Pyrophosphate Selective Fluorescent Chemosensor: A Cascade Recognition of Nuclear Stain Mimicking DAPI

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CONTENTS

General procedure for drawing Job plot by Fluorescence method:

Stock solution of same concentration of **SPHN** and Zn^{2+} were prepared in the order of ≈ 2.0 x 10^{-5} by using CH_3CN-aqueous HEPES buffer (7/3, v/v, 25 °C) at pH =7.4. The intensity in each case with different *host–guest* ratio but equal in volume was recorded. Job plots were drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of intensity of the fluorescent spectrum during titration and X_{host} is the mole fraction of the host).

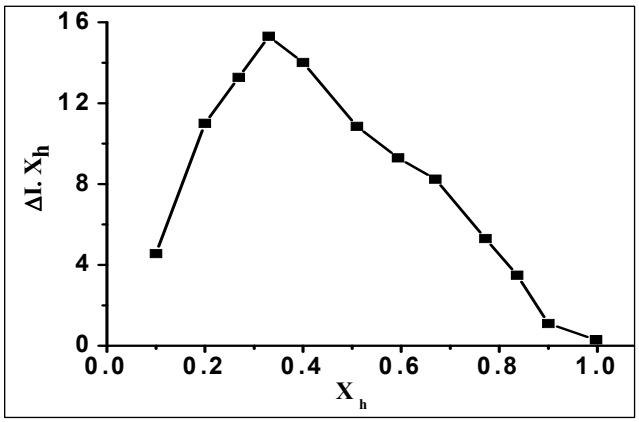


Figure S1. Jobs plot diagram of **SPHN** for Zn^{2+} (where X_h is the mole fraction of host and ΔI indicates the change of the emission intensity).

Association constant determination:

The binding constant value of cation Zn^{2+} with the **SPHN** and PPi with **SPHN-Zn** complex has been determined from the emission intensity data following the modified Benesi–Hildebrand equation, 1/ΔI = 1/ΔI max + (1/K[C])(1/ΔI max). Here ΔI = I-Imin and ΔI max = Imax-Imin, where Imin, I, and Imax are the emission intensities of sensor considered in the absence of guest , at an intermediate concentration and at a concentration of complete saturation of guest where K is the binding constant and [C] is the guest concentration respectively. From the plot of (Imax-Imin)/(I-Imin) against $[C]^{-1}$ for sensor, the value of K has been determined from the slope. The association constant (K_a) as determined by

fluorescence titration method for **SPHN** with Zn^{2+} is found to be 1 x $10^5 M^{-1}$ (error < 10%) and PPi towards **SPHN-Zn** complex is $5x10^4 M^{-1}$.

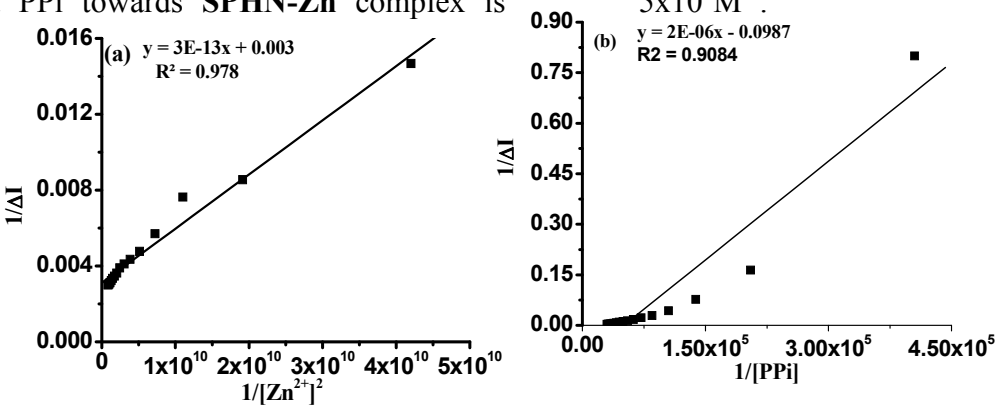


Figure S_2: (a) Benesi–Hildebrand plot from fluorescence titration data of **SPHN** (20μM) with Zn^{2+}. (b) Benesi–Hildebrand plot from fluorescence titration data of **SPHN - Zn** (20μM) with PPi.

Determination of equilibrium competition constant of PPi with SPHN-Zn(Recetor 1):

Solutions of the chemosensing ensemble for competition assays were prepared by using **SPHN-Zn (Recetor 1)** CH_3CN-HEPES buffer (7/3, v/v, pH = 7.4) solution. After standard solutions of PPi weas added to the buffer solution containing the chemosensing ensemble, absorption and emission spectra were measured. The equilibrium competition constant (Kcomp) was calculated based on the titration curve.

$$\mathrm{R{-}A + P \Leftrightarrow R + P{-}A}$$

$$K_{comp} = ([R][P{-}A])/([R{-}A][P])$$

Here, [R] is the concentration of **SPHN**, [R–A] is the concentration of the complex between **SPHN-Zn (Recetor 1).** [P] is the concentration of free PPi and [P–A] is the concentration of the complex between PPi and Zn^{2+}. The fitting of the titration profiles with a non linear leastsquares procedure using a model provides the equilibrium competition constant (Kcomp).[1]

$$[\mathrm{P{-}A}] = \frac{A_T + P_T K_{Comp} + A_T K_{Comp} - \sqrt{-4P_T A_T\left(-1+K_{Comp}\right)K_{Comp} + \left(A_T - P_T K_{Comp} - A_T K_{Comp} - R_T\right)^2} + R_T}{2\left(-1+K_{Comp}\right)}$$

$$F = F_{R-A} - \Delta F * [\mathrm{P{-}A}]$$

where F_{R-A} is the fluorescence of **SPHN - Zn (Recetor 1)**and DF is the change in fluorescence due to the formation of Zn^{2+}-PPi, A_T is the total concentration of Zn^{2+}, R_T is the total concentration of **SPHN** and P_T is the total concentration of PPi.

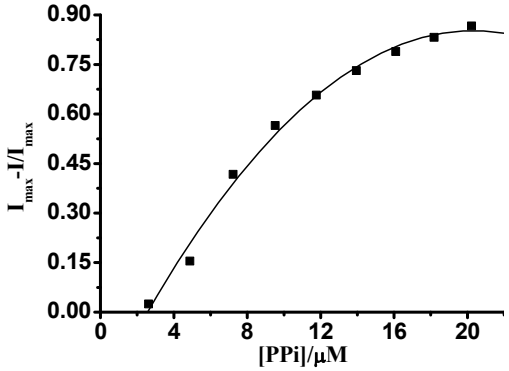


Figure S_3: Fitting of competitive titrations of **SPHN – Zn (Recetor 1)** chemosensing ensembles with PPi in CH_3CN-HEPES buffer (7/3, v/v, 25 °C) at pH =7.4.

Calculation of the detection limit:

The detection limits DL of **SPHN** for Zn^{2+} and **Receptor 1** for PPi were determined from the following equation[1]:

DL = K* Sb1/S

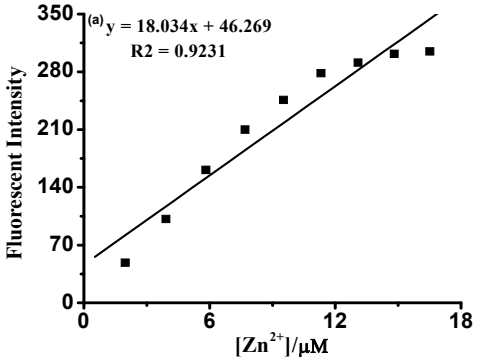
Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

From the graph Fig.S_3(a), we get slope = 18.034, and Sb1 value is 27.678.

Thus using the formula we get the Detection Limit for Zn^{2+} to **SPHN** = 4.6 μM.

From the graph Fig.S_3(b), we get slope = 17.826, and Sb1 value is 36.540.

Thus using the formula we get the Detection Limit for PPi to the **SPHN-Zn (Receptor 1)** = 6.3 μM.



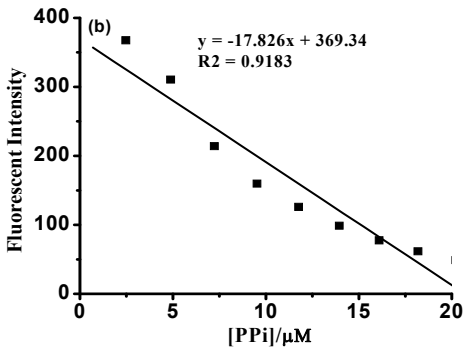


Figure S4: (a) Changes of Fluorescence Intensity of **SPHN** as a function of [Zn^{2+}] at 450 nm.(b) Changes of Fluorescence Intensity of **SPHN-Zn** complex(**Receptor 1)** as a function of [PPi] at 450 nm.

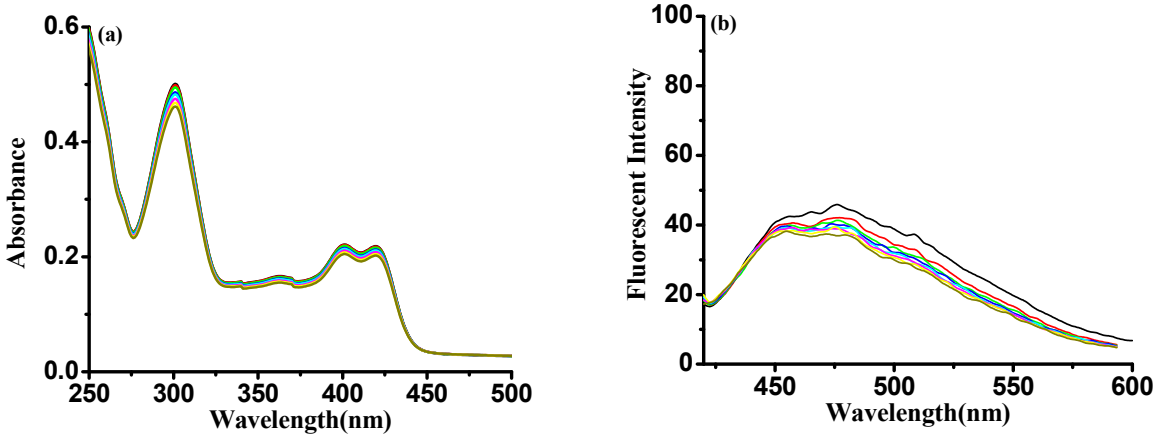


Figure S5 (a) Uv-Vis spectra of receptor **SPHN** (c = 2×10^{-5} M) in CH_3CN-HEPES buffer (7/3, v/v, 25 ° C) upon titration with PPi (c = 2×10^{-4}M) at pH-7.4. (b) (a) Fluorescence spectra of receptor **SPHN** (c = 2×10^{-5} M) in CH_3CN-HEPES buffer (7/3, v/v, 25 ° C) upon titration with PPi (c = 2×10^{-4}M) at pH-7.4.

Reversibility Experiment:

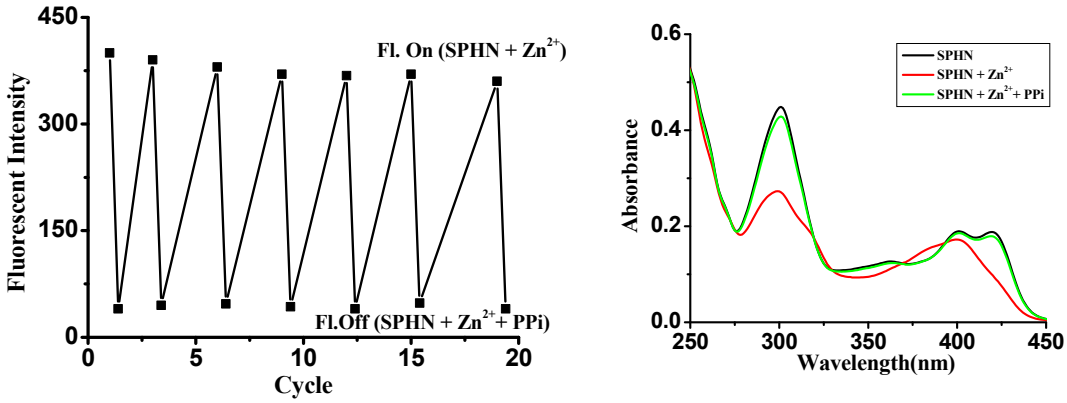


Figure S6: (a) Fluorescence intensity changes of **SPHN**(c = 2.0 × 10^{-5}M) in CH_3CN-HEPES buffer (7/3, v/v, 25 ° C) upon alternate addition of Zn^{2+} and PPi(c = 2.0 × 10^{-4}M). (b) UV-vis absorption spectra of **SPHN** (c = 2.0 × 10^{-5}M) in CH_3CN-HEPES buffer (7/3, v/v, 25 ° C) by alternative addition of Zn^{2+} and PPi(c = 2.0 × 10^{-4}M).

Partial ^{1}H NMR spectra of (a) SPHN (b) SPHN+Zn^{2+} complex (Receptor 1). (c) SPHN+Zn^{2+} + PPi

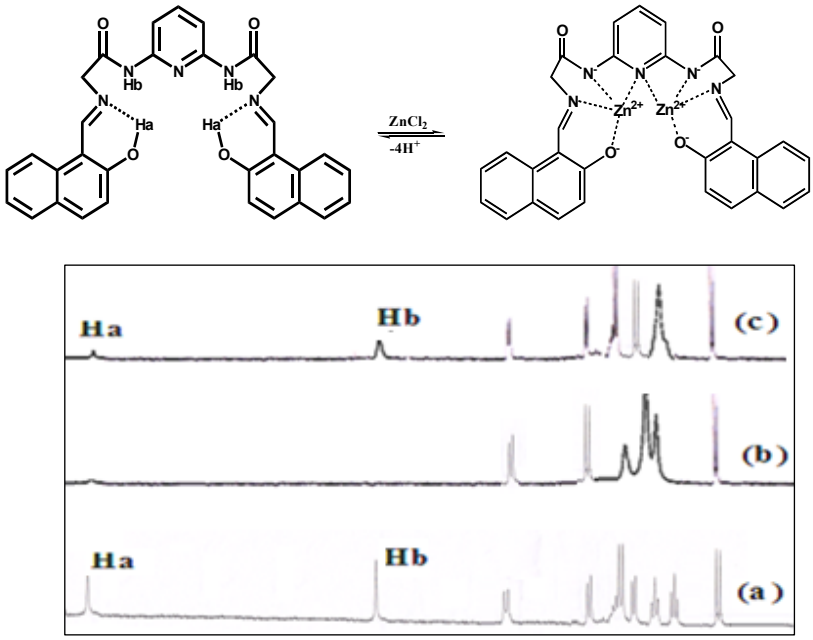
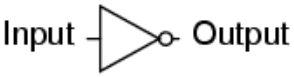


Figure S7. Partial ^{1}H NMR spectra (400 MHz) of **SPHN**(c =2.07x10^{-2} M) in CD_3CN:D_2O (7:3) with Zn^{2+} and PPi : (a) free **SPHN**; (b) **SPHN** + Zn^{2+}; (c) **SPHN** + Zn^{2+} +PPi.

Truth table of Different Gates:



Input	Output
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1	0

Not gate

A	B	Output
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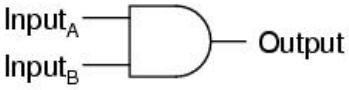
0	1	1
1	0	1
1	1	0

OR gate



A	B	Output
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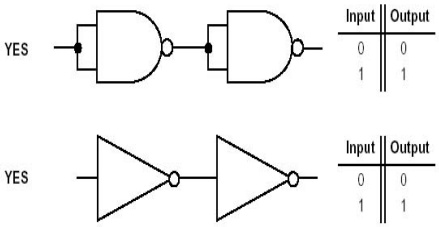
0	1	0
1	0	0
1	1	1

AND gate



YES gate

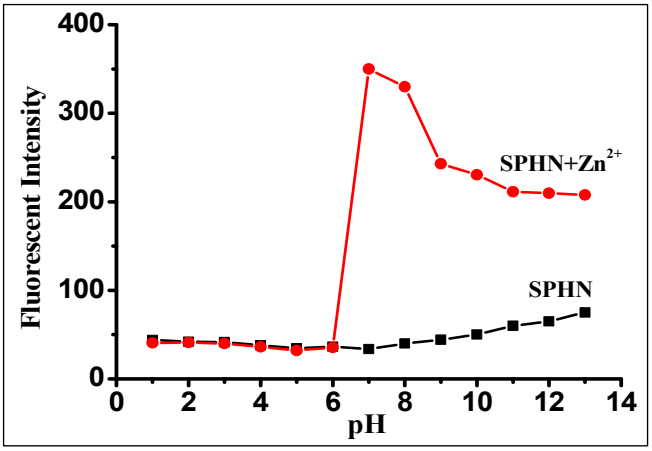
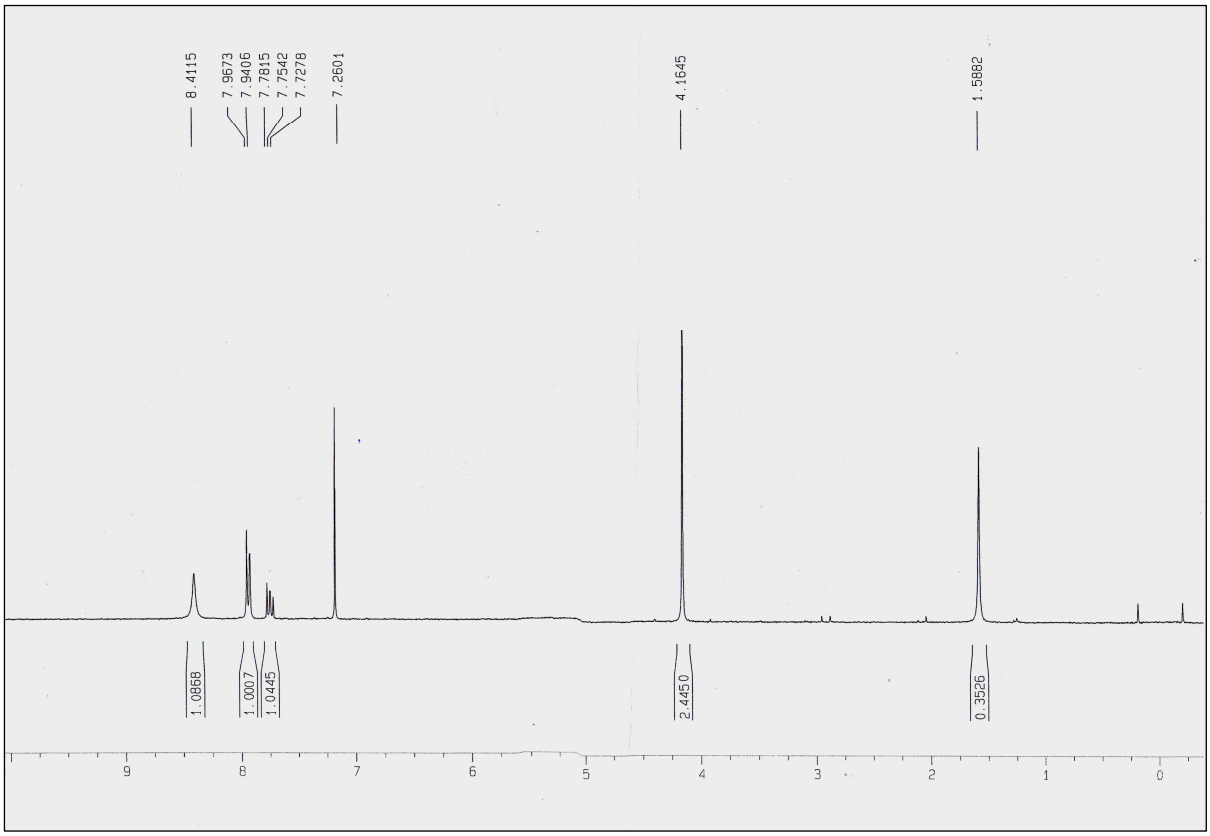
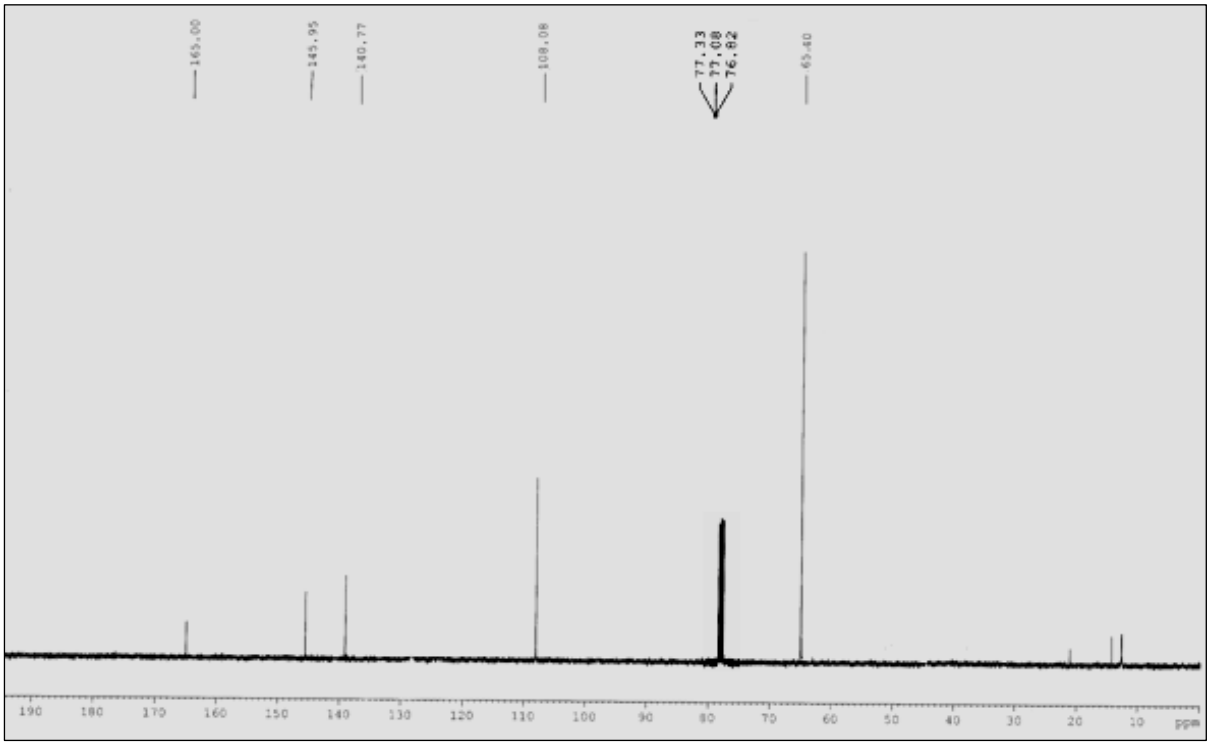


Figure S8. Fluorescence intensity of **SPHN** (c = 2×10^{-5} M) at various pH values in water medium in the absence and presence of Zn^{2+} (c = 2.0 x10^{-4}M). pH of different solution adjusted by using $HClO_4$ and NaOH.

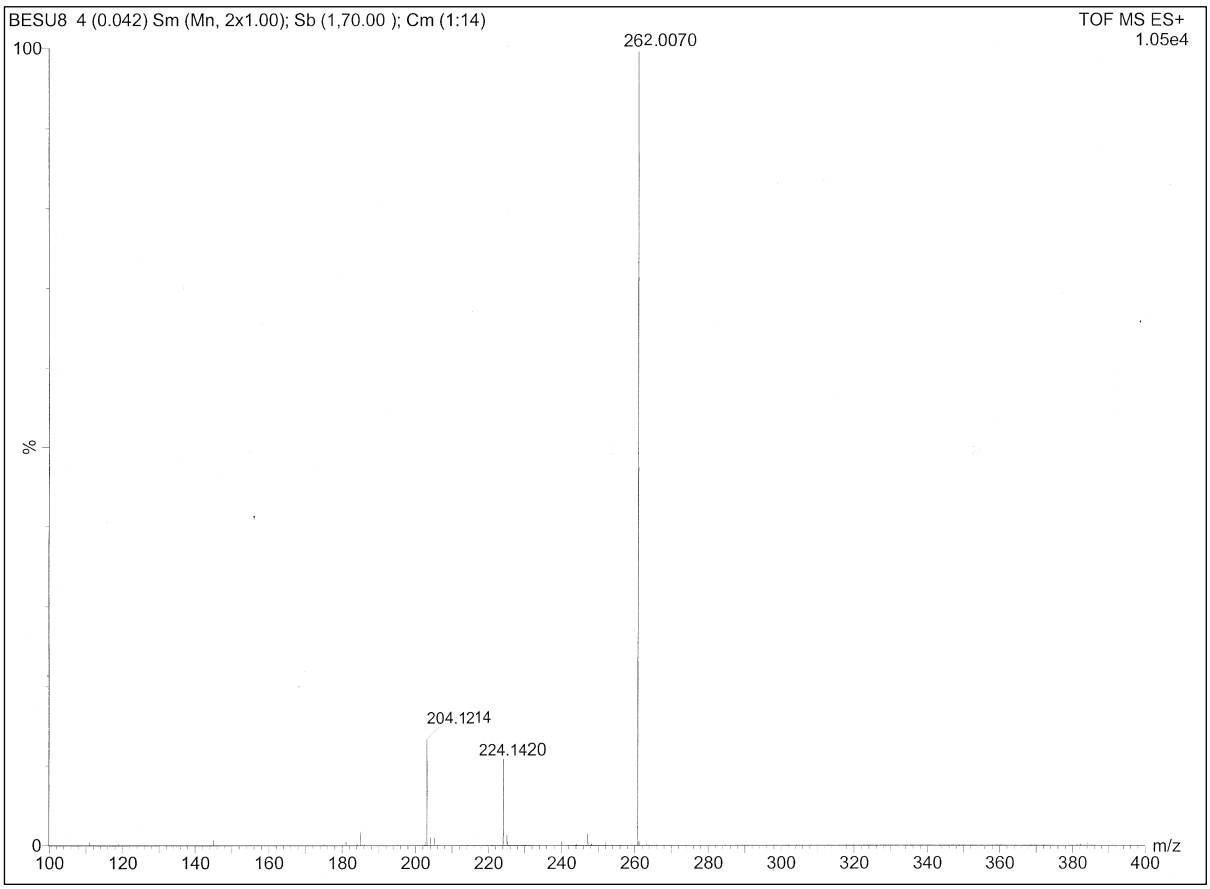
^{1}H NMR spectrum (S_9) of Compound A:



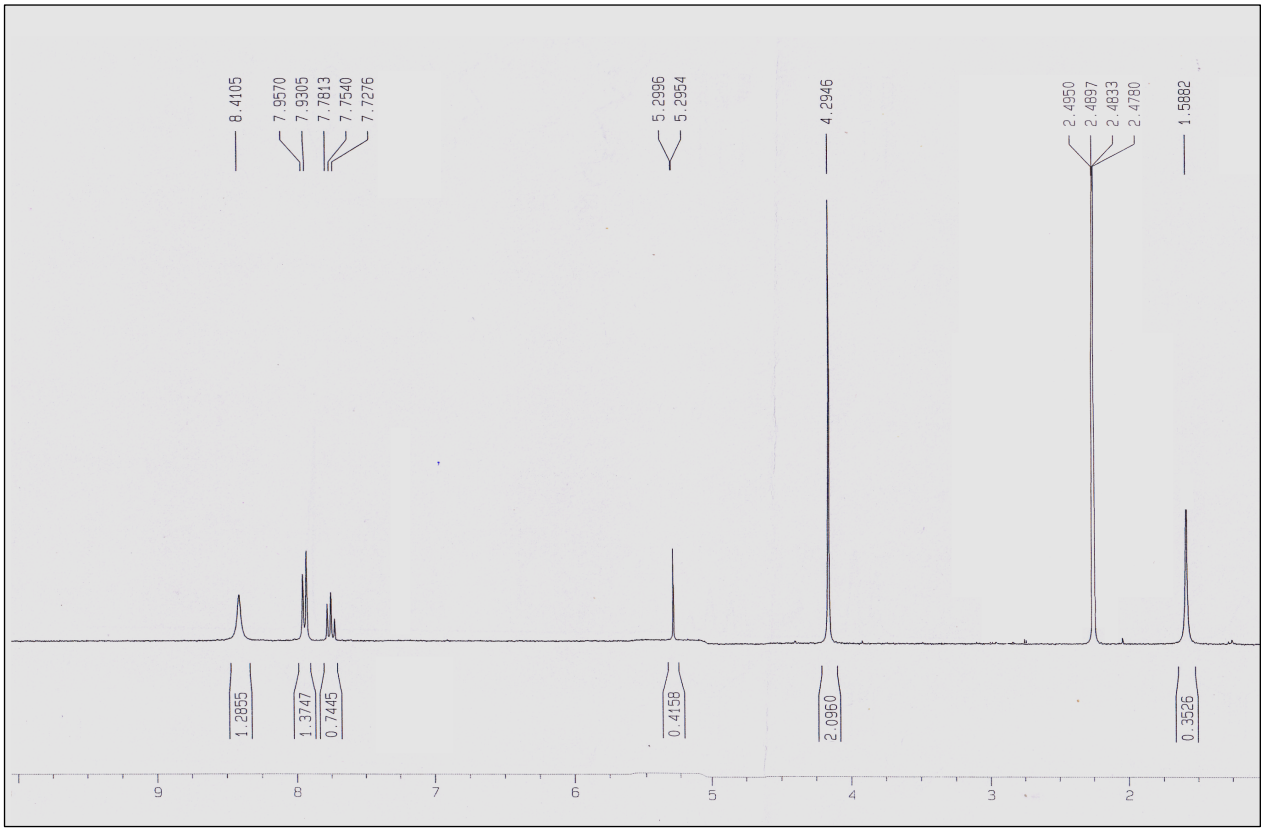
^{13}C NMR spectrum (S_{10}) of Compound A:



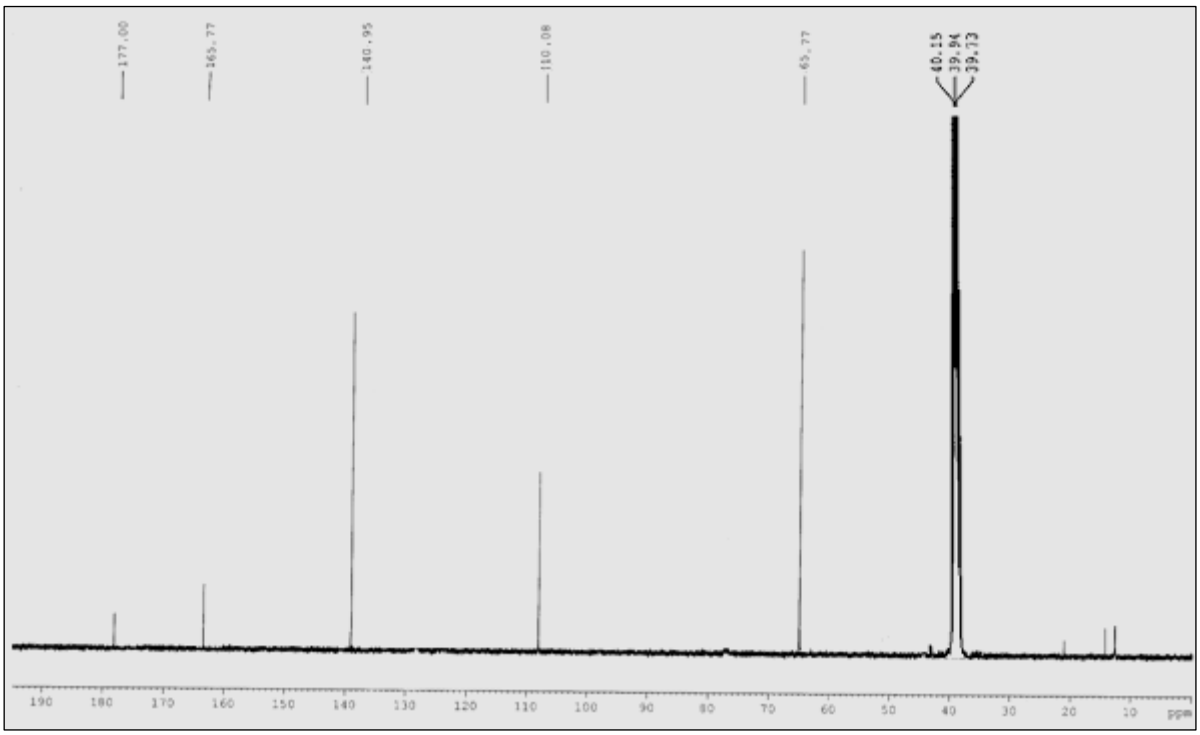
Mass spectrum (S_{11}) of compound A:



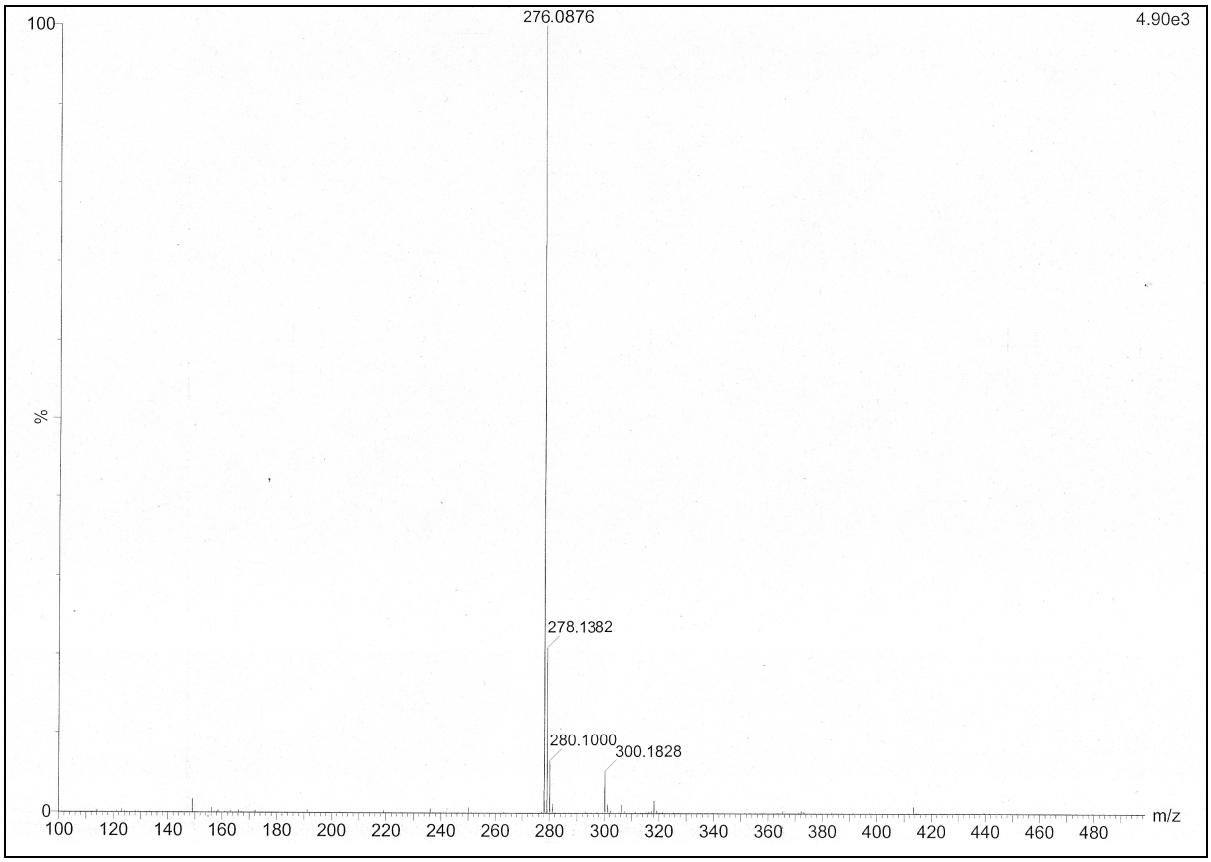
^{1}H NMR spectrum (S_{12}) of Compound B:



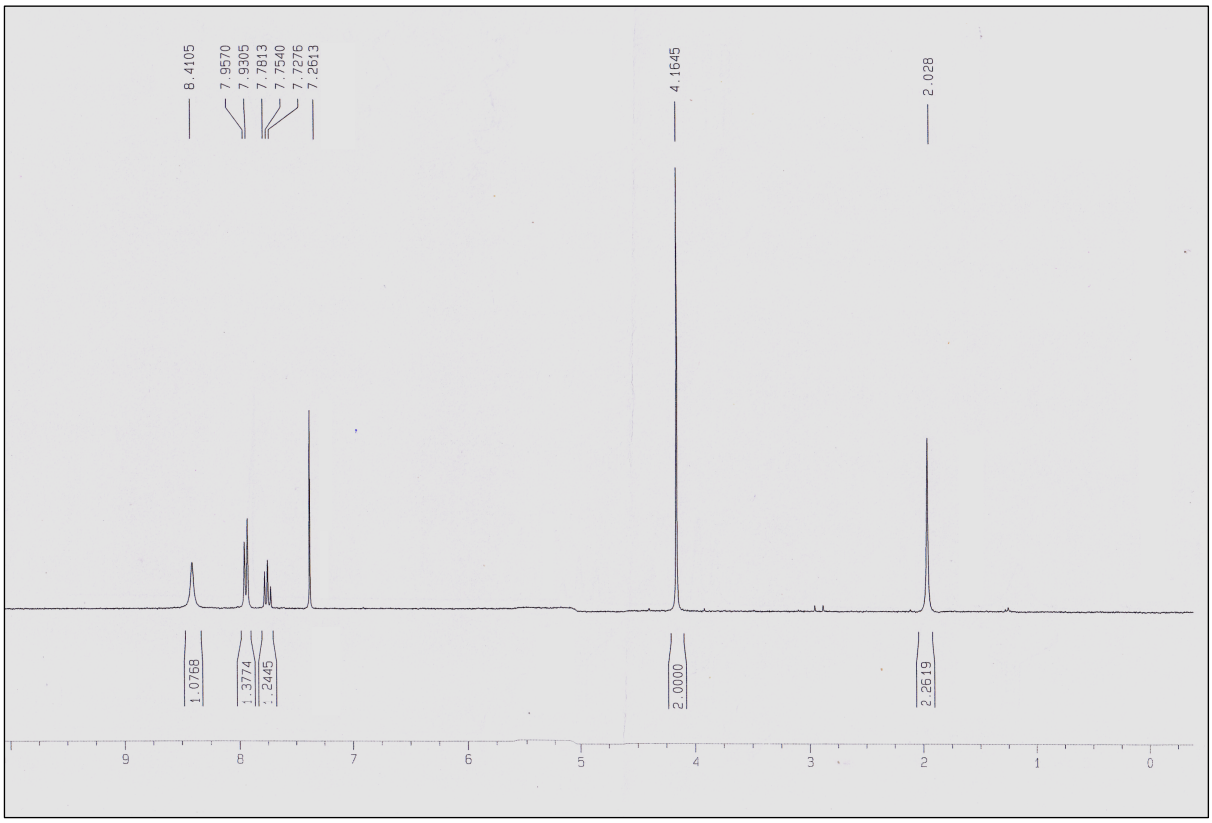
^{13}C NMR spectrum (S_{13}) of Compound B:



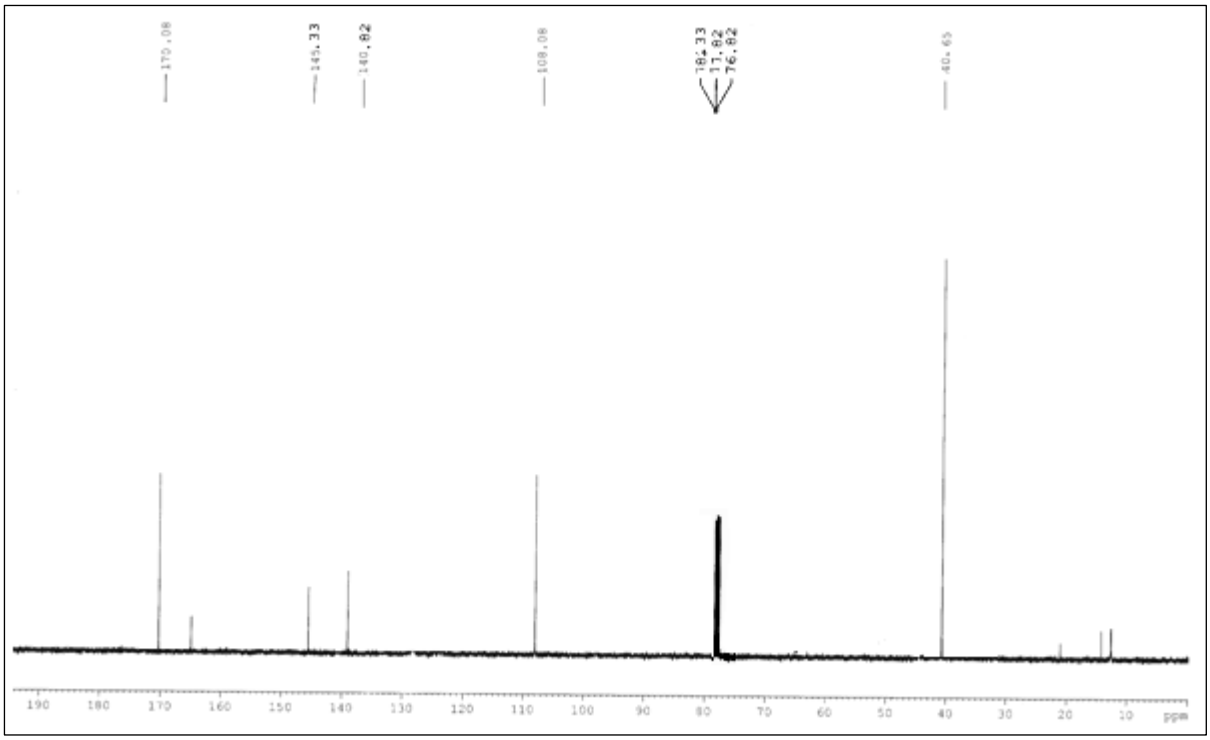
Mass spectrum (S_{14}) of compound B:



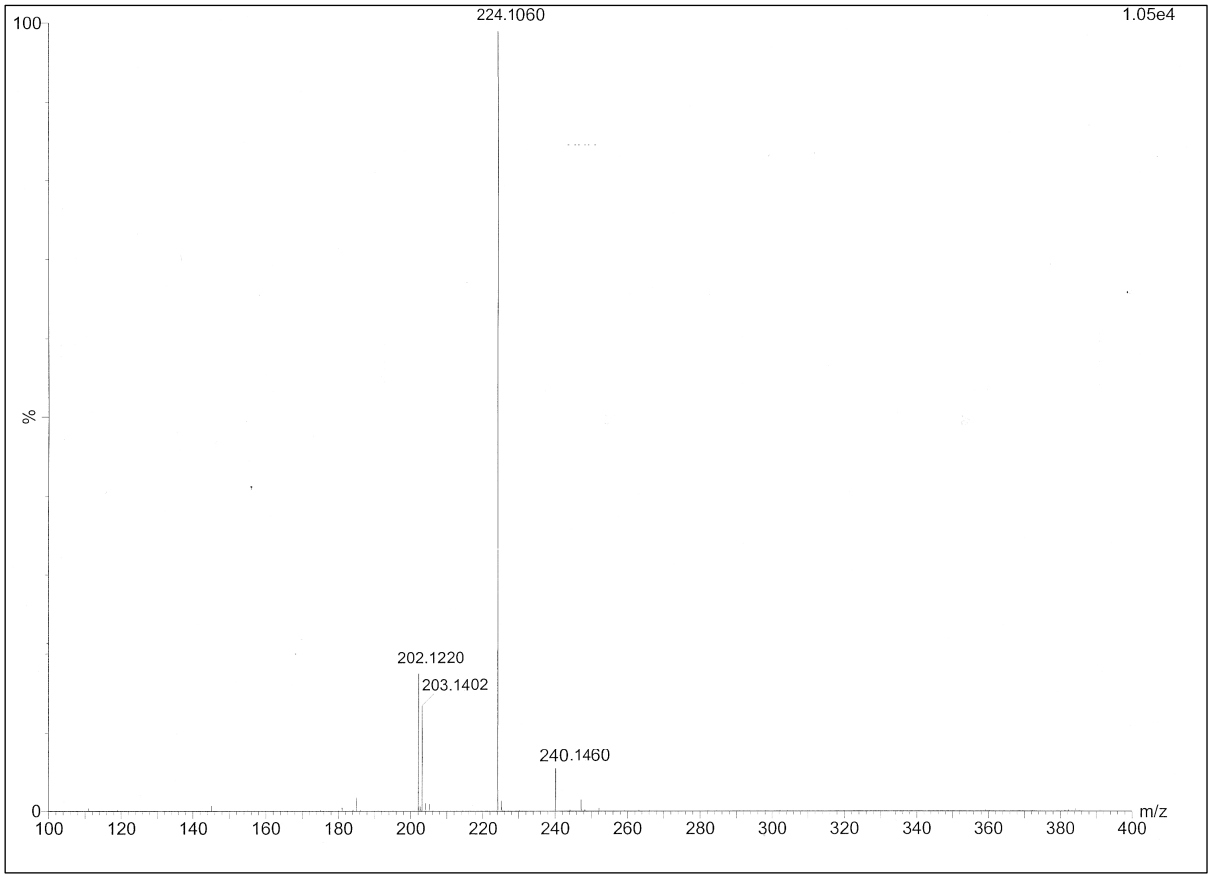
^{1}H NMR spectrum (S_{15}) of Compound C:



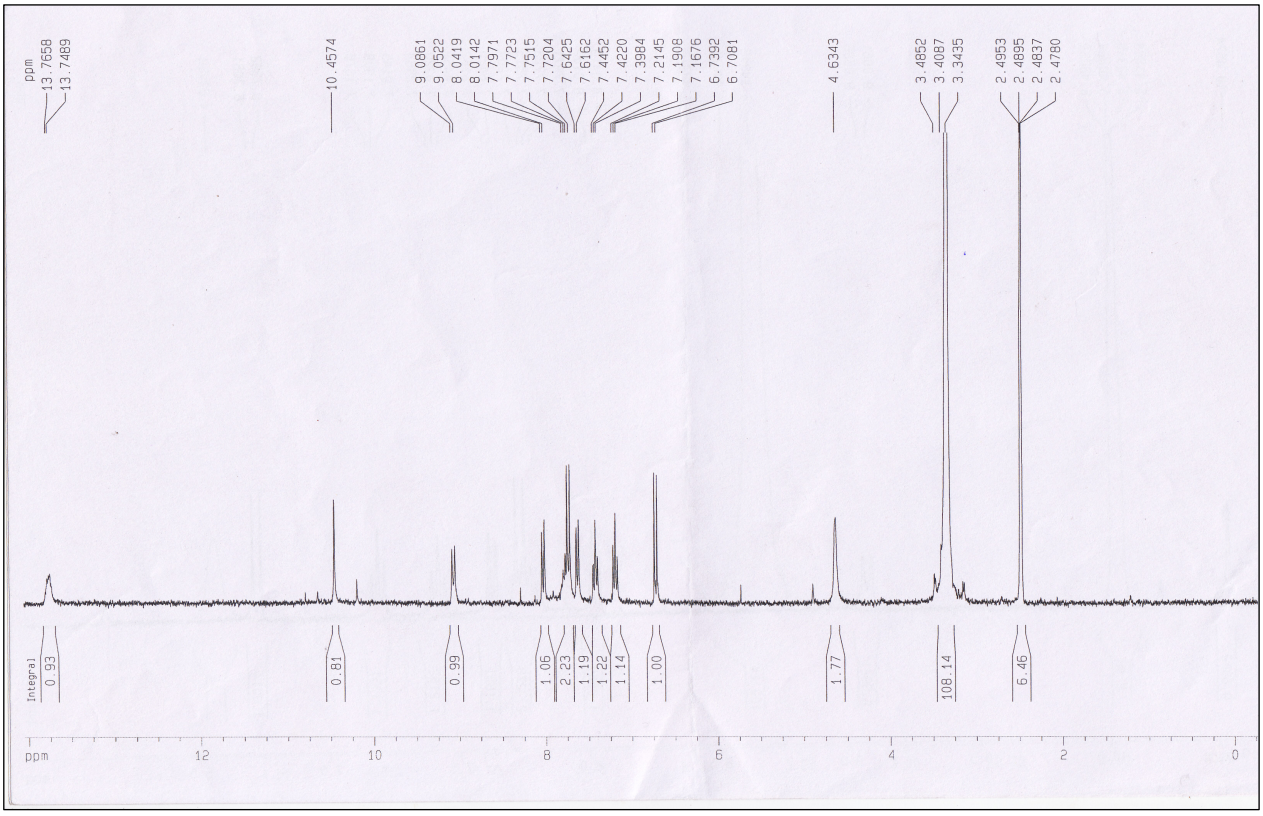
^{13}C NMR spectrum (S_{16}) of Compound C:



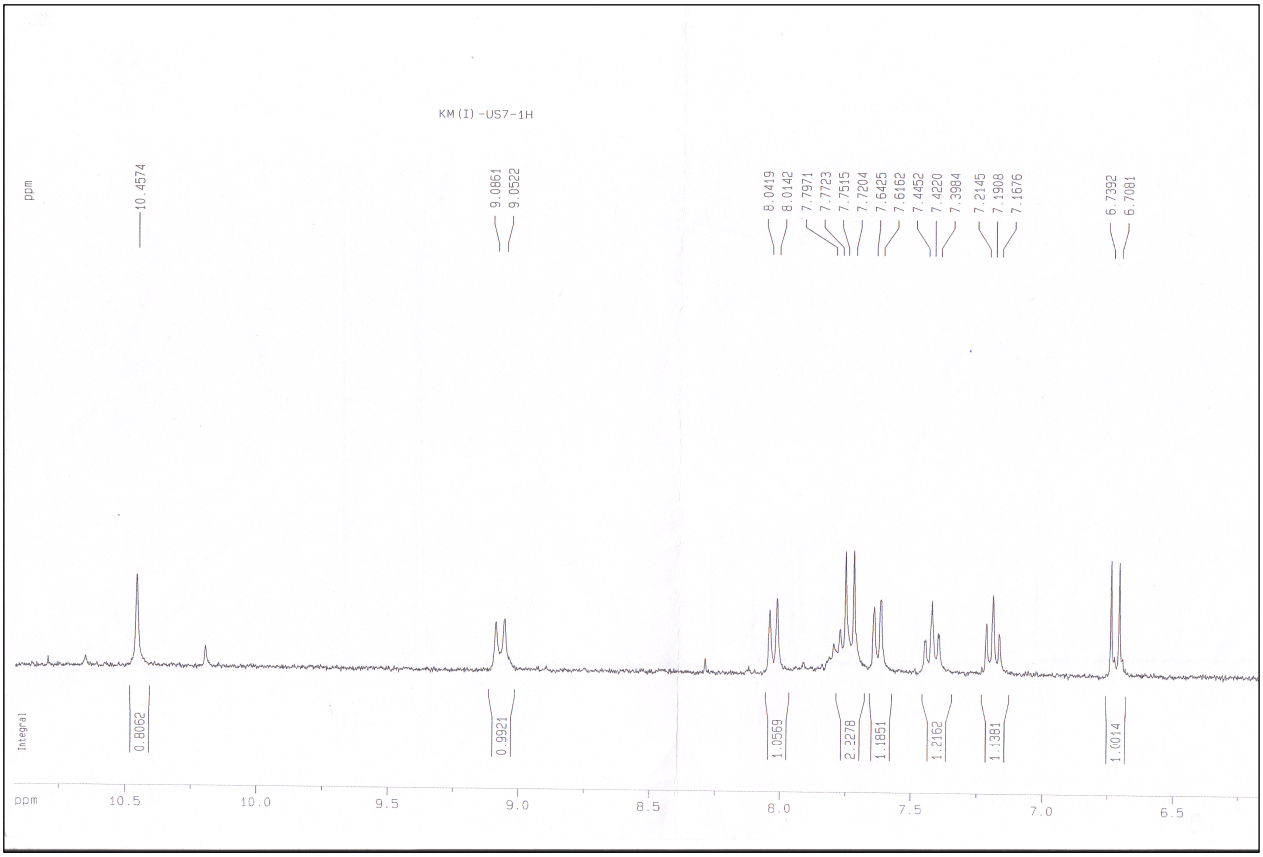
Mass spectrum (S_{17}) of Compound C:



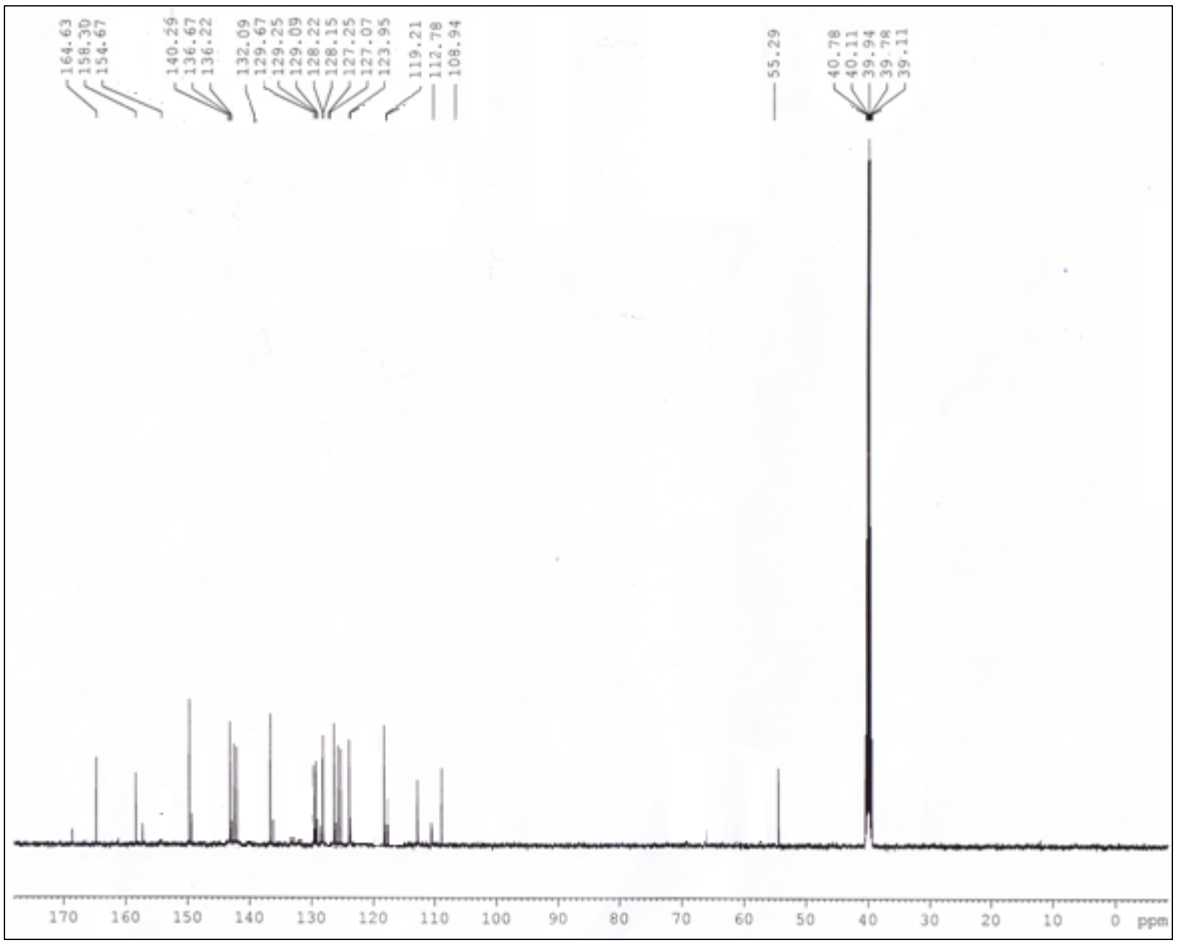
^{1}H NMR spectrum (S_{18}) of Compound SPHN:



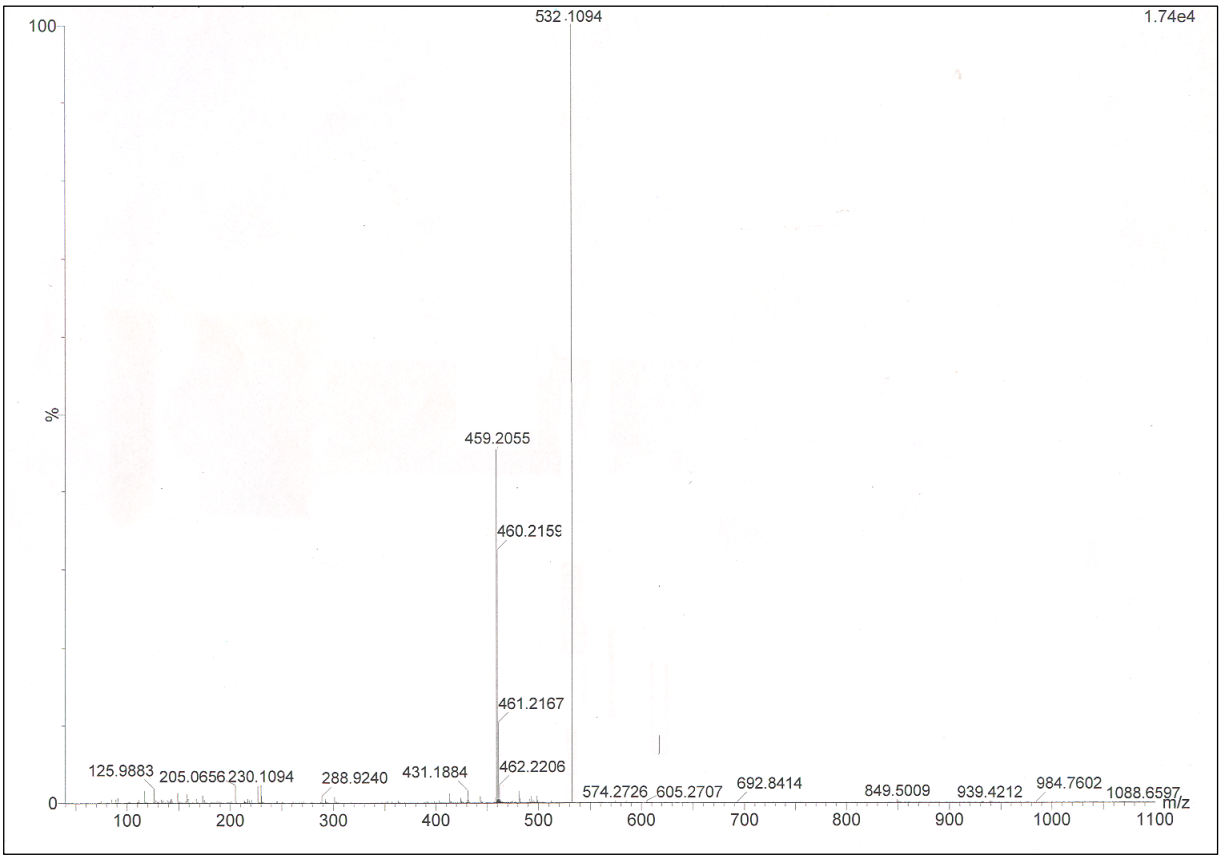
^{1}H NMR spectrum of Compound SPHN (expansion mode)



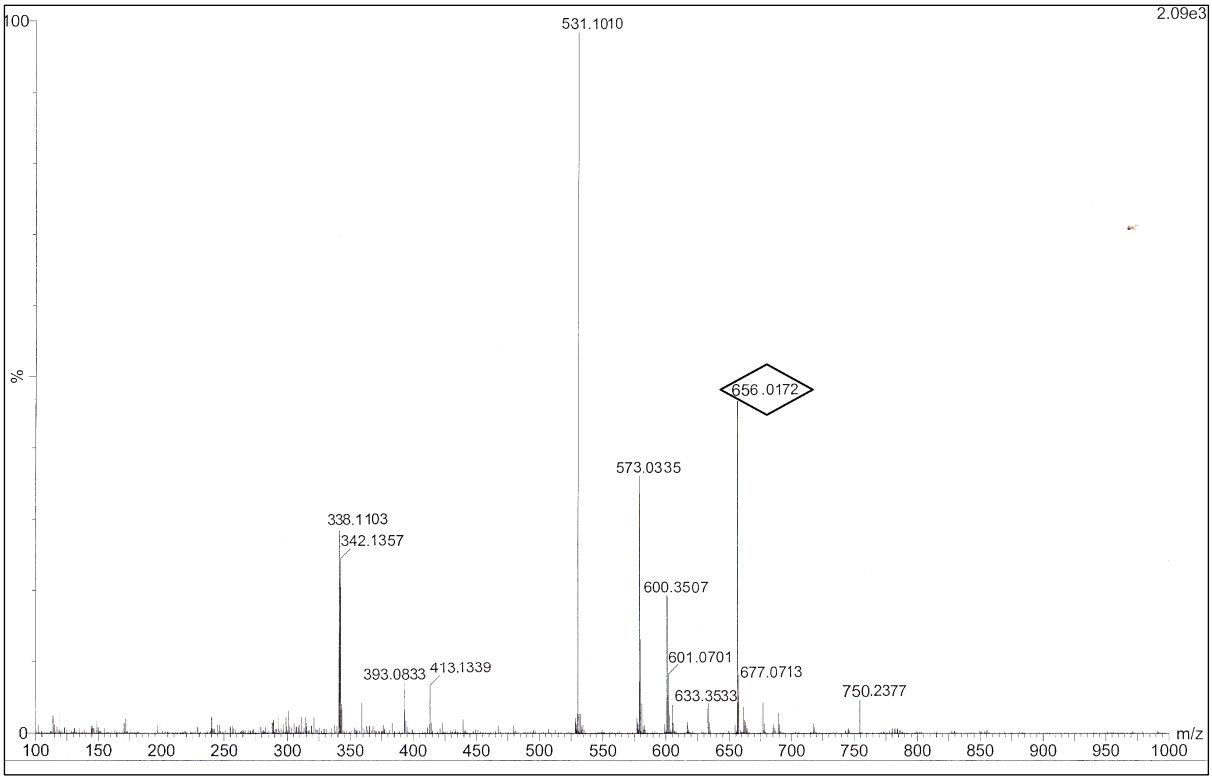
^{13}C NMR spectrum (S_{19}) of Compound SPHN:



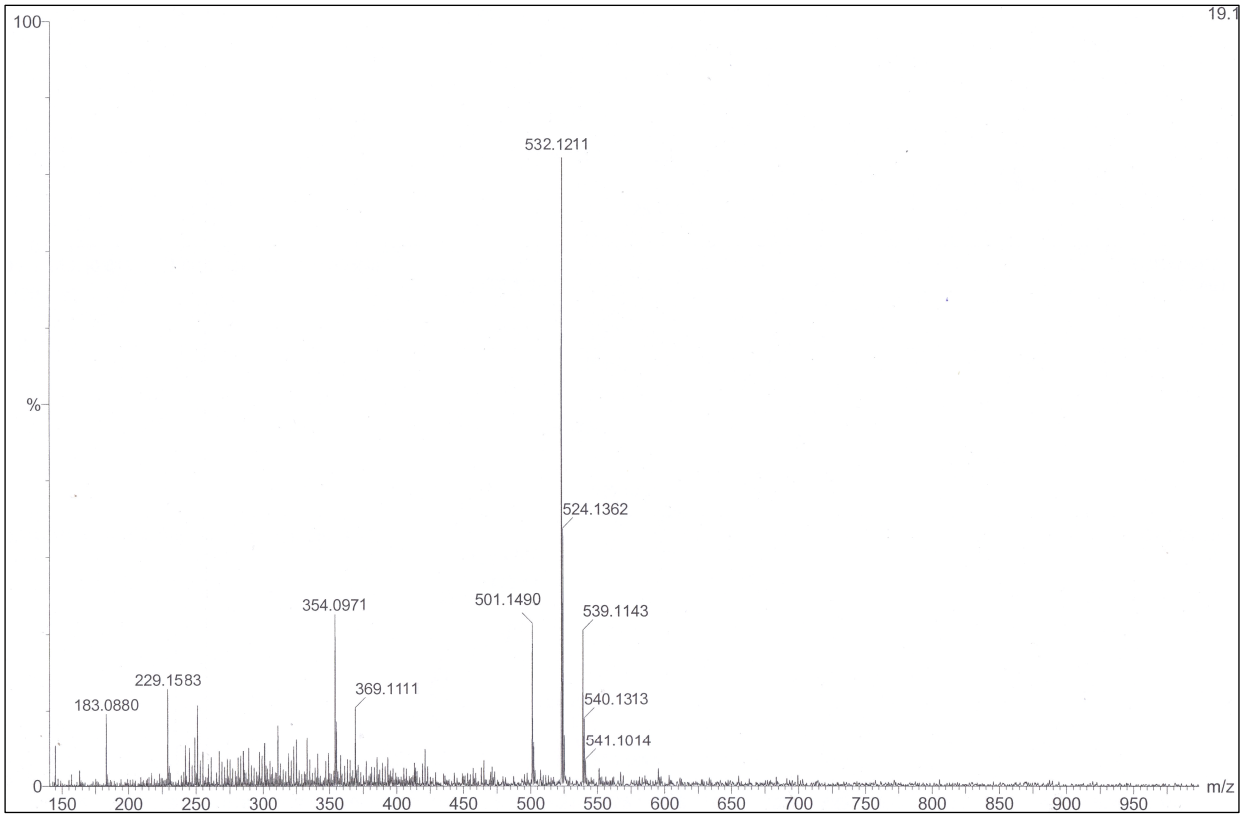
Mass spectrum (S_{20}) of SPHN:



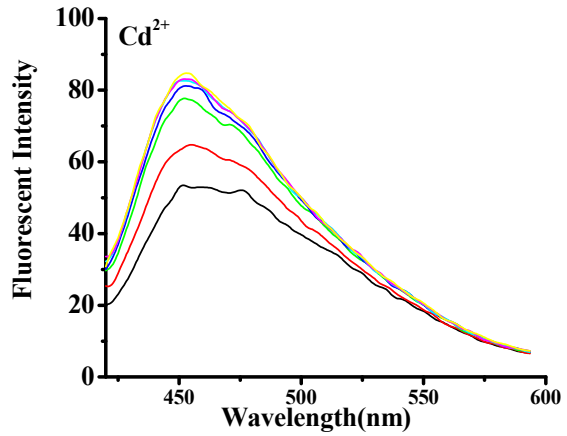
Mass spectrum (S_{21}) of SPHN- Zn complex (Receptor 1):

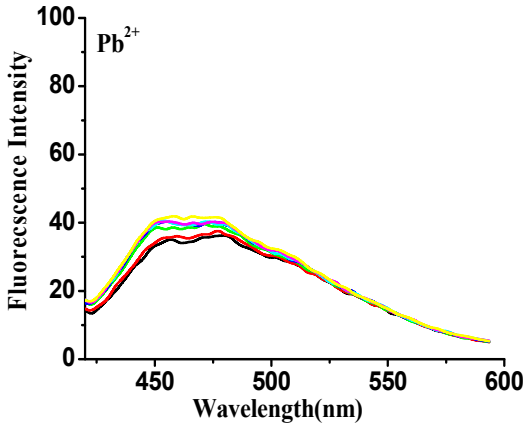


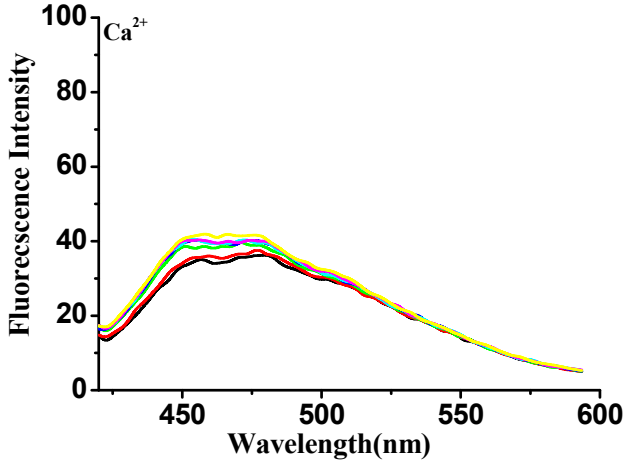
Mass spectrum (S_{22}) of SPHN- Zn complex (Receptor 1)+ PPi:

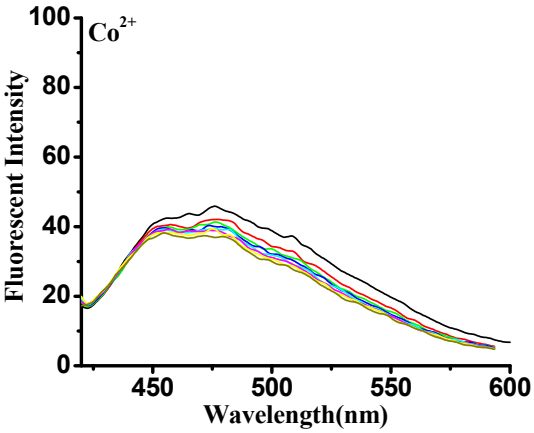


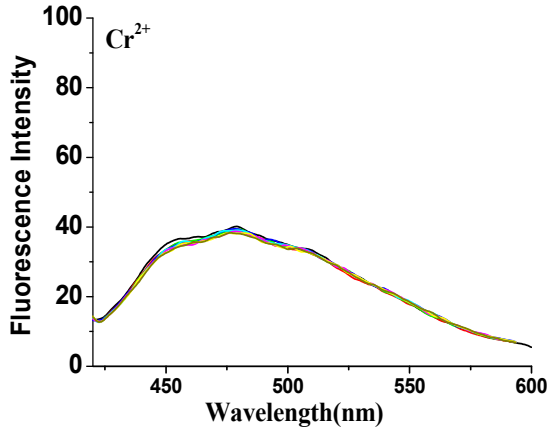
Fluorescence titration spectra (S_{23}) of receptor (c = $2x10^{-5}$ M) with different guest cations (c = $2x10^{-4}$M) in CH_3CN-10 mM aqueous HEPES buffer solution (7/3, v/v, 25°C) at pH-7.4 :

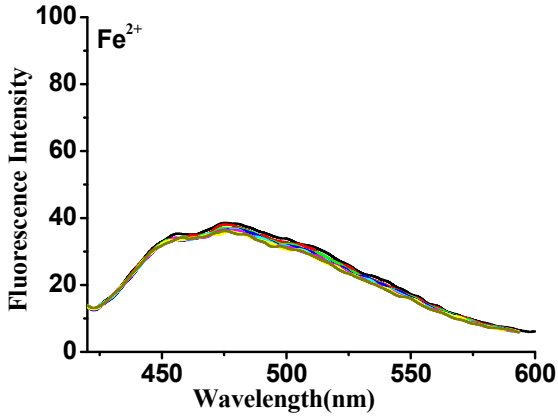


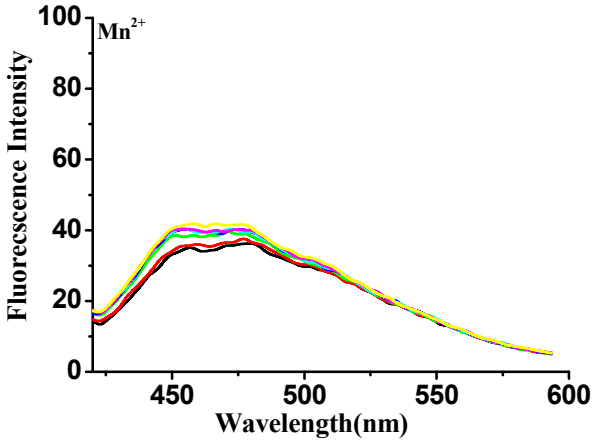


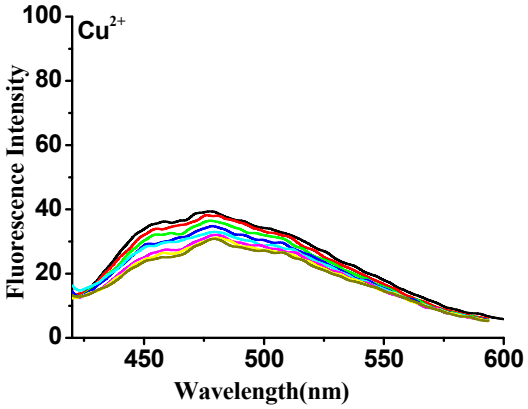


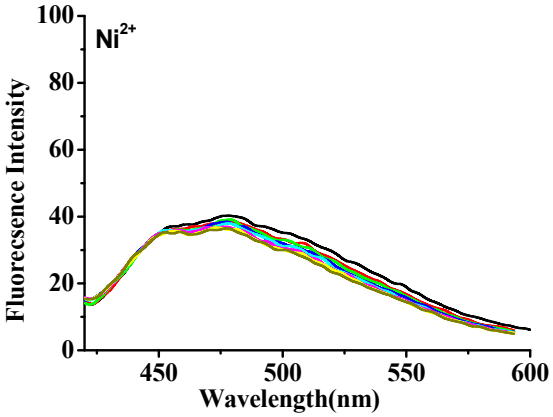


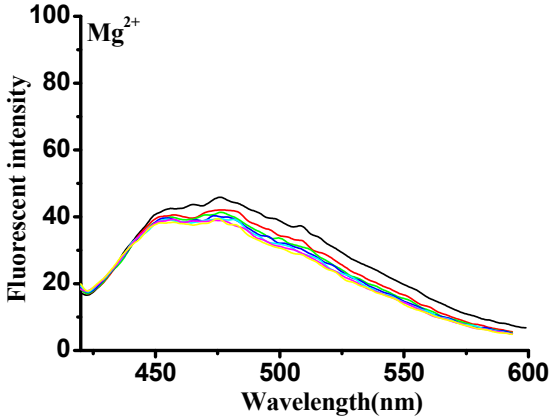


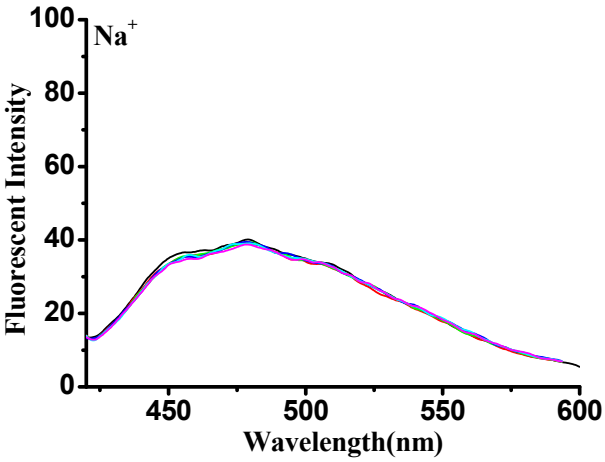


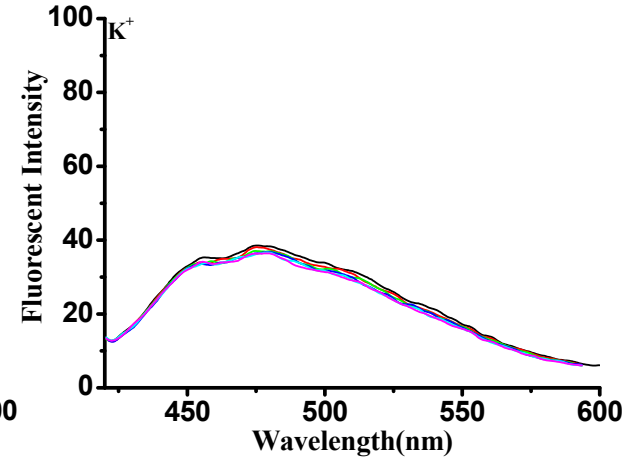

Mn2+
Fluorescence Intensity
Wavelength(nm)

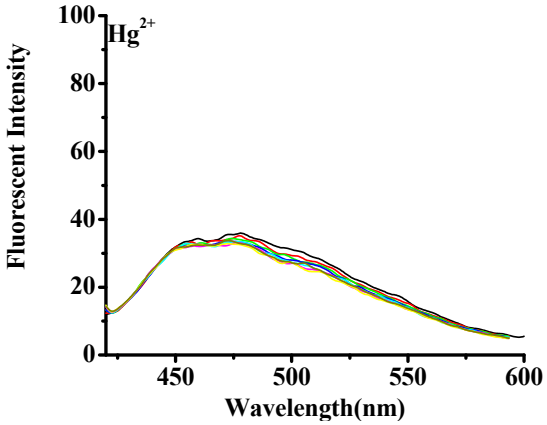

Cu2+
Fluorescence Intensity
Wavelength(nm)


Ni2+
Fluorescence Intensity
Wavelength(nm)


Mg2+
Fluorescent intensity
Wavelength(nm)


Na+
Fluorescent Intensity
Wavelength(nm)


K+
Fluorescent Intensity
Wavelength(nm)



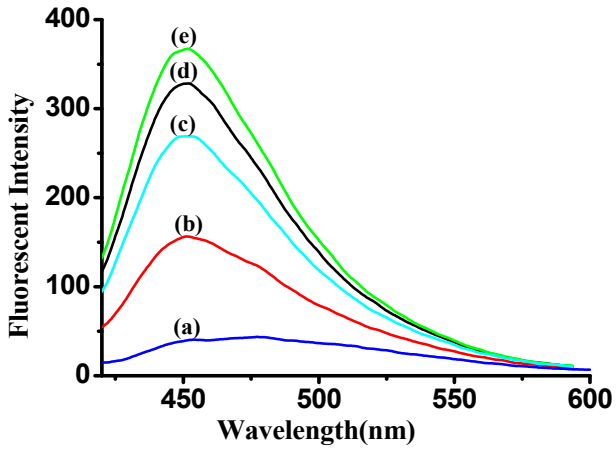


Figure S_{24}: Fluorescence spectra of the receptor **SPHN** (c = 2x10^{-5} M) with 4.0 equiv of zinc chloride (c = 2x10^{-4} M) in different proportions of water in CH_3CN at pH =7.4: (a) **SPHN** itself. (b) H_2O/CH_3CN (8/2, v/v); (c) H_2O/CH_3CN (5/5, v/v); (d) H_2O/CH_3CN (4/6, v/v); (e) H_2O/CH_3CN (3/7, v/v).

❖ **The changes of emission curve of SPHN (c =2x10^{-5}M) at different time interval by addition of Zn^{2+}(c =2x10^{-4}) and calculation of first order rate constant:**

Fig S_{25} (a) represents the changes of fluorescence at different time interval by addition of zinc.

From the time vs. fluorescence plot Fig.$_{25}$ (b) at fixed wavelength at 450 mm by using first order rate equation, we get the rate constant K=slope x 2.303= 0.002 x 2.303=4.6 x10^{-3} Sec^{-1}.

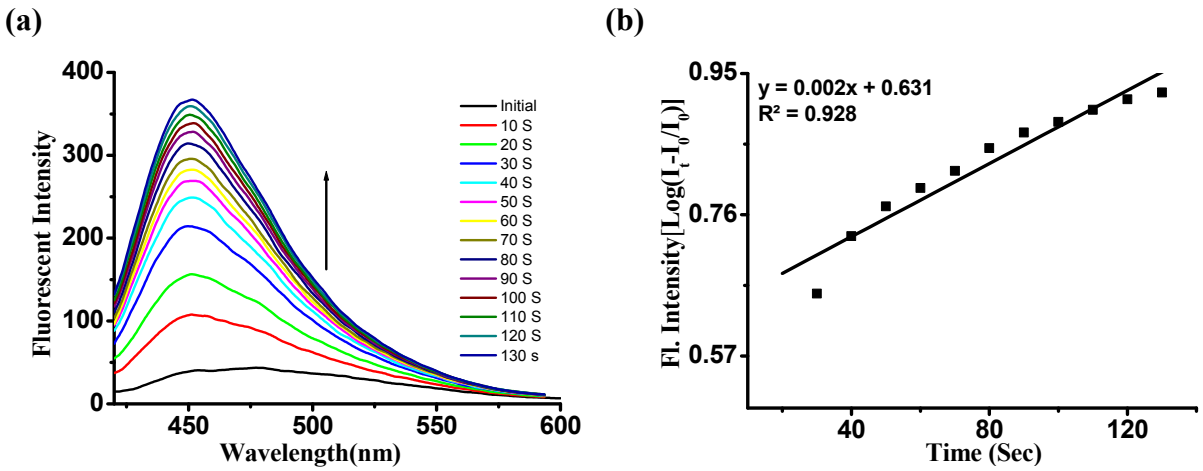


Figure S_{25}: (a) The changes of fluorescence of **SPHN** in presence of Zn^{2+} in CH_3CN- 10 mM aqueous HEPES buffer (7/3, v/v, 25 ºC) at pH =7.4. **Inset**-Different time intervals are shown in the rectangle ('S' denotes Second). (b) The first order rate equation by using Time vs. fluorescence plot at 450 nm (I_t =Maximum intensity, I_0 = Initial Intensity).

Computational Details:

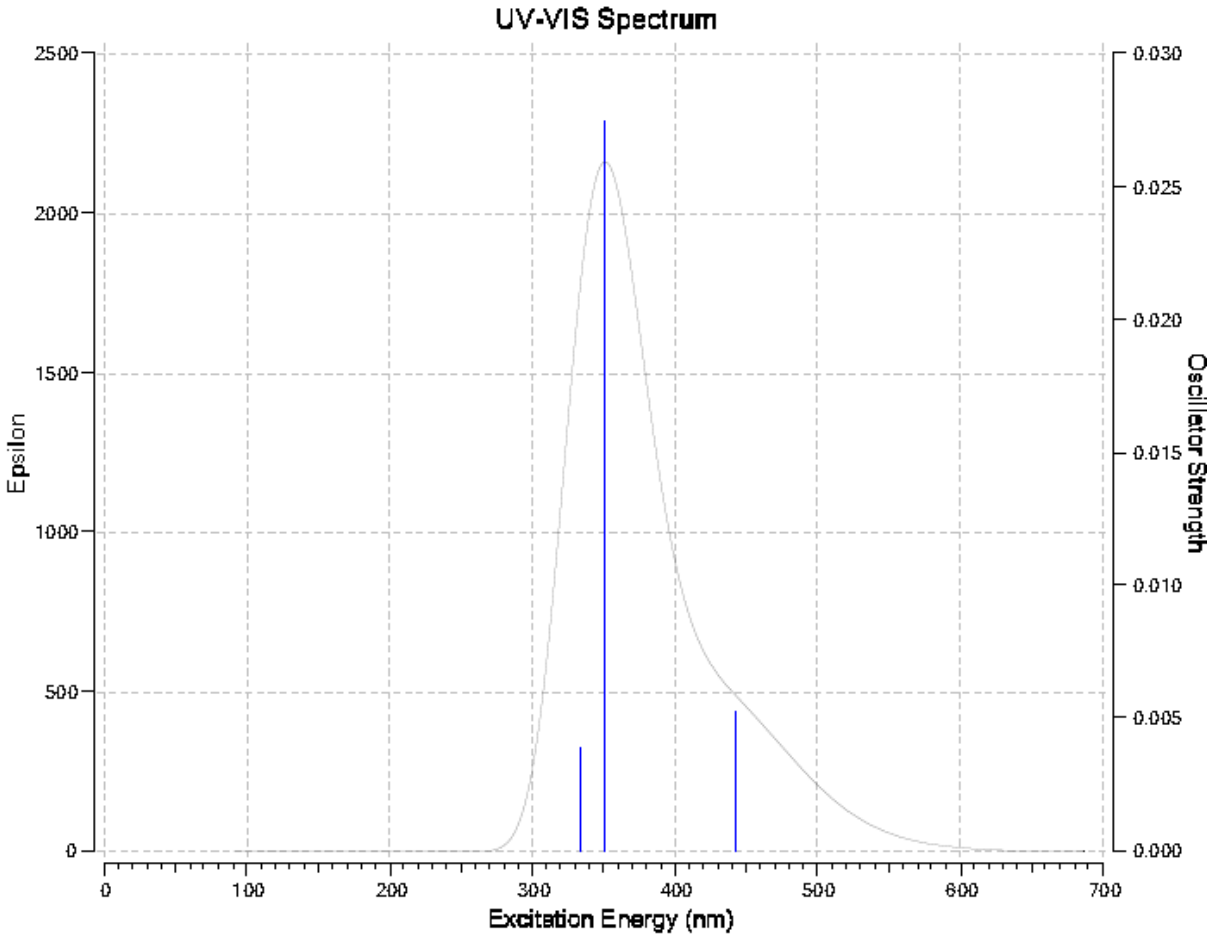


Figure S_{26}: UV-VIS spectrum of SPHN

Three electonic transition that was observed in ligand were given below with oscillator strength.

1.homo-1 to lumo+1 333.99 nm f=0.0039
2.homo-1 to lumo 350.29 nm f=0.0275
3.homo to lumo 438.67 nm f=0.0049

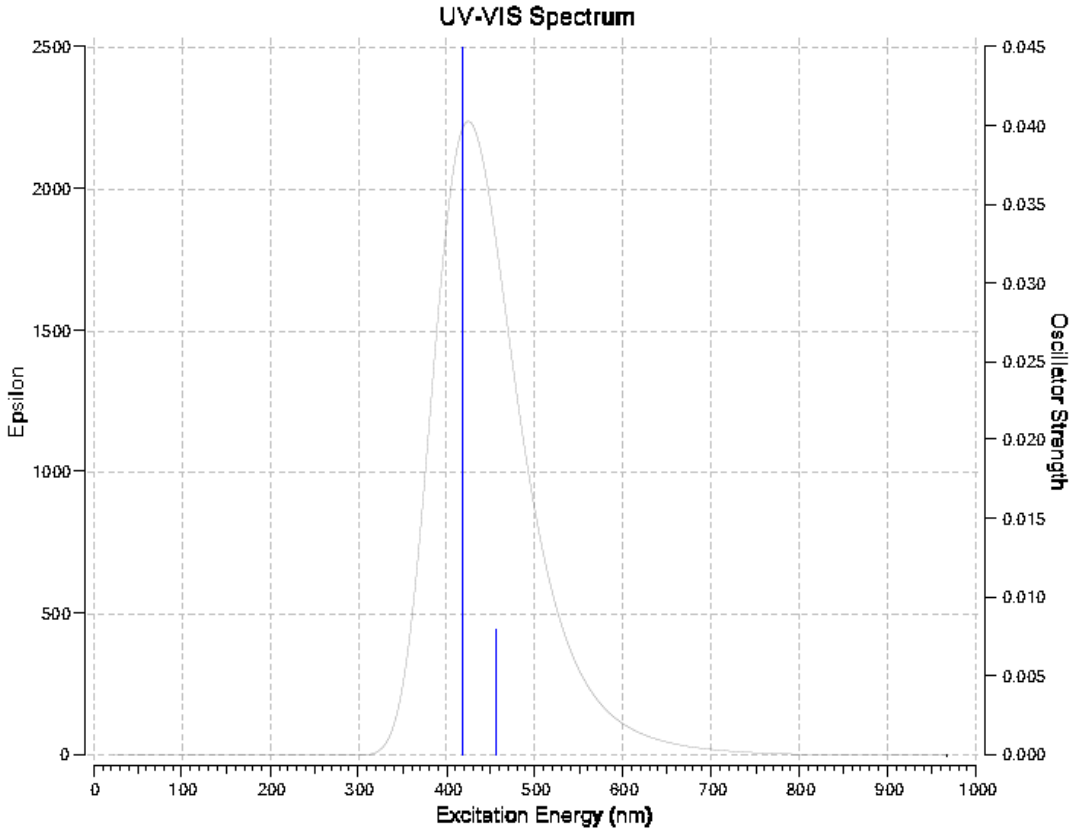


Figure S_{27}: UV-VIS spectra of SPHN – $Zn2^{+}$ complex form TD-DFT calculations.

Form TD-DFT calculations of Zn complex the following electronic tansitions were obberved .

1. homo-1 to lumo+1 417.79 nm f=0.0450
2. homo-4 to lumo 456.17 nm f=0.0080

Pyrophosphate linked compound:

Optimized coordinates of SPHN:

C	-2.53729512	3.96467760	1.78995771
C	-1.13041712	3.96467760	1.78995771
C	-0.46372612	5.19128460	1.78995771
C	-1.21779912	6.36607160	1.78971671
C	-2.62100112	6.26326660	1.78957571
N	-3.28053112	5.08831560	1.78976071
H	0.63481788	5.23106060	1.78900771
H	-0.57568512	3.01777660	1.79022171
H	-0.73376312	7.35099560	1.78961571
N	-3.46381651	7.46765948	1.78946792
C	-3.80680542	8.07722906	0.61499803
H	-3.77250556	7.82077206	2.65697466
O	-3.42574049	7.64469155	-0.48633811
N	-3.28993545	2.70196920	1.79012007
C	-3.16872543	1.81940328	2.82693323
H	-3.88138570	2.51163571	1.02428384
O	-2.42751760	2.04454529	3.79912791
C	-4.69272372	9.32775822	0.76641001
H	-5.62027276	9.05441392	1.22448824
H	-4.88213318	9.74963104	-0.19849795
C	-4.01501486	0.53973507	2.69335380
H	-5.05383358	0.79426795	2.72447745
H	-3.78633167	-0.12493851	3.50008364
N	-4.00385954	10.31739146	1.60723830
N	-3.70984631	-0.11830161	1.41477651
C	-3.39765907	11.18826871	2.34716719
C	-3.97599011	12.60656433	2.50707575
H	-2.49130269	10.92226182	2.84981860
C	-3.23784165	13.70591565	1.98953371
C	-5.17955853	12.81064064	3.13623318
C	-3.75936146	15.01791323	2.13047662
C	-1.98902927	13.52579329	1.33352690
C	-5.70045153	14.11983583	3.27698405
O	-5.92536105	11.70786395	3.65830803
C	-5.00810401	15.19890430	2.78622065
C	-3.02088179	16.11741544	1.61286367
C	-1.29647311	14.60489317	0.84295904
H	-1.59178321	12.50504182	1.22850145
H	-6.66655404	14.25723761	3.78468150
H	-5.90957559	11.73587307	4.61776950
H	-5.40503914	16.21978071	2.89102795
H	-3.43408662	17.13078053	1.72699575
C	-1.81746243	15.91423273	0.98400337
H	-0.33027893	14.46749426	0.33550634
H	-1.24368854	16.76242691	0.58246592
C	-3.44129798	-0.69737389	0.28962850
C	-3.94889982	-2.12448480	0.01160902
H	-2.86450439	-0.18302352	-0.45040721

C	-2.99691415	-3.17075744	-0.13095004
C	-5.29261707	-2.38632278	-0.09760625
C	-3.45133276	-4.49016526	-0.38756684
C	-1.59947057	-2.93020277	-0.02321772
C	-5.74654971	-3.70297004	-0.35357016
O	-6.25365180	-1.33705511	0.04504575
C	-4.84854864	-4.73160077	-0.49532869
C	-2.49901034	-5.53654326	-0.53023991
C	-0.70136648	-3.95878717	-0.16524081
H	-1.25558401	-1.90410849	0.17588254
H	-6.82783464	-3.88715337	-0.43657266
H	-6.65911452	-1.15597578	-0.80607720
H	-5.19205349	-5.75781031	-0.69439927
H	-2.86162706	-6.55623127	-0.72878118
C	-1.15550605	-5.27552658	-0.42145952
H	0.37989337	-3.77453897	-0.08249025
H	-0.41620835	-6.08253778	-0.53125031

Optimized Cordinate of SPHN-Zn^{2+} (Receptor 1) complex:

C	-2.17837303	-1.33849219	3.38825689
C	-1.17691196	-0.39137954	3.63717772
C	-0.84883206	0.55713434	2.65959251
C	-1.51973623	0.55840140	1.43049531
C	-2.52464815	-0.38845185	1.18367232
C	-2.85427107	-1.33530445	2.16234053
H	0.66023168	1.48200240	3.86183463
H	-2.42723844	-2.06387637	4.13446655
H	-0.66021518	-0.39219525	4.57496939
C	0.15075288	1.49729933	2.92077774
C	-1.19746820	1.49391707	0.43710453
H	-3.04143004	-0.38788751	0.24649439
H	-3.62160370	-2.05623831	1.97295565
C	-0.15686879	2.50371303	0.71914950
C	0.49079994	2.45918103	1.96311533
H	1.25610398	3.17372592	2.18353694
O	0.27466734	3.58459081	-0.19155747
C	-1.97189579	1.36198708	-0.92630260
H	-2.61617924	0.53484961	-1.14409198
N	-1.74486167	2.27769840	-1.74996698
O	-2.45220158	4.20849371	-4.81550335
N	-1.67407696	4.92386990	-2.63813469
C	-2.91741855	5.36731801	-1.83160793
C	-4.19322247	5.76199110	-2.25243494
N	-2.59409895	5.12790526	-0.50076766
C	-5.29548543	5.47065149	-1.35861659
H	-4.35325827	6.21128958	-3.21004660
C	-3.74036058	4.99473948	0.38572073
C	-5.06300404	4.97147798	-0.02639065
H	-6.30031791	5.62619265	-1.69327374

H	-5.85894571	4.65157545	0.61377922
N	-3.04486824	5.10169048	1.69834269
C	-3.07221803	5.54552336	3.05685832
O	-4.07547726	5.62088062	3.81228136
C	-1.48713980	5.96591658	3.43006532
H	-0.99133984	5.18777986	3.97279248
H	-1.51846669	6.85268720	4.02864839
N	-0.69814514	6.23702406	2.09006175
C	0.48685545	6.41301886	1.76663795
H	1.31757881	5.97534497	2.27963229
C	0.63322109	7.39976172	0.54947646
C	1.91926093	7.56695671	0.02044321
C	-0.49300171	8.16979942	-0.03711304
C	2.98527754	6.83160892	0.55827058
C	2.14864437	8.46004123	-1.03362250
C	-0.20820126	9.06298795	-1.08159602
C	4.27764103	6.98718954	0.04176884
H	2.81110040	6.15064890	1.36499850
C	3.44221055	8.61355165	-1.55140347
C	1.09519019	9.20495019	-1.57511003
H	-0.99901856	9.64278532	-1.50944501
C	4.50602529	7.87804297	-1.01418928
H	5.09035540	6.42542823	0.45406811
H	3.61669799	9.29421011	-2.35841926
H	1.28755429	9.88914952	-2.37495126
H	5.49293466	7.99656794	-1.41020251
O	-1.91848261	8.07567889	0.37310791
Zn	-2.00430063	6.28227589	0.85062063
Zn	-1.27618994	3.94693289	-1.16121177
C	-1.99175599	3.89282811	-3.68737948
C	-1.74705319	2.34216812	-3.25881140
H	-0.79191169	2.02386479	-3.62196722
H	-2.50774485	1.71086112	-3.66826321

Optimized Cordinate of SPHN-Zn^{2+} + PPi complex:

C	-1.43679	-4.53964	2.2766
C	-1.55806	-3.40751	3.09644
C	-1.61151	-2.12903	2.52326
C	-1.5434	-1.98668	1.13318
C	-1.41958	-3.11612	0.31211
C	-1.36615	-4.39299	0.88433
H	-1.78141	-1.09932	4.40155
H	-1.39825	-5.51575	2.7132
H	-1.61132	-3.51944	4.15945
C	-1.73419	-0.99194	3.33805
C	-1.5989	-0.7146	0.55926
H	-1.36491	-3.00314	-0.75162
H	-1.27155	-5.25613	0.25895
C	-1.72831	0.43072	1.36741

C	-1.79641	0.28609	2.76038
H	-1.89678	1.15033	3.38342
O	-1.81402	1.74471	0.77374
C	-1.55614	-0.56098	-0.96373
H	-1.74549	-1.39981	-1.60021
N	-1.3049	0.59281	-1.46127
C	-1.38878	2.52638	-2.9579
O	-1.38851	3.03886	-4.10716
N	-0.92023	3.32766	-1.78563
C	-1.80689	4.22083	-1.07948
C	-3.07055	4.72566	-1.39952
N	-1.21463	4.57495	0.01396
C	-3.65597	5.62977	-0.4689
H	-3.5754	4.44181	-2.29856
C	-1.63659	5.46638	0.8427
C	-2.91646	6.01498	0.68632
H	-4.63773	6.01915	-0.63729
H	-3.31865	6.70121	1.40233
N	-0.62076	5.7437	1.84843
C	-0.71181	7.00675	2.63581
O	-1.81594	7.49876	2.98055
C	0.66397	7.68616	3.02263
H	1.07652	7.23851	3.90354
H	0.52917	8.73286	3.19805
N	1.53121	7.48282	1.85837
C	2.45385	8.29233	1.4707
H	2.89265	8.98478	2.15821
C	2.90709	8.2566	-0.00412
C	4.10241	8.88255	-0.39094
C	2.10191	7.61609	-0.97395
C	4.89703	9.5346	0.56282
C	4.50424	8.85547	-1.73414
C	2.5157	7.5882	-2.31288
C	6.09041	10.15922	0.17251
H	4.59317	9.55547	1.58835
C	5.69625	9.4805	-2.1232
C	3.71579	8.20409	-2.69151
H	1.91286	7.09617	-3.04761
C	6.48915	10.13282	-1.17061
H	6.69694	10.65615	0.90005
H	6.00072	9.45964	-3.14871
H	4.03027	8.17801	-3.714
H	7.39901	10.61075	-1.46848
O	0.84723	7.00753	-0.61342
Zn	1.08867	5.88368	0.9014
Zn	-0.39752	1.98061	-0.47997
P	2.90735	4.23068	0.46145
O	4.00945	5.08875	-0.09816
O	3.44601	2.59838	0.47575
P	1.96882	1.76067	0.36349

O	2.08387	0.43478	1.06697
O	1.44418	4.4425	-0.34398
O	1.34926	1.52236	-1.18861
O	0.71391	2.69454	0.93101
O	2.36815	4.86652	1.94329
C	-1.82294	1.03746	-2.76376
H	-1.44229	0.43363	-3.56094
H	-2.89083	0.96974	-2.75052

References:

1. (a) Reddi, A. R.; Guzman, T. R.; Breece, R. M.; Tiemey, D. L.; Gibney, B. R.; *J. Am. Chem. Soc.*, **2007**, *129*, 12815–12827. (b) Lohani, C. R.; Kim, J-M.; Chung, S-Y.; Yoon, J.; Lee, K-H.; *Analyst*, **2010**, *135*, 2079-2084.