

Synthesis of ^{13}C and ^{15}N labeled 2,4-dinitroanisole

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Syntheses of [$^{13}\text{C}_6$]-2,4-dinitroanisole (ring- $^{13}\text{C}_6$) from [$^{13}\text{C}_6$]-anisole (ring- $^{13}\text{C}_6$) and [$^{15}\text{N}_2$]-2,4-dinitroanisole from anisole using *in situ* generated acetyl nitrate and [^{15}N]-acetyl nitrate, respectively, are described. Treatment of [$^{13}\text{C}_6$]-anisole (ring- $^{13}\text{C}_6$) with acetyl nitrate generated in 100% HNO_3 gave [$^{13}\text{C}_6$]-2,4-dinitroanisole (ring- $^{13}\text{C}_6$) in 83% yield. Treatment of anisole with [^{15}N]-acetyl nitrate generated in 10 N [^{15}N]- HNO_3 gave [$^{15}\text{N}_2$]-2,4-dinitroanisole in 44% yield after two cycles of nitration. Byproducts in the latter reaction included [^{15}N]-2-nitroanisole and [^{15}N]-4-nitroanisole.

Keywords: [$^{13}\text{C}_6$]-2,4-dinitroanisole; [$^{15}\text{N}_2$]-2,4-dinitroanisole; [^{15}N]-acetyl nitrate; synthesis; insensitive munitions compounds

Introduction

2,4-Dinitroanisole (DNAN, **1**) is an insensitive munitions compound in development to replace conventional energetic compounds (e.g., TNT) because of its improved resistance to heat and shock.¹ Although the technical properties of DNAN are well characterized, its environmental fate is poorly understood. DNAN and other nitroaromatic compounds can undergo extensive transformation in soil due to both microbial and abiotic processes. For example, nitro groups may be reduced in anaerobic soils,² leading to the formation of reactive aminoarene compounds that can undergo further transformations to oligomeric compounds² and/or to humus-bound residues.³ Experiments with ^{13}C and ^{15}N stable isotope-labeled DNAN can help to reveal the products and (bio) transformation mechanisms of this energetic material through the use of ^{13}C -NMR and ^{15}N -NMR techniques⁴ and by liquid chromatography–tandem mass spectrometry (MS) analysis for assignment of degradation product chemical structures. Microorganisms able to utilize carbon and/or nitrogen atoms from ^{13}C - and ^{15}N -labeled DNAN can be identified by a combination of DNA-stable isotope probing and 16S-rRNA gene clone libraries⁵ created from enrichment cultures with universal bacterial primers. The degradation rate and ultimate environmental fate of DNAN and intermediates, transformed into safe end products such as mineralized products and bound residue in humus, can be revealed by incubation experiments designed to determine the biotic and abiotic processes interacting on ^{13}C - and ^{15}N -labeled DNAN. Thus, ^{13}C - and ^{15}N -labeled compounds **2** and **3** were needed in gram quantities, and so a chemical synthesis capable of providing these compounds was developed, executed, and is disclosed herein.

Given the commercial availability of [$^{13}\text{C}_6$]-anisole (ring- $^{13}\text{C}_6$), we limited our literature search to methods that provide DNAN starting from anisole. DNAN (**1**) has been prepared by mononitration of 2-nitroanisole or 4-nitroanisole,^{6–8} or by dinitration of anisole in a single step.^{9–12} The dinitration procedures were more appealing and included treatment of anisole with nitronium tetrafluoroborate,⁹ urea nitrate-sulfuric acid,¹⁰ urea nitrate-polyphosphoric acid,¹¹ and an acidic montmorillonite clay impregnated with anhydrous cupric nitrate (claycop).¹² For obvious reasons, we preferred the single-step route of anisole dinitration. Among the available dinitration procedures, it seemed that only the claycop procedure would be amenable to the synthesis of both ^{13}C -labeled **2** and ^{15}N -labeled **3** (via the use of ^{15}N -enriched claycop).

Experimental

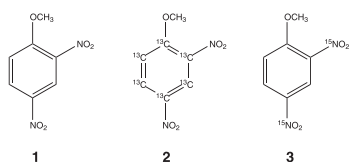
All reagents and solvents were commercially available and were used as received. Solutions were concentrated *in vacuo* using a rotary evaporator. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60 F-254 glass plates. TLC visualization required using UV light and/or staining. Anisaldehyde stain (100 mL anisaldehyde, 50 mL glacial AcOH, 100 mL conc. H_2SO_4 , 1 L 95% EtOH) and phosphomolybdic acid stain (5 g phosphomolybdic acid, 100 mL 95% EtOH) were the most commonly used TLC stains. Flash and gravity chromatography were performed using silica gel 60 (230–400 mesh). Melting points are uncorrected. NMR experiments were performed on a 500 MHz

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spectrometer. NMR spectra were referenced to TMS (0.00 ppm) or CDCl₃ (7.26 ppm, 77.0 ppm). Mass spectrometry was conducted using gas chromatography (GC)/MS (EI, Shimadzu QP 2010 Ultra instrument) on a DB-5 column with a flow rate of 1 mL/min. High resolution mass spectrometry was conducted on an AB Sciex TripleTOF 5600 mass spectrometer (AB SCIEX 500 Old Connecticut Path Framingham, MA 01701 USA) using positive mode electrospray ionization according to methods described in reference 2.

[¹³C₆]-2,4-Dinitroanisole (2)

Nitric acid (100%, 2.96 g, 47 mmol, 2 mL) was added dropwise to acetic anhydride (5 mL, 5.4 g, 53 mmol) at 0 °C with stirring. After 10 min, a solution of **4** (1.14 g, 10 mmol, Sigma-Aldrich) in CCl₄ (3 mL) was added dropwise and the mixture allowed to come to room temperature. After stirring overnight, the reaction mixture was diluted with CH₂Cl₂ (200 mL) and washed with water (4 × 150 mL), brine (100 mL), and dried (MgSO₄). Volatiles were removed under reduced pressure and the residual oil loaded onto a flash silica gel column (100 g). Elution with 5% EtOAc/hexanes gave **5** (0.24 g, 1.50 mmol, 15%) as a light yellow solid. Further elution with 25% EtOAc/hexanes gave **2** (1.70 g, 8.33 mmol) as an off-white crystalline solid in 83% yield. Recrystallization of **2** from 20% EtOAc/hexanes afforded colorless needles that melted at 86–87 °C. ¹H NMR (500 MHz, CDCl₃) δ 4.10 (3H, d, J=4 Hz), 7.23 (1H, merged dd, J=167 Hz), 8.44 (1H, merged dd, J=172 Hz), 8.72 (1H, apparent d, J=173 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 57.4 (s), 113.6 (t, J=62 Hz), 121.8 (t, J=70 Hz), 129.1 (merged t, J=63 Hz), 138.7 (t, J=74 Hz), 140.0 (t, J=69 Hz), 157.2 (merged dt, J=72 Hz); GC/MS (EI, M⁺) 204, t_r=12.4 min; HRMS (ESI⁺) calculated m/z for (M+H)⁺ 205.0551, observed 205.0586; calculated m/z for (M+Na)⁺ 227.0370, observed 227.0372.

[¹⁵N₂]-2,4-Dinitroanisole (3)

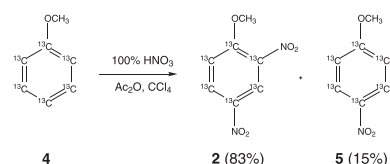
[¹⁵N]-Nitric acid (10 N, 4 mL, 40 mmol, Sigma-Aldrich) was added dropwise to acetic anhydride (21.64 g, 212 mmol, 20 mL) at 0 °C with stirring. After 10 min, a solution of anisole (**6**, 1.08 g, 10 mmol) in CCl₄ (3 mL) was added dropwise and the mixture allowed to come to room temperature. After stirring for 24 h, the reaction mixture was diluted with CH₂Cl₂ (200 mL), washed with water (4 × 150 mL), brine (10 mL), and dried (MgSO₄). Volatiles were removed under reduced pressure and the residue subjected to a second nitration as just described. After the second workup, the residual oil was loaded onto a flash silica gel column (150 g). Elution with 5% EtOAc/hexanes gave **8** (0.19 g, 1.2 mmol, 12%) as a colorless solid. Further elution with 10% EtOAc/hexanes gave **7** (0.56 g, 3.6 mmol, 36%) as a yellow oil. Finally, elution with 25% EtOAc/hexanes gave **3** (0.88 g, 4.4 mmol, 44%) as an off-white crystalline solid. Recrystallization of **3** from 20% EtOAc/hexanes produced colorless crystals that melted at 86–87 °C. ¹H NMR (500 MHz, CDCl₃) δ 4.10 (3H, s), 7.24 (1H, merged dd), 8.43–8.46 (1H, m), 8.72–8.75 (1H, m); ¹³C NMR (125 MHz, CDCl₃) δ 57.4 (s), 113.6 (s), 121.8 (s), 129.1 (s), 138.8 (d, J=16 Hz), 140.1 (d, J=17 Hz), 157.2 (s); GC/MS (EI, M⁺) 200, t_r=12.4 min; HRMS (ESI⁺) calculated m/z for (M+H)⁺ 201.0290, observed 201.0291; calculated m/z for (M+Na)⁺ 223.0110, observed 223.0116.

Results and discussion

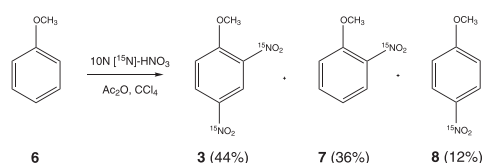
Prior to attempts at syntheses of **2** and **3**, we performed the claycop nitration of anisole according to the published procedure,¹² which involved addition of a solution of anisole (10 mmol) in CCl₄ (30 mL) to a mixture of claycop (4.8 g, Sigma-Aldrich) and acetic anhydride (15 mL, 16 mmol). As described, ~6 equivalents of nitric acid were required to complete the nitration process. Acetic anhydride and nitric acid mixtures generate acetyl nitrate¹³ *in situ*, and many examples of aromatic nitration with acetyl nitrate have been reported.^{14,15} In order to check whether claycop was required for dinitration of anisole,

we attempted its dinitration with *in situ* generated acetyl nitrate. Thus, a solution of anisole (10 mmol) in CCl₄ was added dropwise to an ice-cold mixture of acetic anhydride (53 mmol) and 100% nitric acid (47 mmol). After stirring overnight at room temperature, the reaction mixture was diluted with CH₂Cl₂ and washed several times with water. The organic layer was dried and concentrated under reduced pressure, and the resulting oil was subjected to flash silica gel column chromatography using 5% ethyl acetate/hexanes and 20% ethyl acetate/hexanes as elutants. DNAN (**1**) was obtained as a light yellow solid in 85% yield. ¹³C-labeled **2** was similarly prepared from [¹³C₆]-anisole (ring-¹³C₆, Sigma Aldrich) in 83% yield, along with [¹³C₆]-4-nitroanisole (**5**, 15% yield), as depicted in Scheme 1 and described in the Experimental section. While this work was in progress, Smith, *et al.* reported the synthesis of **1** from anisole by treatment with propanoyl nitrate generated *in situ* over zeolite Hβ catalyst in 97% yield.¹⁶

With ¹³C-labeled DNAN (**2**) in hand, we set about preparing ¹⁵N-labeled **3** from anisole. [¹⁵N]-labeled nitric acid is available commercially as a 10 N solution in water. Therefore, we investigated the synthesis of **1** from anisole using a 10 N solution of nitric acid in water. To account for the presence of water, excess acetic anhydride was used. Thus, a solution of anisole (10 mmol) in CCl₄ was added dropwise to an ice-cold mixture of acetic anhydride (212 mmol) and 10 N nitric acid (40 mmol). After 24 h of stirring at room temperature, TLC analysis of the reaction mixture indicated that 2-nitroanisole was the major product, with **1** and 4-nitroanisole as minor products. The reaction mixture was diluted with CH₂Cl₂ and washed several times with water. The organic layer was dried and concentrated under reduced pressure, and the residue was subjected to a second treatment with acetic anhydride (212 mmol) and 10 N nitric acid (40 mmol) as previously described. TLC analysis indicated the presence of much more **1** after the second exposure to acetyl nitrate. After workup as previously described, the resulting oil was subjected to flash silica gel column chromatography using 5% ethyl acetate/hexanes and 20% ethyl acetate/hexanes as elutants. DNAN (**1**) was obtained in 43% yield, along with 2-nitroanisole (**7**, 31% yield) and 4-nitroanisole (**8**, 12% yield). ¹⁵N-Labeled **3** was similarly prepared in 44% yield from anisole and [¹⁵N]-acetyl nitrate generated *in situ*,



Scheme 1. Synthesis of [¹³C₆]-2,4-dinitroanisole (**2**).



Scheme 2. Synthesis of [¹⁵N₂]-2,4-dinitroanisole (**3**).

along with [¹⁵N]-2-nitroanisole (**7**, 36%) and [¹⁵N]-4-nitroanisole (**8**, 12%) as depicted in Scheme 2 and described in the Experimental section.

Conclusion

We have described a synthesis of 2,4-dinitroanisole (**1**) in good yield from anisole and acetyl nitrate generated *in situ* without the need for solid catalysts. Using this method, gram quantities of [¹³C₆]-2,4-dinitroanisole (**2**) and [¹⁵N₂]-2,4-dinitroanisole (**3**) have been prepared for the first time.

Acknowledgement

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Conflict of Interest

The authors did not report any conflict of interest.

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