[AMT08] Synthesis of ferrocene derivatives and application studies for biosensor materials

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Introduction

Soon after discovery of ferrocene in the early 1950s, it has attracted the attention of scientist worldwide in preparation of bridged ferrocenes or ferrocenophanes with bivalve-like structure (Moline et al., 2001). Ferrocenophanes are ring systems in which the two cyclopentadienyl (Cp) groups of ferrocene are joined by an atomic or a molecular bridge (Heo & Lee, 1999). Variety of ferrocenophanes with numerous modes of ring attachments that influenced the chemistry properties of these molecules had been synthesized (Watt, 1967). Some bridges contain two or more carbon atoms while others contain heteroatomic atoms such as phosphorous, sulfur, silicon and germanium (Osborne & Whiteley, 1975).

Ferrocenophanes have drawn interest of scientists because many of desirable characteristics of the parent ferrocene are retained in the ferrocenophanes. First, unlike many organometallic compounds, ferrocene and ferrocenophanes are stable in air. Consequently, ferrocene-based materials can be used in a wide variety of applications without fear of degradation. Second, ferrocene and ferrocenophanes can reversibly undergo oxidation to give stable, deep green ferrocenium cations (Yamin, 1996). This feature makes ferrocene and ferrocenophanes systems attractive electrochemical agents for using in chemical sensing (Heo & Lee, 1999).

The interest of ferrocene and its derivatives like ferrocenophanes in this research is the present of Fe(II) that will undergo oxidation as a quasi-reversible redox process (Fery-Forgues & Delavaux-Nicot, 2000; Molina et al., 2001) and has a potential in developing electrochemical devices such as biosensor electrode to replace oxygen as electron acceptor (Bu et al., 1995). Nowadays, a fabrication of ferrocene or its derivatives in matrix polymer system has received received attention as one of the methods for developing biosensor electrode and for instance is poly(vinylferrocene-*co*-2-hydroxyethyl methacrylate) (Watanabe & Saito, 1998).

In this work, seven ferrocene derivatives were synthesized such as acetylferrocene (1) and two ferrocenophanes like [4](1)(1,4-dimethyl-2,3-diazabuta-1,3-diene) ferrocenophane (2) and [4](1)(2,3-diazabuta-1,3-diene)ferrocenophane (3). Other ferrocene derivatives are chloroacetylferrocene (4) and arylferrocene like toluiferrocene (5), *m*-nitrophenylferrocene (6) and phenylferrocene (7). Modification of ferrocene to ferrocenophane that has molecular bridge consists of azomethine conjugate group, C=N-N=C will increase the conductivity of one electron transfer in redox process. Due to these electrochemical properties, ferrocenophanes will become as molecular wires (Heo & Lee 1999) that allow the electron transfer between two ferrocene units to produce the current.

Materials and Methods

Ferrocene, chloroacetyl chloride, methanol, neutral Al_2O_3, phosphoric acid, ethyl acetate, and sodium hydroxide were obtained from MERCK-Schuchardt. Anhydrous aluminium chloride, hydrazine, sodium nitrite, anhydrous sodium sulfite, 3-nitroaniline, enzyme glucose oxidase (GOD), glucose monohydrate, phosphate buffer and tetrabutylammonium hexafluorophosphate (NBu_4PF_6) were obtained from Fluka-Chemika. Hexane and tetrahydrofuran (THF) were supplied by J.T. BAKER. Other chemicals used were chloroform (Fisher Chemical), dichloromethane (LAB.SCAN), magnesium sulfate (R&M Chemicals), glacial acetic acid and acetonitrile (BDH Chemicals Ltd.), acetic anhydride and aniline (Riedel-de-Haën), sulphuric acid, 2,2-

dimetoxi-2-phenylacetophenone (DMPP), and *p*-toluidine (Aldrich) and monomer 2-glucose solutions were prepared by dissolving appropriate amounts in 0.1 M phosphate buffer (pH 7).

Preparation of acetylferrocene

Ferrocene (50 g, 270 mmol) was dissolved in acetic anhydride (170 ml). Phosphoric acid was added drop wise to this mixture with constant stirring and the solution was heated to 100ºC in a water bath for ten minutes. The appearance of a deep blue color indicated that the reaction was occurring. The mixture was then poured over 20 g of ice into a large beaker. When the ice had melted, NaOH was added drop wise until the pH of the solution was at least 7. As the solution was neutralized, a precipitate should form which was filtered off and washed with distilled water to remove impurities and allow to air-drying. Recrystallization from hexane produced orange needle crystals of **(1)**.

Preparation of ferrocenophane

Acetylferrocene (0.5 g, 2.2 mmol) was added to 10 ml methanol and stirred at room temperature until completely dissolved. A stoichiometric amount of hydrazine (0.11 g, 2.2 mmol) and 0.5 ml of glacial acetic acid were then poured into the solution in a two-neck round-bottomed flask. The reaction mixture was refluxed for I h. After this period, the reaction mixture was filtered and the filtrate was allowed to cool to room temperature. Slow evaporation of the solvent produced a precipitate, which was filtered off and washed with cold methanol and air-dried. Recrystallization from dichloromethane and hexane produced dark orange crystals of **(2)** and **(3)**.

Preparation of chloroacetylferrocene

Under a nitrogen atmosphere, to the stirred solution of ferrocene (12.00 g, 60 mmol) in 100 ml of dichloromethane, was added drop wise chloroacetyl chloride (13.55 g, 120 mmol) at room temperature. The flask with the solution was immersed in an ice bath. When the solution had been chilled thoroughly, the anhydrous aluminium chloride (8.00 g, 60 mmol) was added and the reaction mixture remained below 5ºC for 2 h. The appearance of a deep blue color hydroxiethyl methacrylate (Sigma). Phosphate buffer was prepared in distilled water and indicated that the reaction was occurring. After its completion, stirring was continued for overnight at room temperature.

The reaction mixture was poured cautiously in 100 ml of distilled water with some ice and the resulting two-phase mixture was stirred vigorously for 30 minutes. The aqueous phase was extracted with (2 x 50 ml) of dichloromethane. The combined dichloromethane extracts were washed with distilled water (50 ml), NaOH (2 x 50 ml) and then dried over magnesium sulfate. The extract was concentrated and chromatographed on Al_2O_3 gel column. Elution with petroleum ether and chloroform gave the desire product. Recrystallization from dichloromethane and hexane produced orange crystals of **(4)**.

Preparation of arylferrocene

Ferrocene (1.86 g, 10 mmol) was dissolved in 50 ml of tetrahydrofuran (THF) and immersed in a beaker that containing some ice. Corresponding amines (10 mmol) were poured to 50 ml of H_2SO_4 in conical flask and also immersed in some ice. Under the nitrogen atmosphere, to the magnetically stirred mixture of amines were added drop wise a solution of sodium nitrite (0.69 g, 10 mmol) in 15 ml of H_2SO_4 and the reaction mixture remained below 0ºC for 30 minutes. Then the ferrocene solution was added to the diazonium salts and kept stirring for overnight.

The appearance of a deep blue color indicated that the reaction was occurring. The reaction mixture was poured cautiously in 150 ml of distilled water with some ice. The solution of sodium sulfite (1.26 g, 10 mmol) was added drop wise until the deep blue color disappeared and then extracted with ethyl acetate (3 x 80 ml). After steam distillation to remove unreacted ferrocene, appeared red oily solution and then became precipitate after cooling at room temperature. That precipitate was dissolved again with ethyl acetate and dried over magnesium sulfate. The extract was concentrated and chromatographed on Al_2O_3 gel column. Elution with ethyl acetate and dichloromethane gave the desire products (5), (6) and (7).

Electrode preparation

An appropriate amount of ferrocene derivatives were added in the mixture of monomer HEMA and DMPP. The Fc/HEMA mixture was drop-coated onto a screen-printed electrode. The screen-printed electrode was then exposed to UV lights to photo cure.

Melting points of the crystals were determined by using Elektroterma 1A 9100 (0-400ºC) instrument. Microelemental analysis had been carried out by using CHNS-O (Thermo Finnigan Flash EA 1112 Series) analyzer. IR spectra were measured by a Perkin Elmer Spectrum GX spectrophotometer using KBr pellets in the 4000-400 cm^{-1} regions. NMR spectra were obtained by using spectrometer JNM-ECP 400 with $CDCl_3$ as the solvent and Me_4Si as an internal standard. X-ray crystallographic analysis was carried out by using BRUKER Smart Apex Diffractometer. The SHELTX program was used for structure solution and refinement. Electrochemical measurements were obtained from Autolab PGSTST 12 Potentiostat/Galvanostat under nitrogen atmosphere and used acetonitrile (HPLC-grade) as solvent and tetrabutylammonium hexafluorophosphate (0.1 M) as supporting electrolyte. We used platinum as working electrode, Ag/AgCl reference electrode and a glassy carbon auxiliary electrode for these cyclic voltammetry measurements. For biosensor studies, we replaced platinum to Fc/HEMA-coated screen-printed electrode as working electrode. The cyclic voltametry of Fc/HEMA-coated screen-printed electrode was studied between 0.0 and 0.75 V versus Ag/AgCl. All experiments were performed in 0.1 M phosphate/0.1 M NaCl buffer (pH 7).

Results and Discussion

Seven ferrocene derivatives were successfully synthesized. The physical properties and microelemental analysis data are in agreement and consistent with the expected molecular formula are listed in Table 1. The IR spectra of ferrocene derivatives are shown in Table 2. The characteristics bands of ferrocene group appear at 3089, 2343, 1104, 1005, 823 and 500 cm^{-1} assignable to ν(C-H), ν(C=C), δ(CH)Cp and metal-ring respectively. The sharp band at 1628 cm^{-1} due to ν(C=N) **(3)** is shifted to higher wave number if compared to its analogue **(2)** because of the bulky substituent. Stretching band of carbonyl group **(1)** and **(4)** are shown at wave number 1654 and 1678 cm^{-1} respectively. The present of nitro group **(6)** is approved with the sharp bands at 1342 and 1524 due to the symmetry and asymmetry stretching of the N-O group. The typical stretching bands of the methyl, fragment at 2922 cm^{-1} for symmetry and 2850 cm^{-1} for asymmetry **(5)**.

TABLE 1 Microelemental analysis and some physical properties of ferrocene derivatives **(1)** to **(7)**

Compound	**Molecular formula**	**Colour**	**Yield (%)**	**Melting point (°C)**	**Microelemental analysis: Found (Calc.) (%)**		
					C	**H**	**N**
(1)	$FeC_{12}H_{12}O$	Orange	47.0	82-84	64.22 (63.20)	5.20 (5.30)	-
(2)	$Fe_2C_{24}H_{24}N_2$	Dark orange	42.0	216-217	63.82 (63.75)	5.93 (5.35)	6.05 (6.20)
(3)	$Fe_2C_{22}H_{20}N_2$	Dark orange	40.0	213-216	61.60 (62.31)	4.60 (4.75)	6.22 (6.61)
(4)	$FeC_{12}H_{11}OCl$	Orange	32.2	91-93	55.53 (55.20)	4.06 (4.13)	-
(5)	$FeC_{17}H_{16}$	Orange	37.0	-	72.21 (73.94)	5.40 (5.84)	-
(6)	$FeC_{16}H_{13}O_2N$	Dark orange	42.0	-	61.89 (62.57)	4.19 (4.27)	4.43 (4.56)
(7)	$FeC_{16}H_{14}$	Orange	51.0	-	72.89 (73.31)	5.20 (5.38)	-

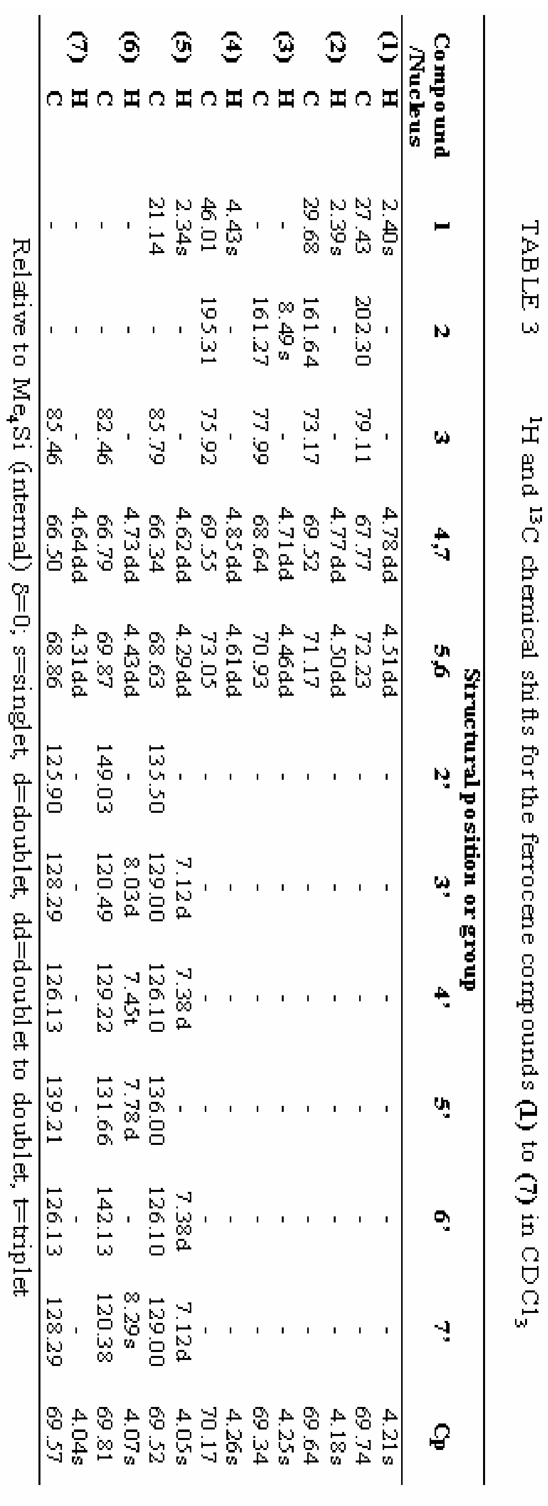
TABLE 2 IR absorption bands of ferrocene derivatives **(1)** to **(7)**

Compound	**ν(C=N)**	**ν(C=C)**	**δ_s(CH$_2$-Cl)**	**δ_s(CH$_3$)**
Ferrocene	-	-	-	-
(1)	-	-	-	1373m 1456m
(2)	1598s	895m	-	1359m
(3)	1628s	-	-	-
(4)	-	-	1452m 1239m	-
(5)	-	1525m 1451m 1451m	-	-
(6)	-	1600m 1496m	-	-
(7)	-	1597m 1450m	-	-

s=sharp, m=medium

Proton NMR spectra of ferrocene derivatives (1) to (7) (Table 3) show two asymmetry triplets in the region 4.30-4.90 ppm for H_α and H_β due to the overlapping by the next proton. These peaks undergo H-decoupling doublet to doublet. The presence of electron pulling groups such as $COCH_3$ (1) and $COCH_2Cl$ (4) had caused H_α slightly deshielded and shifted to lower magnetic field with higher chemical shift compared to H_β.

TABLE 3 ^{1}H and ^{13}C chemical shifts for the ferrocene compounds **(1)** to **(7)** in $CDCl_3$



Compound /Nucleus		**Structural position or group**										
		1	**2**	**3**	**4,7**	**5,6**	**2'**	**3'**	**4'**	**5'**	**6'**	**7'**	**Cp**
(1)	**H**	2.40s	-	-	4.78dd	4.51dd	-	-	-	-	-	-	4.21s
	C	27.43	202.30	79.11	67.77	72.23	-	-	-	-	-	-	69.74
(2)	**H**	2.39s	-	-	4.77dd	4.50dd	-	-	-	-	-	-	4.18s
	C	29.68	161.64	73.17	69.52	71.17	-	-	-	-	-	-	69.64
(3)	**H**	-	8.49s	-	4.71dd	4.46dd	-	-	-	-	-	-	4.25s
	C	-	161.27	77.99	68.64	70.93	-	-	-	-	-	-	69.34
(4)	**H**	4.43s	-	-	4.85dd	4.61dd	-	-	-	-	-	-	4.26s
	C	46.01	195.31	75.92	69.55	73.05	-	-	-	-	-	-	70.17
(5)	**H**	2.34s	-	-	4.62dd	4.29dd	-	7.12d	7.38d	-	7.38d	7.12d	4.05s
	C	21.14	-	85.79	66.34	68.63	135.50	129.00	126.10	136.00	126.10	129.00	69.52
(6)	**H**	-	-	-	4.73dd	4.43dd	-	8.03d	7.45t	7.78d	-	8.29s	4.07s
	C	-	-	82.46	66.79	69.87	149.03	120.49	129.22	131.66	142.13	120.38	69.81
(7)	**H**	-	-	-	4.64dd	4.31dd	-	-	-	-	-	-	4.04s
	C	-	-	85.46	66.50	68.86	125.90	128.29	126.13	139.21	126.13	128.29	69.57

Relative to Me_4Si (internal) δ=0; s=singlet, d=doublet, dd=doublet to doublet, t=triplet

X-ray crystallographic analysis of the compounds (1) to (4) were obtained (Figure 1). Both ferrocenophanes are centrosymmetric about the mid-point at the N-N bond. The Cp rings of both ferrocene moieties are eclipsed and at *trans* position with each other.

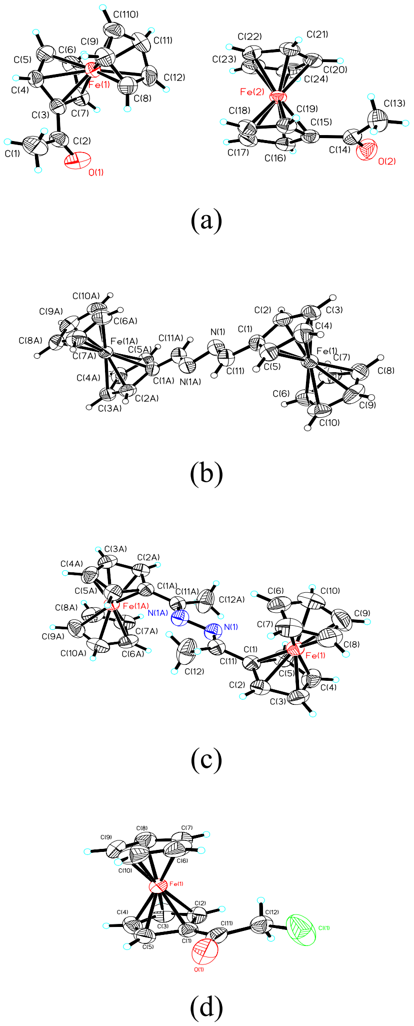


FIGURE 1 The molecular structures of acetylferrocene (a), [4](1)(1,4-dimethyl-2,3-diazabuta-1,3-diene)ferrocenophane (b), [4](1)(2,3-diazabuta-1,3-diene)ferrocenophane (c) and chloroacetylferrocene (d), showing the atom-labelling schemes and 50% probability displacement ellipsoids for non-H atoms.

In order to compare the influence of the substituents on the redox ability of Fe(II) and the electron giving and electron withdrawing power of the nitro, methyl, carbonyl and azomethine groups, we carried out electrochemical studies on compounds (1) to (7). In all cases the cyclic voltamograms exhibited one anodic peak with a directly associated cathodic peak in the reverse scan. The value of half wave potentials $E_{1/2}$ for compounds (1) to (7) were higher than ferrocene itself and are listed in table 4. The propensity of Fe(II) to undergo oxidation is strongly dependent on the nature of the substituents. For instance, it is well known that electron donor groups facilitate the oxidation of Fe(II) like methyl, CH_3 for compound (5) that showed lower potential and nearly to ferrocene value. While electron pulling groups reduce the proclivity of the Fe(II) to undergo oxidation like nitro and acetyl groups for compounds (6) and (1) respectively. They showed higher potential around 0.60V.

For biosensor studies, compounds (5), (6) and (7) had been chosen. These compounds in the biosensor system containing GOD enzyme showed positive responses with good cyclic voltamograms (Table 5). However no current response was observed when glucose was added to the system. The absence of response compare to unsubstituted ferrocene may due to the complicated side interactions involving the polymeric matrix. Further study is required for understanding the interactions.

TABLE 4 Electrochemical data for the ferrocene derivatives **(1)** to **(7)** (V vs. Ag/AgCl)

Compound	E_{pa} (V)	E_{pc} (V)	$E_{1/2}$ ^{a} (V)	ΔE_p ^{b} (mV)	i_{pa} (x 10^{-5} A)	i_{pc} (x 10^{-5} A)	i_{pa}/i_{pc} ^{c}
Ferrocene	0.43	0.34	0.39	90	2.00	2.00	1.0
$FeC_{11}H_{10}O$	0.76	0.66	0.71	100	4.50	3.84	1.2
(1)	0.72	0.62	0.67	100	2.25	1.96	1.1
(2)	0.60	0.44	0.52	160	4.50	3.50	1.3
(3)	0.77	0.63	0.70	140	3.50	2.50	1.2
(5)	0.47	0.39	0.43	80	5.62	4.23	1.3
(6)	0.60	0.47	0.54	130	4.55	3.45	1.3
(7)	0.51	0.37	0.44	140	4.95	3.50	1.4

TABLE 5 Electrochemical data for the Fc/HEMA-coated screen-printed **(5)** to **(7)** (V vs. Ag/AgCl)

Compound	E_{pa} (V)	E_{pc} (V)	$E_{1/2}$ ^{a} (V)	ΔE_p ^{b} (mV)	i_{pa} (x 10^{-7} A)	i_{pc} (x 10^{-7} A)	i_{pa}/i_{pc} ^{c}
(5)	0.38	0.30	0.34	80	7.00	10.00	0.7
(6)	0.51	0.38	0.45	130	4.00	3.00	1.3
(7)	0.39	0.30	0.35	90	6.25	6.10	1.0

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