Quinone Methides as Alkylating and Cross-Linking Agents

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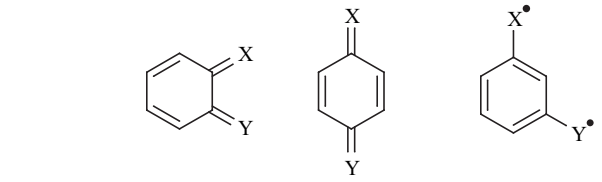
Abstract: Quinone methides (QMs) are reactive intermediates involved in a large number of chemical and biological processes such as enzyme inhibition, DNA alkylation and cross-linking. Their electrophilicity towards amines, thiols, water, amino acids and peptides has been kinetically measured in aqueous solution. The alkylation process is often thermally and photochemically reversible and the resulting adducts may act as QM carriers.

Keywords: Quinone methide electrophilicity, DNA alkylation and enzyme inhibition.

1. INTRODUCTION

This brief review will highlight some mechanistic and kinetic aspects of quinone methides (QMs) reactivity [1,2] and their most important biochemical applications. QMs are methylene cyclohexadienones closely related to both benzoquinone (BQs) [2] and quinone dimethides (QDMs, or xylylenes) (Scheme **1**) [3]. Respect to BQs, one of the carbonyl oxygen is replaced by a methylene group. Like for BQs and QDMs three parent isomers of QMs are known, *ortho* (**1**), *para* (**2**) and *meta* (**3**), but only two (**1** and **2**) are closed shell species, which are reactive towards both nucleophiles and electrophiles, due to their high polarisability. The *meta* isomer **3** displays a dominant diradical character.



X = O
Y = CH_2
X = Y = CH_2
X = Y = O

o-QM (**1**)

p-QM (**2**)
QDMs
BQs

m-QM (**3**)

Scheme 1.

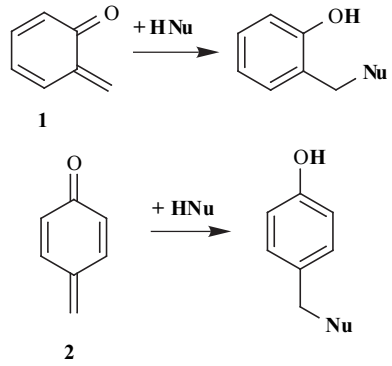
Ortho quinone methides (*o*-QMs, such as **1**) have been used in organic synthesis [4] mainly as heterodienes with electron rich alkenes, to give chromanes [5]. Thermal and photochemical methods for preparing QMs and their applications in inverse demand Diels-Alder reaction have already been reviewed by Boger [6] and Wan [7], respectively. In this review, we focused on the electrophilic character of QMs, which makes them powerful and highly reactive alkylating agents. Since the beginning of QMs discovery their bioactivity has been always associated with their alkylating reactivity toward macromolecular nucleophiles [8]. More recently, QM electrophilicity has been used to achieve (i) the covalent modification of peptides and enzymes where QMs may act as mechanism-based inhibitors (MBIs) [9-13] and (ii) the alkylation and cross-linking of DNA [14a]. Interstrand cross-linking represents the most toxic of all DNA alkylation events, since it results in seizure of the replication fork [14].

Among hydrolase inhibitors [9-13], QMs (and their nitrogen analogues iminoquinone methides) have been used as β-lactamase [11a], serine hydrolase [11b,c], phosphatase [9b,12] and ribonuclease A7 [10] inactivators.

Despite the general knowledge of the biological consequences associated to the alkylation of biological nucleophiles by QMs, a comprehensive and recent review focused on kinetic properties, namely the quantitative measurement of the QM electrophilicity and on thermodynamic stability of the resulting QM-adducts is still unavailable in the current literature.

2. ALKYLATION OF SIMPLE NUCLEOPHILES BY QMS. KINETIC AND MECHANISTIC ASPECTS

QMs are Michael acceptors capable of nucleophilic addition at the *exo*-cyclic methylene group to form benzylic adducts (Scheme **2**).



Scheme 2.

Alkylation of simple sulphur, nitrogen- and oxygen-centred nucleophiles by QMs has been experimentally investigated, under both aqueous and non-aqueous conditions [15-17]. Under non-protic solvents and in absence of nucleophiles unsubstituted QMs are still highly reactive and the formation of dimers and trimers compromise their isolation [18]. Supporting QMs on solid phase (SP),

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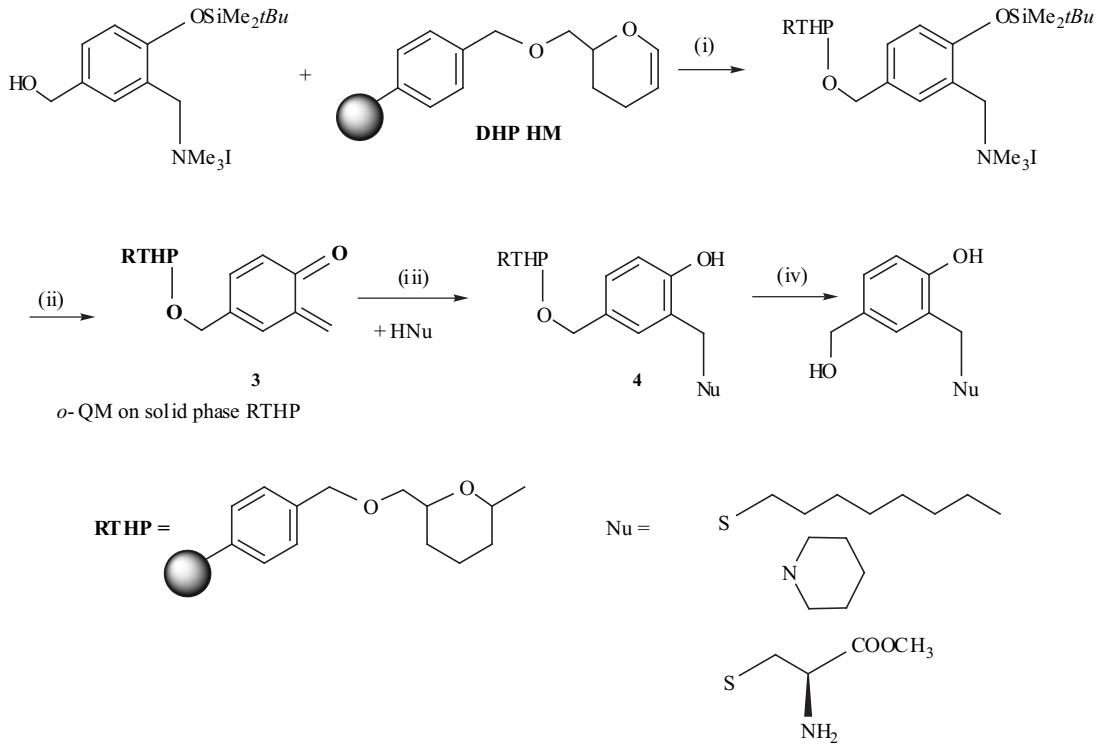


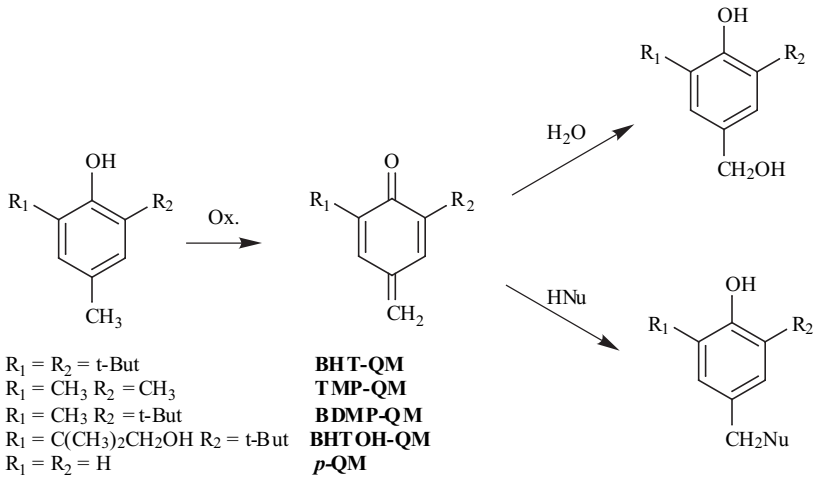
Scheme 3. (i) PTSA, $ClCH_2CH_2Cl$, r.t. (ii) nBu_4NF, in CH_2Cl_2, r.t. (iii) HNu 50 mM, in CH_2Cl_2, r.t. (iv) $CF_3COOH : H_2O = 95 : 5$.

dramatically enhances their stability up to a few days [19]. *o*-QM has been linked to a polystyrene resin with tetrahydropyranyl linker (**RTHP**) by an acid catalysed coupling procedure involving a benzyl precursor of the QM and 3,4-dihydro-2H-pyran-2-ylmethoxymethyl polystyrene (**DHP HM**, in Scheme **3**). The increased stability of *o*-QM on resin (**3**), is truly remarkable, since *o*-QM can only survive for a very short period in solution; i.e. < 10 ms in water [17] and < 1 s in organic non nucleophilic solvents (in the latter case due to dimerisation-polymerisation reactions at r.t.) [18], while supported *o*-QM can be safely stored in a dry and cool place. On the other hand, SP does not preclude the typical reactivity/selectivity of *o*-QM in solution towards nitrogen and sulphur centred nucleophiles. In fact, similarly to free *o*-QM in solution, which is highly selective toward thiols, *o*-QM on SP alkylates selectively only the SH moiety of cysteine methyl ester (to give adduct **4**) and no N-alkylation adduct was detected [19].

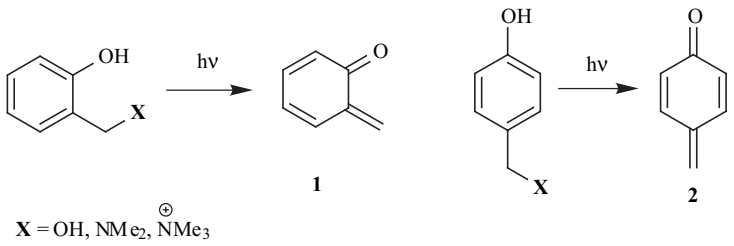
In absence of SH groups, nitrogen alkylation by supported *o*-QM of amino acids occurs in good yield.

The intrinsic reactivity and stability of QMs is also strongly affected by structural features. For example, the presence of bulky hydrophobic groups at both the 2- and 6-positions of the cyclohexadienone ring (Scheme **4**) raises considerably the QM stability in water (TMP-QM, $t_{1/2}$ 26 s; BDMP-QM, $t_{1/2}$ 47 s; BHTOH-QM, $t_{1/2}$ 400 s; BHT-QM, $t_{1/2}$ 3060 s) [20].

By contrast the parent *o*-QM and *p*-QM are transient species in water, where they show half-lives of 2 ms and 208



Scheme 4. *p*-QMs obtained by oxidation of substituted *p*-methyl phenol, and their reactions with water and nucleophiles (HNu).



Scheme 5. *o*-QMs and *p*-QMs obtained by photochemical activation of hydroxybenzyl alcohols (X = OH), phenolic Mannich bases (X = NMe_2) and their benzyl ammonium salts (X = NMe_3).

ms, respectively [17,21]. Since unsubstituted QMs are highly reactive species, they have often been thermally and photochemically generated from stable precursors.

In particular Wan has investigated the photochemical activation of *o*- and *p*- hydroxybenzyl alcohols [22] (Scheme **5**, X = OH) and Saito focused his attention on phenolic Mannich bases (Scheme **5**, X = NMe_2) as QM precursors [23]. Our group showed that actually benzyl ammonium salts of phenolic Mannich bases (Scheme **5**, X = NMe_3^+) are better and preferable QM-precursors than both the above, since (i) they display higher photochemical quantum yield (Φ = 0.98) than the alcohols (Φ = 0.23) and (ii) unlike the Mannich bases, the ammonium salts are not nucleophilic [17].

Therefore, benzyl ammonium salts of phenolic Mannich bases do not undergo alkylation by the QM. In addition, unlike hydroxybenzyl alcohols and phenolic Mannich bases, the benzyl ammonium salts can generate QMs under thermal activation at much lower temperature than any other precursor (80°C at pH < 7.0, and at 37° C at pH 7.8, in water).

Laser flash photolysis (LFP) has often been used by Wan [22], Kresge [21] and our group [17] to investigate the kinetic behaviour in water solution under neutral and acid conditions. LFP allows a (i) photochemical generation of both *o*-QM and *p*-QM in high concentration in water, where they display maximum absorption at 400 and 310 nm, respectively (see Fig. **1**) and a (ii) quantitative evaluation of QM electrophilicity by kinetic measurements (Table **1**).

Table 1. Second Order Alkylation Reaction Rates (k_{Nu}) of Several Nucleophiles by *o*-QM and *p*-QM in Water Solution at 25 °C and their Ratio in Comparison to BDMP-QM Reaction Rates from Literature

Alkylated Substrates [a]	*o*-QM k_{Nu} ($M^{-1}s^{-1}$)	*p*-QM k_{Nu} ($M^{-1}s^{-1}$)	*o*-QM /*p*-QM	BDMP-QM k_{Nu} ($M^{-1}s^{-1}$)[b]
H_2O (I = 0.1 M)	5.8	0.2	30	2.7×10^{-4}
OH^-	3.0×10^4	-	-	50
H_3O^+	8.4×10^5	5.3×10^4	16	200
n-$PrNH_2$ (pH 12.0)	5.5×10^5	2.4×10^4	23	-
t-$BuNH_2$ (pH 12.0)	1.1×10^5	4.3×10^3	26	-
Pyrrolidine (pH 12.0)	-	2.4×10^5	-	
Piperidine (pH 12.0)	1.3×10^6	1.8×10^5	7	-
Morpholine (pH 12.0)	2.3×10^6	2.2×10^5	10	-
Et_3N (pH 12.0)	7.1×10^5	1.1×10^5	6	-
Glycine (pH 12.0)	6.9×10^5	-	-	-
Lysine (pH 12.1)	5.9×10^5	-	-	18.6 ± 2.8
$HO(CH_2)_2SH$ (pH 6.9)	1.9×10^5	1.3×10^3	146	-
$HO(CH_2)_2S^-$ (pH 12.1)	2.8×10^8	5.0×10^7	5.6	-
Tyr-O^- (pH 12.0)	2.3×10^5	-	-	-
Tyr-NH_2 (pH 12.0)	6.9×10^5	-	-	45.0 ± 3
Cysteine (pH 6.8)	1.3×10^5	3.2×10^3	41	3320 ± 66
Cysteine (pH 12.2)	1.3×10^8	3.4×10^7	3	-
Glutathione (pH 7.1)	9.5×10^5	1.9×10^3	500	-

[a] In brackets is reported the pH of the solutions stabilised 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. 25b.

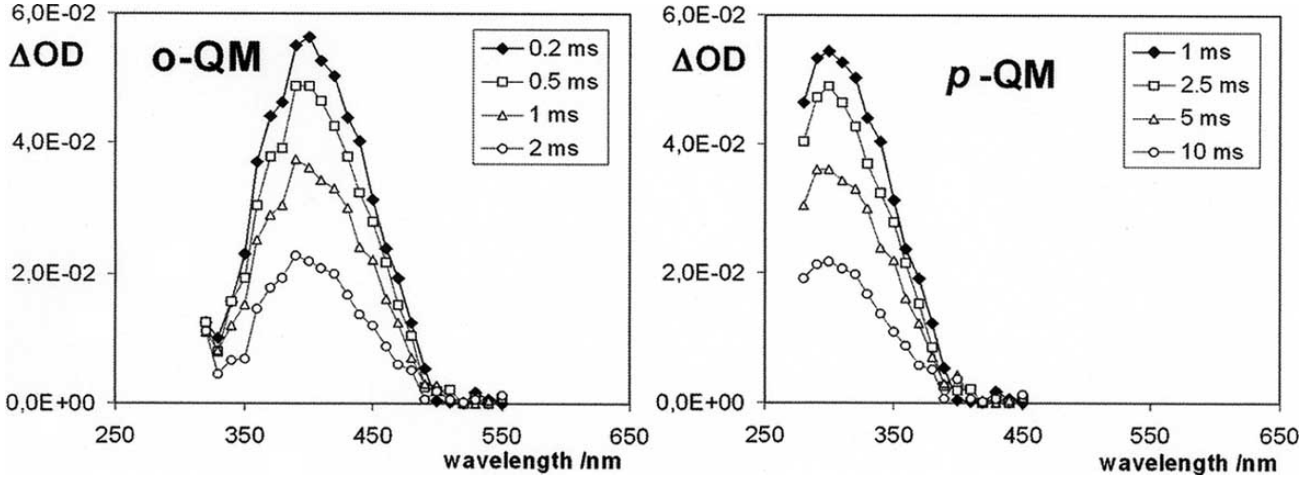


Fig. (1). Transient absorption spectra of *o*-QM (**1**) and *p*-QM (**2**), following 266 nm excitation of an aqueous solution of Mannich base ammonium salt (0.4 mM) at pH 7, recorded 0.2, 0.5, 1.0 and 2 ms for ***o*-QM** and recorded 1.0, 2.5, 5.0 and 10 ms for ***p*-QM** after the laser pulse.

Data from Kresge's [21] and our group [17] suggests the following reactivity scale in water for both QMs: $Cl^- < H_2O <$ thiols < primary amines < cyclic secondary amines < thiolate ions.

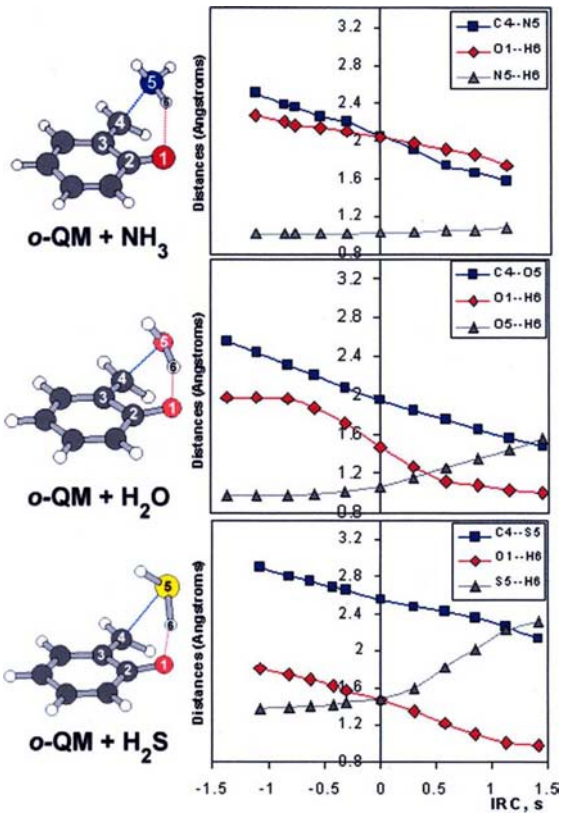
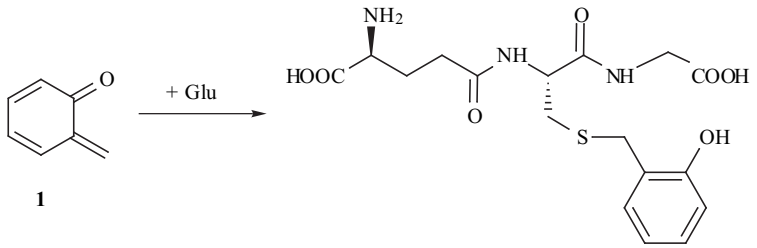


Fig. (2). Change of the forming C--Nucleophile bond (■), breaking Nucleophile--H bond (Δ), and forming O_1--H bond length (♦) along the IRC path starting from reactants [**(a)** alkylation of NH_3, **(b)** hydration reaction of *o*-QM, and **(c)** alkylation of H_2S by *o*-QM] to final products, respectively. The IRC calculation has been performed at B3LYP/6-31+G(d,p) (for N and O nucleophiles) and B3LYP/6-31+G(d, p),S(3df) (for hydrogen sulphide) levels, in gas phase. The IRC length is given by *s* ($amu^{1/2}$ Bohr), where *s*=0 represent the transition structures (**TSs**), s → ∞ the products.

Data in Table **1** clearly display that ***o*-QM** is always more reactive than ***p*-QM**, but the *o*-QM/*p*-QM reactivity gap is a function of the nucleophile structure. In more detail, ***p*-QM** although always less reactive than ***o*-QM**, particularly with thiols, displays a comparable reactivity to ***o*-QM** only with thiolate anions. The reactivity trend outlined in Table **1** suggests that, nucleophiles bearing acidic protons on the nucleophilic centre are more reactive toward ***o*-QM** than toward ***p*-QM**. This evidence shows the greater susceptibility of the electrophilic character by protic assistance of *o*-QM in comparison to *p*-QM due to the peculiar *ortho* geometry of the former. The catalytic effect of an acidic proton on the nucleophilic center and the water catalysis on the reactivity of *o*-QM toward ammonia, water and hydrogen sulfide has been displayed by computational investigation at B3LYP/6-311+G(d, p) level of theory in gas phase and in solvent bulk (by C-PCM model) [24]. In particular the computations show that the proton transfer from prototype nucleophiles such as NH_3, H_2O and H_2S to the *o*-QM oxygen atom occurs much later than the formation of the new C-Nu bond in the alkylation of ammonia, but it becomes progressively more synchronous to the formation of the new C-Nu bond passing from water to H_2S (see Fig. **2**).

In other words, the alkylation of NH_3 (and other NH nucleophiles) by *o*-QM is an example of "pure nucleophilic addition" onto *o*-QM with development of a dipolar TS, which displays a cationic character on the ammonia nitrogen atom and anionic nature on the QM oxygen atom. The alkylation reaction mechanism of water can be defined as "nucleophilic addition assisted by H-bonding", while in the alkylation of H_2S the latter exhibits a dominant electrophilic interaction with *o*-QM at the TS, showing an early transfer of the hydrogen sulphide proton to the oxygen atom. Thus, along the series NH_3, H_2O, H_2S there is a progressive shift from a "nucleophilic" interaction to an "electrophilic" one of the adding reactant [24].

The H-bonding with the solvent, involving the QM oxygen atom is a key aspect in the QM reactivity as electrophile, since in water under neutral conditions; the *o*-QM hydration reaction is a water-catalysed mechanism [24]. In fact, the reduction of such an interaction by bulk effect of the *ortho* substituents (such as those in the BHT and



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Scheme 6.

BDMP-QMs) enhances the stability of the QMs in water solution [20]. These substituents shielding the carbonyl oxygen from protic solvent interactions reduce the catalytic effect of H-bonding at the QM oxygen atom.

3. COVALENT MODIFICATION OF AMINO ACIDS AND PEPTIDES

Reactions of poorly reactive, moderately and highly reactive QMs such as 2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexanedione (BHT-QM), 2-tert-butyl-6-methyl-4-methylene-2,5-cyclohexanedione (BDMP-QM, see Scheme **4**) and the parent QMs (*ortho* and *para*), respectively, have been investigated in aqueous solutions with nucleophilic amino acids and peptides, under physiological conditions and as a function of the pH.

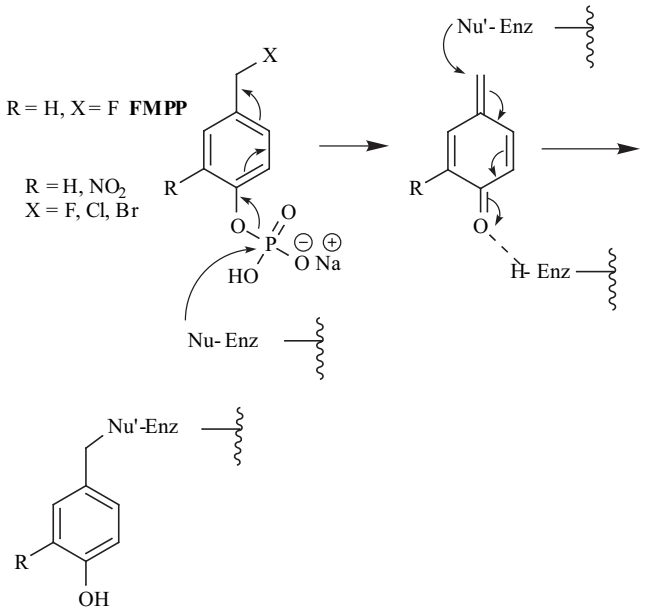
Each QM rapidly formed a thioether derivative with cysteine and cysteine-containing peptides like glutathione (Scheme **6**), with little or no competition from the addition of water (hydration reaction) [17, 25].

The selectivity of the alkylation reaction of nitrogen nucleophiles by QMs, i.e: α-amino group and side chain nitrogen centres of lysine and histidine and its efficiency in comparison to the hydration reaction are both strongly dependent on QM structures. In more detail, the α-amino groups are the primary sites of alkylation for poorly and moderately reactive QMs such as BHT-QM and BDMP-QM, with pseudo-first order rates 5-8-fold greater that the hydration rate [25a]. Alkylation of the side chain nitrogens is much less competitive for BDMP-QM in comparison to the hydration reaction and it is not detectable for the least reactive BHT-QM [25a]. Side chain modifications occur only with more electrophilic QMs such as *o*-QM, which is also capable of undergoing tyrosine oxygen alkylation [17]. The chemoselectivity in the alkylation of lysine and tyrosine is also a function of the pH. Specifically, the α-amino/side-chain ratio rises gradually from basic to neutral conditions [17]. Under neutral conditions, the second order rate constant for the *o*-QM hydration is at least 10^4-10^5 fold smaller than that of the alkylation of amines and thiols [17]. These kinetic data explain the efficiency of QMs as alkylating agent in water solution under physiological conditions. The second-order rate constants ($\mathbf{k_{Nu}}$) with H_3O^+ and OH^- demonstrate the importance of the acid and base catalysis in the hydration process of *o*-QM.

4. ALKYLATION OF ENZYMES: QMS AS MECHANISM-BASED INHIBITORS

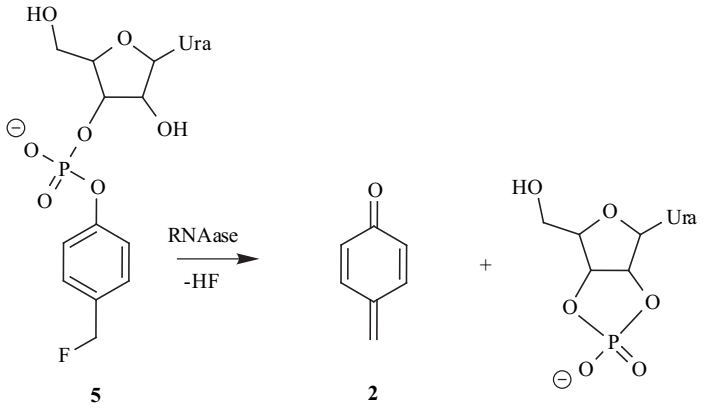
The search for molecules that interact with β-lactamase enzymes is an important part of the quest for new antibiotics. In addition, the synthesis and hydrolysis of phosphate esters by phosphatases and phosphodiesterases control both the structures and the activity of proteins and also DNA processing and replication, including viral reproduction. Therefore, it is not surprising that in the last decade several β-lactamase substrates and phosphate esters have been modified in order to achieve a latent QM electrophile, which could be unmasked, in principle, during enzymatic turnover and react with an active site nucleophile, causing the inactivation of the enzyme (according to the sequence in Scheme **7**) [9-13].

In more detail Widlanski and co-workers investigated the mechanisms of the inactivation of prostatic acid phosphatase by 4-halomethylaryl phosphates such as 4-(fluoromethyl) phenyl phosphate (**FMPP**), which generate *p*-QMs within the active site (Scheme **7**) [9].



Scheme 7.

These studies demonstrate that the rate and the efficiency of the inactivation are dependent on the nature of the leaving group at the benzylic position. Furthermore, the addition of a nitro group at the *ortho* position of the benzene ring of the inhibitor (R = NO_2, in Scheme **7**) results in an improvement of the selectivity between two different phosphatases [9b]. The nitro derivatives (in Scheme **7**, R= NO_2) display higher selectivity for the inactivation of prostatic acid phosphatase than that of protein tyrosine phosphatases (PTPases). On the other hand, no selectivity is shown by **FMPP** and by 4-



Scheme 8.

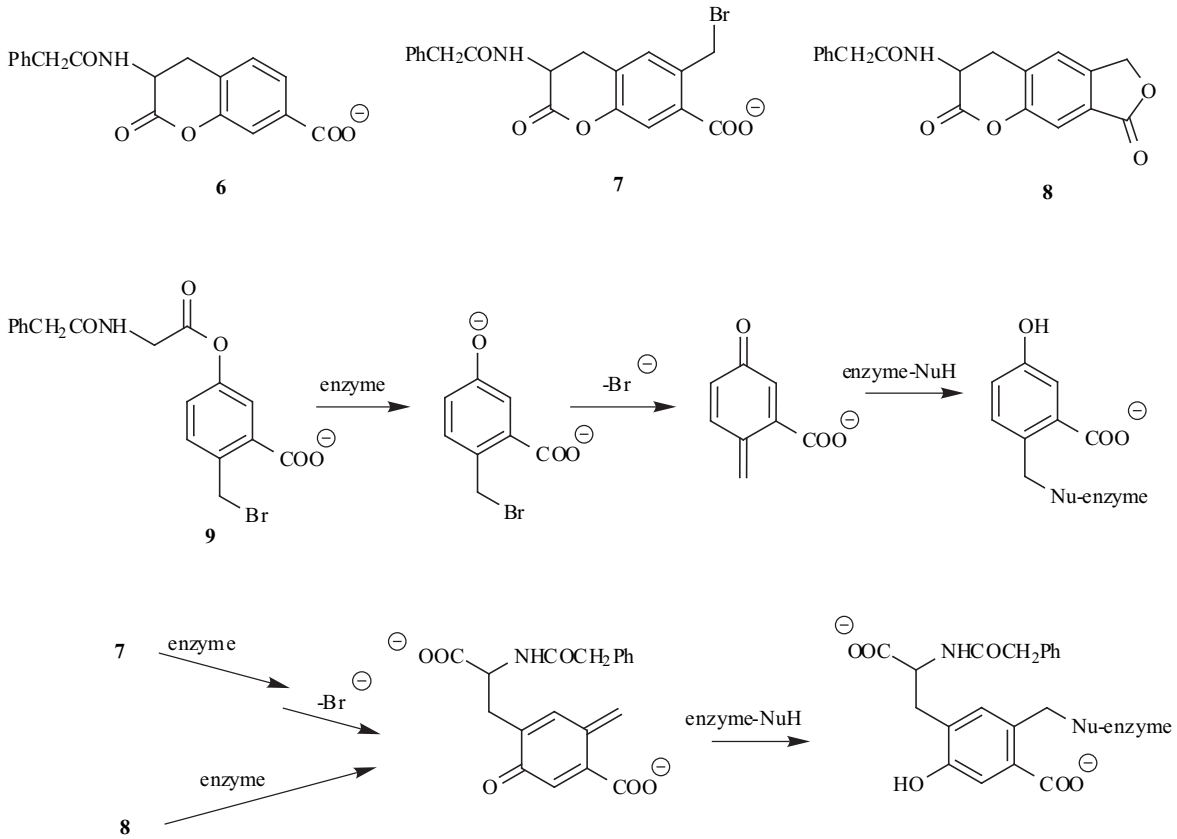
difluoromethylphenyl phosphate (**DFPP**), which inactivate both prostatic acid phosphatase and PTPases.

Widlanski applied an almost similar strategy to ribonuclease A, finding the first example of a mechanism-based-inhibitor of a phosphodiesterase [10]. The 4-(fluoromethyl)phenyl phosphate (**5**) in Scheme 8 induces an enzyme catalysed generation of *p*-QM (**2**), which causes an inhibition of the enzyme itself. Although the inactivation brought about *p*-QM alkylation was not complete (67%), Widlanski's work provides a useful application of QMs as mechanism-based inhibitors of ribonuclease A, as well as inhibitors of other nucleases.

The competition between alkylation of a nucleophile in the enzyme active site by the QM and its diffusion outside the active site has been suggested as a possible limiting factor of the effectiveness of substrates like **5** as inhibitor of β-lactamases.

A few dihydrobenzopyranones **6-8** (Scheme **9**) have been synthesised by Wakselman's group exploring this structural theme as potential substrate (**6**) and/or mechanism-based inhibitors (MBIs) (**7-9**). The extent of irreversible inhibition of β-lactamases caused by **7** and **8** was not significantly greater than that achieved by the acyclic analogue **9**.

MBIs, such as **7** and **8**, are able to generate QMs tethered to the acyl group (Scheme **9**). Such a feature limits the diffusion of QM-like structures from the active site, at least with serine hydrolases [11b,c], reducing the hydrolysis of the substrate and improving efficiency. The modest inhibitor



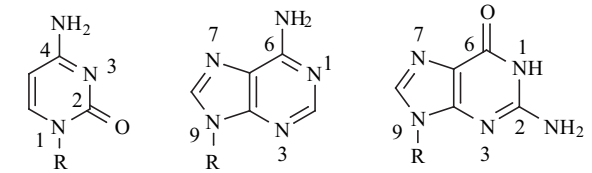
Scheme 9.

activity of the latter compounds (**7** and **8**) thus may have arisen as much from the absence of a suitable active site functional group in the vicinity of the electrophilic methylene of the QM [11].

5. ALKYLATION OF THE DNA BASES, AND THE PHOSPHATE BACKBONE

Quinone methides and related electrophiles are intermediates, formed during the metabolism of drugs and xenobiotics and often may lead not only to protein and enzyme inhibition, but also to DNA alkylation. The intrinsic reactivity of *o*-QM towards DNA bases has been characterised using *O*-(*tert*-butyldimethylsilyl)-2-bromomethyl phenol designed by Rokita to generate *o*-QM in the presence of fluoride anion [26-29]. 2'-Deoxynucleotides have also been alkylated in vitro by two more stable *p*-QMs such as BHT-QM and BHTOH-QM [30]. The deoxynucleotide adducts generated by the *o*-QM covalent modification of deoxycytidine (dC) [26], deoxyadenosine (dA) [27, 28] and deoxyguanosine (dG) [29] were isolated and spectroscopically characterised. The experimental selectivity of QM-like structures obtained from product distribution analysis, appears to be different in comparison to other alkylating agents without H-bonding properties such as diazonium and phenylnitrenium ions [31], carbocations [32] and benzyl halides [33]. In fact, QMs are likely to selectively attack the cyclic N3 position of deoxycytidine and the *exo*-amino groups of guanine (N^2) and adenine (N^6), rather than guanine N7 or adenine N1 [27-29, 34-36], which are generally recognised as the most intrinsic nucleophilic sites (see Scheme **10** for numbering).

Actually, the 2-amino group of guanine is the most reactive site toward activated Mitomycin C (which acts through the formation of a transient QM) among the nucleophilic centres present in the DNA bases [37]. Recently, Rokita and co-workers showed that the "most nucleophilic site of **dA** (deoxyadenosine) -N1 preferentially, but reversibly, conjugates to a model *ortho*-quinone methide".



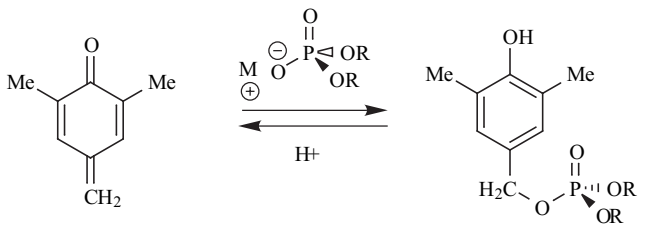
R = H **C** **A** **G**
= Me **1-MeC** **9-MeA** **9-MeG**
= dRib **dC** **dA** **dG**

Scheme 10.

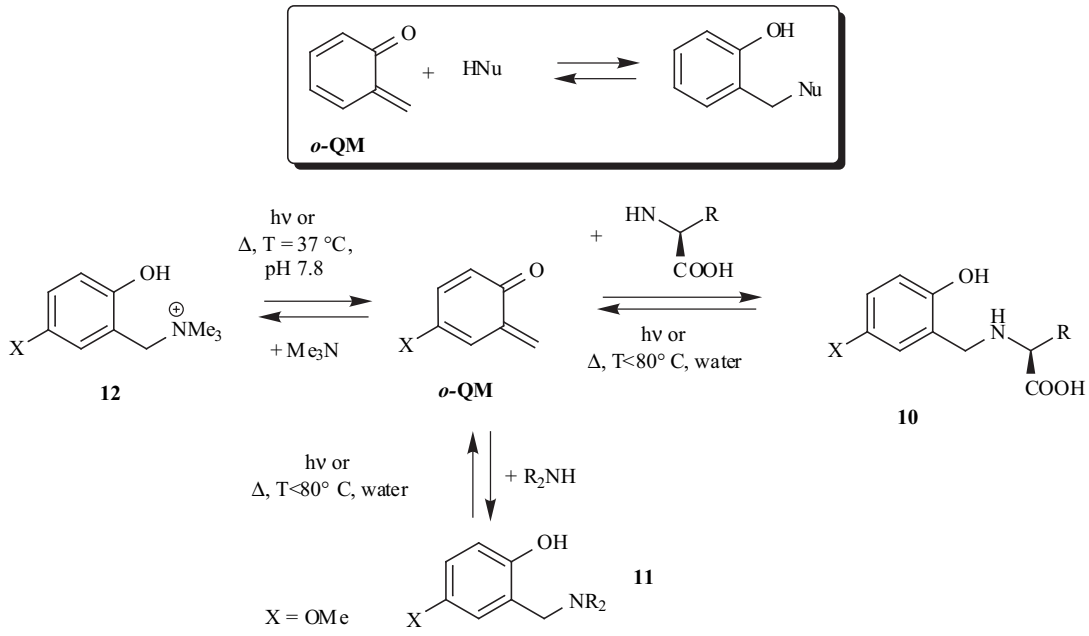
Such a result, which is in apparent contradiction to less recent data, clearly suggests that "thermodynamic rather than kinetic" aspects play an additional and important role in the control of selectivity [28]. Although the same author suggested that "attention to kinetic and thermodynamic selectivity will no doubt enhance our ability to predict modification of DNA" [28], thermodynamic aspects [namely stability of alkylation adduct], have seldom been analysed and thoroughly evaluated beside kinetic parameters (activation free energies for each possible reaction pathways). Only very recently, the issue of the chemoselectivity towards cytosine (**C**), adenine (**A**) and guanine (**G**) together with their N-methylderivatives (**1-MeC**, **9-MeA** and **9-MeG**) has also been addressed computationally by our group in both gas phase and water solution [38,39]. The high reactivity and selectivity of cytosine N3 moiety towards *o*-QM-like structures both in the deoxymononucleoside and in a single stranded DNA, has been rationalised on the basis of kinetic and thermochemical aspects, namely strong H-bonding interactions between reactants and adduct stability.

From a kinetic point of view, two general models of reactivity in water have been shown to be operative: uncatalysed and water catalysed mechanisms for nitrogen and oxygen nucleophiles, respectively [38]. In the water-catalysed mechanism, which is operative in the alkylation of the cytosine oxygen atom, an ancillary water molecule is directly involved in transferring a proton from cytosine to the *o*-QM oxygen atom in a cascade process.

The computational investigation has been extended to adenine, guanine and their 9-methyl derivatives as prototype substrates of deoxy-adenosine and -guanosine [39]. The calculations suggest that the most nucleophilic site of the methyl-substituted nucleobases in the gas phase is the guanine oxygen atom (O^6) ($\Delta G^{\neq}_{gas}$ = 5.6 kcal mol^{-1}), followed by the adenine N1 ($\Delta G^{\neq}_{gas}$ = 10.3 kcal mol^{-1}), while other centres exhibit a substantially lower nucleophilicity. The bulk effect of water as a solvent is the dramatic reduction of the nucleophilicity of both 9-methyladenine N1 ($\Delta G^{\neq}_{solv}$ = 14.5 kcal mol^{-1}) and 9-methylguanine O^6 ($\Delta G^{\neq}_{solv}$ = 17.0 kcal mol^{-1}). As a result there is a reversal of the nucleophilicity order of the purine bases. While O^6 and N7 nucleophilic centres of 9-methylguanine compete almost on the same footing, the reactivity gap between N1 and N7 of 9-methyladenine in solution is highly reduced. Our data clearly show that site alkylations at the adenine N1 and the guanine O6 and N7 in water are the result of kinetically controlled processes and that the selective modification of the *exo*-amino groups of guanine N2 and adenine N6 are generated by thermodynamic equilibration. Not only the alkylation of DNA bases, but also the covalent modifications of the phosphate backbone can be accomplished. In fact, the alkylation of phosphodiesters with a *p*-QM (2,6-dimethyl-*p*-quinone methide, TMP-QM) has been realised by Turnbull and co-workers under anhydrous conditions (promoted by a Bronsted acid) [40], and under aqueous solution (buffured at pH 4.0, 28%, aqueous acetonitrile) (Scheme **11**) [41]. The relative rates of phosphodiester alkylation and hydrolysis have been measured by ^{1}H-NMR analysis. These kinetic studies also prove that the phosphodiester alkylation reaction by *p*-QMs is an acid-catalysed process. The rate constant was found to range from approximately 370-3700 times the rate constant of QM hydrolysis with diethyl and dibenzyl phosphate, respectively.



Scheme 11.



Scheme 12.

6. REVERSIBILITY OF THE ALKYLATION PROCESS. QM-ADDUCTS AT NITROGEN NUCLEOPHILES AS QM CARRIERS

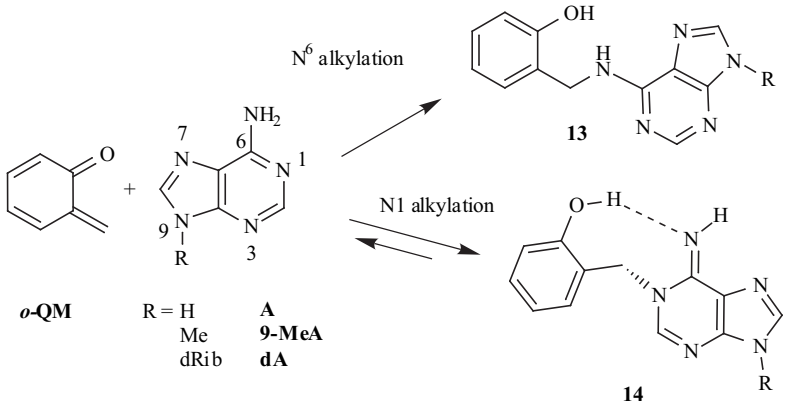
o-QM alkylation adducts with nitrogen nucleophiles are capable of acting as QM precursors under photochemical activation [17]. The alkylation process becomes thermally reversible under mild conditions (water, 7.0 < pH < 7.8) for the adducts with amino acids at the α-amino group (such as **10**) [17], with amines having an electron rich aromatic ring such as **11** [42] and for the ammonium salts **12** (Scheme **12**) [17]. Such reversibility of the alkylation process under mild conditions is actually a general key feature of the QM-alkylation adducts with several nitrogen nucleophiles, including DNA bases.

In fact, it has been shown experimentally by Rokita [28,29] and computationally by our group [39] that the alkylation adduct at dA N1 (deoxyadenosine N1 nucleophilic centre), such as **14**, has a lifetime under physiological conditions of few hours. Concerning adduct-stability, the calculations predict that only two of the 9-methyladenine adducts with *o*-QM, those at NH_2 (**13**) and N1 (**14**) positions, are lower in energy than reactants, in water. However, adduct at N1 can easily dissociate in water with an activation free energy of +19.8 kcal mol^{-1} at 298 K (Scheme **13**).

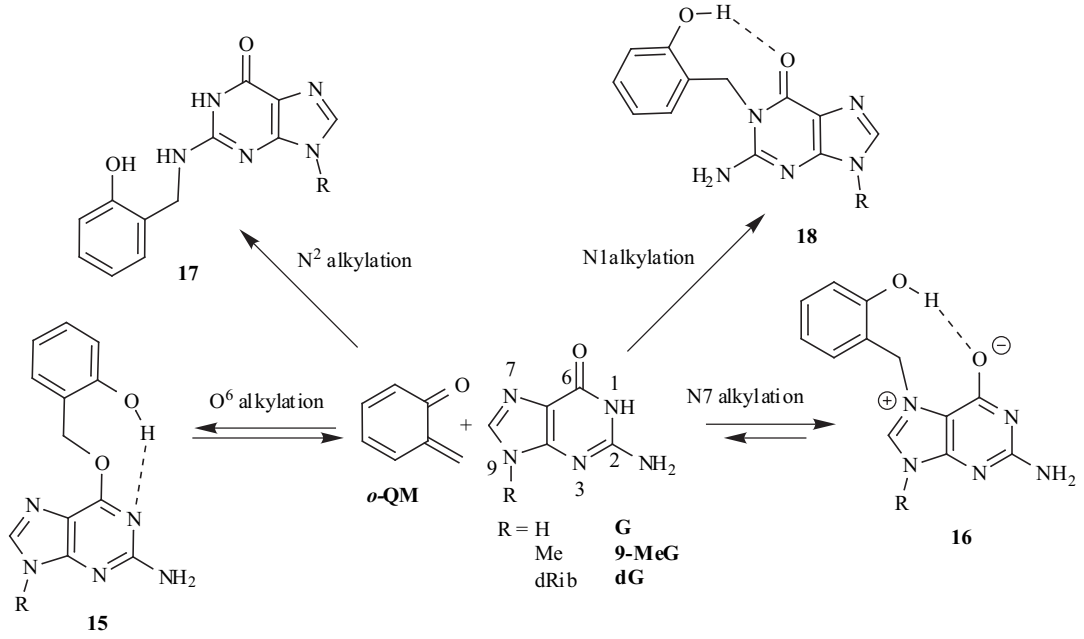
The adducts arising from the covalent modification of 9-methylguanine (**15-18**, in Scheme **14**) are largely more stable than reactants in the gas phase, but their stability is markedly reduced in water. In particular, the oxygen alkylation adduct (**15**) becomes slightly unstable in water in comparison to free reactants (ΔG_{solv} = +1.4 kcal mol^{-1}) and the N7 alkylation product (**16**) remains only moderately more stable (ΔG_{solv} = -2.8 kcal mol^{-1}).

Our computational data display that site alkylations at the adenine N1 and the guanine O^6 and N7 in water are the result of kinetically controlled processes and that the selective modification of the *exo*-amino groups of guanine N2 and adenine N6 are generated by thermodynamic equilibration.

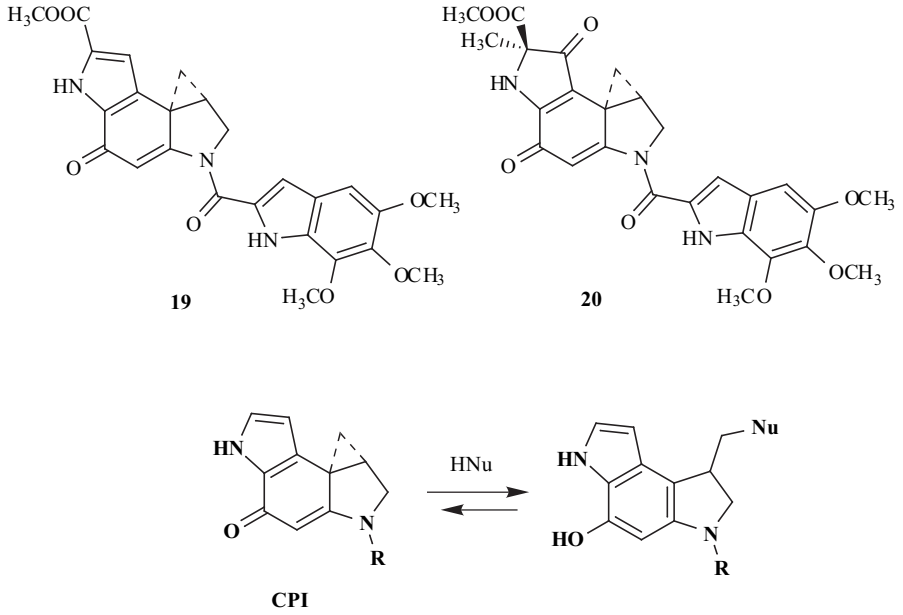
This general reversibility of the alkylation process suggests that the site selectivity as judged from product



Scheme 13.



Scheme 14.

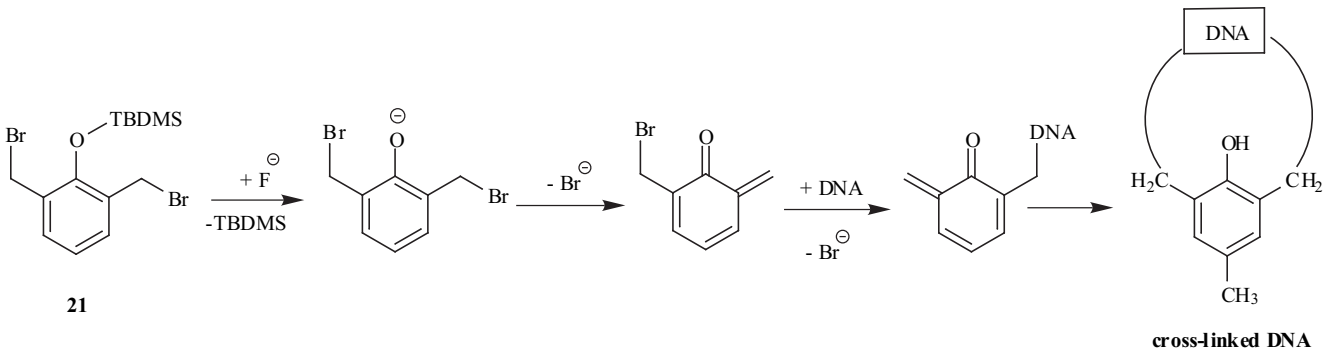


Scheme 15.

distribution analysis may reflect thermodynamic rather than kinetic selectivity [28]. In other words, kinetically favoured adduct with DNA bases such as **14**-**16** cannot be efficiently isolated, because they reversibly regenerate *o*-QM, which is then trapped by less nucleophilic sites, which afford much more stable products that can accumulate (such as **13**, **17** and **18**). The ability of QMs to form metastable adducts with purine nucleobases (at N7 and O^2 of guanine, and at N1 of adenine) and certain amino acids in water suggests that the above adducts may act as *o*-QM carriers [17,38,39]. The formation of metastable adducts with QMs provides a method of dispersing a short-lived electrophile throughout a biological system [28]. The importance of this general principle is becoming increasingly evident, since also anthracyclines and more recently other alkylating agents with anticancer activity such as Ecteinascidin 743 (Et743) [43] and duocarmycins (**19** and **20**, Scheme **15**), reversibly alkylate DNA [44]. Duocarmycins SA and A (**19, 20**) and more generally cyclopropylpyrroloindoles (**CPIs**) are considered homologues of the *para*-quinone methide (*p*-QM, **2**) [45] and like the latter CPIs exhibit a general sensibility to acid catalysis [46].

7. CROSS-LINKING OF DNA DOUBLE STRANDS BY BIFUNCTIONAL QMS

The DNA interstrand cross-linking (ISC) agents represent one of the most potent categories of antitumour antibiotic [14]. This is due to the ability of such compounds to shut down DNA strand separation, which is crucial to replication



Scheme 16.

and transcription. The design of novel dimeric agents targeting DNA has been investigated by several groups [14] and among these antitumour agents, QMs have also been involved as intermediates.

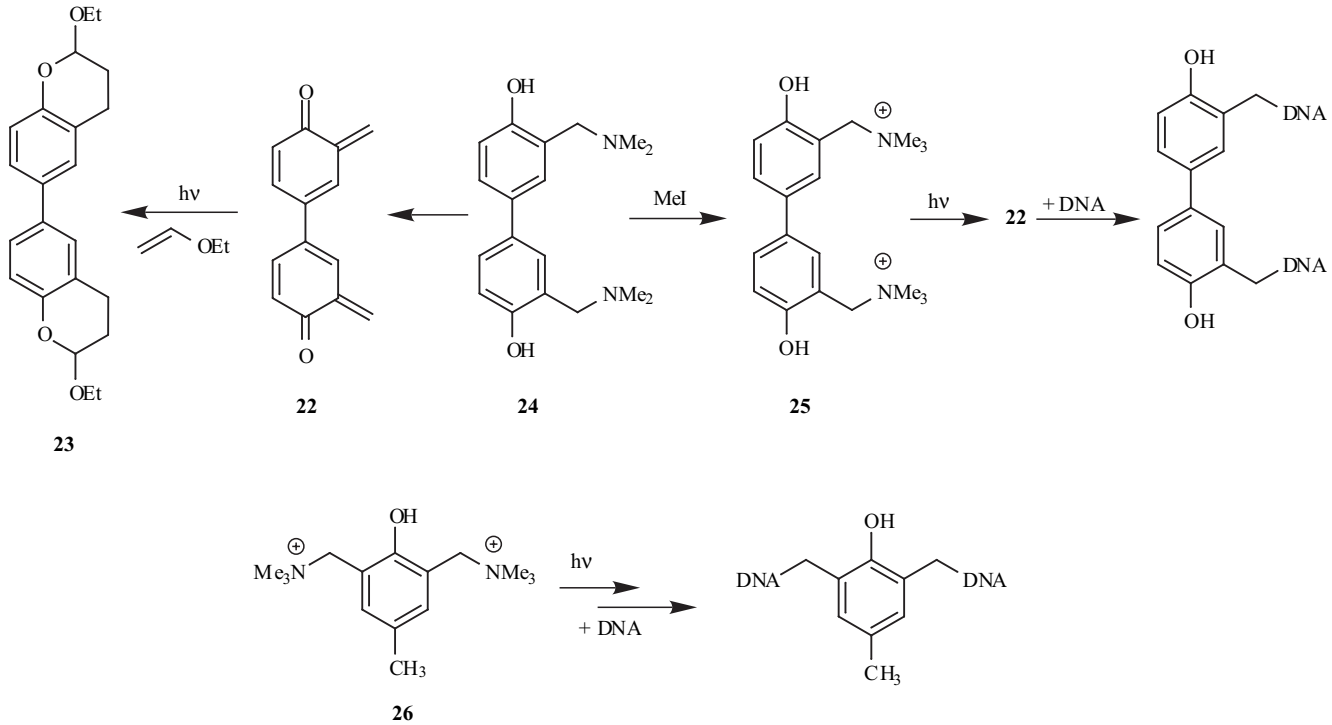
Rokita and coll. developed a derivative designed to yield a tandem quinone methide generation starting from *O*-(*tert*-butyldimethylsilyl)2,6-bis(bromomethyl)phenol (**21**) (Scheme **16**) [47].

Incubation of **21** in the presence of fluoride anion and of two duplex oligodeoxy-nucleotides produced high molecular weight species with electrophoretic mobilities that are expected for cross-linked DNA.

Saito photogenerated a transient "bisquinone methide" **22** (Scheme **17**), which was trapped by electron rich alkenes to give the Diels-Alder adduct **23**, by irradiation of the Mannich base **24** [23]. Very recently Zhou's group showed that **25**, which is the ammonium salt of the Mannich base **24**, is an efficient and photoinducible cross-linking agent at $\lambda > 400$ nm. [48] The DNA cross-linking ability of compounds **25** and its benzo analogue **26** has been investigated using linearised plasmid DNA by denaturing alkaline agarose gel electrophoresis. The experiments have been carried out in buffered solution (pH 7.7), exposing the samples to a 50 W mercury lamp. DNA cross-linking by compound **25** has been observed at concentration as low as 1.0 μM. In comparison with compound **25**, compound **26** has been found 100-fold less potent as a DNA cross-linking agent.

8. QMS FROM ANTHRACYCLINES

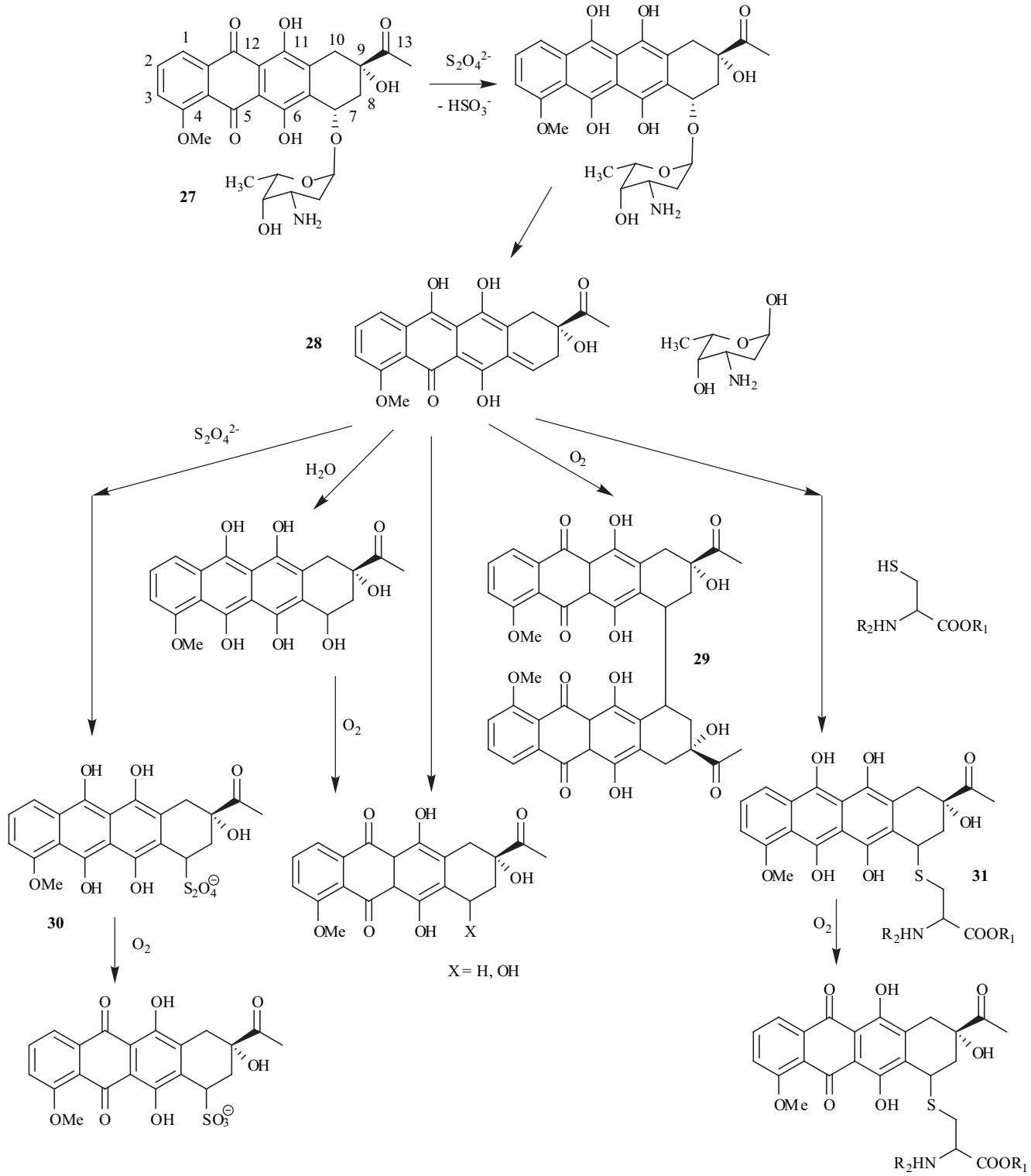
The anthracyclines represent a structurally diverse and important class of antitumour antibiotics [49], exhibiting vastly different chemistries. The aim of this review is not to systematically analyse their reactivity and their structural diversity [50], which has been extensively done in previous reports, [51, 52] but to take into consideration only the chemistry correlated by their ability to undergo redox conversion to quinone methides. Elegant studies by Koch



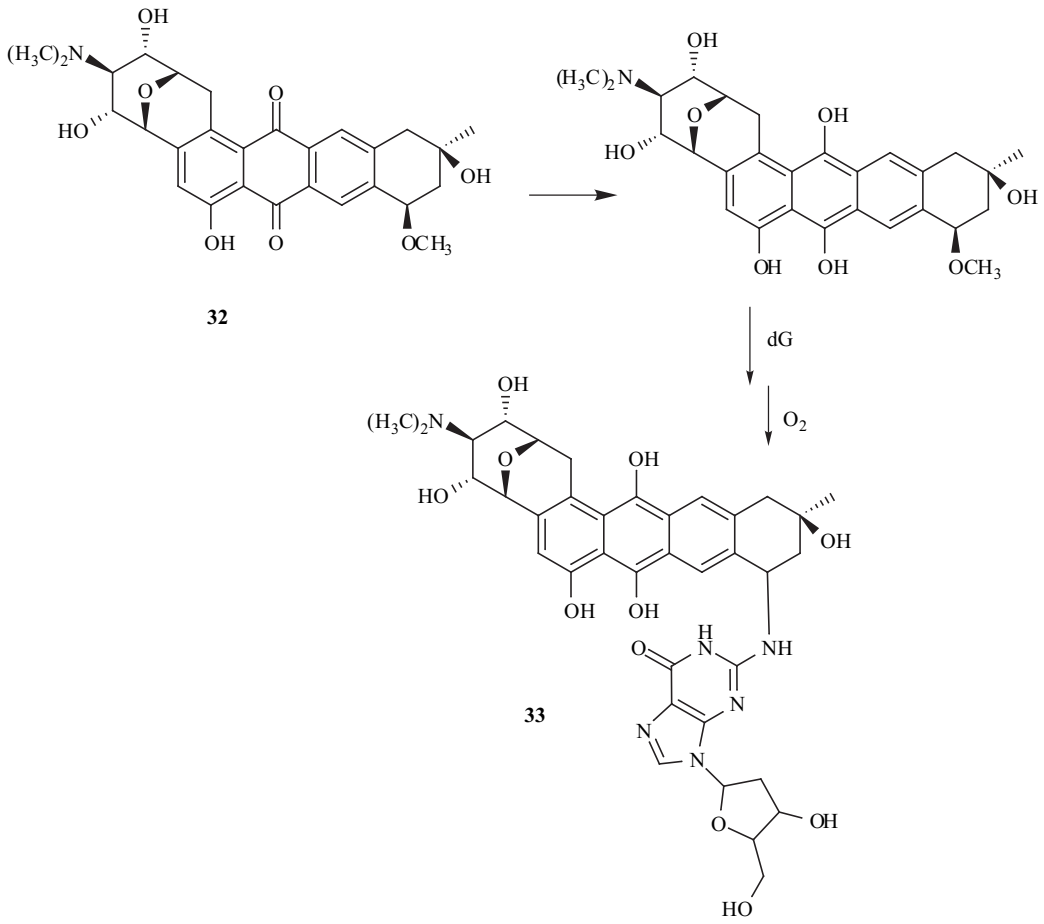
Scheme 17.

[53] have shown that anaerobic reduction of daunomycin (**27**) with sodium dithionite affords a wide array of adducts (Scheme **18**) arising from a highly conjugated QM (**28**). This quinone methide generated upon aglycon release from the reduced daunomycin can be an efficient electrophilic trap of biological nucleophiles at the C7 position [53]. In the absence of nucleophiles **28** generates many adducts such as the dimer **29**.

The QM **28** has been characterised spectroscopically from the absorption bands centred at 310 + 610 nm [54] and at 420 + 680 nm in protic medium and in DMSO respectively [55]. QM **28** has been trapped efficiently by the dithionite anion [53] to give **30** and by thiols such as N-acetylcysteine providing the thioether **31** in fairly good yield [56]. Nucleic acid alkylation has been achieved by Koch by reduction of menogaril (**32**) (Scheme **19**) through its related QM [57].



Scheme 18.



Scheme 19.

Deoxyguanosine yields the nucleoside modified at the NH_2 group (**33**) by reductive activation of **32**.

Although several members of anthracyclines are capable of cross-linking into DNA, the reductive quinone methide formation explains only their ability to induce monofunctional covalent binding, accounting for one of the two alkylation events necessary for the interstrand cross-linking (ISC) [57]. The second alkylation event necessary to achieve cross-linking has been proposed to involve simple Schiff base formation involving the carbonyl group at C13 and the N^2 of deoxyguanosine. However, the DNA lesion is often unstable and it has been difficult to characterise.

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