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The Search for Biochemical Photoprobes. IV[1]. The Photoreactions of 2,6-Difluoro-4-nitroanisole with Nucleophiles.

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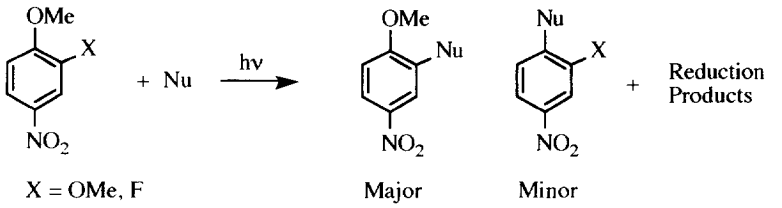
Abstract: The photoreactivity of 2,6-difluoro-4-nitroanisole in the presence of nucleophiles suggests that 2,6-difluoro-4-nitrophenyl ethers can constitute improved biochemical photoprobes for use in photoaffinity labeling reagents, specially for proteins where a number of good nucleophiles are present. Almost no photoreduction processes are observed, and one of the fluorine atoms is never replaced. This would allow the detection of the labeled fragments in a photoaffinity labeling experiment by simple NMR methods.

INTRODUCTION

The use of photochemically activated reagents in biology and medicine has gained big importance in recent years.[2] They can be divided into (i) photolabeling, (ii) photoaffinity labeling[3,4] and (iii) photocrosslinking reagents, and are composed of some or all of the following parts: photoprobes, thermal probes, label, and a linker which may contain a cleavable group. Common and most important to the different parts of these reagents are the photoprobes. A number of photoprobes have been described, some of which are even commercially available in the form of reactive derivatives. In practice, the most used have been aryl azides[2,5,6], with diazo compounds as the second most used type.[2,5,7] In a few cases ketones,[2,8] aryldiazonium salts,[2,9] or aromatic diazirines[10] have been used. Several other potential photoprobes, such as aroyl azides,[11] perfluorophenyl azides,[12] or aroyl nitrogen ylides[13] have been recently proposed in the literature and are currently under scrutiny. In an idealized version, the photoprobe should be thermally stable and the key intermediate created after photolysis would posses global reactivity. That is, its reaction with any chemical entity at the target site should be instantaneous and irreversible. None of the currently popular photoactivatable chemicals have demonstrated such thermal stability and global reactivity. On the contrary the bane of many photolabeling experiments is low efficiency of covalent bond formation after photolysis.[2] In addition, the ideal photoprobe should be easy to detect. The most common approach to this problem consist in introducing a radioactive tag into the molecule.

Cantor *et al* .[14] suggested the use of nitrophenyl ethers as photoprobes acting through nucleophilic aromatic photosubstitution[15] reactions (S_NAr^*). The photoreaction products should be in this case nitrophenyl compounds conjugated to proteins. Such compounds are well known haptens, and antibodies raised against the appropriate nitrophenyl ethers should be of great assistance as analytical tools.[16] Some successful examples of the use of nitrophenyl ethers as biochemical photoprobes have been reported,[17] even though simple nitrophenyl ethers

generally show relatively low efficiencies in photoreactions towards nucleophiles[18] and a certain propensity to photoreduce.[19] Recently, and based on studies on 2-fluoro-4-nitroanisole (Scheme 1), we proposed[20] 2-fluoro-4-nitrophenyl ethers as improved nitrophenyl ethers type photoprobes that can constitute a reasonable alternative to 4-nitrophenylazides, specially for proteins where a number of good nucleophiles are present. The improved photoreactivity and selectivity of monofluoronitrophenyl ethers with respect to simple nitrophenyl ethers was demonstrated in photoreactions with bovine pancretic ribonuclease A and with model nucleophiles.[1] One case of the use of fluoronitrophenoxy derivatives as photolabelling reagents of a model protein has been reported.[21]



Scheme 1

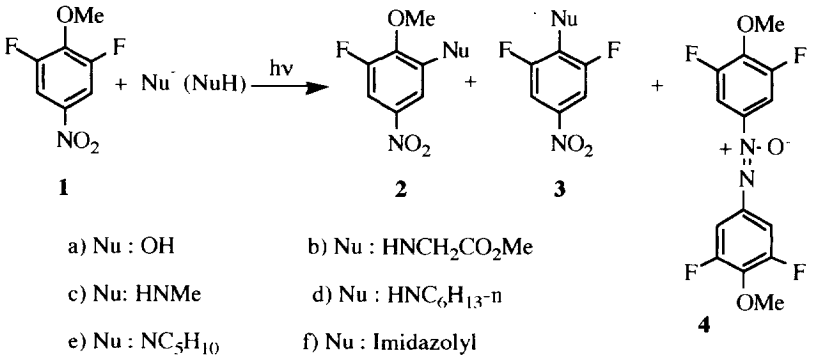
Our research on the reductive reactivity of polyfluoronitroaromatics[22] indicate that on increasing the number of fluorine atoms in the aromatic ring, outer sphere electron transfer reduction leads to longer lived radical anions without transformation into nitro group reduction products (the principal deletereous side reaction for the action of a nitrophenyl ether as a photoprobe). Therefore, we considered 2,6-difluoro-4-nitrophenyl ethers as good candidates for easily detectable biochemical photoprobes. We expected the good properties observed for the monofluoro compounds (enhanced substitution photoreactivity and practically disappearance of photoreduction processes) to be at least maintained, and only one fluorine atom to be substituted. In that case, the second fluorine atom that would remain in the molecule would permit alternative analysis and detection by simple spectroscopic methods (NMR). In the present paper we present a comparative study of the efficiencies of the photoreaction of 2,6-difluoro-4-nitroanisole, **1**, in the presence of different nucleophiles of biological type, and also a comparison of the efficiencies of some of the photoreactions of product **1** with respect to those reported for 4-nitroveratrole (as example of simple nitrophenyl ether) and 2-fluoronitroanisole (as example of monofluorinated nitrophenyl ether). The results are discussed from the viewpoint of their significance for the possible usefulness of 2,6-difluoro-4-nitrophenyl ethers as biological photoprobes.

RESULTS AND DISCUSSION

The photoreactions of 2,6-difluoro-4-nitroanisole, **1**, with several amines and with water and hydroxide ion are described in Scheme 2 and Table 1. A 400W medium pressure Hg lamp was used in a Pyrex inmersion well reactor. Excess of nucleophile was used in all the cases. The photohydrolysis reaction in basic or neutral medium (exp. 1 and 2 Table 1) led the isolation of 3-fluoro-2-methoxy-5-nitrophenol, **2a**, in 84% and 89% yields respectively. No other product (except the recovered starting material) could be detected.

The photoreactions of 2,6-difluoro-4-nitroanisole with amines of different type are described in the experiments 3-8 of Table 1. The reported experiments are organized from hard (exp. 3) to soft (exp. 8) amines.[19a] Thus, the photoreaction between substrate **1** and a hard amine as ethyl glycinate (exp. 3) in aqueous solution, led to 49% yield of the substitution product in the *meta* position with respect to the nitro group (**2b**). No substitution of the methoxy group was observed.On the other hand, when softer amines as methylamine and n-hexylamine were used in the same conditions (exp. 4 and 5), substantial amounts (20% and 22% yields respectively) of the substitution products in *para* position with respect to the nitro group, **3c** and **3d**, were obtained in addition to the corresponding substitution products in the meta position with respect to the nitro group, (N-methyl-3-fluoro-2-methoxy-5-nitroaniline, **2c**, and N-(1-hexyl)-3-fluoro-2-methoxy-5-nitroaniline, **2d**, respectively), and the photohydrolysis product **2a**. This trend is confirmed in the photoreaction of substrate

1 with an even softer amine as piperidine (exp. 7) where only substitution by amine in the *para* position is observed [N-(2,6-difluoro-4-nitrophenyl)piperidine, **3e**].



Scheme 2

From studies carried out mainly with 4-nitroveratrole,[15d,18] we known that in 3,4-disubstituted nitrobenzene systems the *meta* photosusbstitution product comes from a "polar" mechanism (the original S_NAr^* reaction described by Havinga[15a]), whereas the *para* photosubstitution one comes from a mechanism (SNAr*-SET)[18,15e] that includes initial electron transfer from the amine to the substrate excited state and collapse of the resulting radical-ion pair. Hard nucleophiles follow the first mechanism but soft ones tend to react through the single electron transfer mechanism. We have not carried out mechanistic studies on the photoreactions described in Table 1, but the results described in Table 1 fit nicely in the general S_NAr^* / S_NAr^*-SET mechanistic scheme for the nucleophilic aromatic photosusbtitution reactions.

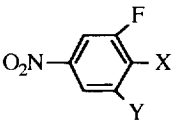
Experiments 6 and 8 show another remarkable result. Thus, when the photoreaction between substrate **1** and *n*-hexylamine is carried out in a polar aprotic solvent such as acetonitrile (exp. 6), the *para* substituted product (SNAR*-SET) is not produced any more, increasing the yield of the *meta* one (S_NAr^*) to 91%. The deletereous effect of acetonitrile on the S_NAr^*-SET process is confirmed by the result of experiment 8 (piperidine as a nucleophile) where no photosubstitution products were obtained. These results confirm earlier reports[1] described for the photoreactions of 2-fluoro-4-nitroanisole, and they can be important in the action of fluorinated nitrophenyl ethers as biochemical photoprobes since their environment in most cases will be lipophilic.[9b]

Imidazole is present in the histidine residues of proteins and these residues are very common in the catalytic sites of many enzymes (i.e. chymotypsin[23] or ribonuclease A[24]). Therefore, it was important to know if imidazole could act as a nucleophile in the presence of excited 2,6-difluoro-4-nitroanisole, **1**. The photoreactions of substrate **1** with imidazole as a nucleophile are described in exp. 9 and 10 (Table 1). When the photoreaction was carried out in water, 14% yield of the *meta* substitution product, 1-(3-fluoro-2-methoxy-5-nitrophenyl)imidazole, **2f**, was obtained together with a certain amount of the photohydrolysis product. The corresponding reaction in anhydrous acetonitrile resulted to be more complicated but the photosubstitution product **2f** was detected in the reaction crude by ^1H NMR. The conclusion from these experiments is that imidazole is much less active as a nucleophile in the presence of the excited substrate **1** than the other tested amines, but it gives covalent bond formation under irradiation. Considering that our model reactions correspond to the worst possible situation (the reactants moving freely in the solution), and that the probe in a photoaffinity labeling experiment is placed in the close vicinity of the nucleophile by the action of the biologically active molecule to which it is linked, these results can be considered as very positive.

Photoreactions described in Table 1 show the very low tendency of 2,6-difluoro-4-nitroanisole, **1**, to undergo photoreduction processes when compared with photoreactions of simple nitrophenyl ethers.[1,19] In experiments 4,5, and 8, 4,4'-dimethoxy-3,3',5,5'-tetrafluoroazoxybenzene, **4**, was obtained in small amounts

(less than 5% yield). This behavior confirms earlier reports[1,22] indicating that the presence of fluorine atoms in the aromatic ring reduces the electronic density in the nitro group of the nitroaromatic radical anion, stabilizing it, and making the nitro group reduction a slower process (reduction of the nitro group in nitrophenyl ethers includes protonation or hydrogen atom transfer to the corresponding radical anion).

Table 1. **Photoreactions of 2,6-Difluoro-4-nitroanisole, 1, with Nucleophiles.**



Exp.	Nucleophile	Reaction Conditions[a]	Substitution X	Products Y		Yield (%)[b]	Other Products (%)[b]
1	OH^-	H_2O/MeOH (4:1) 1h.	OMe	OH	**2a**	84	
2	H_2O	H_2O/CH_3CN (92:2) 1h 15 min.	OMe	OH	**2a**	89	
3	$EtO_2CCH_2NH_2$[c]	H_2O/CH_3CN (4:1) 0.5 h.	OMe	$HNCH_2CO_2Et$	**2b**	49	
4	CH_3NH_2[c]	H_2O/MeOH (4:1) 1 h.	OMe	HNMe	**2c**	20	**4** (4)
			HNMe	F	**3c**	20	
			OMe	OH	**2a**	28	
5	*n*-$C_6H_{13}NH_2$	H_2O/MeOH (4:1) 1 h.	OMe	HNC_6H_{13}-*n*	**2d**	19	**4** (5)
			HNC_6H_{13}-*n*	F	**3d**	22	
			OMe	OH	**2a**	30	
6	*n*-$C_6H_{13}NH_2$	CH_3CN anh., 0.5 h.	OMe	HNC_6H_{13}-*n*	**2d**	91	
7	$C_5H_{10}NH$	H_2O/MeOH (4:1) 0.5 h.	NC_5H_{10}	F	**3e**	12	
			OMe	OH	**2a**	41	
8	$C_5H_{10}NH$	CH_3CN anh., 0.5 h.	---	---	---	---	**4** (5)
9	Imidazole	H_2O, 12 h.	OMe	Imidazolyl	**2f**	14	
			OMe	OH	**2a**	40	
10	Imidazole	CH_3CN anh., 30 h.	OMe	Imidazolyl	**2f**	**d**	

[a]400W Medium pressure Hg lamp with pyrex filter. Room temperature. Excess of nucleophile. [b]Isolated yields with respect to non recovered starting material. [c]The free amine was prepared in situ from the corresponding hydrochloride and a stoichiometric amount of sodium hydroxide. [d]The crude was a complex mixture. Product **1g** could be detected by ^{1}HNMR.

In Table 2 the limiting (in the presence of a large excess of nucleophile) quantum yields for the photoreactions of 2,6-difluoronitroanisole with hydroxide ion and with water are compared with the previously known ones for the corresponding photoreactions of 4-nitroveratrole and 2-fluoro-4-nitroanisole.[20] The results of Table 2 confirm the high photoreactivity of 2,6-difluoro-4-nitroanisole even with neutral nucleophiles. The observed photoreaction with neutral, relatively weak nucleophiles such as H_2O (see also experiment 2 in Table 1) is not a drawback for many applications since the environment is going to be lipophilic.[9] On the contrary, the viability of those photoreactions would ensure bond formation in a wide pH range. This fact, taken together with the thermal inertness of **1**, its low tendency to give photoreduction products, and the observed single fluorine photosubstitution (one fluorine atom always remains in the phenyl ring) makes 2,6-difluoro-4-nitrophenyl ethers a reasonable alternative to other previously published compounds as biochemical photoprobes.

Table 2. **Limiting Quantum Yields for Photohydrolysis Reactions of 2,6-Difluoro-4-nitroanisole, 2-Fluoro-4-nitroanisole , and 4-Nitroveratrole.**

	Photosubstitution Quantum Yield		
Nucleophile	2,6-difluoro-4-nitroanisole	2-fluoro-4-nitroanisole	4-nitroveratrole
OH^{-a}	0.52[b]	0.6[c]	0.09[d]
H_2O	0.12[b]	0.11[c]	$<10^{-3}$[e]

[a]Excess of sodium hydroxide in water. [b]This work. [c]Ref. 25. [d]Ref.26. [e]Ref. 20.

EXPERIMENTAL

All melting points are uncorrected. 1H NMR spectra were recorded at 80 or 400 MHz and the 13C NMR at 20 or 100 MHz. The coupling constants with fluorine are indicated in the ^{13}C NMR spectra. 2,6-Difluoro-4-nitroanisole, 1, was prepared according to the method of Niemann, Benson, and Meal.[27]

Photohydrolysis of 2,6-difluoronitroanisole, 1. a) Neutral medium. In a 600 mL photochemical reactor, 0.183 g (0.87 mmol) of 2,6-difluoro-4-nitroanisole, **1**, 588 mL of water, and 12 mL of acetonitrile were introduced. The solution was irradiated through a pyrex filter, with a 400W medium-pressure Hg lamp for 1h 15 min. at room temperature. Then, the solution was extracted with chloroform, the organic layer dried and the solvent evaporated. Analysis of the residue indicated the absence of starting material. The aqueous layer was acidified and extracted again with chloroform. The new organic layer was dried and evaporated affording 0.162 g (89%) of a residue that was identified as 3-fluoro-2-methoxy-5-nitrophenol, **2a**, mp 53-56°C [chloroform-hexane, or sublimation (10 torr, 80°C)]: IR (KBr) 3700-3100 (broad), 2959, 2924, 1615, 1523, 1507, 1347, 1262, 1234, 1180, 1058, 975, 874 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.17 (d, J=3 Hz, 3H), 6.30 (broad, 1H), 7.59 (dd, J=11 Hz, J=2 Hz, 1H), 7.63 (d, J=2 Hz, 1H); ^{13}C ($CDCl_3$) δ 62.07 (d, J=8 Hz), 105.91 (d, J=25 Hz), 107.61 (d, J=2Hz), 141.03 (d, J=11 Hz), 143.11 (d, J=10 Hz), 150.09 (d, J=6 Hz), 153.78 (d, J=249 Hz); MS m/e (relative intensity) 188 (8) 187 (M^+, 100), 157 (42), 141 (17), 129 (15), 126 (19), 98 (17), 81(18), 70 (47), 69 (31); Calculated for $C_7H_6FNO_4$: C, 44.92; H, 3.21; N, 7.49. Found: C, 44.83; H, 3.22; N, 7.52.

b) *Basic medium.* In a 600 mL photochemical reactor, 0.343 g (1.81 mmol) of 2,6-difluoro-4-nitroanisole, **1**, 2,266 g (59.4 mmol) of sodium hydroxide, 480 mL of water, and 120 mL of methanol were introduced. The solution was irradiated through a pyrex filter with a 400W medium-pressure Hg lamp for 1h at room temperature. Then, the methanol was evaporated under vacuum. The resulting aqueous solution was extracted with chloroform, the organic layer dried and the solvent evaporated obtaining 0.195 g of a residue identified as 2,6-difluoro-4-nitroanisole, **1**, starting material. The aqueous layer was acidified and extracted again with chloroform. The new organic layer was dried and evaporated affording 0.123 g (37% yield, 84% with respect to non recovered starting material) of a residue that was identified as 3-fluoro-2-methoxy-5-nitrophenol, **2a**.

General procedure for the photoreactions of 2,6-difluoro-4-nitroanisole with amines (Table 1, exp. 3-8). In a 600 mL photochemical reactor, 1 mmol of 2,6-difluoro-4-nitroanisole, and 10 mmol of the corresponding amine dissolved in 600 mL of the indicated solvent were introduced. The solution was irradiated through a pyrex filter with a 400W medium-pressure Hg lamp at room temperature for the time indicated. Then, the reaction mixture was extracted between chloroform and water, and the aqueous phase acidified with HCl (1M) and extracted again with chloroform. The two organic layers were dried and the solvent evaporated. Phenols were obtained directly from the acid medium extraction. All other reaction products were obtained from the residue of the initial extraction after column chromatography through silica gel using mixtures chloroform/hexane as eluent. In the different reactions described in the Table 1 the following products (3-fluoro-2-methoxy-5-nitrophenol, **2a** has been just described in a previous paragraph) were obtained:

Ethyl N-(3-fluoro-2-methoxy-5-nitrophenyl)glycinate, **2b**, (exp. 3, Table 1). This product (49% total yield) appeared as an oil. IR (film) 3413, 3095, 2925, 1742, 1623, 1527, 1343, 1295, 1237, 1163 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (t, J=7 Hz, 3H), 3.94 (s, 2H), 4.06 (d, J=3 Hz, 3H), 4.24 (q, J=7 Hz, 2H), 5.00-5.50 (broad,

1H), 7.10 (m, 1H), 7.36 (dd, J=3 Hz, J=11 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.06, 44.97, 61.14 (d, J=8 Hz), 62.00, 101.37 (d, J=2 Hz), 102.85 (d, J=26 Hz), 139.15 (d, J=12 Hz), 141.12 (d, J =6 Hz), 142.88 (d, J=11 Hz), 153.87 (d, J=246 Hz), 169.88; Calculated for $C_{11}H_{13}FN_2O_5$: C, 48.53; H, 4.81; N, 10.29. Found: C, 48.79; H, 4.91; N, 10.12.

N-methyl-3-fluoro-2-methoxy-5-nitroaniline, **2c**, (exp. 4, Table 1). This product was obtained in 20% total yield, mp 72-73°C; IR (KBr) 3407, 2951, 1525, 1359, 1225, 996, 847 cm^{-1}; 1H NMR ($CDCl_3$) δ 2.94 (s, 3H), 4.06 (d, J=2.5 Hz, 3H), 7.26 (broad, 1H), 7.34 (dd, J=3.7 Hz, J=11.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 30.03, 60.93 (d, J=8 Hz), 99.99 (d, J=2 Hz), 101.08 (d, J=26 Hz), 143.21 (d, J=6.5 Hz), 152.84 (d, J=2.5 Hz); MS m/e (relative intensity) 201 (9), 200 (M^+,100), 185 (61), 155 (16), 154 (12), 139 (35), 127 (16), 111 (23), 70 (16); Calculated for $C_8H_9FN_2O_3$: C, 48.00; H, 4.53; N, 13.99. Found: C, 48.16; H, 4.69; N, 13.90.

N-methyl-2,6-difluoro-4-nitroaniline, **3c**, (exp. 4, Table 1). This product was obtained in 14% total yield (20% with respect to non recovered starting material), mp 130-2°C; IR (KBr) 3359, 3093, 2959, 1618, 1552, 1515, 1483, 1314, 1289, 888 cm^{-1}; 1H NMR ($CDCl_3$) δ 3.22 (t, J=2.5 Hz, 3H), 7.65 (dd, J=2.75 Hz, J= 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 62.63 (t, J=3.7 Hz), 108.81 (m), 140.79 (broad), 142.33 (t, J=12.9 Hz), 153.85 (dd, J=252 Hz, J=2.5 Hz) (absorbtions corresponding to carbons 1 and 2 in the phenyl ring appear as ill defined peaks around δ 127); MS m/e (relative intensity) 189 (9), 188 (M^+, 100), 158 (43), 142 (55), 127 (14), 114 (19), 101 (26), 95 (75), 75 (18); Calculated for $C_7H_6F_2N_2O_2$: C, 44.70; H, 3.19; N, 14.89. Found: C, 44.71; H, 3.17; N, 14.85.

Since product **3c** always appeared contaminated with product **2c**, it was synthesized by an independent way. Thus, a mixture of 0.730 g (3.8 mmol) of 2,6-difluoro-4-nitroanisole, **1**, 6.78 g (0.1 mol) of methylamine hydrochloride, 4.1 g (0.1 mol) of sodium hydroxide, 65 mL of water and 15 mL of methanol, was heated to 80°C for 130 h. under magnetic stirring in a stopped vessel. Then, the reaction crude was extracted with chloroform and the organic layer dried and evaporated under vacuum. The residue was identified as N-methyl-2,6-difluoro-4-nitroaniline, **3c**, (0.503 g, 70% yield), mp 130-132°C (methylene chloride-pentane).

N-(1-hexyl)-3-fluoro-2-methoxy-5-nitroaniline, **2d**, (exp. 5 and 6 Table 1). This product was obtained in 14% total yield (19% with respect to non recovered starting material) when the photoreaction was carried out in aqueous solution (exp. 5) and 91% total yield when it was performed in acetonitrile (exp. 6), and appeared as an oil that could not be crystallized; IR (film) 3411, 2950, 1525, 1330, 1210, 847 cm^{-1}; 1H NMR ($CDCl_3$) δ 0.87 (m, 3H), 1.16-1.87 (m, 8H), 3.26 (m, 2H), 3.98 (d, J=2.6 Hz, 3H), 5.45 (broad, 1H), 7.13-7.37 (complex abs. 2H); MS m/e (relative intensity) 271 (M^+, 2), 270 (13), 199 (100), 153 (16), 83 (4), 55 (8); Calculated for $C_{13}H_{19}FN_2O_3$: C, 55.77, H, 7.08; N, 10.36. Found: C, 55.81; H, 6.95; N, 10.20.

N-(1-hexyl)-2,6-difluoro-4-nitroaniline, **3d**, (exp. 5, Table 1). This product was obtained as an oil, in 16% total yield (22% with respect to non recovered starting material); IR 3392, 2930, 2860, 1616, 1525, 1325, 1068, 1018 cm^{-1}; 1H NMR ($CDCl_3$) δ 0.95 (m, 3H), 1.24-1.81 (complex abs. 8H), 3.52 (m, 2H), 7.64 (dd, J=9.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 13.78, 22.34, 26.03, 30.65, 31.30, 45.21 (t, J=4.6 Hz), 108.6 (m), 132.83 (t, J=12.9), 134.81 (t, J=11.5), 149.44 (dd, J=243 Hz, J=9.2 Hz); MS m/e (relative intensity) 258 (M^+, 7), 187 (100), 174 (12), 141 (51), 94 (12); Calculated for $C_{12}H_{16}F_2N_2O_2$: C, 55.81; H, 6.24; N, 10.85. Found: C, 56.08; H, 6.22; N, 10.83.

Since product **3d** always appeared contaminated with product **2d**, it was synthesized by an independed way. Thus, a mixture of 0.530 g (2.8 mmol) of 2,6-difluoro-4-nitroanisole, **1**, 8.51 g (0.084 mol) of *n*-hexylamine, 65 mL of water and 15 mL of methanol, was heated to 75°C for 45 h. under magnetic stirring in a stopped vessel. Then, the reaction crude was extracted with chloroform and the organic layer dried and evaporated under vacuum. The residue was identified as N-(1-hexyl)-2,6-difluoro-4-nitroaniline, **3d**, (0.543 g, 75% yield), as an oil that did not crystallized.

N-(2,6-difluoro-4-nitrophenyl)piperidine, **3e**, (exp. 7, Table 1). This product was obtained as an oil, in 11% total yield (12% with respect to non recovered starting material). IR (film) 3102, 2939, 1607, 1520, 1450, 1392, 1350 cm^{-1}; 1H NMR ($CDCl_3$) δ 1.55 (m, 6H), 3.20 (m, 4H), 7.62 (dd, J=8.5 Hz, J=1.5 Hz, 2H); ^{13}C NMR

($CDCl_3$) δ 23.99, 26.39, 51.93, (t, J=4.6 Hz), 108.86 (m), 135.71 (t, J=12.02 Hz), 138.90, 154.92 (dd, J=248.8 Hz, J=8.33 Hz); MS m/e (relative intensity) 242 (M^+, 58), 241 (100), 201 (15), 195 (28), 186 (13), 139 (10); Calculated for $C_{11}H_{12}F_2N_2O_2$: C, 54.54; H, 4.96; N, 11.57. Found: C, 54.60; H, 5.21; N, 11.42.

In order to completely characterize product **3e**, it was synthetized by an independent way. Thus, a mixture of 0.360 g (1.97 mmol) of 2,6-difluoro-4-nitroanisole, **1**, 5.023 g (59.1 mmol) of piperidine, 65 mL of water and 15 mL of methanol, was heated to 80°C for 96 h. under magnetic stirring in a stopped vessel. Then, the reaction crude was extracted with chloroform and the organic layer dried and evaporated under vacuum. The residue was identified as N-(2,6-difluoro-4-nitrophenyl)piperidine, **3e**, (0.356 g, 75% yield), as an oil, bp (oven temperature) 120°C/0.4 torr.

4,4'-Dimethoxy-3,3',5,5'-tetrafluoroazoxybenzene, **4**, (exp. 4, 5, and 8, Table 1). Mp 160-4°C; IR (KBr) 3100, 2959, 1604, 1569, 1507, 1342, 1250, 1045, 995, 869 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.09 (t, J=1.5 Hz, 3H), 4.14 (t, J=1.5 Hz, 3H), 7.92 (m, 4H); MS m/e (relative intensity) 331 (6), 330 (M^+, 36), 173 (22), 157 (95), 143 (100), 128 (38), 100 (33), 95 (23); Calculated for $C_{14}H_{10}F_4N_2O_3$: C, 50.92; H, 3.05; N, 8.48. Found: C, 50.79; H, 3.10; N, 8.55.

Photoreactions of 2,6-difluoro-4-nitroanisole, 1, with imidazole (exp. 9 and 10, Table 1). In a 600 mL photochemical reactor, a solution of 0.060 g (0.317 mmol) of 2,6-difluoro-4-nitroanisole,**1**, and 0.648 g (9.52 mmol) of imidazole in 600 mL of water. The solution was irradiated through a pyrex filter with a 400W medium-pressure Hg lamp and at room temperature for 12 h. Then, the reaction mixture was extracted between chloroform and aqueous NaOH (0.1M).The aqueous phase was acidified with 1M HCl and extracted again with chloroform. The organic layer corresponding to this extraction was dried and evaporated, affording 0.020 g (34% total yield, 40% with respect to the non recovered starting material) of a residue that was identified as 3-fluoro-2-methoxy-5-nitrophenol, **2a**. The organic layer of the former extraction was washed with 0.1M HCl. The organic layer was dried and the solvent evaporated affording 0.009 g of a residue that was identified as 2,6-difluoro-4-nitroanisole, 1, starting material. The aqueous layer was basified with aqueous NaOH and then extracted with chloroform. The organic layer corresponding to this last extraction was dried and the solvent evaporated. The residue was filtered through a short column of silica-gel using hexane-chloroform as eluent to give 0.009 g (12% total yield, 14% with respect to non recovered starting material) of an oily residue that was identified as 1-(3-fluoro-2-methoxy-5-nitrophenyl)imidazole, **2f**, on the basis of its spectroscopic behavior: IR (film) 3115, 2924, 1589, 1525, 1263, 1056, 860 cm^{-1}; ^1H NMR (acetone-d_6) δ 4.00 (s, 3H), 7.10 (m, 1H), 7.34 (d, J=10.6 Hz, 1H), 7.60 (m, 1H), 7.93 (s, 1H), 8.10 (broad, 1H); MS m/e (relative intensity) 211 (9), 210 (100), 194 (31), 168 (90), 140 (23), 120 (16), 113(17), 100 (17).

This reaction was repeated using acetonitrile as a solvent. The reaction crude was very complex, and by comparison of the ^1H NMR spectrum, product **2f** was detected in the mixture.

Quantum yield measurements (Table 2). Quantum yields for the photosubstitution products were measured using a merry-go-round apparatus. The irradiation source was a 250W medium pressure Hg lamp. The wavelength of excitation (366 nm) was selected using a monochromator. The amounts of photoproducts were determined by GC analyses (internal reference). Actinometry was performed using potassium ferrioxalate,[28] and conversion was kept around 5% in all the cases. Care was taken that >98% of the light was absorbed by the sample and the actinometer. No precautions were taken for the presence of oxygen. All the values are the result of five measurements eliminating the two extremes and averaging the other three.

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