# PREPARATION OF CARBON-14 LABELED HAIR DYES: NITROPHENYLENEDIAMINE

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## SUMMARY

Nitration of N-ethoxycarbonyl-[U-$^{14}$C]aniline with two equivalents of nitric acid in sulfuric acid afforded N-ethoxycarbonyl-2,4-dinitro-[U-$^{14}$C]aniline which was deprotected quantitatively to 2,4-dinitro-[U-$^{14}$C]aniline. Selective hydrogenation provided 99.5% radiochemically pure [U-$^{14}$C]nitrophenylenediamine with specific activity 25.6 mCi/mmol in 20% radiochemical yield.

**Key Words:** Oxidation Base 22, nitrophenylenediamine, 2,4-dinitroaniline, carbon-14

## INTRODUCTION

The C.I. Oxidation Base 22, a member of the group classified as nitro dyes and used in hair colors, is 2-nitro-*p*-phenylenediamine (**1**). It has been recently classified as a carcinogen affecting a single subspecies (1). Since it is used in hair dyes, the FDA required a carbon-14 labeled sample for studies of skin penetration.

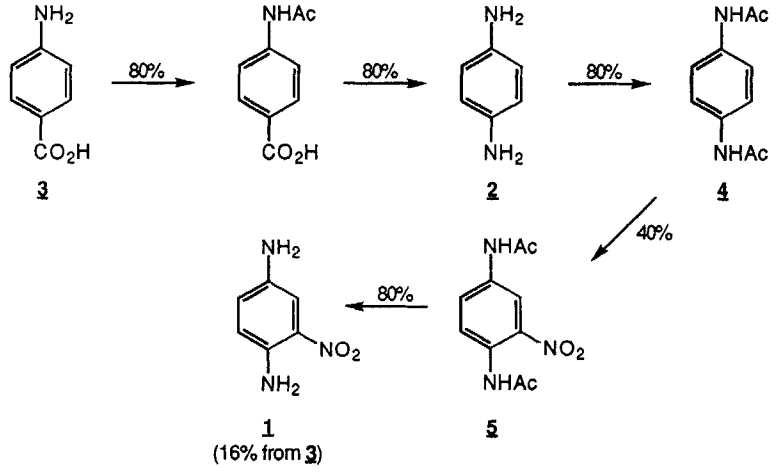
## RESULTS AND DISCUSSION

The usual preparation of 2-nitro-*p*-phenylenediamine (**1**) is by the nitration of derivatives of *p*-phenylenediamine (**2**). Since the carbon-14 label must be in the aromatic ring of **1**, the availability of a relatively inexpensive carbon-14 labeled benzene derivative as starting material is a key consideration. The required carbon-14 labeled *p*-phenylenediamine derivative [$^{14}$C]-**1** could be prepared from commercially available 4-aminobenzoic acid ([$^{14}$C]-**3**) by converting it to the diamino compound [$^{14}$C]-**2**, followed by acetylation (Scheme 1). Nitration of the acetylated diamine [$^{14}$C]-**4** to the 2-nitro-4-acetamidoacetanilide [$^{14}$C]-**5** (2) followed by hydrolysis would then afford the desired [$^{14}$C]-**1**. However, the low radiochemical yield (16%) of [$^{14}$C]-**1** expected for the five-step sequence would require an unacceptably large quantity of the starting 4-aminobenzoic acid, [$^{14}$C]-**3**.

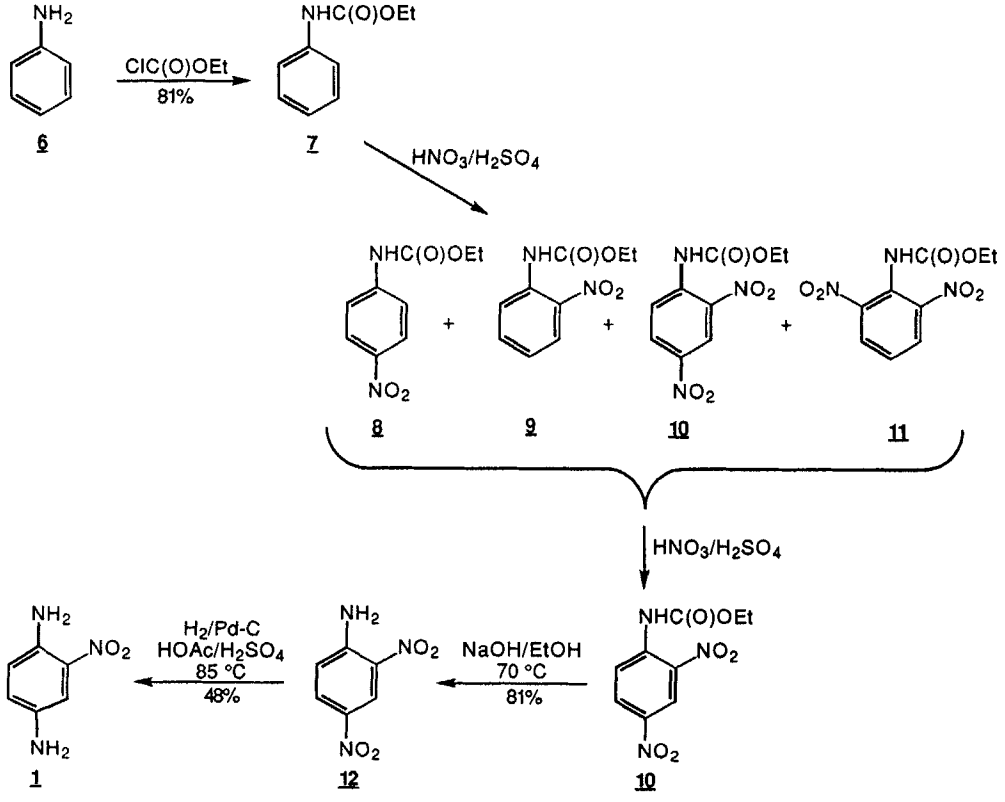
CCC 0362–4803/96/010923–06

Received 12 April 1996
Revised 13 May 1996

Scheme 1




Scheme 2



A relatively inexpensive carbon-14 labeled benzene derivative, which can be used as starting material in an alternative approach to the preparation of [$^{14}$C]-1, is aniline [$^{14}$C]-6. Thus, it has been reported that the nitration of the ethoxycarbonyl derivative of aniline (7) affords primarily N-ethoxycarbonyl-4-nitroaniline (8) and that further nitration gives N-ethoxycarbonyl-2,4-dinitroaniline (10) in 97% yield (3) (Scheme 2). Since selective reduction of the 4-nitro group of 2,4-dinitroaniline (12), in 48% yield, is known (4), and assuming typical experimental yields for the transformation of 6 to 7 and of 10 to 12, this approach, which would afford the product in 31% yield, represents a viable synthesis of 1.

Prior to undertaking the radiosynthesis, the reaction sequence was probed with unlabeled aniline (6). Treatment of the free base 6 with ethyl chloroformate (5) gave N-ethoxycarbonylaniline 7, which was purified by flash chromatography (40 μm $SiO_2$, 80% $CH_2Cl_2$-hexane), in 84% yield. Nitration of 7 with 90% nitric acid in sulfuric acid following the literature (3) yielded four products (TLC, $SiO_2$, 75% $CH_2Cl_2$-pet. ether) which were separated by flash chromatography (40 μm $SiO_2$, 75% pet. ether-$CH_2Cl_2$, 60%-pet. ether-$CH_2Cl_2$). Comparison with authentic samples and analysis by $^1$H NMR showed that the four products were the desired ethyl 2,4-dinitrophenylcarbanilate 10, the 2,6-dinitro analog 11 along with the 2- and 4-mononitro analogs 9 and 8. Treatment of a mixture of the four products with additional 90% nitric acid in sulfuric acid converted 8 and 9 to the desired product 11. Thus, nitration of 7 with two equivalents of 90% nitric acid in sulfuric acid gave 10 in >95% accompanied by a small amount of 11, which was readily removed by flash chromatography. Deprotection of 10 by base (6) afforded 2,4-dinitroaniline 12 in quantitative yield. Selective reduction was carried out by hydrogenation over 10% platinum on carbon in 50% sulfuric acid in acetic acid. Although the reaction failed to go to completion, the desired product 1 could be isolated by flash chromatography (40 μm $SiO_2$, $CH_2Cl_2$).

Starting from commercially available, carbon-14 labeled aniline ([$^{14}$C]-6) hydrochloride (25 mCi, 56 mCi/mmol), and following the above protocol a 76% (19.1 mCi) radiochemical yield of N-ethoxycarbonylaniline ([$^{14}$C]-7) was achieved. Because the specific activity of the final product [$^{14}$C]-1 only needed to be >15 mCi/mmol this product was diluted with authentic, unlabeled 7 (0.39 mmol) and nitrated with two equivalents of 90% nitric acid in sulfuric acid. Flash chromatography afforded pure [$^{14}$C]-10 in 82% yield (15.3 mCi) and deprotection of [$^{14}$C]-10 by saponification gave [$^{14}$C]-12 in quantitative yield. Hydrogenation of the carbon-14 labeled dinitroaniline [$^{14}$C]-12 over platinum in a mixture of sulfuric and acetic acid at 70 °C (4) proceeded very slowly with only 11% of the desired product [$^{14}$C]-1 being apparent after two hours; 84% of the dinitroaniline [$^{14}$C]-12 was also

detected. Additional catalyst only increased the percentage of the product, [$^{14}$C]-1 to 26.5 after a five hour reaction time. The reaction was terminated and the product [$^{14}$C]-1 (3.45 mCi) was separated from the starting [$^{14}$C]-12 (6.91 mCi) by flash chromatography. Although radio-TLC ($SiO_2$, $CH_2Cl_2$) showed radiochemical purity of [$^{14}$C]-1 to be >95%, radio-HPLC (7) showed a substantial impurity (38%). The final product was, therefore, purified by preparative HPLC; it was obtained in 99.5% radiochemical purity and in 25% yield (2.11 mCi).

## CONCLUSIONS

A good yield of carbon-14 labeled 2,4-dinitroaniline (>60%) is obtained from carbon-14 labeled aniline by nitration of the N-ethoxycarbonyl protected derivative followed by deprotection. Selective reduction of the 4-nitro group affords the carbon-14 labeled hair dye nitrophenylenediamine.

## EXPERIMENTAL

NMR spectra were recorded on a Bruker WM-250 or AM-500 spectrometer using tetramethylsilane as internal standard. TLC-radioscan analysis was performed using E. Merck silica gel 60F-254 plates on a Berthold model LB Linear Analyzer. HPLC analyses were carried out on Waters 6000A dual pump system with a Waters U6K injector and a IN/US System, Inc., β-RAM Flow-Through Monitor. Prep-HPLC was carried out on a Thermo Separation Products spectra-SERIES P100 isocratic pump with a Waters U6K injector. Samples were counted using Ultima Gold as scintillant on a Packard Tri-carb 4000 liquid scintillation spectrometer.

**[U-$^{14}$C]-N-Ethoxycarbonylaniline, [U-$^{14}$C]-7.** A chilled solution of ethyl chloroformate (0.45 mmol, 43 μL) in acetone (0.2 mL) was added portionwise over 20 min via gas tight syringe to a stirred ice-cooled solution of [U-$^{14}$C]aniline • HCl, [U-$^{14}$C]-6 • HCl (57.9 mg, 0.45 mmol, 25 mCi, 56 mCi/mmol) in pyridine (0.5 mL). After 1 h of stirring at 0 °C under $N_2$, the mixture was poured over ice containing 2N HCl (2 mL) and extracted with ether (15 mL). The ether extract was washed with $H_2O$ (3 x 20 mL), dried ($Na_2SO_4$), filtered, then concentrated to a yellow oil. Radio-TLC ($SiO_2$:$CH_2Cl_2$) showed [U-$^{14}$C]-7 (19.1 mCi, 76% yield) to be 97% radiochemically pure. The product was diluted with 63.7 mg (0.39 mmol) authentic unlabeled 7 (recrystallized from pet. ether) to give a total weight of 119.7 mg with specific activity 26 mCi/mmol. $^1$H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.3 (t, 3H), 4.2 (q, 2H), 7.0 (m, 1H), 7.3 (m, 4H).

**[U-$^{14}$C]-2,4-Dinitroethylcarbanilate, [U-$^{14}$C]-10.** To a chilled suspended mixture of [$^{14}$C]-7 (119.7 mg, 0.72 mmol, 19.1 mCi) in conc. $H_2SO_4$ (1.1 mL) was added portionwise a chilled solution

of 90% $HNO_3$ (64.3 μL, 2 eq.) in conc. $H_2SO_4$ (0.5 mL). The resulting mixture stirred at 0 °C under $N_2$, and the reaction progress was followed by radio-TLC ($SiO_2$; 75% $CH_2Cl_2$-pet. ether). At 1 h, 36% of [U-$^{14}$C]-7 (radio-TLC, area ratio) remained, so additional 90% $HNO_3$ (10 μL) in $H_2SO_4$ (80 μL, chilled) was added. The reaction was complete at 2 h. The mixture was taken up in ether (20 mL), washed with $H_2O$ (4 x 20 mL), and dried over $Na_2SO_4$. The crude [U-$^{14}$C]-10 (17.4 mCi, 89% radiochemical purity) was purified by flash chromatography (Baker 40 μm flash $SiO_2$, 75% pet. ether-$CH_2Cl_2$, 60% pet. ether-$CH_2Cl_2$, 50% pet. ether-$CH_2Cl_2$). A radiochemical purity of 97.5% was achieved yielding 15.3 mCi (82%). $^1$H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.38 (t, 3H), 4.3 (q, 2H), 8.4 (dd, 1H), 8.9 (d, 1H), 9.1 (d, 1H).

**[U-$^{14}$C]-2,4-Dinitroaniline, [U-$^{14}$C]-12.** Sodium hydroxide (0.5N, 1.16 mL) was added to a solution of [U-$^{14}$C]-10 (15.3 mCi, ~0.58 mmol) in EtOH (12.8 mL), and the mixture was heated to 70 °C. At 1 h, radio-TLC ($SiO_2$; $CH_2Cl_2$) showed [U-$^{14}$C]-10 still present, so additional 0.5N NaOH (200 μL) was added. Upon completion, the reaction mixture was taken up in $H_2O$, extracted with EtOAc (3 x 20 mL), and dried over $Na_2SO_4$. A quantitative yield of [U-$^{14}$C]-12 was achieved. $^1$H NMR (250 MHz, $CD_3OD$) δ (ppm): 7.0 (d, 1H), 8.1 (dd, 1H), 8.9 (d, 1H).

**[U-$^{14}$C]-2-Nitro-1,4-phenylenediamine, [U-$^{14}$C]-1.** A suspension of [U-$^{14}$C]-12 (~15.3 mCi, 0.64 mmol) in 50% $H_2SO_4$ (0.24 mL), HOAc (1.42 mL), and 10% platinum on carbon (16 mg) was exposed to hydrogen at atmospheric pressure. At 2 h, it appeared that hydrogen uptake had ceased; this was confirmed by radio-TLC ($SiO_2$, $CH_2Cl_2$, 11% [U-$^{14}$C]-1, 84% [U-$^{14}$C]-12, area ratio). Additional 10% Pt/C (10 mg) increased the percentage to 26.5% [U-$^{14}$C]-5. The reaction was stopped and filtered through a cotton plug. The filtrate was basified ($NH_4OH$), extracted with $CHCl_3$ (3 x 15 mL), then dried over $Na_2SO_4$. Isolation of [U-$^{14}$C]-1 was carried out by flash chromatography (Baker 40 μm flash $SiO_2$, $CH_2Cl_2$), followed by purification by prep-HPLC using a Waters μ Bondapak C18, 10 μ, 10 x 25 RCM column with a 18 μ Bondapak guard column, eluting with 15% $CH_3CN$-85% (0.005M diaminooctane, 0.02M sodium heptanesulfonate) aq, pH adj to 4.5 with 85% $H_3PO_4$ at 9.0 mL/min, and detecting at 275 nm. A total of 2.11 mCi (12.6 mg, 25%) of the desired product [U-$^{14}$C]-1 with radiochemical purity at 99.5% and with specific activity 25.6 mCi/mmol (167.0 μCi/mg) was isolated. $^1$H NMR (250 MHz, $CDCl_3$): δ (ppm) 6.7 (d, 1H), 6.8 (dd, 1H), 7.4 (d, 1H).

## ACKNOWLEDGMENT

This work was supported in part by Contract No. 223-94-2275 from the Food and Drug Administration, which is gratefully acknowledged. The content of this publication does not necessarily

reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U. S. government.

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