SYNTHESIS OF [3,3-D_2]4-HYDROXY-1-(3-PYRIDYL)-1-BUTANONE, AN INTERNAL STANDARD FOR ANALYSIS OF TOBACCO-SPECIFIC NITROSAMINE HEMOGLOBIN AND DNA ADDUCTS

Jyh-Ming Lin[1], Shantu Amin[1], Sharon E. Murphy[1], Jerome J. Solomon[2], and Stephen S. Hecht[1]

SUMMARY

The synthesis of [3,3-D_2]4-hydroxy-1-(3-pyridyl)-1-butanone ([3,3-D_2]HPB), an internal standard for the analysis of hemoglobin and DNA adducts of tobacco-specific nitrosamines, is reported. 3-Pyridine carboxaldehyde was converted to 2-(3-pyridyl)-1,3-dithiane. This was condensed with 2-(2-bromoethyl)-1,3-dioxalane to yield 2-[2-(3-pyridyl)-1,3-dithiane]ethyl-1,3-dioxalane. Selective hydroylsis of the aldehyde protecting group produced 4-(1,3-dithian-2-yl)-4-(3-pyridyl)-butanal, which was then labeled with deuterium adjacent to the aldehyde carbonyl group. Reduction with $LiAlH_4$ produced the corresponding alcohol. Removal of the dithianyl protecting group required protection of the alcohol as its t-butyldimethylsilyl ether. Treatment with $AgNO_3$/N-chlorosuccinimide in aqueous CH_3CN produced [3,3-D_2]HPB.

Key Words: Deuterium labeled 4-hydroxy-1-(3-pyridyl)-1-butanone; [3,3-D_2]HPB; NNK

INTRODUCTION

The tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN) are believed to play an important role in causing cancers of the lung, oral cavity, esophagus, and pancreas in people who use tobacco products (1,2). NNK and NNN, upon metabolic activation by α-hydroxylation, yield adducts with DNA and globin via intermediates **1-3**. These adducts yield HPB upon hydrolysis (3,4). The released HPB can be derivatized as its pentafluorobenzoate and detected by gas chromatography negative ion chemical ionization

[1]American Health Foundation, Valhalla, New York 10595
[2]Institute of Environmental Medicine, New York University Medical Center, New York, NY 10016

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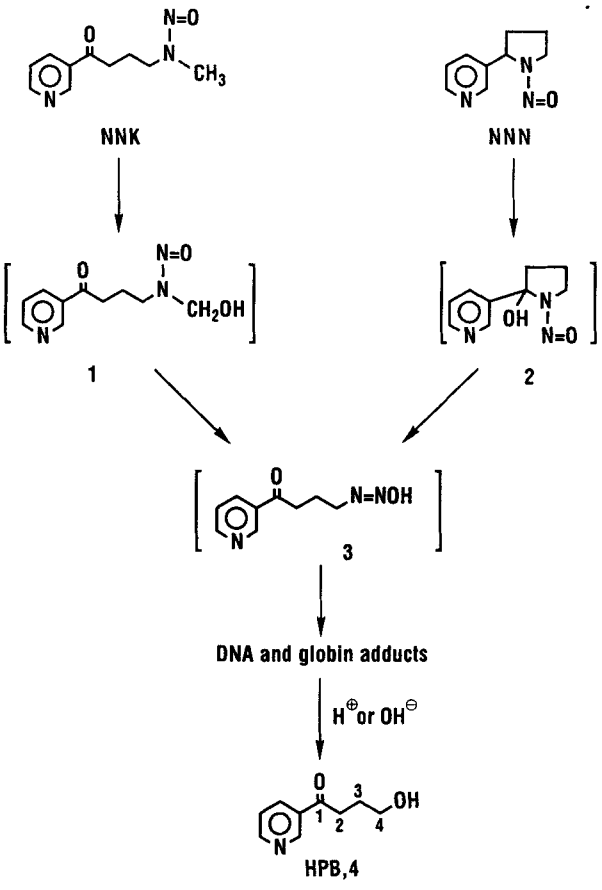
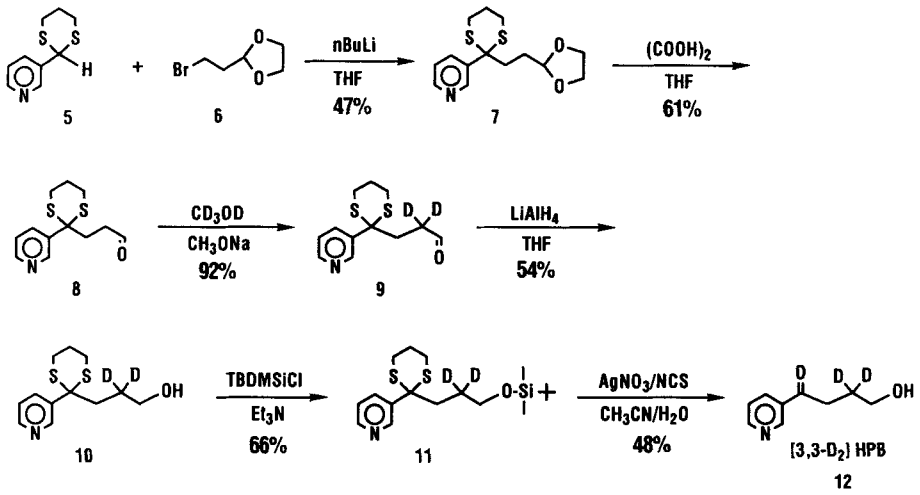
mass spectrometry with selected ion monitoring (GC-NICI-MS-SIM) (5-7).



Quantitation of released HPB is achieved with a deuterated internal standard. In published work, [4,4-D_2]HPB, prepared by *in vitro* metabolism of [4,4-D_2]NNK, has been used as the internal standard (5-7). However, [4,4-D_2]NNK is not widely available and is difficult to synthesize. Its metabolism produces only a low yield of [4,4-D_2]HPB. Therefore, we have developed a synthesis of [3,3-D_2]HPB (12).

RESULTS AND DISCUSSION

The synthesis is summarized in the Scheme. The strategy is similar to that which we have used for synthesis of secondary keto alcohols related to HPB; these compounds all had methyl groups at carbon 4 (8). Condensation of **5** with **6** produced the doubly protected precursor **7** in good yield. Selective deprotection proceeded smoothly to give aldehyde **8**. Deuterium exchange and reduction with LAH were carried out in good yield producing **10**. However, attempted deprotection of **10** with $AgNO_3$ and N-chlorosuccinimide, a



method we have used for analogous secondary alcohols (8), did not produce **12** but rather gave a complex mixture of products. Attempted deprotection of **10** with $AgNO_3$/EtOH (9), $CuCl_2$/CuO (10), and $HgCl_2/CH_3OH/H_2O$ (11) was also unsuccessful. Therefore, the alcohol group was protected as the t-butyldimethylsilyl ether. Removal of the dithiane group of **11**, followed by hydrolysis proceeded smoothly to give the desired product, [3,3-D_2]HPB (**12**).

The structure of **12** was confirmed as 100% D_2 by NMR. The NICI-MS of the pentafluorobenzoate derivative of **12** was virtually identical to the corresponding derivative of [4,4-D_2]HPB. [3,3-D_2]HPB and [4,4-D_2]HPB were compared as internal standards for the analysis of HPB. The level of HPB released from hemoglobin of NNK-treated rats, determined with respect to either standard, was the same, as were the percent recoveries.

EXPERIMENTAL SECTION

NMR spectra were determined in $CDCl_3$ on a Bruker AM 360 WB spectrometer. Chemical shifts are expressed in ppm downfield from

tetramethylsilane. MS were determined with a Hewlett-Packard Model 5988A instrument. High resolution MS were determined on a VG-70SE instrument at the Institute of Enviromental Medicine, New York University. TLC was performed on aluminum supported pre-coated silica gel plates from EM Separations (Gibbstown, NJ). Starting materials were obtained from Aldrich Chemical Co., Milwaukee, WI.

2-(3-Pyridyl)-1,3-dithiane (**5**)

3-Pyridine carboxaldehyde (4.84g, 45.2 mmol) in anhydrous THF (25 mL) was mixed with 1,3-propanedithiol (6.47g, 58.7 mmol) and $BF_3.Et_2O$ (1M, 15 mL) under an N_2 atmosphere. The solution was heated to reflux for 0.5 h, then stirred at room temperature overnight. The mixture was poured into 100 mL of H_2O, and the aqueous layer was adjusted to pH 4.0 with 1N HCl. It was extracted with EtOAc (100 mL × 4), the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield the crude product. This was purified by chromatography on silica gel with elution by hexane/EtOAc:2/1 to give **5** (5.2 g, 58%): mp 69-70°C; R_f= 0.1 (hexane/EtOAc:2/1); ^1H NMR($CDCl_3$) δ 1.88-2.00(m, 1H), 2.16-2.24(m, 1H), 2.89-3.12(m, 4H), 5.18(s, 1H), 7.29(dd, 1H, J=7.9 Hz, 4.8 Hz), 7.84(dt, 1H, J=7.9 Hz, 1.8 Hz), 8.55(dd, 1H, J=4.85 Hz, 1.57 Hz), 8.69 (d, 1H, J=2.05 Hz); ^{13}C NMR($CDCl_3$) δ 24.83, 31.83, 48.36, 123.56, 135.02, 135.37, 149.10, 149.65; MS m/z (rel intensity) 197(M^+,100), 154(10), 124(74), 79(12); HRMS exact mass calc'd for $C_9H_{11}NS_2$ 197.0333, found 197.0336.

2-[2-(3-Pyridyl)-1,3-dithiane]ethyl-1,3-dioxolane (**7**)

A solution of 2-(3-pyridyl)-1,3-dithiane **5** (5.0 g, 25.3 mmol) in anhydrous THF (120 mL) was treated under N_2 at -78°C with n-butyllithium (1.6M, 19 mL, 30.4 mmol). After stirring for 1 h at -78°C, a solution of 2-(2-bromoethyl)-1,3-dioxolane **6** (5.5g, 30.4 mmol) in dry THF (20 mL) was added. This reaction mixture was stirred for 2h and allowed to warm up to room temperature

overnight. The mixture was poured into 200 mL of H_2O and extracted with EtOAc (150 mL × 4). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield the crude product, which was purified by chromatography on silica gel with elution by hexane/EtOAc:1/1 to give **7** (3.57 g, 47%): mp 84-85°C; R_f= 0.1 (hexane/EtOAc:2/1); ^1H NMR($CDCl_3$) δ 1.63-1.74(m, 2H), 1.90-1.99(m, 2H), 2.13-2.18(m, 2H), 2.60-2.74(m, 4H), 3.76-3.91(m, 4H), 4.78(t, 1H, J=4.38 Hz), 7.31(dd, 1H, J=8.1 Hz, 4.8 Hz), 8.20(dq, 1H, J=8.11 Hz, 1.63 Hz), 8.51(dd, 1H, J=4.75 Hz, 1.55 Hz), 9.13(d, 1H, J=2.34 Hz); ^{13}C NMR($CDCl_3$) δ 24.88, 27.47, 28.30, 38.61, 56.14, 64.90, 103.64, 123.16, 123.64, 136.66, 137.48, 148.26, 150.63; MS m/z (rel intensity) 297(M^+, 18), 223(5), 196(34), 122(18), 104(10), 86(100); HRMS exact mass calc'd for $C_{14}H_{19}NO_2S_2$ 297.0857, found 297.0863.

4-(1,3-Dithiane-2-yl)-4-(3-pyridyl)butanal (**8**)

Aryl acetal **7** (3.57 g, 12.0 mmol) was dissolved in THF (5 mL) and mixed with aqueous oxalic acid solution (0.4M, 1.0 L). The mixture was stirred at room temperature for 72 h and extracted with ether (200 mL). The pH of the aqueous layer was adjusted to 4 with 10N NaOH and it was extracted with EtOAc (150 mL × 6). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield a yellowish residue. The residue was purified by chromatography on silica gel with elution by hexane/EtOAc:1/1 to give **8** (1.88 g, 61%): R_f= 0.45 (EtOAc); ^1H NMR($CDCl_3$) δ 1.92-2.03(m, 2H), 2.34-2.38(m, 2H), 2.52-2.56(m, 2H), 2.60-2.77(m, 4H), 7.32(dd, 1H, J=8.12 Hz,4.72 Hz), 8.17-8.20(m, 1H), 8.53(dd, 1H, J=4.68 Hz, 1.34 Hz), 9.11(d, 1H, J=2.0 Hz), 9.66(s, 1H); ^{13}C NMR($CDCl_3$) δ 24.56, 27.43, 36.51, 38.88, 55.73, 123.35, 136.50, 137.23, 148.61, 150.34, 200.12; MS m/z (rel intensity) 253(M^+, 16), 196(62), 180(64), 122(100), 104(38), 78(22), 45(24); HRMS exact mass calc'd for $C_{12}H_{15}NOS_2$ (MH^+) 254.0673, found 254.0672.

[2,2-D_2]4-(1,3-dithian-2-yl)-4-(3-pyridyl)butanal (**9**)

Compound **8** (0.81 g, 3.19 mmol) was dissolved in CD_3OD (8.0 mL) and mixed with sodium methoxide (0.70 g, 13.0 mmol) at 0°C under an N_2 atmosphere. The mixture was stirred for 1.5 h at 0°C, then D_2O (8.0 mL) was added. This mixture was extracted with EtOAc (30 mL × 4), and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield a yellowish residue. The residue was purified by chromatography on silica gel with elution by EtOAc/hexane :2/1 to give **9** (0.75 g, 92%): R_f = 0.45 (EtOAc); ^1H NMR($CDCl_3$) δ 1.90-2.02(m, 2H), 2.36(s, 2H), 2.60-2.80(m, 4H), 7.33(dd, 1H, J=8.07 Hz, 4.69 Hz), 8.20(m, 1H), 8.54(dd, 1H, J=4.69 Hz, 1.54 Hz), 9.12(d, 1H, J=2.48 Hz), 9.67(s, 1H); ^{13}C NMR($CDCl_3$) δ 24.57, 27.44, 36.42, 55.72, 123.35, 136.49, 137.23, 148.64, 150.37, 200.30; MS m/z (rel intensity) 255(M^+, 20), 196(62), 182(40), 122(100), 104(36), 78(24), 45(22); HRMS exact mass calc'd for $C_{12}H_{13}NOS_2D_2$ (MH^+) 256.0799, found 256.0758.

[2,2-D_2]4-(1,3-dithian-2-yl)-4-(3-pyridyl)butan-1-ol (**10**)

Butanal **9** (0.62g, 2.43 mmol) in anhydrous THF (5.0 mL) was added dropwise to an anhydrous THF solution containing LAH (0.21 g, 5.53 mmol) at 0°C under an N_2 atmosphere. The reaction mixture was stirred for 1 h at 0°C, then H_2O (0.21 mL), 15% NaOH (0.21 mL), and H_2O (0.63 mL) were added in sequence. The precipitate was removed by filtration, and the filter cake was washed with ether (50 mL). The organic layer was concentrated *in vacuo* to yield a yellowish residue. The residue was purified by chromatography on silica gel with elution by EtOAc to give **10** (0.34 g, 54%): R_f= 0.24 (EtOAc); ^1H NMR($CDCl_3$) δ 1.87-2.02(m, 2H), 2.08(s, 2H), 2.60-2.74(m, 4H), 3.53(s, 2H), 7.31(dd, 1H, J=8.07 Hz, 4.71 Hz), 8.19-8.24(m, 1H), 8.50(dd, 1H, J=4.82 Hz, J=1.6 Hz), 9.20(d, 1H, J=1.7Hz); ^{13}C NMR($CDCl_3$) δ 24.93, 27.49, 41.35, 55.44, 62.28, 123.18, 136.75, 137.63, 148.19, 150.66; MS m/z (rel intensity) 257(M^+, 10), 196(40), 184(8), 150(100), 137(100), 104(38), 78(30), 45(32).

t-Butyldimethylsilyl[[2,2-D_2]4-(1,3-dithian-2-yl)-4(3-pyridyl)butyl] ether (**11**)

The alcohol **10** (0.40 g, 1.55 mmol) in anhydrous THF (30 mL) was mixed with t-butyldimethylsilyl chloride (0.97 g, 6.45 mmol), 4-dimethylaminopyridine (10 mg) and triethylamine (1.12 g, 10.2 mmol). The reaction mixture was stirred at room temperature overnight. The mixture was poured into saturated aqueous $NaHCO_3$ solution (50 mL), and extracted with ether (30 mL × 3). The combined organic layers were washed with brine (30 mL × 2), dried (Na_2SO_4), and concentrated *in vacuo* to yield a residue, which was purified by chromatography on silica gel with elution by EtOAc to give **11** (0.38 g, 66%): R_f= 0.69 (EtOAc); ^1H NMR($CDCl_3$) δ -0.03(s, 6H), 0.83(s, 9H), 1.94-2.04(m, 2H), 2.06(s, 2H), 2.69-2.75(m, 4H), 3.48(s, 2H), 7.31(dd, 1H, J=8.07 Hz, 4.71 Hz), 8.19-8.24(m, 1H), 8.50(dd, 1H, J=4.70 Hz, 1.46Hz), 9.13(d, 1H, J=2.31 Hz); ^{13}C NMR($CDCl_3$)δ -5.37, 18.24, 24.97, 25.89, 27.49, 41.32, 56.49, 62.41, 123.10, 136.71, 137.70, 148.12, 150.70; MS m/z (rel intensity) 371(M^+, 2), 356(5), 314(100), 240(25), 196(22), 150(72), 75(32).

[3,3-D_2]4-hydroxy-1-(3-pyridyl)-1-butanone ([3,3-D_2]HPB, **12**)

Silyl ether **11** (0.38 g, 1.02 mmol) in CH_3CN (3.0 mL) was added in one portion at 0°C, under an N_2 atmosphere, to a solution of 80% CH_3CN containing N-chlorosuccinimide (0.39 g, 2.92 mmol) and $AgNO_3$ (0.68 g, 4.0 mmol). The reaction mixture was allowed to stir at 0°C for 1 h, then 2 mL of saturated aqueous Na_2SO_3, saturated aqueous $NaHCO_3$, and brine were added in sequence. The mixture was diluted with 50 mL of CH_2Cl_2/hexane:1/1 and the precipitate was removed by filtration. The filter cake was washed with 50 mL of CH_2Cl_2/hexane :1/1. The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to give a yellowish residue. The residue was purified by chromatography on silica gel with elution by EtOAc to give **12** (0.082 g, 48%): R_f= 0.48 (EtOAc/MeOH:10/1); ^1H NMR($CDCl_3$) δ 3.14(s, 2H), 3.76(s, 2H), 7.42(dd, 1H, J=7.93 Hz, 4.72

Hz), 8.24-8.27(m, 1H), 8.78(dd, 1H, J=4.82 Hz, 1.60 Hz), 9.19(d, 1H, J=1.70 Hz); ^{13}C NMR ($CDCl_3$) δ 35.39, 61.86, 123.68, 132.15, 135.40, 149.60, 153.45, 199.19; MS m/z (rel intensity) 167 (M^+, 4), 166 (1), 134 (4), 121 (75), 106 (100), 78 (100), 51 (90); HRMS calc'd for $C_9H_{10}NO_2D_2$ (MH^+) 168.0994, found 168.0992.

[3,3-D_2]HPB was converted to the corresponding pentafluorobenzoate, as described (5). Its NICI-MS, obtained with a methane pressure of 0.8 torr, ionizing energy of 120 eV, and source temperature of 150°C, had m/z (rel intensity) 362[$(M+1)^-$, 18], 361 (M^-, 100), 211 (11), 198 (10), 167 (30).

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