Rhodamine-based turn-on nitric oxide sensor in aqueous medium with endogenous cell imaging: an unusual formation of nitrosohydroxylamine.

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Supporting Information for Publication

17.	Fluorescence response of the ligand **L^3** in presence of various type of amino-acids.	**Fig. S6.**
18.	LOD of **L^3** + NO.	**Fig.S7**.
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25.	Selected parameters for the vertical excitation (UV-VIS absorptions) of **RH-en-ONO(3)**.	**Table S4**

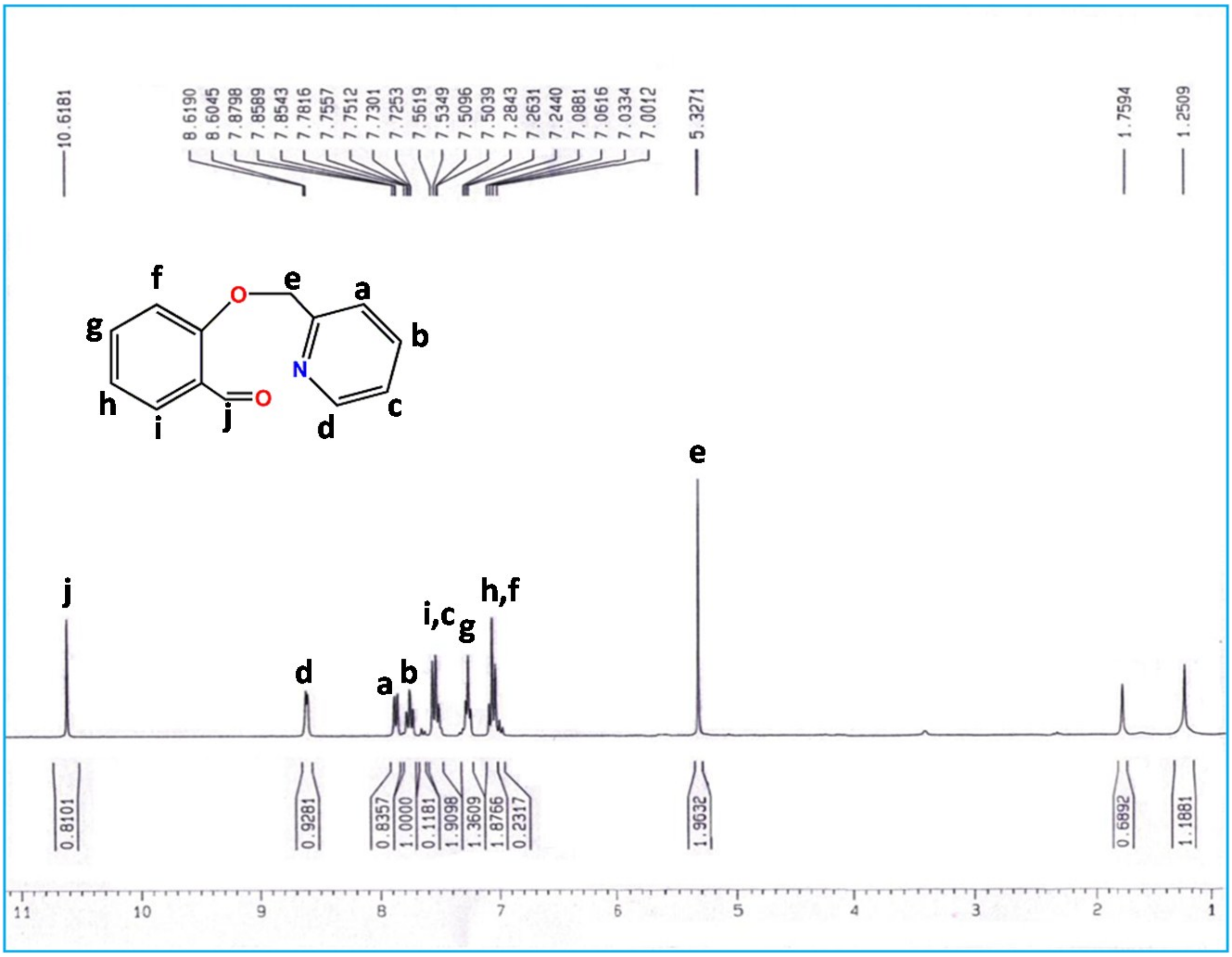


Fig. S1a.^{1}H NMR spectrum of spectrum of (**1**) [aldehyde product(1)] in CDCl$_3$, in Bruker 300 MHz instrument.

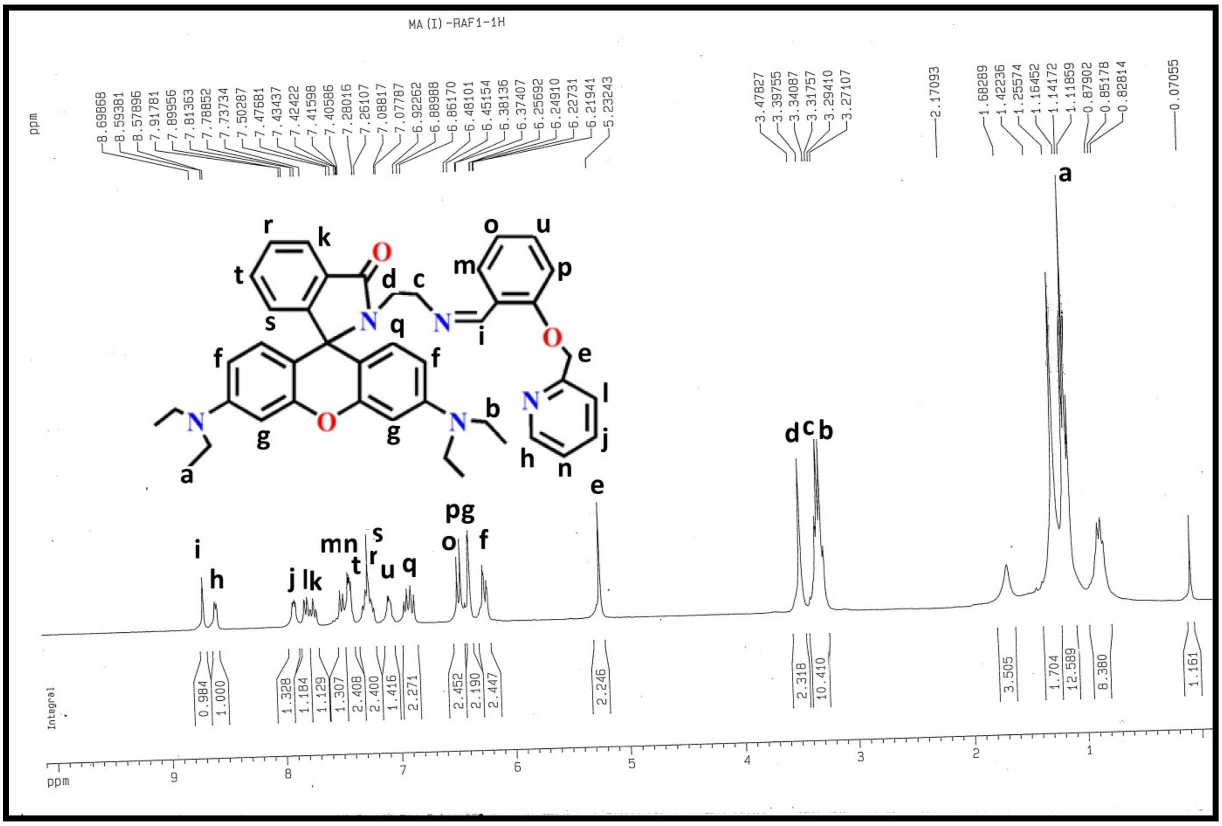


Fig.S1b. ^{1}H NMR spectrum of **L^3** in $CDCl_3$, in Bruker 300 MHz instrument.

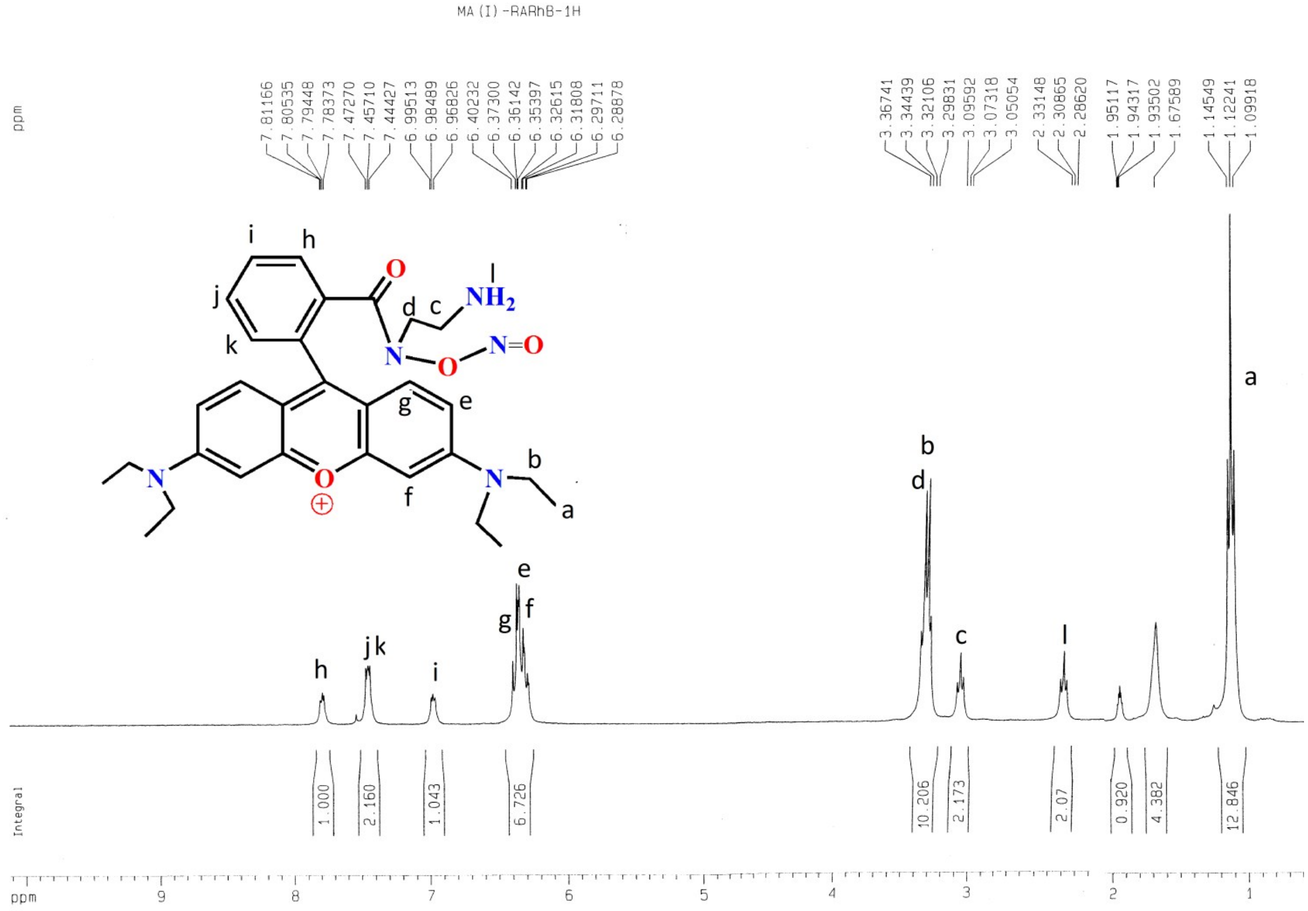


Fig.S1c. ^{1}H NMR spectrum of the amine product **(3)** in CD_3CN solvent after addition of NO solution, in Bruker 300 MHz instrument.

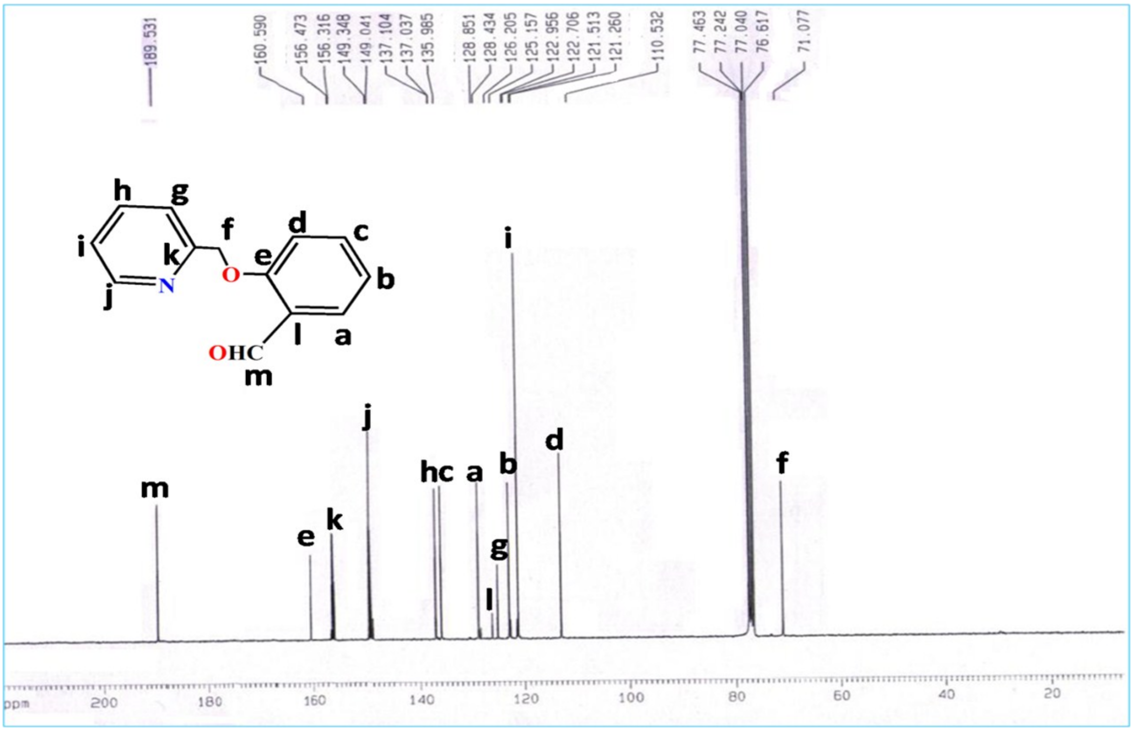
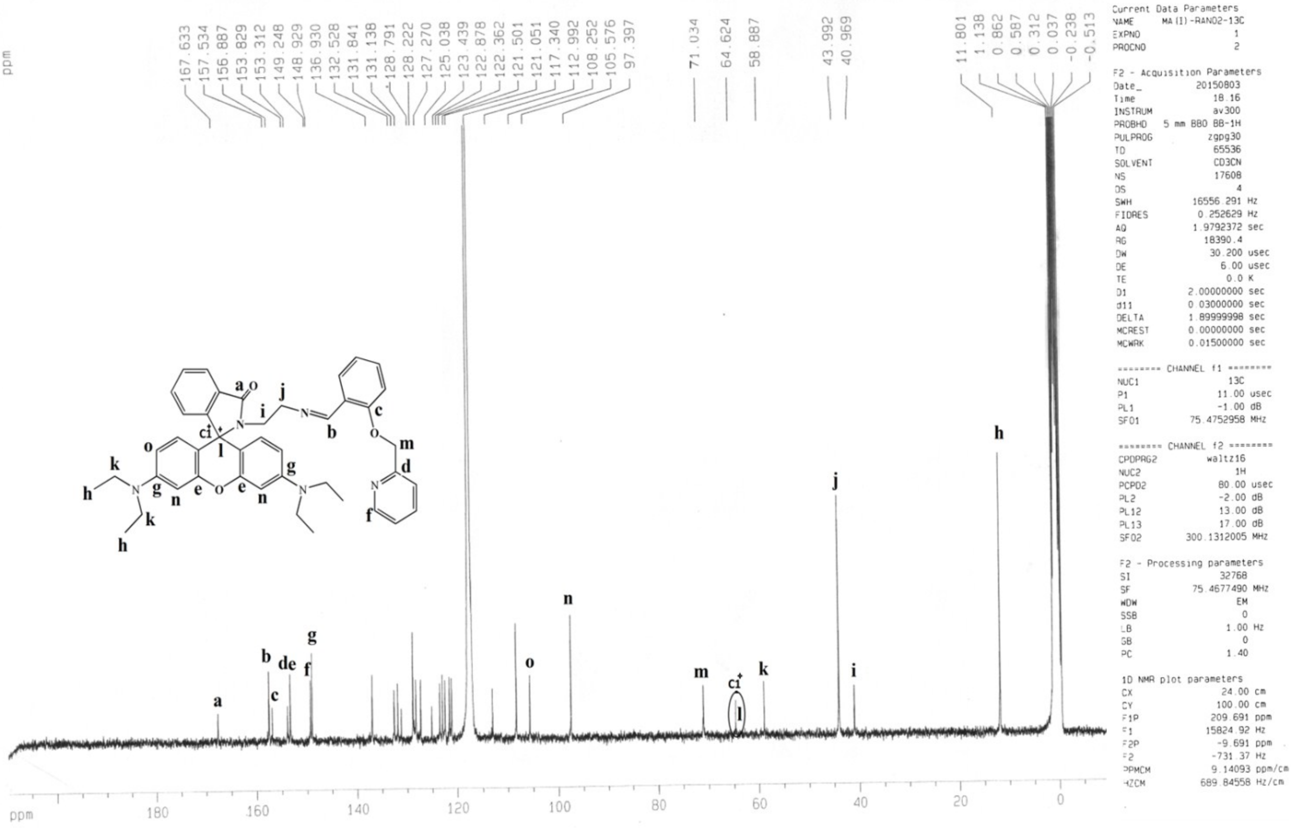


Fig.S2a. ^{13}CNMR spectrum of (**1**) [aldehyde product(1)]in $CDCl_3$, in Bruker 300 MHz instrument.



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FIDRES           0.252629 Hz
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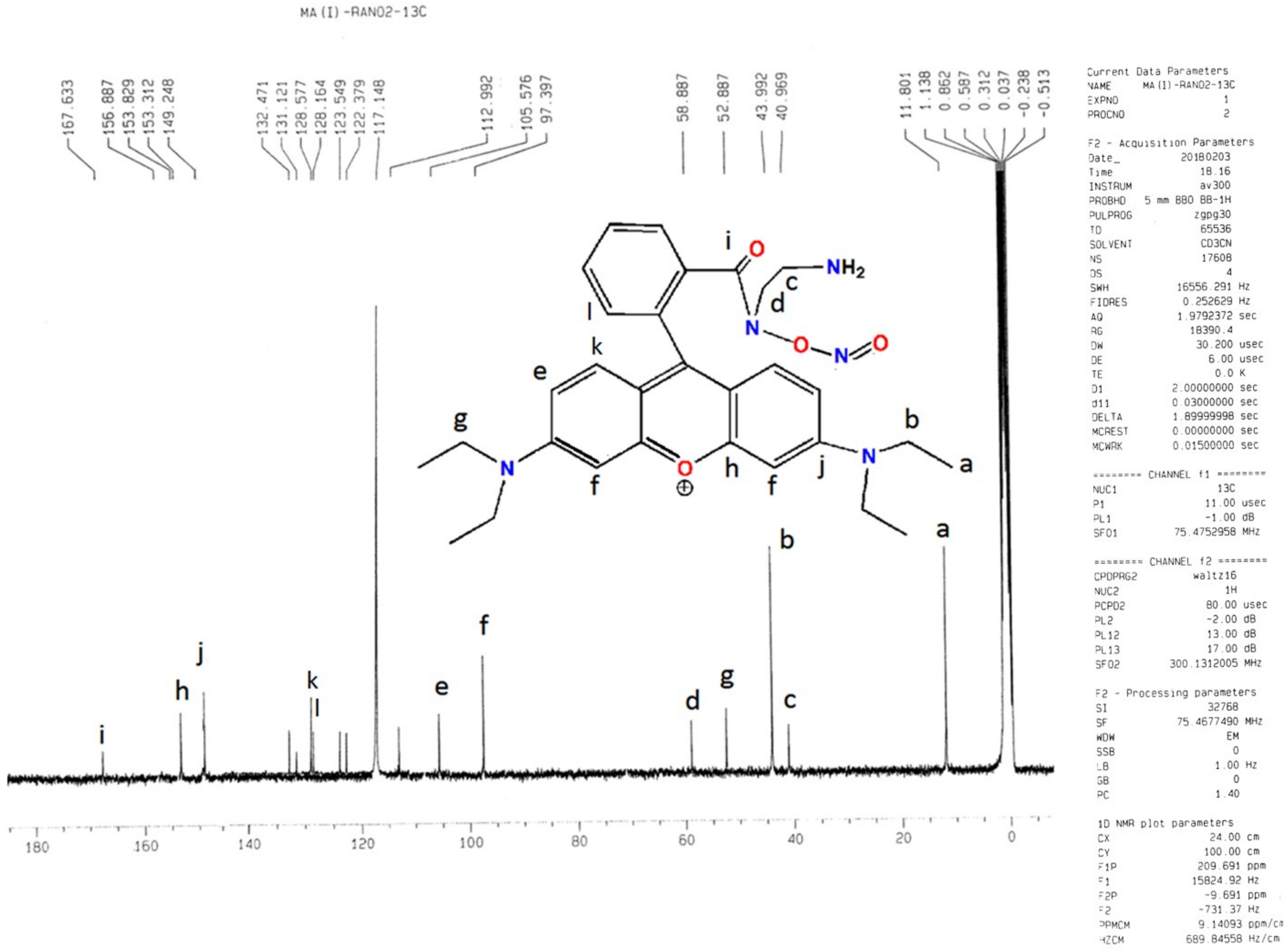
Fig. S2b. ^{13}CNMR spectrum of **L^3** in CD$_3$CN, in Bruker 300 MHz instrument**.**



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SWH             16556.291 Hz
FIDRES           0.252629 Hz
AQ              1.9792372 sec
RG                   18390.4
DW                30.200 usec
DE                  6.00 usec
TE                   0.0 K
D1             2.00000000 sec
d11            0.03000000 sec
DELTA          1.89999998 sec
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MCWRK          0.01500000 sec

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======== CHANNEL f2 ========
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1D NMR plot parameters
CX                  24.00 cm
CY                 100.00 cm
F1P              209.691 ppm
F1              15824.92 Hz
F2P               -9.691 ppm
F2               -731.37 Hz
PPMCM          9.14093 ppm/cm
HZCM         689.84558 Hz/cm
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Fig.S2c. ^{13}C NMR spectra of the amine product(**3**) in CD_3CN solvent after addition of NO solution, in Bruker 300 MHz instrument**.**

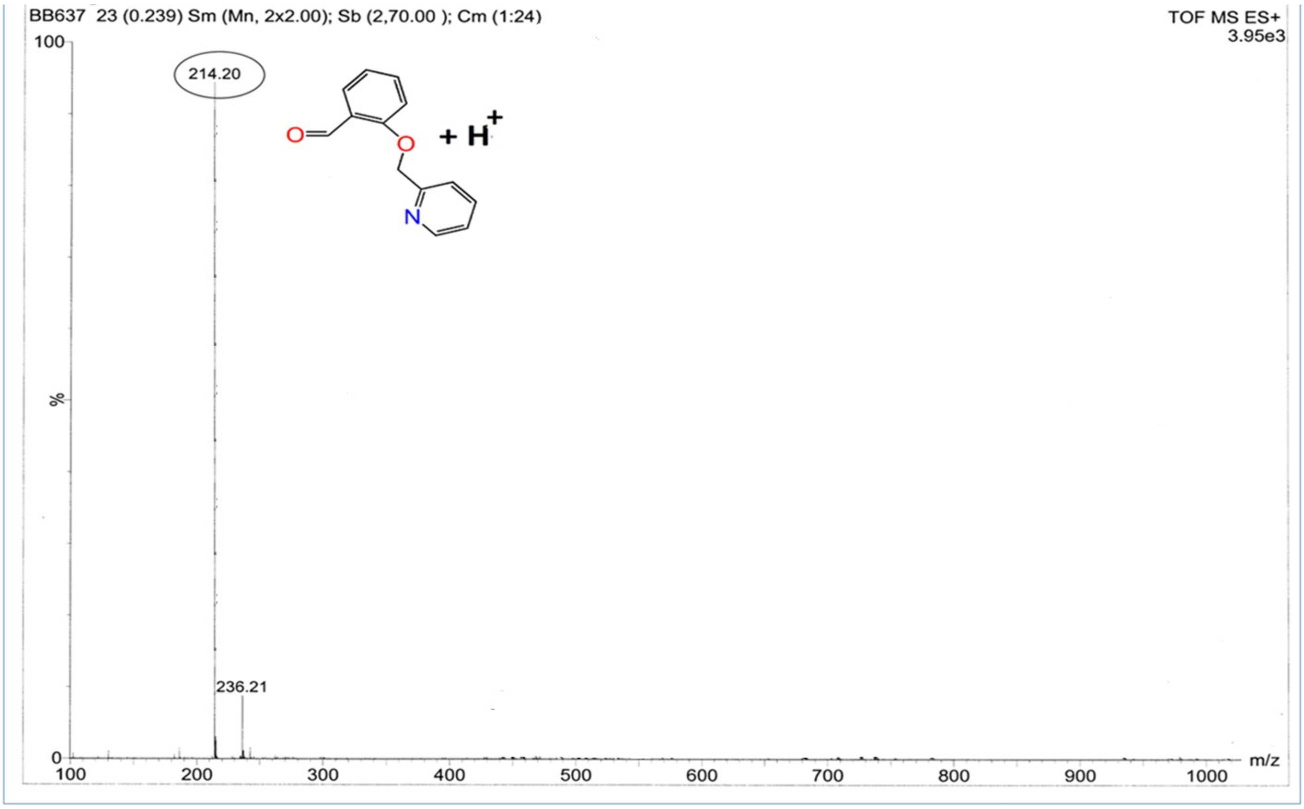

Fig. S3a. Mass spectrum of (**1**) [aldehyde product(1)].

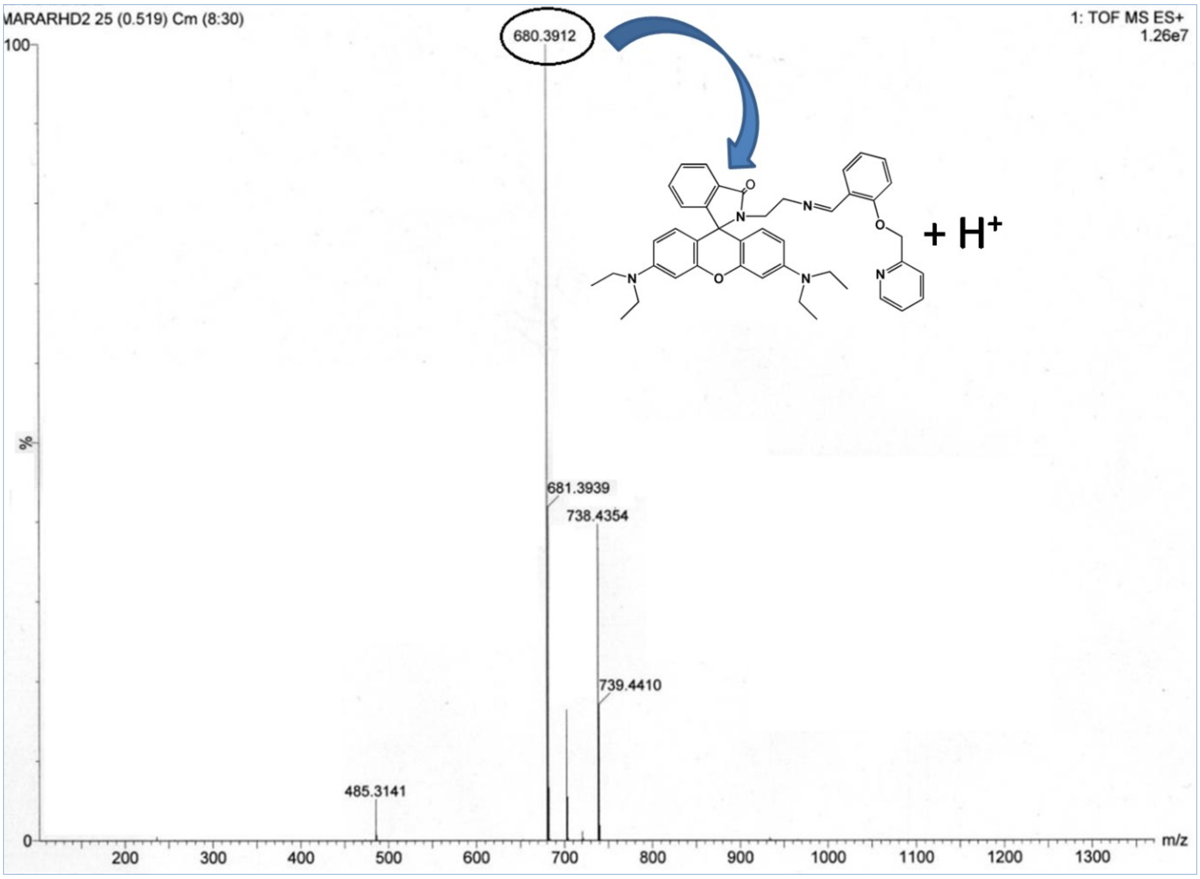

Fig. S3b. Mass spectrum of **L^3**

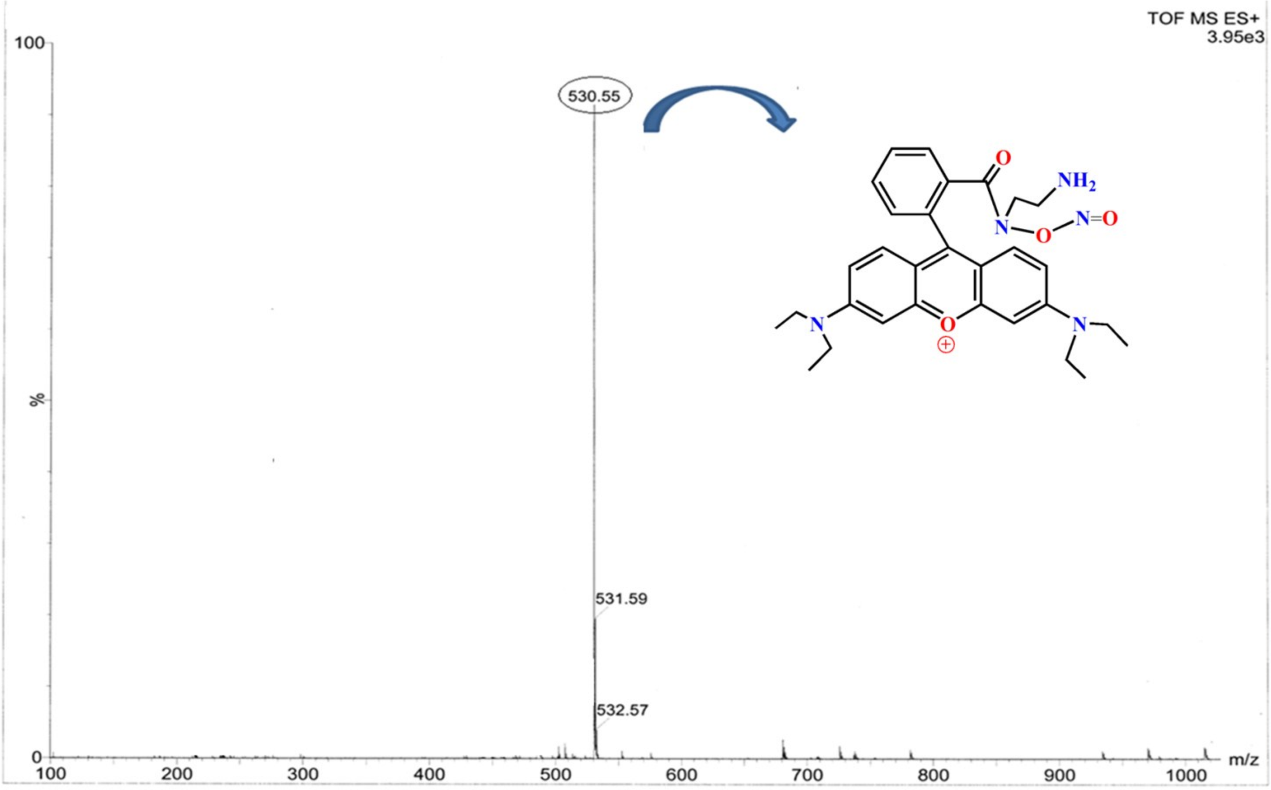

Fig. S3c. Mass spectrum of amine product(**3**) after purging NO in **L^3.**

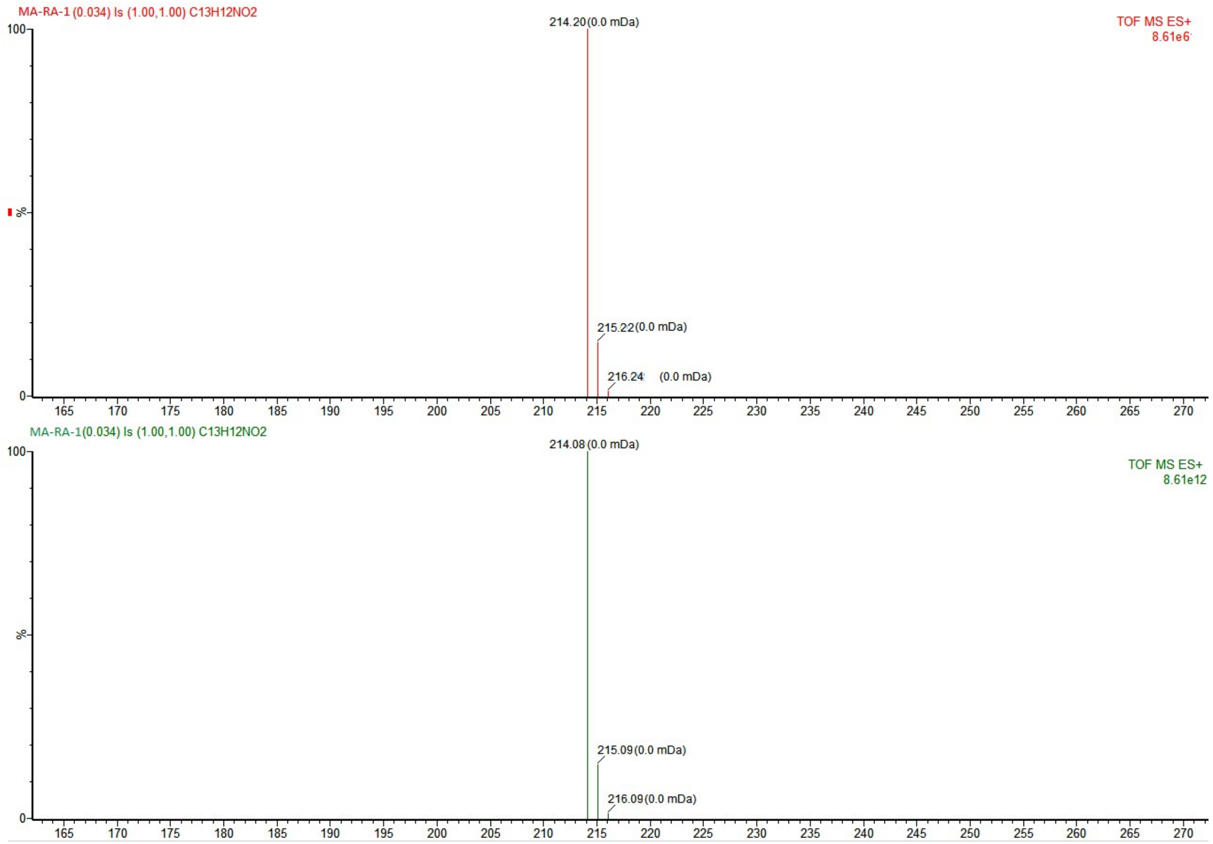

Fig. S3d. HRMS (ESI-MS+, m/z) spectra, both simulated and experimental, for aldehyde product(**1**).

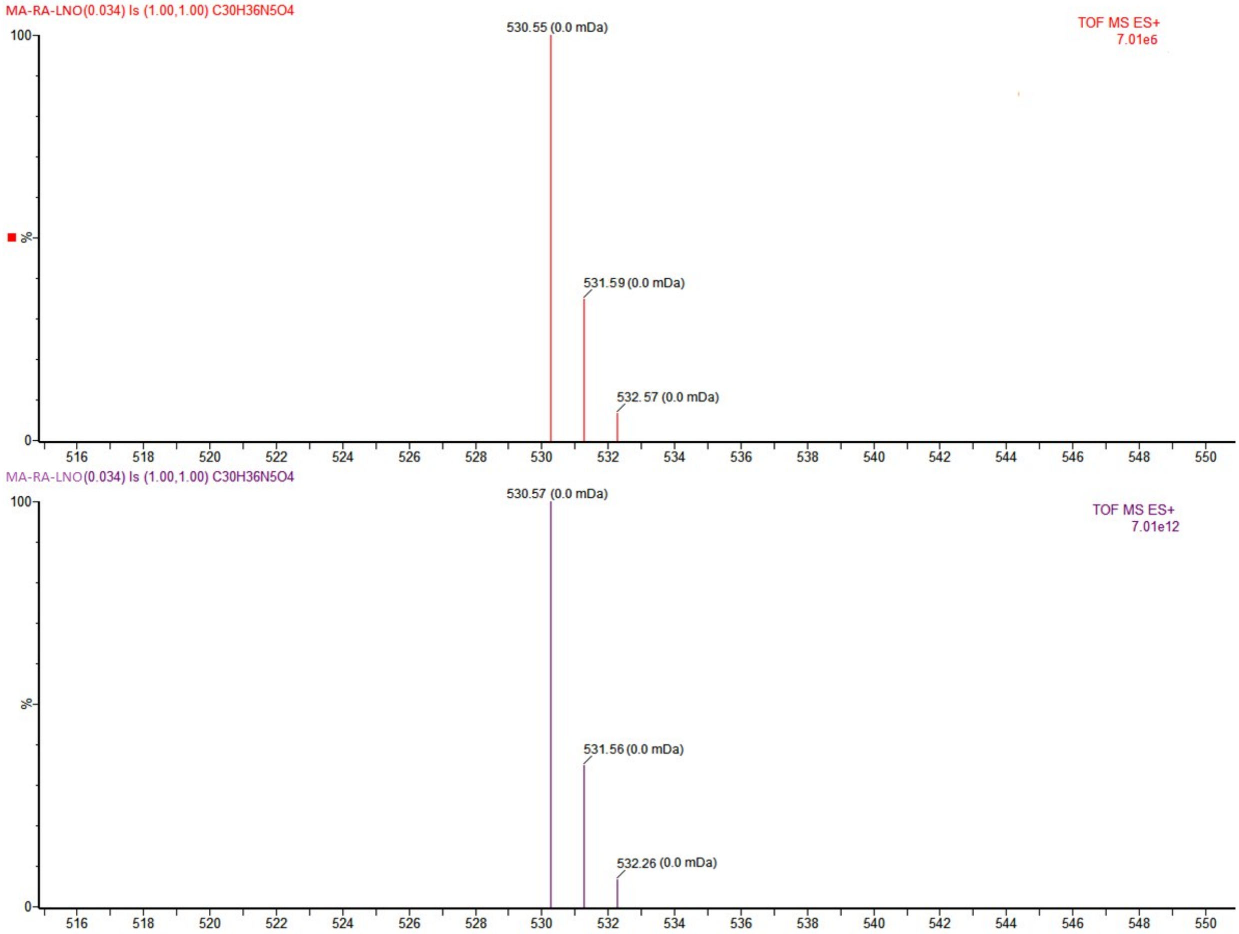

Fig. S3e. HRMS (ESI-MS+, m/z) spectra, both simulated and experimental, for amine product**(3)**.

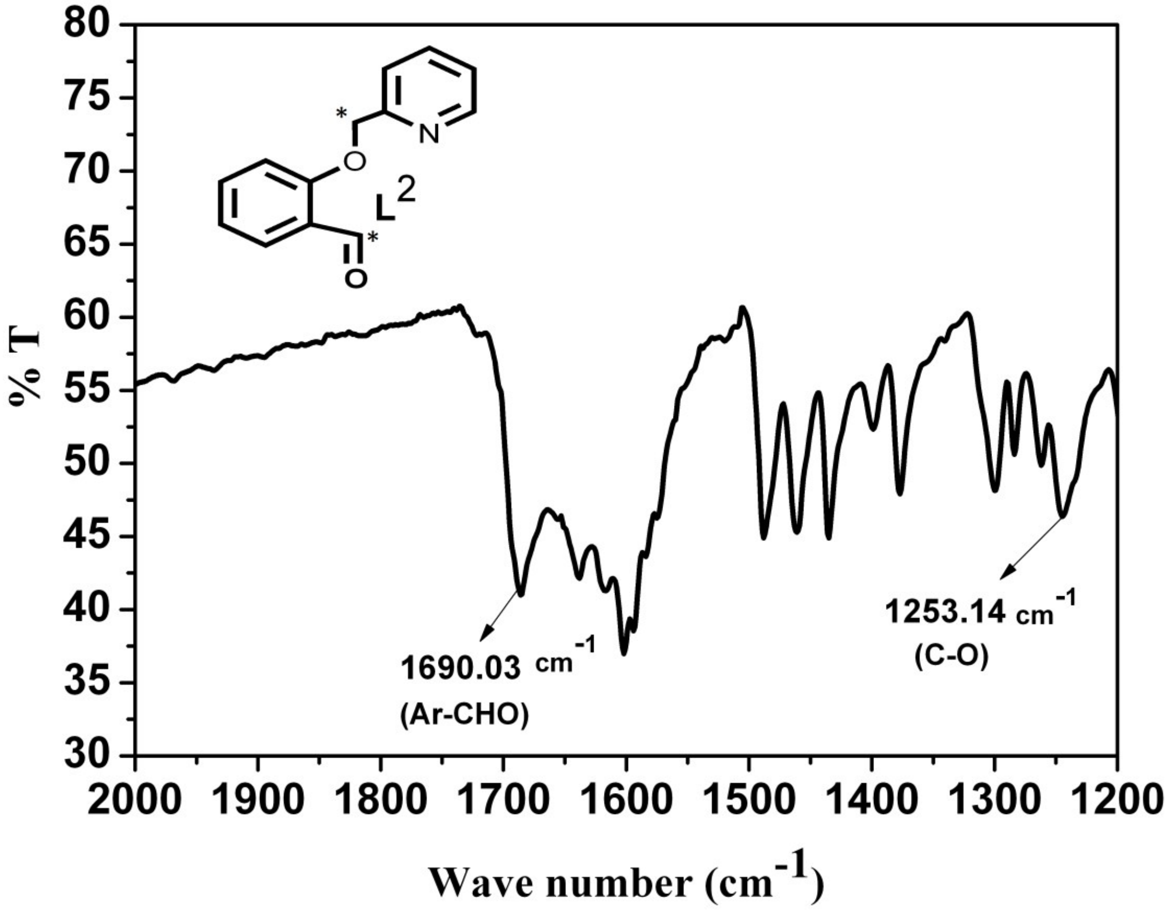

Fig. S4a. IR spectrum of for (**1**) [aldehyde product(1)].

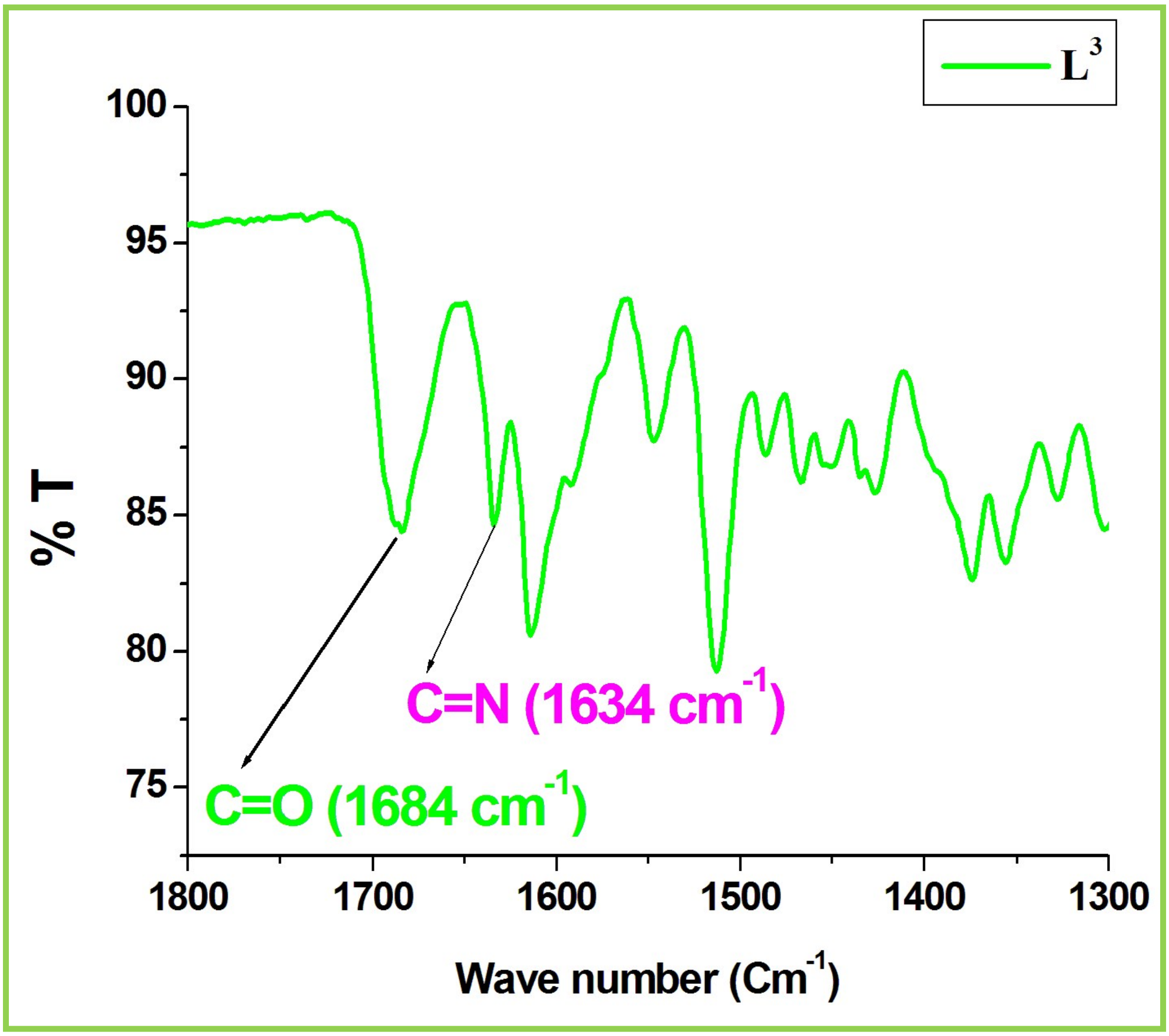

Fig. S4b. IR spectrum of **L^3** in solid state.

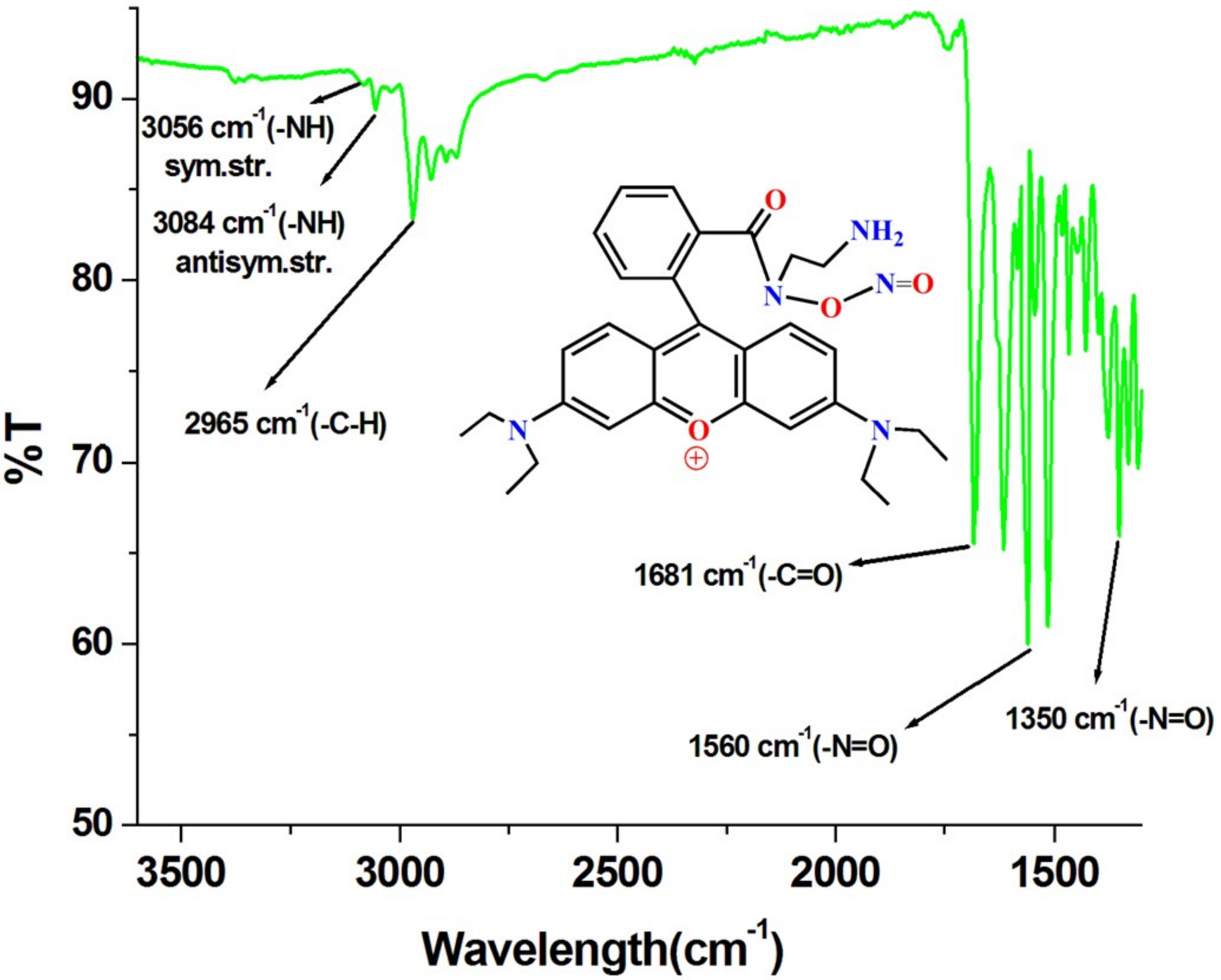

Fig. S4c. IR spectrum for amine product **(3)** in solid state(1000-3700cm^{-1}).

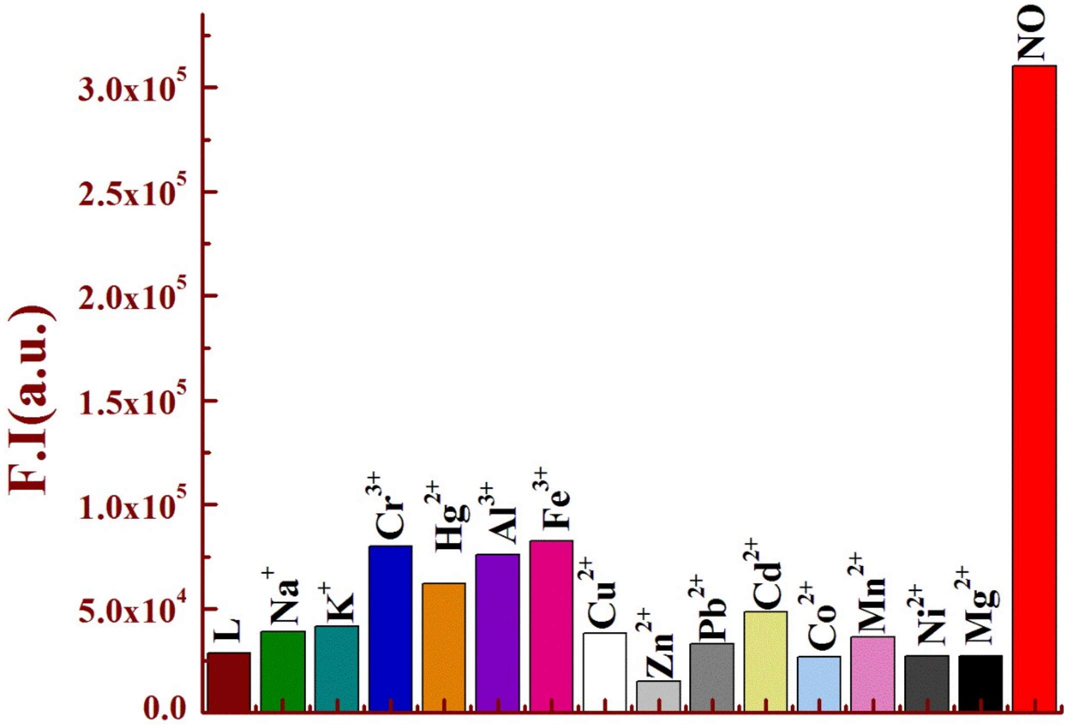

Fig. S5a.Fluorescence response of the ligand **L^3** in presence of different cations (Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cr^{3+} and Zn^{2+}, Pb^{2+}, Cd^{2+}, Al^{3+}, Hg^{2+}, Na^+, K^+ and Mg^{2+}(100μM)into the solution of **L^3**(20 mM) which were added 5 equivalents into the solution of **L^3**(20 μM).(λ_{ex} = 510 nm)

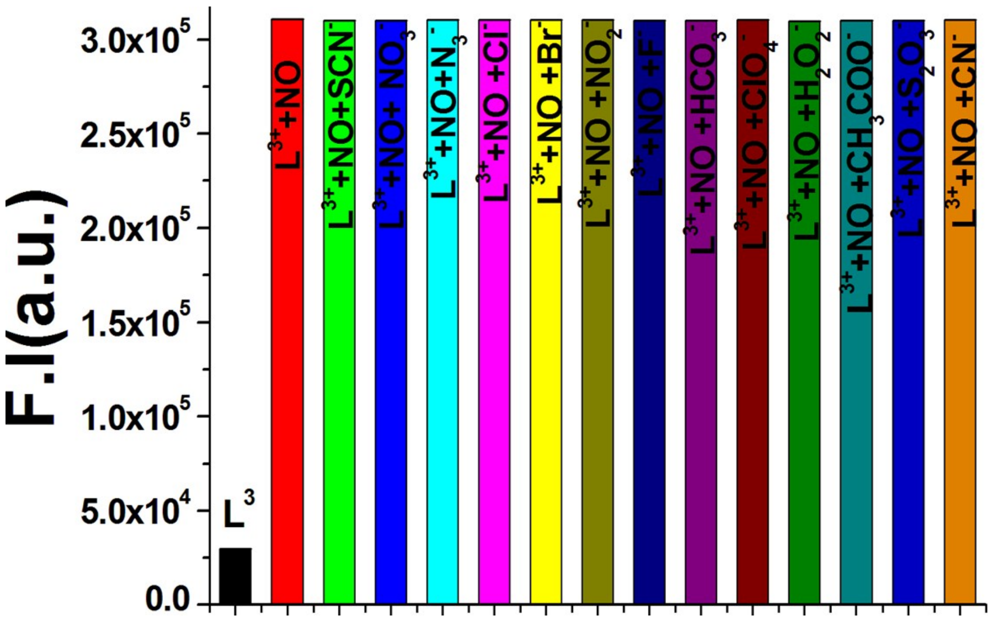

Fig. S5b.Competitive study by fluorescence response of the ligand **L^3** towards NO in presence of different anions (SCN^-, NO_3^-, N_3^- Cl^-, Br^- F^-, HCO_3^-, ClO_4^-, H_2O_2, OAc^-, $S_2O_3^{2-}$,CN^- etc(100μM) which were added 5 equivalents into the solution of **L^3**(20 μM).(λ_{ex} = 510 nm)

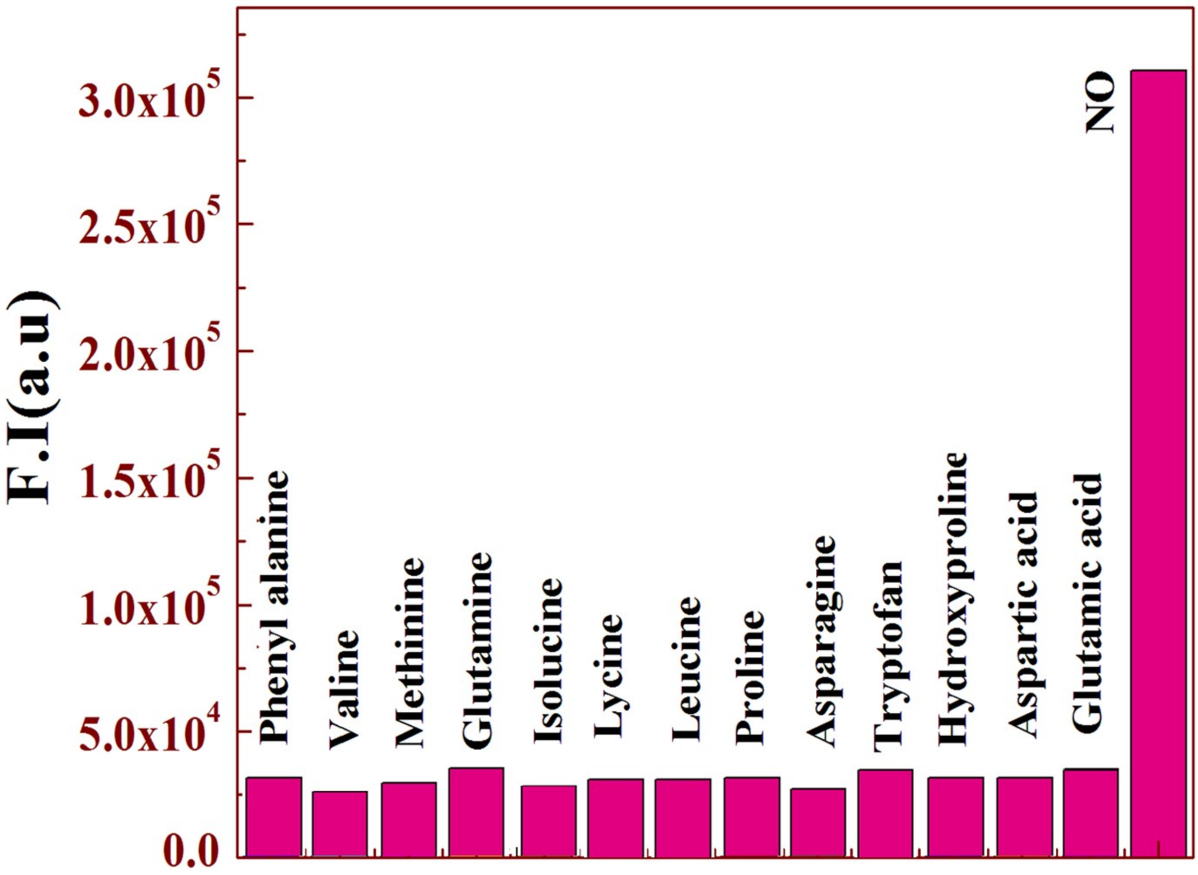

Fig. S6. Fluorescence response of various type of amino-acids(100µM) towards **L³** (20 µM).(λ_{ex} = 510 nm)

Calculation of the detection limit(LOD):

The detection limit DL of **L^3**for NO was determined from3σ method by followingequation:**DL = K* Sb_1/S**

Where K = 2 or 3 (we take 3 in this case); Sb_1 is the standard deviation of the blank solution; S is the slope of
The calibration curve obtained from Linear dynamic plot of FI vs. [**NO**]

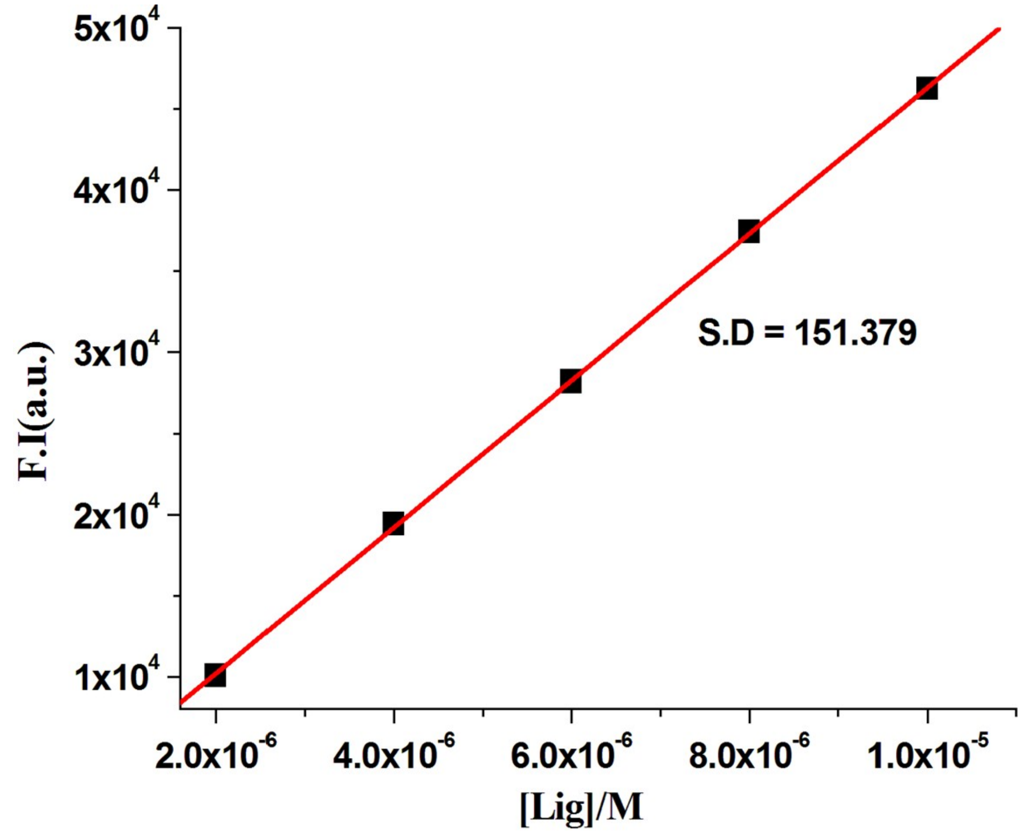

Fig. S7. Determination of Sb_1 of the blank, ligand solution.

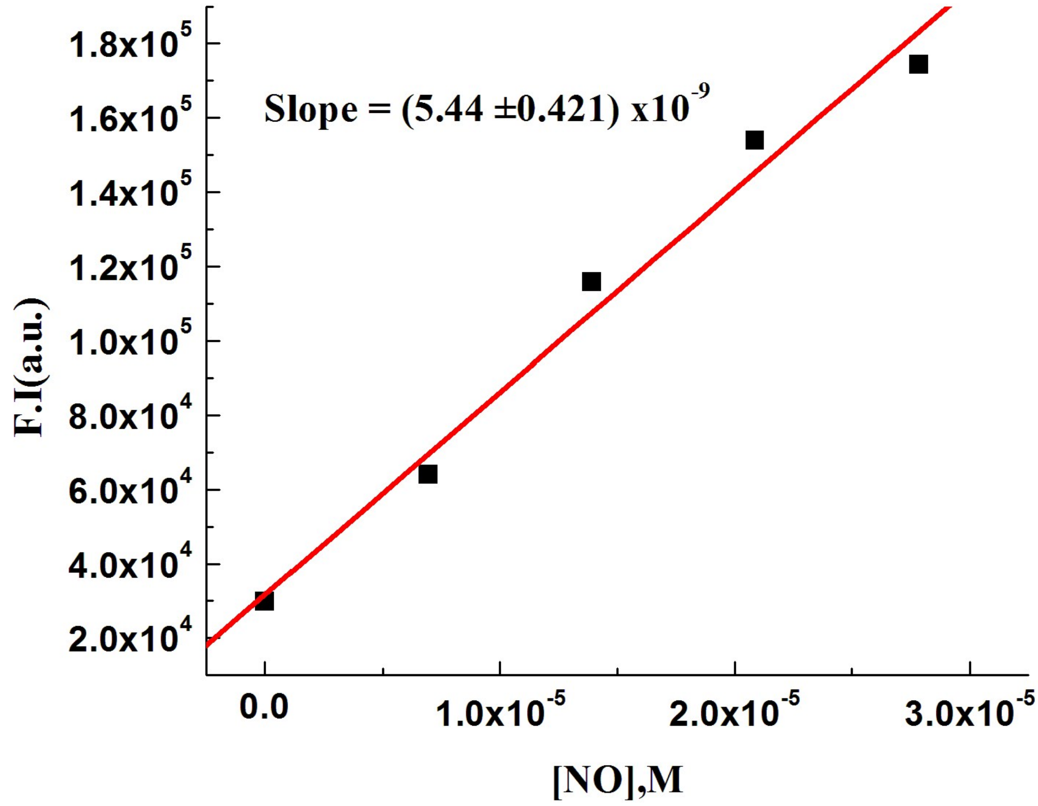

Fig.S7a. Linear dynamic plot of FI at 587 nm vs. [NO] for the determination of S (slope); [**L**^{3}] =20 µM

LOD (NO) = (3 x 151.379)/5.44 x 10^{-9} = 83.4 nM

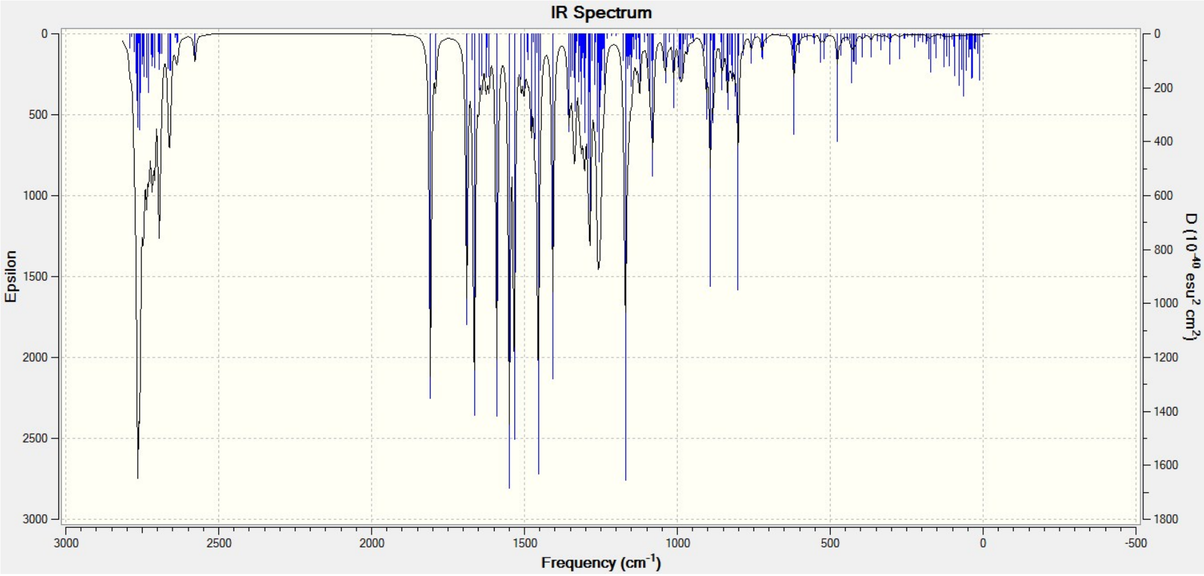

Fig. S8a. Frequency study of **L^3** by DFT method.

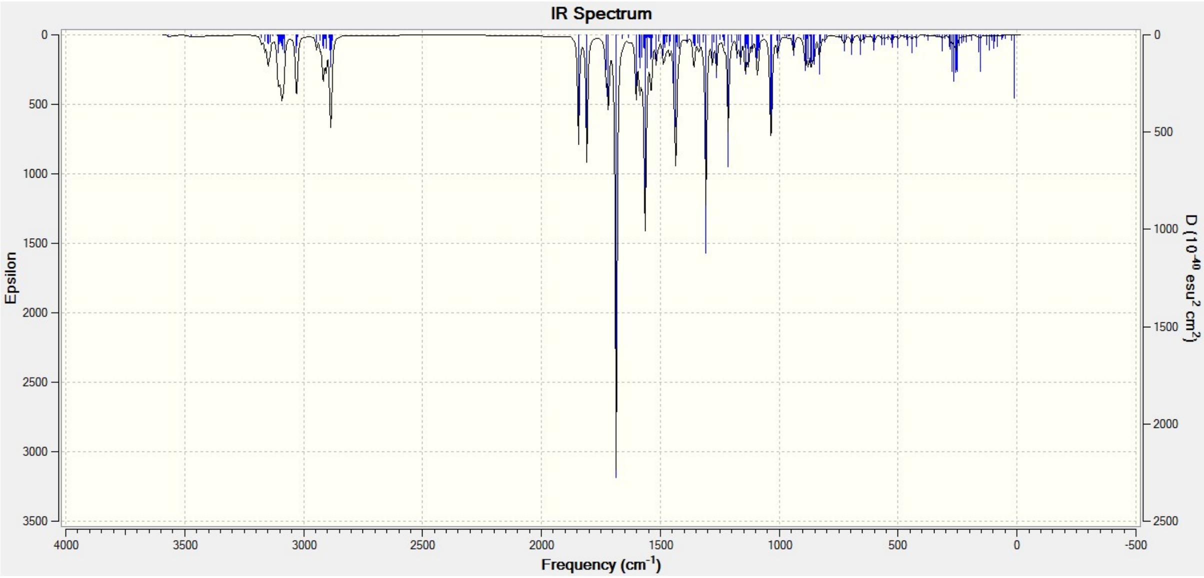

Fig. S8b. Frequency study of **RH-en-ONO(3)** by DFT method.

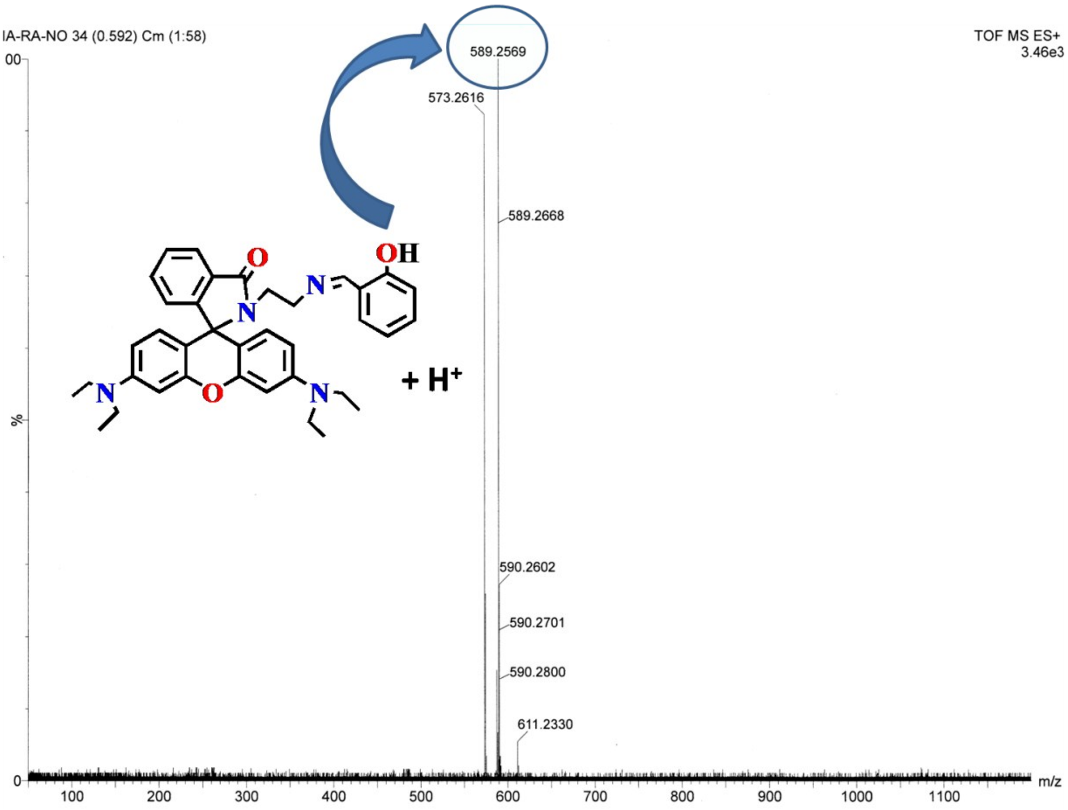

Fig. S9. Mass spectrum of Rhodamine B-en and salisaldehyde based ligand(**L^4**).

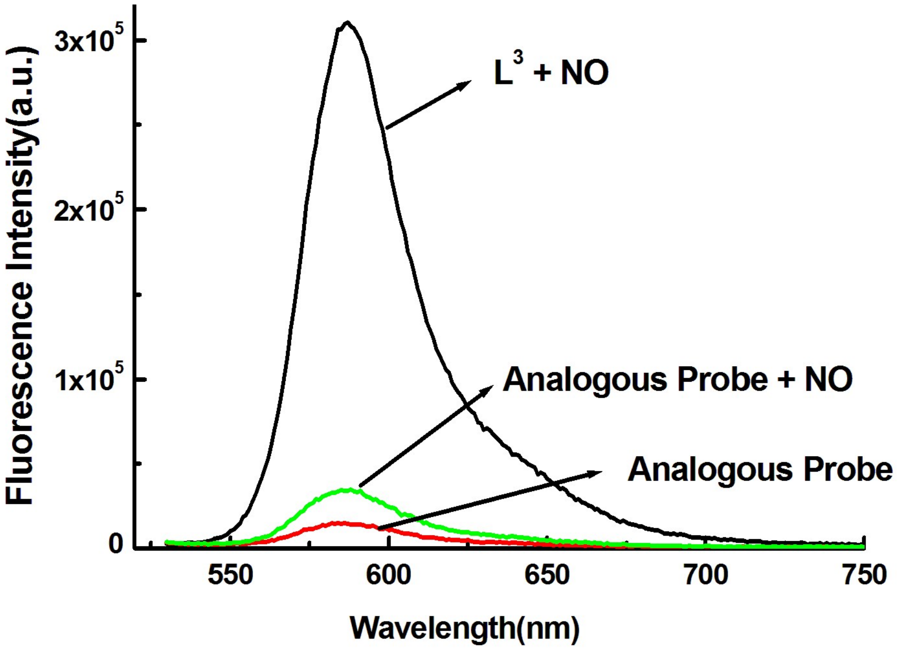

Fig. S10. Fluorescence intensity of analogous Schiff base probe (**L^4**) in comparison with probe **L^3**.

Table S1 : The comparable calculated IR transition with Experimental IR values for the ligand (**L^3**), **RH-en-ONO(3)** compound.

Ligand and **RH-en-ONO** compound**(3)**	Theoretical stretching frequency(cm^{-1})	Experimental stretching frequency (cm^{-1})
Ligand(**L^3**)	1807.79 (amidic "C=O")	1684
Ligand(**L^3**)	1789.91 (C=N)	1634
RH-en-ONO compound**(3)**	1808.82 (amidic "C=O")	1681
RH-en-ONO compound**(3)**	1844.15 (N-O)	1560
RH-en-ONO compound**(3)**	3474.34 (N-H)	2965

Table S2a: Selective bond distance and bond angles of **L^{3}**

Bond distance(Å)		Bond-angles(^{o})	
O2-C14	**1.214**	O2 C14 N3	**124.861**
N3-C14	**1.407**	C51 N54 C55	**121.99**
C51-N54	**1.470**	C58 O65 C82	**118.26**
N94-C87	**1.358**		
O65-C82	**1.456**		
O65-C58	**1.375**		

Table S2b: Selective bond distance and bond angles of **RH-en-ONO(3).**

Bond distance(Å)		Bond-angles(^{o})	
N67-O68	**1.174**	N63 O65 N67	**131.46**
N67-O65	**1.298**	068 N67 O65	**128.74**
C70-N73	**1.490**	O62 C61 N63	**121.53**
C61-O62	**1.213**		
N63-O65	**1.420**		

Table S3: Selected parameters for the vertical excitation (UV-VIS absorptions) of **L^3**; electronic excitation energies (eV) and oscillator strength (f), configurations of the low-lying excited states of **L^3**; calculation of the $S_0 \rightarrow S_n$ energy gaps on optimized ground- state geometries (UV-vis absorption).

Electronic transition	Composition	Excitation energy	Oscillator Strength(f)	CI	Assignment	λ_{exp} (nm)
$S_0 \rightarrow S_{12}$	HOMO-3→LUMO+4	3.7203 eV	0.7284	0.42875	ILCT	315
	HOMO-3→LUMO+6			0.19363	ILCT	
$S_0 \rightarrow S_5$	HOMO→LUMO+5	3.0211 eV	0.1270	0.23945	ILCT	416
	HOMO→LUMO+8			0.42774	ILCT	

Table S4 : Selected parameters for the vertical excitation (UV-VIS absorptions) of **RH-en-ONO(3)**; electronic excitation energies (eV) and oscillator strength (f), configurations of the low-lying excited states of **RH-en-ONO(3)**; calculation of the $S_0 \rightarrow S_n$ energy gaps on optimized ground- state geometries (UV-vis absorption).

Electronic transition	Composition	Excitation energy	Oscillator Strength(f)	CI	Assignment	λ_{exp} (nm)
$S_0 \rightarrow S_5$	HOMO-1→LUMO+3	2.1603 eV	0.196	0.48735	ILCT	561
$S_0 \rightarrow S_6$	HOMO-1→LUMO+1	2.4790 eV	0.2947	0.58294	ILCT	518
$S_0 \rightarrow S_{10}$	HOMO-6→LUMO+6	2.7030eV	0.1087	0.28476	ILCT	476