Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Synthesis and *in Vitro* Cytotoxicity of Cross-Conjugated Prostaglandin A and J Series and Their Hydroxy Derivatives

Remigiusz Żurawiński,*[a] Marian Mikołajczyk,*[a] Marcin Cieślak,[b] Karolina Królewska[b] and Julia Kaźmierczak-Barańska[b]

[a] *Department of Heteroorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland*
[b] *Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza.112, 90-363 Łódź, Poland*
[*] *Corresponding authors. E-mail addresses: remzur@cbmm.lodz.pl (R. Żurawiński); marmikol@cbmm.lodz.pl (M. Mikołajczyk)*

The synthesis of two cross-conjugated prostaglandin analogues of a known neurotrophic activity and their new hydroxy derivatives was accomplished starting from the diastereoisomeric (+)-camphor protected 3-[(dimethoxyphosphoryl)methyl]-4,5-dihydroxycyclopent-2-enones. The cytotoxicity of these compounds was determined against HeLa, K562, HL-60 human cancer cell lines and normal human cells (HUVEC). We found that NEPP11 and its C7-hydroxy derivative demonstrated high anticancer activity against the HeLa and HL-60 human cancer cell lines at concentrations ranging from 1 to 2 μM. Moreover, C7-hydroxy derivative of NEPP11 displayed high cytotoxic selectivity between cancer cell lines and normal human cells. On the other hand, J-type prostaglandin analogue of NEPP11 and its C13-hydroxy derivatives were much less toxic or nontoxic against the cancer and normal cells at concentrations up to 1 mM.

Introduction

Prostaglandins (PGs) are part of a family of naturally occurring biologically active lipids mediators derived from the oxidation of polyunsaturated twenty-carbon fatty acids. These labile, but highly potent molecules, regulate a broad range of physiological processes in animals and humans, including blood circulation, the contraction and relaxation of smooth muscle tissue, renal physiology, the cytoprotection of gastric mucosa, digestion and reproduction.[1,2] They are also involved in many pathophysiological processes associated with inflammation[3] and cancer.[4,5] On the other hand, various members of the cyclopentenone PG family (cyPG), which are characterized by the presence of the α,β-unsaturated carbonyl moiety, exhibit anti-neoplastic, anti-inflammatory and antiviral activities.[6-8] In contrast to other PGs, which exert their effects by binding to G-protein coupled receptors, the cyPGs interact with other cellular targets, including signaling molecules and transcription factors. These interactions are mainly attributed to the presence of α,β-unsaturated carbonyl group which makes these PGs susceptible for the Michael addition reaction with soft nucleophiles, such as the sulfhydryl group of cysteine residues. While formation of covalent adducts of cyPGs with glutathione (GSH) leads to the loss of their antitumor activity, binding with intracellular proteins impairs the function of these proteins, thus affecting cellular viability. Among the known targets for cyPGs there are transcription factors such as NF-κB[9] and AP-1,[10] nuclear receptor PPARγ[11], proteins involved in the regulation of cellular redox status[12,13] and cytoskeletal proteins.[14,15] The cellular mechanism of antitumor activity of cyPGs is complex, multiple and depends among other factors on the cell type, cyPG structure and the concentration used. cyPGs antitumor activity mainly manifests by inducing apoptosis or suppressing the tumor growth by influence on the expression of genes involved in cell growth and cell proliferation, as well as, on stress-induced genes.[6,8] Cell cycle arrest is correlated with modulation of cell cycle regulatory proteins, such as cyclins D1[16] and B1[17], cyclin-dependent kinases[18], or cyclin-dependent kinase inhibitors.[19]

Antitumor activity of cyPGs is inherently connected with the presence of the cyclopentenone backbone and depends on the degree of the olefin-ketone conjugation as well as on accessibility and the nucleophilic reactivity of the endocyclic β-carbon atom of the α,β-unsaturated carbonyl moiety. PGs with a cross-conjugated cyclopentadienone system like Δ^7-PGA_1, Δ^{12}-PGJ_2 and $\Delta^{12,14}$-PGJ_2 (Fig. 1) have considerably higher antitumor activity in comparison with PGs containing a simple enone unit[20] and substantially lower than cross-conjugated dienone punaglandins containing an electron-withdrawing chlorine atom attached to the α-carbon atom of the α,β-unsaturated carbonyl moiety and the hydroxyl group at C12 (Fig. 2).[21]

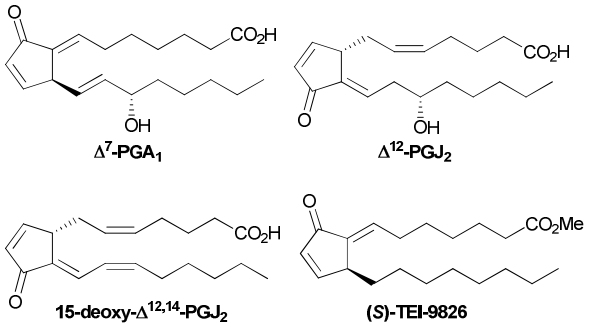


Fig. 1 Structures of cross-conjugated PGs mentioned in the text.

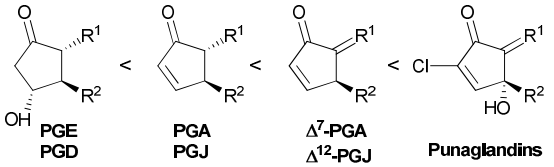


Fig. 2 Order of anticancer activity of PGs and their derivatives.

The evidence that the cyclopentenone ring with the conjugated enone moiety is responsible for the anticancer properties of cyPGs is given by cyclopent-2-enone itself. This compound mimics most of the biological activities of cyPGs.[22,23] It was also shown that it was cytotoxic and pro-apoptotic in cancer cells derived from human malignant tumors.[24] The structure-activity relationship studies on cyPGs also revealed that the hydroxyl group at C15 was not required for a high antitumor activity with both the PGA and PGJ series compounds[20] and in the PGA series, inversion of the configuration on the isomerase-sensitive C12 stereocenter from the natural *S* to the unnatural *R* configuration significantly increases biological stability while maintaining antitumor potency.[25] The above observations led to the synthesis of 13,14-dihydro-15-deoxy-Δ^7-PGA_1 methyl ester (TEI-9826) which exhibited a highly improved stability in serum in comparison to Δ^7-PGA_1. Lipid microsphere-integrated TEI-9826 has been shown to be active against human ovarian carcinoma cells in nude mice and to retain *in vivo* activity against cisplatin-resistant tumors.[26] Moreover, this compound displayed a unique antitumor activity profile by the COMPARE program with 38 tumor cell lines *in vitro*.[27] Although the use of cyPGs and their analogues as anticancer drugs is still a matter of the future, their multiple mechanism of action makes them attractive and promising antitumor agents in human chemotherapy, especially from the viewpoint of overcoming multi-drug chemoresistance.

Recently, in the frame of our research program aimed at the invention and development of general methods for the synthesis of bioactive cyclopentenones and cyclopentanones using phosphorus reagents,[28] the synthesis of both enantiomers of TEI-9826 has been presented based on the diastereoisomeric camphor protected 3-[(dimethoxyphosphoryl)methyl]-4,5-dihydroxycyclopent-2-enones **1** (Fig. 3).[29]

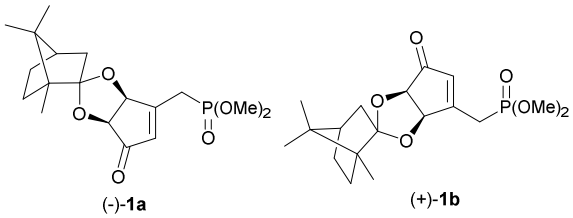


Fig. 3 Structures of the (+)-camphor protected diastereoisomeric 3-[(dimethoxyphosphoryl)methyl]-4,5-dihydroxycyclopent-2-enones **1**.

These chiral cyclopentenone building blocks are easily available in a two-step reaction sequence, which involves complete desymmetrization of *meso*-tartaric acid during the acid-catalyzed reaction with (+)-camphor and methyl orthoformate and the transformation of the camphor protected dimethyl tartrate formed to a separable mixture of the diastereoisomeric **1a** and **1b** upon treatment with an α-phosphonate carbanion.[30] Continuing our interest in this field, we report herein a new approach to the synthesis of the two cross-conjugated analogues of the prostaglandin A and J series, **2** and **3**, respectively, and their stereo-defined hydroxy derivatives *anti*-**4**, *syn*-**5b** and *anti*-**5b**, using chiral building blocks **1** as substrates (Fig. 4).

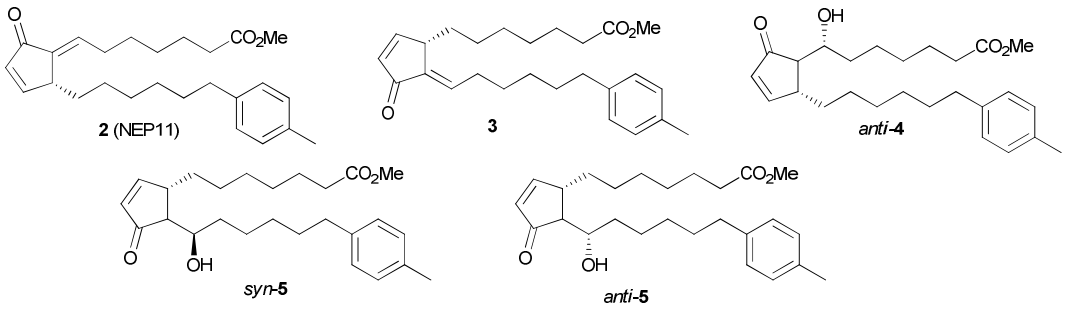


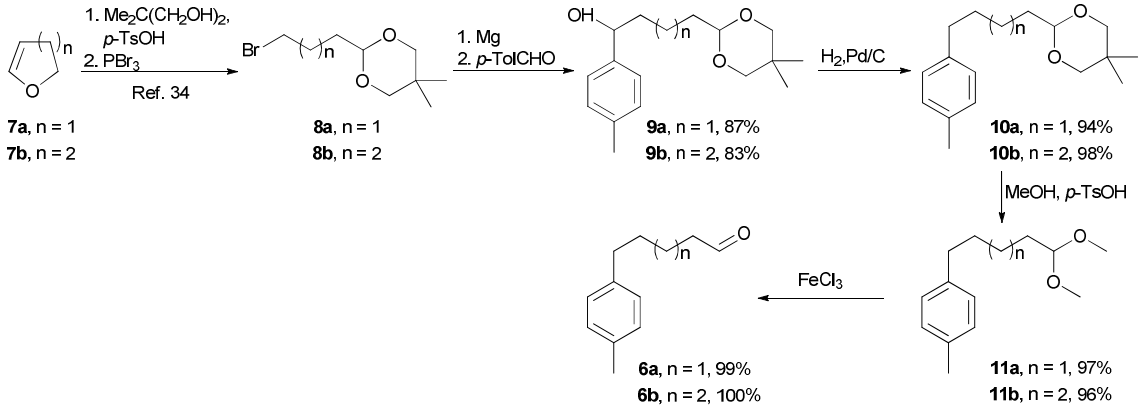
Fig. 4 Structures of the target compounds.

It was demonstrated that **2** (designated NEPP11) and its J-type analogue **3** exhibited neurotrophic activities.[31-33] They protect HT22 neuronal cells against oxidative glutamate toxicity, promote neurite outgrowth from PC12 cells induced by nerve growth factor, and suppress the manganese-induced apoptosis of PC12 cells. In spite of a great attention toward the neuroprotective and neuroregenerative properties of **2** and **3**, no experimental details on their synthesis and information on the expected anticancer activities have been given. Therefore, in this paper we also report the cytotoxicity of these compounds as well as of their new hydroxy derivatives *anti*-**4**, *syn*-**5b** and *anti*-**5b**, determined against human cervical carcinoma (HeLa), chronic leukemia (K562) and human acute leukemia (HL-60) cancer cell lines and normal human umbilical vein endothelial cells (HUVEC). We have identified compounds that were toxic against the HeLa, K562 and HL-60 cells and induced apoptosis. We have also demonstrated that at sublethal doses some of these cyPG analogues induce formation of aneuploid HeLa cells and arrest cells in G1 and G2/M phase of the cell cycle.

Results and discussion

Synthesis of the target compounds

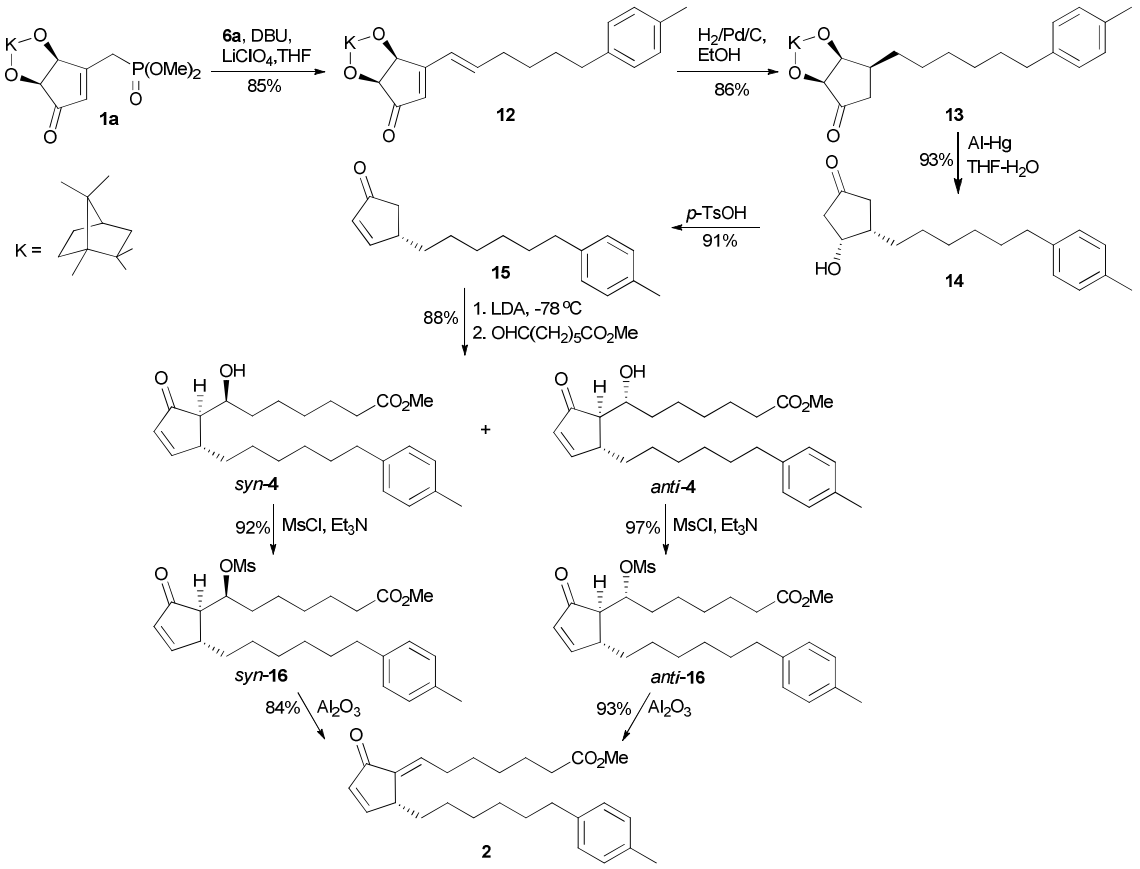
Because our strategy devised for the synthesis of the title compounds assumed installation of a proper ω-chain in the Horner olefination reaction (A-type PGs **2** and *anti*-**3**) or in the aldol condensation (J-type PG derivatives **3**, *syn*-**5**, *anti*-**5**) with appropriate aldehydes, 5-(4-methylphenyl)pentanal (**6a**) and 6-(4-methylphenyl)hexanal (**6b**) were prepared in a five step reaction sequence starting from 2,3-dihydrofuran (**7a**) and 3,4-dihydro-2*H*-pyran (**7b**), respectively (Scheme 1). Thus, the acid catalyzed acetalization of **7a** and **7b** with 2,2-dimethyl-1,3-propanediol, followed by bromination of the hydroxyacetals formed with phosphorus tribromide in one pot afforded the corresponding bromoacetals **8a** and **8b**.[34] The reaction of Grignard reagents generated from **8** with 4-methylbenzaldehyde gave benzyl alcohols **9** which subsequently were subjected to the reductive deoxygenation with hydrogen in the presence of 10%-Pd/C catalyst to yield the appropriate saturated acetals **10**. Because direct acidic deprotection of **10** to the corresponding aldehydes did not give the desired products in a satisfactory yield and with high purity, this transformation was accomplished in two steps involving transacetalization of **10** with methanol followed by deacetalization of dimethyl acetals **11** formed with hydrated iron(III) chloride. This procedure allowed us to prepare the desired aldehydes **6a** and **6b** in very good yields and of satisfactory purity. Therefore, they were used in the next synthetic step without purification.



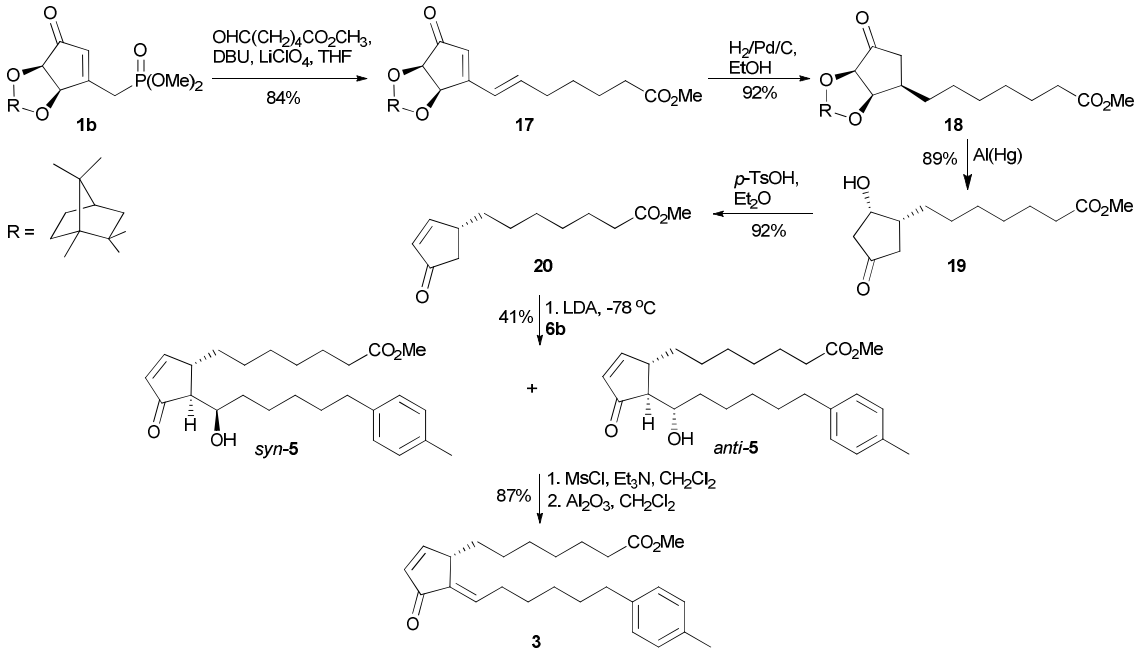
Scheme 1 Synthesis of aldehydes **6a** and **6b**.

Having aldehyde **6a** in hand, we focused on the synthesis of NEPP11 (**2**). The synthetic pathway to this compound is outlined in Scheme 2. The Horner olefination reaction of phosphonate **1a** and aldehyde **6a** carried out in the presence of DBU and $LiClO_4$ gave dienone **12**. Hydrogenation of the latter catalyzed by 10%-Pd/C proceeded with complete diastereoselectivity under stereocontrol of a chiral diol moiety affording **13** as a single diastereoisomer with the *S* configuration at the newly formed stereogenic center. Reductive deacetalization of **13** with aluminum amalgam in aqueous solution followed by water elimination from 3-hydroxycyclopentanone **14** formed produced the enantiopure cyclopentenone **15**. The aldol condensation of **15** with methyl 6-formylhexanoate gave a mixture of *anti*- and *syn*-aldols **4** in a ratio 5.4:1. They were separated by column chromatography and the absolute configuration at the C7 carbon atom of *anti*- and *syn*-aldols **4** was assigned based on the value of the coupling constants between protons at C7 and C8, which were found to be 8.4 and 2.8 Hz, respectively. The formation of aldol *anti*-4 as a major isomer can be rationalized by the steric interaction in the chair-like cyclic transition state involving the lithium enolate and the aldehyde, and is in agreement with the observations described in the literature.[35] Both *anti*- and *syn*-aldols **4** were subjected to mesylation with methanesulfonyl chloride and the corresponding *anti*- and *syn*-mesylates **16** formed were treated with neutral aluminum oxide to afford NEPP11 (**2**). The identical stereochemical outcome of the elimination of the mesyloxy group from *anti*- and *syn*-mesylates **16** can be explained in terms of the *syn* elimination (E1cB) and *anti* elimination (E2), respectively, as proposed by Kobayashi.[35]

The J-type PG derivatives **3**, *syn*-**5** and *anti*-**5** were synthesized using a very similar protocol to that described above in which the order of installation of side-chains was reversed (Scheme 3). Thus, the introduction of the appropriate substituent at the C8 was achieved via the Horner olefination reaction of phosphonate **1b** with methyl 5-formylpentanoate and the subsequent stereocontrolled reduction of the carbon-carbon double bond in the dienone **17** formed. The transformation of the protected diol moiety into an olefinic bond was accomplished in a two-step reaction sequence involving reductive deacetalization of **18** with aluminum amalgam followed by acid catalyzed dehydration of the resulting hydroxycyclopentanone **19**. The aldol condensation of **20** with 6-(4-methylphenyl)hexanal (**6b**) gave a mixture of diastereoisomeric *anti*- and *syn*-aldols **5** (they were separated by column chromatography only for the purpose of their characterization and cytotoxicity determination). The latter were converted in the reaction with methanesulfonyl chloride to the corresponding mesylates which upon treatment with neutral aluminum oxide gave the J-type PG analogue **3**.



Scheme 2 Synthesis of NEPP11 (**2**).



Scheme 3 Synthesis of PGJ derivatives **3** and **5**.

Biological results

Cytotoxicity

The cytotoxicity of the synthetized cyPG derivatives has been determined against human cancer cell lines (HL-60, K562 and HeLa) and human normal cells (HUVEC). The viability of the cells was determined by MTT assay at four different prostaglandin concentrations: 1 mM, $1x10^{-2}$ mM, $1x10^{-4}$ mM and $1x10^{-6}$ mM and IC_{50} values were calculated. We have previously reported that IC_{50} values obtained from 4-point dose-response curves can be reliable and successful for initial screening of cytotoxic compounds.[36,37] As the control cells with 100% viability, HeLa cells treated with 1% DMSO (vehicle control) were used. Under our experimental conditions, cross-conjugated derivatives of PGA and PGJ series NEPP11 (**2**) and **3**, respectively, displayed comparable or higher in vitro cytotoxicity against HL-60 and HeLa cells (IC_{50} of 1-8 μM) than the enantiomers of TEI-9826 (IC_{50} of 5-200 μM), which were used here as references (Table 1). At the same time, **3** was much less toxic towards the normal HUVEC cells, while cytotoxicity of NEPP11 (**2**) was comparable to that of (*R*)-TEI-9826. In contrast to a cross-conjugated J-type PG analogue **3**, the single enone derivatives of PGJ series with a hydroxy substituent at C13 *anti*-**5** and *syn*-**5** were not toxic for any cell line used. On the other hand, a single enone C7-hydroxy derivative of PGA series *anti*-**4** efficiently killed K562 and HeLa cells, while being practically non-toxic for HL-60 cells up to a concentration of 1 mM. After 48h incubation in the cell culture *anti*-**4** displayed high cytotoxicity against HeLa cells with IC_{50} of 2 μM, which was equal to cytotoxicity of a cross-conjugated dienone **2** and was 5-fold higher than that observed for (*S*)-TEI-9826. Moreover, beside selective toxicity against the cancer cell lines, *anti*-**4** did not show any negative effect on the viability of the normal HUVEC cells at concentrations up to 1 mM.

Table 1. The IC_{50} values calculated from the dose-response curves.

Compound	HL-60		K562		HeLa		HUVEC	
	IC_{50} 24h	IC_{50} 48h	IC_{50} 24h	IC_{50} 48h	IC_{50} 24h	IC_{50} 48h	IC_{50} 24h	IC_{50} 48h
(*R*)-TEI-9826	80 μM	5 μM	nd	nd	300 μM	200 μM	nd	100 μM
(*S*)-TEI-9826	80 μM	5 μM	nd	nd	80 μM	10 μM	nd	200 μM
NEP11 (**2**)	40 μM	1 μM	4 μM	1 μM	3 μM	2 μM	100 μM	100 μM
3	> 1 mM	5 μM	nd	nd	> 1 mM	8 μM	nd	> 1 mM
anti-**4**	> 1 mM	> 1 mM	> 1 mM	4 μM	5 μM	2 μM	> 1 mM	> 1 mM
syn-**5**	> 1 mM	> 1 mM	nd	nd	> 1 mM	> 1 mM	nd	> 1 mM
anti-**5**	> 1 mM	> 1 mM	nd	nd	> 1 mM	> 1 mM	nd	> 1 mM

nd – not determined

Determination of apoptosis and effects on cell cycle progression

cyPG derivatives **2**, **3**, *anti*-**4**, and (*S*)-TEI-9828 showing the highest cytotoxicity towards HeLa cells were investigated for their ability to induce apoptosis. Because increased activity of caspases is a hallmark of apoptotic processes, effector caspase

3 and 7 enzymatic cleavage assay that uses fluorescently labeled synthetic peptide as a substrate (Z-DEVD-R110) was applied. The amount of fluorescent product generated is proportional to caspase-3/7 cleavage activity and can be quantified by measurement of fluorescence of an analyzed sample using a spectrofluorometer. Cells exhibiting higher fluorescence values undergo apoptosis to a higher extent. HeLa cells grown in the presence of 1% DMSO (vehicle) served as a negative control and cells treated with staurosporin, a strong inducer of programmed cell death, were used as a positive control. Cells were also incubated with a given cyPG at the concentration of $5xIC_{50}$ for 18h. As shown on Figure 5**,** the highest fluorescence values were detected in the cells which were treated with staurosporin (positive control) and NEPP11 (**2**). This observation suggests that derivative **2** can activate caspases 3 and 7, which in consequence leads to apoptosis in HeLa cells. On the other hand, we did not observe any increase in fluorescence in cells treated with **3,** *anti*-**4** or (*S*)-TEI-9826, which may imply that toxicity of these compounds towards HeLa cells may result from necrosis.

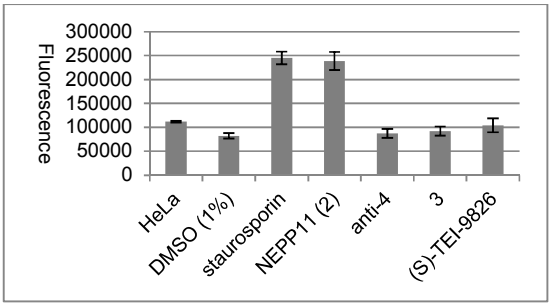


Fig. 5 Activity of caspase 3/7 in HeLa cells treated with test compounds for 18h. Compound concentration: staurosporin 1 μM, NEPP11 (**2**) 15μM, *anti*-**4** 25μM, **3** 40μM, (*S*)-TEI-9826 50μM. Mean values ± SD from 3 experiments are shown.

To determine the influence of the synthesized cyPGs on the cell cycle, HeLa cells were incubated at sublethal concentrations of the tested cyPGs for 24h and their DNA content was analyzed by FACS. First, we examined whether these cyPGs can induce changes in ploidy of HeLa cells. We found that **3**, (*R*)-TEI-9826, NEPP11 (**2**) and *anti*-**4** caused significant increase in the number of aneuploid HeLa cells (Table 2). And thus, for example, after 24h incubation with **3** or (*R*)-TEI-9826 we observed approximately 40% of diploid and 60% of aneuploid HeLa cells. On the contrary, in control or DMSO treated cells we observed 90% of diploid and 10% of tetraploid cells but no aneuploid cells.

Table 2 Influence of cyPG derivatives on ploidity of HeLa cells. Mean values and SD from 3 experiments are shown. cyPG concentration in a cell culture is given in parentheses.

	% Diploid	% Tetraploid	% Aneuploid
HeLa cells	90.4 ± 1.4	9.6 ± 1.4	0
DMSO (0.1%)	92.6 ± 0.9	7.4 ± 0.9	0
(*R*)-TEI-9826 (5 μM)	39.9 ± 1.7	0	60.1 ± 1.7
NEPP11 (**2**) (0.5 μM)	46.3 ± 1.5	0	53.7 ± 1.5
3 (5 μM)	39.8 ± 4.1	0	60.2 ± 4.1
anti-**4** (0.5 μM)	62.7 ± 2.5	0	37.3 ± 2.5
syn-**5** (5 μM)	95.1 ± 0.4	4.9 ± 0.4	0
anti-**5** (5 μM)	95.7 ± 0.8	4.3 ± 0.8	0

We have also analyzed cell cycle distribution of the diploid HeLa cells treated with the tested cyPGs (Table 3). As expected, *syn*-**5** and *anti*-**5** did not induce significant changes in cell cycle distribution as compared to the control or DMSO-treated cells. On the other hand, cross-conjugated PGJ derivative **3** induced accumulation of HeLa cells in G1 and G2/M phase of the cell cycle. In the presence of (*R*)-TEI-9826, we observed significantly increased accumulation of HeLa cells specifically in G2/M phase with no distribution changes in G1 phase. NEPP11 (**2**) and its hydroxy derivative *anti*-**4** with the highest cytotoxicity as determined by MTT assay, did not induced significant changes in HeLa cells distribution in both G1 and G2/M phases of the cell cycle.

Table 3 Influence of cyPG derivatives on the cell cycle distribution of diploid HeLa cells. Mean values and SD from 3 experiments are shown. cyPG concentration in a cell culture is given in parentheses.

	% of diploid in G1 phase	% of diploid in S phase	% of diploid in G2/M phase
HeLa cells	52.8 ± 0,6	39.2 ± 0,6	8.0 ± 0
DMSO (0.1%)	52.9 ± 6.7	36.1 ± 1.7	8.0 ± 0
(*R*)-TEI-9826 (5 μM)	53.9 ± 4.5	17.5 ± 6.3	28.6 ± 1.8
NEPP11 (**2**) (0.5 μM)	51.9 ± 4.8	37.0 ± 3.5	11.1 ± 1.9
3 (5 μM)	71.0 ± 7.5	5.5 ± 8.8	23.5 ± 1,4
anti-**4** (0.5 μM)	48.7 ± 9.1	40.9 ± 8.3	10.4 ± 0.7
syn-**5** (5 μM)	57.3 ± 0.9	34.7 ± 0.9	8.0 ± 0
anti-**5** (5 μM)	53.5 ± 0.3	38.5 ± 0.3	8.0 ± 0

Conclusion

In conclusion, a new approach to the synthesis of NEPP11 (**2**) and its J-type PG analogue **3** has been developed based on the diastereoisomeric (+)-camphor protected 3-[(dimethoxyphosphoryl)methyl]-4,5-dihydroxycyclopent-2-enones **1**. The key steps encompass a fully diastereoselective hydrogenation of the endocyclic carbon-carbon double bond in the cyclopentenone ring proceeding under control of a chiral diol moiety and the conversion of the latter into a new cyclopentenone with a transposed olefinic bond. The cytotoxicity of NEPP11 (**2**) and its J-type PG analogue **3**, as well as their C7- and C13-hydroxy precursors, respectively, were investigated against the HeLa, K562 and HL-60 human cancer cell lines and the normal human cells (HUVEC). NEPP11 (**2**) and its C7-hydroxy derivative *anti*-**4** displayed a high anticancer activity against the HeLa and HL-60 human cancer cell lines at concentrations ranging from 1 to 2 μM. It was also found that NEPP11 (**2**) induced apoptosis in HeLa cells being relatively nontoxic for the normal human cells. Interestingly, C7-hydroxy derivative of NEPP11 *anti*-**4** showed high cytotoxic selectivity between the cancer cell lines and the normal human cells, which makes this compound especially attractive for further investigations. On the other hand, J-type prostaglandin analogue of NEPP11 (**2**), i.e. **3** and its C13-hydroxy derivatives *syn*-**5** and *anti*-**5** were much less toxic or nontoxic against the cancer and normal cells at the concentrations up to 1 mM. At sublethal doses NEPP11 (**2**), **3** and *anti*-**4** induced changes in ploidy of HeLa cells which manifested in notable increase of aneuploid cells with respect to diploid ones. Additionally, in contrast to NEPP11 (**2**) and *anti*-**4**, compound **3** suppressed the growth of HeLa cells at the G1 and G2/M phases of the cell cycle.

Experimental

General remarks

Unless stated otherwise, all air and water sensitive reactions were carried out under an argon atmosphere using freshly distilled dry solvents. All glassware was dried prior to use by heating under vacuum. Commercial grade reagents and solvents were used without further purification except as indicated below. THF and diethyl ether were distilled over Na/benzophenone prior to use. Thin layer chromatography (TLC) was conducted on Silica Gel 60 F_{254} TLC purchased from Merck. Column chromatography was performed using Merck silica gel (70-230 mesh). NMR spectra were recorded on Bruker AV 200, Bruker DRX 500 or Bruker Avance III 600 spectrometers. ^{1}H, ^{13}C and ^{31}P chemical shifts are reported relative to the residual proton resonance in the deuterated solvents or referred to an 85% aqueous solution of H_3PO_4, respectively. All chemical shifts (δ) are given in ppm and the coupling constants (J) in Hz. HRMS were recorded on Finnigan MAT 95 apparatus. Optical rotations were measured using a Perkin-Elmer MC 241 photopolarimeter. Melting and boiling points are uncorrected.

General procedure for the preparation of hydroxyacetals 9. A mixture of activated magnesium turnings (4.88 g, 201 mmol) and bromoacetal **8** (67.5 mmol) in THF (80 mL) was heated in an oil bath at 60 ^{o}C for 3 h. After cooling to 0 ^{o}C, 4-methylbenzaldehyde (7.68 g, 64 mmol) in THF (5 mL) was added. The mixture was stirred for 0.5 h, poured into saturated aqueous ammonium chloride, extracted with chloroform (4 x 30 mL), and dried over anhydrous sodium sulfate. After evaporation of the solvents the crude material was purified by column chromatography using petroleum ether/acetone 10:1 as an eluent to yield the corresponding hydroxyacetals.

4-(5,5-Dimethyl-1,3-dioxan-2-yl)-1-(4-methylphenyl)butan-1-ol (9a). Yield 87%. Colorless oil; R_f = 0.35 (petroleum ether/acetone 5:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.23 (d, J = 8.3 Hz, 2 H, $C_{Ar}H$), 7.14 (d, J = 8.1 Hz, 2 H, $C_{Ar}H$), 4.69-4.57 (m, 1 H, C*H*OH), 4.40 (t, J = 4.8 Hz, 1 H, O-C*H*-O), 3.58 (d, J = 11. 0 Hz, 2 H, OCH_AH$_B$), 3.39 (d, J = 10.9 Hz, 2 H, OCH$_A$$H_B$), 2.33 (s, 3 H, C$H_3C_{Ar}$), 1.92-1.32 (m, 7 H), 1.17 (s, 3 H, CH_3C), 0.71 (s, 3 H, CH_3C); ^{13}C NMR (50 MHz, $CDCl_3$): δ 141.81 (C_{Ar}), 137.00 (C_{Ar}), 129.02 (2 C, C_{Ar}H), 125.78 (2 C, C_{Ar}H), 102.01 (O-*C*H-O), 77.12 (2 C, O*C*H$_2$), 74.15 (*C*OH), 38.79 (*C*H$_2$), 34.48 (*C*H$_2$), 30.06 (*C*(CH$_3$)$_2$), 22.92 (*C*H$_3$), 21.77 (*C*H$_3$), 21.04 (C_{Ar}*C*H$_3$), 20.35 (*C*H$_2$); HRMS (EI) calcd for $C_{17}H_{26}O_3$ 278.1882, found 278.1880; Anal. calcd for $C_{17}H_{26}O_3$: C, 73.34; H, 9.41. Found: C, 73.38; H, 9.50.

5-(5,5-Dimethyl-1,3-dioxan-2-yl)-1-(4-methylphenyl)pentan-1-ol (9b). Yield 83%. Colorless oil; R_f = 0.37 (petroleum ether/acetone 5:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.22 (d, J = 8.0 Hz, 2 H, $C_{Ar}H$), 7.14 (d, J = 8.1 Hz, 2 H, $C_{Ar}H$), 4.62 (t, J = 6.5 Hz, 1 H, C*H*OH), 4.38 (t, J = 4.9 Hz, 1 H, O-C*H*-O), 3.58 (d, J = 11.0 Hz, 2 H, OCH_AH$_B$), 3.39 (d, J = 10.8 Hz, 2 H, OCH$_A$$H_B$), 2.34 (s, 3 H, C$H_3C_{Ar}$), 1.88-1.19 (m, 9H), 1.17 (s, 3 H, CH_3C), 0.71 (s, 3 H, CH_3C); ^{13}C NMR (50 MHz, $CDCl_3$): δ 141.85 (C_{Ar}), 137.06 (C_{Ar}), 129.05 (2 C, C_{Ar}H), 125.82 (2 C, C_{Ar}H), 102.06 (O-*C*H-O), 77.16 (2 C, O*C*H$_2$), 74.27 (*C*HOH), 38.82 (*C*H$_2$), 34.67 (*C*H$_2$), 30.09 (*C*(CH$_3$)$_2$), 25.68 (*C*H$_2$), 23.76 (*C*H$_2$), 22.93 (*C*H$_3$), 21.80 (*C*H$_3$), 21.06 (C_{Ar}*C*H$_3$); HRMS (EI) calcd for $C_{18}H_{28}O_3$ 292.2038, found 292.2041; Anal. calcd for $C_{18}H_{28}O_3$: C, 73.93; H, 9.65. Found: C, 73.97; H, 9.71.

General procedure for the preparation of acetals 10. A mixture of a hydroxyacetal **9** (4.07 mmol) and 10%-Pd/C (0.35 g) in ethanol (40 mL) was vigorously stirred at room temperature under a hydrogen atmosphere for 24 h. Filtration through a pad of Celite and concentration by rotary evaporation furnished, after column chromatography with 100:3 petroleum ether/acetone corresponding 5,5-dimethyl-2-substituted-1,3-dioxane.

5,5-Dimethyl-2-((4-methylphenyl)butyl)-1,3-dioxane (10a). Yield 94%. Colorless liquid; R_f = 0.63 (petroleum ether/acetone 40:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.09 (d, J = 8.7 Hz, 2 H, $C_{Ar}H$), 7.05 (d, J = 8.6 Hz, 2 H, $C_{Ar}H$), 4.40 (t, J = 4.9 Hz, 1 H, O-C*H*-O), 3.60 (d, J = 11.0 Hz, 2 H, OCH_AH$_B$), 3.41 (d, J = 10.8 Hz, 2 H, OCH$_A$$H_B$), 2.57 (t, J = 7.5 Hz, 2 H, C_{Ar}CH_2), 2.31 (s, 3 H, C_{Ar}CH_3), 1.84-1.33 (m, 6 H), 1.19 (s, 3 H, CH_3C), 0.72 (s, 3 H, CH_3C); ^{13}C NMR (50 MHz, $CDCl_3$): δ 139.51 (C_{Ar}), 134.95 (C_{Ar}), 128.89 (2 C, C_{Ar}H), 128.22 (2 C, C_{Ar}H), 102.13 (O-*C*H-O), 77.20 (2 C, O*C*H$_2$C), 35.41 (*C*H$_2$),

34.74 (CH_2), 31.55 (CH_2), 30.11 ($C(CH_3)_2$), 23.73 (CH_2), 22.95 (CH_3), 21.82 (CH_3), 20.94 ($C_{Ar}CH_3$); HRMS (EI) calcd for $C_{17}H_{26}O_2$ 262.1933, found 262.1931; Anal. calcd for $C_{17}H_{28}O_3$: C, 77.82; H, 9.99. Found: C, 77.53; H, 10.12.

5,5-Dimethyl-2-(5-(4-methylphenyl)pentyl-1,3-dioxane (10b). Yield 98%. Colorless liquid; R_f = 0.64 (petroleum ether/acetone 40:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (s, 4 H, $C_{Ar}H$), 4.40 (t, J = 4.9 Hz, 1 H, O-CH-O), 3.60 (d, J = 11.0 Hz, 2 H, OCH_AH_B), 3.41 (d, J = 10.9 Hz, 2 H, OCH_AH_B), 2.56 (t, J = 7.7 Hz, 2 H, $C_{Ar}CH_2$), 2.31 (s, 3 H, $C_{Ar}CH_3$), 1.73-1.23 (m, 8 H), 1.19 (s, 3 H, CH_3C), 0.72 (s, 3 H, CH_3C). ^{13}C NMR (50 MHz, $CDCl_3$): δ 139.66 (C_{Ar}), 134.92 (C_{Ar}), 128.88 (2 C, C_{Ar}H), 128.24 (2 C, C_{Ar}H), 102.21 (O-CH-O), 77.22 (2 C, OCH_2C), 35.34 (CH_2), 34.80 (CH_2), 31.43 (CH_2), 30.13 ($C(CH_3)_2$), 29.13 (CH_2), 23.79 (CH_2), 22.97 (CH_3), 21.83 (CH_3), 20.95 ($C_{Ar}CH_3$). HRMS (EI) calcd for $C_{18}H_{27}O_2$ [M-H]$^+$ 275.2011, found 275.2012; Anal. calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 78.00; H, 10.41.

General procedure for the preparation of dimethyl acetals 11. A solution of 5,5-dimethyl-2-(substituted)-1,3-dioxane **10** (2.33 g, 8.88 mmol) and *p*-toluenesulfonic acid hydrate (25 mg, 0.133 mmol) in methanol (50 mL) was heated under reflux for 6 h. After cooling to room temperature, potassium carbonate was added (30 mg, 0.217 mmol) and methanol was removed by rotary evaporation. To a residue, water (5 mL) was added and a mixture was extracted with petroleum ether (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give a residue. This residue was additionally treated three times in the same way to achieve a full transacetalization. The final dimethyl acetals obtained were used without purification in the next reaction.

1-(5,5-Dimethoxypentyl)-4-methylbenzene (11a). Yield 97%. Colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.10 (d, J = 8.5 Hz, 2 H, $C_{Ar}H$), 7.05 (d, J = 8.7 Hz, 2 H, $C_{Ar}H$), 4.36 (t, J = 5.6 Hz, 1 H, O-CH-O), 3.31 (s, 6 H, OCH_3), 2.58 (t, J = 7.6 Hz, 2 H, $C_{Ar}CH_2$), 2.31 (s, 3 H, $C_{Ar}CH_3$), 1.75-1.52 (m, 4 H), 1.47-1.28 (m, 2 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 139.19 (C_{Ar}), 134.75 (C_{Ar}), 128.75 (2 C, C_{Ar}H), 128.06 (2 C, C_{Ar}H), 104.23 (O-CH-O), 52.29 (2 C, OCH_3), 35.24 ($C_{Ar}CH_2$), 32.17 (CH_2), 31.29 (CH_2), 24.12 (CH_2), 20.76 ($C_{Ar}CH_3$); Anal. calcd for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 75.53; H, 9.88.

1-(6,6-Dimethoxyhexyl)-4-methylbenzene (11b). Yield 96%. Colorless liquid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.09 (d, J = 8.2 Hz, 2 H, $C_{Ar}H$), 7.06 (d, J = 8.2 Hz, 2 H, $C_{Ar}H$), 4.35 (t, J = 5.7 Hz, 1 H, O-CH-O), 3.31 (s, 6 H, OCH_3), 2.57 (t, J = 7.7 Hz, 2 H, $C_{Ar}CH_2$), 2.32 (s, 3 H, $C_{Ar}CH_3$), 1.65-1.52 (m, 2 H), 1.43-1.28 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.60 (C_{Ar}), 134.99 (C_{Ar}), 128.90 (2 C, C_{Ar}H), 128.24 (2 C, C_{Ar}H), 104.44 (O-CH-O), 52.56 (2 C, OCH_3), 35.35 (CH_2), 32.36 (CH_2), 31.50 (CH_2), 29.08 (CH_2), 24.43 (CH_2), 20.98 ($C_{Ar}CH_3$); HRMS (EI) calcd for $C_{15}H_{23}O_2$ [M - H]$^+$ 235.1698, found 235.1696.

General procedure for the preparation of aldehydes 6. To a solution of appropriate dimethyl acetal (8.54 mmol) in dichloromethane (120 mL) and acetone (12 mL) at room temperature was added iron(III) chloride hexahydrate (8.08 g, 29.9 mmol). The resulting mixture was stirred for 3 h and poured into 80 mL of a saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted three times with chloroform, and the combined organic extracts were washed with brine and dried over anhydrous magnesium sulfate. Concentration under reduced pressure yielded corresponding aldehyde which was used to the next reaction without further purification.

5-(4-Methylphenyl)pentanal (6a). Yield 100%. Colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.76 (d, J = 1.6 Hz, 1 H, CHO), 7.10 (d, J = 9.0 Hz, 2 H, $C_{Ar}H$), 7.05 (d, J = 8.9 Hz, 2 H, $C_{Ar}H$), 2.60 (t, J = 6.8 Hz, 2 H, $C_{Ar}CH_2$), 2.44 (dt, J = 6.8 Hz, J = 1.7 Hz, 2 H, CH_2CHO), 2.32 (s, 3 H, $C_{Ar}CH_3$), 1.74-1.58 (m, 4 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 202.24 (CHO), 138.67 (C_{Ar}), 135.00 (C_{Ar}), 128.84 (2 C, C_{Ar}H), 128.07 (2 C, C_{Ar}H), 43.52 (CH_2CHO), 34.98 ($C_{Ar}CH_2$), 30.79 (CH_2), 21.46 (CH_2), 20.79 ($C_{Ar}CH_3$); HRMS (EI) calcd for $C_{12}H_{16}O$ 176.1201, found 176.1199.

6-(4-Methylphenyl)hexanal (6b). Yield 100%. Colorless liquid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.77 (t, J = 1.4 Hz, 1 H, CHO), 7.12 (d, J = 8.0 Hz, 2 H, $C_{Ar}H$), 7.09 (d, J = 8.0 Hz, 2 H, $C_{Ar}H$), 2.60 (t, J = 7.7 Hz, 2 H, $C_{Ar}CH_2$), 2.44 (td, J = 7.4 Hz, J =1.4 Hz, 2 H, CH_2CHO), 2.35 (s, 3 H, $C_{Ar}CH_3$), 1.72-1.61 (m, 4 H), 1.43-1.34 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 202.70 (CHO), 139.19 (C_{Ar}), 135.01 (C_{Ar}), 128.88 (2 C, C_{Ar}H), 128.15 (2 C, C_{Ar}H), 43.72 (CH_2CHO), 35.13 ($C_{Ar}CH_2$), 31.22 (CH_2), 28.63 (CH_2), 21.82 (CH_2), 20.89 ($C_{Ar}CH_3$); HRMS (EI) calcd for $C_{13}H_{18}O$ 190.1358, found 190.1359.

Preparation of dienone 12. Phosphonate **1a** (2.88 g, 7.76 mmol) and lithium perchlorate (0.83 g, 7.99 mmol) were dissolved in THF (11 mL) and cooled to 0 °C. 1,8-Diazabicyclo-[5.4.0]undec-7-ene (DBU, 1.22 g, 7.99 mmol) was added and the mixture was stirred for 15 min. 5-(4-Methylphenyl)pentanal (1.52 g, 8.63 mmol) in THF (2 mL) was added and the resulting solution was stirred at 0-5 °C for 3 h. After evaporation of the solvent under reduced pressure, the residue was subjected to column chromatography (petroleum ether/acetone 5:1) affording **12** (2.80 g, 86%) as a yellowish oil. R_f = 0.48 (petroleum ether/acetone 5:1); $[\alpha]_D^{19}$ -102.1 (*c* 1.5 in CH_2Cl_2); $[\alpha]_D^{19}$ -93.0 (*c* 1.6 in acetone); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.09 (d, J = 8.0 Hz, 2 H, $C_{Ar}H$), 7.06 (d, J = 8.0 Hz, 2 H, $C_{Ar}H$), 6.61 (dt, J = 15.7 Hz, J = 7.2 Hz, 1 H, CH=CHCH$_2$), 6.42 (d, J = 15.7 Hz, 1 H, CH=CHCH$_2$), 5.79 (s, 1 H, C(O)CH=C), 5.27 (d, J = 5.8 Hz, 1 H, C(O)CH-O), 4.34 (d, J = 5.8 Hz, 1 H, C(O)-CH-CH-O), 2.59 (t, J = 7.6 Hz, 2 H, $C_{Ar}CH_2$), 2.32 (s, 3 H, $C_{Ar}CH_3$), 2.32-2.24 (m, 2 H, CH=CHCH_2), 2.08 (ddd, J = 13.1 Hz, J = 4.6 Hz, J = 3.0 Hz, 1 H), 1.93 (ddd, J = 13.2 Hz, J = 9.4 Hz, J = 4.0 Hz, 1 H), 1.75 (t, J = 4.5 Hz, 1 H), 1.72-1.59 (m, 3 H), 1.57-1.47 (m, 3 H), 1.33 (td, J = 12.2 Hz, J = 4.7 Hz, 1 H), 1.20 (ddd, J = 14.3 Hz, J = 7.2 Hz, J = 3.4 Hz, 1 H), 0.95 (s, 3 H, CH_3 (camphor)), 0.82 (s, 3 H, CH_3 (camphor)), 0.61 (s, 3 H, CH_3 (camphor)); ^{13}C NMR (126 MHz, $CDCl_3$): δ 201.19 (C=O), 168.72 (C=CHC(O)), 145.75 (CH=CHCH$_2$), 139.12 ($C_{Ar}CH_2$), 135.17 ($C_{Ar}CH_3$), 128.96 (2 C, C_{Ar}H), 128.23 (2 C, C_{Ar}H), 126.67 (C=CHC(O)), 124,76 (CH=CHCH$_2$), 124.01 (O-C-O), 77.98 (C(O)-CH-O), 76.17 (C(O)-CH-CH-O), 51.55 (4^o C), 47.83 (4^o C), 45.14 (CH), 44.55 (CH_2), 35.26 ($C_{Ar}CH_2$), 33.45 (CH_2), 31.02 (CH_2), 29.31 (CH_2), 28.12 (CH_2), 26.77 (CH_2),

20.97 ($C_{Ar}CH_3$), 20.22 (CH_3 (camphor)), 20.20 (CH_3 (camphor)), 9.37 (CH_3 (camphor)); HRMS (EI) calcd for $C_{28}H_{36}O_3$ 420.2664, found 420.2664; Anal. calcd for $C_{28}H_{36}O_3$: C, 79.96; H, 8.63. Found: C, 79.90; H, 8.47.

Camphor protected 2,3-dihydroxy-4-(6-(4-methylphenyl)hexyl)cyclopentanone (13). A mixture of dienone **12** (3.01 g, 7.16 mmol) and 10%-Pd/C (0.95 g) in ethanol (50 mL) was vigorously stirred at room temperature under a hydrogen atmosphere for 3.5 h. Filtration through a pad of Celite and concentration by rotary evaporation furnished, after column chromatography (petroleum ether/acetone gradient) **13** (2.38 g, 86%) as a white solid. R_f = 0.49 (petroleum ether/acetone 5:1); mp 47 °C; $[\alpha]_D^{19}$ +90.6 (*c* 1.8 in CH_2Cl_2); $[\alpha]_{546}^{19}$ +114.6 (*c* 1.8 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.09 (d, *J* = 8.1 Hz, 2 H, $C_{Ar}H$), 7.07 (d, *J* = 8.1 Hz, 2 H, $C_{Ar}H$), 4.63 (t, *J* = 4.3 Hz, 1 H, O-C*H*-CH-C(O)), 4.03 (d, *J* = 5.1 Hz, 1 H, O-C*H*-C(O)), 2.57 (t, *J* = 7.8 Hz, 2 H, $C_{Ar}CH_2$), 2.32 (s, 3 H, $C_{Ar}CH_3$), 2.30-2.10 (m, 3 H), 2.00-1.90 (m, 2 H), 1.74 (t, *J* = 4.5 Hz, 1 H), 1.71-1.50 (m, 5 H), 1.48-1.29 (m, 8 H), 1.2 (ddd, *J* = 12.6 Hz, *J* = 9.5 Hz, *J* = 4.6 Hz, 1 H), 0.98 (s, 3 H, CH_3 (camphor)), 0.84 (s, 3 H, CH_3 (camphor)), 0.71 (s, 3 H, CH_3 (camphor)); ^{13}C NMR (126 MHz, $CDCl_3$): δ 214.36 (*C*=O), 139.65 ($C_{Ar}CH_2$), 134.99 ($C_{Ar}CH_3$), 128.90 (2 C, $C_{Ar}H$), 128.24 (2 C, $C_{Ar}H$), 119.85 (O-*C*-O), 78.65 (O-*C*H-CH-C(O)), 78.03, (O-*C*HC(O)), 51.64 (4^oC), 47.75 (4^oC) , 45.15 (*C*H), 43.62 (CH_2), 40.06 (CH_2), 35.42 (2 C, CH_2 and *C*H), 31.53 (CH_2), 30.10 (CH_2), 29.53 (CH_2), 29.46 (CH_2), 29.11 (CH_2), 27.37 (CH_2), 26.93 (CH_2), 20.98 ($C_{Ar}CH_3$), 20.24 (2 C, CH_3 (camphor)), 9.18 (CH_3 (camphor)); HRMS (EI) calcd for $C_{28}H_{40}O_3$ 424.2977, found 424.2962; Anal. calcd for $C_{28}H_{40}O_3$: C, 79.20; H, 9.50. Found: C, 79.08; H, 9.61.

(3*R*,4*S*)-3-Hydroxy-4-(6-(4-methylphenyl)hexyl)cyclopentanone (14). The freshly prepared aluminum amalgam from granular aluminum (1.0 g, 37.1. mmol) and the saturated solution of mercuric chloride (15 mL) was added to a solution of **13** (567 mg, 2.07 mmol) in 8:1 THF/H_2O (6 ml). Additional portion of the aluminum amalgam were added after 10 and 17 h. After being stirred for an additional 7 h, the reaction mixture was filtered through a pad of Celite, concentrated under vacuum, and the crude product was purified by column chromatography (petroleum ether/acetone gradient) to yield **14** (317 mg, 87%) as a colorless solid. R_f = 0.27 (petroleum ether/acetone 5:1); mp 52-53 °C; $[\alpha]_D^{19}$ +98.8 (*c* 1.7 in CH_2Cl_2); $[\alpha]_{546}^{19}$ +98.8 (*c* 1.7 in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.10 (d, *J* = 9.0 Hz, 2 H, $C_{Ar}H$), 7.06 (d, *J* = 8.7 Hz, 2 H, $C_{Ar}H$), 4.47 (br s, *J* = 7.6 Hz, 1 H, HOC*H*), 2.57 (t, *J* = 7.5 Hz, 2 H, $C_{Ar}CH_2$), 2.32 (s, 3 H, $C_{Ar}CH_3$), 2.43-2.00 (m, 5 H), 1.72-1.20 (m, 11 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 217.66 (*C*=O), 139.59 (C_{Ar}), 134.96 (C_{Ar}), 128.88 (2 C, $C_{Ar}H$), 128.20 (2 C, $C_{Ar}H$), 70.86 (*C*HOH), 48.72 (C(O)*C*H_2-CH-OH), 42.26 (HO-CH-*C*H-CH_2C(O)), 40.88 (HO-CH-CH-*C*H_2C(O)), 35.41 ($C_{Ar}CH_2$), 31.48 (CH_2), 29.60 (CH_2), 29.38 (CH_2), 29.11 (CH_2), 27.60 (CH_2), 20.93 ($C_{Ar}CH_3$); HRMS (EI) calcd for $C_{18}H_{26}O_2$ 274.1933, found 274.1937; Anal. calcd for $C_{18}H_{26}O_2$: C, 78.79; H, 9.55. Found: C, 78.87; H, 9.70.

(*S*)-4-(6-(4-Methylphenyl))hexyl)cyclopent-2-enone (15). A solution of **14** (284 mg, 1.03 mmol) and *p*-toluenesulfonic acid hydrate (29 mg, 0.16 mmol) in diethyl ether (10 mL) was stirred at room temperature for 24 h. The reaction mixture was poured into diethyl ether and saturated aqueous $NaHCO_3$. The layers were separated, and the aqueous phase was extracted several times with $CHCl_3$. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography using 50:1 petroleum ether/acetone as the eluent yielded **15** (241 mg, 91%) as a colorless liquid. R_f = 0.37 (petroleum ether/acetone 20:3); $[\alpha]_D^{21}$ -100.2 (*c* 1.7 in CH_2Cl_2); $[\alpha]_{546}^{21}$ -121.0 (*c* 1.7 in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.62 (dd, *J* = 5.6 Hz, *J* = 2.5 Hz, 1 H, C*H*=CHC(O)), 7.10 (d, *J* = 9.0 Hz, 2 H, $C_{Ar}H$), 7.05 (d, *J* = 8.7 Hz, 2 H, $C_{Ar}H$), 6.14 (dd, *J* = 5.6 Hz, *J* = 2.0 Hz, 1 H, CH=C*H*C(O)), 3.00-2.81 (m, 1 H, CH=CHC*H*), 2.57 (t, *J* = 7.5 Hz, 2 H, $C_{Ar}CH_2$), 2.47 (dd, *J* = 18.8 Hz, *J* = 6.3 Hz, 1 H, $CH_AH_BC(O)$), 2.32 (s, 3 H, CH_3), 1.98 (dd, *J* = 18.8 Hz, *J* = 2.1 Hz, 1 H, $CH_AH_BC(O)$), 1.70-1.20 (m, 10 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 209.88 (*C*=O), 168.52 (*C*H=CHC(O)), 139.46 ($C_{Ar}CH_2$), 134.88 ($C_{Ar}CH_3$), 133.43 (CH=*C*HC(O)), 128.83 (2 C, $C_{Ar}H$), 128.14 (2 C, $C_{Ar}H$), 41.34 (CH=CH*C*H), 40.91(CH_2C(O)), 35.33 ($C_{Ar}CH_2$), 34.60 (CH_2), 31.39 (CH_2), 29.35 (CH_2), 28.98 (CH_2), 27.41 (CH_2), 20.88 ($C_{Ar}CH_3$); HRMS (EI) calcd for $C_{18}H_{24}O$ 256.1827, found 256.1828; Anal. calcd for $C_{18}H_{24}O$: C, 84.32; H, 9.44. Found: C, 84.09; H, 9.72.

Preparation of aldols 4. To a stirred solution of *i*-Pr_2NH (122 mg, 1.21 mmol) in THF (10 mL), *n*-BuLi (0.42 mL, 2.43 M in hexane, 1.02 mmol) was added at -30 °C under an argon atmosphere. Stirring was continued for 15 min and the mixture was allowed to warm to 0 °C. The resulting solution of LDA was cooled to -78 °C and **15** (238 mg, 0.93 mmol) in THF (2 mL) was added. The mixture was stirred for 10 min and methyl 6-formylhexanoate (176 mg, 1.11 mmol) was added. After 30 min at -78 °C, the solution was poured into an ice-cold saturated aqueous NH_4Cl. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3x30 mL). The combined organic extracts were dried over Na_2SO_4, concentrated under vacuum, and a residue was subjected to flash column chromatography (petroleum ether/acetone gradient) to give *anti*-**4** (287 mg, 75%) and *syn*-**4** (53 mg, 13%) as colorless liquids. *anti*-**4** (less polar): R_f = 0.25 (petroleum ether/acetone 5:1); $[\alpha]_D^{22}$ -88.5 (*c* 2.3 in CH_2Cl_2); $[\alpha]_{546}^{22}$ -106.8 (*c* 2.3 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (dd, *J* = 5.7 Hz, *J* = 2.4 Hz, 1 H, C*H*=CHC(O)), 7.09 (d, *J* = 8.1 Hz, 2 H, $C_{Ar}H$), 7.06 (d, *J* = 8.1 Hz, 2 H, $C_{Ar}H$), 6.13 (dd, *J* = 5.7 Hz, *J* = 1.7 Hz, 1 H, CH=C*H*C(O)), 3.87 (s, 1 H, O*H*), 3.72-3.63 (m, 1 H, C*H*OH), 3.66 (s, 3 H, OCH_3), 2.65-2.59 (m, 1 H, CH=CHC*H*), 2.56 (t, *J* = 7.7 Hz, 2 H, $C_{Ar}CH_2$), 2.31 (s, 3 H, $C_{Ar}CH_3$), 2.31 (t, *J* = 7.5 Hz, 2 H, $CH_2CO_2CH_3$), 2.00 (dd, *J* = 8.4 Hz, *J* = 2.1 Hz, 1 H, CH-C*H*C(O)), 1.72-1.27 (m, 18 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 212.95 (*C*=O), 174.19 (CO_2CH_3), 168.60 (*C*H=CHC(O)), 139.49 ($C_{Ar}CH_2$), 135.01 ($C_{Ar}CH_3$), 132.81 (CH=*C*HC(O)), 128.89 (2 C, $C_{Ar}H$), 128.19 (2 C, $C_{Ar}H$), 72.06 (*C*HOH), 55.86 (CH-*C*HC(O)), 51.44 (OCH_3), 44.87 (CH=CH*C*H), 35.37 (CH_2), 35.29 ($C_{Ar}CH_2$), 33.94 (CH_2), 33.80 (CH_2), 31.46 (CH_2), 29.55 (CH_2), 29.04 (CH_2), 29.00 (CH_2), 26.80 (CH_2), 25.05 (CH_2), 24.84 (CH_2), 20.95 ($C_{Ar}CH_3$); HRMS (EI) calcd for $C_{26}H_{38}O_4$ 414.2770, found 414.2776; Anal. calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.27; H, 9.31. *syn*-**4** (more polar): R_f = 0.18 (petroleum ether/acetone 5:1); $[\alpha]_D^{22}$ -81.0 (*c* 0.9 in CH_2Cl_2); $[\alpha]_{546}^{22}$ -95.0 (*c* 0.9 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.69 (dd, *J* = 5.7 Hz, *J* = 2.4 Hz, 1 H, C*H*=CHC(O)), 7.09 (d, *J* = 8.1 Hz, 2 H, $C_{Ar}H$), 7.06 (d, *J* = 8.1 Hz, 2 H, $C_{Ar}H$), 6.13 (dd, *J* = 5.7 Hz, *J* = 1.8 Hz, 1 H, CH=C*H*C(O)), 4.03 (br s, 1 H, C*H*OH), 3.66 (s, 3 H, OCH_3), 2.88-2.81 (m, 1 H, CH=CHC*H*), 2.56 (t, *J* = 7.6 Hz, 2 H, $C_{Ar}CH_2$), 2.32 (s, 3 H, $C_{Ar}CH_3$), 2.31 (t, *J* = 7.4 Hz, 2 H, $CH_2CO_2CH_3$),

2.30-2.25 (m, 1 H, O*H*), 2.13 (t, *J* = 2.8 Hz, 1 H, CH-C*H*C(O)), 1.70-1.26 (m, 18 H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 211.99 (*C*=O), 174.15 (*C*O_2CH_3), 169.07 (*C*H=CHC(O)) , 139.51 (*C*$_{Ar}CH_2$), 134.99 (*C*$_{Ar}CH_3$), 133.37 (CH=*C*HC(O)), 128.89 (2 C, *C*$_{Ar}$H), 128.19 (2 C, *C*$_{Ar}$H), 71.18 (*C*HOH), 56.80 (CH-*C*HC(O)), 51.46 (O*C*H_3), 42.95 (CH=CH*C*H), 35.38 (C$_{Ar}$*C*H_2), 34.26 (*C*H_2), 34.16 (*C*H_2), 33.90 (*C*$H_2CO_2CH_3$), 31.47 (*C*H_2), 29.57 (*C*H_2), 29.06 (*C*H_2), 28.88 (*C*H_2), 27.13 (*C*H_2), 25.85 (*C*H_2), 24.78 (*C*H_2), 20.95 (C$_{Ar}$*C*H_3). HRMS (EI) calcd for $C_{26}H_{38}O_4$ 414.2770, found 414.2782; Anal. calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.20; H, 9.37.

General procedure for the preparation of mesylates 16. To an ice-cold solution of an *syn*- or *anti*-aldol **4** (0.27 mmol) and Et_3N (273 mg, 2.70 mmol) in CH_2Cl_2 (5 mL) was added methanesulfonyl chloride (217 mg, 1.89 mmol). Stirring was continued for 2 h at the same temperature and the solution was poured into saturated aqueous $NaHCO_3$. The mixture was extracted with dichloromethane and the combined organic layers were dried over anhydrous sodium sulfate. After concentration in vacuo, the residue was subjected to flash chromatography (petroleum ether/acetone 5:1) to give a corresponding mesylate.

***anti*-16.** Yield 97%. Colorless liquid; R_f = 0.20 (petroleum ether/acetone 5:1); $[\alpha]_D^{24}$ -52.2 (*c* 1.4 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (dd, *J* = 5.7 Hz, *J* = 2.4 Hz, 1 H, C*H*=CHC(O)), 7.07 (d, *J* = 8.2 Hz, 2 H, C$_{Ar}$*H*), 7.05 (d, *J* = 8.2 Hz, 2 H, C$_{Ar}$*H*), 6.10 (dd, *J* = 5.7 Hz, *J* = 1.8 Hz, 1 H, CH=C*H*C(O)), 4.98 (dt, *J* = 8.6 Hz, *J* = 4.1 Hz, 1 H, C*H*OMs), 3.65 (s, 3 H, OCH_3), 2.99 (s, 3 H, CH_3S), 2.97-2.92 (m, 1 H, CH-C*H*C(O)), 2.58-2.51 (m, 3 H, C$_{Ar}$CH_2 and C*H*-CHC(O)), 2.30 (s, 3 H, C$_{Ar}$CH_3), 2.29 (t, *J* = 7.5 Hz, 2 H, C$H_2CO_2CH_3$), 1.81-1.70 (m, 1 H), 1.66-1.17 (m, 17 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.88 (*C*=O), 173.93 (*C*$OOCH_3$), 168.18 (*C*H=CHC(O)), 139.48 (*C*$_{Ar}CH_2$), 134.89 (*C*$_{Ar}CH_3$), 133.11 (CH=*C*HC(O)), 128.82 (2 C, *C*$_{Ar}$H), 128.16 (2 C, *C*$_{Ar}$H), 82.18 (*C*HOMs), 54.45 (CH-*C*HC(O)), 51.41 (O*C*H_3), 43.86 (CH=CH*C*H), 38.32 (*C*H_3S), 35.30 (C$_{Ar}$*C*H_2), 34.14 (*C*H_2), 33.72 (*C*H_2), 31.39 (*C*H_2), 30.70 (*C*H_2), 29.41 (*C*H_2), 28.95 (*C*H_2), 28.50 (*C*H_2), 27.04 (*C*H_2), 25.42 (*C*H_2), 24.49 (*C*H_2), 20.88 (C$_{Ar}$*C*H_3); HRMS (EI) calcd for $C_{27}H_{40}O_6$ 492.2546, found 492.2533.

***syn*-16.** Yield 92%. Colorless liquid; R_f = 0.13 (petroleum ether/acetone 5:1); $[\alpha]_D^{22}$ -37.9 (*c* 1.0 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.73 (dd, *J* = 5.7 Hz, *J* = 2.4 Hz, 1 H, C*H*=CHC(O)), 7.08 (d, *J* = 8.1 Hz, 2 H, C$_{Ar}$*H*), 7.06 (d, *J* = 8.1 Hz, 2 H, C$_{Ar}$*H*), 6.17 (dd, *J* = 5.7 Hz, *J* = 1.8 Hz, 1 H, CH=C*H*C(O)), 5.03 (dt, *J* = 7.3 Hz, *J* = 2.6 Hz, 1 H, C*H*OMs , 3.66 (s, 3 H, CH_3O), 3.07-3.00 (m, 1 H, CH=CHC*H*), 2.85 (s, 3 H, CH_3S), 2.56 (t, *J* = 7.7 Hz, 2 H, C$_{Ar}$CH_2), 2.31 (t, *J* = 7.5 Hz, 2 H, C$H_2CO_2CH_3$), 2.31 (s, 3 H, C$_{Ar}$CH_3), 2.22 (t, *J* = 2.6 Hz, 1 H, CH-C*H*C(O)), 2.05-1.95 (m, 1 H), 1.80-1.23 (m, 17 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 208.41 (*C*=O), 173.94 (*C*O_2CH_3), 168.87 (*C*H=CHC(O)), 139.44 (*C*$_{Ar}CH_2$), 135.03 (*C*$_{Ar}CH_3$), 132.89 (CH=*C*HC(O)), 128.90 (2 C, *C*$_{Ar}$H), 128.19 (2 C, *C*$_{Ar}$H), 82.21 (*C*HOMs), 53.50 (CH-*C*HC(O)), 51.49 (O*C*H_3), 42.60 (CH=CH*C*H), 38.00 (*C*H_3S), 35.35 (C$_{Ar}$*C*H_2), 34.17 (*C*H_2), 33.78 (*C*H_2), 33.59 (*C*$H_2CO_2CH_3$), 31.43 (*C*H_2), 29.52 (*C*H_2), 29.03 (*C*H_2), 28.58 (*C*H_2), 26.68 (*C*H_2), 25.15 (*C*H_2), 24.58 (*C*H_2), 20.94 (C$_{Ar}$*C*H_3); HRMS (EI) calcd for $C_{27}H_{40}O_6$ 492.2546, found 492.2549.

General procedure for the preparation of 15-deoxy-13,14-dihydro-19,20-dinor-12-iso-18-(4-methylphenyl)-Δ^7-PGA$_1$ methyl ester (NEPP11) (2). A suspension of a *syn*- or *anti*-mesylate **16** (47 mg, 0.095 mmol) and neutral aluminum oxide (80 mg) in dichloromethane (2.5 mL) was stirred at room temperature for 24 h (additional equal portions of Al_2O_3 were added after 2 and 6 h). The reaction mixture was filtered through a pad of Celite, concentrated, and the residue was purified by column chromatography to afford a corresponding alkene.

From *anti*-**16**. Yield 93%. Colorless liquid; R_f = 0.31 (petroleum ether/acetone 5:1); $[\alpha]_D^{22}$ -127.1 (*c* 1.6 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (dd, *J* = 5.8 Hz, *J* = 2.2 Hz, 1 H, C*H*=CHCO), 7.08 (d, *J* = 8.0 Hz, 2 H, C$_{Ar}$*H*), 7.05 (d, *J* = 8.0 Hz, 2 H, C$_{Ar}$*H*), 6.52 (t, *J* = 7.6 Hz, 1 H, C=C*H*CH$_2$), 6.32 (dd, *J* = 6.0 Hz, *J* = 1.6 Hz, 1 H, CH=C*H*C(O)), 3.66 (s, 3 H, OCH_3), 3.49-3.41 (m, 1 H, C*H*), 2.54 (t, *J* = 7.7 Hz, 2 H, C$_{Ar}$CH_2), 2.31 (t, *J* = 7.4 Hz, 2 H, C$H_2CO_2CH_3$), 2.31 (s, 3 H, C$_{Ar}$CH_3), 2.30-2.18 (m, 2 H, C=CHCH_2), 1.83-1.73 (m, 1 H), 1.70-1.18 (m, 15 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.87 (*C*=O), 173.96 (*C*O_2CH_3), 161.86 (*C*H=CHC(O)), 139.51 (*C*$_{Ar}CH_2$), 138.01 (HC=*C*C(O)), 135.16 (C=*C*HCH$_2$), 134.95 (*C*$_{Ar}CH_3$), 134.75 (CH=*C*HC(O)), 128.86 (2 C, *C*$_{Ar}$H), 128.18 (2 C, *C*$_{Ar}$H), 51.42 (O*C*H_3), 43.22 (CH=CH*C*H), 35.37 (C$_{Ar}$*C*H_2), 33.83 (*C*$H_2CO_2CH_3$), 32.37 (*C*H_2), 31.43 (*C*H_2), 29.60 (*C*H_2), 29.05 (*C*H_2), 28.83 (*C*H_2), 28.82 (*C*H_2), 28.27 (*C*H_2), 25.72 (*C*H_2), 24.66 (*C*H_2), 20.91 (C$_{Ar}$*C*H_3); Anal. calcd for $C_{26}H_{36}O_3$: C, 78.75; H, 9.15. Found: C, 78.82; H, 9.30.

From *syn*-**16**. Yield 84%. Colorless liquid; $[\alpha]_D^{21}$ -126.5 (*c* 0.8 in CH_2Cl_2).

(+)-Camphor protected methyl 7-((4*R*,5*R*)-4,5-dihydroxy-3-oxocyclopent-1-en-1-yl)hept-6-enoate (17). According to the procedure described for **12** phosphonate **1b** (1.95 g, 5.26 mmol) was transformed into dienone **17** (1.71 g, 84%). Pale yellow oil; R_f = 0.41 (petroleum ether/acetone 5:1); $[\alpha]_D^{24}$ +99.3 (*c* 1.9 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.59 (dt, *J* = 15.7 Hz, *J* = 7.0 Hz, 1 H, CH=C*H*CH$_2$), 6.41 (d, *J* = 15.8 Hz, 1 H, C*H*=CHCH$_2$), 5.77 (s, 1 H, C=C*H*C(O)), 5.10 (d, *J* = 5.8 Hz, 1 H, C(O)C*H*-O), 4.42 (d, *J* = 5.8 Hz, 1 H, C(O)CH-C*H*-O), 3.62 (s, 3 H, OCH$_3$), 2.30 (t, *J* = 7.4 Hz, 2 H, CH_2COOCH$_3$), 2.25 (q, *J* = 7.3 Hz, 2 H), 2.05 (ddd, *J* = 13.1 Hz, *J* = 4.3 Hz, *J* = 3.3 Hz, 1 H), 1.79-1.58 (m, 5 H), 1.56-1.41 (m, 3 H), 1.27 (td, *J* = 12.2 Hz, *J* = 4.7 Hz, 1 H), 1.17-1.11 (m, 1 H), 0.94 (s, 3 H, CH_3), 0.77 (s, 3 H, CH_3), 0.58 (s, 3 H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 201.42 (*C*=O), 173.74 (*C*O_2CH_3), 168.16 (*C*=CHC(O)), 144.88 (CH=*C*HCH$_2$), 126.96 (C=*C*HC(O)), 124.86 (*C*H=CHCH$_2$), 124.02 (O-*C*-O), 77.91 (C(O)*C*H-O), 75.93 (C(O)CH-*C*H-O), 51.56 (4^o *C*), 51.41 (O*C*H_3), 47.67 (4^o *C*), 45.09 (C*H*), 44.51 (*C*H_2), 33.62 (*C*H_2), 33.06 (*C*H_2), 29.65 (*C*H_2), 27.72 (*C*H_2), 26.79 (*C*H_2), 24.29 (*C*H_2), 20.37 (*C*H_3), 20.13 (*C*H_3), 9.23 (*C*H_3); HRMS (EI) calcd for $C_{23}H_{32}O_5$ 388.2249, found 388.2241; Anal. calcd for $C_{23}H_{32}O_5$: C, 71.11; H, 8.30. Found: C, 70.98; H, 8.47.

(+)-Camphor protected methyl 7-((1*R*,2*R*,3*R*)-2,3-dihydroxy-4-oxocyclopentyl)heptanoate (18). The hydrogenation reaction of dienone **17** (1.70 g, 4.38 mmol) was performed according to the procedure described for **13** and gave saturated cyclopentanone **18** (1.59 g, 92%) as a colorless oil. R_f = 0.47 (petroleum ether/acetone 5:1); $[\alpha]_D^{20}$ -115.4 (*c* 1.6 in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.47 (dd, *J* = 4.6 Hz, *J* = 3.4 Hz, 1 H, O-C*H*-CHC(O)), 4.16 (d, *J* = 5.1 Hz, 1 H, C*H*C(O)), 3.67 (s, 3 H, OCH_3), 2.32 (t, *J* = 7.4 Hz, 2 H, CH_2CO$_2$CH$_3$), 2.24-2.12 (m, 2 H), 2.04 (ddd, *J* = 13.0 Hz, *J* = 4.3 Hz, *J* = 3.3 Hz, 1 H), 1.94-1.78 (m, 1 H), 1.78-1.52 (m, 7 H), 1.39 (d, *J* = 12.4 Hz, 8 H), 1.25-1.06 (m, 1 H), 0.98 (s, 3 H, CH_3), 0.83 (s, 3 H, CH_3), 0.70 (s, 3 H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 214.01 (*C*=O), 173.97 (*C*O$_2$CH$_3$), 120.23 (O-*C*-O), 79.84 (O-*C*H-CHC(O)), 77.37 (*C*HC(O)), 51.54 (4° *C*), 51.24 (O*C*H$_3$), 47.70 (4° *C*), 45.01 (*C*H), 44.08 (*C*H$_2$), 39.78 (*C*H$_2$), 35.31 (*C*H), 33.82 (*C*H$_2$), 29.94 (*C*H$_2$), 29.60 (*C*H$_2$), 29.11 (*C*H$_2$), 28.76 (*C*H$_2$), 27.21 (*C*H$_2$), 26.61 (*C*H$_2$), 24.65 (*C*H$_2$), 20.28 (*C*H$_3$), 19.91 (*C*H$_3$), 9.52 (*C*H$_3$); HRMS (EI) calcd for $C_{23}H_{36}O_5$ 392.2563, found 392.2560; Anal. calcd for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24. Found: C, 70.36; H, 9.20.

Methyl 7-((1*R*,2*S*)-2-hydroxy-4-oxocyclopentyl)heptanoate (19). The reductive deacetalization reaction of **18** (258 mg, 0.658 mmol) with the aluminum amalgam was carried out according to the procedure described for **14** to give **19** (142 mg, 89%) as a colorless solid. R_f = 0.25 (petroleum ether/acetone 5:1); mp 49-50 °C; $[\alpha]_D^{29}$ -110.1 (*c* 1.4 in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.47 (s, 1 H, C*H*OH), 3.67 (s, 3 H, CO$_2$CH_3), 2.37 (t, *J* = 4.0 Hz, 2 H, CH_2CO$_2$CH$_3$), 2.29 (d, *J* = 7.5 Hz, 2 H), 2.23-2.03 (m, 2 H), 1.71-1.50 (m, 6 H), 1.44-1.26 (m, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 217.66 (*C*=O), 174.31 (*C*O$_2$CH$_3$), 70.76 (*C*HOH), 51.45 (O*C*H$_3$), 48.77 (C(O)*C*H$_2$CHOH), 42.31 (C(O)CH$_2$*C*HCH$_2$), 40.92 (C(O)*C*H$_2$CHCH$_2$), 33.95 (*C*H$_2$), 29.29 (2 C *C*H$_2$), 28.89 (*C*H$_2$), 27.39 (*C*H$_2$), 24.71 (*C*H$_2$); Anal. calcd for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 64.38; H, 9.28.

Methyl (*R*)-7-(4-oxocyclopent-2-en-1-yl)heptanoate (20). The dehydration of **19** (132 mg, 0.54 mmol) was carried out in accordance with the procedure applied for **15** to afford **20** (112 mg, 92%) as a colorless liquid. R_f = 0.38 (petroleum ether/acetone 7:1); $[\alpha]_D^{23}$ +111.8 (*c* 1.7 in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63 (dd, *J* = 5.6 Hz, *J* = 2.5 Hz, 1 H, C*H*=CHC(O)), 6.14 (dd, *J* = 5.6 Hz, *J* = 2.0 Hz, 1 H, CH=C*H*C(O)), 3.67 (s, 3 H, OCH_3), 2.98-2.84 (m, 1 H, C*H*CH$_2$), 2.53 (dd, *J* = 18.8 Hz, *J* = 6.3 Hz, 1 H, C(O)CH$_A$H$_B$), 2.31 (t, *J* = 7.4 Hz, 2 H, CH_2CO$_2$Me), 1.99 (dd, *J* = 18.8 Hz, *J* = 2.1 Hz, 1 H, C(O)CH$_A$*H*$_B$), 1.70-1.25 (m, 10 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 210.10 (*C*=O), 174.15 (*C*O$_2$CH$_3$), 168.63 (*C*H=CHC(O)), 133.52 (CH=*C*HC(O)), 51.43 (O*C*H$_3$), 41.37 (*C*HCH$_2$), 40.94 (CH-*C*H$_2$C(O)), 34.56 (*C*H$_2$), 33.91 (*C*H$_2$CO$_2$Me), 29.15 (*C*H$_2$), 28.88 (*C*H$_2$), 27.34 (*C*H$_2$), 24.74 (*C*H$_2$); HRMS (EI) calcd for $C_{13}H_{20}O_3$ 224.1412, found 224.1410.

Preparation of aldols 5. An aldol condensation of **20** (127 mg, 0.57 mmol) with 6-(4-methylphenyl)hexanal (**6b**) (124 mg, 0.65 mmol) was performed according to the procedure described for **4** to afford *anti*-**5** (82 mg, 35%) and *syn*-**5** (14 mg, 6%) as colorless oils. *anti*-**5** (less polar): R_f = 0.23 (petroleum ether/acetone 20:3); $[\alpha]_D^{24}$ +82.6 (*c* 1.1 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (dd, *J* = 5.7 Hz, *J* = 2.4 Hz, 1 H, C*H*=CHC(O)), 7.08 (d, *J* = 8.0 Hz, 2 H, C$_{Ar}$*H*), 7.06 (d, *J* = 8.2 Hz, 2 H, C$_{Ar}$*H*), 6.14 (dd, *J* = 5.7 Hz, *J* = 1.7 Hz, 1 H, CH=C*H*C(O)), 3.82 (s, 1 H, O*H*), 3.67 (s, 3 H, OCH_3), 3.69-3.61 (m, 1 H, C*H*OH), 2.66-2.60 (m, 1 H, CH=CHC*H*), 2.57 (t, *J* = 7.6 Hz, 2 H, C$_{Ar}$CH_2), 2.31 (s, 3 H, C$_{Ar}$CH_3), 2.31 (t, *J* = 7.4 Hz, 2 H, CH_2CO$_2$CH$_3$), 2.00 (dd, *J* = 8.4 Hz, *J* = 2.1 Hz, 1 H, CH-C*H*C(O)), 1.70-1.26 (m, 18 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 212.92 (*C*=O), 174.17 (*C*O$_2$CH$_3$), 168.50 (*C*H=CHC(O)), 139.60 (*C*$_{Ar}$CH$_2$), 134.96 (*C*$_{Ar}$CH$_3$), 132.89 (CH=*C*HC(O)), 128.89 89 (2 C, *C*$_{Ar}$H), 128.23 89 (2 C, *C*$_{Ar}$H), 72.16 (*C*HOH), 55.91 (CH-*C*HC(O)), 51.50 (O*C*H$_3$), 44.86 (CH=CH*C*H), 35.44 (*C*H$_2$), 35.37 (*C*H$_2$), 33.93 (*C*H$_2$), 33.75 (*C*H$_2$), 31.53 (*C*H$_2$), 29.32 (*C*H$_2$), 29.17 (*C*H$_2$), 28.91 (*C*H$_2$), 26.69 (*C*H$_2$), 25.31 (*C*H$_2$), 24.75 (*C*H$_2$), 20.96 (C$_{Ar}$*C*H$_3$); Anal. calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.25; H, 9.15. *syn*-**5** (more polar): R_f = 0.16 (petroleum ether/acetone 20:3); $[\alpha]_D^{24}$ +71.5 (*c* 0.6 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (dd, *J* = 5.4 Hz, *J* = 2.1 Hz, 1 H, C*H*=CHC(O)), 7.08 (d, *J* = 7.6 Hz, 2 H, C$_{Ar}$*H*), 7.06 (d, *J* = 7.3 Hz, 2 H, C$_{Ar}$*H*), 6.14 (dd, *J* = 5.3 Hz, *J* = 1.1 Hz, 1 H, CH=C*H*C(O)), 4.03 (br s, 1 H, C*H*OH), 3.66 (s, 4 H, O*H* and OCH_3), 2.84 (br s, 1 H, CH=CHC*H*), 2.56 (t, *J* = 7.4 Hz, 2 H, C$_{Ar}$CH_2), 2.31 (s, 3 H, C$_{Ar}$CH_3), 2.30 (t, *J* = 7.4 Hz, 2 H, CH_2CO$_2$CH$_3$), 2.13 (dd, *J* = 2.7 Hz, *J* = 2.0 Hz, 1 H, CH-C*H*C(O)), 1.77-1.18 (m, 18 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 212.02 (*C*=O), 174.18 (*C*O$_2$CH$_3$), 168.98 (*C*H=CHC(O)), 139.51 (*C*$_{Ar}$CH$_2$), 135.00 (*C*$_{Ar}$CH$_3$), 133.45 (CH=*C*HC(O)), 128.90 (2 C, *C*$_{Ar}$H), 128.22 (2 C, *C*$_{Ar}$H), 71.33 (*C*HOH), 56.78 (CH-*C*HC(O)), 51.49 (O*C*H$_3$), 42.93 (CH=CH*C*H), 35.35 (*C*H$_2$), 34.35 (*C*H$_2$), 34.21 (*C*H$_2$), 33.95 (*C*H$_2$CO$_2$CH$_3$), 31.50 (*C*H$_2$), 29.34 (*C*H$_2$), 29.05 (*C*H$_2$), 28.93 (*C*H$_2$), 27.02 (*C*H$_2$), 26.11 (*C*H$_2$), 24.78 (*C*H$_2$), 20.96 (C$_{Ar}$*C*H$_3$); Anal. calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.21; H, 9.11.

15-Deoxy-13,14-dihydro-19,20-dinor-18-(4-methylphenyl)-Δ^{12}-PGJ$_1$ methyl ester (3). To an ice-cold solution of *syn*- and *anti*-aldol **5** (68 mg, 0.164 mmol) and Et$_3$N (166 mg, 1.64 mmol) in CH_2Cl_2 (2 mL) was added methanesulfonyl chloride (131 mg, 1.15 mmol). Stirring was continued for 2 h at the same temperature and the solution was poured into saturated aqueous $NaHCO_3$. The mixture was extracted with dichloromethane and the combined organic layers were dried over anhydrous sodium sulfate. After concentration in vacuo, the residue was dissolved in CH_2Cl_2 (2 mL) and neutral Al_2O_3 (1.2 g) was added in three equal portions after 3 and 6h. The reaction mixture was stirred for 24h at room temperature, filtered through a pad of Celite, concentrated, and the residue was purified by column chromatography to afford **3** (57 mg, 87%) as a colorless liquid. R_f = 0.31 (petroleum ether/acetone 5:1); $[\alpha]_D^{21}$ +119.7 (*c* 1.4 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.52 (ddd, *J* = 6.0 Hz, *J* = 2.6 Hz, *J* = 0.7 Hz, 1 H, C*H*=CHC(O)), 7.08 (d, *J* = 7.9 Hz, 2 H, C$_{Ar}$*H*), 7.06 (d, *J* = 8.1 Hz, 2 H, C$_{Ar}$*H*), 6.54 (t, *J* = 7.7 Hz, 1 H, C=C*H*CH$_2$), 6.32 (dd, *J* = 6.0 Hz, *J* = 1.8 Hz, 1 H,C*H*C(O)), 3.66 (s, 3 H, OCH_3), 3.46-3.43 (m, 1 H, C*H*), 2.56 (t, *J* = 7.7 Hz, 2 H, C$_{Ar}$CH_2), 2.31 (s, 3 H, C$_{Ar}$CH_3), 2.29 (t, *J* = 7.5 Hz, 2 H, CH_2CO$_2$CH$_3$), 2.27-2.19 (m, 2 H, C=CHCH_2), 1.84-1.76 (m, 1 H), 1.65-1.57 (m, 5 H), 1.55-1.47 (m, 3 H), 1.41-1.21 (m, 7 H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 196.93 (*C*=O), 174.13 (*C*O$_2$CH$_3$), 161.77 (*C*H=CHC(O)), 139.42 (*C*$_{Ar}$CH$_2$), 137.84 (HC=*C*C(O)), 135.63 (C=*C*HCH$_2$), 135.07 (*C*$_{Ar}$CH$_3$), 134.86 (CH=*C*HC(O)), 128.95 (2 C, *C*$_{Ar}$-H), 128.23 (2 C, *C*$_{Ar}$H), 51.45 (O*C*H$_3$), 43.24 (CH=CH*C*H), 35.35

($C_{Ar}CH_2$), 33.97 ($CH_2CO_2CH_3$), 32.37 (CH_2), 31.41 (CH_2), 29.43 (CH_2), 29.04 (CH_2), 29.03 (CH_2), 28.97 (CH_2), 28.55 (CH_2), 25.67 (CH_2), 24.80 (CH_2), 20.96 ($C_{Ar}CH_3$); Anal. calcd for $C_{26}H_{36}O_3$: C, 78.75; H, 9.15. Found: C, 78.66; H, 9.10.

Cells and cytotoxicity assays

Human umbilical vein endothelial cells (HUVEC) were isolated from freshly collected umbilical cords as previously described[38], and cultured in plastic dishes coated with gelatin, in RPMI 1640 medium supplemented with 20% FBS (fetal bovine serum), 90 U/ml heparin, 150µg/ml ECGF (Endothelial Cell Growth Factor, Roche Diagnostics, Mannheim, Germany) and antibiotics (100 µg/ml streptomycin and 100 U/ml penicillin). $10x10^3$ cells were seeded on each well on 96-well plate (Nunc). The HeLa (human cervix carcinoma), K562 (chronic leukemia) and HL-60 (acute leukemia), cells were cultured in RPMI 1640 medium supplemented with antibiotics and 10 % fetal calf serum (HeLa, K562) or 20% fetal calf serum (HL-60), in a 5 % CO_2 - 95 % air atmosphere. $7x10^3$ cells were seeded on each well on 96-well plate (Nunc). 24h later cells were exposed to the test compounds. Stock solutions (100mM) of test compounds were freshly prepared in DMSO. The final concentrations of the compounds tested in the cell cultures were: 1mM, $1x10^{-2}$ mM, $1x10^{-4}$ mM and $1x10^{-6}$ mM. The concentration of DMSO in the cell culture medium was 1%. The values of IC_{50} (the concentration of test compound required to reduce the cell survival fraction to 50 % of the control) were calculated from dose-response curves and used as a measure of cellular sensitivity to a given treatment. The cytotoxicity of all compounds was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, St. Louis, MO] assay as described.[39] Briefly, after 24 h or 48 h of incubation with drugs, the cells were treated with the MTT reagent and incubation was continued for 2 h. MTT-formazan crystals were dissolved in 20 % SDS and 50 % DMF at pH 4.7 and absorbance was read at 570 and 650 nm on an ELISA-PLATE READER (FLUOstar Omega). As a control (100 % viability), we used cells grown in the presence of vehicle (1% DMSO) only.

Caspase-3/7 assay

HeLa cells cultured in a RPMI 1640 medium supplemented with antibiotics and 10% fetal bovine serum in a 5% CO_2 at 37 °C were used. $20x10^3$ cells were seeded on each well on 96-well plate. After 24h cells were exposed to the cyPGs at conc. of $5xIC_{50}$ for another 18h. Cells were also exposed to 1% DMSO (in order to check if the reagent used to dissolve the test compounds has any effect to the induction of apoptosis), 1µM staurosporin (Sigma, St.Louis, MO) which is a strong inducer of cell apoptosis. The induction of cell apoptosis was analyzed by Apo-ONE® Homogeneous Caspase-3/7 Assay (Promega, Madison, WI, USA). After 18h of incubation with the test compounds, the cells were treated with the caspase-3/7 reagent (according to manufacturer's instructions) and incubated for additional 1.5 hour at room temperature. The fluorescence of wells was measured at the excitation wavelength 485 nm and emission wavelength 520 nm using microplate reader FLUOStar Omega (BMG-Labtech, Germany).

Cell cycle analysis

HeLa cells were seeded in the 6-well plate at a density of $3.2x10^5$ cells per well. 24 hours later cells were treated with a given PG derivative for additional 24h. After treatment, cells were washed with an ice cold PBS (free of Ca^{2+} and Mg^{2+}), trypsinized, collected and fixed with 70% ethanol at -20 °C overnight. Directly before the flow cytometry assay the cells were washed again with the ice cold PBS and treated with RNase A (50 µg/ml) for 1h at 37 °C. Subsequently, cells were stained with Propidium Iodide (10 µg/ml) for 30 min at room temperature in dark. Flow cytometry was performed on a BD FACS Calibur Flow Cytometry System (Becton Dickinson) using an Ar-ion laser (488 nm). Fluorescence dot plots and histograms were generated using a CellQuest software. The distribution of cells in a given cell cycle phase was analyzed using a ModFit LT software, based on measurements of at least 10^4 cells in each experiment.

Acknowledgements

The authors gratefully acknowledge financial support from the Ministry of Science and Higher Education of Poland (grant number N N204 129737).

Notes and references

1 C. D. Funk, *Science*, 2001, **294**, 1871-1875.
2 T. M. A. Elattar, *J. Oral Pathol.*, 1978, **7**, 239-252.
3 E. Ricciotti and G. A. FitzGerald, *Arterioscler. Thromb. Vasc. Biol.*, 2011, **31**, 986-1000.
4 D. Wang and R. N. DuBois, *Nat. Rev. Cancer*, 2010, **10**, 181-193.
5 D. Wang and R. N. DuBois, *Gut*, 2006, **55**, 115-122.
6 D. S. Straus and C. K. Glass, *Med. Res. Rev.*, 2001, **21**, 185-210.
7 B. Diez-Dacal and D. Perez-Sala, *The Scientific World Journal*, 2010, **10**, 655-675.
8 B. Diez-Dacal and D. Perez-Sala, *Cancer Lett.*, 2012, **320**, 150-157.

9 E. Cernuda-Morollon, E. Pineda-Molina, F. J. Canada and D. Perez-Sala, *J. Biol. Chem.*, 2001, 276, 35530–35536.
10 D. Perez-Sala, E. Cernuda-Morollon and F. J. Canada, *J. Biol. Chem.*, 2003, **278**, 51251-51260.
11 S. A. Kliewer, J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris and J. M. Lehmann, *Cell*, 1995, **83**, 813-819.
12 T. Shibata, T. Yamada, T. Ishii, S. Kumazawa, H. Nakamura, H. Masutani, J. Yodoi and K. Uchida, *J. Biol. Chem.*, 2003, **278**, 26046-26054.
13 P. B. Cassidy, K. Edes, C. C. Nelson, K. Parsawar, F. A. Fitzpatrick and P. J. Moos, *Carcinogenesis*, 2006, **27**, 2538-2549.
14 K. Stamatakis, F. J. Sanchez-Gomez and D. Perez-Sala, *J. Am. Soc. Nephrol.*, 2006, **17**, 89-98.
15 G. Aldini, M. Carini, G. Vistoli, T. Shibata, Y. Kusano, L. Gamberoni, I. Dalle-Donne, A. Milzani and K. Uchida, *Biochemistry*, 2007, **46**, 2707-2718.
16 P. A. Campo, S. Das, C.-H. Hsiang, T. Bui, C. E. Samuel and D. S. Straus, *Cell Growth Differ.*, 2002, **13**, 409-420.
17 C. Kamagata, N. Tsuji, M. Moriai, D. Kobayashi and N. Watanabe, *Breast Cancer Res. Treat.*, 2007, **102**, 263-273.
18 M. Tanikawa, K. Yamada, K. Tominaga, H. Morisaki, Y. Kaneko, K. Ikeda, M. Suzuki, T. Kiho, K. Tomokiyo, K. Furuta, R. Noyori and M. Nakanishi, *J. Biol. Chem.*, 1998, **273**, 18522-18527.
19 U. Munoz, F. Bartolome, N. Esteras, F. Bermejo-Pareja and A. Martin-Requero, *Cell. Mol. Life Sci.*, 2008, **65**, 3507-3519.
20 T. Kato, M. Fukushima, S. Kurozumi and R. Noyori, *Cancer Res.*, 1986, **46**, 3538-3542.
21 K. Ikai, R.Sone and M. Fukushima, *Skin Pharmacol.*, 1991, **4**, 9-13.
22 A. Rossi, G. Elia and M. G. Santoro, *J. Biol. Chem.*, 1996, **271**, 32192-32196.
23 T. Bui and D. S. Straus, *Biochim. Biophys. Acta*, 1998, **1397**, 31-42.
24 M. Rosetti, M. Frasnelli, F. Fabbri, C. Arienti, I. Vannini, A. Tesei, W. Zoli and M. Conti, *Anticancer Res.*, 2008, **28**, 315-320.
25 M. Suzuki, T. Kiho, K. Tomokiyo, K. Furuta, S. Fukushima, Y. Takeuchi, M. Nakanishi and R. Noyori, *J. Med. Chem.*, 1998, **41**, 3084-3090.
26 H. Sasaki, S. Niimi, M. Akiyama, T. Tanaka, A. Hazato, S. Kurozumi, S. Fukushima and M. Fukushima, *Cancer Res.*, 1999, **59**, 3919-3922.
27 S. Fukushima, S., Y. Takeuchi, S. Yamashita, S. Kurozumi and M. Fukushima, *Proc. Am. Assoc. Cancer Res.*, 1998, **39**, 315.
28 M. Mikolajczyk and P. Balczewski, *Top. Curr. Chem.*, 2003, **223**, 161-214.
29 R. Zurawinski, M. Mikina and M. Mikołajczyk, *Tetrahedron: Asymmetry*, 2010, **21**, 2794-2799.
30 M. Mikolajczyk, M. Mikina, M. W. Wieczorek and J. Blaszczyk, *Angew. Chem. Int. Ed. Engl.*, 1996, **35**, 1560-1562.
31 K. Furuta, M. Maeda, Y. Hirata, S. Shibata, K. Kiuchib and M. Suzuki, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5487-5491.
32 K. Furuta, K. Tomokiyo, T. Satoh, Y. Watanabe and M. Suzuki, *ChemBioChem*, 2000, **1**, 283-286.
33 Y. Hirata, K. Furuta, S. Miyazaki, M. Suzuki and K. Kiuchi, *Brain Research*, 2004, **1021**, 241-247.
34 G. Bartels, US Pat., 4 847 391, 1989.
35 Y. Kobayashi, M. G. Murugesh and M. Nakano, *Tetrahedron Lett.*, 2001, **42**, 1703-1707.
36 B. Bachowska, J. Kazmierczak-Baranska, M. Cieslak, B. Nawrot, D. Szczesna, J. Skalik and P. Bałczewski, *ChemistryOpen*, 2012, **1**, 33-38.
37 D. G. Piotrowska, M. Cieslak, K. Krolewska and A. E. Wroblewski, *Eur. J. Med. Chem.*, 2011, **46**, 1382-1389.
38 E. A. Jaffe, R. L. Nachman, C. G. Becker and C. R. Minick, *J. Clin. Invest.*, 1973, **52**, 2745-2756.
39 M. Maszewska, J. Leclaire, M. Cieslak, B. Nawrot, A. Okruszek, A. M. Caminade and J. P. Majoral, *Oligonucleotides*, 2003, **13**, 193-205.