Degradation of pharmaceuticals through sequential photon absorption and photoionization – the case of amiloride derivatives

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Abstract
Photodegradation of pharmaceutical and agrochemical compounds is an important concern for health and the environment. Amiloride derivatives undergo clean photosubstitution in protic solvents. We have studied this apparent photo-SN\textsubscript{Ar} reaction with a range of experimental and computational techniques. Available evidence points to a mechanism starting with photoexcitation followed by absorption of a second photon to eject an electron to give a radical cation intermediate. Subsequent substitution reaction with the protic solvent is assisted by a general base, possibly strengthened by the proximal solvated electron. Final recombination with the solvated electron generates the observed product. Quantum chemical computations reveal that excited state antiaromaticity is relieved when an electron is ejected from the photoexcited molecule by the second photon, leading to a weakly aromatic radical cation. The mechanism indicated here could have wide applicability to photoinduced degradation of similar heteroaromatic compounds in the environment as well as in protic solvents. There are also strong similarities to a class of increasingly popular synthetic photoredox methods.

Introduction
Pharmaceutical research is aimed at increasing quality of life globally by finding cures to debilitating or fatal diseases. The progress in recent years has been astounding, but the route to a new drug still faces many obstacles, in particular regarding safety, that impedes and increases the cost of pharmaceutical development. One important safety factor is drug product degradation, which can give impurities with potentially dangerous properties. The identification and investigation of all possible degradation products forms a significant part of the work that must precede the first dosing to human subjects. In some cases, degradation pathways are well understood and can be predicted using computational tools.\textsuperscript{1} However, photochemical processes, leading to degradation and phototoxicity, are still less well understood than, for example, oxidative processes. Increased understanding of these processes could lead to better predictions of benefit to all drug development. The same processes are also important contributors to degradation of other substances in the environment, such as agrochemicals.
One of the most important photochemical degradation pathways of relevance to many drugs is photodehalogenation, which can occur either by photosubstitution, typically by the solvent, or photoreduction (Figure 1a). For example, chlorpromazine, an antipsychotic drug, reacts by photosubstitution in water, while irradiation in methanol leads to both photoreduction and photosubstitution in a 1:1 ratio (Figure 1b). Reaction in more substituted alcohols favors the reduction product. As with most photodegradations, the complete product profile is complicated and highly dependent on the reaction conditions, e.g., presence of oxygen. Irradiation under conditions simulating the aquatic environment leads to a total of 57 photoproducts, the major ones from photosubstitution and oxidation. Another prominent drug undergoing photodehalogenation is diclofenac (Figure 1b). Upon irradiation, it undergoes ring-closure and loss of one of the chlorine substituents to form a carbazole. Further irradiation leads to both photoreduction (major) and photosubstitution (minor) in water or methanol. Other drugs that undergo photodechlorination include frusemide, chloroquine and hydrochlorothiazide (Figure 1b). In addition, similar photodegradations could occur in compounds used in the agrochemical sector.

Figure 1. (a) Photosubstitution and photoreduction mechanism for dehalogenation of aromatic substrates. (b) Examples of drug molecules undergoing photodehalogenation.

In the process of our development work at AstraZeneca, some of the authors became interested in the photodegradation of compounds related to the diuretic drug amiloride (1, Figure 2a). The photochemistry of 1 is dominated by the pyrazine moiety, and irradiation in water leads to photosubstitution of the chlorine substituent as well as further secondary photoreactions (vide infra). To better understand the photodegradation mechanism of this family of compounds, we investigated the amiloride analogue 2 in water and other protic solvents. We find that the primary photoreaction of 2 is photosubstitution by the solvent. The mechanism likely involves photoionization to the radical cation, induced by sequential absorption of two photons, followed by a concerted SNAr-type reaction in which the chlorine is displaced by attack of solvent. This finding has implications not only for drug development, but also synthetic organic chemistry. In recent years, photochemistry has emerged as a new way to access thermodynamically unfeasible modes of reactivity, opening previously inaccessible routes to target compounds. Of particular interest in this context is the work of Nicewicz, who showed that photoredox chemistry could be harnessed to effect nucleophilic aromatic substitution (SNAr) of nonactivated substrates. The SNAr reaction typically requires activated substrates carrying electron-withdrawing groups (Figure 2b), but Nicewicz and coworkers showed that photooxidation of the substrate to the radical cation can increase its reactivity substantially. Here we show that similar reactivity can also be accessed without the use of a separate photoredox catalyst, by sequential photoabsorption of the substrate to access the radical cation intermediate. This sequential photoabsorption is especially notable in light of recent work of Wenger and König on harnessing the energy of two sequential photoexcitations to perform unprecedented transformations.
Figure 2: (a) Amiloride (1), experimentally studied derivative (2), computational model (2'), and derivative lacking terminal amino group (2"). (b) S$_N$Ar reactions generally require an activated substrate with electron-withdrawing groups. Nicewicz showed that oxidation by a photoredox catalyst can activate the substrate. Here we show that photoionization by sequential absorption of two photons can also activate the substrate, without the need for a separate photoredox catalyst.

**Photochemistry of amiloride**

The primary photoreaction of 1 in water is substitution of Cl to give the hydroxylated product 1–OH (Figure 3). This substitution reactivity contrasts with similar compounds, e.g., frusemide and hydrochlorothiazide, which undergo both photosubstitution and photoreduction. One explanation put forth in the literature is that frusemide and hydrochlorothiazide form an ion-pair complex [Ar–Cl]$^{\cdot+}$ + [Ar–Cl]$^{\cdot-}$ by electron transfer from one molecule in the excited state to another molecule in the ground state. The resulting [Ar–Cl]$^{\cdot+}$ radical cation reacts with solvent to form Ar–OH, while the [Ar–Cl]$^{\cdot-}$ radical anion cleaves off a chloride anion and abstracts a hydrogen atom from the solvent to form Ar–H. As 1 only gives the Ar–OH product, Moore and co-workers argued that the mechanism is different, and suggested that substitution occurs by attack of the water solvent on the radical cation of 1, formed by photoionization. The hypothesis was supported by the observation of photoionization of 1 in aqueous solution accompanied by the formation of solvated electrons at both 265 nm and 353 nm, as shown by Hamoudi et al. The quantum yield of photoionization is 0.011 at pH 7 and increases with pH. The pH dependence of photoionization is similar to that of the quantum yield of amiloride photodegradation which increases from 0.009 at low pH (4.0) to 0.023 at high pH (10.4), with an inflection point at ca pH 8. The location of the inflection point compares well with the pK$_a$ of 1 at 8.7. It is expected that the neutral base form of amiloride would give away an electron more readily than the pronated, cationic form. Interestingly, Hamoudi did not observe the formation of solvated electrons in i-PrOH. Instead, the triplet amiloride was postulated to abstract hydrogen from i-PrOH.
While the primary photoreaction of 1 is photosubstitution, the compound degrades further upon continued irradiation as its photoproduct 1–OH absorbs in the same region. Moore studied photodegradation under oxygen-free conditions using a medium pressure mercury lamp (Pyrex glass filter, >300 nm) and found that 1–OH forms with apparent first-order kinetics over the studied pH range of 4–11. The 1–OH product exists predominately as its keto tautomer. After ca 50% conversion, unidentified secondary photoproducts started to appear. Using similar conditions, Calza and co-workers suggested a dihydroxy-substituted product which degraded upon continued irradiation (Figure 3). De Luca and co-workers studied the pH dependence at 300–800 nm under aerobic conditions and suggested three secondary photoproducts based on LC-MS analysis (Figure 3). An explanation for the discrepancy between these studies may be that Calza and co-workers used anaerobic conditions, promoting reactivity from the triplet state of 1 and preventing singlet oxygen-induced degradation pathways.

**Results and discussion**

To clarify the mechanism of the primary photoreactivity of the amiloride family, we chose to study compound 2 in detail. Compound 2 was synthesized in a single step from commercially available building blocks according to a literature procedure (Scheme 1). The absorption spectrum in methanol shows three main peaks in the UV region, at 210 nm, 273 nm and 360 nm (Figure 4). The spectrum of 2 is very similar to the spectrum of 1 in water, which has main peaks at 212 nm, 285 nm and 362 nm. TD-DFT calculations of 2 show that both the peak at 360 nm (HOMO → LUMO) and at 273 nm (HOMO–2→LUMO+2) are of $\pi\pi^*$ character (see Supporting Information, Section 3).
Figure 4. Absorption spectrum of 2 in methanol.

Initial photochemical experiments at 350 nm in argon-saturated H$_2$O gave the hydroxy-substituted product 2–OH, showing that 2 reacts by photosubstitution in the same way as 1 (Table 1). Unfortunately, we were not able to determine the conversion due to the overlap between the reactant and the product peaks. In methanol, the methoxy-substituted 2–OMe was obtained with 68% conversion after 2 h of irradiation with traces of an unknown by-product. Irradiation in the more substituted alcohol solvents EtOH, i-PrOH and t-BuOH for the same time also resulted in photosubstitution but with lower conversions (60%, 46% and 20% respectively, entry 3–5). As far as we know, this is the first report of photoreactivity in alcoholic solvents for amiloride-like compounds. To test for the influence of the terminal amino group, we synthesized derivate 2” (Figure 2), where the alkyl amine is replaced with a methyl group. Irradiation of 2” under the standard conditions led to 57% conversion to the methoxy-substituted product 2”–OMe, showing that the terminal alkyl amine is not essential for the observed photoreactivity. Varying the light intensity using 2–16 of the lamps in the Rayonet reactor showed consistent reactivity with 2–OMe as the photoproduct (Figure S28).
Table 1. Reaction of 2 with various nucleophilic solvents.

| Entry | Solvents  | Product     | Yield* (%) |
|-------|-----------|-------------|------------|
| 1     | H₂O       | 2-OH        | a          |
| 2     | MeOH      | 2-OMe       | 68         |
| 3     | EtOH      | 2-OEt       | 60         |
| 4     | t-PrOH    | 2-O-t-Pr    | 46         |
| 5     | t-BuOH    | 2-O-t-Bu    | 20         |
| 6     | MeCN      | 2-NHCOCH₃   | b          |

Reaction condition: A 0.002 M solution of 2 was irradiated at 350 nm for 2 hrs using a Rayonet reactor.
a) Not able to determine the conversion as signals from the product and the starting material overlap.
b) Only starting material was observed. * NMR yield.

Mechanistic scenarios
In order to elucidate the reaction mechanism, we investigated several plausible scenarios (Figure 5). After Moore’s hypothesis of photoionization and subsequent nucleophilic attack by the solvent (Figure 5.3) was formulated, another possible mechanism for photoinduced dehalogenation was reported by Fagnoni and co-workers for similar substrates. This mechanism involves initial formation of an aryl cation by heterolytic cleavage of the C–Cl bond in the S₁ or T₁ state (Figure 5.1). The resulting ground state singlet aryl cation then reacts with a nearby solvent molecule. We call this mechanism “photo-S₅₁”, by analogy with its ground-state equivalent. We have further computationally investigated the possibility of a more traditional S₅Ar mechanism in S₁ or T₁ (Figure 5.2). For completeness, we also list photoreduction (Figure 5.4), which is not observed for neither 1, nor 2 and which will not be considered further.
Figure 5. Investigated mechanistic scenarios.

Photo-S_N1

DFT calculations
As an initial plausibility check of heterolytic chloride dissociation and aryl cation formation in S_1 and T_1, we used TD-DFT calculations on the model compound 2^* (Supporting Information, Section 2.1). The calculations showed a barrier for chloride dissociation of only 5.0 kcal/mol in the T_1 state and 5.4 kcal/mol in the S_1 state. These low barriers would make the reaction feasible at least in the T_1 state, given its longer lifetime. However, the calculations also showed that the reaction was endergonic both in T_1 (ΔG = 3.9 kcal/mol) and S_1 (ΔG = 5.0 kcal/mol), raising the question whether
it would be disfavored thermodynamically. Given that dissociation would happen, the resulting aryl cation would relax to a ground state of either singlet or triplet multiplicity.\textsuperscript{23} Our DFT calculations indicate that the triplet is 1.6 kcal/mol more stable, which is confirmed by high-level CASPT2 calculations (2.3 kcal/mol). The calculations show that the singlet aryl cation would add solvent molecules (H\textsubscript{2}O, MeOH, i-PrOH or t-BuOH) without any barrier, while the triplet cation is unreactive. With an energy gap of only ca 1–2 kcal/mol between singlet and triplet, inter-system crossing would be feasible and reaction with nearby solvent molecules should be rapid. In summary, the photo-S\textsubscript{N}1 mechanism seems plausible from a computational point of view, but as we shall see below, it turns out that it is not consistent with experimental findings.

**Competition experiments**

To test the photo-S\textsubscript{N}1 hypothesis experimentally, we turned to competition experiments. We first tested competition in solvent mixtures. The aryl cation intermediate is extremely reactive and would not discriminate between nucleophiles. In MeOH/H\textsubscript{2}O (1:1 v/v), 2-OH and 2-OMe were obtained in an equal ratio (Table 2, entry 1). As the molar ratio of MeOH to H\textsubscript{2}O is 1:2.2, there is a slight preference for MeOH over H\textsubscript{2}O as nucleophile. With MeOH/EtOH (1:1 v/v), a 3:1 mixture of 2-OMe and 2-OEt was obtained (Table 2, entry 2). As the molar ratio is only 1.4:1, MeOH is here preferred over EtOH. In MeOH/i-PrOH (1:1 v/v), eight times as much 2-OMe was obtained than 2-O-i-Pr\textsubscript{2}, showing that MeOH is clearly superior to i-PrOH as nucleophile as the molar ratio is in its favor only by 1.9:1.

| Entry | Solvent ratio (v/v) | Product | Product ratio | Solvent ratio |
|-------|---------------------|---------|---------------|---------------|
| 1     | MeOH/H\textsubscript{2}O (1:1) | 2-OMe:2-OH | 1:1           | 1:2.2         |
| 2     | MeOH/EtOH (1:1)     | 2-OMe:2-OEt | 3:1           | 1.4:1         |
| 3     | MeOH/i-PrOH (1:1)   | 2-OMe:2-O-i-Pr | 8:1          | 1.9:1        |

Reaction condition: A 0.002 M solution of 2 was irradiated at 350 nm for 2 hrs using a Rayonet reactor.

Although the differences in reactivity between the different nucleophiles is strong evidence against the photo-S\textsubscript{N}1 mechanism, they could in principle be consistent with subtle differences in preferential solvation of the aryl cation.\textsuperscript{24} Therefore, we carried out another round of experiments. Aryl cations are known to coordinate to acetonitrile, resulting in the formation of acetamides after attack by water. (Error! Reference source not found.). This reaction, which is the photochemical equivalent of the Ritter reaction, has been observed previously for aryl cation intermediates of other compounds by Albini and co-workers.\textsuperscript{25} Our DFT calculations show that acetonitrile coordination to the aryl cation of 2’ is highly exergonic (\(\Delta G = -53.0\) kcal/mol). Subsequent addition of H\textsubscript{2}O occurs with a barrier of 17.4 kcal/mol, which should be feasible at room temperature. Therefore, acetonamide product 3 would be expected to form upon irradiating 2 in MeCN or MeCN/H\textsubscript{2}O mixtures. However, irradiation in neat MeCN did not result in acetamide 3 or the alternative cyclized imidazopyrazine 4. Irradiation in solvent mixtures of MeCN with MeOH or H\textsubscript{2}O (9:1 v/v) only gave trace amounts of 2-OMe and 2-OH, respectively, and no evidence of
If the aryl cation is formed, it should react statistically as addition of MeOH/H$_2$O and MeCN are all barrierless processes. Furthermore, irradiation of 2 at 365 nm in DMSO in the presence of KCN (10 equiv.) did not result in any cyano-substituted product. Also, no reaction occurred under irradiation in MeCN with tetrabutylammonium cyanide (TBACN, 10 equiv).

Experiments with π-nucleophiles also make the photo-S$_{N}$1 mechanism unlikely. The triplet aryl cation, which is predicted to be the ground state according to our calculations, should readily add π-nucleophiles to give the allylated or vinylated product, respectively. We tested reaction with both allylsilane and styrene in MeCN/MeOH (9:1 v/v) with irradiation for 2 hrs at 365 nm, but no addition product was found. Lack of reactivity with styrene and allylsilane also indicate that radicals are not generated during the reaction.

In conclusion, an array of experiments are inconsistent with the photo-S$_{N}$1 mechanism. The non-statistical outcomes of the competition experiments in mixed solvents and the lack of reactivity with MeCN and CN$^-$ are not consistent with barrierless addition of the closest nucleophile to a very reactive singlet aryl cation intermediate. The lack of reactivity with π-nucleophiles is not consistent with a triplet aryl cation intermediate. One possible explanation why the reaction does not occur despite the calculated small barrier is the endergonicity of the initial dissociation of Cl$^-$ in S$_1$ and T$_1$. 

Figure 6. Experiments excluding aryl cation intermediate. Lack of triplet state reactivity with styrene and allylsilane and lack of singlet state reactivity with CN$^-$ and MeCN. Path A: Proposed mechanisms of the photo-Ritter reaction in acetonitrile/water mixtures. Path B: Proposed reaction pathway for the formation of cyclized product imidazopyrazine 4.
**Concerted or stepwise S$_{\text{N}}$Ar**

We further considered the possibility that the photoreaction proceeds via a S$_{\text{N}}$Ar mechanism in the excited state. However, DFT calculations showed a high barrier for attack by H$_2$O in the T$_1$ state (36.4 kcal/mol, using another H$_2$O as general base). This barrier is much too high for the reaction to take place in this short-lived excited state. Interestingly, the reaction is calculated to be concerted, with displacement of Cl$^-$ occurring simultaneously with C–O bond formation. Considering OH$^-$ as the nucleophile (standard state of 10$^{-7}$ M) lowered the barrier to 18.9 kcal/mol, but as will be shown below, hydroxide is not a plausible nucleophile in the reaction. The barrier is of similar magnitude in the S$_1$ ππ* state (22.1 kcal/mol). For more details, see Supporting Information, Section 2.2. Based on these calculations, the photo-S$_{\text{N}}$Ar mechanisms is highly implausible.

**Photoionization**

**Detection of photocurrent and thermodynamics of photoionization**

Having excluded the photo-S$_{\text{N}}$1 and photo-S$_{\text{N}}$Ar mechanism, we then assessed photoionization followed by nucleophilic attack by the solvent (Figure 5.2). As shown recently by Nicewicz in the context of photoelectrocatalysis, aren radical cations are much more prone to undergo S$_{\text{N}}$Ar reactions than the neutral compounds.\textsuperscript{11,12} We investigated photoionization of 2 by photoelectrochemistry in MeOH, EtOH and i-PrOH. A photocurrent corresponding to the release of solvated electrons was detected in MeOH and was found to correlate with light intensity and applied bias potential (see Supporting Information, Section 9). The formation of solvated electrons is in accordance with the flash photolysis experiments by Hamoudi.\textsuperscript{18} Photocurrent measurements in a series of alcohols (MeOH, EtOH and i-PrOH) detected similar photoinduced currents within experimental error in all tested solvents, in line with the observed formation of substitution products. The detection of a photocurrent for 2 in i-PrOH is different compared to 1, for which Hamoudi detected solvated electrons in H$_2$O but not in i-PrOH.

Hypothesizing that more electron-rich arenes should photoionize more efficiently, we compared the reactivity of 2 with its ester analogue S2 (see Supporting Information, Section 8 and 9). Cyclic voltammetry showed that S2 is more electron-poor than 2, as evidenced by its higher redox potential. Consistent with its electronic properties, we observed smaller generated photocurrent and lower reactivity by a factor of 6 as compared to 2. The monoamino-substituted pyrazine derivative S1 has an even higher redox potential and no detectable photocurrent. Below, we discuss the influence of the frontier orbital energies for the rationalization of photoionization.

While Hamoudi and co-workers argued that photoionization of 1 is a monophotonic process at 353 nm and a biphotonic process at 265 nm,\textsuperscript{18} we find that this is not thermodynamically feasible. The calculated solution-phase vertical ionization potential (IP\textsubscript{vert}) of 2 is 6.08 eV (204 nm), very close to the 5.98 eV (207 nm) calculated for 1. Considering the possibility of adiabatic ionization, and that the electron is transferred to bulk water with a hydration enthalpy of −1.34 eV,\textsuperscript{26} the best-scenario cost of photoionization is reduced to 4.57 eV (271 nm) for 2 and 4.43 (280 nm) for 1. Clearly, these values are still too high for a monophotonic process at 353 nm, even allowing for a large error in the calculated values. As noted by Grabner and El-Gogary,\textsuperscript{27} a linear relationship between laser power and concentration of solvated electrons, as was observed by Hamoudi, is not sufficient evidence to conclude a monophotonic process. Measurements over a wide range of
irradiation powers and correcting for non-linear behavior with pulse energy would be needed, which was not done by Hamoudi.\textsuperscript{18} For a more extensive discussion, see Supporting Information, Section 2.3.2. We therefore conclude that the photoionization of 1 and 2, as shown by the observed photo-current, is a biphotonic process not only at 265 nm but also at 353 nm.

**Identity of the nucleophile**

Having shown that photoionization occurs in all protic solvents investigated, we then turned to the next step of the reaction: nucleophilic attack on the radical cation. The nucleophile could be either a neutral or deprotonated solvent molecule depending on the pH. DFT calculations showed that attack by H$_2$O on the model 2$^{++}$ occurs with a considerable barrier of 27.5 kcal/mol using the SMD implicit solvent model. However, the barrier was lowered considerably to 19.6 kcal/mol by adding another explicit water molecule acting as a general base. Barriers of similar height were obtained for 2$^+$ and was not affected significantly by protonation of the terminal amine (see Supporting Information, Section 2.3.1). Considering the known limitations of DFT for reactions of charged species in solution, we believe that these barriers are consistent with reaction on the microsecond timescale as dictated by the expected lifetime of the radical cation.\textsuperscript{28,29}

A competing pathway in neutral or slightly basic solution is attack by hydroxide or alkoxides. We measured the pH of a 0.8 mM aqueous solution of 2 to 8.5–8.7, which would mean a hydroxide concentration of ca 4 μM (Supporting Information, Section 5). DFT calculations indicate that OH$^-$ and MeO$^-$ react with 2$^{++}$ in a barrierless manner, while EtO$^-$, i-PrO$^-$ and t-BuO$^-$ show manageable barriers of 5.9 kcal/mol, 5.4 kcal/mol and 11.1 kcal/mol, respectively (a standard state of 10$^{-7}$ M is used for the alkoxides). These computational results are consistent with the slower reactivity in EtOH, i-PrOH and t-BuOH as compared to MeOH and H$_2$O (Table 1). However, there are also other plausible explanations for the observed difference in reactivity. One such explanation is the lower rates of autoprotolysis of the more substituted alcohols (Table 3). For example, the ambient concentration of OH$^-$ in H$_2$O is 14 orders of magnitude larger than that of t-BuO$^-$ in t-BuOH. As 2 is a weak base, the alcohols’ pK$_a$ is also relevant (Table 3). The more substituted alcohols are harder to deprotonate, and we therefore expect lower concentrations of alkoxide nucleophile. The nucleophilicities of the alkoxide anions follow the same trend (Table 3).

|      | pK$_a$\textsuperscript{a} | pK$_a$\textsuperscript{b} | N (RO$^-$)\textsuperscript{c} |
|------|----------------|----------------|-----------------|
| H$_2$O | 14.0          | 15.7          | 10.47           |
| MeOH  | 16.5–16.7     | 15.5          | 15.78           |
| EtOH  | 18.7–19.5     | 15.9          | 15.78           |
| i-PrOH| 20.6–20.8     | 17.1          | 17.03           |
| t-BuOH| 28.5          | 19.2          | -               |

\textsuperscript{a} Autoprotolysis constants at 25°C. Ref. 30. \textsuperscript{b} Acid dissociations constants in water. Ref. 31. \textsuperscript{c} Mayr nucleophilicity parameter for alkoxide in the corresponding alcohol.

To test the hypothesis that OH$^-$ is the active nucleophile, we performed competition experiments with AcO$^-$. When both AcOH and H$_2$O are present, the ratio AcO$^-$/OH$^-$ should be determined by their relative pK$_a$, ca. 10\textsuperscript{12}. Detailed calculations show that AcO$^-$ should be present in a concentration of ca 0.02 M with a pH of 1.8, meaning that the ratio of AcO$^-$ to OH$^-$ is 3·10\textsuperscript{10} (see Supporting Information, Section 4). Computationally, we find that attack by AcO$^-$ on 2$^{++}$ has a
barrier of only 8.0 kcal/mol ($10^{-2}$ M standard state). In case the reactive species is the anion, acetate should therefore outcompete hydroxide by a wide margin. From the experiment performed in a 1:1 ratio (v/v) of AcOH and H$_2$O, we see only trace amount of 2-OH by LC-MS (227 m/z) and no acetylated product 2-OAc. General base catalysis is also supported by irradiation of 2 in D$_2$O vs H$_2$O, with an observed rate difference of ca 10%. This is clearly a solvent isotope effect, as expected if a selectivity-determining nucleophilic attack by a solvent molecule is assisted by a general base. The observed rate enhancement is a function of a competition between the rate of nucleophilic attack and the unknown rate of electron recombination (leading back to starting material). We therefore cannot determine an exact value for the KIE, but we can see that it is clearly larger than one. We therefore conclude that the nucleophile must be the neutral species, assisted by a general base in solution. Another piece of evidence against OH$^{-}$ as the active nucleophile comes from experiments by De Luca et al. They observed similar rates of formation of photoproduct at pH 3 and pH 7, even though the concentration of OH$^{-}$ is negligible at pH 3 ($10^{-11}$ M).

As the photochemical reaction is electroneutral, the radical cation must be reduced after the substitution step (Figure 7). This implies that the solvated electrons cannot quantitatively react with solvent to produce dihydrogen and hydroxide (alkoxide). Due to its negative charge, it may instead enhance the ability of the local water molecule to act as a general base towards the nucleophile in the substitution reaction, eventually recombining with the product radical cation to give the observed product. Considering that the solvent act as nucleophile with a high effective concentration, we believe that this process is competitive with decay of the solvated electrons, which likely occurs on the $\mu$s timescale.

![Figure 7](image_url)

**Figure 7.** Suggested mechanism for photosubstitution of 2. Photoionization occurs by sequential absorption of two photons, with the second absorbed either from the $S_1$ state (less likely) or $T_1$ state (more likely). The resulting radical cation is attacked either by solvent or hydroxide/alkoxide with concerted loss of Cl$^{-}$. Deprotonation and recombination with the electron leads to the substitution product 2-OH. The solvated electron plausibly acts as a general base to assist reactivity.

**Excited state antiaromaticity as a potential driving force for photoionization**

Having established that photoionization by sequential absorption of two photons is the most likely mechanism, we now turn to the question of driving force. One possible explanation for the facile photoionization of 2 is based on excited state antiaromaticity. Ground state aromatic compounds become antiaromatic in their lowest singlet and triplet $\pi\pi^*$ states according to Baird’s rule, the excited state equivalent of Hückel’s rule.33 Aromatic compounds are much more reactive when
photoexcited,\textsuperscript{34,35} and it has recently been shown that new photochemical reactions can be developed with relief of excited state antiaromaticity as the driving force.\textsuperscript{36,37} We now hypothesize that excited state antiaromaticity can also be a driving force for photoionization from the excited state.

Aromaticity in the ground state is linked with high ionization potentials (IP),\textsuperscript{38} while non-aromatic and anti-aromatic compounds display lower IPs (Figure 8a). The reason is that antiaromatic compounds have smaller frontier orbital splittings, and their HOMOs are consequently higher in energy than for aromatic compounds. Photoionization could occur either in a monophotonic process directly from the HOMO, or from the LUMO in a biphotonics process via initial excitation to the excited state (HOMO $\rightarrow$ LUMO). For the monophotonic process, ground state aromatic compounds like 2 should have a relatively high IP. For the biphotonic process, on the other hand, excitation occurs from a relatively high-lying LUMO and should be more facile. Within this context, removing an electron from the LUMO could be seen as a way for excited state antiaromatic compounds to relieve their antiaromaticity. This qualitative picture is corroborated by our calculations, showing that the IP decreases much more going from ground to excited state for ground state aromatic compounds than for antiaromatic ones (see Supporting Information Section 2.3.3). Indeed, intramolecular charge transfer of an electron from an excited state antiaromatic benzene ring was recently found to occur during photodissociation of a protecting group.\textsuperscript{39,40}

Now, how does the aromaticity of 2 change upon photoexcitation and subsequent ionization to the radical cation? We investigated this with calculations on the model compound 2'. Ring currents according to the anisotropy of the induced current density (ACID) method\textsuperscript{41} reveal a moderate diatropic (aromatic) ring current in the $S_0$ ground state of 2', while the relaxed $T_1$ state is antiaromatic with an appreciable paratropic ring current (Figure 8a). Photoionization to the radical cation 2'' is accompanied by loss of antiaromaticity and regain of aromaticity. These findings are further corroborated by multiter center index (MCI) calculations and nucleus-independent chemical shift (NICS) scans (Figure 8b).\textsuperscript{42,43,44} Furthermore, the similar MCI values for the relaxed $T_1$ (0.0057) and $S_1$ states (0.0089) indicate that the $S_1$ state of 2' is also antiaromatic. The loss of aromaticity according to MCI is consistent with that for the parent pyrazine going from the ground state (0.0644) to the corresponding $\pi\pi^*$ triplet (0.0019) and singlet (0.0061) excited states. Due to the longer $T_1$ than $S_1$ lifetime, it is likely that photoexcitation occurs from the $T_1$ state. In summary, a contributing factor to the facile photoionization of 2 could therefore be antiaromaticity in the excited state, which is alleviated when going from the $T_1$ state to the radical cation.
Figure 8. (a) Qualitative explanation for why excited state antiaromaticity leads to more facile sequential photoionization as the LUMO level is relatively high for ground state aromatic molecules. (b) ACID plots of $2'$ show a moderate aromatic ring current in the $S_0$ state (left), antiaromatic ring current in the relaxed $T_1$ state (middle) and moderate aromatic ring current in the radical cation $2''^+$ (right). MCI values indicate moderate aromaticity in for $2'$ in $S_0$ and $2''^+$ and antiaromaticity for $2'$ in $T_1$. Reference values for pyrazine is given in parenthesis. (c) NICS scans show
an aromatic minimum in $S_0$ and $2^{\text{1*}}$ and an antiaromatic maximum for the $T_1$ state. Calculations at the B3LYP/6-311+G(d,p) level.

Conclusions and outlook
We have studied the apparent photo-$S_N\text{Ar}$ reaction of amiloride analogs by a combination of experimental and theoretical methods, to evaluate possible reaction mechanisms. The simplest possibility, a conventional $S_N\text{Ar}$ reaction in the excited state, can be excluded by the high calculated barriers in both singlet and triplet states. It is therefore clear that the excited state needs to evolve to a more reactive state. This could occur by dissociation of the chloride in an $S_N1$-type fashion, giving an aryl cation, or by ionization, giving an arene radical cation. Heterolytic chloride dissociation is found to be possible, albeit endergonic. However, the observed reactivity profile does not agree with either a singlet or triplet aryl cation, and we can therefore exclude a photo-$S_N1$ reaction pathway. Due to the anti-aromaticity of the excited state, the energy of the excited electron is high, but not sufficiently high for spontaneous ionization. However, a second photon can eject the electron into the surrounding solvent. The solvated electrons are detected, giving support for the ionization hypothesis. The experimental selectivity profile combined with calculated barriers indicates that the radical cation reacts with a neutral solvent molecule, assisted by another solvent molecule acting as a general base. It is plausible that the basicity of the solvent is strengthened by the nearby solvated electron. We have observed that several protic solvents are competent nucleophiles, but neutral solvents such as acetonitrile are unreactive, giving further support to the hypothesis that a general base is necessary for reactivity. From a pharmaceutical perspective, understanding the degradation mechanism is the first step in creating predictive tools able to alert developers of drugs to potential light sensitivity. The mechanism studied here could also be an important tool to understand the fate of agrochemicals, which generally are exposed to light, water, and air in the environment. In organic synthesis, the reaction studied here is closely related to recent advances allowing mild $S_N\text{Ar}$ reactions of electron-rich substrates.\textsuperscript{11,12}

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Supporting information description
The supporting information contains a detailed description of the materials and methods, calculations of singlet-triplet gap of aryl cations, analysis of the nature of excited state of compound 2, an analysis of mono- or biphotonic photoionization of amiloride, pH measurements, experiments with deuterated solvent, a computational analysis of the effect of protonation on
barrier of attack on radical cations, UV and fluorescence spectra, cyclic voltammetry and photocurrent measurements, NMR spectra and chromatograms. Cartesian coordinates for computed structures are given in a separate file.

Methods

Synthesis
Synthesis of 3,5-diamino-6-chloro-N-(2-(methylamino)ethyl)pyrazine-2-carboxamide (2) was carried out as reported in the literature (Scheme 1). To the methyl 3,5-diamino-6-chloropyrazine-2-carboxylate S2 (4.94 mmol), the 2-(methylamino) ethylamine (24.68 mmol) was added and heated neat for 18 h at 120 °C in a microwave sealed tube. The residual amine was removed under reduced pressure and diluted with acetonitrile. The precipitate formed was filtered and washed with acetonitrile to furnish a brown solid in 60% isolated yield.

Spectroscopy
Absorption spectra were obtained on a scanning UV-Vis spectrometer and a diode-array UV-Vis spectrometer with matched 1.0-cm quartz cells. Fluorescence spectra were recorded on a diode-array automated combined luminescence and UV-Vis spectrometer in 1.0 cm quartz fluorescence cuvettes at 23 ± 1 °C. The sample concentration was set to keep the absorbance below 0.5 at λirr and a correction for self-absorption was applied for each spectrum. Emission spectra were also corrected using standard correction files. Each sample was measured 3–5 times, and the spectra were averaged. Due to the photosensitivity of the compounds, fresh samples were used for each measurement.

Cyclic voltammetry
The electrochemistry was recorded in methanolic solutions of pyrazine derivative (c = 1 mM) using tetrabutylammonium hexafluorophosphate as a conducting salt (c = 0.1 M). The electrochemical measurements were accomplished using a glassy-carbon disc working electrode, a platinum counter electrode, and a silver pseudo-reference electrode. The cyclic voltammograms in the measurement range (+1.2 V to −0.7 V) were recorded three times.

Photochemical experiments
Standard experimental procedure
The photochemical reactions were carried out for 2 hours in a Rayonet reactor with 16 UV lamps (300–460 nm, λmax = 350 nm). Solutions of concentration 2 mM in quartz test tubes were degassed by bubbling with argon for 10 min prior to irradiation. After 2 hours of irradiation, residual solvent was removed under reduced pressure and the crude product was analyzed using 1H NMR and LC-MS.

Experiments with π nucleophiles
Allylsilane and styrene (30 equiv.) were added to two independent quartz test tubes containing 2 in MeCN/MeOH (9:1, v/v). The test tubes were sealed with rubber septa, degassed using argon for 10 mins and irradiated for 2 hours at 350 nm. Excess solvent was removed under reduced pressure and the crude products were analyzed using 1H NMR. In both the cases, only traces of 2–OMe were observed and no addition product could be seen. Most of the starting material remained unreactive. Allylsilane was completely consumed after irradiation, as shown by 1H NMR (see
Supporting Information, Figure S30). The allylsilane was probably consumed by protodesilylation under the slightly acidic conditions to give volatile 1-butene. Importantly, neither styrene nor allylsilane show any absorbance above 300 nm and were therefore not photoexcited.

**Solvent isotopic experiments**
Two separate 2 mM solutions of compound 2 were prepared in H₂O and D₂O. The solutions were then degassed for ca 20 min by bubbling with argon and irradiated simultaneously in the Rayonet reactor with 18 350 nm UV-lamps. After every 30 mins of irradiation, samples were carefully withdrawn from the solutions while maintaining the inert atmosphere in the reaction flask using argon. The residual solvent was removed under reduced pressure, and the crude ¹H NMR spectra were recorded and analyzed for conversion. The solutions were irradiated for a total period of 2 hours.

**Photocurrent experiments**
The photocurrent measurements were accomplished using a spectroelectrochemical cell irradiated with a UV-light source. The light was gradually turned on and off and the induced photocurrent was measured by a chronoamperometric method applying a bias potential to accelerate the ionized electrons towards the electrode. A detailed description of experimental procedure is shown in the Supporting Information.

**Quantum-chemical calculations**
All quantum-chemical calculations, unless otherwise noted, were carried out with Gaussian 16, revisions A.03 and B.01. Geometry optimizations were done at the B3LYP-D3(BJ)/6-31+G(d)/SMD level, i.e., with the B3LYP functional, the 6-31+G(d) basis set, the SMD solvation model and the D3-BJ dispersion model. Stationary points on the potential energy surface were confirmed with frequency calculations and transition states corroborated by quick reaction coordinate calculations. Final energies were obtained from M06-2X-D3/6-311+G(d,p)/SMD single point calculations at the B3LYP geometries, i.e., using the M06-2X functional, the 6-311+G(d,p) basis set, the SMD solvent model and the D3 dispersion model, together with thermal contributions from B3LYP-D3(BJ)/6-31+G(d)/SMD. Standard state corrections were applied to give a standard state of 1 M for reactants and 55.5 M for H₂O, unless otherwise noted. Triplet state calculations used the same levels of theory but with unrestricted DFT.

Optimization and frequency calculations in the excited S₁ state used TD-DFT with B3LYP-D3(BJ)/6-31+G(d)/SMD based on a ground-state restricted reference. Final energies were obtained by combining the thermal corrections at the optimization level of theory with single-point energies at the M06-2X/6-311+G(d,p)/SMD level, enforcing equilibrium solvation treatment with the keyword “IOp(9/73=2)”. MS-CASPT2 calculations of the aryl cations were done with OpenMolcas 18.0 with an active space of 6 electrons in 7 orbitals and the ANO-RCC-VDZP basis set. An imaginary shift of 0.2 a.u. and an IPEA shift of 0.0 a.u. were used. Further details on the reference wave function and active space orbitals can be found in the Supporting Information, Section 2.1.2.

Ionization potentials and electron affinities were calculated with the ΔSCF approach, using the energy of the neutral molecule and its radical anion/cation. Vertical values were obtained by
single-point calculations of the anion/cation at the geometry of the parent molecule, while adiabatic values were obtained by allowing the anion/cation to relax. For vertical values, we used the electronic energy difference and for adiabatic values we used the free energy difference.

NICS scans\textsuperscript{42,43} were performed with Aroma 1.0,\textsuperscript{60} and ACID plots\textsuperscript{41} with the AICD 2.0.0 package, both using Gaussian 09, revision E.01.\textsuperscript{61} MCI values\textsuperscript{44} were obtained using the ESI-3D program,\textsuperscript{62} based on the Quantum Theory of Atoms in Molecules atomic partition and the integration scheme as provided by the AIMAll package.\textsuperscript{63} MCI values for pyrazine in the lowest \( \pi\pi^* \) triplet state and its radical cation were calculated by optimizing the structures in \( D_{2h} \) symmetry with the orbital occupation altered (keyword “guess=alter”) and employing symmetry in the SCF calculation (keyword “scf=symm”). For the \( \pi\pi^* \) singlet excited state, we used TD-DFT, optimizing the third root. For the singlet and triplet states, the \( D_{2h} \)-symmetric geometry was not a minimum.

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