Synthesis and biological evaluation of novel ferrocene–naphthoquinones as antiplasmodial agents

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2013.10.011 · Source: PubMed

6 AUTHORS, INCLUDING:

Pedro Manuel García-Barrantes
Vanderbilt University
10 PUBLICATIONS **99** CITATIONS
SEE PROFILE

Antonio R. Martinez-Fernandez
Complutense University of Madrid
74 PUBLICATIONS **885** CITATIONS
SEE PROFILE

Synthesis and biological evaluation of novel ferrocene-naphthoquinones as antiplasmodial agents

Pedro M. García-Barrantes, Guy V. Lamoureux, Alice L. Pérez, Rory N. García-Sánchez, Antonio R. Martínez, Arturo San Feliciano

PII: S0223-5234(13)00652-1

DOI: 10.1016/j.ejmech.2013.10.011

Reference: EJMECH 6474

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 31 March 2013

Revised Date: 2 October 2013

Accepted Date: 5 October 2013

Please cite this article as: P.M. García-Barrantes, G.V. Lamoureux, A.L. Pérez, R.N. García-Sánchez, A.R. Martínez, A. San Feliciano, Synthesis and biological evaluation of novel ferrocene-naphthoquinones as antiplasmodial agents, *European Journal of Medicinal Chemistry* (2013), doi: 10.1016/j.ejmech.2013.10.011.

Synthesis and biological evaluation of novel ferrocene-naphthoquinones as antiplasmodial agents

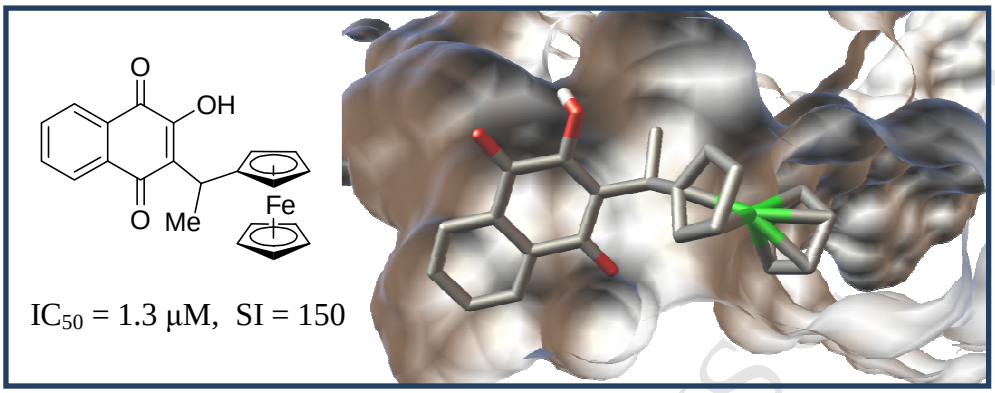
Pedro M. García-Barrantes[a], Guy V. Lamoureux[a,]*, Alice L. Pérez[a], Rory N. García-Sánchez[b], Antonio R. Martínez[b], Arturo San Feliciano[c]

[a]*Centro de Investigaciones en Productos Naturales and Escuela de Química, Universidad de Costa Rica, San Pedro 2060, San José, Costa Rica pedro.garcia_b@ucr.ac.cr, guy.lamoureux@ucr.ac.cr, alice.perez@ucr.ac.cr*
[b]*Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid, España. rgbiounap@yahoo.es, arm@farm.ucm.es*
[c]*Departamento de Química Farmacéutica, Facultad de Farmacia, CIETUS - IBSAL, Universidad de Salamanca, Salamanca, España. asf@usal.es*

Corresponding author

Guy V. LAMOUREUX[a], guy.lamoureux@ucr.ac.cr, phone: +(506)25114091, Fax: +(506) 22535020
Alice L. PEREZ[a], alice.perez@ucr.ac.cr, phone: +(506)25111405
[a]Centro de Investigaciones en Productos Naturales and Escuela de Química, Universidad de Costa Rica, San Pedro 2060, San José, Costa Rica.

Graphical abstract



Highlights

11 new 3-(ferrocenylmethyl)-1,4-naphthoquinone derivatives were synthesized with an "on-water" methodology.

3 compounds showed antiplasmodial activity (IC_{50}<2 μM) against chloroquine-sensitive and resistant strains of *Plasmodium falciparum*.

3 compounds showed selective cytotoxicity (SI>100) towards the parasite strains relative to a mammal cell line.

Abstract

This work deals with the synthesis and evaluation of new compounds designed by combination of 1,4-naphthoquinone and ferrocene fragments in a 3-ferrocenylmethyl-2-hydroxy-1,4-naphthoquinone arrangement. A practical coupling reaction between 2-hydroxy-1,4-naphthoquinone and ferrocenemethanol derivatives has been developed. This procedure can be carried out "on-water", at moderate temperatures and without auxiliaries or catalysts, with moderate to high yields. The synthesized derivatives have shown significant *in vitro* antiplasmodial activity against chloroquine-sensitive and resistant *Plasmodium falciparum* strains and it has been shown that this activity is not related to the inhibition of biomineralization of ferriprotoporphyrin IX. Binding energy calculations and docking of these compounds to cytochrome b in comparison with atovaquone have been performed.

Keywords

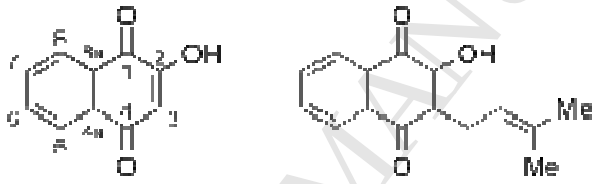
Naphthoquinone, lawsone, ferrocene, antimalarial, on-water reactions

Abbreviations

FBIT: Ferriprotoporphyrin IX Biomineralization Inhibition Test
SI: Selectivity Index

1. Introduction

Quinones are organic molecules that possess a cyclic six membered conjugated planar system of two carbonyls and two double bonds. There are several types of quinones depending on the size of the system, the arrangement of functional groups and substituents and the possibility of heteroatoms involved. A quinone can be reduced reversibly to a hydroquinone, passing through a stable semiquinone form [1]. Redox properties of quinones can be modulated by their substituents. Electron-donating groups decrease the reduction potential while the opposite occurs with electron-withdrawing substituents [2]. Quinones can act as nucleophiles or electrophiles, depending on electronic effects. As electrophiles, attack occurs at the carbonyl group or at the β-position by a Michael-like addition reaction; these reactions are favored by electron-withdrawing groups [3, 4]. Quinones acting as nucleophiles are less common and are favored by electron donors, (hydroxy, methoxy, amino groups). For example, lawsone **1** can be substituted at the C-3 position by various electrophiles [5].

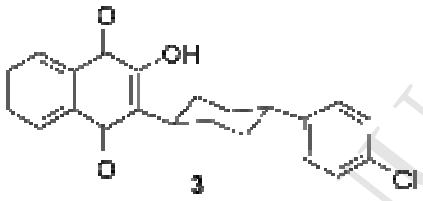


Quinones are known to be electron transporters (e.g. ubiquinone, vitamin K), and are essential for many enzymatic processes [6]. They can act as anti- or pro-oxidants depending on the conditions of the media. This chemical versatility gives them an important role in different biochemical processes that are essential to living organisms. Also, exogenous synthetic quinones (or quinones isolated from natural sources) have been shown to inhibit different physio-pharmacological targets, to cause oxidative stress and cell death of microbes [7]. Furthermore, different naphthoquinones have been reported and clinically used as anticancer, antifungal, antiprotozoal, and antibacterial therapeutic agents, as well as platelet antiaggregants and to prevent the myotoxicity of snake venom [8–14]. Their biological activity has been ascribed to their electronic properties and, in some cases, to specific interactions with defined targets such as DNA and dihydroorotate dehydrogenase [15]. Their readiness to stabilize radical species has been pointed out as the cause of their cytotoxicity, producing oxidative stress and promoting cell death [4, 5, 7, 16, 17].

We are involved in the effort to explore the chemical space of naphthoquinones with selective inhibition properties, with lesser cytotoxic effects, and to develop new derivatives of naphthoquinones with more druglike characteristics [18]. A starting point for the development of analogues of these kinds of compounds is the substitution at the C-3 position, such as the natural product lapachol **2** [19].

Malaria is one of the most important infectious diseases, causing the death of more than 1 million people in 2010 (the majority children under five years old) [20]. In 2011, there were

estimated more than 250 million symptomatic cases. Incidence is greater in poorer regions where control has not been effective and access to health services is scarce [21]. Efforts have been made to combat widespread infection, such as vector control, opportune diagnostics and the treatment of infected patients. However, the therapeutic arsenal is limited and parasites are developing growing resistance to current medications; therefore, new antimalarial drugs are needed. Naphthoquinones have received special attention due to their redox and metal chelating properties [2]. The most successful quinone antimalarial drug is atovaquone (ATV, **3**). It was introduced by Glaxo as Malarone® in 2000, in combination with proguanyl for the treatment and prevention of malaria when dealing with strains resistant to chloroquine, mefloquine, amodiaquine and halofantrine. The main mechanism of action proposed for atovaquone is the inhibition of the cytochrome b subunit of the mitochondrial bc_1 complex involved in the respiratory-chain of the parasite [22, 23].

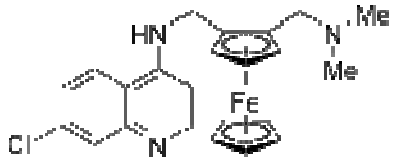


3

Ferrocene is an organometallic compound formed by an iron(II) center surrounded by two cyclopentadiene rings arranged in a sandwich-like structure. The iron(II) can be oxidized to iron(III) generating the bis(η^5-cyclopentadienyl)iron(1+) ion, which possesses a similar geometry [24]. Ferrocene is characterized by possessing aromatic behavior, each ring with six electrons according to Hückel's rule [25].

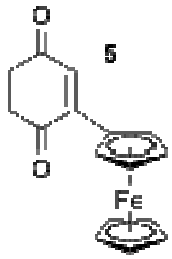
The use of ferrocene in Medicinal Chemistry has increased recently. As a substituent, ferrocene presents high lipophilicity (π 2.46) and can act as an electron donor (σ_m -0.15; σ_p -0.18) similar to cyclohexane (π 2.51; σ_m -0.15; σ_p -0.22), but it presents high molar volume and refractivity [26]. Ferrocene has been proposed as an arene bioisostere to modulate the pharmacokinetic or pharmacodynamic profiles of drugs [27, 28]. Moreover, ferrocene, compared to other organometallic compounds, has many advantages for biological applications, such as chemical modification capacity, low toxicity and stability to water and oxygen [29, 30]. Compounds with ferrocene moieties have been evaluated as antimalarial, anticancer, antifungal, antiviral [31–33], anti-inflammatory [34], anti-tuberculosis [35], and as agonists to receptors like H3, D1, D2, D3, D4, alfa-1 adrenergic and 5-HT1A [36–38].

In malaria research, the incorporation of ferrocene led to ferroquine **4,** a chloroquine (CQ) analogue that advanced to phase II in clinical trials and effective against resistant *Plasmodium falciparum* [39]. The advantages of this analogue have been attributed to the increase in lipophilicity and the decrease in affinity towards the CQ exporter [40–42]. Many quinoline-ferrocene derivatives have been synthesized and tested as antimalarials [43, 44]. Also, ferrocene can act as a catalyst of Fenton-type reactions and promote the generation of reactive oxygen species in the digestive vacuole of the parasite [27, 32].

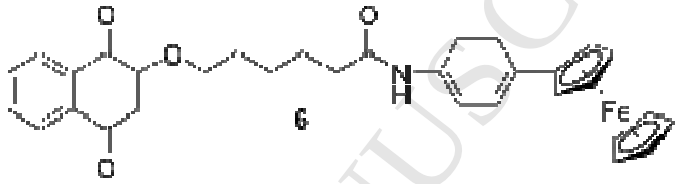


4

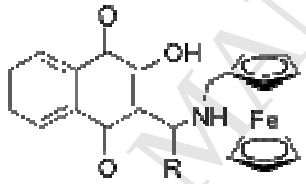
A few reports about ferrocene-quinone conjugates have been published. These substances have been synthesized mainly to explore their electrochemistry (**5** and **6**), but activity against *P. falciparum* and *Toxoplasma gondii* has been reported for aminoquinone derivatives **7** [16, 45, 46].



5



6



7

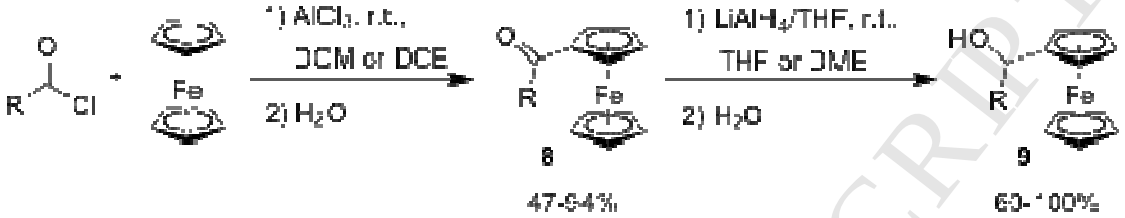
The aim of this work is to develop a synthesis (with a green perspective) of new 3-(ferrocenylmethyl)-1,4-naphthoquinone derivatives, and to characterize these compounds in terms of their antimalarial properties. We were inspired to design these new compounds based on a hybrid molecule approach [47]. Similar to lapachol and atovaquone the 1,4-naphthoquinone moiety was conserved, due to its role as a privileged structure. The ferrocene moiety was introduced to explore its potentially positive influence, such as exists in ferroquine, in the pharmacodynamic profile of the hybrid.

2. Results and discussion

2.1. Chemistry

For the synthesis of the desired ferrocenylnaphthoquinone derivatives, it was necessary to obtain the ferrocenecarbinols **9**. Their preparation was carried out in three steps, using ferrocene and the corresponding carboxylic acid as starting materials (Scheme 1). Acyl chlorides, obtained from the corresponding acids by treatment with thionyl chloride, were used for the acylation of ferrocene, by the classical Friedel-Crafts procedure, using $AlCl_3$ as the Lewis acid and following a literature procedure to minimize diacylation subproducts [48, 49]. Yields for this reaction were moderate to good. Lower yields were attained when

using aromatic acyl chlorides which can react with themselves [50]. For the reduction of the ketones **8**, $LiAlH_4$ in THF was employed and we efficiently obtained the ferrocenecarbinols **9** in high yields, with short reaction times and absence of subproducts. It was observed that these ferrocenecarbinols (ferrocenyl alcohols) tend to oxidize slowly to the corresponding ferrocenylketones when exposed to air. Therefore, these compounds were used immediately after purification.



R:	H-	$Cl(CH_2)_2CH_2$-	Ph-
	CH_3-	$CH_3(CH_2)_9CH_2$-	*p*-ClPh-
	CH_3CH_2-	cHex-	*p*-BrPh-
	$CH_3CH_2CH_2$-	Bn-	

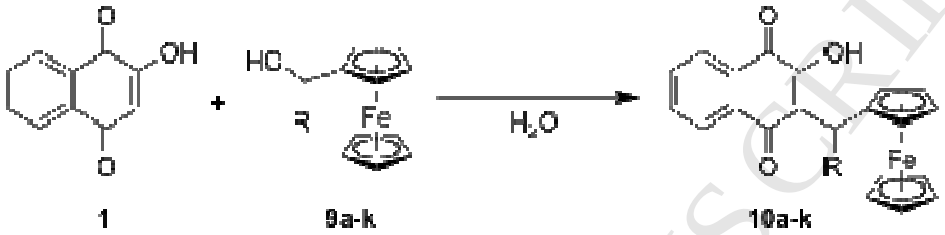
Scheme 1. Synthesis of ferrocenecarbinols.

The coupling reaction between ferrocenecarbinols **9** and lawsone **1** to produce 3-ferrocenylmethyl-1,4-naphthoquinones **10a-k** utilized an heterogeneous "on-water" procedure [51]. In general, ferrocenecarbinols are not reactive to nucleophilic substitution and usually they need to be derivatized to form a better leaving group [52]. When dealing with an S_N1 type reaction, the stability of the generated carbocations should be considered as it relates to its electrophilicity. In the Mayr scale of electrophilicity (*E*), the ferrocenecarbinols generate more electrophilic carbocations than substances like benzhydrol [53]. This reactivity can be justified because of the delocalization of the positive charge [54] and electron density donation from iron *d* orbitals [55]. On the other hand, 2-hydroxy-1,4-naphthoquinone represents a multidentate nucleophile similar to β-dicarbonyl compounds [3]. Selectivity for C-3 alkylation is due to the irreversibility of C-alkylation (thermodynamic product) and is consistent with the Pearson's Hard Soft Acid Base theory [56].

The solvent plays an important role for this reaction, as polar protic solvents better stabilize carbocations and water's capacity to form hydrogen bonds may promote the protonation of the alcohol and the formation of these intermediates. The alcohol and the quinone have a relatively low solubility in water, and they form heterogeneous mixtures while stirring ("on-water" reaction). No addition of Brønsted or Lewis acid was necessary, so it is assumed that water is acting as a catalyst, as demonstrated in other cases. Most alkylation studies have been done in solution, but it was found recently that the rate enhancement tends to be greater with "on-water" reactions [57]. The network of water molecules tends to leave "dangling" protons in the water:oil interphase; it has been hypothesized that these protons could stabilize possible transition states in the reaction [58]. Also, the reaction could be favored by an hydrophobic effect that leads to a closer contact between the alcohols and the quinone [59, 60]. This procedure has the advantage of green reaction conditions and easy workup and purification of products.

The reaction was tested with different ferrocenecarbinol derivatives **9a-k**, producing moderate to high yields (**Table 1**). Better yields were obtained when the substituent R was a short (not bulky) aliphatic group. Competition between nucleophilic substitution and elimination affected the yield in some cases; for instance in the synthesis of **10g** and **10h**, elimination products were isolated and their structures confirmed by NMR.

Table 1. Reaction conditions and yields for the synthesis of ferrocene-naphthoquinones **10a-k**



1 9a-k 10a-k

Compound	R	Temperature / °C	Time / h	Yield / %
10a	H-	40	23	65
10b	Me-	40	4	79
10c	Et-	40	18	75
10d	Pr-	40	18	83
10e	$ClCH_2CH_2CH_2$-	40	18	81
10f	$CH_3(CH_2)_9CH_2$-	70	6	28
10g	cHex-	70	24	11
10h	Bn-	40	6	53
10i	Ph-	40	18	68
10j	*p*-ClPh-	70	6	79
10k	*p*-BrPh-	40	15	73

All compounds were obtained and evaluated as racemic mixtures (when substituted at the linking carbon). Structural characterization was made by spectroscopic methods and mass spectrometry. Infrared spectra of the products show characteristic bands associated to O-H stretching signals (~3350-3400 cm^{-1}), C=O stretching signals (~1640-1670 cm^{-1}) and C=C stretching signals (~1585-1595 cm^{-1}) in all cases. The spectrum of many derivatives of 2-hydroxy-1,4-naphthoquinone shows a band of the intramolecular hydrogen bond between the hydroxyl at C-2 and the carbonyl at C-1; this interaction was observed as a sharp O-H stretching signal (ν: 3350-3370 cm^{-1}) in the IR spectra, except for **10g** and **10k** that show a wider band. The ^{1}H-NMR spectra of the compounds can be subdivided in three regions. The aromatic region related to the quinone is composed of four signals, two doublet of doublets (8.00-8.15 ppm) and two triplets of doublets (7.6-7.8 ppm), and this region shows

little variation between compounds. The ferrocene region can show from two to four signals (around 4.0-4.5 ppm) that correspond to the substituted cyclopentadiene. The number of signals depends on the heterotopicity of the ring protons, with one characteristic singlet corresponding to the five protons of the non-substituted cyclopentadiene, around 4.15 or 4.00 ppm for the cases of aliphatic or aromatic groups bound to the methane bridge, respectively. The third region contains the signals of protons on the bridge and those of the aliphatic substituents R, and it varies depending on the substituent. The ^{13}C-NMR spectra can be analyzed in an analogous manner to that of the proton spectra, with signals corresponding to the naphthoquinone above 120 ppm and to the ferrocene unit between 65 and 90 ppm. Confirmation of the structural scaffold was obtained for **10b** through X-Ray crystallography (Supplementary material) and no significant deviations in angles or bonds distances were observed with respect to those in ferrocene and 2-hydroxy-1,4-naphthoquinone structures.

2.2. Biological section

For the determination of antiplasmodial activity, *in vitro* tests were performed on two cultured strains of *P. falciparum* (3d7, CQ-sensitive; Dd2, CQ-resistant). Most of the compounds showed IC_{50} values under 15 μM in both strains, **10j** being the only exception (**Table 2**). For comparison, a IC_{50} for atovaquone of 0.0008 μM has been measured against cell lines Dd2 [61]. Maximum activity was observed for compounds **10a**, **10b** and **10c** which present an IC_{50} close to 1 μM, and an important decrease in growth inhibition can be observed when the substituent is a propyl or larger group. The use of bulky substituents in this position does not favor antiplasmodial activity; however, compound **10f** does not suffer a dramatic change in its activity despite having an undecyl group as substituent, the most voluminous of the series. As multiple effects could influence this behavior, an explanation is hard to rationalize; the possibility that this compound has higher penetration in the cells and more exposure to the parasite could be important, as well as the different conformations that this lipophilic chain could achieve. Compounds **10a**, **10b**, **10c** might be considered as potential leads for further optimization. Moreover, these compounds have the advantage of inhibiting both CQ-sensitive and CQ-resistant strains at a similar concentration.

Table 2. *In vitro* antiplasmodial and biomineralization activities of the ferrocene-naphthoquinone derivatives **10a-k**.

Compound	*P. falciparum* strain IC_{50} (μM)			FBIT test IC_{50} (mg/mL)
	3D7	Dd2	Resistance index*	
10a	1.40	1.48	1.1	>2
10b	1.30	1.19	0.9	>2
10c	1.60	2.10	1.3	>2
10d	13.2	13.3	1.0	>2
10e	12.1	13.4	1.1	>2

10f	4.01	4.51	1.1	>2
10g	8.35	18.1	2.2	>2
10h	7.34	10.2	1.4	0.79
10i	12.6	14.0	1.1	>2
10j	37.4	22.5	0.6	>2
10k	7.59	4.28	0.6	>2
CQ	0.03	0.50	16.7	0.04

* Values calculated by the equation RI = IC_{50} (DD2)/ IC_{50} (3D7).

To obtain more information about the mechanism of action of these substances, a ferriprotoporphyrin IX biomineralization inhibition test (FBIT) was performed. Ferriprotoporphyrin IX is the heme group product of the digestion of hemoglobin from erythrocytes by the parasite. This substance is toxic to the parasite and its accumulation is prevented by biomineralization, producing hemozoin. Ferriprotoporphyrin IX biomineralization inhibition is the mechanism proposed for the chloroquine antimalarial activity, and this is related to iron coordination [41, 62]. Therefore, as some quinones can coordinate metals, a similar effect was expected for these derivatives; however, no inhibition of biomineralization was observed except for **10h** which showed low activity in the full parasite tests.

Because of their quinonic nature, the antimalarial activity of these compounds might involve the mitochondrial bc1 complex, as in the case of ATV. Malaria parasites contain enzymatic electron-transfer complexes based on cytochromes in the mitochondria and in the apicoplast (a non-photosynthetic plastid derived from alga). Experimentally it has been suggested that the mitochondria and apicoplast are physically and functionally associated in *Plasmodium spp,* both originating from the characteristic symbiont of this apicomplexan group of protozoan [63, 64]. To clarify, ATV has been shown to bind to the mitochondrial bc1 complex and, due to the redox system of the quinone, might disrupt the same electron-transport system in the apicoplast. The proposed activity on the cytochrome bc1 complex is probably extensible to the apicoplast. As a consequence, a pharmacological target based on the plant-like apicoplast would be safer because of its phylogenic distance from the host.

The crystallized structure of this target is not available for *P. falciparum* and molecular docking was carried out using the crystal structure of cytochrome b from *Saccharomyces cerevisiae*; these structures have high homology and the protein from the yeast has been used in the literature to simulate the plasmodial protein complex [23]. We used the LGA search method with the standard parameters in AutoDock using a flexible ligand against a rigid protein target in this study (no flexible side-chain docking was performed).

Interestingly, in these simulations and according to the values found, compounds **10a**, **10b**, **10d**, **10g** seem to display higher affinities (lower ΔG values) than ATV (**Table 3**); two of them, **10a** and **10b** showed IC_{50} values under 2 μM (**Table 2**). It should be mentioned that we do not expect a clear trend between the predicted binding energies and the experimental activities for all the compounds. This lack of a clear correlation is obvious comparing the

ΔG with the results of inhibition for the anomalous compounds **10d** and **10g**. First, the structures for *P. falciparum* and *S. cerevisiae* are not the same and until the former structure is available we can only intuit the binding that occurs in the actual host. Second, the docking studies were performed without the ability to include changes that occur in p*K*a and entropy that are related to the change of the C3 group. Finally, our docking method does not present a flexible protein during docking so that subtle changes in the backbone and side-chains could affect the results.

Table 3. Free energy ΔG values calculated for the simulated binding of ferrocene-naphthoquinones **10a-k** to cytochrome b.

Compound	ΔG (kcal•mol^{-1})
10a	**-9.12**
10b	**-9.29**
10c	-8.85
10d	**-9.05**
10e	-8.16
10f	-7.87
10g	**-9.25**
10h	-8.49
10i	-8.10
10j	-7.43
10k	-6.95
ATV	-8.95

When visualizing the predicted conformations, it is very important to note that only compounds **10a**, **10b** and **10c** share a similar binding mode with ATV (**Figure 1A**), unlike the compounds **10d** and **10g**, which, when accommodated in the lowest energy according to AutoDock, have a different binding orientation. We predict that the binding mode for higher antimalarial activity would permit that the small substituent on the bridge can be accommodated in a pocket of the cytochrome (**Figure 1B**). The substituent on the bridge should not be larger than the ethyl group for proper interaction with the target. In terms of future optimization, other positions should be explored to enhance binding, such as substitutions on either cyclopentadiene ring or the introduction of a larger linker between the ferrocene and the quinone fragments.

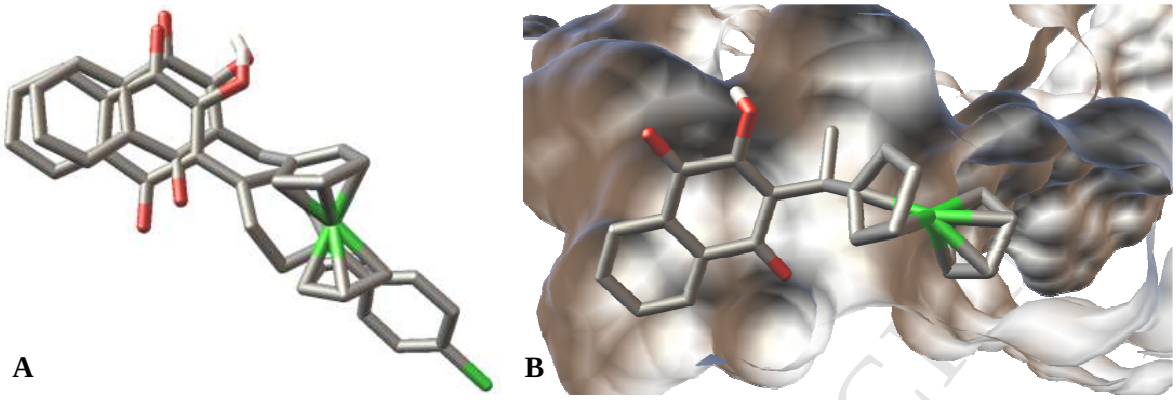


Figure 1. A) Superimposition of the main conformers of **10a** and atovaquone B) Docking simulation of **10b** in cytochrome b of the mitochondrial *bc*$_1$ complex.

The binding of these compounds to the target site is predicted to have similar interactions as atovaquone. It can be distinguished by a hydrogen-bond with His181 (1.998 Å, -4.149 kcal/mol) on the subunit that contains the iron-sulfur cluster of the cytochrome bc1 complex (**Figure 2**). This interaction could be especially important, as histidine could be protonated and generate an electrostatic interaction with the deprotonated 2-hydroxynaphthoquinone.

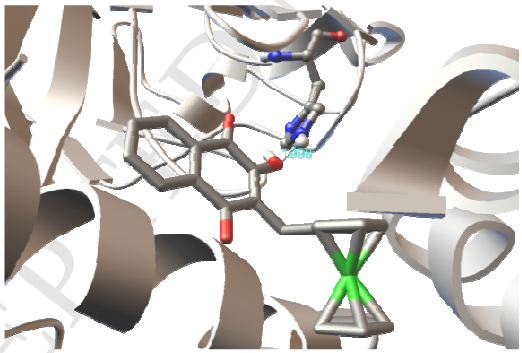


Figure 2. Hydrogen bond between the 2-OH group of **10a** and His181 in the iron sulfur cluster of the cytochrome *bc*$_1$ complex.

Besides being toxic to the parasite, antiplasmodial agents should exert this toxicity selectively, without significantly damaging human cells at therapeutic doses. The cytotoxicity of the ferrocenequinones was tested in mammal cells (murine macrophages JJ74) and CC_{50} values between 45 and 416 μM were observed (**Table 4**). These values are higher than those found for the parasite strains and represent selectivity indexes (SI) between 2 and 260 (**Table 4**). It is remarkable that the most potent antiplasmodial compounds **10a**, **10b** and **10c** also show the highest selectivity indexes.

Table 4. Cytotoxicity of ferrocenenaphthoquinones for JJ74 mouse macrophages.

Compound	CC_{50}(μM)	Selectivity Index*
10a	**167**	**120**
10b	**194**	**150**
10c	**416**	**260**
10d	299	23
10e	318	27
10f	122	31
10g	49.6	6
10h	45.2	6
10i	56.6	5
10j	62.6	2
10k	67.0	9

*Values calculated by the equation SI = CC_{50} (JJ74)/ IC_{50} (*P. falciparum* 3D7).

3. Conclusions

An "on-water" methodology was adapted for the synthesis of eleven new derivatives of 3-(ferrocenylmethyl)-2-hydroxynaphthoquinone. The structures of the compounds were established by spectroscopic techniques. The compounds showed IC_{50} values under 20 μM to *Plasmodium falciparum* CQ-sensitive and CQ-resistant strains, without appreciable inhibition of ferriprotoporphyrin IX biocrystallization. Most substances showed selective toxicity for parasites with respect to mammal cells with SI values higher than 120. Compounds **10a**, **10b**, **10c** showed the best antimalarial activity with IC_{50} values under 2 μM, as well as adequate ΔG values of *in silico* binding to the cytochrome b unit of the mitochondrial bc_1 complex; these compounds could be explored as a scaffold to new and more potent analogues. Further work is needed to determine the *in vivo* efficacy of these compounds and to confirm their mechanism of action.

4. Experimental

4.1.General

All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. Melting points were determined using a Mel-Temp Thomas Scientific, Model IA9100 with a precision of ±1 °C. IR spectra were recorded on Perkin Elmer Spectrum Paragon 1000 using KBr pellets and values were represented in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR were carried out on Varian-Mercury 400 BB (400 MHz and 100 MHz for ^{1}H and ^{13}C, respectively) and a Brucker Ascend 600 (600 and 150 MHz for ^{1}H and ^{13}C, respectively), using TMS as an internal standard and chemical shifts were recorded in ppm on a δ scale. Exact mass of the compounds was determined in a Waters Synapt high resolution mass spectrometer in positive mode and introducing the sample by electrospray. Thin layer chromatography (TLC) on silica gel plates containing UV indicator HF_{254} was employed routinely to follow the course of reactions. When necessary, products were purified by column chromatography using silica gel 230-400 mesh. High-performance liquid chromatography was performed on a Dionex instrument: column, ProteCol GP C18 125 (250 x 4.6 mm, 5 μm). Compounds were detected by UV at 254 nm. The mobile phase was (water /0.1% acetic acid)/methanol (20:80 v/v) with flow rate of 1.2-2.0 mL/min. The purity of all tested compounds was greater than 95% based on analytical HPLC.

4.2. Synthesis of acyl chlorides

To 5 mL of previously distilled thionyl chloride were added 3 mmol of the carboxylic acid, and immediately 3 drops of dimethylformamide (DMF). The reaction mixture was heated at 70 °C for approximately 90 minutes, forming a yellowish translucent solution. Excess of thionyl chloride was eliminated from the mixture with a rotary evaporator connected to a potassium hydroxide trap. Products obtained were pale yellow oils or solids. Acyl chlorides were employed directly for following reactions without further purification.

4.3. Synthesis of ferrocenylketones **8**

A solution 3-5 mmol of ferrocene in dichloromethane (DCM) or dichloroethane (DCE) (3-5 mL) was prepared. A previously prepared solution of 1 equivalent of acyl chloride and 1 equivalent of aluminum chloride in DCM or DCE (3-5 mL) was transferred to the ferrocene solution, while stirring and with positive nitrogen pressure. The mix of the two liquids generated an intense purple or blue mixture. Reaction was stopped after 30-60 minutes of stirring at room temperature. An equal quantity of water was added to the reaction mixture. The organic phase was separated and the aqueous phase was extracted with methyl *t*-butyl ether (MTBE), and the combined organic phase was dried over anhydrous sodium sulfate. The solvent was removed and the crude product (red to orange solid) was purified by column chromatography column on silica gel (230-400 mesh) with a gradient of cyclohexane-benzene as eluent.

4.4. Synthesis of ferrocenylcarbinol derivatives **9**

To a clean and dry round bottom flask with a septum, 3-5 mmol of the ferrocenylketone were added and approximately 20 mL of dimethoxyethane (DME) were transferred with a positive nitrogen pressure; the mixture was stirred to obtain a reddish solution. 0.75

equivalents of lithium aluminum hydride (1 M in tetrahydrofuran) were added, and a change of color from red to yellow in the solution was observed. The reaction was stopped after 30 minutes at room temperature; complete transformation was confirmed with TLC. After this time, Glauber's salt was added and the mixture was stirred until a formation of a granular precipitate was observed. The mixture was filtered, and the solvent was eliminated to obtain yellow to orange oils or solids. The compounds were employed directly for following reactions without further purification.

4.5. Synthesis of 3-(ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinones derivatives **10**

To a clean and dry round bottom flask containing 3-5 mmol of the ferrocenylcarbinol, an equivalent quantity of 2-hydroxy-1,4-naphthoquinone and 20 mL of water were added. The mixture was stirred and heated at 40 °C or 70 °C; the formation of a green solid in the walls of the flask was observed. Reaction was stopped when no change was detected by TLC, reaction time was variable depending on the product. The mixture was extracted with MTBE and the combined organic phase was dried over anhydrous sodium sulfate. The solvent was removed and the crude product (red to orange solid) was purified by column chromatography column on silica gel (230-400 mesh) with a gradient of cyclohexane-benzene as eluent, obtaining green solids.

4.5.1. 3-(ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10a**

10a was obtained from ferrocenemethanol **9a** according to general procedure 4.5. Yield 65%, pale green powder. m.p. = 161-162 °C. IR (KBr) ν/cm^{-1}: 3359 (s, sh), 3084 (w, b), 2928 (w, sh), 1656, 1592 (s, sh), 1457 (w, sh), 1426 (w, sh), 1368 (s, sh), 1344 (s, sh), 1273 (m, sh), 1248 (m, sh).^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.10 (1H, d, *J*=7.6 Hz), 8.03 (1H, d, *J*=7.5 Hz), 7.73 (1H, t, *J*=7.5 Hz), 7.65 (1H, t, *J*=7.5 Hz), 7.25 (1H, s, -OH), 4.25 (2H, s), 4.16 (5H, s), 4.02 (2H; s), 3.65 (2H, s). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.2, 182.2, 153.3, 134.9, 132.8, 132.8, 129.5, 126.8, 126.0, 123.0, 85.9, 69.1, 68.8, 67.4, 23.1. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{21}H_{16}FeO_3$ 373.0527, $[M+H]^+$ Found: 373.0522.

4.5.2. 3-(1-ferrocenylethyl)-2-hydroxy-1,4-naphthoquinone **10b**

10b was obtained from 1-ferroceneethanol **9b** according to general procedure 4.5. Yield 79%, intense green powder. m.p. = 142-143 °C. IR (KBr) ν/cm^{-1}: 3350 (s, sh), 3091 (w, sh), 2930 (w, b), 1667 (s, sh), 1646 (s, sh), 1635 (s, sh), 1561 (s, sh), 1413 (m, sh), 1361 (s, sh), 1344 (s, sh), 1266 (s, sh). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.10 (1H, dd, *J*=7.7 Hz, *J*=0.9 Hz), 8.03 (1H, dd, *J*=7.6 Hz, *J*=0.9 Hz), 7.74 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.3 Hz), 7.65 (1H, td, *J*=7.5 Hz, *J*=7.5 Hz, *J*=1.3 Hz), 7.37 (1H, s, -OH), 4.42 (2H, m), 4.16 (5H, s broad), 4.08 (2H, m), 4.05 (1H, s broad), 1.67 (3H, d, *J*=7.3 Hz).^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.1, 181.9, 152.3, 135.0, 132.9, 132.8, 129.2, 127.2, 127.0, 125.9, 91.7, 68.7, 68.1, 67.2, 66.7, 29.2, 17.2. EI-HRMS (*m/z*) $[M+H]^+$Calcd for $C_{22}H_{18}FeO_3$ 387.0684, $[M+H]^+$ Found: 387.0674.

4.5.3. 3-(1-ferrocenylpropyl)-2-hydroxy-1,4-naphthoquinone **10c**

10c was obtained from 1-ferrocenepropanol **9c** according to general procedure 4.5. Yield 75%, dark green powder. m.p. = 108-110 °C. IR (KBr) ν/cm^{-1}: 3353 (s, sh), 3082 (w, b),

2967 (w, sh), 2919 (w, sh), 1652 (s, sh), 1590 (m, sh), 1458 (w, sh), 1372 (s, sh), 1269 (s, sh), 1249 (m, sh).^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.11 (1H, d, *J*=6.6 Hz), 8.05 (1H, dd, *J*=7.6 Hz, *J*=0.9 Hz), 7.75 (1H, t, *J*=7.3 Hz), 7.67 (1H, td, *J*=7.5 Hz, *J*=7.5 Hz, *J*=1.6 Hz), 7.40 (1H, s, -OH), 4.36 (1H, s broad), 4.20 (1H, d broad), 4.12 (5H, s broad), 4.08 (2H, m), 4.03 (1H, m), 2.3 (1H, m), 2.13 (1H, m), 0.93 (3H, t, *J*=7.5 Hz). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.4, 181.8, 152.6, 135.0, 132.9, 132.8, 129.2, 127.2, 126.0, 125.7, 91.3, 68.7, 67.9, 67.8, 67.1, 66.7, 29.7, 24.9, 12.9. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{23}H_{20}FeO_3$ 401.0840, $[M+H]^+$ Found: 401.0819.

4.5.4. 3-(1-ferrocenylbutyl)-2-hydroxy-1,4-naphthoquinone **10d**

10d was obtained from 1-ferrocenebutanol **9d** according to general procedure 4.5. Yield 83%, dark green powder. m.p. = 136-139 °C. IR (KBr) ν/cm^{-1}: 3351 (s, sh), 3083 (w, b), 2955 (w, sh), 2932 (w, sh), 1657 (s, sh), 1648 (m, sh), 1592 (m, sh), 1460 (w, sh), 1377 (m, sh), 1269 (s, sh). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.11 (1H, s broad), 8.05 (1H, dd, *J*=7.6 Hz, *J*=0.8 Hz), 7.74 (1H, td, *J*=7.4 Hz, *J*=7.4 Hz, *J*=1.2 Hz), 7.66 (1H, td, *J*=7.5 Hz, *J*=7.5 Hz, *J*=1.2 Hz), 7.41 (1H, s, -OH), 4.36 (1H, s broad), 4.30 (1H, s broad), 4.10 (5H, s broad), 4.08 (2H, m), 4.03 (1H, s broad), 2.34 (1H, m broad), 2.07 (1H, m broad), 1.31 (2H, sextet, *J*=7.3 Hz), 0,96 (3H, t, *J*=7.3 Hz).^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.3, 181.8, 152.6, 135.0, 132.9, 132.8, 129.2, 127.1, 126.0, 91.4, 68.7, 68.0, 67.8, 67.1, 66.7, 34.0, 21.4, 21.4, 14.2.EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{24}H_{22}FeO_3$415.0997, $[M+H]^+$ Found: 415.0989.

4.5.5. 3-(4-chloro-1-ferrocenylbutyl)-2-hydroxy-1,4-naphthoquinone **10e**

10e was obtained from 4-chloro-1-ferrocenebutanol **9e** according to general procedure 4.5. Yield 81%, pale green powder. m.p. = 169-171 °C. IR (KBr) ν/cm^{-1}: 3352 (s, sh), 3067 (w, b), 2963 (w, b), 1655 (s, sh), 1634 (m, sh), 1589 (m, sh), 1458 (w, sh), 1376 (m, sh), 1265 (s, sh). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.10 (1H, s broad), 8.06 (1H, dd, *J*=7.6 Hz, *J*=1.0 Hz), 7.76 (1H, td, *J*=7.3 Hz, *J*=7.3 Hz, *J*=1.1 Hz), 7.68 (1H, td, *J*=7.5 Hz, *J*=7.5 Hz, *J*=1.1 Hz), 7.45 (1H, s, -OH), 4.36 (1H, s broad), 4.29 (1H, s broad), 4.14 (5H, s), 4.09 (2H, m), 4.05 (1H, m), 3,59 (2H, m), 2,46 (1H, m), 2,32 (1H, m), 1.78 (2H, m). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.3, 181.6, 152.7, 135.1, 133.0, 132.8, 129.2, 127.1, 126.1, 125.1, 90.8, 68.7, 68.0, 67.7, 67.2, 66.8, 44.8, 31.1, 28.8, 1.1. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{24}H_{21}ClFeO_3$ 449.0607, $[M+H]^+$ Found: 449.0589.

4.5.7. 3-(1-ferrocenyldodecyl)-2-hydroxy-1,4-naphthoquinone **10f**

10f was obtained from 1-ferrocenedodecanol **9f** according to general procedure 4.5. Yield 28%, green oil. IR (KBr) ν/cm^{-1}: 3354 (s, sh), 3092 (w, b), 2919 (s, sh), 1654 (s, sh), 1593 (m, sh), 1458 (w, sh), 1377 (s, sh), 1348 (m, sh), 1267 (s, sh).^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.11 (1H, s broad), 8.05 (1H, dd, *J*=7.6 Hz, *J*=1.0 Hz), 7.74 (1H, td, *J*=7.4 Hz, *J*=7.4 Hz, *J*=1.0 Hz), 7.66 (1H, td, *J*=7.5 Hz, *J*=7.5 Hz, *J*=1.0 Hz), 7.41 (1H, s, -OH), 4.36 (1H, s broad), 4.26 (1H, s broad), 4.11 (5H, s), 4.08 (2H, m), 4.03 (1H, m), 2.32 (1H, m), 2.05 (1H, m), 1,15-1.40 (18H, m), 0.87 (3H, t, *J*=7.1 Hz). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.3, 182.9, 152.6, 135.0, 132.9, 132.8, 129,25, 127.1, 126.1, 126,0, 91.5, 68.7,

68.0, 67.8, 67.1, 66.7, 31.9, 28.4, 29.3, 29.6, 29.7, 22.7, 14.1. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{32}H_{38}FeO_3$ 527.2249, $[M+H]^+$ Found: 527.2247.

4.5.6. 3-(1-cyclohexyl-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10g**

10g was obtained from 1-cyclohexyl-1-ferrocenemethanol **9g** according to general procedure 4.5. Yield 11%, green crystals. m.p. = 97-98 °C (dec). IR (KBr) ν/cm^{-1}: 3404 (s, b), 2925 (s, sh), 1654 (s, sh), 1592 (m, sh), 1364 (m, sh), 1267 (m, sh), 1222 (s, sh).^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm), mixture of major and minor rotameric compounds (minor rotamer indicated as "rotamer")**:** 8.24 (1H, dd, *J*=7.7 Hz, *J*=1.3 Hz; rotamer 8.18, 1H, d, *J*=7.7 Hz), 8.14 (1H, dd, *J*=7.6 Hz, *J*=1.3 Hz; rotamer 8.11, 1H, d, *J*=7.6 Hz), 7.83-7.77 (1H, m), 7.74-7.69 (1H, m), 7.59 (1H, s, -OH; rotamer 7.52, 1H, s), 4.33 (1H, s; rotamer 4.28, 1H, s), 4.15 (1H, s; rotamer 4.11, 1H, s), 4.07 (2H, m broad), 3.93 (1H, d, *J*=10.8 Hz; rotamer 3.60, 1H, d, *J*=10.6 Hz), 3.85 (5H, s; rotamer 3.86, 5H, s), 2.09 (1H, m; rotamer 2.22, 1H, m), 1.56-1.61 (4H, m), 1.19-1.05 (4H, m), 0.76-0.94 (2H, m). ^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.9, 182.2, 152.1, 135.2, 133.1, 132.7, 129.2, 127.4, 126.4, 126.1, 89.5, 71.2, 68.4, 67.7, 67.1,66.0, 41.0, 39.8, 32.5, 32.2, 26.9, 26.4, 26.1. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{27}H_{26}FeO_3$ 455.1310, $[M+H]^+$ Found: 455.1300.

4.5.8. 3-(1-ferrocenyl-2-phenylethyl)-2-hydroxy-1,4-naphthoquinone **10h**

10h was obtained from 2-phenyl-1-ferroceneethanol **9h** according to general procedure 4.5. Yield 53%, dark green powder. m.p. = 68-70 °C (dec). IR (KBr) ν/cm^{-1}: 3363 (m, b), 3084 (w, sh), 2927 (w, b), 1659 (s, sh), 1648 (s, sh), 1592 (m, sh), 1494 (w, sh), 1458 (m, sh), 1367 (s, sh), 1337 (m, sh), 1283 (s, sh), 1263 (m, sh).^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.03 (1H, d, *J*=7.2 Hz), 7.98 (1H, d, *J*=7.5 Hz), 7.70 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.2 Hz), 7.61 (1H, td, *J*=7.5 Hz, *J*=7.5 Hz, *J*=1.1 Hz), 7.19 (4H, m), 7.10 (1H, t, J=7.1 Hz), 4.77 (1H, s broad), 4.40 (1H, s broad), 4.15 (5H, s broad), 4.11 (2H, m broad), 4.07 (1H, m broad), 3.61 (1H, s broad), 3.49 (1H, s broad).^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 183.9, 181.7, 152.4, 140.3, 134.9, 132.7, 132.5, 129.0, 128.6, 128.2, 127.0, 126.0, 125.9, 125.1, 90.6, 68.7, 68.1, 67.9, 67.3, 37.4, 35.5. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{28}H_{22}FeO_3$ 463.0997, $[M+H]^+$ Found: 463.1001.

4.5.9. 3-(1-ferrocenyl-1-phenylmethyl)-2-hydroxy-1,4-naphthoquinone **10i**

10i was obtained from 1-ferrocenyl-1-phenylmethanol **9i** according to general procedure 4.5. Yield 68%, bright green crystals. m.p. = 65-66 °C (dec). IR (KBr) ν/cm^{-1}: 3364 (m, b), 3083 (w, b), 2928 (w, b), 1662 (s, sh), 1594 (m, sh), 1493 (w, sh), 1459 (w, sh), 1371 (s, sh), 1337 (m, sh), 1266 (s, sh). ^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.18 (1H, dd, *J*=7.7 Hz, *J*=0.7 Hz), 8.09 (1H, dd, *J*=7.6 Hz, *J*=0.8 Hz), 7.78 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.3 Hz), 7.70 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.2 Hz), 7.47 (1H, s, -OH), 7.36 (impurity), 7.34 (2H, d, *J*=7.3 Hz), 7.24 (2H, d, *J*=7.8 Hz), 7.18 (1H, t, *J*=7.3 Hz), 5.79 (1H, s), 4.23 (1H, m), 4.19 (1H, m), 4.15 (2H, t, *J*=1.8 Hz), 4.03 (5H, s).^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 184.4, 181.8, 152.6, 135.2, 135.0, 132.9, 132.8, 129.2, 128.8, 127.9, 127.0, 126.3, 126.0, 125.7, 91.3, 68.6, 67.9, 67.1, 66.7, 29.7.EI-HRMS (*m/z*) $[M+H]^+$Calcd for $C_{27}H_{20}FeO_3$ 449.0840, $[M+H]^+$ Found: 449.0827.

4.5.10. 3-(1-(4-chlorophenyl)-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10j**

10j was obtained from 1-(4-chlorophenyl)-1-ferrocenemethanol **9j** according to general procedure 4.5. Yield 79%, bright green crystals. m.p. = 83-84 °C (dec). IR (KBr) ν/cm^{-1}: 3367 (m, b), 3088 (w, b), 1654 (s, sh), 1647 (s, sh), 1593 (m, sh), 1489 (m, sh), 1459 (w, sh), 1370 (s, sh), 1338 (s, sh), 1265 (s, sh).^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.18 (1H, dd, *J*=7.7 Hz, *J*=0.9 Hz), 8.10 (1H, dd, *J*=7.6 Hz, *J*=1.0 Hz), 7.80 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.3 Hz), 7.71 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.3 Hz), 7.49 (1H, s, -OH), 7.25 and 7.20 (4H, AA'BB', *J*=8.3 Hz), 5.74 (1H, s) $H_{1'}$, 4.21 (1H, m), 4.16 (3H, m), 4.03 (5H; m).^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 183.9, 181.9, 152.8, 141.2, 135.4, 133.2, 132.7, 132.1, 130.1, 129.2, 128.0, 127.3, 126.3, 125.3, 87.4, 69.8, 68.8, 68.6, 67.7, 67.6, 40.2. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{27}H_{19}ClFeO_3$ 483.0450, $[M+H]^+$ Found: 483.0431.

4.5.11. 3-(1-(4-bromophenyl)-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10k**

10k was obtained from 1-(4-bromophenyl)-1-ferrocenemethanol **9k** according to general procedure 4.5. Yield 73%, bright green crystals. m.p. = 77-78 °C (dec). IR (KBr) ν/cm^{-1}: 3368 (m, b), 2923 (w, b), 1654 (s, sh), 1594 (w, sh), 1484 (w, sh), 1375 (s, sh), 1339 (s, sh), 1266 (s, sh).^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 8.19 (1H, dd broad, *J*=7.7 Hz, *J*=0.9 Hz), 8.10 (1H, dd, *J*=7.6 Hz, *J*=0.9 Hz), 7.80 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.3 Hz), 7.7 (1H, td, *J*=7.6 Hz, *J*=7.6 Hz, *J*=1.2 Hz), 7.49 (1H, s, -OH), 7.36 and 7.20 (4H, AA'BB', *J*=8.4 Hz), 5.73 (1H, s), 4.20 (1H, m broad), 4.16 (3H, m broad), 4.03 (5H; s).^{13}C-NMR ($CDCl_3$, 150 MHz) δ (ppm): 183.9, 181.9, 152.7, 141.7, 135.4, 133.2, 132.7, 130.9, 130.6, 129.1, 127.3, 126.2, 125.2, 120.2, 87.3, 69.8, 68.8, 68.6, 67.7, 67.5, 40.2. EI-HRMS (*m/z*) $[M+H]^+$ Calcd for $C_{27}H_{19}BrFeO_3$ 526.9945, $[M+H]^+$ Found: 526.9928.

4.6. Biological evaluation

4.6.1. In vitro antimalarial activity screening against *Plasmodium falciparum* [61]

The SYBR® GreenI-based micromethod was followed for testing the antimalarial activity of the compounds. Erythrocytic stages of *P. falciparum* 3D7 strain, CQ-sensitive, and Dd2 strain, CQ-resistant, were maintained in RPMI 1640 culture medium supplemented with 0.5% Albumax II at 37 ºC in an atmosphere with 5% CO_2. An erythrocyte suspension, with initial 1% parasitemia and 4% hematocrit, was prepared using the aforementioned culture and then distributed into a 96-well plate, 50 µL per well. Next, stock solutions of each compound were prepared in DMSO and diluted in RPMI medium. The final DMSO concentration was never higher than 0.1%. 50 µL of each prepared concentration were added per well. DMSO and chloroquine were included as negative and positive controls, respectively. All compounds and controls were placed in triplicate. The plate was incubated under the same conditions and after 48 h, the plate was removed from the incubator and frozen for at least 1 h at -70 °C and then thawed. Finally, 100 µL of SYBR®GreenI in lysis buffer (0.2 µL/mL) was added per well and shaken for 5 minutes or until no precipitated erythrocytes were observed. The plate was left to stand in the dark for 1 h at room temperature. Fluorescence intensity (FI) for each well was measured (exciting wavelength

485 nm, detecting wavelength 535 nm). The background of the nonparasitized erythrocytes was subtracted from each well tested. Percentage inhibition of the parasite growth for each concentration was calculated by using the following formula: % inhibition = 100 × [(F.I.control – F.I.comp)/(F.I.control)]. IC_{50}values were estimated by plotting drug concentration versus percentage inhibition.

4.6.2. Nonspecific cytotoxicity tests [62]

Murine J774 macrophages were maintained in RPMI 1640 medium supplemented with 10% FBS at 37 °C in a 5% CO_2 atmosphere. First, in a flat bottom 96-well microplate, 100 µL of macrophage suspension in RPMI medium, containing 7×10^4 cells, were distributed per well. The cells were allowed to attach for 24 h at 37 °C. Next, the medium was replaced by different concentrations of the compounds in 200 µL of medium, or DMSO at the same concentration as growth control, and the cells were exposed to the compounds solutions for another 24 h. Each concentration was assayed three times. Afterwards, the medium was eliminated and 100 µL/well of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution, 0.4 mg/mL in PBS, was added and the plates were returned to incubator for 1 h. The suspension was removed and the toxic effect of the compounds was assessed by the reduction of MTT to formazan crystals (as a cell viability indicator); said crystals were solubilized by adding 100 µL of DMSO. Finally, the absorbance (A) was measured at 595 nm and the viability percentage was calculated as follows: % viability = [(Acontrol - Acomp)/(Acontrol)] × 100. IC_{50} values were estimated by plotting drug concentration versus percentage of viability.

4.6.3.Ferriprotoporphyrin IX biomineralization inhibition test (FBIT) [65]

The procedure for testing FP biomineralization was carried out according to the method described by Deharo [62]. A mixture containing 50 µL of a 10 mg/mL drug solution or solvent (for control) or chloroquine, 50 µL of 0.5 mg/mL of hemin chloride freshly dissolved in dimethylsulfoxide (DMSO) and 100 µL of 0.5 M sodium acetate buffer pH 4.4 was incubated in anon-sterile flat bottom 96-well plate at 37 °C for 24 hrs. After incubation, the plate was centrifuged at 1,600 G for 5 min and the supernatant was discarded. The remaining pellet was re-suspended with 200 µL of DMSO in order to remove unreacted FP. The plate was then centrifuged once again and the supernatant discarded. The pellet (precipitate of ß-hematin) was dissolved in 150 µL of 0.1 M NaOH and the absorbance (A) read at 405 nm. The data was expressed as the percentage of inhibition of FP biomineralization, calculated using the following equation: % inhibition = 100 × [(Acontrol -Acomp)/(Acontrol)]. IC_{50} values were estimated by plotting drug concentration versus percentage inhibition.

4.6.4. Molecular docking simulation in the cytochrome b of the cytochrome b-c1 complex

The ligands were docked using a regular rigid receptor-flexible ligand docking approach. Molecular docking simulations was performed in the software AutoDockv4.2.3, using PyRx v0.8 as virtual screening tool and AutoDock Tools v1.5.4 for visualization. The crystallized structure of *Saccharomyces cerevisiae* cytochrome b-c1 complex bound to its

inhibitor Stigmatellin A (PDB: 1KYO) was used for the study. Preparation of the protein (elimination of hydration waters, merge non-polar, add polar hydrogens and charges) was performed in Discovery Studio Visualizer v3.1.1.11517. Docking was performed in a grid (18.75 Å x 18.75 Å x 22.5 Å) that enclosed Stigmatellin A binding site generated with Autogrid v4.2.3. After simulation, predicted conformations were analyzed by visual inspections and by their binding free energy.

Acknowledgements

We are thankful to Dr. Grettel Valle (School of Chemistry, Universidad de Costa Rica) and Dr. Thomas Weyhermueller (Max-Planck-Institute für Bioanorganische Chemie) for collaboration on X-Ray Crystallography; to the Vice Presidency of Research, Universidad de Costa Rica and to the National Rectors Council, CONARE, (projects No. 809-A8-001 and FEES-UCR No. 809-A8-518) and to Spanish FIS-ISCIII Program (project No. PI060782) for the financial support. We also wish to thank the referees for their helpful suggestions.

References

[1] H.G. Viehe, R. Merenyi, Z. Janousek, Captodative substituent effects in radical chemistry, Pure App. Chem. 60 (1988) 1635-1644.

[2] M. Uchimiya, A.T. Stone, Reversible redox chemistry of quinones: impact on biogeochemical cycles, Chemosphere 77 (2009) 451-458.

[3] S. Spyroudis, Hydroxyquinones: synthesis and reactivity, Molecules 5 (2000) 1291-1330.

[4] C. Asche, Antitumour quinones, Mini-Reviews Med. Chem. 5 (2005) 449-467.

[5] H. Hussain, K. Krohn, V.U. Ahmad, G.A. Miana, I. R. Greend, Lapachol: an overview, ARKIVOC ii (2007) 145-171.

[6] G. Lenaz, R. Fato, A. Baracca, M.L. Genova, Mitochondrial quinone reductases: complex I, Methods Enzymol. 382 (2004) 3-20.

[7] M.N. da Silva, V.F. Ferreira, and M.C. de Souza, Um panorama atual da química e da farmacologia de naftoquinonas, com ênfase na β-lapachona e derivados, Quim. Nova 26 (2003) 407-416.

[8] E. Pérez-Sacau, R.G. Díaz-Peñate, A. Estévez-Braun, A.G. Ravelo, J.M. García-Castellano, L. Pardo, M. Campillo, Synthesis and pharmacophore modeling of naphthoquinone derivatives with cytotoxic activity in human promyelocytic leukemia HL-60 cell line, J. Med. Chem. 50 (2007) 696-706.

[9] K. Sasaki, H. Abe, F. Yoshizaki, *In vitro* antifungal activity of naphthoquinone derivatives, Biol. Pharm. Bull 25 (2002) 669-670.

[10] K.H. Xu, D.P. Lu, Plumbagin induces ROS-mediated apoptosis in human promyelocytic leukemia cells in vivo, Leuk. Res. 34 (2010) 658-665.

[11] S.C. Cho, M.Z. Sultan, S.S. Moon, Anti-acne activities of pulsaquinone, hydropulsaquinone, and structurally related 1,4-quinone derivatives, Arch. Pharm. Res. 32 (2009) 489-494.

[12] Y.R. Jin, K.A. Hwang, M.R. Cho, S.Y. Kim, J.H. Kim, C.K. Ryu, D.J. Son, Y.H. Park, Y.P. Yun, Antiplatelet and antithrombotic activities of CP201, a newly synthesized 1,4-naphthoquinone derivative, Vascul. Pharmacol. 41 (2004) 35-41.

[13] G.J. Kapadia, M.A. Azuine, V. Balasubramanian, R. Sridhar, Aminonaphthoquinones - a novel class of compounds with potent antimalarial activity against *Plasmodium falciparum*, Pharmacol. Res. 43 (2001) 363-367.

[14] A.J. da Silva, C.D. Buarque, F.V. Brito, L. Aurelian, L.F. Macedo, L.H. Malkas, R.J. Hickey, D.V. Lopes, F. Noel, Y.L. Murakami, N.M Silva, P.A. Melo, R.R. Carusso, N.G. Castro, P.R. Costa, Synthesis and preliminary pharmacological evaluation of new (+/-) 1,4-naphthoquinones structurally related to lapachol, Bioorg. Med. Chem. 10 (2002) 2731-2738.

[15] W. Knecht, J. Henseling, M. Löffler, Kinetics of inhibition of human and rat dihydroorotate dehydrogenase by atovaquone, lawsone derivatives, brequinar sodium and polyporic acid, Chem. Biol. Interact. 124 (2000) 61-76.

[16] L. Pelinski, A. Baramee, A. Coppin, S. Tomavo, J. Brocard, Synthesis and in vitro activities of ferrocenic aminohydroxynaphthoquinones against *Toxoplasma gondii* and *Plasmodium falciparum*, Bioorg. Med. Chem. 14 (2006) 1294-1302.

[17] A.V. Pinto, S. Castro, The trypanocidal activity of naphthoquinones: a review, Molecules (2009) 4570-4590.

[18] J.L. Bolton, M.A. Trush, T.M. Penning, G. Dryhurst, T.J. Monks, Role of quinones in toxicology, Chem. Res. Tox. 13 (2000) 135-160.

[19] D. Marzin, D. Kirkland, 2-Hydroxy-1,4-naphthoquinone, the natural dye of Henna, is non-genotoxic in the mouse bone marrow micronucleus test and does not produce oxidative DNA damage in chinese hamster ovary cells, Mutation. Res. 560 (2004) 41–47.

[20] G. Kokwaro, Ongoing challenges in the management of malaria, Malaria J. 8 (2009) S2-S7.

[21] World Health Organization, World malaria report, (2012).

[22] I.K. Srivastava, J.M. Morrisey, E. Darrouzet, F. Daldal, A.B. Vaidya, Resistance mutations reveal the atovaquone-binding domain of cytochrome b in malaria parasites, Mol. Microbiol. 33 (1999) 704-712.

[23] J. J. Kessl, B.B. Lange, T. Merbitz-Zahradnik, K. Zwicker, P. Hill, B. Meunier, H. Palsdottir, C. Hunte, S. Meshnick, B.L. Trumpower, Molecular basis for atovaquone binding to the Cytochrome bc1 Complex, J. Biol. Chem. 278 (2003) 31312-31318.

[24] G.B. Kauffman, The discovery of ferrocene: the first sandwich compound, J. Chem. Ed. 60 (1983) 185-186.

[25] M. Laskoski, W. Steffen, M.D. Smith, U.H. Bunz, Is ferrocene more aromatic than benzene?, Chem. Commun. 2001 (2001) 691-692.

[26] X. Wu, M. Go, Antimalarial activity of ferrocenyl chalcones, Bioorg. Med. Chem. Lett. 12 (2002) 2299-2302.

[27] N. Chavain, H. Vezin, D. Dive, N. Touati, J.F. Paul, E. Buisine, C. Biot, Investigation of the redox behavior of ferroquine, a new antimalarial, Mol. Pharm. 5 (2008) 710-716.

[28] J. Manosroi, K. Rueanto, K. Boonpisuttinant, W. Manosroi, C. Biot, Novel ferrocenic steroidal drug derivatives and their bioactivities, J. Med. Chem. 53 (2010) 3937-3943.

[29] C.L. Ferreira, C.B. Ewart, C.A. Barta, S. Little, V. Yardley, C. Martins, E. Polishchuk, P.J. Smith, J.R. Moss, M. Merkel, M.J. Adam, C. Orvig Synthesis, structure, and biological activity of ferrocenyl carbohydrate conjugates, Inorg. Chem. 45 (2006) 659-667.

[30] B. Long, S. Liang, D. Xin, Y. Yang, J. Xiang, Synthesis, characterization and in vitro antiproliferative activities of new 13-cis-retinoyl ferrocene derivatives, Eur. J. Med. Chem. 44 (2009) 2572-2576.

[31] M.F.R. Fouda, M.M. Abd-elzaher, R.A. Abdelsamaia, A.A. Labib, On the medicinal chemistry of ferrocene, App. Organometal. Chem. 21 (2007) 613-625.

[32] N. Chavain, E. Davioud-Charvet, X. Trivelli, L. Mbeki, M. Rottmann, R. Brun, C. Biot, Antimalarial activities of ferroquine conjugates with either glutathione reductase inhibitors or glutathione depletors via a hydrolyzable amide linker, Bioorg. Med. Chem. 17 (2009) 8048-8059.

[33] M. Soares, J. Oliveira, M. Santos, R. Ribeiro, P. Pigeon, G. Jaouen, A.E. Santana, M. Goulart, E. Hillard, Antiparasitic and Immunomodulatory Activities of 1,1-bis(4-hydroxyphenyl)-2-phenyl-but-1-ene and its protected and free 2-ferrocenyl derivatives, Drug Develop. Res. 71 (2010) 69–75.

[34] B. Maryanoff, S. Keeley, F. Persico, Replacement of aromatic or heteroaromatic groups in nonsteroidal antiinflammatory agents with the ferrocene group, J. Med. Chem. 26 (1983) 226-229.

[35] A. Mahajan, L. Kremer, S. Louw, Y. Guéradel, K. Chibale, C. Biot, Synthesis and in vitro antitubercular activity of ferrocene-based hydrazones, Bioorg. Med. Chem. Lett. 21 (2011) 2866-2868.

[36] D. Huber, H. Hubner, P. Gmeiner, 1,1'-Disubstituted ferrocenes as molecular hinges in mono- and bivalent dopamine receptor ligands, J. Med. Chem. 52 (2009) 6860-6870.

[37] K. Schlotter, F. Boeckler, H. Hubner, P. Gmeiner, Fancy Bioisosteres□: Metallocene-derived G-protein-coupled receptor ligands with subnanomolar binding affinity and novel selectivity profiles, J. Med. Chem. 48 (2005) 3696-3699.

[38] K. Sander, T. Kottke, C. Hoffend, M. Walter, L. Weizel, J.C. Camelin, X. Ligneau, E.H. Scheneider, R. Seifert, J.C. Schwartz, H. Stark, First metal-containing histamine H3 receptor ligands, Org. Lett. 12 (2010) 2578-2581.

[39] A. Kreidenweiss, P.G. Kremsner, K. Dietz, B. Mordmüller, In vitro activity of ferroquine (SAR97193) is independent of chloroquine resistance in *Plasmodium falciparum*, Am. J. Trop. Med. Hyg. 75 (2006) 1178-1181.

[40] M.A. Blackie, P. Beagley, S.L. Croft, H. Kendrick, R. Moss, K. Chibale, Metallocene-based antimalarials□: An exploration into the influence of the ferrocenyl moiety on in vitro antimalarial activity in chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*, Bioorg. Med. Chem. 15 (2007) 6510-6516.

[41] C. Biot, D. Taramelli, I. Forfar-Bares, L.A. Maciejewski, M. Boyce, G. Nowogrocki, J.S. Brocard, N. Basilico, P. Olliaro, T.J. Egan, Insights into the mechanism of action of ferroquine. Relationship between physicochemical properties and antiplasmodial activity, Mol. Pharm. 2 (2001) 185-193.

[42] F. Dubar, T.J. Egan, B. Pradines, D. Kuter, K.K. Ncozaki, D. Forge, C. Pierrot, H. Kalamou, J. Khalife, E. Buisine, C. Rogier, I. Forfar, C. Slomianny, O.X. Trivelli, S. Kapishnikov, L. Leiserowitz, D. Dive, C. Biot, The antimalarial ferroquine: role of the metal and intramolecular hydrogen bond in activity and resistance, ACS Chem. Biol. 6 (2011) 275-287.

[43] J. Guillon, S. Moreau, E. Mouray, V. Sinou, I. Forfar, S. Belisle, V. Desplat, P. Millet, D. Parzy, C. Jarry, P. Grellier, New ferrocenic pyrrolo [1,2-a] quinoxaline derivatives□: Synthesis, and in vitro antimalarial activity, Bioorg. Med. Chem. 16 (2008) 9133-9144.

[44] C. Biot, W. Daher, N. Chavain, T. Fandeur, J. Khalife, D. Dive, E. Clercq, Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities, J. Med. Chem. 49 (2006) 2845-2849.

[45] S.B. Colbran, S.T. Lee, D.G. Lonnon, F.J.D Maharai, A.M. Mcdonagh, K.A. Walker, R.D. Young, Covalently linked ferrocenyl quinones□: proton-dependent redox behavior and charge redistribution, Organometallics 25 (2006) 2216-2224.

[46] K. Okamoto, H. Imahori, S. Fukuzumi, Metal ion-promoted intramolecular electron transfer in a ferrocene-naphthoquinone linked dyad. Continuous change in driving force and reorganization energy with metal ion concentration, J. Am. Chem. Soc. 4 (2003) 7014-7021.

[47] L. Vendier, J. Brocard, B. Meunier, A. Robert, Trioxaferroquines as new hybrid antimalarial drugs, J. Med. Chem. 53 (2010) 4103-4109.

[48] N. Srouji, T. L. Newirth, Acetylation of ferrocene, J. Chem. Educ. 72 (1995) 454-456.

[49] R.M. Janet, N. Kora, S. Allen, L. Cannon, J. Coughlan, L. Cusumano, B. Nolan, Friedel-Crafts acylation and alkylation with acid chlorides, J. Chem. Educ. 66 (1989) 1056-1057.

[50] P.H. Groggins, S.B. Detwiler, Friedel-Crafts reactions, Ind. Eng. Chem. 43 (1951) 1970-1974.

[51] P.G. Cozzi, L. Zoli, A rational approach towards the nucleophilic substitutions of alcohols “on water”, Angew. Chem. Int. Ed. 47 (2008) 4162 –4166.

[52] G.W. Gokel, D. Marquarding, I.K. Ugi, Stereoselective synthesis. VIII. Retentive nucleophilic displacements of alpha-substituted alkylferrrocenes, J. Org. Chem. 37 (1972) 3052-3057.

[53] H. Mayr, B. Kempf, A.R. Ofial, π-Nucleophilicity in carbon-carbon bond-forming reactions, Acc. Chem. Res. 36 (2003) 66-77.

[54] C. Schindele, K.N. Houk, H. Mayr, Relationships between carbocation stabilities and electrophilic reactivity parameters, E: quantum mechanical studies of benzhydryl cation structures and stabilities, J. Am. Chem. Soc. 124 (2002) 11208-11214.

[55] G.K. Prakash, Electrophilic intermediates and their reactions in superacids, J. Org. Chem. 71 (2006) 3661-3676.

[56] H. Tse-Lok, The hard soft acid bases (HSAB) principle in organic chemistry, Chemical Reviews 75 (1975) 1-20.

[57] S. Narayan, J. Muldoon, M.G. Finn, V.V. Fokin, H.C. Kolb, K.B. Sharpless, “On water”: unique reactivity of organic compounds in aqueous suspension, Angew. Chem. Int. Ed. 44 (2005) 3275-3279.

[58] Y. Jung, R.A. Marcus, On the theory of organic catalysis “on water”, J. Am. Chem. Soc. 129 (2007) 5492-5502.

[59] T. P. Silverstein, The real reason why oil and water don’t mix, J. Chem. Educ. 75 (1998) 116-118.

[60] R. Breslow, Hydrophobic effects on simple organic reactions in water, Acc. Chem. Res. 24 (1991) 159-164.

[61] M. Rottmann, C. McNamara, B.K. Yeung, M.C. Lee, B. Zou, B. Russell, P. Seitz, D.M. Plouffe, N.V. Dharia, J. Tan, S.B. Cohen, K.R. Spencer, G.E. González-Páez, S.B. Lakshminarayana, A. Goh, R. Suwanarusk, T. Jegla, E.K. Schmitt, H.P. Beck,

R. Burn, F. Nosten, L. Renia, V. Dartois, T.H. Keller, D.A. Fidock, E.A. Winzeler, T.T. Diagana. Spiroindolones, a new and potent chemotype for the treatment of malaria. Science 329 (2010) 1175.

[62] S. Pérez-Silanes, L. Berrade, R.N. Garcia-Sankchez, A. Mendoza, S. Galiano, B.M. Perez-Solorzano, J.J. Nogal-Ruiz, A.R. Martinez-Fernandez, I. Aldana, A. Monge, New 1-aryl-3-substituted propanol derivatives as antimalarial agents, Molecules 14 (2009) 4120-4135.

[63] S.A. Ralph, G.G. van Dooren, R.F. Waller, M.J. Crawford, M.J. Fraunholz, B.J. Foth, C.J. Tonkin, D.S. Roos, G.I. McFadden, Metabolic maps and functions of the Plamodium falciparum apicoplast, Nature Rev. Microbiol. 2 (2004) 203-216.

[64] R.F. Waller, G.I. McFadden, The apicoplast: a review of the derived plastid of apicomplexan parasites, Curr. Issues Mol. Biol. 7 (2005) 57-59.

[65] E. Deharo, R.N. García, P. Oporto, A. Gimenez, M. Sauvain, V. Jullian, H. Ginsburg, A nonradiolabeled ferriprotoporphyrin IX biomineralization inhibition test (FBIT) for the high throughput screening of anti malarial compounds, Exp. Parasitol. 100 (2002) 252–256.

Supplemental information

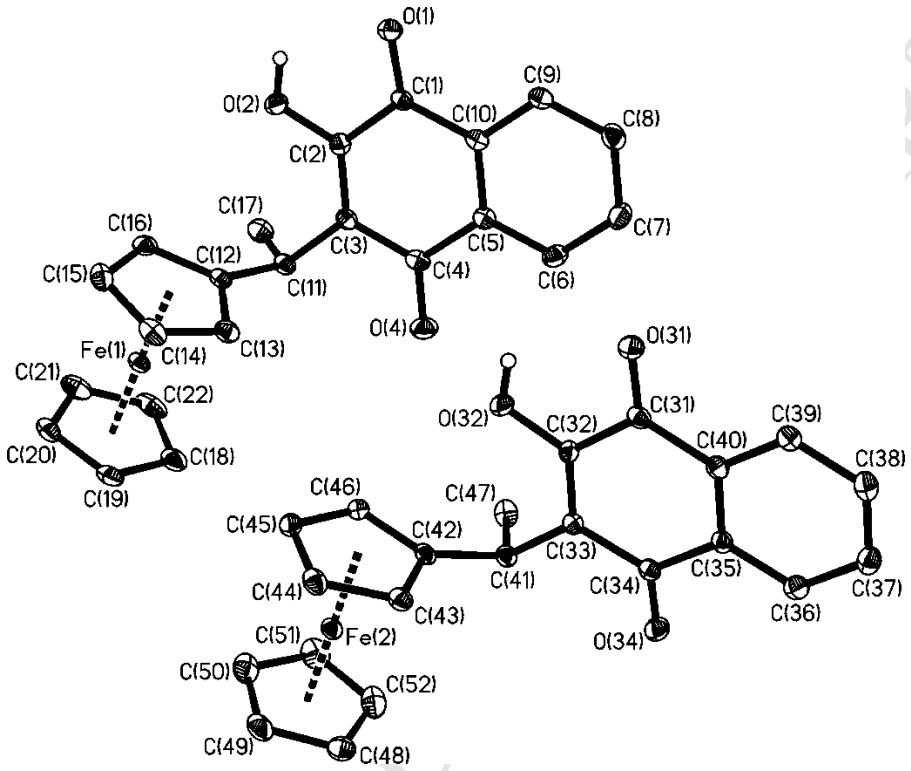


Figure S1. Crystallographic structure of 3-(ferrocenylethyl)-2-hydroxy-1,4-naphthoquinone **10b**.

Characterization of Ferrocenylketones

1.1. Ethyl ferrocenylketone **8c**

8c was obtained from ferrocene (5.00 mmol, 930 mg) according to general procedure 4.3 and 3 hours of reaction time. Yield 1016 mg (4.20 mmol, 84%) of an intense orange solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:** 4.79 (2H, s broad), 4.49 (2H, s broad), 4.19 (5H, s), 2.74 (2H, q, *J*=8.0 Hz), 1.20 (3H, t, *J*=8.0 Hz) H_3. Signals in accordance with Wang, R.; Hong, X.; Shan, Z. 2008.[1]

1.2. Propyl ferrocenylketone **8d**

8d was obtained from ferrocene (2.00 mmol, 372 mg) according to general procedure 4.3 and 0.5 hours of reaction time. Yield 461 mg (1.79 mmol, 90%) of an intense orange solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:** 4.78 (2H, t broad), 4.48 (2H, t broad), 4.19 (5H, s), 2.67 (2H, t, *J*=7.5 Hz), 1.74 (2H, sextet, *J*=7.5 Hz), 1.01 (3H, t, *J*=7.2 Hz). Signals in accordance with Wang, R.; Hong, X.; Shan, Z. 2008.[1]

1.3. 4-Chloropropyl ferrocenylketone **8e**

8e was obtained from ferrocene (2.68 mmol, 500 mg) according to general procedure 4.3 and 1.5 hours of reaction time. Yield 731 mg (2.52 mmol, 94%) of an intense orange solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:** 4.82 (2H, s broad), 4.54 (2H, s broad), 4.22 (5H, s), 3,70 (2H, t broad) H_4, 2.94 (2H, t broad), 2.20 (2H, m broad). Signals in accordance with Vicennati, P.; Cozzi, P. 2007.[2]

1.4.Undecyl ferrocenylketone **8f**

8f was obtained from ferrocene (1.50 mmol, 279 mg) according to general procedure 4.3 and 0.5 hours of reaction time. Yield 432 mg (1.17 mmol, 78%) of an intense red solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:** 4.80 (2H, s broad), 4.48 (2H, s broad), 4.20 (5H, s broad), 2.70 (2H, t, *J*=6.0 Hz), 1.64-1.72 (2H, m), 1.18-1.38 (16H, m), 0.88 (3H, t, *J*=6.0 Hz). Signals in accordance with Shi, J.; Xu, J.; Jiang, X. 2001.[3]

1.5. Cyclohexyl ferrocenylketone **8g**

8g was obtained from ferrocene (5.37 mmol, 1000 mg) according to general procedure 4.3 and 0.5 hours of reaction time. Yield 1461 mg (4.94 mmol, 92%) of an intense orange solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:** 4.78 (2H, s broad), 4.50 (2H, s broad), 4.20 (5H, s), 2.80 (1H, m), 1.86 (2H, m), 1.72 (2H, m), 1.54 (2H, m), 1.22-1.42 (4H, m). Signals in accordance with Wang, R.; Hong, X.; Shan, Z. 2008.[1]

1.6. 2-phenylmethyl ferrocenylketone **8h**

8h was obtained from ferrocene (7.34 mmol, 1366 mg) according to general procedure 4.3 and 2 hours of reaction time. Yield 1784 mg (5.86 mmol, 80%) of an intense orange solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:** 7.24-7.44 (5H, m), 4.84 (2H, s broad), 4.52 (2H, s broad), 4.12 (5H, s), 3.98 (2H, s). Signals in accordance with Wang, R.; Hong, X.; Shan, Z. 2008.[1]

1.7. Phenyl ferrocenylketone **8i**

8i was obtained from ferrocene (5 mmol, 930 mg) according to general procedure 4.3 and 2 hours of reaction time. Yield 1006 mg (3.47 mmol, 69%) of an intense red solid. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ (ppm)**:** 7.90 (2H, dd broad, *J*=7.0 Hz), 7.55 (1H, t, *J*=7.0 Hz), 7.47 (2H, t, *J*=7.0 Hz), 4.91 (2H, t, *J*=2.0 Hz), 4.59 (2H, t, *J*=2.0 Hz), 4.21 (5H, s). Signals in accordance with Yang *et al.* 2007.[4]

1.8. 4-Chlorophenyl ferrocenylketone **8j**

8j was obtained from ferrocene (5.51 mmol, 1026 mg) according to general procedure 4.3 and 0.5 hours of reaction time. Yield 1079 mg (3.32 mmol, 60%) of an intense red solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:**. 7.86 y 7.45 (4H, AA'BB', *J*=8.0 Hz), 4.88 (2H, t broad, *J*=1.5 Hz), 4.61 (2H, t broad, *J*=1.5 Hz), 4.20 (5H, s). Signals in accordance with Yang *et al.* 2007.[4]

1.9. 4-Bromophenyl ferrocenylketone **8k**

8k was obtained from ferrocene (4.30 mmol, 800 mg) according to general procedure 4.3 and 0.5 hours of reaction time. Yield 752 mg (2.04 mmol, 47%) of an intense red solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)**:**. 7.78 y 7.61 (4H, AA'BB', *J*=9.0 Hz), 4.88 (2H, t broad, *J*=2.4 Hz), 4.61 (2H, t broad, *J*=2.4 Hz), 4.20 (5H, s). Signals in accordance with Carollo, L.; Curulli, A.; Floris, B. 2003.[5]

References

[1] R. Wang, X. Hong, and Z. Shan, A novel , convenient access to acylferrocenes : acylation of ferrocene with acyl chlorides in the presence of zinc oxide, Tetrahedron Lett. 49 (2008) 636-639.

[2] P. Vicennati and P. G. Cozzi, Facile Access to Optically Active Ferrocenyl Derivatives with Direct Substitution of the Hydroxy Group Catalyzed by Indium Tribromide., Eur. J. Org. Chem. 14 (2007) 2248 – 2253.

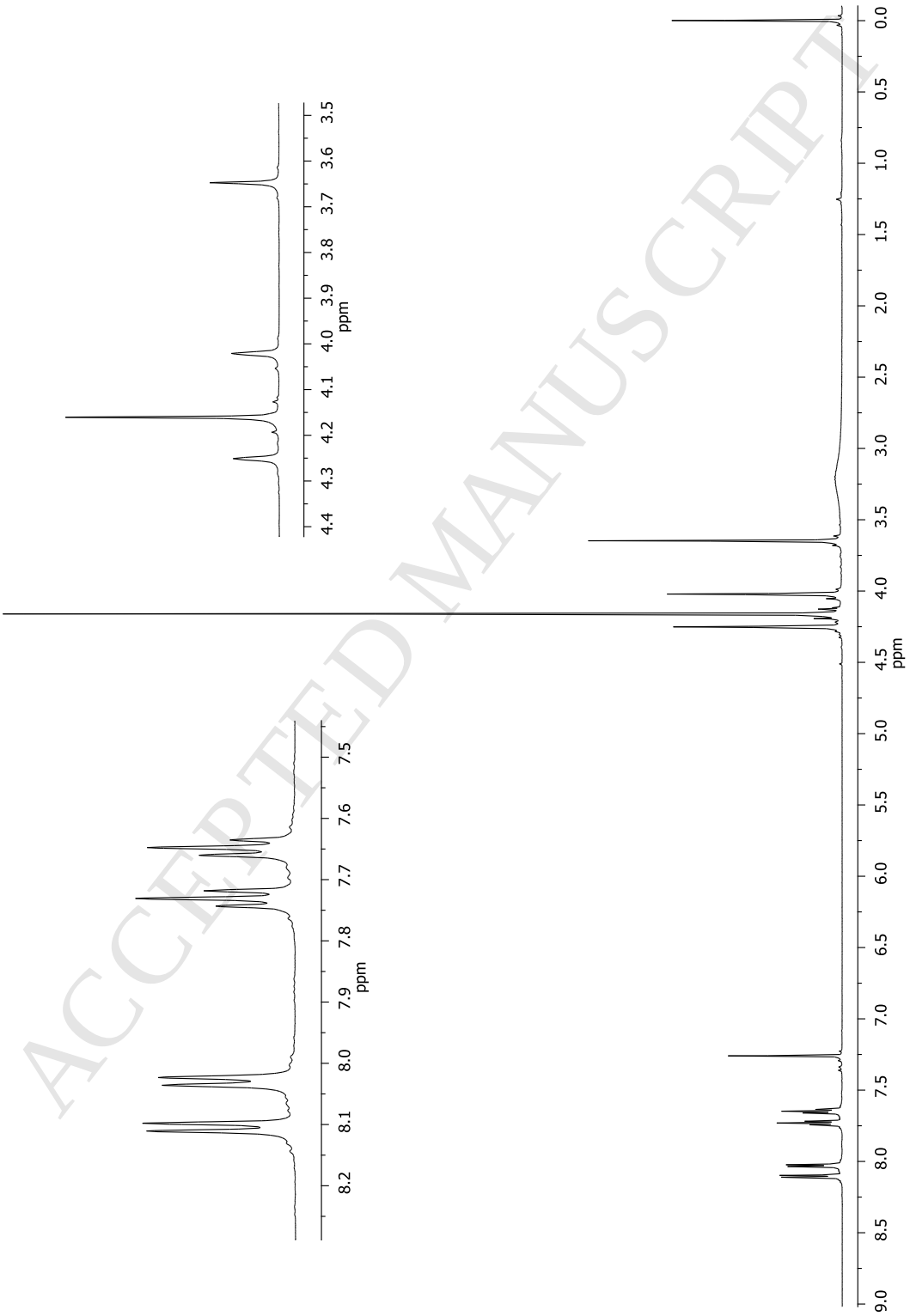
[3] J. Shi, J. Xu, and X. Jiang, Hydrophobic Acceleration of Electron- Transfer Fluorescence Quenching Processes between Excited 1- Alkanoylperylenes and Ferrocene Derivatives, Chin. J. Chem. 19 (2001) 481-487.

[4] D. Yang, Z. Liu, Y. Li, and B. Chen, Convenient Synthesis of Aryl Ferrocenyl Ketone via Palladium-Catalyzed Carbonylation Coupling, Synt. Comm. 37 (2007) 3759-3765.

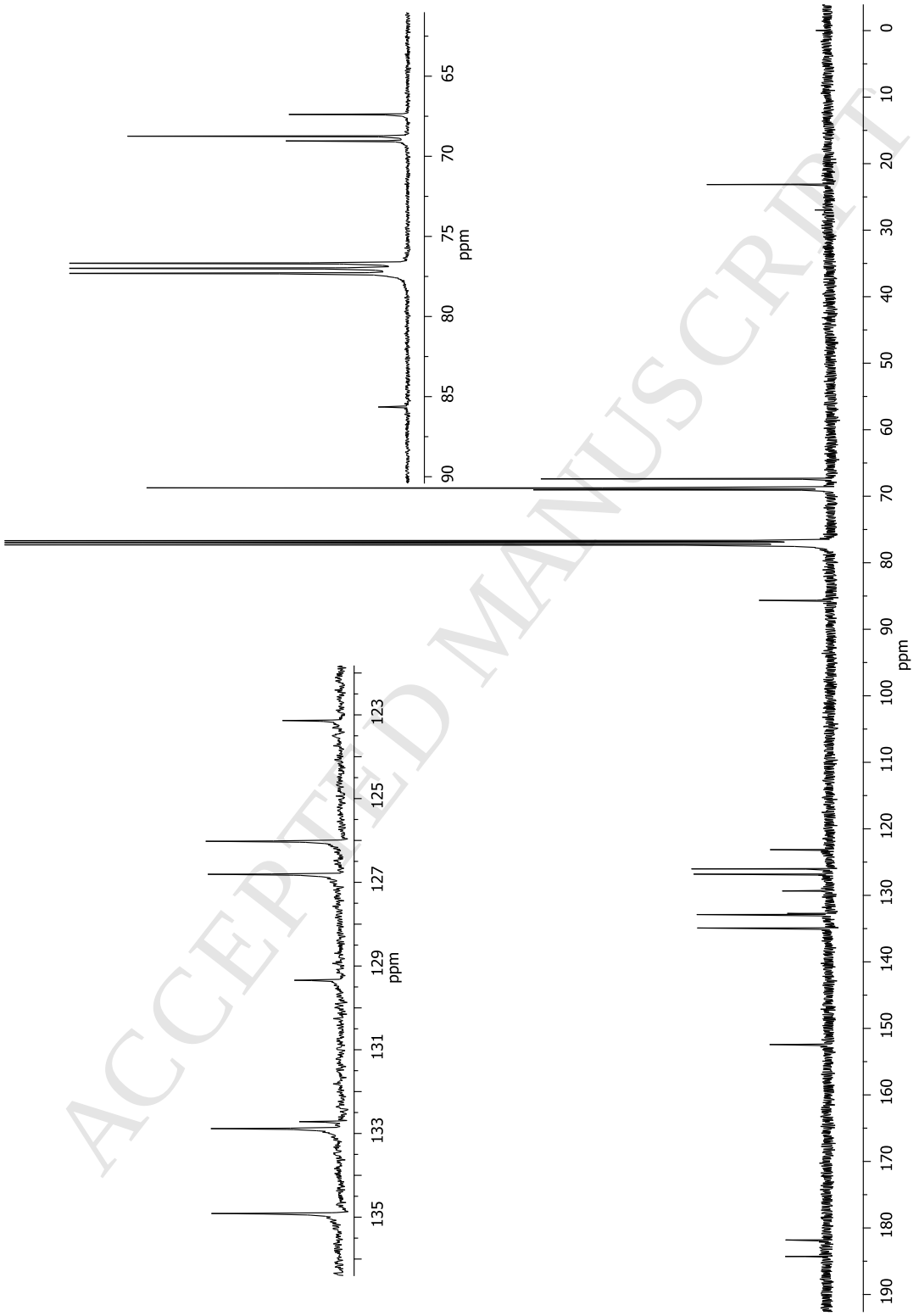
[5] L. Carollo, A. Curulli, and B. Floris, Arylferrocenylmethanols: a new family of ferrocenes to be used as mediators in biosensors, App. Organometal. Chem. 17 (2003) 589-599.

NMR spectra of the 3-(ferrocenylmethyl)-1,4-naphthoquinone 10 derivatives.

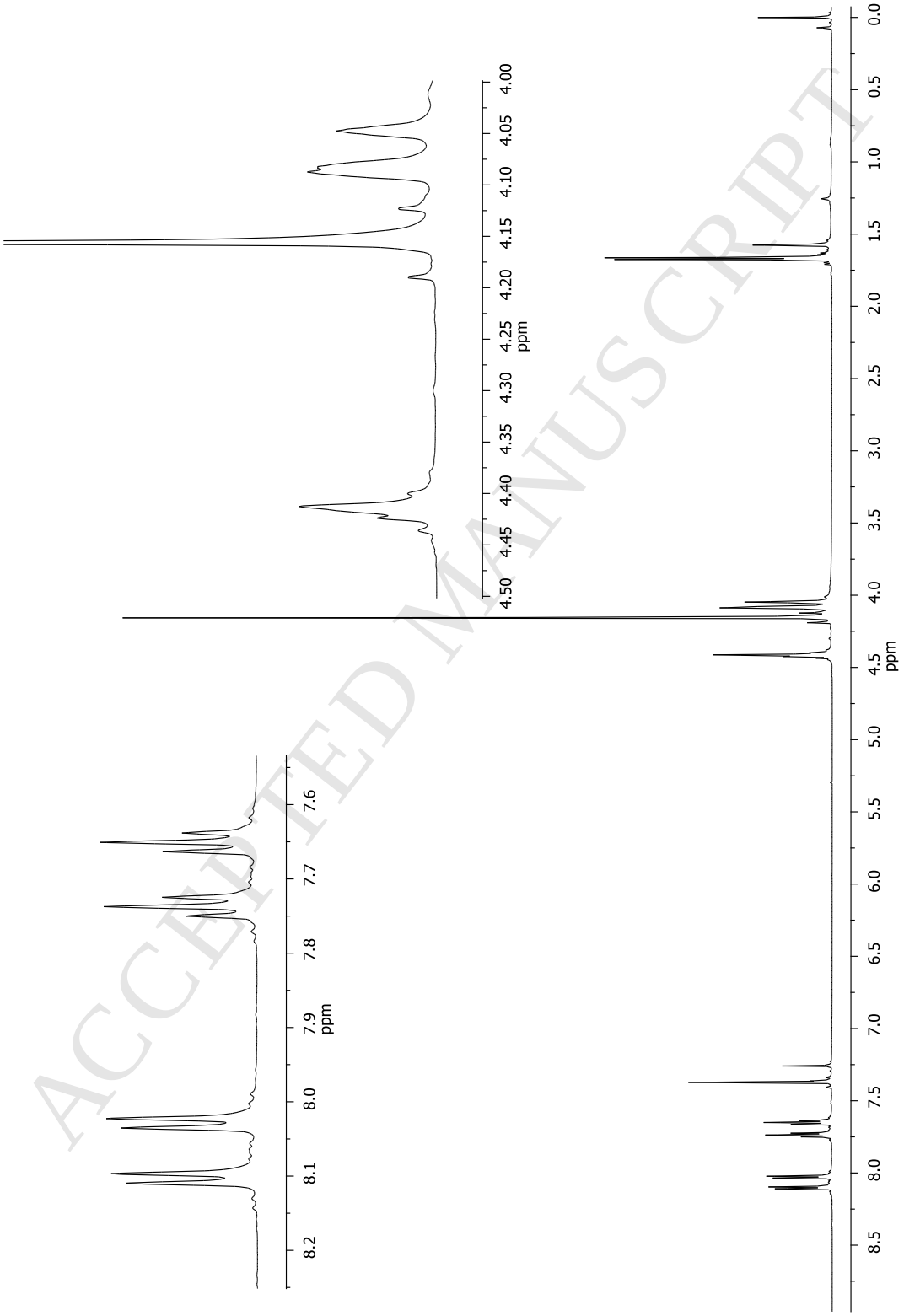
3-(ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10a** ^{1}H-NMR



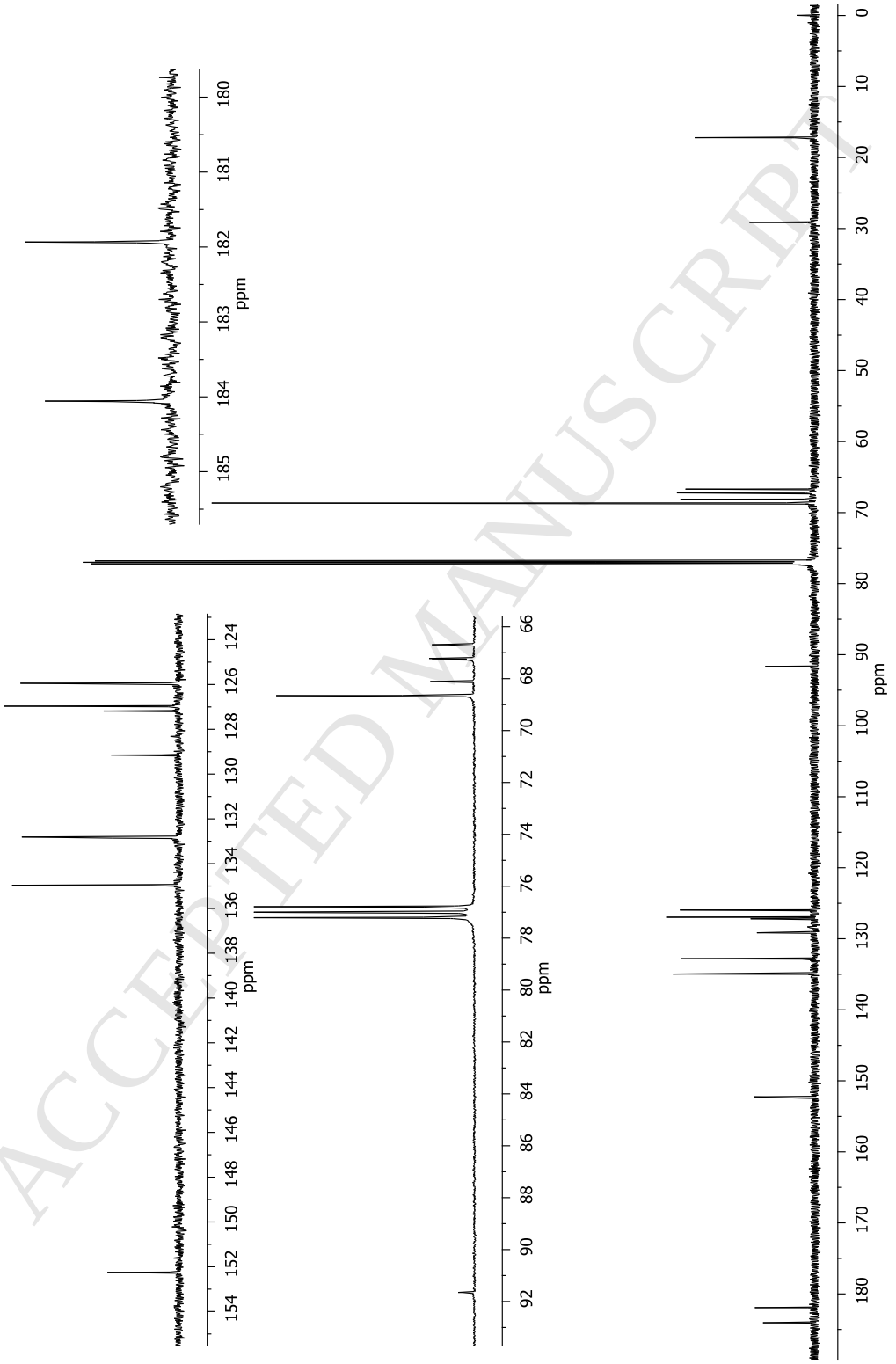
3-(ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10a** ^{13}C- NMR



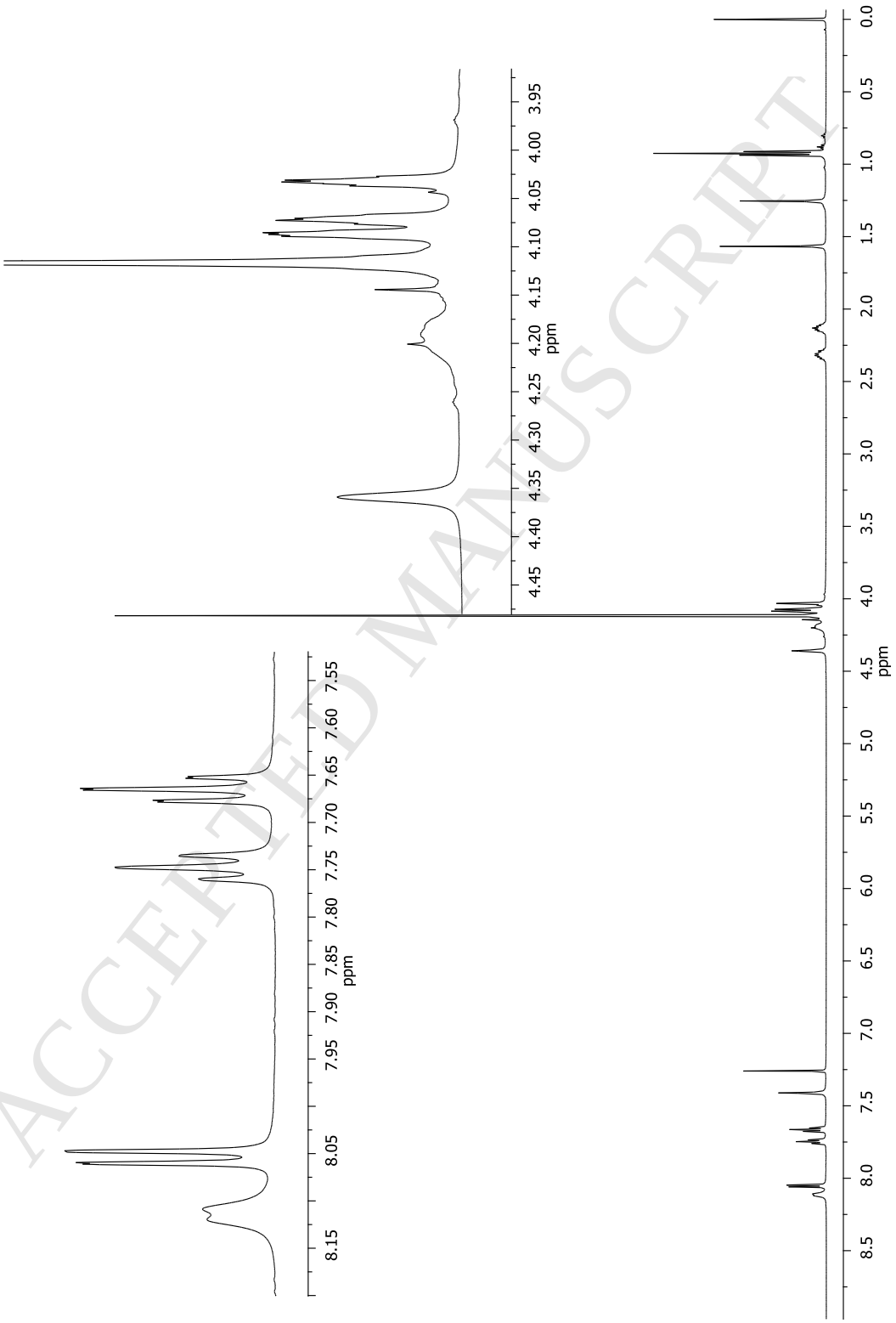
3-(1-ferrocenylethyl)-2-hydroxy-1,4-naphthoquinone **10b** ^{1}H- NMR



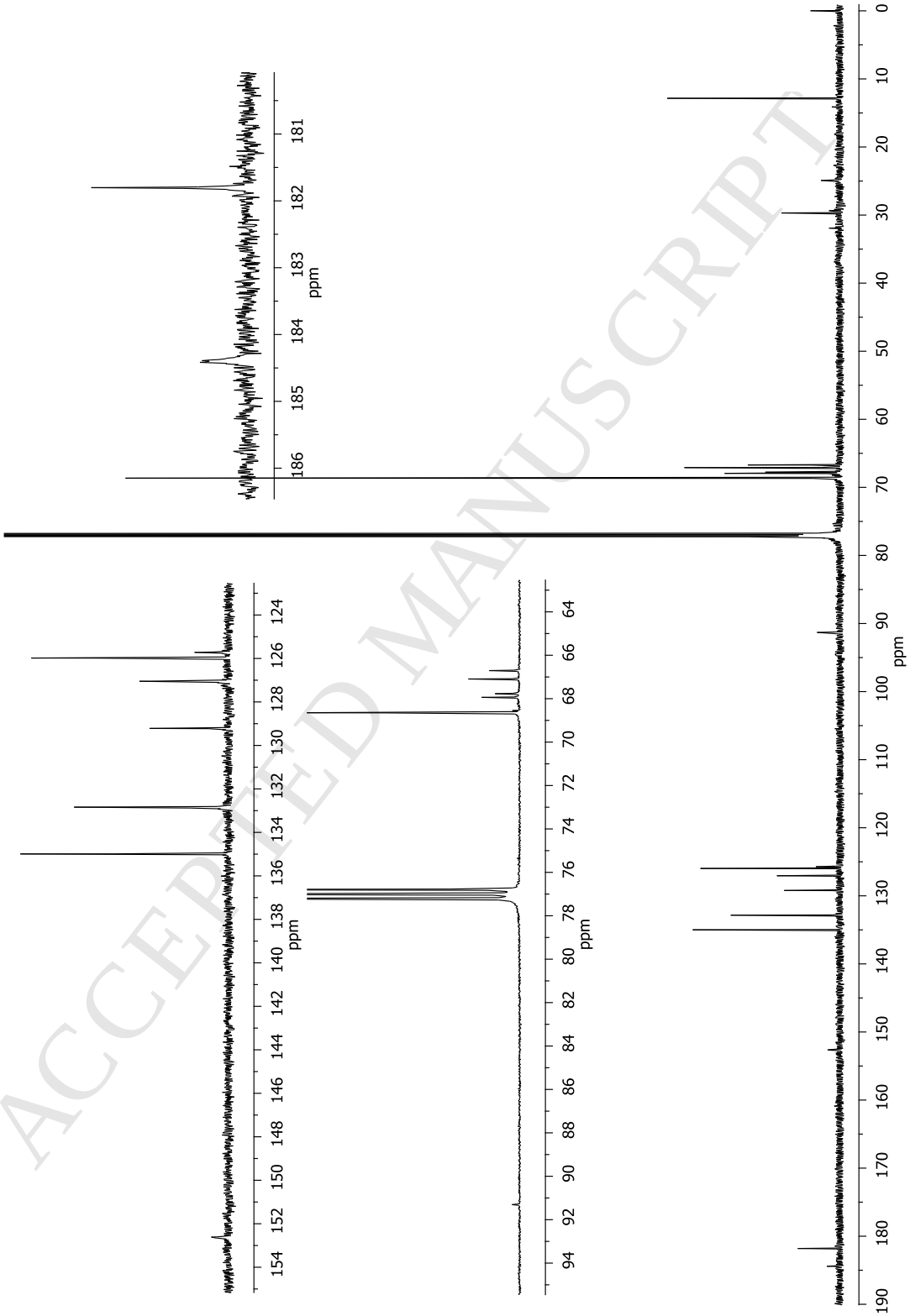
3-(1-ferrocenylethyl)-2-hydroxy-1,4-naphthoquinone **10b** ^{13}C- NMR



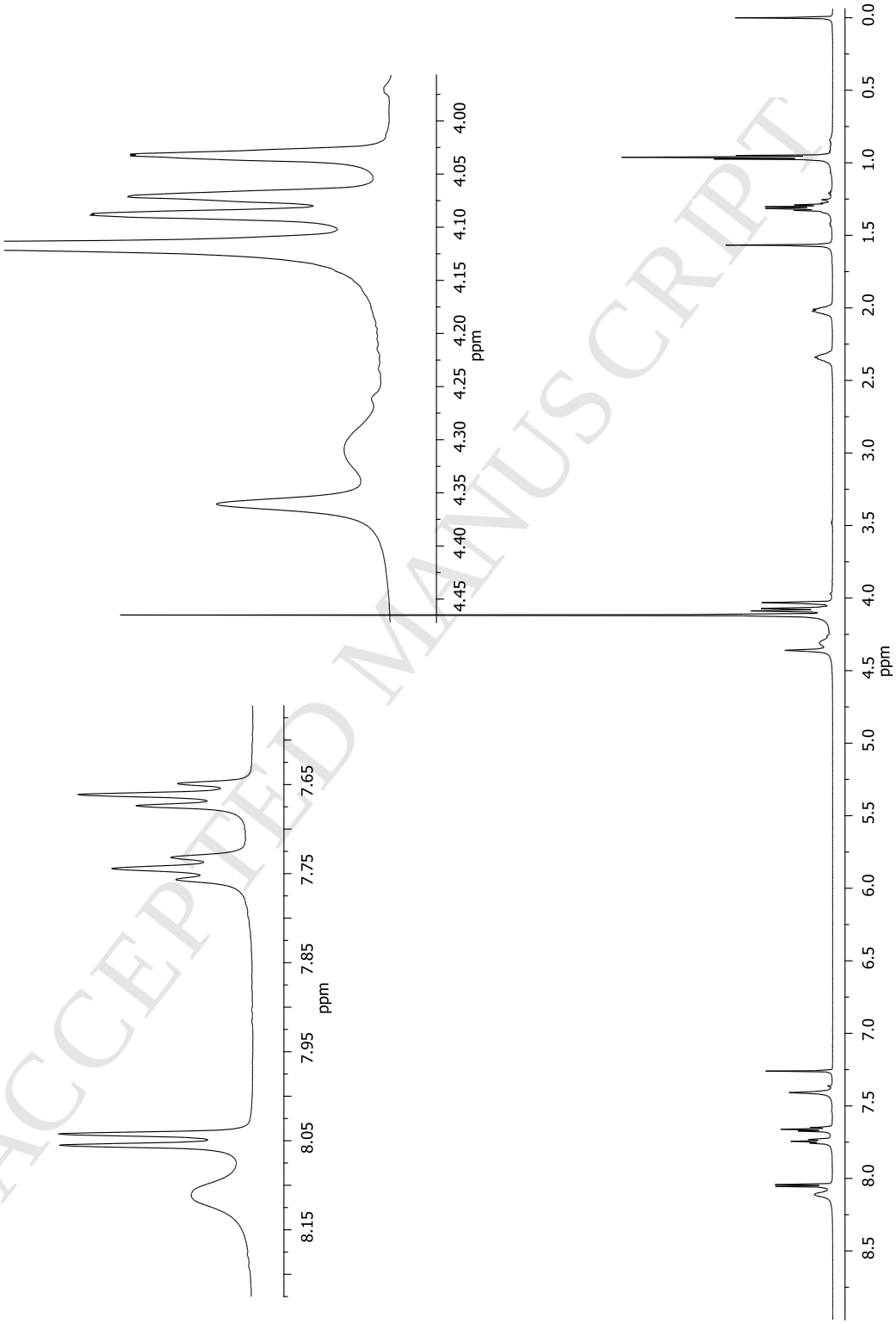
3-(1-ferrocenylpropyl)-2-hydroxy-1,4-naphthoquinone **10c** ^{1}H- NMR



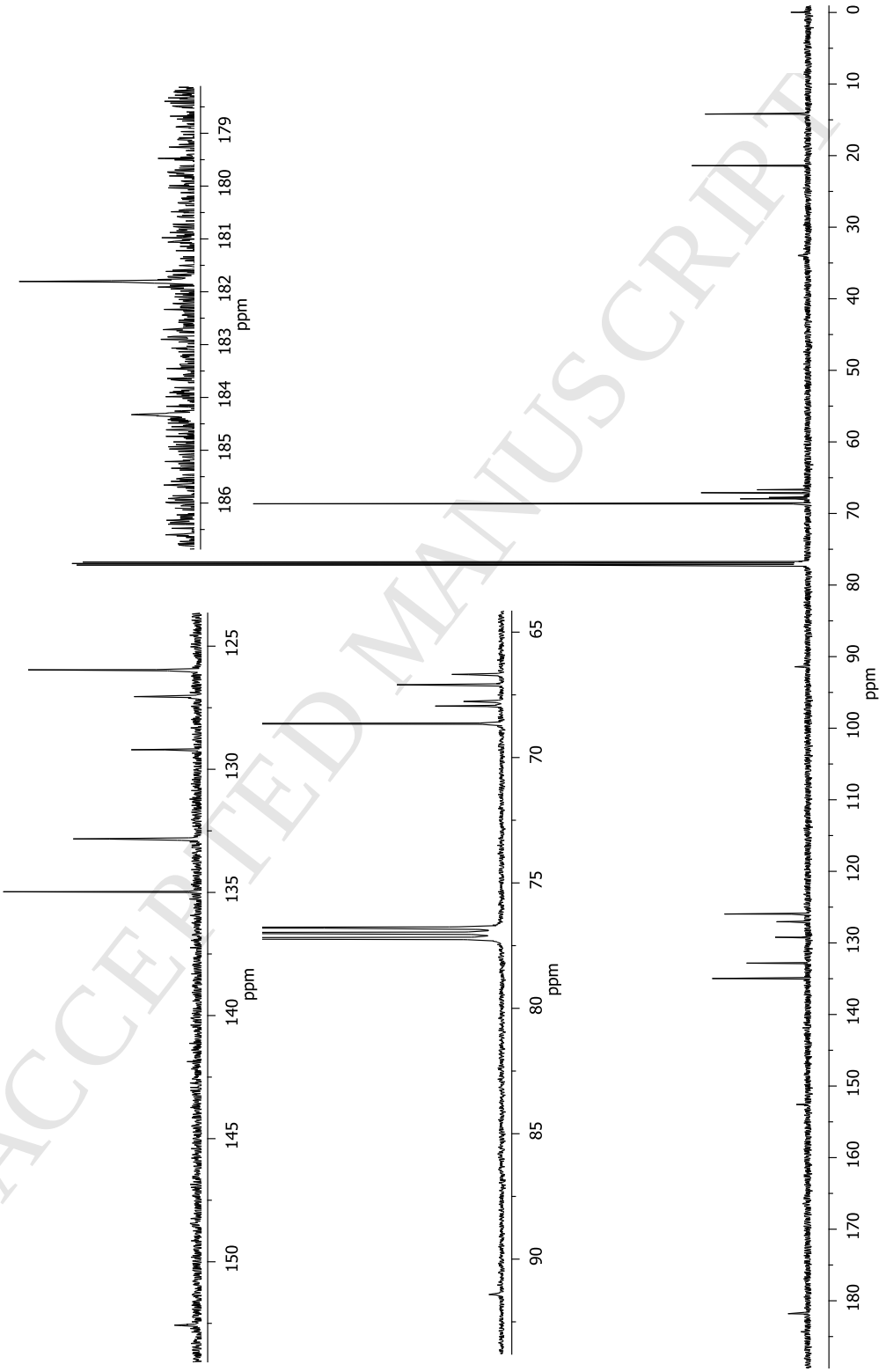
3-(1-ferrocenylpropyl)-2-hydroxy-1,4-naphthoquinone **10c** ^{13}C- NMR



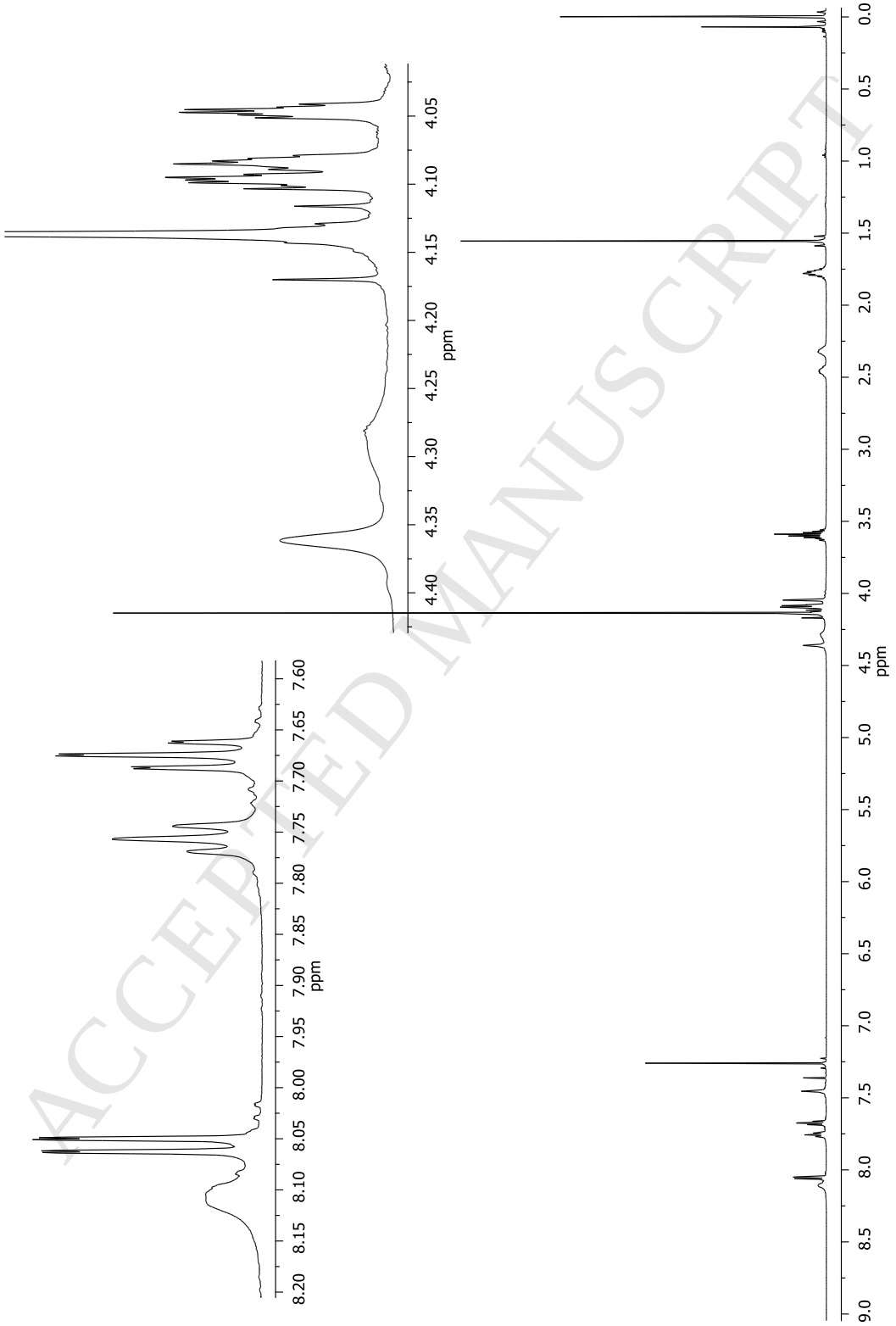
3-(1-ferrocenylbutyl)-2-hydroxy-1,4-naphthoquinone **10d** ^{1}H- NMR



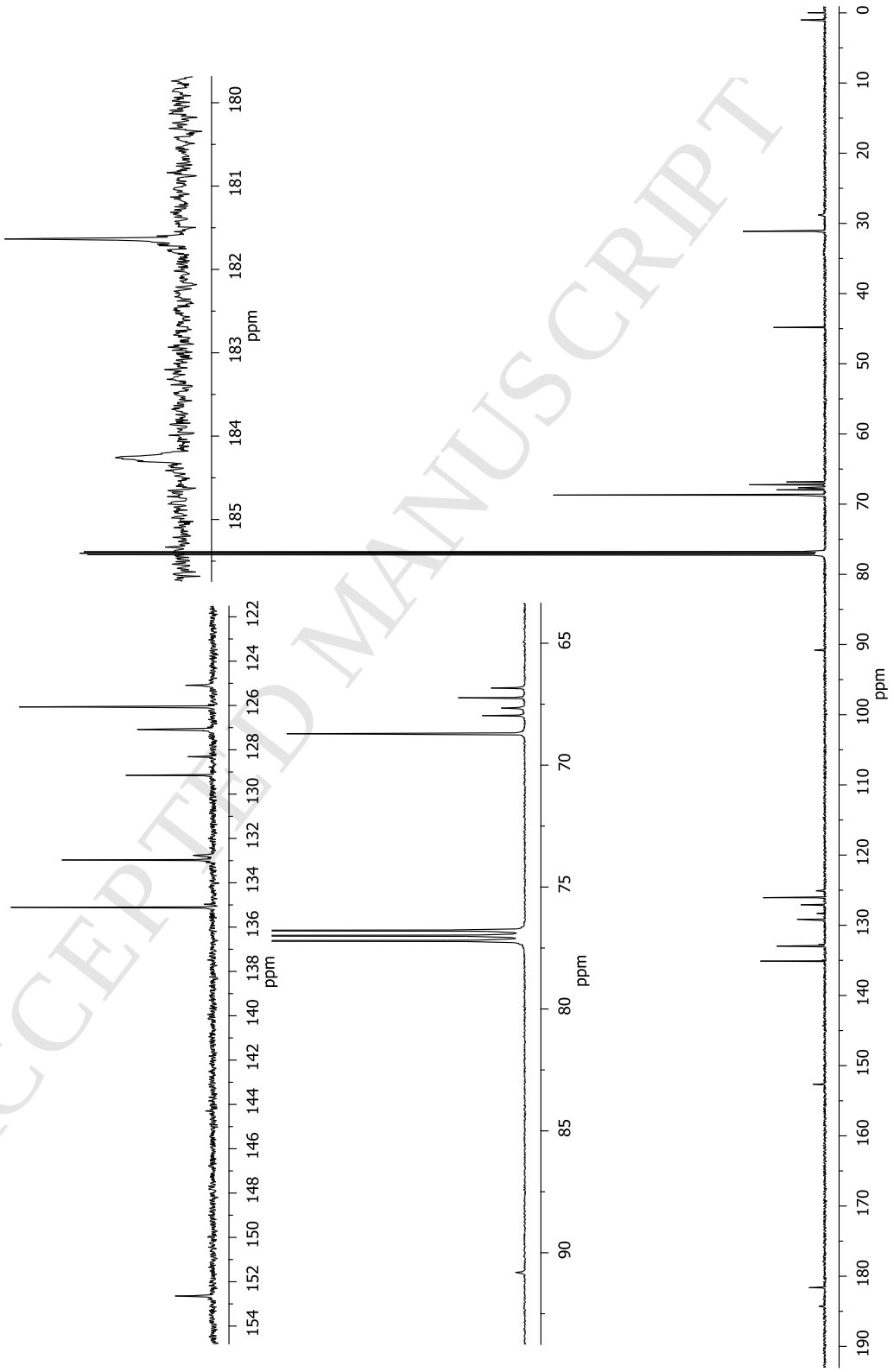
3-(1-ferrocenylbutyl)-2-hydroxy-1,4-naphthoquinone **10d** ^{13}C- NMR



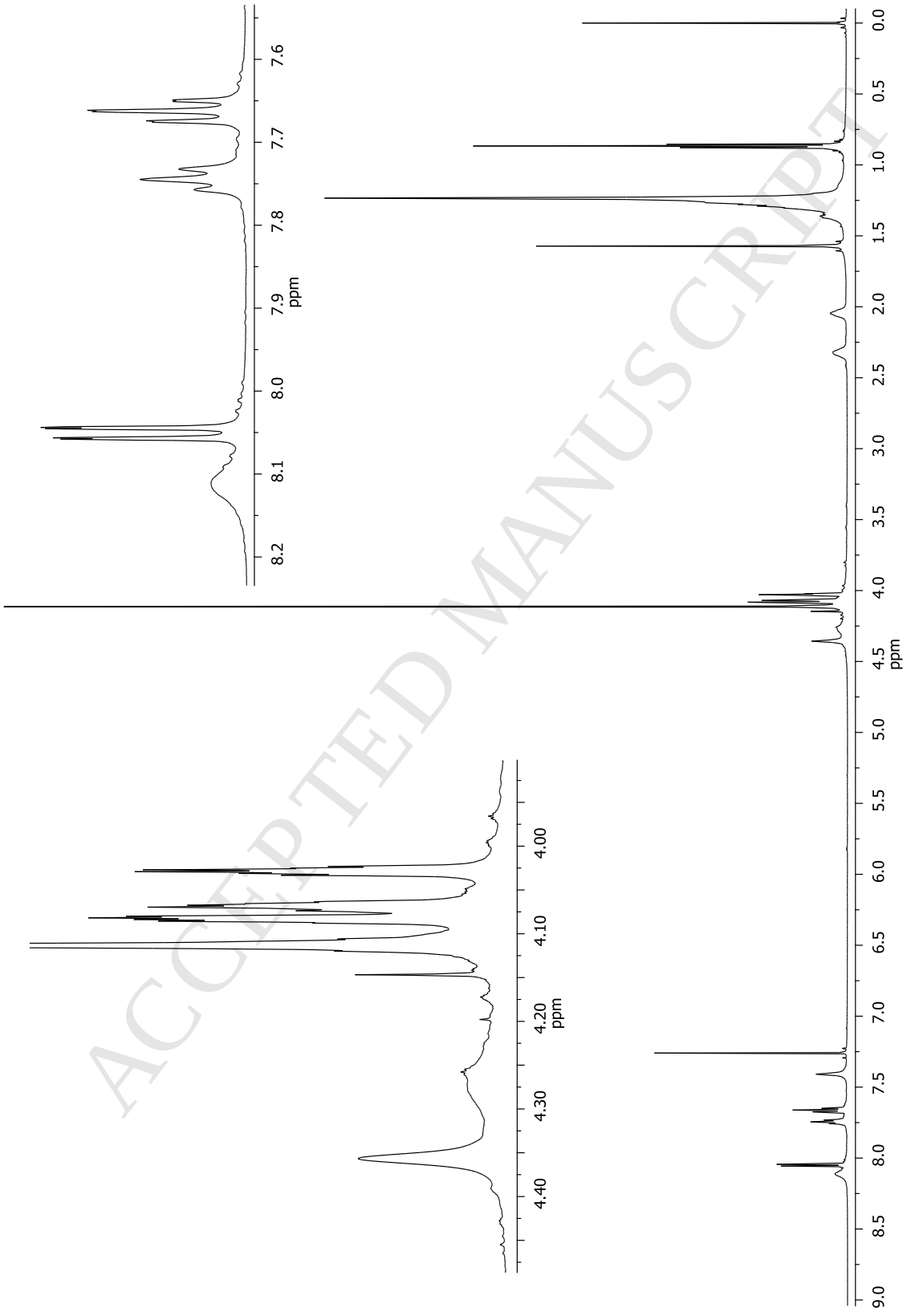
3-(4-chloro-1-ferrocenylbutyl)-2-hydroxy-1,4-naphthoquinone **10e** ^{1}H- NMR



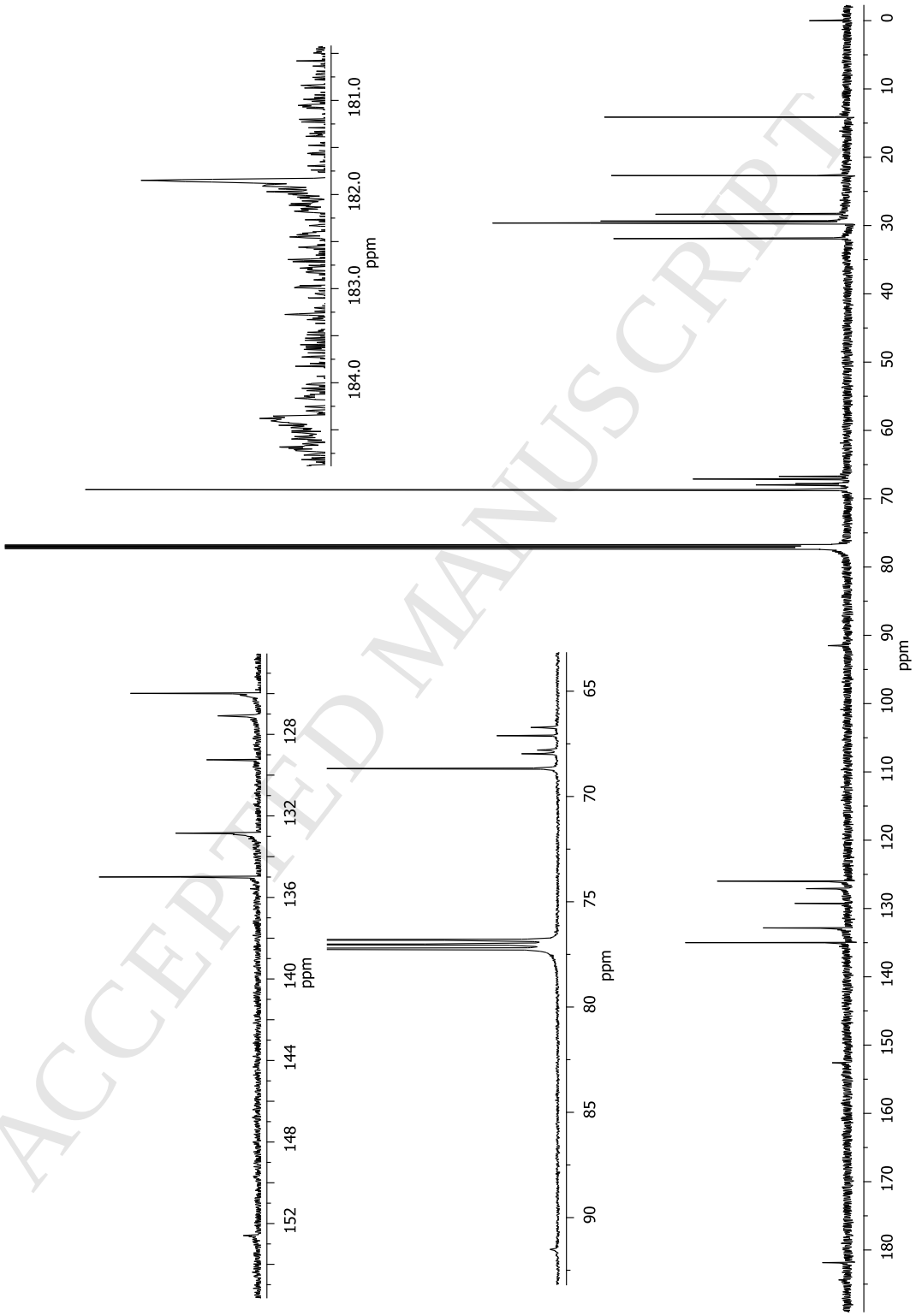
3-(4-chloro-1-ferrocenylbutyl)-2-hydroxy-1,4-naphthoquinone **10e** ^{13}C- NMR



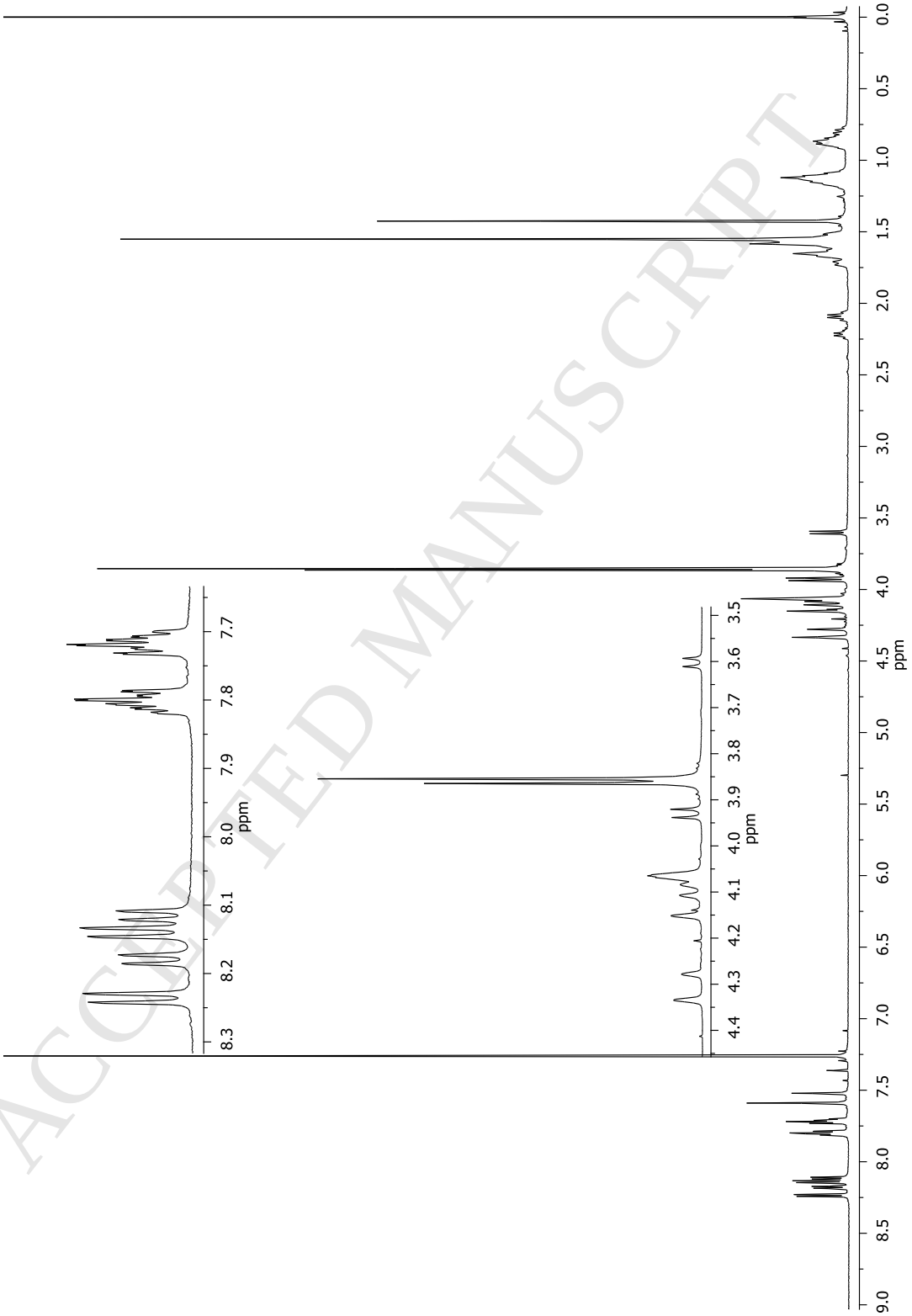
3-(1-ferrocenyldodecyl)-2-hydroxy-1,4-naphthoquinone **10f** ^{1}H- NMR



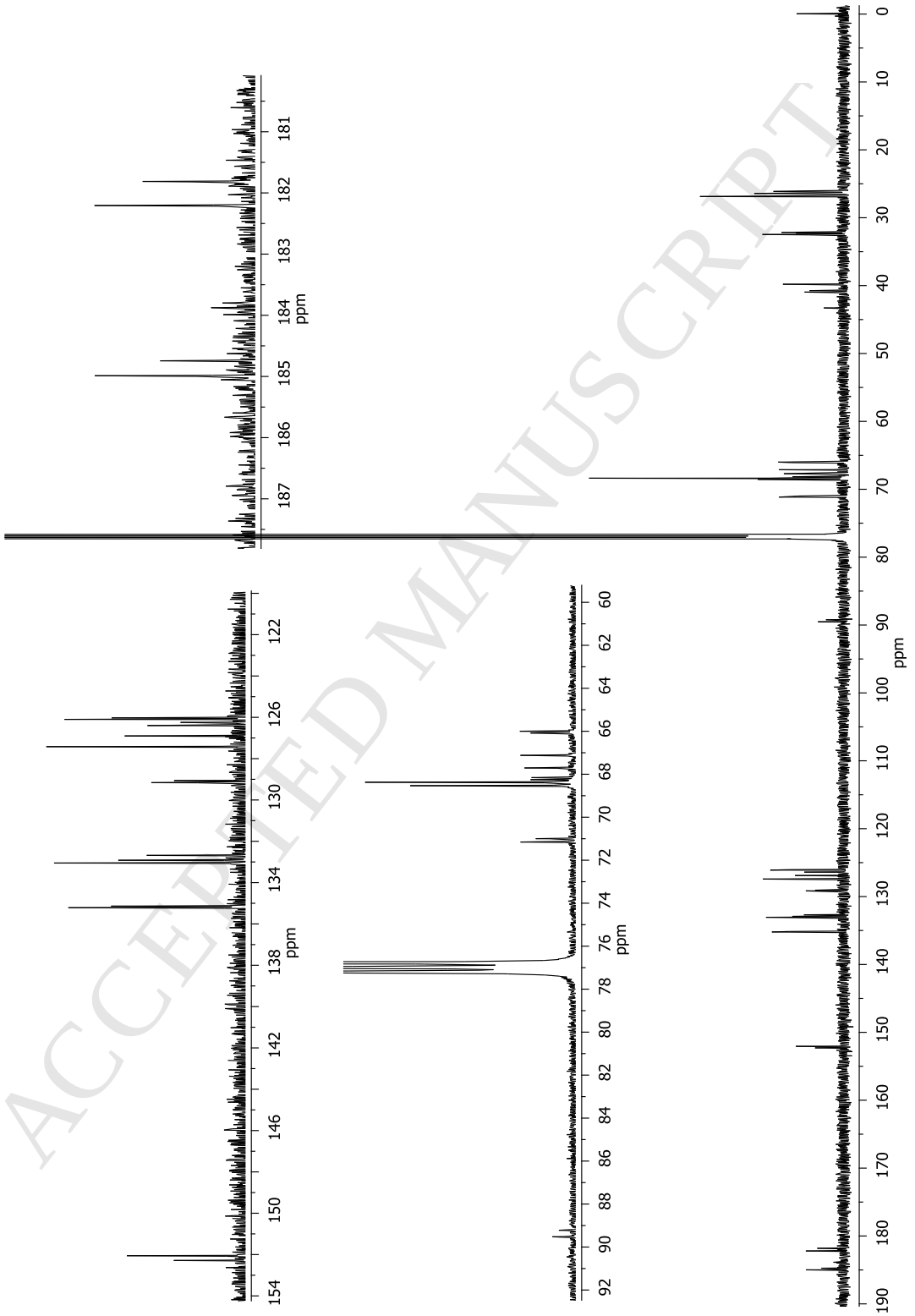
3-(1-ferrocenyldodecyl)-2-hydroxy-1,4-naphthoquinone **10f** ^{13}C- NMR



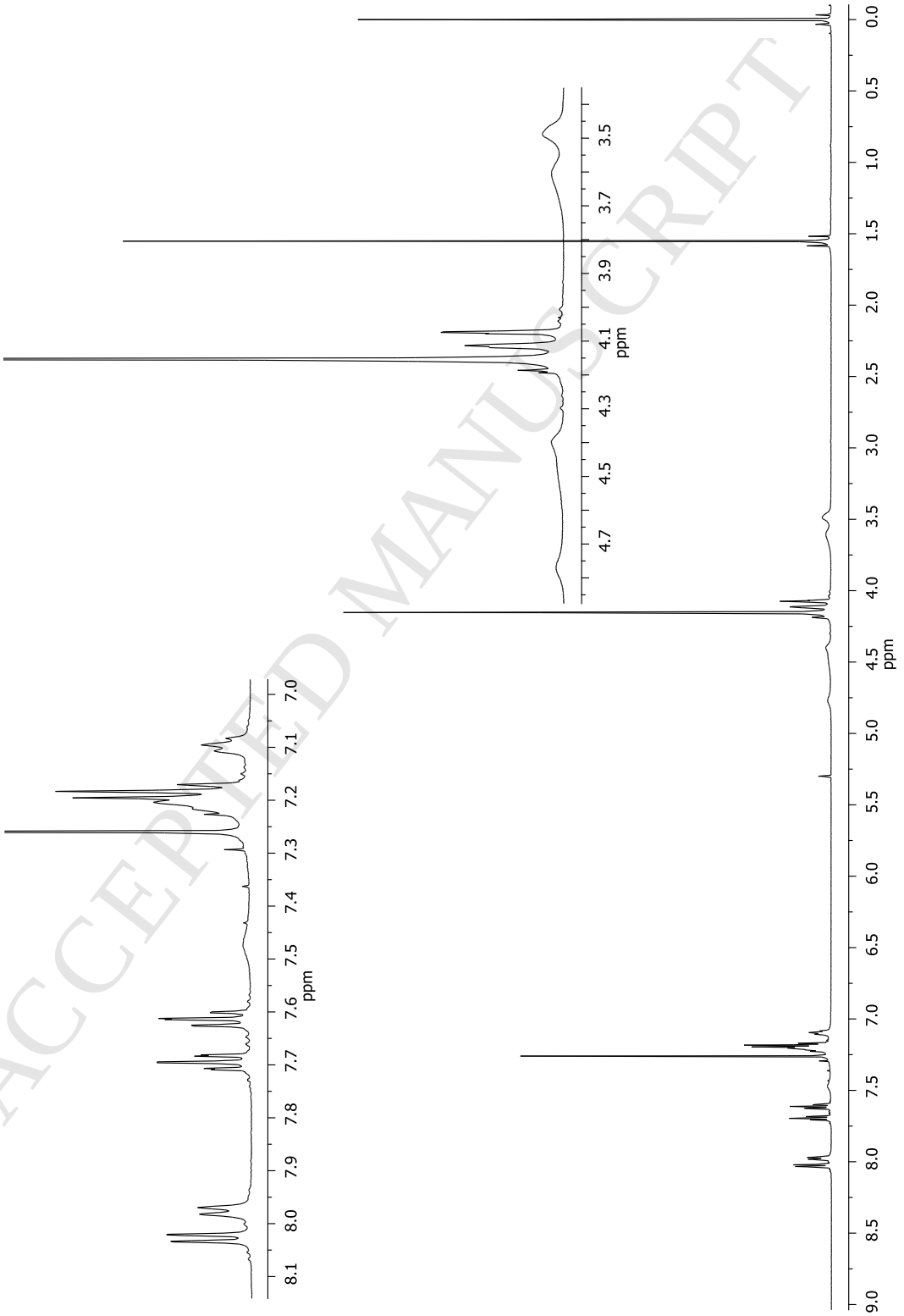
3-(1-cyclohexyl-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10g** ^{1}H- NMR



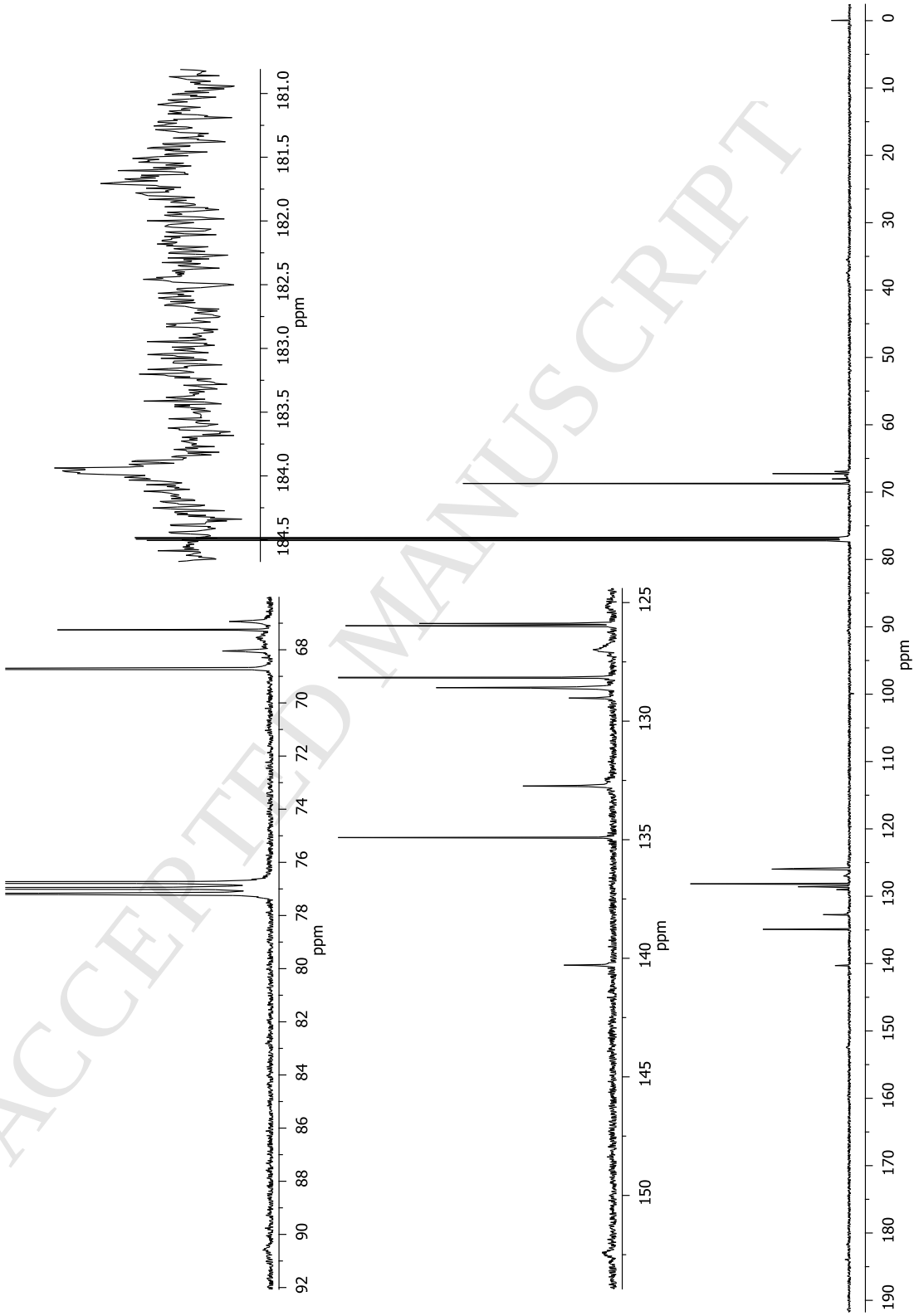
3-(1-cyclohexyl-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10g** ^{13}C- NMR



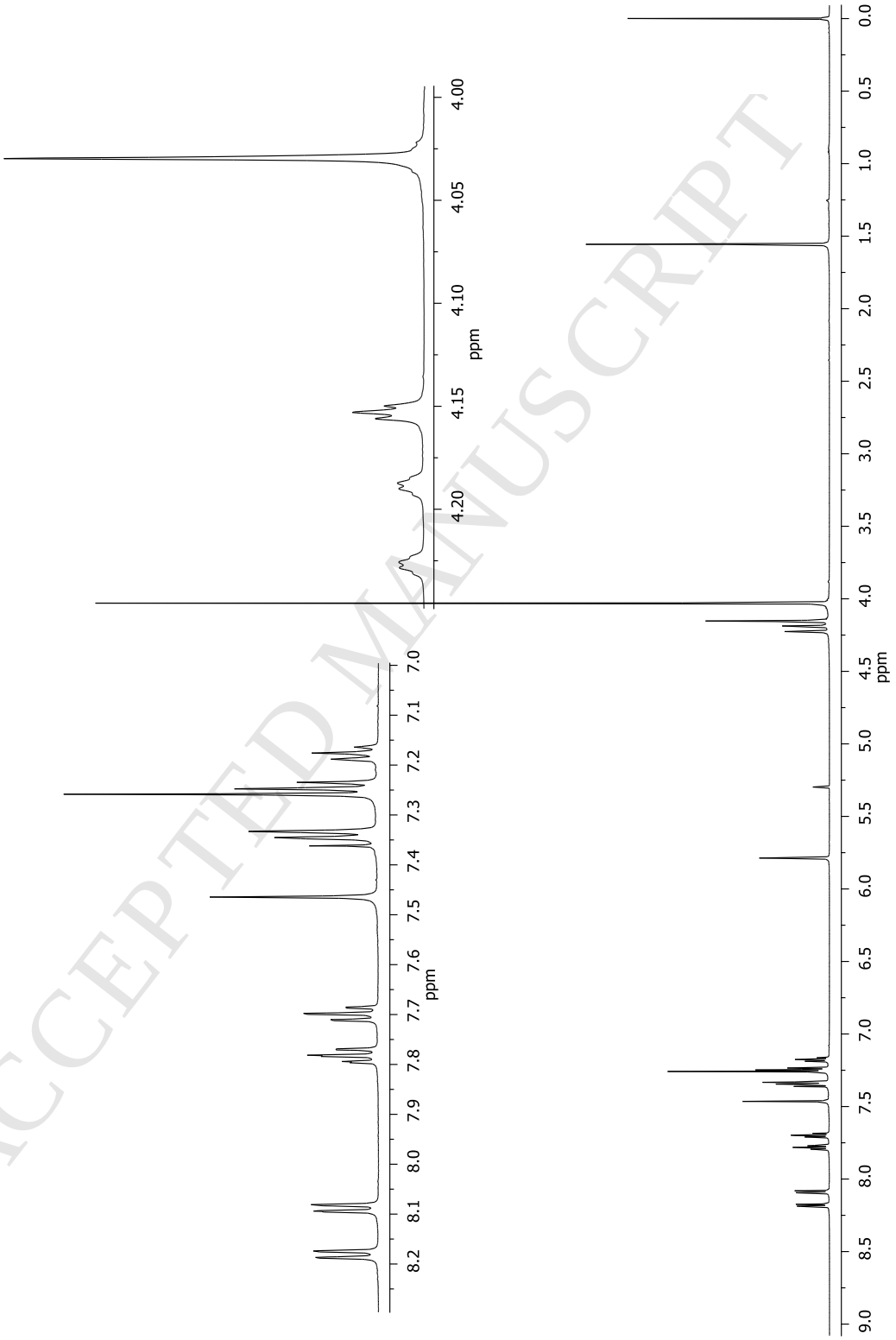
3-(1-ferrocenyl-2-phenylethyl)-2-hydroxy-1,4-naphthoquinone **10h** ^{1}H- NMR



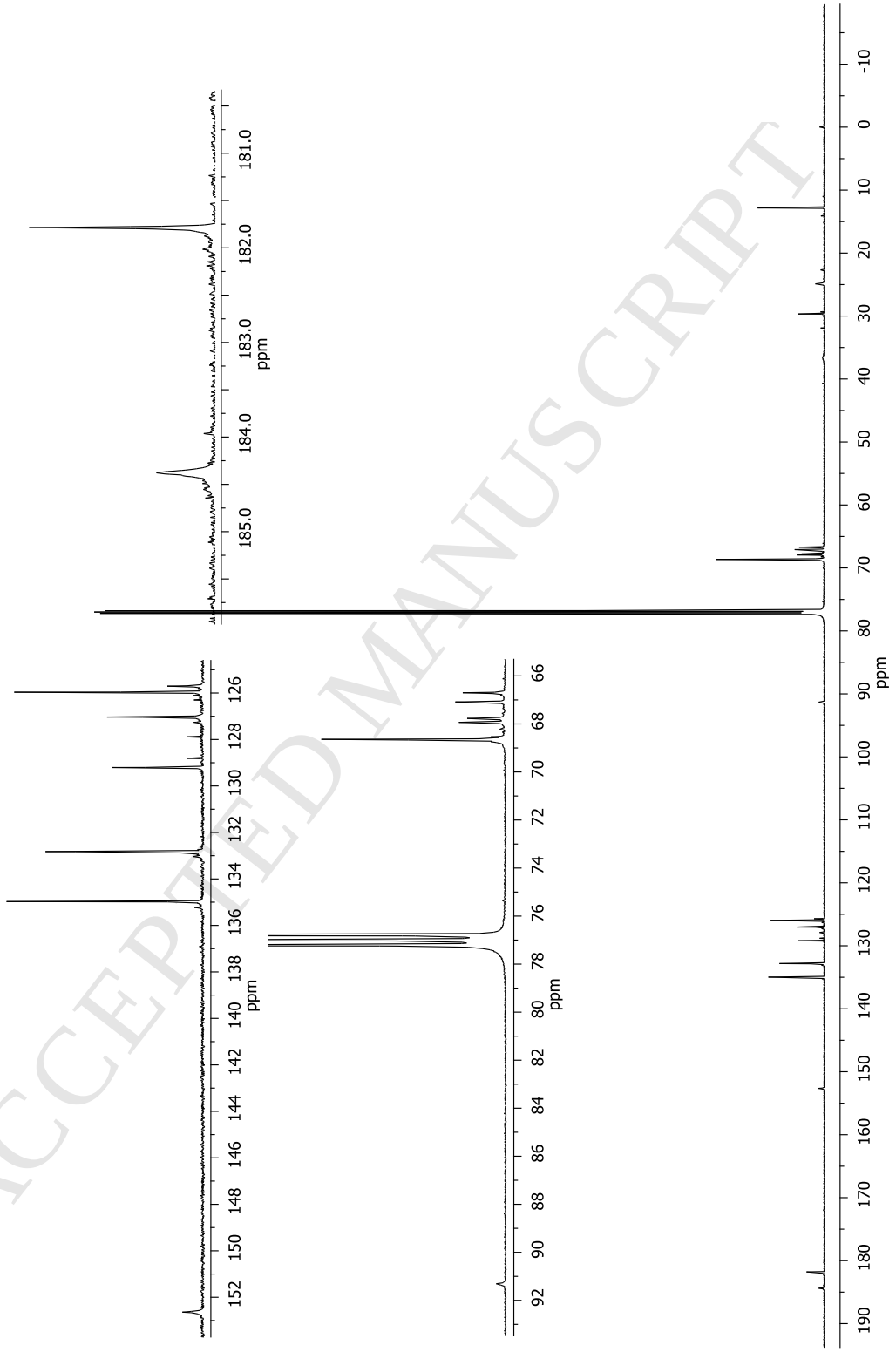
3-(1-ferrocenyl-2-phenylethyl)-2-hydroxy-1,4-naphthoquinone **10h** ^{13}C- NMR



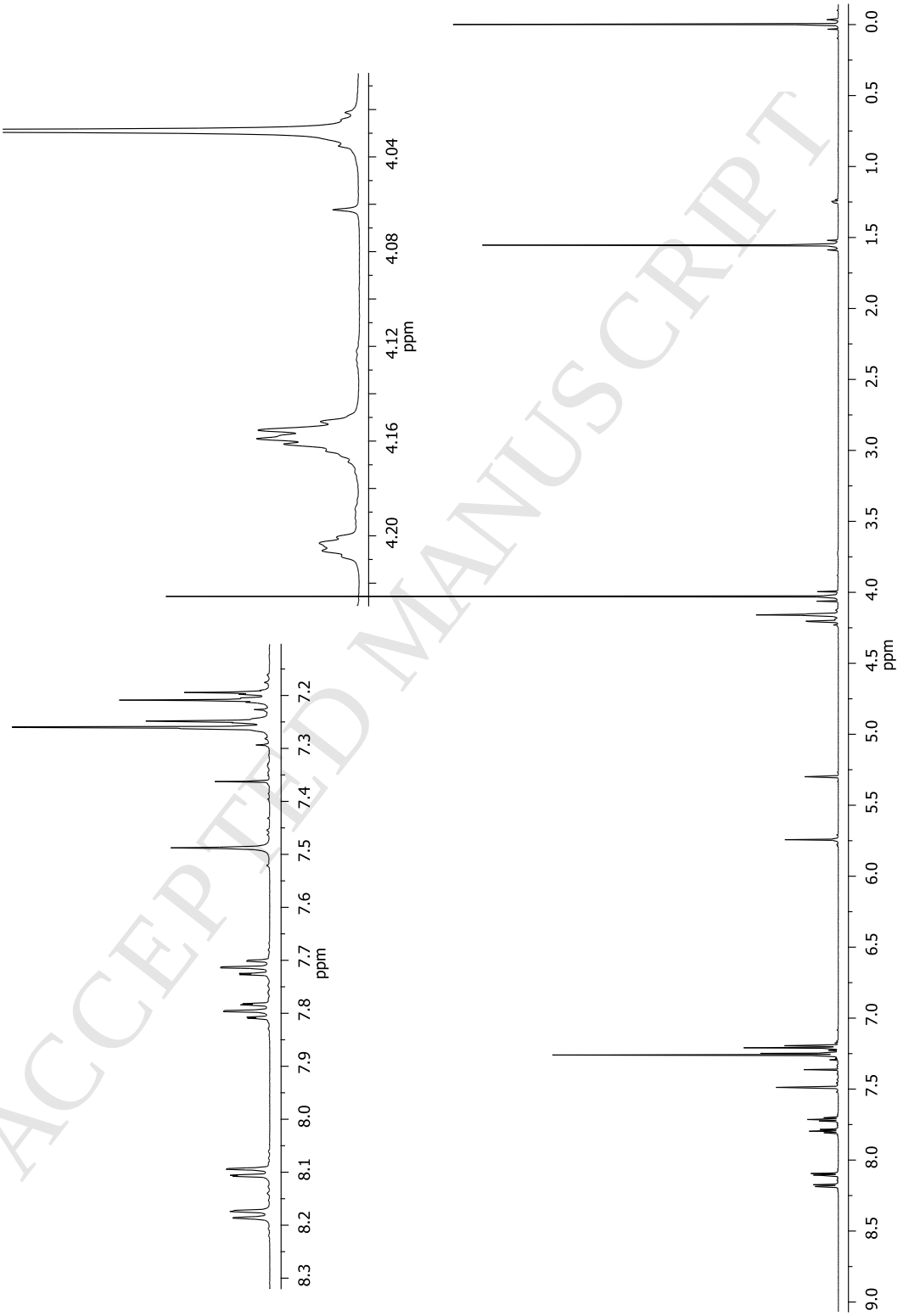
3-(1-ferrocenyl-1-phenylmethyl)-2-hydroxy-1,4-naphthoquinone **10i** ^{1}H- NMR



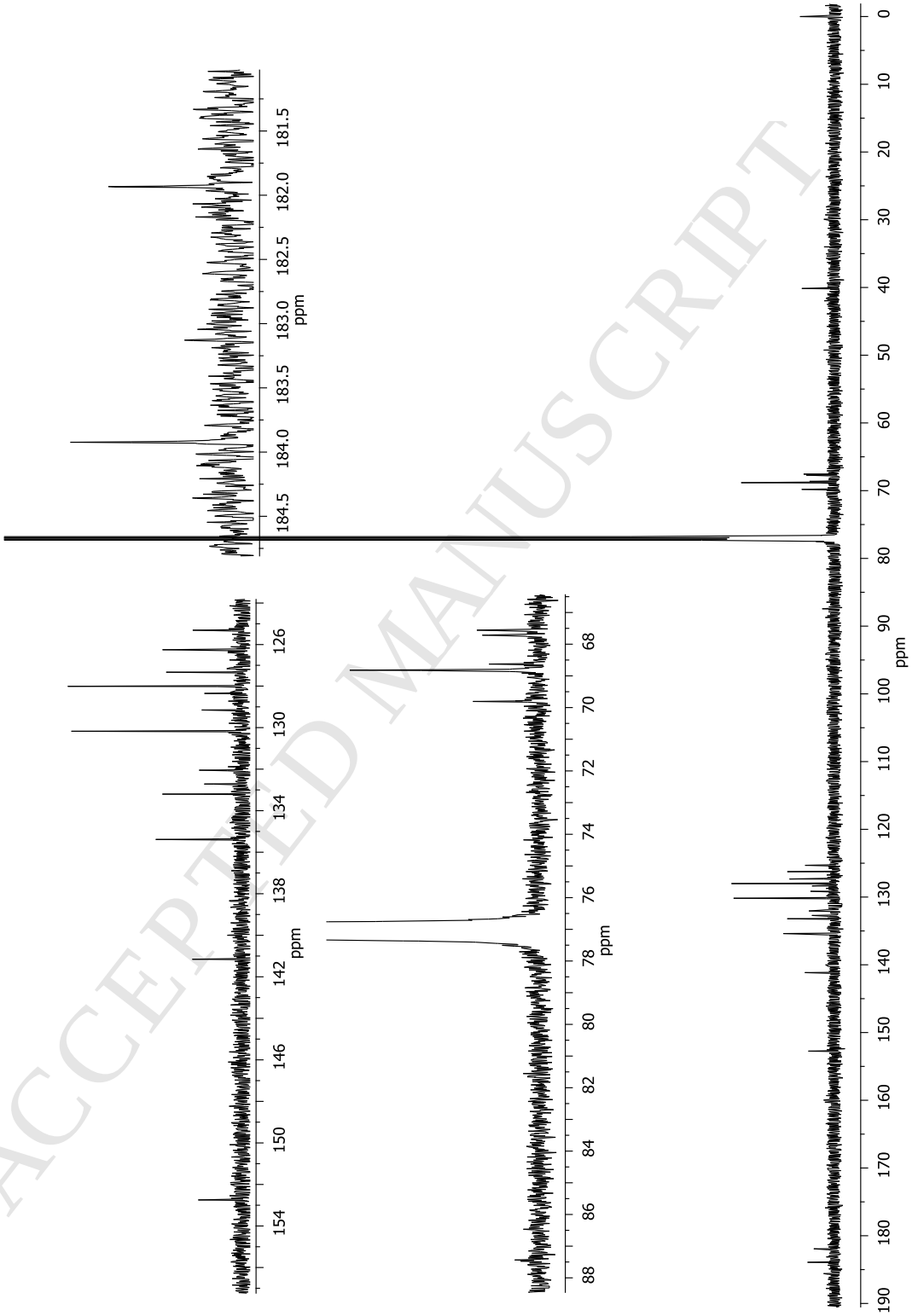
3-(1-ferrocenyl-1-phenylmethyl)-2-hydroxy-1,4-naphthoquinone **10i** ^{13}C- NMR



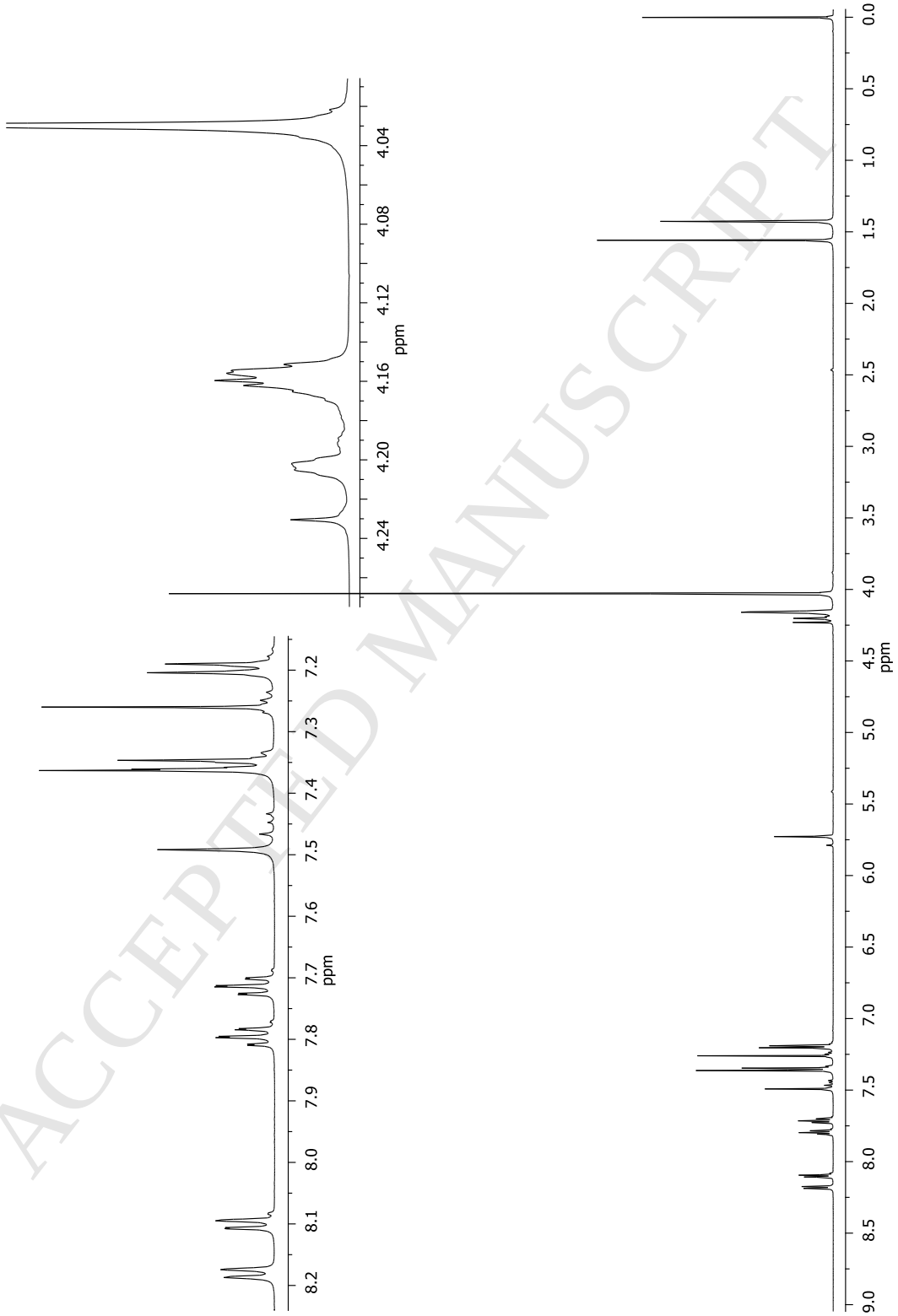
3-(1-(4-chlorophenyl)-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10j** ^{1}H- NMR



3-(1-(4-chlorophenyl)-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10j** ^{13}C- NMR



3-(1-(4-bromophenyl)-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10k** ^{1}H- NMR



3-(1-(4-bromophenyl)-1-ferrocenylmethyl)-2-hydroxy-1,4-naphthoquinone **10k** ^{13}C- NMR