EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201200346

Reactivity of (*E*)-4-Hydroxy-2-nonenal with Fluorinated Phenylhydrazines: Towards the Efficient Derivatization of an Elusive Key Biomarker of Lipid Peroxidation

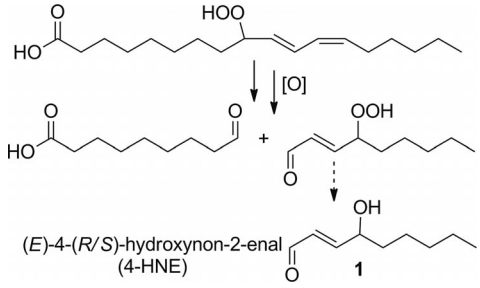
Riccardo Matera,[a] Simone Gabbanini,[a] Alice Valvassori,[b] Mathilde Triquigneaux,[c] and Luca Valgimigli*[b]

Keywords: Synthetic methods / Hydrazones / Cyclization / Lipids / Kinetics

4-Hydroxynonenal (4-HNE) is a major product of the oxidation of ω-6-polyunstaturated lipids and an effector of radical-mediated oxidative damage, whose analytical determination requires chemical derivatization. In this work, its reactivity with fluorinated phenylhydrazines was explored both under preparative and analytical settings. A five-step synthesis of 4-HNE on gram-scale with an overall yield of 30 % is described. Reaction of 4-HNE with *ortho*-, *meta*-, or *para*-CF_3-phenylhydrazine, as well as with the 3,5-di-CF_3, 2,4-di-CF_3, or pentafluoro analogues, in MeCN with 0.5 mM TFA yields the corresponding hydrazones with rate constants k_f of 2.8 ± 0.4, 1.7 ± 0.1, 3.0 ± 0.2, 0.6 ± 0.1, 0.5 ± 0.1, and 3.5 ± 0.5 $M^{-1} s^{-1}$, respectively at 298 K. At higher temperatures, the hydrazones undergo intramolecular cyclization to form 1,6-dihydropyridazines that, depending on the solvent and temperature, may further react with the hydrazine to yield tetrahydropyridazine adducts and their oxidation products. Other reaction products were isolated, depending on the reaction conditions, and the complex reactivity of 4-HNE with the above nucleophiles is discussed. Due to the good yield and rate of formation of the hydrazone adducts, their stability and favorable UV absorbance, 2-(trifluoromethyl)-phenylhydrazine and 2,3,4,5,6-pentafluorophenylhydrazine are the most interesting candidates for the development of rapid and efficient analytical derivatizations of 4-HNE.

Introduction

Lipid peroxidation is one of the most important and detrimental types of radical-mediated biological damage, a chain-reaction sustained by the attack of peroxyl radicals to polyunsaturated lipids.[1] Initially formed lipid hydroperoxides undergo enzymatic transformation or further oxidation and spontaneous Hock-type cleavage to yield a variety of aldehydes.[2] Among them, (*E*)-4-hydroxy-2-nonenal (4-HNE) is a major aldehyde product that originates in vivo during peroxidation of ω-6-polyunsaturated fatty acids (18:2, 20:4; Scheme 1).[3,4] The importance of 4-HNE is, however, not limited to being a major oxidation product but is also related to its primary involvement in inflammatory events, in the regulation of cell signaling, proliferation, and apoptosis, as well as being a chemotactic factor of phagocytosis by polymorphonuclear leukocytes.[5,6] Due to its electrophilic nature, 4-HNE is also a mutagenic agent and is able to alter protein function.[7,8]



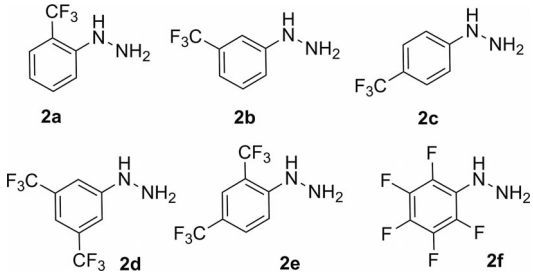
Scheme 1. Formation of 4-HNE during peroxidation of ω-6 polyunsaturated lipids.

In other words, 4-HNE is not only a product of lipid peroxidation but also a key effector of the associated biological damage[9–12] and/or a mediator of the associated cell signaling function.[13] Hence its analytical determination in biological systems as a key biomarker of radical-mediated damage is of major relevance. Its determination would also be relevant in food chemistry to assess preservation of fats and oils and guarantee consumer safety.

Not surprisingly, several investigations have dealt with the development of analytical methods to address this challenging need, none of which, however, appears to be ideally suited to the task. Besides the obvious problem associated

[a] BeC s.r.l., R&D Division,
Via C. Monteverdi 49, 47100 Forlì, Italy
[b] University of Bologna, Department of Organic Chemistry "A. Mangini",
Via S. Giacomo 11, 40126 Bologna, Italy
Fax: +39-051-2095688
E-mail luca.valgimigli@unibo.it
[c] Universités Aix-Marseille I, II & III-CNRS,
UMR 6264: Laboratoire Chimie Provence,
13397 Marseille Cedex 20, France
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201200346.

with its low concentration (micromolar levels are documented in tissues[6]), 4-HNE gives very low sensitivity with the most common analytical approaches (e.g., HPLC–UV, HPLC–MS, GC–MS), which makes chemical derivatization an important preliminary step. Due to the electrophilic nature of 4-HNE, the majority of such methods are based on a reaction with a nucleophile having good UV absorbance or yielding a relatively volatile product, such as benzylamine,[14] *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine,[15] or 2,4-dinitrophenylhydrazine.[16] In most cases, however, conversion into the desired imine, oxime, or hydrazone is largely incomplete, possibly due to the relevant formation of side products – a fact that appears to be overlooked in the current literature – resulting in low recoveries in quantitative analysis.[17,18] Surprisingly, little is known on the reactivity of 4-HNE with nucleophiles, partly owing to its instability[19] and difficult synthetic accessibility on a suitable scale. Therefore, the vast majority of derivatization methods are developed on a trial-and-error basis, limiting the efficient and rational optimization of the methods themselves. In the course of the development of one such analytical approach, based on the quantitation of 4-HNE after derivatization with fluorinated phenylhydrazines, chosen because of their favorable chromatographic behavior, we faced a previously undescribed very complex reactivity of 4-HNE, which we set to explore, by using the nucleophiles shown in Scheme 2, to fill the gap of knowledge and to provide a basis for the rational development of improved analytical methods. We summarize here our results together with an improved method for the synthesis of 4-HNE.

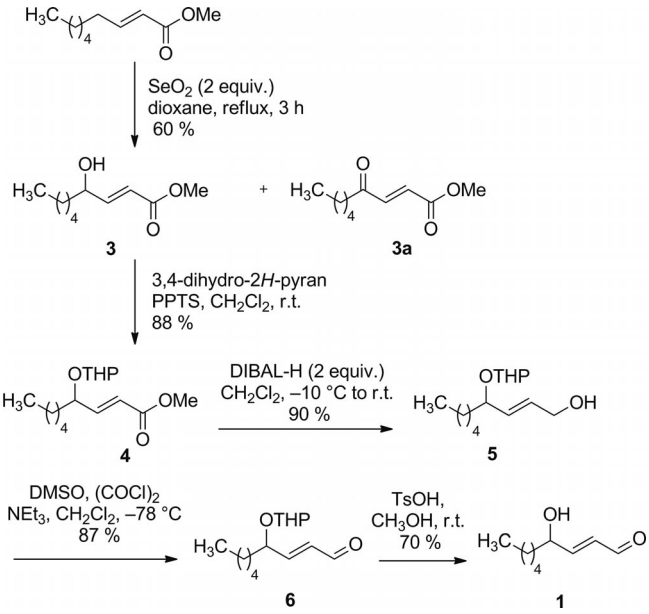


Scheme 2. Fluorinated phenylhydrazines investigated as reagents in this study.

Results and Discussion

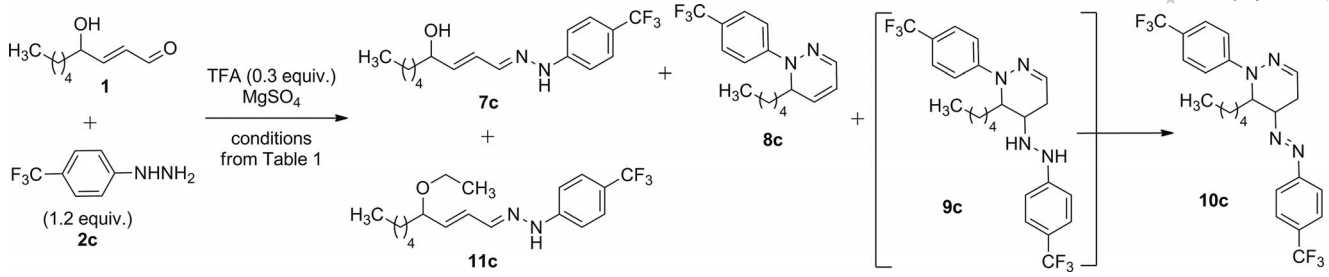
Synthesis of (*E*)-4-Hydroxy-2-nonenal

Synthesis of 4-HNE was accomplished on gram-scale by using the five-step sequence summarized in Scheme 3, with ~30% overall yield. The first step, consisting in the regioselective selenium dioxide mediated allylic hydroxylation of commercially available methyl non-2-enonate, proceeded in ca. 60% yield (in product **3**), affording also as much as 10% of corresponding ketone **3a**. However, the unreacted starting material was easily recovered by flash chromatography and could be recycled in subsequent product batches, whereas ketone **3a** can, in principle, be reduced to desired enol **3** with $NaBH_4$, as originally proposed by Jouanin et al.[20] Indeed, our revised sequence represents a significant advancement over the original proposal, where hydroxylation of the non-2-enonate was obtained in two steps consisting in oxidation to **3a** with SeO_2/TBHP (in reported 25% yield after 72 h) followed by reduction.[20] Any subsequent step proceeded with yields close to 90%, including the oxidation of protected alcohol **5** to the desired aldehyde, which we accomplished under Swern conditions, at variance with the approach previously reported (Dess–Martin periodinane) that showed to be less efficient (reported yield 62%).[20] A lower yield was, however, observed in the last step. Although deprotection of acetal **6** was nearly quantitative, the product [i.e., 4-HNE (**1**)] was unstable even under mild purification conditions, affording 4-HNE in 70% yield after chromatography on silica. Higher yields could be obtained by using alumina as stationary phase; however, the purification was less reproducible and satisfactory. Interestingly, degradation of 4-HNE was observed also on prolonged storage at –20 °C; therefore, we preferred to store protected form **6** and proceed to its rapid hydrolysis/purification in small batches shortly before use.



Scheme 3. Synthesis of (*E*)-4-(*R*/*S*)-hydroxy-2-nonenal.

Other synthetic routes where comparatively tested; however, they were generally less satisfactory. For instance, the two-step sequence consisting in the epoxidation of 3-nonen-1-ol with 3-chloroperbenzoic acid, followed by oxidation with pyridinium chlorochromate, described by Spiteller et al.,[21] yielded overall 16% of 4-hydroxy-2-nonenal selectively in the (unwanted) *Z*-isomer, whereas the five-step sequence from furan described by Grée et al.,[22] in our hands, yielded overall less than 3% of the desired product after a very difficult final purification step. Additional methods for the synthesis of 4-HNE have been proposed in recent years;[23,24] however, they where not comparatively tested.



Scheme 4. Summary of products isolated in the reaction of 4-HNE (**1**) with hydrazine **2c**.

Reactivity of 4-HNE with hydrazines 2a–f

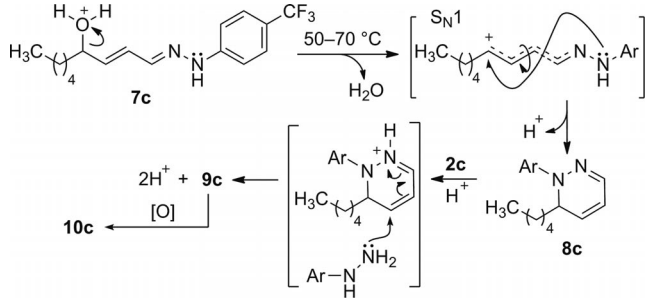
Whereas the reaction of 4-HNE with 4-trifluoromethylphenylhydrazine (**2c**) in dry ethanol proceeded inefficiently and very slowly in the absence of acids, addition of 1 equiv. of sulfuric acid at 50 °C for 1 h yielded a complex multitude of uncharacterized products, of which the expected hydrazone represented only trace amounts. Replacing sulfuric acid with 0.1 to 0.3 equiv. of 4-toluensulfonic acid (TsOH) or trifluoroacetic acid (TFA) afforded a somewhat simpler scenario, as depicted in Scheme 4. The nature and yield of the reaction products significantly depended on the reaction conditions, particularly the solvent and temperature, and allowed some degree of control, as summarized in Table 1.

Table 1. Experimental conditions and associated product yields for the reaction of 4-HNE with 4-trifluomethylphenylhydrazine (**2c**).

Solvent	*T* / °C	*t*	Product (% yield)[a]
EtOH	r.t.	10 min	**7c** (65) + **11c** (trace)
EtOH/H_2O (99:1)	r.t.	10 min	**7c** (70)[b]
MeCN	r.t.	15 min	**7c** (80)[b]
*t*BuOH	25	10 min	**7c** (73)[b]
EtOH	50	6 h	**8c** (30) + **11c** (40)
*t*BuOH	50	3 h	**7c** (32) + **8c** (38)
MeCN	50	3 h	**8c** (32) + **9c** (15) +**10c**[c]
*t*BuOH	70	5 h	**8c** (64)[b]
MeCN	70	7 h	**8c** (3) + **9c** (50) + **10c**[c]

[a] Yield of isolated product. [b] Yield appeared quantitative by TLC analysis of the reaction mixture. [c] Formed at the expense of **9c** during workup and purification.

At room temperature, in ethanol or acetonitrile (or in *tert*-butyl alcohol just above its melting temperature), linear hydrazone **7c** was formed rapidly (10–15 min) and selectively and could be isolated in 65–80% yield. Trace amounts of compound **11c** formed by reaction with the solvent were observed in ethanol; however, its formation can be completely suppressed by the addition of 1% water to the reaction mixture, or in less nucleophilic solvents. On increasing the reaction temperature and time, the hydrazone cyclized to 1,6-dihydropyridazine **8c**, as exemplified in Scheme 5. Evidence for the mechanism to explain the formation of **8c** was obtained by incubating isolated linear hydrazone **7c** with 0.3 equiv. of TFA at 50 or 70 °C in acetonitrile for 5 h, resulting in nearly complete conversion into **8c**. Product **9c** resulting from addition of a second arylhydrazine molecule to **8c** became dominant at 70 °C, when acetonitrile was the solvent or when an excess amount of hydrazine was present in the reaction mixture. Furthermore, **9c** could be isolated in >50% yield by treating isolated **8c** with 1 equiv. of **2c** and 0.3 equiv. of TFA (in MeCN, 70 °C, N_2 atmosphere) envisioning a mechanism of 1,4-addition of the second hydrazine on dihydropyridazine **8c** (Scheme 5). On standing in solution at room temperature, **9c** was rapidly oxidized by atmospheric oxygen to diazenyl derivative **10c**.



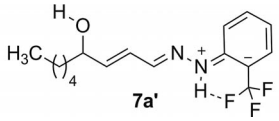
Scheme 5. Proposed mechanism leading to the formation of products **8c**–**10c**.

It is worth noting that with any of the tested hydrazines, the 1,4-addition proceeded in a diastereoselective fashion to furnish predominantly the *anti* adducts (i.e., for **9**–**10c**, *anti*/*syn* = 8:1 *dr*, from ^{1}H NMR spectroscopy), as nucleophilic attack is more favorable from the less hindered side. From racemic **8c**, major *anti* adducts were obtained as a couple of enantiomers. A ^4J coupling can be observed in the COSY spectrum between the H3 and H5 protons of the pyridazine ring due to W-shaped coupling (see Figure S1 and the COSY spectrum of **10c** in the Supporting Information).

Reaction of 4-HNE with *meta*-substituted phenylhydrazines **2b** and **2d** (3-CF_3- and 3,5-di-CF_3-, respectively) afforded similar results. Linear hydrazones **7b** and **7d** were selectively obtained at room temperature (isolated yields were 62 and 80%, respectively, when the reaction occurred in EtOH), whereas formation of dihydropyridazines **8b** and **8d**, together with tetrahydropyridazine adducts **9b** and **9d**, became increasingly relevant at higher temperatures (40–70 °C). Oxidation of these last two substrates to diazenyl derivatives **10** was very rapid for the monotrifluoromethylated compound (i.e., **9b** to **10b**), whereas it occurred only very slowly (about two weeks at r.t.) for the more electron poor disubstituted derivative (i.e., **9d** to **10d**).

Interestingly, the scenario was substantially different when 4-HNE was treated with *ortho*-CF_3-phenylhydrazine **2a**, as linear hydrazone **7a** was the only reaction product

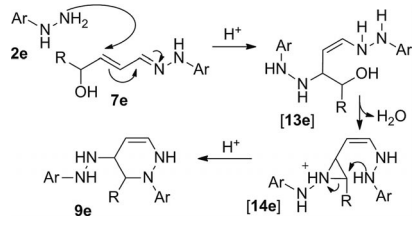
that could be isolated from the reaction mixture (in 60–70% yield), in different solvents in the temperature range 20–70 °C. Lack of formation of cyclic adducts **8**–**10** indicates that intramolecular nucleophilic attack from the inner nitrogen atom in **7a** to the carbon atom in the 4-position is hampered by intramolecular H-bonding (NH···F) to the CF_3 substituent, which stabilizes resonance structure **7a′**.



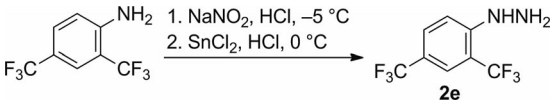
Support to the occurrence of such an interaction is provided by the ^{1}H NMR spectrum, which shows a higher field signal for the (N)H proton in **7a** (δ =9.39 ppm) as compared to *meta* isomer **7b** (δ =10.34 ppm) or *para* isomer **7c** (δ =10.46 ppm).

To check this hypothesis, we prepared 2,4-di-CF_3-phenylhydrazine (**2e**) from the corresponding aniline through diazotization and subsequent $SnCl_2$ reduction according to Scheme 6, and subjected it to reaction with 4-HNE under the usual variety of conditions. In no case could dihydropyridazine **8** be observed among the reaction products, even at temperatures as high as 70 °C. However, by using an excess amount of the hydrazine, minor amounts (ca. 10%) of adduct **9e** could be isolated, as illustrated in Scheme 7. Because formation of **9e** implies the transient formation of dihydropyridazine **8e** (see Scheme 5), this finding suggests that the nucleophilicity of hydrazone **7e** is not completely abolished by the presence of the *ortho*-CF_3 group, but it is, nonetheless, significantly hampered, as can be deduced from comparison with the different behavior recorded with hydrazines **2b**–**d**.[25] Electron-poor **9e** did not spontaneously oxidize in solution.

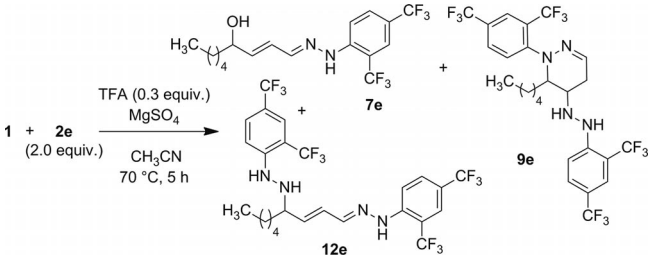
An alternative mechanism to justify the formation of **9e** in the absence of **8e** can also be hypothesized, such as that illustrated in Scheme 8.



Scheme 8. Alternative mechanism to explain the formation of adduct **9e** in the absence of 1,6-piridazine **8e** (see Scheme 7). In this scheme, Michael-type addition of a second molecule of arylhydrazine to hydrazone **7e** results in hypothetical adduct **13e** (not isolated), in which the ring closure upon nucleophilic intermolecular substitution of the OH group is facilitated by anchimeric assistance by the newly entered neighboring NH group (structure **14e**).

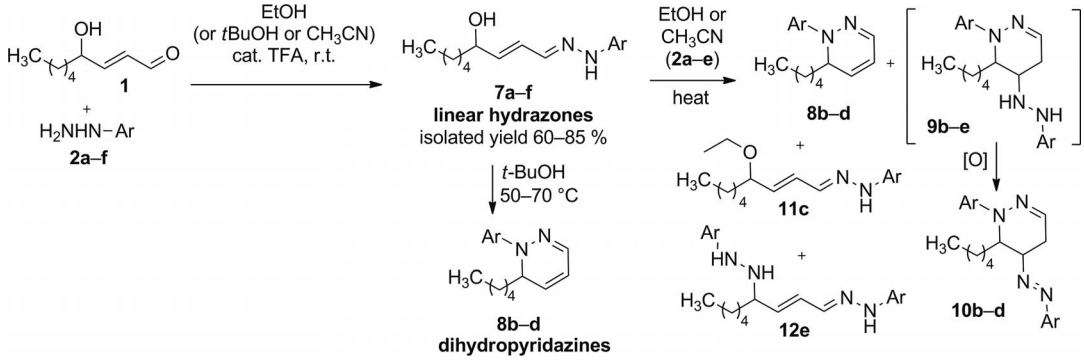


Scheme 6. Synthesis of 2,4-di-CF_3-phenylhydrazine (**2e**).



Scheme 7. Reaction of 4-HNE (**1**) with 2,4-di-CF_3-phenylhydrazine (**2e**).

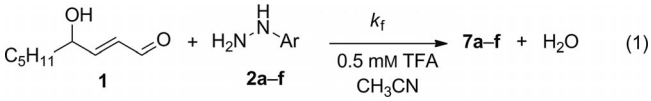
The reactivity of 4-HNE with phenylhydrazines **2a**–**f** is summarized in Scheme 9. Overall, it was possible to selectively prepare linear hydrazones **7a**–**f** in good yield by treating 4-HNE with a slight excess amount (1.2 equiv.) of the corresponding hydrazines at room temperature for a short time (10–20 min), and the reaction was tolerant to the presence of some water (1–2% v/v) and air. This reaction appeared particularly suited to be implemented for analytical purposes. Selective formation of dihydropyridazines **8b**–**d**[26] was also possible by performing the reaction at higher temperature (50–70 °C) for a longer time (5–7 h) in *tert*-butyl alcohol, but a mixture of products was always observed in other solvents. Because the reaction appeared somewhat less useful for 4-HNE derivatization, it was not further optimized.



Scheme 9. Summary of the reactivity of fluorinated phenylhydrazines with 4-HNE.[26]

Optimized Formation and Properties of Linear Hydrazones 7a–f

To be useful for analytical derivatization, a reaction is expected to satisfy some key requisites: (i) The reaction should be sufficiently simple and not overly sensitive to water and air. (ii) The reaction should be as close as possible to being quantitative. (iii) The reaction should be as fast as possible as to allow the screening of many samples in a reasonable time. (iv) The reaction should yield products that are stable under common laboratory conditions and that are easily detected (e.g., by UV/Vis spectroscopy). Although it is clear that Equation (1) satisfies some of these requisites, we sought to optimize the reaction under analytical settings (i.e., at very low concentration of 4-HNE) and investigated some relevant properties of product hydrazones **7a**–**f**.



(1)

When 4-HNE (**1**) was treated at room temperature in the presence of a large excess (10-fold or higher) of hydrazines **2a**–**f**, the best results were always obtained in acetonitrile as the solvent, where the formation of hydrazones **7a**–**f** proceeded quantitatively, with calculated yields (by HPLC analysis) in the range 98 ± 4%. However, when the initial concentration of 4-HNE was in the range 1–50 μM (i.e., of the magnitude expected in oxidized food or in biological tissues), the reaction time to completion was significantly longer than under preparative settings, which prompted a preliminary evaluation of the reaction kinetics. When an excess amount of the arylhydrazine was used, the reaction proceeded with apparent pseudo-first-order kinetics, as illustrated in Figure 1.

Analysis of the kinetic traces under different initial concentrations of the arylhydrazine allowed the apparent second-order rate constants of formation (k_f) to be estimated. Data recorded in the presence of 0.5 mM TFA are collected in Table 2.[27] The recorded rate constants differed by as much as sevenfold among the tested reactions under identical settings. The presence of a moderately electron-withdrawing groups (CF_3 or F) in conjugated positions (i.e., in compounds **2a**, **2c**, and **2f**) slightly accelerated the reaction

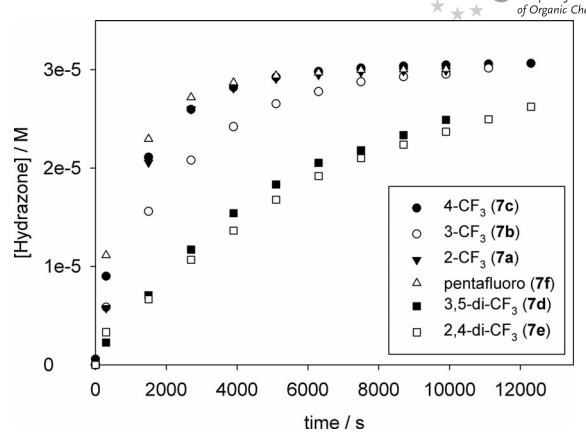
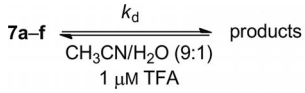


Figure 1. Time course of hydrazones **7a**–**f** formation by reaction of 30 μM 4-HNE with 10-fold excess amount of hydrazines **2a**–**f** in the presence of 0.5 mM TFA at 298 K in MeCN.

(with respect to *meta*-substituted **2b**). On the other hand, di-CF_3-substituted compounds **2d** and **2e** reacted significantly slower, possibly due to higher steric hindrance.

The stabilities of hydrazones **7a**–**f** also depended to some extent on the pattern of substitution in the aromatic ring, although not in an easily predictable manner. Any of the tested hydrazones was perfectly stable in acetonitrile solution in air for 24 h, indicating low susceptibility to air oxidation. On the other hand, some of them were less stable in the presence of water and catalytic amounts of TFA. No significant hydrolysis was recorded for **7c** and **7f**, whereas any other hydrazone gave some degradation over 5 h, which appeared to proceed to equilibrium [Equation (2)] as illustrated in Figure 2. The apparent pseudo-first-order rate constant of decomposition, (k_d, at 298 K) in the presence of 10% water and 1 μM TFA was obtained from traces of the initial slopes of decay and confirmed by numerical fittings by using Gepasi software.[27–29] Results are collected in Table 2. The reasons for the recorded differences among tested compounds are currently not understood. On the other hand, their different kinetic behavior is clearly of major relevance in the design of efficient analytical methods.



(2)

Table 2. UV/Vis spectral properties, rate of formation, and stability of hydrazones **7a**–**f** at 298 K.

Compound	λ_{max}[a] / nm	ε[a] / abs cm^{-1} mol^{-1}	k_f[b] / M^{-1} s^{-1}	k_d[c] / 10^{-5} s^{-1}	% Degrad. (5 h)[d]
7a	310	26000	2.8 ± 0.4	1.4 ± 0.2	8.7
7b	305	18130	1.7 ± 0.1	1.7 ± 0.4	11.0
7c	324	35800	3.0 ± 0.2	ca. 0	ca. 0
7d	306	37960	0.6 ± 0.1	1.9 ± 0.3	10.4
7e	312	21980	0.5 ± 0.1	2.8 ± 0.8	16.9
7f	294	31670	3.5 ± 0.5	ca. 0	ca. 0

[a] Determined in MeCN. [b] Apparent rate constant of formation of the hydrazone in MeCN at 298 K in the presence of 0.5 mM TFA. [c] Apparent pseudo-first-order rate constant of degradation of the hydrazone in MeCN/H_2O (9:1) at 298 K in the presence of 1 μM TFA. [d] Percent degradation at the equilibrium under identical settings as determined from spectrophotometric measurements.

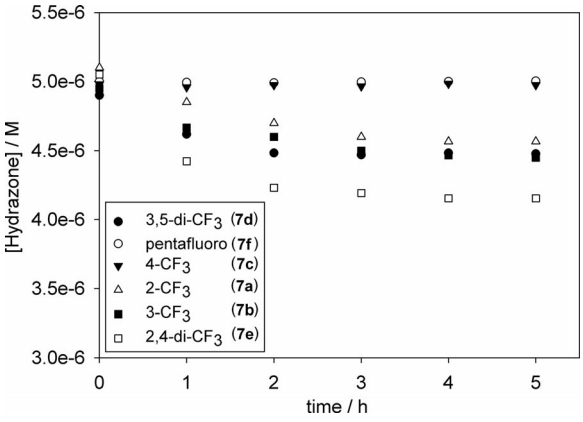


Figure 2. Degradation of hydrazones **7a**–**f** at 298 K in MeCN/H_2O (9:1) and 1 μM TFA.

Indeed, upon consideration of their kinetic behavior, derivatives **7c** and **7f** are clearly the most interesting for further implementation of analytical derivatization methods for 4-HNE. Not only were they perfectly stable to both hydrolysis and air oxidation under common laboratory settings, but they had also the highest rate of formation, being potentially suitable for the rapid screening of many samples (by using initial concentration of hydrazines **2c** and **2f** around 1 mM the reaction was complete after ca. 10–15 min). Of interest, hydrazones **7c** and **7f** also had among the highest UV extinction coefficients (see Table 2) in a spectral region (300–320 nm), perfectly suited to spectrophotometric detection coupled to separation techniques (e.g., HPLC–UV).

Conclusions

An improved strategy for the gram-scale synthesis of (*E*)-4-HNE has allowed detailed investigation of its reactivity with fluorinated phenylhydrazines as promising novel reagents for analytical derivatization of such a relevant, yet unstable, biomarker of lipid peroxidation. The product distribution largely depends on the reaction conditions, strengthening the concept that the reactivity of 4-HNE might be more complex than often supposed in other works dealing with its chemical derivatization. Expected linear hydrazones are formed quantitatively and rapidly at room temperature (in MeCN) in the presence of catalytic amounts of TFA. At higher temperature, intramolecular cyclization products (1,6-dihydropyridazines and tetrahydropyridazine adducts) become prevalent. On the basis of the relative stability of the adducts, their favorable UV absorbance, and the kinetics of the reaction, 4-trifluoromethylphenylhydrazine and 2,3,4,5,6-pentafluophenylhydrazine are the most promising reactants for derivatization of 4-HNE under analytical settings. Comparative evaluation of the reactivity of such derivatizing agents with other relevant aldehyde products formed during lipid peroxidation will also be pursued in future work.

Experimental Section

General: Reagents and solvents were purchased at the highest commercial quality and used without further purification. Flash column chromatography was performed by using silica gel (particle size 40–63 μm, 230–400 mesh) at increased pressure. NMR (^1H, ^{13}C) spectra were recorded with Varian Unity INOVA 600 MHz, Varian Mercury Plus 400 MHz, and Varian Gemini 300 MHz spectrometers. The chemical shifts (δ) are referenced to residual undeuterated solvent as an internal reference. The ^{13}C NMR spectra of fluorinated compounds were registered by using relaxation delay (t_1 = 8 s) for J_{C-F} evaluation. IR spectra were recorded with a Nicolet Protégé 460 FTIR spectrometer. Low-resolution mass spectra (LRMS) were recorded with a Thermo LCQ-Fleet (ESI) or with a Varian Saturn 2000 (EI) instrument, while high-resolution spectra (HRMS) were recorded with a Thermo-Finnigan MAT95 XP instrument. UV/Vis spectra were recorded in MeCN in a Perkin Elmer Lambda-20 double-beam spectrometer with bandwidth of 1 nm.

(*E*)-Methyl 4-Hydroxynon-2-enoate (3) and (*E*)-Methyl 4-Oxonon-2-enoate (3a): A round-bottom flask with a condenser was charged with selenium dioxide (3.26 g, 29.4 mmol, 2.0 equiv.) in dry dioxane (20 mL) and stirred at room temperature under N_2 atmosphere for 15 min before adding (*E*)-methyl non-2-enoate (2.79 mL, 14.7 mmol, 1.0 equiv.). The reaction mixture was heated at reflux for 4 h under N_2 atmosphere. The obtained yellow solution was allowed to cool at room temperature, filtered through a pad of silica and Celite, and concentrated, and the resulting red suspension was washed with brine. The dried (Na_2SO_4) organic layer was concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 9:1 to 8:2). Alcohol **3** was obtained as the main product as a yellow oil (1.64 g, 8.82 mmol, 60%). ^1H NMR (400 MHz, $CDCl_3$): δ = 6.94 (dd, *J* = 15.6, 5.2 Hz, 1 H), 6.01 (dd, *J* = 15.2, 1.6 Hz, 1 H), 4.27 (dt, *J* = 6.4, 1.6 Hz, 1 H), 3.71 (s, 3 H), 2.11 (br. s, 1 H), 1.60–1.50 (m, 2 H), 1.47–1.20 (m, 6 H), 0.86 (t, *J* = 6.4 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3, 150.9, 119.8, 71.3, 51.8, 36.8, 31.8, 25.1, 22.7, 14.2 ppm. LRMS (EI): *m*/*z* (%) = 186 (0.3) $[M]^{+\cdot}$, 157 (16), 125 (28), 115 (51), 98 (49), 87 (50), 55 (100). Amounts of ketone **3a** as white solid (0.27 g, 1.47 mmol, 10) and starting material (0.35 g, 2.06 mmol) were also recovered (spectroscopic data in the Supporting Information).

(*E*)-Methyl 4-(Tetrahydro-2*H*-pyran-2-yloxy)non-2-enoate (4): A dry round-bottomed flask capped with a rubber septum was loaded under N_2 with dry CH_2Cl_2 (25 mL), alcohol **3** (1.05 g, 5.64 mmol, 1.0 equiv.), and 3,4-dihydro-2*H*-pyran (2.57 mL, 28.2 mmol, 5.0 equiv.). The solution was stirred at 0 °C before adding a solution of PPTS (0.140 g, 0.564 mmol, 0.1 equiv.) in CH_2Cl_2 (1 mL) by syringe. The resulting mixture was warmed up to room temperature and stirred for 2 h. The mixture was diluted with CH_2Cl_2 and washed with an aqueous solution of NaCl (10%) and brine. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 9.5:0.5 to 9:1) to afford protected alcohol **4** (1:1 *dr*) as a yellow oil (1.34 g, 4.96 mmol, 88%). ^1H NMR (400 MHz, $CDCl_3$): δ = 6.96 (dd, *J* = 16.0, 5.2 Hz, 1 H), 6.78 (dd, *J* = 16.0, 6.4 Hz, 1 H), 6.06 (dd, *J* = 15.6, 7.6 Hz, 1 H), 5.94 (d, *J* = 15.6 Hz, 1 H), 4.70 (t, *J* = 3.6 Hz, 1 H), 4.56 (t, *J* = 3.2 Hz, 1 H), 4.32–4.21 (m, 2 H), 3.91–3.76 (m, 2 H), 3.74 (s, 3 H), 3.73 (s, 3 H), 3.54–3.42 (m, 2 H), 1.80–1.55 (m, 2 H), 1.54–1.48 (m, 14 H), 1.45–1.20 (m, 12 H), 0.88 (t, *J* = 6.8 Hz, 3 H), 0.87 (t, *J* = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3, 166.9, 149.7, 148.9, 121.8,

120.1, 97.5, 96.3, 75.3, 74.5, 62.6, 62.5, 51.8, 51.7, 35.4, 34.1, 32.0, 31.9, 30.9, 30.8, 25.6, 25.6, 25.2, 24.5, 22.7 (2 C), 19.6, 19.5, 14.2, 14.2 ppm. LRMS (EI): *m*/*z* (%) = $[M]^{+\cdot}$ absent, 169 (9), 152 (10), 137 (10), 128 (13), 125 (22), 115 (20), 113 (35), 111 (33), 85 (47), 84 (40), 55 (100).

(*E*)-4-(Tetrahydro-2*H*-pyran-2-yloxy)non-2-en-1-ol (5): A dry round-bottomed flask capped with a rubber septum was flushed with N_2 and then dry CH_2Cl_2 (40 mL) and ester **4** (1.10 g, 4.07 mmol, 1.0 equiv.) were introduced by syringe. The solution was stirred and cooled down to –10 °C with an ice–NaCl bath, and DIBAL-H (1.0 M in hexanes, 8.95 mL, 8.95 mmol, 2.2 equiv.) was added dropwise by glass syringe. The resulting mixture was allowed to come to room temperature over 1 h. The mixture was quenched with H_2O, stirred, and filtered through a small pad of Celite. The filtrate was washed with water and brine, dried (Na_2SO_4), concentrated. The crude was purified by flash chromatography (petroleum ether/EtOAc, 7:3) to afford allylic alcohol **5**[20] (1:1 *dr*) as a transparent oil (0.89 g, 3.67 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.81 (tdd, *J* = 15.6, 5.2, 0.8 Hz, 1 H), 5.79 (tdd, *J* = 15.6, 5.2, 0.8 Hz, 1 H), 5.73 (ddt, *J* = 15.6, 6.4, 1.2 Hz, 1 H), 5.65 (ddt, *J* = 15.6, 8.0, 1.6 Hz, 1 H), 4.67 (m, 1 H), 4.64 (t, *J* = 4.0 Hz, 1 H), 4.13 (t, *J* = 5.2 Hz, 4 H), 4.12–4.02 (m, 2 H), 3.90–3.82 (m, 2 H), 3.52–3.42 (m, 2 H), 1.84–1.78 (m, 2 H), 1.76–1.60 (m, 4 H), 1.60–1.40 (m, 10 H), 1.38–1.20 (m, 12 H), 0.87 (t, *J* = 6.8 Hz, 3 H), 0.87 (t, *J* = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 133.3, 132.3, 131.9, 129.9, 98.0, 95.0, 77.3, 75.5, 63.4, 63.1, 62.9, 62.3, 36.0, 34.9, 32.1, 32.0, 31.1, 30.9, 25.8, 25.6, 25.4, 24.9, 22.8 (2 C), 19.9, 19.7, 14.3, 14.2 ppm. MS (EI): *m*/*z* (%) = $[M]^{+\cdot}$ absent, 169 (6), 91 (16), 85 (75), 69 (50), 55 (100).

Diastereomeric Mixture of (*E*)-4-(Tetrahydro-2*H*-pyran-2-yloxy)-non-2-enal (6): To a solution of oxalyl chloride (2.0 M in CH_2Cl_2, 3.96 mL, 7.92 mmol, 3.0 equiv.) diluted with CH_2Cl_2 (15 mL) at –78 °C under N_2 atmosphere was dropwise added a solution of DMSO (0.94 mL, 13.2 mmol, 5 equiv.) in CH_2Cl_2 (5.0 mL) in a dry round-bottomed flask capped with a rubber septum. After 10 min, a solution of alcohol **5** (0.63 g, 2.64 mmol, 1 equiv.) in CH_2Cl_2 (10 mL) was added dropwise. After 1 h, Et_3N (3.68 mL, 26.4 mmol, 10 equiv.) was added, and stirring was continued at –78 °C for 30 min. The reaction mixture was allowed to reach room temperature over a period of 1 h. NH_4Cl (aq. sat.) was added, and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo, and the resulting residue was purified by flash chromatography (petroleum ether/EtOAc, 8.5:1.5) to give aldehyde **6**[20] (0.54 g, 2.25 mmol, 87% yield) as a colorless oil (1:1 *dr*). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.58 (d, *J* = 3.6 Hz, 1 H), 9.56 (d, *J* = 4.0 Hz, 1 H), 6.84 (dd, *J* = 16.0, 5.6 Hz, 1 H), 6.68 (dd, *J* = 16.0, 6.4 Hz, 1 H), 6.31 (ddd, *J* = 15.6, 8.0, 0.8 Hz, 1 H), 6.21 (dd, *J* = 15.6, 8.0 Hz, 1 H), 4.71 (t, *J* = 3.6 Hz, 1 H), 4.56 (t, *J* = 3.2 Hz, 1 H), 4.43 (q, *J* = 6.4 Hz, 1 H), 4.36 (q, *J* = 6.0 Hz, 2 H), 3.92–3.84 (m, 1 H), 3.83–3.76 (m, 1 H), 3.54–3.42 (m, 2 H), 1.90–1.78 (m, 2 H), 1.77–1.45 (m, 14 H), 1.43–1.20 (m, 12 H), 0.88 (t, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 194.0, 193.7, 158.6, 157.5, 132.6, 131.3, 98.3, 96.6, 75.7, 74.5, 62.9, 62.7, 35.3, 34.1, 31.9, 31.9, 30.9, 30.8, 25.6, 25.5, 25.2, 24.7, 22.7 (2 C), 19.7, 19.6, 14.2, 14.2 ppm. MS (EI) *m*/*z* (%) = $[M]^{+\cdot}$ absent, 204 (1), 193 (1), 147 (3), 139 (11), 125 (6), 109 (10), 81 (76), 55 (100).

(*E*)-4-Hydroxynon-2-enal (1): To a solution of protected aldehyde **6** (0.40 g, 1.66 mmol, 1 equiv.) in MeOH (5 mL) was added *para*-toluenesulfonic acid monohydrate (TsOH, 32 mg, 0.166 mmol, 0.1 equiv.) solubilized in a small amount of MeOH at 0 °C. The reaction mixture was warmed to room temperature and monitored by TLC until complete consumption of the starting material (about 2 h). The reaction mixture was diluted with CH_2Cl_2 and washed with brine. The organic layers were dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8:2 to 7.5:2.5) to give **1** (0.18 g, 1.15 mmol, 70%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.57 (d, *J* = 8.0 Hz, 1 H), 6.82 (dd, *J* = 15.6, 4.8 Hz, 1 H), 6.30 (ddd, *J* = 16.0, 7.6, 1.6 Hz, 1 H), 4.43 (quint., *J* = 5.6 Hz, 1 H), 1.88 (d, *J* = 4.8 Hz, 1 H), 1.68–1.57 (m, 2 H), 1.50–1.40 (m, 6 H), 0.89 (t, *J* = 5.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 193.8, 159.1, 130.8, 71.4, 36.7, 31.8, 25.1, 22.7, 14.2 ppm. LRMS (EI): *m*/*z* (%) = 157 (1) $[M]^{+\cdot}$, 138 (12), 123 (7), 109 (22), 96 (22), 81 (100), 67 (26), 55 (20), 53 (30).

[2,4-Bis(trifluoromethyl)phenyl]hydrazine (2e): To a fine suspension of 2,4-bis(trifluoromethyl)aniline (0.60 g, 2.62 mmol, 1 equiv.) in conc. HCl (4 mL) cooled in an ice–water bath was added dropwise a solution of sodium nitrite (0.198 g, 2.88 mmol, 1.1 equiv.) in water (1 mL). After 30 min, a chilled solution of $SnCl_2{\cdot}2H_2O$ (1.29 g, 5.76 mmol, 2.2 equiv.) in conc. HCl (1 mL) was then added dropwise to the cooled diazonium salt yellow solution, keeping the reaction below –5 °C. The mixture was stirred an additional 1 h at 0 °C. The pink solid was collected by vacuum filtration, dissolved in hot water, and filtered. The filtrate was basified with NaOH (aq. 40%) and a precipitate suddenly formed. The white solid was recovered by filtration and redissolved in EtOAc. The solution was then washed with brine, dried with Na_2SO_4, and evaporated under reduced pressure to obtain pure hydrazine **2e** (0.25 g, 1.02 mmol, 40%) as white needles. M.p. 46–48 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.67 (s, 1 H), 7.65 (d, *J* = 8.4 Hz, 1 H), 7.46 (d, *J* = 8.4 Hz, 1 H), 6.10 (br. s, 1 H), 3.70 (br. s, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.7, 130.3, 124.4 (q, *J* = 270 Hz, 2 C), 124.2 (q, *J* = 3.2 Hz), 119.3 (q, *J* = 33.0 Hz), 112.3, 111.8 (q, *J* = 30.6 Hz) ppm. LRMS (EI): *m*/*z* (%) = 244 (100) $[M]^{+\cdot}$, 224 (82), 205 (45), 176 (73), 145 (18). HRMS (EI): calcd. for $C_8H_6F_6N_2$ $[M]^{+\cdot}$ 244.0435; found 244.0439.

General Procedure for the Synthesis of Linear Hydrazones 7a–f: A dry round-bottomed flask capped with a rubber septum was charged with aldehyde **1** (0.15 mmol, 1 equiv.), flushed with N_2, and then dry EtOH (or *t*BuOH or MeCN, 2 mL) and solid $MgSO_4$ were added. Neat aromatic hydrazine **2a**–**f** (1.2 equiv.) and a solution of TFA (5% v/v in the chosen solvent, 0.3 equiv.) were successively added by syringe at room temperature. The reaction was judged complete within 10–15 min by TLC. The solvent was evaporated under reduced pressure maintaining the rotary evaporator water bath at room temperature. The obtained crude was purified directly by flash chromatography (petroleum ether/EtOAc, from 9:1 to 7:3) to afford title compounds **7a**–**f**.

(1*E*,2*E*)-1-{2-[2-(Trifluoromethyl)phenyl]hydrazono}non-2-en-4-ol (7a): In EtOH, 30.2 mg of a yellow oil (0.096 mmol, 64%); in CH_3CN, 36 mg (0.115 mmol, 76%) ^{1}H NMR (400 MHz, [D_6]-DMSO): δ = 9.39 (s, 1 H), 8.01 (d, *J* = 9.6 Hz, 1 H), 7.54 (d, *J* = 8.8 Hz, 1 H), 7.50–7.43 (m, 2 H), 6.87 (t, *J* = 7.6 Hz, 1 H), 6.27 (dd, *J* = 16.0, 9.6 Hz, 1 H), 5.96 (dd, *J* = 15.6, 5.6 Hz, 1 H), 4.83 (d, *J* = 4.4 Hz, 1 H) 4.07 (quint., *J* = 5.6 Hz, 1 H), 1.50–1.37 (m, 2 H), 1.35–1.20 (m, 6 H), 0.89 (t, *J* = 6.4 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 145.5, 143.1, 134.1, 126.7 (q, *J* = 5.7 Hz, 2 C), 126.6, 125.0 (q, *J* = 270 Hz), 118.9, 115.2, 111.5 (q, *J* = 29.8 Hz), 70.8, 37.6, 32.0, 25.3, 22.8, 14.6. MS (EI) *m*/*z* (%) = $[M]^{+\cdot}$ absent, 296 (17), 239 (24), 225 (51), 212 (100), 145 (34), 114 (29) ppm. LRMS (ESI+) = 315.0 $[M + H]^+$. HRMS (ESI+): calcd. for $C_{16}H_{22}F_3N_2O$ $[M + H]^+$ 315.1684; found 315.1689. IR ($CDCl_3$):

$\tilde{\nu}$ = 3607, 3383, 1612, 1594, 1524, 1469, 1323, 1277, 1138, 1109, 1082 cm^{-1}.

(1*E*,2*E*)-1-{2-[3-(Trifluoromethyl)phenyl]hydrazono}non-2-en-4-ol (7b): In EtOH, 30 mg of a yellow oil (0.095 mmol, 63%. GC–MS calculated yield = 83%). ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.34 (s, 1 H), 7.58 (d, J = 9.6 Hz, 1 H), 7.36 (t, J = 7.6 Hz, 1 H), 7.18 (s, 1 H), 7.12 (d, J = 8.4 Hz, 1 H), 6.98 (d, J = 7.6 Hz, 1 H), 6.28 (dd, J = 15.6, 9.2 Hz, 1 H), 5.97 (dd, J = 15.6, 5.6 Hz, 1 H), 4.80 (d, J = 4.4 Hz, 1 H), 4.05 (quint., J = 5.6 Hz, 1 H), 1.46–1.34 (m, 2 H), 1.32–1.18 (m, 6 H), 0.84 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 146.5, 142.1, 141.5, 130.8, 130.6 (q, J = 33.8 Hz), 126.6, 125.0 (q, J = 270 Hz), 116.1, 115.0 (q, J = 3.5 Hz), 108.1 (q, J = 3.5 Hz), 70.8, 37.7, 32.0, 25.3, 22.8, 14.6. LRMS (EI): m/z (%) = $[M]^{+\cdot}$ absent, 296 (8), 277 (2), 239 (16), 225 (100), 160 (2), 145 (5) ppm. LRMS (ESI+) = 315 $[M + H]^+$. HRMS (ESI+): calcd. for $C_{16}H_{22}F_3N_2O$ $[M + H]^+$ 315.1684; found 315.1678.

(1*E*,2*E*)-1-{2-[4-(Trifluoromethyl)phenyl]hydrazono}non-2-en-4-ol (7c): In EtOH, 31 mg of a white solid (0.098 mmol, 65% GC–MS calculated yield = 87%): in MeCN, 37.7 mg (0.120 mmol, 80%). M.p. 118–120 °C. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.46 (s, 1 H), 7.60 (d, J = 9.6 Hz, 1 H), 7.46 (d, J = 8.8 Hz, 1 H), 7.02 (d, J = 8.4 Hz, 2 H), 6.27 (dd, J = 16.0, 9.6 Hz, 1 H), 5.99 (dd, J = 15.6, 5.6 Hz, 1 H), 4.81 (d, J = 4.8 Hz, 1 H) 4.06 (quint., J = 5.6 Hz, 1 H), 1.50–1.40 (m, 2 H), 1.40–1.27 (m, 6 H), 0.84 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 148.9, 142.1, 142.1, 127.1 (q, J = 4.0 Hz, 2 C), 126.5, 125.7 (q, J = 269 Hz), 118.7 (q, J = 31.4 Hz), 112.1 (2 C), 70.8, 37.7, 32.0, 25.3, 22.8, 14.6 ppm. HRMS (ESI+): calcd. for $C_{16}H_{22}F_3N_2O$ $[M + H]^+$ 315.1684; found 315.1690. IR ($CDCl_3$): $\tilde{\nu}$ = 3608, 3343, 1616, 1531, 1467, 1325, 1264, 1165, 1121, 1064 cm^{-1}.

(1*E*,2*E*)-1-{2-[3,5-Bis(trifluoromethyl)phenyl]hydrazono}non-2-en-4-ol (7d): In EtOH, 46.8 mg of a yellowish solid (0.120 mmol, 80%). M.p. 82–84 °C. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.70 (s, 1 H), 7.63 (d, J = 9.2 Hz, 1 H), 7.40 (s, 2 H), 7.25 (s, 1 H), 6.30 (dd, J = 15.6, 9.2 Hz, 1 H), 6.07 (dd, J = 15.6, 5.6 Hz, 1 H), 4.83 (d, J = 4.4 Hz, 1 H) 4.07 (quint., J = 5.6 Hz, 1 H), 1.50–1.40 (m, 2 H), 1.39–1.20 (m, 6 H), 0.84 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 145.6, 141.6, 141.3, 132.7 (q, J = 33.0 Hz, 2 C), 126.9, 123.6 (q, J = 270 Hz, 2 C), 113.0 (q, J = 3.0 Hz), 112.4 (q, J = 3.7 Hz), 72.6, 37.3, 31.9, 25.2, 22.8, 14.2 ppm. HRMS (ESI+): calcd. for $C_{17}H_{21}F_6N_2O$ $[M + H]^+$ 383.1558; found 383.1562.

(1*E*,2*E*)-1-{2-[2,4-Bis(trifluoromethyl)phenyl]hydrazono}non-2-en-4-ol (7e): A yellowish solid (35.5 mg, 0.093 mmol, 62%). M.p. 79–81 °C. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 9.92 (s, 1 H), 8.13 (d, J = 9.2 Hz, 1 H), 7.79 (d, J = 8.8 Hz, 1 H), 7.72 (s, 1 H), 7.70 (d, J = 8.8 Hz, 1 H), 6.30 (dd, J = 15.6, 9.6 Hz, 1 H), 6.08 (dd, J = 15.6, 5.6 Hz, 1 H), 4.83 (d, J = 4.8 Hz, 1 H) 4.09 (quint., J = 5.6 Hz, 1 H), 1.50–1.40 (m, 2 H), 1.39–1.17 (m, 6 H), 0.84 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 144.6, 143.6, 142.4, 130.4 (q, J = 3.0 Hz), 126.5, 124.2 (q, J = 270 Hz), 124.1 (q, J = 270 Hz), 124.0 (q, J = 3.2 Hz), 121.1 (q, J = 33.8 Hz), 114.8, 111.7 (q, J = 31.4 Hz), 72.3, 37.3, 31.9, 25.2, 22.8, 14.2 ppm. HRMS (ESI+): calcd. for $C_{17}H_{21}F_6N_2O$ $[M + H]^+$ 383.1558; found 383.1553.

(1*E*,2*E*)-1-[2-(Perfluorophenyl)hydrazono]non-2-en-4-ol (7f): A white solid (42.8 mg, 0.128 mmol, 85%). M.p. 110–112 °C. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 9.91 (s, 1 H), 7.76 (d, J = 9.6 Hz, 1 H), 6.18 (dd, J = 16.0, 9.6 Hz, 1 H), 5.98 (dd, J = 15.6, 5.6 Hz, 1 H), 4.81 (d, J = 4.8 Hz, 1 H), 4.04 (quint., J = 5.2 Hz, 1 H), 1.35–1.27 (m, 2 H), 1.27–1.18 (m, 6 H), 0.84 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 145.0, 142.3, 138.6 (dm, J = 247 Hz, 2 C), 138.4 (dm, J = 250 Hz, 2 C), 136.1 (dm, J = 246 Hz), 126.4, 120.5 (tm, J = 10.0 Hz), 72.4, 37.2, 31.9, 25.2, 22.7, 14.2 ppm. HRMS (ESI+): calcd. for $C_{17}H_{21}F_6N_2O$ $[M + H]^+$ 337.1339; found 337.1345.

General Procedure for the Synthesis of Substituted 1,6-Dihydropyridazines 8b–d and Michael Addition Products 9b–d and 10b–d: A dry round-bottomed flask was charged with a magnetic stirrer and capped with a rubber septum. The reaction vessel was charged with aldehyde **1** (0.25 mmol, 1 equiv.) and flushed with N_2 and then anhydrous MeCN (8 mL) and solid $MgSO_4$ were added. Neat aromatic hydrazine **2b–d** (2.0 equiv.) and a solution of TFA (5% in MeCN, 0.3 equiv.) were successively added by syringe at room temperature. The reaction was heated at 70 °C for 6 h. The solvent was evaporated under reduced pressure to give a crude that was purified by flash chromatography (petroleum ether/EtOAc, from 95:5 to 7:3) to afford 1,6-dihydropyridazine **8b–d** and Michael addition products **9b–d**. The *anti* isomers were predominantly formed, as judged by ^1H NMR spectroscopy (≥8:1 *dr*). The isolated hydrazinyl products were moderately stable and were rapidly or slowly oxidized by O_2/air towards oxidized diazenyl derivatives upon standing at room temperature. Diazenyl products **10b–d** were purified again by flash column chromatography (petroleum ether/EtOAc, from 85:15 to 75:25). To obtain selectively substituted 1,6-dihydropyridazines **8b–d**, the reaction was performed in *t*BuOH by using a solution of 5% TFA in *t*BuOH (0.3 equiv.) and flash chromatography (petroleum ether/EtOAc, from 95:5 to 85:15).

6-Pentyl-1-[3-(trifluoromethyl)phenyl]-1,6-dihydropyridazine (8b): In MeCN, 14.8 mg of a yellow oil (0.050 mmol, 20%); in *t*BuOH, 44.4 mg (0.15 mmol, 60%). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.54 (br. s, 1 H), 7.38 (dd, J = 4.8, 1.5 Hz, 2 H), 7.16 (m, 1 H), 7.06 (dd, J = 3.3, 2.1 Hz, 1 H), 6.05 (ddd, J = 8.7, 6.0, 2.0 Hz, 1 H), 5.96 (dd, J = 9.6, 3.0 Hz, 1 H), 4.78 (m, 1 H), 1.80–1.65 (m, 1 H), 1.58–1.42 (m, 1 H), 1.40–1.20 (m, 6 H), 0.87 (t, J = 6.9 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 146.5, 136.3, 131.6 (q, J = 32.2 Hz), 129.7, 127.0, 124.5 (q, J = 271 Hz), 118.1, 116.9 (q, J = 3.6 Hz, 2 C), 111.2 (q, J = 3.6 Hz), 52.1, 31.9, 31.3, 23.6, 22.7, 14.1 ppm. HRMS (ESI+): calcd. for $C_{16}H_{20}F_3N_2$ $[M + H]^+$ 297.1579; found 297.1575.

Mixture of 6-Pentyl-1-[3-(trifluoromethyl)phenyl]-5-{2-[3-(trifluoromethyl)phenyl]hydrazinyl}-1,4,5,6-tetrahydropyridazine (9b): ^1H NMR (400 MHz, $CDCl_3$, **9b**/**10b** = 1.7:1): δ = 7.80 (s, 0.41) minor, 7.67 (d, J = 8.0 Hz, 0.45 H) minor, 7.64 (d, J = 8.0 Hz, 0.43 H) minor, 7.50 (t, J = 7.2 Hz, 0.55) minor, 7.44 (m, 1.13 H) overlapped, 7.32 (m, 1.35 H) overlapped, 7.25 (m, 1.78 H) overlapped, 7.15 (t, J = 8.0 Hz, 1 H) major, 7.08 (d, J = 7.2 Hz, 1 H) major, 7.03 (m, 0.78 H) minor, 6.96–6.89 (m, 2 H) overlapped, 6.81 (m, 1 H) major, 4.55 (br. t, 0.60 H) minor, 4.46 (m, 0.53 H) minor, 4.18 (m, 1 H) major, 3.41 (m, 1 H) major, 2.74 (d, J = 18.8 Hz, 0.71 H) minor, 2.64 (dd, J = 19.4, 4.8 Hz, 0.65 H) minor, 2.50 (dd, J = 17.6, 4.0 Hz, 1 H) major, 2.20 (d, J = 19.2 Hz, 1 H) major, 1.85 (m, 0.76 H) minor, 1.90–1.75 (m, 1 H), 1.70–1.60 (m, 1.78 H) overlapped, 1.60–1.40 (m, 6.06 H) overlapped, 1.42–1.30 (m, 3.15 H) overlapped, 1.28–1.25 (m, 4.23 H) overlapped, 0.92 (t, 1.71 H) minor, 0.86 ppm (t, J = 6.8 Hz, 3 H) major. ^{13}C NMR spectrum could not be recorded because **9b** was oxidizing to **10b** in the NMR tube the during analysis time. LRMS (EI+): m/z (%) = $[M]^{+\cdot}$ absent, 312 (12), 241 (100), 213 (8), 145 (11). LRMS (ESI+) = 473 $[M + H]^+$. HRMS (ESI+): calcd. for $C_{23}H_{27}F_6N_4$ $[M + H]^+$ 473.2140; found 473.2131.

(*E*)-6-Pentyl-1-[3-(trifluoromethyl)phenyl]-5-{[3-(trifluoromethyl)-phenyl]diazenyl}-1,4,5,6-tetrahydropyridazine (10b): In MeCN, 37.6 mg of a yellow oil (0.080 mmol, 32%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80 (s, 1 H), 7.67 (d, J = 7.6 Hz, 1 H), 7.64 (d, J = 7.2 Hz, 1 H), 7.50 (t, J = 7.2 Hz, 1 H), 7.44 (s, 1 H), 7.31 (t, J = 7.6 Hz, 1 H), 7.25 (m overlapped, 1 H), 7.05 (d, J = 7.2 Hz, 1 H), 6.90 (br. s, 1 H), 4.55 (br. t, 1 H), 4.46 (m, 1 H), 2.75 (d, J = 18.8 Hz, 1 H), 2.64 (dd, J = 19.4, 4.8 Hz, 1 H), 1.90–1.75 (m, 1 H), 1.70–1.60 (m, 1 H), 1.60–1.45 (m, 2 H), 1.42–1.30 (m, 4 H), 0.90 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.7, 147.4, 134.9, 131.8 (q, J = 33.0 Hz), 131.5 (q, J = 33.0 Hz), 129.8, 129.6, 127.4 (q, J = 3.2 Hz), 125.3, 124.6 (q, J = 270 Hz), 123.8 (q, J = 270 Hz), 119.9 (q, J = 4.0 Hz), 116.4, 116.1 (q, J = 3.2 Hz), 110.7 (q, J = 4.0 Hz), 67.5, 54.9, 31.8, 29.6, 25.6, 23.4, 22.7, 14.1 ppm. LRMS (EI): m/z (%) = 470 (20) $[M]^{+\cdot}$, 451 (6), 297 (55), 225 (60), 213 (40), 145 (100). LRMS (ESI–) = 469.13 $[M - H]^-$. HRMS (EI): calcd. for $C_{23}H_{24}F_6N_4$ $[M]^{+\cdot}$ 470.1905; found 470.1910.

6-Pentyl-1-[4-(trifluoromethyl)phenyl]-1,6-dihydropyridazine (8c): In *t*BuOH, 47.4 mg of a yellow oil (0.160 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 (d, J = 8.4 Hz, 2 H), 7.30 (d, J = 8.8 Hz, 2 H), 7.07 (m, 1 H), 6.07 (td, J = 6.4, 1.6 Hz, 1 H), 5.96 (dd, J = 9.2, 3.2 Hz, 1 H), 4.79 (m, 1 H), 1.82–1.72 (m, 1 H), 1.54–1.45 (m, 1 H), 1.38–1.22 (m, 6 H), 0.87 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.5, 136.7, 127.5, 126.5 (q, J = 4.1 Hz, 2 C), 126.4 (q, J = 269 Hz), 122.0 (q, J = 32.2 Hz), 118.0, 113.7 (2 C), 52.0, 31.9, 31.5, 23.6, 22.7, 14.1 ppm. LRMS (EI+): m/z (%) = 296 (0.5) $[M]^{+\cdot}$, 277 (5), 251 (2), 225 (100). LRMS (ESI+) = 297.05 $[M + H]^+$. HRMS (EI): calcd. for $C_{16}H_{19}F_3N_2$ 296.1502; found 296.1510.

6-Pentyl-1-[4-(trifluoromethyl)phenyl]-5-{2-[4-(trifluoromethyl)-phenyl]hydrazinyl}-1,4,5,6-tetrahydropyridazine (9c): In MeCN, 65 mg of a yellow oil (0.138 mmol, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, J = 8.4 Hz, 2 H), 7.58 (d, J = 8.4 Hz, 2 H), 7.46 (d, J = 9.2 Hz, 2 H), 7.19 (d, J = 8.8 Hz, 2 H), 6.92 (m, 1 H), 4.57 (br. t, 1 H), 4.45 (m, 1 H), 2.74 (d, J = 19.2 Hz, 1 H), 2.65 (dd, J = 18.4, 5.2 Hz, 1 H), 1.90–1.74 (m, 1 H), 1.73–1.64 (m, 1 H), 1.52–1.45 (m, 2 H), 1.42–1.30 (m, 4 H), 0.91 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.7, 150.0, 135.4, 130.0, 126.5 (m, 4 C), 123.6 (2 C), 112.9, 112.7 (2 C), 111.9 (2 C), 53.4, 51.6, 31.9, 29.4, 25.7, 24.5, 22.7, 14.2 ppm. LRMS (ESI+): m/z = 473 $[M + H]^+$. HRMS (ESI+): calcd. for $C_{23}H_{27}F_6N_4$ $[M + H]^+$ 473.2140; found 473.2144.

(*E*)-6-Pentyl-1-[4-(trifluoromethyl)phenyl]-5-{[4-(trifluoromethyl)-phenyl]diazenyl}-1,4,5,6-tetrahydropyridazine (10c): In MeCN, 41 mg of a yellow oil (0.087 mmol, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, J = 8.4 Hz, 2 H), 7.58 (d, J = 8.4 Hz, 2 H), 7.46 (d, J = 9.2 Hz, 2 H), 7.19 (d, J = 8.8 Hz, 2 H), 6.92 (m, 1 H), 4.57 (br. t, 1 H), 4.45 (m, 1 H), 2.74 (d, J = 19.2 Hz, 1 H), 2.65 (dd, J = 18.4, 5.2 Hz, 1 H), 1.90–1.74 (m, 1 H), 1.73–1.64 (m, 1 H), 1.52–1.45 (m, 2 H), 1.42–1.30 (m, 4 H), 0.91 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.6, 149.3, 135.3, 132.6 (q, J = 32.3 Hz), 126.5 (m, 4 C), 123.9 (q, J = 276 Hz), 125.0 (q, J = 276 Hz), 122.7 (2 C), 121.2 (q, J = 33.0 Hz), 112.9 (2 C), 67.5, 54.7, 31.9, 29.7, 25.6, 23.5, 22.7, 14.2 ppm. LRMS (EI+): m/z (%) = 470 (3) $[M]^{+\cdot}$, 451 (15), 397 (26), 297 (100), 295 (25), 225 (25), 213 (15), 145 (36). HRMS (EI): calcd. for $C_{23}H_{24}F_6N_4$ $[M]^{+\cdot}$ 470.1905; found 470.1909.

1-[3,5-Bis(trifluoromethyl)phenyl]-6-pentyl-1,6-dihydropyridazine (8d): In MeCN, 13 mg of a yellow oil (0.036 mmol, 14%); in *t*BuOH, 56 mg (0.15 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (s, 2 H), 7.36 (s, 1 H), 7.10 (dd, J = 2.8, 1.6 Hz, 1 H), 6.11 (ddd, J = 9.2, 6.0, 1.6 Hz, 1 H), 5.99 (dd, J = 9.6, 3.2 Hz, 1 H), 4.80 (m, 1 H), 1.80–1.70 (m, 1 H), 1.55–1.45 (m, 1 H), 1.40–1.25 (m, 6 H), 0.87 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.1, 137.3, 132.4 (q, J = 32.2 Hz, 2 C), 128.1, 123.7 (q, J = 271 Hz, 2 C), 118.0, 113.5 (q, J = 3.5 Hz, 2 C), 113.2 (q, J = 4.1 Hz), 52.1, 31.8, 31.4, 23.6, 22.7, 14.1 ppm. HRMS (ESI+): calcd. for $C_{17}H_{19}F_6N_2$ $[M + H]^+$ 365.1452; found 365.1461.

1-[3,5-Bis(trifluoromethyl)phenyl]-5-{2-[3,5-bis(trifluoromethyl)-phenyl]hydrazinyl}-6-pentyl-1,4,5,6-tetrahydropyridazine (9d): In MeCN, 78 mg of a yellow oil (0.128 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45 (s, 2 H), 7.25 (s, 1 H), 7.13 (s, 2 H), 7.11 (s, 1 H), 6.88 (s, 1 H), 5.49 (s, 1 H), 4.18 (br. t, 1 H), 3.61 (br. s, 1 H), 3.44 (m, 1 H), 2.57 (dd, J = 19.2, 5.2 Hz, 1 H), 2.16 (dd, J = 19.6 Hz, 1 H), 1.70–1.60 (m, 1 H), 1.55–1.40 (m, 1 H), 1.40–1.20 (m, 6 H), 0.85 (t, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.2, 147.6, 135.8, 132.5 (q, J = 33.0 Hz, 2 C), 132.4 (q, J = 33.0 Hz, 2 C), 123.7 (q, J = 271 Hz, 2 C), 123.4 (q, J = 271 Hz, 2 C), 112.4 (m, 4 C), 112.1 (m, 2 C), 52.6, 52.1, 31.8, 29.3, 25.7, 24.7, 22.6, 14.0 ppm. HRMS (ESI+): calcd. for $C_{25}H_{25}F_{12}N_4$ $[M + H]^+$ 609.1888; found 609.1893.

(*E*)-1-[3,5-Bis(trifluoromethyl)phenyl]-5-{[3,5-bis(trifluoromethyl)-phenyl]diazenyl}-6-pentyl-1,4,5,6-tetrahydropyridazine (10d): A yellow oil (18 mg, 0.029 mmol) ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.96 (s, 2 H), 7.91 (s, 1 H), 7.54 (s, 2 H), 7.28 (s, 1 H), 6.98 (m, 1 H), 4.65 (br. t, J = 6.8 Hz, 1 H), 4.57 (m, 1 H), 2.83 (dd, J = 19.2, 4.0 Hz, 1 H), 2.70 (ddd, J = 19.2, 6.0, 1.6 Hz, 1 H), 1.90–1.75 (m, 1 H), 1.75–1.62 (m, 1 H), 1.60–1.48 (m, 2 H), 1.45–1.30 (m, 4 H), 0.91 (t, J = 6.4 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.8, 147.8, 136.4, 132.9 (q, J = 33.1 Hz, 2 C), 132.5 (q, J = 33.1 Hz, 2 C), 124.3, 123.4 (q, J = 270 Hz, 2 C), 123.0 (q, J = 270 Hz, 2 C), 122.8 (2 C), 113.0 (2 C), 112.7, 67.7, 54.6, 31.7, 29.6, 25.6, 23.2, 22.7, 14.0 ppm. LRMS (ESI–): m/z = 605 $[M - H]^-$. HRMS (EI): calcd. for $C_{25}H_{22}F_{12}N_4$ $[M]^{+\cdot}$ 606.1653; found 606.1559.

(*E*)-1-[(*E*)-4-Ethoxynon-2-enylidene]-2-[4-(trifluoromethyl)phenyl]-hydrazine (11c): The reaction was run according to the general procedure in EtOH (2.0 mL) with aldehyde **1** (0.020 g, 0.128 mmol, 1 equiv.), hydrazine **2c** (1.1 equiv.), and a solution of TFA (5% in EtOH, 0.088 mL, 0.3 equiv.) at 50 °C for 5 h. The crude residue was purified by flash chromatography (petroleum ether/EtOAc, 9:1 to 8:2) to afford ethyl ether **11c** (40%) and 1,6-dihydropyridazine **8c** (30%).

11c: Yellow oil (17 mg, 0.078 mmol, 40%). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 10.5 (s, 1 H), 7.61 (d, J = 9.2 Hz, 1 H), 7.47 (d, J = 8.4 Hz, 2 H), 7.03 (d, J = 8.4 Hz, 2 H), 6.31 (dd, J = 15.6, 9.6 Hz, 1 H), 5.85 (dd, J = 15.6, 7.2 Hz, 1 H), 3.79 (q, J = 6.4 Hz, 1 H), 3.44 (m, 1 H), 3.30 (m overlapped, 1 H), 1.60–1.45 (m, 2 H), 1.44–1.25 (m, 6 H), 1.08 (t, J = 7.2 Hz, 3 H), 0.84 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 148.8, 141.5, 138.9, 129.3, 127.1 (q, J = 4.0 Hz, 2C), 125.6 (q, J = 270 Hz), 118.9 (q, J = 31.4 Hz), 112.2 (2C), 79.6, 63.8, 35.7, 31.9, 25.1, 22.7, 16.0, 14.6 ppm. LRMS (EI): m/z (%) = 342 (2) $[M]^{+\cdot}$, 323 (3), 296 (10), 239 (8), 212 (56), 185 (10), 145 (10), 85 (18), 57 (100). HRMS (EI): calcd. for $C_{1.8}H_{25}F_3N_2O$ $[M]^{+\cdot}$ 342.1919; found 342.1923.

Reactions of (*E*)-4-Hydroxynon-2-enal (1) with [2,4-Bis(trifluoromethyl)phenyl]hydrazine (2e): Reaction followed the general procedure with aldehyde **1** (0.020 g, 0.128 mmol, 1 equiv.) hydrazine **2e** (2.0 equiv.) in CH_3CN (3 mL) with TFA (5% in CH_3CN, 0.156 mL, 0.3 equiv.) and solid $MgSO_4$. The reaction was heated at 70 °C for 5 h. The crude was purified by flash chromatography (petroleum ether/EtOAc, 95:5) to afford 1,6-disubstituted product **12e**. Further

elution (petroleum ether/EtOAc, 85:15) furnished linear hydrazone **7e** and addition product **9e**.

1-[2,4-Bis(trifluoromethyl)phenyl]-5-{2-[2,4-bis(trifluoromethyl)-phenyl]hydrazinyl}-6-pentyl-1,4,5,6-tetrahydropyridazine (9e): Yellow oil (15 mg, 0.025 mmol, 13%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.94 (s, 1 H), 7.72 (dd, J = 8.8, 1.6 Hz, 1 H), 7.66 (s, 1 H), 7.59 (d, J = 9.2 Hz, 2 H), 7.56 (d, J = 9.2 Hz, 2 H), 7.39 (d, J = 8.4 Hz, 1 H), 6.94 (m, 1 H), 6.30 (s, 1 H), 4.01 (d, J = 1.8 Hz, 1 H), 3.88 (br. t, 1 H), 3.30 (br. t, J = 1.5 Hz, 1 H), 2.47 (dd, J = 19.6, 8.4 Hz, 1 H), 2.24 (dm, J = 19.6 Hz, 1 H), 1.55–1.40 (m, 1 H), 1.30–1.20 (m, 6 H), 0.80 (t, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.1, 149.6, 139.0, 130.2, 129.4, 126.7, 126.3 (q, J = 33.1 Hz), 125.8 (q, J = 7.2 Hz), 124.4 (q, J = 271 Hz), 124.3 (q, J = 271 Hz), 124.1 (m), 123.8 (q, J = 271 Hz), 123.7 (q, J = 271 Hz), 122.8 (q, J = 31.4 Hz), 119.7 (q, J = 33.0 Hz), 111.7 (q, J = 31.4 Hz), 58.6, 52.9, 31.6, 29.9, 25.5, 24.2, 22.5, 14.0 ppm. LRMS (ESI+): m/z = 609.1 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{25}H_{25}F_{12}N_4$ [M + H]$^+$ 609.1888; found 609.1883.

(*E*)-1-[2,4-Bis(trifluoromethyl)phenyl]-2-[(*E*)-4-{2-[2,4-bis(trifluoromethyl)phenyl]hydrazinyl}non-2-enylidene]hydrazine (12e): Bright yellow oil (7 mg, 0.078 mmol, 8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (s, 1 H), 7.75–7.71 (m, 2 H), 7.68–7.61 (m, 2 H), 7.61–7.56 (m, 2 H), 7.51 (d, J = 9.2 Hz, 1 H), 6.39 (dd, J = 15.6, 9.2 Hz, 1 H), 5.97 (br. s, 1 H), 5.78 (dd, J = 15.6, 8.8 Hz, 1 H), 3.53 (br. s, 1 H), 3.39 (q, J = 7.2 Hz, 1 H), 1.66–1.58 (m, 1 H), 1.55–1.45 (m, 1 H), 1.45–1.20 (m, 6 H), 0.89 (t, 3 H) ppm. HRMS (ESI+): calcd. for $C_{25}H_{25}F_{12}N_4$ [M + H]$^+$ 609.1888; found 609.1896.

HPLC Analysis: The reaction mixture during the synthesis or degradation of hydrazones **7a–f** was analyzed by HPLC-DAD (injection volume: 10 μL) by using a C18 stationary phase (150 mm × 4.6 mm × 5 μm particle size), eluting at 500 μL/min with a gradient mixture of A (0.5% formic acid in MeCN) and B (0.5% formic acid in water) with the following programming: t = 0 min, A/B 60:40; t = 10 min, A/B 80:20; t = 15 min A/B 80:20; t = 20 min A/B 60:40, detecting in the wavelength range 230–450 nm. Calibration curves (8 levels) were built for each analyte at the band maxima by using authentic standards prepared as described above.

Kinetics of Formation and Degradation of Hydrazones 7a–f: To study the kinetics of formation of the hydrazones, a solution of 4-HNE in MeCN (1–50 μM) was incubated with 10-fold or higher excess of the hydrazines **2a–f** in the presence of an excess amount of trifluoroacetic acid (TFA, 0.01–1 mM) at 25 °C. The progress of the reaction was monitored at time intervals by HPLC analysis of the reaction mixture as indicated above. The growth of the concentration of hydrazones **7a–f** vs. time was first analyzed against first-order curves. The pseudo-first-order rate constant was then plotted vs. the initial concentration of the hydrazine (4 data points or more for each hydrazine) at fixed concentration of TFA to obtain the apparent second-order rate constant under the fixed experimental conditions as reported in Table 2. To study the decomposition of hydrazones **7a–f** solutions in MeCN, either in the presence or absence of water and TFA, were incubated at 25 °C in air and were monitored with time, both continuously by spectrophotometry at the λ_{max} reported in Table 2 and at time intervals by HPLC analysis (vide supra). Decay plots were first analyzed against first-order decay curves and the initial first-order decomposition rate constants k_d were then used as inputs for numerical fittings using Gepasi 3.30 software[30] and a reversible hydrolysis model to obtain the optimized k_d values collected in Table 2.

Supporting Information (see footnote on the first page of this article): Additional spectroscopic data and copies of the ^{1}H NMR and ^{13}C NMR spectra.

Acknowledgments

Authors are grateful to BeC s.r.l (Forlì, Italy), the University of Bologna (Italy) and Ministero dell'Università e della Ricerca (MIUR) (Rome, Italy; project PRIN2008: Processi Radicalici e di Trasferimento Elettronico in Chimica e Biologia: Applicazioni nella Sintesi, nella Catalisi e nelle Scienze dell'Ambiente e dei Materiali) for funding, and to the University of Orléans (France) for a grant to M. T. We thank Sébastien Guesné (Queen Mary, University of London) for helpful discussion on cyclization products formation.

[1] L. Valgimigli, D. A. Pratt in *Encyclopedia of Radicals in Chemistry, Biology and Materials* (Eds.: C. Chatgilialoglu, A. Studer), John Wiley & Sons, Ltd., **2012**, vol. 3, pp. 1623–1677.
[2] C. Schneider, K. A. Tallman, N. A. Porter, A. R. Brash, *J. Biol. Chem.* **2001**, *276*, 20831–20838.
[3] G. Poli, R. J. Schaur, W. G. Siems, G. Leonarduzzi, *Med. Res. Rev.* **2008**, *28*, 569–631.
[4] H. Esterbauer, R. J. Schaur, H. Zollner, *Free Radical Biol. Med.* **1991**, *11*, 81–128.
[5] Y. C. Awasthi, R. Sharma, J. Z. Cheng, Y. Yang, A. Sharma, S. S. Singhal, S. Awasthi, *Mol. Aspects Med.* **2003**, *24*, 219–230.
[6] W. Siems, C. Crifo, E. Capuozzo, K. Uchida, T. Grune, C. Salerno, *Arch. Biochem. Biophys.* **2010**, *503*, 248–252.
[7] G. Aldini, I. Dalle-Donne, R. M. Facino, A. Milzani, M. Carini, *Med. Res. Rev.* **2007**, *27*, 817–868.
[8] I. Dalle-Donne, G. Aldini, M. Carini, R. Colombo, R. Rossi, A. Milzani, *J. Cell. Mol. Med.* **2006**, *10*, 389–406.
[9] S. Pennathur, J. W. Heinecke, *Antioxid. Redox Signaling* **2007**, *9*, 955–969.
[10] T. Nystrçm, *EMBO J.* **2005**, *24*, 1311–1317.
[11] R. M. LoPachin, D. S. Barber, T. Gavin, *Toxicol. Sci.* **2008**, *104*, 235–249.
[12] K. Uchida, *Free Radical Biol. Med.* **2000**, *28*, 1685–1696.
[13] K. Uchida, *Progr. Lipid Res.* **2003**, *42*, 318–343.
[14] Y. Kim, D. Kim, J. Hwang, D.-H. Lee, M. Kim, *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1238–1242.
[15] a) D. Spies-Martin, O. Sommerburg, C.-D. Langhans, M. Leichsenring, *J. Chromatogr. B* **2002**, *774*, 231–239; b) A. Stopforth, B. V. Burger, A. M. Crouch, P. Sandra, *J. Chromatogr. B* **2006**, *834*, 134–140.
[16] I. H. Han, A. S. Csallany, *J. Am. Oil Chem. Soc.* **2008**, *85*, 777–782.
[17] Low recoveries are typically overcome in analytical chemistry by using isotopically labeled analytes as internal standards;[18] however, this approach is unpractical in the case of highly unstable 4-HNE due to the difficult accessibility of suitably labeled standards in pure form.
[18] X. P. Luo, M. Yazdanpanah, N. Bhooi, D. C. Lehotay, *Anal. Biochem.* **1995**, *228*, 294.
[19] Solutions need to be stored at –160 to –80 °C, and as much as 25% degradation is observed in 1 h at 4 °C.[15a]
[20] I. Jouanin, V. Sreevani, E. Rathahao, F. Guéraud, A. Paris, *J. Labelled Compd. Radiopharm.* **2008**, *51*, 87–92.
[21] G. Spiteller, W. Kern, P. Spiteller, *J. Chromatogr. A* **1999**, *843*, 29–98.
[22] R. Grée, H. Tourbah, R. Carrié, *Tetrahedron Lett.* **1986**, *27*, 4983–4986.
[23] A. Vila, K. A. Tallman, A. T. Jacobs, D. C. Liebler, N. A. Porter, L. J. Marnett, *Chem. Res. Toxicol.* **2008**, *21*, 432–444.
[24] L. Soulère, Y. Queneau, A. Doutheau, *Chem. Phys. Lipids* **2007**, *150*, 239–243.
[25] The ^{1}H NMR signal for the hydrazone (N)H in **7e** was 9.93 ppm as compared to the value of 10.70 ppm for disubstituted **7d**.
[26] The reaction of **2f** with 4-HNE at higher temperature to provide the corresponding dihydropyridazine was not investigated.
[27] The apparent value of the rate constants varied with the concentration of the acid catalyst, in line with expectations for

a general acid catalysis mechanism. A more detailed kinetic investigation was outside the scope of this work, and kinetic data have been determined for comparative evaluation only at fixed TFA concentrations.

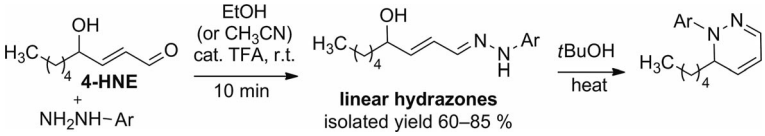
[28] L. Valgimigli, R. Amorati, M. G. Fumo, G. A. DiLabio, G. F. Pedulli, K. U. Ingold, D. A. Pratt, *J. Org. Chem.* **2008**, *73*, 1830–1841.

[29] a) R. Amorati, P. T. Lynett, L. Valgimigli, D. A. Pratt, *Chem. Eur. J.* **2012**, *18*, 6370–6379; b) R. F. Enes, A. C. Tomé, J. A. S. Cavaleiro, R. Amorati, M. G. Fumo, G. F. Pedulli, L. Valgimigli, *Chem. Eur. J.* **2006**, *12*, 4646–4653.

[30] a) P. Mendes, *Comput. Appl. Biosci.* **1993**, *9*, 563; b) P. Mendes, *Trends Biochem. Sci.* **1997**, *22*, 361; c) P. Mendes, D. B. Kell, *Bioinformatics* **1998**, *14*, 869.

Received: March 20, 2012
Published Online: ■

Lipid Peroxidation



4-Hydroxynonenal reacts rapidly at room temperature with 2-, 3-, or 4-CF_3-phenylhydrazine, or with the 3,5-di-CF_3, 2,4-di-CF_3, or pentafluoro analogues, to form hydrazones, which may undergo cyclization to 1,6-dihydropyridazines and other addition/oxidation products. The product distribution can be controlled by solvent and temperature to develop rapid derivatization assays.

Keywords: Synthetic methods / Hydrazones / Cyclization / Lipids / Kinetics

