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Facile Preparation of 9-*H*-Pyrimido[4,5-*b*][1,4]diazepine Derivatives from 4,5-Diaminopyrimidines and Ethyl Pyruvate.

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Abstract: A facile novel procedure to obtain (±)-6,8-diethoxycarbonyl-6-methyl-9-*H*-pyrimido[4,5-*b*][1,4]diazepines from 4,5-diaminopyrimidines and ethyl pyruvate is described. The structure of pyrimido[4,5-*b*][1,4]diazepine derivatives for the products of this reaction was confirmed by single crystal X-ray diffraction analysis. The procedure proved to be of wide scope with reference to the substituent of the starting pyrimidines. The results of the biological test as anticancer and antiviral agents performed with several of the newly synthesized compounds are presented.

INTRODUCTION

Treatment of 4,5-diaminopyrimidine derivatives with 1,2 dicarbonyl compounds is a classical procedure, known as "the Gabriel-Colman Synthesis", to obtain pteridines (pyrazino[2,3-*b*]pyrimidines). In fact, it has been considered as the most widely employed route in the synthesis of pteridines[1] . A particular case, is the use of an α-keto acid or one of its esters as the 1,2-dicarbonyl compound to obtain a 6-alkylpteridin-7(8*H*)-one or the isomeric 7-alkylpteridin-6(5*H*)-one. Many examples of this later case can be found in the literature[2], including the use of pyruvic esters as the α-keto ester reagent. However, an unexpected result emerged during our investigations of the synthesis of pteridines, when formation of 9*H*-pyrimido[4,5-*b*][1,4]diazepine derivatives as main products arose upon treatment of 4,5-diaminopyrimidines with excess ethyl pyruvate in refluxing ethanol. This constitutes, to the best of our knowledge, a type of reactivity not previously reported.

With reference to the products of these reactions, the pyrimido[4,5-*b*][1,4]diazepine is a ring system of rare natural occurrence. Only two compounds of natural origin containing this structure have been isolated at present, both from *Drosophyla melanogaster*: one of them is a complex pentacyclic structure[3]; the other one is 6-acetyl-2-

amino-7,8-dihydro-9*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one (6-acetyldihydrohomopterin)[4], whose synthesis has been reported by Boyle *et al.* in 1987[5]. On the other hand, the literature dealing with the synthesis of derivatives of the referred heterocyclic system is surprisingly scarce[6], in contrast with the great efforts devoted to the synthesis of benzodiazepines and annelated benzodiazepines[7].

In any case, the potential biological interest of new 9*H*-pyrimido[4,5-*b*][1,4]diazepine derivatives is obvious in view of the recognized biological activities of annelated benzodiazepines, the more recent finding of anti-HIV activity for several compounds having nitrogen-containing heterocycles annelated to 1,4-diazepines, such as the TIBO[8] and Nevirapine[9] classes of compounds, and the evident character of pyrimido[4,5-*b*][1,4]diazepines as seven-membered homologues of the widely natural occurring pteridines.

The present paper contains the results of our investigations on the formation of pyrimido[4,5-*b*][1,4]diazepine derivatives by reaction between 4,5-diaminopyrimidines and ethyl pyruvate, together with the results of antiviral and anticancer tests performed with several new compounds containing the referred skeleton of 9*H*-pyrimido[4,5-*b*][1,4]diazepine.

RESULTS AND DISCUSSION

Treatment of 4,5-diamino-2-methylthiopyrimidin-6(1*H*)-one, **1a**, with excess ethyl pyruvate, **2**, (4 mol of **2** per mol of **1a**) in ethanol under reflux gave a complex mixture of products, but the presence of a non-fluorescent[10] main product was detected by tlc monitoring. Isolation of this product, and application of the usual spectroscopic techniques for structural elucidation (p-nmr, c-nmr, ms, uv and ir) led to the proposal of a 6,8-diethoxicarbonyl-6-methyl-2-methylthio-5,6-dihydro-9*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one, **3a**, as the structure of this product. Single crystal X-ray diffraction analysis of compound **3a** unambiguously confirmed the structure proposed for it (see Figure 1).

Table 1. X-Ray Diffraction Analysis of **3a**. Selected Bond Lengths and Angles[a].

Bond length, Å[b]				Angle, deg.[c]					
C1-S1	1.753	C5-N11	1.371	S1-C1-N2	113.34	C5-C4-N7	123.66	C81-C8-C84	108.16
C1-N2	1.352	N7-C8	1.484	S1-C1-N6	122.83	C4-C5-N6	122.74	C81-C8-C9	112.56
C1-N6	1.292	C8-C81	1.533	N2-C1-N6	123.83	C4-C5-N11	124.44	C84-C8-C9	108.98
N2-C3	1.377	C8-C84	1.527	C1-N2-C3	121.49	N6-C5-N11	112.80	C8-C9-C10	128.86
C3-O3	1.236	C8-C9	1.489	N2-C3-O3	119.80	C1-N6-C5	117.48	C9-C10-C101	120.81
C3-C4	1.424	C9-C10	1.326	N2-C3-C4	116.00	C4-N7-C8	120.60	C9-C10-N11	129.31
C4-C5	1.370	C10-C101	1.500	O3-C3-C4	124.17	N7-C8-C81	107.02	C101-C10-N11	109.87
C4-N7	1.403	C10-N11	1.387	C3-C4-C5	118.41	N7-C8-C84	107.04	C5-N11-C10	129.74
C5-N6	1.374			C3-C4-N7	117.45	N7-C8-C9	112.84		

[a] Numbering of atoms refers only to X-ray atom labels used in Figures 1 and 2.
[b] Error is 0.003 Å for all bond lengths.
[c] Errors are between 0.15-0.19 deg. for bond angles.

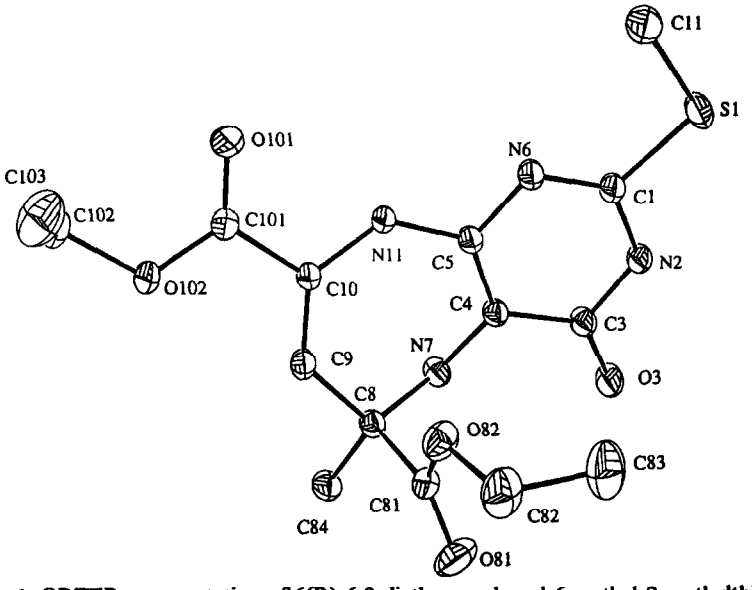


Figure 1. ORTEP representation of 6(R)-6,8-diethoxycarbonyl-6-methyl-2-methylthio-5,6-dihidro-9*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one, **3a**. (Atom numbers are for X-ray structural purposes only and are used also in Table 1 and in X-ray structural discussion in the text).

Relevant features of this structure are the coplanarity of the pyrimidine ring and the C(9)-C(10)-N(11) fragment, and the double bond character evident for C(9)-C(10) (bond length = 1.326 Å)[11]. This latter feature confirms the preference for the enamine tautomeric form in the C(9)-C(10)-N(11) fragment of the diazepine ring. On the other hand, the crystal packing is based on hydrogen bonded pairing of enantiomeric molecules, as shown in Figure 2, thus confirming the racemic composition of the crystalline sample.

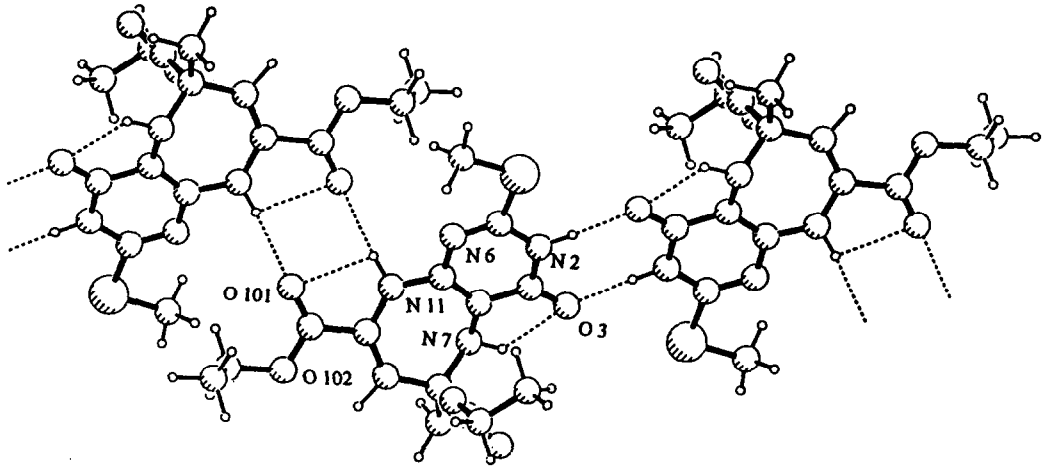
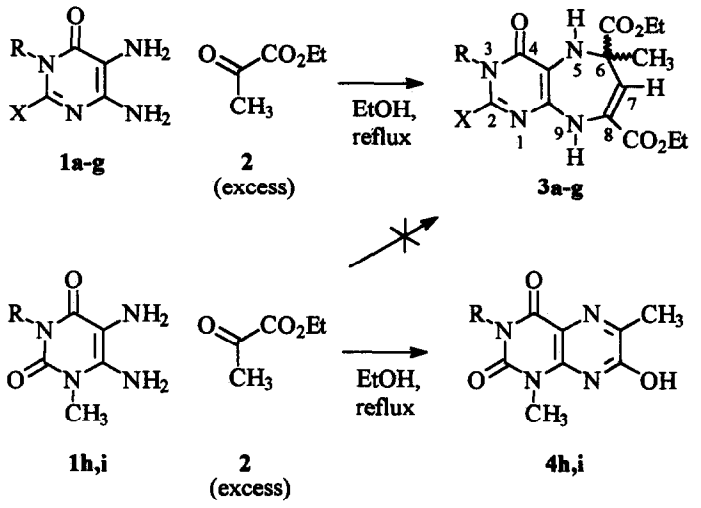


Figure 2. Hydrogen bond pairing between enantiomers of **3a** in the crystalline structure.



1, 3, 4	X	R
a	CH_3S	H
b	CH_3S	CH_3
c	CH_3O	H
d	CH_3O	CH_3
e	NH_2	H
f	OH	H
g	OH	CH_3
h	--	H
i	--	CH_3

Scheme 1.

This unexpected result prompted us to explore the scope of this reaction. Thus, the reactivity of several 4,5-diaminopyrimidin-4(3*H*)-ones, **1b-i**, towards ethyl pyruvate under similar conditions, was tested. The results for diaminopyrimidines **1b-g** were quite similar to that obtained for **1a**, leading to the isolation of the pyrimido[4,5-*b*][1,4]diazepine derivatives **3b-g** in moderate to good yields, while the 3-methylpyrimidine derivatives **1h,i** gave the corresponding 7-hydroxy-6-methylpteridines **4h,i** (see Scheme 1).

The spectroscopic properties of compounds **3b-g** are consistent with the structures of pyrimido[4,5-*b*] [1,4]diazepine derivatives proposed for all of them. Thus, the fragmentation patterns in MS of every compound **3** are closely parallel (see Table 2). In addition, their UV spectra show a characteristic absorption band of medium intensity (log ϵ values 3.46-3.60) with a λ_{max} between 342-346 nm (see Table 2). The reason for this long wavelength band seems to be the π-electron delocalization along the moiety formed by the pyrimidine ring, the N(9) atom and the C(8)=C(7) fragment. This idea is supported by the geometry found for **3a** through its X-ray diffraction analysis, in which the whole pyrimidine ring, the N(9) atom and the C(8)=C(7) fragment are in a same plane, as discussed above. This geometry and the electron delocalization associated with it, also accounts for the observation of a long distance spin coupling (J = 1.7 Hz) between N(9)H and C(7)H protons observed in p-nmr spectra of compounds **3** (see Table 4). This is consistent with the preference for the enamine tautomeric form of the C(7)=C(8)-N(9) fragment in the diazepine ring also in solution.

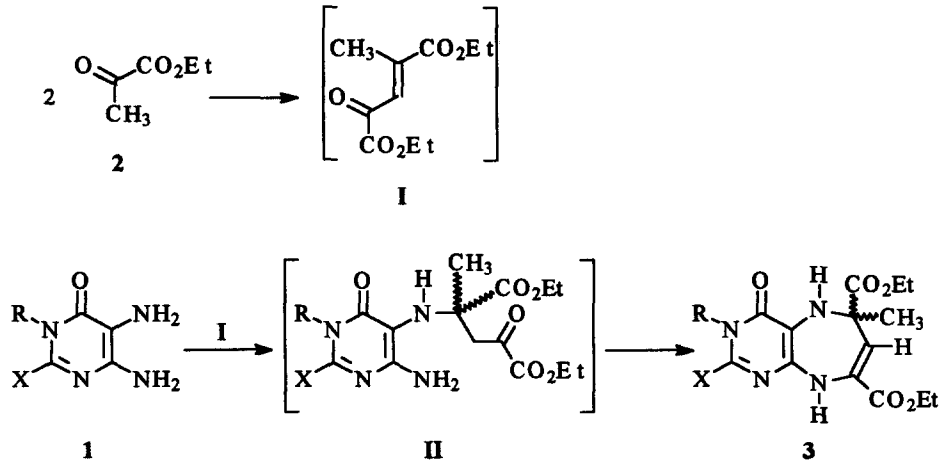
On the other hand, the 8-hydroxy-7-methylpteridine derivatives **4h,i**, were characterized by elemental analysis and comparison of their UV spectra with those previously reported the literature[12] (see experimental part).

Table 2. Physical Data for Compounds 3.

Compd.	m.p. (°C)	UV (MeOH)					Mass spectra fagments[a], m/z (% rel.abund.)				
		c (mol/l)	λ_{max} (log ε)				M^+	M-73	M-101	M-119	M-147
3a	180	$5.2\cdot10^{-5}$	231 (4.14)	271 (4.37)	[307][b] (3.67)	345 (3.51)	368 (16)	295 (100)	267 (18)	249 (11)	221 (9)
3b	126-7	$4.7\cdot10^{-5}$	235 (4.14)	273 (4.35)	[308][b] (3.66)	346 (3.50)	382 (17)	309 (100)	281 (21)	263 (11)	235 (7)
3c	196	$5.7\cdot10^{-5}$	218 (4.15)	262 (4.22)		343 (3.51)	352 (12)	279 (100)	251 (19)	233 (14)	205 (12)
3d	125-6	$5.5\cdot10^{-5}$	220 (4.17)	263 (4.20)		343 (3.48)	366 (15)	293 (100)	265 (21)	247 (12)	219 (9)
3e	171	$4.7\cdot10^{-5}$	223 (4.18)	269 (4.22)		346 (3.46)	337 (16)	264 (100)	236 (19)	218 (18)	190 (11)
3f	203 (dec.)	$4.4\cdot10^{-5}$	208 (4.02)	253 (4.04)		343 (3.53)	338 (15)	265 (100)	237 (21)	219 (12)	191 (20)
3g	171-2 (dec.)	$5.2\cdot10^{-5}$	215 (4.09)	253 (4.11)		342 (3.60)	352 (10)	279 (100)	251 (23)	233 (9)	205 (27)

[a] E.I. 70 eV. Probable assignment of fragments: M-73: $[M - CO_2Et]^+$; M-101: $[M - CO_2Et - C_2H_4]^+$; M-119: $[M - CO_2Et - C_2H_4 - H_2O]^+$; M-147: $[M - CO_2Et - C_2H_4 - H_2O - CO]^+$.
[b] Shoulder

The reaction giving rise to pyrimido[4,5-*b*][1,4]diazepines, **3**, can be formally interpreted in terms of Michael-type addition of the 5-amino group of **1** to the α,β-unsaturated ketone **I**, which should arise from condensation of two molecules of ethyl pyruvate, followed by cyclization, as outlined in Scheme 2.



Scheme 2

However, this does not seem to be the actual path leading to the observed results, because no reasonable explanation for the marked differences in reactivity shown by the 3-methyl-4,5-diaminopyrimidines **1h,i**, with reference to the rest of 4,5-diaminopyrimidines studied, **1a-g**, can be found. That is, assuming non reversibility for the alkylation of the 5-amino group obtained by Michael-type addition of **1** to **I**, the nucleophilicity of the 5-amino group in each diaminopyrimidine, **1**, should control the obtaining of a pyrimido[4,5-*b*][1,4]diazepine derivative as final product. But this is not the case, as revealed by the finding that diaminouracil derivatives **1f** and **1i** lead to different products (see Scheme 1) while the steric environment of their 5-amino groups is very similar and the basic pKa values measured for them (actually for their 5-amino groups) are very close[13] (4.56 for **1f** and 4.44 for **1i**). Thus, a different sequence of reactions should be proposed in order to explain the observed differences in reactivity between the pyrimidines used as starting material.

The results obtained for the four pyrimidines of the 4,5-diaminouracil series, **1f-i**, (see Scheme 1) seem to indicate that the differences in reactivity are directly related to the presence of a methyl group at N(3) in the starting pyrimidine derivative. This methyl group seems to exert no significant influence on the reactivity of the 5-amino group (as discussed in the paragraph above), but on the less reactive 4-amino. Consequently, an explanation for the formation of pyrimido[4,5-*b*][1,4]diazepine derivatives from 4,5-diaminopyrimidines and ethyl pyruvate should be based on a sequence in which the key step involves reaction of the 4-amino group of a starting pyrimidine with a pyruvate fragment. Unfortunately, no direct experimental results were found which would lead to a definitive explanation.

BIOLOGICAL RESULTS

Biological tests were made in order to establish the properties of several 9-*H*-pyrimido[4,5-*b*][1,4]diazepines as antitumor and antiviral agents. Compounds **3a-e** were tested "in vitro" by the NCI (Bethesda, Maryland, USA), against a cell panel containing 60 different tumor cell lines, in accordance with the current protocol of preclinical antitumor drug discovery screen of this Institution[14]. No significant antitumor activity was found for any of these compounds.

Assays of "in vitro" antiviral activity were performed with compunds **3a-e** against several types of viruses[15]. The assays revealed some moderate activity with compounds **3a**, **3b** and **3d** against the arenaviruses Junin and Tacaribe, as summarised in Table 3. No activity was found with compounds **3a-e** against any of the other viruses tested.

In summary, the reaction of 4,5-diaminopyrimidines with excess ethyl pyruvate gave easy access to 9-*H*-pyrimido[4,5-*b*][1,4]diazepine derivatives, a heterocyclic ring system of evident biological interest. This constitutes a novel reactivity scheme that is of wide scope with reference to the substituents in the pyrimidine ring of the

starting material, although limitations were found for those starting pyrimidine derivatives carrying a methyl group at N(3). From the biological point of view, some moderate antiviral activity was found for three of the novel compounds reported against the arenaviruses Junin and Tacaribe.

Table 3. Activity for Compounds **3a**, **3b** and **3d** against Junin Virus and Tacaribe Virus.

Compound	MTC[a] (μg/ml)	MIC[b] (μg/ml)	
		Junin	Tacaribe
3a	40	20	11
3b	10	4.3	5.0
3d	40	10	8.8

[a] Minimum toxic concentration to cause a microscopically detectable change in morphology of normal uninfected cells treated with the compounds and run in parallel with the infected treated cells.
[b] Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity by 50 %. Compounds were added inmediatly after virus adsorption and remained on the cells thereafter. Viral cytopathogenicity was recorded at day 5 p.i.

Table 4. ^{1}H-NMR Data for Compounds **3** [δ (ppm), Integral, Multiplicity, J (Hz)][a].

Compd.			**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**
Solvent			$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	DMSO-d_6	DMSO-d_6	DMSO-d_6
Groups	6-CH_3		1.66, s	1.64, s	1.64, s	1.62, s	147, s	1.55, s	1.56, s
	CO_2Et	CH_3	1.17, 3H, t, J=7.2 1.37, 3H, t, J=7.2	1.15, 3H, t J=7.2 1.37, 3H, t, J=7.2	1.15, 3H, t J=7.2 1.37, 3H, t, J=7.2	1.17, 3H, t J=7.2 1.37, 3H, t, J=7.2	1.10, 3H, t J=7.2 1.29, 3H, t, J=7.2	1.16, 3H, t J=7.2 1.36, 3H, t, J=7.2	1.15, 3H, t J=7.2 1.36, 3H, t, J=7.2
		CH_2	3.85-4.65, 4H, m	3.85-4.65 4H, m	3.80-4.60 4H, m	3.85-4.65 4H, m	3.70-4.60 4H, m	3.85-4.60 4H, m	3.80-4.75 4H, m
	C(7)H[b]		6.22, d, J=1.7	6.20, d, J=1.7	6.20, d, J=1.7	6.20, d, J=1.7	6.10 [d]	6.19, d, J=1.7	6.23, d, J=1.7
	N(5)H[c]		Zone 3.85-465	Zone 3.85-4.65	Zone 3.80-4.60	Zone 3.85-4.65	Zone 370-4.60	4.45, bs	Zone 3.80-4.75
	N(9)H[c]		7.55, bs	7.40, bs	7.45, bs	7.34, bs	7.10, bs	8.18, bs	8.19, bs
	2-X		2.54, 3H, s S-CH_3	2.52, 3H, s S-CH_3	3.91, 3H, s O-CH_3	3.95, 3H, s O-CH_3	6.10, 2H, bs NH_2	---	---
	3-R		12.9, 1H, bs, D_2O ex. N(3)H	3.54, 3H, s N(3)-CH_3	12.4, 1H, bs,D_2O ex. N(3)H	3.42, 3H, s N(3)-CH_3	[e] N(3)H	11.1, 1H, bs,D_2O ex. N(3)H	3.22, 3H, s N(3)-CH_3
	Other		---	---	---	---	----	10.45, 1H, bs, D_2O ex. N(1)H	10.70, 1H, bs, D_2O ex. N(1)H

Abreviations: s = singlet; d = doublet; t = triplet; bs = broad singlet; D_2O ex.= D_2O exchangeable.
[a] Spectra obtained in a "Hitachi-Perkin-Elmer R-600" instrument at 60 MHz; TMS as internal standard. [b] Singlet after D_2O exchange. [c] D_2O exchangeable. [d] Coupling not observed. [e] Not observed.

EXPERIMENTAL

Melting Points were determinated in a Melting Points Apparatus Gallenkamp and are uncorrected. Proton nuclear Magnetic Resonance (^{1}H-NMR) spectra were recorded in a Perkin-Elmer R-600 Spectrometer, using tetramethylsilane as internal standard; the following abbreviations are used to described signal multiplicity: s= singlet; bs=broad singlet. ^{13}C-NMR spectra were recorded in a Bruker AM-300 Spectrometer from "Servicios Técnicos de la Universidad de Granada" (STUGRA). Ultraviolet and Visible (UV) spectra were recorded in a Perkin-Elmer Lambda 19 spectrophotometer. Infrared spectra were recorded in a Beckman 4250 spectrophotometer (potassium bromide pellets). Mass spectra were recorded in a Hewlett-Packard HP-5988-A from STUGRA. Elemental analyses, C, H and N, have been performed in a Perkin-Elmer 240 C from STUGRA. Flash column chromatography have been performed on Merck Silica Gel 60 (0.040-0.063 mm) using the solvent system indicated in each case. Reaction progress and products purity were monitorized by thin layer chromatography (tlc) on Merck Silica Gel $60GF_{254}$ (0.2 mm) aluminium precoated sheets with fluorescent indicator, the spots were visualized by ultraviolet irradiation. Ethyl pyruvate (98%) was purchased from Aldrich, and directly used without further purification.

General procedure for the preparation of (±)-6,8-diethoxycarbonyl-6-methyl-9-*H*-pyrimido[4,5-*b*][1,4]diazepine derivatives, 3a-g, from 4,5-diaminopyrimidines, 1a-g, and ethyl pyruvate. To a suspension of the corresponding diaminopyrimidine, **1**, in ethanol, excess ethyl pyruvate (aprox. 4 mol of pyruvate per mol of **1**) was added and the mixture refluxed with continuous stirring for the appropriate time (as indicated in each case). The solvent was removed under reduced pressure, the resulting residue applied on a silicagel column for flash chromatograpy, except for compounds **3a** and **3g** (see below), and eluted with the appropriate mixture of solvents (see each case for column dimensions and eluents). Appropriate fractions were collected, pooled and evaporated to give the corresponding 9-*H*-pyrimido[4,5-*b*][1,4]diazepine derivative, **3**.

(±)-6,8-diethoxycarbonyl-6-methyl-2-methylthio-5,6-dihydro-9-*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one (3a). A mixture of 2.00 g (11.61 mmol) of 4,5-diamino-2-methylthiopyrimidin-6(1*H*)-one, **1a**, and 5.19 ml (46.45 mmol) of ethyl pyruvate in 50 ml of EtOH was refluxed for 1h. The solvent was partially removed under reduced pressure until the volume was ca. 20 ml. The solution was kept in the refrigerator at 0 °C for 12 h. The crystalline solid was collected by filtration, washed with MeOH and dried in vacuo at 78 °C to give 1.999 g (5.43 mmol, 47 %) of **3a**. Anal. calcd. for $C_{15}H_{20}N_4O_5S$ (368.41): C, 48.90; H, 5.47; N, 15.21. Found: C, 48.86 ; H, 5.51; N, 15.36. IR (solid, KBr Pellets), ν (cm^{-1}): 3344, 3328, 2831, 2788, 2729, 1733, 1709, 1664, 1622, 1605, 1578, 1523.

(±)-6,8-diethoxycarbonyl-3,6-dimethyl-2-methylthio-5,6-dihydro-9-*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one (3b). A mixture of 2.00 g (10.74 mmol) of 4,5-diamino-1-methyl-2-methylthiopyrimidin-6(1*H*)-one, **1b**, and 4.80 ml (42.96 mmol) of ethyl pyruvate in EtOH (25 ml) was refluxed for 1 h 45 min. Flash chromatography of the crude residue (column: ϕ = 4 cm, l = 15; eluent: CH_2Cl_2/Acetone, 98:2, v/v, 1600 ml) afforded 3.090 g of material which after recrystallization from MeOH gave, in two crops, 2.383 g (6.23 mmol, 58 %) of **3b**. Anal. calcd. for $C_{16}H_{22}N_4O_5S$ (382.43): C, 50.25; H, 5.80; N, 14.65. Found: C, 50.23; H, 5.84; N, 14.90. IR (solid, KBr Pellets), ν (cm^{-1}): 3390, 3294, 1735, 1716, 1671, 1647, 1630, 1546, 1517.

(±)-6,8-diethoxycarbonyl-6-methyl-2-methoxy-5,6-dihydro-9-*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one (3c). A mixture of 1.50 g (8.61 mmol) of 4,5-diamino-2-methoxypyrimidin-6(1*H*)-one hydrate, **1c**, and 4.30 ml (38.43 mmol) of ethyl pyruvate in EtOH (25 ml) was refluxed for 1 h. Flash chromatography of the crude residue (column: ϕ = 4 cm, l = 15; eluent: CH_2Cl_2/Acetone mixtures, 1500 ml of 9:1, v/v, and 500 ml of 8:1, v/v) afforded 1.908 g of material which after recrystallization from MeOH gave 1.605 g (4.56 mmol, 53 %) of **3c**. Anal. calcd. for $C_{15}H_{20}N_4O_6$ (352.35): C, 51.13; H, 5.72; N 15.90. Found: C, 51.03; H, 5.80; N, 15.96. IR (solid, KBr Pellets), ν (cm^{-1}): 3387, 3324, 2872, 2793, 2738, 2642, 1734, 1714, 1637, 1606, 1531, 1500.

(±)-6,8-diethoxycarbonyl-3,6-dimethyl-2-methoxy-5,6-dihydro-9-*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one (3d). A mixture of 1.50 g (8.81 mmol) of 4,5-diamino-1-methyl-2-methoxypyrimidin-6(1*H*)-one, **1d**, and 3.94 ml (35.25 mmol) of ethyl pyruvate in EtOH (25 ml) was refluxed for 1 h. Flash chormatography of the crude residue (column: ϕ = 4 cm, l = 15 cm; eluent: CH_2Cl_2/Acetone, 9:1, v/v, 1300 ml) afforded 2.432 g of material which after recrystallization from MeOH gave, in two crops, 2.175 g (5.94 mmol, 67 %) of **3d**. Anal. calcd. for $C_{16}H_{22}N_4O_6$ (366.37): C, 52.45; H, 6.05; N, 15.29. Found: C, 52.34; H, 6.12; N, 15.40. IR (solid, KBr Pellets), ν (cm^{-1}): 3360, 3296, 1725, 1705, 1652, 1636, 1576, 1525.

(±)-2-amino-6,8-diethoxycarbonyl-6-methyl-5,6-dihydro-9-*H*-pyrimido[4,5-*b*][1,4]diazepin-4(3*H*)-one (3e). A mixture of 1.50 g (10.63 mmol) of 2,4,5-triaminopyrimidin-6(1*H*)-one, **1e**, and 4.75 ml (42.51 mmol) of ethyl pyruvate in EtOH (25 ml) was refluxed for 50 min. Flash chromatography of the crude residue (column: ϕ = 4 cm, l = 15 cm; eluent: CH_2Cl_2/MeOH, 9:1, v/v, 1000 ml) afforded 1.519 g of material which after recrystallization from EtOH gave 1.274 g (3.78 mmol, 35 %) of **3e**. Anal. calcd. for $C_{14}H_{19}N_5O_5$ (337.34): C, 49.85; H, 5.68; N, 20.75. Found: C, 49.69; H, 5.72; N, 20.93. IR (solid, KBr Pellets), ν (cm^{-1}): 3484, 3388, 3375, 3327, 2822, 2700, 1724, 1709, 1642, 1626, 1594, 1536.

(±)-6,8-diethoxycarbonyl-6-methyl-5,6-dihydro-9-*H*-pyrimido[4,5-*b*][1,4]diazepine-2,4(1*H*,3*H*)-dione (3f). A mixture of 1.56 g (10.99 mmol) of 4,5-diaminouracil and 4.19 ml (43.96 mmol) of ethyl pyruvate in EtOH

(25 ml) was refluxed for 8 h. Flash chromatography of the crude residue (column: ϕ = 5.5 cm, l = 15 cm; eluent CH_2Cl_2/MeOH mixtures, 1500 ml of 95:5 v/v, and 250 ml of 9:1 v/v) afforded 1.706 g (5.04 mmol, 46 %) of **3f** as a chromatographically pure solid. A sample of it was recrystallized from MeOH for analytical purposes. Anal. calcd. for $C_{14}H_{18}N_4O_6$ (338.32): C, 49.70; H, 5.36; N, 16.57. Found: C, 49.49; H, 5.24; N, 16.33. IR (solid, KBr Pellets), ν (cm^{-1}): 3333, 3288, 3159, 3030, 2810, 1731, 1654, 1555.

(±)-6,8-diethoxycarbonyl-3,6-dimethyl-5,6-dihydro-9-*H*-pyrimido[4,5-*b*][1,4]diazepine-2,4(1*H*,3*H*)-dione (3g). A mixture of 1.04 g (6.67 mmol) of 4,5-diamino-1-methylpyrimidine-2,6(3*H*,1*H*)-dione, **1g**, and 2.98 ml (26.69 mmol) of ethyl pyruvate in EtOH (15 ml) was refluxed for 2 h 40 min. The reaction mixture was kept at rt for 12 h. The solid in suspension was collected by filtration, washed with EtOH and Et_2O, and dried in vacuo at 78 °C to give 1.523 g (4.32 mmol, 65 %) of chromatographically pure **3g**. A sample was recrystallized from MeOH for analytical purposes. Anal. calcd. for $C_{15}H_{20}N_4O_6$ (352.35): C, 51.13; H, 5.72; N, 15.90. Found:C, 51.04; H, 5.59; N, 16.03. IR (solid, KBr Pellets), ν (cm^{-1}): 3324, 3286, 3263, 1736, 1723, 1698, 1652, 1630, 1556, 1519.

1,6-dimethyl-7-hydroxypteridine-2,4(1*H*,3*H*)-dione (4h). To a suspension of 4,5-diamino-3-methylpyrimidine-2,6(3*H*,1*H*)-dione, **1h**, (1.51 g, 9.67 mmol) in EtOH (20 ml), ethyl pyruvate (4.32 ml, 38.68 mmol) was added and the mixture refluxed with continuous stirring for 1h 10 min. The solid in the suspension was collected by filtration, washed with EtOH and Et_2O, and dried in vacuo at 78 °C to give 0,920 g (4.07 mmol) of **4h**·H_2O. Anal. calcd. for $C_8H_8N_4O_3 \cdot H_2O$ (226.19); C, 42.48; H, 4.45; N, 24.77. Found: C, 42.26; H, 4.41; N, 24.50. UV (H_2O, pH=1.O) λ_{max} (log ϵ): 267 (3.89), 283 (3.83), 327 (4.09); Lit[12]: 267 (3.91), 283 (3.85), 327 (4.09). The mother liquors were evaporated to dryness an the residue treated with boiling MeOH (10 ml). The solid in suspension was collected by filtration, washed with MeOH and Et_2O, and dried in vacuo to give another 0.550 g (2.43 mmol) of **4h**·H_2O (global yield: 1.470 g, 6.50 mmol, 67 %).

1,3,6-trimethyl-7-hydroxypteridine-2,4(1*H*,3*H*)-dione (4i). To a suspension of 4,5-diamino-1,3-dimethylpyrimidine-2,6(3*H*,1*H*)-dione, **1i**, (1.54 g, 9.02 mmol) in EtOH (25 ml), ethyl pyruvate (4.03 ml, 36.08 mmol) was added and the mixture refluxed with continuous stirring for 4h 15 min. The solid in suspension was collected by filtration, washed with EtOH and Et_2O, and dried in vacuo at 78 °C to give 0,707 g (4.07 mmol) of **4i**. Anal. calcd. for $C_9H_{10}N_4O_3$ (222.20): C, 48.65; H, 4.54; N, 25.21. Found: C, 48.51; H, 4.61; N, 25.17. UV (H_2O, pH=1.5) λ_{max} (log ϵ): 268 (3.81), 283 (3.79), 327 (4.08); Lit[12]: 269 (3.82), 284 (3.79), 327 (4.09). The mother liquors were evaporated to a final volume of ca. 10 ml and the solid in suspension collected by filtration, washed and dried as before to give another 0.190 g (0.85 mmol) of **1i** (global yield: 0.897 g, 4.04 mmol, 45 %).

X-ray analysis of 3a[16]: Diffraction data were collected on a "Nicolet P3" difractometer at 293 K, using graphite monochromated MoK_α radiation (λ = 0.70930 Å). Data were obtained from a crystal grown from methanol with sizes 0.40 x 0.80 x 0.60 mm. Unit cell parameters were obtained by least square refinement of 14 reflections in the range 21° < 2Θ < 23°. Crystals are monoclinic, space group $P2_1/n$, with 4 formula units of $C_{15}H_{20}N_4O_5S$ (368.40) in a unit cell with parameters a = 15.153(12) Å, b = 7.798(4) Å, c = 15.681(16) Å, β = 110.57(7)°, V = 1734.8(24) Å³, d_{calc} = 1.411 g/cm³. Intensity data were collected using the Θ/2Θ scan mode in the range 0° < 2Θ < 50.0°. From a total of 3181 mesured reflections 3065 were unique and 2774 were considered as observed following the criterion $I > 2.5\sigma(I_o)$. No correction was made for absorption. The structure was solved with direct methods and refined with the least squares method[17]. Non-hydrogen atoms were anisotropically refined, while hydrogen atoms were treated as "riding" (C-H distance 1.08 Å). The last least square cycle was calculated with 45 atoms, 226 parameters and 2774 reflections. The calculation resulted in final $R_F = \Sigma(F_o - F_c)/\Sigma(F_o)$ = 0.047, $R\omega = [(\Sigma\omega(F_o - F_c)^2)/\Sigma(\omega F_o^2)]^{1/2}$ = 0.067 and GoF = $[(\Sigma\omega(F_o - F_c)^2)/(\text{No. of refIns - No. of params.})]^{1/2}$ = 3.71.

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REFERENCES AND NOTES

1. Review: Brown, D. J. "Pteridines" (vol. 24, part 3 of "The Chemistry of Heterocyclic Compounds"); Taylor E.C. ed.; Wiley, 1988.
2. (a) Reference 1, pags. 92-105.
   (b) Melguizo, M.; Nogueras M.; Sanchez A. *Heterocycles*, **1991**, *32*, 1719-1728.
3. Theobald, N.; Pfleiderer W. *Chem. Ber.*, **1978**, *111*, 3385-3402.
4. Jacobson, K. B.; Dorsett, D.; Pfleiderer, W; McCloskey, J. A.; Sethi, S. K.; Buchanan, M. W.; Rubin J. B. *Biochemistry*, **1982**, *21*, 5700-5706.
5. (a) Boyle, P. H.; Hughes, E. M.; Kahttab, H. A.; Lockhart, R. J. *Tetrahedron Lett.*, **1987**, *28*, 5331-5334.
   (b) Boyle, P. H.; Hughes, E. M.; Kahttab, H. A.; Lockhart, R. J. *J. Chem. Soc, Perkin I*, **1990**, 2071-2077.
6. (a) Reference 5.
   (b) Review: Frier R. I.; Walser A. in *Bicyclic Diazepines*; Frier, R. I. ed. (vol. 50 of the series "The Chemistry of Heterocyclic Compounds"; E. C. Taylor, ed.); John Wiley & Sons, 1991.
   (c) Pike, D. C.; Hora, M. T.; Bailey, S. W.; Ayling J. E. *Biochemistry*, **1986**, *25*, 4762-4771.
   (d) Boyle, P. H.; Hughes, E. M.; Kahttab, H. A. *Tetrehedron*, **1991**, *47*, 5259-5268.

7. (a) Review on annelated benzodiazepines: Chimirri, A.; Gitto, R.; Grasso, S.; Monforte, A. M.; Romeo, G.; Zappalà, M. *Heterocycles*, **1993**, *36*, 601-637.
(b)Part 2 of ref. 7(a): Chimirri, A.; Gitto, R.; Grasso, S.; Monforte, A. M.; Romeo, G.; Zappalà, M. *Heterocycles*, **1993**, *36*, 865-890.

8. Pauwels R.; Andires, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *Nature*, **1990**, *343*, 470-474.

9. Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Woo, J. C.; Shih, C.-K.; Eckner, K.; Hattox, S.; Adams, J.; Rosenthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, E. A.; Sullivan, J. L. *Science*, **1990**, *250*, 1411-1413.

10. The 6-methyl-2-methylthiopteridine-4,7(3*H*,8*H*)-dione that might be formed in a Grabriel-Colman reaction between the diaminopyrimidine **1a** and ethyl pyruvate possesses a remarkable fluorescence. For the synthesis of that pteridine derivative see ref. 2(b).

11. Bond lengths tables of X-ray diffraction resolved structures from: Allen, F. H.; Kennard O.; Watson, D. G.; Brammer, L.; Orpen A. G.; Taylor R. *J. Chem. Soc. Perkin Trans II*, **1987** S1-S19; consulted in *Handbook of Chemistry and Physics, 73rd eddition (1992-93)*, Lide D. R. ed, CRC Press, Ann Arbor ,(1992).

12. Pfleiderer, W. *Chem. Ber.*, **1957**, *90*, 2588-2603.

13. Barlin, G. B.; Pfleiderer, W. *J. Chem. Soc. (B)*, **1971**, 1425-1432.

14. Boyd, M. R. *Principles & Practices of Oncology*, **1989**, *3*, 1-12.

15. The viruses tested were: Influenza A, Influenza B, Respiratory Syncytial virus, Herpes Simplex virus-1 (KOS), Herpes Simplex virus-2 (G), Herpes Simplex virus-1 TK$^-$ B2006, Herpes Simplex virus-1 TK$^-$ VMW1837, Vaccinia virus, Vesicular Stomatitis virus, Coxsackie virus B4, Polio virus-1, Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Semliki Forest virus, Cytomegalovirus AD-169 stain, Cytomegalovirus Davis stain, the arenaviruses Junin and Tacaribe, HIV-1 and HIV-2.

16. Full experimental details of the X-ray analysis, tables of atomic coordinates, thermal parameters, bond lengths, bond angles, torsion angles and least square planes are supplementary material to this paper.

17. **Software:** Data reduction: NRCVAX DATRD2; structure solution: SHELXS86; structure refinement: NRCVAX LSTSQ. References relevant to the NRCVAX system: (a) Full system reference: Gabe, E. J.; Le Page, Y.; Charland, J.-P.; Lee, F. L.; White, P.S. *J. Appl. Cryst.*, **1989**, *22*, 384-387. (b) Scattering Factors from Int. Tab. Vol. 4 : International Tables for X-ray Crystallography, Vol. IV, Kynoch Press, Birmingham, England (1974).

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