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Conjugation, Substituent, and Solvent Effects on the Photogeneration of Quinone Methides

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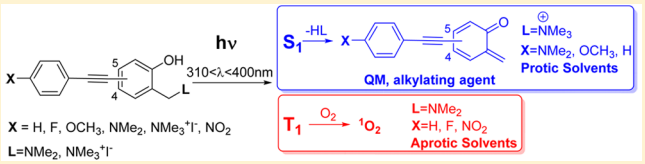
Supporting Information

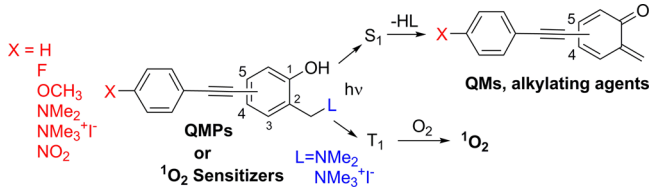
ABSTRACT: 4- and 5-arylethynyl water-soluble Mannich bases and related quaternary ammonium salts were synthesized and investigated as a model of conjugated quinone methide precursors (QMPs) by UV−vis light activation. Preparative photohydration and trapping reactions by thiols were studied, together with the detection of both transient QMs and competing QMP lowest triplet excited states (T_1), by laser flash photolysis. The efficiency of the arylethynyl derivatives as QMPs was remarkably affected by structural features (i.e., conjugating arylethynyl moieties, substituents, and leaving groups) and protic vs aprotic solvation. Our collective data clarify the dichotomy in the photoreactivity of conjugated Mannich bases and related quaternary ammonium salts as alkylating agents and singlet oxygen sensitizers.



INTRODUCTION

Quinone methides (QMs) are reactive carbon electrophiles, frequently involved in chemical and biological processes, targeting amino acids,[1,2] proteins,[3,4] and nucleic acids,[5−12] which are generated from stable and suitable precursors (QMPs), upon activation. Consequently, several strategies have been successfully developed for biocompatible generation of QMs,[13] including tautomerization,[14] oxidation,[15−18] reduction,[19] acid or base catalysis, and photolysis.[20−25] Concerning recent biological applications, a mild QMs generation was exploited to achieve bioorthogonal ligations, which is very useful in the labeling of biomolecules in living systems.[26,27] Photogeneration of QMs has been a thoroughly investigated area, as (i) it can be performed under very mild conditions in the absence of activating reactants and (ii) the QMs can be spectroscopically detected and kinetically characterized by transient absorption techniques such as laser flash photolysis (LFP). According to current literature, QM-photogeneration occurs from the lowest singlet excited state (S_1) of the QMP, by excited-state proton transfer (ESPT) to the solvent or excited state intramolecular proton transfer (ESIPT).[28−30] The generation of QMs by ESIPT from Mannich bases is a pH-dependent process, being very efficient under aqueous solution when the QMPs are in their dipolar form.[31] This evidence corroborates the idea that Mannich bases are effective QMPs under physiological conditions as, exhibiting pK_a values lower than 8.5, they frequently exist in their zwitterionic forms.[31] Several groups have been involved in the photochemical generation of QMs from Mannich bases exhibiting potential biological applications as alkylating and cross-linking agents over the past decade. The resulting photoinduced antiproliferative activity was reported by us for naphthol,[7,32] 1,1′-bi-2-naphthol (BINOL),[7,33,34] and bis-pirydyl derivatives.[8] More recently, the photogeneration and detection of QMs from 2-hydroxy-3-(diphenylhydroxymethyl)-anthracenanthrol precursors was achieved by Basaric irradiating at 350 nm, addressing the issue of the QM photogeneration at longer wavelength.[20] Concerning this aspect, it is important to keep in mind that the exploitation of QM photogeneration and reactivity within living cells or tissue requires the development of QMPs absorbing visible light ($\lambda \geq 400$ nm), in order to limit potential direct or sensitized photodamages with the biological matrix. In fact, it is well-known that UV irradiation triggers competitive ds (double-stranded) DNA thymine-thymine cross-linking.[35] Electronic conjugation is the most straightforward strategy to reach an effective red shift of the absorbing QMP. Unfortunately, π-conjugation might also lower the energy of the lowest triplet state (T_1), increasing the efficiency of the competing intersystem crossing (ISC). In this context, it is not clear how the effect of conjugation, substituents, and solvent may affect the efficiency of the S_1 population, which directly translates into QM generation efficiency. In order to study the combined effect of electronic conjugation, substituents, and aqueous solvation on QM generation, we synthesized several water-soluble 4- and 5-arylethynyl Mannich bases as models of photoreactive and electronically conjugated QMPs (Scheme 1) by Sonogashira cross-coupling reactions. Preparative photohydrations and

Scheme 1



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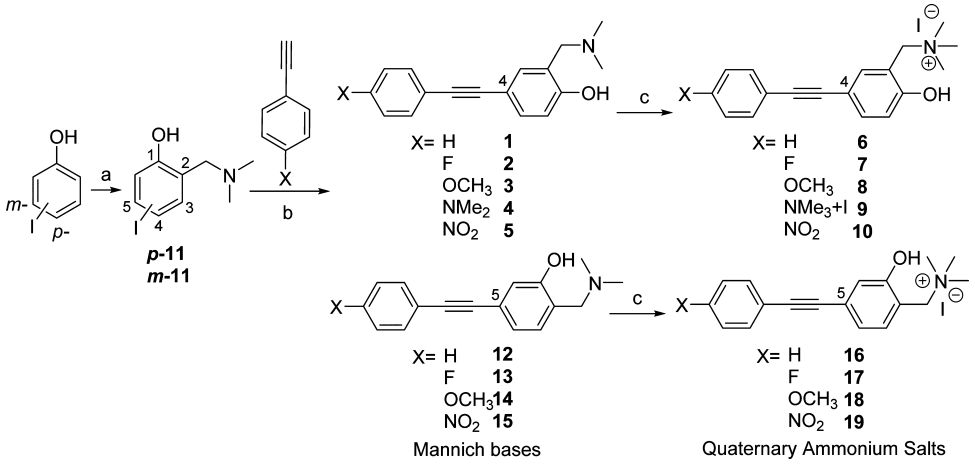
Scheme 2. Synthesis and Structures of 4- and 5-Arylethynyl Mannich Bases and Related Quaternary Ammonium Salts[a]



[a](a) EtOH abs, *para*-formaldehyde (PFA), $NHMe_2$ in EtOH (33%), reflux; (b) $Pd(PPh_3)_4$ (3%), CuI (17%), TEA, rt, 2.5 h (yields: 70−94%); (c) CH_3I, ACN, rt 24 h (yields: 95−99%).

detection of both the transient QMs and the competing QMP lowest T_1 have been investigated by LFP experiments, as a function of the QMP structure (conjugating arylethynyl on C4 or C5 and X substituents), leaving group (L), and protic vs aprotic conditions. Our collective data clarify the photoreactivity dichotomy of conjugated Mannich bases as both alkylating agents and singlet oxygen sensitizers in aqueous solution, improving our general understanding of the photocytotoxic effects associated with these compounds.

RESULTS AND DISCUSSION

Synthesis of Substituted Arylethynyl Mannich Bases. Synthesis of the 4-arylethynyl Mannich bases **1**−**5** and their ammonium salts **6**−**10** (Scheme 2) was accomplished following a three steps protocol. We started from the commercially available *p*-iodophenol, which was converted to its Mannich base ***p*-11** in good yield, via a published procedure.[36] The following Sonogashira cross-coupling was carried out on ***p*-11** in the presence of a moderate excess (1.2:1) of commercially available 1-ethynyl-4-substituted benzenes (Scheme 2) in neat trimethylamine (TEA) with $Pd(PPh_3)_4$ (3% mol) and CuI (17% mol). These reaction conditions provided **1**−**5** in good yields (70−94%). Methylation of the resulting Mannich bases in ACN (acetonitrile) at rt quantitatively yielded the quaternary ammonium salts **6**−**10**. Similarly, the synthesis of isomeric 5-arylethynyl Mannich bases (**12**−**15**) and their quaternary ammonium salts **16**-**19** was achieved starting from *m*-iodophenol (***m*-11**, Scheme 2).

UV−vis Absorption and Photoreactivity. The absorption spectra of the arylethynyl derivatives are affected by the substituent X, with bands centered from 285 nm (**13**) to 348 nm (**10**) in aqueous ACN. The arylethynyl derivatives most bathochromically shifted are the yellow nitro-derivatives (**5**, **10**, **15**, and **19**), which exhibit an absorption tail up to 450 nm (Tables 1 and 2). Particularly interesting for the absorption properties is the conjugate base of the acidic quaternary ammonium **10** (pK_a = 7.53 ± 0.05), which exhibits a λ_{max} = 405 nm and a 100 nm red-shifted absorption tail (Figure S1).

Photohydrolysis reactions were investigated by irradiation at 310 nm (with four 15 W lamps) in ACN:H_2O = 1:1 and in buffered solutions at pH 7.4 (phosphate buffer, without organic cosolvent) in the presence of molecular oxygen. The products

Table 1. Absorption (λ_{max}, in ACN:H_2O = 1:1) of 4-Arylethynyl Mannich Bases (1−5) and Their Quaternary Ammonium Salts (6−10)

X	Mannich base	λ_{max} (nm)	quaternary ammonium	λ_{max} (nm)
H	**1**	308	**6**	292
F	**2**	305	**7**	285, 302
OCH_3	**3**	313	**8**	320
NMe_2	**4**	326	–	–
NMe_3^+	–	–	**9**	296, 308
NO_2	**5**	335	**10**	348[a]

[a]The conjugate base exhibits maximum absorption at 405 nm in aqueous solution (see Figure S1).

Table 2. Absorption (λ_{max}, in ACN:H_2O = 1:1) of the 5-Arylethynyl Mannich Bases (12−15) and Their Quaternary Ammonium Salts (16−19)

X	Mannich base	λ_{max} (nm)	quaternary ammonium	λ_{max} (nm)
H	**12**	289	**16**	290
F	**13**	285	**17**	285, 301
OCH_3	**14**	317	**18**	320
NO_2	**15**	340	**19**	330

were isolated by preparative chromatography, measuring the conversion by HLPC. We also measured the efficiency of the photohydration (Φ_R: quantum yield) for **1**, **6**, **12**, and **16** by ferrioxalate actinometry.[37] Although the irradiation of **1** and **6** (10^{-4} M) gave clean conversions to product **20** (Scheme 3), the quaternary ammonium salt (**6**) was a much more efficient QMP than its Mannich base (**1**) (86% reaction yield, Φ_R = 0.48 ± 0.02 vs 35% yield, Φ_R = 0.06 ± 0.01; the reaction yields were measured after 5 min of irradiation). In buffered solution the photoreactivity of both compounds was very similar, yielding identical photoproducts with a slightly lower efficiency for the precursor **6** (Φ_R = 0.30 ± 0.08). Conversely, **1** exhibited a higher Φ_R in buffer than in aqueous ACN (Φ_R = 0.08 ± 0.01).

Similarly, the irradiation of **16** gave an almost quantitative conversion to product **21**, with a lower efficiency (Φ_R = 0.25 ± 0.01 in aqueous ACN; Φ_R = 0.06 ± 0.01 in water at pH 7.4) than **6**. The related Mannich base **12** gave the photohydration product **21** in both very low reaction yield (10%) and efficiency (Φ_R = 0.02 ± 0.01 in aqueous ACN; Φ_R = 0.04 ± 0.01 in water at pH

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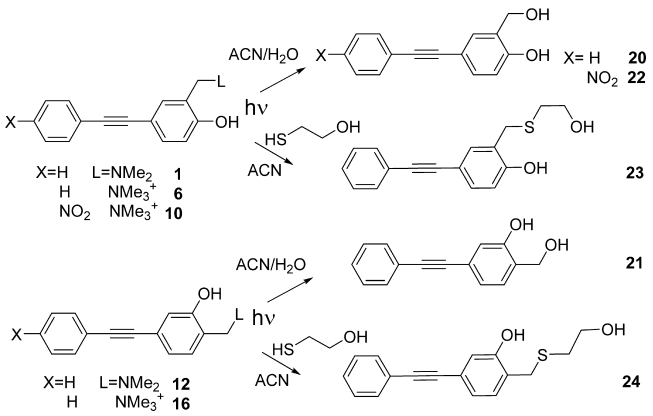
Scheme 3. QMP Photohydrolysis and QM Trapping Products by 2-Mercaptoethan-1-ol



7.4). The nitro derivative **5** was recovered unreacted after 2 h of irradiation (at 360 nm, with four 15 W lamps, in ACN:H_2O = 1:1). Contrary to **5**, the quaternary ammonium salt **10** gave the photoproduct **22** (Scheme 3) with a good chemical yield (85%, after 7.5 h irradiation time) and very low efficiency ($\Phi_R = 7 \pm 1 \times 10^{-4}$, measured at 360 nm). **10** was also reactive using visible light at a longer wavelength ($\lambda > 400$ nm) under buffered conditions (pH 7.6), where the reactive QMP was the zwitterionic conjugate base of **10**. The Mannich base of the isomeric 5-alkynyl derivatives **15** was unreactive at 310 nm under prolonged irradiation (5 h).

The general photoreactivity was further investigated in the presence of thiols, which are a well-known efficient QM trap. Thus, thioethers **23** and **24** were isolated after irradiation of **6** and **16**, respectively, in ACN:H_2O = 9:1 and in the presence of freshly distilled 2-mercaptoethan-1-ol.

Generation, Detection, and Assignment of the Transient Species by LFP. To probe for QMs and additional competitive transients, such as the lowest triplet excited state (T_1), involved in the photochemistry and photophysics of compounds **1**−**19** (Scheme 2), we performed LFP measurements. 1×10^{-4} M ACN and aqueous ACN (1:1) solutions of the potential QMP **1**−**19** were irradiated by use of a Nd:YAG laser at both 266 and 354 nm. The longer excitation wavelength was used for the pale-yellow-colored nitro-derivatives (**5**, **10**, **15**, and **19**). The measurements were performed in Ar- and O_2-purged ACN, aqueous ACN, and buffered water solution (pH 7.4 for **1** and **10**). In Ar-purged ACN solutions of all derivatives, we observed a transient absorbing with a maximum at 430−600 nm (as a function of the substituent X, Table 3), which was effectively quenched by O_2. Based on the quenching by O_2 and similarity with the published spectra of phenol and naphthol triplets,[29] we assigned the observed transients to triplet−triplet (T-T) absorptions.

Irradiation of the Unsubstituted QMPs (X = H): Effects of the Arylethynyl Delocalization, Leaving Group (NMe_2 vs NMe_3^+), and Water on QM Generation. Besides the fast decaying triplet in the transient spectra of **1** obtained in Ar-purged ACN solution (λ_{max} 435 nm, Figure 1a, red line), we observed the parallel generation of an intense absorption with a maximum at 365 nm (Figure 1a, blue line), which was stable within 400 μs time scale (data not shown).

The second transient was not affected by O_2, but it was efficiently quenched by 2-mercaptoethan-1-ol with a second-order rate constant of $k_2 = 1.18 \pm 0.02 \times 10^5$ M^{-1} s^{-1} in 1:1 aqueous ACN. HPLC product distribution analysis of the LFP irradiated solution in the presence of thiol (after 50 pulses) revealed the formation of the adduct **23** (Scheme 3). This evidence, undoubtedly allowed the assignment of the transient at 365 nm to the quinone metide **QM1** (Table 4), suggesting the role of **1** as QMP.

The transient spectra of the isomeric **12** exhibited a much more intense absorption with maxima at 410 and 430 nm (Figure 1b), which was assigned to the T_1, due to the efficient quenching by O_2. A second residual absorption centered at 350 nm was detected as well, but it was too weak to be further investigated in the presence of trapping nucleophiles.

We ran the very same LFP irradiation in aqueous ACN (1:1) and in buffered water solution (pH 7.4). The protic solvent (both with and without the organic cosolvent) remarkably reduces the efficiency of **1** and **12** triplet generation (absorbing at 435 and 410−430 nm; Figure S2a and S2b, respectively), leaving the efficiency of **QM1** generation (at 380 nm) substantially unaffected. Moreover, we recorded negligible differences between the transient detected in aqueous ACN and buffered water solution (pH 7.4; Figure S3)

The quaternary ammonium salt **6** generated an identical absorption at 380 nm (Figure S4), but the intensity of the signal ascribed to **QM1** was roughly 5 times more intense, confirming that **6** is a more efficient QMP than its Mannich base. **QM1** was less stable in aqueous ACN than in neat ACN solution. Therefore, we were able to measure an observed rate constant $k_{obs} = 5.9 \pm 0.1 \times 10^2$ s^{-1}. The QM generated from **12** (**QM12**, Table 4b) became detectable as a stable species within 100 μs, exhibiting a maximum absorption centered at 350 nm in aqueous

Table 3. T-T Absorption Maxima (λ_{max}, in ACN) of 4- and 5-Arylethynyl Mannich Bases and Their Quaternary Ammonium Salts (in ACN:H_2O = 1:1)

	X	Mannich base	λ_{max} (nm)	quaternary ammonium	λ_{max} (nm)
4-arylethynyl	H	**1**	435	**6**	430
	F	**2**	440	**7**	440
	OCH_3	**3**	450, 480	**8**	nd
	NMe_2	**4**	545	–	–
	NMe_3^+	–	–	**9**	440
	NO_2	**5**	600	**10**	560−580
5-arylethynyl	H	**12**	410, 430	**16**	330
	F	**13**	400−430	**17**	420
	OCH_3	**14**	440	**18**	460
	NO_2	**15**	590	**19**	550 broad

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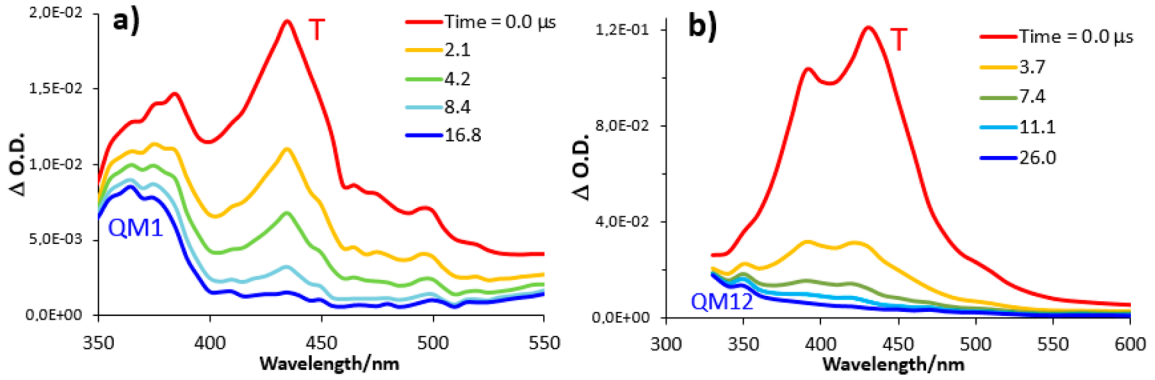

Figure 1. Transient absorption spectra of Ar-purged ACN solutions of (a) **1** and (b) **12** (both 10^{-4} M) irradiated at 266 nm by LFP.

Table 4. Structures and Absorption Maxima (λ_{max}) of (a) 4- and (b) 5-Arylethynyl Quinone Metides (QMs) Photogenerated Irradiating the QMPs 1−10 and 12−19 at 266 and 354 nm in ACN and 1:1 Aqueous ACN

	X	QM	λ_{max}/nm		k_{obs} (H_2O)/s^{-1}	k_2 (RSH)/$M^{-1}s^{-1}$
			ACN	ACN:H_2O=1:1	ACN:H_2O=1:1	
(a)	H	**QM1**	365, 375	380	$5.9 \pm 0.1 \times 10^2$	$1.18 \pm 0.02 \times 10^5$
	F	**QM2**	370, 530, 620	380, 540, 630	$3.5 \pm 0.2 \times 10^2$	-
	OCH_3	**QM3**	390	410	$2.6 \pm 0.2 \times 10^2$	$1.29 \pm 0.1 \times 10^3$
	NMe_2	**QM4**	425, 475	440, 485	$2.0 \pm 0.3 \times 10^2$	$7.0 \pm 0.2 \times 10^2$
	NO_2	**QM5**	420	420	$3.4 \pm 0.08 \times 10^4$	$1.45 \pm 0.1 \times 10^5$
	NMe_3^+	**QM9**	350, 380	340, 350	$1.8 \pm 0.05 \times 10^3$	-
(b)	H	**QM12**	350	350	9.8 ± 0.1	-
	F	**QM13**	350	350	$6.6 \pm 0.3 \times 10^2$	$7.7 \pm 0.1 \times 10^2$
	OCH_3	**QM14**	370	360	53.1 ± 0.4	-
	NO_2	**QM15**	370	400	$4.4 \pm 0.08 \times 10^4$	$3.5 \pm 0.1 \times 10^4$

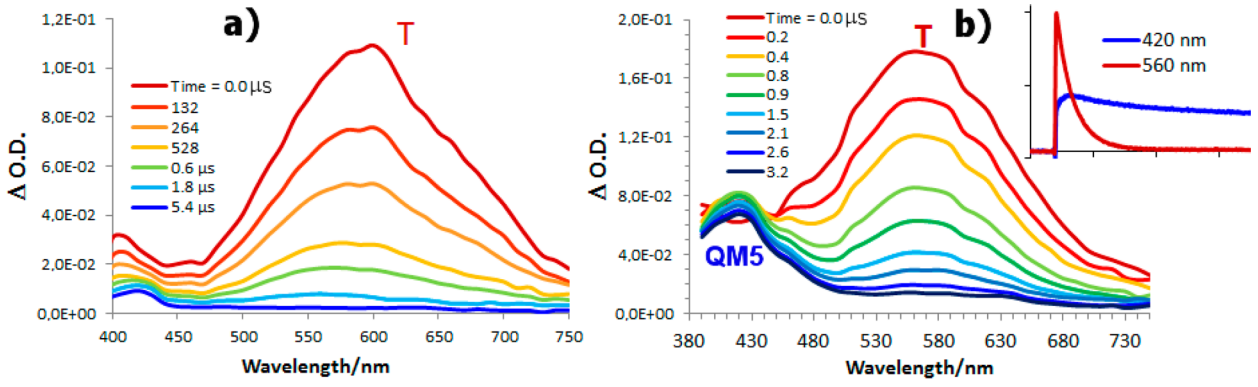

Figure 2. Transient absorption spectra of Ar-purged ACN:H_2O = 1:1 solutions of (a) **5** and (b) **10** (both 1×10^{-4} M) irradiated at 354 nm. Inset: decay traces monitored at 420 and 560 nm.

ACN. The quaternary ammonium salt **16** generated a similar spectrum (data not shown) with an intensity of the signal ascribed to **QM12** immediately after a laser pulse roughly 3.8 times more intense, suggesting that **16** is a more efficient QMP than its Mannich base. In addition, the observed hydration rate [k_{obs}(H_2O)] in Table 4 describes a much higher electrophilicity of **QM1** in comparison to **QM12** in aqueous ACN.

For both the 4- and 5-substituted QMPs, the water effect on the efficiency of the T_1 generation was remarkable. In fact, the reduced intensity of the T-T absorption after the laser pulse suggests a depletion of the T_1 population caused by water. On the contrary, we never recorded a weaker absorbance of the QM generation after the laser pulse under protic conditions.

Irradiation of Mannich Bases vs Quaternary Ammonium Salts for Electron-Poor QMPs (X = NO_2): Effect of the Leaving Group on QM Generation. In the previous paragraph, we described the quaternary ammonium effects on the prototypes 4- and 5-arylethynyl QMP (**1**, **6**, **12**, and **16**),

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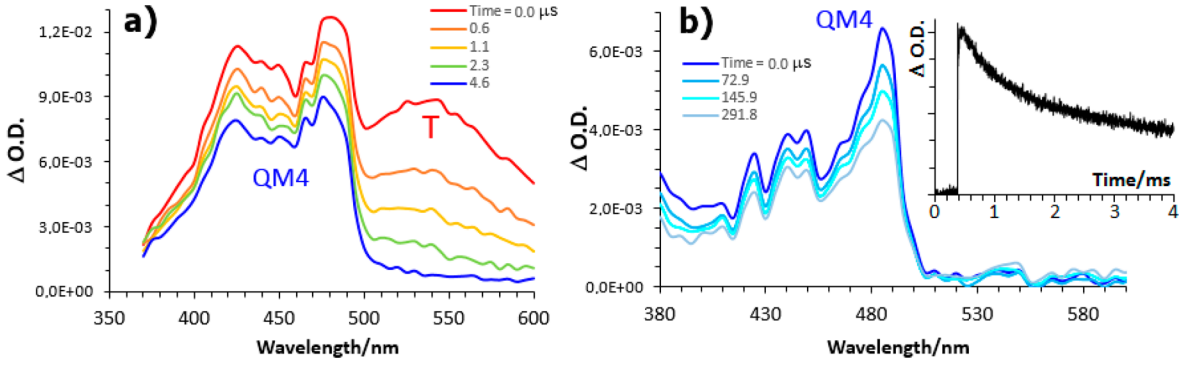

Figure 3. Transient absorption spectra of Ar-purged solutions of **4** in (a) neat ACN and (b) ACN:H_2O 1:1 (both 1×10^{-4} M). Inset: decay trace monitored at 490 nm in ACN:H_2O = 1:1.

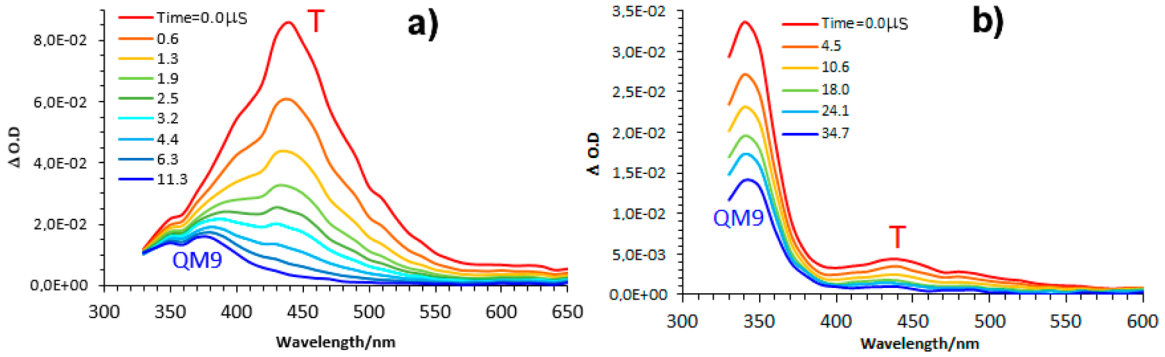

Figure 4. Transient absorption spectra of Ar-purged solutions of **9** 1×10^{-4} M in (a) neat ACN and (b) ACN-H_2O 1:1.

which enhanced the QM generation efficiency in aqueous ACN (1:1) and in buffered water. The role of quaternary ammonium salt in QMP containing the strong electron-withdrawing moiety NO_2 is even more remarkable. In fact, both Mannich bases **5** and **15** generate a strongly absorbing transient ascribed to a T-T transition (Figure 2a for **5**). Beside the T_1, we detected an additional weak absorbing species at 420 nm (Figure 2a) irradiating **5** at 354 nm in aqueous ACN. The very same transient became 10 times more intense irradiating the quaternary ammonium salt **10** (Figure 2b). The new species was assigned to **QM5** (Table 4) based on the position of the absorption maximum, the lack of influence of O_2, and the quenching effect of 2-mercaptoethan-1-ol (Figure S5). Surprisingly, the decay trace monitored at 420 nm (colored in blue, in the inset of Figure 2b) exhibits a rising paralleling the decay trace of the T_1. This evidence suggests a possible cogeneration of **QM5** from the T_1.

The photogeneration of **QM5** was not significantly affected passing from aqueous ACN to a buffered solution at pH 7.4 (Figure S6)

We observed a comparable behavior for the compounds **15** and **19**, with the latter being the only one acting as QMP. The transient absorbing at 400 nm in aqueous ACN (Figure S7) has been assigned to **QM15** (Table 4).

Substituents Effects (X) of the Arylethynyl Moiety on the QM Photogeneration and Reactivity. Among the 4-substituted arylethynyl Mannich bases **1**−**5**, **4** was the most effective QMP. In fact, the main detected species generated in neat ACN by LFP was the **QM4**, absorbing at 425 and 475 nm (Figure 3a). **QM4** became the only detectable species in aqueous solution (at 440 and 485 nm), as the T_1 (at 600 nm) was completely erased by water (Figure 3b). The collective LFP data suggest that electronically conjugated Mannich bases are effective QMPs (without competing T_1 generation) only in the presence of strong electron-donating substituents. As expected, the electron-rich **QM4** was the longest leaving transient in aqueous solution with a $k_{obs} = 2.0 \pm 0.3 \times 10^2 s^{-1}$, even in the presence of trapping thiol (Figure S8). In fact, it was 168 times less reactive than the prototype **QM1**, with a $k_2 = 7.0 \pm 0.1 \times 10^2$ M^{-1} s^{-1} (Table 4).

The photochemical behavior of the Mannich base **3** was similar to **4**, as **QM3** was effectively generated in neat ACN (Figure S9a), where the triplet was detectable only under Ar-purged conditions at shortened delay time. **QM3** became the dominant photogenerated transient, in aqueous ACN, with a negligible presence of the fast decaying triplet (Figure S9b).

Electron-withdrawing substituents X embedded on the Mannich bases have a detrimental effect on QM generation. In fact, we were able to generate and detect the QMs by LFP by only using the quaternary salts (**7**, **9**, **10**, **17**, and **19**), as their related Mannich bases (**2**, **5**, **13**, and **15**) exclusively photogenerated the T_1. Water solvation regains the full QMP character of quaternary ammonium salts bearing strong electron-withdrawing substituents such as NMe_3^+ (**9**, Figure 4) and NO_2 (**10**, Figure 2). In fact, we observed an enhanced signal ascribed to the QM, paralleling a remarkable quenching of T_1 under aqueous conditions for **7** (Figure S10), **9** (Figure 4a vs b), and **10**.

The pseudo-first-order rate constants $k_{obs}(H_2O)$, listed in Table 4a, for the hydration reaction of the 4-arylethynyl QMs (**QM1**-**5** and **QM9**) suggest a moderate reduction of the electrophilicty for the QMs containing electron-donating groups in comparison to **QM1**, with **QM4** being the least reactive. In contrast, electron-withdrawing moieties NMe_3^+ and NO_2 enhanced the reactivity. The effect is moderate with the former, acting through an inductive effect (−*I*), and it is more robust with

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an electron-withdrawing moiety by resonance effect ($-M$). The $+M$ effect of the fluorine atom rationalizes the moderate reduction of the **QM2** reactivity in comparison to **QM1** (Table 4a). The hydration reaction kinetics of 5-arylethynyl QMs (**QM12-15**) highlight a lower electrophilicity in comparison to the isomeric 4-arylethynyl QM counterparts for the substituents X = H, CH_3O. The exception to this trend was **QM13**. In fact, there is no direct conjugation between fluorine and the exocyclic methylene moiety, unlike in **QM2**, and therefore F atom should act as $-I$ substituent in 5-arylethynyl QMs.

Arylethynyl Derivatives as Singlet Oxygen Photosensitizers. 4- and 5-arylethynyl Mannich bases embedding a nitro-substituent (**5** and **15**) and to a lesser extent their quaternary ammonium salts (**10** and **19**) are ineffective QMPs in ACN solution, as they mainly generate the T_1. In addition, they exhibit an absorption tail in the visible range (up to 420 nm) and a fairly long living triplet excited state ($\tau = 4\ \mu s$). Thus, as a proof of concept, we decided to investigate **10** and **19** as singlet oxygen sensitizers in the photo-oxidation of 1,5-dihydroxynaphthalene (DHN) in ACN, irradiating at 360 nm. DHN is in fact oxidized by $^1O_2$ to the naphthoquinone derivative 5-hydroxy-1,4-naphthalenedione (juglone) through the endoperoxide.[38] The resulting naphthoquinone absorbing at 360−440 nm has been efficiently monitored by both UV−vis absorption (Figures S29 and S30) and HPLC. We did not measured any concentration changes of **10** nor **19** during 1 h of irradiation, thus demonstrating the photochemical stability of these quaternary ammonium salts. 5-hydroxy-1,4-naphthalenedione was the only product detected by HPLC in the irradiated solutions of both **10** and **19**. The kinetics of the photooxidation of DHN was studied by fitting the concentration change of DHN against the irradiation time. The logarithm of the DHN concentration vs irradiation time (t) plot was linear. The pseudo-first-order rate constant (k_{obs}) and the initial consumption rate of DHN (ν_i) were determined by eqs 1 and 2, respectively:

$$\ln[\mathrm{DHN}]_t = \ln[\mathrm{DHN}]_0 - k_{obs}t \quad (1)$$

$$\nu_i = k_{obs}[\mathrm{DHN}]_0 \quad (2)$$

The DHN consumption was monitored by HPLC, as DHN and **10** absorption spectra were partially overlapping. Similarly, k_{obs} was determined by measuring the juglone production by the increase in the absorption at 427 nm and independently by HPLC (Figures S29 and S30) from eq 3:

$$\ln[\mathrm{juglone}]_t = k_{obs}t \quad (3)$$

The quantum yield for singlet oxygen generation (Φ_Δ) was determined using eq 4:

$$\Phi_\Delta = \Phi_\Delta(\mathrm{std})[\nu_i I(\mathrm{std})/\nu_i(\mathrm{std})I] \quad (4)$$

where Φ_Δ(std) was the singlet oxygen generation quantum yield of a reference tetra-substituted naphthalediimide quaternary ammonium salts used as standard sensitizer [Φ_Δ(std) = 0.30],[38] ν_i(std) is the initial rate of the DHN consumption for the photooxidation with the standard sensitizer, and ν_i is the initial rate of the DHN consumption with **10** or **19**. I(std) is the number of photons absorbed by the standard sensitizers, and I is the number of photons absorbed by sensitizers **10** or **19**.

Based on the calculated Φ_Δ for **10** and **19** (0.017 vs 0.028), the former, which is the least effective QMP, acts as the best singlet oxygen sensitizer among the tested arylethynyl derivatives in neat ACN.

DISCUSSION AND CONCLUSIONS

Eighteen 4- and 5-arylethynyl-substituted Mannich bases and their quaternary ammonium salts have been synthesized and investigated as QMPs, with the aim to clarify the role of the electronic conjugation on the efficiency of QM photogeneration, competing with T_1 generation. Steady-state preparative photohydrations, QM trapping experiments with 2-mercaptoethan-1-ol, 1,5-dihydroxynaphthalene-sensitized photo-oxidations, and LFP detection suggested that QMs and the T_1 of the QMPs are often cogenerated transient species. Both QMP structural features and water solvation tune the photogeneration of QMs and T_1. In more detail, the conjugating arylethynyl moiety on C4 (Scheme 1, for numbering), together with strong electron-donating X substituents ($+M$), good leaving group (L; i.e., NMe_3^+), and protic conditions favor the selective photogeneration of the electrophilic intermediate QM, suppressing the T_1 generation. On the contrary, conjugated arylethynyl moiety at C5, electron-withdrawing X substituents ($-M$ and to lower extent $-I$), bad leaving group (L; i.e., NMe_2), and polar aprotic solvent (i.e., ACN) populate effectively the T_1 of the precursors, with negligible or absent QM generation. Consequently, the isomeric 5-arylethynyl derivatives are worse QMPs than the 4-isomer analogues, acting mainly as singlet oxygen sensitizers. Concerning the 4-arylethynyl derivatives, the most effective structural feature controlling QM vs T competition is the X substituent. In fact, it has been possible to switch the quantitative and effective photogeneration of QM (for X = NMe_2), even in the presence of a bad leaving group to a selective population of the T_1, replacing the NMe_2 substituent with the strong electron-withdrawing NO_2. Indeed, 4-arylethynyl quaternary ammonium salts embedding a NO_2 group are photostable under nonprotic conditions (ACN), acting as singlet oxygen sensitizers, as both ESIPT and ESPT cannot take place. They become QMPs in aqueous solutions, albeit with low quantum yields, as ESPT to water is possible. We took advantage of the red-shifted absorption of the yellow zwitterionic precursor (λ_{max} = 405 nm), which is the main reactive species at pH ≥ 7.6, to generate an electrophilic QM by visible light.

Summing up, the presence of both a good leaving group (L; i.e., NMe_3^+) and aqueous solutions is mandatory to achieve QM photogeneration from colored and electron-poor 4-arylethynyl derivatives.

EXPERIMENTAL SECTION

Synthesis and Purification. ***p*-11** and ***m*-11** Mannich bases have been previously synthesized and characterized.[36] In the present investigation, we optimized a synthetic protocol of the arylethynyl Mannich bases **1**−**10** and **12**−**19**, starting from the precursors ***p*-11** and ***m*-11**. The presence of OH groups is seldom compatible with the Sonogashira cross-coupling reaction conditions; therefore, these moieties are commonly protected. In the presence of the Mannich base, the cross-coupling could be carried out using the free phenol derivatives (***p*-11**, ***m*-11**), due to the formation of an intramolecular H-bond within the Mannich bases, which introduces a sort of "self-protective" effect on the OH phenol moiety.

We performed HPLC analysis using analytical HPLC, with a CSH C18 column (150 × 4.6 mm). Analytical method: flow 1.0 mL/min; aqueous solvent: 0.1% trifluoroacetic acid in water; organic solvent: acetonitrile; isocratic flow over 2 min 95% aqueous, gradient: 95% aqueous, gradually to 0% aqueous over 8 min and at the end an isocratic flow over 2 min. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer, and the chemical shifts are reported relative to TMS. The structures of new compounds were assigned by ^1H and ^{13}C NMR.

General Sonogashira Cross-Coupling Procedure for the Synthesis of 1−5 and 12−15. The aryl iodide- ***p*-11**, or ***m*-11** (0.72

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mmol, 0.200 g), was dissolved in neat triethylamine (25 mL) and Pd(tetrakis) (3 mol %, 25.0 mg, 0.022 mmol) in the presence of CuI (17 mol %, 23 mg, 0.12 mmol). We added the reactant and catalysts under stirring while bubbling the solution with argon. After a few minutes, 1-ethynyl-4-substitutedbenzenes (1.3 equiv, 0.9 mmol) was added, and the mixture was stirred at rt keeping the reaction under Ar. After 2.5 h, the reaction mixture was poured into water (100 mL), the aqueous solution was extracted three times with DCM (100 mL), and the organic phases were collected and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford a brown-yellow oil. The crude products were purified by flash chromatography (eluent:cyclohexane/ethyl acetate = 1:1).

*2-((Dimethylamino)methyl)-4-(phenylethynyl)phenol (**1**).* Yield = 94% (0.170 g); yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): 10.86 (bs, 1OH), 7.57−7.54 (m, 2H), 7.44−7.38 (m, 4H), 7.21 (s, 1H), 6.86 (d, J = 8.3 Hz, 1H), 3.62 (s, 2H), 2.30 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 158.6; 132.3; 131.6; 131.3; 128.2; 127.7; 123.6; 121.9; 116.2; 113.3; 89.5; 87.4; 62.4; 44.3. Anal. calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57; O, 6.37. Found: C, 81.19; H, 6.89; N, 5.61.

*2-((Dimethylamino)methyl)-4-((4-fluorophenylethynyl)phenol (**2**).* Yield = 88% (0.170 g); yellow solid; mp = 88−90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.51−7.46 (m, 2H), 7.36 (dd, J = 2.0 Hz, J = 8.3 Hz, 1H), 7.18 (d, J = 2.0 Hz, 1H), 7.07−7.01 (m, 2H), 6.82 (d, J = 8.3 Hz, 1H), 3.66 (s, 2H), 2.35 (s, 6H). ^{13} C NMR (75 MHz, $CDCl_3$): 161.7 (d, J_{CF} = 246.7 Hz), 158.2; 132.7 (d, J_{CF} = 8.2 Hz); 131.9; 131.2; 121.5; 119.3 (d, J_{CF} = 3 Hz); 115.9; 115.1 (d, J_{CF} = 21.7 Hz); 112.7; 88.8; 85.9; 61.9; 43.9. Anal. calcd for $C_{17}H_{16}FNO$: C, 75.82; H, 5.99; F, 7.05; N, 5.20; O, 5.94. Found: C, 75.84; H, 6.05; N, 5.18.

*2-((Dimethylamino)methyl)-4-((4-methoxyphenylethynyl)phenol (**3**).* Yield = 70% (0.142 g); yellow solid; mp = 122−124 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.45 (AA′XX′ system, J = 8.8 Hz, 2H), 7.36 (dd, J = 2.1 Hz, J = 8.3 Hz, 1H), 7.18 (d, J = 2.1 Hz, 1H), 6.88 (AA′XX′ system, J = 8.8 Hz, 2H), 6.84 (d, J = 8.3 Hz, 1H), 3.84 (s, 3H); 3.67 (s, 2H), 2.37 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 159.1; 158.2; 132.7; 132.2; 131.6; 121.7; 116.3; 115.7; 113.8; 113.7; 88.0; 87.3; 62.2; 55.1; 44.3. Anal. calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98; O, 11.37. Found: C, 76.89; H, 6.76; N, 5.05.

*2-((Dimethylamino)methyl)-4-((4-(dimethylamino)-phenyl)-ethynyl)phenol (**4**).* Yield = 71% (0.150 g); yellow solid; mp = 149−150 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.39 (AA′XX′ system, J = 9 Hz, 2H), 7.35 (dd, J = 1.8 Hz, J = 8.3 Hz, 1H), 7.18 (d, J = 1.8 Hz, 1H), 6.83 (d, J = 8.3 Hz, 1H), 6.67 (AA′XX′ system, J = 9 Hz, 2H), 3.69 (s, 2H), 3.00 (s, 6H), 2.35 (s, 6H). ^{13} C NMR (75 MHz, $CDCl_3$): 157.7; 149.7; 132.4; 132.2; 131.7; 121.3; 116.4; 114.5; 111.8; 110.4; 88.5; 87.0; 61.9; 44.1; 40.1. Anal. calcd for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52; O, 5.43. Found: C, 77.54; H, 7.58; N, 9.49.

*2-((Dimethylamino)methyl)-4-((4-nitrophenylethynyl)phenol (**5**).* Yield = 77% (0.164 g); yellow solid; mp = 111−113 °C. ^{1} H NMR (300 MHz, $CDCl_3$): 11.19 (bs, OH); 8.17 (AA′XX′ system, J = 9 Hz, 2H), 7.58 (AA′XX′ system, J = 9 Hz, 2H), 7.37 (dd, J = 1.8 Hz, J = 8.3 Hz, 1H), 7.19 (d, J = 1.8 Hz, 1H), 6.82 (d, J = 8.3 Hz, 1H), 3.66 (s, 2H), 2.34 (s, 6H). ^{13} C NMR (75 MHz, $CDCl_3$): 159.6; 146.5; 132.7; 131.9; 131.7; 130.7; 123.5; 116.5; 113.8; 112.1; 95.5; 86.1; 62.3; 44.3. Anal. calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45; O, 16.20. Found: C, 68.81; H, 5.47; N, 9.49.

*2-((Dimethylamino)methyl)-5-(phenylethynyl)phenol (**12**).* Yield = 93% (0.168 g); yellow solid; mp = 66−68 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.86 (bs, 1OH), 7.58−7.55 (m, 2H), 7.38−7.35 (m, 3H), 7.06 (s, 1H), 7.02−6.94 (m, 2H), 3.66 (s, 2H), 2.34 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 157.8; 131.5; 128.2; 128.0; 123.4; 123.3; 122.4; 122.3; 118.8; 89.3; 88.8; 62.5; 44.3. Anal. calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57; O, 6.37. Found: C, 81.28; H, 6.79; N, 5.59.

*2-((Dimethylamino)methyl)-5-((4-fluorophenylethynyl)phenol (**13**).* Yield = 85% (0.165 g); yellow solid; mp = 104−106 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.54−7.49 (m, 2H), 7.08−6.96 (m, 5H), 3.68 (s, 2H), 2.36 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 162.3 (d, J_{CF} = 247.5 Hz), 157.8; 133.3 (d, J_{CF} = 8.2 Hz); 128.3; 123.2; 122.4; 112.3; 119.4; 118.9; 115.4 (d, J_{CF} = 21.7 Hz); 88.9; 87.8; 62.4; 44.3. Anal. calcd for $C_{17}H_{16}FNO$: C, 75.82; H, 5.99; F, 7.05; N, 5.20; O, 5.94. Found: C, 75.81; H, 6.00; N, 5.22.

*2-((Dimethylamino)methyl)-5-((4-methoxyphenylethynyl)phenol (**14**).* Yield = 70% (0.142 g); yellow solid; mp = 89−91 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.48 (AA′XX′ system, J = 8.8 Hz, 2H), 7.00 (s, 1H), 6.96−6.95 (m, 2H); 6.89 (AA′XX′ system, J = 8.8 Hz, 2H), 3.84 (s, 3H); 3.67 (s, 2H), 2.36 (s, 6H). ^{13} C NMR (75 MHz, $CDCl_3$): 159.4; 157.7; 132.9; 128.2; 123.7; 122.2; 122.1; 118.7; 115.4; 113.8; 88.9; 87.9; 62.4; 55.2; 44.3. Anal. calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98; O, 11.37. Found: C, 76.92; H, 6.88; N, 4.95.

*2-((Dimethylamino)methyl)-5-((4-nitrophenylethynyl)phenol (**15**).* Yield = 75% (0.160 g); yellow solid; mp = 89−91 °C. ^{1}H NMR (300 MHz, CD_3OD): 8.39 (AA′XX′ system, J = 8.9 Hz, 2H), 7.87 (AA′XX′ system, J = 8.9 Hz, 2H), 7.50 (d, J = 7.3 Hz, 1H), 7.29−7.25 (m, 2H), 4.45 (s, 2H), 2.99 (s, 6H). ^{13}C NMR (75 MHz, CD_3OD): 158.1; 149.1; 134.1; 133.9; 131.1; 127.1; 125.1; 124.9; 119.5; 119.4; 94.2; 89.5; 58.2; 43.6. Anal. calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45; O, 16.20. Found: C, 68.95; H, 5.42; N, 9.49.

General Procedure for the Synthesis of Quaternary Ammonium Salts 6−10 and 16−19. The arylethynyl Mannich base [**1** or **2−10**, **12−19** (0.72 mmol)] was dissolved in CH_3CN and CH_3I (1.5 mmol). The resulting solution was stirred under N_2. After 24 h the solvent was removed under vacuum to give the quaternary ammonium salts **6** (or **2−10**, **16−19**) as pure yellow crystals, in quantitative yield.

*1-(2-Hydroxy-5-(phenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**6**).* Yield = 99% (0.280 g); yellow solid; mp = 218−220 °C. ^{1}H NMR (300 MHz, CD_3OD): 7.68 (d, J = 1.59 Hz, 1H), 7.57−7.48 (m, 3H), 7.39−7.36 (m, 3H), 7.01 (d, J = 8.4 Hz, 1H), 4.58 (s, 2H), 3.18 (s, 9H). ^{13} C NMR (75 MHz, CD_3OD): 157.4; 137.3; 135.2; 130.7; 127.9; 127.7; 122.9; 116.0; 114.6; 114.5; 87.7; 87.6; 63.3; 52.1; 52.0; 51.9. Anal. calcd for $C_{18}H_{20}INO$: C, 54.97; H, 5.13; I, 32.27; N, 3.56; O, 4.07. Found: C, 54.94; H, 5.19; N, 3.54.

*1-(2-Hydroxy-5-(4-fluorophenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**7**).* Yield = 99% (0.293 g); yellow solid; mp = 209−211 °C. ^{1}H NMR (300 MHz, CD_3OD): 7.65 (d, J = 2.02 Hz, 1H), 7.57−7.50 (m, 3H), 7.16−7.10 (m, 2H), 7.02 (d, J = 8.5 Hz, 1H), 4.57 (s, 2H), 3.18 (s, 9H). ^{13} C NMR (75 MHz, CD_3OD): 164.2 (d, J_{CF} = 246.7 Hz), 159.4; 139.2; 137.1; 134.7 (d, J_{CF} = 8.2 Hz); 121.1; 117.9; 116.9 (d, J_{CF} = 22.5 Hz); 116.5; 116.3; 89.3; 88.5; 65.3; 53.8. Anal. calcd for $C_{18}H_{19}FINO$: C, 52.57; H, 4.66; F, 4.62; I, 30.86; N, 3.41; O, 3.89. Found: C, 52.59; H, 4.71; N, 3.44.

*1-(2-Hydroxy-5-(4-methoxyphenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**8**).* Yield = 97% (0.295 g); yellow solid; mp = 197−200 °C. ^{1}H NMR (300 MHz, CD_3OD): 7.61 (d, J = 2.0 Hz, 1H); 7.53 (dd, J = 2.0 Hz, J = 8.5 Hz, 1H), 7.42 (AA′XX′ system, J = 8.9 Hz, 2H), 7.00 (d, J = 8.5 Hz, 1H), 6.94 (AA′XX′ system, J = 8.9 Hz, 2H), 4.55 (s, 2H); 3.83 (s, 3H); 3.17 (s, 9H). ^{13} C NMR (75 MHz, CD_3OD): 161.5; 159.1; 138.9; 136.9; 134.1; 117.8; 117.0; 116.8; 116.4; 115.4; 89.7; 88.0; 65.1; 56.1; 53.8; 53.7. Anal. calcd for $C_{19}H_{22}INO_2$: C, 53.91; H, 5.24; I, 29.98; N, 3.31; O, 7.56. Found: C, 53.94; H, 5.22; N, 3.35.

*4-((4-Hydroxy-3-((trimethylammonio)methyl)phenyl)ethynyl)-N,N,N-trimethylbenzenaminium Iodide (**9**).* Yield = 95% (0.395 g); yellow solid; mp dec >150 °C. ^{1}H NMR (300 MHz, CD_3OD): 7.99 (AA′XX′ system, J = 8.6 Hz, 2H), 7.77 (AA′XX′ system, J = 9 Hz, 2H), 7.75 (s, 1H), 7.62 (dd, J = 2.0, J = 8.6 Hz, 1H), 7.04 (d, J = 8.6 Hz, 1H), 4.60 (s, 2H), 3.74 (s, 9H), 3.21 (s, 9H). ^{13} C NMR (75 MHz, CD_3OD): 160.0; 147.9; 139.7; 137.4; 134.5; 127.5; 122.1; 118.0; 116.7; 115.4; 92.6; 87.4; 65.2; 58.2; 54.0; 53.9. Anal. calcd for $C_{21}H_{28}I_2N_2O$: C, 43.62; H, 4.88; I, 43.89; N, 4.84; O, 2.77. Found: C, 43.64; H, 4.51; N, 4.87.

*1-(2-Hydroxy-5-(4-nitrophenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**10**).* Yield = 98% (0.310 g); yellow solid; mp = 220−222 °C. ^{1}H NMR (300 MHz, DMSO): 11.05 (bs, OH); 8.27 (AA′XX′ system, J = 8.9 Hz, 2H), 7.77 (AA′XX′ system, J = 8.9 Hz, 2H), 7.72 (d, J = 2.1 Hz, 1H), 7.63 (dd, J = 2.1 Hz, J = 8.5 Hz, 1H), 7.07 (d, J = 8.5 Hz, 1H), 4.48 (s, 2H), 3.08 (s, 9H). ^{13} C NMR (75 MHz, DMSO): 158.7; 146.6; 138.4; 135.6; 132.2; 129.4; 123.9; 116.8; 115.6; 111.9; 94.3; 86.7; 62.5; 52.2. Anal. calcd for $C_{18}H_{19}IN_2O_3$: C, 49.33; H, 4.37; I, 28.96; N, 6.39; O, 10.95. Found: C, 49.37; H, 4.36; N, 6.41.

*1-(2-Hydroxy-4-(phenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**16**).* Yield = 99% (0.280 g); yellow solid; mp = 189−191 °C. ^{1}H NMR (300 MHz, CD_3OD): 7.55−7.51 (m, 2H), 7.48 (d, J = 8.4 Hz, 1H), 7.42−7.39 (m, 3H), 7.16−7.13 (m, 2H), 4.58 (s, 2H), 3.32

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(s, 9H). ^{13}C NMR (75 MHz, CD_3OD): 158.9; 136.2; 132.9; 130.2; 129.9; 128.9; 124.2; 119.8; 116.4; 91.9; 89.4; 65.4; 65.3; 53.9; 53.4. Anal. calcd for $C_{18}H_{20}INO$: C, 54.97; H, 5.13; I, 32.27; N, 3.56; O, 4.07. Found: C, 54.91; H, 5.19; N, 3.57.

*1-(2-Hydroxy-4-(4-fluorophenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**17**).* Yield = 98% (0.290 g); yellow solid; mp = 187−190 °C. ^{1}H NMR (300 MHz, CD_3OD): 7.58 (m, 2H), 7.47 (d, J = 7.7 Hz, 1H), 7.19−7.12 (m, 4H); 4.57 (s, 2H), 3.17 (s, 9H). ^{13}C NMR (75 MHz, CD_3OD): 164.5 (d, J_{CF} = 247.5 Hz), 159.0; 136.2; 135.1 (d, J_{CF} = 8.2 Hz); 128.8; 124.4; 120.5 (d, J_{CF} = 3.7 Hz); 119.8; 117.1 (d, J_{CF} = 22.5 Hz); 116.5; 90.8; 89.1; 65.4; 53.8; 53.7. Anal. calcd for $C_{18}H_{19}FINO$: C, 52.57; H, 4.66; F, 4.62; I, 30.86; N, 3.41; O, 3.89. Found: C, 52.59; H, 4.64; N, 3.44.

*1-(2-Hydroxy-4-(4-methoxyphenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**18**).* Yield = 99% (0.302 g); yellow solid; mp = 198−200 °C. ^{1}H NMR (300 MHz, CD_3OD): 7.46 (AA′XX′ system, J = 8.8 Hz, 2H), 7.43 (d, J = 7.9 Hz,1H), 7.11 (d, J = 7.9 Hz, 1H); 7.10 (s, 1H); 6.96 (AA′XX′ system, J = 8.8 Hz, 2H), 4.55 (s, 2H); 3.84 (s, 3H), 3.16 (s, 9H). ^{13}C NMR (75 MHz, CD_3OD): 162.0; 159.0; 136.1; 134.5; 129.5; 124.3; 119.7; 116.2; 116.0; 115.6; 92.3; 88.1; 65.5; 56.2; 53.8; 53.7. Anal. calcd for $C_{19}H_{22}INO_2$: C, 53.91; H, 5.24; I, 29.98; N, 3.31; O, 7.56. Found: C, 53.95; H, 5.28; N, 3.35.

*1-(2-Hydroxy-4-(4-nitrophenylethynyl)phenyl)-N,N,N-trimethylmethanaminium Iodide (**19**).* Yield = 97% (0.306 g); yellow solid; mp = 212−215 °C. ^{1} H NMR (300 MHz, CD_3OD): 8.29 (AA′XX′ system, J = 8.9 Hz, 2H), 7.77 (AA′XX′ system, J = 8.9 Hz, 2H), 7.49 (d, J = 7.8 Hz, 1H), 7.21−7.17 (m, 2H), 4.57 (s, 2H), 3.17 (s, 9H). ^{13}C NMR (75 MHz, CD_3OD): 159.2; 149.1; 136.4; 134.0; 131.0; 127.9; 125.1; 124.6; 120.2; 117.4; 94.0; 89.9; 65.4; 53.9; 53.8. Anal. calcd for $C_{18}H_{19}IN_2O_3$: C, 49.33; H, 4.37; I, 28.96; N, 6.39; O, 10.95. Found: C, 49.37; H, 4.35; N, 6.41.

General Procedure for the Preparative Irradiation of 6, 10, and 16. The photochemical reactions were performed by using argon-purged solutions in quartz tubes in a multilamp reactor equipped with two lamps (15 W each, emission centered at 310/360 nm) for the irradiation. Quantitative determination for compounds of interest was carried out by means of HPLC (**20**−**24**) calibration curves.

Preparative Photohydration of 6 and 16. An argon-purged solution of **6** or **16** (0.1 mmol) in ACN:H_2O 1:1 (100 mL) was irradiated for 5 min, 10 Pyrex tubes (20 mL) using a multilamp reactor fitted with two 15 W lamps, with maximum emission centered at 310 nm. Then, solvent was removed under vacuum, and the crude residue was purified by chromatographic separation (cyclohexane:ethyl acetate = 1:1) affording **20** and **21** (89% and 64% yields, starting from **6** and **16**, respectively).

*2-(Hydroxmethyl)-4-(phenylethynyl)phenol (**20**).* Yield = 89% (0.020 g); yellow solid; mp = 149−151 °C. ^{1}H NMR (300 MHz, CD_3COCD_3): 9.00 (s, OH), 7.62−7.58 (m, 3H), 7.50−7.44 (m, 3H), 7.38 (dd, J = 2.1 Hz, J = 8.3 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 4.84 (s, 2H), 4.60 (bs, OH). ^{13} C NMR (75 MHz, CD_3COCD_3): 156.8; 132.6; 132.4; 132.0; 129.7; 129.6; 129.1; 125.0; 116.6; 115.1; 91.1; 88.3; 61.3. Anal. calcd for $C_{15}H_{12}O_2$: C, 80.34; H, 5.39; O, 14.27. Found: C, 80.37; H, 5.41.

*2-(Hydroxymethyl)-5-(phenylethynyl)phenol (**21**).* Yield = 64% (0.014 g); yellow solid; mp = 135−138 °C. ^{1}H NMR (300 MHz, CD_3COCD_3): 8.79 (s, OH), 7.65−7.62 (m, 2H), 7.52−7.50 (m, 3H), 7.43 (d, J = 7.7 Hz, 1H), 7.13 (dd, J = 1.6 Hz, J = 7.7 Hz, 1H), 7.09 (s, 1H); 4.86 (d, J = 5.3 Hz, 2H), 4.58 (t, J = 5.3 Hz, OH). ^{13} C NMR (75 MHz, CD_3COCD_3): 156.2; 132.6; 130.2; 129.8; 129.6; 128.8; 124.5; 124.1; 123.6; 118.8; 90.5; 89.6; 61.6. Anal. calcd for $C_{15}H_{12}O_2$: C, 80.34; H, 5.39; O, 14.27. Found: C, 80.32; H, 5.35.

Preparative Photohydration of 10. An argon-purged solution of **10** (0.1 mmol) in ACN:H_2O 1:1 (100 mL) was irradiated for 450 min, 10 Pyrex tubes (20 mL) using a multilamp reactor fitted with two 15 W lamps, with maximum emission centered at 360 nm. Then, solvent was removed under vacuum, and the crude residue was purified by chromatographic separation (cyclohexane:ethyl acetate = 1:1) affording **22** (97.5% yield).

*2-(Hydroxmethyl)-4-(4-nitrophenylethynyl)phenol (**22**).* Yield = 97.5% (0.026 g); yellow solid; mp = 148−150 °C. ^{1}H NMR (300 MHz, CD_3COCD_3): 8.36 (AA′XX′ system, J = 8.9 Hz, 2H), 7.84 (AA′XX′ system, J = 8.9 Hz, 2H), 7.68 (d, J = 1.9 Hz, 1H), 7.47 (dd, J = 1.9 Hz, J = 8.3 Hz, 1H), 7.00 (d, J = 8.3 Hz, 1H), 4.85 (s, 2H), 3.04 (bs, OH).^{13} C NMR (75 MHz, CD_3COCD_3): 157.5; 148.1; 133.3; 133.1; 132.4;131.9; 129.9; 124.9; 116.6; 113.9; 96.7; 87.1; 61.0. Anal. calcd for $C_{15}H_{11}NO_4$: C, 66.91; H, 4.12; N, 5.20; O, 23.77. Found: C, 66.94; H, 4.11; N, 5.24.

Preparative Irradiation of 6 and 16 in the Presence of 2-Mercaptoethan-1-ol. An argon-purged solution of **6** or **16** (0.1 mmol) together with 2-mercaptoethan-1-ol (1 mmol) in a solution of ACN:H_2O 9:1(100 mL) was irradiated for 5 min, 10 Pyrex tubes (20 mL) using a multilamp reactor fitted with two 15 W lamps, with maximum emission centered at 310 nm. Then, solvent was removed under vacuum, and the crude residue was purified by chromatographic separation (cyclohexane:ethyl acetate = 1:1) affording **23** and **24** (73% and 38% yields, starting from **6** and **16**, respectively).

*2-(((2-Hydroxyethyl)thio)methyl)-4-(phenylethynyl)phenol (**23**).* Yield = 73% (0.021 g); colorless oil; ^{1} H NMR (300 MHz, $CDCl_3$): 7.54−7.50 (m, 2H), 7.40−7.32 (m, 5H), 6.87 (d, J = 8.1 Hz, 1H), 3.87−3.83 (m, 4H), 2.69 (t, J = 5.8 Hz, 2H). ^{13} C NMR (75 MHz, $CDCl_3$): 155.1; 133.9; 132.5; 131.3; 128.2; 127.9; 123.3; 116.9; 115.4; 88.9; 88.1; 61.5; 33.4; 31.6. Anal. calcd for $C_{17}H_{16}O_2S$: C, 71.80; H, 5.67; O, 11.25; S, 11.27. Found: C, 71.77; H, 5.68.

*2-(((2-Hydroxyethyl)thio)methyl)-5-(phenylethynyl)phenol (**24**).* Yield = 38% (0.011 g); white solid; mp = 83−85 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.56−7.51 (m, 2H), 7.37−7.35 (m, 3H), 7.15−7.07 (m, 3H), 3.94−3.81 (m, 4H), 2.68 (t, J = 5.8 Hz, 2H). ^{13} C NMR (75 MHz, $CDCl_3$): 154.5; 131.5; 130.6; 128.2; 124.1; 123.8; 123.7; 123.0; 119.6; 89.5; 88.7; 61.5; 33.4; 31.6. Anal. calcd for $C_{17}H_{16}O_2S$: C, 71.80; H, 5.67; O, 11.25; S, 11.27. Found: C, 71.83; H, 5.65.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00331.

Additional UV−vis absorptions, transient absorptions, HPLC purity data and NMR spectra of the new 4- and 5-arylethynyl Mannich bases and quarternary ammonium salts (PDF)

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Notes

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