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# 1,2-Dihydro-1,2-Dihydroxynaphthalene Dehydrogenase Containing Recombinant Strains: Preparation, Isolation and Characterisation of 1,2-Dihydroxynaphthalenes and 1,2-Naphthoquinones

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*Abstract*: 1,2-Dihydroxynaphthalenes are produced by dehydrogenation of the corresponding 1,2-dihydro-1,2-dihydroxynaphthalenes using an *Escherichia coli* recombinant strain containing the dihydrodiol naphthalene dehydrogenase gene cloned from *Pseudomonas fluorescens* N3. Conversions are led in carefully controlled conditions to minimise product polymerisation. A multistep procedure using a weakly basic resin permits isolation of good product amounts, solving the toxicity problem. Products are isolated and characterised as t-butyldimethylsilyl derivatives that are stable compounds. The transformation of the 1,2-dihydroxynaphthalenes into the corresponding 1,2-naphthoquinones is also reported. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords*: 1,2-Dihydroxynaphthalene, 1,2-naphthoquinone, bioconversion, t-butyldimethylsilyl derivative, recombinant strain

## INTRODUCTION

The interest in 1,2-dihydroxynaphthalenes can be ascribed to their importance in both material science and pharmacology. 1,2-Dihydroxynaphthalene itself is commonly used in the manufacturing of polystyrene and epoxy resins.[1,2] 1,2-Dihydroxynaphthalenes show high cytotoxic activity and related compounds (e.g. apomorphine) show pharmacological activity as emetic and anti-Parkinsonism drugs.[3] 1,2-Naphthoquinones are also important for their biological activity, having fungicide,[4] cytotoxic,[5] antitumoral,[6] antiviral,[7] and pesticide[8] effects.

However, 1,2-dihydroxynaphthalenes and 1,2-naphthoquinones are generally difficult to prepare with the classic methods of organic synthesis. The most used preparation of 1,2-dihydroxynaphthalenes is undoubtedly the reduction of the corresponding quinones using sodium dithionite[9] or sodium bisulphite.[10] Likewise preparative methods of 1,2-naphthoquinones are scarcely represented in the literature, in contrast with 1,4-naphthoquinones. Examples are the oxidation of β-naphthols with Fremy's salt[11] or with other oxidising agents,[12] or the condensation of pyruvic acid with benzene derivatives[4], or the oxidation of 1,2-dihydroxy naphthalenes using sodium persulfate (but these latter compounds are usually obtained from the quinones, thus this preparation is of limited utility).[13] In addition, the yields are quite often scarce; therefore their production

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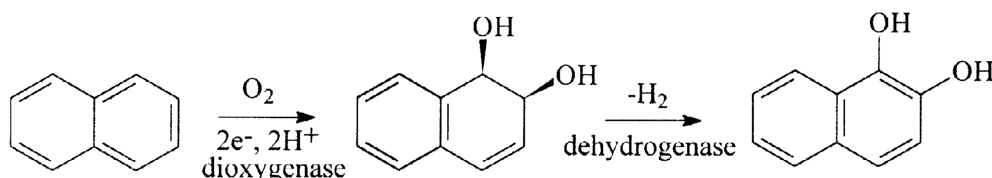
by bioconversion is appealing.

Microorganisms of the *Pseudomonas* type are well known for their capacity of degrading aromatic hydrocarbons, that are important xenobiotic compounds. Recently, we have turned our attention to the study of *Pseudomonas fluorescens* N3,[14] a microorganism grown using naphthalene as the sole energy and carbon source, degrading it to pyruvate, acetaldehyde, and $CO_2$ following the salicylic acid pathway.[15] In particular we studied the first two steps of the metabolic pathway (Scheme 1), the first transforming naphthalene into 1,2-dihydro-1,2-dihydroxynaphthalene[16] and the second transforming this latter compound into 1,2-dihydroxynaphthalene.[17]




**Scheme 1**. Two step oxidation of naphthalene: dioxygenation and dehydrogenation

The DNA genes responsible for the first two metabolic steps were localized and cloned in an *E.coli* JM109 recipient and two recombinant strains were obtained. The first, JM109 (pPS1778), contains the gene coding for the dioxygenase and showed the ability of converting naphthalenes to the chiral 1,2-dihydro-1,2-dihydroxynaphthalenes (diols), offering the possibility to obtain different optically active diols. The other, JM109 (pVL2028), contains the gene for the dehydrogenase. This second strain quantitatively converted 1,2-dihydro-1,2-dihydroxynaphthalenes into the corresponding 1,2-dihydroxynaphthalenes (catechols).

In a previous work we described the transformation system used to operate this bioconversion. The produced 1,2-dihydroxynaphthalenes are not stable in air and oxidise giving unidentifiable polymers in addition to the corresponding quinones. Since the net reaction does not need oxygen, we therefore utilised the anaerobic facultative nature of *E.coli*, conducting the bioconversions under inert atmosphere ($N_2$) in properly modified experimental conditions: deareation of the medium by $N_2$ insufflation, addition of pyruvate to favour the recycling of NADH to $NAD^+$, and strict control of all the bioconversion phases by continuously maintaining the inert atmosphere ($N_2$).

Nevertheless we still had some problems to solve because of the great difficulty in isolating and conserving the bioconversion products. In this paper, we are going to address these problems; in particular we will discuss: a) the rate of bioconversion of different substrates into corresponding 1,2-dihydroxy derivatives; b) the increase of production yield of 1,2-dihydroxynaphthalenes obtained from the corresponding 1,2-dihydro-1,2-dihydroxynaphthalenes; c) the isolation of 1,2-dihydroxynaphthalenes in spite of their easy atmospheric oxidation; d) the synthesis of 1,2-naphtoquinones from corresponding 1,2-dihydroxynaphthalenes.

## RESULTS AND DISCUSSION

*Enzyme recognition*

The recombinant strain JM109 (pVL2028) was utilised for the study of the biotransformation of several

1,2-dihydro-1,2-dihydroxynaphthalenes. (Figure 1)

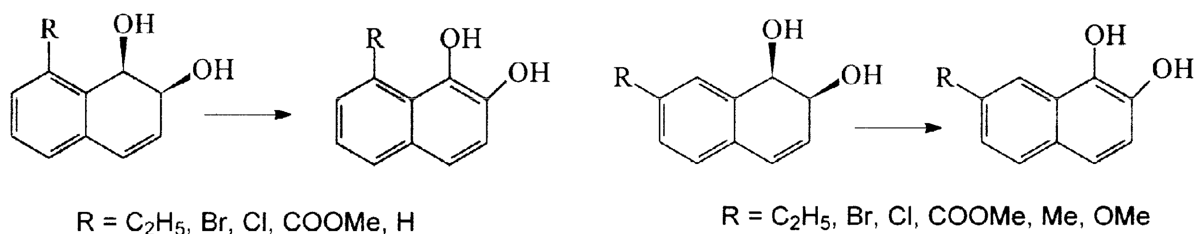



**Fig. 1.** 1,2-Dihydroxynaphthalenes obtained by bioconversion

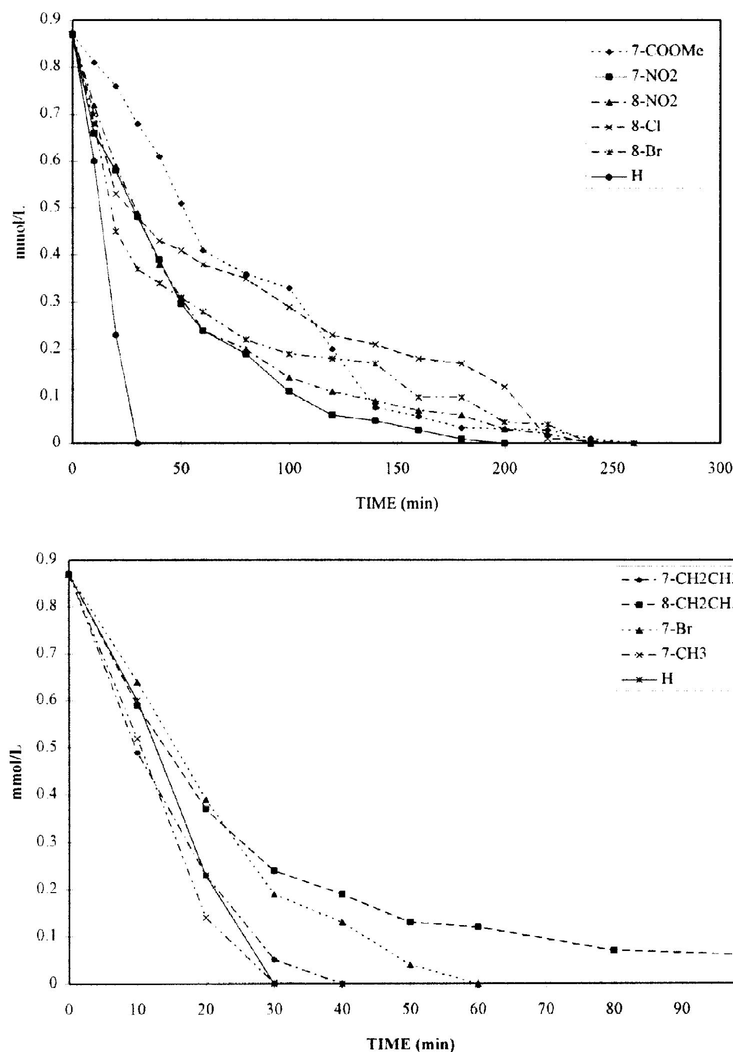



**Fig. 2.** Rate of conversion of variously substituted 1,2-dihydro-1,2-dihydroxynaphtalenes

In order to analyse the influence of the substrate substitution on the microorganism recognition we must ensure that the operative conditions are well suited. Because we know through toxicity experiments,[18] that the produced catechol is toxic for the microorganism, we should select a substrate concentration that can be confidently used. 1,2-Dihydroxynaphthalene itself is toxic at a concentration equal to ~1.25 mmol/L[19] when the

solution contains a fixed amount of cells (measured by an O.D. equal to 1), and other naphthalene derivatives (e.g. containing a halogen atom or a nitro group) are more toxic. Consequently, we chose to operate at initial concentration of the substrates of about 0.9 mmol/L (concentration that is well below toxicity limits and that permits a complete conversion in a reasonable amount of time, i.e. ~100-250 min) in our experiments, in order to obtain complete bioconversions and reliable transformation periods. The results are shown in Figure 2 and deserve some comment.

It can be seen that the conversion time depends on the nature and position of the substituent and in particular that: alkyl substituents (7-$CH_2CH_3$, 8-$CH_2CH_3$, 7-$CH_3$) or substituents in position 7 (7-Br) have a fast bioconversion, comparable to that of the parent compound,[20] while electron withdrawing substituents (7-COOMe, 7-$NO_2$, 8-$NO_2$) or substituents in position 8 (8-Cl, 8-Br) show a bioconversion rate slower than that of 1,2-dihydro-1,2-dihydroxynaphthalene.

We also tested other compounds, e.g. *cis*-3,5-cyclohexadiene 1,2-diol and 1,2-dihydro-1,2-dihydroxy phenanthrene. The first substrate shows a slow bioconversion (the conversion is ~80% after 300 min) whilst the second one has a behaviour comparable to that of 1,2-dihydro-1,2-dihydroxynaphthalene. All this data is in complete agreement with the recognition capability of the dioxygenase, as already discussed.[21]

*Catechol production*

The second point of the present work concerns the attempt to increase the yield of 1,2-dihydroxynaphthalene per unit of volume and of cells which was unsatisfactory mainly because of the above reported toxicity of the bioconversion products. To solve this problem we decided to separate the catechols from the reaction medium during their production by carrying out the bioconversion in the presence of a weakly basic IRA68 resin. This resin is known to react with acid molecules like 1,2-dihydroxynaphthalenes. In connection with the use of the resin some other modifications of the reported procedure helped the transformation: the IRA68 resin must be treated under vacuum/$N_2$ before bioconversion and the cultural medium must be buffered with a solution of $H_3PO_4$ to pH 7 because the resin causes an increase of the pH (to about 9), conditions not favourable for the microorganism.

The efficiency of the product adsorption by the resin depends on several factors, the most important of which are the contact time and surface. As we can neither effectively influence the time of product formation which depends on the microorganism, nor the resin surface which depends on the shape and size of the commercially available resin, the only chance is increase of the resin quantity. After several modifications, we selected an amount of 15 g/100mL of IRA68 resin that allows a fast bioconversion (85%, 1h; 100%, ~2h; Figure 3) and an increase of the yield from 1.25 mmol/L to 3.1 mmol/L. The second step was the use of an iterative procedure. In light of the previous data we furthered the system by restoring the initial bioconversion conditions. Thus, after a first bioconversion in the presence of IRA68 resin, we moved the cultural medium, containing the cells but not the resin, to a new reaction vessel using nitrogen pressure, added new resin, buffered the pH with a solution of $H_3PO_4$, and added more substrate (3.1 mmol/L). In this way, we could make three subsequent bioconversions increasing the quantity of converted 1,2-dihydro-1,2-dihydroxynaphthalene up to 9 mmol/L (i.e. ~1.5 g/L) (Figure 3).

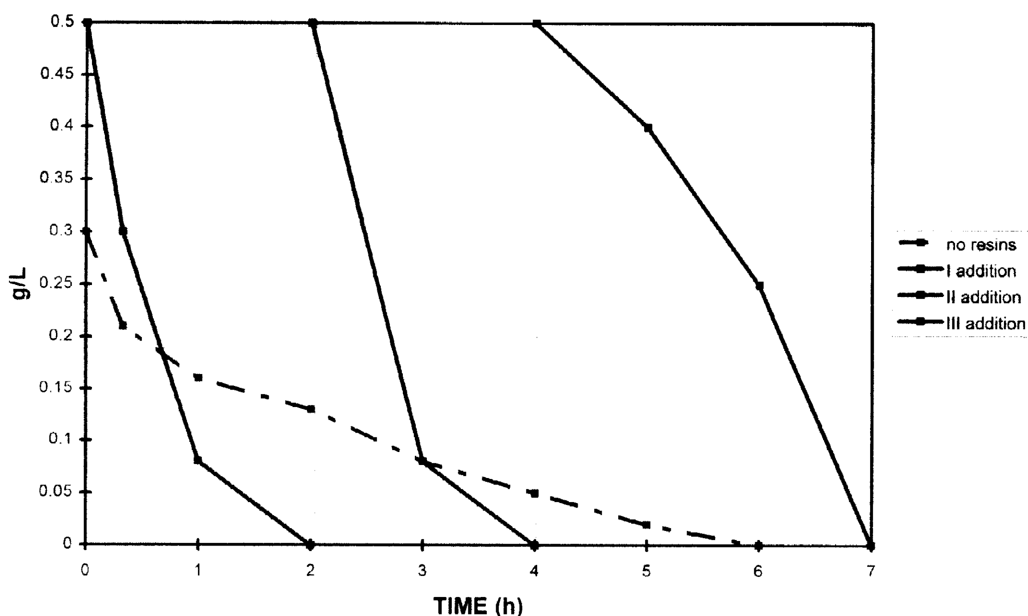



**Fig. 3.** Rate of conversion without resin, and with consecutive additions of resin and substrate

In principle, using this methodology it would still be possible to increase the quantity of produced 1,2-dihydroxynaphthalene operating more transfers and more bioconversions, the limit being the cell efficiency.

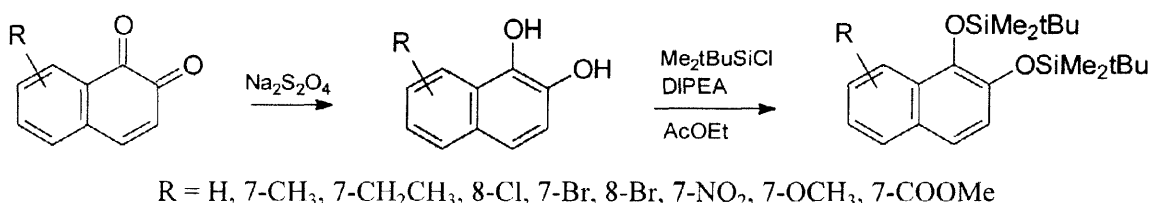
The release of the adsorbed product from the resin is achieved using a buffer solution [TRIS·HCl (tris(hydroxymethyl)aminomethane hydrochloride) 50mM pH 8.5 and NaCl 1M (HPLC estimated recover yields 80-90 %)] in the presence of AcOEt to immediately extract the catechols from the water solution. During all these operations an inert atmosphere ($N_2$) must be assured. The alternative use of a strongly basic resin (DOWEX 1X8) proved impossible because they failed to give the 1,2-dihydroxynaphthalenes with any work-up procedure.

*Catechol isolation*

1,2-Dihydroxynaphthalenes, obtained using the recombinant strain JM109 (pVL2028) as shown before and extracted with AcOEt, cannot be directly recovered from the solution, isolated and characterised because of their easy atmospheric oxidation that gives unidentifiable polymers as the only products on solvent evaporation. Therefore our interest was in their transformation into products more stable in the air. The protected catechols should have been easily isolated but they would be equally easily transformed back to the starting compounds. As a consequence, their cleavage should be as fast and clean as possible in order to make them available when desired.

We chose to protect the 1,2-dihydroxynaphthalenes as tert-butyldimethylsilyl derivatives.[22] The reference reaction, i.e. the protection of 1,2-dihydroxynaphthalene, occurs in good yield (90%), but it was only possible with the commercial substrate. In fact, even if the products obtained by bioconversion and extracted with AcOEt always remain in a nitrogen atmosphere, we could not avoid, for most of them, the partial oxidation to the corresponding quinones. Thus, we should previously reduce the product mixture coming from the culture

with sodium dithionite to assure the sole presence of catechols at the time of the reaction with the silyl chloride. Obviously, the final solution containing only the 1,2-dihydroxynaphthalene in AcOEt is directly used for the reaction with tert-butyldimethylchlorosilane as shown in Scheme 2 without catechol isolation. In this way, we obtained stable products that we could characterise by standard spectroscopic methods.




R = H, 7-$CH_3$, 7-$CH_2CH_3$, 8-Cl, 7-Br, 8-Br, 7-$NO_2$, 7-$OCH_3$, 7-COOMe

**Scheme 2**

Yields are around 50-70% for the two steps. In this case too, yields are exactly calculated only for the commercial 1,2-naphthoquinone without isolation of the corresponding 1,2-dihydroxynaphthalene. For the other substrates, we can weigh the starting compounds, the diols, and calculate their transformation into the catechols by HPLC; consequently, the yields are calculated comparing the starting diol moles and the final silyl derivative moles. In this way the yields are never overestimated.

Table 1. Silyl derivatives of 1,2-dihydroxynaphthalenes

(Structure: $R^1$, $R^2$-substituted naphthalene with $OSiMe_2tBu$, $OSiMe_2tBu$)

| | 1 | 5a | 6 | 7 | 8 | 10 |
|---|---|---|---|---|---|---|
| $R^1$ | H | H | Br | Cl | H | H |
| $R^2$ | H | Br | H | H | $NO_2$ | $COOCH_3$ |

(Structure: $R^1$, $R^2$-substituted naphthalene with $OR^3$, $OR^4$)

$R^3$= $tBuMe_2Si$, $R^4$= H or
$R^3$= H, $R^4$= $tBuMe_2Si$

| | 1a | 2 | 3 | 4 | 5 | 9 |
|---|---|---|---|---|---|---|
| $R^1$ | H | H | H | $CH_3CH_2$ | H | H |
| $R^2$ | H | $CH_3$ | $CH_3CH_2$ | H | Br | $OCH_3$ |

We obtained different silyl derivatives depending on the nature and position of the substituents (Table 1). In general: 1,2-dihydroxynaphthalene itself gives a mixture of the mono- and di-protected derivatives; electron withdrawing substituents or substituents in position 8 give the di-substituted derivatives; electron donating substituents or substituents in position 7 give the mono-substituted derivatives. However, we think that by increasing the reaction time and forcing the conditions it should be possible to transform all the compounds into the di-silyl derivatives, but this is not required for isolation and storage.

8-Br and 8-Cl substituted naphthalenes show the presence of the other regioisomer deriving from the bioconversion of the corresponding naphthalenes into the 1,2-dihydro-1,2-dihydroxynaphthalenes; in the former case the isomer is present only in traces, whilst in the latter it is present in a 6:4 ratio. (Figure 4)

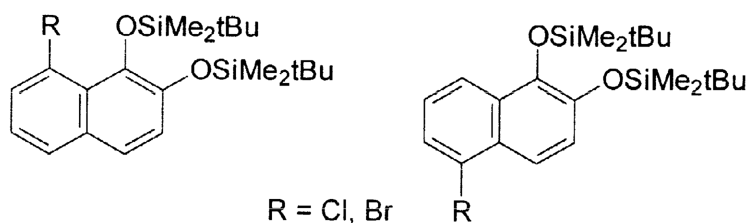
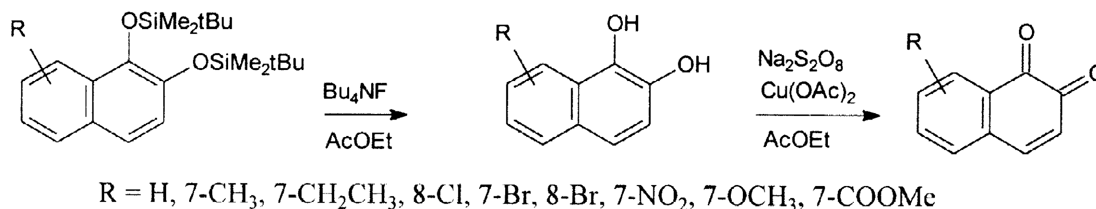



**Fig. 4.** Regioisomers of 8-Cl (**7, 7a**) and 8-Br derivatives

*Quinone preparation*

The protected 1,2-dihydroxynaphthalenes were then used to synthesise the corresponding 1,2-naphthoquinones as shown in Scheme 3.




R = H, 7-$CH_3$, 7-$CH_2CH_3$, 8-Cl, 7-Br, 8-Br, 7-$NO_2$, 7-$OCH_3$, 7-COOMe

**Scheme 3**

Yields are around 50-60% for the two steps. Again, the direct oxidation of the parent compound to 1,2-naphthoquinone proceeds quantitatively, confirming that the demanding step in Scheme 3 is the deprotection to catechol that is extremely sensitive to the basic conditions required in the reaction with $Bu_4NF$. The 1,2-naphthoquinones are quite stable compounds and can be characterised by spectroscopic methods (Table 2). In addition to the reported naphthalenes we also obtained 1,2-acenaphtenequinone (**20**).

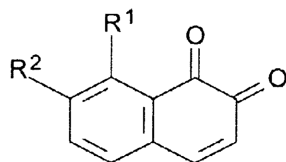



Table 2. 1,2-Naphthoquinones

| | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** |
|---|---|---|---|---|---|---|---|---|---|
| **$R^1$** | H | H | H | H | Br | Cl | H | H | H |
| **$R^2$** | H | $CH_3$ | $CH_3CH_2$ | Br | H | H | $NO_2$ | $OCH_3$ | $COOCH_3$ |

In order to obtain the quinones we can also directly oxidise the catechols obtained from the bioconversion (knowing that the commercial 1,2-dihydroxynaphthalene oxidises quantitatively). In fact, we easily obtained the corresponding 1,2-naphthoquinones, e.g. from 7-methoxy-1,2-dihydro-1,2-dihydroxynaphthalene and 7-

carbomethoxy-1,2-dihydro-1,2-dihydroxynaphthalene but, when the substrate of the bioconversion, the diol, was present in the AcOEt solution the reaction did not occur.

## CONCLUSION

In conclusion we can affirm that we have positively answered all the questions with which we began the work. We can now obtain a good quantity of 1,2-dihydroxynaphthalenes by bioconversion; we can transform them into stable compounds that can be characterised and stored; finally, we can recover them and obtain the corresponding quinones. Taking into account that no effort has been done to transfer the method to a larger scale we can nevertheless get a good amount of the desired compounds.

## EXPERIMENTAL

*General*

Proton nuclear magnetic resonance ($^1H$ NMR) spectra were obtained with Varian L-200, Bruker AC-200 and Bruker AC-300 instruments. I.R. spectra were recorded on a Perkin-Elmer 681 spectrophotometer. Unless otherwise stated, spectra are registered in $CH_2Cl_2$ solutions in KBr cells. Mass spectra were run on a VG 7070 EQ spectrometer. Thin-layer chromatography was carried out on silica gel plates (60 $F_{254}$, Merck): spots were detected visually by ultraviolet irradiation (254 nm) or using iodine as stainer. All products were chromatographated over silica gel (*n*-hexane/chloroform/isopropanol 95/5/0.5% for silyl derivatives or *n*-hexane/ethyl acetate 6/4 for quinones).

*Biotransformation procedure*

Biotransformations using *E. coli* JM109 (pVL2028) were carried out as described below. The culture was prepared in 100 mL M9 medium containing: glucose 10mM; thiamine 0.05 mM; kanamycin 50 μg/mL; IPTG (Isopropyl-β-d-thiogalactopyranoside) 1mM as inducer; sodium pyruvate 10 mM as oxidative source. Then the culture was deareated with $N_2$ for 60 min. and incubated overnight in nitrogen atmosphere on a shaker at 30 °C. After the growth, OD 0.8-1.0 (λ 600nm), glucose 10mM, sodium pyruvate 10mM and the substrate (concentration of 3.1 mmol/L) were directly supplied to the culture. The transformation was carried out under nitrogen atmosphere at 30 °C.

*Product Extraction.* Under nitrogen atmosphere a solution of 0.3N HCl was added until acid pH. The medium was then extracted three times with 100 mL ethyl acetate. The organic solvent was dried ($Na_2SO_4$) and concentrated to about 2 mL at reduced pressure.

*Biotransformation procedure (resin method)*

Biotransformations using *E. coli* JM109 (pVL2028) were carried out as described in "*Biotransformation procedure*". After the growth, OD 0.8-1.0 (λ 600nm), glucose 10mM, sodium pyruvate 10mM, IRA68 resin (15g) deareated by multiple vacuum/$N_2$ cycles and the substrate (concentration of 3.1 mmol/L) were directly

supplied to the culture. The transformation was carried out under nitrogen atmosphere at 30 °C. After 1 h, the cultural medium was removed from the resin with a double-tipped needle using nitrogen pressure. A new amount (15g) of IRA68 resin was added to the medium buffered with a solution of $H_3PO_4$ (0.4 mL) until pH neutral and other substrate (3.1 mmol/L) was supplied. The procedure was repeated two times.

*Product Extraction from resin.* Ethyl acetate (80 mL) and TRIS·HCl buffer pH 8.5 (80 mL, containing NaCl 1M and deareated by multiple vacuum/$N_2$ cycles) were added to the resin recovered from the medium. The mixture was then shaken for 20 min under $N_2$.

*Typical synthetic procedures*

*Protection reactions with tert-butyldimethylchlorosilane*

*A: From 1,2-dihydroxynaphthalene*

To a solution of 1,2-dihydroxynaphthalene (0.02 g, 0.125 mmol) in ethyl acetate (1 mL) under nitrogen atmosphere were added 4 Å molecular sieves and acetonitrile (1.5 mL) as cosolvent followed by diisopropylethylamine (0.2 mL, 1.147 mmol) and *tert*-butyldimethylchlorosilane (0.04 g, 0.25 mmol). The reaction was stirred for 10 min and monitored by thin-layer chromatography (silica gel, *n*-hexane/ethyl acetate 6/4). The reaction mixture was then evaporated under reduced pressure to afford the crude mixture of isomeric mono- and disilyl derivatives (0.106 mmol, 0.073 mmol of mono derivatives and 0.033 mmol of di derivative as estimated by NMR spectroscopy).

*B: From 1,2-naphthoquinone*

*Reduction with sodium dithionite.* To a solution of 1,2-naphthoquinone (0.200 g, 1.27 mmol) in ethyl acetate (2 mL) under nitrogen atmosphere was added a solution of $Na_2S_2O_4$ (0.440 g, 2.53 mmol) in $H_2O$ (3.3 mL). The reaction was monitored by thin-layer chromatography (silica gel, *n*-hexane/ethyl acetate 6/4). The biphasic mixture was vigorously stirred for 15 min. The reaction was diluted with a saturated solution of NaCl (3 mL) and with ethyl acetate (3 mL). The aqueous phase was extracted three times with ethyl acetate (3 x 3 mL). The combined organic phases were carefully dried ($Na_2SO_4$) and the solvent was reduced under vacuum approximately to 1.5 mL. The obtained 1,2-dihydroxynaphthalene was not isolated because of its instability; the solution in ethyl acetate was immediately used for the next step.

*Protection with tert-butyldimethylchlorosilane.* To a solution of 1,2-dihydroxynaphthalene (0.203 g, 1.27 mmol) in ethyl acetate (1.5 mL) obtained from the previous reaction under nitrogen atmosphere were added 4 Å molecular sieves and acetonitrile (1.5 mL) as cosolvent followed by diisopropylethylamine (2.2 mL, 12.63 mmol) and *tert*-butyldimethylchlorosilane (0.380 g, 2.5 mmol). The reaction was stirred for 10 min and monitored by thin-layer chromatography (silica gel, *n*-hexane/ethyl acetate 6/4). The reaction mixture was then evaporated under reduced pressure to afford the crude mixture of isomeric mono- and disilyl derivatives (0.706 mmol, 0.485 mmol of mono and 0.221 mmol of di derivative estimated by NMR spectroscopy, 56% yield).

*Oxidation reactions with sodium persulfate*

*C: From 7-COOMe-1,2-dihydroxynaphthalene*

To a solution under nitrogen of 7-COOMe-1,2-dihydroxynaphthalene (0.070 g; 0.32 mmol) extracted with ethyl acetate from the bioconversion and concentrated to about 4.3 mL at reduced pressure was added a phosphate buffer, 0.5M pH 6.5 (4.3 mL), containing: sodium persulfate (0.304 g, 1.277 mmol), tetrabutylammonium hydrogensulfate (0.003 g, 0.008 mmol), *o*-phenanthroline (0.003 g, 0.014 mmol), previously dissolved in methanol (0.06 mL) and copper(II) acetate monohydrate (0.003 g, 0.016 mmol). The reaction was vigorously stirred for 20 min and monitored by thin-layer chromatography (silica gel, *n*-hexane/ethyl acetate 6/4). The reaction mixture was then extracted, dried ($Na_2SO_4$) and the solvent removed under reduced pressure obtaining a crude mixture, which was chromatographated over silica gel (*n*-hexane/ethyl acetate 6/4) to afford 7-COOMe-1,2-naphthoquinone (0.055 g, 0.256 mmol, 80% yield).

*D: From tert-butyldimethylsilyl derivative*

*Cleavage with tetrabutylammonium fluoride.* To a solution of the three derivatives (0.219 g, 0.706 mmol) obtained from procedure *B* in ethyl acetate (5.5 mL) under nitrogen atmosphere was added a solution of tetrabutylammonium fluoride (0.292 g, 0.927 mmol) in ethyl acetate (4 mL) deareated by multiple vacuum/$N_2$ cycles. The reaction was vigorously stirred for 10 min and monitored by thin-layer chromatography (silica gel, *n*-hexane/ ethyl acetate 6/4). The reaction mixture was then neutralised by a solution of 0.3N HCl. The obtained 1,2-dihydroxynaphthalene was not isolated because of its instability; the solution in ethyl acetate was immediately used for the next step.

*Oxidation with sodium persulfate.* To a solution of 1,2-dihydroxynaphthalene (0.113 g, 0.706 mmol) in ethyl acetate obtained from the previous reaction under nitrogen atmosphere was added a phosphate buffer, 0.5M pH 6.5 (9.5 mL), containing: sodium persulfate (0.672 g, 2.824), tetrabutylammonium hydrogensulfate (0.006 g, 0.018 mmol), *o*-phenanthroline (0.006 g, 0.031 mmol), previously dissolved in methanol (0.12 mL) and copper(II) acetate monohydrate (0.007 g, 0.031 mmol). The reaction was vigorously stirred for 20 min and monitored by thin-layer chromatography (silica gel, *n*-hexane/ethyl acetate 6/4). The reaction mixture was then extracted, dried ($Na_2SO_4$) and the solvent removed under reduced pressure to afford 1,2-naphthoquinone (0.058 g, 0.37 mmol, 52% yield).

*Compound characterisation*

**1,2-Bis(tert-butyldimethylsilyloxy)-naphthalene (1)**

White solid; $R_f$ (6:4 hexane:AcOEt) 0.75; $\delta_H$ (300MHz, $CDCl_3$) 0.15 (6H, s), 0.34 (6H, s), 1.0 (9H, s), 1.15 (9H, s), 7.1 (1H, d, J= 8.5 Hz), 7.25-7.4 (3H, m), 7.7 (1H, d, J= 7.7 Hz), 8.1 (1H, d, J= 7.7 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$, selected peaks) -4 (q), 18 (s), 27 (q), 121 (d), 122 (d), 122.5 (d), 124 (d), 125(d), 127 (d), 129(s), 130(s), 142(s); m/z (EI) 388 ($M^{+\cdot}$,72), 373 (6), 331(50), 273 (11), 258 (11), 216 (100 %); HRMS (EI): $M^+$, found 388.2266. $C_{22}H_{36}O_2Si_2$ requires 388.2254.

**1(or 2)- tert-Butyldimethylsilyloxy-naphthalene (1a)**

White solid; $R_f$ (6:4 hexane:AcOEt) 0.67; $\delta_H$ (200MHz, $CDCl_3$) 0.35 (6H, s), 1.1 (9H, s), 5.8 (1H, s, $D_2O$), 7.1 (1H, d, J= 8.5 Hz), 7.25-7.55 (3H, m), 7.75 (1H, d, J= 7.7 Hz), 8.15 (1H, d, J= 7.7 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) -4 (q), 18 (s), 26 (q), 119 (d), 121 (d), 121.5 (d), 124 (d), 125(d), 127 (d), 129 (s), 130 (s), 137 (s), 142 (s); m/z

(EI) 274 ($M^{+\cdot}$, 6), 259 (11), 217 (100), 202 (22), 187 (20), 186 (90 %); HRMS (EI): $M^+$, found 274.1394. $C_{16}H_{22}O_2Si$ requires 274.1389.

**1(or 2)-tert-Butyldimethylsilyloxy-7-methyl-naphthalene (2)**
Brown oil; $R_f$ (6:4 hexane:AcOEt) 0.7; $\delta_H$ (200MHz, $CDCl_3$) 0.3 (6H, s), 1.05 (9H, s), 2.5 (3H, s), 5.8 (1H, s, $D_2O$), 7.05 (1H, d, J= 8.6 Hz), 7.2 (1H, dd, J= 8 Hz, J= 1.5 Hz), 7.25 (1H, d, J= 8.6 Hz), 7.65 (1H, d, J= 7 Hz), 7.9 (1H, bs); $\delta_C$ (75.5 MHz, $CDCl_3$) -4 (q), 18 (s), 21(q), 26 (q), 118 (d), 119 (d), 120 (d), 126 (d), 127(d), 127.5 (s), 128 (s), 135 (s), 137 (s), 140 (s); m/z (EI) 288 ($M^{+\cdot}$, 23), 273 (4), 231 (100), 216 (55), 200 (15), 173 (4), 155 (27), 141 (9 %); HRMS (EI): $M^+$, found 288.1543. $C_{17}H_{24}O_2Si$ requires 288.1546.

**1(or 2)-tert-Butyldimethylsilyloxy -7-ethyl-naphthalene (3)**
Brown oil; $R_f$ (6:4 hexane:AcOEt) 0.73; $\delta_H$ (300MHz, $CDCl_3$) 0.3 (6H, s), 1.05 (9H, s), 1.35 (3H, t, J=9 Hz), 2.85 (2H, q, J=9 Hz), 5.8 (1H, s, $D_2O$), 7.05 (1H, d, J= 8.5 Hz), 7.25 (1H, d, J= 8.5 Hz), 7.3 (1H, d, J= 8.5 Hz), 7.7 (1H, d, J= 8.5 Hz), 7.9 (1H, s); $\delta_C$ (75.5 MHz, $CDCl_3$, selected peaks) -4 (q), 18 (s), 26 (q), 29 (t), 31 (q), 118 (d), 119 (d), 125 (d), 126 (d), 127(d), 129 (s), 130 (s), 141 (s); m/z (EI) 302 ($M^{+\cdot}$, 36), 286 (13), 245 (100), 229 (34), 216 (37), 187 (3); HRMS (EI): $M^+$, found 302.1695. $C_{18}H_{26}O_2Si$ requires 302.1702.

**1(or 2)-tert-Butyldimethylsilyloxy -8-ethyl-naphthalene (4)**
Brown oil; $R_f$ (6:4 hexane:AcOEt) 0.8; $\delta_H$ (200MHz, $CDCl_3$) 0.3 (6H, s), 1.05 (9H, s), 1.35 (3H, t, J=7.7 Hz), 3.05 (2H, q, J=7.7 Hz), 5.8 (1H, s, $D_2O$), 7.1 (1H, d, J= 9 Hz), 7.2 (1H, d, J= 7.7 Hz), 7.3 (1H, t, J= 7.7 Hz), 7.45 (1H, d, J= 9 Hz), 8 (1H, d, J=7.7 Hz).

**1(or 2)-tert-Butyldimethylsilyloxy -7-bromo-naphthalene (5)**
Brown oil; $R_f$ (6:4 hexane:AcOEt) 0.7; $\delta_H$ (200MHz, $CDCl_3$) 0.35 (6H, s), 1.1 (9H, s), 5.8 (1H, s, $D_2O$), 6.9 (1H, d, J= 7.7 Hz), 7.3 (1H, d, J= 7.7 Hz), 7.5 (1H, dd, J= 8.6, J= 1.5 Hz), 7.65 (1H, d, J= 8.6 Hz), 8.3 (1H, d, J=1.5 Hz); m/z (EI) 354 (3), 352 ($M^{+\cdot}$, 2), 297 (10), 295 (9), 281 (2), 279 (1), 266 (4), 264 (3), 216 (26 %); HRMS (EI): $M^+$, found 352.0487. $C_{16}H_{21}BrO_2Si$ requires 352.0494.

**1,2-Bis(tert-butyldimethylsilyloxy)-7-bromo-naphthalene (5a)**
Brown oil; $R_f$ (6:4 hexane:AcOEt) 0.75; $\delta_H$ (200MHz, $CDCl_3$) 0.15 (6H, s), 0.25 (6H, s), 1 (9H, s), 1.1 (9H, s), 7.1 (1H, d, J= 8.6 Hz), 7.32 (1H, d, J= 8.6 Hz), 7.35 (1H, dd, J= 8.6, J= 1.5 Hz), 7.55 (1H, d, J= 8.6 Hz), 8.2 (1H, bs); m/z (EI) 468 (100), 466 (93), 411 (57), 409 (53), 354 (17), 352 (16), 273 (44 %).

**1,2-Bis(tert-butyldimethylsilyloxy)-8-bromo-naphthalene (6)**
Colourless oil; $R_f$ (6:4 hexane:AcOEt) 0.81; $\delta_H$ (200MHz, $CDCl_3$) 0.15 (6H, s), 0.3 (6H, s), 1.1 (9H, s), 1.15 (9H, s), 7.2-7.3 (2H, m), 7.65 (1H, d, J= 7.3 Hz), 7.8 (1H, d, J=9.5 Hz), 8.3 (1H, d, J=8.8 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$, selected peaks) -4 (q), 19 (s), 26 (q), 120 (d), 122 (d), 124 (d), 125 (d), 128 (d); m/z (EI) 468 (80), 466 (75), 411 (100), 409 (87), 296 (65), 294 (58), 273 (36 %); HRMS (EI): $M^+$, found 466.1364. $C_{22}H_{35}BrO_2Si_2$ requires 466.1359.

**1,2-Bis(tert-butyldimethylsilyloxy)-8-chloro-naphthalene (7)**
Colourless oil; $R_f$ (6:4 hexane:AcOEt) 0.81; $\delta_H$ (200MHz, $CDCl_3$) 0.15 (6H, s), 0.3 (6H, s), 1.1 (9H, s), 1.1 (9H, s), 7.2-7.4 (3H, m), 7.8 (1H, d, J=8.9 Hz), 8.05 (1H, d, J=6 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) -4 (q), 19 (s), 27 (q), 30 (s), 122 (d), 123 (d), 124 (d), 124.5 (d), 129 (d), 127 (s), 128 (s), 131 (s), 132 (s), 140 (s); m/z (EI) 424 (36), 422 ($M^{+\cdot}$, 74), 367 (14), 365 (38), 294 (18), 292 (52), 252 (36), 250 (94), 237 (37), 235 (100), 200 (39 %); HRMS (EI): $M^+$, found 422.1870. $C_{22}H_{35}ClO_2Si_2$ requires 422.1864.

**1,2-Bis(tert-butyldimethylsilyloxy)-5-chloro-naphthalene (7a)**
Colourless oil; $R_f$ (6:4 hexane:AcOEt) 0.67; $\delta_H$ (200MHz, $CDCl_3$) 0.15 (6H, s), 0.3 (6H, s), 1.1 (9H, s), 1.1 (9H, s), 7.1 (1H, d, J=8.9 Hz), 7.2-7.45 (2H, m), 7.65 (1H, d, J=8.9 Hz), 8.05 (1H, d, J=6 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$, selected peaks) -4 (q), 19 (s), 27 (q), 30 (s), 118 (d), 121 (d), 121.5 (d), 123 (d), 124.5 (d); m/z (EI) 424 (36),

422 ($M^{+\cdot}$, 74), 367 (14), 365 (38), 294 (18), 292 (52), 252 (36), 250 (94), 237 (37), 235 (100), 200 (39 %); HRMS (EI): $M^+$, found 422.1867. $C_{22}H_{35}ClO_2Si_2$ requires 422.1864.

**1,2-Bis(tert-butyldimethylsilyloxy)-7-nitro-naphthalene (8)**
Yellow solid; $R_f$ (6:4 hexane:AcOEt) 0.63; $\delta_H$ (200MHz, $CDCl_3$) 0.2 (6H, s), 0.3 (6H, s), 1.05 (9H, s), 1.15 (9H, s), 7.3 (1H, d, J= 8.5 Hz), 7.45 (1H, d, J= 8.5 Hz), 7.6 (1H, d, J= 9.2 Hz), 8.05 (1H, dd, J= 9, J= 1.5 Hz), 9.1 (1H, d, J= 1.5 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) -4 (q), 19 (s), 26 (q), 117 (d), 120 (d), 121 (d), 126 (d), 128(s), 129 (d), 132(s), 142(s), 144 (s), 145 (s); m/z (EI) 433 ($M^{+\cdot}$, 9), 418 (3), 376 (100), 262 (20), 216 (4), 215 (13 %); HRMS (EI): $M^+$, found 433.2078. $C_{22}H_{35}NO_4Si_2$ requires 433.2105.

**1(or 2)-tert-Butyldimethylsilyloxy -7-methoxy-naphthalene (9)**
Brown oil; $R_f$ (6:4 hexane:AcOEt) 0.76; $\delta_H$ (200MHz, $CDCl_3$) 0.31 (6H, s), 1.08 (9H, s), 4 (3H, s), 5.8 (1H, s, $D_2O$), 6.96 (1H, d, J= 8 Hz), 7.03 (1H, dd, J= 9, J= 2.5 Hz), 7.2 (1H, d, J= 8 Hz), 7.4 (1H, d, J= 2.5 Hz), 7.64 (1H, d, J=9 Hz).

**1,2-Bis(tert-butyldimethylsilyloxy)-7-carbomethoxy-naphthalene (10)**
Brown oil; $R_f$ (6:4 hexane:AcOEt) 0.71; $\delta_H$ (200MHz, $CDCl_3$) 0.16 (6H, s), 0.25 (6H, s), 1.01 (9H, s), 1.17 (9H, s), 3.97 (3H, s), 7.23 (1H, d, J= 8.6 Hz), 7.44 (1H, d, J= 8.6 Hz), 7.77 (1H, d, J= 8.7 Hz), 7.91 (1H, dd, J= 8.7, J= 1.5 Hz), 8.93 (1H, d, J= 1.5 Hz); m/z (EI) 446 ($M^{+\cdot}$, 36), 431 (4), 389 (38), 274 (22), 243 (100), 215 (38 %).

**1,2-Naphthoquinone (11)**
Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.26; $\nu_{max}$ (KBr) 1674 $cm^{-1}$; $\delta_H$ (300MHz, $CDCl_3$) 6.4 (1H, d, J= 10.3 Hz), 7.35 (1H, d, J= 7.7 Hz), 7.4 (1H, d, J= 10.3 Hz), 7.5 (t, 1H, J=7.7 Hz), 7.65 (1H, t, J=7.7 Hz), 8.1 (1H, d, J=7.7 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) 128 (d), 130 (d), 130.1 (d), 130.8 (d), 131 (s), 135 (s), 136 (d), 145 (d), 179 (s), 181 (s); m/z (EI) 160 (5), 158 ($M^{+\cdot}$,%), 130 (100), 102 (69); HRMS (EI): $M^+$, found 158.0374. $C_{10}H_6O_2$ requires 158.0368.

**7-Methyl-1,2-naphthoquinone (12)**
Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.34; $\nu_{max}$ (KBr) 1667 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 2.4 (3H, s), 6.4 (1H, d, J= 9.8 Hz), 7.28 (1H, d, J=7 Hz), 7.42 (1H, d, J=9.8 Hz), 7.44 (d, 1H, J=7 Hz), 7.9 (1H, s).

**7-Ethyl-1,2-naphthoquinone (13)**
Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.34; $\nu_{max}$ (KBr) 1667 $cm^{-1}$; $\delta_H$ (300MHz, $CDCl_3$) 1.35 (2H, s), 2.7 (3H, t, J=7 Hz), 6.34 (1H, d, J= 10 Hz), 7.2 (1H, d, J=9 Hz), 7.4 (1H, d, J=10 Hz), 7.44 (d, 1H, J=9 Hz), 7.95 (1H, s).

**7-Bromo-1,2-naphthoquinone (14)**
Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.26; $\nu_{max}$ (KBr) 1674 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 6.45 (1H, d, J= 10 Hz), 7.25 (1H, d, J= 7.9 Hz), 7.4 (1H, d, J= 10 Hz), 7.78 (1H, dd, J= 7.9, J= 1.5 Hz), 8.2 (1H, d, 1.5 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) 126 (s), 128 (d), 131 (d), 133 (s), 133.5 (d), 138.5 (d), 144 (d), 178 (s), 180 (s); m/z (EI) 240 (42), 238 (42), 236 (3), 210 (100), 208 (99), 182 (34), 180 (34), 159(10), 101 (13 %); HRMS (EI): $M^+$, found 235.9454. $C_{10}H_5BrO_2$ requires 235.9473.

**8-Bromo-1,2-naphthoquinone (15)**
Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.34; $\nu_{max}$ (KBr) 1674 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 6.55 (1H, d, J= 10.5 Hz), 7.4 (1H, t, J= 7.7 Hz), 7.88 (1H, d, J= 7.7 Hz), 7.97 (1H, d, J= 10.5 Hz), 8.1 (1H, d, 7.7 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) 125 (s), 128.5 (d), 129 (d), 131 (d), 133 (s), 140 (d), 143 (d), 178 (s), 180 (s); m/z (EI) 240 (10), 238 (9), 236 (8), 210 (65), 208 (100), 182 (30), 180 (20), 127 (43), 101 (13 %); HRMS (EI): $M^+$, found 235.9473. $C_{10}H_5BrO_2$ requires 235.9473.

**8-Chloro-1,2-naphthoquinone (16)**
Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.4; $\nu_{max}$ (KBr) 1674 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 6.55 (1H, d, J= 10.5 Hz),

7.41 (1H, d, J= 10.5 Hz), 7.45 (1H, t, J= 7.5 Hz), 7.68 (1H, dd, J= 7.5, J= 1.5 Hz), 8.05 (1H, dd, 7.5, J= 1.5 Hz); m/z (EI) 196 (10), 194 (28), 192 ($M^{+\cdot}$, 3), 166 (33), 164 (100), 138 (23), 136 (65), 101 (23 %).

**5-Chloro-1,2-naphthoquinone (16a)**

Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.4; $\nu_{max}$ (KBr) 1674 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 6.45 (1H, d, J= 9.8 Hz), 7.3 (1H, t, J= 7.5 Hz), 7.42 (1H, d, J= 7.5 Hz), 7.6 (1H, dd, J= 7.5, J= 1.5 Hz), 8 (1H, d, 9.8 Hz); m/z (EI) 196 (10), 194 (28), 192 ($M^{+\cdot}$, 3), 166 (33), 164 (100), 138 (23), 136 (65), 101 (23 %).

**7-Nitro-1,2-naphthoquinone (17)**

Brown solid; $R_f$ (6:4 hexane:AcOEt) 0.18; $\nu_{max}$ (KBr) 1673 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 6.62 (1H, d, J=10 Hz), 7.43 (1H, d, J= 10 Hz), 7.61 (1H, d, J=8 Hz), 7.65 (1H, dd, J= 8, J= 2 Hz), 8.9 (1H, d, J=2 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) 124.7 (d), 129.9 (d), 130.7 (d), 132 (s), 139 (s), 142.5 (d), 149 (s), 176.8 (s), 179.5 (s); m/z (EI) 205 (100), 203 ($M^{+\cdot}$, 5), 175 (14), 147 (23), 131 (23), 101 (7 %); HRMS (EI): $M^+$, found 203.0205. $C_{10}H_5NO_4$ requires 203.0219.

**7-Methoxy-1,2-naphthoquinone (18)**

Red solid; $R_f$ (6:4 hexane:AcOEt) 0.4; $\nu_{max}$ (KBr) 1666 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 3.9 (3H,s), 6.3 (1H, d, J= 10.2 Hz), 7.13 (1H, dd, J= 8.36, J= 2.8 Hz), 7.29 (1H, d, J= 8.3 Hz), 7.39 (1H, d, J= 10.2 Hz), 7.62 (1H, d, 2.8 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) 55.8(q), 115(d), 122(d), 125(d), 128(s), 132(d), 133(s), 146(d), 162 (s), 179 (s), 181 (s); m/z (EI) 190 (6), 188 ($M^{+\cdot}$, 49), 160 (100), 145 (23), 117 (34 %); HRMS (EI): $M^+$, found 188.0465. $C_{11}H_8O_3$ requires 188.0473.

**7-Carbomethoxy-1,2-naphthoquinone (19)**

Orange solid; $R_f$ (6:4 hexane:AcOEt) 0.26; $\nu_{max}$ (KBr) 1727, 1673 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 4.0 (3H, s), 6.6 (1H, d, J= 10 Hz), 7.5 (1H, d, J= 8 Hz), 7.53 (1H, d, J= 10 Hz), 8.35 (1H, d, J= 8, J= 2 Hz), 8.75 (1H, d, J= 2 Hz) ; $\delta_C$ (75.5 MHz, $CDCl_3$) 52.6 (q), 129.5 (d), 129.8 (d), 130.9 (d), 131.5 (s), 132.3 (s), 136.1 (s), 136.5 (d), 137.9 (s), 143.9 (d), 177.9 (s), 180.3 (s); m/z (EI) 218 (34), 216 ($M^{+\cdot}$, 3), 188 (60), 157 (100), 129 (42), 101(35 %); HRMS (EI): $M^+$, found 216.0421. $C_{12}H_8O_4$ requires 216.0423.

**Acenaphthenequinone (20)**

Brown solid; $R_f$ (6:4 hexane:AcOEt) 0.2; $\nu_{max}$ (nujol) 1722 $cm^{-1}$; $\delta_H$ (200MHz, $CDCl_3$) 7.9 (2H, t, J= 7.7 Hz), 8.15 (2H, d, J= 6.87 Hz), 8.3 (2H, d, J= 8.55 Hz); m/z (EI) 182 ($M^{+\cdot}$, 54), 154 (94), 126 (100 %).

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18. Data not shown.
19. Reported concentrations must be understood as mmol per cell amount in volume unit, as specified in the Experimental.
20. Conversion is very fast also with 7-$OCH_3$ about 30 min, data not reported.
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22. Acetylation occurs quantitatively but the back reaction needs strong basic conditions, conditions that favour the oxidative degradation of the products. A second possibility is the protection of the catechols as ketals, reaction that requires acid conditions for the cleavage, but in our experiments the yields were very low (37%), probably because the preparation needs heating and basic conditions ($Cs_2CO_3$).