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Synthesis of 9- and 12-nitro conjugated linoleic acid: Regiospecific isomers of naturally occurring conjugated nitrodienes

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ABSTRACT

Conjugated diene-containing fatty acids (rumenic and rumelenic acids) are major substrates for nitration under physiological conditions. Their nitrated products are present in human urine. These nitrodiene-containing lipid electrophiles contain a strongly electron-withdrawing pair of conjugated double bonds amenable to nucleophilic attack in biological milieu, which affords them pluripotent signaling capabilities. We report synthetic methods to obtain useful quantities of three main biological nitrated fatty acids (9- and 12-nitro-conjugated linoleic acids and 9-nitro-conjugated linolenic acid) in six or seven steps from commercially available starting materials, for biological evaluation of these naturally occurring biomolecules.

Conjugated fatty acids are common dietary components enriched in dairy products composed mainly of rumenic acid (conjugated linoleic acid, 9,11-CLA) [1–4] and, to a lesser extent, rumelenic acid (conjugated α-linolenic acid, 9,11,15-CLNA). Under normal physiological conditions, they are nitrated in the gastric compartment [5–6] producing 9- and 12-nitrooctadeca-9,11-dienoic acids (i.e., 9-NO_2-CLA (**5**) and 12-NO_2-CLA (**11**), Fig. 1), 9- and 12-nitrooctadeca-9,11,15-trienoic acids (i.e., 9-NO_2-CLNA (**15**) and 12-NO_2-CLNA), and are commonly detected in human plasma and urine [6,7]. These electrophilic lipids participate in Michael addition reactions with cysteine thiolates *in vivo* to regulate their enzymatic activity and function [8]. As naturally occurring biomolecules with unusual electrophilic signaling activities, we viewed it important to develop methods to obtain useful quantities of single-regioisomer nitro conjugated linoleates and linolenates for biological and pharmacological evaluation.

Nitroalkene-containing fatty acids (nitrolipids, nitro fatty acids, NO_2-FA) have been of considerable recent interest owing to their detection in plants and mammals, cell signaling, and pharmacological value [9]. Initial work with nitro-oleic [10] (NO_2-OA) and nitro-linoleic [11,12] (NO_2-LA) acids demonstrated the modulation of the activity of key homeostatic and inflammatory regulatory proteins, including PPAR-γ [13], NFκB [14], Keap1-Nrf2 [15,16], and STING [17] through reversible thia-Michael addition [18] of critical cysteine residues of these proteins to the nitroalkene [19]. Standards for both compounds were initially produced by nonspecific methods that gave mixed regioisomers. The biological detection and signaling effects motivated developing a synthetic strategy for specific regioisomers, which we [20] and others [21–24] accomplished for several nitrated lipids. The evaluation of single-isomers [13,25] have demonstrated differing activity in various biological contexts, with some isomers being highly unstable [26]. Recent work on the biological role of these NO_2-FA has shown them to have unique pluripotent effects on STING [17,27] and certain cancer cell types [28,29]. More definitive mechanisms of cell signaling and gene expression responses should come from an ongoing blinded, placebo-controlled evaluation of 10-nitro oleic acid in a Phase II clinical trial for the treatment of obesity-related asthma [30,31].

The highest yields for the physiological nitration of fatty acids are obtained in the stomach during digestion and occurs almost exclusively in fatty acids containing conjugated double bonds [5,32]. Nitration is highly prevalent during gastric digestion given the combination of saliva-derived nitrite in the acidic milieu and the availability of dietary conjugated fatty acids, forming mainly 9-NO_2-CLA and 12-NO_2-CLA (Fig. 1: **5** and **11**), each containing a 1-nitro-1,3-diene. The metabolism reported for other nitrated fatty acids—a combination of enzymatic nitroalkene to nitroalkane

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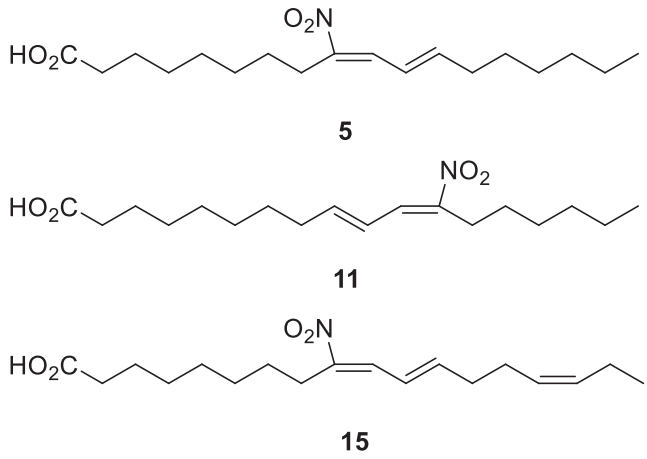


Fig. 1. Structures of synthetic targets 9- and 12-NO_2-CLA (**5** and **11**) and 9-NO_2-Rumelenic acid (**15**).

reduction [33], addition to glutathione and metabolic β-oxidation [34]—are also major inactivation and excretion mechanisms of these compounds as evidenced by their presence in human urine [7,32]. Nitrodienes [35] themselves are unusually reactive motifs in biological systems; strongly electrophilic and prone to add to available thiols by reversible kinetically and thermodynamically-driven reactions [8].

A prior report on NO_2-CLA synthesis included a direct biomimetic conversion of conjugated (9,11)-linoleic acid to nitro conjugated linoleic acid [36], which produced NO_2-CLA as a mixture of two positional isomers. The need to further evaluate the role of specific NO_2-CLA isomers motivates the synthesis of 9-NO_2-CLA (**5**) and 12-NO_2-CLA (**11**), the two most common isomers of NO_2-CLA [5] found *in vivo*. Moreover, rumelenic acid is not commercially available to be used as a substrate for biomimetic nitration reactions. In this work, we describe our regiospecific synthesis of the three major biologically-detectable nitro fatty acids.

The core of our synthetic design was the assembly of the nitrodiene and protection/deprotection of the free fatty acid. The major functional group is the nitrodiene (1-nitro-1,3-diene) moiety present in both isomers, which would be the product of condensation between a nitroalkane and an α,β-unsaturated aldehyde (Fig. 2).

Nitroalkenes are frequently produced from β-nitro-alcohols, themselves the products of nitroaldol condensation between a primary nitroalkane and an aldehyde. Literature methods [37–39] for nitroaldol-type reactions only infrequently used α,β-unsaturated aldehydes, and were frequently reacted with high molar excess of a simple nitroalkane (often nitromethane). The less common nitrodiene has been formed by this approach but the intermediates as well as the final product are noticeably less stable, and there are additional side reactions available at each step of the synthesis, such as the 1,4-conjugate addition as an unproductive side reaction (see discussion below).

Previous work with nitro-fatty acids utilized allyl esters as protecting groups for the carboxylic acid, however to avoid potential complications with a nitrodiene under palladium-catalyzed conditions this work instead utilized *t*-butyl esters which could be removed under mild acidic conditions [40,41].

(9*E*,11*E*)-9-Nitrooctadeca-9,11-dienoic acid (**5**): Key intermediate *t*-butyl 9-nitrononanoate (**1**) was generated (Scheme 1) in two steps from commercially available 9-bromononanoic acid. The acid was esterified *via* oxalyl chloride activation to the acyl chloride and subsequent addition of *t*-butanol in the presence of catalytic DMAP (4-dimethylaminopyridine). Then, the primary bromide of the resulting ester was displaced with silver nitrite in ether over one to two weeks of stirring at room temperature. Purification to remove unreacted starting material (and a nitrite side product, not shown) afforded useful amounts of **1** in two steps, 40% overall yield. Nitroalkyl ester **1** was subsequently coupled to commercially available 2-*trans*-nonenal (**2**) using triethylamine (TEA) as both base and solvent, stirred at rt for two days then cooled to −20 °C and stirred for two additional days. While many literature methods use a large excess of nitroalkane, this was inefficient for our purposes. The desired nitro-allyl alcohol **3** was isolated in 35% yield after chromatographic purification without 1,4 addition product observed. Next, the allylic alcohol group was activated by exposure to trifluoroacetic anhydride (TFAA) in dichloromethane, yielding allylic trifluoroacetyl ester **4**. Upon isolation of crude **4** (95%), the activated trifluoroacetate group could be eliminated by exposure to potassium acetate or propionate (yields 30–40%); however, upon consideration that the efficiency could be enhanced by increased solubility of the carboxylate salt, we tested tetrabutylammonium acetate and obtained an 82% yield of the desired nitrodiene ester, which was deprotected with neat formic acid (51%) to afford free 9-nitro-conjugated linoleic acid (**5**).

(9*E*,11*E*)-12-Nitrooctadeca-9,11-dienoic acid (**11**): The key unsaturated aldehyde starting material for 12-nitro isomer **11**, *t*-butyl (*E*)-11-oxo-undec-9-enoate (**7**), was obtained from 9-decenoic acid in four steps (Scheme 2). Free 9-decenoic acid was readily esterified with *t*-butanol [42] then oxidized to a 9,10-diol in quantitative yield with catalytic osmium tetroxide and *N*-methylmorpholine *N*-oxide (NMO). Given the poor chromatographic behavior of these polar intermediates, the crude diol was preferably used directly in the next step. Oxidative cleavage with sodium metaperiodate afforded the much more tractable *t*-butyl 9-oxononanoate (**6**) for a total of 45% yield over three steps. This nine-carbon aldehyde was then homologated to an eleven-carbon α,β unsaturated aldehyde by a Wittig-type reaction with a stabilized ylide (formylmethylene-triphenylphosphorane), which afforded the desired **7** in 70% yield.

Unsaturated aldehyde ester **7** was subsequently condensed with a two-fold excess of 1-nitroheptane (**8**) using TEA as base and solvent (Scheme 2), affording purified nitro-allyl alcohol **9** in 36% yield. The allylic alcohol group was subsequently trifluoroacetylated (**10**, 77%) and then eliminated with tetrabutylammonium acetate to give the desired nitrodiene ester in 84% yield. Deprotection with neat formic acid produced the desired 12-nitro conjugated linoleic acid **11** in an improved 79% yield.

The final individual NO_2-CLA isomers **5** and **11** were obtained in overall yields of 5.6% and 5.5% over six and seven steps, respectively. The products were identical in all spectroscopic respects

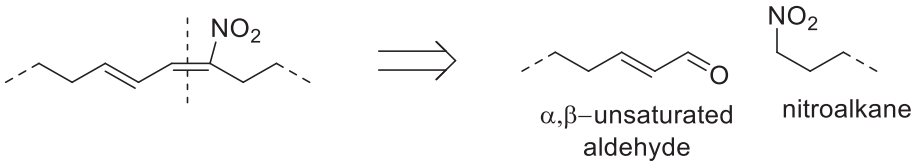
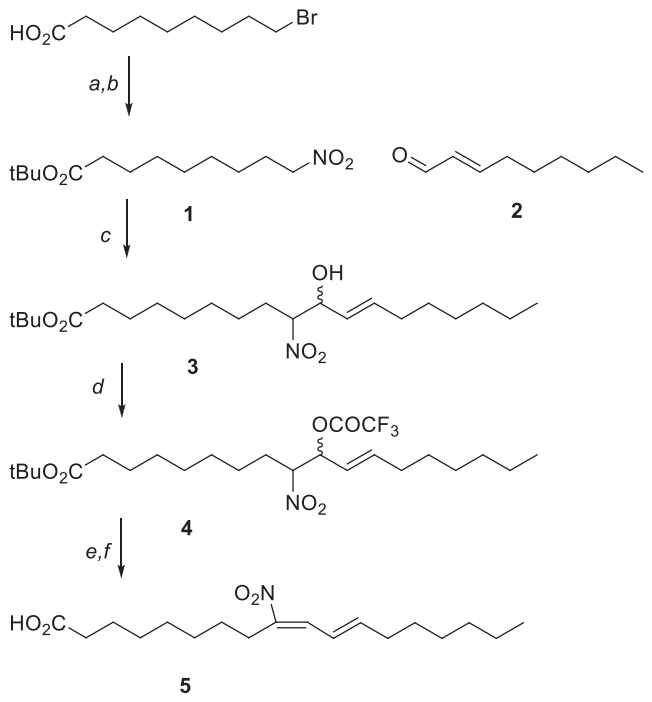


Fig. 2. Retrosynthetic strategy.

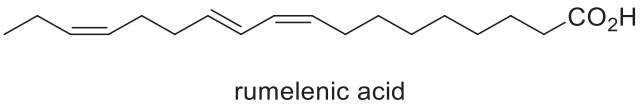


Scheme 1. *Reagents and conditions: a*) $(COCl)_2$, cat. DMF; then *t*-BuOH; *b*) $AgNO_2$/Et_2O, 40% for two steps; *c*) TEA, 35%; *d*) TFAA/CH_2Cl_2, −20 °C, 95% (crd); *e*) tetrabutylammonium acetate (Bu_4NOAc)/Et_2O, 82%; *f*) HCO_2H, 51%.

to the mixture produced by direct nitration of dienes [36]. As discussed in prior work (see Ref. [36]) the nitrodiene was assigned (*E*, *E*) based on the α-proton downfield shift from (*E*)-nitroalkene (δ 7.1 ppm) to δ7.5 ppm as opposed to upfield (*Z*)-nitroalkene (δ 5.6 ppm). Both positional isomers have the same relative configuration and display nearly identical stereoscopic data, reflecting the relative isolation of the nitrodiene moiety from the carboxylate or methyl terminus.

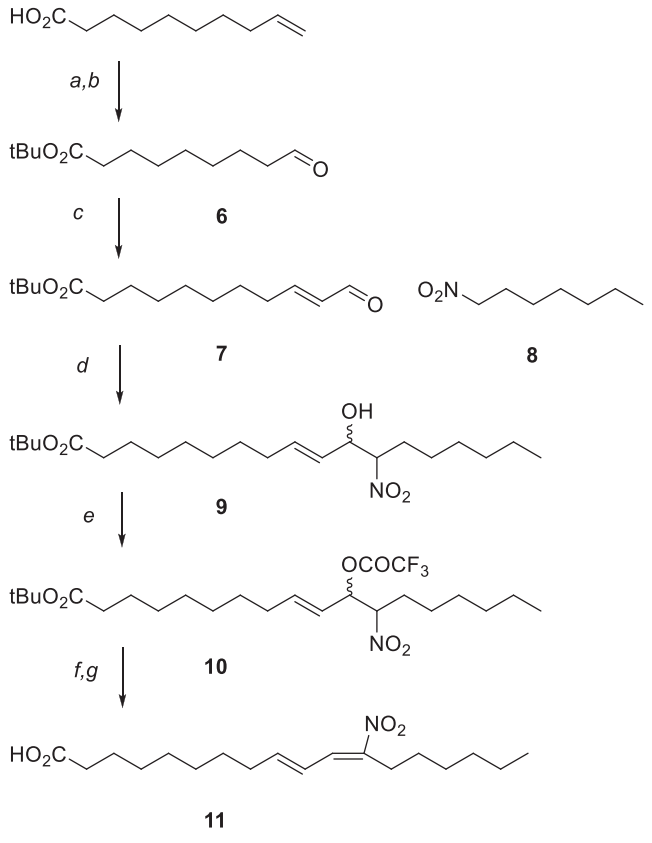
Rumelenic acid (9*Z*,11*E*,15*Z*-octadecatrienoic acid) [43,44] is a triene derived from α-linolenic acid and produced in the rumen of several animals [45–46]. Rumelenic acid contains a (9*Z*,11*E*) diene motif and its nitration products have been identified in human urine, making it an attractive candidate to extend synthetic approaches to include exemplary nitro conjugated linolenic acids (Fig. 3).

(9*E*,11*E*,15*Z*)-9-Nitrooctadeca-9,11,15-trienoic acid (15): Commercially available *cis*-4-heptenal was homologated to *trans, cis*-2,6-nonadienal (**12**) in 59% yield by the same Wittig-type reaction used to afford **7** (Scheme 3). This dienal **12** was condensed with nitroalkyl ester **1** in TEA over 3–5 days. Nitro-allyl alcohol **13** was isolated in 36% yield after workup and chromatography.

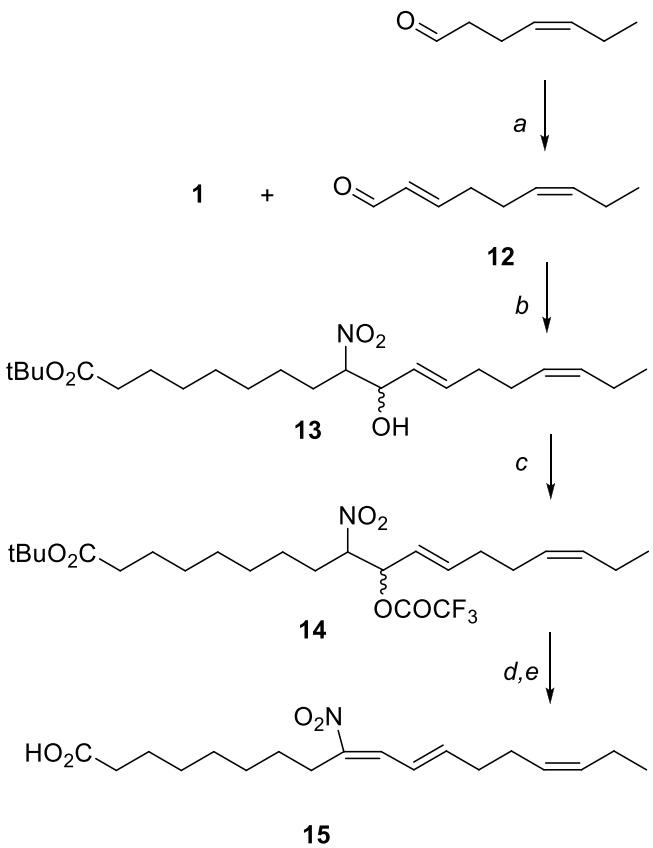


rumelenic acid

Fig. 3. Naturally occurring (9*Z*,11*E*,15*Z*)-rumelenic acid containing a (9*Z*,11*E*) conjugated diene.



Scheme 2. *Reagents and conditions: a*) *t*-BuOH/$MgSO_4$/H_2SO_4 (cat.), 68%; *b*) *i.* OsO_4 (cat.), NMO/Et_2O, quant.; *ii.* $NaIO_4$, 71%; *c*) PPh_3=CHCHO, MeCN, 70%; *d*) TEA, 36%; *e*) TFAA/CH_2Cl_2, 77% (crd); *f*) Bu_4NOAc/Et_2O, 84%; *g*) HCO_2H, 79%.



Scheme 3. *Reagents and conditions: a*) Ph_3P=CHCHO/MeCN, 59%; *b*) *t*-butyl 9-nitrononanoate (**1**)/TEA, 36%; *c*) TFAA/CH_2Cl_2, 95% (crd); *d*) potassium propionate with approx. 10 mol% Bu_4NOAc/Et_2O, 49%; *e*) HCO_2H, 81%.

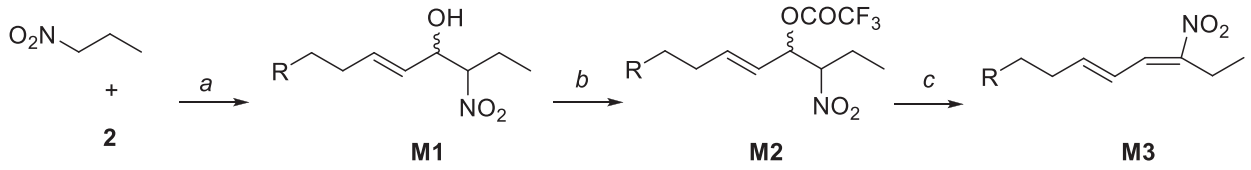
It was activated as trifluoroacetyl ester **14** with TFAA (quantitative), then the crude trifluoroacetyl ester eliminated with potassium propionate (with a catalytic amount of tetrabutylammonium acetate) to produce a 49% yield of the nitrodiene-alkene. Finally, the ester was deprotected with neat formic acid to afford free 9-nitro rumelenic acid **15** in 81% yield (8.0% overall for five steps). The additional nonconjugated double bond was unreactive to these conditions, and no bond migration or oxidation was observed.

The three required steps, condensation, activation and elimination, were also screened on a model nitrodiene (3-nitrododeca-3,5-diene) *via* condensation of readily available *trans*-2-nonenal (**2**) and 1-nitropropane (Scheme 4; also see ESI).

Nitroaldol condensation *via* a 1,2-addition was optimally accomplished using TEA as solvent and base, despite a typical 30–40% yield, with suppressed production of the potential 1,4-product (Fig. 4: 5% or less detected by ^{1}H NMR spectroscopy) and without decomposition or need for large stoichiometric excesses of the nitroalkane. A number of combined acylation/elimination strategies for activation of the β-nitro-allylic alcohol intermediate were explored, such as acetyl and mesyl adducts, which were unsatisfactory either in formation or elimination. Instead, our approach developed out of our previous nonspecific nitrodiene syntheses [36] built on the prior work of Bloom and Mellor [47,48], in which activated β-nitro trifluoroacetyl intermediates were reacted with mild carboxylate bases, and was incorporated into the current design by converting the β-nitro alcohols to β-nitro trifluoroacetates.

The β-nitro-alcohol intermediates were formed in a roughly 2:3 diastereomeric ratio (Fig. 4) as indicated by ^{1}H NMR spectroscopy. The major and minor diastereomers appear to be respectively *syn*- or *anti*-orientation, and the resulting gauche interactions and optimal orientation for E_2 elimination (*anti*-elimination: leaving-group alignment opposite the α-proton being lost) meant one diastereomer would eliminate quite rapidly while the other would be forced into a slower E_{1cb} pathway (Fig. 5). Possibly the trifluoroacetate intermediates could partially eliminate before isolation, as the dr indicated relative loss of the more active minor (*anti*-) diastereomer. Incomplete elimination step reaction mixtures contained unreacted (*syn*-) diastereomer and (*E*,*E*) nitroalkenes. Since both the starting material and products were base-sensitive, optimizing the reaction conditions required encouraging the slower but more abundant diastereomer to react before product decomposition or hydrolysis became competitive. Note the (*E*,*E*) configuration appears to be a thermodynamic product, favored over potential alternative isomers (δ 7.9 ppm) with a greater than 12:1 ratio by ^{1}H NMR spectroscopy.

This approach produced the desired products in 5–6% overall yields and shows potential scalability. Concerns about both the bulk stability of the intermediates and final products encourages



Scheme 4. *Reagents and conditions: a*) TEA, 39%; *b*) TFAA/CH_2Cl_2, 85% (crd); *c*) potassium propionate/Et_2O, 45%. R = $-C_5H_{11}$.

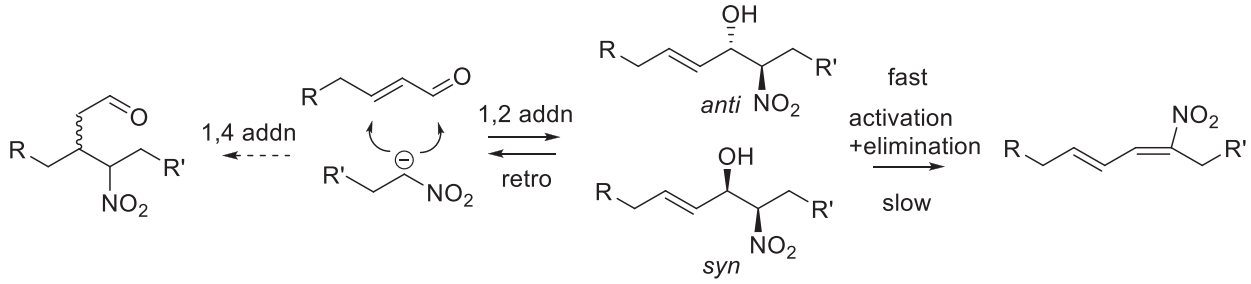


Fig. 4. Stepwise synthesis of the conjugated nitrodiene.

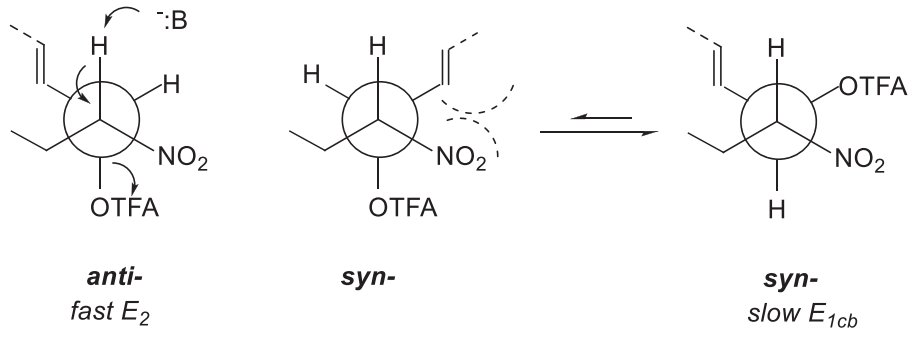


Fig. 5. Diastereomeric considerations in elimination of β-nitro trifluoroacetate intermediates.

caution in large-scale synthesis. The products and intermediates can decompose, oxidize or polymerize upon extended storage without solvent. Particularly notable is the loss of the 5–6 ppm region alkene peaks relative to other features by ^{1}H NMR spectroscopy, that could be used to assess the integrity of the intermediate products. The nitroalcohol and nitro-trifluoroacetate intermediates were reactive but less so than the nitrodiene. It is recommended to move rapidly through the synthetic sequence to minimize opportunities for decomposition. The nitrodiene-containing compounds were sensitive to high concentration and thermal conditions, including rotary evaporation to dryness at elevated temperatures. Importantly, the nitrodienes are stable long-term in dilute solution at low temperatures and the overall synthetic conditions are mild, so this should be extendable to larger-scale work.

This synthetic approach provides additional value by being amenable to isotopic labeling to generate mass spectrometry standards and support metabolic studies. Nonspecific nitration using [^{15}N]-nitrates yields single mass-unit difference mass spectrometry standards. Also, multiple commercially available deuterated analogs of starting materials for **2** and **8** (e.g. heptanal and *n*-bromoheptane) allow for the synthesis of a variety of isotopomers with significant and specific molecular weight variations. These approaches could also be extended to several potential conjugated diene nitro linolenic or other polyunsaturated fatty acids, as well as non-naturally occurring isomers of variable chain length. Future directions include the synthesis of isotopically labeled NO_2-CLA to support analytical method developments and clinical evaluation and quantification of these species, mechanism and products of thia-Michael addition to these nitrodienes, and characterization of nitration products of additional related conjugated diene or triene (CLNA) containing fatty acids.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bruce A. Freeman reports a relationship with Creegh Pharmaceuticals, Inc that includes: equity or stocks. Francisco J. Schopfer reports a relationship with Creegh Pharmaceuticals, Inc that includes: equity or stocks.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.153371.

References

[1] M.W. Pariza, Y. Park, M.E. Cook, The biologically active isomers of conjugated linoleic acid, Prog. Lipid Res. 40 (4) (2001) 283–298.
[2] J.K.G. Kramer, P.W. Parodi, R.G. Jensen, M.M. Mossoba, M.P. Yurawecz, R.O. Adlof, Rumenic acid: A proposed common name for the major conjugated linoleic acid isomer found in natural products Lipids 33 (8) (1998)835–835.
[3] A. Bhattacharya, J. Banu, M. Rahman, J. Causey, G. Fernandes, Biological effects of conjugated linoleic acids in health and disease, J. Nutr. Biochem. 17 (12) (2006) 789–810.
[4] M.A. Belury, Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action, Annu. Rev. Nutr. 22 (1) (2002) 505–531.
[5] G. Bonacci, P.R.S. Baker, S.R. Salvatore, D. Shores, N.K.H. Khoo, J.R. Koenitzer, D. A. Vitturi, S.R. Woodcock, F. Golin-Bisello, S. Watkins, C. St Croix, C.I. Batthyany, B.A. Freeman, F.J. Schopfer, Conjugated linoleic acid is a preferential substrate for fatty acid nitration, J. Biol. Chem. 287 (53) (2012) 44071–44082.
[6] S.R. Salvatore, P. Rowart, F.J. Schopfer, Mass spectrometry-based study defines the human urine nitrolipidome, Free Radical Biol. Med. 162 (2021) 327–337.
[7] S.R. Salvatore, D.A. Vitturi, P.R.S. Baker, G. Bonacci, J.R. Koenitzer, S.R. Woodcock, B.A. Freeman, F.J. Schopfer, Characterization and quantification of endogenous fatty acid nitroalkene metabolites in human urine, J. Lipid Res. 54 (7) (2013) 1998–2009.
[8] L. Turell, D.A. Vitturi, E.L. Coitino, L. Lebrato, M.N. Moller, C. Sagasti, S.R. Salvatore, S.R. Woodcock, B. Alvarez, F.J. Schopfer, The chemical basis of thiol addition to nitro-conjugated linoleic acid, a protective cell-signaling lipid, J. Biol. Chem. 292 (4) (2017) 1145–1159.
[9] A.G.M. Barrett, G.G. Graboski, Conjugated nitroalkenes: versatile intermediates in organic synthesis, Chem. Rev. 86 (5) (1986) 751–762.
[10] P.R.S. Baker, Y. Lin, F.J. Schopfer, S.R. Woodcock, A.L. Groeger, C.I. Batthyany, S. Sweeney, M.H. Long, K.E. Iles, L.M.S. Baker, B.P. Branchaud, Y.E. Chen, B.A. Freeman, Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands, J. Biol. Chem. 280 (51) (2005) 42464–42475.
[11] D.G. Lim, S. Sweeney, A. Bloodsworth, C.R. White, P.H. Chumley, N.R. Krishna, F. Schopfer, V.B. O'Donnell, J.P. Eiserich, B.A. Freeman, Nitrolinoleate, a nitric oxide-derived mediator of cell function: Synthesis, characterization, and vasomotor activity, Proc. Nat. Acad. Sci. USA 99 (25) (2002) 15941–15946.
[12] B. Coles, A. Bloodsworth, S.R. Clark, M.J. Lewis, A.R. Cross, B.A. Freeman, V.B. O'Donnell, Nitrolinoleate inhibits superoxide generation, degranulation, and integrin expression by human neutrophils, Circ. Res. 91 (5) (2002) 375–381.
[13] M.J. Gorczynski, P.K. Smitherman, T.E. Akiyama, H.B. Wood, J.P. Berger, S.B. King, C.S. Morrow, Activation of peroxisome proliferator-activated receptor gamma (PPAR gamma) by nitroalkene fatty acids: importance of nitration position and degree of unsaturation, J. Med. Chem. 52 (15) (2009) 4631–4639.
[14] T. Cui, F.J. Schopfer, J. Zhang, K. Chen, T. Ichikawa, P.R.S. Baker, C.I. Batthyany, B. K. Chacko, X. Feng, R.P. Patel, A. Agarwal, B.A. Freeman, Y.E. Chen, Nitrated fatty acids: endogenous anti-inflammatory signaling mediators, J. Biol. Chem. 281 (47) (2006) 35686–35698.
[15] E. Kansanen, G. Bonacci, F.J. Schopfer, S.M. Kuosmanen, K.I. Tong, H. Leinonen, S.R. Woodcock, M. Yamamoto, C. Carlberg, S. Yla-Herttuala, B.A. Freeman, A.-L. Levonen, Electrophilic nitro-fatty acids activate NRF2 by a KEAP1 cysteine 151-independent mechanism, J. Biol. Chem. 286 (16) (2011) 14019–14027.
[16] E. Kansanen, H.-K. Jyrkkanen, O.L. Volger, H. Leinonen, A.M. Kivela, S.K. Hakkinen, S.R. Woodcock, F.J. Schopfer, A.J. Horrevoets, S. Yla-Herttuala, B.A. Freeman, A.-L. Levonen, Nrf2-dependent and -independent responses to nitro-fatty acids in human endothelial cells: identification of heat shock response as the major pathway activated by nitro-oleic acid, J. Biol. Chem. 284 (48) (2009) 33233–33241.
[17] A.L. Hansen, G.J. Buchan, M. Ruhl, K. Mukai, S.R. Salvatore, E. Ogawa, S.D. Andersen, M.B. Iversen, A.L. Thielke, C. Gunderstofte, M. Motwani, C.T. Moller, A.S. Jakobsen, K.A. Fitzgerald, J. Roos, R.T. Lin, T.J. Maier, R. Goldbach-Mansky, C.A. Miner, W. Qian, J.J. Miner, R.E. Rigby, J. Rehwinkel, M.R. Jakobsen, H. Arai, T. Taguchi, F.J. Schopfer, D. Olagnier, C.K. Holm, Nitro-fatty acids are formed in response to virus infection and are potent inhibitors of STING palmitoylation and signaling, Proc. Nat. Acad. Sci. USA 115 (33) (2018) E7768–E7775.
[18] L.M.S. Baker, P.R.S. Baker, F. Golin-Bisello, F.J. Schopfer, M. Fink, S.R. Woodcock, B.P. Branchaud, R. Radi, B.A. Freeman, Nitro-fatty acid reaction with glutathione and cysteine, J. Biol. Chem. 282 (42) (2007) 31085–31093.
[19] C.I. Batthyany, F.J. Schopfer, P.R.S. Baker, R. Duran, L.M.S. Baker, Y. Huang, C. Cervenansky, B.P. Branchaud, B.A. Freeman, Reversible post-translational modification of proteins by nitrated fatty acids in vivo, J. Biol. Chem. 281 (29) (2006) 20450–20463.
[20] S.R. Woodcock, A.J.V. Marwitz, P. Bruno, B.P. Branchaud, Synthesis of nitrolipids. all four possible diastereomers of nitrooleic acids: (E)- and (Z)-, 9- and 10-nitro-octadec-9-enoic acids, Org. Lett. 8 (18) (2006) 3931–3934.
[21] M.J. Gorczynski, J. Huang, S.B. King, Regio- and stereospecific syntheses and nitric oxide donor properties of (E)-9- and (E)-10-nitrooctadec-9-enoic acids, Org. Lett. 8 (11) (2006) 2305–2308.
[22] E. Dunny, P. Evans, Stereocontrolled synthesis of the PPAR-gamma agonist 10-nitrolinoleic acid, J. Org. Chem. 75 (15) (2010) 5334–5336.
[23] G. Zanoni, M. Valli, L. Bendjeddou, A. Porta, P. Bruno, G. Vidari, Improved synthesis of (E)-12-nitrooctadec-12-enoic acid, a potent PPAR-gamma activator. development of a "buffer-free" enzymatic method for hydrolysis of methyl esters, J. Org. Chem. 75 (23) (2010) 8311–8314.
[24] K.J. Hock, J. Grimmer, D. Göbel, G.G.T. Gasaya, J. Roos, I.V. Maucher, B. Kühn, J. Fettel, T.J. Maier, G. Manolikakes, Modular regiospecific synthesis of nitrated fatty acids, Synthesis 49 (03) (2017) 615–636.
[25] R.L. Alexander, M.W. Wright, M.J. Gorczynski, P.K. Smitherman, T.E. Akiyama, H.B. Wood, J.P. Berger, S.B. King, C.S. Morrow, Differential potencies of naturally occurring regioisomers of nitrolinoleic acid in PPAR-gamma activation, Biochemistry 48 (2) (2008) 492–498.
[26] P. Manini, L. Capelli, S. Reale, M. Arzillo, O. Crescenzi, A. Napolitano, V. Barone, M. d'Ischia, Chemistry of nitrated lipids: remarkable instability of 9-nitrolinoleic acid in neutral aqueous medium and a novel nitronitrate ester product by concurrent autoxidation/nitric oxide-release pathways, J. Org. Chem. 73 (19) (2008) 7517–7525.

[27] A.L. Hansen, K. Mukai, F.J. Schopfer, T. Taguchi, C.K. Holm, STING palmitoylation as a therapeutic target, Cell. Mol. Immunol. 16 (3) (2019) 236–241.
[28] B. Kuhn, C. Brat, J. Fettel, N. Hellmuth, I.V. Maucher, U. Bulut, K.J. Hock, J. Grimmer, G. Manolikakes, M. Ruhl, A. Kuhn, K. Zacharowski, C. Matrone, A. Urbschat, J. Roos, D. Steinhilber, T.J. Maier, Anti-inflammatory nitro-fatty acids suppress tumor growth by triggering mitochondrial dysfunction and activation of the intrinsic apoptotic pathway in colorectal cancer cells, Biochem. Pharmacol. 155 (2018) 48–60.
[29] C.S.C. Woodcock, Y. Huang, S.R. Woodcock, S.R. Salvatore, B. Singh, F. Golin-Bisello, N.E. Davidson, C.A. Neumann, B.A. Freeman, S.G. Wendell, Nitro-fatty acid inhibition of triple-negative breast cancer cell viability, migration, invasion, and tumor growth, J. Biol. Chem. 293 (4) (2018) 1120–1137.
[30] C.M. Arbeeny, H. Ling, M.M. Smith, S. O'Brien, S. Wawersik, S.R. Ledbetter, A. McAlexander, F.J. Schopfer, R.N. Willette, D.K. Jorkasky, CXA-10, a nitrated fatty acid, is renoprotective in deoxycorticosterone acetate-salt nephropathy, J. Pharmacol. Exp. Ther. 369 (3) (2019) 503–510.
[31] F.J. Schopfer, D.A. Vitturi, D.K. Jorkasky, B.A. Freeman, Nitro-fatty acids: New drug candidates for chronic inflammatory and fibrotic diseases, Nitric Oxide 79 (2018) 31–37.
[32] M. Delmastro-Greenwood, K.S. Hughan, D.A. Vitturi, S.R. Salvatore, G. Grimes, G. Potti, S. Shiva, F.J. Schopfer, M.T. Gladwin, B.A. Freeman, S.G. Wendell, Nitrite and nitrate-dependent generation of anti-inflammatory fatty acid nitroalkenes, Free Radical Biol. Med. 89 (2015) 333–341.
[33] D.A. Vitturi, C.-S. Chen, S.R. Woodcock, S.R. Salvatore, G. Bonacci, J.R. Koenitzer, N.A. Stewart, N. Wakabayashi, T.W. Kensler, B.A. Freeman, F.J. Schopfer, Modulation of nitro-fatty acid signaling: prostaglandin reductase-1 is a nitroalkene reductase, J. Biol. Chem. 288 (35) (2013) 25626–25637.
[34] V. Rudolph, F.J. Schopfer, N.K.H. Khoo, T.K. Rudolph, M.P. Cole, S.R. Woodcock, G. Bonacci, A.L. Groeger, F. Golin-Bisello, C.-S. Chen, P.R.S. Baker, B.A. Freeman, Nitro-fatty acid metabolome: saturation, desaturation, beta-oxidation, and protein adduction, J. Biol. Chem. 284 (3) (2009) 1461–1473.
[35] R. Ballini, N. Araujo, M.V. Gil, E. Roman, J.A. Serrano, Conjugated nitrodienes, synthesis and reactivity, *Chem. Rev.* 113 (5) (2013) 3493–3515.
[36] S.R. Woodcock, S.R. Salvatore, G. Bonacci, F.J. Schopfer, B.A. Freeman, Biomimetic nitration of conjugated linoleic acid: formation and characterization of naturally occurring conjugated nitrodienes, J. Org. Chem. 79 (1) (2014) 25–33.
[37] G. Blay, V. Hernández-Olmos, J.R. Pedro, Enantioselective Henry addition of methyl 4-nitrobutyrate to aldehydes. Chiral building blocks for 2-pyrrolidinones and other derivatives, Org. Lett. 12 (13) (2010) 3058–3061.
[38] D. Scharnagel, F. Prause, J. Kaldun, R.G. Haase, M. Breuning, (2S,5R)-2-Methylaminomethyl-1-methyl-5-phenylpyrrolidine, a chiral diamine ligand for copper(II)-catalysed Henry reactions with superb enantiocontrol, Chem. Commun. 50 (50) (2014) 6623–6625.
[39] Y. Zhou, Y. Zhu, S. Yan, Y. Gong, Copper-catalyzed enantioselective Henry reaction of enals and subsequent iodocyclization: stereoselective construction of chiral azatricyclic frameworks, Angew. Chem. 52 (39) (2013) 10265–10269.
[40] T.W. Greene, P.G.M. Wuts, Greene's Protective Groups in Organic Synthesis, 4th ed.;., John Wiley & Sons:, New York, 2006, p. 1082.
[41] P.J. Kocienski, Protecting Groups, Georg Thieme Verlag, New York, 2005.
[42] S.W. Wright, D.L. Hageman, A.S. Wright, L.D. McClure, Convenient preparations of t-butyl esters and ethers from t-butanol, Tetrahedron Lett. 38 (42) (1997) 7345–7348.
[43] F. Destaillats, O. Berdeaux, J.-L. Sébédio, P. Juaneda, S. Grégoire, J.-M. Chardigny, L. Bretillon, P. Angers, Metabolites of conjugated isomers of α-linolenic acid (CLnA) in the rat, J. Agric. Food Chem. 53 (5) (2005) 1422–1427.
[44] P. Gómez-Cortés, C. Tyburczy, J.T. Brenna, M. Juárez, M.A. de la Fuente, Characterization of cis-9 trans-11 trans-15 C18:3 in milk fat by GC and covalent adduct chemical ionization tandem MS, J. Lipid Res. 50 (12) (2009) 2412–2420.
[45] G.-F. Yuan, X.-E. Chen, D. Li, Conjugated linolenic acids and their bioactivities: a review, Food Funct. 5 (7) (2014) 1360–1368.
[46] M. Cholewski, M. Tomczykowa, M. Tomczyk, A Comprehensive review of chemistry, sources and bioavailability of omega-3 fatty acids, Nutrients 10 (11) (2018) 1662.
[47] A.J. Bloom, J.M. Mellor, Synthesis of 1-nitro-1,3-dienes via nitrotrifluoroacetoxylation of 1,3-dienes, Tetrahedron Lett. 27 (7) (1986) 873–876.
[48] A.J. Bloom, J.M. Mellor, Preparation of 1-nitro-1,3-dienes via nitrotrifluoroacetoxylation of 1,3-dienes, J. Chem. Soc., Perkin Trans. 1 (1987) 2737–2741.