Total Synthesis and Biological Activities of (+)- and (−)-Boscialin and Their 1′-Epimers

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Natural (−)-boscialin [(−)-**1**] has recently been described as one of the constituents of various medicinal plants. To obtain more material for investigations of its biological activities, we carried out the synthesis of (−)-**1** and its isomers. Starting from the chiral building block **2**, the key steps of the synthesis involved a regioselective reduction and a nucleophilic addition. The enantiomer of the natural product, (+)-boscialin [(+)-**1**], could be obtained via acid-catalyzed epimerization of hydroxyketone **4** to (+)-**3**. Starting the synthesis with (−)-**3** led to (−)-boscialin [(−)-**1**] with the natural absolute configuration. In addition to (+)- and (−)-boscialin, the corresponding 1′-epimers (+)- and (−)-epiboscialin were also obtained. In vitro assays with (−)-boscialin [(−)-**1**] and its three stereoisomers were carried out to test for activity against microbes, parasites, and human fibroblasts. The investigations revealed activity against various microbes and against *Trypanosoma brucei rhodesiense* and also revealed cytotoxicity against human cancer cells.

Due to increasing interest in volatile aroma components, many C_{13}-norisoprenoids have recently been isolated and identified from various plants.[1] As some of these plants are used in traditional medicine,[2,3] there is also much interest in the biological properties of their constituents. As an example, some β-ionone derivatives, isolated from *Cinnamomum cassia* Blume, showed potent antiulcerogenic activity.[4]

The natural β-ionone derivative (−)-boscialin [(−)-**1**] and its β-D-glucopyranoside were first isolated from the methanolic extract of the leaves of the African tree *Boscia salicifolia* Oliv.[5] The constitutions and relative configurations of these compounds were assigned from spectroscopic measurements, especially from NOE experiments. Later, Pérez et al. were able to determine the absolute configuration. They obtained natural (−)-boscialin [(−)-**1**] through selective oxidation of (3*S*,5*R*,6*S*,9*R*)-3,6-dihydroxy-5,6-dihydro-β-ionol,[2] a natural product isolated from *Apollonias barbujana*.

In many African countries, extracts from aerial parts of *B. salicifolia* Oliv. are used in traditional medicine for wound healing.[6] Alcoholic extracts of the bark and leaves were shown to exhibit antibacterial and antifungal activity.[7,8] Recently, boscialin [(−)-**1**] and its glucoside were also obtained from *Dendranthema shiwogiku* (Kitam.) Kitamara,[9] *Prunus prostrata* Labill.,[3] and *Averrhoa carambola* L.[10] To our knowledge, no investigation of the biological activities of boscialin has so far been reported. To get more material for in vitro tests, we developed a synthesis of **1**, which, in addition, led to some novel stereoisomers.

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Results and Discussion

Regioselective reduction of diketone **2** with H_2/Raney-nickel (Scheme 1) afforded a mixture of the hydroxyketones (−)-**3** and **4** with preferential formation of **4**; the diastereomers could be separated by MPLC. Acid-catalyzed epimerization of **4** gave access to (+)-**3**, the enantiomer of hydroxyketone (−)-**3**. Protection of the alcoholic function of both enantiomeric hydroxyketones with the TBDMS-group led to the ketones (+)- and (−)-**5**, the substrates for the attachment of the side chain. The synthesis of these enantiomeric cyclohexanone building blocks was the key to the synthesis of both enantiomeric boscialin isomers.

Reactivity and stereoselectivity of nucleophilic additions to hindered cyclohexanones are limited by steric effects.[11] Reactions with acetylenic nucleophiles take place exclusively under axial attack, whereas mixtures are obtained with vinyllithium derivatives.[12]

Nucleophile **9**, the side chain synthon (Scheme 2), was built up starting with the acetylenic alcohol **6**, which was trimethylsilylated to **7** and subsequently hydrostannated with Bu_3SnH−AIBN. During hydrostannation, a maximum *E*/*Z* ratio of 87:11 could be achieved by thermodynamic reaction control; the product ratio was determined by GC. In the ^1H NMR spectrum, the vinyl protons of the main product showed a coupling constant of $J = 19$ Hz, which proved the *E*-configuration of this compound. Because separation of the two isomers was not possible, the mixture was used for the next reaction step. Transmetalation of **8** with *n*-BuLi at −78 °C lead to the vinyllithium derivative **9**. Subsequent nucleophilic addition of **9** to (−)-**5** (Scheme 3) resulted in the formation of the diastereomeric alcohols (−)-**10** and (−)-**11** in a ratio of approximately 2:5. The diastereomeric mixture was then subjected to oxidation with pyridinium fluorochromate, which deprotected the allylic alcohol and oxidized it in one step without cleavage of

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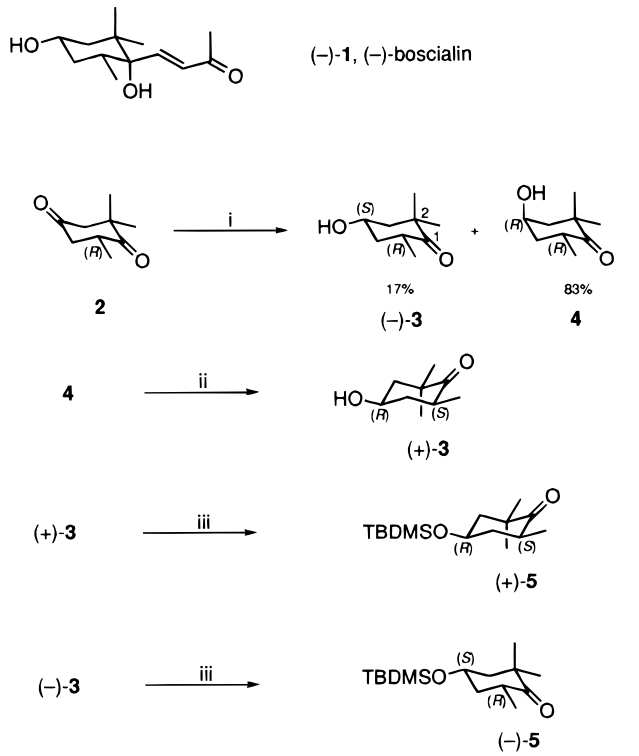
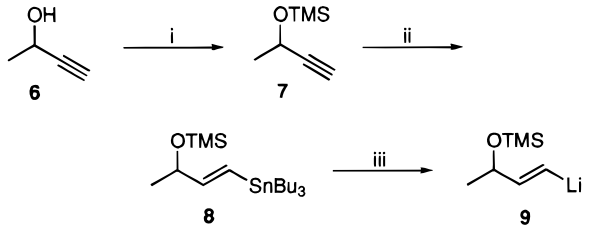
Scheme 1[a]



[a] Key: (i) H_2, Raney-Ni, rt, MeOH, 1 h; (ii) HCl, CH_2Cl_2; (iii) TBDMSCl, DMF, imidazole, 2 h.

Scheme 2[a]

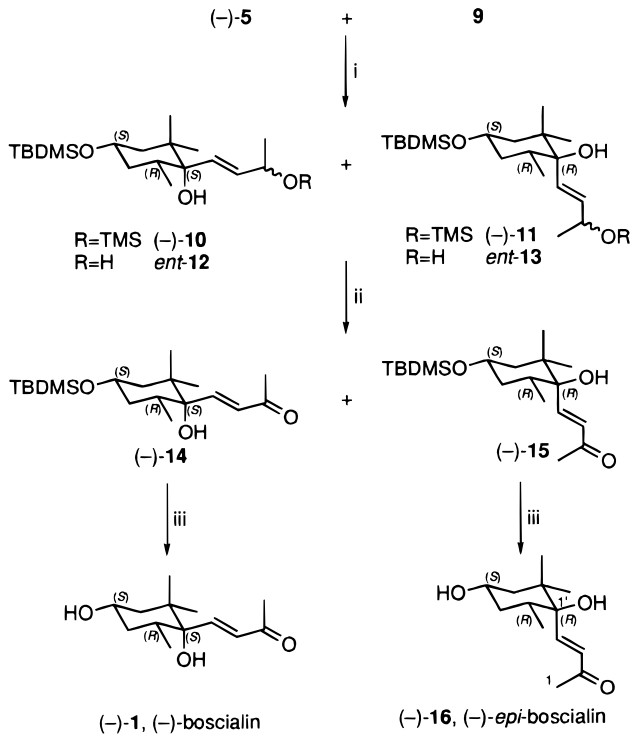


[a] Key: (i) hexamethyldisilazane, 110 °C, 16 h; (ii) Bu_3SnH, AIBN, 130 °C, 16 h; (iii) *n*-BuLi, THF, −78 °C, 1.5 h.

the *tert*-butyldimethylsilyl (TBDMS) group. After separation of the two epimers on Si gel, the TBDMS group was finally removed using Bu_4NF to give (−)-boscialin [(−)-**1**] and (−)-epiboscialin [(−)-**16**]. The spectral data of (−)-boscialin [(−)-**1**] were identical with those of the natural product. Its optical rotation corroborated the absolute configuration of the natural enantiomer to be (1′*S*,4′*S*,6′*R*). Using the same synthetic steps as outlined above, but starting from the hydroxyketone (+)-**5**, the unnatural (+)-boscialin [(+)-**1**] and its epimer (+)-**16** were readily obtained.

Compounds (+)-**1**, (+)-**16**, (−)-**1**, and (−)-**16** were tested against some protozoan parasites and against a human cancer cell line (Table 1). Whereas (+)-**1** and (+)-**16** were found to inhibit the growth of the bloodstream forms of *Trypanosoma brucei rhodesiense* only at a high concentration (100 μg/mL for 100% inhibition) and therefore were considered as inactive, the natural boscialin [(−)-**1**] and its epimer (−)-**16** gave more interesting results. For compound (−)-**1**, a minimum inhibitory concentration (MIC) of 11 μg/mL and a 50% inhibitory concentration (IC_{50}) of 1.9 μg/mL were found. The most active compound, (−)-**16**, showed a MIC of 3.7

Scheme 3[a]



[a] Key: (i) THF, −78 °C; (ii) pyridinium fluorochromate, 16 h; (iii) Bu_4NF.

Table 1. Activity of Compounds (+)-**1**, (+)-**16**, (−)-**1**, and (−)-**16** Against *Trypanosoma brucei rhodesiense* and Human HT-29 Cells

	T. b. rhodesiense		HT-29 cells		selectivity index[a]
	MIC[b]	IC_{50}[b]	MIC[b]	IC_{50}[b]	
melarsoprol	0.011	0.0004	11	3.6	9000
(+)-**1** [(+)-boscialin]	100	31	56	8.1	0.3
(+)-**16** [(+)-epiboscialin]	100	18	>100	17	1.0
(−)-**1** [(−)-boscialin]	11	1.9	>100	43	23
(−)-**16** [(−)-epiboscialin]	3.7	0.9	56	8.5	9.4

[a] IC_{50} (HT-29 cells)/IC_{50} (*T. b. rhodesiense*). [b] In μg/mL.

μg/mL and an IC_{50} of 0.9 μg/mL against *T. b. rhodesiense*. No inhibition of the growth of *T. cruzi* could be observed up to 100 μg/mL for all four compounds tested. Against *Leishmania donovani*, only compound (+)-**16** showed any activity with an IC_{50} value of 77 μg/mL; the other compounds were not active. All four compounds showed cytotoxic activity against the HT-29 cell line. To assess the cytotoxicity in relation to the activity against the parasites, selectivity indices were calculated by dividing the IC_{50} values for mammalian cells by the IC_{50} values for the parasites. The selectivity index for *T. b. rhodesiense* was 23 for compound (−)-**1** and 9.4 for compound (−)-**16**. In contrast, melarsoprol, one compound of choice against *T. b. rhodesiense* infections, has a selectivity index of 9000, which is significantly better than the values determined here for (−)-**1** and (−)-**16**. All four compounds tested proved to be more toxic against the human HT-29 cells than against *T. cruzi* and *L. donvani*. All this indicates the rather high general toxicity of these compounds.

Compounds (+)-**1**, (+)-**16**, (−)-**1**, and (−)-**16** were also tested by agar diffusion method for their antimicrobial activity against Gram-positive and Gram-negative bacteria and the yeast *Candida albicans* (Table 2). The test organisms particularly represent the microorgan-

Table 2. Microbiostatic Activity of Compounds (+)-**1**, (+)-**16**, (−)-**1**, and (−)-**16**

	inhibition zone (in mm)			
	(+)-**1**	(−)-**1**	(+)-**16**	(−)-**16**
Staphylococcus aureus ATCC[a] 9144	10.5	2.5	3	10
Staphylococcus epidermidis ATCC 12228	8	0	0	8
Corynebacterium xerosis ATCC 373	13	2	3	12
Corynebacterium minutissimum ATCC 23348	18	9.5	5	20
Escherichia coli NCTC[b] 8196	2	1	1	1.5
Candida albicans ATCC 10231	4.5	5	4	15.5

[a] ATCC = American Type Culture Collection. [b] NCTC = National Collection of Type Cultures.

isms of the resident skin flora (Staphylococci and Corynebacteria) and typical organisms of the transient skin flora (*Escherichia coli* and *Candida albicans*). All isomers showed a broad-spectrum antimicrobial activity. The strongest efficacy was observed against the Gram-positive Corynebacteria strains, whereas the activity against the Gram-negative *Escherichia coli* was the weakest. In general, (+)-boscialin [(+)-**1**] and (−)-epiboscialin [(−)-**16**] showed a better activity than (−)-boscialin [(−)-**1**] and (+)-epiboscialin [(+)-**16**]. The latter two epimers were not active against *Staphylococcus epidermidis* in the test method used.

Experimental Section

General Experimental Procedures. All solvents were dried and distilled before use. The chemicals were obtained either from Fluka or Aldrich. Melting points were measured on a Kofler hot stage and are uncorrected. Optical rotations were determined with a Perkin−Elmer polarimeter 141. UV spectroscopy was carried out with a Beckmann UV 25 spectrometer. IR spectra were obtained on a FT-IR Perkin−Elmer 1600. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300, and chemical shifts are in parts per million using Me_4Si as internal standard. MS were measured on a Hewlett−Packard 5970A GC/MS at 70 eV.

Regioselective Reduction of Diketone 2. *(R)*-2,2,6-Trimethyl-1,4-cyclohexanedione (**2**, 5.86 g, 38.0 mmol) was dissolved in MeOH (50 mL) and Raney-Ni (W2-activity) was added. After evacuation, the suspension was hydrogenated under shaking for 1 h. After filtration through Celite, the MeOH solution was evaporated. The product mixture was separated by MPLC (SiO_2, Et_2O−*n*-hexane 1:1) to give (−)-**3** (1.01 g, 6.45 mmol, 17%) and **4** (4.92 g, 31.5 mmol, 83%).

(4*S*,6*R*)-4-Hydroxy-2,2,6-trimethylcyclohexanone [(−)-3]: colorless needles (Et_2O−hexane); mp 51.9−52.6 °C (lit.[13] 51.5−52.5 °C); $[\alpha]^{25}_D$ −52.5 (*c* 0.75, MeOH; ee ca. 50%) [Lit.[13] −105 (*c* 0.8, MeOH)]; IR (KBr) ν_{max} 3700−3100 (OH), 2966, 2929 (C−H), 1698 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.32 (1H, m, H-4), 2.72 (1H, m, H-6), 2.28 (1H, m, H_{eq}-5), 2.21 (1H, s, OH), 2.07 (1H, m, H_{eq}-3), 1.58 (1H, m, H_{ax}-3), 1.41 (1H, m, H_{ax}-5), 1.21 (3H, s, CH_3-2), 1.07 (3H, s, CH_3-2), 1.03 (3H, d, J = 6.6 Hz, CH_3-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.8 (C-1); 65.6 (C-4); 49.4 (C-3); 44.6 (C-2, C-5); 37.9 (C-6); 26.4 (*C*H_{3eq}-C-2); 25.8 (*C*H_{3ax}-C-2); 14.8 (*C*H_3−C-6); EIMS *m*/*z* 156 [M]$^+$ (13), 138 [M−H_2O]$^+$ (6), 110 (5), 95 (11), 83 (100), 69 (51), 57 (64), 41 (60). Anal. C, 69.15%; H, 10.32%; O, 20.52%; calcd for $C_9H_{16}O_2$: C, 69.19%; H, 10.32%; O, 20.48%.

(4*R*,6*R*)-4-Hydroxy-2,2,6-trimethylcyclohexanone (4): colorless needles (Et_2O−hexane); mp 45.8−46.6 °C (lit.[14] 45.0−47.5 °C); $[\alpha]^{25}_D$ −110.1 (*c* 0.70, MeOH); [lit.[14] −108.9 (*c* 1.06, MeOH)]; IR (KBr) ν_{max} 3700−3100 (OH), 2966, 2929 (C−H), 1698 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.22 (1H, m, H-4), 3.18 (1H, m, H-6), 2.14 (1H, m, H_{eq}-5), 2.00 (1H, m, H_{eq}-3), 1.93 (1H, s, OH), 1.80 (1H, m, H_{ax}-3), 1.69 (1H, m, H_{ax}-5), 1.36 (3H, s, CH_3-2), 1.05 (3H, s, CH_3-2), 1.04 (3H, d, J = 6.6 Hz, CH_3-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 217.7 (C-1), 66.1 (C-4), 47.5 (C-3), 44.6 (C-2), 42.7 (C-5), 35.5 (C-6), 26.3 (*C*H_{3eq}-C-2), 28.5 (*C*H_{3ax}-C-2), 14.7 (*C*H_3−C-6); EIMS *m*/*z* 156 [M]$^+$ (11), 138 (6, [M−H_2O]$^+$), 110 (4), 95 (10), 83 (100), 69 (48), 57 (62), 41 (49). Anal. C, 69.19%; H, 10.24%; O, 20.56%; calcd for $C_9H_{16}O_2$: C, 69.19%; H, 10.32%; O, 20.48%.

Epimerization of Hydroxyketone 4. *(4R,6R)*-4-Hydroxy-2,2,6-trimethylcyclohexanone (**4**, 15.99 g, 102.4 mmol) was dissolved in CH_2Cl_2 (180 mL), and 37% HCl (7.5 mL) was added. After 70 min of stirring at room temperature, saturated aqueous $NaHCO_3$ solution (90 mL) was added. The organic layer was separated, and the aqueous layer was washed with CH_2Cl_2 (2 × 25 mL). The organic layers were evaporated under reduced pressure, and the product was purified by MPLC (SiO_2, *n*-hexane−Et_2O 1:1) to give 12.99 g (83.17 mmol, 81.2%) of (4*R*,6*S*)-4-hydroxy-2,2,6-trimethylcyclohexanone [(+)-**3**]: colorless needles (Et_2O−*n*-hexane); mp 51.8−52.8 °C (lit.[14] 48.0−50.0 °C); $[\alpha]^{25}_D$ +91.3° (*c* 0.88, MeOH) [lit.[14] $[\alpha]^{25}_D$ +95.0° (*c* 0.88, MeOH)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.33 (1H, m, H-4), 2.72 (1H, m, H-6), 2.27 (1H, m, H_{eq}-5), 2.07 (1H, m, H_{eq}-3), 1.84 (1H, s, OH), 1.58 (1H, m, H_{ax}-3), 1.42 (1H, m, H_{ax}-5), 1.21 (3H, s, CH_3-2), 1.07 (3H, s, CH_3-2), 1.04 (3H, d, J = 6.6 Hz, CH_3-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.7 (C-1), 65.6 (C-4), 49.4 (C-3), 44.6 (C-2, C-5), 37.9 (C-6), 26.4 (*C*H_{3eq}-C-2), 25.8 (*C*H_{3ax}-C-2), 14.8 (*C*H_3−C-6).

Protection of Hydroxyketone (+)-3. (4*R*,6*S*)-4-Hydroxy-2,2,6-trimethylcyclohexanone [(+)-**3**, 7.78 g, 49.8 mmol], imidazole (6.80 g, 99.9 mmol), and (*tert*-butyl)dimethylsilyl chloride (14.41 g, 90.7 mmol) were dissolved in DMF (200 mL) and stirred for 90 min. The reaction mixture was hydrolyzed by adding H_2O (180 mL). The layers were separated, and the aqueous layer was washed three times with Et_2O. The organic layer was washed with brine and evaporated under reduced pressure. Purification of the product was carried out by vacuum distillation (138 °C/12−14 mbar) to give (+)-**5** (11.50 g, 42.6 mmol, 85.5%): colorless oil; mp 4−5 °C; $[\alpha]^{25}_D$ +58.2 (*c* 0.99, MeOH) [lit.[14] $[\alpha]^{25}_D$ +58.0 (*c* 0.98, MeOH)]; IR (NaCl) ν_{max} 2957, 2929, 2885, 2857 (C−H), 1712 (C=O), 1256 (Si−C), 1086 (Si−O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27 (1H, m, H-4), 2.70 (1H, m, H-6), 2.14 (1H, m, H_{eq}-5), 1.92 (1H, m, H_{eq}-3), 1.60 (1H, m, H_{ax}-3), 1.43 (1H, m, H_{ax}-5), 1.19 (3H, s, CH_3-2), 1.05 (3H, s, CH_3-2), 1.02 (3H, d, J = 6.5 Hz, CH_3-6), 0.90 [9H, s, CH_3(*tert*-butyl)], 0.10 (6H, s, CH_3−Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.6 (C-1), 66.3 (C-4), 49.9 (C-3), 44.7 (C-5), 44.3 (C-2), 38.1 (C-6), 26.4 (*C*H_{3eq}-C-2), 25.8 (*C*H_{3ax}-C-2, *C*H_3-(*tert*-butyl), 18.1 ((CH_3)$_3$*C*-Si), 14.8 (*C*H_3−C-6), −4.7 (*C*H_3−Si); EIMS *m*/*z* 213 [M−C_4H_9]$^+$ (28), 171 (100), 157 (15), 143 (49), 121 (11), 75 (42), 59 (8), 41 (11). Anal. C, 66.65%; H, 11.18%; calcd for $C_{15}H_{30}O_2Si$: C, 66.61%; H, 11.18%.

Protection of Hydroxyketone (−)-3. As for (+)-**3**, but with (4*S*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone [(−)-**3**, 4.06 g, 26.0 mmol], imidazole (3.55 g, 52.2 mmol), and (*tert*-butyl)dimethylsilyl chloride (7.52 g, 47.4 mmol). Purification of the product was carried out by vacuum distillation (135−137 °C/12 mbar) to give (−)-**5** (6.24 g, 23.1 mmol, 89.0%): colorless oil; $[\alpha]^{25}_D$ −36.8 (*c* 1.18, MeOH; ee ca. 50%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.26 (1H, m, H-4), 2.69 (1H, m, H-6), 2.14 (1H, m, H_{eq}-5), 1.92 (1H, m, H_{eq}-3), 1.60 (1H, m, H_{ax}-3), 1.44 (1H, m, H_{ax}-5), 1.19 (3H, s, CH_3-2), 1.05 (3H, s, CH_3-2), 1.02 (3H, d, J = 6.5 Hz, CH_3-6), 0.90 [9H, s, CH_3(*tert*-butyl)], 0.10 (6H, s, CH_3−Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.6 (C-1); 66.3 (C-4); 49.9 (C-3); 44.7 (C-5); 44.3 (C-2); 38.1 (C-6); 26.4 (CH_{3eq}-C-2); 25.8 [CH_{3ax}-C-2, CH_3-(*tert*-butyl)]; 18.1 [$(CH_3)_3C$-Si]; 14.8 (CH_3−C-6); −4.7 (CH_3−Si).

(*R*/*S*)-3-(Trimethylsiloxy)-1-butyne (7). To (*R*/*S*)-3-butyn-2-ol (**6**, 10.25 g, 0.1466 mol) was added under argon hexamethyldisilazane (12.19 g, 75.58 mmol) and the mixture refluxed for 16 h at 110 °C. After cooling, the reaction mixture was filtered through SiO_2 to give **7** (20.63 g, 0.1450 mol, 99%): colorless liquid (bp: 110 °C); the spectroscopic data of the product were identical with those in the literature.[15]

Hydrostannation of Alkyne 7. To (*R*/*S*)-3-(trimethylsiloxy)-1-butyne (**7**, 18.81 g, 132.2 mmol) was added under argon tri-*n*-butylstannane (45 mL, 0.17 mol) and azobis(isobutyronitrile) (AIBN, 150 mg) and the mixture refluxed for 16 h at 130 °C. After cooling to room temperature, the excess of tri-*n*-butylstannane was removed at 75−80 °C/0.05 mbar. The product mixture was distilled at 126−130 °C/0.04 mbar to give stannane **8**: colorless liquid (54.95 g, 126.8 mmol, 86.5%); GC/EIMS *m*/*z* *E*-isomer: 377 $[M-C_4H_8]^+$ (100), 369 (3), 321 (6), 291 (3), 249 (5), 235 (4), 209 (28), 193 (49), 175 (15), 143 (20), 121 (18), 73 (42), 41 (14); GC/EIMS *m*/*z* *Z*-isomer: 377 $[M-C_4H_8]^+$ (18), 365 (2), 323 (100), 319 (42), 305 (33), 281 (5), 267 (69), 249 (13), 207 (62), 193 (23), 177 (15), 135 (13), 121 (17), 73 (12), 41 (25). Anal. C, 52.60%; H, 9.62%; calcd for $C_{19}H_{42}$-OSiSn: C, 52.67%; H, 9.77%.

Transmetalation to 9 and Subsequent Nucleophilic Addition to (+)-5. Vinylstannane **8** (3.20 g, 7.38 mmol) was dissolved in THF (10 mL) and cooled to −78 °C; *n*-BuLi (1.6 M in hexane, 4.60 mL, 7.36 mmol) was added slowly, and the reaction mixture was stirred for 90 min at −78 °C. (4*R*, 6*S*)-4-[(*tert*-Butyl)-dimethylsiloxy]-2,2,6-trimethylcyclohexanone ((+)-**5**, 0.995 g, 3.67 mmol) was dissolved in THF (15 mL) and added within 30 min to the reaction flask. After stirring for 2 h at −78 °C, the solution was allowed to warm to 0 °C and was quenched with saturated aqueous NH_4Cl solution (10 mL). The layers were separated, and the aqueous layer was washed with Et_2O (3 × 10 mL). The combined organic layers were dried over Na_2SO_4. The product ratio was determined by GC. After evaporation of the solvent, the diastereomers (+)-**10** and (+)-**11** were separated by MPLC (SiO_2−*n*-hexane−Et_2O gradient). During chromatography on SiO_2, the TMS group was cleaved off to some extent and the allylic alcohols **12** and **13** were also obtained.

(1*R*,4*R*,6*S*)-4-[(*tert*-Butyl)dimethylsiloxy]-2,2,6-trimethyl-1-[(*E*,*R*/*S*)-3′-(trimethylsiloxy)-1′-butenyl]-cyclohexan-1-ol [(+)-10, 380 mg, 0.918 mmol, 25%]: obtained as a colorless oil; $[\alpha]^{25}_D$ +6.1 (*c* 0.94, $CHCl_3$); IR (NaCl) ν_{max} 3620 (OH), 2957, 2858 (C−H), 1082 (C−O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.71−5.65 (1H, m, H-2′), 5.52−5.45 (1H, m, H-1′), 4.34 (1H, qui, J = 5.4 Hz, H-3′), 3.84 (1H, m, H-4), 1.88 (1H, m, H-6), 1.69−1.26 (5H, m, H-5, H-3, OH), 1.23 (3H, d, J = 6.3 Hz, CH_3-4′), 0.96 (3H, s, CH_3-2), 0.94−0.74 (3H, m, CH_3-6, CH_3-2), 0.89 [9H, s, CH_3(*tert*-butyl)], 0.11 (9H, s, CH_3−Si_{TMS}), 0.06 (6H, s, CH_3−Si_{TBDMS}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.5 (C-1′), 131.9, 131.7 (C-2′), 76.8 (C-1), 68.9 (C-3′), 67.3 (C-4), 45.8, 45.7 (C-3), 39.6 (C-5), 39.5 (C-2), 34.2, 34.1 (C-6), 25.9, 25.1, 25.0 24.6 [C-4′, CH_3(*tert*-butyl), CH_{3eq}-C-2, CH_{3ax}-C-2], 18.2 [$(CH_3)_3$C−Si], 16.0, 15.9 (CH_3−C-6), 0.1 (CH_3−Si_{TMS}), −4.6 (CH_3−Si_{TBDMS}); EIMS *m*/*z* 399 ($[M-CH_3]^+$ (<1), 381 (<1), 357 (<1), 309 (<1), 282 (1), 267 (2), 225 (1), 201 (43), 175 (12), 143 (15), 117 (25), 95 (19), 75 (78), 73 (100), 69 (10), 55 (12), 43 (20). Anal. C, 63.51%; H, 10.95%; calcd for $C_{22}H_{46}O_3Si_2$: C, 63.71%; H, 11.18%.

(1*S*,4*R*,6*S*)-4-[(*tert*-Butyl)dimethylsiloxy]-2,2,6-trimethyl-1-[(*E*,*R*/*S*)-3′-(trimethylsiloxy)-1′-butenyl]-cyclohexan-1-ol [(+)-11, 835 mg, 2.02 mmol, 55%]: obtained as a colorless oil; $[\alpha]^{25}_D$ +26.5 (*c* 0.48, $CHCl_3$); IR (NaCl) ν_{max} 3512 (OH); 2958, 2859 (C−H); 1251, 1038 (C−O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90 (1H, d, J = 15.6 Hz, H-1′), 5.69 (1H, m, H-2′), 4.36 (1H, qui, J = 6.2 Hz, H-3′), 3.93 (1H, m, H-4), 1.95 (1H, m, H-6), 1.75−1.42 (5H, m, H-5, H-3), 1.29 (s, OH), 1.23, 1.23 (3H, 2d, J = 6.3 Hz, CH_3-4′), 1.05 (3H, s, CH_3-2), 0.89 [9H, s, CH_3(*tert*-butyl)], 0.81−0.74 (3H, d, CH_3-6, CH_3-2), 0.12, 0.12 (9H, 2s, CH_3−Si_{TMS}), 0.06 (6H, s, CH_3−Si_{TBDMS}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.1 (C-1′), 128.1 (C-2′), 78.0 (C-1), 69.4 (C-3′), 67.3 (C-4), 47.4, 47.3 (C-3), 41.9, 41.8 (C-5), 39.2, 39.1 (C-2), 35.7, 35.5 (C-6), 26.2 (C-4′), 25.9 [CH_3(*tert*-butyl)], 25.3, 25.1 (CH_{3eq}-C-2), 22.8 (CH_{3ax}-C-2), 18.2 [$(CH_3)_3C$-Si], 15.7 (CH_3−C-6), 0.2 (CH_3−Si_{TMS}), −4.6 (CH_3−Si_{TBDMS}); EIMS *m*/*z* 414 $[M]^+$ (<1), 399 $[M-CH_3]^+$ (<1), 357 (<1), 324 (<1), 267 (2), 225 (1), 201 (40), 171 (7), 143 (7), 119 (27), 95 (17), 75 (84), 73 (100), 69 (15), 55 (13), 43 (22). Anal. C, 63.53%; H, 11.04%; calcd for $C_{22}H_{46}O_3Si_2$: C, 63.71%; H, 11.18%.

(1*R*,4*R*,6*S*)-4-[(*tert*-Butyl)dimethylsiloxy]-2,2,6-trimethyl-1-[(*E*,*R*/*S*)-3′-hydroxy-1′-butenyl]cyclohexan-1-ol (12, 25 mg, 0.73 mmol): obtained as a colorless oil; IR (NaCl) ν_{max} 3650−3100 (OH), 2958, 2857 (C−H), 1071 (C−O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80−5.73 (1H, m, H-2′), 5.58−5.53 (1H, d, J = 15.7 Hz, H-1′), 4.38 (1H, m, H-3′), 3.84 (1H, m, H-4), 1.90 (1H, m, H-6), 1.70−1.18 (8H, m, H-5, H-3, OH, CH_3-4′), 0.97, 0.95 (3H, 2s, CH_3-2), 0.89 [9H, s, CH_3(*tert*-butyl)], 0.88−0.77 (6H, m, CH_3-6, CH_3-2), 0.06 (6H, s, CH_3−Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.3 (C-1′), 133.1, 133.0 (C-2′), 76.8 (C-1), 68.6 (C-3′), 67.2 (C-4), 45.7 (C-3), 39.6 (C-5), 39.4 (C-2), 34.1 (C-6), 25.9 [CH_3(*tert*-butyl)], 25.8, 25.1, 24.6, 23.9 (C-4′, CH_{3eq}-C-2, CH_{3ax}-C-2), 18.2 [$(CH_3)_3C$-Si], 15.9 (CH_3−C-6), −4.6 (CH_3−Si). EIMS *m*/*z* 267 (3), 238 (5), 215 (2), 201 (100), 185 (8), 173 (64), 159 (12), 135 (38), 119 (21), 109 (30), 95 (63), 75 (99), 57 (21), 43 (69). Anal. C, 66.64%; H, 11.04%; calcd for $C_{19}H_{38}O_3Si_2$: C, 66.61%; H, 11.18%.

(1*S*,4*R*,6*S*)-4-[(*tert*-Butyl)dimethylsiloxy]-2,2,6-trimethyl-1-[(*E*,*R*/*S*)-3′-hydroxy-1′-butenyl]cyclohexan-1-ol (13, 22 mg, 0.064 mmol): obtained as a

colorless oil; IR (NaCl) ν_{max} 3600−3100 (OH), 2958, 2859 (C−H), 1037 (C−O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.01−5.95 (1H, m, H-1′), 5.80−5.73 (1H, m, H-2′), 4.40 (1H, m, H-3′), 3.93 (1H, m, H-4), 1.96 (1H, m, H-6), 1.76−1.20 (8H, m, H-5, H-3, OH, CH_3-4′), 1.05 (3H, s, CH_3-2), 0.89 [9H, s, CH_3(*tert*-butyl)], 0.81−0.76 (6H, m, CH_3-6, CH_3-2), 0.06 (6H, s, CH_3−Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.8 (C-1′), 129.2 (C-2′), 78.0 (C-1), 68.9 (C-3′), 67.2 (C-4), 47.3, 47.2 (C-3), 41.8 (C-5), 39.0 (C-2), 35.6 (C-6), 26.1 (C-4′), 25.9 [*C*H_3(*tert*-butyl)], 24.0, 23.9 (*C*H_{3eq}-C-2), 22.7 (*C*H_{3ax}-C-2), 18.2 [$(CH_3)_3$*C*-Si], 15.7 (*C*H_3−C-6), −4.7 (*C*H_3−Si); EIMS *m/z* 324 $[M-H_2O]^+$ (1), 308 (1), 293 (2), 267 (8), 238 (6), 211 (11), 201 (70), 175 (9), 159 (8), 137 (11), 123 (20), 109 (51), 95 (41), 85 (33), 75 (100), 69 (51), 55 (35), 43 (83). Anal. C, 66.35%; H, 10.90%; calcd for $C_{19}H_{38}O_3Si_2$: C, 66.61%; H, 11.18%.

Oxidation of (+)-10. (1*R*,4*R*,6*S*)-4-[(*tert*-Butyl)dimethylsiloxy]-2,2,6-trimethyl-1-[(*E*,*R*/*S*)-3′-(trimethylsiloxy)-1′-butenyl]cyclohexane-1-ol [(+)-**10**, 0.132 g, 0.319 mmol] was dissolved in THF (10 mL) and pyridinium fluorochromate (0.50 g, 2.5 mmol) was added. The suspension was stirred for 16 h at room temperature. After a crude filtration through SiO_2, the product was purified by MPLC (SiO_2−*n*-hexane−Et_2O gradient) and crystallized from Et_2O−*n*-hexane 4:1 to give (*E*)-4-{(1′*R*,4′*R*,6′*S*)-4′-[(*tert*-butyl)dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohexyl}-3-buten-2-one [(+)-**14**, 0.090 g, 0.264 mmol, 83.0%]: colorless crystals; mp 98.8−99.0 °C; $[\alpha]^{25}_D$ +19.7 (*c* 0.64, CH_3OH); IR (KBr) ν_{max} 3600−3250 (OH), 2952, 2856 (C−H), 1679, 1624 (C═O), 1150, 1093 (C−O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.74 (1H, d, *J* = 15.8 Hz, H-4), 6.38 (1H, d, *J* = 15.8 Hz, H-3), 3.86 (1H, m, H-4′), 2.27 (3H, s, H-1), 2.03 (1H, m, H-6′), 1.72−1.24 (5H, m, H-5′, H-3′, OH), 1.03 (3H, s, CH_3-2′), 0.89 [9H, s, CH_3(*tert*-butyl)], 0.86 (3H, s, CH_3-2′), 0.78 (3H, d, *J* = 6.8 Hz, CH_3-6′), 0.06 (6H, s, CH_3−Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.6 (C-2), 150.5 (C-3), 130.2 (C-4), 77.8 (C-1′), 66.9 (C-4′), 45.5 (C-3′), 39.8 (C-2′), 39.3 (C-5′), 33.9 (C-6′), 28.3 (C-1), 25.9 [*C*H_3(*tert*-butyl)], 25.2 (*C*H_{3eq}-C-2′), 24.6 (*C*H_{3ax}-C-2′), 18.2 [$(CH_3)_3$*C*-Si], 16.0 (*C*H_3−C-6′), −4.6 (*C*H_3−Si); EIMS *m/z* 284 $[M-C_4H_8]^+$ (13), 265 (3), 227 (11), 208 (27), 191 (22), 173 (27), 159 (8), 149 (14), 135 (17), 111 (55), 93 (28), 75 (85), 73 (50), 59 (20), 43 (100). Anal. C, 67.12%; H, 10.54%; calcd for $C_{19}H_{36}O_3Si$: C, 67.01%; H, 10.65%.

Oxidation of (+)-11. (1*S*,4*R*,6*S*)-4-[(*tert*-Butyl)dimethylsiloxy]-2,2,6-trimethyl-1-[(*E*,*R*/*S*)-3′-(trimethylsiloxy)-1′-butenyl]cyclohexane-1-ol [(+)-**11**, 0.252 g, 0.609 mmol] was dissolved in THF (10 mL) and pyridinium fluorochromate (0.50 g, 2.5 mmol) was added. The suspension was stirred for 16 h at room temperature. After a quick first separation on SiO_2 the product was purified by MPLC (SiO_2−*n*-hexane−Et_2O gradient) and crystallized from Et_2O−*n*-hexane 4:1 to give (*E*)-4-{(1′*S*,4′*R*,6′*S*)-4′-[(*tert*-butyl)dimethylsiloxy]-1′-hydroxy-2′,2,′6′-trimethylcyclohexyl}-3-buten-2-one [(+)-**15**, 0.163 g, 0.479 mmol, 78.7%]: colorless crystals; mp 115.5−116.0 °C; $[\alpha]^{25}_D$ +44.8 (*c* 0.76, CH_3OH); IR (KBr) ν_{max} 3600−3200 (OH), 2954, 2859 (C−H), 1685, 1615 (C═O), 1078 (C−O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 (1H, d, *J* = 15.9 Hz, H-4), 6.38 (1H, d, *J* = 15.7 Hz, H-3), 3.95 (1H, m, H-4′), 2.29 (3H, s, H-1), 2.00 (1H, m, H-6′), 1.80−1.26 (5H, m, H-5′, H-3′, OH), 1.08 (3H, s, CH_3-2′), 0.90 [9H, s, CH_3(*tert*-butyl)], 0.80 (3H, s, CH_3-2′), 0.79 (3H, d, *J* = 6.4 Hz, CH_3-6′), 0.07 (6H, s, CH_3−Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.8 (C-2), 146.9 (C-3), 130.8 (C-4), 78.9 (C-1′), 67.0 (C-4′), 47.3 (C-3′), 41.8 (C-5′), 39.4 (C-2′), 36.3 (C-6′), 28.1 (C-1), 26.3 (*C*H_{3eq}-C-2′), 25.9 [*C*H_3(*tert*-butyl)], 22.8 (*C*H_{3ax}-C-2′), 18.2 [$(CH_3)_3$*C*-Si], 15.8 (*C*H_3−C-6′), −4.6 (*C*H_3−Si); EIMS *m/z* 340 $[M]^+$ (<1), 325 (1), 307 (1), 283 (26), 227 (16), 209 (18), 191 (15), 173 (12), 143 (57), 133 (30), 111 (34), 75 (100), 73 (60), 59 (24), 55 (21), 43 (80). Anal. C, 67.16%; H, 10.56%; calcd for $C_{19}H_{36}O_3Si$: C, 67.01%; H, 10.65%.

Deprotection of (+)-14. (*E*)-4-{(1′*R*,4′*R*,6′*S*)-4-[(*tert*-Butyl)dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohex-1-yl}-3-buten-2-one [(+)-**14**, 53.0 mg, 0.156 mmol] was dissolved in THF (5 mL), and Bu_4NF (350 mg, 1.11 mmol) dissolved in THF (6 mL) was added. After 16 h of stirring at room temperature, H_2O (8 mL) was added, and the layers were separated. The aqueous layer was washed exhaustively with Et_2O (10 × 8 mL). The combined organic layers were evaporated under reduced pressure, and the residue was purified by MPLC (SiO_2−*n*-hexane−EtOAc gradient) to yield (*E*)-4-[(1′*R*,4′*R*,6′*S*)-1′,4′-dihydroxy-2′,2′,6′-trimethylcyclohexyl]-3-buten-2-one ((+)-boscialin) [(+)-**1**, 32.7 mg, 0.145 mmol, 92.8%]: colorless amorphous solid; $[\alpha]^{25}_D$ +22.0 (*c* 0.70, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 232 (4.01); IR (NaCl) ν_{max} 3700−3050 (OH), 2935 (C−H), 1671, 1625 (C═O), 1040 (C−O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.76 (1H, d, *J* = 15.8 Hz, H-4), 6.37 (1H, d, *J* = 15.7 Hz, H-3), 3.90 (1H, m, H-4′), 2.28 (3H, s, H-1), 2.07 (1H, m, H-6′), 1.87−1.23 (6H, m, H-5′, H-3′, 2 OH), 1.04 (3H, s, CH_3-2′), 0.88 (3H, s, CH_3-2′), 0.81 (3H, d, *J* = 6.8 Hz, CH_3-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.6 (C-2), 150.6 (C-3), 130.3 (C-4), 77.8 (C-1′), 66.4 (C-4′), 45.0 (C-3′), 39.9 (C-2′), 38.9 (C-5′), 33.9 (C-6′), 28.2 (C-1), 25.1 (*C*H_{3eq}-C-2′), 24.5 (*C*H_{3ax}-C-2′), 15.9 (*C*H_3−C-6′); EIMS *m/z* 208 $[M-H_2O]^+$ (8), 170 (30), 140 (4), 126 (35), 111 (100), 98 (26), 82 (22), 71 (21), 55 (31), 43 (95). Anal. C, 69.15%; H, 9.90%; O, 21.03%; calcd for $C_{13}H_{22}O_3$: C, 68.99%; H, 9.80%; O, 21.21%.

Deprotection of (+)-15. As for (+)-**14** but with (*E*)-4-{(1′*S*,4′*R*,6′*S*)-4-[(*tert*-butyl)dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohex-1-yl}-3-buten-2-one [(+)-**15**, 93.0 mg, 0.274 mmol] in THF (5 mL) and Bu_4NF (690 mg, 2.18 mmol) in THF (6 mL). (*E*)-4-[(1′*S*,4′*R*,6′*S*)-1′,4′-dihydroxy-2′,2′,6′-trimethylcyclohexyl]-3-buten-2-one ((+)-*epi*-boscialin) [(+)-**16**, 49.1 mg, 0.217 mmol, 79.3%] was obtained: colorless crystals; mp 151.9−152.3 °C; $[\alpha]^{25}_D$ +49.7 (*c* 0.92, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 232 (4.08); IR (KBr) ν_{max} 3700−3100 (OH), 2951, 2860 (C−H), 1667, 1643 (C═O), 1029 (C−O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.11 (1H, d, *J* = 15.7 Hz, H-4), 6.41 (1H, d, *J* = 15.7 Hz, H-3), 4.02 (1H, m, H-4′), 2.30 (3H, s, H-1), 2.05 (1H, m, H-6′), 1.98−1.23 (6H, m, H-5′, H-3′, 2OH), 1.10 (3H, s, CH_3-2′), 0.83 (3H, s, CH_3-2′), 0.82 (3H, d, *J* = 6.7 Hz, CH_3-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.7 (C-2), 146.5 (C-3), 130.8 (C-4), 78.9 (C-1′), 66.3 (C-4′), 46.8 (C-3′), 41.4 (C-5′), 39.4 (C-2′), 36.2 (C-6′), 28.2 (C-1), 26.2 (*C*H_{3eq}-C-2′), 22.8 (*C*H_{3ax}-C-2′), 15.7 (*C*H_3−C-6′); EIMS *m/z* 208 $[M-H_2O]^+$ (6), 193 (1), 170 (19), 156 (7), 152 (4), 137 (4), 126 (32), 111 (96), 98 (32), 82 (24), 71 (24), 57 (16), 55 (35), 43 (100). Anal. C, 69.11%; H, 10.01%; O, 21.25%; calcd for $C_{13}H_{22}O_3$: C, 68.99%; H, 9.80%; O, 21.21%.

(*E*)-4-{(1′*S*,4′*S*,6′*R*)-4′-[(*tert*-Butyl)dimethylsiloxy]-

1′-hydroxy-2′,2′,6′-trimethylcyclohexyl}-3-buten-2-one ((−)-14) and (*E*)-4-{(1′*R*,4′*S*,6′*R*)-4′-[(*tert*-Butyl)-dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohex-1-yl}-3-buten-2-one [(−)-15]. Vinylstannane **8** (26.0 g, 59.9 mmol) was dissolved in THF (60 mL) and cooled to −78 °C. Then *n*-butyllithium (1.6 M in hexane, 37.5 mL, 60.0 mmol) was added dropwise. After 2 h of stirring at −78 °C, (4*S*, 6*R*)-4-[(*tert*-butyl)-dimethylsilyloxy]-2,2,6-trimethylcyclohexanone [(−)-**5**, 6.24 g, 23.0 mmol] dissolved in THF (18 mL) was added within 30 min. The solution was stirred for another 2 h at −78 °C, before it was allowed to warm to 0 °C and quenched with saturated aqueous NH_4Cl solution (60 mL). The layers were separated, and the aqueous layer was washed with Et_2O (4 × 50 mL). The combined organic layers were dried over Na_2SO_4. After evaporation of the solvent, the product mixture was prepurified by column chromatography on SiO_2 (*n*-hexane−Et_2O gradient).

The mixture of (−)-**10** and (−)-**11** thus obtained (32.79 g, 23 mmol) was dissolved in CH_2Cl_2 (300 mL), pyridinium fluorochromate (24.66 g, 123.3 mmol) was added, and the suspension was stirred for 20 h. The reaction mixture was filtered through SiO_2, and the solvent was evaporated. Further purification and separation was carried out by MPLC (SiO_2−*n*-hexane−Et_2O gradient). (*E*)-4-{(1′*S*,4′*S*,6′*R*)-4′-[(*tert*-Butyl)dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohexyl}-3-buten-2-one [(−)-**14**, 1.54 g, 4.53 mmol, 19.7% from (−)-**5**] was obtained as colorless crystals: mp 98.8−99.0 °C; $[\alpha]^{25}_D$ −15.5 (*c* 0.88, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (1H, d, J = 15.9 Hz, H-4), 6.38 (1H, d, J = 15.8 Hz, H-3), 3.86 (1H, m, H-4′), 2.27 (3H, s, H-1), 2.03 (1H, m, H-6′), 1.67−1.34 (5H, m, H-5′, H-3′, OH), 1.03 (3H, s, C*H*$_3$-2′), 0.89 [9H, s, C*H*$_3$(*tert*-butyl)], 0.86 (3H, s, C*H*$_3$-2′), 0.78 (3H, d, J = 6.8 Hz, C*H*$_3$-6′), 0.06 (6H, s, C*H*$_3$−Si); ^{13}C NMR (300 MHz, $CDCl_3$) δ 197.6 (C-2), 150.5 (C-3), 130.1 (C-4), 77.8 (C-1′), 66.9 (C-4′), 45.5 (C-3′), 39.8 (C-2′), 39.3 (C-5′), 33.9 (C-6′), 28.3 (C-1), 25.9 [*C*H$_3$(*tert*-butyl)], 25.2 (*C*H$_{3eq}$-C-2′), 24.6 (*C*H$_{3ax}$-C-2′), 18.2 [(CH$_3$)$_3$*C*-Si], 15.9 (*C*H$_3$−C-6′), −4.6 (*C*H$_3$−Si).

(*E*)-4-{(1′*R*,4′*S*,6′*R*)-4′-[(*tert*-Butyl)dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohexyl}-3-buten-2-one [(−)-15, 2.72 g, 8.00 mmol, 34.8% from (−)-5]: obtained as colorless crystals; mp 115.5−116.0 °C; $[\alpha]^{25}_D$ −30.2 (*c* 0.86, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (1H, d, J = 15.9 Hz, H-4), 6.38 (1H, d, J = 15.7 Hz, H-3), 3.96 (1H, m, H-4′), 2.29 (3H, s, H-1), 2.00 (1H, m, H-6′), 1.77−1.29 (5H, m, H-5′, H-3′, OH), 1.08 (3H, s, C*H*$_3$-2′), 0.90 [9H, s, C*H*$_3$(*tert*-butyl)], 0.80 (3H, s, C*H*$_3$-2′), 0.79 (3H, d, J = 6.4 Hz, C*H*$_3$-6′), 0.07 (6H, s, C*H*$_3$−Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.8 (C-2), 146.9 (C-3), 130.8 (C-4), 78.9 (C-1′), 67.0 (C-4′), 47.3 (C-3′), 41.8 (C-5′), 39.4 (C-2′), 36.3 (C-6′), 28.1 (C-1), 26.3 (*C*H$_{3eq}$-C-2′), 25.9 [*C*H$_3$(*tert*-butyl)], 22.9 (*C*H$_{3ax}$-C-2′), 18.2 [(CH$_3$)$_3$*C*-Si], 15.8 (*C*H$_3$−C-6′), −4.7 (*C*H$_3$−Si).

Deprotection of (−)-14. (*E*)-4-{(1′*S*,4′*S*,6′*R*)-4-[(*tert*-Butyl)dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohex-1-yl}-3-buten-2-on [(−)-**14**, 1.19 g, 3.50 mmol] was dissolved in THF (25 mL) and Bu_4NF (12.0 g, 38.1 mmol) dissolved in THF (60 mL) was added. After 18 h stirring at room temperature, H_2O (50 mL) was added, and the layers were separated. The aqueous layer was washed exhaustively with Et_2O (10 × 50 mL). The combined organic layers were evaporated under reduced pressure, and the residue was purified by MPLC (SiO_2−*n*-hexane−EtOAc gradient) to give (*E*)-4-[(1′*S*,4′*S*,6′*R*)-1′,4′-dihydroxy-2′,2′,6′-trimethylcyclohexyl]-3-buten-2-one [(−)-boscialin] [(−)-**1**, 0.698 g, 3.09 mmol, 88%]: colorless amorphous solid; $[\alpha]^{25}_D$ −18.6 (*c* 0.84, $CHCl_3$), [lit.[5] $[\alpha]^{25}_D$ −19 ± 5 (*c* 0.14, $CHCl_3$)];[2] −21.1 (*c* 0.31, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (1H, d, J = 15.9 Hz, H-4), 6.38 (1H, d, J = 15.9 Hz, H-3), 3.90 (1H, m, H-4′), 2.28 (3H, s, H-1), 2.07 (1H, m, H-6′), 2.05 (1H, s, OH), 1.87−1.24 (5H, m, H-5′, H-3′, OH), 1.04 (3H, s, C*H*$_3$-2′), 0.88 (3H, s, C*H*$_3$-2′), 0.81 (3H, d, J = 6.8 Hz, C*H*$_{3eq}$-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.7 (C-2), 150.7 (C-3), 130.3 (C-4), 77.7 (C-1′), 66.3 (C-4′), 45.0 (C-3′), 39.9 (C-2′), 38.8 (C-5′), 33.9 (C-6′), 28.1 (C-1), 25.1 (*C*H$_3$-C-2′), 24.5 (*C*H$_{3ax}$-C-2′), 15.9 (*C*H$_3$−C-6′). Anal. C, 68.70%; H, 9.93%; O, 21.09%; calcd for $C_{13}H_{22}O_3$: C, 68.99%; H, 9.80%; O, 21.21%.

Deprotection of (−)-15. As above for (−)-**14** but with (*E*)-4-{(1′*R*,4′*S*,6′*R*)-4-[(*tert*-butyl)dimethylsiloxy]-1′-hydroxy-2′,2′,6′-trimethylcyclohex-1-yl}-3-buten-2-one [(−)-**15**, 2.08 g, 6.11 mmol] dissolved in THF (40 mL) and Bu_4NF (20.0 g, 63.5 mmol) dissolved in THF (100 mL) to yield (*E*)-4-[(1′*R*,4′*S*,6′*R*)-1′,4′-dihydroxy-2′,2′,6′-trimethylcyclohexyl]-3-buten-2-one [(−)-epiboscialin] [(−)-**16**, 1.10 g, 4.87 mmol, 79.7%]: colorless crystals; mp 152.0−152.3 °C; $[\alpha]^{25}_D$ −62.6 (*c* 0.91, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (1H, d, J = 15.7 Hz, H-4), 6.41 (1H, d, J = 15.7, Hz H-3), 4.02 (1H, m, H-4′), 2.30 (3H, s, H-1), 2.05 (1H, m, H-6′), 1.98−1.23 (6H, m, H-5′, H-3′, 2OH), 1.10 (3H, s, C*H*$_3$-2′), 0.83 (3H, s, C*H*$_3$-2′), 0.82 (3H, d, J = 6.7 Hz, C*H*$_3$-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.8 (C-2), 146.5 (C-3), 130.8 (C-4), 78.9 (C-1′), 66.3 (C-4′), 46.8 (C-3′), 41.4 (C-5′), 39.4 (C-2′), 36.2 (C-6′), 28.2 (C-1), 26.2 (*C*H$_{3eq}$-C-2′), 22.8 (*C*H$_{3ax}$-C-2′), 15.7 (*C*H$_3$-C-6′). Anal. C, 68.89%; H, 10.00%; O, 21.21%; calcd for $C_{13}H_{22}O_3$: C, 68.99%; H, 9.80%; O, 21.21%.

Parasite Strains. *Trypanosoma brucei rhodesiense* STIB 900 is a cloned population isolated in 1982, from a patient in Tanzania. The strain was adapted to grow under axenic culture conditions according to Baltz et al.[16] The minimum essential medium (MEM) was supplemented with 15% heat-inactivated horse serum. Stock cultures were kept in 24-well tissue culture plates at 37 °C in an atmosphere of 5% CO_2 in air. *Trypanosoma cruzi* MHOM/Br/OO/Y was cultivated in MEM supplemented with 10% heat-inactivated fetal calf serum in L6 myoblasts (ECACC 92102118). Stock cultures were maintained in T-12.5 flasks at 37 °C in an atmosphere of 5% CO_2 in air. *Leishmania donovani* MHOM/ET/67/L82 was maintained in hamsters.

Human Cell Line. The human cell line HT-29 derived from an isolated primary case of adenocarcinoma was obtained from the American Type Culture Collection (HTB 38). Cells were cultivated in MEM supplemented with 10% heat-inactivated fetal calf serum in T-25 flasks at 37 °C in an atmosphere of 5% CO_2 in air.

Bioassays. The test compounds were dissolved in DMSO as a 100-fold stock solution leading to a final DMSO concentration of 1% in the assay system. The highest concentration of the test compounds was 100 μg/mL (500 μg/mL for tests with HT-29 cells), with

dilution steps of three in the assays. The *T. b. rhodesiense* and HT-29 assays were performed as previously described by Räz et al.[17] and Kaminsky et al.,[18] respectively. Briefly, trypanasomes (200 cells/100 μL) or HT-29 cells (5000 cells/100 μL) were incubated in the presence of various drug concentrations in 96-well microtiter plates. After 72 h of drug exposure, the minimum inhibitory concentration (MIC) was determined microscopically. MIC was defined as the lowest concentration at which no cells with normal morphology or motility could be found. Additionally, 50% inhibitory concentrations (IC_{50}) were determined by adding the fluorochrome Alamar Blue (Laboserve). The plate was incubated for further 2 h, then the fluorescence was quantified with a Cyto Fluor plate reader (model 2300; Millipore, Bedford, MA) and IC_{50} values of fluorescence were calculated by linear interpolation.[19] For the *T. cruzi* assay, L6 cells (50 000 cells/500 μL) were incubated in 48-well microtiter plates. After 24 h of incubation, the cells were infected with trypomastigotes from the supernatant of stock cultures at a ratio of five parasites per cell and incubated for another 48 h. The parasites were then exposed to serial drug dilutions for 5 d in total, with a medium change with drug after 3 d. A MIC was determined microscopically. The *L. donovani* assay was performed as decribed by Neal et al.[20] Isolated mouse peritoneal macrophages were cultivated in tissue chamber slides (50 000 cells/500 μL) in RPMI 1640 plus 10% heat inactivated fetal calf serum. After 24 h of incubation they were infected with a suspension of amastigotes from hamster spleen at a ratio of 10 amastigotes per macrophage. Again 24 h later, the amastigote suspension was replaced by medium containing serial dilutions of drug. After 5 d of drug exposure with a medium change with drug after 3 d, the macrophages were fixed with MeOH and stained with Giemsa. The proportion of infected to uninfected macrophages was determined microscopically and IC_{50} values were calculated.

Agar Diffusion Test.[21] Gram-positive bacteria (*Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 12228, *Corynebacterium xerosis* ATCC 373, and *Corynebacterium minutissimum* ATCC 23348), Gram-negative bacteria (*Escherichia coli* NCTC 8196), and a yeast (*Candida albicans* ATCC 10231) were used as test organisms for a qualitative assessment of the antimicrobial activity of the boscialin isomers. Overnight cultures of the respective test strains in casein peptone soymeal peptone medium (Merck) were diluted in sterile 0.9% NaCl in distilled H_2O and subsequently added to molten casein peptone soymeal peptone agar (Merck) resulting in a final concentration of 5×10^4–10^5 colony-forming units per mL. The inoculated agar (6 mL) was equally distributed on the surface of sterile casein soymeal peptone agar plates (Petri dishes with a diameter of 9 cm). After solidification of the agar medium, circular holes with a diameter of 1 cm were cut out, and 100 μL of the test substances (1 mg/mL in H_2O) were given in each hole. The plates were incubated at 37 °C for 24 h. For the assessment of the microbiostatic activity, the diameter of the inhibition zone around the holes was measured.

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