Supporting Information for:

2-Hydroxy-5-nitrobenzyl as a Diazeniumdiolate Protecting Group: Application in NO-Releasing Polymers with Enhanced Biocompatibility

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General Methods. ^{1}H NMR spectra were collected on a Bruker AMX 300 (300 MHz) or a Varian Unity Plus 400 (400 MHz) Fourier transform NMR spectrometer and were referenced to the residual proton solvent resonance. UV-vis spectra were monitored on a Hewlett-Packard 8453 diode array spectrophotometer.

Thermal Gravimetric Analysis. Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer DSC/TGA by increasing the temperature under a nitrogen environment to mimic a standard extrusion profile for polyurethanes. Specifically, the temperature was initially held at 50 °C for 1 min followed by a rapid ramping of the temperature at a rate of 40 °C /min up to 100 °C. The temperature was held steady at 100 °C for 2 hours. Another rapid temperature ramp of 40 °C /min up to 170 °C was followed by a 3 min hold at 170 °C. The sample was then rapidly cooled to 25 °C. The data were recorded on Perkin-Elmer DSC/TGA 7.

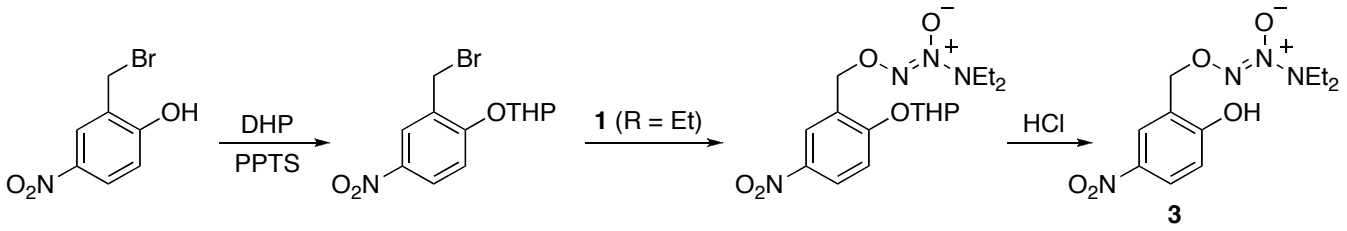
Quantification of Nitric Oxide Release. Nitric oxide (NO) measurements were performed using a Sievers Nitric Oxide Analyzer (NOA), model 280. The instrument was calibrated before each experiment using an internal two-point calibration (zero gas and 45 ppm). The measurement was performed by inserting the NO donors or polymeric films into a clean, dry NOA measurement cell, sealing the cell with a rubber septum, and collecting a baseline level of nitric oxide. Nitrogen-purged PBS buffer was then injected via a syringe

through a septum into the NOA measurement cell. The NO generated from the sample was removed from the solution via a constant nitrogen purge. The data were recorded as a concentration of NO in ppb or ppm.

Implantation procedure. A 25 kg juvenile farm swine was anesthetized using 1-4% isoflurane and was ventilated with 100% oxygen. The stomach and urinary bladder were surgically drained. Both groins as well as the bilateral neck were cut down to the femoral and carotid artery level, respectively, using electro-cautery. The proximal and distal portions of each artery were controlled with a vascular clamp and silk suture, respectively, and a 14-gauge angiocatheter was inserted pointing downstream (distal). Four PVC coated rods were placed in the arteries (one in each of the femoral arteries and one in each of the carotid arteries) via the angiocatheters such that 1-cm of the rod protruded past the end of the catheter. The arteries were not ligated so that blood was allowed to flow past the rods. Heparinized lactated Ringer's (1U/mL) drips were attached to each device and allowed to drip slowly (0.25 mL/min) to prevent blood from flowing retrograde into the catheters. The positioning of the rods ensured that the heparinized lactated Ringer's was being washed distally from the catheter and did not make contact with the coated surface of the rods.

Eight hours after implantation, the angiocatheter, rod, and the surrounding artery were excised. The rods were carefully dissected from the arteries without pulling them out of the vessel or through the angiocatheter to prevent scraping off any surface clots or protein film. The rods were then gently rinsed in PBS and inspected visually. Following removal of the rods, the animal was euthanized with 1mL/4kg I.V. Fatal Plus (Vortech Pharmaceutical, Dearbon, MI).

Synthesis of *O*^{2}-(2-hydroxy-5-nitrobenzyl)-1-(*N*,*N*-diethylamino)diazen-1-ium 1,2-diolate (3). NO precursor **3** was synthesized from commercially available 2-hydroxy-5-nitrobenzyl bromide (Scheme S1). The hydroxyl group of 2-hydroxy-5-nitrobenzyl bromide was protected as the tetrahydropyranyl ether[1] and the protected compound was coupled with diazeniumdiolate **1** (R = Et). The protecting group was then removed by treatment with acid[2] to yield precursor **3**.



DHP = dihydropyran; PPTS = pyridinium *p*-toluenesulfonate

Scheme S1. Synthesis of NO precursor **3**.

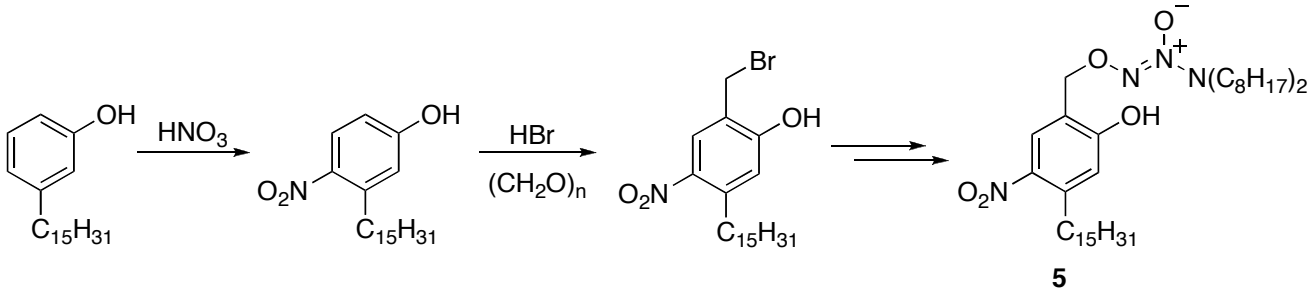
A solution of 2-hydroxy-5-nitrobenzyl bromide (232mg, 1 mmol), dihydropyran (168 mg, 2 mmol) and catalytic amount of pyridinium *p*-toluenesulfonate in anhydrous dichloromethane (10 ml) was stirred for four hours at room temperature. The reaction solution was then diluted with ether and washed once with half-saturated brine to remove the catalyst. After evaporation of the solvent, the residue was further purified by flash chromatography on silica gel column using ethyl acetate/petroleum ether (1:19) as the eluent to yield 2-(2-bromomethyl-4-nitro-phenoxy)-tetrahydro-pyran as a colorless oil (25%). ^{1}H NMR ($CDCl_3$) δ 1.4-1.9 (6H, m), 2.8-3.0 (2H, m), 3.6-3.7 (1H, m), 3.8-3.9 (1H, m), 4.4-4.6 (2H, m), 5.67 (1H, s), 7.23 (1H, s), 8.16-8.26 (2H, m).

To a stirred mixture of 2-(2-bromomethyl-4-nitro-phenoxy)-tetrahydro-pyran (200 mg, 0.638 mmol) and a catalytic amount of 15-crown-5 in 10 mL of anhydrous tetrahydrofuran at 0 °C, was added sodium 1-(*N*,*N*-diethylamino)-diazen-1-ium-1,2-diolate (**1**, R = Et) (400 mg). The reaction mixture was stirred at room temperature for two days. The solid precipitate was filtered. After evaporation of the solvent from the filtrate under reduced pressure, the residue was dissolved in ether, washed with water, and dried with anhydrous magnesium sulfate. The solvent was evaporated to yield *O*^{2}-((5-nitro-2-(tetrahydro-pyran-2-yloxy)-phenyl)-methyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (70%). ^{1}H NMR ($CDCl_3$) δ 1.06 (6H, t, *J* = 7 Hz), 1.4-1.9 (6H, m), 2.8-3.0 (2H, m), 3.1(4H, q, *J* = 7.1 Hz), 3.5-3.8 (2H, m), 5.39 (2H, s), 5.61 (1H, m), 7.36 (1H, s), 8.16-8.26 (2H, m).

The THP-protected diazeniumdiolate derivative was then dissolved in 10 mL of ether saturated with hydrogen chloride. The reaction mixture was stirred overnight. After evaporation of the solvent under reduced pressure, the residue was further purified by flash chromatography on silica gel column using ethyl acetate/petroleum ether (15:85) as the eluent

to yield O^2-(2-hydroxy-5-nitrobenzyl)-1-(*N,N*-diethylamino)diazen-1-ium-1,2-diolate (**3**) as a white solid (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (6H, t, J = 7.1 Hz), 3.24 (4H, q, J = 7.1 Hz), 5.31 (2H, s), 6.94 (1H, d, J = 9 Hz), 8.09 (1H, dd, J_1 = 8.9 Hz, J_2 = 2.8 Hz), 8.16 (1H, d, J = 2.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.35, 48.04, 71.45, 116.58, 121.43, 126.55, 127.18, 140.38, 161.86; MS(FAB, MH^+) 285.1195 (285.1199 cal)

Synthesis of O^2-(2-hydroxy-5-nitro-4-pentadecyl-benzyl)-1-(*N,N*-dioctylamino) diazen-1-ium-1,2-diolate (5). Lipophilic NO precursor **5** was synthesized (Scheme S2) by nitration of 3-pentadecylphenol with nitric acid which yielded both the 4- and 6-nitro isomers.[3] The desired isomer (4-nitro-3-pentadecylphenol) was isolated by recrystallization from petroleum ether. A bromomethyl group was introduced onto the aromatic ring by treatment of 4-nitro-3-pentadecylphenol with hydrobromic acid and paraformaldehyde.[4,5] The synthesis of lipophilic precursor **5** was then completed according to the Scheme S1 shown above for **3**, except that the diazeniumdiolate coupling was carried out with the newly synthesized hydrophobic diazeniumdiolate 1 (R = C_8H_{17}), prepared by exposing methanolic solution of *n*-dioctylamine and sodium methoxide to 40 psi NO for 24 hours.



Scheme S2. Synthesis of lipophilic NO precursor 10.

A solution of nitric acid (1.775 g, 70%) was added dropwise into an ice-cooled solution of 3-pentadecylphenol (5 g) in 20 mL chloroform. After stirring for 30 minutes, the reaction mixture was poured into water. The chloroform layer was separated, washed with water, and passed through a short plug of silica gel. After evaporation of the solvent under reduced pressure to yield a mixture of 4-nitro-3-pentadecyl phenol and 6-nitro-3-pentadecyl phenol, the desired compound 4-nitro isomer was recrystallized in petroleum ether as light brown color crystals with a melting point of 70-72 °C.

A mixture of 4-nitro-3-pentadecyl phenol (1 g) and paraformaldehyde (98 mg) were dissolved in 10 mL HBr/acetic acid solution (33 wt %). The reaction mixture was heated to 70 °C and was allowed to stand at this temperature for overnight. The solid that precipitated from the solution was collected and recrystallized in petroleum ether to yield 2-bromomethyl-4-nitro-5-pentadecyl-phenol (30%) as a pink solid with a melting point of 91-92 °C. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, *J* = 6.5 Hz), 1.2-1.5 (26 H, m), 2.89 (2H, t, *J* = 7.7 Hz), 4.52 (2H, s), 6.75 (1H, s), 8.04 (1H, s).

A solution of 2-bromomethyl-4-nitro-5-pentadecyl-phenol (442 mg, 1 mmol), dihydropyran(168 mg, 2 mmol), and a catalytic amount of pyridinium *p*-toluenesulfonate in anhydrous dichloromethane (10 ml) was stirred for four hours at room temperature. The reaction solution was then diluted with ether and washed once with half-saturated brine to remove the catalyst. After evaporation of the solvent, the residue was further purified by flash chromatography on silica gel column using ethyl acetate/petroleum ether (1:19) as the eluent to yield 2-(2-bromomethyl-4-nitro-5-pentadecyl-phenoxy)-tetrahydro-pyran as a colorless oil (32%). ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, *J* = 6.5 Hz), 1.2-1.4 (26H, m), 1.4-1.9 (6H, m), 2.8-3.0 (2H, m), 3.6-3.7 (1H, m), 3.8-3.9 (1H, m), 4.4-4.6 (2H, m), 5.65 (1H, s), 7.04 (1H, s), 8.04 (1H, s).

To a stirred mixture of 2-(2-bromomethyl-4-nitro-5-pentadecyl-phenoxy)-tetrahydro-pyran (336 mg, 0.638 mmol) and a catalytic amount of 15-crown-5 in 10 mL of anhydrous THF under ice cooling, was added sodium 1-(*N*,*N*-dioctylamino)-diazen-1-ium-1,2-diolate (**1**, R = C_8H_{17}) (400 mg), which was prepared by exposing methanolic solution of *n*-dioctylamine and sodium methoxide to 40 psi NO for 24 hours. The reaction mixture was stirred at room temperature for two days. The solid precipitate was filtered. After evaporation of the solvent from the filtrate under reduced pressure, the residue was dissolved in ether, washed with water, and dried with anhydrous magnesium sulfate. The solvent was evaporated to yield O^2-((5-nitro-4-pentadecyl-2-(tetrahydro-pyran-2-yloxy)-phenyl)-methyl)-1-(*N*,*N*-dioctylamino)diazen-1-ium-1,2-diolate (40%). ^{1}H NMR ($CDCl_3$) δ 0.8-1.9 (59H, m), 2.8-3.0 (2H, m), 3.0 (2H, m), 3.5-3.8 (2H, m), 5.31 (2H, s), 5.59 (1H, m), 7.03 (1H, s), 8.03 (1H, s).

The THP-protected diazeniumdiolate derivative was then dissolved in 10 mL ether saturated with hydrogen chloride. The reaction mixture was stirred overnight. After

evaporation of the solvent under reduced pressure, the residue was further purified by flash chromatography on silica gel column using ethyl acetate/petroleum ether (15:85) as the eluent to yield *O*^{2}-(2-hydroxy-5-nitro-4-pentadecyl-benzyl)-1-(*N*,*N*-dioctylamino)diazen-1-ium-1,2-diolate (**5**) as light yellow oil (90%). ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, *J* = 6.5 Hz), 1.1-1.7 (50H, m), 2.86 (2H, t, *J* = 7.6 Hz), 3.15 (4H, t, *J* = 7.1 Hz), 5.23 (2H, s), 6.77 (1H, s), 7.96 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.07, 14.10, 22.62, 22.68, 26.30, 26.77, 29.15, 29.22, 29.35, 29.38, 29.59, 29.65, 29.67, 29.69, 30.36, 31.75, 31.92, 33.63, 53.73, 71.4, 119.37, 119.59, 128.49, 141.17, 142.53, 159.82; MS (ESI): 685.5255 (M+Na)(685.5244 cal.).

References

(1) Miyashita, M.; Yoshikoshi, A.; Grieco, P. A. *J. Org. Chem.* **1977**, *42*, 3772-3774.

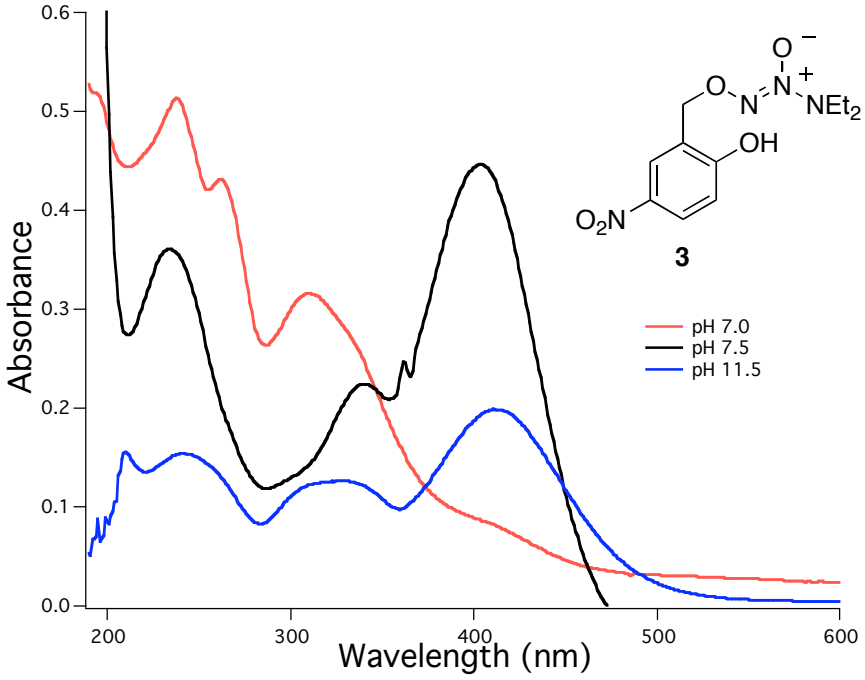
(2) Carato, P.; Laconde, G.; Ladjel, C.; Depreux, P.; Henichart, J.-P. *Tetrahedron Lett.* **2002**, *43*, 6533-6535.

(3) Wasserman, D.; Dawson, C. R. *J. Am. Chem. Soc.* **1950**, *72*, 4994-4997.

(4) Gates, M. *J. Org. Chem.* **1982**, *47*, 578-82.

(5) Boehmer, V.; Marschollek, F.; Zetta, L. *J. Org. Chem.* **1987**, *52*, 3200-3205.

Spectra.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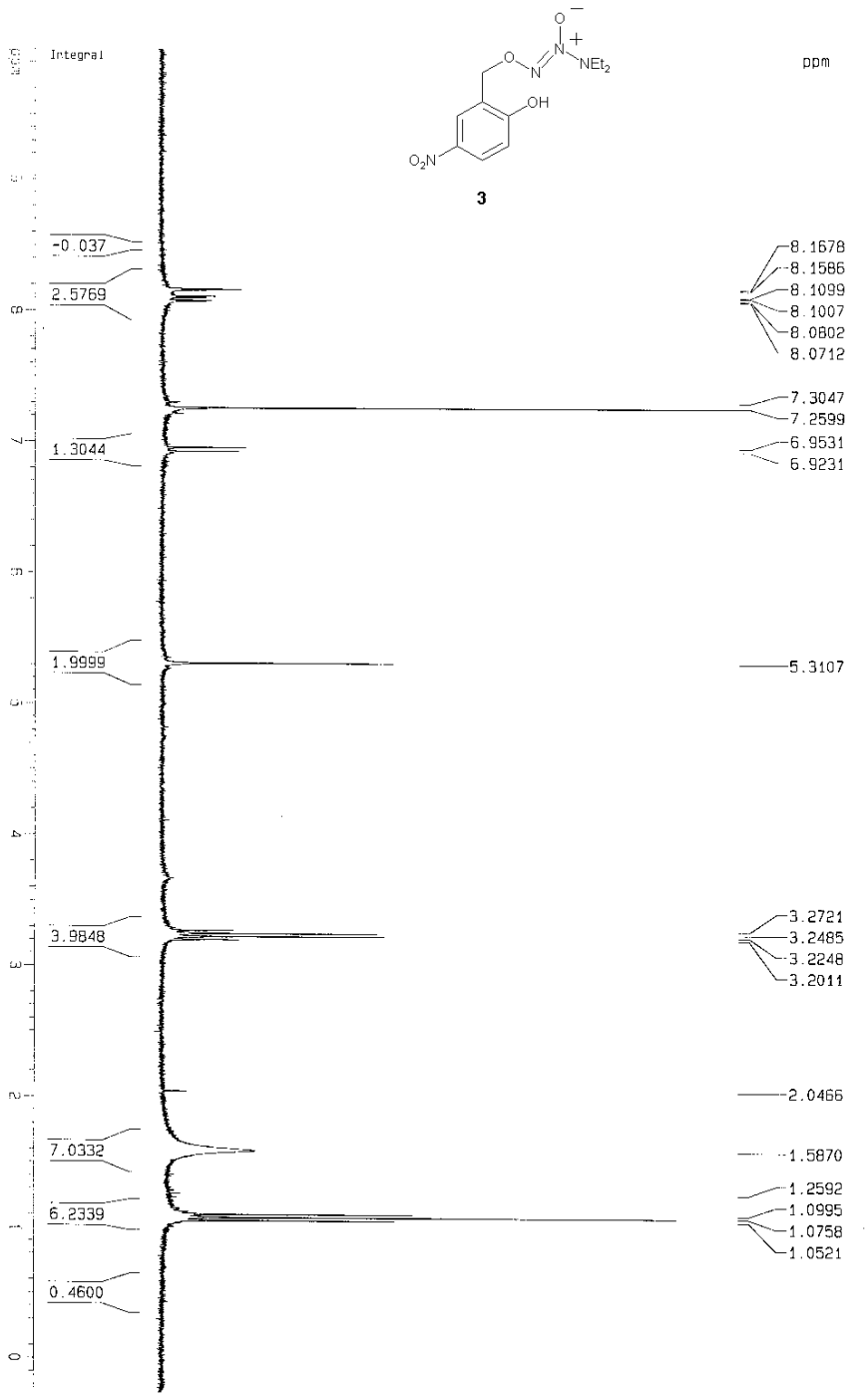
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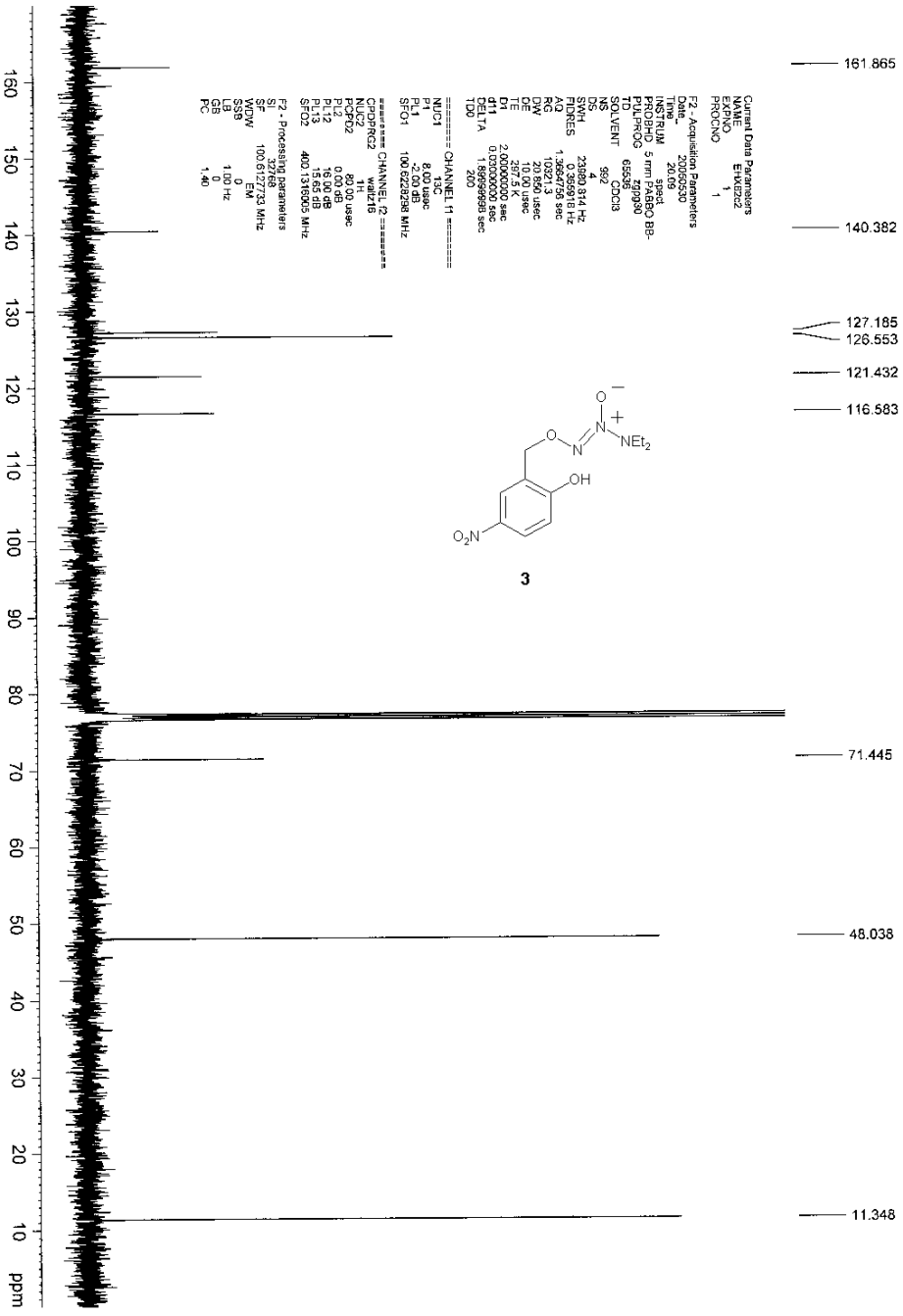
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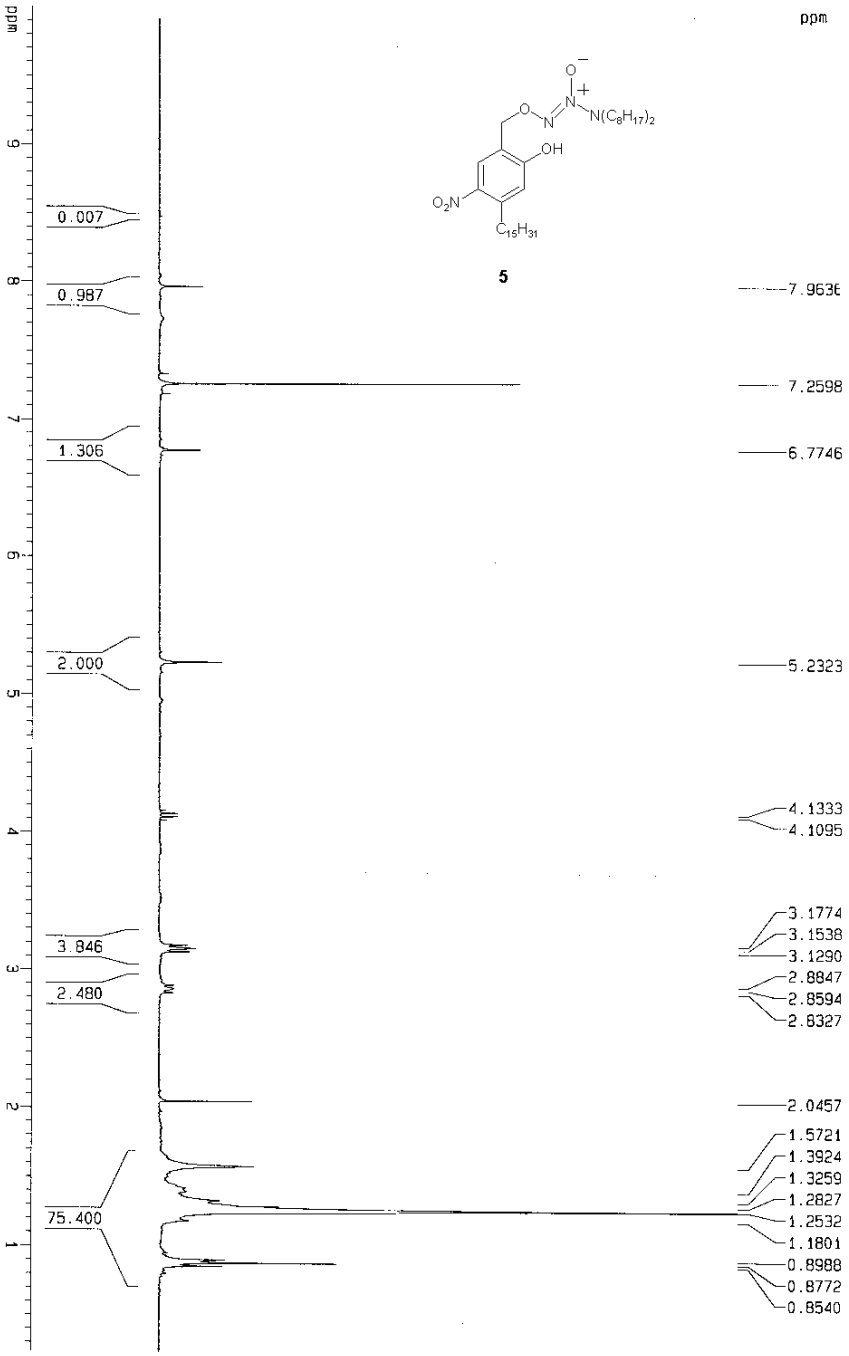
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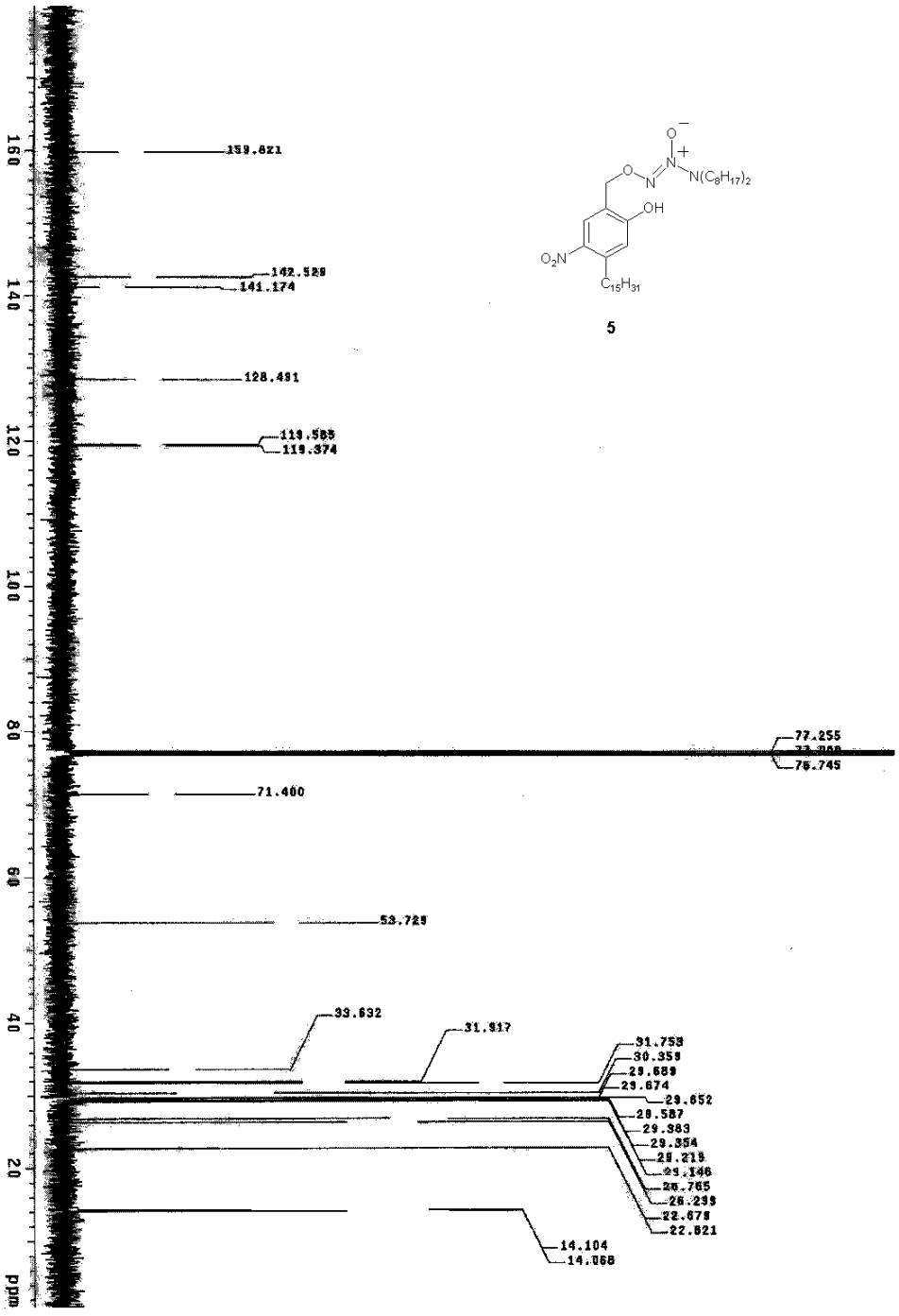
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