Aminolysis of Pyrido[2,1-*i*]purines

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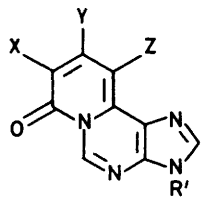
7-Oxo-3-(tetrahydropyran-2-yl)-7*H*-pyrido[2,1-*i*]purines (**1**) are highly fluorescent molecules with absorption and emission in the visible region. Under mild conditions these compounds react with primary and secondary amines and the products (**2**) formed are non-fluorescent. Kinetic studies reveal a mechanism, which resembles ester aminolysis and is completely second order in monoamine concentration. Diazabicyclo-octane accelerates the reaction as does 2-pyridone. In addition kinetics of reactions with diamines are described.

In previous publications we presented the preparation and photophysical properties of a series of pyrido[2,1-*i*]purines (**1**).[1,2] These novel purine analogues exhibit strong fluorescence



(**1**)

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, R' = tetrahydropyranyl

in the visible region and their fluorescence is quenched by tertiary amines in an electron-transfer process obeyed by the Stern–Volmer equation (1).

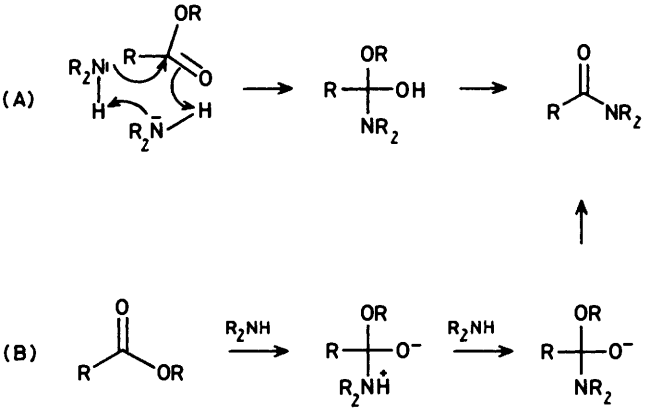
$$F_0/F = 1 + k_{SV}[Q] \qquad (1)$$

In this paper we report on the products and kinetics of aminolysis of pyrido[2,1-*i*]purines with different primary and secondary amines. The progress of aminolysis could be followed very accurately by measuring the decrease in fluorescence intensity.

Ester aminolysis is a very well documented subject and considerable evidence is available for bifunctional and/or general base catalysis (Scheme 1).[3–12] Pseudo-first-order kinetics for ester aminolysis are described in terms of second- and third-order processes in the presence or absence of a catalyst [equation (2)].

$$k' = k_2[A] + k_3[A]^2 + k_4[A][cat.] \qquad (2)$$

A study of the aminolysis of pyrido[2,1-*i*]purines (tetrahydropyranyl protected) may contribute to the understanding of the behaviour of their corresponding ribosides as tracers in biological systems.[13]



Scheme 1.

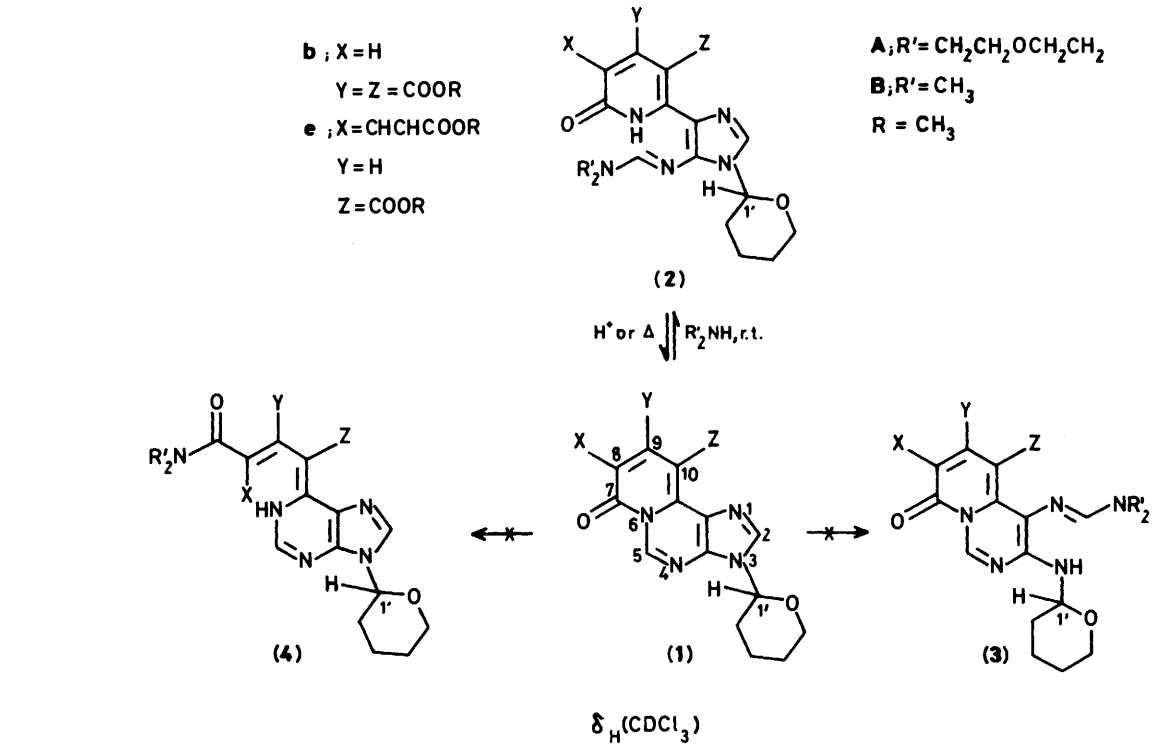
Product Identification.—For product identification the aminolysis of the pyridopurines (**1b** and **e**) with morpholine (**A**) and dimethylamine (**B**) was carried out in acetone at room temperature (Scheme 2). After column purification yields were nearly quantitative, but the products were always to some extent contaminated with starting material (1—5%). Addition of acid to a solution of the product reversed the reaction and resulted in the production of starting materials. Because of thermal lability m.p.s could not be determined and mass spectra were identical with those of (**1b** or **e**).

Pyridopurines contain three different sites which can be attacked by a nucleophile, C(2), C(5), and C(7) (Scheme 2). Attack at C(2) or C(7), leading to compound (**3**) or (**4**), can be excluded on the basis of i.r. and ^{1}H n.m.r. spectral data. I.r. spectra show absorption near 1 660 and 1 550 cm^{-1}, indicating a secondary amide. (2) Attack at C(2), leading to (**3**), leaves the pyridopyrimidine moiety intact; this is not in agreement with an upfield chemical shift of *ca.* 2 p.p.m. for 5-H in the ^{1}H n.m.r. spectrum. An upfield shift of *ca.* 0.9 p.p.m. for 1'-H is not in agreement with an intact purine.

Reaction of (**1b**) with 1,3-diaminopropane at room temperature yielded a product (**5**) (Scheme 3) which lacks one of the downfield protons in ^{1}H n.m.r. The product is stable (m.p. 93—95 °C) and heating (**5**) in the presence of trimethyl orthoformate produces (**1b**) again.

Kinetics.—Pseudo-first-order conditions are created by adding a large excess of amine to the pyridopurine studied (*ca.* 0.25 *versus* 10^{-5}M). By measuring the fluorescence intensity at different time intervals the pseudo-first-order rate constant k' can be determined for a particular amine concentration. Dividing k' by the amine concentration and plotting the result (k'/[A]) against the amine concentration [A] gives second- and third-order rate constants k_2 and k_3. Figure 1 shows a typical plot for the n-butylaminolysis of (**1b**).

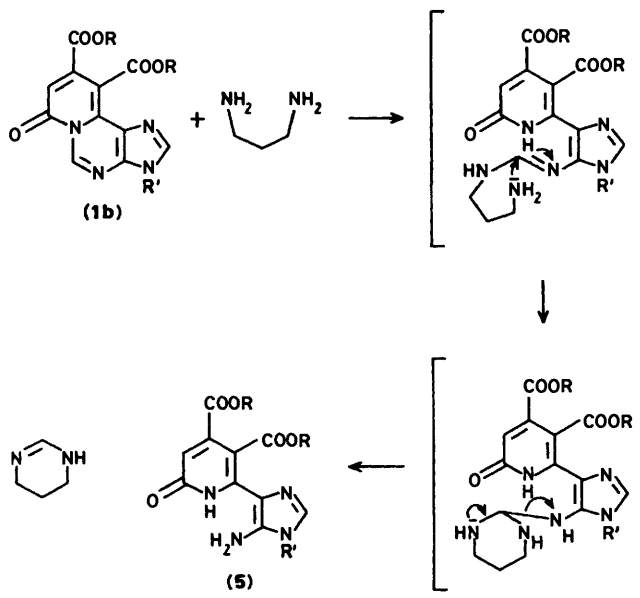
Clearly a second-order term is negligible as it is for the other pyridopurines for CH_3CN and CH_2Cl_2 solutions (Table 1).



$\delta_H(CDCl_3)$

	(1b)	(1e)	(2bA)	(2bB)	(2eA)	(2eB)
low-field protons	7.07	8.22	6.65	6.70	7.76	7.78
	8.15	8.27	7.45	7.45	7.96	7.94
	9.72	9.95	7.67	7.75	8.33	8.31
vinylic		7.04			7.05	7.05
protons		7.78			7.56	7.58
1'-H	5.78	5.88	4.90	4.90	4.90	4.90

Scheme 2.



Scheme 3.

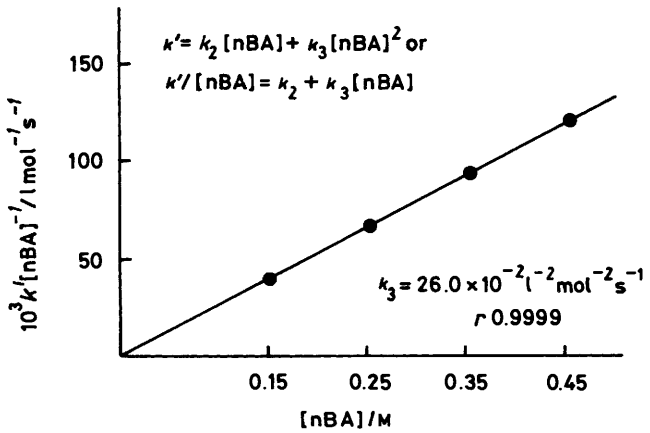


Figure 1. n-Butylaminolysis of (1b) in CH_3CN at 25 °C

From Table 1 it can be concluded that aminolysis of pyridopurines is a process with a polar transition state. Independent of the substituents on pyridopurine, k_3 values in CH_3CN are *ca.* 20 times as high as those in CH_2Cl_2. With the exception of (1d) the character and position of substituents are reflected in the value of k_3. Because the electron-withdrawing properties of an ester function at C(10) are much more effective

Table 1. n-Butylaminolysis of (**1a**—**f**) at 25 °C

	CH_3CN		CH_2Cl_2	
	$10^2\ k_3$/ $l^2\ mol^{-1}\ s^{-1}$	k_{sv}/l mol^{-1}	$10^{-2}\ k_3$/ $l^2\ mol^{-1}\ s^{-1}$	k_{sv}/l mol^{-1}
(**1a**)	2.8	0.1	0.1	
(**1b**)	26	0.2	1.6	
(**1c**)	580	0.5	30	
(**1d**)	4.6		0.2	
(**1e**)	250		12	
(**1f**)	2 270		104	

Table 2. Aminolysis of (**1b**) in CH_3CN at 25 °C

Amine	$10^2\ k_3/l^2\ mol^{-2}\ s^{-1}$	k_{sv}/l mol^{-1}	pK_a
n-Butylamine	26	0.2	10.77
Cyclopentylamine	9	0.4	10.7
Diethylamine		15.0	10.49
Di-isopropylamine		7.9	10.96
Piperidine	9.6	26.4	11.12
Morpholine	0.8	10.3	8.33

morpholine. In this case there is a correlation between reaction rate and basicity. This correlation does not exist for the primary amines n-butylamine and cyclopentylamine. Cyclopentylamine reacts slower, but its basicity is comparable to that of n-butylamine.

Table 2 exemplifies the differences between nucleophilicity (k_3), electron-donating ability (k_{sv}), and basicity (pK_a). Clearly aminolysis is dominated by steric rather than by electronic effects.

In the case of general base catalysis, aminolysis should be accelerated by the addition of a tertiary amine. Triethylamine does not accelerate or initiate the reaction (Table 2). DABCO (diazabicyclo-octane) is a less hindered base and the aminolysis of (**1b**) with n-butylamine or cyclopentylamine in the presence of DABCO as a catalyst fits expression (3) or (4). Plotting

$$k' = k_3[A]^2 + k_4[A][DABCO] \quad (3)$$

$$k'/[A]^2 = k_3 + k_4[DABCO]/[A] \quad (4)$$

$k'/[A]^2$ against [DABCO]/[A] results in the k_3 values already in Table 2 and the k_4 values representing DABCO catalysis (Figure 2). Earlier we stated that aminolysis was governed by

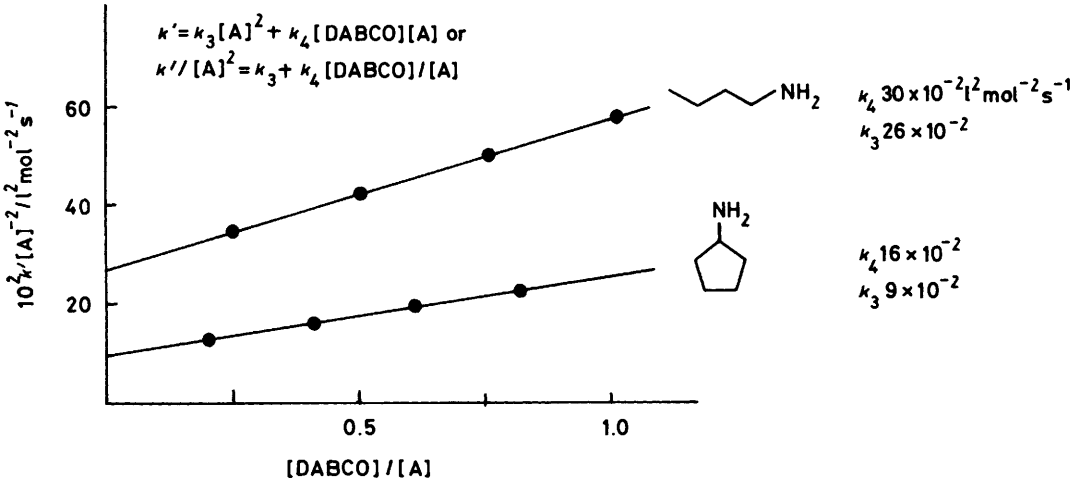


Figure 2. DABCO-catalysed aminolysis of (**1b**) in CH_3CN at 25 °C

than at C(9) [compare (**1a** and **b**)] we expected a k_3 value for (**1d**) close to that of (**1b**). Because of the low electron-donating ability of a phenyl group at C(9), we must assume that the phenyl ring at C(9) forces the ester at C(10) out of plane of the pyridopurine and in this way influences the electron-withdrawing effect of the ester function.

Another phenomenon we encountered is the quenching of fluorescence by electron transfer as described for tertiary amines.[2] Extrapolating the intensities of fluorescence, measured during aminolysis, to t_0, does not result in a value that equals the intensity in the absence of amine. The differences thus observed fit the Stern–Volmer equation (1).

In addition to aminolysis by n-butylamine, the kinetics of reaction (**1b**) with other amines were studied (Table 2). Again we did not find evidence for a second-order process and again k_3 values could be determined with great accuracy from pseudo-first-order reaction rates. Addition of diethylamine or di-isopropylamine to a solution of (**1b**) did not alter the fluorescence intensity after longer periods of time (except for Stern–Volmer quenching). On the other hand k_3 values could be determined for other secondary amines, like piperidine and steric effects. The steric hindrance of the cyclopentyl ring in comparison with the n-butyl chain is not just shown in a lower value for k_3, but it also affects DABCO catalysis (compare k_4 values for n-butyl- and cyclopentyl-amine). The basicity of DABCO (pK_a 8.6[14]) is much lower than the basicity of n-butyl- or cyclopentyl-amine. Nevertheless in both cases k_4 exceeds k_3.

These arguments support general base catalysis, but bifunctional catalysis cannot be excluded. 2-Pyridone functions as a bifunctional catalyst.[15] We have studied the n-butylaminolysis of (**1b**) in the presence of 2-pyridone. Again, from pseudo-first-order rate constants, we derived k_3 26 × 10^{-2} and for 2-pyridone catalysis k_4 17 × 10^{-2}. In comparison Su and Watson[7] found 1 000 × 10^{-2} (k_3 6 × 10^{-2}) for 2-pyridone-catalysed n-butylaminolysis of *p*-nitrophenyl acetate k_4; for DABCO they found k_4 8 × 10^{-2}.

Bifunctional catalysis cannot be excluded for pyridopurine aminolysis, but the contribution is far smaller than in the case of ester aminolysis. On the other hand the effect of DABCO on pyridopurine or ester aminolysis is of the same magnitude.

In aminolysis with diamines the second amine group functions as a base. This will be expressed in a second-order

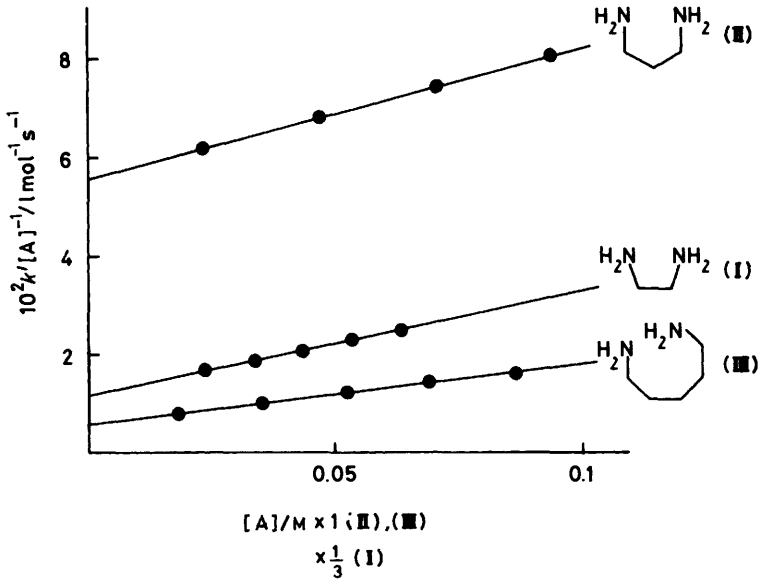


Figure 3. Aminolysis of (**1b**) by diamines in CH_3CN at 25 °C

Table 3.

	$10^{-2}\ k_2$/ l mol^{-1} s^{-1}	$10^{2}\ k_3$/ l^2 mol^{-2} s^{-1}	$k_2\ k_3^{-1}$/ mol l^{-1}
Diaminoethane	11	70	0.16
Diaminopropane	55	263	0.21
Diaminopentane	5	122	0.04

term k_2 (first-order in diamine concentration). We have studied the kinetics of the aminolysis of (**1b**) with diamino-ethane, -propane, and -pentane (Figure 3, Table 3).

For comparison of the second- and third-order process we derived the effective amine concentration at which the contribution of the second-order process is half the observed rate constant and thus equals the contribution of the third-order process [equation (5)]. If $k_2[A] = \frac{1}{2}k'$ or $k_2[A] = k_3[A]^2$ then

$$k' = k_2[A] + k_3[A]^2 \qquad (5)$$

$[A]_{eff.} = k_2/k_3$. From the values of k_2/k_3 we can conclude that intramolecular catalysis is most efficient in the case of diaminopropane.

Experimental

All amines were distilled from CaH_2 and stored under N_2. DABCO was recrystallized from benzene (m.p. 157—158 °C) and 2-pyridone twice from alcohol (m.p. 107—108 °C). CH_3CN was distilled from P_2O_5 and CH_2Cl_2 from $CaCl_2$.

Kinetics were performed in triplicate in stoppered cuvettes placed in the thermostatted (25.0 ± 0.1 °C) cell holder of a Spex Fluorolog instrument. The reactions were initiated by adding the appropriate quantity of amine to a 10^{-5}M solution of pyridopurine. The wavelength of emission and amine concentration were based on the pyridopurine and amine studied. In all runs the amine was in large excess over the pyridopurine, so that pseudo-first-order conditions prevailed. Pseudo-first-order plots of ln *F versus* time were linear in all cases, with a slope taken as k'.

For product analysis reactions were carried out with (**1b** and **e**) and a small excess of morpholine or dimethylamine in acetone at room temperature. After column purification (silica; EtOAc) yields were nearly quantitative, but always contaminated (1—5%) with starting material.

I.r. spectra were recorded on a Perkin-Elmer 257 spectrometer for $CHCl_3$ solutions. ^{1}H N.m.r. spectra were run on a Varian Associates model XL-100 instrument, using Me_4Si as internal standard.

Dimethyl 2-[5-morpholinomethyleneamino-1-(tetrahydropyran-2-yl)imidazol-4-yl]-6-oxopyridine-3,4-dicarboxylate (**2bA**): $\nu_{max.}$($CHCl_3$) 3 350 (NH), 1 730 (ester), 1 660, 1 545 (sec. amide), and 1 630, 1 585 cm^{-1} (pyridone, imidazole); δ_H($CDCl_3$) 3.4—3.7 (8 H, m, morph.), 3.80 (3 H, s, OCH_3), 3.83 (3 H, s, (OCH_3), 4.90 (1 H, m, NCHO), and 6.65, 7.45, 7.67 (3 × 1 H, 3 s, 3 =CH).

(**2bB**): $\nu_{max.}$($CHCl_3$) 3 400 (NH), 1 730 (ester), 1 665, 1 550 (sec. amide), and 1 640, 1 590 cm^{-1} (pyridone, imidazole); δ_H($CDCl_3$) 3.08 (3 H, s, NCH_3), 3.17 (3 H, s, NCH_3), 3.85 (6 H, s, OCH_3), 4.90 (1 H, m, NCHO), and 6.70, 7.45, 7.75 (3 × 1 H, 3 s, 3 =CH).

(**2eA**): $\nu_{max.}$($CHCl_3$) 3 400 (NH), 1 705 (ester), 1 660, 1 550 (sec. amide), and 1 630, 1 590 cm^{-1} (pyridone, imidazole); δ_H($CDCl_3$) 3.4—3.7 (8 H, m, morph.), 3.65 (3 H, s, OCH_3), 3.71 (3 H, s, OCH_3), 4.90 (1 H, m, NCHO), 7.05 (1 H, d, *J* 16 Hz, C=CHC=O), 7.56 (1 H, d, *J* 16 Hz, CH=CC=O), and 7.76, 7.96, 8.33 (3 × 1 H, 3 s, 3 =CH).

(**2eB**): $\nu_{max.}$($CHCl_3$) 3 400 (NH), 1 710 (ester), 1 660, 1 550 (sec. amide), and 1 630, 1 585 cm^{-1} (pyridone, imidazole); δ_H($CDCl_3$) 3.06 (3 H, s, NCH_3), 3.15 (3 H, s, NCH_3), 3.65 (3 H, s, OCH_3), 3.73 (3 H, s, OCH_3), 4.90 (1 H, m, NCHO), 7.05 (1 H, d, *J* 16 Hz, C=CHC=O), 7.58 (1 H, d, *J* 16 Hz, CH=CC=O), and 7.78, 7.94, 8.31 (3 × 1 H, 3 s, 3 =CH).

Reaction of (**1b**) *with Diaminopropane.*—To (**1b**) (1 mmol) in methanol (3 ml) was added 1,3-diaminopropane (2 mmol) in methanol (3 ml) at room temperature. After a short period the solid material completely dissolved and fluorescence disappeared. After addition of water the mixture was neutralized with acetic acid. Extraction with EtOAc followed by column purification (silica; EtOAc) yielded (**5**) (357 mg, 95%), m.p. 93—95 °C; $\nu_{max.}$($CHCl_3$) 3 450, 3 360 (NH), 1 735 (ester), 1 665, 1 545 (sec. amide), and 1 610 cm^{-1} (aromatic); δ_H([$^2H_6$]DMSO) 3.72 (3 H, s, OCH_3), 3.82 (3 H, s, OCH_3), 5.22 (1 H, dd, *J* 9, 2 Hz,

NCHO), 6.42 (NH, disappears with D_2O), and 6.55, 7.34 (2 × 1 H, 2 s, 2 =CH). Compound (**5**) (50 mg, 0.13 mmol) is dissolved in dimethoxyethane (1 ml). To this solution one drop of trimethyl orthoformate was added. After heating at 80 °C for a short period, the starting material disappeared. The solvent was evaporated and the residue purified over a short column (silica; EtOAc), yielding (**1b**) (35 mg, 70%).

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