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An Improved Synthesis of (±)-*N*′-Nitrosonornicotine 5′-Acetate

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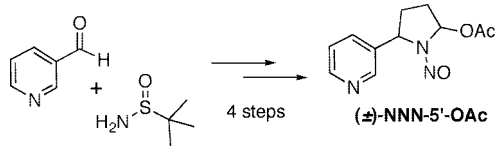
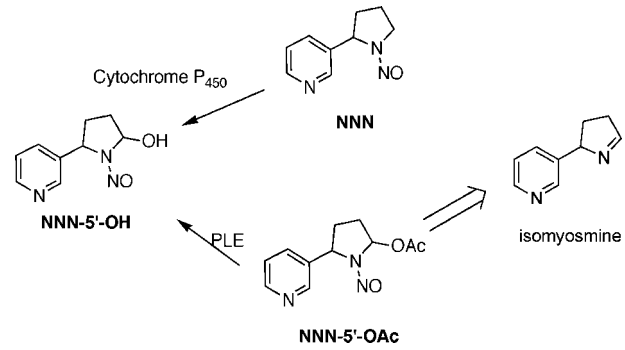


A concise and efficient route to (±)-*N*′-nitrosonornicotine 5′-acetate (NNN-5′-OAc), a stable precursor of the active metabolite 5′-hydroxy(±)-*N*′-nitrosonornicotine NNN-5′-OH is reported. The synthesis utilizes sulfinimine chemistry to give (±)-NNN-5′-OAc in 26% yield over 4 steps.

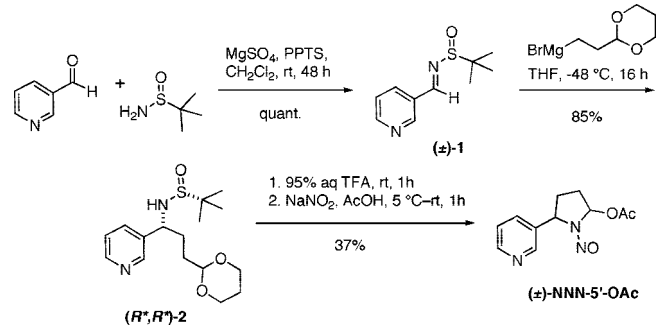
N′-Nitrosonornicotine (NNN) is a tobacco-specific *N*-nitrosamine that is known to cause esophageal cancer in animals.[1] Metabolic activation of NNN occurs by P_{450} α-oxidation to form NNN-5′-OH (Scheme 1) and other metabolites.[1,2] Unlike the other metabolites of NNN, there have been very few studies of the interaction of metabolically activated NNN-5′-OH with biological macromolecules,[3,4] which can at least partly be attributed to a lack of ready synthetic access to a suitable stable precursor of NNN-5′-OH. Although previous work by Hecht and co-workers has demonstrated that *N*′-nitrosonornicotine 5′-acetate (NNN-5′-OAc) can be deprotected by using pig liver esterase under physiological conditions to afford the NNN-5′-OH metabolite[3] (Scheme 1), no efficient synthesis of NNN-5′-OAc has been published. The only previously reported synthesis affords an NNN-5′-OAc in less than 1% overall yield.[3] Here we report a much improved synthesis of NNN-5′-OAc that employs sulfinimide chemistry.

We reasoned that NNN-5′-OAc could be prepared through nitrosation of isomyosmine in the presence of acetic acid (Scheme 1).[5] Few previous routes to isomyosmine have been reported.[6] Nakane and Hutchinson obtained isomyosmine through the reduction and subsequent, relatively inefficient deprotection/cyclization of a 4-imino-4-(pyridin-3-yl)butanal thioacetal.[7] More recently, Campos and co-workers reported the preparation of isomyosmine by photolysis of *N*-cyclopropyl-3-pyridinecarboxaldhyde imine,[8] but this did not appear amenable to scale-up. Recently, Brinner and Ellman reported a route to 5-substituted-1-pyrrolines employing sulfinimide precursors,[9] and we employed this approach to access isomyosmine.

SCHEME 1. Metabolic Activation of NNN to NNN-5′-OH and the Proposed Route to NNN-5′-OAc, a Precursor for NNN-5′-OH, from Isomyosmine



SCHEME 2. Synthesis of (±)-NNN-5′-OAc



Starting with commercially available 3-nicotinaldehyde and racemic *tert*-butyl sulfinamide, chromatographically stable sulfinimine **1** was formed in quantitative yield (Scheme 2). Addition of the Grignard reagent derived from 2-(2′-bromoethyl)-1,3-dioxane as a masked propanal homoenolate afforded the desired sulfinamide **2**. The level of relative stereochemical control in this addition is high; the sulfinamine **2** was isolated as a single diasteromer, which was found to have the (*R**,*R**) configuration by X-ray crystallography (see the Supporting Information). Deprotection of **2** with aqueous trifluoroacetic acid followed by nitrosoacylation generates (±)-NNN-5′-OAc as a mixture of diastereomers in 26% overall yield over three steps.

(1) For reviews see: (a) Hoffmann, D.; Brunnemann, K. D.; Prokopczyk, B.; Djordjevic, M. V. *J. Toxicol. Environ. Health* **1994**, *41*, 1–52. (2) (b) Hecht, S. H. *Chem. Res. Toxicol.* **1998**, *11*, 559–603. (c) Pfeifer, G. P.; Denissenko, M. F.; Olivier, M.; Tretyakova, N.; Hecht, S. S.; Hainut, P. *Oncogene* **2002**, *21*, 7435–7451. McIntee, E. J.; Hecht, S. S. *Chem. Res. Toxicol.* **2000**, *13*, 192–199, and references cited therein.

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(4) (a) Lao, Y.; Yu, N.; Kassie, F.; Villalta, P. W.; Hecht, S. S. *Chem. Res. Toxicol.* **2007**, *20*, 246–256. (b) Upadhyaya, P.; Hecht, S. S. *Chem. Res. Toxicol.* **2008**, *21*, 2164–2171.

(5) Mueller, E.; Kettler, R.; Wiessler, M. *Liebigs Ann. Chem.* **1984**, 1468–1493.

(6) There is some confusion in the older literature as some authors mistakenly attributed the 3-(3,4-dihydro-2*H*-pyrrol-2-yl)pyridine structure to myosmine, see for example: Jarboe, C. H.; Rosene, C. J. *J. Chem. Soc.* **1961**, 2455–2458.

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(8) Campos, P. J.; Soldevilla, A.; Sampedro, D.; Rodrigues, M. A. *Tetrahedron Lett.* **2002**, *43*, 8811–8813.

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Although not separable by HPLC (see the Supporting Information), the (±)-NNN-5′-OAc appears to consist of a ca. 4:1 mixture of (*E*)- and (*Z*)-*N*-nitroso rotamers[10] of a 1:1 mixture of *cis*-/*trans*-2,5-pyrrolidine diasteromers by NMR. Isolation of the intermediate isomyosmine is possible by chromatography on Fluorisil or basic alumina; however, isolation does not improve the overall yield of NNN-5′-OAc. Yields remain consistent on scales ranging from milligram to multigram quantitites.[11]

This route should enable studies on the biological effects of NNN-5′-OH. The synthesis is technically simple, uses readily available starting materials, and provides the desired product in satisfactory overall yields. An additional advantage of this route is that by using nonracemic *tert*-butyl sulfinamide, it should be possible to selectively access precursors of NNN metabolites derived from both enantiomers of nicotine.

Experimental Section

Caution. Nitrosamines are potent carcinogens. Care should be taken in handling NNN-5′-OAc to avoid exposure and possible environmental contamination.

(±)-*tert*-Butylsulfinamide.[12,13] A variation on the published route to the racemic sulfinamine was followed: oxidation of di-*tert*-butyldisulfide by hydrogen peroxide in AcOH[14] afforded the *tert*-butylthiosulfinate, which was then transformed to the *tert*-butyl sulfinyl chloride with SO_2Cl_2. Subsequent reaction with NH_4OH as reported[12] gave the desired product.

***N*-(3-Pyridinemethylidene)-2-methylpropane-2-sulfinamide (1).**[15] (±)-*tert*-Butylsulfinamide (400 mg, 3.3 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (6.6 mL) in a 10 mL oven-dried, Ar-flushed round-bottomed flask. $MgSO_4$ (2.0 mg, 16.5 mmol, 5 equiv) was added, followed by pyridinium *p*-toluenesulfonate (42 mg, 0.17 mmol, 0.05 equiv). Nicotinaldehyde (354 mg, 3.3 mmol, 1 equiv) was added as a liquid. This cloudy colorless solution was stirred at room temperature for 16 h. The reaction mixture was filtered through Celite, washed with CH_2Cl_2, and concentrated to yield a yellow oil that was purified by flash chromatography (SiO_2, 10−50% EtOAc/Hex) to yield the sulfinimine **1** as a yellow oil (680 mg, 3.2 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (1H, br s), 8.69 (1H, d, J = 4.8 Hz), 8.59 (1H, s), 8.11 (1H, d, J = 8.2 Hz), 7.37 (1H, dd, J = 7.9, 5.2 Hz), 1.19 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.32, 152.79, 150.86, 135.60, 129.54, 123.83, 57.99, 22.48. LRMS (CI^+) 211 (M + 1), 155 (M − ^{t}Bu). HRMS (CI^+) calcd for $C_{10}H_{15}N_2OS$ 211.0905, found 211.0903; IR (thin film) 3411, 2964, 2860, 1652, 1429, 1380 cm^{-1}.

(±)-*N*-(3-[1,3]Dioxan-2-yl-1-pyridin-3-yl-propyl)-2-methylpropanesulfinamide (2). Freshly ground magnesium turnings (1.56 g, 64.2 mmol, 15 equiv) were placed in a 10 mL oven-dried, Ar-flushed, round-bottomed flask. THF (7.1 mL, 3 M) was added. A 3 M solution of 2-(2′-bromoethyl)-1,3-dioxane (1.62 mL, 21.4 mmol, 5 equiv) in THF (21.4 mL) was added dropwise. With intermittent heating and the addition of a catalytic amount of iodine (1 small crystal) to initiate the reaction, the mixture was stirred at room temperature for 1 h. The resulting solution of Grignard reagent was separated from the excess Mg metal by removal via syringe to a clean 10 mL oven-dried, Ar-flushed, round-bottomed flask. This solution was cooled to −48 °C, and the sulfinimine **1** (900 mg, 4.3 mmol, 1 equiv) was added as a solution in THF (4.3 mL) at −48 °C. This mixture was stirred at this temperature overnight. After 16 h, the reaction was warmed to room temperature and quenched with satd NH_4Cl (10 mL). The reaction mixture was partitioned between satd NH_4Cl (50 mL) and EtOAc (50 mL). The aqueous layer was extracted (2 × 50 mL of EtOAc), and the combined organic layers were washed with satd NaCl (50 mL) and dried (Na_2SO_4). After evaporation of the solvent, the product was purified by flash chromatography (SiO_2, 10% MeOH in CH_2Cl_2) to yield **2** as a yellow oil (930 mg, 3.6 mmol, 85%). Crystallization from EtOAc/Hex yielded a white crystalline solid with mp 99.0−99.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (1H, br s), 8.51 (1H, d, J = 4.5 Hz), 7.65 (1H, d, J = 7.9 Hz), 7.26 (1H, dd, J = 7.7, 5.5 Hz), 4.48 (1H, t, J = 5.0 Hz), 4.38 (1H, q, J = 6.8 Hz), 4.04 (2H, dd, J = 11.7, 4.8 Hz), 3.70 (2H, t, J = 11.7 Hz), 3.57 (1H, d, J = 4.8 Hz), 2.05−2.15 (1H, m), 1.95−2.05 (1H, m), 1.81−1.92 (1H, m), 1.53−1.63 (1H, m), 1.40−1.50 (1H, m), 1.31 (1H, d of m, J = 13.3 Hz), 1.20 (9H, s). ^{13}C NMR δ (75 MHz, $CDCl_3$) 149.1, 148.7, 137.8, 134.8, 123.6, 101.4, 66.8, 57.1, 56.0, 31.2, 30.9, 30.7, 25.6, 22.5. LRMS (CI^+) 327 (M + 1), 231 (M − pyr). HRMS (CI^+) calcd for $C_{16}H_{27}N_2O_3S$ 327.1742, found 327.1746. IR (thin film) 3414, 3225, 3051, 2964, 2858, 1710, 1644, 1592, 1578 cm^{-1}.

(±)-5-(3-Pyridyl)-1-pyrroline ((±)-Isomyosmine).[7,8] The sulfinamide **2** (4.34 g, 13.3 mmol, 1 equiv) was dissolved in 95% aq TFA (67 mL, 0.2 M) and stirred at room temperature for 1 h. The reaction was then quenched by pouring over satd $NaHCO_3$ (150 mL) then extracted (4 × 80 mL of $CHCl_3$). The organic layers were combined, dried over Na_2SO_4, and concentrated by rotary evaporation. Chromatography on Fluorisil (5% MeOH/$CHCl_3$) yielded a brown oil (1.87 g, 12.8 mmol, quant.) whose ^{1}H NMR spectral data were in accordance with literature values.[7,8] ^{13}C NMR ($CDCl_3$) δ 168.3, 148.2, 139.3, 133.9, 123.4, 73.5, 61.5, 37.5, 30.1. LRMS (CI^+) 147 (M + 1). HRMS (CI^+) calcd for $C_9H_{11}N_2$ 147.0922, found 147.0923. IR (thin film) 3019, 2968, 1215 cm^{-1}.

(±)-*N*′-Nitrosonornicotine 5′-Acetate.[3] Isomyosmine (87 mg, 0.60 mmol, 1 equiv), prepared as above, but used immediately without any chromatographic purification, was dissolved in glacial acetic acid (1 mL) and cooled to 5 °C. $NaNO_2$ (53 mg, 0.77 mmol, 1.3 equiv) was added, and the reaction mixture was allowed to warm to room temperature for 1 h (until complete as judged by TLC). The reaction was neutralized by pouring over satd $NaHCO_3$ (20 mL) then extracted (3 × 15 mL $CHCl_3$), dried (Na_2SO_4), and concentrated. The resulting brown oil was passed immediately through a Fluorisil column with EtOAc as the eluent. The resulting yellow oil was purified by flash chromatography (SiO_2, 50−75% EtOAc/Hex) to yield a light yellow oil (48.2 mg, 0.22 mmol, 37%) whose ^{1}H NMR, IR, and MS spectral data were in accordance with the literature values.[3] Subsequent chromatography (SiO_2, 75% EtOAc/Hex) yielded an inseparable mixture of (±)-NNN-5′-OAc diastereomers as a pale yellow solid, mp 57.4−64.1 °C. ^{13}C NMR ($CDCl_3$) δ 169.5, 169.4, 148.8, 148.7, 147.8, 147.1, 135.0, 133.9, 133.5, 132.7, 123.5, 123.4, 85.0, 83.8, 59.3, 58.5, 31.1, 30.5, 29.6, 29.5, 21.1, 21.0. Analysis by LC-MS (see the Supporting Information for details): 92.5% pure (diastereomers not resolved).

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Supporting Information Available: X-ray structural information for **2**, LC-MS chromatogram for (±)-NNN-5′-OAc, and NMR spectra for isomyosmine, NNN-5′-OAc, and compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) At the largest scale attempted (15.7 mmol), the yield from **2** is 18%. See the Supporting Information for details.

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