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Mass spectrometric studies on 4-aryl-1-cyclopropyl-1,2-dihydropyridinyl derivatives: an examination of a novel fragmentation pathway

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Examination of the electrospray ionization product ion spectra of 1,2-dihydropyridinyl and 4-aryl-1,2-dihydropyridinyl derivatives bearing a 1-cyclopropyl or 1-*trans*-2-phenylcyclopropyl group has led to the characterization of unexpected fragment ions. For example, the base peak at *m/z* 156 present in the product ion spectrum of *trans*-1-(2-phenylcyclopropyl)-4-phenyl-1,2-dihydropyridine proved not to be the expected 4-phenylpyridinium species but rather the isomeric 3-phenyl-5-azoniafulvenyl species. The results of studies with a series of structural and isotopically labeled analogs require a novel fragmentation pathway to account for the formation of this and related fragment ions. One possible pathway is based on an initial 1,5-sigmatropic shift of a cyclopropylmethylene hydrogen atom that is accompanied by opening of the cyclopropyl ring. The resulting eniminium intermediates then fragment to yield the 5-azoniafulvenyl species. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: 1-cyclopropyl-4-aryl-1,2-dihydropyridines; fragmentation mechanism; 5-azoniafulvenyl species; stable isotopes; 1,5-sigmatropic hydrogen migration

INTRODUCTION

Interest in 1,4-disubstituted-1,2,3,6-tetrahydropyridines is derived in part from the potent and selective neurodegenerative properties of the parkinsonian-inducing 'street drug' 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**1**)].[1,2] The neurotoxicity of MPTP is reported to be mediated by the corresponding pyridinium metabolite $\mathbf{4}^+$, which is formed via monoamine oxidase[3]- and cytochrome P450[4]-catalyzed oxidations that generate the 2,3-dihydropyridinium intermediate $\mathbf{3H}^+$ (Scheme 1). One mechanism proposed to account for this 2-electron α-carbon oxidation proceeds via an initial single electron transfer (SET) step to form the corresponding aminyl radical cation $\mathbf{1}^{\bullet+}$. Ring α-carbon deprotonation of $\mathbf{1}^{\bullet+}$ yields the neutral radical $\mathbf{2}^{\bullet}$ that undergoes further oxidation to the 2,3-dihydropyridinium product $\mathbf{3H}^+$.[5,6] An alternative proposal assumes an initial hydrogen atom transfer (HAT) step to yield $\mathbf{2}^{\bullet}$ directly.[7–9] The second 2-electron oxidation ($\mathbf{3} \rightarrow \mathbf{4}^+$) has not been characterized fully.

As part of our efforts to characterize the pathway of these enzyme catalyzed reactions, we have investigated the chemical fate of tetrahydropyridinyl radical cations in a model of the SET reaction that involves the electrochemical (EC) 1-electron oxidation of the substrate molecules. The reaction products were detected by an on-line electrospray ionization-mass spectrometric (ESI/MS) assay.[10] The fates of the EC-generated 1-cyclopropyltetrahydropyridinyl radical cations have been particularly informative. For example, MH^+ of the first product detected by ESI/MS following the EC 1-electron oxidation of *trans*-1-(2-phenylcyclopropyl)-4-phenyl-1,2,3,6-tetrahydropyridine (**5**) at an EC cell potential of 100 mV has *m/z* 274. It should be clear that the ion at *m/z* 274 is detected only after the substrate molecule **5** has undergone EC oxidation. The full triple-quadrupole tandem mass spectrum of unoxidized **5** displayed an ion at 276 ($\mathbf{5H}^+$, 100%) and only one significant fragment ion at *m/z* 146 (10%). No ion was detected at *m/z* 275 (corresponding to $\mathbf{5}^+$). Consequently, products observed in the EC-ESI/MS experiment are not formed by oxidation of the analyte molecule **5** during the course of the ESI/MS analysis. The full MS^1 spectrum of this species shows MH^+ at *m/z* 274 (100%) and in-source fragment ions at *m/z* 272 (10%) and 117 (5%).

One possible structure for this ion is $\mathbf{6H}^+$, a product that would be expected to form enzymatically by the HAT pathway.[11] In the EC chemical model, the formation of $\mathbf{6H}^+$ would proceed via ring α-carbon deprotonation of the EC-generated cyclopropylaminyl radical cation $\mathbf{5a}^{\bullet+}$ to form $\mathbf{6}^{\bullet}$ followed by a second 1-electron oxidation. A second possible structure of the product detected at *m/z* 274 in the EC-ESI/MS experiment is the ring-opened eniminium species $\mathbf{7}^+$ (one or more diastereomers) that would be generated via ring opening of $\mathbf{5a}^{\bullet+}$ and subsequent 1-electron oxidation

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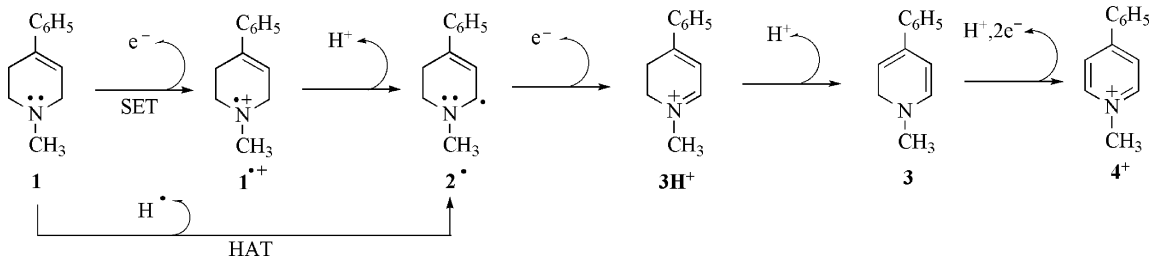
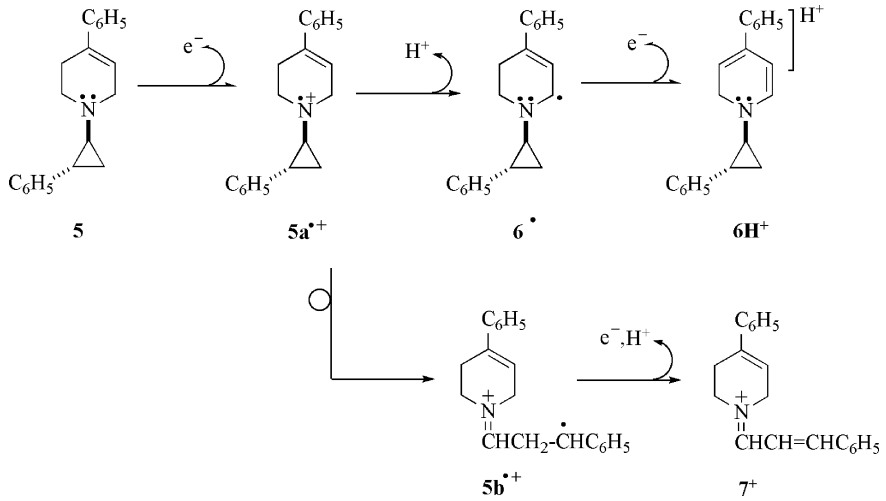


Scheme 1. Proposed SET and HAT pathways for the enzyme catalyzed oxidation of the parkinsonian-inducing neurotoxin MPTP (**1**).



Scheme 2. Pathways leading to the proposed oxidation products **6H$^+$** and/or **7$^+$** following the EC 1-electron oxidation of **5**.

of the resulting distonic radical cation **5b$^{\bullet+}$** (Scheme 2).[12] The characterization of the product(s) obtained from the 1-electron oxidation of **5** required product ion analyses by direct infusion into the ESI source of authentic samples of **6H$^+$** and **7$^+$**. This report describes the MS^2 obtained by ESI/MS analysis of synthetic dihydropyridinyl compounds, including **6**. These studies have led to the identification of unexpected fragment ions and to the proposal of a possible fragmentation pathway to account for their formation.

EXPERIMENTAL

Important notice: Some 1,4-disubstituted-1,2,3,6-tetrahydropyridines are known neurotoxins. Compounds of this general type should be handled with disposable gloves in a properly ventilated hood following good laboratory practices. Detailed procedures for the safe handling of MPTP have been reported.[13]

Chemistry

*1-Cyclopropyl-4-phenylpyridinium perchlorate (**25$^+$ClO$_4^-$**)*

The known[14] hydrochloride salt **25$^+$Cl$^-$** was converted into the more stable perchlorate salt **25$^+$ClO$_4^-$** in methanolic $HClO_4$: mp 131–132 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 1.42 (m, 4H), 4.35 (m, 1H), 7.62 (m, 3H), 7.97 (m, 2H), 8.32 (d, J = 7.0 Hz, 2H), 8.97 (d, J = 7.0 Hz, 2H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 6.8, 42.1, 124.5, 127.9, 129.6, 132.1, 134.0, 145.3, 157.1. Anal. Calcd for $C_{14}H_{14}ClNO_4$ (295.72): C, 56.86; H, 4.77; N, 4.74. Found: C, 56.75; H, 4.70; N, 4.87.

*1-Cyclopropyl-4-phenyl-1,2-dihydropyridine (**24**)*

A mixture of **25$^+$ClO$_4^-$** (5.0 g, 17 mmol) and $NaBH_4$ (3.2 g. 85 mmol) in 100 ml MeOH containing 2 g KOH was shaken vigorously for 5 min. Short path distillation (60 °C/10^{-5} mmHg) of the organic isolate gave 2.7 g (81%) of pure **24** as an orange oil: UV (MeOH) λ_{max} 249 nm (ε 13 200); ^{1}H NMR (500 MHz, C_6D_6) δ 0.16 (m, 2H), 0.28 (m, 2H), 1.85 (m, 1H), 3.85 (dd, J = 1.0, 4.0 Hz, 2H), 5.24 (dd, J = 2.0, 7.5 Hz, 1H), 5.31 (dt, 1.0, 4.0 Hz, 1H), 6.14 (dd, J = 1.0, 7.5 Hz, 1H), 7.12 (m, 1H), 7.18 (m, 2H), 7.45 (m, 2H); ^{13}C NMR (125.8 MHz, C_6D_6) δ 5.8, 34.5, 49.3, 97.6, 109.1, 125.7, 127.1, 128.4, 137.0, 139.3, 140.5. Anal. Calcd for C_{14} H_{15} N (197.28): C, 85.24; H, 7.66; N, 7.10. Found: C, 85.07; H, 7.71; N, 7.17. The corresponding hydrochloride salt, prepared in methanolic HCl, proved to be identical to an authentic sample of 1-cyclopropyl-4-phenyl-1,2-dihydropyridinium chloride (**24H$^+$Cl$^-$**).[14]

*4-Phenyl-1-(2-*trans*-phenylcyclopropyl)pyridinium perchlorate (**12$^+$ClO$_4^-$**)*

The known,[12] hygroscopic **12$^+$Cl$^-$** was converted into its stable perchorate salt **12$^+$ClO$_4^-$** in methanolic $HClO_4$. The solid that separated upon addition of ether was analytically pure: mp 167–168 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.85 (m, 1H), 2.21 (m, 1H), 3.07 (m, 1H), 4.65 (m, 1H), 7.34 (m, 5H), 7.66 (m, 3H), 8.12 (m, 2H), 8.49 (d, J = 6.5 Hz, 2H), 9.17 (d, J = 6.5 Hz, 2H); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 16.4, 26.0, 49.6, 124.7, 127.3, 127.5, 128.8, 129.0, 130.3, 132.8, 134.0, 138.8, 145.4, 155.7. Anal. Calcd for $C_{20}H_{18}ClNO_4$ (371.81): C, 64.61; H, 4.88; N, 3.77. Found: C, 64.40; H, 4.63; N, 3.82.

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*4-Phenyl-1-(2-*trans*-phenylcyclopropyl)-1,2-dihydropyridine (**6**)*

The pyridinium perchlorate **12**$^+$**ClO$_4$**$^-$ was converted into the oily **6** in the same manner as described above for the preparation of **24**: UV (MeOH) λ_{max} 249 nm (ε 16 900); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15 (m, 1H), 1.26 (m, 1H), 2.08 (m, 1H), 2.46 (m, 1H), 4.02 (m, 2H), 5.05 (dd, J = 2.0, 7.5 Hz, 1H), 5.46 (m, 1H), 6.35 (dd, J = 0.5, 7.5 Hz, 1H), 7.27 (m, 10H); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 16.1, 24.3, 45.1, 49.1, 96.8, 109.6, 125.5, 126.3, 126.5, 127.7, 128.8, 129.0, 135.9, 139.4, 139.5, 141.6. FAB-HRMS: Calcd for $C_{20}H_{20}N^+$: 274.1596. Found: 274. 1577.

*4-Phenyl-1-(2-*trans*-phenylcyclopropyl)-1,2-dihydropyridine-d_1 (**6-d_1**)*

In a similar manner, **12**$^+$**ClO$_4$**$^-$ was converted into the oily diastereomeric mixture **6-d_1**: ^{1}H NMR (500 MHz, C_6D_6) δ 0.70 (m, 1H), 0.91 (m, 1H), 1.81 (m, 1H), 2.13 (m, 1H), 3.83 (m, 1H), 5.28 (dd, J = 2.5, 7.0 Hz, 1H), 5.33 (m, 1H), 6.16 (m, 1H), 7.81 (m, 2H), 7.12 (m, 6H), 7.48 (m, 2H); ^{13}C NMR (125.8 MHz, C_6D_6) δ 15.9, 16.0, 44.4, 48.6 (t), 48.7 (t), 97.9, 109.2, 125.7, 125.9, 126.1, 127.2, 128.4, 128.5, 137.0, 138.6, 138.7, 140.4, 141.1. FAB-HRMS: Calcd for $C_{20}H_{19}DN^+$: 275.1659. Found: 275.1646.

*4-(4-Methylphenyl)pyridine (**16**)*[15]

The published procedure was followed except that [Pd(PPh_3)$_4$] was used as catalyst: mp 86–87 °C (lit.[12] 88–90 °C).

*1-(2,4-Dinitrophenyl)-4-(4-methylphenyl)pyridinium chloride (**17**$^+$**Cl**$^-$)*[12]

A solution of 4-(4-methylphenyl)pyridine[15] (6.2 g, 37 mmol) and 2,4-DNCB (11.2 g, 55 mmol) in dry acetone (160 ml) was heated under reflux for 48 h. The insoluble product was collected and recrystallized from MeOH to give **17**$^+$**Cl**$^-$ as hygroscopic light yellow crystals (9.2 g, 67%): mp 197–199 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.45 (s, 3H), 7.51 (d, J = 8.0 Hz, 2H), 8.21 (d, J = 8.0 Hz, 2H), 8.52 (d, J = 8.5 Hz, 1H), 8.86 (d, J = 7.0 Hz, 2H), 8.99 (dd, J = 2.5, 8.5 Hz, 1H), 9.12 (d, J = 2.4 Hz, 1H), 9.48 (d, J = 7.0 Hz, 2H); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 21.7, 122.0, 124.1, 129.3, 130.7, 130.8, 131.1, 132.8, 139.2, 143.8, 144.6, 146.4, 149.5, 157.6.

*4-(4-Methylphenyl)-1-(*trans*-2-phenylcyclopropyl)-pyridinium perchlorate (**18**$^+$**ClO$_4$**$^-$)*[12]

A solution of crude **17**$^+$**Cl**$^-$ (2.7 g, 7.5 mmol) and *trans*-2-phenylcyclopropylamine (2.0 g, 15 mmol) in anhydrous 1-butanol (90 ml) was heated under reflux for 12 h. The solvent was removed under reduced pressure and the residue was partitioned between water and CH_2Cl_2. Treatment of the residue obtained after removing the water under reduced pressure with ethereal methanolic $HClO_4$ gave 920 mg (32%) of the white, crystalline **18**$^+$**ClO$_4$**$^-$: mp 135–137 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 1.86 (m, 1H), 2.12 (m, 1H), 2.45 (s, 3H), 3.01 (m, 1H), 4.52 (m, 1H), 7.34 (m, 5H), 7.44 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 6.5 Hz, 2H), 8.33 (d, J = 6.5 Hz, 2H), 8.96 (d, J = 6.5 Hz, 2H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 15.4, 20.2, 25.3, 49.2, 124.0, 126.4, 127.0, 127.9, 128.5, 130.4, 131.0, 137.8, 143.6, 144.8, 157.1. Anal. Calcd for $C_{21}H_{20}ClNO_4$ (385.84): C, 65.37; H, 5.22; N, 3.63. Found: C, 65.29; H, 5.12; N, 3.66.

*4-(4-Methylphenyl)-1-(2-*trans*-phenylcyclopropyl)-1,2-dihydropyridine (**19**)*

Reduction of **18**$^+$**ClO$_4$**$^-$ in methanolic KOH with $NaBH_4$ gave **19** as a dark yellow oil (53 mg, 71%): UV (MeOH) λ_{max} 253 nm (ε 11 200), 319 nm (ε 5800); ^{1}H NMR (500 MHz, C_6D_6) δ 0.72 (m, 1H), 0.93 (m, 1H), 1.83 (m, 1H), 2.14 (s, 3H), 2.15 (m, 1H), 3.87 (m, 2H), 5.32 (dd, J = 7.5, 2.0 Hz, 1H), 5.38 (m, 1H), 6.17 (d, J = 3.0 Hz, 1H), 6.83 (d, J = 8.0 Hz, 2H), 7.10 (m, 5H), 7.43 (d, J = 8.0 Hz, 2H); ^{13}C NMR (125.8 MHz, C_6D_6) δ 15.9, 20.9, 24.5, 44.5, 49.1, 98.2, 108.7, 125.6, 125.9, 126.1, 128.4, 129.2, 136.6, 136.8, 137.6, 138.5, 141.1. FAB-HRMS: Calcd for $C_{21}H_{22}N^+$: 288.1752. Found: 288.1729.

*1-Formyl-4-phenyl-1,2,3,6-tetrahydropyridine-1-^{13}C (**21-^{13}C**)*[16]

A mixture of 4-phenyl-1,2,3,6-tetrahydropyridine (**20**, 3.3 g, 21 mmol), H^{13}COOEt (2.0 g, 27 mmol) in CH_3CN (30 ml) was heated at 60 °C for 36 h to give **21-^{13}C** (mixture of rotomers) as a pale yellow waxy solid (3.55 g, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.55 (m, 4H), 3.61 (m, 4H), 3.77 (m, 1H), 4.04 (m, 1H), 4.16 (m, 4H), 6.02 (m, 2H), 7.31 (m, 10H), 8.14 (d, $^1J_{CH}$ = 182 Hz, 1H), 8.20 (d, $^1J_{CH}$ = 182 Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 26.8, 28.1, 37.2, 40.4, 42.8, 42.9, 45.2, 45.3, 119.4, 119.5, 119.7, 125.0, 125,1, 127.7, 127.8, 128.6, 135.4, 136.8, 140.3, 140.4, 161.2 (^{13}C), 161.6 (^{13}C). FAB-HRMS: Calcd for $C_{11}{}^{13}CH_{14}NO^+$: 189.1109. Found: 189.1101.

*1-Cyclopropyl-1-^{13}C-2,2,3,3-d_4-4-phenyl-1,2,3,6-tetrahydropyridinium oxalate salt (**22-^{13}C-d_4 · $C_2H_2O_4$**)*[16]

Ethyl bromide-d_5 (3.6 ml, 48 mmol) was added dropwise to a suspension of magnesium turnings in THF (50 ml). The mixture was heated under reflux for 3 h and, after cooling, was added slowly via a cannula to 50 ml THF containing **21-^{13}C** (3.55 g, 19 mmol) and Ti(iOPr)$_4$ (6.1 ml, 21 mmol). By stirring at 60 °C for 18 h, the reaction mixture was worked up to give 3.0 g (54%) of the oxalate salt **22-^{13}C-d_4** · $C_2H_2O_4$: mp 181–182 °C (lit[13] mp 185–186 °C for **22** · **$C_2H_2O_4$**); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.43 (d, $^1J_{CH}$ = 178 Hz, 1H), 2.65 (m, 2H), 3.25 (m, 2H), 3.67 (m, 2H), 6.16 (m, 1H), 7.28 (m, 1H), 7.36 (m, 2H), 7.45 (m, 2H); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 25.0, 37.9 (^{13}C), 49.8, 51.6, 118.4, 125.3, 128.2, 129.1, 134.6, 139.5, 164.8. FAB-HRMS: Calcd for $C_{13}{}^{13}CH_{14}D_4N^+$: 205.1724. Found: 205.1724.

*1-Cyclopropyl-1-^{13}C-2,2,3,3-d_4-4-phenyl-2,3-dihydropyridinium perchlorate (**24**$^+$**-^{13}C-d_4-ClO$_4$**$^-$)*[14]

A mixture of 70% *m*-chloroperoxobenzoic acid *m*-CPBA (*m*-CPBA, 157 mg, 0.64 mmol) **22-^{13}C-d_4** (100 mg, 0.49 mmol) in CH_2Cl_2 (5 ml) was stirred at 0 °C for 20 min. The product was passed through basic alumina [CH_2Cl_2 followed by CH_2Cl_2: MeOH (97 : 3)] to give 98 mg (91%) of crude **23-^{13}C-d_4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.79 (m, 1H), 2.99 (d, $^1J_{CH}$ = 173 Hz, 1H), 3.05 (m, 1H), 3.50 (m, 2H), 4.03 (m, 2H), 6.00 (m, 1H), 7.30 (m, 1H), 7.35 (m, 2H), 7.42 (m 2H). TFAA (0.29 ml, 2.1 mmol) was added dropwise to a solution of **23-^{13}C-d_4** (90 mg, 0.41 mmol) in CH_2Cl_2 (5 ml) at 0 °C. After 15 min at 0 °C, a methanolic solution of 70% $HClO_4$ (3 ml, 0.3 M) was added and the mixture was stirred for 1 h. The solvent was removed under reduced pressure. Addition of a few drops of ether to a methanolic solution of the residue

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gave 80 mg (65%) of **24$^+$-^{13}C-d_4-ClO$_4^-$**: mp 125–127 °C (dec.) (lit.[11] mp 138–139 °C for **24$^+$ClO$_4^-$**); ^{1}H NMR (500 MHz, CD_3OD) δ 3.32 (m, 2H), 3.60 (d, $^1J_{CH} = 187$ Hz, 1H), 4.12 (m, 2H), 6.95 (m, 1H), 7.55 (m, 3H), 7.84 (m, 2H), 8.51 (m, 1H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 24.9, 40.5 (^{13}C), 47.3, 113.3, 127.2, 129.1, 132.6, 134.8,160.2, 163.4. FAB-HRMS: Calcd for $C_{13}{}^{13}CH_{12}D_4N^+$: 203.1567. Found: 203.1562.

*1-Cyclopropyl-4-phenyl-2,6-$^{13}C_2$-1,2,3,6-tetrahydropyridinium oxalate salt (**22-$^{13}C_2 \cdot C_2H_2O_4$**)*[17]

A 50/50 mixture of ^{13}C-labeled and unlabeled formaldehyde was used. Mass selection allowed us to examine specifically the fragmentation properties of the 2,6-$^{13}C_2$ product.

A mixture of cyclopropylamine hydrochloride (486 mg, 5.2 mmol), H^{13}CHO (200 mg, 6.5 mmol) and H^{12}CHO (200 mg, 6.5 mmol) was heated at 60 °C for 10 min at which time α-methylstyrene (0.35 ml, 2.6 mmol) was added. After 8 h at 60 °C, MeOH (5 ml) was added and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue in 37% HCl (5 ml) was heated under reflux for 1 h. The reaction mixture was poured slowly into a saturated aqueous solution of K_2CO_3; the crude CH_2Cl_2 soluble product was purified on basic alumina chromatography (hexanes : ethyl acetate-9 : 1). The oxalate salt **22-$^{13}C_2 \cdot C_2H_2O_4$** was obtained from methanol: mp 183–184 °C (lit[14] mp 185–186 °C for **22 · $C_2H_2O_4$**); ^{1}H NMR (500 MHz, CD_3OD) δ 1.01 (m, 4H), 2.88 (m, 3H), 3.64 (dt, $J = 10$ Hz, $^1J_{CH} = 146$ Hz, ~1H), 3.65 (t, $J = 10$ Hz, ~1H), 4.03 (m, ~1H), 4.11 (d, $^1J_{CH} = 146$ Hz, ~1H), 6.14 (m, 1H), 7.35 (m, 3H), 7.46 (m, 2H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 3.3, 24.5, 38.3, 50.1 (^{13}C), 51.6 (^{13}C), 115.3, 124.7, 128.0, 128.4, 135.6, 138.7, 163.7. Calcd for $C_{12}{}^{13}C_2H_{18}N^+$: 202.1506. Found: 202.1510.

*1-Cyclopropyl-4-phenyl-2,6-$^{13}C_2$-2,3-dihydropyridinium perchlorate (**24H$^+$-$^{13}C_2$-ClO$_4^-$**)*

The procedure described for the synthesis of **24-^{13}C-d_4** gave **24H$^+$-$^{13}C_2 \cdot ClO_4^-$** in 60% yield: mp 128–130 °C (dec.) (lit[11] mp 138–139 °C for **24H$^+ \cdot$ ClO$_4^-$**); ^{1}H NMR (500 MHz, CD_3OD) δ 1.16 (m, 2H), 1.27 (m, 2H), 3.33 (m, 2H), 3.63 (m, 1H), 4.12 (dt, $J = 10$ Hz, $^1J_{CH} = 146$ Hz, ~1H), 4.13 (t, $J = 10$ Hz, ~1H), 6.94 (m, 1H), 7.57 (m, 3H), 7.85 (m, 2H), 8.52 (m, 0.5H), 8.61 (m, 0.5H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 5.4, 24.9, 40.8, 47.5 (^{13}C), 113.2, 127.2, 129.1, 132.7, 134.8, 160.2, 163.4 (^{13}C). Calcd for $C_{12}{}^{13}C_2H_{16}N^+$: 200.1350. Found: 200.1353.

*1-Cyanomethyl-3-phenylpyrrole (**30**)*[18]

Small pieces of potassium (168 mg, 4.3 mmol) were added to 3-phenylpyrrole[19] (**29**, 614 mg, 4.3 mmol). After heating at 50 °C for 3 h, toluene (2 ml) was added, which was followed, under reflux, by the dropwise addition of chloroacetonitrile (1.4 ml, 21 mmol). After 48 h, the reaction mixture was partitioned between water and ethyl acetate. Work up of the organic phase gave an oil that was subjected to chromatography on silicagel (hexanes : ethyl acetate 7 : 3) to give 15 mg (2%) of **30** as a white solid: mp 92–93 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.82 (s, 2H), 6.54 (dd, $J = 3.0$, 1.5 Hz, 1H), 6.74 (t, $J = 2.5$ Hz, 1H), 7.00 (t, $J = 2.0$ Hz, 1H), 7.20 (m, 1H), 7.35 (m, 2H), 7.49 (m, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) 37.2, 108.9, 114.6, 117.5, 122.1, 125.4, 126.2, 128.7, 128.8, 134.9. FAB-HRMS: Calcd for $C_{12}H_{11}N_2^+$: 183.0922. Found: 183.0916.

Mass spectrometry

A TSQ equipped with an ESI source (Thermo Electron, San Jose, CA, USA) was used for the MS^1 and MS^2 analyses. Sample solutions (final analyte concentration 5 μM) in acetonitrile containing 1% formic acid were infused into the TSQ ion source at a total flow rate of 28 μl/min using a syringe pump (Harvard Apparatus, Holliston, MA). The following ESI operating conditions were used: The spray voltage was 4 kV, the temperature of the capillary inlet was 270 °C and the nitrogen sheath gas pressure was 8 psi. Collision induced dissociation (CID) studies were run at an isolation width of m/z 0.7 with argon as the collision gas at a pressure of approximately 1 mTorr. Data acquisition was performed with Xcalibur 1.2 software (Thermo Electron).

MS^3 spectra were acquired on a LTQ mass spectrometer (Thermo Electron, San Jose, CA) equipped with an in-house built nano-ESI source (LTQ). Samples, prepared in CH_3CN acidified with acetic acid (0.1%), were infused at 0.3 μl/min with the aid of a syringe pump (Harvard Apparatus, Holliston, MA) and electrosprayed at 1700 V. The ion source capillary inlet temperature was set at 200 °C. CID parameters were set at isolation width 1.4 m/z, normalized collision energy 50%, activation Q 0.25 and activation time 30 ms. Ion accumulation was allowed for 500 ms with enabled automatic gain control. Ten microscans were averaged to generate a mass spectrum. Data acquisition was performed with Xcalibur 1.2 software (Thermo Electron).

RESULTS AND DISCUSSION

Initial attempts to prepare the dihydropyridinium target compound **6H$^+$** by treatment of the diastereomeric *N*-oxides **8/9** with trifluoroacetic anhydride (TFAA), a reaction that has been employed successfully in the synthesis of other 1,2-dihydropyridinium analogs,[20] led unexpectedly and exclusively to the eniminium isomers **10$^+$** and **11$^+$** (Scheme 3).[10]

An alternative approach to this system, involving treatment of 4-phenyl-*trans*-1-(2-phenylcyclopropyl)pyridinium perchlorate (**12$^+$ClO$_4^-$**) with $NaBH_4$ in methanolic KOH,[21] gave the free base **6** in good yield (Scheme 4). The ^{1}H and ^{13}C NMR spectra are fully consistent with the assigned structure.

The TSQ MS^2 of **6** obtained at a collision induced dissociation energy (CIDE) of 23 V is shown in Fig. 1; the proposed rationalizations for the formation of the observed product ions are presented in Schemes 5 and 6. *C*-Protonation of **6** gives the thermodynamically preferred 2,3-dihydropyridinium species **6aH$^+$** (Scheme 5), which rearranges to yield the fused bicyclo system **13H$^+$**. Loss of H_2 from **13H$^+$** gives **i$^+$**, the structure assigned to the ion of low abundance at m/z 272. Alternatively, cleavage of the N–C bond of the cyclopropylaminyl group, accompanied by opening of the cyclopropyl ring, yields the phenyl vinyl carbocation **ii$^+$** (m/z 117) with 4-phenyl-2,3-dihydropyridine (**A**) as the neutral loss species. Cyclization of **ii$^+$** followed by dehydrogenation of the resulting fused

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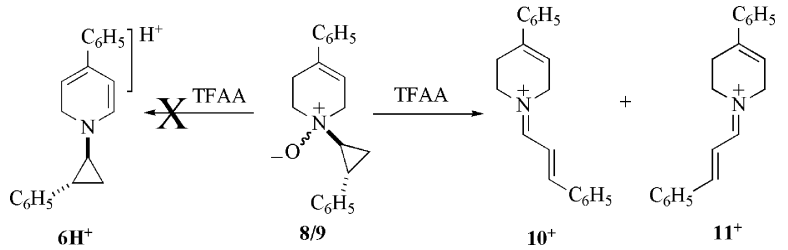
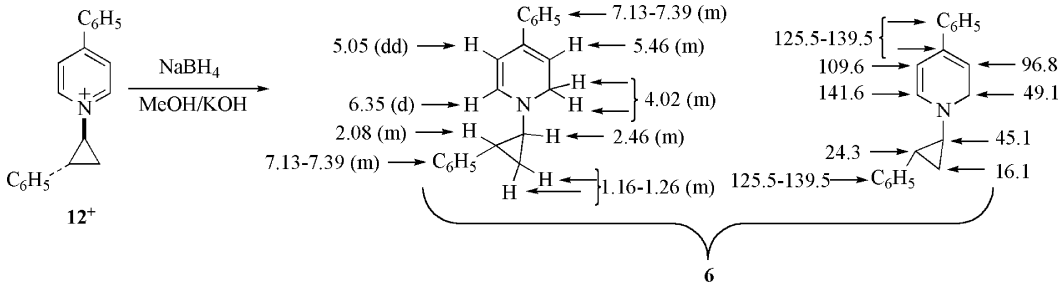


Scheme 3. Reaction of N-oxides **8/9** with TFAA.



Scheme 4. Synthesis and NMR characterization of **6**.

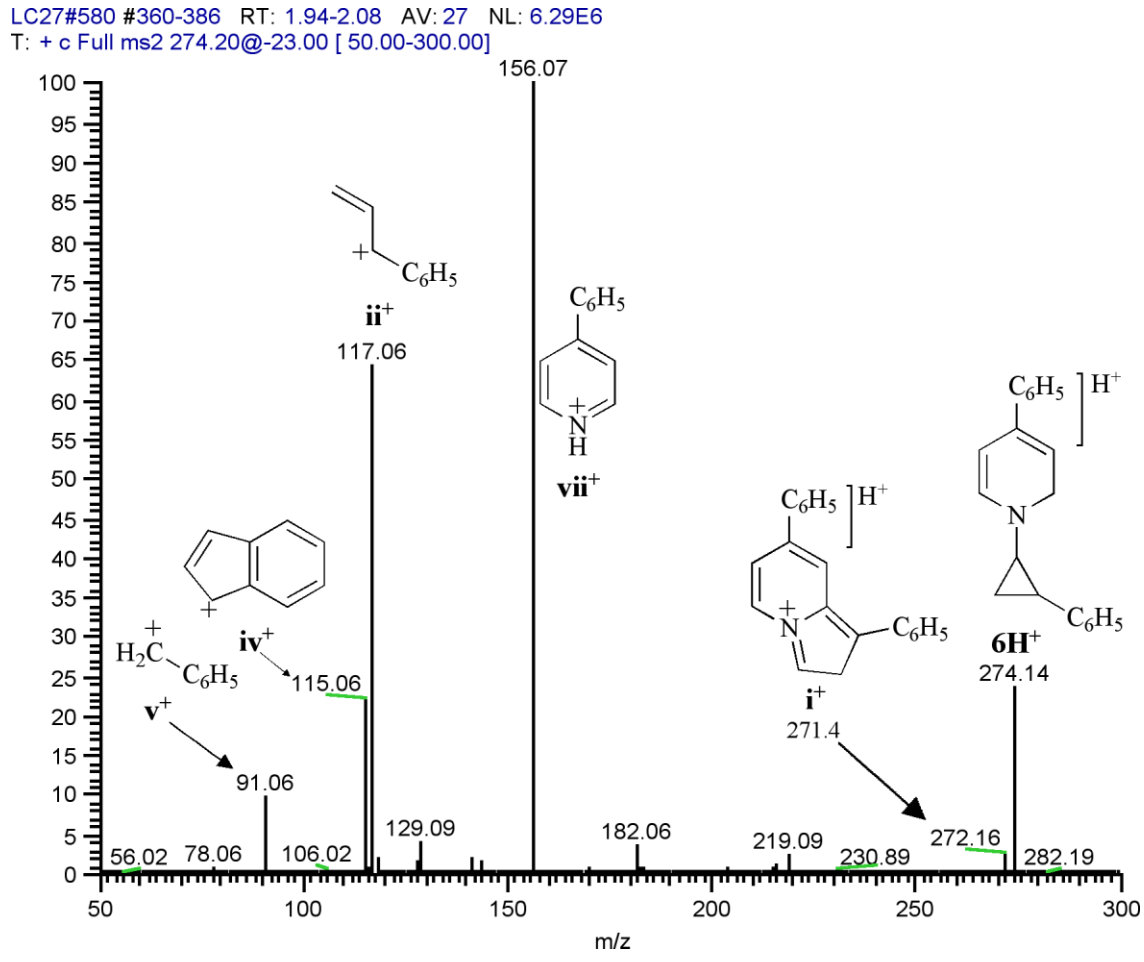


Figure 1. TSQ MS^2 of **6H+** obtained at a CIDE of 23 V.

bicyclo intermediate **iii**$^+$ generates **iv**$^+$ (m/z 115). Fragment **ii**$^+$ also may lose acetylene to form the benzylcarbocation **v**$^+$ (m/z 91). The formation of the fragment ion at m/z 129 is visualized as proceeding via **6a′H**$^+$ to yield **vi**$^+$ with the iminocyclopropanyl species **B** as the neutral loss.

Formation of the base peak fragment ion at m/z 156 is proposed to proceed via the kinetically preferred *N*-protonated species **6bH**$^+$ (Scheme 6). Cleavage of the *N*-cyclopropyl bond, this time with transfer of a proton from C(2) of the 1,2-dihydropyridinium moiety,

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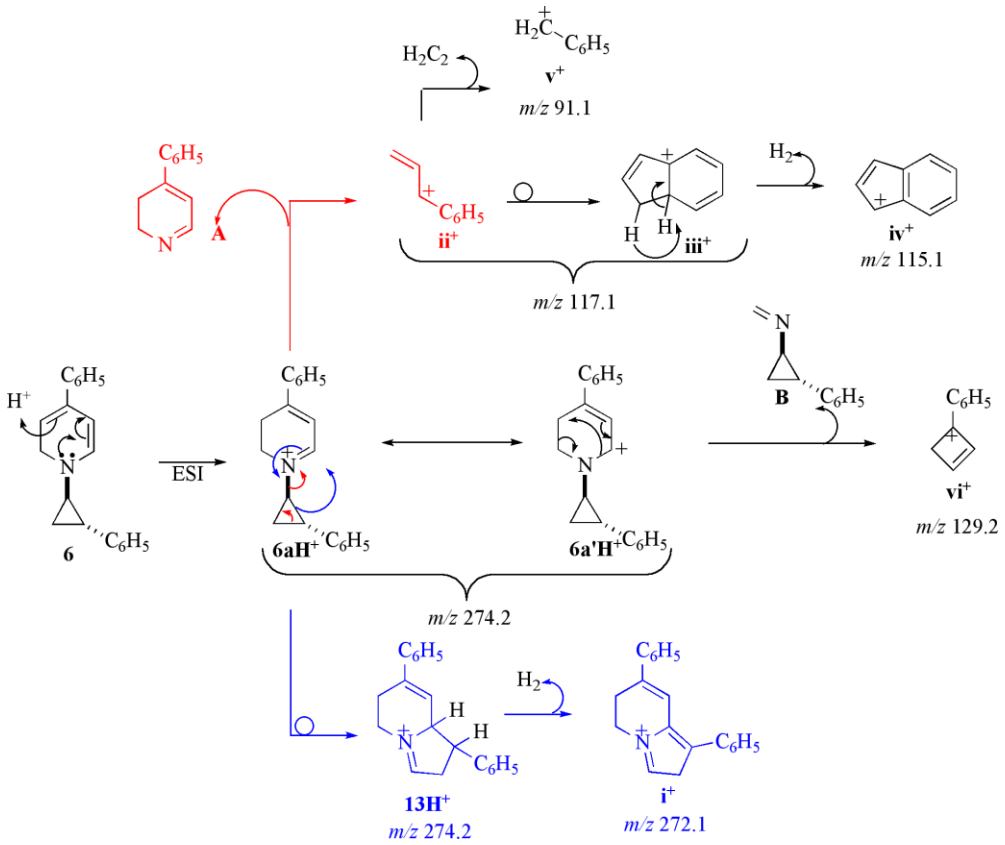
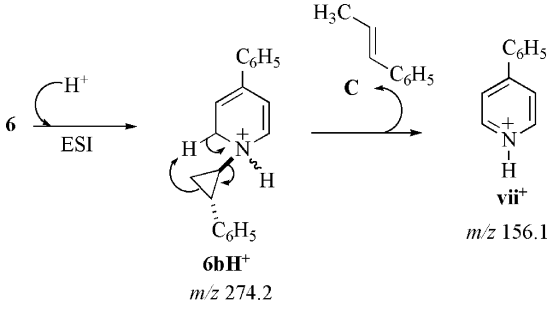


Scheme 5. Proposed scheme to account for the fragment ions observed in the TSQ MS^2 of **6**.

yields **vii**$^+$ and 1-phenylpropene (**C**) as the neutral loss species.

The C(2)-d_1 analog **6-d_1** was prepared by reaction of **12**$^+$ with $NaBD_4$. The TSQ MS^2 (CIDE 25 V) of **6-d_1** [**6H**$^+$**-d_1** m/z 275 (8%) → 273 (2%), 157 (100%), 130 (3%), 117 (70%), 115 (35%) and 91 (12%)] was consistent with the pathways shown in Scheme 5. However, the fragmentation pathway proposed for the formation of **vii**$^+$ [**6** → **6bH**$^+$ → **vii**$^+$] (Scheme 6) should lead, in the case of **6-d_1**, to a mixture of undeuterated and monodeuterated fragment ions at m/z 156 and m/z 157, respectively (Scheme 7). The ion current intensity at m/z 156 in the TSQ MS^2 of **6-d_1**, however, was below the limits of detection of the triple quadrupole instrument. Furthermore, the MH^+ ion of a synthetic sample of 4-phenylpyridinium chloride (m/z 156) was stable when exposed in the LTQ to a normalized collision energy of 40%, whereas an MS^3 experiment established that the fragment ion in the LTQ MS^2 of **6** at m/z 156 fragmented at a normalized collision energy of 25%. These results forced us to consider an alternative structure for the m/z 156 fragment ion.



Scheme 6. Pathway leading to the proposed fragment ion **vii**$^+$.

The presence of a phenyl substituent on both the dihydropyridinyl and cyclopropyl groups of **6** introduced some ambiguity with respect to the identity of the phenyl group present in the m/z 156 fragment ion. Consequently we examined the TSQ MS^2 of 4-(4-methylphenyl)-1-(2-*trans*-phenylcyclopropyl)-1,2-dihydropyridine (**19**). The synthesis of **19** (Scheme 8) was achieved via a reaction sequence involving the coupling of 4-methylphenylboronic acid (**14**) and 4-bromopyridine (**15**) in the presence of $Pd(PPh_3)_4$ to give 4-(4-methylphenyl)pyridine (**16**).[15] Reaction of **16** with 2,4-dinitrochlorobenzene (2,4-DNCB) led to the pyridinium intermediate **17**$^+$**Cl**$^-$, which formed, upon heating with *trans*-2-phenylcyclopropylamine (*t*-2-PCA) in BuOH, the phenylcyclopropylpyridinium product **18**$^+$ and 2,4-dinitroaniline (2,4-DNA).[12] The perchlorate salt of **18**$^+$ underwent smooth reduction with $NaBH_4$ in methanolic KOH to give the desired dihydropyridine **19**.

The base peak present in the TSQ MS^2 of **19** (CIDE 23 V) [**19H**$^+$ m/z 288 (10%) → 286 (2%), 156 (100%), 129 (2%) 117 (45%), 115 (8%) and 91 (2%)] again appeared at m/z 156. Therefore, the phenyl group present in this fragment ion is derived from the phenyl group of the cyclopropyl moiety. This outcome ruled out the pathway **6** → **6bH**$^+$ → **vii**$^+$ (Scheme 6).

Additional studies were undertaken with the aid of the known[14] 1-cyclopropyl-4-phenyl-2,3-dihydropyridinium species **24H**$^+$ and two stable isotopically labeled analogs, namely, **24H**$^+$**-^{13}C$_2$** and **24H**$^+$**-^{13}C-d_4**. The synthetic sequence

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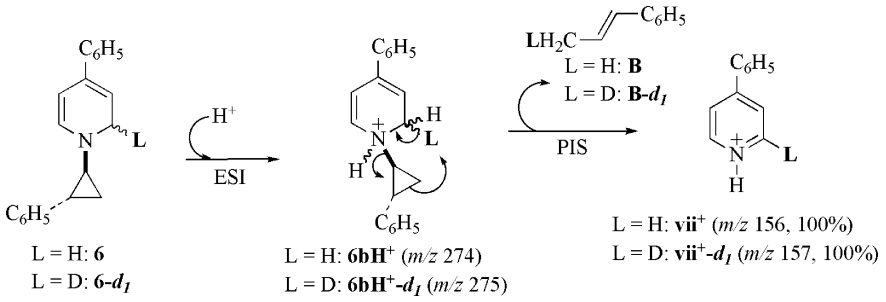
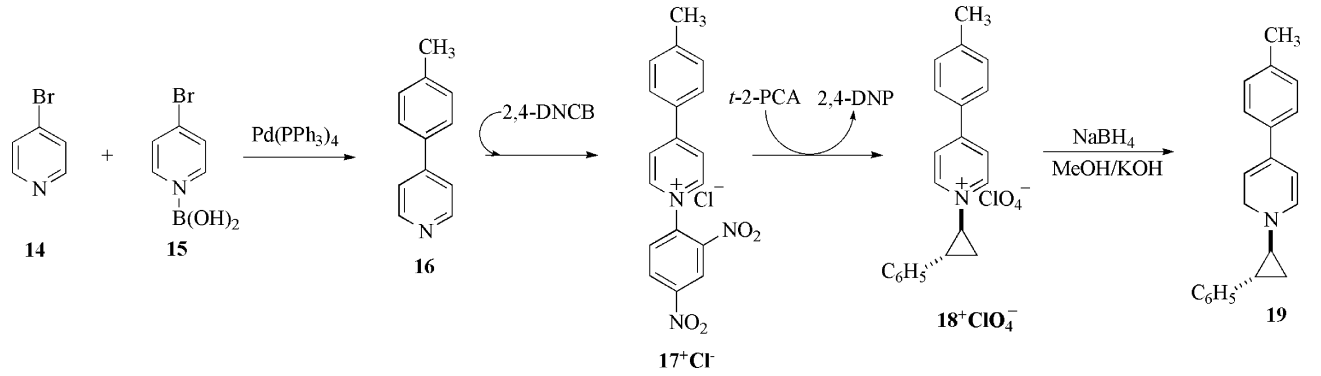


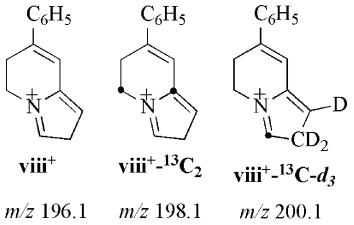
Scheme 7. Fragmentation of the putative MH^+ ions **6bH⁺** and **6bH⁺**$-d_1$ to yield **vii⁺** and **vii⁺**$-d_1$.



Scheme 8. Synthesis of the 4-(4-methylphenyl)dihydropyridinyl analog **19**.

to **24H⁺-^{13}C$_2$** started with **22-^{13}C$_2$**, which was obtained from the condensation of α-methylstyrene, cyclopropylamine and ^{13}C-labeled formaldehyde (Scheme 9).[17] The preparation of **24H⁺-^{13}C-d_4** required **22-^{13}C-d_4**, which was obtained by treatment of 4-phenyl-1,2,3,6-tetrahydropyridine (**21**) with H^{13}COOEt followed by construction of the cyclopropyl ring from the resulting formamide **22-^{13}C** by reaction with C_2D_5MgBr in the presence of titanium tetraisopropoxide [$Ti(OPr_i)_4$].[16] Treatment of these tetrahydropyridinyl intermediates with *m*-CPBA gave the corresponding *N*-oxides **23, 23-^{13}C$_2$** and **23-^{13}C-d_4**, which were converted into the desired *N*-cyclopropyldihydropyridinium products **24H⁺**, **24H⁺-^{13}C$_2$** and **24H⁺-^{13}C-d_4** with TFAA. The unlabeled dihydropyridinyl analog **24** was prepared by reduction of the known[14] pyridinium species **25⁺** with $NaBH_4$ in methanolic KOH. Treatment of **24** with methanolic HCl gave a good yield of **24H⁺Cl⁻**. The conversion of **24** into **24H⁺** documents that the protonation of 1-cyclopropyl-1,2-dihydropyridines in methanol yields the thermodynamically preferred *C*-protonated products.

As expected, the TSQ MS^2 of synthetic **24** and synthetic **24H⁺** obtained at a CIDE of 24 V were identical – **24H⁺** m/z 198 (10%) → 196 (5%), 156 (6%), 141 (6%), 128 (6%), 115 (7%), 91 (10%) and 80 (100%). Therefore, protonation of **24** under ESI conditions also takes place on carbon. Structure **viii⁺** was assigned to the ion of low abundance at m/z 196 by analogy with the sequence **6aH⁺** → **13H⁺** → **i⁺** + H_2 (Scheme 4). Confirmation of this assignment was obtained with the spectra of **24H⁺-^{13}C$_2$** (MH^+ at m/z 200) and **24H⁺-^{13}C-d_4** (MH^+ at m/z 203) that displayed the corresponding weak signals at m/z 198 (**viii⁺-^{13}C$_2$**) and 200 (m/z **viii⁺-^{13}C-d_3**), respectively, which is fully consistent with the proposed ring expansion-dehydrogenation pathway.



The molecular composition of the base peak in the TSQ MS^2 of **24H⁺** at m/z 80 could be assigned tentatively to $C_5H_6N^+$. The mass of this ion shifted to m/z 82 in the corresponding spectrum of **24H⁺-^{13}C$_2$**, establishing that both C(2) and C(6) of the dihydropyridinyl ring are retained in the base fragment ion peaks in this series. The corresponding fragmentation in the TSQ MS^2 of **24H⁺-^{13}C-d_4** led to a 1 : 1 doublet at m/z 83 and 84 (Fig. 2).

It is apparent from the available evidence that the carbon atoms identified in structures **24** and **6** are retained in the fragment ions at m/z 80 and 156, respectively. Furthermore, in order to accommodate the fragment ion at m/z 84, three of the four deuterium atoms colored blue in **24-^{13}C-d_4** must be retained. As this fragment ion also appears at m/z 83, the fragmentation pathway also must include a mechanism to account for loss of one of these three deuterons. Structures

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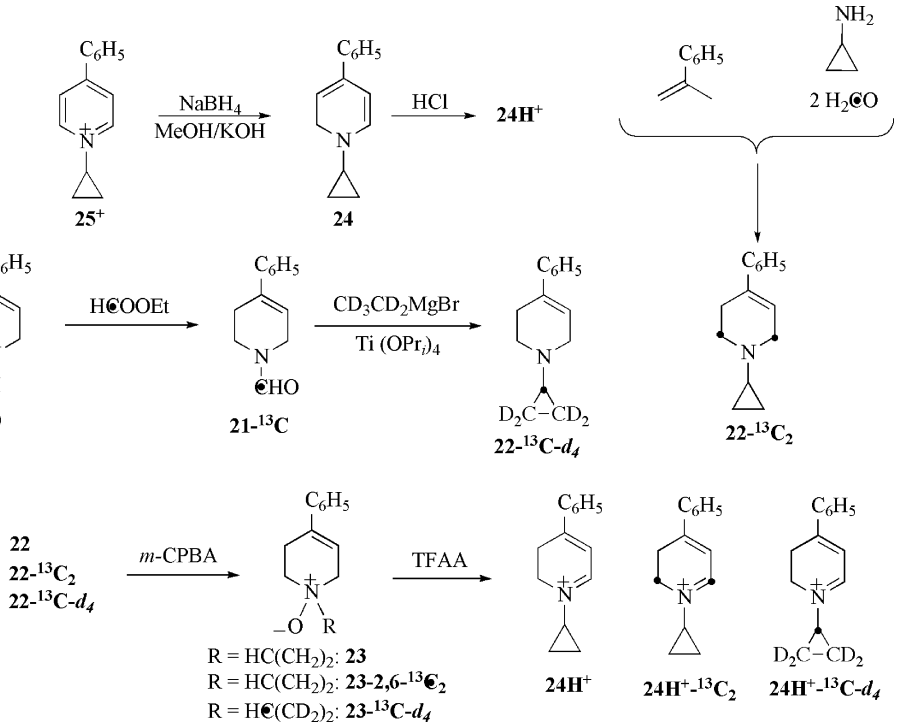

Scheme 9. Synthetic pathways to **24H⁺**, **24H⁺-$^{13}C_2$** and **24H⁺-^{13}C-d_4**.

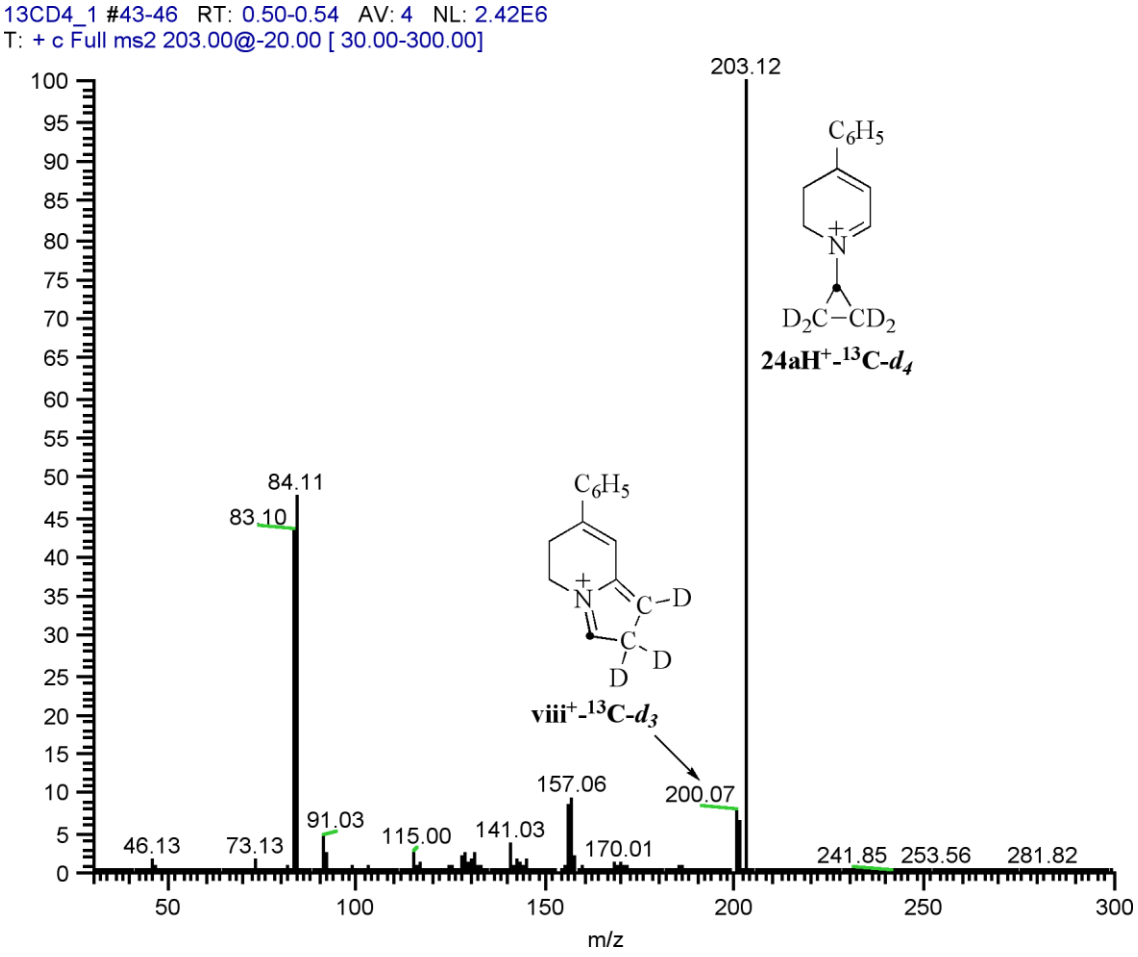

Figure 2. The TSQ MS^2 of **24aH⁺-^{13}C-d_4** obtained at a collision energy of 20 V.

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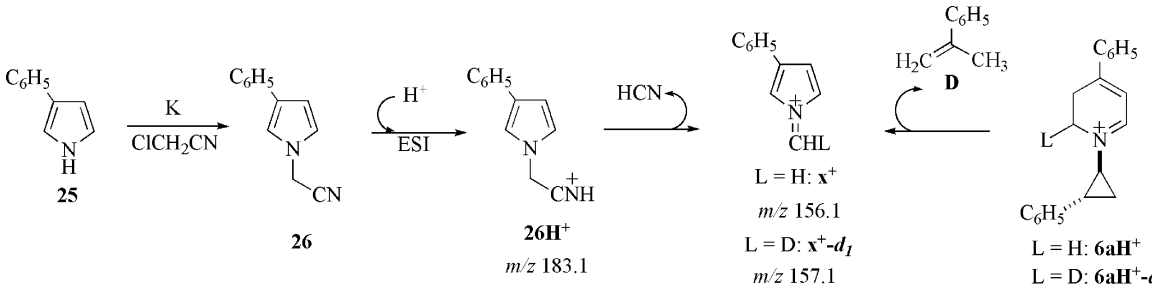
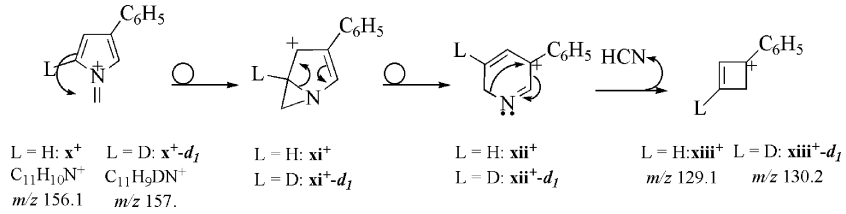
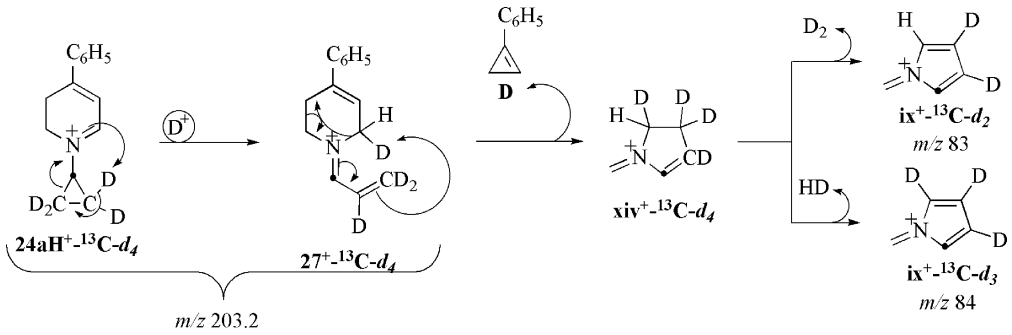


Scheme 10. Synthetic pathway to 1-cyanomethyl-3-phenylpyrrole and the convergent fragmentation of **26H**$^+$ and **6aH**$^+$ to **x**$^+$ and of **6aH**$^+$-d_1 to **x**$^+$-d_1.

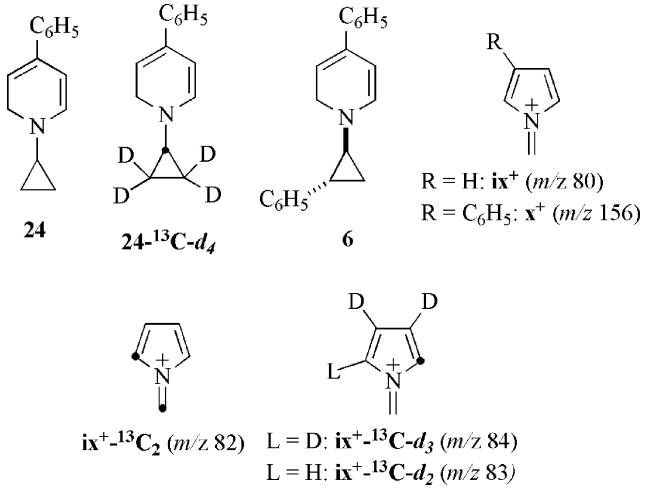


Scheme 11. Pathway to account for the LTQ MS^3 fragmentation of 5-azoniafulvenyl ions **x**$^+$ and **x**$^+$-d_1.



Scheme 12. Proposed pathway to account for the formation of azoniafulvenyl fragment ions **x**$^+$-^{13}C-d_3 and **x**$^+$-^{13}C-d_2 observed in the TSQ MS^2 of **24aH**$^+$-^{13}C-d_4.

that are consistent with these data are the 5-azoniafulvenyl species shown below, right.



Evidence supporting the 5-azoniafulvenyl assignment was sought with the aid of 1-cyanomethyl-3-phenylpyrrole (**26**), a compound that could be prepared, albeit in poor yield, by treatment of the potassium salt of the known 3-phenylpyrrole (**25**)[19] with chloroacetonitrile (Scheme 10).[18] As expected, the TSQ MS^2 spectrum of **26** displayed a major fragment ion at m/z 156 for the 5-azoniafulvenyl species **x**$^+$. We reasoned that identical MS^3 fragmentation patterns of the m/z 156 ions derived from **6** and **26** would provide convincing evidence supporting the structure of **x**$^+$.[22]

The LTQ MS^3 tracings for m/z 156 derived from the LTQ MS^2 of **26** and **6** are presented in Fig. 3 together with the corresponding MS^3 tracing of m/z 157 present in the MS^2 of **6**-d_1 (discussed below). The only significant MS^3 fragment ion of m/z 156 derived from **26** appears at m/z 129. The MS^3 of m/z 156 derived from **6** also displays m/z 129 as the only significant fragment ion. We conclude, therefore, that the m/z 156 fragment ions derived from **26** and from **6** must be the same, i.e. **x**$^+$!

The fragmentation m/z 156 → m/z 129 may proceed via the fused bicyclo species **xi**$^+$, which ring expands to **xii**$^+$. Loss of HCN from **xii**$^+$ gives **xiii**$^+$ (Scheme 11). This analysis is consistent with the LTQ MS^3 features of the fragment ion **x**$^+$-d_1 [**x**$^+$-d_1 → **xi**$^+$-d_1 → **xii**$^+$-d_1 → **xiii**$^+$-d_1] generated in the LTQ MS$^+$ of **6**-d_1 and is analogous to the pathway proposed for the formation of the cyclobutenyl cation observed in the EI-MS spectrum of 1-butylpyrrole.[23]

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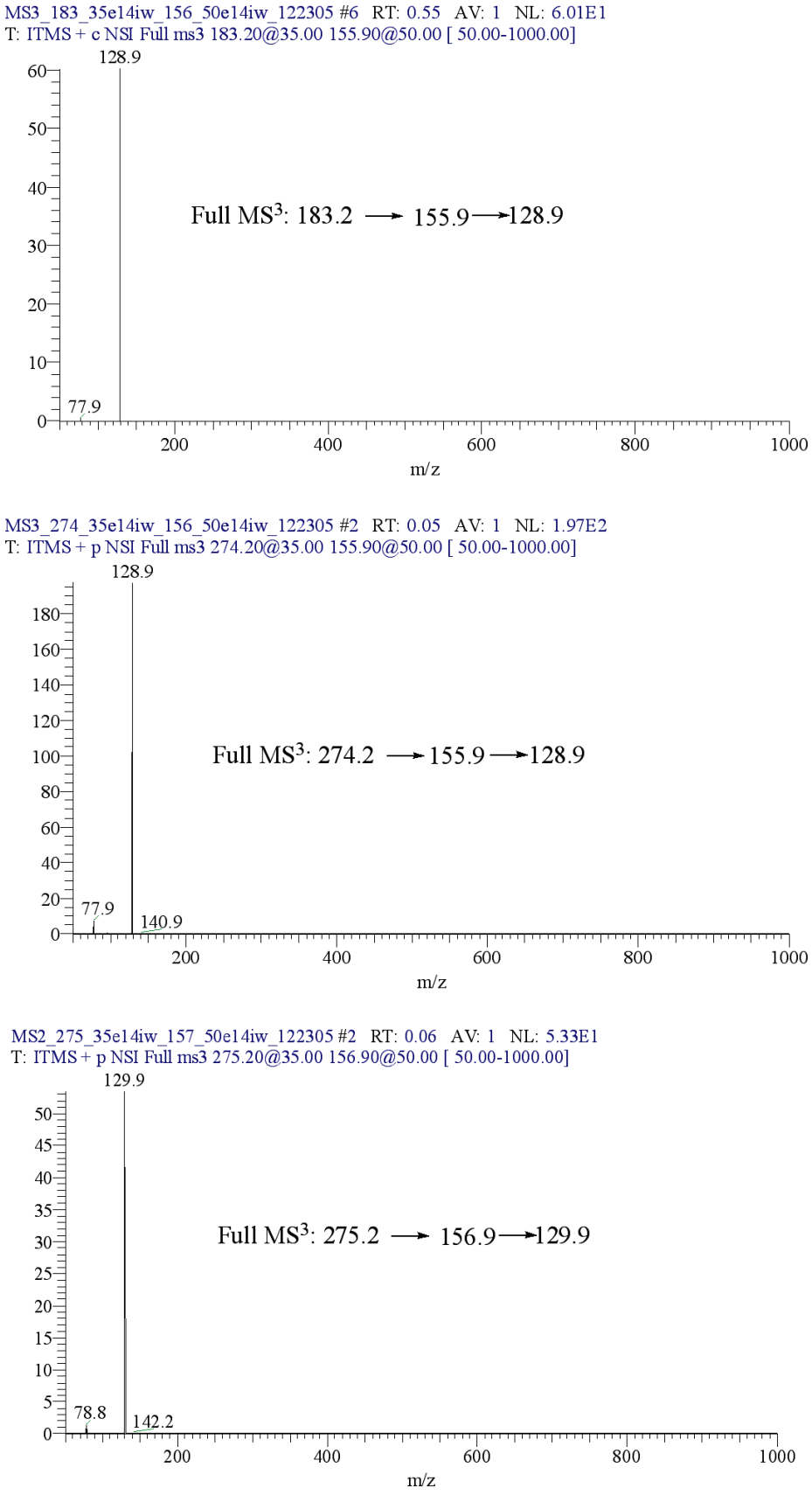

Figure 3. Full LTQ MS^3 tracings of ions at *m/z* 156 derived from **26H$^+$** (top) and **6H$^+$** (middle) and of *m/z* 157 derived from **6H$^+$-*d*$_1$** (bottom).

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An attempt to devise a pathway to accommodate the CID fragmentation of these dihydropyridinium species is presented in Scheme 12 with **24aH$^+$-^{13}C-d_4**. The initial reaction involves deuteride transfer from the methylene of the cyclopropyl group to C(6) of the dihydropyridinium species with concomitant ring opening to give the eniminium species **27H$^+$**. This intermediate then fragments to give **xiv$^+$-^{13}C-d_4** with 1-phenylcyclopropene (**D**) as the neutral loss species. Loss of D_2 from **xiv$^+$-^{13}C-d_4** leads to **ix$^+$-d_2** (m/z 83) whereas loss of HD leads to **ix$^+$-d_3** (m/z 84). This analysis predicts structure **x$^+$** for the m/z 156 fragment ion observed in the TSQ MS^2 of *trans*-(2-phenylcyclopropyl)-4-phenyl-1,2-dihydropyridine (**6**) and structure **x$^+$-d_1** for the m/z 157 fragment ion observed in the TSQ MS^2 of **6-d_1** (Scheme 10).

CONCLUSIONS

The structures of the base peaks observed in the TSQ MS^2 of various 1-cyclopropyl-1,2-dihydropyridines proved not to be the expected pyridinium species. Instead, a rather complex fragmentation process appears to take place leading to 5-azoniafulvenyl products. With the aid of structural analogs and a comparison of the MS^3 of the m/z 156 base peak present in the LTQ MS^2 of the dihydropyridinyl analog **6** with the corresponding MS^3 of the m/z 156 base peak present in the LTQ MS^2 of the 1-cyanomethylpyrrolyl compound **26**, it was possible to assign the structure of this fragment ion as **x$^+$**. Analogous 5-azoniafulvenyl structures account for the corresponding base peaks observed in the TSQ MS^2 of the other 1,2-dihydropyridines reported in this paper. A possible pathway to account for the formation of these fragment ions is presented in Scheme 12. This pathway, while consistent with the results obtained with various structural and isotopically labeled substrates, may not be exclusive. A critical step involves an initial 1,5-sigmatropic hydrogen migration from one of the cyclopropyl methylene groups to C(6) of the protonated dihydropyridine, a proposal that may merit further exploration.

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