A LINEAR SYNTHESIS OF 1-(β-D-GLUCOPYRANOSYL)BRASSININ, -BRASSENIN A, -BRASSENIN B AND 9-(β-D-GLUCOPYRANOSYL)-CYCLOBRASSININ

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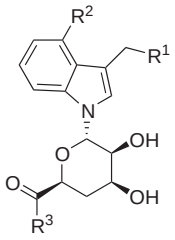
Received October 15, 2003
Accepted November 10, 2003

The first synthesis of 1-(β-D-glucopyranosyl)brassinin, 1-(β-D-glucopyranosyl)brassenin A, 1-(β-D-glucopyranosyl)brassenin B and 9-(β-D-glucopyranosyl)cyclobrassinin, nucleoside analogs derived from indole phytoalexins, was achieved by linear approach, using the 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carbaldehyde as a starting compound. Antiproliferative and antimicrobial activity of synthesized compounds against murine leukaemia tumor cell line L1210 and selected bacteria and fungi was examined and compared with the corresponding phytoalexin aglycons.

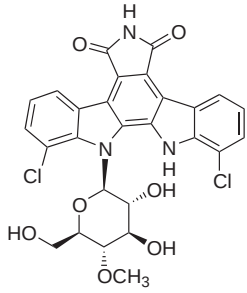
Keywords: Indoles; Phytoalexins; Glucosides; Glycosides; Nucleosides; Nucleoside analogs; Alkaloids; Antiproliferative activity; Antimicrobial activity.

Indole nucleoside antibiotics are a rare type of natural products. The first compound of this group, neosidomycin (**1**), possessing a weak activity against Gram-negative bacteria was isolated from the fermentation broth of a *Streptomyces hygroscopicus*[1a] strain and its structure was finally confirmed by synthesis[1b].

Another indole nucleoside termed SF-2140 (**2**) with antiviral properties was obtained from *Actinomadura* species from a Japanese soil sample[1c] and later synthesized[1b]. Recently, kahakamide A (**3**) and B (**4**) possessing the same 4-deoxy-α-D-*lyxo*-hexonopyranoside moiety, were isolated from the *Norcadiopsis dassonwillei* actinomycete from a shallow water sediment collected on the Kauai island, Hawaii[1d]. The antitumor nucleoside antibiotic rebeccamycin (**5**) isolated from actinomycete *Nocardia aerocoligenes*[1e] con-



1, $R^1 = CONH_2$, $R^2 = H$, $R^3 = OCH_3$
2, $R^1 = CN$, $R^2 = R^3 = OCH_3$
3, $R^1 = CONH_2$, $R^2 = R^3 = OCH_3$
4, $R^1 = CONH_2$, $R^2 = OCH_3$, $R^3 = NH_2$



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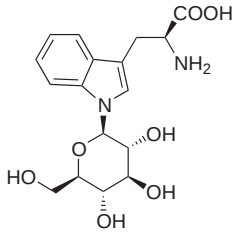

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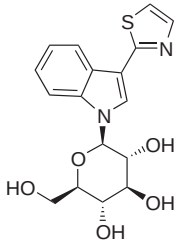
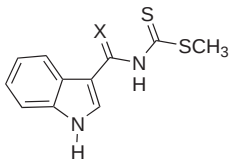
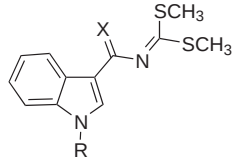


7

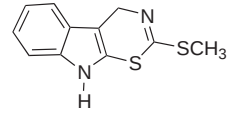
tains fused indolocarbazole moiety, linked with a β-glycosidic bond to 4-*O*-methyl-D-glucose residue. Rebeccamycin and its analogs, acting as DNA topoisomerase I inhibitors have been identified as attractive cancer chemotherapy agents[1f]. Interestingly, the N^1-(β-D-glucopyranosyl)-L-tryptophan (**6**) has not been isolated from microorganisms. It was recently found in pear juice and was also detected in other fruits and juices[1g]. Compound **6**, belonging to a new group of tryptophan glycoconjugates found in proteins[1h] is biosynthesized by enzymatic glycosylation of L-tryptophan[1g]. The role of nucleoside **6** is not clear; however, it should be noted that tryptophan serves as a precursor for the plant hormone indole-3-acetic acid, phytoalexins, glucosinolates and alkaloids. Thus tryptophan plays an important role in the regulation of plant development, in pathogen defence response, and plant-insect interactions[1i]. Very recently 1-(β-D-glucopyranosyl)camalexin (**7**), the first naturally occurring nucleoside analog derived from indole phytoalexin, has been isolated as a detoxification product of indole phytoalexin camalexin, produced by fungus *Sclerotinia sclerotiorum*[1j].



8, X = H, H
9, X = O



10, R = H, X = H, H
11, R = OCH_3, X = H, H
12, R = H, X = O
13, R = OCH_3, X = O

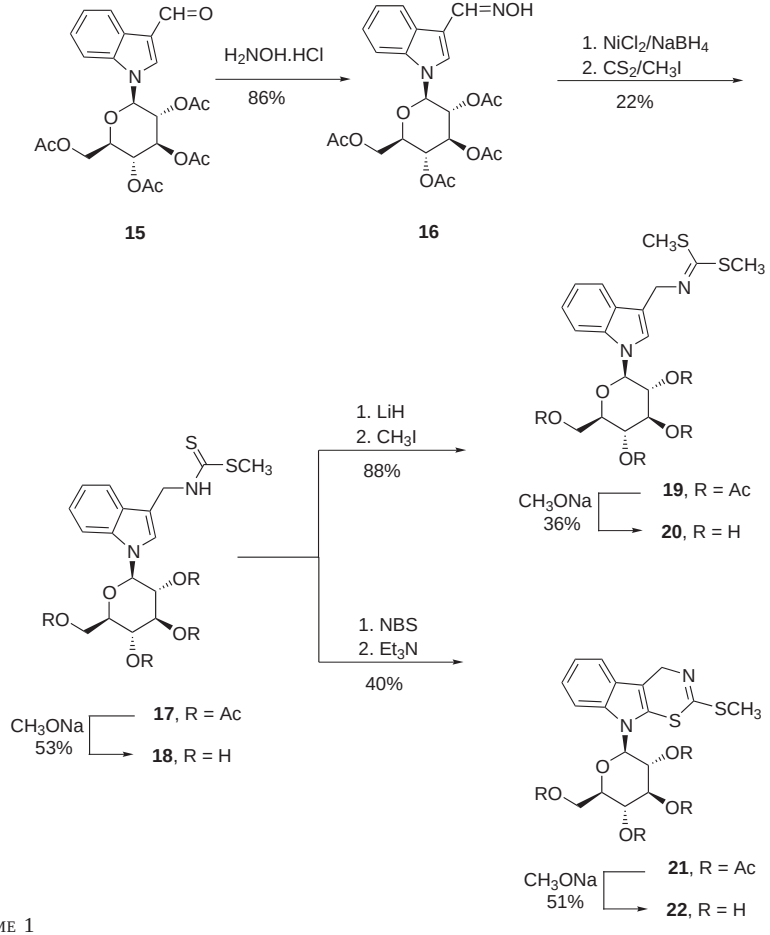


14

Within our continuing investigation of the synthesis of tryptophan-derived cruciferous phytoalexins[2a–2e] and their analogs[2f,2g], we have also focused our attention on the synthesis and biological activity of *N*-glucosides derived from indole phytoalexins brassinin (**8**)[3a], methoxybrassenin A (**11**)[3b], methoxybrassenin B (**13**)[3b] and cyclobrassinin (**14**)[3a]. Previously brassinin (**8**) and cyclobrassinin (**14**) were found to exhibit cancer chemopreventive activity against 7,12-dimethylbenz[*a*]anthracene (DMBA) induced mammary gland lesions[4a] and skin carcinogenesis[4b] in mice, and antiproliferative activity against murine leukaemia L1210 tumor cell line[4c]. Our recent syntheses of brassinin (**8**)[2a], cyclobrassinin (**14**)[2a] as well as demethoxybrassenin A (**10**)[2a] and demethoxybrassenin B (**12**)[2f] have created a starting position for the present investigation of convergent and linear synthesis of *N*-glucosides of phytoalexins **8**, **11**, **13** and **14**. With brassinin (**8**) possessing two reactive sites, at indole nitrogen and thiocarbamoyl grouping, the convergent synthesis has not been studied; however, with **10**, **12** (demethoxy analogs of **11** and **13**) and **14**, a convergent approach was attractive, since only the indole nitrogen is available for a reaction with electrophilic glycosylating reagents. Although, the nucleophilicity of indole nitrogen is in general low and standard methods, used in nucleoside chemistry fail to afford indole nucleosides[5], several successful glucosylations of indoles are described. 1-(β-D-Glucopyranosyl)indole derivatives were obtained by glycosylation of 7-chloroindole-3-acetamide with 1,2-anhydro-2,3,6-tri-*O*-benzyl-4-*O*-methyl-α-glucopyranose[6a], of 5-methoxy-2,3-diphenylindole with 2,3,4,6-tetra-*O*-acetyl-1-*O*-(trichloroacetimidoyl)-α-D-glucopyranose[6b], of indolocarbazoles with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide or 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride[6c,6d] and derivatives of 4-bromo-3-(1*H*-indol-3-yl)-1-methyl-2,5-dihydro-1*H*-pyrrole-2,5-dione via Mitsunobu reaction (PPh_3, diethyl azodicarboxylate (DEAD))[6c,6e]. In our case, however, these methods or their modifications failed to afford the desired glucosides from compounds **10**, **12**

and **14**, probably because of the low reactivity of starting indoles, and either no reaction or decomposition was observed. In the case of successful glucosylation with benzylated glucosyl donors, another problem would be expected: the sulfur-containing phytoalexin moieties are unstable under reductive conditions required for the removal of benzyl protecting groups. Therefore in further investigation of the synthesis of the target compounds, we focused our attention on the linear synthesis, using the acetyl protecting groups, easily removable under mild conditions by treatment with a catalytic amount of sodium methoxide in methanol[7].

For this purpose 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carbaldehyde (**15**, Scheme 1), readily available by the indoline–indole method[5], was selected as a starting compound.

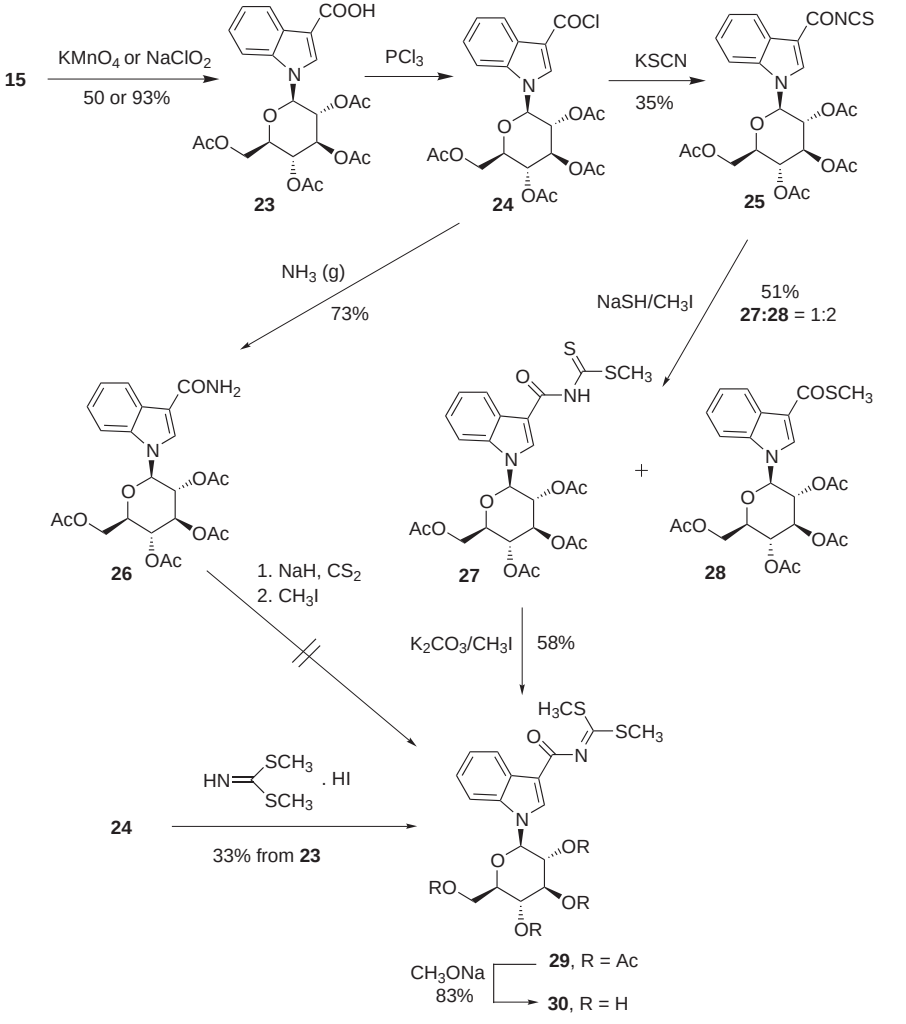


SCHEME 1

Aldehyde **15** is formed in a high yield by glycosylation of indoline with 1,2,3,4,6-penta-*O*-acetyl-α-(or β)-D-glucopyranose, subsequent oxidation of glucosylindoline with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[8a–8c] and Vilsmeier formylation[8d]. Reaction of aldehyde **15** with hydroxylamine afforded the corresponding oxime **16** as a mixture of *Z* and *E* isomers. Its reduction by a method elaborated for 1-methylindole-3-carbaldehyde oxime[2a] resulted in the formation of a highly unstable amine, which was in dichloromethane immediately transformed to 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)brassinin (**17**; Scheme 1) in 22% yield. Next, we have studied the methylation of protected glucosylbrassinin **17** with methyl iodide according to the method used for methylation of brassinin (**8**) to demethoxybrassenin A (**10**) in 40% yield by treatment with methyl iodide and lithium hydride as a base in dimethylformamide at room temperature[2a]. Compound **10** was previously also prepared by the methylation of brassinin with methyl iodide in the presence of sodium hydride (solvent and experimental details not given)[9]. However, we have found that in the presence of sodium hydride at room temperature or 0 °C, the brassinin had decomposed to 3-[(methylsulfanyl)methyl]indole to a significant extent. This type of decomposition of brassinin in a strongly basic medium was already observed in our laboratory[2a]. With the aim to improve the yield, we have first optimized the methylation of brassinin (**8**). It has been found that the yield can be raised up to 76% by using lithium hydride and methyl iodide in dry acetonitrile and performing the reaction at 0 °C for 25 min and then at room temperature for 7 h. Applying these optimized conditions, glucosylbrassinin (**17**) afforded 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)brassenin A (**19**) in 88% yield (Scheme 1). The protected glucosylbrassinin (**17**) appeared to be a suitable starting compound for the preparation of 9-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)cyclobrassinin (**21**) in 40% yield by cyclization with *N*-bromosuccinimide, the reagent previously used for cyclization of brassinin to cyclobrassinin, in 35% yield[4b]. Removal of the acetyl groups from protected glucosides **17**, **19** and **21** with sodium methoxide in dry methanol[7a,7b] and subsequent neutralization with an ion exchange resin[7c–7e] (IR-120 H^+) resulted in the preparation of 1-(β-D-glucopyranosyl)brassinin (**18**), 1-(β-D-glucopyranosyl)brassenin A (**20**) and 9-(β-D-glucopyranosyl)cyclobrassinin (**22**), the first synthesized glucosides of indole phytoalexins in 36–53% yields.

The synthesis of 1-(β-D-glucopyranosyl)brassenin B (**30**; Scheme 2) was started by oxidation of aldehyde **15** to carboxylic acid **23**. After some attempts to oxidize aldehyde **15** with potassium permanganate in alkaline medium, according to the procedure for indole-3-carboxylic acid[10a], afford-

ing only 12% yield, we have found that the acid **23** can be advantageously prepared by the oxidation with potassium permanganate (50% yield) or sodium chlorite (93% yield) under neutral conditions, using modifications of the procedures previously applied to preparation of 2-chloro-1-methylindole-3-carboxylic acid[10b] and 1-methoxyindole-3-carboxylic acid[10c]. Further treatment of acid **23** with phosphorus trichloride afforded an unstable acid chloride **24**, which was successfully used in the subsequent reactions as a crude product.



SCHEME 2

In a previous paper[2f] we have described the reaction of indol-3-ylcarbonyl isothiocyanate with NaSH and methyl iodide in a mixture of dimethylformamide and methanol, resulting in the formation of oxobrassinin (**9**) in 31% yield (based on starting indole-3-carboxylic acid), which was methylated to demethoxybrassenin B (**12**) in 87% yield. Therefore, the same approach was examined for the synthesis of target compound **30**. Transformation of acid chloride **24** to isothiocyanate **25** proceeded smoothly and compound **25** appeared to be stable enough to be isolated by column chromatography and crystallization, albeit in low 35% yield because of decomposition during chromatography separation (red column). Therefore, the crude product obtained in a substantially higher yield was used in the next reaction. However, reaction of glucosyl isothiocyanate **25** with NaSH and methyl iodide afforded only 17% yield of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxobrasinin (**27**) and the corresponding thioester **28** was isolated as the main product in 34% yield. The formation of thioester **28** can be explained by instability of isothiocyanate **25** and its decomposition to corresponding ketene like intermediate, as it was described previously for analogous indole-3-carbonyl isothiocyanates[2e,2f]. Variation of the solvents (acetonitrile, *tert*-butyl alcohol, acetone, toluene and tetrahydrofuran) and temperatures from room temperature to –20 °C, using a methanolic solution of sodium hydrogen sulfide freshly prepared by bubbling of sulfane through a methanolic solution of sodium hydroxide, or commercially available $NaSH \cdot xH_2O$, resulted only in a moderate improvement. The best results, affording 17% of glucosylated oxobrassinin **27**, were obtained by using $NaSH \cdot xH_2O$ in THF at room temperature. Methylation of **27** lead to the protected 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)brassenin B (**29**) and its deprotection with sodium methoxide afforded 1-(β-D-glucopyranosyl)-brassenin B (**30**) in 83% yield.

Because of the low yield of intermediate **27**, other ways of utilization of chloride **24** for the synthesis of target compound **30** have been investigated. In its reaction with methyl aminomethanedithioate in dry tetrahydrofuran in the presence of 4-(dimethylamino)pyridine[11a], no expected oxobrassinin glucoside **27** was formed and the starting compound decomposed within several hours. Reaction of a benzene solution of **24** with gaseous ammonia smoothly afforded the corresponding carboxamide **26**. Its treatment with sodium hydride, carbon disulfide and methyl iodide, according to the method previously used for the preparation of the dimethyl *N*-acylcarbonimidodithioates derived from 3-phenylacrylamide[11b], furan-2-carboxamide[11c], 3-chloro-1-benzothiophene-2-carboxamide[11d], pyridine-2-carboxamide[11e] and benzothiazole-2-carboxamide[11e] did not afford the

desired **29**, and only decomposition products were formed. Finally we succeeded by the reaction of chloride **24** as the acylating reagent, with dimethyl carbonimidodithioate hydroiodide[11f] in pyridine, previously used in the case of pyridine-4-carbonyl chloride[11g]. Thus protected derivative **29** was obtained in 33% yield from acid **23**.

The structure of the prepared nucleoside analogs was confirmed by spectral methods. In the electron impact mass spectra of compounds **17**–**20**, **22**, **23**, **29**, **30**, it was not possible to detect their unstable molecular ions. Therefore MALDI-TOF spectra were measured, in which the presence of quasimolecular $[M + Na]^+$ ions confirmed the expected molecular weights. In ^1H and ^{13}C NMR spectra, the signals were assigned on the basis of 2D H-H COSY and HSQC heterocorrelated spectra. The β-anomeric configuration of all compounds was confirmed by vicinal *trans*-diaxial coupling constants $J(1',2') = 8.0–9.1$ Hz.

It was our final goal to obtain an information about antitumor and antimicrobial activities of the synthesized glucosides and compare them with brassinin (**8**), cyclobrassinin (**14**) and phytoalexin analogs **9**, **10** and **12**. Antiproliferative activity at concentration 10^{-5} mol l^{-1} was examined using the murine leukaemia tumor cell line L1210 under the conditions described previously[4c]. Antimicrobial activity was tested by the standard disc diffusion method against selected bacteria (*Pseudomonas aeruginosa* ULM 162/78, *Enterococcus faecalis* CCM 1875, *Escherichia coli* CCM 3954 and *Staphylococcus aureus* CCM 4223) and fungi (*Candida albicans* ATCC 60193, *Candida crusei* CCM 29748 and *Candida tropicalis* CCM 29927). None of the synthesized compounds exhibited considerable antitumor or antimicrobial activity.

In conclusion, a new linear synthesis of nucleoside analogs derived from indole phytoalexins has been elaborated, using hitherto unknown glucosylated (indol-3-ylmethyl)amine and indole-3-carboxylic acid as a key intermediates. It was found that convergent synthesis using the known glucosylating methods fails to afford target nucleosides because of a low reactivity of the indole nitrogen to used glycosylating agents. Biological screening of antiproliferative and antimicrobial activity revealed no significant activity of the prepared compounds.

EXPERIMENTAL

The infrared absorption spectra were recorded on an IR-75 spectrometer (Zeiss Jena); the wavenumbers (ν) are given in cm^{-1}. ^1H and ^{13}C NMR spectra were measured on a Bruker Avance 300 DPX FT NMR spectrometer, using tetramethylsilane as an internal standard. Chemical shifts (δ) are reported in ppm, downfield from tetramethylsilane, coupling con-

stants (*J*) in Hz. The assignment of proton and carbon atom signals is based on H-H COSY, DEPT and HSQC spectra of compounds **16**, **18**–**21**, **25**, **26**, **28**–**30**. Microanalyses were performed with a Perkin–Elmer, Model 2400 analyzer. The EI mass spectra were recorded on a Finigan SSQ 700 spectrometer at an ionization energy of 70 eV, whereas MALDI-TOF mass spectra were measured on a Compact Kratos Maldi III spectrometer. The analyzed sample was dissolved in acetonitrile and water mixture (1:1). Matrix 2,4-dihydroxybenzoic acid (DHB) was dissolved in the same mixture. The solutions of sample and matrix were mixed in the 1:10 ratio. After drying on target, the samples were bombarded with the 3 ns dose (100 doses) of N_2 laser (λ = 337 nm). Optical rotations were determined on a digital polarimeters POLAR L-mP (IBZ Messtechnik) and P3002 (Kruess); specific rotations are given in 10^{-1} deg cm^2 g^{-1}. The reaction course was monitored by thin layer chromatography, using Silufol plates (Kavalier®). The preparative column chromatography (flash chromatography) was performed on the Kieselgel Merck Type 9385, 230–400 mesh.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)indol-3-carbaldehyde Oxime (**16**)

To a stirred solution of aldehyde **15** [8d] (1.423 g, 3 mmol) in ethanol (165 ml) was added a solution of hydoxylamine hydrochloride (0.417 g, 6 mmol) and sodium carbonate (0.318 g, 3 mmol) in water (3 ml), and the mixture was stirred under reflux for 45 min. After addition of water (21 ml), ethanol was evaporated, the precipitated product filtered off with suction, washed with water and dried. Yield 1.260 g (80%), m.p. 208–210 °C (ethanol–water), $[\alpha]_D^{25}$ –52.5 (*c* 0.236, dichloromethane). For $C_{23}H_{26}N_2O_{10}$ (490.5) calculated: 56.32% C, 5.34% H, 5.71% N; found: 56.09% C, 5.44% H, 5.98% N. IR ($CHCl_3$): 3597 (O–H); 1753 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6): 11.65 s, 0.42 H and 10.84 s, 0.58 H (=CH); 8.45 s, 0.42 H and 8.35 s, 0.58 H (H-2); 8.09 d, 0.58 H, *J*(4,5) = 7.9 and 7.99 d, 0.42 H, *J*(4,5) = 7.9 (H-4); 7.90–7.78 m, 2 H (H-7, OH); 7.42–7.28 m, 2 H (H-5, H-6); 6.41 d, 0.42 H, *J*(1′,2′) = 8.0 and 6.35 d, 0.58 H, *J*(1′,2′) = 8.0 (H-1′); 5.68–5.66 m, 2 H (H-2′, H-3′); 5.39–5.33 m, 1 H (H-4′); 4.43–4.40 m, 1 H (H-5′); 4.22–4.20 m, 2 H (H-6′$_a$, H-6′$_b$); 2.15 s, 3 H; 2.09 s, 3 H; 2.07 s, 3 H and 1.73 s, 3 H (CH_3CO). EI MS, *m/z* (%): 490 (M^+, 22), 330 (4), 169 (45), 160 (8), 127 (9), 109 (30), 43 (100).

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)brassinin (**17**)

To a solution of nickel(II) chloride hexahydrate (0.615 g, 2.58 mmol) in methanol (30 ml) was added sodium tetrahydridoborate (0.195 g, 5.15 mmol) within 5 min. To the formed black suspension of nickel boride (Ni_2B), a solution of oxime **16** (1.050 g, 2.19 mmol) in methanol (350 ml) prepared by heating and then cooled to 30–35 °C was added. Then sodium tetrahydridoborate (0.750 g, 19.8 mmol) was added in two portions during 1 min, and after the gas evolution ceased (5 min), the reaction mixture was filtered, concentrated to approximately 50 ml until turbidity appeared and poured into a mixture of water (185 ml) and 25% ammonium hydroxide (6 ml). The product was extracted with ethyl acetate (1 × 400 and 1 × 200 ml), the extract dried with anhydrous sodium sulfate and solvent evaporated. The obtained unstable {[1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)indol-3-yl]methyl}amine was immediately dissolved in dichloromethane (25 ml). Triethylamine (0.305 ml, 0.221 g, 2.19 mmol) and carbon disulfide (0.39 ml, 0.494 g, 6.48 mmol) were added with stirring at room temperature, followed after 5 min by methyl iodide (0.27 ml, 0.617 g, 4.34 mmol) and stirring was continued for 35 min. The reaction mixture was diluted with 10 ml of dichloromethane, a small amount of silica gel (particle size 100–250 μm) was added, solvent evapo-

rated and the product preabsorbed on silica gel was chromatographed on silica gel (120 g, eluent benzene–acetone 7:1). Yield 263 mg (22%), m.p. 165–167 °C (chloroform–light petroleum), $[\alpha]_D^{25}$ –15.5 (*c* 0.21, dichloromethane). For $C_{25}H_{30}N_2O_9S_2$ (566.7) calculated: 52.99% C, 5.30% H, 4.94% N; found: 53.12% C, 5.41% H, 4.78% N. IR ($CHCl_3$): 3387 (N–H); 1753 (C=O); 1457 (NHCS); 1217 (C–O). ^{1}H NMR (300 MHz, $CDCl_3$): 7.60 d, 1 H, *J*(4,5) = 7.8 (H-4); 7.42 d, 1 H, *J*(6,7) = 8.3 (H-7); 7.32–7.26 m, 2 H and 7.20 t, 1 H, *J* = 7.3 (H-2, H-4, H-5); 7.07 s, 1 H (NH); 5.62 d, 1 H, *J*(1′,2′) = 8.67 (H-1′); 5.51–5.41 m, 2 H (H-2′, H-3′); 5.30–5.24 m, 1 H (H-4′); 5.03 d, 1.6 H, *J* = 4.4 and 4.75 d, 0.4 H, *J* = 4.4 (CH_2N); 4.30 dd, 1 H, *J*(6′$_b$,6′$_a$) = 12.5, *J*(6′$_b$,5′) = 4.9 (H-6′$_b$); 4.14 dd, 1 H, *J*(6′$_a$,6′$_b$) = 12.5, *J*(6′$_a$,5′) = 2.5 (H-6′$_a$); 4.20–3.98 m, 1 H (H-5′); 2.75 s, 0.6 H and 2.66 s, 2.4 H (SCH_3); 1.65 s, 3 H; 2.03 s, 3 H and 2.06 s, 6 H (4 × CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 198.30 (C=S); 170.53, 170.09, 169.35 and 168.72 ($CH_3\mathbf{C}O$); 136.59 (q), 127.59, 124.11 (q), 123.20, 121.21, 119.38, 112.71 (q) and 109.84 (C-arom.); 83.01 (C-1′); 74.70, 73.09, 70.55 and 68.01 (C-2′–C-5′); 61.76 (C-6′); 42.79 (CH_2N); 20.66, 20.53 and 20.05 ($\mathbf{C}H_3CO$); 18.10 (SCH_3). MALDI-TOF MS, *m/z* (%): 605.7 $[M + K]^+$ (63), 589.1 $[M + Na]^+$ (100), 558.3 (7), 543.4 (14), 505.8 (8), 160.2 (16), 331.0 (17).

1-(β-D-Glucopyranosyl)brassinin (**18**)

To a stirred suspension of **17** (0.057 g, 0.1 mmol) in dry methanol (1 ml) was added 0.1 M methanolic solution of sodium methoxide (0.1 ml, 0.01 mmol) and the reaction mixture was stirred at room temperature for 100 min. After addition of Amberlite IR-120 H^+ (0.040 g) to a clear solution and 5 min stirring, the reaction mixture was filtered, solvent evaporated and the obtained residue chromatographed on silica gel (10 g, eluent dichloromethane–methanol 8:1), affording the product as a white crystalline powder. Yield 0.021 g (53%), m.p. 105–109 °C, $[\alpha]_D^{25}$ –5.5 (*c* 0.16, methanol). For $C_{17}H_{22}N_2O_5S_2$ (398.5) calculated: 51.24% C, 5.56% H, 7.03% N; found: 51.36% C, 5.40% H, 6.87% N. IR (KBr): 3400 (br, O–H); 1453 (NHCS). ^{1}H NMR (300 MHz, $(CD_3)_2CO$): 9.08 br s, 1 H (D_2O exchangeable, NH); 7.65 d, 1 H, *J*(4,5) = 7.7 (H-4); 7.55 d, 1 H, *J*(6,7) = 8.2 (H-7); 7.51 s, 1 H (H-2); 7.17 t, 1 H, *J* = 7.2 and 7.07 t, 1 H, *J* = 7.5 and 7.0 (H-5, H-6); 5.55 d, 1 H, *J* = 9.0 (H-1′); 5.11–5.08 m, 2 H (CH_2N); 4.48 d, 1 H, *J* = 4.0 (OH-3′); 4.41 d, 1 H, *J* = 4.8 (OH-2′); 4.35 d, 1 H, *J* = 4.0 (OH-4′); 3.96–3.91 m, 1 H (H-2′); 3.87–3.84 m, 1 H (H-6′$_b$); 3.72–3.56 m, 5 H (H-3′, H-4′, H-5′, H-6′$_a$, OH-6′); 2.61 s, 3 H (SCH_3). ^{13}C NMR (75 MHz, $(CD_3)_2CO$): 198.55 (C=S); 137.82 (q), 128.77 (q), 126.31, 122.73, 120.69, 119.87, 112.01 (q) and 111.67 (C-arom.); 86.25 (C-1′); 80.20, 79.04 and 71.49 (C-3′, C-4′, C-5′); 73.44 (C-2′); 62.81 (C-6′); 43.13 (CH_2N); 17.52 (SCH_3). MALDI-TOF MS, *m/z* (%): 420.9 $[M + Na]^+$ (52), 292.7 (38).

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)brassenin A (**19**)

To a stirred suspension of lithium hydride (0.027 g, 3.34 mmol), cooled with ice to 0 °C was added protected 1-(β-D-glucopyranosyl)brassinin (**17**; 0.2 g, 0.35 mmol), followed after 10 min by methyl iodide (0.26 ml, 0.593 g, 4.2 mmol). After stirring at 0 °C for 25 min and at room temperature for 7 h, the mixture was poured into cold water (100 ml), the product was extracted with ethyl acetate (2 × 50 ml) and the extract dried over anhydrous sodium sulfate. Evaporation of solvent afforded pure product as a pale yellow crystals. Yield 0.18 g (88%), m.p. 93–95 °C, $[\alpha]_D^{25}$ –19.6 (*c* 0.20, dichloromethane). For $C_{26}H_{32}N_2O_9S_2$ (580.7) calculated: 53.78% C, 5.55% H, 4.85% N; found: 53.50% C, 5.81% H, 4.68% N. IR ($CHCl_3$): 1753 (C=O);

1213 (C–O). ^{1}H NMR (300 MHz, $CDCl_3$): 7.73–7.70 m, 1 H, 7.41 d, 1 H, *J* = 8.2 and 7.28–7.18 m, 3 H (H-arom.); 5.64 d, 1 H, *J* = 9.3 (H-1′); 5.62–5.58 m, 1 H (H-2′); 5.45 t, 1 H, *J* = 9.3 and 8.7 (H-3′); 5.30 t, 1 H, *J* = 9.8 and 9.5 (H-4′); 4.28 s, 2 H (CH_2N); 4.30 dd, 1 H, $J(6'_b,6'_a)$ = 12.3, $J(6'_b,5')$ = 4.8 (H-6′$_b$); 4.16 d, 1 H, $J(6'_a,6'_b)$ = 12.4 (H-6′a); 4.02–3.98 m (H-5′); 2.63 s, 3 H and 2.54 s, 3 H (2 × SCH_3); 2.09 s, 3 H; 2.08 s, 3 H; 2.05 s, 3 H and 1.76 s, 3 H (CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 170.65, 170.20, 169.47 and 168.87 (CH_3CO); 156.60 (C=N); 136.77 (q), 128.22 (q), 124.38, 122.64, 120.61, 120.08, 113.14 (q) and 109.71 (C-arom.); 83.20 (C-1′); 74.50 (C-5′); 73.62 (C-3′); 70.08 (C-2′); 68.23 (C-4′); 61.98 (C-6′); 48.11 (CH_2N); 20.74, 20.63 and 20.27 (CH_3CO); 15.00 (SCH_3). MALDI-TOF MS, *m/z* (%): 619 $[M + K]^+$ (52), 603 $[M + Na]^+$ (100). EI MS, *m/z* (%): 581 (M^+, 5), 460 (12), 169 (14), 109 (12), 43 (100).

1-(β-D-Glucopyranosyl)brassenin A (**20**)

To a stirred solution of tetraacetyl derivative **19** (0.174 g, 0.3 mmol) in dry methanol was added 0.1 M methanolic solution of sodium methoxide (0.3 ml, 0.03 mmol) and the mixture was stirred at room temperature for 20 min. After addition of Amberlite IR-120 H^+ (0.210 g) and 5 min stirring, the resin was filtered off, washed with methanol, the filtrate was evaporated and the obtained residue was chromatographed on silica gel (19 g, eluent dichloromethane–methanol 8:1). The eluate was evaporated to approximaltely 1 ml, diethyl ether added until turbidity appeared and set aside at 3 °C overnight. Separated yellow crystals were filtered, washed with diethyl ether and dried. Yield 0.045 g (36%), m.p. 88–90 °C, $[\alpha]_D^{25}$ –8.7 (*c* 0.17, methanol). For $C_{18}H_{24}N_2O_5S_2$ (412.5) calculated: 52.41% C, 5.86% H, 6.79% N; found: 52.11% C, 5.51% H, 7.07% N. IR (KBr): 3420 (br, O–H); 1610 (C=N); 1066 (C–O). ^{1}H NMR (300 MHz, $(CD_3)_2CO$): 7.62 d, 1 H, *J*(6,7) = 7.9 (H-7); 7.53 d, 1 H, *J*(4,5) = 8.1 (H-4); 7.40 s, 1 H (H-2); 7.11 t, 1 H, *J* = 8.2 and 7.1 and t, 1 H, *J* = 7.7 and 7.1 (H-5, H-6); 5.51 d, 1 H, *J*(1′,2′) = 9.0 (H-1′); 4.76 s, 2 H (CH_2N); 4.50 br s, 2 H (OH-2′, OH-4′); 4.37 br s, 1 H (OH-3′); 3.97 t, 1 H, *J* = 8.7 and 8.8 (H-2′); 3.81 dd, 1 H, $J(6'_b,6'_a)$ = 11.6, $J(6'_b,5')$ = 1.8 (H-6′$_b$); 3.71–3.55 m, 5 H (H-3′, H-4′, H-5′, H-6′$_a$, OH-6′); 2.62 s, 3 H and 2.38 s, 3 H (2 × SCH_3). ^{13}C NMR (75 MHz, $(CD_3)_2CO$): 151.83 (C=N); 138.19 (q), 129.17 (q), 124.48, 122.51, 120.33, 120.16, 115.42 (q) and 111.59 (C-arom.); 86.26 (C-1′); 80.18 (C-3′); 79.70 (C-5′); 73.33 (C-2′); 71.48 (C-4′); 62.83 (C-6′); 48.48 (CH_2N); 14.80 and 14.65 (SCH_3). MALDI-TOF MS, *m/z* (%): 413 $[M + K]^+$ (100).

9-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)cyclobrassinin (**21**)

To a solution of 1-(tetraacetyl-β-D-glucopyranosyl)brassinin (**17**; 0.057 g, 0.1 mmol) in dichloromethane (1.2 ml) stirred at 30 °C was added a solution of *N*-bromosuccinimide (0.018 g, 0.1 mmol) in dichloromethane (1.2 ml). After stirring for 5 min, triethylamine (0.028 ml, 0.024 g, 0.2 mmol) was added, solvent evaporated and the residue chromatographed on silica gel (10 g, eluent cyclohexane–acetone 4:1). During evaporation the product separated as colorless crystals. Yield 0.022 g (40%), m.p. 140–142 °C, $[\alpha]_D^{25}$ –29.5 (*c* 0.19, dichloromethane). For $C_{25}H_{28}N_2O_9S_2$ (564.6) calculated: 53.18% C, 5.00% H, 4.96% N; found: 52.93% C, 4.76% H, 6.96% N. IR ($CHCl_3$): 1757 (C=O); 1220 (C–O). ^{1}H NMR (300 MHz, $CDCl_3$): 7.46–7.44 m, 2 H (H-5, H-8); 7.26–7.15 m, 2 H (H-6, H-7); 5.63–5.60 m, 1 H (H-2′); 5.59 d, 1 H, *J* = 9.0 (H-1′); 5.42–5.33 m, 2 H (H-3′, H-4′); 5.19 d, 1 H and 4.83 d, 1 H, *J* = 18.0 (CH_2N); 4.29–4.26 m, 2 H (H-6′a, H-6′$_b$); 3.98–3.95 m, 1 H (H-5′);

2.58 s, 3 H (SCH_3); 2.16 s, 3 H; 2.11 s, 3 H; 2.04 s, 3 H and 1.58 s, 3 H (CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 170.54, 170.01, 169.36 and 168.22 (CH_3CO); 153.02 (C=N); 136.98 (q), 126.22 (q), 123.93 (q), 122.29, 121.01, 117.61, 110.09 (q) and 106.06 (C-arom.); 83.50 (C-1′); 74.95 (C-5′); 72.87 (C-3′); 69.99 (C-2′); 67.81 (C-4′); 61.59 (C-6′); 48.55 (CH_2N); 20.38, 20.64 and 19.89 (CH_3CO); 15.53 (SCH_3). EI MS, *m/z* (%): 564 (M^+, 14), 272 (12), 230 (12), 201 (100), 169 (46), 109 (20), 43 (82).

9-(β-D-Glucopyranosyl)cyclobrassinin (**22**)

To a stirred solution of tetraacetyl derivative **21** (0.110 g, 0.2 mmol) in dry methanol (2 ml) was added 0.1 M methanolic solution of sodium methoxide (0.4 ml, 0.04 mmol) and stirring was continued at room temperature for 25 min. After neutralization with Amberlite IR-75 H^+ (0.100 g) and stirring for 5 min, the resin was filtered off, washed with methanol, the filtrate was evaporated and the obtained residue chromatographed on silica gel (13 g, eluent dichloromethane–methanol, 8:1). Yield 0.039 g (55%), m.p. 218–220 °C (acetone–hexane), $[\alpha]_D^{25}$ 84.9 (*c* 0.17, methanol). For $C_{17}H_{20}N_2O_5S_2$ (396.5) calculated: 51.50% C, 5.08% H, 7.07% N; found: 51.78% C, 5.35% H, 6.90% N. IR ($CHCl_3$): 3400 (br, O–H); 1617 (C=N); 1017 (C–O). ^1H NMR (300 MHz, $(CD_3)_2CO$): 7.57 d, 1 H, *J* = 7.3 and 7.51 d, 1 H, *J* = 8.5 (H-5, H-8); 7.16–7.07 m, 2 H (H-6, H-7); 5.50 d, 1 H, *J* = 9.0 (H-1′); 5.31 d, 1 H and 4.77 d, 1 H, *J* = 17.7 (CH_2N); 4.60 d, 1 H, *J* = 4.9, 4.44 d, 1 H, *J* = 4.4 and 4.37 d, 1 H, *J* = 3.2 (D_2O exchangeable, 3 × OH); 4.07–4.03 m, 2 H (H-2, OH); 3.92 d, 1 H, $J(6'_b,6'_a)$ = 10.7 (H-6′a); 3.78 dd, 1 H, $J(6'_a,6'_b)$ = 10.4, $J(6'_b,5')$ = 4.8 (H-6′b); 3.69–3.61 m, 3 H (H-3′, H-4′, H-5′). ^{13}C NMR (75 MHz, $(CD_3)_2CO$): 150.43 (C=N); 138.51 (q), 125.16 (q), 123.39 (q), 122.41, 120.97, 117.87, 112.35 (q) and 108.50 (C-arom.); 87.27 (C-1′); 80.89, 78.61 and 71.39 (C-3′, C-4′, C-5′); 63.60 (C-2′); 61.59 (C-6′); 49.05 (CH_2N); 15.24 (SCH_3). MALDI-TOF MS, *m/z* (%): 395.3 $[M - H_2 + H]^+$ (100).

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carboxylic Acid (**23**)

*A. By oxidation of aldehyde **15** with potassium permanganate*: To a solution of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carbaldehyde (**15**; 1.900 g, 4 mmol) in acetone (80 ml) was added a solution of potassium permanganate (1.800 g, 11.4 mmol) in distilled water (36 ml). The reaction mixture was stirred at room temperature for 3.5 h, decolorized by addition of 3% solution of hydrogen peroxide (53 ml) and filtered. From the filtrate, the acetone was evaporated thoroughly, the resulting solution diluted with distilled water (30 ml), filtered and acidified with 2 M hydrochloric acid (2.7 ml). The separated precipitate of acid **23** was filtered off with suction and dried. Yield 0.98 g (50%), m.p. 224–226 °C (methanol).

*B. By oxidation of aldehyde **15** with sodium chlorite*: A solution of sodium chlorite (1.809 g, 20 mmol) and sodium dihydrogenphosphate dihydrate (2.940 g, 15 mmol) in water (10 ml) was added at 0 °C to a suspension of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carbaldehyde (**15**; 0,475 g, 1 mmol) in a mixture of *tert*-butyl alcohol (10 ml) and 2-methylbut-2-ene (10 ml). Dioxane (12 ml) was added to dissolve starting aldehyde **15** and the mixture was stirred at room temperature for 2 h. After dilution with water (30 ml), the product was extracted with chloroform (2 × 20 ml), the extract was washed with brine (30 ml) and water (50 ml), dried over anhydrous sodium sulfate, and solvent was evaporated under reduced pressure. Yield 0.460 g (93%), m.p. 234–237°C (ethyl acetate–hexane), $[\alpha]_D^{25}$ –34.4

(*c* 0.20, dichloromethane). For $C_{23}H_{25}NO_{11}$ (491.5) calculated: 56.21% C, 5.13% H, 2.85% N; found: 56.37% C, 4.90% H, 2.61% N. IR ($CHCl_3$): 3650 (O–H); 1750 and 1666 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 8.27–8.24 m, 1 H, 8.09 s, 1 H, 7.50–7.48 m, 1 H and 7.35–7.28 m, 2 H (H-arom.); 5.68 d, 1 H, *J* = 8.6 (H-1′); 5.56–5.51 m, 2 H (H-2′, H-3′); 5.37–5.34 m, 1 H (H-4′); 4.34 dd, 1 H, $J(6'_b,6'_a)$ = 12.6, $J(6'_b,5')$ = 4.8 (H-6′$_b$); 4.21 d, 1 H, $J(6'_b,6'_a)$ = 12.3 (H-6′$_a$); 4. 05–4.02 m, 1 H (H-5′); 2.12 s, 3 H; 2.11 s, 3 H; 2.05 s, 3 H and 1.71 s, 3 H (4 × CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 170.65, 170.21, 169.60 169.40 and 168.57 (CH_3CO, C=O); 136.32 (q), 132.54, 126.93 (q), 123.72, 123.09, 122.27, 110.42 and 109.19 (q) (C-arom.); 83.76 (C-1′); 75.12 (C-5′); 73.02 (C-3′); 70.77 (C-2′); 67.94 (C-4′); 61.77 (C-6′); 20.75, 20.61 and 20.06 (CH_3CO). MALDI-TOF MS, *m/z* (%): 530.6 $[M + K]^+$ (36), 514.7 $[M + Na]^+$ (76), 492 $[M + H]^+$ (30), 474.1 (100).

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carbonyl Chloride (**24**)

To a suspension of carboxylic acid **23** (0.492 g, 1 mmol) in a mixture of dry benzene (10 ml) and dry acetonitrile (1.6 ml) was added phosphorus trichloride (0.270 g, 0.175 ml, 2 mmol) and the mixture was stirred at 50 °C (bath temperature) for 35 min. The resulting solution was decanted from phosphorous acid deposited on the flask walls, the flask was washed with dry benzene (12 ml) and the obtained solution concentrated to ca. 1/6 of its original volume (bath temperature up to 30 °C) to remove excess of phosphorus trichloride. The obtained solution of the unstable crude product was used in the next reactions.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carbonyl Isothiocyanate (**25**)

The solution of crude acid chloride **24** freshly prepared from 1 mmol of acid **23** was diluted with dry acetone (9 ml) and added in one portion to a solution of potassium thiocyanate (0.097 g, 1 mmol) in dry acetone (4 ml). The mixture was stirred at room temperature for 1 h, filtered and the flask washed with dry acetone (10 ml). After evaporation of acetone the crude isothiocyanate **25** is pure enough for the next reaction; however, it can be isolated in pure crystalline form as follows: to an acetone solution of isothiocyanate prepared from 0.25 mmol of acid **23** was added a small amount of silica gel (100/200 μm) and, after evaporation of acetone, the preadsorbed isothiocyanate was chromatographed on silica gel (40 g, eluent hexane–ethyl acetate 1:1). Yield 0.080 g (25% based on **23**), m.p. 175–178 °C (acetone–cyclohexane), $[\alpha]_D^{25}$ –33.0 (*c* 0.09, dichloromethane). For $C_{24}H_{24}N_2O_{10}S$ (532.5) calculated: 54.13% C, 4.54% H, 5.26% N; found: 54.42% C, 4.20% H, 4.93% N. IR ($CHCl_3$): 1966 (N=C=S); 1750 and 1706 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 8.24–8.20 m, 1 H (H-4); 8.03 s, 1 H (H-2); 7.49–7.46 m, 1 H (H-7); 7.49–7.27 m, 2 H (H-5, H-6); 5.67 d, 1 H, *J* = 8.9 (H-1′); 5.47 m, 2 H (H-2′, H-3′); 5.35–5.32 m, 1 H (H-4′); 4.34 dd, 1 H, $J(6'_b,6'_a)$ = 12.5, $J(6'_b,5')$ = 4.8 (H-6′$_b$); 4.22–4.18 m (H-6′$_a$); 4.05–4.00 m, 1 H (H-5′); 2.11 s, 3 H; 2.10 s, 3 H; 2.03 s, 3 H and 1.68 s, 3 H (4 × CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 170.58, 170.12, 169.37 and 168.56 (CH_3CO); 136.77 (q), 134.05, 128.41 (q), 124.69, 123.97, 122.32, 112.43 (q) and 110.57 (C-arom.); 126.32 (NCS); 83.64 (C-1′); 75.34 (C-5′); 72.79 and 70.97 (C-2′, C-3′); 67.86 (C-4′); 61.73 (C-6′); 20.76, 20.60 and 20.04 (CH_3CO). EI MS, *m/z* (%): 532 (M^+, 23), 474 $[M – NCS]^+$ (100), 472 (53), 331 (91), 271 (14), 229 (13), 211 (13), 186 (17).

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carboxamide (**26**)

The freshly prepared crude acid chloride **24** obtained from acid **23** (0.500 g, 0.102 mmol) after evaporation of the reaction mixture to dryness was dissolved in dry benzene (8 ml) and gaseous ammonia was bubbled through the solution until the formation of precipitate stopped. The dissolved portion of product was precipitated by adding hexane, the precipitate was filtered off, dried and thoroughly washed with water to remove ammonium chloride and then dried. Yield 0.366 g (73% based on **23**), m.p. 216–219 °C (ethanol), $[\alpha]_D^{25}$ –33.8 (*c* 0.45, chloroform). For $C_{23}H_{26}N_2O_{10}$ (490.5) calculated: 56.32% C, 5.34% H, 5.71% N; found: 56.19% C, 5.48% H, 5.66% N. IR ($CHCl_3$): 3417 (N–H); 1750 and 1653 (C=O). ^{1}H NMR (300 MHz, $(CD_3)_2SO$): 8.28–8.24 m, 2 H (H-2, H-4); 7.80 d, 1 H, *J* = 7.9 (H-7); 7.53 br s, 1 H (NH); 7.27–7.36 m, 2 H (H-5, H-6); 7.06 br s, 1 H (NH); 6.38 d, 1 H, *J* = 8.5 (H-1′); 5.69–5.65 m, 1 H (H-3′); 5.59–5.56 m, 1 H (H-2′); 5.34–5.26 m, 1 H (H-4′); 4.46–4.44 m, 1 H (H-5′); 4.26–4.19 m, 2 H (H-6′$_a$, H-6′$_b$); 2.15 s, 3 H; 2.10 s, 3 H; 2.07 s, 3 H and 1.73 s, 3 H (4 × CH_3CO). ^{13}C NMR (75 MHz, $(CD_3)_2SO$): 170.21, 169.66, 168.57, 167.27 and 165.87 (CH_3CO, C=O); 136.22 (q), 128.74, 126.86 (q), 122.84 (double intensity), 121.71, 112.25 (q) and 111.04 (C-arom.); 81.88 (C-1′); 73.38 (C-5′); 72.55 (C-3′); 70.45 (C-2′); 68.09 (C-4′); 62.13 (C-6′); 20.66, 20.57, 20.42 and 19.92 (CH_3CO). EI MS, *m/z* (%): 490 (M^+, 22), 331 (14), 169 (47), 144 (14), 109 (30), 43 (100).

Reaction of 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)indole-3-carbonyl Isothiocyanate (**25**) with Sodium Hydrogensulfide

To a solution of crude isothiocyanate **25**, prepared from 1 mmol of acid **23**, in dry tetrahydrofuran (12 ml) was added methyl iodide (426 mg, 0.118 ml, 3 mmol) and sodium hydrogensulfide hydrate ($NaSH \cdot xH_2O$, 0.222 g, ca. 2.5 mmol) and the mixture was stirred at room temperature for 3 h. To the resulting mixture was added a small amount of aluminium oxide and solvent evaporated. Chromatography of the preadsorbed product on aluminium oxide (Brockmann II, 105 g, eluent benzene–acetone 7:1) afforded compounds **27** and **28**.

*1-(2,3,4,6-Tetra-O-acetyl-*β*-D-glucopyranosyl)oxobrassinin* (**27**). Yield 0.099 g (17% based on **23**), m.p. 152–155 °C (acetone–cyclohexane), $[\alpha]_D^{25}$ –52.0 (*c* 0.19, dichloromethane). For $C_{25}H_{28}N_2O_{10}S_2$ (580.6) calculated: 51.71% C, 4.86% H, 4.82% N; found: 51.46% C, 4.57% H, 5.08% N. IR ($CHCl_3$): 3400 (N–H); 1753 and 1683 (C=O); 1453 (NHCS). ^{1}H NMR (300 MHz, $CDCl_3$): 10.05 s, 1 H (NH); 8.18–8.15 m, 1 H, 7.97 s, 1 H, 7.49–7.46 m, 1 H and 7.37–7.35 m, 2 H (H-arom.); 5.75 d, 1 H, *J* = 8.6 (H-1′); 5.54–5.42 m, 2 H (H-2′, H-3′); 5.34–5.28 m, 1 H (H-4′); 4.38–4.28 m, 2 H (H-6′$_a$, H-6′$_b$); 4.20–4.16 m, 1 H (H-5′); 2.72 s, 3 H (SCH_3); 2.12 s, 3 H; 2.11 s, 3 H; 2.05 s, 3 H and 1.63 s, 3 H (4 × CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 185.63 (C=S); 170.50, 170.21, 169.30 and 168.51 (CH_3CO, C=O); 136.14 (q), 129.80, 125.59 (q), 123.91, 123.25, 122.30, 118.13 (q) and 110.18 (C-arom.); 83.56 (C-1′); 75.02 (C-5′); 72.91 (C-3′); 70.56 (C-2′); 67.87 (C-4′); 61.69 (C-6′); 20.65, 20.51 and 19.96 (CH_3CO); 10.97 (SCH_3). EI MS, *m/z* (%): 532 [M – CH_3SH]$^+$ (24), 474 (80), 331 (17), 169 (91), 144 (22), 109 (38), 43 (100).

*Methyl 1-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)indole-3-carbothioate* (**28**). Yield 0.18 g (34% based on **23**), m.p. 144–146 °C (toluene–light petroleum), $[\alpha]_D^{25}$ –30.4 (*c* 0.10, dichloromethane). For $C_{24}H_{27}NO_{10}S$ (521.5) calculated: 55.27% C, 5.22% H, 2.69% N; found: 55.56% C, 5.41% H, 2.80% N. IR ($CHCl_3$): 1753 and 1640 (C=O); 1213 (C–O). ^{1}H NMR (300 MHz, $CDCl_3$): 8.28–8.25 m, 1 H, 8.01 s, 1 H, 7.47–7.44 m, 1 H and 7.35–7.18 m, 2 H (H-arom.); 5.65 d, 1 H, *J* = 8.7 (H-1′); 5.55–5.43 m, 2 H (H-2′, H-3′); 5.35–5.28 m, 1 H (H-4′); 4.32 dd,

1 H, $J(6'_b,6'_a)$ = 12.5, $J(6'_b,5')$ = 4.9 (H-$6'_b$); 4.19 dd, 1 H, $J(6'_b,6'_a)$ = 12.5, $J(6'_b,5')$ = 2.0 (H-$6'_a$); 4. 05–4.01 m, 1 H (H-5′); 2.49 s, 3 H (SCH_3); 2.10 s, 3 H; 2.09 s, 3 H; 2.03 s, 3 H and 1.69 s, 3 H (4 × CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 170.57, 170.08, 169.45 and 168.56 (CH_3CO); 159.88 (C=O); 136.50 (q), 128.63, 126.05 (q), 124.49, 123.23, 121.80, 110.68 (q) and 110.02 (C-arom.); 83.00 (C-1′); 75.34 (C-5′); 72.59 (C-3′); 71.20 (C-2′); 68.07 (C-4′); 62.12 (C-6′); 20.61 (triple intensity), 19.96 (CH_3CO, SCH_3); 10.97 (SCH_3). EI MS, *m/z* (%): 521 $[M]^+$ (24), 474 (36), 331 (9), 169 (100), 144 (35), 109 (34), 43 (44).

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)brassenin B (**29**)

*A. By reaction of oxobrassinin **27** with methyl iodide:* To a solution of oxobrassinin **27** (0.1 g, 0.17 mmol) in dry acetone (6 ml) was added methyl iodide (0.072 g, 0.032 ml, 0.51 mmol) and anhydrous potassium carbonate (0.024 g, 0.17 mmol) and the mixture was stirred at room temperature for 1 h. After pouring into cold water (20 ml), the product was extracted with chloroform (3 × 25 ml), the extract was dried with anhydrous sodium sulfate and solvent evaporated. The precipitate, formed after addition of diethyl ether to the oily residue, was filtered off and dried. Yield 0.059 g (58%).

*B. By the reaction of acid chloride **24** with dimethyl carbonimidodithioate*: To a stirred solution of crude acid chloride **24** (the reaction mixture concentrated to approximately 1/4 of its original volume), freshly prepared from 0.4 mmol of acid **23**, was added a solution of dimethyl carbonimidodithioate hydroiodide (0.100 g, 0.4 mmol) in pyridine (4 ml, stored over sodium hydroxide) and the reaction mixture was stirred at room temperature for 1.5 h. After pouring into water (70 ml), the product was extracted with diethyl ether (3 × 30 ml), the extract was washed with saturated solution of sodium hydrogencarbonate (3 × 50 ml), dried over anhydrous sodium sulfate, solvent was evaporated and the obtained residue was chromatographed on silica gel (30 g, eluent hexane–ethyl acetate 1:1). Yield 0.078 g (33%, from acid **23**), m.p. 175–177 °C (acetone–cyclohexane), $[\alpha]_D^{25}$ –56.4 (*c* 0.21, dichloromethane). For $C_{26}H_{30}N_2O_{10}S_2$ (594.7) calculated: 52.51% C, 5.08% H, 4.71% N; found: 52.77% C, 5.19% H, 4.53% N. IR ($CHCl_3$): 1750 and 1623 (O=C–N=C); 1212 (C–O). ^{1}H NMR (300 MHz, $CDCl_3$): 8.38–8.36 m, 1 H, 8.00 s, 1 H, 7.46–7.44 m, 1 H and 7.35–7.28 m, 2 H (H-arom.); 5.69 d, 1 H, $J(1',2')$ = 9.1 (H-1′); 5.60 t, $J(1',2')$ = 9.1, $J(2',3')$ = 9.2 (H-2′); 5.48 t, 1 H, $J(2',3')$ = 9.2, $J(3',4')$ = 9.2 (H-3′); 5.32 t, 1 H, $J(4',5')$ = 9.7, $J(3',4')$ = 9.7 (H-4′); 4.32 dd, 1 H, $J(6'_b,6'_a)$ = 12.5, $J(6'_b,5')$ = 5.1 (H-$6'_b$); 4.19 d, 1 H, $J(6'_b,6'_a)$ = 12.1 (H-$6'_a$); 4.08–4.04 m, 1 H (H-5′); 2.63 s, 6 H (2 × SCH_3); 2.10 s, 3 H; 2.09 s, 3 H; 2.05 s, 3 H and 1.74 s, 3 H (4 × CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$): 176.79 and 172.25 (O=C–N=C); 170.55, 170.11, 169.44 and 168.76 (CH_3CO); 136.83 (q), 132.06, 127.20 (q), 123.76, 123.04, 122.68, 115.57 (q) and 110.09 (C-arom.); 83.44 (C-1′); 74.90 (C-5′); 73.27 (C-3′); 70.15 (C-2′); 68.03 (C-4′); 61.83 (C-6′); 20.72, 20.61 and 20.17 (CH_3CO); 16.11 (SCH_3). MALDI-TOF MS, *m/z* (%): 634.6 $[M + K]^+$ (19), 617.8 $[M + Na]^+$ (63), 595.4 $[M + H]^+$ (76), 474.1 (100).

1-(β-D-Glucopyranosyl)brassenin B (**30**)

To a stirred solution of tetraacetyl derivative **29** (0.059 g, 0.1 mmol) in dry methanol (1 ml) was added 0.1 M methanolic solution of sodium methoxide (0.1 ml, 0.001 mmol) and stirring was continued at room temperature for 20 min. After neutralization with Amberlite IR-75 H^+ (0.050 g) and stirring for 5 min, the resin was filtered off, washed with methanol, the filtrate was evaporated and the obtained residue chromatographed on silica gel (7 g, eluent dichloromethane–methanol 8:1). The oily product obtained after evaporation of

eluate crystallized after addition of diethyl ether. Yield 0.035 g (83%), m.p. 108–112 °C, $[\alpha]_D^{25}$ –7.7 (*c* 0.14, methanol). For $C_{18}H_{22}N_2O_6S_2$ (426.5) calculated: 50.69% C, 5.20% H, 6.75% N; found: 50.42% C, 4.99% H, 6.81% N. IR ($CHCl_3$): 3420 (br, O–H); 1606 (O=C–N=C). ^{1}H NMR (300 MHz, $(CD_3)_2CO$): 8.26–8.23 m, 2 H, 7.68–7.66 m, 1 H and 7.27–7.24 m, 2 H (H-arom.); 5.61 d, 1 H, *J*(1′,2′) = 9.0 (H-1′); 4.69 d, 1 H, *J* = 4.8, 4.53 s, 1 H and 4.39 s, 1 H (D_2O exchangeable, 3 × OH); 4.72–4.66 m, 1 H (H-2′); 4.00–3.75 m, 4 H (H-4′, H-5′, H-6′$_a$, H-6′$_b$); 3.67–3.62 m, 1 H (H-3′); 2.60 s, 6 H (2 × SCH_3). ^{13}C NMR (75 MHz, $(CD_3)_2CO$): 175. 22 and 174.12 (O=C–N=C); 138.18 (q), 135.34, 127.76 (q), 124.21, 123.34, 122.18, 113.72 (q) and 112.85 (C-arom.); 86.67 (C-1′); 80.13 (C-3′); 78.12 (C-5′); 73.02 (C-2′); 70.57 (C-4′); 62.02 (C-6′); 16.07 (SCH_3). MALDI-TOF MS, *m/z* (%): 465.5 $[M + K]^+$ (48), 449.7 $[M + Na]^+$ (100), 427.6 $[M + H]^+$ (70).

We thank the Slovak Grant Agency for Science (grant No. 1/9246/02) for financial support of this work.

REFERENCES

1. a) Furuta A., Naruto S., Tamura A., Yokogawa K.: *Tetrahedron Lett.* **1979**, 1704; b) Buchanan J. G., Stoddard J., Wightman R. H.: *J. Chem. Soc., Perkin Trans. 1* **1994**, 1417; c) Ito T., Ohbai K., Koyama M., Sezaki M., Tohyama H., Shomura T., Fukuyasu H., Kazuno Y., Niwa T., Kojima M., Niida T.: *J. Antibiot.* **1984**, *37*, 931; d) Schumacher R. W., Harrington B. L., Davidson B. S.: *Tetrahedron Lett.* **2001**, *42*, 5133; e) Nettleton D. E., Doyle T. W., Kirshnan B., Matsumoto G. K., Clardy J.: *Tetrahedron Lett.* **1985**, *26*, 4011; f) Prudhomme M.: *Curr. Med. Chem.* **2000**, *7*, 1189; g) Diem S., Bergmann J., Herderich M.: *J. Agric. Food Chem.* **2000**, *48*, 4913; h) Hofsteenge J., Blommers M., Hess D., Furmanek A., Miroshnichenko O.: *J. Biol. Chem.* **1999**, *274*, 32786; i) Herderich M., Gutsche B.: *Food Rev. Int.* **1997**, *13*, 103; j) Pedras M. S. C., Ahiahonu P. W. K.: *Bioorg. Med. Chem.* **2002**, *10*, 3307.
2. a) Kutschy P., Dzurilla M., Takasugi M., Török M., Achbergerová I., Homzová R., Rácová M.: *Tetrahedron* **1998**, *54*, 3549; b) Suchý M., Kutschy P., Monde K., Goto K., Harada N., Takasugi M., Dzurilla M., Balentová E.: *J. Org. Chem.* **2001**, *66*, 3940; c) Suchý M., Kutschy P., Dzurilla M., Kováčik V., Andreani A., Alföldi J.: *Tetrahedron Lett.* **2001**, *42*, 6961; d) Kutschy P., Suchý M., Monde K., Harada N., Marušková R., Čurillová Z., Dzurilla M., Miklošová M., Mezencev R., Mojžiš J.: *Tetrahedron Lett.* **2002**, *43*, 9489; e) Kutschy P., Suchý M., Andreani A., Dzurilla M., Kováčik V., Alföldi J., Rossi M., Gramatová M.: *Tetrahedron* **2002**, *58*, 9029; f) Kutschy P., Dzurilla M., Takasugi M., Sabová A.: *Collect. Czech. Chem. Commun.* **1999**, *64*, 348; g) Kutschy P., Suchý M., Andreani A., Dzurilla M., Rossi M.: *Tetrahedron Lett.* **2001**, *42*, 9281.
3. a) Takasugi M., Monde K., Katsui N., Shirata A.: *Bull. Chem. Soc. Jpn.* **1988**, *61*, 285; b) Monde K., Sasaki K., Shirata A., Takasugi M.: *Phytochemistry* **1991**, *30*, 3921.
4. a) Mehta G., Liu J., Constantinou A., Hawthorne M., Pezzuto J. M., Moon R. C., Moriarty R. M.: *Anticancer Res.* **1994**, *14*, 1209; b) Mehta G., Liu J., Constantinou A., Thomas C. F., Hawthorne M., Gerhäuser C., Pezzuto J. M., Moon R. C., Moriarty R. M.: *Carcinogenesis* **1995**, *16*, 399; c) Sabol M., Kutschy P., Siegfried L., Miroššay A., Suchý M., Hrbková H., Dzurilla M., Marušková R., Starková J., Paulíková E.: *Biologia* **2000**, *55*, 701.

5. Preobrazhenskaya M. N., Korbukh I. A. in: *Chemistry of Nucleosides and Nucleotides* (L. B. Townsend, Ed.), Vol. 3, p. 1. Plenum Press, New York 1994.
6. a) Faul M. M., Winneroski L. L., Krumrich C. A.: *J. Org. Chem.* **1999**, *64*, 2465; b) El-Sayed I., El-Desoky, Abdel-Rahman H. A. R., Schmidt R. R.: *Liebigs Ann. Chem.* **1990**, 877; c) Ohkubo M., Kawamoto H., Ohno T., Nakano M., Morishima H.: *Tetrahedron* **1997**, *53*, 585; d) Ohkubo M., Nishimura I., Kawamoto H., Nakano M., Honma T., Yoshinari T., Arakawa H., Suda H., Morishima H., Nishimura S.: *Bioorg. Med. Chem. Lett.* **2000**, *10*, 419; e) Ohkubo M., Nishimura T., Honma T., Nishimura I., Ito S., Yoshinari T., Arakawa H., Suda H., Morishima H., Nishimura S.: *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3307.
7. a) Zemplen G., Gerecs A., Hadacsy J.: *Ber. Dtsch. Chem. Ges.* **1936**, *69*, 1927; b) Suvorov N. N., Preobrazhenskaya M. N.: *Zh. Obshch. Khim.* **1961**, *31*, 2832; *Chem. Abstr.* **1962**, *56*, 14386; c) Yartseva Y. E., Ektova L. V., Sakharova V. I., Dobrynin J. V., Yavorskaya N. P., Nikolaeva T. G., Sofina Z. P., Preobrazhenskaya M. N.: *Zh. Org. Khim.* **1977**, *13*, 1743; *Chem. Abstr.* **1978**, *88*, 7227; d) Wieland I., Kluge M., Schneider B., Schmidt J., Sicker D., Schulz M.: *Phytochemistry* **1998**, *49*, 719; e) Fuentes J., Molina J., Pradera M. A.: *Tetrahedron: Asymmetry* **1998**, *9*, 2518.
8. a) Suvorov N. N., Preobrazhenskaya M. N.: *Zh. Obshch. Khim.* **1960**, *30*, 2434; *Chem. Abstr.* **1961**, *55*, 8388; b) Tolkachev V. N., Preobrazhenskaya M. N.: *Zh. Org. Khim.* **1975**, *11*, 658; *Chem. Abstr.* **1975**, *83*, 677; c) Sokolova T. N., Yarzeva I. V., Preobrazhenskaya M. N.: *Carbohydr. Res.* **1981**, *93*, 19; d) Preobrazhenskaya M. N., Tolkachev V. N., Kudryaskova V. A., Yarzeva I. V.: *Zh. Org. Khim.* **1975**, *11*, 199; *Chem. Abstr.* **1975**, *82*, 140433.
9. Pedras M. S. C., Taylor J. L.: *J. Org. Chem.* **1991**, *56*, 2619.
10. a) Schallenberg J., Meyer E.: *Z. Naturforsch., B* **1983**, *38*, 108; b) Marchetti L., Andreani A.: *Ann. Chim. (Rome)* **1973**, *63*, 681; c) Somei M., Tanimoto A., Orita H., Yamada F., Ohta T.: *Heterocycles* **2001**, *54*, 425.
11. a) Dzurilla M., Kutschy P., Árvai R.: Unpublished results; b) Richter M., Herrmann C., Augustin M.: *J. Prakt. Chem.* **1980**, *322*, 434; c) Sato M., Fukuda N., Kurauchi M., Takeshima T.: *Synthesis* **1981**, 554; d) Richter M., Augustin M., Strauss K.: *J. Prakt. Chem.* **1984**, *326*, 633; e) Richter M., Richter W.: *Z. Chem.* **1988**, *28*, 292; f) Masataka Y., Hidekatsu H.: *Synthesis* **1985**, 891; g) Richter M., Richter W.: *Z. Chem.* **1988**, *8*, 292.