

Multitarget Antioxidant NO-Donor Organic Nitrates: A Novel Approach to Overcome Nitrates Tolerance, an Ex Vivo Study

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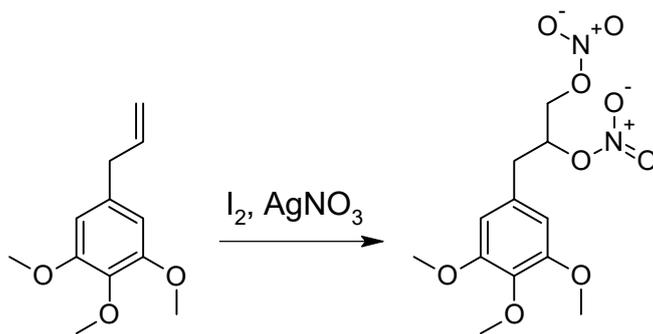
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Supporting Materials

Chemistry. ¹H and ¹³C NMR spectra were recorded on a BrukerAvance 300, at 300 and 75 MHz, respectively, using SiMe₄ as internal standard. The following abbreviations indicate peak multiplicity: *s* = singlet, *d* = doublet, *t* = triplet, *m* = multiplet. Low resolution mass spectra were recorded with a Finnigan-Mat TSQ-700. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh ASTM). The progress of the reactions was followed by thin-layer chromatography (TLC) on 5 × 20 cm plates Merck Kieselgel 60 F254, with a layer thickness of 0.20 mm. Anhydrous sodium sulfate (Na₂SO₄) was used as drying agent for the organic phases. Organic solvents were removed under reduced pressure at 30 °C. Synthetic purity solvents dichloromethane (DCM), acetonitrile (CH₃CN), ethyl acetate (EtOAc), diethyl ether (Et₂O), 40–60 petroleum ether (PE) were used. Dry tetrahydrofuran (THF) was distilled immediately before use from Na and benzophenone under positive N₂ pressure. Commercial starting materials were purchased from Sigma-Aldrich, Alfa Aesar, and TCI Europe.

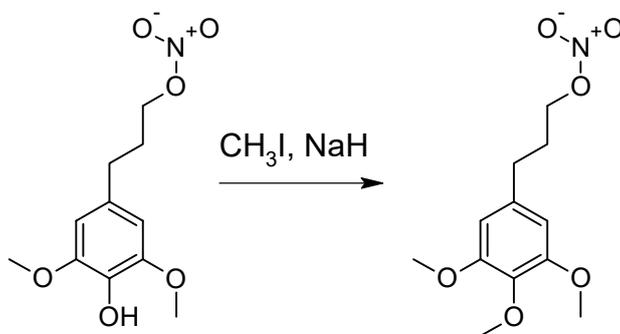
Synthesis of 3-(3,4,5-trimethoxyphenyl)propane-1,2-diyl dinitrate (7)



To a stirred solution of 5-allyl-1,2,3-trimethoxybenzene (1.80 g, 8.70 mmol) and AgNO₃ (4.50 g, 26.0 mmol) in CH₃CN (50 ml) iodine (2.20 g, 8.70 mmol) was added in one portion at rt. The reaction mixture was stirred at rt until all I₂ dissolved and then under reflux for 48 h. After cooling, bine (10 mL) was added to the mixture and precipitate was filtered off and washed with EtOAc (100 mL). Filtrate was extracted with EtOAc (2 × 50 ml). The combined organic layers were washed with water, then brine, dried and evaporated. The resulting yellow oil was partly purified by flash chromatography (PE/EtOAc, 80/20 v/v). Obtained yellow oil was further purified by preparative HPLC (Lichrospher 250-25 C₁₈, CH₃CN/H₂O, 5/5 v/v mobile phase, flow-rate 25 mL/min, λ 254 nm) to give the title product as a yellow oil.

Yield: 24%. ¹H-NMR (CDCl₃) δ: 2.93 (*dd*, 1H, ¹J_{HH} = 14.1 Hz, ³J_{HH} = 7.2 Hz, ArCHH), 3.05 (*dd*, 1H ¹J_{HH} = 14.1 Hz, ³J_{HH} = 6.9, ArCHH), 3.84 (*s*, 3H, CH₃O), 3.86 (*s*, 6H, 2CH₃O), 4.46 (*dd*, 1H, ¹J_{HH} = 12.9 Hz, ³J_{HH} = 6.3 Hz, CHHONO₂), 4.75 (*dd*, 1H ¹J_{HH} = 12.9 Hz, ³J_{HH} = 3.0, CHHONO₂), 5.41 – 5.49 (*m*, 1H, CHONO₂), 6.43 (*s*, 2H, Ar); ¹³C-NMR (CDCl₃) δ: 35.9, 56.2, 60.9, 70.2, 79.5, 106.1, 129.7, 137.5, 153.6. MS (EI) *m/z* 332 (M⁺).

Synthesis of 3-(3,4,5-trimethoxyphenyl)propanyl nitrate (5)



To a stirred solution of 3-(4-hydroxy-3,5-dimethoxyphenyl)propyl nitrate (330 mg, 1.28 mmol) in dry THF (10 mL) under positive N₂ pressure NaH 60% in mineral oil suspension (50 mg, 1.28 mmol)

was added, followed after 10 min by CH₃I (480 μL, 7.68 mmol). The reaction mixture was stirred at rt until completed (TLC control). Then it was diluted with Et₂O (20 mL) and organic layer was washed with water, then brine, dried and evaporated. The resulting yellow oil was purified by flash chromatography (PE/EtOAc, 90/10 v/v). to give the title product as a yellow oil. Yield: 55%.

¹H-NMR (CDCl₃) δ: 2.06 (*m*, 2H, CH₂CH₂CH₂), 2.70 (*t*, 2H, ³*J*_{HH} = 7.7, ArCH₂), 3.84 (*s*, 3H, CH₃O), 3.86 (*s*, 6H, 2CH₃O), 4.47 (*t*, 2H, ³*J*_{HH} = 6.3 Hz, CH₂ONO₂), 6.40 (*s*, 2H, Ar); ¹³C-NMR (CDCl₃) δ: 25.9, 29.2, 56.2, 60.9, 71.3, 106.1, 129.