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REGIOSELECTIVE SYNTHESIS OF DEUTERATED ANALOGS OF THE NEUROTOXIN MPTP

Stéphane Mabic and Neal Castagnoli, Jr.*

Department of Chemistry

Virginia Tech, Blacksburg VA 24061

SUMMARY

1-Methyl-4-phenyl-2-pyridone has been used as starting material for the efficient and regioselective synthesis of deuterated analogues of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP-2,2-d_2, MPTP-6,6-d_2 and MPTP-2,2,6,6-d_4 were obtained in good yield through a combination of alkaline deuterium exchange and selective $LiAlH_4$ and $LiAlD_4$ reduction reactions.

Keywords: MPTP, deuteration, reduction, pyridone, oxidation

INTRODUCTION

The flavoenzymes monoamine oxidases A and B catalyze the α-carbon oxidation of various 1,4-disubstituted tetrahydropyridine derivatives. In particular, the parkinsonian inducing 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP **1**), an excellent substrate for MAO-B, is initially oxidized to the corresponding 1-methyl-4-phenyl-2,3-dihydropyridinium species[1] which undergoes further oxidation to the 1-methyl-4-phenylpyridinium species,[2] the ultimate toxin. In an effort to obtain a better understanding of the MAO-B pathway that catalyzes this bioactivation, we plan to undertake detailed kinetic isotope effect studies which will require selected deuterated

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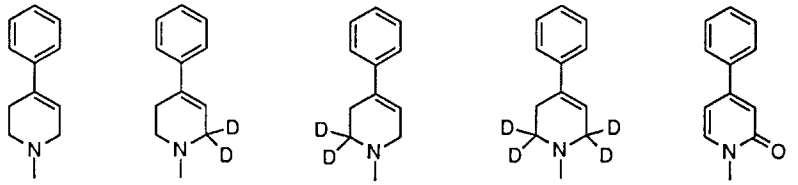
analogs of MPTP. Specifically we require the deuterated MPTP analogs MPTP-6,6-d_2 (**2**), MPTP-2,2-d_2 (**3**) and MPTP-2,2,6,6-d_4 (**4**). Deuterated analogs **2** and **4** have been prepared previously but in low overall yield.[3,4] In this paper we report convenient and high yield syntheses of **2**, **3** and **4** starting from 1-methyl-4-phenyl-2-pyridone **5**, a compound which has been prepared previously[3] but again in poor overall yield.

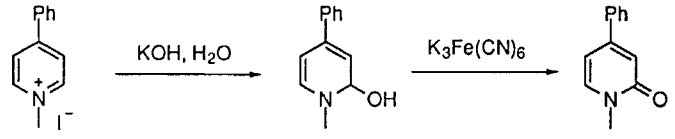


MPTP **1** MPTP-6,6-d_2 **2** MPTP-2,2-d_2 **3** MPTP-2,2,6,6-d_4 **4** 2-pyridone **5**

RESULTS AND DISCUSSION

Pyridone **5** was previously obtained by K_3FeCN_6 oxidation of the pseudo base **6b** which is derived by treatment of the pyridinium species **6** with KOH (Scheme 1). Under the reported reaction conditions[5] (alternating additions of the KOH and ferricyanide over a 2 hour period followed by stirring for 2-3 hours at 25 °C) the average yield of the final product is only 25%. However, employing conditions reported by Fujii for analogous reactions[6] (dropwise addition of both reagents to the pyridinium substrate at 0 °C followed by stirring in the presence of toluene for 18 hours) provided the desired 2-pyridone **5** in 83% yield.

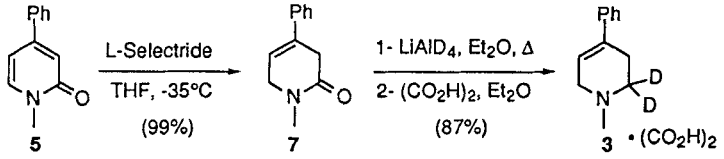
Scheme 1. Oxidation of the pyridinium iodide **6** to the 2-pyridone **5**



6 **6b** **5**

We previously have shown that treatment of pyridone **5** with $LiAlH_4$ leads exclusively to 1-methyl-4-phenyl-3,6-dihydro-2-pyridone **7**.[7] An alternative reductant, L-Selectride in THF at -35 °C, gave a quantitative yield of **7**. This intermediate is converted efficiently to MPTP-2,2-d_2 (**3**) by reduction with $LiAlD_4$ in refluxing diethyl ether. The reduction proceeds quantitatively in less than one hour. The resulting amine was characterized as its oxalate salt **3**[8] which can be obtained under anhydrous conditions in 87% yield (Scheme 2).

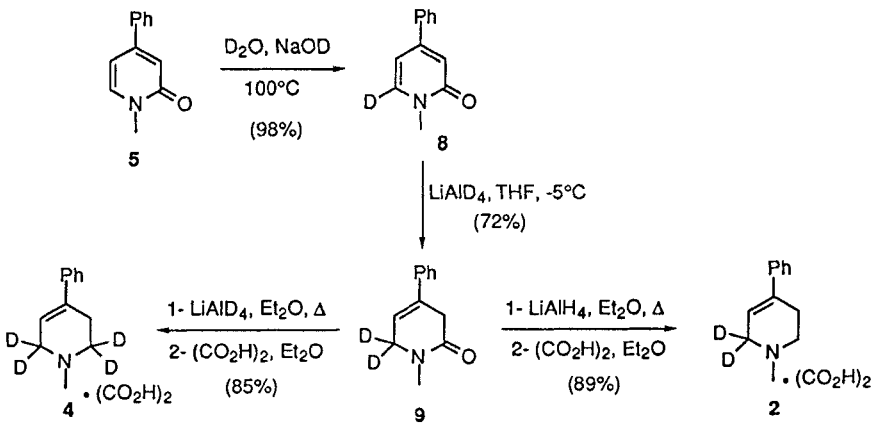
Scheme 2. Synthesis of MPTP-2,2-d_2 (**3**)



5 **7** **3**

The syntheses of MPTP-6,6-d_2 (**2**) and MPTP-2,2,6,6-d_4 (**4**) (Scheme 3) were achieved in good yields via the monodeuterated 1-methyl-4-phenyl-2-pyridone-6-d_1 (**8**). The deuterium was selectively introduced in the C-6 position by treatment of the 2-pyridone **5** in refluxing D_2O with one equivalent of NaOD, a procedure originally described by Beak.[9] The reaction proceeds slowly. GC-EIMS and ^1H NMR analyses following one day under reflux indicated 92% deuterium incorporation. Since the

Scheme 3. Synthesis of MPTP-6,6-d_2 (**2**) and MPTP-2,2,6,6-d_4 (**4**)



5 **8** **4** **9** **2**

proton-deuterium exchange is an equilibrium reaction under thermodynamic control, it was necessary to perform a second exchange with fresh D_2O which resulted in > 98% deuterium incorporation. The incorporation is completely selective and no by-products could be detected by GC-EIMS. The subsequent reduction of **8** with $LiAlD_4$ in THF at -5 °C afforded a 72% yield of the 6,6-dideuterated 2-pyridone derivative **9**. Finally, treatment of **9** with $LiAlH_4$ gave MPTP-6,6-d_2 (**2**) which was isolated as its oxalate salt in 89% overall yield. Alternatively treament of **9** with $LiAlD_4$ gave MPTP-2,2,6,6-d_4 (**4**) in 85% yield as its oxalate salt.

In conclusion, we have reported an improved synthesis of 1-methyl-4-phenyl-2-pyridone **5** and have been able to convert this key intermediate by efficient and regioselective reductions and proton-deuteron exchange reactions to three specifically deuterium labeled 1,2,3,6-tetrahydropyridines of biological interest.

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Caution: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine **1** is a known nigrostriatal neurotoxin and should be handled using disposable gloves in a properly ventilated hood. Detailed procedures for the safe handling of MPTP have been reported.[10]

EXPERIMENTAL

General. Reagents and starting materials were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone ketyl. All reactions were conducted using flame dried glassware under an atmosphere of dry nitrogen. Chromatography refers to flash column chromatography on silica gel unless otherwise noted. Melting points were performed on a Thomas-Hoover melting point apparatus and are uncorrected.

Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. Proton and carbon spectra were recorded on a Bruker WP 270-MHz spectrometer. Exponential function (LB= 0.1-0.2) was applied to the FID to obtain integrals and gaussian function (LB= -1, GB= 0.25) to record coupling constants. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane (d=0). Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet) or m (multiplet). Coupling constants (*J*) values are given in hertz (Hz). Gas chromatography-electron ionization mass spectrometry (GC-EIMS) was performed on a Hewlett Packard 5890 GC fitted with an HP-1 capillary column which was coupled to a Hewlett Packard 5870 mass-selective detector. Data were acquired using an HP 5970 Chemstation. Normalized peak heights are reported as a percentage of the base peak. UV-vis absorption spectra were recorded on a Beckman DU Series 50 spectrophotometer.

1-Methyl-4-phenyl-2-pyridone (5). Prepared according the cited literature. mp 139-139.5°C; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 7.56 (m, 2H), 7.43 (m, 3H), 7.33 (d, J = 7.0 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 6.42 (dd, J = 7.0 Hz, J = 2.0 Hz, 1H), 3.52 (s, 3H); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 163.2, 151.7, 138.0, 137.4, 129.3, 128.9, 126.6, 116.9, 105.3, 88.6, 37.2; MS (EI) m/z (rel int) 185 (100), 157 (80), 142 (9), 128 (13), 115 (50), 89 (5), 77 (6); IR ($CHCl_3$, cm^{-1}) 3019, 1658, 1591, 1477, 1212; UV (MeOH, nm) 324, 258, 232, 209.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-2,2-*d*$_2$ (3). A suspension of 41 mg (1 mmole) of $LiAlD_4$ in ether (8 mL) was heated to reflux and 94 mg (0.5 mmole) of 1-methyl-4-phenyl-3,6-dihydro-2-pyridone **7** in ether (5 mL) was added dropwise. The reaction mixture was heated for 45 min after which the excess $LiAlD_4$ was decomposed with cooling by addition of H_2O (50 μL) and 1N NaOH (50 μL). The solution was filtered and dried over $MgSO_4$. Ethereal oxalic acid (54 mg, 0.6 mmoles) was added under anhydrous conditions and the resulting white precipitate was filtered and recrystallized from methanol/ether to give 114 mg (87 %) of the oxalate salt of **3**: mp 165-165.5°C; ^{1}H-NMR (DMSO-*d*$_6$, 270 MHz) δ 7.47 (m, 2H), 7.35 (m, 3H), 6.19 (bs, 1H), 3.76 (m, 2H), 3.16 (s, 3H), 2.73 (bs, 2H); ^{13}C NMR (DMSO-*d*$_6$, 67.5 MHz) δ 164.7, 164.6, 138.7, 134.0, 128.8, 128.1, 125.0, 117.2, 52.0, 42.0, 24.0; MS (EI) m/z (rel int)

175 (100), 174 (66), 159 (0.5), 144 (25), 129 (50), 115 (34), 98 (61), 77 (5). UV (MeOH, nm) 242, 213. Estimated deuterium incorporation > 98 %. Anal. Calcd for $C_{14}H_{15}D_2NO_4$: C, 63.38; H+D, 6.46; N, 5.28. Found: C, 63.29; H+D, 6.46; N, 5.34.

1-Methyl-4-phenyl-2-pyridone-6-*d*$_1$ (8). Under anhydrous conditions, D_2O (3 mL), CD_3OD (1 mL), NaOD (2 mmoles) and 1-methyl-4-phenyl-2-pyridone **5** (370 mg, 2 mmoles) were heated under reflux for 48 h. After cooling, the partially deuterated pyridone was extracted with ether. After drying over $MgSO_4$, the solvent was removed under reduced pressure and the product was dried under vacuum. The same procedure was then repeated to give 371 mg of **8** as a white solid: mp 139.5-140°C; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 7.57 (m, 2H), 7.45 (m, 3H), 6,80 (d, J = 2.0 Hz, 1H), 6.43 (d, J = 2.0 Hz, 1H), 3.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 163.2, 151.8, 137.4, 129.3, 128.9, 126.6, 116.8, 105.2, 88.6, 37.1; MS (EI) m/z (rel int) 186 (100), 158 (76), 143 (7), 129 (11), 116 (35), 89 (10). UV (MeOH, nm) 314, 257, 233, 210. Estimated deuterium incorporation > 98 %. Anal. Calcd for $C_{12}H_{10}DNO$: C, 77.40; H+D, 5.89; N, 7.52. Found: C, 77.31; H+D, 5.91; N, 7.44.

1-Methyl-4-phenyl-3,6-dihydro-2-pyridone-6,6-*d*$_2$ (9). To a solution of pyridone **8** (93 mg, 0.5 mmole) in THF at -5 °C was added $LiAlD_4$ (41 mg, 1 mmole). After 2.5 h, the reaction mixture was cooled to -78 °C and methanol was added. After warming to ambient temperature NaOH (500 mL) was added and then H_2O (2 mL). The solution was extracted with ether (3 x 15 mL) and the organic phase was washed with brine, dried over $MgSO_4$ and evaporated. The crude extract was chromatographied (SiO_2, EtOH/ EtOAc/ Hex 10:40:50) to give pyridone-6,6-*d*$_2$ **9** (68 mg, 0.36 mmole) in 72% yield as a white solid: mp 89.5-91°C; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 7.35 (m, 5H), 6.09 (bs, 1H), 3.34 (bs, 2H), 3.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 167.3, 138.2, 133.2, 128.6, 128.0, 124.82, 116.0, 33.7; MS (EI) m/z (rel int) 189 (100), 160 (23), 144 (73), 131 (17), 116 (55), 115 (54), 102 (11), 97 (10), 77 (15); UV (MeOH, nm) 248, 210. Estimated deuterium incorporation > 98 %. Anal. Calcd for $C_{12}H_{11}D_2NO$: C, 76.16; H+D, 6.92; N, 7.40. Found: C, 76.02; H+D, 6.97; N, 7.47.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-6,6-*d*$_2$ (2). The same procedure as described for **3** was followed with **9** and $LiAlH_4$: mp 165 °C; ^{1}H-NMR

(DMSO-d_6, 270 MHz) δ 7.48 (m, 2H), 7.35 (m, 3H), 6.18 (bs, 1H), 3.34 (t, J = 5.8 Hz, 2H), 2.81 (s, 3H), 2.75 (t, J = 5.8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 165.5, 138.6, 134.1, 128.1, 128.0, 125.0, 117.0, 49.9, 42.0, 24.2; MS (EI) m/z (rel int) 175 (100), 174 (45), 146 (36), 131 (53), 117 (29), 116 (30), 98 (71), 83 (18), 77 (24); UV (MeOH, nm) 241, 211. Estimated deuterium incorporation > 98 %. Anal. Calcd for $C_{14}H_{15}D_2NO_4$: C, 63.38; H+D, 6.46; N, 5.28. Found: C, 63.23; H+D, 6.46; N, 5.32.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-2,2,6,6-d_4 (4). The same procedure as described for **3** was carried out with **9** and $LiAlD_4$: mp 164-165°C; ^{1}H-NMR (DMSO-d_6, 270 MHz) δ 7.47 (m, 2H), 7.35 (m, 3H), 6.17 (bs, 1H), 2.85 (s, 3H), 2.74 (bs, 2H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 165.1, 138.6, 134.0, 128.4, 128.0, 125.0, 117.1, 42.0, 24.1; MS (EI) m/z (rel int) 177 (100), 176 (41), 146 (32), 133 (48), 117 (25), 116 (32), 98 (57), 77 (19); UV (MeOH, nm) 242, 210. Estimated deuterium incorporation > 98 %. Anal. Calcd for $C_{14}H_{13}D_4NO_4$: C, 62.91; H+D, 7.90; N, 5.24. Found: C, 62.65; H+D, 8.05; N, 5.34.

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