Protection and Labelling of Thymidine by a Fluorescent Photolabile Group

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Dedicated to Professor *André M. Braun* on the occasion of his 60th birthday

A fluorescent photolabile group including coumarin and MeNPOC moieties was synthesized to protect 5′-OH terminal function of thymidine (T). Its photochemical and photophysical properties were studied, in particular the photocleavage (photodeprotection under a 365-nm irradiation) is only lowered by a factor of two by addition of the fluorophore. Fluorescence properties of the coumarin probe are not changed upon irradiation, which is satisfactory for the application required, *i.e.*, *in situ* synthesis of DNA microarrays.

Introduction.[1]) – Over the past few years, the fabrication of high-density DNA microarrays has been of central importance in the biotechnology field [1], especially for biological analysis and the development of new DNA-sequencing methods. Within this context, we first focused our research on the synthesis of 5′-OH terminal function photolabile protecting groups of deoxynucleosides, particularly useful in light-directed combinatorial synthesis [2][3].

Second, and especially with the detection aspects in mind, we started to develop new photolabile groups with fluorescent properties [4][5]. In fact, photolabile protecting groups with a permanent fluorescent moiety might allow visualization of support grafting during *in situ* synthesis of oligonucleotides, and quantification of photocleavage after irradiation at 365 nm (the usual wavelength used with the classical photolabile groups). Moreover, if the fluorophores do not show the same photophysical properties, the resulting probes might encode for each deoxynucleoside.

At the beginning of our project, only rare examples of fluorescent photolabile protectors were described in the literature. Among them, *Burgess et al.* [6] have described a dansyl derivative as a fluorophore, whose excitation domain was around the irradiation wavelength required for the deprotection photolysis (*i.e.*, 365 nm); despite low photolysis efficiency rate and yield, these authors have enlarged their studies on the synthesis of nucleoside triphosphates with 3′-*O*-blocking photolabile fluorescent groups [7][8].

In our work, we chose to develop a synthetic pathway to assess fluorescent photolabile groups by using four fluorophores bound to the same photolabile group *via* a route parallel to chemistry developed for solid-phase photolabile linkers [9]. The

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[1]) *Abbreviations:* Cou-MNPEOC: 1-(4-{5-[7-(diehtylamino)coumarin-3-carboxamido]pent-1-yloxy}-5-methoxy-2-nitrophenyl)-ethoxycarbonyl. MeNPOC: [(α-methyl-2-nitropiperonyl)oxy]carbonyl. T: thymidine.

retained photolabile group was of MeNPOC type, because it exhibits good photolytic properties in DNA microarrays application [2]. The fluorophores have been selected according to their absorption and emission wavelengths which is to be longer than the λ_{exc} for photolysis (365 nm). A coumarin (=2*H*-1-benzopyran-2-one) fluorophore was previously studied in our laboratory [10][11], and, by the synthetic route shown in the *Scheme*, which implies coupling the modified MeNPOC group to the coumarin derivative *via* an amino linker, it was possible to prepare a fluorescent photolabile group, which could be used for the protection of the 5′-OH terminal function of thymidine.

In this paper, we present both, the study of the photochemical deprotection of the deoxynucleoside and the photophysical behavior, before and after photocleavage.

Results and Discussion. 1. *Chemical Syntheses.* The target molecule, the fluorescent photolabile deoxynucleoside Cou-MNPEOC-T (**8**), was synthesized in a convergent way between photolabile and fluorescent parts, which were separately functionalized as pictured in the *Scheme*.

*Scheme Synthesis of the Fluorescent Photolabile Nucleoside Cou-MNPEOC-T (**8**)*
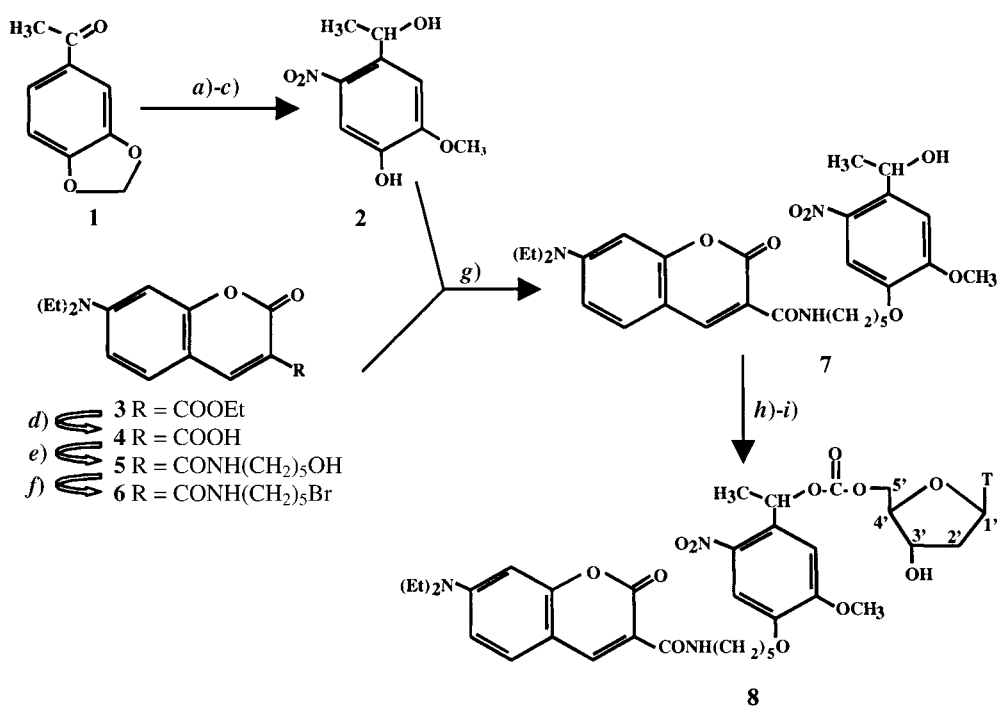
a) 70% HNO_3, 10–15°, 1 h; 68%. *b*) $NaBH_4$, MeOH, 10°, 4 h; 98%. *c*) Na, MeOH, DMSO, 80°, 2 h; 80%. *d*) KOH, EtOH/H_2O; 96%. *e*) $H_2N(CH_2)_5OH$, HOBT-DCCI, CH_2Cl_2, DMF, 0°, 0.5 h, then r.t., 24 h; 91%. *f*) CBr_4, PPh_3, CH_2Cl_2, 0°, 2 h, then r.t., 2 h; 90%. *g*) K_2CO_3, DMF, r.t., 72 h; 75%. *h*) $(Cl_3CO)_2CO$, CH_2Cl_2, 0°, 4 h. *i*) Thymidine, pyridine, 0°, then r.t., 24 h; 36%.

1.1. *Photolabile Part.* Starting from the commercial compound 5-acetyl-1,3-benzodioxole (**1**), the photolabile moiety was synthesized in three steps in 53% overall yield: electrophilic aromatic nitration was performed with concentrated HNO_3 at 10–15° as described by *Fetscher* [12]; the oxo group was reduced with $NaBH_4$ in MeOH, at 10° [2]; finally, regioselective aromatic nucleophilic displacement with methoxide according to the *Teague* procedure [13] led to the compound **2**, which was used in the fluorescent grafting reaction without further purification.

1.2. *Fluorescent Part.* The basic structure of coumarin derivative Cou-COOEt (**3**) was obtained by a *Knoevenagel* condensation of commercial 4-(diethylamino)-2-hydroxybenzaldehyde and diethyl malonate in the presence of piperidine [14]. Saponification of the ester function with KOH led to the corresponding acid **4**, which allowed grafting of the linker *via* an amide bond to yield the compound **5**. The primary terminal alcohol function of the spacer was then converted to the corresponding bromide **6** by treatment with CBr_4 and PPh_3.

1.3. *Fluorescent Photolabile Deoxynucleoside Cou-MNPEOC-T* (**8**). – Alkylation of the phenoxy moiety of the photolabile part **2** with the alkylbrominated fluorescent derivative **6**, realized in the presence of K_2CO_3 in DMF, yielded 75% of the fluorescent photolabile group **7**.

The chloroformate prepared from triphosgene in CH_2Cl_2 was then condensed on T dissolved in pyridine: the 5′-*O*-protected T, Cou-MNPEOC-T (**8**), purified by silica-gel column chromatography, was obtained with 36% yield.

2. *Photocleavage Studies.* The nucleoside T protected by the new fluorescent photolabile group was subjected to irradiation at 365 nm (see the *Exper. Part* for the conditions). Progress of the reaction (disappearance of **8** and subsequent free thymidine formation) was monitored by analytical HPLC. The results were compared with those obtained after irradiation of the 5′-*O*-protected thymidine with MeNPOC. *Fig. 1*, which shows the parallel decrease of **8** and MeNPOC-T, and the formation of free thymidine (same concentration and photolysis conditions), indicates similar photolytic behaviors for the two compounds (in the presence or absence of the coumarin dye).
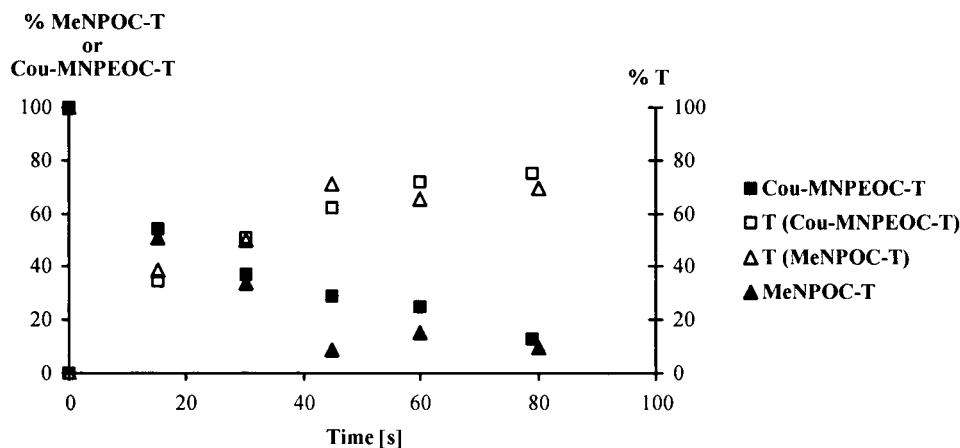

Fig. 1. *Photolysis of Cou-MNPEOC-T* (**8**) *and MeNPOC-T*

The corresponding photolytic data are collected in *Table 1*. The quantum yield Φ of the photolysis of compound **8** is half of that of MeNPOC-T. This might be due to the dissipation of part of the absorbed energy *via* fluorescence pathways that compete with the photocleavage reaction.

Table 1. *Comparison of Photolytic Data of MeNPOC-T with those of Cou-MNPEOC-T* (**8**)

5'-*O*-Protected thymidine	ε_{365}[a] $[1 \cdot mol^{-1} \cdot cm^{-1}]$	$t_{1/2}$[b] [s]	T [%, after 5 min]	Φ[c] (photolysis)
MeNPOC-T	3750	19	98	0.008
Cou-MNPEOC-T (**8**)	6980	21	98	0.004

[a]) Molar absorption coefficient at 365 nm. [b]) Half-life under experimental photolysis conditions, *i.e.*, time required for a 50% conversion by photocleavage. [c]) Quantum yield of the photocleavage reaction.

3. *Photophysical Properties*. With an application aimed at optimum visualization, it became very important to study the photophysical properties of the coumarin derivatives **3** and **5**, and to test the influence of grafting. Absorption and fluorescence spectra of **3**, **5**, and **8** were similar to the spectra of the parent coumarin and other coumarin dyes. Fluorescence spectra are characteristic of donor-acceptor compounds, with only one single emission band, which is red-shifted with increasing solvent polarity (see *Table 2*). The analysis of the photophysical data obtained for the coumarin derivatives in homogeneous solution indicates that the coupling between the fluorescent coumarin part to the photolabile group associated to thymidine does not change the emission properties, hence the fluorophore plays its labelling role.

Table 2. *Absorption and Fluorescence Maximum Wavelengths of Coumarin Derivatives in Solvents of Various Polarities, at 25°*

		Toluene	MeCN	MeOH	MeOH/H$_2$O 3:2 (*v/v*)
$E_T(30)$[a])		33.9	45.6	55.4	58.5 [b])
Cou-COOEt (**3**)	$\lambda_{a\,max}$	404	407	414	423
	$\lambda_{f\,max}$	439	457	466	473
Cou-CONH(CH$_2$)$_5$OH (**5**)	$\lambda_{a\,max}$	412	414	418	427
	$\lambda_{f\,max}$	437	462	465	473
Cou-MNPEOC-T (**8**)	$\lambda_{a\,max}$	412	414	418	427
	$\lambda_{f\,max}$	439	461	467	472

[a]) $E_T(30)$ [kcal $\cdot$ mol^{-1}]: polarity parameter, values defined by *Reichardt* [15]. $\lambda_{a\,max}$ [nm]: absorption wavelength at its maximum. $\lambda_{f\,max}$ [nm]: emission wavelength at its maximum. [b]) The value was calculated based on the assumption of a linear relationship for the two solvents and a value of 63.1 for the $E_T(30)$ of H$_2$O.

The fluorescence spectra (*Fig. 2*) indicate that the emission of the fluorescent part of the fluorescent photolabile probe **8** is not altered by the photolysis. This observation suggests that the resulting photolysis compound (*i.e.*, nitroso-substituted aromatic ketone derivative) has similar fluorescent properties as the parent photolabile part.

We also checked that irradiation at the wavelength of excitation (425 nm) for fluorescence analysis (maximum time for the assay was 7 min) does not cleave the photolabile part; HPLC analyses of aliquots (0.5, 1, 2, 7 min) do not show any
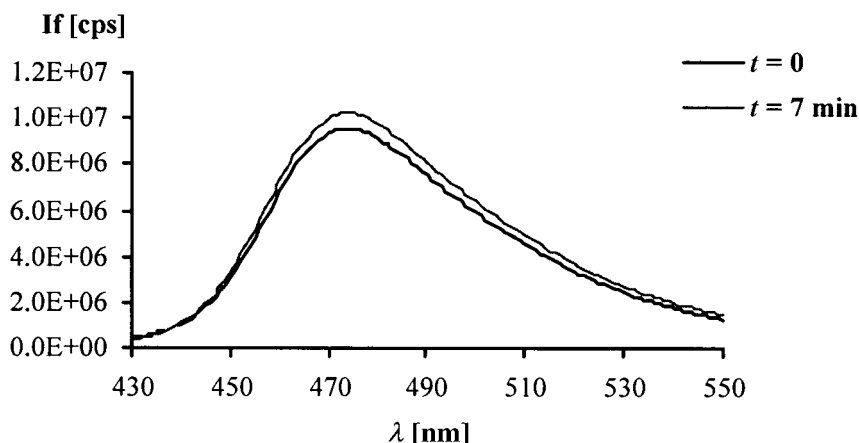
Fig. 2. *Fluorescence spectra of a solution of Cou-MNPEOC-T (**8**; $3.6 \cdot 10^{-6}$ M, MeOH/H_2O 3:2 (v/v); $\lambda_{exc} = 425$ nm) before and after 7 min of irradiation*

significant changes, neither in the shape nor in the integration of the peak corresponding to the starting fluorescent photolabile protected thymidine **8**.

Conclusion. – A coumarin-labelled thymidine protected by a MeNPOC derivative was synthesized as a fluorescent photolabile probe with very interesting properties, from both a photophysical and a photochemical point of view. Actually, even if the coupling of the fluorophore to the photolabile molecule lowers the photolysis quantum yield by a factor of two, the results are satisfying with respect to an analytic application, because the specific behavior of the two parts remains unchanged: the MNPEOC plays its photolabile role, whereas the coumarin derivative retains its fluorescent properties during and after irradiation. In addition, recent results [3], concerning a new photolabile group with higher photolytic efficiency, suggest a potential improvement of the method.

Syntheses of other fluorescent photolabile groups, which will present different photophysical properties over a large wavelength range to allow the identification of each nucleoside, are in progress.

The characteristics of these probes are promising for the development of very powerful tools, not only for DNA microarray approaches, but also for many other applications in biosciences.

Experimental Part

General. Chemicals were purchased from *Aldrich*, *Fluka*, *Lancaster*, and *Peninsula Laboratories, Inc.* Solvents were purchased from *SdS, France Chemical*. Pyridine, Et_3N, and MeCN were dried over 4-Å molecular sieves. Org. solns. were concentrated by rotary evaporation below 50°. M.p.: uncorrected. Column chromatography (CC): silica gel (*Merck 60*, 70–230 mesh). TLC: precoated aluminium silica-gel plates *60 F254* (*Merck*, 0.25 mm, 230–400 mesh); visualization of the chromatogram developed was performed either under UV light at 254 or 360 nm, by I_2 vapors, or by spraying with H_2SO_4 followed by heating on a hot plate.

HPLC: reversed-phase *C18* column, *Shimadzu LC-10AS* pump, variable-wavelength *Shimadzu SPD-10A* UV/ VIS detector set at 260 nm; *Spectra-Physics Chromjet* integrator. UV Spectra: *Perkin-Elmer Lambda 2* spectrometer; $\lambda_{a\ max}$ in nm. Fluorescence spectra: *Fluorolog 2* spectrofluorimeter; $\lambda_{f\ max}$ in nm. IR Spectra: *Perkin-Elmer Spectrum-RXI FT-IR* spectrometer; KBr pellets or thin films on NaCl; ν in cm^{-1}. ^{1}H-NMR Spectra: *Bruker AC-200 P* spectrometer operating at 200 MHz; chemical shifts (δ) in ppm (referenced internally to solvent signals), and coupling constant J in Hz. Elemental analyses were performed by CNRS Laboratory (Vernaison, France).

Syntheses. Ethyl 7-(Diethylamino)-2-oxo-2H-[1]benzopyran-3-carboxylate (**3**). Compound **3** was prepared according to the procedure described for the synthesis of Coumarin 343 methyl ester [14]. Diethyl malonate (16.5 ml, 150 mmol) and 4-(diethylamino)-2-hydroxybenzaldehyde (20 g, 100 mmol) were dissolved in piperidine (30 ml) and MeCN (300 ml). The soln. was heated at reflux for 3 h and then slowly distilled. The remaining crude product was purified by chromatography: AcOEt/CH$_2$Cl$_2$ 1:2 (*v/v*): 26 g (90%) of **3**. M.p.: 90 – 92° ([16]: 80 – 82°). IR: 1735 (C=O, ester). ^{1}H-NMR (CDCl$_3$): 8.43 (*s*, H−C(4)); 7.37 (*d*, *J* = 9, H−C(5)); 6.60 (*dd*, *J* = 2.5, 9, H−C(6)); 6.47 (*d*, *J* = 2.5, H−C(8)); 4.39 (*q*, MeC*H*$_2$O); 3.45 (*q*, (MeC*H*$_2$)$_2$N); 1.39 (*t*, *Me*CH$_2$O); 1.23 (*t*, (*Me*CH$_2$)$_2$N)). Anal. calc. for C$_{16}$H$_{19}$NO$_4$: C 66.42, H 6.63, N 4.84; found: C 66.53, H 6.64, N 5.18.

7-(Diethylamino)-2-oxo-2H-[1]benzopyran-3-carboxylic Acid (**4**). A soln. of **3** (17 g, 59 mmol) and KOH (5.3 g, 94.4 mmol) in EtOH/H$_2$O 3:2 (*v/v*, 250 ml) was heated under reflux for 6 h. EtOH was evaporated *in vacuo*, the remaining aq. phase was acidified with 1.2N HCl to pH 6. The orange solid that precipitated was removed by filtration, washed with H$_2$O and pentane, and finally dried *in vacuo* (crude yield 14.8 g (96%)). M.p. 228°. IR: 3459 (OH), 1736 (C=O, acid). ^{1}H-NMR (CDCl$_3$): 12.4 (*s*, COOH); 8.65 (*s*, H−C(4)); 7.45 (*d*, *J* = 9, H−C(5)); 6.73 (*dd*, *J* = 2.3, 9, H−C(6)); 6.53 (*d*, *J* = 2.3, H−C(8)); 3.49 (*q*, (MeC*H*$_2$)$_2$N); 1.27 (*t*, (*Me*CH$_2$)$_2$N). Anal. calc. for C$_{14}$H$_{15}$NO$_4$: C 64.36, H 5.79, N 5.36; found: C 64.53, H 6.01, N 5.56.

7-(Diethylamino)-N-(5-hydroxypentyl)-2-oxo-2H-[1]benzopyran-3-carboxamide (**5**). To a soln. of **4** (7.8 g, 30 mmol) in CH$_2$Cl$_2$ at 0° were added HOBT (4.8 g, 36 mmol) in DMF (10 ml) and DCCI (6.2 g, 30 mmol) in CH$_2$Cl$_2$ (15 ml). The mixture was stirred for 0.5 h at r.t. under N$_2$. A soln. of 5-aminopentanol (3.1 g, 30 mmol) in CH$_2$Cl$_2$ (6 ml) was added dropwise, and the mixture was stirred for 24 h at r.t. Dicyclohexylurea was removed by filtration and washed with a small amount of CH$_2$Cl$_2$. The solvents were evaporated, and the residue was purified by chromatography (AcOEt/CH$_2$Cl$_2$ 1:3 (*v/v*)): 9.3 g (91%) of **5**. M.p. 212°. IR: 3333 (OH), 2974 (NH), 1702 (C=O, amide). ^{1}H-NMR (CDCl$_3$): 8.84 (*m*, NH); 8.70 (*s*, H−C(4)); 7.43 (*d*, *J* = 8.76, H−C(5)); 6.65 (*dd*, H−C(6)); 6.50 (*d*, H−C(8)); 4.22 (*m*, C*H*$_2$OH); 3.67 (*t*, C*H*$_2$NH); 3.46 (*m*, (MeC*H*$_2$)$_2$N, O*H*); 1.65 (*m*, (C*H*$_2$)$_3$CH$_2$OH); 1.29 (*t*, (*Me*CH$_2$)$_2$N). Anal. calc. for C$_{19}$H$_{26}$N$_2$O$_4$: C 65.89, H 7.51, N 8.09; found: C 65.06, H 7.38, N 8.53.

N-(5-Bromopentyl)-7-(diethylamino)-2-oxo-2H-[1]benzopyran-3-carboxamide (**6**). To **5** (4.8 g, 14 mmol), dissolved in CH$_2$Cl$_2$ (25 ml) under N$_2$, at r.t., was added CBr$_4$ (6.5 g, 19.6 mmol). After stirring for 10 min, the soln. was cooled to 0°, and PPh$_3$ (10.3 g, 39.2 mmol) was added. The mixture was stirred 1 h at 0°, then 2 h at r.t. After concentration of the solvent, the mixture was filtered through silica gel (CH$_2$Cl$_2$) to eliminate the undesired by-products. Evaporation of the solvent provided **6** (5.1 g, 90%). M.p. 148°. ^{1}H-NMR (CDCl$_3$): 8.83 (*m*, NH); 8.71 (*s*, H−C(4)); 7.44 (*d*, *J* = 8.77, H−C(5)); 6.65 (*dd*, *J* = 8.77, 2.92, H−C(6)); 6.50 (*d*, *J* = 2.92, H−C(8)); 3.44 (*m*, (MeC*H*$_2$)$_2$N, NHC*H*$_2$, CH$_2$Br); 1.91 (*m*, C*H*$_2$CH$_2$Br); 1.58 (*m*, (C*H*$_2$)$_2$CH$_2$CH$_2$Br); 1.24 (*t*, (*Me*CH$_2$)$_2$N). Anal. calc. for C$_{19}$H$_{25}$BrN$_2$O$_3$: C 55.74, H 6.11, N 6.84, Br 19.54; found: C 55.80, H 6.22, N 6.89, Br 18.95.

7-(Diethylamino)-N-[5-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]pentyl]-2-oxo-2H-[1]benzopyran-3-carboxamide (**7**). A slurry of **2** (213 mg, 1 mmol), **6** (409 mg, 1 mmol), and K$_2$CO$_3$ (208 mg, 1.5 mmol) in 5 ml DMF was stirred at r.t. for 72 h. H$_2$O was added to the mixture, until all the salts were dissolved, and the soln. was extracted with CH$_2$Cl$_2$. The org. phase was dried (MgSO$_4$), filtered, and evaporated to dryness to give crude compound. Chromatography on silica gel (AcOEt/CH$_2$Cl$_2$ 1:3 (*v/v*)) gave **7** (405 mg, 75%). Light yellow solid. M.p. 168°. IR: 3386 (OH). ^{1}H-NMR (CDCl$_3$): 8.82 (*m*, NH); 8.70 (*s*, H−C(4) of Cou); 7.56 (*s*, 1 arom. H); 7.43 (*d*, *J* = 8.76, H−C(5) of Cou); 7.29 (*s*, 1 arom. H); 6.65 (*dd*, H−C(6) of Cou); 6.50 (*d*, H−C(8) of Cou); 5.56 (*m*, C*H*(Me)OH); 4.09 (*t*, ArOC*H*$_2$); 3.98 (*s*, MeO); 3.46 (*m*, (MeC*H*$_2$)$_2$N, NHC*H*$_2$); 2.35 (*m*, CH(Me)O*H*); 1.92 (*m*, ArOCH$_2$C*H*$_2$); 1.68 (*m*, NHCH$_2$(C*H*$_2$)$_2$); 1.56 (*d*, CH(*Me*)OH); 1.27 (*t*, (*Me*CH$_2$)$_2$N). Anal. calc. for C$_{28}$H$_{35}$N$_3$O$_8$: C 62.10, H 6.28, N 7.76; found: C 62.15, H 6.56, N 7.66.

5'-O-[[1-(4-[5-[7-(Diethylamino)-2-oxo-2H-[1]benzopyran-3-carboxamido]pentyloxy]-5-methoxy-2-nitro-phenyl)-ethoxy]carbonyl]thymidine (**8**). Compound **7** (640 mg, 1.2 mmol) and pyridine (94 mg, 96 μl, 1.2 mmol) in 20 ml of CH$_2$Cl$_2$ was added dropwise under N$_2$ to bis(trichloromethyl) carbonate (118 mg, 0.4 mmol) in 2 ml CH$_2$Cl$_2$ cooled at 0°. After the mixture was stirred for 4 h, thymidine (291 mg, 1.2 mmol; co-evaporated three

times with 6 ml of anh. pyridine) dissolved in anh. pyridine (6 ml) was added at 0°. After 30 min, the ice-bath was removed, and the soln. was allowed to stir overnight at r.t. After evaporating the solvents, the crude material was diluted with H_2O and extracted three times with CH_2Cl_2. The org. extracts were combined and dried (Na_2SO_4), filtered, and the solvent was removed *in vacuo*. The residue was purified on a silica-gel column (EtOH/CH_2Cl_2 5 : 95 (*v/v*)): 350 mg (36%) of **8**. M.p. 135°. ¹H-NMR (CDCl₃): 8.88 (*m*, NH); 8.74 (*s*, H−C(4) of Cou); 8.70 (*s*, H−N(3)); 7.56, 7.53 (2*s*, 1 arom. H); 7.44 (*d*, *J* = 8.77, H−C(5) of Cou); 7.31, 7.12 (2*s*, H−C(6)); 6.99, 6.93 (2*s*, 1 arom. H); 6.64 (*d*, *J* = 8.77, H−C(6) of Cou); 6.50 (*d*, H−C(8) of Cou); 6.34 (*m*, Ar*CH*Me, H−C(1′)); 4.22 (*m*, ArOC*H₂*, H−C(3′), H−C(4′), H−C(5′), with 2*s* at 3.93 and 3.90, MeO); 3.48 (*m*, (MeC*H₂*)₂N), NHC*H₂*); 2.08 (*m*, OH, H−C(2′), NHCH₂(C*H₂*)₃ with *s* at 1.91, Me−C(5), and 2*d* at 1.71 and 1.68, CH(*Me*)O); 1.24 (*t*, (*Me*CH₂)₂N. Anal. calc. for $C_{39}H_{47}N_5O_{14}$: C 57.84, H 5.82, N 8.60; found: C 56.79, H 6.00, N 7.87.

Photolysis Conditions. Cou-MNPEOC-T (**8**; $7.2 \cdot 10^{-5}$ M) was dissolved in MeOH/H_2O 3 : 2 (*v/v*), and irradiated in a 2-mm path-length quartz cuvette with a 500-W Hg arc lamp (*Osram*) at 365 nm, selected by a *UG11* filter, which gave a photon flux of $1.26 \cdot 10^{17}$ photon $\cdot$ s⁻¹ $\cdot$ cm⁻², determined by chemical actinometry (potassium ferrioxalate and uranyl oxalate methods [17]).

Photodeprotection *vs.* time was followed by HPLC with a reversed-phase column (5 μm C_{18} silica, 4.6-mm internal diameter × 250 mm) eluted with a linear gradient of MeCN in 0.1M aq. Et₃NHOAc, pH 7.3 (5 – 80% MeCN over 25 min) at a flow rate of 1 ml $\cdot$ min⁻¹ and monitoring absorbance of the eluent at 260 nm. By HPLC analysis, we followed **8** and free thymidine concentrations variations *vs.* irradiation time.

The half-lives ($t_{1/2}$) were calculated from the plot of concentration *vs.* the irradiation time (peak area at time t (A_t) divided by peak area at time zero (A_0) multiplied with 100%).

The quantum yields (Φ) were determined from the following equation [18]:

$$\Phi = \frac{(0.693)(6.02 \times 10^{20})}{2.303 t_{1/2} \varepsilon_\lambda I_0}$$

where I_0 is the incident photon flux [photon $\cdot$ s⁻¹ $\cdot$ cm⁻²], $t_{1/2}$ the half-life [s], and ε_λ the molar absorption coefficient at 365 nm [l $\cdot$ mol⁻¹ $\cdot$ cm⁻¹].

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