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Total Synthesis of 15-D_{2t}- and 15-*epi*-15-E_{2t}-Isoprostanes

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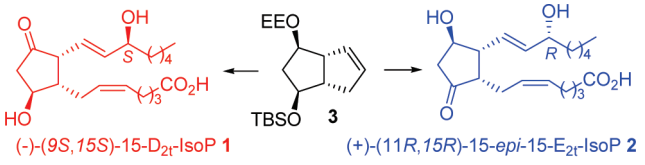
Received January 8, 2010



A bicyclic precursor makes all the difference

The first total synthesis of 15-D_{2t}-isoprostane is described. (−)-(9*S*,15*S*)-15-D_{2t}-IsoP **1** and (+)-(11*R*,15*R*)-15-*epi*-15-E_{2t}-IsoP **2** have been obtained in 15 steps from orthogonally protected enantiopure bicycle **3**. Key features include an easy introduction of the *cis* side chains via ozonolysis, a highly selective enzymatic chemical differentiation of a non-meso-1,5-diol, and the use of a common synthetic intermediate allowing a stereodivergent approach to the target molecules.

Isoprostanes,[1] isomeric to the enzymatically formed prostaglandins, bear their lateral side chains with a *cis* relationship on the prostane ring. They are formed in vivo as a racemic mixture by free radical nonenzymatic peroxidation of membrane-bound arachidonic acid (Figure 1). The pathway leads to five classes of isoprostanes (F-, D-, E-, A-, and J-types) that differ in the substitution pattern of the cyclopentane ring. Since their discovery in 1990 by Morrow[2] and co-workers, F-IsoPs that incorporate two hydroxyl groups in the 1,3 position on their ring system have been studied extensively. They are used as markers of oxidative stress,[3] show biological activity as vasoconstrictors, and have been shown to possess platelet aggregation properties and to act as smooth muscle growth factors.[4] D- and E-IsoPs possess one carbonyl and one hydroxyl group in the 1,3 position on the prostane ring. They are less stable than F-isoprostanes and may undergo further transformations by dehydration to the corresponding J and A derivatives.[5]

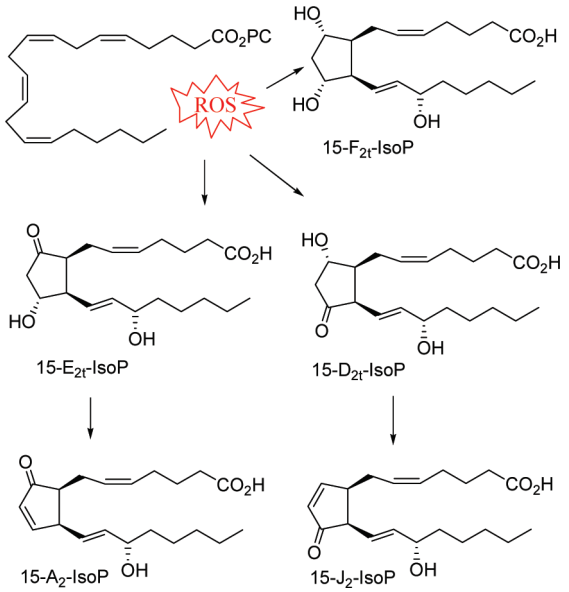


FIGURE 1. Different types of isoprostanes generated by reactive oxygen species (ROS) from membrane-bound AA: only the 15 series is represented here for clarity.

The biological activities of E-IsoPs have been examined to a limited extent.[6] Recent reports have shown that the E-type isoprostanes are potent vasoconstrictors at low nanomolar concentrations.[6a] The biological functions of D-IsoPs have

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DOI: 10.1021/jo1000274 Published on Web 03/10/2010

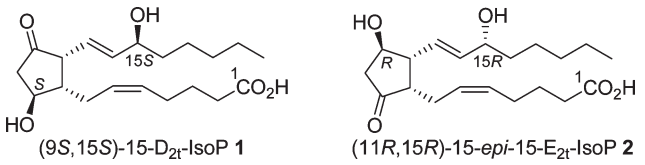
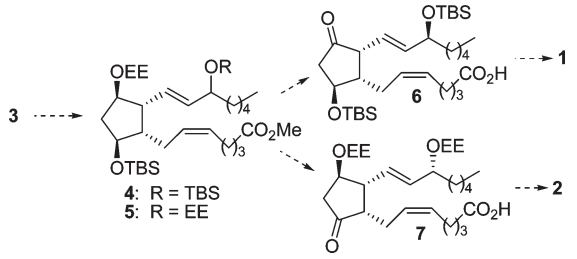


FIGURE 2. Structure of (−)-(9*S*,15*S*)-15-D_{2t}-IsoP **1** and (+)-(11*R*,15*R*)-15-*epi*-15-E_{2t}-IsoP **2**.

SCHEME 1. Access to 15-D_{2t}-IsoP 1 and to 15-*epi*-15-E_{2t}-IsoP 2



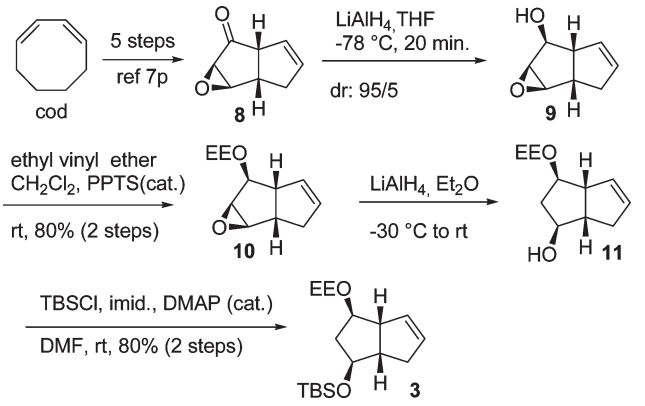
not yet been determined. For the evaluation of the roles of these metabolites, synthetic reference material is needed since isolation from natural sources is scarcely possible. Furthermore, detailed analytical investigation requires enantiopure synthetic compounds; therefore, the development of stereoselective total synthesis is of great interest. Whereas several total synthesis of the different types of isoprostanes has been accomplished,[1b,7] to date no total synthesis of D-IsoPs has been realized. Here we report the first total synthesis of (−)-(9*S*,15*S*)-15-D_{2t}-IsoP **1** as well as the total synthesis of (+)-(11*R*,15*R*)-15-*epi*-15-E_{2t}-IsoP **2** (Figure 2) generated from a common, late-stage synthetic intermediate.

We envisioned the total synthesis of 15-D_{2t}- and 15-*epi*-15-E_{2t}-isoprostanes from enantiopure bicycle **3** incorporating an orthogonally protected 1,3-diol functionality (Scheme 1). Ozonolysis of **3** would reveal the 1,2-*cis* stereochemistry necessary for side chain functionnalization via HWE and Wittig reactions. Selective removal of one protecting group of compounds **4** and **5**, ester hydrolysis, and oxidation of the free hydroxyl to the ketone should afford **6** and **7**, respectively. Final deprotection should then provide the target molecules **1** and **2**.

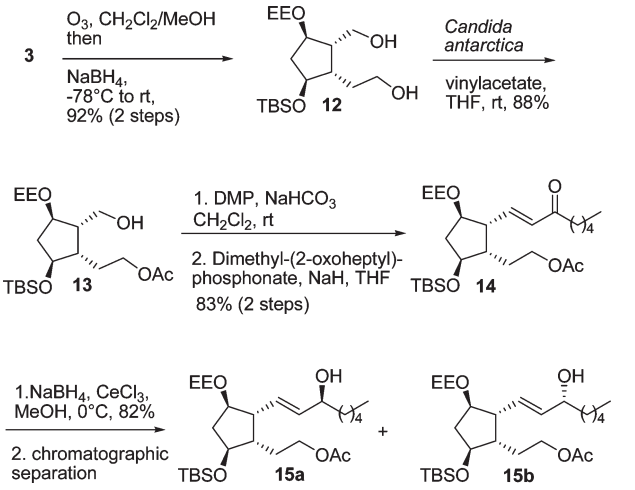
We recently developed a new synthetic strategy that permits the rapid access to different types of iso-, neuro-, and phytoprostanes. Following our previously published results,[7p] enantiopure bicyclic ketoepoxide **8** (>99% ee) was obtained from commercially available 1,3-cyclooctadiene in five steps on a multigram scale.

In order to access orthogonally protected bicycle **3**, which should serve as a common synthetic intermediate for the total synthesis of 15-D_{2t}- and 15-*epi*-15-E_{2t}-isoprostanes, the keto functionality of epoxyketone **8** was reduced chemoselectively by treatment of **8** with $LiAlH_4$ at low temperature and short reaction times ($LiAlH_4$ (2.3 equiv per hydride), THF, −78 °C, 20 min, dr 95:5) giving compound **9** (Scheme 2). The observed diastereoselectivities can be explained by the presence of the epoxide functionality that leads to an attack of the hydride source at the sterically less hindered back site of the ketone. We first protected the alcohol functionality of **9** as TBS and TPS ethers, but in both cases further reaction with $LiAlH_4$ to open the epoxide led to deprotection of the silyl ether.

SCHEME 2. Synthesis of Common Synthetic Precursor 3



SCHEME 3. Synthetic Pathway toward 15a and 15b



Taking into account these results, we next decided to introduce an ethoxyethyl ether protection (EE) (ethyl vinyl ether, CH_2Cl_2, PPTS cat., rt) affording **10**. Epoxide opening proceeded smoothly giving rise to the exclusive formation of **11**. Hydride attack occurred selectively at one of the two theoretically possible positions due to the bended structure of the bicycle. TBS protection of the resulting alcohol afforded orthogonally protected bicycle **3**.

Ozonolysis of **3** followed by $NaBH_4$ reduction gave diol **12** (Scheme 3). Chemical differentiation of the primary alcohols of **12** under classical protection conditions (TESCl (1 equiv) or Ac_2O (1 equiv)) failed to provide the regioisomerically pure monoprotected alcohol. Indeed, an approximately 1/1 mixture of isomers was obtained together with recovered starting material and bisprotected compound. We were pleased to find that high selectivity was achieved by using lipase B from *Candida antarctica* in the presence of vinyl acetate in THF leading to monoacetylated compound **13** (88%) together with bis-acetylated side product (<10%). The reaction proceeded with an unprecedented regioselectivity favoring acetylation of the alcohol attached to the longer side chain.[8] The first side-chain introduction was

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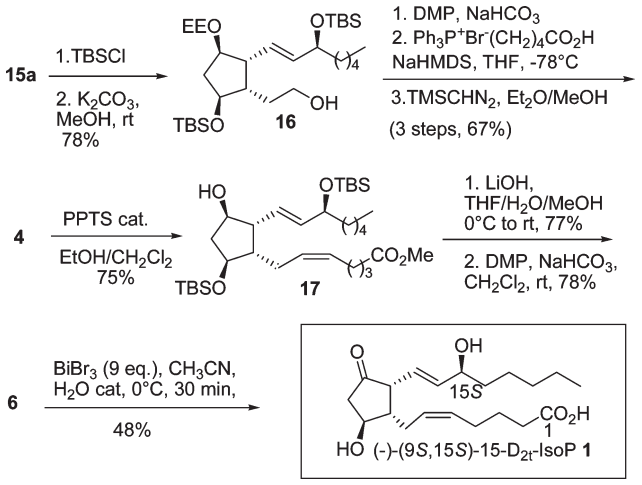
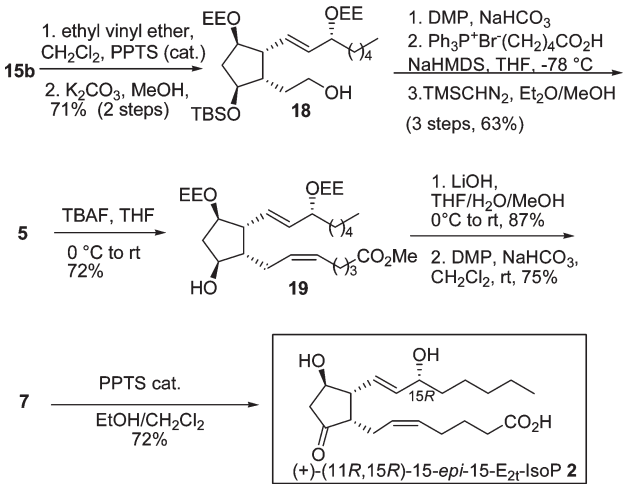
SCHEME 4. Total Synthesis of 15-D_{2t}-isoprostane



SCHEME 5. Total Synthesis of 15-*epi*-15-E_{2t}-isoprostane



carried out by DMP oxidation[9] of **13** and subsequent HWE reaction of the resulting aldehyde with the phosphoryl-stabilized anion of dimethyl (2-oxoheptyl)phosphonate. Enone **14** was obtained in 82% yield possessing the alkene functionality with the desired *trans* stereochemistry. Carbonyl reduction under Luche conditions afforded the corresponding allylic alcohols as an equimolar epimeric mixture. The epimers were separated by flash chromatography giving enantiopure **15a** and **15b**.

The relative configuration of the stereogenic center of **15a** and **15b** was determined by a variation[10a] of Mosher's method.[10b] Each epimer was esterified with *R*-(−)-α- and *S*-(+)-α-acetoxyphenylacetic acid. Analysis of ^{1}H NMR spectra allowed an unambiguous assignment of the configuration at the allylic stereocenter in each of the alcohols.

For the synthesis of 15-D_{2t}-IsoP, **15a** was protected as TBS ether and the acetate group was cleaved to produce alcohol **16** (Scheme 4). DMP oxidation and subsequent Wittig reaction of intermediate aldehyde with (4-carboxybutyl)triphenylphosphonium bromide in the presence of NaHMDS permitted the introduction of the second side chain. Wittig olefination proceeded selectively to produce exclusively the *Z*-olefin stereochemistry at the newly formed C5−C6 double bond. For ease of purification, the carboxylic acid functionality was esterified with $TMSCHN_2$ affording **4**. Selective removal of the ethoxyethyl ether protecting group was achieved by treatment of **4** with a catalytic amount of PPTS in $EtOH/CH_2Cl_2$ giving rise to **17** in 70% yield. Ester hydrolysis and subsequent oxidation of the free hydroxyl to the ketone afforded **6**. All that remained to access to (9*S*,15*S*)-15-D_{2t}-IsoP **1** was the removal of two TBS protecting groups;[11] however, this proved to be no simple task. Attempts to deprotect **6** by TBAF/AcOH, TASF, HF/pyr, HF aq, HCl (1 N, 0.1 N) under various conditions led to formation of undesired elimination side products, decomposition, or unreacted starting material. Deprotection was finally achieved by treatment of **6** with $BiBr_3$ (9 equiv, CH_3CN, H_2O cat., 0 °C, 30 min) leading to desired compound (−)-**1** (48% yield, >99%ee) and minor amounts of deoxygenated side products.[12]

For the synthesis of 15-*epi*-15-E_{2t}-IsoP, allylic alcohol **15b** was protected as EE, and the acetate group was cleaved to produce alcohol **18**. DMP oxidation, Wittig reaction, and subsequent esterification gave **5** (Scheme 5). Selective removal of the TBS protecting group was realized by reaction of **5** with TBAF (1.3 equiv, THF, 72%), affording **19**. Saponification of the methyl ester followed by DMP oxidation led to **7**. Final deprotection was easily achieved by treatment of **7** with a catalytic amount of PPTS in $EtOH/CH_2Cl_2$ giving rise to (+)-**2** (72% yield, >99% ee, white solid). Properties of (+)-(11*R*,15*R*)-15-*epi*-15-E_{2t}-IsoP **2** were identical to published analytical and spectroscopic data described for the enantiomer except for the sign of the optical rotation.[7m]

In summary, the first total synthesis of 15-D_{2t}-IsoP is described. This opens access to investigation of the not yet determined biological activities of the D-class isoprostanes.

(−)-(9*S*,15*S*)-15-D_{2t}-IsoP **1** and (+)-(11*R*,15*R*)-15-*epi*-15-E_{2t}-IsoP **2** have been synthesized in 15 steps from orthogonally protected diol **3**. Key features include an easy introduction of *cis* side chains via ozonolysis, a highly selective enzymatic chemical differentiation of a non-meso-1,5-diol, and the use of a common late-stage synthetic intermediate allowing a stereodivergent approach to the target molecules.

Experimental Section

(−)-(9*S*,15*S*)-15-D_{2t}-IsoP 1. Compound **6** (90 mg, 0.155 mmol, 1 equiv) was dissolved under nitrogen in 5 mL of CH_3CN. The solution was cooled to 0 °C, and $BiBr_3$ (400 mg, 0.89 mmol, 5.7 equiv) was added in one portion under stirring. Then H_2O (40 μL) was added in one portion. After 20 min at the same temperature another portion of $BiBr_3$ (226 mg, 0.50 mmol, 3.3 equiv) and H_2O (30 μL) was added. After 30 min, TLC analysis indicated the complete disappearance of the starting material. The reaction was quenched at 0 °C by dropwise addition of a saturated solution of $NaHCO_3$ until pH = 4 was reached. Layers were separated, and the aqueous one was extracted three

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(11) Since 15-D_{2t}-IsoP is extremely labile under basic conditions, it is absolutely necessary to carry out final cleavage of the protective groups under mild acidic conditions.

(12) Epimerization of (−)-(9*S*,15*S*)-15-D_{2t}-IsoP **1** leading to non-natural 9β-PGD_2 is unlikely because enolization of PGD_2 gives rise to α,β-unsaturated ketone isomers and/or unstable trienone (deoxygenated compounds).

times with an excess of AcOEt. The combined organic layers were washed with a saturated solution of NaCl, dried over $MgSO_4$, and filtered, and the solvent was evaporated under reduced pressure using a cold water bath. The crude was purified using demetalated silica gel (solvent: petroleum spirit/AcOEt 3:7, AcOEt−AcOEt/MeOH 9:1) to afford the desired **1** (26 mg, colorless oil, 48%) and elimination side products **1b** and **1c** (14 mg, light yellow-orange oil). **1**: R_f 0.64 (AcOEt/MeOH 9:1); $[\alpha]^{20}{}_D = -17.5$ (c 0.4 10^{-2}, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.66 (dd, 1H, J = 15.6 Hz, J = 6.5 Hz), 5.52 (m, 3H), 4.41 (m, 1H), 4.17 (m, 1H), 3.39 (t, 1H and 2 OHs, J = 7.8 Hz), 2.59 (dd, 1H, J = 19.1 Hz, J = 6.5 Hz), 2.30 (m, 5H), 2.11 (m, 2H), 1.80 (m, 1H), 1.67 (m, 2H), 1.52 (m, 2H), 1.29 (m, 6H and H_2O), 0.88 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.6, 176.7, 138.3, 131.0, 128.1, 123.7, 73.0, 71.0, 54.0, 50.0, 44.6, 37.0, 32.5, 31.7, 26.2, 25.9, 25.0, 24.3, 22.6, 14.0; IR ν_{max} (cm^{-1}) 3390, 2930, 2860, 1710,1404, 1189, 1013, 972; HRMS (ESI) calcd for $C_{20}H_{31}O_5$ $[M - H]^-$ 351.2171, found 351.2162.

(+)-(11*R*,15*R*)-15-*epi*-15-E_{2t}-IsoP 2. Compound **7** (61.8 mg, 0.125 mmol) was dissolved in EtOH (4.5 mL) and CH_2Cl_2 (0.9 mL) under nitrogen, and a catalytic amount of PPTS (10 mol %) was added in one portion. The resulting reaction mixture was stirred at room temperature for 1 h 30 min until complete disappearance of the starting material. Solid $NaHCO_3$ was added, and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography using demetalated silica gel (solvent: AcOEt/cyclohexane 8/2 → AcOEt) giving pure **2** (31.6 mg, white solid, 72%): R_f 0.50 (AcOEt/MeOH 9:1); $[\alpha]^{20}{}_D = +68$ (c 10^{-2}, MeOH); ^{1}H NMR (300 MHz, MeOD) δ 5.70 (dd, 1H, J = 15.5 Hz, J = 6.7 Hz), 5.42 (m, 3H), 4.31 (m, 1H), 4.06 (m, 1H), 3.06 (t, 1H, J = 8.8 Hz), 2.74 (m, 1H), 2.61 (dd, 1H, J = 18.9 Hz, J = 6.1 Hz), 2.48 (m, 1H), 2.34 (t, 2H, J = 7.1 Hz), 2.25−1.96 (m, 4H), 1.72 (m, 2H), 1.54 (m, 2H), 1.36 (m, 6H), 0.95 (m, 3H); ^{13}C NMR (75 MHz, MeOD) δ 219.5, 177.4, 138.5, 131.0, 129.0, 128.1, 73.3, 72.8, 52.4, 51.7, 45.5, 38.4, 34.4, 32.9, 27.8, 26.2, 26.0, 24.2, 23.7, 14.4.; IR ν_{max} (cm^{-1}) 3394, 2929, 2859, 1728, 1710, 1238, 971; HRMS (ESI) calcd for $C_{20}H_{31}O_5$ $[M - H]^-$ 351.2171, found 351.2169; mp 101.2 °C.

Acknowledgment. We gratefully acknowledge generous funding by Prof. Jean-Yves Lallemand (ICSN). We also thank University Montpellier 1 (Grant No. BQR-2009) for support. Y.B. thanks the Centre National de la Recherche Scientifique for postdoctoral fellowships. We thank Prof. Rita S. Majerle (University of Minnesota) for proofreading.

Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.