5-F_{2t}-Isoprostane, A Human Hormone?

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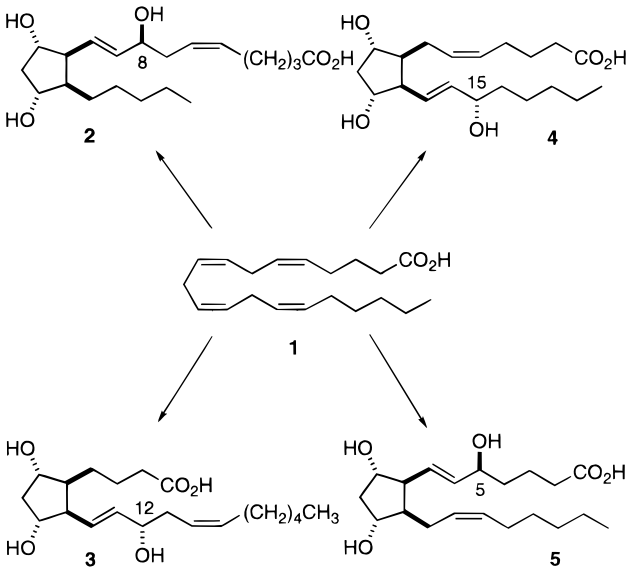
Abstract: Syntheses of the four enantiomerically pure diastereomers of 5-F_{2t}-isoprostane (**5**−**8**) are described. The key step is the lipase-catalyzed chemo-enzymatic resolution of the racemic diol **40** to give the monoacetates **41** and **42**. The enantiomerically pure diastereomers of 5-F_{2t}-isoprostane (**5**) may be human hormones.

Introduction

In 1990, prostaglandin (PG) F_2-like compounds were discovered to be produced in abundance in vivo by free radical-induced peroxidation of arachidonic acid (**1**), independent of the cyclooxygenase enzymes.[1] Because these compounds are isomeric to the $PGF_{2\alpha}$ derived by the action of cyclooxygenase, they were named F_2-isoprostanes.[2] Subsequently, it was demonstrated that D_2-isoprostanes and E_2-isoprostanes are also produced in vivo as products of this pathway.[3] Four different regioisomers of each of these classes of isoprostanes are formed (e.g., 8-F_{2t}-isoprostane **2**, 12-F_{2t}-isoprostane **3**, 15-F_{2t}-isoprostane **4**, 5-F_{2t}-isoprostane **5**). 15-F_{2t}-Isoprostane (**4**), prepared by total synthesis,[4−11] has been shown to have hormonal activity, with a receptor in the kidney vasculature.[12] To investigate the biological activity[4] of the other isoprostanes, it will be necessary to devise synthetic routes to them. We report herein the first preparation of each of the four enantiomerically pure isomers of 5-F_{2t}-isoprostane (**5**−**8**).



Results and Discussion

The 5-isoprostanes are produced in vivo as racemic mixtures of C-5 diastereomers. Rather than design an independent synthesis of each of the four enantiomerically pure diastereomers of a particular isoprostane, it seemed more sensible to develop a stereodivergent synthesis that would lead to each of the four from a common intermediate.

We proposed (Scheme 1) to prepare 5-F_{2t}-isoprostane (**5**), 5-epi-5-F_{2t}-isoprostane (**6**), *ent*-5-F_{2t}-isoprostane (**7**), and 5-epi-*ent*-5-F_{2t}-isoprostane (**8**) by aldol condensation of the diazoketone **9** with the aldehyde **10**, followed by cyclization of the silyloxy ketone **11**. The relative configuration of the alkyl side chains on the ring would then be established by kinetic opening of the activated cyclopropane of the bicyclic ketone **12** with thiophenol and $BF_3{\cdot}OEt_2$. A key question was whether the cyclization of the diazoketone **11**, having a trans,cis conjugated side chain, would give the desired bicyclic ketone **12** efficiently.

(1) Morrow, J. D.; Hill, K. E.; Burk, R. F.; Nammour, T. M.; Badr, K. F.; Roberts, L. J., II. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9383.

(2) (a) For a summary of isoprostane nomenclature, see Taber, D. F.; Morrow, J. D.; Roberts, L. J., II. *Prostaglandins* **1997**, *53*, 63. (b) For an alternative nomenclature system for the isoprostanes, see Rokach, J.; Khanapure, S. P.; Hwang, S. W.; Adiyaman, M.; Lawson, J. A.; FitzGerald, G. A. *Prostaglandins* **1997**, *54*, 853.

(3) Morrow, J. D.; Minton, T. A.; Mukundan, C. R.; Campbell, M. D.; Zackert, W. E.; Daniel, V. C.; Badr, K. F.; Blair, I. A.; Roberts, L. J., II. *J. Biol. Chem.* **1994**, *269*, 4317.

(4) Morrow, J. D.; Roberts, L. J., II. *Biochem. Pharmacol.* **1996**, *51*, 1.

(5) For synthetic routes to 15-F_{2t}-isoprostane: (a) Corey, E. J.; Shih, C.; Shih. N.-Y.; Shimoji, K. *Tetrahedron Lett.* **1984**, *25*, 5013. (b) Hwang, S. W.; Adiyaman, M.; Khanapure, S.; Schio, L.; Rokach, J. *J. Am. Chem. Soc.* **1994**, *116*, 10829. (c) Taber, D. F.; Herr, R. J.; Gleave, D. M. *J. Org. Chem.* **1997**, *62*, 194. (d) Taber, D. F.; Kanai, K. *Tetrahedron* **1998**, *54*, 11767.

(6) For synthetic routes to 15-F_{2c}-isoprostane: (a) Larock, R. C.; Lee, N. H. *J. Am. Chem. Soc.* **1991**, *113*, 7815. (b) Vionnet, J.-P.; Renaud, P. *Helv. Chim. Acta* **1994**, *77*, 1781. (c) Hwang, S. W.; Adiyaman, M.; Khanapure, S. P.; Rokach, J. *Tetrahedron Lett.* **1996**, *37*, 779.

(7) (a) For a synthetic route to 15-E_{2t}-isoprostane: Taber, D. F.; Hoerrner, R. S. *J. Org. Chem.* **1992**, *57*, 441. (b) See also ref 5d.

(8) For a synthetic route to 5-F_{2t}-isoprostane: Adiyaman, M.; Lawson, J. A.; Hwang, S.-W.; Khanapure, S. P.; FitzGerald, G. A.; Rokach, J. *Tetrahedron Lett.* **1996**, *37*, 4849.

(9) For a synthetic route to 5-F_{2c}-isoprostane: Adiyaman, M.; Lawson, J. A.; FitzGerald, G. A.; Rokach, J. *Tetrahedron Lett.* **1998**, *39*, 7039.

(10) For a synthetic route to 8-F_{2t}-isoprostane: Adiyaman, M.; Li, H.; Lawason, J. A.; Hwang, S.-W.; Khanapure, S. P.; FitzGerald, G. A.; Rokach, J. *Tetrahedron Lett.* **1997**, *38*, 3339.

(11) For a synthetic route to 12-F_{2t}-isoprostane: Pudukulathan, Z.; Manna, S.; Hwang, S.-W.; Khanapure, S. P.; Lawson, J. A.; FitzGerald, G. A.; Rokach, J. *J. Am. Chem. Soc.* **1998**, *120*, 11953.

(12) (a) Morrow, J. D.; Minton, T. A.; Roberts, L. J., II. *Prostaglandins* **1992**, *44*, 155. (b) Fukunaga, M.; Makita, N.; Roberts, L. J., II.; Morrow, J. D.; Takahashi, K.; Badr, K. F. *Am. J. Physiol (Cell Physiol. 33)* **1993**, *264*, C1619. (c) Takahashi, K.; Nammour, T. M.; Fukunaga, M.; Ebert, J.; Morrow, J. D.; Roberts, L. J., II; Hoover, R. L.; Badr, F. K. *J. Clin. Invest.* **1992**, *990*, 136. (d) Longmire, A. W.; Roberts, L. J., II.; Morrow, J. D. *Prostaglandins* **1994**, *48*, 247. (e) Fukunaga, M.; Takahashi, K.; Badr, K. F. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 507.

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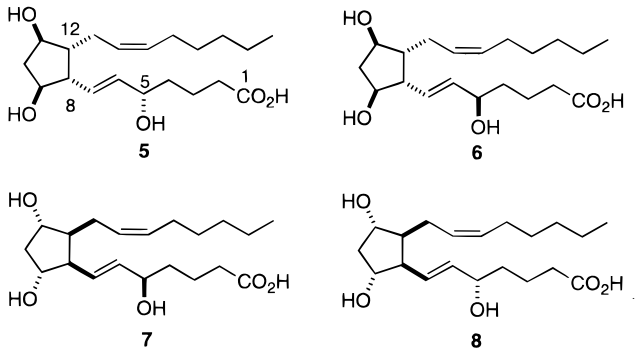
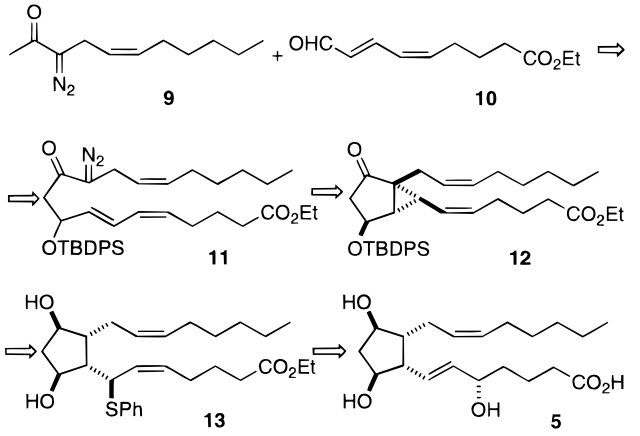


Scheme 1

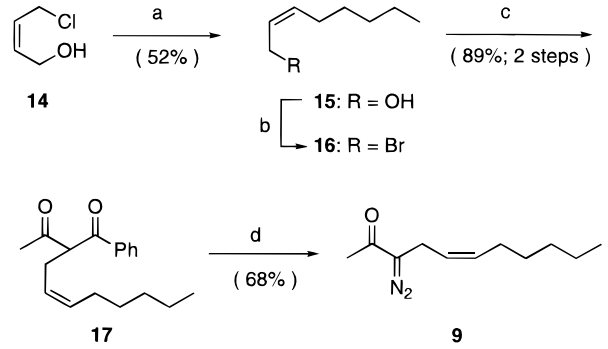


The diazoketone **9** was prepared (Scheme 2) by homologation[13] of the chloro alcohol **14** with butylmagnesium bromide, to give the alcohol **15**. Alkylation of benzoylacetone with the derived bromide **16** gave the diketone **17**. Diketone **17** was smoothly converted to the diazoketone **9** on exposure to *p*-nitrobenzenesulfonyl azide (*p*-NBSA) and DBU.[14]

The requisite (*Z*,*E*)-conjugated dienal **10** (Scheme 3) was prepared by a modification of the method of Kobayashi.[15] Wittig reaction of the phosphonium salt **18** and ethyl 5-oxopentanoate[16] *with one molar equivalent of KHMDS* proceeded smoothly to give dienol **19** (*Z*,*E*/*E*,*E* = >95:<5). Oxidation of **19** with MnO_2 furnished the dienal **10**.[17]

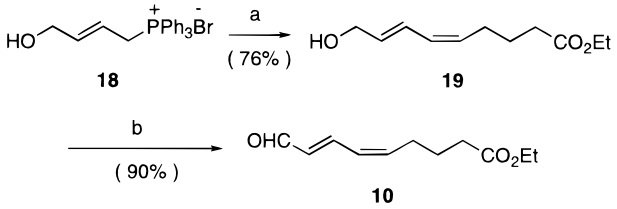
To be sure of the geometry of **19**, we also prepared the isomeric dienols **20**−**22** (Table 1). Dienol **20** was prepared by oxidation of **19** with PCC followed by reduction with $NaBH_4$. A inseparable mixture of **21** and **22** was prepared by condensation of the phosphonium salt derived from **14** with ethyl 4-oxopentanoate. The geometry of the conjugated dienes was easily assigned by comparison of the ^{13}C NMR chemical shifts of the C-4 and C-9 methylenes.[18] The ^{13}C chemical shift of the C-4 methylenes of compounds **20** and **22**, having an (*E*)-double bond next to the C-4 methylene, are downfield compared to the (*Z*)-isomers **19** and **21**. The ^{13}C chemical shifts for the C-9 methylenes of compounds

Scheme 2[a]



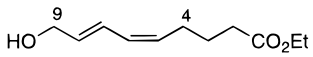
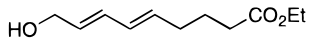
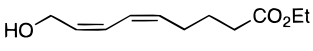
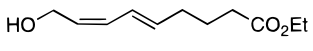
[a] Reagents and conditions: (a) C_4H_9MgBr, Et_2O, 0 °C ∼ rt; (b) CBr_4, Ph_3P, CH_2Cl_2, 0 °C ∼rt; (c) benzoylacetone, K_2CO_3, $^nBu_4NBr$, toluene, 90 → 40 °C; (d) p-NBSA, DBU, CH_2Cl_2, 0 °C.

Scheme 3[a]



[a] Reagents and conditions: (a) KHMDS; ethyl 5-oxopentanoate, THF, −78 ∼0 °C; (b) MnO_2, CH_2Cl_2, rt.

Table 1. ^{13}C Chemical Shifts of C-4 and C-9 Methylenes

Compounds	Chemical Shift[a] (ppm) C-4	C-9
 **19**	26.8	63.0
 **20**	31.8	61.9
 **21**	26.5	58.3
 **22**	31.9	58.4

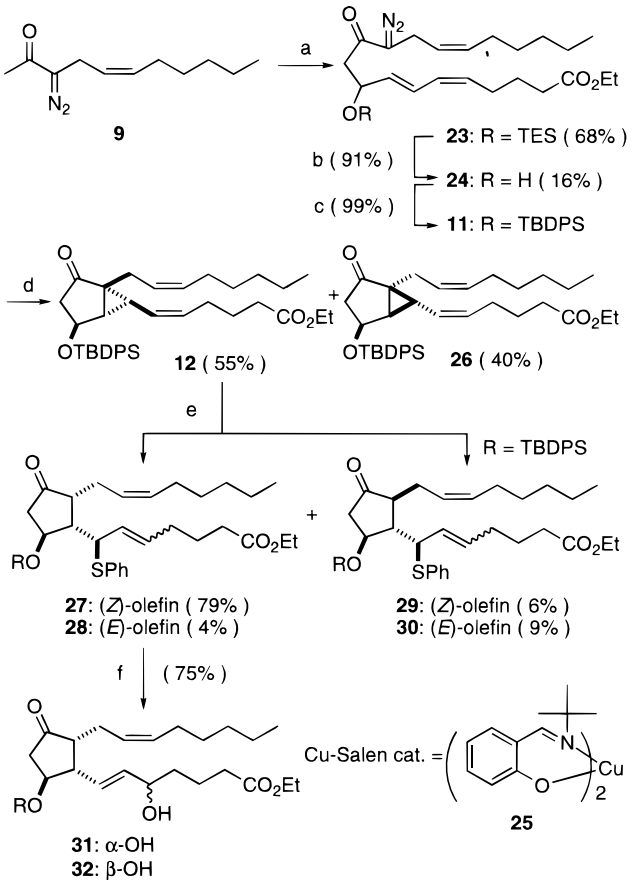
[a] In $CDCl_3$.

19 and **20**, having an (*E*)-double bond next to the C-9 methylene, are also downfield compared to the (*Z*)-isomers **21** and **22**.

With the requisite components **9** and **10** in hand, we embarked on the preparation of the four enantiomerically pure diastereomers of 5-F_{2t}-isoprostane (Scheme 4). Aldol condensation[5d] of the potassium enolate of the diazoketone **9** with the dienal **10** in the presence of triethylchlorosilane (TESCl) in toluene gave the TES-protected aldol **23** together with a small amount of the free aldol **24** (hydrolysis on work up) in good yield. The TES group of **23** does not survive under the conditions for cyclopropane ring opening with thiophenol and $BF_3{\cdot}OEt_2$, so it was necessary to change the protecting group from TES to *tert*-butyldiphenylsilyl (TBDPS). The diazoketone **11** was then

(13) Taber, D. F.; Louey, J. P. *Tetrahedron* **1995**, *51*, 4495.
(14) Taber, D. F.; Gleave, D. M.; Herr, R. J.; Moody, K.; Hennessy, M. J. *J. Org. Chem.* **1995**, *60*, 2283.
(15) Hosoda, A.; Taguchi, T.; Kobayashi, Y. *Tetrahedron Lett.* **1987**, *28*, 65.
(16) Ethyl 5-oxopentanoate was prepared by ethanolysis of δ-valerolactone followed by PCC oxidation. For full characterization, see Penn, J. H.; Liu, F. *J. Org. Chem.* **1994**, *59*, 2608.
(17) For a complementary approach to the *methyl* esters of **19** and **10**, see Pohnert, G.; Boland, W. *Tetrahedron* **1996**, *52*, 10073.
(18) For a detailed discussion of the ^{13}C chemical shifts of allylic methylenes, see Taber, D. F.; You, K. *J. Org. Chem.* **1995**, *60*, 139 and references therein.

Scheme 4[a]



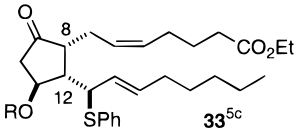
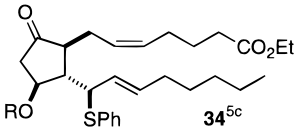
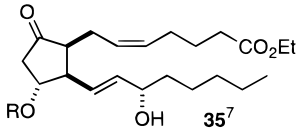
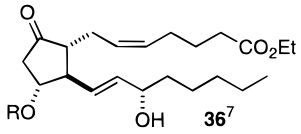
[a] Reagents and conditions: (a) KHMDS, toluene, −78 °C; **10**, TESCl, toluene, −78 °C; (b) TBAF, NH_4Cl (solid), THF, 0 °C; (c) TBDPSCl, imidazole, 4-DMAP, CH_2Cl_2, rt; (d) Cu-Salen cat., toluene, 100 °C; (e) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −30 °C; (f) mCPBA, CH_2Cl_2, −78 °C; $(MeO)_3P$, EtOH, −78 °C ∼rt.

cyclized[19] with the Cu−Salen catalyst **25**[20] in toluene to provide the bicyclic ketones **12** and **26**.

The structures of the bicyclic ketones **12** and **26** were assigned by comparing the ^1H and ^{13}C NMR spectra to those for the analogous bicyclic ketones that are intermediates in the synthesis of the 15-F_{2t}-isoprostanes.[7] In particular, the oxygenated methine of **12** (^{13}C δ 69.3, ^1H δ 4.46, d, J = 4.9 Hz) is almost exactly congruent with the analogous 15-F_{2t}-isoprostane precursor **12** (^{13}C δ 69.6, ^1H δ 4.41, d), while the oxygenated methine of **26** (^{13}C δ 68.0, ^1H δ 4.59, dt, J = 5.1, 7.8 Hz) is quite different.

Difficulties were initially encountered in the kinetic cyclopropane ring opening of **12**. We eventually found that treatment of **12** with an excess of thiophenol and $BF_3{\cdot}OEt_2$ in CH_2Cl_2 (0.4 M concentration) at −30 °C for 4 h gave the desired thioether **27** in 79% yield, accompanied by three other isomers (**28**, **29**, and **30**). ^{13}C NMR has proven to be an effective tool for the assignment of the relative configuration of the isoprostanes (Table 2).[21] The ring methines (C-8 and C-12) are particularly distinctive. Ketones **28** and **30** could easily be assigned on the basis of these correlations. We confirmed that the side chains of ketone **27** were cis one to another by converting both **27** and **28** to the allylic alcohols **31** and **32**. On the basis of the data in Table 2,[21] both **31** and **32** have side chains cis to each other on the ring. These observations support the structures of **27**, **28**, **29**, and **30** depicted in Scheme 4. In each case, we have assumed that the opening with thiophenol proceeded with inversion at the reacting center, as we have previously observed.[7]

Table 2. ^{13}C Chemical Shifts of C-8 and C-12 Methines

Compounds[a]	Chemical Shifts (ppm)[b]	
27	45.9	50.4
28	50.5	51.2
29	48.8	50.2
30	50.3	53.1
31	50.6	51.3
32	50.7	51.4
 **33**[5c]	50.3	51.4
 **34**[5c]	50.0	53.0
 **35**[7]	50.6	51.6
 **36**[7]	53.4	54.0
R = TBDPS		

[a] References. [b] In $CDCl_3$.

Reduction of the ketone **27** produced the epimeric alcohols **37** and **38** (Scheme 5). Again, the relative configurations of **37** (^1H NMR δ 4.74, dt, J = 2.6, 6.2 Hz, 1H; 4.29, m, 1H) and **38** (^1H NMR δ 4.44, dt, J = 3.6, 6.6 Hz, 1H; 4.08, m, 1H) were assigned by analogy to the chemical shifts of the H's at C-9 and C-11 in the corresponding diastereomers of the 15-isoprostane precursors (^1H NMR δ 4.64, m, 1H; 4.24, m, 1H) and (^1H NMR δ 4.38, m, 1H; 3.99, m, 1H).[5c,d] The undesired alcohol **37** was recycled by oxidation with Dess−Martin periodinane,[22] followed by reduction with $NaBH_4$. Desilylation of the β-alcohol **38** with TBAF in THF afforded the diol **13**.

At first, we attempted the chemo-enzymatic resolution[23] at this stage. Unfortunately, under the conditions we screened, the diol **13** was not resolved efficiently. We reasoned that the *Z*-side chain might be presenting too much steric bulk to be accommodated easily in the binding site of the lipase. We therefore

(19) With $Rh_2(OAc)_4$, $Rh_2(oct)_4$, and $Rh_2(piv)_4$, the unstable tetraene resulting from β-hydride elimination was the major product.

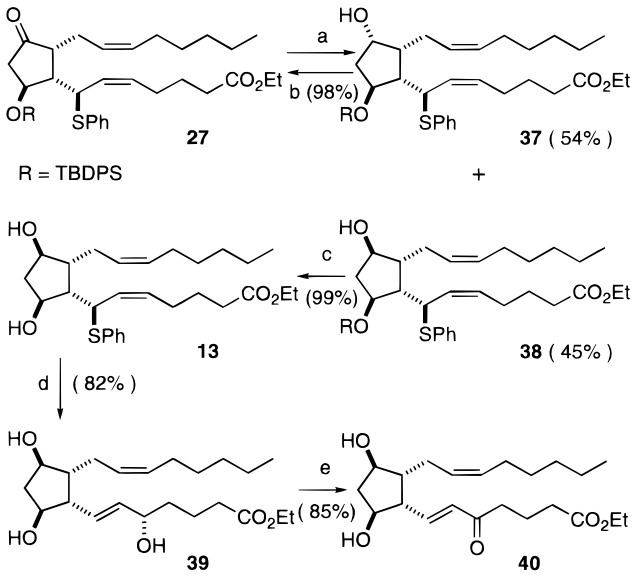
(20) (a) Charles, R. G. *J. Org. Chem.* **1957**, *22*, 677. (b) Sacconi, L.; Ciampolini, M. *J. Chem. Soc.* **1964**, 276. (c) Corey, E. J.; Myers, A. G. *Tetrahedron Lett.* **1984**, *25*, 3559.

(21) Taber, D. F.; Kanai, K. *J. Org. Chem.* **1998**, *63*, 6607.

(22) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

(23) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon Press: Oxford, U.K., 1994; and references therein.

Scheme 5[a]



[a] Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C; (b) Dess-Martin periodinane, CH_2Cl_2, rt; (c) TBAF, THF, rt; (d) mCPBA, CH_2Cl_2, −78 °C; $(MeO)_3P$, EtOH, −78 °C ∼rt; (e) DDQ, 1,4-dioxane − CH_2Cl_2, 40 °C.

decided to change the substrate of the enzyme reaction. To this end, we effected oxidation and Mislow rearrangement[24] of the thioether **13** to give the allylic alcohol **39**, which on further treatment with DDQ[5a,5d,25] in 1,4-dioxane-CH_2Cl_2 (1:1) afforded the enone **40**.

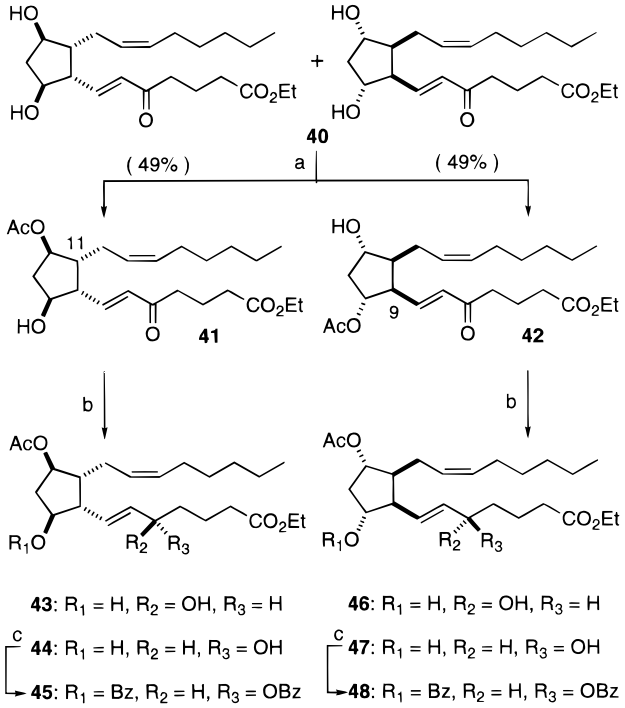
After some exploration, we found (Scheme 6) that the pseudo-meso enone **40** was effectively resolved by Amano lipase AK in neat vinyl acetate, to furnish the monoacetates **41** and **42**.

Reduction of the enantiomerically enriched enone **41** with $NaBH_4$ gave the alcohols **43** and **44**, which were separable by TLC. Racemic **44** and **47** were prepared from **13** (Scheme 5) by acetylation followed by oxidation and Mislow rearrangement. After separation of **43** and **44** by silica gel chromatography, alcohol **44** was converted to the dibenzoate **45**. The dibenzoate **48** was also prepared in the same way. The ee's of the dibenzoates **45** and **48** were determined to be >98% and 91%, respectively, by chiral HPLC analysis.[26]

To investigate the biological activity of the isoprostanes, it is necessary to prepare each of these in high enantiomeric excess. Therefore, the enantiomerically enriched acetate **42** (only 91% ee), after conversion (Scheme 7) to the diol **40a** by reduction of the enone, hydrolysis of the acetyl group, and oxidation of the allylic alcohol with DDQ,[5a,5d,25] was again subjected to the enzymatic resolution[23] to give the monoacetate **42a**. Reduction of the enone **42a** with $NaBH_4$ gave the epimeric alcohols **46a** and **47a**. Diol **47a** was converted to the dibenzoate **48a**, the ee of which was determined to be >99% by chiral HPLC analysis.[26]

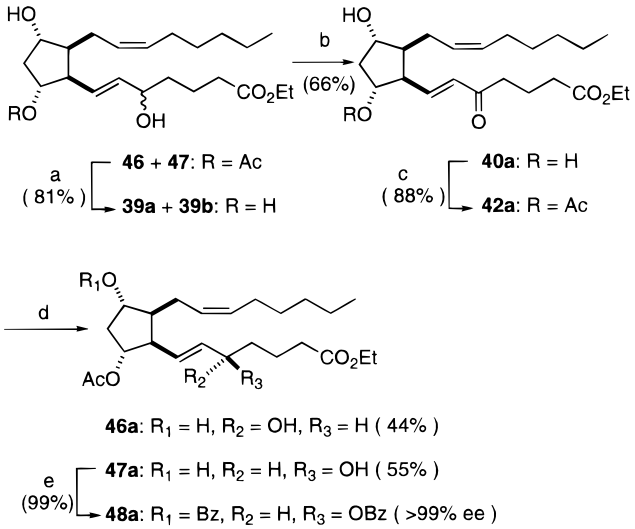
The absolute configuration of the enzymatically resolved compounds was determined by comparison of the chiral HPLC retention time of the dibenzoate **45** with that of the structually

Scheme 6[a]



[a] Reagents and conditions: (a) Amano lipase AK, vinyl acetate, rt; (b) $NaBH_4$, MeOH, 0 °C, (**43**, 37%, **44**, 44%; **46**, 42%, **47**, 51%); (c) BzCl, Et_3N, 4-DMAP, CH_2Cl_2 (**45**, 89%, >98% ee; **48**, 99%, 91% ee).

Scheme 7[a]



[a] Reagents and conditions: (a) K_2CO_3, EtOAc, MS 4A, EtOH, 50 °C; (b) DDQ, 1,4-dioxane − CH_2Cl_2, 40 °C; (c) amano lipase AK, vinyl acetate, rt; (d) $NaBH_4$, MeOH, 0 °C; (e) BzCl, Et_3N, 4-DMAP, CH_2Cl_2, rt.

defined dibenzoate **45b** (Scheme 8). This dibenzoate **45b** was prepared by acetylation of the racemic alcohol **38** to give the acetate **49**, which was then subjected to oxidation and Mislow rearrangement[24] to give the alcohol **50**. After oxidation of the alcohol with Dess−Martin periodinane,[22] the enone **51** was reduced enantioselectively with (*S*)-BINAL-H under conditions already reported,[5d,6a,6c,8,9,10,11,27] to give the alcohols **52** and **53** as an inseparable mixture. Desilylation followed by acid treatment gave the diols **44b** and **46b**, which were separated. Diol **44b** was converted to the dibenzoate **45b**, the ee of which

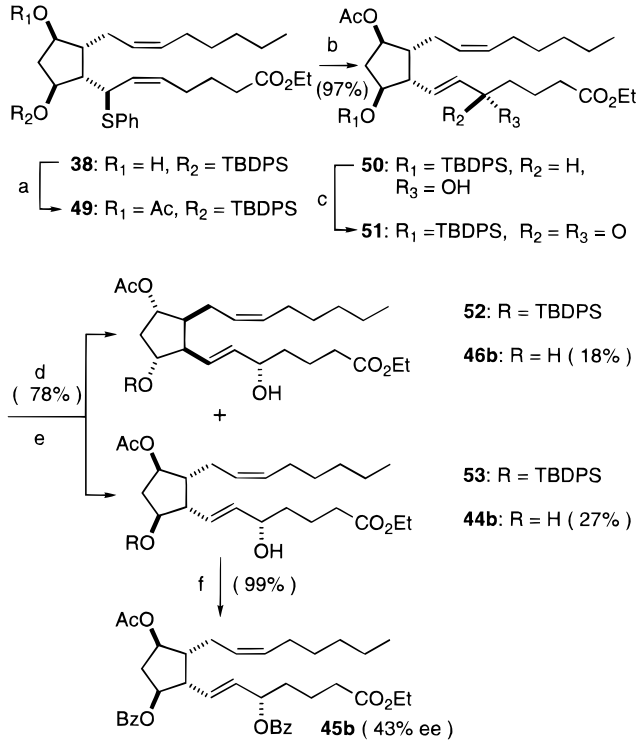
(24) (a) Bickart, P.; Carson, F. W.; Jacobus, J.; Miller, E. J.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4869. (b) Tang, R.; Mislow, K. *J. Am. Chem. Soc.* **1970**, *92*, 2100.

(25) (a) Becker, H.-D.; Björk, A.; Alder, E. *J. Org. Chem.* **1980**, *45*, 1596. (b) Tsubuki, M.; Kanai, K.; Keino, K.; Kakinuma, N.; Honda, T. *J. Org. Chem.* **1992**, *57*, 2930.

(26) The ee's were determined by HPLC analyses with a CHIRALCEL OD column (Daicel Chemical Industries Ltd.): detector, UV (254 nm); flow rate, 1 mL/min; mobile phase, hexane/2-PrOH = 95/5 for **45** and **45b**, hexane/2-PrOH = 99/1 for **48** and **48a**.

(27) Noyori, R.; Tomino, I.; Tanimoto, Y.; Nishizawa, M. *J. Am. Chem. Soc.* **1984**, *106*, 6709.

Scheme 8[a]

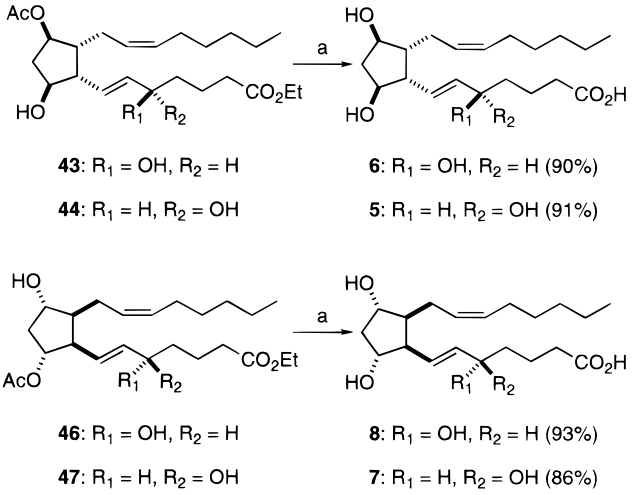


[a] Reagents and conditions: (a) Ac_2O, Py, 4-DMAP, CH_2Cl_2, rt, (99%); (b) mCPBA, CH_2Cl_2, −78 °C; $(MeO)_3P$, EtOH, −78 °C ∼rt; (c) Dess-Martin periodinane, rt, (84%); (d) (*S*)-BINAL-H, THF, −78 °C; (e) TBAF, THF, rt; cat. H_2SO_4, EtOH, rt; (f) BzCl, Et_3N, 4-DMAP, CH_2Cl_2, rt.

was determined to be 43% by chiral HPLC.[26] The major enantiomer of the dibenzoate **45b** had a retention time of 10.8 min, and its minor enantiomer had a retention time of 8.0 min. The dibenzoate **45**, derived by the enzymatic resolution, had a retention time of 10.8 min. Thus, we established the absolute configurations of the enzymatically resolved acetates **41** and **42** to be as shown in Scheme 6. This result is consistent both with our previous observations[5d] and with those of others.[23]

The acetates **43**, **44**, **46**, and **47** were separately hydrolyzed (Scheme 9) with LiOH in THF−H_2O (1:1) to furnish 5-*epi*-F_{2t}-isoprostane (**6**), 5-F_{2t}-isoprostane (**5**), *ent*-5-*epi*-F_{2t}-isoprostane (**8**), and *ent*-5-F_{2t}-isoprostane (**7**).

Scheme 9[a]



[a] Reagents and conditions: (a) LiOH·H_2O, THF − H_2O (1:1), rt.

Conclusion

We have developed a practical synthesis of the potential human hormones **5**−**8** using the chemo-enzymatic resolution of the pseudo-meso diol **40** as a key step. We have also established what should be a general strategy for the assignment of relative configurations in this series. This synthesis will make **5**−**8** available in sufficient quantity to allow the detailed assessment of their physiological activity.

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

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