Photophysical Properties of Fluorescent Pyrido[2,1-*i*]purines

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7-Oxo-3-(tetrahydropyranyl-2-yl)-7*H*-pyrido[2,1-*i*]purines (**1**) are highly fluorescent molecules with absorption and emission in the visible region. Quantum yields range from 0.33 to 0.64, while fluorescence lifetimes are rather short (2—6 ns). An increase in solvent polarity shifts absorption to shorter and emission to longer wavelengths. From this a change in dipole moment of 5.5 D could be estimated between ground and excited states of (**1b**). Fluorescence is quenched by tertiary amines *via* electron transfer. Hydrolysis of the protecting tetrahydropyranyl function results in a pyridopurine with somewhat different properties.

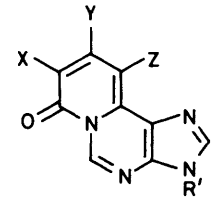
In a previous publication we presented the preparation of a series of pyrido[2,1-*i*]purines, (**1**) and (**2**).[1] These novel purine analogues exhibited strong fluorescence in the visible region, λ_{em} ranging from 460 to 500 nm (absorption at 400—460 nm). Fluorescent nucleosides are of interest as substrates or inhibitors of enzymes, since they can be easily monitored in the biological system of choice.[2–4]

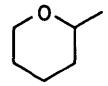
In this paper we report on a study of their photophysical properties, such as absorption, emission, quantum yields, and fluorescence lifetimes and the influence of the surrounding molecules on these properties.

Absorption and Emission.—Table 1 summarizes the absorption characteristics of (**1a**—**f**). As shown, the fluorescent 7-oxopyrido[2,1-*i*]purines absorb at rather long wavelengths. Comparable absorption maxima were found by Acheson and Woollard for the non-fluorescent oxoquinolizines (**3**) and (**4**).[5] On the other hand the oxopyrimidoisoquinolizine (**5**),[6] the oxopyrimidopurine (**6**),[7] and the oxopyridopurine (**7**)[8] absorb at much shorter wavelengths. We cannot present a reasonable explanation for this substantial difference in absorption behaviour in terms of electron-withdrawing effects or π-system elongation by substituents. Figure 1 shows emission and excitation spectra of (**1a**—**f**). From the expected mirror-image relation between emission and the $S_0 \longrightarrow S_1$ transition we assign the absorption at 330—350 nm to excitation of levels higher than S_1 [see (**1a**—**d**)]. For (**1e,f**) these bands are shifted to longer wavelengths and are only visible as shoulders.

Lifetimes and Quantum Yields.—We measured lifetimes for (**1b** and **e**) employing the pulse sampling method (N_2 laser, FWHM *ca.* 8 ns) and found 6 and 2 ns, respectively, by comparison with calculated decay curves. Because the decay of fluorescence follows the pulse rather closely, the values are inaccurate and only indicative.

Quantum yields are measured with biphenylanthracene in cyclohexane[9] and with 9-aminoacridine in ethanol[10] as references. According to the literature both compounds possess quantum yields close to unity in the solvents used. The results are summarized in Table 2. For compounds containing lone pair electrons the lowest energy transition is often of the n–π^* type. It is known that π^*–n fluorescence is generally not observed because of radiationless deactivation to the triplet level.[11] However, in complex chromophores like conjugated ketones,[12] the first π–π^* transition tends to pass or hide the n–π^* transition, leading to chromophores capable of strong fluorescence. In these terms we explain the very high quantum yields of our pyridopurines.



(**1**) R′ = 

(**2**) R′ = H

a; X = Z = H, Y = COOR
b; X = H, Y = Z = COOR
c; X = H, Y = COOR, Z = CN
d; X = H, Y = Ph, Z = COOR
e; X = CHCHCOOR, Y = H, Z = COOR
f; X = CHCHCOOR, Y = H, Z = CN, R = CH_3

The Solvent.—The interactions between the solvent and fluorophore molecules affect the energy difference between the ground and the excited state. To a first approximation this energy difference (in cm^{-1}) is a function of the refractive index (n) and dielectric constant (ε) of the solvent, and is described by the Lippert equation (1).[13–15] In this equation, h is Planck's

$$\bar{\nu}_a - \bar{\nu}_f \cong \frac{2}{hc}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right)\frac{(\mu^* - \mu)^2}{a^3} + \text{const.} \quad (1)$$

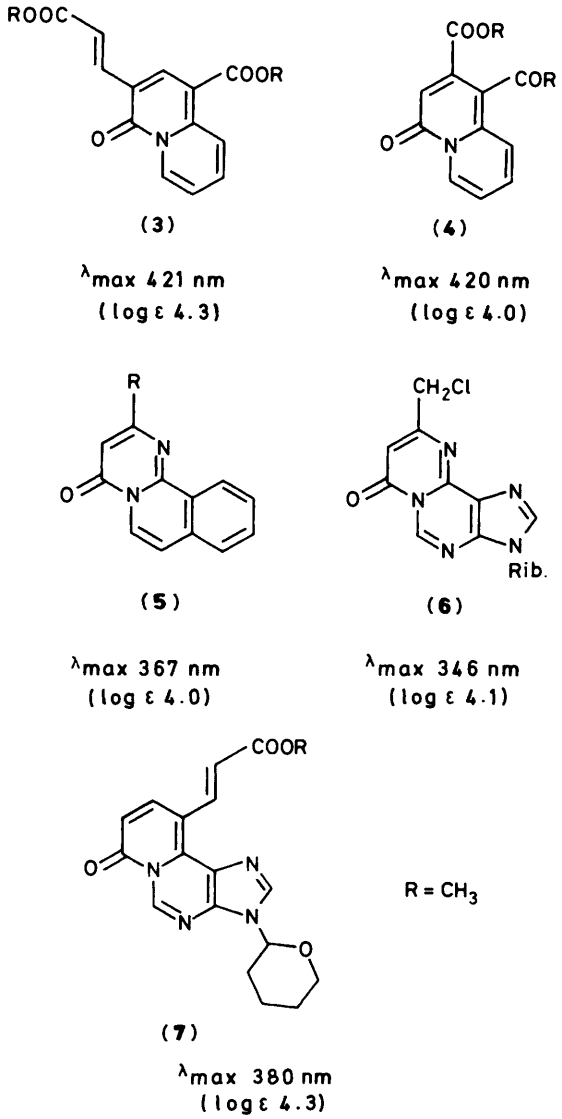
constant, c is the velocity of light, and a is the radius of the cavity in which the fluorophore resides. (If this cavity has a pronounced ellipsoidal shape, a is usually taken to be 40% of its long axis.) The wavenumbers in cm^{-1}, of the absorption and the emission, are $\bar{\nu}_a$ and $\bar{\nu}_f$ respectively. We recorded absorption and emission spectra of (**1b**) in different solvents and plotted $\bar{\nu}_a$—$\bar{\nu}_f$ against Δf (Figure 2).

Clearly the Lippert equation only accounts for a general solvent effect. Protic solvents do not fit. The intercept of this Figure, 1 900 cm^{-1}, corresponds acceptably with vibrational relaxation and from the slope we derive the difference in dipole moment between ground and excited state. Estimating a to be 5 Å $\Delta\mu$ is 5.5 D. With increasing polarity of the solvent the absorption shifts to shorter and the emission, to a small extent, to longer wavelengths. From this we conclude that it is the ground state, rather than the excited state, which is stabilized to a larger extent in more polar solvents.

Quenching by Tertiary Amines.—Quenching of fluorescence of aromatic hydrocarbons by tertiary amines has been studied

Table 1. Absorption and extinction of (**1a**—**f**) in CH_2Cl_2

Compound	$\lambda_{max.}$/nm	log ε	$\lambda_{max.}$/nm	log ε	$\lambda_{max.}$/nm	log ε
(**1a**)	457	3.9	354	3.4	266	3.9
	432	3.9	335	3.4		
(**1b**)	451	4.0	350	3.7	268	4.0
	428	4.1	335	3.7		
(**1c**)	415	4.1	345	3.9	273	3.9
			332	3.9		
(**1d**)	417	4.2	347	4.0	267	4.5
	400	4.2	332	3.9		
(**1e**)	442	4.5			277	4.3
	425	4.5				
(**1f**)	440	4.5			267	4.4
	432	4.4				
	417	4.4				



extensively.[16–18] Considerable evidence is collected for an electron transfer mechanism and in many cases exciplex emission was observed. Since pyridopurines are capable of accommodating a negative charge their interaction with tertiary amines was studied.

Figure 3 shows typical plots of Stern–Volmer quenching by triethylamine of pyridopurine fluorescence. In all solutions the

Table 2. Quantum yields of (**1a**—**f**). A, 9-Aminoacridine as reference in ethanol; λ_{ex} 423 nm. B, Diphenylanthracene as reference in cyclohexane; λ_{ex} 330 nm

	A	B
(**1a**)	0.56	0.47
(**1b**)	0.64	0.55
(**1c**)	0.50	0.43
(**1d**)	0.33	0.35
(**1e**)	0.40	0.40
(**1f**)	0.40	0.38

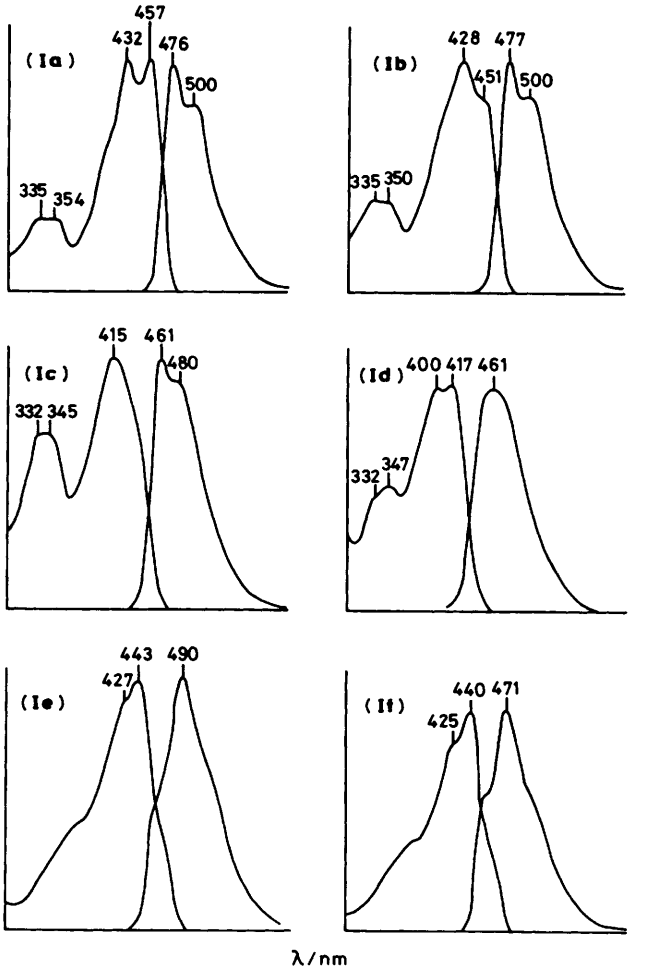


Figure 1. Excitation and emission (**1a**—**f**) in CH_2Cl_2. Efficiency in arbitrary units

absorption spectra of the pyridopurines (**1a**—**f**) are not significantly changed by triethylamine, excluding the possible formation of bound ground-state molecular complexes. No exciplex emission is observed under any condition. An increase of k_{SV} with increasing temperature or increasing polarity of the solvent (Tables 3 and 4) again suggests a dynamic process of electron transfer. Quenching parameters of pyridopurines (**1a**—**f**) with four different tertiary amines are summarized in Table 5.

If we assume the lifetimes of (**1a**—**d**) and of (**1e, f**) to be respectively comparable, *viz.* 6 and 2 ns respectively, we can derive the quenching constant k_q from equation (2). The

$$k_q = k_{SV}/\tau_o \qquad (2)$$

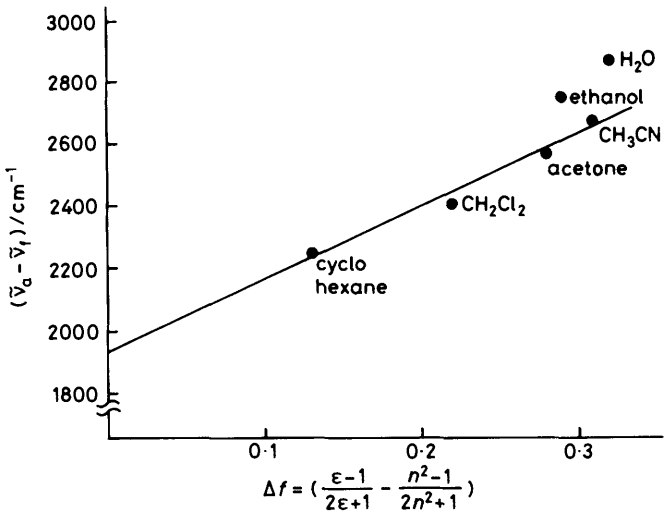


Figure 2. Stokes' shifts ($\tilde{\nu}_a$—$\tilde{\nu}_f$) *versus* orientation polarizability (Δf)

Table 3. Quenching of fluorescence of (**1b**) by triethylamine as a function of temperature in CH_2Cl_2

	20 °C	30 °C	40 °C	50 °C
k_{SV}/l mol^{-1}	19.5	22.9	26.8	30.0

collisional frequency, $k_{diff.}$, is related to the bimolecular quenching constant by the quenching efficiency α [equation (3)]. We estimate $k_{diff.}$ to be 2×10^{10} l mol^{-1} s^{-1} in CH_2Cl_2

$$k_q = \alpha \cdot k_{diff.} \quad (3)$$

using the combined Stokes–Einstein and Smoluchowski equation (4) where N is Avogadro's number, k is the Boltzmann

$$k_{diff.} = \frac{2NkT}{\eta}(R_f + R_q)(1/R_f + 1/R_q) \quad (4)$$

constant, and η is the solvent viscosity. R_f and R_q are the radii of fluorophore and quencher respectively.

Table 5 shows a higher quenching efficiency for ethyldiisopropylamine compared with triethylamine in accordance with the lower I_{pot} of the former. Analogous observations are made for the tertiary amines *N*-methylmorpholine and *N*-ethylpiperidine. The low reactivity of the cyclic amines compared with the open chain amines is a phenomenon already encountered in quenching of fluorescence of naphthalenes.[18]

We find an increase in quenching efficiency with an extra ester function (**1a**) ⟶ (**1b**) or by exchanging an ester for a nitrile (**1b**) ⟶ (**1c**), (**1e**) ⟶ (**1f**). Compound (**1d**) deviates the series and we assume geometric factors to play a role [if τ_0(**1d**) = 6 ns!]. Sterical interaction of the phenyl group with the ester function decreases the contribution of the ester in stabilizing a negative charge.

For comparison, Meeuws *et al.*[17] found in the protic solvent ethanol (no exciplexes) a value α of 0.043 in *N*-methylpiperidine and 0.10 in triethylamine quenching of 2-methylnaphthalene.

Other Quenchers.—As a consequence of our interest in biological applications of fluorescent pyridopurines, we have studied the possibility of quenching by cellular components. Emission spectra of (**1b**) (10^{-5}M) did not show any alteration in 10^{-3}M protein or DNA (molarity based on average molecular weight amino acid or nucleotide) in comparison with the spectra recorded in demineralized water. No quenching was found in 10^{-3}M of adenosine or thymidine. The amino acids phenylalanine, cysteine, or serine did not quench either. However, tryptophan quenches fluorescence of (**1b**) as does indole. Quenching fits a Stern–Volmer plot and we found k_{SV}(tryptophan) 96 and k_{SV}(indole) 76.

Recording spectra before and after degassing a solution of (**1b**) did not result in spectral changes. Apparently, quenching by oxygen does not play a significant role.

From these results and in line with quenching by tertiary amines in aprotic solvents it can be concluded that pyridopurines in the excited state are electron acceptors and only quenched by fairly strong electron-donating species.

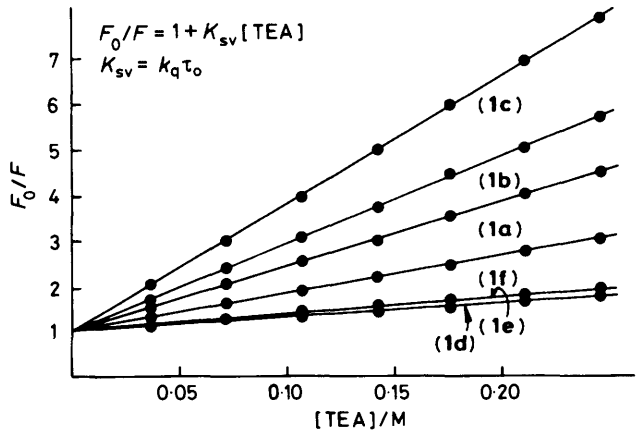


Figure 3. Quenching of fluorescence of (**1a**—**f**) by triethylamine in CH_2Cl_2 at 20 °C

Table 4. Quenching of fluorescence of (**1b**) by triethylamine in different solvents at 20 °C

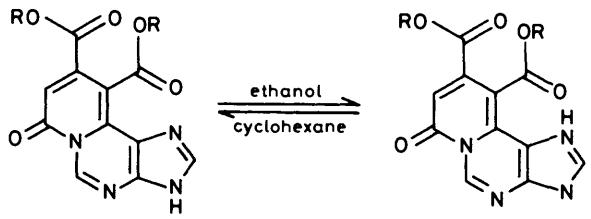
	Cyclohexane	CH_2Cl_2	Acetone	CH_3CN
k_{SV}/l mol^{-1}	17.4	19.5	34.3	42.4

Unprotected Pyridopurine (**2b**).—The unprotected pyridopurine (**2b**) possesses a proton, which can dissociate from the molecule. The fluorescence spectra of most aromatic compounds containing acidic or basic functional groups are very sensitive to the pH and hydrogen-bonding ability of the solvent. As emission of (**1b**) (protected form) shows a red shift of 2 nm on changing the solvent from cyclohexane to ethanol, emission of (**2b**) shifts 26 nm in this direction (Table 6). On the other hand, absorption of (**1b**) is blue shifted, while absorption of (**2b**) shows a red shift of 25 nm. However, the structure of our pyridopurines is rather complex and we cannot draw any conclusions without taking the possibility of intramolecular hydrogen-bonding into consideration (Scheme). Because absorption characteristics of (**2b**) (α) are almost identical to those of (**1b**) in ethanol, we assume intermolecular hydrogen-bonding of ethanol with carbonyl functions of the pyridopurines to be a serious possibility. From shifts in absorption and emission of (**2b**), which are both in the same direction and to the same extent, we conclude that the degree of hydrogen-bonding is the same in ground and excited state.

So far we have discussed hydrogen-bonding in which the proton is delivered by the solvent. Absorption and emission spectra recorded in aprotic solvents of different hydrogen-bond accepting ability did not give significant differences (Table 7). Goldman and Wehry[19] describe comparable results for 8-hydroxyquinoline (capable of intra- and inter-molecular hydrogen-bonding) but found the expected differences for 5-

Table 5. k_{SV}/l mol^{-1}, k_q/l mol^{-1} s^{-1}, and quenching efficiency α for quenching of (**1a**—**f**) by tertiary amines in CH_2Cl_2 at 20 °C

	(1a)			(1b)			(1c)			(1d)			(1e)			(1f)		
	k_{SV}	$10^9 k_q$	α	k_{SV}	$10^9 k_q$	α	k_{SV}	$10^9 k_q$	α	k_{SV}	$10^9 k_q$	α	k_{SV}	$10^9 k_q$	α	k_{SV}	$10^9 k_q$	α
Triethylamine	14.4	2.4	0.13	19.5	2.3	0.18	28.3	4.7	0.25	3.1	0.5	0.03	3.7	1.9	0.10	8.3	4.2	0.21
Ethyldi-isopropylamine	31.3	5.2	0.27	40.5	6.7	0.36	41.5	6.9	0.37	9.8	1.6	0.08	11.7	5.9	0.30	18.0	9.0	0.46
N-Methylmorpholine	2.0	0.3	0.02	4.6	0.8	0.04	9.8	1.6	0.09	0.2			0.2	0.1	0.01	1.2	0.6	0.03
N-Ethylpiperidine	6.9	1.2	0.07	12.0	2.0	0.11	16.5	2.8	0.16	1.4			2.0	1.0	0.05	4.4	2.2	0.12



Scheme.

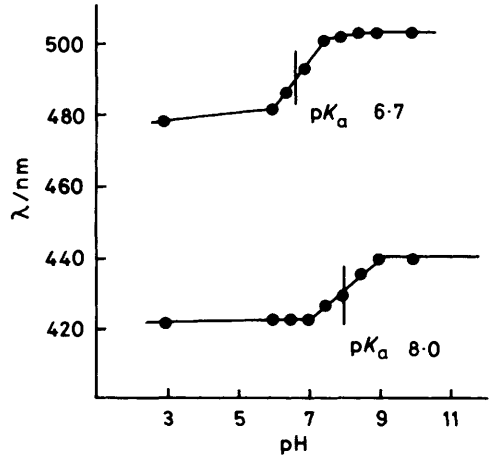


Figure 4. Absorption and emission shifts of (**2b**) at different pH values

hydroxyquinoline (capable only of intermolecular hydrogen-bonding). We therefore consider the data in Table 7 to be additional evidence for intramolecular hydrogen-bonding in (**2b**).

Most proton-transfer reactions in polar solvents are very fast, such that Brønsted acid–base reactions can occur during the lifetime of an excited-singlet state. Generally molecules are more basic or acidic in the excited state. Figure 4 shows shifts in absorption and emission of (**2b**) as a function of pH. There is a difference of 1.3 pK units between ground state and lowest singlet acidity. This is a rather small effect compared with a range of 4—9 pK units observed for various other systems.[20–22]

Table 6. Fluorometric characteristics of (**2b**) in comparison with (**1b**)

(2b)				(1b)			
Ethanol		Cyclohexane		Ethanol		Cyclohexane	
λ_{abs}/nm	log ε	λ_{abs}/nm	log ε	λ_{abs}/nm	log ε	λ_{abs}/nm	log ε
442(sh)	3.9	450(sh)	3.7	448(sh)	4.0	455(sh)	4.4
425	4.0	425(sh)	3.9	423	4.1	430	4.5
336	3.9	400	4.0	345	3.8	355	4.2
332	3.9	340	3.9	330	3.8	330	4.2
λ_{em} 497 nm Q_f 0.68		λ_{em} 471 nm Q_f 0.72		λ_{em} 478 nm Q_f 0.64		λ_{em} 476 nm Q_f 0.55	

Table 7. Absorption and emission of (**2b**) in different aprotic solvents

	λ_{abs}/nm	λ_{em}/nm
Cyclohexane	400	471
CH_2Cl_2	398	471
Acetone	400	472
CH_3CN	399	471

Experimental

Measurements.—Electronic absorption, excitation, and emission spectra were recorded on Cary 17D and Shimadzu RF500 instruments. All samples were diluted to 10^{-5}M. Quantum yields were determined relative to 9,10-diphenylanthracene in cyclohexane and 9-aminoacridine in ethanol,[9,10] using the same excitation wavelengths and by comparison of the total emission intensities. For measurement of fluorescence lifetimes excitation by a nitrogen laser (337 nm) was employed (Lambda Physik EMG101 filled with N_2, FWHM 8 ns). The fluorescence was detected at right angles to the laser beam with an RCA C-31025C GaAs photomultiplier *via* a Zeiss M4QIII monochromator. The transients were digitized by a Biomation 6500 transient digitizer and fed to a Tandy TRS-80 model III microcomputer. The lifetimes were obtained by comparing the decays with convoluted decays with known lifetimes.[23]

Materials.—Spectrograde solvents were obtained from Merck and used without further purification. The pyridopurines (**1a**—**f**) and (**2b**) were synthesized as described previously.[1] Tertiary amines were distilled over CaH_2 and freshly used.

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