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Improved Synthesis of (*E*)-12-Nitrooctadec-12-enoic acid, a Potent PPARγ Activator. Development of a "Buffer-Free" Enzymatic Method for Hydrolysis of Methyl Esters

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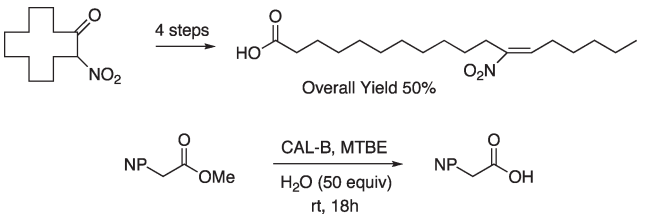

Endogenous nitro-fatty acids, acting as partial agonist of PPARγ, are able to lower the insulin and glucose levels without the side effects associated with common antidiabetic drugs. (*E*)-12-Nitrooctadec-12-enoic acid, a potent activator of this peroxisome receptor, was synthesized in a very efficient sequence via a Henry−retro-Claisen ring fragmentation, followed by a novel enzymatic cleavage of methyl esters. The latter method was then applied in the last step of the synthesis of a few labile natural products, such as prostaglandins, isoprostanes, and phytoprostanes.

Nitro-fatty acids (O_2N-FAs) such as nitrolinoleic (**1**) and nitrooleic acid (**2**) are endogenously generated electrophilic byproducts of nitric oxide and nitrite-dependent oxidative inflammatory reactions (Figure 1).

They are implicated in the modulation of multiple signaling pathways resulting in several important bioactivities including vasodilation, inhibition of inflammation, and inhibition of platelet activation and function.[1] In addition, these nitrated unsaturated fatty acids are among the most potent endogenous ligand activators of peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor involved in the transcription of genes associated with carbohydrate and lipid homeostasis, cellular differentiation, and inflammation.[2] PPARγ is, therefore, the biological target of antidiabetic type 2 drugs, such as rosiglitazione, pioglitazone. and troglitazone.[3]

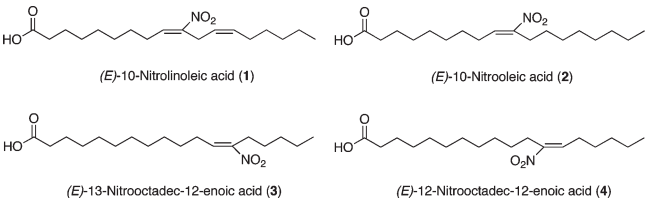

FIGURE 1. Structure of representative nitro-fatty acids: natural (*E*)-10-nitrolinoleic and (*E*)-10-nitrooleic acids. Synthetic (*E*)-13-nitrooctadec-12-enoic and (*E*)-12-nitrooctadec-12-enoic acids.

In 2009, Morrow and co-workers carried out a structure−activity relationship study on a series of O_2N-FAs, aimed at finding the best ligand for PPARγ activation.[4] They found that synthetic (*E*)-13-nitrooctadec-12-enoic acid (**3**) and, even better, (*E*)-12-nitrooctadec-12-enoic acid (**4**) were the most potent ligands for PPRγ, showing IC_{50} values of 190 and 39 nM, respectively. These potencies were, thus, higher not only than endogenous **1** (IC_{50} 220 nM)[5a] and **2** (IC_{50} 980 nM), but even than rosiglitazone itself (IC_{50} 250 nM).[5b]

The importance of O_2N-FAs as signaling molecules has stimulated the development of different synthetic routes, whose high efficiency and stereoselectivity were the main goals. Indeed, the high electrophilic nature of the nitroolefin moiety, which is highly vulnerable to Michael addition even under physiologically aqueous conditions, seriously jeopardizes any synthetic approach to O_2N-FAs. In particular, one of the last key steps, involving the hydrolysis of an ester group, has often been marred by low yields.[6] For example, methyl esters of nitro-fatty acids depicted in Figure 1 were uncleaved by prolonged exposure to 1 M NaOH at room temperature, while they suffered severe decomposition at higher temperatures.[7] Similarly, methyl ester cleavage by trimethyliodosilane in refluxing MeCN met with no success. On the other hand, acidic hydrolysis of the same esters in 6 N HCl at reflux delivered desired nitro-fatty acids only in modest yields.[4,7]

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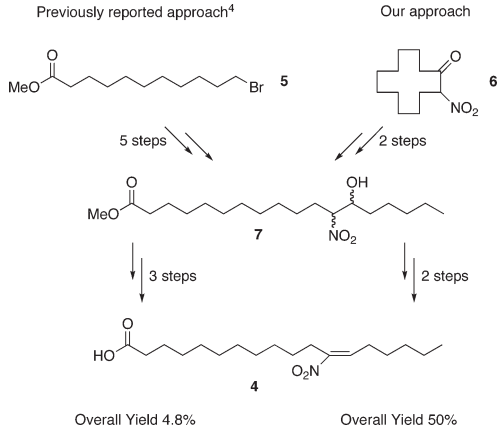
SCHEME 1. Comparison of Synthetic Approaches to the Potent PPARγ Activator O_2N-FA 4



To surmount the difficulties associated with the hydrolytic step, D'Ischia and collaborators,[6] as well as Branchaud and collaborators,[8] replaced the methyl esters with the corresponding allyl derivatives, which were smoothly cleaved by using Pd(0) complexes. This procedure required, however, a longer number of steps, resulting in lower overall yields. Very recently, acid **1** was obtained in a good yield of 73% by enzymatic hydrolysis of the corresponding methyl ester in a phosphate buffer; however, despite the high enzyme–substrate ratio (25:1), hydrolysis of the ester was incomplete.[5a]

In this paper, we describe a very efficient preparation of O_2N-FA **4** along with a new procedure for the enzymatic cleavage of methyl esters under "buffer-free" conditions. Nitrated fatty acid **4** was prepared by Morrow et al. from commercially available methyl 11-bromoundecanoate (**5**), in *eight* steps and 4.8% overall yield.[4] Notably, our approach delivered, instead, compound **4** in only *four* steps and 50% overall yield from commercially available 2-nitrocyclododecanone (**6**) (Scheme 1).

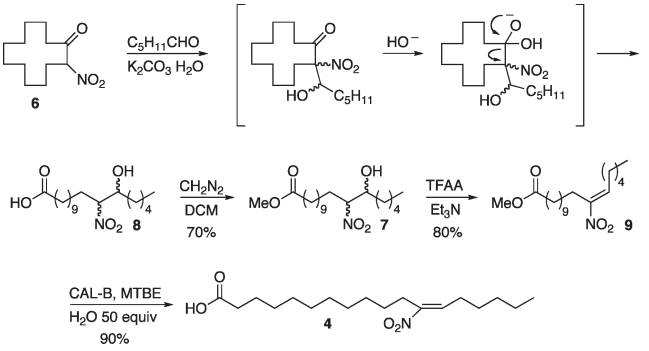
Key features of our synthetic route comprise (i) a new efficient protocol for the preparation of nitro alcohol **7**, (ii) a stereoconvergent dehydration of alcohol **7** to give nitroolefin **9**, (iii) a smooth enzymatic cleavage of methyl ester **9** to afford acid **4**.

Indeed, one-pot consecutive nitroaldol addition (Henry reaction) of 2-nitrocyclododecanone **6** to hexanal (3 equiv), followed by retro-Claisen ring cleavage, promoted by aqueous K_2CO_3 (2 equiv) at 35 °C, smoothly afforded the corresponding hydroxy-nitro acid **8** as a mixture of stereomers (Scheme 2).

The only other example of consecutive reactions of this kind was documented by Ballini and collaborators in 1997. Indeed, with formaldehyde as the electrophilic partner of the anions of different 2-nitrocycloalkanones, the corresponding 2-nitro-1,3-diol-ω-alkanoic acids were produced via a double aldol addition.[9] The authors also nicely clarified the mechanism of this synthetic sequence;[10] however, the same reaction with other aldehydes has not been explored so far.

Direct esterification of crude acid **8** with diazomethane in dichloromethane delivered methyl ester **7** in 70% isolated

SCHEME 2. High-Yield Synthesis of Nitro-Fatty Acid 4 (TFAA, trifluoroacetic anhydride; CAL-B, *Candida antartica* lipase B; DCM, dichloromethane; MTBE, methyl *tert*-butyl ether)



yield over two steps. Subsequently, we tried to obtain the methyl ester **7** directly from 2-nitrocycloalkanone **6** and hexanal by substituting H_2O with MeOH as the solvent of the nitroaldol–retro-Claisen reaction sequence, according to a previous report by Ballini and collaborators.[11] However, for still unclear reasons, substrate **6** was recovered unchanged. Subsequently, the mixture of stereomers **7** was converted to the sole (*E*)-nitroolefin **9** in 80% isolated yield via E_2 elimination of the corresponding trifluoroacetates, prepared in situ by exposing **7** to trifluoroacetic anhydride and Et_3N in DCM at 0 °C.[12] The desired *E*-configuration of nitroolefin **9** was firmly established by the characteristic ^{1}H NMR signal for the olefinic proton (triplet at δ 7.07 ppm, J = 7.9 Hz).[4]

To unmask the methyl ester **9**, we imagined a new mild enzymatic procedure. In fact, the expected pK_a of the carboxylic acid product[13] suggested to us that the medium would remain only slightly acidic throughout the reaction, thus avoiding enzyme inactivation. Moreover, using a pH "insensitive" enzyme, such as lipase B from *Candida antarctica* (CAL-B),[14] hydrolysis could proceed in an organic solvent, containing only the minimum amount of H_2O necessary to cleave intermediate acyl enzyme.[15] In fact, the Gibbs free energy difference between reactants and products in lipase-catalyzed reactions is quite small; therefore, a clever change of the reaction environment allows the equilibrium position to be readily shifted to either direction.[16] Moreover, we anticipated to apply this mild protocol to substrates, such as ester **9** (vide infra), sensitive not only to basic or acidic

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(9) Ballini, R.; Barboni, L.; Pintucci, L. *Synlett* **1997**, 1389. For a review on 2-nitrocycloalkanones chemistry, see: Ballini, R. *Synlett* **1999**, 1009.

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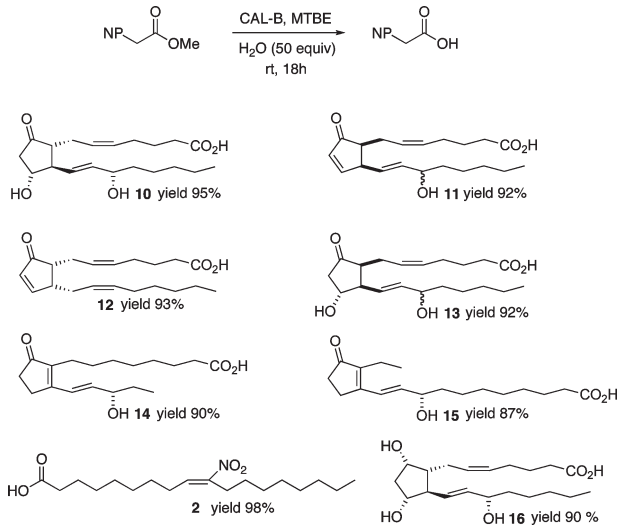
SCHEME 3. General Application of CAL-B-Mediated Enzymatic Hydrolysis of a Few Labile Natural Product (NP) Methyl Esters



hydrolytic conditions but even to a neutral medium. In the event, after a few preliminary experiments, exposure of ester **9** to CAL-B (**9**/CAL-B 5:1 w/w) in MTBE, in the presence of H_2O (50 equiv), afforded, after 18 h at 35 °C, the desired acid **4** in 90% isolated yield as a single *E*-stereomer (NMR spectra). MTBE was revealed to be the solvent of choice for the reaction of ester **9** since in dichloromethane conversion to **4** was very low (40%), while in THF, acid **4** was formed in merely 30% isolated yield, contaminated by an unidentified side product.

Carboxylic acids are usually protected as methyl esters, thanks to the easy formation, little steric hindrance, and clear NMR spectra of these derivatives.[17] Thus, to prove its reliability, our *buffer-free* procedure was employed to cleave a methyl ester group in the last step of the synthesis of a few labile natural products.[18]

In the event, prostaglandin-E_2 (**10**), isoprostane-A_2 (**11**), preclavulone-A (**12**), isoprostane-E_{2t} (**13**), phytoprostanes-B_1 type I (**14**) and type II (**15**), prostaglandin-$F_{2\alpha}$ (**16**), and (*E*)-10-nitrooleic acid **2** were produced as free acids in high isolated yields upon treatment of the corresponding methyl esters with CAL-B (5:1 ratio, w/w) in MTBE−H_2O (50 equiv) for 18 h at 35 °C (Scheme 3). Compared with existing approaches to **10**, **13**, and **2**, this method clearly performed much better, affording PGE_2 (**10**) in 95% yield vs 60%,[19a] $isoPGE_{2t}$ (**13**) in 92% yield vs 50%,[19b] and nitrooleic acid (**2**) in 98% yield vs 42%.[7]

This scenario was even more intriguing considering the two phytoprostanes **14** and **15** which, indeed, occur in nature as free carboxylic acids. It is, thus, quite surprising that the "total synthesis" of phytoprostanes **14** and **15** has been claimed by all authors achieving, instead, only the methyl ester precursors,[20] possibly due to difficulties encountered in the final hydrolytic step. Actually, the biological activity of the esters may be quite different from the free acids, due to likely interactions of the carboxylic group with biomolecules.

According to our novel procedure, the two phytoprostanes **14** and **15** were obtained, for the first time, from corresponding methyl esters in gratifying 90 and 87% isolated yields, respectively. The ^1H and ^{13}C NMR spectra were consistent with literature.[21]

In summary, we have described a very efficient route to the potent PPARγ partial agonist (*E*)-12-nitrooctadec-12-enoic acid **4**, requiring only four steps and proceeding in 50% overall yield. This new synthesis of compound **4** was about 10 times more efficient than that reported in literature, which proceeded in eight steps and in 4.8% overall yield.[4] A one-pot Henry−retro-Claisen ring cleavage and a high yield, buffer free, CAL-B-mediated enzymatic methyl ester cleavage are the salient features of our approach to **4**. Extending this extremely mild hydrolysis procedure to other methyl esters, we obtained phytoprostanes **14** and **15** for the first time as the naturally occurring free acids. The merits of the new enzymatic procedure are especially valuable with labile substrates under standard hydrolytic conditions.

Experimental Section

Methyl 13-Hydroxy-12-nitrooctadecanoate (7). To a stirred solution of K_2CO_3 (605 mg, 4.4 mmol) in H_2O (7.5 mL) were added, in the order, hexanal (0.8 mL, 6.6 mmol) followed by 2-nitrocyclododecanone (500 mg, 2.2 mmol). The reaction was stirred at 35 °C for 18 h, then diluted with H_2O (30 mL), and quenched at rt with 3 M HCl (1.5 mL, 4.62 mmol). The solution was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried on anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was dissolved in CH_2Cl_2 (25 mL) and treated with an ethereal solution of CH_2N_2 at 0 °C until a pale yellow color persisted. The mixture was concentrated under vacuum to give a crude product which was purified on silica gel. Elution with 9:1 hexane−EtOAc (R_f = 0.30) gave **7** (554 mg 70%) as a colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, br t, J = 6.8 Hz), 1.25−1.85 (26H, m), 2.00−2.20 (1H, m), 2.30 (2H, t, J = 7.4 Hz), 3.65 (3H, s), 3.85 (0.5H, m), 4.05 (0.5H m), 4.50 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4 (s), 92.9 (d), 92.3 (d), 72.4 (d), 72.0 (d), 51.4 (q), 34.1 (t), 33.5 (t), 33.1 (t), 31.5 (t), 31.4 (t), 30.4 (t), 29.3 (t), 29.1 (t), 29.1 (t), 28.9 (t), 28.9 (t), 27.9 (t), 25.9 (t), 25.6 (t), 25.3 (t), 24.9 (t), 22.5 (t), 13.9 (q); IR (CH_2Cl_2) 3448, 2928, 2856, 1739, 1549, 1438, 1364, 1173 cm^{-1}; HRMS $C_{19}H_{37}NO_5$ calcd 359.2672, found 359.2683.

(*E*)-Methyl 12-Nitrooctadec-12-enoate (9). Trifluoroacetic anhydride (0.305 mL, 2.19 mmol) was added to a solution of nitro-alcohol **7** (750 mg, 2.09 mmol) in dry CH_2Cl_2 (30 mL) under Ar, followed by the addition of Et_3N (0.62 mL, 4.4 mmol) dropwise at 0 °C. The reaction was allowed to reach room temperature and after 4 h was quenched with aqueous saturated NH_4Cl (10 mL). The mixture was diluted with CH_2Cl_2 (50 mL),

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(21) Thoma, I.; Krischke, M.; Loeffler, C.; Mueller, M. J. *Chem. Phys. Lipids* **2004**, *128*, 135.

and the two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), and the organic phases were combined, washed with brine, and dried over Na_2SO_4. Solvent removal under vacuum afforded a crude residue which was purified by column chromatography on silica gel. Elution with 95:5 hexane−EtOAc (R_f = 0.28) delivered ester **9** (450 mg, 80%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, br t), 1.25−1.70 (16H, m), 1.45 (4H, m), 1.60 (2H, m), 2.25 (2H, dt, J_1 = 7.4 Hz, J_2 = 7.7 Hz), 2.35 (2H, t, J = 7.4 Hz), 2.60 (2H, t, J = 7.3 Hz), 3.65 (3H, s), 7.07 (1H, t, J = 7.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (s), 151.9 (s), 136.3 (d), 51.4 (q), 34.1 (t), 31.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.1 (t), 28.2 (t), 27.9 (t), 27.9 (t), 26.3 (t), 24.9 (t), 22.4 (t), 13.9 (q); IR (CH_2Cl_2) 2928, 2855, 1740, 1522, 1336, 1198, 1171 cm^{-1}; ESI-MS calcd for $C_{19}H_{35}NO_4$ 341.26 [M], found 342.3 [$(M + H)^+$, 65], 359.3 [$(M + H_2O)^+$, 25], 705.2 [$(2M + Na)^+$, 100]; HRMS $C_{19}H_{35}NO_4$ calcd 341.2566, found 341.2571.

Representative Procedure of Methyl Ester Enzymatic Hydrolysis: (*E*)-12-Nitrooctadec-12-enoic acid (4). Nitro-acid methyl ester **9** (200 mg, 0.58 mmol) was dissolved in HPLC grade MTBE (40 mL), and HPLC grade H_2O (0.53 mL, 29 mmol) was added. To the resulting stirred solution was added solid-supported CAL-B (40 mg), and the suspension was gently stirred at 35 °C for 18 h. The enzyme was filtered off over a sintered glass funnel, and the solid was carefully washed with MeCN−MTBE (1:1, 4 × 15 mL). Filtrates were collected and evaporated under vacuum (*CAUTION: without heating*); silica gel column chromatography (7:3 hexane−EtOAc, R_f = 0.25) of the residue afforded pure acid **4** (171 mg, 90%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, t, J = 6.8 Hz), 1.30 (16H, m), 1.50 (4H, m), 1.65 (2H, m), 2.25 (2H, dt, J_1 = 7.4 Hz, J_2 = 7.7 Hz), 2.45 (2H, br t), 2.60 (2H, t, J = 7.3 Hz), 7.06 (1H, t, J = 7.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.7 (s), 136.2 (d), 31.3 (t), 29.2 (t), 29.1 (t), 29.0 (t), 28.9 (t), 28.0 (t), 27.8 (t), 27.7 (t), 26.1 (t), 24.5 (t), 22.2 (t), 13.7 (q); IR (CH_2Cl_2) 2927, 1710, 1520, 1460, 1336, 1112, 725 cm^{-1}; LCMS m/z 326.6 [$(M - H)^-$, 22], 653.6 [$(2M - H)^-$, 100], 350.3 [$(M + Na)^+$, 25], 677.2 [$(2M + Na)^+$, 100], 693.3 [$(2M + K)^+$, 40]; HRMS $C_{18}H_{33}NO_4$ calcd 327.2410, found 327.2422.

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Supporting Information Available: Experimental procedures and spectral data for acids **10**−**16** and **2**, including ^{1}H and ^{13}C NMR. This material is available free of charge via the Internet at http://pubs.acs.org.