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The Search for Biochemical Photoprobes. III[1]. The Photoreactions of 4-Nitroveratrole and 2-Fluoro-4-nitroanisole with Bovine Pancreatic Ribonuclease A and with Model Nucleophiles.

Jorge Marquet,*[a] Lourdes Rafecas,[a] Albert Cantos,[a] Marcial Moreno-Mañas,[a] Maria Cervera,[a] Francisco Casado,[a] M. Victòria Nogués,[b] and Claudi M. Cuchillo[b]

[a]Department of Chemistry. [b]Department of Biochemistry and Molecular Biology.. Universitat Autònoma de Barcelona. 08193 Bellaterra. Barcelona. Spain

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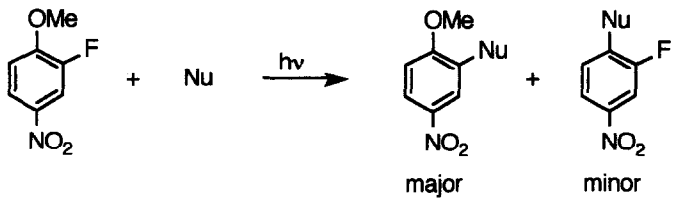
Abstract: The photoreactions of 4-nitroveratrole (NVT) and 2-fluoro-4-nitroanisole (FNA) with bovine pancreatic ribonuclease A (RNAase A) and with model nucleophiles are described. From them it is concluded that 2-fluoro-4-nitrophenyl ethers constitute improved alternative biochemical photoprobes, specially for proteins where a number of good nucleophiles are present.

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) photolabelling, (ii) photoaffinity labelling 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. Photolabelling reagents are used to attach a label (tag) on biological macromolecules and are usually composed of a photoprobe and a label. Photoaffinity labelling reagents are used to identify and analyze receptors on biological macromolecules by photochemical attachment of a label to the target.[3] They are, as a rule, modified receptor substrates with an auxiliary group (the photoprobe)[4] or with their own structure slightly modified to become photoactive by itself.[5] Photocrosslinking reagents are used to study interactions between two or more biological macromolecules. They usually contain one thermal probe and one photoprobe (or two photoprobes) bound together by a linker, ideally cleavable. Common and most important to the different parts of the commented 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 arylazides[2,6,7], with diazo compounds as the second most used type.[2,6,8] In a few cases ketones[2,9] or aryldiazonium salts[2,10] have been used. Several other potential photoprobes, such as aroylazides[11] and perfluorophenyl azides[12] have

been recently proposed in the literature. However, no complete studies have been performed on them yet. It is known that aroylazides would produce, under irradiation, long lived isocyanates as side products that will almost certainly cause unacceptable pseudolabelling. 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 photolabelling experiments is low efficiency of covalent bond formation after photolysis.[2] Cantor *et al*.[13] suggested the use of nitrophenyl ethers as photoprobes acting through nucleophilic aromatic photosubstitution[14] reactions (S_NAr^*). Some successful examples of their use have been reported,[15] even though simple nitrophenyl ethers generally show relatively low efficiencies in photoreactions towards nucleophiles[16] and a certain propensity to photoreduce.[17] Recently, and based on studies on 2-fluoro-4-nitroanisole (Scheme 1), we proposed[1,18] 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. One case of the use of fluoronitrophenoxy derivatives as photolabelling reagents of a model protein has been reported.[19]



Scheme 1

Since nothing was known, from the chemical point of view, about the photoreactivity of simple nitrophenyl ethers *versus* biological macromolecules of interest such as proteins, we have decided to compare the photoreactions of 4-nitroveratrole (2-methoxy-4-nitroanisole, NVT), as a model for simple nitrophenyl ethers, and 2-fluoro-4-nitroanisole (FNA), as a representative of the new fluoinated nitrophenyl ethers photoprobes, with bovine pancreatic ribonuclease A, an enzyme that hydrolyzes RNA and consists of a single polypeptide chain of 124 residues.[20] The particular photoselectivities of the photoprobes *vs.* the different nuleophiles present in the amino acid residues have been elucidated for this particular protein. Finally, and with the help of model photoreactions, the chemoselectivity (photosubstitution *vs.* photoreduction) in each case has also been established.

RESULTS AND DISCUSSION

Photoreactions of 4-nitroveratrole (NVT) and 2-fluoro-4-nitroanisole (FNA) with pancreatic bovine ribonuclease A (RNAase A).

RNAA was irradiated in the presence of excess NVT or FNA in water/methanol solution at pH 10. After irradiation, the samples were freeze-dried and chromatographed through Bio-gel P-6 (exclusion limit 6000) to separate the non

reacted NVT or FNA. The resulting proteins were hydrolyzed and the total amino acid contents of the samples analyzed. The results were compared with the corresponding amino acid contents of the starting RNAase A, analyzed following exactly the same procedure. In that way, the decrease percentage for each particular amino acid after irradiation was obtained. The results are summarized in Table 1. Thus, in spite of its reasonable photoreactivity with amine nucleophiles in model reactions,[14,16] NVT does not seem to photoreact with the lysine residues present in RNAase A. In fact, the photoreactivity of NVT *versus* RNAA is very limited, only three amino acid residues showing a significant contents decrease, His (imidazole in the side chain), Met (thioether) and Tyr (phenol). On the other hand, and as it was predicted from model experiments, FNA shows a much higher and broader photoeactivity versus the nucleophiles present in the different amino acid residues of RNAase A. Thus, a significant contents decrease after irradiation is observed for Asp and Glu (carboxylic acids), His (imidazole), Lys (primary amino group), Met (thioether), and Ser (primary alcohol). Curiously enough, no photoreaction is observed between FNA and the tyrosine (Tyr) residues of RNAase A.

Table 1.- Irradiation of the Bovine Pancreatic Ribonuclease A (RNAase A) in the presence of 4-Nitroveratrole (NVT) or 2-Fluoro-4-nitroanisole (FNA) at pH 10.

Amino acid residues[a]	Amino acid residues number			
	Theoretical[b]	Actual[c]	NVT hv (decrease %)[d]	FNA hv (decrease %)[e]
Asp	15	12.2	f	6.9 (44)
Glu	12	10.7	f	5.7 (47)
His	4	3.1	1.8 (42)	0.8 (74)
Lys	10	7.6	f	5.0 (34)
Met	4	3.9	3.3 (16)	2.4 (38)
Ser	15	12.2	f	9.0 (26)
Tyr	6	5.7	3.4 (40)	f

[a]Only the significantly altered (>15%), after irradiation, amino acid residues are indicated. The cystine bridges are not hydrolyzed in the followed procedure and therefore no information about their possible modification is available. [b]Theoretical amino acid contents in the pure RNAA (after ref. 18). [c]Amino acid contents in the actually used RNAase A, after two concordant analysis. [d]Amino acid contents in the RNAase A after irradiation in the presence of NVT. [e]Amino acid contents in the RNAase A after irradiation in the presence of FNA. [f]Not significantly different from the value in RNAase A prior to irradiation.

Analysis of the results reported in Table 1 indicate that for NVT, the photoreactions were produced on the amino acid residues more prone to undergo redox photochemistry (Met and Tyr), and on a non particularly nucleophilic amino acid residue like His. No model studies on the photoreactivity of NVT in front of phenols, thioethers, and imidazoles were known. Useful photolabelling should involve covalent bond formation between the biological macromolecule and the photoprobe (photosubstitution reaction in the case of the nitrophenyl ethers). Therefore, we considered necessary to complete the model studies in order to establish the nature of the observed photoreactions (photoredox or photosubstitution) with the protein.

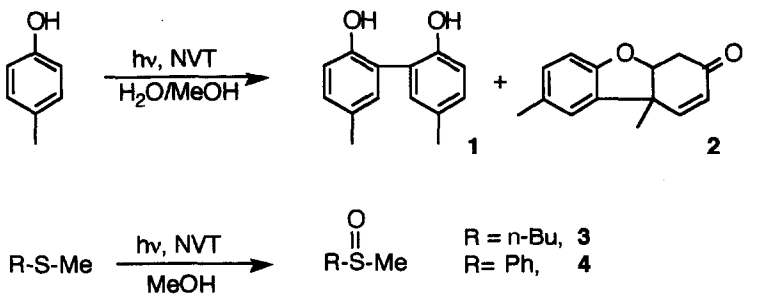
The case of FNA is clearly more promising, all the modified amino acid residues, except Met, should show a higher photosubstitution than photoredox reactivity. Nevertheless, only model photoreactions with amines and alkoxide groups have been previously reported with this substrate. It was therefore convenient, also in this case, to complete the model studies. Remarkably, both probes show an interesting very high photoreactivity *versus* the amino acid residue histidine (His), not predictable on the bases only of its nucleophilicity.

Model photoreactions of NVT in polar protic solvents.

The irradiation of NVT in the presence of excess p-cresol (as a model for tyrosine), in water-methanol as a solvent for 24 h led to a mixture where only photoredox products, the dimer 1, and the Pummerer's ketone 2, could be isolated in small amounts (Scheme 2). No photosubstitution products were identified in the reaction crude. The irradiation was carried out filtering the light for wavelenghts lower than 370 nm, so that only the NVT was excited.

In a similar way, n-butyl methyl sulfide, and thioanisole, were irradiated in the presence of NVT in methanol for 30 minutes. The crude was directly analyzed by GC/MS showing, in both cases, the formation of a unique photochemical product, identified as the corresponding sulfoxide (n-butyl methyl sulfoxide, 3, and methyl phenyl sulfoxide, 4, scheme 2) on the basis of their molecular peak (for 3 the chemical ionization mass spectrum was necessary) and fragmentations.

These results suggest that NVT acts only as a redox photosensitizer in front of phenols (like tyrosine residues) and sulfides (like methionine residues).



Scheme 2

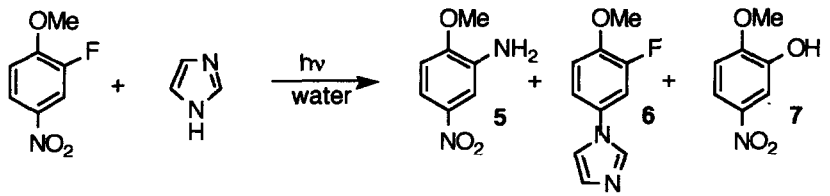
The corresponding irradiation of imidazole in the presence of NVT in water/methanol led to the recovering of the starting materials, leaving open the question about the photoreactivity of histidine residues in front of NVT.

Model photoreactions of FNA in polar protic solvents.

Considering the amino acid residues that had been altered by irradiation of RNAase A in the presence of FNA (Table 1), model photoreactions for those bearing amine (Lys) and hydroxyl (Ser) nucleophiles in the side chain are already known[1,18] (Scheme 1). These photoreactions suggest that covalent bond formation is very likely between RNAase A and the probe on these amino acid residues.

We have studied the model photoreactions of the other nucleophiles involved in the results of Table 1 with FNA. Thus, irradiation of a solution of imidazole in the

presence of FNA in water for 6 hours, led to a mixture from which 2-methoxy-5-nitroaniline, 5 (13% yield), 1-(3-fluoro-4-methoxyphenyl)imidazole, 6 (13% yield) and 2-methoxy-5-nitrophenol, 7 (31% yield) could be isolated (Scheme 3; yields based in non recovered starting material). Product 7 comes from the well known ability of FNA to photoreact with water. Product 6 is a photosubstitution product, and product 5 seems to come from photosubstitution to produce 1-(2-methoxy-5-nitrophenyl)imidazole and its photodegradation in the reaction conditions. In any case FNA is able to form covalent bond with imidazole although the photoreaction does not seem to be very efficient.



Scheme 3

No photoreaction was achieved between model carboxylates and FNA. This was surprising considering that the Glu (glutamic acid) and Asp (aspartic acid) residues had been modified when RNAase A was irradiated in the presence of FNA (Table 1).

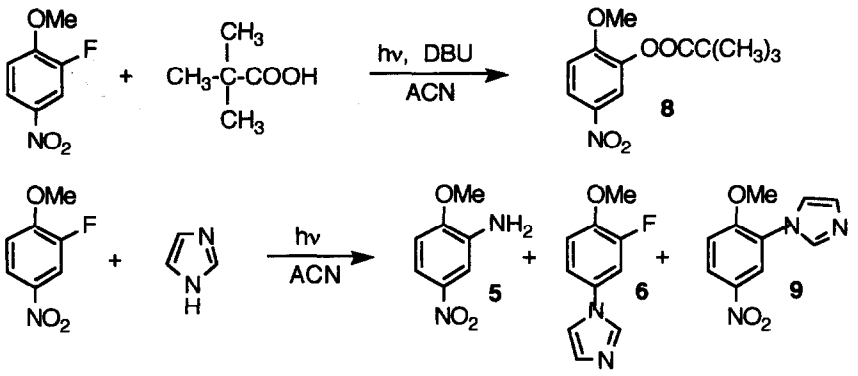
An interesting feature from the results of Table 1 is the unreactivity of Tyr residues *versus* FNA. From our previous experiments with amines,[1,16] it was already evident that FNA was less prone than NVT to undergo redox photochemistry, and this result seems to support this observation. In a model experiment, FNA was irradiated in the presence of *p*-cresol in methanol-water and no photoreaction (apart from the known photoreaction of FNA with the solvent) was observed. However, when FNA was irradiated in the presence of thioanisole (as a model for MET) for 30 m in similar conditions, methyl phenyl sulphoxide, 4 (photoredox product) was the only identified photoproduct.

Model photoreactions of FNA in polar aprotic solvents.

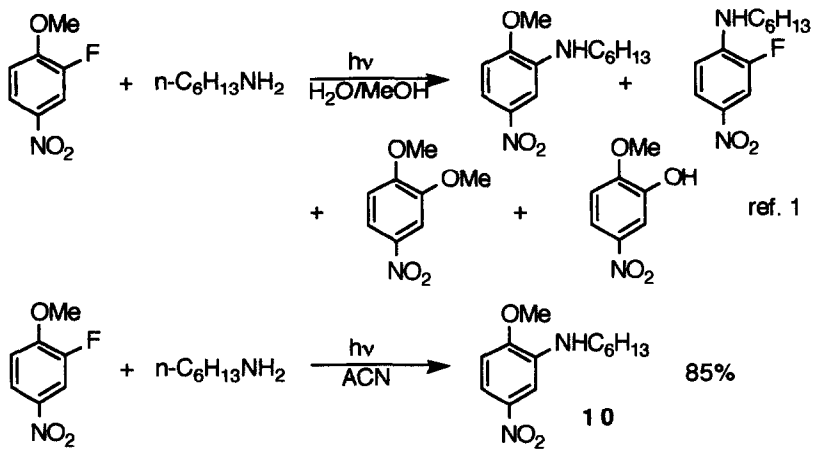
In the previously discussed model photoreactions carried out in water, reaction with the solvent was always an important side reaction. Also, the unreactivity of carboxylate derivatives was surprising considering the results of Table 1. We considered that a protein like RNAase A has hydrophobic microenvironments. The stability of the carboxylate groups in model photoreactions could be due to the strong solvation in water that would reduce their nucleophilicity. Thus, an equimolecular solution of pivalic acid, DBU (diazobicycloundecene, as a non nucleophilic base), and FNA in anhydrous acetonitrile was irradiated for 6 h. 2-Methoxy-5-nitrophenyl 2,2-dimethylpropionate, 8, was produced in 29% yield base on non consumed starting material (Scheme 4), thus confirming the highest nucleophilicity of carboxylates in non protic media and justifying the modification of Asp and Glu residues in RNAA upon irradiation in the presence of FNA (Table1).

When the photoreaction between FNA and imidazole was carried out in anhydrous acetonitrile, trace amounts of a new photoproduct, tentatively formulated as 1-(2-methoxy-5-nitrophenyl)imidazole, 9, were obtained (Scheme 4).

The improved properties of acetonitrile over water or methanol-water mixtures, from the photosubstitution point of view, were tested carrying out the photoreaction of FNA with n-hexylamine (well studied in water-methanol[1,18]) in anhydrous acetonitrile as a solvent. In this case the *meta* photosubstitution product 10, was the only identified photoproduct (55% preparative yield, 85% over non consumed starting material). The results of both photoreactions, in water-methanol and in anhydrous acetonitrile are compared in Scheme 5.



Scheme 4



Scheme 5

CONCLUSIONS

From the reported results we can conclude that FNA shows a higher and broader photoreactivity than NVT against the nucleophiles present in the amino acid residues of RNAase A. Model studies suggest that photoredox processes are predominant in the case of NVT. On the other hand, model studies suggest that for FNA nearly all the observed photochemistry is of the photosubstitution type. Therefore,

fluoronitrophenyl ethers are predicted to be good choices as alternative biochemical photoprobes. In addition, the medium effects observed in our model photoreactions indicate the existence of a different environement from water around the modified Glu and Asp residues in RNAase A.

Finally, in spite of some covalent bond formation in the model photoreaction between imidazole and FNA, the high photoreactivity of the His residues against both photoprobes has not been explained. An attractive hypothesis could be the existence of some sort of recognition of the aromatic photoprobe by the RNAase A.

EXPERIMENTAL

All melting points are uncorrected. ^{1}H NMR were recorded at 80 or 400 MHz. The GC analysis were performed using a HP-Crosslinked Dimethylsilicone Gum 12m x 0.2mm x 0.33m film thickness capillary column. 4-Nitroveratrole was prepared by nitration of veratrole.[21] 2-Fluoro-4-nitroanisole was prepared by nitration of 2-fluoroanisole.[22]

Protein amino acid analysis. Aprox. 2 nmol of the protein was hydrolyzed with 50 μl of 6N constant-boiling-point HCl for 48 h at 105°C in vacuo. The resulting solution was freeze-dried and the analysis was performed in a Rank Hilger Chromaspek J 180 ion-exchange chromatograph with fluorescence detector. The results elucidation was performed following the method of Murren *et al.*.[23]The amino acid fluorescent derivatives were obtained following the method of Drescher *et al.*.[24] Norleucine was used as internal standard.

Photoreactions of Bovine Pancretic Ribonuclease A (RNAase A) with 4-nitroveratrole (NVT) and 2-fluoro-4-nitroanisole (FNA) (Table 1). A solution of 0.15 mmol of NVT or FNA and 34.7 mg (0.0025 mmol) of RNAase A in 24 ml of a 1/2 (v/v) mixture of methanol/20mM ammonium carbonate (pH10) water solution), was irradiated for 4 h with a 125 W high pressure Hg vapor lamp in a pyrex photochemical reactor. Prior to irradiation, the pH of the solution was adjusted to pH10 with sodium hydroxide. After irradiation the sample was freeze-dried. The non-reacted nitrophenyl ether was separated by gel filtration chromatography through a water equilibrated column of Bio-gel P-2 (1 x 90 cm column, 6 ml/h flow rate, exclusion limit 6000). The protein-containing fractions were collected and the corresponding amino acid analysis carried out. The results are indicated in Table 1.

Photoreactions of 4-nitroveratrole (NVT) and 2-fluoro-4-nitroanisole (FNA) with p-cresol in methanol-water. Irradiation of a solution of NVT (0.0285 g, 0.156 mmol), *p*-cresol (4.87 g, 45.1 mmol) in a mixture of 160 mL of methanol and 320 mL of water for 24 h in a Pyrex immersion well reactor using a 400-W medium pressure Hg lamp afforded, after isolation by column chromatography through silica-gel (hexane/$CHCl_3$): the starting materials (p-cresol, 3.39 g; NVT, 0.026 g); 2,2'-dihydroxy-5,5'-dimethylbiphenyl, 1 (0.069 g, 4.7% yield based on non recovered starting *p*-cresol), mp 153-154 C (lit[25] 153.5 C) : IR (KBr) 3130, 2920, 1480, 1445, 1400, 1335, 1230, 1110, 805 cm^{-1}; ^{1}H NMR ($CDCl_3$) 2.32 (s, 6H), 5.26 (broad, 2H), 6.87 (d, J=8.2 Hz, 2H), 7.06 (d, J=2.6 Hz, 2H), 7.20 (dd, J=8.2 Hz, J=2.6 Hz, 2H); MS, m/e (relative intensity) 214 (110, M^+), 199 (23), 115 (22), 77 (13); and the Pummerer's ketone, 2 (0.078 g, 2.7% yield based on non recovered starting *p*-cresol), mp 122.5-124 C (lit[26] 124 C): IR (KBr) 2910, 1660, 1610, 1230, 1100, 1045, 990, 935, 895, 795, 750; ^{1}H NMR ($CDCl_3$) 1.57 (s, 3H), 2.35 (s, 3H), 2.78 (dd, J=17.7 Hz, J=4.1 Hz, 1H), 3.05 (ddd, J=17.7 Hz, J=2.8 Hz, J=0.8 Hz, 1H), 4.70 (ddd, J=4.1 Hz, J=2.8 Hz, J=1.5 Hz, 1H),

5.91 (dd, J=0.8 Hz, J=10.0, 1H), 6.44 (dd, J=1.5 Hz, J=10.0 Hz, 1H), 6.69 (d, J=7.5 Hz, 1H), 6.95 (dd, J=7.5 Hz, J=2.3 Hz, 1H), 7.00 (d, J=2.3 Hz, 1H); MS, m/e (relative intensity) 214 (11, M^+), 171 (10), 159 (10), 145 (43), 128 (52), 115 (46), 102 (22), 91(57), 77 (86), 63 (78), 51(100), 42(86).

This procedure was repeated using FNA instead of NVT. In this case no photoproducts derived from interaction of *p*-cresol and FNA were detected by GC analysis of the reaction crude. Only peaks corresponding to the starting materials, 2-methoxy-4-nitrophenol and 4-nitroveratrole (photoreactions with the solvent) were observed.

Photoreactions of 4-nitroveratrole (NVT) and 2-fluoro-4-nitroanisole (FNA) with thioethers in methanol. 2 mL of a 0.018M NVT and 0.3M n-butyl methyl thioether methanolic solution were placed in a quartz cuvette and irradiated for 30 m with a 125-W high pressure Hg lamp through a filter of 2-(2-hydroxy-5-methylphenyl)-2-benzotriazole (TINUVIN-P™, $\lambda > 370$ nm). Direct analysis of the crude (after filtration through a column of silica-gel using CH_2Cl_2 as eluent) by GC/MS showed the presence of the starting materials and a new peak identified by comparison with an authentical sample as n-butyl methyl sulfoxide, 3: MS m/e (relative intensity) 78 (90), 63 (32) , 57(38), 41(100); MS m/e chemical ionization (NH_3) 121 (M+1), 138 (M+18), 155 (M+35).

2 mL of a 0.018M NVT and 0.3M thioanisole methanolic solution were placed in a quartz cuvette and irradiated for 30 m with a 125-W high pressure Hg lamp through a filter of 2-(2-hydroxy-5-methylphenyl)-2-benzotriazole (TINUVIN-P™, $\lambda > 370$ nm). Direct analysis of the crude (after filtration through a column of silica-gel using CH_2Cl_2 as eluent) by GC/MS showed the presence of the starting materials and a new peak identified by comparison with an authentical sample as methyl phenyl sulfoxide, 4 : MS m/e (relative intensity) 140 (M^+, 36), 125 (63), 109 (29), 97 (58), 91 (22), 77 (43), 65 (24), 51 (100). The same result was obtained when this procedure was followed using FNA instead NVT.

Photoreactions of 2-fluoro-4-nitroanisole with imidazole in water and in acetonitrile. A solution of FNA (0.230 g, 1.35 mmol), and imidazole (2.74 g, 40.4 mmol) in 740 mL of water was irradiated for 6 h in a Pyrex immersion well reactor using a 400-W medium pressure Hg lamp. After irradiation, the solution was basified with aqueous NaOH and extracted with chloroform. The aqueous phase was acidified with 1M HCl and extracted with chloroform to afford 40 mg of 2-methoxy-5-nitrophenol,[1,27] 7 (31% yield based on consumed FNA). The first chloroform phase was washed with HCl 1M, to afford there 100 mg of the starting FNA. The aqueous layer was brought to neutrality and then extracted with chloroform. The crude (58 mg) thus obtained was chromatographed through neutral alumina using hexane/$CHCl_3$ as eluent. The following compounds were eluted: 2-Methoxy-5-nitroaniline (compared with a commercially available sample), 5 (17 mg, 13% yield based on non consumed starting material), and 1-(3-fluoro-4-methoxyphenyl)imidazole, 6 (19 mg, 13% yield based on non consumed starting material): IR (film) 3115, 2924, 1525, 1263, 1056; ^1H NMR ($CDCl_3$) 4.00 (s, 3H), 7.14 (m, 2H), 7.27 (broad, 3H), 7.90 (broad, 1H); MS m/e (relative intensity) 192 (M^+, 100), 177 (21), 165 (9), 150 (62), 122 (20), 95 (18); calculated for $C_{10}H_9FN_2O$: C, 62.45; H, 4.72; N, 14.58; found: C, 62.30; H, 4.91; N, 14.63.

When the same procedure was followed, but using anhydrous acetonitrile as a solvent, trace amounts (less than 1 mg) were isolated of a compound that was

tentatively formulated as 1-(2-methoxy-5-nitrophenyl)imidazole, 9, on the bases of its spectroscopic behaviour: ^{1}H NMR (Acetone-d_6) 4.09 (s, 3H), 7.15 (broad, 1H), 7.37 (m, 2H), 8.03 (broad, 1H), 8.37 (m, 2H); MS m/e (relative intensity) 220 (M+1, 13), 219 (M^+, 100), 192 (76), 191 (45), 158 (11), 146 (11), 103 (12), 79 (15), 77(11).

Photoreaction of 2-fluoro-4-nitroanisole (FNA) with 2,2-dimethylpropanoate (pivalate) in anhydrous acetonitrile. Irradiation of a solution of FNA (0.171 g, 1 mmol), pivalic acid (0.102 g, 1 mmol) and DBU (0.152 g, 1 mmol) in 600 ml of anhydrous acetonitrile for 6 h in a Pyrex immersion well reactor using a 400-W medium pressure Hg lamp afforded, after isolation by column chromatography through silica-gel (hexane/$CHCl_3$): FNA (108 mg) and 2-methoxy-5-nitrophenyl 2,2-dimethylpropanoate, 8 (27 mg, 29% yield over non consumed starting material), mp 71-73 C; IR (KBr) 3097, 3076, 2983, 2927, 1749, 1604, 1520, 1343; ^{1}H NMR ($CDCl_3$) 1.39 (s, 9H), 3.94 (s, 3H), 7.00 (d, J=10 Hz, 1H), 7.97 (d, J=2.5 Hz, 1H), 8.16 (dd, J=10 Hz, J=2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 26.94, 39.02, 56.36, 111.28, 118.89, 122.76, 139.88, 140.93, 156.77, 175.80; Calculated for $C_{12}H_{15}NO_5$: C, 56.69; H, 5.92; N, 5.53. Found: C, 56.70; H, 6.26; N, 5.28.

Photoreaction of 2-fluoro-4-nitroanisole with n-hexylamine in anhydrous acetonitrile. Irradiation of a solution of FNA (0.085 g, 0.5 mmol) and n-hexylamine (0.759 g, 7.5 mmol) in 40 ml of anhydrous acetonitrile for 30 m in a Pyrex immersion well reactor using a 125-W high pressure Hg lamp afforded, after isolation by column chromatography through silica-gel (hexane/AcOEt): FNA (0.031 g) and N-(1-hexyl)-2-methoxy-5-nitroaniline, 10 (0.068 g, 85% yield over non recovered starting material), mp 34-36°C (lit[16] 34-36°C), this product is fully described in ref 16.

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REFERENCES AND NOTES

1. Part 2. Pleixats, R.; Figueredo, M.; Marquet, J.; Moreno-Mañas, M.; Cantos, A. *Tetrahedron* 1989, *45*, 7817.
2. *Photochemical Probes in Biochemistry*; Nielsen, P.E. Ed. NATO ASI Series C vol 272. Kluver 1989.
3. Bayley, H.; Knowles, J.R. Photoaffinity Labelling . In *Methods in Enzymology*, 1977, *46*, 69; Jakoby W.B.; Wilcheck, M. Eds. Academic Press, New York.
4. See for instance Castelló, A.; Cervelló, J.; Marquet, J.; Moreno-Mañas, M.; Sirera, X. *Tetrahedron* 1986, *42*, 4073.
5. See for instance Marquet, J.; Teixidó, M.; Cantos, A.; Moreno-Mañas, M. *Steroids* 1989, *54*, 441.
6. Knowles, J.R. *Acc. Chem. Res.* 1972, *5*, 155.
7. a) Hixson, S.H.; Hixon, S.S. *Biochemistry* 1975, *14*, 4251. b) Nielsen, P.E.; Buchardt, O.; *Photochem. and Photobiol.* 1982, *35*, 317. c) Nielsen P.E. *Eur. J. Biochem.* 1982, *122*, 283. d) Buchardt, O.; Ehrbar, U.; Larsen, C.; Moller, J.; Nielsen, P.E.; Thomsen, T.; Watjer, F.; Hansen, J.B. *J. Org. Chem.* 1984, *49*, 4123. e) Earl, C.Q.; Patel, A.; Craig, R.H.; Daluge, S.M.; Linden, J. *J. Med. Chem.* 1988, *31*, 752. f) Henriksen, U.; Buchardt, O. *Tetrahedron Lett.* 1990, *31*, 2443.

8. a) Sen, R.; Widlanski, T.S.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* 1983, *105*, 5160. b) Sen, R.; Singh, A.K.; Balogh-Nair, V.; Nakanishi, K. *Tetrahedron* 1984, *40*, 493. c) Kessler, P.; Ehret-Sabatier, L.; Goeldner, M.; Hirth, C. *Tetrahedron Lett.* 1990, *31*, 1275.
9. a) Galardy, R.E.; Craigh, L.C.; Jamieson, J.D.; Printz, M.P. *J. Biol. Chem.* 1974, *249*, 3510. b) Blaas, D.; Patzelt, E.; Kuechler, E. *Nucleic Acids Res.* 1983, *11*, 5821. c) Barta, A.; Kuechler, E. *FEBS Lett.* 1983, *163*, 319. d) Patzelt, E.; Blaas, D.; Kuechler, E. *Nucleic Acids Res.* 1983, *11*, 5821.
10. Goeldner, M.P.; Hirth, C.G, *Proc. Natl. Acad. Sci. USA* 1980, *77*, 6439. b) Keifer, B.; Goeldner, M.P.; Hirth, C.G. *J.C.S. Chem. Commun.* 1981, 398.
11. a)Woelfle, I.; Sauerwein, B.; Autrey, T.; Schuster, G.B. *Photochem. Photobiol.* 1988, *47*, 497. b) Sigman, M.E.; Autrey, T.; Schuster, G.B. *J. Am. Chem. Soc.* 1988, *110*, 4297.
12. Keana, J.F.W.; Cai, S.X. *J. Org. Chem.* 1990, *55*, 3640.
13. Jelenc, P.C.; Cantor, C.R.; Simon, S.R. *Proc. Natl. Acad. Sci. USA* 1978, *75*, 3564.
14. a) Cornelisse, J.; Havinga, E. *Chem. Rev.* 1975, *75*, 353. b) Cornelisse, J.; Lodder, G.; Havinga, E.; *Rev. Chem. Intermed.* 1979, *2*, 231. c) Varma, C.A.G.O.; Taminga, J.J.; Cornelisse, J. *J.C.S. Faraday Trans. 2* 1982, *78*, 265. d) Van Zeijl, P.H.M.; Van Eijk, L.M.J.; Varma, C.A.G.O. *J. Photochem.* 1985, *29*, 415. e) Van Eijk, L.M.J.; Huizer, A.H.; Varma, C.A.G.O.; Marquet, J. *J. Am. Chem. Soc.* 1989, *111*, 88. f) Cantos, A.; Marquet, J.; Moreno-Mañas, M.; González-Lafont, A.; Lluch, J.M.; Bertrán, J *J. Org. Chem.* 1990, *55*, 3303.
15. a) Gozlan, H.; Homburger, V.; Lucas, M.; Bockaert, J. *Biochem. Pharmacol.* 1982, *31*, 2879. b) Arévalo, M.A.; Tejedor, F.; Polo, F.; Ballesta, J.P.G. *J. Med. Chem.* 1989, *32*, 2200.
16. Cantos, A.; Marquet, J.; Moreno-Mañas, M.; Castelló, A. *Tetrahedron* 1988, *44*, 2607.
17. Marquet, J.; Moreno-Mañas, M.; Vallribera, A.; Virgili, A.; Bertrán, J.; González-Lafont, A.; Lluch, J.M. *Tetrahedron* 1987, *43*, 351.
18. Figueredo, M.; Marquet, J.; Moreno-Mañas, M.; Pleixats, R. *Tetrahedron Lett.* 1989, *30*, 2427.
19. Hatanaka, Y.; Yoshida, E.; Taki, M.; Nakayama, H.; Kanaoka, Y. *Photomed. Photobiol.* 1988, *10*, 215.
20. a) Richards, F.M.; Wyckoff, H.W. Bovine Pancreatic Ribonuclease. In *The Enzymes*; Boyer, P.D. Ed.. 3rd. Ed. Academic Press, New York, 1971; vol. 4, pp. 647-806. b) Blackburn, P.; Moore, S. Pancreatic Ribonuclease. In *The Enzymes*; Boyer, P.D. Ed.. 3rd. Ed. Academic Press, New York, 1982; vol. 15, pp. 317-433.
21. Vermeulen, M.H. *Rec. Trav. Chim. Pays-Bas* 1906, *25*, 12.
22. Elderfield, R.C.; Gensler, W.J.; Williamson, T.A.; Griffing, J.M.; Kupchan, S.M.; Maynard, J.T.; Kreysa, F.J.; Wright, J.B. *J. Am. Chem. Soc.* 1946, *68*, 1584.
23. Murren, C; Stelling, D.; Felstead, G.; *J. Chromatog.* 1975, *115*, 236.
24. Drescher, M.J.; Medina, J.E.; Drescher, D.G. *Anal. Biochem.*1981,, *116*, 280.
25. Pummerer, R.; Puttfarcken, H.; Schopflocher, P *Ber.* 1925, *58*, 1808.
26. Barton, D.H.R.; Deflorin, A.M.; Edwards, D.E. *J. Chem. Soc.* 1956, 531.
27. Cardwell, D; Robinson, R.J. *J. Chem. Soc.* 1915, *107*, 255.