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Graphical Abstract

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ABSTRACT

Substituted goniothalamins containing cyclopropane-groups were efficiently prepared in high yields and good selectivity. Antiproliferative activity was measured on three human cancer cell lines (A549, MCF-7, HBL-100), to show which of the structural elements of goniothalamins is mandatory for cytotoxicity. We found that the configuration of the stereogenic centre of the δlactone plays an important role for cytotoxicity. In our studies only (*R*)-configured goniothalamins showed antiproliferative activity, whereby (*R*)-configuration accords to natural goniothalamin (*R*)-**1**. Additionally, the δlactone needs to be unsaturated whereas our results show that the vinylic double bond is not mandatory for cytotoxicity. Furthermore, with a two-fold *in vitro* and *in vivo* strategy, we determined the inhibitory effect of the compounds to the yeast protein Pdr5. Here, we clearly demonstrate that the configuration seems to be of minor influence, only, while the nature of the substituent of the phenyl ring is of prime importance.

1. Introduction

In the past, the natural product (*R*)-(+)-goniothalamin and its derivatives were shown to display biological activity against a broad range of cell lines,[1-5] bacteria[6] and fungi.[6,7] Goniothalamin can be isolated from plants of the genus of *Goniothalamus*, which grow in tropic and subtropic areas of Asia and Oceania.[8] In 1967 *Hlubucek et al.* isolated goniothalamin for the first time from *Cryptocarya caloneura* and determined the stereogenic centre to be (*S*)-configured.[9] This was revealed in 1979, when *Meyer et al.* elucidated the structure of natural occurring goniothalamin as (*R*)-configured by the synthesis of both enantiomers.[10]

Goniothalamin, (*R*)-**1**, and its derivatives have an αβunsaturated δlactone as a central element, which can be used as a *Michael*-acceptor system with corresponding nucleophiles. Furthermore it seems, that the vinylic double bond and the configuration of the stereogenic centre in the lactone is essential for its cytotoxicity (Fig. 1).

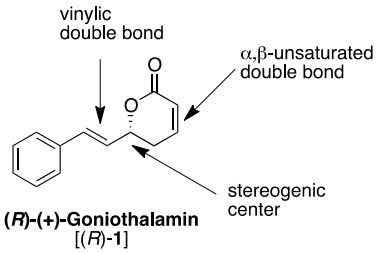


Figure 1. Structure of the natural (*R*)-(+)-goniothalamin and its structural elements.

In addition to the naturally-occurring (*R*)-(+)-goniothalamin (**1**), *Fátima et al.* showed that the (*S*)-enantiomer [(*S*)-**1**] and the two derivatives, (*S*,*E*)-6-(2-cyclohexylvinyl)-5,6-dihydro-2*H*-pyran-2-one [(*S*)-**2**] and (*S*,*E*)-6-(4-methoxystyryl)-5,6-dihydro-2*H*-pyran-2-one [(*S*)-**3**], have a higher potential towards the inhibition of kidney cell proliferation in comparison to the

natural goniothalamin (*R*)-**1**, with IC_{50}-values in the micromolar range (Fig. 2).[1,2,3,4,5] They could also show, that the fully hydrogenated derivatives (*R*)-6-phenethyltetrahydro-2*H*-pyran-2-one and (*S*)-6-phenethyltetrahydro-2*H*-pyran-2-one have no cytotoxic effect.[1]

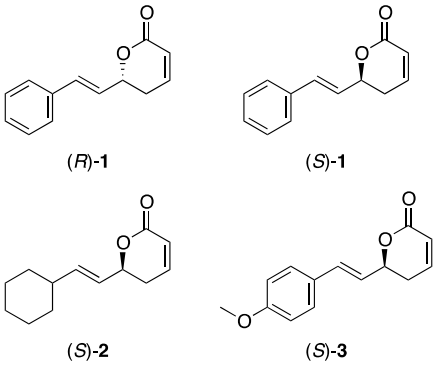


Figure 2. Structure of goniothalamin (*R*)-**1** and some derivatives.

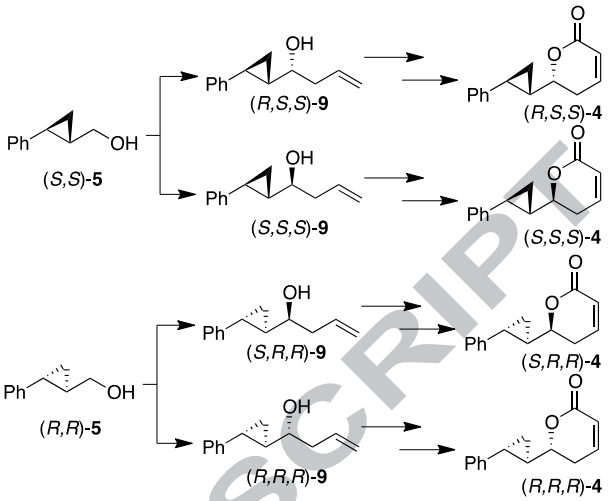
Further biological studies were reported by *Wach* et al. in 2010: They showed that naturally occurring (*R*)-goniothalamin [(*R*)-**1**] can inhibit the nucleocytoplasmatic transport.[11] In 2013 *Bruder* et al. synthesized new goniothalamin derivatives, with an additional methyl groups at the now quaternary stereogenic centre of γ and δlactones and substituents at the phenyl group. No increase in anti-proliferative activity of (*E*)-6-methyl-6-styryl-5,6-dihydro-2*H*-pyran-2-one and (*E*)-5-methyl-5-styrylfuran-2(5*H*)-one relative to natural product (*R*)-**1** was observed. By adding a trifluoromethyl groups to the phenyl group of the methylated compounds, they could improve biological activity.[12] *Pilli* et al. synthesized 29 novel goniothalamin analogues and reported that aza-analogues and γpyrones had no increased antiproliferative activity, while tri- and tetra-methoxylated goniothalamin derivatives showed a promising increase in cytotoxicity.[13] All in all goniothalamin and its derivatives show a wide variety of biological effects, e.g., anti-inflammatory,[14] anti-nociceptive[14] anti-tumour activity[15] as well as plant growth inhibition.[15,16]

Here we would like to present our enantioselective synthetic route towards goniothalamin and derivatives. In addition, the corresponding results of cytotoxicity tests of these compounds on human lung adenocarcinoma cell line A549, human breast adenocarcinoma cell line MCF-7 as well as on the triple negative human breast cancer cell line HBL-100 demonstrate a first hint for a structure-activity relationship. With these cytotoxicity tests, we want to elucidate, which structural elements of the vinyl-lactone are essential for biological activity. Furthermore, we also performed assays with the *Saccharomyces cerevisiae* multidrug exporter Pdr5. Pdr5 is a functional homologue of Cdr1, an important drug efflux pump of the clinical relevant fungi *Candida albicans.* Consequently, the development of new inhibitors against these multidrug exporter proteins plays an important role in our daily fight against resistance fungi. Therefore, we analyzed goniothalamin derivatives with respect to their inhibitory capacity against Pdr5.

2. Results

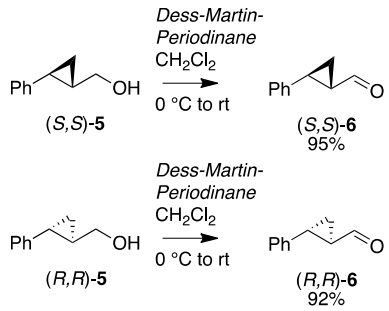
2.1. Synthesis of Cyclopropane Derivatives

To show the importance of different structural elements of goniothalamin, we decided to synthesize different derivatives and demonstrate the influence of the vinylic double bond on the cytotoxicity of the compound; more specifically, we synthesized cyclopropane derivatives **4** with the cyclopropyl unit instead of the vinylic double bond.



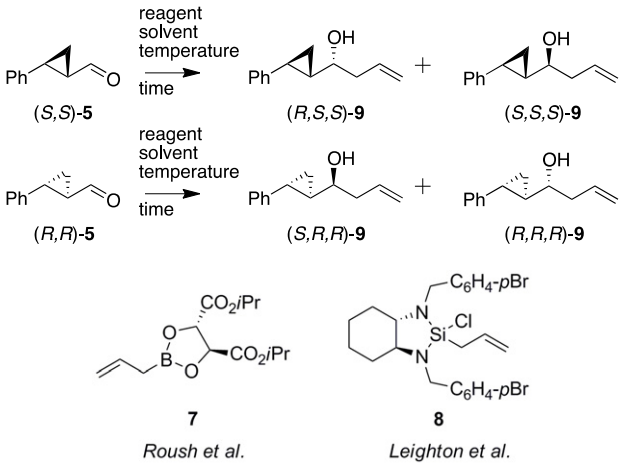
Scheme 1. Synthesis scheme for cyclopropane derivatives **4**.

Synthesis of goniothalamin derivatives **4** was performed starting from cyclopropyl alcohol **5**, which can be synthesized from corresponding cinnamic alcohol using a previously reported enzymatic kinetic resolution (Scheme 1).[17] Both diastereoisomers of cyclopropyl alcohol **5** could readily be converted into the corresponding aldehydes (*S,S*)-**6** and (*R,R*)-**6** with the use of *Dess-Martin*-periodinane[18-22] in very good yields of 95% and 92% (Scheme 2). It has to be mentioned that the cyclopropyl aldehydes (*S,S*)-**6** and (*R,R*)-**6** are not stable against oxygen and directly oxidize to the corresponding acids under air, they have to be stored under an argon atmosphere.



Scheme 2. Oxidation of alcohol **5** to the corresponding aldehydes **6**.

Stereoselective allyl addition of the enantiomerically-pure aldehydes **6** with allylboronic acid ester **7**[24,25] and Leighton reagent **8**[26,27] gave the homoallylic alcohols **9** in good yields. Using the allylboronate **7** from *Roush et al.*, we found a 78:22-mixture of diastereoisomers (*R,S,S*)-**9** and (*S,S,S*)-**9** in 93% yield (Table 1, entry 1), with the *anti*-product (*R,S,S*)-**9** being the major product, due to the control of reagent **7**. To improve the diastereomeric ratio we used the Leighton reagent **8**, which gave the *anti*-product (*R,S,S*)-**9** >99:1 in 75% yield (Table 1, entry 2). Due to the fact of good separation of diastereoisomers (*R,S,S*)-**9** and (*S,S,S*)-**9** by column chromatography and shorter reaction times, we decided to use allylboronic acid ester **7** for the synthesis of homoallylic alcohols (*S,R,R*)-**9** and (*R,R,R*)-**9**. With the allylboronic acid ester **7** from *Roush et al.* we could obtain the major *syn*-product (*R,R,R*)-**9** in a diastereomeric ratio of 77:23 and a yield of 72%.



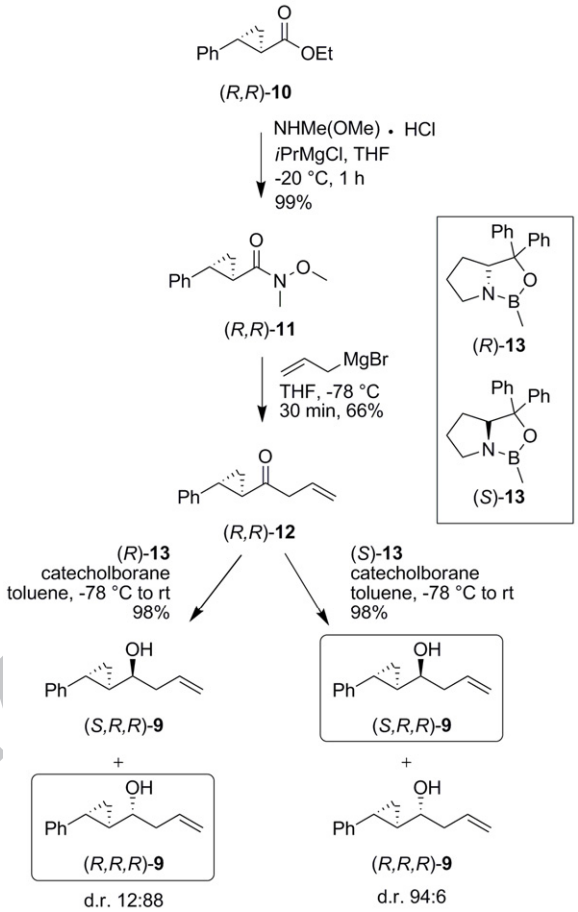
Scheme 3. Stereoselective allyl addition towards homoallylic alcohols **9**.

Table 1. Conditions for stereoselective allyl addition of aldehydes **6** towards homoallylic alcohols **9**.

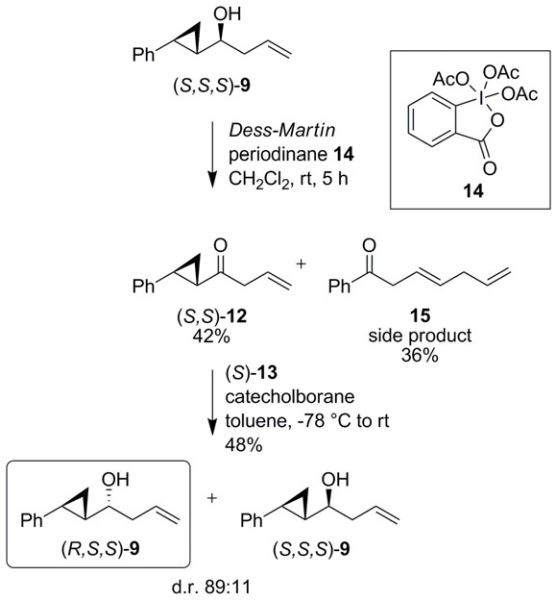
Starting-material	Reagent	Conditions	d.r. (*anti*:*syn*)	Yield
(*S*,*S*)-**6**	**7**	Toluene -78 °C to rt 5 h	78:22 (*R*,*S*,*S*)-**9**:(*S*,*S*,*S*)-**9**	93%
(*S*,*S*)-**6**	**8**	CH_2Cl_2 -12 °C, 20 h	>99:1 (*R*,*S*,*S*)-**9**:(*S*,*S*,*S*)-**9**	75%
(*R*,*R*)-**6**	**7**	Toluene -78 °C to rt 5 h	23:77 (*S*,*R*,*R*)-**9**:(*R*,*R*,*R*)-**9**	72%

Additionally, we started an alternative synthesis furnishing homoallylic alcohols (*S*,*R*,*R*)-**9** and (*R*,*R*,*R*)-**9** from enantiomerically-pure ethyl ester (*R*,*R*)-**10**,[23] also assessable by kinetic resolution:[28] First we converted enantiomerically pure ethyl ester (*R*,*R*)-**10** (>98% ee) into the corresponding *Weinreb*-amide (*R*,*R*)-**11** in 99% yield, followed by a *Grignard* reaction to the ketone (*R*,*R*)-**12**.[29,30,31,32] As a side product we obtained the alcohol, (1*R*,2*R*)-4-(2-phenylcyclopropyl)hepta-1,6-dien-4-ol (**16**), from a reaction of ketone **12** with two equivalents of *Grignard*-reagent. Subsequently, the ketone (*R*,*R*)-**12** could be reduced by CBS-reagent (*R*)-**13** or (*S*)-**13** in the presence of catecholborane to the homoallylic alcohols (*S*,*R*,*R*)-**9** and (*R*,*R*,*R*)-**9**.[33,34,35,36] In the reaction with (*S*)-**13** the *syn*-product (*R*,*R*,*R*)-**9** is preferred (d.r. 12:88). The *anti*-product (*S*,*R*,*R*)-**9** as the major product can be obtained by use of (*R*)-CBS reagent (*R*)-**13** (d.r. 94:6) (Scheme 4).

We used the homoallylic alcohols **9** as perfect precursors for the synthesis of natural products like constanolactone A-F,[37,38] halicholactone,[39] neohalicholactone,[39] and solandelactone A-H.[40] Accordingly we had no use for homoallylic alcohol (*S*,*S*,*S*)-**9**, but we could readily convert it into the desired homoallylic alcohol (*R*,*S*,*S*)-**9** by the previously established method. Starting from (*S*,*S*,*S*)-**9** (>98% ee), the reaction pathway was performed for the (*S*,*S*)-configured homoallylic alcohols (*R*,*S*,*S*)-**9** and (*S*,*S*,*S*)-**9**: First the (*S*,*S*,*S*)-**9** alcohol was oxidized to the corresponding ketone (*S*,*S*)-**12** by *Dess-Martin* periodinane (**14**) (42% yield); as a side-product upon prolonged reaction time and acid formation, the ring-opening product **15** could be identified. Subsequent CBS-reduction was conducted with the (*S*)-**13** enantiomer of the CBS-reagent, to obtain the *anti*-diastereomer (*R*,*S*,*S*)-**9** (d.r. 89:11) in 48% yield (Scheme 5). Homoallylic alcohols (*R*,*S*,*S*)-**9** and (*S*,*S*,*S*)-**9** could readily be separated by flash column chromatography.

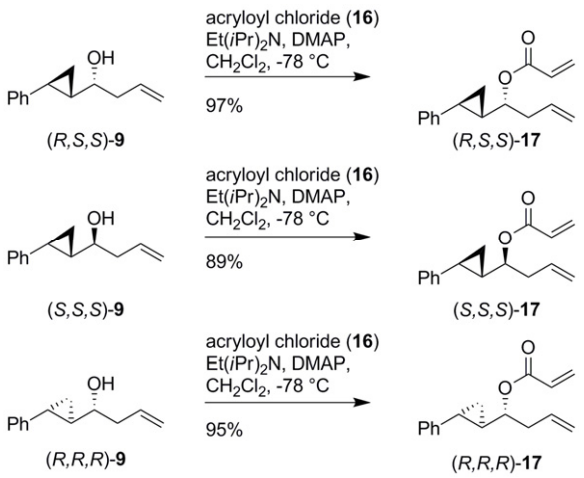


Scheme 4. Reaction scheme for the synthesis of homoallylic alcohols (*S*,*R*,*R*)-**9** and (*R*,*R*,*R*)-**9** *via* CBS-reduction.



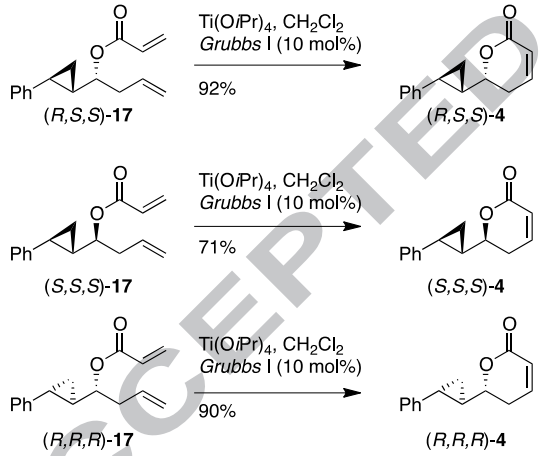
Scheme 5. Reaction scheme for the synthesis of homoallylic alcohols (*R*,*S*,*S*)-**9** and (*S*,*S*,*S*)-**9** *via* oxidation-reduction sequence.

To establish the unsaturated lactone unit, we focused on a ring closing metathesis starting from the homoallylic alcohols **9**. The first step was the esterification with acryloyl chloride (**16**) providing dienes **17** in very good yields (89-95%) (Scheme 6).[41]



Scheme 6. Esterification of alcohols **9** with acryloyl chloride (**16**).

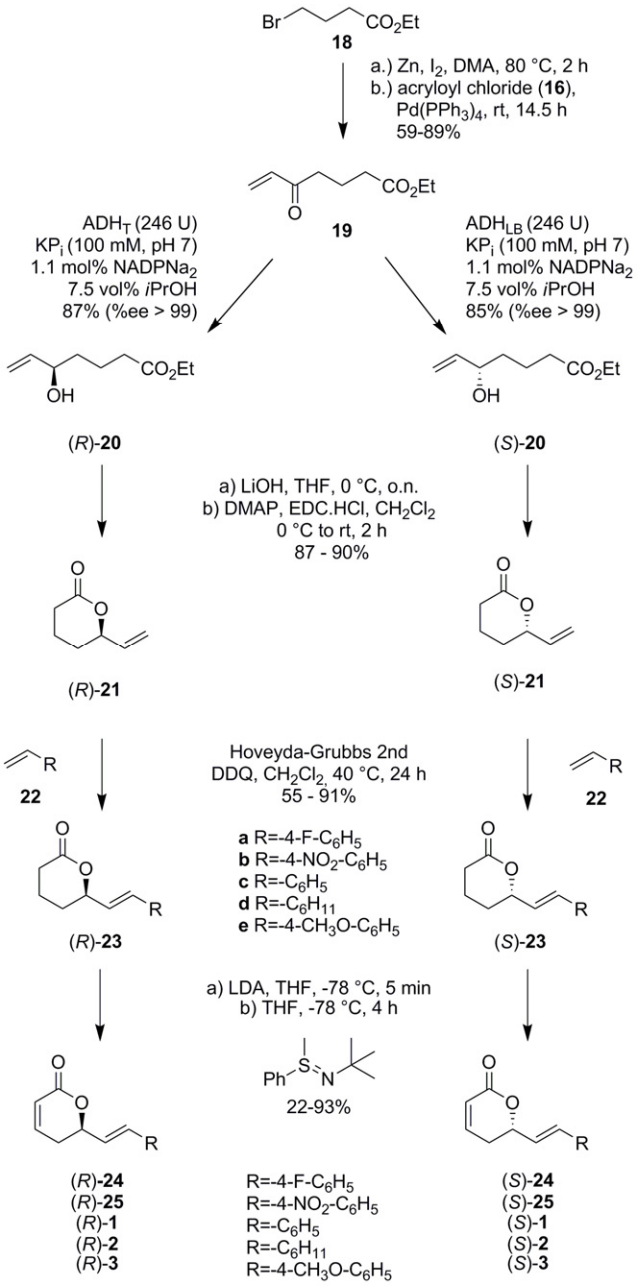
Subsequently, dienes **17** were converted into the corresponding α,β-unsaturated δ-lactones **4** with Grubbs 1st generation catalyst (Scheme 7). We used titaniumtetra-isopropylate[41] as a Lewis acid to avoid the formation of a ruthenium-chelate complex, which makes the catalyst unavailable for the metathesis. The ring closing metathesis was conducted in moderate to excellent yields (71-92%),[42] providing the target cyclopropyl lactones **4**, which are versatile precursors for the synthesis of other natural products.[37-40]



Scheme 7. Ring closing metathesis towards the desired cyclopropyl goniothalamin derivatives **4**.

2.2. Synthesis of Substituted Goniothalamins

To show the influence of different substituents on the aromatic ring and of a cyclohexane derivatives, we synthesized the compounds according to a previously reported chemoenzymatic approach.[43,44] The synthesis started with a *Negishi*-coupling of bromide **18** to acid chloride **16** yielding the corresponding vinylketone **19**. This was followed by a stereoselective alcoholdehydrogenase [from *Lactobacillus brevis* (ADH_{LB}) and *Thermoanaerobacter sp*. (ADH_T)] based reduction furnishing the two enantiomerically alcohols **20**. Cyclisation yielded the desired lactones **21**. Subsequent cross-metathesis with different styrene derivatives **22** led to saturated goniothalamin derivatives **23**. Finally, oxidation to the α,β-unsaturated lactones **1**, **2**, **3**, **24** and **25** was performed by using *N-tert*-butylphenylsulfinimidoyl chloride (Scheme 8). With this approach, we could synthesize the ten enantiomerically pure goniothalamin derivatives depicted in Figure 3.



Scheme 8. Chemoenzymatic route towards goniothalamin derivatives **24, 25, 1, 2** and **3**.

2.3. Biological activities of goniothalamin derivatives

a) Inhibition of the multidrug exporter Pdr5: With the *in vivo* liquid drug assay we demonstrated that all of the tested compounds are cytotoxic to yeast up to a concentration of 100 µg/mL except for compound *(R)*-**3** which showed no cytotoxicity at all (data not shown). First, we compared a *S. cerevisiae* strain containing the *wild-type* protein (Pdr5 *wt*) to a yeast strain expressing the dead mutant (Pdr5 EQ). We were able to show that with compound *(R)*- and *(S)*-**2** the Pdr5 *wt* strain was more resistant than the mutant strain. For *(R)*-**1**, *(R)*-**3**, *(R)*-**24** and *(S)*-

25 there was no obvious difference detectable between both strains (Table 2).

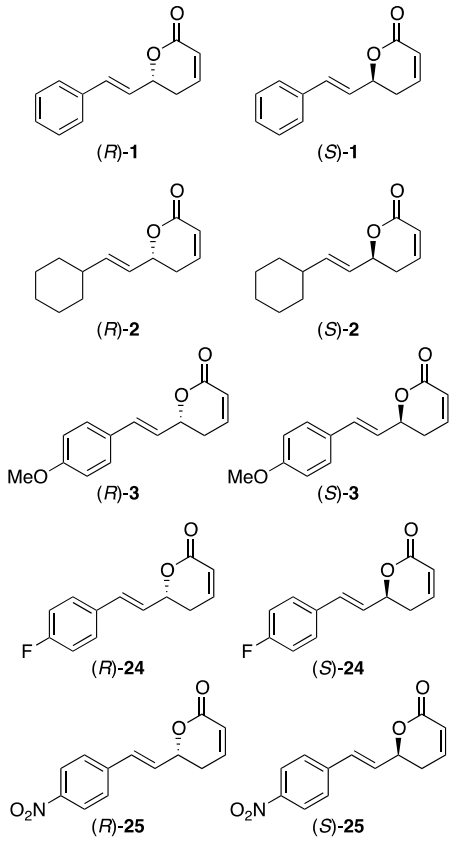


Figure 3. Synthesized enantiomerically-pure goniothalamin derivatives

Table 2. Relative transport activity of Pdr5 *wt*

compound	relative fluorescence intensity [%]		
	500 µg/mL (*125 µg/mL)	50 µg/mL	5 µg/mL (*13 µg/mL)
(R)-**1**	-2	10	60
(R)-**2**	-8	18	91
(S)-**2**	0	37	86
(R)-**3**	0	4	44
(R)-**25**	*21	-	*54
(S)-**24**	10	75	99

In the transport assay the tested compounds were compared to Pdr5 *wt* without additives, which was set to 100% transport activity. All of them displayed inhibitory effects at the highest tested concentration (Table 3). The test with lower concentrations showed that only compounds (*R*)-**1** and (*R*)-**3** were able to inhibit the transport activity clearly. (*R*)- and (*S*)-**2** only inhibit the transport efficiency with the 500 µg/mL. However, the lowest concentration did not affect the transport activity. Compound (*S*)-**24** showed the same behavior, while (*R*)-**25** showed an inhibition to 50% transport activity with the 500 µg/mL and 50 µg/mL and no inhibitory effect with the lowest concentration.

Only compound *(R)*-**3** was able to inhibit the ATPase activity of Pdr5 completely and the lowest concentration of this compound reduced ATPase activity by 75%. Both, *(R)*- and *(S)*-**2** showed the same inhibition profile for the ATPase activity, independently of their configuration. The highest concentration is only able to inhibit the ATPase activity to 50% and at the lowest concentration used in the assay no inhibitory effect was detected at all. *(R)*-**1**, *(R)*-**25** and *(S)*-**24** demonstrated an identical behavior. Higher concentrations inhibited ATPase activity to 30-50% while at lower concentrations nearly no influence was detected.

Table 3. ATPase activity of Pdr5 wt

compound	[%] ATPase activity		
	500 µg/mL (*250 µg/mL)	50 µg/mL (*125 µg/mL)	5 µg/mL (*13 µg/mL)
(R)-**1**	24,3 ± 6,5	30,0 ± 1,2	71,7 ± 2,1
(R)-**2**	56,0 ± 11,1	79,0 ± 4,4	98,0 ± 1,0
(S)-**2**	53,0 ± 2,6	74,7 ± 9,0	88,0 ± 1,7
(R)-**3**	0,0 ± 0	8,7 ± 2,1	25,7 ± 3,1
(R)-**25**	*50,0 ± 1,0	*51,0 ± 0	*79,3 ± 7,6
(S)-**24**	26,3 ± 2,5	40,7 ± 1,5	71,0 ± 0,0

Data represent mean values ± SD of at least three independent experiments

In summary, we demonstrated that the *(R)*- or *(S)*-configuration showed cytotoxic and inhibitory effects, respectively. No differences were detected between *(R)*- and *(S)*-**2** in all three assays using Pdr5 *wt*. The higher resistance of the *wildtype yeast* strain against this compound and a comparison of ATPase and transport assay leads to the conclusion that this compound is a substrate of Pdr5. Compound *(R)*-**1**, *(R)*-**25** and *(S)*-**24** showed only a cytotoxic effect in the *in vivo* assay and the two *in vitro* assays demonstrated no big differences concerning their behavior as an inhibitor of Pdr5. Only compound *(R)*-**3**, which showed no cytotoxicity in the *in vivo* assay, displayed a quantitative inhibition in both *in vitro* assays. This leads to the obvious conclusion that an inhibition of Pdr5 is independent of the stereogenic configuration [*(R)* or *(S)*] rather the substituent of the phenyl ring plays the dominant role.

b) Cytotoxicity assessment: The results for the cytotoxicity assessment are summarized in Table 4. Due to different sensitivities of cancer entities towards cytotoxic compounds, we used three different cell lines of various histological origin [A549 (human lung adenocarcinoma cell line), MCF-7 (estrogen receptor positive human breast adenocarcinoma cell line), HBL-100 (triple negative human breast cancer cell line)].

First, we evaluated the racemic mixture of natural goniothalamin (**1**) (Table 4, entry 1). It displayed acceptable antiproliferative activity on the lung carcinoma cell line A549 as well as on the triple negative breast cancer cell line HBL-100. By characterizing the cytotoxicity of enantiomerically-pure (*R*)- and (*S*)-goniothalamin [(*R*)-**1** and (*S*)-**1**] (entry 2 and 3, Table 4), respectively, the natural occurring (*R*)-goniothalamin **1** demonstrated higher efficacy in all three cell lines, in comparison to its enantiomer (*S*)-goniothalamin **1**.

To explore the influence of the phenyl ring on cell viability, we synthesized the cyclohexyl derivative **2**. The (*R*)-enantiomer showed a six-fold lower cytotoxicity in A549 human lung adenocarcinoma cells in comparison to the natural goniothalamin (*R*)-**1** (Table 4, entry 4). Moreover, in the MCF-7 and HBL-100 the natural product displays reduced cytotoxicity. The (*S*)-**2** shows even lower cytotoxicity in comparison to the natural goniothalamin (*R*)-**1** (Table 4, entry 5). Comparing cyclohexyl derivative (*S*)-**2** with the phenyl-derivative (*S*)-**1**, the cytotoxicity is roughly on the same low level.

Table 4. Cytotoxicity against human cancer cell lines after 48 h compound incubation

Compound	IC_{50} (µM)		
	A549	**MCF-7**	**HBL-100**
rac-**1**	34.7 ± 0.8	>100	22.4 ± 3.8
(*R*)-**1**	5.7 ± 1.5	55.5 ± 6.4	20.9 ± 5.4
(*S*)-**1**	70.3 ± 14.4	>100	>100
(*R*)-**2**	31.0 ± 0.3	>100	29.0 ± 3.7
(*S*)-**2**	68.6 ± 0.5	>100	48.2 ± 4.8
(*R*)-**25**	15.4 ± 0.2	28.8 ± 2.3	6.7 ± 0.5
(*S*)-**25**	>100	>100	>100
(*R*)-**3**	>100	>100	>100
(*S*)-**3**	>100	88.7 ± 5.6	>100
(*R*)-**24**	10.2 ± 0.3	76.2 ± 6.3	14.1 ± 2.3
(*S*)-**24**	>100	>100	>100
(*R,S,S*)-**4**	11.6 ± 0.2	48.2 ± 2.0	14.4 ± 0.5
(*R,R,R*)-**4**	62.8 ± 1.6	>100	43.4 ± 2.8
(*S,S,S*)-**4**	15.2 ± 0.3	68.3 ± 9.7	34.9 ± 1.5
(*R,R,R*)-**26**	>100	>100	>100
(*R,R*)-**27**	>100	>100	>100
(*S,S,S*)-**26**	>100	>100	>100
(*R,S,S*)-**26**	>100	>100	>100
(*R,S*)-**27**	>100	>100	>100

Data represent mean values ± SEM of at least three independent experiments.

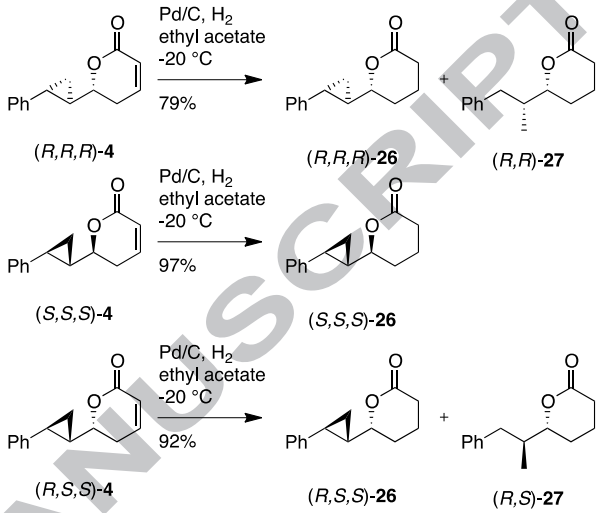
Antiproliferative activity of more electron-poor *p*-nitrophenyl-substituted goniothalamins **25** could not increase cytotoxicity for the (*S*)-compound **25** (Table 4, entry 7). The enantiomer (*R*)-**25** demonstrates a three-fold increased cytotoxicity against human breast cancer cell line HBL-100, in contrast to the natural (*R*)-enantiomer **1** (Table 4, entry 6).

The highly-activated *p*-methoxyphenyl goniothalamins **3** did not affect cell viability in any of the tested cell lines (Table 4, entry 8 and 9). Using deactivated *para*-fluorinated goniothalamins **24** only the (*R*)-enantiomer showed notable IC_{50} values (Table 4, entry 10), which are less cytotoxic compared to the naturally-occurring (*R*)-enantiomer **1**. In summary, we demonstrated the (*R*)-configuration of the lactone ring to be mandatory for cytotoxic activity of the compound cluster. Furthermore, our data suggest that the substituent does not significantly influence the identified structure activity relationship.

Additionally, we characterized cyclopropane derivatives **4** of the natural goniothalamin. The derivatives **4** demonstrated clear cytotoxicity, except for compound (*R,R,R*)-**4**, which did not influence cell viability of breast adenocarcinoma cell line MCF-7 (Table 4, entry 13). In contrast, it displayed moderate activity on the two other cancer cell lines. Cyclopropane derivative (*R,S,S*)-**4** showed comparable biological activity to (*R*)-**1**.

Hydrogenation of lactone (*R,R,R*)-**4** and (*R,S,S*)-**4** led not only to the corresponding saturated lactone (*R,R,R*)-**26** and (*R,S,S*)-**26**, but also to compounds (*R,R*)-**27** and (*R,S*)-**27** derived from the ring-opening reaction as a side product (Scheme 9). The last entry of Table 4 shows no detectable cytotoxicity for cyclopropanes (*R,R,R*)-**26**, (*S,S,S*)-**26** and (*R,S,S*)-**26** on any cell line tested. The same holds true for the goniothalamin derivatives (*R,R*)-**27** and (*R,S*)-**27**. In conclusion, our data demonstrate a saturated lactone moiety to be compulsory for antiproliferative activity of goniothalamins.

Furthermore, our cytotoxicity results of cyclopropane-containing goniothalamins **4** confirm the external double bond not to be mandatory for the biological activity of the compound. There is evidence that the molecule needs a certain conformational rigidity to show cytotoxic activity; however, in our case this rigidity is provided from either a cyclopropane ring or a (*E*)-double bond.



Scheme 9. Hydrogenation of unsaturated lactone **19a, 19b** and **18** to corresponding saturated lactone (*R,R,R*)-**26** (79%), (*S,S,S*)-**26** (97%) and (*R,S,S*)-**26** (92%) with methyl-compound (*R,R*)-**27** (3%) and (*R,S*)-**27** (4%) as a side product.

3. Conclusion

Comparing the cytotoxicity results the following three conclusions can be drawn:

First, the configuration of the stereogenic centre plays an important role for cytotoxicity. Only the (*R*)-configured goniothalamins with the vinylic double bond demonstrated cytotoxicity. The (*R*)-configuration is in accordance to natural goniothalamin (*R*)-**1**. However, looking at the inhibition of the multidrug exporter Pdr5, no pronounced configurational preference was observed, while the substitution pattern mattered.

Second, the double bond in the lactone ring is essential for the biological activity. With saturated lactones, no antiproliferative activity could be detected (derivatives **26**).

Third, the vinylic double bond is not mandatory, but a certain rigidity is required for cytotoxicity. However, this rigidity is not absolute, which is exemplified by the achieved biological activity of the cyclopropane derivatives **4**. These results are in agreement with literature, where also goniothalamin epoxides show good biological activity.[51-54] *Chandraratna et al.* reported in 1996, that cyclopropyl groups can be used as double bond isosteres for retinoid analogues, which show activity towards the retinoic acid receptors (RARs) and retinoid X receptors (RXRs).[55] These results show the same effect of a mandatory rigidity for biological activity and is in perfect accordance with our findings.

4. Experimental

4.1. General procedures

Unless specified, the reactions were carried out by standard *Schlenk*-technique under dry Ar/N_2 and magnetic stirring.

Glassware was oven-dried at 120 °C overnight. Solvents were dried and purified by conventional methods prior to use; tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. Solvents for chromatography (petroleum ether, ethyl acetate) were distilled prior to use. Column and flash column chromatography were performed on silica gel 60, 0.040-0.063 mm (230-400 mesh). TLC was performed on pre-coated plastic sheets (Polygram SIL G/UV254, Macherey-Nagel) with detection by coloration with ceric phosphomolybdic acid solution [phosphomolybdic acid (25 g), $Ce(SO_4)_2{\cdot}H_2O$ (10 g), concentrated H_2SO_4 (60 mL), H_2O (940 mL)]. Compounds **1-3** and **24-25** were synthesized according to a reported protocol.[43,44]

*4.2. Synthesis of (S,S)-(2-Phenylcyclopropyl)carbaldehyde [(S,S)-**6**] and (R,R)-(2-Phenylcyclopropyl)carbaldehyde [(R,R)-**6**]*

To a solution of 3.00 g (20.2 mmol, 1.0 equiv.) enantiomerically pure (*S,S*)-(2-phenylcyclopropyl)methanol [(*S,S*)-**5**][17] (>98% ee) in 230 mL CH_2Cl_2 under a nitrogen atmosphere at 0 °C was added 12.0 g (28.3 mmol, 1.4 equiv.) *Dess-Martin*-periodinane (**14**).[18-23] The solution was stirred 1 h at 0 °C and 14 h at room temperature. After complete conversion of the starting material (TLC), the solution was hydrolysed with a 1:1-mixture of 1 M sodium thiosulfate solution and saturated sodium bicarbonate solution. The mixture was stirred until both layers were clear. The aqueous layer was extracted several times with dichloromethane. The organic layers were combined, dried with $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography. After purification 2.8 g (19.2 mmol, 95%, 98% *ee*) of the (*S,S*)-aldehyde **6** could be isolated. The spectroscopic data is in agreement with literature data.[56]

(*R,R*)-(2-Phenylcyclopropyl)methanol [(*R,R*)-**5**] has been used according to the same protocol, using 1.4 g (9.3 mmol, 1.0 equiv.) (*R,R*)-(2-phenylcyclopropyl)methanol (**5**) (> 98% ee) in 106 mL CH_2Cl_2, 5.5 g (13.0 mmol, 1.4 equiv.) *Dess-Martin*-periodinane, resulting in 1.3 g (8.6 mmol, 92%) (*R,R*)-(2-phenylcyclopropyl)carbaldehyde (**6**) (> 98% ee).

R_f = 0.6 (PE : EE = 75 : 25). (*S,S*)-**6** $[\alpha]_D^{20}$= +395 (c = 1.5, $CHCl_3$, >98% ee).[57] (*S,S*)-**6** $[\alpha]_D^{20}$= +391 (c = 1.5, $CHCl_3$, >98% ee). (*R,R*)-**6** $[\alpha]_D^{20}$= -411 (c = 1.2, $CHCl_3$, >98% ee)[58,59]. MS (EI, 70 eV): m/z (%) = 146 (25) [$(M+H)^+$], 145 (34) [M^+], 117 (100) [$(M\text{-}CHO)^+$], 115 (68) [$(C_9H_7)^+$], 91 (26) [$(C_7H_7)^+$]. IR (ATR, film): 3032, 2832, 2732, 1702 (C=O), 1605, 1499, 1458, 1400, 1334, 1181, 1080, 1055, 1029, 965, 940, 922, 859, 759, 697 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ [ppm] = 1.53 (ddd, $^3J_{3a,1}$ = 8.3 Hz, $^3J_{3a,2}$ = 6.7 Hz, $^2J_{3a,3b}$ = 5.0 Hz, 1 H, 3-Ha), 1.75 (ddd, $^3J_{3b,2}$ = 9.2 Hz, $^3J_{3b,1}$ = 5.2 Hz, $^2J_{3b,3a}$ = 5.0 Hz, 1 H, 2-Hb), 2.18 (ddddd, $^3J_{1,3a}$ = 8.3 Hz, $^3J_{1,3b}$ = 5.2 Hz, $^3J_{1,CHO}$ = 4.6 Hz, $^3J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 2.63 (ddd, $^3J_{2,3b}$ = 9.2 Hz, $^3J_{2,3a}$ = 6.7 Hz, $^3J_{2,1}$ = 4.0 Hz, 1 H, 2-H), 7.10-7.32 (m, 5 H, arom. CH), 9.33 (d, $^3J_{CHO,1}$ = 4.6 Hz, 1 H, CHO). ^{13}C-NMR ($CDCl_3$, 151 MHz): δ [ppm] = 16.5 (C-3), 26.6 (C-1), 33.8 (C-2), 126.7 (o-C), 127.1 (p-C), 128.6 (m-C), 139.0 (i-C), 199.7 (CHO).

4.3 Synthesis of (1R,1'S,2'S)-1-(2-Phenylcyclopropyl)but-3-en-1-ol [(*R,S,S*)-**9**] *and (1S,1'S,2'S)-1-(2-Phenylcyclopropyl)but-3-en-1-ol* [(*S,S,S*)-**9**]

Method A: Allyl addition with *Roush*-reagent

In a *Schlenk*-flask was dissolved 10.8 g (38.3 mmol, 2.0 equiv.) allylboronic ester **7** in 100 mL toluene. The solution was coold to -78 °C and a solution of 2.6 g (17.7 mmol, 1.0 equiv., >98% ee) aldehyde (*S,S*)-**6** in 20 mL toluene was added *via* syringe. The solution was stirred for 5 h at -78 °C. At this temperature the reaction-mixture was washed with 50 mL of a saturated ammonium-carbonate solution. The solution was warmed to room temperature and the aqueous phase was extracted three times with Et_2O and two times with ethyl acetate. The combined organic layers were dried with $MgSO_4$, filtered and the solvent was evaporated under vacuum. The diastereomeric ratio was determined by ^{1}H-NMR (78:22). The crude product was purified by column chromatography (PE : EE = 90 : 10, then 80 : 20). 2.5 g (13.2 mmol, 71%, >98% ee) of (*1R,1'S,2'S*)-1-(2-Phenylcyclopropyl)but-3-en-1-ol [(*R,S,S*)-**9**], 324 mg (1.7 mmol, 10%) of a diastereomeric mixture and 386 mg (2.1 mmol, 12%, 98% ee) (*1S,1'S,2'S*)-1-(2-phenylcyclopropyl) but-3-en-1-ol [(*S,S,S*)-**9**] could be isolated (93%).

Method B: Allyl addition with *Leighton*-reagent[60]

To a solution of 309.1 mg (0.6 mmol, 1.5 equiv.) of the *Leighton*-reagent (*S,S*)-**8** in 3 mL dichloromethane, 54.8 mg of aldehyde (*S,S*)-**6** dissolved in 1 mL dichloromethane was slowly added at -10 °C under an argon atmosphere. The reaction mixture was stirred 20 h at -10 °C. Afterwards at -10 °C, 5 mL of ethyl acetate and 5 mL of a 1 M HCl solution was added to hydrolyse. The mixture was stirred for 10 min at -10 °C and allowed to warm to room temperature. The reaction mixture was extracted three times with ethyl acetate. The combined organic layers were dried with $MgSO_4$, filtered and the solvent was evaporated under vacuum. The diastereomeric ratio was determined by ^{1}H-NMR (>99:1).

After column chromatography (PE:EE 70:30) 68 mg (0.3 mmol, 75%, 98% ee) of homoallylic alcohol (*R,S,S*)-**9** could be isolated.

Method C: CBS-reduction[61] of ketone (*S,S*)-**12**

To a solution of 276 mg (1.48 mmol, 1.00 equiv.) of ketone (*S,S*)-**12** in 4.14 mL toluene was added under an argon atmosphere 185 mg molecular sieve (powder, 4 Å) and 3 mL (2.96 mmol, 2.0 equiv. 1 M in toluene) (*S*)-CBS-reagent (*S*)-**13**. The reaction mixture was cooled to -78 °C and 3 mL (2.96 mmol, 2.0 equiv., 1 M in toluene) of catecholborane was slowly added. The mixture was stirred overnight, where it warms to room temperature. After full conversion of starting material (TLC), the mixture was again cooled to -78 °C and 600 µL of absolute methanol was added. The mixture was warmed to room temperature and 10 mL of Et_2O and 10 mL of a 2:1 mixture of 1 M NaOH and saturated $NaHCO_3$ solution was added. The reaction mixture was extracted four times with Et_2O. The combined organic layers were washed with 1 M HCl and brine. The organic layers were dried with $MgSO_4$, filtered and the solvent was evaporated under vacuum. The crude product was purified by column chromatography (PE : EE = 90 : 10). 121 mg (0.64 mmol, 43%, >98% ee) of homoallylic alcohol (*R,S,S*)-**9** and 15 mg (80 µmol, 5%, 98% ee) of homoallylic alcohol (*S,S,S*)-**9** could be isolated.

R_f = 0.4 (PE : EE = 75 : 25). Rf = 0.6 (PE : EE = 70 : 30). (*R,S,S*)-**9** $[\alpha]_D^{20}$= +84 (c = 1.0, $CHCl_3$, >98% ee). (*S,S,S*)-**9** $[\alpha]_D^{20}$= +94 (c = 0.9 $CHCl_3$, >98% ee).

(*1R,1'S,2'S*)-*1-(2'-Phenylcyclopropyl)but-3-en-1-ol* [(*R,S,S*)-**9**]

HPLC: column: Chiracel OB (250 mm · 4.6 mm, Fa. Daicel); solvent: heptane : 2-propanol = 90 : 10, flowrate: 0.5 mL/min; pressure: 23 bar, detection:

UV 220 nm; t_R [(*R,S,S*)-**9**] 10.8 min; t_R [(*S,R,R*)-**9**] 13.4 min. MS (EI, 70 eV): m/z (%) = 188 (<5) [M^+], 170 (8) [$(M\text{-}H_2O)^+$], 147 (20) [$(M\text{-}C_3H_5)^+$], 142 (7) [$(C_{11}H_{10})^+$], 129 (100) [$(C_{10}H_9)^+$], 107 (54) [$(C_8H_{11})^+$], 104 (69) [$(C_7H_4O)^+$], 91 (69) [$(C_7H_7)^+$], 77 (20)

$[(C_6H_5)^+]$. Elemental analysis $C_{13}H_{16}O$ (188.27 g/mol): calc.: C 82.94 H 8.57; found: C 82.68 H 8.68. IR (ATR, film): 3387 (OH), 3072, 3002, 2978, 2903, 1641, 1604, 1497, 1464, 1431, 1413, 1277, 1090, 1067, 1047, 1030, 991, 913, 749, 697 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ [ppm] = 0.95 (m_c, 2 H, 3'-H), 1.28 (dddd, $^3J_{1',3'}$ = 8.0 Hz, $^3J_{1',1}$ = 7.6 Hz, $^3J_{1',3'}$ = 6.3 Hz, $^3J_{1',2'}$ = 4.6 Hz, 1 H, 1'-H), 1.76 (d, $^3J_{OH,1}$ = 3.6 Hz, 1 H, OH), 1.94 (ddd, $^3J_{2',3'}$ = 8.2 Hz, $^3J_{2',3'}$ = 6.0 Hz, $^3J_{2',1'}$ = 4.6 Hz, 1 H, 2'-H), 2.35 (dddt, $^2J_{2a,2b}$ = 14.0 Hz, $^3J_{2a,1}$ = 7.7 Hz, $^3J_{2a,3}$ = 7.6 Hz, $^4J_{2a,4}$ = 1.2 Hz, 2-Ha), 2.46 (dddt, $^2J_{2b,2a}$ = 14.0 Hz, $^3J_{2b,3}$ = 6.7 Hz, $^3J_{2b,1}$ = 4.6 Hz, $^4J_{2b,4}$ = 1.4 Hz, 1 H, 2-Hb), 3.26 (dddd, $^3J_{1,2a}$ = 7.7 Hz, $^3J_{1,1'}$ = 7.6 Hz, $^3J_{1,2b}$ = 4.6 Hz, $^3J_{1,OH}$ = 3.6 Hz, 1 H, 1-H), 5.1 (ddt, $^3J_{4a,3}$ = 10.2 Hz, $^2J_{4a,4b}$ = 2.0 Hz, $^4J_{4a,2}$ = 1.1 Hz, 1 H, 4-Ha), 5.17 (ddt, $^3J_{4b,3}$ = 17.1 Hz, $^2J_{4b,4a}$ = 2.0 Hz, $^4J_{4b,2}$ = 1.4 Hz, 1 H, 4-Hb), 5.90 (dddd, $^3J_{3,4b}$ = 17.1 Hz, $^3J_{3,4a}$ = 10.2 Hz, $^3J_{3,2a}$ = 7.6 Hz, $^3J_{3,2b}$ = 6.7 Hz, 1 H,3-H), 7.07-7.27 (m, 5 H, arom. CH). ^{13}C-NMR ($CDCl_3$, 151 MHz): δ [ppm] = 13.4 (C-3'), 20.9 (C-2'), 28.9 (C-1'), 41.6 (C-2), 74.4 (C-1), 118.0 (C-4), 125.6 (arom. CH), 126.1 (arom. CH), 128.3 (arom. CH), 134.6 (C-3), 142.6 (i-C).

(1S,1'S,2'S)-1-(2'-Phenylcyclopropyl)but-3-en-1-ol [(*S,S,S*)-**9**]

R_f = 0.3 (PE : EE = 75 : 25), m.p.: 35 °C. [(*S,S,S*)-**9**] $[\alpha]_D^{20}$= +94 (c = 0.9 $CHCl_3$ >98% ee); HPLC: column: Chiracel OB (250 mm·4.6 mm, Fa. Daicel) ; solvent: heptane : 2-propanol = 90 : 10; flowrate 0.5 mL/min; pressure: 23 bar; detection: UV 220 nm; t_R [(*S,S,S*)-**9**]10.4 min; t_R [(*R,R,R*)-**9**] 16.2 min. MS (EI, 70 eV): m/z (%) = 188 (<5) $[M^+]$, 170 (12) $[(M-H_2O)^+]$, 147 (18) $[M-C_3H_5)^+]$, 142 (17) $(C_{11}H_{10})^+]$, 129 (100) $[(C_{10}H_9)^+]$, 107 (54) $[(C_8H_{11})^+]$, 104 (54) $[(C_7H_4O^+)]$, 91 (65) $[(C_7H_7)^+]$, 77 (18) $[(C_6H_5)^+]$. Elemental analysis: $C_{13}H_{16}O$ (188.27 g/mol): calc: C 82.94 H 8.57; found: C 82.32 H 8.51. IR (ATR, film): 3387 (OH), 3072, 3002, 2978, 2903, 1641, 1604, 1497, 1464, 1431, 1413, 1277, 1090, 1067, 1047, 1030, 991, 913, 749, 697 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ [ppm] = 0.97 (ddd, $^3J_{3'a,1'}$ = 8.4 Hz, $^3J_{3'a,2'}$ = 5.1 Hz, $^2J_{3'a,3'b}$ = 5.1 Hz, 1 H, 3'-Ha), 1.04 (ddd, $^3J_{3'b,2'}$ = 8.9 Hz, $^3J_{3'b,1'}$ = 5.6 Hz, $^2J_{3'b,3'a}$ = 5.1 Hz, 1 H, 3'-Hb), 1.28 (dddd, $^3J_{1',3'a}$ = 8.4 Hz, $^3J_{1',1}$ = 7.8 Hz, $^3J_{1',3'b}$ = 5.6 Hz, $^3J_{1',2'}$ = 4.5 Hz, 1 H, 1'-H), 1.69 (d, $^3J_{OH,1'}$ = 3.5 Hz, 1 H, OH), 1.84 (ddd, $^3J_{2',3'b}$ = 8.9 Hz, $^3J_{2',3'a}$ = 5.1 Hz, $^3J_{2',1'}$ = 4.5 Hz, 1 H, 2'-H), 2.37 (dddt, $^2J_{2a,2b}$ = 15.2 Hz, $^3J_{2a,3}$ = 7.8 Hz, $^3J_{2a,1}$ = 7.8 Hz, $^4J_{2a,4}$ = 1.2 Hz, 1 H, 2-Ha), 2.48 (dddt, $^2J_{2b,2a}$ = 15.2 Hz, $^3J_{2b,3}$ = 6.4 Hz, $^3J_{2b,1}$ = 4.6 Hz, $^4J_{2b,4}$ = 1.3 Hz, 1 H, 2-Hb), 3.25 (dddd, $^3J_{1,1'}$ = 7.8 Hz, $^3J_{1,2a}$ = 7.8 Hz, $^3J_{1,2b}$ = 4.6 Hz, $^3J_{1,OH}$ = 3.5 Hz 1 H, 1-H), 5.13 (ddt, $^3J_{4a,3}$ = 10.3 Hz, $^2J_{4a,4b}$ = 2.0 Hz, $^5J_{4a,2}$ = 1.0 Hz, 1 H, 4-Ha), 5.14 (ddt, $^3J_{4b,3}$ = 16.9 Hz, $^2J_{4b,4a}$ = 2.0 Hz, $^5J_{4b,2}$ = 1.4 Hz, 1 H, 4-Hb), 5.87 (dddd, $^3J_{3,4b}$ = 16.9 Hz, $^3J_{3,4a}$ = 10.3 Hz, $^3J_{3,2a}$ = 7.8 Hz, $^3J_{3,2b}$ = 6.4 Hz, 1 H, 3-H), 7.05-7.27 (m, 5 H, arom. CH). ^{13}C-NMR ($CDCl_3$, 151 MHz): δ [ppm] = 13.5 (C-3'), 20.9 (C-2'), 28.9 (C-1'), 41.9 (C-2), 74.5 (C-1), 118.1 (C-4), 125.7 (arom. CH), 126.0 (arom. CH), 128.3 (arom. CH), 134.5 (C-3), 142.3 (i-C).

4.4. Synthesis of (1S,1'R,2'R)-1-(2-Phenylcyclopropyl)but-3-en-1-ol [(*S,R,R*)-**9**] *and (1R,1'R,2'R)-1-(2-Phenylcyclopropyl)but-3-en-1-ol* [(*R,R,R*)-**9**]

Method C: CBS-reduction[54] of ketone (*R,R*)-**12**

84 mg (451 µmol, 1.0 equiv.) of ketone (*R,R*)-**12** with 0.90 mL (0.90 mmol, 2.0 equiv. 1 M in toluene) of (*S*)-CBS-reagent (*S*)-**13** and 0.90 mL (0.90 mmol, 2.0 equiv. 1 M in toluene) catecholborane have been reduced *via* method C for (*R,S,S*)-**9** and (*S,S,S*)-**9**. After purification 10 mg (53 µmol, 11%, 81% ee) of homoallylic alcohol (*S,R,R*)-**9** and 75 mg (398 µmol, 88%, 95% ee) of homoallylic alcohol (*R,R,R*)-**9** have been isolated. The diastereomeric ratio was 12:88. The spectroscopic data accords to (*R,S,S*)-**9** and (*S,S,S*)-**9**.

Analogously, 100 mg (540 mmol, 1.0 Äq) of ketone (*R,R*)-**12** was reduced with 1.08 mL (1.08 mmol, 2.0 equiv. 1 M in toluene) of (*R*)-CBS-reagent (*R*)-**13** and 0.54 mL (1.08 mmol, 2.0 equiv. 2 M in toluene) catecholborane. After purification 60 mg (319 µmol, 93%, 81% ee) of homoallylic alcohol [(*S,R,R*)-**9**] and 6.0 mg (32 µmol, 5%, 98% ee) of homoallylic alcohol (*R,R,R*)-**9** have been isolated. The spectroscopic data accords to (*S,R,R*)-**9** and (*R,R,R*)-**9**.

4.5. Synthesis of (1S,2S)-N-Methoxy-N-methyl-2-phenylcyclopropanecarboxamide [(*S,S*)-**11**] *and (1R,2R)-2-N-Methoxy-N-methyl-2-phenylcyclopropanecarboxamide* [(*R,R*)-**11**]

Under an argon atmosphere 198 mg (1.0 mmol, 1.0 equiv.) of ester (*S,S*)-**10** was dissolved in 4 mL abs. THF and 152 mg (1.6 mmol, 1.5 equiv.) *N*-methoxy-*N*-methylamino hydrochloride was added and cooled to -20 °C. With the use of a syringe pump 1.6 mL of an *iso*-propylmagnesium chloride solution (2 M in THF, 3.1 mmol, 3.0 equiv.) was added over 45 min. After full addition the mixture stirred for 20 min at -20 °C (control *via* TLC). After full conversion, the solution was hydrolysed with saturated ammonium carbonate solution. The mixture was warmed to room temperature and diethylether and water was added until both layers were clear. The layers were separated and the aqueous layer was extracted three times with diethylether. The combined organic layers were dried with $MgSO_4$, filtered and the solvent was evaporated. After column chromatography (PE : EE 80 : 20, then 70:30) 160 mg (0.8 mmol, 78%) oft he *Weinreb*-amide (*S,S*)-**11** could be isolated as a colourless oil. The spectroscopic data accord to literature.[62]

Analogous to this method, 400 mg (2.1 mmol, 1.0 equiv.) of ester (*R,R*)-**10** was dissolved in 7.2 mL abs. THF. Afterwards, 312 mg (3.2 mmol, 1.5 equiv.) *N*-methoxy-*N*-methylamino hydrochloride was added. At -20 °C 3.2 mL of an *iso*-propylmagnesium chloride solution (2 M in THF, 6.3 mmol, 3.0 equiv.) was added over 45 min. After 30 min complete conversion was detected. After workup, 426 mg (2.1 mmol, 99%) of (*R,R*)-**11** was isolated.

R_f = 0.35 (PE : EE = 70 : 30); (*S,S*)-**11** $[\alpha]_D^{20}$= +228 (c = 1.0, $CHCl_3$, 97% ee); (*R,R*)-**11** $[\alpha]_D^{20}$= -233 (c = 0.8 $CHCl_3$, 98% ee); HPLC: column: Chiracel OD-H (250 mm · 4.6 mm, Fa. Daicel); solvent: heptane : 2-propanol = 98 : 2; flowrate: 0.5 mL/min; pressure: 33 bar; detection: UV 225 nm; t_R[(*R,R*)-**11**]: 26.2 min; t_R[(*S,S*)-**11**]: 29.1 min; MS (EI, 70 eV): m/z (%) = 205 (25) $[M^+]$, 145 (100) $[(C_{10}H_9O)^+]$, 127 (84) $[(C_6H_9NO_2)^+]$, 117 (72) $[(C_9H_9)^+]$, 115 (71) $[(C_9H_7)^+]$, 91 (33) $[(C_7H_7)^+]$. HRMS (ESI, positiv-Ion): calc.: 228.1001 ($C_{12}H_{15}NO_2Na$) $[(M^+Na)^+]$ found: 228.0995 ($C_{12}H_{15}NO_2Na$) $[(M^+Na)^+]$. Elemental analysis: $C_{12}H_{15}NO_2$ (205.25 g/mol): calc.: C 70.22 H 7.37 N 6.82; found: C 69.90 H 7.48 N 6.85; IR (ATR, film): 3029, 3005, 2967, 2937, 1651 (C=O), 1605, 1498, 1460, 1439, 1421, 1394, 1368, 1174, 1119, 1097, 1022, 1004, 995, 939, 749, 698 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ [ppm] = 1.31 (ddd, $^3J_{3a,1}$ = 8.4 Hz, $^3J_{3a,2}$ = 6.4 Hz, $^2J_{3a,3b}$ = 4.3 Hz, 1 H, 3-Ha), 1.63 (ddd, $^3J_{3b,2}$ = 9.1 Hz, $^3J_{3b,1}$ = 5.4 Hz, $^2J_{3b,3a}$ = 4.3 Hz, 1 H, 3-Hb), 2.42 (br, 1 H, 1-H), 2.51 (ddd, $^3J_{2,3b}$ = 9.1 Hz, $^3J_{2,3a}$ = 6.4 Hz, $^3J_{2,1}$ = 4.2 H, 1 H, 2-H), 3.24 (s, 3 H, CH3), 3.69 (s, 3 H, OCH3), 7.12-7.30 (m, 5 H, arom. CH). ^{13}C-NMR ($CDCl_3$, 151 MHz): δ [ppm] = 16.5 (C-3), 21.6 (C-1), 25.9 (C-2), 32.6 (NCH3), 61.7 (OCH3), 126.3 (arom. CH), 128.3 (arom. CH), 128.6 (arom. CH), 140.8 (i-C), 173.1 (NCO).

4.6. Synthesis of (1'S,2'S)-1-(2'-Phenylcyclopropyl)but-3-en-1-on [(*S,S*)-**12**] *and (1'R,2'R)-1-(2'-Phenylcyclopropyl)but-3-en-1-on* [(*R,R*)-**12**]

According to a procedure from *Datta*[63] et al. and *Lee*[64] et al. 100 mg (487 µmol, 1.0 equiv.) of *Weinreb*-amide (*S,S*)-**11** was dissolved in 2.5 mL abs. THF. The mixture was cooled to -78 °C

and 0.6 mL (584 µmol, 1.2 equiv.) of a 1 M allylmagnesium bromide solution in diethylether was added slowly. The mixture was stirred for 30 min at -78 °C (reaction control by TLC). After full conversion it was hydrolysed with 5 mL of water and 0.8 mL of 1 M HCl. The mixture was warmed to room temperature and the two layers have been separated. The aqueous layer was extracted with diethylether three times. The combined organic layers were dried with $MgSO_4$, filtered and the solvent was evaporated. The crude product was purified by column chromatography (PE : EE 95 : 5). 70 mg (376 µmol, 77%, 97% ee) of ketone (*S,S*)-**12** could be isolated as a colourless oil.

(*R,R*)-**12** could be synthesized with the same protocol, using 119 mg (580 µmol, 1.0 equiv.) of (*1R,2R*)-*N,N*-methoxymethyl-2-phenylcyclopropancarboxamide [(*R,R*)-**11**] at -78 °C with 1.16 mL (1.16 mmol, 2.0 equiv.) of a 1 M allylmagnesium bromide solution in diethylether. The mixture was stirred for 30 min at -78 °C and hydrolysis with saturated ammonium carbonate solution at -60 °C. The mixture of two layers was warmed to room temperature and 2 mL of 1 M HCl was added. It was added water and ether until both layers were clear. The layers have been separated and the organic layer was extracted with diethylether a couple of times, followed by two times with ethyl acetate. The combined organic layers were dried with $MgSO_4$, filtered and the solvent was evaporated. The crude product was purified by column chromatography (PE : EE 95 : 5). 70 mg (38 µmol, 66%, 98% ee) of ketone (*R,R*)-**12** could be isolated as a colourless oil.

R_f = 0.2 (PE : EE = 70 : 30); (*S,S*)-**12** $[\alpha]_D^{20}$= +475 (c = 1.2, $CHCl_3$, 97% ee); (*R,R*)-**12** $[\alpha]_D^{20}$= -479 (c = 0.6, $CHCl_3$, >98% ee); HPLC: column: Chiracel OD-H (250 mm · 4.6 mm, Fa. Daicel); solvent: heptane : 2-propanol = 99.8 : 0.2; flowrate: 0.5 mL/min, pressure: 33 bar; detection: UV 225 nm; t_R[(*S,S*)-**12**]: 29.4 min; t_R[(*R,R*)-**12**]: 32.3 min. MS (EI, 70 eV): 186 (8) [M^+], 145 (91) [(M-C_3H_5)$^+$], 127 (78), 117 (100) [(C_9H_9)$^+$], 115 (85) [(C_9H_7)$^+$], 91 (45) [(C_7H_7)$^+$], 69 (19) [(C_4H_5O)$^+$]. Elemental analyse: $C_{13}H_{14}O_2$ (186.25 g/mol) calc.: C 83.83 H 7.58; found: C 83.40 H 7.63, IR (ATR, film): 3081, 3031, 1698 (C=O), 1636, 1604, 1497, 1457, 1432, 1398, 1342, 1212, 1180, 1120, 1058, 1044, 1016, 993, 918, 837, 749, 698 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ [ppm] = 1.39 (ddd, $^3J_{3'a,1'}$ = 8.1 Hz, $^3J_{3'a,2'}$ = 6.6 Hz, ^2J 3'a,3'b = 4.2 Hz 1 H, 3'-H_a), 1.69 (ddd, $^3J_{3'b,2'}$ = 9.1 Hz, $^3J_{3'b,1'}$ = 5.3 Hz, $^2J_{3'b,3'a}$ = 4.2 Hz, 1 H, 3'-H_b) 2.23 (ddd, $^3J_{1',3'a}$ = 8.1 Hz, $^3J_{1',3'b}$ = 5.3 Hz, ^3J1',2' = 4.0 Hz, 1 H, 1'-H), 2.53 (ddd, $^3J_{2',3'b}$ = 9.1 Hz, $^3J_{2',3'a}$ = 6.6 Hz, $^3J_{2',1'}$ = 4.0 Hz, 1 H, 2'-H), 3.36 (dt, $^3J_{2,3}$ = 6.8 Hz, $^4J_{2,4}$ = 1.4 Hz, 2 H, 2-H), 5.17 (ddt, $^3J_{4a,3}$ = 17.2 Hz, $^2J_{4a,4b}$ = 3.0 Hz, $^4J_{4a,2}$ = 1.4 Hz, 1 H, 4-H_a) 5.20 (ddt, $^3J_{4b,3}$ = 10.2 Hz, $^2J_{4b,4a}$ = 3.0 Hz, $^4J_{4b,2}$ = 1.4 Hz, 1 H, 4-H_b) 5.97 (ddt, $^3J_{3,4a}$ = 17.2 Hz, $^3J_{3,4b}$ = 10.2 Hz, $^3J_{3,2}$ = 6.8 Hz, 1 H, 3-H), 7.08-7.29 (m, 5 H, arom. CH). ^{13}C-NMR ($CDCl_3$, 151 MHz): δ [ppm] = 19.2 (C-3'), 29.2 (C-2') 31.8 (C-1'), 48.7 (C-2), 119.0 (C-4), 126.1 (arom. CH), 126.6 (arom. CH), 128.5 (arom. CH), 130.5 (C-3), 140.3 (i-C), 206.6 (C-1).

4.7. Synthesis of (1R,1'S,2'S)-1-(2'-Phenylcyclopropyl)but-3-en-1-yl acrylate [(*R,S,S*)-**17**]

850 mg (4.51 mmol, 1.00 equiv.) of homoallyl alcohol (*R,S,S*)-**9** was dissolved in 15 mL absolute dichloromethane under an argon atmosphere. The solution was cooled to -78 °C when 2.32 mL ethyldiisopropylamine (13.5 mmol, 3.00 equiv.) and 55 mg (0.45 mmol, 0.1 equiv.) DMAP was added. To this mixture 550 µl (6.77 mmol, 1.50 equiv.) acryloyl chloride was added slowly. The mixture was stirred 2 h at -78 °C until no starting material could be detected (TLC control). After hydrolysis with a saturated ammonium chloride-solution at -78 °C the mixture was warmed to RT and the two layers were separated. The aqueous phase was extracted three times with dichloromethane. The organic layers were combined, dried with $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified *via* column chromatography (PE : EE = 90 : 10). The clean product (*R,S,S*)-**17** could be isolated as a colourless oil [1.06 g (4.37 mmol, 97%, >98% ee)].

Dienes (*S,S,S*)-**17** and (*R,R,R*)-**17** have been synthesized according to the same protocol.

(1R,1'S,2'S)-1-(2'-Phenylcyclopropyl)but-3-en-1-yl acrylate [(*R,S,S*)-**17**]

R_f = 0.8 (PE : EE = 70 : 30); $[\alpha]_D^{20}$= +147 (c = 1.0, $CHCl_3$); MS (EI, 70 eV): m/z (%) = 241 (<5) [M^+], 207 (<5) [($C_{13}H_{13}O_2$)$^+$], 187 (<5) [($C_{13}H_{15}O$)$^+$], 170 (19) [($C_{13}H_{14}$)$^+$], 161 (28) [($C_{11}H_{13}O$)$^+$], 142 (10) [($C_{11}H_{10}O$)$^+$], 129 (56) [($C_{10}H_9$)$^+$], 117 [(C_9H_9)$^+$], 104 (40) [(C_8H_8)$^+$], 91 (34) [(C_7H_7)$^+$], 70 (15) [(C_6H_7)$^+$], 55 (100) [(C_3H_5O)$^+$]. Elemental analysis: $C_{16}H_{18}O_2$ (242.31 g/mol): calc.: C 79.31 H 7.49; found: C 79.22 H 7.58. IR (ATR, film): 3077, 3028, 2944, 1721 (C=O), 1619, 1638, 1605, 1498, 1404, 1295, 1270, 1195, 1092, 1047, 983, 916, 809, 751, 698 cm^{-1}. ^{1}H-NMR (CDCl3, 600 MHz): δ [ppm] = 0.96 (ddd, $^3J_{3'''a,2''}$ = 8.9 Hz, $^3J_{3''a,1''}$ = 5.5 Hz, $^2J_{3''a,3''b}$= 5.3 Hz, 1 Hz, 3''-H_a), 1.03 (ddd, $^3J_{3''b,1''}$ = 8.7 Hz, $^3J_{3''b,2''}$ = 5.3 Hz, $^2J_{3''b,3''a}$ = 5.3 Hz, 1 H, 3''-H_b), 1.35 (dddd, $^3J_{1'',3''b}$ = 8.7 Hz, $^3J_{1'',1'}$ = 8.6 Hz, $^3J_{1'',3''a}$ = 5.5 Hz, $^3J_{1'',2''}$ = 4.5 Hz, 1 H, 1''-H), 2.07 (ddd, $^3J_{2'',3''a}$ = 8.9 Hz, $^3J_{2'',3''b}$ = 5.3 Hz, $^3J_{2'',1''}$ = 4.5 Hz, 1 H, 2''-H), 2.49 (ddddd, $^2J_{2'a,2'b}$ = 14.3 Hz, $^3J_{2'a,3'}$ = 7.5 Hz, $^3J_{2'a,1'}$ = 6.8 Hz, $^4J_{2'a,4'a}$ = 1.2 Hz, $^4J_{2'a,4'b}$ = 1.2 Hz, 1 H, 2'-H_a), 2.54 (ddddd, $^2J_{2'b,2'a}$ = 14.3 Hz, $^3J_{2'b,3'}$ = 6.6 Hz, $^3J_{2'b,1'}$ = 5.5 Hz, $^4J_{2'b,4'a}$ = 1.3 Hz, $^4J_{2'b,4'b}$ = 1.3 Hz, 1 H, 2'-H_b), 4.62 (ddd, $^3J_{1',1''}$ = 8.6 Hz, $^3J_{1',2'a}$ = 6.8 Hz, $^3J_{1',2'b}$ = 5.5 Hz, 1 H, 1'-H), 5.08 (dddd, $^3J_{4'a,3'}$ = 10.2 Hz, $^2J_{4'a,4'b}$ = 2.1 Hz, $^4J_{4'a,2'b}$ = 1.3 Hz, $^4J_{4'a,2'a}$ = 1.2 Hz, 1 H, 4'-H_a), 5.21 (dddd, $^3J_{4'b,3'}$ = 17.0 Hz, $^2J_{4'b,4'a}$ = 2.1 Hz, $^4J_{4'b,2'b}$ = 1.3 Hz, $^4J_{4'b,2'a}$ = 1.2 Hz, 1 H, 4'-H_b), 5.81 (dd, $^3J_{3a,2}$ = 10.4 Hz, $^2J_{3a,3b}$ = 1.5 Hz, 1 H, 3-H_a), 5.83 (dddd, $^3J_{3',4'b}$ = 17.0 Hz, $^3J_{3',4'a}$ = 10.2 Hz, $^3J_{3',2'a}$ = 7.5 Hz, $^3J_{3',2'b}$ = 6.6 Hz, 1 H, 3'-H), 6.11 (dd, $^3J_{2,3b}$ = 17.3 Hz, $^3J_{2,3a}$ = 10.4 Hz, 1 H, 2-H), 6.40 (dd, $^3J_{3b,2}$ = 17.3 Hz, $^2J_{3b,3a}$ = 1.5 Hz, 1 H, 3-H_b), 7.04-7.29 (m, 5 H, arom. CH). ^{13}C-NMR ($CDCl_3$, 151 MHz):δ [ppm] = 13.3, 21.5, 26.3, 38.9, 76.8, 117.9,125.7, 126.1, 128.3, 128.7, 130.7, 133.4, 142.2, 165.9.

(1S,1'S,2'S)-1-(2'-Phenylcyclopropyl)but-3-en-1-yl acrylate [(*S,S,S*)-**17**] *and (1R,1'R,2'R)-1-(2'-Phenylcyclopropyl)but-3-en-1-yl acrylate* [(*R,R,R*)-**17**]

(*S,S,S*)-**17**: 514 mg (2.12 mmol, 89%), colourless oil. [(*R,R,R*)-**17**: 1.3 g (5.4 mmol, 95%), colourless oil. R_f = 0.8 (PE : EE = 70 : 30). [(*R,R,R*)-**17** $[\alpha]_D^{20}$= -38 (c = 1.0, $CHCl_3$). MS (EI, 70 eV): m/z (%) = 242 (<5) [M^+], 201 (<5) [(M-C_3H_5)$^+$], 187 (<5) [($C_{13}H_{15}O$)$^+$], 170 (26) [($C_{13}H_{14}$)$^+$], 161 (51) [($C_{11}H_{13}O$)$^+$], 142 (13) [($C_{11}H_{10}O$)$^+$], 129 (69) [($C_{10}H_9$)$^+$], 104 (44) [(C_8H_8)$^+$], 91 (41) [(C_7H_7)$^+$], 55 (100) [(C_3H_5O)$^+$]. Elemental analysis: $C_{16}H_{18}O_2$ (242.31 g/mol): calc.: C 79.31 H 7.49; found: C 79.07 H 7.50. IR (ATR, film): 3078, 3028, 3008, 2942, 1720 (C=O), 1637, 1605, 1498, 1465, 1404, 1295, 1270, 1195, 1044, 983, 919, 808, 752, 697 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ [ppm] = 0.97 (ddd, $^3J_{3''a,1''}$ = 8.5 Hz, $^3J_{3''a,2''}$ = 5.3 Hz, $^2J_{3''a,3''b}$ = 5.3 Hz, 1 H, 3''-Ha), 1.12 (ddd, $^3J_{3''b,2''}$ = 8.9 Hz, $^3J_{3''b,1''}$ = 5.5 Hz, $^2J_{3''b,3''a}$ = 5.3 Hz, 1 H, 3''-Hb), 1.37 (dddd, $^3J_{1'',1'}$ = 8.6 Hz, $^3J_{1'',3''a}$ = 8.5 Hz, $^3J_{1'',3''b}$ = 5.5 Hz, $^3J_{1'',2''}$ = 4.5 Hz, 1 H, 1''-H), 1.88 (ddd, $^3J_{2'',3''b}$ = 8.9 Hz, $^3J_{2'',3''a}$ = 5.3 Hz, $^3J_{2'',1''}$ = 4.5 Hz, 1 H, 2''-H), 2.50-2.53 (m, 2 H, 2'-H), 4.61 (ddd, $^3J_{1',1''}$ = 8.6 Hz, $^3J_{1',2'}$ = 6.4 Hz, $^3J_{1',2'}$ = 5.9 Hz, 1 H, 1'-H), 5.05 (dddd, $^3J_{4'a,3'}$ = 10.2 Hz, $^2J_{4'a,4'b}$ = 2.0 Hz, $^4J_{4'a,2'}$ = 1.1 Hz, $^4J_{4'a,2'}$ = 1.1 Hz, 1 H, 4'-Ha), 5.07 (dddd, $^3J_{4'b,3'}$ = 17.1 Hz, $^2J_{4'b,4'a}$ = 2.0 Hz, $^4J_{4'b,2'}$ = 1.5 Hz, $^4J_{4'b,2'}$ = 1.5 Hz, 1 H, 4'-Hb), 5.80

(dddd, $^3J_{3',4'b}$ = 17.1 Hz, $^3J_{3',4'a}$ = 10.2 Hz, $^3J_{3',2'}$ = 7.0 Hz, $^3J_{3',2'}$ = 6.9 Hz, 1 H, 3'-H), 5.84 (dd, $^3J_{3a,2}$ = 10.4 Hz, $^2J_{3a,3b}$ = 1.4 Hz, 1 H, 3-Ha), 6.15 (dd, $^3J_{2,3b}$ = 17.3 Hz, $^3J_{2,3a}$ = 10.4 Hz, 1 H, 2-H), 6.42 (dd, $^3J_{3b,2}$ = 17.3 Hz, $^2J_{3b,3a}$ = 1.4 Hz, 1 H, 3-Hb), 7.05-7.28 (m, 5 H, arom. CH). ^{13}C-NMR ($CDCl_3$, 151 MHz): δ [ppm] = 14.1 (C-3''), 21.6 (C-1''), 26.5 (C-2''), 39.2 (C-2'), 76.8 (C-1'), 117.9 (C-4'), 125.8 (arom. CH), 126.0 (arom. CH), 128.4 (arom. CH), 128.7 (C-2), 130.7 (C-3), 133.3 (C-3'), 141.8 (i-C), 165.6 (C-1).

4.8. Synthesis of (6R,1'S,2'S)-6-(2'-Phenylcyclopropyl)-5,6-dihydro-2H-pyran-2-one [(*R,S,S*)-**4**]

According to *Gosh et al.*[65] 1.06 g (4.37 mmol, 1.00 equiv.) diene (*R,S,S*)-**17** was dissolved under an argon atmosphere in 700 mL dichloromethane. Subsequently 388 µL (1.31 mmol, 0.30 equiv.) titantetraisopropylate was added and the solution was refluxed for 30 minutes. Afterwards, 360 mg (0.44 mmol, 0.10 equiv.) Grubbs-I catalyst was added and the purple solution was refluxed for 16 h under absence of oxygen (control by TLC). After complete conversion the solution was distilled until 10 mL of solution were left over. The crude product was then transferred to a silica column (PE : EE = 95 : 5, then 90 : 10, then 80 : 20) and purified twice *via* column chromatography. The product (*R,S,S*)-**4** could be isolated as a colourless solid [859 mg (4.00 mmol, 92%)].

Lactones (*S,S,S*)-**4** and (*R,R,R*)-**4** have been synthesized according to the same protocol.

(6R,1'S,2'S)-6-(2'-Phenylcyclopropyl)-5,6-dihydro-2H-pyran-2-one [(*R,S,S*)-**4**]

R_f = 0.2 (PE : EE = 75 : 25). Melting point: 65 °C. $[\alpha]_D^{20}$= +264 (c = 0.9, $CHCl_3$). MS EI (70 eV): m/z (%) = 214 (16) [M^+], 169 (6) [$(M\text{-}CHO_2)^+$], 145 (8) [$(C_{10}H_9O)^+$], 129 (26) [$(C_{10}H_9)^+$],117 (43) [$(C_9H_9)^+$], 103 (14) [$(C_6H_8)^+$], 97 (100) [$(C_5H_5O_2)^+$], 69 (23) [$(C_4H_4O)^+$]. IR (ATR, film): 3059, 3026, 2898, 1710 (C=O), 1605, 1498, 1415, 1381, 1246, 1152, 1062, 1021, 950, 937, 876, 816, 761, 698 cm^{-1}. ^{1}H-NMR (CDCl3, 600 MHz): δ [ppm] = 1.02 (ddd, ^3J = 8.6 Hz, ^2J = 5.5 Hz, ^3J = 5.5 Hz, 1 H), 1.05 (ddd, ^3J = 8.9 Hz, ^2J = 5.5 Hz, ^3J = 5.5 Hz, 1 H), 1.46 (dddd, ^3J = 8.6 Hz, ^3J = 7.7 Hz, ^3J = 5.5 Hz, ^3J = 4.5 Hz, 1 H), 2.12 (ddd, ^3J = 8.9 Hz, ^3J = 5.5 Hz, ^3J = 4.5 Hz, 1 H), 2.25-2.53 (m, 2 H), 4.05 (ddd, ^3J = 7.7 Hz, ^3J = 7.7 Hz, 3J6,5b = 7.7 Hz, 1 H), 6.05 (ddd, ^3J = 9.8 Hz, 4J3,5 = 1.9 Hz, ^4J = 1.9 Hz, 1 H), 6.90 (ddd, ^3J = 9.8 Hz, ^3J = 4.8 Hz, ^3J = 4.0 Hz, 1 H), 7.10-7.28 (m, 5 H, arom. CH). ^{13}C-NMR (CDCl3, 151 MHz): δ [ppm] = 12.1, 21.3, 25.8, 29.4, 80.8, 121.7, 125.7, 126.3, 128.3, 141.4, 144.8, 164.3.

(S)-6-((1S,2S)-2-Phenylcyclopropyl)-5,6-dihydro-2H-pyran-2-one [(*S,S,S*)-**4**]*and (R)-6-((1R,2R)-2-phenylcyclopropyl)-5,6-dihydro-2H-pyran-2-one* [(*R,R,R*)-**4**]

[(*S,S,S*)-**4**]: (322 mg, 1.51 mmol, 71%), colourless oil. [(*R,R,R*)-**4**]: 1.04 g (4.30 mmol, 90%), colourless oil. R_f = 0.2 (PE : EE = 75 : 25). R_f = 0.2 (PE : EE = 80 : 20). (*R,R,R*)-**4** $[\alpha]_D^{20}$= +0.7 (c = 0.9, $CHCl_3$, 97% ee). (*S,S,S*)-**4** $[\alpha]_D^{20}$= -0.8 (c = 1.1, $CHCl_3$, 94% ee). HPLC: column: Chiralpak AS (250mm · 4.6 mm, Fa. Daicel); solvent: heptane : 2-propanol = 80 : 20; flowrate: 0.5 mL/min, pressure: 10 bar; detection: UV 205 nm; t_R [(*R,R,R*)-**4**] 42.2 min; t_R [(*S,S,S*)-**4**] 48.1 min. MS (EI, 70 eV): m/z (%) = 214 (10) [M^+], 169 (6) [$(M\text{-}CHO_2)^+$], 145 (8) [$(C_{10}H_9O)^+$], 129 (23) [$(C_{10}H_9)^+$], 117 (44) [$(C_9H_9)^+$], 104 (41) [$(C_6H_9)^+$], 97 (100) [$(C_5H_5O_2)^+$], 91 (32) [$(C_7H_7)^+$], 77 (10)[$(C_6H_5)^+$], 69 (25) [$(C_4H_5O)^+$], 68 (35) [$(C_4H_5O)_+$]. HRMS (ESI, positiv-Ion): calc.: 237.0886 ($C_{14}H_{14}O_2Na$) [$(M+Na)^+$] found: 237.0884 ($C_{14}H_{14}O_2Na$) [$(M+Na)_+$]. Elemental analysis: $C_{14}H_{14}O_2$ (214.26 g/mol): calc.: C 78.48 H 6.59; found: C 78.14 H 6.67. IR (ATR, film): 3027, 1713 (C=O), 1605, 1499, 1465, 1417, 1383, 1247, 1153, 1076, 1063, 1031, 938, 916, 754, 698 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ [ppm] = 1.11 (ddd, $^3J_{3'a,1'}$ = 8.5 Hz, $^3J_{3'a,2'}$ = 5.5 Hz, $^2J_{3'a,3'b}$ = 5.5 Hz, 1 H, 3'-Ha), 1.21 (ddd, $^3J_{3'b,2'}$ = 9.0 Hz, $^3J_{3'b,1'}$ = 5.5 Hz, $^2J_{3'b,3'a}$ = 5.5 Hz, 1 H, 3'-Hb), 1.49 (dddd, $^3J_{1',3'a}$ = 8.5 Hz, $^3J_{1',6'}$ = 8.0 Hz, $^3J_{1',3'b}$ = 5.5 Hz, $^3J_{1',2'}$ = 4.5 Hz, 1 H, 1'-H), 1.96 (ddd, $^3J_{2',3'b}$ = 9.0 Hz, $^3J_{2',3'a}$ = 5.5 Hz, $^3J_{2',1'}$ = 4.5 Hz, 1 H, 2'-H), 2.50-2.59 (m, 2 H, 5-H), 4.04 (ddd, $^3J_{6,5}$ = 9.5 Hz, $^3J_{6,1'}$ = 8.0 Hz, $^3J6_{,5}$ = 5.8 Hz, 1 H, 6-H), 6.04 (ddd, $^3J_{3,4}$ = 9.7 Hz, $^3J_{3,5}$ = 2.3 Hz, $^3J_{3,5}$ = 1.4 Hz, 1 H, 3-H), 6.89 (ddd, $^3J_{4,3}$ = 9.7 Hz, $^3J_{4,5}$ = 5.1 Hz, $^3J_{4,5}$ = 3.1 Hz, 1 H, 4-H), 7.06-7.29 (m, 5 H, arom. CH). ^{13}C-NMR ($CDCl_3$, 151 MHz): δ [ppm] = 13.8 (C-3'), 20.5 (C-1'), 26.2 (C-2'), 29.6 (C-5), 80.7 (C-6), 121.6 (C-3), 125.9 (arom. CH), 126.1 (arom. CH), 128.5 (arom. CH), 141.4 (i-C), 144.9 (C-4), 164.2 (C-2).

4.9 *Biological Methods*

Cell culture

Human lung adenocarcinoma cell line A549 (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) was cultured in DMEM medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% heat inactivated fetal bovine serum (h.i. FBS; Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA).

Human breast adenocarcinoma cell line MCF-7 (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) was cultured in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% h.i. FBS, 1x MEM non-essential amino acids (Biochrom, Berlin, Germany), 1 mM sodium pyruvate (Biochrom, Berlin, Germany), 10 µg/mL human insulin (Biochrom, Berlin, Germany), 100 U/mL penicillin and 100 µg/mL streptomycin.

Triple negative human breast cancer cell line HBL-100 (Cell Lines Service, Eppelheim, Germany) was cultured in Mc Coy's 5A medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented 10% h.i. FBS, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin.

Cell lines were cultured at 37 °C and 5% CO_2 in a humidified atmosphere.

Cytotoxicity assay

Cytotoxicity was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA) according to the manufacturer's protocol. This assay is based on a luciferase reaction to determine the ATP content, which is proportional to the rate of living cells. Cells were seeded in white 384-well plates (A549, HBL-100: $2{\cdot}10^3$ cells/well; MCF-7: $5{\cdot}10^3$ cells/well) in cell culture medium with the CyBi-Well 96-channel simultaneous pipettor (Analytik Jena AG, Jena, Germany). After 24 h incubation at 37 °C and 5% CO_2, the test compounds were added and the cells were further incubated for 48 h. Afterwards, the CellTiter-Glo reagent was added and luminescence was measured using the Infinite m200 microplate reader (Tecan Group AG, Maennedorf, Switzerland).

IC_{50} values were determined by plotting the luminescence values against the logarithmic molar concentration of the compounds and subsequent curve fitting (nonlinear regression) using GraphPad Prism v. 6.07 (GraphPad Software, San Diego, CA, USA). The results are mean values of at least three independent experiments

Yeast Strains. *S. cerevisiae* was cultured in YPD medium (10 g/liter yeast extract, 20 g/liter peptone, 2% glucose). The following *S. cerevisiae* yeast strain was used: YRE1001 (*MATa ura3-52 trp1-1 leu2-3,112 his3-11,15 ade2-1 pdr1-3 pdr5pdr5promΔ::TRP1*).

Liquid Drug Assay. The test compounds were diluted in DMSO to final concentration of 20 mg/mL (except of **25** was diluted to a final concentration of 10 mg/mL). The assay was carried out in sterile 96-well microtiter plates (Falcon) with 6,25 µl of serial dilution of the test compounds, 193,75 µl of YPD medium and 50 µl of yeast culture at an OD_{600} of 0.15. Plates were incubated for 48 h at 30 °C, and OD_{600} was measured with an ELISA plate reader (BioRad).

Isolation of Plasma Membranes. Yeast cells were cultured to an OD_{600} of 1.5 in YPD at 25 °C. At this time point, the nitrogen sources was replenished by addition of a 10th volume 5x YP (50 g/liter yeast extract, 100 g/liter peptone). Cells were harvested at an OD_{600} of 3.5. The isolation of plasma membranes (PM) was performed as described.[66,71]

Rhodamine 6G Transport Assay. Active transport of rhodamine 6 G (R6G) was measured, using a Tecan Infinite 200 PRO reader (Tecan), according to the protocol developed by Kolaczkowski *et al*.[66] Isolated PM (6 µL of a 1 mg/mL stock solution) were resuspended in 200 µl of transport buffer (50 mM Hepes, pH 7.0, 5 mM $MgCl_2$, 10 mM NaN_3, and 150 nM R6G) and incubated at 30 °C in a 96-well-FIA-plate (Greiner). In addition, 5 µL of 3 different concentrations (500 µg/mL, 50 µg/mL and 5 µg/mL) (except of **25**: 125 µg/mL, 13 µg/mL) of the test compounds were added. The active transport was started by addition of 10 mM ATP and the fluorescence was recorded for 20 min (excitation at 524 nm, emission at 558 nm; number of flashes: 30, integration time 2000 µs).

ATPase Activity Assay. Oligomycin (OM)-sensitive ATPase activity of Pdr5 in highly enriched PM was determined by a colometric assay in 96-well microtiter plate.[67-69] 40 µL of the plasma membrane solution (20 µg/mL stock solution) were incubated with 2 mM ATP, 5 mM $MgCl_2$ in 270 mM Tris-glycine buffer (pH 9.5) and 5 µL of the indicated test compound concentrations in a total volume of 100 µL. To reduce the background activities 0.2 mM ammonium molybdate, 10 mM NaN_3 and 50 mM KNO_3, respectively, were added.[67,70] In a second assay, OM (20 µg/mL) was added to the experiment under same conditions. After incubation at 30 °C for 20 min, the reaction was stopped by adding 25 µL of the reaction to 175 µL 40 mM H_2SO_4. The amount of released inorganic phosphate was determined by a colorimetric assay, using Na_2HPO_4 as standard. The difference of both assays corresponds to OM-sensitive ATPase activity of Pdr5.[71]

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Supplementary Material

Supplementary material is available.