Materials:

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Debromodeformylflustrabromine (starting material for halogenation to produce **3)** was synthesized according to previous reports.[1] Deuterated solvents were obtained from Cambridge Isotope labs. Silicycle silica gel plates (250 mm, 60 F254) were used for analytical TLC, and preparative chromatography was performed using SiliCycle SiliaFlash silica gel (230-400 mesh). Oligonucleotides were purchased from Integrated DNA Technologies (San Diego, CA). Plasmids pET-28a/RebF and pET-28a/RebH in BL-21 DE3 *E. coli* were provided by the Walsh group of Harvard Medical School, Boston, MA.[2] The pLIC-MBP plasmid was provided by the Bottomley group of Monash University, Clayton, Australia.[3] The pGro7 plasmid encoding the groES and groEL chaperone set was purchased from Takara (Otsu, Shiga, Japan). BL21(DE3) *E. coli* cells were purchased from Invitrogen (Carlsbad, CA). T7 DNA ligase, Taq DNA polymerase, and Phusion HF polymerase were purchased from New England Biolabs (Ipswitch, MA). Luria Broth (LB) and Terrific Broth (TB) media were purchased from Research Products International (Mt. Prospect, IL). Library colonies were picked using an automated colony picker (Norgren Systems). Qiagen Miniprep Kits were purchased from QIAGEN Inc. (Valencia, CA) and used according to the manufacturer's instructions. All genes were confirmed by sequencing at the University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility (900 E. 57th Street, Room 1230H, Chicago, IL 60637). Electroporation was carried out on a Bio-Rad MicroPulser using method Ec2. Ninitrilotriacetic acid (Ni-NTA) resin and Pierce® BCA Protein Assay Kits were purchased from Fisher Scientific International, Inc. (Hampton, NH), and the manufacturer's instructions were following when using both products (for Ni-NTA resin, 5 mL resin were used, with buffers delivered by a peristaltic pump at a rate of 1 mL/min, in a 4 °C cold cabinet). Amicon® 30 kD spin filters for centrifugal concentration were purchased from EMD Millipore (Billerica, MA) and used at 4,000 g at 4 °C. The glucose dehydrogenase GDH, FAD, and NAD were purchased from Codexis (Redwood City, CA). NADH was purchased from Chem-Impex International (Wood Dale, IL). Biotage reverse phase columns (SNAP KP-C18-HS) were purchased from Biotage.

General Procedures:

Standard molecular cloning procedures were followed.[4] Reactions were monitored using UPLC (Agilent 1200 UPLC with an Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 µM particle size; C18 4.6 x 50 mm column, 3.5 µM particle size; and C18 2.1 x 50 mm column, 1.8 µM particle size; solvent A = H2O/0.1% TFA, solvent B = CH3CN). Gel filtration was performed using a HiLoad 16/600 Superdex 200 column (GE Healthcare Life Sciences). Reverse phase preparative chromatography was carried out using a Biotage Isolera One. 1H spectra were recorded at 500 MHz on a Bruker DMX-500 or DRX-500 spectrometer, and chemical shifts are reported relative to residual solvent peaks.[5]

Experimental Procedures:

Library construction, expression, and screening: The procedures used for library construction, expression, and screening were adapted from those previously reported for the evolution of RebH for increased thermostability.[7] All genes encoding RebH were cloned into pET-28a between the NdeI and HindIII digestion sites. Mutant libraries were constructed by error-prone PCR, using Taq polymerase with 150 μM $MnCl_2$. PCR was performed in a volume of 50 μL with conditions of 95 °C 30 s, (95 °C 30 s, 55 °C 30 s, 72 °C 90 s) for 20 cycles, 72 °C 10 min. Beneficial mutations were recombined via overlap extension[6] with PCR conditions of 98 °C 30 s, (98 °C 10 s, 72 °C 50 s) for 35 cycles, 72 °C 10 min. Plasmids were transformed by electroporation into *E. coli* containing the chaperone pGro7. Library colonies were picked using an automated colony picker (Norgren Systems) and arrayed in 1-ml 96-well plates containing 300 μL LB with 50 μg/mL kanamycin and 20 μg/mL chloramphenicol. Cells were grown overnight at 37 °C, 250 rpm, and 50-100 μL of overnight culture was used to inoculate 1 mL TB (with 50 μg/mL kanamycin and 20 μg/mL chloramphenicol) in 2-mL 96-well plates. Following growth at 37 °C, 250 rpm, to an OD_{600} = 0.9-1.0, enzyme expression was induced with IPTG and arabinose to final concentrations of 10 μM and 0.2 mg/mL, respectively. Protein expression continued for ~20 h at 30 °C, 250 rpm, after which cultures were harvested by centrifugation and stored at -80 °C until use.

Cell pellets were thawed and suspended in 100 μL HEPES buffer (25 mM, pH 7.4) containing 0.75 mg/mL lysozyme. After incubation at 37 °C, 250 rpm, cells were flash frozen in liquid nitrogen and thawed in a 37 °C water bath. Ten microliters of DNaseI at 1 mg/mL were added and the cells incubated at 37 °C, 250 rpm, for 15 min. After centrifugation, 50 μL of supernatant were transferred to a microtiter plate for screening.

Similar to what was previously described for the evolution of RebH for increased thermostability,[7] a combined stock solution containing MBP-RebF (2.5 μM final concentration) and glucose dehydrogenase (9 U/mL final concentration) was added to 50 μL lysate. A second combined stock solution containing all small molecule components, including NaCl (10 mM final concentration), FAD (100 μM final concentration), NAD (100 μM final concentration), substrate (from a 10 mM stock solution in MeOH, 0.5 mM final concentration), and glucose (20 mM final concentration), all in 25 μM HEPES buffer (pH 7.4), was added to the reaction mixtures to initiate. Reactions were mixed, the plates sealed, and left overnight on the benchtop (increased activity on L-tryptophan and tryptoline) or shaken at 600 rpm (increased activity on debromodeformylflustrabromine). Reactions were quenched with an equal volume of methanol and centrifuged, and the supernatant was filtered and analyzed for chlorination of substrate via UPLC (Agilent 1200 UPLC with an Agilent Eclipse Plus C18 2.1 x 50 mm column, 1.8 μM particle size; solvent A = H_2O/0.1 % TFA, solvent B = CH_3CN; 0-0.5 min, B = 16%; 0.5-1.5 min, B = 16-80%).

Enzyme purification: The MBP-RebF and RebH variants used for analytical and 10 mg bioconversions was grown, expressed, lysed and purified according to a previous report.[7] An overnight starter culture was used to inoculate 50 mL TB (with 50 μg/mL kanamycin and 20 μg/mL chloramphenicol). Following

growth at 37 °C, 250 rpm, until OD_{600} = 0.6-0.8, enzyme expression was induced with IPTG and arabinose to final concentrations of 100 µM and 2 mg/mL, respectively. Protein expression continued for ~20 h at 30 °C, 250 rpm, after which cultures were harvested by centrifugation and stored at -80 °C until use. Cell pellets were thawed, suspended in 30 mL 25 mM HEPES (pH 7.4) and lysed by sonication while kept on ice (Qsonica S-4000 with a 0.5" horn; 5 x 1 min with 1 min rests, 20 % duty cycle delivering 40-50 W). After clarification by centrifugation, MBP-RebF and RebH variants were purified by Ni-NTA affinity chromatography and exchanged into a buffer of 25 mM HEPES (pH 7.4) and 10 % glycerol. Protein concentrations were measured using the Pierce® BCA Protein Assay Kit and protein stocks were then stored at -20 °C until use.

General Procedure for Analytical Bioconversions[7]: Substrate (37.5 nmol) was added to a 1.5 mL Eppendorf tube as a 10 mM solution in MeOH (evodiamine, **8**, was added as a solution in DMSO). Solutions of NAD (0.2 equiv., 100 µM final concentration), FAD (0.2 equiv., 100 µM final concentration), NaCl (20 equiv., 10 mM final concentration), and glucose dehydrogenase (9 U/mL final concentration GDH) were added to the reaction. This was diluted such that the final reaction volume was 75 µL with HEPES buffer, and RebH (0.005-0.05 equiv., 2.5-25 µM final concentration) and MBP-RebF (0.005 equiv., 2.5 µM final concentration) were added as solutions of HEPES/glycerol buffer (25 mM HEPES, pH 7.4, 10% glycerol v/v). The reaction was initiated with a solution of 1 M glucose (40 equiv., 20 mM final concentration), the tube was closed, and incubated at 25 °C at 600 rpm. Reactions were quenched by addition of a reaction volume of MeOH after 12 hours. These reactions were analyzed by UPLC (Agilent 1200 UPLC with an Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 µM particle size; solvent A = H_2O/0.1% TFA, solvent B = CH_3CN). The following method was used for all substrates: 0-10 min, B = 15%; 10-20 min, B = 15-100%; 20-24 min, B = 100%. Alternate buffer salts, concentrations, and pHs were tested as well, and we observed that up to 2.5-fold increased conversions could be obtained on relative to the conditions described above by using 300 mM HEPES, pH 7.4 for certain substrates (L-tryptophan, tryptoline, pindolol, and carazolol all showed increased conversions with this buffer substitution).

General Procedure for 10 mg Bioconversions[8]: Substrate (10.0 mg) was added to a crystallization dish (100 x 50 mm) as a solution in MeOH. Solutions of NAD (0.2 equiv., 100 µM final concentration), FAD (0.2 equiv., 100 µM final concentration), NaCl (20 equiv., 10 mM final concentration), and a glucose dehydrogenase (9 U/mL final concentration GDH) were added to the reaction. This was diluted to the appropriate volume with HEPES buffer, and RebH (0.01-0.05 equiv., 5-25 µM final concentration) and MBP-RebF (0.005 equiv., 2.5 µM final concentration) were added as solutions of HEPES/glycerol buffer (25 mM HEPES, pH 7.4, 10% glycerol v/v). The reaction was initiated with a solution of 1 M glucose (40 equiv., 20 mM final concentration), sealed with an AeraSeal film, and left on the benchtop at room temperature without shaking. These reactions were analyzed by UPLC (Agilent 1200 UPLC with an Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 µM particle size; solvent A = H_2O/0.1% TFA, solvent B = CH_3CN). The following method was used for all substrates: 0-10 min, B = 15%; 10-20 min, B = 15-100%; 20-24 min, B = 100%. Each reaction was allowed to continue for 3 days, at which time no additional

conversion was seen by UPLC and enzyme had visibly precipitated out. The bioconversions were quenched with HCl (5 M, until pH<2) and saturated with NaCl. Precipitated protein was filtered out through a pad of Celite and the filtrate brought to pH>12 through addition of NaOH (5M). The filtrate was extracted into CH_2Cl_2. The crude material was purified by reverse phase chromatography (Biotage SNAP-KP-C18-HS, gradient from pure H_2O to 40% CH_3CN/H_2O).

Determination of Kinetic Parameters for RebH Variants: Kinetic parameters were determined as was previously described.[8] Rates were determined by monitoring the conversion of 75-215 μM tryptoline in the presence of NAD (100 μM final concentration), FAD (100 μM final concentration), NaCl (10 mM final concentration), MBP-RebF (2.5 μM final concentration), glucose dehydrogenase (9 U/mL final concentration), glucose (20 mM final concentration), and phenol as an internal standard (10 mM in MeOH, 0.5 mM final concentration) at a final volume of 75 μL in a microtiter plate. RebH variants were added at a final concentration of either 25 μM for wild-type RebH and 1-PVM, 15 μM for 2-T, or 2.5 μM for 3-SS. The reactions were left shaking at 600 rpm at room temperature, then quenched at 15-60 minutes (all time points were collected in triplicate) by addition of 75 μL of MeOH. The precipitated protein was then removed by centrifugation and the reactions were filtered and analyzed by UPLC using the method described in the General Procedures. Product formation was quantitated by calculating the ratio of product to internal standard and fitting that value to a calibration curve prepared from known concentrations of each material. The kinetic parameters (K_m and k_{cat}) for each substrate were determined using the Hanes-Woolf plots (see p. 9-10) constructed from the substrate concentrations and the observed initial rates.

Detailed Isolation and Characterization:

6-chlorotryptoline (1): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of tryptoline. RebH variant 3-SS was added to a final concentration of 5 µM (0.01 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **1** in 78% yield (14.5 mg of **1**·TFA, 0.045 mmol). ^{1}HNMR spectrum was consistent with previous reports of this compound.[8] ^{1}H NMR (500 MHz, MeOD) δ 7.47 (d, *J* = 1.7 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.11 (dd, *J* = 8.6, 1.8 Hz, 1H), 4.44 (s, 2H), 3.58 (t, *J* = 6.1 Hz, 2H), 3.05 (t, *J* = 6.0 Hz, 2H). Only trace (<5%) 7-chlorotryptoline could be observed (consistent with previous reports of this compound[8]), but the low abundance (in an already small amount of material) and the fact that one of the 7-chlorotryptoline peaks is partially obscured by an impurity makes precise quantitation difficult. HRMS (ESI-TOF) calc'd for $C_{11}H_{11}N_2Cl$ [M + H]+: 207.0689 and 209.0660, found: 207.0645 and 209.0626.

6-chloroeleagnine (5): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of eleagnine. RebH variant 3-SS was added to a final concentration of 5 µM (0.01 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **5** in 83% yield (14.9 mg of **5**·TFA, 0.045 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.48 (d, *J* = 1.8 Hz, 1H), 7.33 (d, *J* = 8.6 Hz, 1H), 7.12 (dd, *J* = 8.6, 2.0 Hz, 1H), 4.78 (q, *J* = 6.6 Hz, 1H), 3.73 (m, 1H), 3.47 (m, 1H), 3.14 – 2.96 (m, 2H), 1.73 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 161.88, 161.61, 138.47, 128.35, 123.89, 120.44, 116.58, 111.55, 106.67, 42.05, 21.37, 18.83. HRMS (ESI-TOF) calc'd for $C_{12}H_{13}N_2Cl$ [M + H]+: 221.0846 and 223.0816, found: 221.0799 and 223.0795. Note: NOESY for this compound was performed using the free base of **5**, not the TFA salt.

7-chloropinoline (6): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of pinoline. RebH variant 3-SS was added to a final concentration of 10 µM (0.02 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **6** in 80% yield (13.9 mg of **6**·TFA, 0.040 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.37 (s, 1H), 7.09 (s, 1H), 4.41 (s, 2H), 3.88 (s, 3H), 3.58 (t, *J* = 6.0 Hz, 2H), 3.05 (t, *J* = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 151.75, 136.08, 120.36, 118.68, 105.09, 94.89, 55.43, 49.24, 41.11, 29.26, 22.80, 18.07, 16.32. HRMS (ESI-TOF) calc'd for $C_{12}H_{13}N_2OCl$ [M + H]+: 237.0795 and 239.0765, found: 237.0762 and 239.0747.

6-chlorotetrahydroharmine (7): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of tetrahydroharmine. RebH variant 3-SS was added to a final concentration of 25 µM (0.05 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **7** in 79% yield (13.3 mg of **7**·TFA, 0.037 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.46 (s, 1H), 7.01 (s, 1H), 4.74 (q, *J* = 6.6 Hz, 1H), 3.88

(s, 3H), 3.70 (m, 1H), 3.44 (m, 1H), 3.12 – 2.91 (m, 2H), 1.71 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 153.16, 137.49, 121.77, 120.09, 106.50, 96.30, 56.84, 50.64, 42.51, 30.67, 24.21, 19.47, 17.73. HRMS (ESI-TOF) calc'd for $C_{13}H_{15}N_2OCl$ [M + H]+: 251.0951 and 253.0922, found: 251.0897 and 253.0895.

6-chlorodebromodeformyllflustrabromine (3): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of debromodeformylflustrabromine. RebH variant 4-V was added to a final concentration of 25 μM (0.05 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **3** in 68% yield (11.0 mg of **3**·TFA, 0.028 mmol). ^{1}H NMR (500 MHz, MeOD) δ 7.45 (d, *J* = 8.4 Hz, 1H), 7.34 (s, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.19 (m, 1H), 5.17 (m, 2H), 3.15 (m, 4H), 2.74 (s, 3H), 1.54 (d, *J* = 1.5 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 145.86, 127.34, 126.67, 119.15, 117.77, 110.96, 110.48, 104.12, 49.26, 38.75, 32.27, 29.25, 26.90, 22.80, 21.54. HRMS (ESI-TOF) calc'd for $C_{16}H_{21}N_2Cl$ [M + H]+: 277.1472 and 279.1442, found: 277.1432 and 279.1422.

10-chloroyohimbine and 11-chloroyohimbine (referred to together as 4): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of yohimbine. RebH variant 4-V was added to a final concentration of 25 μM (0.05 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **4** in 54% yield (7.65 mg of **4**·TFA, 0.015 mmol). ^{1}H NMR (500 MHz, MeOD) δ 11.11 (s, 0.5H), 11.07 (s, 0.2H), 7.51 (d, *J* = 1.5 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 0.4H), 7.38 (s, 0.4H), 7.35 (d, *J* = 8.6 Hz, 1H), 7.15 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.08 (dd, *J* = 8.4, 1.6 Hz, 0.4H), 4.77 (d, *J* = 11.9 Hz, 1H), 4.34 (d, *J* = 2.1 Hz, 1H), 3.87 – 3.82 (m, 1H), 3.81 (s, 3H), 3.66 – 3.55 (m, 2H), 3.21 (t, *J* = 12.1 Hz, 2H), 3.10 (dd, *J* = 16.5, 5.3 Hz, 1H), 2.93 (d, *J* = 14.5 Hz, 1H), 2.47 (dd, *J* = 11.6, 2.4 Hz, 1H), 2.38 – 2.28 (m, 1H), 1.99 (dd, *J* = 13.8, 3.0 Hz, 1H), 1.74 (t, *J* = 13.5 Hz, 2H), 1.63 (m, 1H), 1.57 – 1.43 (m, 2H). ^{13}C NMR (126 MHz, MeOD) δ 172.92, 122.36, 118.90, 117.29, 112.44, 66.74, 61.52, 57.97, 52.67, 51.40, 50.91, 38.18, 34.24, 31.66, 31.49, 30.53, 24.47, 23.93, 21.99, 18.64. The previously described peak assignments represent a roughly 3:1 mixture of 10-chloroyohimbine and 11-chloroyohimbine, respectively – note the peaks in the aryl region of ^{1}H NMR (7.51 (d, *J* = 1.5 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 0.4H), 7.38 (s, 0.4H), 7.35 (d, *J* = 8.6 Hz, 1H), 7.15 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.08 (dd, *J* = 8.4, 1.6 Hz, 0.4H)). All peaks observed in the ^{13}C NMR observed with a signal:noise of >2:1 are reported – higher quality ^{13}C NMR data were not able to be obtained in a reasonable number of scans, given the small amount of isolated material available and the high percentage of tertiary and quarternary carbons present in these two compounds. HRMS (ESI-TOF) calc'd for $C_{21}H_{25}N_2O_3Cl$ [M + H]+: 389.1632 and 391.1602, found: 389.1601 and 391.1596.

7-chloropindolol (9): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of pindolol. RebH variant 4-V was added to a final concentration of 5 μM (0.01 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **9** in 93% yield (14.9 mg of **9**·TFA, 0.037 mmol). ^{1}H

NMR (500 MHz, MeOD) δ 7.21 (d, *J* = 3.0 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.62 (d, *J* = 3.1 Hz, 1H), 6.51 (d, *J* = 8.3 Hz, 1H), 4.36 – 4.25 (m, 1H), 4.15 (m, 2H), 3.47 (m, 1H), 3.37 – 3.17 (m, 2H, partially obscured by methanol residual peak), 1.37 (dd, *J* = 6.3, 5.1 Hz, 7H). ^{13}C NMR (126 MHz, MeOD) δ 150.79, 123.96, 120.58, 120.18, 109.60, 100.88, 99.39, 69.83, 65.59, 50.68, 47.22, 17.93, 17.40. HRMS (ESI-TOF) calc'd for $C_{14}H_{19}N_2O_2Cl$ [M + H]+: 283.1213 and 285.1184, found: 283.1181 and 285.1159.

8-chlorocarazolol (10): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of carazolol. RebH variant 4-V was added to a final concentration of 5 µM (0.01 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **10** in 79% yield (11.8 mg of **10**·TFA, 0.027 mmol). ^{1}H NMR (500 MHz, MeOD) δ 8.27 (d, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.44 – 7.37 (m, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 7.20 (t, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 4.44 (m, 1H), 4.30 (m, 2H), 3.49 (m, 1H), 3.44 – 3.27 (m, 2H, partially obscured by methanol residual peak), 1.38 (dd, *J* = 6.4, 5.0 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 154.88, 140.86, 139.40, 126.58, 126.25, 123.88, 123.57, 120.57, 111.96, 109.94, 102.64, 71.14, 67.01, 52.04, 48.61, 19.34, 18.78. HRMS (ESI-TOF) calc'd for $C_{18}H_{21}N_2O_2Cl$ [M + H]+: 333.1370 and 335.1340, found: 333.1337 and 335.1315.

8-chlorocarvedilol (11): The 10 mg bioconversion was conducted according to the general procedure, using 10.0 mg (1 equiv.) of carvedilol. RebH variant 4-V was added to a final concentration of 10 µM (0.02 equiv.). The reaction was monitored by UPLC. After reaction completion, the reaction mixture was purified according to the general procedure to afford **11** in 69% yield (9.4 mg of **11**·TFA, 0.017 mmol). ^{1}H NMR (500 MHz, MeOD) δ 8.24 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.99 (m, 3H), 6.93 – 6.85 (m, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 4.53 - 4.51 (m, 1H), 4.41 – 4.22 (m, 4H), 3.77 (s, 3H), 3.68 – 3.41 (m, 4H). ^{13}C NMR (126 MHz, MeOD) δ 153.47, 149.63, 146.98, 139.42, 137.99, 125.12, 124.81, 122.78, 122.54, 122.14, 120.91, 119.20, 115.08, 113.62, 111.86, 110.48, 108.55, 101.21, 69.82, 65.32, 64.74, 54.95, 50.21, 46.91, 29.26, 22.80. HRMS (ESI-TOF) calc'd for $C_{24}H_{25}N_2O_4Cl$ [M + H]+: 441.1537 and 443.1524, found: 441.1537 and 443.1552.

Demonstrations of Novel Selectivity:

Enzyme-Free Chlorinations: To demonstrate that the selectivity and/or reactivity we observed using our RebH variants 3-SS and 4-V (see main text, Table 2), we attempted to perform chlorinations in the absence of our enzymes to compare the activities and selectivities observed.

Three substrates were tested: **2**, tryptoline; **6**, pinoline; and **11**, carvedilol. Three chlorination conditions were initially tested, the first using N-chlorosuccinimide in MeCN[9] and the latter two using sodium hypochlorite in aqueous buffer (one in 25 mM HEPES, pH 7.4, and the second in 25 mM HEPES, pH 5.0).[10] In all three reaction conditions on all three substrates, 1 equiv. of the chlorinating reagent was added and the reactions proceeded at room temperature, at 600 rpm, for 12 hours. After addition of an equal volume of MeOH and analysis by LCMS, no conversion of **2** or **6** was observed, but low conversion of **11** was seen to at least two monochlorinated products and at least two dichlorinated products.

To try to see conversion of **2** or **6**, the same reaction chlorinating reagents described above were again employed, but this time under harsher reaction conditions. 10 equivs. of the chlorinating reagent were added to each reaction, and the reactions were heated to 50 °C, at 600 rpm, for 2 days. After addition of an equal volume of MeOH and analysis by LCMS, higher consumption of **11** was seen with the same product distribution described above with additional appearance of trichlorinated product as well (see chromatograms shown below). However, there were still no mono-, di-, or trichlorinated products observed in any of the reactions of **2** or **6**.

Fig. S1: *Shown above are the UV (280 nm) chromatogram and extracted ion chromatograms (for starting material, monochlorination, dichlorination, and trichlorination) for the chlorination of* ***11****, carvedilol, using 10 equiv. NaOCl in 25 mM HEPES, pH 7.4 at 50 °C for 2 days. Majority of starting material still remains, but at least two resolved monochlorination products, as well as di- and trichlorination products are visible.*

We therefore conclude from this experiment that with at least substrate **11** we observe novel selectivity, as we have isolated and characterized only a single monochlorinated product whereas at least two were observed in chlorinations in the absence of enzyme. Furthermore, our trials on substrates **2** and **6** were not able to produce cleanly chlorinated derivatives of these substrates, even at

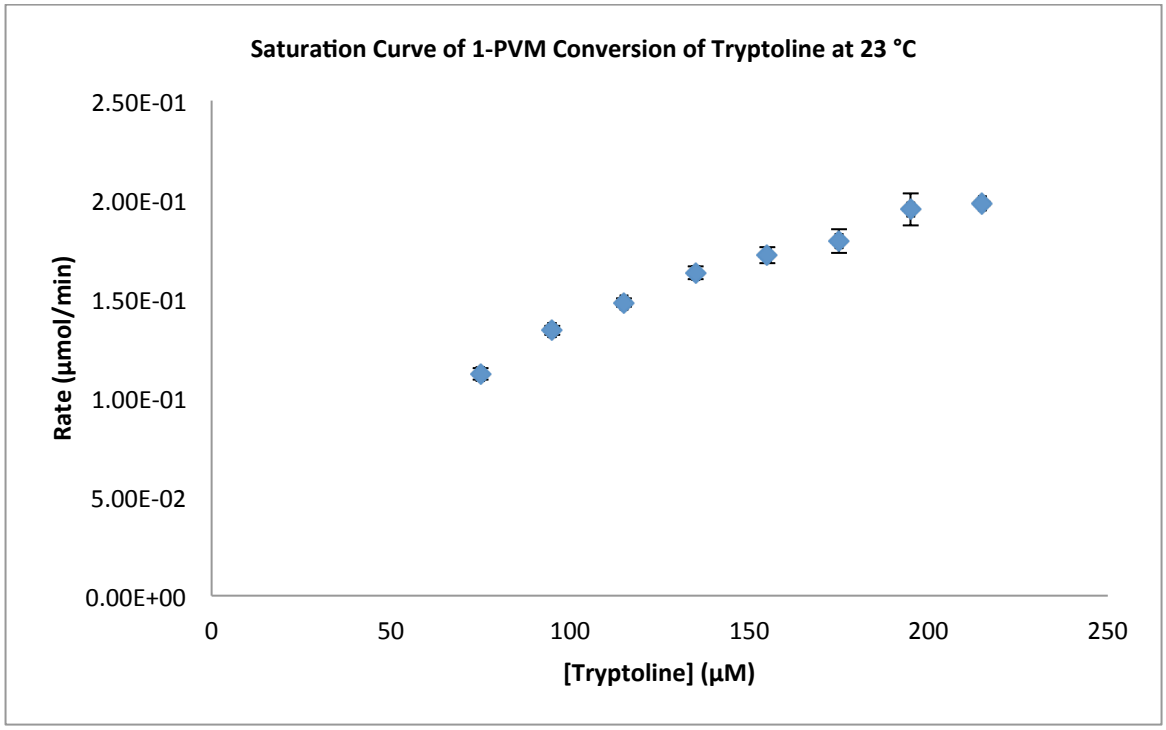
high loadings of chlorinating agents at elevated temperatures for long reaction times, whereas we saw immediate conversion to a single monochlorinated derivative of these substrates at room temperature.

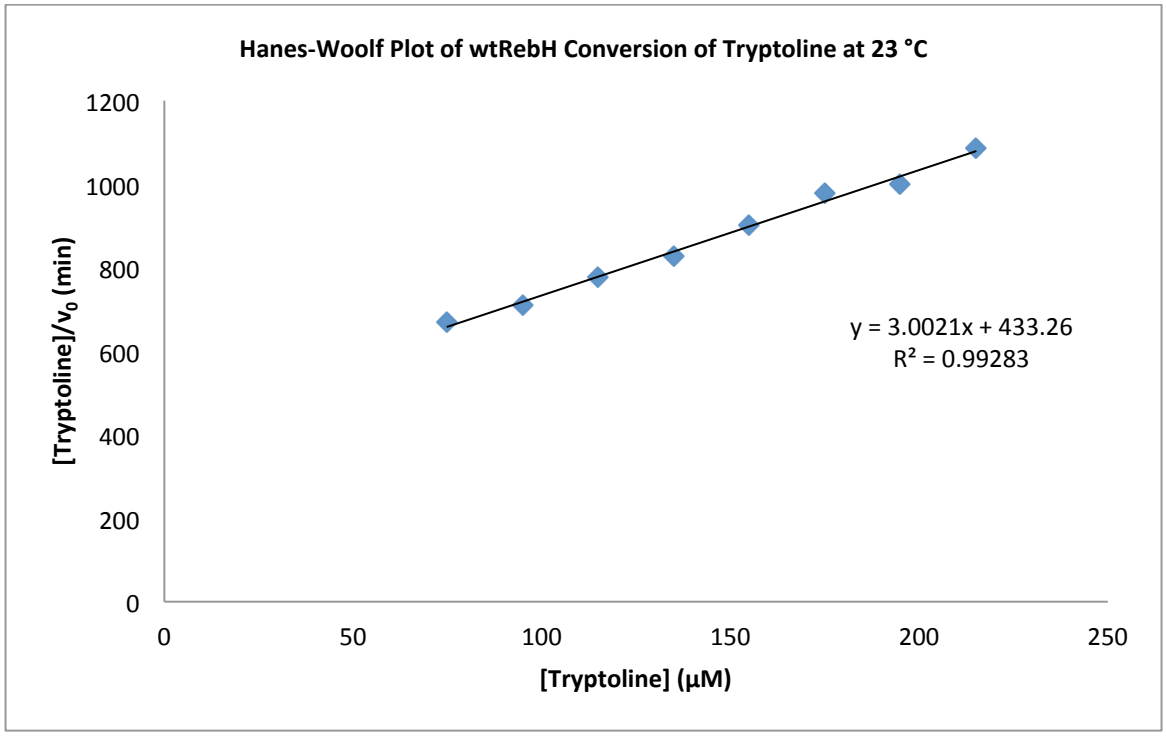
Preserved Regioselectivity on Small Substrates:

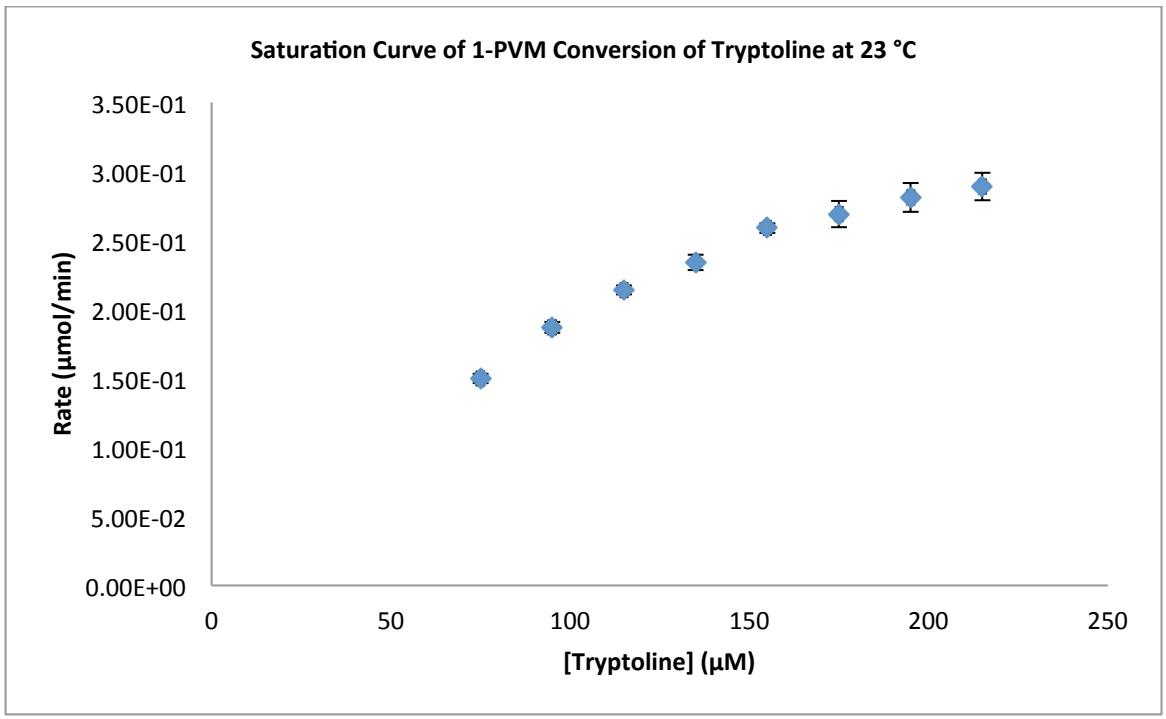
As an additional test that we have not reduced the selectivity imparted by our enzyme variants 3-SS and 4-V relative to that established for wild-type RebH (we speculated that by expanding the active site to accommodate larger substrates, we might have reduced substrate binding such that we would only halogenate the most electronically activated site(s)), we retested halogenations of two small substrates with which we reported novel regioselectivity with wild-type RebH. Chlorination reactions of both tryptamine and tryptophol (both of which we previously reported[8] and both of which are unprotected at the 2-position, which is by far the most electronically activated position on these substrates) were set up with wild-type RebH, 3-SS, and 4-V. After the reactions were quenched and analyzed by UPLC, the products were seen to perfectly coelute to the best of our ability to resolve any potential regioisomers by HPLC. We therefore conclude that 3-SS and 4-V are providing the same regioselectivity we previously reported on these substrates with wild-type RebH, 7-chlorination, and therefore we are not simply halogenating the most electronically activated site with these enzyme variants.

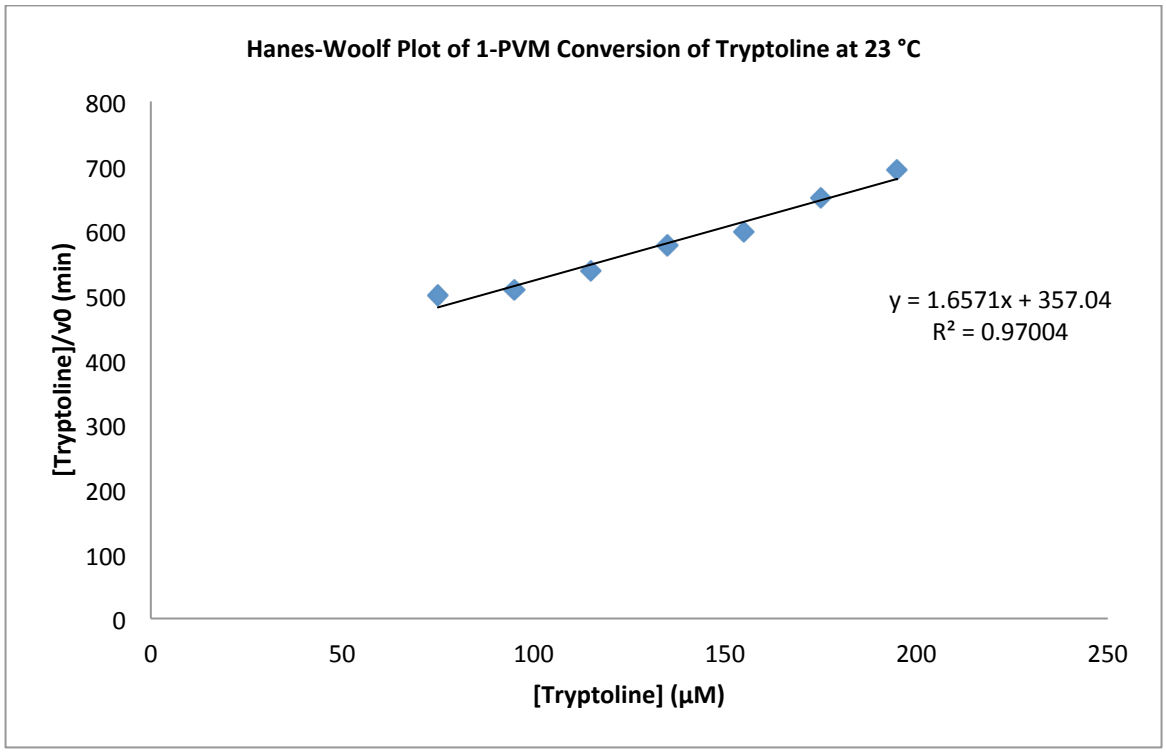
Kinetics:

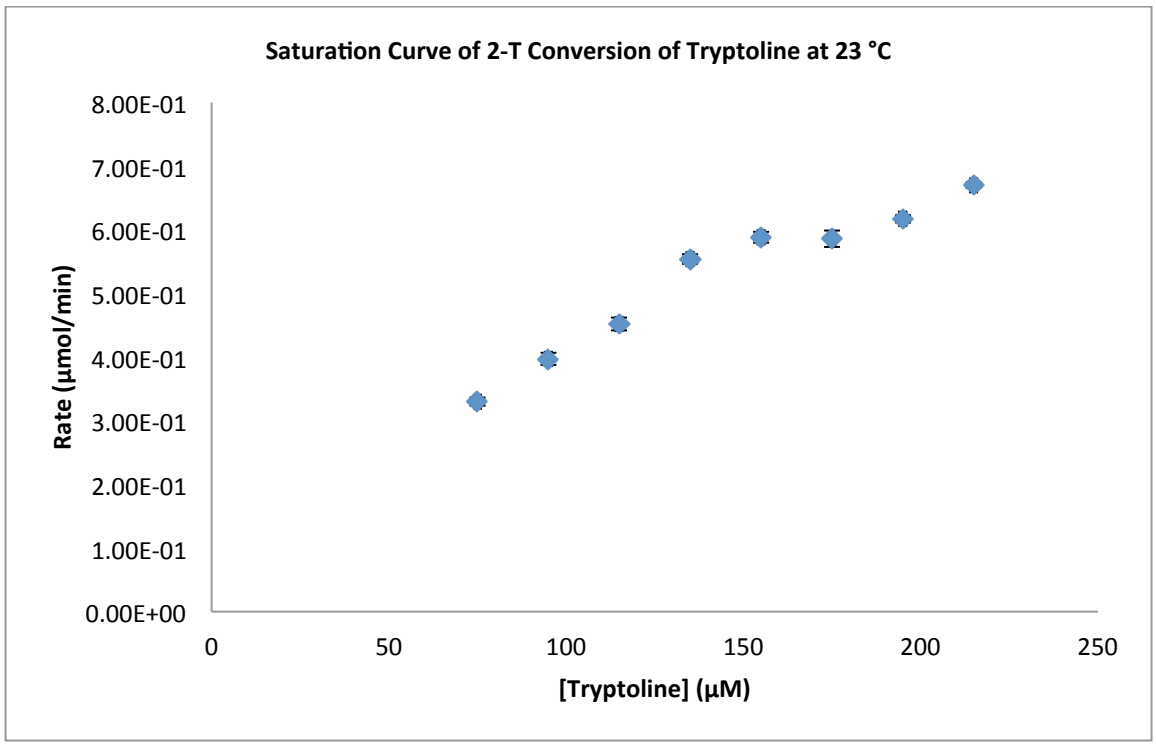
All measurements were conducted in triplicate. Error bars on saturation curves represent standard deviations of the triplicate measurements of rate. Hanes-Woolf plots were then constructed from the averages of these three rates.

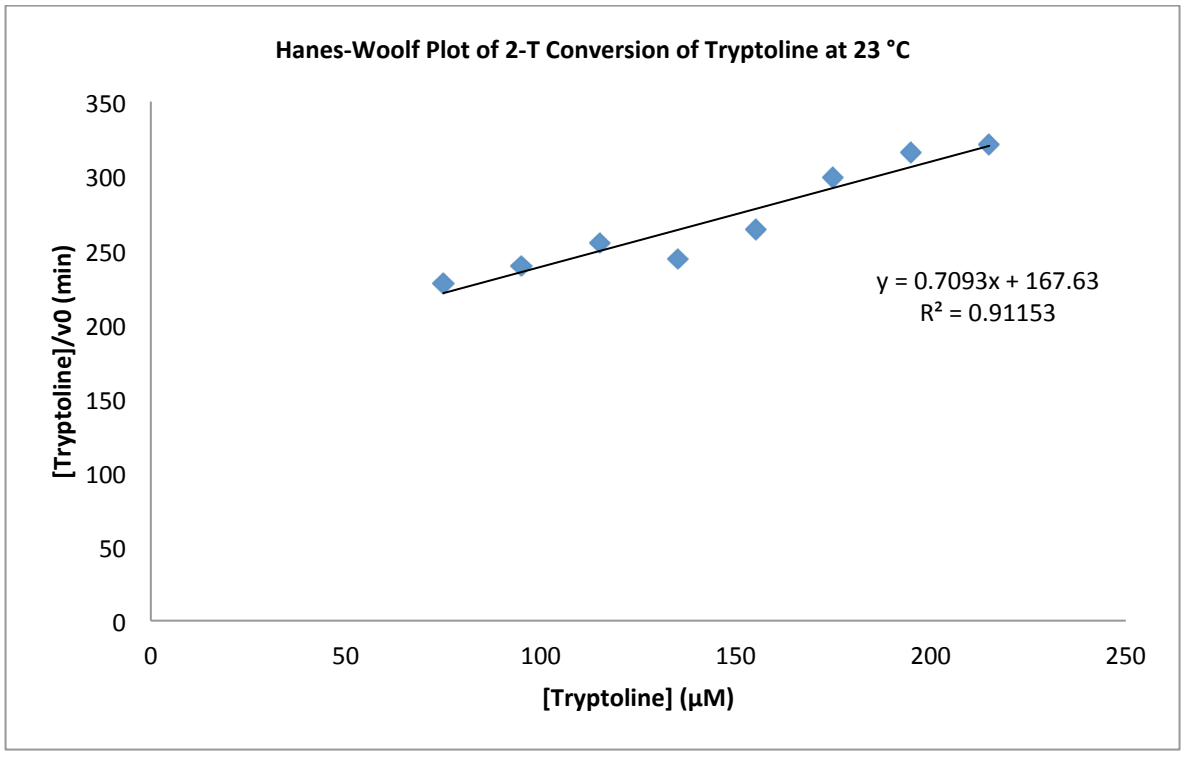


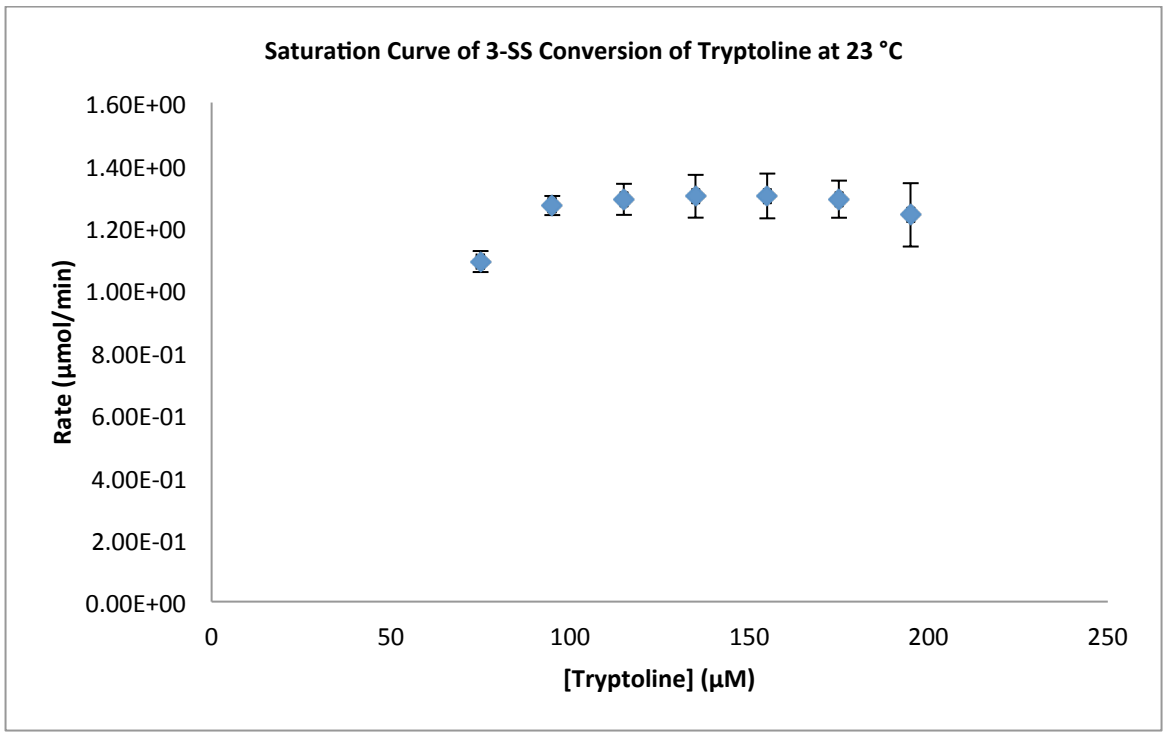


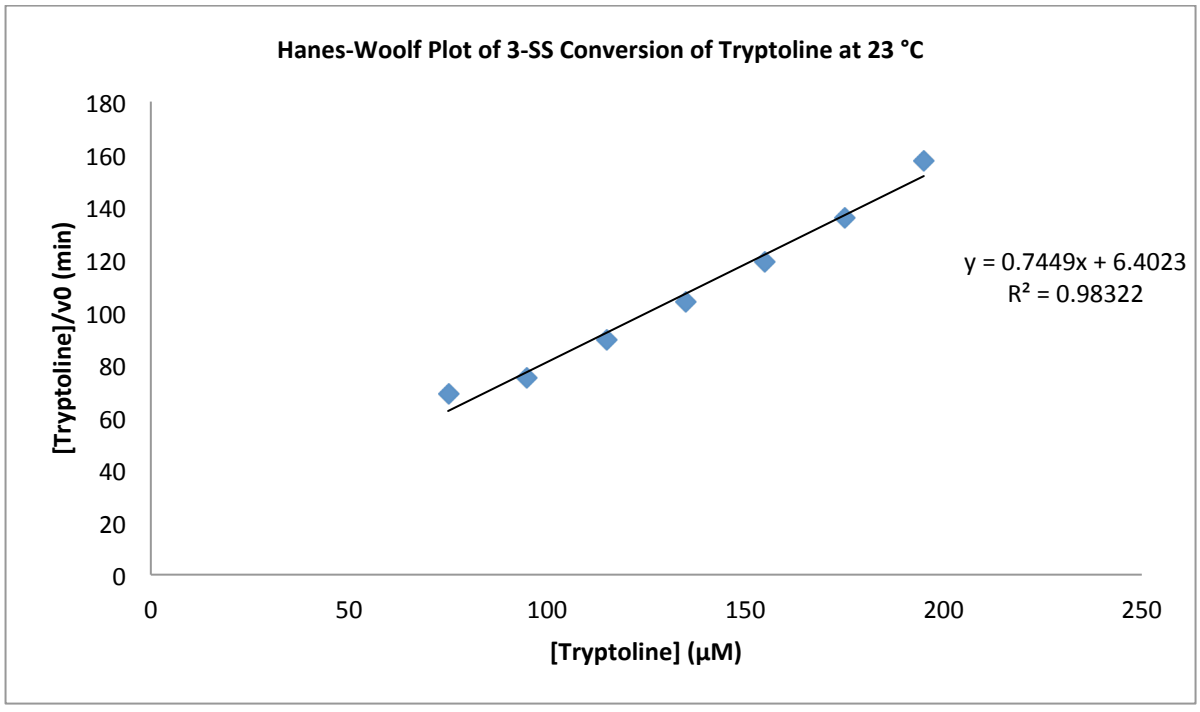

Saturation Curve of 1-PVM Conversion of Tryptoline at 23 °C
Rate (µmol/min)
3.50E-01
3.00E-01
2.50E-01
2.00E-01
1.50E-01
1.00E-01
5.00E-02
0.00E+00
0
50
100
150
200
250
[Tryptoline] (µM)


Hanes-Woolf Plot of 1-PVM Conversion of Tryptoline at 23 °C
[Tryptoline]/v0 (min)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
[Tryptoline] (µM)
y = 1.6571x + 357.04
R² = 0.97004


Saturation Curve of 2-T Conversion of Tryptoline at 23 °C
Rate (µmol/min)
8.00E-01
7.00E-01
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
0
50
100
150
200
250
[Tryptoline] (µM)


Hanes-Woolf Plot of 2-T Conversion of Tryptoline at 23 °C
[Tryptoline]/v0 (min)
350
300
250
200
150
100
50
0
0
50
100
150
200
250
[Tryptoline] (µM)
y = 0.7093x + 167.63
R² = 0.91153


Saturation Curve of 3-SS Conversion of Tryptoline at 23 °C
Rate (µmol/min)
1.60E+00
1.40E+00
1.20E+00
1.00E+00
8.00E-01
6.00E-01
4.00E-01
2.00E-01
0.00E+00
0
50
100
150
200
250
[Tryptoline] (µM)


Hanes-Woolf Plot of 3-SS Conversion of Tryptoline at 23 °C
[Tryptoline]/v0 (min)
180
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
[Tryptoline] (µM)
y = 0.7449x + 6.4023
R² = 0.98322

Location of mutations in 3-SS and 4-V:

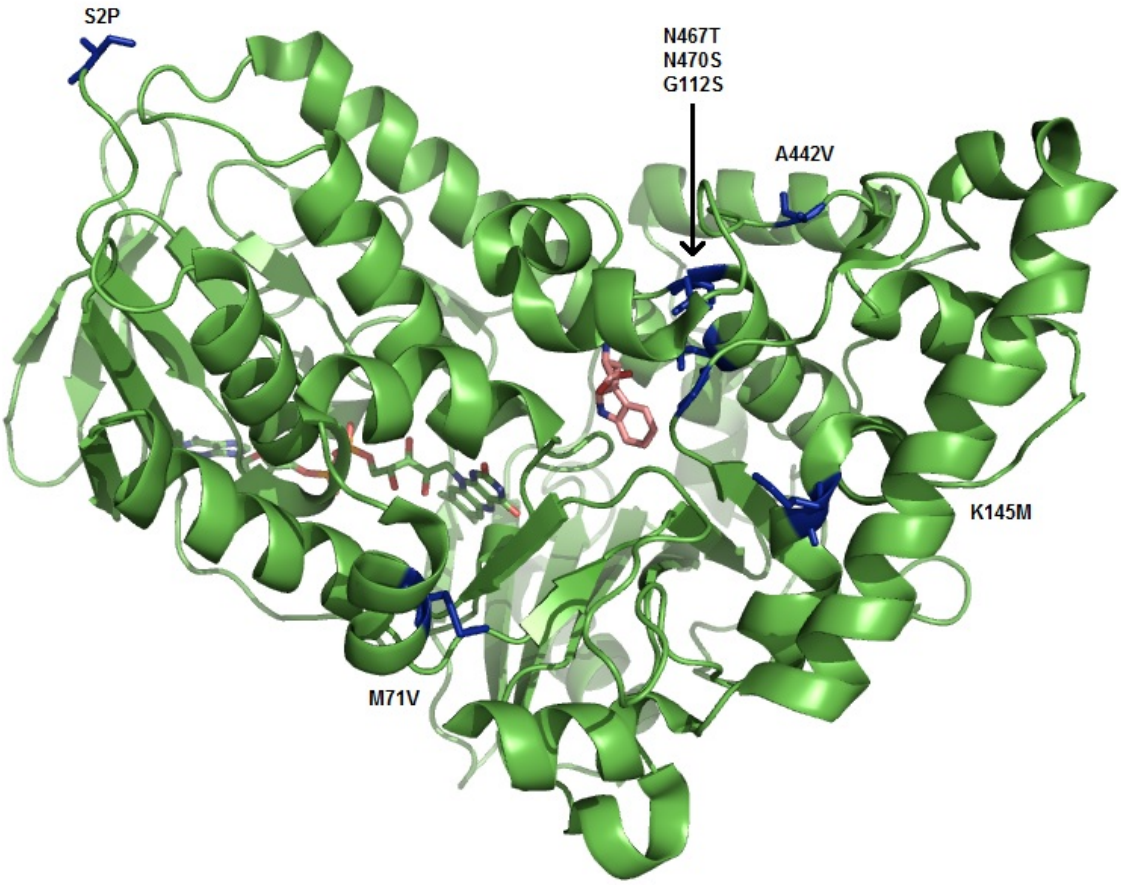
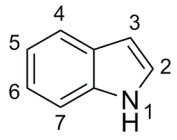


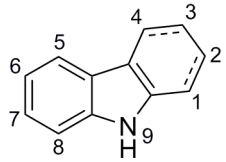
Fig. S2: *Pymol structure of PDB entry 2OA1[11], shown with residues that are mutated in variants 3-SS and 4-V shown in blue (bound L-tryptophan as substrate is shown in pink).*

Numbering Used for Compounds:

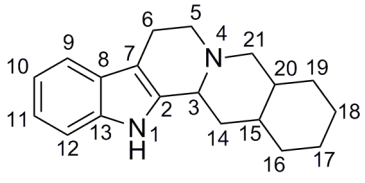
Indoles:

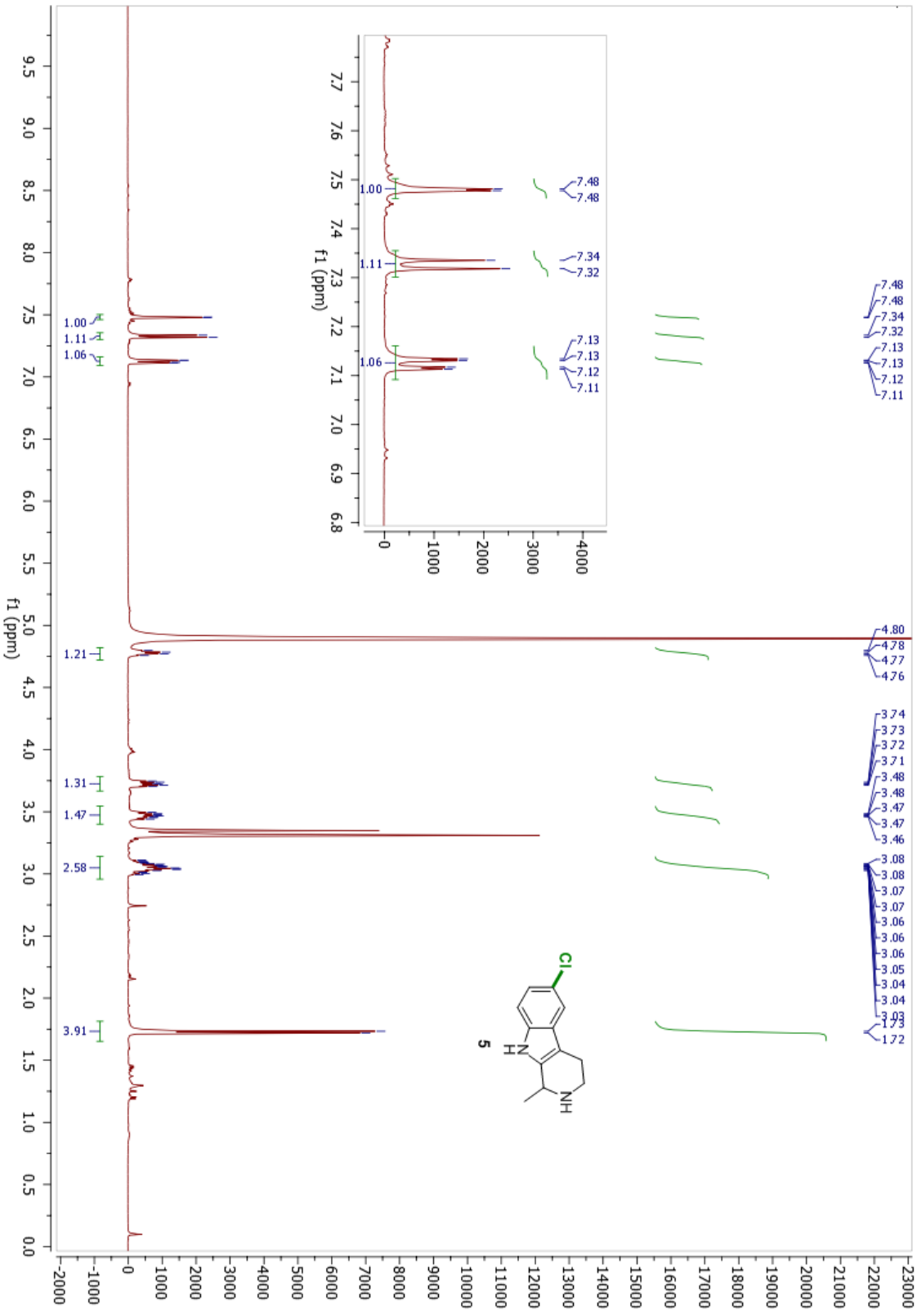


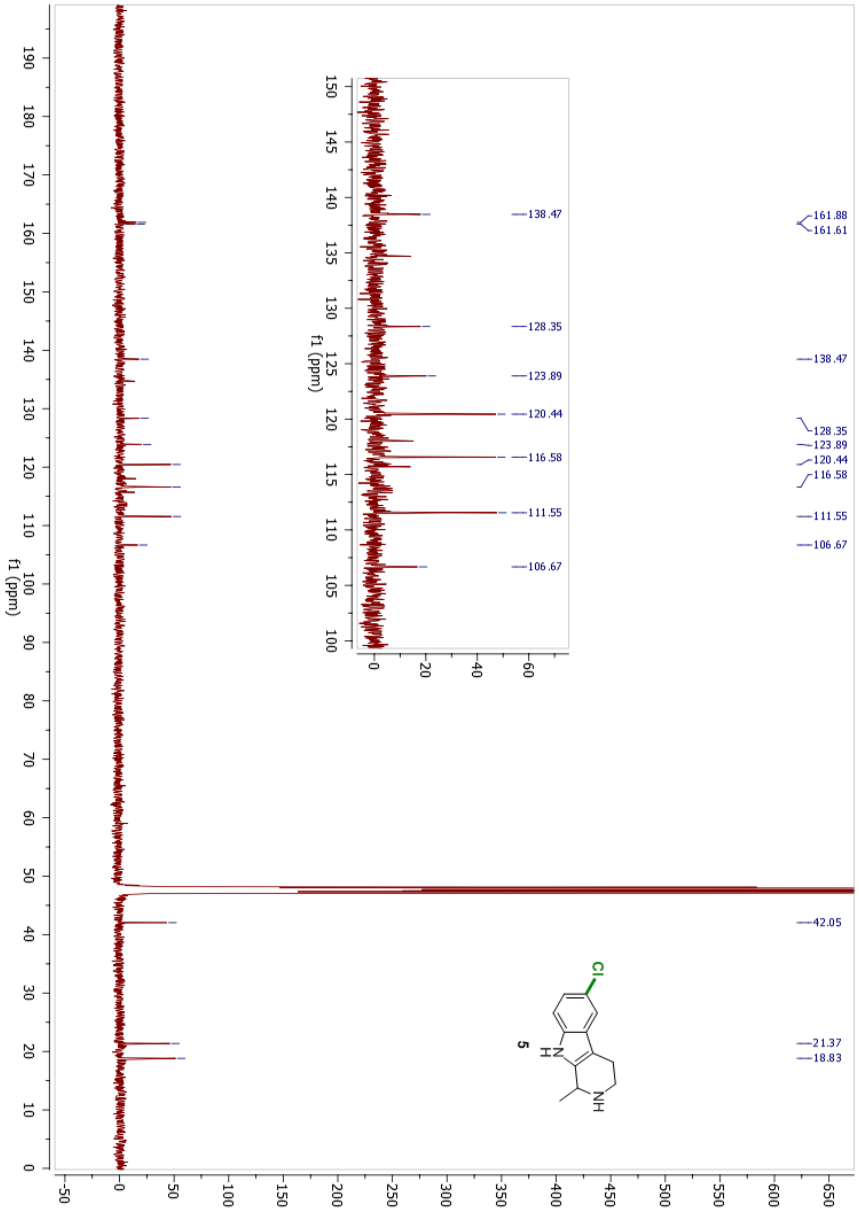
Carbazoles/Tryptoline Derivatives:

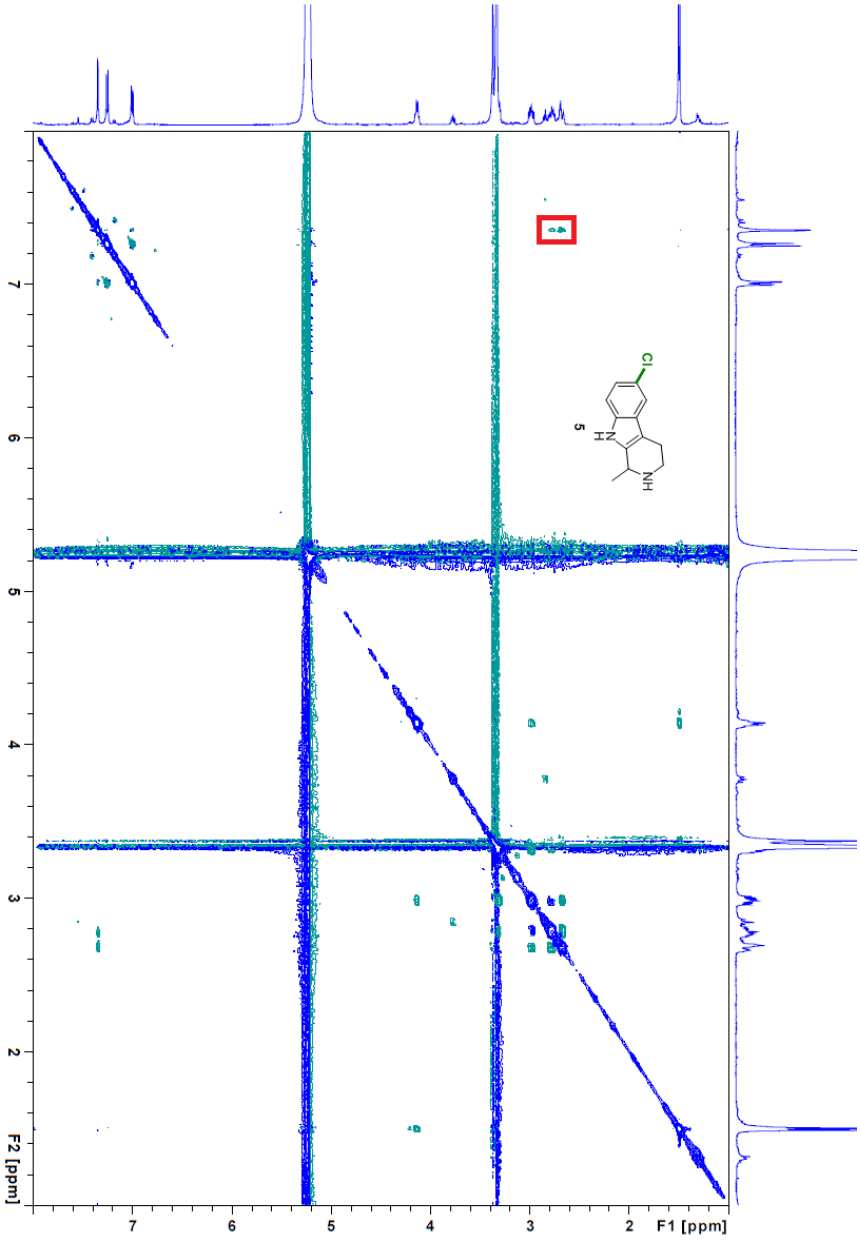


Yohimban Derivatives:

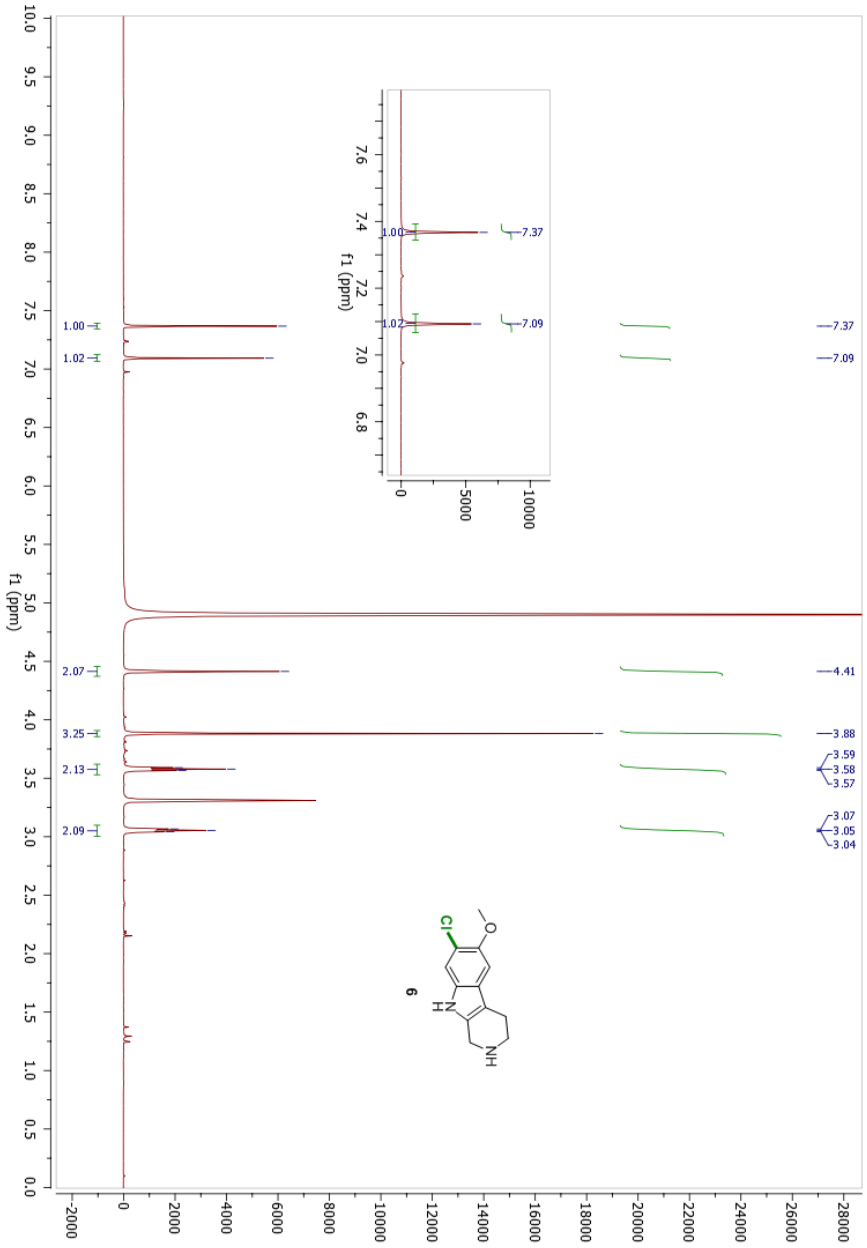


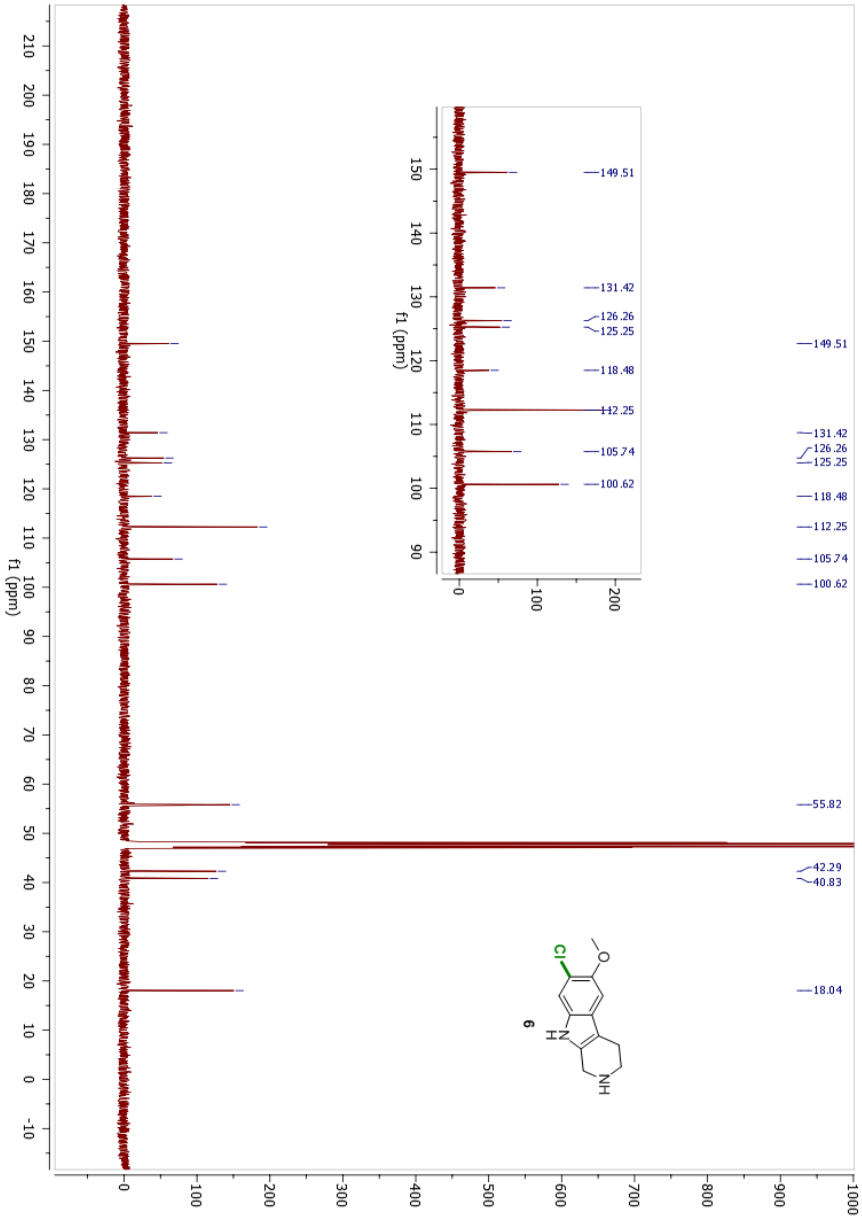

f1 (ppm)
1.00
1.11
1.06
1.21
1.31
1.47
2.58
3.91
7.48
7.48
7.34
7.32
7.13
7.13
7.12
7.11
4.80
4.78
4.77
4.76
3.74
3.73
3.72
3.71
3.48
3.48
3.47
3.47
3.46
3.08
3.08
3.07
3.07
3.06
3.06
3.06
3.05
3.04
3.04
3.03
1.73
1.72
Cl
NH
NH
5

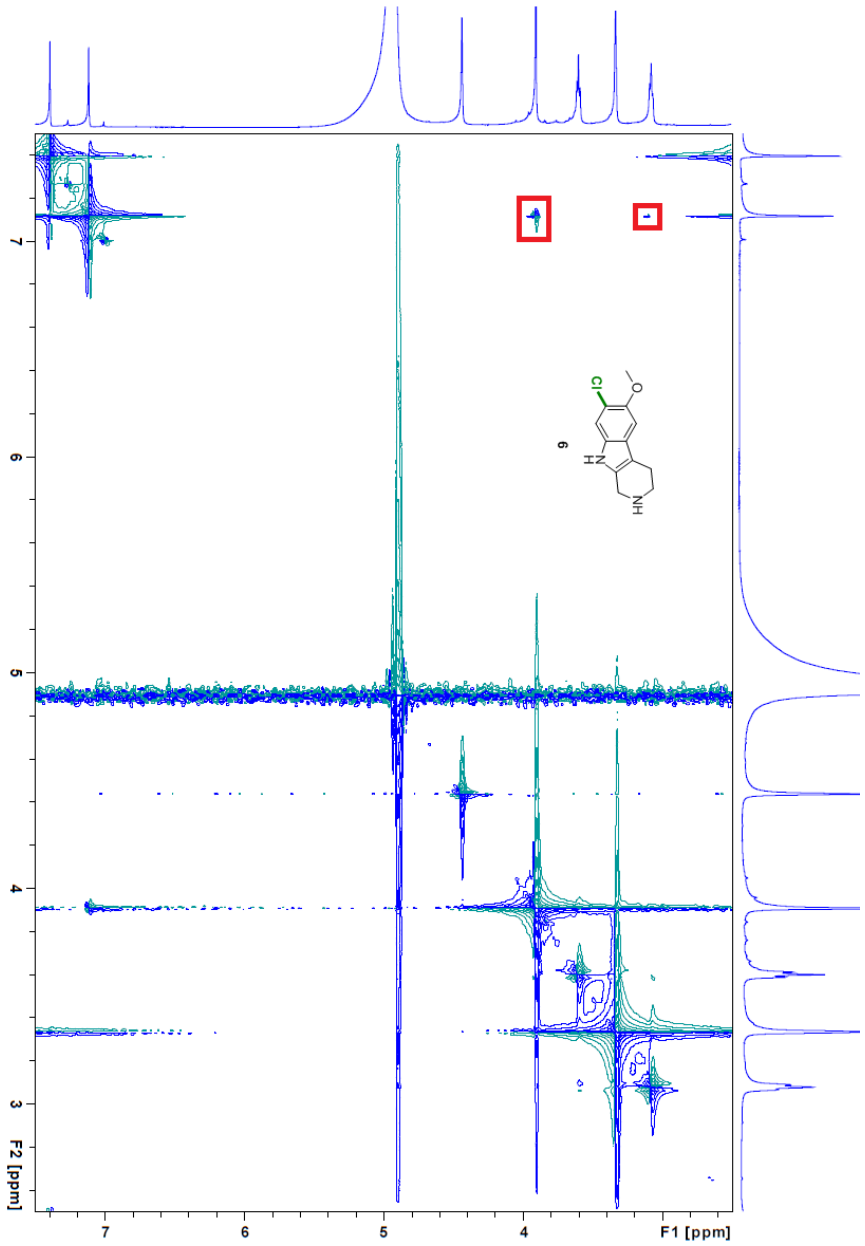

f1 (ppm)
161.88
161.61
138.47
128.35
123.89
120.44
116.58
111.55
106.67
42.05
21.37
18.83
Cl
NH
NH
5



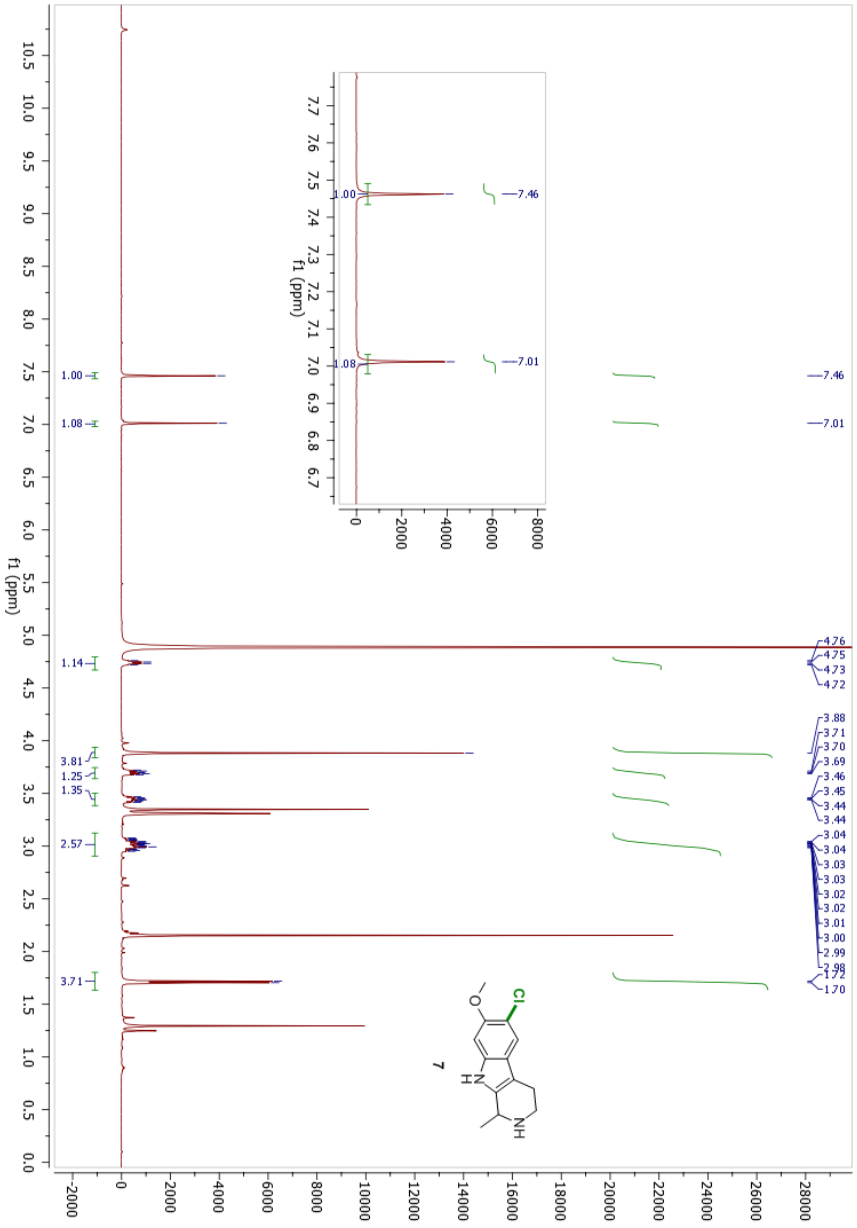
NOESY of **5**: Cross-peak between peaks at ~7.4 ppm (carbazole 5-position) and ~2.9 ppm (carbazole 4-position) demonstrates chlorination at the carbazole 6-position.

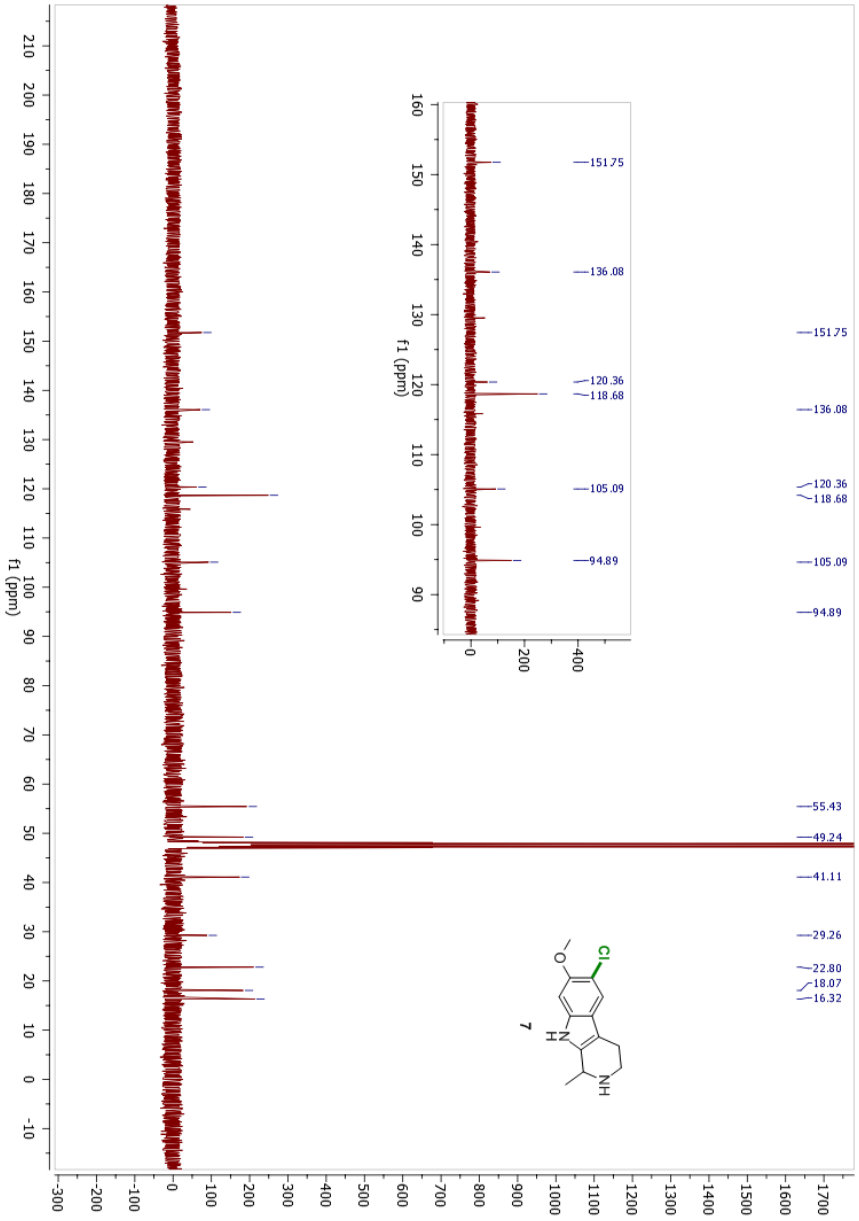

f1 (ppm)
1.00
1.02
2.07
3.25
2.13
2.09
7.37
7.09
4.41
3.88
3.59
3.58
3.57
3.07
3.05
3.04
Cl
O
N
H
NH
6

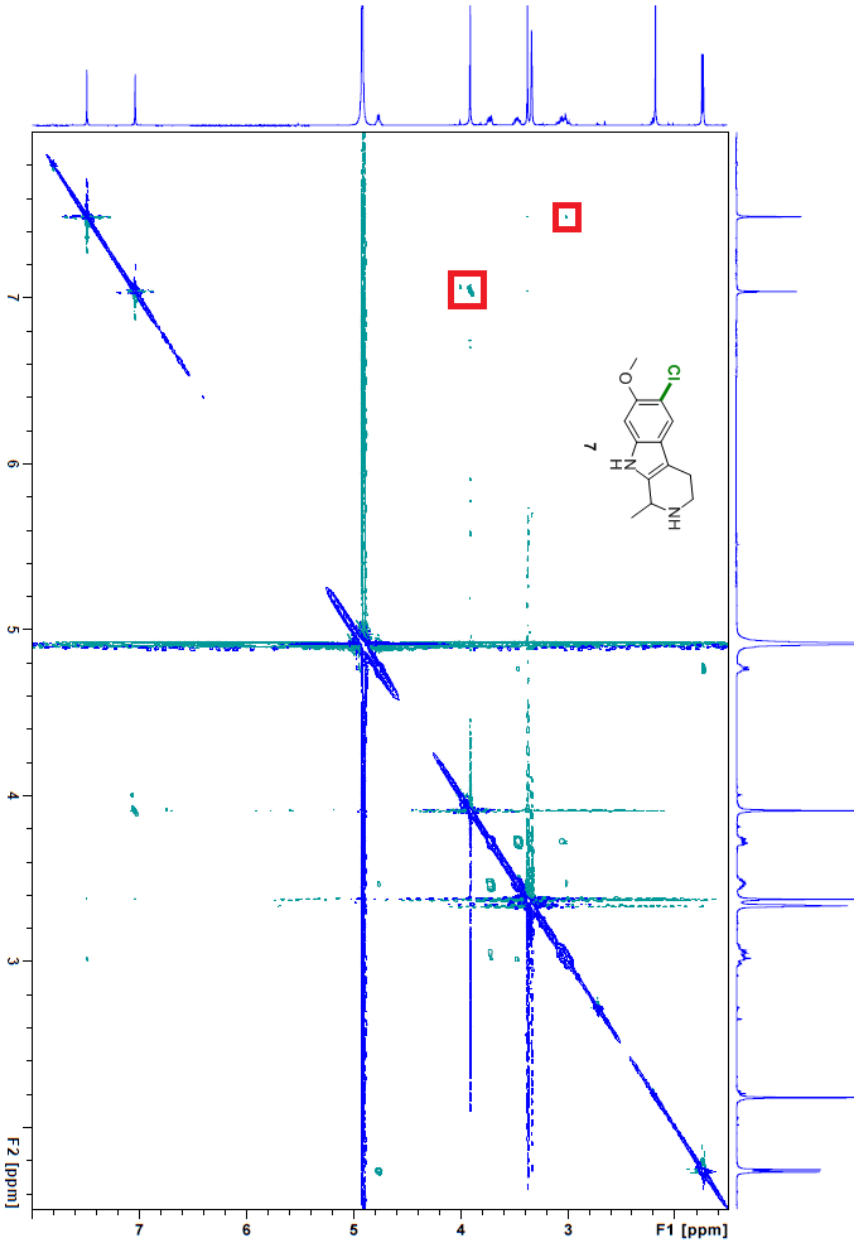

f1 (ppm)
149.51
131.42
126.26
125.25
118.48
112.25
105.74
100.62
55.82
42.29
40.83
18.04
Cl
O
H
N
NH
6



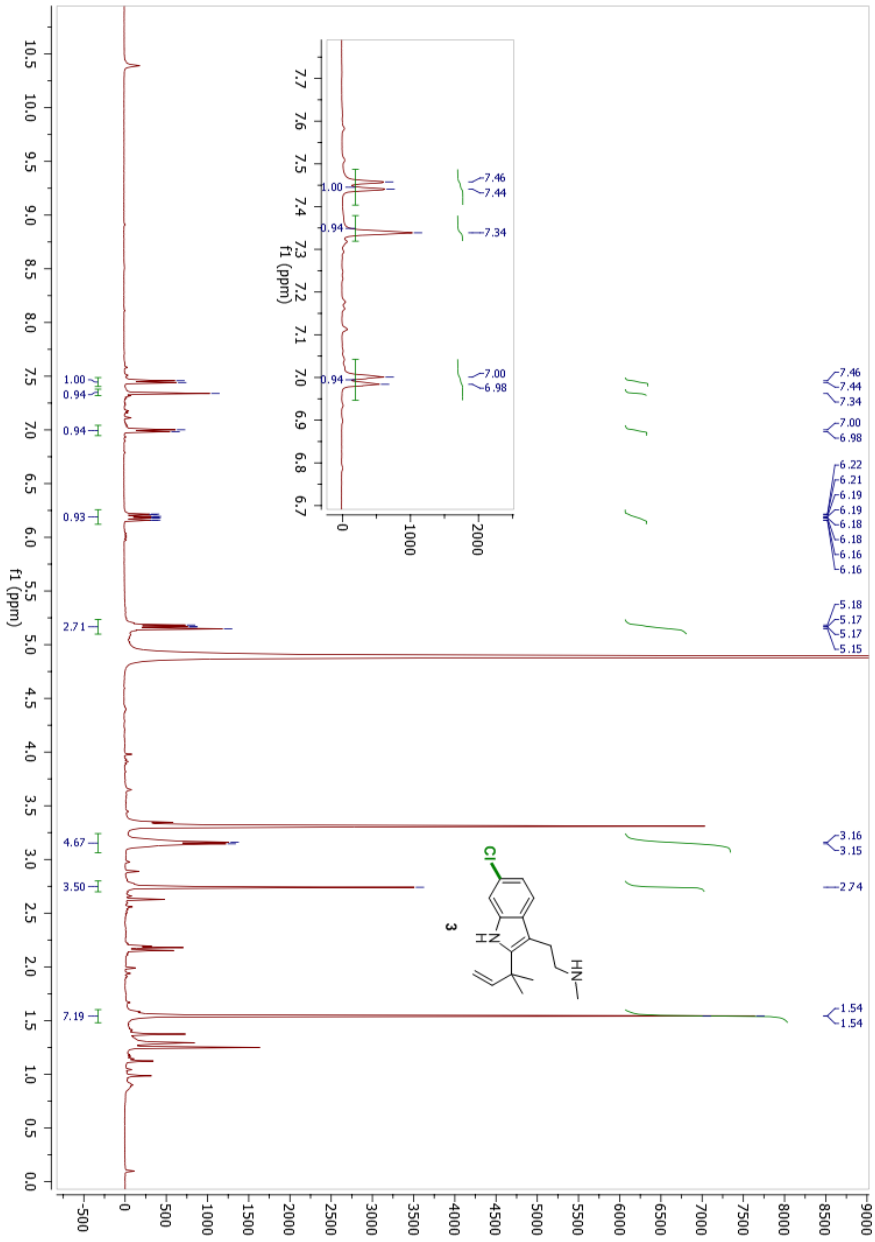
NOESY of **6**: Cross-peak between peaks at ~7.1 ppm (carbazole 5-position) and ~3.1 ppm (carbazole 4-position), along with absence of cross-peak between peaks at ~3.9 ppm (carbazole 6-methoxy protons) and ~7.4 ppm (which we therefore conclude to be the carbazole 8-position), demonstrates chlorination at the carbazole 7-position.

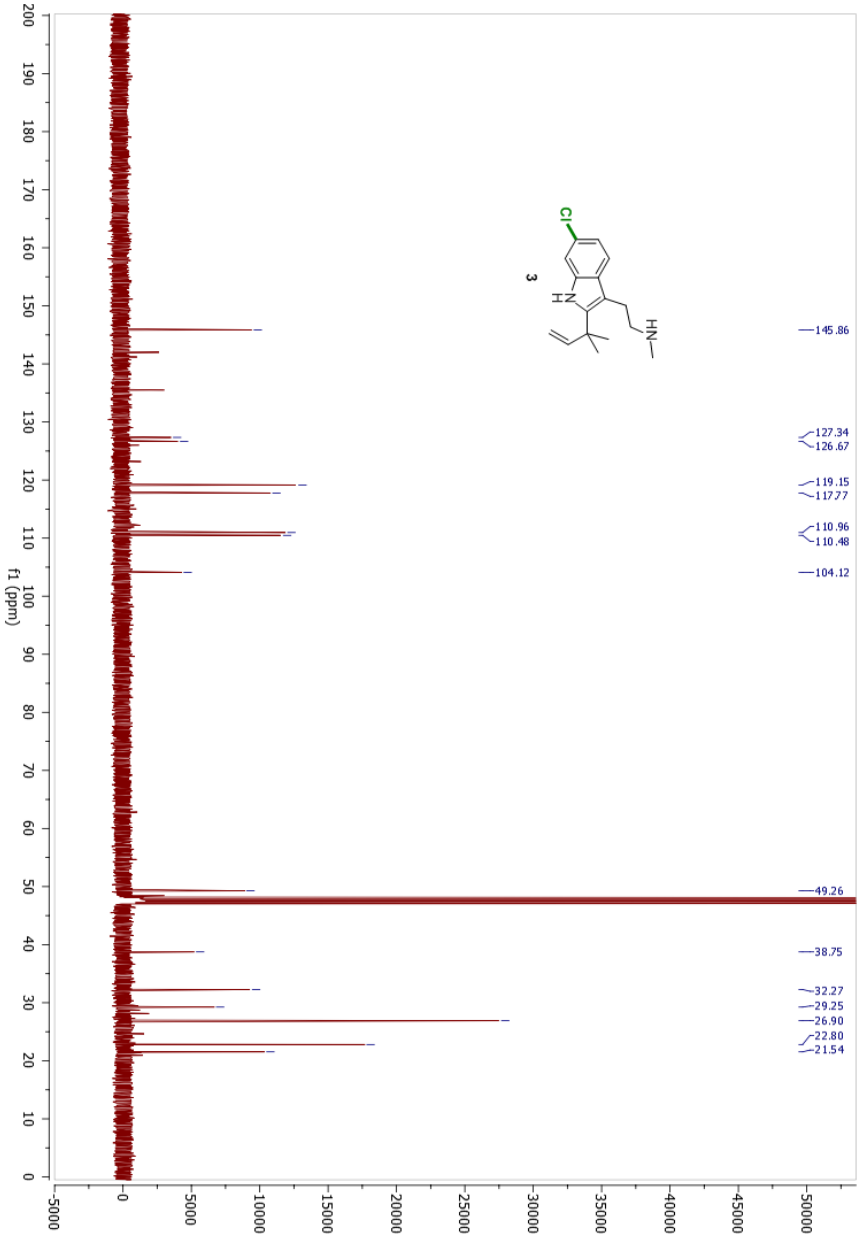

f1 (ppm)
1.00
1.08
1.14
3.81
1.25
1.35
2.57
3.71
7.46
7.01
4.76
4.75
4.73
4.72
3.88
3.71
3.70
3.69
3.46
3.45
3.44
3.44
3.04
3.04
3.03
3.03
3.02
3.02
3.01
3.00
2.99
2.98
1.72
1.70
Cl
O
N
H
NH
7

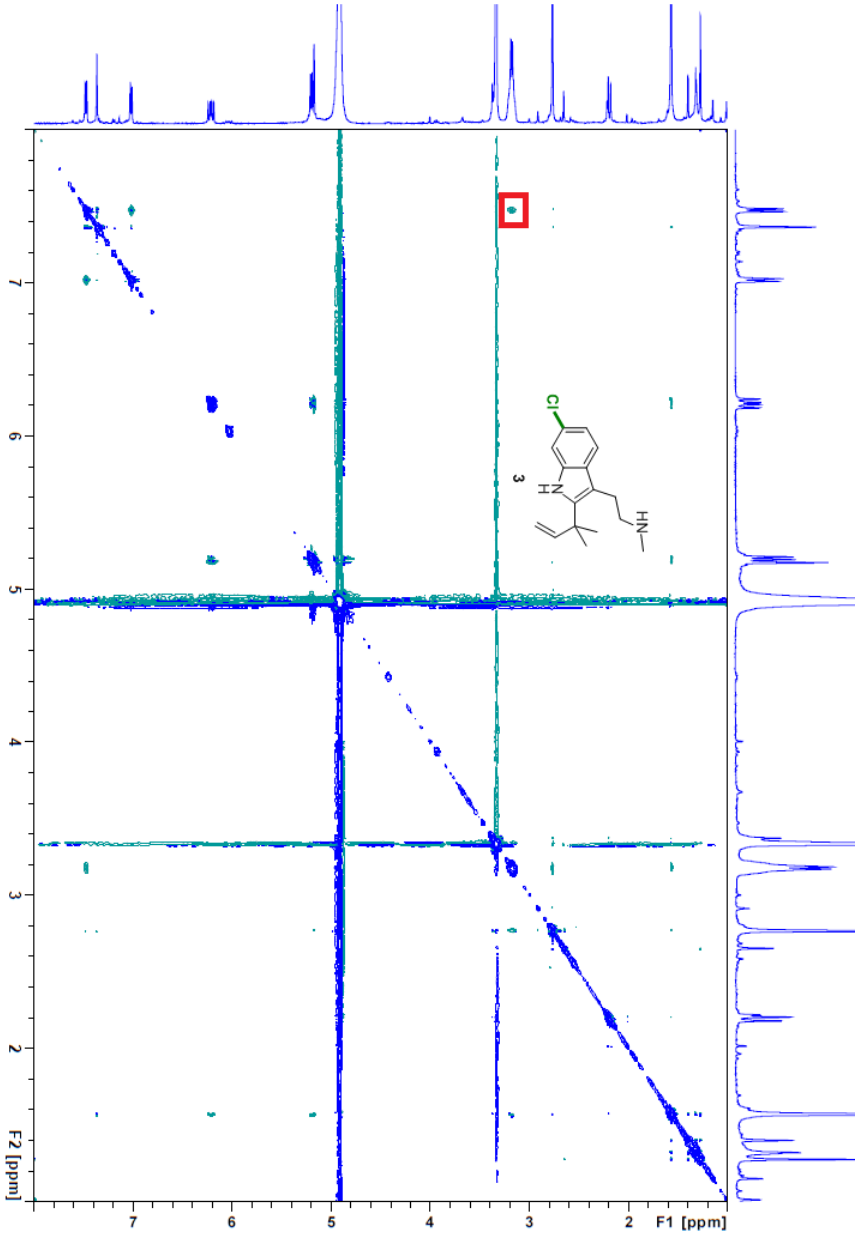

f1 (ppm)
151.75
136.08
120.36
118.68
105.09
94.89
55.43
49.24
41.11
29.26
22.80
18.07
16.32
Cl
O
H
N
NH
7



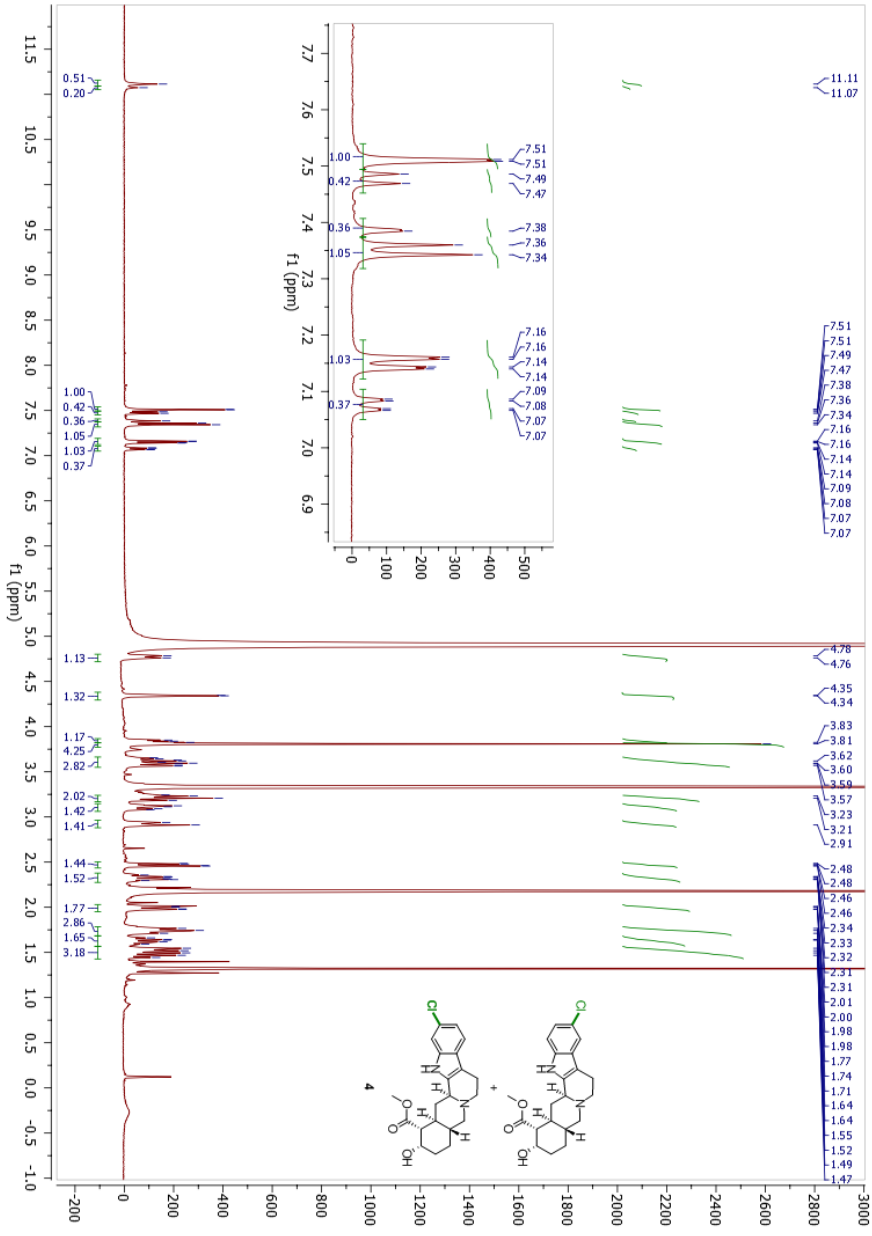
NOESY of **7**: Cross-peak between peaks at ~7.5 ppm (carbazole 5-position) and ~3.0 ppm (carbazole 4-position), along with cross-peak between peaks at ~3.9 ppm (carbazole 6-methoxy protons) and ~7.1 ppm (carbazole 8-position), along with concurrent absence of cross-peak between peaks at ~3.9 ppm (carbazole 6-methoxy protons) and ~7.5 ppm (further corroborating that this is the carbazole 5-position), demonstrates chlorination at the carbazole 7-position.

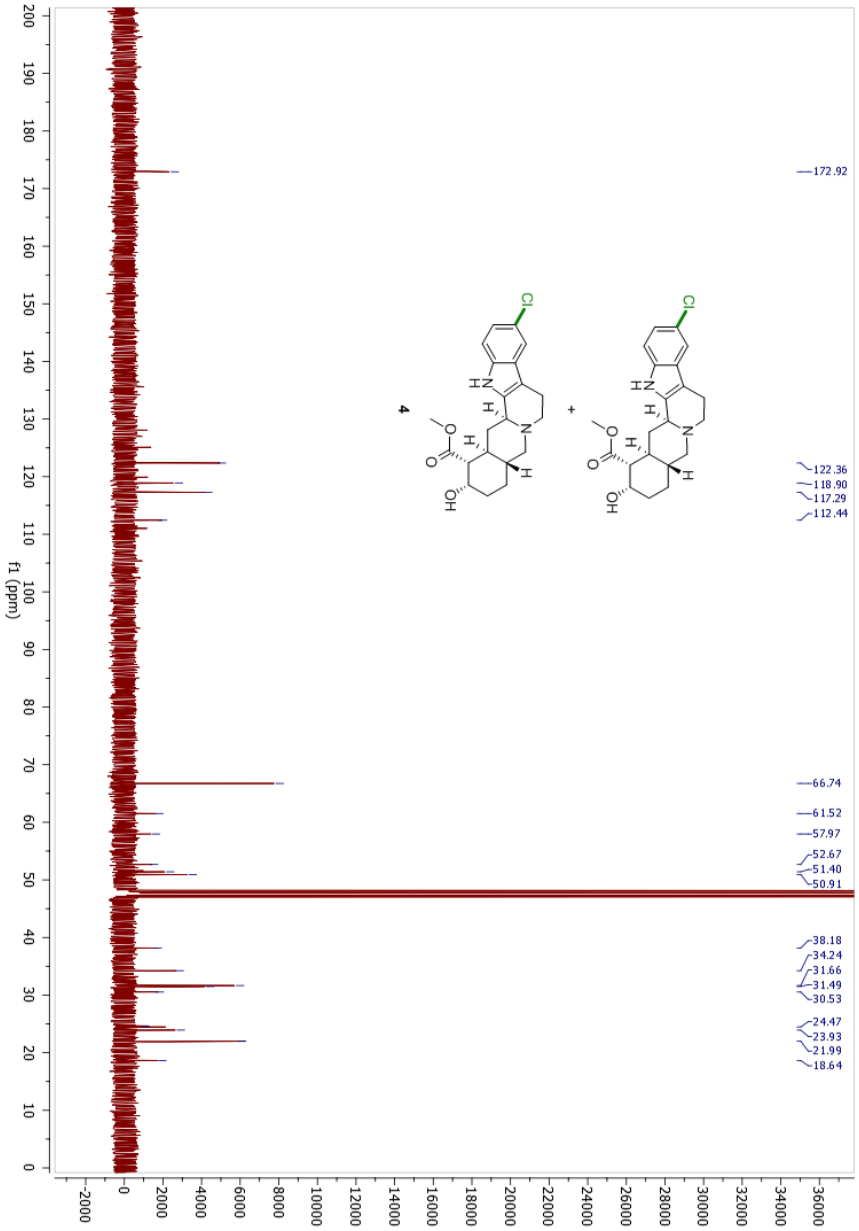

f1 (ppm)
1.00
0.94
0.94
0.93
2.71
4.67
3.50
7.19
7.46
7.44
7.34
7.00
6.98
6.22
6.21
6.19
6.19
6.18
6.18
6.16
6.16
5.18
5.17
5.17
5.15
3.16
3.15
2.74
1.54
1.54
Cl
HN
NH
3

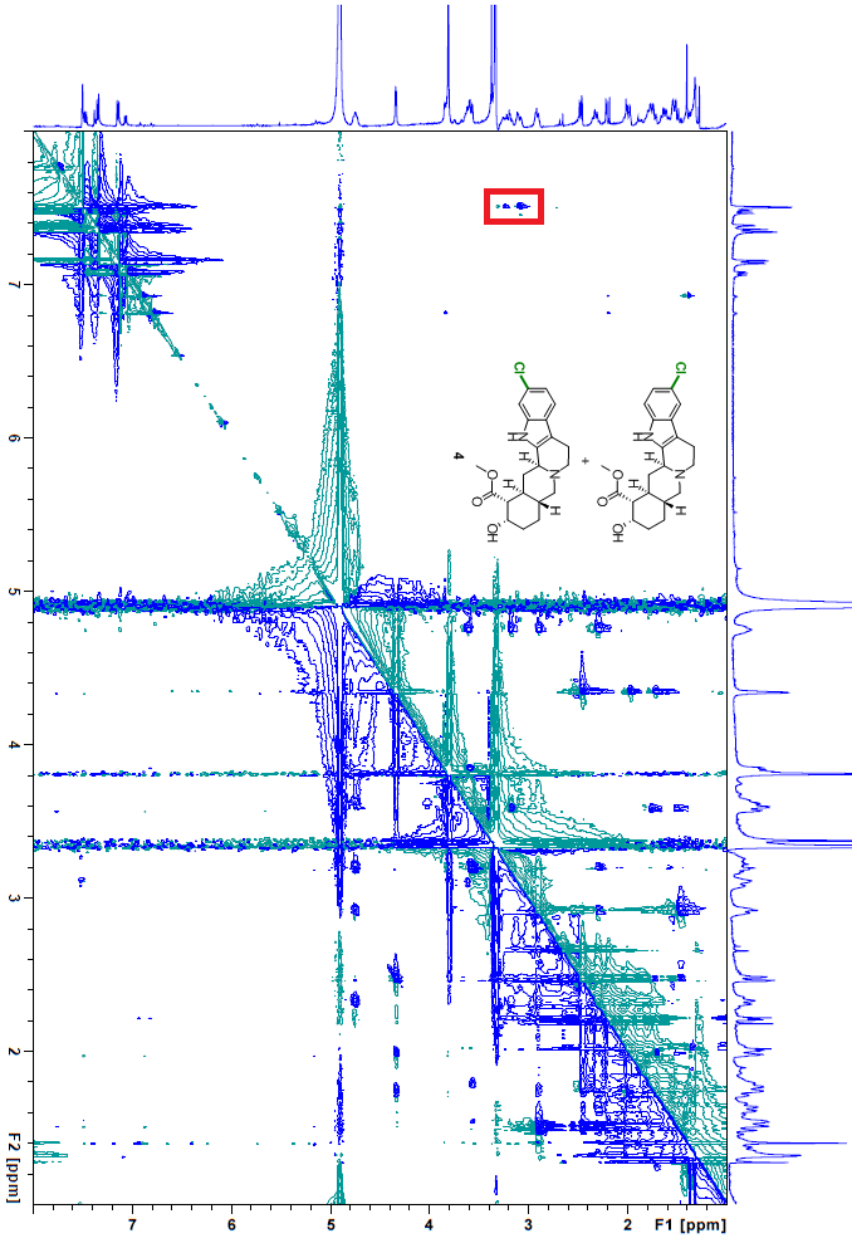

Cl
N
H
HN
3
145.86
127.34
126.67
119.15
117.77
110.96
110.48
104.12
49.26
38.75
32.27
29.25
26.90
22.80
21.54
f1 (ppm)



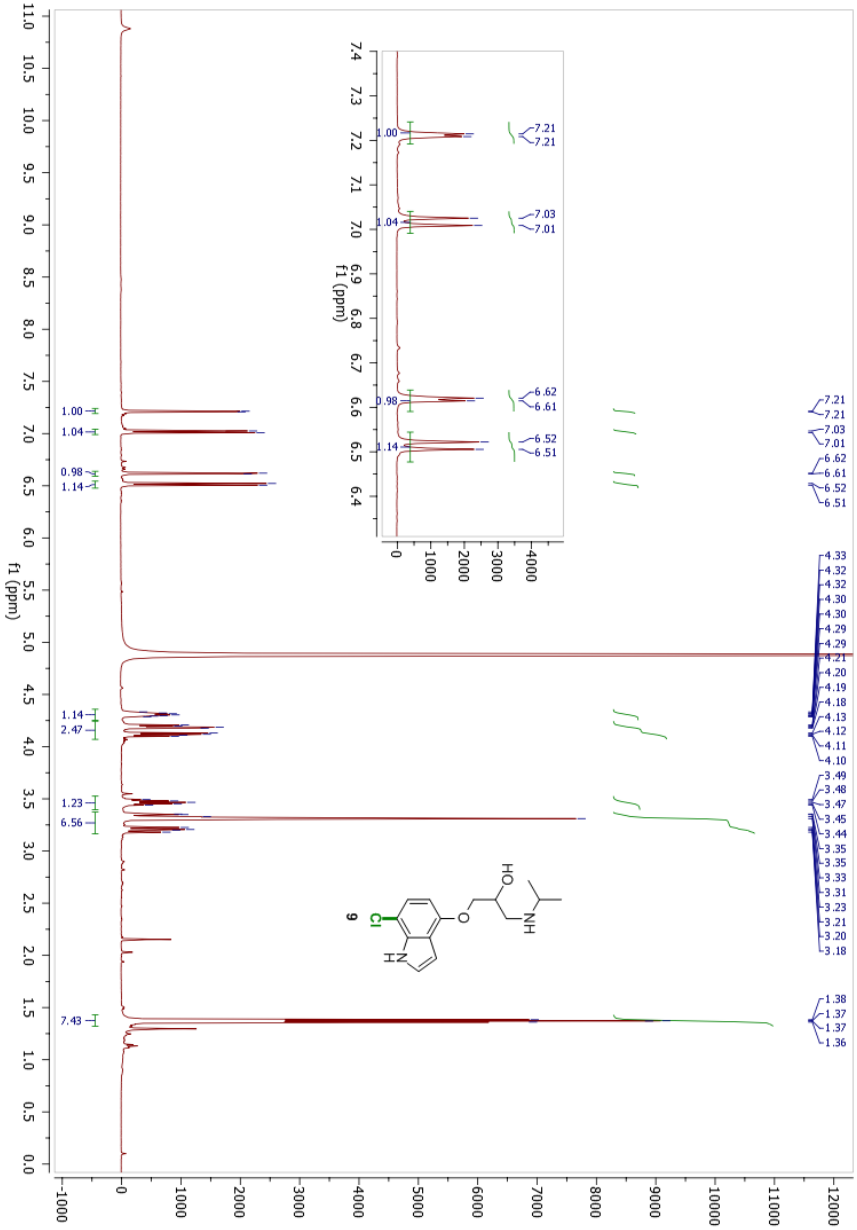
NOESY of **3**: Cross-peak between peaks at ~7.5 ppm (tryptamine 4-position) and ~3.2 ppm (tryptamine β-position) demonstrates chlorination at the tryptamine 6-position.

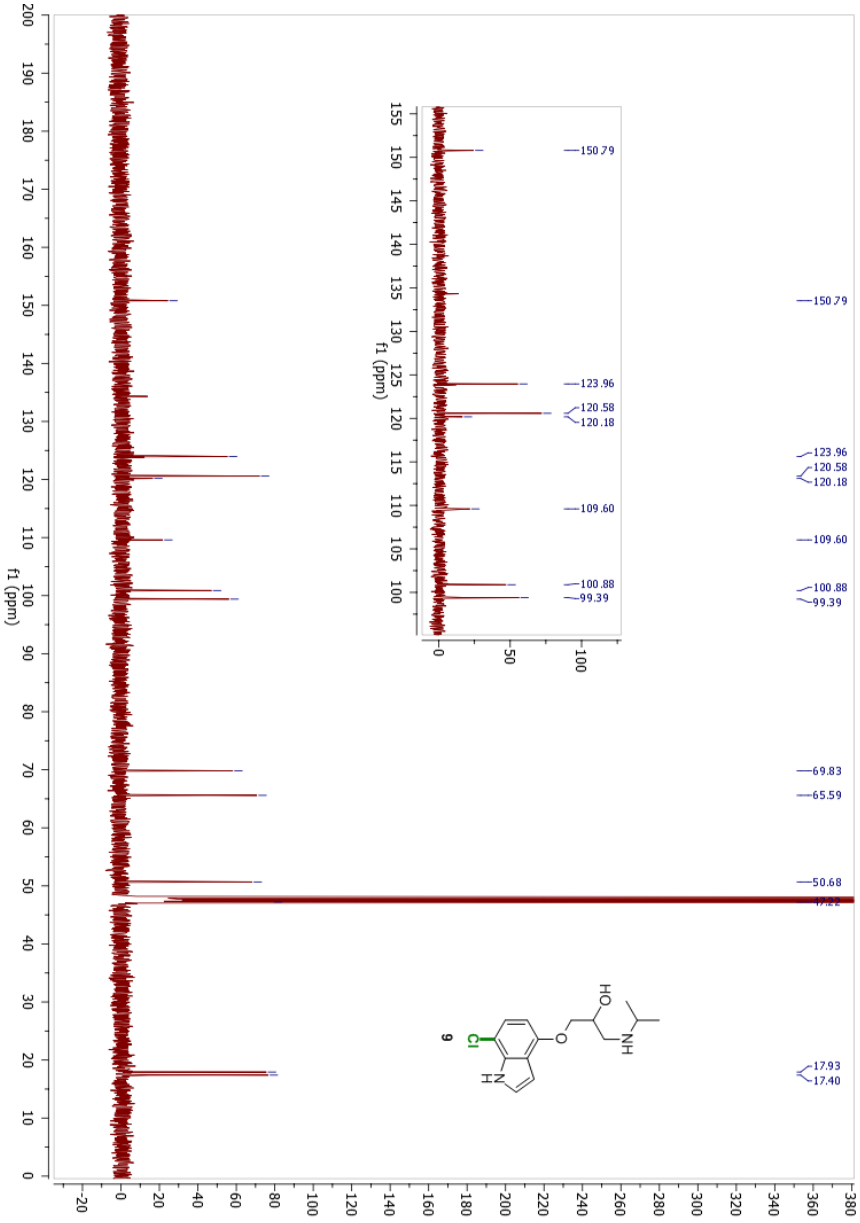

f1 (ppm)
4

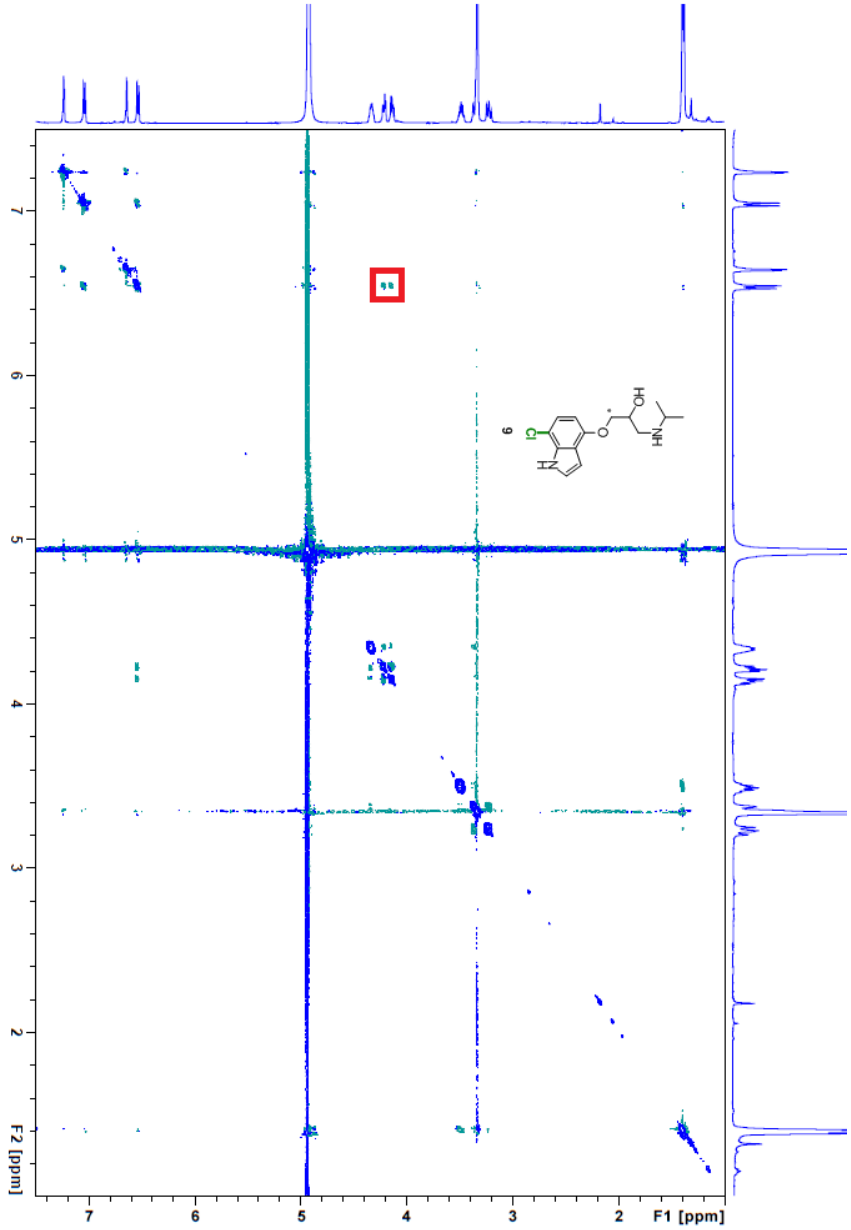

f1 (ppm)
172.92
122.36
118.90
117.29
112.44
66.74
61.52
57.97
52.67
51.40
50.91
38.18
34.24
31.66
31.49
30.53
24.47
23.93
21.99
18.64
4
+
Cl
Cl
N
H
O
OH



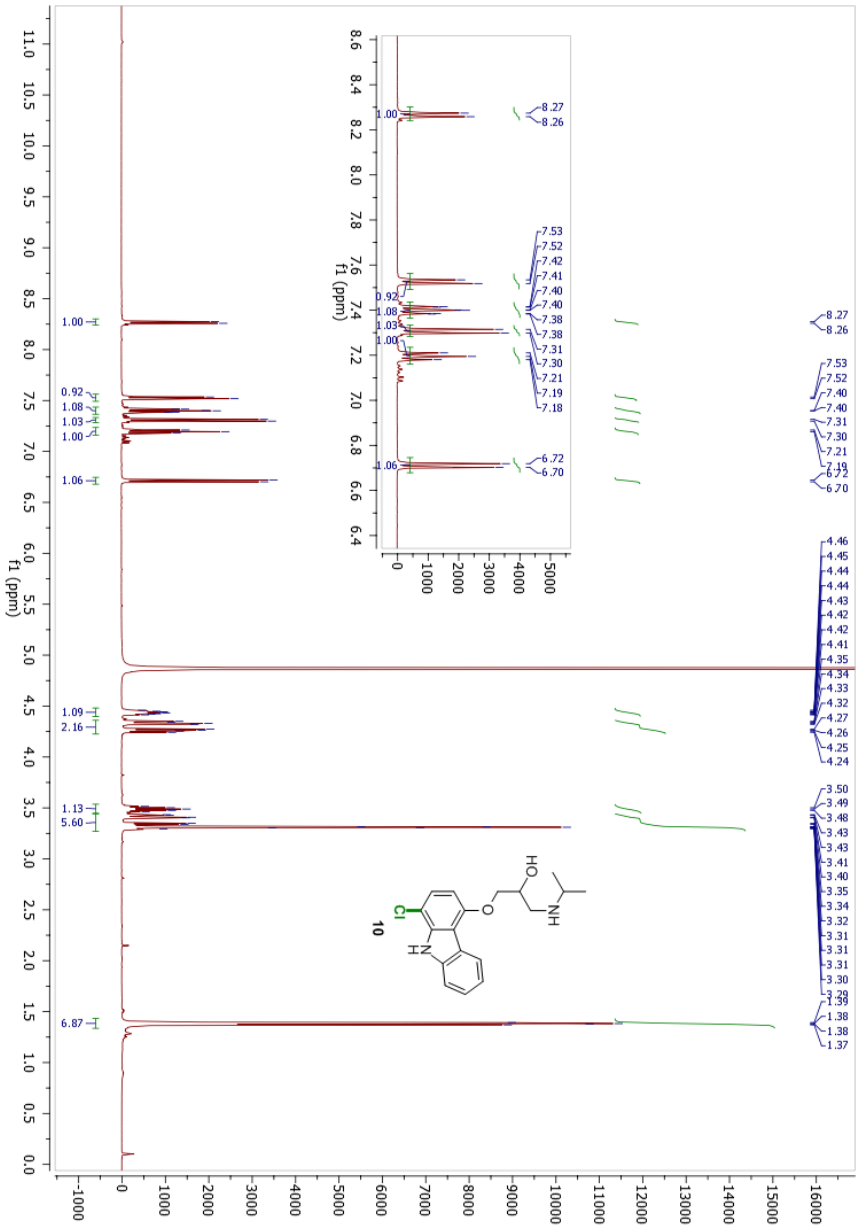
NOESY of**4**: Cross-peaks between peaks at ~7.5 ppm (yohimban 9-position) and ~3.1 ppm (yohimban 6-position) demonstrates chlorination of major product occurs at the yohimban 10-position and chlorination of minor product occurs at the yohimban 11-position.

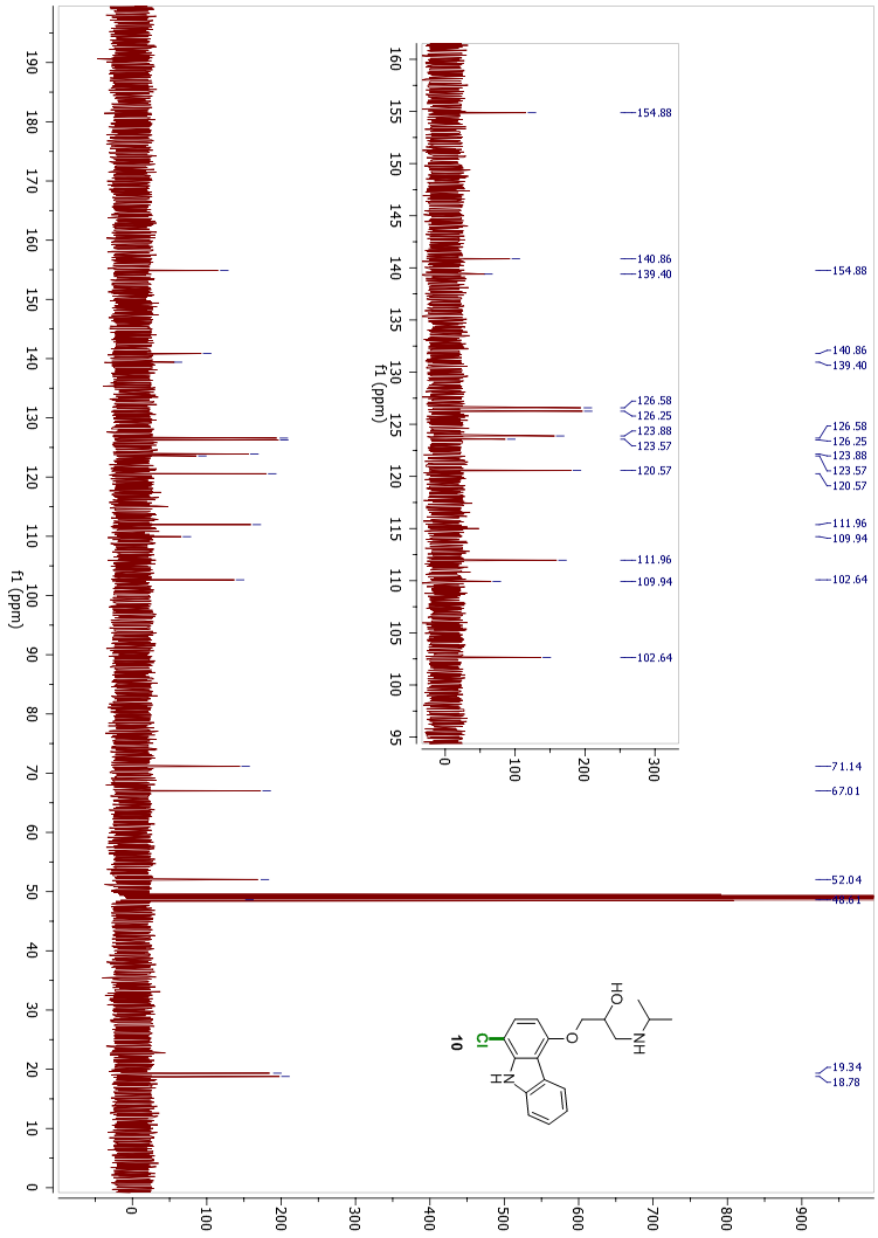

f1 (ppm)
1.00
1.04
0.98
1.14
2.47
1.23
6.56
7.43
7.21
7.21
7.03
7.01
6.62
6.61
6.52
6.51
4.33
4.32
4.32
4.30
4.30
4.29
4.29
4.21
4.20
4.19
4.18
4.13
4.12
4.11
4.10
3.49
3.48
3.47
3.45
3.44
3.35
3.35
3.33
3.31
3.23
3.21
3.20
3.18
1.38
1.37
1.37
1.36
HO
NH
O
Cl
H
N
9

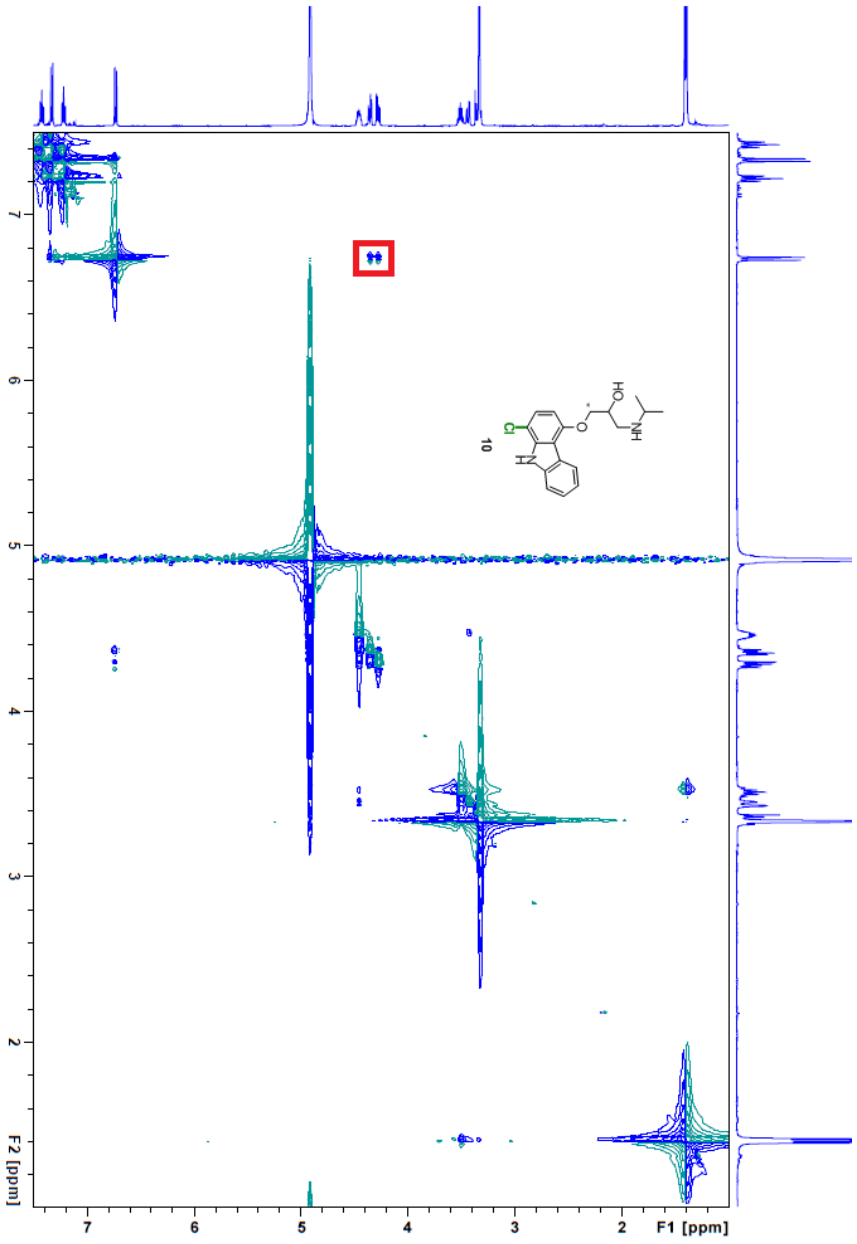

f1 (ppm)
150.79
123.96
120.58
120.18
109.60
100.88
99.39
69.83
65.59
50.68
17.93
17.40
Cl
HO
NH
O
N
H
9



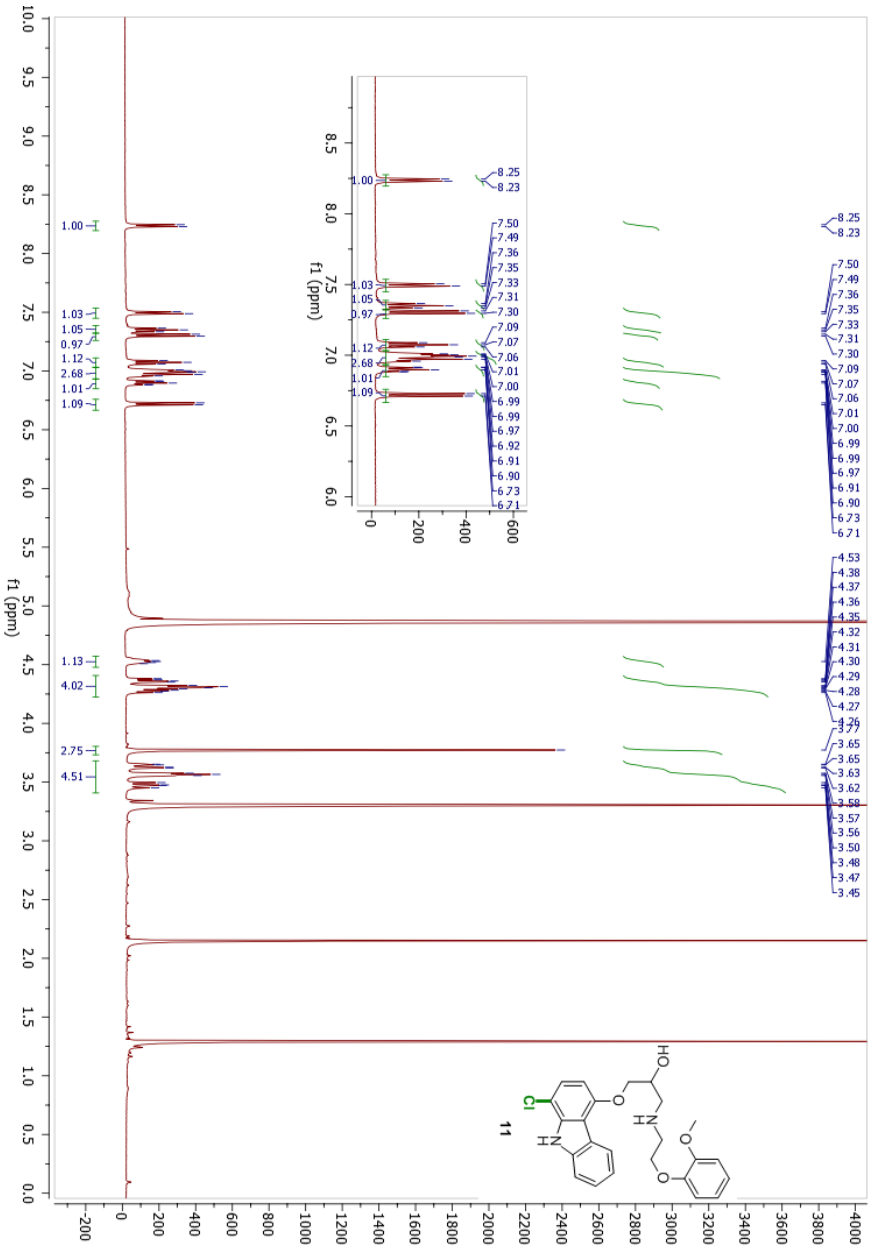
NOESY of **9**: Cross-peak between peaks at ~6.5 ppm (indole 5-position) and ~4.2 ppm (position marked by *) demonstrates chlorination occurs at the indole 7-position.

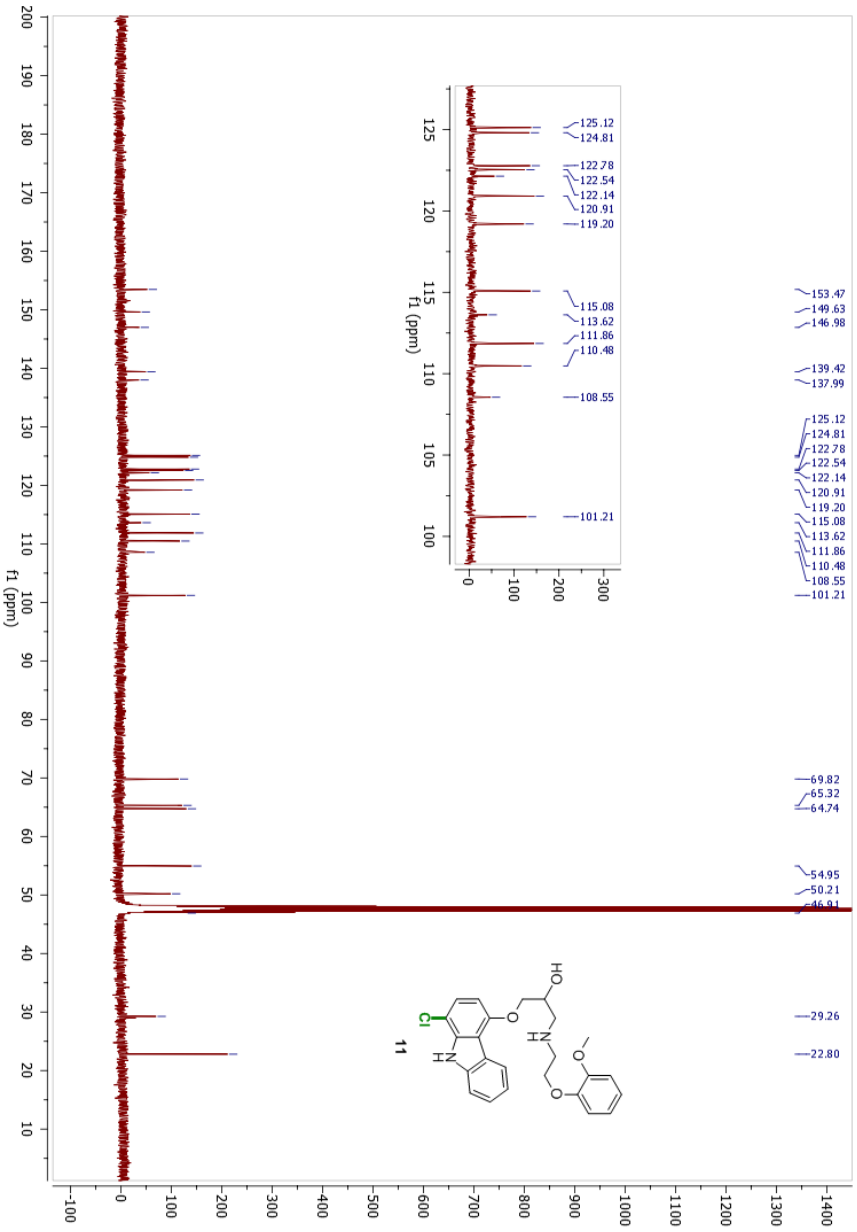

f1 (ppm)
1.00
0.92
1.08
1.03
1.00
1.06
1.09
2.16
1.13
5.60
6.87
8.27
8.26
7.53
7.52
7.42
7.41
7.40
7.40
7.38
7.38
7.31
7.30
7.21
7.19
7.18
6.72
6.70
4.46
4.45
4.44
4.44
4.43
4.42
4.42
4.41
4.35
4.34
4.33
4.32
4.27
4.26
4.25
4.24
3.50
3.49
3.48
3.43
3.43
3.41
3.40
3.35
3.34
3.32
3.31
3.31
3.31
3.30
1.39
1.38
1.38
1.37
HO
NH
O
Cl
H
N
10

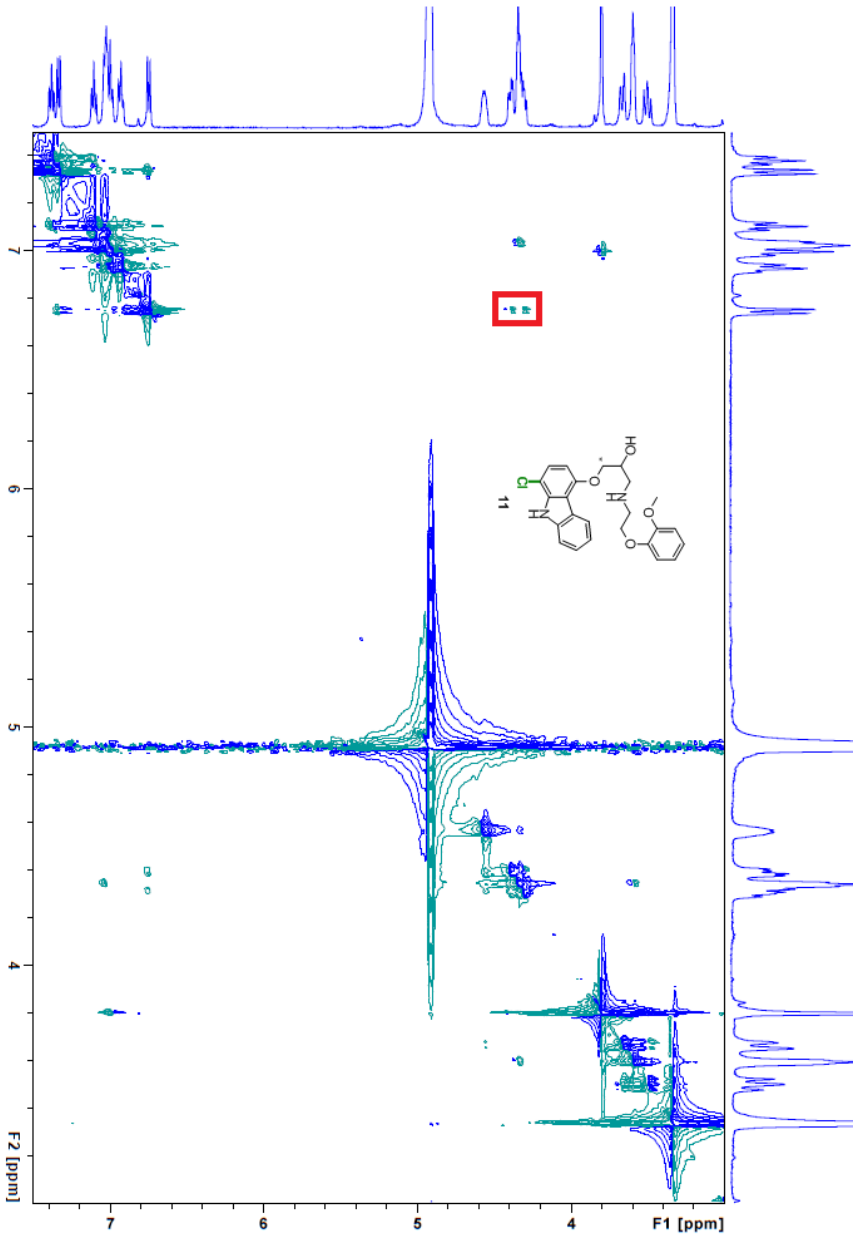

f1 (ppm)
154.88
140.86
139.40
126.58
126.25
123.88
123.57
120.57
111.96
109.94
102.64
71.14
67.01
52.04
19.34
18.78
10
Cl
HO
O
NH
H
N



NOESY of **10**: Cross-peak between peaks at ~6.7 ppm (carbazole 6-position) and ~4.4 ppm (position marked by *) demonstrates chlorination occurs at the carbazole 8-position.


f1 (ppm)
11


f1 (ppm)
125.12
124.81
122.78
122.54
122.14
120.91
119.20
115.08
113.62
111.86
110.48
108.55
101.21
153.47
149.63
146.98
139.42
137.99
69.82
65.32
64.74
54.95
50.21
29.26
22.80
Cl
HO
O
N
H
O
O
11



NOESY of **11**: Cross-peak between peaks at ~6.8 ppm (carbazole 6-position) and ~4.4 ppm (position marked by *) demonstrates chlorination occurs at the carbazole 8-position.

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