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A new approach to the synthesis of polyunsaturated deuterated isoprostanes: Total synthesis of d_4-5-*epi*-8,12-*iso*-$iPF_{3\alpha}$-VI and d_4-8,12-*iso*-$iPF_{3\alpha}$-VI

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ABSTRACT

The total and stereospecific synthesis of d_4-5-*epi*-8,12-*iso*-$iPF_{3\alpha}$-VI **55** and d_4-8,12-*iso*-$iPF_{3\alpha}$-VI **64**, EPA-derived all-*syn*-isoprostanes (iPs), has been accomplished. Because of issues related to volatility and yield with some of the primary deuterated synthons an improved synthesis is presented. These two deuterated analogs were used to discover and quantify the presence of the corresponding endogenous isoprostanes in human urine. These assays may serve as a valuable index of oxidative stress in population with omega-3 fatty acid enriched diets containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and may also be useful as an index of the severity of inflammatory diseases such as atherosclerosis and Alzheimer's disease.

Isoprostanes (iPs) are natural products of non-enzymatic free radical peroxidation of polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA) and eicosapentaenoic acid (EPA).[1,2] Isoprostanes are isomeric with prostaglandins (PGs), which are natural products generated by the action of enzymes. The mechanisms of formation of isoprostanes have been discussed in some detail.[3–5] Several syntheses of iPs have been reported.[3,6] These compounds have been widely used as markers of oxidative stress in various diseases. We and others have shown that the measurement of iPs in urine can be used as an index of the severity of inflammatory diseases such as Alzheimer's disease (AD) and atherosclerosis.[2,7]

Isoprostanes generated from EPA have received less attention than AA-derived isoprostanes.[8,9] However, the AA-derived iPs we and others have measured so far as an index of oxidative stress[10–12] may not be appropriate for individuals in which the major PUFAs in the phospholipids are EPA and DHA. A more appropriate index in these cases might be the measurement of EPA- and/or DHA-derived iPs, for example, **24** and **25** (Scheme 2A). For these reasons we have synthesized these two all-*syn* Group VI isoprostanes and identified their occurrence in human urine.[13] The reason for choosing Group VI isoprostanes derived from EPA stems from our experience that the presence of a hydroxyl group in the 5-position of these iPs causes them to be resistant to β-oxidation,[8] facilitating their detection in urine. For example, we have shown that $iPF_{3\alpha}$-VI is highly resistant to β-oxidation and we have been able to detect appreciable amounts of this substance in human urine.[8] Recent studies from our laboratory suggest that 5-*epi*-8,12-*iso*-$iPF_{3\alpha}$-VI **24** and 8,12-*iso*-$iPF_{3\alpha}$-VI **25** are considerably more abundant in urine than $iPF_{3\alpha}$-VI and may therefore serve as excellent markers for oxidative stress involving degenerative diseases of the brain, which contains high levels of DHA, as well as in individuals who consume large amounts of ω3-PUFA. To provide the deuterium-labeled standards required for the quantitation of these major EPA-derived iPs in biological fluids[14] we are reporting here the total synthesis of deuterated analogs of **24** and **25**, namely d_4-5-*epi*-8,12-*iso*-$iPF_{3\alpha}$-VI **55** and d_4-8,12-*iso*-$iPF_{3\alpha}$-VI **64** (Scheme 4).

Site of deuteration: We have previously developed methods to introduce four deuterium atoms into the lower side chains of iPs, as this would avoid the loss of deuterium as a result of β-oxidation. Although this is much less of an issue with Group VI iPs, as

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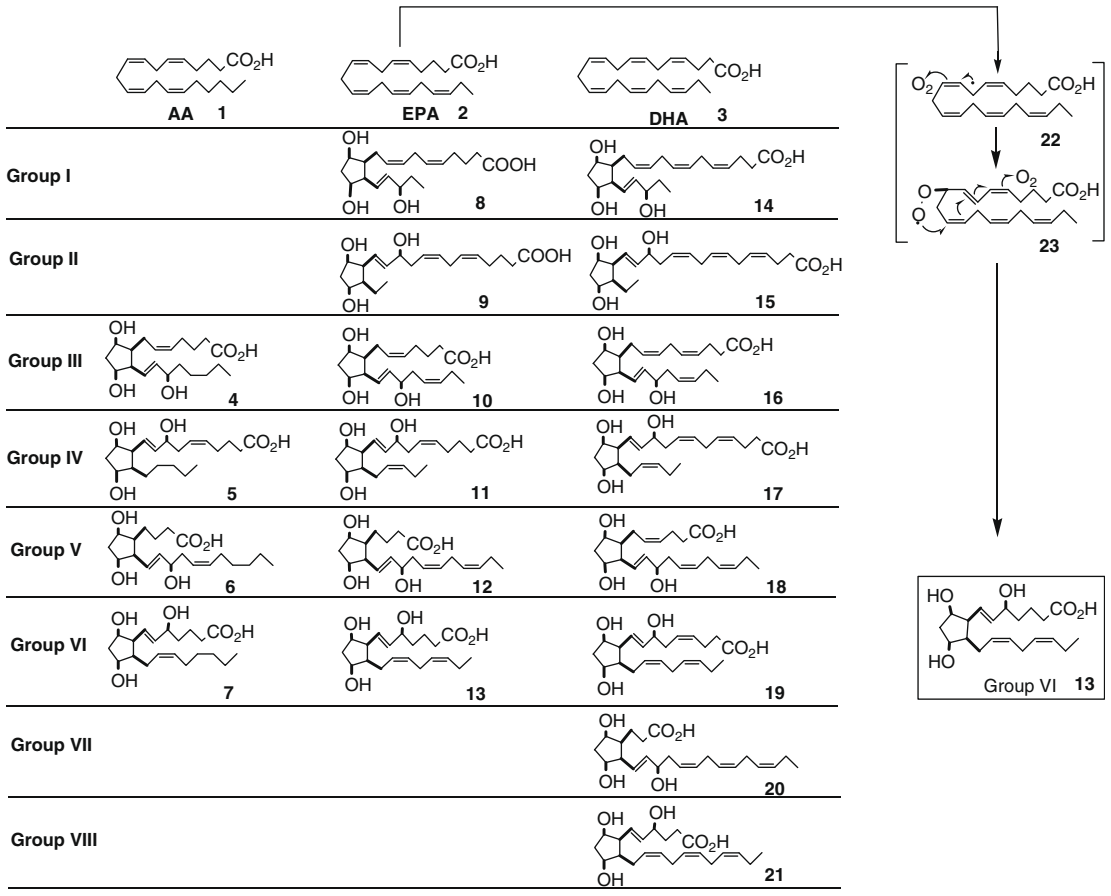
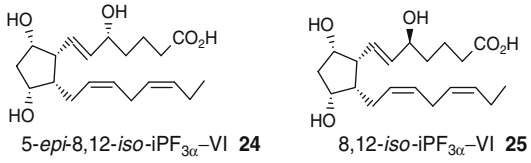


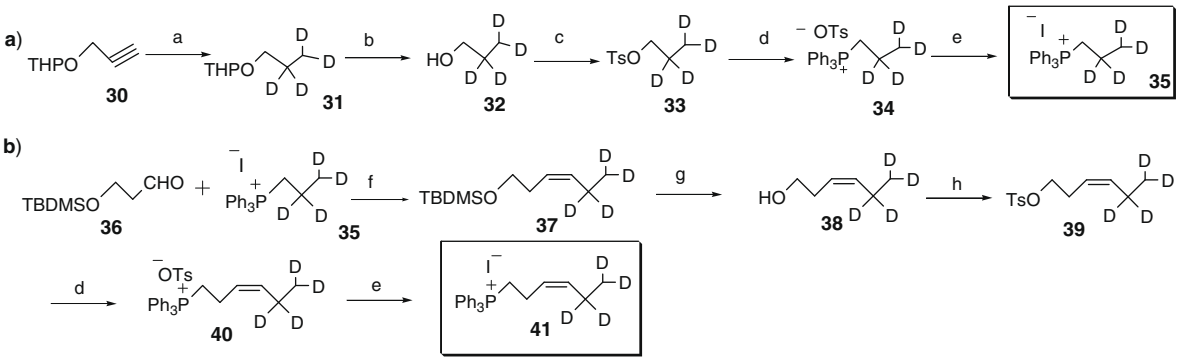
Scheme 1. iPs and nPs derived from AA, EPA, and DHA by free radical peroxidation.



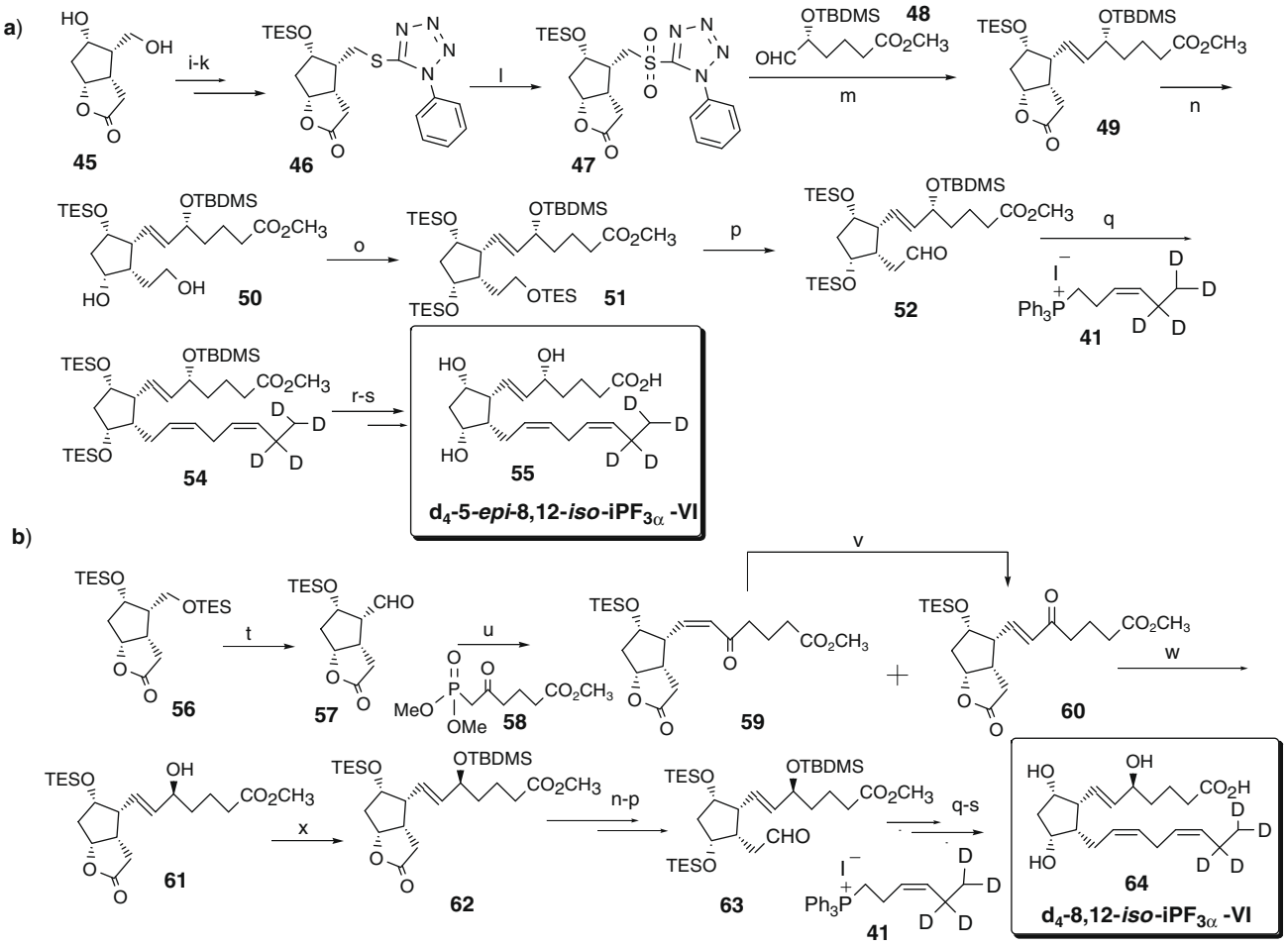
Scheme 2. Structures of the major EPA-derived iPs 5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI and 8,12-*iso*-iPF$_{3\alpha}$-VI.

discussed above, there is an additional advantage in avoiding the addition of deuterium to the upper side chain due to the risk of scrambling in the synthetic oxo precursors of this side chain. Because of the presence of the ω3-double bond in **24** and **25**, we elected to introduce four deuterium atoms in positions 19 and 20 of these molecules.

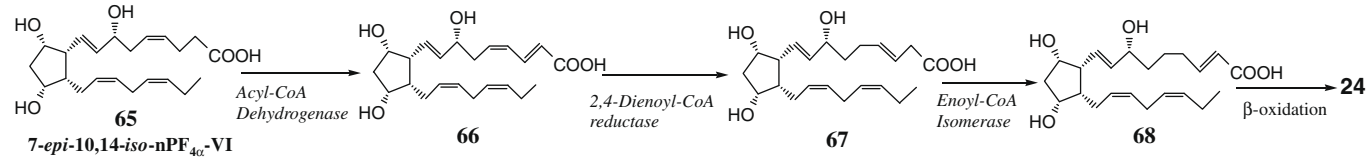
Synthesis: The most difficult step in our prior synthesis of tetradeutero iPF$_{4\alpha}$-VI was the isolation of deuterated propanol **32** (Scheme 3).[15] The continuation of the synthesis to obtain derivative **38** was impractical at that point because of the dwindling yields due to the multistep synthesis. We elected at that time to redesign the synthesis by extending the isoprostane synthon by three additional carbons and reacting it with deuterated synthon **35**.[15] This was hardly a fruitful alternative since the isoprostane synthon requires a multistep synthesis, making the synthesis completely linear rather than convergent, which was our original intention.



Scheme 3. Synthesis of deuterated side chain. Reagents and conditions: (a) Wilkinson's catalyst, benzene, D_2, 97%; (b) Me_2AlCl, CH_2Cl_2, −25 °C to rt, 8 h; (c) TsCl, pyridine, CH_2Cl_2, 0 °C to rt, 6 h, 75% (two steps); (d) PPh_3, CH_3CN, 70 °C, 96%; (e) NaI, CH_3CN, rt, 97%; (b) (f) KHMDS, THF, −90 °C to rt, 85%; (g) 1 N HCl, THF, rt; (h) TsCl, pyridine, CH_2Cl_2, rt, 6 h, 80% (two steps).



Scheme 4. Stereospecific synthesis of d_4-5-*epi*-8,12-*iso*-$iPF_{3\alpha}$-VI **55** and d_4-8,12-*iso*-$iPF_{3\alpha}$-VI **64**. Reagents and conditions: (a) (i) TESCl, Et_3N, DMAP, CH_2Cl_2, rt, 20 h, 98%; (j) Wilkinson's catalyst, catechol borane, THF, 0 °C to rt, overnight, 91%;[16] (k) PT-SH, Ph_3P, DIAD, THF, 0 °C to rt, 4.5 h, 97%; (l) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, rt, overnight, 96%; (m) KHMDS, **48**, DME, −60 °C to rt, 8 h, 52% (*E*:*Z* = 63:37), separated by silica gel chromatography; (n) $NaBH_4$, ethylether/MeOH (2:1), rt, 35 min, 70%; (o) TESCl, Et_3N, DMAP, CH_2Cl_2, 20 h, 95%; (p) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −70 °C to −20 °C, 1.5 h; (q) LiHMDS, **41**, THF, −78 °C to rt, 2 h, (two steps 73%); (r) TBAF, THF, rt, overnight; (s) LiOH, *i*PrOH/H_2O (1:1), rt, 4 h 93% (two steps); (b) (t) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −70 °C to −20 °C, 1.5 h; (u) LiHMDS, **48**, THF, −78 °C to rt, 2 h, (**59**, 8.4%, **60**, 73%) (80% after I_2 treatment); (v) I_2, CH_2Cl_2, rt, 15 h, 87%; (w) EtOH, $LiAlH_4$, (*S*)-Binal-H, THF, −100 °C, 45 min, 92%; (x) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, rt, overnight, 85%.



Scheme 5. β-Oxidation of neuroprostane to EPA-derived isoprostane.

In the present study we circumvented the volatility issue in two ways. We used a non-volatile tosylate intermediate and a very efficient procedure to convert a tosylate phosphonium salt into an iodide derivative, as the use of the phosphonium tosylate by itself results in low yields in a Wittig reaction. Transformation of tosylate salts **34** and **40** to iodides **35** and **41**, respectively, proceeds in quantitative yields. We were surprised to note that the literature does not contain examples of such transformations. This can be a useful general procedure to deal with deuterium labeled small molecules.

Scheme 3 describes the synthesis of the deuterated side chain, which was subsequently connected to the 5-membered all-*syn* isoprostane ring. Using commercial propargyl alcohol as our starting material, the alcohol was protected using THP. The acetylene derivative **30** (Scheme 3) was then treated with Wilkinson's catalyst in the presence of deuterium gas to generate the deuterated intermediate **31**, which on further treatment with dimethyl aluminum chloride generated the low-boiling volatile three-carbon alcohol **32**. Attempts to isolate this alcohol resulted in very low recovery. The crude **32**, which was used without purification or workup in the next step, was treated with TsCl to generate tosylate **33**. After workup and purification, **33** was treated with triphenyl phosphine in the presence of CH_3CN to generate the phosphonium salt **34**, which was purified by column chromatography. We attempted to react **34** with aldehyde **36** and obtained a low yield of the desired product **37** (22–38%). By changing the tosylate to an iodide using sodium iodide, we obtained the iodo phosphonium salt **35**.[17] This was then used in a Wittig reaction with aldehyde **36** to obtain the desired product in 85% isolated yield. A cursory look at the literature reveals that tosylate salts, when used in Wittig reactions, afford low yields of the desired products. It is worth mentioning that an attempt to prepare the tetradeutero propyl iodide from

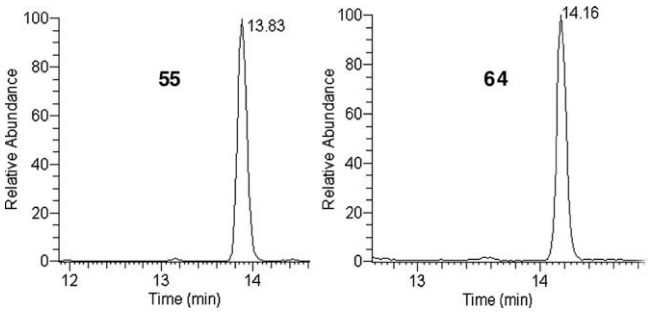


Figure 1. High Performance Liquid Chromatography: The UHPLC was performed using an Accela solvent delivery system (Thermo, Waltham, MA) and a column of Hypersil GOLD C18 (2) (200 mm × 2.1 mm; 1.9 μm particle size column; Thermo). The mobile phase consisted of water (solvent A) and acetonitrile:methanol (95:5, solvent B), both with 0.005% acetic acid adjusted to pH 5.7 with ammonium hydroxide. The flow rate was 350 μl/min. The separations involved various linear solvent gradient programs.

32 resulted in very low yield of the iodo derivative, probably due to volatility problems. As shown in Scheme 3, phosphonium salt **41** was prepared in good yield by a similar sequence (Panel B). Scheme 4 describes the stereospecific synthesis of d_4-5-*epi*-8,12-*iso*-iPF$_{3\alpha}$-VI **55** and d_4-8,12-*iso*-iPF$_{3\alpha}$-VI **64**.[18] For reasons of clarity we have included the first few structures, which we have previously disclosed.[15] Step u, Scheme 4B afforded in addition to the desired product **60**, 8–9% of the *cis* derivative **59**. The isomerization procedure works well also on the crude mixture and no special purification and isolation of **59** is necessary. The transformation of bis-TES **56** to a TES aldehyde **57** was accomplished in high yields using Spur's procedure.[19]

Metabolism: The iPs **24** and **25** (Scheme 2) can be formed by the direct autooxidation of EPA as shown in Scheme 1. Alternatively, these iPs can be formed as a result of β-oxidation of DHA-derived neuroprostanes (nPs) as shown in Scheme 5, which we have previously demonstrated in the case of isomers of **65**.[8] Furthermore we and others have shown the DHA-derived nPs could not be detected in urine, a fact we interpreted as being due to β-oxidation.[8,9] Since iPs such as **24** and **25** are resistant to β-oxidation we were readily able to measure them in urine. Hence the measurement of **24** and **25**, which we have accomplished in human and murine urine,[14] is an indication of the in vivo formation of either or both EPA-derived iPs and DHA-derived nPs that have been converted to the corresponding C_{20} derivatives by endogenous β-oxidation. Figure 1 shows LC/MS/MS chromatogram of tetradeutero isoprostones **55** and **64**.

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16. Patel, P.; Chang, C. T.; Kang, N.; Lee, G. J.; Powell, W. S.; Rokach, J. *Tetrahedron Lett.* **2007**, *48*, 5289.
17. Experimental procedure for Scheme 3a: Step a: To the solution of acetylene **30** (4 g, 28.54 mmol) in 70 mL benzene was added 1 g of Wilkinson's catalyst (25 wt %) at room temperature. The mixture was purged with argon gas. The deuterium gas was passed in the flask until deuterium gas absorption stopped. The crude mixture was filtered through celite and florisil layers to afford the desired deuterium product **31** in quantitative yields. Sometimes the deuteration did not go to completion. In this case, the crude mixture was filtered and the deuteration process repeated.Steps b and c: To the solution of deuterium **31** (311 mg, 2.10 mmol) in CH_2Cl_2 (15 mL), Me_2AlCl (6.29 mL, 6.29 mmol) was added dropwise at −25 °C and stirred for 30 min at −25 °C. The reaction mixture was gradually allowed to reach room temperature and the reaction continued for 12 h. After all the starting material was consumed the reaction was quenched using aqueous saturated $NaHCO_3$ solution at 0 °C. The aqueous layer was extracted with CH_2Cl_2 and the organic layers combined and dried using Na_2SO_4. The solvent was cautiously evaporated up to the point where approximately 2 mL of CH_2Cl_2 was left inside the flask. The deuterium compound **32** was used without further purification in the next step. To the solution of crude alcohol **32**, TsCl was added at 0 °C followed by pyridine and stirred for 10 min. The reaction mixture was gradually allowed to reach room temperature and the stirring continued for 12 h. The reaction was quenched by adding water and extracted using CH_2Cl_2, then dried and purified by silica gel chromatography to afford tosylate **33** in 75% combined yield.Step d: To the solution of tosylate **33** (660 mg, 3 mmol) in 5 mL of CH_3CN was added Ph_3P (3 g, 11.4 mmol) and the reaction was continued at 70 °C for 24 h. The solvent was evaporated and the crude compound purified using silica gel column chromatography to afford **34** in quantitative yields.Step e: To the solution of the salt **34** (774 mg, 1.60 mmol) in 15 mL of CH_3CN was added NaI (1.2 g, 8.05 mmol) at room temperature and stirred for 12 h. The solvent was evaporated and the crude mixture purified using column chromatography to afford the desired iodo salt **35** (683 mg) in 97% yield.
18. *Spectral data of d_4-5-epi-8,12-iso-iPF$_{3\alpha}$-VI* **55**: ^{1}H NMR (methanol-d_4, 400 MHz) δ 5.75 (1H, dd, J = 15.3, 10.6), 5.44–5.32 (2H, m), 5.28–5.13 (3H, m), 4.09–3.92 (3H, m), 2.72–2.64 (2H, m), 2.57–2.49 (1H, m), 2.36–2.27 (1H, m), 2.26–2.02 (4H, m), 1.77–1.68 (1H, m), 1.68–1.42 (5H, m), 0.81 (1H, s); ^{13}C NMR (methanol-d_4, 100 MHz) δ 177.59, 137.46, 132.48, 130.08, 129.60, 129.41, 128.69, 74.83, 73.09, 72.80, 52.03, 49.86, 43.49, 37.83, 35.13, 26.64, 25.10, 22.37, 13.90(m); *spectral data of d_4-8,12-iso-iPF$_{3\alpha}$-VI* **64**: ^{1}H NMR (methanol-d_4, 400 MHz) δ 5.72 (1H, dd, J = 15.4, 10.6), 5.47–5.132 (2H, m), 5.27–5.16 (3H, m), 4.05–3.96 (3H, m), 2.75–2.67 (2H, m), 2.56–2.52 (1H, m), 2.38–2.30 (1H, m), 2.22–1.99 (4H, m), 1.78–1.68 (1H, m), 1.63–1.36 (5H, m), 0.81 (1H, s); ^{13}C NMR (methanol-d_4, 100 MHz) δ 177.58, 137.62, 132.65, 131.11, 130.10, 129.59, 128.75, 74.97, 73.96, 72.93, 52.09, 48.63, 43.70, 37.74, 35.06, 26.81, 25.26, 22.41, 13.92 (m).
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