Minimal Pharmacophoric Elements and Fragment Hopping, an Approach Directed at Molecular Diversity and Isozyme Selectivity. Design of Selective Neuronal Nitric Oxide Synthase Inhibitors

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Experimental Section

Computer Modeling

Protein Structures

The crystallographic coordinates for NOS from the Research Collaboratory for Structural Bioinformatics (RCSB) protein database are as follows: rat nNOS[1]:1p6h (1.98 Å resolution, R_{cryst} = 0.231); 1p6i (1.90 Å resolution, R_{cryst} = 0.228); 1p6j (2.00 Å resolution, R_{cryst} = 0.225); 1qw6 (2.1 Å resolution, R_{cryst} = 0.20), human eNOS[2]: 3nos (2.4 Å resolution, R_{cryst} = 0.193), bovine eNOS[1]: 1p6l (2.35 Å resolution, R_{cryst} = 0.214); 1p6m (2.27 Å resolution, R_{cryst} = 0.215); 1p6n (2.5 Å resolution, R_{cryst} = 0.219), human iNOS:[3] 1nsi (2.55 Å resolution, R_{cryst} = 0.209), murine iNOS[4]: 1qw4 (2.3 Å resolution, R_{cryst} = 0.213). The amino acid sequences of NOS were retrieved from the PIR protein sequence database. The sequences are human nNOS (entry P29475), rat nNOS (entry P29476), human eNOS (entry P29474), bovine eNOS (entry P29473), human iNOS (entry P35228), and murine iNOS (entry P29477). All of the computational work was performed on Silicon Graphics Octane 2 Workstations with an IRIX 6.5 operating system. The molecular modeling was achieved with commercially available InsightII 2000[5] and SYBYL 6.8[6] software packages. The NOS model was prepared by first adding H-atoms using the Accerlyrs/InsightII 2000 software, and the protonation states of the residues were set to pH 7.0. The cvff force field of the Discover 98.0 program within Insight II was used to optimize the orientation of hydrogen atoms of the protein and of structural waters. The ligands and solvent molecules of the protein structures were removed, but the heme and H_4B were retained near the active site. The size and spatial orientation of the active site was analyzed by the Binding Site

Analysis module within Insight II. The grid size for searching the proteins was set to 1 Å ×1 Å ×1 Å. All of the solvent-accessible surfaces in the proteins were filled with grid points, and only those having at least 125 grid points were accepted as possible ligand binding sites.

GRID Calculations

The calculations were performed with version 22 of the GRID software.[7] Hydrogens were added with the program GRIN. The GRID box dimensions were chosen to encompass all of the active site residues shown in Figure 1. This results in a box size of 31 × 28 × 31 Å. The grid spacings were set to 1 Å (directive NPLA = 1) and 0.5 Å (directive NPLA = 2), respectively. The amino acids in the active site were considered rigid (directive move = 0). The directives NETA and ALMD were set to 120 and 1, respectively, to include the atoms of heme and H_4B in calculations and to interpret which atom(s) in the active site contribute(s) to the interaction with a specific probe atom. The following single atom probes were used in the calculation: DRY, C3, NM3, N1+, N3+, N1, NH=, O, O1, and the multi-atom probes COO^-, Amidine, and ARamidine.

MCSS Calculations

The functional groups chosen for the MCSS calculations were benzene (all hydrogen model), cyclohexane, isobutene, *N*-methylacetamide, methanol, ether, acetate ion, methylammonium, trimethylamine cation, and pyrrolidine cation. The binding site area in the MCSS simulation was defined as an approximately 27 Å × 26 Å × 31 Å box, which was centered around the grid points searched by InsightII/Binding Site Analysis. Replicas of a given functional group were randomly distributed inside the binding site and then simultaneously and independently energy minimized. Pairs of molecules were considered to be identical if the root-mean-square deviation (RMSD) between them was less than 0.2 Å, and in such cases one of the pairs was eliminated. The above protocol was repeated 10 times for each of the functional groups to allow complete searching of the active site. The minima with the binding energy lower than 0 kcal/mol were retrieved in the analysis. All of the above calculations were performed using the CHARMM 22 force field and MCSS 2.1 program.[8]

LUDI Ligand Design

The constructed targeted fragment libraries were converted into a LUDI user library. To determine the appropriate position and orientation of the fragments from the LUDI user library in the active site of NOS, LUDI was first applied to generate the interaction sites for each pharmacophore. Four different types of the interaction sites are defined in the LUDI program: lipophilic–aliphatic, lipophilic–aromatic, hydrogen donor, and hydrogen acceptor. The residues inside an 8.0 Å radius sphere, which centered on the centroid of the minimal pharmacophoric elements, were used to generate the interaction sites. The link library switch was turned off, and the target mode switch was turned on. The LUDI scoring function was set to Energy-estimate-2.[9] Other settings were kept with the standard default parameters. To convert the above determined fragments into a molecule, the side chain library in Supporting Information Figure 5 was converted into a LUDI link library and was used further in the connection operation. The hydrogen atoms in the above fragment structure were replaced by a link fragment to create a new substructure. The switch for the target mode was turned off, but the switch for the link library was turned on. The linkage parameter can be set to 1 (the link fragment fit at least one link site), 2 (the link fragment simultaneously fit at least two link sites), or be specified (the link site was

specifically assigned) according to the actual requirements. The other settings are the standard default parameters.

AutoDock Analysis

AutoDock 3.0 was employed to perform the docking calculations, as reported in our previous paper.[10] The ligands and solvent molecules of the protein structures were removed, but the cofactors heme and H_4B were retained near the active site. For each protein structure, polar hydrogen atoms were added, and Kollman united atom charges were assigned.[11] Hydrogens were also added to the heme and H_4B, and charges were calculated by the Gasteiger–Marsili method.[12] The charge of the Fe atom bound to heme was assigned +3. The nonpolar hydrogen atoms of heme and H_4B were removed manually and their charges were united with the bonded carbon atoms. Atomic solvation parameters and fragmental volumes were assigned using the AddSol utility. The 3D structures of the ligands were built using SYBYL 6.8. Partial atomic charges were also calculated using the Gasteiger–Marsili method. The rotatable bonds in the ligands were defined using another AutoDock 3.0 auxiliary program, AutoTors, which also unites the nonpolar hydrogens and partial atomic charges to the bonded carbon atoms. The grid maps were calculated using AutoGrid. The dimensions of the grid box was 27 × 26 × 31 Å and the grid spacing was set to 0.375 Å. Docking was performed using the Lamarckian genetic algorithm (LGA), and the pseudo-Solis and Wets method were applied for the local search. Each docking experiment was performed 100 times, yielding 100 docked conformations. Parameters for the docking experiments were as follows: initial population size of 200; random starting position and conformation; maximal mutation of 0.2 Å in translation and 5º in orientation and rotation; elitism of 5; mutation rate of 0.02 and crossover rate of 0.8; local search rate of 0.06 and maximal iteration per local search of 300. Simulations were performed with a maximum of 1.5×10^6 energy evaluations and a maximum of 27,000 generations. Other settings were the standard default parameters. All of the ligands followed the same docking protocol. The results of the docking experiments were evaluated by calculating the positional root-mean-square deviation (RMSD) of the corresponding atoms of each conformation[13] and/or by a visual comparison to determine if the orientation of the inhibitor matches the pharmacophores determined.

Chemical Synthesis

General Methods, Reagents and Materials

All reagents were purchased from Aldrich, Acros Organics, and Fisher Scientific and were used without further purification unless stated otherwise. NADPH, calmodulin, and human ferrous hemoglobin were obtained from Sigma Chemical Co. Tetrahydrobiopterin (H_4B) was purchased from Alexis Biochemicals. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz or a Varian Inova 500 MHz spectrometer (100.6, or 125.7 MHz, for ^{13}C NMR spectra, respectively) in $CDCl_3$, DMSO-d_6, CD_3OD or D_2O. Chemical shifts are reported as values in parts per million and the reference resonance peaks set at 0 ppm [TMS($CDCl_3$)], 2.50 ppm [$(CD_2H)_2SO$], 3.31 ppm (CD_2HOD), and 4.80 ppm (HOD) respectively for ^{1}H NMR and 77.23 ppm ($CDCl_3$), 39.52 ppm (DMSO-d_6) and 49.00 ppm (CD_3OD) for ^{13}C NMR spectra. An Orion research model 701H pH meter with a general combination electrode was used for pH measurements. Mass spectra were performed on a Micromass Quattro II triple quadrupole HPLC/MS mass spectrometer with an electrospray ionization (ESI) source or atmospheric pressure chemical ionization (APCI) source. High-resolution mass spectra were carried out

using a VG70-250SE mass spectrometer. Chemical ionization (CI) or electron impact (EI) was used as the ion source. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Thin-layer chromatography was carried out on E. Merck pre-coated silica gel 60 F254 plates with visualization accomplished with phosphomolybdic acid or ninhydrin spray reagent or with a UV–visible lamp. Column chromatography was performed with E. Merck silica gel 60 (230–400 mesh) or Sorbent Technologies 250 mesh silica gel. Tetrahydrofuran (THF) and ethyl ether were redistilled under nitrogen using sodium and benzophenone as the indicator. Dichloromethane was redistilled from CaH_2 under nitrogen. Other dry solvents were directly purchased from the companies.

***tert*-Butyl 6-methylpyridin-2-ylcarbamate (16).** A solution of 2-amino-6-picoline (2.702g, 0.025 mol) in 50 mL of melted *tert*-butanol was treated with di-*tert*-butyl dicarbonate (Boc_2O) (6.00 g, 0.0275 mol). The temperature was kept about 60 °C. After the solution was stirred for 48 h, the solvent was evaporated in vacuo. The residue was purified directly by silica gel column chromatography (hexanes : ethyl acetate = 8 : 2) to afford a white solid (4.42 g, 85%). mp 57.9 –58.6 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.717 (d, 1H, J=8.8Hz), 7.540 (t, 1H, J=8Hz), 6.804 (d, 1H, J=7.6Hz), 2.432 (s, 3H), 1.501 (s, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 156.8 (1C), 152.6 (1C), 151.3 (1C), 138.5 (1C), 118.0 (1C), 109.2 (1C), 80.9 (1C), 28.6 (3C), 24.3 (1C). MS (ESI, CH_3OH): *m/z* = 209.2 ($[M+H]^+$).

3-Benzyl-6-oxa-3-azabicyclo[3.1.0]hexane (17). To an ice-cooled solution of 1-benzyl pyrroline (1.591 g, 0.01 mol), 98% H_2SO_4 (1.200 g, 0.012 mol), H_2O (1.5 g), and acetone (10 mL) in a round bottom flask was added MCPBA (maximum 77% pure, 2.904 g, 0.013 mol) with stirring and allowed to react for about 50 h at room temperature. After completion of the reaction (TLC monitored the consumption of the starting material), acetone was evaporated under reduced pressure, the mixture was neutralized with 1M NaOH (20 mL), and extracted with toluene (30 mL × 3). The precipitates that appeared were filtered, and the filtrate was washed with water (30 mL × 2). After the solvent was evaporated under reduced pressure, the residue was purified by silica gel column chromatography (CH_2Cl_2 : EtOAc : MeOH = 7.5 : 2.00 : 0.5) to afford a pure colorless oil (1.35 g , 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.328-7.231 (m, 5H), 3.724 (s, 2H), 3.623 (s, 2H), 3.211 (s, 1H), 3.182 (s,1H), 2.561 (s,1H), 2.532 (s,1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 138.6 (1C), 128.8 (2C), 128.3 (2C), 127.1 (1C), 60.3 (1C), 56.2 (1C), 53.7 (1C).

(±)-*tert*-Butyl 6-[(*trans*-1'-benzyl-4'-hydroxypyrrolidin-3'-yl)methyl]pyridin-2-ylcarbamate (18). A solution of **16** (1.301 g, 0.00625 mol) in 10 mL of dry THF was cooled in a –78 °C bath. n-BuLi (1.6 M in hexanes, 7.81 mL, 0.0125 mol) was added during 15 min under N_2. The color of the solution changed from colorless to orange, then the cooling bath was removed. After 45 min stirring at room temperature, the colored solution changed to dark red. The solution was then returned to the –78 °C bath. **17** (0.876 g, 0.005 mol) in 10 mL of dry THF was added over 1 h. After 1 h, the cooling bath was removed, and the solution was stirred for 1 h more at room temperature. The reaction was quenched by the addition of ice-cold water (50 mL). The mixture was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2 : MeOH = 9:1) to afford a pale-green oil (1.724 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.739 (d, 1H, J=8.4 Hz), 7.561 (t, 1H, J=7.2Hz), 7.319-7.202 (m,

6H), 6.8065 (d, 1H, J=7.6Hz), 4.131-4.096 (m, 1H), 3.654 (d, 1H, J=12.8Hz), 3.580 (d, 1H, J=12.8Hz), 3.007-2.964 (m, 1H), 2.901-2.705 (m, 4H), 2.538-2.506 (m, 1H), 2.192-2.149 (m,1H), 1.501 (s, 9H). ^{13}C NMR($CDCl_3$, 100.6 MHz): δ 158.8 (1C), 152.2 (1C), 151.2 (1C), 138.8 (2C), 128.8 (2C), 128.3 (2C), 127.0 (1C), 118.2 (1C), 109.9 (1C), 81.2 (1C), 77.1 (1C), 61.9 (1C), 60.6 (1C), 59.3 (1C), 47.1 (1C), 40.9 (1C), 28.6 (3C). MS (ESI, CH_3OH): *m/z* = 384.4 ($[M+H]^+$).

(±)-*tert*-Butyl 6-[(1'-benzyl-4'-oxopyrrolidin-3'-yl)methyl]pyridin-2-ylcarbamate (19). To a solution of dimethyl sulfoxide (DMSO, 0.313 g, 0.284 mL, 0.004 mol) in 30 mL of dry CH_2Cl_2 was added dropwise oxalyl chloride (0.381 g, 0.285 mL, 0.003 mol). The mixture was stirred at –78 °C for 10 min. After this time a solution of **18** (0.766 g, 0.002 mol) in 10 mL of anhydrous CH_2Cl_2 was added dropwise at a rate to keep the reaction temperature below –60 °C. Upon complete addition, the mixture was allowed to stir at –78 °C for 2 h. Anhydrous triethylamine (0.607 g, 0.836 mL, 0.006 mol) was added dropwise to the mixture, then the reaction mixture was allowed to warm to room temperature. The resulting solution was partitioned between 1 M NaOH (40 mL) and CH_2Cl_2, and the product was extracted with CH_2Cl_2 (30 mL × 2). All organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated in vacuo to yield the crude product, which was purified using silica gel column chromatography (CH_2Cl_2 : EtOAc = 4 : 1) to afford a pale-yellow oil (0.66g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.735 (d, 1H, J=8 Hz), 7.561 (t, 1H, J=8Hz), 7.322-7.263 (m, 5H), 7.142 (brs,1H), 6.7745 (d, 1H, J=7.6Hz), 3.730 (d, 1H, J=12.8Hz), 3.617 (d, 1H, J=12.8Hz), 3.316-3.121 (m, 3H), 3.008-2.958 (m, 1H), 2.795-2.688 (m, 2H), 2.470-2.424 (m, 1H), 1.521 (s, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 215.3 (1C), 157.5 (1C), 152.4 (1C), 151.4 (1C), 138.7 (1C), 137.7 (1C), 128.8 (2C), 128.6 (2C), 127.5 (1C), 118.0 (1C), 109.8 (1C), 81.1 (1C), 61.9 (1C), 60.8 (1C), 57.2 (1C), 48.7 (1C), 36.1 (1C), 28.5 (3C).

(±)-*tert*-Butyl 6-{[*cis*-1'-benzyl-4'-(3"-phenylpropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-ylcarbamate (20a) and (±)-*tert*-butyl 6-{[*trans*-1'-benzyl-4'-(3"-phenylpropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-ylcarbamate (21a). To a solution of **19** (0.381 g, 0.001 mol), a substituted amine such as 3-phenyl-1-propylamine (0.203 g, 0.0015 mol), acetic acid (0.090 g, 0.086 mL, 0.0015 mol), and 3 Å molecular sieves (1 g) in dry MeOH (20 mL) was added $NaBH_3CN$ (0.126 g, 0.002 mol). The reaction was stirred at room temperature under a N_2 atmosphere for 36 h. The reaction mixture was then filtered, and the filtrate was concentrated in vacuo. The residue was diluted with 1M NaOH (30 mL) and extracted with CH_2Cl_2 (30 mL × 2). The organic layers were combined, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give the crude product, which was purified by silica gel column chromatography (hexanes : EtOAc : Et_3N = 6 : 4 : 0.5) to afford a pale-yellow oil (0.325 g, 65%). The *cis* isomer (**20a**) and the *trans* isomer (**21a**) can be separated with the above eluent. The ratio of *cis* and *trans* isomers was 45 : 55.

20a: (pale-yellow oil, 0.146 g): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.714 (d, 1H, J=8 Hz), 7.520 (t, 1H, J=8Hz), 7.307-7.118 (m, 11H), 6.776 (d, 1H, J=7.2Hz), 3.609 (d, 1H, J=12.8Hz), 3.520 (d, 1H, J=12.8Hz), 2.967-2.929 (m, 1H), 2.853-2.688 (m, 4H), 2.571-2.533 (m, 2H), 2.458-2.327 (m,4H), 2.261-2.223 (m,1H), 1.732-1.660 (m, 2H), 1.504 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 159.2 (1C), 152.4 (1C), 151.2 (1C), 142.2 (1C), 139.2 (1C), 138.5 (1C), 128.9 (2C), 128.5 (2C), 128.4 (2C), 128.330 (2C), 127.0 (1C), 125.9 (1C), 118.1 (1C), 109.6 (1C), 81.0 (1C), 63.6 (1C), 60.9 (1C), 60.5 (1C), 59.4 (1C), 47.8 (1C), 45.4 (1C), 42.6 (1C), 33.8 (1C), 32.0 (1C), 28.6 (3C). MS (APCI, CH_3OH): *m/z* = 501.5 ($[M+H]^+$).

21a: (pale-yellow oil, 0.179 g): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.704 (d, 1H, J=8.4 Hz), 7.522 (t, 1H, J=8Hz), 7.299-7.150 (m, 11H), 6.796 (d, 1H, J=7.2Hz), 3.635 (d, 1H, J=12.8Hz), 3.592 (d, 1H, J=12.8Hz), 3.274-3.228 (m, 1H), 2.947-2.894 (m, 2H), 2.750-2.558 (m, 6H), 2.511-2.447 (m, 1H), 2.393-2.358 (m,2H), 1.861-1.766 (m, 2H), 1.506 (s, 9H). ^{13}C NMR (125.7MHz, $CDCl_3$): δ 159.8 (1C), 152.5 (1C), 151.3 (1C), 142.3 (1C), 139.4 (1C), 138.5 (1C), 128.8 (2C), 128.5 (2C), 128.5 (2C), 128.3 (2C), 127.0 (1C), 125.9 (1C), 118.2 (1C), 109.4 (1C), 81.0 (1C), 60.8 (1C), 60.4 (1C), 59.2 (1C), 58.7 (1C), 48.3 (1C), 41.5 (1C), 36.7 (1C), 33.9 (1C), 32.1 (1C), 28.5 (3C). MS (APCI, CH_3OH): *m/z* = 501.5 ($[M+H]^+$).

(±)-*tert*-Butyl 6-{[*cis*-1'-benzyl-4'-(3"-hydroxypropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-ylcarbamate (20b) and (±)-*tert*-Butyl 6-{[*trans*-1'-benzyl-4'-(3"-hydroxypropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-ylcarbamate (21b). The procedure to prepare **20b** and **21b** is the same as that to prepare **20a** and **21a** except using 3-amino-1-propanol (0.113 g, 0.0015 mol) instead of 3-phenyl-1-propylamine. The yield was 55% (0.242 g). The *cis* isomer (**20b**) and the *trans* isomer (**21b**) can be separated by silica gel column chromatography (CH_2Cl_2 : Me_2CO : Et_3N = 9 : 1 : 0.5). The ratio of the *cis* isomer to the *trans* isomer was 45 : 55.

20b: (pale-yellow oil, 0.109 g): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.709 (d, 1H, J=8.5 Hz), 7.534 (t, 1H, J=7.5Hz), 7.477 (brs, 1H), 7.323-7.226 (m, 5H), 6.780 (d, 1H, J=7.5Hz), 3.871-3.788 (m, 2H), 3.652 (d, 1H, J=13Hz), 3.601 (d, 1H, J=13Hz), 3.201-3.169 (m, 1H), 2.921-2.839 (m, 3H), 2.737-2.617 (m, 4H), 2.498-2.470 (m, 1H), 2.412-2.385 (m, 1H), 1.726-1.705 (m, 2H), 1.519 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 159.5 (1C), 152.7 (1C), 151.5 (1C), 139.4 (1C), 138.8 (1C), 128.8 (2C), 128.5 (2C), 127.1 (1C), 118.0 (1C), 109.6 (1C), 81.0 (1C), 64.2 (1C), 60.6 (1C), 59.6 (1C), 59.4 (1C), 58.3 (1C), 48.6 (1C), 41.9 (1C), 36.7 (1C), 31.3 (1C), 28.5 (3C). MS (ESI, CH_3OH): *m/z* = 441.3 ($[M+H]^+$).

21b: (pale-yellow oil, 0.133 g): ^{1}H NMR (500MHz, $CDCl_3$): δ 7.762 (brs, 1H), 7.721 (d, 1H, J=8 Hz), 7.529 (t, 1H, J=8Hz), 7.311-7.242 (m, 5H), 6.765 (d, 1H, J=7.5Hz), 3.786 (t, 2H, J=5Hz), 3.600 (d, 1H, J=13Hz), 3.546 (d, 1H, J=13Hz), 2.959-2.940 (m, 1H), 2.864-2.619 (m, 5H), 2.562-2.506 (m, 2H), 2.466-2.390 (m, 1H), 2.162-2.131 (m,1H), 1.607-1.588 (m, 2H), 1.521 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 158.9 (1C), 152.9 (1C), 151.8 (1C), 139.2 (1C), 138.5 (1C), 128.8 (2C), 128.5 (2C), 127.2 (1C), 118.1 (1C), 109.7 (1C), 80.8 (1C), 64.5 (1C), 63.4 (1C), 60.4 (1C), 59.6 (1C), 59.4 (1C), 48.3 (1C), 44.5 (1C), 42.2 (1C), 30.7 (1C), 28.5 (3C). MS (ESI, CH_3OH): *m/z* = 441.3 ($[M+H]^+$).

(±)-6-{[*cis*-1'-Benzyl-4'-(3"-phenylpropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-amine trihydrochloride (8). A solution of 4M HCl in 1,4-dioxane (4 mL) was added to **20a** (0.100 g, 0.0002 mol) at 0 ºC under argon. The ice-water bath was removed after 3 h, and the reaction mixture was stirred at room temperature 48 h. After the completion of the reaction, liquids were evaporated under reduced pressure, and the residue was partitioned between water (10 mL) and ethyl acetate (10 mL). The aqueous layer was then washed with ethyl acetate (5 mL × 2). After evaporation of water by high-vacuum rotory evaporation, the residue was dried with a lyophilizer to afford a hygroscopic white solid (0.102 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O,): δ 7.792 (t, 1H, J=8.5Hz), 7.533-7.482 (m, 5H), 7.390-7.287 (m, 5H), 6.893 (d, 1H, J=9Hz), 6.7285 (d, 1H, J=7.5Hz), 4.489 (s, 2H), 4.306-4.292 (m, 1H), 4.058-4.017 (m, 1H), 3.672-3.641 (m, 1H), 3.598-3.560 (m, 1H), 3.412-3.370 (m, 1H), 3.218-3.049 (m, 4H), 2.889-2.833 (m, 1H), 2.794-2.692 (m, 2H), 2.116-2.066 (m, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.8 (1C), 144.7 (1C),

144.0 (1C), 140.5 (1C), 130.7 (2C), 129.7 (2C), 129.0 (2C), 128.6 (2C), 126.8 (2C), 112.7 (1C), 112.4 (1C), 59.3 (1C), 56.5 (1C), 55.2 (1C), 53.2 (1C), 47.3 (1C), 37.5 (1C), 31.8 (1C), 29.3 (1C), 27.0 (1C). MS (ESI, CH_3CN–H_2O): *m/z* = 401.4($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 401.2700, Found: 401.2701.

(±)-6-{[*trans*-1'-Benzyl-4'-(3''-phenylpropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-amine trihydrochloride (14). The procedure to prepare **14** is the same as that to prepare **8** except using **21a** instead of **20a**, affording a hygroscopic white solid (0.102 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.783 (t, 1H, J=8.5Hz), 7.560-7.507 (m, 5H), 7.390-7.258 (m, 5H), 6.886 (d, 1H, J=9Hz), 6.719 (d, 1H, J=7Hz), 4.496 (s, 2H), 4.038-4.000 (m, 2H), 3.755-3.650 (m, 2H), 3.405-3.366 (m, 1H), 3.225-3.185 (m, 1H), 3.060-2.910 (m, 4H), 2.736-2.692 (m, 2H), 2.048-1.977 (m, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.7 (1C), 144.7 (1C), 144.4 (1C), 140.3 (1C), 130.7 (2C), 129.6 (2C), 129.0 (2C), 128.6 (2C), 126.8 (2C), 112.6 (2C), 59.0 (1C), 59.0 (1C), 56.3 (1C), 54.2 (1C), 46.6 (1C), 39.5 (1C), 34.1 (1C), 31.7 (1C), 27.1 (1C). MS (ESI, CH_3CN–H_2O): *m/z* 401.4 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 401.2700, Found: 401.2697.

(±)-3-{*cis*-4'-[(6''-Aminopyridin-2''-yl)methyl]-1'-benzylpyrrolidin-3'-ylamino}propan-1-ol trihydrochloride (9). The procedure to prepare **9** is the same as that to prepare **8** except using **20b** (0.090 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.090 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.818 (t, 1H, J=8.5Hz), 7.549-7.508 (m, 5H), 6.916 (d, 1H, J=9Hz), 6.764 (d, 1H, J=7 Hz), 4.528 (s, 2H), 4.384-4.374 (m, 1H), 4.169-4.127 (m, 1H), 3.743-3.674 (t, 2H, J=6Hz), 3.635-3.598 (m, 1H), 3.465-3.398 (m, 2H), 3.327-3.218 (m, 4H), 2.931-2.876 (m, 1H), 2.054-1.954 (m, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.9 (1C), 144.7 (1C), 144.0 (1C), 130.7 (2C), 129.7 (2C), 129.0 (1C), 112.7 (1C), 112.4 (1C), 59.4 (1C), 58.9 (1C), 56.6 (1C), 55.2 (1C), 53.3 (1C), 46.1 (1C), 37.6 (1C), 29.4 (1C), 27.0 (1C). MS (ESI, CH_3CN–H_2O): *m/z* = 341.3 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 341.2336, Found: 341.2330.

(±)-3-{*trans*-4'-[(6''-Aminopyridin-2''-yl)methyl]-1'-benzylpyrrolidin-3'-ylamino}propan-1-ol trihydrochloride (15). The procedure to prepare **15** is the same as that to prepare **8** except using **21b** (0.090 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.090 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.827 (t, 1H, J=8.5Hz), 7.539-7.516 (m, 5H), 6.917 (d, 1H, J=9Hz), 6.766 (d, 1H, J=7Hz), 4.509 (s, 2H), 4.067-4.004 (m, 1H), 3.753-3.721 (m, 2H), 3.687 (t, 2H, J=6Hz), 3.467-3.118 (m, 6H), 3.016-2.966 (m, 1H), 1.958-1.919 (m, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.8 (1C), 144.7 (1C), 144.5 (1C), 130.6 (2C), 129.7 (2C), 129.0 (1C), 112.6 (2C), 59.2 (1C), 59.1 (1C), 58.8 (1C), 56.2 (1C), 54.1 (1C), 45.4 (1C), 40.0 (1C), 34.0 (1C), 27.995 (1C). MS (ESI, CH_3OH): *m/z* = 341.3 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calc.: 341.2336, Found: 341.2329.

***tert*-Butyl 6-oxa-3-azabicyclo[3.1.0]hexane-3-carboxylate (22).** Boc_2O (3.274 g, 0.015 mol) was added in portions to a solution of 3-pyrroline (65% pure, 1.063 g, 0.01 mol) in 20 mL of MeOH at 0 °C. The reaction mixture was then stirred at room temperature for 24 h. After the reaction was completed (TLC monitored using 9:1 hexanes : EtOAc), the solvent was evaporated in vacuo. The residue was then dissolved in 30 mL of CH_2Cl_2. The reaction mixture was cooled to 0 °C, and *m*-CPBA (maximum 77% pure, 2.904 g, 0.013 mol) was added in portions. After the mixture was stirred at room temperature for 48 h, 20% Na_2SO_3 (30 mL) was

added, and the two layers were separated. The aqueous layers were extracted with CH_2Cl_2 (20 mL × 2). The combined organic extracts were washed with 20% Na_2SO_3 (30 mL × 2) and water (30 mL × 2). The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (hexanes : EtOAc = 7:3) to give a colorless oil (71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.829-3.672 (m, 4H), 3.333-3.295 (m, 2H), 1.449 (s, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 154.8 (1C), 79.9 (1C), 55.8 (1C), 55.3 (1C), 47.5 (1C), 47.1 (1C), 28.7 (3C).

(±)-*trans*-*tert*-Butyl 3-{[6'-(tert-butoxycarbonylamino)pyridin-2'-yl]methyl}-4-hydroxypyrrolidine-1-carboxylate (23). The procedure to prepare **23** is the same as that to prepare **18**. **16** (1.041 g, 0.005 mol) was treated with n-BuLi (1.6 M in hexanes, 7.81 mL, 0.0125 mol), and then with **22** (1.158 g, 0.00625 mol) to afford a colorless oil (1.475 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.806 (d, 1H, J=8Hz), 7.606 (t, 1H, J=8Hz), 7.211 (brs, 1H), 6.843 (d, 1H, J=7.2Hz), 5.231 (brs, 1H), 4.180-4.096 (m, 1H), 3.840-3.631 (m, 2H), 3.229-3.066 (m, 2H), 2.866-2.851 (m, 2H), 2.445-2.389 (m, 1H), 1.507 (s, 9H), 1.464 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ (157.9+157.8) (1C), (154.6+154.5) (1C), (152.4+152.3) (1C), (151.6+151.5) (1C), 139.0 (1C), 118.0 (1C), 110.1 (1C), (80.9+80.8) (1C), (79.35+79.33) (1C), (74.7+74.1) (1C, 10), (52.5+52.2) (1C, 9), (49.5+49.0) (1C, 8), (45.3+44.9) (1C, 7), (39.2+39.0) (1C, 6), 28.4 (3C, 17), 28.2 (3C, 13). MS (ESI, CH_3OH): *m/z* = 416.4 ($[M+Na]^+$).

(±)-*tert*-Butyl 3-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}-4-oxopyrrolidine-1-carboxylate (24). To a suspension of Dess–Martin periodinane (0.594 g, 0.0014 mol) in 10 mL of dry CH_2Cl_2 was added a solution of **23** (0.393 g, 0.001 mol) in 5 mL of dry CH_2Cl_2 *via* cannula. The reaction mixture was stirred at room temperature for 18 h. $Na_2S_2O_3$ (1 M, 10 mL) was added to the reaction, and after stirring for 10 min, the reaction mixture was extracted with CH_2Cl_2 (10 mL × 3). The combined organic layers were washed with 5% aqueous $NaHCO_3$ (20 mL × 3) and brine (20 mL × 2). The organic layer was dried over Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (hexanes : EtOAc = 8 : 2) to give a pale-yellow oil (0.376 g, 96%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.756 (d, 1H, J=8Hz), 7.561 (t, 1H, J=8Hz), 7.061 (brs, 1H), 6.7985 (d, 1H, J=7.5Hz), 3.950-3.911 (m, 2H), 3.836-3.725 (m, 1H), 3.353 (m, 1H), 3.197-3.171 (m, 1H), 3.058 (m,1H), 2.897 (m, 1H), 1.526 (s, 9H), 1.469 (s, 9H). ^{13}C NMR(125.7 MHz, $CDCl_3$): δ 212.8 (1C), 156.3 (1C), 154.6 (1C), 152.4 (1C), 151.5 (1C), 138.9 (1C), 118.2 (1C, 4), 110.1 (1C), 81.3 (1C), 80.5 (1C), 53.4 (1C), 48.3 (1C), 46.8 (1C), 35.9 (1C), 28.6 (3C), 28.5 (3C).

(±)-*cis*-*tert*-Butyl 3-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}-4-(3'-phenylpropylamino)pyrrolidine-1-carboxylate (25a) and (±)-*trans*-*tert*-Butyl 3-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}-4-(3'-phenylpropylamino)pyrrolidine-1-carboxylate (26a). The procedure to prepare **25a** and **26a** is the same as that to prepare **20a** and **21a** except using **24** (0.391 g, 0.001 mol) instead of **19**. The yield was 65% (0.332 g). The *cis* isomer (**25a**) and the *trans* isomer (**26a**) can be separated by silica gel column chromatography (CH_2Cl_2 : Me_2CO : Et_3N = 8 : 2 : 0.5). The ratio of the *cis* isomer and the *trans* isomer was 45 : 55.

25a: (pale-yellow oil, 0.149 g): ^{1}H NMR(500 MHz, $CDCl_3$): δ 7.753-7.720 (m, 1H), 7.570-7.519 (m, 1H), 7.273-7.124 (m, 6H), 6.797 (d, 1H, J=7.5Hz), 3.428-3.357 (m, 2H), 3.266-3.132 (m, 2H), 2.865-2.814 (m, 1H), 2.668-2.469 (m, 7H), 1.804-1.793 (m, 2H), 1.514 (s, 9H), 1.447 (s,

9H). ^{13}C NMR(125.7 MHz, $CDCl_3$,): δ 158.9 (1C), 155.0 (1C), 152.5 (1C), 151.5 (1C), 142.2 (1C), 138.7 (1C), 128.6 (2C), 128.6 (2C), 126.0 (1C), (118.23+118.18) (1C), 109.7 (1C), 81.1 (1C), 79.4 (1C), (59.8+58.7) (1C), (50.9+50.4) (1C), (49.6+49.3) (1C), 47.8 (1C), (42.4+41.6) (1C), (35.4+35.3) (1C), 33.8 (1C), 32.1 (1C), 28.7 (3C), 28.5 (3C). MS (ESI, CH_3OH): *m/z* = 511.3 ($[M+H]^+$).

26a: (pale-yellow oil, 0.182 g): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.742 (m, 1H), 7.552-7.538 (m, 1H), 7.273-7.141 (m, 6H), 6.7725 (d, 1H, J=7.5Hz), 3.698-3.686 (m, 0.5H), 3.632-3.615 (m, 0.5H), 3.588-3.553 (m, 0.5H), 3.489-3.457 (m, 0.5H), 3.141-2.852 (m, 4H), 2.630-2.581 (m, 5H), 2.426-2.415 (m, 0.5H), 2.349-2.338 (m, 0.5H), 1.805-1.742 (m, 2H), 1.514 (s, 9H), 1.446 (s, 9H). ^{13}C NMR(125.7 MHz, $CDCl_3$): δ 158.4 (1C), 154.8 (1C), 152.5 (1C), 151.5 (1C), 142.2 (1C), 138.7 (1C), 128.6 (4C), 126.0 (1C), 118.1 (1C), 109.8 (1C), 81.1 (1C), 79.4 (1C), (62.6+61.8) (1C), (52.2+51.6) (1C), (50.1+49.9) (1C), 47.8 (1C), (44.2+43.6) (1C), (40.14+40.05) (1C), 33.7 (1C), 32.1 (1C), 28.7 (3C), 28.5 (3C). MS (ESI, CH_3OH): *m/z* = 511.3 ($[M+H]^+$).

(±)-*cis*-*tert*-Butyl 3-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl)methyl)-4-(3'-hydroxypropylamino)pyrrolidine-1-carboxylate (25b) and (±)-*trans*-*tert*-Butyl 3-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl)methyl)-4-(3'-hydroxypropylamino)pyrrolidine-1-carboxylate (26b). The procedure to prepare **25b** and **26b** is the same as that to prepare **20a** and **21a** except using **24** (0.391 g, 0.001 mol) and 3-amino-1-propanol (0.113 g, 0.0015 mol) instead of **19** and 3-phenyl-1-propylamine. The yield was 50% (0.225 g). The *cis* isomer (**25b**) and the *trans* isomer (**26b**) can be separated by silica gel column chromatography (CH_2Cl_2 : EtOAc : Et_3N = 4 : 6 : 0.5). The ratio of the *cis* isomer and the *trans* isomer was 45 : 55.

25b: (colorless oil, 0.101 g): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.769-7.739 (m, 1H), 7.588-7.559 (m, 2H), 6.8055 (d, 1H, J=7.5Hz), 3.865-3.804 (m, 2H), 3.500-3.406 (m, 1H), 3.337-3.274 (m, 1H), 3.240-3.067 (m, 2H), 2.952-2.834 (m, 2H), 2.730-2.584 (m, 4H), 1.769-1.728 (m, 2H), 1.524 (s, 9H), 1.454 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 158.4 (1C), 155.0 (1C), 152.7 (1C), 151.7 (1C), 138.9 (1C), (118.0+117.9) (1C), 109.9 (1C), 81.0 (1C), 79.6 (1C), 63.6 (1C), (59.7+58.7) (1C), (50.3+50.0) (1C), (49.5+49.3) (1C), 47.7 (1C), (43.0+41.9) (1C), 35.5 (1C), 31.6 (1C), 28.7 (3C), 28.5 (3C). MS (ESI, CH_3OH): *m/z* = 451.2 ($[M+H]^+$).

26b: (colorless oil, 0.124 g): ^{1}H NMR (500 MHz, $CDCl_3$,): δ 7.750-7.735 (m, 1H), 7.626 (brs, 1H), 7.576-7.531 (m, 1H), 6.768 (d, 1H, J=7.5Hz), 3.828 (m, 2H), 3.729-3.669 (m, 1H), 3.604-3.567 (m, 0.5H), 3.517-3.480 (m,0.5H), 3.151-3.002 (m, 3H), 2.938-2.827 (m, 3H), 2.701-2.646 (m, 1H), 2.503-2.464 (m, 0.5H), 2.416-2.389 (m, 0.5H), 1.704-1.693 (m, 2H), 1.525 (s, 9H), 1.451 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 157.8 (1C), 155.0 (1C), 152.7 (1C), 151.7 (1C), 138.7 (1C), 118.1 (1C), 110.0 (1C), 81.0 (1C), 79.6 (1C), 64.3 (1C), (62.4+61.6) (1C), (51.7+51.0) (1C), (50.0+49.8) (1C), 48.2 (1C), (43.8+43.1) (1C), 39.5 (1C), (31.3+31.2) (1C), 28.7 (3C, 20), 28.5 (3C, 16). MS (ESI, CH_3OH): *m/z* = 451.3 ($[M+H]^+$).

(±)-*cis*-*tert*-Butyl 3-[2'-(*tert*-butoxycarbonylamino)ethylamino]-4-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}pyrrolidine-1-carboxylate (25c) and (±)-*trans*-*tert*-Butyl 3-[2'-(*tert*-butoxycarbonylamino)ethylamino]-4-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}pyrrolidine-1-carboxylate (26c). The procedure to prepare **25c** and **26c** is the same as that to prepare **20a** and **21a** except using **24** (0.391 g, 0.001 mol) and *N*-Boc-ethylenediamine (0.240 g, 0.0015 mol) instead of **19** and 3-phenyl-1-propylamine. The yield was 60% (0.321 g). The *cis* isomer (**25c**) and the *trans* isomer (**26c**) can

be separated by silica gel column chromatography (CH_2Cl_2 : EtOAc : Et_3N = 7 : 3 : 0.5). The ratio of the *cis* isomer and the *trans* isomer was 45 : 55.

25c: (colorless oil, 0.145 g): ^{1}H NMR (500 MHz, $CDCl_3$,): δ 7.769 (m, 1H), 7.592-7.544 (m, 1H), 7.339 (m, 1H), 6.8215 (d, 1H, J=6.5Hz), 5.336 (brs, 1H), 3.466-3.350 (m, 3H), 3.225-3.078 (m, 4H), 2.911-2.809 (m, 2H), 2.661-2.516 (m, 3H), 1.525-1.455 (m, 27H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 159.0 (1C), 156.4 (1C), 155.0 (1C), 152.6 (1C), 151.7 (1C), 138.8 (1C), 118.0 (1C), 110.0 (1C), 81.1 (1C), 79.5 (2C), (59.0+58.0) (1C), (51.1+50.7) (1C), (49.6+49.3) (1C), 47.5 (1C), (42.9+42.1) (1C), 40.7 (1C), 35.3 (1C), 28.7 (3C), 28.6 (3C), 28.5 (3C). MS (ESI, CH_3OH): *m/z* = 536.4 ($[M+H]^+$).

26c: (colorless oil, 0.177 g): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.755 (m, 1H), 7.589-7.550 (m, 1H), 7.271 (m, 1H), 6.7775 (d, 1H, J=7.5Hz), 5.006 (brs, 1H), 3.734-3.702 (m, 0.5H), 3.676-3.633 (m, 0.5H), 3.588-3.553 (m, 0.5H), 3.491-3.476 (m, 0.5H), 3.180-2.983 (m, 4H), 2.883-2.860 (m, 1H), 2.712-2.508 (m, 4H), 2.411-2.334 (m, 1H), 1.523-1.448 (m, 27H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 158.3 (1C), 156.3 (1C), 154.8 (1C), 152.5 (1C), 151.7 (1C), 138.7 (1C), 118.1 (1C), 110.0 (1C), 81.1 (1C), 79.5 (2C), (62.0+61.2) (1C), (52.1+51.6) (1C), (50.0+49.7) (1C), 47.5 (1C), (44.2+43.7) (1C), 40.7 (1C), 39.7 (1C), 28.7 (3C), 28.6 (3C), 28.5 (3C). MS (ESI, CH_3OH): *m/z* = 536.4 ($[M+H]^+$).

(±)-6-{[*cis*-4'-(3"-phenylpropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-amine trihydrochloride (5). The procedure to prepare **5** is the same as that to prepare **8** except using **25a** (0.102 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.084 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.839 (t, 1H, J=8.5Hz), 7.402-7.281 (m, 5H), 6.924 (d, 1H, J=9Hz), 6.7935 (d, 1H, J=7.5Hz), 4.274-4.234 (m, 1H), 3.937-3.895 (m, 1H), 3.649-3.609 (m, 1H), 3.537-3.500 (m, 1H), 3.360-3.322 (m, 1H), 3.239-3.125 (m, 4H), 2.867-2.749 (m, 3H), 2.119-2.075 (m, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.9 (1C), 144.7 (1C), 144.2 (1C), 140.5 (1C), 129.0 (2C), 128.6 (2C), 126.7 (1C), 112.6 (2C), 57.6 (1C), 47.5 (1C), 47.4 (1C), 45.3 (1C), 38.1 (1C), 31.8 (1C), 29.0 (1C), 27.1 (1C). MS (ESI, CH_3OH): *m/z* = 311.4 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 311.2230, Found: 311.2227. Anal. ($C_{19}H_{26}N_4 \cdot 3HCl \cdot 2.1H_2O$), Calcd.: C, 49.86; H, 7.31; N, 12.24, Found: C, 49.61; H, 6.95; N, 11.92.

(±)-6-{[*trans*-4'-(3"-phenylpropylamino)pyrrolidin-3'-yl]methyl}pyridin-2-amine trihydrochloride (11). The procedure to prepare **11** is the same as that to prepare **8** except using **26a** (0.102 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.084 g, quantitative yield). ^{1}H NMR(500 MHz, D_2O): δ 7.8 (t, 1H, J=8Hz), 7.401-7.271 (m, 5H), 6.905 (d, 1H, J=9Hz), 6.770 (d, 1H, J=7Hz), 3.997-3.909 (m, 2H), 3.706-3.621 (m, 2H), 3.349-3.311 (m, 1H), 3.182-3.140 (m, 1H), 3.073-2.904 (m, 4H), 2.739-2.711 (m, 2H), 2.074-1.986 (m, 2H). ^{13}C NMR(125.7 MHz, D_2O): δ 154.8 (1C), 144.7 (1C), 144.6 (1C), 140.4 (1C), 129.0 (2C), 128.6 (2C), 126.7 (1C), 112.8 (1C), 112.6 (1C), 59.8 (1C), 48.8 (1C), 46.8 (1C), 46.6 (1C), 40.2 (1C), 33.8 (1C), 31.8 (1C), 27.1 (1C). MS (ESI, CH_3OH): *m/z* = 311.4 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 311.2230, Found: 311.2229. Anal. ($C_{19}H_{26}N_4 \cdot 3HCl \cdot H_2O$), Calcd: C, 52.12; H, 7.14; N, 12.80; Found: C, 52.40; H, 7.10; N, 12.50.

(±)-3-{*cis*-4'-[(6"-aminopyridin-2"-yl)methyl]pyrrolidin-3'-ylamino}propan-1-ol trihydrochloride (6). The procedure to prepare **6** is the same as that to prepare **8** except using **25b** (0.090 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.072 g,

quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.851 (t, 1H, J=8 Hz), 6.932 (d, 1H, J=9Hz), 6.823 (d, 1H, J=7Hz), 4.324-4.314 (m, 1H), 3.991-3.949 (m, 1H), 3.752-3.671 (m, 3H), 3.565-3.527 (m, 1H), 3.374-3.172 (m, 5H), 2.903-2.846 (m, 1H), 2.077-1.953 (m, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 155.0 (1C), 144.7 (1C), 144.3 (1C), 112.7 (1C), 112.6 (1C), 58.9 (1C), 57.7 (1C), 47.4 (1C), 46.0 (1C), 45.3 (1C), 38.3 (1C), 29.1 (1C), 27.8 (1C). MS (ESI, CH_3OH): *m/z* = 251.3 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 251.1866, Found: 251.1866. Anal. ($C_{13}H_{22}N_4O\cdot 3HCl\cdot 0.625H_2O$), Calcd.: C, 42.07; H, 7.13; N, 15.09, Found: C, 42.46; H, 7.17; N, 14.70.

(±)-3-{*trans*-4'-[(6''-aminopyridin-2''-yl)methyl]pyrrolidin-3'-ylamino}propan-1-ol trihydrochloride (12). The procedure to prepare **12** is the same as that to prepare **8** except using **26b** (0.090 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.072 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.853 (t, 1H, J=8.5 Hz), 6.933 (d, 1H, J=9Hz), 6.819 (d, 1H, J=6.5Hz), 4.024-4.011 (m, 2H), 3.748-3.676 (m, 4H), 3.360-3.174 (m, 4H), 3.104 (m, 1H), 2.984-2.936 (m, 1H), 1.963-1.952 (m, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.9 (1C), 144.8 (1C), 144.7 (1C), 112.8 (1C), 112.6 (1C), 60.0 (1C), 58.7 (1C), 48.7 (1C), 46.7 (1C), 45.3 (1C), 40.3 (1C), 33.8 (1C), 28.0 (1C). MS (ESI, CH_3OH): *m/z* = 251.3 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 251.1866, Found: 251.1866.

(±)-N^1-{*cis*-4'-[(6''-aminopyridin-2''-yl)methyl]pyrrolidin-3'-yl}ethane-1,2-diamine tetrahydrochloride (4). The procedure to prepare **4** is the same as that to prepare **8** except using **25c** (0.107 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.076 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.847 (t, 1H, J=8Hz), 6.920 (d, 1H, J=9Hz), 6.825 (d, 1H, J=7.5Hz), 4.333-4.013 (m, 1H), 3.953-3.753 (m, 1H), 3.751-3.578 (m, 1H), 3.553-3.515 (m, 1H), 3.513-3.170 (m, 7H), 2.909-2.857 (m, 1H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.8 (1C), 144.8 (2C), 112.6 (1C), 112.4 (1C), 58.3 (1C), 47.4 (1C), 46.2 (1C), 44.2 (1C), 39.1 (1C), 36.3 (1C), 29.4 (1C). MS (ESI, CH_3OH-H_2O): *m/z* = 236.3 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 236.1870, Found: 236.1873. Anal. ($C_{12}H_{21}N_5\cdot 4HCl\cdot 2H_2O$), Calcd.: C, 34.55; H, 7.01; N, 16.79, Found: C, 34.83; H, 6.88; N, 16.46.

(±)-N^1-{*trans*-4'-[(6''-aminopyridin-2''-yl)methyl]pyrrolidin-3'-yl}ethane-1,2-diamine tetrahydrochloride (10). The procedure to prepare **10** is the same as that to prepare **8** except using **26c** (0.107 g, 0.0002 mol) instead of **20a**, affording a hygroscopic white solid (0.076 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.850 (t, 1H, J=8.5Hz), 6.927 (d, 1H, J=9Hz), 6.818 (d, 1H, J=7Hz), 4.011-4.004 (m, 2H), 3.705-3.666 (m, 2H), 3.447-3.315 (m, 6H), 3.154-3.024 (m, 1H), 2.965-2.914 (m, 1H). ^{13}C NMR (125.7 MHz, D_2O): δ 154.8 (1C), 144.8 (2C), 112.7 (1C), 112.5 (1C), 60.7 (1C), 48.5 (1C), 46.6 (1C), 43.9 (1C), 40.6 (1C), 35.9 (1C), 33.6 (1C). MS (ESI, CH_3OH-H_2O): *m/z* = 236.3 ($[M+H]^+$). HRMS (CI+, CH_3OH) Calcd.: 236.1870, Found: 236.1871. Anal. ($C_{12}H_{21}N_5\cdot 4HCl\cdot 1.33H_2O$), Calcld: C, 35.58; H, 6.88; N, 17.29, Found: C, 35.95; H, 6.87; N, 16.89.

***tert*-Butyl cyclopent-3-enylcarbamate (27).** To a solution of triphenyl phosphine (Ph_3P, 3.93 g, 0.015 mol) in dry THF (10 mL) was added a solution of 3-cyclopenten-1-ol (1.053 g, 0.012 mol) in dry THF (10 mL) and a solution of *N*-Boc ethyl oxamate (3.30 g, 0.015 mol) in dry THF (10 mL) at 0 ºC. Diethyl azodicarboxylate (DEAD, 2.611 g, 2.4 mL, 0.015 mol) was added dropwise.

The mixture was stirred at 0 °C for 2 h and then allowed to stir at room temperature for 48 h. The solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (30 mL) and washed with water and brine (20 mL × 3). The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (100% CH_2Cl_2) to yield a mixture of **27** and *N*-Boc ethyl oxamate, which was used without further purification in the next reaction.

To a stirred solution cooled in an ice-water bath of the crude **27** (3.80 g) in THF (35 mL) was added a solution of LiOH (1.82 g, 0.0765 mol) in water (35 mL). The mixture was stirred in an ice-water bath for 3 h. The organic material was extracted with CH_2Cl_2 (30 mL × 3), the organic layers were combined and washed with brine (30 mL × 2), and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (100% CH_2Cl_2) to obtain a white solid (1.168 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.69 (s, 2H), 4.68 (s, 1H), 4.28 (s, 1H), 2.75-2.69 (m, 2H), 2.17 (d, 2H, *J* = 14.4Hz), 1.44 (s, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 155.55 (1C), 128.83 (2C), 79.04 (1C), 50.12 (1C), 40.42 (2C), 28.54(1C). MS (CI, CH_3OH) *m/z* =184.1 $([M+H])^+$. HRMS (CI+, CH_3OH) calcd. 184.1332, found 184.1331.

***tert*-Butyl (1*R*,3*R*,5*S*)-6-oxabicyclo[3.1.0]hexan-3-ylcarbamate (28).** Solid $NaHCO_3$ (1.42 g, 0.0167 mol) and MCPBA (2.174 g, 0.0128 mol) were added in portions to a stirred solution of **27** (1.693 g, 0.0093 mol) in CH_2Cl_2 (60 mL). The mixture was stirred at 0 °C for 2 h and then was allowed to stir for about 48 h at room temperature. Aqueous 20% Na_2SO_3 (30 mL) was added, and the two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 3), and the combined organic layers were washed with 20% Na_2SO_3 (30 mL), 5% $NaHCO_3$ (30 mL), and water (30 mL). The combined organic phase was concentrated in vacuo, and the residue was purified by silica gel column chromatography (100% CH_2Cl_2) to give **28** as a colorless oil (2.136 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.96 (d, 1H, *J* = 8.7 Hz), 4.15 (dd, 1H, *J* = 16.5, J = 9.6 Hz), 3.54 (s, 2H), 2.07-1.92 (m, 4H), 1.42 (s, 9H); MS (ESI) *m/z* 200.1 $([M+H])^+$; HRMS (CI+, CH_3OH) calcd.: 200.1281, found: 200.1277. The trans isomer, *tert*-butyl (1*R*,3*S*,5*S*)-6-oxabicyclo[3.1.0]hexan-3-ylcarbamate (**28a**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.37 (s, 1H), 3.78 (s, 1H), 3.47 (s, 2H), 2.53-2.46 (m, 4H), 1.43 (s, 9H).

A mixture of (1*S*,3*R*,4*S*)- and (1*S*,3*S*,4*R*)-*tert*-butyl 3-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}-4-hydroxycyclopentyl-1-carbamate and (29). The procedure to prepare **29** is the same as that to prepare **18** except that 3.5 equivalents of n-BuLi (1.6 M in hexanes) were used in the preparation of **31** instead of 2.5 equivalents of n-BuLi (1.6 M in hexanes) that was used in the preparation of **20**. The amounts of each component used in this reaction are **16** (0.588 g, 2.8 mmol), *n*-BuLi (1.6 M in hexanes, 6.1 mL, 9.7 mmol) and **28** (0.643 g, 3.2 mmol). The product, purified by silica gel column chromatography (hexanes : EtOAc = 5 : 5), was obtained as a white-yellow solid (0.562 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, 1H, *J* = 8 Hz), 7.58 (t, 1H, *J* = 8 Hz), 6.82 (d, 1H, *J* = 7.2 Hz), 7.03 (s, 1H), 4.85 (s, 1H), 4.13-3.95 (m, 2H), 2.44- 2.32 (m, 2H), 2.04 (s, 1H), 1.91 (m, 1H) 1.55 (s, 9H), 1.43 (s, 9H), 1.73-1.66 (m, 1H); MS (EI, CH_3OH) *m/z* 407.1, M^+.

A mixture of (1*S*,3*R*)- and (1*S*,3*S*)-*tert*-butyl 3-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}-4-oxocyclopentyl-1-carbamate (30). TPAP (0.024 g, 0.07 mmol) was added to a stirred solution of **29** (0.562 g, 1.38 mmol), NMO in CH_3CN (1 mL), and CH_2Cl_2 (9 mL) at room temperature and was allowed to react overnight. When complete, the solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2 : EtOAc = 8 :

2) to afford **30** as a white solid (0.380 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, 1H, *J* = 8 Hz), 7.55 (t, 1H, *J* = 7.6 Hz), 7.12 (s, 1H), 6.78 (d, 1H, *J* = 7.6 Hz), 4.76 (s, 1H), 4.13 (s, 1H), 3.19-3.08 (m, 1H), 2.83-2.35 (m, 4H), 2.14-1.95 (m, 2H), 1.49 (s, 9H), 1.40 (s, 9H); MS (ESI, CH_3OH) *m/z* 406.1 $([M+H])^+$.

A mixture of *cis*-(1*S*,3*R*,4*R*)- and (1*S*,3*S*,4*S*)-*tert*-Butyl 3-[2'-(*tert*-butoxycarbonylamino)ethylamino]-4-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}cyclopentyl-1-carbamate (31) and the *trans* mixture of (1*S*,3*R*,4*S*)- and (1*S*,3*S*,4*R*)-*tert*-Butyl 3-[2'-(*tert*-butoxycarbonylamino)ethylamino]-4-{[6'-(*tert*-butoxycarbonylamino)pyridin-2'-yl]methyl}cyclopentyl-1-carbamate (32). The procedure to prepare **31** and **32** is the same as that to prepare **20a** and **21a**. The amount of each component used in this reaction is **30** (0.372 g, 0.9 mmol), *N*-Boc-ethyldiamine (0.233 g, 1.4 mmol), 5 drops of acetic acid, 3 Å molecular sieves (0.43 g), dry MeOH (10 mL), and $NaBH_3CN$ (0.115g, 1.8 mmol). The yield was 21% (0.106 g, 0.225 g). The *cis* isomer (**31**) and the *trans* isomer (**32**) can be separated by silica gel column chromatography (,CH_2Cl_2 : EtOAc : Et_3N = 8 : 2 : 0.5). The ratio of the *cis* isomer to the *trans* isomer was 60 : 40.

For *cis*-**31**: (colorless oil, 0.064 g), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 1H, *J* = 7.6 Hz), 7.57 (t, 1H, *J* = 7.6 Hz), 6.81 (d, 1H, *J* = 7.6 Hz), 7.41 (s, 1H), 6.05 (s, 1H), 5.19 (s, 1H), 4.05 (s, 1H), 3.25 (s, 2H), 2.92 (s, 2H), 2.82-2.65 (m, 4H), 2.24-2.03 (m, 4H), 1.53 (s, 9H), 1.51 (s, 9H), 1.46 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 159.4 (1C), 156.5 (1C), 155.6 (1C), 152.2 (1C), 151.5 (1C), 138.9 (1C), 117.9 (1C), 109.8 (1C), 81.5 (1C), 79.5 (1C), 78.9 (1C), 60.0 (1C), 49.4 (1C), 47.3 (1C), 43.5 (1C), 40.5 (1C), 39.5 (1C), 37.3 (1C), 37.1 (1C), 28.7 (3C), 28.6 (3C), 28.5 (3C); MS (ESI, CH_3OH) *m/z* 550.3 $([M+H]^+)$.

For *trans*-**32:** (colorless oil 0.042 g), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, 1H, *J* = 8 Hz), 7.54 (t, 1H, 7.8 Hz), 7.42 (s, 1H), 6.80 (d, 1H, *J* = 7.2 Hz), 5.30 (s, 1H), 4.51 (s, 1H), 4.13-4.09 (m, 1H), 3.22-3.07 (m, 3H), 2.81-2.74 (m, 2H), 2.61-2.51 (m, 3H), 2.08-1.85 (m, 4H), 1.52 (s, 9H), 1.43 (s, 9H), 1.33 (s, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 159.6 (1C), 156.4 (1C), 155.5 (1C), 152.7 (1C), 151.6 (1C), 138.6 (1C), 118.1 (1C), 109.8 (1C), 79.4 (3C), 59.6 (1C), 49.1 (1C), 47.3 (1C), 42.7 (1C), 40.6 (1C), 39.4 (1C), 37.6 (1C), 37.0 (1C), 28.6 (6C), 28.5 (3C); MS (ESI, CH_3OH) *m/z* 550.3 $([M+H]^+)$.

A mixture of *cis*-(1*S*,3*S*,5*S*)- and (1*R*,3*S*,5*R*)-N^1-(2'-aminoethyl)-5-[(6'-aminopyridin-2'-yl)methyl]cyclopentane-1,3-diamine (7). The procedure to prepare **7** is the same as that to prepare **8**, affording a hygroscopic white solid (0.039 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.84 (t, 1H, *J* = 8.0 Hz), 6.89 (d, 1H, *J* = 9 Hz), 6.81 (d, 1H, *J* = 7 Hz), 4.02-4.00 (m, 1H), 3.71-3.63 (m, 1H), 3.59-3.55 (m, 1H), 3.49-3.45 (m, 3H), 3.23 (d, *J* = 11.5 Hz), 2.93-2.86 (m, 1H), 2.83-2.78 (m, 2H), 2.27-2.22 (m, H), 2.05-1.98 (m, 1H), 1.68-1.65 (m, 1H). ^{13}C NMR (500 MHz, D_2O): δ 154.7 (1C), 145.7 (1C), 144.8 (1C), 112.8 (1C), 112.0 (1C), 59.0 (1C), 48.0 (1C), 43.8 (1C), 37.9 (1C), 35.6 (1C), 33.6 (1C), 32.5 (1C), 32.2 (1C). MS (ESI, CH_3OH) *m/z* 250.5 $([M+H])^+$.

A mixture of *trans*-(1*R*,3*S*,5*S*)- and (1*S*,3*S*,5*R*)-N^1-(2'-aminoethyl)-5-[(6'-aminopyridin-2'-yl)methyl]cyclopentane-1,3-diamine (13). The procedure to prepare **13** is the same as that to prepare **8**, affording a hygroscopic white solid (0.029 g, quantitative yield). ^{1}H NMR (500 MHz, D_2O): δ 7.85, (dd, 1H, *J* = 8.5, J = 1 Hz), 6.91 (d, 1H, *J* = 9 Hz), 6.81 (d, 1H, *J* = 7 Hz), 4.44-4.12 (m, 2H), 3.61-3.44 (m, 4H), 3.14-3.02 (m, 2H), 2.68 (t, 1H, *J* = 13.5 Hz), 2.59-2.52 (m, 1H),

2.16-2.10 (m, 1H), 2.42-2.36 (m, 1H), 1.96-1.84 (m, 1H); ^{13}C NMR (500 MHz, D_2O): δ 154.7 (1C), 145.6 (1C), 144.8 (1C), 112.9 (1C), 112.1 (1C), 60.5 (1C), 47.5 (1C), 44.0 (1C), 39.3 (1C), 35.6 (1C), 32.6 (1C), 31.2 (1C), 30.6 (1C); MS (ESI, CH_3OH) *m/z* 250.6 $([M+H])^+$.

Enzyme and assay

All of the NOS isoforms used were recombinant enzymes overexpressed in *E.coli*. The murine macrophage iNOS was expressed and isolated according to the procedure of Hevel et al.[14] The rat nNOS was expressed[15] and purified as described.[16] The bovine eNOS was isolated as reported.[17] Nitric oxide formation from NOS was monitored by the hemoglobin capture assay as described.[18] NOS assays were recorded on a Perkin–Elmer Lamda 10 UV–visible spectrophotometer.

Determination of K_i values

The apparent K_i values were obtained by measuring the percent enzyme inhibition in the presence of 10 μM L-arginine with at least five concentrations of inhibitor. The parameters of the following inhibition equation[19] were fitted to the initial velocity data: % inhibition = $100[I]/\{[I] + K_i(1+[S]/K_m)\}$. K_m values for L-arginine were 1.3 μM (nNOS), 8.2 μM (iNOS), and 1.7 μM (eNOS). The selectivity of an inhibitor was defined as the ratio of the respective K_i values.

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Supplementary Figure 1

The following basic fragment library was constructed based upon the literatures below:

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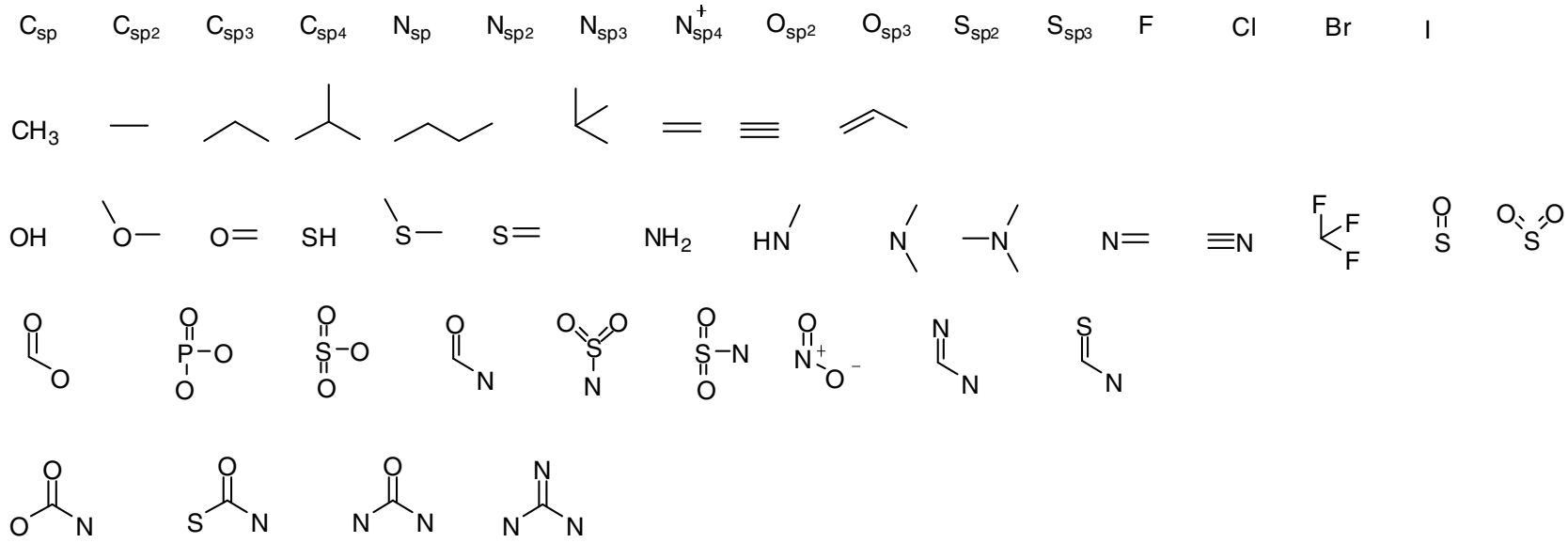
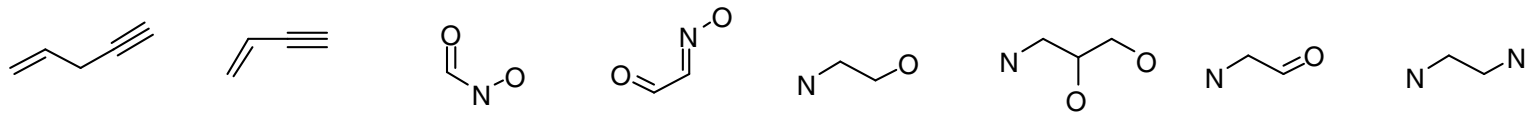
1. Basic atoms and functional groups



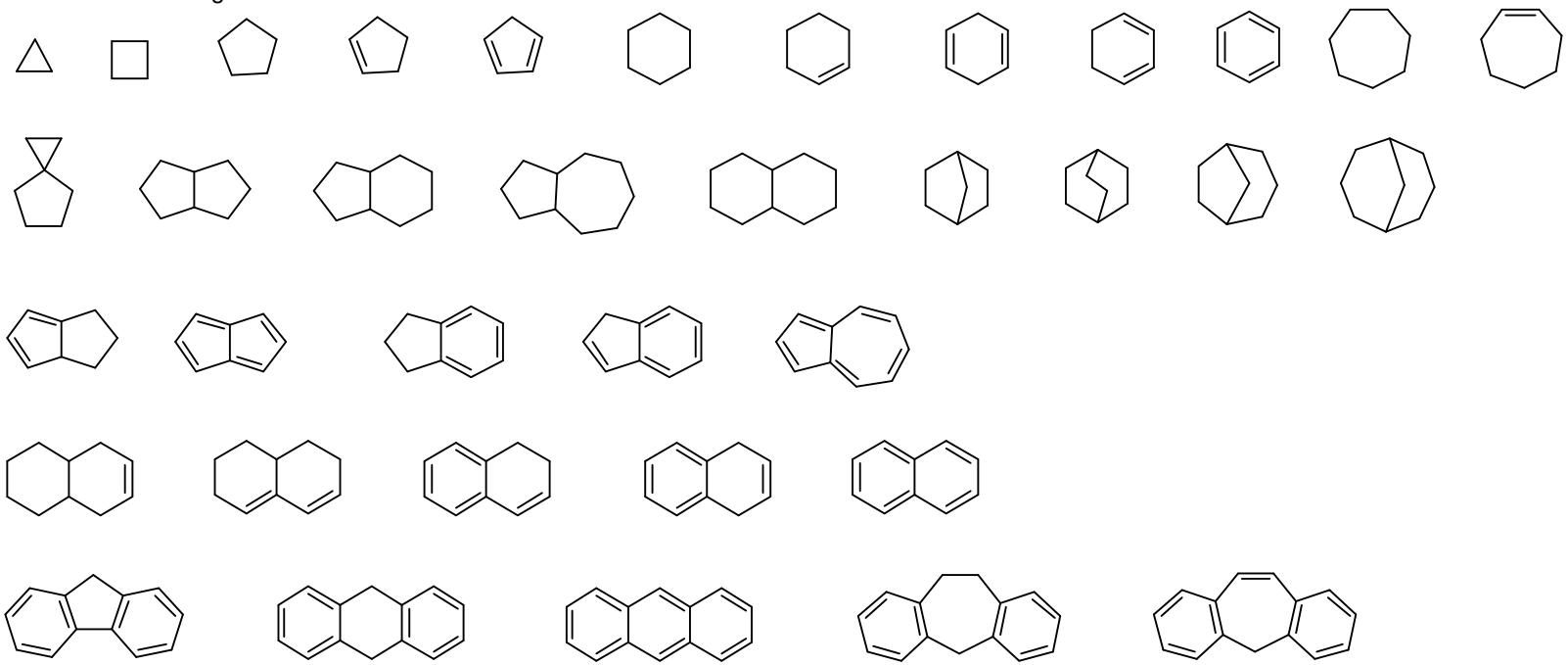
Some common alphatic fragments used in drug candidates



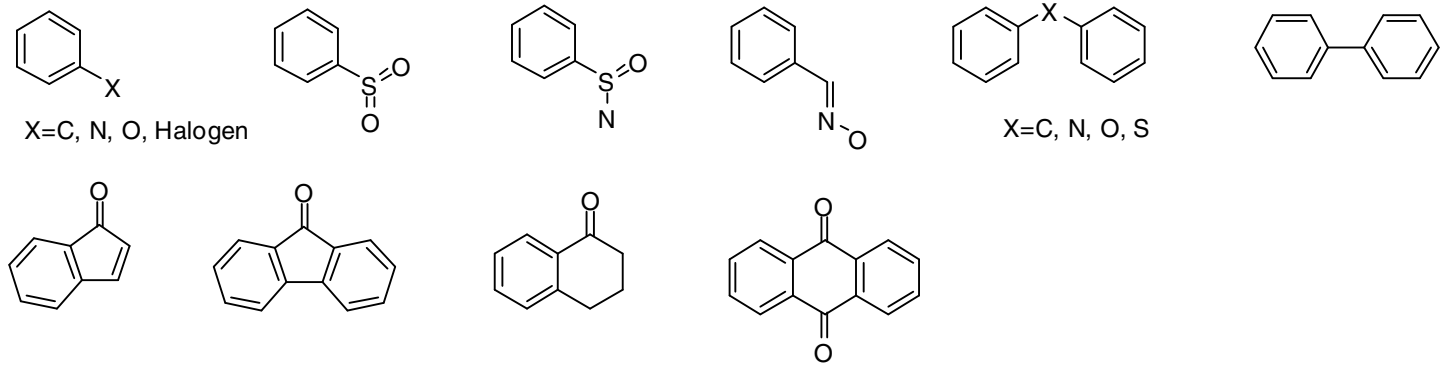
Supplementary Figure 1. Basic fragment library

Supplementary Figure 1 (continued)

2. Basic carbon rings



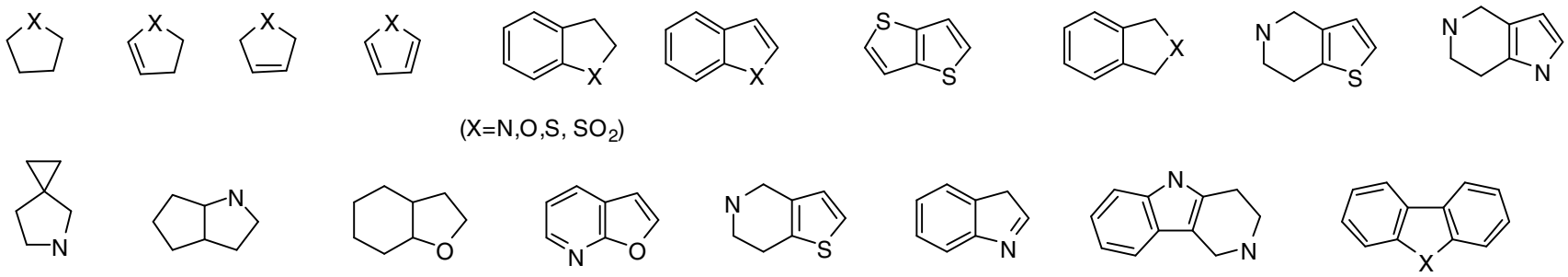
Some common fragments used in drug candidates



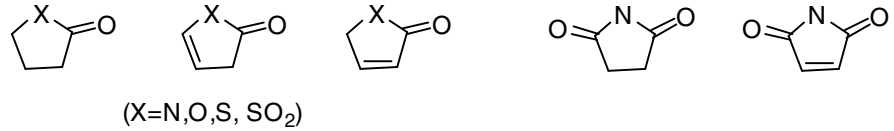
Supplementary Figure 1 (continued)

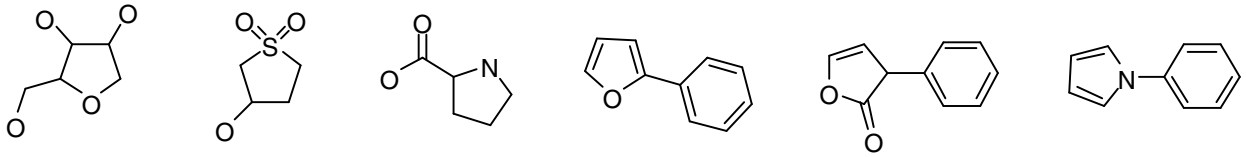
3. Five-membered heterocyclic rings

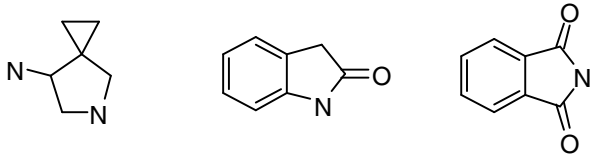
(1) Basic rings containing one heteroatom



Some common fragments used in drug cadidates

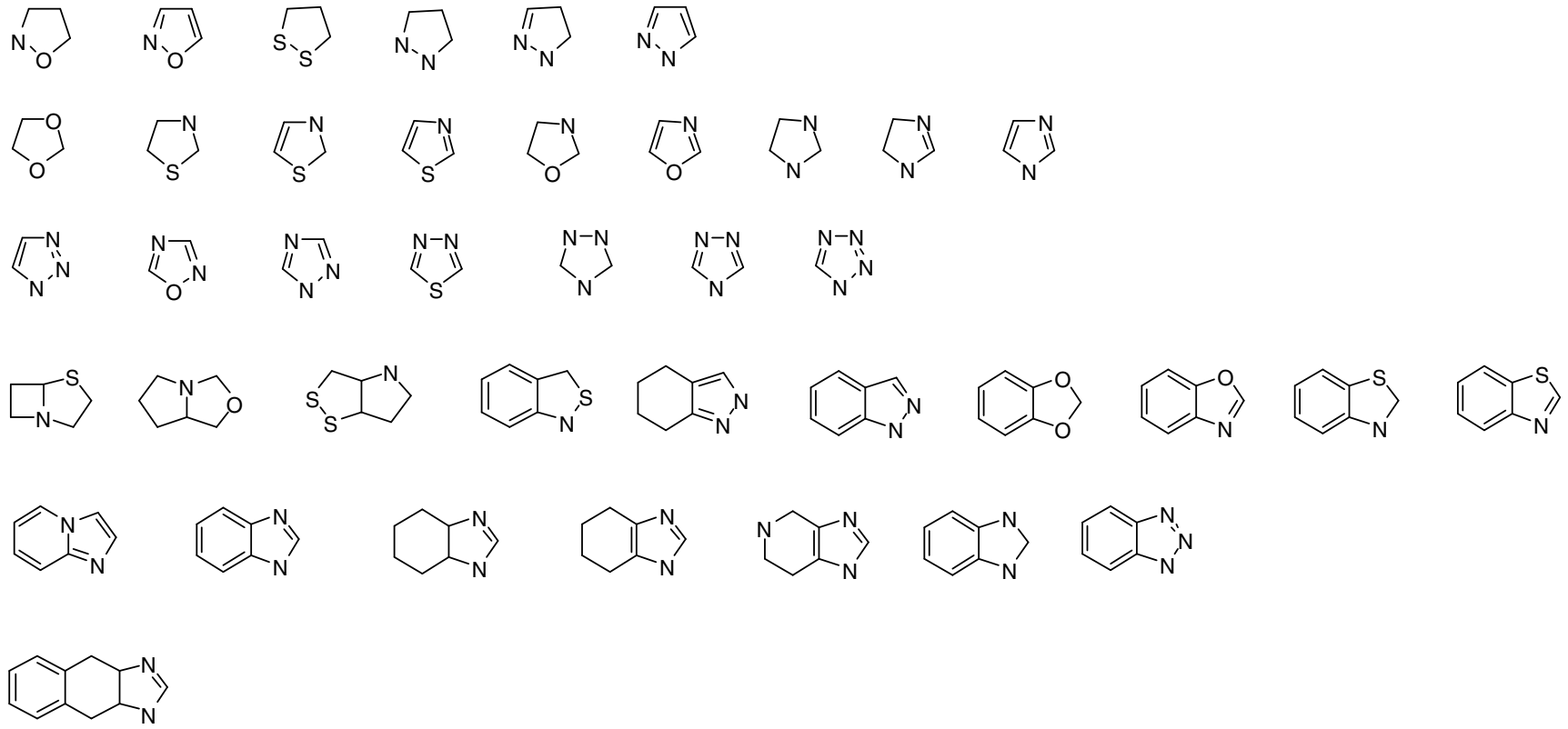




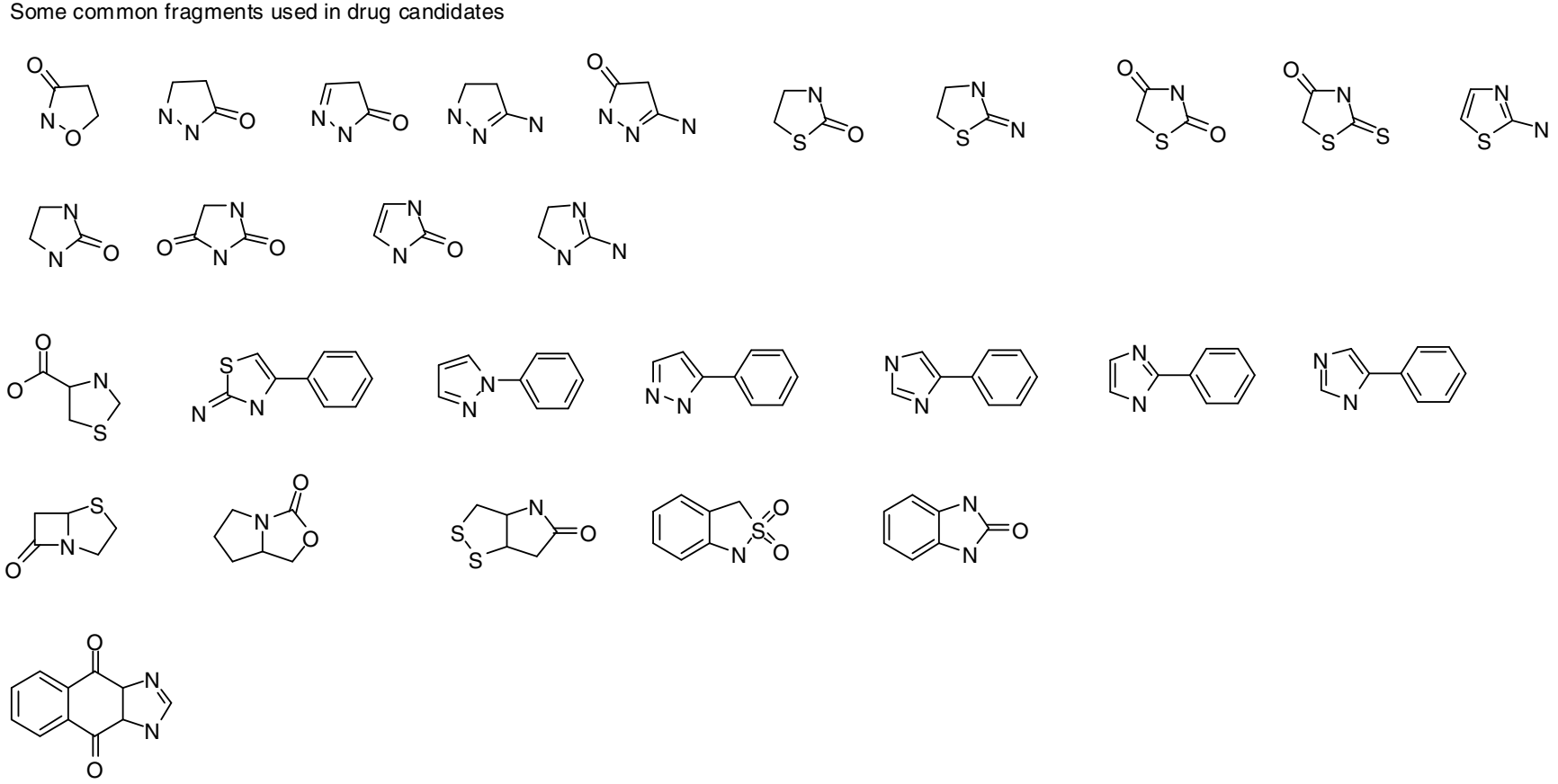


Supplementary Figure 1 (continued)

(2) Basic rings containing more thant one heteroatom



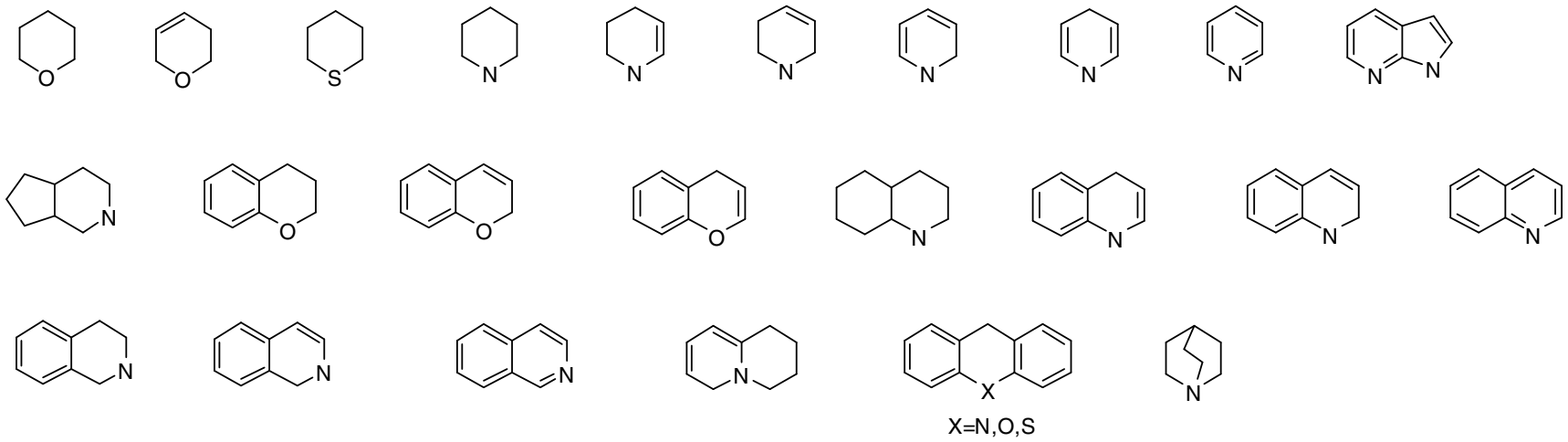
Supplementary Figure 1 (continued)



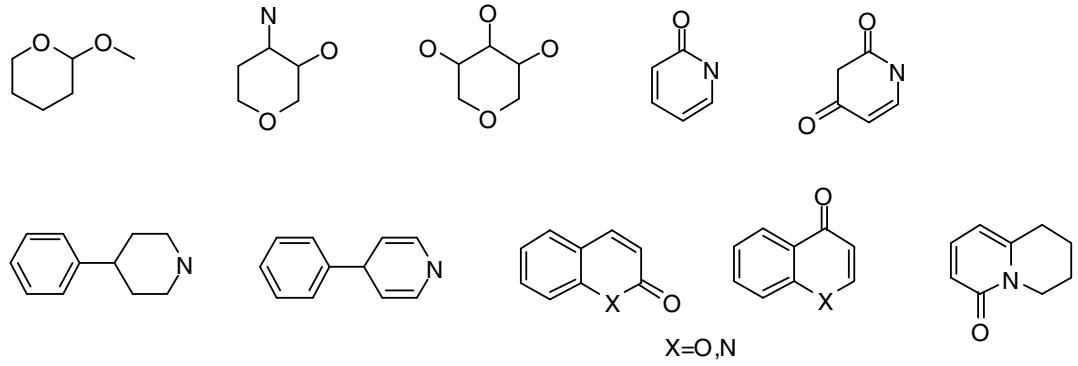
Supplementary Figure 1 (continued)

4. Six-membered heterocyclic rings

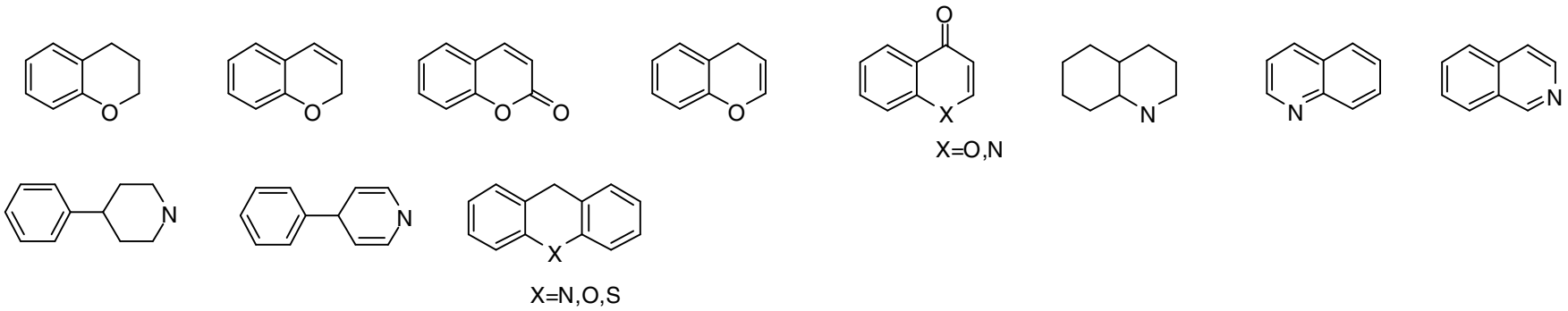
(1) Basic rings containing one heteroatom



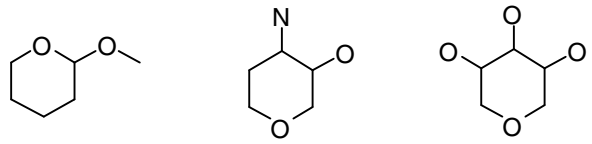
Some common fragments used in drug candidates



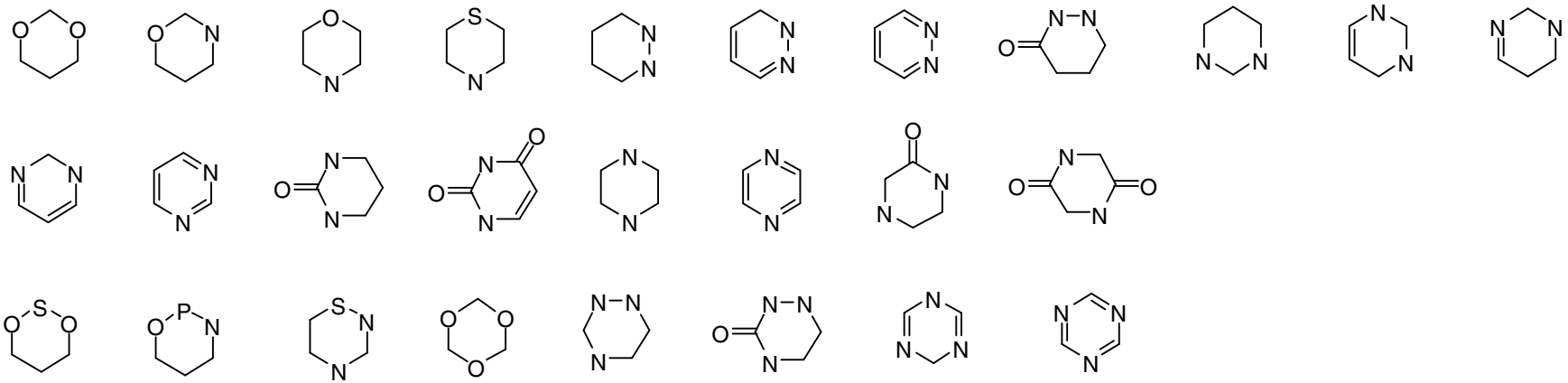
Supplementary Figure 1 (continued)



Some common fragments used in drug candidates

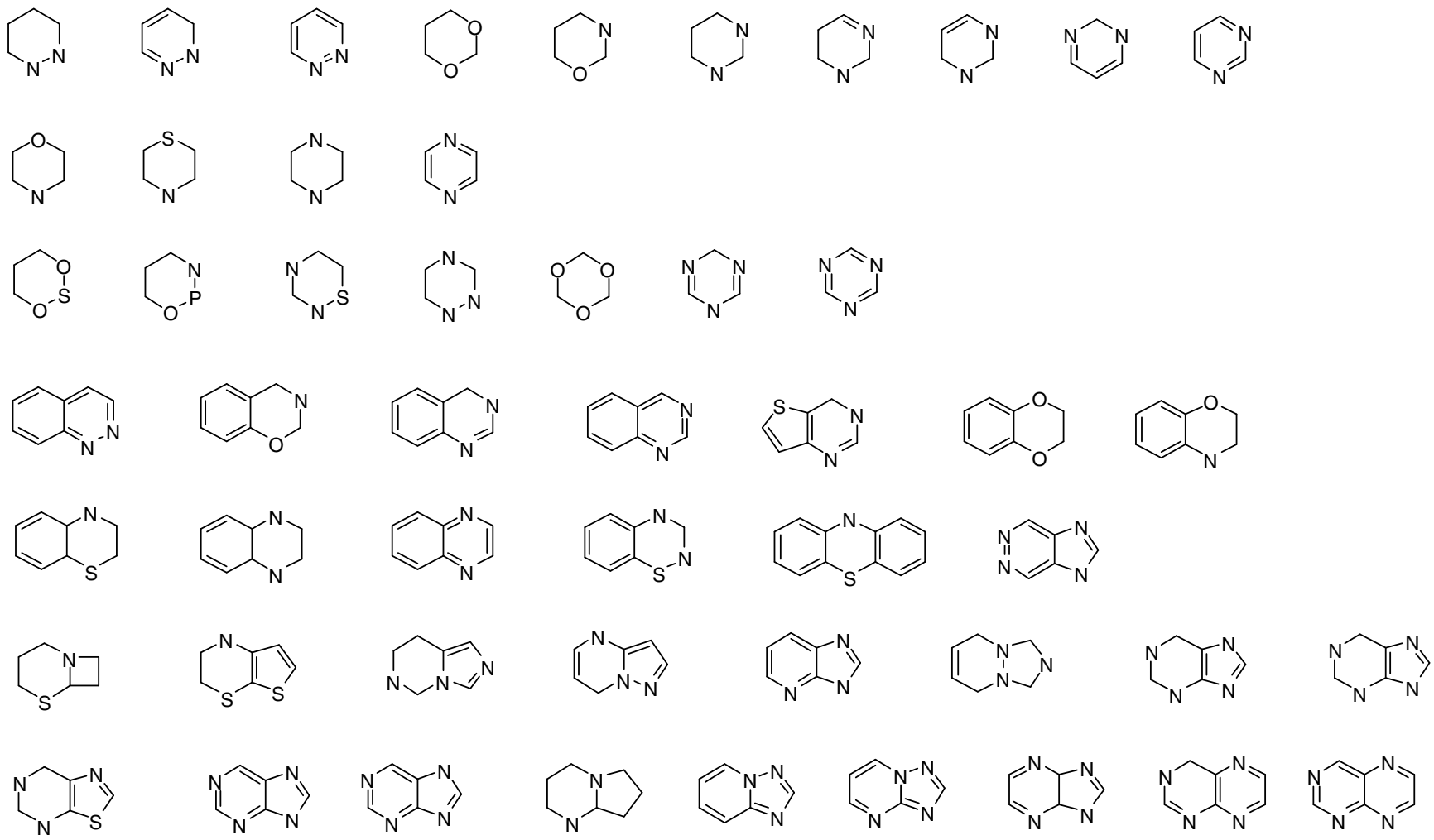


(2) Basic rings containing more than one heteroatom



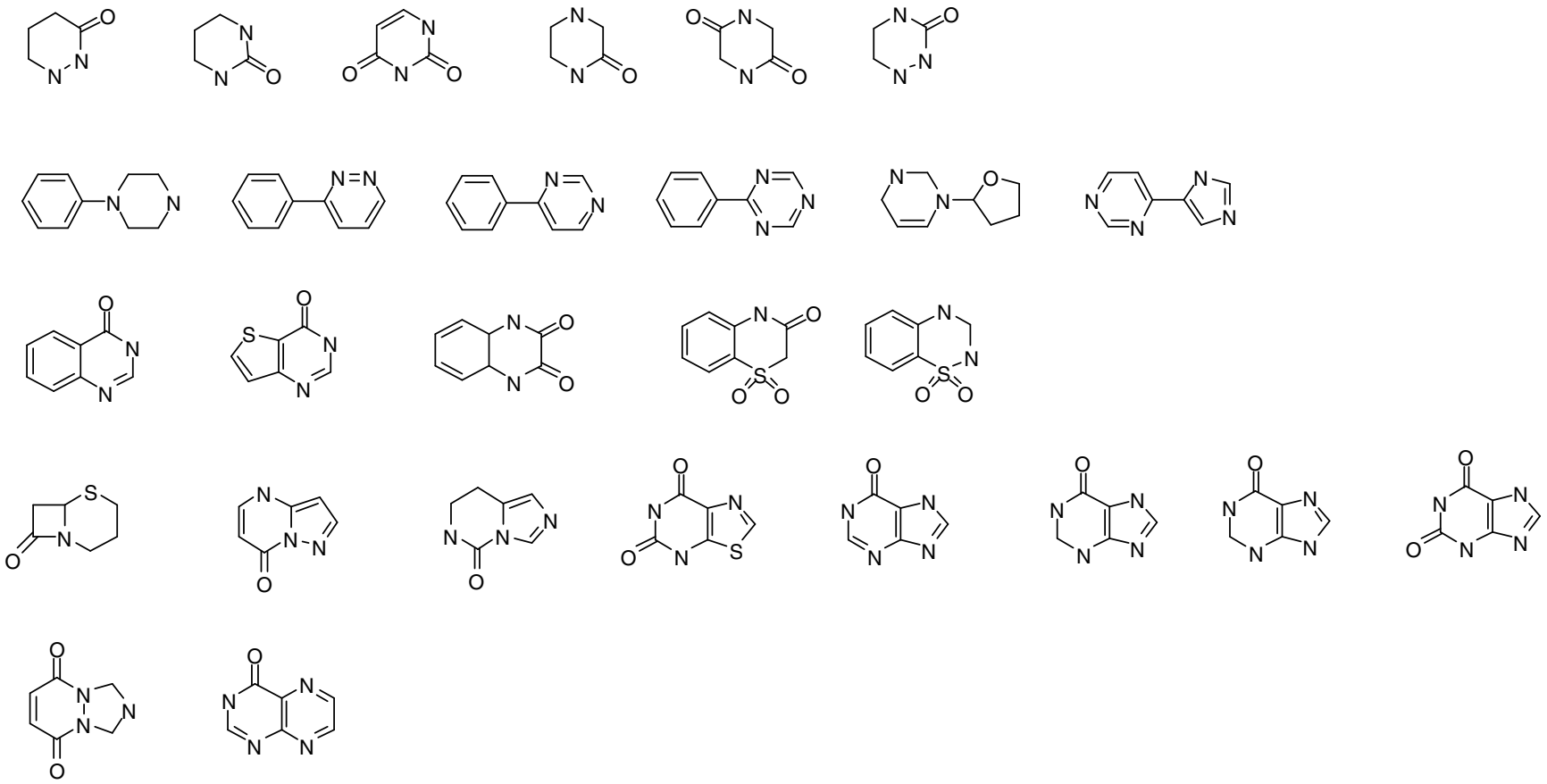
Supplementary Figure 1 (continued)

(2) Basic rings containing more than one heteroatom



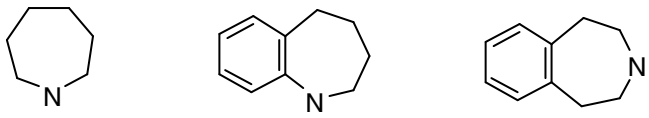
Supplementary Figure 1 (continued)

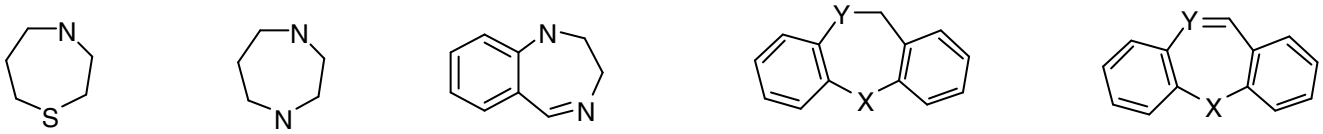
Some common fragments used in drug candidates



Supplementary Figure 1 (continued)

5. Seven-membered heterocyclic rings

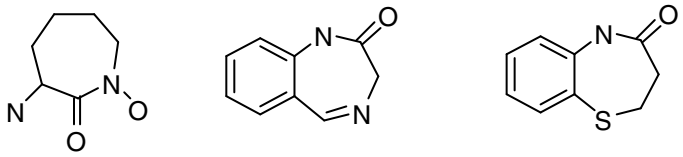




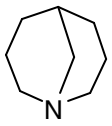
X=N,O,S; Y=C, N, O

X=N,O,S; Y=C, N

Some common fragments used in drug candidates



6. Eight-membered heterocyclic rings



Supplementary Figure 2

The following bioisostere library was constructed based upon the literatures below:

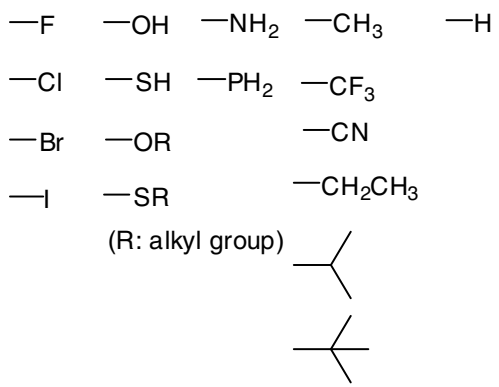
1. Patani, G. A.; LaVoie, E. J. Bioisosterism: A rational approach in drug design. *Chem. Rev.* **1996**, *96(8)*, 3147–3176.
2. Lima, L. M.; Barreiro, E. J. Bioisosterism: a useful strategy for molecular modification and drug design. *Curr. Med. Chem.* **2005**, *12(1)*, 23–49.
3. Thornber, C. W. Isosterism and molecular modification in drug design. *Chem. Soc. Rev.* **1979**, *8*, 563–580;
4. Wermuth, C. G. The practice of medicinal chemistry. 2nd Edition Academic Press, San Diego, **2003**; Chapter 13.Wermuth C. G. "Molecular variations based on isosteric replacements." pp 189–214.
5. Abraham D. J. Burger's medicinal chemistry and drug discovery vol. 1 drug discovery. 6th Edition, A John Wiley and sons, Inc., Publication, **2003**; Chapter 16. Cannon J. G. "Analog design" pp 687–714.

In making a bioisosteric replacement the following parameters of the group being changed could be considered:
a. Structural requirements (size, shape: bond angles, hybridization)
b. Electronic distribution (polarizability, inductive effects, charge, dipoles)
c. Lipophilicity and hydrophilicity
d. pKa
e. Chemical reactivity (metabolism)
f. Hydrogen bond capacity

The extent to which the replacement is useful depend upon which of these parameters is important and which ones the bioisostere can best mimic

I. Classical bioisosteres

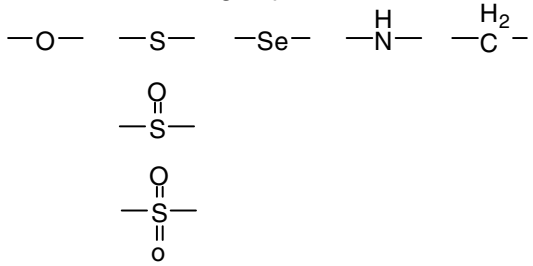
A. Monovalent atoms or groups



Especially,

1. H vs F; CH_3 vs CF_3
2. -OH vs -SH, $-NH_2$
3. -F vs -OH, $-NH_2$, -Cl, $-CH_3$
4. -Cl vs -Br, -SH, -OH

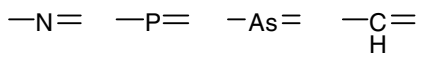
B. Divalent atoms or groups



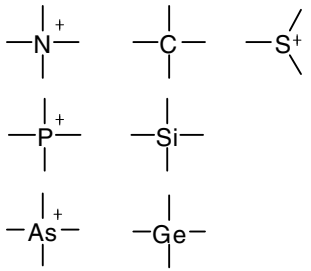
Supplementary Figure 2. Bioisostere library

Supplementary Figure 2 (continued)

C. Trivalent atoms or groups



D. Tetrasubstituted atoms

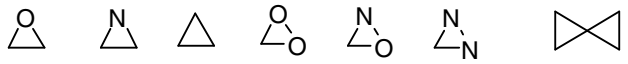


E. Ring equivalents

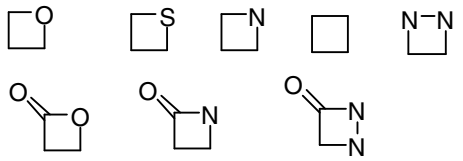
Basic theory: The exchange of the above heavy atoms leads to the formation of different ring systems (aliphatic ring or aromatic ring equivalents)
The selection criteria of ring equivalents
a. bioling point: If two heterocyclic ring have the same geometry, the rings with the same boiling point (correlated to the dipolar moment) are picked up preferentially
b. electrostatic potential surface map

The common rings

1. Three-membered heterocycles



2. Four-membered heterocycles

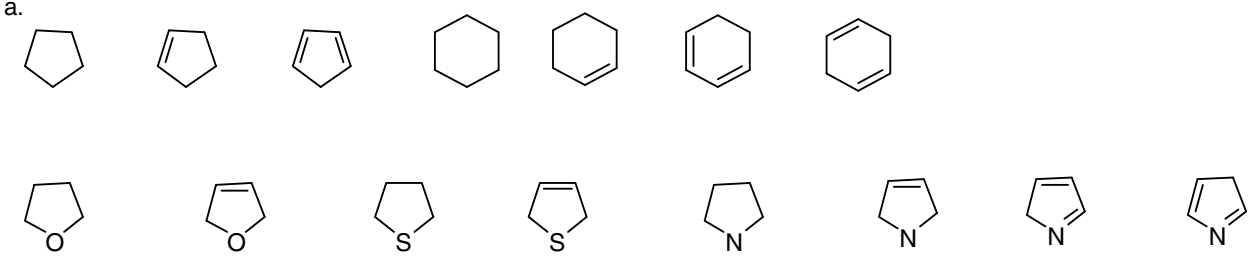


Supplementary Figure 2 (continued)

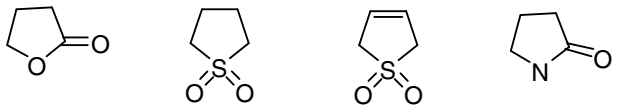
3. Five-membered heterocycles

(1) nonaromatic rings

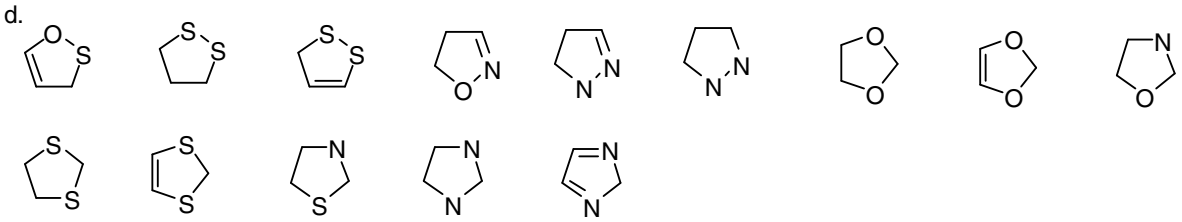
a.



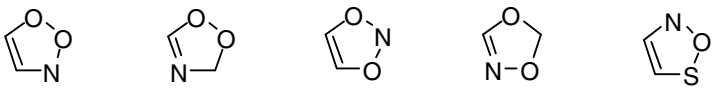
b.



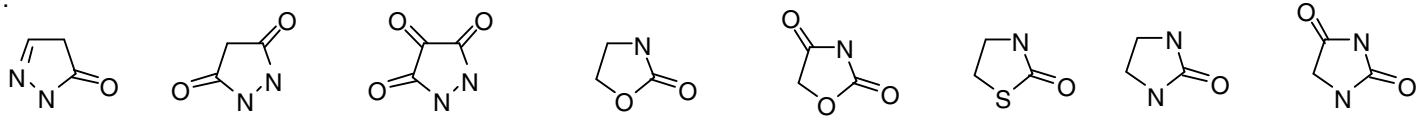
d.



e.

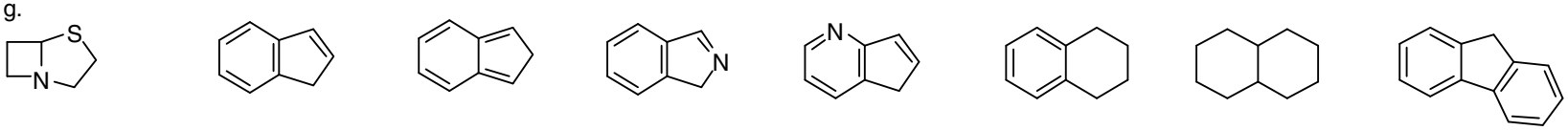


f.



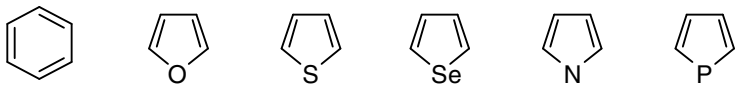
Supplementary Figure 2 (continued)

g.

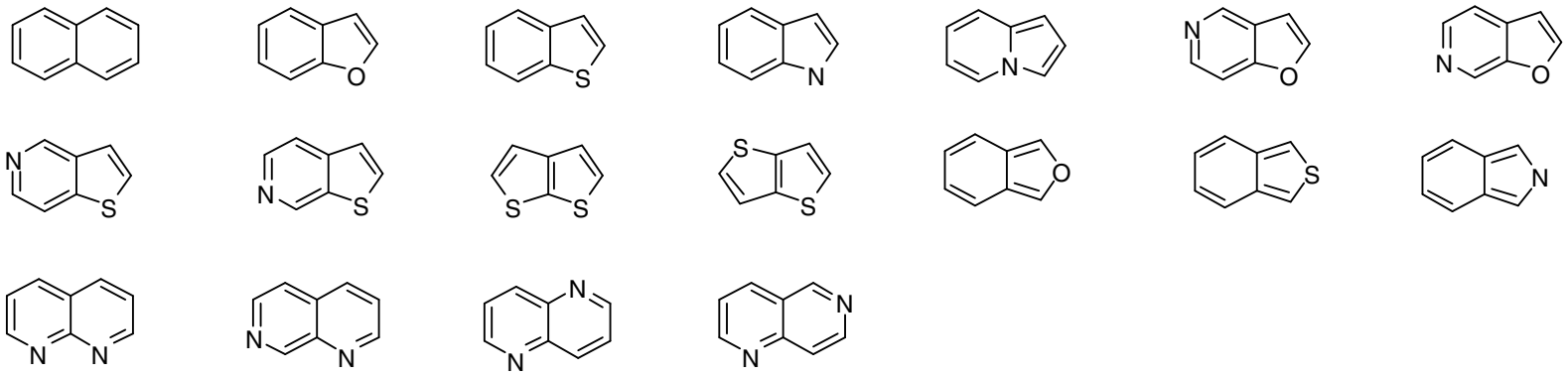


(2) aromatic rings

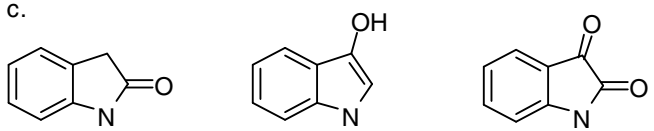
a.



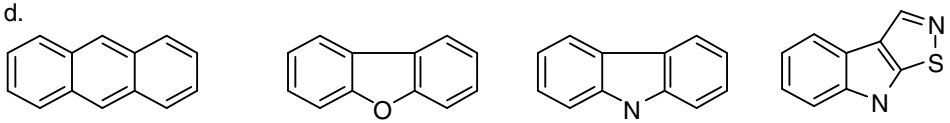
b.



c.



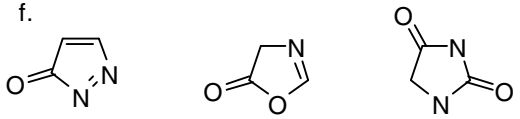
d.



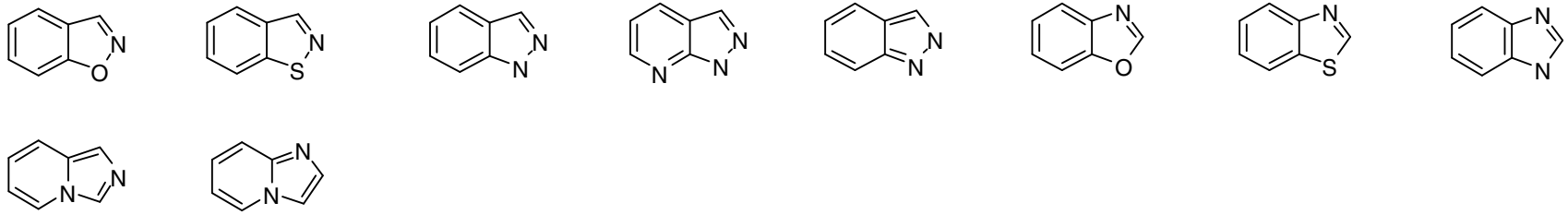
Supplementary Figure 2 (continued)

e.

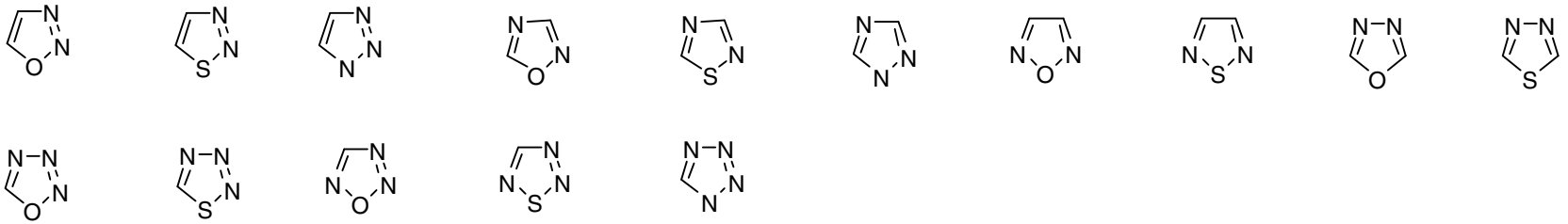
f.



g.



h.



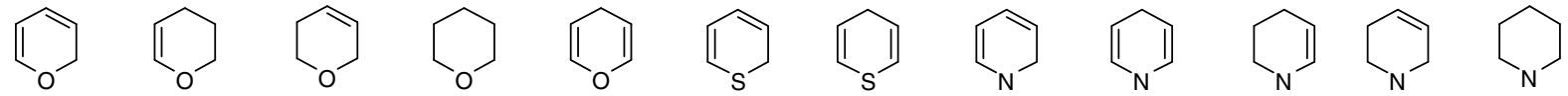
i.

Supplementary Figure 2 (continued)

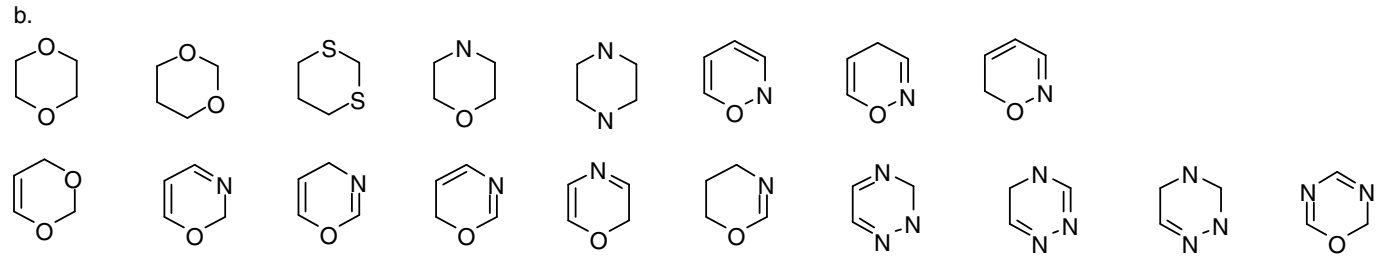
4. Six-membered heterocycles

(1) nonaromatic rings

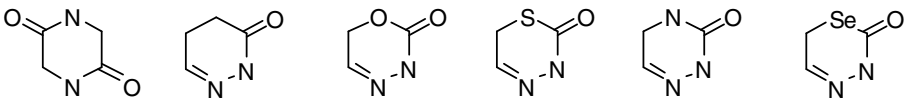
a.



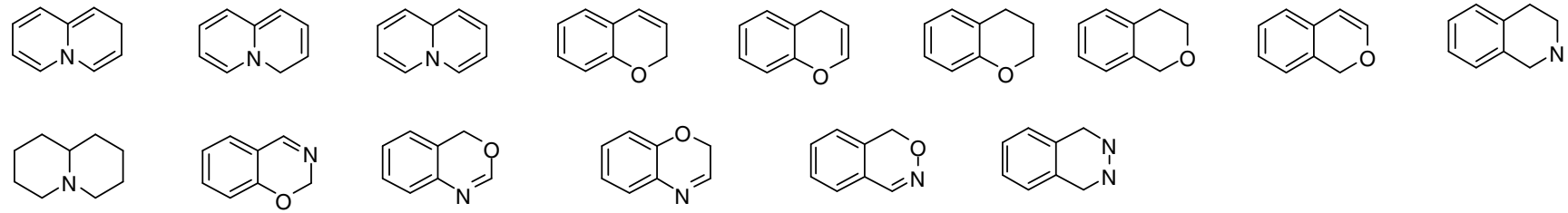
b.



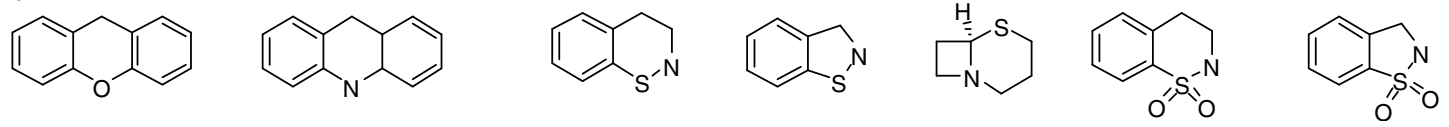
c.



d.



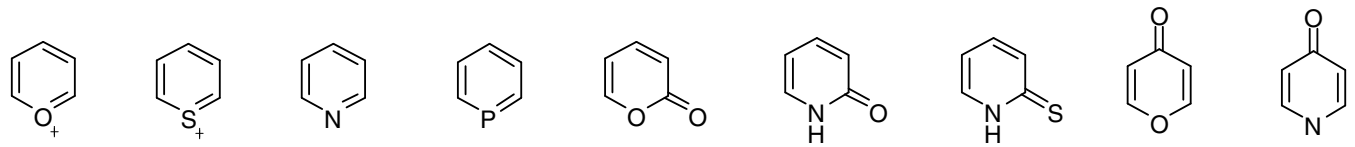
e.



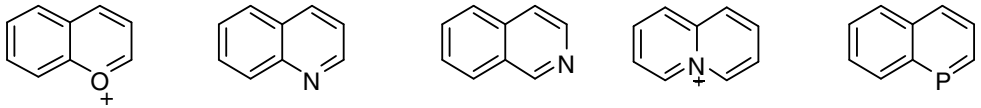
Supplementary Figure 2 (continued)

(2) aromatic rings

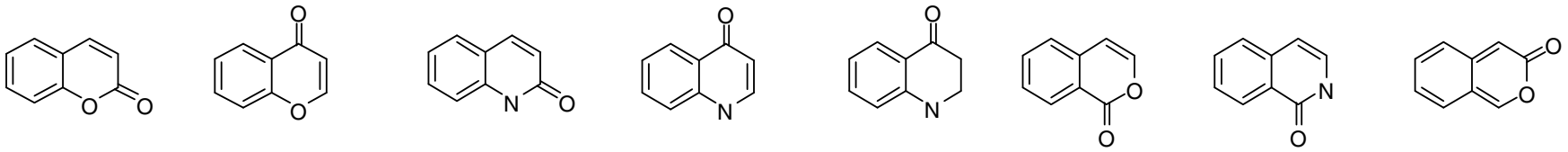
a.



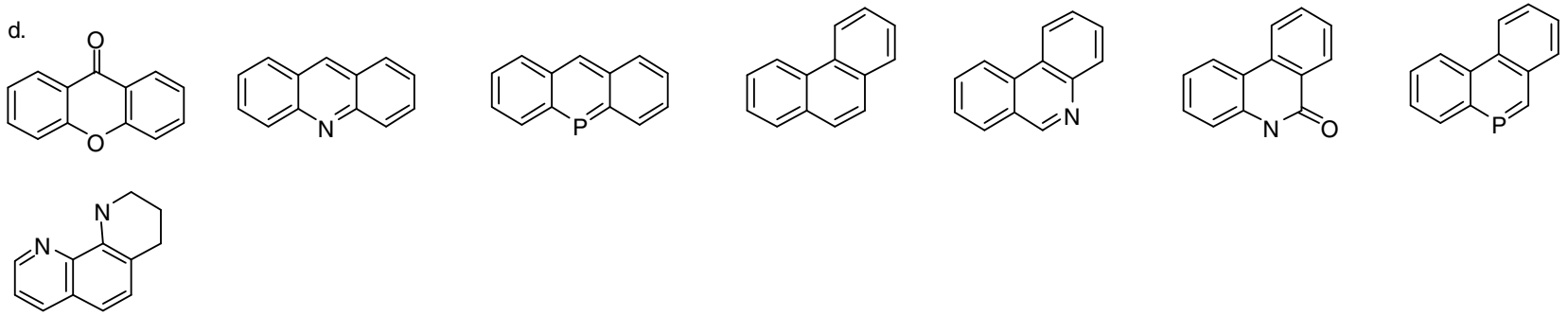
b.



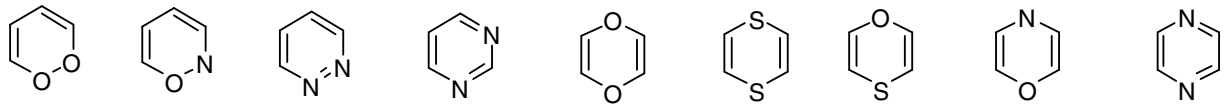
c.



d.

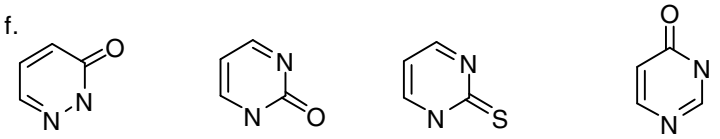


e.

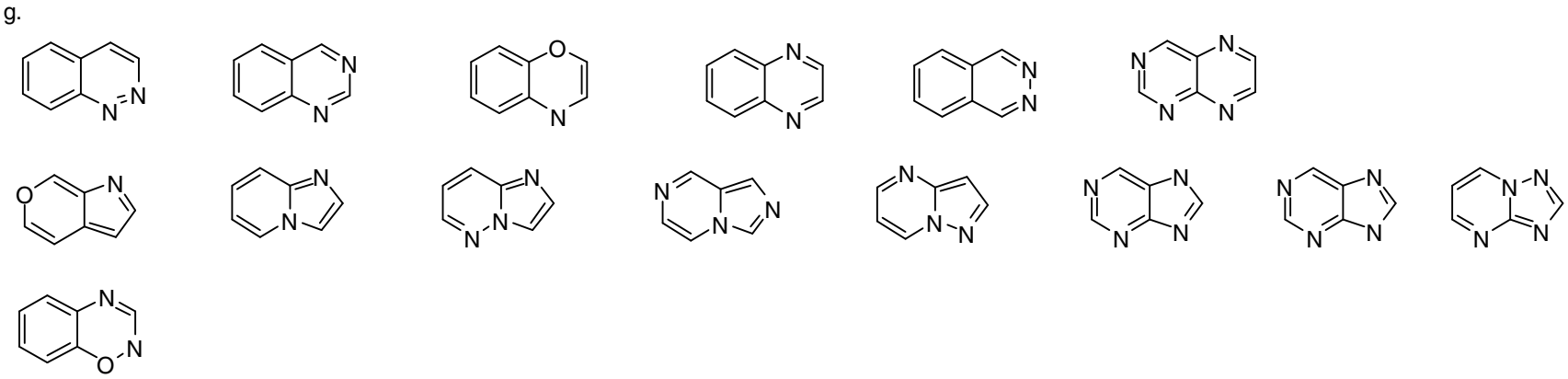


Supplementary Figure 2 (continued)

f.



g.



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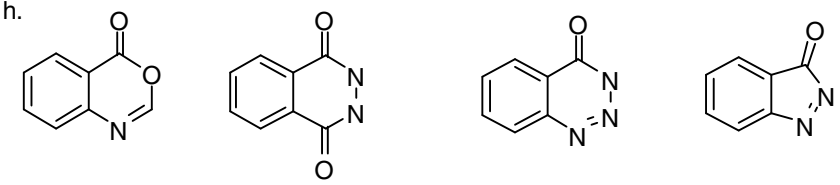

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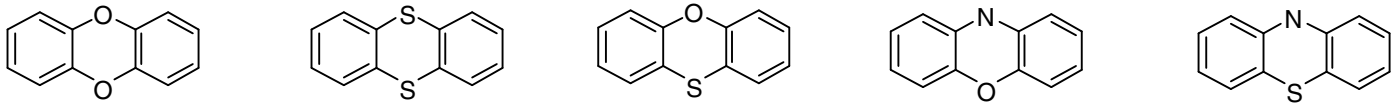

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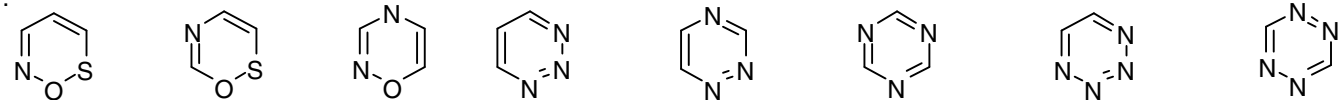
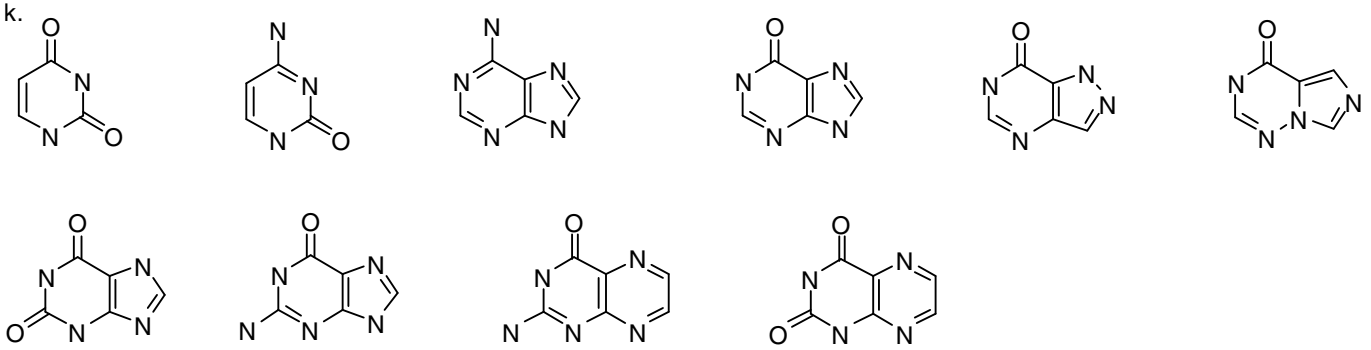
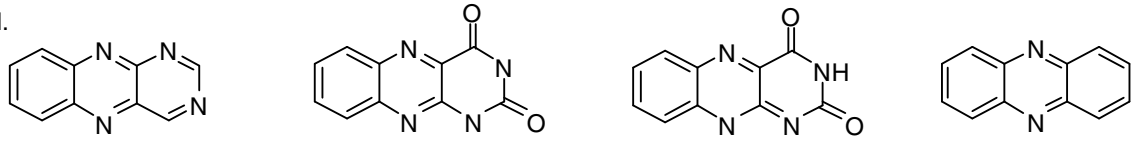


Supplementary Figure 2 (continued)

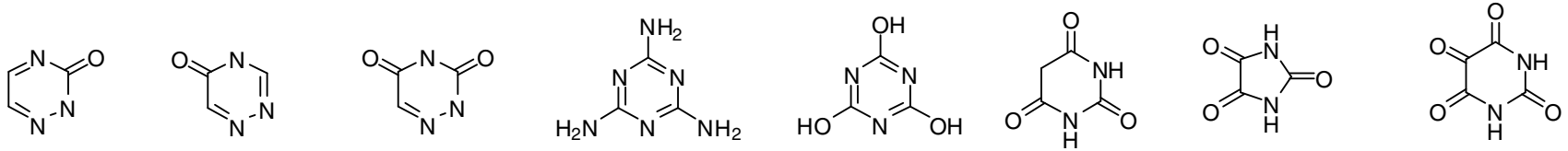
k.



l.

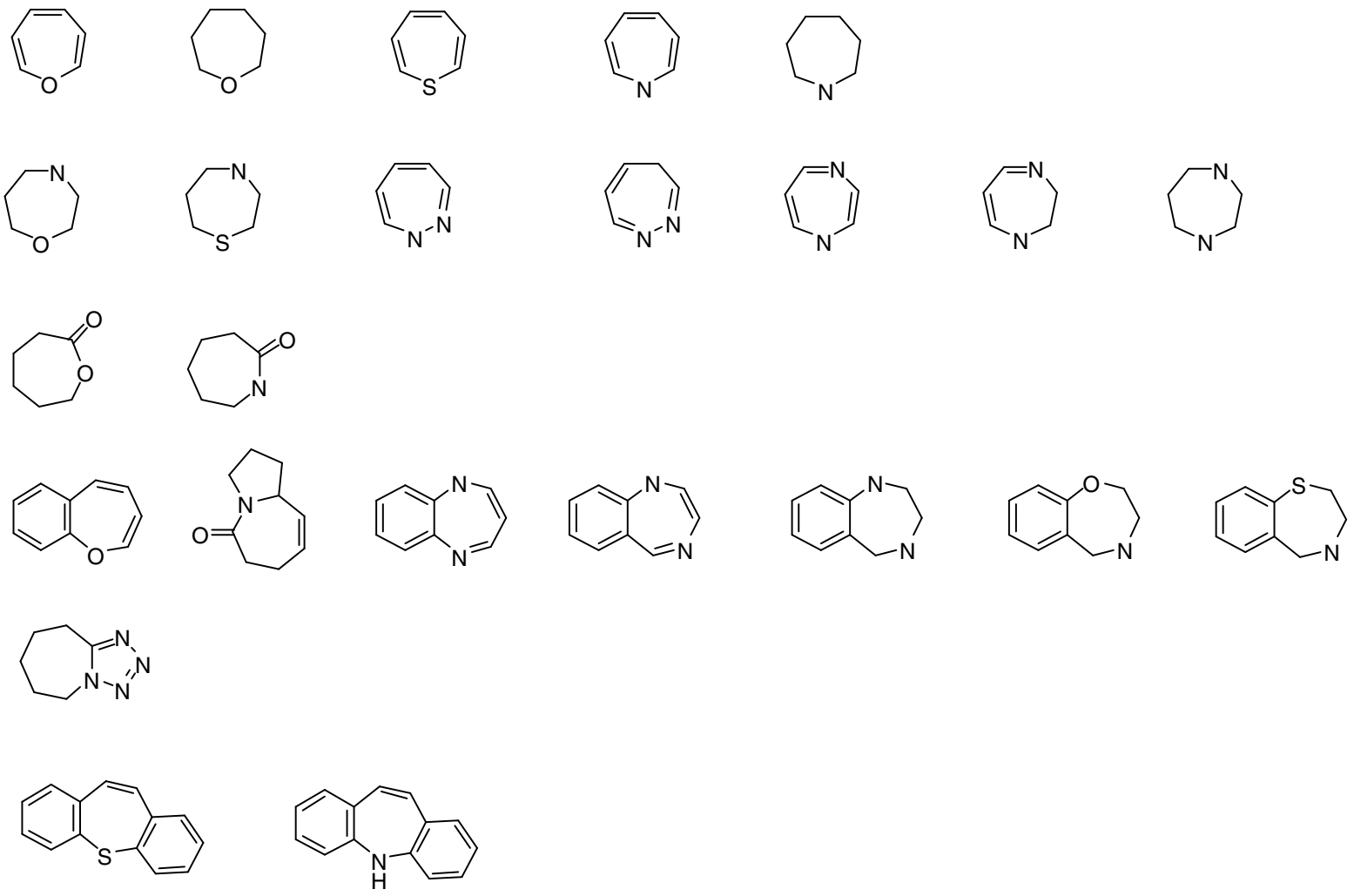


m.



Supplementary Figure 2 (continued)

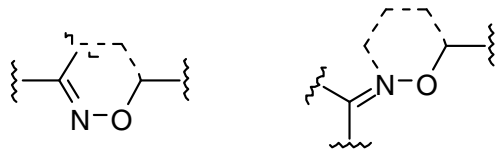
5. Seven-membered heterocycle



Supplementary Figure 2 (continued)

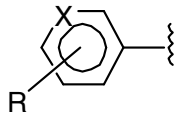
II. Nonclassical bioisosteres

A. Cyclic vs noncyclic: conformationally constrained analogues

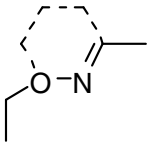


(Methyleneaminoxy methyl moiety, MAOMM)

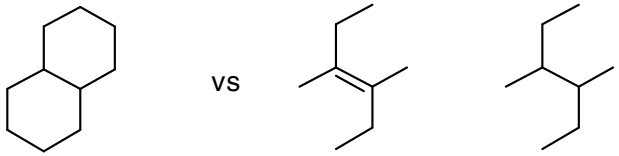
vs

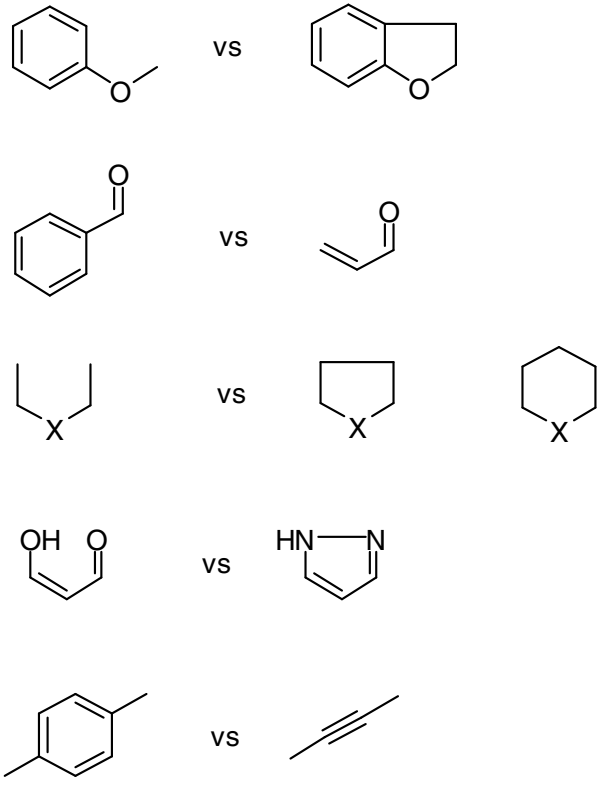


(Aryl and other aromatic groups)



[(Methyloxy)imino]methyl moiety, MOIMM)





Supplementary Figure 2 (continued)

B. Nonclassical bioisosteric replacement of functional groups

1. Halogens

—H vs —F

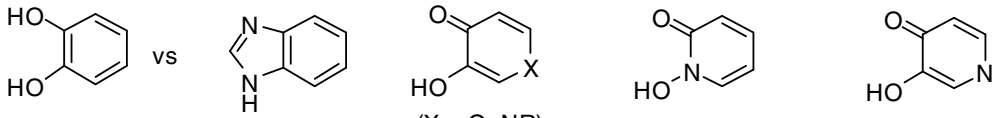
—X vs —CH_3 —CF_3 —CN —$N(CN)_2$ —$C(CN)_3$ —SCN

2. Hydroxyl group

—OH vs —CH_2OH —$NHCONH_2$ —NHCOR —$NHSO_2R$ —NHCN —$CH(CN)_2$

a. Phenol

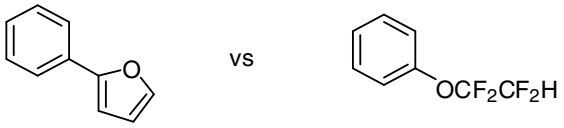
b. Catechol



(X = O, NR)

3. Ether group

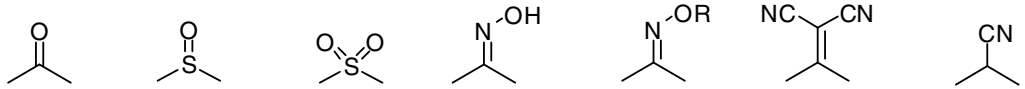
—O— vs —S—  —C=C— (H H)



(Biorg. Med. Chem. Lett. 2001, 11, 1625-1628; ChemBioChem, 2004, 5, 637-643)

Supplementary Figure 2 (continued)

4. Carbonyl group

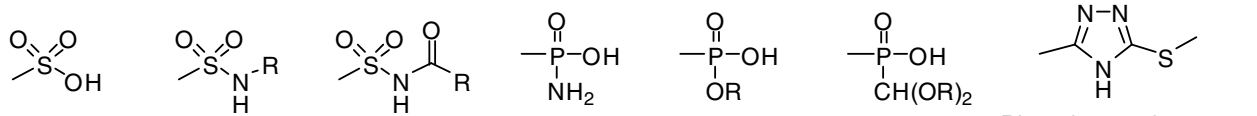


5. Carboxylic acid group: considering liphophilicity

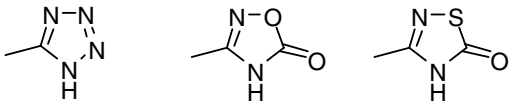
High chelating power

lipophilic

lipophilic



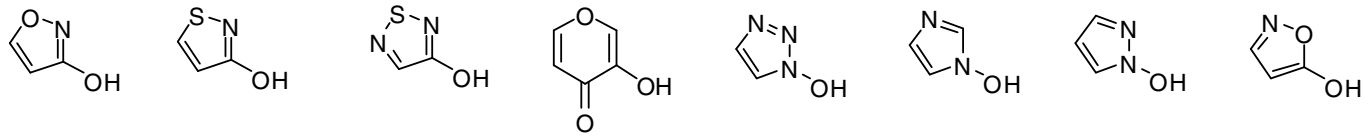
Phosphonate isosteres



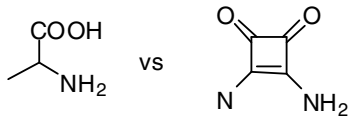
lipophilic

lipophilic

lipophilic

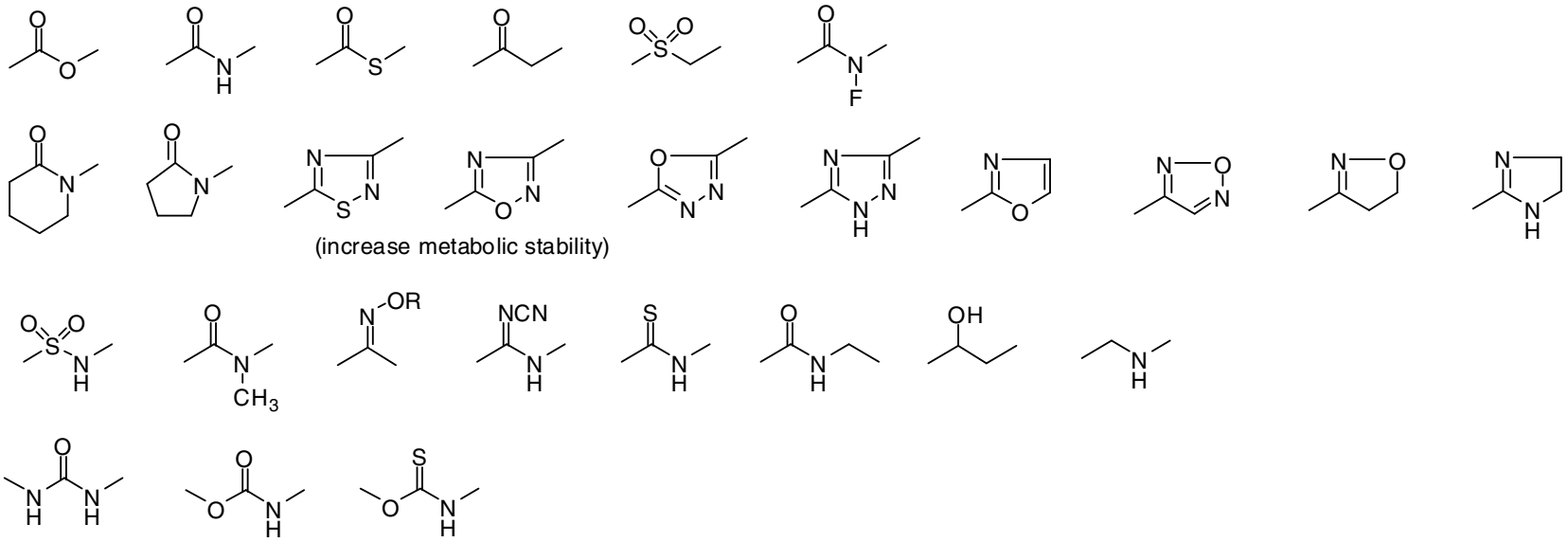


α-Amino acid vs Diamino-cyclobutene-dione

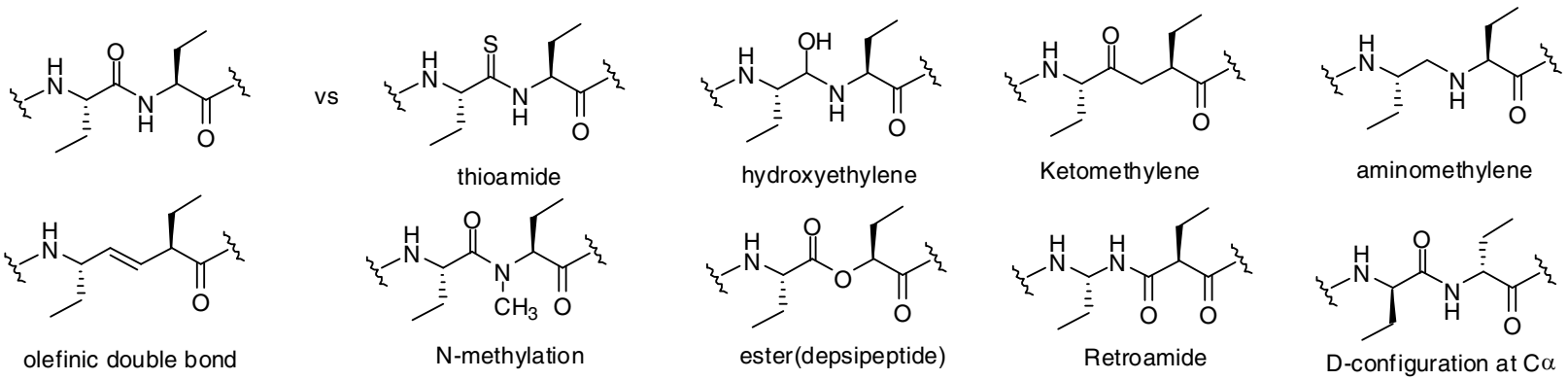


Supplementary Figure 2 (continued)

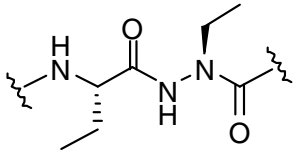
6. Ester group or amide group: considering metabolic stability



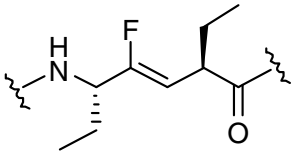
Isosteric replacement of peptide bonds



Supplementary Figure 2 (continued)

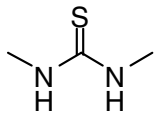


α-azapeptide

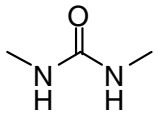


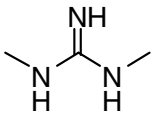
olefinic double bond

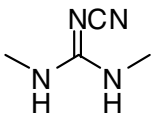
7. Thiourea

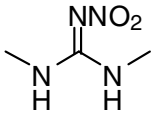


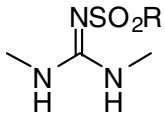
vs

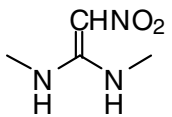


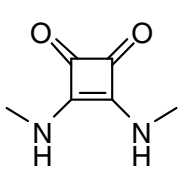


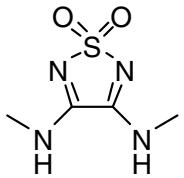


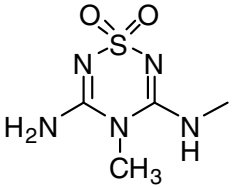




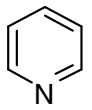




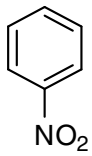


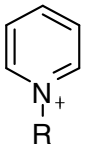


8. Pyridines

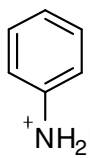


vs





vs

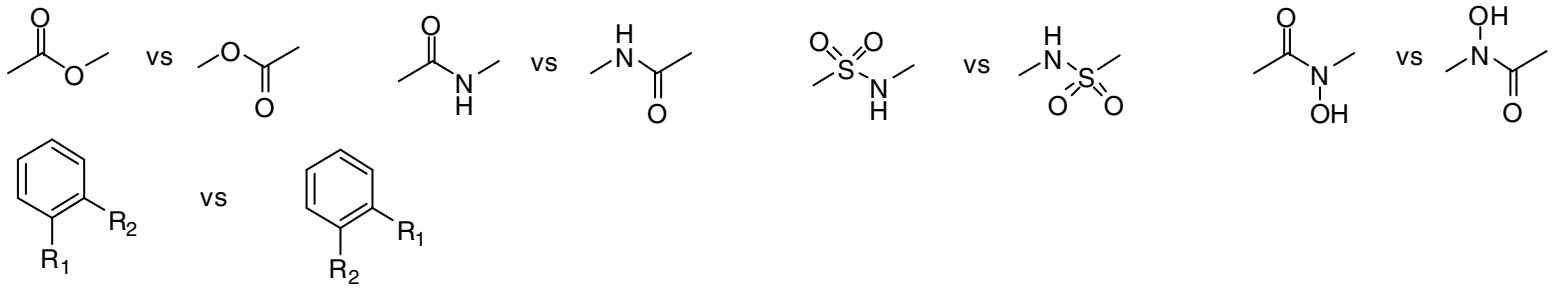


9. Azomethine

vs

Supplementary Figure 2 (continued)

10. Retroisoterism



Supplementary Figure 3

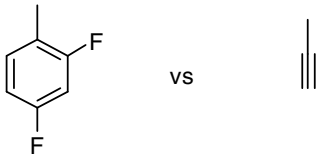
The following rules of metabolism stability were constructed based upon the literatures below:

1. van De Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. Property-based design: optimization of drug absorption and pharmacokinetics. *J. Med. Chem.* **2001**, *44(9)*, 1313-1333.
2. Nassar, A.-E. F.; Kamel, A. M.; Clarimont, C. Improving the decision-making process in the structural modification of drug candidates: enhancing metabolic stability. *Drug Discov. Today* **2004**, *9(23)*, 1020-1028.
3. Rishton, G. M. Reactive compounds and in vitro false positives in HTS. *Drug Discov. Today*, **1997**, *2(9)*, 382-384.
4. Rishton, G. M. Nonleadlikeness and leadlikeness in biochemical screening. *Drug Discov. Today* **2003**, *8(2)*, 86-96.
5. Böhm, H.-J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. Fluorine in medicinal chemistry. *ChemBioChem*, **2004**, *5*, 637-643.

The strategies to increase metabolic stability:

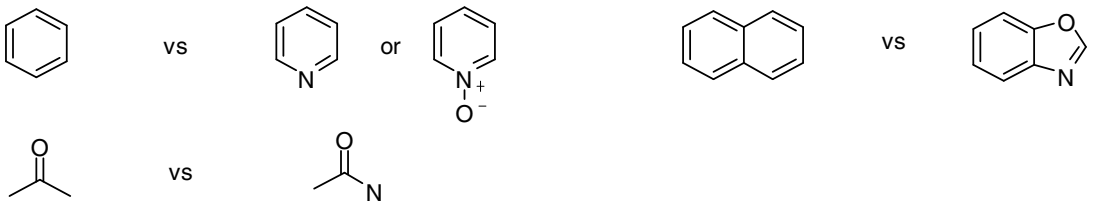
1. To reduce the overall lipophilicity: because the binding site of metabolizing enzyme is generally lipophilic in nature
(1) Introduction of substituents with reduced lipophilicity

e.g.



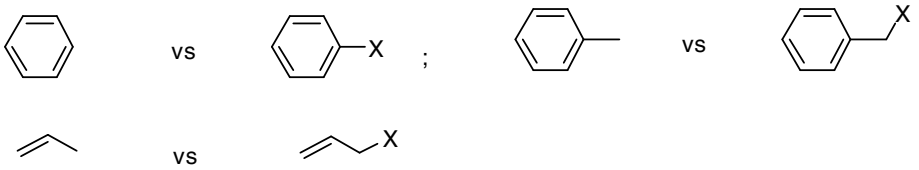
(2) Introduction of isosteric atoms or functional groups to increase polarity

e.g.

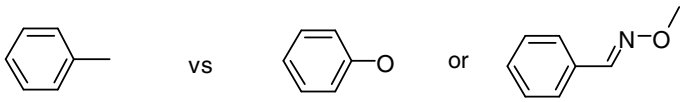


2. Block metabolically labile sites

(1) Introduction of a halogen atom (particularly fluorine and chlorine)



(2) The replacement of benzylic CH_2 with an isostere such as an oxygen atom or methoxime



Supplementary Figure 3. Rules of metabolic stability

Supplementary Figure 3 (continued)

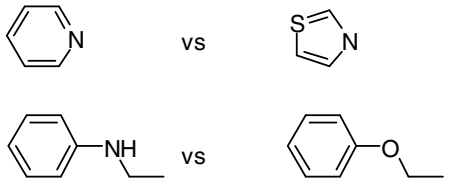
(3) The replacement of allylic group with acetylene

e.g.

(4) Deactivation of aromatic rings to facilitate oxidation through substitution with strongly electron-withdrawing groups (e.g. CF3, F, Cl).

e.g.

(5)



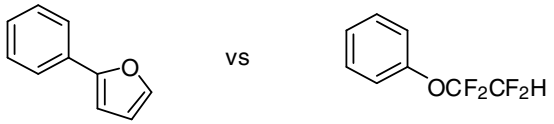
3. Modification of metabolically labile groups

(1) Constraining the molecule in an unfavorable conformation to the metabolic pathway, more typically, protecting the labile moiety by steric shielding. e.g. Introduction of an N-*tert*-butyl group to prevent N-dealkylation.

(2) Replacement of a labile ester linkage with an amide group, or cyclic bioisostere

(3)The phenolic function has consistently been shown to be rapidly glucuronidated. Thus, avoidance of this moiety in a sterically unhindered position. Instead

(4) monosubstituted furan is generally undesirable due to its metabolic instability and its potential to generate reactive metabolites

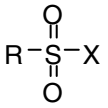


(more stable)

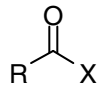
Supplementary Figure 3 (continued)

The following reactive functional groups responisible for in vitro false positives.
1. These reactive functional groups are generally prone to decompostion under hydrolytic conditions.
2. They are reactive towards protein and biological nucleophiles
3. They are exhibit poor stability in serum

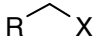
X=F,Cl,Br,I, tosyl,mesyl
R=alkyl, aryl,heteroalkyl,heteroaryl



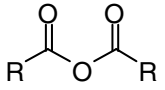
Sulfonyl halides



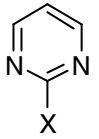
Acyl halides



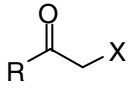
Alkyl halides



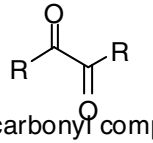
Anhydrides



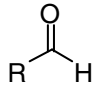
Halopyrimidines



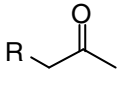
α-Halocarbonyl compounds



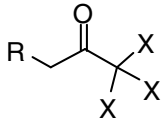
1,2-Dicarbonyl compounds



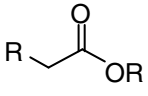
Aldehyde



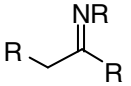
Aliphatic ketones



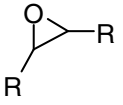
Perhalo ketones



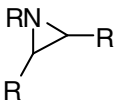
Aliphatic ketones



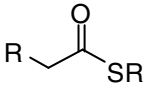
Imines



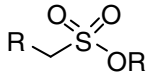
Epoxides



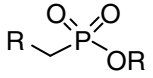
Aziridines



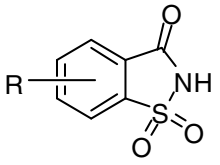
Thioesters



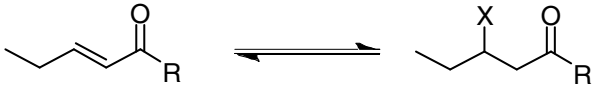
Sulfonate esters



Phosphonate esters



Saccharinoids



Michael acceptors and β-heterosubstituted carbonyl compounds

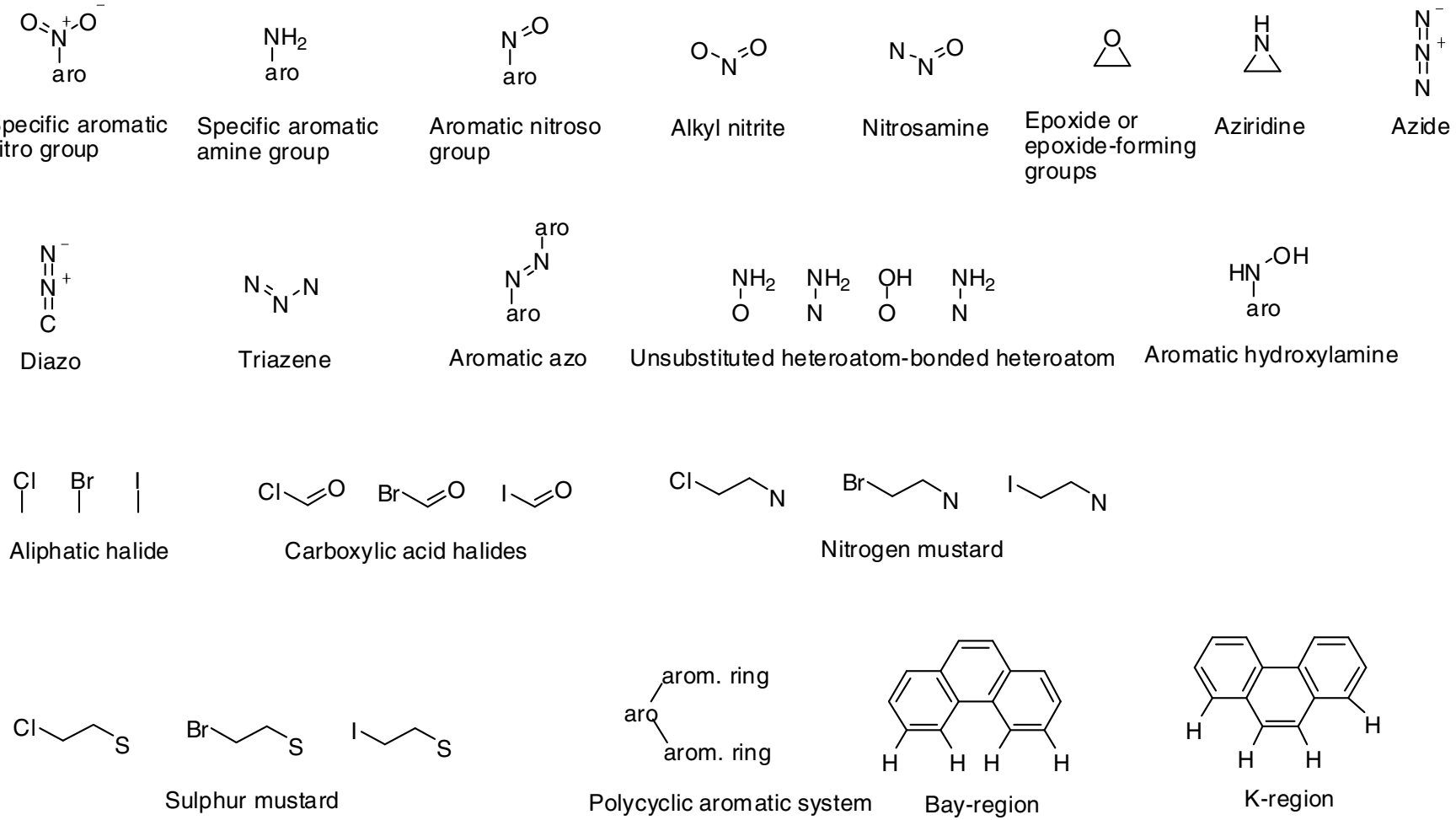
Heteroatom-heteroatom single bonds

Supplementary Figure 4

The following toxicophore library was constructed based upon the literatures below:

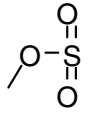
1. Kazius, J.; McGuire, R.; Bursi, R. Derivation and validation of toxicophores for mutagenicity prediction. *J. Med. Chem.* **2005**, *48(1)*, 312-320.
2. Nassar, A.-E. F.; Kamel, A. M.; Clarimont, C. Improving the decision-making process in structural modification of drug candidates: reducing toxicity. *Drug Discov. Today*, **2004**, *9(24)*, 1055-1064.
3. Williams, D. P.; Naisbitt, D. J. Toxicophores: groups and metabolic routes associated with increased safety risk. *Curr. Opin. Drug Discov. Devel.* **2002**, *5(1)*, 104-115.
4. Llorens, O.; Perez, J. J.; Villar, H. O. Toward the Design of Chemical Libraries for Mass Screening Biased against Mutagenic Compounds. *J. Med. Chem.* **2001**, *44(17)*, 2793-2804.

"aro" indicates an aromatic atom. "arom. rings" indicates an atom that is part of multiple aromatic rings

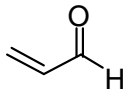


Supplementary Figure 4. Toxicophore library

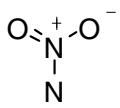
Supplementary Figure 4 (continued)



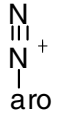
Sulfonate-bonded carbon atom



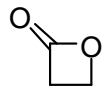
α,β–Unsaturated aldehyde



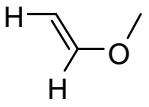
Aliphatic N-nitro



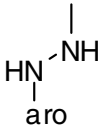
Aromatic diazonium



β-Propiolactone

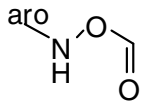


Unsubstituted α,β–unsaturated alkoxy

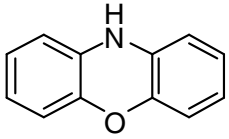


1-Aryl-2-monoalkyl hydrazine

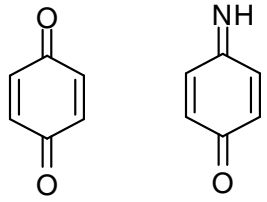
Aromatic methylamine



Aromatic hydroxylamine ester



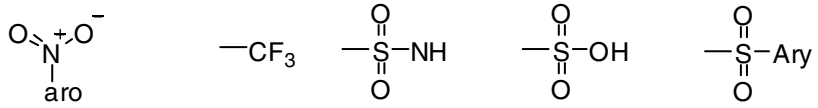
Polycyclic planar system



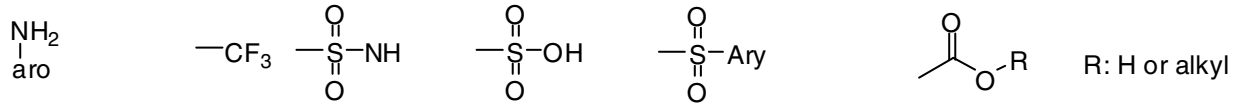
quinone- or quinone imine-forming groups

Supplementary Figure 4 (continued)

However, the introduction of the following groups to aromatic nitro group, aromatic amine group or aromatic azo group can decrease or eliminate toxicities



Specific aromatic nitro group



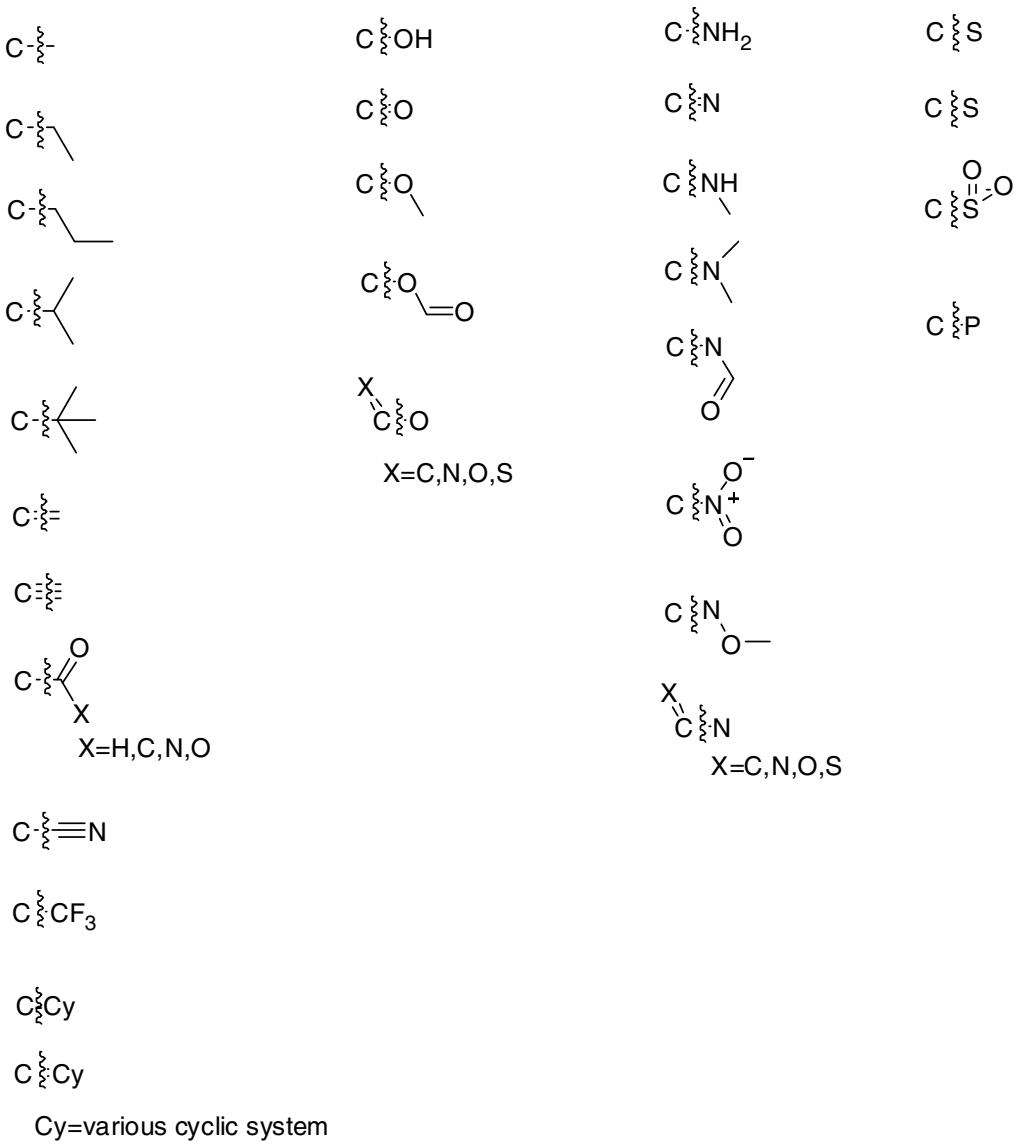
Specific aromatic amine group

Aromatic azo group

Supplementary Figure 5

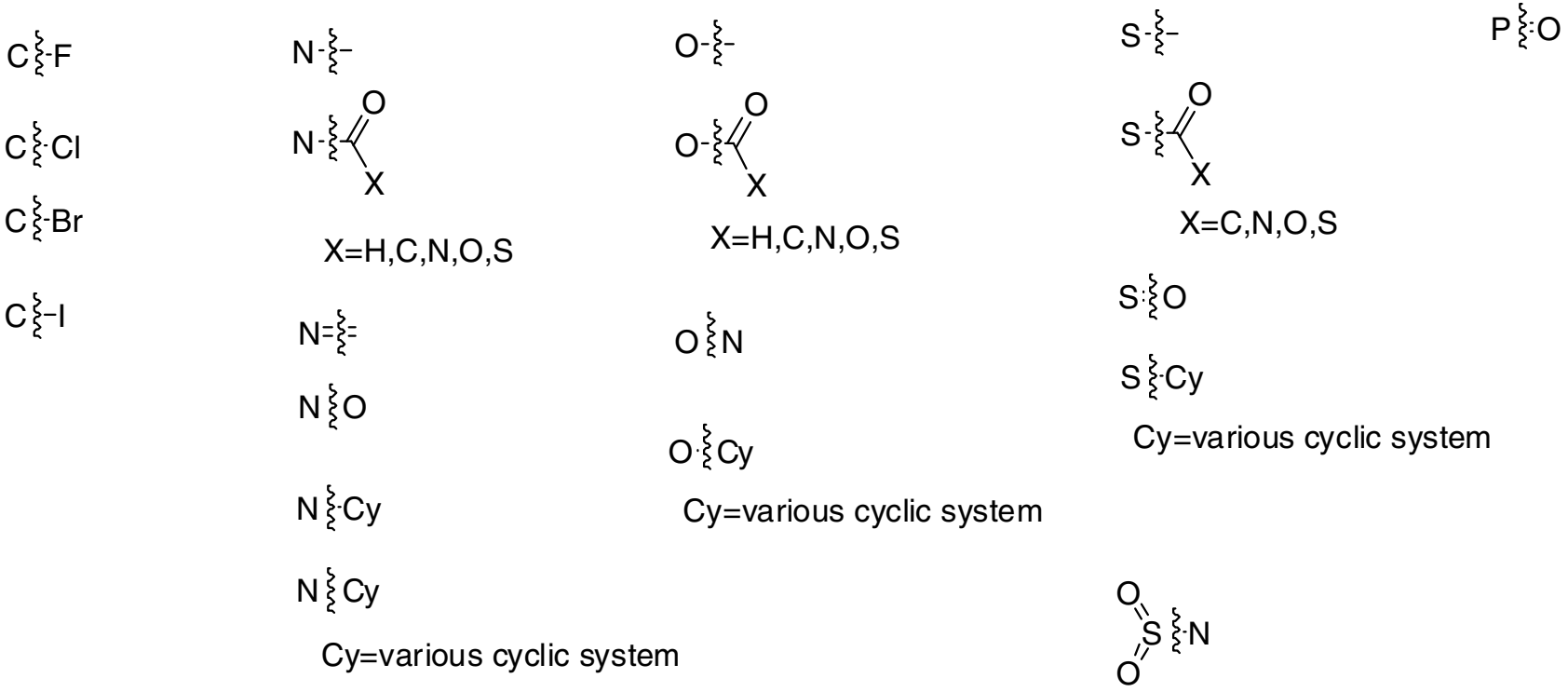
The following side chain library was constructed based upon the literatures below:

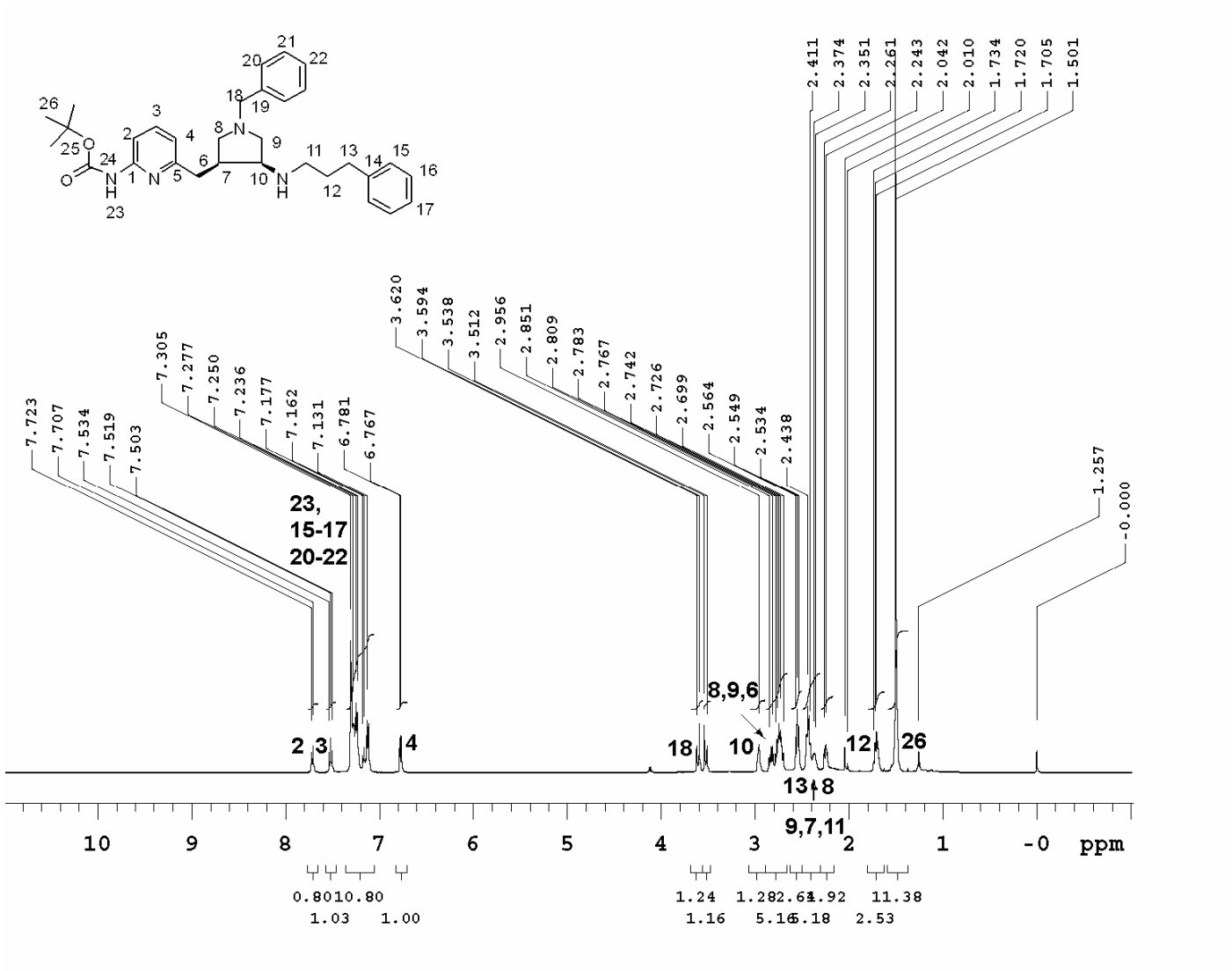
1. Ertl, P. Cheminformatics analysis of organic substituents: identification of the most common substituents, calculation of substituent properties, and automatic identification of drug-like bioisosteric groups. *J. Chem. Inf. Comput. Sci.* **2003**, *43(2)*, 374-380.
2. Lewell, X. Q.; Judd, D. B.; Watson, S. P.; Hann, M. M. RECAP–retrosynthetic combinatorial analysis procedure: a powerful new technique for identifying privileged molecular fragments with useful applications in combinatorial chemistry. *J. Chem. Inf. Comput. Sci.* **1998**, *38(3)*, 511-522.
3. Bemis, G. W.; Murcko, M. A. Properties of known drugs. 2. Side chains. *J. Med. Chem.* **1999**, *42(25)*, 5095-5099.



Supplementary Figure 5. Side chain library

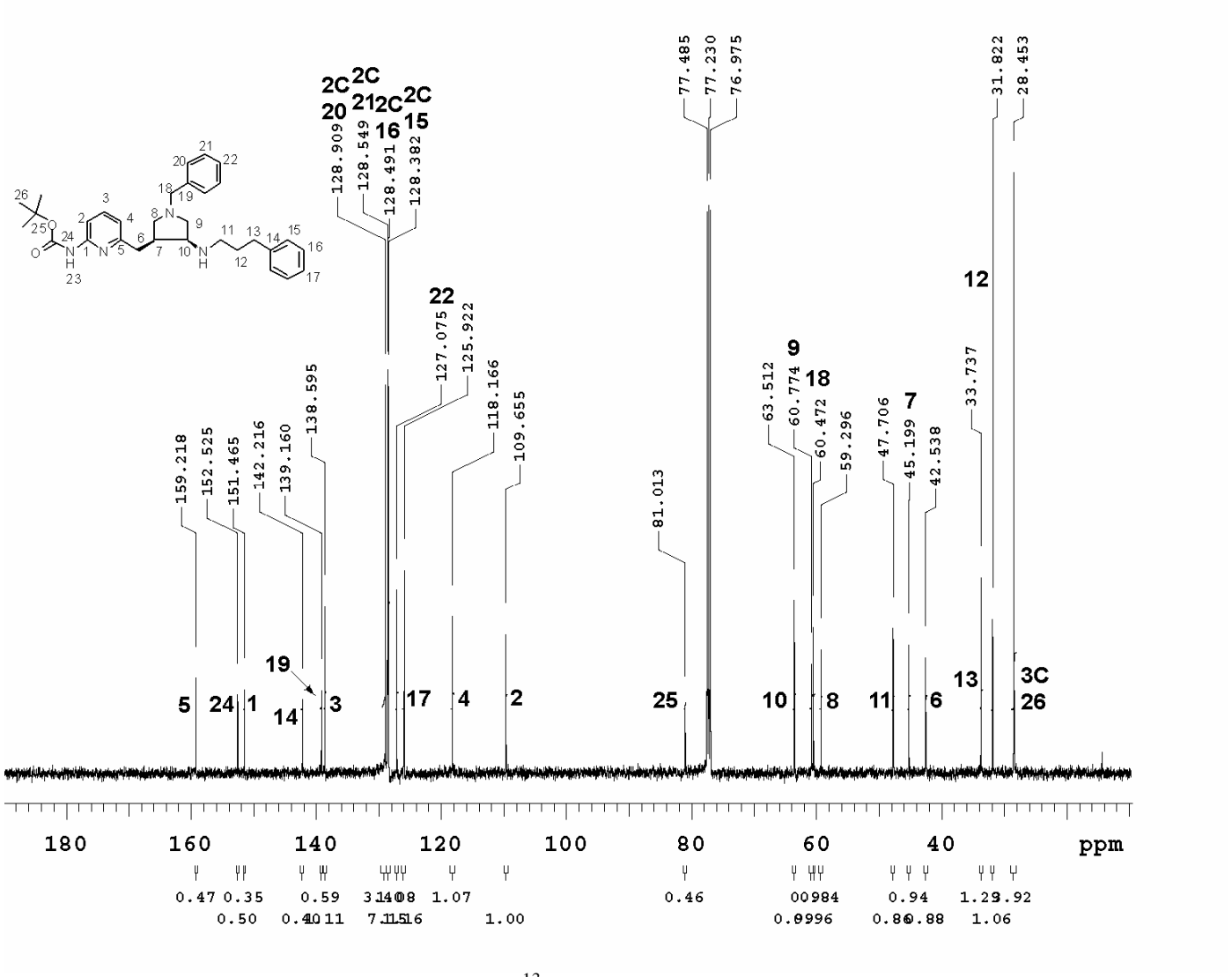
Supplementary Figure 5 (continued)





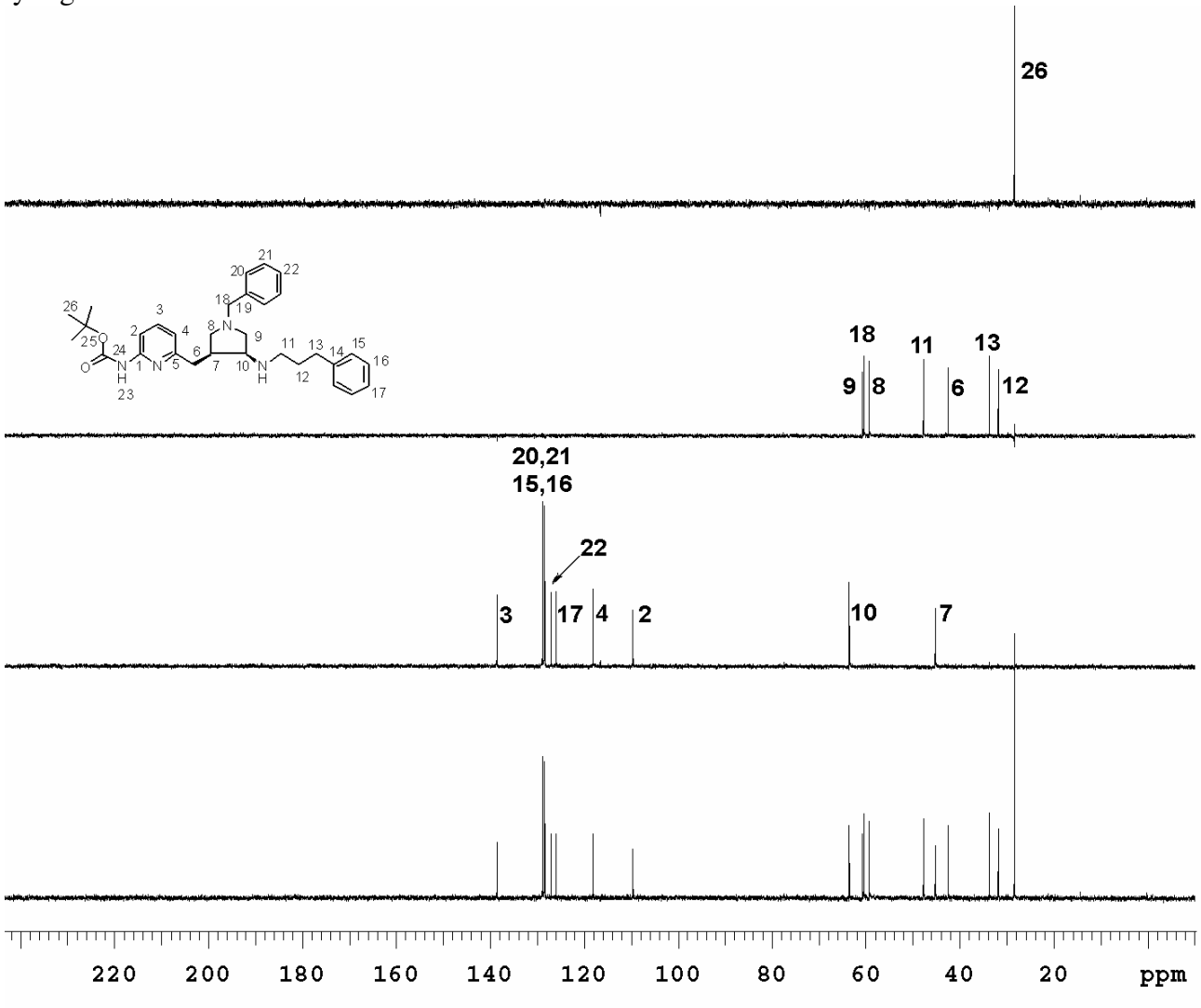
Supplementary Figure 6 ^{1}H NMR of **20a**

Supplementary Figure 6 continued



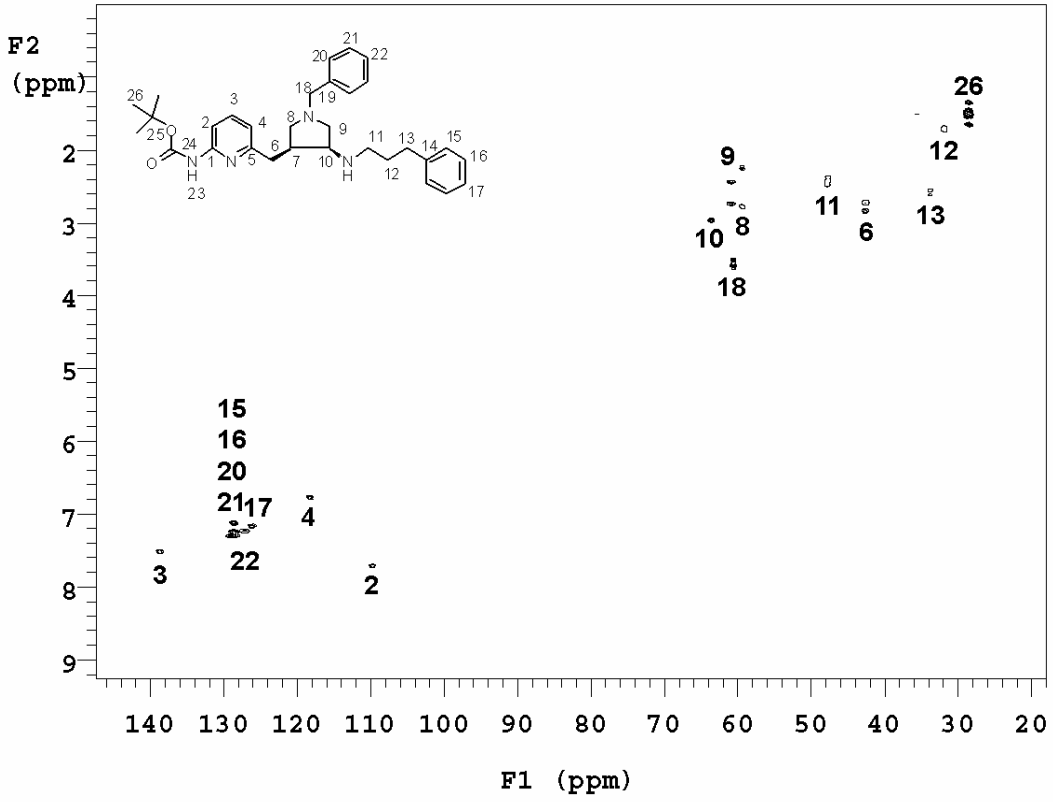
^{13}C NMR of **20a**

Supplementary Figure 6 continued



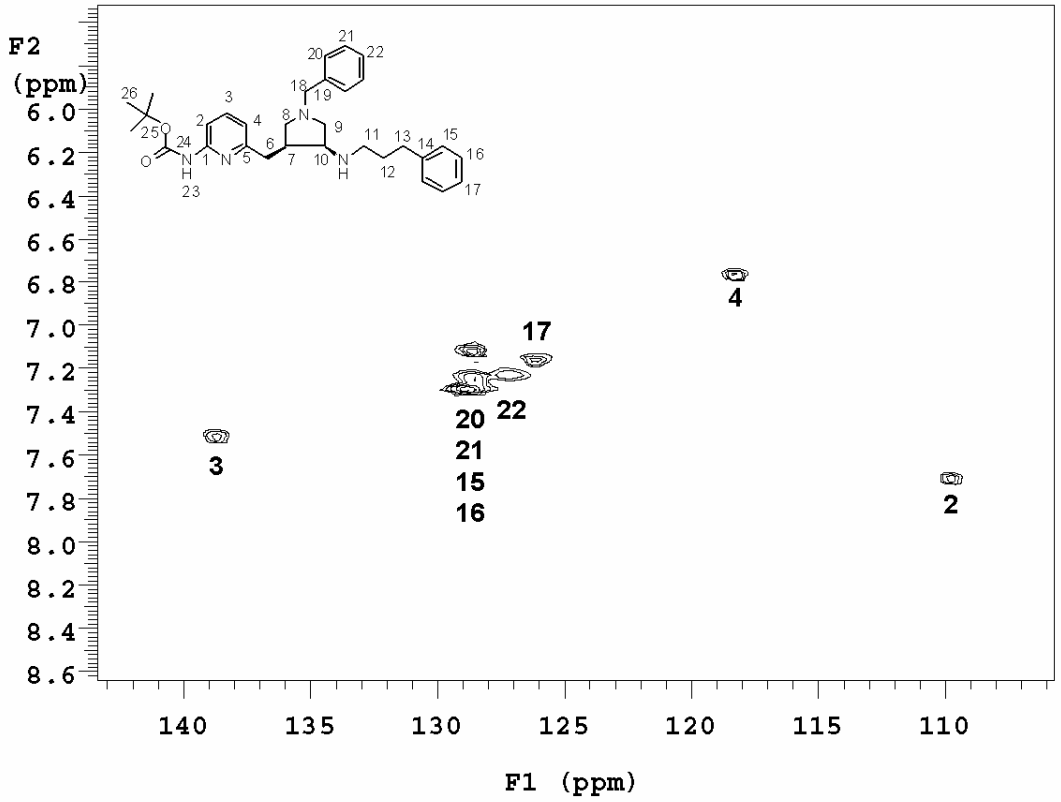
^{13}C NMR-DEPT of **20a**

Supplementary Figure 6 continued



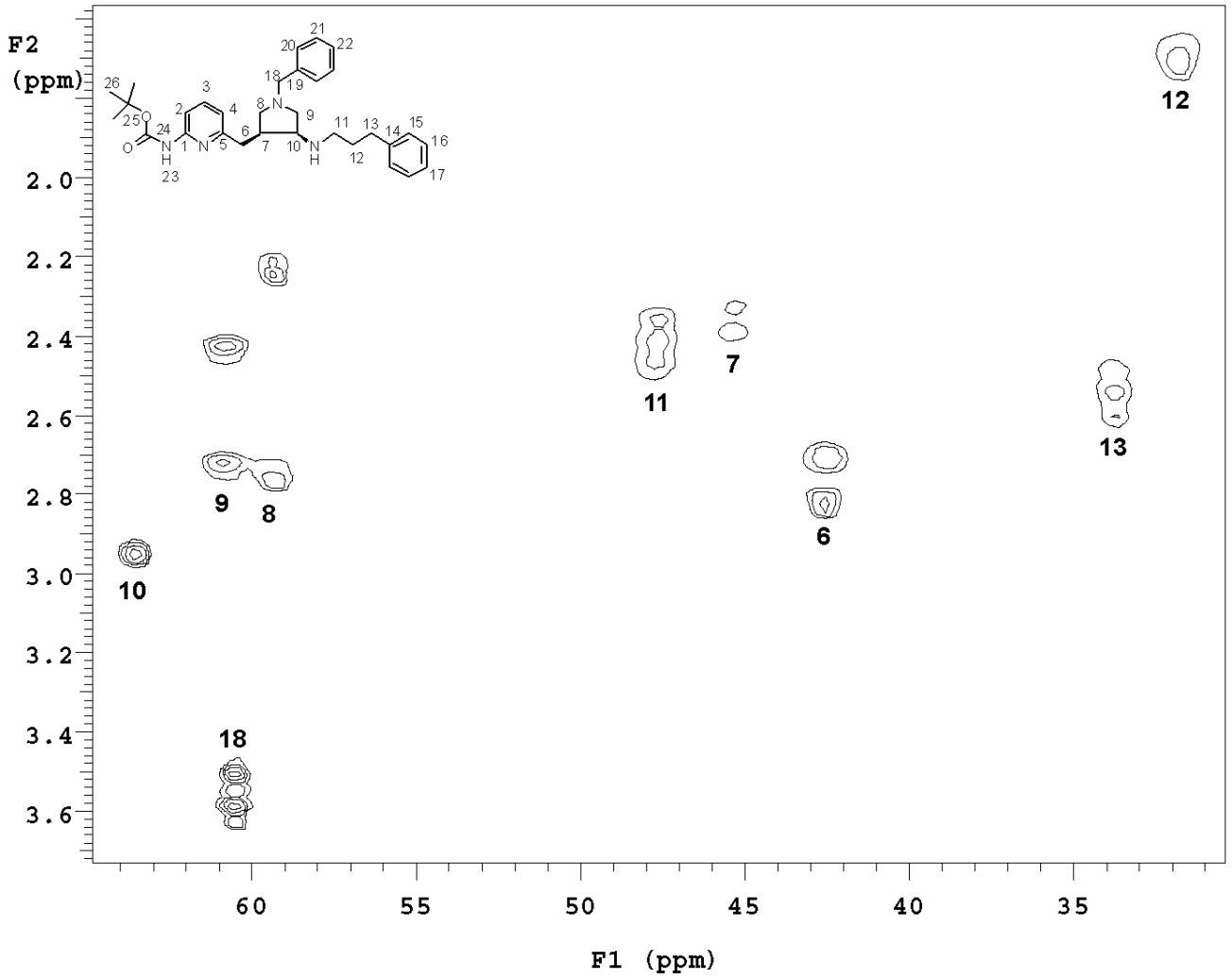
^{1}H-^{13}C HMQC of **20a**

Supplementary Figure 6 continued



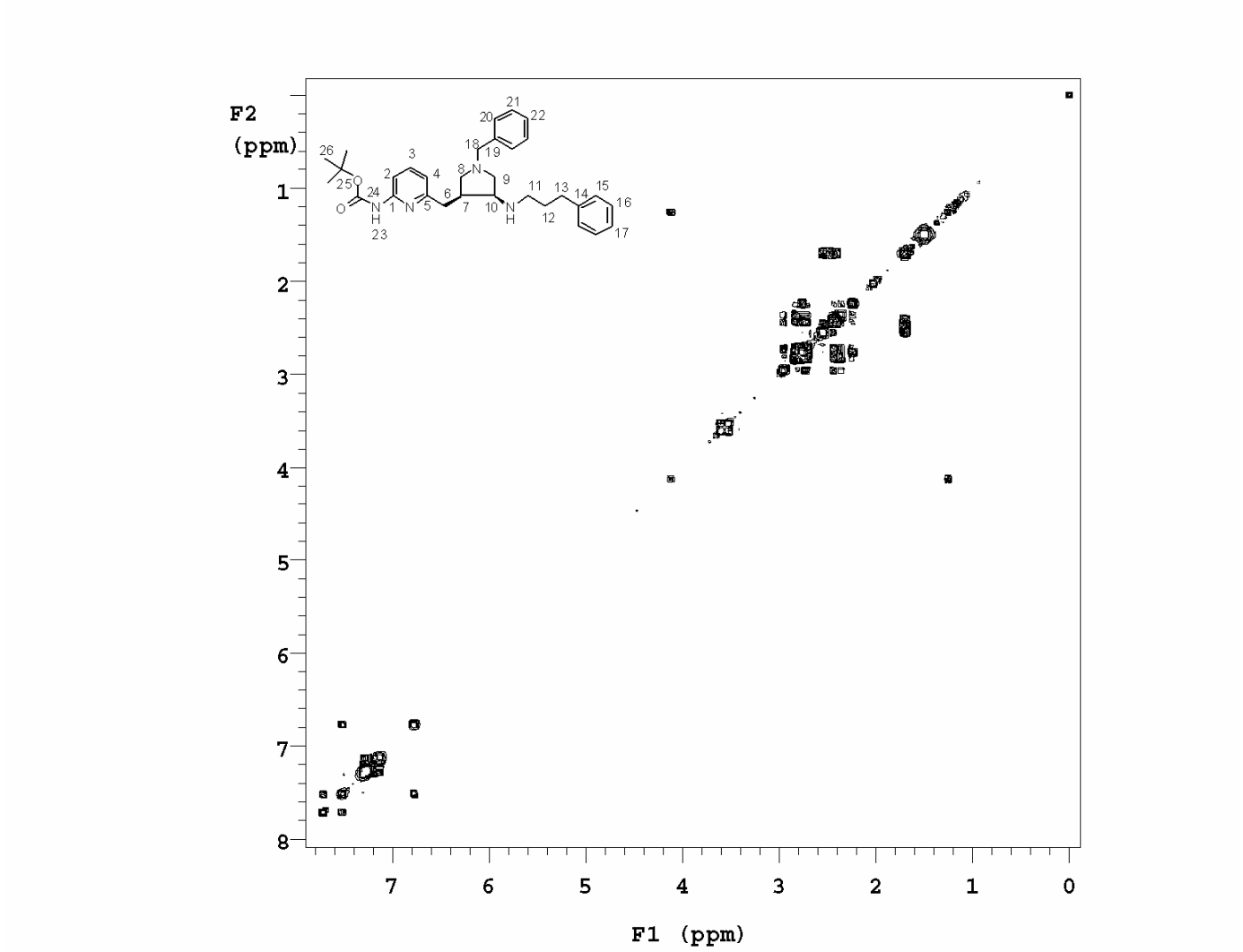
^{1}H-^{13}C HMQC of **20a**

Supplementary Figure 6 continued



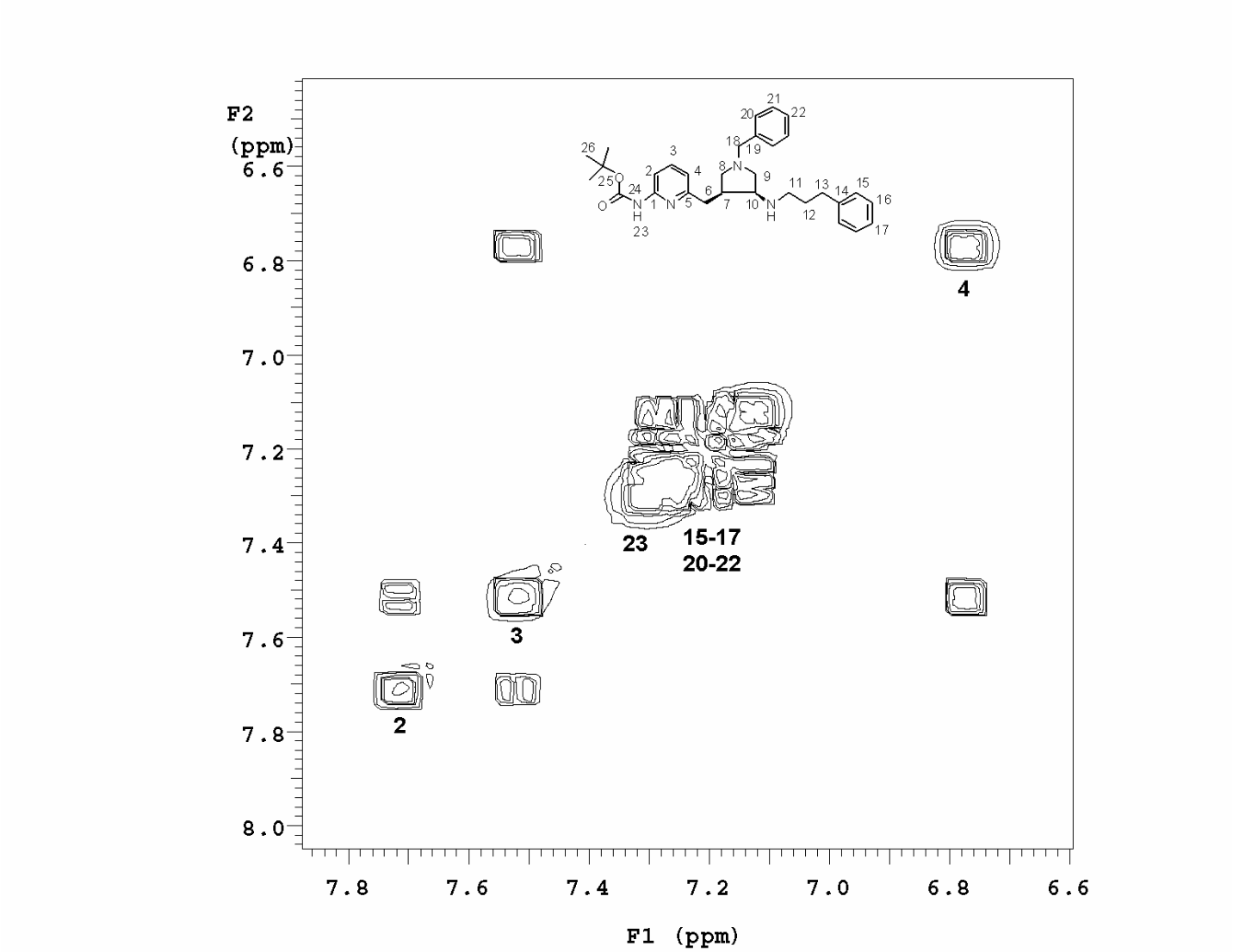
^{1}H-^{13}C HMQC of **20a**

Supplementary Figure 6 continued



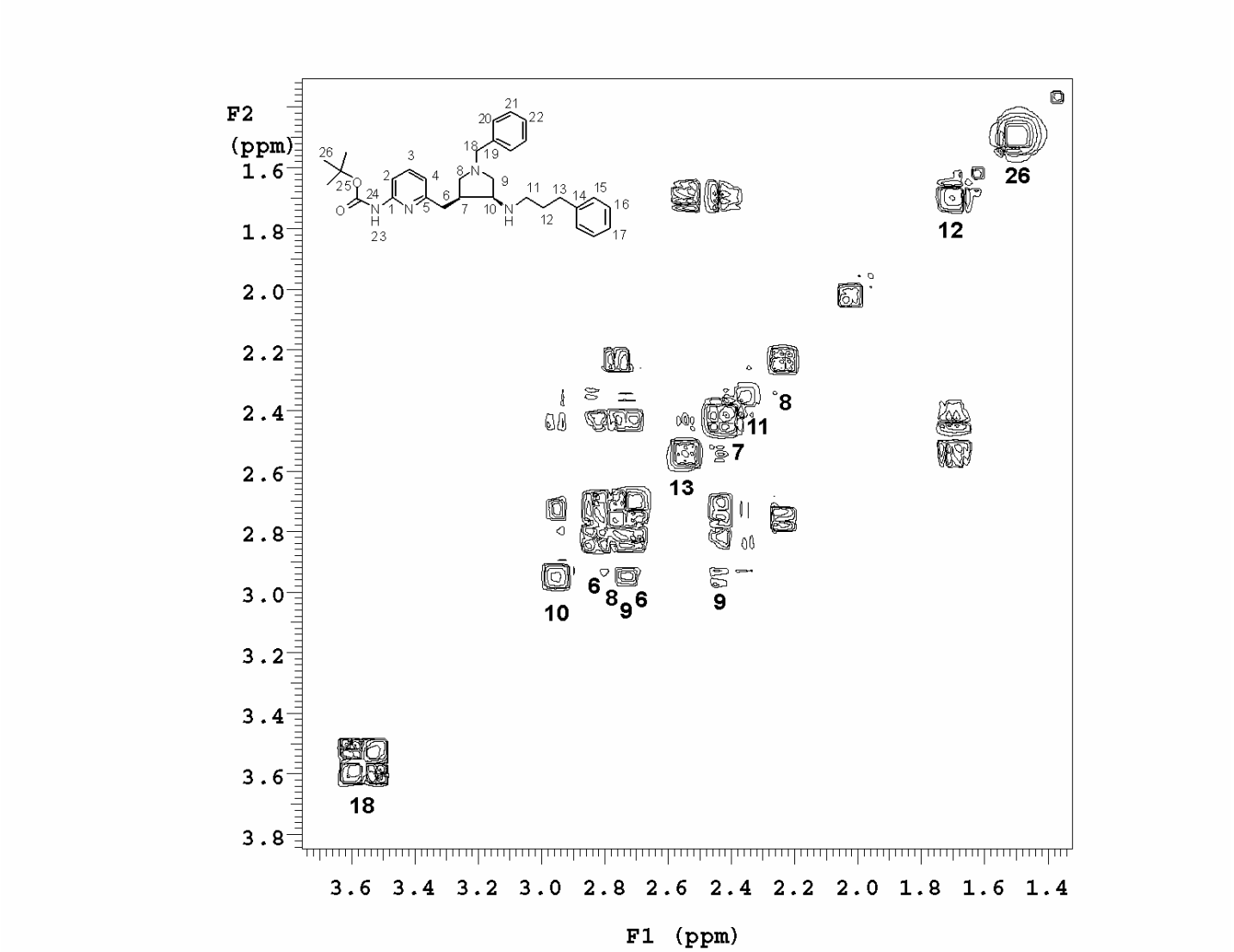
^{1}H-^{1}H COSY of **20a**

Supplementary Figure 6 continued



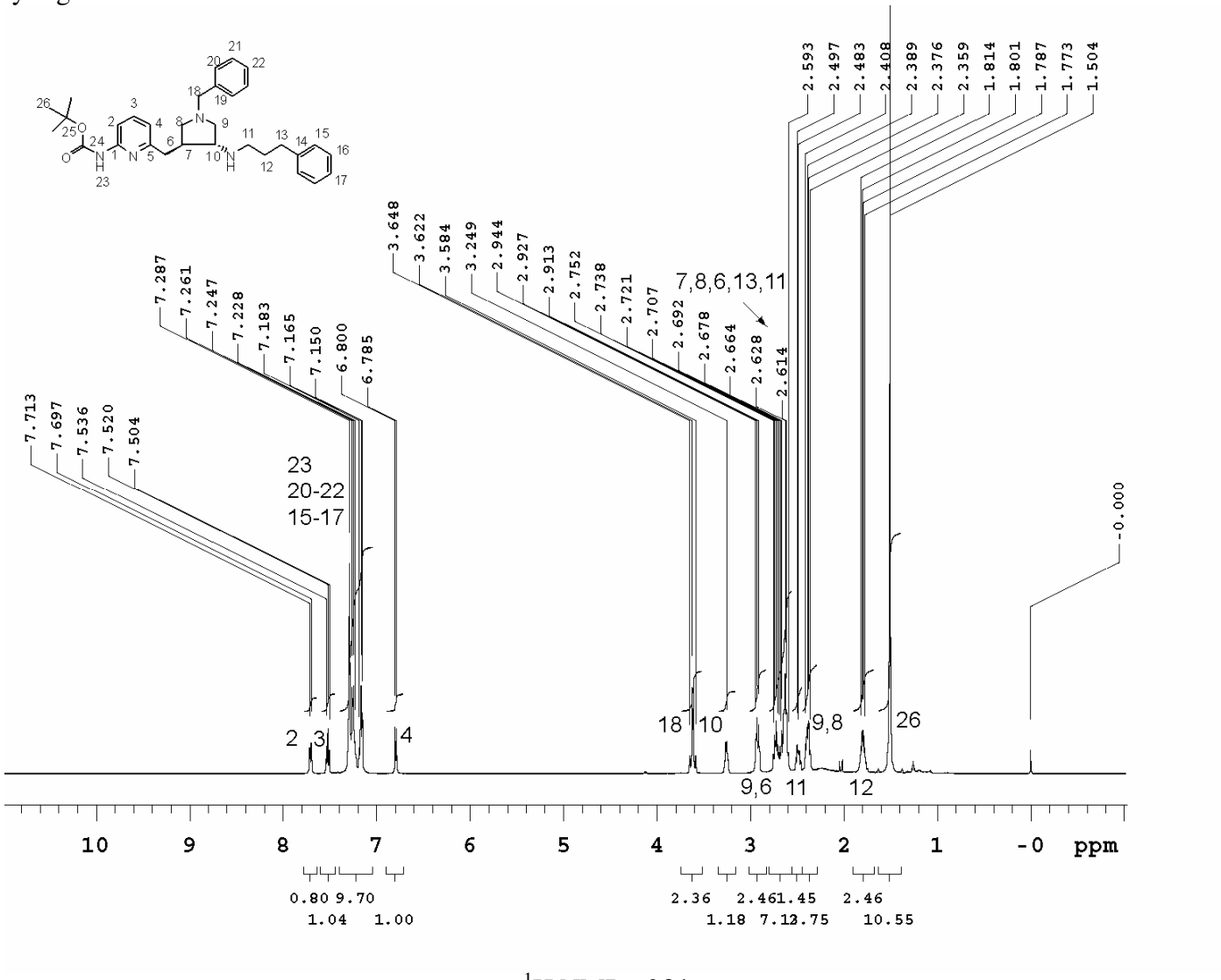
^{1}H-^{1}H COSY of **20a**

Supplementary Figure 6 continued



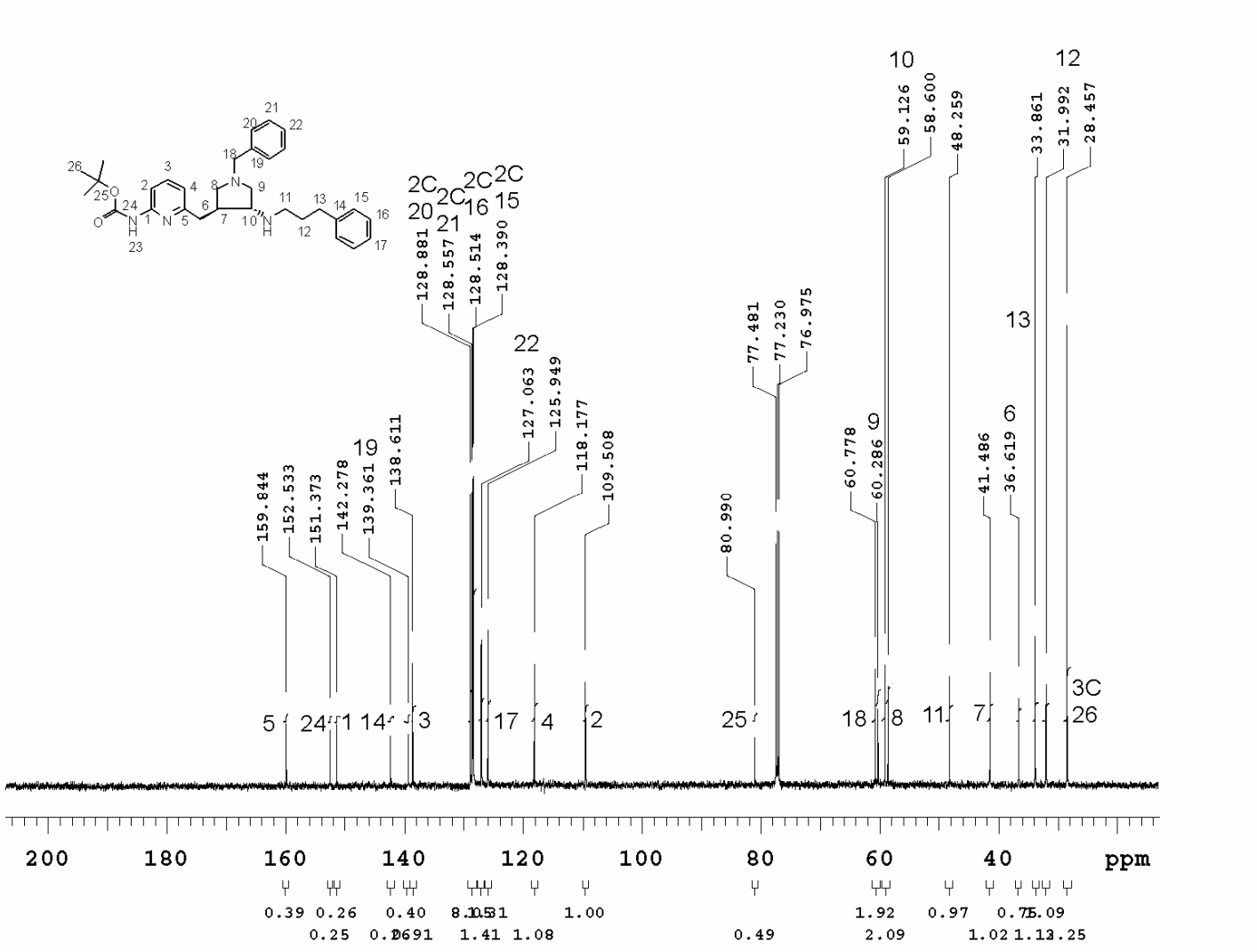
^{1}H-^{1}H COSY of **20a**

Supplementary Figure 6 continued



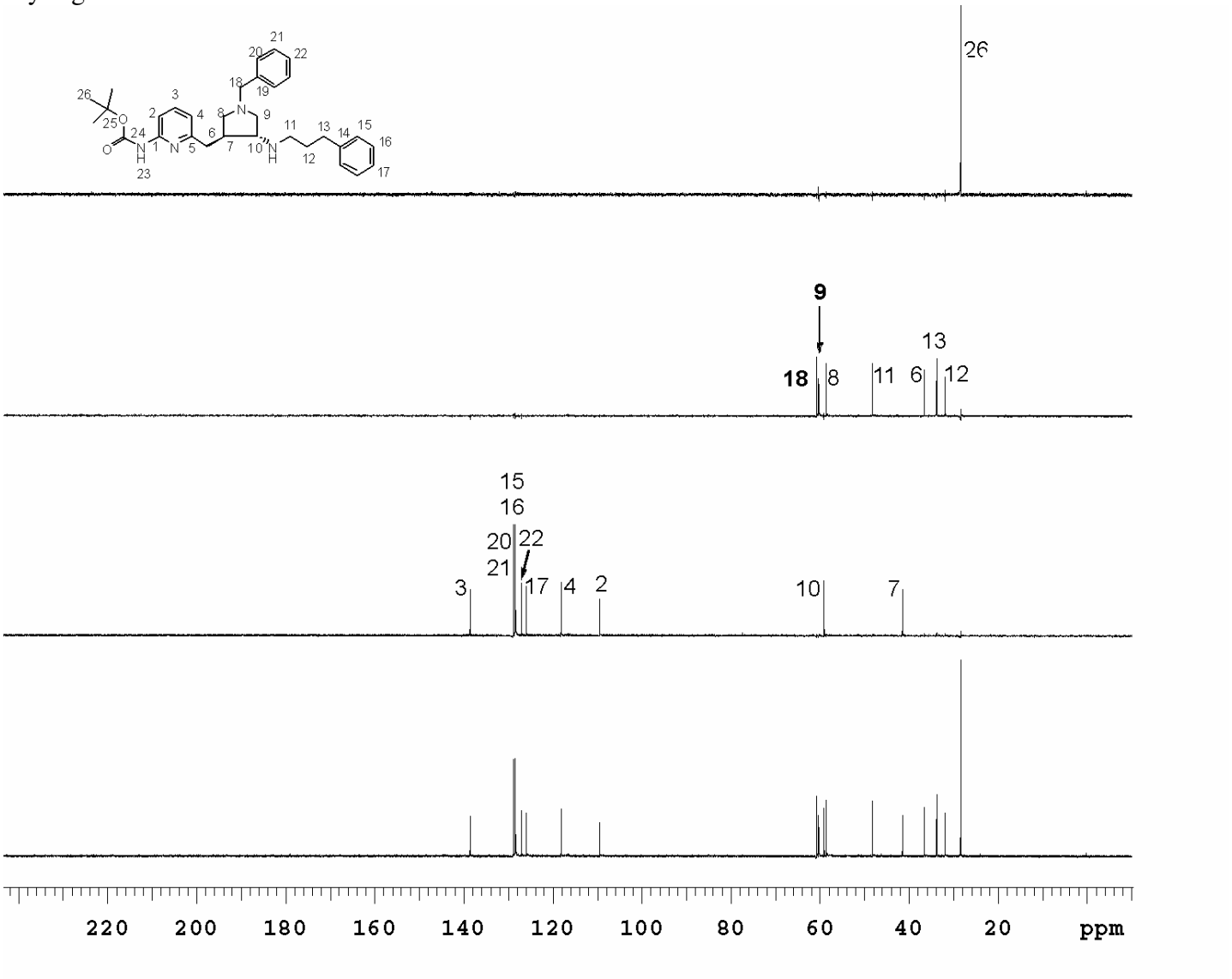
^{1}H NMR of **21a**

Supplementary Figure 6 continued



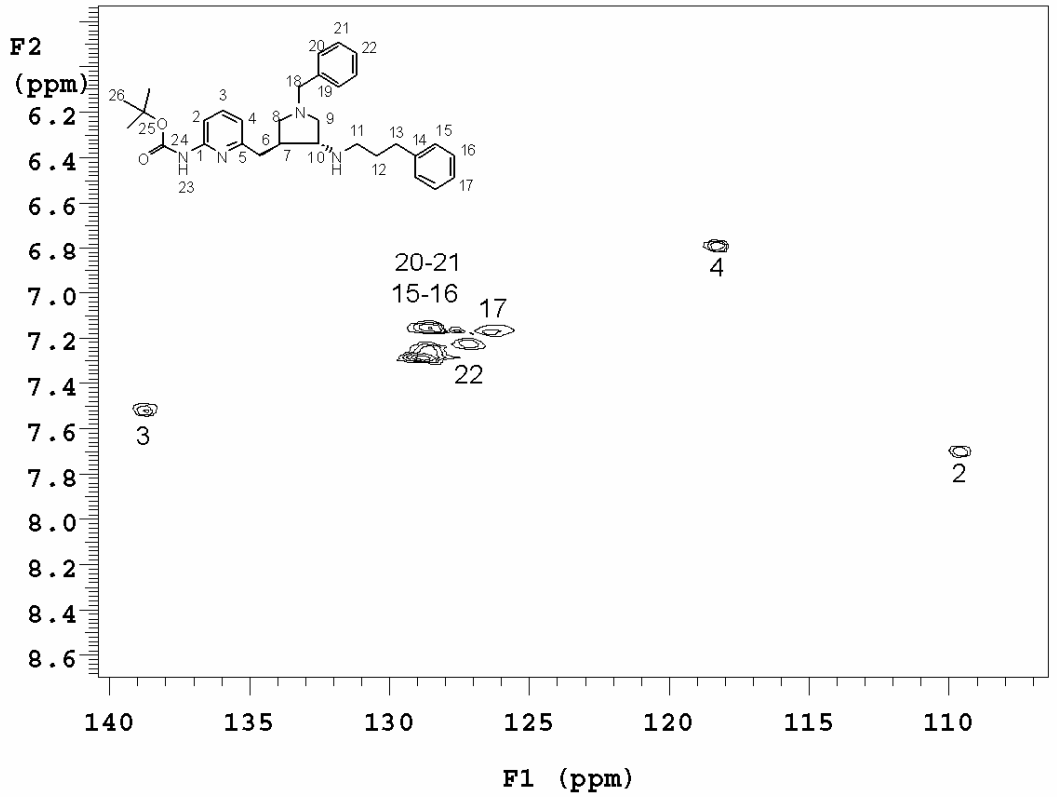
^{13}C NMR of **21a**

Supplementary Figure 6 continued



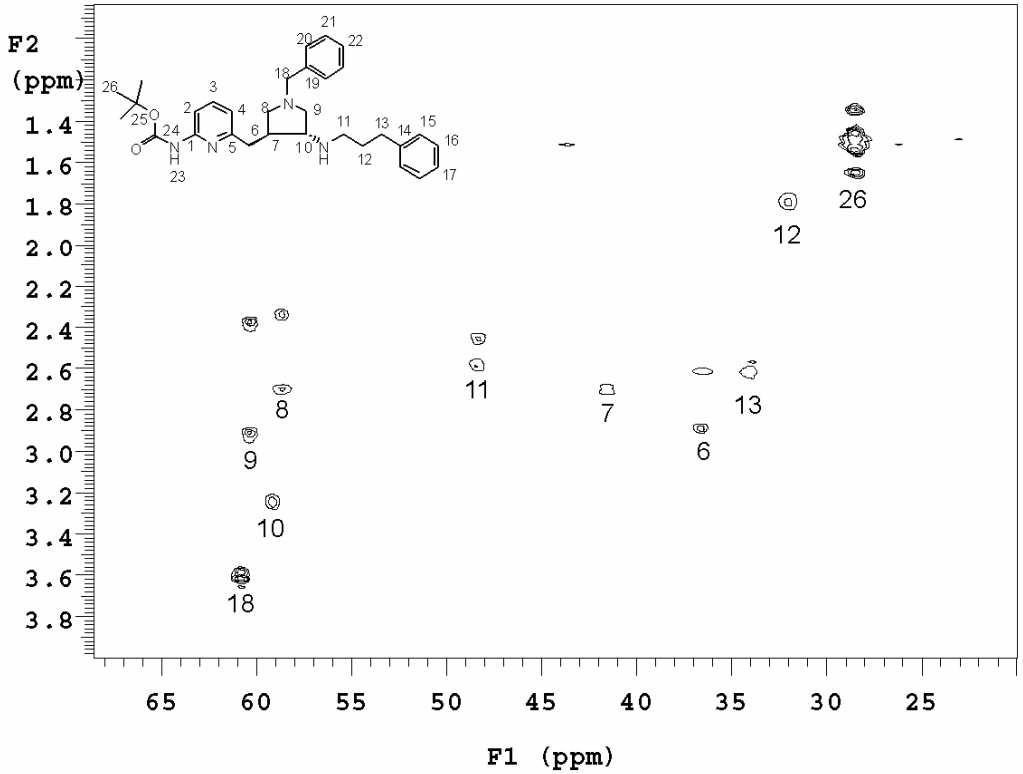
^{13}C NMR-DEPT of **21a**

Supplementary Figure 6 continued



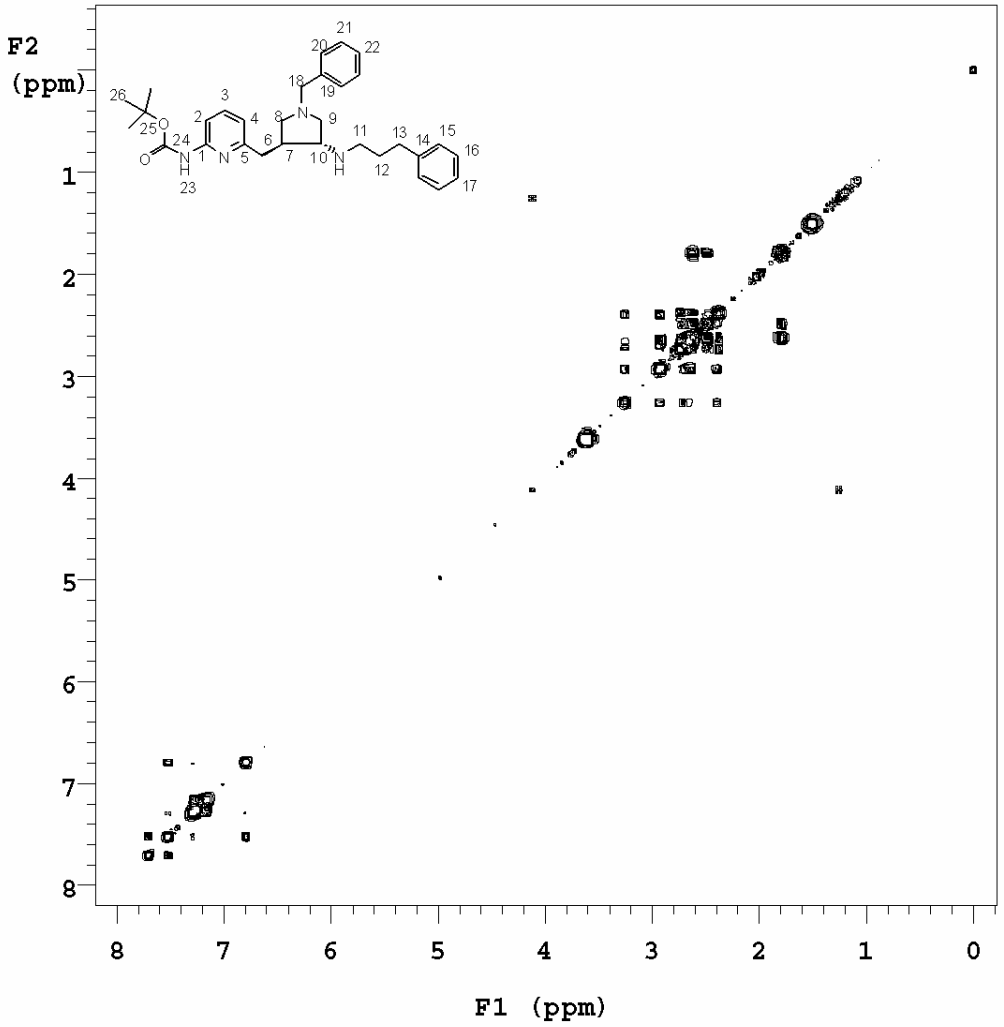
^{1}H-^{13}C HMQC of **21a**

Supplementary Figure 6 continued



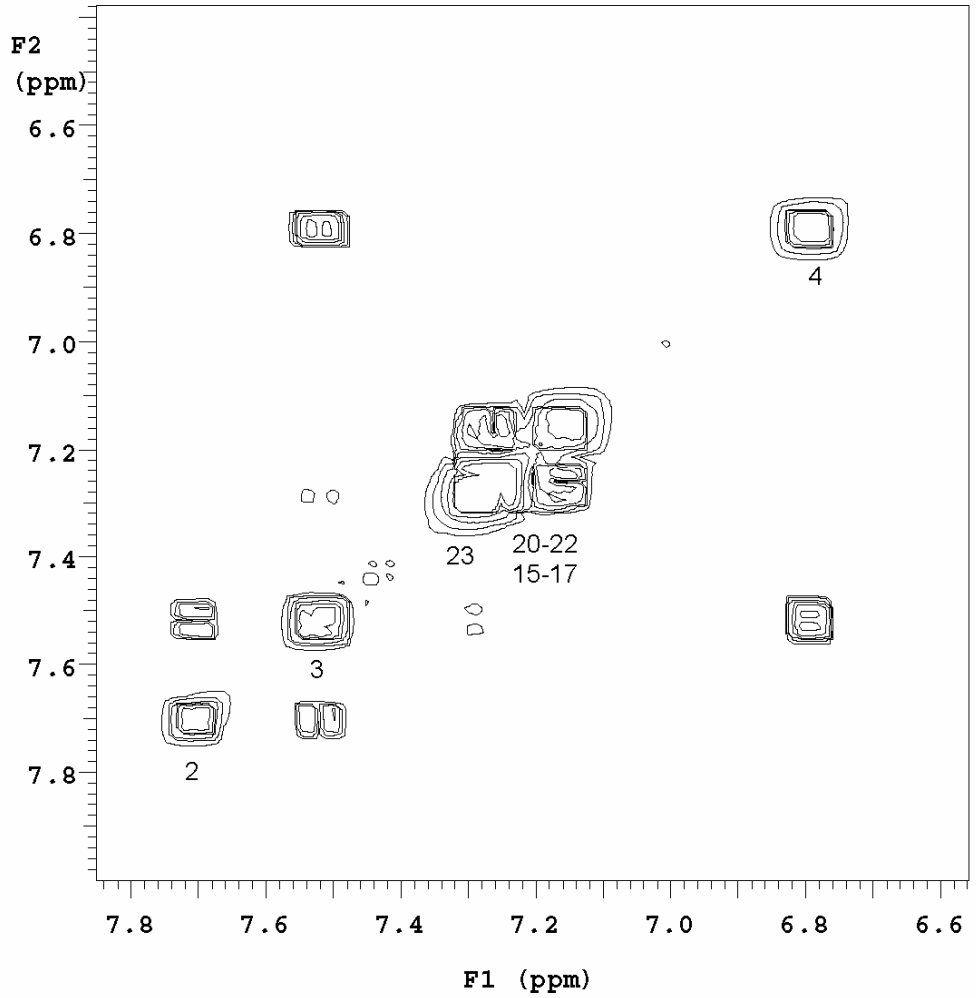
^{1}H-^{13}C HMQC of **21a**

Supplementary Figure 6 continued



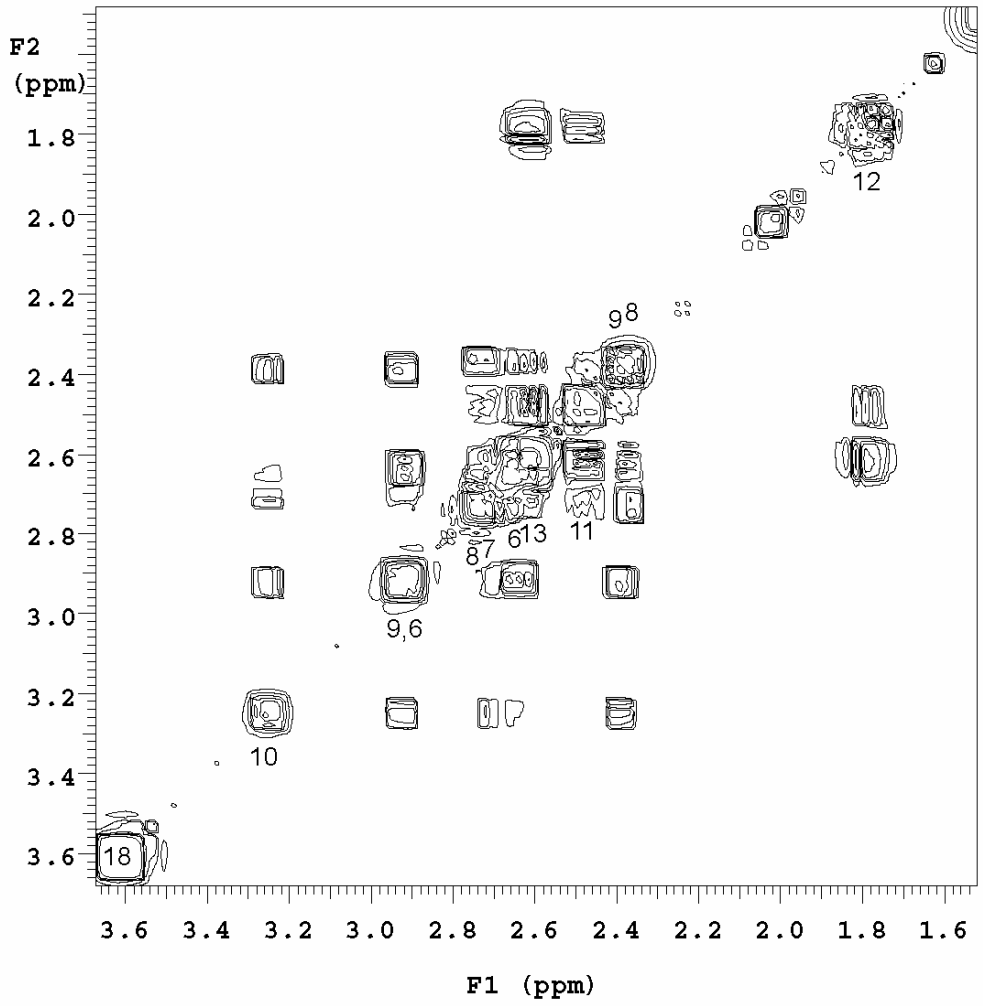
^{1}H-^{1}H COSY of **21a**

Supplementary Figure 6 continued

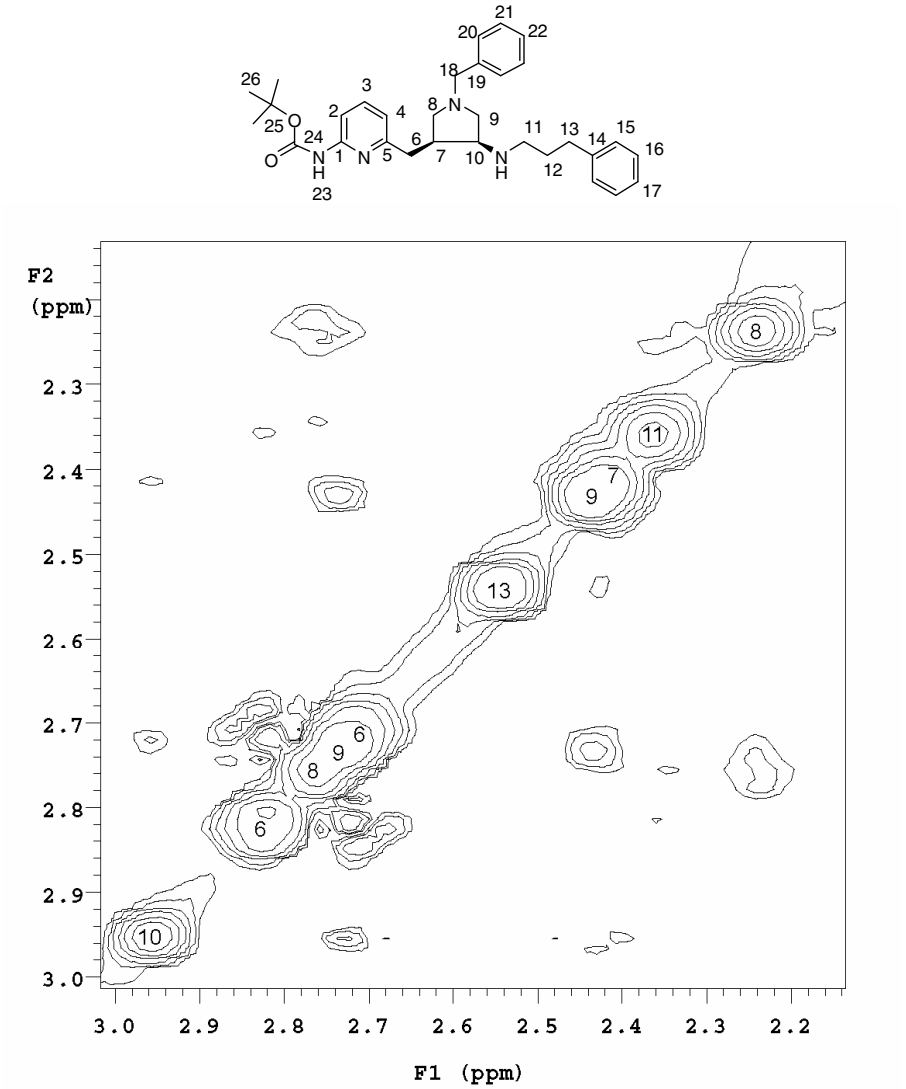


^{1}H-^{1}H COSY of **21a**

Supplementary Figure 6 continued



^{1}H-^{1}H COSY of **21a**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(ppm)
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2.5
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2.8
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3.0
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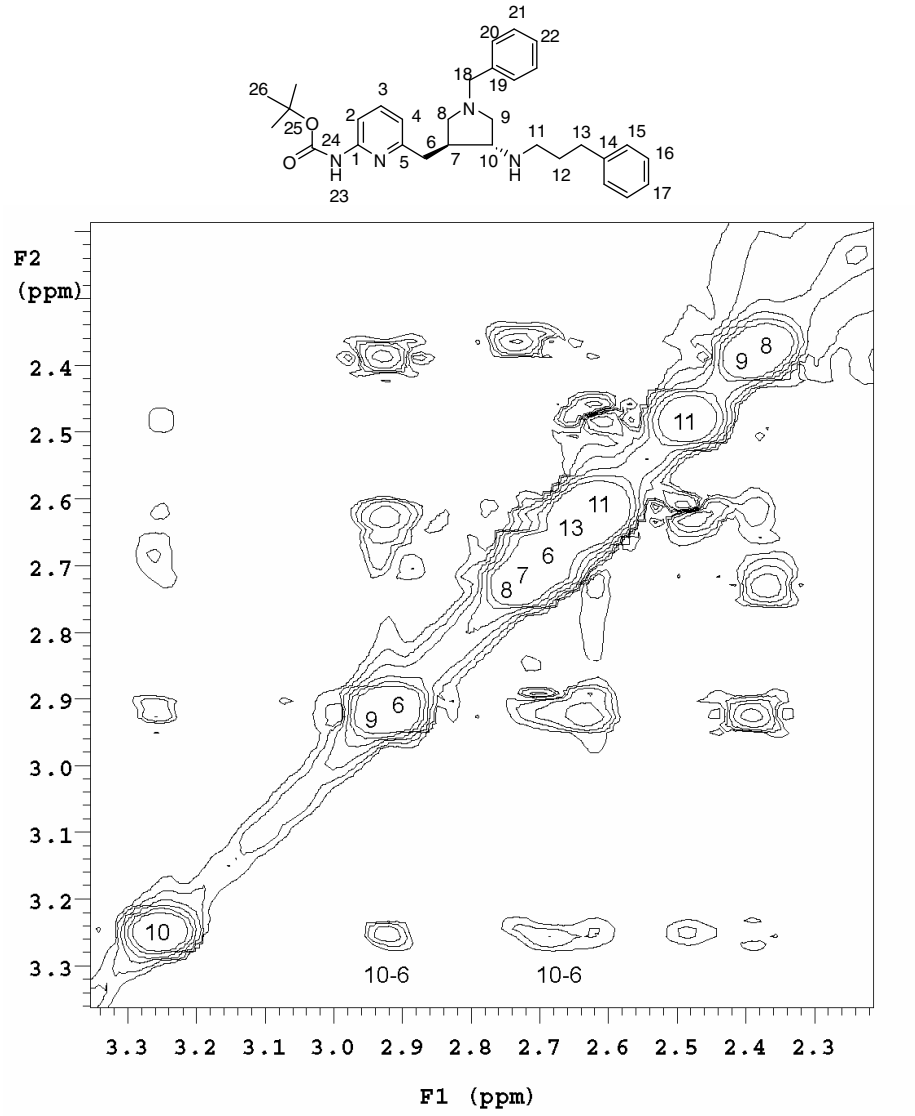
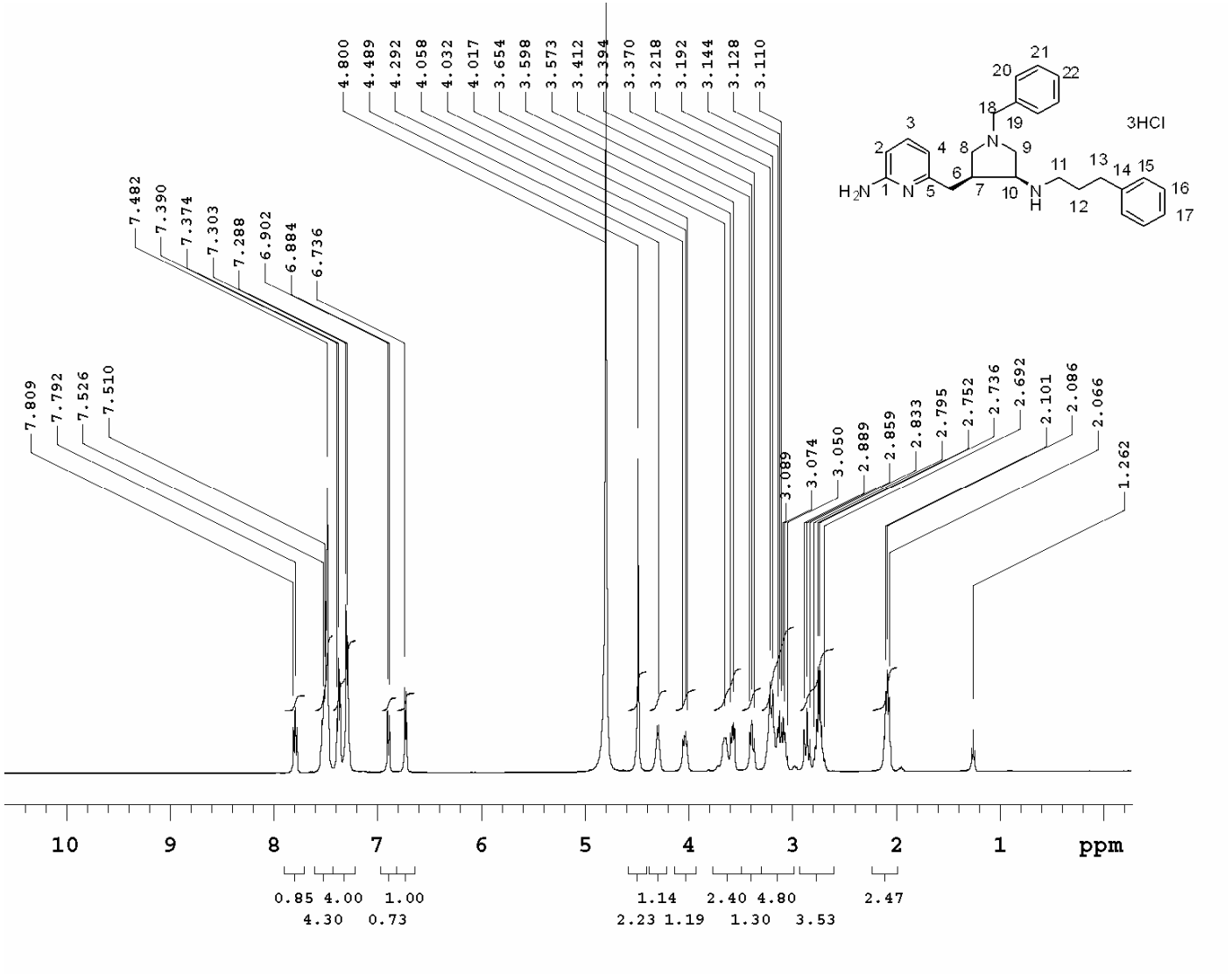
2.7
2.6
2.5
2.4
2.3
2.2
F1 (ppm)
8
11
7
9
13
6
9
8
6
10

A



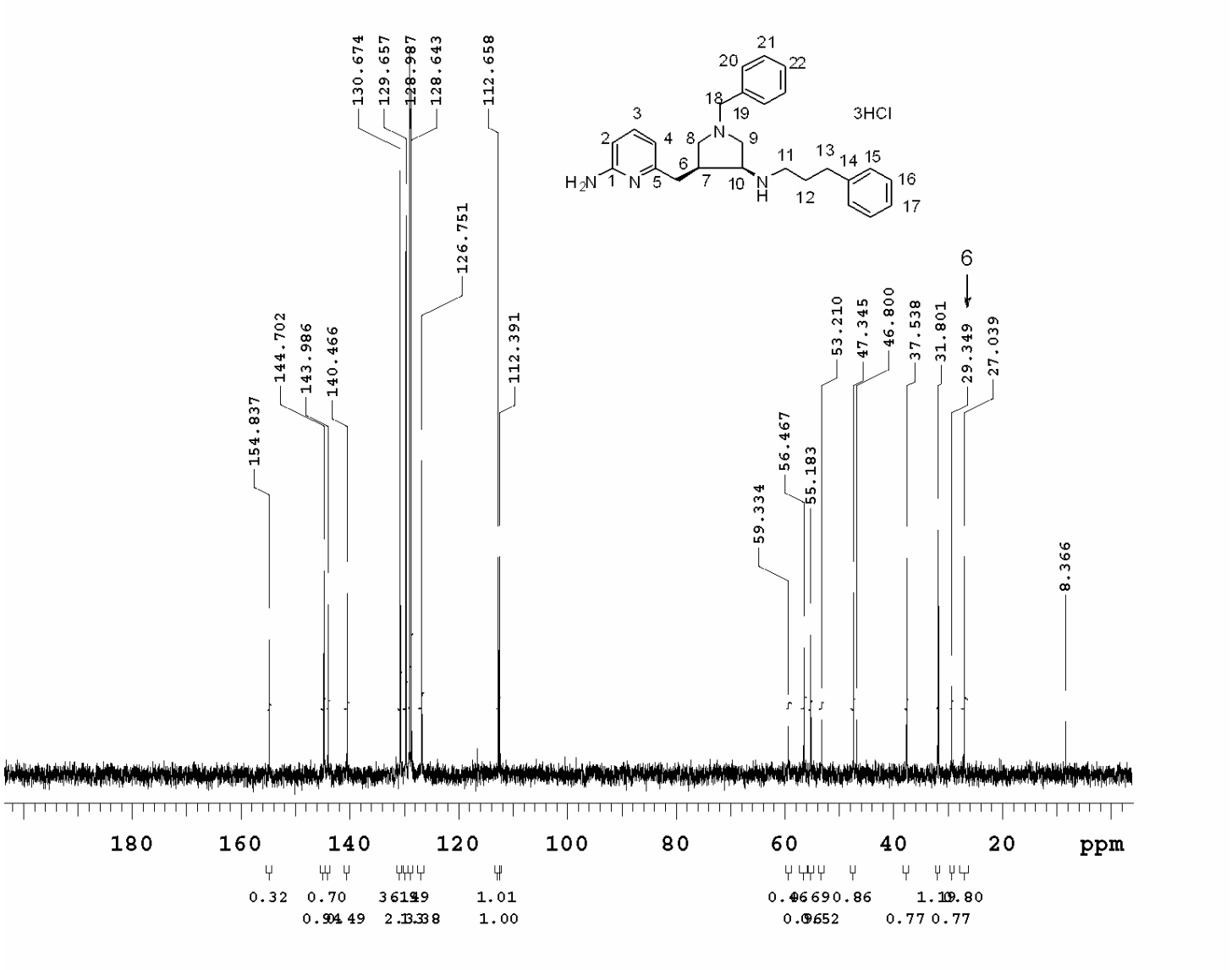
B

Supplementary Figure 7. NOESY spectra discriminating the *cis* isomer (**20a**, panel A) and the *trans* isomer (**21a**, panel B) after the reductive amination reaction between ketone intermediate **19** and 3-phenyl-propylamine.

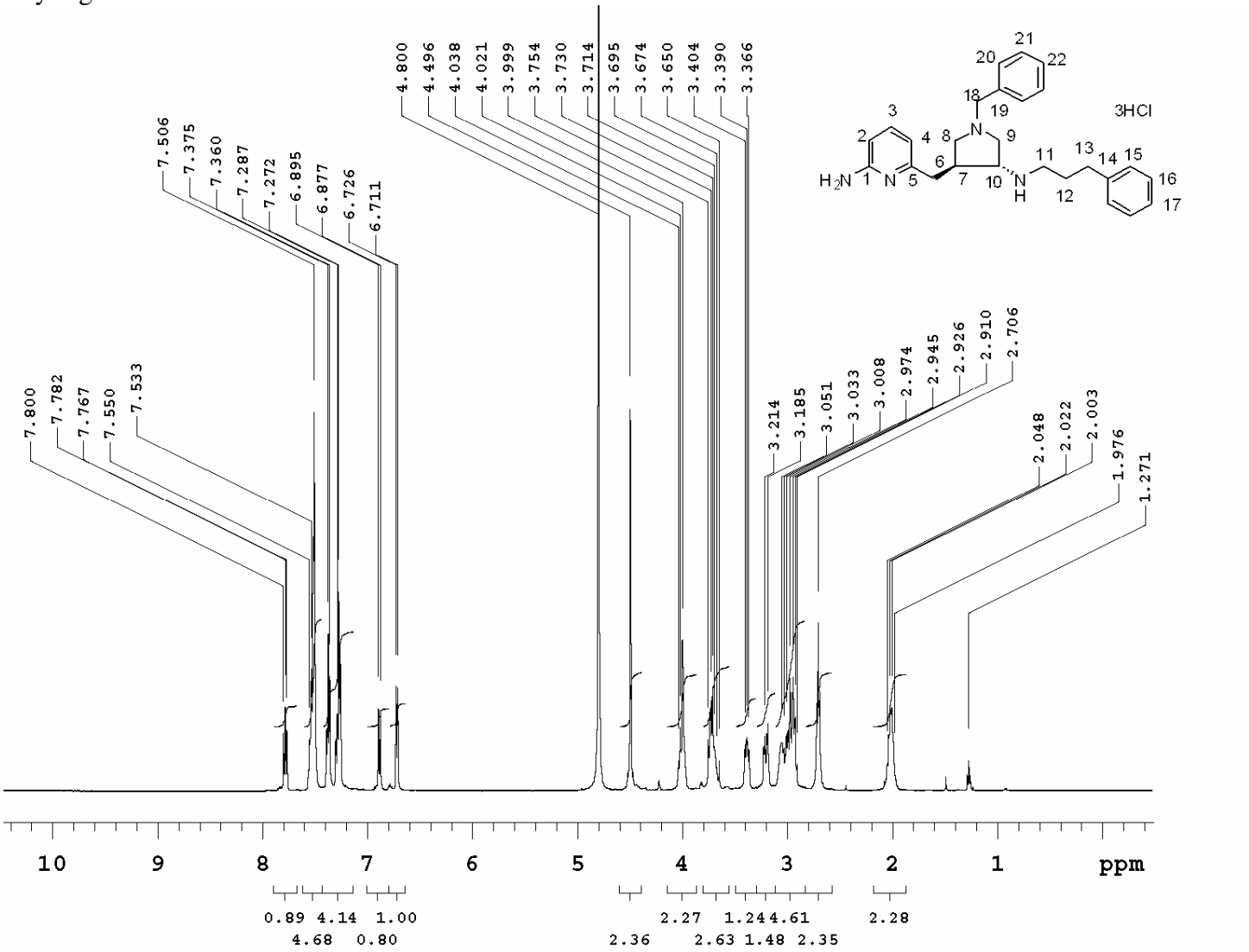


Supplementary Figure 8

Supplementary Figure 8 continued



Supplementary Figure 8 continued



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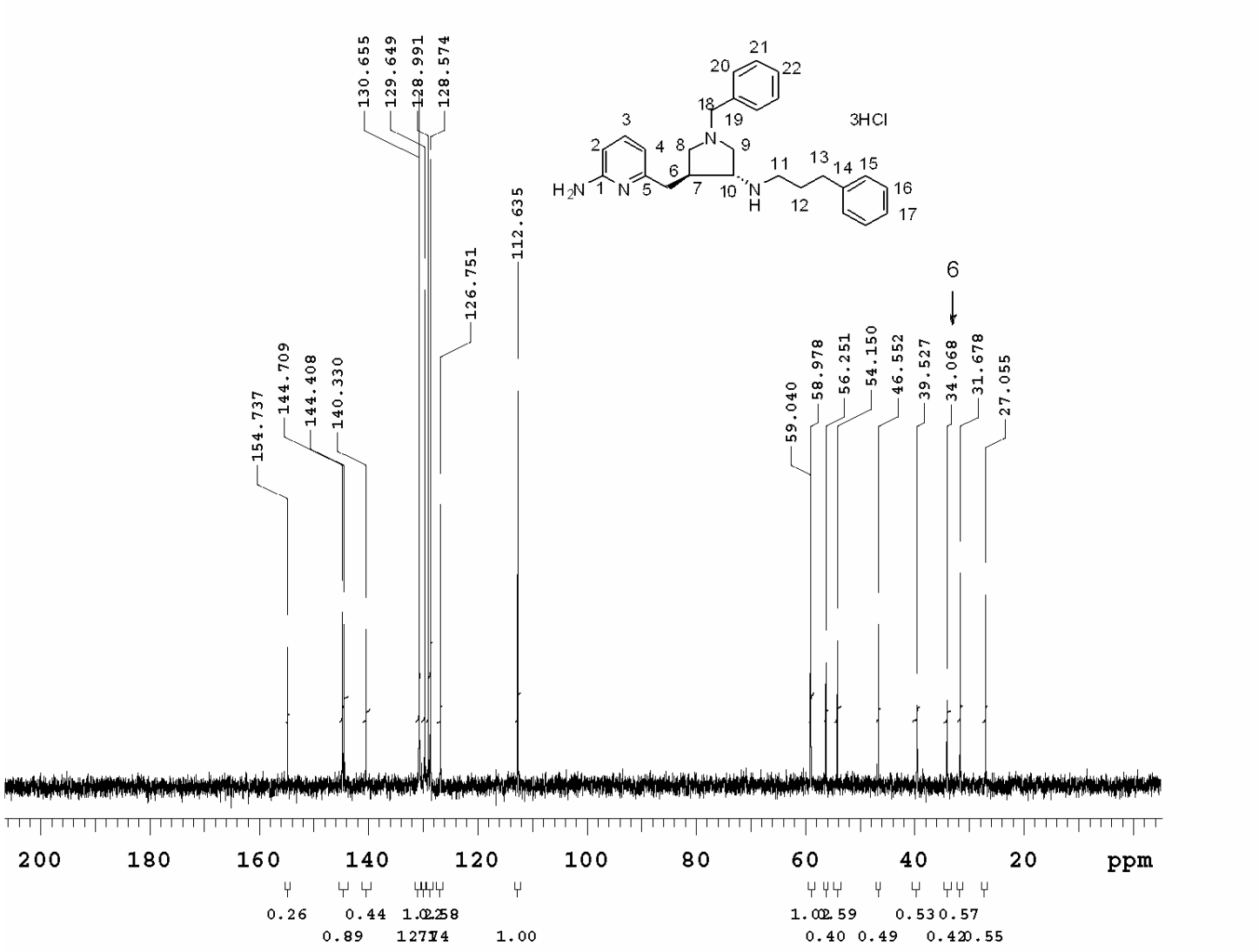
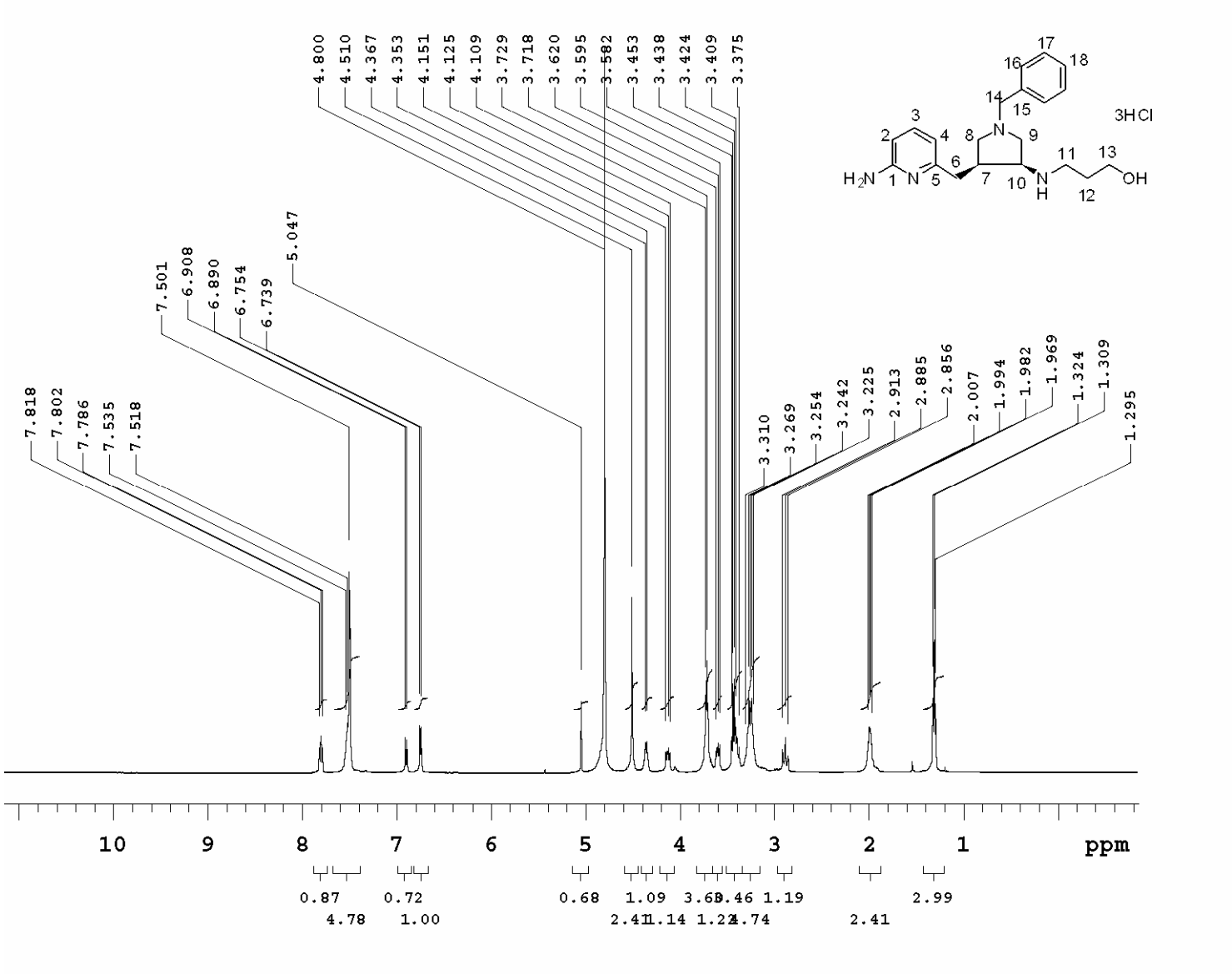


Supplementary Figure 8 continued



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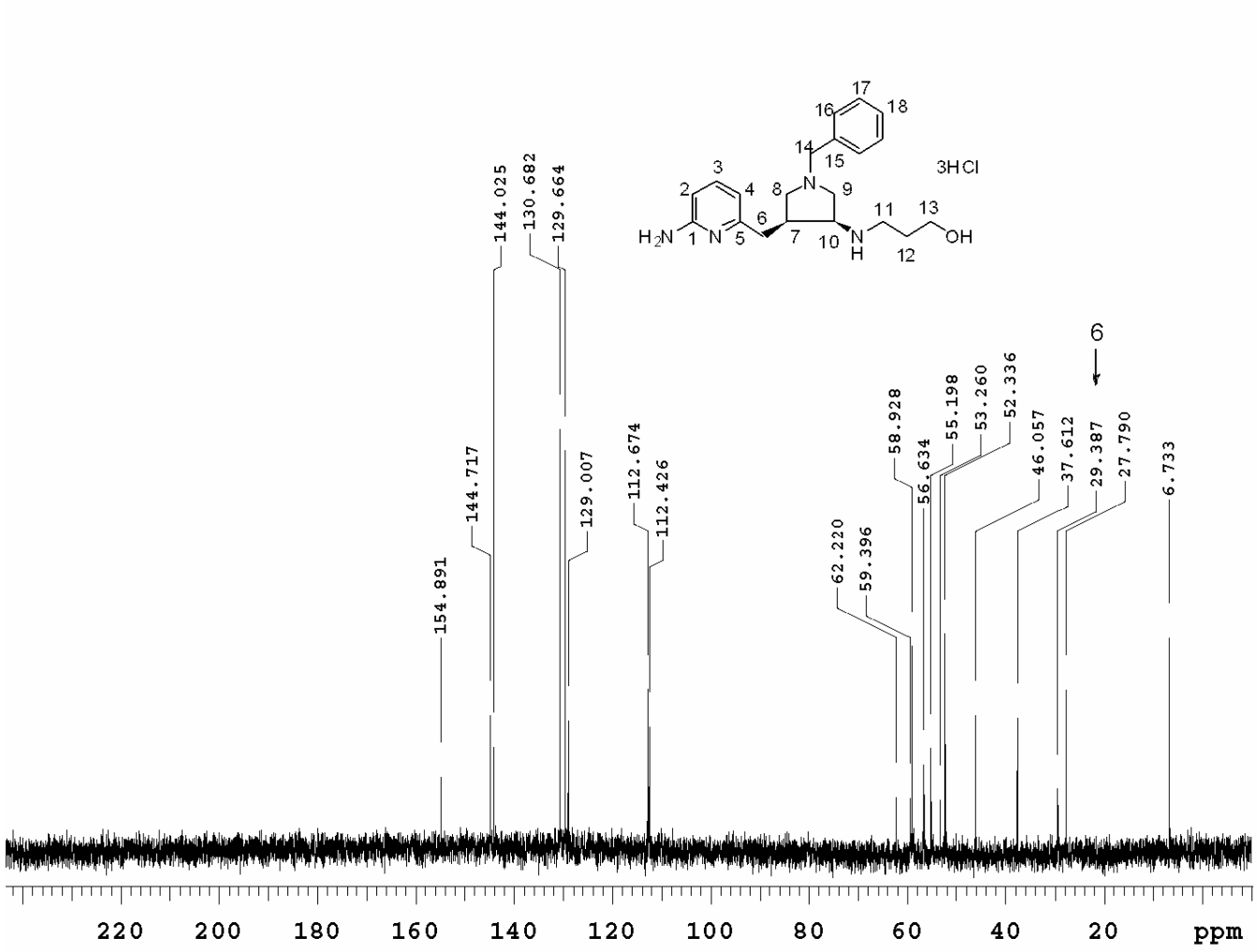

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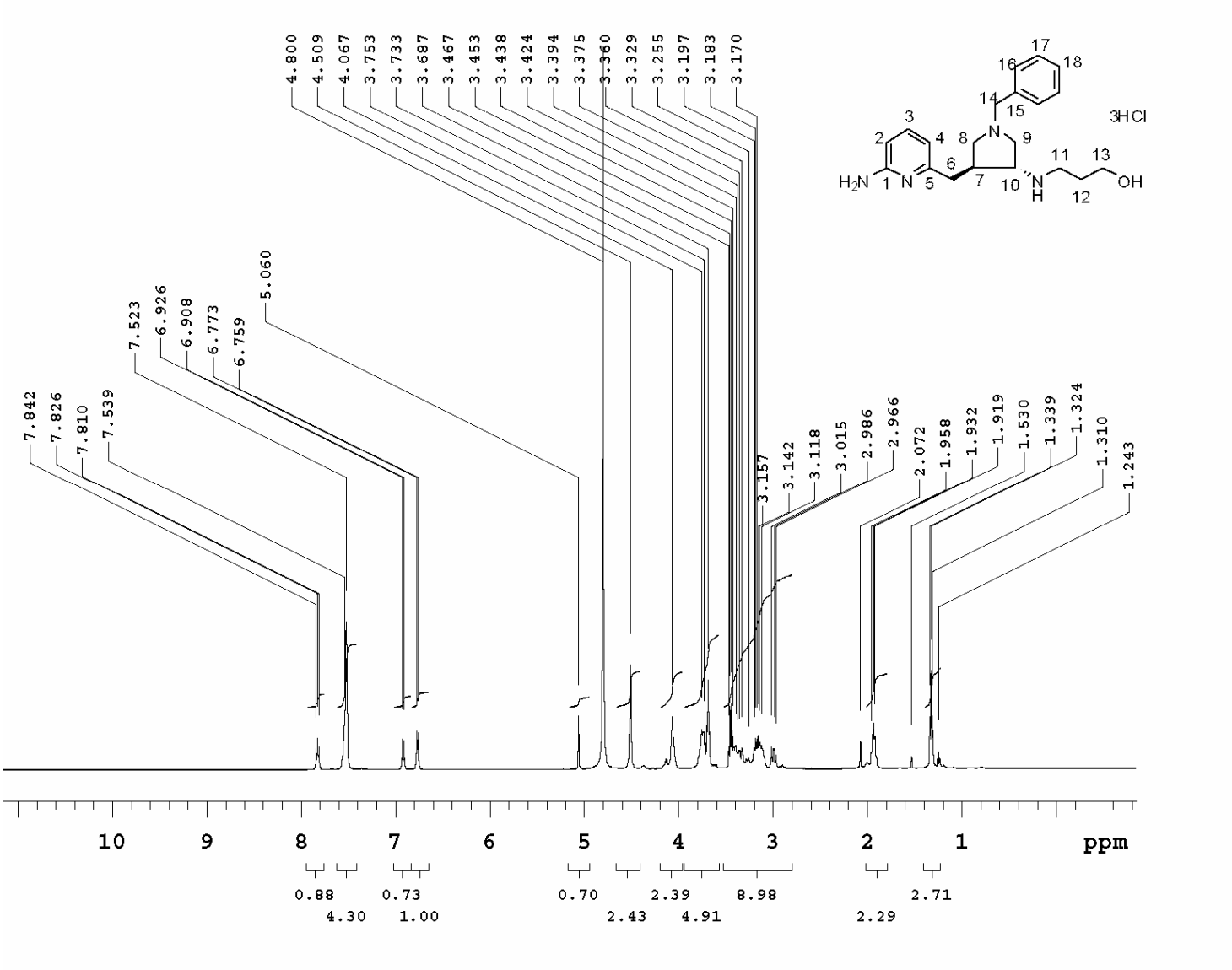

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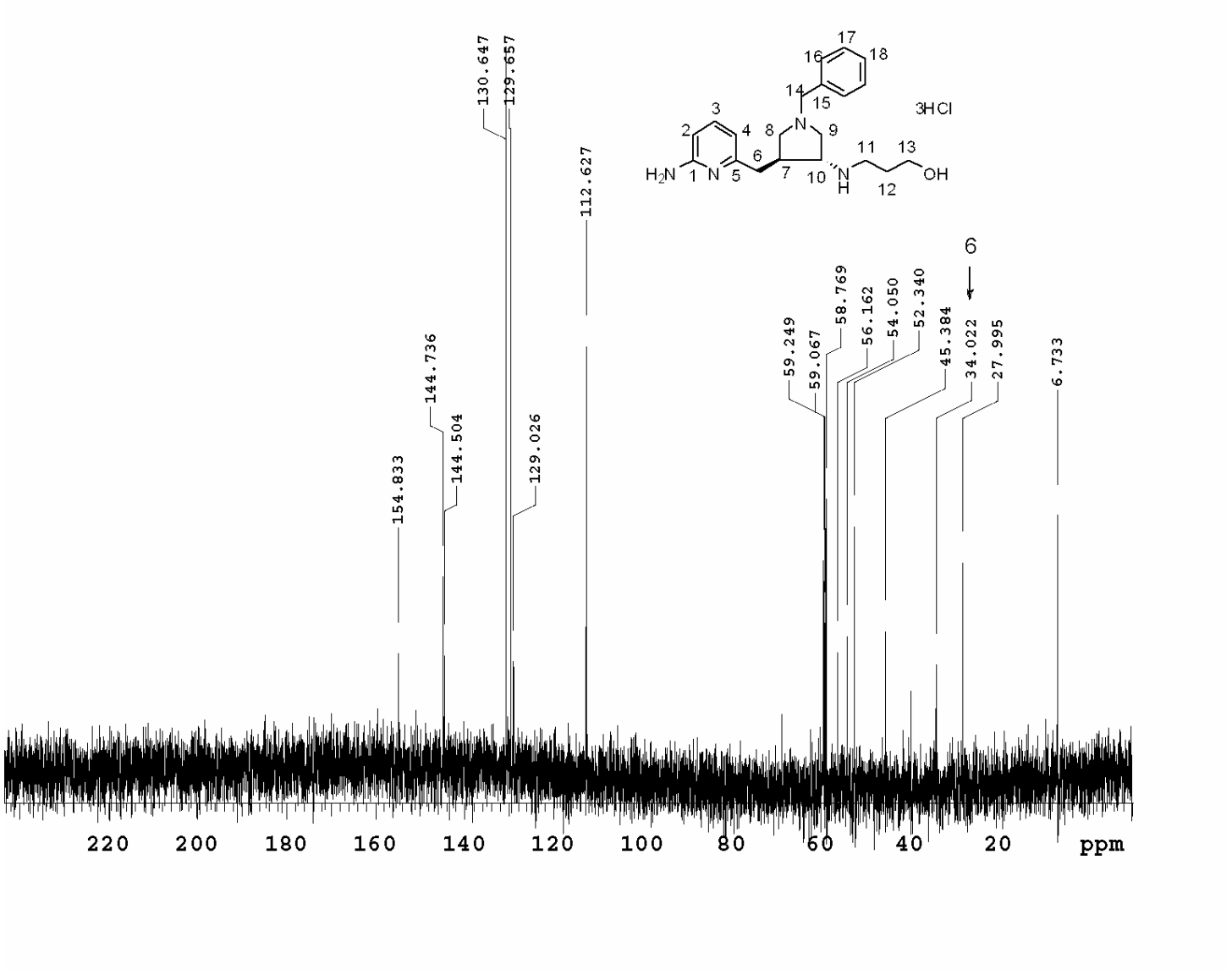

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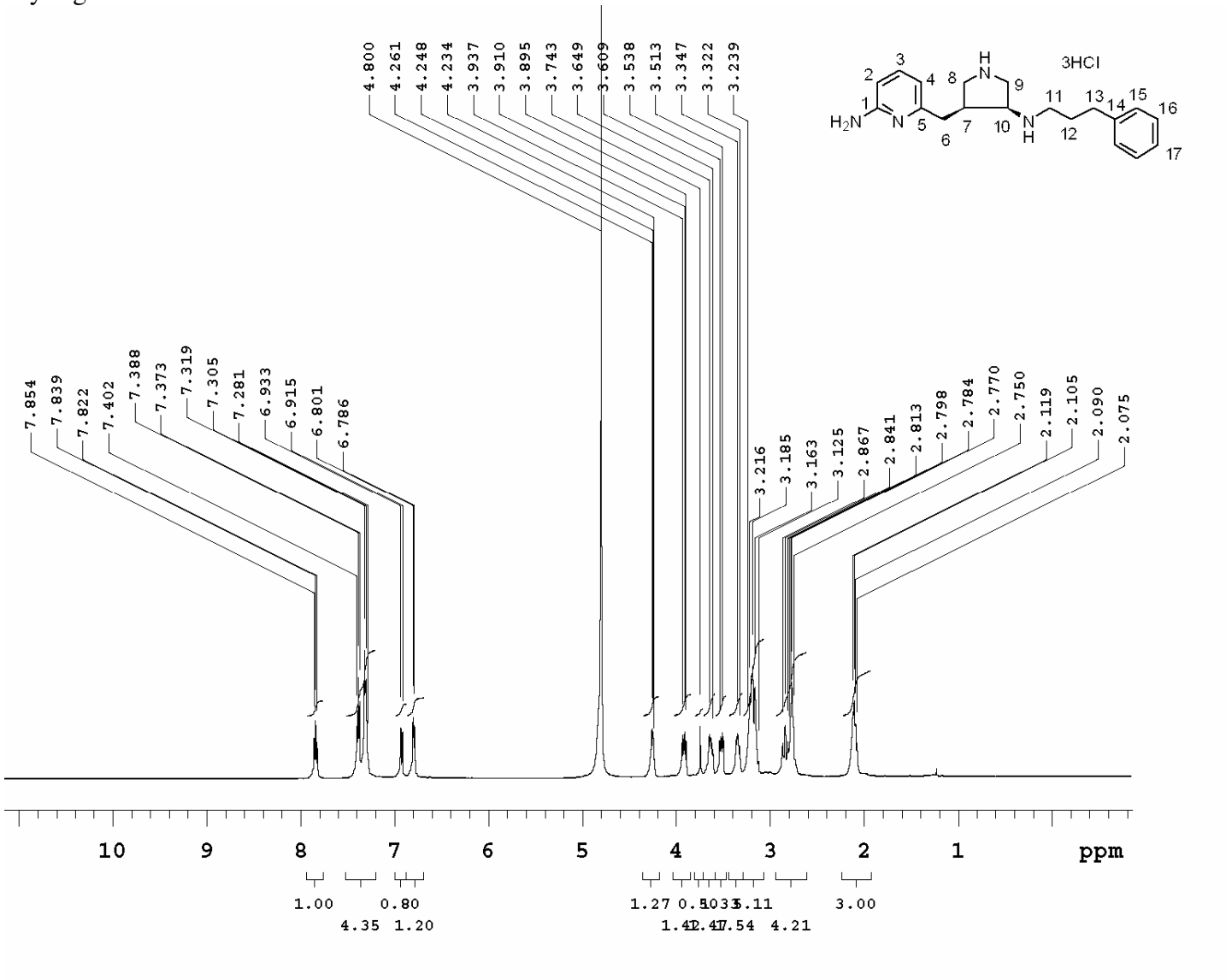

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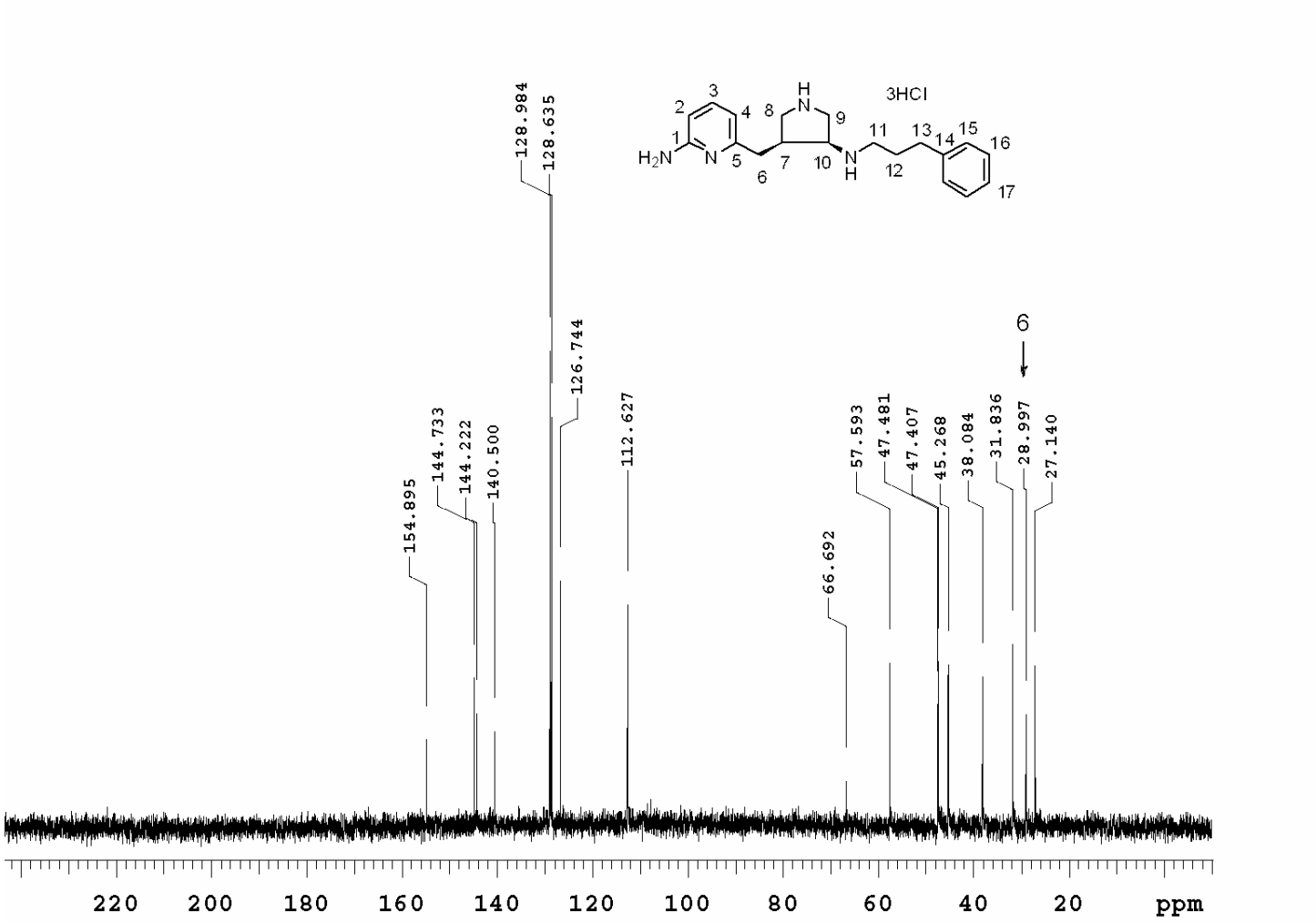

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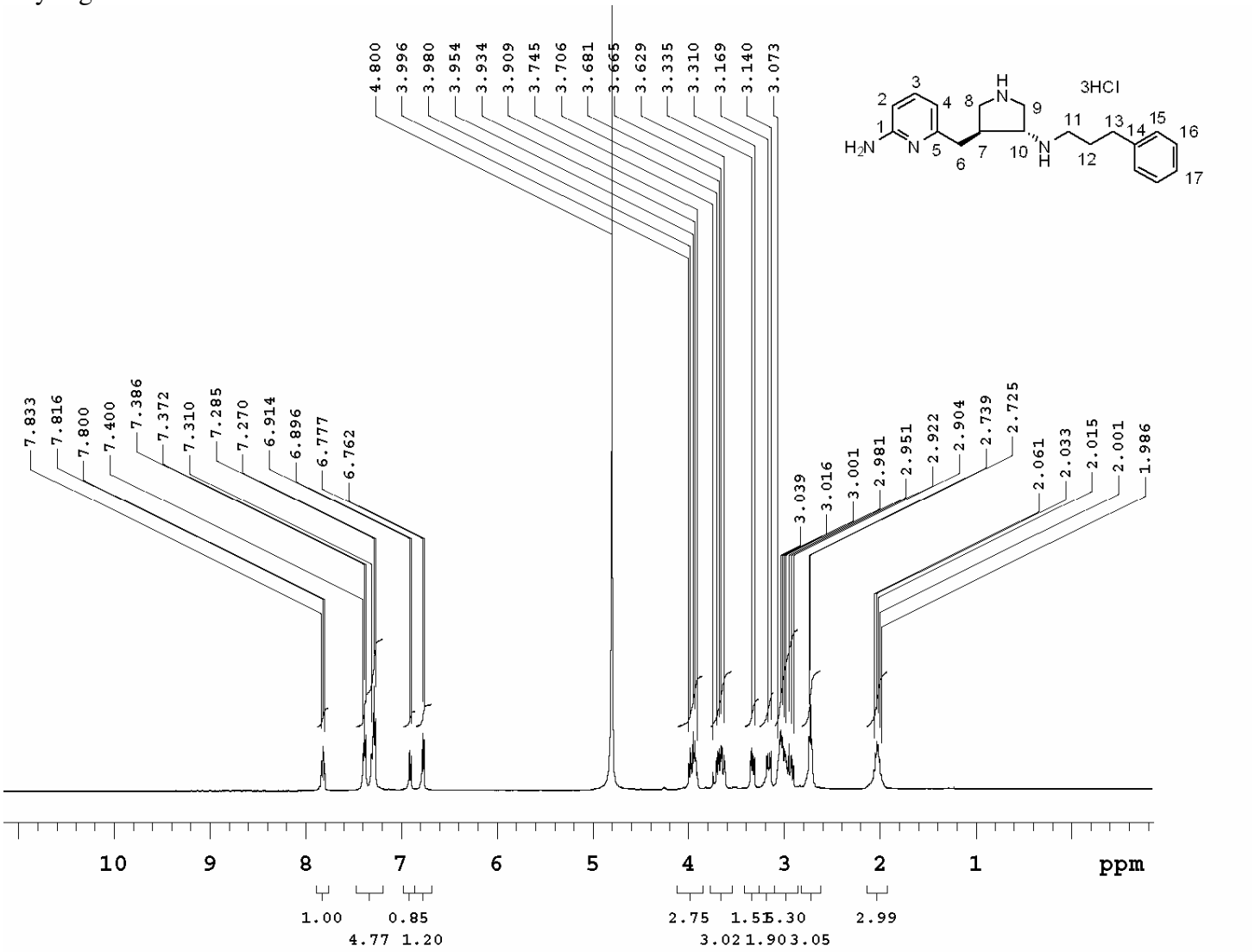

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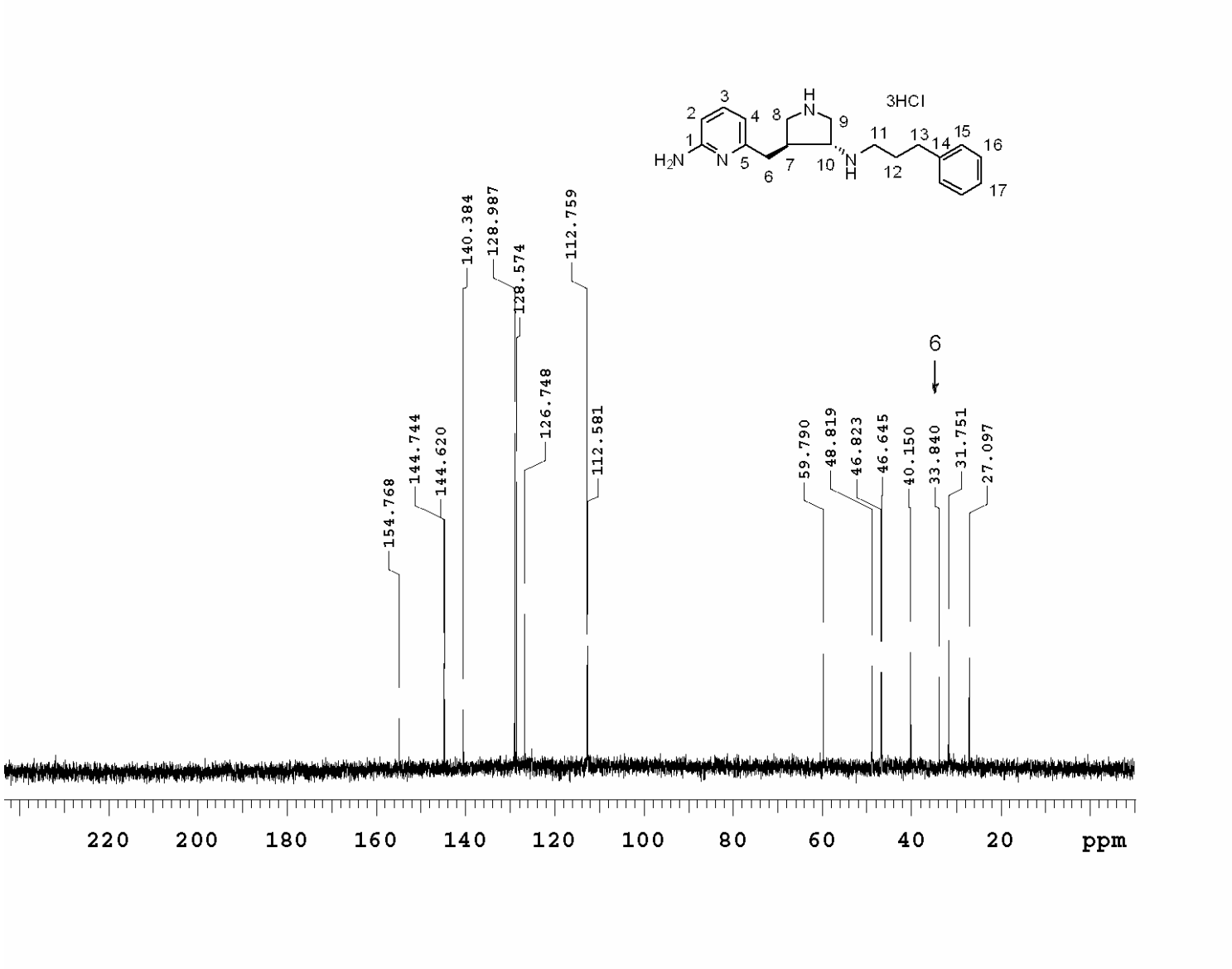

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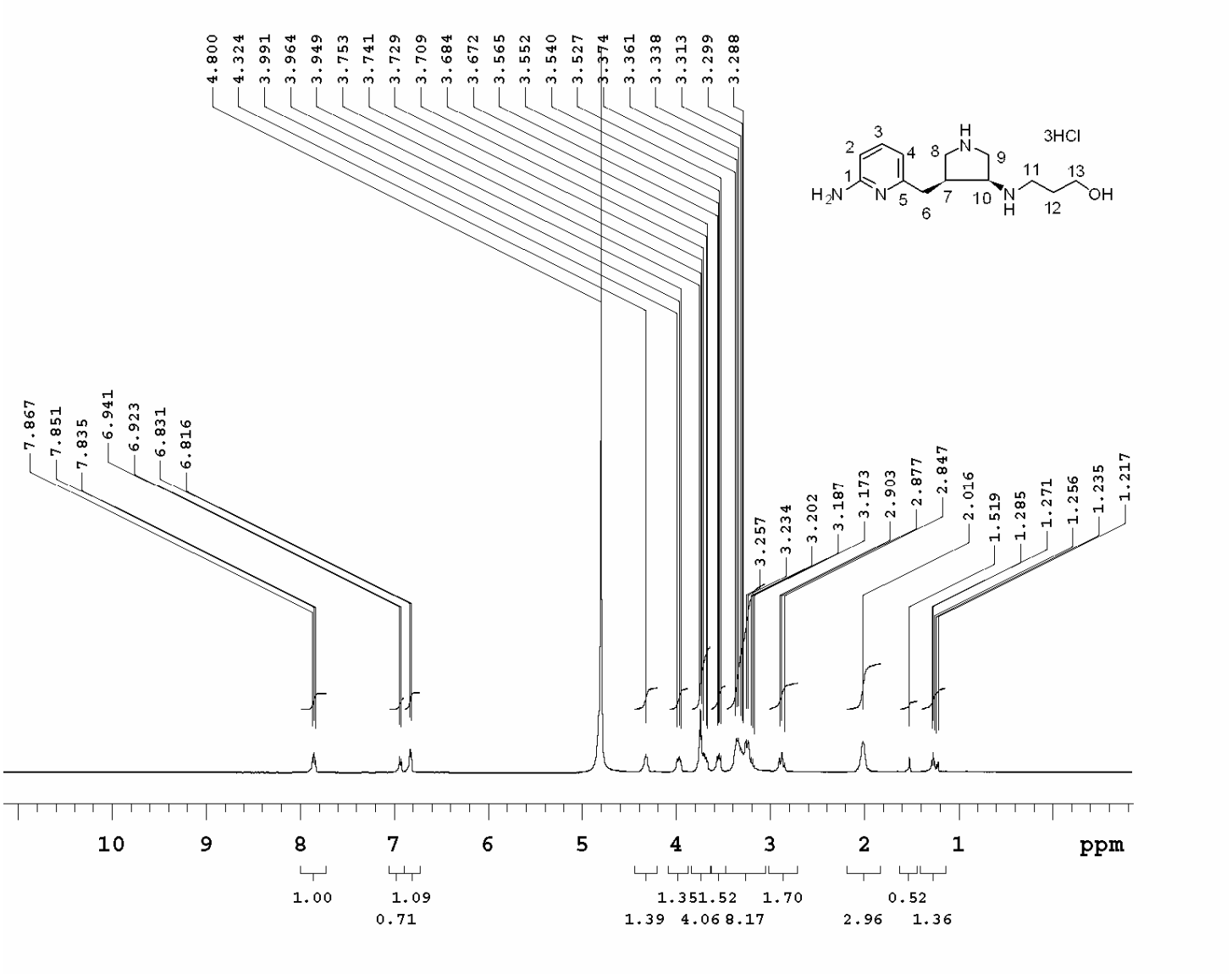

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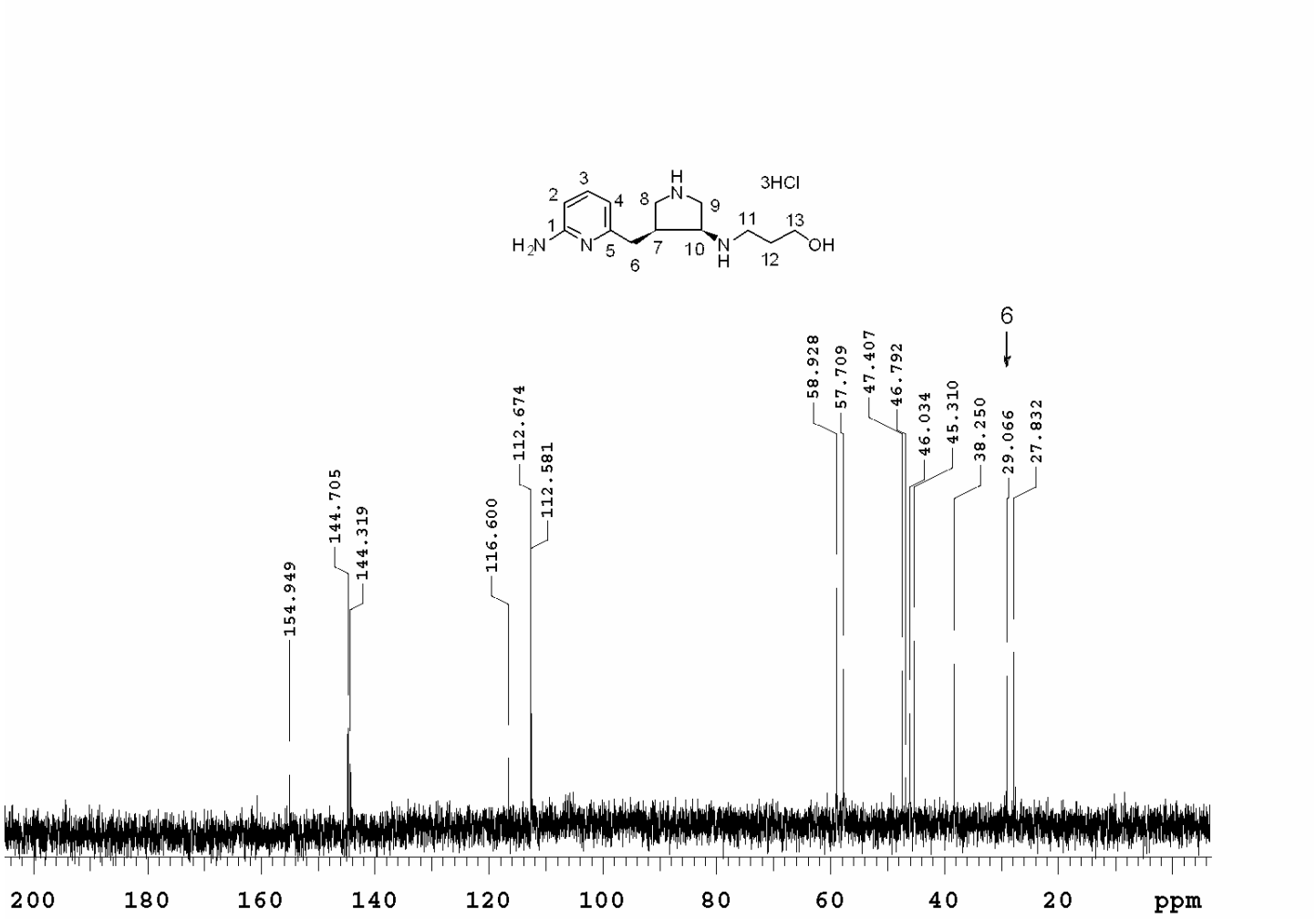

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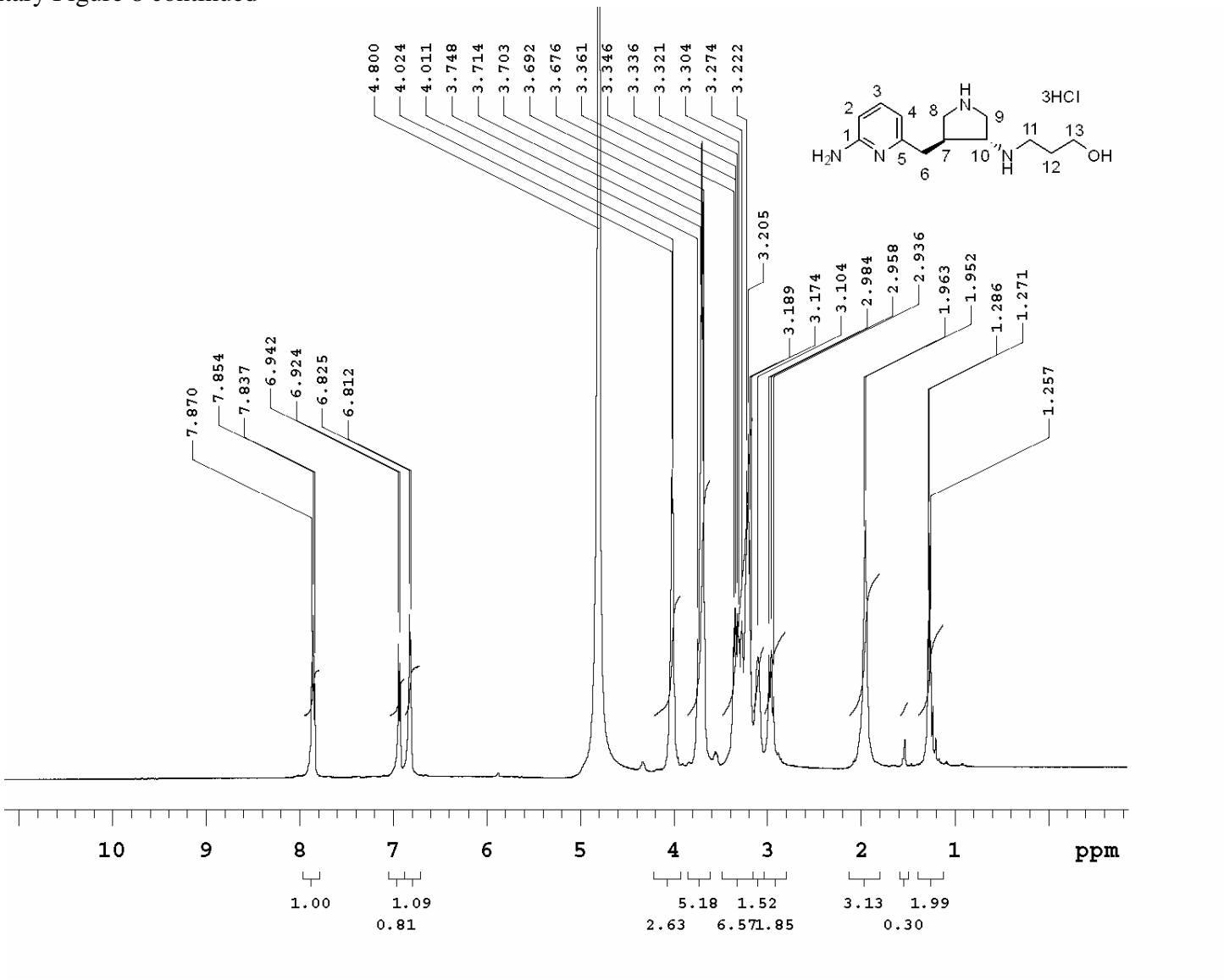
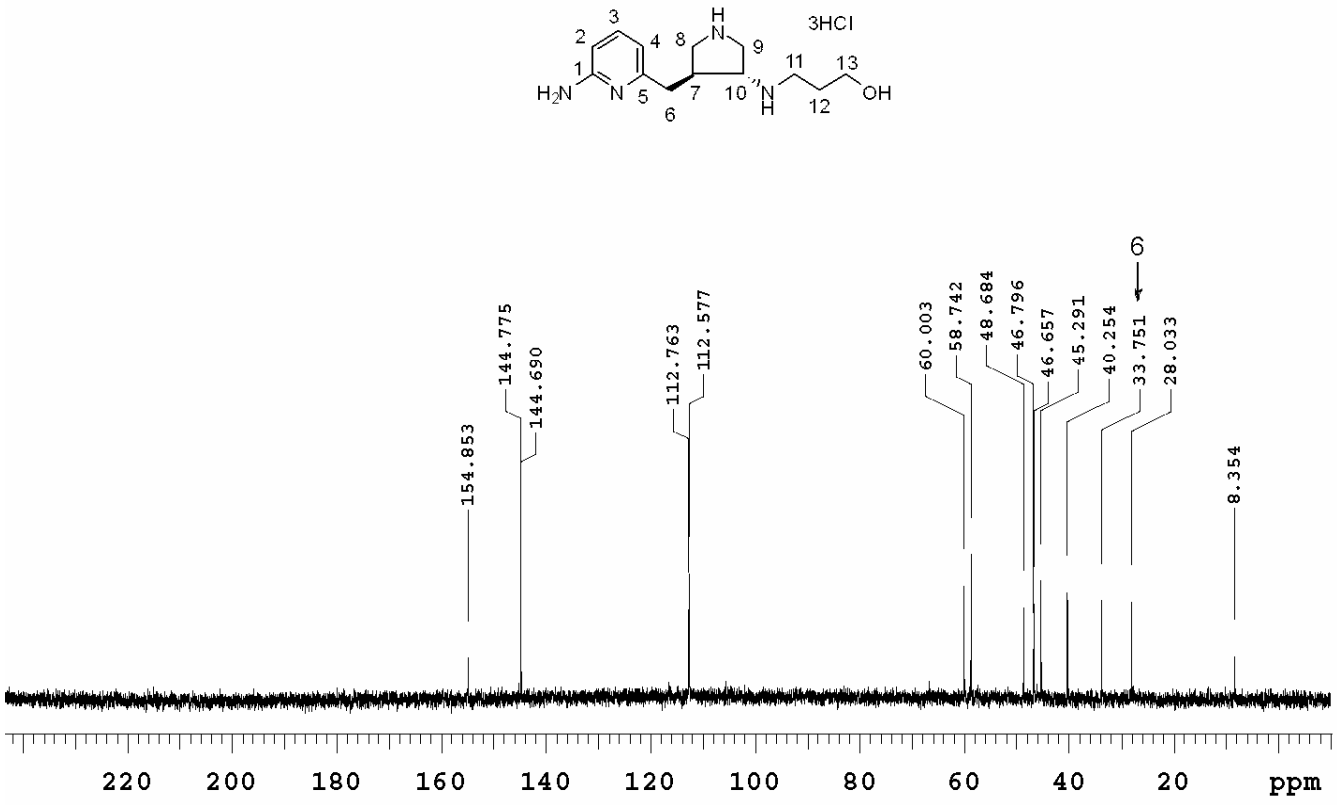
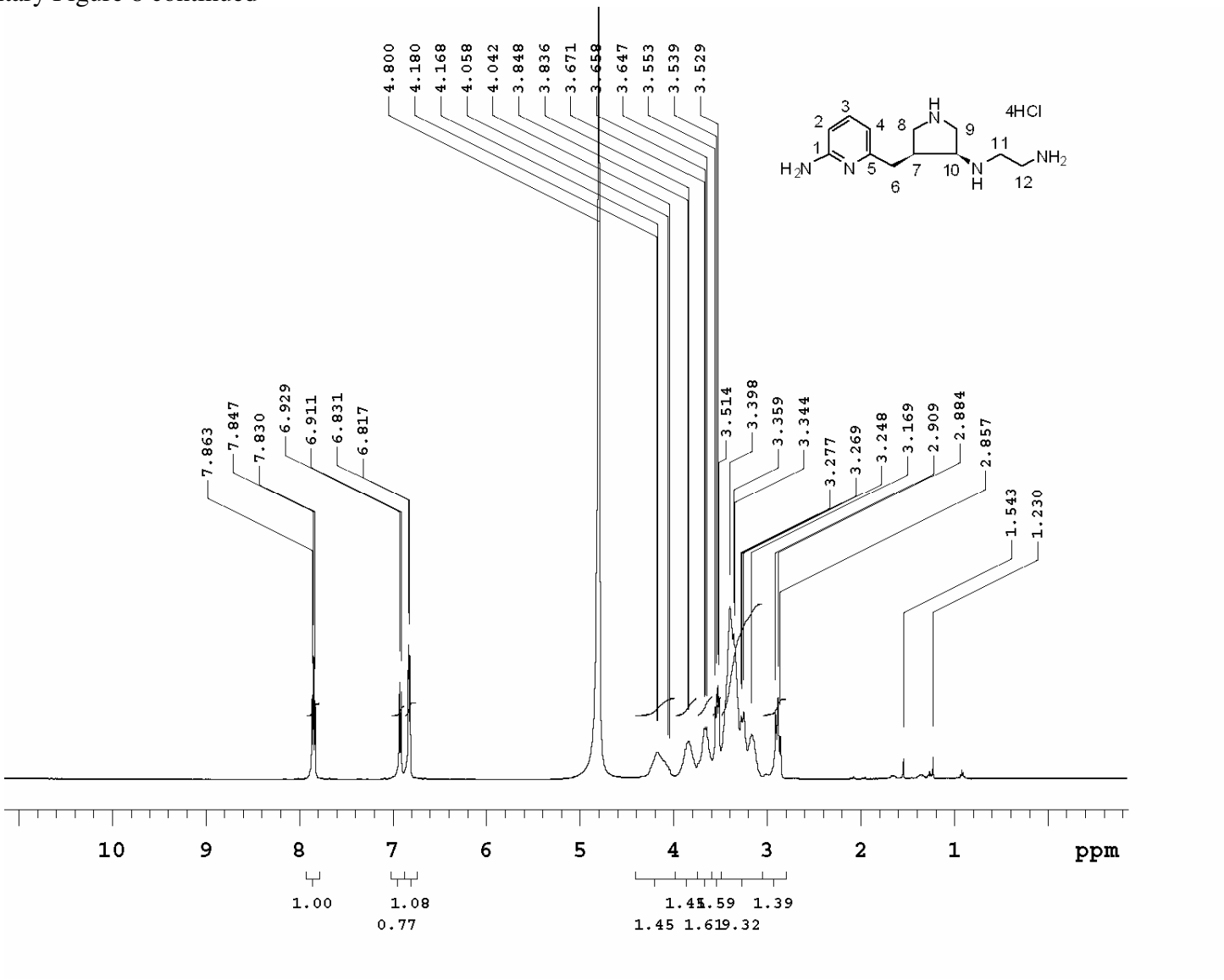


Supplementary Figure 8 continued



Supplementary Figure 8 continued



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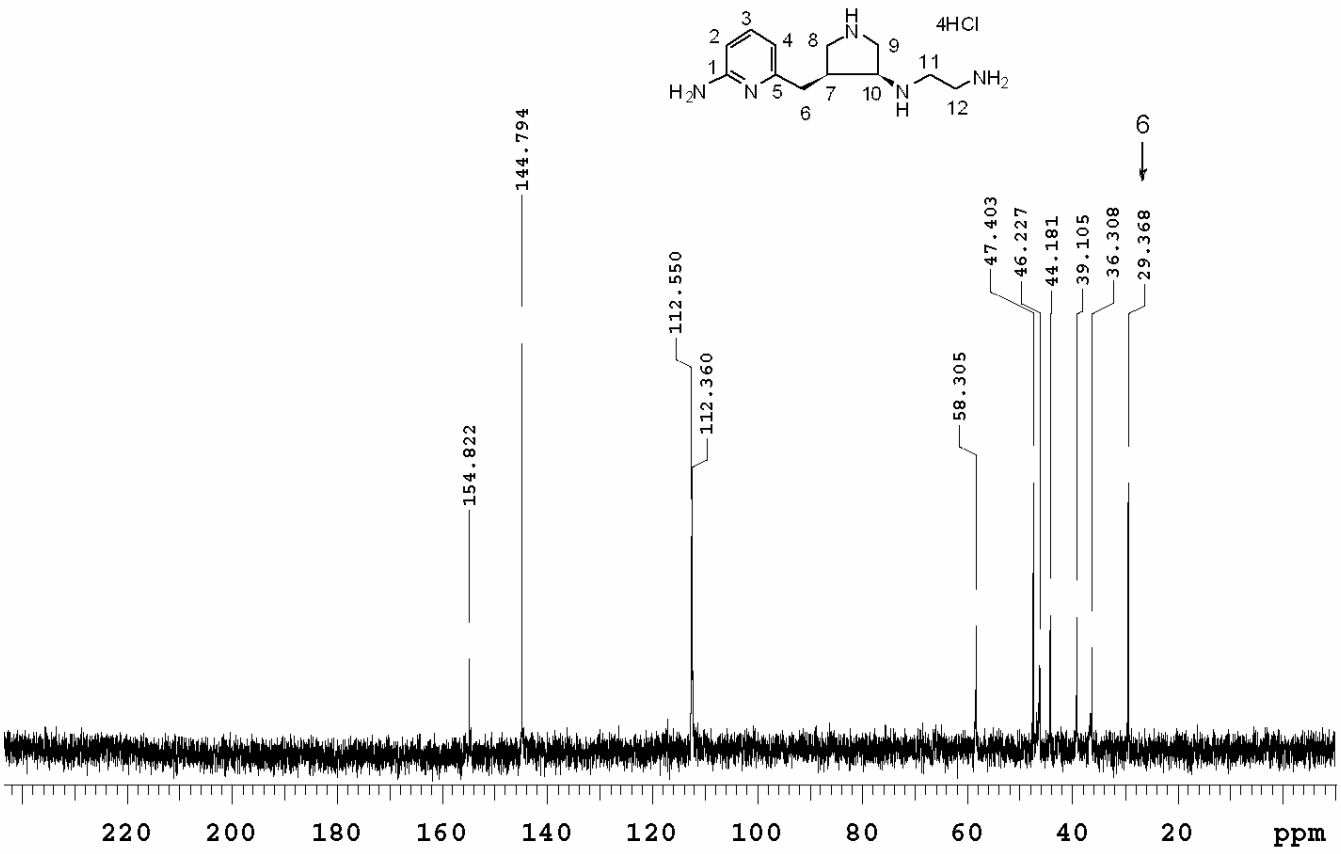

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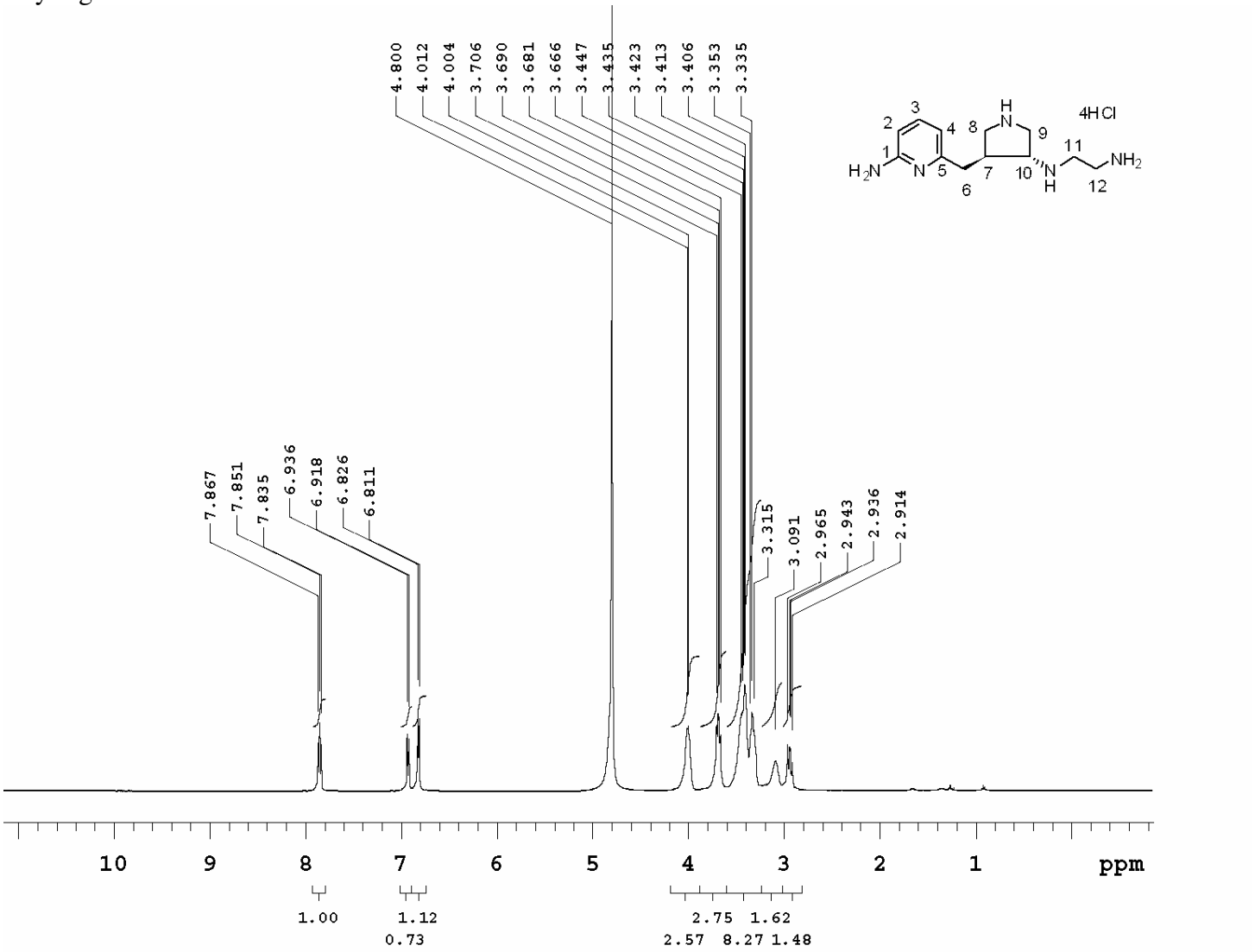

Supplementary Figure 8 continued