Alkylation of Amino Acids and Glutathione in Water by *o*-Quinone Methide. Reactivity and Selectivity

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o-Quinone methide (**1**) has been produced in water both thermally and photochemically from (2-hydroxybenzyl)trimethylammonium iodide (**2**). Michael addition reactions of **1** to various amines, and sulfides, including amino acids and glutathione have been carried out, obtaining alkylated adducts (**3**−**16**) in fairly good to quantitative yields. The reaction rate and selectivity of **1** toward nitrogen and sulfur nucleophiles, in competition with the hydration reaction, have been investigated at different pH by laser flash photolysis technique. The observed reactivity spans 7 orders of magnitude on passing from water ($k_{Nu} = 5.8\ M^{-1}\ s^{-1}$) to the most reactive nucleophile ($2.8 \times 10^8\ M^{-1}\ s^{-1}$, 2-mercaptoethanol under alkaline conditions). These are the first direct reaction rate measurements of nucleophilic addition to the parent *o*-quinone methide (**1**). Competition experiments provided strong kinetic support to the involvement of free **1** as an intermediate in both thermal and photochemical reactions. Furthermore, several alkylation adducts regenerate **1** either by heating (**9**, **10**, **13**, and **14**) or by irradiation (**9**, **11**−**13**, **16**). Such a thermal and photochemical reversibility of the alkylation process opens a new perspective for the use and application of such adducts as *o*-QM molecular carriers.

Introduction

Quinone methides (QMs) have been proposed as intermediates in a large number of chemical and biological processes.[1] The reactivity of QMs is mainly due to their electrophilic nature, which can also be directly correlated to their toxicological properties. QMs have been proposed as intermediates in the biosynthesis of lignin[2] and enzyme inhibition.[1,3−7] Among hydrolase inhibitors,[4−7] QMs have been recently used as covalent β-lactamase,[5] phosphatase,[4,6] and ribonuclease A[7] inactivators. It has been suggested that quinone methides play a key role in the chemistry of several classes of antibiotic drugs and antitumor compounds such as mitomycin C[8] and anthracyclines.[9,10]

o-QM (**1**) is the prototype of more complex *o*-quinone methide-like structures. With simple modifications suggested by Rokita[11] and others,[12] *o*-QM could cross-link two biologically useful molecules, such as nucleic bases or peptides and proteins. If the alkylation process is achieved under mild (ideally, biological) reaction conditions, it could be used in a number of biomolecular applications.

Numerous investigations on nucleic acid base alkylations with *o*-QMs have appeared in the past decade, mainly published by the Rokita's group.[11,13−15] In fact these reactive intermediates have been used as DNA alkylating agents and cross-linkers. Recently, the same author used parent *o*-QM **1**, generated by fluoride induced desilylation of *O*-(*tert*-butyldimethylsilyl)-2-(bromomethyl)phenol at 37−50 °C in aqueous DMF or acetonitrile, to alkylate dC,[14] dA,[15] and dG.[15]

More recently Turnbull investigated the possibility of alkylation of the phosphodiester functional group by *p*-QMs in the presence of a Brønsted acid.[16] He concluded that in the absence of an acid catalyst the potential alkylation of DNA through phosphodiester groups is unlikely.

QM reactions with various nucleophiles, often under nonaqueous conditions, have been studied.[17,18] More

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(1) Peter, M. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 555 and references therein.
(2) *Lignins: Occurrence, Formation, Structure and Reactions*; Sarkanen, K., V.; Ludving, C., Eds.; Wiley: New York, 1971.
(3) McDonald, I. A.; Nyce, P. L.; Jung, M. J.; Sabol, J. S. *Tetrahedron Lett.* **1991**, *32*, 887.
(4) Wang, Q.; Dechert, U.; Jirik, F.; Withers, S. G. *Biochem. Biophys. Res. Commun.* **1994**, *200*, 577.
(5) Cabaret, D.; Adediran, S. A.; Garcia Gonzales, M. J.; Pratt, R. F.; Wakselman, M. *J. Org. Chem.* **1999**, *64*, 713.
(6) Myers, J. K.; Cohen, J. D.; Widlanski, T. S. *J. Am. Chem. Soc.* **1995**, *117*, 11049.
(7) Stowell, J. K.; Widlanski, T. S., Kutateladze, T. G.; Raines, R. T. *J. Org. Chem.* **1995**, *60*, 6930.
(8) (a) Han, I.; Russell, D. J.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 1799. (b) Li, V.-S.; Kohn, H. *J. Am. Chem. Soc.* **1991**, *113*, 275. (c) Tomasz, M.; Das, A.; Tang, K. S.; Ford, M. G. J.; Minnock, A.; Musser, S.; Waring, M. J. *J. Am. Chem. Soc.* **1998**, *120*, 11581.
(9) (a) Egholm, M.; Koch, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 8291. (b) Gaudiano, G.; Frigerio, M.; Bravo, P.; Koch T. H. *J. Am. Chem. Soc.* **1990**, *112*, 6704.
(10) (a) Angle, S. R.; Rainier, J. D.; Woytowicz C. *J. Org. Chem.* **1997**, *62*, 5884. (b) Angle, S. R.; Yang, W. *J. Org. Chem.* **1992**, *57*, 1092.
(11) Zeng, Q.; Rokita, S. E. *J. Org. Chem.* **1996**, *61*, 9080.
(12) Nakatani, K.; Higashida, N.; Saito, I. *Tetrahedron Lett.* **1997**, *38*, 5005.
(13) Chatterejee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1994**, *116*, 1690.
(14) Rokita, S. E.; Yang, J.; Pande, P.; Shearer, J.; Greenberg, W. A. *J. Org. Chem.* **1997**, *62*, 3010.
(15) Pande, P.; Shearer, J.; Yang, J.; Greenberg, W. A.; Rokita, S. E. *J. Am. Chem. Soc.* **1999**, *121*, 6773.
(16) (a) Zhou, Q.; Turnbull, K. D. *J. Org. Chem.* **1999**, *64*, 2847. (b) Zhou, Q.; Turnbull, K. D. *J. Org. Chem.* **2000**, *65*, 2022.
(17) Leary, G.; Miller, I. J.; Thomas, W.; Woolhouse, A. D. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1737.
(18) Gardner, P. D.; Sarrafizadeh Rafsanjani, H.; Rand, L. *J. Am. Chem. Soc.* **1959**, *81*, 3364.

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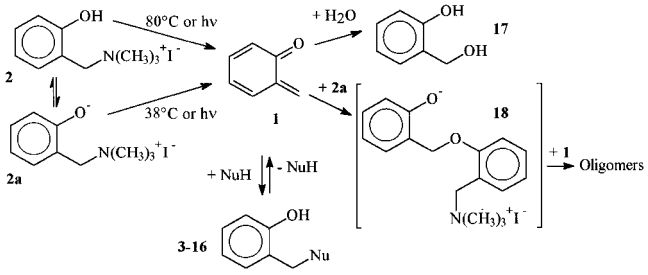
recently, a few investigations have focused on alkylation of cellular nucleophiles in water, such as amino acids and peptides, but only moderately reactive QMs such as 2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone (BHT-QM) and 2-*tert*-butyl-6-methyl-4-methylene-2,5-cyclohexadienone (BDMP-QM) have been used in these studies.[19,20] The comparison of the results concerning the alkylation of purine amines by BHT-QM, obtained by Thompson's group,[21] to those obtained by Rokita's group (which used *o*-QM **1**) seems to suggest that bulky *p*-QM intermediates react with deoxynucleosides with lower selectivity. If we assume the reactivity−selectivity principle to be operative, the above finding is surprising, because the prototype *o*-QM **1** is expected to be more reactive than the alkyl-substituted analogues above.[15] This aspect of the *o*-QM reactivity/selectivity is peculiar and, from our point of view, it should be more thoroughly investigated. Low reactivity of crowded QMs has mainly been attributed to the shielding of the carbonyl oxygen from solvent interactions (including hydrogen bonding) by the hydrophobic substituents at the 2- and 6-positions.[20,21] Other intrinsic structural features, pointed out by Soucek, such as the distortion of the quinonoid ring,[22] and electronic delocalization in benzoannelated QMs[23] play an important role in lowering the reactivity of 2,6-disubstituted and other QMs in comparison to unsubstituted QMs.

Although the research on enzyme inhibition and DNA cross-linking by QMs seeks a rationalization of the covalent labeling process, Wakselman notes that, "there is only a limited amount of data in the literature on the lifetime of quinone methides in aqueous solution"[5] in the presence of biological nucleophiles. Surprisingly, reported data are related only to 2,6-disubstituted QMs, and only reasonable hypotheses have been made on the reactivity of **1**.[15]

Due to their synthetic utility and biological importance, several methods for *o*-QM generation (i.e., high-temperature dehydration of *o*-hydroxybenzyl alcohols and amino derivatives,[24a−c] thermal extrusion of small molecules,[24a,d] oxidation of phenols,[24a,e] and photochemical deprotonation of *o*-hydroxybenzyl alcohols[25] or Mannich bases[12]) have been published. Unfortunately, only a few of these methods are suitable for studying reaction dynamics under mild conditions using water as solvent. We thought that an elimination reaction from a benzylammonium salt[26] could be the opportune strategy for *o*-QM generation in the presence of biological nucleophiles. Both thermal and photochemical (steady state and laser) activation processes of the salt were successful in water. We presently report a study on the reactivity and selectivity of **1**, in Michael addition reactions to nitrogen, oxygen, and sulfur nucleophiles of amino acids in competition with hydration under acidic, neutral, and basic conditions. The aim of this work is to measure and rationalize the electrophilic nature of **1** toward simple bifunctional and polyfunctional biological nucleophiles.

To the best of our knowledge neither adduct isolation nor direct measurement of alkylation rates have previously been reported for the reaction of the parent *o*-QM **1** with free amino acids and peptides in water. The data obtained from product distribution analysis, competition alkylation experiments, and laser flash photolysis (LFP) kinetic measurements will contribute to those investigations focused on QM-based enzyme inhibition and DNA alkylation studies.

Scheme 1



Results

Alkylation by Thermally Generated *o*-QM (1). The chosen precursor was (2-hydroxybenzyl)trimethylammonium iodide (**2**) easily available from the phenol. When this compound was heated at 38 °C and 80 °C in aqueous solution, *o*-QM **1** was generated and trapped by N, O, and S nucleophiles (see Scheme 1).

We initially investigated the alkylation of *n*-PrNH$_2$, *t*-BuNH$_2$, aniline, piperidine, morpholine, and 2-mercaptoethanol by quinone methide **1** obtaining addition products (**3**−**8**) (Scheme 2) in good to quantitative yields (see Table 1). The study was then extended to the product distribution (**9**−**16**, Scheme 2) arising from the alkylation, by the electrophile **1**, of free amino acids such as glycine (Gly), L-serine (Ser), L-cysteine (Cys), L-lysine (Lys), L-tyrosine (Tyr), and glutathione (Glu) in water solution from slightly acidic to basic conditions (5 < pH < 12).

Glycine, serine, and lysine were selectively alkylated at the N-atom(s) to give adducts **9**, **10** and **12**, **13**. Lysine was alkylated both at chain amino (Nϵ) and the α-amino groups (Nα) to give adducts **12** and **13**, respectively. No adducts arising from an *O*-alkylation of the carboxy or of the hydroxy (in the case of serine) groups were detected, by ^{1}H NMR or by HPLC analysis, in the reaction mixture. Under neutral or slightly acidic conditions *o*-hydroxybenzyl alcohol (**17**), resulting from the hydration of **1**, was always present as a byproduct. Increasing the pH of the solution (by NaHCO$_3$/Na$_2$CO$_3$

(19) McCracken, P. G.; Bolton, J. L.; Thatcher, G. R. J. *J. Org. Chem.* **1997**, *62*, 1820.

(20) (a) Bolton, J. L.; Turnipseed, S. B.; Thompson, J. A. *Chem.-Biol. Interact.* **1997**, *107*, 185. (b) Bolton, J. L.; Valerio, L. G.; Thompson, J. A. *Chem. Res. Toxicol.* **1992**, *5*, 816. (c) Thompson, D. C.; Perera, K.; Krol, E. S.; Bolton, J. L. *Chem. Res. Toxicol.* **1995**, *8*, 323. (21) Lewis, M. A.; Graff Yoerg, D.; Bolton, J. L. Thompson, J. A. *Chem. Res. Toxicol.* **1996**, *9*, 1368.

(22) Velek, J.; Koutek, B.; Musil, L.; Vasickova, S.; Soucek, M. *Collect. Czech. Chem. Commun.* **1981**, *46*, 873.

(23) (a) Musil, L.; Koutek, B.; Pisova, M.; Soucek, M. *Collect. Czech. Chem. Commun.* **1981**, *46*, 1148−1159. (b) Koutek, B.; Pisova, M.; Krupicka, J.; Lycka, A.; Snobl, D.; Soucek, M. *Collect. Czech. Chem. Commun.* **1982**, *47*, 1645.

(24) (a) Grünanger, P. *Methoden Org. Chem.* **1979**, *VII/3b*, 395. (b) Dorrestijn, E.; Kranenburg, M.; Ciriano, M. V.; Mulder, P. *J. Org. Chem.* **1999**, *64*, 3012. (c) Qiao, G. G.-H.; Lenghaus, K.; Solomon, D. H.; Reisinger, A.; Bytheway, I.; Wentrup, C. *J. Org. Chem.* **1998**, *63*, 9806. (d) Yato, M.; Ohwada, T.; Shudo, K. *J. Am. Chem. Soc.* **1990**, *112*, 5341. (e) Bolon, D. A. *J. Org. Chem.* **1970**, *35*, 3666.

(25) (a) Wan, P.; Barker, B.; Diao, L.; Fisher, M.; Shi, Y.; Yang, C. *Can. J. Chem.* **1996**, *74*, 465. (b) Diao, L.; Cheng, Y.; Wan, P. *J. Am. Chem. Soc.* **1995**, *117*, 5369. (c) Brousmiche, D.; Wan, P. *Chem. Commun.* **1998**, 491.

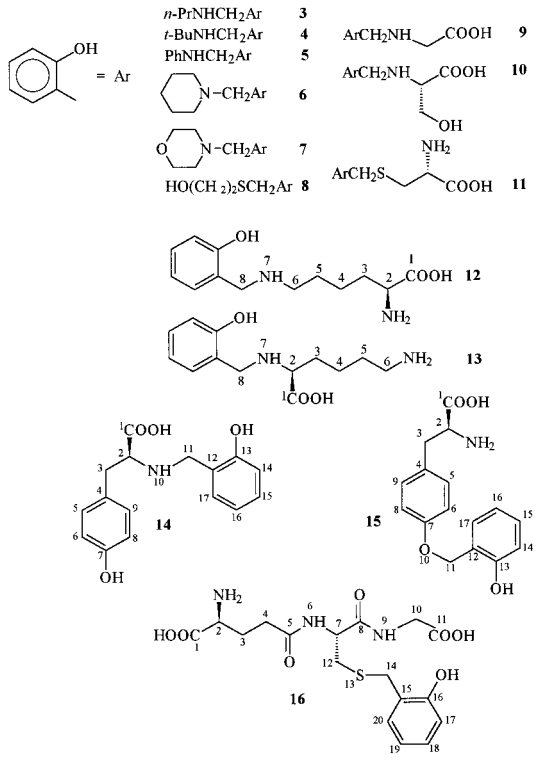
(26) (a) Gutsche, C. D.; Chun Nam, K. *J. Am. Chem. Soc.* **1988**, *110*, 6153. (b) Bladé-Font, A.; de Mas Rocabayera, T. *J. Chem. Soc., Perkin Trans. 1* **1982**, 841. (c) Breuer, E.; Melumad, D.; *Tetrahedron Lett.* **1969**, *23*, 1875.

Table 1. Products from Preparative Experiments, Generating *o*-QM 1 Thermally from 2, in the Presence of Amines, Thiols, Amino Acids, and Glutathione

nucleophiles	conditions (pH,[a] temp/°C, reaction time/h)	alkylation adducts (%, yield)[b]	hydroxybenzyl alcohol (**17**) (%, yield)[b]
n-$PrNH_2$	pH 12, 80 °C, 1 h	**3** (65)	-
t-$ButNH_2$	pH 12, 80 °C, 1 h	**4** (87)	-
$PhNH_2$	CH_3CN, 80 °C, 12 h	**5** (81)	-
piperidine	pH 12, 80 °C, 1 h	**6** (84)	-
morpholine	pH 12, 80 °C, 1 h	**7** (>99)	-
$HO(CH_2)_2SH$	pH 7, 80 °C, 3.5 h	**8** (95)	-
glycine	pH 7, 80 °C, 12 h	**9** (29)	70
	pH 10, 80 °C, 2 h	**9** (99)	-
serine	pH 6, 80 °C, 15 h	**10** (60)	36
	pH 7, 80 °C, 15 h	**10** (74)	21
	pH 10, 80 °C, 2 h	**10** (84)	4
cysteine	pH 7, 80 °C, 2 h	**11** (98)	-
	pH 8, 38 °C, 36 h	**11** (10)	-
	pH ≥ 10, 80 °C, 0.5 h	**11** (99)	-
		side-chain, α-amino	
lysine	pH 5.0, 80 °C, 3 h	**12** (−), **13** (−)	-
	pH 6.0, 80 °C, 3 h	**12** (10), **13** (30)	39
	pH 7.0, 80 °C, 1 h	**12** (17), **13** (32)	23
	pH 7.6, 80 °C, 1 h	**12** (33), **13** (40)	12
	pH 9.6, 80 °C, 1 h	**12** (35), **13** (39)	-
tyrosine	pH 7.0, 80 °C, 1 h	**15** (−), **14** (−)	32
	pH 8.5, 80 °C, 1 h	**15** (1.6), **14** (57)	23
	pH 9.0, 80 °C, 1 h	**15** (1.9), **14** (60)	17
	pH 9.5, 80 °C, 1 h	**15** (5.5), **14** (58)	14
	pH 10, 80 °C, 1 h	**15** (12), **14** (64)	10
	pH 12, 80 °C, 1 h	**15** (18), **14** (53)	8
glutathione	pH 7.0, 80 °C, 0.5 h	**16** (84)	-
	pH 7.8, 38 °C, 24 h	**16** (20)	-

[a] pH was maintained constant by use of the following buffers: CH_3COOH/CH_3COO^- (pH 5.0), KH_2PO_4/Na_2HPO_4 (pH 6.0−7.8), H_3BO_3/NaOH (pH 8.0−9.0), $NaHCO_3/Na_2CO_3$ (pH 9.5−10.4). pH 12.0 was adjusted by addition of NaOH 0.1 M solution. pH was measured in reference solutions, which were prepared to be identical to the corresponding solution from preparative irradiation. [b] Yield has been determined by HPLC analysis of the reaction mixture, after 10-fold dilution, comparing peak areas to standard solution (at known concentration) prepared from purified adducts.

Scheme 2



buffer) led to improved yields of alkylation products (Table 1). Thermal decomposition of **2** was inefficient at pH lower than 5, and under such conditions neither alkylation adducts arising from **1** nor **17** were detected.

The chemoselectivity in the alkylation of lysine and tyrosine was also a function of the solution pH. Specifically, α-amino/side chain product ratios (**13**/**12** and **14**/**15**) rose gradually from basic to neutral conditions (see Table 1). L-Tyrosine was not reactive under neutral conditions and after 1 h at 80 °C **17** was the only product detected in the reaction mixture. Nevertheless, two adducts, arising from a *N*-alkylation (**14**) and from an *O*-alkylation (**15**), were isolated and identified at higher pH (8.5 by borate buffer) at 80 °C. Similar to lysine, the chemoselectivity of the tyrosine alkylation, evaluated by the **14**/**15** ratio, is strongly affected by pH (see data in Table 1).

Cysteine and a cysteine-containing peptide such as glutathione were both alkylated, with complete selectivity, at the S atom, by **1** (pH 7 by KH_2PO_4/Na_2HPO_4 buffer, at 80 °C) and gave adducts **11** and **16** in quantitative yields. Under mildly basic conditions (38 °C and pH 7.8), only a small amount of the substrate was alkylated, but a higher pH (pH ≥ 10) allowed a quantitative transformation. No adducts arising from an *N*-alkylation of these substrates or from a competitive hydration were in any case detected in the crude reaction mixture by ^1H NMR or by HPLC analysis. Adduct **16** is the first example, to the best of our knowledge, of a fully characterized *o*-QM-glutathione type adduct.

The structures of the adducts **9**−**16** were firmly established on the basis of spectroscopic evidences, in particular on ^{13}C NMR chemical shifts, and $^1H-^{13}C$ short and long-range correlation experiments (HSQC and HMBC, see Experimental Section).

Table 2. Products from Preparative Experiments, Generating *o*-QM 1 Photochemically (at 254 nm) from 2, in the Presence of Free Amino Acids and Glutathione in Water

nucleophiles	conditions (pH,[a] $\lambda = 254$ nm, reaction time/h)	alkylation adducts (%, yield)[b]	hydroxybenzyl alcohol (**17**) (%, yield)[b]
glycine	pH 2, *hν*, 2 h	**9** (−)	6
	pH 7, *hν*, 2 h	**9** (25)	75
serine	pH 2, *hν*, 2 h	**10** (−)	45
	pH 7, *hν*, 2 h	**10** (9)	77
	pH 10, *hν*, 2 h	**10** (84)	-
cysteine	pH 2, *hν*, 1 h	**11** (20)	55
	pH 7, *hν*, 0.16 h	**11** (10)	-
	pH 7, *hν*, 1 h	**11** (45)	40
lysine	pH 12, *hν*, 2 h	**12** (16), **13** (64)	-
glutathione	pH 7, *hν*, 2 h	**16** (86)	12

[a] Constant pH was maintained by use of the following buffers: KH_2PO_4/Na_2HPO_4 (pH 6.0−7.8), $Na_2CO_3/NaHCO_3$ (pH 9.5−10.4). pH 2.0 and 12.0 were adjusted by addition of HCl and NaOH 0.1 M solutions, respectively. The pH was measured in reference solutions, which were prepared to be identical to the corresponding solution from preparative irradiation. [b] Yield has been determined by HPLC analysis of the reaction mixture, after 10-fold dilution, comparing peak areas to standard solution (at known concentration) prepared from purified adducts.

The decomposition of the ammonium salt **2** was also explored in the absence of added nucleophiles. Under slightly basic conditions (pH ≤ 10) the only product detected was **17**. At pH > 10 the thermal decomposition of **2** produced a mixture of insoluble oligomers which could not be fully characterized. The spectroscopic properties (IR and ^{1}H NMR, see Experimental Section) of this mixture are consistent with its formation from the anionic polymerization of **1** initiated by a Michael addition on **2a** through the intermediate **18** (according to Scheme 1). No detectable amounts of dimers (or trimers) arising from either a Diels−Alder dimerization of **1**[27] or a Michael addition between **1** and the phenolic OH group of the precursor **2**[28] were detected at pH lower than 10.

Alkylation by Photochemically Generated *o*-QM. We found that *o*-QM **1** could also be generated photochemically from (2-hydroxybenzyl)trimethylammonium iodide (**2**) or from the corresponding dipolar ion **2a** under basic conditions by irradiation at $\lambda = 254$ nm (see Scheme 1). Irradiation of **2** in the presence of glycine and under acidic conditions (pH 2 by 0.01 M HCl) afforded **17** in a low yield (6%) leaving **2** largely (86%) unreacted. No alkylation adduct was detected. When the pH of the solution was increased to 7, the precursor was quantitatively transformed, and the alkylated adduct **9** accounted for 25% of the product mixture (Table 2).

Likewise, irradiation of **2** at pH 2 in the presence of serine afforded **17** (45%), unreacted starting material (42%) and no alkylation adduct. The preparative photochemical alkylation of serine to give **10** became progressively more efficient when we passed from neutral to basic conditions (see data in Table 2). At pH 10, adduct **10** was the only product detected and isolated (84% yield).

In the case of lysine at pH 12, a mixture of adducts **12** and **13** (16% and 64% yield, respectively) and unreacted precursor (20%) was obtained. The **12/13** ratio was not constant with the irradiation time. Specifically, it dropped from 0.38 to 0.25 increasing the irradiation time from 1 min up to 2 h. With cysteine (after 1 h of irradiation of **2** at pH 7), adduct **11** and **17** were present in almost equimolar amount, together with unreacted starting material **2**. In preparative irradiation the ratio **11**/**17** changed with time. In the initial stage this ratio was very high (in fact, after a few minutes no hydroxybenzyl alcohol could be detected), and the ratio increased to approximately 1 after 1 h.

The alkylation of glutathione to give **16** proceeded efficiently under photochemical conditions (86% yield). A preparative photochemical alkylation of cysteine or glutathione under basic conditions was not feasible because such substrates in the anionic form absorbed at 254 nm precluding excitation of **2a**.

Photochemical and Thermal Reactivity of the Alkylation Adducts. As mentioned in the previous paragraph, it was noted that alkylation adducts/**17** and **12**/**13** ratios (in preparative photochemical experiments) depended on the irradiation time. The above ratios were also different from those obtained in thermal experiments. This evidence suggested that a photoinduced elimination process could have taken place from adducts **9**−**13** and **16** with reformation of *o*-QM **1**. In fact, the irradiation at 254 nm of **9**, **11**−**13**, and **16** in diluted aqueous solution (concentration 10^{-3} M and pH ≤ 7) at low conversion afforded **17** and the corresponding free amino acid (see Table 3). Surprising and noteworthy is the high photoreactivity of adducts **12** and **13**, which both efficiently decompose under acid conditions (3 < pH < 7) to give **17** (see data in Table 3) with high and very similar quantum yields ($\Phi = 0.78$ and $\Phi = 0.73$, respectively). Under basic condition (pH 12) **12** and **13** were still photoreactive, though with lower quantum yields. The photogenerated **1** could be trapped by adding cysteine at low concentration (10^{-3} M). In particular, the quantum yield of *o*-QM generation from **12** ($\Phi = 0.38$) was three times higher than that one from **13** ($\Phi = 0.13$). The higher photofragmentation efficiency of **12** in basic solution, rationalizes the chemoselectivity (**13**:**12** = 4:1) observed in the preparative photochemical alkylation of lysine.

As far as thermal stability is concerned, all the adducts alkylated at the α-amino acid groups, i.e., **9**, **10**, **13**, and **14**, decomposed at 80 °C under neutral conditions within a few hours, affording **17** (see Table 3) and the corresponding free amino acid. The other adducts (**8**, **11**, **12**, and **15**) were stable in water at pH ≤ 7, and 80 °C for at least 2 h.

Absolute Rate Constants for the Alkylation of *o*-QM As Measured by LFP. Laser flash photolysis (LFP) was used to investigate the formation, as intermediate, of *o*-QM **1** and to measure its alkylation rate constants with S (thiol) and N (amine) nucleophiles, including amino acids and glutathione. A Nd:YAG laser (power 2−8 mJ, pulse duration 10 ns, $\lambda_{exc} = 266$ nm) was used for the measurements, and the transient absorption was recorded between 300 and 550 nm. As a diluted solution of **2** in water was flashed, a transient with absorption maximum at 400 nm was detected (Figure 1). This absorption was attributed to **1** on the basis of the following evidences: (a) the absorption maximum was very close to that produced from hydroxybenzyl alcohol

(27) (a) Letulle, M.; Guenot, P.; Ripoll, J.-L. *Tetrahedron Lett.* **1991**, *32*, 2013. (b) Gardner, P. D.; Sarrafizadeh Rafsanjani, H.; Brandon, R. L. *J. Am. Chem. Soc.* **1959**, *81*, 5515. (c) Cavitt, S. B.; Sarrafizadeh Rafsanjani, H.; Gardner, P. D. *J. Org. Chem.* **1962**, *27*, 1211.

(28) (a) Chiba, K.; Hirano, T.; Kitano, Y.; Tada, M. *Chem. Commun.* **1999**, 691. (b) Inoue, T.; Inoue, S.; Sato, K. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1062.

Table 3. Thermal and Photochemical (at 254 nm) Reactivity of the Alkylated Adducts

alkylated adducts	conditions (pH,[a] thermal or photochemical activation,[b] reaction time/h)	unreacted alkylation adduct (%, yield)[c]	**17** or **11** (%, yield)[c]
9	pH 3, 80 °C, 1 h	100	-
	pH 7, 80 °C, 1 h	96	**17** (3)
	pH 12, 80 °C, 1 h	97	**17** (2)
10	pH 7, 80 °C, 3 h	70	**17** (28)
12	pH 3, 80 °C, 1 h	100	-
	pH 7, 80 °C, 1 h	100	-
	pH 12, 80 °C, 1 h, + cysteine[d]	95	**11** (5)
13	pH 3, 80 °C, 1 h	100	-
	pH 7, 80 °C, 1 h	80	**17** (20)
	pH 12, 80 °C, 1 h, + cysteine[d]	83	**11** (17)
14	pH 7, 80 °C, 3 h	65	**17** (32)
9	pH 3, *hν*, 0.08 h	75	**17** (25)
	pH 7, *hν*, 0.16 h	86	**17** (14)
	pH 12, *hν*, 0.16 h, + cysteine[d]	90	**11** (10)
11	pH 3, *hν*, 0.16 h	30	**17** (68)
	pH 7, *hν*, 0.16 h	80	**17** (16)
12	pH 3, *hν*, 0.16 h	-	**17** (99)
	pH 7, *hν*, 0.16 h	-	**17** (99)
	pH 12, *hν*, 0.16 h, + cysteine[d]	-	**11** (10)
13	pH 3, *hν*, 0.16 h	-	**17** (99)
	pH 7, *hν*, 0.16 h	-	**17** (99)
	pH 12, *hν*, 0.16 h, + cysteine[d]	-	**11** (15)
16	pH 7, *hν*, 0.25 h	78	**17** (19)

[a] Constant pH was maintained by use of the following buffer: KH_2PO_4/Na_2HPO_4 (pH 6.0−7.8). pH 3.0 and 12.0 were adjusted by addition of HCl (0.01 M) and NaOH (0.1 M) solutions, respectively. The pH was measured in reference solutions, which were prepared to be identical to the corresponding solution from preparative irradiation. [b] Concentration of the alkylation adducts **9**, **11**−**13** were 10^{-3} M; 10-fold smaller than in preparative photochemical experiments (in Table 2). [c] Yield has been determined by HPLC analysis of the reaction mixture, without further dilution, comparing peak areas to standard solution (at known concentration) prepared from purified adducts. [d] Cysteine was added as *o*-QM trap, to avoid polymerization of **1**, which occurs at pH 12 in absence of efficient nucleophiles.

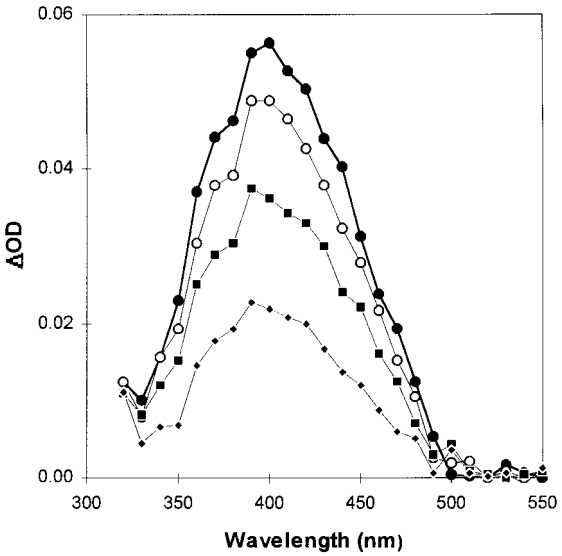


Figure 1. Transient absorption spectrum of *o*-QM **1**, following 266 nm excitation of an aqueous solution of **2** (0.4 mM) at pH 7, recorded 0.2 (●), 0.5 (○), 1.0 (■), and 2 ms (◆) after the laser pulse.

both by high-temperature dehydration in the gas phase[27a] and by photolysis in acetonitrile solution,[25] (b) it showed a 40 nm blue shift with respect to that reported by Wan for a phenyl-substituted *o*-QM,[25a] and (c) its lifetime was reduced by the addition of N and S nucleophiles. *o*-QM **1** was a long-lived species in neat acetonitrile ($\tau > 1$ s, not measurable with a fast technique like LFP) but in water (pH 7) it decayed with a medium-long lifetime [$\tau = 2.30$ (±0.05) ms], which was unaffected by the presence of oxygen, in accordance with what has been reported by Wan.[25b]

Photogeneration of **1** from **2** at various pHs showed that its formation was more efficient at pH 7, but it remains high also under alkaline conditions. After 50 laser shots, **17** was detected in the solution as the only product. In the presence of the nucleophiles, the corresponding alkylation adducts were identified by comparison with authentic samples obtained from thermal preparative experiments, as described above.

o-QM **1** was highly reactive (Table 4), particularly with sulfur and nitrogen nucleophiles. On the other hand hydration of *o*-QM was a much slower process (by at least 10^4-10^5 times) under neutral conditions, but the rate of this reaction increased under both acid and alkaline conditions.

The direct measurement of the second-order rate constants of the alkylation and hydration reactions by LFP allowed us a quantitative evaluation and comparison of the reactivity and selectivity of **1** toward free amino acids and the solvent at different pH. Plots of the pseudo-first-order rate constant vs nucleophile concentration yielded linear correlations. The second-order rate constants (k_{Nu}, see Table 4) for the addition reaction were obtained from the slopes of these plots.

o-QM lifetime was not reduced by addition of acetate, chloride, and perchlorate ions in 0.1 M concentration. In particular *o*-QM became slightly more stable increasing the ionic strength to 0.1 M by NaCl [$\tau = 3.1$ (±0.05) ms].

Furthermore, the determination of the **14**/**15** product ratio, at pH 12, afforded the opportunity to calculate the second-order rate constant for the alkylation of the hydroxy group of tyrosine in the anionic form: $k_{Nu} = 2.3 \times 10^5\ M^{-1}\ s^{-1}$. This value was obtained by assuming that the absolute rate constant for the alkylation of the tyrosine amino group was identical to that of the glycine NH_2.

Table 4. Second Order Rate Constants (k_{Nu}), Measured in Water by LFP, for the Alkylation Reactions by *o*-QM 1

alkylated substrates[a]	pK_a	*o*-QM k_{Nu} (M^{-1} s^{-1})	BDMP-QM k_{Nu} (M^{-1} s^{-1})[b]	relative (to water) *o*-QM reactivity scale	relative (to water) BDMP-QM reactivity scale
H_2O ($I = 0.1$ M)	-	5.8[c,d]	2.7×10^{-4}	1	1
H_2O ($I = 0.0$)		7.8[c,e]			
OH^-	15.74[f]	6.3×10^4 [g]	50	1.1×10^4	1.8×10^5
H_3O^+	-	1.4×10^6 [h]	200	2.4×10^5	7.4×10^5
n-$PrNH_2$ (pH 12.0)	10.68[i]	5.5×10^5	-	9.5×10^4	-
t-$BuNH_2$ (pH 12.0)	10.34[j]	1.1×10^5	-	1.9×10^4	-
piperidine (pH 12.0)	11.24[k]	1.3×10^6	-	2.2×10^5	-
morpholine (pH 12.0)	8.78[k]	2.3×10^6	-	4.0×10^5	-
Et_3N (pH 12.0)	10.9[l]	7.1×10^5	-	1.2×10^5	-
glycine (pH 12.0)	9.68[m]	6.9×10^5	-	1.2×10^5	-
lysine (pH 12.1)	10.8[n] 9.16[n]	5.9×10^5	18.6 ± 2.8	1.0×10^5	6.9×10^4
$HO(CH_2)_2SH$ (pH 6.9)	9.17[o]	1.9×10^5	-	3.3×10^4	-
$HO(CH_2)_2S^-$ (pH 12.1)	-	2.8×10^8	-	4.8×10^7	-
Tyr-O^- (pH 12.0)	9.99[p]	2.3×10^5 [q]	-	3.6×10^4	-
Tyr-NH_2 (pH 12.0)	8.94[p]	6.9×10^5	45.0 ± 3	1.2×10^5	1.7×10^5
cysteine (pH 6.8)	8.2[r]	1.3×10^5	3320 ± 66	2.2×10^4	1.2×10^7
cysteine (pH 12.2)	-	1.3×10^8	-	1.9×10^7	-
glutathione (pH 7.1)	8.72[r]	9.5×10^5	-	1.6×10^5	-

[a] In brackets is reported the pH of the solutions stabilized by KH_2PO_4/Na_2HPO_4 buffer (pH 6.8−7.1). pH 12.0 was adjusted by addition of NaOH 0.1 M. [b] From ref 19. [c] Second-order rate constant for the hydration process (k_{H_2O}) at pH 7 has been calculated from the measured pseudo-first-order rate constant and the H_2O concentration in pure water. [d] k_{H_2O} in a solution with constant ($I = 0.1$ M) ionic strength by NaCl. Identical value has been obtained using $NaClO_4$. [e] k_{H_2O} in a solution of pure water ($I = 0.0$). [f] Reference 52. [g] Obtained monitoring the lifetime of **1** as a function of HO^- concentration, adding NaOH, and keeping the ionic strength constant ($I = 0.1$ M) with NaCl. [h] Obtained monitoring the lifetime of **1** as a function of H_3O^+ concentration, adding HCl, and keeping the ionic strength constant ($I = 0.1$ M) with NaCl. [i] Reference 53. [j] Reference 54. [k] Reference 55. [l]Reference 56. [m] Reference 57. [n] Reference 34. [o] Reference 58. [p] Reference 35, 36. [q] Calculated k_{Nu} value, from **14**/**15** product ratio, assuming the alkylation rate to the N atom identical to the alkylation rate of glycine. [r] Reference 59.

Table 5. Competition Alkylation Experiments Involving $PrNH_2$/*t*-$BuNH_2$, Morpholine/Piperidine, and *t*-$BuNH_2/OH^-$ as Couples of Nucleophiles

competing nucleophiles	couple of adducts	adducts ratio		second-order rate constant ratios ($k_{Nu'}/k_{Nu''}$)[a]
		$h\nu$[b]	Δ[c]	
n-$PrNH_2$ *t*-$BuNH_2$	**3/4**	4.6 (±0.3)	4.9 (±0.1)	5.0
morpholine piperidine	**6/7**	1.5 (±0.2)	1.6 (±0.1)	1.7
t-$BuNH_2$ (pH 12)[d] OH^- (pH 12)[d]	**4/17**	1.6 (±0.2)	1.7 (±0.1)	1.8

[a] From absolute second-order rate constants (k_{Nu}) in Table 4. [b] Photochemical competition experiments were performed at 254 nm, at low conversion, in the presence of a 10-fold excess of nucleophiles, which were in equimolecular ratio (for more detail, see Experimental Section, competition experiments). The values were obtained as an average of five different experiments. [c] Thermal competition experiments at low conversion (see Experimental Section, competition experiments). The values were obtained as an average of three different experiments. [d] pH was adjusted by NaOH solution (0.1 M).

In the absence of nucleophiles the decay of *o*-QM **1** consistently followed a pseudo-first-order kinetic. This kinetic behavior provided convincing evidence that the dimerization of **1**, through a [4 + 2] Diels−Alder reaction, was not significant under these conditions. At pH > 10, the decay rate of **1** became a linear function of the precursor (**2a**) concentration. This suggested that under alkaline conditions the dimerization process was occurring via Michael addition of the precursor **2a** on *o*-QM (**1**) (see Scheme 1). This kinetic evidence fits with the formation of a mixture of oligomers in preparative experiments under the same conditions.

When a diluted solution of adducts **9**−**13** and **16** was flashed at 266 nm, *o*-QM (**1**) was generated as transient with an identical spectrum as that in Figure 1. This unequivocally supports that the alkylation adducts **9**−**13** and **16** are also photoprecursors of **1**.

Competition Alkylation Experiments. Although quinone methides have been proposed as the intermediates involved in thermally induced decomposition reactions of phenolic Mannich base methiodides[25] no direct experimental proof has been produced so far. To produce such an evidence, we carried out a set of competition alkylation experiments involving a few couples of nucleophiles, generating **1** with both a thermal and photochemical activation process. We used *n*-$PrNH_2$/*t*-$BuNH_2$, morpholine/piperidine, and *t*-$BuNH_2/OH^-$, at pH 12, in equimolecular ratio, as couples of nucleophiles. We generated **1** both under thermal and photochemical conditions (at low conversion <10%). The product ratios of the thermal and photochemical alkylations were very similar to each other. Moreover, their values were almost identical to the ratios calculated from the measured (by LFP) absolute rate constants. The results are summarized in Table 5.

Discussion

Other studies have used the approach of benzylammonium thermal elimination[26] and the photochemically induced deprotonation of *o*-hydroxybenzyl alcohols[25] and (2-hydroxybenzyl)dimethylamine[12] to achieve *o*-QM generation. Nevertheless, we feel that some aspects of our investigation are novel and they could be useful for further applications involving *o*-QM **1** as biological alkylating agent. They will be discussed in more detail in the following paragraphs.

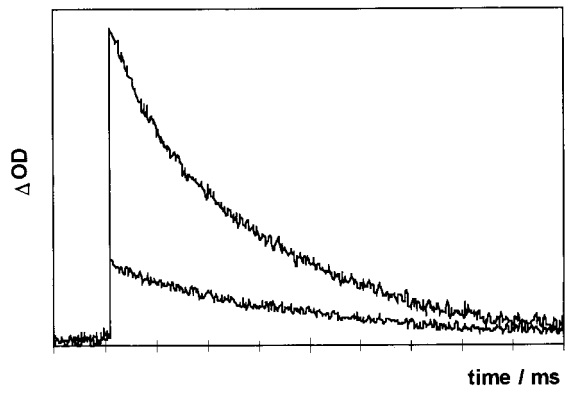


Figure 2. Decay curves for *o*-QM **1** generated from **2** (upper trace) and from hydroxybenzyl alcohol (**17**) (lower trace) at 400 nm, in water.

***o*-QM (1) Generation.** Our investigation has revealed that *o*-QM **1** can be generated not only in organic solvents, but also in water by a thermally induced elimination at 80 °C at pH > 5, using salt **2** as precursor. Generation of **1** from **2** is easier under slightly basic conditions as revealed from more efficient alkylation reactions. In fact, we obtained higher alkylated product yields in shorter reaction times at pH ≥ 8. Thus, the results of preparative experiments can be rationalized by assuming that compound **2a** is the actual precursor of **1** under basic conditions (pH 12), where its concentration can be significant. Noteworthy, the activation of the substrate (Scheme 1) can be thermally achieved under almost physiological conditions (38 °C and pH 7.8) where *o*-QM can be trapped by an efficient nucleophile such as cysteine.

Since proteins, peptides, purine, and pyrimidine nucleobases all contain basic functionalities and the acidity of **2** is not negligible [pK_a(**2**) = 8.74],[29] they could induce formation of **1** from **2** (through **2a**) by base catalysis. Thus, it is reasonable to consider **2** a leading candidate for alkylation of biological nucleophiles by *o*-QM **1** through a pH-activated process.

Passing to the photogeneration of **1** from **2**, it should be stressed that it was not unexpected. Indeed, Wan had previously studied the photoreactivity of substituted hydroxybenzyl alcohols,[25] and even more recently Saito used (2-hydroxybenzyl)dimethylamine as a photoprecursor of **1**, trapping it by electron rich alkenes in a hetero Diels−Alder reaction.[12] Nevertheless, with LFP of **2**, we managed to achieve, for the first time, a full spectroscopic characterization of **1** in water (see Figure 1). LFP of hydroxybenzyl alcohol, as stated by Wan, "gave a long lived species but the spectrum was much weaker and not as well-defined".[25b] We were able to record the full spectrum of **1**, because its quantum yield from **2** was 4.3 times higher [Φ = 0.98 (±0.02) at pH 7] than that from the alcohol (Φ = 0.23).[25b] The decay traces of **1** generated, respectively, from hydroxybenzyl alcohol and from salt **2** (Figure 2), clearly indicated the latter as a more efficient precursor of **1** than the alcohol. The above quantum yield was calculated from the ratio of the absorption of **1** (at time zero) obtained by flashing a solution of **2** and a solution of hydroxybenzyl alcohol (keeping the laser power and the absorbance of both solutions constant) and then multiplying it by the quantum yield determined by Wan.[25b]

Comparison of *o*-QM photochemical generation from **2** and (2-hydroxybenzyl)dimethylamine (used by Saito)[12] demonstrates that both precursors have identical efficiency at pH 7, each showing quantum yield Φ ∼ 1. Nevertheless, our *o*-QM photochemical generation from **2** remains advantageous for two reasons: (i) lower nucleophilicity of the precursor and (ii) higher quantum yield under alkaline conditions. The scarce nucleophilic character of **2** prevents its alkylation by **1**, while the opposite occurs with Saito's amine.[30] Both aspects make salt **2** an ideal *o*-QM photoprecursor for LFP kinetic measurements of alkylation process of several nucleophiles even under basic conditions.

The fact that product ratio in both thermal and photochemical alkylations was almost identical to the ratio calculated from the absolute rate constants measured by LFP (see Table 5) provides compelling kinetic evidence that *o*-QM **1** is the actual intermediate in both processes. Therefore, an alternative mechanism involving direct displacement of trimethylamine from the salt **2**, or **2a**, is unlikely.

Reactivity of *o*-QM as Alkylating Agent. The absolute second-order rate constants (k_{Nu}) for alkylation reactions by QMs present in the literature are sporadic and have often been derived from product ratios.[19] One could also be tempted to apply such an approach to **1**. However, due to the variable thermal and photochemical instability of some *o*-QM alkylation adducts, such an approach could give erroneous results. On the other hand, we have shown that the generation of *o*-QM **1** starting from **2** by LPF afforded a way to measure its k_{Nu} with several nucleophiles. The above technique allows a quantitative analysis of the reactivity of **1** and, in principle, of other highly reactive QMs.

o-QM reactivity (see data in Table 4) spans seven magnitude orders on passing from water (k_{Nu} = 5.8 M^{-1} s^{-1}) to the most reactive nucleophiles (2.8 × 10^8 M^{-1} s^{-1}, 2-mercaptoethanol; 1.3 × 10^8 M^{-1} s^{-1} Cys; under strongly alkaline conditions). Thiols are the only nucleophiles that can be quantitatively alkylated also under neutral or slightly acidic conditions (3 < pH < 7). In fact, under such conditions they still react with k_{Nu} higher than 1 × 10^5 M^{-1} s^{-1} (see data in Table 4). Unprotonated NH_2 groups are also highly reactive toward **1**, showing k_{Nu} from 1.1 × 10^5 to 2.3 × 10^6 M^{-1} s^{-1}.

Our results show that *o*-QM **1** is the most reactive alkylating agent in water among QMs.

In fact, *o*-QM **1** appears to be much more reactive than 2-*tert*-butyl-6-methyl-4-methylene-2,5-cyclohexadienone (BDMP-QM),[19] which has been defined as "highly reactive" in previous investigation.[20a] The former reacts 2.1 × 10^4, 1.3 × 10^3 and 39-fold faster than the latter toward H_2O, OH^-, and cysteine, respectively (see Table 4 for more details).

Even the highly electrophilic 4-[bis(trifluoromethyl)-methylene]cyclohexa-2,5-dienone[31] studied by Richard is

(29) Epstein, J.; Plapinger, R. E.; Michel, H. O.; Cable, J. R.; Stephani, R. A.; Hester, R. J.; Billington, C., Jr.; List, G. R. *J. Am. Chem. Soc.* **1964**, *86*, 3075.

(30) *o*-QM (**1**) lifetime is at least 10 times shorter if it is generated starting from (2-hydroxybenzyl)dimethylamine. The lifetime is also linearly dependent on the precursor concentration. This kinetic evidence show that (2-hydroxybenzyl)dimethylamine competes for *o*-QM (**1**) more efficiently than water, even at low concentration (∼10^{-4} M).

(31) Richard, J. P.; Toteva, M. M.; Crugeiras, J. *J. Am. Chem. Soc.* **2000**, *122*, 1664.

60- and 130-fold slower than *o*-QM **1** in the addition to thiolates (propanethiolate, $k_{Nu} = 4.6 \times 10^6$ M^{-1} s^{-1})[32] and primary amines (ethylamine, $k_{Nu} = 4.1 \times 10^3$ M^{-1} s^{-1}),[32] respectively. At present no other data on *o*-QM alkylation rates are available in the current literature. Our results in addition to those results obtained for BDMP-QM[19,20] and 4-[bis(trifluoromethyl)methylene]cyclohexa-2,5-dienone[31] represent, to our knowledge, the only kinetic data present in the literature on QMs reactivity in water.

The fact that *o*-QM reactivity with water is the highest of all known QMs may have an important consequence in limiting the pharmacological side effects of *o*-QM-based drugs. In fact, it is commonly believed that the shorter QM half-lives the lower their hepatotoxic effects.[19,20b,c]

Selectivity of *o*-QM in Alkylation Reactions. Although *o*-QM **1** is the most reactive among QMs, it is also, surprisingly, a quite selective alkylating agent. Comparing the second-order rate constants measured by LFP (Table 4) we can compile a nucleophilicity scale toward *o*-QM: $RS^- \gg R_3N > PhO^- > RSH \approx RNH_2$(bulky R substituents) $> HO^- \gg H_2O \geq Cl^-$, AcO^-, ROH.[33]Such a scale can be used also to rationalize the selectivity of **1** in alkylation reactions.

Obviously, the pH of the solution has an important role in controlling the selectivity of the alkylation, particularly when the competing nucleophiles have different basicity, as in the case of the alkylation of Nϵ versus Nα amino groups of lysine and amino versus hydroxy groups of tyrosine. The selectivity enhancement of *o*-QM, for the α-amino versus the ϵ-amino group of lysine, on passing from basic to neutral conditions, can be rationalized by the different degree of protonation of the former relative to the latter ($pK_a(2) = 9.16$, $pK_a(3) = 10.81$).[34] The above explanation for such a selectivity of the alkylation reaction of lysine by BDMP-QM (at pH 7.4) has also been suggested by Thompson.[20] A convincing support concerning the role of selective protonation in the control of the selectivity comes from the comparison of second-order rate constants for the alkylation of n-$PrNH_2$ ($k_{Nu} = 5.5 \times 10^5$ M^{-1} s^{-1}) and glycine ($k_{Nu} = 6.9 \times 10^5$ M^{-1} s^{-1}). Such rate constants have been measured under strongly alkaline conditions (pH 12) where protonation of both nucleophiles ($pK_a = 10.68$[54] and 9.68,[58] respectively) is negligible. Second-order rate constants suggest that unprotonated side-chain and α-amino groups compete for *o*-QM at the same rate.

Although a fairly good alkylation selectivity of lysine by *o*-QM can be achieved under neutral condition (Nα/Nϵ = 3 at pH 6.0), its selectivity is at least 10 times smaller than the BDMP-QM selectivity (Nα/Nϵ = 30 at pH 7.4), where no side chain alkylation adduct was detected.[20] Similarly to lysine, the product ratio **14**/**15** from the alkylation of tyrosine decreases with the increase of the pH of the solution (see in more detail data in Table 1). In fact, the phenolic and amino acidic pK_a of the tyrosine are respectively 9.99 (OH)[35,36] and 8.94 (NH_3^+).[36]

The observed competition between the anionic form of tyrosine ($k_{Nu} = 2.3 \times 10^5$ M^{-1} s^{-1}) and the unprotonated NH_2 group of the amino acid ($k_{Nu} = 6.9 \times 10^5$ M^{-1} s^{-1}) toward **1** provides the only example of *O*-alkylation by QMs with amino acids. Moreover, it stands in striking contrast with the fully selective alkylation at the nitrogen atom of tyrosine by BDMP-QM reported by Bolton, Thatcher, and Thompson.[19,20] This reactivity trend is not a peculiarity of tyrosine. In fact, *o*-QM **1** alkylates **2a** in the absence of both N and S nucleophiles, confirming that the PhO^- nucleophilic system competes efficiently with the solvent and N nucleophiles (under basic conditions) for *o*-QM **1**.

It is well-known that in an aqueous intracellular environment the strongest nucleophile with quinones and QMs is the cysteinyl SH group.[1] Actually, UV-spectroscopic evidences for the reaction between cysteine and various quinones,[37] and the characterization of the cysteinyl-Dopa conjugates[38] support such a high nucleophilicity. However, to the best of our knowledge, *adduct **16** is the first example of a fully characterized o-QM-glutathione like adduct*, as the addition of the thiol group, in cysteine-containing peptides, to *o*-QM-like structures has not been documented so far. Only recently few *p*-QM glutathionyl-SH adducts have been isolated from in vitro experiments.[39] Thus, it should be appreciated that through the photochemical activation method it is possible to achieve the alkylation of cysteine and glutathione even under acidic conditions, even if the same results cannot be obtained through a thermal reaction. The latter observation is consistent with the finding by Wan that the efficiency of the *o*-QM formation is controlled by the deprotonation of the singlet excited state of the phenol ($pK_a = 2.5$),[25a] which is much more acidic than its own ground state.

(32) Richard, J. P.; Amyes, T. L.; Bei, L.; Stubblefield, V. *J. Am. Chem. Soc.* **1990**, *112*, 9513.
(33) Second-order rate constants for Cl^-, AcO^-, ROH alkylation reactions by **1** were too small, in comparison to k_{H_2O}, to be measured in water solution.
(34) Ellenbogen, E. *J. Am. Chem. Soc.* **1952**, *74*, 5198.
(35) (a) Kim, K.; Cole, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 6851. (b) Castro, E. A.; Pavez, P.; Santos, J. G. *J. Org. Chem.* **1999**, *64*, 2310.
(36) Ishihama, Y.; Oda, Y.; Asakawa, N. *J. Pharm. Sci.* **1994**, *83*, 1500.
(37) Mason, H. S.; Peterson, E. V. *Biochim. Biophys. Acta* **1965**, *111*, 134.
(38) Ito, S.; Prota, G. *Experentia* **1977**, *33*, 1118.
(39) Thompson, D. C.; Perera, K.; Krol, E. S.; Bolton, J. L. *Chem. Res. Toxicol.* **1995**, *8*, 323.
(40) Bordwell, F. G.; Cripe, T. A.; Hughes, D. L. *Nucleophilicity, Basicity, and the Brønsted Equation* in *Nucleophilicity*; Harris, J. M.; McManus, S. P., Eds.; American Chemical Society: Washington, DC, 1987; Chapter 9, pp 137−153.
(41) Pearson, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 6109.
(42) Et_3N ($\Delta G^o_{Nu} = -1.1$ kcal mol^{-1}) and morpholine are 1.4 and 4 times, respectively, more reactive than $PrNH_2$ ($\Delta G^o_{Nu} = +2.4$ kcal mol^{-1}), even if the latter is less solvated by water. $TyrO^-$ ($\Delta G^o_{Nu} \sim -4.6$ kcal mol^{-1}; $TyrO^-$ solvation energy was assumed to be identical to PhO^- solvation energy) is 3.6 times as reactive as HO^- ($\Delta G^o_{Nu} = -4.4$ kcal mol^{-1}),[41] and they have similar solvation.
(43) In preparation.
(44) Pochini, A.; Puglia, G.; Ungaro, R. *Synthesis* **1983**, *11*, 906. (b) Stedman, E. *J. Chem. Soc.* **1927**, 1904.
(45) Baires, S. V.; Ivanov, V. B.; Ivanov, B. E.; Zyablikova, T. A.; Efrimov, Y. Y. *Bull. Acad. Sci. USSR (Engl. Transl.)* **1987**, *36*, 1881.
(46) Maroni, P.; Cazaux, L.; Tisnes, P.; Zambeti, M. *Bull. Soc. Chim. Fr.* **1980**, *2*, 179.
(47) Schepartz, A.; Breslow, R. *J. Am. Chem. Soc.* **1987**, *109*, 1814.
(48) Steevens, J. B.; Pandit, U. K. *Tetrahedron* **1983**, *39*, 1395.
(49) Hodgkin, J. H. *Aust. J. Chem.* **1984**, *37*, 2371.
(50) Ranganathan, S.; Singh, W. P. *Tetrahedron Lett.* **1988**, *29*, 1435.
(51) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(52) Harned, R. *Trans. Faraday Soc.* **1940**, *36*, 973.
(53) Battye, P. J.; Ihsan, E. M.; Moodie, R. B. *J. Chem. Soc., Perkin Trans. 2* **1980**, 741.
(54) Hine, J.; Chou, Y. *J. Org. Chem.* **1981**, *46*, 649.
(55) Castro, E. A.; Cubillos, M.; Santos, J. G. *J. Org. Chem.* **1994**, *59*, 3572.
(56) Emly, M.; Leussing, D. L. *J. Am. Chem. Soc.* **1981**, *103*, 628.
(57) Li, N. C.; White, J. M.; Yoest, R. L. *J. Am. Chem. Soc.* **1956**, *78*, 5218.
(58) Eldin, S.; Jencks, W. P. *J. Am. Chem. Soc.* **1995**, *117*, 4851.
(59) Patel, H. M. S.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1990**, 37.

Both product distribution analysis of lysine (and tyrosine) in alkylation reaction and LFP measurements show, without doubt, that **1** is less selective and much more reactive than BHP-QM and BDMP-QM, at least in the alkylation of amino acids. As far as selectivity is concerned, our results show an unexpected different behavior of *o*-QM **1** toward amino acids and nucleosides. In fact, as also mentioned in the Introduction, Rokita stated that *o*-QM shows a "greater selectivity in nucleoside modification."[15] Our result suggests that, in addition to cysteine thiols, protein alkylation by *o*-QM **1** should occur not only at the N-terminal residue but also at the side-chain groups of lysine and tyrosine. This remarkable difference between *o*-QM and BDMP-QM chemical reactivity suggests that the former could find different applications as an enzyme inhibitor in comparison to the latter.

The rate of addition of **1** to water, amines, and sulfides does not correlate with the pK_a of the conjugated acid of the nucleophiles, as apparent from the data collected in Table 4. Thus, a simple parallelism between basicity (measured by the pK_a values) and nucleophilicity (measured by the second-order rate constant of the alkylation process) of the substrates is not possible. The lack of linearity in the plot ln(k_{Nu}) vs pK_a is expected for different classes of nucleophiles (S, N, and O nucleophiles),[40] but it is surprising within a more homogeneous nucleophile family such as alkylamines. Solvation of the nucleophile by the protic medium is not the main controlling factor of nucleophile reactivity toward **1**, because there is no correlation between stabilization of nucleophile by hydration (as described by ΔG^{o}_{Nu}[41]) and ln(k_{Nu}).[42] Therefore, it is likely that other factors related to the peculiar *ortho* geometry of **1**, such as steric effects and electrostatic repulsion between the *o*-QM oxygen atom and the nucleophile could also be important. In fact, *o*-QM **1** appears to be slightly sensitive to the steric requirements of the nucleophile, as $PrNH_2$ is 5 times more reactive than *t*-$BuNH_2$, and twice less reactive than piperidine.

Alkylation vs Hydration. The Role of Acid and Base Catalysis. The second-order rate constants (k_{Nu}) with H_3O^+ and OH^- demonstrate the importance of the acid and base catalysis in the hydration process of *o*-QM. The above k_{Nu} values have been measured without buffers, controlling the pH of the solution by HCl or NaOH addition. Therefore the acid- and base-catalyzed hydration process has been studied without the interference of buffer acids or buffer anions. The *o*-QM rate vs pH dependence is qualitatively similar to other QMs previously described, such as BDMP-QM.[19] H_3O^+ reacts with **1** 22 times faster than OH^-. The reactivity of OH^- in water is always lower than that of unprotonated amines (even in the case of the bulky *t*-$BuNH_2$) and thiols. Thus, it is not surprising that in alkaline aqueous solution (pH 10) hydroxybenzyl alcohol has never been detected, even at low nucleophile concentration (10^{-3} M). The hydration of **1** is competitive under acidic conditions, as shown qualitatively by thermal and photochemical preparative experiments, for two different reasons. These reasons are acid catalysis, which increases the hydration rate, and protonation of the N nucleophiles. The only substrates which can compete efficiently with water at pH lower than 7 are cysteine and cysteine-containing peptides. In fact, the SH group shows second-order rate constants higher than 10^5 M^{-1} s^{-1}, under neutral conditions (cysteine, $k_{Nu} = 1.3 \times 10^5$ M^{-1} s^{-1}; 2-mercaptoethanol, 1.9×10^5 M^{-1} s^{-1} glutathione, $k_{Nu} = 9.5 \times 10^5$ M^{-1} s^{-1}), while k_{H_2O} is 5.8 M^{-1} s^{-1}.

Currently, we are investigating in more detail the role of hydrogen-donor solvents in the activation of *o*-QM, and in the solvation of the nucleophile.[43]

Alkylated Adducts as *o*-QM Molecular Carriers. In our study we have been able to identify in the *o*-QM alkylation adducts at the NH_2 amino acidic group (**9**, **10**, **13**, and **14**) a new class of compounds which are capable of a thermal generation of *o*-QM from neutral to basic conditions. Such a reactivity contrasts with the thermal stability of all the other products obtained in this study and suggests that *the above compounds and similar peptide like adducts* (where the *o*-QM is linked to the N-terminal amino acid) *could be used as o-QM molecular carriers.* The peculiar thermal behavior of **9**, **10**, **13**, and **14** is probably due to the presence, of a proximal $-COO^-$ to the phenolic OH group, which may catalyze (through an intramolecular deprotonation) the decomposition to *o*-QM. Work is still in progress in our laboratory to clarify this aspect, and to decrease the activation temperature of the above presursors.[43]

The photoreactivity of all isolated alkylation adducts, including those with cysteine and glutathione, shows that the photoalkylation by *o*-QM **1** is a reversible process. This general photoreactivity explains (i) the higher amount, in comparison to thermal alkylation, of hydroxybenzyl alcohol formed as byproduct and (ii) the different selectivity of lysine alkylation in steady-state preparative irradiations. Adducts photoreactivity provides a wider and milder approach, in comparison to the thermal one, to efficiently generate alkylating agents.

Actually, photoactivation of *o*-QM-amino acid and *o*-QM-oligopeptides conjugates such as *o*-QM-glutathione (**16**) is certainly one of the most interesting finding of the present study. It suggests that any alkylation adduct of biological nucleophiles is a potential candidate in delivering, under irradiation, a highly reactive alkylating agent such as *o*-QM **1**.

Conclusion

The investigation described above has provided a useful quantitative characterization of the electrophilic character of *o*-QM **1** under neutral, acid, and basic aqueous conditions. Several interesting findings of our study can be summarized as follows:

(i) We reported one the most efficient thermal and photochemical approaches to *o*-QM **1** generation in water, starting from the same precursor (**2**).

(ii) Thermal generation from **2** is achievable under "almost" physiological conditions by pH activation to give alkylation products in good yields.

(iii) Salt **2** shows properties that make it a more preferable *o*-QM photoprecursor than *o*-hydroxybenzyl alcohols or (2-hydroxybenzyl)dimethylamines for kinetic measurements.

(iv) This study is the first quantitative investigation on the electrophilic character of parent *o*-QM **1**. To our knowledge there are no studies either on the selectivity or reactivity of a quinone methide with an *ortho* geometry as alkylating agent of amino acids and peptides.

(v) Quantitative comparison (based on absolute second-order rate constants) of *o*-QM and BDMP-QM reactivity (provided mainly by Thompson, Bolton, and Thatcher)[19,20]

shows the huge reactivity gap between 2,6-disubstituted-*p*-QMs and *o*-QM **1**.

(vi) *o*-QM should alkylate the side-chains of lysine and tyrosine containing peptides much more efficiently than other less reactive QMs, which are capable of linking mainly the N-terminal residues of peptides.

(vii) Several alkylation adducts regenerate **1** by heating or by irradiation. Thus, they represent a new and a broad class of compounds which could be used as molecular alkylating carriers which generate *o*-QM by pH-triggered processes. Additional studies are being carried out in our laboratory in the attempt to modify such precursors with the aim of activating them in even milder conditions (photochemically using a longer wavelength and thermally at lower temperature).

Thus, we feel confident that such results will facilitate further study and hopefully result in the use of *o*-QM as covalent protein linker and enzyme inhibitor.

Experimental Section

General Methods. Melting points are uncorrected. Elemental analyses were performed using a standard analyzer. Infrared spectra were recorded as KBr disks, Nujol, or films on a FT spectrophotometer. ^{1}H, ^{13}C, ^{13}C-DEPT, and 2D-correlated NMR spectra were recorded in D_2O solutions (unless otherwise stated) on a 300 MHz spectrometer. Chemical shifts are reported in ppm. Protons were correlated by decoupling and ^{1}H−^{13}C COSY experiments (HSQC and HMBC). Reaction products were separated and quantified analytically by reverse-phase (Intersil ODS-2, 5 μm, column dimension: ϕ = 4.6 mm, length = 250 mm, ϕ = 10.0 mm, length = 250 mm, Micro-Column) HPLC chromatography using a variable-wavelength detector.

Materials. Chemicals and solvents were purchased from Aldrich and Sigma and used without further purification. All aqueous solutions were made with distilled water.

(2-Hydroxybenzyl)trimethylammonium Iodide (2). (2-Hydroxybenzyl)trimethylammonium iodide was prepared, in almost quantitative yield, from 2-(dimethylaminomethyl)-phenol.[44] Using the general protocol described in the literature, the Mannich base (50 g, 33 mmol) was treated with methyl iodide (4.27 mL, 69 mmol) in acetonitrile (15 mL) for 2 h. After addition of 100 mL of diethyl ether, the insoluble quaternary ammonium salt was filtered. The product was crystallized from ethanol: diethyl ether = 8:2, to give 8.29 g (92% yield) of white crystals, mp = 169 ± 0.5 °C (literature 169−170 °C[45]). ^{1}H NMR (D_2O) δ 2.97 (s, 9 H, NMe_3), 4.35 (s, 2 H, CH_2Ph), 6.88−6.95 (m, 2 H, aromatics), 7.25−7.35 (m, 2 H, aromatics).

Adducts **3**,[46] **4**,[46] **5**,[47,48] **6**,[49] and **7**[50] were identified by comparison of their spectroscopic properties with published data.

2-(2-Hydroxy-ethylsulfanylmethyl)phenol (8). A solution of 2-mercaptoethanol (40 mg, 0.51 mmol) and (2-hydroxybenzyl)trimethylammonium iodide (**2**) (100 mg, 0.34 mmol) in 5 mL of KH_2PO_4/Na_2HPO_4 buffer adjusted at pH 7.0 (2.0 mL of a KH_2PO_4 0.1 M and 3.0 mL of a Na_2HPO_4 0.1 M) was heated at 80 °C for 3.5 h and then cooled. The solution was acidified with 0.5 M HCl and extracted with diethyl ether. The organic phase was washed with water and dried over $MgSO_4$. Evaporation of the solvent followed by column chromatography on silica gel (eluent, diethyl ether:hexane = 6:4) gave 59.5 mg (95%, yield) of **8** as colorless oil. IR (Nujol) (cm^{-1}) 3330 (broad), 1596, 1456, 1247. ^{1}H NMR ($CDCl_3$) δ 2.20−2.60 (very broad, 1 H), 2.70 (t, J = 6.8 Hz, 2 H), 3.80 (t, J = 6.8 Hz, 2 H), 3.90 (s, 2 H), 6.00−6.75 (very broad, 1 H), 6.70−6.85 (m, 2 H, aromatics), 7.10−7.25 (m, 2 H, aromatics). ^{13}C NMR δ 31.67, 33.46, 61.32, 116.70, 120.65, 123.12, 129.01, 130.59, 154.70. Anal. Calcd for $C_9H_{12}O_2S$: C, 58.67; H, 6.56; S, 17.40. Found: C, 58.60; H, 6.52; S, 17.35.

***N*-(2-Hydroxybenzyl)glycine Hydrochloride (9).** A solution of glycine (64 mg, 0.85 mmol) and (2-hydroxybenzyl)-trimethylammonium iodide (**2**) (50 mg, 0.17 mmol) in 5 mL of $NaHCO_3/Na_2CO_3$ buffer adjusted at pH 10 (2.6 mL of a $NaHCO_3$ 0.1 M and 2.4 mL of a Na_2CO_3 0.1 M) was heated at 80 °C for 2 h, cooled, and then purified by preparative HPLC (eluent, H_2O: CH_3CN = 85:15 + 0.1% CF_3COOH, flux = 4.0 mL/min, t_R = 32.0 min). To exchange the trifluoroacetate anion with chlorine anion the product was dissolved in 1 mL of a 0.1 M HCl solution. The solution was stirred, and the solvent was evaporated at room temperature in a vacuum (0.1 mmHg). 36.7 mg (99% yield) of adduct **9** was obtained as a colorless oil. IR (Nujol) (cm^{-1}) 3400−2400 (broad), 1681, 1557, 1206, 1143. ^{1}H NMR (D_2O) δ 3.90 (s, 2 H), 4.35 (s, 2 H), 6.90−7.00 (m, 2 H, aromatics), 7.30−7.45 (m, 2 H, aromatics). ^{13}C NMR δ 47.55, 48.06, 116.38, 117.88, 121.38, 132.39, 132.69, 155.96, 170.82. Anal. Calcd for $C_9H_{12}NO_3Cl$: C, 49.67; H, 5.56; N, 6.44; Cl, 16.29. Found: C, 49.75; H, 5.59; N, 6.40; Cl, 16.35.

***N*-(2-Hydroxybenzyl)serine Hydrochloride (10).** A solution of L-serine (220 mg, 2.10 mmol) and (2-hydroxybenzyl)-trimethylammonium iodide (**2**) (100 mg, 0.34 mmol) in 5 mL of $NaHCO_3/Na_2CO_3$ buffer pH 10 (2.6 mL of a $NaHCO_3$ 0.1 M and 2.4 mL of a Na_2CO_3 0.1 M) was heated at 80 °C for 2 h, cooled, and then purified by preparative HPLC (eluent, H_2O: CH_3CN = 85:15 + 0.1% CF_3COOH, flux = 4.2 mL/min, t_R = 27 min). Trifluoroacetate anion of the adduct was replaced by chlorine anion according to the procedure described above for glycine adduct to give 71.0 mg (84% yield) of adduct **10** as colorless crystals, which decomposes at T > 150 °C. IR (Nujol) (cm^{-1}) 3417 (very broad), 2520 (broad), 1688, 1597, 1203, 1143. ^{1}H NMR (D_2O) δ 3.98−4.05 (m, 1 H), δ 4.05−4.10 (m, 2 H), 4.30−4.40 (m, 2 H), 6.90−7.00 (m, 2 H, aromatics), 7.30−7.45 (m, 2 H, aromatics). ^{13}C NMR δ 46.97, 59.65, 62.15, 116.36, 117.80, 121.43, 132.38, 132.68, 155.98, 171.32. Anal. Calcd for $C_{10}H_{14}NO_4Cl$: C, 48.48; H, 5.70; N, 5.66; Cl, 14.31. Found: C, 48.50; H, 5.69; N, 5.69; Cl, 14.35.

***S*-(2-Hydroxybenzyl)cysteine Hydrochloride (11).** A solution of L-cysteine (50 mg, 0.41 mmol) and (2-hydroxybenzyl)trimethylammonium iodide (**2**) (100 mg, 0.34 mmol) in 5 mL of $NaHCO_3/Na_2CO_3$ buffer (pH 10) was heated at 80 °C for 30 min, cooled, and then purified by preparative HPLC (eluent = H_2O:CH_3CN = 85:15 + 0.1% CF_3COOH, flux = 4.0 mL/min, t_R = 18.0 min). Trifluoroacetate anion of the adduct was replaced by chlorine anion according to the procedure described above for glycine adduct to give 89.1 mg (99% yield) of adduct **11** as colorless crystals, which decomposes at T > 145 °C. IR (Nujol) (cm^{-1}) 3395, 1694, 1598, 1207, 1188, 1149. ^{1}H NMR (D_2O) δ 2.95 (dd, J = 15.2 Hz, J = 8.3 Hz, 1 H), 3.13 (dd, J = 15.2 Hz, J = 4.1, 1 H), 3.76 (d, J = 13.2 Hz, 1 H), 3.83 (d, J = 13.2 Hz, 1 H), 4.20 (dd, J = 8.3 Hz, J = 4.1 Hz, 1 H), 6.90−7.00 (m, 2 H, aromatics), 7.15−7.25 (m, 2 H, aromatics). ^{13}C NMR δ 30.90, 31.59, 52.10, 118.73, 121.57, 124.86, 130.15, 131.8, 154.62, 171.43. Anal. Calcd for $C_{10}H_{14}NO_3SCl$: C, 45.54; H, 5.35; N, 5.31; S, 12.16; Cl, 13.44. Found: C, 45.49; H, 5.30; N, 5.25; S, 12.13; Cl, 13.40.

ϵ-*N*-(2-Hydroxybenzyl)-L-lysine Hydrochloride (12) and *N*-(2-Hydroxybenzyl)-L-lysine Hydrochloride (13). A solution of L-lysine (144 mg, 0.99 mmol) and (2-hydroxybenzyl)-trimethylammonium iodide (**2**) (60 mg, 0.20 mmol) in 5 mL of $NaHCO_3/Na_2CO_3$ buffer adjusted at pH 9.6 was heated at 80 °C for 1 h and then cooled. Two alkylation adducts **12** and **13** were isolated and purified by preparative HPLC (eluent, H_2O: CH_3CN = 90:10 + 0.1% CF_3COOH, flux = 4.0 mL/min, t_R = 14.1 min and t_R = 12.2 min). Trifluoroacetate anion of the adducts was replaced by chlorine anion according to the procedure described above for glycine adduct to give adducts **12** (22.8 mg, 35% yield), and **13** (25.4 mg, 39% yield) as hydrochlorides both as colorless crystals, which both decomposed at T = 155 °C and 147 °C, respectively.

12. IR (Nujol) (cm^{-1}) 3389, 1747, 1603. ^{1}H NMR (D_2O) δ 1.50 (m, 2 H), 1.75 (m, 2 H), 1.90 (m, 2 H), 3.05 (t, J = 8.0 Hz, 2 H, CH_2N), 3.97 (t, J = 6.0 Hz, 1 H, CHCOO), 4.20 (s, 2 H, CH_2-Ph), 6.90−7.00 (m, 2 H, aromatics), 7.15−7.25 (m, 2 H, aromatics). ^{13}C NMR δ 21.35 (CH_2), 24.73 (CH_2), 29.18 (CH_2), 46.22 (CH_2), 46.59 (CH_2), 52.88 (CH), 115.38 (CH), 117.27 (C), 120.40 (CH), 131.31 (CH), 131.57 (CH), 154.85 (C), 172.38 (C).

Anal. Calcd for $C_{13}H_{22}N_2O_3Cl_2$: C, 48.01; H, 6.82; N, 8.61; Cl, 21.28. Found: C, 48.11; H, 6.80; N, 8.59; Cl, 21.30.

13. IR (Nujol) (cm^{-1}) 3572, 1735, 1615. ^1H NMR (D_2O) δ 1.40 (m, 2 H), 1.65 (m, 2 H), 1.95 (m, 2 H), 2.95 (t, $J = 7.1$ Hz, 2 H, CH_2N), 3.85 (t, $J = 6.0$ Hz, 1 H, CHCOO), 4.29 (s, 2 H, CH_2-Ph), 6.90−7.00 (m, 2 H, aromatics), 7.25−7.40 (m, 2 H, aromatics). ^{13}C NMR δ 21.23 (CH_2), 26.09 (CH_2), 28.49 (CH_2), 38.82 (CH_2), 45.93 (CH_2), 59.07 (CH), 115.40 (CH), 116.64 (C), 120.47 (CH), 131.58 (CH), 131.89 (CH), 155.09 (C), 171.40 (C). Anal. Calcd for $C_{13}H_{22}N_2O_3Cl_2$: C, 48.01; H, 6.82; N, 8.61; Cl, 21.28. Found: C, 48.09; H, 6.77; N, 8.55; Cl, 21.33.

The structure of the adducts **12** and **13** have been firmly based on spectroscopic evidences, in particular on ^{13}C NMR chemical shifts, DEPT and $^1H-^{13}C$ long-range correlation experiments (HMBC). In more detail, on passing from **12** to **13** both the carbon atoms at the α position (C-2) and that at position C-6 (see Scheme 2 for numbering) of the amino acid moiety experience a diagnostic chemical shift change. C-2 is more deshielded (59.07 ppm) in the adduct **13** than the corresponding carbon atom in the adduct **12** (52.88 ppm). Vice versa, C-6 in the adduct **13** is more shielded (38.82 ppm) than the corresponding carbon atom in the adduct **12** (46.59 ppm). This difference is likely due to the fact that alkylated amino group gives rise to higher deshielding effect than the corresponding NH_2.

After a full assignment of ^1H and ^{13}C spectra by HSQC, the identity of **12** and **13** have been definitely established by long range $^1H-^{13}C$ coupling detected through a HMBC protocol,[51] since each one exhibits a unique connectivity between the benzylic protons (H-8) and lysine carbon atoms (C-6 in **12** or C-2 in **13**).

***N*-(2-Hydroxybenzyl)-L-tyrosine Hydrochloride (14) and *O*-(2-Hydroxybenzyl)-L-tyrosine Hydrochloride (15).** A solution of L-tyrosine (87 mg, 0.48 mmol), and (2-hydroxybenzyl)trimethylammonium iodide (**2**) (50 mg, 0.18 mmol) in 5 mL of $NaHCO_3/Na_2CO_3$ buffer (pH 10) was heated at 80 °C for 1 h. Then, two alkylated products **14** and **15** were isolated and purified by preparative HPLC (eluent = H_2O:CH_3CN = 80:20 + 0.1% CF_3COOH, flux = 4.0 mL/min, $t_R = 10.3$ min and $t_R = 20.5$ min, respectively). Trifluoroacetate anion of the adducts was replaced by chlorine anion according to the procedure described for glycine adduct, to give **14** (37 mg, 64% yield), **15** (7 mg, 12% yield) both as hydrochloride, and **17** (2 mg, 10% yield, $t_R = 11.5$ min).

14. White crystals, mp = 114 ± 1 °C followed by decomposition. IR (Nujol) (cm^{-1}) 3290, (very broad), 1730, 1518. ^1H NMR (D_2O) δ 3.06 (dd, $J = 14.7$ Hz, $J = 7.5$ Hz, 1 H, H-3), 3.15 (dd, $J = 14.7$ Hz, $J = 6.6$ Hz, 1 H, H-3′), 3.99 (dd, $J = 7.5$ Hz, $J = 6.6$ Hz, 1 H, H-2), 4.15 (d, $J = 13.2$ Hz, 1 H, H-11), 4.22 (d, $J = 13.2$ Hz, 1 H, H-11′), 6.73−6.89 (m, 4 H, aromatics), 6.99−7.14 (m, 2 H, aromatics), 7.14−7.18 (m, 1 H, aromatic), 7.22−7.29 (m, 1 H, aromatic). ^{13}C NMR δ 34.39 (C-3), 46.35 (C-11), 60.21 (C-2), 115.26 (C-14), 115.74 (C-6 and C-8), 116.24 (C-12), 120.42 (C-16), 125.23 (C-4), 130.49 (C-5 and C-9), 131.54 (C-15), 131.76 (C-17), 154.86 (C-7), 154.97 (C-13), 170.88 (C-1). Anal. Calcd for $C_{16}H_{18}NO_4Cl$: C, 58.35; H, 5.60; N, 4.33; Cl, 10.95. Found: C, 58.30; H, 5.62; N, 4.27; Cl, 10.85.

15. Colorless oil. IR (Nujol) (cm^{-1}) 3270, (very broad), 1725, 1502. ^1H NMR (D_2O) δ 2.90 (dd, $J = 14.2$ Hz, $J = 7.7$ Hz, 1 H, H-3), 3.15 (dd, $J = 14.2$ Hz, $J = 5.3$ Hz, 1 H, H-3′), 3.82 (s, 2 H, H-11), 3.89 (dd, $J = 7.7$ Hz, $J = 5.3$ Hz, 1 H, H-2), 6.79−6.90 (m, 4 H, aromatics), 6.93−6.99 (m, 1 H, aromatic), 7.03−7.14 (m, 3 H, aromatics). ^{13}C NMR δ 35.12 (C-3), 55.38 (C-2), 69.41 (C-11), 115.45 (CH), 115.85 (CH), 120.76 (CH), 126.55 (C), 126.83 (C), 127.67 (CH), 127.83 (CH), 128.35 (CH), 130.52 (CH), 131.09 (CH), 152.71 (C-7), 153.65 (C-13), 171.48 (C-1). Anal. Calcd for $C_{16}H_{18}NO_4Cl$: C, 58.35; H, 5.60; N, 4.33; Cl, 10.95. Found: C, 58.20; H, 5.67; N, 4.25; Cl, 10.86.

Tyrosine alkylation products on N and O atoms are distinguishable through the ^{13}C chemical shift and the multiplicity of the ^1H signals of the methylene group, i.e., CH_2 at position 11, deriving from the former *o*-quinone methide moiety. C-11 in **14** is more shielded (46.35 ppm) than the corresponding carbon atom in **15** (69.41 ppm). H-11 protons resonate as an AB system in **14** and as a singlet in **15**, indicating that the stereogenic center is far in **14** and close in **15** to the CH_2. We confirmed the structures by HSQC and HMBC experiments.

***S*-(2-Hydroxybenzyl)glutathione (16).** A solution of glutathione (50 mg, 0.16 mmol) and (2-hydroxybenzyl)trimethylammonium iodide (**2**) (45 mg, 0.15 mmol) in 5 mL of KH_2PO_4/Na_2HPO_4 buffer pH 7.0 (2.0 mL of a KH_2PO_4 0.1 M and 3.0 mL of a Na_2HPO_4 0.1 M) was heated at 80 °C for 30 min, cooled, and then purified by preparative HPLC (eluent, H_2O:CH_3CN = 80:20 + 0.1% CF_3COOH, flux = 4.0 mL/min, t_R = 10.37 min). Trifluoroacetate anion of the adducts was replaced by chlorine anion according to the procedure described above for glycine adduct to give adduct **16** (57 mg, 84% yield). Colorless crystals mp = 51 ± 1 °C. IR (Nujol) (cm^{-1}) 3410 (broad), 1732, 1652, 1547, 1244. ^1H NMR (D_2O) δ 2.11 (dt, $J = 7.1$ Hz, 6.7 Hz, 2 H, H-3), 2.44 (t, $J = 7.1$ Hz, H-4, 2 H), 2.72 (dd, $J = 14.3$ Hz, $J = 8.7$ Hz, 1 H, H-12), 2.91 (dd, $J = 14.3$ Hz, $J = 5.2$ Hz, 1 H, H-12′), 3.67 (d, $J = 13.0$ Hz, 1 H, H-14), 3.73 (d, $J = 13.0$ Hz, 1 H, H-14′), 3.89 (s, 2 H, H-10), 3.97 (t, $J = 6.7$ Hz, 1 H, H-2), 4.41 (dd, $J = 8.7$ Hz, $J = 5.2$ Hz, 1 H, H-7), 6.81−6.88 (m, 2 H, H-17, H-19, aromatics), 7.10−7.17 (m, 1 H, H-18, aromatic), 7.17−7.20 (m, 1 H, H-20 aromatic). ^{13}C NMR δ 25.30 (C-3), 30.17 (C-14), 30.70 (C-4), 32.24 (C-12), 40.90 (C-10), 52.06 (C-2), 52.83 (C-7), 115.71 (C-17), 120.50 (C-19), 124.39 (C-15), 128.97 (C-18), 130.76 (C-20), 153.53 (C-16), 171.40 (C-1), 172.64 (C-8 and C-11), 174.03 (C-5). Anal. Calcd for $C_{17}H_{24}N_3O_7ClS$: C, 45.38; H, 5.38; N, 9.34; Cl, 7.88; S, 7.13. Found: C, 45.29; H, 5.35; N, 9.30; Cl, 7.89; S, 7.09.

HSQC and HMBC experiments were used to fully assign every signal in both ^1H and ^{13}C spectra. In the alkylated product **16** (Scheme 2), the connectivity between the benzylic carbon (C-14) and protons (H-14) with C-12 and H-12 of peptide cysteine moiety was established by the combined used of HSQC and HMBC technique.

Oligomers. We isolated an oligomeric mixture as a pale yellow powder, mp = 177−185 °C. IR (KBr) (cm^{-1}) 3261 (broad), 1607, 1453, 1256. ^1H NMR (DMSO) δ 2.80−3.10 (NMe_3^+), 3.60−3.90 (CH_2O), 4.10−4.30 (CH_2N), 6.50−7.10 (aromatics), 9.4−10.2 (m, broad, phenolic OH). The integral ratio of the signals at δ 3.60−3.90 and 4.10−4.30 was 6.5. This value suggest that the average of the *o*-QM units involved in the oligomeric structures is 6−7.

Preparative Photochemical Reactions. General Procedure. A phosphate buffer solution (10 mL, adjusted at pH 7.0) of (2-hydroxybenzyl)trimethylammonium iodide (28 mg, 0.1 mmol, 10^{-2} M) and cysteine (24 mg, 0.2 mmol) was placed in a quartz tube (20 mL capacity) which was then sealed with a rubber serum cap and purged with nitrogen. The sample was then irradiated at 254 nm for 1 h at room temperature, by means of two 15 W low-pressure mercury lamps. After irradiation and 10-fold dilution, the alkylation product **11** was identified and quantified by HPLC analysis by comparison with a solution containing an authentic sample (obtained from thermal experiments) in a known concentration.

The other reactions with glycine, serine, lysine, and glutathione were similarly carried out.

Photochemical Stability of the Adducts. General Procedure. A phosphate buffer solution (10 mL, adjusted at pH 7.0) of ϵ-*N*-(2-hydroxybenzyl)-L-lysine hydrochloride (**12**) (3.3 mg, in 10 mL phosphate buffer, 10^{-3} M) was placed in a quartz tube (20 mL capacity) which was then sealed with a rubber serum cap and purged with nitrogen. The sample was then irradiated at 254 nm for 10 min at room temperature. After irradiation without further dilution, hydroxybenzyl alcohol (**17**) was measured by HPLC analysis by comparison with a solution containing an authentic sample.

Photochemical Stability of the Adducts at pH 12. General Procedure. ϵ-*N*-(2-hydroxybenzyl)-L-lysine hydrochloride (**12**) (3.2 mg, 1×10^{-2} mmol) and cysteine (1.3 mg, 1.1×10^{-2} mmol) were dissolved in 10 mL of water in order to obtain a 10^{-3} M solution in both reagents. The pH of the solution was then adjusted to 12 by NaOH addition (0.5 M). The sample was then irradiated at 254 nm for 10 min at room temperature. After irradiation, alkylation adduct **11** was

measured by HPLC analysis by comparison with a solution containing an authentic sample.

Competition Experiments. General Procedure. An aqueous solution (5 mL) of (2-hydroxybenzyl)trimethylammonium iodide (**2**, 10 mg, 0.034 mmol), *t*-$BuNH_2$ (50 mg, 0.68 mmol), $PrNH_2$ (40 mg, 0.68 mmol), and Na_2CO_3 (144 mg, 1.35 mmol) was kept for 5 min at 80 °C in order to achieve a conversion yield of the substrate **2** lower than 30%. The ratio **3**/**4** = 4.9 of the resulting alkylated products has been measured by HPLC.

A water solution (15 mL) obtained by mixing a *t*-$BuNH_2$ solution (5 mL, 0.18 M), a *n*-$prNH_2$ solution (5 mL, 0.18 M), a (2-hydroxybenzyl)trimethylammonium iodide solution (5 mL, 0.018 M), and Na_2CO_3 (360 mg, 3.40 mmol) in a quartz tube (20 mL capacity) was flushed with nitrogen and then irradiated at 254 nm for 45 min at room temperature. The ratio **3**/**4** = 4.6 of the resulting alkylated products has been measured by HPLC.

The other competition experiments with morpholine/piperidine, and *t*-$BuNH_2/OH^-$, were similarly carried out.

Kinetic Studies. Nucleophile Reactivity. Kinetic studies of nucleophiles addition to *o*-QM (with the exception of hydration), were carried out at 25 °C, keeping constant at 7.0 or 12.0 the pH (according to Table 4). Phosphate buffer or a NaOH solution were used to adjust the pH. The pH of each solution was measured using an Orion SA520 pH meter with a 8102 Ross electrode. Ionic strength was also kept constant at $I = 0.1$ M by NaCl, since Cl^- is unreactive with *o*-QM in water. **1** was produced by LFP, flashing a dilute solution of **2** (5×10^{-4} M). The disappearance of **1** was followed, under pseudo first-order conditions, by monitoring the absorbance decrease at 400 nm. Pseudo-first-order rate constant (k_{obsd}) were obtained from the fit of the absorbance data to a single-exponential function and were reproducible to ± 2%. The second-order rate constants k_{Nu} (M^{-1} s^{-1}) for the reaction of nucleophiles with **1** were determined as the least-squares slopes of linear plots of k_{obsd} against the total concentration of the nucleophile, used in 5×10^{-2} to 2×10^{-4} M concentration range.

Kinetic Studies. Hydration. Kinetic studies of acid- and base catalyzed *o*-QM hydration were similarly carried out. No buffers were used to change the pH, which was adjusted in the range $1 < pH < 3.4$ or $11 < pH < 13$ by HCl or NaOH addition. In a fashion similar to nucleophile kinetic studies ionic strength was kept constant at $I = 0.1$ M by NaCl.

Laser Flash Photolysis. The laser pulse photolysis apparatus consisted of a Nd:YAG laser used at the fourth harmonic of its fundamental wavelength. It delivered a maximum power of 10 mJ at 266 nm with 10 ns pulse duration. The monitor system, arranged in a cross-beam configuration, consisted of a 275 W Xe arc lamp, an F/3.4 monochromator, and a five-stage photomultiplier supplied by Applied Photophysics. The signals were captured by means of a Hewlett-Packard 54510A digitizing oscilloscope, and the data was processed on a 486-based computer system using software developed in-house. Solutions for analysis were placed in a fluorescence cuvette ($d = 10$ mm). The absorbance of each solution was adjusted to 1.5.

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