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The first total synthesis of iPF$_{4\alpha}$-VI and its deuterated analog

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Abstract—The total and stereospecific syntheses of iPF$_{4\alpha}$-VI and d$_4$-iPF$_{4\alpha}$-VI have been accomplished. iPF$_{4\alpha}$-VI is a docosahexanenoic acid (DHA)-derived isoprostane. DHA is the most abundant polyunsaturated fatty acid in the brain. Different synthetic designs have been used for the two syntheses. In the d$_4$-iPF$_{4\alpha}$-VI design, the deuterated part of the molecule was introduced last in the synthesis.

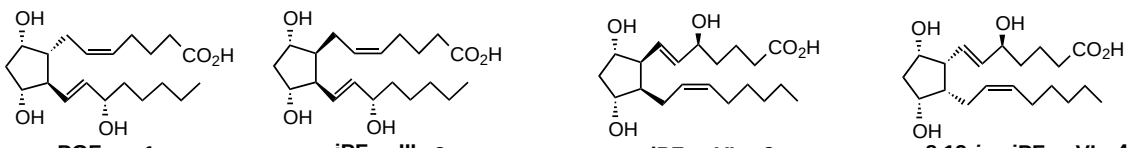
Isoprostanes (iPs) are a class of natural products generated by the action of free-radicals on polyunsaturated fatty acids (PUFA).[1,2] We have previously reported on the total synthesis of isoprostanes and metabolites derived from free-radical peroxidation of arachidonic acid (AA) **5**.[3–7] We have used these synthetic standards to discover and characterize four classes of iPs in biological fluids[2,8,9] and designed a GC and LC/MS/MS methodology to analyze for the distribution of iPs within each class.[10,11]

We wish to report here on the first synthesis of an iP derived from docosahexaenoic acid (DHA) **18**, namely iPF$_{4\alpha}$-VI. DHA is a 22:6ω3 fatty acid (ω3 = the first double bond from the ω end of the molecule, 22 = the number of carbons, 6 = the number of double bonds).[12]

Alzheimer's disease (AD) includes a group of dementing neurodegenerative disorders that have diverse etiologies but the same hallmark brain lesions. Since oxidative stress may play a role in the pathogenesis of AD and iPs are chemically stable peroxidation products of AA, our lab measured both iPF$_{2\alpha}$-III **2** and iPF$_{2\alpha}$-VI **3** using GC/MS in AD and control brains.[13] The levels of both iPs were markedly elevated in both frontal and temporal poles of AD brains compared to the corresponding cerebella and controls. The next phase was to attempt to show this correlation in a non-invasive fashion by measuring iPs in urine. Our group has made some preliminary measurements of iPs level in urine of AD patients and was able to show a substantial increase of 8,12-*iso*-iPF$_{2\alpha}$-VI **4** (Scheme 1).[14]

Human phospholipids normally contain large amounts of arachidonic acid with only low levels of ω–3 polyunsaturated fatty acids. The brain, however, is extremely rich in DHA, which makes up to 37% of its total fatty acids. Because of the preponderance of DHA in brain tissue, we decided to take a closer look at iPs derived from DHA, with the ultimate goal of providing a selective index of the severity of AD. Some GC/MS studies have in fact provided evidence for the formation of DHA-derived isoprostanes.[15,16]

Because of the preponderance of DHA in the brain, the iPs derived from this ω-3 *n*–6 PUFA are expected to be



Scheme 1.

Keywords: isoprostanes, iP; arachidonic acid, AA; docosahexaenoic acid, DHA; iPF$_{4\alpha}$-VI; d$_4$-iPF$_{4\alpha}$-VI.

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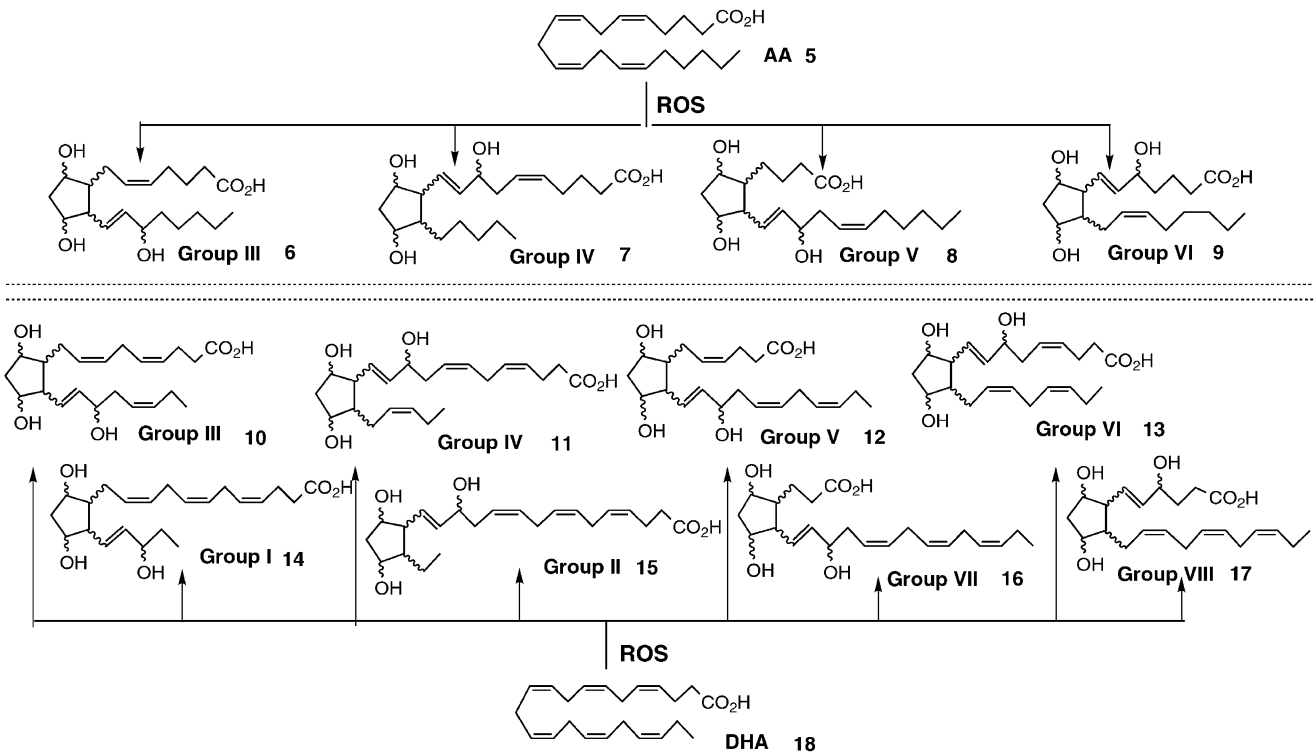
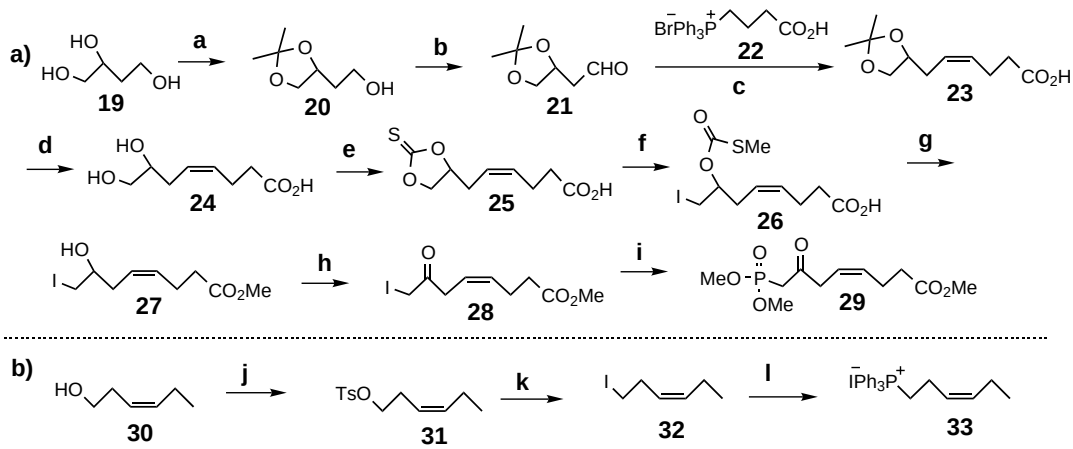
major iPs. In addition, the types and distribution of iPs derived from this PUFA will be different. It is anticipated that eight groups of iPs can be derived from DHA (Scheme 2).[17] We have devised a formula to predict the number of groups of F_2 iPs as a function of PUFA double bonds $(n-2)\times 2 =$ number of groups, n being the number of skipped double bonds. For example, DHA has six skipped double bonds. This translates to eight groups of iPs (Scheme 2). As a first step in this area we performed the total and stereospecific synthesis of $iPF_{4\alpha}$-VI (Scheme 4) and its tetradeutero derivative (Scheme 7).

The new synthon **29** was selected and prepared as shown in Scheme 3. 1,2,4-Butanetriol **19** was converted to the aldehyde **21** in two steps. Variable amounts of the six-membered ring acetonide were formed. These can be purified by flash chromatography. Alternatively, the oxidation of the six-membered isopropylidene alcohol is much slower than the required **20** and can be purified at that stage. Then the Wittig reaction with commercial (3-carboxylpropyl)triphenylphosphonium bromide in THF gave the *cis* olefin **23** in 78% yield. After removal of the 7,8-isopropylidene group in **23** with 4% aqueous H_2SO_4, the 7,8-dihydroxy compound



Scheme 2. Expected iPs from AA and DHA. (ROS: reactive oxygen species).



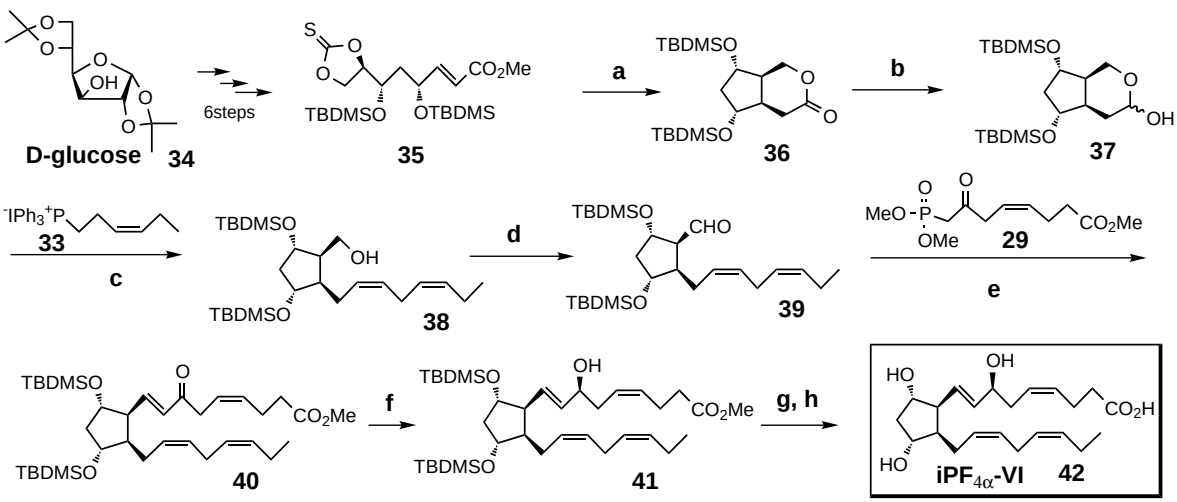
Scheme 3. *Reagents and conditions*: (a) 2,2-dimethoxy propane, TsOH, CH_3CN, 1 h 65%; (b) PCC, camphorsulfonic acid, CH_2Cl_2, 3 h, 74%; (c) LiHMDS, HMPA, −78°C, 3 h, 78%; (d) 4% H_2SO_4, THF, rt, 8 h; (e) $S{=}C(Im)_2$, CH_3CN, rt, 8 h, 51%; (f) MeI, $ClCH_2CH_2Cl$, 70°C, 6 h, 87%; (g) DIBAL-H, CH_2Cl_2, −78°C, 30 min, CH_2N_2, ether, 97% (two steps); (h) periodinane, CH_2Cl_2, rt, 6 h; (i) $P(OMe)_3$, THF, rt, 6 h, 62% (two steps); (j) TsCl, pyridine, rt, 8 h, 60%; (k) NaI, CH_3CN, 70°C, 3 h; (l) Ph_3P, CH_3CN, 80°C, 8 h, 87%.

24 was treated with thiocarbonylbis(imidazole) in acetonitrile to afford the thionocarbonate **25**. The conversion of thionocarbonate **25** to the iodohydrin **26** was performed by a method described by us recently.[18] The reduction of the methylthiocarbonyl group in **26** with DIBAL-H, followed by an acidic work-up and diazomethane treatment furnished the iodohydrin **27** in 97% yield. After oxidation with Dess–Martin periodinane, the treatment of the β-keto iodide **28** with trimethylphosphite in THF at room temperature afforded the β-keto phosphonate **29** in 62% yield for the two steps.

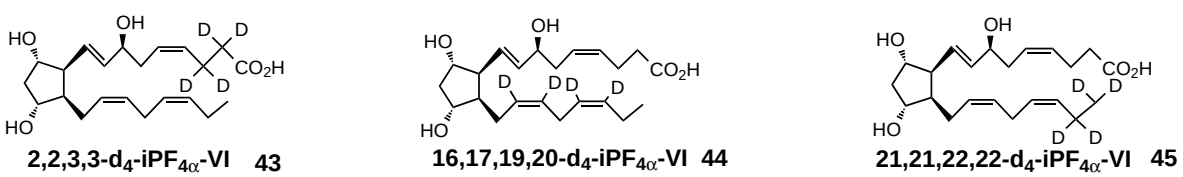
The precursor **35** for the cyclization was prepared in six steps from diacetone D-glucose **34**.[3] The lactol **37** was reacted with the phosphonium derivative **33** to afford the alcohol **38** in 78% yield. Dess–Martin oxidation gave aldehyde intermediate **39**. The β-keto phosphonate **29** was reacted in a Horner–Emmons reaction with aldehyde **39** to introduce the upper side chain, affording derivative **40**. As shown in Scheme 4, the (S)-BINAL-H reduction was carried out at −100°C to afford **41** in 87% yield. We have been unable to detect any of the *R*-isomer, indicating that the e.e. is >95%. iPF$_{4\alpha}$-VI **42** was obtained after the deprotection of silyl and hydrolysis.[19] We can perform steps g and h as a one-pot reaction. We found the use of formic acid in the deprotection step convenient[20] and, in some cases, competitive with TBAF or HF methods.

We also needed to synthesize the tetradeutero derivative of iPF$_{4\alpha}$-VI for its eventual quantitation by GC and LC/MS in biological fluids. With the extra double bonds in the molecule, as compared with AA-derived iP (Scheme 2), our choices for the position of four deuterium atoms in the molecule were limited. Scheme 5 shows some choices of deuterated derivatives we have considered. The tetradeutero compound **43** is not appropriate because the deuterium would be positioned α to a carboxylate group. This could result in a loss of deuterium during the synthesis. Analog **44**, while practically feasible, appeared more challenging. We elected to proceed with the synthesis of the tetradeutero analog **45** (Scheme 5). The synthesis of the deuterated synthon **56** (Scheme 6) was our initial target. We planned to use **56** in a synthesis similar to that shown in Scheme 4.

The synthesis of **56** as it turned out is not without problems. The main problem is the small size of alcohol **49** which is very volatile. In addition, we could not find any precedent for the deuteration of a primary acetylene. We were worried that the acetylenic proton, being acidic, would cause partial hydrogen–deuterium exchange.[21] The synthon **51** we needed for the synthesis was prepared from the O-THP derivative of propagyl alcohol **46**. The transformation of **47** to **48** proceeded smoothly and a high incorporation of D_2 (>99.7%) has been achieved, no scrambling was observed. The deprotection of the O-THP, however, was difficult (low yield)



Scheme 4. *Reagents and conditions*: (a) *n*-Bu_3SnH, AIBN, benzene, reflux, 5 h, 52%; (b) DIBAL-H, CH_2Cl_2, −78°C, 97%; (c) *t*-BuOK, HMPA, THF, −78°C to rt, 3 h, 78%; (d) periodinane, CH_2Cl_2, rt, 4 h, 97%; (e) LiHMDS, THF, −78°C, 69%; (f) S-BINAL-H, THF, −100°C, 4 h, 87%; (g) 6:3:1 = THF:formic acid:H_2O, rt, 3 h, 72%; (h) 5% KOH, THF, rt, 2 h, 97%.



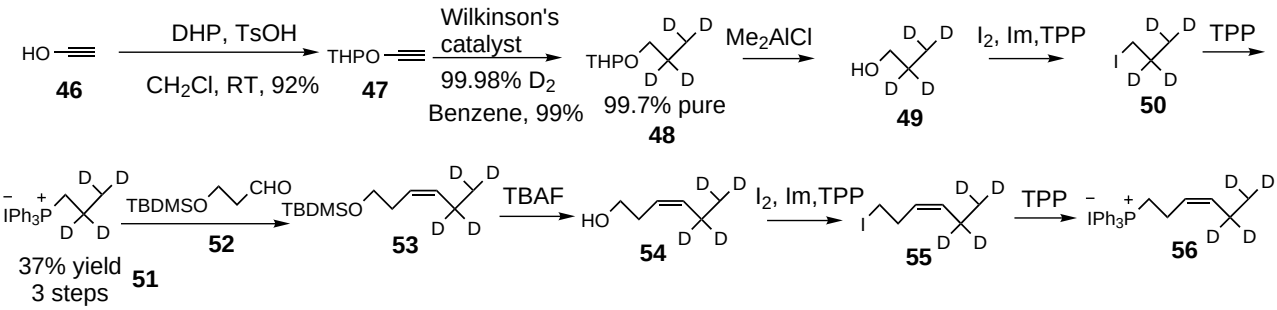
Scheme 5.

because of the volatility of tetradeutero propanol. We have managed to obtain the phosphonium derivative **51** in 15–37% yield, as shown in Scheme 6.

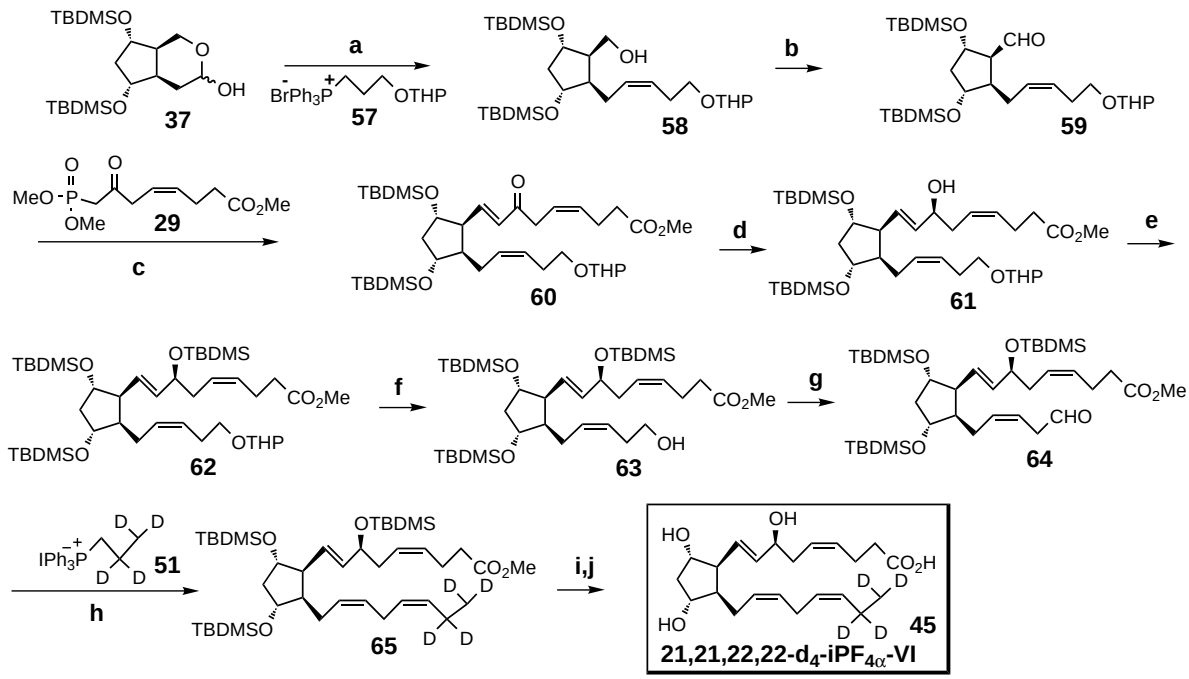
In addition, we have tried different protections on propagyl alcohol such as silyl groups. The reduction with Wilkinson's catalyst was very sluggish and mostly starting material was recovered. It is possible that the bulky silyl group interferes with the reduction.

As mentioned above, the most difficult step was the isolation of deuterated propanol **49**. The continuation of the synthesis to obtain the phosphonium derivative **56**, beyond alcohol **54**, was impractical at that point because of the dwindling yield due to the multi-step synthesis. We elected instead to redesign the synthesis of **45**, which is shown in Scheme 7. The major advantage of the new design is the fact that we performed the addition of the three-carbon synthon **51** at the last step of the synthesis.

The bicyclic lactol **37** was coupled with 3-(tetrahydropyran-2-yloxy)-propyl-phosphonium bromide **57** by Wittig reaction and **58** was obtained in 82% yield. The synthesis of **59** was achieved by a Dess–Martin oxidation. The aldehyde **59** was used in a Horner–Emmons reaction at −78°C to introduce the upper side chain using the anion of β-ketophosphonate **29** and afforded **60** in 76% yield. The enantioselective reduction of the C7 keto group **60** with chiral reducing agent (S)-BINAL-H proceeded well and afforded the desired pure 7(S) derivative **61** in 97% yield. Compound **61** was protected with the TBDMS group, and then the tetrahydro pyranyl group deprotection was carried out selectively using Me_2AlCl in 72% yield. Aldehyde **64** was reacted with the tetradeutero phosphonium derivative **51** as shown. The deprotection of *tris*-silyl groups in **65** was carried out using formic acid. Finally, hydrolysis with aqueous KOH afforded the desired d_4-iPF$_{4\alpha}$-VI **45**.[22]



Scheme 6.



Scheme 7. *Reagents and conditions*: (a) *t*-BuOK, THF, −20°C, 5 h, 82%; (b) periodinane, CH_2Cl_2, rt, 2 h, 97%; (c) LiHMDS, THF, −78°C, 76%; (d) S-BINAL-H, THF, −100°C, 5 h, 97%; (e) TBDMSCl, Im, CH_2Cl_2, rt, 8 h, 75%; (f) Me_2AlCl, CH_2Cl_2, −20°C to rt, 3 h, 72%; (g) periodinane, *t*-BuOH, CH_2Cl_2, rt, 5 h; (h) LiHMDS, HMPA, −78°C, 3 h, 68%; (i) 6:3:1 = THF:formic acid:H_2O, rt, 3 h; (j) KOH, THF, 1.5 h 85% (two steps).

Acknowledgements

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19. Mass spec. analysis of compound **42**: MS (EI) methyl ester, TMS deriv. m/z 608 (M^+), 593 (M–15), 481 (M–127) base peak, 391 (M–217), 365 (M–243), 301 (M–307), 275 (M–333).
    The NMR data of compound **42**: ^{1}H NMR ($CDCl_3$) δ 5.67–5.22 (m, 8H), 4.14 (q, 1H, $J=5.4$ and 12.2), 4.05–3.93 (m, 2H), 2.76 (m, 4H), 2.46–2.28 (m, 7H), 2.16 (m, 1H), 2.03 (m, 4H), 0.96 (t, 3 H, $J=7.5$).
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22. Mass spec. analysis of compound **45**: d_4 content (LC/MS): 99.85%.
    The NMR data of compound **45**: ^{1}H NMR (CD_3OD): δ 5.55–5.23 (m, 8H), 4.04 (q, 1H, $J=5.3$ and 10.3), 3.93 (q, 1H, $J=6.6$ and 11.8), 3.82 (q, 1H, $J=6.9$ and 11.3), 2.76 (m, 2H), 2.65 (m, 2H), 2.44 (m, 1H), 2.30 (m, 4H), 2.03 (m, 2H), 1.58 (m, 3H), 1.21 (s, 1H).