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Stereocontrolled Synthesis of the PPAR-γ Agonist 10-Nitrolinoleic Acid

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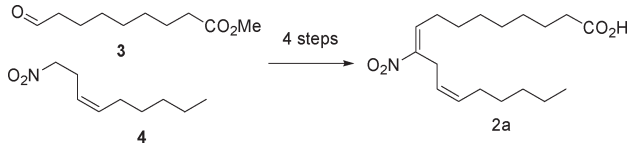

The naturally occurring PPAR-γ ligand 10-nitrooctadeca-9(*E*),12(*Z*)-dienoic acid (10-nitrolinoleic acid) (**2a**) was prepared as a single regio- and geometrical isomer in a practical eight-step, convergent sequence. The synthetic route featured a nitro aldol reaction between 9-oxononanoic acid methyl ester (**3**) and 1-nitronon-3(*Z*)-ene (**4**) in the key carbon−carbon bond forming step. The ability of **2a** (and its methyl ester **9**) to bind to PPAR-γ in a ligand-binding assay is reported.

The peroxisome proliferator activated receptor-γ (PPAR-γ), first identified in the late 1980s, is a transcription factor belonging to the steroid, thyroid, and retinoid receptor super family.[1] Members of this class control lipid and glucose cellular homeostasis, and as such, PPAR-γ has clinical relevance since it is the biological target for the thiazolidindione (TZD) class of drugs, which are widely prescribed to combat the symptoms of type 2 diabetes.[2] In 1995, two groups independently suggested that its natural ligand was the cyclopentenone prostanoid $\Delta^{12,14}$-15-deoxy-PGJ$_2$.[3] However, this proposal has not been universally accepted since the in vivo detectable levels of this prostanoid are significantly lower than the dose required to affect its appreciable activation.[4] More recently, it has been shown that unsaturated nitrated lipids (for example, **2a** and **2b**, Figure 1), which have been postulated to form from nitric oxide by an unknown biochemical pathway, are potent PPAR-γ activators at concentrations below those detected within cells.[5] In 2008, an X-ray crystallographic study demonstrated that a mixture of 10-nitrolinoleic acid (**2a**) and 12-nitrolinoleic acid (**2b**) bind within the same groove in PPAR-γ as the TZD rosiglitazone (for further discussion, see below).[6] Syntheses of the regioisomers and geometrical isomers of the monounsaturated nitro olefins stemming from oleic acid have been reported.[7] However, to date, a regiodefined and stereoselective synthesis of the linoleic acid derived congener has not. The reported synthesis, relying on the mercury(II)- and selenium-based nitration of **1**, is cumbersome and requires preparative HPLC separation of the regioisomeric 9-, 10-, 12-, and 13-nitro olefinic products.[5b,8]

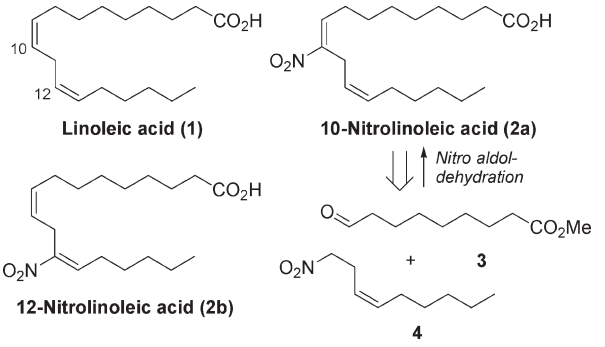

FIGURE 1. Structures of linoleic acid (**1**) and 10-nitrolinoleic acid (**2a**) and its retrosynthesis based on the nitro aldol reaction.

As a consequence of the potent reported biological activities of **2**, our interest[9] in the activation of PPAR-γ, and the unmet need for chemically pure samples of **2a**, we designed a synthesis based on the classic nitro aldol (Henry) reaction. Thus, it was envisaged that **2** might be assembled in a straightforward manner from aldehyde **3** and (*Z*)-olefin **4**.

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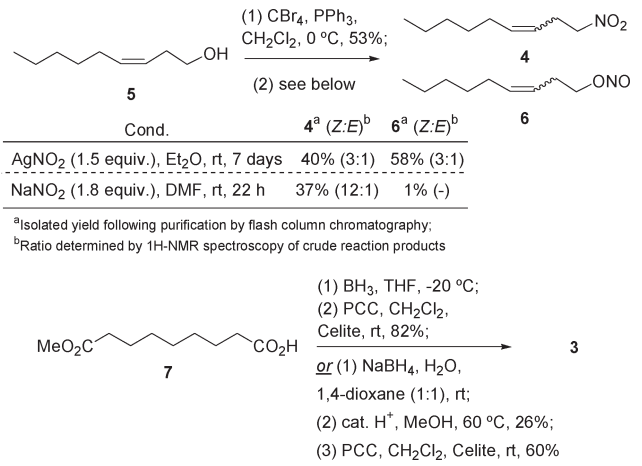
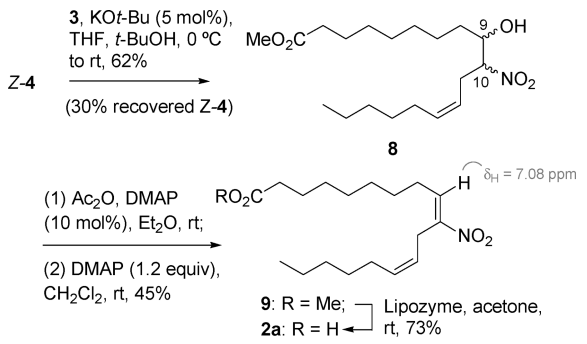
SCHEME 1. Nitration of Alcohol 5 and Preparation of Aldehyde 3



SCHEME 2. Formation of 10-Nitrolinoleic Acid (2a)



As shown in Scheme 1, the required (*Z*)-nitro olefin **4** was prepared in two steps from the commercially available alcohol **5**. Thus, bromination of **5** followed by treatment with $NaNO_2$ in DMF (often termed the Kornblum reaction) gave **4**. In relation to this sequence, negligible amounts of the nitrite ester **6** were detected under these reaction conditions, and only minor amounts of alkene isomerization were detected. However, this observation was in contrast with the corresponding $AgNO_2$-mediated process (Victor Meyer reaction) which gave both **4** and **6**, which proved chromatographically separable, and more significantly double-bond isomerization ((*Z*)-**4**:(*E*)-**4**; 3:1).

Resubmission of **6** to the $NaNO_2$−DMF conditions did not result in formation of **4**, suggesting a lack of reversibility of this process under the reaction conditions (Scheme 1).

The aldehyde aldol reaction partner **3** was prepared from mono methyl azelate **7** by either a borane reduction−oxidation sequence, or following a reduction−esterification−oxidation sequence (Scheme 1).

The pivotal nitro aldol reaction was performed according to Denmark's protocol using substoichiometric amounts of KO-*t*-Bu.[10] This gave **8** in reasonable yield as a mixture of diastereoisomers (Scheme 2). Optimal conditions for the formation of **9** (R = Me) from **8** were found to be acetylation followed by solvent exchange and addition of 1.2 equiv of DMAP to facilitate acetoxy elimination.[7] Thus, **9** could be isolated in 45% yield as a single geometrical isomer based on the characteristic vinylic chemical shift for the nitro olefin proton.[7b] Following chromatographic purification, the intermediate acetoxy derivative was also isolated (26%) and could be resubmitted to the elimination process. In relation to this process, the use of 3 equiv of DMAP in the second step, acetoxy elimination, gave **9** in diminished yield (30%). The sensitive methyl ester **9** was then converted into 10-nitrolinoleic acid (**2a**) following an enzyme-mediated ester hydrolysis protocol in 73% yield.[9]

Apart from the issues surrounding the previously described methods for the synthesis of nitro olefins derived from linoleic acid, inspection of the X-ray crystal structure of PPAR-γ bound to a mixture of **2a** and **2b**[6] indicated that **2a** was oriented in an arrangement in which the nitro group interacted electrostatically with an arginine residue (Arg288). This in turn served to place the electrophilic β-carbon of **2a** in close proximity to a cysteine residue (Cys285) within the binding cleft. Since it is widely appreciated that nitro olefins undergo rapid conjugate addition with nucleophilic reagents such as thiol-containing species, etc.,[11,12] the fact that the crystal structure, solved at 2.4 Å resolution, does not detect a covalent linkage between PPAR-γ and **2a** seems surprising (Scheme 3). In relation to this point, it is argued that the formation and reservoir of such nitrated lipids reside in the cell membrane, which protects them from reaction with adventitious nucleophilic species such as water and glutathione.[5,8] The nature of this type of ligand−PPAR-γ binding mode is extremely relevant since it has been suggested that the conformation adopted by the ligand and the way it interacts with PPAR-γ, on a molecular scale, may determine the precise nature and level of the subsequent gene transcription.[6,13]

With samples of **9** and **2a** in hand, the ability of these lipids to bind to human PPAR-γ was investigated in a cell-free PPAR-γ binding assay.[14] On the basis of the displacement of radiolabeled rosiglitazone, IC_{50} values of 0.22 and 0.11 μM were calculated for **2a** and **9**, respectively.

Experimental Section

(*Z*)-Methyl 9-hydroxy-10-nitrooctadec-12-enoate (8). Under nitrogen, potassium *tert*-butoxide (0.02 g, 0.18 mmol) was added to a solution of methyl 9-oxononanoate (**3**) (0.38 g, 2.04 mmol), (*Z*)-1-nitronon-3-ene (**4**) (0.34 g, 1.99 mmol) in THF (1 mL), and 2-methyl-2-propanol (1 mL) at 0 °C. The mixture was stirred for 15 min at 0 °C and then allowed to stir for

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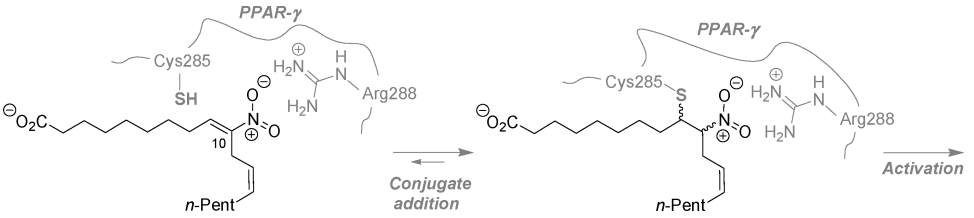
SCHEME 3. Plausible Mechanism for the Activation of PPAR-γ by the Electrophilic Nitro Lipid 2a



24 h at room temperature. Water (10 mL) and diethyl ether (10 mL) were added, and the solution was transferred to a separating funnel where it was washed with a saturated aqueous solution of $NaHCO_3$ (10 mL), brine (10 mL), and diethyl ether (2×10 mL). The combined organic layers were dried with $MgSO_4$ and filtered, and the solvent was removed in vacuo. Purification by silica gel column chromatography (pentane/diethyl ether 19:1 to 9:1) yielded the product **8** (0.45 g, 62%) as a mixture of diastereoisomers. Nitro compound **4** (0.11 g, 30%) was also recovered: $R_f = 0.03$ (pentane/diethyl ether 9:1); IR (film) 3450, 3100, 2929, 2857, 1739, 1551, 1438, 1377, 1201, 1169, 1107, 1030, 877 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, $J = 8$ Hz, 3H), 1.29−1.63 (m, 18H), 2.03 (broad q, $J = 8$ Hz, 2H), 2.28 (t, $J = 8$ Hz, 2H), 2.54−2.61 (m, 1H), 2.78−2.92 (m, 1H), 3.67 (s, 3H), 3.85−3.94 (m, 0.6H), 4.03−4.09 (m, 0.4H), 4.41−4.47 (m, 1H), 5.25−5.33 (m, 1H), 5.54−5.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 24.8, 25.3, 26.3, 27.2, 27.25, 28.6, 28.95, 29.0, 29.05, 29.10, 29.15, 31.45, 31.50, 33.2, 33.6, 34.0, 51.5, 71.6, 72.1, 91.9, 92.2, 121.5, 122.1, 135.0, 135.3, 174.3; HRMS $C_{19}H_{36}NO_5$ calcd 358.2593, found 358.2579.

(9*E*,12*Z*)-Methyl 10-Nitrooctadeca-9,12-dienoate (9). Under nitrogen, a solution of (*Z*)-methyl 9-hydroxy-10-nitrooctadec-12-enoate (**8**) (169 mg, 0.49 mmol), DMAP (6 mg, 0.05 mmol), and acetic anhydride (0.06 mL, 0.63 mmol) was stirred in diethyl ether (1 mL) for 6 h and then concentrated. DMAP (73 mg, 0.60 mmol) was added to a solution of the crude diastereoisomeric nitroacetates in CH_2Cl_2 (2 mL), and the solution was stirred at room temperature for 24 h. The solution was diluted with CH_2Cl_2 (10 mL) followed by washing with water (10 mL), 0.1 M HCl (10 mL), brine (10 mL), and CH_2Cl_2 (2×10 mL). The combined organic layers were dried with $MgSO_4$ and filtered, and the solvent was removed in vacuo. Purification by silica gel column chromatography (pentane/diethyl ether 15:1) yielded the product **9** (72 mg, 45%) as a clear oil. Further elution yielded the nitroacetates (42 mg, 26%) (HRMS $C_{21}H_{38}NO_6$ calcd 400.2699, found 400.2679): $R_f = 0.5$ (pentane/diethyl ether; 9:1); IR (film) 3050, 2928, 2857, 1742, 1643, 1556, 1436, 1371, 1227, 1162, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, $J = 8$ Hz, 3H), 1.26−1.66 (m, 16H), 2.12 (q, $J = 8$ Hz, 2H), 2.24 (q, $J = 8$ Hz, 2H), 2.31 (t, $J = 8$ Hz, 2H), 3.34 (d, $J = 8$ Hz, 2H), 3.67 (s, 3H), 5.21−5.29 (m, 1H), 5.46−5.54 (m, 1H), 7.08 (t, $J = 8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 24.82, 24.83, 27.4, 27.9, 28.4, 28.93, 28.95, 29.0, 29.1, 31.5, 34.0, 51.4, 123.2, 133.0, 136.4, 150.6, 174.1; HRMS $C_{19}H_{34}NO_4$ calcd 340.2488, found 340.2471.

(9*E*,12*Z*)-10-Nitrooctadeca-9,12-dienoic Acid (2a). Lipozyme TL 100 L (400 mg) was added to a solution of nitrated linoleic acid methyl ester **9** (16 mg, 0.049 mmol) in acetone (0.6 mL) and aqueous phosphate buffer (6 mL, pH 7.4). The solution was vigorously stirred at room temperature for 24 h before acidification with 1 M HCl solution (pH 3) and extraction with diethyl ether (5×10 mL). The combined organic layers were dried with $MgSO_4$ and filtered, and the solvent was removed in vacuo. Purification by silica gel column chromatography (pentane/diethyl ether 5:1) yielded the title compound **2a** (11 mg, 73%) as a yellow oil.[8] Methyl ester **9** (4 mg, 25%) was also recovered: $R_f = 0.07$ (pentane/diethyl ether 5:1); IR (film) 3278, 3017, 2918, 2850, 1710, 1522, 1336, 911 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83−0.95 (m, 3H), 1.18−1.67 (m, 16H), 2.13 (q, $J = 8$ Hz, 2H), 2.24 (q, $J = 8$ Hz, 2H), 2.36 (t, $J = 8$ Hz, 2H), 3.34 (d, $J = 8$ Hz, 2H), 5.21−5.30 (m, 1H), 5.46−5.54 (m, 1H), 7.08 (t, $J = 8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 24.6, 24.9, 27.4, 27.9, 28.4, 28.9, 28.95, 29.0, 29.1, 31.6, 33.4, 123.2, 133.0, 136.4, 150.7, 176.9; HRMS $C_{18}H_{32}NO_4$ calcd 326.2331, found 326.2318.

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Supporting Information Available: Experimental details and copies of proton, carbon NMR spectra and biological data. This material is available free of charge via the Internet at http://pubs.acs.org.