Synthesis of the Eight Enantiomerically Pure Diastereomers of the 12-F₂-isoprostanes

Douglass F. Taber,* Ming Xu, and John C. Hartnett†

Contribution from the Department of Chemistry and Biochemistry, University of Delaware,
Newark, Delaware 19716

Received June 7, 2002

Abstract: Syntheses of the eight enantiomerically pure diastereomers of the 12-F₂-isoprostanes (4–11) are described. The key steps included rhodium-mediated intramolecular cyclopropanation and enzymatic resolution of the racemic diol 12.

Introduction

The isoprostanes (e.g. 1–4), a new family of prostaglandin-like compounds, were recently discovered to be produced in vivo in humans, independent of the cyclooxygenase enzymes, by free radical mediated oxidation of membrane-bound arachidonic acid. There are D-ring, E-ring, and F-ring isoprostanes. By free radical mediated oxidation of membrane-bound arachidonic acid, there are D-ring, E-ring, and F-ring isoprostanes. Four different regiosomers of each of these classes of isoprostanes are formed. Interestingly, levels of F₂-isoprostanes (1–4) in normal human biological fluids exceed levels of prostaglandins. Several synthesis routes to particular isoprostanes have been reported. Since we are interested in the physiological activity of each of the isoprostanes, we thought it more attractive to prepare, through a common advanced intermediate, the several diastereomers of an isoprostane family. Herein, we report the preparation of all eight of the enantiomerically pure diastereomers of the 12-F₂-isoprostanes (4–11) from the racemic diol 12 (Scheme 1). This is the first preparation of the 12-F₂ series.

Results and Discussion

The 12-F₂-isoprostanes were first identified in human plasma samples by Roberts. To screen the physiological activity of the eight enantiomerically pure diastereomers of the 12-F₂-isoprostanes, it will be necessary to individually prepare each of them. One synthesis, specifically of 12-F₂-isoprostane 4, was reported by Rokach in 1998. It seemed more attractive to develop a stereodivergent synthesis scheme toward these targets from a common intermediate, rather than design different syntheses of each. We envisioned (Scheme 1) that the eight target molecules could be prepared from the same racemic intermediate 12. The two enantiomers of 12, which could be obtained by enzymatic resolution, could each be converted to two of the four

(* To whom correspondence should be addressed. E-mail taberdf@udel.edu.)
enantiomerically pure diastereomers 4–7. Mitsunobu inversion\(^\text{13}\) of the enantiomers of 12 would lead to the four enantiomerically pure cis isomers 8–11.

Pursuing the retrosynthetic analysis (Scheme 2), the key diol 12 could be generated by kinetic opening of the activated bicyclic ketone 13 with thiophenol and BF\(_3\)\(\cdot\)OEt\(_2\). The bicyclic ketone 13 could be constructed by rhodium-mediated cyclopropanation of the diazoketone 14. The aldol condensation of diazoketone 15 and aldehyde 16 would provide the desired diazoketone 14.

The aldehyde 16, a natural odorant,\(^\text{14}\) was prepared from the commercially available 1,2,4-butanetriol 17 (Scheme 3). On exposure to acetone, 17 was converted to 18a and 18b (ratio 18a/18b = 3:1) as a mixture, which was further transferred to the mixture of p-toluenesulfonates 19a and 19b. These were tedious to separate by column chromatography, especially on a large scale. Fortunately, on exposure to NaI in acetone, both 19a and 19b were converted smoothly to 20. We also found that the ratio of 18a to 18b increased when the mixture was stored at 5 °C for several weeks. Using this approach, substantial quantities of the valuable phosphonium salt 21\(^\text{15}\) could be conveniently prepared.

Wittig coupling of 21 with hexanal resulted in the cis-alkene 22, which was hydrolyzed with 80% aqueous acetic acid to

provide the diol 23. Expecting the $\beta,\gamma$-unsaturated aldehyde 24 to be unstable, we carried the crude product from the Vo-Quang periodate cleavage 16 directly into the modified Wittig reaction, 17 to afford the trienol 25. Oxidation of the trienol 25 with the Dess–Martin periodinane 18 provided the aldehyde.

The diazoketone 1519 (Scheme 4) was prepared from the commercially available diketone 26 following the procedure we


**Scheme 7**

(-)-35 \[\rightarrow\] AcO \[\rightarrow\] AcO \[\rightarrow\] SPh (+)-40

\[\rightarrow\] 88%

\[\rightarrow\] 99%

\[\rightarrow\] b
d (90%)

\[\rightarrow\] d (90%)

\[\rightarrow\] d (91%)

\[\rightarrow\] d (89%)

\[\rightarrow\] c

\[\rightarrow\] c

\[\rightarrow\] a

**Scheme 8**

(-)-36 \[\rightarrow\] AcO \[\rightarrow\] AcO \[\rightarrow\] SPh (-)-40

\[\rightarrow\] 98%

\[\rightarrow\] b
d (90%)

\[\rightarrow\] d (90%)

\[\rightarrow\] d (89%)

\[\rightarrow\] c

\[\rightarrow\] c

\[\rightarrow\] a

**Reagents and conditions:** (a) Ac₂O, pyr., CH₂Cl₂, room temp.; (b) (i) mCPBA, CH₂Cl₂, −78 °C; (ii) (MeO)₃P, EtOH, −78 °C to room temp.; (c) Dess−Martindale periodinane, CH₂Cl₂, room temp.; (d) NaBH₄, EtOH, 0 °C; (e) LiOH, THF/H₂O, room temp.

previously developed.²⁹ Alkylation of benzoylacetonate 26 with ethyl 4-bromobutyrate gave the diketone 27. The diazo transfer of 27 with p-nitrobenzenesulfonyl azide provided the diazoketone 15.

Aldol condensation of the diazoketone 15 and the aldehyde 16 was carried out in toluene in the presence of triethylsilyl (TES) chloride with potassium bis(trimethylsilyl)amide at −16 °C. Apart from the desired TES-protected aldol 29 with 27, we changed the protecting group to t-butyldiphenylsilyl (TBDPS), to give 14.

The rhodium catalyzed cyclopropanation of diazoketone 14 afforded the bicyclic ketone 13 and its diastereomer 30. To optimize this intramolecular cyclopropanation, we screened several variables of solvent, temperature, and catalyst loading. The results are summarized in Table 1. We found that the optimal concentration was around 0.05 M (entries 1–5). The yield decreased dramatically when the solution of 14 was added to the solution of Rh₂(Oct)₄ in CH₂Cl₂ (entry 4 vs 3). Temperature had a modest affect on the ratio of 13 to 30. The mixed solvent of toluene and dichloromethane gave the best yield of 13 (58%) and 30 (22%) (entry 11). A catalyst loading of 0.5 mol % also gave good results (entry 12). The reaction did not go to completion with 0.2 mol % of Rh₂(Oct)₄, resulting in a low chemical yield (entry 13).

**Scheme 8**

(-)-36 \[\rightarrow\] AcO \[\rightarrow\] AcO \[\rightarrow\] SPh (-)-40

\[\rightarrow\] 98%

\[\rightarrow\] b
d (90%)

\[\rightarrow\] d (89%)

\[\rightarrow\] c

\[\rightarrow\] c

\[\rightarrow\] a

**Reagents and conditions:** (a) Ac₂O, pyr., CH₂Cl₂, room temp.; (b) (i) mCPBA, CH₂Cl₂, −78 °C; (ii) (MeO)₃P, EtOH, −78 °C to room temp.; (c) Dess−Martindale periodinane, CH₂Cl₂, room temp.; (d) NaBH₄, EtOH, 0 °C; (e) LiOH, THF/H₂O, room temp.

The structures of the bicyclic ketones 13 and 30 were assigned by comparing the 1H and 13C NMR spectra to those for analogous bicyclic ketones that were intermediates in the synthesis of the 5-F₂t-isoprostanes⁴⁸ and the 8-F₂t-isoprostanes." In particular, the oxygenated methine of 13 (13C δ 69.5; 1H δ 4.47, d, J = 4.9 Hz) is congruent with the analogous 5-F₂t-isoprostane precursor (13C δ 69.3; 1H δ 4.46, d, J = 4.9 Hz) and 8-F₂t-isoprostane precursor (13C δ 69.3; 1H δ 4.46, d, J = 4.9 Hz), while the oxygenated methine of 30 (13C δ 68.1; 1H δ 4.60, d, J = 5.1, 7.9 Hz) is quite different.

Initially, difficulties were encountered in the cyclopropane ring opening of 13 (Scheme 5) with thiophenol and BF₃·OEt₂. Low temperature (<−30 °C) or low concentration (<0.1 M) resulted in incomplete reaction. Warmer temperatures (0–30 °C) generated several side products. We found that treatment of 13 with 3 equiv of thiophenol and 4 equiv of BF₃·OEt₂ in CH₂Cl₂ (0.2 M) at −30 to −20 °C for 6 h gave smooth conversion to the ketone 31, which was used directly in the subsequent reduction. We found that some deprotection of the TBDPS group occurred when the reduction was carried out in MeOH or EtOH over 1 h. Fortunately, this reaction could be finished in a mixed solvent system of MeOH/EtOH (1:1) with NaBH₄ within 20 min without causing silyl group deprotection.

The undesired alcohol 33 could be transferred to the mixture of 33 and 34 by oxidation with Dess−Martin periodinane.¹⁸

**Notes:**


(21) From ketone 13 on, all reactions (except for the enzymatic resolution and the final hydrolysis steps) were run in the presence of a trace amount of methylene blue to inhibit isomerization of the side chain. For the use of methylene blue to stabilize 1,4-dienes, see: Taber, D. F.; Phillips, M. A.; Hubbard, W. C. Prostaglandins 1981, 22, 349.
followed by reduction. The relative configuration of 34 (1H NMR $\delta$ 4.47, dt, $J$ = 2.5, 6.4 Hz, 1H, 4.08, m, 1H) was also assigned by comparing the 1H and 13C NMR spectra to those precursors for the 5-F$_2$-isoprostanes (1H NMR $\delta$ 4.44, dt, $J$ 3.6, 6.6 Hz, 1H, 4.08, m, 1H) and the 8-F$_2$-isoprostanes (1H NMR $\delta$ 4.48, dt, $J$ = 2.5, 6.5 Hz, 1H, 4.05, m, 1H). Desilylation of 34 with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) provided the key intermediate 12.

The racemic diol 12 (Scheme 6) when treated with Amano lipase AK in neat vinyl acetate at 40 °C for 48 h provided monoacetates (+)-35 (53% yield) and (−)-36 (34% yield) along with the diacetate (−)-40 (8% yield with 86% ee). The monoacetates (+)-35 and (−)-36 were converted to their diacetates (+)-40 and (−)-40 in quantitative yield. Their ee values were determined to be 85 and >98% respectively, by chiral HPLC. The enantiomerically enriched monoacetate (+)-35 was hydrolyzed to diol 12 and subjected to enzyme resolution again to give the monoacetate (+)-35 with >98% ee in 41% overall yield (82% of theoretical) from the racemic diol 12.

The structure of the monoacetate (+)-35 was established by conversion to 34 by protection followed by hydrolysis. The same transformation converted the monoacetate (−)-36 to 39. Since the same enzyme and a very similar substrate were employed in this resolution, the absolute configurations of (+)-35 and (−)-36 were assigned by analogy to our recent synthesis of the enantiomerically pure diastereomers of 15-F$_2$-isoprostane.8d

Reagents and conditions: (a) K$_2$CO$_3$, EtOH, 60 °C, 1.5 h; (b) PPh$_3$, DEAD, p-NO$_2$C$_6$H$_4$COOH, benzene; (c) mCPBA, CH$_2$Cl$_2$, −78 °C; (ii) (MeO)$_3$P, EtOH, −78 °C to room temp; (d) (i) Dess−Martin periodinane, CH$_2$Cl$_2$, room temp; (ii) NaBH$_4$, EtOH, 0 °C; (e) K$_2$CO$_3$, EtOH; (f) LiOH, THF/H$_2$O, room temp.
With the requisite enantiomerically pure acetates (+)-35 and (-)-36 in hand, the four enantiomerically pure trans isomers of 12-F₂t-isoprostane were prepared. The diacetate (±)-40 (Scheme 7) was obtained in quantitative yield by treating the monoacetate (±)-35 with acetic anhydride. Oxidation and Mislow rearrangement²³ of (±)-40 provided the allylic alcohol (±)-41, which on treatment with Dess–Martin periodinane, followed by reduction with NaBH₄, afforded the epimeric allylic alcohol (±)-41 and (±)-42. These were readily separated by column chromatography. They were separately hydrolyzed with LiOH in THF/H₂O (1:1) to furnish ent-12-F₂-isoprostane 6 and its 12-epimer 7 in 90 and 91% yields, respectively. The same procedures were carried out (Scheme 8) with the monoacetate (±)-36 to complete the preparation of 12-F₂t-isoprostane 4 and its 12-epimer 5. The spectroscopic data for 4 (¹H and ¹³C NMR) were consistent with those previously reported.⁹,²⁴

We thought that perhaps Mitsunobo inversion¹³ of 12 could lead to the all-cis 12-F₂-isoprostane derivatives. In fact, Mitsunobo coupling of the diol (±)-12 (Scheme 9) afforded the 12-F₂t-isoprostane derivative (±)-43 in 45% yield, accompanied by elimination products. That the reaction had proceeded with inversion at each of the reacting stereogenic centers was confirmed by comparing the ¹³C NMR chemical shifts of the non-oxygenated methines of (±)-43 (δ 43.1, 44.6, 49.4) with those of (±)-12 (δ 49.6, 50.5, 53.1). As can be seen by comparing 33 (δ 46,3, 47.9, 55.2) with 34 (δ 47.3, 50.4, 54.6), the methine adjacent to a cis OH resonates at higher field than the methine adjacent to a trans OH. Since both the methines of (±)-43 were shifted upfield, both of the reacting centers must have inverted. This assignment was confirmed by comparing the ¹³C NMR chemical shifts of the non-oxygenated methines of 6 (δ 50.1, 54.2) with those of 8 (δ 46.9, 50.1).

Oxidation and Mislow rearrangement²² of (±)-43 provided the allylic alcohol (±)-44. Oxidation of (±)-44 followed by reduction gave an inseparable mixture of (±)-44 and 45. Fortunately, transesterification of (±)-44 and 45 with K₂CO₃ at room temperature in EtOH gave (−)-46 and (−)-47, which could be separated by column chromatography. Hydrolysis of (−)-46 or (−)-47 individually with LiOH furnished the 12-F₂c-isoprostane diastereomers 8 and 9. The same strategy was applied to the enantiomerically pure monoacetate (−)-36 (Scheme 10), leading to the two other 12-F₂c-isoprostane diastereomers 10 and 11. The 12-F₂c diastereomers had never previously been prepared.²⁵

**Conclusion**

A stereodivergent and practical synthesis of the eight enantiomerically pure diastereomers of the 12-F₂-isoprostanes has been developed. The key steps include rhodium-mediated intramolecular cyclopropanation and enzymatic resolution. This approach has provided a sufficient quantity of 4–11 to assess their physiological activity.

**Acknowledgment.** We thank the National Institutes of Health (Grant GM42056) for support for this work.

**Note Added after Print Publication:** Due to a production error, Table 1 and Scheme 5 contained errors in the version published on the Web 10/11/2002 (ASAP) and in the November 6, 2002 issue (Vol. 124, No. 44, pp 13121–13126); the correct electronic version of the paper was published on 11/27/2002 and an Addition and Correction appears in the December 25, 2002 issue (Vol. 124, No. 51).

**Supporting Information Available:** Text giving detailed experimental procedures and figures showing spectra for all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA020816V