Supporting Information

Biosynthetic crossover of 5-lipoxygenase and cyclooxygenase-2 yields 5-hydroxy-PGE_2 and 5-hydroxy-PGD_2

Fumie Nakashima[1], Takashi Suzuki[1], Odaine N. Gordon[1], Dominic Golding[1], Toshiaki Okuno[2], Juan A. Giménez-Bastida[1], Takehiko Yokomizo[2], and Claus Schneider[1]

[1]Division of Clinical Pharmacology, Department of Pharmacology, and Vanderbilt Institute of Chemical Biology, Vanderbilt University Medical School, Nashville, TN 37232, U.S.A.
[2]Department of Biochemistry, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan

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General Information

Materials. Prostaglandin standards (PGD_2, PGE_2, $PGF_{2\alpha}$, $PGF_{2\beta}$, 11β-$PGF_{2\alpha}$), NS-398, and indomethacin were obtained from Cayman Chemical. Other reagents and solvents were obtained from Acros, Fischer, Sigma-Aldrich, or TCI America. Recombinant human COX-2 (1) and recombinant human H-PGDS (2) were expressed and purified as described. 5*S*-HETE was prepared from arachidonic acid according to a described procedure (3,4).

HPLC. Samples were analyzed using an Agilent 1200 HPLC system equipped with a diode array detector. RP-HPLC used a Waters Symmetry 5-μm column (4.6 x 250 mm). Solvents A (water/acetonitrile/HOAc 80/20/0.01, by vol.) and B (water/acetonitrile/HOAc 20/80/0.01, by vol.) were mixed to generate linear gradients for elution (1 ml/min flow rate).

Spectroscopy. NMR spectra were recorded using a Bruker AV-II 600 MHz spectrometer equipped with a cryoprobe. Chemical shifts (δ value) are given relative to the residual protiated solvent and are reported in parts per million (ppm). Coupling constants (*J*) are given in Hertz (Hz). Pulse frequencies were taken from the Bruker library. UV spectra were recorded during on-line HPLC-diode array analyses using an Agilent 1200 system or off-line using a Perkin Elmer PE-35 spectrophotometer. The LC-MS instrument was a Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer. LC-mass spectra were recording using electrospray ionization and detection in the negative ion mode. Instrument and ionization parameters were optimized by infusion of a solution of PGD_2. For high-resolution mass spectrometry an LTQ Orbitrap 3 XL system (Thermo Scientific) was used.

Safety Statement. No unexpected or unusually high safety hazards were encountered.

Naming of Compounds. Known compounds are identified by their trivial names. The novel 5-hydroxy-prostaglandins have been named in accord with their arachidonic acid-derived analogues.

Experimental Procedures

Singlet oxidation of PGD_2 and PGE_2

Reactions appeared to give higher overall yield when conducted in multiple small aliquots in parallel rather than combined into a single large reaction. PGD_2 or PGE_2 (100 μg each) were placed in conical 1 mL glass vials containing 75 μg methylene blue (from a 5 mg/ml stock solution in methanol) in a total volume of 100 μL methanol. The vials were placed on a sheet of aluminum foil on ice. A desk lamp with a 100 W bulb was placed about 20 cm above the vials and served as the light source. For reaction times >10 h light exposure was interrupted and samples were stored in a freezer overnight before re-starting the reaction. Reaction progress was monitored by analyzing 1 μL aliquots by RP-HPLC using a Waters Symmetry C18 5 μm-column, 250 x 4.6 mm, eluted with a solvent of acetonitrile/water/acetic acid (37.5/62.5/0.01, by vol.) at 1 mL/min flow rate and UV detection using an Agilent 1200 diode array detector. After about 10-20% conversion to products (for PGE_2: 17 h; for PGD_2: 4 h) an excess of TPP was added, and the solvent was evaporated to 50 μL volume under a

stream of nitrogen. The residue was dissolved in 1 mL of ethyl acetate to remove excess methylene blue that has limited solubility that solvent, transferred to a new vial and evaporated. Products were isolated by RP-HPLC using a Waters Symmetry C18 5 μm-column, 450 x 4.6 mm, eluted with acetonitrile/water/acetic acid (for PGE_2: 30/70/0.01, by vol.; for PGD_2: 25/75/0.01, by vol.) at 1 mL/min flow rate. The collected products were evaporated to remove the organic solvent, diluted with water, and extracted using a 30 mg Waters HLB cartridge.

Methyl esterification

A solution of diazomethane in ether (2 drops, ca. 20 μL) was added to 20 μg of 5-OH-PG or 5-HETE dissolved in 20 μL of acetonitrile. The sample was mixed and immediately evaporated under a stream of nitrogen.

Derivatization with (-)-menthyl chloroformate

(-)-Menthoxycarbonyl derivatives were prepared by treatment of methyl-esterified 5-OH-eicosanoids in 40 μL of dry benzene and 12 μL of pyridine with 60 μL of a solution containing 1 μmol/μL (-)-menthyl chloroformate in toluene at room temperature for 18 h. Samples were diluted with 2 mL of water, and the mixture was acidified by adding HCl. The mixture was extracted twice with 500 μL of methylene chloride and evaporated to dryness under a stream of nitrogen. The residue was dissolved in chloroform for ozonolysis.

Oxidative ozonolysis

Ozonolysis was performed by bubbling O_3 into a solution of the (-)-menthoxycarbonyl derivatives dissolved in 0.5 mL of chloroform at -20°C for 1.5 h. After the reaction, the solvent was evaporated under a stream of nitrogen, and the sample was treated with 1 mL of glacial acetic acid containing 30% hydrogen peroxide at 50°C for 18 h. The sample was diluted with 3 mL of water and extracted using a 30 mg Waters HLB cartridge. Products were eluted using methanol, evaporated, and dissolved in 50 μL acetonitrile/water 1:1 (by vol.) for LC-MS analysis.

Reduction of PGD_2 and PGE_2

PGD_2 and PGE_2 dissolved in PBS (500 nM, 1 mL) were reduced by addition of 20 μL of 1 M $NaBH_4$ in 0.1 N NaOH. After 5 min reaction at room temperature the samples were acidified with acetic acid (pH 4). The products were recovered by extraction using a 30-mg Waters HLB cartridge. Products were eluted from the cartridge using 0.25 mL of ethyl acetate and 1 mL of methanol and evaporated under a stream of nitrogen.

Derivatization with AMPP

Products formed in leukocyte incubations were derivatized with N-(4-aminomethylphenyl)pyridinium (AMPP) to increase sensitivity using positive ion LC-MS analysis (5). The eluates from the HLB cartridges were evaporated under a stream of nitrogen, and the following reagents were added: 10 μL of ice-cold acetonitrile/dimethyoformamide (4:1, v/v), 10 μL of 1-ethyl-3-(e-dimethylamino-propyl) carbodiimide (640 mM in water), and 20 μL of a solution containing 5 mM N-hydroxylbenzotriazole and 15 mM AMPP (in acetonitrile). Sample tubes were briefly vortex mixed and incubated at 60°C for 30 min. The samples were diluted with 40 μL of acetonitrile/water (1:1, v/v) and analyzed using LC-MS on the same day while maintained at 4°C in the autosampler during analysis.

LC-MS analysis

Products formed by the reaction of recombinant human COX-2 with 5-HETE (Fig. 1), samples treated with $SnCl_2$ (Fig. 2A) and $NaBH_4$ (Fig. 4) were analyzed using a Thermo TSQ Vantage triple quadrupole MS instrument (Mass Spectrometry Core, Vanderbilt University) equipped with a heated electrospray interface operated in negative ion mode. The instrument parameters were optimized by infusion of a solution of PGD_2 in acetonitrile/water 1:1 (by vol.). The electrospray needle was maintained at 4.0 kV. The ion transfer tube was operated at 300°C. A Zorbax Eclipse Plus C18 1.8-μm column (2.1 x 50 mm; Agilent Technologies, Santa Clara, CA, USA) was used for the separation. Water and acetonitrile containing 0.01% formic acid were the mobile phases (A and B, respectively) used at a flow rate of 0.4 mL/min. Samples were analyzed in negative ion mode, and a linear gradient was used starting with 80% of solvent A, reaching 40% of solvent B at 4 min. Samples were analyzed in the negative ion Full Scan mode. The signal intensities obtained for each ion chromatogram are given in exponential format with the maximum intensity set as 100%. Data acquisition and spectral analysis were performed using Thermo Scientific Xcalibur software.

Hematin treated samples (Fig. 2B and 2C) and leukocyte samples (Fig. 5) were analyzed using a Thermo TSQ Quantis Triple Quadrupole MS instrument (Mass Spectrometry Core, Vanderbilt University) equipped with a heated electrospray interface operated in negative ion mode. A Zorbax Eclipse Plus C18 1.8-μm column (2.1 x 50 mm; Agilent Technologies, Santa Clara, CA, USA) was used for the separation. Water and acetonitrile containing 0.01% formic acid were the mobile phases (A and B, respectively) used at a flow rate of 0.4 mL/min. For the hematin-treated sample, a linear gradient was used starting with 100% of solvent A, reaching 50% of solvent B at 4 min. For the leukocyte samples a linear gradient was used starting with 95% of A, reaching 50% of B at 4 min,

100% of B at 4.5 min, and held for 0.8 min. Data acquisition and spectral analysis were performed using Thermo Scientific Xcalibur software.

The following transitions were recorded in the selected reaction monitoring (SRM)-positive ion mode for AMPP-derivatized compounds: HKD_2 *m/z* 567->369 (+ 35 eV), HKE_2, *m/z* 567->381 (+ 35 eV); 5-OH-PGD_2 *m/z* 535->183 (+ 42 eV); 5-OH-PGE_2 *m/z* 353->183 (+ 45 eV); hexa-hydroxy C20:2 *m/z* 571->341 (+ 45 eV); 5-OH-PGF_2 *m/z* 537->183 (+ 45 eV).

References

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Supplementary Figures

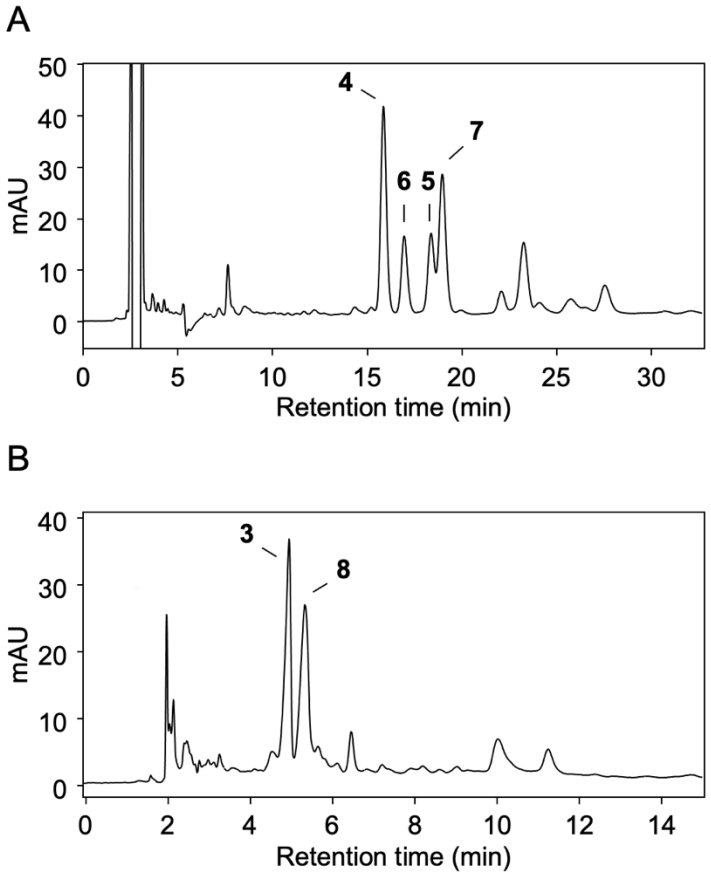


Fig. S1. Products from singlet oxidation of PGD_2 and PGE_2. RP-HPLC analysis of (A) PGD_2 and (B) PGE_2 reacted with methylene blue in methanol under a 100 W light bulb on ice for 4 h or 17 h, respectively. Samples were reduced with an excess of triphenylphosphine and injected on a Waters Symmetry C18 5 μm-column, 450 x 4.6 mm, eluted with acetonitrile/water/acetic acid (30/70/0.01, by vol. in panel A and 25/75/0.01, by vol. in panel B) at 1 ml/min flow rate and UV detection at 205 nm. The equivalent of 100 μg starting material was injected.

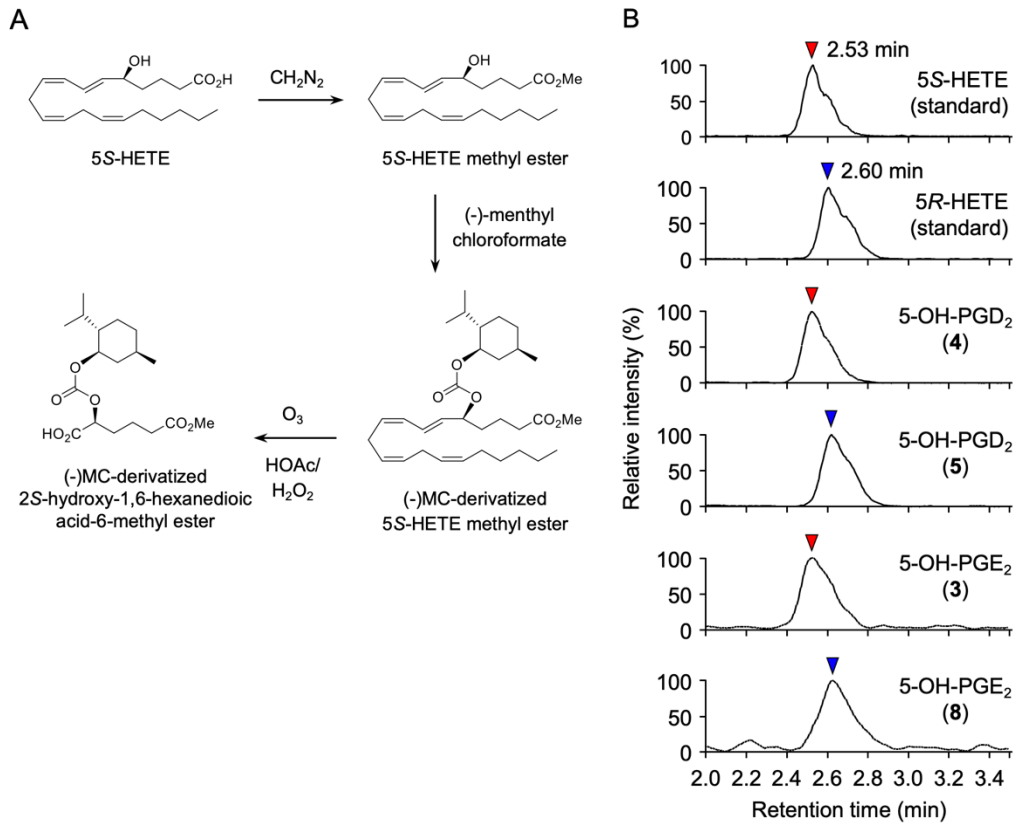


Fig. S2. Analysis of the configuration of C-5 of 5-OH-PGD$_2$ and 5-OH-PGE$_2$ diastereomers prepared by singlet oxidation of the respective prostaglandin precursor. (A) Transformation of 5*S*- and 5*R*-HETE standards and isolated 5-OH-PGs to diastereomeric 2*R*- and 2*S*-(-)-menthoxycarbonyl (MC) 2-hydroxy-1,6-hexanedioic acid-6-methyl esters. (B) LC-MS negative ion traces (*m/z* 357.4) of (-)MC-derivatized 2*S*- and 2*R*-hydroxy-1,6-hexanedioic acid-6-methyl ester formed upon methyl esterification (CH_2N_2), (-)MC derivatization, and oxidative ozonolysis of 5-HETE enantiomers of known configuration and of 5-OH-PG diastereomers **3**, **4**, **5**, and **8**.

Samples were analyzed using a Waters Xevo TQ-S MS instrument (Eicosanoid and Neurochemistry Core, Vanderbilt University) equipped with a heated electrospray interface operated in negative ion mode. The instrument parameters were optimized by infusion of a solution of PGD$_2$ in acetonitrile/water 1:1 (by vol.). A Zorbax Eclipse Plus C18 1.8-µm column (2.1 x 50 mm; Agilent Technologies, Santa Clara, CA, USA) was used for the separation. Water and acetonitrile containing 0.1% formic acid were the mobile phases (A and B, respectively) used at a flow rate of 0.5 ml/min. A linear gradient was used starting with 50% of solvent A, reaching 55% of solvent B at 4.5 min. The ion traces for *m/z* 357.4 for derivatized 2*R* or 2*S*-hydroxyhexanedioic methyl ester were acquired in negative ion MS1 mode. The signal intensities obtained for each ion chromatogram are given in exponential format with the maximum intensity set as 100%. Data acquisition and spectral analysis were performed using Waters MassLynx software.

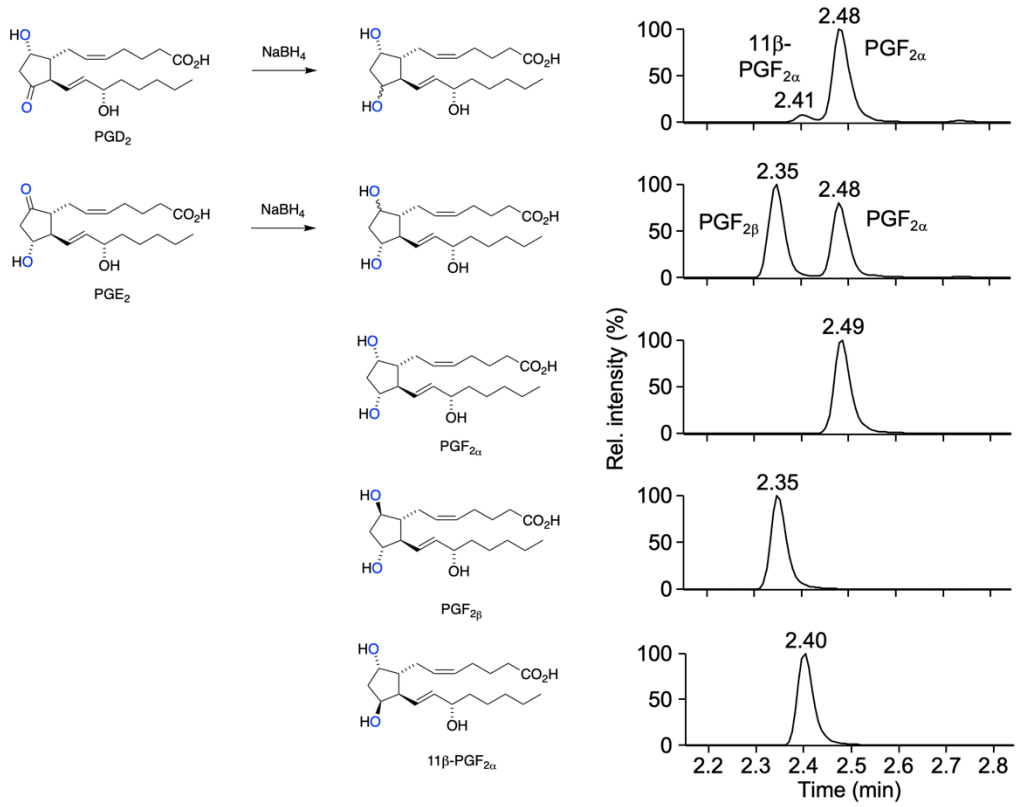


Fig. S3. Reduction of PGD_2 and PGE_2 with $NaBH_4$ yields three PGF_2 diastereomers that were identified by co-elution with authentic standards, $PGF_{2\alpha}$, $PGF_{2\beta}$, and 11β-$PGF_{2\alpha}$. Diastereomers were analyzed using LC-MS, and the ion traces for *m/z* 353 acquired in negative ion MS1 mode are shown to the right of each reaction or standard. Reduction of PGD_2 yielded $PGF_{2\alpha}$ as a prominent product whereas 11β-$PGF_{2\alpha}$ was about 5% in abundance. The same preference for reduction was assumed to have occurred with 5-OH-PGD_2. Reduction of PGE_2, in contrast, gave the expected ≈1:1 mixture of $PGF_{2\alpha}$ and $PGF_{2\beta}$ diastereomers, showing no stereoselectivity in the reduction of this compound. Samples were analyzed using a Thermo TSQ Vantage triple quadrupole MS instrument equipped with a heated electrospray interface operated in negative ion mode. The same conditions as in Fig. 4 were used.

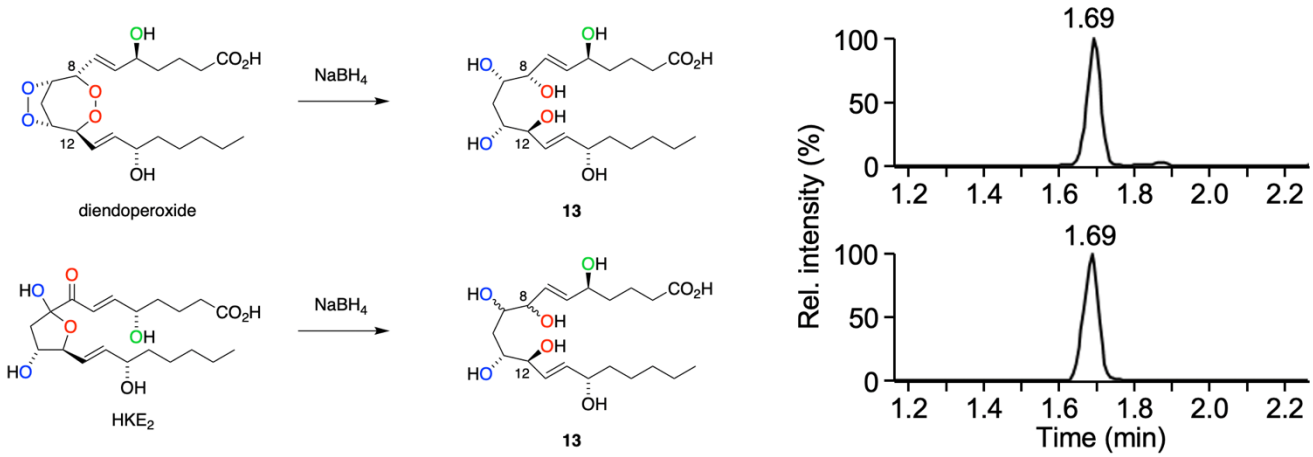


Fig. S4. Reduction of a crude reaction of 5-HETE with COX-2 (top) and of isolated HKE_2 (bottom) with $NaBH_4$ yields 5,8,9,11,12,15-hexahydroxy-eicosadienoic acid **13**. Reaction products were analyzed using LC-MS, and the ion traces for *m/z* 403 acquired in negative ion MS1 mode are shown to the right of each reaction. Samples were analyzed using a Thermo TSQ Vantage triple quadrupole MS instrument equipped with a heated electrospray interface operated in negative ion mode. The same conditions as in Fig. 4 were used.

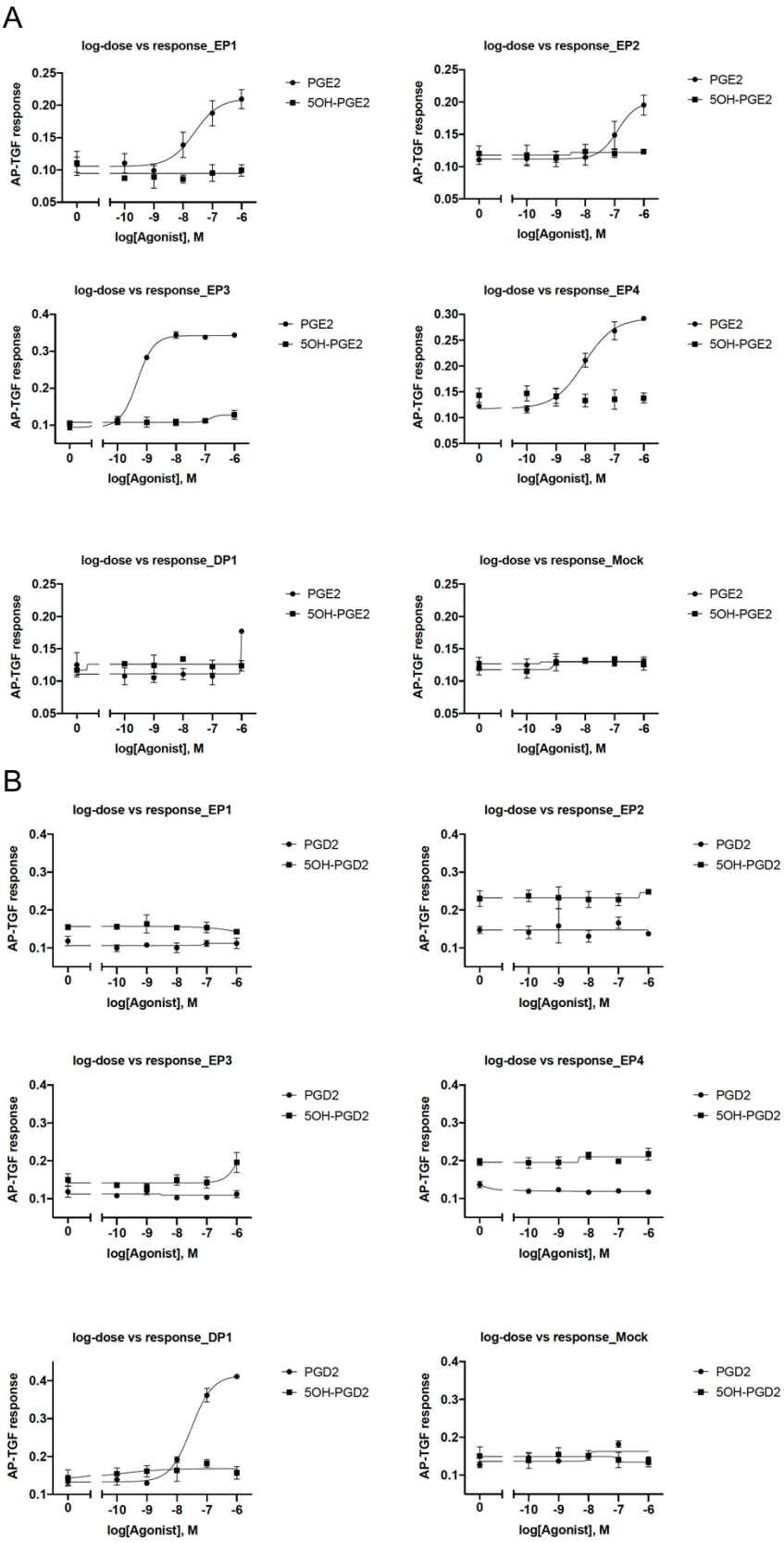


Fig. S5. Analysis of EP1-EP4 and DP1 prostanoid receptor activation by (A) 5-OH-PGE_2 and (B) 5-OH-PGD_2 in comparison to PGE_2 and PGD_2.

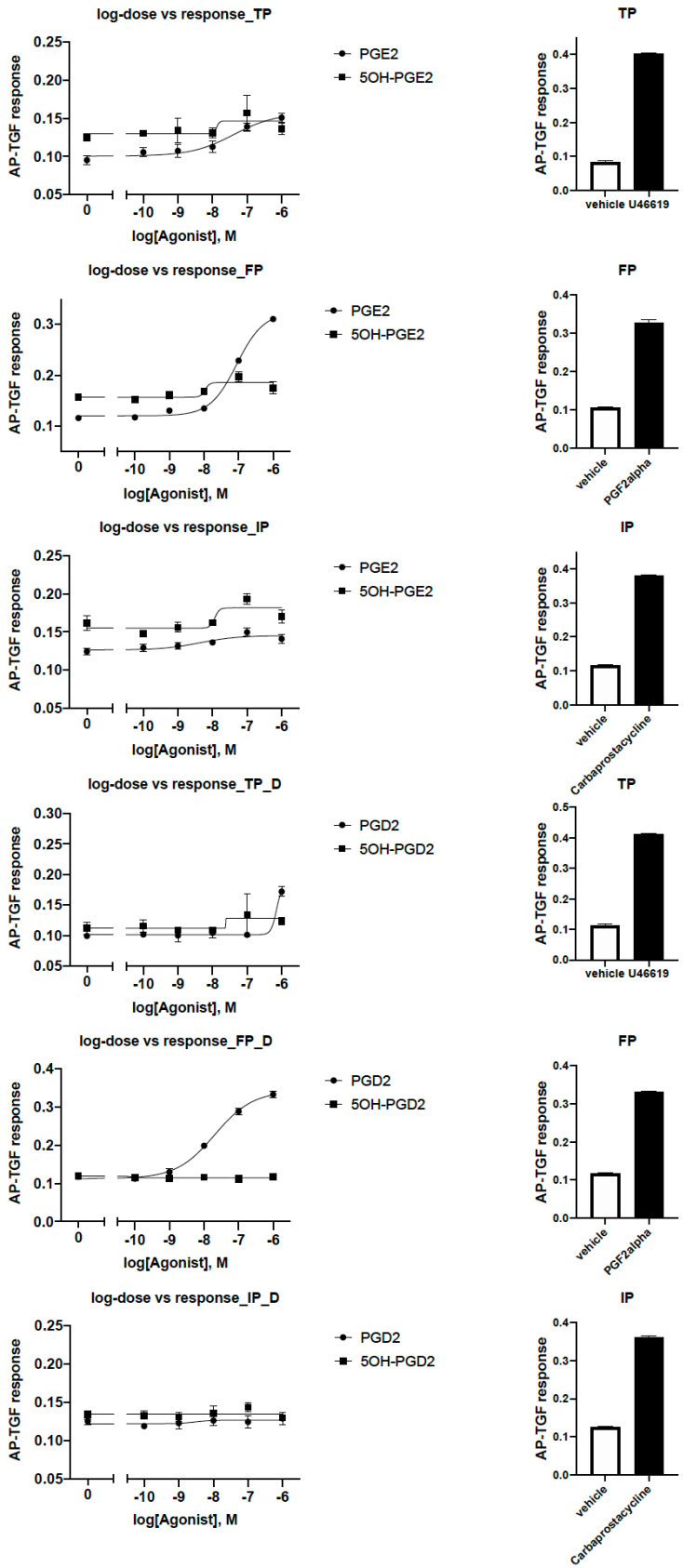


Fig. S6. Analysis of TP, FP, and IP prostanoid receptor activation by 5-OH-PGE_2 and 5-OH-PGD_2 in comparison to PGE_2, PGD_2, and known receptor activators (1 μM).

Table S1: NMR chemical shifts for 5*S*-OH-PGD$_2$ (4) (600.13 MHz, $CDCl_3$) and 5*R*-OH-PGD$_2$ (5) (600.13 MHz, CD_3CN).

	5*S*-OH-PGD$_2$ (**4**)					5*R*-OH-PGD$_2$ (**5**)		
Proton No.	δ ^{1}H (ppm)	Multi-plicity	Coupling constant (Hz)	δ ^{13}C[b] (ppm)	HMBC	δ ^{1}H (ppm)	Multi-plicity	Coupling constant (Hz)
2	2.34	t	$J_{2,3}$ = 7.7	33.0	1, 3, 4	2.28	t	$J_{2,3}$ = 7.4
3	1.69	m		20.0	2	1.53-1.67	m	
4	1.61-1.67[a]	m		36.4	2, 3	1.46	dt	$J_{4,5}$ = 6.4; $J_{4,3}$ = 7.9
5	4.23	m		71.9	7	4.03	dt	$J_{5,4}$ = 6.2; $J_{5,6}$ = 6.3
6	5.72	dd	$J_{6,7}$ = 15.7; $J_{5,6}$ = 6.2	136.5	5, 7, 8	5.57	dd	$J_{6,7}$ = 15.3; $J_{5,6}$ = 6.2
7	5.83	dd	$J_{6,7}$ = 15.7; $J_{7,8}$ = 7.1	127.6	5, 6, 8	5.73	dd	$J_{7,6}$ = 15.7; $J_{7,8}$ = 7.5
8	2.76	ddd	$J_{8,12}$ = 11.5; $J_{8,9}$ = 3.3; $J_{8,7}$ = 6.8	50.3		2.65	ddd	$J_{8,12}$ = 11.2; $J_{7,8}$ = 7.7; $J_{8,9}$ = 3.4
9	4.48	dd	$J_{9,10b}$ = 3.7; $J_{9,8}$ = 3.7	69.9		4.31	dd	$J_{9,10a}$ = 4.2; $J_{9,8}$ = 4.2
10a	2.50	d	$J_{10a,10b}$ = 18.9	46.7	8, 9, 11	2.43	dd	$J_{10a,10b}$ = 18.3; $J_{10a,9}$ = 4.9
10b	2.45	dd	$J_{10b,10a}$ = 18.8 $J_{10b,9}$ = 4.5	46.7	11	2.24	d	$J_{10a,10b}$ = 18.4
11	(keto)			215.5		(keto)		
12	3.10	dd	$J_{8,12}$ = 12.1; $J_{12,13}$ = 5.1	51.5	13, 14	2.91	dd	$J_{8,12}$ = 11.9; $J_{12,13}$ = 7.4
13	5.62	dd	$J_{13,14}$ = 15.7 $J_{13,12}$ = 5.5	124.9	12, 15	5.41	dd	$J_{13,14}$ = 15.5; $J_{13,12}$ = 7.4

14	5.58	dd	$J_{14,13}$ = 15.7 $J_{14,15}$ = 5.7	136.4	12, 15	5.52	dd	$J_{14,13}$ = 15.5; $J_{14,15}$ = 6.4
15	4.10	dt	$J_{15,14}$ = 6.3; $J_{15,16}$ = 6.3	72.9	13	3.94	dt	$J_{15,16}$ = 6.7; $J_{14,15}$ = 6.3
16	1.48	m		36.8		1.35-1.43	m	
17, 18, 19	1.25-1.35	m		22.6/25.3/31.7	20	1.23-1.29	m	
20	0.86	t	$J_{19,20}$ = 6.7	14.2	18, 19	0.87	t	$J_{19,20}$ = 6.7

[a] the chemical shift was estimated from the H,H-COSY experiment.

[b] the chemical shift was derived from the HSQC and HMBC experiments.

Table S2: NMR chemical shifts for the 6-OH-PGD$_2$ diastereomers (6 and 7) (600.13 MHz, CD_3CN).

	6-OH-PGD$_2$, (**6**)			6-OH-PGD$_2$, (**7**)		
Proton No.	δ ^{1}H (ppm)	Multi-plicity	Coupling constant (Hz)	δ ^{1}H (ppm)	Multi-plicity	Coupling constant (Hz)
2	2.33	t	$J_{2,3} = 6.7$	2.34	t	$J_{2,3} = 7.0$
3	2.25	q	$J = 6.8$	2.26	q	$J = 6.9$
4	5.61	dt	$J_{4,5} = 15.4$; $J_{4,3} = 6.5$	5.63	dt	$J_{4,5} = 15.5$; $J_{4,3} = 6.5$
5	5.48	dd	$J_{5,4} = 15.1$; $J_{5,6} = 6.1$	5.53	dd	$J_{5,4} = 15.4$; $J_{5,6} = 6.1$
6	4.13	dt	$J_{6,7} = 6.8$; $J_{6,5} = 6.7$	4.21	m	
7(a)	1.71	ddd	$J_{7a,7b} = 13.9$; $J_{7a,8} = 10.6$; $J_{7a,6} = 7.3$	1.71	ddd	$J_{7a,7b} = 14.2$; $J_{7a,8} = 10.4$; $J_{7a,6} = 3.8$
7b	1.51	ddd	$J_{7a,7b} = 13.9$; $J_{7b,8} = 5.8$; $J_{7b,6} = 4.4$	1.54	ddd	$J_{7a,7b} = 13.7$; $J_{7b,8} = 7.7$; $J_{7b,6} = 4.1$
8	1.98	dt	$J_{8,12} = 10.9$; $J_{7,8} = 4.2$	2.14[a]	m	
9	4.38	dd	$J_{9,10a} = 4.4$; $J_{9,8} = 4.4$	4.40	dd	$J_{9,10a} = 4.2$; $J_{9,8} = 4.2$
10a	2.41	dd	$J_{10a,10b} = 18.5$; $J_{10a,9} = 5.2$	2.42	dd	$J_{10a,10b} = 18.4$; $J_{10a,9} = 5.0$
10b	2.19[a]	m		2.21[a]	m	
11	(keto)			(keto)		
12	2.60	dd	$J_{8,12} = 11.6$; $J_{12,13} = 8.6$	2.63	dd	$J_{8,12} = 11.9$; $J_{12,13} = 8.4$
13	5.34	dd	$J_{13,14} = 15.5$; $J_{13,12} = 8.3$	5.33	dd	$J_{13,14} = 15.5$; $J_{13,12} = 8.3$
14	5.46	dd	$J_{14,13} = 15.1$; $J_{14,15} = 7.0$	5.48	dd	$J_{14,13} = 15.4$; $J_{14,15} = 6.5$
15	3.96	dt	$J_{15,16} = 6.3$; $J_{14,15} = 6.5$	3.95	dt	$J_{15,16} = 6.2$; $J_{14,15} = 6.5$
16	1.36-1.47	m		1.35-1.46	m	
17, 18, 19	1.23-1.31[a]	m		1.23-1.32	m	
20	0.87	t	$J_{19,20} = 6.8$	0.87	t	$J_{19,20} = 6.7$

[a] the chemical shift was estimated from the H,H-COSY experiment.

Table S3: NMR chemical shifts for 5*S*-OH-PGE$_2$ (3) and 5*R*-OH-PGE$_2$ (8) (600.13 MHz, $CDCl_3$).

	5*S*-OH-PGE$_2$ (**3**)			5*R*-OH-PGE$_2$ (**8**)		
Proton No.	δ ^{1}H (ppm)	Multi-plicity	Coupling constant (Hz)	δ ^{1}H (ppm)	Multi-plicity	Coupling constant (Hz)
2	2.36	t	$J_{2,3}$ = 6.9	2.37	t	$J_{2,3}$ = 6.7
3	1.65-1.71[a]	m		1.68-1.72[a]	m	
4	1.46-1.52[a]	m		1.47-1.53[a]	m	
5	4.11-4.17	m		4.11-4.18	m	
6	5.60	dd	$J_{6,7}$ = 15.7; $J_{5,6}$ = 6.5	5.65	dd	$J_{6,7}$ = 15.8; $J_{5,6}$ = 5.6
7	5.54	dd	$J_{6,7}$ = 15.7; $J_{7,8}$ = 7.0	5.61	dd	$J_{6,7}$ = 15.7; $J_{7,8}$ = 5.4
8	2.75	dd	$J_{8,12}$ = 12.4; $J_{7,8}$ = 7.0	2.75	dd	$J_{8,12}$ = 12.2; $J_{7,8}$ = 5.6
9	(keto)			(keto)		
10a	2.81	dd	$J_{10a,10b}$ = 18.8 $J_{10a,11}$ = 7.7	2.81	dd	$J_{10a,10b}$ = 18.7 $J_{10a,11}$ = 7.4
10b	2.32	dd	$J_{10b,10a}$ = 18.8 $J_{10b,11}$ = 9.7	2.32	dd	$J_{10b,10a}$ = 18.7 $J_{10b,11}$ = 9.7
11	4.11-4.17	m		4.11-4.18	m	
12	2.52	ddd	$J_{8,12}$ = 12.2; $J_{12,11}$ = 8.3; $J_{12,13}$ = 8.3	2.57	ddd	$J_{8,12}$ = 12.4; $J_{12,11}$ = 8.3; $J_{12,13}$ = 8.3
13	5.63	dd	$J_{13,14}$ = 15.6 $J_{13,12}$ = 8.0	5.65	dd	$J_{13,14}$ = 15.6 $J_{13,12}$ = 7.2
14	5.72	dd	$J_{14,13}$ = 15.5 $J_{14,15}$ = 6.0	5.73	dd	$J_{14,13}$ = 15.4 $J_{14,15}$ = 5.9
15	4.11-4.17	m		4.11-4.18	m	
16	1.46-1.52[a]	m		1.47-1.53[a]	m	
17, 18, 19	1.26-1.29[a]	m		1.25-1.29[a]	m	

20	0.86	t	$J_{19,20} = 6.6$		0.87	t	$J_{19,20} = 6.6$

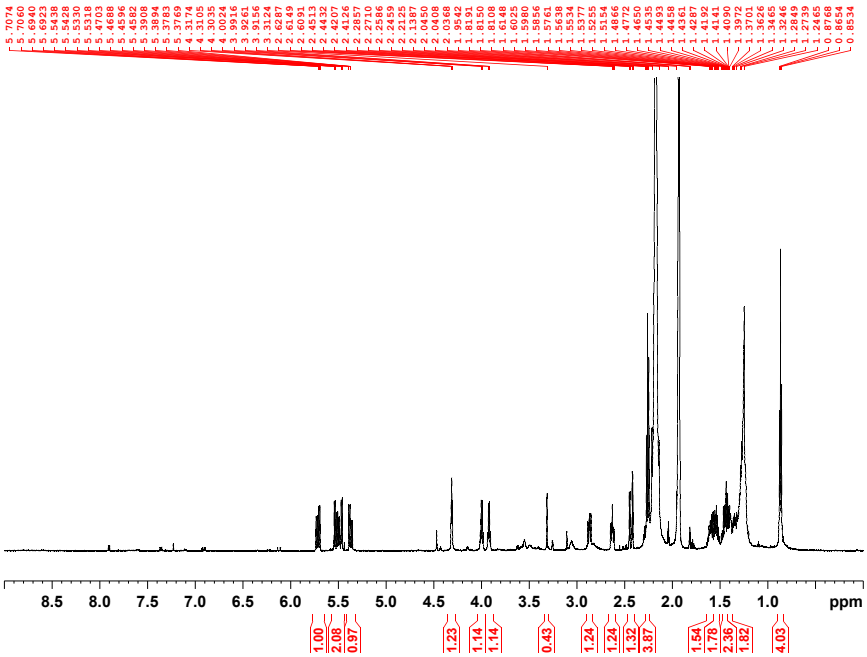
[a] the chemical shift was estimated from the H,H-COSY experiment.

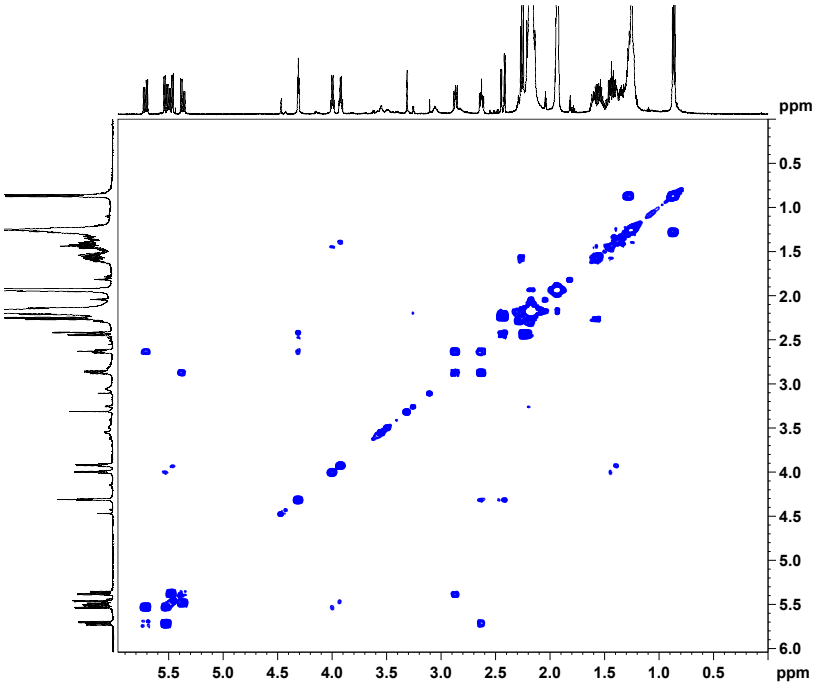
Table S4: HR-MS analysis of 5*S*-OH-PGD$_2$, 5*S*-OH-PGE$_2$, and 5*S*-OH-PGF$_{2\alpha}$.

Compound	*m/z* calculated [a]	*m/z* observed	δ (ppm)	Derived molecular formula
5*S*-OH-PGD$_2$ **4**	367.2126	367.2124	0.5	$C_{20}H_{32}O_6$
5*S*-OH-PGE$_2$ **3**	367.2126	367.2123	0.8	$C_{20}H_{32}O_6$
5*S*-OH-PGF$_{2\alpha}$ **9**	369.2283	369.2279	1.1	$C_{20}H_{34}O_6$

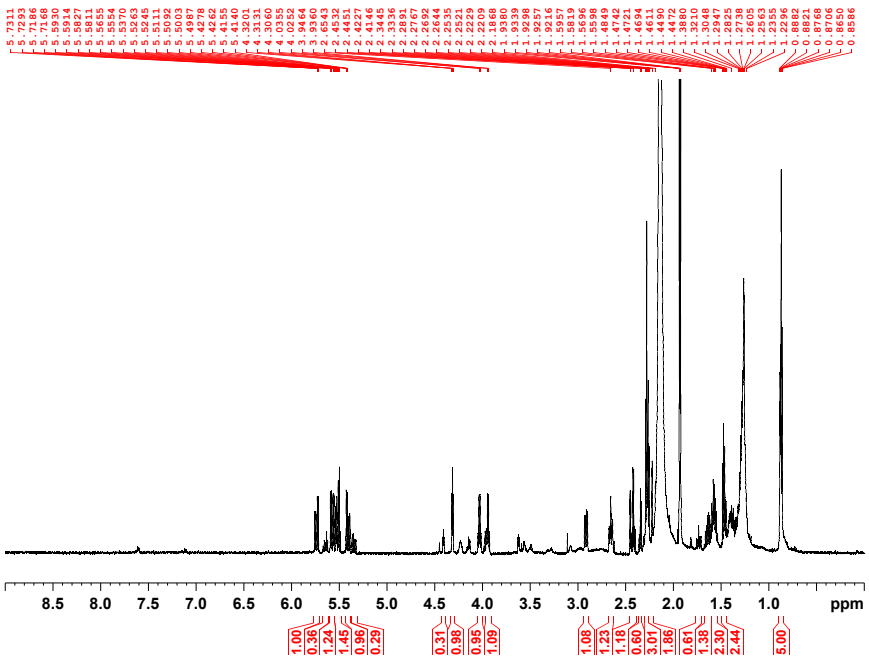
[a] calculations refer to the $[M-H]^-$ molecular ion.

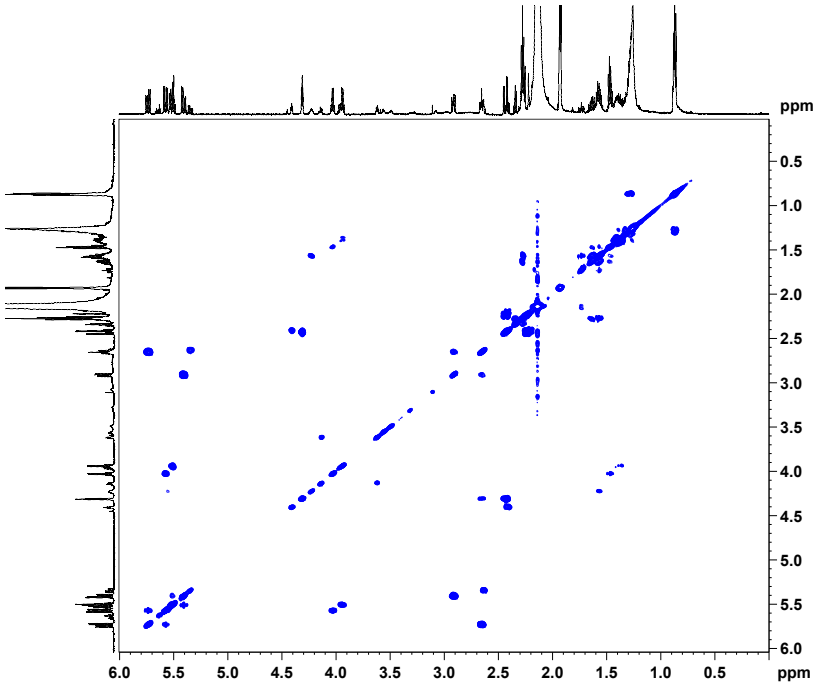
5*S*-OH-PGD$_2$, product **4**, 600 MHz, CD_3CN



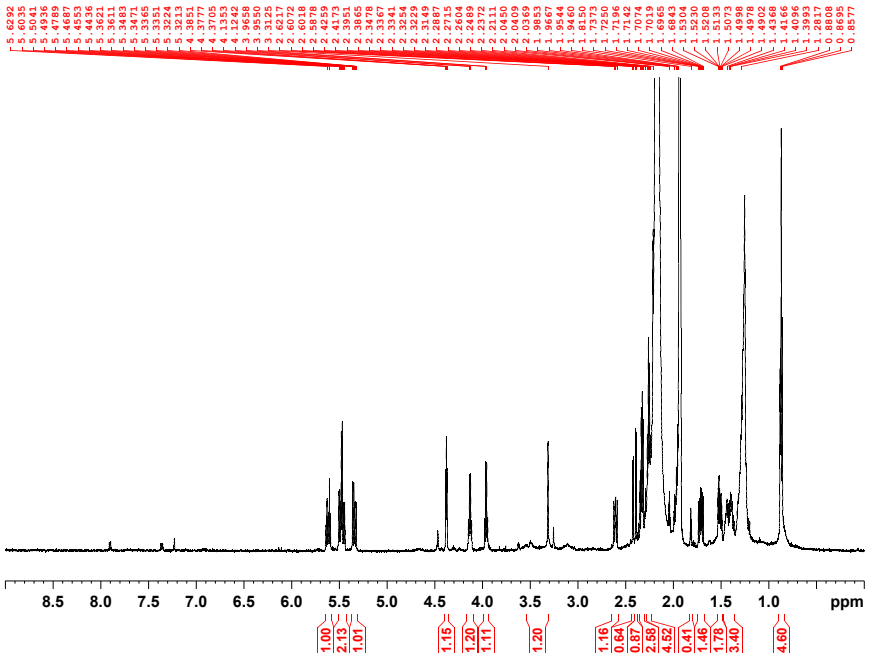


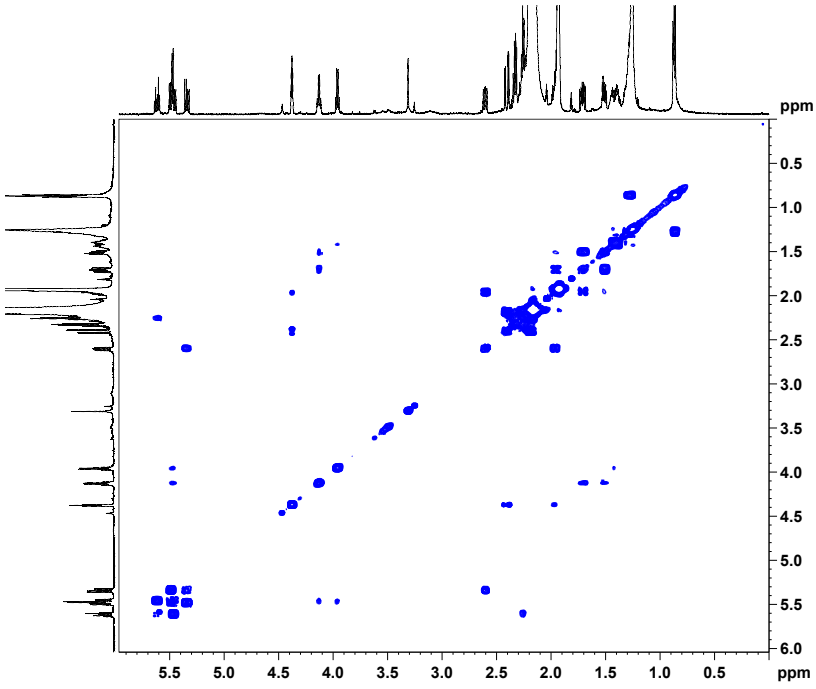
5*R*-OH-PGD$_2$, product **5**, 600 MHz, CD_3CN



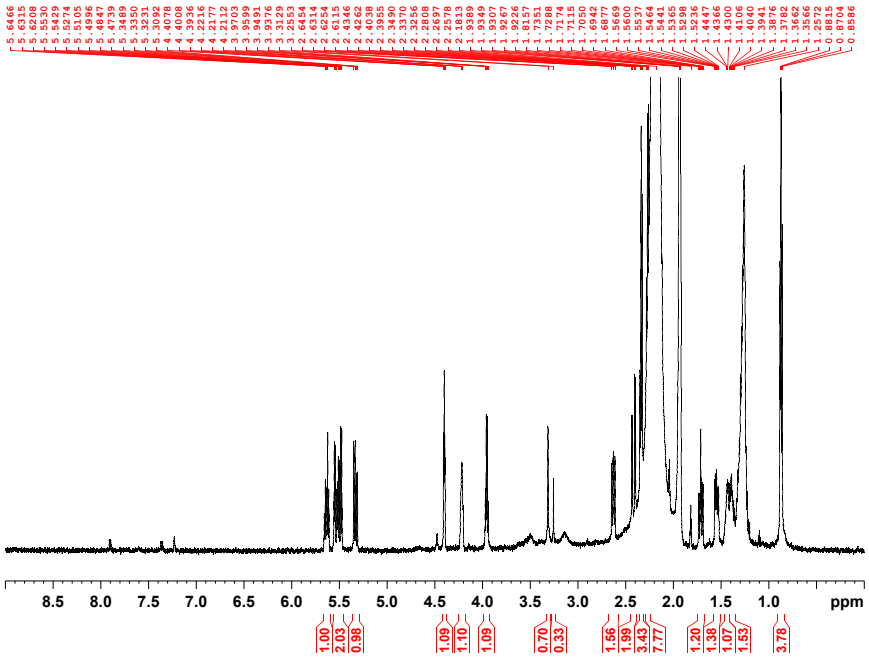


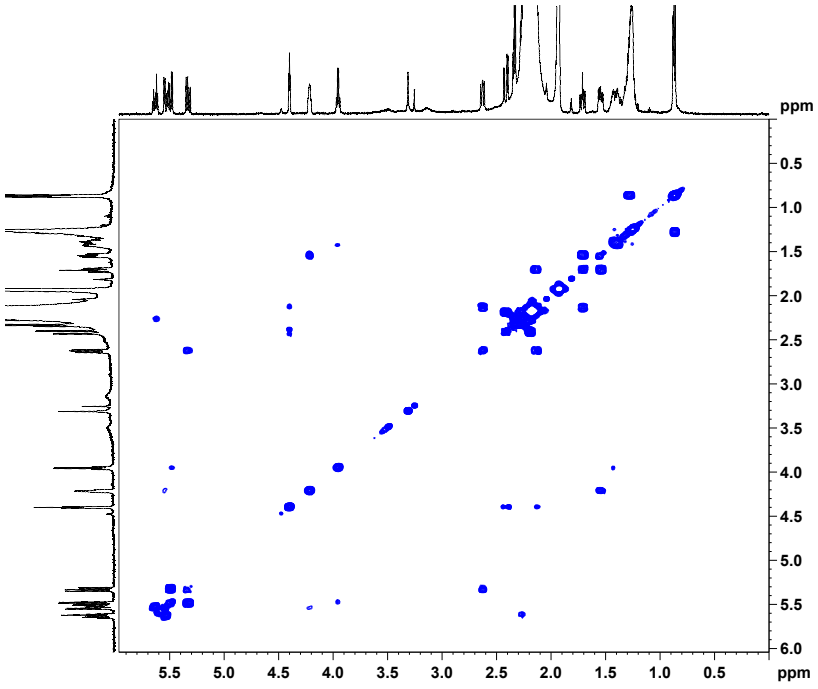
6-OH-PGD$_2$, product **6**, 600 MHz, CD_3CN



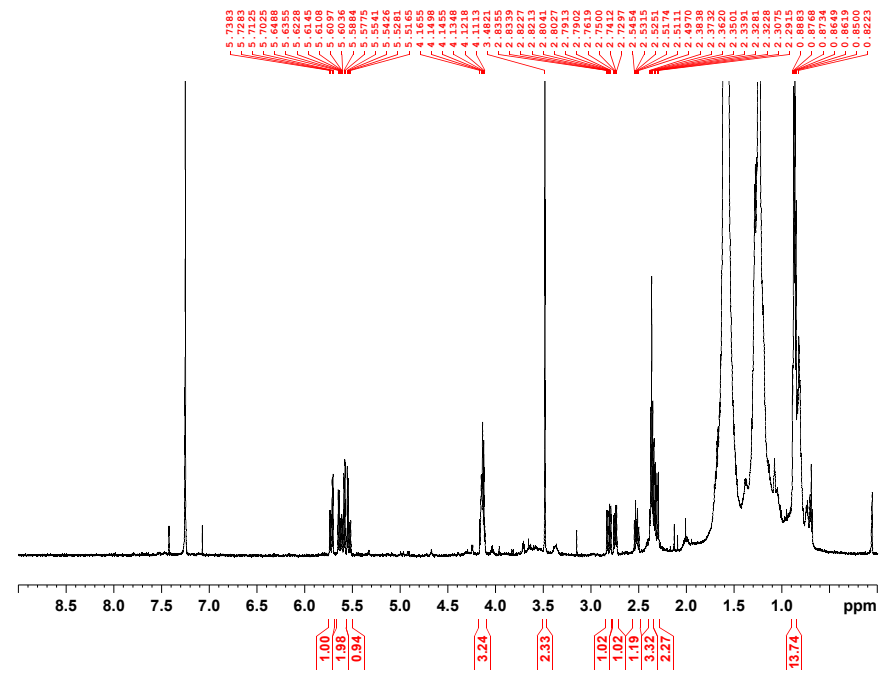


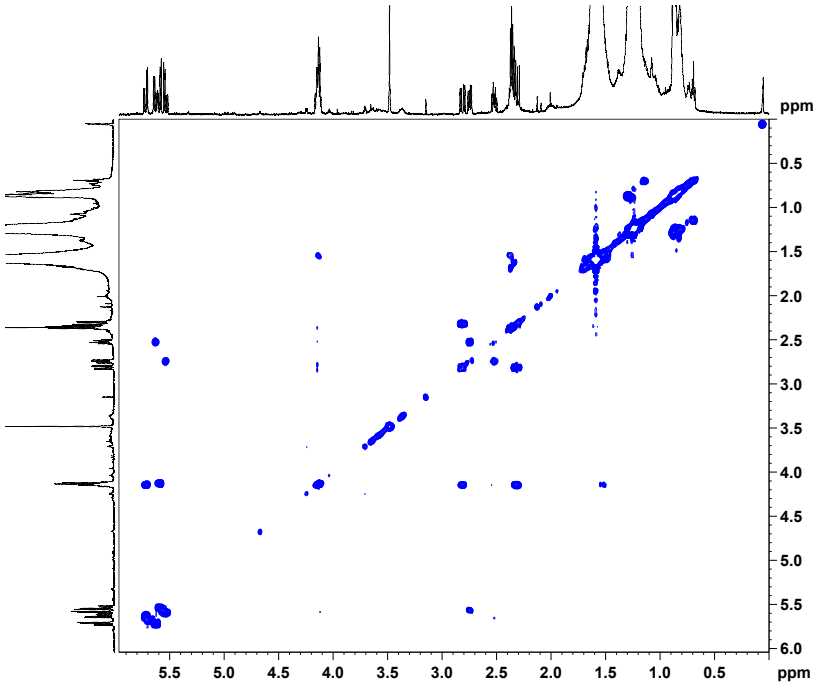
6-OH-PGD$_2$, product **7**, 600 MHz, CD_3CN





5*S*-OH-PGE$_2$, product **3**, 600 MHz, CDCl$_3$





5*R*-OH-PGE$_2$, product **8**, 600 MHz, $CDCl_3$