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A Hypervalent Bismuth(III) Derivative of Tetrakis-4-Methoxyphenyl porphyrin: Synthesis, Spectroscopic Characterisation and Morphological Investigations Padma Dechan and Gauri Devi Bajju*

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Abstract

In this study, we aimed to synthesize as well as spectroscopically characterize a sitting-atop (SAT) macro molecule of Tetrakis-4-methoxyphenylporphyrinato Bi(III). The bismuth porphyrin was prepared by the reaction of free base macrocycle, 5, 10, 15, 20-Tetrakis-4methoxyphenylporphyrin H_2TMP (I) with bismuth nitrate pentahydrate, Bi(NO₃)₃·5H₂O in dry pyridine. The spectroscopic data demonstrates the existence of a binuclear moiety [Bi(TMP)NO₃]₂ in the solid state and even in solution in which two bismuth porphyrins are interlinked by a bridging bidentate nitrate. Self dimerisation of bismuth porphyrin is expected to arise due to strong tendency of Bi^{3+} ions to become hypervalent. The electronic absorption as well emission bands in complex (II) are bathochromically shifted relative to compound (I) with emission bands significantly quenched in the metal chelate. At pH ~5, complex (II) is demetallized to form the dicationic analogs of I which tend to undergo prominent J-type supramolecular aggregation. Demetallation is also followed when a dichloromethane solution of metal chelate (II) is kept for a long period at room temperature. The macro rings were also studied by nano technology like SEM, TEM, EDX and powder XRD. Thermal analysis and invitro cytotoxicity screening of the macro rings against leukaemia K₅₆₂ strains have been further demonstrated.

Keywords: sitting-atop (SAT), binuclear moiety, bismuth porphyrin, bidentate nitrate, hypervalent

1. Introduction

The first bismuth porphyrins, mesoporphyrin IX dimethyl ester and etioporphyrin bismuth (III) was reported back in 1969 [1]. However, the UV-visible and elemental analysis data alone could not establish the complete structure of these complexes. Later some additional spectroscopic measurement data like IR, ¹H NMR and mass spectrometry aided the enhanced characterisation of third bismuth porphyrin i.e. bismuth octaethylporphyrin (OEP), Bi(OEP)(O)NO₂ [2]. When the size of a central metal ion is higher than that of porphyrinic core, the metal ion sits above the N₄ core plane of ligand thereby distorting it. The first evidence for out of plane coordination of Bi³⁺ ion was reported by Tanya Barbour and coworkers on the basis of variable-temperature ¹H NMR investigation of [Bi(TTP)]NO₃ [3]. The out of plane coordination mode of bismuth porphyrin owing to size mismatch between

the central metal ion and porphyrin core was later supported by the first single crystal XRD report of another bismuth porphyrin, Bi(OEP)SO₃CF₃ [4]. In the solid state, this complex exists as dimer with two $[(OEP)Bi]^+$ cations inter linked via three oxygen atoms belonging to two symmetrically related SO₃CF₃⁻ anions [4]. Solid state dinuclear bismuth porphyrins containing halide anion, Bi(tpClpp)(X), X = Cl, Br and nitrate anion, Bi(tpClpp)(NO₃) were also reported by Bernard and co-workers [5]. The halide complexes Bi(tpClpp)(X), X = Cl, Br crystallizes as centrosymmetric dimers doubly bridged through Cl or Br, whereas the nitrate complexes, Bi(tpClpp)(NO₃) crystallises as a less symmetric dimer in which one NO₃ ligand bridges the two bismuth centres and the second NO₃ ligand interacts with only one bismuth atom. In its dinuclear form, bismuth centre acts as strong Lewis-acid and hence become 'hypervalent', a tendency satisfied by self association and sometimes by solvent coordination [6-9].

In this report we present similar hypervalent interaction of a Bi^{3+} derivative of Tetrakis-(4methoxyphenyl) porphyrin and on the basis of our findings we suggest that the judicious choice of chelating ligands (very less sterically demanding) may allow identification of 'hypervalency' even in those systems for which crystallographic analyses are not forthcoming. The remarkable history of medicinal use of bismuth compounds [10-14] led us investigate the antitumor activity of the synthesized macro ring **II** against Leukaemia K₅₆₂ strains and compare the results with its free base analog **I**. Thermal stability of both the macrocycles has been further demonstrated. The proposed structure of synthesized complex **II** is given in Scheme 1. The scheme explains that the Lewis acidity of Bi^{3+} centre is satisfied by interactions with two oxygen atoms of a bridging bidentate nitrate from neighbouring Bi atom, giving a dimer moiety.



Scheme 1 Proposed structure of II

2. Experimental

2.1. Materials and methods

The analytical reagent grades chemicals were used for the synthesis of **I** and **II**. The AR grade Chloroform, dichloromethane, pyridine, pyrrole, 4-methoxybenzaldehyde, propionic acid and conc. HCl were purchased from Sigma-Aldrich and were used as received. The metal carrier, $Bi(NO_3).5H_2O$ and K_{562} cell lines established from human chronic myelogenous leukaemia (CML) cells in blastic crisis were also purchased from Sigma Aldrich.

2.2. Analytical methods

FT-IR spectra of the compounds were recorded between 4000 cm⁻¹ to 400 cm⁻¹ using KBr pellets on Perkin Elmer Spectrum. ¹H NMR spectra were recorded on Bruker spectrometer, model AV 400 N (400 MHZ), using CDCl₃ as solvent and TMS as internal reference at room temperature. UV-Visible spectra were recorded on PG spectrophotometer in dichloromethane ranging from 300 to 700 nm. To introduce acidic conditions in the organic solvent containing porphyrinic dye, stock aqueous solutions of 0.1M HCl were used. The pH values of the solution were measured with a pH paper. Photoluminescence emission studies were recorded on Cary eclipse Agilent using dichloromethane as solvent. Mass spectra were acquired on Bruker Compass Data Analysis 4.1. TGA were recorded on Perkin Elmer under nitrogen atmosphere. The phase purity and crystallite sizes of the as-prepared samples were determined from powder X-ray diffraction (PXRD) using XPERT-PRO Diffractometer in the 2θ range of 4° to 80°, with CuK α radiation ($\lambda = 1.540$ Å). Scanning electron microscopy (SEM) micrographs of the samples were recorded on FEI Nova Nano SEM 450. Transmission electron microscopy (TEM) was recorded on Tecnai G2 20 S-TWIN transmission electron microscope with a field emission gun operating at 200 kV. The samples for TEM measurements were prepared by evaporating a drop of the colloid onto a carboncoated copper grid.

2.3. Cytotoxicity assay against leukaemia K562 cells

Cell culture: The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100μ g/ml penicillin, 10μ g/ml streptomycin and 2mmol/l L- glutamine. The cells were maintained in log phase growth at 37 °c in a humidified atmosphere containing 5% CO₂. The leukaemia K₅₆₂ cells were treated with **I** and **II** at different concentrations of 1, 5, 10, 20, 30, 40 and 50 μ M for 48h. In vitro cytotoxicity against leukaemia K₅₆₂ cells was determined using sulforhodamine B dye assay [15, 16].

Synthetic procedure

2.4. Synthesis of I

The starting free base porphyrin H_2TMP , I was synthesized according to modified Adler's method [17]. Equivalent moles of pyrrole (2.08 mL, 20mmol) and 4-methoxybenzaldehyde (3.65 mL, 20mmol) were added to the propionic acid solution (50 mL) in a 250 mL round bottomed flask fitted with water condenser (scheme 2). The reaction mixture was then refluxed at 60°C for half an hour in dark. At the end of the reaction, the mixture was allowed to cool at room temperature and then filtered. The residues were allowed to dry in vaccum dessicator overnight and the crude product was chromatographed on a silica gel column using pure methanol as eluent. The porphyrin elutes from the column as purple band. The solid compound obtained after evaporating the organic solvent was finally recrystallized from ethylacetate/petroleum ether mixtures (8:2).

I was obtained as purple solid with 60% yield. FT-IR (KBr, v_{max} , cm⁻¹): 3317.66 v(N-H)pyrrole, 730.46 γ(C-H)ph, 800.04 γ(C-H)pyrrole, 1341.22 v(C-N), 1595.22 v(C=N), 2913.18 v(C-H) OCH₃, 1023.84 v(C-O-C)_{sym}, 1233.98 v(C-O-C)_{asym}.¹H NMR (CDCl3, 400MHz, δ, ppm): -2.74 (s., 2H, NH), 4.12 (s., 12H, OCH₃), 7.31-7.33 (d., 8H, ArH_m), 8.14-8.16 (d., 8H, ArH_o), 8.89 (s., 8H, βH). UV-Vis (λ_{max} , nm, CH₂Cl₂): 421 (B band), 519, 555, 594, 651 (Q bands).

2.5. Synthesis of II

Bismuth porphyrin was synthesized according to the method provided in the literature [4]. In a typical reaction, 0.0813 mmol of H_2TMP and 0.813 mmol of Bi(NO₃)·5H₂O were added to a basic pyridine solution (30 mL) in a 250 mL round bottomed flask fitted with water condenser (scheme 2). The mixture was then refluxed at 65°C for 1 hour in dark under inert atmospheric conditions. As the reaction progresses the solution color changed from dark purple to dark green. The progress of the reaction was also determined spectrophotometrically (by noting the position of B and Q bands). As the reaction completed, the mixture was allowed to cool at room temperature and the solvent was completely removed using rotary vapour. The crude green residues were then chromatographed on silica gel column. Elution with dichloromethane first removed a faint purple fraction containing unreacted H₂TMP. The desired compound **II** eluted later from the column as a dark green band using methanol/dichloromethane fractions (80:20).

Complex **II** was obtained as green solid with 40% yield. FT-IR (KBr, v_{max} , cm⁻¹): 1349.92 v(C-N), 1602.55 v(C=N), 2834.84 v(C-H), 1035.58 v(C-O-C)_{sym}, 1245.78 v(C-O-C)_{asym}, 989.30 (Bi-N). ¹H NMR (CDCl3, 400MHz, δ , ppm): 4.15/4.19 (s., 12H, OCH₃), 7.35-7.37/7.55-7.58 (d., 8H, ArH_{3,5}), 8.57-8.59/8.73-8.75 (d., 8H, Ar_{2,6}), 9.28 (s., 8H, β H). UV-Vis

 $(\lambda max, nm, CH_2Cl_2)$: 454, 471 (soret bands) and 605, 680 (Q bands). MS (intensity): m/z 941.27 [Bi(III)(TMP)]⁺.

3. Results and Discussions

3.1. Syntheses

The starting material **I** was prepared according to the modified Adler's method [17]. For the preparation of complex **II**, we followed the same procedure as described in the literature [4]. However, in our method we have used only 30 mL of basic solvent i.e. pyridine against 100 mL used in the previous method for the same molar ratios (~ 1:10) of the reactants i.e. free base and Bi(NO₃).5H₂O mixtures. Also in the previous method, the free ligand, Tetramesitylporphyrin and Bi(NO₃).5H₂O were needed to reflux for 5 days. However, in the present method, the chelating ligand **I** was subjected to Bi³⁺ insertion by refluxing the two reactants i.e. Tetra-para-methoxyphenylporphyrin and Bi(NO₃).5H₂O for just one hour under dark in an inert atmospheric conditions. Inert atmosphere was used since bismuth is highly sensitive towards reactive oxygen [18]. Dark environment during the entire refluxing period was maintained to avoid the effect of light on the highly conjugated π system. The crude metal porphyrin like in previous method was purified on silica gel column using CH₂Cl₂/CH₃OH mixtures. However in our case, we had to slightly increase the polarity of the eluents as the metal complex was reluctant to elute from the column using low polar eleunts. As a result, we were needed to reconsider the CH₂Cl₂/CH₃OH ratio as 20:80.



Scheme 2 Synthetic route for I and II

FT-IR spectroscopy, ¹H NMR spectroscopy, Mass spectrometry, Powder XRD data and UV– Vis spectroscopy aided the full characterization of Tetra-para-methoxy phenyl porphyrin and its Bi³⁺ analogue.

3.2. FT-IR spectroscopy

The selected regions of the FT-IR spectra of the synthesized porphyrins are presented in Fig. 2. The full spectrum is provided in the supplementary file as Fig. S1. The free base porphyrin **I** exhibit bands occurring due to N–H stretching and bending vibrations at 3315.03 and 981.17 cm⁻¹ [Fig. 2b]. These bands are however absent in the bismuth porphyrin due to the replacement of two imino protons by the metal ion [19]. The bismuth porphyrin in fact exhibits characteristics bands due to Bi–N stretching [Fig. 2b] at 989.30 cm⁻¹. A band due to aromatic ring vibration at 964.23 cm⁻¹ in **I** appear as diminished broad band in **II**. FT-IR spectroscopy also helped in determining the mode in which the nitrate anions coordinate with the Bi³⁺ ion in complex **II**. Nitrate ion can coordinate to a metal in three different modes as shown in Fig. 1. In particular the ability of nitrate to act as a bridge between two metal ions plays important role in the formation of unique multinuclear coordination complexes [20-22].



Fig. 1 Different coordination modes of the nitrate ion: (a) monodentate, (b) bidentate bridging and (c) bidentate chelating.

The FT-IR spectra of **II** indicate the presence of bidendate nitrates. Peak due to N=O stretching mode (v₁) appear at 1465.63 cm⁻¹ [Fig. 2c]. The asymmetric stretching mode (v₅) of the $-NO_2$ group in the complex appear at 1286.28 cm⁻¹ [Fig. 2c]. The symmetric stretching mode (v₂) of the $-NO_2$ group in the complex appear at 1018.23 cm⁻¹ [Fig. 2b]. The separation between the two high frequency bands is ~180 cm⁻¹ indicating bidendate mode of coordination of nitrate group with the metal ions. The symmetrical in plane bending (v₄) of NO₂ coordinated with the metal ion is split into two bands [Fig. 2b] and can be assigned at 711.60 and 725.10 cm⁻¹ or 740.53 and 755.95 cm⁻¹ [23]. The FT-IR spectroscopy also helps in determining the dinuclear bismuth porphyrin. The new bands ~560-580 cm⁻¹ [Fig. 2a] and ~636-680 cm⁻¹ (Fig. 2b) in the metal complex **II** are attributed to symmetric and asymmetric

stretching vibrations of Bi-O-Bi bonds [24, 25]. A small peak ~850 cm⁻¹ [Fig. 2b] may be due to Bi-O bending vibration in the metal complex [26].



Fig. 2 The FT-IR spectra of I (magenta) and II (blue).

3.3. ¹H NMR spectroscopy

Fig. 3 and Fig. 4 represent the selected regions of the ¹H NMR spectra of **I** and **II** in CDCl₃ at room temperature. The complete spectra are given in the supplementary file as Fig. S2 and S3 respectively. The pmr spectra of **I** consists of singlets due to β -pyrrole proton (**Ha**), para phenyl methoxy proton (**Hd**) and imino proton (**He**) resonances accompanied by a pair of

doublets each consistent with ortho phenyl proton (**Hc**) and meta phenyl proton (**Hb**) resonances. Upon Bi³⁺ insertion, the signal due to highly shielded imino protons in **I** disappear due to the replacement of pyrrolic hydrogens by the metal ion confirming the successful metallation of the chelating ligand [Fig. S3]. The characteristic feature is the splitting of the signals due to (**Hb**) and (**Hc**) in bismuth porphyrin, **II**. This splitting of signals may be ascribed to magnetic inequivalence of ortho and meta aryl protons due to out of plane coordination of metal ion with ligand molecule **I** [3]. Complex **II** further shows splitting of methoxy proton (**Hd**) signal which are not originated due to out of plane coordination of metal ion and in fact may be attributed to the existence of a binuclear moiety with the two bismuth (III) centres having different coordination numbers. The split signal is the pretence of 'hypervalent' interaction in action which leads to self dimerisation even in solution. The molecular complexation of **I** with Bi³⁺ ion causes downfield shifts of resonance signals. This is due to increase in ring current produced by extension of the aryl π system.



Fig. 3 The selected regions of the ¹H NMR spectra (400 MHz pulse FT) I (~4.0 X 10⁻⁵ M) in CDCl₃.



Fig. 4 The selected regions of the ¹H NMR spectra (400 MHz pulse FT) II (~4.0 X 10^{-5} M) in CDCl₃.

3.4. Mass spectrometry

Electrospray ionization mass spectrometry (ESI/MS) also allowed the characterisation of bismuth porphyrin dimer. Fig. 5 represents the mass spectrum of metalloporphyrin **II**. The dissociation of $[(BiTMP)NO_3]_2$ (molecular weight 2007.64g/mol) produce $(BiTMP)^+$ and $[Bi(TMP)(NO_3)_2]^+$ ions with m/z 941.27 and 1065.46 (calculated value 940.82, 1064.82). The bismuth porphyrin $[(BiTMP)NO_3]_2$ also dissociates to produce ions with m/z 1470.62 and 561.11 (calculated value 1465.68 and 540.96) which may correspond to $(TMP)_2^+$ and $(BiNO_3)_2^+$ species. The peak with m/z 1676.58 (calculated value 1673.66) may be due to the formation of $[Bi(TMP)_2]^+$. The highest intensity peak with m/z 735.31 (calculated value 733.84) correspond to $(TMP)^+$.



4. Powder XRD analysis

The structure and phases of the resulting macro rings **I** and **II** were measured using powder X-ray diffraction (PXRD). Fig. 6 shows the X-ray diffraction patterns of **I** and **II**. The peaks at $2\theta 6.35^{\circ}$, 12.57°, 14.71°, 19.06, 25.24° and 28.38° in **I** [Fig. 6a] corresponds to diffraction from (111), (310), (321), (422), (620) and (711) planes respectively. Similarly the XRD peaks at $2\theta 6.45^{\circ}$, 10°, 12.72° 17.72°, 19.66°, 32.88° and 47.08° in **II** [Fig. 6b] are consistent with diffraction from (211), (321), (422), (622), (633), (1055) and (1297) planes respectively. The diffraction peaks corresponding to metal complex **II** [Fig. 6b] are significantly different from that of **I** suggesting appreciable change introduced in the crystal structure of **I** upon incorporation of metal ion. The XRD patterns indicate that **I** crystallize in face centred cubic geometry (JCPCDS card number 77-0437) whereas **II** crystallizes in the body centred cubic geometry (JCPCDS card number 45-0109). The high intensities of the diffraction peaks in **I** indicate high crystalline nature of the compound. The diffraction peaks in **II** are broader especially at larger 2θ values. The average crystallite sizes of the macro rings were measured from the characteristic peaks using Scherer's equation (1)

 $D=0.9\lambda/\beta \cos\theta$

Where D is the crystallite size, λ is the wavelength of the X- ray, β is the FWHM (full width at half maximum) of the diffraction peak, θ is the diffraction angle and k is the Scherer's constant of the order of unity. The average crystallite sizes calculated using three higher intensity peaks at 2θ 6.35°/18.85°/25.18° (I) and 6.42°/17.72°/32.88° (II) was found to be 60.45 (I) and 31.11 nm (II). The calculated result indicates that metal coordinated porphyrin particles are of very small crystallite size. The smaller interplanar spacing (Table S1) in II may ascribe to close intermolecular interaction induced by bridging bidentate nitrate group. The scattering angles and calculated *d*-spacings of the most intense scattering peaks are summarised in Table S1.



Fig. 6 X-ray powder diffraction patterns of (a) I and (b) II.

^{5.} Absorption spectroscopy

The UV-Visible absorption and excitation spectra of **I** in CH₂Cl₂ are given in Fig. S4. The absorption spectrum of **I** manifest five characteristic bands [Fig. S4 (left)]. The most intense band at 422 nm is the B band and the four weak bands between 518 to 652 nm are the Q bands. The B band originates because of π , π^* transition from the ground singlet state to the second excited singlet state (S₀ \rightarrow S₂), while the Q bands arise due to π , π^* transitions from the ground singlet state to the first excited singlet state (S₀ \rightarrow S₁) [27]. Both B and Q bands arise from π - π electronic transitions. In comparison to the spectra of tetraphenyl porphyrin (B band at 416 nm) under the same solvent [28], the absorption bands in tetra para methoxyphenyl porphyrin are shifted towards longer wavelengths. This is attributed to increased π conjugation and reduction of the HOMO–LUMO energy gap induced by the electron donating para methoxy substituents in **I** [29, 30]. The excitation spectra of **I** [Fig. S4 (right)] is in good agreement with its corresponding absorption spectra.

The coordination of Bi^{3+} ion to the nitrogen atoms of **I** results in the marked splitting of B band followed by reduction in the number of Q bands to only two [Fig. 7]. The split B bands appear at 453 and 472 nm, while as the two Q bands are centred at 605 and 680 nm respectively. The splitting of B band and whole spectral broadening reflects the existence of binuclear motifs in the solution. The splitting occurs due to the exciton coupling between the two porphyrin macrocycles in the ground state [31, 32, 33]. The split bands are separated by $\lambda_{max} \sim 18$ nm [Fig. 7 (right)]. The reduced number of Q bands in metal porphyrin is due to the increase in the symmetry (D₄h) of the macro ring. The presence of two diagonally positioned hydrogens within the porphyrin core of free base ligand decreases the symmetry of macro ring to D₂h [34].

The bathochromic shift of absorption bands in **II** relative to **I** is ascribed to electronic as well as steric effect induced by complexation of **I** with heavy metal ion (Bi³⁺). These effects can be explained as the atomic orbitals of weakly bonded metal centre affects the unoccupied metal orbitals of porphyrin more than the occupied ones resulting in the reduction of the HOMO-LUMO energy gap (electronic factor) while as distortion of ligand plane due to sitting atop coordination of metal ion results in twisting of phenyl rings from almost perpendicular orientation to the planar ones favouring strong interaction to occur between the peripheral aryl rings and porphyrin π system (steric factor) [35, 36, 37]. In addition to B and Q bands, the metalloporphyrin **II** manifests an extra absorption band at 328 nm characteristic of a hyper p type porphyrin [Fig. 8 (left)]. This band appear due to charge transfer electronic transition from filled 6p orbitals of Bi³⁺ centre to empty π^* orbitals of porphyrin [38].

Out of plane (OOP) metalloporphyrins are kinetically labile in nature due to the fact that the weakly bonded metal ion in OOP complexes lies out of ligand plane and can be easily demetallized under certain conditions like presence of acid [39]. Similar behaviour is also reflected by the bismuth porphyrin in the present case. At pH ~ 5, notably a split B band appears at 422 and 454 nm accompanied by a high energy red shifted QI band centred at 691 nm which is forbidden otherwise [Fig. 8 (left)]. The splitting of B band into two individual Lorentzian bands and appearance of high energy red shifted QI band signifies metal removal followed by formation of dications which tend to undergo J-type (head to tail) intermolecular aggregation [40, 41, 42]. The dications are generated due to the replacement of Bi³⁺ ion from the porphyrin ring of **I** by the protons of the acid. The lower energy band at 422 nm is consistent with that of electrically neutral free base monomer, whereas the higher energy band at 454 nm (J-band) corresponds to the J aggregated dications. The small bands at 517 and 557 nm further correspond to the Q bands of neutral free base monomer. The band at 333 nm (marked with star) is attributed to charge transfer transition from peripheral para methoxy phenyl rings (π) to protonated porphyrin core (π^*) [42].

A dichloromethane solution of macro ring **II** is initially green in color characteristic of bismuth porphyrins. However, when the solution is kept for a long period at room temperature, the solution becomes acidic (turns litmus paper to pink) followed by precipitation of a highly shine blue crystals. When the blue crystals separated by filteration method were redissolved in dichloromethane, notably the solution becomes deep green. The UV-Visible spectrum of solid blue precipitates measured in dichloromethane [Fig. 8 (right)] is consistent with the absorption spectrum of **II** measured in acidic media [Fig. 8 (left)]. This entire phenomenon may be attributed to dichloromethane induced demetallation of II followed by self association of the demetallized porphyrins. Bismuth with vacant 6p and 4d orbitals acts as strong lewis acid and accepts the lone pair electrons of porphyrin N4 core. When the metal complex is kept dissolved in dichloromethane for a longer time, it is possible that the out of plane and weakly coordinated metal ion with vacant 6p and 4d orbitals interacts with the lone electron pair of chlorine atoms of CH₂Cl₂ moiety more strongly than that of nitrogen atoms of porphyrin core, leading to demetallation followed by protonation of the inner nitrogen atoms of porphyrinic core. The protonated analog of I then undergoes aggregation in J-type fashion as reflected by the appearance of J and QI band in the absorption spectrum (Scheme 3).



Fig. 7 [left] The UV/Vis absorption spectra of **II** in CH_2Cl_2 at increasing concentration [right] enhanced view of B bands.



Fig. 8 [left] The UV/Vis absorption spectra of II in CH_2Cl_2 at pH ~ 5 [right] absorption spectra of blue precipitates.



Scheme 3 Demetallation pathway of **II** in CH₂Cl₂.

6. Emission spectroscopy

Fig. 9 represents the emission spectra of macro rings at two different excitation wavelengths i.e. 390 and 454 nm. At 390 nm excitation [Fig. 9 (a)], the macro rings reflects the usual S_1 emissions i.e. Q(0,0) and Q(0,1) in the visible region and the less common S_2 fluorescence (with a small shoulder band) designated as B(0,0) in the near UV region marked with star. The Q(0,0)/Q(0,1) bands for the macro rings appear at 655/724 (I) and 660/728 nm (II) whereas, B(0,0) bands are centred at 436 nm with the shoulder bands appearing at 458 (I) and 471 nm (II) respectively. The shoulder peak in II is bathochromically shifted by λ_{max} 13 nm relative to I. The presence of less common S_2 -S₀ emission in both the macro rings indicates good mirror image relationship between their corresponding absorption and emission bands. The intensity distributions of S₁ emissions in I and II are consistent with the intensity distributions of their corresponding S₁ electronic absorption bands.

At 454 nm excitation [Fig. 9 (b)], the S₁ emissions in **I** occurs at 656 and 720 nm whereas S₂ emission (marked with star) appears at 480 nm. The Q(0,0) in **II** split into two bands appearing at 725 and 743 nm. The split bands are separated by $\lambda_{max} \sim 18$ nm. The other bands i.e. Q (0,1) and B(0,0) bands are centred at 779 and 487 nm respectively. It can be observed from Fig. 10 that the emission peaks in bismuth porphyrin are bathochromically shifted relative to free base ligand. The bathochromic shift is more pronounced at higher excitation wavelength. The phenomenon is attributed to distortion of macrocyclic ring due to out of plane coordination of Bi³⁺ ion with the ligand molecule.



Fig. 9 The emission spectra of I and II at (a) 390 and (b) 454 nm excitations in CH_2Cl_2 .

In case of compound **I** on absorbing the energy from UV-Visible radiation, the π electrons of the conjugated system undergo excitation from singlet ground state S₀ to first excited singlet

state S_1 or second excited singlet state S_2 [Fig. 10]. After a very short time span in the excited state, the π electrons return to the ground state by emitting the fluorescence ~ 400 and 600– 750 nm respectively. In case of metalloporphyrin **II**, Bi³⁺ ion (heavy metal ion) induces orbit coupling which results in the acceleration of the intersystem crossing $S_1 \rightarrow T_1$ [43]. Thus the density of electrons accumulated in the triple state increases and since many electrons hop to the excited triplet state, the emitting fluorescence drop dramatically. As a result due to combination of non planarity, heavy atom effect and efficient energy transfer between the two porphyrin molecules in the dimer moiety due to strong intermolecular interactions, the emission bands in **II** are quenched relative to that in **I** [44].



Fig. 10 The energy level diagram of fluorescence of porphyrins

The lifetime of an excited state is the average time a fluorophore spends in the excited state before returning to the ground state by emitting a photon. The fluorescence lifetimes for the macro ring at 390 and 454 nm excitations (Fig. S5-S6) were found to be 7.56 (I)/7.0(II) and 7.58(I)/3.03(II) ns respectively. Since the lifetime measurements of the fluorophores were recorded in uniform solvent (CH₂Cl₂), the decay was found to be monoexponential in nature. The coordination of Bi³⁺ ion to the nitrogen atoms of I reduces the excited state lifetime of the ligand by ~ 0.5 ns (λ_{exc} 390 nm) and ~ 4.5 ns (λ_{exc} 454 nm) which is again attributed to heavy atom effect generated by metal ion of large ionic radius.

7. SEM and TEM analysis

The morphological features of the synthesized porphyrins were investigated by SEM and TEM analysis. Fig. 11 depicts the typical SEM micrographs of **I** and **II**. The SEM image of **I** [Fig. 11 (left)] shows plate-like morphology with rough surface. After metallation with Bi^{3+} ion an appreciable change in surface morphology takes place [Fig. 11 (right)]. The surface morphology in **II** is fairly uniform and smoother relative to **I**.



Fig. 11 SEM micrographs of I (a) and II (b).

TEM image of **I** [Fig. 12 (left)] shows that the monomers are stacked one above another in a linear fashion leaving significant interplanar distance and imparting leave shaped morphology or one can simply assume a linear extended aggregations of monomers of **I** as represented in [Fig. 12 (right)]. The TEM micrographs of bismuth porphyrin [Fig. 13 (left)] clearly reflect parallel displaced monomeric sub units of binuclear motifs forming nanosheet like structures. A representation of parallel displaced mononuclear rings of **II** is illustrated by [Fig. 13 (right)]. Fig. 14 shows another TEM micrograph of **II** in which the monomers are involved in the formation of number of slipped stacked multilayers. Some other TEM images showing layered structures of **II** have been included in supplementary file as Fig. S7.



Fig. 12 TEM micrograph of I (a) and representation of supramolecular aggregation of mononuclear rings of I (b).



Fig. 13 TEM micrograph of **II** (a) and representation of head to tail aligned monomeric subunits of binuclear motifs of **II** (nitrate groups have been omitted for clarity) (b).



Fig. 14 TEM micrograph of **II** (a) representation of J-type aggregation of mononuclear rings of **II** (nitrate groups have been omitted for clarity) (b).

8. EDS analysis

EDS analysis (Fig. S8-S9) allows determining the chemical compositions of **I** and **II**. In the EDX profiles of porphyrins, the peaks of essential elements like C, N, O [**I**] and C, N, O, Bi [**II**] which constitute the molecules of chelating ligand **I** and its metal chelate **II** are clearly identified supporting the proposed structures.

9. Thermal analysis

In order to determine the temperature stability of the free base porphyrin **I** and its metal derivative **II**, thermogravimetric analyses (TGA) have been carried out. Compound **I** is stable up to ~250°C [Fig. 15 (left)]. After this, the graph shows constant mass loss till temperature reaches around ~ 450°C. A drastic weight loss associated with the decomposition of peripheral phenyl rings occurs at ~ 501.87 °C. The residual mass percent (45.929 %) at ~ 800°c is in agreement with the mass percent of highly stable tetrapyrrole macro ring (42.78%). Compound **II** is stable up to ~ 150°C [Fig. 15 (right)]. The compound then undergoes constant weight loss up to ~ 400°C. A sharp weight loss due to demetallation leading to the decomposition of entire porphyrin scaffold takes place ~ 480°C. The residual mass (27.929%) of the compound at ~ 550°c is comparable with the calculated weight of BiN (23.69%). A comparison especially of the onset temperatures (OT) of decomposition of the two macrocycles reflects that macro ring **I** is thermally more stable than **II**. The poor thermal stability of **II** can be attributed to weaker bonds in the out of plane coordinated metal porphyrin. Hence the obtained results confirm that the insertion of heavy metals like Bi³⁺ within the porphyrin ring system of **I** greatly affects their thermal robustness.



Fig. 15 Thermogravimetric analysis curves of I (red) and II (blue)



The invitro cytotoxicity assay of **I** and **II** was investigated against leukaemia K_{562} cell lines for 48 h. Bismuth porphyrin exhibited much higher potency (IC₅₀ = 40.08) as compared to free base porphyrin having (IC₅₀ > 100) classifying the former as chemotherapeutically significant dye against leukaemia K_{562} strains [Fig. 16]. The greater antitumor activity of **II** might be attributed to an increased cellular uptake of Bi^{3+} centre. The invitro cytotoxicity data of porphyrins are summarised in Table S4.



Fig. 16 Cell inhibition at different concentrations of I (green) and II (dark blue) against leukaemia K_{562} cell lines.

11. Conclusion

A Bi^{3+} derivative of Tetra-para-methoxyphenylporphyrin has been synthesized and characterised with the aid of spectroscopic measurement and nanotechnology data. Due to strong hypervalent nature of the central metal ion, Bismuth porphyrin tends to undergo dimerisation in the solid state and to certain extend in solution. A dichloromethane solution of **II** when kept for long time causes remarkable demetallation followed by simultaneous protonation of the demetallized porphyrin. Demetallation also occurs when the media is acidic in nature. The XRD result confirms that **I** and **II** belongs face centred and body centred cubic geometries. SEM images reflect plate like morphology of the macro rings whereas, TEM micrographs reveal the linear associations of monomers of **I** and slipped (head to tail) stacked layers of particles of **II**. The EDX data confirms the chemical compositions of porphyrins. TGA analysis reveals weak thermal stability of **II**. In the end, the incorporation of Bi^{3+} ion into the porphyrinic core of **I** remarkably enhances the biological activity of **I** against Leukaemia K₅₆₂ strains.

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- A sitting-atop (SAT) macro molecule of Tetrakis-4-methoxyphenylporphyrinato ٠ Bi(III) has been synthesized.
- Bismuth porphyrin exists as binuclear moiety owing to hypervalent nature of Bi³⁺ ٠ centre
- Dinuclear bismuth porphyrin lie in head to tail stacking fashion ٠
- TEM images reflects mutilayered nanosheets of synthesized complex •
- Bismuth complex exhibit high antitumor activity against leukaemia K₅₆₂ strains •
- Due to out of plane coordination mode, the synthesized complex have low thermal • stability

Declaration of interests

 \square The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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