Supporting Information (7 pages)

N-Methylputrescine Oxidation During Cocaine Biosynthesis: Study of Prochiral Methylene Hydrogen Discrimination Using the Remote Isotope Method

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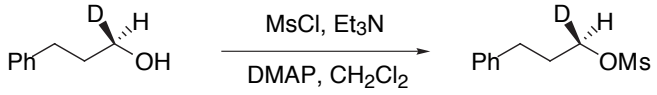
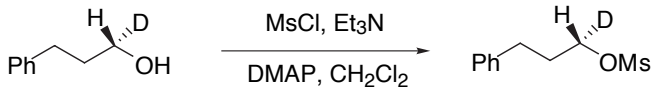
(1*R*)-Methanesulfonyl 1-^{2}H-3-Phenyl-1-propanol (101R)



11R **101R**

Alcohol **11R** (270 mg, 2.0 mmol) and triethylamine (450 μL, 3.2 mmol, 1.6 equiv) were dissolved in methylene chloride (10 mL) and cooled in an ice bath. Methanesulfonyl chloride (220 μL, 2.8 mmol, 1.4 equiv) was added, and the mixture was stirred at 0 °C for 5 minutes. The reaction mixture was warmed to room temperature and stirred overnight and poured into aqueous sodium bicarbonate. The mixture was stirred for 20 min, separated, and extracted with methylene chloride (2x10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to leave a yellow liquid (365 mg), which was purified by MPLC (2:1 Hx:EtOAc) to give **101R** as a colorless liquid (342 mg, 81%). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 7.17-7.35 (m, 5H, Ar-H), 4.21 (t, 1H, J = 6.5 Hz, $CH_2CHDOMs$), 2.99 (s, 3H, OSO_2CH_3), 2.75 (t, 2H, J = 7.5 Hz, $PhCH_2CH_2$), and 2.07 (q, 2H, J = 7.5 Hz, $CH_2CH_2CHDOMs$). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 140.38, 128.65, 128.50, 126.37, 68.89 (t, J = 23 Hz), 37.43, 31.59, and 30.60. **GC/ LRMS** (EI, 70 eV): *m/z* (rel int) 120 (M^+ - OMs, 9), 119 (83), 118 (100), 92 (13), 91 (44), 79 (15), and 77 (10).

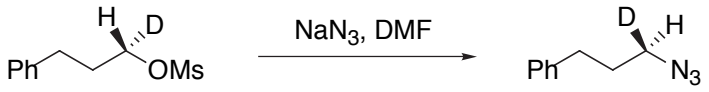
(1*S*)-Methanesulfonyl 1-^{2}H-3-Phenyl-1-propanol (101S)



11S **101S**

Compound **101S** was prepared from **11S** using a procedure analogous to the preparation of **101R** (120 mg, 86%). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 7.17-7.35 (m, 5H, Ar-H), 4.21 (t, 1H, *J* = 6.5 Hz, $CH_2CHDOMs$), 2.99 (s, 3H, OSO_2CH_3), 2.76 (t, 2H, *J* = 7.5 Hz, $PhCH_2CH_2$), and 2.07 (q, 2H, *J* = 7.5 Hz, $CH_2CH_2CHDOMs$). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 140.37, 128.65, 128.50, 126.37, 68.88 (t, *J* = 23 Hz), 37.44, 31.59, and 30.60. **GC/ LRMS** (EI, 70 eV): *m/z* (rel int) 120 (M^+ - OMs, 11), 119 (85), 118 (100), 92 (13), 91 (43), 79 (14), and 77 (10).

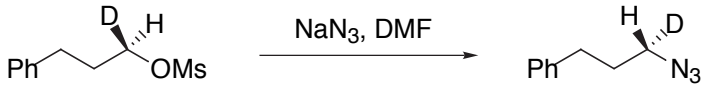
(1*R*)-1-Azido-1-^{2}H-3-phenylpropane (102R)



101S **102R**

Mesylate **101S** (218 mg, 1.01 mmol) was dissolved in DMF (5 mL) and stirred at 0 °C. Sodium azide (325 mg, 5 mmol) was added, and the suspension was stirred for 40 h at room temperature. Water (10 mL) was added, and the mixture was extracted with hexanes (3x20 mL). The combined extracts were washed with water, dried over $MgSO_4$, and evaporated to afford azide **102R** as a clear liquid (141 mg, 86%). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 7.16-7.33 (m, 5H, Ar-H), 3.27 (t, 1H, *J* = 6.5 Hz, CH_2CHDN_3), 2.71 (t, 2H, *J* = 7.5 Hz, $PhCH_2CH_2$), and 1.92 (q, 2H, *J* = 7.5 Hz, $CH_2CH_2CHDN_3$). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 140.88, 128.54, 128.47, 126.18, 50.34 (t, *J* = 21.8 Hz), 32.76, and 30.36. **GC/ LRMS** (EI, 70 eV): *m/z* (rel int) 134 (M^+ - N_2, 25), 133 (75), 132 (7), 118 (10), 105 (25), 104 (100), 103 (23), 91 (71), 79 (28), and 63 (11).

(1*S*)-1-Azido-1-^{2}H-3-phenylpropane (102S)

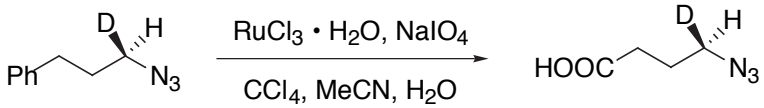


101R **102S**

Azide **102S** was prepared from mesylate **101R** using a procedure analogous to the preparation of **102R** from **101S** (1.12 g, 63%). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 7.16-7.33 (m, 5H, Ar-H), 3.27 (t, 1H, *J* = 6.5 Hz, CH_2CHDN_3), 2.70 (t, 2H, *J* = 7.5 Hz, $PhCH_2CH_2$), and 1.91 (q, 2H, *J* = 7.5 Hz, $CH_2CH_2CHDN_3$). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 140.92, 128.58, 128.50, 126.21, 50.40 (t, *J* = 21.5 Hz), 32.82, and 30.39. **GC/ LRMS** (EI, 70 eV): *m/z* (rel int) 134 (M^+ - N_2,

27), 133 (80), 132 (7), 118 (11), 106 (24), 105 (49), 104 (100), 103 (24), 91 (78), 79 (26), 78 (35), 77 (45), 65 (33), and 63 (13).

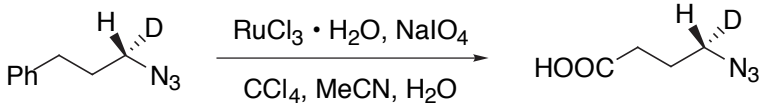
(4*R*)-4-Azido-4-^{2}H-butyric Acid (12R)



102R **12R**

Azide **102R** (866 mg, 5.34 mmol) was dissolved in carbon tetrachloride (21 mL), acetonitrile (21 mL), and water (40 mL). The biphasic mixture was stirred and cooled to 0 °C, and sodium periodate (17g, 79 mmol, 15 equiv) was added. To this vigorously stirred suspension ruthenium chloride trihydrate (75 mg, 0.29 mmol, 0.05 mol %) was added. The mixture was warmed to room temperature, stirred for 40 h, and suction-filtered. The filtered solid was washed with methylene chloride, and the filtrate layers were separated. The aqueous layers were extracted with methylene chloride (3x25 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated to a dark greenish-purple liquid. Ether was added to precipitate remaining ruthenium salts, and the mixture was eluted through a column of Celite. The filtrate was again dried over $MgSO_4$ and concentrated to give acid **12R** as a colorless liquid (535 mg, 77%). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.37 (t, 1H, *J* = 6.5 Hz, $CH_2C\underline{H}DN_3$), 2.48 (t, 2H, *J* = 7.5 Hz, $HOOCC\underline{H}_2CH_2$), and 1.92 (q, 2H, *J* = 7.5 Hz, $CH_2C\underline{H}_2CHDN_3$). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 178.82, 50.25 (t, *J* = 21.5 Hz), 30.94, and 23.92.

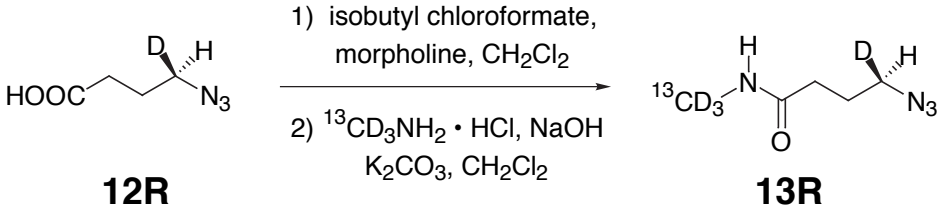
(4*S*)-4-Azido-4-^{2}H-butyric Acid (12S)



102S **12S**

Acid **12S** was prepared from phenylazide **102S** using a procedure analogous to the preparation of **12R** from **102R** (505 mg, 97%). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 3.37 (t, 1H, *J* = 6.5 Hz, $CH_2C\underline{H}DN_3$), 2.48 (t, 2H, *J* = 7.5 Hz, $HOOCC\underline{H}_2CH_2$), and 1.92 (q, 2H, *J* = 7.5 Hz, $CH_2C\underline{H}_2CHDN_3$). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 179.24, 50.25 (t, *J* = 21.5 Hz), 31.01, and 23.96.

(4*R*)-4-^{2}H-4-Azidobutyric Acid, (^{13}C^{2}H$_3$-Methyl)amide (13R)

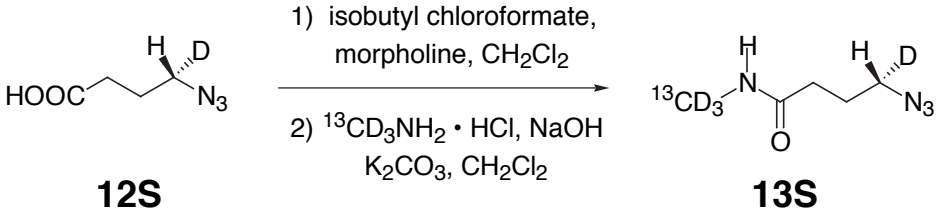


12R **13R**

4-Azidobutyric acid **12R** (336 mg, 2.5 mmol) was dissolved in methylene chloride (20 mL) under argon and cooled to 0 °C in an ice bath. Added dropwise to the stirred solution were N-methylmorpholine (300 μL, 2.73 mmol, 1.1 equiv) and isobutyl chloroformate (355 μL, 2.73 mmol, 1.1 equiv), which resulted in formation of a white precipitate. The mixture was stirred for 15 min. ^{13}C^{2}H$_3$-Methylamine hydrochloride (250 mg, 3.5 mmol) was suspended in methylene chloride (2 mL). Added to this suspension was 50% NaOH (300 μL), and the suspension was stirred gently. After 20 min K_2CO_3 (500 mg) was added, and the organic solution was transferred dropwise to the previously described mixture containing the mixed anhydride. The resulting mixture was stirred in the ice bath for 30 min and warmed to room temperature over 1.5 h. The reaction was quenched with 1M NH_4Cl (10 mL), and the layers were separated and extracted with methylene chloride (4x5 mL). The combined organics were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure (room temperature) to leave a yellow liquid (474 mg). The liquid was purified by MPLC (99:1 EtOAc:MeOH) to provide amide **13R** as a colorless liquid (266 mg, 73%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.78 (br s, 1H, CON<u>H</u>CH$_3$), 3.36 (t, 2H, J = 6.5 Hz, CH$_2$C<u>H</u>$_2$N$_3$), 2.28 (t, 2H, J = 7.5 Hz, CH$_2$C<u>H</u>$_2$CON), and 1.92 (tt, 2H, J = 6.5 and 7.5 Hz, CH$_2$C<u>H</u>$_2$CH$_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.52, 50.83 (t, J = 21.5 Hz), 33.04, 25.72 (septet, J = 21.5 Hz) and 24.83. **Anal.**: Calcd for C$_4$^{13}CH$_6$^{2}H$_4$N$_4$O: C, 41.49; H, 9.54. Found: C, 41.15; H, 6.60.*

* Discussion with the head analyst at MHW Laboratories (Phoenix, AZ) indicates that the amount of water and carbon dioxide is determined on a molar basis (thermal conductivity GC detection) and is largely insensitive to their isotopic composition (i.e., natural vs. non-natural abundance). We cannot entirely correct for this since the detector response for H_2O vs. HDO vs. D_2O and for $^{12}CO_2$ vs. $^{13}CO_2$ is not known.

(4*S*)-4-^{2}H-4-Azidobutyric Acid, (^{13}C^{2}H$_3$-Methyl)amide (13S)

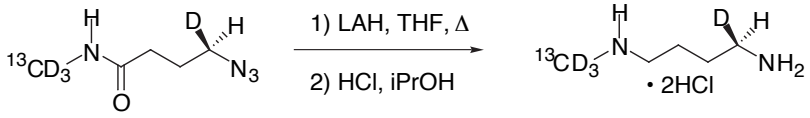


12S **13S**

Amide **13S** was prepared from 4-azidobutyric acid **12S** and ^{13}C^{2}H$_3$-methylamine hydrochloride using a procedure analogous to the preparation of **13R** (225 mg, 68%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.75 (br s, 1H, CONHCD$_3$), 3.36 (t, 2H, J = 6.5 Hz, CH$_2$CH$_2$N$_3$), 2.28 (t, 2H, J = 7.5 Hz, CH$_2$CH$_2$CON), and 1.93 (tt, 2H, J = 6.5 and 7.5 Hz, CH$_2$CH$_2$CH$_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 174.53, 50.80 (t, J = 21.5 Hz), 33.05, 25.64 (septet, J = 21 Hz), and 24.83. **Anal.**: Calcd for C$_4$^{13}CH$_6$^{2}H$_4$N$_4$O: C, 41.49; H, 9.54. Found: C, 40.97; H, 6.30.*

* Discussion with the head analyst at MHW Laboratories (Phoenix, AZ) indicates that the amount of water and carbon dioxide is determined on a molar basis (thermal conductivity GC detection) and is largely insensitive to their isotopic composition (i.e., natural vs. non-natural abundance). We cannot entirely correct for this since the detector response for H_2O vs. HDO vs. D_2O and for $^{12}CO_2$ vs. $^{13}CO_2$ is not known.

(4*R*)-(^{13}C^{2}H$_3$-Methyl)-4-^{2}H-putrescine dihydrochloride (3R)

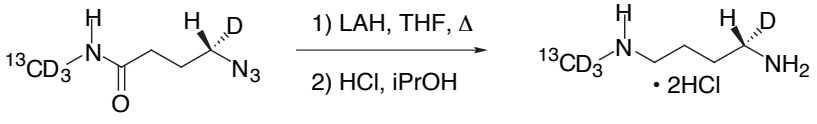


13R **3R**

Lithium aluminum hydride (235 mg, 6.2 mmol, 4.1 equiv) was dissolved in tetrahydrofuran (5 mL) and stirred in an ice bath. Added to the hydride solution was amide **13R** (223 mg, 1.5 mmol) in tetrahydrofuran (2 mL). The mixture was stirred in the ice bath for 5 min, warmed to room temperature over 25 min, and refluxed for 4.5 h. The mixture was then cooled in an ice bath, quenched (230 μL water, 230 μL 15% NaOH, and 700 μL water), and refluxed for 20 min. The mixture was suction filtered, and the aluminum cake was washed with ether (50 mL). To the combined ether layers was added a saturated solution of HCl in isopropyl alcohol until the ether layer indicated acidic to pH paper. The solution was stored at 0 °C overnight, and the precipitated diamine hydrochloride **3R** was collected by suction filtration (188 mg, 69%). A second crop of crystals was obtained (34 mg, 81%, mp 173-6 °C). **^{1}H NMR** (CD_3OD, 500 MHz) δ 3.04-2.97 (m, 3H) and

1.81-1.74 (m, 4H). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 41.27 (t, J = 21.5 Hz), 33.12 (septet, J = 21 Hz), 25.62, 25.57, and 24.26. **HRMS** (Electrospray FTMS) Calcd for $C_4{}^{13}CH_{10}{}^{2}H_4N_2$: 108.1516 ($M+H^+$). Found: 108.1510 ($M+H^+$).

(4*S*)-(^{13}C^{2}H$_3$-Methyl)-4-^{2}H-putrescine dihydrochloride (3S)



13S **3S**

Compound **3S** was prepared from **13S** using a procedure analogous to the preparation of **3R** from **13R** (133 mg, 58%, mp 177-8 °C). **^{1}H NMR** (CD_3OD, 500 MHz) δ 3.05-2.95 (m, 3H) and 1.81-1.73 (m, 4H). **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 41.35 (t, J = 21.5 Hz), 33.10 (septet, J = 21 Hz), 25.63, 25.57, and 24.32. **HRMS** (Electrospray FTMS) Calcd for $C_4{}^{13}CH_{10}{}^{2}H_4N_2$: 108.1516 ($M+H^+$). Found: 108.1514 ($M+H^+$).

Mass-Spectrometry Data. Mass-spectrometry data (both high and low resolution) for cocaine samples were obtained on a Finnigan MAT 95 double-focusing mass spectrometer with BE-geometry attached to a Hewlett-Packard Series II Model 5890 Gas Chromatograph with use of either EI or CI ionization capabilities. Samples were introduced as chloroform solutions. High resolution mass-spectrometry data for deuterated amine hydrochloride salts **3R** and **3S** were obtained using Analytica electrospray ionization source interface to a Finnigan 2001 FTMS instrument. Samples were introduced as aqueous solutions. Low resolution data for synthetic intermediates were obtained in electron impact (70 eV) mode on a HP-5971 MSD interfaced with an HP 5890 GC.

Feeding Experiments. A young *Erythroxylum coca* plant was chosen for the experiment and defoliated by cutting leaves off the tree with scissors. New leaves were allowed to grow to approximately the normal size. Precursor dihydrochloride salt **3** (20 mg) was dissolved in water (5 mL) and a drop of non-ionic detergent (Tween 80) was mixed with the solution. The resulting solution was applied twice daily on both top and bottom faces of leaves (~20 leaves for each precursor) until the solution was used up (~one week). After one additional week the leaves were harvested, cut in pieces with scissors, and mixed in a mortar with some sand. Liquid nitrogen was poured on top of this mixture. After one minute the leaves were crushed with a pestle. After evaporation of the nitrogen, a light-green powder remained. The powder was transferred into a 100 mL Erlenmeyer flask. Chloroform (~30 mL) and 10% $NaHCO_3$ solution (~6 mL) were added, and the contents were stirred in darkness for three days. The chloroform layer was concentrated *in vacuo*, and the contents were dissolved in diethyl ether and extracted with 3 x 10 mL of 0.5N HCl. The aqueous layer was basified with K_2CO_3 and extracted with 3 x 20 mL of chloroform. The organic layer was dried with $MgSO_4$ and evaporated to give crude cocaine (~20 mg), which was 50-90% pure by GC/MS and ^{1}H NMR analyses. The sample was subjected to GC/MS analysis [low resolution, EI

(70 eV) for natural cocaine and the cocaine resulting from the feeding of **3S** and low and high resolution for cocaine resulting from feeding of **3R**] without further purification. The relative peak intensity data reported in Table 1 are from the low resolution EI spectra.

Natural cocaine (**1**) **^{1}H NMR** ($CDCl_3$, 300 MHz, Varian VI-300, Me_4Si internal standard){COSY}: 8.03 d, 2H, J = 6.5 Hz, *ortho*-ArH {7.41}; 7.53 t, 1H, J = 6.0 Hz, *para*-ArH {7.41}; 7.41 t, 2H, J = 6.0 Hz, *meta*-ArH {8.03, 7.53}; 5.24 ddd, 1H, J = 12.0, 6.0 and 6.0 Hz, C<u>H</u>-OBz {3.02, 2.44, 1.87}; 3.72 s, 3H, COOMe {none}; 3.56 ddd, 1H, J = 6.7, 3.3 and 1.6 Hz, bridgehead H next to COOMe {3.02, 2.20 - 2.05}; 3.30 dddd, 1H, J = 7.0,3.4, 3.4 and 1.8 Hz bridgehead H away from COOMe {2.44, 2.20 - 2.05, 1.87}; 3.02 ddd, 1H, J = 5.9, 3.3 and 1.0 Hz, C<u>H</u>-COOMe {3.56}; 2.44 ddd 1H, J = 11.8, 11.8 and 3.1 Hz CH_α<u>H_β</u>-CHOBz (endo) {3.56, 1.87}; 2.23 s, 3H, N-Me, {none}; 2.20 - 2.05 m, 2H, C<u>H_α</u>H_β-C<u>H_α</u>H_β (exo){3.56, 3.30, 1.80 - 1.65}; 1.87 dddd, 1H, J = 11.9, 6.1, 3.3 and 1.2, C<u>H_α</u>H_β-CHOBz (exo) {3.30, 2.44}; 1.80 - 1.65 m, 2H, CH_α<u>H_β</u>-CH_α<u>H_β</u> (endo){2.20 - 2.05}. **LRMS**: *m/z* 306 (0.07%), 305 (0.76%), 304 (5.49%), 303 (9.85%, $M^{\cdot+}$), 302 (0.32%), 273 (1.68%), 272 (9.09%, $M^{\cdot+}$-$OMe^{\cdot}$), 199 (1.15%), 198 (9.51%), 184 (0.85%), 183 (9.35%), 182 (94.32%, $M^{\cdot+}$-$OBz^{\cdot}$), 181 (3.83%), 166 (1.24%), 155 (2.82%), 154 (1.22%), 152 (2.25%), 150 (1.22%), 123 (1.14%), 122 (5.82%), 120 (1.07%), 119 (1.78%), 110 (1.09%), 108 (2.60%), 106 (2.32%),105 (28.41%, Bz^+), 98 (1.15%), 97 (14.76%, $M^{\cdot+}$- MeOOC-CH_2-$CH^{\cdot}$-OBz), 96 (26.08%, $M^{\cdot+}$- MeOOC-CH=CH-OBz), 95 (3.10%), 94 (29.15%), 93 (1.97%), 91 (2.53%), 84 (2.68%), 83 (44.40%), 82 (100.00%, *N*-Methyl-Δ^1-pyrrolinium$^+$, 5-yl$^{\cdot}$), 81 (8.66%), 80 (1.77%), 79 (1.69%), 78 (2.43%), 77 (25.26%, Ph^+), 76 (1.63%), 70 (1.96%), 68 (3.44%), 67 (2.75%), 65 (1.48%).

Cocaine isolated from feeding 3S. **LRMS** (only listed are the peaks for the isotopic distribution patterns for the parent ions and selected key fragment ions that contain both the *N*-methyl and C(4)-hydrogen groups): *m/z* (rel int. as % of base peak) 308 (0.15%), 307 (0.52%), 306 (0.13%), 305 (1.12%), 304 (8.36%), 303 (48.36%), 302 (0.51%), ... 276 (0.17%), 275 (0.08%), 274 (0.39%), 273 (2.35%), 272 (13.54%), ... 187 (0.29%), 186 (1.26%), 185 (0.18%), 184 (1.24%), 183 (3.67%), 182 (100.00%), 181 (4.57%), ... 87 (0.42%), 86 (0.92%), 85 (0.27%), 84 (2.12%), 83 (35.36%), 82 (71.26%), and 81 (6.47%).

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Cocaine isolated from feeding 3R. **LRMS** (only listed are the peaks for the isotopic distribution patterns for the parent ions and selected key fragment ions that contain both the *N*-methyl and C(4)-hydrogen groups): *m/z* (rel int. as % of base peak) 308 (0.52%), 307 (0.05%), 306 (0.11%), 305 (1.00%), 304 (7.01%), 303 (49.61%), 302 (0.42%), ... 277 (0.17%), 276 (0.02%), 275 (0.06%), 274 (0.33%), 273 (2.08%), 272 (11.70%), ... 187 (1.46%), 186 (0.15%), 185 (0.12%), 184 (1.21%), 183 (12.07%), 182 (100.00%), 181 (4.61%), ... 87 (1.40%), 86 (0.25%), 85 (0.31%), 84 (2.58%), 83 (42.80%), 82 (89.67%), 81 (7.90%), ... High-resolution mass-spectrum: *m/z* calculated for $C_{16}{}^{13}C_1H_{17}{}^2H_4NO_4$: 308.1755, found 308.1731 (0.82%), *m/z* calculated for $C_{17}H_{21}NO_4$: 303.11471, found 303.1475 (27.09%).