*Research Article*

# Mimicking Peroxidase Activity by a Manganese(II) Complex Involving a New Asymmetric Tetradentate Ligand Containing Both Amino and Imino Groups

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The asymmetric ligand (*E*)-4-bromo-2-(((2-((5-bromo-2-hydroxybenzyl)(methyl)amino)ethyl)imino)methyl)phenol has been prepared by a novel seven-step route. All organic compounds isolated in each step have been characterised by elemental analysis, infrared and $^1$H NMR spectroscopy, and mass spectrometry. Interaction of this ligand with manganese has been investigated employing an electrochemical method. This method leads to the formation of a neutral manganese(II) complex **7** in high yield and purity. The complex has been thoroughly characterised by elemental analysis, infrared spectroscopy, mass spectrometry, magnetic susceptibility measurements, and cyclic voltammetry. Complex **7** behaves as peroxidase mimic in the presence of the water-soluble trap ABTS, probably due to its ease to coordinate the substrate molecule.

*Dedicated to Professor Manuel R. Bermejo on the occasion of his 70th birthday*

## 1. Introduction

Oxidative stress causes uncontrolled oxidation of organic molecules with secondary alteration of their structure and biological functions, leading to the progressive deterioration and the collapse of organs and systems in the living organisms [1–3]. The superoxide dismutase, catalase, and peroxidase enzymes are part of the defense mechanisms against the reactive oxygen species generated by oxidative stress [4–7].

Vast interest has been developed to devise small molecule models, the properties of which can be compared with different metalloproteins [8–11]. Biomimetic modeling of metal complexes has denoted a better catalytic behaviour when the complex is able to easily coordinate the substrate molecule, and this is favoured when the catalyst has either a vacancy in the coordination sphere or a labile ligand [12–15]. To achieve this, a careful design of the ligand is necessary. In this connection, unsymmetrical ligands are also required to imitate many of the active sites of the metalloproteins [16–18].

Tetradentate ONNO Schiff bases are well-known systems for coordination of metal ions, and their synthesis is easily accomplished by condensation of salicylaldehyde and diamines [19, 20]. However, this condensation favours symmetrization processes, and symmetric ligands are systematically obtained even using a mixture of different salicylaldehydes in solution [21]. Consequently, alternative synthetic routes should be explored to achieve unequivocally unsymmetrical ligands.

We report here on the synthesis of a new type of unsymmetrical ONNO tetradentate imino-amino *bishydroxy* ligand following a novel seven-step synthetic route. The ability of the new ligand to coordinate a redox-active metal ion as manganese is checked by an electrochemical synthesis procedure. Finally, catalytic studies to determine the peroxidase

activity have been carried out using the obtained manganese compound.

## 2. Materials and Methods

*2.1. Materials.* All the starting materials (Aldrich) and solvents (Probus) used for the synthesis were of commercially available reagent grade and were used without further purification.

*2.2. Physical Measurements.* Elemental analyses were performed on a Carlo Erba Model 1108 CHNS-O elemental analyser. The IR spectra were recorded on KBr pellets on a Bio-Rad FTS 135 spectrophotometer in the range 4000–400 cm$^{-1}$. $^1$H spectra were recorded on a Bruker AC-300 spectrometer using DMSO-d$_6$ (296 K) as solvent and SiMe$_4$ as an internal reference. FAB mass spectra were recorded on a Kratos MS50TC spectrometer connected to a DS90 data system, using meta-nitrobenzyl alcohol as a matrix. Electrospray ionization mass spectra of the compounds were obtained on a Hewlett-Packard model LC-MSD 1100 instrument (positive-ion mode, 98 : 2 CH$_3$OH/ HCOOH as the mobile phase, 30–100 V). Room-temperature magnetic susceptibilities were measured using a digital measurement system MSB-MKI, calibrated using mercury tetrakis(isothiocyanato)cobaltate(II), Hg[Co(NCS)$_4$], as a susceptibility standard. Magnetic data for **7** at different applied field strengths was obtained with a Quantum Design PPMS susceptometer. Conductivities of 10$^{-3}$ M solutions in DMF were measured on a Crison microCM 2200 conductivimeter.

Cyclic voltammetry was performed using an EG&G PAR model 273 potentiostat, controlled by EG&G PAR model 270 software. A Metrohm model 6.1204.000 graphite disc coupled to a Metrohm model 628-10 rotating electrolyte device was used as a working electrode. A saturated calomel electrode was used as a reference and a platinum wire as an auxiliary electrode. All measurements were made with ca. 10$^{-3}$ mol dm$^{-3}$ solutions of the complexes in dimethylformamide using 0.2 mol dm$^{-3}$ NBu$_4$PF$_6$ as a supporting electrode. Cyclic voltammetry measurements were performed with a static graphite electrode.

*2.3. Preparation of the Organic Ligand.* The asymmetrical ligand *(E)-4-bromo-2-(((2-((5-bromo-2-hydroxybenzyl)(methyl)amino)ethyl)imino)methyl)phenol* (**H$_2$L$^1$**) has been successfully obtained by means of a seven-step synthetic route (see Scheme 1).

*Phthalimido-acetal* (**1**). Potassium phthalimide (5 g, 26.5 mmol), bromoacetal (4.1 mL, 26.5 mmol), and hexadecyltributylphosphonium bromide (13.8 g, 26.5 mmol) were heated together in toluene (200 mL) for 24 h at 111°C under N$_2$ atmosphere. The product was then filtered, and the filtrate was concentrated on a rotary evaporator to give yellow oil. Column chromatography using hexane : ether (1:1) allowed obtaining the desired compound. Yield 70%; Elemental analysis calculated for C$_{14}$H$_{17}$NO$_4$ (263.29): C 63.8, H 6.4,

N 5.3; found: C 64.6, H 6.5, N 5.3. $^1$H NMR (300 MHz, CDCl$_3$): δ 7.8–7.6 (m, 4H), 4.79 (t, 1H), 3.75 (d, 2H), 3.62–3.42 (m, 4H), 1.06 (t, 6H). ES-MS: *m/z* 264. IR: $\nu$(C-O-C) 2800, $\nu$(C=O) 1721 cm$^{-1}$.

*Phthalimidoacetaldehyde* (**2**). The foregoing compound (3 g) and hydrochloric acid (15 mL) were heated at 100°C for 30 minutes with stirring. The product crystallised on cooling. It was collected and purified from diethyl ether. Yield 80%; Elemental analysis calculated for C$_{10}$H$_7$NO$_3$ (189.17): C 63.4, H 3.7, N 7.4; found: C 62.4, H 3.4, N 7.5. $^1$H NMR (300 MHz, CDCl$_3$): δ 9.62 (s, 1H), 7.85–7.65 (m, 4H), 4.45 (s, 2H). ES-MS: *m/z* 190. IR: $\nu$(C=O) 1721 cm$^{-1}$.

*4-Bromo-2-((methylamino)methyl)phenol* (**3**). To an ethanolic solution (100 mL) of 4-bromo-salicylaldehyde (5 mL, 46.7 mmol), methylamine (8.06 mL, 93.4 mmol) was added. The mixture was heated under reflux in a round-bottom flask fitted with a Dean-Stark trap to remove the water produced during the reaction. After heating for 3 h, the solution is left at room temperature and then sodium borohydride (0.90 g, 23.3 mmol) was added. The solution was stirred for 2 h before adding a second fraction of sodium borohydride (0.90 g, 23.3 mmol). After stirring for 2 h, the solution was cooled in an ice bath and acidified to pH 2. The solvent was evaporated and the residue was extracted with distilled water/diethyl ether (3 × 20 mL). The combined aqueous phases were adjusted to pH 11 by addition of aqueous potassium hydroxide and extracted with dichloromethane (3 × 30 mL). The combined organic phases were dried with anhydrous sodium sulphate, and the solvent was evaporated. The solid was dried in vacuo. Yield 35%; Elemental analysis calculated for C$_8$H$_{10}$BrNO (263.29): C 44.4, H 4.6, N 6.5; found: C 44.2, H 4.4, N 6.5. $^1$H NMR (300 MHz, CDCl$_3$): δ 7.19–6.63 (m, 3H), 4.8–4.2 (a, 1H), 3.86 (s, 2H), 2.40 (s, 3H). ES-MS: *m/z* 264. IR: $\nu$(O-H) 3014, $\nu$(N-H) 2736 cm$^{-1}$.

*2-((6-Bromo-3-methyl-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-yl)methyl)isoindoline-1,3-dione* (**4**). A 100 mL Schlenk flask was charged under N$_2$ with a solution of 0.72 g (3.33 mmol) of salicylamine **3** in THF. Under N$_2$ stream, phthalimidoacetaldehyde **2** was added (0.95 g, 5.03 mmol). The flask, saturated with N$_2$, is left to stir for 20 h at room temperature. Then, a white product was collected by filtration, washed with diethyl ether, and dried in air. Yield 65%; Elemental analysis calculated for C$_{18}$H$_{15}$BrN$_2$O$_3$ (387.22): C 55.9, H 3.9, N 7.2; found: C 55.0, H 4.2, N 7.4. $^1$H NMR (300 MHz, CDCl$_3$): δ 7.90-7.71 (m, 4H), 7.26–6.64 (m, 3H), 5.14 (t, 1H), 4.08–3.95 (m, 4H), 2.51 (s, 3H). IR: $\nu$(C=O) 1719 cm$^{-1}$.

*2-(2-((5-Bromo-2-hydroxybenzyl)(methyl)amino)ethyl) isoindoline-1,3-dione* (**5**). Benzoxazine **4** (2 g, 5.17 mmol) was stirred in a mixture of dichloromethane (25 mL) and acetonitrile (20 mL). Sodium cyanoborohydride (0.43 g, 6.46 mmol, 1.25 eq) was added and the mixture was acidified with hydrochloric acid to pH of ca. 2. After 6 h, the mixture was acidified with hydrochloric acid to pH ca. 1. After 1 h of stirring, the solvent was removed. The residue

was dried in vacuo and extracted with a 9 : 1 mixture of dichloromethane/methanol (saturated with ammonia). The solvent was evaporated and the residue again vacuum-dried. Recrystallization of the crude product from absolute methanol afforded the desired product. Yield 70%; Elemental analysis calculated for $C_{18}H_{17}BrNO_3$ (389.24): C 55.5, H 4.4, N 7.2; found: C 55.6, H 4.9, N 7.6. $^1$H NMR (300 MHz, DMSO): $\delta$ 7.89–7.80 (m, 4H), 7.19 (d, 2H), 6.49 (d, 1H), 3.77 (t, 2H), 3.60 (s, 2H), 2.70 (s, 2H), 2.30 (s, 3H). IR: $\nu$(C=O) 1709 cm$^{-1}$.

*2-(((2-Aminoethyl)(methyl)amino)methyl)-4-bromophenol (6).* Under nitrogen, a 100 mL round-bottom flask was charged with a solution of 0.31 g (0.79 mmol) of 5 in 10 mL of absolute tetrahydrofuran and 10 mL of absolute ethanol. Hydrazine hydrate (99%, 0.26 mL, 5 mmol) was added. After stirring at room temperature for 24 h, the reaction mixture was acidified with concentrated hydrochloric acid to pH of ca. 1 and stirred for another 10 min. The solvent was evaporated, and the residue was extracted with water (3 × 20 mL). The combined aqueous phases were adjusted to pH 11 by addition of anhydrous potassium hydroxide and extracted with dichloromethane (3 × 30 mL). The combined organic phases were dried with anhydrous sodium sulphate, and the solvent was evaporated. The solid residue was washed with diethyl ether and then dried in vacuo. Yield 45%; Elemental analysis calculated for $C_{10}H_{15}BrN_2O$ (389.24): C 46.3, H 5.8, N 10.8; found: C 47.3, H 5.6, N 10.1. $^1$H NMR (300 MHz, DMSO): $\delta$ 7.21–6.62 (m, 3H), 4.1–3.7 (a, 2H), 3.59 (s, 2H), 2.84 (t, 2H), 2.49 (t, 2H), 2.23 (s, 3H). ES-MS: $m/z$ 390. IR: $\nu$(O-H) 3473, $\nu$(N-H) 3280 cm$^{-1}$.

*(E)-4-Bromo-2-(((2-((5-bromo-2-hydroxybenzyl)(methyl)amino)ethyl)imino)methyl)phenol ($H_2L^1$).* To a solution of primary amine **6** (0.105 g, 0.405 mmol) in chloroform, 4-bromo-salicylaldehyde (0.081 g, 0.405 mmol) was added. The mixture was heated under reflux in a round-bottom flask fitted with a Dean-Stark trap to remove the water produced during the reaction. After heating for 3 h, the solution was concentrated to yield a yellow solid. The product was collected by filtration, washed with diethyl ether, and dried in air. Yield 85%; Elemental analysis calculated for $C_{17}H_{18}Br_2N_2O_2$ (442.14): C 46.1, H 4.1, N 6.3; found: C 46.4, H 4.1, N 6.3. $^1$H NMR (300 MHz, DMSO): $\delta$ 8.12 (s, 1H), 7.34–6.60 (m, 6H), 3.71 (t, 2H), 3.65 (s, 2H), 2.78 (t, 2H), 2.29 (s, 3H). ES-MS: $m/z$ 443. IR: $\nu$(C=N) 1634 cm$^{-1}$; $\nu$(C-O) 1270 cm$^{-1}$.

### 2.4. Complex Preparation.

$[MnL^1(OH_2)_2]\cdot H_2O$ (**7**) was obtained following an electrochemical procedure (see Figure 1). The cell was a beaker (100 cm$^3$) fitted with a rubber bung, through which the electrochemical leads entered the cell [22]. A manganese metal platelet was suspended from a platinum wire and a platinum wire was used as a cathode. The ligand $H_2L^1$ (0.1 g) was dissolved in acetonitrile (70 cm$^3$) and a small amount of tetramethylammonium perchlorate was added as a supporting electrolyte. During electrolysis, nitrogen was bubbled through the solution
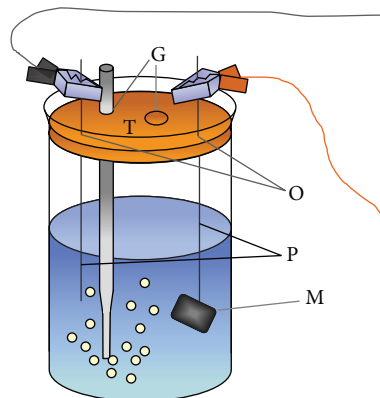


FIGURE 1: Scheme of electrochemical cell. T: rubber stopper; G: inert gas entry/exit; P: platinum cathode; M: manganese anode.

to ensure the presence of an inert atmosphere, with the current being provided by a purpose built DC power supply. The cells can be summarized as follows: Pt(−)/MeCN + $H_2L^n$/Mn(+). During electrolysis, hydrogen gas was evolved at the cathode. The solid was collected by filtration and washed successively with acetonitrile and diethyl ether before drying in vacuo. Yield: 75%. Elemental analysis calculated for $C_{17}H_{22}MnBr_2N_2O_5$ (549.11): C 37.2, H 4.0, N 5.1; found: C 36.9, H 4.3, N 5.2. FAB: $m/z$ 494.9 [MnL]$^+$. IR: $\nu$(C=N) 1627 cm$^{-1}$. $\nu$(C-O) 1273 cm$^{-1}$. $\mu$ = 5.94 B. M. Conductivity (in DMF) $\Lambda_M$ = 9 $\mu$S. $E_{ox}$ = 0.488 V. $E_{red}$ = 0.150 V. $E_{1/2}$ = 0.319 V.

### 2.5. Peroxidase Probes.

Oxidation of 2,2′-azinobis-(3-ethyl-benzothiazoline)-6-sulfonic acid (ABTS) with $H_2O_2$ at ca. pH 6.8 in the presence of complex **7** was tested in the following manner. An aqueous solution of ABTS (50 $\mu$L; 0.009 M; 4.5 × 10$^{-7}$ mol) and a methanolic solution of the complex (10 $\mu$L; 10$^{-3}$ M; 10$^{-8}$ mol) were added to water (3 mL). The intensity of the UV absorption bands of ABTS started to increase immediately after addition of an aqueous solution of $H_2O_2$ (50 $\mu$L; 10 M; 5 × 10$^{-4}$ mol).

## 3. Results and Discussion

Our synthetic sequence leading to the tetradentate ligand $H_2L^1$ is summarized in Scheme 1. All the precursors and organic compounds were characterised by elemental analysis, $^1$H NMR and IR spectroscopies, and MS spectrometry.

In the first step, precursor **1** was obtained through Gabriel synthesis [23]. The potassium salt of phthalimide was N-alkylated with a primary alkyl halide such as bromoacetal. The $^1$H NMR doublet signal at 3.75 ppm, corresponding to the methylene group next to the nitrogen atom (R1-N-C$H_2$-R2), confirmed the formation of the phthalimido-acetal. Figure 2 shows the $^1$H NMR spectrum for **1** and for other organic compounds of the synthetic route (**2**, **4**, and the final ligand $H_2L^1$).

Upon workup by acidic hydrolysis with aqueous hydrochloric acid, phthalimidoacetaldehyde **2** was liberated
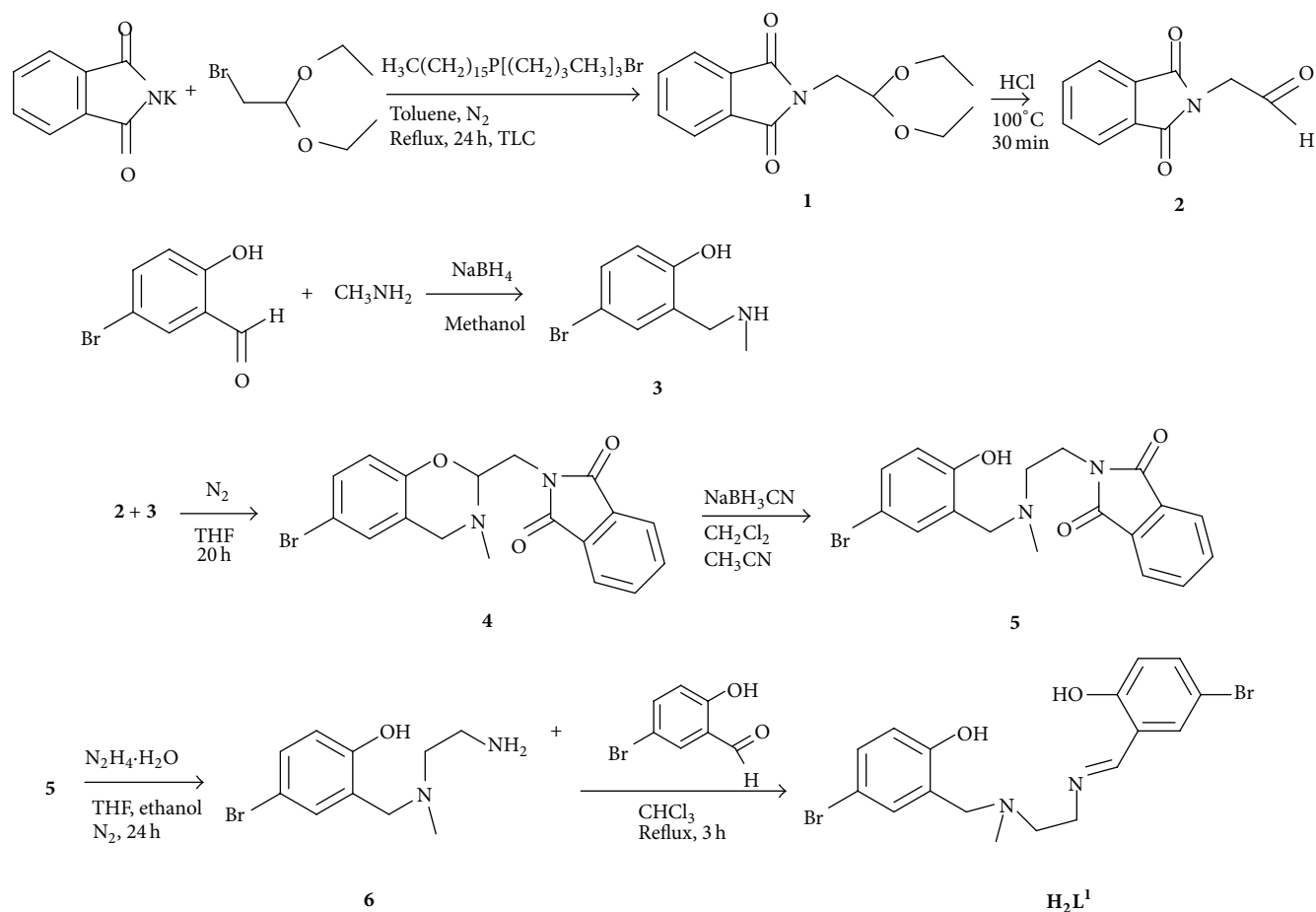
SCHEME 1: Synthetic route to obtain $H_2L^1$.

[24]. A singlet signal in $^1$H NMR at 9.6 ppm was assigned to the aldehyde proton (see Figure 2).

For the further course of the synthesis, 4-bromo-salicylaldehyde reacted with methylamine in the presence of a reducing agent such as sodium borohydride [25]. This led to formation of primary amine 3. The salicylamine was identified by the broad signal at about 4.8–4.2 ppm of the amine proton and also by the singlet at 3.8 ppm corresponding to the protons of the methylene group.

In a fourth step, aminophenol 3 was condensed with phthalimidoacetaldehyde 2, affording benzoxazine 4. The $^1$H NMR triplet at 5.1 ppm was characterised as the signal from the proton of the carbon between N and O atoms (see Figure 2). The absence in 4 of the IR band at about 2600 cm$^{-1}$ corresponding to the phenolic and aminic vibrational modes (showed in 2 and 3) also confirmed the progress of the reaction.

Reductive cleavage of the acetalic C-O bond of 4 using sodium cyanoborohydride gave N-phthaloyl-protected ethylene diamine 5. The benzoxacine signals disappeared in the $^1$H NMR spectrum, and the triplets at 3.8 ppm and 2.7 ppm from the methylene chain formed [26]. Deprotection of 5 by hydrazinolysis proceeded smoothly, showing the disappearance of the phthalimide signals in $^1$H NMR and the formation of a new broad signal at 4.1–3.7 ppm from the amine protons [27].

Primary amine 6 was finally condensed with 4-bromo-salicylaldehyde, affording the Schiff base $H_2L^1$ ((E)-4-bromo-2-(((2-((5-bromo-2-hydroxybenzyl)(methyl)amino)eth-yl)imino)methyl)phenol). The imine group from $H_2L^1$ is identified by the singlet peak at 8.12 ppm (see $^1$H NMR in Figure 2) and also by the strong IR band at 1634 cm$^{-1}$. Other $^1$H NMR signals, elemental analysis, and ES spectrometry confirm the formation and purity of the $H_2L^1$ amine-imine ligand.

The reaction of $H_2L^1$ with a manganese plate in an electrochemical cell yields complex 7 with formula $[MnL^1(OH_2)_2]\cdot H_2O$ [28]. The electrochemical efficiency of the cell, around 0.5 mol F$^{-1}$, is in accordance with the following mechanism of the reaction:

$$\text{Cathode: } H_2L + 2e^- \longrightarrow H_2\,(g) + L^{2-}$$
$$\text{Anode: } L^{2-} + Mn \longrightarrow MnL + 2e^- \tag{1}$$
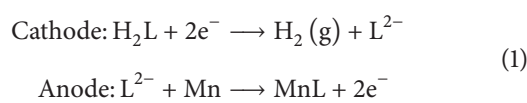
This synthetic procedure allowed us to obtain the neutral complex with high purity and in very good yield. Complex 7 seems to be stable in air and thermally stable, melting above
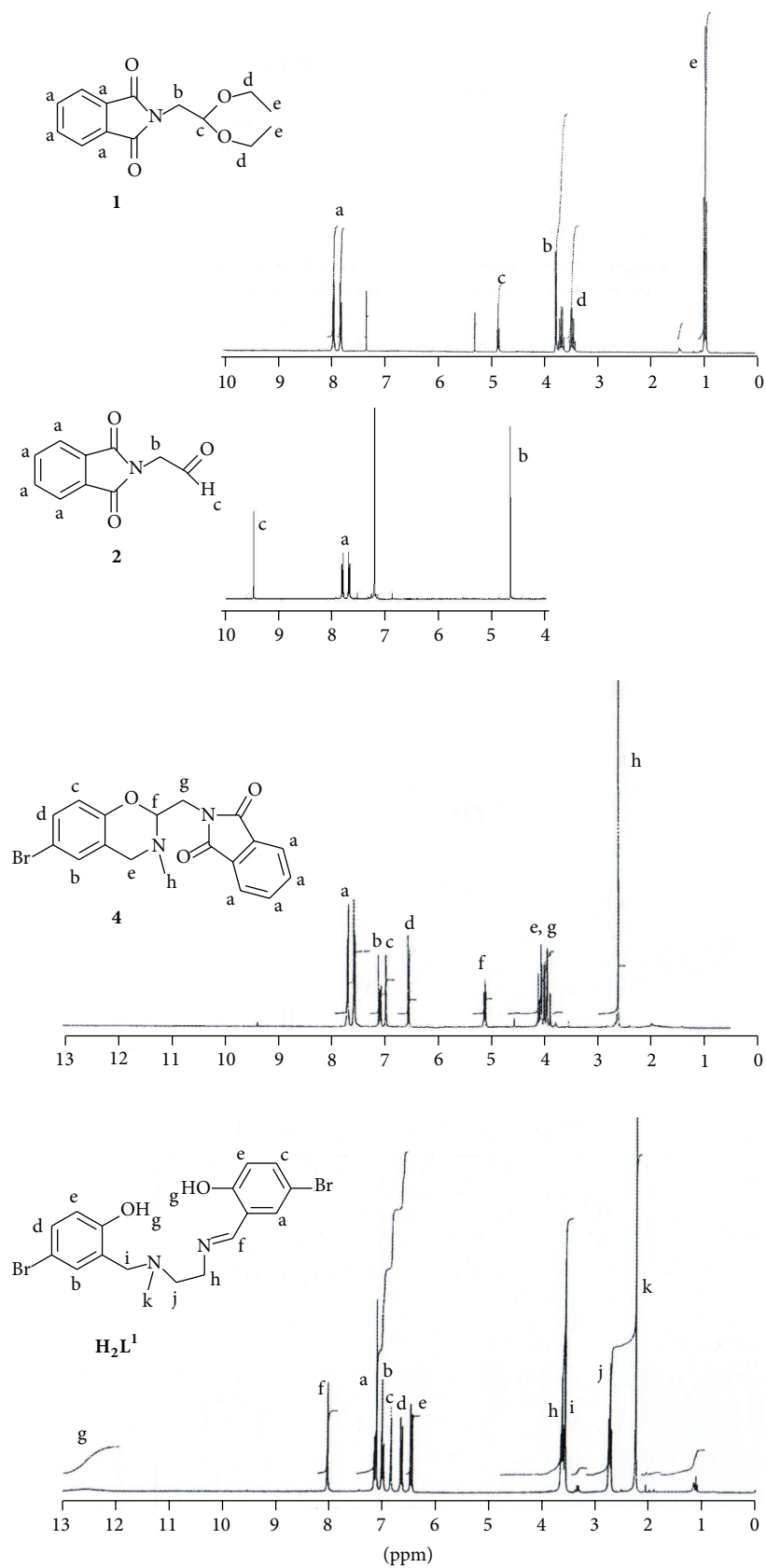
FIGURE 2: $^1$H NMR spectra and assignments for **1**, **2**, **3**, and the ligand **H$_2$L$^1$**.

TABLE 1: Magnetic susceptibility and magnetic moment for **7** at different applied field strengths.

| Field (G) | $\chi$ (uem/g) | $\mu$ (B. M.) |
|---|---|---|
| 4696 | 0.3260 | 5.83 |
| 5017 | 0.3373 | 5.94 |
| 5251 | 0.3464 | 6.19 |
| 5486 | 0.3555 | 6.33 |
| 5718 | 0.3579 | 6.40 |
| 5952 | 0.3637 | 6.51 |



FIGURE 3: Cyclic voltammogram for **7** at scan rate of $0.02\,\mathrm{V\,s^{-1}}$.



FIGURE 4: UV-Vis absorption spectrum of ABTS + $H_2O_2$ + **7**, recorded 10 min after mixing the solution.

$300°C$ without decomposition. The complex is insoluble or sparingly soluble in water and common organic solvents, but soluble in polar coordinating solvents such as DMF, DMSO, and pyridine.

The IR spectrum of **7** shows a strong band at $1627\,\mathrm{cm^{-1}}$ attributable to $\nu$(C=N). This band appears $7\,\mathrm{cm^{-1}}$ shifted to lower frequency with respect to the free ligand. Moreover, $\nu$(C–O) band is also shifted $3\,\mathrm{cm^{-1}}$ to higher frequency with respect to the free ligand. This behaviour is compatible with the participation of both imine nitrogen and phenolic oxygen atoms in the coordination to the manganese ion, illustrating the dianionic and tetradentate character of this ligand in the complex [29].

The FAB mass spectra show a peak due to the fragment $[MnL]^+$, which thus further corroborate coordination of the ligand to the metal centre. The conductivity value ($9\,\mu$S) suggests the nonionogenous nature for **7** [30].

Magnetic measurements were performed at room temperature for **7** with diamagnetic corrections. The magnetic moment, $\mu = 5.94$ B. M., is close to those expected for magnetically dilute Mn(II) ions, confirming the oxidation state II for the central atom and, therefore, confirming the notion that the ligand acts as dianion. The study of the magnetic behaviour at different applied field strengths (Table 1) confirms the values expected for magnetically dilute Mn(II) ions. Thus, magnetic data suggest the presence of manganese(II) ion in an octahedral environment with little or no interaction between neighbouring metal centres.

Cyclic voltammogram of **7** was measured in dimethylformamide (DMF) over a potential range from +1.00 to −1.00 V. In DMF solution, the complex exhibits a quasi-reversible one-electron reduction-oxidation wave, which at slow scan rates of about $0.02\,\mathrm{V\,s^{-1}}$ is reversible (Figure 3; $E_{ox} = 0.488$ V; $E_{red} = 0.150$ V). Nevertheless, the peak-to-peak separation (0.338 V) is slightly longer than in other series of manganese(II) complexes derived from tetradentate Schiff base ligands [12, 13], highlighting a poorer reversible character for the present complex.

*3.1. Peroxidase Studies.* ABTS (diammonium salt of 2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid) is a colourless water-soluble compound used for clinical peroxidase assays because of its excellent characteristics [31–33]. The peroxidase-like activity of **7** was followed by the oxidation of the ABTS at pH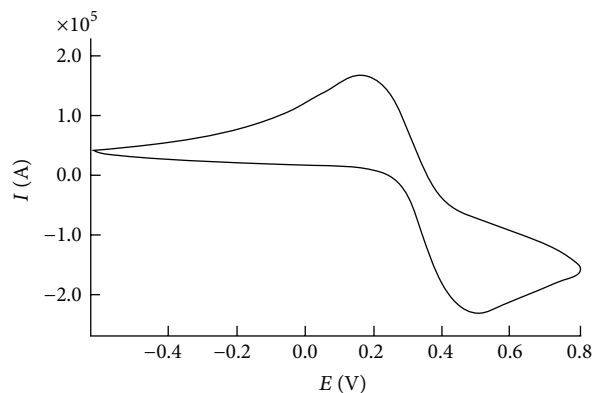 6.8. ABTS is colourless and it reacts readily with hydrogen peroxide in the presence of a peroxidase catalyst to yield a stable green coloured radical cation which absorbs light at 415, 650, 735, and 815 nm [34].
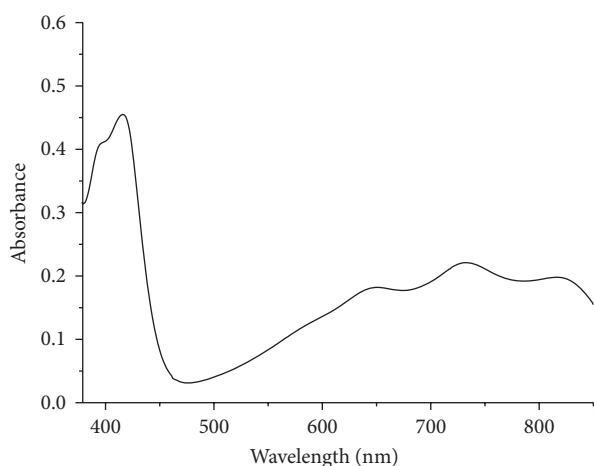
The reaction of ABTS with $H_2O_2$ in the presence of **7** (complex concentration: $3.3\ 10^{-6}$ M) generates ABTS$^{\bullet+}$. A reduction of one mole of $H_2O_2$ requires two moles of ABTS, according to the mechanism

$$2\mathrm{ABTS} + H_2O_2 \xrightarrow{\text{Mn-SB Complex}} 2\mathrm{ABTS}^{\bullet+} + 2H_2O \quad (2)$$
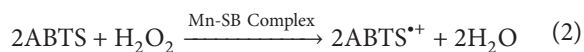
The oxidised form, ABTS$^{\bullet+}$, can be clearly identified by its characteristic absorption bands (Figure 4) and measured quantitatively at $\lambda = 650$ nm, since $\varepsilon = 12000\,\mathrm{M^{-1}\,cm^{-1}}$ has been determined. Further oxidation to the corresponding dication was not observed. In the absence of the complex, a solution of ABTS and $H_2O_2$ is stable for several hours without showing any formation of ABTS$^{\bullet+}$.

The rate of formation of ABTS$^{\bullet+}$ of about 11% shows a significant capacity of hydrogen peroxide decomposition by **7**. The activity is comparable to that of a previous series of Mn-Schiff base complexes, reported by us [12, 13], but is much higher than an earlier Mn-Schiff base series with high symmetrical octahedral geometries [35].

With regard to the peroxidase activity, two of the most important questions that are of concern are (a) the rate of reversibility of the Mn(II)/Mn(III) redox potentials and (b) the ease of coordination of the substrate molecule to the manganese ion. The peak-to-peak separation of 0.338 V for the oxidation-reduction wave of **7** is slightly longer than in other series of efficient peroxidase mimics, and even longer than in other series of Mn(II) inactive complexes. Despite this limited reversible character, the behaviour of complex **7** as peroxidase mimic may be attributable to its capacity to coordinate the substrate molecule. Our strategy in the design of the ligand $H_2L^1$ sought adequate conformations and flexibility to coordinate metal ions in a way that two adjacent positions of the coordination sphere may be vacant or filled with labile ligands. The different hybridization mode for the amino ($sp^3$) and imino ($sp^2$) groups may favour the tetracoordination of the organic ligand outside from the equatorial plane of the metal ion. In this case, two of the *cis* positions of a hypothetical first coordination sphere would not be occupied by the ligand and, subsequently, these two *cis* positions would be able to coordinate a suitable substrate molecule for catalysis.

On the other hand, the reversible character of the redox potentials for this new type of complexes may be increased using different electron donor/withdrawing substituents on the phenyl rings of the ligand [36–38]. Thus, more efficient peroxidase mimics would be achieved using this new type of asymmetric ligands, containing both amino and imino groups, by employing appropriate substituents on the phenyl rings.

## 4. Conclusions

Complex **7**, which behaves as a manganese(II) peroxidase mimic, incorporates a new asymmetric ligand $H_2L^1$ containing both amino and imino groups. The seven-step synthetic route for $H_2L^1$ afforded successfully the desired asymmetric product with a reasonable yield. This synthetic strategy may be used to obtain a new family of organic ligands suitable for coordination of different metal ions. The variety of this novel family of ligands may be envisioned by the chance of replacing the methylamine used in the third step of the synthetic route by different amines or amino acids. The peroxidase activity of this type of complexes would be enhanced by employing electron donor/withdrawing substituents on the phenyl rings to get complexes with better reversible character for the electrochemical processes.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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