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PAPER

A one-pot synthesis of 1,4-naphthoquinone-2,3-bis-sulfides catalysed by a commercial laccase

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Oxidative C–S bond formation with aryl and alkyl thiols was catalysed under mild conditions in a reaction vessel open to air at pH 4.5 and 7.15 in the presence of a commercial laccase (Novozym 51003) and a co-solvent (DMF) to afford 1,4-naphthoquinone-2,3-bis-sulfides. The synthesis of 1,4-naphthoquinone-2,3-bis-sulfides from two different 1,4-naphthohydroquinone substrates was investigated with regard to pH and number of equivalents of the thiol.

Introduction

Laccases are a group of enzymes that have a multinuclear copper-containing active site and have been classified as oxidoreductases. They use non-toxic atmospheric oxygen to catalyse the monoelectronic oxidation of substrates and produce only water as a by-product.[1–4] By abstracting hydrogen from phenolic hydroxyl groups and by using atmospheric oxygen as an electron acceptor, laccases are able to generate phenoxy radicals that undergo a broad range of reactions.[1–3] The substrate spectrum of laccases is broad and include phenols, *o*- and *p*-diphenols, methoxyphenols, aminophenols, polyphenols, aryl thiols, anilines, polyamines, and lignin-related molecules.[1–3]

Features of laccases that have made them attractive for application in organic synthesis and green chemistry include: (i) the use of non-toxic atmospheric oxygen to catalyse the monoelectronic oxidation of substrates; (ii) the production of only water as a by-product; (iii) high oxidative selectivity; and (iv) they can function under mild and environmentally friendly reaction conditions.[5]

Interest in the application of laccase-catalysed oxidations has grown significantly in recent years and has led to several reports in organic synthesis. These reports show that laccases catalyse C–N[6] or C=N[7] bond formation in their reactions with amines and C–C,[8] C–O[9] and C=C[10] bond formations in their reactions with phenolic substrates. Laccase-catalysed bond formation has been successfully applied in green chemistry for the dimerisation of various compounds such as penicillin X,[11] bisphenol A,[12] salicyclic acids,[13] estradiol,[14] oxidative domino reactions of dibenzofuranones,[15] oxidative coupling of hydroquinone and mithramicine[16] or (+)-catechin,[17] oxidation of substituted imidazoles,[18] derivatisation of L-tryptophan,[19] dihydrocaffeic acid[20] and *para*-dihydroxylated compounds,[21] the synthesis of substituted triazolobenzothiadiazinones,[22] naphthoquinone,[23] cinnabarinic acid[24] and actinocin.[25] The synthesis of cycloheptenes, cyclooctenes, diazaspiro cyclohexenes, and phenazines was also accomplished[26] as well as the regioselective synthesis of substituted *para*-benzoquinones.[27]

A class of natural and synthetic compounds that have several beneficial effects are the quinones.[28] They improve general health conditions by their antioxidant activity and as vitamins they are used to prevent and treat several illnesses such as osteoporosis and cardiovascular diseases. Quinones are found in some anticancer,[29] antibacterial,[30] antifungal[31] and antimalarial agents.[32] Many of the drugs that have been clinically approved or are still in clinical trials against cancer are quinone related compounds. A subgroup of quinones are the 1,4-naphthoquinones that have displayed anticancer activity.[33]

In our laboratories we have been interested in the use of enzymes to develop green methods of synthesis and also to access compounds that may have potential pharmaceutical value. Since laccase-catalysed C–S bond formation was not reported in the literature we decided to investigate whether laccase could catalyse this type of bond formation. In this paper we report on the synthesis of 1,4-naphthoquinone-2,3-bis-sulfides, which is, to the best of our knowledge, the first using the enzyme laccase. We have previously reported on the synthesis of diaminobenzoquinones and aminonaphthoquinones using the commercial laccases Denilite® II Base and Novozymes 51003 respectively.[6]

Results and discussion

The 1,4-naphthohydroquinones and the aryl and alkyl thiols used for the syntheses of the 1,4-naphthoquinone-2,3-bis-sulfides are depicted in Fig. 1.

The initial goal of our investigation was to synthesise the 1,4-naphthoquinone monosulfide **12** (Scheme 1).

In our initial investigation, the synthesis entailed the reaction of hydroquinone **1** with one equivalent of thiol **3–9** in succinate-lactate buffer (pH 4.5) and DMF using the laccase Novozym 51003 (Scheme 1). Novozym 51003 is a robust, stable laccase

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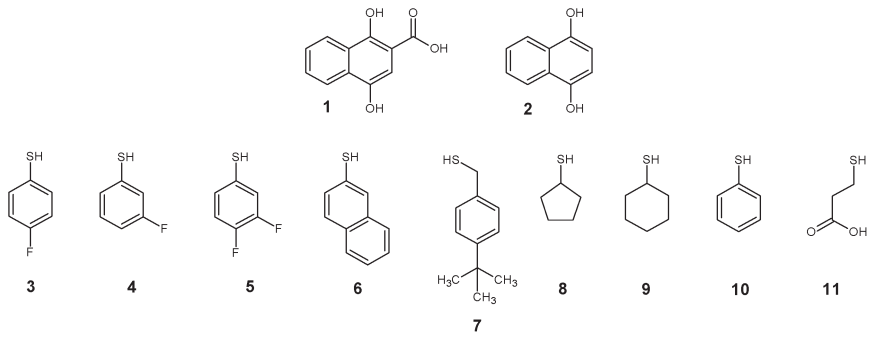
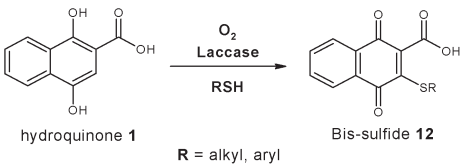


Fig. 1 The 1,4-naphthohydroquinones and the aryl and alkyl thiols used in this study.



Scheme 1

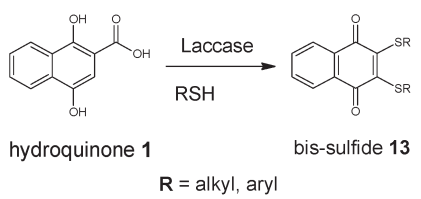
Table 1 Synthesised 1,4-naphthoquinone-2,3-bis-sulfides (yield in parentheses) at 37 °C in aqueous DMF using Method A[a]

Entry	Hydroquinone	Thiol (1 eq.)	Reaction time (h)	Product
1	**1**	**3**	72	**14** (6%)
2	**1**	**4**	72	**15** (4%)
3	**1**	**5**	72	**16** (8%)
4	**1**	**6**	72	**17** (5%)
6	**1**	**7**	72	**18** (10%)
7	**1**	**8**	72	**19** (20%)
8	**1**	**9**	72	**20** (13%)

[a] Method A: 37 °C, 5.0 mL (5550 U) laccase, 1 eq. thiol, 1,4-naphthohydroquinone **1** (0.6 mmol), 1.0 mL DMF, 2.0 mL succinate-lactate buffer (pH 4.5), 2.0 mL H_2O.

used for lignin modification within pulps and effluents. It is produced by submerged fermentation of genetically modified *Aspergillus* sp. and has a molecular weight of 56 000 Da. The results of these investigations are shown in Table 1. From the analysis of the data for the isolated products of these reactions reported in Table 1 it was found that only the 1,4-naphthoquinone-2,3-bis-sulfide **13** was formed (Scheme 2). This product was obtained in very low yield as can be seen in entries 1–8 in Table 1.

The low yields are attributed to the formation of the bis-sulfide product **13** instead of the anticipated monosulfide **12**. This is because the formation of the bis-sulfide **13** resulted in unreacted 1,4-naphthoquinone remaining since two molecules of thiol reacts with one molecule of the 1,4-naphthoquinone. The highest yield obtained was for **19** (20%, entry 7, Table 1) and the lowest for **15** (4%, entry 2 Table 1). A negative control reaction was also conducted from which it was evident that the reaction did not proceed without laccase.



Scheme 2

From the results in Table 1 it can be seen that the reaction of the 1,4-naphthoquinone **1** with the smallest thiol **8** resulted in the highest yield of bis-sulfide, **19**, while the reaction with the largest thiol **6** afforded the lowest yield of bis-sulfide, **17**. It appears that as the bulk of the thiol increases the yield of the bis-sulfide **13** decreases.

The structures of the 1,4-naphthoquinone-2,3-bis-sulfides **14–20** are shown in Fig. 2.

From these results it was apparent that the yield of the bis-sulfide **13** had to be increased and several parameters were taken into account for developing synthesis methods *i.e.* the pH, the number of equivalents of thiol, reaction time, and units of enzyme. Among these parameters, the one that could lead to a definite increase in yield of bis-sulfide **13** was the number of equivalents of thiol.

This led us to investigate selected reactions with two (Method B), three (Methods C, D, E) and five equivalents (Method F) of thiol. Three and five equivalents of thiol were used because an excess could promote the formation of the bis-sulfide **13**. Parameters such as reaction time and units of enzyme were also varied in the different methods that were used.

It was also decided to investigate the synthesis of the bis-sulfide **13** at pH 7.15 (Method G) to determine whether the laccase could also oxidise the hydroquinone **1** at this pH and thus catalyse the formation of the bis-sulfide **13**. It was postulated that at a higher pH, the formation of the product (C–S bond formation) would be favoured since the thiols would be less protonated. A possible drawback at this pH was that the laccase might not have oxidised hydroquinone **1**. The results of the investigations are shown in Table 2 below.

From the results in Table 2 it can be seen that the yield of the product was still low when two equivalents of the thiol was used.

Better yields were obtained when three equivalents of thiol was used. The highest yield was for **22** (69%, entry 26, Table 2) and the lowest for **21** (6%, entry 24, Table 2). The yield of **15** (18%, entry 4, Table 2), using Method E, increased at least 4-fold when compared to Method A (4%, entry 2, Table 1). The yield of **17** (29%, entry 11, Table 2), using Method C, was also

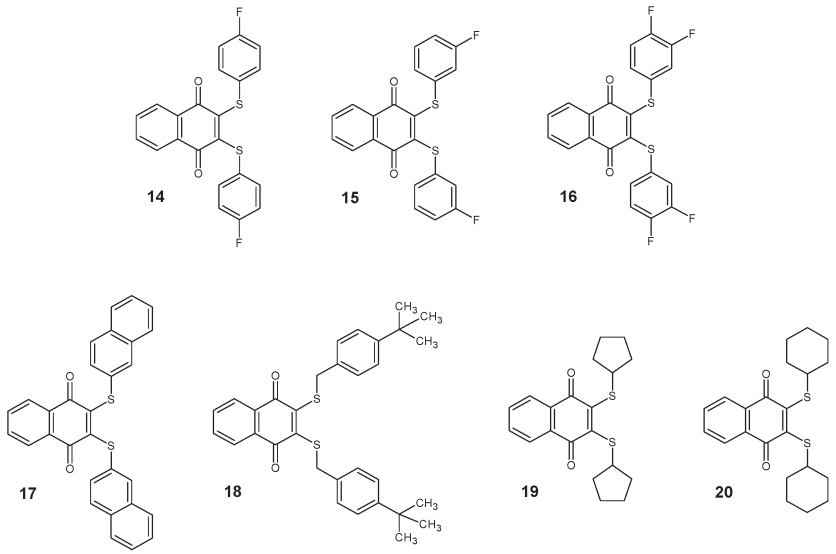


Fig. 2 The synthesised 1,4-naphthoquinone-2,3-bis-sulfides.

Table 2 Synthesised 1,4-naphthoquinone-2,3-bis-sulfides (yield in parentheses) at 35 °C in aqueous DMF using Methods B, C, D, E, F and G[a]

Entry	Hydroquinone	Thiol (eq.)	pH	Reaction time (h)	Method	Product
1	**1**	**3** (2)	4.5	52	B	**14** (17%)
2	**1**	**3** (5)	4.5	49	F	**14** (38%)
3	**1**	**3** (3)	7.15	48	G	**14** (30%)
4	**1**	**4** (3)	4.5	48	E	**15** (18%)
5	**1**	**4** (3)	7.15	48	G	**15** (12%)
6	**1**	**5** (2)	4.5	52	B	**16** (8%)
7	**1**	**5** (3)	4.5	45	C	**16** (21%)
8	**1**	**5** (3)	4.5	72	D	**16** (18%)
9	**1**	**5** (3)	7.15	48	G	**16** (14%)
10	**1**	**6** (2)	4.5	52	B	**17** (8%)
11	**1**	**6** (3)	4.5	45	C	**17** (29%)
12	**1**	**6** (3)	7.15	48	G	**17** (27%)
13	**1**	**7** (3)	4.5	72	D	**18** (21%)
14	**1**	**7** (3)	4.5	48	E	**18** (14%)
15	**1**	**7** (5)	4.5	49	F	**18** (26%)
16	**1**	**7** (3)	7.15	48	G	**18** (18%)
17	**1**	**8** (3)	4.5	48	E	**19** (19%)
18	**1**	**8** (3)	7.15	48	G	**19** (43%)
19	**1**	**9** (2)	4.5	48	B	**20** (18%)
20	**1**	**9** (3)	4.5	45	C	**20** (15%)
21	**1**	**9** (3)	4.5	72	D	**20** (37%)
22	**1**	**9** (5)	4.5	49	F	**20** (24%)
23	**1**	**9** (3)	7.15	48	G	**20** (16%)
24	**1**	**10** (3)	4.5	48	E	**21** (6%)
25	**1**	**10** (5)	4.5	49	F	**21** (32%)
26	**1**	**11** (3)	4.5	48	E	**22** (69%)
27	**1**	**11** (3)	7.15	48	G	**22** (30%)

[a] Method B: 35 °C, 4.0 mL (4440 U) laccase, 2 eq. thiol, 1,4-naphthohydroquinone **1** (0.6 mmol), 1.0 mL DMF, 2.0 mL succinate-lactate buffer (1.0 M, pH 4.5), 2.0 mL H_2O. Method C: 35 °C, 6.0 mL (6660 U) laccase, 3 eq. thiol, 1,4-naphthohydroquinone **1** (0.6 mmol), 1.0 mL DMF, 2.0 mL succinate-lactate buffer (1.0 M, pH 4.5), 2.0 mL H_2O. Method D: 37 °C, 6.0 mL (6660 U) laccase, 3 eq. thiol, 1,4-naphthohydroquinone **1** (0.6 mmol), 1.0 mL DMF, 2.0 mL succinate-lactate buffer (1.0 M, pH 4.5), 2.0 mL H_2O. Method E: 35 °C, 3.5 mL (3 885 U) laccase, 3 eq. thiol, 1,4-naphthohydroquinone **1** (0.6 mmol), 1.0 mL DMF, 2.0 mL succinate-lactate buffer (1.0 M, pH 4.5), 2.0 mL H_2O. Method F: 35 °C, 4.0 mL (4 440 U) laccase, 5 eq. thiol, 1,4-naphthohydroquinone **1** (0.6 mmol), 1.0 mL DMF, 2.0 mL succinate-lactate buffer (1.0 M, pH 4.5), 2.0 mL H_2O. Method G: 35 °C, 3.0 mL (3 330 U) laccase, 3 eq. thiol, 1,4-naphthohydroquinone **1** (0.6 mmol), 1.0 mL DMF, 2.0 mL potassium phosphate buffer (0.1 M, pH 7.15), 2.0 mL H_2O.

improved. It increased almost 6-fold when compared to Method A (5%, entry 4, Table 1) and almost 4-fold when compared to Method B (8%, entry 10, Table 2). Similarly the yield of **18** (21%, entry 13, Table 2), using Method D, was increased at least 2-fold when compared to Method A (10%, entry 6, Table 1). The yield of **20** (37%, entry 21, Table 2), also using Method D,

increased 3-fold when compared to Method A (13%, entry 8, Table 1) and also 2.5-fold when compared to Method C (15%, entry 20, Table 2).

The additional synthesised 1,4-naphthoquinone-2,3-bis-sulfides, **21** and **22**, are shown in Fig. 3.

The yield of the bis-sulfide was much better when five equivalents of thiol (Method F) was used as can be seen in Table 2. The highest yield was obtained for **14** (38%, entry 2) and the lowest for **20** (24%, entry 22).

The yield for **14** (38%, entry 2, Table 2) increased at least 6-fold when compared to Method A (6%, entry 1, Table 1). For **18** (26%, entry 15, Table 2) there was at least a 2.5-fold increase when compared to Method A (10%, entry 6, Table 1) and almost a 2-fold increase when compared to Method E (14%, entry 14, Table 2). There was also almost a 2-fold increase in yield for **20** (24%, entry 22, Table 2) when compared to Method A (13%, entry 8, Table 1) and for **21** (32%, entry 25, Table 2) there was at least a 5-fold increase when compared to Method E (6%, entry 24, Table 2).

From the results in Table 2 it can also be seen that the bis-sulfides can also be synthesised at pH 7.15 using Method G. The yields were compared to Methods C, D and E where three equivalents of thiol were also used. It was found that the yield of the product using Method G was generally slightly lower except for **22** (30%, entry 27, Table 2) where the yield was much lower *i.e.* by 29%. In the case of **19** (43%, entry 18, Table 2), the yield increased at least 2-fold when compared to Method E (19%, entry 17, Table 2).

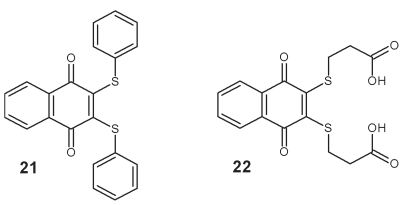
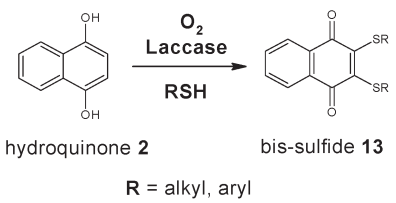


Fig. 3 Additional synthesised 1,4-naphthoquinone-2,3-bis-sulfides.



Scheme 3

The success of the synthesis of the bis-sulfides **14**–**22** from the hydroquinone **1** led us to investigate the synthesis from hydroquinone **2** (Scheme 3).

The reactions were investigated using Methods H (pH 4.5) and I (pH 7.15) each of which utilised three equivalents of thiol. The results of these investigations are shown in Table 3.

From the results in Table 3 it can be seen that the bis-sulfides can also be accessed from the hydroquinone **2** at pH 4.5 and 7.15.

The results obtained using Method I were compared to those obtained using Method G. The yield of **19** (9%, entry 8) was less, by at least 4.5-fold, when compared to that obtained using Method G (43%, entry 18, Table 2). The yield of **22** (56%, entry 11) was almost 2-fold higher than that obtained using Method G (30%, entry 27, Table 2).

Overall, at pH 7.15, the yields of the bis-sulfides from hydroquinone **2** (Method I) were lower than those from hydroquinone **1** (Method G) and it may thus be concluded that hydroquinone **1** may be a better substrate for laccase for the synthesis of the bis-sulfides at this pH.

β-Keto acid decarboxylation is known in organic chemistry[34] as well as in biological systems.[35] The key step in the biosynthesis of terpenoids, steroids, and neurotransmitter amino compounds is the decarboxylation step.[36] Decarboxylations of a variety of β-keto acid systems have also been used as models for enzymatic reactions.[37]

A proposed mechanism for the formation of the 1,4-naphthoquinone-2,3-bis-sulfides **14**–**22** from the 1,4-naphthohydroquinone **1** is shown in Fig. 4a and from the 1,4-naphthohydroquinone **2** in Fig. 4b. For the proposed mechanism in Fig. 4a, the 1,4-naphthohydroquinone carboxylic acid **1** is oxidised by laccase to the 1,4-naphthoquinone-2-carboxylic acid (a β-keto acid). The latter then undergoes a Michael addition by a thiol to give a keto-enol intermediate. This then undergoes oxidation by

Table 3 Synthesised 1,4-naphthoquinone-2,3-bis-sulfides (yield in parentheses) at 35 °C using Methods H and I[a]

Entry	Hydroquinone	Thiol (3 eq.)	pH	Reaction time (h)	Method	Product
1	**2**	**3**	4.5	48	H	**14** (34%)
2	**2**	**4**	4.5	48	H	**15** (16%)
3	**2**	**5**	4.5	48	H	**16** (6%)
4	**2**	**6**	4.5	48	H	**17** (12%)
5	**2**	**7**	4.5	48	H	**18** (34%)
6	**2**	**7**	7.15	48	I	**18** (15%)
7	**2**	**8**	4.5	48	H	**19** (4%)
8	**2**	**8**	7.15	48	I	**19** (9%)
9	**2**	**9**	4.5	48	H	**20** (14%)
10	**2**	**10**	4.5	48	H	**21** (12%)
11	**2**	**11**	7.15	48	I	**22** (56%)

[a] Method H: 35 °C, 6.0 mL (6660 U) laccase, 3 eq thiol, 1,4-naphthohydroquinone **2** (0.6 mmol), 1.0 mL DMF, 2.0 mL succinate-lactate buffer (1.0 M, pH 4.5), 2.0 mL H_2O. Method I: 35 °C, 6.0 mL (6660 U) laccase, 3 eq thiol, 1,4-naphthohydroquinone **2** (0.6 mmol), 1.0 mL DMF, 2.0 mL potassium phosphate buffer (0.1 M, pH 7.15), 2.0 mL H_2O.

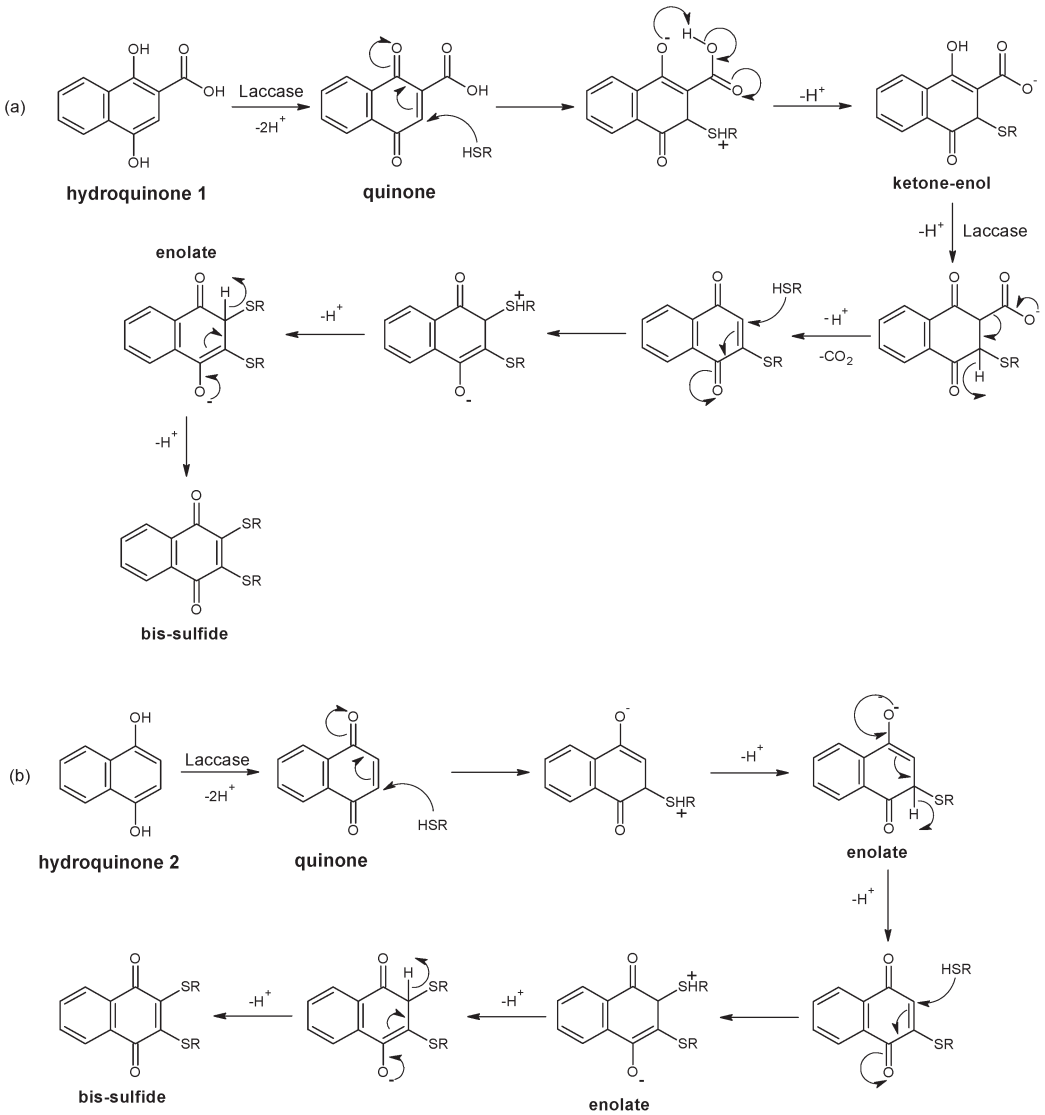


Fig. 4 Proposed mechanisms for the formation of 1,4-naphthoquinone-2,3-bis-sulfides from (a) 1,4-naphthohydroquinone **1** and (b) 1,4-naphthohydroquinone **2**.

laccase followed by acid catalysed decarboxylation to afford a 1,4-naphthoquinone monosulfide. The latter then undergoes a second thiol Michael addition resulting in a keto-enol intermediate which subsequently affords the 1,4-naphthoquinone-2,3-bis-sulfides **14**–**22**.

For the proposed mechanism in Fig. 4b, the 1,4-naphthohydroquinone **2** is oxidised by laccase to the 1,4-naphthoquinone which then undergoes Michael addition by a thiol to afford an enolate before forming a naphthoquinone monosulfide as intermediate. The latter then undergoes a second thiol addition resulting in an enolate intermediate before affording the 1,4-naphthoquinone-2,3-bis-sulfides **14**–**22**.

Several chemical methods for the synthesis of 1,4-naphthoquinone-2,3-bis-sulfides have been reported in the literature. 2,3-Bis(arylthio)-1,4-naphthoquinones were obtained by Tandon *et al.*,[38] Ryu *et al.*,[39] Fieser *et al.*[40] and Tjepkema[41] by a substitution reaction with 2,3-dichloro-1,4-naphthoquinone derivatives and the appropriate arylthiol in ethanol by heating to reflux or stirring at room temperature. Fieser obtained **17** and Tandon *et al.* obtained **21** by this route.[38] Ibis *et al.* obtained **20** by reacting 2,3-dichloro-1,4-naphthoquinone with **9** and stirring in an ethanolic solution of sodium carbonate at room temperature.[42] Blackhall *et al.* synthesised **22** by reacting 2,3-dibromo-1,4-naphthoquinone with **11** in ethanol and pyridine.[43]

The 1,4-naphthoquinone-2,3-bis-sulfides are classically synthesised from 2,3-dichloro-1,4-naphthoquinone, also known as dichlone.[40] This compound is harmful if swallowed, an irritant to the eyes and skin, extremely toxic to aquatic organisms, and may cause long-term adverse effects in the aquatic environment.[44] 2,3-Dichloro-1,4-naphthoquinone is synthesised in three steps from 1,4-naphthohydroquinone. The first step entails methylation (sulphuric acid in methanol) of the hydroxyl groups, the second entails chlorination (sulfuryl chloride and chloroform), and the third entails oxidation (cerium ammonium nitrate) of the methoxy groups to the ketones resulting in 2,3-dichloro-1,4-naphthoquinone. Sulfuryl chloride is toxic, corrosive and is a

lachrymator. In addition, it can form explosive mixtures with water and donor solvents such as DMSO and DMF.[45]

When 2,3-dichloro-1,4-naphthoquinone is reacted with an alkyl thiol by refluxing in an alcoholic solution, only one of the chlorine atoms is replaced and affords the 1,4-naphthoquinone monosulfide even in the presence of excess alkyl thiol. In order to obtain the 1,4-naphthoquinone-2,3-bis-sulfide, sodium salts of the alkyl thiols have to be prepared to react with the 2,3-dichloro-1,4-naphthoquinone.[40] When aryl thiols are reacted with 2,3-dichloro-1,4-naphthoquinone, the 1,4-naphthoquinone-2,3-bis-sulfide is readily obtained.[40] A limitation of this approach is that a sodium salt of the alkyl thiol first has to be prepared and that it cannot react directly with the latter. In light of the above, new methods are required for the synthesis of 1,4-naphthoquinone-2,3-bis-sulfides. The enzymatic synthesis of these compounds was therefore explored in order to develop a safer, shorter and environmentally friendly synthetic route to 1,4-naphthoquinone-2,3-bis-sulfides.

Our laccase method offers several advantages. For example, 1,4-naphthohydroquinone can be used as the starting point. Other advantages are that a chemical oxidant (*e.g.* sodium periodate), the halogenation step (*e.g.* chlorination or bromination), and the activation of alkyl thiols by sodium, are not required. In addition, less organic solvent is used and the reactions can be conducted under mild conditions (35 or 37 °C).

Free thiols (RSH) are known to be potent inhibitors of laccases. Inhibition occurs presumably by co-ordination of the thiol to the copper atoms in the enzyme active site.[46] Halides are also known to inhibit laccase and the fluoro substituents could also have contributed to the low yields for **14–16**.[1a,47] Aryl thiols are also substrates of laccases.[1b] In spite of this, we have shown that 1,4-naphthoquinone-2,3-bis-sulfides can be synthesised in the presence of free thiols using laccase.

In summary, this one-pot laccase method allows direct access to 1,4-naphthoquinone-2,3-bis-sulfides from both the 1,4-naphthohydroquinone-2-carboxylic acid **1** and the 1,4-naphthohydroquinone **2** in the reaction with an aryl or alkyl thiol at both pH 4.5 and 7.15. The use of a chemical oxidant and a chlorinated intermediate has been circumvented. In addition, there is also no need for the activation of alkyl thiols with sodium. Green solvents such as *N*,*N*′-dimethylpropyleneurea (a DMF equivalent), 1,3-propanediol, 1,3-dioxolane and 2-methyltetrahydrofuran can also be used as co-solvents in place of DMF.

Conclusions

We have developed, *via* C–S bond formation, a one-pot laccase method that allows direct access to 1,4-naphthoquinone-2,3-bis-sulfides from both the 1,4-naphthohydroquinone-2-carboxylic acid **1** and the 1,4-naphthohydroquinone **2** in a reaction with an aryl or alkyl thiol at pH 4.5 and 7.15.

This method has eliminated the use of a chemical oxidant, a chlorinated intermediate and there is also no need for the activation of alkyl thiols with sodium. Product formation is affected by factors such as the pH of the reaction mixture, the solubility of the starting materials, nucleophilicity, and the number of equivalents of thiol.

This work is a proof-of-principle and therefore further improvement is required to optimise the yields of these C–S bond forming reactions. Other laccases may be more resistant to thiol inhibition and thus could be better for the synthesis of 1,4-naphthoquinone-2,3-bis-sulfides. There is also an opportunity to develop laccases which are resistant to thiol inhibition through rational design and/or directed evolution.

The application of laccases in organic chemistry has been broadened and constitutes an additional tool for organic chemists, particularly in the area of fine chemical synthesis.

Experimental

General

^{1}H NMR spectra were recorded on a Varian Mercury 200 MHz spectrometer. ^{13}C NMR spectra were recorded on the same instruments at 50 MHz. Chemical shifts are reported in ppm relative to the solvent peaks. High-resolution mass spectra were recorded on a Waters HPLC coupled to a Synapt HDMS mass spectrometer. Reactions were monitored by thin layer chromatography (TLC) on aluminium-backed Merck silica gel 60 F_{254} plates. Gravity column chromatography was done using Merck silica gel 60 (70–230 mesh). Melting points were determined using a Glassco melting point apparatus and are uncorrected.

Materials

All chemicals were reagent grade materials.

Substrates

The 1,4-naphthohydroquinones and the aryl and alkyl thiols were obtained from Sigma-Aldrich South Africa.

Enzymes

The laccase, Novozyme 51003 (1 110.00 LAMU/G), was obtained from Novozymes SA.

Synthesis

Several methods (Methods A–I) were used for the synthesis of the 1,4-naphthoquinone-2,3-bis-sulfides and are depicted in Table 4. A procedure for conducting the reactions is shown for Method A below:

Method A. The laccase, Novozymes 51003 (2.0 mL), was added to a mixture of the thiol (0.6 mmol, 1 equivalent), the 1,4-naphthohydroquinone **1** (0.6 mmol), succinate-lactate buffer (2.0 mL, 1.0 M, pH 4.5), ddH_2O (2.0 mL) and DMF (1.0 mL). The reaction mixture was heated at 37 °C while stirring and monitored by TLC (silica/EtOAc) to check for the disappearance of the 1,4-naphthohydroquinone **1**. More enzyme (1.0 mL) was added after 1.5, 3, and 4.5 h. After heating for 72 h the reaction mixture was transferred to a separating funnel (250 mL) to which ddH_2O (60.0 mL) was added. The resulting mixture was

Table 4 Reaction methods used for the synthesis of 1,4-naphthoquinone-2,3-bis-sulfides

Method	HQ[a] (mmol)	Thiol (mmol)	Buffer conc. (M)	Buffer (mL)	Buffer (pH)	DMF (mL)	Water (mL)	Enzyme vol. at T_0 (mL)	Enzyme addition (h)	Enzyme volume (mL)	Total enzyme vol. (mL)	Reaction time (h) temp. (°C)	Purif. & method[b]
A	**1** (0.6)	(0.6)	s–l[c] (1.0)	4.5	2.0	1.0	2.0	2.0	1.5, 3, 4.5	1.0	5.0	72 (37)	fc[e]
B	**1** (0.6)	(1.2)	s–l[c] (1.0)	4.5	2.0	1.0	2.0	1.5	2, 4	1.0	4.0	48–52 (35)	fc[e]
									20	0.5			
C	**1** (0.6)	(1.8)	s–l[c] (1.0)	4.5	2.0	2.0	2.0	2.0	19, 26	1.0	4.0	45 (35)	fc[e]
D	**1** (0.6)	(1.8)	s–l[c] (1.0)	4.5	2.0	1.0	2.0	2.0	1.5, 3, 27, 54	1.0	6.0	72 (37)	fc[e]
E	**1** (0.6)	(1.8)	s–l[c] (1.0)	4.5	2.0	1.0	2.0	1.5	1.5, 3	1.0	3.5	48 (35)	fc[e]
F	**1** (0.6)	(5.0)	s–l[c] (1.0)	4.5	2.0	1.0	2.0	2.0	1, 2	1.0	4.0	49 (35)	fc[e]
G	**1** (0.6)	(1.8)	pp[d] (0.1)	7.15	2.0	1.0	2.0	2.0	3, 24	0.5	3.0	48 (35)	plc[f]
H	**2** (0.6)	(1.8)	s–l[c] (1.0)	4.5	2.0	1.0	2.0	2.0	1, 2, 21, 26	1.0	6.0	48 (35)	fc[e]
I	**2** (0.6)	(1.8)	pp[d] (0.1)	7.15	2.0	1.0	2.0	2.0	3, 24	0.5	3.0	48 (35)	plc[f]

[a] HQ: hydroquinone. [b] Purif.: purification. [c] s–l: Succinate–lactate. [d] pp: Potassium phosphate. [e] fc: Flash chromatography. [f] plc: Preparative layer chromatography.

extracted with EtOAc, the solvent evaporated, and the residue purified by flash chromatography.

This procedure was also followed for Methods B to I and the differences in the methods can be seen in Table 4.

A. Dithiolation of 1,4-naphthohydroquinone 1 with aryl and alkyl thiols. Methods A–G were used for the synthesis of the 1,4-naphthoquinone-2,3-bis-sulfides from the 1,4-naphthohydroquinone **1**.

B. Dithiolation of 1,4-naphthohydroquinone 2 with aryl and alkyl thiols. Methods H and I were used for the synthesis of the 1,4-naphthoquinone-2.3-bis-sulfides from 1,4-naphthohydroquinone **2**. Method I is the same as Method G except that 1,4-naphthohydroquinone **2** was used as substrate. For both methods H and I the reactions were monitored by TLC (silica: EtOAc/hexane, 1 : 1) to check for the disappearance of the 1,4-naphthohydroquinone **2**.

2,3-Bis[(4-fluorophenyl)thio]naphthoquinone 14

Method A. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40 and 1 : 20) to afford an orange-brown solid (0.0144 g, 6%). R_f = 0.19 (EtOAc/hexane, 1 : 24).

Method B. Stirring time = 52 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40, 1 : 20 and 1 : 10) to afford an orange-brown solid (0.0430 g, 17%).

Method F. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 90, 1 : 80, 1 : 70, 1 : 60 and 1 : 50) to afford an orange-brown solid (0.0929 g, 38%).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 2) to afford a light-brown solid (0.0741 g, 30%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 75, 1 : 50 and 1.5 : 50) to afford a light-brown solid (0.0289 g, 6%). mp: 153–154 °C; TLC (EtOAc/hexane, 1 : 19 v/v): R_f = 0.25; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.95 (2H, m, ArH), 7.66 (2H, m, ArH), 7.38 (4H, m, ArH), 7.00 (4H, t J = 8.4, 8.8 Hz, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.6, 165.0, 160.1, 147.9, 138.2, 133.9, 133.8, 133.6, 132.6, 128.3, 127.1, 116.6, 116.1; HRMS (*m*/*z*): $[M - H]^+$ calcd for $C_{22}H_{11}F_2O_2S_2$, 409.0169); found: 409.0178.

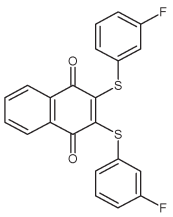
2,3-Bis[(3-fluorophenyl)thio]naphthoquinone 15

Method A. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40 and 1 : 20) to afford a yellow-brown solid (0.0094 g, 4%). R_f = 0.14 (EtOAc/hexane, 1 : 24).

Method E. Stirring time = 48 h. Purification by preparative layer chromatography (silica: EtOAc/hexane, 1 : 12) to afford a yellow-brown solid (0.0443 g, 18%).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 2.5, 1 : 1.5) to afford a dark-brown solid (0.0291 g, 12%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 80, 1 : 60, 1 : 40, 1 : 30 and 1 : 20) to afford an orange solid (0.0401 g, 16%). mp: 118–120 °C; TLC (EtOAc/hexane, 1 : 19 v/v): R_f = 0.13; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.00 (2H, m, ArH), 7.69 (2H, m, ArH), 6.92–7.36 (8H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.4, 165.0, 160.2, 148.2, 138.4, 135.3, 135.2, 134.0, 132.5, 130.5, 130.4, 127.3, 126.6, 126.5, 118.1, 117.7, 115.2, 114.9. HRMS (*m*/*z*): $[M - H]^+$ calcd for $C_{22}H_{11}F_2O_2S_2$, 409.0169; found, 409.0157.



2,3-Bis[(3,4-difluorophenyl)thio]naphthoquinone 16

Method A. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 50 and 1 : 25) to afford a yellow solid (0.0222 g, 8%).

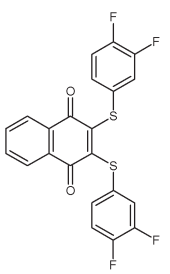
Method B. Stirring time = 52 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40, 1 : 20 and 1 : 10) to afford a yellow solid (0.0217 g, 8%).

Method D. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 50 and 1 : 25) to afford a red-brown solid (0.0385 g, 14%). R_f = 0.31 (EtOAc/hexane, 1 : 9).

Method C. Stirring time = 45 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 60, 1 : 50, 1 : 40, 1 : 30 and 1 : 20) to afford a yellow solid (0.0560 g, 21%). R_f = 0.17 (EtOAc/hexane, 1 : 19).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 2) to afford a yellow-brown solid (0.0494 g, 18%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 1 : 60, 1 : 40, 1 : 30 and 1 : 20) to afford a yellow solid (0.0168 g, 6%). mp: 123–125 °C; TLC (EtOAc/hexane, 1 : 19 v/v): R_f = 0.16; ^{1}H NMR (200MHz, $CDCl_3$): δ 7.97 (2H, m, ArH), 7.69 (2H, m, ArH), 7.06–7.38 (6H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 181.8, 178.2, 167.1, 160.0, 152.8, 152.6, 148.0, 147.8, 147.6, 134.5, 134.1, 133.5, 132.4, 128.3, 2 × 128.2, 128.1, 128.0, 127.3, 126.9, 126.6, 121.0, 120.6, 118.2, 117.9. HRMS (*m*/*z*): $[M - H]^+$ calcd for $C_{22}H_9O_2F_4S_2$, 444.9980; found, 444.9970.



2,3-Bis-(naphthalen-2-ylsulfanyl)-[1,4]naphthoquinone 17

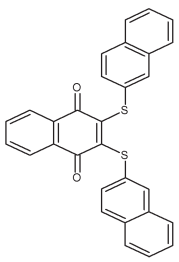
Method A. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 50, 1 : 25 and 1 : 13) to afford a red-brown powder (0.0151 g, 5%).

Method B. Stirring time = 52 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 50, 1 : 40, 1 : 30, 1 : 20, 1 : 10, 1 : 5) to afford a red-brown powder (0.0216 g, 8%). R_f = 0.28 (EtOAc–hexane, 1 : 49).

Method C. Stirring time = 45 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 60, 1 : 50, 1 : 40, 1 : 30, 1 : 20, 1 : 10 and 1 : 5) to afford a red-brown powder (0.0799 g, 29%). R_f = 0.13 (EtOAc/hexane, 1 : 19).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 2, 1 : 1.5) to afford a red solid (0.0752 g, 27%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 60, 1 : 50, 1 : 40, 1 : 30 and 1 : 20) to afford a red-brown powder (0.0340 g, 12%). mp: 165–167 °C (lit.[48] 196–197 °C); TLC (EtOAc/hexane, 1 : 19): R_f = 0.14; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.98 (2H, m, 2 × ArH), 7.62–7.86 (10H, m, ArH), 7.35–7.45 (6H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.9, 147.8, 133.8, 133.5, 132.7, 132.5, 130.7, 130.2, 128.7, 128.4, 127.8, 127.6, 127.2, 126.7, 126.6. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{30}H_{19}O_2S_2$, 475.0826; found, 475.0763.



2,3-Bis-(4-tert-butyl-benzylsulfanyl)-[1,4]naphthoquinone 18

Method A. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 50 and 1 : 25) to afford a red solid (0.0304 g, 10%). R_f = 0.35 (EtOAc/hexane, 1 : 24).

Method C. Stirring time = 45 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 40, 1 : 30 and 1 : 20) to afford a red crystalline solid (0.0089 g, 3%).

Method D. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 75, 1 : 50 and 1 : 25) to afford a red solid (0.0659 g, 21%).

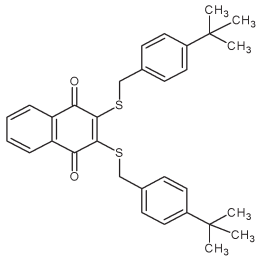
Method E. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 75, 1 : 50 and 1 : 25) to afford a red solid (0.0413 g, 14%).

Method F. Stirring time = 49 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 75, 1 : 50 and 1 : 25) to afford a red solid (0.0813 g, 26%).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 3) to afford a red solid (0.0405 g, 13%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 100, 1 : 80, 1 : 60, 1 : 50 and 1 : 40) to afford a red solid (0.1067 g, 34%). R_f = 0.40 (EtOAc/hexane, 1 : 19).

Method I. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 4) to afford an orange-red solid (0.0495 g, 15%). mp: 125–126 °C; TLC (EtOAc/hexane, 1 : 19 v/v): R_f = 0.16; ^{1}H NMR (200MHz, $CDCl_3$): δ 8.00 (2H, m, ArH), 7.65 (2H, m, ArH), 7.17–7.34 (8H, m, ArH), 4.44 (4H, s, 2 × CH_2), 1.26 (18H, m, 6 × CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.9, 179.2, 150.4, 147.7, 133.9, 133.3, 132.9, 129.0, 126.7, 125.5, 39.0, 34.5, 31.3; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{32}H_{35}O_2S_2$, 515.2078; found: 515.2078.



2,3-Bis(cyclopentylthio)naphthoquinone 19

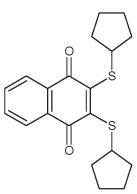
Method A. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 50 and 1 : 25) to afford a red solid (0.0429 g, 20%). R_f = 0.30 (EtOAc/hexane, 1 : 24).

Method E. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40 and 1 : 20) to afford a red solid (0.0420 g, 19%).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 4) to afford a dark orange-brown solid (0.0935 g, 43%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40, 1 : 30, and 1 : 20) to afford a yellow-brown crystalline material (0.0089 g, 4%).

Method I. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 4) to afford a dark orange-brown solid (0.0191 g, 9%). mp: 67–69 °C; TLC (EtOAc/hexane, 1 : 19 v/v): R_f = 0.41; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.05 (2H, m, 2 × ArH), 7.68 (2H, m, 2 × ArH), 4.29 (2H, m, 2 × CH), 1.86–2.10 (4H, m, 2 × CH_2), 1.72–1.88 (4H, m, 2 × CH_2), 1.50–1.69 (8H, m, 6 × CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 179.2, 148.9, 133.3, 133.0, 127.1, 47.1, 34.4, 24.9. HRMS (*m*/*z*) $[M + H]^+$ calcd for $C_{20}H_{23}O_2S_2$, 359.1139; found, 359.1113.



2,3-Bis-cyclohexylsulfanyl-[1,4]naphthoquinone.[42] 20

Method A. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 75, 1 : 50 and 1 : 25) to afford a dark-red semi-solid (0.0300 g, 13%).

Method B. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 75) to afford a dark-red semi-solid (0.0413 g, 18%). R_f = 0.26 (EtOAc/hexane, 1 : 49).

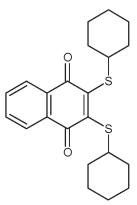
Method C. Stirring time = 45 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 75, 1 : 50 and 1 : 25) to afford a dark-red semi-solid (0.0861 g, 15%). R_f = 0.28 (EtOAc/hexane, 1 : 49).

Method D. Stirring time = 72 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 75, 1 : 50 and 1 : 25) to afford a dark-red semi-solid (0.0347 g, 37%). R_f = 0.27 (EtOAc/hexane, 1 : 49).

Method F. Stirring time = 72 h. Purification by flash chromatography (silica: hexane; EtOAc/hexane, 1 : 90) to afford a dark-red semi-solid (0.0545 g, 24%).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: DCM/hexane, 1 : 2) to afford a dark-red semi-solid (0.0379 g, 16%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40, 1 : 30 and 1 : 20) to afford a red-black semi-solid (0.0333 g, 14%). TLC (EtOAc/hexane, 1 : 49 v/v): R_f = 0.26; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.05 (2H, m, 2 × ArH), 7.67 (2H, m, 2 × ArH), 3.90 (2H, m, 2 × CH), 1.89–2.09 (4H, m, 2 × CH_2), 1.70–1.86 (4H, m, 2 × CH_2), 1.02–1.69 (12H, m, 6 × CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 179.2, 148.7, 133.3, 133.0, 126.9, 47.0, 34.1, 25.9, 25.6. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{27}O_2S_2$, 387.1452; found, 387.1451.

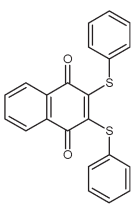


2,3-Bis-phenylsulfanyl-[1,4]naphthoquinone 21

Method E. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60 and 1 : 40) to afford a black solid (0.0630 g, 6%).

Method F. Stirring time = 49 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 70, 1 : 60 and 1 : 50) to afford a dark blue-black solid (0.0728 g, 32%).

Method H. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/hexane, 1 : 80, 1 : 60, 1 : 40, 1 : 30 and 1 : 20) to afford a light red-brown solid (0.0269 g, 12%). mp: 137–139 °C (lit.[49] 136 °C); TLC (EtOAc/hexane, 1 : 49 v/v): R_f = 0.29; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.97 (2H, m, ArH), 7.65 (2H, m, ArH), 7.24–7.42 (10H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 178.7, 148.3, 138.2, 133.8, 133.6, 132.7, 131.1, 129.1, 127.8, 127.2. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{15}O_2S_2$, 375.0513; found, 375.0421.

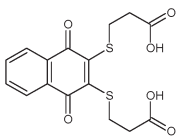


3-[3-(2-Carboxy-ethylsulfanyl)-1,4-dioxo-1,4-dihydro-naphthalen-2-ylsulfanyl]-propionic acid 22

Method E. Stirring time = 48 h. Purification by flash chromatography (silica: EtOAc/MeOH, 19 : 1, 9 : 1, 4 : 1 and 2 : 1) to afford a brown solid (0.0766 g, 69%). R_f = 0.15 (MeOH/EtOAc, 1 : 2).

Method G. Stirring time = 48 h. Purification by preparative layer chromatography (silica: MeOH/DCM, 1 : 25, 1 : 12) to afford a brown solid (0.0669 g, 30%).

Method I. Stirring time = 48 h. Purification by preparative layer chromatography (silica: MeOH/DCM, 1 : 25, 1 : 20, 1 : 15) to afford a brown solid (0.1234 g, 56%). mp 171–173 °C (lit.[43] 204 °C); TLC (MeOH/EtOAc, 1 : 10): R_f = 0.30. ^{1}H NMR (400 MHz, MeOH-d_4): δ 8.04 (2H, m, 2 × ArH), 7.75 (2H, m, 2 × ArH), 3.43 (4H, t J = 7.2, 6.8 Hz, 2 × CH_2), 2.67 (4H, t J = 7.2 Hz, 2 × CH_2); ^{13}C NMR (50 MHz, MeOH-d_4): δ 180.3, 149.2, 134.9, 134.6, 127.9, 37.0, 31.3; HRMS (m/z): $[M - H]^+$ calcd for $C_{16}H_{13}O_6S_2$, 365.0154; found, 365.0187.



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