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Synthesis of Nitrolipids. All Four Possible Diastereomers of Nitrooleic Acids: (*E*)- and (*Z*)-, 9- and 10-Nitro-octadec-9-enoic Acids

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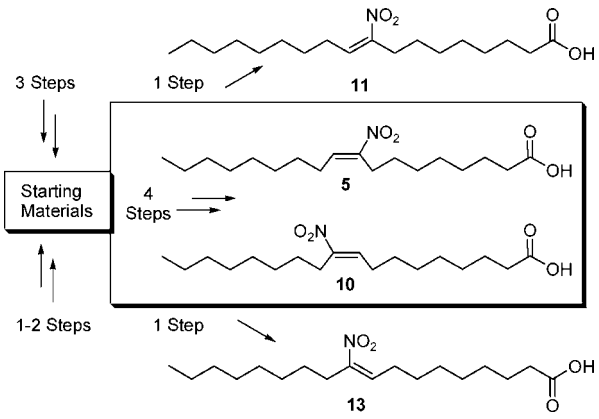
ABSTRACT



Unsaturated fatty acids are nitrated endogenously to produce nitrated lipids. Recent studies have shown that these nitrated lipids have high chemical reactivity and profound biological implications. We report an efficient, scalable synthesis which is regiospecific and stereoselective for all possible isomers of nitrated oleic acid: (*E*)- and (*Z*)-, 9- and 10-octadec-9-enoic acids.

Nitric oxide[1] and oxidative stress generate reactive nitrating species (RNS).[2,3] Nitrated lipids are produced by the reaction of RNS with lipids. These nitrated lipids have been shown to transduce nitric oxide signaling[4] and to react via a Michael addition to form reversible adducts with nucleophiles including thiols and imidazoles.[5] We recently reported[6] that nitrated lipids can act as hormone-like signaling molecules in specific PPAR-γ activation and demonstrated nitrated lipids as a natural ligand of this key homeostatic and metabolic control point. As part of our comprehensive effort to study the chemistry and biochemistry of nitrated lipids, we have been

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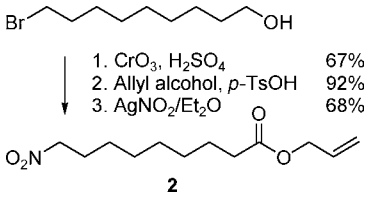
developing concise regio- and stereochemically pure syntheses of all possible isomers, which are essential to dissect the biological targets of these compounds. The synthesis of two of the natural isomers of nitrooleic acid (OA−NO_2, **5** and **10**) was recently reported.[7] Herein, we discuss our regiospecific and stereoselective general strategy for all four possible nitrated oleic acids: (*E*)- and (*Z*)-, 9- and 10-octadec-9-enoic acids (**5**, **10**, **11**, **13**).

Direct nitration of lipids by production of radical NO_2 or cationic $^+NO_2$ has been used to synthesize nitrated linoleic and oleic acids.[8] These methods, although useful, produce a mixture of isomers in low yield, along with byproducts such as nitronate esters and allylic nitroalkanes. Workers more recently synthesized nitrooleic acid by phenylselenation and nitration of oleic acid followed by oxidative elimination to produce a statistical distribution of (*E*)-9- and 10-nitro regioisomers (**5** + **10**).[6] Our current approach is built around a nitroaldol addition (Henry reaction),[9] which fixes the regiochemistry of the nitro group by combining known precursors. Activation/dehydration then forms the nitroalkene moiety by activating the hydroxyl group and eliminating via catalytic base.[10] Under mild conditions, this reaction gives clean stereoisomers and few side products. Finally, an additional isomerization step affords the alternative stereoisomers.

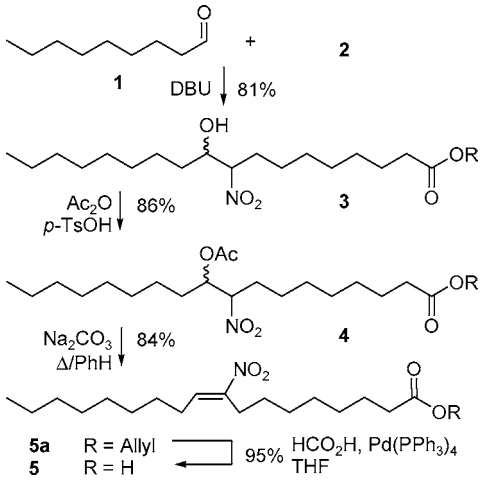
Synthesis of (*E*)-9-Nitro-octadec-9-enoic Acid (5). Synthesis of **5** began with commercially available nonyl aldehyde (**1**) and 9-bromononanol (Scheme 1). 9-Bromononanol was

Scheme 1. Synthesis of Precursor **2**



oxidized[11] (Jones' reagent, 67%) to the carboxylic acid, protected as the allyl ester (92%), and nitrated by the method of Kornblum[12] (68%) to yield 9-nitro-nonanoic acid, allyl ester (**2**), in overall 42% yield.

Scheme 2. Synthesis of (*E*)-9-Nitro-octadec-9-enoic Acid (**5**)



The nitroaldol condensation of **1** and **2** (Scheme 2) was performed neat[13] with a catalytic amount (10 mol %) of DBU as base affording β-hydroxynitro **3** in good yield (81%) as a 1:1 mixture of diastereomers.[14] β-Hydroxynitro ester **3** was acetylated[10a] in acetic anhydride with a catalytic amount of *p*-toluenesulfonic acid to produce β-acetoxynitro ester **4** in high yield. Finally, the protected nitroalkene[15] (**5a**) was generated by base-induced (0.5 equiv of sodium carbonate) elimination with azeotropic removal of water, to give stereoselectively clean (*E*)-isomer nitroalkenes in 84% yield without isomerization or deconjugation of the double bond to form allylic nitroalkanes. The elimination occurs by an E2 or E1cb mechanism. Although the reaction produced a clean (*E*)-isomer, this could also be the result of rapid isomerization of any (*Z*)-isomer formed to an (*E*)-isomer during the long reaction time. However, observation of the reaction progress by ^{1}H NMR (a tube-scale reaction) showed no intermediate formation of a (*Z*)-isomer.

The base sensitivity of both nitroalkanes and nitroalkenes led us to use consistently acidic conditions when possible throughout the reaction series, in both reaction and workup. Common deallylation methods[16] and reagents are incompatible with nitroalkenes (basicity and/or nucleophilicity). We found that palladium-catalyzed isomerization in the presence of formic acid[17] as a hydride donor promoted facile ester cleavage and recovery of the free acid in nearly quantitative yield (95%), affording **5** in 56% yield for four steps.

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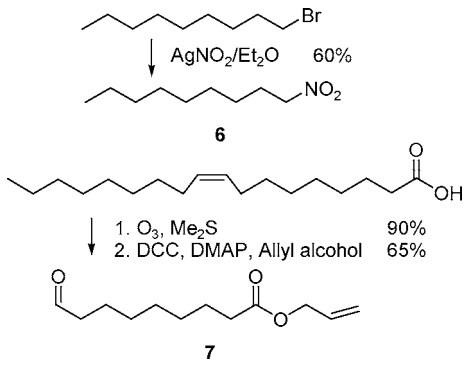
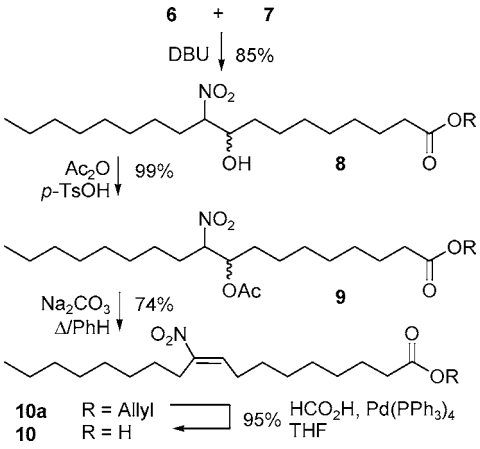
Synthesis of (*E*)-10-Nitro-octadec-9-enoic Acid (10). For the alternative regioisomer, we began with conversion of 1-bromononane to 1-nitrononane[12] (**6**, 60%) and ozonolysis of oleic acid to afford 9-oxononanoic acid[18] and nonyl aldehyde (**1**). The acid was isolated and then protected as the allyl ester via a DCC/DMAP-promoted esterification to afford 9-oxononanoic acid, allyl ester (**7**), in 59% yield for two steps (Scheme 3).

Scheme 3. Synthesis of Precursors **6** and **7**



The nitroalkene moiety is isolated from any other functional group in the molecule, allowing both regioisomers to react completely in parallel. A DBU-promoted nitroaldol condensed **6** and **7** (Scheme 4) to afford **8**, which was

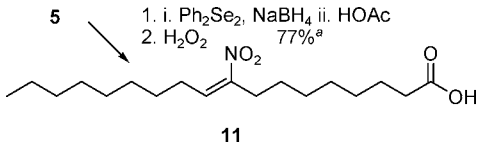
Scheme 4. Synthesis of (*E*)-10-Nitro-octadec-9-enoic Acid (**10**)



subsequently acetylated (**9**), dehydrated, and deprotected to afford free acid **10** in 59% yield for four steps. Upon deprotection, we confirmed our product nitrooleic acids **5** and **10** to be identical with previous synthetic samples.[6,8]

Synthesis of (*Z*)-9-Nitro-octadec-9-enoic Acid (11). Next, we focused on the two (*Z*)-stereoisomers (**11** and **13**, 9- and 10-nitro-elaidic acids). The (*Z*)-isomers can be produced endogenously and may be used to probe the geometry of specific binding/activity sites. Although many nitroalkene-forming elimination methods produce a mixture of stereoisomers, the poor control and difficulty of separation of isomers make stepwise isomerization preferable. Mixtures of isomers can be preferentially converted to the (*E*)-isomer via known methods.[19] The (*E*)-isomers of nitroalkenes are known to be thermodynamically more stable. This is a result of the 1,3 allylic strain imposed by the large nitro group's steric interaction with the opposite allylic position, which is minimized in the (*E*) conformation. Controlled conversion of (*E*) to (*Z*) is complementary to the clean formation of the initial (*E*)-nitroalkene. Michael addition of phenyl selenide to the nitroalkene followed by low-temperature protonation yielded a predominantly *anti*-*β*-nitroselenide intermediate. Subsequent oxidation of the phenylselenide resulted in regiospecific syn elimination of phenylselenic acid, producing the (*Z*)-nitroalkene. Protected (*E*)-isomer **5a** was isomerized via a modification of the method of Ono[20] to the corresponding (*Z*)-isomer **11a**.[21] Unfortunately, final deprotection of **11a** by the standard deallylation method proceeded with complete reversion of the stereochemistry of the nitroalkene to the (*E*)-isomer **5**. Subsequent investigation showed this to be the result of the triphenylphosphine present in the palladium catalyst. A catalytic amount of a nucleophile is sufficient to reisomerize the (*Z*)-isomer to (*E*), by reversible Michael addition to the nitroalkene which then permits free rotation to the less sterically crowded (*E*)-isomer upon elimination.[19] Instead, the most direct route[22] (Scheme 5) was to first

Scheme 5. Synthesis of (*Z*)-(9)-Nitro-octadec-9-enoic Acid (**11**)



[a] Isolated yield; isomer ratio of 82:18 by ^{1}H NMR.

deprotect the nitroalkene (to **5**) and then isomerize the free acid. This approach produced (*Z*)-nitrolipid **11** in reasonable yields (77% yield, (*Z*) content typically 80−90% by ^{1}H NMR). The mixed-isomer product was purified by repeated flash chromatography to provide analytically pure samples of the (*Z*)-isomer which were used for characterization.

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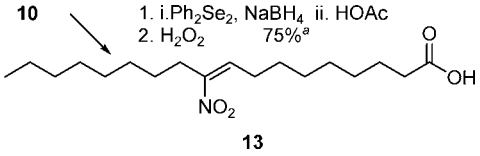
(21) Note that in contrast to their IUPAC nomenclature the (*Z*)-isomers are sometimes referred to as "trans" nitrated fatty acids to identify the parent fatty acid structural motif: see ref 6.

(22) It is also possible to form the *β*-nitroselenide, deprotect, then oxidize and eliminate the phenylselenous acid.

Greater efficiency in separation is expected for preparatory HPLC.

Synthesis of (*Z*)-10-Nitro-octadec-9-enoic Acid (13). The alternative regioisomer again reacted in parallel. The phenylselenide addition/elimination strategy was repeated with **10** (Scheme 6) to afford **13** directly in 75% isolated yield

Scheme 6. Synthesis of (*Z*)-(10)-Nitro-octadec-9-enoic Acid (**13**)



13

[a] Isolated yield; isomer ratio of 85:15 by ^{1}H NMR.

and 85% (*Z*)-isomer by ^{1}H NMR. Again, an analytical sample of the pure (*Z*)-isomer was isolated by flash chromatography.

In summary, we have developed a modular, scalable synthesis which is mild and efficient for all desired nitrooleic acids with regio- and stereochemical control, with yields of 56−59% (*E*) and 42−44% (*Z*) from the nitroaldol step. Current work to extend this methodology to the synthesis of additional nitrated fatty acids as molecular probes and their application for the study of their biological activity is underway.

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Supporting Information Available: Complete synthetic methods along with ^{1}H and ^{13}C spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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