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Photogeneration and Reactivity of Naphthoquinone Methides as Purine Selective DNA Alkylating Agents

Daniela Verga,[†] Matteo Nadai,[‡] Filippo Doria,[†] Claudia Percivalle,[†] Marco Di Antonio,[§] Manlio Palumbo,[§] Sara N. Richter,*[,‡] and Mauro Freccero*[,†]

Dipartimento di Chimica Organica, Università di Pavia, V. le Taramelli 10, 27100 Pavia, Italy, Dipartimento di Istologia, Microbiologia e Biotecnologie Mediche, Università di Padova, via Gabelli, 63, 35121 Padova, Italy, and Dipartimento di Scienze Farmaceutiche, Università di Padova, Via Marzolo, 5, 35131 Padova, Italy

Received July 19, 2010; E-mail: mauro.freccero@unipv.it (M.F.); sara.richter@unipd.it (S.R.)

Abstract: A one-step protecting-group-free synthesis of both 6-hydroxy-naphthalene-2-carbaldehyde and the bifunctional binaphthalenyl derivative afforded 6-hydroxymethylnaphthalen-2-ol, 6-methylaminomethyl-naphthalen-2-ol, [(2-hydroxy-3-naphthyl)methyl]trimethyl ammonium iodide, and a small library of bifunctional binol analogues in good yields. Irradiation of naphthol quaternary ammonium salt and binol-derivatives (X = OH, NHR, NMe_3^+, $OCOCH_3$, and L-proline) at 310 and 360 nm resulted in the photogeneration of the 2,6-naphthoquinone-6-methide (**NQM)** and binol quinone methide analogues (**BQMs**) by a water-mediated excited-state proton transfer (ESPT). The hydration, the mono- and bis-alkylation reactions of morpholine and 2-ethanethiol, as N and S prototype nucleophiles, by the transient **NQM** (λ_{max} 310, 330 nm) and **BQMs** (λ_{max} 360 nm) were investigated in water by product distribution analysis and laser flash photolysis (LFP). Both the photogeneration and the reactivity of **NQM** and **BQMs** exhibited striking differences. **BQMs** were at least 2 orders of magnitude more reactive than **NQM**, and they were generated much more efficiently from a greater variety of photoprecursors including the hydroxymethyl, quaternary ammonium salt and several binol-amino acids. On the contrary, the only efficient precursor of **NQM** was the quaternary ammonium salt. All water-soluble **BQM** precursors were further investigated for their ability to alkylate and cross-link plasmid DNA and oligonucleotides by gel electrophoresis: the **BQMs** were more efficient than the isomeric *o*-**BQM** (binol quinone methide analogue of 2,3-naphthoquinone-3-methide). Sequence analysis by gel electrophoresis, HPLC, and MS showed that the alkylation occurred at purines, with a preference for guanine. In particular, a **BQM** was able to alkylate N7 of guanines resulting in depurination at the oligonucleotide level, and ribose loss at the nucleotide level. The photoreactivity of **BQM** precursors translated into photocytotoxic and cytotoxic effects on two human cancer cell lines: in particular, one compound showed promising selectivity index on both cell lines.

Introduction

DNA interstrand cross-linking (ISC) effectively inhibits both DNA replication and gene expression by preventing the melting of the two strands. Such an event, shutting down the DNA replication process, represents by far the most cytotoxic of all the alkylations. Therefore, ISC has considerable potential in molecular biology and human medicine for pharmacological applications.[1] The mechanism of action of interstrand cross-linking agents, including their activation, and the design of new types of cross-linking agents has been extensively investigated. The compounds capable of DNA ISC include antitumor antibiotics, such as mitomycins,[2] azinomycins,[3] and synthetic antitumor agents, such as aziridinomitosenes[4] and bizelesin,[5] nitrogen mustard derivatives,[6] and cis-platinum-like organometallics.[7] The photoactivated agents are probably the less developed class, with the exception of psoralens.[8] Only quite recently bifunctional quinone methides (QMs) have been

[†] Università di Pavia.
[‡] Dipartimento di Istologia, Università di Padova.
[§] Dipartimento di Scienze Farmaceutiche, Università di Padova.

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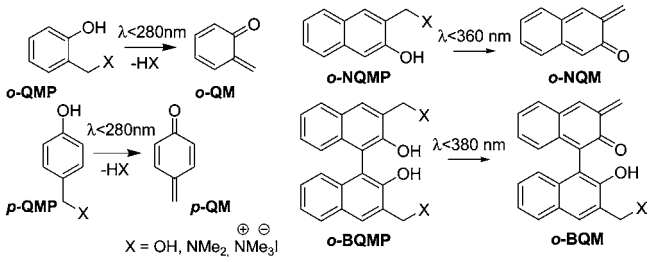
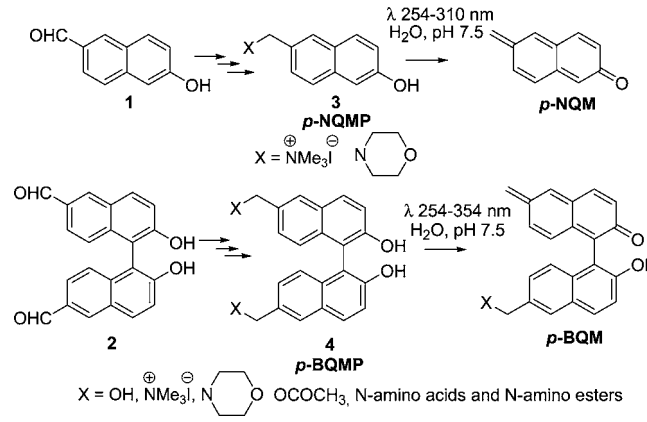
Scheme 1



successfully used to accomplish nucleoside alkylation and DNA ISC[9] by photochemical,[10] fluoride-induced activations,[11] thermal digestion,[12] and oxidation.[13] Concerning the photochemical activation, *o*-QM and *p*-QM have been generated by Wan,[14] Kresge,[15] Popik,[16] and our group[10c,d,12,17] using benzyl alcohols (X = OH), Mannich bases (X = NMe_2), and quaternary ammonium salts (X = $NMe_3^+I^-$) (Scheme 1).

It has been shown that the efficiency of the photoactivation process and the water solubility of the quinone methide precursors (**QMPs**) are both a function of the leaving groups (X).[18] In fact, quaternary ammonium salts of phenolic Mannich bases (Scheme 1, X = $NMe_3^+I^-$) are better and preferable **QMPs** than the alcohol analogues (X = OH, photochemical quantum yield Φ 0.98 vs 0.23).[19] The prototype ***o*-QMPs** and ***p*-QMPs** have little absorbance above 310 nm, and therefore they are not suitable for biological applications as photoactivated alkylating agents under mild conditions. The absorbance of naphthols derivatives and binol analogues as quinone methide precursors (***o*-NQMPs** and ***o*-BQMPs**, respectively, see Scheme 1) on the other hand, extends beyond 310 nm, and they still exhibit excellent solubility under physiological conditions. In fact, we have shown that ***o*-BQMPs**, such as the bis-quaternary ammonium salt, are capable of undergoing bis-alkylation in water and DNA cross-linking with promising potency by UV−vis photoactivation.[10d] The high efficiency of the *o*-QM photogeneration has been associated to the higher excited state acidity of phenol, 2-naphthol (pK_a* 2.8),[20] and 3-hydroxy-2-naphthalenemethanol (pK_a* 0.77),[16a] which should facilitate the excited state intramolecular proton transfer (ESIPT) to the leaving group X at the ortho position. In the case of ***p*-QMPs** the distance between the phenol and the hydroxyl group is too large to undergo ESIPT, and although excited state proton transfer (ESPT) can occur in the presence of a protic solvent the efficiency for the ***p*-QM** photogeneration is often much lower.[21] This is probably the reason why ***p*-QMs** have been almost completely neglected in the recent literature as photoactivatable alkylating agents. In this paper, we report (i) the efficient generation of 2,6-naphthoquinone-6-methide (***p*-NQM**) and binol quinone methide analogues (***p*-BQM**) (Scheme 2) by irradiation of suitable precursors in water, (ii) the original protecting-group-free synthesis of both ***p*-NQMPs**, and ***p*-BQMPs**, (iii) the ability of ***p*-BQMs** to efficiently alkylate and cross-link single- (ss) and double-stranded (ds) DNA at the purine level, and (iv) their promising photocytotoxic versus cytotoxic properties.

Scheme 2. Synthesis of the 6-CH_2X-Substituted-2-naphthol- and Binol-Analogues As Precursors of ***p*-NQM** and ***p*-BQM** Quinone Methides (***p*-NQMP** and ***p*-BQMP**)



Results and Discussion

Synthesis of the Naphtho- and Binol-Quinone Methide Precursors *p*-NQMP and *p*-BQMPs. Two small libraries of compounds (**3a**−**3d** and **4a**−**4j**, Scheme 3) have been investigated as photoprecursors of the 2,6-naphthoquinone-6-methide (***p*-NQM**) and binol quinone methide analogues (***p*-BQMs**) (***p*-NQMP** and ***p*-BQMPs,** respectively Scheme 2). The key intermediates for the synthesis are the 6-hydroxynaphthalene-2-carbaldehyde (**1**) and the 2,2′-dihydroxy-[1,1′]binaphthalenyl-6,6′-dicarbaldehyde (**2**). Although these aldehydes are known in the literature, they have always been prepared through a four-

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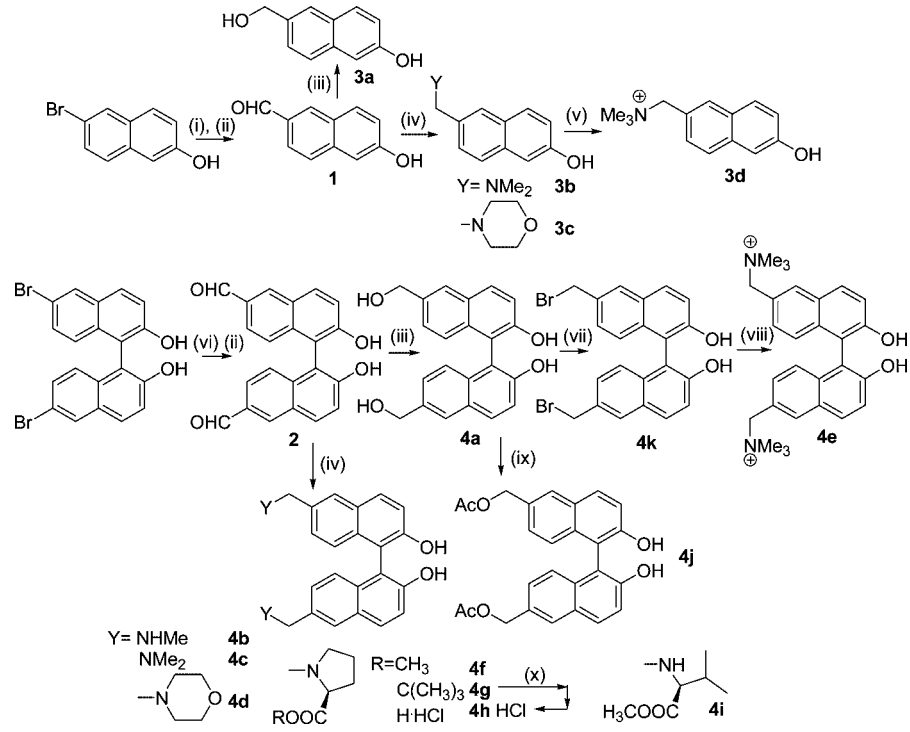
Scheme 3. Synthesis of the 6-Hydroxynaphthalene-2-carbaldehyde (**1**), the 2,2′-Dihydroxy-[1,1′]binaphthalenyl-6,6′-dicarbaldehyde (**2**), and the ***p*-QMPs 3a−3d** and **4a−4j**[a]



[a] (i) *n*-BuLi 4.4 equiv, THF, −78°C, 5 h. (ii) DMF, −50°C 1 h. −50 °C → 0 °C, 1 h. Workup H_2O/HCl, 0°C (iii) $NaBH_4$, EtOH/THF = 7:3, 5 h. (iv) Morpholine (or proline methyl and *t*-butyl esters and valine methyl ester) $NaBH(AcO)_3$, EtOH, 24 h, r.t. NH_2Me (or $NHMe_2$), CH_3OH, 20 h, r.t. and then $NaBH_4$ r.t. 3 h. (v) CH_3I, CH_2Cl_2 r.t. 24 h. (vi) *n*-BuLi 8.0 equiv, THF, −78°C, 5 h. (vii) CH_3COOH, HBr, r.t.. 5 h. (viii) NMe_3, THF, r.t., 15 min. (ix) CH_3COOH, Δ, 48 h. (x) $CH_2Cl_2/CF_3COOH/Et_3SiH$ = 20.5:10:4, r.t. 1 h.

step synthesis.[22] We have been able to synthesize **1** and **2** with a novel one-step protecting-group-free synthetic protocol, in quantitative yields, starting from 6-bromo-2-naphthol and 6,6′-dibromobinol (Scheme 3). The lithiation and the following formylation by DMF have been performed on both racemic and enantiomerically pure (*R*)- and (*S*)-6,6′-dibromobinols. The 6,6′-dibromobinol enantiomers have been prepared starting from enantiomerically pure [1,1′]binaphthalenyl-2,2′-diol (binols) according to published procedures, with 98% ee.[23] Both enantiomers react with retention of the configuration giving (*R*)-**2** and (*S*)-**2** at −78 °C. In fact, (*R*)-**2** and (*S*)-**2** have been recovered from the formylation workup at 0 °C, with a 96% and 94% ee, respectively, measured by chiral HPLC (chiralcel OD-H 0.46 × 25 cm).

Both the aldehydes **1** and **2** have been quantitatively reduced to the alcohols **3a** and **4a**, by $NaBH_4$ in EtOH/THF = 7:3, or transformed into the amines **3b−c** and **4b−c** by a one-pot reductive amination performed in methanol. Similarly, **4d−i** have been synthesized in good yield using $NaBH(AcO)_3$, in ethanol as solvent. **4d** has been recovered and purified as hydrochloride. The binol amino acid (binolam) **4h** has been obtained as hydrochloride by acid deprotection of **4g** in quantitative yield. The reductive amination of (*R*)-**2** using proline methyl and *t*-butyl esters and valine methyl ester give the diastereoisomers (*R*,*S*,*S*)-**4f**, **4g**, and **4i** in good yields (>75%). The quaternary ammonium salts **3d** and **4e** have been synthesized from 6-dimethylaminomethylnaphthalen-2-ol (**3b**) by CH_3I methylation and from an anhydrous THF solution of 6,6′-bis-bromomethyl-[1,1′]binaphthalenyl-2,2′-diol (**4k**), bubbling gaseous NMe_3.

Photoreactivity of *p*-NQMPs. The possibility to exploit **3a−3d** and **4a−4j** as phototriggerable mono- and bis-alkylating agents, respectively, through the generation of transient **QMs** has been investigated in aqueous acetonitrile solutions (water/ACN = 7:3), irradiating at 310 nm (1×10^{-3} M, in a photoreactor with 4 or 2 lamps 15 W, ca. 25 °C, 10 and 30 min, under Ar and O_2 purged solutions), in the presence of morpholine or 2-ethanethiol, as prototypes of water-soluble nucleophiles.

Although the naphthol **3a** was quantitatively recovered after 3 h of irradiation, photolysis of the amine **3b** in the presence of 2-ethanethiol gave **3f** as the main adduct (in low yield), with traces of **3a** and **3e** (Table 1). The photoactivation afforded a quantitative conversion of **3c** into the hydration adduct **3a**. The same photochemical reaction performed in the presence of 2-ethanethiol produced a mixture containing the alkylated adduct **3e**, together with **3a** and **3f** in lower yields (Table 1). The quaternary ammonium salt was the most photoreactive naphthol, generating the hydration adduct **3a** or the alkylation adduct **3e**, without or with 2-ethanethiol, respectively.

The product distribution was slightly affected by the presence of oxygen. In fact, the irradiation of **3d** showed a higher conversion into the alkylated adduct **3e**, in air-saturated solution, with the generation of the aldehyde **1** as a byproduct. A longer irradiation time (≥2 h) revealed that the adduct **3e** was also photoreactive, generating **3f** or **1** as the main products in sloppy reaction mixtures, performed in the absence or in the presence of oxygen, respectively. Therefore, the photoproducts **1** and **3f** were both secondary photoproducts arising from the irradiation

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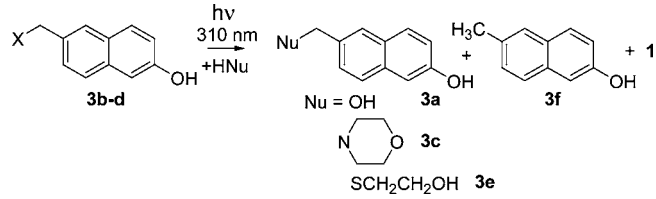
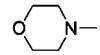
Table 1. Photoreactivity of Naphthols in Water/Acetonitrile (7:3) with and without Added Nucleophiles (Morpholine and 2-Ethanethiol)



Naphthol[a]	X	Nu[b]	Conversion (%)[c,d]	Ar/O_2[e,f]	Time min	Products (Yield %)[c,d]
3b	Me_2N-	$HOCH_2CH_2S$-	48	Ar	120	**3e**(<2), **3a**(8), **3f** (35), **1**(-)
3c		HO	77	Ar	30	**3a**(72), **3f** (-), **1**(-)
3c		$HOCH_2CH_2S$-	80	Ar	120	**3e**(39), **3a**(2), **3f** (15), **1**(-)
3d	Me_3N^+-	HO-	40	Ar	5	**3a**(35), **3f** (-), **1**(-)
3d	Me_3N^+-	$HOCH_2CH_2S$-	70	Ar	10	**3e**(52), **3a**(3), **3f** (11), **1**(-)
3d	Me_3N^+-	$HOCH_2CH_2S$-	93	O_2	10	**3e**(73), **3a**(4), **3f** (-), **1**(16)
3d	Me_3N^+-		79	Ar	10	**3a**(75), **3c** (4), **3f** (-), **1**(-)
3e	$HOCH_2CH_2S$-	HO-	70	Ar	120	**3a**(10), **3f** (31), **1**(-)
3e	$HOCH_2CH_2S$-	HO-	72	O_2	120	**3a**(11), **3f** (-), **1**(32)

[a] [**3b**−**3e**] = 10^{-3} M. [b] [HNu] = 5×10^{-2} M. [c] Measured by HPLC. [d] Irradiated at 310 nm in a photoreactor with 4 lamps, 15 W. $H_2O/CH_3CN \cong 7:3$, $T \cong 25$ °C. [e] Argon purged for 5 min. [f] Oxygen purged for 10 s.

of the primary adduct **3e**. Their formation suggests the generation of 6-naphthylmethyl radical as an intermediate in the photolysis of **3e**. The product analysis of the photolyzed mixtures has been performed by HPLC using purified samples, isolated from preparative photolysis, as standards (Table 1).

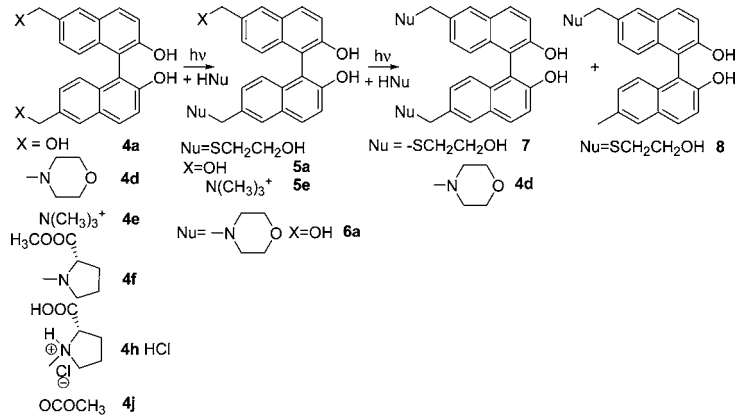
Photoreactivity of *p*-BQMPs. Similarly **4a**−**4j** were irradiated at 310 nm in water/ACN (7:3 or 1:1) and in the presence of 2-ethanethiol or morpholine (Table 2). The irradiation of **4d**−**f**, **4j**−**k**, performed in the absence of an added nucleophile, afforded the alcohol **4a** from good to modest yields (Table 2). **4b** and **4c** are essentially photostable for 3 h. Photolysis of **4a** and **4e** in aqueous solutions containing 2-ethanethiol gave the bifunctional thioether **7**, together with the monofunctional derivatives **5a** and **5e**. The latter were clearly precursors of the former, which became the main adduct at long (>2 h) irradiation times (Table 2). Similarly, the irradiation of **4a** in the presence of morpholine gave a clean conversion (75%) into **6a** and **4d**, after 1 h of irradiation. These results suggest that the 6,6′-CH_2X-bis-substituted binols **4a**, **4d**, **4e**, **4h**, and **4j** act as efficient phototriggerable mono- and bis-alkylating agents, through a stepwise generation of two monoalkylating conjugated QMs (such as ***p*-BQM-4** and ***p*-BQM-5** in Scheme 4). The binolam **4f** was much less efficient as a photoalkylating agent than the above, and although it is slightly photoreactive **4i** did not undergo a clean photohydration in aqueous solution.

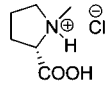
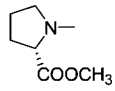
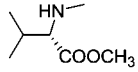
All the QM-precursors **4a**−**4j** investigated and the resulting alkylation adducts **5a**, **5e**, **6a**, **7**, and **8** are thermodynamically stable upon digestion at 40 °C for several days.

Efficiency of the QM-Photogeneration. The quantum yields (Φ = photoproducts/absorbed photons) listed in Table 3 revealed that the efficiency of the QMPs photoactivation process is affected by the nature of both the chromophore (naphthol vs binol) and the leaving groups (Scheme 4). Indeed, the binol quaternary ammonium salt **4e** was twice more efficient than the naphthol analogue **3d**. Furthermore, the highest photoefficiency of the binol **4a** (Φ = 0.87) contrasts to the lack of reactivity of the naphthol **3a**, which was photostable. Unexpectedly, **4a** exhibited a higher efficiency in the generation of the ***p*-BQM-4a** compared to the quaternary ammonium salt **4e**, which still releases the monoalkylating ***p*-BQM-4e** with high quantum yield (Table 3). Therefore, the lower conversion of **4a** in comparison to **4e,** measured from product distribution analysis as a function of the irradiation time (Table 2), has to be ascribed to a lower absorption (see Supporting Information). Although the generation of the second quinone methide ***p*-BQM-5** (Scheme 4) is always less efficient than the first one (***p*-BQM-4**), the QMP **5e** is almost twice more efficient than **5a** (Table 3).

Comparison between *p*-BQMPs and *p*-NQMPs. The product distribution analysis and quantum yield measurements suggest that in general ***p*-BQMPs** are much better photoalkylating agents than ***p*-NQMPs**. Such a large difference in reactivity may be related to the intramolecular H-bonding in the binol derivatives, which is lacking in the naphthol analogues. Such interaction should improve the efficiency of the ESIPT for the binols in comparison to the naphthols, which can only undergo intermo-

Table 2. Photoreactivity of Binols in Water/Acetonitrile (7:3) with and without Added Nucleophiles (Morpholine and 2-Ethanethiol)



Binol[a]	X	Nu[b]	Conv.(%)[c,d]	Ar/O_2[e,f]	Time (min)	Products (Yield %)[c,d]
4a	HO-	$HOCH_2CH_2S$-	10	Ar	2	**5a**(10), **7**(-)
			34		10	**5a**(32), **7**(2)
			54		20	**5a**(44), **7**(10)
			63		30	**5a**(49), **7**(23)
			84		60	**5a**(53), **7**(31)
			95		120	**5a**(8), **7**(81), **8**(5)
4a	HO-		75		60	**6a**(45), **4d**(30)
4e	Me_3N^+-	HO-	99	Ar or O_2	60	**4a**(99)
4e	Me_3N^+-	$HOCH_2CH_2S$-	31	Ar	2	**5e**(37), **7**(-)
			76		10	**5e**(57), **7**(18)
			83		20	**5e**(48), **7**(35)
			93		30	**5e**(36), **7**(57)
			99		60	**5e**(14), **7**(85)
			100		90	**5e**(2), **7**(95), **8**(3)
4d		HO-	82	Ar	60	**4a**(63), **6a**(19)
4h HCl			95	Ar	60	**4a**(93)
4j	CH_3COO-	HO-	95	Ar	90	**4a**(93)
4j	CH_3COO-	$HOCH_2CH_2S$-	96	Ar	90	**7**(90), **8**(4)
4f		HO-	55	Ar[g]	90	**4a**(30)
4i		HO-	45	Ar[g]	90	**4a**(<5)
7	$HOCH_2CH_2S$-	$HOCH_2CH_2S$-	14	Ar	120	**8**(11)

[a] [**4a**, **4d**−**j**] = 10^{-3} M. [b] [HNu] = 5×10^{-2} M. [c] Reactant conversion measured by HPLC. [d] Irradiated at 310 nm in a photoreactor with 2 lamps, 15 W. $H_2O/CH_3CN \cong 7:3$, $T \cong 25$ °C. [e] Argon purged for 5 min. [f] Oxygen purged for 10 s. [g] NaPi buffered water:acetonitrile (pH 6).

lecular ESPT to the solvent. This explanation is consistent with the reduced emission of the binol derivatives in comparison to the naphthol analogues (see Supporting Information). In addition, among the binols, **4a** is more prone to act as a monoalkylating agent than the quaternary ammonium salt **4e**, which in turn should act as a better bis-alkylating agent.

Further differences between the photoreactivity of binols and naphthol analogues have been provided by product distribution analysis. In fact, although the naphthols **3c** and **3d** act as photoactivatable monoalkylating agents, the formation of the byproducts **1** and **3f** reveals a side reactivity which can be ascribed to the 6-naphthylmethyl radical as a reactive intermediate. Such a side naphthylmethyl-like radical reactivity is lacking in the ***p*-BQMPs**, which act as mono- and bis-alkylating agents, without any detectable competing reaction pathway. The behavior of the ***p*-BQMPs** is quite similar to the previously investigated ***o*-BQMPs** (Scheme 1). The only remarkable difference is the efficiency of the second QM

Scheme 4

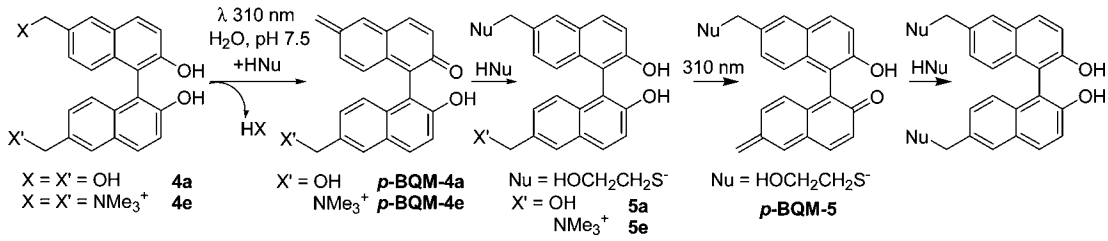


Table 3. Efficiency of the QM-Photogeneration Evaluated from the Quantum Yield Measurements (Φ) at 310 nm, Measured in 7:3 H_2O/CH_3CN Solutions of the Naphthol **3d** and Binols **4a**, **5a**, **4e**, and **5e** at Low Conversion (<23%)[a]

adduct	[adduct]	conversion %	irrad time (min)	Φ
3d	1×10^{-3}	21.2	5	0.28
4a	8.37×10^{-4}	22.7	2	0.87
5a	1.08×10^{-3}	15.9	8	0.15
4e	1.03×10^{-3}	18.1	2	0.61
5e	9.92×10^{-4}	18.7	5	0.24

[a] Photon flow = 3.23×10^{-7} E/min, measured by potassium ferrioxalate actinometry (monitored by UV-vis).

generation (***p*-BQM-5**, Scheme 4), which is always lower for ***p*-BQMPs** than ***o*-BQMPs**.

Detection and Reactivity of Transient *p*-NQM and *p*-BQM by Laser Flash Photolysis. Our group and others have shown that laser flash photolysis (LFP) is an effective method for direct detection of QMs, using Mannich bases, their quaternary ammonium salts and *o*-hydroxybenzyl alcohols as phototriggerable precursors.[10,14,15,17,19,20] Excitation of the naphthol **3d** with a 266 nm pulses of a Nd:YAG laser resulted in the formation of two transient absorbances centered at 370 nm, and at 310−330 nm (Figure 1). Time evolution of the transient absorbance monitored at 370 nm occurred by biexponential kinetics. Neither of them was appreciably affected by O_2 or 2-ethanethiol. Unfortunately, the naphthol **3d** was strongly fluorescent in the 330−480 nm spectral window and the strong emission compromised the possibility to perform reliable kinetic measurements on the fastest decay at 370 nm. Therefore, our attention has been focused mainly on the second transient at 310−330 nm, in which the rise was monoexponential and occurred within 20 μs. Such an intermediate decayed at a much slower rate, exhibiting a $t_{1/2} > 1$ ms, which was too long-lived to be measured by our laser equipment, but it was quenched by 2-ethanethiol, with a second-order reaction rate very similar to that of the prototype ***p*-QM** ($k_2 = 1.3 \times 10^3$ M^{-1} s^{-1}).[24]

After 30−40 laser shots (8 mJ power) in the presence of 2-ethanethiol (10^{-2} M), **3e** was detected as the main product of the photolysis of **3d** in aqueous solution, by HPLC. Furthermore, the UV absorbance of the above transient was very similar to that of the isomeric 2,3-naphthoquinone-3-methide ***o*-NQM** (λ_{max} 330 nm).[16a] Therefore, we were able to identify the second transient as 2,6-naphthoquinone-6-methide ***p*-NQM** (Scheme 2). Since saturation of the solution with O_2 did not significantly change the distribution of the alkylation products in the preparative experiments, we can conclude that **3d** triplet excited state was not involved in the formation of ***p*-NQM**. On the other hand, ESPT from the phenolic OH, in the singlet excited state, to the vicinal sp^2 carbon atoms is well-documented for both for phenols and naphthols.[14c,25] Such a protonation should generate the keto-tautomer of the naphthol, which could be responsible for the transient absorbance centered at 370 nm. The aromatization of the above should lead to C-deuterated naphthols running the photohydration at 310 nm (4 lamps, 15 W), in deuterated protic solvents.[26] ^{1}H NMR analysis of the alcohol **3a** recovered after 1 h irradiation of the quaternary ammonium salt **3d** in ACN/D_2O = 7:3 did not show any detectable deuterium enrichment on the aromatic ring. This observation enable us to rule out the generation of keto tautomer as an intermediate (**3d-K**, Scheme 5). On the other hand, ***p*-NQM**, unlike ***o*-NQM**, cannot reversibly isomerize to benzoxete derivative,[16a,27] and therefore the latter intermediate cannot be associated with the transient absorbance at 370 nm. In addition, the zwitterionic specie **3d-ZW** (Scheme 5) resulting from the dissociation of the highly acidic excited states of the 2-naphthol derivative **3d**, which could be the precursors of the 2,6-naphthoquinone-6-methide, is unlikely to be the most reactive transient of the biexponential decay at 370 nm, since the lifetime of **3d-ZW** should be much shorter than a few microseconds in aqueous solution. As mentioned above, the strong emission compromises a reliable investigation of the nature of the 2,6-naphthoquinone-6-methide precursor, which is probably embedded in the fast decay of the biexponential kinetics at 370 nm. Therefore, no further LFP investigation on the naphthols was taken into consideration.

Unlike naphthols **3a** and **3d**, binols **4a** and **4e** were much less fluorescent in the 320−400 nm spectral window (see Supporting Information). Therefore, LFP measurements on both **4a** and **4e** had been performed without artifacts due to the saturation of the detector. Excitation of the binols **4a** and **4e** with 10 ns 266 nm pulses of a Nd:YAG laser, in argon purged buffered solutions (pH 7), yielded the formation of a short-lived transients ($t_{1/2} = 2$ μs) with λ_{max} centered at 360 and 355 nm, respectively, and a broad absorption extending to 440 nm for the transient generated from **4e** (Figure 2).

Although the molecular oxygen did not quench these transient species generated from **4a** and **4e**, it slightly bleached the shoulders stretching above 380 nm, that were more persistent and may be associated with long-living naphthoxy radicals generated in the photolysis. Both the transients were efficiently quenched by both morpholine and 2-ethanethiol. In the presence of the latter, the transients generated by flashing solutions of **4a** and **4e** were quenched with second-order rate constants (k_2) 1.95(±0.1) × 10^7 M^{-1} s^{-1} and 2.1(±0.1) × 10^7 M^{-1} s^{-1},

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(27) (a) Qiao, G. G.; Lenghaus, K.; Solomon, D. H.; Reisinger, A.; Bytheway, I.; Wentrup, C. *J. Org. Chem.* **1998**, *63*, 9806. (b) Tomioka, H. *Pure Appl. Chem.* **1997**, *69*, 837. (c) Sauter, M.; Adam, W. *Acc. Chem. Res.* **1995**, *28*, 289. (d) Shanks, D.; Frisell, H.; Ottosson, H.; Engman, L. *Org. Biomol. Chem.* **2006**, *4*, 846.

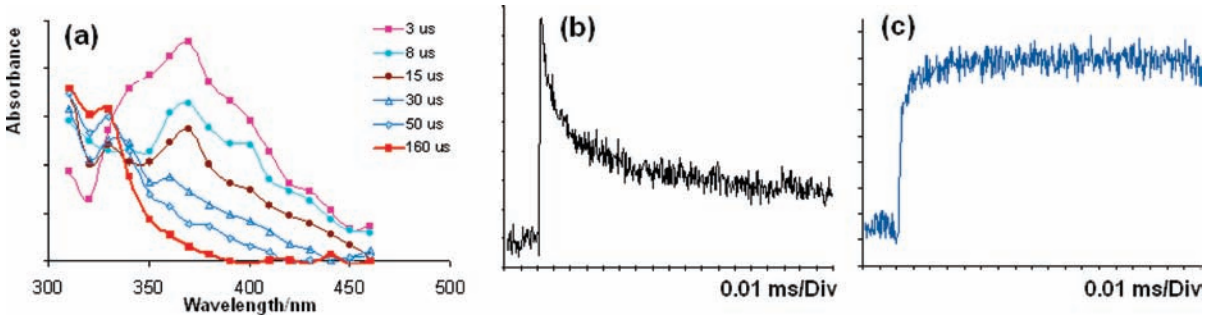
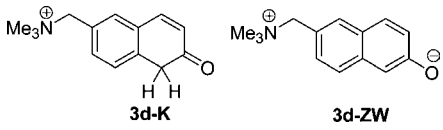


Figure 1. Transient absorptions upon 266 nm excitation of an oxygen purged water solution of **3d**. (a) Full spectra recorded 3, 8, 15, 30, 50, 160 μs after the laser pulse. (b) Decay trace recorded at 370 nm (c) Decay trace recorded at 310 nm.

Scheme 5



respectively (see the effect of 2-ethanethiol in the inset of Figure 2a). Considering that thiols are efficient quenchers of QMs and that after 20 laser shots **5a** and **5e** were detected by HPLC as the main products of the photolysis of **4a** and **4e** in aqueous solution containing 2-ethanethiol (10^{-2} M), we have been able to assign the transients centered at 360 (Figure 2a,b) and 355 nm (Figure 2c) to the binol-QM structures ***p*-BQM-4a** and ***p*-BQM-5a,** respectively (Scheme 4). It is interesting to note that the ***p*-BQMs** exhibit a very similar maximum absorbance to the ***o*-BQMs**[10d] and that both are 30 nm red-shifted in comparison to the ***o*-NQM** and ***p*-NQM** spectra. The similarity between ***p*-BQMs** and ***o*-BQMs** is extended to their reactivity, since the pseudo-first-order decay with water [$k_{obs} = 7.1(\pm 0.2 \times 10^5\ s^{-1})$] and the second-order rate constants with thiols [$k_2 = 2(\pm 0.2) \times 10^7\ M^{-1}\ s^{-1}$)] are very similar. In addition, they are both much more reactive than the ***o*-NQMs** and ***p*-NQMs** by almost two magnitude orders, probably as a result of the specific intramolecular acid catalysis due to the presence of the 2′-naphthol hydroxyl group.

DNA Alkylation and Cross-Linking Experiments. The water solubility of the binols **4a**, **4e**−**i** ($\sim 10^{-2}$ M), together with their bis-alkylating reactivity, suggests evaluating them as DNA cross-linking agents triggerable upon photolysis. The DNA cross-linking ability of these compounds was investigated using a negatively supercoiled plasmid DNA (pBR322) in an alkaline agarose gel assay (Figure 3).

The nonreacted plasmid was mainly present (95%) in its supercoiled (C) form and partly (5%) as open circular (OC) DNA. Interstrand cross-linking (XL) activities of tested compounds were evident as XL-bands of both initial forms (XL C and XL OC), which run slightly slower than the open circular form or just below gel wells, respectively (Figure 3). Activities were measured as the minimal compound concentration required to obtain XL-effects.

Both binols **4a** and **4e** showed remarkable reactivity: **4a** XL effect was evident at 1.25 μM (lane 1, Figure 3A), while **4e** induced detectable XL at concentration as low as 0.3 μM (lane 2, Figure 3B), hence being at least 4 times more potent than its ***o*-BQM** analogue.[10d] In addition, both compounds were able to cross-link more than 90% DNA at 40 μM (lanes 6 and 9, Figure 3, panel A and B, respectively). Incubation of DNA in the dark with **QMPs** was not performed as the control experiment, since the **QMPs 4a**−**4j** investigated were stable in the dark at 40 °C for several days.

Testing the binolam derivatives **4f**−**4i** we found that **4h** and its methyl ester **4f** were both able to induce XL at a concentration of 1.25 μM (Table 1). The binolams **4g** and **4i** were the less active beginning to induce detectable XL at a concentration higher than 5 μM (Table 4). It is interesting to note that the most efficient DNA XL-agents (**4a** and **4e**) are the most efficient mono- and bis-alkylating agents with simple nucleophiles (Table 2). All the 6,6′-disubstituted binol derivatives investigated, which act through the sequential generation of two ***p*-BQMs**, always display higher XL potency (by 2−16 fold) than the 3,3′-disubstituted binol derivatives, generating the ***o*-BQMs** isomers.[10d]

Next, the alkylation properties of the most active compound **4e** were tested on short ds- and ss-random oligonucleotides. As shown in Figure 4, **4e** was able to alkylate both ds- and ss-DNA after exposure to UV light (lanes 3 and 6, respectively, Figure 4). In more detail, **4e** induced both alkylation products and interstrand DNA cross-linking (ALK and XL band, respectively, in Figure 4) on the ds-oligonucleotide. Both adduct bands were multiple and slightly smeared, indicating that the alkylation reaction likely occurred at several different sites. On the ss-DNA, the alkylation band was extremely intense and smeared (lane 6, Figure 4) indicating the presence of different species such as mono- and multiple-alkylated DNA and intrastrand alkylated oligo. In addition, a weak cleavage of both the ds- and ss-alkylated oligos was observed. To detect the alkylation sites, the ss-alkylated oligo was further treated with hot piperidine, which induces cleavage at the alkylated base according to the Maxam and Gilbert reaction mechanism.[28] As shown in Figure 4, lane 9, major cleavage sites following hot alkali treatment were found at the guanine level and, to a lesser extent, at adenines, indicating alkylation reaction at purines.

Adduct characterization was performed by reacting **4e** with single 2′-deoxy-oligonucleosides (dNTs) in solution upon UV exposure. Adducts were separated by means of HPLC analysis, which revealed one new adduct peak with dG, dA, and dC, while no adducts were observed upon reaction with dT. Percentages of adduct formation, with respect to total **4e** amount, were 5.2%, 4.5%, and 0.8% for reaction with dG, dA, and dC, respectively. The purine selectivity, with a preference for dG, is quite surprising for a quinone methide, since it is known that the prototype benzo *o*-QM exhibits a defined selectivity for dC.[29] This could be the result of two combined effects: (i) the ***p*-BQM**

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(29) Veldhuyzen, W. F.; Shallop, A. J.; Jones, R. A.; Rokita, S. E. *J. Am. Chem. Soc.* **2001**, *123*, 11126–11132.

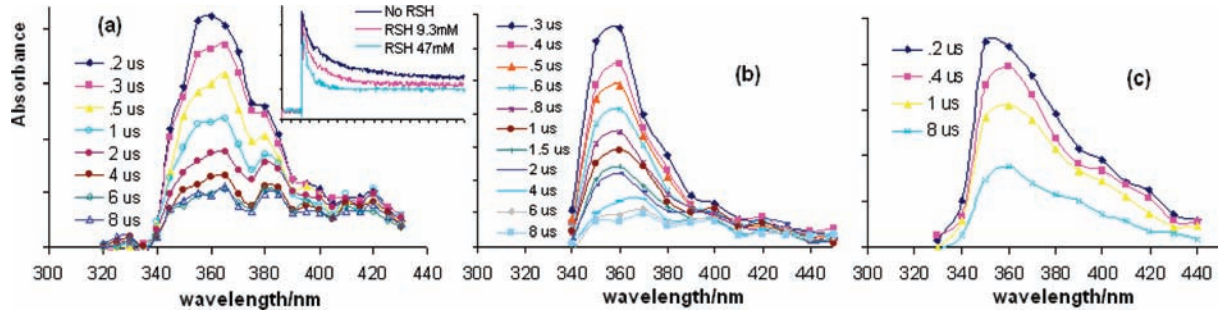

Figure 2. Transient absorptions of ***p*-BQM-4a** in an (a) argon purged, (b) oxygen purged water solution of **4a**. Transient absorption of ***p*-BQM-5a**, upon 266 nm excitation of (c) an oxygen purged water solution of **4e**. Panel (a), the inset shows the quenching effect of 2-ethanethiol on the transient centered at 360 nm [without (dark blue line) and with 2-ethanethiol 9.3 mM (fuchsia line) and 47 mM (light blue line)]. Time scale 1 μs/Div.

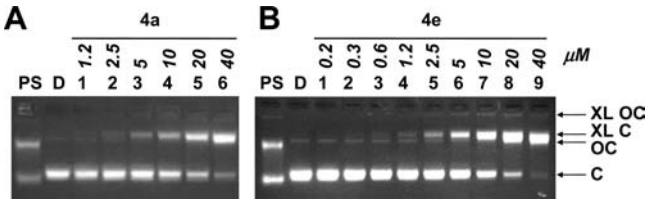

Figure 3. DNA cross-linking (XL) concentration-dependent activity of binol−amino acid conjugates. Plasmid DNA was mixed with increasing amounts of each compound (as indicated above each gel image), in phosphate buffer (50 mM, pH 7.5). Reaction mixtures were irradiated at 360 nm for 20 min and loaded on 1% alkaline agarose gel. Gels were stained with ethidium bromide. Lane D is nontreated, irradiated DNA; lane PS is a control for XL species induced by 4,5′,8-trimethylpsoralen at 360 nm. Nonreacted circular and open circular forms of plasmid are indicated as C and OC; their XL species are indicated as XL C and XL OC, respectively, on the right of gel images.

nature which is a harder electrophile in comparison to the prototype *o*-QM, exhibiting a much shorter lifetime in water, and (ii) the thermodynamic stability of the resulting alkylation adducts arising from all the binol-precursors. Such stability is in striking contrast to the reversibility of the alkylation process involving the prototype *o*-QM.[9,18,29,30] Fractions corresponding to the new peaks formed during the alkylation reactions with dG and dA were collected and analyzed by mass spectrometry based on electrospray ionization coupled with a time-of-flight analyzer (ESI-TOF MS). Peak formed in the reaction with dC was not collected due to the small amount observed. Multiple mass peaks were obtained for each analyzed adduct: molecular masses obtained for the two main peaks matched 1:1 covalent adducts of 2′-deoxy-purines with **4e** without proton loss and with a double positive charge (m/z = 319.1 and 311.1 for dG and dA, respectively) or with one proton loss and a single positive charge (m/z = 637.3 and 621.3 for dG and dA, respectively). In addition, a third major peak was detected, corresponding to a deglycosilated adduct (m/z = 521.2 and 505.2 for dG and dA, respectively) (Figure 5A,B). Interestingly, when the **4e-dG** HPLC-collected peak was subjected to heat treatment (i.e., 95 °C for 30 min), this latter adduct lacking ribose became the highly prevalent species, while the peaks retaining ribose either disappeared or were greatly reduced (Figure 5C). A plausible mechanism would involve the initial attack of the ***p*-BQM-4e** to the nucleophilic N7 of the purine base followed by the cleavage of the N-glycosidic bond at N9. Since the depurination process is a direct and unique consequence of alkylation at N7 of purines (and as such it is routinely used in biochemical sequencing of DNA based on the Maxam and Gilbert protocol, by the hard electrophile dimethyl sulfate) once again, the harder nature of the ***p*-BQM** is evident, which behaves more like a stabilized carbocation than a prototype QM.

Photocytotoxic and Cytotoxic Effects of Binol-Amino Acid Conjugates. 4a−b and **4e−i** were investigated on two human cancer epithelial cell lines: A549 (lung adenocarcinoma) and HT-29 (colorectal adenocarcinoma). These two cell lines were chosen since they represent common human tumors and are routinely used to test the effects of anticancer drugs. Results were reported as effective drug concentration able to kill 50% of cell population after photoirradiation (EC_{50}) and cytotoxic concentration that killed 50% of cell population without photoirradiation (CC_{50}). Selectivity indexes (SI) were measured as the ratio of CC_{50} over EC_{50} values. As shown in Table 4, compounds were in general more active and less cytotoxic on the HT-29 cell line. The most active compounds on both cell lines were binol-derivative **4g** and **4i** (EC_{50} in the high nanomolar range); however, they also showed remarkable cytotoxicity prior to UV irradiation at low doses (SI = 5−10). Compounds **4e** and **4f** were slightly less active, displaying EC_{50} in the low micromolar range. **4f** was also fairly cytotoxic (SI 10−18), while **4e** was remarkably less cytotoxic (SI 22−33). Compound **4b** exhibited a high SI (SI 33) only on HT-29 cells, but poor on the A549 cell line (SI 9). The other binolams **4a** and **4h** exhibited higher EC_{50} paralleled by higher CC_{50}; however, SI remained in general low (4−18). **PS** (4,5′,8-trimethylpsoralen), used as a control, in these conditions displayed lower SI than the analyzed compounds.

Conclusion

We have described the photogeneration of new and detectable bifunctional binol-quinone methides (***p*-BQMs**), by laser flash photolysis at 360 nm, which are capable of undergoing mono- and bis-alkylation of simple nucleophiles, oligonucleotides, ss-DNA and ds-DNA in water. The ***p*-BQMs** are more efficiently generated and much more reactive transient electrophiles than the monoalkylating naphtho-QM analogue (***p*-NQM**), exhibiting also a much harder electrophilic nature than the prototype ***o*-QM**. Since the resulting ***p*-BQM**−conjugate adducts are very stable and do not release free ***p*-BQMs** at $T < 40$ °C for several days, unlike the ***o*-QM**, their reactivity has been exploited to achieve stable and efficient DNA cross-linking with sub-micromolar potency by UV−visible activation. The study highlights that the water-soluble 6,6′-bis(trimethylaminomethyl)-[1,1′]binaphthalenyl-2,2′-diol (**4e**) is the most promising photocross-linking

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Table 4. Biological in Vitro and Cellular Properties of the Photoreactive ***p*-BQMPs** and **PS** (4,5′,8-trimethylpsoralen)

		EC_{50} (μM)		CC_{50} (μM)		SI (CC_{50}/EC_{50})	
photoreactive alkylating agent	DNA XL MC (μM)	cell line → A549	HT29	A549	HT29	A549	HT29
PS	<0.5	27 ± 14	20 ± 4	>200	35 ± 7	>7	2
4a	1.25	33 ± 11	11 ± 2	>200	>200	>6	18
4b	5	2.3 ± 0.6	0.6 ± 0.1	21 ± 1	>200	9	33
4e	0.3	9 ± 1	6 ± 0.5	200	200	22	33
4f	1.25	2 ± 0.04	1.5 ± 0.08	20 ± 1	27 ± 3	10	18
4g	5	0.9 ± 0.03	0.5 ± 0.02	4.5 ± 0.3	5 ± 0.2	5	10
4h	1.25	35 ± 11	26 ± 3	>200	>100	6	>4
4i	5−10	1.2 ± 0.1	0.2 ± 0.02	6.5 ± 0.8	1.4 ± 0.03	5	7

agents exhibiting a significant purine selectivity and promising photocytotoxicity versus cytotoxicity.

Experimental Section

General Procedures. 2-Naphthol and [1,1′]binaphthalenyl-2,2′-diol are commercially available products. The substituted naphthols: 6-Br-2-naphthol,[31] **3f**,[32] **1**,[33] and **3a**[34] and the substituted binols: 6,6′-diBr-[1,1′]binaphthalenyl-2,2′-diol,[35] **2**,[36] **4k**[37] are known products. They have been synthesized via standard published procedures, with the exception of **1** and **2**.

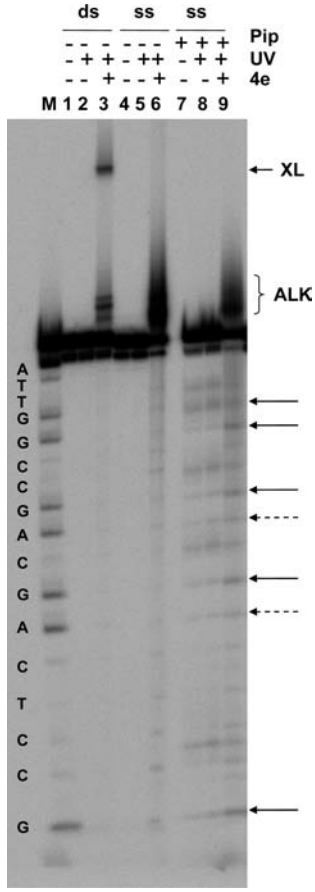

Figure 4. Reactivity of **4e** toward ds- and ss-DNA. The oligonucleotide 5′-AAC AGC CTC AGC AGC CGG TTA-3′ was 5′-end-labeled and annealed to its complementary strand. Both the single ds- and ss-oligonucleotides were reacted with 10 mM **4e** in 50 mM phosphate buffer, pH 7.4, and ethanol precipitated after 24 h. The ss-DNA was further treated with piperidine 1 M at 90 °C for 30 min (Pip), vacuum-dried, and then loaded on 20% denaturing acrylamide gels. Gels were run at 80 W for 2.5 h and autoradiographed. Control lanes: lanes 1 and 4 are the nontreated ds- and ss-oligos, respectively; lanes 2 and 5 indicate the nonreacted oligos exposed to UV but not to **4e**. Lanes 7 and 8 represent ss-DNA treated with piperidine and with piperidine and UV, respectively, but not exposed to **4e**. Lane M is a marker for purines (G + A) obtained by the Maxam and Gilbert reaction. Piperidine-cleaved G and A bases are indicated on the right side with continuous and discontinuous arrows, respectively, while the oligonucleotide resolved sequence is reported on the left.

6-Hydroxynaphthalene-2-carbaldehyde (1). A 0.500 g sample of 6-bromo-2-naphthol (2.24 mmol) was dissolved in 26.9 mL of dry THF under an argon atmosphere. The magnetically stirred

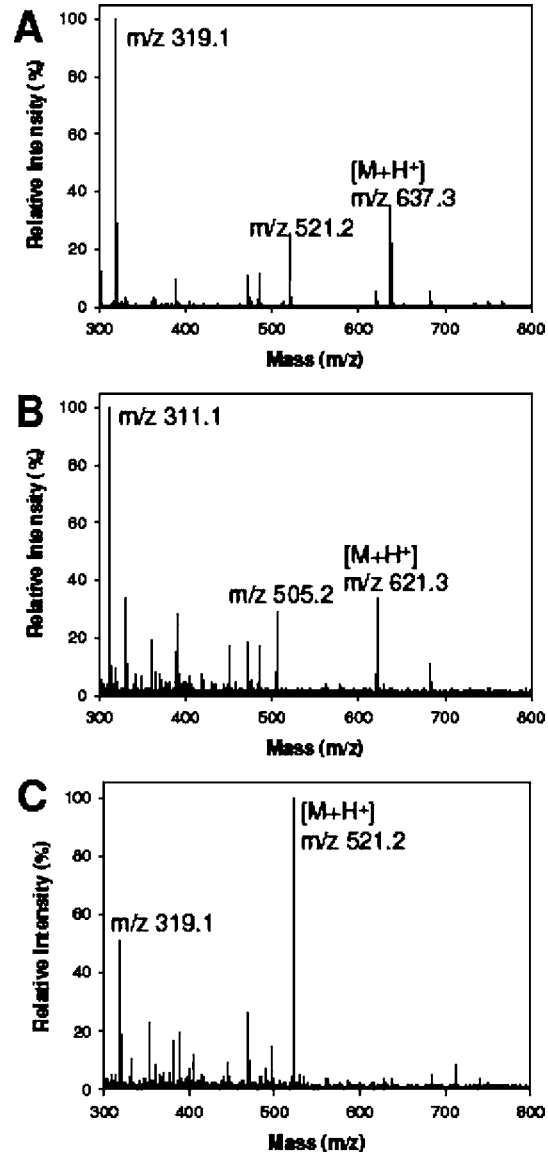

Figure 5. ESI-TOF MS spectrum of **4e-dG** and **4e-dA** conjugates. The adduct chromatographic peaks obtained in the reaction of **4e** with dG (A) or dA (B) were collected and analyzed by ESI-MS in positive ion mode to determine the molecular masses of the reaction products. (C) The **4e-dG** HPLC-collected peak was treated at 95 °C for 30 min prior to ESI-MS analysis. The *m*/*z* values for significant adduct ions are shown.

solution was cooled to −78 °C and 6.16 mL of *n*-BuLi in *n*-hexane (1.6 M) (9.85 mmol) was added at such a rate in order to not allow the temperature to exceed −70 °C. After 5 h of stirring at this temperature, 1.27 mL of dry *N*,*N*-dimethylformamide (16.4 mmol) was added so that the temperature remained below −50 °C. After stirring for 45 min at this temperature, the reaction mixture was poured into HCl/ice (pH < 1) under vigorous stirring. It was allowed to reach rt overnight and extracted 3 × CH_2Cl_2. The combined organic phases were washed twice with water and dried over $MgSO_4$, and the solvent was removed under reduced pressure after filtration to give an oil. The oil was purified by MPLC column chromatography (cyclohexane:ethyl acetate 8/2) to give 0.330 g of **1** (85.5%) as a white solid.[33] Mp 178−180 °C: ^{1}H NMR (DMSO): δ 7.20−7.23 (*m*, 2H), 7.76−7.84 (*m*, 2H), 8.02 (*d*, *J* = 9.8 *Hz*, 1H), 8.43 (*s*, 1H), 10.04 (*s*, 1H), 10.35 (*s*, 1H). ^{13}C NMR (DMSO): 109.2, 119.8, 122.7, 126.6, 127.0, 131.3, 131.6, 134.7, 138.1, 158.5, 192.4. Anal. Calcd. For. $C_{11}H_8O_2$: C, 76.73; H, 4.68; O, 18.58. Found: C, 76.69; H, 4.70.

6-[(Dimethylamino)methyl]naphthalen-2-ol (3b). To 0.250 g (1.45 × mmol) of **1** 10 mL of a solution of dimethylamine (33% in absolute ethanol) was added. The solution was kept under argon atmosphere and stirred for 20 h at rt. $NaBH_4$ (0.165 g, 4.35 mmol) was added to the solution and stirred for 3 h at rt. The solution was dried under a vacuum and water was added. The aqueous solution was extracted with $CHCl_3$ (3 × 30 mL). The organic layers were combined and dried over Na_2SO_4. Solvent was removed under reduced pressure and a white solid was obtained (0.248 g, 85% yield). Mp 154−156 °C: ^{1}H NMR ($CDCl_3$): δ 2.42 (*s*, 6H), 3.71 (*s*, 2H), 6.97 (*d*, *J* = 2.2 Hz, 1H), 7.04 (*dd*, *J* = 8.8, 2.2 Hz, 2H), 7.34 (*dd*, *J* = 8.4, 1.4 Hz, 1H), 7.41 (*d*, *J* = 8.4 Hz, 1H), 7.51 (*d*, *J* = 8.8 Hz, 1H), 7.62 (*dd*, *J* = 1.4 Hz, 1H), 7.91 (*bs*, 1H). ^{13}C NMR ($CDCl_3$): 44.4, 63.6, 109.4, 119.0, 126.5, 127.7, 128.0, 128.6, 129.2, 130.2 134.3, 155.1. Anal. Calcd. For. $C_{13}H_{15}NO$: C, 77.58; H, 7.51; N, 6.96; O, 7.95. Found: C, 77.53; H, 7.54, N, 6.98.

6-(Morpholinomethyl)naphthalen-2-ol (3c). Morpholine (0.272 g, 3.12 mmol) and **1** (0.215 g, 1.25 mmol) were mixed in dry THF (5 mL) at rt under argon atmosphere. Sodium triacetoxyborohydride (0.377 g, 1.70 mmol) was added and the mixture stirred at rt under an argon atmosphere for 24 h. The reaction mixture color became pale yellow at the end of the reaction time. The reaction mixture was quenched by adding aqueous K_2CO_3 (the solution became pale pink), and the product was extracted with Et_2O. The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography cyclohexane/ethyl acetate 1/1. The product was obtained as a white solid 0.304 g (90% yield). White solid. Mp 100−102 °C: ^{1}H NMR ($CDCl_3$): δ 2.59 (*t*, *J* = 4.3 Hz, 4H), 3.68 (*s*, 2H), 3.79 (*t*, *J* = 4.3 Hz, 4 H), 6.00 (*bs*, 1H), 6.99−7.05 (*m*, 2H), 7.40 (*dd*, *J* = 8.4, 1.6 Hz, 1H), 7.51 (*d*, *J* = 8.4 Hz, 1H), 7.61−7.65 (*m*, 2H). ^{13}C NMR ($CDCl_3$): 53.4, 63.5, 66.6, 109.4, 118.3, 126.3, 128.0, 128.2, 128.3, 129.4, 131.4 134.0, 153.9. Anal. Calcd. For. $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76; O, 13.15. Found: C, 74.10; H, 7.05, N, 5.68.

1-(6-Hydroxynaphthalen-2-yl)-*N*,*N*,*N*-trimethylmethanaminium Iodide (3d). Compound **3b** (0.137 g, 0.681 mmol) was dissolved in 5 mL of CH_2Cl_2 and 0.483 g (0.340 mmol, 0.212 mL) of CH_3I was added dropwise. The solution was stirred at rt for 5 h and a white solid precipitated. The solid was filtered and dried under a vacuum (0.190 g, 81% yield). White solid. Mp 187−189 °C: ^{1}H NMR (DMSO): δ 3.08 (*s*, 9H), 4.65 (*s*, 2H), 7.17−7.19 (*m*, 2H), 7.50 (*d*, *J* = 8.3 Hz, 1H), 7.79−7.87 (*m*, 2H), 7.98 (*s*, 1H), 10.01 (*bs*, 1H). ^{13}C NMR (DMSO): 51.7, 68.1, 108.5, 119.5, 122.3, 126.6, 127.1, 129.5, 130.0, 132.8 135.2, 156.7. Anal. Calcd. For. $C_{14}H_{18}INO$: C, 48.99; H, 5.29; I, 36.98; N, 4.08; O, 4.66. Found: C, 48.96; H, 5.31, I, 36.99; N, 4.06.

2,2′-Dihydroxy-1,1′-binaphthyl-6,6′-dicarbaldehyde (2). A 2.000 g sample of 6,6′-dibromobinol (4.50 mmol) was dissolved in 54 mL of dry THF under an argon atmosphere. The magnetically stirred solution was cooled to −78 °C and 14.4 mL of *n*-BuLi in *n*-hexane (2.5 M) (36.0 mmol) was added at such a rate in order to not allow the temperature to exceed −70 °C. After 5.5−6 h of stirring at this temperature, 2.60 mL of dry *N*,*N*-dimethylformamide (33.6 mmol) was added so that the temperature remained below −50 °C. After being stirred for 45 min at this temperature, the reaction mixture was poured into HCl/ice (pH < 1) under vigorous stirring. It was allowed to reach rt overnight. The fair yellow solid formed was filtered. The solid was dried under vacuum, washed with CH_2Cl_2 and filtered. Product **2** was obtained as a pale yellow solid (1.29 g, 84% yield).[36] Mp: 179−181 °C. ^{1}H NMR (DMSO): δ 7.16 (*d*, *J* = 8.8 Hz, 2H), 7.61 (*d*, *J* = 8.8 Hz, 2H), 7.72 (*d*, *J* = 8.8 Hz, 2H), 8.25 (*d*, *J* = 8.8 Hz, 2H), 8.63 (*s*, 2H), 10.16 (*s*, 2H), 10.17 (*s*, 2H). ^{13}C NMR (DMSO): 115.5, 119.6, 122.7, 125.1, 127.1, 131.1, 131.2, 135.2, 137.3, 156.4, 192.4. Anal. Calcd. For. $C_{22}H_{14}O_4$: C, 77.18; H, 4.12; O, 18.69. Found: C, 77.15; H, 4.15.

6,6′-Bis(hydroxymethyl)-1,1′-binaphthyl-2,2′-diol (4a). To a solution of **2** (0.500 g, 1.46 mmol) in a mixture of absolute ethanol (20.7 mL) and THF (3.46 mL) $NaBH_4$ (0.450 g, 11.8 mmol) was added at rt. After being stirred for 5 h the reaction mixture was dried under reduced pressure. The residue was taken up in 3 N HCl and vigorous stirred until all salts were dissolved and pH solution was acid. The white solid was filtered and washed with CH_2Cl_2. The solid was dried to give **4a** (0.340 g, 77% yield). White solid. Mp: 123−125 °C. ^{1}H NMR (acetone d_6): δ 4.25 (*t*, *J* = 4.1 *Hz*, 2H), 4.73 (*d*, *J* = 4.1 Hz, 4H), 7.04 (*d*, *J* = 8.6 Hz, 2H), 7.24 (*dd*, *J* = 8.7, 1.6 Hz, 2H), 7.35 (*d*, *J* = 8.7 Hz, 2H), 7.85−7.91 (*m*, 6H). ^{13}C NMR (Acetone d_6): 65.2, 115.1, 119.9, 125.8, 126.5, 127.0, 130.1, 130.7, 135.0, 138.1, 154.6. Anal. Calcd. For. $C_{22}H_{18}O_4$: C, 76.29; H, 5.24; O, 18.48. Found: C, 76.33; H, 5.22.

6,6′-Bis[(methylamino)methyl]-1,1′-binaphthyl-2,2′-diol (4b). Methylamine (6.8 mL, 33% by weight, 8 M solution in absolute ethanol, 54.4 mmol) was added under nitrogen atmosphere to 2,2′-dihydroxy-1,1′-binaphthyl-6,6′-dicarbaldehyde (**2**) (0.200 g, 5.84 mmol), the reaction mixture was stirred at rt for 24 h. Then $NaBH_4$ (0.332 g, 8.76 mmol) was added in one portion. The solution was stirred for another 3 h until the Schiff base was completely reduced to amine. The solution was concentrate under reduced pressure and the residue was mixed with 20 mL of water. The aqueous solution was extracted with $CHCl_3$ (3 × 40 mL). The combined organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the amine as pale yellow solid **4b** (0.142 g, 65% yield). Pale yellow solid. Mp > 115 °C dec. ^{1}H NMR (DMSO): δ 2.30 (*s*, 6H), 3.72 (*s*, 4H), 6.88 (*d*, *J* = 8.6 Hz, 2H), 7.15 (*d*, *J* = 8.6 Hz, 2H), 7.29 (*d*, *J* = 8.8 Hz, 2H), 7.74 (*s*, 2H), 7.80 (*d*, *J* = 8.8 Hz, 2H). ^{13}C NMR (DMSO): 35.5, 55.0, 115.5, 118.5, 124.4, 126.4, 126.6, 127.9, 128.3, 133.2, 134.2, 152.7. Anal. Calcd. For. $C_{24}H_{24}N_2O_2$: C, 77.39; H, 6.49; N, 7.52; O, 8.59. Found: C, 77.43; H, 6.47; N, 7.53.

1,1′-(2,2′-Dihydroxy-1,1′-binaphthyl-6,6′-diyl)bis(*N*,*N*-dimethylmethanaminium) Chloride (4c HCl). Dimethylamine (8 mL, 33% by weight, 8 M solution in absolute ethanol, 64.0 mmol) was added under nitrogen atmosphere to 2,2′-dihydroxy-1,1′-binaphthyl-6,6′-dicarbaldehyde (**2**) (0.200 g, 5.84 mmol), and the reaction mixture was stirred at rt for 24 h. Then $NaBH_4$ (0.332 g, 8.76 mmol) was added in one portion. The solution was stirred for another 3 h until the Schiff base was completely reduced to amine. The solution was concentrated under reduced pressure and the residue was mixed with 20 mL of water. The aqueous solution was extracted with

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$CHCl_3$ (3 × 40 mL). The combined organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The obtained reaction mixture was purified by reverse phase MPLC H_2O/MeOH 7/3 TFA 0.1% to give the amine hydrochloride as a white solid **4c HCl** (0.200 g, 72.2% yield), after treatment with a diluted solution of HCl. White solid. Mp > 138 °C dec. ^{1}H NMR (DMSO): δ 2.71 (*s*, 12H), 4.35 (*d*, J = 4.1 Hz, 4H), 6.00 (*bs*, 2H), 6.98 (*d*, J = 8.6 Hz, 2H), 7.37 (*d*, J = 8.7 Hz, 2H), 7.44 (*d*, J = 8.9 Hz, 2H), 7.90 (*d*, J = 8.9 Hz, 2H), 8.02 (*s*, 2H), 10.65 (*bs*, 2H). ^{13}C NMR (DMSO): 41.5, 59.6, 115.52 119.3, 124.1, 124.8, 127.5, 127.9, 129.1, 131.1, 134.2, 154.1. Anal. Calcd. For. $C_{26}H_{30}Cl_2N_2O_2$: C, 65.96; H, 6.39; Cl, 14.98; N, 5.92; O, 6.76. Found: C, 65.92; H, 6.43; Cl, 15.00; N, 5.90.

1,1′-(2,2′-Dihydroxy-1,1′-binaphthyl-6,6′-diyl)bis(*N*,*N*,*N*-trimethylmethanaminium) Bromide (4e). Compound **4k** (0.250 g, 0.530 mmol) was dissolved in 9 mL of dry THF. A slow stream of dry Et_3N was bubbled through at the previously stirred solution. The bubbled solution was stirred at rt for 15 min and a white solid precipitated. The obtained product was filtered under nitrogen atmosphere and dried as product **4e** (0.297 g, 95% yield). White solid. Mp > 110 °C dec. ^{1}H NMR (DMSO): δ 3.08 (*s*, 18H), 4.66 (*s*, 4H), 7.03 (*d*, J = 8.8 Hz, 2H), 7.34 (*d*, J = 8.8 Hz, 2H), 7.46 (*d*, J = 8.8 Hz, 2H), 7.99 (*d*, J = 8.8 Hz, 2H), 8.09 (*s*, 2H), 9.64 (*bs*, 2H). ^{13}C NMR (DMSO): 51.7, 67.9, 115.1, 119.4, 122.2, 124.8, 127.5, 129.4, 129.6, 133.2, 134.5, 154.4. Anal. Calcd. For. $C_{28}H_{34}Br_2N_2O_2$: C, 56.96; H, 5.80; Br, 27.07; N, 4.74; O, 5.42. Found: C, 56.92; H, 5.84; Br, 27.08; N, 4.74.

General Procedure for the Synthesis of Binolams Derivatives. The desired amino acid (2.63 mmol) and (*R*)-**2** (0.300 g, 0.876 mmol) were mixed in anhydrous ethanol (20 mL) at rt under argon atmosphere. Sodium triacetoxyborohydride (0.186 g, 2.63 mmol) was added and the mixture stirred at rt under an argon atmosphere for 24 h. The reaction mixture color became pale orange at the end of the reaction time. The reaction mixture was quenched by adding aqueous $KHCO_3$ and the product was extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography cyclohexane/ethyl acetate.

(*R*,*S*,*S*,)-Dimethyl-1,1′-(2,2′-dihydroxy-1,1′-binaphthyl-6,6′-diyl)-bis(methylene)dipyrrolidine-2-carboxylate (4f). The oily residue was purified by column chromatography (cyclohexane/ethyl acetate 6/4). White solid. (0.458 g, 92% yield). Mp 64−66 °C: ^{1}H NMR ($CDCl_3$): δ 1.80−2.0 (*m*, 6H), 2.13−2.18 (*m*, 2H), 2.43−2.46 (*m*, 2H), 3.07−3.10 (*m*, 2H), 3.28−3.33 (*m*, 2H), 3.62 (*s*, 6H), 3.70 (*d*, J = 12.8 *Hz*, 2H), 4.02 (*d*, J = 12.8 *Hz*, 2H), 7.09 (*d*, J = 8.6 *Hz*, 2H), 7.27−7.32 (*m*, 2H), 7.34−7.38 (*m*, 2H), 7.82 (*s*, 2H), 7.91−7.95 (*m*, 2H). ^{13}C NMR ($CDCl_3$): 22.9, 29.2, 51.7, 53.2, 58.4, 65.2, 111.0, 117.7, 124.1, 128.1, 128.9, 129.1, 131.0, 132.6, 133.9, 152.5, 174.4. Anal. Calcd. For. $C_{34}H_{36}N_2O_6$: C, 71.81; H, 6.38; N, 4.93; O, 16.88. Found: C, 71.85; H, 6.37; N, 4.91.

(*R*,*S*,*S*)-*tert*-Butyl 1,1′-(2,2′-dihydroxy-1,1′-binaphthyl-6,6′-diyl) bis(methylene) dipyrrolidine-2-carboxylate (4g). The oily residue was purified by column chromatography (cyclohexane/ethyl acetate 7/3). Pale orange solid (0.475 g, 83% yield). Mp: 92−94 °C: ^{1}H NMR ($CDCl_3$): δ 1.43−1.44 (*s*, 18H), 1.67−1.78 (*m*, 4H), 1.87−1.97 (*m*, 2H), 2.06−2.10 (*m*, 2H), 2.38−2.46 (*m*, 2H), 3.03−3.08 (*m*, 2H), 3.17−3.22 (*m*, 2H), 3.67 (*d*, J = 13.1 Hz, 2H), 4.10 (*d*, J = 13.1 Hz, 2H), 7.10 (*d*, J = 8.6 Hz, 2H), 7.28−7.38 (*m*, 4H), 7.83−7.85 (*m*, 2H), 7.93 (*d*, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$): 22.8, 26.8, 28.0, 29.1, 30.1, 43.4, 53.1, 58.2, 65.8, 80.4, 110.9, 117.6, 124.1, 128.0, 129.0, 129.2, 131.0, 132.6, 134.5, 152.3, 173.2. Anal. Calcd. For. $C_{40}H_{48}N_2O_6$: C, 73.59; H, 7.41; N, 4.29; O, 14.70. Found: C, 73.57; H, 7.45; N, 4.33.

(*R*,*S*,*S*)-1,1′-(2,2′-Dihydroxy-1,1′-binaphthyl-6,6′-diyl)bis(methylene)bis(2-carboxy-1-methyl pyrrolidinium) Chloride (4h HCl). The *t*-butyl amino ester (1 mmol) was deprotected by dissolving it in a solution of trifluoroacetic acid (1.94 mL, 13 mmol) and dichloromethane (10 mL) in the presence of triethylsilane (0.8 mL, 2.5 mmol) at rt. After stirring for 2 h **4g**, 1 M HCl (1 mL) was added and the solvent was removed in a vacuum. The residue was suspended in diethyl ether, and the product was isolated by filtration. The yields were almost quantitative (93%) only using triethylsilane, as the carbocation scavenger. White solid. Mp > 110 °C Dec. ^{1}H NMR (CD_3OD): δ 2.03−2.04 (*m*, 2H), 2.17−2.19 (*m*, 4H), 2.62 (m, 2H), 3.36−3.54 (*m*, 4H), 4.44 (*m*, 4H), 4.69 (*d*, J = 12.0 Hz, 2H), 7.10−7.15 (*m*, 2H), 7.32 (*m*, 2H), 7.41 (*d*, J = 8.8 Hz, 2H), 7.99 (*d*, J = 8.9 Hz, 2H), 8.07 (*s*, 2H). ^{13}C NMR (CD_3OD): 23.58, 23.63, 29.6, 55.8, 55.9, 60.2, 67.9, 116.5, 118.5, 120.7, 125.77, 125.81, 127.2, 128.78, 128.84, 130.2, 131.4, 132.54, 132.58, 136.53, 136.56, 156.0, 171.3. Anal. Calcd. For. $C_{34}H_{38}Cl_2N_2O_6$: C, 63.65; H, 5.97; Cl, 11.05; N, 4.37; O, 14.96. Found: C, 63.60; H, 5.98; Cl, 11.07; N, 4.36.

(*R*,*S*,*S*)-Dimethyl-2,2′-(2,2′-dihydroxy-1,1′-binaphthyl-6,6′-diyl)bis-(methylene)bis(azanediyl) bis(3-methylbutanoate) (4i). The oily residue was purified by column chromatography (cyclohexane: ethyl acetate 65/35). Product **4i** was isolated as pale yellow solid (0.376 g, 75% yield). Pale yellow solid. Mp: 69 − 71 °C. ^{1}H NMR ($CDCl_3$): δ 0.93−0.97 (*m*, 12H), 1.88−1.97 (*m*, 2H), 3.07 (*d*, J = 5.9 Hz, 2H), 3.70 (*d*, J = 13.2 Hz, 2H), 3.73 (*s*, 6H), 3.95 (*d*, J = 13.2 Hz, 2H), 7.09−7.13 (*m*, 2H), 7.29 (*dd*, J = 8.8 Hz, 2H), 7.36 (*d*, J = 9.0 Hz, 2H), 7.81 (*s*, 2H), 7.92 (*d*, J = 9.0 Hz, 2H). ^{13}C NMR ($CDCl_3$): 18.6, 19.2, 31.6, 51.3, 52.2, 66.4, 111.1, 117.8, 124.3, 127.1, 128.2, 129.2, 130.9, 132.6, 135.5, 152.4, 175.6. Anal. Calcd. For. $C_{34}H_{40}N_2O_6$: C, 71.31; H, 7.04; N, 4.89; O, 16.76. Found: C, 71.33; H, 7.01; N, 4.92.

(2,2′-Dihydroxy-1,1′-binaphthyl-6,6′-diyl)bis(methylene) diethanoate (4j). 0.150 g (0.581 mmol) of compound **4a** were dissolved in 7 mL of glacial acetic acid. The solution was refluxed for 48 h under nitrogen atmosphere, and after this period the solvent was removed under reduced pressure. The oily residue was purified by column chromatography (cyclohexane/ethyl acetate 7/3). Compound **4j** was obtained as colorless oil (74 mg, 40% yield). Colorless oil. ^{1}H NMR (Acetone d_6): δ 2.05 (*s*, 6H), 5.20 (*s*, 4H), 4.73 (*d*, J = 4.1 Hz, 4H), 7.09 (*d*, J = 8.7 Hz, 2H), 7.27 (*dd*, J = 8.7, 1.6 Hz, 2H), 7.39 (*d*, J = 8.9 Hz, 2H), 7.91−7.96 (*m*, 4H), 8.02 (*s*, 2H). ^{13}C NMR (Acetone d_6): 21.2, 67.0, 115.3, 120.2, 126.1, 127.8, 128.9, 130.0, 131.0, 132.2, 135.5, 155.2, 172.3. Anal. Calcd. For. $C_{26}H_{22}O_6$: C, 72.55; H, 5.15; O, 22.30. Found: C, 72.50; H, 5.17.

Photochemical Synthesis. General Procedure for the Synthesis of Naphthol Derivatives. The typical procedure for the photochemical synthesis of **3e** is as follows. A solution of naphthol derivatives (**3b**−**d**, 1×10^{-3} M) in 300 mL of CH_3CN/H_2O 3:7 in the presence of mercaptoethanol (5×10^{-2} M) was poured into Pyrex tubes, flushed with argon for 5 min, and externally irradiated by means of four 15 W phosphor-coated lamps (center of emission 310 nm) for 10−120 min in a merry-go-round apparatus. The irradiated solution was evaporated under reduced pressure, and the residue was purified by preparative HPLC. The product was obtained as a white solid.

6-((2-Hydroxyethylthio)methyl)naphthalen-2-ol (3e). White solid. Mp: 117−119 °C. ^{1}H NMR (Acetone d_6): δ 2.56 (*t*, J = 6.7 Hz, 2H), 3.65 (*t*, J = 6.7 Hz, 2H), 3.70 (*bs*, 1H), 3.88 (*s*, 2H), 7.13 (*dd*, J = 8.8, 2.4 Hz, 1H), 7.18 (*d*, J = 2.4 Hz, 1H), 7.42 (*dd*, J = 8.5, 1.7 Hz, 1H), 7.65 (*d*, J = 8.5 Hz, 1H), 7.68 (*d*, J = 1.7 Hz, 1H), 7.57 (*d*, J = 8.8 Hz, 1H), 8.59 (*bs*, 1H). ^{13}C NMR (Acetone d_6): 34.8, 37.2, 62.6, 110.2, 119.8, 127.8, 128.4, 128.9, 129.5, 130.4, 134.5, 135.4, 156.6. Anal. Calcd. For. $C_{13}H_{14}O_2S$: C, 66.64; H, 6.02; O, 13.66; S, 13.68. Found: C, 66.68; H, 6.01; S, 13.64.

General Procedure for the Synthesis of Binol Derivatives. The typical procedure for the photochemical synthesis of **5a**, **5e**, **6a**, **7**, **8** is as follows. A solution of binol derivatives (**4a**−**4j**, 1×10^{-3} M) in 300 mL of CH_3CN/H_2O in the presence of nucleophile (5×10^{-2} M) was poured into Pyrex tubes, flushed with argon for 5 min, and externally irradiated by means of two 15 W phosphor-coated lamps (emission 310 nm) for 20−60 min in a merry-go-round apparatus. The irradiated solution was evaporated under reduced pressure, and the residue was purified by chromatography on silica gel 60 HR or by reverse phase MPLC. The products were

obtained as solids or oils from the fractions (by repeating the separation with HPLC in the case of unsatisfactory separation).

6-(Hydroxymethyl)-6′-(morpholinomethyl)-1,1′-binaphthyl-2,2′-diol (6a). Compound **6a** was obtained after irradiation of **4a** at 310 nm, as described above, for 1 h. Compound **6a** was separated by silica gel chromatography and eluted with pure ethyl acetate. A pale yellow oil was isolated as product (30% yield). Pale yellow oil. ^{1}H NMR (Acetone d_6): δ 2.42 (*m*, 4H), 2.86 (*bs*, 1H), 3.59−3.62 (*m*, 6H), 4.72 (*d*, J = 5.2 Hz, 2H), 7.02−7.06 (*m*, 2H), 7.21−7.27 (*m*, 2H), 7.31−7.36 (*m*, 4H), 7.80−7.91 (*m*, 4H). ^{13}C NMR (Acetone d_6): 54.9, 64.3, 65.1, 67.8, 115.38, 115.40, 119.8, 125.8, 126.5, 127.0, 128.9, 129.1, 130.16, 130.66, 130.77, 133.9, 135.0, 135.1, 138.2, 152.2, 154.6, 154.6. Anal. Calcd. For. $C_{26}H_{25}NO_4$: C, 75.16; H, 6.06; N, 3.37; O, 15.40. Found: C, 75.19; H, 6.04; N, 3.36.

4,4′-(2,2′-Dihydroxy-1,1′-binaphthyl-6,6′-diyl)bis(methylene)dimorpholin-4-ium chloride (4d HCl). Compound **4d** was obtained after irradiation of **4e** at 310 nm, as described above, for 1 h 45 min. Compound **4d** was separated by silica gel chromatography and eluted with pure ethyl acetate, followed by HPLC purification: T = 8.43 s. Flow 1.40 mL/min; gradient elution: eluent A% MeCN, eluent B% H_2O 0.1% TFA: 0 min (5/95), 3 min (5/95), 13 min (100/0), 16 min (100/0), 22 min (5/95). After HPLC elution, trifluoroacetate ion was exchanged with chloride ion: the solid was dissolved in 3 mL of HCl 1 M and dried under reduced pressure. Compound **4d HCl** was isolated as pale orange solid (60% yield). Pale orange solid. Mp > 200 dec ^{1}H NMR (D_2O): δ 3.18−3.21 (*m*, 4H), 3.34−3.38 (*m*, 4H), 3.64−3.72 (*m*, 4H), 3.99−4.03 (*m*, 4H), 4.40 (*s*, 4H), 7.06 (*d*, J = 8.7 Hz, 2H), 7.20 (*d*, J = 8.7 Hz, 2H), 7.40 (*d*, J = 8.7 Hz, 2H), 8.01−8.04 (*m*, 4H). ^{13}C NMR (D_2O): 51.0, 60.6, 63.5, 114.5, 118.9, 122.8, 124.7, 125.1, 128.0, 128.4, 130.6, 131.9, 134.1, 152.9. Anal. Calcd. For. $C_{30}H_{34}Cl_2N_2O_4$: C, 64.63; H, 6.15; Cl, 12.72; N, 5.02; O, 11.48. Found: C, 64.61; H, 6.16; Cl, 12.75; N, 4.99.

6,6′-Bis[(2-hydroxyethylthio)methyl]-1,1′-binaphthyl-2,2′-diol (7). Compound **7** was obtained after irradiation of **4e** at 310 nm, as described above, for 45 min. Compound **7** was separated by MPLC reverse phase chromatography and eluted with H_2O/MeOH 6: 4 TFA 0.1%. Compound **7** was separated as white solid (73.6% yield). White solid. Mp: 100−102 °C. ^{1}H NMR (CD_3OD): δ 2.66 (*t*, J = 6.9 Hz, 4H), 3.74 (*t*, J = 6.9 Hz, 4H), 3.96 (*s*, 4H), 7.11 (*d*, J = 8.7 Hz, 2H), 7.33 (*dd*, J = 8.7, 1.7 Hz, 2H), 7.40 (*d*, J = 8.9 Hz, 2H), 7.85 (*d*, J = 1.7 Hz, 2H), 7.95 (*d*, J = 8.9 Hz, 2H). ^{13}C NMR (CD_3OD): 34.5, 37.3, 62.6, 116.6, 119.8, 126.6, 128.8, 129.0, 130.4, 130.6, 134.4, 135.2, 154.5. Anal. Calcd. For. $C_{26}H_{26}O_4S_2$: C, 66.92; H, 5.62; O, 13.72; S, 13.74. Found: C, 66.90; H, 5.64; S, 13.75.

6-[(2-Hydroxyethylthio)methyl]-6′-methyl-1,1′-binaphthyl-2,2′-diol (8). Compound **8** was obtained after irradiation of **4a** at 310 nm, as described above, for 2 h. Compound **8** was separated by silica gel chromatography and eluted with $CHCl_3$/MeOH 99/1 and followed by HPLC purification: T = 11.48 s. Flow 1.40 mL/min; gradient elution: eluent A% MeOH, eluent B% H_2O: 0 min (50/50), 3 min (50/50), 13 min (100/0), 16 min (100/0), 21 min (50/50). Compound **8** was separated as white solid (10% yield). Pale green oil. ^{1}H NMR (acetone d_6): δ 2.43 (*s*, 3H), 2.59 (*t*, J = 6.9 Hz, 2H), 3.68 (*t*, J = 6.9 Hz, 2H), 3.90 (*s*, 4H), 6.96−7.05 (*m*, 2H), 7.10 (*dd*, J = 8.6, 1.7 Hz, 1H), 7.26 (*dd*, J = 8.7, 1.8 Hz, 1H), 7.30−7.36 (*m*, 2H), 7.66 (*d*, J = 1.8 Hz, 1H), 7.81−7.90 (*m*, 3H). ^{13}C NMR (acetone d_6): 21.6, 34.9, 37.0, 62.4, 115.1, 119.7, 120.0, 125.8, 126.2, 128.3, 128.8, 129.6, 130.1, 130.3, 130.6, 133.2, 134.0, 134.4, 134.9, 154.1, 154.7. Anal. Calcd. For. $C_{24}H_{22}O_3S$: C, 73.82; H, 5.68; O, 12.29; S, 8.21. Found: C, 73.79; H, 5.65; S, 8.25.

6-[(2-Hydroxyethylthio)methyl]-6′-(hydroxymethyl)-1,1′-binaphthyl-2,2′-diol (5a). Compound **5a** was obtained after irradiation of **4a** at 310 nm, as described above, for 1 h. Compound **5a** was purified by reverse phase MPLC and eluted with H_2O/MeOH 4: 6 TFA 0.1%. Compound **5a** was separated as colorless oil (53.5% yield). ^{1}H NMR (Acetone d_6): δ 2.58 (*t*, J = 6.8 Hz, 2H), 3.68 (*t*, J = 6.8 Hz, 2H), 3.90 (*s*, 2H), 7.04 (*d*, J = 8.6 Hz, 2H), 7.23−7.28 (*m*, 2H), 7.35 (*dd*, J = 8.9, 1.9 Hz, 2H), 7.81−7.91 (*m*, 4H). ^{13}C NMR (Acetone d_6): 34.8, 37.0, 62.4, 65.0, 115.3, 115.5, 119.8, 120.0, 125.8, 126.2, 126.5, 127.0, 128.8, 130.0, 130.1, 130.6, 130.8, 134.4, 134.9, 135.0, 138.2, 154.5, 154.7. Anal. Calcd. For. $C_{24}H_{22}O_4S$: C, 70.91; H, 5.46; O, 15.74; S, 7.89. Found: C, 70.94; H, 5.42; S, 7.93.

[2,2′-Dihydroxy-6′-(2-hydroxy-ethylsulfanylmethyl)-[1,1′]binaphthalenyl-6-ylmethyl]-trimethyl-ammonium (5e). Compound **5e** was obtained after irradiation of **4e** at 310 nm, as described above, for 20 min. Compound **5e** was purified by reverse phase MPLC and eluted with H_2O/MeOH 7: 3 TFA 0.1%. Compound **5e** was separated as pale orange oil (53.5% yield). ^{1}H NMR (CD_3OD): δ 2.68 (*bs*, 2H), 3.20 (*s*, 9H), 3.74 (*t*, J = 6.8 Hz, 2H), 3.96 (*bs*, 2H), 4.68 (s, 2H), 7.09 (*d*, J = 8.6 Hz, 1H), 7.26−7.41 (*m*, 4H), 7.50 (*d*, J = 8.6 Hz, 1H), 7.84 (*s*, 1H) 7.95 (*d*, J = 8.0 Hz, 1H), 8.09 (*d*, J = 8.6 Hz, 1H), 8.16 (*s*, 1H). ^{13}C NMR (CD_3OD): 34.5, 37.2, 62.6, 71.3, 116.0, 117.0, 119.8, 120.9, 123.2, 126.3, 127.2, 128.9, 129.1, 130.1, 130.4, 130.9, 131.4, 135.0, 137.0, 154.7, 156.3. Anal. Calcd. For. $C_{27}H_{30}ClNO_3S$: C, 66.99; H, 6.25; Cl, 7.32; N, 2.89; O, 9.92; S, 6.62. Found: C, 67.02; H, 6.22; Cl, 7.35; N, 2.85; S, 6.59.

Cross-Linking of Plasmid DNA. Compounds **4a**, **4b**, **4e**−**4i** were dissolved in DMSO to a final concentration of 20 mM. These stock solutions were diluted in mQ-grade H_2O and used for reactions. Plasmid pBR322 (0.5 μg/sample) was mixed with increasing amounts (0.2−40 μM, 2-fold serial dilutions, as indicated in each figure) of each compound, in phosphate buffer (50 mM, pH 7.5). A saturated water solution of PS was used to get a 0.5 μM concentration of drug in the control samples. Reaction mixtures were irradiated on ice for 20 min at 360 nm at 120 W in a photochemical multirays reactor (Helios Italquartz, Italy). Irradiated solutions were added to alkaline agarose gel loading buffer [50 mM NaOH, 1 mM ethylenediaminetetraacetic acid (EDTA), 3% Ficoll, and 0.02% bromophenol blue] and loaded on a 1% alkaline agarose gel containing 50 mM NaOH and 1 mM EDTA. Gels were run in 50 mM NaOH and 1 mM EDTA at 12 V for 15−16 h, stained with ethidium bromide (0.5 μg/mL) for 10 min, and subsequently washed in water for 10 min. Stained gels were visualized in Gel-Doc 1000 (Bio-Rad, Italy), and DNA bands were quantified by Quantity One software (Bio-Rad).

Reaction with Oligonucleotides. The ability of binol derivatives to damage single-stranded DNA was tested at the sequencing level using a short oligonucleotide whose sequence is 5′-AAC AGC CTC AGC AGC CGG TTA-3′ (prF ACCM). The oligonucleotide was obtained in desalted and lyophilized form and was gel purified before use. It was then 5′-end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase and purified by Micro-Spin G-25 desalting columns (Amersham Biosciences, Europe). For reactions with ds-DNA, the labeled oligonucleotide prF ACCM was annealed to equimolar amounts of its complementary cold oligonucleotide (prF ACCM comp), whose sequence is 5′-TAA CCG GCT GCT GAG GCT GTT-3′. Drug reactions with the labeled ss- or ds-oligonucleotides (4 pmol/sample) in 50 mM phosphate buffer, pH 7.4, were irradiated on ice for 20 min at 360 nm at 120 W in a photochemical multirays reactor (Helios Italquartz, Italy). Samples were immediately ethanol precipitated to eliminate nonreacted drug, then resuspended and split into two aliquots, one of which was kept on ice, while the other was treated at 90 °C for 30 min with 1 M piperidine to complete strand scission according to the Maxam and Gilbert protocol.[28] Samples were finally lyophilized twice, resuspended in formamide gel loading buffer, heated at 90 °C for 5 min and cleavage products were resolved in 20% denaturing polyacrylamide gels. Gels were visualized by phosphorimaging analysis (Molecular Dynamics, Amersham Biosciences Europe). Quantification was performed by Image-Quant software (Molecular Dynamics).

HPLC-MS and MS/MS. The following reaction mixtures were prepared to measure reactivity of **4e** with nucleosides: (i) **4e** 5 mM

and nucleosides 8.9 mM in phosphate buffer 50 mM, pH 7.4; (ii) **4e** 5 mM in phosphate buffer 50 mM, pH 7.4; (iii) nucleosides 8.9 mM in phosphate buffer 50 mM, pH 7.4. Each mixture was irradiated on ice for 30 min at 360 nm, filtrated with 0.45 mm filters (Sartorius Stedim Biotech, Goettingen, Germany), and loaded on a HPLC C18 reverse phase column (Eclipse XDB C18 column, Agilent Technologies, Milan, Italy). The HPLC system consisted of an Agilent 1200 series liquid chromatography, equipped with a binary pump, standard autosampler, thermostatted column compartment, diode-array detector, and ChemStation software (Agilent Technologies). To separate the adducts solvents A (CH_3CN) and B (H_2O/0.05% TFA) were used, delivered at a flow rate of 1 mL/min with the following gradient: A from 10% to 48.5% in 15 min; A from 48.5% to 10% in 1 min; A 10% for 4 min (postrun equilibration time). Peaks were detected at 260 nm, collected in Eppendorf tubes, dried at rt in Speed Vac UniVapo 100 H (UniEquip, Martinsried, Germany), and stored at 4 °C if necessary. Positive ion mass spectra were acquired on a time-of-flight (TOF) mass analyzer (Mariner ESI-TOF, Applied Biosystems, CA) by directly injecting 10 μL of analyte solutions in CH_3CN/H_2O/HCOOH (50:49.5:0.5) at rt and with a 0.16 μL/min flow rate. The nozzle temperature was 140 °C, while a constant flow of N_2 gas was kept at 0.35 L min^{-1} to facilitate the spray. A three-point external calibration provided typical 100 ppm accuracy.

Photocytotoxicity and Cytotoxicity Assays. Human lung adenocarcinoma cells A549 and human colorectal adenocarcinoma cells HT-29 were obtained from ATCC. A549 and HT29 cells were grown as monolayers in Dulbecco's Modified Eagle Medium (D-MEM) (Invitrogen, Italy) with 10% fetal bovine serum (FBS) supplemented with penicillin (100 units/mL) and streptomycin (100 μg/mL) in a humidified atmosphere with 5% CO_2 at 37 °C. Cytotoxic effects on tumor cell growth were determined by a MTT assay. QM compounds and TMP were dissolved and diluted into working concentrations with DMSO. Cells (1.75×10^4 cells/well) were plated onto 96-microwell plates to a final volume of 100 μL and allowed an overnight period for attachment. At day 1, 1 μL of each dilution of tested compounds was added per well to get a 1% final concentration of drug solvent per well; at day 2, medium was removed, cells were washed with phosphate-buffered saline (PBS), and fresh medium was added. Immediately after, QM- and TMP-treated cells were irradiated for 20 min at 360 nm in a photochemical multirays reactor and incubated at 37 °C for an additional 24 h. Control cells (without any compound but with 1% drug solvent) were treated under the exact same conditions. Cell survival was evaluated by an MTT assay: 10 μL of freshly dissolved solution of MTT (5 mg/mL in PBS) was added to each well, and after 4 h of incubation, 100 μL of solubilization solution [10% sodium dodecyl sulfate (SDS) and 0.01 M HCl] was added. After overnight incubation at 37 °C, absorbance was read at 540 nm. Data were expressed as mean values of three individual experiments conducted in triplicate. The percentage of cell survival was calculated as follows: cell survival $= (A_{well} - A_{blank})/(A_{control} - A_{blank}) \times 100$, where blank denotes the medium without cells.

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Supporting Information Available: General procedure and materials, together with ^1H NMR and ^{13}C NMR, LC/MS, and DEPT for the following 2-naphthol and binol derivatives: **1**−**2**, **3a**−**f**, **4a**−**k**, **5a**, **5e**, **6a**, **7**, **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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