were terminated by the addition of 2 volumes of ice-cold acetonitrile followed by centrifugation. Preparative-scale incubations were run for 2 h with 1 mM MPTP and mitochondrial or microsomal incubations preparations containing 3–5 mg of protein/mL and were terminated by the addition of 2 volumes of cold acetonitrile.

HPLC Separations. The acetonitrile fractions from the mitochondrial incubation experiments were chromatographed with acetonitrile/100 mM acetic acid (85:15, v/v) treated with triethylamine to pH 5.6. Metabolite isolation was accomplished as described previously.[6] The aqueous acetonitrile phases obtained in the microsomal incubation experiments were chromatographed with acetonitrile/100 mM acetic acid (65:35, v/v) treated with triethylamine to pH 5.6. Isolation of the microsomal metabolites was achieved by 35 400-μL injections. The effluents contained in the 3–4 and 5–6-min windows were collected, and the solvent was removed under vacuum followed by a stream of nitrogen at 40 °C. The CI mass spectrum of the fraction isolated from the mitochondrial incubation mixture was obtained at a source temperature of 130 °C and displayed a single intense ion at m/z 156. The ^{1}H NMR spectrum displayed all of the signals characteristic of the 1-methyl-4-phenylpyridinium species.[6] The two principal fractions separated by HPLC of the microsomal incubation mixture extract (see Figure 1) also were analyzed by mass spectrometry. Fraction A (4-phenyl-1,2,3,6-tetrahydropyridine, **10**) displayed a strong CIMS ion at m/z 160 (MH^+) and the following GC–EI mass spectrum: 159 (M^+, 100), 158 (70), 156 (98), 130 (75), 129 (68), 128 (68), 115 (80), 102 (13), 91 (19), which was essentially identical with the corresponding spectrum of authentic **10**. Fraction B (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine *N*-oxide, **11**) displayed a CI mass spectrum similar to that of the synthetic *N*-oxide and the following ^{1}H NMR signals: δ 3.55 (s, NCH_3), 6.0 [br s, C(5)-H], 7.4 (m, Ar H). All signals were enhanced by the addition of authentic **11**. Biological contaminants prevented the unambiguous assignments of the remaining signals. The CI, EI, and GC–EI mass spectra of the cyano adduct isolated from microsomal incubation mixtures of MPTP and sodium cyanide were identical with the corresponding spectra reported above for synthetic 1-(cyanomethyl)-4-phenyl-1,2,3,6-tetrahydropyridine (**14**).

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Psoralenamines. 3.[1] Synthesis, Pharmacological Behavior, and DNA Binding of 5-(Aminomethyl)-8-methoxy-, 5-[[(3-Aminopropyl)oxy]methyl]-, and 8-[(3-Aminopropyl)oxy]psoralen Derivatives

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A series of derivatives of 5-(aminomethyl)-8-methoxypsoralens, 8-[(3-aminopropyl)oxy]psoralens, and 5-[[[3-(trimethylammonio)propyl]oxy]methyl]-8-methoxypsoralen has been synthesized and their potential as PUVA reagents examined. While the DNA association constants of selected psoralens were found to be 10^5–10^6 L mol^{-1}, corresponding to efficient binding, flow linear dichroism studies indicated that only the 8-substituted psoralens bind to DNA by intercalation. Furthermore, the ability to photoinduce interstrand cross-links in calf thymus DNA, in vitro, was as efficient as that of 8-methoxypsoralen for the 8-substituted psoralens, which were up to 25 times as efficient as the 5-substituted psoralens. Four of the psoralens studied were radiolabeled and used to study photobinding to DNA. Analogously to the cross-binding results, the 8-substituted psoralens were more efficiently photobound than the 5-substituted, while only slight differences were found in the photobinding–cross-linking ratio. The photoreactivity of the aminopsoralens toward cyclohexene and 2′-deoxythymidine was enhanced compared to that of 8-methoxypsoralen, the effect being most pronounced when the amino group is close to the furocoumarin ring system. Most of the new compounds were less photocytotoxic than 8-methoxypsoralen to NHIK 3025 cells, in vitro, and they caused less light-induced DNA interstrand cross-linking, in situ, in these cells. A clear correlation between the photocytotoxicity and the DNA cross-linking ability of the psoralens was observed. Several of the derivatives showed more pronounced effects in the light-dependent skin thickening (inflammatory) test on mice than 8-methoxypsoralen. No correlation between DNA cross-linking capacity, in vitro, and skin phototoxicity was found for this series of psoralens.

Psoralen derivatives are currently used as photochemotherapeutical drugs in the PUVA (psoralen-ultraviolet A) therapy of dermatological disorders like psoriasis, vitiligo, and alopecia, as well as mycosis fungoides (a malignant cutaneous lymphoma).[3–6] Such compounds are also useful as tools for studying the structure of nucleic acids in molecular biology.[7–9]

The pharmacological mechanism of action of these drugs has not yet been established. However, psoralens photoreact with nucleic acids, in particular DNA, forming both mono- and diadducts (interstrand cross-links). These

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(1) Previous papers consist of the following: Hansen, J. B.; Koch, T.; Buchardt, O.; Nielsen, P. E.; Wirth, M.; Nordén, B. *Biochemistry* **1983**, *22*, 4878 and ref 2.
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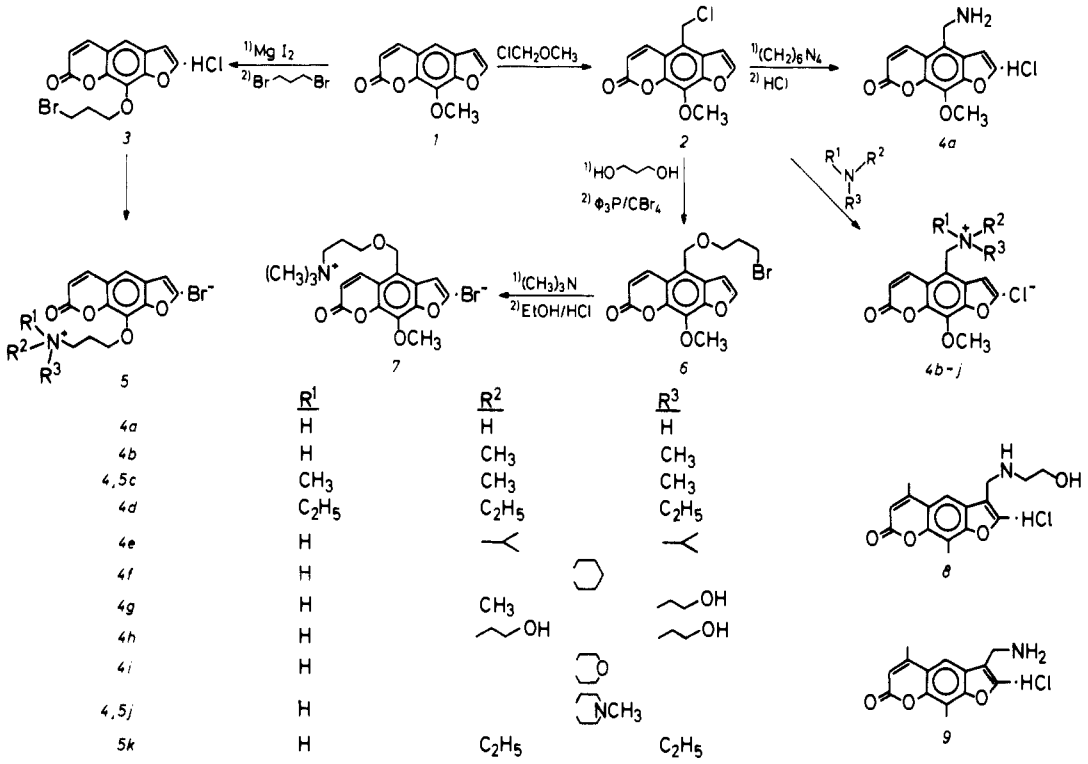
Scheme I

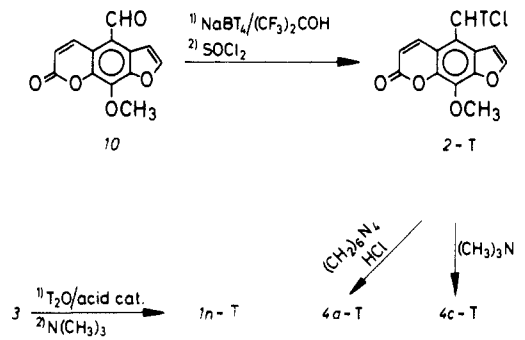


	R^1	R^2	R^3
4a	H	H	H
4b	H	CH_3	CH_3
4,5c	CH_3	CH_3	CH_3
4d	C_2H_5	C_2H_5	C_2H_5
4e	H		
4f	H		
4g	H	CH_3	
4h	H		
4i	H		
4,5j	H		
5k	H	C_2H_5	C_2H_5

photoreactions have been demonstrated to occur both with isolated DNA and with DNA, in situ, in cells in culture.[7,10-13] Furthermore, it is well documented that the photocytotoxic effects of the psoralens on cell cultures are due to the formation of DNA adducts, especially the DNA interstrand cross-links.[14-18] The therapeutic action has been suggested to be due to the formation of DNA adducts,[6] to singlet oxygen,[19,20] or to effects on the immune system.[4]

8-Methoxypsoralen (8-MOP), which is a naturally occurring compound, is currently the only PUVA drug in general clinical use. However, the use of 8-MOP causes unwarranted side effects in the patients, e.g., gastrointestinal problems and undesirably long-lasting photosensitizing effects.[4] Furthermore, 8-MOP in combination with UV-A irradiation has moderate mutagenic properties[21-24] and was found to be carcinogenic at least in mice.[25-27] An increased rate of skin tumors in patients receiving PUVA therapy has also been reported.[28,29]

Scheme II



Therefore, the need for psoralens with improved therapeutical properties and diminished side effects is apparent. Other psoralens, 3-carbethoxypsoralen,[30,31] and angelicins[32] have been suggested as safer, and thus better,

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photochemotherapeutical drugs, partly on the basis of their greatly diminished, if existing, capacity to induce DNA cross-links. Averbeck et al.[33] suggested that the carcinogenic effect of the psoralens is due to cross-links, while Hanawalt et al.[34] attribute the beneficial effects to these. Clinical investigations have indicated that 5-methoxypsoralen[29] and 3-carbethoxypsoralen[30] are efficient as PUVA drugs.

It has previously been found that the substitution pattern of psoralens strongly influences their DNA association (K_{eq}) and photoreactivity (pyrone vs. furan addition).[35]

As part of our studies to learn more about the structure–activity relations of psoralens in terms of photochemotherapy, we now report the synthesis of a new series of psoralens containing amino substituents either in the 5- or in the 8-position. We also report data on the phototoxicity of these compounds on NHIK 3025 cells in culture, as well as their photosensitizing capacity in hairless mice, and their ability to act as singlet-oxygen photosensitizers, in order to evaluate their potential use as photochemotherapeutics. Furthermore, we describe the DNA interaction of a slightly extended series of psoralens in the absence and presence of long-wavelength ultraviolet light, particularly in order to examine the effects of amino substituents in the 5- and 8-positions. The DNA cross-linking efficiency, in vitro, of all of the psoralens was determined, and the DNA binding constants (K_{eq}) as well as the ability to intercalate in DNA were examined for selected compounds.

The binding constants were estimated from equilibrium analysis in a two-phase system consisting of aqueous dextran/polyethylene glycol. The DNA intercalation was examined by flow linear dichroism measurements. The DNA photobinding measurements were undertaken with tritiated psoralen derivatives whose syntheses are also reported in this paper. Relative quantum yields for the photoreactions of selected aminopsoralens with cyclohexene and 2′-deoxythymidine have been determined.

Results

Chemistry (Schemes I and II). In each case, the starting material was the naturally occurring 8-MOP (1). This was either converted to the 5-chloromethyl derivative **2** with chloromethyl methyl ether[36] or to 8-[(3-bromopropyl)oxy]psoralen (**3**) by demethylation with magnesium iodide, followed by reaction with 1,3-dibromopropane.[37] Compound **2** was transformed to 5-(aminomethyl)-8-methoxypsoralen (**4a**) by the Dēlepine procedure,[1] while the other 5-aminomethyl derivatives of 8-MOP (**4b–j**) were prepared from compound **2** and the corresponding secondary or tertiary amines. Attempts to prepare type **4** derivatives from **2** and primary amines gave mixtures of compounds, probably due to ring opening of the lactone moiety.[38] Alternatively, **4a** could be prepared by hydrogenation of 5-(azidomethyl)-8-methoxypsoralen with use of PtO_2 as a catalyst.[1] Attempts to prepare **4a** by hydrazinolysis of 5-phthalimido-8-methoxypsoralen have been unsuccessful in our hands as well as in others.[39]

Compounds **5** were obtained from **3** and the corresponding secondary or tertiary amines. Compound **7** was prepared from **2**, which was transformed via 5-[[(3-hydroxypropyl)oxy]methyl]-8-methoxypsoralen to 5-[[(3-bromopropyl)oxy]methyl]-8-methoxypsoralen (**6**),[1] which in turn was reacted with trimethylamine to give **7**. The syntheses of the remaining psoralens were performed by analogous reactions.

All of the new aminopsoralens were highly soluble in water. The aqueous solubility at 20 °C was as follows: **4a**, 0.3 M;[2] **4c**, 0.1 M; **4d**, 0.25 M; **5c**, 0.18 M, and thus larger than that of 8-MOP, 1.7×10^{-4} M.

The tritiated 5-substituted psoralens were prepared by reducing 5-formyl-8-methoxypsoralen (**10**) with sodium tritioborate ($NaBT_4$) in the rather unusual solvent hexafluoro-2-propanol, which, in model reactions with $NaBH_4$, was found to be superior to more traditional solvents in terms of yield and purity. The resulting tritiated 5-(hydroxymethyl)-8-methoxypsoralen was transformed to the corresponding 5-chloromethyl derivative with thionyl chloride, which in turn was reacted with trimethylamine to give tritiated **4c** (**4c**-T) or with hexamethylenetetramine followed by hydrolysis to give tritiated **4a** (**4a**-T) (Scheme II).

5-Tritio-8-methoxypsoralen can be prepared by acid-catalyzed exchange of the parent compound with tritium oxide. Attempts to undertake tritium exchange in a similar manner with **5c** were unsuccessful, and instead such exchange was performed on 8-[(3-bromopropyl)oxy]psoralen (**3**), which was subsequently reacted with trimethylamine to give **5c**-T.

In Vitro Photocytotoxicity. A photocytotoxicity screening of the aminopsoralens was performed. In this assay, growth inhibition of the human epithelial cancer cell line, NHIK 3025,[41] was used as a measure of photocytotoxicity. All compounds except **5k** were less active than 8-MOP, which was phototoxic at 100 ng/mL (0.8 J/cm^2). Quantitatively similar results were obtained with use of the ciliate *Tetrahymena thermophila* in the assay (Table I).

DNA Photo-cross-linking in Cells. The ability of the psoralens to photo-cross-link DNA in situ in the cells was also assessed with use of both NHIK 3025 cells and *Tetrahymena thermophila*. The results showed, analogously to the phototoxicity assay, that all aminopsoralens except **5k** were significantly less active than 8-MOP.

Photoreactions of the Psoralens with DNA. The ability of the psoralens to photo-cross-link calf thymus DNA was measured. The results, which are presented in Table I, show that the psoralens containing an amino function cross-link DNA more efficiently than the analogous psoralens without such a function, e.g., compound **4a** compared to compounds 4l and 4m and compound **7** compared to compound **4n**. Furthermore, the results clearly indicate that psoralens with amino substituents in the 8-position cross-link DNA more efficiently than those having the substituents in the 5-position. This is most directly appreciated when comparing the result for compound **5c** with that for compound **7**, but it appears to be a general rule, at least for bulky substituents, i.e., com-

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Table I. Photochemical and Photobiological Behavior of Psoralen Derivatives

psoralen[1]	R^1	R^2	photo-cross-linking of DNA	rel quantum yield		phototoxic effect in vitro[a]		DNA photo-cross-linking in cells[b]		photosensitization in vivo[c]
				cyclohexene	thymidine	NHIK	T. th.	NHIK	T. th.	
8-MOP	H	OCH_3	1.0	1.0	1.0	100	100	100	100	100
4a	CH_2NH_2	OCH_3	2.1	4.1	5.6	1	5	8	3	172 ± 25
4b	$CH_2N(CH_3)_2$	OCH_3	0.6	6.6	10.3	7	15	24	14	194 ± 21
4c	$CH_2N^+(CH_3)_3$	OCH_3	0.1	6.6		1	0.3	3	2	140 ± 20
4d	$CH_2N^+(Et)_3$	OCH_3	0.05			3	5	8	14	133 ± 27
4e	$CH_2N(i\text{-}Pr)_2$	OCH_3	0.15			2	10	12	nd	87 ± 17
4f		OCH_3	0.3			3	nd	19	8	238 ± 17
4g	$CH_2N(CH_3)CH_2CH_2OH$	OCH_3	0.5			2	3	15	nd	306 ± 12
4h	$CH_2N(CH_2CH_2OH)_2$	OCH_3	0.2	3.6	12.4	3	0.3	1	3	339 ± 45
4i		OCH_3	0.1			0.3	0.3	<1	6	154 ± 44
4j		OCH_3	0.2			5	10	9	nd	325 ± 51
4l	CH_2OH	OCH_3	0.07	1.5						
4m	CH_2OCH_3	OCH_3	0.04							
4n	$CH_2O(CH_2)_3OH$	OCH_3	<0.07	1.8						
5c	H	$O(CH_2)_3N^+(CH_3)_3$	1.1	1.4	1.6	15	3	3	5	477 ± 177
5j	H		2.5	1.4		10	10	7	2	463 ± 115
5k	H	$O(CH_2)_3NEt_2$	1.8	1.2		100	150	190	170	357 ± 16
5p	H	OCH_2CH_2OH	1.0							
7	$CH_2O(CH_2)_3N^+(CH_3)_3$	OCH_3	0.1	2.5	1.0	3	<0.3	1	4	nd
8	H	OH	<0.03	<0.2						

[a] NHIK: on human cell line NHIK 3025. T. th.: on the ciliate *Tetrahymena thermophila*. The assay was performed with use of threefold dilution steps, and the results were reproducible by one dilution step. Values relative to 8-MOP that caused inhibition of cell growth at 100 ng/mL (NHIK) and 3 μg/mL (T. th.), respectively. nd: not determined. [b] Relative cross-linking efficiency of DNA in situ measured by the alkaline elution technique. [c] Increase in skin thickness relative to that of 8-MOP. The values were measured 24-h postirradiation (4 J cm^{-1}).

Table II. Interaction of Psoralens with DNA

psoralen	assoc constant, L mol^{-1}	angle to DNA helix upon binding,[a] deg	n^d	photobinding yield (rel)[c]	ratio of monoadducts to cross-links
8-MOP	$10^{3\,d}$	90	nd	1.0	0.7
4a	1.2×10^5	80	1.2	1.5 ± 0.5	2.6
4b	7×10^5	70	0.7		
4c	6×10^5	70	0.6	0.15 ± 0.05	4.7
4f	1.1×10^6	65	1.1		
5c	1.1×10^6	90	1.1	2.5 ± 1.0	2.0
5j	1.1×10^6	90	0.3		
5k	1.1×10^6	90	1.1		

[a] These values were obtained at a psoralen–base pair ratio (r) of 0.05. [b] Base pairs per psoralen at saturation. [c] Mean of two determinations. [d] This value is estimated from dextran/polyethylene glycol two-phase partition measurements, assuming $n \sim 0.1$. The previously published values are between 10^2 and 10^4 L mol^{-1}; cf. ref 58.

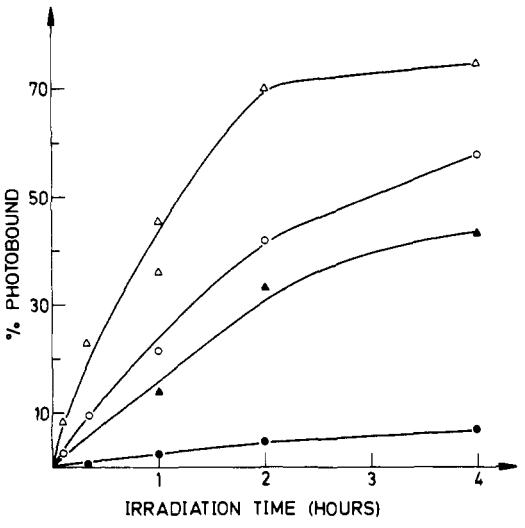

Figure 1. Photoaddition of tritiated psoralens to calf thymus DNA: (▼) 8-methoxypsoralen, (○) **4a**, (●) **4c**, (△) **5c**.

pounds **5j** and **5k** compared with compounds **4c–e** and **4j**.

A few psoralens were selected for photobinding studies with use of their tritiated derivatives. The relative yields and reaction rates for the photochemical binding of these psoralens to DNA were found to be approximately parallel to the values for photo-cross-linking when determined at comparable DNA concentrations (Figure 1, Table II).

By measuring both photo-cross-linking and photobinding in the same samples, we were also able to estimate the absolute values of the ratio between monoadducts and cross-links produced by these psoralens (Table II). It is noteworthy that this property of the compounds seems to vary less than both the photo-cross-linking and photobinding efficiencies.

Interactions of the Psoralens with DNA in the Absence of Light. The affinity of the psoralens toward DNA was measured by the dextran/polyethylene glycol partition method.[42] The results, which are presented in Table II, show that the presence of the amino functions, which are protonated under the reaction conditions, greatly enhances the affinity to DNA compared to 8-methoxypsoralen. This is probably due to electrostatic interactions between the ammonio groups and the DNA phosphate backbone.

Flow linear dichroism measurements were performed on the psoralen–DNA complex in order to examine the intercalation of the psoralens in the DNA helix. By this method the angle between the plane of the psoralen ring system and the longitudinal axis of the DNA helix can be calculated.[42,43] Classical intercalation would require an angle of ca. 90°.

The results (Table II) indicate that of the compounds investigated only **5c**, **5j**, and **5k** bind to DNA by perfect intercalation, while **4a** may be intercalating. In contrast, predominant nonintercalative DNA binding is indicated for compounds **4b**, **4c**, and **4f** by this method. However, at very low dye to base pair ratio ($r < 0.02$), compound **4f** gave results in agreement with intercalation. This will be discussed in more detail elsewhere.[44] The influence of selected aminopsoralens on the viscosity of sonicated DNA was measured, but little effect was observed.

Photoreactivity of the Psoralens with Cyclohexane and 2′-Deoxythymidine. A comparison of the photoreactivity of the psoralens was determined by measuring the rate of photoaddition to cyclohexene and 2′-deoxythymidine (Table I). An increased photoreactivity of the aminopsoralens as compared to 8-methoxypsoralen was found. Furthermore, this effect of the amino groups only appears significant if the nitrogen atom is close to the psoralen nucleus (compare compounds **4a**, **4b**, **4c**, and **4h** to compounds **7**, **5k**, **4e**, and **4g**).

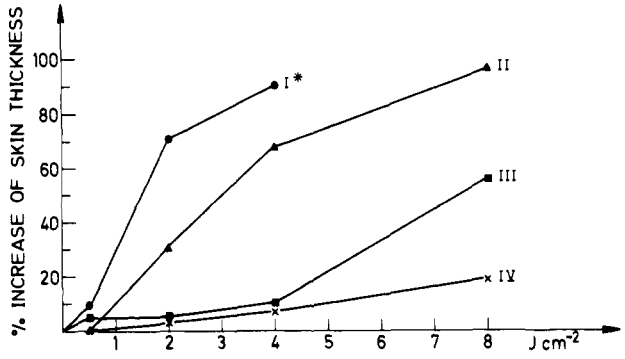

Figure 2. Skin-thickness increase for different concentrations of 8-MOP in hairless mice (Hr/Hr). I (●), 60 mg/kg; II (▲), 6 mg/kg; III (■), 0.6 mg/kg; IV (x), light only. (*) No data on high light and 8-MOP dose due to blistering and desquamation of the skin; SEM ≤ 28%.

Photohemolysis. The ability of compounds **4a** and **5c** to photohemolyze normal human erythrocytes was determined.[45,46] In the photohemolytic assay, the known membrane toxic compound benoxaprofen, 2-(4-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid, was used as a positive drug control, giving a photohemolysis of 30% relative to a totally hemolyzed standard. Neither of the two aminopsoralens showed any photohemolytic effect.

Singlet-Oxygen Photosensitization. The method proposed by Kraljic and Mohsni[47] was used to determine the ability of the psoralens as photosensitizers for singlet oxygen. The tested aminopsoralens, **4a**, **4d**, **4h**, **5c**, and **5k**, showed activities that were of the same order of magnitude as that of 8-MOP. The $O_2{}^1$-photosensitizing activities range from 68% to 170% of that of 8-MOP, and no correlation to skin photosensitizing, in vivo, could be seen.

In Vivo Photosensitization. Skin fold thickness, measured 24-h postirradiation, was used as an indicator of skin toxicity.[48] In preliminary experiments, skin thickness was measured at varying intervals after irradiation. Maximum increase in skin thickness was observed after 24 h. Table I shows the increase in skin fold thickness relative to 8-MOP (8-MOP = 100% 24 h after irradiation). The compounds were tested at the following concentrations by intraperitoneal (ip) injections: 0.6, 6, and 60 mg/kg. At 6 mg/kg doses, no acute dark toxicity was observed, and a linear light response relationship was obtained with light intensities comparable to doses given in human therapeutic situations (Figure 2). Results in Table I are shown for 4 J/cm^2 and 6 mg/kg. The absolute increase in skin thickness with 8-MOP was 68% when the animals were irradiated on the ventral part of the body and 133% when irradiated on the dorsum. The compounds **5c** and **5j** had more than 4 times the skin-thickening effect of 8-MOP;

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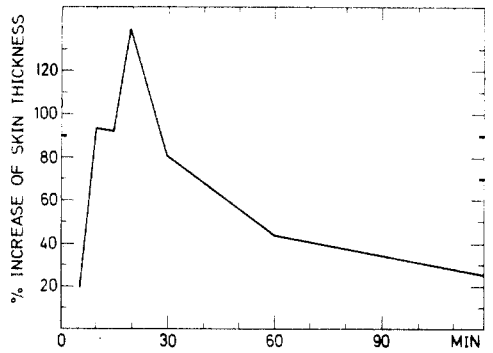

Figure 3. Effect of time elapse after injection on the skin thickening. The animals were injected with compound **5a** (0.25 mg/mouse) at time zero and irradiated after the indicated times. The increase in skin thickness was measured 24 h after irradiation.

4g, **4h**, and **5k** were ca. 3 times as efficient as 8-MOP; **4a**, **4b**, and **4f** were ca. twice as efficient as 8-MOP, in vivo. The effect of light without administration of drugs or of drugs without light was between 3% and 15%.

Acute toxicity studies (LD_{50}, without light) were not generally performed due to lack of sufficient amount of materials. In the skin phototoxicity experiments conducted, **4b** showed less toxicity than 8-MOP, which was lethal in three of 28 animals given 60 mg/kg i.p. Compounds **4a** and **5c** showed similar toxicity to that of 8-MOP, while LD_{50} was observed at 60 mg/kg for compound **4c**. The animals were observed for more than 1-year posttreatment. No macroscopic tumors were observed. The maximum doses were 100-fold that of normal human dosage.

The time relationship between ip injection and the effect of irradiation is shown for compound **5c** (Figure 3), where injection 15–20 min prior to the start of irradiation gives maximal phototoxic effect.

Discussion

A generally accepted animal model system for testing of antipsoriatic drugs has not yet been established. Therefore, it is extremely difficult to perform preclinical evaluation of potential photochemotherapeutics for the treatment of psoriasis. However, photocytotoxicity and skin photosensitization, measured as induction of erythema and edema, are prominent and measurable properties of 8-MOP, the drug of choice at present. However, these effects are not necessarily connected to the therapeutical effect. Thus a comparison of the new psoralens to 8-MOP in terms of these effects could give an indication of their potential as PUVA drugs or at least provide a basis for selection of compounds for further experiments.

All the new aminopsoralens presented in this paper have much higher water solubilities than that of 8-MOP due the the amino groups, which provide the psoralens with one or two cationic charges at physiological pH. The aqueous solubility should facilitate the medical use of the drugs, where reproducible and optimized serum levels during PUVA treatment are important.

The aminopsoralens of this series except one, **5k**, are less efficient than 8-MOP in the in vitro photocytotoxicity assay. When compared to the DNA photo-cross-linking efficiency on cells, a considerable correlation between the data is seen; cf. ref 15. The general trend is that low photo-cross-linking ability gives low photocytotoxicity, with **4h**, **4i**, and **7** having the lowest efficiencies and **5k** the highest in both tests.

The DNA photo-cross-linking efficiency of the psoralens on cellular DNA must reflect both cellular uptake, DNA affinity, and overall photoreactivity. Thus it is of interest to examine psoralen–DNA interactions. The in vitro DNA photo-cross-linking assay was performed at a DNA concentration where a binding constant exceeding 6×10^3 L mol^{-1} would result in greater than 90% association of the psoralen with the DNA. Our binding studies (Table II) indicate that the association constants for the aminopsoralens are well above this value ($K_a > 10^5$ L mol^{-1}), and we believe that this may be extrapolated to the aminopsoralens on which we have not obtained binding data.

The observed cross-linking efficiencies of the various psoralens could be directly reflecting the relative quantum yields of the two necessary consecutive cycloadditions. This would be the case when the irradiation was stopped before most of the psoralen was used. However, under circumstances where most or all of the psoralen is used up, the case is more complicated, and the measurements simply reflect effective chemical yields.

Irradiation time studies with the psoralens 8-MOP, **4a**, and **4c** showed that the photo-cross-linking reaction had reached a plateau before the end of the irradiation time employed in this cross-linking assay. Consequently, we believe that the cross-linking data directly express the cross-linking efficiencies of the psoralens. Thus the results indicate that the introduction of bulky groups in the 5-position greatly diminishes the psoralen cross-linking efficiency. This is most convincingly seen for the series **4a–d**. Conversely, quite large substituents may be introduced into the 8-position without loss of activity (compounds **5c**, **5j**, **5k**). The photobinding to DNA of the tritiated psoralens similarly was more efficient for the 8-substituted compounds as compared to the 5-substituted ones (Table II).

As seen from Table I no correlation between the photoreactions of the psoralens with either cyclohexene or thymidine and their DNA cross-linking efficiency is observed. Thus the different DNA photoreactivity of the 5- and the 8-substituted psoralens does not appear to be directly related to the quantum yield for the [2 + 2] cycloaddition reactions. However, analysis by flow linear dichroism of the binding geometry of the psoralen–DNA association complex gives a clue to the answer. The results (Table II) clearly indicate that while the 8-substituted psoralens bind to DNA by intercalation, this is not the case for the 5-substituted compounds. Since intercalation is regarded as a prerequisite for photo-cross-linking of DNA, poor intercalation would result in poor photobinding. The pronounced difference in behavior of the 8- and the 5-substituted aminopsoralens presumably reflects a steric constraint for the intercalation in DNA. Photo-cross-linking requires that the 8-substituent protrudes into the minor groove.[12,13]

It is interesting that the ratio between monoadducts and cross-links varies significantly less between the psoralens than either of these parameters alone. This indicates that a similar DNA–psoralen (intercalative) association complex is responsible for both of these adducts, although possibly operating at different sites.

Concerning the intercalative binding of the psoralens to DNA, our data may appear inconsistent at first sight. For compounds **5c**, **5j**, and **5k**, the linear dichroism measurements clearly indicate DNA binding by an intercalative mechanism. On the other hand, little extension of the DNA helix upon binding of the psoralens was indicated by viscometry measurements (~25% of that expected for full intercalative binding).[44] However, this apparent discrepancy may be understood when considering the differencies of the two techniques. While the flow linear dichroism measurements may be performed at very low

psoralen to base pair ratios ($r < 0.02$), much higher ratios are needed for viscosimetric analysis. We thus interpret these results to mean that only a very limited number ($r \ll 0.5 \sim$ neighbor exclusion[49]) of intercalation binding sites in the DNA exist for these psoralens. This is also consistent with our flow linear dichroism data, which show that the angle of the psoralen plane to the DNA helix axis decreases with increasing psoralen to base-pair ratios.[44]

From these results on psoralen–DNA interactions we conclude that psoralens with high DNA affinity, preferentially intercalating, and with high DNA photoreactivity may be obtained by placing amino functions in the 8-position. Unfortunately, no other simple relations between the substitution pattern of the psoralens and their photoreactivity are apparent from our data.

The low cellular DNA photo-cross-linking efficiencies and in vitro phototoxicity of **5c,j** therefore might be due to low cellular uptake, while those of compounds **4a** and **7** probably are due to a combination of reduced cellular uptake as well as decreased DNA intercalative binding, compared to those of 8-MOP.

Most of the aminopsoralens were superior to 8-MOP in the in vivo photosensitizing assay, the most efficient being **4g**, **4h**, **4j**, **5c**, **5j**, and **5k**. From a clinical point of view, the in vivo results are important, since they show that the compounds reach the skin and are capable of exerting photobiological effects there. It was found for **5c** that the maximal in vivo effect was obtained when irradiation of the animals was started ca. 20 min after ip administration. This response, which is approximately twice as fast as that found in similar experiments with 8-MOP in rats,[50] indicates that the maximal concentration of drug in the skin is reached at that time.

The high in vivo effect of several of these drugs, e.g., **4a**, **4h**, and **5c**, is interesting. It has previously been suggested that skin phototoxicity is connected with bifunctional lesions in DNA,[51] with singlet-oxygen formation,[19,20] or with a combination of these effects.[20] The present results do not allow such a straightforward comparison, but they clearly show no apparent correlation between the clinical results and the in vitro results.

The two psoralens tested, **4a** and **5c**, were nonphotohemolytic, similar to that previously reported for 8-MOP.[46] The lack of photohemolytical activity, which is an indicator of membrane degradation through singlet-oxygen or radical species, is in agreement with the low ability of the aminopsoralens to induce singlet-oxygen formation in solution. Since all of the aminopsoralens except one, **5k**, show lower photo-cross-linking ability and lower in vitro phototoxicity than 8-MOP, a plausible mechanism for the skin-photosensitizing effects of these aminopsoralens is not apparent from the present data.

Nothing is known about the metabolism of the present compounds. However, the rapid response after ip administration of **5c** indicating that the maximal level of the photoactive substance in skin and in plasma is reached very fast[52] and the high in vivo activity of structurally different aminopsoralens, e.g., **4a**, **4h**, and **5c**, suggest that no common degradation product is responsible for the in vivo phototoxicity.

Table III. Analytical Data of New Compounds

compd	yield, %	mp, °C	formula	anal.
4b	75	228–229	$C_{15}H_{15}NO_4 \cdot HCl \cdot H_2O$ (327.8)	C, H, N, Cl
4c	85	>255 dec	$C_{16}H_{18}NO_4Cl \cdot {}^1/_4H_2O$ (328.3)	C, H, N, Cl
4d	66	176–177	$C_{19}H_{24}NO_4Cl \cdot H_2O$ (383.9)	C, H, N, Cl
4e	38	203–204	$C_{19}H_{23}NO_4 \cdot HCl \cdot {}^1/_2H_2O$ (374.8)	C, H, N, Cl[a]
4f	92	210–211	$C_{18}H_{19}NO_4 \cdot HCl \cdot H_2O$ (367.8)	C, H, N, Cl
4g	76	172–173	$C_{16}H_{17}NO_5 \cdot HCl \cdot H_2O$ (357.8)	C, H, N, Cl[b]
4h	68	203–204	$C_{17}H_{19}NO_6 \cdot HCl \cdot {}^1/_4H_2O$ (374.3)	C, H, N, Cl
4i	78	235–237	$C_{17}H_{17}NO_5 \cdot HCl \cdot {}^1/_4H_2O$ (356.3)	C, H, N, Cl
4j	34	235–240	$C_{18}H_{20}N_2O_5 \cdot 2HCl \cdot {}^1/_2H_2O$ (426.28)	C, H, N
5c	60	234–240 dec	$C_{17}H_{20}NO_4Br$ (382.2)	C, H, N, Br
7	71	197–201	$C_{19}H_{24}NO_5Br$ (426.3)	C, H, N, Br

[a] Cl: calcd 9.45, found 9.93. [b] Cl: calcd 9.90, found 10.33.

The strong photosensitizing effects of **4g** and **4h** might be due to special properties of the (2-hydroxyethyl)amino groups. It has analogously been shown[53] that 4′-[[(2-hydroxyethyl)amino]methyl]-4,5′,8-trimethylpsoralen (**8**) is strongly photosensitizing on guinea pig skin, with an activity superior to that of 4′-(aminomethyl)-4,5′,8-trimethylpsoralen (**9**) and 8-MOP.

The biological properties of **5k** have been investigated previously.[36,54] It was found that the compound in combination with UV-A caused inhibition of the DNA and RNA synthesis in *Ehrlich ascites* cells, in vitro. The photosensitizing effect of **5k** administered topically on guinea pig skin was furthermore found to be superior to that of 8-MOP.

It remains to be seen if the effects of the psoralens described in this paper relate to their therapeutical efficiency. The use of skin photosensitization as a test for therapeutic potency has been questioned, as some psoralen derivatives, e.g., 3-carbethoxypsoralen, which were nonphotosensitizing in animal models, were therapeutically active.[30,55] However, the results obtained after treatment of psoriatic patients with 3-carbethoxypsoralen are ambiguous, since several patients were strongly photosensitized.[30]

The examined aminopsoralens appear to possess no acute toxicity when given orally to mice and the use of some of the derivatives as photochemotherapeutics should be investigated.

Conclusion

These results show the existence of a good correlation between the binding mode and the photochemical behavior of the psoralens toward DNA. Furthermore, the ability of the psoralens to introduce DNA interstrand cross-links in cultured cells can be clearly correlated to the photocytotoxicity of the psoralens on these cells. However, the

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photosensitization results obtained with animals can neither be explained from the binding mode of the psoralens to DNA, their DNA photo-cross-linking ability, nor their capacity for the generation of singlet oxygen.

Experimental Section

Chemistry. Melting points, uncorrected, were determined on a Büchi melting point apparatus; IR spectra were recorded on a Perkin-Elmer 157 spectrometer; ^{1}H NMR spectra were recorded on a JEOL FRPOQ spectrometer. The elemental analyses were within ±0.4% unless otherwise specified. All new compounds had spectroscopic and analytical properties as expected (Table III).

Compounds **2**,[34] **3**,[35] **4a**,[2] **5j**,[1] **5k**,[1] **4l**,**m**,[36] **6**,[1] **8**,[36] and **10**[36] were synthesized as described previously. Compounds **4c**, **4d**, **7**, and **5c** were prepared as described for **4d**. Compounds **4h–j** were prepared as described for **4i**. The aminopsoralens are light sensitive in solution, and the preparation should be carried out in the dark.

5-[(Dimethylamino)methyl]-8-methoxypsoralen Hydrochloride (4b). Compound **2** (0.53 g, 2 mmol) was dissolved in 25 mL of acetone containing 0.4 g of K_2CO_3 (anhydrous). The mixture was purged with dimethylamine (gas) at room temperature and stirred for 0.5 h and then concentrated in vacuo. The resulting oil was triturated with water, giving 0.55 g of crude aminopsoralen, which was converted to its hydrochloride (**4b**), 0.42 g (75%).

5-[(Triethylammonio)methyl]-8-methoxypsoralen Chloride (4d). Compound **2** (1.0 g, 3.8 mmol) was dissolved in 30 mL of acetone and 10 mL of triethylamine. The mixture was refluxed for 24 h and cooled, and the precipitated white crystals were isolated by filtration, giving 0.92 g (66%) of **4d**, which was recrystallized from ethanol.

5-[(Diisopropylamino)methyl]-8-methoxypsoralen Hydrochloride (4e). Compound **2** (0.4 g, 1.5 mmol) was dissolved in diisopropylamine (10 mL) and acetonitrile (40 mL). The mixture was refluxed for 40 h under nitrogen, allowed to cool to room temperature, filtered, and concentrated in vacuo. The product was taken up in acetone (10 mL), filtered, and concentrated in vacuo. The resulting psoralenamine was recrystallized from hexane to give 0.2 g of **4e** (~42%). The base was converted to its hydrochloride and recrystallized from acetone to give 0.2 g of **4e**.

5-(*N*-Piperidylmethyl)-8-methoxypsoralen Hydrochloride (4f). Compound **2** (1 g, 3.8 mmol), piperidine (0.4 g, 4.7 mmol), and K_2CO_3 (anhydrous) (0.8 g) were stirred in 60 mL of acetone for 70 h at room temperature. The light yellow solution was filtered and concentrated in vacuo and subsequently chromatographed by column chromatography (silica gel 60, 2.5 cm × 14 cm, acetone), giving 1.1 g of **4f** as white crystals (92%). The amine was converted to its hydrochloride and recrystallized from ethanol (absolute) to give 0.98 g of **4f** (70%). An analytically pure sample was obtained by sublimation.

5-(Morpholinomethyl)-8-methoxypsoralen Hydrochloride (4i). Compound **2** (0.25 g, 0.9 mmol), morpholine (0.1 g, 1.1 mmol), and K_2CO_3 (anhydrous) (0.2 g) was refluxed in acetone (10 mL) for 6 h. The mixture was poured on ice and extracted with $CHCl_3$. The organic phase was dried with $MgSO_4$ and concentrated in vacuo to give 0.2 g of **4i** (~71%). The amine was converted to its hydrochloride and recrystallized from acetone to give 0.12 g of **4i**.

5-(Hydroxy[^{3}H]methyl)-8-methoxypsoralen (4l-T). A mixture of 5-formyl-8-methoxypsoralen purified by sublimation (25.0 mg, 0.103 mmol) in hexafluoro-2-propanol (3 mL) was added to NaB^3H_4 (0.05 mmol, 5 Ci/mmol), and the heterogeneous mixture was stirred overnight at room temperature. The solvent was evaporated, the yellow residue was redissolved in $CHCl_3$ and the solution was purified by preparative TLC on silica gel, with $CHCl_3$/EtOH (99:1) as eluent. The product (4 mCi) was isolated by Soxhlet extraction with $CHCl_3$.

5-(Chloro[^{3}H]methyl)-8-methoxypsoralen (2-T). A 2% solution of $SOCl_2$ in $CHCl_3$ (40 mL) was added to **4l-T** (2 mCi) diluted with unlabeled 5-(hydroxymethyl)-8-methoxypsoralen (11.2 mg, 4.55×10^{-2} mmol). The light yellow solution was stirred at room temperature for 24 h in the dark. The solvent and excess $SOCl_2$ were evaporated. The product was not further purified but redissolved in $CHCl_3$ and divided into three portions, two of which were used for synthesis of **4a**-T and **4c**-T.

5-[(Trimethylammonio)[^{3}H]methyl]-8-methoxypsoralen Bromide (4c-T). To one-third of the crude 5-(chloro[^{3}H]-methyl-8-methoxypsoralen (0.7 mCi) in dry $CHCl_3$ (5 mL) was added trimethylamine (1.2 g, 0.02 mol) in dry $CHCl_3$ (5 mL) and the mixture was stirred for 24 h at room temperature in the dark, with use of a flask equipped with a $CaCl_2$ tube.

A white precipitate formed quickly, and the reaction was almost complete after 10 h. The solvent and excess trimethylamine were evaporated, and the residue was resuspended in $CHCl_3$ and collected on a paper filter (Whatman 50) as a white powder. The purity was >95% as tested by two-dimensional chromatography in Me_2SO/MeOH (50:50) and EtOH/triethylamine (97:3, dry); yield 60%; sp act. 61 Ci/mol.

5-(Amino[^{3}H]methyl)-8-methoxypsoralen (4a-T). To another third of the crude 5-(chloro[^{3}H]methyl-8-methoxypsoralen (0.7 mCi) in $CHCl_3$ (5 mL) was added hexamethylenetetramine (2.3 g, 16.4 mmol) and the mixture was stirred for 4 days at 30 °C. The conversion to the hexamethyltetraammonium ion was monitored by TLC (silica, Merck) in $CHCl_3$. The solvent was removed in an air stream, giving an off-white residue. This residue was dissolved in a mixture of EtOH and HCl (3:1, 96% EtOH, 37% HCl) and the solution stirred for 24 h at room temperature. The solvent was removed in an air stream at 80 °C, and the white solid residue was resuspended in $CHCl_3$ and collected by filtration (Whatman No. 50, hardened); yield 80%; sp act. 32 Ci/mol.

5-Tritio-8-[(3-bromopropyl)oxy]psoralen (3-T) was prepared by catalytical exchange. The unlabeled compound (40.3 mg, 0.125 mmol), tritium oxide (50 μL, 2.7 mmol, 25 Ci/mL), dioxane (1.7 mL), and fuming sulfuric acid (33% in SO_3) (190 μL) was stirred for 24 h at 50 °C and then for 3 days at room temperature. During this time the color changed from light yellow to dark amber. The reaction mixture was cooled in crushed ice and brought to pH 6 by sodium hydroxide (33%). The mixture was warmed to remove the dioxane and then extracted 10 times with 1 mL of $CHCl_3$. The combined extracts were reduced to 3.5 mL and worked up by TLC (silica gel 60, Merck, 20 × 20) in $CHCl_3$/1% EtOH. The silica gel was extracted by boiling $CHCl_3$ (3 × 30 mL + 2 × 10 mL). The extract was used without further purification; yield 8.2 mCi.

5-Tritio-[[3-(trimethylammonio)propyl]oxy]psoralen Bromide (5c-T). 5-Tritio-8-[(3-bromopropyl)oxy]psoralen (2.9 mCi) in $CHCl_3$ was magnetically stirred in a solution of trimethylamine (1.0 g, 17 mmol in 5 mL of $CHCl_3$, absolutely dry) for 24 h. The solvent was removed in an air stream on a water bath, and the white residue was twice resuspended in $CHCl_3$ (4 mL) and finally dried under reduced pressure. Purity was checked by TLC in $CHCl_3$ and EtOH/water (95:5) and saturated NaAc. The yield was 97% and sp act. 54 Ci/mol.

Aqueous Solubility. The aminopsoralens were suspended in distilled water, the mixture was stirred at 20 °C for 24 h and filtered, and the concentration of the drugs was measured by absorption spectroscopy with use of the following coefficients: **4a**, $\epsilon_{307} = 1.07 \times 10^4$; **4b**, $\epsilon_{306} = 1.25 \times 10^4$; **4c**, $\epsilon_{307} = 1.20 \times 10^4$; **5c**, $\epsilon_{302} = 1.20 \times 10^4$.

DNA Binding Data. Equilibrium analysis was carried out by using two-phase partition in aqueous 6% dextran (M_r 500 000) and 4% polyethylene glycol (M_r 6000) as described elsewhere.[42] The ionic strength was in all experiments 1 mM NaCl and 1 mM Na_2EDTA. The free reagent concentration and the DNA concentration were determined spectrophotometrically in top and bottom phases, respectively.

Determination of Psoralen Plane to Helix Axis Angle of DNA-Bound Psoralen. Linear dichroism (LD) and absorbance (A) spectra were recorded on a Jasco 500 spectropolarimeter converted to LD mode according to Davidsson and Nordén[43] and on a Cary 219 spectrophotometer. The angle between the DNA helix and the ligand transition moment of the absorption at about 315 nm, which is polarized in the molecular plane, was calculated as $\alpha = \arccos [1/3 - (LD/A)_{315}/3(LD/A)_{260}]^{1/2}$, where the reduced dichroisms (LD/A) are obtained by dividing the respective linear dichroism by the absorption of DNA (260 nm) and the absorption (315 nm) of bound ligand. All LD spectra were run on a Couette flow cell at a spinning rate corresponding to the gradient 1366 s^{-1}. The temperature in equilibrium and spectroscopy studies was 25 °C.

Viscometry. This was done analogously to the method described in ref 1.

Photo-cross-linking of Calf Thymus DNA. To the psoralen (0.1–100 μg/mL) dissolved in 200 μL of 10 mM Tris-HCl, 1 mM EDTA, pH 7.0, was added 200 μg of sonicated calf thymus DNA dissolved in 200 μL of the same buffer. The solution was irradiated for 30 min (λ ~365 nm, 24 kJ/m²), heated at 100 °C for 5 min, and quickly cooled in ice. The relative amounts of double- and single-stranded DNA were determined by chromatography on hydroxylapatite (Bio-Rad) (1 × 5 cm column eluted with a 100-mL linear gradient of 50–500 mM sodium phosphate, pH 7). For each psoralen the concentration resulting in 50% cross-linked DNA was determined. With use of this value, the cross-linking capacity relative to 8-MOP was calculated.

Photobinding of [³H]Psoralens to Calf Thymus DNA. A mixture of 1 mg of calf thymus DNA and the tritiated psoralen (8-MOP, 5.5 × 10⁵ cpm; **4a**, 2.8 × 10⁵ cpm; **4c**, 1.1 × 10⁶ cpm; **5c**, 2.8 × 10⁵ cpm) in 1 mL of 10 mM Tris-HCl, 1 mM EDTA (pH 7.4) was irradiated in Pyrex tubes equipped with a magnetical stirrer. At the times indicated in Figure 1, 100-μL aliquots were withdrawn, and the DNA was precipitated with 250 μL of 0.2 M NaOAc in 96% ethanol. The pellet was redissolved in 100 μL of H_2O, precipitated again, and finally dissolved in 100 μL of H_2O. The amount of ^3H (cpm) was determined by liquid scintillation counting in 3 mL of Instagel (Packard).

Determination of the Ratio between Mono- and Diadducts. The tritriated psoralen (8-MOP, 7 × 10⁴ cpm; **4a**, 1.5 × 10⁵ cpm; **4c**, 2.8 × 10⁶ cpm; **5c**, 1.1 × 10⁶ cpm) dissolved in 100 μL of 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, was mixed with 900 μg of sonicated calf thymus DNA dissolved in 900 μL of the same buffer. The mixture was irradiated for 45 min and applied to a 1 × 5 cm hydroxylapatite column. The DNA was eluted by a 100-mL 50–500 mM sodium phosphate gradient and detected by the UV absorbance (254 nm). Single- and double-stranded DNA was collected and the amount of bound psoralen determined by scintillation counting (4-mL sample, 8 mL of H_2O/Insta-gel). For calculation of the ratio between mono- and diadducts it was assumed that the monoadducts are distributed equally in all of the DNA, i.e., identical monoadducts to base-pair ratio in the single-stranded as well as in the double-stranded (cross-linked) DNA fraction. Thus, the ratio between monoadducts and cross-links may be calculated according to the following equation:

$$\frac{\text{monoadducts}}{\text{cross-links}} = \frac{(\text{cpm(ss)}/\text{OD}_{254}\text{(ss)}) \times \text{OD}_{254}\text{(ds)}}{\text{cpms(ds)} - (\text{cpm(ss)}/\text{OD}_{254}\text{(ss)})\text{OD}_{254}\text{(ds)}}$$

where cpm(ss) = ^3H (cpm) in the single-stranded (ss) DNA fraction, cpm(ds) = ^3H (cpm) in the double-stranded (ds) DNA fraction, OD_{254}(ss) = OD_{254} units (E_{254} × volume in milliliters) in ss-DNA fraction, and OD_{254}(ds) = OD_{254} units in ds-DNA fraction.

Photoreaction with Cyclohexene and 2′-Deoxythymidine. For estimation of photoreactivity, light from an Osram SP200 superpressure mercury lamp was used, and the light was filtered through an acetone filter to eliminate light of wavelength shorter than 325 nm.

The samples, which were about 1 × 10⁻⁴ M of the psoralens in 1 M cyclohexene in ethanol or in 0.4 M 2′-deoxythymidine in 10 mM Tris-buffer, pH 7.0, were irradiated in quartz cuvettes at room temperature. The photoreactions were followed spectrophotometrically as functions of the disappearance of the psoralen absorptions at 305 nm. The relative photoreactivity of the psoralens was determined from their half-life relative to that of 8-MOP. The half-live of 8-MOP in the photoreactions with cyclohexene was 3.3 min and with 2′-deoxythymidine 31 min.

In Vitro Phototoxicity. The human epithelial cell line NHIK 3025[41] was grown at 37 °C in RPMI 1640 medium (Gibco) containing 10% fetal calf serum and penicillin/streptomycin in a CO_2 incubator (5% CO_2).

For measurements of phototoxicity, the cells were seeded in microtiter wells (100 μL/36 mm²) at ~5% confluence. Eighteen hours after seeding, the medium was aspirated off and replaced with phosphate buffered saline (PBS) (37 °C) containing the psoralen. The psoralens were tested in concentrations ranging from 0.03 to 30 μg/mL. Following a 10-min equilibration period, the cells were irradiated for 10 min with a Philips TL 20 W/09 fluorescent light tube (0.8 J/cm², λ ~365 nm), and the PBS was aspirated off. Fresh medium was added, and the cells were reincubated at 37 °C. After 3 days, cell growth was scored visually and the lowest psoralen concentration causing growth inhibition was estimated.

The ciliate *Tetrahymena thermophila*, strain B1868-VII, was also used for the phototoxicity test. The cells were grown as described[56] and the phototreatment was performed in 50 μL of 10 mM Tris-HCl, pH 7.4 (2 × 10³ cells/mL). After addition of 150 μL of medium, the cells were incubated at 28 °C, and cell growth was scored visually after 48 h.

Photo-cross-linking Assay. Measurement of psoralen photoinduced DNA interstrand cross-links in situ in NHIK 3025 was performed by the alkaline elution technique as previously described.[17,57] Briefly, ~10⁶ cells were labeled with [³H]thymidine (1 μCi/mL, 20 Ci/mmol, New England Nuclear) for 16 h. The medium was aspirated off and replaced with a solution of the psoralen dissolved in PBS (concentrations, 1–90 μg/mL). Following a 10-min equilibration period, the cells were irradiated (0.8 J/cm², λ ~365 nm). The PBS was then aspirated off, and the cells were trypsinized for 5 min. One volume of medium was added, and the cells were isolated by centrifugation and finally resuspended in 500 μL of cold PBS.

For alkaline elution analysis, the cells were caught on a 2-μm PVC filter (Millipore) and lyzed in 100 μL of 0.2% Sarcosyl, 2 M NaCl, 40 mM EDTA, pH 10. The lysis solution (5 mL) was pumped through the filters at 1 mL/min, and the DNA was eluted at 6 mL/h with 0.7% tetraethylammonium hydroxide, 0.01 M EDTA, pH 12.2.

Cross-linking efficiencies relative to 8-MOP were determined from the psoralen concentrations that caused similar retardation of the DNA elution.[17]

Analogous experiments were performed on *Tetrahymena thermophila*: 2 × 10⁵ cells (prelabeled with [³H]thymidine[56]) were phototreated with the psoralens in 500 μL of 10 mM Tris-HCl, pH 7.4, and analyzed as described above.

Skin Phototoxicity. Hr/Hr female mice of 4–6 weeks of age (Bomholtgaard, Rye, Denmark) were used throughout the experiments. The animals (5–28/group) were injected ip with compounds of concentrations of 0.6, 6, and 60 mg/kg (results are shown for 6 mg/kg). After incubation periods ranging from 5 min to 2 h, the mice were either anesthetized with pentobarbital and strapped in a positioning device during irradiation or were kept in a glass cage and irradiated on the ventral side of the body. In both instances, the photochemotherapeutic effect was registered on a visual scale, evaluating degrees of erythema, edema, and vesiculation. Skin-fold thickness was measured by a caliper. Measurements were performed 4 and 24 h after irradiation.

The mice were irradiated by a bank of four fluorescence tubes (Philips TL20W/09). The light was filtered through a plate glass filter to remove traces of UV-B. Intensity was 2.4 mW/cm² (PUVA meter, H. Waldmann, West Germany). The doses delivered were between 0.5 and 16 J/cm². Irradiation was performed at 20 °C; the temperature was kept stable by filtered streaming air.

Photohemolysis Assay. Normal human erythrocytes were isolated, washed, and incubated with the test compound, 10 μg/mL in PBS. Samples were irradiated with UV-A, 1 mW/cm² for various time schedules (maximum 4 h), and the temperature was kept at 25–28 °C. Photohemolysis was determined spectroscopically at 546 nm,[45,46] relative to a totally hemolyzed solution. Benoxaprofen was used as a positive drug control.

Singlet-Oxygen Photosensitization. The method described by Kraljic and Moshni[47] was used. Solutions of *p*-nitroso-*N*,*N*-dimethylaniline (4.0 × 10⁻⁵ M), histidine (1 × 10⁻² M), and the aminopsoralens (5 × 10⁻⁶ M) in 2 × 10⁻² M phosphate buffer (pH 7.0) were irradiated at room temperature with an SP 200 superpressure mercury lamp equipped with a Pyrex filter. The decrease in absorbance at 440 nm (ΔA_{440}) was followed spectrophotometrically as a function of time of irradiation. The O_2^1-

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photosensitizing activities, which are given as ΔA_{440} relative to that of 8-MOP (100%) were as follows: **4a**, 86; **4d**, 170; **4h**, 150; **5c**, 68; and **5k**, 80. The values of **4a**, **5c**, and **5k** were insignificantly different from the blind values obtained upon irradiation of the solution containing no psoralens.

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Registry No. 1, 298-81-7; 2, 43111-03-1; 2-T, 96616-33-0; 2-T·$(CH_2)_6N_4$, 96632-97-2; 3, 96616-34-1; 3-T, 96616-35-2; 4a, 78827-46-0; 4a·HCl, 78827-47-1; 4a-T, 96616-36-3; 4b, 96616-37-4; 4b·HCl, 96616-38-5; 4c, 96616-39-6; 4c-T, 96616-40-9; 4d, 96616-41-0; 4e, 96616-421; 4e·HCl, 96616-43-2; 4f, 73166-85-5; 4f·HCl, 96616-44-3; 4g, 96616-45-4; 4g·HCl, 96616-46-5; 4h, 96616-47-6; 4h·HCl, 96616-48-7; 4i, 73166-84-4; 4i·HCl, 96616-49-8; 4j, 96616-50-1; 4j·HCl, 96616-51-2; 4l, 43111-10-0; 4l-T, 96616-52-3; 4m, 50639-52-6; 4n, 86863-32-3; 5c, 96616-53-4; 5c-T, 96616-54-5; 5j, 86863-15-2; 5k, 85079-39-6; 5p, 96616-55-6; 6, 86863-17-4; 7, 96616-56-7; 8, 96616-57-8; 10, 43111-11-1; $ClCH_2OCH_3$, 107-30-2; $Br(CH_2)_3Br$, 109-64-8; $HO(CH_2)_3OH$, 504-63-2; 5-(azidomethyl)-8-methoxypsoralen, 78827-45-9; 5-[[(3-hydroxypropyl)oxy]methyl]-8-methoxypsoralen, 86863-32-3.

Synthesis and Biological Activity of 6-Azacadeguomycin and Certain 3,4,6-Trisubstituted Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides[1,2]

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Several 3,4,6-trisubstituted pyrazolo[3,4-*d*]pyrimidine ribonucleosides were prepared and tested for their biological activity. High-temperature glycosylation of 3,6-dibromoallopurinol (**6**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of BF_3·OEt_2, followed by ammonolysis, provided 6-amino-3-bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**7a**). Similar glycosylation of either 3-bromo-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidin-6-yl methyl sulfoxide (**8**) or 6-amino-3-bromopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**11**), and subsequent ammonolysis, also gave **7a**. The structural assignment of **7a** was on the basis of spectral studies, as well as its conversion to the reported guanosine analogue **1d**. Application of this glycosylation procedure to 6-(methylthio)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**14**) gave the corresponding N-1 glycosyl derivative (**16a**). Dethiation and debenzoylation of **16a** provided an alternate route to the recently reported 3-carbamoylallopurinol ribonucleoside (**17**), thus confirming the structural assignment of **16a** and the nucleosides derived therefrom. Oxidation of **16a** and subsequent ammonolysis afforded 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**15a**). Alkaline treatment of **15a** gave 6-azacadeguomycin (**18**). Acetylation of **15a**, followed by dehydration with phosgene, provided the versatile intermediate 6-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**19**). Deacetylation of **19** gave 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**20a**). Reaction of **19** with H_2S gave 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (**20b**). All of these compounds were tested in vitro against certain viruses and tumor cells. Among these compounds, the guanosine analogues **7a** and **20a** showed significant activity against measles in vitro and were found to exhibit moderate antitumor activity in vitro against L1210 and P388 leukemia. 6-Azacadeguomycin (**18**) and all other compounds were inactive against the viruses and tumor cells tested in vitro.

Pyrazolo[3,4-*d*]pyrimidines have received renewed attention in recent years owing to the discovery of certain derivatives possessing antiparasitic activity. Since there is an absence of de novo purine biosynthesis in most parasites,[3-8] these organisms are wholly dependent on the salvage pathway for purine nucleoside metabolism and will accept certain pyrazolo[3,4-*d*]pyrimidines in place of purines.[3] Allopurinol (pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one, **1a**)[9] was the first such analogue shown to be active against several leishmania[10-14] and trypanosoma[13-15] species in vitro. In both leishmania[12] and trypanosoma[16] species, allopurinol is converted to allopurinol ribonucleoside 5′-phosphate (HPPR-MP) by a unique enzyme of the parasite, nucleoside phosphoribosyltransferase. Sequential conversion of HPPR-MP by the parasite enzymes adenylosuccinate synthetase and succino-AMP lyase gives 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) ribonucleoside

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