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SYNTHESIS OF NEW HETEROCYCLIC RING SYSTEMS VIA NUCLEOPHILIC SUBSTITUTION OF PYRIMIDO[4,5-d]PYRIDAZINES

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Abstract - Hydroxy groups at positions C^5 and C^8 were replaced in 2-phenyl- and 2-aminopyrimido[4,5-d]pyridazines by better leaving groups (Cl, $OSiMe_3$ and SMe, $OSiMe_3$ respectively), and the 5,8-disubstituted compounds were substituted by aminoalkanols. The 5- and 8--mono(ω-hydroxyalkylamino) derivatives obtained in regioselective reactions were cyclized into imidazo[1,2-b]pyrimido[5,4-d]pyridazine, imidazo[1,2-b]pyrimido[4,5-d]pyridazine, dipyrimido[1,2-b:5',4'-d]pyridazine and dipyrimido[1,2-b:4',5'-d]pyridazine derivatives containing new heterocyclic ring systems. Kinetic measurements and MNDO calculations showed that C^8 site at the pyridazine ring is more reactive than C^5, and aminolysis follows by usual two-step S_NAr mechanism.

The aim of the present work was to investigate the reactions of pyrimido[4,5-d]pyridazine derivatives leading to new tricyclic compounds in which the pyrimido-pyridazine skeleton is fused with partially saturated imidazole or pyrimidine ring. Such compounds seem to be useful for testing in cancer and virus therapies as their structure and properties resemble those of certain purines and pteridines.

Starting from 2-phenyl-5,8-dihydroxypyrimido[4,5-d]pyridazine[1] and from the analogous 2-amino derivative[2], our strategy was te replace the OH substituents by more reactive leaving groups (e.g. with Cl, SMe or $OSiMe_3$) which allow nucleophilic replacements at the pyridazine ring. Using aminoalkanol reagents, an ω-hydroxyalkylamino group was introduced into

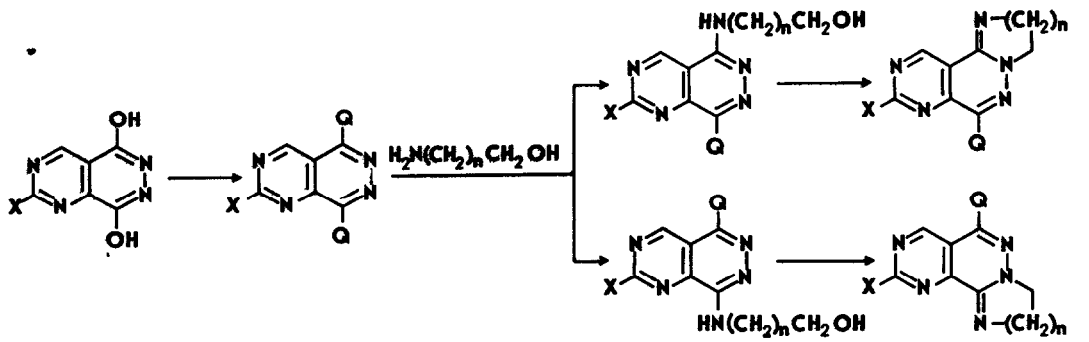


Fig.1. General scheme of reactions investigated.

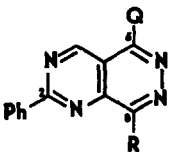
the positions 5 or 8 in regioselective reactions, with a subsequent submission of the obtained intermediates to cyclization (Fig. 1).

Although several papers have dealt with the synthesis of 2-phenyl- and 2-aminopyrimido[4,5-d]pyridazine derivatives[1-3], nucleophilic displacements occurring on the former type compounds were only mentioned by Yurugi et al.[1,4,5]. There has been, however, no experience for the conversion of 2-amino derivatives, which may be ascribed either to preparative difficulties in obtaining suitable substrates, or to the very poor reactivity of pyridazine derivatives toward nucleophiles (cf. Ref. 6). For ring closures shown in Fig. 1 some analogy can be found in the literature. The formation of a dihydroimidazole ring via cyclization of ω-hydroxyalkylamino side chain was first reported by Castle et al.[7] This type of reaction was discussed thoroughly by Körmendy et al. who built up tricyclic systems with fused dihydroimidazole and dihydropyrimidine rings from phthalazine and pyrido-pyridazine derivatives[8].

RESULTS AND DISCUSSION

From 2-phenyl- and 2-amino-5,8-dihydroxypyrimido[4,5-d]pyridazine (1 and 14) were synthesized reactive derivatives (2, 4, 16 and 17); from the latter hydroxyalkylamino compounds were obtained (5-12 and 18-21); transformed subsequently into tricyclic compounds with new heterocyclic ring systems, including 2,3-dihydroimidazo[1,2-b]pyrimido[5,4-d]pyridazines (22-23), 2,3-dihydroimidazo[1,2-b]pyrimido[4,5-d]pyridazines (24-27), a 2*H*-3,4-dihydrodipyrimido[1,2-b:5',4'-d]pyridazine (28) and 2*H*-3,4-dihydrodipyrimido[1,2-b:4',5'-d]pyridazines (29-31). The structure of all compounds discussed are shown in Fig. 2. Ring numbering follow the IUPAC rules; capital letters in circle point to the different structural positions of methylene- and methin-hydrogens playing a part in the ^{1}H NMR studies. In every case structure and purity were checked by elemental (C, H, N) analysis and ^{1}H NMR spectroscopy. Data for new compounds are listed in Tables 1 and 2. Attention may be drawn to the relatively large coupling constants (J_{BC}) of methylene hydrogens which are characteristic of the dihydroimidazole ring. Constitutional problems associated with regioselective aminations and ring closures were solved by dNOE method.

Activation by OH → Cl change. Using the method in Ref. 1 we prepared 2-phenyl-5,8-dichloropyrimido[4,5-d]pyridazine (2) from the corresponding dihydroxy compound (1) without difficulty. In a similar way, we also tried to convert the analogous 2-amino-5,8-dihydroxy derivative (14 → 15) by different chlorinating agents ($POCl_3$-PCl_5, $POCl_3$-DMF, $POCl_3$-pyridine). However, we obtained a mixture of 8-10 compounds (as shown by TLC) probably formed by partial chlorination and decomposition of the starting material. To explain this phenomenon Yurugi et al. suggested[1] that the 2-phenyl group in compound 1 stabilizes the 5,8-dihydroxy form essential for chlorination. With other 2-substituents (e.g. with 2-amino group) this stabilizing effect disappears and one of the monooxo-monohydroxy tautomeric forms (14B and 14C in Fig. 3) becomes the most stable. Some objections may, however, be raised against this argument. Tautomerism is an equilibrium process and the various tautomers can easily



Q; R

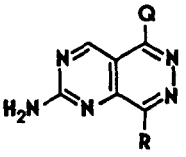
1[a] bis-OH
2[a] bis-Cl
3[b] bis-SMe
4[c] bis-$OSiMe_3$

Q; R

5[d] $NH(CH_2)_2OH$; Cl
6[d] Cl; $NH(CH_2)_2OH$
7 $NHCH_2CHMeOH$; Cl
8 Cl; $NHCH_2CHMeOH$

Q; R

9 $NH(CH_2)_3OH$; Cl
10 Cl; $NH(CH_2)_3OH$
11 OH; $NH(CH_2)_2OH$
12 OH; $NH(CH_2)_3OH$
13[c] bis-$NH(CH_2)_3OH$

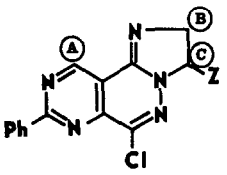


Q; R

14[e] bis-OH
15[b] bis-Cl
16 bis-SMe
17[c] bis-$OSiMe_3$

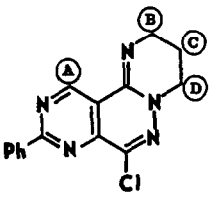
Q; R

18 $NH(CH_2)_2OH$; SMe
19 OH; $NH(CH_2)_2OH$
20 OH; $NH(CH_2)_3OH$
21 bis-$NH(CH_2)_2OH$



Z

22 H
23 Me



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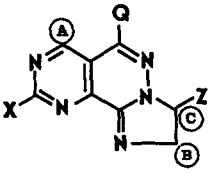
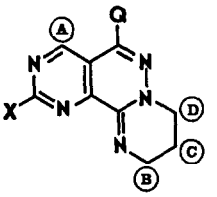


	X	Q	Z
24	Ph	Cl	H
25	Ph	Cl	Me
26	Ph	OH	H
27	NH_2	OH	H



	X	Q
29	Ph	Cl
30	Ph	OH
31	NH_2	OH

Fig. 2. Intermediates and products in synthesis of new tricyclic heteroaromatic compounds.

(a) Ref. 1. (b) Compounds 3 and 15 were not prepared. (c) Compounds 4, 13 and 17 were not isolated. (d) Ref. 4. (e) Ref. 2.

Table 1. Physical data for bicyclic compounds

	m.p. °C	Analysis						4-H[a] s (1H)	dNOE %
		calc			found				
		C	H	N	C	H	N		
7	208-10	57.1	4.5	22.2	57.2	4.5	22.3	10.02	-
8	205-6	57.1	4.5	22.2	57.1	4.5	22.1	9.64	-
9	182-3	57.1	4.5	22.2	57.0	4.7	21.8	10.00	11[b]
10	188-9	57.1	4.5	22.2	56.8	4.4	22.0	9.58	4[c]
11	283-5	59.4	4.6	24.7	59.1	4.3	24.5	9.65	0[b]
12	232-3	60.6	5.1	23.6	60.4	5.0	23.6	9.67	3[c]
16	278-9	40.2	3.8	29.3	40.4	3.3	29.3	9.17	0[b]
18	237-9	42.9	5.0	33.3	43.3	4.9	33.3	9.38	8[b]
19	285-7	43.2	4.5	37.8	43.0	4.7	37.4	9.01	0[b]
20	265-7	45.8	5.1	35.6	45.5	5.2	35.8	9.08	0[b]
21	202-3	45.3	5.7	36.7	45.0	5.7	36.3	9.28	-

(a) Characteristic ^1H NMR shift in DMSO D_6 (ppm). (b) DNOE between N-H of hydroxyalkylamino side chain and C^4-H. (c) DNOE between N-H of hydroxyalkylamino side chain and ortho hydrogens of phenyl group.

Table 2. Physical data for tricyclic compounds

	m.p.	Analysis calc./found C	H	N	A-H[a] s(1H)	B-H,C-H 2xt(2x2 H)	J_{BC}	B-H,D-H 2xt(2x2 H)	C-H m(2H)	NH_2 s(2H)	Ph m(2H)	m(3H)
22	226-7	59.3	3.6	24.7	9.41	4.15-4.39[b]		-	-	-	8.51-8.62	7.49-7.60
		59.3	3.4	24.9								
23	169-71	60.5	4.0	23.5	9.38	c	10	-	-	-	8.45-8.55	7.60-7.70
		60.2	4.1	22.8								
24	216-8	59.3	3.5	24.7	9.20	4.11-4.25[b]		-	-	-	8.51-8.62	7.49-7.60
		58.8	3.4	24.4								
25	194-6	60.5	4.1	23.5	9.26	d	10	-	-	-	8.45-8.50	7.55-7.70
		60.3	4.2	23.8								
26[e]	265-8	53.5	3.9	22.3	10.05	4.78,4.48	11	-	-	-	8.40-8.51	7.46-7.81
		53.5	4.2	22.4								
27	> 320	47.1	4.0	41.2	9.50	4.68,4.36	11	-	-	8.30	-	
		47.1	4.2	41.1								
28	205-6	60.5	4.1	23.5	9.53	-	-	4.01,3.59	2.08	-	8.54-8.58	7.50-7.83
		60.7	4.1	23.6								
29	205-6	60.5	4.1	23.5	9.19	-	-	4.06,3.80	2.11	-	8.61-8.65	7.49-7.53
		60.7	4.2	23.6								
30	> 320	64.7	4.7	25.2	9.92	-	-	4.40,3.79	2.49	-	8.49-8.29	7.45-7.71
		64.9	4.8	25.5								
31	> 320	49.5	4.6	38.5	9.41	-	-	4.21,3.70	2.31	8.32	-	
		49.3	4.9	38.5								

(a) Detailed ^{1}H NMR analysis (in TFA); for the hydrogen positions see Fig.2. (b) Unresolved multiplet. (c) B-H 4.16, 3.54 2xdd(2x1H); C-H 4.40 m(1H); $-CH_3$ 1.38 d(3H). (d) B-H 4.20, 3.58 2xdd(2x1H); C-H 4.40 m(1H); $-CH_3$ 1.38 d(3H). (e) Sulphate salt of 26 ($26\frac{1}{2}H_2SO_4$).

convert into each other during the chlorination process (cf. for example the conversion of barbituric acid into trichloropyrimidine). Furthermore, the starting material has completely been converted (although not into the compound expected), suggesting that the 2-amino derivative (14) can also be chlorinated, but this process is accompanied or followed by decomposition.

To exclude the role of tautomeric distributions in controlling the different chlorination reactions, we performed MNDO calculations for the tautomeric forms of compound 14 (see 14A-14D in Fig. 3). Complete geometry optimization was carried out in each cases to obtain reliable results for energy and electronic properties. Relative energy values computed for the different tautomeric forms, 14A 0.0, 14B 39.1, 14C 44.7 and 14D 91.7 kJ/mol unequivocally indicate that the 5,8-dihydroxy tautomer is (at least in matrix isolated environment) the most stable also in the case of 2-amino derivatives.

Net atomic charges calculated for the different tautomers of compound 14 (Table 3) point to extreme separation of charges in the fused ring skeleton. Relatively large positive charges on the pyrimidine ring (at C^2 and C^4) suggest that the nucleophilic attack may occur not only at atoms C^5 and C^8 bearing hydroxyl substituents, but on the pyrimidine ring as well.

Table 3. Net atomic charges calculated for the tautomeric forms of compound 14

atom[a]	14A	14B	14C	14D
N^1	-0.271	-0.275	-0.258	-0.266
C^2	0.267	0.282	0.266	0.285
N^3	-0.294	-0.310	-0.297	-0.306
C^4	0.195	0.205	0.188	0.204
C^{4a}	-0.219	-0.289	-0.202	-0.277
C^5	0.182	0.394	0.164	0.374
N^6	-0.181	-0.275	-0.157	-0.242
N^7	-0.146	-0.143	-0.294	-0.247
C^8	0.129	0.130	0.371	0.341
C^{8a}	0.134	0.182	0.103	0.123
N^9	-0.245	-0.250	-0.246	-0.251
H^{10}	0.171	0.177	0.173	0.182
H^{11}	0.174	0.179	0.176	0.183
H^{12}	0.109	0.111	0.110	0.114
O^{13}	-0.237	-0.341	-0.244	-0.321
O^{14}	-0.210	-0.214	-0.302	-0.256
H^{15}	0.226	0.214	0.219	0.179
H^{16}	0.217	0.220	0.229	0.178

(a) For the numbering of atoms see Fig. 3.

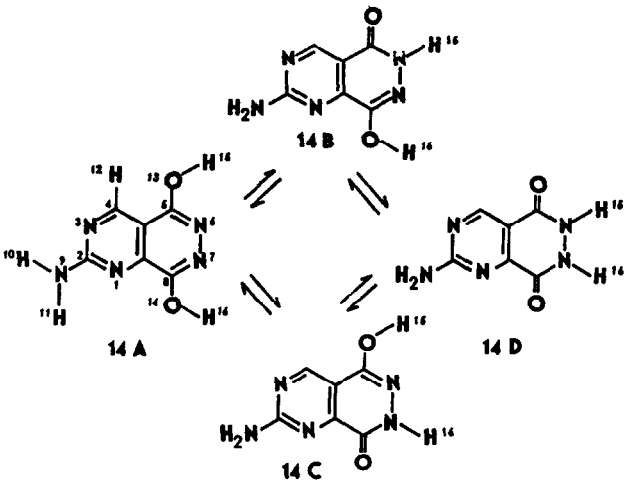


Fig. 3. Tautomeric forms of compound 14

Activation by OH → SMe change. – OH → Cl and OH → OTs displacements could not be realized because the 2-amino derivative 14 exhibited very low solubility, therefore we investigated the OH → SH → SMe route in order to activate the substrate[9] for nucleophilic substitution on the pyridazine ring. Since the Lawesson reagent was ineffective in our case, we used phosphorus pentasulphide in hot pyridine. We obtained the 5,8-dimercapto compound which partially decomposed, however, during the purification process. Thus the crude product was directly methylated with methyl iodide to give the 5,8-bis(methylthio) derivative 16 in rather poor yield.

Amination of 2-phenyl-5,8-dichloropyrimido[4,5-d]pyridazine. – It has been shown earlier[4], that the dichloro compound 2 reacts with 2-aminoethanol to give a mixture of monoamino-substituted derivatives (5 and 6). In a similar way, we obtained analogous compounds with 1-amino-2-propanol (2 → 7 and 8) and with 3-amino-1-propanol (2 → 9 and 10). The regioselectivity in aminations is rather small, but nucleophilic attack at C^8 seems to be more preferred than at C^5. The separation of isomers is easier for 5/6 and 7/8 pairs than for 9/10. If the amination were carried out under usual mild conditions, the formation of 5,8-disubstituted compounds could not be detected. This may be ascribed to the strong electron-releasing effect of the hydroxyalkylamino group joined to the pyridazine ring in the first reaction step, deactivating the possible site of the second nucleophilic attack (cf. Ref. 5).

S_NAr mechanism of amination. - The reaction between 2-phenyl-5,8-dichloropyrimido-[4,5-d]pyridazine (2) and 3-amino-1-propanol was studied by kinetic methods; in interpretations the results of our earlier MNDO calculations[10] were also used. The question was how the four nitrogen atoms located asymmetrically in the pyrimido-pyridazine ring skeleton and the entering hydroxyalkylamino group control the regioselectivity and the rates of chlorine → hydroxyalkylamino displacements. Under pseudo-first-order conditions, i.e. when applying a large excess of 3-amino-1-propanol, the progress of reactions (shown in Fig. 4) was followed by HPLC. Kinetic data are listed in Table 4 (intervals refer to 95% confidence limit). The reaction showed first-order kinetics for both the heterocyclic substrate and the 3-amino-1-propanol reagent. The latter was established by measuring the rate of reaction with two different starting concentrations of amine at 332.9 K. The pseudo-first-order rate constants (k') were found different, while the second-order rate constants (k") were equal within the confidence limits (see Table 4). Since k_{1a}" and k_{1b}" considerably differs from both k_2" and k_3", we were not able to measure the corresponding reaction rates at the same temperature. For comparison k_{1a}" and k_{1b}" was extrapolated to T = 367.2 K by means of activation parameters ($\Delta E^{\neq}$ and $\Delta S^{\neq}$) (see also the note in Table 5).

All these facts suggest that the reaction of chloropyridazines and aminoalkanols follows the usual S_NAr mechanism. The second-order rate constants which are independent of the concentration of aminoalkanol reagent, and the negative values obtained for the entropy of activation (Table 5) both support the assumption that the formation of a neutral Meisenheimer complex is rate-determining (cf. Ref. 11).

Activation energy values in Table 5 obtained from kinetic experiments and MNDO calculations[10] agree well, showing that a properly chosen theoretical model can also give reliable results even for the solution of such a "laboratory smell" problem.

Table 4. Pseudo-first-order (k') and second-order (k") rate constants for the reaction of compound 2 with 3-amino-1-propanol[a]

T K	[s][b] 10^4 mol/l	[ap][d] 10^2 mol/l	k_{1a}/k_{1b}	k_1'	k_{1a}'	k_{1b}'	k_2'	k_3'	k_1"	k_{1a}"	k_{1b}"	k_2"	k_3"
						10^5 1/s						10^5 1/mol/s	
332.9	4.33	4.03	1.33	16.5±0.4	9.39	7.08	-	-	409±10	233	176	-	-
332.9	4.40	6.92	1.34	29.9±0.9	16.98	12.64	-	-	429±12	246	183	-	-
328.1	5.36	6.63	1.38	16.8±1.2	9.77	7.07	-	-	254±18	147	107	-	-
323.3	4.01	7.37	1.41	12.6±0.7	7.37	5.25	-	-	171±9	100	71	-	-
318.2	5.23	7.66	1.37	9.8±0.5	5.72	4.16	-	-	128±12	74	54	-	-
367.2	55[c]	e	-	-	-	-	4.15 ±0.2	4.35 ±0.2	4425[f]	2412[f]	2013[f]	0.309 ±0.01	0.327 ±0.01

(a) Reaction paths are depicted in Figure 4. The pseudo-first-order rate constants were obtained either by measuring the appearance of products (for reactions 1a and 1b) or by detecting the decrease of substrate concentration (for reactions 2 and 3) (b) Concentration of 2-phenyl-5,8-dichloropyrimido[4.5-d]pyridazine. (c) Concentration of substrate 10 or 9 (for reactions 2 or 3). (d) Concentration of 3-amino-1-propanol. (e) Solvent. (f) Extrapolated by means of activation parameters.

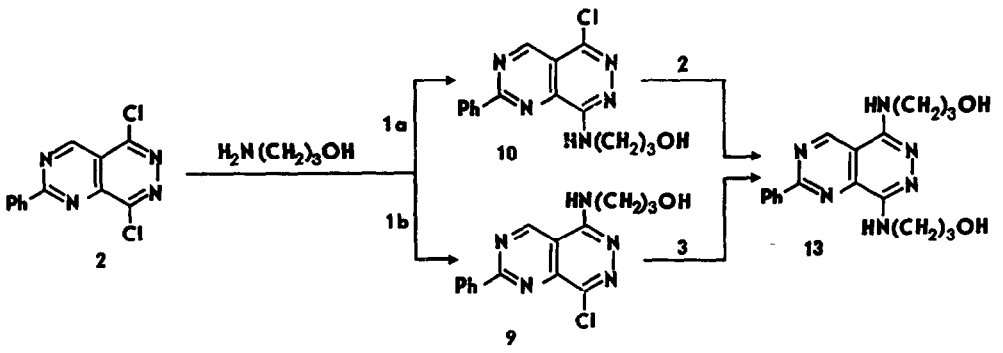


Fig. 4. Reactions examined by kinetic measurements

Table 5. $\Delta S^{\neq}$ (J/mol/K) and $\Delta E_i^{\neq}$ (kJ/mol) data for the reaction of compound 2 with 3-amino-1-propanol

path[a] (i)	$\Delta S_i^{\neq}$ exp.	$\Delta E_i^{\neq b}$ exp.	$\Delta E_i^{\neq b}$ calc.[c]	$\Delta E_i^{\neq} - \Delta E_{1a}^{\neq b}$ exp.	$\Delta E_i^{\neq} - \Delta E_{1a}^{\neq b}$ calc.[c]
1a	-95±20	67.2±6.5	102.0	0	0
1b	-91±26	69.0±8.2	105.4	1.8	3.4
2	-	-	118.1	27.4[d]	16.1
3	-	-	127.8	27.2[d]	25.8

(a) See Fig. 4. (b) Supposing that $\Delta H^{\neq} \approx \Delta E^{\neq}$. (c) Taken from Ref. 10. (d) Obtained by assuming that $\Delta E_i^{\neq} - \Delta E_{1a}^{\neq} \approx RT\ln(k_{1a}/k_i)$, where k_{1a} and k_i are rate constants measured at the same temperature, meaning that the difference between entropies of activation is close to zero. Several papers dealing with these types of reactions support this assumption.[12]

Amination of 2-amino-5,8-bis(methylthio)pyrimido[4,5-d]pyridazine. - The bis(methylthio) compound 16 proved to be rather resistant toward aminoalkanol nucleophiles. Using 2-aminoethanol solvent, the reaction occurred only at 140° yielding a number of different compounds (as shown by TLC) from which only the 2-amino-5-(2-hydroxyethylamino)-8-(methylthio) derivative could be isolated in a very poor yield. That is why a similar conversion of the 2-phenyl-5,8-dihydroxy compound (1) via 5,8-bis(methylthio) derivative (3) was not carried out.

Activation by OH → $OSiMe_3$ change; regioselective "one pot" amination. – We found that both starting materials, 2-pheny- and 2-amino-5,8-dihydroxypyrimido[4,5-d]pyridazine (1 and 14) were best activated for nucleophilic substitution when hydroxyl groups were converted into trimethylsilyloxy groups, using the hexamethyl-disilazan (HMDSA)[13] reagent. ^{1}H NMR spectra exhibiting 18 protons at 0.45 ppm indicated that silylation yielded the 5,8-bis(trimethylsilyloxy) derivatives (4 and 17) without affecting the nitrogen atoms (e.g. in the 2--amino group). For preparations it was favorable that the incorporation of six methyl groups remarkably enhanced the solubility of the substrates.

The 5,8-bis(trimethylsilyloxy) intermediates were allowed to react directly with an excess of 2-aminoethanol and 3-amino-1-propanol. The aminolysis proceeded rather slowly, but side-reactions could be suppressed by diluting the reaction mixture with collidine. Surprisingly, the conversion proved completely regioselective. Only the 5-hydroxy-8-(hydroxyalkylamino) derivatives (11/12 and 19/20) were formed and prepared in good yield, showing that the C^8 atom in the pyrimido[4,5-d]pyridazine skeleton is more reactive than the C^5. These findings are in full accordance with the results of kinetic measurements shown in Table 4.

The progress of reactions of bis(trimethylsilyloxy) derivatives 4 and 17 with 3-amino--1-propanol was also followed by TLC. Qualitative results showed that the rate of conversion was practically independent of the nature of C^2-substituent.

When collidine was omitted and the reaction was conducted for a longer time at higher temperature, the 5,8-bis(2-hydroxyethylamino) derivative 21 accompanied by a large amount of other products was formed from the bis(trimethylsilyloxy) derivative 17 and 2-aminoethanol. The compound 21 could be purified only by chromatography.

Structure determination for 2-(X)-5(Q)-8(R)-pyrimido[4,5-d]pyridazine isomers. – The structure of 2-phenyl compounds 9-12, and 2-amino compounds 18-20 were determined by dNOE analysis (Table 1, Fig. 5). The irradiation of C^4-H hydrogen in compounds 9 and 18 generated 11 and 8% dNOE, respectively, on the amino-hydrogen of the neighbouring (3-hydroxypropyl)amino moiety, so this effect has been accepted as a proof for the C^5-$NH(CH_2)_3OH$ arrangement. On the other hand, 4 and 3% dNOE was observed on the ortho-hydrogen of 2-phenyl group in compounds 10 and 12, respectively, when the amino-hydrogen in the proximal (3-hydroxypropyl)amino group was irradiated. Thus the latter effect may be considered as an evidence for the C^8-$NH(CH_2)_3OH$ alternative. With compounds 11, 19 and 20 the assignation of structure was based on the close analogy in preparation of these compounds and compound 12, where 2-aminoethanol and 3-amino-1-propanol was used as reagent.

As it was expected from the 8-(hydroxyalkyl)amino structure, no dNOE was observed when C^4-H was irradiated in compounds 11, 19 and 20.

Cyclization of 2-phenyl-5/8-mono(hydroxyalkylamino) derivatives. – Boiling of the monochloro-mono(hydroxyalkylamino) derivatives 5/6 and 9/10 in thionyl chloride afforded the corresponding isomeric tricyclic compounds 22/24 and 28/29, respectively. The first step in

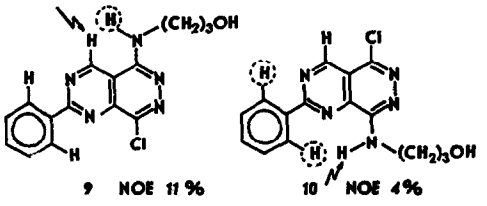


Fig. 5. DNOE analysis of 5- and 8-hydroxyalkylamino derivatives
Selected ^{1}H NMR data for 9 and 10 (DMSO D_6; ppm from TMS): 4.62 (t, OH), 8.28 (t, NH), 8.68 (d, Ar) and 4.72 (t, OH), 8.23 (t, NH), 8.55 (d, Ar), respectively.

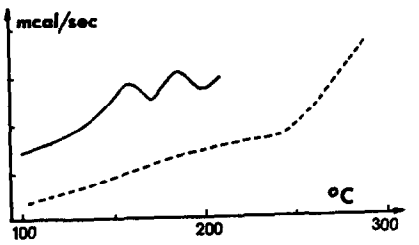


Fig. 6. DSC curves of cyclization of compounds 7 and 8

this process is obviously the replacement of the ω-hydroxy group by chlorine which is then followed by ring closure. The latter step involves a nucleophilic attack of a pyridazine-nitrogen atom at the side chain ω-carbon yielding the hydrochloride of the tricyclic product (cf. Ref. 8). The cyclization leading to six-membered dihydropyrimidine ring (9/10 → 28/29) proved to be considerably faster than the formation of the five-membered dihydroimidazole ring (5/6 → 22/24). For the cyclization of isomeric models with secondary hydroxyl group in side chain (7 and 8) there can be found no analogy in the literature. We observed that these compounds did not cyclize in boiling thionyl chloride, though the hydroxyl group was replaced by chlorine. Ring closure only occurred on heating at 190° (7/8 → 23/25). The DSC curves shown in Fig. 6 support this conclusion. The first-heating curve (solid line) of the sample containing the ω-chloro substituted compound exhibits two exothermic peaks in the range of 150-190°. The other curve (broken line) was obtained when the sample was cooled and heated again. The different shapes of the curves indicate that irreversible conversion occurred during the first heating. For cyclization of monohydroxy-mono(hydroxyalkylamino) derivatives (11 → 26, 12 → 30) heating (100°C) in concentrated sulfuric acid was used.

Cyclization of 2-amino-8-mono(hydroxyalkylamino) derivatives. - All of the tricyclic compounds with a phenyl group are insoluble in water and also exhibit very low solubility in other usual solvents. For this reason these compounds are not suitable for any biological testing. If the 2-phenyl group is replaced by a 2-amino group an increase in solubility can be expected. Therefore we tried to cyclize the monohydroxy-mono(hydroxyalkylamino) derivatives 19 and 20 by the methods mentioned in the previous section. When heated in thionyl chloride (or with PCl_3, PBr_3, HCl, HBr, etc.) these compounds undergo decomposition. These findings provide a further assumption that the 2-amino group is responsible for the very low stability of pyrimido-pyridazine ring toward nucleophiles. On the other hand, ring closure occurred when the substrates were heated at 100°C in concentrated sulfuric acid (19 → 27 and 20 → 31). The tricyclic compounds obtained are soluble in water, and exhibit particularly high solubility in acidic media.

EXPERIMENTAL PART

2-Phenyl-5-(hydroxyalkylamino)-8-chloropyrimido[4,5-d]pyridazines (5, 7, 9) *and 2-phenyl-5-chloro-8-(hydroxyalkylamino)pyrimido[4,5-d]pyridazine* (6, 8, 10) - 15 mmol of 2-phenyl-5,8-dichloropyrimido[4,5-d]pyridazine (2) was suspended in ethanol (150 ml). Aminoalkanol (30 mmol) in ethanol (10 ml) was added to the boiling suspension over 10 min. The mixture was boiled for 3 hr, then cooled. The crystals were filtered off and recrystallized from ethanol, giving the mixtures of monosubstituted isomers (90%).

The mixture of compounds 5 and 6 (4.5 g) was separated by extraction using a Soxhlet extractor and chloroform as solvent. After extraction (72 hr) the remaining solid was crystallized from ethanol yielding 5 (1.47 g, 32%). The soln was evaporated, the residue was crystallized from ethanol, giving 6 (1.53 g, 34%).

For the separation of compounds 7 and 8 fractional crystallization was used. The mixture of isomers (3.0 g) was suspended in chloroform and boiled for 10 min, then slowly cooled to 40°, and the crystals were filtered off. Repeating the procedure pure 7 was obtained (1.3 g, 43%). The solid product obtained from the filtrates was crystallized from ethanol to yield pure 8 (1.0 g, 30%).

The mixture of compounds 9 and 10 (2.8 g) was separated by vacuum chromatography using solid sample and benzene-acetone (5:1) eluent system. The first fractions gave pure 9 (0.8 g, 28%); pure 10 (0.3 g, 11%) was obtained from the subsequent fractions.

2-Amino-5,8-bis(methylthio)pyrimido[4,5-d]pyridazine (16) - Phosphorus pentasulphide (4.7 g) was dissolved in abs. pyridine (140 ml). 2.0 g of 2-amino-5,8-dihydroxypyrimido[4,5-d]pyridazine (14) was added to the hot (100°) mixture while stirred vigorously. Stirring was continued at 100° for 3 hr. The reddish-brown mixture was evaporated and cold water (100 ml) was added to the solid residue. The suspension was allowed to stand at room temp for 24 hr, then it was boiled for 1 hr. After cooling the light-brown residue was filtered off and dissolved in the mixture of 4% NaOH aq (40 ml) and pyridine (10 ml). The cooled soln was acidified to pH 4.0 by HCl aq. The brown precipitate was separated and the obtained red soln was acidified to pH 2.0. The yellow precipitate was filtered off and dried, yielding crude 2-amino-5,8-dimercaptopyrimido[4,5-d]pyridazine, which was methylated without further purification.

The 5,8-dimercapto derivative (0.5 g) was dissolved in the mixture of 4% NaOH aq (10 ml) and ethanol (10 ml). To the red soln methyl iodide (0.3 ml) was added and the mixture was stirred for 5 hr at room temp; yellow crystals separated from the mixture. The crude product was filtered off and recrystallized from pyridine-water (1:1), yielding pure 16 (0.3 g, 11%).

2-Amino-5-(2-hydroxyethylamino)-8-methylthiopyrimido[4,5-d]pyridazine (18) - The bis(methylthio) derivative 16 (0.5 g) was heated in 2-aminoethanol (50 ml) to 120° for 5 hr. The solvent was evaporated in vacuum and the compound 18 was isolated from residue by vacuum chromatography (0.08 g, 14%).

2-Phenyl- and 2-amino-5-hydroxy-8-(hydroxyalkylamino)pyrimido[4,5-d]pyridazine (11/12 *and 19/20*) - The mixture of 2-phenyl- or 2-amino-5,8-dihydroxypyrimido[4,5-d]pyridazine (1 g of 1 or 14), p-toluenesulphonic acid hydrate (0.03 g), HMDSA (8 ml), collidine (5 ml) and 2-aminoethanol (1.7 ml) or 3-amino-1-propanol (2.1 ml) was heated to 120° under vigorous stirring. After the mixture became homogenous the temperature was raised to 140°, and kept at 140° for 24 hr. The mixture was evaporated in vacuum. The solid residue was boiled in methanol-water (9:1) for 3 hr. The yellow crystals were filtered off and recrystallized from pyridine-water (1:1, for 11) or water-dioxan (3:2, for 12) or 4% HCl aq (for 19 and 20) to yield pure 11 (0.41 g, 34%), 12 (0.5 g, 40%), 19 (0.75 g, 60%) and 20 (0.65 g, 50%).

2-Amino-5,8-bis(2-hydroxyethylamino)pyrimido[4,5-d]pyridazine (21) - 2-Amino-5,8-dihydroxypyrimido[4,5-d]pyridazine (14, 1.0 g), HMDSA (9.0 ml), p-toluenesulphonic acid hydrate (0.03 g) and 2-aminoethanol (1.7 ml) were heated to 120° under vigorous stirring. After the mixture cleared up, the temperature was risen to 150° and kept at 150° for 30 hr. The mixture

was evaporated and the residue was boiled in methanol for 3 hr. The solid precipitate was the monosubstituted derivative 19, which was purified by recrystallization. The filtrate was evaporated and the residue was purified by vacuum chromatography, using dichloromethane--methanol (3:2) eluent, to yield 21 (0.3 g; 20%).

6-Chloro-8-phenyl-2,3-dihydroimidazo[1,2-b]pyrimido[5,4-d]pyridazine (22) *and 6-chloro--9-phenyl-2,3-dihydroimidazo[1,2-b]pyrimido[4,5-d]pyridazine* (24) - 2-Phenyl-5-chloro-8--(2-hydroxyethylamino) (5; 0.3 g) or 2-phenyl-5-(2-hydroxyethylamino)-8-chloro derivative (6; 0.3 g) boiled in thionyl chloride (10 ml) for 2 hr. The solvent was evaporated and the residue was mixed with cold water. To the suspension cc NH_3 aq was added (pH 9). The yellow crystals were filtered off and dried to yield 22 (0.18 g; 60%) and 24 (0.27 g; 95%).

7-Chloro-9-phenyl-2H-3,4-dihydrodipyrimido[1,2-b:5',4'-d]pyridazine (28) *and 7-chloro--10-phenyl-2H-3,4-dihydrodipyrimido[1,2-b:4',5'-d]pyridazine* (29) - Starting from 2-phenyl--5-(3-hydroxypropylamino)-8-chloro derivative (9; 0.3 g) or 2-phenyl-5-chloro-8-(3-hydroxypropylamino) derivative (10; 0.3 g) the above procedure yielded 28 (0.15 g; 52%) and 29 (0.12 g; 43%).

3-Methyl-6-chloro-8-phenyl-2,3-dihydroimidazo[1,2-b]pyrimido[5,4-d]pyridazine (23) *and 3-methyl-6-chloro-9-phenyl-2,3-dihydroimidazo[1,2-b]pyrimido[4,5-d]pyridazine* (25) - 1.0 g of 2-phenyl-5-(2-hydroxypropylamino)-8-chloro (7) or 2-phenyl-5-chloro-8-(2-hydroxypropylamino) (8) derivative was boiled in thionyl chloride for 1 hr. After evaporation water and NaOH aq was added to the reaction mixture and the alkaline suspension was filtered. The solid residue separated was dried to give the ω-chloro derivative of the starting material. The crude product was heated in an evacuated glass tube at 190° for 30 min. The color of crystals turned orange. The mixture was basified by 0.4% NaOH aq, when the color of the crystals turned yellow again. The precipitate was filtered off, washed with water and dried to yield 23 (0.32 g, 35%) and 25 (0.36 g, 39%).

6-Hydroxy-9-phenyl-2,3-dihydroimidazo[1,2-b]pyrimido[4,5-d]pyridazine (26) - 0.5 g of 2-phenyl-5-hydroxy-8-(2-hydroxyethylamino) derivative (11) was heated in concentrated H_2SO_4 (5 ml) to 100° for 3 hr. By adding ether (60 ml) to the reaction mixture crystalline product was precipitated and then separated. The yellow solid was recrystallized from 0.01% H_2SO_4 aq to give the sulphate of 26 (0.25 g; 30%).

7-Hydroxy-10-phenyl-2H-3,4-dihydrodipyrimido[1,2-b:4',5'-d]pyridazine (30) - Starting from 0.5 g of 2-phenyl-5-hydroxy-8-(2-hydroxyethylamino) derivative (12), the compound 30 was obtained by using the above method (0.33 g, 71%).

6-Hydroxy-9-amino-2,3-dihydroimidazo[1,2-b]pyrimido[4,5-d]pyridazine (27) - 0.5 g of 2-amino-5-hydroxy-8-(2-hydroxyethylamino) derivative (19) was dissolved in concentrated H_2SO_4 and heated at 100° for 2 hr. To the cooled reddish-brown mixture ether (25 ml) was poured slowly. The precipitate was separated then washed with ether. The dry residue was mixed with water then basified with concentrated NH_3 aq (pH 9). The solid product was filtered off, recrystallized from water, and dried (P_2O_5) to give 27 (0.21 g; 42%).

7-Hydroxy-10-amino-2H-3,4-dihydrodipyrimido[1,2-b:4',5'-d]pyridazine (31) - 0.5 g of 2-amino-5-hydroxy-8-(3-hydroxypropylamino) derivative (20) was cyclized by the above procedure yielding 31 (0.23 g; 42%).

Kinetic procedures - Reactions 1a, 1b, 2 and 3 shown in Fig. 4 were examined by HPLC. Samples were put in sealed bulbs and immersed in a thermostatically controlled vessel. Chloroform-methanol (9:1) and 3-amino-1-propanol were used as solvents for reactions 1a-1b and 2-3 respectively.[14] Before HPLC separation the samples were cooled and properly diluted, k' was 0.25 and 0.45 for the 8- and 5-substituted derivative 10 and 9 respectively, providing a baseline separation (α 1.79) required for quantitative analysis. The pseudo-first-order rate

constants were obtained either by measuring the appearance of products (for reactions 1a and 1b) or by detecting the decrease of the substrate concentration (for reactions 2 and 3). Each measurement took over at least two half lives. The rate constants and confidence intervals were calculated by non-linear parameter estimation.

Spectra - ^{1}H NMR spectra were recorded on Varian 90 and Bruker FT 250 (dNOE measurements) spectrometer.

HPLC - The chromatograph was a laboratory assembled instrument. Column: Chromfer-Sil (Labor-MIM Hungary); 9 μm particle size; 250x4 mm. Eluent: chloroform-methanol (9:1). Flow rate: 1 ml/min (maintained with Liquopump 312, Labor-MIM Hungary). Injection: 20 μl of the diluted reaction mixture c ≈ 0.1 mg/ml ($4 \cdot 10^{-4}$ mol/l for the substrate). Detection: UV absorption of the column effluent were monitored at 289 nm. Recording: peaks were recorded on a chart recorder (type: OH-841/1, Radelkis Hungary) and areas under them were calculated with application of Simpson's rule.

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14. Supposing that marked charge separation is not taking place during the formation of the guessed transition state in these reactions (see Ref. 10 for charges calculated by MNDO), it is assumed that the inevitable change of reaction media (compounds 9 and 10 react very slowly in chloroform-methanol) does not make meaningless the comparisons either of reaction rates or of activation energies measured.