1-[4-(*N*-Chlorocarbonyl-*N*-methylamino)phenyl]-2-(phenylsulfonyl)diazene, a Bifunctional Reagent with a Protected Diazonium Function[1]

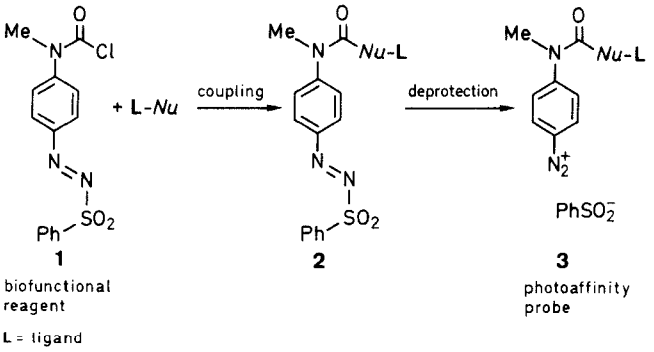
Pascal Kessler, Benoît Chatrenet, Maurice Goeldner,* Christian Hirth*

Laboratoire de Chimie Bio-organique, URA CNRS 1386, Université Louis Pasteur Strasbourg, Faculté de Pharmacie, B.P. 24, F-67401 Illkirch Cedex, France

1-[4-(*N*-Chlorocarbonyl-*N*-methylamino)phenyl]-2-(phenylsulfonyl)diazene is the first bifunctional reagent containing a potential photoactivatable arenediazonium function. The synthesis and the chemical properties of the reagent are described, in particular coupling reactions with series of nucleophiles (primary and secondary amines and a phenolate derivative) and the subsequent deprotection reaction to the corresponding diazonium salt.

The interest in bifunctional reagents has increased considerably in recent years because of their applicability for biological purposes. These reagents are generally used as crosslinkers between two biological entities; thus, they can provide information on the oligomeric structure of a given protein and they also render possible the analysis of the direct surrounding of a protein in more complex structures.[2] Frequently, these reagents contain two electrophilic functions which are selectively coupled with nucleophilic sites on polypeptides or oligonucleotides. Further, bifunctional photochemical reagents[3] have been introduced which render possible controlled two-step cross-linking processes. These reagents have also been used for the synthesis of photoaffinity reagents via chemical coupling of their electrophilic part with a ligand molecule, the photoactivatable moiety remaining unaffected so that it is suitable for the photoaffinity labelling experiment. As such bifunctional reagents, mainly a series of substituted azidoarenes[4] have been described. The purpose of the present work was to synthesize a new bifunctional reagent which would incorporate, in addition to a classical electrophilic function (carbamic chloride), an arenediazonium function. This latter moiety has been shown to be suitable for photoaffinity labelling experiments.[5,6]

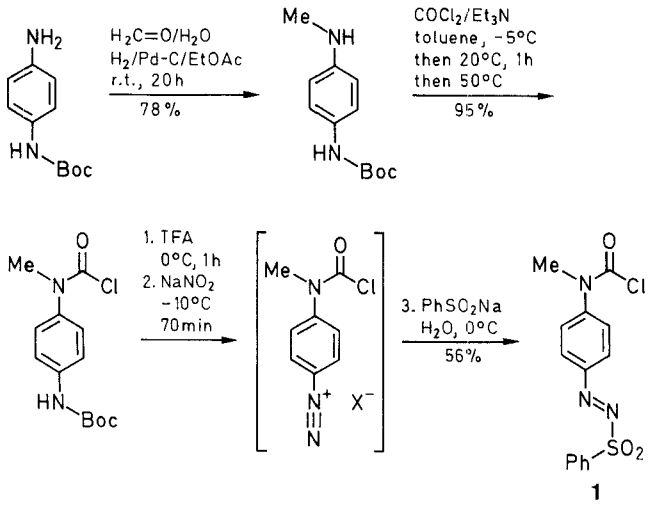
However, the strong electrophilic properties associated to the diazonium function required a prior protection of this group to avoid undesirable side reactions during the coupling to the ligand molecule. Scheme **A** illustrates the



Scheme **A**

strategy of incorporation of the reagent to give a potential photoaffinity label.

The nucleophilic addition of a number of chemical functions to the arenediazonium moiety are known but few are nondestructive and easily removable to allow their use as protecting group.[7] Although a series of protecting groups of arenediazonium salts have been described recently,[8,9] the well known 1-aryl-2-(phenylsulfonyl)diazene[10] group proved to be very convenient both for the synthesis (addition of phenylsulfinate to the diazonium salt) and the deprotection step (aqueous treatment). We therefore elaborated a synthesis of the title reagent **1**, using the following three-step sequence (Scheme **B**).



Scheme **B**

The monomethylation of the starting *N*-Boc-*p*-phenylenediamine was performed by several methods. We found that the classical reductive amination of formaldehyde gave the most satisfactory yields (up to 78%). Treatment of the monomethylated amine with phosgene gave the expected *N,N*-disubstituted carbamic chloride in almost quantitative yield. The conversion of the Boc-protected amino group into the desired *N*-(phenylsulfonyl)diazene moiety was achieved by a one-pot, three-step sequence: deprotection of the Boc-amino group in trifluoroacetic acid, diazotization, and protection of the resulting diazonium function by addition of aqueous sodium benzenesulfinate. Reagent **1** precipitates as an orange solid which is purified to give bright yellow crystals by silica gel chromatography and recrystallization. The satisfactory yields of this synthesis (35% overall) allows the preparation of larger batches of reagent **1**.

Reagent **1** is stable in most organic solvents (dichloromethane, chloroform, ethyl acetate, diethyl ether, acetone, tetrahydrofuran, dioxane) so that the coupling reaction with a nucleophile at the carbamic chloride moiety can be performed in such solvents (e.g., in THF). Although the reagent itself is stable in alcohols (methanol, ethanol), partial ionization of the sulfonyldiazene moiety ($-N=N-SO_2-$) occurs upon reaction of the

Table 1. Coupling of Reagent **1** with Nucleophiles, Deprotection of the Coupling Products **2**, and Anion Exchange of the Resultant Diazonium Benzenesulfinates **3** to Diazonium Chlorides **4**

HNu	Products	Yield (%)	HPLC retention time (min)	Molecular Formula	UV (solvent)[f] λ_{max} (nm)
$BuNH_2$	**2a** (orange gum)	66[a]		$C_{18}H_{22}N_4O_3S$ (374.4)	dioxane: 379 (13500)
	3a	quant.[b]			H_2O:[f] 363 (21000)
	4a	quant.	29.7		
$PhCH_2NH_2$	**2b** (orange crystals)	92		$C_{21}H_{20}N_4O_3S$ (408.4)	dioxane: 373 (11600)
	3b	quant.[b]	33.4		H_2O: 362 (22800)
	4b	quant.	32.2		
piperidine	**2c** (orange gum)	80[a]		$C_{19}H_{22}N_4O_3S$ (386.4)	dioxane: 388 (25500)
	3c	quant.[b]	25.7		H_2O: 364 (31100)
	4c	quant.	25.6		
PhNHMe	**2d** (orange crystals)	55[a]		$C_{21}H_{20}N_4O_3S$ (408.4)	dioxane: 384 (17400)
	3d	quant.[b]			H_2O: 365 (28500)
	4d	quant.	31.4		
cystine dimethyl ester[c]	**2e** (yellow solid)	65[a]		$C_{36}H_{38}N_8O_{10}S_4$ (870.8)	dioxane: 375 (17000)
	3e	quant.[b]	29.5		H_2O: 360 (26000)
	4e	quant.	30.6		
4-$MeOC_6H_4OH$[d]	**2f** (orange gum)	80[a]		$C_{21}H_{19}N_3O_5S$ (425.4)	dioxane: 345 (15400)
	3f	65[b,e]			H_2O: 340 (18300)
	4f	quant.	37.0		

[a] Yield of product isolated by column chromatography on silica gel.

[b] Yields are estimated by UV spectrometry. The purity of the diazonium salts in solution was checked by analytical HPLC: C_{18} reverse phase; flow rate 1 ml/min, gradient 40 min: H_2O (+0.05% TFA) → H_2O (+0.05% TFA)/MeCN (7:3).

[c] Reaction carried out in a 0.06 M solution of THF/water (95:5).

[d] Reaction carried out in 0.1 M solution of THF/water (9:1) containing NaOH (1 equiv).

[e] The low yields observed in this conversion are related to the low solubility of the sulfonyl-diazene **2f** in water.

[f] The UV data for compounds **3** and **4** are identical.

carbamic chloride moiety with a nucleophile. Complete ionization takes place in the presence of water; this property is utilized in the deprotection step.

The chemical reactivity of reagent **1** towards nucleophiles is typical of *N*-arylcarbamic chlorides[11] (cf. Table 1). Primary and secondary aliphatic amines react very rapidly, thus allowing, if necessary, the use of water (up to 10–15%) as co-solvent as illustrated by the coupling of **1** with cystine methyl ester to give **2e**. Secondary aromatic amines react more slowly (formation of **2d**), as expected, and phenols react exclusively via their anion (formation of **2f**). The progress of the coupling reactions can be followed spectrophotometrically: the UV absorption maximum (at λ = 313 nm for reagent **1**) is shifted to higher wavelengths after coupling with amines or a phenol derivatives (Table 1).

After the coupling reaction (Scheme **C**), we used water as a mild agent for the quantitative deprotection of the coupling product **2**. This deprotection reaction can be easily monitored by UV spectrometry: maximum absorptions (λ_{max}) with shorter wavelengths and higher extinction coefficient Δ are observed for the diazonium species (Table 1). Figure 1 shows the change of the UV spectrum during deprotection of the benzyl derivative **2b** to the corresponding diazonium salt **3b** in dioxane/water (7:3). The presence of isosbestic points agrees with the formation of a single transformation product. Quantitative conversion **2b** → **3b** was proven by comparison of the obtained UV characteristics with those of a pure synthetic sample of **3b** (not shown).

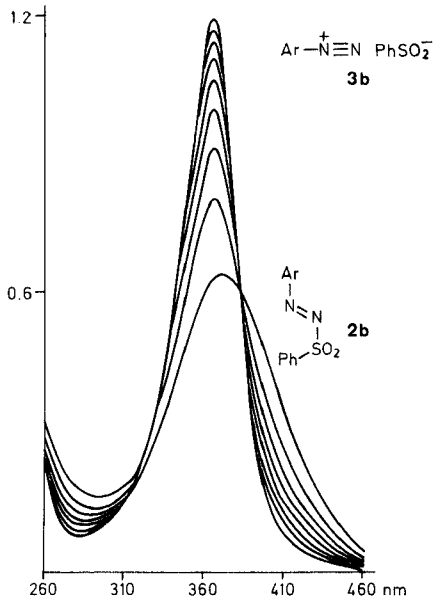
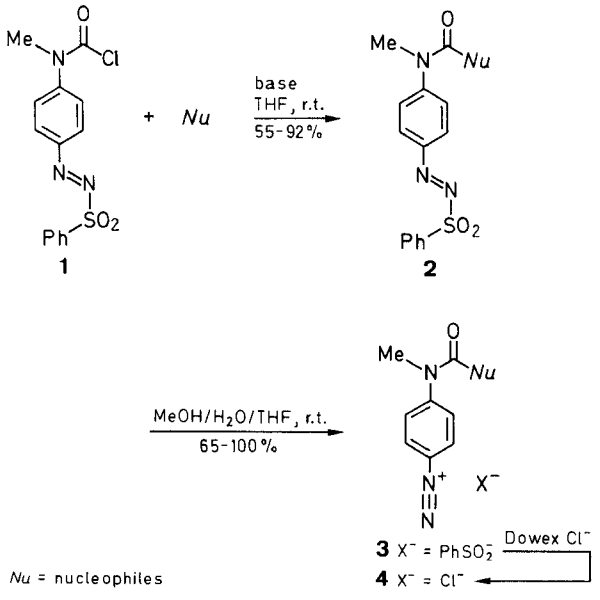


Figure. Deprotection of the Sulfonyldiazene **2b** to the Diazonium Salt **3b** in Dioxane/Water (7:3)

Table 2. ^{1}H-NMR-Spectral Data of Compounds **2** and **4**

Compound	^{1}H-NMR (Solvent/TMS) δ, J(Hz)
2a	($CDCl_3$): 0.89 (t, 3H, J = 7.2), 1.20–1.52 (m, 4H), 3.24 (m, 2H), 3.33 (s, 3H), 4.67 (t, 1H, J = 5.2), 7.38 (d, 2H, J = 8.0), 7.85 (d, 2H, J = 8.0), 7.56–8.00 (m, 5H)
4a	(D_2O): 0.77 (t, 3H, J = 7.2), 1.16–1.46 (m, 4H), 3.15 (t, 2H, J = 6.7), 3.28 (s, 3H), 7.52 (d, 2H, J = 9.5), 8.33 (d, 2H, J = 9.5)
2b	($CDCl_3$): 3.57 (s, 3H), 4.43 (d, 2H, J = 5.6), 4.80–4.90 (br s, 1H), 7.28–8.01 (m, 10H), 7.39 (d, 2H, J = 8.8), 7.85 (d, 2H, J = 8.8)
4b	(D_2O): 3.41 (s, 3H), 4.50 (s, 2H), 7.15–7.25 (m, 5H), 7.56 (d, 2H, J = 5.3), 8.32 (d, 2H, J = 5.3)
2c	($CDCl_3$): 1.50–1.54 (br, 6H), 3.26 (s, 3H), 3.27–3.31 (br, 4H), 7.01 (d, 2H, J = 9.0), 7.50–8.00 (m, 5H), 7.79 (d, 2H, J = 9.0)
4c	(D_2O): 1.52 (br, 6H), 3.22 (s, 3H), 3.37 (br, 4H), 7.04 (d, 2H, J = 8.2), 8.18 (d, 2H, J = 8.2)
2d	($CDCl_3$): 3.16 (s, 3H), 3.31 (s, 3H), 6.86–7.16 (m, 7H), 7.55–7.71 (m, 5H), 7.97 (d, 2H, J = 7.1)
4d	(D_2O): 3.05 (s, 3H), 3.31 (s, 3H), 7.11–7.21 (m, 7H), 8.15 (d, 2H, J = 5.5)
2e	($CDCl_3$): 3.02–3.23 (m, 4H), 3.35 (s, 6H), 3.72 (s, 6H), 4.70–4.79 (m, 2H), 5.47 (d, 2H, J = 7.3), 7.42–8.01 (m, 18H)
4e	(D_2O): 2.94–3.25 (m, 4H), 3.37 (s, 6H), 3.67 (s, 6H), 4.71 (m, 2H), 7.63 (d, 4H, J = 9.2), 8.34 (d, 4H, J = 9.2)
2f	($CDCl_3$): 3.50 (s, 3H), 3.78 (s, 3H), 6.87 (d, 2H, J = 9.1), 7.03 (d, 2H, J = 9.1), 7.60–8.01 (m, 9H)
4f	(D_2O): 3.50 (s, 3H), 3.69 (s, 3H), 6.88 (d, 2H, J = 9.1), 7.04 (d, 2H, J = 9.1), 7.94 (d, 2H, J = 9.3), 8.47 (d, 2H, J = 9.3)

The two species **2** and **3** are in fact in an equilibrium which depends on solvent polarity. A comparative study of the photostability of the two species **2b** and **3b** was carried out by irradiating the isolated compounds under identical conditions (same concentration, λ_{irr} = isosbestic point, i.e. 382 nm for compounds **b**). As expected, the diazonium salt showed higher photosensitivity than the sulfonyldiazene species (ratio of half-lives **3b**:**2b** = 1:3); however, the sulfonyldiazenes are photosensitive[12] and should be handled accordingly.



Scheme **C**

Due to the low solubility of compounds **2** in water, deprotection reactions were satisfactory only in dilute solutions (see experimental). These compounds were identified by UV, ^{1}H-NMR (Table 2), HPLC after quantitative exchange of the benzenesulfinate counterion for the less nucleophilic chloride anion (**3** → **4**) by anion exchange on a resin.

Reagent **1** is the first bifunctional reagent possessing a potential arenediazonium function. The synthesis of photoaffinity probes incorporating such a function will be facilitated by using the present convenient coupling-deprotection procedures which avoid a diazotization step. This may be particularly interesting either for complex molecules possessing other diazotizable functions or for molecules which do not survive the acidic conditions of the diazotization reaction. On the other hand, further developments of photoaffinity labelling experiments require a radioactive source. In this respect, the synthesis of a tritiated reagent **1** is currently in progress.

1-[*N*-Chlorocarbonyl-*N*-methylamino)phenyl]-2-(phenylsulfonyl)diazene (Reagent 1):

N-tert.-Butoxycarbonyl-N'-methyl-p-phenylenediamine: N-tert-Butoxycarbonyl-*p*-phenylenediamine (1.04 g, 5 mmol) is dissolved in EtOAc (20 mL). Formaldehyde (460 μL of a 37% aq solution, 6.25 mmol), and Pd–Cl (10%) are added. The mixture is hydrogenated at atmospheric pressure for 20 h, then filtered. The solvent is evaporated and the residual oil is column-chromatographed on silica gel [100 g Silica gel; eluent: gradient EtOAc/ hexane (1:9 → 4:6)] to give the *N*-methylation product of sufficient purity; yield: 867 mg (78%).

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.50 [s, 9H, $OC(CH_3)_3$], 2.81 (s, 3H, NCH_3), 3.50 (br s, 1H, NHMe), 6.26 (br s, 1H, NHBoc), 6.56 (d, 2H_{arom}, J = 8.7 Hz), 7.17 (d, 2H_{arom}, J = 8.7 Hz).

N-[4-(tert-Butoxycarbonylamino)phenyl]-N-methylcarbamic Chloride: A mixture of *N-tert*-butoxycarbonyl-*N'*-methyl-*p*-phenylenediamine (444 mg, 2 mmol) and Et_3N (500 μL, 3.6 mmol) in dry toluene (20 mL) is added dropwise at −5 °C to a stirred 20% solution of $COCl_2$ in toluene (5.25 mL, 10 mmol). After the addition, the solution is warmed up to 20 °C over 1 h and then heated at about 50 °C. Excess $COCl_2$ vapors are trapped in aqueous NaOH. The mixture is then extracted with H_2O (3 × 20 mL) and brine (20 mL), dried ($MgSO_4$), filtered, and evaporated. The residue is recrystallized from EtOAc/hexane; yield: 541 mg (95%); mp 137–138 °C.

$C_{13}H_{17}ClN_2O_3$	calc.	C 54.83	H 6.02	N 9.84
(284.5)	found	54.97	6.07	9.86

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.53 [s, 9H, $OC(CH_3)_3$], 3.35 (s, 3H, NCH_3), 6.58 (br s, 1H, NHBoc), 7.16 (d, 2H_{arom}, J = 8.7 Hz), 7.43 (d, 2H_{arom}, J = 8.7 Hz).

Reagent **1**: *N*-[4-(*tert*-Butoxycarbonylamino)phenyl]-*N*-methylcarbamic chloride (852 mg, 3 mmol) is stirred with TFA (1 mL) at 0 °C for 1 h. The mixture is then cooled to −10 °C, stirred vigourously, and placed in the dark. Solid $NaNO_2$ (224 mg, 3.3 mmol) is added portionwise over a period of 30 min, and the mixture is stirred for 40 min. The acid is removed under reduced pressure and the diazonium salt is taken up in H_2O (1 mL). This solution is cooled to 0 °C, an aqueous solution (3 mL) of sodium benzenesulfinate (541 mg, 3.3 mmol) is added. The resultant yellow precipitate is isolated by suction and recrystallised from EtOAc. Better yields are obtained by extraction of the suspension with EtOAc (2 × 5 mL). The dried (Na_2SO_4) organic solvent is evaporated and the yellow crystalline residue is column-chromatogaphed on silica gel [80 g silica gel; eluent: gradient EtOAc/hexane (3:7 → 10:0)]. The pure reagent **1** is then obtained by recrystallisation from EtOAc; yield: 550 mg (56%); mp 79 °C.

$C_{14}H_{12}ClN_3O_3S$	calc.	C 49.78	H 3.58	N 12.44
(337.5)	found	49.95	3.53	12.25

UV (dioxane): λ_{max} = 313 nm (ε = 13600).

^{1}H-NMR ($CDCl_3$/TMS): δ = 3.45 (s, 3H, NCH_3), 7.45 (d, 2H, J = 8.8 Hz, H_{arom} *ortho* to MeNCOCl), 7.90 (d, 2H, J = 8.8 Hz, H_{arom} *ortho* to N_2), 7.58–8.02 (m, 5H, $SO_2C_6H_5$).

N'-Substituted-4-(N-Aminocarbonyl-N-methylamino)benzenediazonium Salts [N'-Substituted N-(4-Diazoniophenyl)-N-methylurea Salts 3a–e, 4a–e] and 4-[N-(4-Methoxyphenyl)-N-methylamino]-benzenediazonium Salts [4-Methoxyphenyl N-(4-Diazoniophenyl)-N-methylcarbamate Salts 3f, 4f]; General Procedure:

Coupling Reaction of Reagent **1** *with Nucleophiles:* A 0.2–0.02 M solution of the nucleophile (amines: 2.2 equiv; phenols: 1.1 equiv + 1.1 equiv of Et_3N) is added to a stirred 0.2 M solution of reagent **1** (1.0 equiv) in THF. The progress of the reaction is followed by UV spectrometry (Table 1). When the reaction is complete, the solvent is evaporated under reduced pressure and the remaining coupling product **2** is taken up in EtOAc. This solution is washed with H_2O, dried (Na_2SO_4), and evaporated. The residue is purified either by column chromatography on silica gel or by recrystallization.

Deprotection of the Coupling Products **2** *to the Diazonium Benzenesulfinates* **3**:

To a stirred THF (0.02 M) solution of a coupling product **2** are added MeOH (20- fold excess) and then H_2O (until the solubility limit of **2** is reached). The progress of the reaction is followed by UV spectrometry. The reaction time depends on solvent composition. The final solution is analyzed by HPLC (Table 1).

Anion Exchange **3** → **4**: The previously obtained solution is stirred with Dowex 1 × 2 resin (1–2 g). The anion exchange is followed by UV spectrometry. The final mixture is filtered and the filtrate lyophilized to give the pure diazonium chloride **4**.

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