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A New Photoremovable Protecting Group Containing 2-Methyl-1,4-Naphthoquinone: Steady State and Transient Spectroscopic Studies

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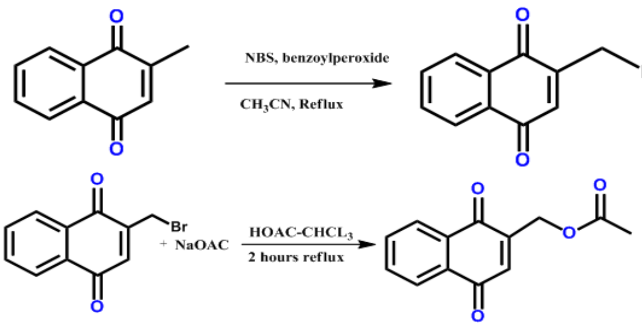
ABSTRACT

The 2-methyl-1, 4-naphthoquinone moiety has been proposed as a photoremovable protecting group when substituted with carboxylate in different solvents upon irradiation. Picoseconds pump-probe investigations in different solvents afforded the rate constants of intersystem crossing from singlet to triplet to be around 10^{11} s^{-1} and the lifetime of the triplet state was about 0.7 ns in methanol and 0.12 µs in the mixture water-acetonitrile.

Keywords: Pump-probe spectroscopy, Photoremovable protecting group, Steady state.

INTRODUCTION

Photoremovable protecting groups (PPGs) known as photolabile protecting groups have gained particular attention of photochemists because of their importance in biochemistry, in biology [1, 2, 3], and in organic synthesis including other field of life science [4, 5, 6]. These compounds consist of chromophores which can protect different chemical functionalities and release them upon selective irradiation. Requirements for being good Photoremovable protecting group are known and reported [5, 6]. Several functionalities such as carboxylates, sulfonates, phosphates, amines have been successfully protected by *O*-Nitrobenzyl [7, 8, 9], 4-hydroxyphenacyl [10], 2-hydroxyphenacyl [11], 2, 5-dimethylphenacyl[12], and Coumarin[13] chromophores and different mechanisms of the release of these functionalities by using UV light was investigated by the cited authors. 2-(1-hydroxyethyl)-anthraquinone has been proposed as a photolabile protecting group for carboxylique acids by Ming-Guang Ren *et al* [14]. In this paper we report the ability of 2-methyl-1,4-naphthoquinone moiety of protecting carboxylates and to release them in different solvents such as methanol, ethanol, n-hexane, acetonitrile, mixture of acetonitrile-water (1:9) and (1:1). In addition, we report a study of the chromophore by picoseconds pump-probe spectroscopy. The rate constants of intersystem crossing including lifetimes of striplet states have been measured at room temperature. The absorption properties of 2-methyl-1,4-naphthoquinone moiety in different solvent have been also investigated.



Scheme 1 Synthesis of 2-(acetoxymethyl)-1, 4-naphthoquinone

Material and Methods

General

2-methyl-1, 4-naphthoquinone (Menadione), N-bromosuccinimide(NBS), benzoylperoxide, and sodium acetate were purchased from sigma Aldrich and used without any purification. Different solvents used were distilled before the use in order to increase the purity. 2-(1-bromomethyl)-1.4-naphtoquinone was prepared following the literature [15], and without any purification the crude reaction mixture was subject of S_N2 reaction according to the literature [16]. 2-(acetoxymethyl)-1, 4-naphthoquinone was obtained after purification by column chromatography using hexane-ethyl acetate (10:1) and recrystallisation with ethanol to afford a yellow solid mp 89-90 °C. ^{1}H NMR ($CDCl_3$) : □(ppm) 2.18(s, 3H), 5.07(s, 2H), 6.83(s, 1H),7.70(d, *J*=7.5 Hz, 1H), 8.10(d, *J*=1.8 Hz, 1H). ^{13}C NMR ($CDCl_3$) : □ (ppm) 20.44, 60.04, 126.48, 131.81,133.38, 133.94, 144.93, 169.85, 183.87, 184.52. MS (EI) : 230, 207, 188, 159,131, 115,105, 105, 76, 63, 51. The GC retention time was about 15.44 minutes comparing 12.08 minutes for Menadione. NMR spectra were recorded on Bruker 300 MHz spectrometers and were referenced to the residual peak of a solvent or TMS and Hitachi Model U-3900/3900H spectrophotometer was used to record UV spectra of compounds in different solvent systems.

Photochemical and steady state experiments

Using NMR tubes, solutions of deuterated solvents containing 2-(acetoxymethyl)-1.4-naphthoquinone were irradiated either by 200 W mercury lamp equipped with a quartz cooling jacket and a condenser at 300 nm or with LED reactor at 405 nm. ^{1}H NMR spectra were taken after each time of measurement and the release of the leaving group was monitored. Steady state exhaustive irradiation was carried out using exclusively LED reactor at 405 nm.

Quantum yields measurements

2-Nitrobenzaldehyde [17] was used as actinometer and different sealed capillary containing TMS in deuterated solvent systems were used as internal standards for each ^{1}H NMR experiment. Conversion of ≤ 10 % was kept for all the samples to avoid other kind of photoreactions to occur. Three independent measurements were carried out and standard deviations are provided in Table 2.

Picosecond pump-probe spectroscopy

Details for the apparatus used for Picosecond pump-probe spectroscopy are given in our previous paper [18]. A Ti:Sa laser system with 775 nm, 1.05 mJ, and full width at half-maximum 150 fs operating at 426 Hz were used. Global analysis was used in order to analyze data and fit the kinetics.

RESULTS AND DISCUSSION

Absorption spectra

Ground state absorption spectra of compounds are shown in Figure 1 and 2. For the parent compound (2-methyl-1,4-naphthoquinone) the maximum of the first band is around 330 nm and the tail goes over 380 nm. However, the maximum for the second band is around 252 nm and the photochemistry was done using the first band. For the 2-(acetoxymethyl)-1,4-naphthoquinone one can notice that the maximum of the first band is about the same with the parent compound but around 300 nm there is a change in absorbance compare to 2-methyl-1,4-naphthoquinone. Figure 3 provide the Steady state irradiation of 2-(acetoxymethyl)-1, 4-naphthoquinone (8mM) in MeCN:H_2O (9:1) in different irradiation time. The UV spectra of 2-methyl-1, 4-naphthoquinone in deuterated water was already reported and the tail were over 420 nm [19]. To the best of our knowledge the UV spectra of 2-(acetoxymethyl)-1, 4-naphthoquinone has not been reported before. We used violet light to show the advantage of using this chromophore as a photoremovable protecting group.

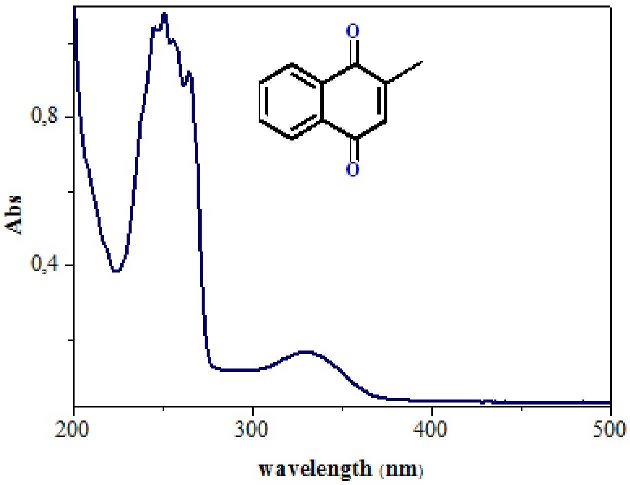


Figure 1 Ground state absorption spectra of 2-methyl-1, 4-naphthoquinone in acetonitrile (2×10-4 M).

Photochemistry

2-(acetoxymethyl)-1,4-naphthoquinone was dissolved in different solvents systems (c ~3 mM) and solutions were irradiated until the disappearance of the starting material (around 90 %). The acetic acid was released (Scheme 2 and table 1) in methanol, ethanol, and in the acetonitrile-water mixtures (1:9) and (1:1). However, in pure acetonitrile, pure n-hexane the starting materiel was vanished but the release of the acetate was not noticed. We assume that aprotic polar and non polar solvents are not convenient for the release of the leaving group. This behavior was also reported when *p*-hydroxyphenacyl and *o*-hydroxyphenacyl are used as photoremovable protecting groups [11, 20] a small amount of water was sufficient for speeding up the photorelease of leaving groups. 2-methyl-1, 4-naphthoquinone moiety was first proposed as Bioreductive alkylating agents by Ian Wilson *et al* using pulse radiolysis [21] and the ability of the chromophore to sensitize singlet oxygen was reported [19].

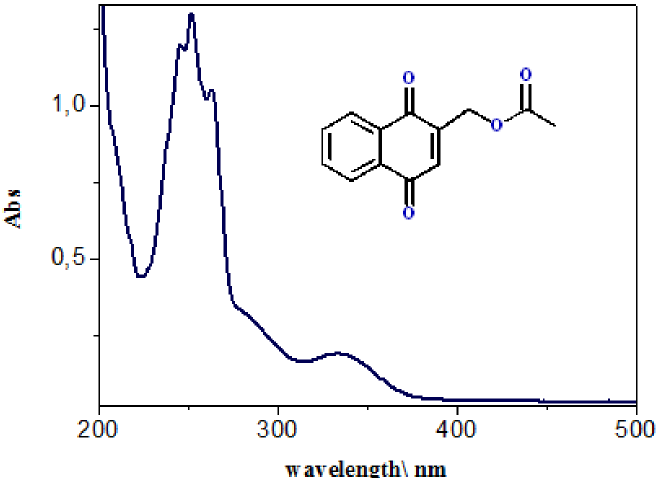
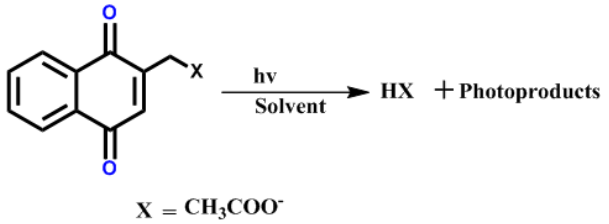


Figure 2 Ground state absorption spectra of 2-(acetoxymethyl)-1, 4-naphthoquinone in acetonitrile (3×10-4 M).



Scheme 2 Photochemistry of 2-(acetoxymethyl) -1,4-naphthoquinone

Using the same solvents systems and taking in account the ground state absorption spectra, steady state irradiation of 2-(acetoxymethyl)-1,4-naphthoquinone solutions at 405 nm was carried out at different times by monitoring the change in absorbance at 330 and 380 nm. It was shown that at 330 nm when the compound is disappearing there was a new band rising at 380 nm in methanol, and in the acetonitrile-water mixtures (1:1) but the behavior was not the same for n-hexane where at 330 nm the compound is disappearing faster and at 380 nm there is no rise at the same irradiation time as shown in Figures 4 and 5.

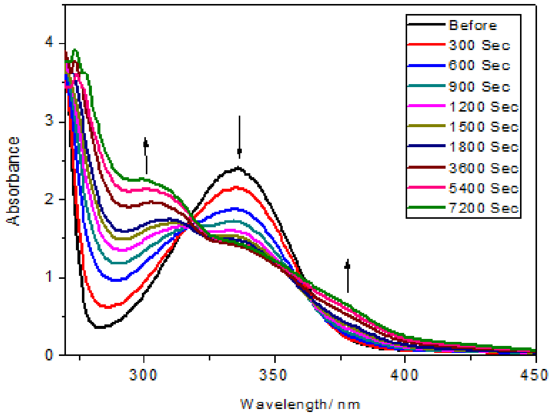


Figure 3 Steady state irradiation of 2-(acetoxymethyl)-1, 4-naphthoquinone (3mM) in MeCN:H2O (1:1)

Chemical yields of acetic acid appearance are shown in Table 1 and it is clear that in methanol acetic acid is produced in good yield compare to other solvent system. The same behavior for quantum yields of disappearance.

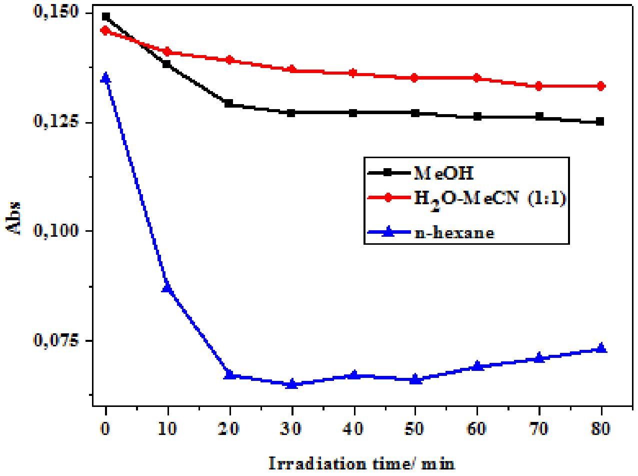


Figure 4 Change in absorbance after exhaustive irradiation of 2-(acetoxymethyl)-1,4-naphthoquinone (2×10-4 M) in MeOH (black square), MeCN:H2O (1:1)(red circle), and n-hexane(blue triangle) at 330 nm.

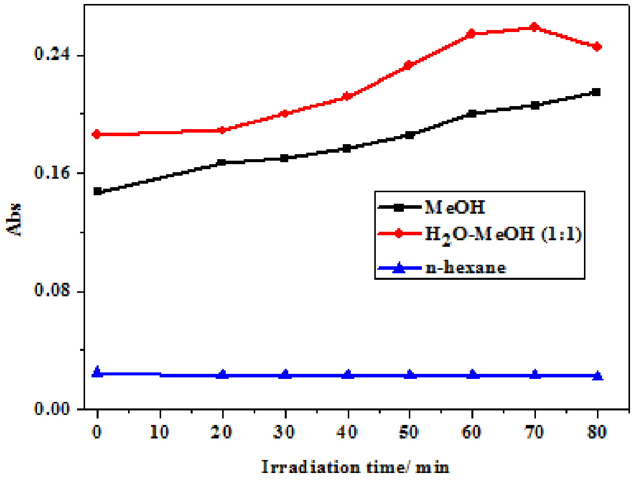


Figure 5 Change in absorbance after exhaustive irradiation of 2-(acetoxymethyl)-1, 4-naphthoquinone (2×10-4 M) in MeOH (black square), MeCN:H2O (1:1)(red circle), and n-hexane(blue triangle) at 380 nm.

Table 1 Chemical yield of acetic acid appearance

Compound	Solvent[a]	Chemical yield[b] % of HX
2-(acetoxymethyl)-1,4-naphthoquinone	MeOH	90 ± 3
	EtOH	79 ± 2
	MeCN:H_2O(1:1)	85 ± 3
	MeCN:H_2O(1:9)	63 ± 1

[a] Deuterated commercially available solvents. [b] Irradiation carried out in non degassed solvent. CH_3COOH was detected by NMR after irradiation and the increasing of its concentration was function of irradiation time. Three or two independent measurements were carried out and standard deviations are provided.

Table 2 Quantum yield of disappearance

Compound	Solvent[a]	Φ
2-(acetoxymethyl)-1,4-naphthoquinone	MeOH	0.31 ± 0.03
	EtOH	0.25 ± 0.02
	MeCN:H_2O(1:1)	0.15 ± 0.01
	MeCN:H_2O(1:9)	0.11 ± 0.02

[a] Aerated solutions of 2-(acetoxymethyl)-1,4-naphthoquinone (3×10^{-4} M) were irradiated at 405 nm to ≤ 10% conversion. 2-nitrobenzaldehyde (Φ = 0.41 ± 0.02 in water or organic solvents)(Edward. 2010) was used as actinometer. ^{1}H NMR was used in order to probe the acetate concentrations. Three independent measurements were carried out and standard deviations are provided.

Solutions were kept in dark twenty four hours and the thermal stability of 2-(acetoxymethyl)-1,4-naphthoquinone was demonstrated along with quantum yields of degradation as shown in Table 2 which are also sufficient to prove that the compound can be used for physiological use.

Picosecond Pump-probe spectroscopy

Picosecond pump-probe spectroscopy of 2-methyl-1, 4-naphthoquinone in acetonitrile gave two different bands ($\square_{max}$= 360 nm and $\square_{max}$= 425nm) within 4 ps which were seen until 20 ps and after 1.8 ns the first band disappeared and the second persisted. The first band ($\square_{max}$= 425nm) was assigned to short lived singlet state of the 2-methyl-1, 4-naphthoquinone (^{1}**Q***) and the second band ($\square_{max}$= 360 nm) to the triplet (^{3}**Q***).

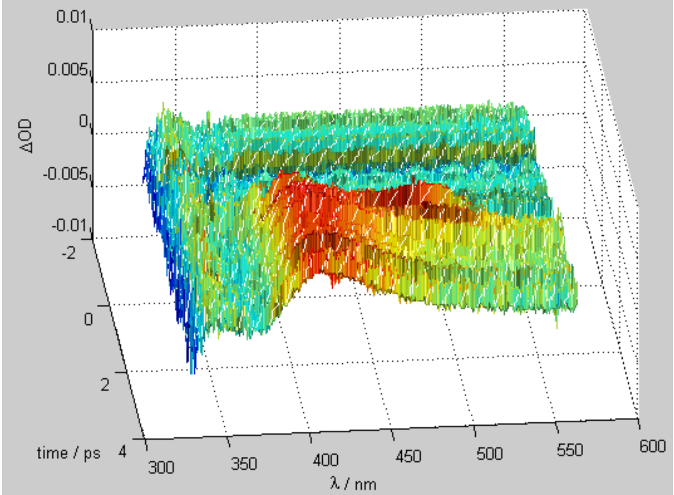


Figure 5 Pump-probe spectra of 2-methyl-1,4-naphthoquinone in MeCN taken within 4 ps.

The rate constant of intersystem crossing was about 4.5 $\times10^{11}$ s^{-1} in methanol. Picosecond pump-probe spectroscopy of 2-(acetoxymethyl)-1, 4-naphthoquinone was carried out in different solvent systems such as methanol, acetonitrile and the mixture of water -acetonitrile (9:1). The outcome is that the two bands observed for the parent compound are still the same with a small red shift ($\square_{max}$= 375 nm and $\square_{max}$= 450nm) in acetonitrile (Figure 5-7). We think that the first band ($\square_{max}$= 450 nm) may be assigned to the short lived singlet state of the 2-(acetoxymethyl)-1, 4-naphthoquinone (^{1}**AQ***) and the second band ($\square_{max}$= 375 nm) to the triplet (^{3}**AQ***). These results are not far away from those found by Ian Wilson *et al*. They used pulse radiolysis and found the Transient absorption spectra of 2-methyl-1, 4-naphthoquinone around 390 nm in 2-propanol [22]. The lifetimes of the triplet manifold in this work were about 0.7 ns in methanol and 0.12 $\square$

in water -acetonitrile (9:1). Triplet manifold lives longer in water -acetonitrile (9:1) compare to methanol. We think that the change in pH or pKa of the medium plays an important role as we might know from the behavior of *aci* nitro intermediates [23]. Rate constants of intersystem crossing are consigned in Table 3 and they are all almost in the same range according to the fitting and global analysis.

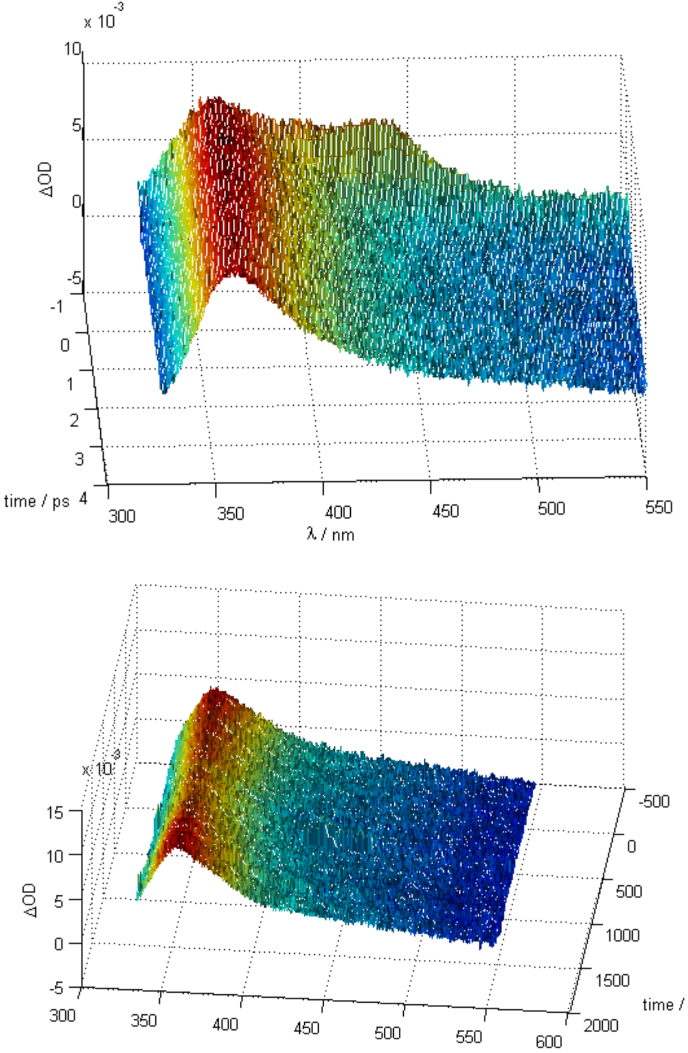


Figure 6 Pump-probe spectra of 2-(acetoxymethyl)-1,4-naphthoquinone in acetonitrile taken within 4 ps(up) and after 1.8 ns (down).

Table 3 The observed rate constants of intersystem crossing from singlet to triplet state in different solvent systems upon excitation at 266 nm

Compound	Solvent	$k_{isc}(10^{11}\ s^{-1})$
2-(acetoxymethyl)-1,4-naphthoquinone	MeOH	2.45 ± 0.01
	MeCN	2.40 ± 0.02
	MeCN:H_2O(1:9)	4.90 ± 0.02
2-methyl-1,4-naphthoquinone	MeOH	4.50 ± 0.03
	MeCN	1.80 ± 0.01
	MeCN:H_2O(1:9)	3.70 ± 0.01

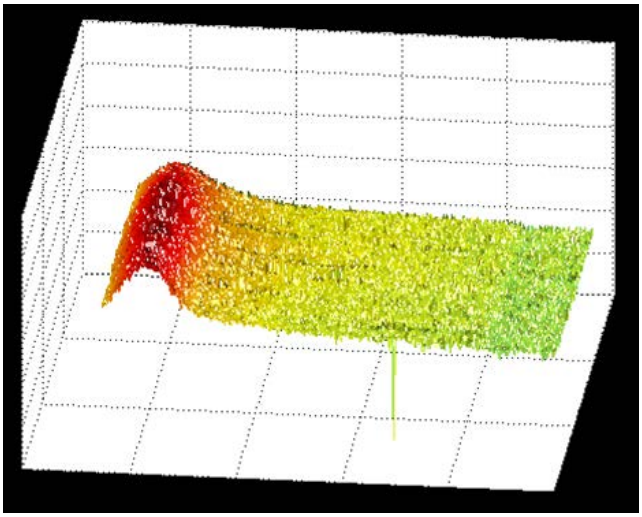


Figure 7 Pump-probe spectra of 2-(acetoxymethyl) -1,4-naphthoquinone in MeOH taken within 1,8 ns.

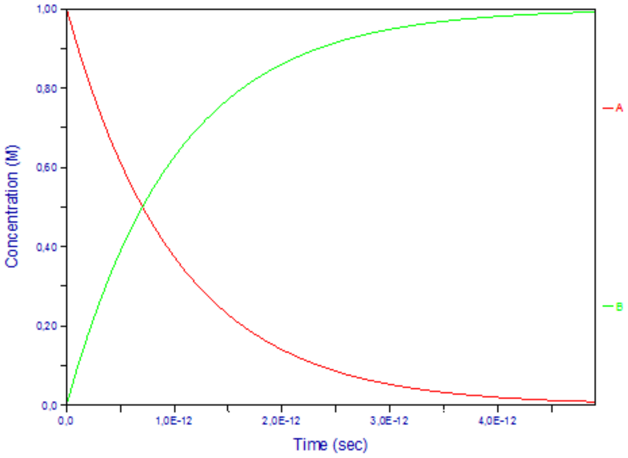


Figure 8 Kinetic trace showing the decay of singlet (A) and the rise of the triplet (B) of 2-(acetoxymethyl) -1,4- naphthoquinone in acetonitrile.

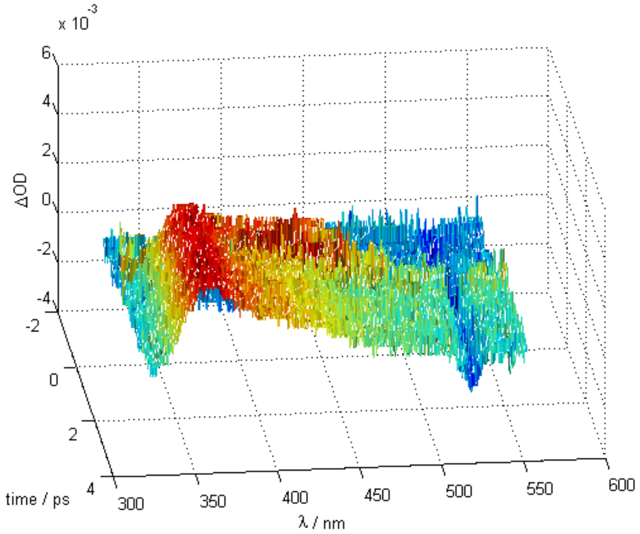


Figure 9 Picosecond pump-probe spectra of 2-(acetoxymethyl) -1,4-naphthoquinone in water-acetonitrile (9:1) within 4 ps.

CONCLUSION

To conclude this paper, it is demonstrated that 2-methyl-1.4-naphthoquinone can be used as a photoremovable protecting group for carboxylates if methanol, ethanol, and a mixture containing water and acetonitrile are used as solvent system. The mechanistic study is under investigations in order to know which state between singlet and triplet is responsible of the release.

Acknowledgements

This project was carried out because of the involvement of the Laboratory of Organic Photochemistry at Masaryk University in Brno, Czech Republic and the Laboratory of Photochemistry at University of Kwazulu Natal in Durban, South Africa for GC-MS, NMR, and elementary analysis. The authors thank Petr Klán for donation of LED reactors.

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Supporting information for:

A New Photoremovable Protecting Group Containing 2-Methyl-1,4-Naphthoquinone: Steady State and Transient Spectroscopic Studies

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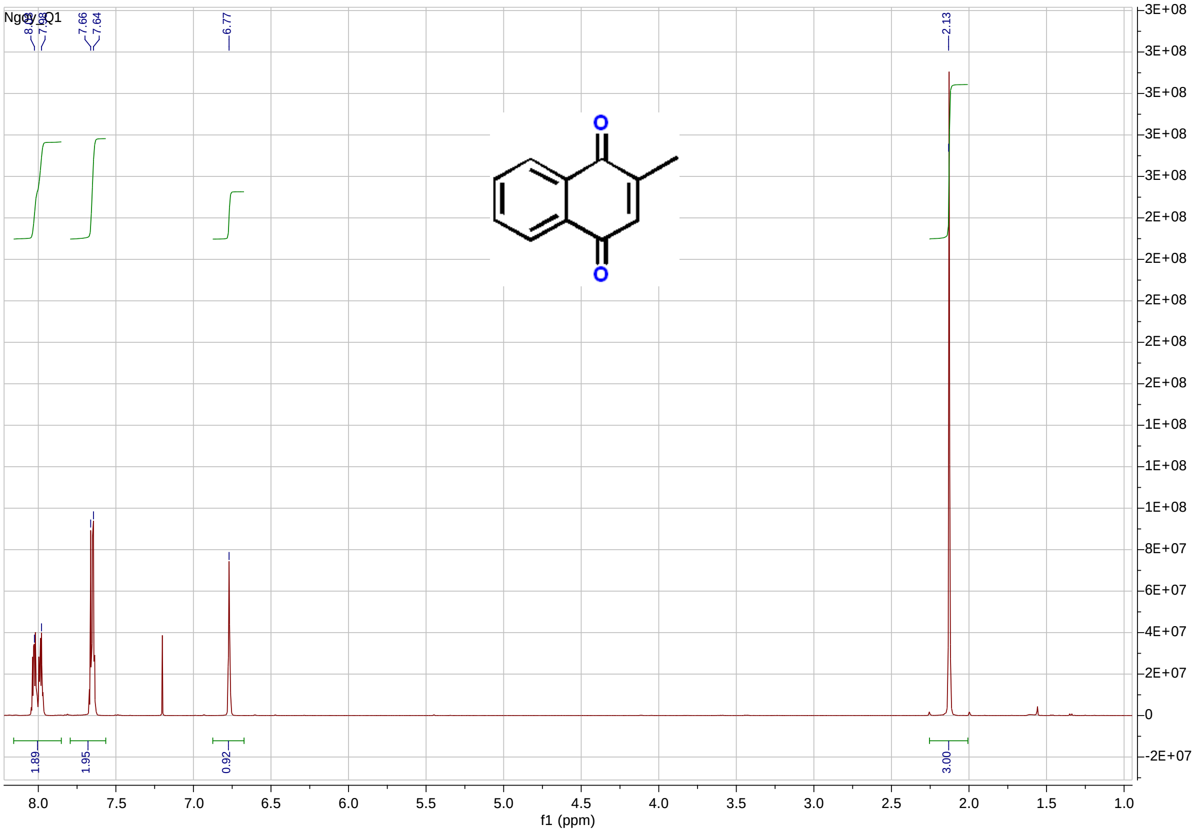


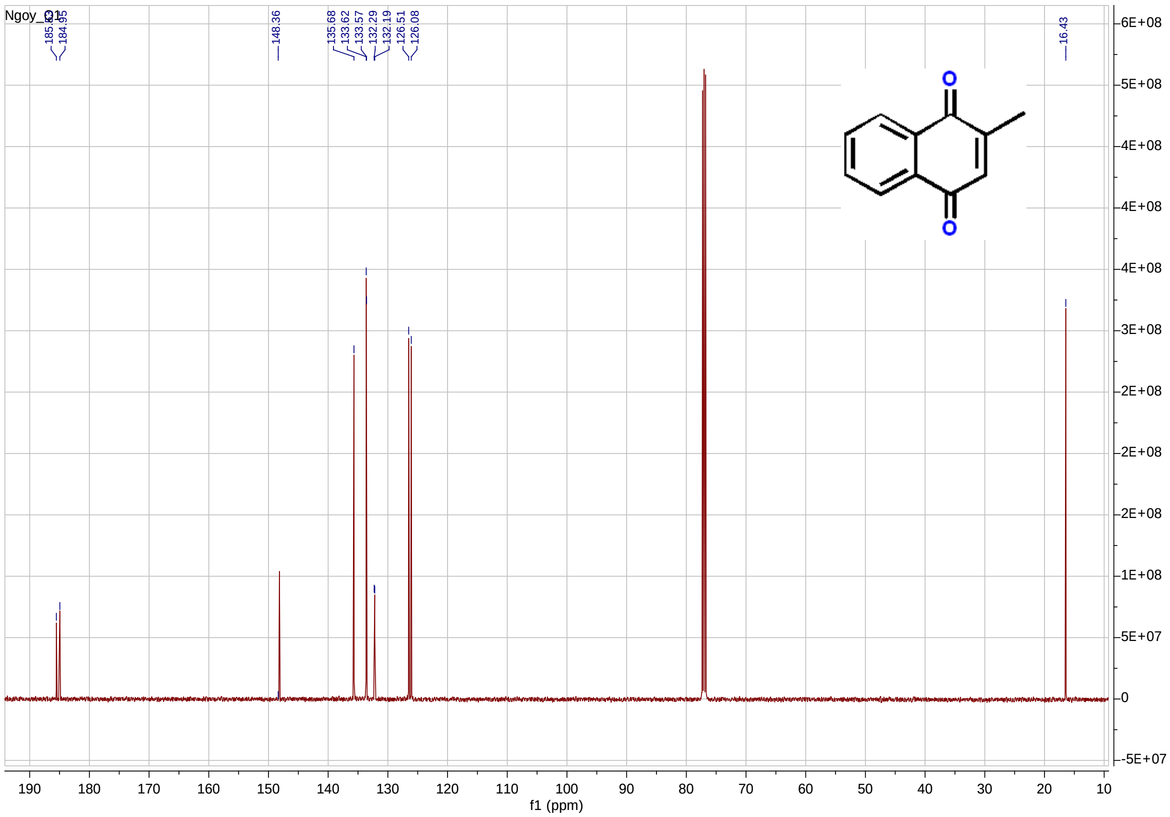
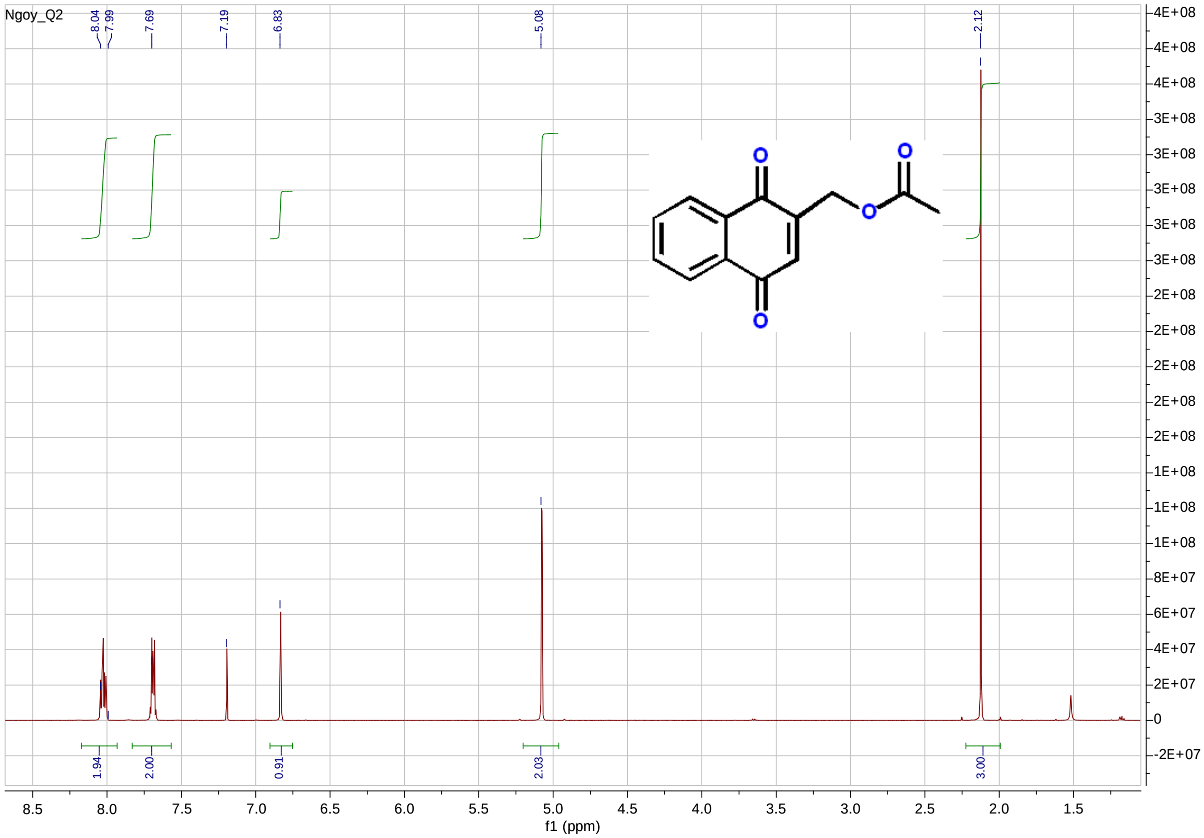


Figure S1 NMR spectra of parent compounds



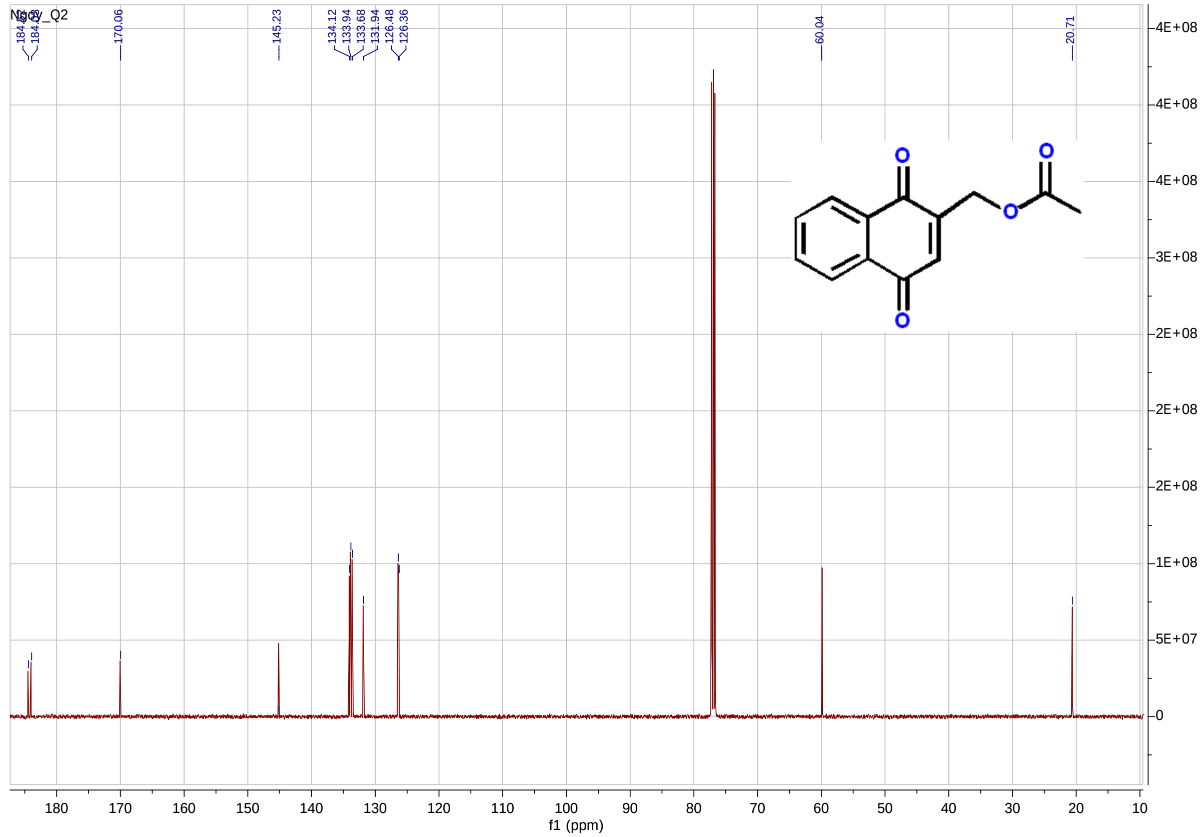


Figure S2 NMR spectra of 2-(acetoxymethyl)-1,4-naphthoquinone

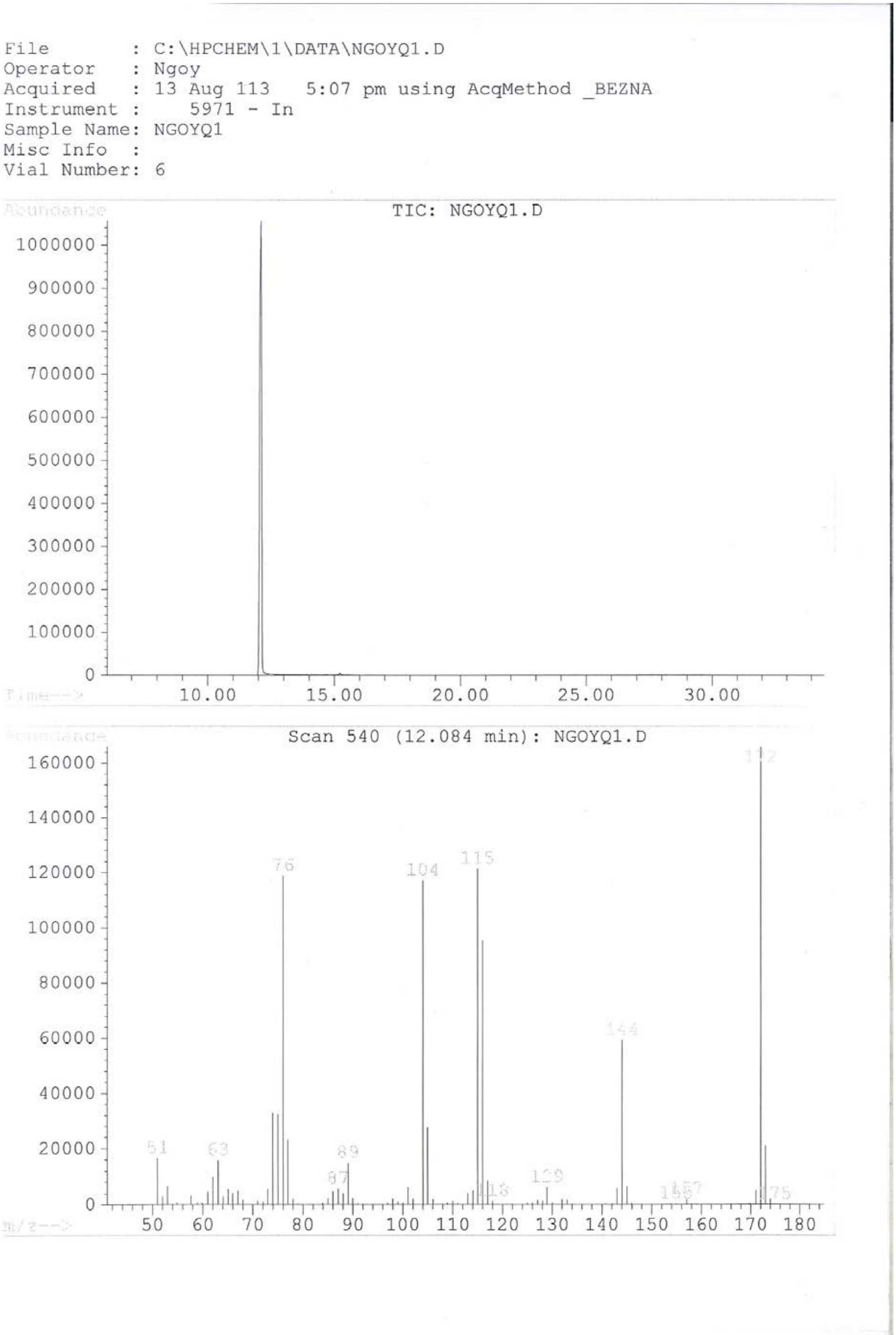


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Figure S3 GC-MS spectra of 2-methyl-1,4-naphthoquinone

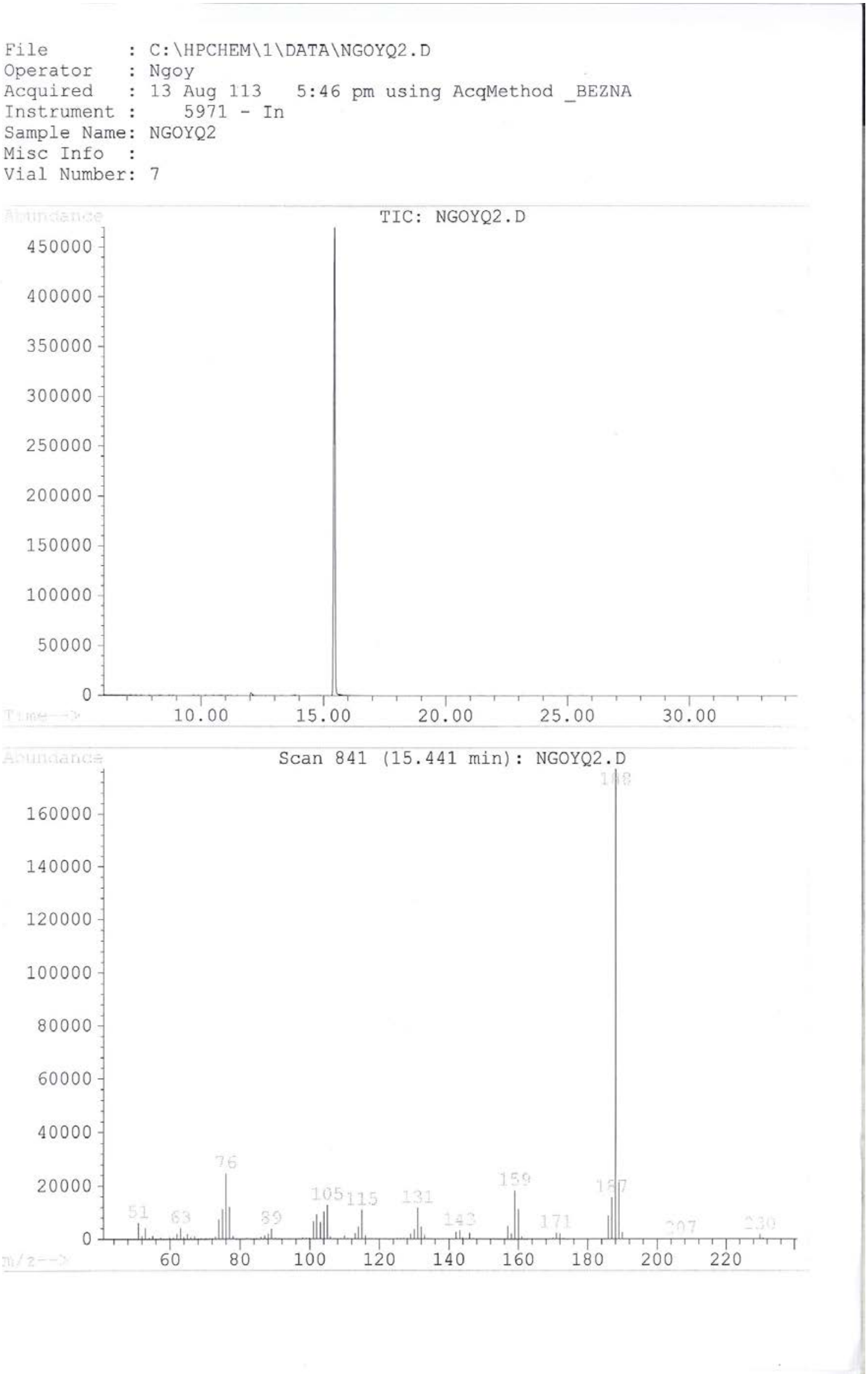

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Figure S4 GC-MS spectra of 2-(acetoxymethyl)-1,4-naphthoquinone

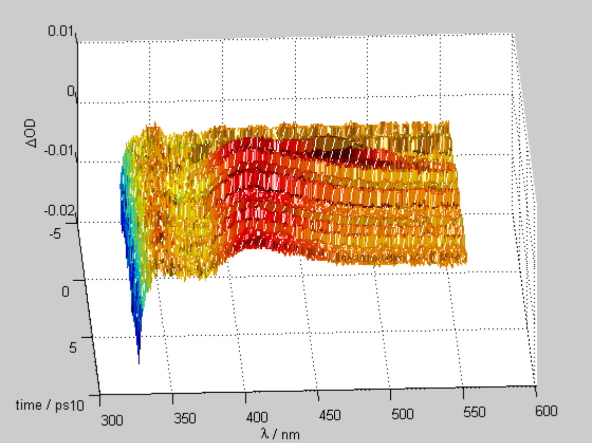

Figure S 5 Pump-probe spectra of 2-methyl-1,4-naphthoquinone in MeCN taken within 10 ps.

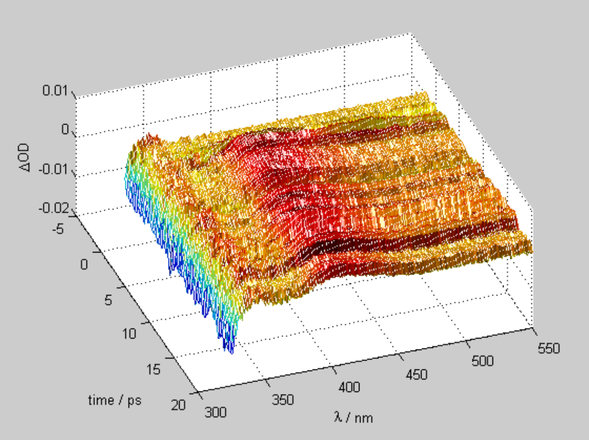

Figure S 6 Pump-probe spectra of 2-methyl-1,4-naphthoquinone in MeCN taken within 20 ps.

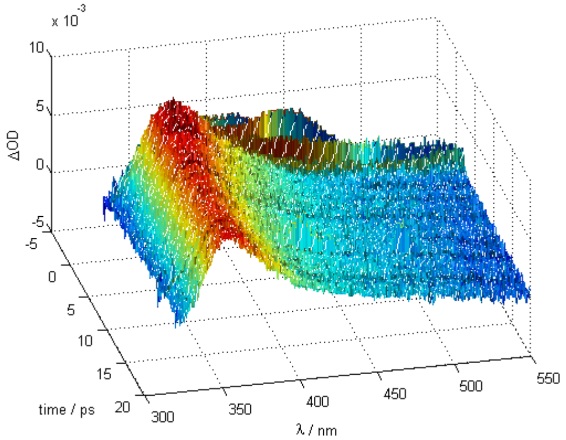

Figure S7 Pump-probe spectra of 2-(acetoxymethyl)-1,4-naphthoquinone in acetonitrile taken within 20 ps.