SAR studies on hydropentalene derivatives – important core units of biologically active tetramic acid macrolactams and ptychanolides

Vanessa Lutz^a, Fabian Mannchen^a, Michael Krebs^a, Natja Park^a, Claudia Krüger^a, Aruna Raja^b, Florenz Sasse^{b,*}, Angelika Baro^a, Sabine Laschat^{a,*}

^a *Institut für Organische Chemie der Universität Stuttgart, Pfaffenwaldring 55, 70569 Stuttgart, Germany*

^b *Department of Chemical Biology, Helmholtz Centre for Infection Research, Inhoffenstr. 7, 38124 Braunschweig, Germany*

ABSTRACT

Structurally diverse bicyclo[3.3.0]octanes were prepared and tested for their biological activity. Both the antiproliferative activity and the results of phenotypic characterization varied with the substitution patterns. Two derivatives displayed high inhibitory ($IC_{50} \leq 3 \mu M$) activity against the L-929 cell line, but differed in their mode of action. A cluster analysis with impedance profiling data showed the two compounds in relationship to microtubule interfering compounds. In PtK₂ cells treated with both derivatives a perturbing effect on the microtubular network was observed, whereas the actin cytoskeleton in incubated PtK₂ cells was disturbed only by one compound. The effects on tubulin and actin polymerization could be confirmed by *in vitro* polymerization experiments.

Keywords: Biological activity, Click chemistry, Hydropentalene, Structure-activity relationships, Synthesis

* Corresponding authors. Tel.: +49 711 685 64565, fax: +49 711 685 64285 (S.L.); tel.: +49 531 6181 3429; fax: +49 531 6181 3499 (F.S.).

E-mail addresses: sabine.laschat@oc.uni-stuttgart.de (S. Laschat), florenz.sasse@helmholtz-hzi.de (F. Sasse).

1. Introduction

Highly substituted bicyclo[3.3.0]octanes (hydropentalenes) represent the core unit of several biologically active natural product families such as tetramic acid macrolactams containing, for example, cylindramide (**1**),^{1,2} alteramide A (**2**),³ geodin A (**3**),^{4,5} and aburatubolactam A (**4**),^{6,7} or ptychanolides **5**, **6**^{8,9} (Figure 1).

Figure 1. Hydropentalene core containing natural products **1**–**6**.

The class of tetramic acid lactams displays a variety of biological activities. Cylindramide (**1**), isolated from the marine sponge *Halichondria cylindrata* in 1989, for example, exhibits pronounced cytotoxicity against B16 melanoma cells.¹ Alteramide A (**2**)³ from a bacterium *Alteromonas* sp. associated with the sponge *Halichondria okadai* is cytotoxic against epidermal carcinoma cell lines. Cylindramide's close relative geodin A (**3**) being isolated as Mg^{2+} salt in 1999 from the Southern Australian marine sponge *Geodia*,⁴ differs from **1** only in the exocyclic methylene group instead of methyl. Biological studies revealed its strong nematocidal activity against the parasitic nematode *Haemonchus contortus*. Aburatubolactam A (**4**) isolated in 1996 from the marine bacterium *Streptomyces* sp. SCRC A-20⁶ inhibits the formation of anionic superoxides which are relevant in both inflammatory and degenerative processes and tumor promotion.¹⁰ Whereas natural products **1**–**4** are of marine origin, a bicyclo[3.3.0]octane skeleton has also been detected in terrestrial sources. For example, the sesquiterpenoids ptychanolide **5** and **6** have been isolated in 1981 from the liverwort *Ptychanthus striatus* (Lehm. et Lindenb.) Nees.⁸

Besides some initial experiments on the biological activity of these natural product classes, however, only limited systematic structure-activity relationship (SAR) studies have been carried out. The influence of the tetramic acid moiety on cytotoxicity and antimicrobial properties of macrocyclic acyltetramates has been investigated by Schobert.¹¹ Previously we aimed towards elucidation of the mode of action of cylindramide (**1**).¹² For this purpose a series of derivatives of **1**, including analogues where a simple cyclopentane replaced the functionalized hydropentalene moiety, were prepared. A broad biological screening revealed promising anti-proliferative activity against several tumor cell lines. The cytotoxicity of **1** was found to be calcium dependent and we observed vacuolisation and vesicle formation in the endoplasmic reticulum of PtK₂ (potoroo kidney) cells that were incubated with **1**. A decrease of activity for the cyclopentane analogue of cylindramide (**1**) indicated that the activity is strongly correlated to the intact functionalized hydropentalene system.

SAR studies of substituted bicyclo[3.3.0]octanes, however, are rarely reported. Most of them focused on orally active PDE4 inhibitors,¹³ carbacycline derivatives such as TXA₂/PGH₂ receptor antagonists and prostaglandin analogues,¹⁴ or mammalian squalene synthase inhibitors.¹⁵ Therefore, we were motivated to investigate the biological properties of the hydropentalene subunit in more detail. Besides studying analogues of the core units of tetramic acid lactams we were interested in a more general insight into the biological properties of various functionalized bicyclo[3.3.0]octanes **A–C**. Our results are reported below.

2. Results and discussion

2.1. Chemistry

In order to supply compounds for SAR studies, a series of bicyclo[3.3.0]octanes was prepared from the easily accessible Weiss diketone (**7a**),¹⁶ its dimethyl analogue **7b**¹⁶ and the known cylindramide core precursor **8**^{2b} (Figure 2) leading to derivatives of structural diversity.

Figure 2. Starting materials and positions for functionalization leading to structures **A–C**.

The hydropentalene derivatives **11**–**18** are based on Weiss diketone (**7a**) (Scheme 1). The synthesis of alkynes **11a** and **11b** which were envisaged for "click chemistry" possibly allowing visualization of the cellular target, commenced with the reduction of monoketal **9a**^{17,18} to give **10**.¹⁸ The latter was either reacted with propionic acid under Mitsunobu conditions to **11a** or converted in three steps to **12** which was esterified analogously to **11b**. Direct esterification of alcohol **10** with propionyl chloride gave derivative **11c**. Alkenes **17** and **18**, providing key structural motifs of the bicyclo[3.3.0]octene core of the natural products **1**–**4**, were prepared from monoketal **9a** by a sequence of double deprotonation/electrophilic trapping followed by swapping of protecting groups to yield compounds **13**, **14**. Derivatives **13b**, **14b** were submitted to Shapiro reaction to give **15b**, **16b**, which were subsequently functionalized to **17**, **18**.¹⁹

Scheme 1. Reagents and conditions: (a) 4.0 equiv PPh₃, 4.0 equiv propionic acid, 4.0 equiv DEAD, 1.0 equiv **10** or **12**, Et₂O, r.t., 24 h, 40% for **11a**, 37% for **11b**; (b) 1.0 equiv 5-[(*tert*-butyl(diphenyl)silyl)oxy]hexahydropentalen-2(1*H*)-one,¹⁸ 3.0 equiv NaBH₄, MeOH, 0 °C, 3 h, quant.; (c) 1.0 equiv **9a**, 2.0 equiv propionyl chloride, Et₃N, CH₂Cl₂, 0 °C, 14 h, 73%. Numbering for NMR assignment.

The synthesis of hydropentalenes **21**–**23** based on bicyclo[3.3.0]octane-1,4-dione **8**^{2b} is shown in Scheme 2. Monoketal **19** was converted to silyl enol ether **20** which was submitted to a Shi epoxidation.²⁰ Reductive epoxide opening of the resulting intermediate with BH₃·THF provided the *trans* configured hydropentalenes **21a** and **21b**. Dienedione **22**^{2b} yielded the alkylated bicyclo[3.3.0]octene-1,4-dione **23a** by Cu-catalyzed Grignard reaction with 3-butenylmagnesium-

bromide in the presence of TMSCl. Ketalization of **23a** with ethylene glycol gave the monoketal **23b** (Scheme 2).

Scheme 2. Reagents and conditions: (a) 1.0 equiv KHMDS, 1.0 equiv **19**, THF, $-78\text{ }^{\circ}\text{C}$, 20 min, 1.1 equiv TBDPSCl, $-78\text{ }^{\circ}\text{C}$ to r.t., 3 h; (b) aq. $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}/\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 0.3 equiv Shi catalyst,²⁰ 0.04 equiv Bu_4NHSO_4 , 1.4 equiv oxone in aq. $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 5.8 equiv K_2CO_3 , 3 h; (c) 2.5 equiv $\text{BH}_3 \cdot \text{THF}$, THF, 3 h, 15% for **21a**, 38% for **21b**; (d) 1.4 equiv CuCN, 1.0 equiv TMEDA, 1.1 equiv 3-butenylmagnesiumbromide, THF, $-78\text{ }^{\circ}\text{C}$, 1.2 equiv TMSCl, 1.0 equiv **22**, 30 min, 63%; (e) 2.0 equiv ethylene glycol, 0.09 equiv *p*-TsOH, reflux, 7 h, 60%. Only one diastereomer of **23a** was detected, its configuration was determined by NOESY (see supplementary data). Numbering for NMR assignment.

Hydropentalenes of the ptychanolide series were prepared from 3a,6a-dimethyltetrahydropentalene-2,5(1*H*,3*H*)-dione (**7b**)¹⁶ following known procedures^{17,18b,21} (Scheme 3).

Scheme 3. Reagents and conditions: (a) 2,2-dimethyl-1,3-propanediol, *p*-TsOH in toluene, reflux, 2 h;^{18b} (b) 1. *n*-BuLi/bis[(*R,R*)-1-phenylethyl]ammonium chloride, $-100\text{ }^{\circ}\text{C}$; then $-78\text{ }^{\circ}\text{C}$, ClSiEt_3 ; 2. MeLi, MeI according to ref.;¹⁷ (c) 3 equiv NaBH_4 , 1 equiv **24**, MeOH, $0\text{ }^{\circ}\text{C}$, 15 min; (d) 1 equiv **25a,b** in DMF, 1.2 equiv TBDPSCl, 2.5 equiv imidazole according to ref.;^{18b} (e) catalytic amounts of *p*-TsOH according to ref.²¹

The ratio of the diastereomeric ketones **24a** and **24b** depended on the temperature. At room temperature, **24a,b** were isolated in 87% yield with *dr* 39:61 whereas the reaction at $-20\text{ }^{\circ}\text{C}$ provided **24a,b** quantitatively with *dr* 85:15. The obtained enantioselectivity (10–12% ee)

could not be improved by trapping the intermediate enolate as silyl enol ether prior to alkylation as previously reported.¹⁷ Presumably the steric bias of the *axial* methyl groups on top of the *convex* bicyclic roof interfered with the enantiodiscrimination of the chiral lithium amide base at the ketone moiety. Nevertheless, ketones **24** were reduced to alcohols **25a,b** which were protected to yield silyl ethers **25c,d**. Final acidic ketal cleavage provided ketones **26a,b**.

2.2. Biological studies – proliferation tests

The ability of the set of nearly 30 hydropentalene derivatives to inhibit the proliferation of various transformed cell lines including human tumor cell lines was investigated in an MTT assay.²² The results are summarized in Tables 1 and 2.

Table 1

Cytotoxicity of bicyclo[3.3.0]octanes against different transformed cell lines^a

Entry	Compounds	L-929 (mouse) connective tissue IC ₅₀ (μM)	KB-3-1 (human) cervix carcinoma IC ₅₀ (μM)
1	11a	2.2	21.6
2	11b	9.3	–
3	11c	>142	>142
4	13a	7.4	15.2
5	13b	32.4	16.0
6	13c	17.8	6.9
7	13d	23.9	28.2
8	14a	14.8	7.4
9	14b	16.2	20.3
10	14c	5.1	16.2
11	14d	5.6	21.7
12	15b	20.0	13.3
13	16b	>67	12.5
14	17	20.4	>96
15	18	7.2	>96
16	21a	41.0	–
17	21b	38.8	–
18	22	3.0	6.0
19	23a	20.0	16.3
20	23b	25.6	32.0

^a Further compounds in supplementary data. IC₅₀ values are means of two assays in parallel.

As shown in Table 1, the antiproliferative activities of most compounds varied in a micromolar range. Exceptions were the alkyne **11a** and dienedione **22** showing promising potencies ($IC_{50} \leq 3 \mu M$) against L-929 mouse fibroblasts. The cytotoxicity of ester-substituted hydropentalenes **11** was found to depend strongly on the ester function. In contrast to both propiolates **11a** and **11b**, the corresponding propionate **11c** was completely inactive. We were worried that the cytotoxicity of the propiolate derivatives **11a,b** might be due to a subsequent hydrolysis product generated by esterase activity rather than caused by **11a** and **11b** themselves. Therefore, the corresponding pentalene alcohol was studied in comparison (supplementary data), but no cytotoxicity was observed for the alcohol, indicating that indeed the intact ester seemed to be the active species. Bicyclo[3.3.0]octenes **17** and **18**¹⁹ displayed cytotoxicities ($IC_{50} = 7.2\text{--}20.4 \mu M$) against L-929 mouse fibroblasts but poor inhibitory ($IC_{50} > 96 \mu M$) activity against the KB-3-1 cell line (Table 1, Entries 14, 15). Unexpectedly, silyl-protection had a significant effect on the antiproliferative activity. The unprotected analogues of **13**, **14** and **21** were completely inactive against all tested cell lines (supplementary data).

A similar effect was observed for unprotected ptychanolide derivatives **25a,b** and their silyl-protected counterparts **25c,d** (Table 2, Entries 2–5). In order to study the influence of the silyl protecting group, the cytotoxic effect of O-silylated cyclopentanol^{23–25} for the two cell lines was investigated. We observed an increased activity in the order of silyl protecting groups TBS < TIPS < TBDPS (Table 2, Entries 8–11).

Table 2

Cytotoxicity of 3a,6a-dimethylhydropentalenes **24–26** and cyclopentanol^as against different transformed cell lines^a

Entry	Compound	L-929 IC_{50} (μM)	KB-3-1 IC_{50} (μM)
1	24a	>150	–
2	25a	>150	–
3	25b	>150	–
4	25c	15.8	–
5	25d	11.8	–
6	26a	14.3	–
7	26b	35.7	–
8	<i>t</i> -butyl(cyclopentyloxy)dimethylsilane	>200	>200
9	(cyclopentyloxy)(triisopropyl)silane	111.4	123.7
10	<i>t</i> -butyl(cyclopentyloxy)diphenylsilane	61.6	–

^a IC_{50} values are means of two assays in parallel.

2.3. Biological studies – target identification

Alkyne **11a** and dihydropentalenedione **22** with the highest potency were selected for further biological studies. In order to get hints about the mode of action we employed a newly developed impedance profiling method which uses time-dependent impedance curves as a fingerprint for the mode of action.²⁶ In L-929 cell cultures that were incubated with **11a** and **22**, impedance was monitored over time (Figure 3).

Figure 3. Impedance curves of L-929 cells incubated with **11a** ($0.6 \mu\text{g mL}^{-1}$) and **22** ($0.4 \mu\text{g mL}^{-1}$). Tubulysin B (3 ng mL^{-1}) was used as one of the reference compounds.

The obtained curves were compared with those of a set of reference compounds using statistical methods. Cluster analysis of the resulting data showed **11a** and **22** in a group of reference compounds such as nocodazole, tubulysin B and griseofulvin that interfere with microtubule polymerization and spindle formation (Figure 4).

Figure 4. Cluster analysis of data obtained by impedance monitoring of incubated cell cultures. Derivatives **11a** (c9a) and **22** (c19) are related to compounds that interfere with tubulin polymerization and mitotic spindle formation.

Based on the results obtained from the impedance profiling, PtK₂ (potoroo kidney) cells which showed in MTT assays an IC₅₀ of approximately 3 μ M with **11a** and **22**, respectively, were incubated with both compounds and stained for effects on microtubules (Figure 5). For both **11a** and **22** a perturbing effect on the microtubular network in the cells and altered mitotic spindles together with an unusual distribution of chromosomes was visible.

Figure 5. Effect of **11a** and **22** on the microtubule cytoskeleton and mitotic spindle. PtK₂ cells were treated with **11a** (4 μ g mL⁻¹; C) and **22** (4 μ g mL⁻¹; D) overnight and stained for microtubules (green) and DNA (blue). Control cells were incubated with methanol only (A and B).

To further verify the effect of **11a** and **22**, tubulin polymerization assays were carried out *in vitro* (Figure 6). The effect of **11a** and **22** was compared to that of methanol and nocodazole, a known inhibitor of tubulin polymerization.²⁷ The basis of this assay is the incorporation of a fluorescent reporter into the developing microtubules. While nocodazole is only inhibiting the polymerization process, **11a** and **22** induce a decrease in fluorescence. The effect of **22** was much stronger than that of **11a**, which still allowed a delayed tubulin polymerization.

Figure 6. *In vitro* tubulin polymerization assay (37 °C). Compound **11a** and **22** (500 $\mu\text{g mL}^{-1}$ each) induced a decrease in fluorescence while the rate of tubulin polymerization progressed in the methanol control. While **11a** still allowed some delayed polymerization, this process was completely blocked by **22**. Fluorescence was monitored at 460 nm (excitation at 340 nm).

We also checked for the influence of **11a** and **22** on the F-actin cytoskeleton, the microfilaments (Figure 7). Whereas compound **22** did not show a perturbing influence on the actin cytoskeleton (Figure 7D), this was indeed the case with **11a** (Figure 7C).

Figure 7. Effect of **11a** and **22** on the F-actin cytoskeleton. PtK₂ cells were incubated with **11a** (2 $\mu\text{g mL}^{-1}$; C) and **22** (3 $\mu\text{g mL}^{-1}$; D) overnight and stained for F-actin (green) and DNA (blue). Control cells were treated with methanol only (A and B).

For **11a**, further studies were carried out that should possibly show co-localization with F-actin. We used a "click chemistry" approach, which is based on a copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.^{28,29} PtK₂ cells were treated with terminal alkyne **11a** (3 $\mu\text{g mL}^{-1}$) for 12 h and then fixed. The fixed cells were permeabilized with Triton X-100 and incubated with a click reagent mixture for 30 min. Further staining for F-actin was carried out using fluorescently labeled phalloidin which binds selectively to F-actin. A blue fluorescence was observed that was co-localized with the green phalloidin label depicting the micro-filaments.

In order to check the effect of **11a** on actin *in vitro*, a polymerization assay with pyrene muscle actin was carried out, wherein the environmentally sensitive fluorophore pyrene indicates the polymerization state of actin. Thus, an increase in fluorescence is directly proportional to an increase in F-actin. As shown in Figure 8, an increase of the polymerization rate was measured with increasing concentration of **11a**.

Figure 8. *In vitro* actin polymerization assay. Compound **11a** induced an increased polymerization rate of pyrene actin *in vitro*. The actin fluorescence baseline was first read without any drug addition for 3 min. Then methanol (control) and **11a** (at different concentrations) were added and fluorescence read for a further 20 min to test if the drug itself has an effect before the polymerization reaction was started by addition of 10X actin polymerization buffer. Fluorescence was monitored at 420 nm (excitation 360 nm).

3. Conclusions

We have synthesized a series of hydropentalene derivatives starting from hexahydropentalene-1,4-dione (**8**), *cis*-tetrahydropentalene-2,5(1*H*,3*H*)-dione **7a** (Weiss diketone) or its 3a,6a-dimethyl analogue **7b**. Hydropentalenes are not only biologically important parts of natural macrolactams but show pharmacophoric properties themselves as was demonstrated by biological studies. Their antitumor activities on mammalian cell lines varied with the substitution patterns. In the series of 1,4-dione **8** derived compounds **21–23** the presence of

unsaturated rings seemed to play a key role. Consequently, the cytotoxicity against the tested L-929 cell line increased in the order saturated **21** (IC_{50} ca. 40 μ M) < single unsaturated **23** (IC_{50} ca 26 μ M) < double unsaturated **22** (IC_{50} 3 μ M). The latter was also most potent within all investigated compounds. In the Weiss diketone **7a** based series, this structural element may be negligible although derivatives **17** and **18** displayed antiproliferative activities against L-929 cells comparable to **23**. The most active compound in this group, however, was the alkyne **11a**. A hierarchical cluster analysis of impedance monitoring data measured with **11a** and **22** suggested a mode of action that might be similar to microtubule affecting compounds. During further investigations with **11a**, tubulin and also actin could be identified as the probable target proteins. Both actin filaments and tubulin network in PtK₂ cells were disturbed when treated with **11a**, and *in vitro* experiments also showed that the polymerization of tubulin and of actin is impaired. With actin we measured an enhancing effect, with tubulin an inhibition of polymerization. An incubation of PtK₂ cells with **22** only induced a perturbing effect on the microtubules, not on the microfilaments. Tubulin polymerization assays showed a strong inhibitory effect *in vitro* also. Future work should reveal whether pentalene-containing tetramic acid macrolactams **1–4** are indeed binding to the same target proteins or whether the tetramic acid moiety results in binding to a completely different target.

4. Experimental

4.1. General

NMR spectra were recorded on a Bruker Avance 500 spectrometer in CDCl₃ with TMS as an internal standard. IR spectra were recorded on a Bruker FT-IR-spectrometer Vektor 22 with MKII golden gate single reflection Diamant ATR-system. Mass spectra were recorded on a Varian MAT 711 (EI, 70 eV) and a Bruker Daltonics micrOTOF_Q (ESI) with nitrogen as carrier gas. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20 °C. Flash chromatography was performed on silica gel, grain size 40–63 μ m (Fluka). Moisture-sensitive reactions were performed under nitrogen atmosphere in oven-dried glassware. All reagents were used as purchased unless otherwise noted. Solvents used for chromatography were distilled. THF was distilled from sodium/benzophenone, CH₂Cl₂ and toluene from CaH₂. The reactions were monitored by TLC (Merck 60 F₂₅₄ plates).

Cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) or American Type Culture Collection (ATCC) and cultivated in the media recommended by the supplier at 37 °C and 10% CO₂.

4.2. (3a'R,6a'S)-5,5-Dimethylhexahydro-1'H-spiro[1,3-dioxane-2,2'-pentalen]-5'-yl propiolate (**11a**)

A suspension of propiolic acid (248 mg, 3.52 mmol) and PPh₃ (923 mg, 3.52 mmol) in abs. Et₂O (10 mL) under N₂ atmosphere was stirred for 15 min. Then DEAD (0.56 mL, 3.52 mmol) was added followed by a solution of **10**¹⁸ (200 mg, 0.88 mmol) in abs. Et₂O (5 mL). The reaction mixture was stirred for 24 h, filtered off, and the precipitate was washed with Et₂O (15 mL). The filtrate was concentrated and the residue purified by flash chromatography on SiO₂ (hexanes/EtOAc, 20:1) to give **11a** (98 mg, 0.35 mmol, 40%) as a white solid. *R*_f 0.40 (hexanes/EtOAc, 10:1). ¹H NMR (500 MHz, CDCl₃): δ 0.96 [s, 6H, C(CH₃)₂], 1.67–1.79 (m, 4H, 1'-H_a, 3'-H_a, 4'-H_a, 6'-H_a), 1.98–2.07 (m, 2H, 4'-H_b, 6'-H_b), 2.17 (dd, *J* = 13.5, 8.9 Hz, 2H, 1'-H_b, 3'-H_b), 2.67–2.77 (m, 2H, 3a'-H, 6a'-H), 2.83 (s, 1H, CCH), 3.46 (s, 2H, OCH₂), 3.47 (s, 2H, OCH₂), 5.31–5.38 (m, 1H, 5'-H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ 22.5 [C(CH₃)₂], 30.1 [C(CH₃)₂], 38.2 (C-3a', C-6a'), 39.0 (C-4', C-6'), 39.8 (C-1', C-3'), 72.0, 72.1 (OCH₂), 74.0 (CCH), 75.1 (CCH), 80.9 (C-5'), 110.0 (C-2), 152.4 [(CO)O] ppm; FT-IR (ATR): $\tilde{\nu}$ = 3246 (w), 2954 (m), 2865 (w), 2359 (w), 2112 (w), 1706 (s), 1472 (w), 1434 (w), 1395 (w), 1352 (w), 1334 (w), 1299 (w), 1233 (s), 1220 (vs), 1121 (s), 1105 (s), 1053 (m), 1011 (m), 993 (m), 966 (w), 943 (w), 926 (w), 905 (w), 877 (w), 792 (w), 758 (m), 731 (w), 694 (w), 594 (w), 561 (w), 528 (w) cm⁻¹; MS (EI): *m/z* (%) = 278.2 (61) [M]⁺, 263.1 (2), 235.1 (10), 225.1 (8), 209.2 (56), 193.1 (26), 181.1 (7), 168.1 (72), 155.1 (19), 141.1 (9), 123.1 (36), 105.1 (4), 95.1 (23), 81.1 (17), 69.1 (100), 53.0 (30), 41.0 (44); HRMS (ESI): calcd. for C₁₆H₂₂O₄Na⁺ [M + Na]⁺ 301.1410; found 301.1421.

4.3. (3aR,6aS)-5-[[*tert*-Butyl(diphenyl)silyl]oxy]octahdropentalen-2-ol (**12**)

NaBH₄ (90.0 mg, 2.38 mmol) was added portionswise to a solution of (3aR,6aS)-5-[[*tert*-butyl(diphenyl)silyl]oxy]hexahdropentalen-2(1*H*)-one¹⁸ (300 mg, 0.79 mmol) in dry MeOH (40 mL) at 0 °C and the reaction mixture stirred at 0 °C for 2 h. Then the reaction mixture was hydrolyzed with H₂O (15 mL), warmed to room temperature, and extracted with Et₂O (3 × 20 mL). The combined organic layers were washed with brine (20 mL) and dried (MgSO₄). After removal of the solvent under reduced pressure, **12** (300 mg, 0.79 mmol, quant.) was obtained as a colorless oil. ¹H NMR (500 MHz, CDCl₃): δ 0.96 [s, 6H, C(CH₃)₂], 1.73–1.90 (m, 4H, 1'-H_a, 3'-H_a, 4'-H_a, 6'-H_a), 2.15–2.33 (m, 4H, 1'-H_b, 3'-H_b, 4'-H_b, 6'-H_b), 2.45–2.62 (m, 2H, 3a'-H, 6a'-H), 2.99 (s, 3H, SO₂-CH₃), 3.48 [s, 4H, (OCH₂)₂], 5.02 (tt, *J* = 6.7, 5.9 Hz, 1H, 5'-H) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 22.5 [C(CH₃)], 22.6 [C(CH₃)],

30.1 (C-3a', C-6a'), 30.5 [$C(CH_3)_2$], 38.2 (OCH₃), 40.2 (C-4'), 40.3 (C-6'), 48.3 (C-1', C-3'), 109.8 [(OCH₂)₂] ppm; FT-IR (ATR): $\tilde{\nu}$ = 3071 (w), 2957 (m), 2929 (m), 2855 (m), 1590 (w), 1487 (w), 1471 (w), 1461 (w), 1427 (m), 1390 (w), 1360 (w), 1262 (w), 1188 (w), 1108 (vs), 1039 (m), 999 (m), 975 (w), 938 (w), 898 (w), 821 (m), 801 (w), 740 (m), 701 (vs), 610 (m) cm⁻¹; MS (ESI): m/z = 403.21 [M + Na]⁺, 381.22 [M]⁺, 365.19, 248.09, 226.95; HRMS (ESI): calcd. for C₂₄H₃₂O₂SiNa⁺ [M + Na]⁺ 403.2064; found 403.2074.

4.4. (3a*R*,6a*S*)-5-[[*tert*-Butyl(diphenyl)silyl]oxy]octahydropentalen-2-yl propiolate (11b)

As described for **11a**, from propiolic acid (130 μ L, 2.10 mmol), PPh₃ (551 mg, 2.10 mmol) in abs. Et₂O (10 mL), DEAD (0.33 mL, 2.10 mmol) and **12** (200 mg, 0.53 mmol) in abs. Et₂O (5 mL), flash chromatography on SiO₂ (hexanes/EtOAc, 100:1, R_f 0.37), colorless needles, yield: 84 mg (0.35 mmol, 37%). ¹H NMR (500 MHz, CDCl₃): δ 1.03 [s, 9H, C(CH₃)₃], 1.45–1.52 (m, 2H, 1'-H_a, 3'-H_a), 1.79–1.89 (m, 2H, 1'-H_b, 3'-H_b), 1.92–2.07 (m, 4H, 4'-H_a, 6'-H_a, 4'-H_b, 6'-H_b), 2.49–2.59 (m, 2H, 3a'-H, 6a'-H), 2.81 (s, 1H, CCH), 4.23 (dt, J = 10.7, 5.4 Hz, 1H, 2'-H), 5.40–5.45 (m, 1H, 5'H), 7.34–7.39 (m, 4H, *m*-H), 7.39–7.45 (m, 2H, *p*-H), 7.62–7.67 (m, 4H, *o*-H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ 19.0 [C(CH₃)₃], 26.9 [C(CH₃)₃], 39.4 (C-3a', C-6a'), 39.7 (C-4', C-6'), 41.8 (C-1', C-3'), 73.9 (CCH), 75.2 (CCH), 81.5 (C-5'), 127.6 (C-*m*), 129.6 (C-*p*), 134.2 [(CO)O], 135.8 (C-*o*) ppm; FT-IR (ATR): $\tilde{\nu}$ = 3268 (w), 3071 (w), 2962 (m), 2929 (w), 2856 (w), 2114 (w), 1711 (s), 1589 (w), 1472 (w), 1461 (w), 1427 (m), 1391 (w), 1373 (w), 1360 (w), 1304 (w), 1260 (s), 1262 (vs), 1175 (w), 1106 (vs), 1044 (s), 1019 (vs), 1001 (s), 940 (w), 902 (w), 861 (w), 820 (s), 798 (s), 758 (m), 740 (m), 701 (vs), 611 (m) cm⁻¹; MS (ESI): m/z = 455.20 [M + Na]⁺, 248.09. HRMS (ESI): calcd. for C₂₇H₃₂O₃SiNa⁺ [M + Na]⁺ 455.2013; found 455.2007.

4.5. (1*R*,3a*S*,4*R*,5*R*,6a*S*)-5-[[*tert*-Butyl(diphenyl)silyl]oxy]-4-methyl-1-(3-methylbut-2-enyl)hexahydropentalen-2(1*H*)-one (13d) and (1*S*,3a*S*,5*R*,6*R*,6a*S*)-5-[[*tert*-butyl(diphenyl)silyl]oxy]-6-methyl-1-(3-methylbut-2-enyl)hexahydropentalen-2(1*H*)-one (14d)

According to GP1 in ref.,¹⁹ from bis(1-phenylethyl)ammonium chloride (213 mg, 0.81 mmol), BuLi (0.99 mL, 1.59 mmol), (3a*S*,4*R*,5*R*,6a*R*)-5-[[*tert*-butyl(diphenyl)silyl]oxy]-4-methylhexahydropentalen-2(1*H*)-one (266 mg, 0.68 mmol) and prenyl bromide (0.18 mL, 269 mg, 1.60 mmol). **13d**: colorless oil, yield: 113 mg (36%), R_f 0.46 (hexanes/EtOAc, 15:1), $[\alpha]_D^{20}$ -52.7 (c = 5.1, CH₂Cl₂). ¹H NMR (500 MHz, CDCl₃): δ 0.86 (d, J = 6.8 Hz, 3H, 4-CH₃), 1.05 [s, 9H, SiC(CH₃)₃], 1.51 (ddd, J = 13.6, 7.0, 4.7 Hz, 1H, 6-H_a), 1.60 (q, J = 1.2 Hz,

3H, 4'-H or 5'-H), 1.65 (q, $J = 1.2$ Hz, 3H, 4'-H or 5'-H), 1.71 (ddq, $J = 9.2, 7.0, 6.8$ Hz, 1H, 4-H), 1.96 (dq, $J = 9.1, 3.9$ Hz, 1H, 3a-H), 2.00–2.07 (m, 1H, 1'-H_a), 2.03 (ddd, $J = 13.6, 8.5, 7.0$ Hz, 1H, 6-H_b), 2.15 (dq, $J = 8.5, 4.7$ Hz, 1H, 6a-H), 2.18–2.24 (m, 1H, 1-H), 2.28 (ddd, $J = 19.1, 3.9, 1.6$ Hz, 1H, 3-H_a), 2.31–2.38 (m, 1H, 1'-H_b), 2.36 (dd, $J = 19.1, 9.3$ Hz, 1H, 3-H_b), 3.80 (q, $J = 7.0$ Hz, 1H, 5-H), 4.98–5.04 (m, 1H, 2'-H), 7.35–7.39 (m, 4H, *m*-H, *m'*-H), 7.41–7.45 (m, 2H, *p*-H, *p'*-H), 7.63–7.68 (m, 4H, *o*-H, *o'*-H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ 17.0 (4-CH₃), 17.8 (C-4' or C-5'), 19.2 [SiC(CH₃)₃], 25.8 (C-4' or C-5'), 27.0 [SiC(CH₃)₃], 28.5 (C-1'), 41.4 (C-6), 42.4 (C-6a), 42.6 (C-3, C-3a), 49.2 (C-4), 56.3 (C-1), 81.6 (C-5), 121.1 (C-2'), 127.5, 127.6 (C-*m*, C-*m'*), 129.61, 129.62 (C-*p*, C-*p'*), 133.3 (C-3'), 134.1, 134.4 (C-*i*, C-*i'*), 135.90 (C-*o*, C-*o'*), 221.4 (C-2) ppm; FT-IR (ATR): $\tilde{\nu} = 3071$ (w), 2958 (s), 2929 (s), 2858 (m), 1737 (vs), 1589 (w), 1460 (m), 1428 (m), 1378 (w), 1110 (vs), 1033 (m), 874 (m), 822 (m), 740 (m), 702 (s), 613 (m), 548 (w), 526 (w) cm⁻¹; MS (ESI): $m/z = 483.3$ [M + Na]⁺, 461.3, 383.2 [M + Na – C₆H₆]⁺, 205.2, 187.2, 86.1; HRMS (ESI): calcd. for C₃₀H₄₀O₂SiNa⁺ [M + Na]⁺ 483.2692; found 483.2692. **14d**: colorless oil, yield: 64 mg (20%), R_f 0.46 (hexanes/EtOAc, 15:1), $[\alpha]_D^{20} -4.0$ ($c = 4.1$, CH₂Cl₂). ¹H NMR (500 MHz, CDCl₃): δ 0.82 (d, $J = 6.6$ Hz, 3H, 6-CH₃), 1.04 [s, 9H, SiC(CH₃)₃], 1.50 (dq, $J = 13.3, 6.6$ Hz, 1H, 4-H_a), 1.58 (q, $J = 1.1$ Hz, 3H, 4'-H or 5'-H), 1.67 (q, $J = 1.1$ Hz, 3H, 4'-H or 5'-H), 1.77–1.86 (m, 2H, 6a-H, 6-H), 2.04–2.11 (m, 2H, 1'-H_a, 4-H), 2.20–2.35 (m, 3H, 1-H, 1'-H_b, 3-H_a), 2.43–2.49 (m, 2H, 3a-H, 3-H_b), 3.80 (q, $J = 6.4$ Hz, 1H, 5-H), 5.02–5.07 (m, 1H, 2'-H), 7.35–7.39 (m, 4H, *m*-H, *m'*-H), 7.41–7.45 (m, 2H, *p*-H, *p'*-H), 7.62–7.68 (m, 4H, *o*-H, *o'*-H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ 17.6 (6-CH₃), 17.8 (C-4' or C-5'), 19.1 [SiC(CH₃)₃], 25.8 (C-4' or C-5'), 27.0 [SiC(CH₃)₃], 29.4 (C-1'), 33.9 (C-3a), 42.0 (C-4), 45.2 (C-3), 49.0 (C-6), 51.0 (C-6a), 54.4 (C-1), 81.8 (C-5), 121.3 (C-2'), 127.53, 127.54 (C-*m*, C-*m'*), 129.59, 129.61 (C-*p*, C-*p'*), 133.4 (C-3'), 134.1, 134.3 (C-*i*, C-*i'*), 135.87, 135.91 (C-*o*, C-*o'*), 222.0 (C-2) ppm; FT-IR (ATR): $\tilde{\nu} = 3070$ (w), 2956 (s), 2930 (s), 2860 (m), 1736 (vs), 1454 (w), 1429 (w), 1380 (w), 1110 (vs), 867 (w), 821 (w), 740 (m), 703 (s), 613 (m), 504 (m) cm⁻¹; MS (ESI): $m/z = 483.3$ [M + Na]⁺, 461.3, 383.2 [M + Na – C₆H₆]⁺, 205.2, 187.2, 86.1; HRMS (ESI): calcd. for C₃₀H₄₀O₂SiNa⁺ [M + Na]⁺ 483.2692; found 483.2683.

4.6. {[(2*R*,3*R*,3*aS*,4*R*,6*aR*)-3-Allyl-4-methyl-1,2,3,3*a*,4,6*a*-hexahydropentalen-2-yl]oxy}-(*tert*-butyl)diphenylsilane (**18**)

In analogy to ref.,¹⁹ a 1.6 M solution of *n*-BuLi in hexane (0.29 mL, 460 μ mol) was slowly added dropwise to a solution of **16b** (115 mg, 190 μ mol) in abs. THF (5 mL) and TMEDA (0.5 mL) at –78 °C. Then the reaction mixture was warmed to room temperature and stirred

for 2.5 h. A satd NaHCO₃ solution (4 mL) was added, the organic solvent removed and the remaining aqueous layer extracted with CH₂Cl₂ (3 × 30 mL). The combined extracts were dried (MgSO₄) and concentrated. The residue was purified by flash chromatography on SiO₂ (hexanes/EtOAc, 10 : 1, *R_f* 0.91) to give **18** (67.0 mg, 85%, purity >95 % by ¹H NMR) as a yellowish oil. $[\alpha]_D^{20}$ -14.6 (*c* = 1.0, CH₂Cl₂). ¹H NMR (500 MHz, CDCl₃): δ 0.94 (d, *J* = 6.9 Hz, 3H, 4-CH₃), 1.04 [s, 9H, SiC(CH₃)₃], 1.25 (dt, *J* = 12.2, 8.6 Hz, 1H, 1-H_a), 1.61 (dt, *J* = 2.5, 8.9 Hz, 1H, 3a-H), 1.70 (ddt, *J* = 12.9, 8.6, 4.4 Hz, 1H, 3-H), 1.84 (ddd, *J* = 12.2, 8.6, 6.4 Hz, 1H, 1-H_b), 1.87–1.91 (m, 1H, 1'-H_a), 2.36–2.42 (m, 1H, 1'-H_b), 2.51–2.57 (m, 1H, 4-H), 2.80–2.87 (m, 1H, 6a-H), 3.67 (dt, *J* = 6.4, 8.6 Hz, 1H, 2-H), 4.90–4.98 (m, 2H, 3'-H), 5.47 (dt, *J* = 5.5, 2.2 Hz, 1H, 5-H), 5.52 (dt, *J* = 5.5, 2.0 Hz, 1H, 6-H), 5.71 (dddd, *J* = 14.5, 10.1, 7.7, 6.8 Hz, 1H, 2'-H), 7.34–7.38 (m, 4H, *m*-H, *m'*-H), 7.39–7.44 (m, 2H, *p*-H, *p'*-H), 7.64–7.69 (m, 4H, *o*-H, *o'*-H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ 19.2 [SiC(CH₃)₃], 21.5 (4-CH₃), 27.0 [SiC(CH₃)₃], 37.0 (C-1'), 39.8 (C-1), 45.5 (C-6a), 46.8 (C-4), 51.4 (C-3a), 53.7 (C-3), 78.3 (C-2), 115.5 (C-3'), 127.4, 127.5 (C-*m*, C-*m'*), 129.4, 129.5 (C-*p*, C-*p'*), 133.5 (C-6), 134.2 (C-5), 134.3, 134.7 (C-*i*, C-*i'*), 136.0 (C-*o*, C-*o'*), 137.5 (C-2') ppm; FT-IR (ATR): $\tilde{\nu}$ = 3050 (w), 2953 (m), 2928 (m), 2859 (m), 2359 (w), 1640 (w), 1427(w), 1373 (w), 1264 (s), 1109 (s), 997 (w), 909 (m), 866 (w), 822 (w), 734 (vs), 701 (vs), 612 (m) cm⁻¹; MS (ED): *m/z* (%) = 401.2 (8) [M – Me]⁺, 359.2 (100) [M – *t*-Bu]⁺, 281.1 (21), 199.1 (68); HRMS (ESI): calcd. for C₂₈H₃₆O₂SiNa⁺ [M + Na]⁺ 439.2428; found 439.2427.

4.7. *tert*-Butyl(diphenyl)[(3a'S,6a'S)-3',3a',6',6a'-tetrahydro-2'H-spiro[1,3-dioxolane-2,1'-pentalen]-4'-yloxy]silane (**20**)

A solution of **19** (72.0 mg, 0.22 mmol) in THF (2 mL) was slowly added dropwise to a solution of KHMDS (64.0 mg, 0.22 mmol) in THF (3 mL) at -78 °C and the mixture stirred for 20 min. After addition of TBDPSCl (76.0 μL, 72.0 mg, 0.24 mmol), the reaction mixture was allowed to warm to room temperature and stirred for a further 1 h. The solvent was removed under vacuum, the residue taken up with pentane and filtered through Celite. The filtrate was concentrated under vacuum and the crude product purified by chromatography on SiO₂ with hexanes/EtOAc (10:1, *R_f* 0.75) to give **20** (72.0 mg, 0.17 mmol, 85%). ¹H NMR (500 MHz, CDCl₃): δ 1.03 [s, 9H, SiC(CH₃)₃], 1.58–1.62 (m, 2H, 2'-H), 1.70–1.77 (m, 1H, 3'-H_a), 1.89–1.93 (m, 1H, 3'-H_b), 2.12–2.16 (m, 2H, 6'-H), 2.41–2.45 (m, 1H, 6a'-H), 3.01–3.13 (m, 1H, 3a'-H), 3.75–3.80 (m, 1H, OCH₂), 3.83–3.87 (m, 3H, OCH₂), 4.07–4.09 (m, 1H, 5'-H), 7.36–7.41 (m, 4H, *o*-H), 7.42–7.45 (m, 2H, *p*-H), 7.68–7.71 (m, 4H, *m*-H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ 19.9 [SiC(CH₃)₃], 26.4 (C-3'), 26.8 [SiC(CH₃)₃], 30.0 (C-6'),

32.9 (C-2'), 45.2 (C-6a'), 48.8 (C-3a'), 63.9 (OCH₂), 65.2 (OCH₂), 102.9 (C-5'), 119.8 (C-1'), 128.1 (C-*o*), 130.2 (C-*p*), 133.4 (C-*i*), 135.9 (C-*m*) ppm; FT-IR (ATR): $\tilde{\nu}$ = 3071 (w), 2955 (s), 2941 (s), 2889 (m), 2858 (s), 2357 (w), 1650 (s), 1588 (w), 1472 (m), 1428 (m), 1391 (w), 1348 (s), 1302 (m), 1269 (m), 1239 (m), 1202 (s), 1183 (m), 1109 (vs), 1030 (s), 1009 (m), 946 (s), 889 (w), 861 (m), 840 (s), 822 (s), 794 (m), 741 (m), 701 (vs), 651 (w), 613 (m), 574 (w) cm⁻¹; MS (ESI): m/z = 443.20 [M + Na]⁺, 421.22 [M + H]⁺, 358.15, 304.26, 282.27, 239.11; HRMS (ESI): calcd. for C₂₆H₃₂O₃Si [M + H]⁺ 420.2193; found 420.2199.

4.8. (3a'S,4'R,5'R,6a'S)- and (3a'S,4'S,5'S,6a'S)-4'-[*tert*-Butyl(diphenyl)silyl]oxy}hexahydro-2'H-spiro[1,3-dioxolane-2,1'-pentalen]-5'-ol (**21a**) and (**21b**)

a) Silyl enol ether **20** (210 mg, 0.50 mmol) was added to a solution of MeCN (2.4 mL), dimethoxymethane (4.80 mL) and stock solution I (4.80 mL) [prepared from sodium tetraborate decahydrate (19.1 g, 50.0 mmol) and disodium ethylenediaminetetraacetate dihydrate (149 mg, 0.40 mmol) in H₂O (1 L)] at 0 °C followed by addition of Shi catalyst (50.0 mg, 0.20 mmol) and Bu₄NHSO₄ (61.7 mg, 0.20 mmol). To this reaction mixture ice-cold solutions of oxone (427 mg, 0.70 mmol) in stock solution II (3 mL) [prepared from disodium ethylenediaminetetraacetate dihydrate (149 mg, 0.40 mmol) in H₂O (1 L)] and K₂CO₃ (402 mg, 2.80 mmol) in H₂O (3 mL) were added successively over 2 h. After complete addition, the two-phase mixture was stirred at 0 °C for a further 3 h and diluted with H₂O (30 mL) to dissolve the precipitated salts. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3 × 30 mL). The combined organic layers were washed with brine, dried (MgSO₄) and concentrated under vacuum to 5 mL. Crude *tert*-butyl[(1b'S,4a'S)-hexahydro-1a'H-spiro[1,3-dioxolane-2,4'-pentaleno[1,2-*b*]oxiren]-1a'-yloxy]diphenylsilane was used without further purification.

b) To a solution of crude *tert*-butyl[(1a'R,1b'S,4a'S)-hexahydro-1a'H-spiro[1,3-dioxolane-2,4'-pentaleno[1,2-*b*]-1a'-yloxy]diphenylsilane (0.25 mmol) in THF (2 mL) at 0 °C was added BH₃·THF (0.64 mL, 0.64 mmol, 1.0 M in THF) and the reaction mixture stirred for 1 h. After complete conversion (GC control), a 1 M tris(hydroxymethyl)aminomethane hydrochloride solution (5 mL) was added (gas formation!). The bilayer system was warmed to room temperature and stirred for 30 min. The layers were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (MgSO₄) and the solvent removed under vacuum. The residue was purified by chromatography on SiO₂ (hexanes/EtOAc, 4:1→3:1→2.5:1) to give **21a** (14 mg, 15%), **21b** (38 mg, 38%) and the respec-

tive *cis*-compound (19 mg, 19%). **21a**: R_f 0.8 (hexanes/EtOAc, 2:1). ^1H NMR (500 MHz, CDCl_3): δ 1.10 (s, 9H, $\text{SiC}(\text{CH}_3)_3$), 1.53–1.59 (m, 1H, 3'- H_a), 1.59–1.62 (m, 1H, 2'- H_a), 1.68–1.74 (m, 1H, 6'- H_a), 1.87–1.91 (m, 1H, 6'- H_b), 2.10–2.16 (m, 1H, 3'- H_b), 2.16–2.24 (m, 2H, 2'- H_b , 6a'-H), 2.41–2.47 (m, 1H, 3a'-H), 2.78–2.80 (d, $J = 2.9$ Hz, 1H, OH), 3.82–3.85 (m, 1H, 5'-H), 3.87–3.94 (m, 4H, $\text{OCH}_2\text{CH}_2\text{O}$), 4.05–4.08 (m, 1H, 4'-H), 7.36–7.49 (m, 4H, *o*-H), 7.42–7.46 (m, 2H, *p*-H), 7.66–7.71 (m, 4H, *m*-H) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ 19.4 ($\text{SiC}(\text{CH}_3)_3$), 22.3 (C-3'), 27.1 ($\text{SiC}(\text{CH}_3)_3$), 31.8 (C-6'), 33.6 (C-2'), 44.2 (C-3a'), 46.9 (C-6a'), 64.0 ($\text{OCH}_2\text{CH}_2\text{O}$), 64.9 ($\text{OCH}_2\text{CH}_2\text{O}$), 74.0 (C-5'), 77.2 (C-4'), 119.4 (C-1'), 127.7 (C-*o*), 127.8 (C-*o*), 129.87 (C-*p*), 129.94 (C-*p*), 133.4 (C-*i*), 133.6 (C-*i*), 135.6 (C-*o*), 135.7 (C-*o*) ppm; FT-IR (ATR): $\tilde{\nu} = 2952$ (m), 2889 (m), 2858 (m), 1472 (w), 1428 (w), 1362 (w), 1332 (w), 1213 (w), 1110 (vs), 1031 (m), 903 (vs), 854 (w), 822 (w), 729 (vs), 703 (vs), 649 (m), 614 (w), 537 (m) cm^{-1} ; MS (ESI): $m/z = 461.21$ $[\text{M} + \text{Na}]^+$, 439.23 $[\text{M} + \text{H}]^+$, 361.18 $[\text{M} - \text{Ph}]^+$, 331.14, 317.16, 283.14, 265.12, 239.11 $[\text{M} - \text{TBDPS}]^+$, 211.08, 197.06, 165.09, 155.09, 121.06; HRMS (ESI): calcd. for $\text{C}_{26}\text{H}_{34}\text{O}_4\text{SiNa}^+ [\text{M} + \text{Na}]^+$ 461.2119; found 461.2117. **21b**: R_f 0.70 (hexanes/EtOAc, 2:1). ^1H NMR (500 MHz, CDCl_3): δ 1.10 (s, 9H, $\text{SiC}(\text{CH}_3)_3$), 1.36–1.42 (m, 1H, 6'- H_a), 1.58–1.65 (m, 2H, 2'- H_a , 3'- H_a), 1.76–1.81 (m, 1H, 2'- H_b), 1.89–1.98 (m, 2H, 6'- H_b , 3'- H_b), 2.29–2.34 (m, 1H, 6a'-H), 2.45–2.52 (m, 1H, 3a'-H), 3.86–3.92 (m, 4H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.96–4.00 (m, 2H, 4'-H, 5'-H), 7.36–7.40 (m, 4H, *o*-H), 7.41–7.44 (m, 2H, *p*-H), 7.66–7.72 (m, 4H, *m*-H) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ 19.4 ($\text{SiC}(\text{CH}_3)_3$), 22.6 (C-3'), 27.1 ($\text{SiC}(\text{CH}_3)_3$), 30.4 (C-6'), 35.1 (C-2'), 42.8 (C-3a'), 44.7 (C-6a'), 64.1 ($\text{OCH}_2\text{CH}_2\text{O}$), 65.0 ($\text{OCH}_2\text{CH}_2\text{O}$), 77.4 (C-4'/C-5'), 81.4 (C-4'/C-5'), 118.7 (C-1'), 127.7 (C-*o*), 127.8 (C-*o*), 129.8 (C-*p*), 129.9 (C-*p*), 133.7 (C-*i*), 134.5 (C-*i*), 135.8 (C-*o*), 135.9 (C-*o*) ppm; FT-IR (ATR): $\tilde{\nu} = 2955$ (m), 2889 (m), 2857 (m), 1472 (w), 1427 (w), 1362 (w), 1104 (m), 1041 (m), 942 (w), 906 (vs), 855 (m), 821 (m), 729 (vs), 701 (vs), 649 (m), 612 (m) cm^{-1} ; MS (ESI): $m/z = 439.23$ $[\text{M} + \text{H}]^+$, 361.18 $[\text{M} - \text{Ph}]^+$, 317.16, 299.15, 283.14, 239.11 $[\text{M} - \text{TBDPS}]^+$, 235.11, 183.10, 165.09, 139.07, 121.06, 105.04; HRMS (ESI): calcd. for $\text{C}_{26}\text{H}_{34}\text{O}_4\text{SiNa}^+ [\text{M} + \text{Na}]^+$ 461.2119; found 461.2125.

4.9. *rac* 3-But-3-enyl-2,3,3a,6a-tetrahydropentalene-1,4-dione (23a)

To a suspension of CuCN (0.55 g, 6.16 mmol) in freshly distilled THF was added TMEDA (0.66 mL, 0.75 g, 4.40 mmol) and the reaction mixture cooled to -78 °C. A solution of 3-butenylmagnesiumbromide, freshly prepared from Mg (0.43 g, 17.6 mmol) and 4-bromo-1-butene (0.49 mL, 0.65 g, 4.80 mmol), was slowly added dropwise and the reaction mixture stirred for 20 min at -78 °C. Then TMSCl (0.68 mL, 0.58 g, 5.28 mmol) was added followed

by a cold solution of **22** (0.59 g, 4.40 mmol) in THF (5 mL). After stirring for 30 min, the reaction mixture was hydrolyzed with a mixture from a satd. NH_4Cl solution/25%ic NH_3 solution (10:1, 15 mL). The layers were separated and the aqueous layer was extracted with Et_2O (3×10 mL). A 1 N HCl solution was added to the combined organic layers, and the mixture stirred for 30 min to hydrolyze the formed silyl enol ether. The layers were separated and the organic layer was dried (MgSO_4) and concentrated. The residue was purified by flash chromatography (hexanes/ EtOAc, 4:1) to give **23a** (530 mg, 2.77 mmol, 63%) as a yellow oil. R_f 0.5 (hexanes/EtOAc, 2:1). ^1H NMR (500 MHz, CDCl_3): δ 1.45–1.54 (m, 1H, 7- H_a), 1.69–1.78 (m, 1H, 7- H_b), 2.05–2.20 (m, 3H, 2- H_a , 8-H), 2.30–2.38 (m, 1H, 3-H), 2.53 (ddt, $J = 17.8, 8.0, 0.8$ Hz, 1H, 2- H_b), 2.77 (ddt, $J = 6.4, 3.6, 0.8$ Hz, 1H, 3a-H), 3.59–3.63 (m, 1H, 6a-H), 4.95–5.05 (m, 2H, 10-H), 5.72–5.82 (m, 1H, 9-H), 6.20–6.23 (m, 1H, 5-H), 7.56–7.58 (m, 1H, 6-H) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ 31.6 (C-8), 35.6 (C-7), 35.8 (C-3), 43.2 (C-2), 52.6 (C-3a), 55.7 (C-6a), 115.6 (C-10), 134.9 (C-5), 137.6 (C-9), 159.5 (C-6), 210.0 (C-4), 212.2 (C-1) ppm; FT-IR (ATR): $\tilde{\nu} = 3076$ (w), 2926 (m), 1740 (s), 1703 (vs), 1640 (m), 1580 (m), 1452 (w), 1409 (w), 1334 (w), 1173 (m), 1073 (w), 995 (w), 912 (m), 788 (m) cm^{-1} ; MS (EI, 70 eV): m/z (%) = 190.1 (2) $[\text{M}]^+$, 162.1 (2), 148.1 (3), 135.0 (15) $[\text{M} - \text{C}_4\text{H}_7]$, 108.1 (100) $[\text{C}_6\text{H}_4\text{O}_2]$, 91.1 (6), 81.1 (32), 67.1 (3), 55.0 (9); HRMS (EI): calcd. for $\text{C}_{12}\text{H}_{14}\text{O}_2$ $[\text{M}]^+$ 190.0994; found 190.0989.

4.10. *rac* 3'-But-3-enyl-2',3',3a',6a'-tetrahydro-4'*H*-spiro[1,3-dioxolane-2,1'-pentalen]-4'-one (**23b**)

To a solution of toluene (5.00 mL), ethylene glycol (0.10 mL, 91 mg, 1.47 mmol) and *p*-TsOH (13 mg, 0.07 mmol) was added **23a** (140 mg, 0.74 mmol) and the reaction mixture heated at reflux for 7 h. After cooling to room temperature, a satd. NaHCO_3 solution (5 mL) was added and the layers were separated. The organic layer was washed with brine (4 mL) and then extracted with EtOAc (10 mL), dried (MgSO_4) and the solvent removed under reduced pressure. The residue was purified by preparative HPLC on a Kromasil column (250 $\times$ 20 mm, 5 μm pore size; MZ Analysentechnik GmbH) with hexanes/EtOAc (4:1) to give **23b** (100 mg, 0.44 mmol, 60%) as a yellow oil. R_f 0.6 (hexanes/EtOAc, 1:1). ^1H NMR (500 MHz, CDCl_3): δ 1.54–1.65 (m, 2H, 2- H_a , 7- H_a), 1.75–1.83 (m, 1H, 7- H_b), 1.93 (dd, $J = 13.3, 7.4$ Hz, 1H, 2- H_b), 2.00–2.07 (m, 1H, 3-H), 2.12 (q, $J = 7.4$ Hz, 2H, 8-H), 2.51 (dd, $J = 6.5, 4.3$ Hz, 1H, 3a-H), 3.36–3.40 (m, 1H, 6a-H), 3.86–4.05 (m, 4H, $\text{OCH}_2\text{CH}_2\text{O}$), 4.94–4.97 (m, 1H, 10- H_a), 5.01–5.06 (m, 1H, 10- H_b), 5.78–5.87 (m, 1H, 9-H), 6.14 (ddd, $J = 5.9, 2.2, 0.7$ Hz, 1H, 5-H), 7.57 (ddd, $J = 5.6, 2.9, 0.7$ Hz, 1H, 6-H) ppm; ^{13}C NMR (125 MHz, CDCl_3): δ

32.1 (C-8), 35.0 (C-7), 38.5 (C-3), 40.7 (C-2), 54.0 (C-3a), 54.6 (C-6a), 64.7, 64.8 (OCH₂CH₂O), 114.9 (C-10), 116.1 (C-1), 134.2 (C-5), 138.5 (C-9), 162.7 (C-6), 211.7 (C-4) ppm; FT-IR (ATR): $\tilde{\nu}$ = 3074 (w), 2923 (m), 1702 (vs), 1640 (m), 1585 (m), 1437 (w), 1341 (m), 1175 (m), 1113 (m), 1066 (m), 1018 (m), 947 (m), 911 (m), 884 (w), 842 (w), 798 (w), 769 (w) cm⁻¹; MS (EI, 70 eV): m/z (%) = 234.1 (33) [M]⁺, 179.1 (25) [M – C₄H₇], 153.1 (100), 125.1 (6), 107.0 (31), 99.0 (8), 86.0 (19), 79.0 (10); HRMS (EI): calcd. for C₁₄H₁₈O₃ [M]⁺ 234.1256; found: 234.1251.

4.11. Cytotoxicity assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to measure growth and viability of cells which are capable of reducing it to a violet formazan product. 60 μ L of serial dilutions of the test compounds were added to 120 μ L aliquots of a cell suspension (50 000 mL⁻¹) in 96-well microplates. Blank and solvent controls were incubated under identical conditions for 5 d. MTT in phosphate buffered saline (PBS) (20 μ L) was added to a final concentration of 0.5 mg mL⁻¹. After 2 h, the precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed with PBS (100 μ L) and dissolved in isopropanol containing 0.4% hydrochloric acid (100 μ L). The microplates were gently shaken for 20 min to ensure a complete dissolution of the formazan and finally measured at 595 nm using an ELISA plate reader. All experiments were carried out in two parallel experiments. Activity values were calculated as the mean with respect to the controls set to 100%.

4.12. Cell staining

PtK₂ cells (ATCC CCL-56) were grown in 750 μ L medium in 4-well plates (Nunc) on glass coverslips, and incubated with the test compound overnight. For F-actin staining cells were fixed with formalin (3.7%) for 10 min, permeabilized with 0.1% Triton X-100 for 5 min, and then incubated with Alexa Fluor 488 phalloidin (1:100; Molecular Probes) for 1 h. For ER and α -tubulin staining cells were fixed with cold (–20°C) MeOH/acetone (1:1) for 10 min and later incubated with a primary antibody against GRP-94 (1:1000; Affinity Bioreagents) and α -tubulin (1:100; Sigma), respectively, and then with a secondary Alexa Fluor 488 goat anti-rat IgG antibody (1:200; Molecular Probes) and Alexa Fluor 488 goat anti-mouse IgG antibody (1:200; Molecular Probes), respectively, and mounted in ProLong Antifade Gold (Molecular Probes), which included DAPI to stain the nuclei.

4.13. Click chemistry

PtK₂ cells (ATCC CCL-56) were grown in 750 μ L medium in 4-well plates (Nunc) on glass coverslips, and incubated with **11a** (3 μ g mL⁻¹) overnight. Cells were fixed with formalin (3.7%) for 10 min and permeabilized with 0.1% Triton X-100 for 5 min. Cells were blocked with 3% FBS in PBS at 37 °C for 5 min. The Click-iT cocktail was prepared as per the manufacturer's protocol just prior its usage and cells were incubated for 30 min. For co-localization studies, further staining was carried out for F-actin using Alexa Fluor 488 phalloidin (Ph488, 1:100 with 10% FBS, Molecular Probes), and mounted in ProLong Anti-fade Gold (Molecular Probes) without DAPI.

4.14. Impedance measurement profiling

The impedance of incubated cell cultures was monitored on a RT-CES system (xCelligence) from Acea Biosciences (Roche). The resulting impedance curves were used for a hierarchical cluster analysis of reference compounds together with compound of unknown mode of action. Co-clustering of the compound of unknown mode of action with reference compounds with known activity class label is used to predict the mode of action. The method was described previously.²⁶

4.15. Tubulin polymerization assay

This assay was carried out according to manufacturer's protocol (Tubulin Polymerization Assay Kit, Catalog #: BK0011P; Cytoskeleton). Tubulin master mix was prepared which included buffer, glycerol, GTP stock (100 mM) and porcine tubulin (10 mg mL⁻¹) according to the manufacturer's guidelines – all were provided in the kit. 5 μ L of the compounds were added to 50 μ L of tubulin mix in a 96-well plate which was immediately placed in a thermo-regulated fluorimeter maintained at 37 °C. Fluorescence was measured for about an hour at 460 nm (excitation: 340 nm).

4.16. Actin polymerization assay

This assay was carried out according to manufacturer's protocol (Actin Polymerization Biochem Kit, Catalog #: BK003; Cytoskeleton). According to the manufacturer's guidelines buffer and actin were prepared. The actin stock was added to a 96-well plate and fluorescence was read at 410 nm (excitation: 360 nm) for 3 min for a baseline reading. After 3 min test

compounds (20 μ L) were added and read for another 20 min. This was followed by addition of 10X actin polymerization buffer and readings were taken for an hour.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://■>.

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