Symptomatic Relief of Botulinum Neurotoxin/A Intoxication with Aminopyridines - A New Twist on an Old Molecule

Supporting Information

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CONTENTS

1. Synthetic procedures for compounds **5**-**12**.

Pyridine-3,4,5-triamine (5).

Pyridine-3,4,5-triamine (**5**) was prepared using the reported synthetic procedure.(*1*) The NMR and MS analyses of the product were found to match those reported in the literature.

MS (ESI-TOF): MH^+ 125.0824, calcd MH^+ 125.0822.

***N*^{4}, *N*^{4}-Dimethylpyridine-3,4-diamine (6).**

N,*N*-Dimethyl-3-nitropyridin-4-amine was obtained using the procedure reported by Burton, *et al*.(*2*) The nitro-intermediate (280 mg) was dissolved in methanol (10 mL), and 10% Pt/C (90 mg) was added. The mixture was agitated by shaking under hydrogen atmosphere (35 psi) for 24 h. After the reaction was complete, as determined by TLC, the catalyst was filtered off and the filtrate was concentrated under reduced pressure. Flash chromatography on silica gel (dichloromethane/methanol, 9:1) yielded **6** as brown oil (115 mg, 50%).

MS (ESI-TOF): MH^+ 138.1026, calcd MH^+ 138.1026.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.94 (d, *J* = 5.3 Hz, 1H), 6.77 (d, *J* = 5.3 Hz, 1H), 4.02-3.33 (br.s, 2H), 2.72 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 147.3, 141.3, 137.3, 136.6, 113.2, 41.9.

5-Azabenzimidazole (**7)** was used as commercially available (Aldrich).

4-Amino-3-(dimethylamino)pyridine 1-oxide (8).

3-Bromo-4-nitropyridine 1-oxide (300 mg) was dissolved in THF, and dimethylamine (2M solution in methanol, 4 mL) was added. Stirring was continued for 14 h, after which the volatile components were evaporated. The residue was partitioned between saturated aqueous $NaHCO_3$ and dichloromethane.

Extraction was repeated thrice, the combined extracts were dried over anhydrous Na_2SO_4, and concentrated under vacuum. The crude residue was dissolved in methanol (3 mL), and 10% Pd/C catalyst (20 mg) was added. The mixture was stirred under atmosphere of hydrogen (1 atm) for 14 h. After the reaction was complete, as determined by TLC, the catalyst was filtered off and the filtrate was concentrated under reduced pressure. Flash chromatography on silica gel (dichloromethane/methanol, 9:1) yielded **8** as amber solid (44 mg, 21%).

MS (ESI-TOF): MH^+ 154.0974, calcd MH^+ 154.0975.

^{1}H NMR (400 MHz, CD_3OD): δ 7.73 (s, 1H), 7.72 (d, *J* = 2.1 Hz, 1H), 6.69 (d, *J* = 7.0 Hz, 1H), 2.69 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 148.0, 138.6, 135.6, 131.9, 109.8, 42.9.

1*H*-Imidazo[4,5-c]pyridine 5-oxide (9).

A solution of methyltrioxorhenium (5 mg, 0.02 mol) and 30% aqueous H_2O_2 in methanol(*3*) (6 mL) was stirred at ambient temperature (15 min). 1*H*-Imidazo[4,5-*c*]pyridine solution in methanol (4 mL) was added dropwise to the oxidant while stirring. The reaction mixture was allowed to stir for 6 h, while monitoring the progress by TLC. Upon completion of the reaction, the volatile components were evaporated under vacuum, the residue was dissolved in ethyl acetate and passed through a plug of silica gel. The filtrate was concentrated in vacuo to yield **9** (130 mg, 96%).

MS (ESI-TOF): MH^+ 136.0507, calcd MH^+ 136.0505. The NMR analyses of the product were found to match those reported in the literature.(*4*)

1,2,3,4-Tetrahydropyrido[4,3-b]pyrazine (10) was prepared using the reported synthetic procedure.(*5*) The NMR and MS analyses of the product were found to match those reported in the literature.

MS (ESI-TOF): MH^+ 136.0872, calcd MH^+ 136.0869.

3,4-Diamino-1-(prop-2-ynyl)pyridinium bromide (11).

Propargyl bromide (599 mg, 80% solution in toluene, 5.04 mmol) was added to a solution of 3,4-diaminopyridine (550 mg, 5.04 mmol) in a mixture of anhydrous DMF (1.35 mL) and acetonitrile (1.35 mL), and the reaction mixture was stirred at ambient temperature for 6 h. Another equivalent of propargyl bromide was added and stirring was continued for 16 h. Ethyl ether (10 mL) was slowly added while stirring, the resulting white precipitate was filtered off and washed generously with ether. The product was dried under vacuum (yield 1.08 g, 94%).

MS (ESI-TOF): M^+ 148.0871, calcd M^+ 148.0869.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (dd, J = 1.8, 6.8 Hz, 1H), 7.76-7.21 (br.s, 2H), 7.68 (d, J = 1.8 Hz, 1H), 6.78 (d, J = 6.8 Hz, 1H), 5.70 (s, 2H), 5.13 (d, J = 2.5 Hz, 2H), 3.83 (t, J = 2.5 Hz, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 148. 7, 134.5, 133.3, 123.3, 107.5, 79.9, 77.7, 47.0.

***N*,*N*,*N*-Triethyl-3-phenylpropan-1-aminium bromide (12).**

3-Phenylpropyl bromide (100 mg) was added slowly to a solution of triethylamine (1.27 mL) in acetonitrile (12 mL) and the mixture was stirred for 4 h. The volatile components were evaporated under reduced pressure and the residue was triturated with diethyl ether to yield **12** as amber solid (100 mg, 66%).

MS (ESI-TOF): M^+ 220.2061, calcd M^+ 220.2065.

^{1}H NMR (400 MHz, D_2O): δ 7.44 (m, 2H), 7.35 (m, 3H), 3.24 (dq, J = 1.2, 7.2 Hz, 6H), 3.13 (m, 2H), 2.75 (t, J = 7.0 Hz, 2H), 2.02 (m, 2H), 1.20 (m, 9H).

^{13}C NMR (100 MHz, CD_3OD): δ 141.4, 129.8, 129.7, 127.7, 57.3, 57. 3, 53.9, 53.9, 53.9, 33.1, 24.5, 7.7.

2. Computational procedures for pK_a estimate calculations.

Molecular modeling experiments employed MacroModel v. 9.1 and Jaguar v.7 equipped with Maestro v. 7.5 graphical interface (Schrödinger, LLC, New York, NY, 2005) installed on a Linux Red Hat 9.0 system. Compound structures were built with standard bond lengths and angles using the Maestro graphical interface, and were subsequently minimized using the OPLS_2005 force field and the Polak-Ribier conjugate gradient (PRCG). Optimizations were converged to a gradient RMSD less that 0.05 kJ/Å mol or continued until a limit of 5,000 iterations was reached. Aqueous solution conditions were simulated using the continuum dielectric water solvent model (GB/SA). Extended cut-off distances were defined at 8Å for Van der Waals, 20Å for electrostatics and 4 Å for H-bonds.

Following the molecular mechanics minimization, the geometry was optimized further with the "fully analytic" method (B3LYP/6-31G**), as implemented in Jaguar. The Jaguar pK_a calculations also employed the "fully analytic" preset setting and the "water" solvent correction factor.

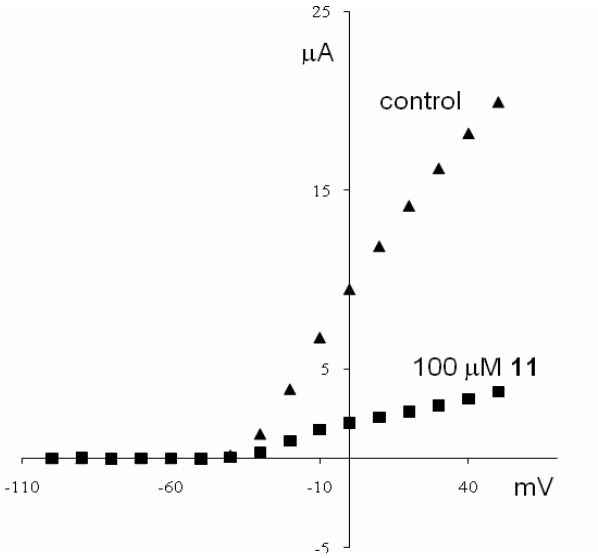
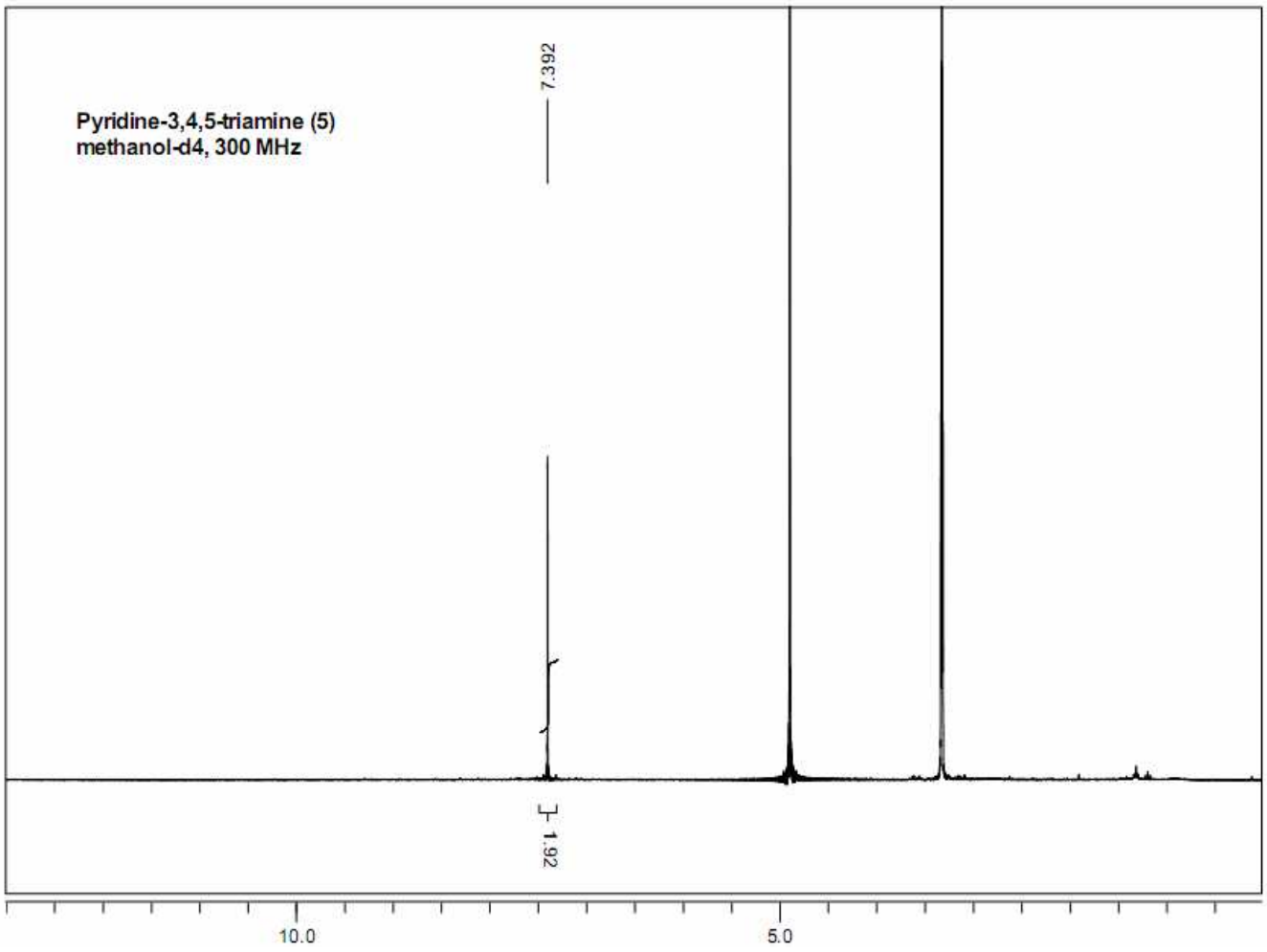
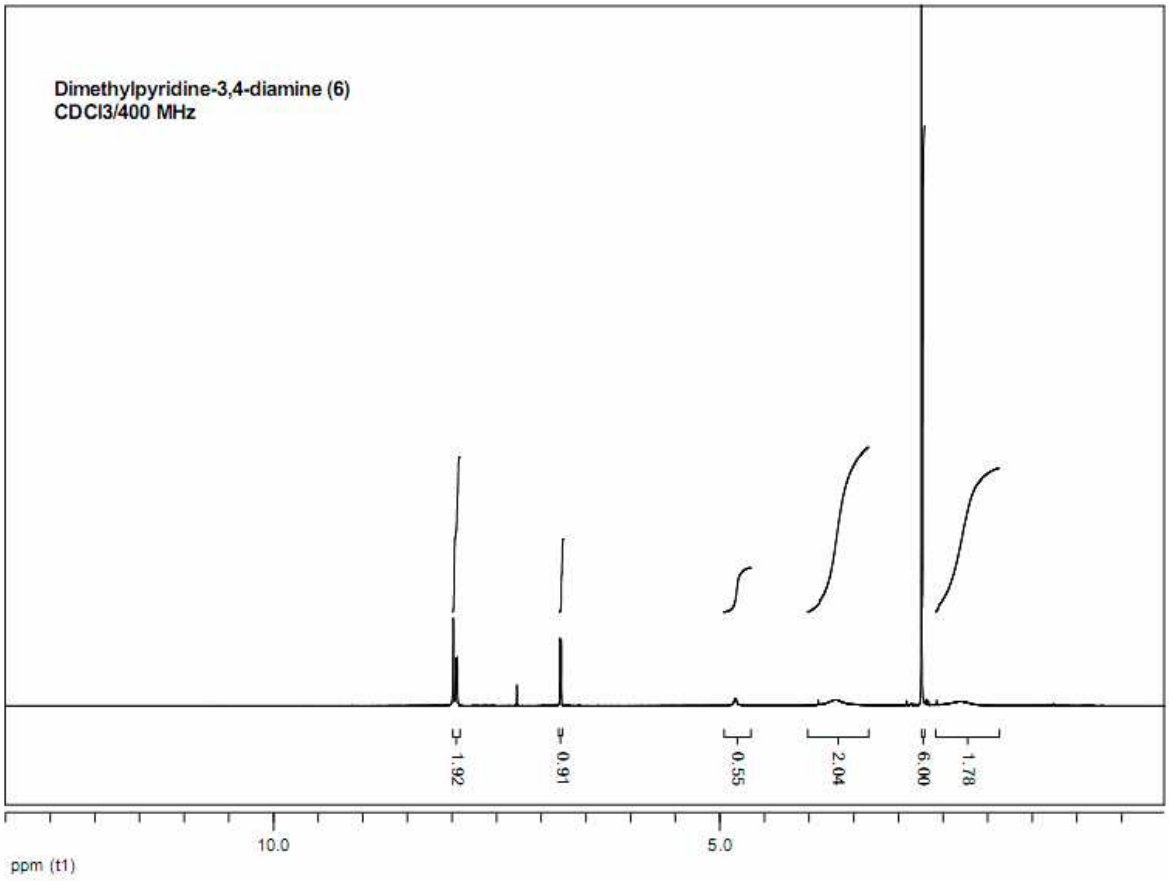
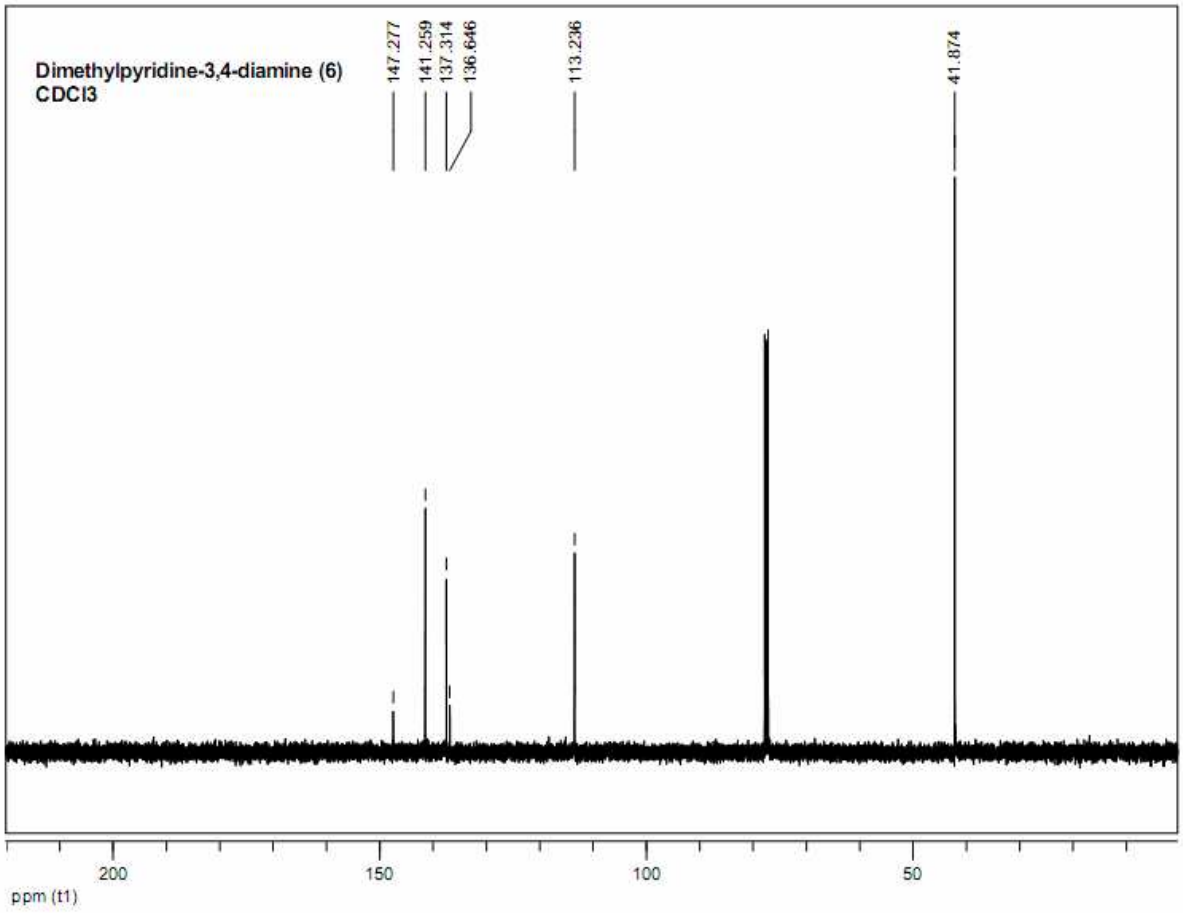
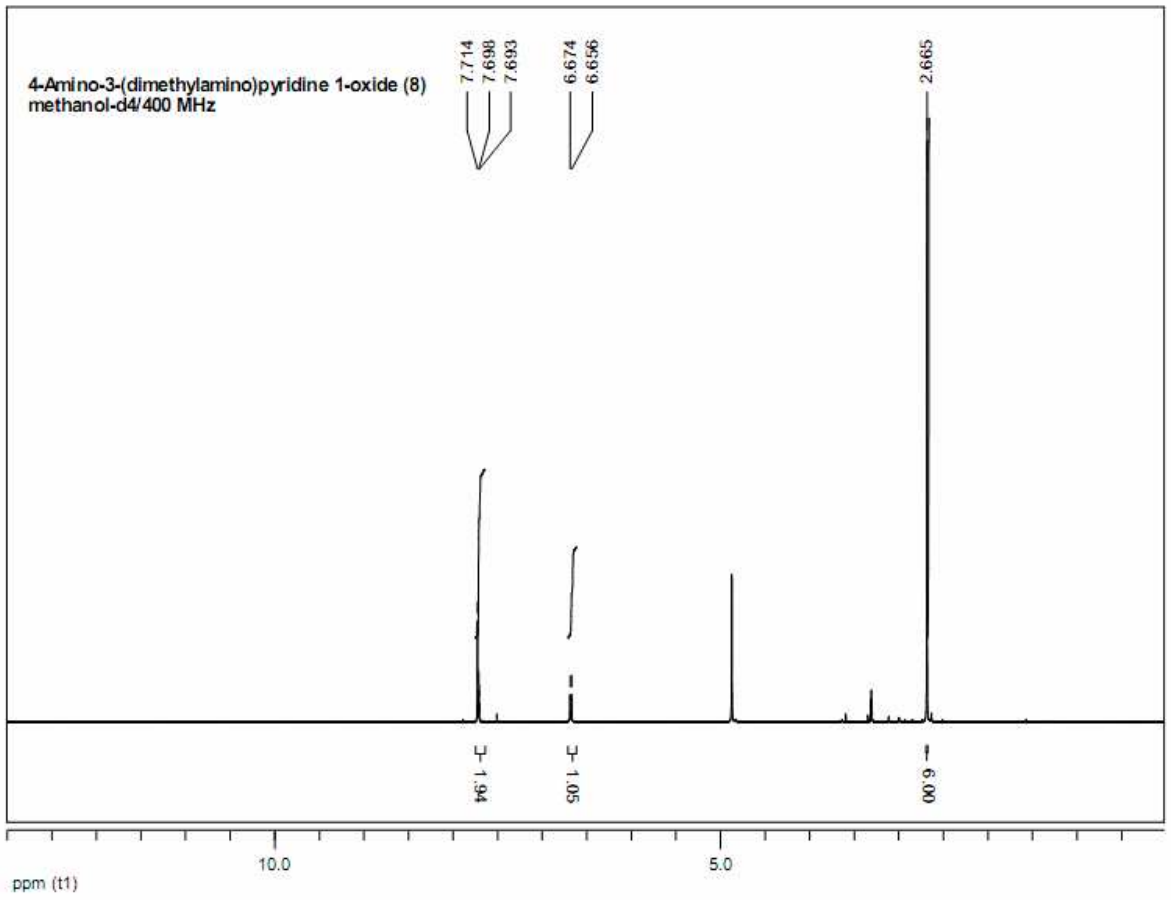


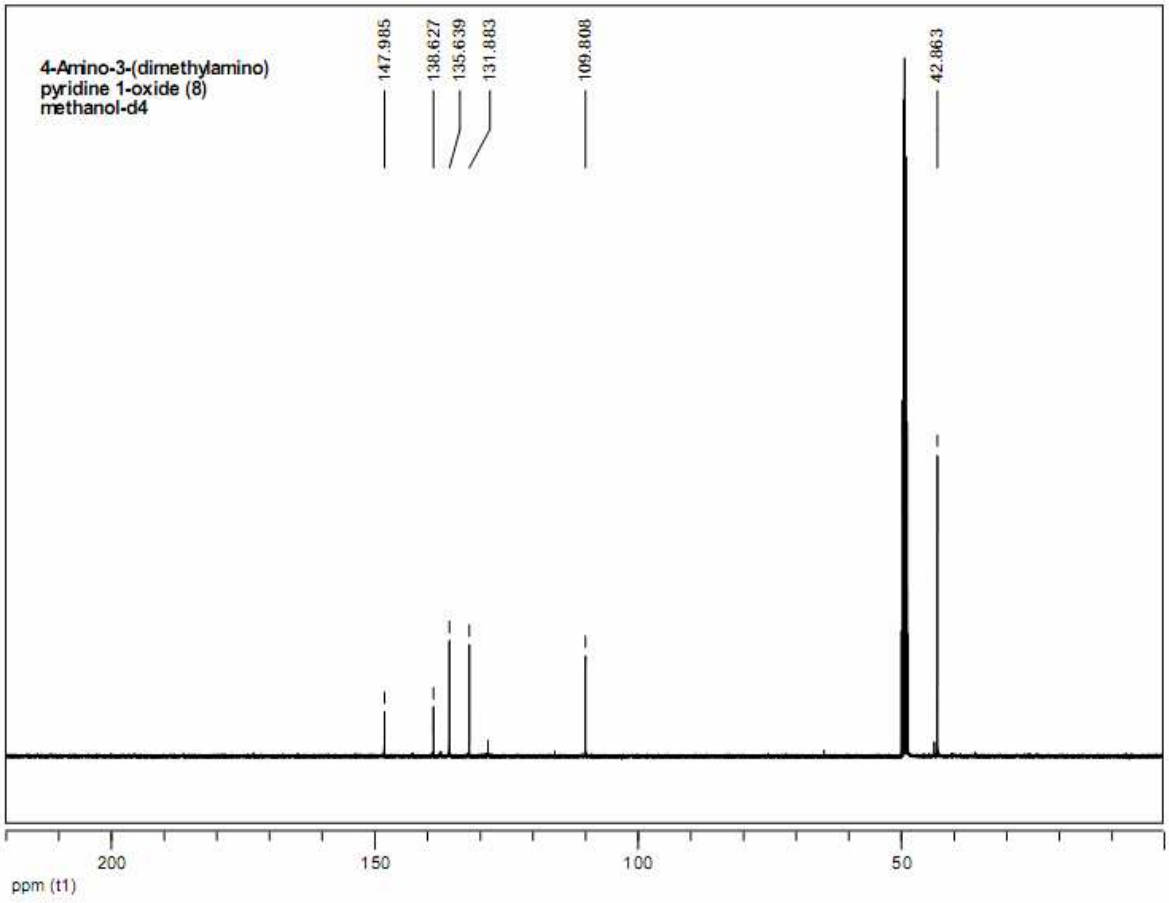
Figure S1. *Shaker* steady state current–voltage relationships for a representative oocyte before (control, triangles) and after (squares) 30 minutes-long incubation with 100 μM **11**. Oocyte membrane potential was held at −80 mV and pulsed from −100 to +50 mV in 10 mV voltage steps for 100 ms with 2 s interpulse intervals. The P/4 protocol was used to subtract nonspecific leaks.

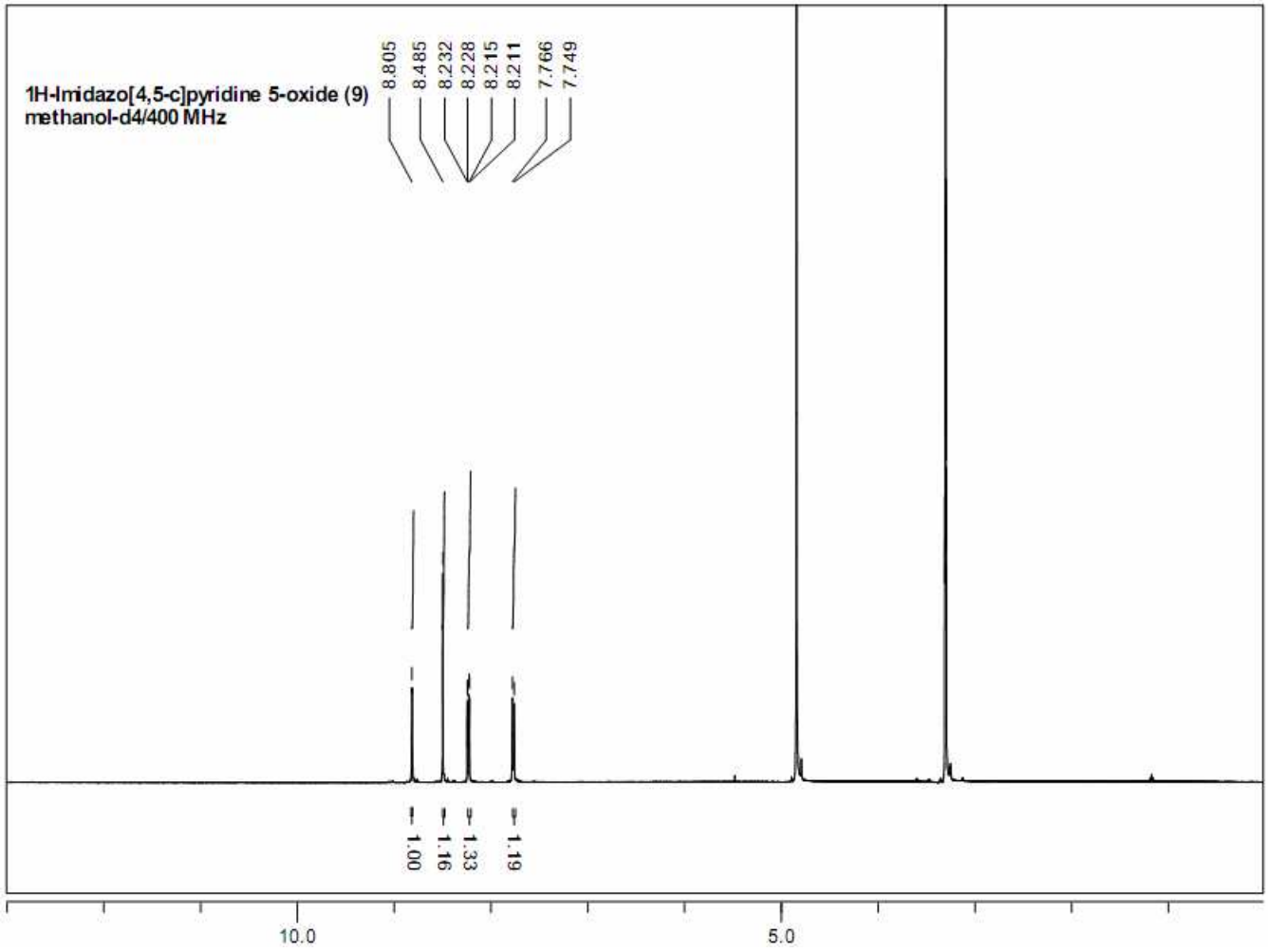

Pyridine-3,4,5-triamine (5)
methanol-d4, 300 MHz
7.392
1.92
10.0
5.0

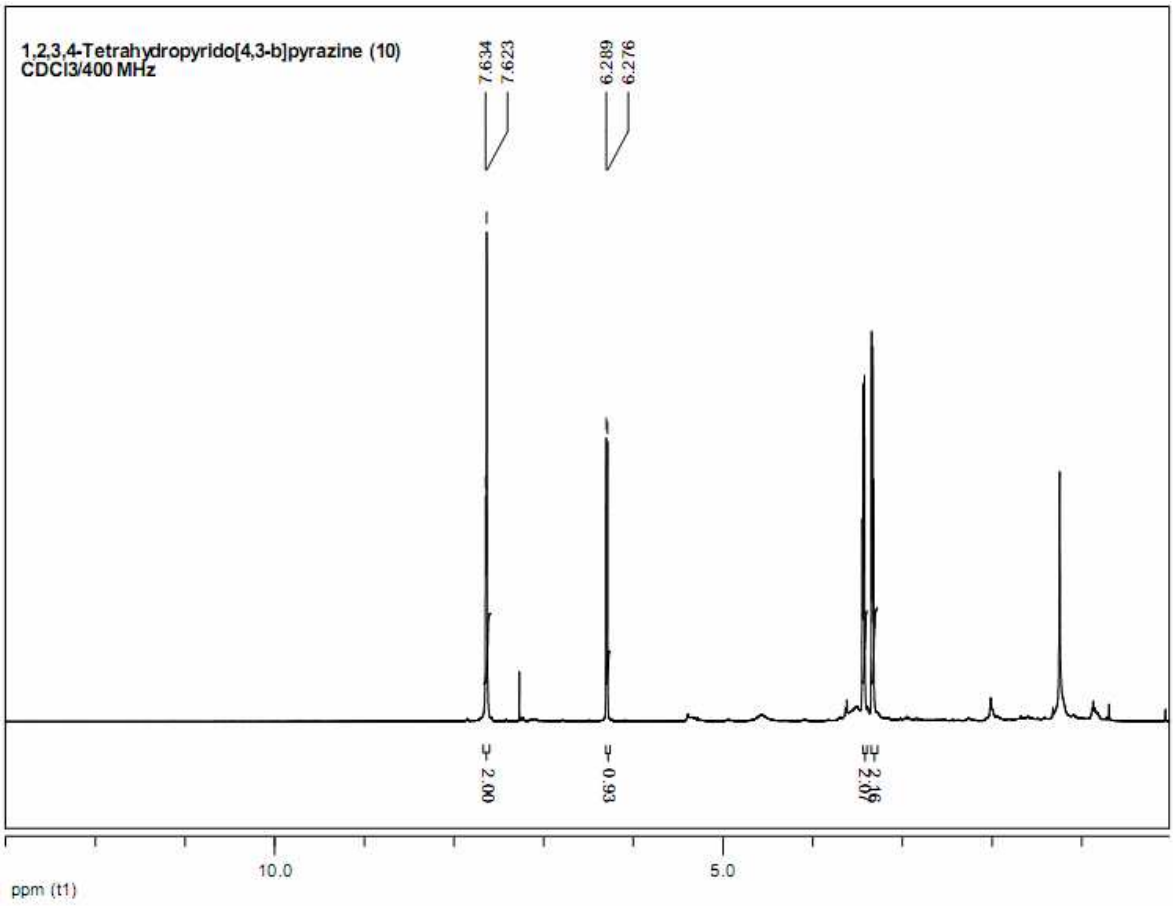

Dimethylpyridine-3,4-diamine (6)
CDCl3/400 MHz
1.92
0.91
0.55
2.04
6.00
1.78
10.0
5.0
ppm (t1)

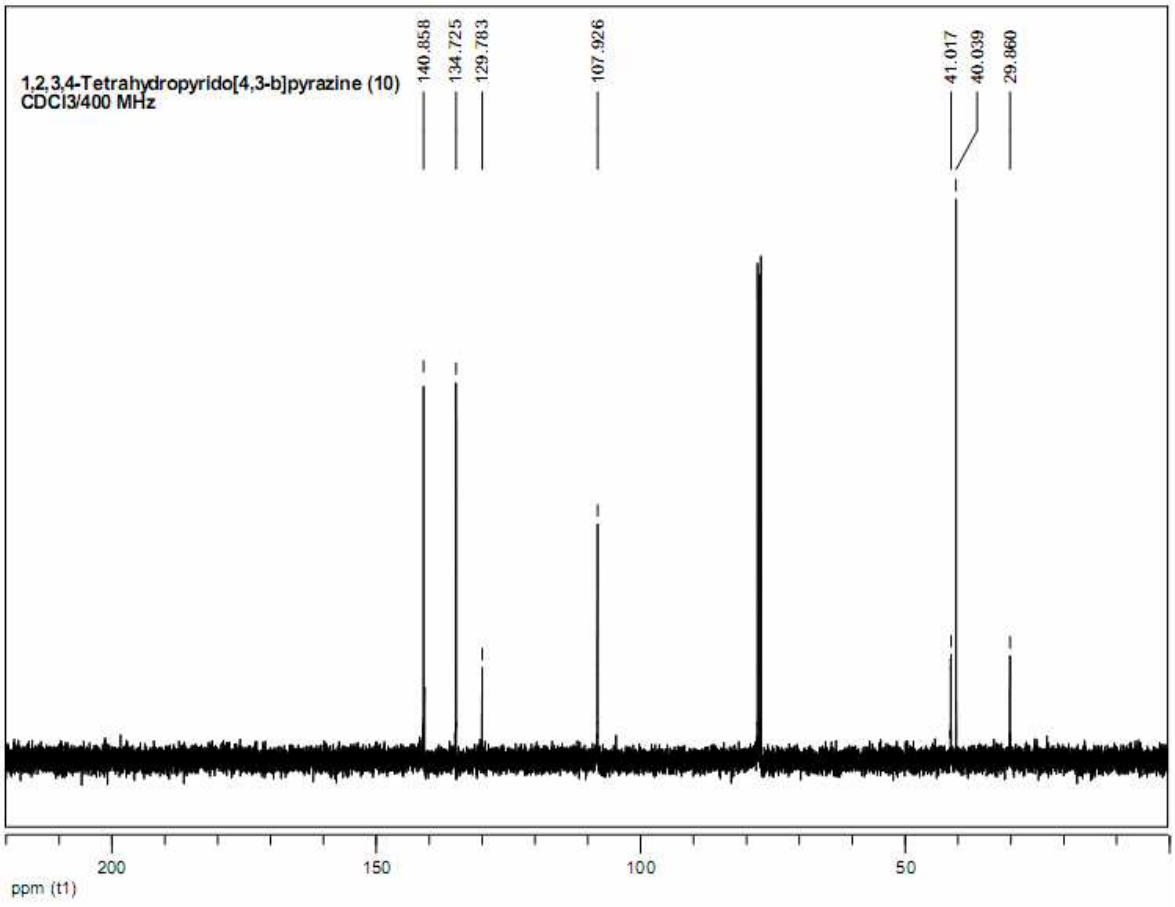

Dimethylpyridine-3,4-diamine (6)
CDCl3
147.277
141.259
137.314
136.646
113.236
41.874
200
150
100
50
ppm (t1)

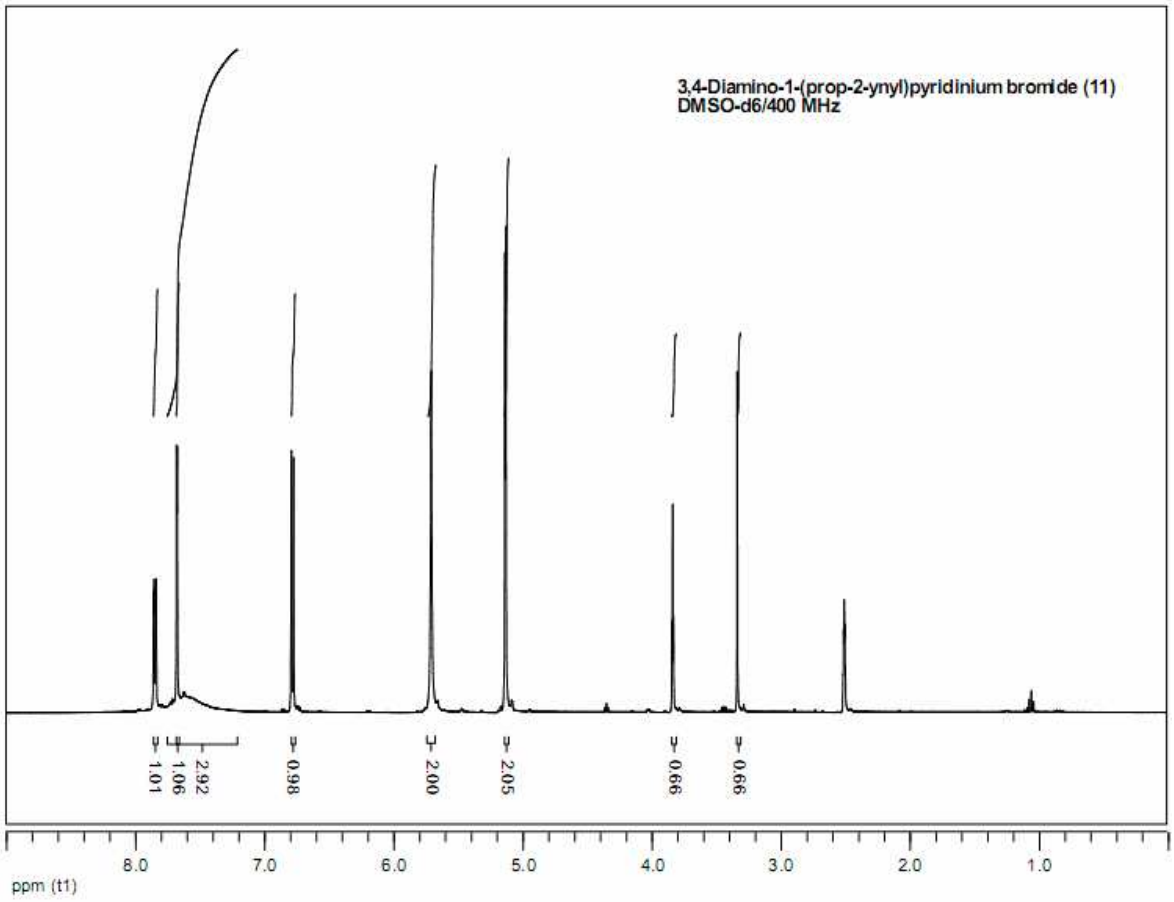

4-Amino-3-(dimethylamino)pyridine 1-oxide (8)
methanol-d4/400 MHz
7.714
7.698
7.693
6.674
6.656
2.665
1.94
1.05
6.00
10.0
5.0
ppm (t1)

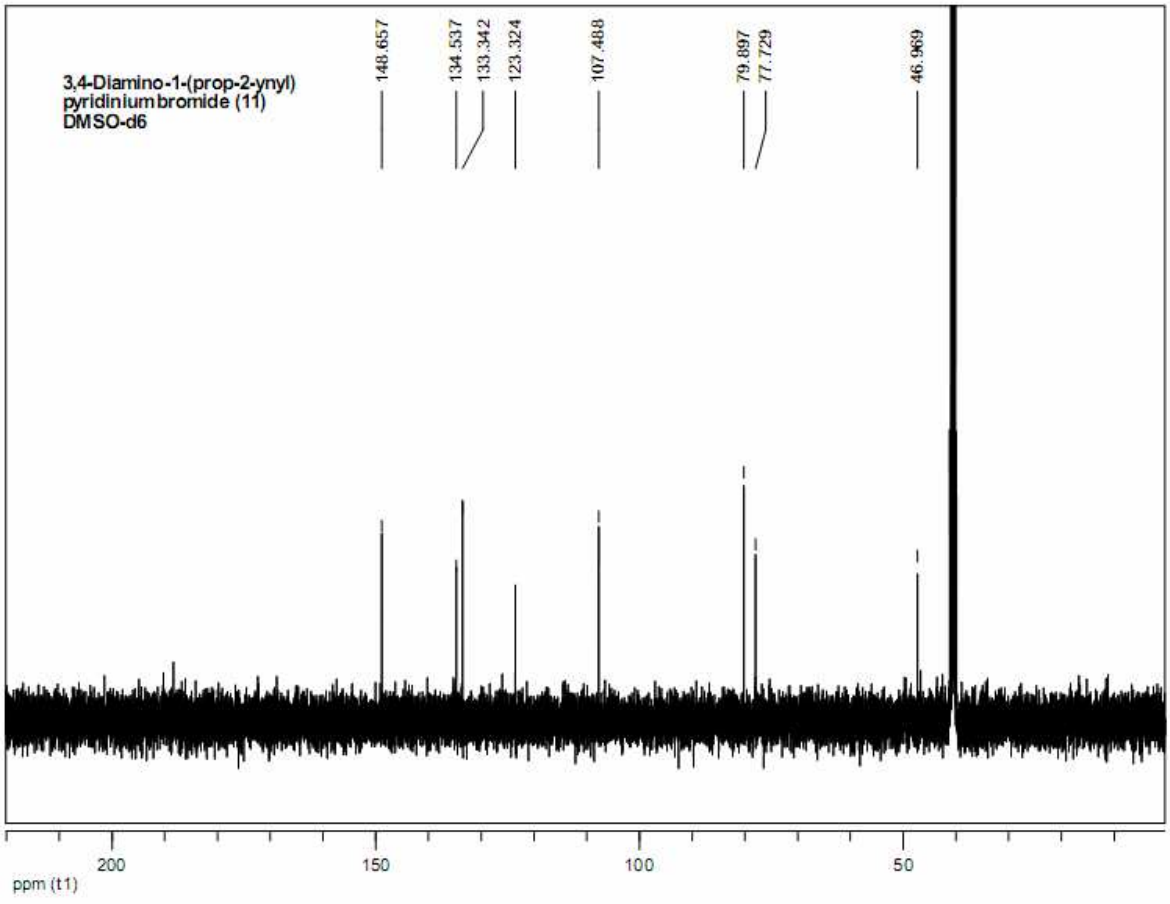

4-Amino-3-(dimethylamino)
pyridine 1-oxide (8)
methanol-d4
147.985
138.627
135.639
131.883
109.808
42.863
200
150
100
50
ppm (t1)

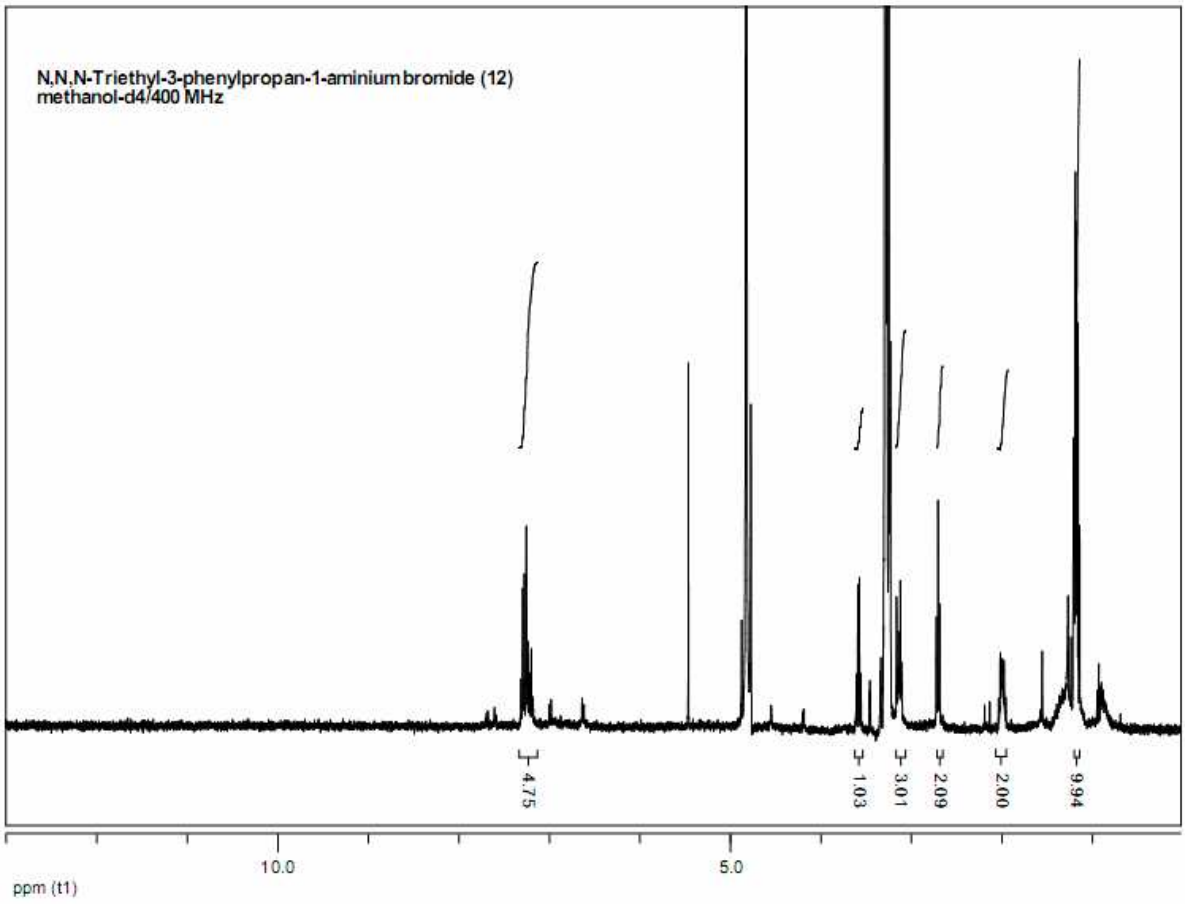

1H-Imidazo[4,5-c]pyridine 5-oxide (9)
methanol-d4/400 MHz
8.805
8.485
8.232
8.228
8.215
8.211
7.766
7.749
1.00
1.16
1.33
1.19
10.0
5.0

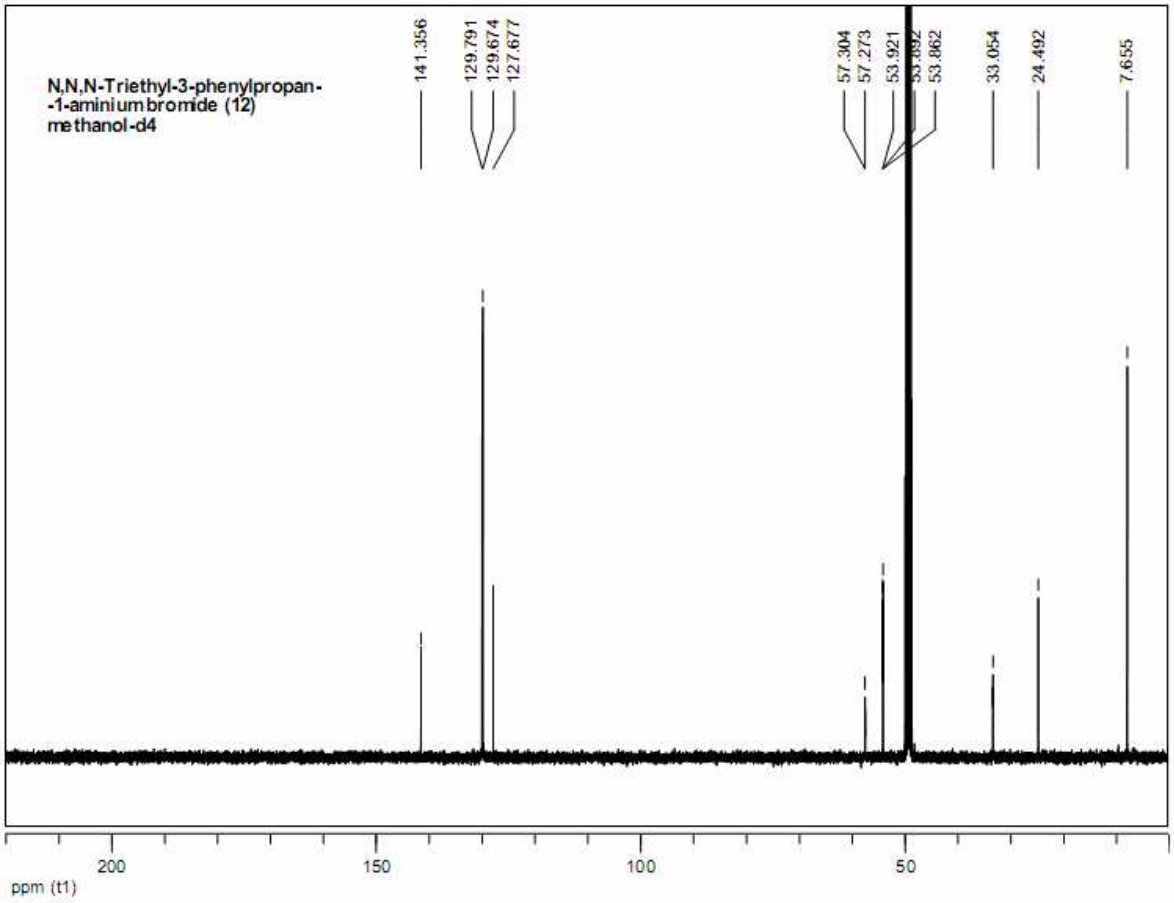

1,2,3,4-Tetrahydropyrido[4,3-b]pyrazine (10)
CDCl3/400 MHz
7.634
7.623
6.289
6.276
2.00
0.93
2.16
10.0
5.0
ppm (t1)


1,2,3,4-Tetrahydropyrido[4,3-b]pyrazine (10)
CDCl3/400 MHz
140.858
134.725
129.783
107.926
41.017
40.039
29.860
200
150
100
50
ppm (t1)


3,4-Diamino-1-(prop-2-ynyl)pyridinium bromide (11)
DMSO-d6/400 MHz
1.01
1.06
2.92
0.98
2.00
2.05
0.66
0.66
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

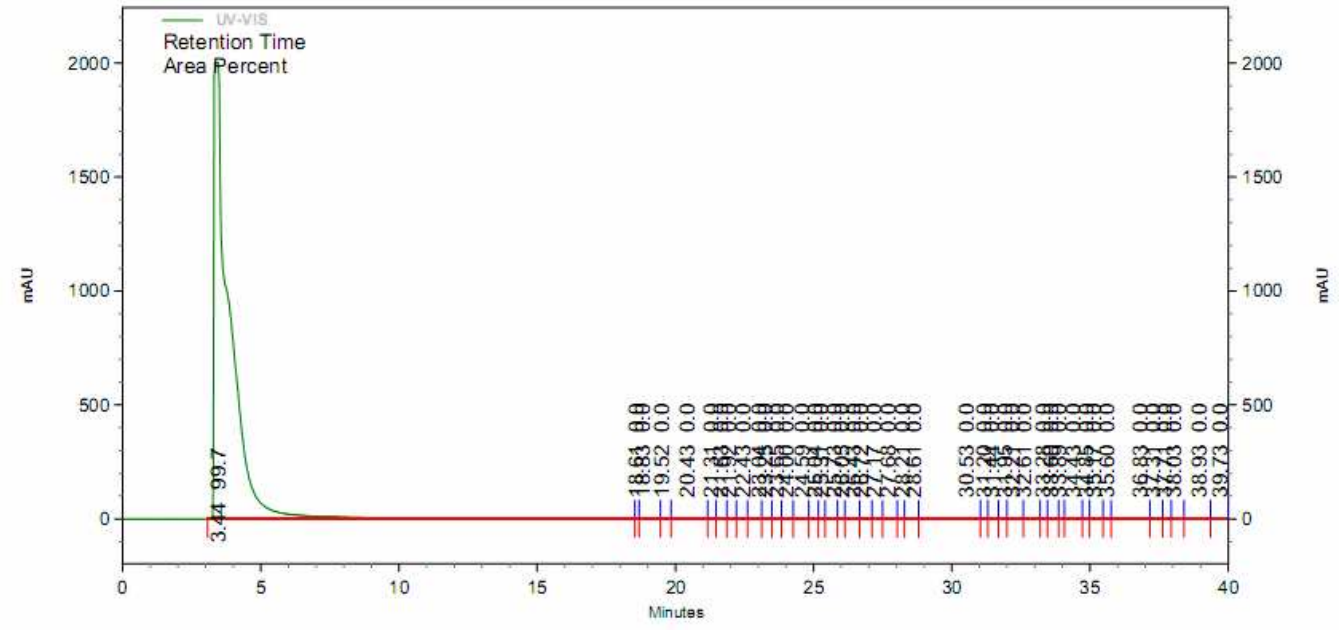

3,4-Diamino-1-(prop-2-ynyl)
pyridinium bromide (11)
DMSO-d6
148.657
134.537
133.342
123.324
107.488
79.897
77.729
46.969
200
150
100
50
ppm (t1)


N,N,N-Triethyl-3-phenylpropan-1-aminium bromide (12)
methanol-d4/400 MHz
4.75
1.03
3.01
2.09
2.00
9.94
10.0
5.0
ppm (t1)


N,N,N-Triethyl-3-phenylpropan-
-1-aminium bromide (12)
methanol-d4
141.356
129.791
129.674
127.677
57.304
57.273
53.921
53.892
53.862
33.054
24.492
7.655
200
150
100
50
ppm (t1)

4. HPLC conditions and gradients. The purity of the compounds **1**-**12** was checked by analytical reverse-phase HPLC using a Vydac C_{18} 218TP104 column (Western Analytical Products) monitored at 230 and 254 nm. A linear gradient of 0.5-10% (v/v) of solvent B (0.09% v/v TFA/acetonitrile) in solvent A (0.1% v/v TFA/water) over 20 min, then 10-90% (v/v) B over 10 min was used for all compounds, except compound **12**, for which a linear gradient of 10-50% (v/v) B over 20 min, then 50-90% (v/v) B over 10 min was used.

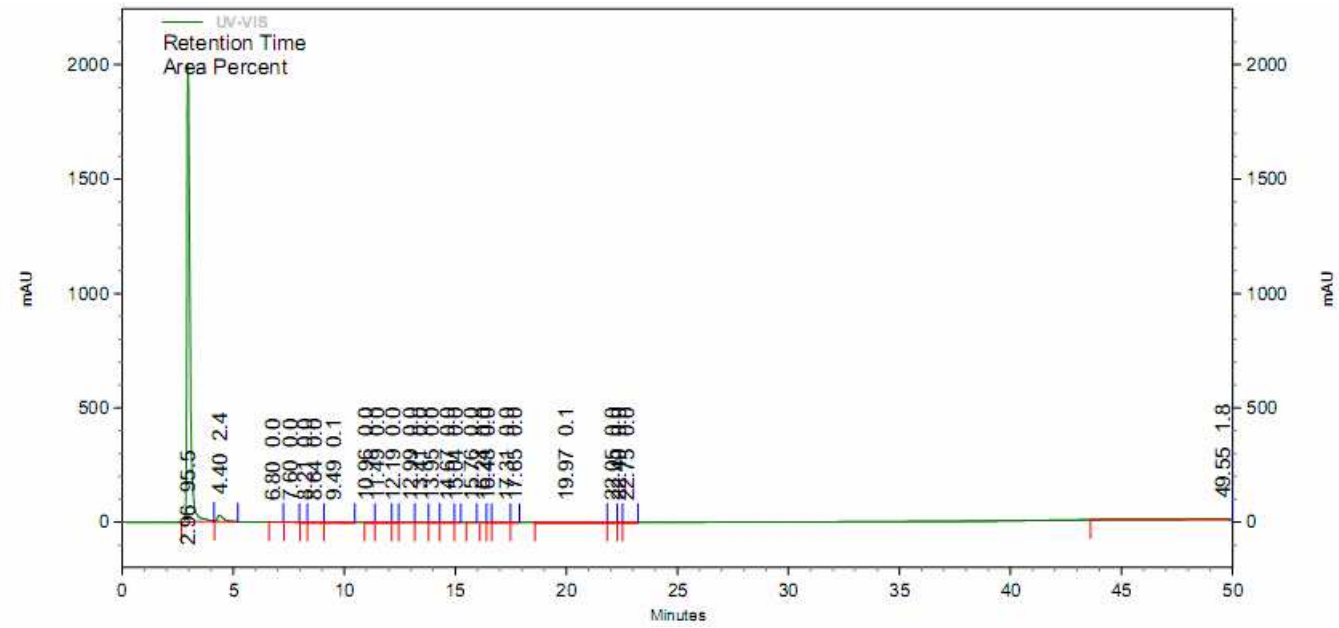
3,4-DAP (**3**)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	3.440	**99.696**
2	18.613	**0.005**

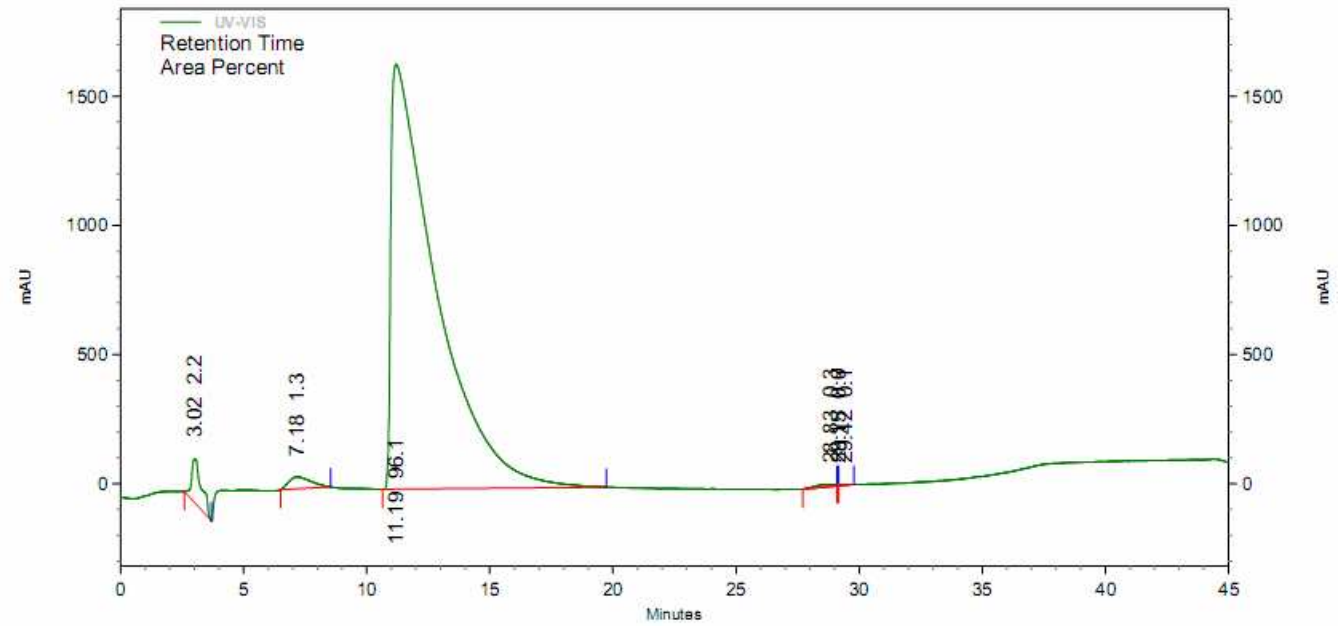
Pyridine-3,4,5-triamine (5)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	2.960	**95.533**
2	4.400	**2.377**
3	6.800	**0.013**

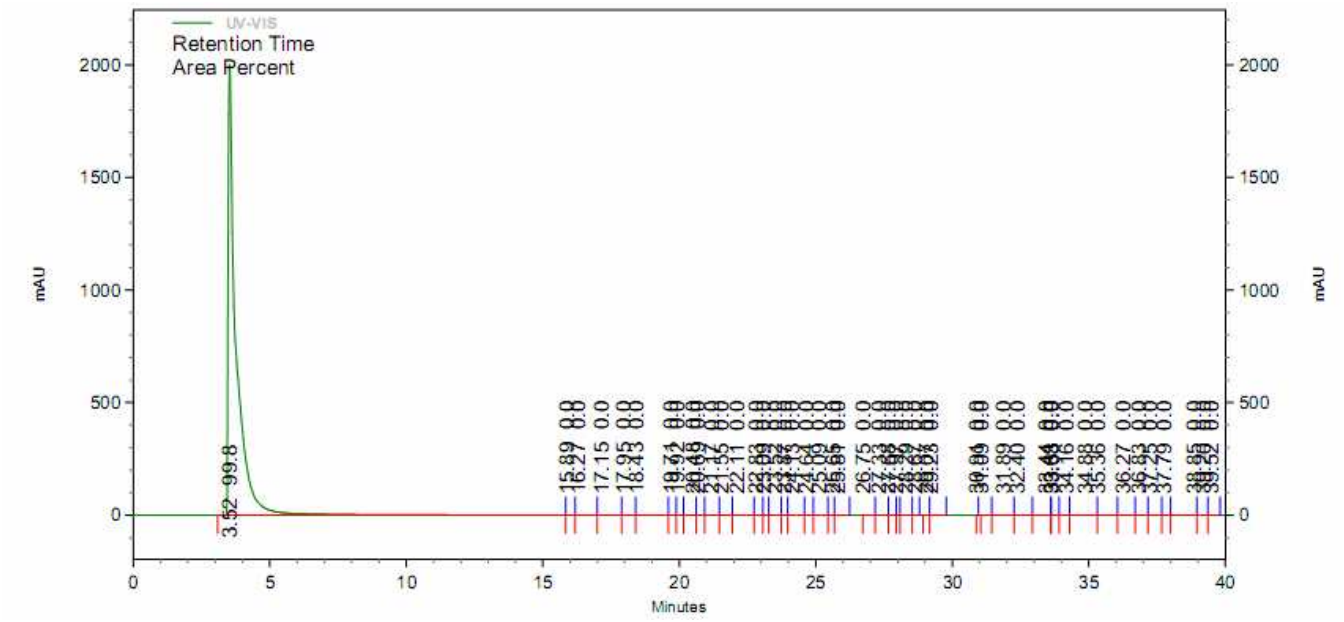
N^4,N^4-Dimethylpyridine-3,4-diamine (6)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	3.020	**2.234**
2	7.177	**1.307**
3	11.193	**96.147**

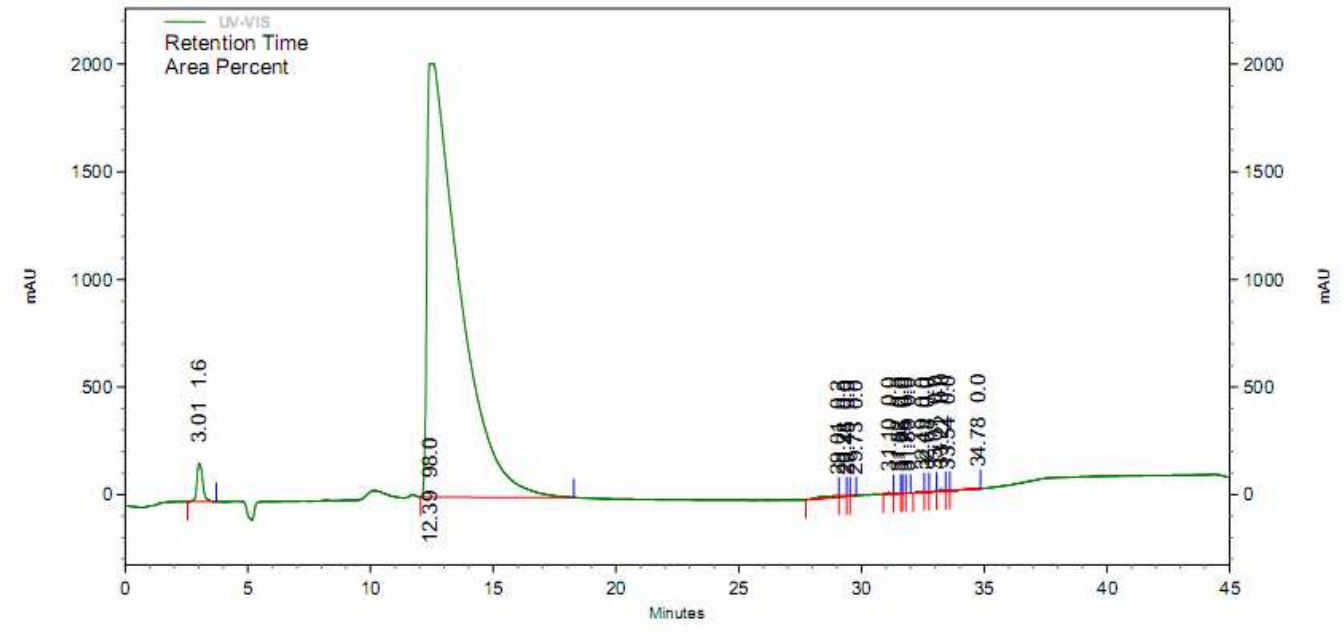
5-Azabenzimidazole (7)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	3.520	**99.795**
2	15.893	**0.014**
3	16.267	**0.029**

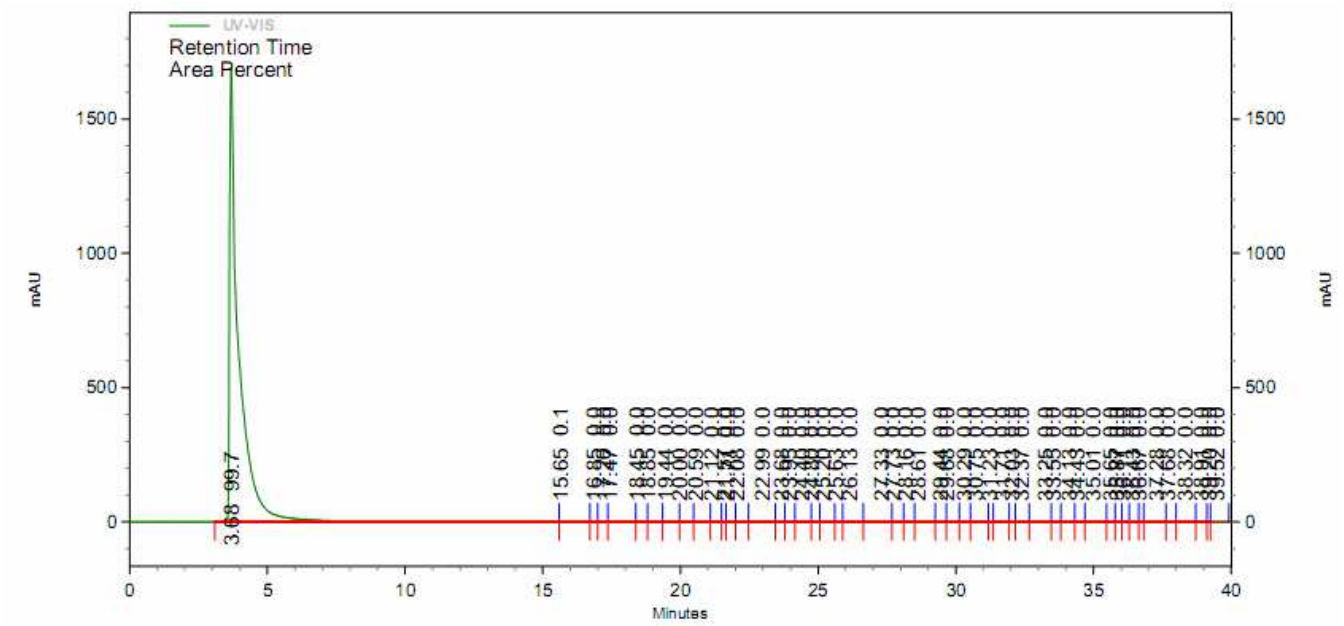
4-Amino-3-(dimethylamino)pyridine 1-oxide (8)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	3.010	**1.567**
2	12.390	**97.977**

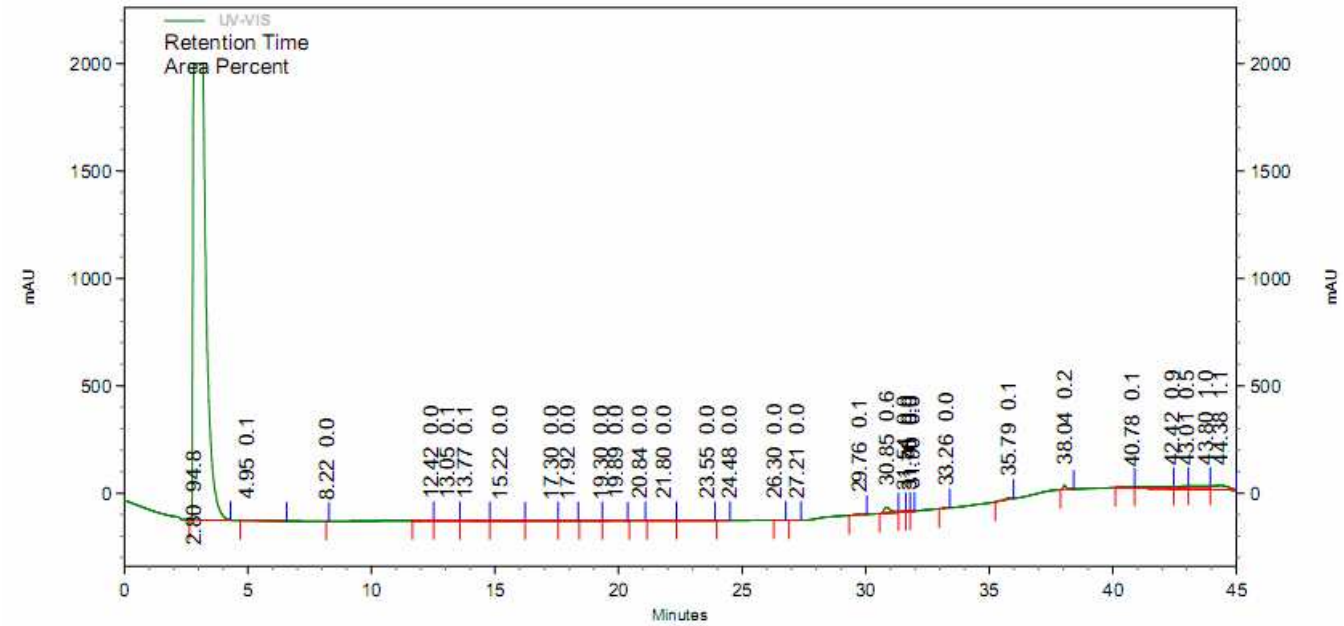
1*H*-Imidazo[4,5-c]pyridine 5-oxide (9)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	3.680	**99.660**
2	15.653	**0.054**
3	16.853	**0.011**

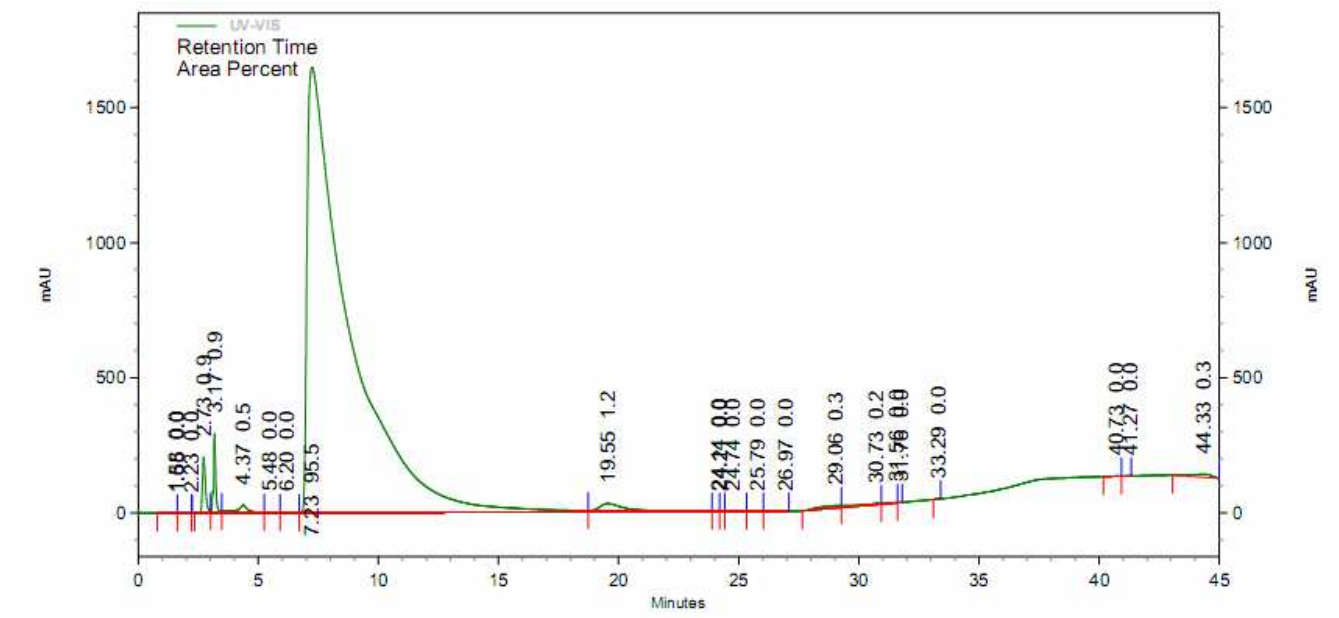
1,2,3,4-Tetrahydropyrido[4,3-b]pyrazine (10)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	2.797	**94.829**
2	4.947	**0.063**

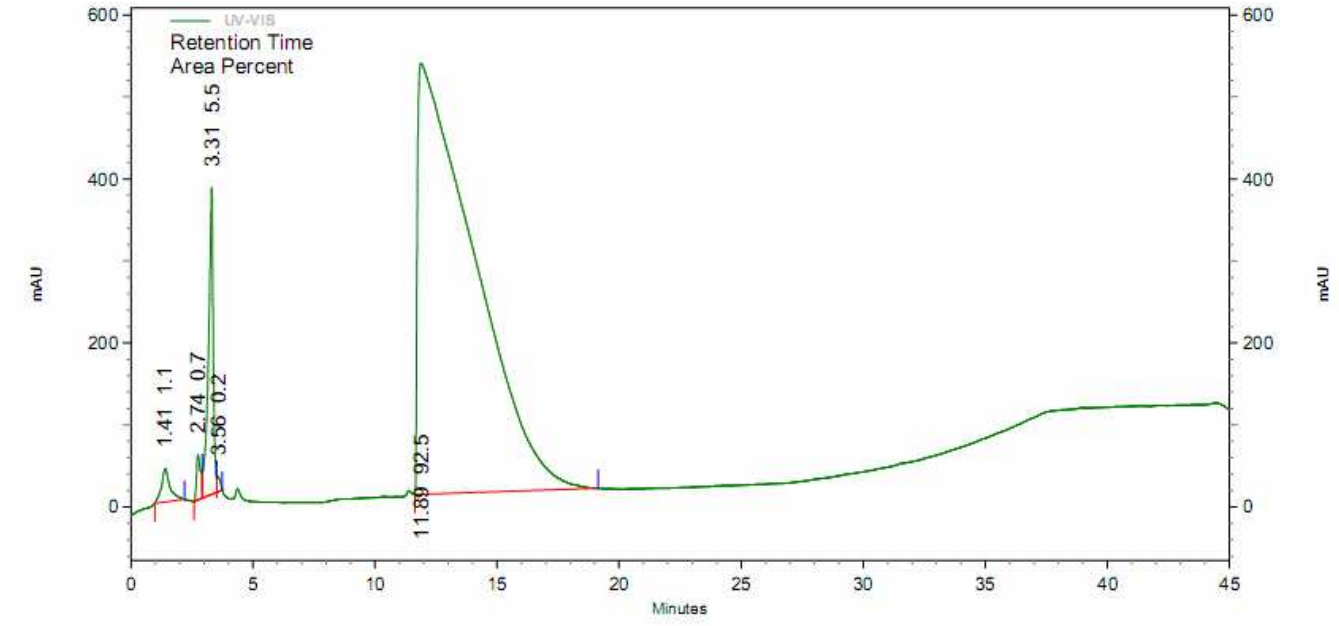
3,4-Diamino-1-(prop-2-ynyl)pyridinium bromide (11)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	1.580	**0.004**
2	1.650	**0.002**
9	7.233	**95.532**

N,*N*,*N*-Triethyl-3-phenylpropan-1-aminium bromide (12)



UV-VIS Results

Pk #	Retention Time	Area Percent
1	1.407	**1.074**
2	2.743	**0.722**
3	3.307	**5.545**
4	3.557	**0.186**
5	11.887	**92.474**

6. REFERENCES

1. Pelletier, J. C., and Rogers, J. F. (2006) Preparation of piperazinyl imidazo[4,5-c]pyridines as gonadotropin releasing hormone receptor antagonists for the treatment of cancer, in *U.S. Pat. Appl. Publ.*, p 30 pp, USA.
2. Burton, A. G., Frampton, R. D., Johnson, C. D., and Katritzky, A. R. (1972) The kinetics and mechanism of the electrophilic substitution of heteroaromatic compounds. Part XXVIII. The preparation and kinetic nitration of 2-, 3-, and 4-dimethylaminopyridines and their 1-oxides in sulphuric acid, *J. Chem. Soc., Perkin Trans. 2*, 1940-1949.
3. Jiao, Y., and Yu, H. (2001) Methyltrioxorhenium (MeReO3) Catalyzed Selective Oxidation of Purine and Related Compounds into Their N-Oxides, *Synlett 2001*, 0073-0074.
4. Crey-Desbiolles, C., Lhomme, J., Dumy, P., and Kotera, M. (2004) 3-Nitro-3-deaza-2'-deoxyadenosine as a Versatile Photocleavable 2'-Deoxyadenosine Mimic, *J. Am. Chem. Soc. 126*, 9532-9533.
5. Singh, S., Das, G., Singh, O. V., and Han, H. (2007) Development of More Potent 4-Dimethylaminopyridine Analogues, *Org. Lett. 9*, 401-404.