Electronic Supporting Information

A Ratiometric Fluorescent Probe for Detection of Biogenic Primary Amines with Nanomolar Sensitivity

Suman Mallick,[a] Falguni Chandra [a] and Apurba L. Koner[a]*

Experimental Section

(A) Materials

All the chemicals except ANH (8) were purchased from commercial sources and used as received without any further purification. Tryptamine, Phenetthyleneamine, 1,6-Diaminohexane and Spermidine were purchased from Alfa-aesar, and 1,3-Diaminopropane, Butylamine, Diethylamine, Diisopropylamine, Triethylamine, 1,4-Butanediamine and Diisopropylethylamine from Spectrochem. The solvents used for spectroscopic measurements were of spectroscopy grade and purchased from Sisco Research Laboratory (SRL), India. All the experiments were carried out at (298 ± 2) K.

(B) Methods

(a) Steady-State Measurements

Steady state absorption measurements were done with Shimadzu UV-spectrophotometer using 10 mm path length quartz cuvettes. All steady state fluorescence measurements were carried out in DMA as a solvent using HORIBA Jobin Yvon Fluorimax-4 Fluorimeter. A dilute solution of ANH 3.6 μM was taken for all the measurements to keep the absorption value low to avoid inner filter effect. Fluorescence spectra for the titration experiments were recorded using 10 mm path length quartz cuvette in the region from 400 nm to 650 nm range by exciting at 385 nm with excitation slit 3 nm and emission slits with a width of 3 nm for all the measurements. For the titration experiments in case of primary amines, successive data points were recorded giving a 5 min interval after each and every amine addition, and for secondary and tertiary amines the intervals were 15 minutes. For kinetic measurements initially a 3.6 μM solution of ANH in DMA

was placed in a 10 mm path length quartz cuvette, excited at 385 nm and the emission intensity was recorded at 575 nm wavelength in kinetics mode for 1 minute , then amine solution was added in stirring condition and kinetics was measured for required time frame. All the experiments were carried out at room temperature (298 K).

(b) X-ray crystallography:

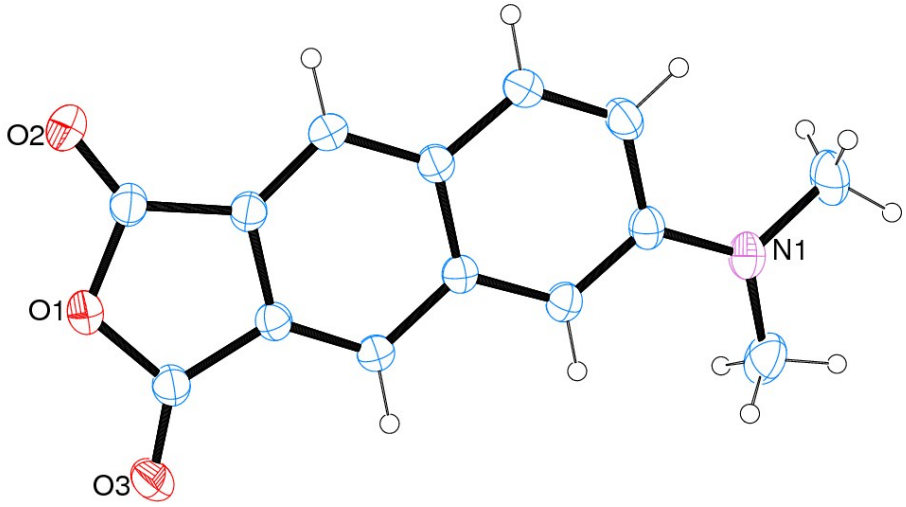
Suitable crystals for X-ray crystallography were obtained by slow evaporation of a solution of ANH in DCM and Hexane solvent mixture (9:1). Single-crystal X-ray diffraction data were collected using a Bruker SMART APEX II CCD diffractometer with graphite monochromated Mo K_α (λ = 0.71073 Å) radiation at 218 K.

Table S1. Crystal data and structure refinement parameters of ANH

Identification code	shelx	
Empirical formula	$C_{14}\ H_{11}\ N\ O_3$	
Formula weight	241.24	
Temperature	218(2) K	
Wavelength	71.073 pm	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 691.60(7) pm	α= 76.992(3)°.
	b = 831.88(8) pm	β= 76.986(3)°.
	c = 1041.44(9) pm	γ = 86.369(4)°.
Volume	0.56875(9) nm^3	
Z	2	
Density (calculated)	1.409 mg/m^3	
Absorption coefficient	0.100 mm^{-1}	
F(000)	252	
Crystal size	0.250 x 0.120 x 0.090 mm^3	
Theta range for data collection	2.882 to 24.987°.	
Index ranges	-8<=h<=8, -9<=k<=10, 0<=l<=11	
Reflections collected	2002	
Independent reflections	2002 [R(int) = 0.00]	
Completeness to theta = 25.242°	97.3 %	

Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.985 and 0.963
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2002 / 0 / 163
Goodness-of-fit on F^2	1.077
Final R indices [I>2sigma(I)]	R1 = 0.0398, wR2 = 0.1103
R indices (all data)	R1 = 0.0516, wR2 = 0.1182
Largest diff. peak and hole	0.170 and -0.159 e.Å^{-3}
CCDC number:	1418667

ORTEM Diagram of compound ANH



(ORTEP diagram)

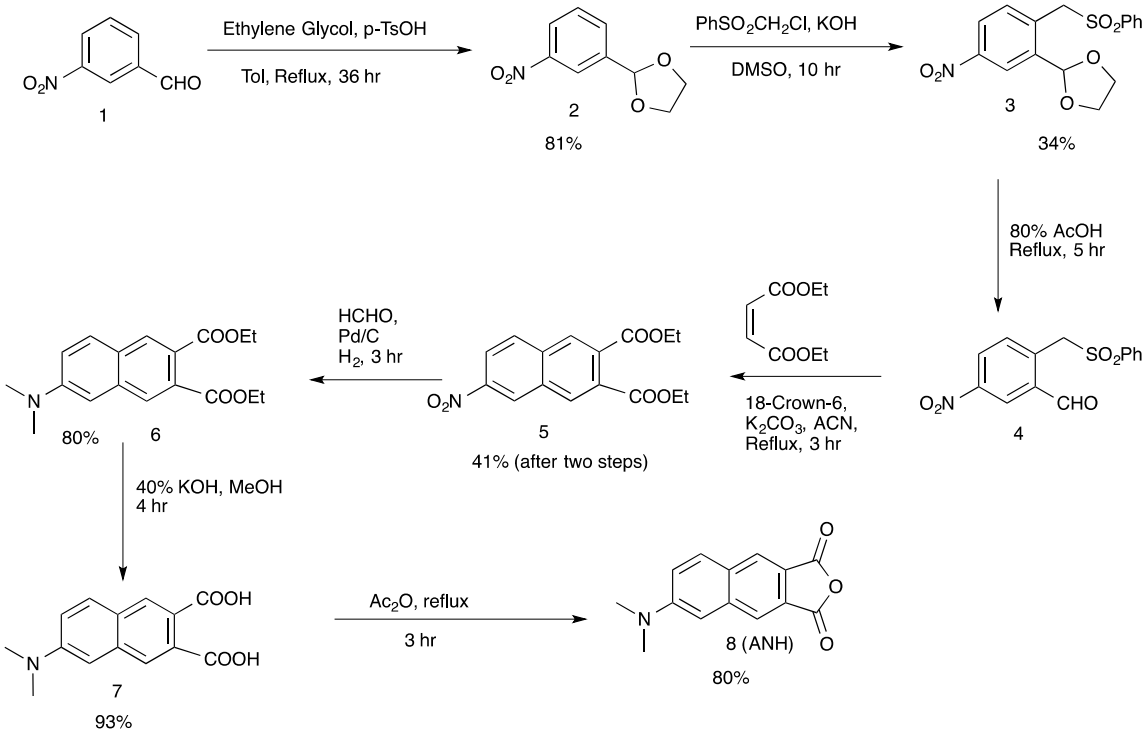
(C) Nuclear magnetic resonance (NMR) spectra and Mass spectrometry

^{1}H NMR spectra were recorded Bruker Avance III 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in ppm relative to the residual solvent ($CDCl_3$) signal (δ = 7.26 for ^{1}H NMR). Data for ^{1}H NMR spectra are reported as follows: chemical shift (multiplicity, coupling constants, number of hydrogen). Abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublets) t (triplet), q (quartet), m (multiplet), td (triplet of doublets). Low-resolution mass spectra were collected on an Agilent Technologies 1200 series HPLC paired to a 6130 mass spectrometer. Bruker Daltonics MicroTOF-Q-II with electron spray ionization (ESI) was used for HRMS data.

(D) Synthesis of ANH

ANH was synthesized following the combined literature protocols,[1,2] as depicted below

Scheme 1



(E) Excitation spectra of ANH

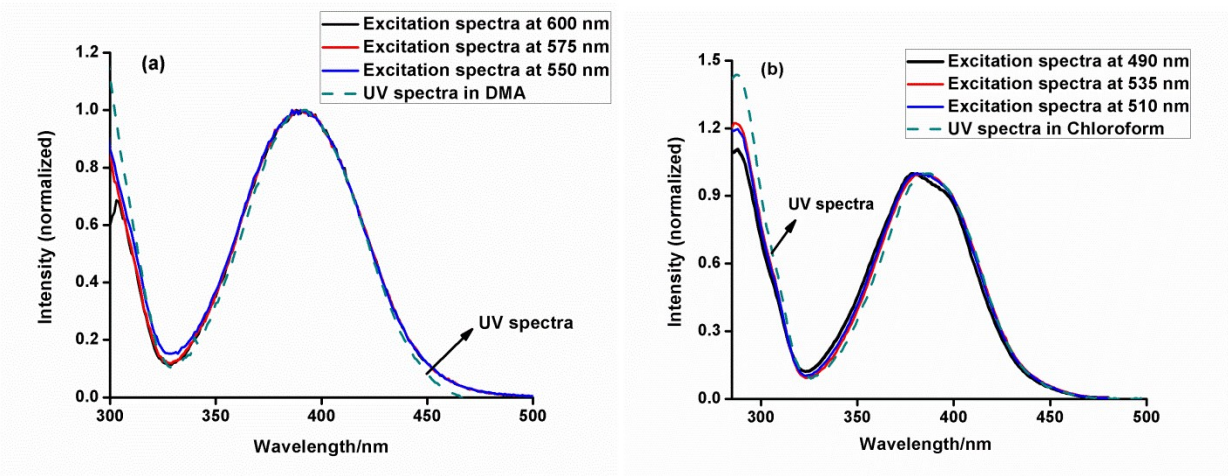


Figure S1 (a) Excitation spectra of ANH combined with its UV spectra in DMA (b) Excitation spectra of ANH combined with its UV spectra in Chloroform

Table S2: Comparison of reported methods related to detection of biogenic primary amines

Reported work	Detection limit	Solvent/support	Response time (min)	Method used	Reference
Detection of biogenic amines	500 μM	HEPES buffer with 6.0 mM SDS	~ 120 min at 50 ºC	UV-Vis and Fluorescence	Chem. Commun. 2011, 47, 9639
Sensing of Biogenic Amines with Aggregation-Induced Emission	10 μM	Dichloromethane	~ 25 min at room temperature	Fluorescence	Chem. Eur. J. 2011, 17, 5344
Indicators for Primary Amines	25 μM	Dichloromethane or Acetonitrile	~ 80 min at room temperature	Fluorescence	J. Am. Chem. Soc. 2014, 136, 15493
Chromogenic Sensing of Biogenic Amines	20 μM	Filter paper	20 min at 60 ºC	Absorption based	Anal. Chem. 2010, 82, 8402
Ratiometric Fluorescent Probe for Detection of Biogenic Primary	0.002 μM	Dimethyl acetamide	10 min at room temperature	Absorption and fluorescence based	This work

Amines					

(F) Titration of ANH by UV with different primary amines including BAs

(i) **Titration of ANH with 1,4-Butanediamine**

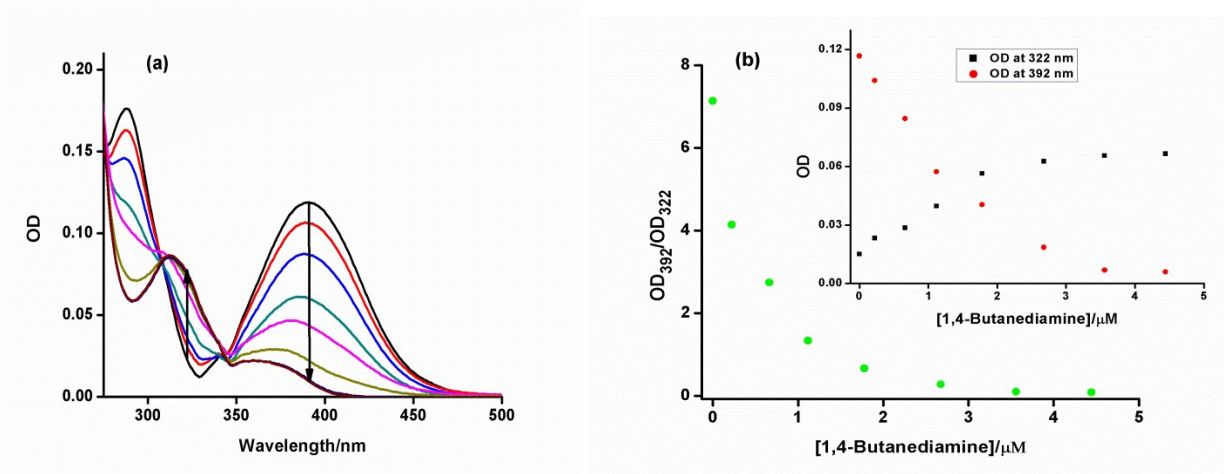


Fig S2 (a) Titration of 3.6 μM ANH with 1,4-Butanediamine (b) Ratiometric plot of titration using OD at two different wavelengths, inset shows the changes in OD at selected wavelengths

(ii) **Titration of ANH with 1,6-Hexanediamine**

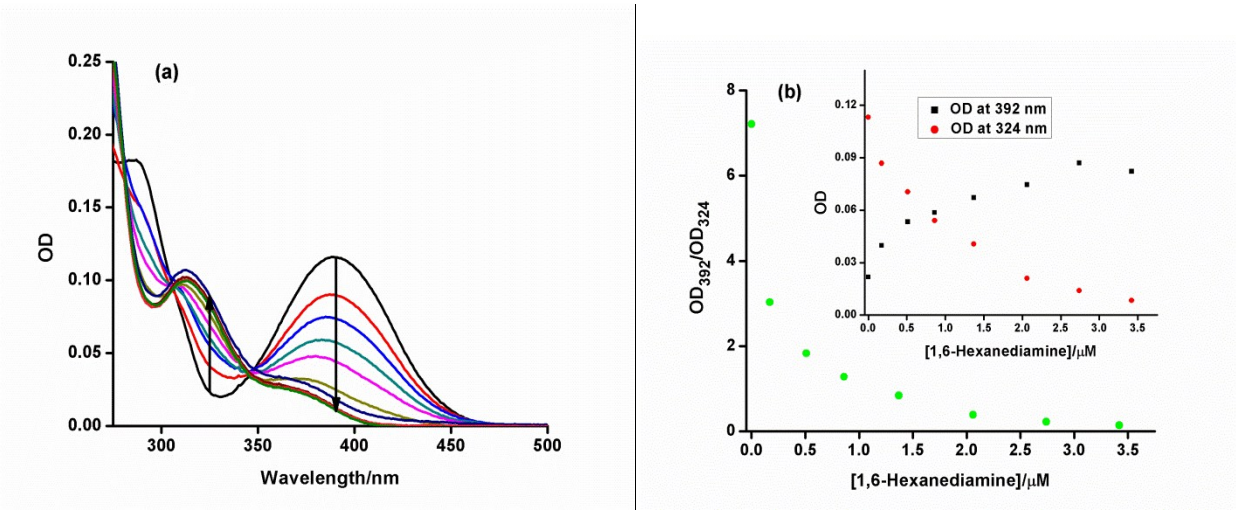


Fig S3 (a) Titration of 3.6 μM ANH with 1,6-Hexanediamine (b) Ratiometric plot using OD at two different wavelengths, inset shows the changes in OD at selected wavelengths

(iii) Titration of ANH with Spermidine

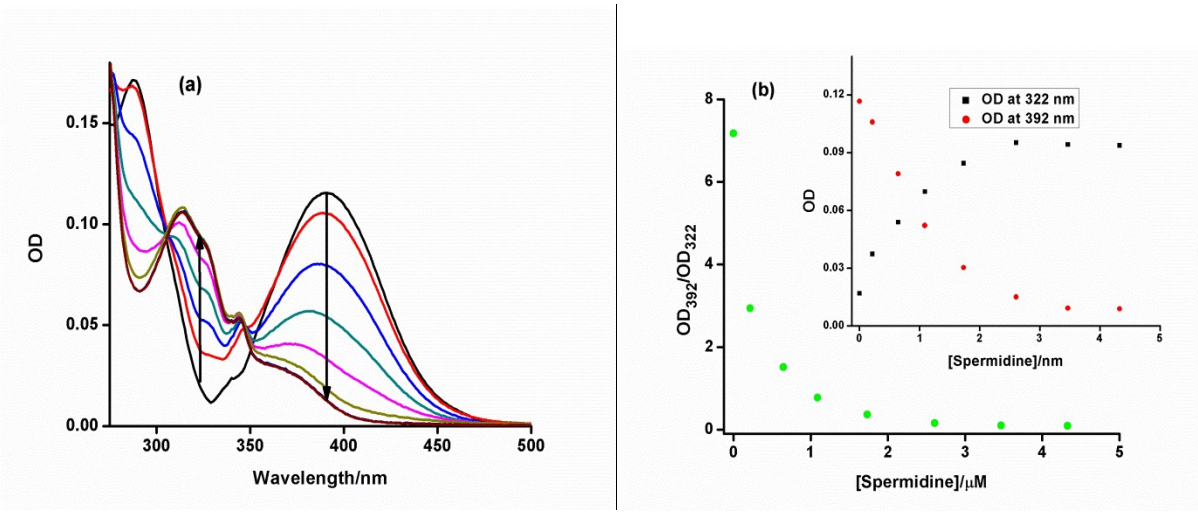


Fig S4 (a) Titration of 3.6 μM ANH with Spermidine (b) Ratiometric plot using OD at two different wavelengths, inset shows the changes in OD at selected wavelengths

(iv) Titration of ANH with Tryptamine

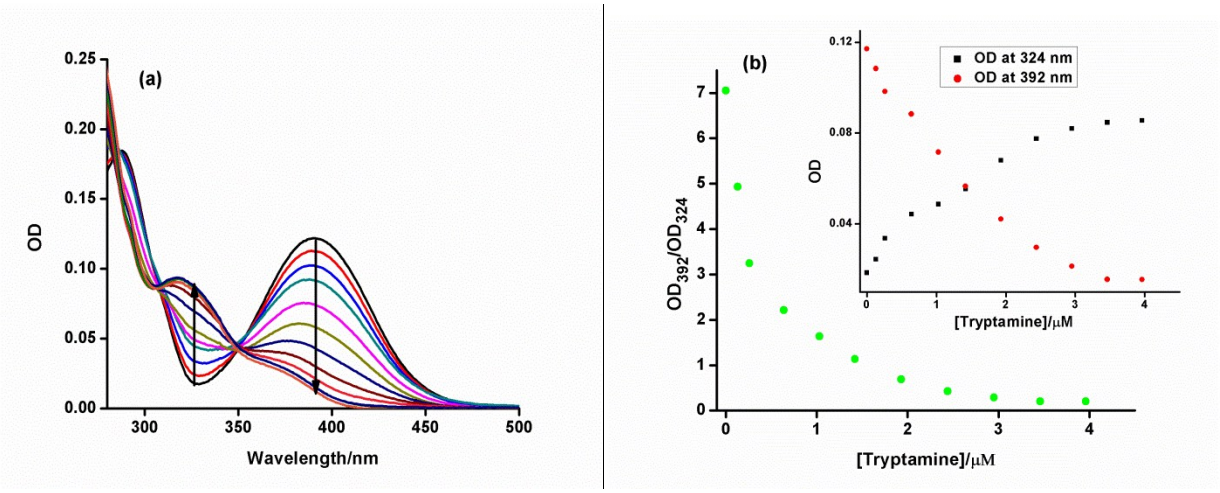


Fig S5 (a) Titration of 3.6 μM ANH with Tryptamine (b) Ratiometric plot using OD at two different wavelengths, inset shows the changes in OD at selected wavelengths

(v) Titration of ANH with Butylamine

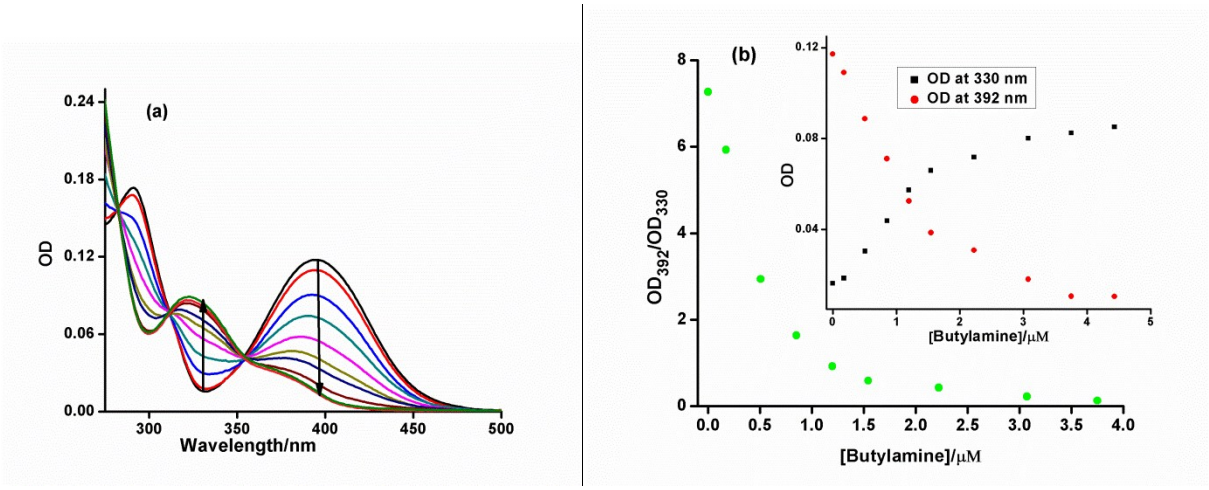


Fig S6 (a) Titration of 3.6 μM ANH with Tryptamine (b) Ratiometric plot using OD at two different wavelengths, inset shows the changes in OD at selected wavelengths

(G) Titration of ANH by UV with secondary amine (Diethylamine)

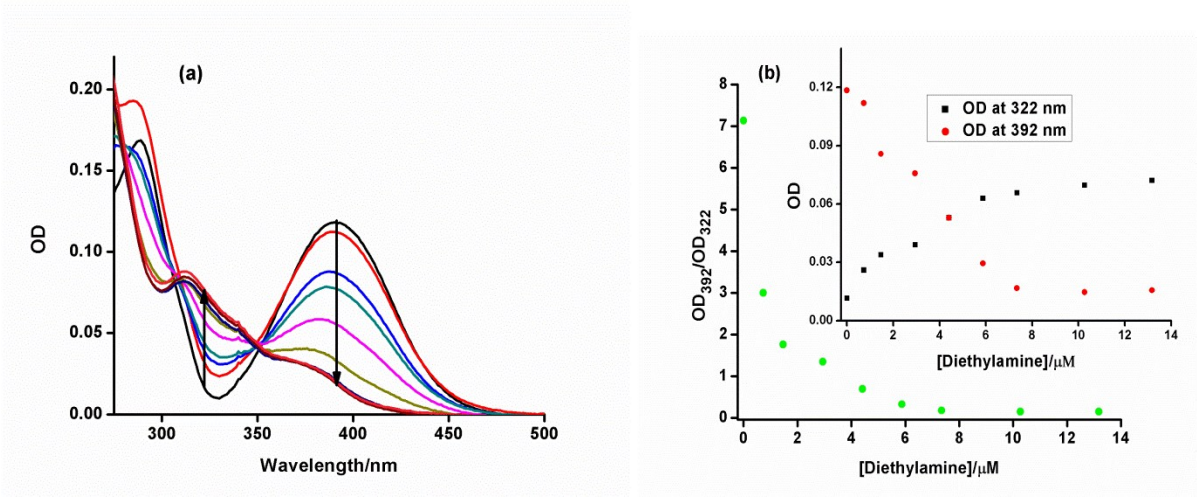


Fig S7 (a) Titration of 3.6 μM ANH with Diethylamine (b) Ratiometric plot using OD at two different wavelengths, inset shows the changes in OD at selected wavelengths

(H) Titration of ANH by UV with tertiary amine (Triethylamine)

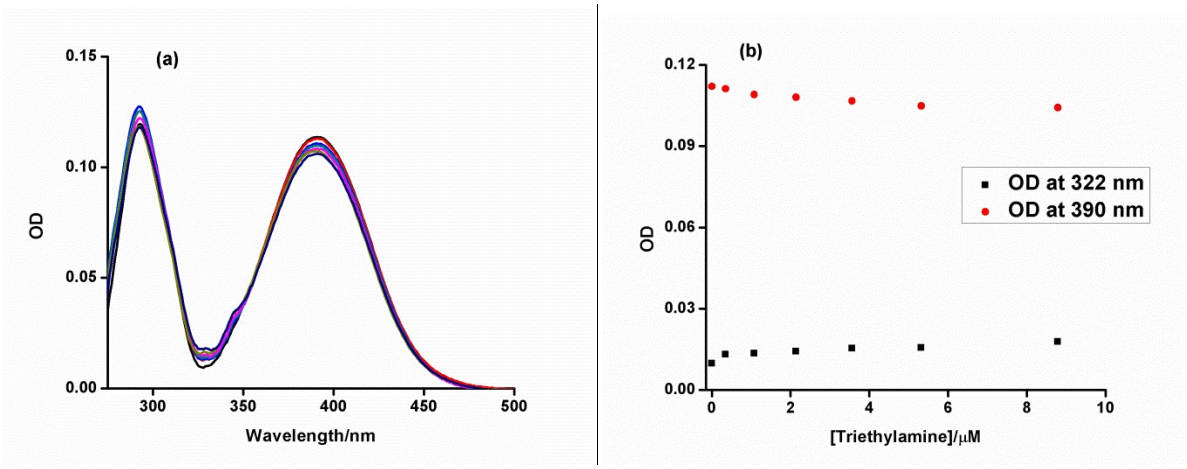


Fig S8 (a) Titration of 3.6 μM ANH with Triethylamine (b) the changes in OD at selected wavelengths

(I) Titration of ANH by fluorescence with different primary amines including BAs

(i) Titration of ANH with 1,4-Butanediamine

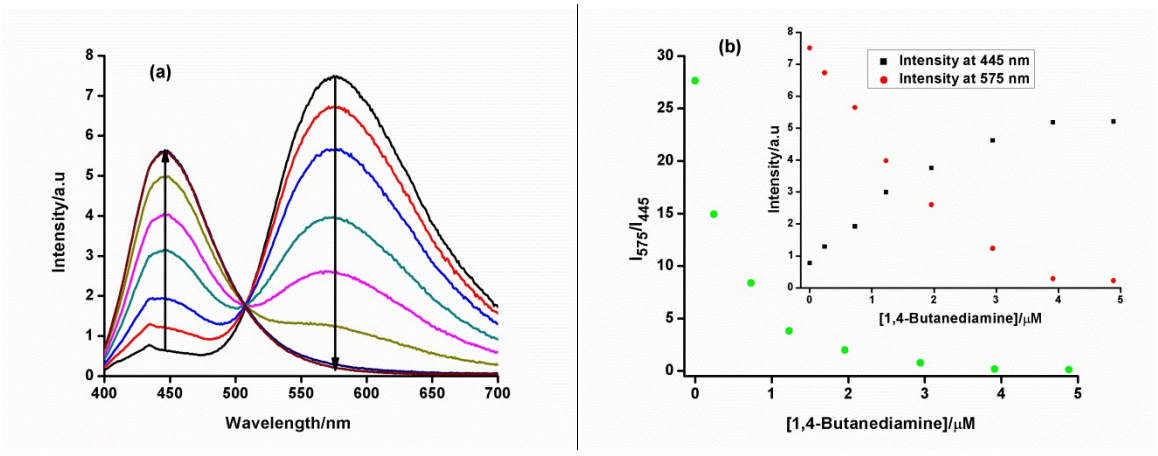


Fig S9 (a) Titration of 3.6 μM ANH with 1,4-Butanediamine (b) Ratiometric plot using fluorescence intensity at two different wavelengths, inset shows the changes in fluorescence intensity at selected wavelengths

(ii) Titration of ANH with 1,6-Hexanediamine

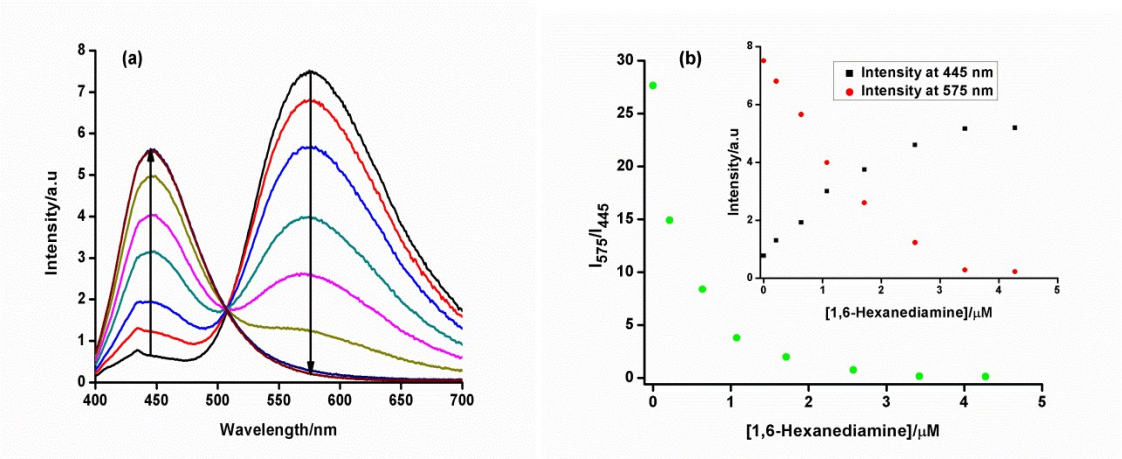


Fig S10 (a) Titration of 3.6 μM ANH with 1,6-Hexanediamine (b) Ratiometric plot using fluorescence intensity at two different wavelengths, inset shows the changes in fluorescence intensity at selected wavelengths

(iii) Titration of ANH with Spermidine

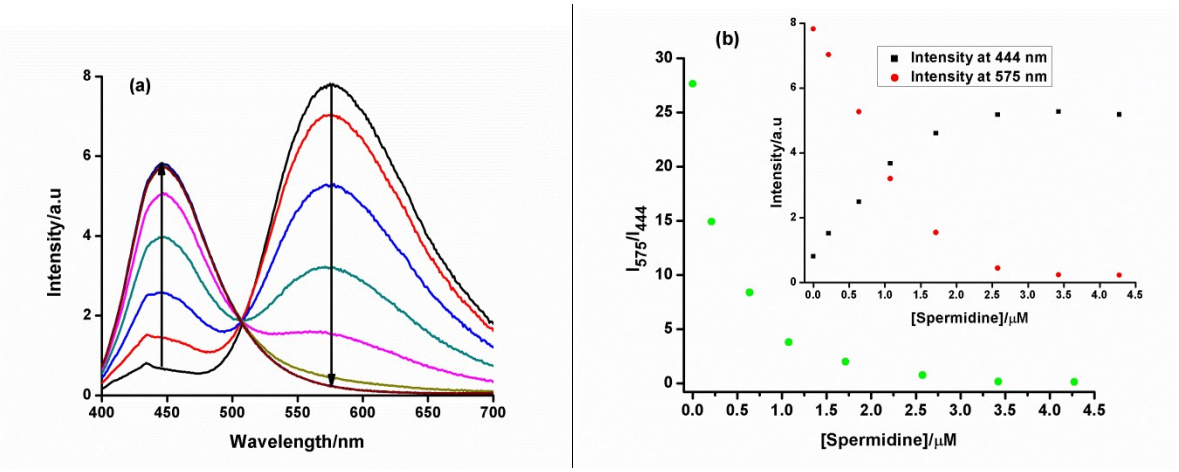


Fig S11 (a) Titration of 3.6 μM ANH with Spermidine (b) Ratiometric plot using fluorescence intensity at two different wavelengths, inset shows the changes in fluorescence intensity at selected wavelengths

(iv) Titration of ANH with Tryptamine

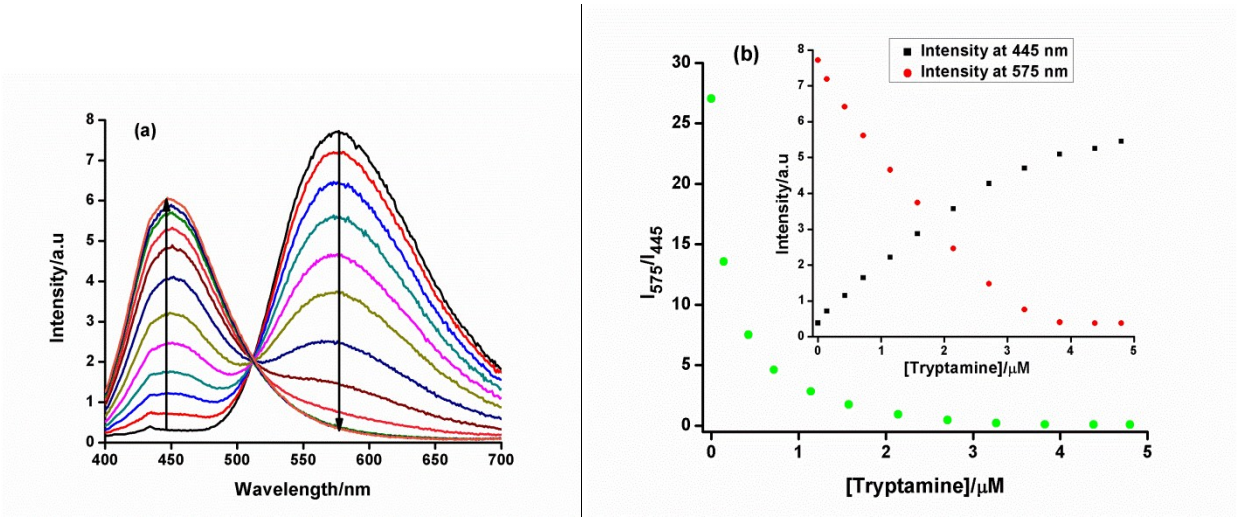


Fig S12 (a) Titration of 3.6 μM ANH with Tryptamine (b) Ratiometric plot using fluorescence intensity at two different wavelengths, inset shows the changes in fluorescence intensity at selected wavelengths

(v) Titration of ANH with Butylamine

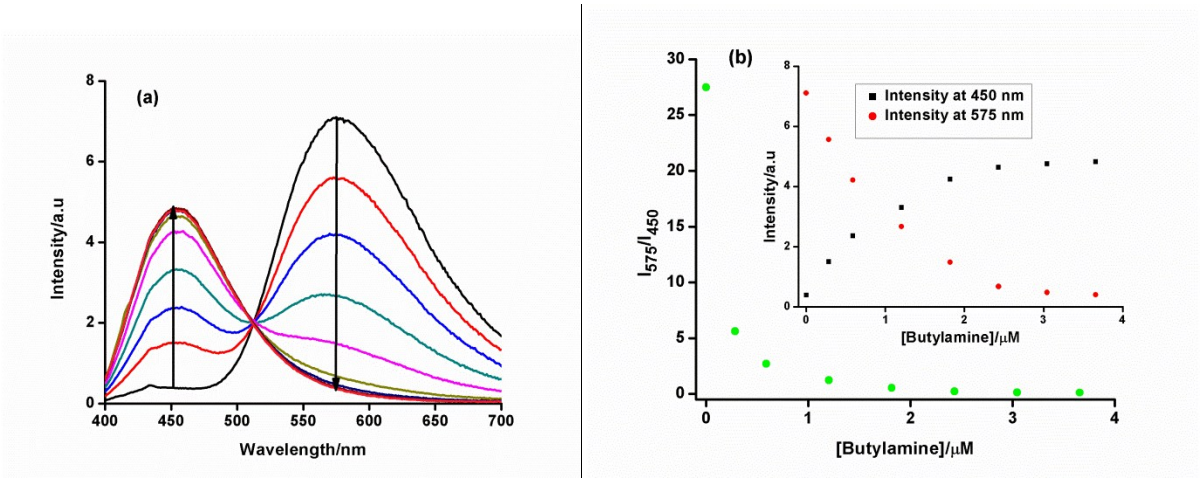


Fig S13 (a) Titration of 3.6 μM ANH with Butylamine (b) Ratiometric plot using fluorescence intensity at two different wavelengths, inset shows the changes in fluorescence intensity at selected wavelengths

(J) Titration of ANH by fluorescence with secondary amine (Diethylamine)

Titration of ANH with Diethylamine

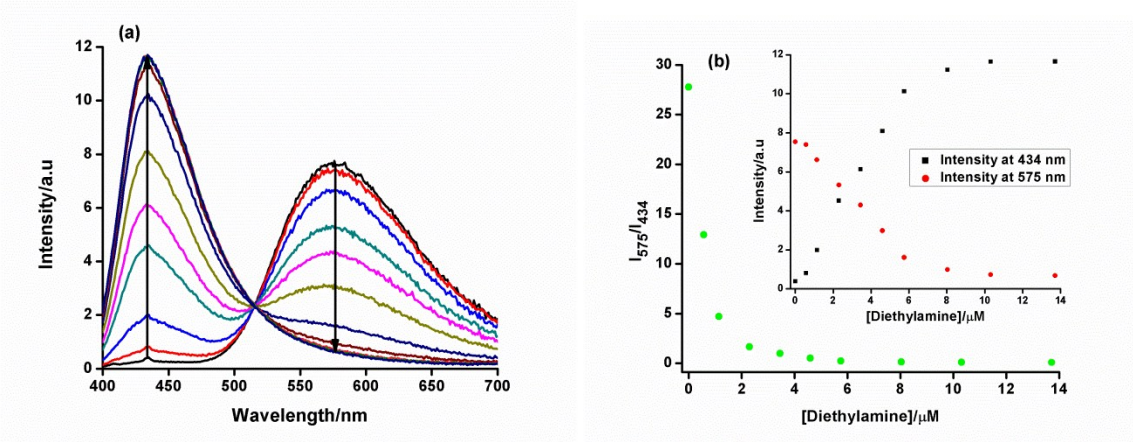


Fig S14 (a) Titration of 3.6 μM ANH with Diethylamine (b) Ratiometric plot using fluorescence intensity at two different wavelengths, inset shows the changes in fluorescence intensity at selected wavelengths

(K) Titration of ANH by fluorescence with tertiary amine (Triethylamine)

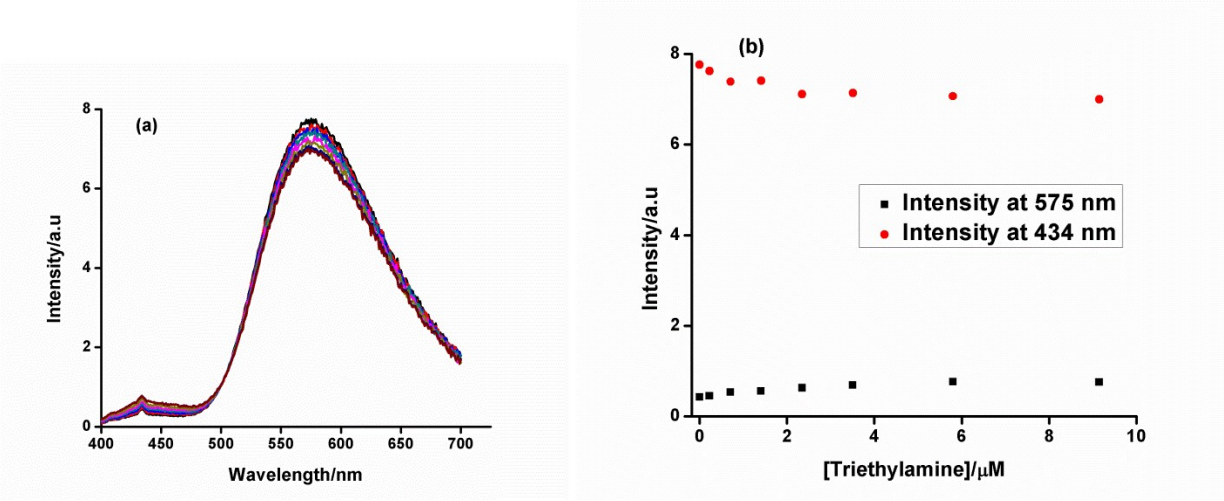


Fig S15 (a) Titration of ANH with Triethylamine (b) The changes in fluorescence intensity at selected wavelengths

(L) Testing the effect of other functional groups on ANH fluorescence

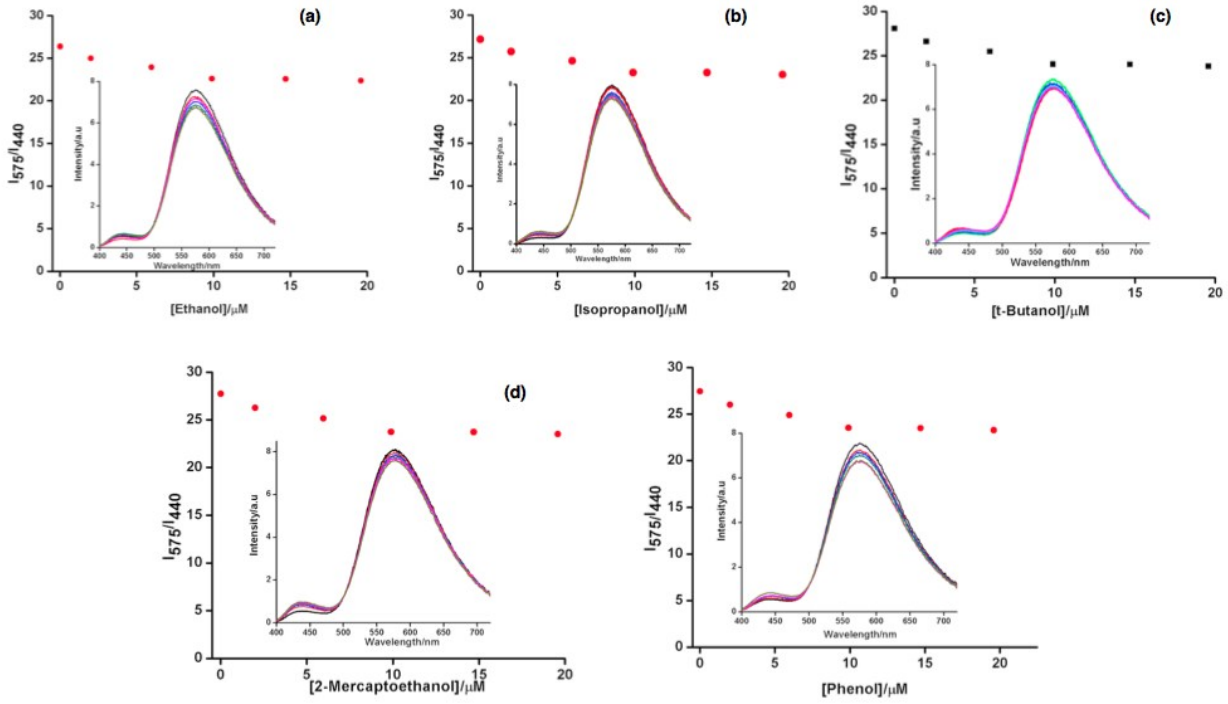


Fig S16 (a) Titration of ANH in DMA with (a) primary alcohol (ethanol); (b) secondary alcohol (isopropanol); (c) tertiary alcohol (*tert*-butanol); (d) aliphatic primary thiol (2-mercaptoethanol); and (e) aromatic alcohol (phenol).

(M) Kinetic evaluation of the reaction of ANH with primary and secondary amines

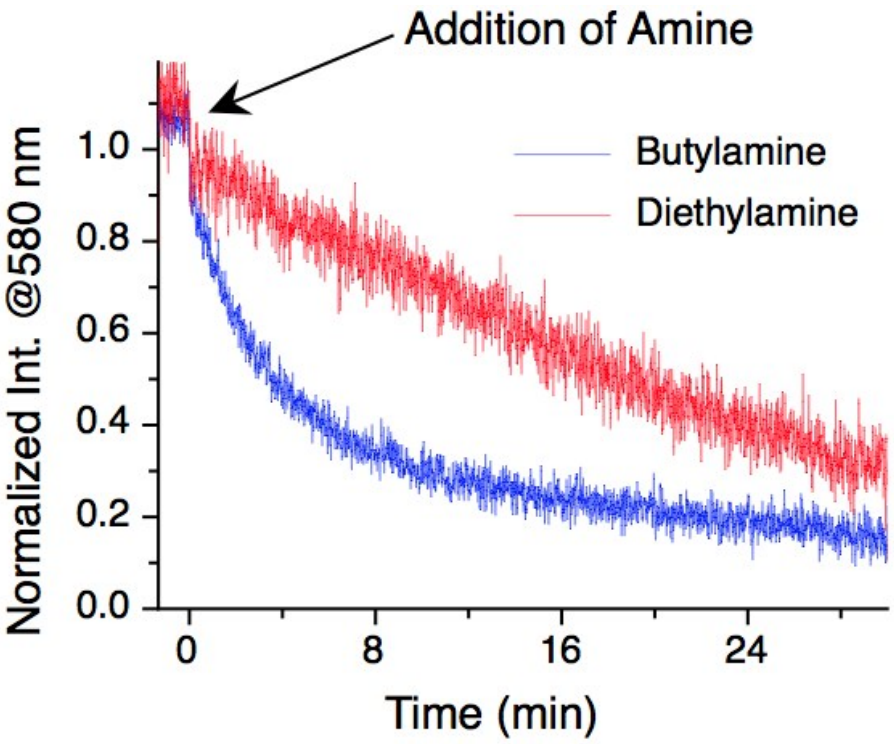
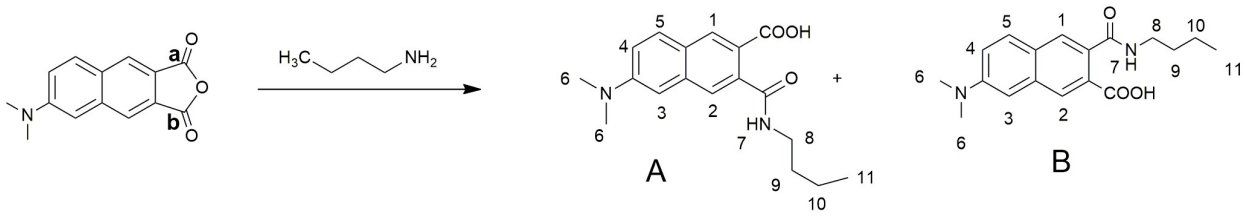


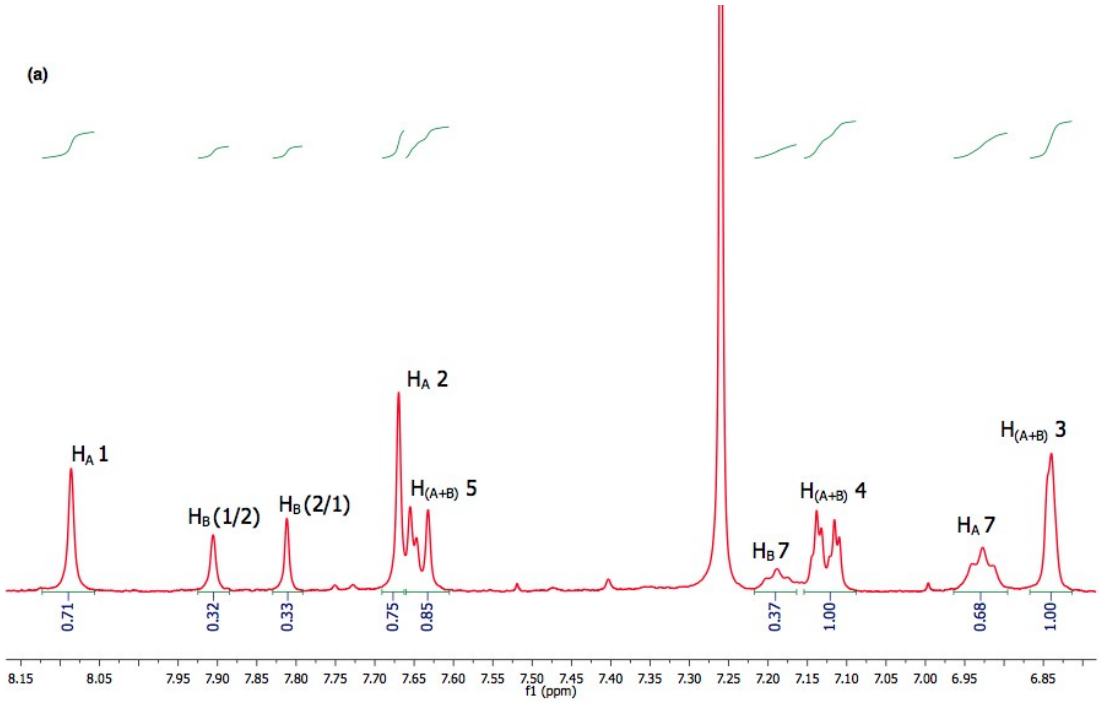
Fig S17 Kinetic monitoring of ANH intensity at 575 nm upon addition of 3.5 μM of primary amine (Butylamine) and 10 μM secondary amine (Diethylamine)

(N) NMR spectrum of the product formed by the interaction of ANH with Butylamine

Structure of regioisomeric products obtained from the reaction between ANH and Butylamine:



From the structure of ANH it is evident that the carbonyl carbon centre at **a** position (as mentioned above in the scheme) is less electrophilic due to participating in direct conjugation, as it is situated at *para* position with respect to the *N,N*-dimethylamine functional group, than the carbonyl carbon centre at the **b** position. So, a favorable attack of the primary amine group at the carbonyl carbon centre at **b**, leads to the major product (A) to be formed than (B) with *ca.* 2:1 ratio.


(a)
H_A 1
H_B (1/2)
H_B (2/1)
H_A 2
$H_{(A+B)}$ 5
H_B 7
$H_{(A+B)}$ 4
H_A 7
$H_{(A+B)}$ 3
0.71
0.32
0.33
0.75
0.85
0.37
1.00
0.68
1.00
8.15 8.05 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.15 7.10 7.05 7.00 6.95 6.85
f1 (ppm)

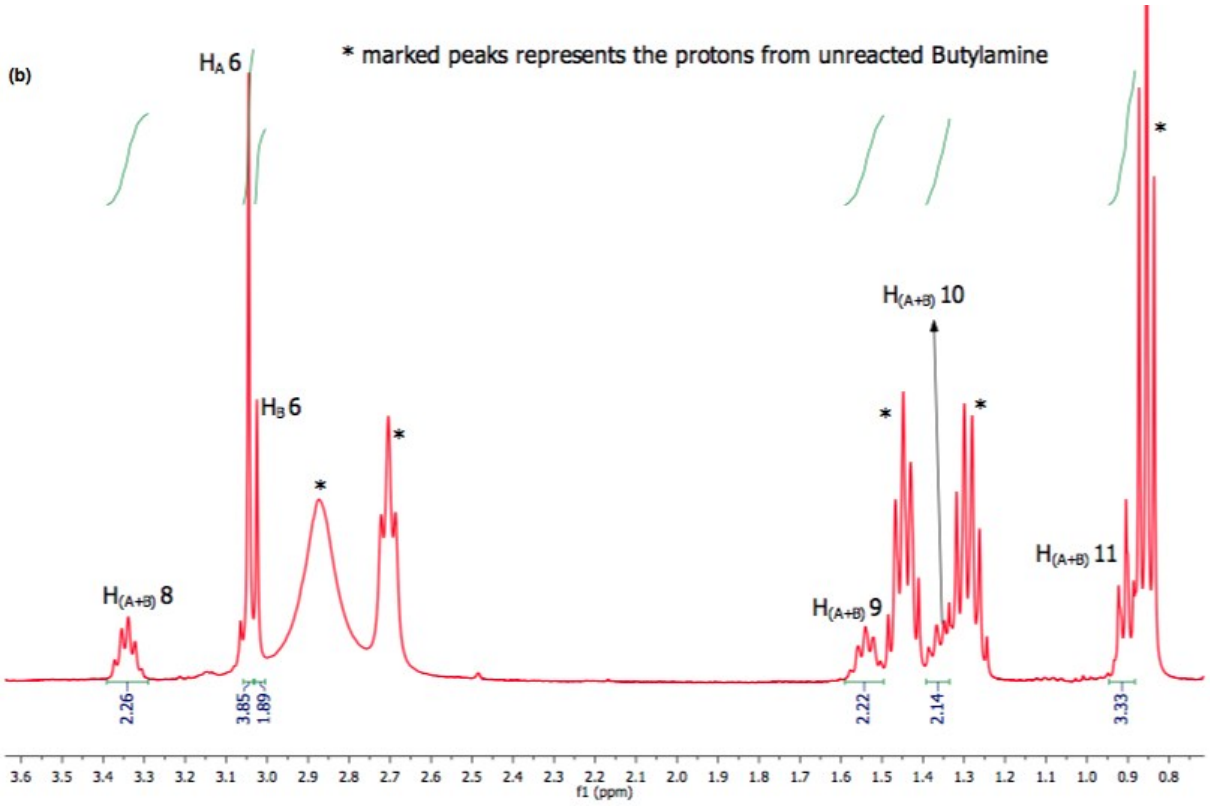
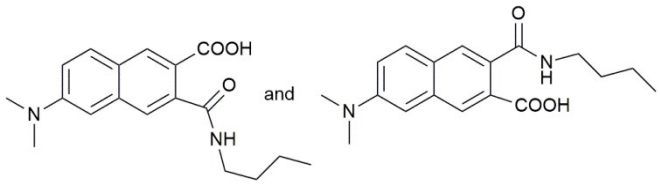


Fig S18: NMR spectra of regioisomeric products obtained after the reaction of ANH with Butylamine in $CDCl_3$ (a) aromatic part and (b) aliphatic part; from the integration *ca.* 2:1 ratio of the products are obtained.

(O) Mass spectra of the products when ANH reacted with Butylamine

Regioisomeric structures of ANH-Butylamine products:



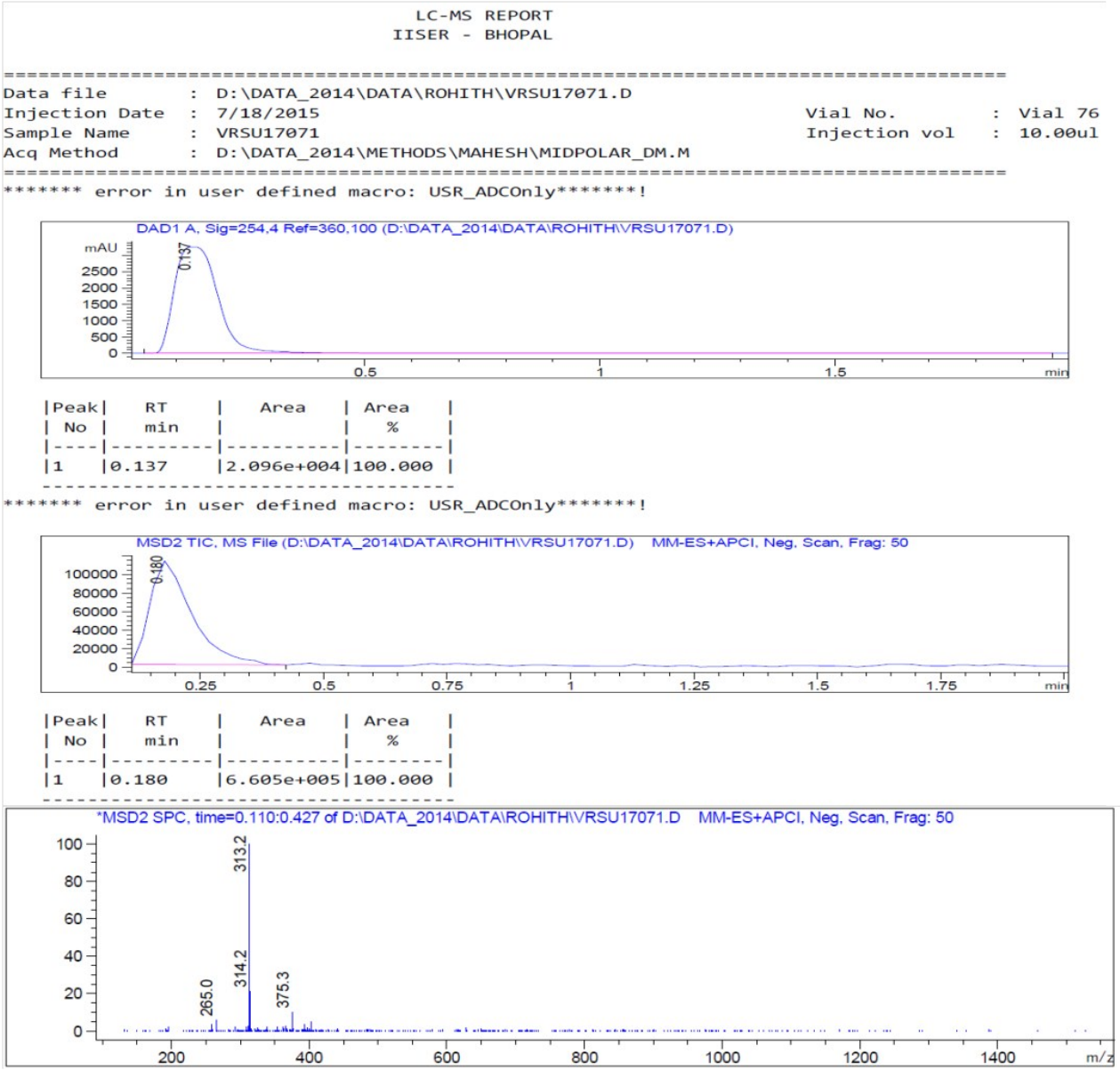
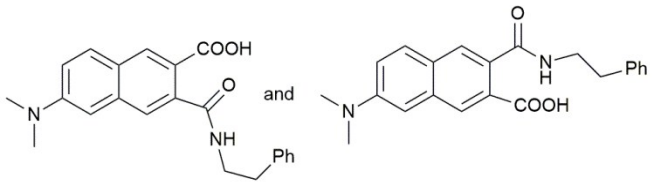


Fig S19: Exact mass (M) calculated 314.16, mass obtained in negative scan mode 314.2 (molecular ion peak) and 313.2 (M^- ion peak).

(P) Mass spectra of the products when ANH reacted with Phenethylamine

Regioisomeric structures of ANH-Phenethylamine products:



LC-MS REPORT
IISER - BHOPAL

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Sample Name      :  VRSUPH                             Injection vol   :  10.00u
Acq Method       :  D:\DATA_2014\METHODS\SRINIVAS\MIDPOLAR_DM.M
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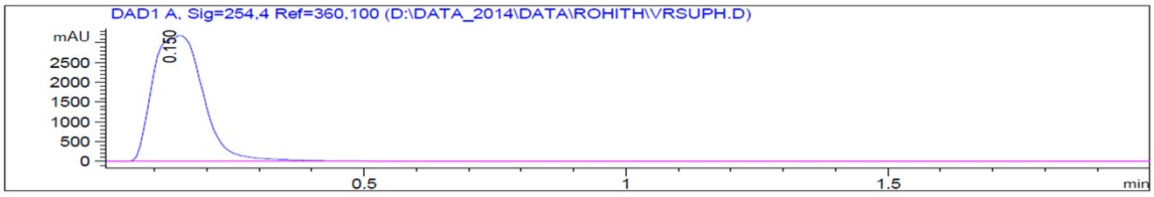

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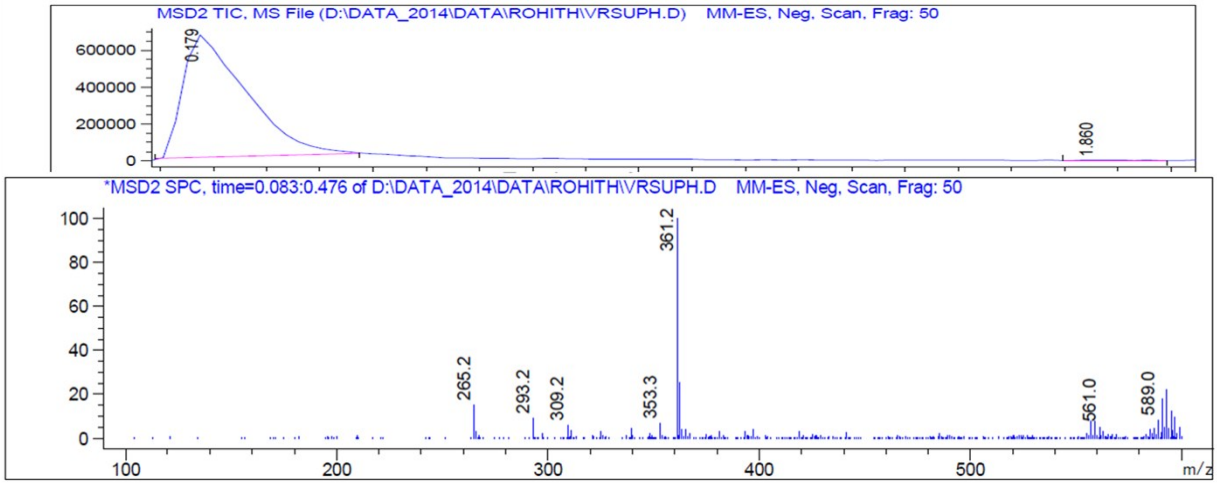
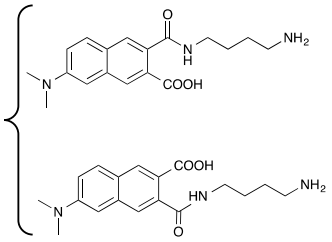


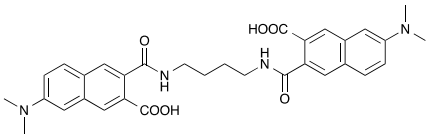
Fig S20: Exact mass (M) calculated 362.16, mass obtained in negative scan mode 361.2 (M^- ion peak).

(Q) Mass spectra of the products when ANH reacted with 1,4-Butanediamine

Monomeric adduct



Chemical Formula: $C_{18}H_{23}N_3O_3$
Exact Mass: 329.174
Molecular Weight: 329.400



Not observed

Chemical Formula: $C_{32}H_{34}N_4O_6$
Exact Mass: 570.248
Molecular Weight: 570.646

LC-MS REPORT
IISER - BHOPAL

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Sample Name     : VRSU14                             Injection vol  : 10.00ul
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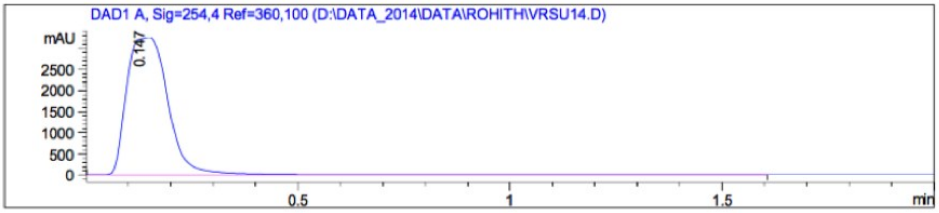

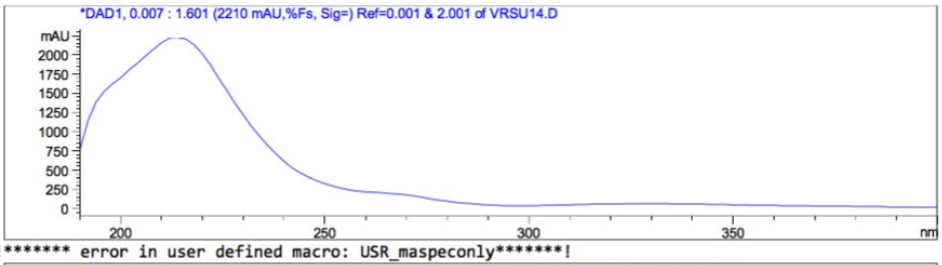


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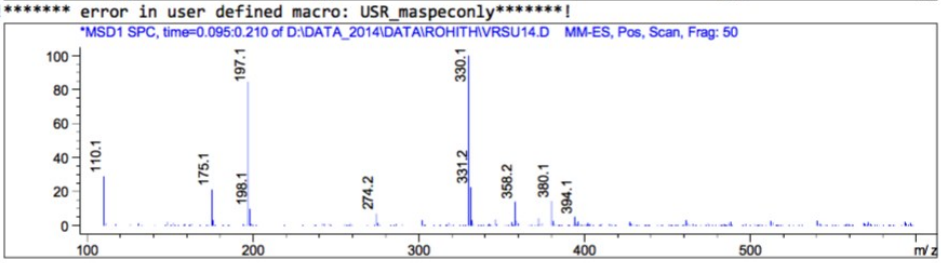
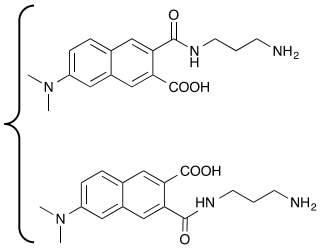


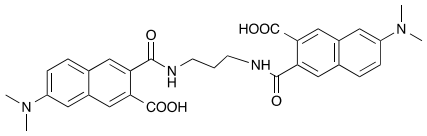
Fig S21: Exact mass (M) calculated 329.1, mass obtained in positive scan mode 330.1 (MH^+ ion peak).

(R) Mass spectra of the products when ANH reacted with 1,3-Propanediamine

Monomeric adduct



Chemical Formula: $C_{17}H_{21}N_3O_3$
Exact Mass: 315.158
Molecular Weight: 315.373



Not observed

Chemical Formula: $C_{31}H_{32}N_4O_6$
Exact Mass: 556.232
Molecular Weight: 556.619

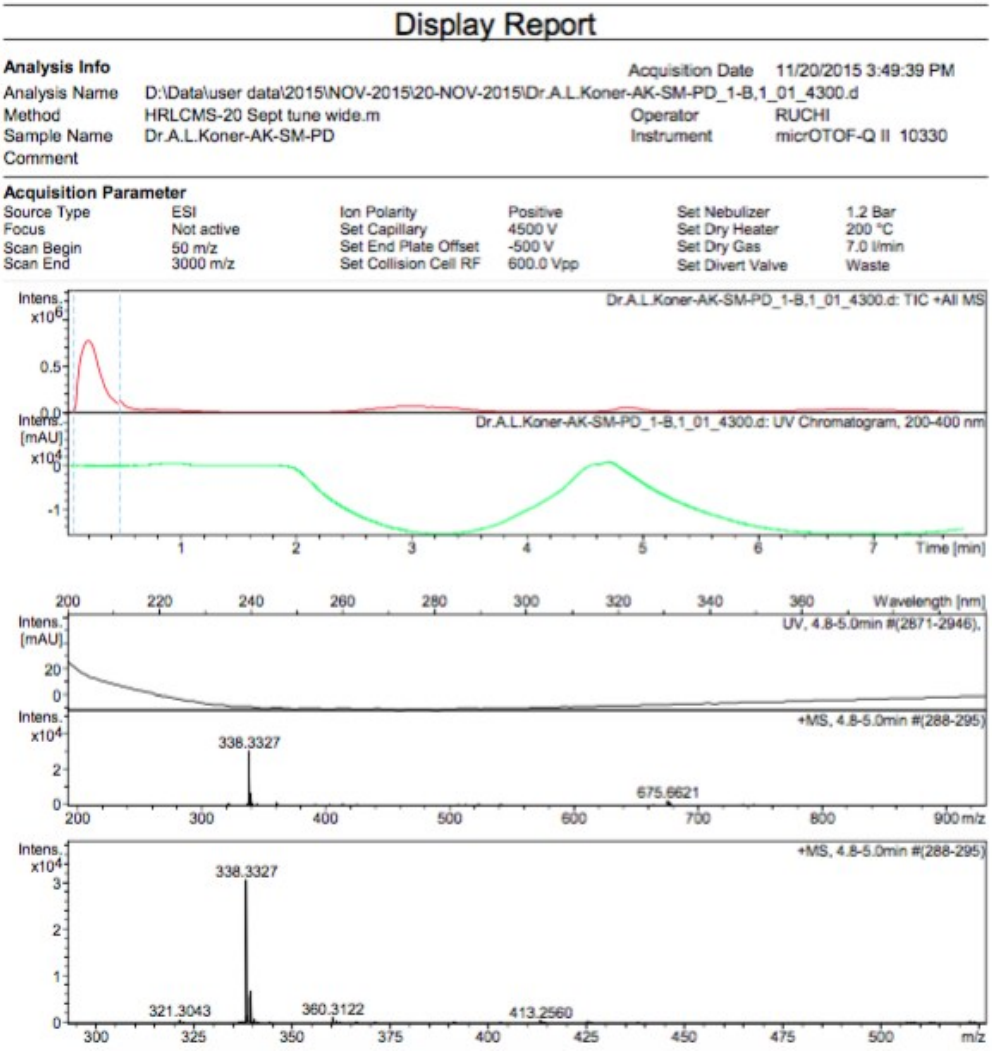
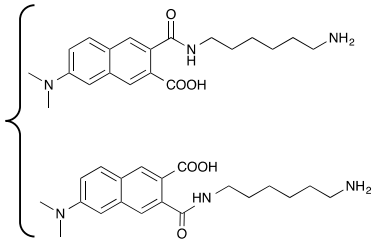


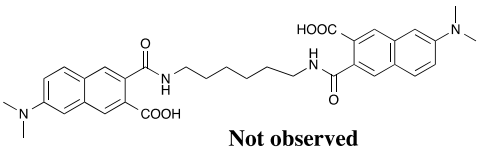
Fig S22: Exact mass (M) calculated 315.158, mass obtained in positive scan mode 338.3327 ($M+Na^+$ ion peak).

(S) Mass spectra of the products when ANH reacted with 1,6-Hexanediamine

Monomeric adduct



Chemical Formula: $C_{20}H_{27}N_3O_3$
Exact Mass: 357.205
Molecular Weight: 357.454



Not observed

Chemical Formula: $C_{34}H_{38}N_4O_6$
Exact Mass: 598.279
Molecular Weight: 598.700

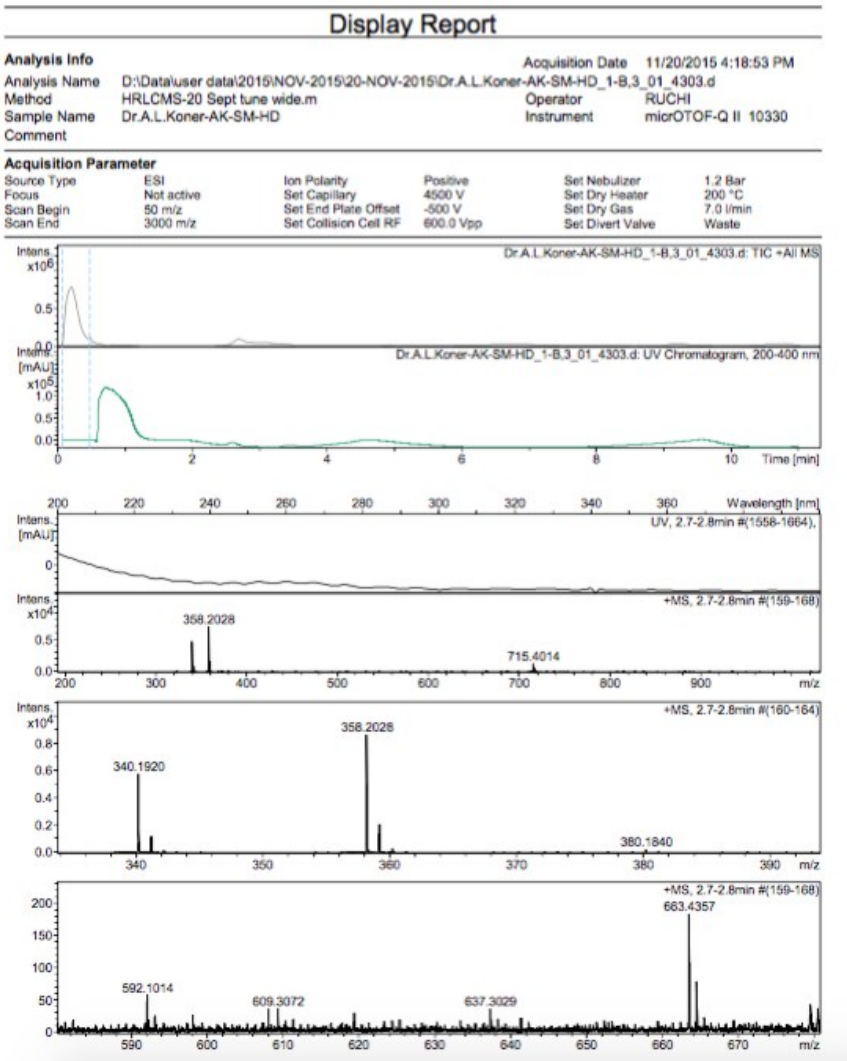
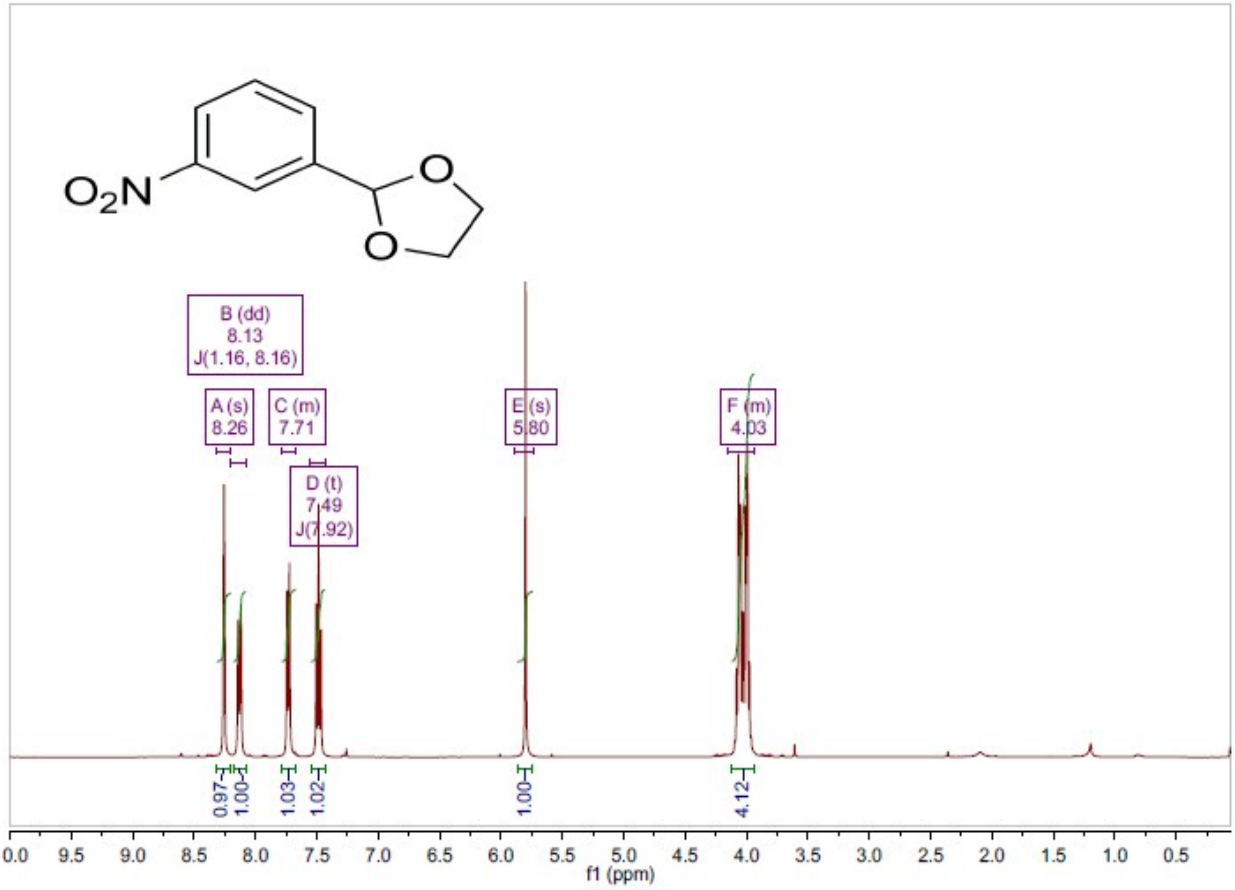


Fig S23: Exact mass (M) calculated 357.205, mass obtained in positive scan mode 358.203 (MH^+ ion peak).

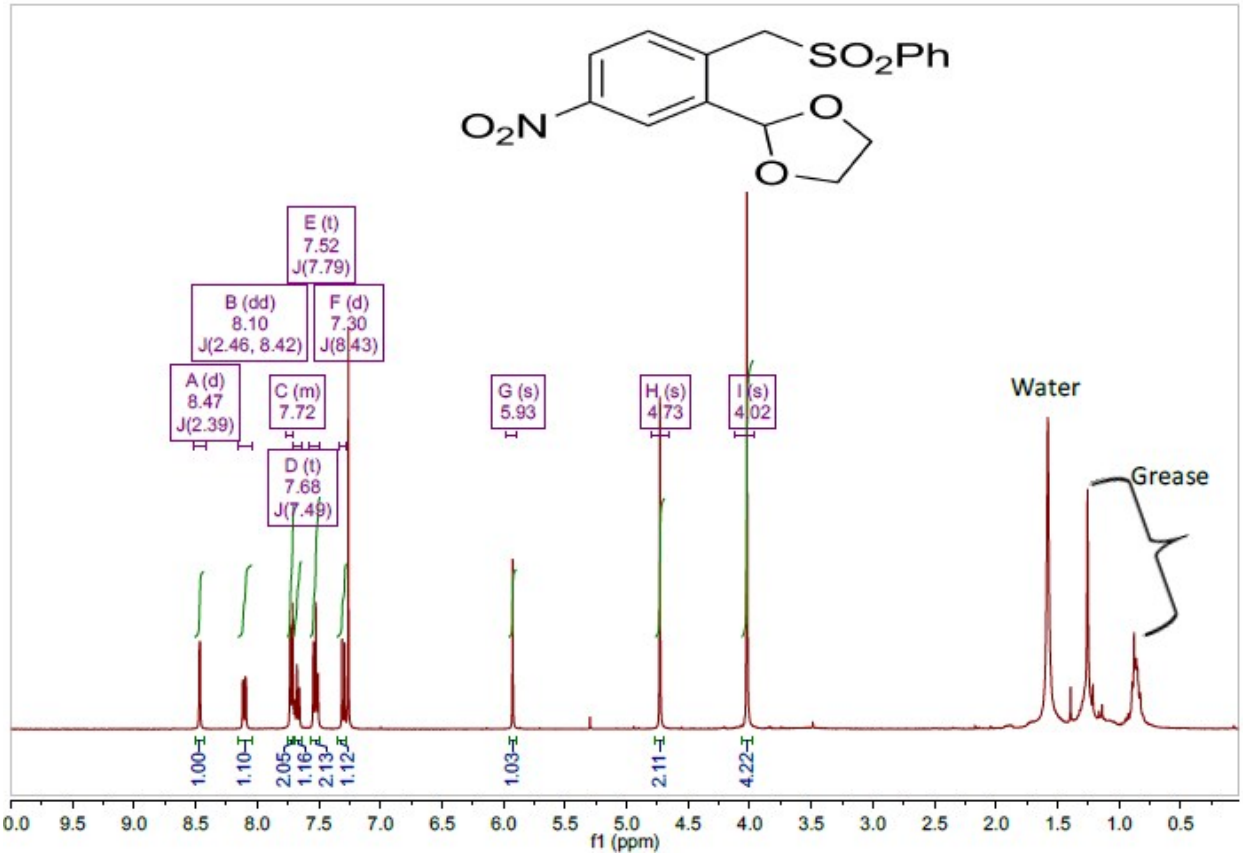
Characterization of synthesized compounds (2, 3, 5, 6, and 8) by ^{1}H NMR:

Compound 2



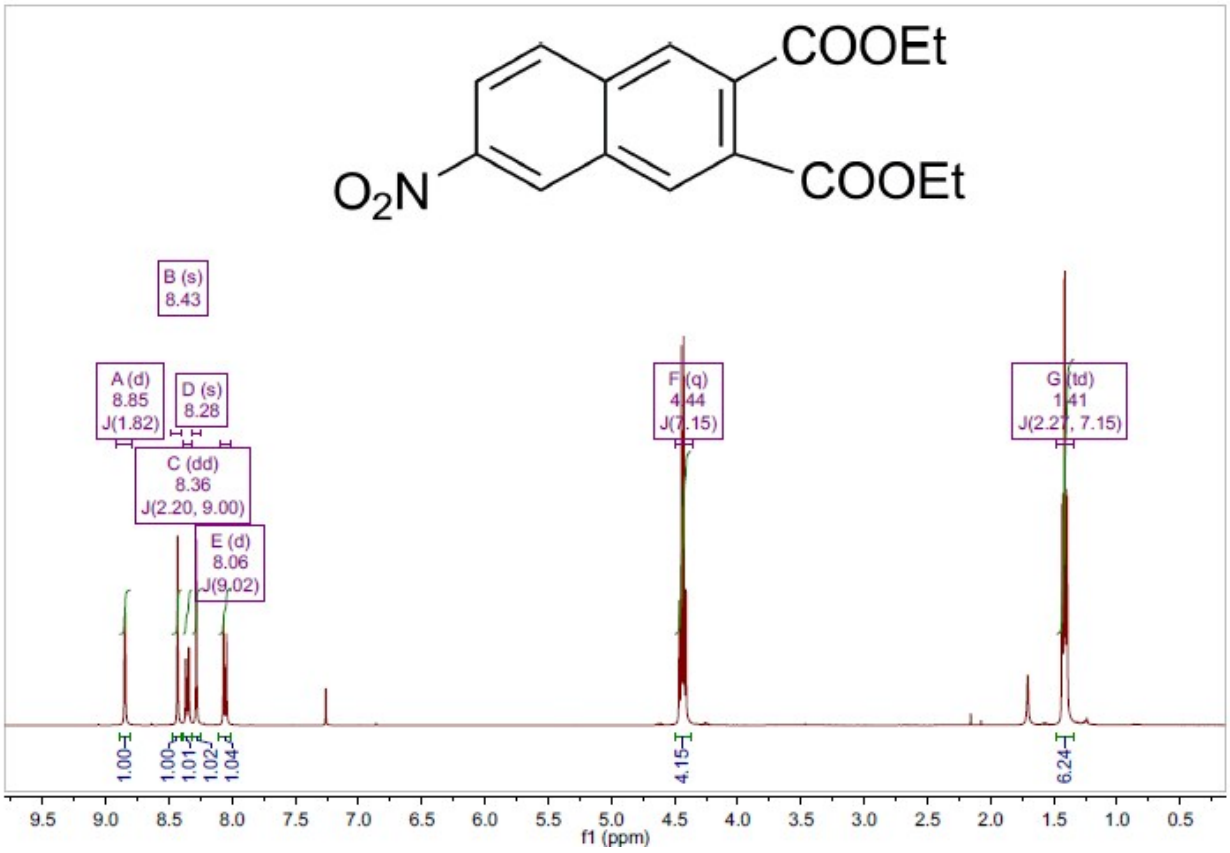
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 8.13 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.79 – 7.68 (m, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 5.80 (s, 1H), 4.15 – 3.94 (m, 4H).

Compound 3



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 2.4 Hz, 1H), 8.10 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.76 – 7.70 (m, 2H), 7.68 (t, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 1H), 5.93 (s, 1H), 4.73 (s, 2H), 4.02 (s, 4H).

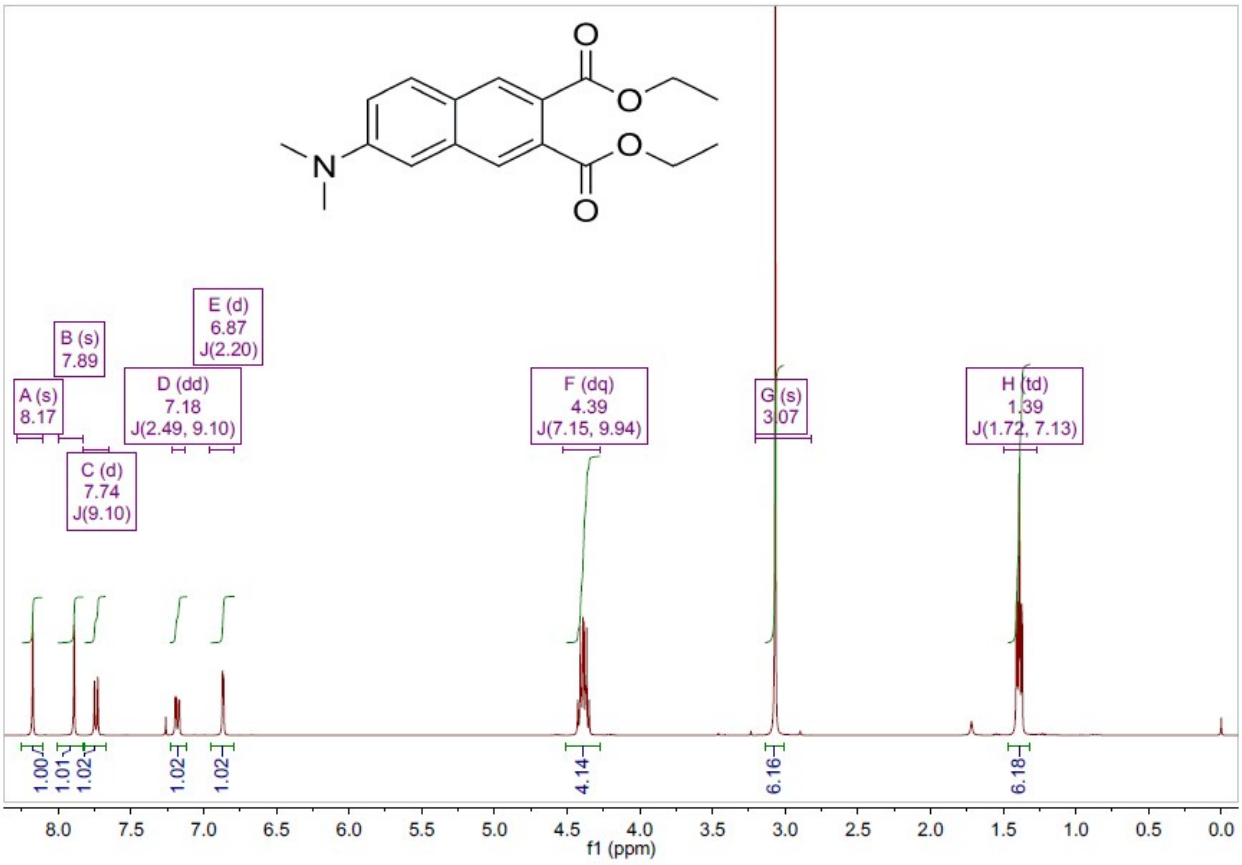
Compound 5:



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 1.8 Hz, 1H), 8.43 (s, 1H), 8.36 (dd, *J* = 9.0, 2.2 Hz, 1H), 8.28 (s, 1H), 8.06 (d, *J* = 9.0 Hz, 1H), 4.44 (q, *J* = 7.1 Hz, 4H), 1.41 (td, *J* = 7.1, 2.3 Hz, 6H).

Compound 6

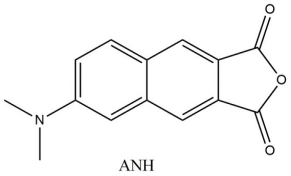
^{1}H NMR



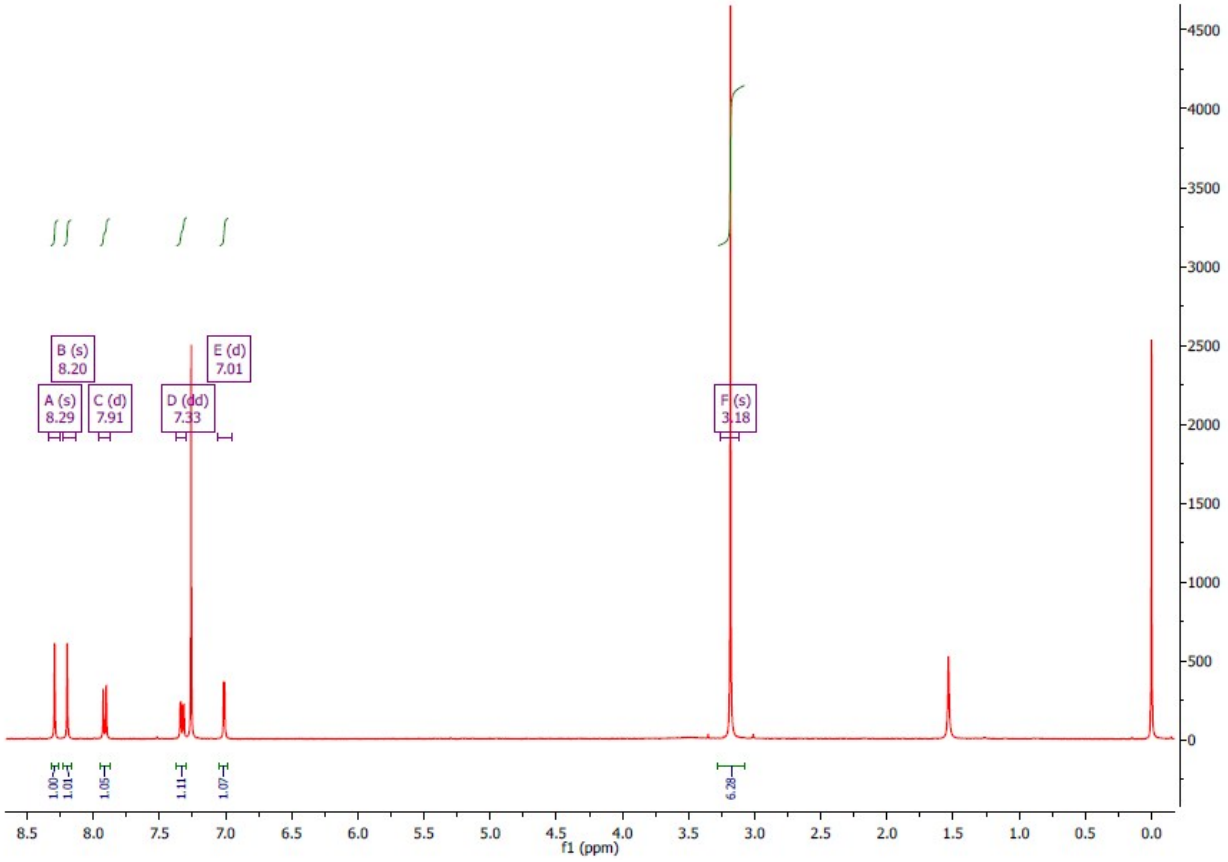
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (1 H, s), 7.89 (1 H, s), 7.74 (1 H, d, *J* 9.1), 7.18 (1 H, dd, *J* 9.1, 2.5), 6.87 (1 H, d, *J* 2.2), 4.39 (4 H, dq, *J* 9.9, 7.1), 3.07 (6 H, s), 1.39 (6 H, td, *J* 7.1, 1.7).

Compound ANH (8)

^{1}H NMR

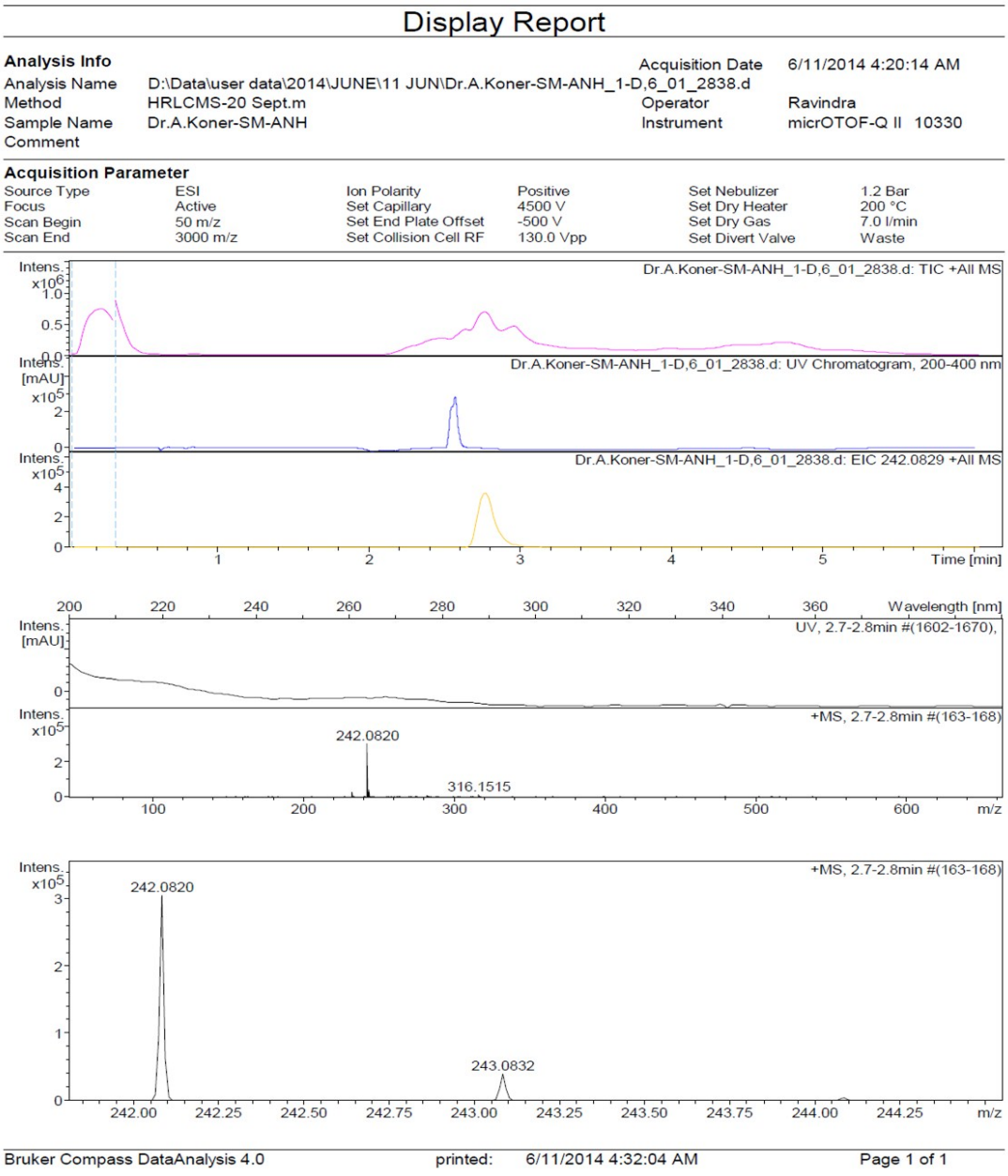


ANH



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (1 H, s), 8.20 (1 H, s), 7.91 (1 H, d, *J* 9.2), 7.33 (1 H, dd, *J* 9.2, 2.6), 7.01 (1 H, d, *J* 2.4), 3.18 (6 H, s).

Mass spectra of ANH (8)



Exact mass (M) calculated 241.0739, Mass obtained (+MS) 242.0820 *i.e* mass of MH^+ was obtained.

References:

1. M. E. Vazquez, J. B. Blanco and B. Imperiali, *J. Am. Chem. Soc.,* 2005, **127**, 1300-1306.

2. K. Baathulaa, Y. Xu and X. Qian, *Nat. Protoc.,* 2011, **6**, 1990-1997.