Synthesis of Enantiomerically Pure Nitronyl Nitroxide Radicals Through Chiral Pool

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Abstract: Two pairs of new optically active nitronyl nitroxides derived from *N*-Boc-*D*- or *L*- prolinol are described. The synthetic route consist of (1) the synthesis of chiral aryl aldehydes by Mitsunobu reaction, (2) the condensation of the 2,3-bis(hydroxylamino)-2,3-dimethylbutane with chiral aldehydes to give 1,3-dihydroxyimidazolidine, and (3) the final oxidation of 1,3-dihydroxyimidazolidine with aqueous $NaIO_4$ at 0 ˚C. These two pairs have been specifically designed for further assessing the differences in activity of chiral nitronyl nitroxides and for developing chiral molecular magnetic material by the metal-radical complexes approach.

Keywords: Chiral nitronyl nitroxides, *N*-Boc-*D*- or *L*- prolinol, chiral pool.

INTRODUCTION

In recent years, stable nitroxyl nitroxide radicals, with membrane-permeable, low molecular weight, and mimic metal-independent superoxide dismutase [1, 2], have been used as biophysical tools for many years. A biologically relevant effect of nitroxides includes their ability to protect cardiomyocytes [3], to inhibit the formation of DNA strand breaks [4], and to be employed as radioprotectors [5], anticancer agents [6]. However differences in the activity of chiral nitroxyl nitroxides have not been reported, as biological molecules can discriminate between enantiomers. There are only few examples of chiral nitroxides [7-11], although chiral nitroxides are well chosen as potential precursors of chiral molecule-based magnets. From these perspectives, in this article we reported using *N*-Boc-*D*-or *L*-prolinol as chiral pool to synthesize three pairs of chiral nitronyl nitroxide radicals.

RESULTS AND DISCUSSION

In general, chiral nitronyl nitroxides can be achieved either by [12] (i) the incorporation of asymmetric groups, (ii) the isolation of atropoisomeric or conformational enantiomers, or (iii) a combination of the two. Among these three methods, to incorporate chirality with the use of asymmetric groups is relatively simple. According to Ullman's pioneering work [13], any aldehydes may give rise to a nitronyl nitroxide. So, chiral aldehydes should be obtained firstly. By the Mitsunobu reaction [14], intermolecular dehydration reactions occurred as *N*-protected-*L* or *D*-prolinol was reacted with phenolic or benzoic acid components through treatment with diethyl azodicarboxylate and triphenylphosphine under mild, neutral conditions (Scheme **1**). The results are shown in Table **1**. However, it was quite difficult to separate and purify production. In order to purify the production, the side product triphenylphosphine oxide and diethyl hydrazinedicarboxylate must be firstly precipitated by ether after the reaction and then filtered. The filtrate was evaporated in vacuum and the crude product was purified by chromatography on silica. The experimental results show that the yields decrease as steric hindrance increases and the yields of esterification of carboxylic acids are less than those of alkylation of phenols.

By condensation of the chiral aryl aldehydes described above with 2,3-bis(hydroxylamino)-2,3-dimethylbutane in methanol solution at room temperature, 1,3-dihydroxyimidazolidines were slowly obtained as stable white solids and then treated with aqueous $NaIO_4$ to yield stable blue nitronyl nitroxides (Scheme **2**).

EPR Spectra of Monoradicals

Due to its sensitivity and accuracy, EPR spectroscopy is the best tool for the study of free radical [13]. As shown in Fig. (**1**), the EPR spectra of all nitronyl nitroxides in dichlormethane show 5 major lines similar to each other in the ratio 1∶2∶3∶2∶1, which is expected due to coupling with two identical nitrogens (Fig. **1**).

Optical Spectra

The electronic absorption of the nitronyl nitroxides is identical to that of the tetramethylated analogues [13]. The n→π* transitions of the radical moiety of the aryl nitronyl nitroxides centered at about 600 nm, while the π→π* transition occurs at 360 nm.

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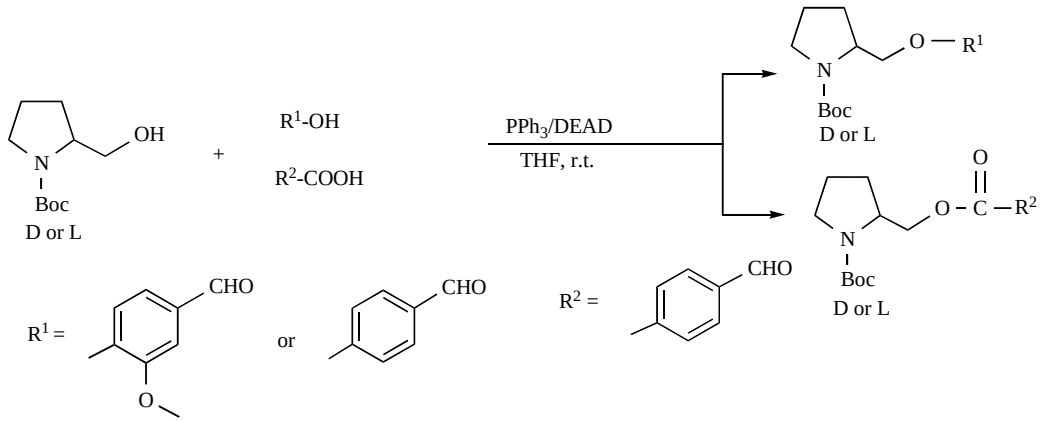
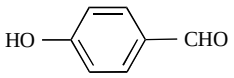
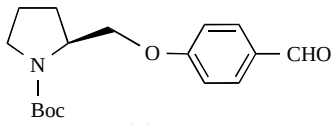
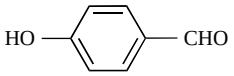
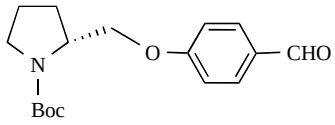
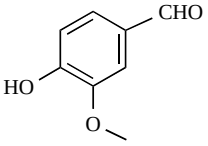
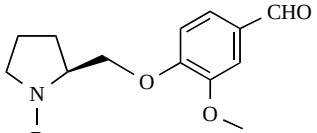
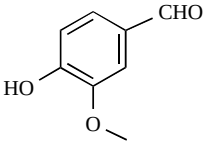
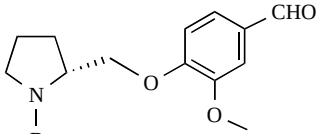
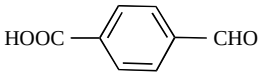
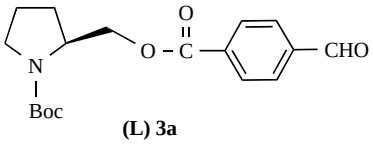
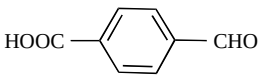
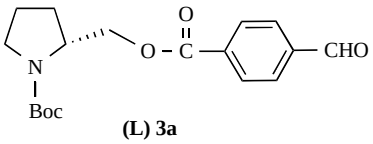
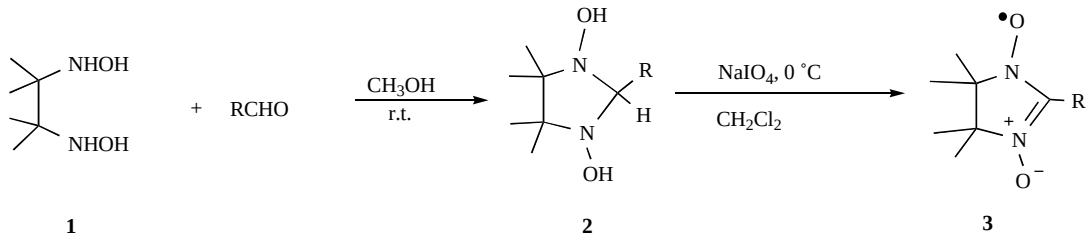


Scheme 1. Synthesis of chiral aryl aldehydes.

Table 1. The Mitsunobu Reaction of N-protected-L or D-prolinol

Entry	Aromatic Aldehydes	Product	Time(h)	Yield(%)	Optical Rotation*
1		 **(L) 1a**	13	84.8	-66.5 [c]
2		 **(D) 1b**	13	87.3	+66.4 [d]
3		 **(L) 2a**	13	69.2	-67.2
4		 **(D)2b**	13	68.1	+67.2
5		 **(L) 3a**	13	62.3	-54.6 [e]
6		 **(L) 3a**	13	63.5	+54.4 [f]

*$[\alpha]_D^{20}$, (c=1.1,CH_2Cl_2).



1 **2** **3**

Scheme 2. Synthesis of chiral nitronyl nitroxides.

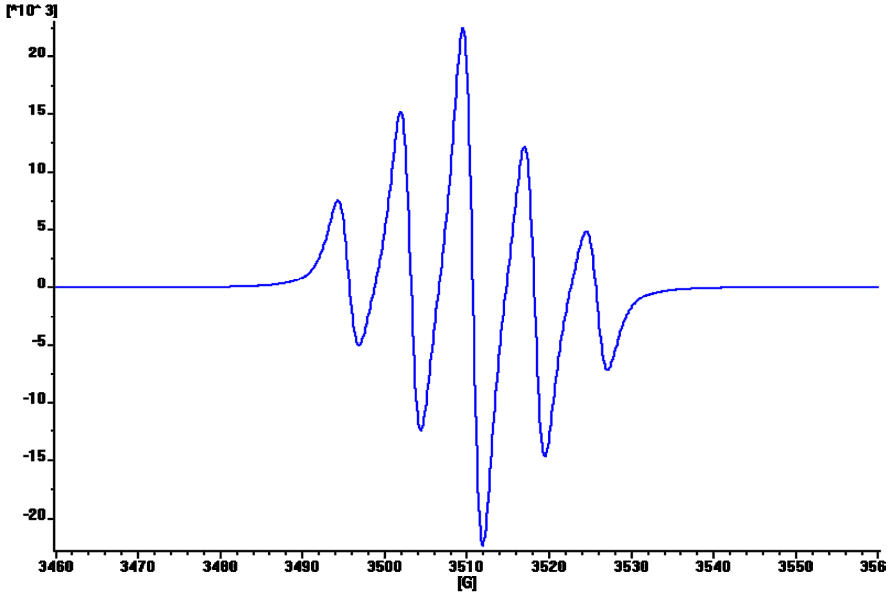


Fig. (1). Typical ESR spectra of nitroxyls in dichloromethane.

The infrared spectra of the nitronyl nitroxides are noteworthy in that the characteristic bands were observed at 1350-1375 cm^{-1}, arising from N-O stretching frequency [15].

CONCLUSION

In summary, a group of chiral nitronyl nitroxide radicals were synthesized through a chiral pool. The isolated radicals were quite stable and, in most cases, could be stored at room temperature for months without decomposition. The chemical structures of the radicals were characterised by UV-Vis, IR, HMRS, and EPR. These chiral nitronyl nitroxide radicals could be used as biophysical tools to assess the differences in activity of chiral nitronyl nitroxides and as new chiral ligands to synthesize metal-nitroxide complexes with MchD effect. This method represents new thinking for the synthesis of chiral nitronyl nitroxide radicals, In other words, by modifying natural chiral molecule into chiral pool, plenty of chiral nitronyl nitroxide radicals could be easily obtained. The results are shown in Table **2**.

EXPERIMENTAL

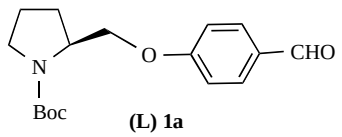
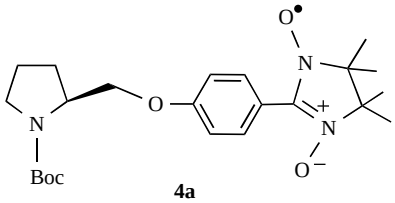
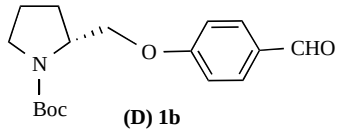
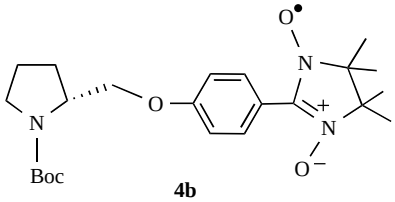
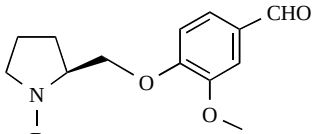
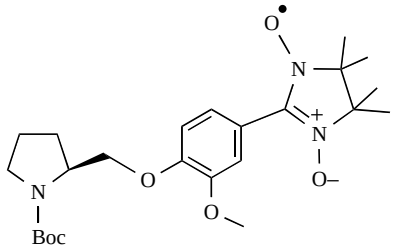
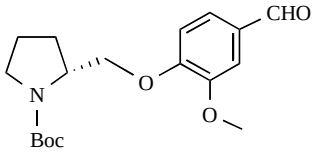
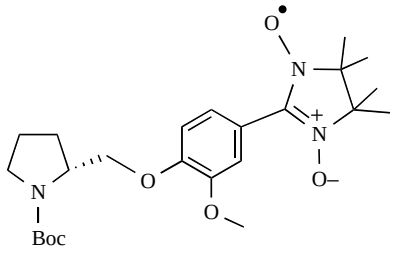
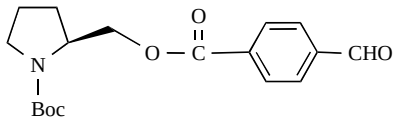
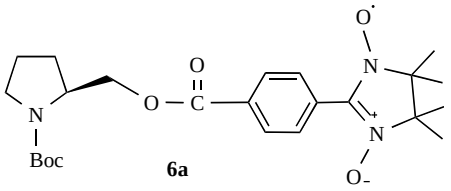
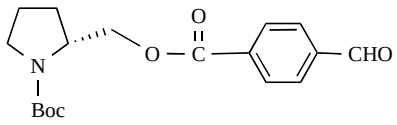
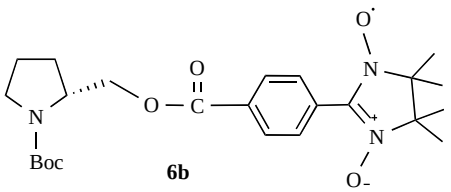
^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-300 NMR spectrometer (400 MHz) (Bruker, Karlsruhe, Germany), with $CDCl_3$ as solvent and TMS as internal reference. Optical rotation values were measured at 20 ^{0}C on a PerkinElmer 343 polarimeter (PerkinElmer Bodenseewerk, Überlingen, Germany). High resolution mass spectroscopy (HRMS) was carried out on a Varian 7.0T ESI-FTICR-MS (Varian. U.S.A.) instument. IR was recorded on a Bruker tensor 27 (Bruker Optics, German) instument. Ultra-violet absorption spectra were recorded on a Jasco V-570 UV/vis/NIR spectrophotometer (Jasco, Japan). The EPR spectra were obtained using a Bruker ESP 300E spectrometer on solutions of radicals dissolved in dichloromethane.

2, 3-bis (hydroxylamino)-2, 3-dimethylbutane was prepared by a published method [16]. *N*-Boc-*L* or *D*-prolinol was prepared in our laboratory. All other chemical reagents were purchased from the Nanjing Tianzun Zezhong Chemical Limited Company (Nanjing, China). THF was distilled under N_2 from Na/benzophenone and CH_2Cl_2 was distilled from CaH_2. The other chemical reagents were used without further purification.

Synthesis of *L* or *D*-*tert*-butyl 2-((4-Formylphenoxy) Methyl) Pyrrolidine-1-carboxylate [1a and 1b] (General Procedure for Synthesis of Chiral Aromatic Aldehydes)

Triphenyl phosphine (1.55 g, 6 mmol), *p*-Hydroxy-benzaldehyde (0.61 g, 5 mmol) and *N*-Boc- *L*- prolinol (1.2 g, 6 mmol) were dissolved in dry THF (70 ml) with vigorous stirring under an atmosphere of nitrogen at 0 ^{0}C. A solution of diethyl azodicarboxylate (1 g, 6 mmol) in dry THF (15 ml) was added dropwise over a period of 1 h at 0 ^{0}C. The mixture was warmed to room temperature and stirred for

Table 2. The Chiral Nitronyl Nitroxide Radicals

Entry	Chiral Aldehydes	Product	Time(h)	Yield(%)	Color
1	 **(L) 1a**	 **4a**	24	46.4	deep blue
2	 **(D) 1b**	 **4b**	24	46.9	deep blue
3	 **(L) 2a**	 **5a**	24	27.6	deep blue
4	 **(D) 2b**	 **5b**	24	28.1	deep blue
5	 **(L) 3a**	 **6a**	24	36.3	deep blue
6	 **(D) 3b**	 **6b**	24	36.9	deep blue

12 h. THF was removed in vacuo. Ether was added to the residue to precipitate triphenylphosphine oxide and diethyl hydrazinedicarboxylate, which were then filtered off. The filtrate was evaporated. The crude product wad purified by column chromatography on silica gel using hexane/acetone (4:1) as eluant, giving a colorless oil product (1.29 g, 84.8%). MS(m/z): 328.15 (MNa^+); IR(KBr): 1395, 1366, 2878, 2975 (ν CH_3), 1162, 1109, 1027 (ν C-C ring of pyrrolidine), 1694, 2732 (ν CHO), 1310, 1256, 1694 (ν ester bond and amide bond), 1601, 1509, 1578, 1456, 833 (ν

benzene ring); ^{1}H NMR($CDCl_3$): 9.82 (s,1H, -CHO), 7.78 (d, 2H, *J*=6.0 Hz, -ArH), 7.01 (d, 2H, *J*=9.0 Hz, -ArH), 4.20 (d, 2H, -CH_2), 3.87 (m, 1H, -CH), 3.35 (t, 2H, -CH_2), 1.97 (m, 2H,-CH_2), 1.77 (m, 2H, -CH_2), 1.42(s, 9H, -CH_3); ^{13}C NMR δ($CDCl_3$): 190.7, 163.9, 154.7, 131.9, 129.9, 114.8, 79.8, 60.0, 55.7, 46.9, 28.4, 24.0, 22.6.

D-tert-butyl 2-((4-formyl-2-methoxyphenoxy) Methyl) Pyrrolidine-1-Carboxylate [2b]

A colorless oil product; MS(m/z): 358.16(MNa^+); IR(KBr): 1396, 1366, 2974, 2878 (ν CH_3), 1167, 1136, 1024, 910 (ν C-C ring of pyrrolidine), 1688, 2833, 2723 (ν CHO), 1688, 1342, 1310, 1270 (ν ester bond and amide bond), 1587, 1510, 866, 811, 731 (ν benzene ring); ^{1}H NMR($CDCl_3$): 9.80 (s, 1H, -CHO), 7.37 (d, 1H, *J*=3.0 Hz, -ArH), 7.28 (s, 1H, -ArH), 7.04 (d, 1H, *J*=6.0 Hz, -ArH), 4.27 (d, 2H, -CH_2), 4.04 (m, 1H, -CH), 3.87(s, 3H, -CH_3), 3.38 (t, 2H, -CH_2), 2.06 (m, 2H, -CH_2), 1.83 (m, 2H, -CH_2), 1.43 (s, 9H, -CH_3); ^{13}C NMR δ($CDCl_3$): 190.98, 154.8, 153.9, 149.8, 129.9, 126.5, 111.8, 109.0, 79.9, 68.7, 60.0, 55.9, 46.9, 28.4, 23.6, 22.6. *L* configuration product **2a** was synthesized by a published method [17].

***L* or *D-tert*-Butyl 2-((4-Formylbenzoyloxy)Methyl)Pyrrolidine-1-Carboxylate [3a and 3b]**

A colorless oil product; MS(m/z): 356.14 (MNa^+); IR(KBr): 1394, 1366, 2879, 2975 (ν CH_3), 1168, 1104, 1016, 908 (ν C-C ring of pyrrolidine), 1696, 2732, 2825 (ν CHO), 1696, 1274, 1021 (ν ester bond and amide bond), 1600, 1577, 1477, 1508, 1455, 855, 759 (ν benzene ring); ^{1}H NMR($CDCl_3$): 10.06 (s,1H, -CHO), 8.18 (d, 2H, *J*=9.0 Hz, -ArH), 7.94 (d, 2H, *J*=6.0 Hz, -ArH), 4.38 (d, 2H, -CH_2), 3.94 (m, 1H, -CH), 3.40 (t, 2H, -CH_2), 1.96 (m, 2H, -CH_2), 1.78 (m, 2H, -CH_2), 1.44 (s, 9H, -CH_3); ^{13}C NMR δ($CDCl_3$): 191.6, 165.3, 154.6, 139.1, 135.1, 130.2, 129.5, 79.8, 65.7, 55.5, 47.5, 28.4, 24.0, 23.0.

Synthesis of *L-tert*-butyl 2- [(4- (4, 5-Dihydro-4, 4, 5, 5-Tetramethyl-3-Oxido-1 H-Imidazol-3-Ium-1-oxyl-2-yl) Phenoxy) Methyl] Pyrrolidine-1-Carboxylate [4a and 4b] (General Procedure for Synthesis of Chiral Nitronyl Nitroxide Radicals)

A solution of *L-tert*-butyl 2-((4-formylphenoxy) methyl) pyrrolidine-1-carboxylate (1.12 g, 3.68 mmol) and 2,3-bis (hydroxylamino) -2,3-dimethylbutane (0.54 g, 3.68 mmol) in methanol (20 ml) was stirred at room temperature for 24 h . After the reaction, the methanol was removed and the residue was suspended in 40 ml dichlormethane, aqueous $NaIO_4$ (0.71 g, 3.35 mmol in 20 ml) was added dropwise over a period of 5 min at 0 ^{0}C, and the mixture was stirred for a further 5 min at 0 ^{0}C. The organic layer was dried over anhydrous Na_2SO_4. The deep blue solution was then evaporated. The crude product was purified by column chromatography on silica gel using absolute ether/petroleum/ acetone (3:2:0.5) as eluent, giving a deep blue solid product (740 mg, 46.4 %). MS (m/z): 455.23 (MNa^+); IR (KBr): 1133, 1363 (ν NO), 2976, 2877, 1393 (ν CH_3), 1254, 1302, 1693, 1683 (ν ester bond and amide bond), 1029, 1167, 909 (ν C-C ring of pyrrolidine), 834, 1605, 1529, 1454 (ν benzene ring); UV($\lambda_{max}^{CH_2Cl_2}$): 285 (benzene ring, $\pi\rightarrow\pi^*$), 368 (ONCNO, $\pi\rightarrow\pi^*$,ε=6.65×10^3 L/(mol·cm)), 550-600 (n→ π^*). EPR(CH_2Cl_2): g factor, 2.0032; a_N, 7.684. As anticipated, nitronyl nitroxide radicals showed no nmr signal. *D* configuration product was synthesized by the same method.

D-tert-butyl 2-[(4-(4,5–Dihydro-4, 4, 5, 5–Tetramethyl–3–Oxido-1H-Imidazol-3-Ium-1-Oxyl-2-yl)-2-Methoxyphenoxy)Methyl] Pyrrolidine-1-Carboxylate [5b]

Deep blue solid product; MS (m/z): 485.24 (MNa^+); IR(KBr): 1358, 1125, 1168 (ν NO), 1391, 2976, 2876 (ν CH_3), 1232, 1026, 909 (ν C-C ring of pyrrolidine), 1692, 1327,1267 (ν ester bond and amide bond), 1599, 1491, 1530, 1455, 867, 810 (ν benzene ring); UV-vis($\lambda_{max}^{CH_2Cl_2}$): 289 (benzene ring, $\pi\rightarrow\pi^*$), 342 (ε=4.4×10^3 L/(mol·cm)), 368 (ONCNO, $\pi\rightarrow\pi^*$), 560—630 (n→π^*). EPR (CH_2Cl_2): g factor, 2.0032; a_N, 7.684.

L configuration product **5a** was synthesized by a published method [17].

***L* or *D-tert*-butyl 2-[(4-(4,5-Dihydro-4,4,5,5–Tetramethyl–3–Oxido-1H-Imidazol-3-Ium-1-oxyl-2-yl) benzoyloxy) Methyl] Pyrrolidine-1-Carboxylate [3a and 3b]**

Deep blue solid product ; MS(m/z): 483.23 (MNa^+); IR (KBr): 1365, 1168 (ν NO), 1394, 2879, 2976(ν CH_3), 1051, 1019, 908 (ν C-C ring of pyrrolidine), 1309, 1273, 1107, 1724, 1693 (ν ester bond and amide bond), 1608, 1508, 1453, 858 (ν benzene ring); UV($\lambda_{max}^{CH_2Cl_2}$): 294(benzene ring, $\pi\rightarrow\pi^*$), 380 (ONCNO, $\pi\rightarrow\pi^*$,ε=4.2×10^3 L/(mol·cm)), 550, 553, 560 (n→π^*). EPR (CH_2Cl_2): g factor, 2.0032; a_N, 7.572.

CONFLICT OF INTEREST

Declared none.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

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