Photoinduced C−F Bond Cleavage in Some Fluorinated 7-Amino-4-quinolone-3-carboxylic Acids

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The photochemistry of some fluorinated 7-amino-4-quinolone-3-carboxylic acids used in therapy as antibacterials and known to be phototoxic has been investigated in water. All of them undergo heterolytic defluorination, and this appears to be a path for the generation of aryl cations in solution. 6-Fluoro derivatives such as norfloxacin (Φ_{dec} = 0.06) and enoxacin (Φ_{dec} = 0.13) give the corresponding phenols. Insertion of an electron-donating substituent makes defluorination inefficient; thus, ofloxacin, an 8-alkoxy derivative, is found to be rather photostable (Φ_{dec} = 0.001) and reacts in part via a process different from defluorination (degradation of the *N*-alkyl side chain). With a 6,8-difluoro derivative, lomefloxacin, the reaction is more efficient (Φ = 0.55) and selective for position 8. Contrary to the previous cases, the aryl cation undergoes insertion in the neighboring *N*-ethyl group rather than solvent addition (a carbene-like chemistry). With all of the above fluoroquinolones an intensive triplet−triplet absorption is detected and is quenched by sulfite (k_q = (1−5) × 10^8 M^{-1} s^{-1}). Under this condition, reductive defluorination via the radical anion takes place. The relation of the above chemistry to the phototoxicity of these drugs is commented upon briefly.

The great chemical inertness of the carbon−fluorine bond allows the use of fluorinated derivatives, e.g., fluoroaromatic or heteroaromatic compounds, as drugs. Although reaction of the C−F bond has been obtained in these derivatives only under strong reductive conditions, the situation may be different in a photochemical reaction. We were attracted to this field by the numerous recent reports of light-related adverse effects of fluoroquinolones (FQs), a family of drugs that have excellent antibacterial properties and are frequently prescribed. Unfortunately, these have a phototoxic[1] and photoallergenic effect[2] as well as, in some cases, a photomutagenic and phototumorigenic effect.[3] This is a major limitation to their use in therapy.

The chemical mechanism underlying such effects has not been clarified up to now. Evidence has been reported for the role of reactive oxygen species such as singlet oxygen, the superoxide anion, or the hydroxyl radical[4,5] to the FQs induced phototoxicity. However, a recent study by Chignell et al.[6,7] has shown that both $^1O_2$ and $O_2^{\bullet-}$ are indeed produced, but rather inefficiently, and at any rate that there is no correlation between the relative efficiency in activating oxygen and the phototoxic potential of the same series of FQs.

Another possibility is that FQs react photochemically with some cell component. Evaluation of this path requires that the photochemistry of these drugs is ascertained. At present there are sparse reports about some FQ photoreactions, in some case involving the isolation of the photoproducts, in other cases only partial evidence about the type of reaction occurring. Reported reactions include dimerization for ciprofloxacin[8] and degradation of the alkylamino side chain for levofloxacin.[9] Recently, indication that defluorination occurs for FQs containing a fluorine atom in position 8, such as lomefloxacin,[10,11] fleroxacin,[11] and orbifloxacin,[5,12] has been reported.

Thus, it appeared worthwhile to establish the photoreactivity of a series of FQs in aqueous solution under uniform conditions. The present work has been carried out on four drugs of this family[13] and shows that heterolytic defluorination is consistently the main pro-

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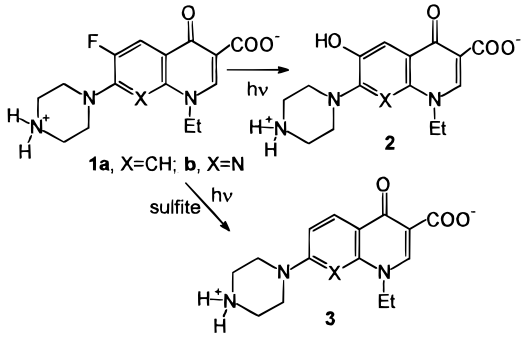
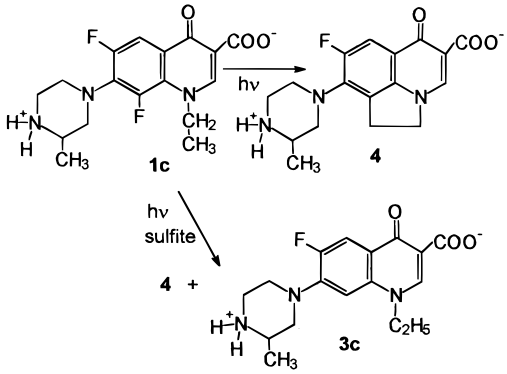
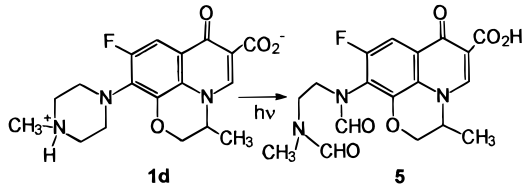
Scheme 1



Scheme 2



Scheme 3



cess. In one case, the resulting aryl cation undergoes an unexpected "carbene-like" chemistry.

Results

The quinolones used in therapy are 1,4-dihydro-1-alkyl-4-oxoquinoline-3-carboxylic acids. More precisely, the drugs most largely used at present (third-generation quinolones) all bear a fluoro in position 6 and a β-aminoethylamino group in position 7 as additional substituents.[14] Therefore, we chose for the present study the 1-ethyl-6-fluoro-7-piperazinyl derivative (norfloxacin, **1a**) as the reference term as well as some of its simple derivatives. These were selected as examples of the structural variations adopted in pharmacological research with the aim of modulating the activity. Apart from a modification of the alkyl chains, presumed not to affect the photochemical behavior, such elaboration mostly involves position 8 in the quinolone ring. Therefore, we chose an 8-aza derivative (enoxacin, **1b**), an 8-fluoro derivative, (lomefloxacin, **1c**), and an 8-alkoxy derivative (ofloxacin, **1d**) (see Schemes 1−5). This choice allowed the exploration of the structure effect on the photoreactivity as well as comparing four out of five of the FQs commonly used in present clinical practice.

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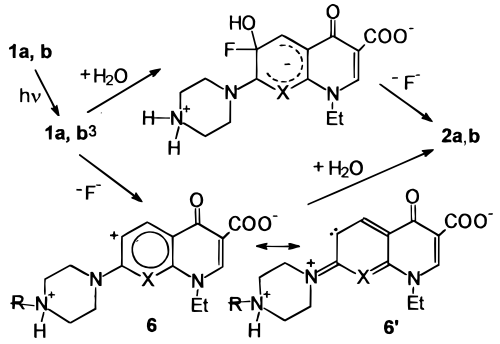
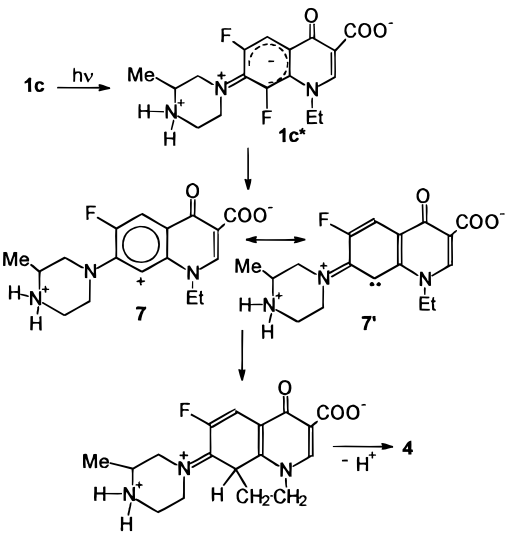
Scheme 4



Scheme 5



The study was carried out in dilute ($(1-2) \times 10^{-4}$ M) neutral aqueous solution. The fluoroquinolones are amino acids and are present as zwitterions in the ca. pH 6−8 range.[15] Product isolation and characterization involved previous functionalization of the photolysis mixture (in most cases by acylation of the amino group, extraction, methylation of the carboxylic group, and chromatographic separation, see Experimental Section). Smaller scale, but otherwise identical, experiments were also carried out and monitored by HPLC. These served for measuring the quantum yield of reaction (Φ_{dec}), as well as for the potentiometric measure of the fluoride released (Φ_{F^-}).

Irradiation of an argon-flushed solution of **1a** led to liberation of fluoride and formation essentially of a single compound. This was derivatized as indicated above and isolated. The product thus obtained was identified as a derivative of phenol **2a** from its analytical and spectroscopic properties (see Scheme 1; in this case, as well as in the following ones, the nonderivatized compounds are

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Table 1. Parameters for the Photochemical Reactions of Fluoroquinolones 1a−c

substrate	reaction quantum yield[a] (%) H_2O	H_2O/air	$NaClO_4$	Na_2SO_3/$NaHSO_3$	τ_F (ns)	$k_q(SO_3^{2-})$[b] ($M^{-1}\,s^{-1}$)
1a	0.06	0.01	0.09	0.004	<1	1.2×10^8
1b	0.13	0.08	0.19	0.027	<1	4.8
1c	0.55	0.5	0.5	0.28	<1	>1
1d	0.0012[c]	0.0016[d]	0.0013[c]	≤0.0001[e]	4	1.0

[a] Quantum yield for the substrate consumption. Unless otherwise noted, an equimolecular amount of fluoride (±5%) is liberated. [b] Quenching rate by sulfite of the transient absorption at 600 nm. [c] 80% fluoride liberated. [d] 85% fluoride liberated. [e] Amount of fluoride too low for an accurate measurement.

indicated in the schemes; for the actually characterized compounds, see the Experimental Section). The quantum yield for the consumption of **1a** was 0.06 (see Table 1), and the molar amount of fluoride set free was equal within 5% to consumed **1a**. The solutions used were buffered at pH 7.2 by adding 5×10^{-4} M $NaHCO_3$ (the natural pH of **1a** and of the other FQs is 6.1−6.5). In this way, the pH did not change appreciably during the experiments. With an unbuffered solution hydrogen fluoride release caused a pH drop down to ca. 4.5 at complete conversion, but the reaction course did not change significantly.

Irradiation of an air-equilibrated solution led to the same process, but with a diminished quantum yield (0.01). The effect of salts was also tested (with Ar-flushed solutions). In the presence of 0.1 M $NaClO_4$, the quantum yield for defluorination to give **2a** was enhanced to 0.09. In 0.02 M $NaHSO_3$−$NaSO_3$ buffer (pH 7), on the other hand, a different defluorination process occurred. This led to the 6-unsubstituted quinolone **3a** with a low quantum yield (0.004).

The naphthyridine derivative **1b** likewise underwent clean defluorination (100% of the substrate consumption) to give phenol **2b** in neat water (or $NaHCO_3$ buffer) with $\Phi = 0.13$, decreasing to 0.08 in air-equilibrated solution and increasing to 0.19 in the presence of 0.1 M $NaClO_4$. In sulfite buffer reductive defluorination to give **3b** was observed instead ($\Phi = 0.027$, see Scheme 1 and Table 1).

The 6,8-difluoro derivative **1c** turned out to react more efficiently than the previous derivatives ($\Phi = 0.55$). The process involved mono-defluorination selectively from position 8; no phenol was isolated, contrary to the case of **1a**,**b**, and the main product was the tricyclic derivative **4** (see Scheme 2). In this case, the effect of both oxygen and $NaClO_4$ on the reaction quantum yield was minimal (10%). In sulfite buffer, the efficiency of the photoreaction was diminished, though not as strongly as in the previous cases ($\Phi = 0.28$), and gave two mono-defluorinated derivatives, the above tricyclic compound **4** and quinolone **3c**. Increasing the sulfite buffer concentration up to 0.1 M caused neither a further drop of the quantum yield nor a change in the product distribution.

The 8-alkoxyquinolone **1d** was the most photostable of the series. The decomposition quantum yield in Ar-flushed water was 0.0012 and changed only marginally in air-equilibrated solution or in the presence of 0.1 M $NaClO_4$ (Table 1). Contrary to the above cases, defluorination was not the only process, though it remained predominant, since the fluoride liberated corresponded to 80% of the substrate decomposed. However, the only product isolated and recognized from preparative irradiation was a fluorine-containing derivative, diamide **5** (Scheme 3). In the presence of sulfite, the quantum yield dropped by 1 order of magnitude, and we were unable to identify any photoproduct.

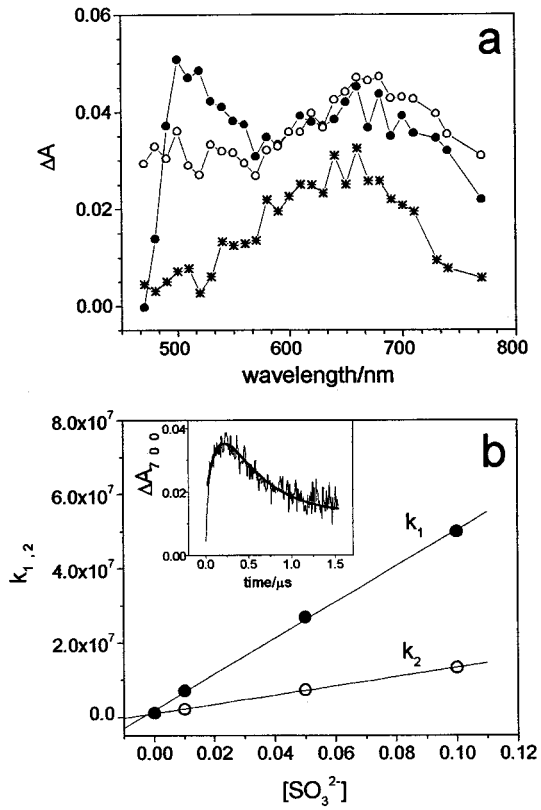


Figure 1. (a) Absorption changes observed in a 7.3×10^{-5} M solution of enoxacin in Ar-saturated sulfite buffer 0.1 M, pH 7.06, upon excitation with a 2 mJ, 20 ns laser pulse at 355 nm: (●) 20 ns, end-of-pulse; (○) 35 ns and (*) 250 ns after time "zero"(see Experimental Section). (b) Dependence of the rate constants extracted by biexponential treatment of the time profiles of ΔA at 700 nm, k_1 and k_2, on the sulfite anion concentration. Inset: time profile of ΔA at 700 nm at 0.01 M sulfite and biexponential fit corresponding to $1/\tau_1 = k_1 = 0.71 \times 10^7\ s^{-1}$ and $1/\tau_2 = k_2 = 0.22 \times 10^7\ s^{-1}$.

The above quinolones showed a blue fluorescence with a maximum around 380 nm, with the difference that lifetime was <1 ns for **1a**−**c**, while **1d** showed $\tau = 4$ ns. Addition of 0.02 M sulfite caused only a minor effect on the fluorescence.

Laser flash photolysis of the four quinolones **1a**−**d** in aqueous solutions led in every case to transient absorption in the 500−650 nm region, which was attributed to the population of the triplet state.[16a] The transients, with lifetimes in the microsecond or submicrosecond range, were quenched in the presence of sulfite, and rate constants in the order of $10^8\ M^{-1}\ s^{-1}$ were measured (see Table 1).

With **1b** a more detailed investigation of the transient behavior in aqueous solution and in the presence of sulfite at pH 7 was performed. The transient spectra observed with 0.1 M sulfite at several delays after the laser are shown in Figure 1a. At the end of the 20 ns laser pulse the absorption spectrum showed two bands. The first one, with a maximum at 500 nm, was attributed to the triplet state (compare ref 16b), while the latter band (maximum at 670 nm) was due to a second transient resulting from sulfite quenching of the triplet

state during the laser pulse. During the following 15 ns, in fact, the 500 nm band disappeared, while the 670 nm band further increased. The kinetics of the absorbance changes over the whole wavelength range was well described by a biexponential function. The time constants τ_1 and τ_2 were ca. 20 and 75 ns, respectively. After 230 ns, the transient absorption was characterized by a large band with maximum at ca. 650 nm, which decayed in the scale of tens of microseconds. In the absence of sulfite the 500 nm absorption decay was monoexponential with a time constant of 850 ns; during the triplet decay no increase of the absorption in the region 600−800 nm or formation of the longer-lived 650 nm species was observed.

The study of the effect of the sulfite concentration on the kinetics of the fast processes showed that, besides the triplet, the 670 nm transient was also quenched by sulfite. In Figure 1b, the linear dependence of the two rate constants, extracted by biexponential analysis of the absorbance changes, on the sulfite concentration is reported. The values of $k_q = 4.8 \times 10^8$ M^{-1} s^{-1} for the triplet and $k_q = 1.2 \times 10^8$ M^{-1} s^{-1} for the 670 nm transient were derived. In the inset of Figure 1b the time profile at 700 nm and the corresponding best fitting for 0.01 M sulfite are reported.

The photochemical behavior of **1b** in phosphate buffer 0.01 M had been previously investigated.[16b] The triplet decay rate had been reported to be 1.1×10^7 s^{-1}. Since in aqueous solution, in the absence of phosphate, the triplet lifetime of **1b** is 850 ns, it is concluded that quenching of the triplet by the phosphate ions is also operative. This effect, which is determinant for the medium-dependent photodecomposition of fluoroquinolones,[10b] is presently under investigation.

The above fluoroquinolones are soluble only in polar media. The photochemical behavior in organic solutions was not systematically investigated. However, explorative irradiations of **1b** and **1c** in trifluoroethanol and in methanol showed that the photoreactivity was much lower than in water.

Discussion

Reaction Observed. The present data show that fluoroquinolones are photosensitive, though with largely different efficiency. The present investigation was carried out with lamps centered at 320 nm or with a Pyrex-filtered mercury arc ($\lambda > 300$ nm), but the absorption of these drugs extended to ca. 360 nm, and thus, the UV-A component of both natural and artificial indoor light is active. Indeed, dilute solutions of the most sensitive of these drugs, viz. **1c**, decomposed in hours under the ordinary laboratory illuminations, and it is not surprising that a phototoxic effect is so easily observed. The photochemistry of these compounds is quite varied as far as both the product distribution and the reaction quantum yield (the latter changing over 4 orders of magnitude depending on structure and medium) are concerned. The observed photoreactions were as follows: (a) nucleophilic substitution of fluoride, observed with both **1a** and **1b** and yielding the corresponding phenols (defluorination occurred also with **1d**, as apparent from the fluoride set free, though with much lower quantum yield than with the other FQs (in this case, however, no defluorinated product was isolated)); (b) substitution of fluoride through an intramolecular alkylation, as observed with **1c**; (c) reductive defluorination, observed in sulfite buffer with all quinolones except **1d**; and (d) oxidative degradation of the alkylamino side chain, observed with **1d** in a slow process.

Defluorination according to paths a or b (or c in the presence of sulfite) was the only reaction observed for **1a**−**c**. In every case, the amount of HF released was identical (±5%) to the extent of the photodecomposition. The quantum yield for this process was diminished under air with **1a** and **1b**, but only slightly in the case of **1c**. On the contrary, a significant fraction of the (slow) photoreaction of **1d** led to a product conserving the fluorine (path d), and in this case the quantum yield was higher under air.

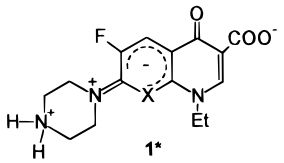
Excited States Involved. Light excitation of the quinolones under study populated the triplet by ISC from the singlet state, as indicated both by the observation of phophorescence in a glassy matrix and by the transient observed by nanosecond flash photolysis. The case of **1b** has been examined in some detail. The strong absorption with a maximum at 500 nm corresponds to the T−T absorption and has a lifetime of 850 ns in water at pH 7.2. As expected, it was quenched by oxygen in the same way as the photochemical reaction. The same transient had been previously reported in phosphate buffer, where the lifetime is shorter, and evidence for its attribution to the triplet and it being the photoreactive state had been reported.[16b] A value of $\Phi_{ISC} = 0.85$ was evaluated, though we must caution that this may be overestimated.[16b]

We observed that the intensity of the end-of-pulse transient absorption was similar with all the quinolones, except for **1c** where it is weaker. Furthermore, there are some differences in the reaction of **1c** on one hand and **1a**,**b** on the other one.

First, the photodecomposition of **1a** and **1b** was only moderately efficient, and both the transient and the photoreaction were quenched by oxygen, while **1c** decomposed quite efficiently and oxygen quenching was limited. Second, sulfite quenched both the transient and process a (for **1a**,**b**) or b (for **1c**), but to a different degree. With 0.02 M sulfite buffer, the reaction quantum yield for **1c** dropped by 24% and a new product, compound **3c**, was formed *in addition* to that formed in water (compound **4**). An increase of the sulfite concetration up to 0.1 M caused no further change. With **1a** and **1b**, on the other hand, the drop in quantum yield was much larger (by a factor of 15 and 5, respectively) and the reduced quinolones **3a**,**b** *substituted* the phenols (**2a**,**b**) formed in water. Third, with none of the above substrates the absorption spectrum as well as the fluorescence spectrum and efficiency were significantly affected by the addition of 0.02 M sulfite, while as seen above the reaction quantum yield dropped, and dramatically so, with **1a** and **1b**.

Therefore, the substitution of a hydroxyl for a fluoro group in quinolones **1a**,**b** (reaction a) undoubtedly occurred via the triplet state; the more efficient defluorination of **1c** (reaction b) arose again from the triplet (shorter lived and thus less easily quenched) and in part from the singlet state. As for process c, the reductive defluorination observed with all three of the above quinolones in the presence of sulfite, this involved in every case the triplet as demonstrated by the effect of this ion on the decay of the T−T absorption (see below for the path involved).

Defluorination via the Excited State: Aryl Cation Reactivity. As seen above, C−F bond cleavage was the only primary photochemical event with **1a**−**c**. This cannot be a homolytic process, since the BDE of the aromatic C−F bond (ca. 120 kcal M^{-1}) largely exceeds that of the singlet (ca. 82 kcal M^{-1}, from fluorescence) and the triplet state (ca. 69 kcal M^{-1}, from phosphorescence) of these molecules. Two paths are viable, viz. the addition−elimination mechanism via a σ complex and heterolytic fragmentation (see Scheme 4 for the case of **1a**,**b**). The former mechanism has been demonstrated for several photoinduced aromatic substitutions, usually occurring via the triplet state.[18] The latter one has been recently suggested for a few electron-donating substituted fluoroaromatics[19] and is favored with the present quinolones by the internal charge-transfer character toward the ring in the $\pi\pi^*$ excited state (see formula **1***). This is due to the presence of both electron-donating (amino) and electron-withdrawing (fluorine) substituents and is evidenced by the considerable Stokes shift of the fluorescence (e.g., for **1b** 5000 cm^{-1} in dioxane, growing to 8000 in D_2O)[17] and the low fluorescence lifetime (<1 ns for **1a**−**c**) typical of such push−pull states.



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The medium and structure effects on the reaction were in accord with both mechanisms. That a ionic mechanism was involved was indicated by the occurrence of the photocleavage in water but only to a much lesser degree in a solvent with a lower dielectric constant such as methanol or trifluoroethanol, and by the reactivity increase upon increasing the ionic strength (see the experiments with $NaClO_4$). Furthermore, the reaction quantum yield (in argon flushed water) decreased in the order Φ_{dec}(**1c**) = 4 × Φ_{dec}(**1b**) = 9 × Φ_{dec}(**1a**) = 460 × Φ_{dec}(**1d**), showing a correlation with the electronegativity of the group in position 8, respectively =C(−F)−, =N−, =C(−H)−, and =C(−OR)−. This may be rationalized as due either to electron-withdrawing groups making nucleophile attack on the excited-state easier or to these groups increasing charge-transfer toward the ring in the excited state and, thus, the incipient heterolytic weakening of the C−F bond. On the other hand, a mesomeric donating group such as the alkoxy group in **1d** both slows down the addition of nucleophiles and balances the charge transfer.

Since the fluoroquinolones are amphoteric, it is impossible to explore the dependence of the photosubstitution quantum yield on [OH^-], as has been done for known bimolecular photosubstitution. However, the fact that defluorination is most efficient in the case of **1c**, where no hydroxyl group is incorporated, supports that unimolecular cleavage is the mechanism.

The photochemistry of quinolone **1c** differs from that of analogues **1a**,**b** in that defluorination is not only more effective but also selective for position 8, not position 6, and that the final product arises through cyclization onto the *N*-ethyl group, not by solvolysis. The regioselectivity can be qualitatively understood by considering the structure of the intermediate cation. An aryl cation in position 6 (**6**, see Scheme 4) receives no stabilization from the neighboring amino group, since this is only possible in mesomeric formulas such **6′**, which are unimportant because they are no more aromatic. However, the situation is different for a cation in position 8, such as **7** from **1c** (Scheme 5), since this gains a significant stabilization through the contribution of structure **7′**, where the charge is on the nitrogen and aromaticity is conserved in the pyridone moiety.[20] The participation of this mesomer explains why C−F cleavage is so much faster from position 8 than from position 6. As a consequence, defluorination is regioselective and more effective with **1c**.

Furthermore, the structure of the cation allows us to understand the different chemistry observed. The obvious reaction from σ cation **6** (X = CH, N) is water addition, and thus, if this is the intermediate, it is reasonable that phenols **2a** and **2b** are the exclusive products (Scheme 4). In the case of cation **7**, however, the charge is distributed between the carbon and the nitrogen atoms through the contribution of mesomeric formula **7′**. This is a π cation where the carbon atom in position 8 is a carbene. Indeed, in this case, the exclusive process is a typical carbene reaction, intramolecular insertion into a C−H bond of the CH_3 in the neighboring *N*-ethyl group (Scheme 5). This may be envisaged as a two-step radical or a concerted reaction; in any case, the attack occurs at the more accessible position (i.e., at the unactivated methyl group, not at the methylene α to the amino group), as it is general in carbene reactions.[21]

Aryl cations are known to be high-energy, and thus quite unselective, intermediates[22] and have been spectroscopically characterized in a rigid matrix.[23] In solution,[24] these are intermediates in the displacement reactions of arenediazonium cations (in the absence of strong bases, reducing agents, or light)[25] and in a few solvolyses.[26] There is both computational and experimental evidence for mesomeric[25a,27] and hyperconjugative[26] stabilization of such species, but no indication of a change in the reaction course. The present data offer a new insight, by demonstrating a clear-cut change of the chemical behavior in a 2-aminoaryl cation where π

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(21) See, e.g.: Majerski, Z.; Hamersak, Z.; Sarac-Arneri, R. *J. Org. Chem.* **1988**, *53*, 5053. Paquette, L. A.; Wilson, J. E.; Menzal, R. P.; Allen, G. R. *J. Am. Chem. Soc.* **1972**, *94*, 7761. Sasaki, T.; Eguchi, S.; Kiriyama, T. *J. Am. Chem. Soc.* **1963**, *91*, 212.

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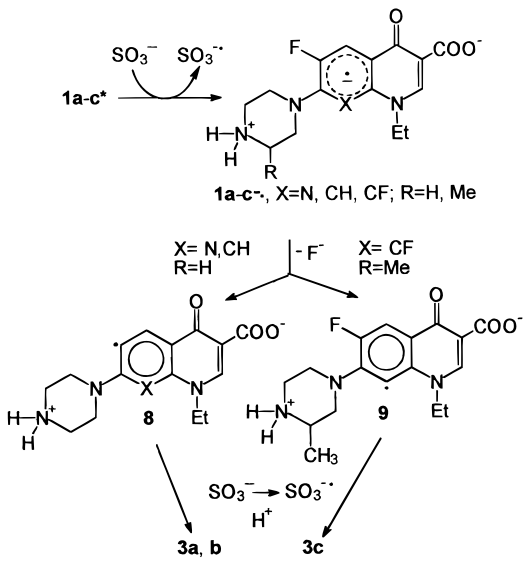
Scheme 6



delocalization is possible: in this case, a carbene-like chemistry is observed instead of the "normal" nucleophile addition.

Defluorination via the Radical Anion. The sulfite anion quenches the triplet state and causes reductive defluorination of the quinolones. These heterocycles are expected to be easily reduced in the excited state. As an example, for **1b**, E_{red}= −1.30 V vs SCE in water,[28] and thus $E_{red}(\mathbf{1b}^{3*}) = E_{red} + E_{exc} = 2.70$ V. Thus, it is reasonable that the efficient triplet quenching (rate constant $>10^8$ M^{-1} s^{-1}) is an electron-transfer process leading to the radical anion (**1a**−**c**$^{\bullet-}$). This species fragments to the corresponding σ aryl radical (**8** or **9**, see Scheme 6) with structure-dependent efficiency. The fluorobenzene radical anion has been characterized,[29] and there are few known defluorinations via the radical anion,[30] although in polysubstituted aromatics other substituents are as a rule lost in preference to fluorine.[31,32] Thus, defluorination from the radical anion is expected to occur inefficiently, even though it is facilitated with the present substrates by the substituent stabilization of the resulting aryl radicals. Indeed, electron-transfer quenching of the excited states by sulfite mainly leads to physical decay via back-electron transfer rather than to chemical reaction. The decomposition quantum yield in sulfite buffer 0.02 M is lower than in neat water (where defluorination proceeds directly from the excited state), and the decrease is the stronger the less electron-withdrawing is the substituent. With the less quenchable **1c** the decrease is moderate (by a factor of 2). Among the other fluoroquinolones, Φ decreases by a factor of 5 for **1b**, of 15 for **1a,** and of ≫10 for **1d**. Thus, when the radical anion is more stabilized (and presumably longer-lived), defluorination by this mechanism is more competitive. In the difluoro derivative **1c**, the cleavage is again selective for position 8, since the same factors that stabilize the cation in position 8 similarly operate on the radical.

The thus-formed σ aryl radicals **8** and **9**, respectively, are then reduced by sulfite to the corresponding anion, and the ensuing protonation completes the observed overall process, viz. reductive defluorination to give quinolones **3a**−**c** (see Scheme 6).

Contrary to the photochemistry in neat water, further transients are observed in the presence of sulfite and can be assigned with reference to Scheme 6. The 670 nm transient (empty circles in Figure 1a) formed by quenching of the triplet state of **1b** by sulfite ($k_1 = 4.8 \times 10^8$ M^{-1} s^{-1}, see Figure 1b) and growing within 15 ns reasonably is the radical anion **1b**$^{\bullet-}$. This species is relatively long-lived, and the strong C−F bond cleaves inefficiently. The fragmentation of this species presumably occurs as a unimolecular process leading to radical **8b**. The long-living (tenths of microseconds) species absorbing at 650 nm that remains after decay of the two first transients (see the lower spectrum in Figure 1a) is thus reasonably radical **8b**. In the final step, the radical is then further reduced as mentioned above. It should be noticed that the second transient is also quenched by sulfite ($k_1 = 1.2 \times 10^8$ M^{-1} s^{-1}, see Figure 1b), though the significance of this phenomenon is uncertain at the moment.

Piperazinyl Side Chain Oxidation. The photochemistry of **1d** clearly differs from that of the other quinolones. This is due to the fact that the alkoxy group in position 8 strongly limits the charge transfer to the ring in the excited state (see above). In fact, in contrast to the other three quinolones considered, the much less reactive **1d** has $\tau_{fluo} = 4$ ns, and thus, the singlet—and presumably also the triplet—state come nearer to "normal" $\pi\pi^*$ states, with low ICT character. This hinders defluorination, and the quantum yield of decomposition drops in the order of 10^{-3}. Potentiometric analysis reveals that fluoride is released also in this case, but the amount of F^- observed is less than 100% of decomposed **1d**. We did not isolate fluorine-free photoproducts in this case, nor did Yoshida et al. in a previous study in air-equilibrated water.[9] The product isolated arises from oxidative degradation of the piperazinyl side chain. Such a degradation is a common occurrence in the photochemistry of dialkyarylamines and often involves triplet-sensitized hydrogen abstraction followed by oxygen addition or further oxidation of the α-amino radical; quantum yields generally are in the same order as observed here.[33] Since long irradiation times are required (notice also that this is the only quinolone for which the degradation in the presence of air is somewhat faster than under deoxygenated condition), it appears likely that in pre-

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parative experiments on dilute solutions traces of air penetrate into the vessel and oxygen is involved in the oxidation leading to the observed product **5**. Therefore, we have no firm evidence for a detailed mechanism in this case, but it is apparent that the photochemistry of **1d** can be classed along with that of other aromatic amines, while it is very different from the peculiar reactivity of **1a**−**c** having an ICT state.

Conclusion. This work reveals that irradiation in water of amino-substituted (hetero)aryl fluorides leads, in some case quite efficiently, to heterolytic fragmentation of the C−F bond, a process for which there is little precedent.

From the chemical point of view, the main interest is that a new access to aryl cations in solution is thus opened as an alternative to the decomposition of diazonium salts. From the present study, it appears that the chemistry of such cations is not uniform, varying between water addition and carbene-like intramolecular insertion depending on the structure. It may be that a similar study of simpler models will afford new evidence about the structure and reactivity of aryl cations. Furthermore, a different pathway for defluorination, in this case via the radical anion, has been evidenced in the presence of sulfite.

From the pharmaceutical point of view, the present data suggest that incorporating a fluorine in a drug, a practice often advantageously used in optimizing the pharmacological profile and technical characteristics, may on the other hand introduce an undesired photoreactivity, at least in aromatics. The correlation of the photolability with the charge transfer to the ring in the excited state suggests a way for predicting the significance of such a side effect on the basis of the substituents.

The observed reactions may be relevant for the reported phototoxic effects. Since, as seen above, oxygen activation is not the mechanism, phototoxicity depends on a photoreaction of the drug with a component of the cell. We observed an efficient carbene-like chemistry with the defluorinated cation from **1c** (actually the most phototoxic of the quinolones we studied).[2a,3a,4c,34,35] In water, this occurs intramolecularly and involves the *N*-ethyl chain, but in the cell it is likely that such an intermediate can find other C−H bonds for which insertion is convenient, thus initiating the degradation of some cell component.

Experimental Section

Materials and Instruments. Nofloxacin (**1a**), enoxacin (**1b**), and ofloxacin (**1d**) were purchased from Sigma Chemical Co. (Milan) and used without further purification. Lomefloxacin hydrochloride, from the same supplier, was dissolved in water (0.02 M solution), and 1 M NaOH was added to neutrality. The free base (**1c**, mp 231−4 °C**)** was extracted with chloroform. All other chemicals and solvents were reagent grade or better. The pH 7 sulfite buffer was prepared from 1.26 g of Na_2SO_3 and 0.95 g of $Na_2S_2O_5$ per liter for 0.02 M solution.

Fluorescence lifetimes were measured (in phosphate buffer) by Dr. R. Argazzi (University of Ferrara) by means of a single-photon-counting apparatus. The other data were obtained from standard instruments.

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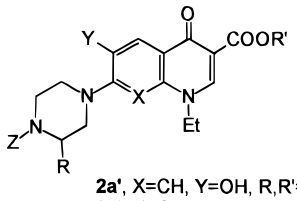
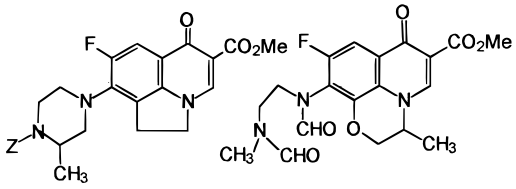
Scheme 7



2a', X=CH, Y=OH, R,R'=H, Z=COOEt
3a', X=CH, Y,R,R'=H, Z=COOEt
2b', X=N, Y=OCOOEt, R,R'=H, Z=COOEt
3b', X=N, Y,R,R'=H, Z=COOEt
3c', X=CF, Y=H, R,R'=Me, Z=COOEt



4', Z=COOEt **5'**

Preparative Irradiations. Solutions (1 to 2 × 10^{-4} M) of the drugs in water or the appropriate buffer (1.4 L) were irradiated in an immersion well apparatus by means of a Pyrex-filtered 500 W medium-pressure mercury arc (Helios Italquartz) at 17 °C. The course of the reaction was monitored by HPLC (see below). When a convenient (50−90%) fraction of the starting material had been consumed, the aqueous solution was brought to neutrality and treated as follows.

Method A. The solution was extracted by 3 × 0.45 L of chloroform with 1% ethyl chloroformate, and the organic phase was washed with $NaHCO_3$ to neutrality and then with water, and then dried. Freshly prepared ethereal diazomethane was added; after 30 min, the excess reagent was decomposed with AcOH and the solution evaporated. Chromatography of the residue on a silica gel column (120 g, 35−70 μm, chloroform−methanol mixture from 99:1 to 90:10 as eluant) gave the products.

Method B. Same as in A, omitting the treatment with diazomethane.

Method C. The aqueous solution was evaporated under reduced pressure.

The method used and the products characterized are given in the following text. See Scheme 7 for the formulas of the derivatized photoproducts.

1a in Water (Method B). 1-Ethyl-1,4-dihydro-6-hydroxy-4-oxo-7-(4-ethoxycarbonyl-1-piperazinyl)quinoline-3-carboxylic acid (2a′): colorless crystals; mp > 260 °C (nitroethane); ^{1}H NMR [$(CD_3)_2SO$] δ 1.2 (t, 3H, J = 7 Hz), 1.5 (t, 3H, J = 7 Hz), 3.25 (m, 4H), 3.6 (m, 4H), 4.08 (q, 2H, J = 7 Hz), 4.55 (q, 2H, J = 7 Hz), 7.08 (s, 1H), 7.68 (s, 1H), 8.82 (s, 1H), 10.2 (broad s, exch., 1H), 15.6 (broad s, exch, 1H); ^{13}C NMR [$(CD_3)_2SO$] δ 18.5, 18.55, 47.0, 53.0, 53.3, 54.8, 109.3, 110.4, 112.6, 124.4, 137.9, 150.3, 152.5, 153.0, 158.5, 170.7, 175.8. Anal. Calcd for $C_{19}H_{23}N_3O_6$: C, 58.60; H, 5.95; N, 10.79. Found: C, 58.7; H, 5.9; N, 10.6.

1a in Sulfite Buffer (Method B). 1-Ethyl-1,4-dihydro-4-oxo-7-(4-ethoxycarbonyl-1-piperazinlyl)quinoline-3-carboxylic acid (3a′): colorless solid from chromatography, containing a small amount of the ethylcarbamate of the starting substrate **1a**; mass 373 *m/e* M^+; ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 3H, J = 7 Hz), 1.6 (t, 3H, J = 7 Hz), 3.35 (m, 4H), 3.75 (m, 4H), 4.2 (q, 2H, J = 7 Hz), 4.45 (q, 2H, J = 7 Hz), 6.6 (d, 1H, J = 1 Hz), 7.06 (dd, 1H, J = 1, 9 Hz), 8.2 (d, 1, J = 9 Hz), 8.55 (s, 1H).

1b in Water (Method C). 1-Ethyl-6-hydroxy-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (2b): colorless crystals; mp > 260 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 1.45 (t, 3H, J = 7 Hz), 3.0 (m, 4H), 3.9 (m, 4H), 4.5 (q, 2H, J = 4 Hz), 7.65 (s, 1H), 8.7 (s, 1H); ^{13}C NMR [$(CD_3)_2SO$] δ 18.9, 48.6, 50.6, 50.8, 111.3, 117.9, 118.6, 145.9, 147.1, 149.3, 156.3, 170.4, 179.8. Anal. Calcd for $C_{15}H_{18}N_4O_4$: C,

56.59; H, 5.70; N, 17.60. Found: C, 56.7; H, 5.8; N, 17.3. When treated with ethyl chloroformiate in chloroform, this compound gave a material identical to that obtained by method B (see below).

Method B. 1-Ethyl-6-ethoxycarbonyloxy-1,4-dihydro-4-oxo-7-(4-ethoxycarbonyl-1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (2b′): colorless crystals; mp 159 °C (methanol); ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 3H, J = 7 Hz), 1.42 (t, 3H, J = 7 Hz), 1.5 (t, 3H, J = 7 Hz), 3.68 (m, 4H), 3.78 (m, 4H), 41.18 (q, 2H, J = 7 Hz), 4.38 (q, 2H, J = 7 Hz), 4.42 (q, 2H, J = 7 Hz), 8.7 (s, 1H), 8.8 (s, 1H), 14.9 (s, exch, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.5, 14.7, 43.2, 47.3, 47.6, 61.7, 65.9, 109.5, 114.5, 129.4, 135.9, 145.5, 146.2, 146.9, 152.2, 154.2, 166. 6, 177.2. Anal. Calcd for $C_{18}H_{22}N_4O_6$: C, 55.38; H, 5.68; N, 14.35. Found: C, 55.3; H, 5.8; N, 14.2.

1b in Sulfite Buffer (Method B). 1-Ethyl-1,4-dihydro-4-oxo-7(4-ethoxycarbonyl-1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (3b′): light yellow solid from chromatography; mass 374 *m/e* M^+; ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 3H, J = 7 Hz), 1.6 (t, 3H, J = 7 Hz), 3.65 (m, 4H), 3.75 (m, 4H), 4.2 (q, 2H, J = 7 Hz), 4.45 (q, 2H, J = 7 Hz), 6.82 (d, 1H, J = 9 Hz), 8.45 (d, 1H, J = 9 Hz), 8.7 (s, 1H). Anal. Calcd for $C_{18}H_{22}N_4O_5$: C, 57.74; H, 5.92; N, 14.93. Found: C, 57.8; H, 5.9; N, 14.8.

1c in Water (Method A). 8-Fluoro-1,2-dihydro-9-(4-ethoxycarbonyl-3-methyl-1-piperazinyl)-6-oxo-6H-pyrrolo-[3,2,1-ij]quinoline-5-carboxylic acid methyl ester (4′): light yellow crystals; mp 233−234 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 3H, J = 7 Hz), 1.4 (d, 3H, J = 7 Hz), 3.1(m, 2H), 3.4 (m, 2H), 3.3 and 4.0 (AB part of an ABX system, 2H), 3.6 (t, 2H, J = 8 Hz), 3.9 (s, 3H), 4.15 (q, 2H, J = 7 Hz), 4.4 (X part, 1H), 4.55 (t, 2H, J = 8 Hz), 7.6 (d, 1H, J = 12 Hz), 8.45 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.6, 15.2, 22.3, 39.2, 47.1, 50.3, 51.7, 51.8, 55.0, 61.4, 110.2 (d, J_{C-F} = 25 Hz), 110.7, 120.7 2 (d, J_{C-F} = 7 Hz), 123.9 2 (d, J_{C-F} = 7 Hz), 140.6, 140.65 2 (d, J_{C-F} = 15 Hz), 142.9, 155.3, 156.3 (d, J_{C-F} = 250 Hz), 166.4, 173.0. Anal. Calcd for $C_{21}H_{24}FN_3O_5$: C, 60.42; H, 5.80; N, 10.07. Found: C, 60.3; H, 5.8; N, 10.0.

1c in Sulfite Buffer (Method A) (Besides 4′). 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-ethoxycarbonyl-3-methyl-1-piperazinyl)quinoline-3-carboxylic acid methyl ester (3c′): colorless crystals; mp 220 °C (methanol); ^{1}H NMR ($CDCl_3$) δ 1.38 (t, 3H, J = 7 Hz), 1.4 (d, 3H, J = 7 hz), 1.55 (t, 3H, J = 7 Hz), 2.85 and 3.4 (two m, 2H), 3.0 and 3.5 (two m, 2H), 3.45 and 4.05 (two m, 2H), 3.9 (s, 3H), 4.2 (m, 4H), 4.4 (m, 1H), 6.7 (d, J = 7 Hz), 8.05 (d, 1H, J = 14 Hz), 8.4 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 14.5, 15.3, 38.6, 46.9, 48.8, 49.6 (d, J_{C-F} = 2 Hz), 51.9, 54.8 (d, J_{C-F} = 2 Hz), 61.4, 103.8 (d, J_{C-F} = 2 Hz), 110.2, 113.7 (d, J_{C-F} = 22 Hz), 123.9 (d, J_{C-F} = 6 Hz), 135.9, 145.1 (d, J_{C-F} = 11 Hz), 151.5, 153.2 (d, J_{C-F} = 246 Hz), 155.2, 166.5, 172.9. Anal. Calcd for $C_{21}H_{26}FN_3O_5$: C, 60.13; H, 6.25; N, 10.02. Found: C, 59.9; H, 6.3; N, 9.9.

1d in Water (Extraction with Chloroform and Treatment with CH_2N_2). 9-Fluoro-2,3-dihydro-3-methyl-10-(*N*″-methyl-*N*′,*N*″-diformyl-2-aminoethylamino)-7*H*-pyrido-[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid methyl ester (5′): colorless crystals; mp >260 °C (nitroethane); ^{1}H NMR ($CDCl_3$) δ (the NC*H*O signals are split in two due to hindered rotation of the amide groups) 1.6 (d, 3H, J = 7 Hz), 2.7 (s, 3H), 3.0 (s, 3H), 3.4−3.7 (m, 2H), 3.8−4.1 (m, 2H), 3.9 (s, 3H), 4.35−4.55 (m, 3H), 7.8 (d, 1H, J = 10 Hz), 7.9/7.92 (s, 1H), 8.18/8.2 (d, 1H, J = 2 Hz), 8.45 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (almost all signals split in two) 17.7/17.9, 29.0/34.6, 41.8/40.9, 43.0/47.3 (d, J_{C-F} = 3 Hz), 52.25/52.2, 54.3/57.5, 54.3/57.5, 69.2/68.9, 105.8/105.2 (d, J_{C-F} = 23 Hz), 110.8/110.7, 120.0/119.9 (d, J_{C-F} = 18 Hz), 123.6, 128.8/128.7 (d, J_{C-F} = 7 Hz), 142.4 (d, J_{C-F} = 67 Hz), 145.6, 155.1/155.6 (d, J_{C-F} = 250 Hz), 162.6/162.5, 163.3, 165.6/165.8, 172.0/172.1. Anal. Calcd for $C_{19}H_{22}FN_3O_4$: C, 60.79; H, 5.91; N, 11.19. Found: C, 60.6: H, 6.0: N, 11.0.

Small Scale Experiments. Photochemical reactions were carried out on 10 mL portions of aqueous solution of the drugs (1×10^{-4} M) in serum-capped quartz tubes. These were irradiated in a merry-go-round apparatus by means of two 15 W phosphor-coated lamps (center of emission 313 nm). When required, deoxygenation of the solution was obtained by flushing for 1 h with argon passed through a Supelco carrier gas purifier.

The reactions were also carried out in spectrophotometric quartz couvettes on an optical bench, in that case using 3 mL samples of 0.5 or 1×10^{-4} M drug solution and irradiating by a focused beam from an Osram 200 W high-pressure mercury arc and an interference filter (transmission peak at 317 nm, bandwidth 8 nm). These experiments as well as those above were carried out at low conversion (<30, in most cases <20%), and the reported Φ values are the average of at least three independent measurements (±10%).

The substrate decomposition was determined by HPLC (Waters model 501 apparatus) with optical detection (Waters 490E, λ 276 nm). An inverse-phase Merck Purosphere RP-18 e (5 μm) column (3 × 125 mm) was used. The eluant was an 85:15 mixture of pH 3 buffer (prepared by adding phosphoric acid to a 0.75% solution of triethylamine until pH 3 was reached) and acetonitrile. A few microliters of a solution of an appropriate standard (**1c** for **1a**, **1d** for **1b** and **1c**, **1b** for **1d**) were added to the photolyzate before analysis.

The fluoride concentration was measured by means of an Orion SA520 potentiometer using a selective electrode (Orion F-94-09) after addition to the 10 mL photolysate of 2 mL of Orion TISAB III buffer (ammonium acetate, ammonium chloride, 1,2-cyclohexylidene-α,δ-dinitrilotetraacetic acid) and dilution to 22 mL with distilled water.

The light flux was measured by ferrioxalate actinometry.[36]

Laser Flash Photolysis. Nanosecond laser flash photolysis experiments were performed by means of a Nd:YAG JK lasers (pulse 20 ns full width at half-maximum (fwhm), 355 nm). The setup for the nanosecond absorption measurements has been described previously.[16b] The laser beam was focused on a 3 mm high and 10 mm wide rectangular area of the cell, and the first 2 mm was analyzed at a right angle geometry. The energy used was ca. 2 mJ/pulse. Time "zero" was taken at the onset of the laser pulse. Spectral resolution was 2 nm. The sample absorbance was ca. 0.6 at 355 over 1 cm. Oxygen was removed by vigorously bubbling the solutions with a constant flux of argon, previously passed through a water trap to prevent evaporation of the sample. Care was taken to renew the solution at each laser shot. Temperature was 295 ± 2 K.

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