

Article

Synthesis of Amino-Acid-Based Nitroalkenes

Velisaria-Eleni Gerogianni ^{1,2} , Giorgos S. Koutoulougenis ^{1,2}, Dimitrios Triantafyllos Gerokonstantis ^{1,2} and George Kokotos ^{1,2,*} 

¹ Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, 15771 Athens, Greece; gvelisaria@chem.uoa.gr (V.-E.G.); gkoutoul@chem.uoa.gr (G.S.K.); akisgerok@chem.uoa.gr (D.T.G.)

² Center of Excellence for Drug Design and Discovery, National and Kapodistrian University of Athens, 15771 Athens, Greece

* Correspondence: gkokotos@chem.uoa.gr; Tel.: +30-210-727-4462

Abstract: Fatty-acid-based nitroalkenes have recently received great attention because of their bioactivities. On the contrary, peptide- or amino-acid-based nitroalkenes have been scarcely explored so far, although they may exhibit interesting biological properties, for example, as enzyme inhibitors. In this work, we study protocols for the efficient synthesis of nitroalkenes based on natural amino acids. A variety of *N*-protected amino alcohols and Weinreb amides, derived from α -amino acids, were converted to the corresponding *N*-protected amino aldehydes, and, through a Henry reaction with nitroalkanes, produced the corresponding nitro alcohols. The subsequent elimination reaction led to the (*E*)-isomer of amino-acid-based nitroalkenes in moderate to high yields.

Keywords: amino acids; elimination reaction; Henry reaction; nitroalkenes; synthesis



Citation: Gerogianni, V.-E.; Koutoulougenis, G.S.; Gerokonstantis, D.T.; Kokotos, G. Synthesis of Amino-Acid-Based Nitroalkenes. *Organics* **2022**, *3*, 137–149. <https://doi.org/10.3390/org3020011>

Academic Editors: Wim Dehaen, Michal Szostak and Huaping Xu

Received: 20 March 2022

Accepted: 7 June 2022

Published: 14 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Covalent inhibitors constitute an interesting class of marketed drugs. Although the first examples of such inhibitors appeared in the 19th century, the field has advanced slowly due to concerns regarding potential off-target toxicity. However, today it is estimated that about 30% of the marketed drugs are covalent inhibitors [1,2]. Covalent inhibitors offer prolonged duration of interaction between a drug and its target, because of the covalent bond formation. They are designed to contain a reactive functionality, called warhead, appropriate to offer them the ability of the formation of a covalent bond with a molecular target protein [3]. Various reactive functionalities have been used in covalent inhibitors and some of the most important functionalities are epoxides, α,β -unsaturated esters or amides, nitriles, activated ketones, etc. [2,4]. Examples of marketed drugs containing reactive warheads are illustrated in Figure 1.

The nitroalkene functionality is a less explored warhead in candidate drugs. The most important example of a candidate drug containing such a functionality is 10-nitro-oleic acid (CXA-10, Figure 1), which is in phase II clinical trials [5]. Nitro fatty acids are a class of compounds which are formed endogenously and present various bioactivities [6]. They exhibit anti-inflammatory and anticancer activities [6], which are attributed to the ability of their nitroalkene functionality to interact with the nucleophilic -SH group of various protein cysteine residues via a reversible Michael reaction [7–10]. A structure–activity relationship study for the ability of fatty acid nitroalkenes to regulate Nrf2 and NF- κ B signaling has been recently reported [11], while other derivatives, such as 6-methylnitroarachidonate [12], a nitroalkene- α -tocopherol analog [13], and a nitroalkene vitamin E analog [14] have been shown to exert interesting biological properties.

There is only one example in the literature demonstrating the use of a nitroalkene functionality in peptide inhibitors, targeting the enzymes cruzain and rhodesain [15], which are present in parasites causing trypanosomiasis [16]. These dipeptidyl nitroalkenes present

selectivity for the inhibition of cruzain and rhodesain over the human cathepsins B and L. Recently, the mechanism of cysteine protease inhibition by dipeptidyl nitroalkenes has been studied by quantum mechanics/molecular mechanics (QM/MM) [17]. Another very recent QM/MM study explored the interaction of inhibitors, including a nitroalkene, with SARS-CoV-2 main protease [18], which is a cysteine protease.

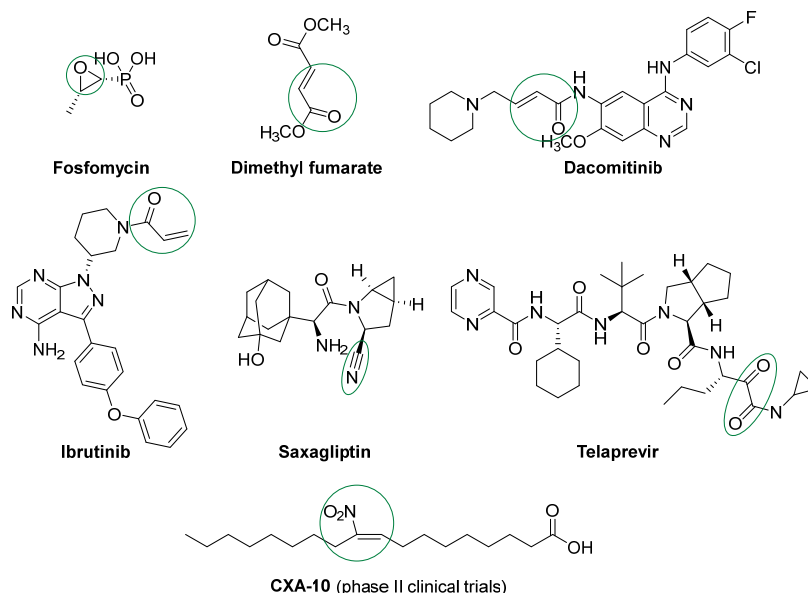


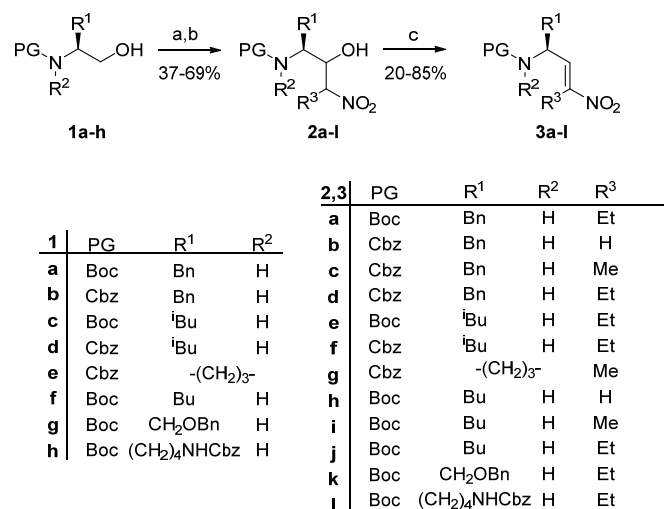
Figure 1. Examples of marketed drugs containing functionalities enabling them to act as covalent inhibitors.

Various synthetic routes have been developed for the synthesis of nitroalkenes, following either a direct alkene nitration approach or a step-by-step approach, aiming to the formation of a certain regio-isomer [19,20]. However, the majority of them refer to the synthesis of nitro fatty acids [21–24]. An example for the synthesis of an amino-acid-based nitroalkene has been reported, where a proline-based nitroalkene has been used as an intermediate for the synthesis of natural products [25,26]. The condensation of *N,N*-dibenzyl α -amino aldehydes with nitroalkanes mediated by tetra-*n*-butylammonium fluoride was reported in 1996 [27], while, later on, the reaction between a limited number of *N,N*-dibenzyl α -amino aldehydes with nitromethane was demonstrated [28,29]. In addition, the reaction of a limited number of Boc-protected α -amino aldehydes with (4-tolylthio) nitromethane in the presence of potassium *tert*-butoxide has been reported [30].

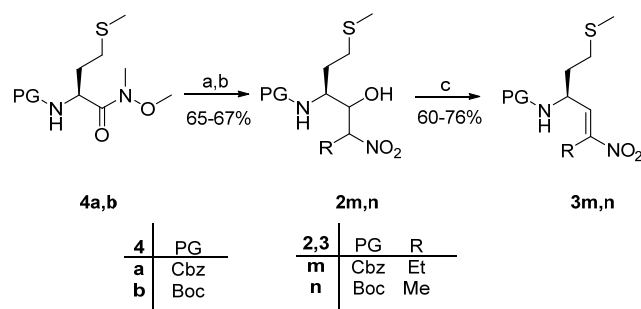
The aim of our work was to study the synthesis of amino-acid-based nitroalkenes, which can be used as cornerstones for the synthesis of either peptide nitroalkenes or natural products. We present herein a detailed study for the synthesis of such compounds, starting from various amino acids protected by various protecting groups.

2. Results and Discussion

N-protected amino alcohols **1a–h**, easily derived from *N*-protected amino acids [31,32], such as phenylalanine, leucine, proline, norleucine, serine and lysine, were oxidized to aldehydes by the treatment with an aqueous solution of NaOCl and NaBr in the presence of a catalytic amount of 4-AcNH-TEMPO, and the formed aldehydes were used for the upcoming Henry reaction without further purification (Scheme 1). As shown in the past [33], this method of oxidation avoids any racemization of the stereogenic center. In the case of methionine and to avoid sulfur oxidation under oxidative conditions, Weinreb amides **4a,b** were used as starting materials, which were reduced to aldehydes by treatment with LiAlH₄ in dry toluene (Scheme 2).



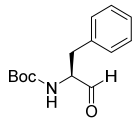
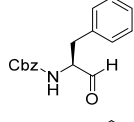
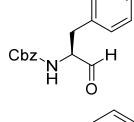
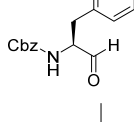
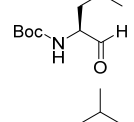
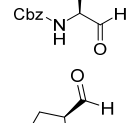
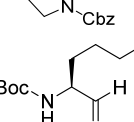
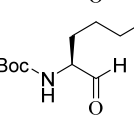
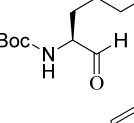
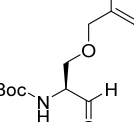
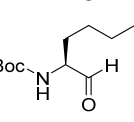
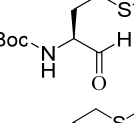
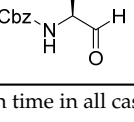
Scheme 1. General procedure for the synthesis of nitroalkenes starting from *N*-protected amino alcohols. Reagents and conditions: (a) NaOCl, 4-AcNH-TEMPO (1 mol%), NaHCO₃, NaBr, H₂O/EtOAc/PhCH₃, −5 to 0 °C, 1 h. (b) Method A: R³CH₂NO₂, DBU, dry THF, 0 °C to r.t., 16 h. Method B: R³CH₂NO₂, aqueous 3N KOH, MeOH, 0 °C to r.t., 16 h. (c) Method C: (i) Ac₂O, DMAP (10 mol%), Et₂O, r.t., 5 h, (ii) DMAP (3.0 eq.), dry CH₂Cl₂, r.t., 2–3 d. Method D: MsCl, DIPEA, dry CH₂Cl₂, 0 °C to r.t., 2 h. Method E: Et₃N, MsCl, dry CH₂Cl₂, 0 °C to r.t., 2–24 h.



Scheme 2. General procedure for the synthesis of nitroalkenes starting from Weinreb amides. Reagents and conditions: (a) LiAlH₄, dry toluene, 0 °C, 30 min. (b) RCH₂NO₂, DBU, dry THF, 0 °C to r.t., 16 h. (c) MsCl, Et₃N, dry CH₂Cl₂, 0 °C to r.t., 1–24 h.

Nitro alcohols **2a–n** were synthesized by a Henry reaction, exploring two different procedures (Schemes 1 and 2, Table 1). In method A, the nitroalkane was diluted in extra dry tetrahydrofuran (THF) and 1,8-diazabicyclo (5.4.0)undec-7-ene (DBU) was added as the base, followed by the addition of the aldehyde. In method B, the nitroalkane was added in an aqueous solution of 3N KOH and MeOH [25,26]. This procedure was tested for compounds **2f**, **2g** and **2k**. Compound **2k** was synthesized by both methods (method A and B) and no significant differences were observed in the yield (36 and 42%, respectively). Method A is characterized by mild conditions and method B should be avoided where an ester group or other base-sensitive groups are present in the substrate, because the aqueous 3N KOH solution might cause unwanted side reactions. In general, products **2a–n** of the Henry reaction were obtained in moderate to high yields. All the products of the Henry reaction were isolated as mixtures of diastereomers.

Table 1. Synthesis of nitro alcohols by the Henry reaction ^a.

Entry	Aldehyde	Method	Nitroalkane	Product	Isolated Yield (%)
1		A	1-Nitropropane	2a	37
2		A	Nitromethane	2b	69
3		A	Nitroethane	2c	40
4		A	1-Nitropropane	2d	57
5		A	1-Nitropropane	2e	51
6		B	1-Nitropropane	2f	42
7		B	Nitroethane	2g	- ^b
8		A	Nitromethane	2h	58
9		A	Nitroethane	2i	46
10		A	1-Nitropropane	2j	40
11		A or B	1-Nitropropane	2k	36 or 42
12		A	1-Nitropropane	2l	36
13		A	Nitroethane	2m	67
14		A	1-Nitropropane	2n	65

^a The reaction time in all cases was 16 h; ^b this nitro alcohol was used in the next step without any purification.

Different synthetic procedures for the final elimination step of the intermediate nitro alcohols were carried out in order to understand the optimized way to synthesize this interesting series of compounds (Table 2). In method C, nitro alcohol **2** was diluted in Et₂O, and Ac₂O and 4-dimethylaminopyridine (DMAP) were added [22]. After the acetylation reaction was over after approximately 5 h, the solvent Et₂O was switched to CH₂Cl₂, along with an addition of an excess of DMAP. The result of this method was the formation of the (*E*)-nitroalkene after 2–3 d. Monitoring the reaction mixture by ¹H-NMR spectroscopy, we observed the initial formation of a mixture of (*E*)- and (*Z*)-isomers, which finally resulted in a clean (*E*)-isomer (thermodynamically more stable), as time was passing by. (*E*)- and (*Z*)-isomers can be identified by ¹H-NMR spectroscopy based on their characteristic different chemical shifts at 7.00–6.75 ppm and around 5.70 ppm, respectively [6,15,20].

However, alternative methods D and E were also tested in order to decrease the reaction time. Both methods were based on examples of elimination reaction in similar substrates. According to method D, nitro alcohol **2** was diluted in CH₂Cl₂, and then MsCl was slowly added at 0 °C, followed by the addition of *N,N*-diisopropylethylamine (DIPEA) [15]. Method E is much like method D, with the only difference that the organic base used for this elimination reaction was triethylamine (Et₃N) [25,26]. However, there are no significant differences in the yields, which are also mediocre to high, nor in the regio-isomer formed. Furthermore, the reaction time of the elimination step is similar to methods D and E in the substrates which were subjected to Henry reaction with the use of nitromethane [28–30]. Method E was used for compounds **3c**, **3f–g**, **3i**, **3k** and **3m–n**. Using method D, the (*E*)-nitroalkene was isolated selectively after almost one hour, while, following method E, the reaction time varied from 2 h to 1 day, based on the substitution of the nitrated double bond and the stereochemical hindrance of the vicinal bulky substituent. Methods D and E outperformed method C, since the desired (*E*)-nitroalkene was formed selectively and the reaction time was much lower. Finally, the yields of the final products were low to very high, depending on the aforementioned parameters. Better yields were observed for the compounds based on norleucine **3h–j** and methionine **3m–n**.

Table 2. Synthesis of amino-acid-based nitroalkenes utilizing different methods.

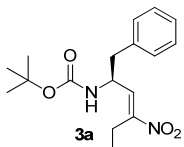
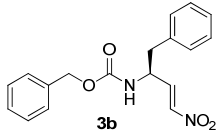
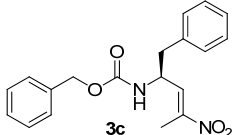
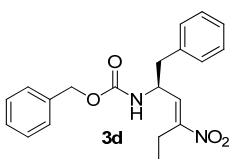
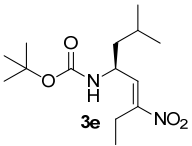
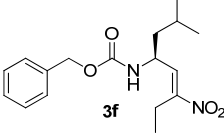
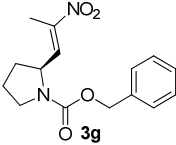
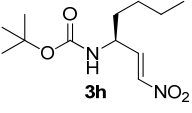
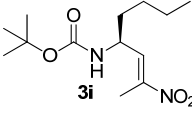
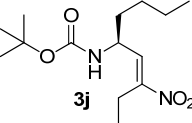
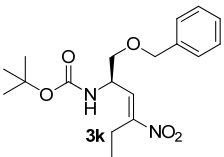
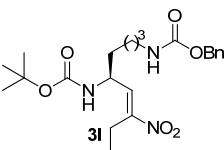
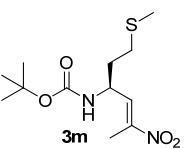
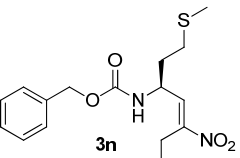
Entry	Nitro Alcohol	Method	Nitroalkene	Reaction Time (h)	Isolated Yield (%)
1	2a	C		72	47
2	2b	D		1	42
3	2c	E		2	42
4	2d	C		72	53

Table 2. Cont.

Entry	Nitro Alcohol	Method	Nitroalkene	Reaction Time (h)	Isolated Yield (%)
5	2e	C		72	58
6	2f	E		7	41
7	2g	E		5	20 ^a
8	2h	D		1	82
9	2i	E		2	85
10	2j	C		72	72
11	2k	C or E		72	35 or 35
12	2l	C		48	51
13	2m	E		24	60
14	2n	E		1.5	76

^a Overall yield over two steps.

3. Conclusions

In summary, we have demonstrated efficient protocols for the synthesis of (*E*)-nitroalkenes starting from *N*-protected amino alcohols or Weinreb amides, studying different synthetic routes. The formation of *N*-protected aldehydes from either the precursor alcohols or the

Weinreb amides of methionine, by an oxidative or a reductive reaction, respectively, was followed by a Henry reaction and an upcoming final elimination reaction, affording this way the final amino-acid-based nitroalkenes. We have overall demonstrated five different methods, the first two applying different conditions for the Henry reaction and the last three for the elimination step reaction. Each time, the advantages and disadvantages of each method were described. Finally, methods D and E seemed to be the best alternatives for the elimination step, due to the optimized reaction time and the selective formation of the thermodynamically stable (*E*)-nitroalkene.

4. Materials and Methods

4.1. General Information

All reagents and solvents were purchased from commercial sources (Fluorochem, Glossop, UK; Alfa-Aesar, Haverhill, MA, USA; Sigma-Aldrich, Saint Louis, MO, USA; Acros Organics, Geel, Belgium; Merck, Darmstadt, Germany and Fischer Scientific, Waltham, MA, USA) and used without further purification.

Chromatographic purification of products was accomplished using forced-flow chromatography on Merck® (Merck, Darmstadt, Germany) Kieselgel 60 F₂₅₄ 230–400 mesh. Thin-layer chromatography (TLC) was performed on aluminum backed silica plates (0.2 mm, 60 F₂₅₄). Visualization of the developed chromatograms was performed by fluorescence quenching using phosphomolybdic acid, ninhydrin or potassium permanganate stains. Melting points were determined on a Buchi® 530 apparatus (Buchi, Flawil, Switzerland) and were uncorrected. Optical rotations were measured on an AA-65 series (Optical Activity Ltd., Bury, UK) polarimeter. ¹H and ¹³C NMR spectra were recorded on a Varian® Mercury (Varian, Palo Alto, CA, USA) (200 MHz and 50 MHz, respectively) or a Bruker Avance Neo (Bruker, Faellanden, Switzerland) (400 MHz and 100 MHz, respectively) and are internally referenced to residual solvent signals. Data for ¹H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad signal), coupling constant, integration and peak assignment. Data for ¹³C NMR are reported in terms of chemical shift (δ ppm). High-resolution mass spectrometry (HRMS) spectra were recorded on a Bruker® Maxis Impact QTOF (Bruker Daltonics, Bremen, Germany) spectrometer.

4.2. General Synthetic Procedures

4.2.1. General Method for the Oxidation of *N*-Protected 2-Amino Alcohols **1a–h** to Aldehydes

To a solution of *N*-protected 2-amino alcohol **1a–h** (1.0 mmol) in a mixture of PhCH₃/EtOAc 1:1 (6.0 mL), a solution of NaBr (1.1 mmol, 108 mg) in H₂O (0.5 mL) was added, followed by 4-AcNH-TEMPO (0.01 mmol, 2 mg). The reaction mixture was cooled to −5 °C and an aqueous solution of 0.35 M NaOCl (1.1 mmol, 3.1 mL) containing NaHCO₃ (3.0 mmol, 252 mg) was added dropwise over 1 h under vigorous stirring, keeping the temperature constantly at −5 °C. After additional stirring for 15 min at 0 °C, a mixture of EtOAc (6.0 mL) and H₂O (2.0 mL) was added. The combined organic layers were washed with a 5% aqueous solution of citric acid (6.0 mL) containing KI (0.04 g), a 10% aqueous solution of Na₂S₂O₃ (6.0 mL), and brine (5.0 mL), and were dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure and the crude aldehyde was immediately used as is for the next step.

4.2.2. General Methods for the Reduction of Weinreb Amides **4a,b** to Aldehydes

To a solution of the Weinreb amide **4a,b** of *N*-protected-L-Met (1.0 mmol) in dry toluene (8.0 mL), a solution of 1M LiAlH₄ in THF (1.5 eq., 1.5 mL) was added dropwise at 0 °C under argon. The reaction mixture was left stirring at 0 °C for 30 min. After the reaction was completed, an aqueous solution of 1M KHSO₄ (15.0 mL) was added and the reaction mixture was left stirring at 0 °C for 5 min. The organic phase was collected, and then the aqueous phase was extracted with EtOAc (3 × 10.0 mL). The combined organic

phases were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The formed aldehyde was used immediately as is for the next step.

4.2.3. General Methods for the Henry Reaction

Method A: To a solution of nitroalkane (3.0 mmol) in extra dry THF (5.0 mL) in a flame-dried flask, DBU (1.0 mmol, 151 mg) was added dropwise at 0 °C and the reaction mixture was left stirring for 15 min at 0 °C. Then, a solution of the aldehyde (1.0 mmol) in extra dry THF (5.0 mL) was added dropwise and the reaction mixture was left stirring for 30 min at 0 °C, and then for 24 h at r.t. After the completion of the reaction, the solvent was removed under reduced pressure and the residue was diluted in CH_2Cl_2 (5.0 mL). The organic phase was washed with an aqueous solution of 5% citric acid (3.0 mL) and brine (3.0 mL), dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The nitro alcohol was purified by column chromatography using petroleum ether: EtOAc 8:2. All the isolated products were mixtures of diastereomers.

Method B: To a stirred solution of nitroalkane (11.0 mmol) in MeOH (5.0 mL), an aqueous solution of 3N KOH (3 mL) was added and the reaction mixture was left stirring for 10 min at r.t. Then, a solution of the aldehyde (1.0 mmol) in MeOH (5.0 mL) was added and the reaction mixture was left stirring at r.t. for 16 h. After the reaction was completed, the mixture was acidified with c. H_2SO_4 (pH 3), diluted with water (20.0 mL), and then extracted with EtOAc (3×25.0 mL). The organic phases were collected, washed with brine (2×25.0 mL), dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The nitro alcohol was purified by column chromatography using petroleum ether: EtOAc 8:2. All the isolated products were mixtures of diastereomers.

tert-Butyl ((2S)-3-hydroxy-4-nitro-1-phenylhexan-2-yl) carbamate (2a). Synthesized according to Method A; yellow solid; ^1H -NMR (200 MHz, CDCl_3): δ 7.36–7.07 (m, 5H), 5.03 (d, $J = 9.9$ Hz, 1H), 4.54–4.37 (m, 1H), 4.02–3.82 (m, 2H), 3.53–3.40 (m, 1H), 2.98–2.76 (m, 2H), 1.98–1.66 (m, 2H), 1.39 (s, 9H), 0.85 (t, $J = 7.3$ Hz, 3H); ^{13}C -NMR (50 MHz, CDCl_3): δ 156.1, 137.5, 129.4, 128.9, 127.0, 93.6, 80.4, 72.0, 52.6, 38.9, 28.5, 23.6, 10.0; HRMS (ESI) m/z 361.1734 $[\text{M} + \text{Na}]^+$ (calculated for 361.1734 $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_5\text{Na}^+$).

Benzyl ((2S)-3-hydroxy-4-nitro-1-phenylbutan-2-yl) carbamate (2b). Synthesized according to Method A; colorless oil; ^1H -NMR (400 MHz, CDCl_3): δ 7.41–7.16 (m, 10H), 5.23 (d, $J = 9.5$ Hz, 0.6H), 5.13–5.02 (m, 2H), 4.90 (d, $J = 8.5$ Hz, 0.4H), 4.54–4.42 (m, 2H), 4.38–4.28 (m, 1H), 4.02–3.89 (m, 1H), 3.61 (br s, 0.4H), 3.37 (br s, 0.6H), 3.05–2.88 (m, 2H); ^{13}C -NMR (100 MHz, CDCl_3): δ 156.4, 136.9, 136.3, 136.0, 129.3, 129.2, 128.9, 128.6, 128.4, 128.1, 127.1, 127.0, 79.0, 78.4, 70.8, 68.2, 67.3, 67.2, 54.5, 54.4, 38.2, 36.0; HRMS (ESI) m/z 367.1261 $[\text{M} + \text{Na}]^+$ (calculated for 367.1264 $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_5\text{Na}^+$).

Benzyl ((2S)-3-hydroxy-4-nitro-1-phenylpentan-2-yl) carbamate (2c). Synthesized according to Method A; white solid; ^1H -NMR (400 MHz, CDCl_3): δ 7.41–7.17 (m, 10H), 5.34 (d, $J = 9.7$ Hz, 1H), 5.11 (d, $J = 13.6$ Hz, 1H), 5.06 (d, $J = 13.6$ Hz, 1H), 4.65–4.58 (m, 1H), 4.10–3.99 (m, 1H), 3.97–3.90 (m, 1H), 3.37 (s, 1H), 3.03–2.86 (m, 2H), 2.73 (d, $J = 6.8$ Hz, 3H); ^{13}C -NMR (100 MHz, CDCl_3): δ 156.4, 137.0, 136.1, 129.2, 128.8, 128.6, 128.3, 128.0, 127.0, 86.7, 72.7, 67.2, 52.7, 38.8, 16.1; HRMS (ESI) m/z 381.1424 $[\text{M} + \text{Na}]^+$ (calculated for 381.1421 $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_5\text{Na}^+$).

Benzyl ((2S)-3-hydroxy-4-nitro-1-phenylhexan-2-yl) carbamate (2d). Synthesized according to Method A; colorless oil; ^1H -NMR (200 MHz, CDCl_3): δ 7.45–7.07 (m, 10H), 5.38 (d, $J = 9.6$ Hz, 1H), 5.09 (d, $J = 13.4$ Hz, 1H), 4.99 (d, $J = 13.4$ Hz, 1H), 4.50–4.37 (m, 1H), 4.08–3.86 (m, 2H), 3.50–3.37 (m, 1H), 3.03–2.77 (m, 2H), 1.93–1.66 (m, 2H), 0.83 (t, $J = 7.3$ Hz, 3H); ^{13}C -NMR (50 MHz, CDCl_3): δ 156.7, 137.3, 136.3, 129.4, 129.0, 128.8, 128.5, 128.2, 127.2, 93.3, 71.9, 67.4, 53.1, 38.9, 23.6, 9.9; HRMS (ESI) m/z 395.1577 $[\text{M} + \text{Na}]^+$ (calculated for 395.1577 $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_5\text{Na}^+$).

tert-Butyl ((4S)-5-hydroxy-2-methyl-6-nitrooctan-4-yl) carbamate (2e). Synthesized according to Method A; yellowish oil; ^1H -NMR (200 MHz, CDCl_3): δ 4.81 (d, $J = 9.9$ Hz, 1H), 4.48–4.29 (m, 1H), 3.99–3.85 (m, 1H), 3.82–3.64 (m, 1H), 3.25 (d, $J = 6.5$ Hz, 1H), 2.18–1.71 (m, 3H), 1.67–1.51 (m, 2H), 1.41 (s, 9H), 0.91 (d, $J = 6.0$ Hz, 9H); ^{13}C -NMR (50 MHz, CDCl_3):

δ 156.1, 93.9, 80.0, 74.2, 49.1, 42.0, 28.5, 24.9, 23.7, 23.0, 22.4, 10.3; HRMS (ESI) m/z 327.1892 $[M + Na]^+$ (calculated for 327.1890 $C_{14}H_{28}N_2O_5Na^+$).

Benzyl ((4S)-5-hydroxy-2-methyl-6-nitrooctan-4-yl) carbamate (2f). Synthesized according to Method B; yellowish oil; 1H -NMR (200 MHz, $CDCl_3$): δ 7.42–7.16 (m, 5H), 5.24 (d, J = 9.6 Hz, 1H), 5.07 (s, 2H), 4.46–4.29 (m, 1H), 3.99–3.74 (m, 2H), 3.59–3.47 (br s, 1H), 2.08–1.75 (m, 2H), 1.60–1.46 (m, 1H), 1.38–1.12 (m, 2H), 0.97–0.64 (m, 9H); ^{13}C -NMR (50 MHz, $CDCl_3$): δ 156.7, 136.4, 128.8, 128.5, 128.1, 93.6, 74.1, 67.3, 49.7, 42.0, 24.8, 23.7, 23.1, 22.5, 10.2; HRMS (ESI) m/z 361.1724 $[M + Na]^+$ (calculated for 361.1734 $C_{17}H_{26}N_2O_5Na^+$).

Benzyl (2S)-2-(1-hydroxy-2-nitropropyl)pyrrolidine-1-carboxylate (2g). Synthesized according to Method B. The product was used as is for the next step.

tert-Butyl ((3S)-2-hydroxy-1-nitroheptan-3-yl) carbamate (2h). Synthesized according to Method A; white solid; 1H -NMR (400 MHz, $CDCl_3$): δ 4.95 (d, J = 9.7 Hz, 0.7H), 4.76 (s, 0.3H), 4.54–4.33 (m, 2H), 4.26 (s, 0.4H), 4.16 (s, 0.2H), 3.99 (s, 0.4H), 3.65–3.49 (m, 1H), 1.69–1.22 (m, 15H), 0.89 (t, J = 6.6 Hz, 3H); ^{13}C -NMR (100 MHz, $CDCl_3$): δ 156.7, 156.3, 80.4, 80.0, 79.4, 78.7, 72.1, 70.2, 53.6, 52.5, 31.9, 30.0, 28.3, 28.1, 27.9, 22.4, 13.9; HRMS (ESI) m/z 299.1577 $[M + Na]^+$ (calculated for 299.1577 $C_{12}H_{24}N_2O_5Na^+$).

tert-Butyl ((4S)-3-hydroxy-2-nitrooctan-4-yl) carbamate (2i). Synthesized according to Method A; white solid; 1H -NMR (400 MHz, $CDCl_3$): δ 5.02–4.92 (m, 1H), 4.70–4.48 (m, 1H), 4.12–4.01 (m, 1H), 3.95–3.76 (m, 1H), 3.71–3.37 (m, 1H), 1.68–1.48 (m, 5H), 1.41 (m, 9H), 1.36–1.20 (m, 4H), 0.88 (s, 3H); ^{13}C -NMR (100 MHz, $CDCl_3$): δ 156.7, 156.3, 156.1, 156.0, 87.0, 85.8, 84.9, 84.3, 81.1, 80.2, 79.9, 79.8, 75.3, 75.1, 74.5, 73.7, 52.9, 51.6, 50.6, 32.6, 31.7, 31.1, 28.3, 28.2, 28.1, 28.0, 27.9, 27.7, 22.4, 22.3, 16.1, 16.0, 14.0, 13.9; HRMS (ESI) m/z 313.1734 $[M + Na]^+$ (calculated for 313.1736 $C_{13}H_{26}N_2O_5Na^+$).

tert-Butyl ((5S)-4-hydroxy-3-nitrononan-5-yl)carbamate (2j). Synthesized according to Method A; colorless oil; 1H -NMR (200 MHz, $CDCl_3$): δ 4.88 (d, J = 10.0 Hz, 1H), 4.47–4.31 (m, 1H), 4.03–3.88 (m, 1H), 3.73–3.56 (m, 1H), 3.46–3.34 (m, 1H), 2.07–1.72 (m, 2H), 1.63–1.10 (m, 15H), 1.00–0.77 (m, 6H); ^{13}C -NMR (50 MHz, $CDCl_3$): δ 156.3, 93.8, 80.1, 73.7, 51.0, 32.7, 28.5, 28.3, 23.7, 22.7, 14.2, 10.2; HRMS (ESI) m/z 327.1871 $[M + Na]^+$ (calculated for 327.1880 $C_{14}H_{28}N_2O_5Na^+$).

tert-Butyl ((2S)-1-(benzyloxy)-3-hydroxy-4-nitrohexan-2-yl) carbamate (2k). Synthesized according to Method A and Method B; yellow oil; 1H -NMR (400 MHz, $CDCl_3$): δ 7.41–7.29 (m, 5H), 5.37–5.00 (m, 1H), 4.70–4.50 (m, 2H), 4.45–4.40 (m, 0.8H), 4.34–4.29 (m, 0.6H), 4.25–4.00 (m, 0.6H), 3.98–3.82 (m, 1H), 3.73–3.57 (m, 2H), 2.02–1.80 (m, 2H), 1.47 (s, 9H), 1.01–0.86 (m, 3H); ^{13}C -NMR (100 MHz, $CDCl_3$): δ 155.8, 140.9, 137.1, 128.7, 128.6, 128.5, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.1, 92.8, 80.3, 73.8, 73.7, 73.5, 72.9, 71.6, 65.2, 49.6, 28.3, 28.3, 28.3, 28.2, 10.1, 10.1, 10.0; HRMS (ESI) m/z 391.1840 $[M + Na]^+$ (calculated for 391.1840 $C_{18}H_{28}N_2O_6Na^+$).

Benzyl *tert*-butyl ((5S)-6-hydroxy-7-nitrononane-1,5-diyl) dicarbamate (2l). Synthesized according to Method A; yellow oil; 1H -NMR (200 MHz, $CDCl_3$): δ 7.39–7.16 (m, 5H), 5.13–4.98 (m, 4H), 4.50–4.28 (m, 1H), 4.14–3.81 (m, 2H), 3.73–3.52 (m, 1H), 3.30–2.94 (m, 2H), 2.05–1.73 (m, 2H), 1.67–1.03 (m, 15H), 0.96–0.78 (m, 3H); ^{13}C -NMR (50 MHz, $CDCl_3$): δ 157.3, 156.3, 136.5, 128.7, 128.4, 93.8, 80.0, 73.0, 67.1, 50.8, 40.2, 32.0, 29.8, 28.5, 23.6, 22.5, 10.3; HRMS (ESI) m/z 498.2464 $[M + HCOOH-H]^-$ (calculated for 498.2457 $C_{23}H_{36}N_3O_9^-$).

tert-Butyl ((3S)-4-hydroxy-1-(methylthio)-5-nitrohexan-3-yl) carbamate (2m). Synthesized according to Method A; yellow oil; 1H -NMR (400 MHz, $CDCl_3$): δ 5.02 (d, J = 9.9 Hz, 0.75H), 4.82 (d, J = 9.2 Hz, 0.25H), 4.70–4.52 (m, 1H), 4.17–4.05 (m, 0.25H), 4.04–3.94 (m, 0.75H), 3.91–3.50 (m, 2H), 2.65–2.44 (m, 2H), 2.10 (s, 2.25H), 2.09 (s, 0.75H), 1.99–1.63 (m, 2H), 1.61–1.54 (m, 3H), 1.42 (s, 9H); ^{13}C -NMR (100 MHz, $CDCl_3$): δ 156.0, 86.9, 86.6, 84.2, 80.4, 80.2, 74.6, 74.3, 52.0, 49.8, 32.3, 30.8, 30.4, 30.3, 28.3, 16.1, 16.0, 15.5, 15.4, 12.6; HRMS (ESI) m/z 331.1298 $[M + Na]^+$ (calculated for 331.1298 $C_{12}H_{24}N_2NaO_5S^+$).

Benzyl ((3S)-4-hydroxy-1-(methylthio)-5-nitroheptan-3-yl) carbamate (2n). Synthesized according to Method A; yellow oil; 1H -NMR (400 MHz, $CDCl_3$): δ 7.42–7.32 (m, 5H), 5.36 (d, J = 9.9 Hz, 0.8H), 5.24 (d, J = 8.8 Hz, 0.2H), 5.11 (s, 2H), 4.52–4.37 (m, 1H), 4.19–3.98

(m, 1H), 3.98–3.85 (m, 1H), 3.84–3.64 (m, 1H), 2.54 (t, $J = 7.2$ Hz, 2H), 2.10 (s, 3H), 2.05–1.64 (m, 4H), 1.04–0.73 (m, 3H); CH

^{13}C -NMR (100 MHz, CDCl_3): δ 156.5, 156.3, 136.2, 128.6, 128.6, 128.3, 128.1, 128.1, 128.0, 127.7, 127.1, 93.3, 74.0, 73.2, 67.2, 65.3, 50.6, 32.1, 30.4, 23.5, 15.5, 10.0; HRMS (ESI) m/z 379.1298 $[\text{M} + \text{Na}]^+$ (calculated for 379.1298 $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_5\text{SNa}^+$).

4.2.4. General Methods for the Synthesis of Nitroalkenes 3a–n

Method C: To a solution of the nitro alcohol **2** (1.0 mmol) in Et_2O (10.0 mL), Ac_2O (1.3 mmol, 126 mg) was added, followed by DMAP (0.1 mmol, 2 mg), and the reaction mixture was left stirring at r.t. for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (10.0 mL). DMAP (3.0 mmol, 367 mg) was then added and the reaction mixture was left stirring at r.t. for 48 h. The reaction mixture was then diluted with CH_2Cl_2 (8.0 mL) and the organic phase was washed with an aqueous solution of 0.1N HCl (8.0 mL), H_2O (8.0 mL) and brine (8.0 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by column chromatography using petroleum ether: EtOAc 8:2.

Method D: To a solution of the nitro alcohol **2** (1.0 mmol) in CH_2Cl_2 (10.0 mL), MsCl (2.0 mmol, 0.16 mL) was added dropwise at 0 °C and the reaction mixture was left stirring for 20 min. DIPEA (4.0 mmol, 0.69 mL) was then added and the reaction mixture was left stirring at 0 °C for 2 h. The reaction was quenched by the addition of an aqueous solution of saturated NH_4Cl (25.0 mL) and the aqueous phase was extracted with CH_2Cl_2 (3×15.0 mL). The organic phases were collected, washed with an aqueous solution of 1N HCl (15.0 mL), an aqueous solution of saturated NaHCO_3 (15.0 mL) and brine (15.0 mL), dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by column chromatography using petroleum ether: EtOAc 8:2.

Method E: To a solution of the nitro alcohol **2** (1.0 mmol) in CH_2Cl_2 (20.0 mL), MsCl (2.0 mmol, 228 mg) was added dropwise at 0 °C and the reaction mixture was left stirring for 20 min. Then, Et_3N (7.0 mmol, 708 mg) was added and the reaction mixture was left stirring at 0 °C for 2–5 h. The reaction was quenched by the addition of an aqueous solution of 2N HCl (15.0 mL) and the aqueous phase was extracted with CH_2Cl_2 (2×25.0 mL). The organic phases were collected, washed with brine (2×25.0 mL), dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by column chromatography using petroleum ether: EtOAc 8:2.

tert-Butyl (*S,E*)-(4-nitro-1-phenylhex-3-en-2-yl)carbamate (**3a**). Synthesized according to Method C; yellow solid; m.p. = 76–78 °C; $[\alpha]_{\text{D}}^{25} = +3$ ($c = 1.0$, CHCl_3); ^1H -NMR (200 MHz, CDCl_3): δ 7.36–7.10 (m, 5H), 6.77 (d, $J = 9.7$ Hz, 1H), 4.70 (d, $J = 8.1$ Hz, 1H), 4.65–4.44 (m, 1H), 3.00 (dd, $J_1 = 13.5$ and $J_2 = 5.8$ Hz, 1H), 2.81 (dd, $J_1 = 13.5$ and $J_2 = 7.8$ Hz, 1H), 2.50 (q, $J = 7.4$ Hz, 2H), 1.40 (s, 9H), 0.83 (t, $J = 7.4$ Hz, 3H); ^{13}C -NMR (50 MHz, CDCl_3): δ 155.9, 154.5, 136.0, 133.5, 129.6, 129.0, 127.4, 80.5, 50.1, 41.6, 28.5, 20.4, 12.4; HRMS (ESI) m/z 343.1624 $[\text{M} + \text{Na}]^+$ (calculated for 343.1628 $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_4\text{Na}^+$).

Benzyl (*S,E*)-(4-nitro-1-phenylbut-3-en-2-yl) carbamate (**3b**). Synthesized according to Method D; white solid; m.p. = 92–94 °C; $[\alpha]_{\text{D}}^{25} = +4$ ($c = 1.0$, CHCl_3); ^1H -NMR (400 MHz, CDCl_3): δ 7.39–7.26 (m, 8H), 7.22–7.13 (m, 3H), 6.94 (d, $J = 13.1$ Hz, 1H), 5.08 (s, 2H), 4.91 (d, $J = 8.0$, 1H), 4.81–4.67 (m, 1H), 3.02–2.92 (m, 2H); ^{13}C -NMR (100 MHz, CDCl_3): δ 155.5, 141.0, 140.3, 135.9, 135.1, 129.3, 129.1, 128.8, 128.6, 128.3, 127.6, 67.5, 50.6, 40.4; HRMS (ESI) m/z 349.1158 $[\text{M} + \text{Na}]^+$ (calculated for 349.1159 $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_4\text{Na}^+$).

Benzyl (*S,E*)-(4-nitro-1-phenylpent-3-en-2-yl)carbamate (**3c**). Synthesized according to Method E; white solid; m.p. = 76–78 °C; $[\alpha]_{\text{D}}^{25} = +12$ ($c = 1.0$, CHCl_3); ^1H -NMR (400 MHz, CDCl_3): δ 7.43–7.26 (m, 8H), 7.17–7.15 (m, 2H), 6.88 (d, $J = 9.6$ Hz, 1H), 5.14–5.05 (m, 3H), 4.74–4.55 (m, 1H), 3.05–3.03 (m, 1H), 2.88–2.83 (m, 1H), 2.01 (s, 3H); ^{13}C -NMR (100 MHz, CDCl_3): δ 155.6, 149.3, 136.1, 135.5, 133.6, 129.5, 129.0, 128.7, 128.5, 128.3, 127.4, 67.3, 50.7, 40.8, 12.7; HRMS (ESI) m/z 363.1315 $[\text{M} + \text{Na}]^+$ (calculated for 363.1315 $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_4\text{Na}^+$).

Benzyl (*S,E*)-(4-nitro-1-phenylhex-3-en-2-yl) carbamate (**3d**). Synthesized according to Method C; yellow solid; m.p. = 72–74 °C; $[\alpha]_{\text{D}}^{25} = +10$ ($c = 1.0$, CHCl_3); ^1H -NMR (200 MHz,

CDCl₃): δ 7.45–7.03 (m, 10H), 6.78 (d, J = 10.1 Hz, 1H), 5.14–4.98 (m, 3H), 4.71–4.54 (m, 1H), 3.01 (dd, J_1 = 13.5 and J_2 = 6.0 Hz, 1H), 2.83 (dd, J_1 = 13.5 and J_2 = 7.9 Hz, 1H), 2.62–2.44 (m, 2H), 0.86 (t, J = 7.8 Hz, 3H); ¹³C-NMR (50 MHz, CDCl₃): δ 155.4, 154.6, 136.0, 135.4, 132.8, 129.4, 128.9, 128.6, 128.4, 128.2, 127.4, 67.2, 50.4, 41.1, 20.2, 12.2; HRMS (ESI) m/z 377.1471 [M + Na]⁺ (calculated for 377.1472 C₁₇H₂₄N₂O₄Na⁺).

tert-Butyl (*S,E*)-(2-methyl-6-nitrooct-5-en-4-yl) carbamate (3e). Synthesized according to Method C; yellow solid; m.p. = 53–55 °C; $[\alpha]_D^{25}$ = −5 (c = 1.0, CHCl₃); ¹H-NMR (200 MHz, CDCl₃): δ 6.70 (d, J = 9.7 Hz, 1H), 4.64–4.54 (m, 1H), 4.50–4.27 (m, 1H), 2.83–2.57 (m, 2H), 1.75–1.51 (m, 3H), 1.40 (s, 9H), 1.12 (t, J = 7.4 Hz, 3H), 0.92 (d, J = 3.2 Hz, 3H), 0.90 (d, J = 3.2 Hz, 3H); ¹³C-NMR (50 MHz, CDCl₃): δ 155.1, 154.1, 135.1, 80.2, 46.9, 44.4, 28.5, 24.8, 22.9, 22.4, 20.5, 12.9; HRMS (ESI) m/z 309.1785 [M + Na]⁺ (calculated for 309.1785 C₁₄H₂₆N₂O₄Na⁺).

Benzyl (*S,E*)-(2-methyl-6-nitrooct-5-en-4-yl)carbamate (3f). Synthesized according to Method E; yellowish solid; m.p. = 58–60 °C; $[\alpha]_D^{25}$ = −5 (c = 0.25, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ 7.40–7.31 (m, 5H), 6.74 (d, J = 9.7 Hz, 1H), 5.15–5.05 (m, 2H), 4.96 (d, J = 8.3 Hz, 1H), 4.56–4.45 (m, 1H), 2.87–2.67 (m, 2H), 1.72–1.52 (m, 2H), 1.42–1.31 (m, 1H), 1.18 (t, J = 7.4 Hz, 3H), 0.99 (t, J = 6.9 Hz, 6H); ¹³C-NMR (100 MHz, CDCl₃): δ 155.6, 154.2, 136.1, 134.3, 128.6, 128.3, 128.2, 67.1, 47.4, 44.0, 24.6, 22.7, 22.2, 20.2, 12.7; HRMS (ESI) m/z 343.1624 [M + Na]⁺ (calculated for 343.1628 C₁₇H₂₄N₂O₄Na⁺).

Benzyl (*S,E*)-2-(2-nitroprop-1-en-1-yl)pyrrolidine-1-carboxylate (3g) [25,26]. Synthesized according to Method E; yellow oil; $[\alpha]_D^{25}$ = −5 (c = 0.25, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ 7.46–7.27 (m, 5H), 6.94 (d, J = 9.4 Hz, 0.5H), 6.89 (d, J = 9.9 Hz, 0.5H), 5.18–4.98 (m, 2H), 4.60–4.50 (s, 0.5H), 4.50–4.40 (s, 0.5H), 3.64–3.52 (m, 2H), 2.35–2.16 (m, 2.5H), 2.11–1.76 (m, 4.5H); ¹³C-NMR (100 MHz, CDCl₃): δ 154.9, 154.6, 148.3, 147.7, 136.5, 136.0, 135.2, 135.0, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 67.6, 67.0, 55.1, 54.7, 47.0, 46.5, 32.4, 31.5, 24.4, 23.8, 12.8, 12.3; HRMS (ESI) m/z 313.1152 [M + Na]⁺ (calculated for 313.1159 C₁₅H₁₈N₂O₄Na⁺).

tert-Butyl (*S,E*)-(1-nitrohept-1-en-3-yl)carbamate (3h). Synthesized according to Method D; white solid; m.p. = 62–64 °C; $[\alpha]_D^{25}$ = −17 (c = 1.0, CH₃OH); ¹H-NMR (400 MHz, CDCl₃): δ 7.12 (dd, J_1 = 13.4 and J_2 = 5.6 Hz, 1H), 7.03 (d, J = 13.4 Hz, 1H), 4.88–4.80 (m, 1H), 4.41–4.13 (m, 1H), 1.65–1.55 (m, 2H), 1.46–1.39 (s, 9H), 1.35–1.26 (m, 4H), 0.88 (t, J = 6.9 Hz, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 155.1, 142.6, 139.7, 80.2, 49.1, 33.9, 28.2, 27.7, 22.2, 13.8; HRMS (ESI) m/z 281.1473 [M + Na]⁺ (calculated for 281.1472 C₁₂H₂₂N₂O₄Na⁺).

tert-Butyl (*S,E*)-(2-nitrooct-2-en-4-yl)carbamate (3i). Synthesized according to Method E; white solid; m.p. = 61–63 °C; $[\alpha]_D^{25}$ = −4 (c = 1.0, CH₃OH); ¹H-NMR (400 MHz, CDCl₃): δ 6.82 (d, J = 9.8 Hz, 1H), 4.80–4.70 (m, 1H), 4.35–4.12 (m, 1H), 2.29 (s, 3H), 1.69–1.58 (m, 1H), 1.55–1.48 (m, 1H), 1.42 (s, 9H), 1.35–1.24 (m, 4H), 0.89 (t, J = 6.9 Hz, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 155.1, 148.5, 135.5, 79.9, 48.8, 34.4, 28.3, 27.6, 22.3, 13.8, 12.9; HRMS (ESI) m/z 295.1625 [M + Na]⁺ (calculated for 295.1628 C₁₃H₂₄N₂O₄Na⁺).

tert-Butyl (*S,E*)-(3-nitronon-3-en-5-yl) carbamate (3j). Synthesized according to Method C; yellow oil; $[\alpha]_D^{25}$ = −5 (c = 1.0, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ 6.71 (d, J = 9.9 Hz, 1H), 4.61 (d, J = 8.2 Hz, 1H), 4.40–4.21 (m, 1H), 2.84–2.53 (m, 2H), 1.75–1.20 (m, 15H), 1.13 (t, J = 7.3 Hz, 3H), 0.88 (t, J = 6.6 Hz, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 155.0, 154.2, 134.6, 80.0, 48.4, 34.8, 28.3, 27.7, 22.3, 20.3, 13.8, 12.7; HRMS (ESI) m/z 309.1785 [M + Na]⁺ (calculated for 309.1785 C₁₄H₂₆N₂O₄Na⁺).

tert-Butyl (*S,E*)-(1-(benzyloxy)-4-nitrohex-3-en-2-yl)carbamate (3k). Synthesized according to Method C and E; yellow oil; $[\alpha]_D^{25}$ = −3 (c = 1.0, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ 7.41–7.27 (m, 5H), 6.98 (d, J = 9.6 Hz, 1H), 5.20–5.10 (m, 1H), 4.62–4.51 (m, 3H), 3.62 (dd, J_1 = 9.4 and J_2 = 4.3 Hz, 1H), 3.53 (dd, J_1 = 9.5 and J_2 = 4.1 Hz, 1H), 2.79–2.66 (m, 2H), 1.45 (s, 9H), 1.13 (t, J = 7.4 Hz, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 154.9, 154.8, 137.2, 131.8, 128.6, 128.1, 127.8, 80.2, 73.5, 71.3, 48.3, 28.4, 20.4, 12.8; HRMS (ESI) m/z 373.1735 [M + Na]⁺ (calculated for 373.1734 C₁₈H₂₆N₂O₅Na⁺).

Benzyl *tert*-butyl (7-nitronon-6-ene-1,5-diyl)(*S,E*)-dicarbamate (3l). Synthesized according to Method C; colorless oil; $[\alpha]_D^{25}$ = −4 (c = 1.0, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ 7.40–7.30 (m, 5H), 6.75 (d, J = 9.9 Hz, 1H), 5.11 (s, 2H), 4.97–4.79 (m, 2H), 4.41–4.24 (m, 1H),

3.28–3.12 (m, 2H), 2.84–2.64 (m, 2H), 1.58–1.34 (m, 15H), 1.15 (t, $J = 7.4$ Hz, 3H); ^{13}C -NMR (100 MHz, CDCl_3): δ 156.6, 155.1, 154.1, 136.5, 134.4, 128.5, 128.1, 128.1, 80.1, 66.7, 48.3, 40.4, 34.4, 29.7, 28.3, 22.5, 20.4, 12.8; HRMS (ESI) m/z 480.2364 $[\text{M} + \text{HCOOH} - \text{H}]^-$ (calculated for 480.2351 $\text{C}_{23}\text{H}_{34}\text{N}_3\text{O}_8^-$).

(*S,E*)-*tert*-Butyl (1-(methylthio)-5-nitrohex-4-en-3-yl) carbamate (3m). Synthesized according to Method E; yellowish oil; $[\alpha]_{\text{D}}^{25} = -4$ ($c = 1.0$, CHCl_3); ^1H -NMR (400 MHz, CDCl_3): δ 6.84 (d, $J = 9.8$ Hz, 1H), 5.08–4.76 (m, 1H), 4.63–4.41 (m, 1H), 2.59–2.44 (m, 2H), 2.28 (s, 3H), 2.10 (s, 3H), 1.99–1.90 (m, 1H), 1.86–1.75 (m, 1H), 1.42 (s, 9H); ^{13}C -NMR (100 MHz, CDCl_3): δ 155.0, 149.1, 134.4, 80.2, 47.9, 33.9, 30.1, 28.3, 15.5, 13.0; HRMS (ESI) m/z 313.1192 $[\text{M} + \text{Na}]^+$ (calculated for 313.1192 $\text{C}_{12}\text{H}_{22}\text{N}_2\text{NaO}_4\text{S}^+$).

(*S,E*)-Benzyl (1-(methylthio)-5-nitrohept-4-en-3-yl) carbamate (3n). Synthesized according to Method E; yellow oil; $[\alpha]_{\text{D}}^{25} = -5$ ($c = 1.0$, CHCl_3); ^1H -NMR (400 MHz, CDCl_3): δ 7.41–7.32 (m, 5H), 6.77 (d, $J = 10.0$ Hz, 1H), 5.23–5.07 (m, 3H), 4.70–4.49 (m, 1H), 2.87–2.67 (m, 2H), 2.61–2.46 (m, 2H), 2.11 (s, 3H), 2.04–1.91 (m, 1H), 1.89–1.79 (m, 1H), 1.18 (t, $J = 7.3$ Hz, 3H); ^{13}C -NMR (100 MHz, CDCl_3): δ 155.6, 154.8, 136.0, 133.0, 128.6, 128.4, 128.2, 67.2, 48.1, 34.0, 30.1, 20.4, 15.5, 12.8; HRMS (ESI) m/z 361.1192 $[\text{M} + \text{Na}]^+$ (calculated for 361.1192 $\text{C}_{16}\text{H}_{22}\text{N}_2\text{NaO}_4\text{S}^+$).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/org3020011/s1>. ^1H NMR and ^{13}C NMR spectra of the compounds synthesized.

Author Contributions: Conceptualization, G.K.; experimental investigation, V.-E.G., G.S.K. and D.T.G.; writing—original draft preparation, G.S.K. and G.K.; writing—review and editing, G.S.K. and G.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a Stavros Niarchos Foundation (SNF) grant to the National and Kapodistrian University of Athens.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are available in the main text or the Supplementary Materials.

Acknowledgments: V.E.G. would like to thank Eugenides Foundation for a scholarship. G.S.K. would like to thank SNF for a doctoral fellowship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sutanto, F.; Konstantinidou, M.; Dömling, A. Covalent inhibitors: A rational approach to drug discovery. *RSC Med. Chem.* **2020**, *11*, 876–884. [\[CrossRef\]](#)
2. Ghosh, A.K.; Samanta, I.; Mondal, A.; Liu, W.R. Covalent inhibition in drug discovery. *ChemMedChem* **2019**, *14*, 889–906. [\[CrossRef\]](#)
3. Martin, J.S.; MacKenzie, C.J.; Fletcher, D.; Gilbert, I.H. Characterising covalent warhead reactivity. *Bioorg. Med. Chem.* **2019**, *27*, 2066–2074. [\[CrossRef\]](#)
4. Dalton, S.E.; Campos, S. Covalent small molecules as enabling platforms for drug discovery. *ChemBioChem* **2020**, *21*, 1080–1100. [\[CrossRef\]](#)
5. Garner, R.M.; Mould, D.R.; Chieffo, C.; Jorkasky, D.K. Pharmacokinetic and pharmacodynamic effects of oral CXA-10, a nitro fatty acid, after single and multiple ascending doses in healthy and obese subjects. *Clin. Transl. Sci.* **2019**, *12*, 667–676. [\[CrossRef\]](#)
6. Koutoulogenis, G.S.; Kokotos, G. Nitro Fatty Acids (NO_2 -FAs): An emerging class of bioactive fatty acids. *Molecules* **2021**, *26*, 7536. [\[CrossRef\]](#)
7. Batthyany, C.; Schopfer, F.J.; Baker, P.R.S.; Durán, R.; Baker, L.M.S.; Huang, Y.; Cervenansky, C.; Branchaud, B.P.; Freeman, B.A. reversible post-translational modification of proteins by nitrated fatty acids in vivo. *J. Biol. Chem.* **2006**, *281*, 20450–20463. [\[CrossRef\]](#)
8. Baker, L.M.S.; Baker, P.R.S.; Golin-Bisello, F.; Schopfer, F.J.; Fink, M.; Woodcock, S.R.; Branchaud, B.P.; Radi, R.; Freeman, B.A. Nitro-fatty acid reaction with glutathione and cysteine. Kinetic analysis of thiol alkylation by a Michael addition reaction. *J. Biol. Chem.* **2007**, *282*, 31085–31093. [\[CrossRef\]](#)

9. Turell, L.; Vitturi, D.A.; Coitiño, E.L.; Lebrato, L.; Möller, M.N.; Sagasti, C.; Salvatore, S.R.; Woodcock, S.R.; Alvarez, B.; Schopfer, F.J. The chemical basis of thiol addition to nitro-conjugated linoleic acid, a protective cell-signaling lipid. *J. Biol. Chem.* **2017**, *292*, 1145–1159. [[CrossRef](#)]
10. Fazzari, M.; Vitturi, D.A.; Woodcock, S.R.; Salvatore, S.R.; Freeman, B.A.; Schopfer, F.J. Electrophilic fatty acid nitroalkenes are systemically transported and distributed upon esterification to complex lipids. *J. Lipid Res.* **2019**, *60*, 388–399. [[CrossRef](#)]
11. Khoo, N.K.H.; Li, L.; Salvatore, S.R.; Schopfer, F.J.; Freeman, B.A. Electrophilic fatty acid nitroalkenes regulate Nrf2 and NF- κ B signaling: A medicinal chemistry investigation of structure-function relationships. *Sci. Rep.* **2018**, *8*, 2295. [[CrossRef](#)]
12. Blanco, F.; Ferreira, A.M.; López, G.V.; Bonilla, L.; Gonzalez, M.; Cerecetto, H.; Trostchansky, A.; Rubbo, H. 6-Methylnitroarachidonate: A novel esterified nitroalkene that potently inhibits platelet aggregation and exerts cGMP-mediated vascular relaxation. *Free Rad. Biol. Med.* **2011**, *50*, 411–418. [[CrossRef](#)]
13. Rodriguez-Duarte, J.; Galliussi, G.; Dapuerto, R.; Rossello, J.; Malacrida, L.; Kamaid, A.; Schopfer, F.J.; Escande, C.; López, G.V.; Batthyany, C. A novel nitroalkene- α -tocopherol analogue inhibits inflammation and ameliorates atherosclerosis in Apo E knockout mice. *Br. J. Pharmacol.* **2019**, *176*, 757–772. [[CrossRef](#)]
14. Dapuerto, R.; Rodriguez-Duarte, J.; Galliussi, G.; Kamaid, A.; Bresque, M.; Batthyany, C.; López, G.V.; Escande, C. A novel nitroalkene vitamin E analogue inhibits the NLRP3 inflammasome and protects against inflammation and glucose intolerance triggered by obesity. *Redox Biol.* **2021**, *39*, 101833. [[CrossRef](#)]
15. Latorre, A.; Schirmeister, T.; Kesselring, J.; Jung, S.; Johé, P.; Hellmich, U.A.; Heilos, A.; Engels, B.; Krauth-Siegel, R.L.; Dirdjaja, N.; et al. Dipeptidyl nitroalkenes as potent reversible inhibitors of cysteine proteases rhodesain and cruzain. *ACS Med. Chem. Lett.* **2016**, *7*, 1073–1076. [[CrossRef](#)]
16. Lecaille, F.; Kaleta, J.; Brömme, D. Human and parasitic papain-like cysteine proteases: Their role in physiology and pathology and recent developments in inhibitor design. *Chem. Rev.* **2002**, *102*, 4459–4488. [[CrossRef](#)]
17. Arafet, K.; González, F.V.; Moliner, V. Quantum Mechanics/Molecular Mechanics studies of the mechanism of cysteine proteases inhibition by dipeptidyl nitroalkenes. *Chem. A Eur. J.* **2020**, *26*, 2002–2012. [[CrossRef](#)]
18. Arafet, K.; Serrano Aparicio, N.; Lodola, A.; Mulholland, A.J.; González, F.V.; Świderek, K.; Moliner, V. Mechanism of inhibition of SARS-CoV-2 Mpro by N3 peptidyl Michael acceptor explained by QM/MM simulations and design of new derivatives with tunable chemical reactivity. *Chem. Sci.* **2021**, *12*, 1433–1444. [[CrossRef](#)]
19. Hassan, M.; Nde, C.N.; Manolikakes, G. Synthesis of nitroolefins via the direct nitration of alkenes. *SynOpen* **2021**, *5*, 229–231.
20. Fioravanti, S.; Pellacani, L.; Tardella, P.A.; Vergari, M.C. Facile and highly stereoselective one-pot synthesis of either (E)- or (Z)-nitro alkenes. *Org. Lett.* **2008**, *10*, 1449–1451. [[CrossRef](#)]
21. Woodcock, S.R.; Marwitz, A.J.V.; Bruno, P.; Branchaud, B.P. Synthesis of nitrolipids. All four possible diastereomers of nitrooleic acids: (E)- and (Z)-, 9- and 10-nitro-octadec-9-enoic acids. *Org. Lett.* **2006**, *8*, 3931–3934. [[CrossRef](#)]
22. Gorczynski, M.J.; Smitherman, P.K.; Akiyama, T.E.; Wood, H.B.; Berger, J.P.; King, S.B.; Morrow, C.S. Activation of Peroxisome Proliferator-Activated Receptor γ (PPAR γ) by nitroalkene fatty acids: Importance of nitration position and degree of unsaturation. *J. Med. Chem.* **2009**, *52*, 4631–4639. [[CrossRef](#)]
23. Hock, K.J.; Grimmer, J.; Göbel, D.; Gasaya, G.G.T.; Roos, J.; Maucher, I.V.; Kühn, B.; Fettel, J.; Maier, T.J.; Manolikakes, G. Modular regiospecific synthesis of nitrated fatty acids. *Synthesis* **2016**, *49*, 615–636.
24. Hassan, M.; Krieg, S.C.; Nde, C.N.; Roos, J.; Maier, T.J.; El Rady, E.A.; Raslan, M.A.; Sadek, K.U.; Manolikakes, G. Streamlined one-pot synthesis of nitro fatty acids. *Eur. J. Org. Chem.* **2021**, *15*, 2239–2252. [[CrossRef](#)]
25. Bhat, C.; Tilve, S.G. Synthesis of (-)-hygrine, (-)-norhygrine, (-)-pseudohygrine and (-)-hygrine via Nef reaction. *Tetrahedron Lett.* **2011**, *52*, 6566–6568. [[CrossRef](#)]
26. Bhat, C.; Tilve, S.G. Henry-Nef reaction: A practical and versatile chiral pool route to 2-substituted pyrrolidine and piperidine alkaloids. *Tetrahedron* **2013**, *69*, 6129–6143. [[CrossRef](#)]
27. Hanessian, S.; Devasthale, P.V. Generation of functional diversity via nitroaldol condensations of α -amino acid aldehydes—A new and stereocontrolled route to acyclic 1,3-diamino-2-alcohols. *Tetrahedron Lett.* **1996**, *37*, 987–990. [[CrossRef](#)]
28. Pereira, V.L.P.; Moura, A.L.; Vieira, D.P.; de Carvalho, L.L.; Torres, E.R.; da Costa, J.S. A versatile and efficient approach for the synthesis of chiral 1,3-nitroamines and 1,3-diamines via conjugate addition to new (S,E)- γ -aminated nitroalkenes derived from L- α -amino acids. *Beilstein J. Org. Chem.* **2013**, *9*, 832–837. [[CrossRef](#)]
29. Meirelis, F.P.; Vieira, B.G.N.; Pereira, V.L.P. Divergent and diastereoselective synthesis of α -monosubstituted and trans- α,β -disubstituted γ -lactams from (S)-N,N-dibenzyl- α -amino aldehydes via Henry and Michael reactions. *Synthesis* **2020**, *52*, 3650–3656.
30. Ambroise, L.; Jackson, R.F.W. Stereoselective synthesis of anti- β -amino- α -hydroxy acid derivatives using nucleophilic epoxidation of 1-arylthio-1-nitroalkenes. *Tetrahedron Lett.* **1996**, *37*, 2311–2314. [[CrossRef](#)]
31. Kokotos, G. A convenient one-pot conversion of N-protected amino acids and peptides into alcohols. *Synthesis* **1990**, *1990*, 299–301. [[CrossRef](#)]
32. Kokotos, G.; Noula, C. Selective one-pot conversion of carboxylic acids into alcohols. *J. Org. Chem.* **1996**, *61*, 6994–6996. [[CrossRef](#)] [[PubMed](#)]
33. Loukas, V.; Noula, C. Efficient protocols for the synthesis of enantiopure γ -amino acids with proteinogenic side chains. *J. Peptide Sci.* **2003**, *9*, 312–319. [[CrossRef](#)] [[PubMed](#)]