data for **16c**,**16d**: Yield: 0.099 g (48%); pale yellow oil; R_f 0.37 (*n*-hexane/ethyl acetate 6:1); IR (neat) ν_{max} 3400 (OH), 2954, 2925, 2868, 1708 (C=O), 1597, 1565 (C=N), 1478, 1385, 1277 cm^{-1}; Anal. Calcd for $C_{22}H_{29}BrN_2O_3S_2$ requires (513.51): C, 51.46; H, 5.69; N, 5.46. Found: C, 51.39; H, 5.58; N, 5.41; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (br s, 1H, H-7), 7.25–7.17 (m, 2H, H-4, H-5), 5.95 (s, 1H, H-2), 5.04 (d, 1H, *J* = 15.6 Hz, H-4b´´), 4.82 (dt, 1H, *J* = 10.9 Hz, *J* = 4.3 Hz, H-1´), 4.26 (d, 1H, *J* = 15.6 Hz, H-4a´´), 2.57 (s, 3H, SCH_3), 2.23–2.12 (m, 1H, H-6´), 2.07–1.85 (m, 1H, H-8´), 1.80–1.70 (m, 2H, H-3´, H-4´), 1.62–1.48 (m, 2H, H-2´, H-5´), 1.30–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.94 (d, 3H, *J* = 6.6 Hz), 0.93 (d, 3H, *J* = 7.3 Hz) [H-9´, H-10´], 0.82 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.5 (C-2´´), 150.1 (C=O), 140.6 (C-7a), 130.0 (C-3a), 126.7 (C-5), 125.2 (C-4), 123.7 (C-6), 118.2 (C-7), 91.9 (C-2), 77.2 (C-1´), 70.1 (C-3), 67.7 (C-4´´), 47.3 (C-2´), 41.3 (C-6´), 34.0 (C-4´), 31.5 (C-5´), 26.5 (C-8´), 23.3 (C-3´), 21.9, 20.8 (C-9´, C-10´), 16.4 (C-7´), 15.2 (SCH_3).

4.1.4.3. *cis*- and *trans*-(±)-6-Methoxy-1-(*tert*-butoxycarbonyl)spirobrasinol [(±)-**23a** and (±)-**23b**]. Following the general procedure, products (±)-**23a**, (±)-**23b** and **24** were obtained using brassinin **22** (0.147 g, 0.402 mmol) and separated on silica gel (30 g, *n*-hexane/ethyl acetate 2:1).

Data for (±)-**23a**: Yield: 0.026 g (17%); colourless oil; R_f 0.42 (*n*-hexane/ethyl acetate 2:1); IR (neat) ν_{max} 3356 (OH), 2971, 2926, 2849, 1701 (C=O), 1560 (C=N), 1488, 1368, 1155 cm^{-1}; Anal. Calcd for $C_{17}H_{22}N_2O_4S_2$ requires (382.50): C, 53.38; H, 5.80; N, 7.32. Found: C, 53.28; H, 5.76; N, 7.28; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (br s, 1H, H-7), 7.26 (d, 1H, *J* = 8.4 Hz, H-4), 6.59 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz, H-5), 5.61 (s, 1H, H-2), 4.36 (d, 1H, *J* = 15.2 Hz,

H-4b´), 4.03 (d, 1H, *J* = 15.2 Hz, H-4a´), 3.80 (s, 3H, OCH_3), 2.58 (s, 3H, SCH_3), 1.61 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.4 (C-2´), 161.3 (C-6), 144.6 (C=O), 140.6 (C-7a), 124.6 (C-4), 122.3 (C-3a), 110.0 (C-5), 100.9 (C-7), 88.2 (C-2), 83.0 [$\underline{C}(CH_3)_3$], 75.4 (C-4´), 66.8 (C-3), 55.5 (OCH_3), 28.4 [$C(\underline{C}H_3)_3$], 15.1 (SCH_3).

Data for (±)-**23b**: Yield: 0.047 g (31%); colourless oil; R_f 0.23 (*n*-hexane/ethyl acetate 2:1); IR (neat) ν_{max} 3354 (OH), 2974, 2926, 1703 (C=O), 1568 (C=N), 1479, 1374, 1159 cm^{-1}; Anal. Calcd for $C_{17}H_{22}N_2O_4S_2$ requires (382.50): C, 53.38; H, 5.80; N, 7.32. Found: C, 53.26; H, 5.72; N, 7.26; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (br s, 1H, H-7), 7.26 (d, 1H, *J* = 8.4 Hz, H-4), 6.60 (dd, 1H, *J* = 8.4 Hz, *J* = 2,3 Hz, H-5), 5.94 (s, 1H, H-2), 5.02 (d, 1H, *J* = 15.5 Hz, H-4b´), 4.23 (d, 1H, *J* = 15.5 Hz, H-4a´), 3.80 (s, 3H, OCH_3), 2.57 (s, 3H, SCH_3), 1.61 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6 (C-2´), 161.4 (C-6), 144.2 (C=O), 141.3 (C-7a), 124.6 (C-4), 121.9 (C-3a), 109.7 (C-5), 100.9 (C-7), 92.4 (C-2), 83.0 [$\underline{C}(CH_3)_3$], 69.8 (C-3), 67.6 (C-4´), 55.5 (OCH_3), 28.4 [$C(\underline{C}H_3)_3$], 15.1 (SCH_3).

4.1.4.4. 7-Methoxy-9-(*tert*-butoxycarbonyl)cyclobrassinin (**24**). Yield: 0.059 g (40%); pale yellow crystals; mp 87–89 °C (ethyl acetate/*n*-hexane); R_f 0.87 (*n*-hexane/ethyl acetate 2:1); IR (neat) ν_{max} 2950, 2920, 2848, 1716 (C=O), 1625, 1487, 1367, 1157 cm^{-1}; Anal. Calcd for $C_{17}H_{20}N_2O_3S_2$ requires (364.48): C, 56.02; H, 5.53; N, 7.69. Found: C, 55.92; H, 5.47; N, 7.53; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (br s, 1H, H-8), 7.27 (d, 1H, *J* = 8.6 Hz, H-5), 6.88 (dd, 1H, *J* = 8.6 Hz, *J* = 2.3 Hz, H-6), 4.96 (s, 2H, H-4), 3.86 (s, 3H, OCH_3), 2.52 (s, 3H, SCH_3), 1.70 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.6 (C-7), 155.8 (C-2), 150.0 (C=O), 137.5 (C-8a), 122.8 (C-9a), 121.5 (C-4b), 117.5 (C-5), 112.0 (C-6), 108.6 (C-4a), 99.8 (C-8), 85.4 [$\underline{C}(CH_3)_3$], 55.6 (OCH_3), 47.9 (C-4), 28.2 [$C(\underline{C}H_3)_3$], 15.2 (SCH_3).

4.1.5. 6-Bromo-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]spirobrassinin (**17a**, **17b**).

To a solution of corresponding mixture *cis*-**16a**,**16b** or *trans*-diastereoisomers **16c**, **16d** (0.1 g, 0.195 mmol) in dichloromethane (3.4 mL) was added pyridinium chlorochromate (0.294 g, 1.365 mmol) and the reaction mixture was vigorously stirred for 24 h at room temperature. After diluting of mixture with dichloromethane (20 mL) and adding a small amount of silica gel, the solvent was evaporated and the residue preabsorbed on silica was subjected to silica gel column chromatography (20 g, *n*-hexane/ethyl acetate 6:1) affording a mixture of **17a** and **17b** in a 50:50 ratio (0,061 g, 61% from a mixture **16a**, **16b** or 0,076 g, 76% from a mixture **16c**, **16d**). The pure isomer **17a** was obtained by repeated chromatography on silica gel (10 g, *n*-hexane/ethyl acetate 6:1).

Data for **17a**: Yield: 0.032 g (32%) from a mixture **16a**, **16b**, 0.041 g (41%) from a mixture **16c**, **16d**; pale yellow oil; $[\alpha]_D^{27}$ –40.6 (*c* 0.28, $CHCl_3$); R_f 0.52 (*n*-hexane/ethyl acetate 6:1); IR (neat) ν_{max} 2956, 2925, 2858, 1777 and 1725 (C=O), 1581 (C=N), 1463, 1269 cm^{-1}; Anal. Calcd for $C_{22}H_{27}BrN_2O_3S_2$ requires (511.50): C, 51.66; H, 5.32; N, 5.48. Found: C, 51.47; H, 5.27; N, 5.39; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (br s, 1H, H-7), 7.37 (dd, 1H, *J* = 8.1 Hz, *J* = 1.2 Hz, H-5), 7.27 (d, 1H, *J* = 8.1 Hz, H-4), 4.89 (dt, 1H, *J* = 11.0 Hz, *J* = 4.3 Hz, H-1´), 4.72 (d, 1H, *J* = 15.3 Hz, H-4b´´), 4.48 (d, 1H, *J* = 15.3 Hz, H-4a´´), 2.62 (s, 3H, SCH_3), 2.25–2.16 (m, 1H, H-6´), 2.15–2.06 (m, 1H, H-8´), 1.82–1.73 (m, 2H, H-3´, H-4´), 1.72–1.60 (m, 2H, H-2´, H-5´), 1.30–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.95 (d, 3H, *J* = 6.0 Hz), 0.93 (d, 3H, *J* = 7.4 Hz) [H-9´, H-10´], 0.80 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8 (C-2), 163.6 (C-2´´), 150.0 (C=O), 139.2 (C-7a), 128.6 (C-5), 128.2 (C-3a), 125.2 (C-4), 123.7 (C-6), 118.8 (C-7), 79.2 (C-1´), 75.8 (C-4´´), 64.6 (C-3), 46.7 (C-2´), 40.6 (C-6´), 34.0 (C-4´), 31.5 (C-5´), 25.9 (C-8´), 23.1 (C-3´), 21.9, 20.8 (C-9´, C-10´), 16.0 (C-7´), 15.7 (SCH_3).

Data for **17b**: Yield: 0.029 g (29%) from a mixture **16a**, **16b**, 0.035 g (35%) from a mixture **16c**, **16d**; pale yellow oil; R_f 0.50 (*n*-hexane/ethyl acetate 6:1); R_f 0.50 (*n*-hexane/ethyl acetate 6:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (br s, 1H, H-7), 7.37 (dd, 1H, *J* = 8.1 Hz, *J* = 1.2 Hz, H-5), 7.27 (d, 1H, *J* = 8.1 Hz, H-4), 4.89 (dt, 1H, *J* = 11.0 Hz, *J* = 4.3 Hz, H-1´), 4.71 (d, 1H, *J* = 15.3 Hz, H-4b´´), 4.48 (d, 1H, *J* = 15.3 Hz, H-4a´´), 2.62 (s, 3H, SCH_3), 2.25–2.15 (m, 1H, H-6´), 2.15–2.06 (m, 1H, H-8´), 1.82–1.73 (m, 2H, H-3´, H-4´), 1.72–1.60 (m, 2H, H-2´, H-5´), 1.30–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.95 (d, 3H, *J* = 6.0 Hz), 0.93 (d, 3H, *J* = 7.4 Hz) [H-9´, H-10´], 0.80 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8 (C-2), 163.6 (C-2´´), 150.0 (C=O), 139.0 (C-7a), 128.6 (C-5), 128.1 (C-3a), 125.2 (C-4), 123.7 (C-6), 118.8 (C-7), 79.2 (C-1´), 75.8 (C-4´´), 64.6 (C-3), 46.7 (C-2´), 40.6 (C-6´), 34.0 (C-4´), 31.5 (C-5´), 25.9 (C-8´), 23.1 (C-3´), 21.9, 20.8 (C-9´, C-10´), 16.0 (C-7´), 15.7 (SCH_3). Spectral data of **17b** were identified from a mixture of **17a** and **17b**.

4.1.6. (*S*)-(–)-6-Bromospirobrassinin [(*S*)-(–)-**18**].

To a stirred solution of **17a** (0.050 g, 0.098 mmol) in dry methanol (1.8 mL) was added CH_3ONa (0.005 g, 0.294 mmol) and the reaction mixture was stirred at room temperature for 20 min. Then the solvent was evaporated and the residue was purified by silica gel column flash chromatography (10 g, *n*-hexane/ethyl acetate 6:1) affording enantiomeric product (*S*)-(–)-**18**. Yield: 0.029 g (89%); pale yellow oil; $[\alpha]_D^{27}$ = –50.0 (*c* 0.16, $CHCl_3$); 87% ee; ECD (CH_3OH, c ~ 0,075 mM) λ_{ext} ($\Delta\varepsilon$): 210 (–13.9), 228 (+25.9), 240 (+9.5), 249 (+13.1), 271

(–6.9), 287 (–4.1), 315 (–9.3) nm; R_f 0.35 (*n*-hexane/ethyl acetate 2:1); IR (neat) ν_{max} 3368 (NH), 3199, 2925, 2847, 1714 (C=O), 1578 (C=N) cm^{-1}; Anal. Calcd for $C_{11}H_9BrN_2OS_2$ requires (329.24): C, 40.13; H, 2.76; N, 8.51. Found: C, 40.02; H, 2.69; N, 8.39; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (br s, 1H, NH), 7.23–7.21 (m, 2H, H-4, H-5), 7.09 (d, 1H, J = 1.8 Hz, H-7), 4.66 (d, 1H, J = 15.2 Hz, H-4b´), 4.47 (d, 1H, J = 15.2 Hz, H-4a´), 2.62 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.7 (C-2), 164.0 (C-2´), 140.4 (C-7a), 130.2 (C-3a), 126.7 (C-5), 125.7 (C-4), 123.2 (C-6), 113.7 (C-7), 74.9 (C-4´), 64.2 (C-3), 15.7 (SCH_3).

4.1.7. (±)-6-Methoxy-1-(*tert*-butoxycarbonyl)spirobrassinin [(±)-**25**].

To a solution of mixture of diastereoisomers **23a** and **23b** (0.050 g, 0.131 mmol) in acetic acid (4 mL) was added CrO_3 (0.065 g, 0.655 mmol) and the reaction mixture was vigorously stirred at room temperature for 2 h. After pouring of mixture into water (17 mL), the product was extracted with diethyl ether (2 × 10 mL), washed with 10% NaOH (2 × 7 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by chromatography on silica gel (5 g, *n*-hexane/ethyl acetate 2:1) to afford product (±)-**25**. Yield: 0.033 g (66%); pale yellow oil; R_f 0.60 (*n*-hexane/ethyl acetate 2:1); IR (neat) ν_{max} 2917, 2849, 1768 and 1727 (C=O), 1579 (C=N), 1462, 1275, 1124 cm^{-1}; Anal. Calcd for $C_{17}H_{20}N_2O_4S_2$ requires (380.48): C, 53.66; H, 5.30; N, 7.36. Found: C, 53.47; H, 5.23; N, 7.27; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (s, 1H, H-7), 7.53 (d, 1H, J = 8.4 Hz, H-4), 6.73 (dd, 1H, J = 8.4 Hz, J = 2.3 Hz, H-5), 4.68 (d, 1H, J = 15.3 Hz, H-4b´), 4.45 (d, 1H, J = 15.3 Hz, H-4a´), 3.94 (s, 3H, OCH_3), 2.61 (s, 3H, SCH_3), 1.64 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5 (C-2), 163.6 (C-2´), 157.1 (C-6), 148.8 (C=O), 138.8 (C-7a), 128.2 (C-4), 121.7 (C-3a), 107.6 (C-5), 100.3 (C-7), 85.3 [$\underline{C}(CH_3)_3$], 75.9 (C-4´), 64.7 (C-3), 56.6 (OCH_3), 28.0 [$C(\underline{C}H_3)_3$], 15.6 (SCH_3).

4.1.8. Capparine A [(±)-**1**].

To a stirred solution of compound (±)-**25** (0.044 g, 0.116 mmol) in dichloromethane (0.4 mL) at room temperature was added trifluoroacetic acid (0.291 g, 0.19 mL, 2.552 mmol). After stirring for 5 min, the reaction mixture was poured into solution of $NaHCO_3$ (0.216 g, 2.552 mmol in 0.86 mL of water) cooled at 0 °C. Then the mixture was diluted with dichloromethane (1 mL), the organic layer was removed and dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was further crystallized from dichloromethane/*n*-hexane to give racemic product (±)-**1**.

Yield: 0.029 g (91%); white crystals; mp 116–118 °C (dichloromethane/*n*-hexane); R_f 0.35 (*n*-hexane/acetone 1:1); IR (neat) ν_{max} 3426 (NH), 3211, 2956, 2856, 1715 (C=O), 1626, 1580 (C=N), 1456, 1277 cm^{-1}; Anal. Calcd for $C_{12}H_{12}N_2O_2S_2$ requires (280.37): C, 51.41; H, 4.31; N, 9.99. Found: C, 51.27; H, 4.29; N, 9.78; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (br s, 1H, NH), 7.25 (d, 1H, J = 8.4 Hz, H-4), 6.59 (dd, 1H, *J* = 8.4 Hz, *J* = 2.3 Hz, H-5), 6.48 (d, 1H, *J* = 2.3 Hz, H-7), 4.65 (d, 1H, *J* = 15.2 Hz, H-4b´), 4.46 (d, 1H, *J* = 15.2 Hz, H-4a´), 3.80 (s, 3H, OCH_3), 2.62 (s, 3H, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.7 (C-2), 164.1 (C-2´), 161.2 (C-6), 140.6 (C-7a), 125.3 (C-4), 122.7 (C-3a), 108.5 (C-5), 97.4 (C-7), 75.0 (C-4´), 64.5 (C-3), 55.6 (OCH_3), 15.6 (SCH_3).

4.1.9. 6-Methoxy-1-[(1*R*,2*S*,5*R*)-menthoxycarbonyl]spirobrassinin (**9a**, **9b**). To a solution of 6-methoxyspirobrassinin [(±)-**1**; 0.050 g, 0.178 mmol] in THF (0.9 mL) at 0 °C was added triethylamine (0.019 g, 0.03 mL, 0.187 mmol) and the reaction mixture was stirred at 0 °C for 10 min. (1*R*,2*S*,5*R*)-(–)-Menthyl chloroformate (0.041 g, 0.04 mL, 0.187 mmol) was then added and the mixture was stirred at 0 °C for 20 min and for 30 min at room temperature. After the reaction was finished, THF was evaporated. The residue obtained after evaporation of the solvent was subjected to column chromatography on silica gel (15 g, *n*-hexane/ethyl acetate 6:1) affording a mixture of isomers **9a** and **9b** in a 50:50 ratio. The pure isomer **9a** and enantiomer-enriched isomer **9b** were obtained by repeated chromatography on silica gel (10 g, *n*-hexane/ethyl acetate 6:1).

Data for **9a**: Yield: 0.037 g (45%); pale yellow crystals; mp 161–163 °C (ethyl acetate/*n*-hexane); $[\alpha]_D^{27}$ –69.2 (*c* 0.12, $CHCl_3$); R_f 0.50 (*n*-hexane/ethyl acetate 6:1); IR (neat) ν_{max} 2954, 2926, 2869, 1749 and 1686 (C=O), 1546, 1422, 1271, 1171 cm^{-1}; Anal. Calcd for $C_{23}H_{30}N_2O_4S_2$ requires (462.63): C, 59.71; H, 6.54; N, 6.06. Found: C, 59.58; H, 6.41; N, 5.91; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (br s, 1H, H-7), 7.31 (d, 1H, *J* = 8.5 Hz, H-4), 6.75 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, H-5), 4.89 (dt, 1H, *J* = 10.9 Hz, *J* = 4.3 Hz, H-1´), 4.70 (d, 1H, *J* = 15.2 Hz, H-4b´´), 4.46 (d, 1H, *J* = 15.2 Hz, H-4a´´), 3.83 (s, 3H, OCH_3), 2.61 (s, 3H, SCH_3), 2.21–2.12 (m, 1H, H-8´), 2.00–1.93 (m, 1H, H-6´), 1.70–1.58 (m, 2H, H-3´, H-4´), 1.50–1.34 (m, 2H, H-2´, H-5´), 1.20–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.93 (d, 3H, *J* = 6.9 Hz), 0.91 (d, 3H, *J* = 6.3 Hz) [H-9´, H-10´], 0.81 (d, 3H, *J* = 6.9 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.6 (C-2), 165.8 (C-2´´), 161.2 (C-6), 149.5 (C=O), 139.4 (C-7a), 130.8 (C-3a), 124.7 (C-4), 111.3 (C-5), 101.7 (C-7), 78.7 (C-1´), 75.5 (C-4´´), 64.6 (C-3), 55.6 (OCH_3), 50.2 (C-2´), 45.1 (C-6´), 34.5 (C-4´), 31.6 (C-5´), 25.9 (C-8´), 23.2 (C-3´), 22.2, 21.0 (C-9´, C-10´), 16.1 (C-7´), 15.4 (SCH_3).

Data for **9b**: Yield: 0.027 g (33%); pale yellow oil; $[\alpha]_D^{27}$ +48.3 (*c* 0.12, $CHCl_3$); R_f 0.48 (*n*-hexane/ethyl acetate 6:1); IR (neat) ν_{max} 2954, 2925, 2869, 1748 and 1683 (C=O), 1548, 1423, 1274, 1173 cm^{-1}; Anal. Calcd for $C_{23}H_{30}N_2O_4S_2$ requires (462.63): C, 59.71; H, 6.54; N, 6.06. Found: C, 59.62; H, 6.48; N, 5.92; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (br s, 1H, H-7), 7.31 (d, 1H, *J* = 8.5 Hz, H-4), 6.75 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, H-5), 4.89 (dt, 1H, *J* = 10.9 Hz, *J* = 4.3 Hz, H-1´), 4.69 (d, 1H, *J* = 15.2 Hz, H-4b´´), 4.46 (d, 1H, *J* = 15.2 Hz, H-4a´´), 3.83 (s, 3H, OCH_3), 2.61 (s, 3H, SCH_3), 2.21–2.12 (m, 1H, H-8´), 2.00–1.93 (m, 1H, H-6´), 1.70–1.58 (m, 2H, H-3´, H-4´), 1.50–1.34 (m, 2H, H-2´, H-5´), 1.20–0.90 (m, 3H, H-3´, H-4´, H-6´), 0.93 (d, 3H, *J* = 6.9 Hz), 0.91 (d, 3H, *J* = 6.3 Hz) [H-9´, H-10´], 0.81 (d, 3H, *J* = 7.0 Hz, H-7´); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.6 (C-2), 165.8 (C-2´´), 161.1 (C-6), 149.5 (C=O), 139.2 (C-7a), 130.8 (C-3a), 124.9 (C-4), 111.3 (C-5), 101.7 (C-7), 78.7 (C-1´), 75.5 (C-4´´), 64.6 (C-3), 55.6 (OCH_3), 50.2 (C-2´), 45.1 (C-6´), 34.5 (C-4´), 31.6 (C-5´), 25.9 (C-8´), 23.2 (C-3´), 22.2, 21.0 (C-9´, C-10´), 16.1 (C-7´), 15.4 (SCH_3).

4.1.10. Capparine A [(*S*)-(–)-**1** and (*R*)-(+)-**1**].

To a stirred solution of **9a** or **9b** (0.1 g, 0.216 mmol) in dry methanol (4 mL) was added CH_3ONa (0.035 g, 0.648 mmol) and the reaction mixture was stirred at room temperature for 20 min. Then the methanol was evaporated and the residue was purified by silica gel (10 g, *n*-hexane/acetone 2:1) affording enantiomeric products (*S*)-(–)-**1** or (*R*)-(+)-**1**.

Data for (*S*)-(–)-**1**: Yield: 0.045 g (75%); pale yellow oil; $[\alpha]_D^{27}$ –10.6 (*c* 0.16, MeOH)}; $[\alpha]_D^{27}$ = –11.9 (c 0.16, $CHCl_3$); 92% ee; ECD (CH_3OH, c ~ 0.075 mM) λ_{ext} ($\Delta\varepsilon$): 210 (–15.0), 228 (+30.9), 240 (+11.5), 249 (+15.2), 271 (–8.2), 287 (–5.0), 315 (–11.1) nm. NMR, IR, UV and EIMS data were fully identical with those of natural (*S*)-(–)-capparine A.[1]

Data for (*R*)-(+)-**1**: Yield: 0.044 g (73%); pale yellow oil; $[\alpha]_D^{27}$ = +3.7 (*c* 0.10, MeOH); $[\alpha]_D^{27}$ = +4.3 (*c* 0.10, $CHCl_3$); 60% ee; ECD (CH_3OH, c ~ 0.075 mM) λ_{ext} ($\Delta\varepsilon$): 210 (+7.4), 228 (–10.2), 240 (–1.6), 249 (–5.0), 271 (+2.8), 287 (+1.9), 315 (+3.7) nm. NMR, IR, UV and EIMS data were fully identical with those of natural (*S*)-(–)-capparine A.[1]

4.2. Anti-proliferative activity

Cell culture

Cancer cell lines

Cell lines HCT116 (human colorectal carcinoma), CCRF-CEM (acute T-lymphoblastic leukemia), A2780 (ovarian carcinoma) and HeLa (human cervical cancer) were cultured in

RPMI 1640 medium (PAA Laboratories, Pasching, Austria). CaCo-2 (human colorectal carcinoma), MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human mammary gland adenocarcinoma), A549 (human alveolar adenocarcinoma), U-87 MG (human glioblastoma), cell lines were maintained in a growth medium consisting of high glucose Dulbecco's Modified Eagle Medium with sodium pyruvate (Invitrogen, Carlsbad, CA, USA). The growth media were supplemented with a 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 µg/mL) (all from Invitrogen). Cells were cultured in an atmosphere containing 5% CO_2 in humidified air at 37 °C. Cell viability, estimated by trypan exclusion, was greater than 95% before each experiment.

3T3 (murine fibroblasts) cell line

Cells were maintained in a growth medium consisting of high glucose Dulbecco's Modified Eagle Medium with sodium pyruvate (GE Healthcare, Piscataway, NJ, USA). The growth medium was supplemented with a 10% fetal bovine serum (FBS), penicillin (100 IU/mL) and streptomycin (100 µg/mL) (all Invitrogen, Carlsbad, CA, USA) in an atmosphere containing 5% CO_2 in humidified air at 37 °C. Cell viability, estimated by trypan exclusion, was greater than 95% before each experiment.

Cytotoxicity assay

The cytotoxic effects of compounds were studied using the colorimetric microculture assay with the MTT endpoint.[25] Briefly, cells were seeded at a density of 5×10^3 cells/well in 96-well polystyrene microplates (SARSTEDT, Nümbrecht, Germany). 24 hours after cell seeding, tested compounds at final concentrations of 10^{-6}-10^{-4} mol × L^{-1} were added. After 72 h, cells in each well were incubated with 10 µL of MTT (5mg/ml, Sigma-Aldrich Chemie, Steinheim, Germany) at 37 ° C. After an additional 4 h, during which insoluble formazan was produced, 100 µl of a 10% sodium dodecyl sulphate (SDS) was added in each well and another 12 h were allowed for the formazan to dissolve. The absorbance was measured at 540 nm using the automated Cytation™ 3 Cell Imaging Multi-Mode Reader (Biotek, Winooski, VT, United States). Three independent experiments were performed for each test. IC_{50} (50% inhibitory concentration) values were determined by 4 parameter logistic non-linear regression model using normalized concentration-response data obtained by MTT (Thiazolyl Blue Tetrazolium Bromide) assay.

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Highlights

- The first synthesis of natural alkaloid capparine A was accomplished.
- Developed strategy relied on bromine-mediated spirocyclization and enantioresolution.
- The antiproliferative activity against ten human cancer cell lines of the racemic (±)- and natural (−)-capparine A was also evaluated.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: