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Synthesis and Spin Trapping Properties of Polystyrene Supported Trifluoromethylated Cyclic Nitrones

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Synthesis and Spin Trapping Properties of Polystyrene Supported Trifluoromethylated Cyclic Nitrones

Polystyrene supported fluorinated cyclic nitrone spin-traps: Resin-2-HFDMPO (2-hydroxymethyl-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2H-pyrrole-1-oxide) and Resin-2-PFDMPO (2-(3-hydroxypropyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2Hpyrrole 1-oxide) containing a trifluoromethyl pyrroline-N-oxide core were developed to detect free radicals under flow conditions. A continuous flow EPR technique was used to evaluate the spin trapping properties of these tethered nitrones. While both resins trapped radicals, polymer supported nitrone Resin-2-PFDMPO with a longer and more flexible linker showed a more information rich spectrum than Resin-2-HFDMPO.

Keywords: nitrone; fluorinated; polymer supported; spin trapping; free radicals

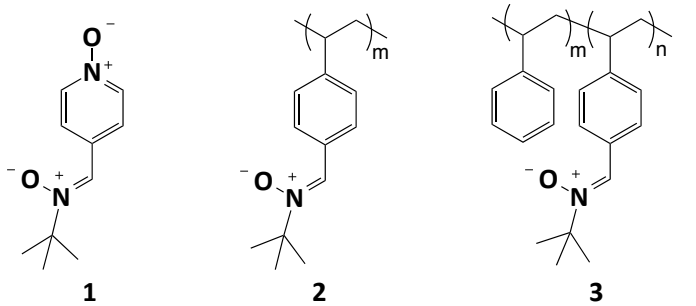
Introduction

Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) play major roles in physiological, pathological, environmental and atmospheric processes. They are also important intermediate species in a variety of chemical processes such as catalysis, batteries and polymerization reactions. As such, radicals can exist in all phases of matter, with their detection restricted many times by short lifetimes. The lifetime of a radical in liquid solution is often controlled by the reaction rate with other constituents. One detection strategy for solution state radicals is spin trapping: forming a secondary stable product that is amenable to both quantitative and qualitative analysis. In most cases this product is detected in its radical form, usually by Electron Paramagnetic Resonance (EPR). In other cases, the trapped radical can be analyzed by NMR or Mass Spectrometry after the radical has been quenched. One advantage of detecting the radical form by EPR is the ability to do *in situ* or operando experiments and collect real time data during a process. This can include not only chemically generated radicals, but also products from photolysis/radiolysis and electrochemistry.

The chemistry and ultimate fate of free radicals play a role in many aspects of the environment: the control of greenhouse gases (climate change), the formation of atmospheric acids (acid rain), and the production of ozone and secondary organic aerosols (photochemical smog, air quality and air pollution) [1]. The reactions of gas phase HO•, NO_3• and O_3 with volatile organic compounds such as terpenes [2-4], and alkanes and aromatics [5] result in the formation of secondary organic aerosols (SOA) in the atmosphere. SOA account for a major fraction of the fine particulate matter in the atmosphere, affecting the climate by scattering sunlight and serving as nuclei for cloud droplets and ice crystals [6]. UV irradiation promotes free radical processes on the surface of aerosol particles, contributing to the aging of aerosols [7], which changes their climate influencing properties such as hygroscopicity [8], light scattering [9], and absorption of the particles as well as their cloud forming abilities [10-14]. Despite large differences in molecular composition, concentration, and conditions among atmospheric, environmental, and biological systems, the underlying chemistry of ROS has many

similarities. The coupling and exchange of atmospheric and physiological ROS and RNS proceed through various bio-surfaces such as the animal respiratory tracts, skin, and plant leaves [15]. ROS and RNS in the atmosphere can deposit onto surfaces, causing oxidative stress and nitrosative stress. Epidemiological studies show a clear correlation between air pollutants and adverse health effects, including cardiovascular [16], respiratory, and allergic diseases in humans [17,18].

Several sampling and analytical methods have been developed to identify and quantify ROS in air samples. The most common methods include low matrix isolation and electron spin-resonance (MI-ESR) [19], chemical ionization mass spectrometry (CIMS) [20], laser-induced fluorescence (LIF) [21-24], and peroxyl radical chemical amplification (PERCA) [25-27]. These methods have been applied to various outdoor and indoor air samples, such as laboratory-generated tobacco smoke and secondary organic aerosol particles from ozone chemistry. However, the accurate analytical determination of ROS is still challenging, due to the high reactivity of the radicals and the instability of probes. Furthermore, methods used successfully to determine high ROS concentrations (such as those present in tobacco smoke and other combustion sources) may not be directly applicable to lower levels found in the atmosphere. While spin trapping of short-lived free radicals using nitrones coupled with EPR has been widely used in biomedical research, employment of this method for the detection of free radicals in the atmosphere is less common. Watanabe et al. [28] succeeded in trapping HO• from the atmosphere using the nitrone alpha-(4-pyridyl-1-oxide)-*N*-tert-butylnitrone (4-POBN) **1** (**Figure 1**). An airborne sampler, mounted with a filter paper impregnated with 4-POBN was used to trap HO•. The resulting 4-POBN-OH adduct was isolated after washing the filter paper with acetone. The acetone was evaporated and the residue was dissolved in benzene and analyzed by EPR. However, this process does not provide an accurate measurement of the HO• in the atmosphere due to the multiple manipulations. Sakakibara et al. [29] reported the use of poly[*N*-(*p*-vinylbenzylidene)-*tert*-butylamine oxide] (PolyPBN) **2,** originally developed by Janzen [30], and copolymers of PBN and styrene (Poly(St-co-PBN)) **3** (**Figure 1**) in reactions with alkyl radicals in the gas phase. The spin-trap polymers were deposited on glass wool or on a solid foam in a reaction tube. Alkyl radicals were generated via photolysis of the corresponding alkyl chlorides. The resultant spin-adducts were characterized and quantified by EPR. The peaks of the PolyPBN adducts were considerably broader than those of the monomeric PBN, due to the restricted motion of the nitroxide moiety on the polymeric support. The broadening was decreased to some extent in the spectrum of Poly(St-co-PBN) **3** adducts, presumably due to a decrease in the steric congestion around the nitroxide moiety. Other examples of the attachment of spin traps to solid supports include decorating the surface of a Au nanoparticle with a thiol-derivatized cyclic nitrone by Liu [31], and the covalently embedding a phosphorylated cyclic nitrone into mesopourous silica by Besson and Hardy [32].



1 **2** **3**

4-POBN **PolyPBN** **Poly(St-co-PBN)**

Figure 1. Spin trap 4-POBN (Watanabe) [28] and polymeric spin traps PolyPBN and Poly(St-co-PBN) (Sakakibara) [29]

The direct detection of the nitroxide spin-adducts by ^{1}H NMR is difficult due to the broadening of the NMR signal by the unpaired electron as well as the instability of most spin-adducts. Therefore, nitroxides are usually reduced using phenyl hydrazine or ascorbic acid to form diamagnetic hydroxylamines. However, the use of ^{1}H NMR or ^{13}C NMR in detection of free radicals is significantly limited by the complexity of multiple overlapping signals. The stable isotope ^{19}F, when incorporated into the spin-trap should give resolvable spectra of the diamagnetic hydroxylamine adducts. The concept of NMR spin-trapping (ST-NMR) was introduced in the 1980s, when Motten et al. [33] synthesized five fluorinated analogues of PBN **4** and evaluated detection of free radicals in organic reactions using ^{19}F NMR spectroscopy (**Figure 2**). The introduction of the NMR-sensitive label ^{19}F into PBN enabled the selective monitoring of chemical reactions associated with phenyl radical after the reduction of spin-adducts with phenylhydrazine.

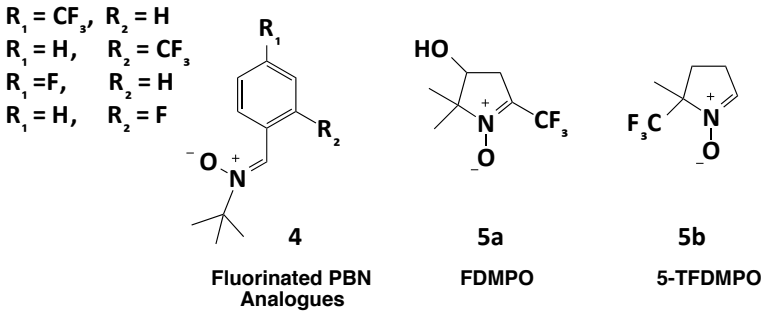


Figure 2. Fluorinated PBN spin traps **4** (Motten) [33], trifluoromethylated FDMPO **5a** (Clanton) [34] and 5-TFDMPO **5b** (Tordo) [36] spin traps

In 2001, Clanton et al. [34] synthesized a water soluble fluorinated analogue of DMPO (5,5-dimethyl-1-pyrroline *N*-oxide), 4-hydroxy-5,5-dimethyl-2-trifluormethyl-pyrroline-1-oxide (FDMPO) **5a**, to study EPR and NMR spin trapping of free radicals. Not only does the nitroxide adduct of FDMPO **5a** have superior stability (up to 24 h), but following nitroxide reduction, the original radical can be identified via ^{19}F NMR. This provides two important advantages: the data can be acquired long after the radical has been trapped, and a lower average concentration of radicals can be detected because the concentration of nitroxide spin-trap adduct can be built up over time. Likewise, ^{31}P NMR with 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO) cyclic nitrone has been used to monitor several biologically relevant radicals. [35] In 2014, Tordo et al. [36] prepared 5-methyl-5-trifluoromethyl-1-pyrroline *N*-Oxide (5-TFDMPO) **5b**, and demonstrated trapping of radicals in both aqueous buffer and organic solution.

We envisioned developing a trifluoromethylated cyclic nitrone spin trap tethered to a polymer support for the detection of free radicals in both the gas phase and the liquid phase. The trifluoronated trap should both give increased radical stability for EPR measurements, and may also allow for detection via ^{19}F MAS-NMR, increasing the ability to detect gas phase radicals. However, the tethered spin trap will also enable new liquid phase experiments. Continuous flow NMR and EPR capabilities have been developed at Pacific Northwest National Laboratory (PNNL), which allows the monitoring of chemistry on a solid phase while a gas or liquid mobile phase flows through the sample cell. Herein is described the application of continuous flow technique to study

reactive radical species using polymer-supported fluorinated cyclic nitrone spin-traps as the solid phase. As shown in **Figure 3**, radicals were either dissolved chemical species or created in situ via photolysis via UV illumination at the center of the EPR resonator. The mobile phase carried solution state radicals, which were trapped by the fluorinated nitrone column (right). The contents of the spin-trap column were then analyzed by EPR.

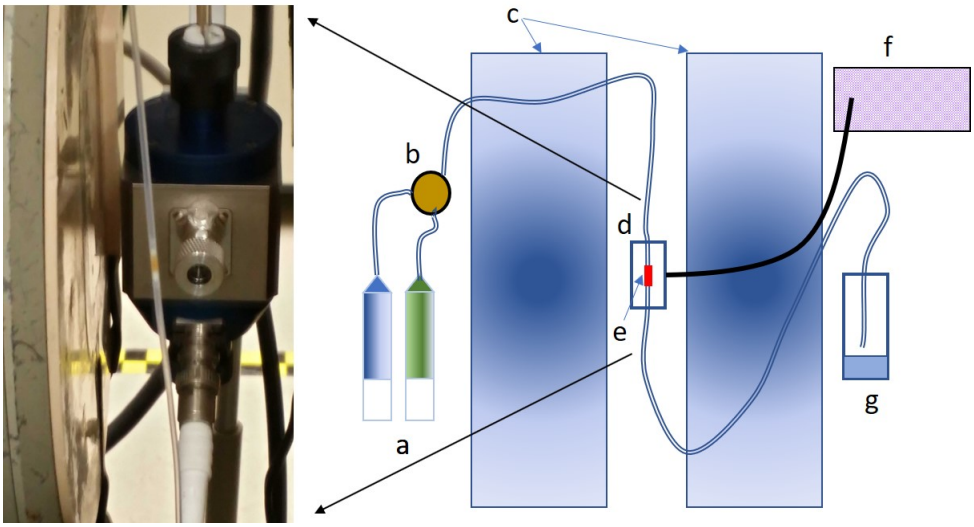


Figure 3. Generalized scheme for Flow Spin-trapping Experiments.
Left side, photograph of spin trap column in front of EPR resonator. Right, instrument setup: a) Double syringe pump, b) mixer, c) EPR electromagnet, d) EPR resonator, e) spin trap column, f) UV lamp connected to resonator with flexible light pipe, g) solvent waste.

FDMPO **5** is the only fluorinated nitrone spin-trap commercially available that can be tethered to a solid support. FDMPO **5** is extremely expensive ($83 for 10 mg from Enzo Life Sciences) and the synthesis is not straightforward. Attempts in our lab to replicate Janzen's synthesis [37] of 2-TFDMPO **6** failed, and the synthetic steps described in the literature are enigmatic. Therefore, a new synthetic route to prepare 2-TFDMPO **6** was developed, and then modified to develop two hydroxyl analogues: 2-hydroxymethyl-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2H-pyrrole 1-oxide (2-HFDMPO) **7** and 2-(3-hydroxypropyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2Hpyrrole 1-oxide (2-PFDMPO) **8** (**Figure 4**). These hydroxyl-substituted analogues **7** and **8** were then attached to a polystyrene solid support to form Resin 2-HFDMPO **9** and Resin 2-PFDMPO **10** with a shorter (**9**) and longer (**10**) linker. Continuous flow EPR under conditions generating free hydroxy radicals probed the EPR spectra of the resulting solid supported nitroxides. The synthesis of these trifluoromethylated nitrone derivatives and their covalent attachment to polystyrene, and the EPR spectra of the resulting nitroxide spin-adducts are presented.

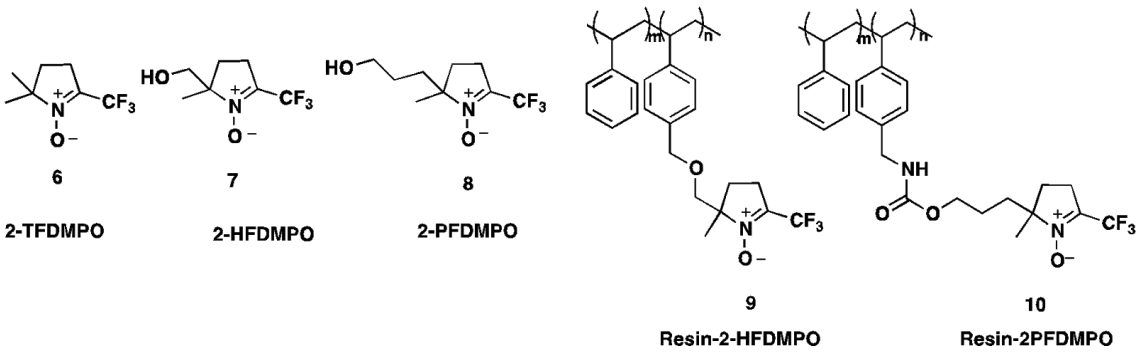


Figure 4. Monomeric and polymer-supported cyclic fluorinated nitrones investigated in this work

Materials and Methods

Tetrahydrofuran (Fisher Scientific) was dried over sodium and benzophenone when anhydrous conditions were required. All other chemicals were used as received. The following chemicals were purchased from Sigma-Aldrich: sodium hydride (60% in mineral oil, used without removal of the oil), triethylamine (99%), benzylamine (99%), paraformaldehyde (95%), Merrifield resin (3.0-4.0 mmol/g of chloride), glacial acetic acid (17.4 N), and *N*,*N*-dimethyformamide. The following chemicals were purchased from Acros: Dess-Martin periodinane (97%), 3,4-dihydro(2H)pyran (99%), tetrabutylammonium fluoride (1 M in THF), and cesium fluoride (99%). 2-nitropropanol (98%) was purchased from Alfa Aesar. The following reagents were purchased from Fischer Scientific: magnesium sulfate (98%), sodium bicarbonate (98%), chloroform, methanol, ethanol, magnesium turnings, zinc (99%), sodium borohydride (98%), and concentrated hydrochloric acid (12M). Trifluoromethyltrimethylsilane (99%) was purchased from Oakwood Chemicals. 2-nitropropane (98%) was purchased from Spectrum Chemicals. Aminomethylated polystyrene was purchased at 100-200 mesh size. Silica gel column chromatography was performed on a Biotage Isolera™ Prime automated flash purification system. Flash chromatography was performed using premium grade 60, 40 - 75 mesh silica gel from Sorbent Technologies. Analytical thin layer chromatography (TLC) was carried out on Whatman silica gel plates (0.25 mm thick). NMR spectra were recorded at ambient temperature on a Varian 500 MHz spectrometer or INOVO 500 MHz in $CDCl_3$ as solvent unless otherwise noted. The spectra were recorded with the residual $CHCl_3$ peak (δ 7.27 ppm) as internal standard for ^{1}H NMR and $CDCl_3$ central peak of 1:1:1 "triplet" (δ 77.27 ppm) for ^{13}C NMR. ^{19}F SSNMR spectra were obtained at 19.9 T (850 MHz for proton) on an Agilent VNMRS spectrometer using a 4 mm $^{1}H^{19}FXY$ MAS probe. FTIR spectra were recorded on a Perkin-Elmer spectrometer as a neat film on a KBr cell. High resolution mass spectra (HRMS) were recorded either on a benchtop Mariner electrospray ionization time-of-flight (ESITOF) mass spectrometer or on LTQ Orbitrap. All EPR measurements were performed on Bruker ELEXSYS E580 spectrometer equipped with an SHQe resonator. A capillary with ID 0.8 mm and OD 1 mm was used to hold the solution in the EPR cavity with both ends sealed by Critoseal. The typical settings for the static spectra were microwave frequency = 9.86 GHz, sweep width = 150 G, sweep time = 20.9 s, power = 20 mW, field modulation amplitude = 0.5 G. Time resolved data were obtained by recording EPR spectrum continually (one spectrum every 20.9 s) during and after UV irradiation. For flow EPR, a two syringe pump with separate control for each syringe (KD Scientific) was used to inject solutions into a mixer and then to a flow cell in EPR cavity.

The flow cell was created by containing the resin beads in 1.8 mm OD x 0.75 mm ID FEP tubing (McMaster-Carr) between frits. A Spectrum 100a UV source (Lesco) was used for *in situ* photolysis of EPR samples. It is equipped with an 18 W mercury vapor lamp, a shutter controlled with a switch and timer, an iris, and 280-400 nm bandpass filters. The light is directed to the sample using a liquid filled light guide connected to the light access port on the EPR resonator.

Synthesis of 2-nitropropan-1-ol 11 [38]. To a stirred mixture of nitroethane (10.26 g, 136.7 mmol) and paraformaldehyde (4.105 g, 136.7 mmol) in 100 mL of acetonitrile was added 0.2 mL of triethylamine at room temperature. The reaction mixture was stirred for 72 h and the solvent was evaporated *in vacuo*. The resulting yellow oil was diluted with 100 mL of chloroform and filtered. The filtrate was concentrated *in vacuo* to give the title compound as a red oil (5.805 g, 40.42% yield).
^{1}H NMR (500 MHz, $CDCl_3$): δ 4.67 (ddq, J = 7.0, 3.3 Hz, 1H), 4.00 (dd, J = 12.4, 7.5 Hz, 1H), 3.92 (dd, J = 12.4, 3.3 Hz, 1H), 2.22 (br s, 1H, OH), 1.55 (d, J = 7.0, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 84.6 (CH), 64.2 (CH_2) 15.3 (CH_3).

Synthesis of 4-nitropentan-1-ol 12 [39]. To a stirred solution of nitroethane (21.08 g, 280.8 mmol) and acrolein (18.8 mL, 280 mmol) in 300 mL of acetonitrile was added 0.1 mL of DBU at 0 °C. The reaction mixture was stirred for 15 minutes and then 2 mL of acetic acid was added to quench the reaction. The reaction mixture was concentrated *in vacuo* to give 15.12 g of an orange crude oil. Methanol (350 mL) was added and the reaction mixture was cooled to 0 °C. Sodium borohydride (4.172 g, 110.3 mmol) was added in portions over 20 minutes. The reaction mixture was stirred for 1 h and the solvent was evaporated *in vacuo*. The resulting orange oil was dissolved in 500 mL of dichloromethane, washed with 100 mL of water, dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound as a pale yellow oil (16.82 g, 46.99% yield).
TLC: 50:50 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.51.
^{1}H NMR (500 MHz, $CDCl_3$): δ 4.65 – 4.58 (m, 1H), 3.71 – 3.63 (m, 2H), 2.11 – 2.03 (m, 1H), 1.90 – 1.82 (m, 1H), 1.66 (br s, 1H), 1.61 – 1.56 (m, 2H), 1.54 (d, J = 7Hz, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 80.3 (CH), 60.2 (CH_2), 30.2 (CH_2), 28.1 (CH_2), 19.8 (CH_3).
FTIR: 3369 (O-H stretch), 1547 (N-O stretch), 1391 (N=O stretch), 1278 (C-N stretch), 1064 (C-O stretch) cm^{-1}.
HRMS: calcd. for $C_5H_{11}NO_3$ [M-H] : 132.0666: found 132.0666.

Synthesis of 2-(2-nitropropoxy)tetrahydro-2H-pyran 13b [40]. To a stirred solution of 2-nitropropan-1-ol **11** (10.08 g, 95.87 mmol) and 3,4-dihydro-2H-pyran (8.871 g, 10.55 mmol) in 100 mL of dichloromethane was added 1 mL of concentrated hydrochloric acid at room temperature. The reaction mixture was stirred for 21 h and then washed sequentially with 30 mL of water, 30 mL of saturated $NaHCO_3$ solution, and 20 mL of brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude orange oil (17.24 g) was purified by silica gel column chromatography with 50:50 hexanes/ethyl acetate as eluent, to give the title compound as a pale yellow oil (15.18 g, 83.66% yield).
^{1}H NMR (500 MHz, $CDCl_3$, two diastereomers): δ 4.84 – 4.72 (m, 1H), 4.66 (q, J = 3.0 Hz, 1H), 4.60 (q, J = 3.0 Hz, 1H), 4.17 – 4.09 (m, 1H), 3.95 – 3.83 (m, 1H), 3.82 – 3.77 (m, 1H), 3.76 – 3.70 (m, 1H), 3.69 – 3.64 (m, 1H), 3.56 – 3.49 (m, 2H), 1.83 – 1.56 (m, 2H), 1.56 – 1.45 (m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$, DEPT, two diastereomers): δ 99.4 (CH), 98.0 (CH), 82.8 (4°), 82.2 (4°), 68.9 (CH_2), 68.0 (CH_2), 62.2 (CH_2), 61.7 (CH_2), 30.2 (CH), 30.1 (CH), 25.3 (CH_2), 19.1 (CH_2), 18.7 (CH_2), 15.8 (CH_3), 15.7 (CH_3).

Synthesis of 2-((4-nitropentyl)oxy)tetrahydro-2*H*-pyran 13c. To a stirred solution of 4-nitropentan-1-ol **4.83** (5.326 g, 40.01 mmol) and 3,4-dihydro-2*H*-pyran (4.035 g, 48.01 mmol) in 100 mL of dichloromethane was added 1 mL of concentrated hydrochloric acid at room temperature. The reaction mixture was stirred for 24 h and then washed sequentially with 30 mL of water, 30 mL of saturated $NaHCO_3$ solution, 20 mL of brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude orange oil (8.326 g) was purified by silica gel column chromatography with 75:25 hexanes/ethyl acetate as eluent, to give the title compound as a clear oil (6.065 g, 67.78% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde stain, R_f: 0.64.
^{1}H NMR (500 MHz, $CDCl_3$, two diastereomers): δ 4.62 (dddd, *J* = 8.2, 6.8, 5.5, 1.4 Hz, 1H), 4.46 (dt, *J* = 5.5, 2.1 Hz, 1H), 3.86 – 3.70 (m, 1H), 3.70 – 3.61 (m, 1H), 3.40 (dt, *J* = 10.0, 6.0 Hz, 1H), 3.31 (dt, *J* = 10.0, 6.0 Hz, 1H), 1.98 (dddd, *J* = 16.7, 14.2, 8.2, 4.1 Hz, 1H), 1.82 – 1.66 (m, 2H), 1.65 – 1.56 (m, 1H), 1.56 – 1.50 (m, 2H), 1.45 (m, 7H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT, two diastereomers): δ 98.9 (CH), 83.4 (CH), 83.3 (CH), 66.4 (CH_2), 66.3 (CH_2), 62.2 (CH_2), 62.3 (CH_2), 32.2 (CH_2), 32.1 (CH_2), 30.6 (CH_2), 25.9 (CH_2), 25.4 (CH_2), 25.3 (CH_2), 19.5 (CH_2), 19.2 (CH_3). FTIR: 1548 (N-O stretch), 1391 (N=O stretch), 1278 (C-N stretch), stretch), 1064 (C-O stretch) cm^{-1}.
HRMS: calcd. for $C_{10}H_{19}NO_4$ [M-H] : 216.1214: found 216.1237.

Synthesis of 4-methyl-4-nitropentanal 14a [41]. The following procedure using the 2-nitropropane to is representative of the general procedure for the addition of dialkynitro anion to acrolein. To a solution of freshly distilled acrolein (14.6 mL, 219 mmol) under ambient pressure and 2-nitropropane (15.9 mL, 175 mmol) in 60 mL of acetonitrile was added triethylamine (4.5 mL g, 50 mmol) at room temperature. The reaction mixture was stirred for 22 h and concentrated *in vacuo*. The resulting crude yellow oil (20.03 g) was purified by silica gel column chromatography with 85:15 hexanes/ethyl acetate as eluent, to give the title compound as a colorless oil (15.33 g, 60.19% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.46.
^{1}H NMR (500 MHz, $CDCl_3$): δ 9.77 (s, 1H), 2.50 (t, *J* = 7.5 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.59 (s, 6H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 199.9 (C=O), 87.3 (4°), 38.9 (CH_2), 32.3 (CH_2), 25.9 (CH_3).
FTIR: 1721 (C=O stretch), 1536 (N-O stretch), 1349 (N=O stretch), 1278 (C-N stretch) cm^{-1}.

Synthesis of 4-nitro-4-((tetrahydro-2*H*-pyran-2-yl)oxy)pentanal 14b: pale yellow oil (11.39 g, 82.69% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.36.
^{1}H NMR (500 MHz, $CDCl_3$, two diastereomers): δ 9.77 (s, 1H), 9.75 (s, 1H), 4.84 – 4.72 (m, 2H), 4.59 (dt, *J* = 10.5, 3.4 Hz, 2H), 4.06 (d, *J* = 10.5 Hz, 1H), 3.95 (d, *J* = 10.5 Hz, 1H), 3.77 – 3.66 (m, 3H), 3.63 (d, *J* = 10.5 Hz, 1H), 3.51 (dt, *J* = 11.0, 4.4 Hz, 2H), 2.54 (dt, *J* = 11.1, 7.7 Hz, 3H), 2.35 (ddt, *J* = 23.0, 15.2, 7.5 Hz, 2H), 2.17 (ddt, *J* = 23.0, 15.2, 7.5 Hz, 2H), 1.77 – 1.61 (m, 2H), 1.61 (s, 4H), 1.59 – 1.55 (m, 3H), 1.53 – 1.44 (m, 8H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT, two diastereomers): δ 199.9 (CHO), δ 199.8 (CHO), 98.9 (CH), 89.7 (4°), 71.7 (CH_2), 71.6 (CH_2), 62.2 (CH_2), 62.0 (CH_2), 38.5 (CH_2), 38.3

(CH_2), 30.2 (CH), 30.1 (CH), 28.3 (CH_2), 28.1 (CH_2), 25.2 (CH_2), 20.8 (CH_3), 20.5 (CH_3), 18.9 (CH_2), 18.8 (CH_2).

Synthesis of 4-methyl-4-nitro-7-((tetrahydro-2*H*-pyran-2-yl)oxy)heptanal 14c: clear oil (1.754 g, 37.16% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.26.
^{1}H NMR (500 MHz, $CDCl_3$, two diastereomers): δ 9.77 (s, 1H), 4.54 (m, 1H), 3.84 - 3.81 (m, 1H), 3.79 - 3.71 (m, 1H), 3.49 – 3.47 (m, 1H), 3.39 – 3.36 (m, 1H), 2.52 – 2.46 (m, 2H), 2.37 – 2.32 (m, 1H), 2.16 – 2.08 (m, 2H), 1.94 – 1.91 (m, 1H), 1.80 – 1.78 (m, 1H), 1.73 – 1.24 (m, 10H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT, two diastereomers): δ 199.2 (HC=O) 99.0 (CH), 98.9 (CH), 90.0 (4Åã), 66.7 (CH2), 62.5 (CH2), 62.4 (CH2), 38.6 (CH2), 36.6 (CH2), 36.5 (CH2), 31.0 (CH2), 30.6 (CH2), 25.4 (CH2), 24.4 (CH2), 22.0 (CH3), 19.5 (CH2).
FTIR: 1725 (C=O stretch), 1537 (N-O stretch), 1391 (N=O stretch), 1033 (C-O stretch) cm^{-1}.

Synthesis of 1,1,1-trifluoro-5-methyl-5-nitrohexan-2-ol 15a. To a stirred mixture of 4-methyl-4-nitropentanal **14a** (3.898 g, 26.85 mmol) and cesium fluoride (1.273 g, 0.8381 mmol) in 30 mL of anhydrous tetrahydrofuran was added trifluoromethyltrimethylsilane (4.3 mL, 29 mmol) dropwise over 10 minutes at 0 °C. The reaction mixture was stirred for 30 minutes, and then 20 mL of 4 N hydrochloric acid was added. The layers were separated and the aqueous layer was extracted three times with 30 mL of dichloromethane. The combined organic layer was washed with 30 mL of saturated $NaHCO_3$ solution, 20 mL of brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude yellow oil (4.023 g) was purified by silica gel column chromatography with 85:15 hexanes/ethyl acetate as eluent, to give the title compound as a colorless oil (2.633 g, 46.17% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.48.
^{1}H NMR (500 MHz, $CDCl_3$): δ 3.44 (dd, J = 10.0 Hz, 2.0 Hz, 1H), 2.28 (m, 2H), 2.23 (m, 2H), 1.64 (s, 3H), 1.63 (s, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 105.4 (CF_3), 87.8 (4°), 82.6 (CH), 37.2 (CH_2), 26.3 (CH_3), 26.2 (CH_2), 25.3 (CH_3).
FTIR: 3468 (O-H stretch), 1536 (N-O stretch), 1349 (N=O stretch), 1278 (C-N stretch), 1127 (C-F stretch) cm^{-1}.
HRMS: calcd. for $C_7H_{12}F_3NO_3$ [M-H] : 214.0696: found 214.0607.

Synthesis of 1,1,1-trifluoro-5-methyl-5-nitro-6-((tetrahydro-2H-pyran-2-yl)oxy)hexan-2-ol 15b. The following procedure using 4-nitro-4-((tetrahydro-2*H*-pyran-2-yl)oxy)pentanal **14b** is representative of the general procedure for the addition of trifluoromethyl to the aldehyde. To a stirred mixture of 4-methyl-4-nitro-5-((tetrahydro-2H-pyran-2-yl)oxy)pentanal **14b** (7.068 g, 28.82 mmol) and cesium fluoride (0.6566 g, 4.323 mmol) in 50 mL of dry tetrahydrofuran was added trifluomethyltrimethylsilane (5.1 mL, 34 mmol) dropwise for 10 minutes at 0 °C. The reaction mixture was stirred for 1 h and tetrabutylammonium fluoride (28 mL, 1M in THF) was added. The reaction mixture was stirred for 10 minutes and diluted with 100 mL of dichloromethane. The organic layer was washed with 50 mL of water and 50 mL of brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude yellow oil (6.238 g) was purified by silica gel column chromatography with 60:40 hexanes/ethyl acetate as eluent, to give the title compound as a colorless oil (4.295 g, 47.27% yield).
TLC: 60:40 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.62.

^{1}H NMR (500 MHz, $CDCl_3$, multiple diastereomers): δ 4.69 – 4.56 (m, 1H), 4.16 – 3.99 (m, 1H), 3.83 – 3.67 (m, 2H), 3.60 – 3.47 (m, 1H), 3.22 – 3.05 (m, 1H), 2.95 – 2.80 (m, 1H), 2.57 – 2.38 (m, 1H), 2.38 – 2.18 (m, 1H), 1.82 – 1.45 (m, 10H), 1.29 (d, *J* = 24.9 Hz, 1H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT, multiple diastereomers): δ 127.1- 120.3 (m, CF_3), 98.10 (CH), 97.12 (CH), 97.8 (CH), 97.8 (CH), 89.0 (4°), 88.8 (4°), 88.76 (4°), 70.8 (CH_2), 70.4 (CH_2), 70.1 (CH_2), 69.9 (CH_2), 69.2 - 68.3 (m, CH-CF_3), 61.5 (CH_2), 61.3 (CH_2), 61.0 (CH_2), 60.9 (CH_2), 30.7 (CH_2), 30.6 (CH_2), 30.4 (CH_2), 30.0 (CH_2), 29.0 (CH_2), 28.95 (CH_2), 28.91 (CH_2), 23.9 (CH_2), 23.00 (CH_2), 22.7 (CH_2), 22.6 (CH_2), 19.6 (CH_3), 19.5 (CH_3), 18.9 (CH_3), 18.6 (CH_3), 17.9 (CH_2), 17.8 (CH_2), 17.7 (CH_2), 17.6 (CH_2).
FTIR: 3393 (O-H stretch), 1544 (N-O stretch), 1352 (N=O stretch), 1278 (C-N stretch), 1121(C-F stretch), 1074 (C-O stretch), 1005 (C-O stretch) cm^{-1}.

Synthesis of 1,1,1-trifluoro-5-methyl-5-nitro-8-((tetrahydro-2*H*-pyran-2-yl)oxy)octan-2-ol 15c: pale yellow oil (74.63% yield) was used without purification.

Synthesis of 1,1,1-trifluoro-5-methyl-5-nitrohexan-2-one 16a. The following procedure using **1,1,1-**trifluoro-5-methyl-5-nitro-6-((tetrahydro-2H-pyran-2-yl)oxy)hexan-2-ol **15a** is representative of the general procedure for the oxidation of the alpha-trifluoromethyl alcohol to the corresponding ketone. To a stirred solution of 1,1,1-trifluoro-5-methyl-5-nitrohexan-2-ol **15a** (3.764 g, 17.49 mmol) in 50 mL of dichloromethane was added Dess-Martin periodinane (8.903 g, 20.99 mmol) at room temperature. The reaction mixture was stirred for 18 h, concentrated *in vacuo*, diluted with 150 mL of hexanes, and filtered. The filtrate was concentrated *in vacuo* to give the title compound as a pale yellow oil (2.761 g, 74.07% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.40.
^{1}H NMR (500 MHz, $CDCl_3$): δ 2.79 (t, *J* = 7.5 Hz, 2H), 2.31 (t, *J* = 7.5 Hz, 2H), 1.64 (s, 6H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT):.δ 189.9 (q, J_{CF} = 35.5 Hz, CF3-C=O), 115.4 (q, J_{CF} = 290 Hz, CF3), 86.7 (4Åã), 32.8 (CH2), 31.6 (CH2), 25.9 (CH3).
FTIR: 1761 (C=O stretch), 1541 (N-O stretch), 1351 (N=O stretch), 1271 (C-N stretch), 1154 (C-F stretch) cm^{-1}.
HRMS: calcd. for $C_7H_{10}F_3NO_3$ [M-H] : 212.0540: found 212.0534.
1,1,1-trifluoro-5-methyl-5-nitro-6-((tetrahydro-2H-pyran-2-yl)oxy)hexan-2-one 16b: pale yellow oil (8.064 g, 77.96% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.40.
^{1}H NMR (500 MHz, $CDCl_3$, two diastereomers): δ 4.69 – 4.56 (m, 1H), 4.16 – 3.99 (m, 1H), 3.83 – 3.67 (m, 2H), 3.60 – 3.47 (m, 1H), 3.22 – 3.05 (m, 1H), 2.95 – 2.80 (m, 1H), 2.57 – 2.38 (m, 1H), 2.38 – 2.18 (m, 1H), 1.82 – 1.45 (m, 10H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT, two diastereomers): δ 191.7 - 186.04 (m, CF_3-C=O), 116.6 - 114.3 (m, CF_3), 98.9 (CH), 98.8 (CH), 89.4 (4°), 89.2 (4°), 71.7 (CH_2), 71.6 (CH_2), 62.1 (CH_2), 62.0 (CH_2), 31.2 (CH_2), 31.1 (CH_2), 30.09 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 25.20 (CH_2), 21.2 (CH_3), 21.0 (CH_3), 18.9 (CH_2), 18.8 (CH_2).
FTIR: 1716 (C=O stretch), 1546 (N-O stretch), 1348 (N=O stretch), 1241 (C-N stretch), 1183 (C-F stretch) cm^{-1}.

1,1,1-trifluoro-5-methyl-5-nitro-8-((tetrahydro-2*H*-pyran-2-yl)oxy)octan-2-one 16c: pale yellow oil (1.545 g, 74.96% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.25.

^{1}H NMR (500 MHz, $CDCl_3$, two diastereomers): δ 4.51 (m, 1H), 3.82 – 3.79 (m, 1H), 3.73 – 3.70 (m, 1H), 3.48 – 3.47 (m, 1H), 3.39 – 3.36 (m, 1H), 2.76 – 2.75 (m, 2H), 2.45 – 2.35 (m, 1H), 2.19 – 2.15 (m, 2H), 2.09 – 2.04 (m, 2H), 1.96 – 1.93 (m, 1H) 1.79 – 1.77 (m, 1H), 1.70 – 1.68 (m, 1H), 1.67 – 1.49 (m, 6H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT, two diastereomers): δ 190.0 (q, J_{CF} = 30 Hz, C-CF_3), 115.2 (q, J_{CF} = 240 Hz, CF_3), 99.1 (CH), 99.0 (CH), 89.9 (4°), 66.8 (CH_2), 66.5 (CH_2), 62.8 (CH_2), 62.5 (CH_2), 36.5 (CH_2), 31.2 (CH_2), 31.1 (CH_2), 30.5 (CH_2), 25.3 (CH_2), 24.3 (CH_2), 24.2 (CH_2), 22.1 (CH_2), 21.9 (CH_2), 19.6 (CH_3).
FTIR: 1766 (C=O stretch), 1539 (N-O stretch), 1351 (N=O stretch), 1278 (C-N stretch), 1175(C-F stretch), 1024 (C-O stretch) cm^{-1}.
HRMS: calcd. for $C_{14}H_{22}F_3NO_5$ [M-H] : 340.1377: found 340.1364.

Synthesis of 2,2-dimethyl-5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole 1-oxide 6 [37]. To a stirred mixture of 1,1,1-trifluoro-5-methyl-5-nitrohexan-2-one **16a** (2.761 g, 12.95 mmol) and zinc (2.424 g, 38.87 mmol) in 70 mL of ethanol was added acetic acid (4.5 mL, 78 mmol) in an ice bath to keep the temperature below 10 °C. The reaction mixture was allowed to warm to room temperature and stirred for 24 h, filtered over Celite, concentrated *in vacuo*, and 150 mL of dichloromethane was added. The organic layer was washed sequentially with 20 mL of water, 20 mL of saturated $NaHCO_3$, and 20 mL of brine. The organic layer was concentrated *in vacuo* to give the title compound as a pale yellow oil (1.082 g, 46.14% yield).
TLC: 75:25 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.25.
^{1}H NMR (500 MHz, $CDCl_3$): δ 2.82 (t, *J* = 7.0 Hz, 2H), 2.16 (t, *J* = 7.0 Hz, 2H), 1.45 (s, 6H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 129.3 (q, J_{CF} = 35.1 Hz, C-CF_3), 119.7 (q, J_{CF} = 271 Hz, CF_3), 77.7 (4°), 32.1 (CH_2), 25.1 (CH_3), 23.9 (CH_2).
^{19}F NMR (470 MHz, C_6F_6): δ -68.6 (s, CF_3).
FTIR: 1581 (N-O stretch), 1424 (C=N stretch), 1258 (C-N stretch), 1144 (C-F stretch) cm^{-1}.
HRMS: calcd. for $C_7H_{10}F_3NO$ [M-H] : 181.0641: found 181.0480.

Synthesis of 2-methyl-2-(((tetrahydro-2H-pyran-2yl)oxy)methyl)-5-(trifluoromethyl)-3,4-dihydro-2H-pyrrole 1-oxide 17b: yellow oil (6.811 g, 94.07% yield).
TLC: 60:40 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.33.
^{1}H NMR (500 MHz, $CDCl_3$, two diastereomers): δ 4.98 – 4.78 (m, 2H), 4.11 - 3.98 (m, 2H), 3.82 – 3.66 (m, 4H), 3.58 – 3.46 (m, 2H), 3.17 – 3.05 (m, 1H), 2.92 – 2.79 (m, 2H), 2.53 – 2.36 (m, 2H), 2.35 – 2.16 (m, 2H), 1.79-1.42 (m, 18H), 1.27 – 1.12 (m, 1H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT, two diastereomers): δ δ 131.4 - 129.7 (m, C-CF_3), 121.7- 116.6 (m, CF_3), 98.5 (CH), 96.1 (CH), 79.1 (4°), 78.7 (4°), 73.1 (CH_2), 72.0 (CH_2), 70.3 (CH_2), 68.9 (CH_2), 61.6 (CH_2), 61.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 26.9 (CH_2), 26.5 (CH_2), 24.1 (CH_2), 23.9 (CH_2), 20.2 (CH_3), 19.9 (CH_3), 18.5 (CH_2), 18.3 (CH_2).
FTIR: 1589 (N-O stretch), 1421 (C=N stretch), 1378 (N=O stretch), 1245 (C-N stretch), 1135 (C-F stretch) cm^{-1}.

Synthesis of 2-(3-(((tetrahydro-2H-pyran-2yl)oxy)methyl)-5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole-1-oxide 17c: red oil (75.56% yield) was used in next step without purification.

Synthesis of 2-hydroxymethyl-2methyl-5-(trifluoromethyl)-3,4-dihydro-2H-pyrrole 1-oxide 7: This procedure using 2-methyl-2-(((tetrahydro-2H-pyran-2yl)oxy)methyl)-5-

(trifluoro-methyl)-3,4-dihydro-2H-pyrrole 1-oxide **17b** is representative for the reductive cyclization to form the nitrone. To a stirred solution of 2-methyl-2(((tetrahydro-2H-pyran-2yl)oxy)methyl)-5-(trifluoromethyl)-3,4-dihydro-2H-pyrrole 1-oxide **17b** (6.811 g, 24.22 mmol) in 70 mL of methanol was added 3 mL of concentrated hydrochloric acid at room temperature. The reaction mixture was stirred for 1 h and then sodium bicarbonate (3.089 g, 36.77 mmol) was added. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The resulting crude brown oil was purified by silica gel column chromatography using 50:50 hexanes/ethyl acetate as eluent, to give the title compound as a white solid (1.719 g, 37.33% yield, m.p: 70 – 72 °C).
TLC: 50:50 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.22.
^{1}H NMR (500 MHz, $CDCl_3$): δ 3.94 (d, *J* = 12.1 Hz, 1H), 3.56 (d, *J* = 12.1 Hz, 1H), 2.96 - 2.97 (m, 2H), 2.43 (m, 1H), 2.04 (m, 1H), 1.44 (s, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 133.2 (q, J_{CF} = 36.1 Hz, C=N), 119.3 (q, J_{CF} = 271 Hz, CF_3), 81.2 (4°), 66.1 (CH_2), 26.7 (CH_2), 24.9 (CH_2), 20.6 (CH_3).
^{19}F NMR (470 MHz, C_6F_6): δ -68.7 (s, CF_3).
FTIR: 3392 (O-H stretch), 1610 (N-O stretch), 1417 (C=N stretch), 1376 (N=O stretch), 1238 (C-N stretch), 1135 (C-F stretch) cm^{-1}.
HRMS: calcd. for $C_7H_{10}F_3NO_2$ [M-H] : 196.0590: found 196.0580.

Synthesis of 2-(3-hydroxypropyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole 1-oxide 8: pale yellow oil (0.3733 g, 36.76% yield).
TLC: ethyl acetate, UV, *p*-anisaldehyde, R_f: 0.37.
^{1}H NMR (500 MHz, $CDCl_3$): δ 3.59 (td, *J* = 6.3, 2.6 Hz, 2H), 2.85 – 2.70 (m, 2H), 2.81 (s, 4H), 2.60 (br s, 1H), 2.25 (m, 1H), 2.04 (m, 1H), 1.88 – 1.74 (m, 2H), 1.70- 1.59 (m, 2H), 1.58 (m, 1H), 1.41 (s, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 131.9 (q, J_{CF} = 36 Hz, C-CF_3), 119.5 (q, J_{CF} = 270 Hz, CF_3), 80.6 (4°), 62.5 (CH_2), 34.0 (CH_2), 28.8 (CH_2), 26.3 (CH_2), 24.6 (CH_3), 24.5 (CH_2).
^{19}F NMR (470 MHz, C_6F_6): δ -68.4 (s, CF_3).
FTIR: 3401 (O-H stretch), 1590 (N-O stretch), 1352 (N=O stretch), 1278 (C-N stretch), 1146 (C-F stretch), 1058 (C-O stretch) cm^{-1}.
HRMS: calcd. for $C_9H_{14}F_3NO_2$ [M-H] : 224.0903: found 224.0900.

Synthesis of 2-((benzyloxy)methyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole 1-oxide 18. To a stirred solution of 2-hydroxymethyl-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2H-pyrrole 1-oxide **7** (0.511 g, 0.2587 mmol) in 3 mL of dimethylformamide was added sodium hydride (0.0093 g, 0.39 mmol) at room temperature. The reaction mixture was stirred for 1 h and then quenched with 1 mL of saturated ammonium chloride solution. The reaction mixture was diluted with 5 mL of dichloromethane. The organic layer was separated and washed one time with 3 mL of brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude brown oil was purified by silica gel column chromatography using 50:50 hexanes/ethyl acetate as eluent, to give the title compound as a yellow oil (0.0236 g, 37.6% yield).
TLC: 50:50 hexanes/ethyl acetate, *p*-anisaldehyde, R_f: 0.64.
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 – 7.34 (m, 2H), 7.32 – 7.20 (m, 3H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H), 3.87 (d, *J* = 10.1 Hz, 1H), 3.36 (d, *J* = 10.1 Hz, 1H), 2.95 – 2.81 (m, 1H), 2.73 (m, 1H), 2.57 (m, 1H), 2.01 (m, 1H), 1.38 (s, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 137.9 (4°), 131.8 (q, J_{CF} = 36.1 Hz, C=N), 128.6 (2 CH), 127.9 (CH), 127.6 (2 CH), 119.5 (q, J_{CF} = 275 Hz, CF_3), 80.3 (4°), 73.6 (CH_2), 73.4 (CH_2), 27.6 (CH_2), 25.1 (CH_2), 21.4 (CH_3).

FTIR: 1610 (N-O stretch), 1417 (C=N stretch), 1376 (N=O stretch), 1238 (C-N stretch), 1135 (C-F stretch) cm^{-1}.
HRMS: calcd. for $C_{14}H_{16}F_3NO_2$ [M+H] : 288.1167: found 288.1198.

Synthesis of Merrifield resin supported nitrone (Resin-2HFDMPO) 9. To a stirred solution of 2-hydroxymethyl-2methyl-5-(trifluoromethyl)-3,4-dihydro-2H-pyrrole 1-oxide **7** (0.3879 g, 1.969 mmol) in 4 mL of DMF was added sodium hydride (0.1889 g, 10.23 mmol) at room temperature and stirred for 30 minutes. Merrifield resin (0.7501 g) was added and the reaction mixture was stirred at 50 °C for 1h. The reaction was cooled to room temperature and then filtered. The resulting cake was washed three times with 10 mL of dimethylformamide, one time 20 mL of water, 20 mL of methanol, 20 mL of chloroform, and 20 mL of hexanes. The resulting cake was dried *in vacuo* to yield 0.8121 g of a brown solid.

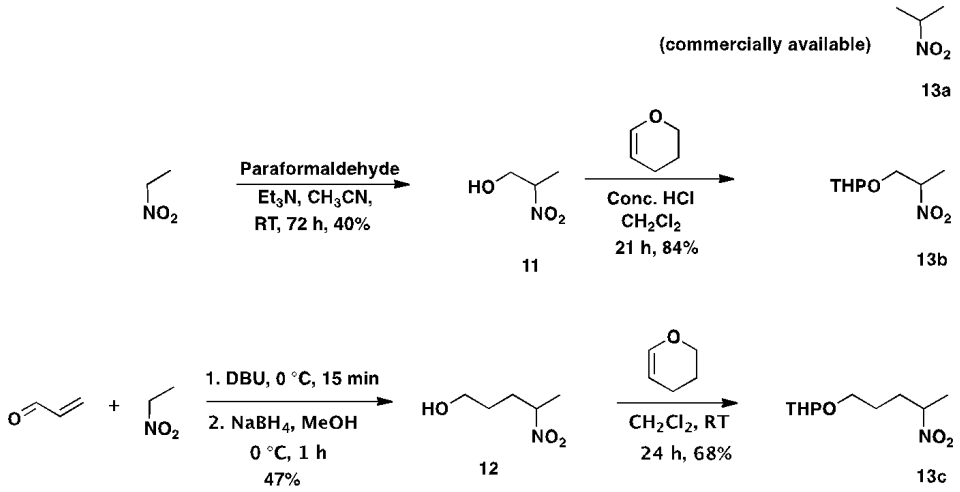
Synthesis of 2-(3-((((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)oxy)propyl)-2-methyl -5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole 1-oxide 21. To a stirred solution of 2-(3-hydroxypropyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2Hpyrrole 1-oxide (2-PFDMPO) (0.6085 g, 2.702 mmol) and *N,N*'-disuccinimidyl carbonate (0.6922 g, 2.702 mmol) in 15 mL of acetonitrile was added triethylamine (0.38 mL, 2.7 mmol) at room temperature. The reaction mixture was stirred for 24 h and then concentrated *in vacuo*. The resulting red oil (1.235 g) was purified by silica gel column chromatography with 50:50 hexanes/ethyl acetate as eluent, to give the title compound as a pale yellow oil (0.6341 g, 64.10% yield).
TLC: ethyl acetate, UV, *p*-anisaldehyde, R_f: 0.73.
^{1}H NMR (500 MHz, $CDCl_3$): δ 4.34 (m, 1H), 4.29 (m, 1H), 2.81 (s, 4H), 2.22 (m, 1H), 2.08 (m, 1H), 1.95 – 1.86 (m, 1H), 1.86 – 1.74 (m, 2H), 1.70 – 1.59 (m, 1H), 1.44 (s, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 168.6 (C=O), 151.4 (C=O), 130.0 (q, J_{CF} = 35 Hz, C-CF_3), 119.5 (q, J_{CF} = 270 Hz, CF_3), 79.7 (4°), 70.7 (CH_2), 33.9 (CH_2), 28.9 (CH_2), 25.4 (CH_2), 24.4 (CH_3), 24.2 (CH_2), 22.8 (CH_2).
FTIR: 1788 (C=O stretch), 1740 (C=O stretch), 1587 (N-O stretch), 1352 (N=O stretch), 1144 (C-F stretch), 1095 (C-O stretch) cm^{-1}.
HRMS: calcd. for $C_{14}H_{17}F_3N_2O_6$ [M-H] : 365.0965: found 365.0934.

Synthesis of 2-(3-((benzylcarbamoyl)oxy)propyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole 1-oxide 22. To a stirred solution of nitrone (0.0512 g, 0.1398 mmol) in 5 mL of acetonitrile was added benzyl amine (0.0149 g, 0.1398 mmol) at room temperature and stirred for 10 minutes. The reaction mixture was concentrated *in vacuo*. The resulting yellow oil (0.0632 g) was purified by silica gel column chromatography with 50:50, ethyl acetate/hexanes as eluent to give the title compound as a colorless oil (0.0313 g, 62.47% yield).
TLC: 50:50 ethyl acetate/hexanes, UV, *p*-anisaldehyde, R_f: 0.73.
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33 (t, *J* = 7.4 Hz, 2H), 7.30 – 7.24 (m, 3H), 5.09 (br s, 1H, NH), 4.35 (d, *J* = 5.8 Hz, 2H), 4.10 (m, 2H), 2.88 – 2.67 (m, 4H), 2.27 – 2.15 (m, 2H), 1.92 -1.58 (m, 2H), 1.44 (s, 3H).
^{13}C NMR (125 MHz, $CDCl_3$, DEPT): δ 156.5 (C=O), 138.5 (4°), 128.7 (CH), 127.5 (CH), 130.0 (q, J_{CF} = 35 Hz, C-CF_3), 119.5 (q, J_{CF} = 270 Hz, CF_3), 80.1 (4°), 64.4 (CH_2), 45.1 (CH_2), 34.4 (CH_2), 29.0 (CH_2), 24.5 (CH_3), 24.3 (CH_2), 23.5 (CH_2).
FTIR: 1788 (C=O stretch), 1587 (N-O stretch), 1352 (N=O stretch), 1144 (C-F stretch), 1095 (C-O stretch) cm^{-1}.
HRMS: calcd. for $C_{17}H_{19}F_3N_2O_3$ [M+H] : 359.1538: found 359.1567.

Synthesis of Polystyrene supported 2-PFDMPO (Resin-2-PFDMPO) 10. To a stirred solution of aminomethylated polystyrene (0.4063 g, 0.3657 mmol) in 5 mL of dimethylformamide was added nitrone (0.1339 g, 0.3657 mmol) at room temperature, stirred for 3 h, and then filtered. The resulting cake was washed three times with 10 mL of methanol, one time with 10 mL of hexanes, and dried to give 0.6013 g of Resin-2-PFDMPO as a pale yellow solid.

Results and Discussion

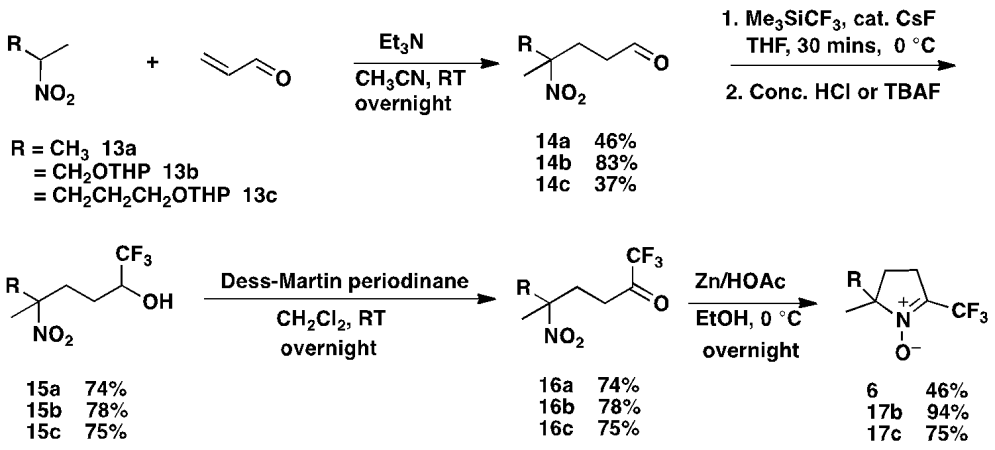
Commercially available 2-nitropropane **13a** was used as the starting materials for our alternate synthesis of 2-TFDMPO **6**. For the synthesis of 2-HFDMPO **7**, the starting material, THP protected 2-nitropropanol **13b**, was synthesized using nitroethane and paraformaldehyde in acetonitrile with triethylamine as a base, followed by the protection of alcohol group in **11** using 3,4-dihydropyran in the presence of a catalytic amount of hydrochloric acid (**Scheme 1**). The starting material for 2-PFDMPO **8** was obtained by



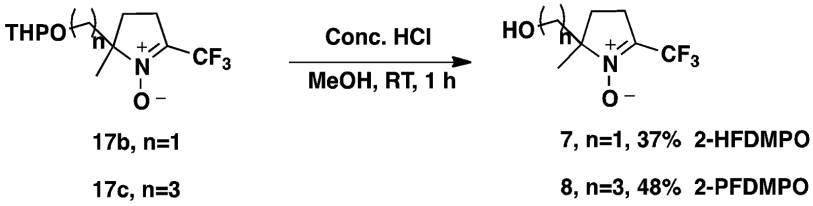
Scheme 1. Synthesis of THP-protected alcohol starting materials for 2-HFDMPO **7** and 2-PFDMPO **8**

Michael addition of the anion of nitroethane to acrolein using catalytic 1,8-diazabicycloundec-7-ene (DBU) as base in acetonitrile, to give the corresponding nitroaldehyde, which was reduced using sodium borohydride in methanol to yield alcohol **12** in 47% yield. The alcohol was protected using 3,4-dihydropyran to give the nitro product **13c** in 68% yield (**Scheme 1**). The nitroalkanes **13a**, **13b**, and **13c** were subjected to Michael addition to acrolein using triethylamine as base to give nitroaldehydes **14a**, **14b**, and **14c** in 46, 83 and 37% yields, respectively (Scheme 2). Trifluoromethylation of the nitroaldehydes using the Ruppert-Prakash reagent ($TMS\text{-}CF_3$) and catalytic cesium fluoride followed by desilyation using concentrated hydrochloric acid for **14a** and tetrabutylammonium fluoride for **14b** and **14c** gave the corresponding trifluoromethylated alcohols **15a**, **15b**, and **15c** in 74, 47, and 75% yields, respectively. Dess-Martin periodinane oxidation of trifluoromethylated alcohols produced trifluoromethyl ketones, which underwent reductive cyclization in the presence of zinc and acetic acid, via the hydroxylamine generated in situ, to yield nitrones **6**, **17b**, and **17c** in 46, 94, and 75% yields, respectively (**Scheme 2**). Final deprotection of the THP group

of **17b** and **17c** using hydrochloric acid in methanol to gave 2-HFDMPO **7** and 2-PFDMPO **8** (**Scheme 3**).

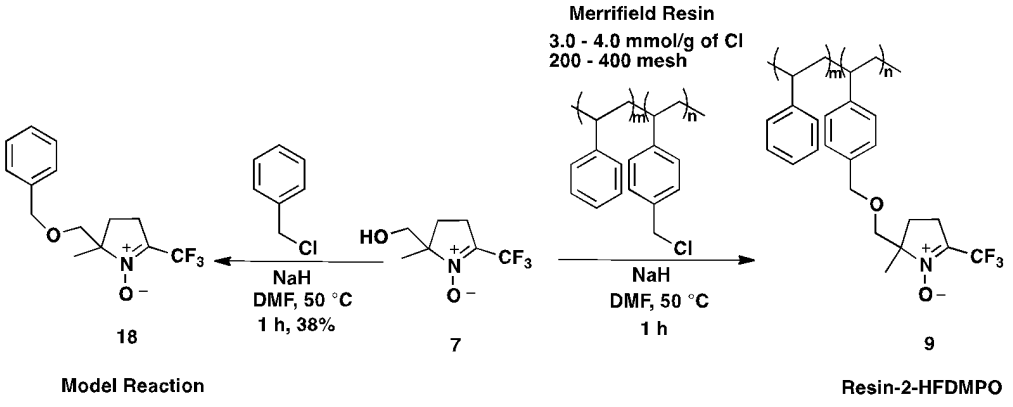


Scheme 2. Preparation of trifluoromethylated nitrones



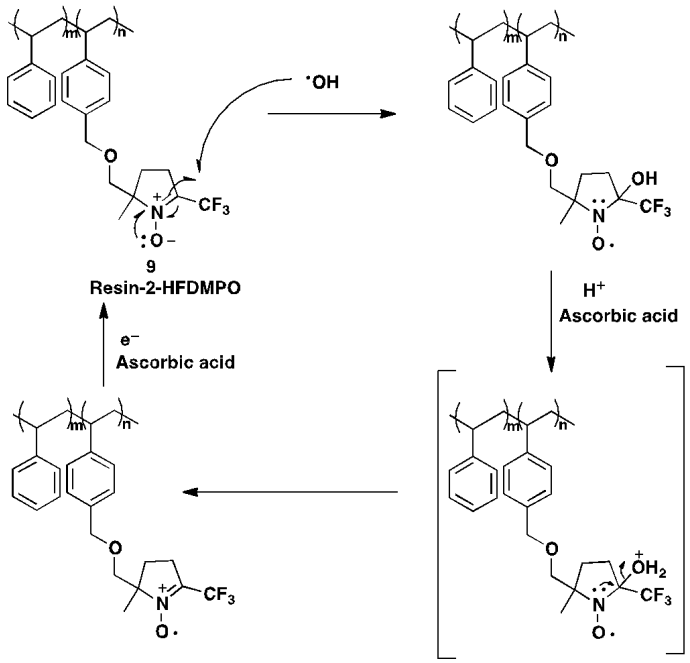
Scheme 3. Deprotection of hydroxyl groups to prepare 2-HFDMPO **7** and 2-PFDMPO **8**

Benzyl chloride was selected as a model compound to test the viability of nucleophilic substitution of the benzylic chloride in Merrifield resin by the alcohol of 2-HFDMPO **7** (**Scheme 4**). The reaction of benzyl chloride with 2-HFDMPO in DMF using sodium hydride at 50 °C afforded the desired ether **18** in 38% yield. To append 2-HFDMPO to the Merrifield resin, a mixture of resin, 2-HFDMPO, and sodium hydride was stirred in anhydrous dimethylformamide at 50 °C for 1 h. The progress of the reaction was monitored by thin layer chromatography to observe the disappearance of 2-HFDMPO (**Scheme 4**).



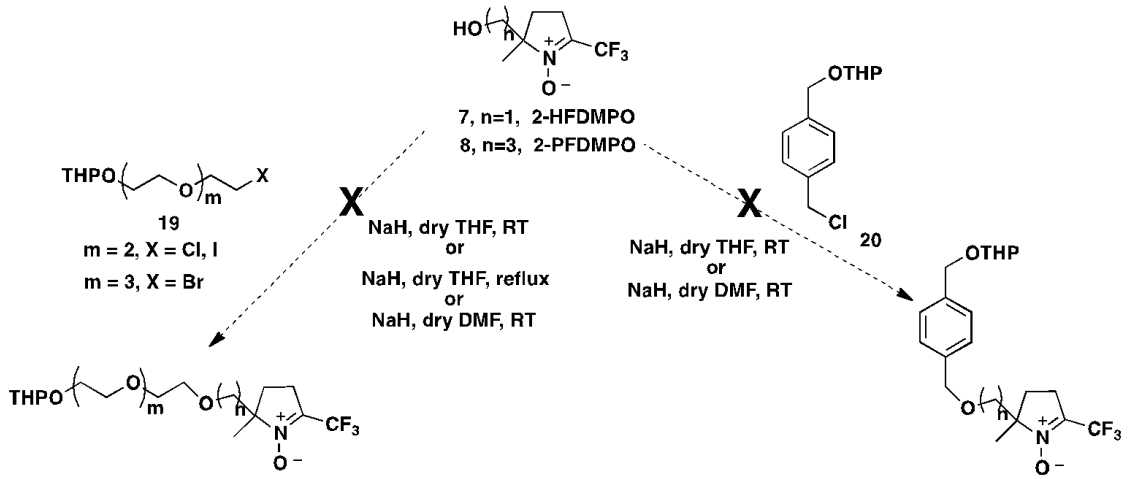
Scheme 4. Model reaction of benzyl chloride with 2-HFDMPO **7** and preparation of spin trap **7** tethered to Merrifield resin: Resin-2-HFDMPO **9**

The presence of the nitrone spin-trap on the resin was confirmed by EPR of the HO• trapped adduct. Hydroxyl radical was generated by photolysis of 3% hydrogen peroxide solution in water using a UV lamp; addition to the Merrifield resin supported 2-HFDMPO gave the EPR active nitroxide. The spin-trap nitrone resin was rejuvenated by reducing it with ascorbic acid for the next experiment (**Scheme 5**). When reduction was attempted using ascorbic acid in water, the EPR spectrum would reappear once the solvent was changed back to water alone. However, when the ascorbic acid was introduced in an aprotic solvent, the trap remained in the reduced state.



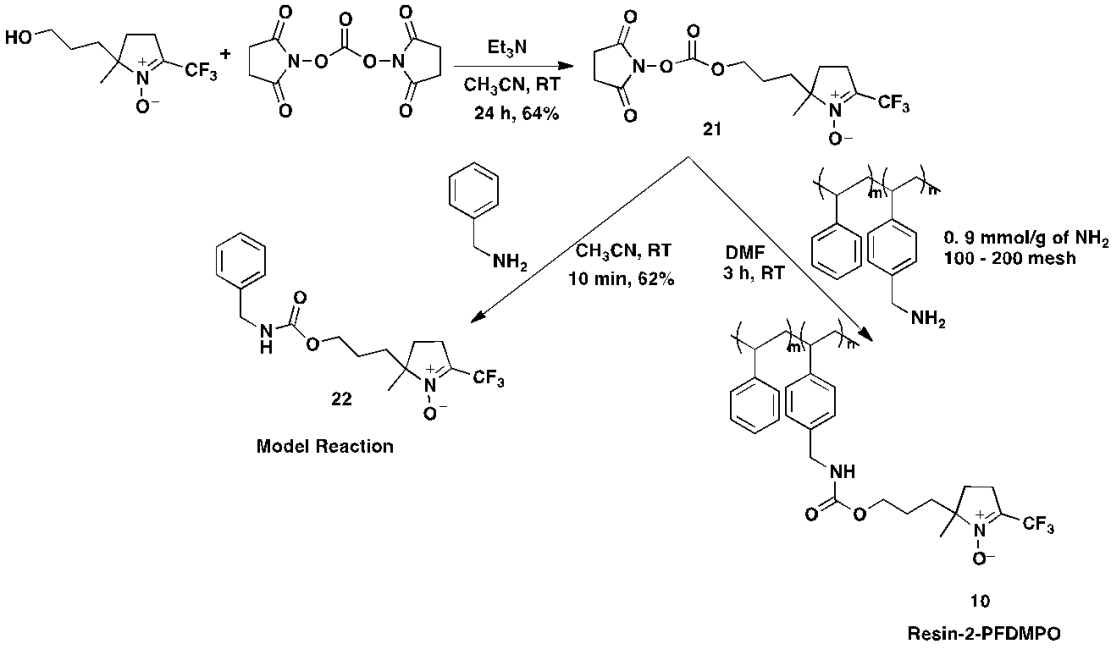
Scheme 5. Rejuvenation of Resin-2-HFDMPO **9**

The EPR spectrum of the hydroxyl adduct of Resin-2-HFDMPO **9** gave broad peaks compared to the EPR spectra of hydroxyl adducts of 2-TFDMPO **6** and 2-HFDMPO **7** (**Figure 5a**). The absence of hyperfine splitting makes the identification of the original HO• radical unclear. Significant peak broadening is likely due to the short tether between the nitrone spin-trap and the polymer, which results in restricted motion of the nitroxide adduct. A longer linker between the nitrone and resin should provide more rotational freedom, resulting in spectra with narrow peaks, providing more information about the initial radical species. Triethylene glycol was investigated as a potential linker. However, 2-HFDMPO failed to undergo S_N2 reaction with the chloro- or iodo-triethylene glycol derivatives **19**, probably due to the steric hindrance of the neopentyl alcohol nucleophile (n = 1) (**Scheme 6**). Therefore, a nitrone with a tether to a primary alcohol, 2-PFDMPO **8**, was developed. The alkoxide anion of 2-PFDMPO was generated in dry tetrahydrofuran using sodium hydride at room temperature, with the intention to use it as a nucleophile with the bromo-derivative of THP-protected tetraethylene glycol. Attempted nucleophilic substitution reactions of 2-HFDMPO and 2-PFDMPO were not successful, and even benzylic chloride **20** failed to react with the sodium alkoxides of 2-HFDMPO and 2-PFDMPO.



Scheme 6. Attempted S_N2 reactions of chloro- and iodo-triethylene glycol derivatives, a bromo-tetraethylene glycol derivative, and a benzylic chloride derivative with 2-HFDMPO **7** and 2-PFDMPO **8**

S_N2 substitution by alcohols at an sp^3 carbon bearing a leaving group is much more sterically demanding than nucleophilic acylation at a flat sp^2 carbon. Thus an acylation approach was pursued, in which the hydroxyl group of 2-PFDMPO was acylated by addition to *N,N*'-disuccinimidyl carbonate (DSC), followed by subsequent coupling with a primary amine to form a carbamate. Nitrone 2-PFDMPO was activated with DSC in acetonitrile using triethylamine as a base to afford the desired activated alcohol acyl succinate of 2-PFDMPO **21** in 64% yield (**Scheme 7**). As a model reaction, the activated acyl succinate of 2-PFDMPO **21** was treated with benzylamine in acetonitrile at room temperature to give the isolated carbamate **22** in 62% yield (**Scheme 7**). To append 2-PFDMPO onto an aminomethylated polystyrene resin, a mixture of resin and acyl succinate nitrone **21** was stirred in dimethylformamide for 3 h. The progress of the reaction was monitored by thin layer chromatography to observe the disappearance of the activated nitrone **21**. The presence of the nitrone on the resin was confirmed by ^{19}F MAS-NMR and by EPR of the HO• adduct via photolysis of hydrogen peroxide (**Figure 5**). The ^{19}F chemical shift was δ -64.91 ppm, similar to that reported for FDMPO at -66.0 ppm [34]. The EPR spectrum of the HO• adduct of **10** in dichloromethane (DCM) was fit using EasySpin [42] using starting parameters typical for a nitroxide in the slow motion regime. Conversion of the anisotropic results to the isotropic values typically reported for spin traps yields a_F=2.46 G and a_N=13.54, comparable to values reported for FDMPO-HO• in water of a_F=2.75 G and a_N=13.90 [34].



Scheme 7. Activation of 2-PFDMPO **8**, model reaction with benzyl chloride and synthesis of Resin-2-PFDMPO **10**

For spin-trapping experiments using Resin-2-PFDMPO **10** as the solid phase, the radical HO• was generated by the photolysis of HOOH. The EPR spectrum of the adduct was compared to the EPR spectrum of 2-HFDMPO-OH. As expected, the broadening of peaks was reduced in the EPR spectrum of the adduct Resin-2-PFDMPO-OH with the longer, more flexible linker compared to Resin-2-HFDMPO-OH (**Figure 5**). Although the spectrum of the spin-adduct Resin-2-PFDMPO-OH is not as narrow as that of the adduct of the free small molecule 2-HFDMPO, it is narrow enough to distinguish between different radical adducts in solvents such as dichloromethane (DCM). Future plans are to investigate the addition of other radical species.

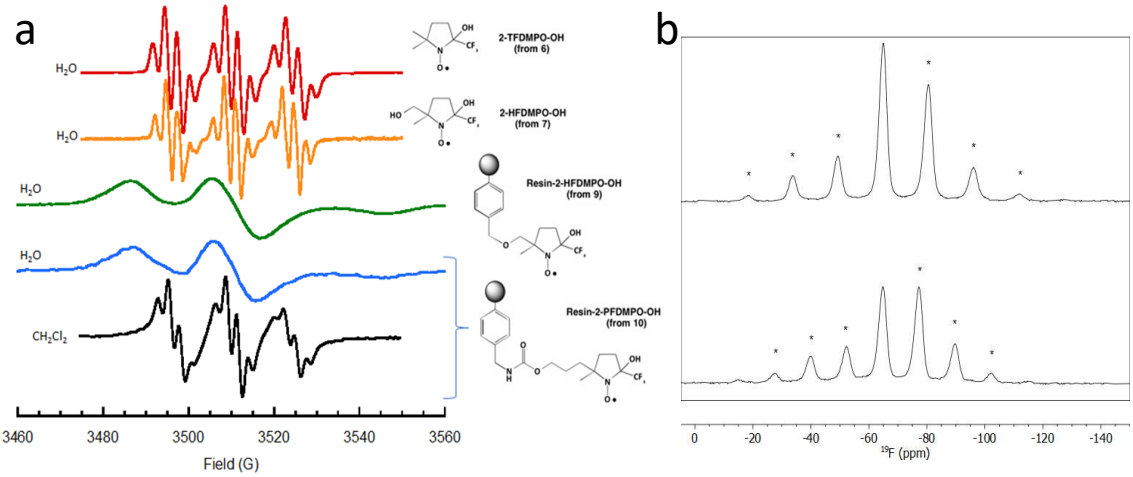


Figure 5. a) EPR spectra of nitroxides 2-TFDMPO-OH (red), 2-HFDMPO-OH (orange), and Resin-2-HFDMPO-OH (green), all three in water, EPR spectra of Resin-2-PFDMPO-OH in water (blue) and in dichloromethane (black). b) ^{19}F MAS-NMR of **10**,

asterisks indicate spinning side bands. Top, 12.5 kHz spinning speed, bottom, 10 kHz spinning speed.

Flow Experiments

With the nitrones successfully attached to resin beads, continuous flow experiments were performed. In order to test whether a radical in the mobile phase could be detected, a miniature column was created in 1/16" ptfe tubing containing 3-4 mg of Resin-2-HFDMPO **9**, Figure 6. This column was centered in the EPR resonator and attached to a syringe pump with the flow set at 100 ul/min; EPR spectra were continually acquired during the course of the experiment. A background radical signal from the resin was first removed by passing a solution of ascorbic acid in DCM/DMF over the column The column was then washed with water for several column volumes. At this point the mobile phase was switched to a 3% H_2O_2 solution and the column allow to equilibrate. The resonator was illuminated with a UV lamp for a series of 20 second flashes, upon each flash, the EPR spectrum increased in magnitude as increasing amounts of Resin-2-HFDMPO-OH was formed. An experiment was then run to ascertain whether the resin could also trap superoxide, Figure 6. First the resin was rejuvenated by removing the radical from the previous experiment to reform the nitrone. In this instance, ascorbic acid dissolved in DMSO was the reductant. Then a superoxide solution, created by dissolving KO_2 in DMSO and 18-crown-6 ether (18-C-6) was flowed over the column. The spectrum of Resin-2-HFDMPO-OOH was then detected. However, in both cases, because of the low mobility of the radical, there was poor spectral resolution.

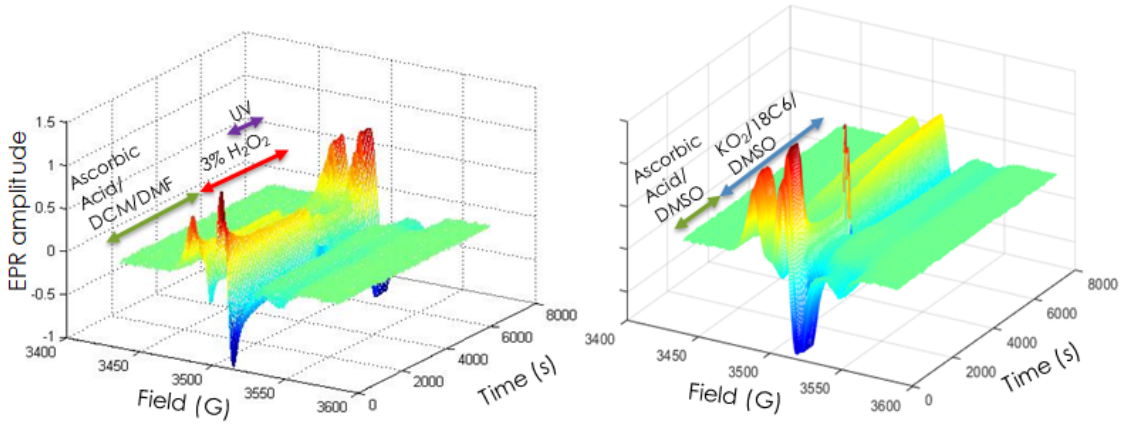


Figure 6. **Flow experiments with Resin-2-HFDMPO-OH.** Left, the column is prepared for the experiment by reducing the radical form of the spin trap with 200 mM ascorbic acid in 80:20 DCM/DMF. A 3% aqueous hydrogen peroxide solution is pumped through the spin trap column. After equilibration, the EPR resonator is then illuminated with a UV lamp creating hydroxyl radicals. Right, the column is again reduced with ascorbic acid, this time in DMSO, to remove the trapped radicals from the previous experiment. The mobile phase is then switched to KO_2 dissolved in DMSO with 18-crown-6 ether to aid solubility. The sharp doublet at ~1000s is from solution state ascorbic acid radicals.

Similar experiments were performed with Resin-2-PFDMPO **10**, Figure 7, where hydroxyl radicals were again formed from H_2O_2 using photolysis. In this case, after the hydroxyl radicals were trapped, the mobile phase was switched to methylene chloride.

The combination of resin swelling and reduced viscosity both contributed to mobility and therefore a much narrower spectrum. This may be the best experimental strategy: monitor build-up of signal in the systems solvent, then switch to methylene chloride to increase spectral resolution.

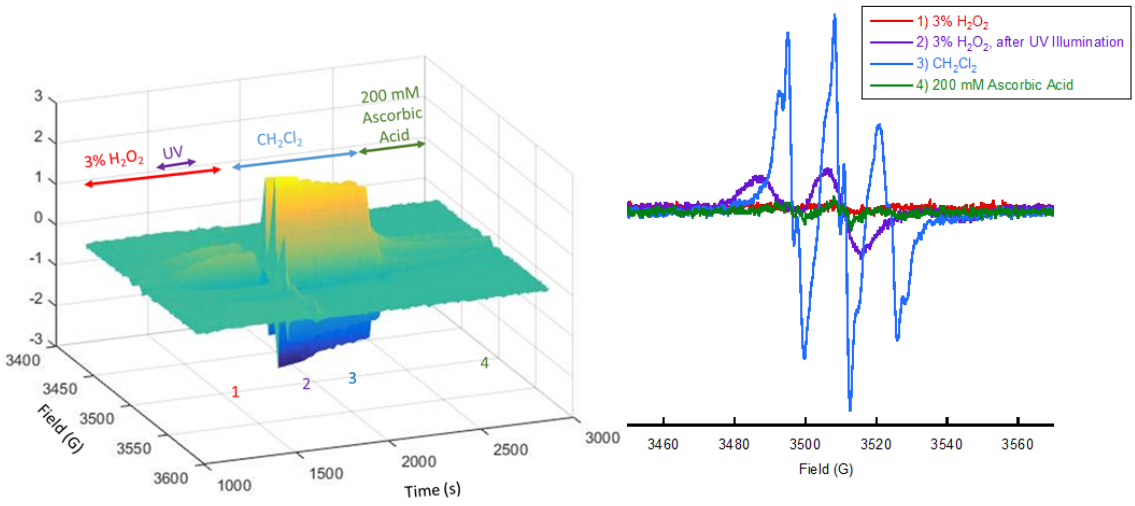


Figure 7. **Flow experiments with Resin-2-PFDMPO-OH.** Left, time resolved EPR spectra. Right, EPR spectra at indicated time points. 1) A 3% aqueous hydrogen peroxide solution is pumped through the spin trap column at a rate of 200 ul/min using a syringe pump. 2) The EPR resonator is illuminated with a UV lamp creating hydroxyl radicals in the flowing hydrogen peroxide solution. Radicals are trapped by the column, but the EPR spectrum is broad because of low mobility. 3) The mobile phase is changed from water to dichloromethane. The EPR spectrum narrows and increases in amplitude because of the increased mobility, allowing for analysis of the spectrum. 4) The column is prepared for the next experiment by reducing the radical form of the spin trap with 200 mM ascorbic acid in 80:20 DCM/DMF.

Conclusion

A practical and reliable synthesis of 2-TFDMPO **6** was developed, and two new amphiphilic nitrones, 2-(hydroxymethyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole 1-oxide (2-HFDMPO) **7**, and 2-(3-hydroxypropyl)-2-methyl-5-(trifluoromethyl)-3,4-dihydro-2*H*-pyrrole 1-oxide (2-PFDMPO) **8** were prepared. These fluorinated spin-traps will be useful in biological studies of free radicals in aqueous environments as well as in subcellular lipophilic environments. Additionally, two polymer-supported, fluorinated spin-traps **9** and **10** were successfully synthesized and then utilized to detect free radicals in solution. There are several advantages to these supported traps, with the main one demonstrated here: these spin trap resins can be packed in columns. This allows for several unique experiments, such as the ability to monitor external reaction vessels. By supporting the nitrone on a polymer, this prevents the trap from becoming a reactant in the system, particularly important with electrochemical systems due to the multiple redox states of nitrones. Likewise, a tethered trap is restricted to detecting radicals that can diffuse to it. This could provide a means of distinguishing the site of radical production: for a mixed phase system, if the radical species is trapped by a traditional small molecule spin trap but not a tethered trap, this would mean that the site of reaction

is not the solution but a surface or confined space. There is also the potential to greatly increase the detection limit in large volume systems: if a large volume of solution is passed over the column, the radicals will be captured and concentrated in the much smaller active volume of the EPR resonator. The same could be accomplished by adding the loose resin to a reaction vessel, stirring, and then filtering it out. The spin trap columns could also be used to perform stopped flow experiments: with the set-up shown in Figure 3, two reactants could be mixed and a reaction time determined by the flow rate and distance between the mixer and the column. Another advantage of having spin traps tethered to a solid phase support is the ability to rejuvenate the trap and reuse the resin (at least with oxygen-based radicals, covalent attachment of carbon-based radicals is likely to be permanent). Besides the attachment to solid phase resin as shown here, the hydroxyl functional groups on 2-HFDMPO **7** and 2-PFDMPO **8** can provide a functional handle to enable the tethering of spin-traps to other macromolecules such as peptides and cyclodextrins, enabling site-specific detection of free radicals in the cell.

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Declaration of Interest Statement

The authors declare that they have no conflict of interest.

Supplemental Material

Characterization (a collection of ^{1}H NMR, ^{13}C NMR, DEPT, ^{19}F NMR and IR spectra) for compounds **6, 7, 8, 12, 13b, 13c, 14c, 15a, 16a, 16c, 17b**, and **21**.

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