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Cycloreversion of Azetidines via Oxidative Electron Transfer. Steady-State and Time-Resolved Studies

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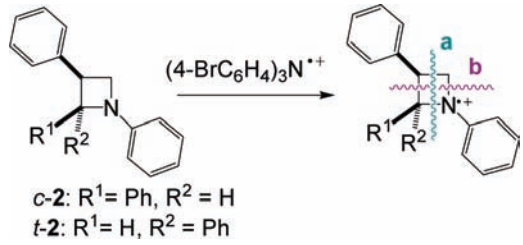
ABSTRACT



Cycloreversion of *cis*- and *trans*-1,2,3-triphenylazetidine (*c*-2 and *t*-2) is achieved by electron transfer to (tris(4-bromophenyl)aminium radical cation ($5^{\bullet+}$). Stepwise C−N and C−C bond cleavage of azetidine radical cations leads to *cis*- and *trans*-stilbene, together with *N*-benzylideneaniline, as final products. Mechanistic evidence is provided by quenching studies, using laser flash photolysis to generate $5^{\bullet+}$ from its neutral precursor.

The azetidine ring can be found in a wide number of compounds with different properties and applications. Thus, it is present in a variety of natural products and in biologically or pharmacologically active substances.[1,2] It is also a key substructure of useful synthetic intermediates[3] and conformationally constrained amino acids,[4] which are used as basic tools for the design of novel peptides and peptidomimetics. Moreover, the use of modified nucleosides containing the azetidine ring as constrained RNA or DNA building blocks has also been reported.[5]

Perhaps one of the most relevant areas of interest for azetidines is related to UVB-induced DNA damages, which include cyclobutane pyrimidine dimers (CPD, 80−90% of the observed lesions) and pyrimidine (6-4) pyrimidones (6-4 photoproducts, 10−20% of the lesions).[6] In a number of biological systems, the enzymatic repair that protects from accumulation of CPD lesions is provided by DNA photolyases, through formation of CPD radical anions, which readily fragment into closed-shell monomeric pyrimidines. Concerning formation of the 6-4 photoproducts, it is assumed

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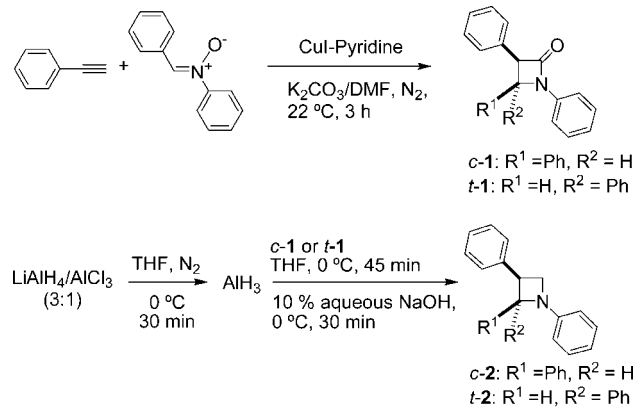
to proceed via cyclic oxetane/azetidine intermediates resulting from photocycloaddition of the C_5═C_6 and $C_{4'}$═O/NH bonds of two bases (in a Paterno−Büchi [2 + 2] cycloaddition). It has been demonstrated that in many organisms 6-4 photoproducts can be efficiently repaired by regeneration of the oxetane/azetidine moiety, in an electron transfer mechanism resembling that employed by CPD photolyases; this is not unexpected in view of the similarity between CPD and 6-4 photoproducts in terms of structure and binding of cofactors. Thus, cycloreversion (CR) of azetidine and oxetane radical ions appears to be involved in the enzymatic repair of DNA. Moreover, the possible involvement of both the anionic and the cationic pathways in the regeneration of native pyrimidines from azetidine and oxetane lesions has been recently studied by means of theoretical calculations.[7]

In this context, the electron transfer CR of model oxetanes has been previously described;[8] however, there seems to be no report dealing with the same type of process in azetidines. Hence, the goal of the present work was to gain insight into the mechanistic aspects of azetidine CR via electron transfer. For this purpose 1,2,3-triphenylazetidines *c*-**2** and *t*-**2** were chosen as models and tris(4-bromophenyl)aminium hexachloroantimonate (also named BAHA or magic blue)[9] as one-electron oxidizing agent.

Although the synthesis of azetidines **2** has been the subject of a previous publication, their isolation and characterization as pure separated stereoisomers has not been completely reported. In the present work, *c*-**2** and *t*-**2** were prepared as depicted in Scheme 1: copper-catalyzed reaction of phenylacetylene with α,*N*-diphenylnitrone to the corresponding 2-azetidinones *c*-**1** and *t*-**1**,[10] followed by reduction with alane[11] (see Supporting Information for further experimental details and spectroscopic characterization).

Scheme 1. Synthesis of *c*-**2** and *t*-**2**



For the electron transfer experiments, a solution of *c*-**2** or *t*-**2** in acetonitrile was reacted with an equimolar amount of BAHA, which was added dropwise in the same solvent. At the end of the addition the initial blue color, typical of tris(4-bromophenyl)aminium radical cation, changed to red-brown. Analysis of the reaction mixture by GC−MS showed the presence of *cis*-stilbene (*c*-**3**) and/or *trans*-stilbene (*t*-**3**), toghether with lower amounts of imine **4** (Scheme 2). For quantitation, known amounts of appropriate standards were added prior to analysis. The structures of all of the products were confirmed by comparison with authentic samples.

The obtained results can be explained by initial electron transfer from *c*-**2** or *t*-**2** to BAHA. Subsequent C_2−N/C_3−C_4 bond cleavage (pathway a) of the generated azetidine radical cation would lead to formation of the two stilbene isomers. Alternatively, the initial azetidine radical cation could undergo C_2−C_3/C_4−N cleavage (pathway b), ultimately resulting in formation of **4**. The proposed mechanism is illustrated in Scheme 3. A control experiment showed that no cycloreversion takes place in the absence of BAHA.

To rule out a possible interconversion of the stilbenes via radical cation under the employed reaction conditions, *c*-**3**

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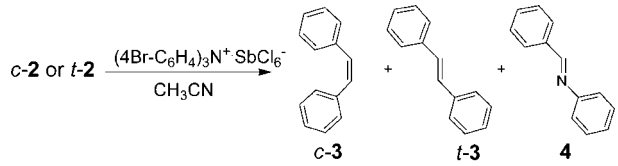
Scheme 2. Reactivity of **2** in the Presence of BAHA

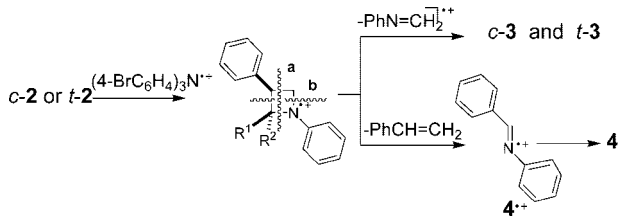


c-**2** (75% C, 60% MB)[a]	37%	58%	traces
t-**2** (100% C, 80% MB)[a]	traces	80%	16%

[a] C: Conversion, MB: Mass Balance

and *t*-**3** were also treated with BAHA. As expected from consideration of the free energy changes associated with the involved redox process,[12] this did not lead to any detectable degree of isomerization, suggesting a stepwise CR mechanism for *c*-**2** or *t*-**2** with formation of a distonic 1,4 radical cationic intermediate, where free rotation around the C_2-C_3 bond would be possible.

Scheme 3. Mechanistic Pathways in the Cycloreversion of Azetidine Radical Cations



To obtain clear-cut experimental evidence in support of the proposed reaction mechanism and specifically to make sure that the observed cycloreversion actually occurs via electron transfer from the azetidine to tris(4-bromophenyl)-aminium radical cation (**5**$^{\bullet+}$), transient absorption spectroscopic studies were undertaken. For this purpose, a pulsed laser was used to generate **5**$^{\bullet+}$ in the absence of a stabilizing counteranion. This way, its lifetime was expected to be much shorter, allowing us to employ time-resolved detection techniques for performing quenching experiments and for determining reaction rate constants. As a matter of fact, laser flash photolysis (LFP) of the neutral precursor **5** (0.25 mM, 355 nm, acetonitrile, air) led to photoionization, giving rise to a transient absorption spectrum, which was coincident in shape and position with the visible band recorded for an acetonitrile solution of BAHA (Figure 1). The UV spectrum of **5** (not shown) displays bands with maxima at 214 and 300 nm and no absorption above 400 nm. Hence, the species obtained by LFP was safely assigned to radical cation **5**$^{\bullet+}$. Its lifetime was 36 μs, a sufficiently long value as to provide a wide dynamic range for further studies.

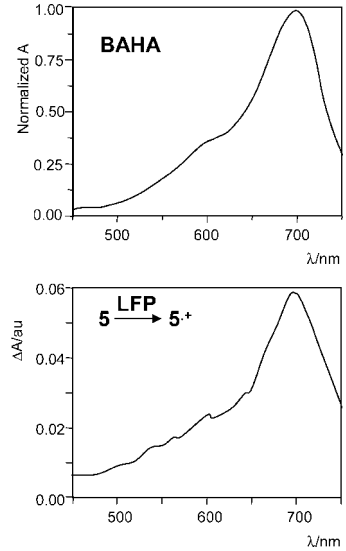


Figure 1. (Top) UV−vis absorption spectrum of BAHA. (Bottom) Transient absorption spectrum obtained after LFP (355 nm, 2.5 × 10^{-4} M) of **5**.

Once the methodology to generate **5**$^{\bullet+}$ as a transient species was available, the next step was to carry out quenching studies. Thus, LFP of **5** in the presence of increasing amounts of *c*-**2** or *t*-**2** generated the same signal at 680 nm attributed to **5**$^{\bullet+}$, but the remarkable shortening of its lifetime with azetidine concentration revealed a strong quenching (Figure 2). Upon application of the Stern−Volmer relationship,[13] the rate constant was estimated to be 2×10^9 M^{-1} s^{-1} for both stereoisomers. This dynamic quenching is assumed to occur by electron transfer, on the basis of the favorable free energy changes associated with the process.[12] By contrast, no quenching of **5**$^{\bullet+}$ was observed in the presence of *c*-**3** or *t*-**3** (see Supporting Information), ruling out electron transfer between **5**$^{\bullet+}$and the stilbenes. These results are consistent with the fact that *c*-**3** and *t*-**3** did not undergo interconversion when BAHA was used as oxidazing agent (see above) and further support that twisting around the C_2-C_3 bond must occur in a distonic 1,4 intermediate, through a stepwise cycloreversion of the azetidine radical cation.

Upon quenching of **5**$^{\bullet+}$ by *c*-**2** or *t*-**2**, no new transient absorption spectrum was observed at 515 or 470 nm that could correspond to *c*- or *t*-**3**$^{\bullet+}$.[14] This indicates that, upon CR of the azetidines along pathway a, spin and charge are mainly located at the more easily oxidizable imine fragment. The same would be true for CR through pathway b, which

(12) The E_{ox} value for *N*,*N*-dimethylaniline (as a simple model for azetidines *c*-**2** and *t*-**2** is 0.7 V versus SCE, whereas that of stilbenes **3** is 1.5 V versus SCE, as reported in *Technique of Electroorganic Synthesis, part 2*; Weinberg, N. L., Ed.; John Wiley & Sons: New York, 1975. As regards the E_{red} value of BAHA, it has been found to be 1.17 V versus SCE.[9] Thus, oxidation of azetidines by BAHA would be exergonic; however, the analogous process with stilbenes is thermodynamically disfavored.

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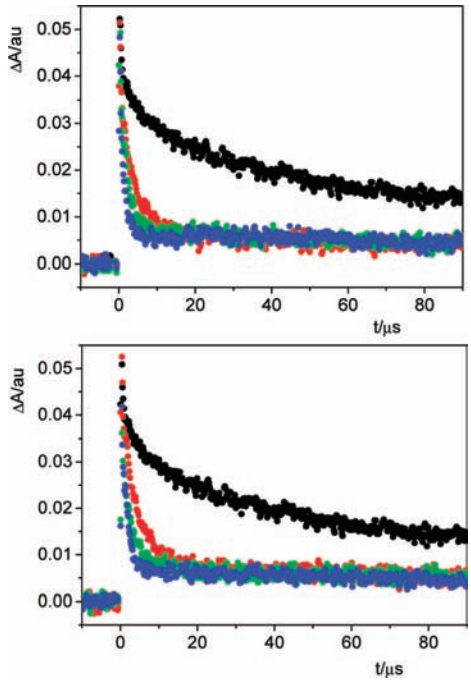

Figure 2. Quenching of $\mathbf{5}^{\bullet+}$ in the presence of increasing amounts of **2**: *c*-**2** (top), *t*-**2** (bottom). Concentration of **5** was 2.5×10^{-4} M in all cases. The decay traces correspond to a [**5**]/[**2**] ratio equal to 1:0 (black), 1:0.5 (red), 1:1 (green), and 1:2 (blue), respectively.

should lead preferentially to $\mathbf{4}^{\bullet+}$. Unfortunately, no transient absorption spectra attributable to the imine radical cations were detected in the LFP experiments, probably because these species are not compatible with the employed wavelength/time scale windows. In this context, it is remarkable that cycloreversion of *c*-$\mathbf{2}^{\bullet+}$ and *t*-$\mathbf{2}^{\bullet+}$ was also observed in the gas phase, upon electron impact ionization. Figure 3 shows the fragmentation pattern obtained for *t*-**2**, as an example; a similar spectrum was obtained for *c*-**2**.

In conclusion, electron transfer cycloreversion of azetidines *c*-**2** and *t*-**2** can be achieved by oxidation with tris(4-bromophenyl)aminium radical cation. The process occurs by stepwise cleavage of the C_2-N/C_3-C_4 or the C_2-C_3/C_4-N bonds, to give stilbenes and *N*-benzylideneaniline as final products. These results, obtained with model systems, confirm the feasibility of the radical ionic pathways to achieve the enzymatic repair of 6-4 photoproducts in damaged DNA. At the same time, the developed experimental approach provides a new entry to undertake similar studies with more complex azetidines, closer to the real biological systems.

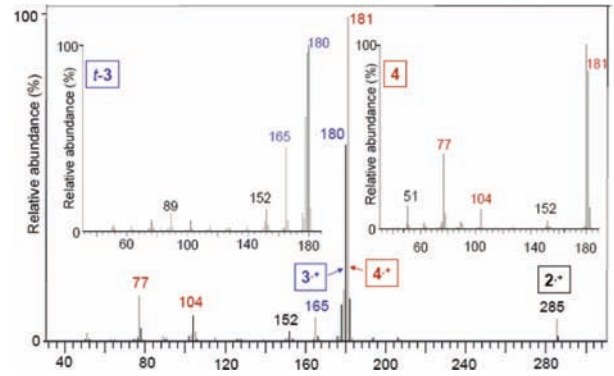

Figure 3. Mass spectrum (electron impact) of *t*-**2**. Insets: spectra of *t*-**3** (left) and **4** (rigth).

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Supporting Information Available: Experimental details, spectroscopic characterization of *c*-**2** and *t*-**2**, and additional figures.This material is available free of charge via the Internet at http://pubs.acs.org.

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