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Photochemical Reactivity of Trifluoromethyl Aromatic Amines: The Example of 3,5-diamino-trifluoromethyl-benzene (3,5-DABTF)

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ABSTRACT

This work presents the application of an on-line photoreactor to the detection of 3,5-diamino-trifluoromethyl-benzene (3,5-DABTF) in aqueous solutions. When irradiated at 310 nm, this compound is defluorinated to 3,5-diaminobenzoic acid by a nucleophilic substitution of the fluoride by water. Concomitantly, defluorination intermediates polymerize through amide bonds to give dark-colored compounds. We take advantage of the photocatalyzed defluorination and the subsequent decrease in pH to develop an original and specific photoreactor. Continuous recording of pH and temperature in the outlet of the reactor by a dual electrode gives us an opportunity to optimize the system. In the photoreactor, 3,5-DABTF is immediately and totally transformed as attested by the rapid drop of the flowing solution pH from 6.2 to 3.2 and the chromatographic analysis of the outgoing solutions. The detection remains effective from 1 to 1000 parts per million.

INTRODUCTION

Trifluoromethyl substituted aromatic compounds are extensively used as agrochemicals (1,2), pharmaceuticals (3) and polymers (4). Due to the steric and electronic properties of fluorine (5), this halogen is often used to replace hydrogen in biologically active organic compounds. Such substitution dramatically modifies their physical, chemical and biological properties, but results in only a slight steric modification. However, fluorine is highly electronegative, and as a consequence, the replacement of a methyl group by a trifluoromethyl increases the lipophilicity of active compounds, leading to efficient internalization into target cells. Such substitution also modifies hydrogen bonding, which often governs enzyme-substrate interactions (6). These properties have been used to design efficient drugs and pesticides (7,8).

The C-F bond is highly resistant to hydrolysis compared to that of other halogens (9). As a consequence, the trifluoromethyl substituent resists drastic chemical treatments as well as biological degradation (10). However, there are a few exceptions, particularly in the case of substituted benzotrifluoromethyl derivatives bearing electrodonor groups such as primary amines. In this case, the trifluoromethyl group is converted to its corresponding carboxylic acid or nitrile (11). Bosca *et al.* (12) reported the photocatalyzed nucleophilic substitution of trifusal, which leads to the replacement of the trifluoromethyl group by a carboxyl. This defluorination is accompanied by the production of fluoride ion.

3,5-Dinitro-trifluoromethylbenzene (3,5-DNBTF) is a fluorinated nitro-aromatic compound currently under evaluation for specific military applications. To prevent contamination of effluents by this molecule, and to ascertain its presence in complex mixtures, a selective continuous sensor remains essential. We have investigated a specific enzymatic biosensor based on the nitroreduction of 3,5-DNBTF to its corresponding diamine, 3,5-diamino-trifluoromethyl-benzene (3,5-DABTF), by a *Bacillus sp.* nitroreductase. 3,5-DABTF is easily defluorinated when irradiated at 310 nm; this particular reactivity was retained as a tool for its detection.

In the present paper we describe the photochemical reactivity of 3,5-DABTF in aqueous solutions and elucidate the mechanisms involved. A continuous-flow, postreaction photoreactor was developed and tested.

MATERIALS AND METHODS

3,5-DNBTF and 3,5-diaminobenzoic acid were from Aldrich. Acetonitrile (SDS, Peypin, France) and formic acid (ACROS, Noisy le Grand, France) were high-performance liquid chromatography (HPLC) grade.

Synthesis of 5-nitro-3-hydroxylamino-benzenetrifluoride (5-N-3-HBTF)

Two grams (8.5 mmol) of 3,5-DNBTF was dissolved in 50 ml of methanol, then 100 mg of Pd/C catalyst was added. The mixture was gently mixed using a magnetic stir bar. Hydrogen was introduced through a septum at room temperature and atmospheric pressure. The reaction was closely monitored by thin-layer chromatography (TLC) and stopped when the starting 3,5-DNBTF was completely converted to 3-hydroxylamino-5-nitro-trifluoromethylbenzene (TLC on silica-gel plates 60F; Merck, Lyon, France, eluted with heptane/ethyl acetate 4:6, UV light at 250 nm). The mixture was filtered and the solvent removed under vacuum. The residue was filtered on silica-gel 70–200 μm (Prolabo) to eliminate the catalyst. The target 5-N-3-HBTF was obtained pure in 82% yield (1.54 g, 6.9 mmol).

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Abbreviations: 3,5,-DABA, 3,5-diaminobenzoic acid; 3,5-DABTF, 3,5-diamino-trifluoromethyl-benzene; DMSO, dimethyl sulfoxide; 3,5-DNBTF, 3,5-dinitro-trifluoromethylbenzene; 5-N-3-HBTF, 5-nitro-3-hydroxylamino-benzenetrifluoride; HPLC, high-performance liquid chromatography; MALDI-Tof, matrix-assisted laser desorption/ionization time of flight; MS, mass spectroscopy; NMR, nuclear magnetic resonance; ppm, parts per million; TLC, thin-layer chromatography.

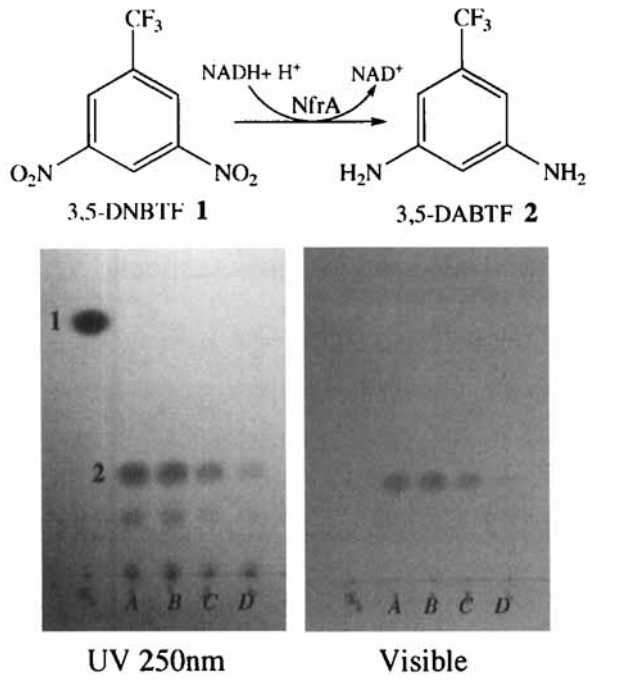

Figure 1. Nitroreduction of 3,5-DNBTF **1** by *Bacillus* nitroreductase represent the biological compartment of the biosensor. The reaction is routinely monitored by TLC (silica-gel plates 60F, Merck; solvent heptane/ethyl acetate 4:6, UV light at 250 nm). Among the spots observed under UV illumination (250 nm) only the 3,5-DABTF spot turned brown when exposed to light. Coloration is accelerated by illumination at 310 nm.

^{1}H Nuclear magnetic resonance (NMR) ($CDCl_3$ 300 MHz): H_5: 8.04 parts per million (ppm) (1H, s); H_7: 8.01 ppm (1H, s); H_3: 7.53 ppm (1H, s); H(NH): 7.16 ppm (1H, s); H(OH): 5.54 ppm (1H, s).

^{13}C NMR ($CDCl_3$ 75 MHz): C_1: 117.53 + 121.14 + 124.75 + 128.36 ppm (q, J = 271 Hz); C_2: 132.26 + 132.73 + 133.16 + 133.62 ppm (q, J = 34 Hz); C_3: 113.32 + 113.39 + 113.44 + 113.51 ppm (q, J = 5 Hz); C_7: 115.91 + 115.98 + 116.04 + 116.11 ppm (q, J = 5 Hz); C_4: 149.22 ppm (s); C_6: 151.93 ppm (s); C_5: 111.64 ppm (s).

Mass spectroscopy (MS): ES^-220.7.

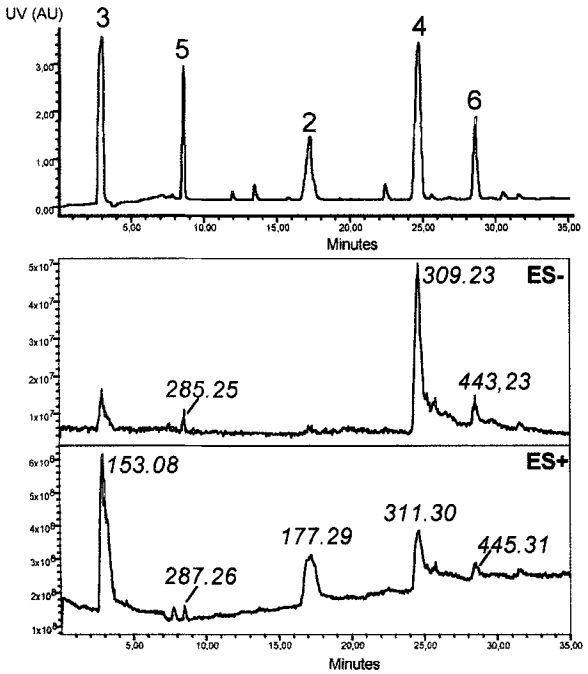

Figure 2. HPLC-MS analysis obtained with a Micromass ZQ mass spectrometer (Waters) equipped with an electrospray ionization source operated in both positive and negative modes. The quality of the response gives us confidence in structure assignments.

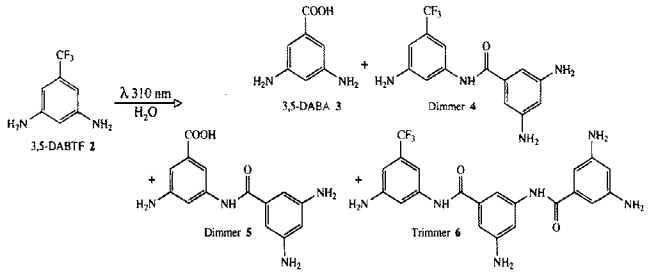

Figure 3. Phototransformation products of 3,5-DABTF.

Synthesis of 3,5-DABTF

3,5-DABTF was synthesized under the same conditions as 5-N-3-HBTF, but the reaction was prolonged until 5-N-3-HBTF was totally transformed. Target 3,5-DABTF was obtained in 92% yield (1.38 g, 7.8 mmol).

^{1}H NMR ($CDCl_3$ 300 MHz): 3.77 (4H, s), 6.09 (1H, s) and 6.32 ppm (2H, s).

^{13}C NMR ($CDCl_3$ 75 MHz): C_1: 117.81 + 122.13 + 126.45 + 130.80 ppm (q, J = 326 Hz); C_2: 131.83 + 132.33 + 132.83 + 133.33 ppm (q, J = 37.5 Hz); $C_3 + C_7$: 102.34 + 102.32 + 102.41 + 102.43 ppm (dd, J = 1.5 and 6.75 Hz); $C_4 + C_6$: 147.98 ppm (s); C_5: 104.07 (s) ppm.

MS: ES^+177.

N-acetylation of 3,5-DABTF

Two grams of 3,5-DABTF was dissolved in 15 ml of acetic acid, then 50 ml of acetic anhydride was added dropwise while stirring. The reaction was monitored by TLC until the complete transformation of DABTF. The solvent was removed and the residue dried under vacuum. The target diamide was obtained in 95% yield (2.80 g, 10.7 mmol).

^{1}H NMR (dimethyl sulfoxide [DMSO] 300 MHz): NH: 9.41 ppm (s, 2H, J = 9 Hz); H_5: 7.18 ppm (s, 1H); $H_3 + H_7$: 6.83 ppm (s, 2H); $H_9 + H_{11}$: 1.19 ppm (s, 6H).

^{13}C NMR (DMSO 75 MHz): C_1: 128.89 + 129.29 + 129.73 + 130.13 ppm (q, J = 25 Hz); C_2: 122.16 ppm; $C_3 + C_7$: 109.51 ppm; $C_4 + C_6$: 140.38 ppm; C_5: 112.14 ppm; $C_8 + C_{10}$: 168.83 ppm; $C_9 + C_{11}$: 23.96 ppm.

MS: ES^+283.

Reactor and light source

Aqueous 3,5-DABTF solution (1 g/L) was placed in a Pyrex cylindrical flask (~100 ml volume, 0.1 cm thickness and 2.2 cm external diameter). Four 8 W lamps positioned in a Rayonnet apparatus were used for external irradiation between 280 and 320 nm. Flowing water was used to minimize heating. Twenty-five milliliters of solution was introduced into the photoreactor. Before irradiation, the solution was extensively flushed with

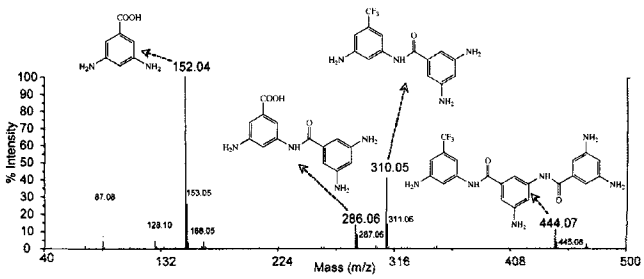

Figure 4. MALDI-Tof analysis of the reaction mixture. The compounds were directly deposited on the MALDI plate without any matrix. For the four major metabolites, peak abundances obtained in MALDI are similar to those observed by HPLC.

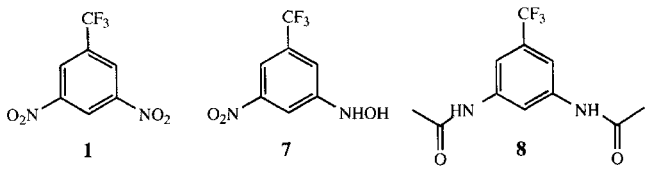

Figure 5. Analogs that are stable when irradiated in the same conditions than 3,5-DABTF.

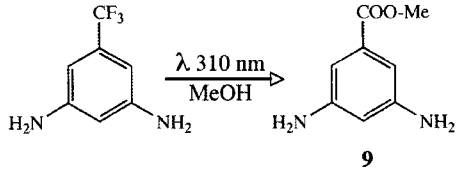

Figure 6. Irradiation of 3,5-DABTF in methanol. Formation of the methyl ester 9 confirmed the nucleophilic substitution of the fluoride by the solvent.

the desired gas (dioxygen or argon) and the mixture stirred by bubbling with the same gas.

Analytical methods

NMR. ^{1}H and ^{13}C NMR spectra were recorded in a Bruker 300 MHz spectrometer (Bruker, Wissembourg, France), in $CDCl_3$ or DMSO as indicated.

HPLC. The liquid chromatograph comprised an Alliance Waters 2695 (Milford, MA) (flow rate, 1 ml/min) and a Waters 996 photodiode array detector with Millennium software to control the analytical system and data processing. The Waters Symmetry C18, 5 μm, 4.6 × 250 mm column was eluted with the following gradient of 0.05% formic acid: t_0: 100% A (0.05% formic acid in water), $t_{0-10min}$: 15% B (100% acetonitrile + 0.05% formic acid), $t_{10-40min}$: 100% B, t_{55min}: 100% B, t_{60min}: 100% A.

HPLC/MS. A Micromass ZQ mass spectrometer (Waters) equipped with an electrospray ionization source was used in positive and negative ionization mode. Mass spectrometry conditions were as follows: cone voltage, 30 V; source temperature, 150°C; desolvation gas flow, 400 L/h; probe temperature, 450°C; Corona current, 7 μA.

Matrix-assisted laser desorption/ionization time of flight (MALDI-Tof). MALDI-Tof mass spectra were acquired on a Voyager DE STR (Applied Biosystems, Foster City, CA) fitted with a pulsed nitrogen laser (337 nm). Mass spectra were acquired in the reflectron mode. The total acceleration voltage was 20 kV with a grid voltage of 68% and a delayed extraction time of 240 ns.

pH and temperature recording

We used a coupled pH and temperature electrode (Lambda, Zurich, Switzerland). Values were continuously recorded on a Minifor unit using Siam software (Lambda).

RESULTS

3,5-DNBTF **1** is a potentially toxic compound that may contaminate aqueous industrial wastes. A specific and sensitive detection technique is essential for continuous monitoring of effluents and triggering of appropriate safety procedures. Biosensors represent a method of choice. The biochemical reactant constitutes the challenge in this approach, and we chose an enzyme-based detector. We have previously shown that *Bacillus subtilis* nitroreductase NfrA catalyzes the reduction of the two nitro groups of 3,5-DNBTF, leading to the corresponding diamine 3,5-DABTF **2**. Further investigation of the biosensor calls for the conversion of the reaction product 3,5-DABTF to easily detected and quantified derivatives.

Our evaluation of the photochemical behavior of 3,5-DABTF stemmed from our investigations of the nitroreduction of 3,5-DNBTF. This reaction was routinely monitored by TLC analysis, and the 3,5-DABTF spots turned brown in a few minutes when the TLC plate was exposed to UV light at 310 nm (Fig. 1). The spot under 3,5-DABTF **2** correspond to the transitional 3-hydroxylamino-5-aminobenzene-trifluoride, which transforms rapidly to 3,5-DABTF.

An aqueous solution of 3,5-DABTF (1 mg/mL) was irradiated in a Rayonnet apparatus under an air stream as described in Materials and Methods. Samples of the reaction mixture were recovered every hour, and the irradiation was stopped and analyzed after 8 h. The first observation was a pH shift from 5.5 to 3. HPLC analysis of the reaction mixture showed the total disappearance of the starting 3,5-DABTF. Four peaks were obtained and their structures deduced from their mass spectra. Figure 2 shows a representative chromatogram. The compounds gave an unambiguous response in HPLC-MS equipped with an electrospray source, either in positive or negative mode. Besides the starting compound **2**, the major irradiation product was 3,5-diaminobenzoic acid **3** (3,5-DABA), which at the end of the reaction, represented more than 70% of the total compounds. The second product in terms of abundance was the dimer **4**, formed between 3,5-DABTF and 3,5-DABA moieties, through an amide bond. Two minor compounds were characterized and correspond to the dimer **5** and the trimer **6** (Fig. 3). These results were confirmed by MALDI-Tof analysis of the reaction mixture (Fig. 4). For the four major metabolites, peak abundances obtained in MALDI-Tof are similar to those observed via HPLC. This photoreactivity was observed only with compound **2** with two primary amine substituents. Under similar irradiation conditions, 3,5-DNBTF **1**, the monoamine 3-hydroxylamino-5-aminobenzene trifluoride **7** as well as the protected derivative N,N-diacethyl-3,5-diaminobenzene trifluoride **8** were unchanged (Fig. 5). This sup-

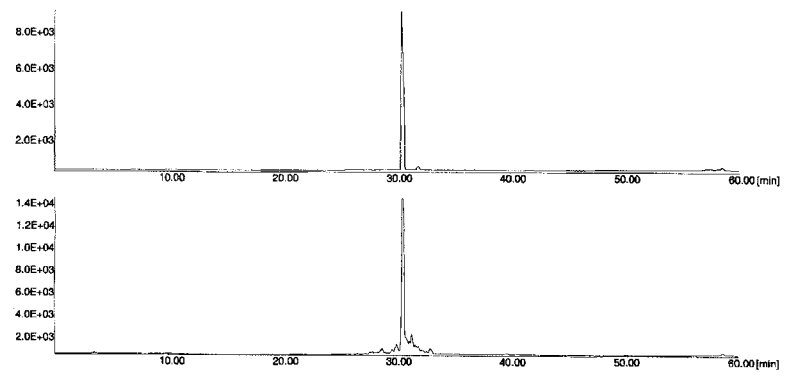

Figure 7. Irradiation 3,5-DABA under standard conditions. The solutions were oxygen-free and maintained under a nitrogen stream, to avoid any interference with oxidation reactions. After 8 h of irradiation, the starting 3,5-DABA (upper chromatogram) was recovered unchanged (down chromatogram).

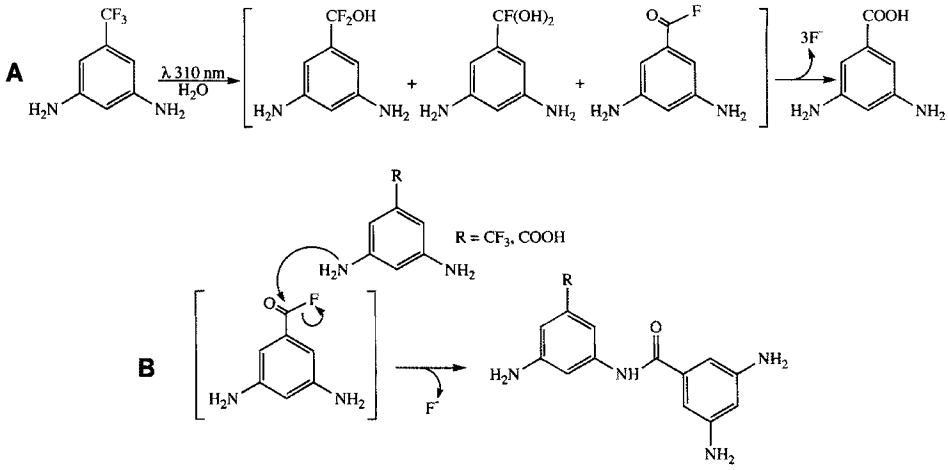

Figure 8. Proposed mechanisms for the formation of 3,5-DABA and polymers. During these reactions fluorine was removed into the medium, causing a rapid and easily measured acidification.

ports the role of the electron-donating primary amines in facilitating photoinduced dehalogenation.

According to these observations, the key step is the 3,5-DABTF defluorination to 3,5-DABA **3**. The removed fluoride ions are responsible for medium acidification. To distinguish between oxidative and hydrolytic mechanisms of defluorination, irradiation of 3,5-DABTF was conducted in deaerated solutions and under a nitrogen stream. This treatment did not affect the nature or proportion of compounds. This observation excludes an oxidative mechanism and indicates nucleophilic substitution of the fluoride by the solvent, water in this case (Fig. 8A). This was confirmed by irradiation in absolute methanol. In these conditions, the major derivative obtained was the methyl ester **9** (Fig. 6). Such a nucleophilic substitution of the fluoride by the solvent was reported in the case of the prodrug triflusal. The defluorination intermediates interact with proteins that account for the photoallergenic properties of triflusal (12).

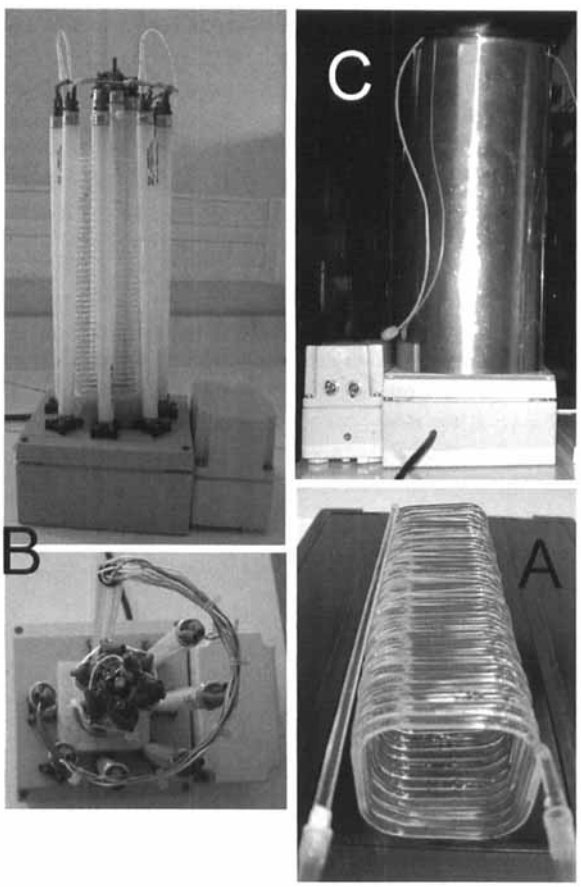

Figure 9. Various views of the photoreactor elements.

DISCUSSION

To our knowledge, the amide bond formation observed in our experiments has not been reported before. Our first hypothesis is that a UV-catalyzed simple interaction between a primary amine and the carboxylic acid of 3,5-DABA formed through the defluorination reaction. To confirm such a mechanism, we irradiated 3,5-DABA under standard conditions. The solutions were oxygen-free and maintained under a nitrogen stream to avoid interference with potential oxidation derivatives. After 8 h of irradiation, the starting 3,5-DABA was recovered unchanged (Fig. 7). This was confirmed by HPLC, HPLC-MS and MALDI-Tof analysis. This suggests an interaction between a defluorination intermediate and a primary amine as proposed in Fig. 8B.

The aim of this study was the development of a biosensor for 3,5-DNBTF, based on coupling between its enzymatic nitroreduction to 3,5-DABTF and irradiation of the latest at 310 nm. To achieve that, we need a continuously operating photoreactor with a light source set at 310 nm. The commercially available Phred system (Aura Industries Incorporation, USA) is not appropriate. The emission wavelength (254 nm) and the polytetrafluoroethylene tube constitute the major limitations. Thus, we developed a device fully adapted to our application. This device is presented in Fig. 9. It consists of a 10 m quartz streamer with 5 mm external diameter and approximately 1.5 mm internal diameter. The coiled streamer has a squared section of 7 cm side length and 23.5 cm height. The measured capacity is 12.5 ml, which differs from the calculated value (17.7 ml) because of the irregular internal diameter due to quartz handling. This tube can be continuously supplied with any liquid at a flow rate ranging from 1 to 5 ml/min. Two series of 15 W and 310 nm irradiating lamps were placed inside and outside the streamer (six internal and six external). The distance between the UV tubes and the streamer was about 7 mm. Internal and external lamps can be switched on separately to control the delivered energy. At maximum energy (180 W), aqueous solutions delivered at 1 ml/min never exceeded a temperature of 29°C.

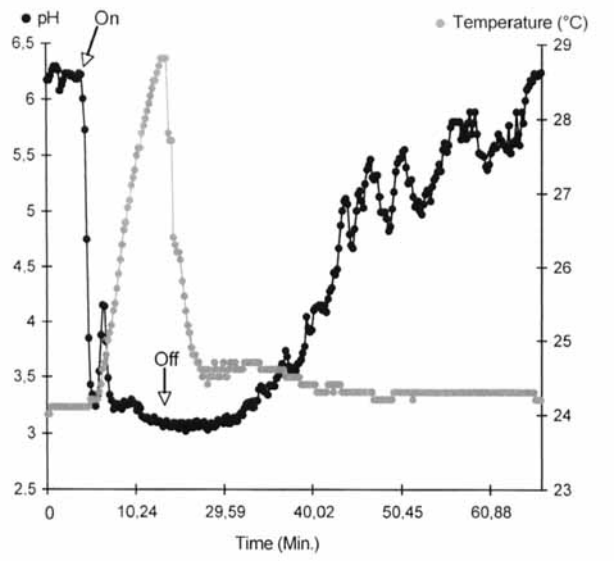

Figure 10. Reactor operations. A coupled pH and temperature electrode was used. Values were continuously recorded on a Minifor unit using Siam software (Lambda).

The first experiment was conducted under static conditions. The tube was filled with a 1 g/L solution of DABTF and irradiated at full power for various periods of time (1 to 10 min). The solution was then checked chromatographically for the presence of residual 3,5-DABTF. The result is that 5 min irradiation is sufficient for the total conversion of 3,5-DABTF. The compounds formed are similar in structure and proportion to those formed in the Rayonnet experiments.

In continuous experiments, 1 g/L, 10 mg/L or 1 mg/L of 3,5-DABTF was continuously passed through the streamer at 1 ml/min. The pH and temperature were continuously recorded as reported in the experimental section (Fig. 10). The results were the same for the three tested initial 3,5-DABTF concentrations (1000, 10 and 1 ppm). The starting pH and temperature were stabilized by flowing the solution through the streamer. When the system was turned on, we observed a rapid drop in pH from 6.22 to 3.24. This was concomitant with a rapid and complete defluorination of 3,5-DABTF to 3,5-DABA in 4.3 min, as confirmed by TLC and HPLC analysis of the collected solutions. The pH remained stable, while the solution temperature increased from 24.1 to 28.8°C in 11.5 min. When the system was turned off, the temperature rose to initial values in 7 min, while the pH returned to the initial values after 42 min.

We are currently optimizing the system (flow rate, energy power) to accelerate the comeback to initial pH values. To adapt the photoreactor to the nitroreductase-based unit, we are trying now to develop a miniaturized system based on laser-rays and capillary quartz tubes.

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REFERENCES

1. Nizamani, S.M. and R.M. Hollingworth (1980) Fentrifanil: a diarylamine acaricide with potent mitochondrial uncoupling activity. *Biochem. Biophys. Res. Commun.* **96**, 704–710.
2. Tingle, C.C., J.A. Rother, C.F. Dewhurst, S. Lauer and W.J. King (2003) Fipronil: environmental fate, ecotoxicology, and human health concerns. *Rev. Environ. Contam. Toxicol.* **176**, 1–66.
3. Wong, D.T., F.P. Bymaster and E.A. Engleman (1995) Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. *Life Sci.* **57**, 411–441.
4. Huff, J. (2003) Does exposure to bisphenol A represent a human health risk? *Regul. Toxicol. Pharmacol.* **37**, 407–408.
5. Welch, J.T. and S. Eswarakrishnan (1991) *Fluorine in Bioorganic Chemistry*. Wiley, New York.
6. Naal Z., J.H. Park, S. Bernhard, J.P. Shapleigh, C.A. Batt and H.D. Abruna (2002) Amperometric TNT biosensor based on the oriented immobilization of a nitroreductase maltose binding protein fusion. *Anal. Chem.* **74**, 140–148.
7. Van Der Stelt C., H.M. Tersteege and W.T. Nauta (1964) The effect of alkyl substitution in drugs. XII. Synthesis and properties of a number of trifluoromethyl-substituted alpha-(aminoalkyl)benzhydrols with their respective dehydrated products. *Arzneimittelforschung* **14**, 1324–1326.
8. Magueur G., B. Crousse, S. Charneau, P. Grellier, J.P. Begue, and D. Bonnet-Delpon (2004) Fluoroartemisinin: trifluoromethyl analogues of artemether and artesunate. *J. Med. Chem.* **47**, 2694–2699.
9. Quistad G.B. and K.M. Mulholland (1983) Metabolism of p-chlorobenzotrifluoride by rats. *J. Agric. Food Chem.* **31**, 585–589.
10. Engesser K.H., R.B. Cain and H. J. Knackmuss (1988) Bacterial metabolism of side chain fluorinated aromatics: cometabolism of 3-trifluoromethyl (TFM)-benzoate by *Pseudomonas putida* mt-2 and *Rhodococcus rubropertinctus* N 657. *Arch. Microbiol.* **149**, 188–197.
11. Kobayashi Y. and I. Kumadaki (1978) Reactions of aromatic trifluoromethyl compounds with nucleophilic reagents. *Acc. Chem. Res.* **11**, 197–204.
12. Bosca F., M.C. Cuquerella, M.L. Marin and M.A. Miranda (2001) Photochemistry of 2-hydroxy-4-trifluoromethylbenzoic acid, major metabolite of the potosensitizing platelet antiaggregant drug triflusal. *Photochem. Photobiol.* **73**, 463–468.