RIBONUCLEOSIDES OF 3-AMINO- AND 3,5-DIAMINOPYRAZOLE-4-CARBOXYLIC ACID AND THEIR OPEN-CHAIN ANALOGUES: SYNTHESIS AND REACTIONS

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Bis(trimethylsilyl) derivative of ethyl 3-aminopyrazole-4-carboxylate (*VI*) and tris(trimethylsilyl) derivative of ethyl 3,5-diaminopyrazole-4-carboxylate (*VII*) on reaction with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride and subsequent debenzoylation afforded the respective β-D-ribofuranosyl derivatives *VIIIa* and *Xa*. Their alkaline hydrolysis led to 1-(β-D-ribofuranosyl)-3-aminopyrazole-4-carboxylic acid (*VIIIc*) and 1-(β-D-ribofuranosyl)-3,5-diaminopyrazole-4-carboxylic acid (*Xb*). The esters *VIIIa* and *Xa* were not ammonolyzed under normal conditions. Contrary to nucleosidation of the silyl derivatives *VI* and *VII*, sodium salt of ethyl 3-aminopyrazole-4-carboxylate was alkylated with 4-chloromethyl-2,2-dimethyl-1,3-dioxolane (*XI*) or 5-(*p*-toluenesulfonyloxy)-1,3-dioxane (*XVIIb*) to give a mixture of the N-isomeric derivatives *XIIIa*, *XIXa* and *XIIa*, *XVIIIa*, respectively; sodium salt of the 3,5-diamino derivative *V* reacted with these synthons under formation of the corresponding compounds *XIIIb* and *XXa*. Subsequent alkaline and acid hydrolysis of *XIIa* and *XIIIb* gave the open-chain analogs of nucleosides *XV* and *XVI*. The N-(1,3-dioxan-5-yl) derivatives *XVIIIc* and *XXa* resisted acid hydrolysis, giving rise only to carboxylic acids *XVIIIb* and *XXb*.

Nucleosides derived from 3-aminopyrazole-4-carboxylic acid and its derivatives (*I*) are isomeric with compounds belonging to the series of the key-metabolite of purine synthesis *de novo* — AICA riboside. In this respect, they are of interest, since they may act as antimetabolites, *e.g.* with inosine 5'-phosphate dehydrogenase as the target enzyme. Such effect has been observed *e.g.* for the as-triazole derivative ribavirin[1], the thiazole derivative thiazofurin[2], its selenium analogue[3] or the nucleoside antibiotic bredynin[4]. Of no less importance is the utilization of compounds *I* for the synthesis of pyrazolo[3,4*d*]pyrimidine nucleosides[5,6], many of which show cytostatic activity (for a review see ref.[7]), and particularly of allopurinol riboside (*II*) which has a specific antiparasitic effect (against *Leischmania* and *Trypanosoma*[8]; for a review see ref.[9]). The nucleoside *II* was also isolated from natural material[10].

N-Glycosylation of the pyrazole system is not a simple matter; depending on the method and conditions it affords mixtures of position isomers or anomers[7]. There-

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fore, an alternative method, consisting in construction of the pyrazole ring from protected ribose hydrazones, has been elaborated[6,11]. In this way, derivatives *Ib*–*Id* were prepared in yields ranging from 25 to 50%. The effectiveness of this method is influenced by the necessary synthesis of the starting hydrazone of the protected D-ribose[11].

Sugar halogenoses react with silyl derivatives of substituted pyrazoles to give mixtures of N^1- and N^2-derivatives. It is assumed that, whereas glycosylation of silyl derivatives of purine bases proceeds with $N^3 \rightarrow N^9$ isomerization[12], the reaction with pyrazoles is accompanied by substitution at the free nitrogen atom and removal of the silyl group from the adjacent atom[13]. Since the silylated 3-triazene derivative *III* reacts with the halogenose to give the N^1-riboside as principal product[5], the silylation affords preferentially the sterically unfavourable N^2-trimethylsilyl derivative.

In our present work we studied the course of nucleosidation reaction of the halogenose with silyl derivatives of esters of 3-aminopyrazole-4-carboxylic acid (*IV*) and 3,5-diaminopyrazole-4-carboxylic acid (*V*) as a possible simpler access to both series of nucleosides. We investigated also potential utilization of the prepared compounds in the synthesis of other derivatives, *e.g.* the amide *Id*, by transformation of the alkoxycarbonyl functionality in position 4. The starting bases are easily accessible by the previously described procedures[14,15]. Both weakly inhibit the growth of *Neurospora crassa*[16,17] and the monoamino derivative *IV* actually inhibits the incorporation of [^{14}C] formate into IMP in pidgeon liver cell-free system[18]. We studied therefore not only the synthesis and biological activity of ribonucleosides of both these bases but also of their so-called open-chain analogues that might be also biologically active[19,20].

The heterocyclic bases *IV* and *V* react with hexamethyldisilazane to give the bis- and tris(trimethylsilyl) derivatives *VI* and *VII*, respectively. Compound *VI* was treated with a small excess of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride in acetonitrile at room temperature and the reaction mixture after deprotection by methanolysis was chromatographed on Dowex 50 or Amberlite IRC 50 to remove a small amount of the unreacted base *IV*. The obtained mixture of nucleosides of *IV* was shown by HPLC to contain only insignificant amounts of the isomer *IX*, the ribosides *VIII* being the main product. The ethyl ester *VIIIa* was accompanied by approximately the same amount of the methyl ester *VIIIb*, the latter arising apparently by base-catalyzed transesterification during methanolysis. The structure of *VIIIa* and *VIIIb* was determined by ^{1}H NMR spectra (comparison with those of N-substituted derivatives of the base *IV*), UV spectra (characteristic maximum at 263 nm, typical of isomers of the type *VIII*), and also by mass spectra (the presence of the expected molecular peaks and corresponding fragments). The reaction mixture after ribosylation of compound *VI* was therefore treated with sodium ethoxide in ethanol giving, as expected, the ethyl ester *VIIIa* as the principal product.

Ribosylation of the symmetrical silyl derivative *VII* can afford only one isomer. The overall conversion to the nucleoside was about the same as in the case of the monoamino derivative *VI* but there had been no reesterification in the methanolysis so that the ethyl ester *Xa* was obtained as the sole product. Its structure was confirmed by the ^{1}H NMR, mass and UV spectra. The ^{1}H NMR spectroscopy also proved that the product was more than 95% pure β-anomer.

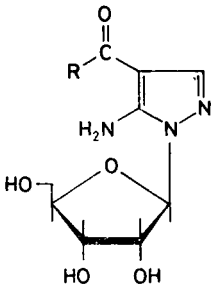
Alkaline hydrolysis of the ester functionality in the nucleosides *VIIIa,b* and *Xa* proceeded with difficulty, requiring 0·4 mol l^{-1} sodium hydroxide for the esters *VIII* and 1 mol l^{-1} for compound *Xa*. Even under these conditions the reaction time was several hours at room temperature.* The products of hydrolysis, carboxylic acids *VIIIc* and *Xb*, were purified first by chromatography on DEAE-Sephadex and then on octadecyl-silica gel. Although their structure followed from the starting esters, it was confirmed independently by spectral methods (^{1}H NMR, mass and UV spectra).

Attempted ammonolysis of esters *VIII* and *Xa*, leading to amides of the type *Id*, failed. Compound *VIIIa* did not change on heating with 30% methanolic ammonia at 120°C or in the presence of 5 mol % of sodium methoxide[22]; in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene[23] under the same reaction conditions the reaction mixture contained (according to HPLC) a significant amount of the carboxylic acid *VIIIc* but only traces of another product, possibly the amide *Id*. The ester bond in *Xa* is also highly resistant to ammonolysis: methanolic ammonia can be therefore used instead of sodium methoxide or ethoxide in working up the crude reaction mixture after ribosylation without affecting the heterocyclic skeleton of the nucleoside molecule.

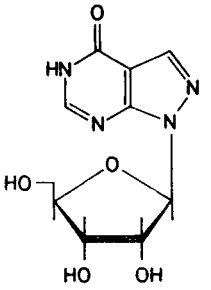
We tried to prepare also the so-called open-chain analogues of the nucleosides *VIII* and *X*. As models we chose two isomeric types: 2,3-dihydroxypropyl and 1,3-dihydroxypropyl derivatives of both 3-amino- and 3,5-diaminopyrazole-4-carboxylic acid. Attempts to prepare these compounds from the trimethylsilyl derivatives *VI* and *VII* failed. Thus, compound *VI* did not react with 4-chloromethyl-2,2-dimethyl-1,3-dioxolane (*XI*) in acetonitrile at room or elevated temperature, even in the presence of sodium iodide. However, compound *XI* reacted smoothly with sodium salts of *IV* and *V* (generated *in situ* by treatment with sodium hydride) in dimethylformamide at elevated temperature, or simply with the mentioned bases and potassium carbonate in the same solvent (see ref.[24]). The monoamino derivative *IV* gave in both cases (in comparable yields) a mixture of the isomers *XIIa* and *XIIIa*. As expected, the diamino compound *V* under the same conditions afforded compound *XIIIb* as the sole product.

* A similar situation is known for esters of anthranilic acid which are hydrolyzed very sluggishly; introduction of another amino group *ortho* to the alkoxycarbonyl slows down the reaction by several orders of magnitude[21].

The NMR spectra of the isomeric pyrazole derivatives were measured in deuteriochloroform and hexadeuteriodimethyl sulfoxide; the amine protons were replaced with deuterium by adding several drops of tetradeuterioacetic acid. Signals were ascribed to the individual protons on the basis of chemical shifts, multiplicities and



I a, R = OH
I b, R = OCH_3
I c, R = OC_2H_5
I d, R = NH_2



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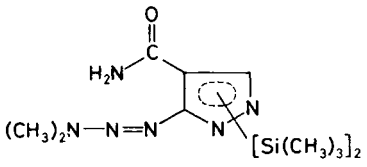
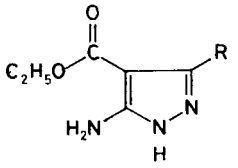
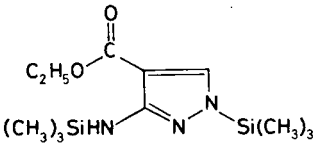


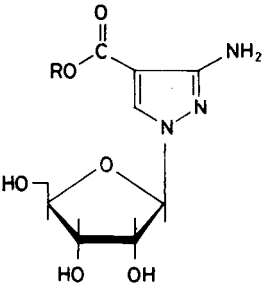
III



IV, R = H
V, R = NH_2

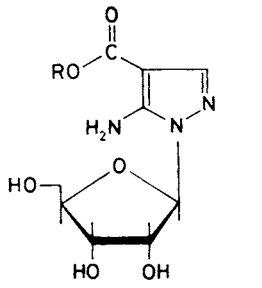


VI, R = H
VII, R = $NHSi(CH_3)_3$

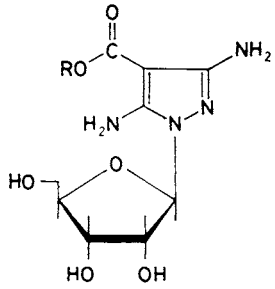


VIII a, R = C_2H_5
VIII b, R = CH_3
VIII c, R = H

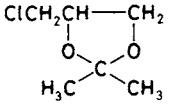
decoupling experiments. Chemical shifts and coupling constants were obtained by first-order analysis from the expanded spectra. The proton spectrum of *XIIa* in deuteriochloroform exhibits a singlet of H-3 (δ 7·86), a pentet H-2′ (δ 4·42) and two doublets of doublets of H-3′ and H-3″ (δ 4·07 and 3·76) with coupling constants $J_{3',2'} = 6{\cdot}3$, $J_{3'',2'} = 5{\cdot}9$, and $J_{3',3''} = 8{\cdot}8$ Hz. Protons H-1′ and H-1″ form a strongly interacting system appearing as a doublet (δ 4·00; $J = 5{\cdot}5$). Similarly, compound *XIIIa* has a singlet of H-3 (δ 7·60), a multiplet of H-2′ (δ 4·42) and doublets of doublets of H-1′ and H-1″ protons (δ 4·23 and 4·05) with coupling constants $J_{1',2'} = 2{\cdot}8$, $J_{1'',2'} = 5{\cdot}0$ and $J_{1',1''} = -15{\cdot}2$. Protons H-3′ and H-3″ form doublets of doublets (δ 4·10 and 3·78; $J_{3',2'} = 6{\cdot}5$, $J_{3'',2'} = 7{\cdot}4$, and $J_{3',3''} = -8{\cdot}7$). Addition of non-deuterated trifluoroacetic acid shifts the singlet of H-3 markedly downfield (0·4 ppm for *XIIa* and 0·7 ppm for *XIIIa*). The remaining protons in both isomers occur only as undistinguished multiplets.



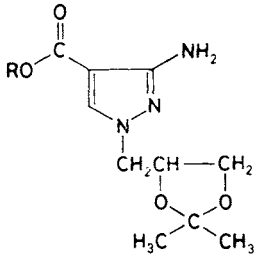
IX, R = CH_3, C_2H_5



X a, R = C_2H_5
X b, R = H



XI



XII a, R = C_2H_5
XII b, R = NH_2

Both the isomers were assigned structure on the basis of their ^{13}C spectra. The chemical shifts of all carbon signals are given in Fig. 1. The signal of the $C_{(3)}$ carbon in compound *XIIa* appears as a doublet of triplets with coupling constants $^1J_{C-H} =$

= 190·0 Hz and $^3J_{C-H}$ = 3·0 Hz for coupling with both protons of the methylene attached to $N_{(1)}$. On the other hand, the signal of $C_{(3)}$ in *XIIIa* occurs as a doublet with $^1J_{C-H}$ = 188·5 Hz showing that the side-chain is bonded to the heterocyclic system at the $N_{(2)}$ nitrogen (Fig. 2).

The chemical shifts and coupling constants in the proton spectra of these and other pyrazole derivatives (in hexadeuteriodimethyl sulfoxide) are given in Tables I–III. It is evident that, in comparison with their N^2-isomers, derivatives with the side-chain on the nitrogen adjacent to the CH group of the heterocycle have their H-3 and H-1′ proton signals shifted downfield and upfield, respectively. These facts

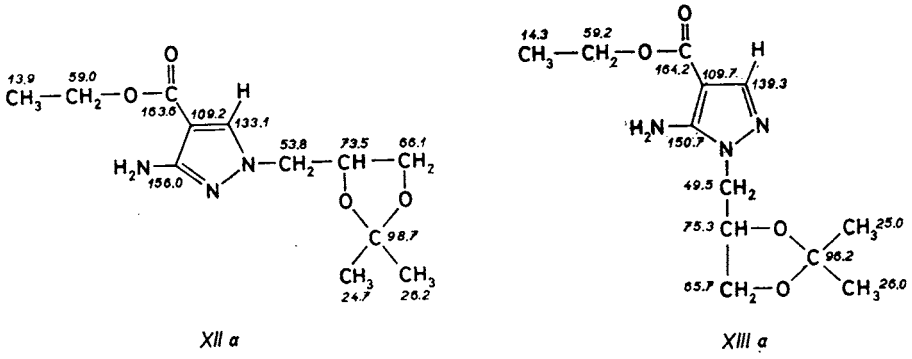


FIG. 1

^{13}C NMR chemical shifts in compounds *XIIa* and *XIIIa*

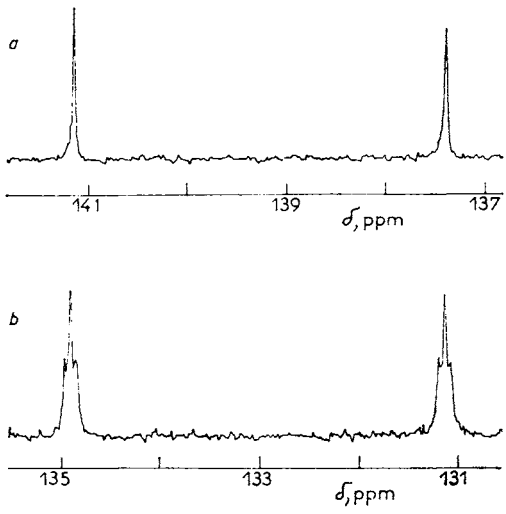
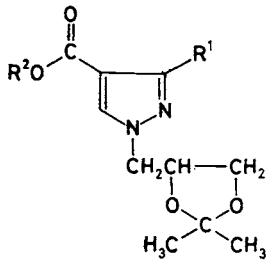


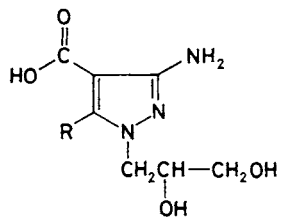
FIG. 2

Signals of $C_{(3)}$ in proton-coupled ^{13}C NMR spectra of compounds *a*) *XIIIa*, *b*) *XIIa*

agree with the rules generally valid for glycosides and other derivatives of five-membered nitrogen heterocycles[5,25].



XIII a, $R^1 = H$; $R^2 = C_2H_5$
XIII b, $R^1 = NH_2$; $R^2 = C_2H_5$
XIV a, $R^1 = R^2 = H$
XIV b, $R^1 = NH_2$; $R^2 = H$



XV, R = H
XVI, $R = NH_2$

TABLE I

Proton NMR parameters of pyrazole derivatives *VIIIa*—*Xb* in hexadeuteriodimethyl sulfoxide

Compound	Chemical shifts (δ)								
	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	H-3	COOR	NH_2
VIIIa	5·48	4·30	4·11	3·92	3·65	3·48	8·20	4·18 1·24	5·43
VIIIb	5·48	4·28	4·09	3·89	3·63	3·47	8·20	3·72	5·45
VIIIc	5·47	4·29	4·10	3·90	3·62	3·43	8·14	—	5·20
Xa	5·57	4·41	4·09	3·84	3·58	3·41	—	4·18 1·24	6·39 5·16
Xb	5·49	4·33	4·08	3·86	3·62	3·50	—	—	5·24

Compound	Coupling constants (Hz)					
	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	$J_{5'5''}$
VIIIa	4·2	5·0	5·0	3·8	5·0	−12·0
VIIIb	4·2	5·0	5·0	3·8	5·0	−12·0
VIIIc	4·0	4·8	4·8	3·8	4·8	−12·0
Xa	4·2	5·0	5·0	3·8	5·0	−12·0
Xb	5·2	5·4	3·9	3·4	4·3	−12·2

Once the structure of *XIIa* and *XIIIa* has been unequivocally determined by the ^{13}C NMR spectroscopy, the structure of other N-substituted pyrazole derivatives prepared in this work can be assigned also using their characteristic UV spectra (Table IV): chromophores of the type *XIIa* (*VIII*, *XVIII* and derived compounds) display an absorption maximum at 263 nm, with a hypsochromic shift of 7 nm for

TABLE II

Proton NMR parameters of pyrazole derivatives *XIIa*—*XVI* in hexadeuteriodimethyl sulfoxide

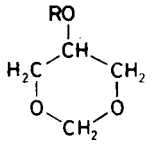
Compound	Chemical shifts (δ)								
	H-1′	H-1″	H-2′	H-3′	H-3″	H-3	COOR	NH_2	$C(CH_3)_2$
XIIa	4·00	4·00	4·36	4·00	3·75	7·91	4·17 1·25	5·37	1·32 1·27
XIIa[a]	4·00	4·00	4·42	4·07	3·76	7·86	4·26 1·33	4·80	1·39 1·34
XIIIa	4·10	3·99	4·35	4·00	3·78	7·47	4·16 1·24	6·23	1·31 1·26
XIIIa[a]	4·23	4·05	4·42	4·10	3·78	7·60	4·26 1·33	5·45	1·35 1·33
XIIIb	3·88	3·73	4·29	3·98	3·78	—	4·15 1·24	6·05 5·04	1·33 1·25
XV	3·38	3·38	3·85[b]	3·85[b]	3·85[b]	7·77	—	5·58	—
XVI	3·32	3·32	3·70[b]	3·70[b]	3·70[b]	—	—	5·20 4·86	—

Compound	Coupling constants (Hz)					
	$J_{1'2'}$	$J_{1''2'}$	$J_{1'1''}$	$J_{2'3'}$	$J_{2'3''}$	$J_{3'3''}$
XIIa	5·5	5·5	[b]	6·2	5·8	−8·5
XIIa[a]	5·5	5·5	[b]	6·3	5·9	−8·8
XIIIa	5·5	6·0	−14·5	6·0	5·8	−8·5
XIIIa[a]	2·8	5·0	−15·2	6·5	7·4	−8·7
XIIIb	5·4	6·0	−14·4	6·2	6·0	−8·5
XV	4·8	4·8	[b]	[b]	[b]	[b]
XVI	4·5	4·5	[b]	[b]	[b]	[b]

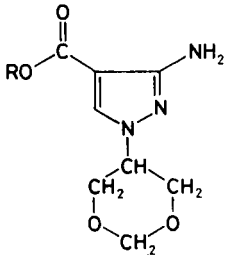
[a] Measured in C^2HCl_3-solution; [b] the value was not determined (unresolved multiplet).

carboxylic acids in a neutral medium, whereas the isomeric compounds (*XIIIa*, *XIXa* and related compounds) show a typical double maximum at about 227 and 254 nm in a neutral medium. Introduction of a second amino group into position 5 of the chromophore *XIIIa* results in a hypsochromic shift to 242–246 nm throughout the whole pH range, accompanied by another maximum at 218–220 nm in an acid medium (compounds *Xa*, *XIIIb*, and *XXa*).

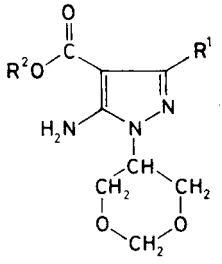
Mass spectra of all the above-mentioned esters of N-substituted aminopyrazole-carboxylic acids exhibit molecular peaks of the corresponding elemental composition (Table V) whereas the free acids show characteristic fragments at $m/z = M^+ - CO_2$. The heterocyclic base in the nucleosides as well as the open-chain analogues is characterized by the fragments B, BH and B—OC_2H_5*, the side-chain by the usual fragmentation pattern of ribonucleosides or fragments arising by loss of methyl or acetone from the isopropylidene derivatives *XII*–*XIV*. Spectra of these compounds display also typical peaks due to B + CH_2 fragments.



XVII a, R = H
XVII b, R = $SO_2C_6H_4CH_3(4\text{-})$



XVIII a, R = C_2H_5
XVIII b, R = H



XIX a, R^1 = H; R^2 = C_2H_5
XIX b, R^1 = R^2 = H
XX a, R^1 = NH_2; R^2 = C_2H_5
XX b, R^1 = NH_2; R^2 = H

Successive alkaline and acid hydrolysis of compounds *XIIa*, *XIIIa,b* afforded the unprotected open-chain analogues. The alkaline hydrolysis of the ester functionality

* B denotes the heterocyclic base.

required hard reaction conditions, similar to those applied to nucleosides (1 – 2 mol . $.l^{-1}$ sodium hydroxide), under which already a more profound destruction of the heterocyclic nucleus partly occured. Acid hydrolysis (removal of the isopropylidene group) of the thus-obtained derivatives of carboxylic acids afforded the final products *XV* and *XVI* whose properties corresponded to the anticipated structure.

The previous syntheses of 1,3-dihydroxy-2-propyl derivatives of heterocyclic bases consist in condensation of these bases with bromomalonic acid esters, followed by reduction[26]. We tried an alternative synthesis starting from the easily accessible[27] 5-*p*-toluenesulfonyloxy-1,3-dioxane (*XVIIb*). Sodium salt of the monoamino derivative *IV* reacted with compound *XVIIb* to give a mixture of isomers *XVIIIa* and *XIXa* which were identified analogously as the isomers *XIIa* and *XIIIa*. Sodium salt of the diamino derivative *V* afforded the single isomer *XXa*. The 1,3-dioxane derivatives *XVIIIa* and *XXa* were converted to salts of the carboxylic acids *XVIIIb* and *XXb*, which, without isolation, were subjected to acid cleavage. However, the

TABLE III

^{1}H NMR parameters of pyrazole derivatives *XVIIIa*—*XXb* in hexadeuteriodimethyl sulfoxide

Compound	Chemical shifts (δ)							
	H-1′	H-2′	H-2″	H-5′	H-5″	H-3	COOR	NH_2
XVIIIa	4·12[a]	4·12[a]	4·12[a]	4·88	4·84	8·05	4·19 1·26	5·41
XVIIIb	4·13[a]	4·13[a]	4·13[a]	4·89	4·82	8·01	—	5·0
XIXa	4·49	4·13	3·90	4·86	4·69	7·52	4·17 1·23	6·56
XXb	4·20	3·99	3·80	4·92	4·54	—	—	5·09 4·58

Compound	Coupling constants (Hz)			
	$J_{1'2'}$	$J_{1'2''}$	$J_{2'2''}$	$J_{5'5''}$
XVIIIa	[a]	[a]	[a]	−6·0
XVIIIb	[a]	[a]	[a]	−6·2
XIXa	4·0	10·0	−10·6	−6·3
XXb	4·7	10·0	−11·0	−6·3

[a] The value was not determined (unresolved multiplet).

compounds isolated by ion exchange chromatography were still 1,3-dioxane derivatives, characterized as the free carboxylic acids *XVIIIb* and *XXb*, whereas products of cleavage of the 1,3-dioxane ring were not detected. Thus, this method of preparing 1,3-dihydroxy-2-propyl derivatives of heterocyclic bases is prohibited by the high stability of the cyclic formal protecting group.

In conclusion, we can summarize that trimethylsilyl derivatives of 3-aminopyrazole-4-carboxylic acid esters are ribosylated analogously to the silyl derivative *III*, containing the 3-triazene group; this procedure is not suitable for preparing compounds of the type *IX* (or *I*). Under S_N2 reaction conditions, alkaline salts of the base *IV* are alkylated to give approximately equal amounts of both isomers.

TABLE IV
Ultraviolet absorption spectra (in aqueous solutions)

Formula	pH 2		pH 7	
	λ_{max}(nm)	ε_{max}	λ_{max}(nm)	ε_{max}
VIIIa	263	5 700	263[a]	6 300
VIIIb	263	5 700	263[a]	6 100
VIIIc	263	5 000	256[a]	6 100
Xa	218	12 800	244[a]	14 300
	242	27 200		
Xb	248	22 600	—	—
XIIa	—	—	263[b]	6 350
XIIIa	—	—	227[b]	6 000
			254	9 100
XIIIb	—	—	245[b]	15 000
XIVb	—	—	239[b]	—
XVI	239	22 400	—	—
XVIIIa	—	—	263[b]	—
XVIIIb	265	5 400	258	5 600
XIXa	—	—	228[b]	8 500
			256	8 000
XXa	—	—	246[b]	14 700
XXb	240	—	223	—

[a] Identical at pH 12; [b] in methanol solution.

TABLE V

Analysis and mass spectrometry data

Compound	Formula (mol. weight)	M^{+a} (*m/z*)	Calculated/found		
			% C	% H	% N
VIIIa	$C_{11}H_{17}N_3O_6$ (287·4)	287	45·99 45·91	5·96 5·93	14·63 14·37
VIIIb	$C_{10}H_{15}N_3O_6$ (273·3)	273	43·95 44·10	5·53 5·73	15·28 14·98
VIIIc	$C_9H_{13}N_3O_6$ (259·2)	—	41·70 41·96	5·05 5·29	16·21 16·14
Xa	$C_{11}H_{18}N_4O_6$ (302·3)	302	43·70 43·60	6·00 5·81	18·54 18·29
Xb	$C_9H_{14}N_4O_6$ (274·2)	—	39·41 39·55	5·15 5·45	20·43 20·87
XIIa	$C_{12}H_{19}N_3O_4$ (269·3)	269	53·51 53·66	7·11 7·01	15·60 15·77
XIIIa	$C_{12}H_{19}N_3O_4$ (269·3)	269	53·51 53·45	7·11 6·95	15·60 15·87
XIIIb	$C_{12}H_{20}N_4O_4$ (284·3)	284	50·69 50·27	7·09 6·89	19·71 19·96
XV	$C_7H_{11}N_3O_4$ (201·2)	—	41·79 42·02	5·51 5·38	20·89 21·03
XVI	$C_7H_{12}N_4O_4$ (216·2)	—	38·88 39·04	5·60 5·32	25·92 26·18
XVIIIa	$C_{10}H_{15}N_3O_4$ (241·3)	241	49·78 50·12	6·27 6·35	17·42 17·25
XVIIIb	$C_8H_{13}N_3O_5$[b] (231·2)	213	41·55 41·34	5·67 5·84	18·18 18·09
XIXa	$C_{10}H_{15}N_3O_4$ (241·3)	241	49·78 50·15	6·27 6·43	17·42 16·62
XXa	$C_{10}H_{16}N_4O_4$ (256·3)	256	46·86 46·99	6·29 6·27	21·87 21·53
XXb	$C_8H_{12}N_4O_4$ (228·2)	228	42·10 41·99	5·30 5·15	24·55 24·76

[a] Mass spectrum (mol. peak); [b] monohydrate.

Both ribosylation of silyl derivative of 3,5-diaminopyrazole-4-carboxylic acid (*VII*) and alkylation of sodium salt of the ester *V* give a single product, thus opening the way toward further, hitherto undescribed, pyrazolo[3,4*d*]pyrimidine compounds.

Biological activity. Compounds *VIIIa*–*VIIIc*, *IX*, *Xa*, *Xb*, *XV*, *XVI*, *XVIIIa*, and *XXa* were tested for antibacterial activity against *Escherichia coli* B in a synthetic medium with glucose. Neither of these compounds showed biological effect at concentrations up to 1 mg/ml of the medium. Compounds *VIIIa*, *VIIIc*, *Xa*, and *Xb* had no cytostatic effect on murine leukemic cells L-1210 grown in a tissue culture, at concentration 10^{-4} mol l^{-1}.

EXPERIMENTAL

Unless otherwise stated, the solvents were evaporated at 40°C/2 kPa and the compounds were dried at 13 Pa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected. Liquid chromatography (Table V) was carried out on 3·3 × 150 mm columns packed with Separon SIX C 18 (5 μ) (Laboratorní přístroje, Prague) in 0·1 mol l^{-1} triethylammonium acetate, pH 7·0, containing the following amounts of methanol (v/v): S1 0%, S2 10%, S3 40%; flow rate 0·4 ml/min, detection at 254 nm (an LDC instrument with an EZ 11 recorder; Laboratorní přístroje, Prague). Thin-layer chromatography (TLC) on silica gel was performed on Silufol UV 254 plates in chloroform–methanol 95 : 5 (S4), preparative TLC on 40 × 16 × 0·4 cm loose layers of Silpearl UV 254 (Kavalier, Czechoslovakia). Column chromatography was done on the same silica gel. Paper chromatography was carried out on a Whatman No 1 paper in 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2; S5), paper electrophoresis (40 V/cm, 1 h) on a Whatman No 3 MM paper in 0·1 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5. Chromatography on octadecyl silica gel (20 μ) was performed on a 30 × 1·5 cm column, chromatography on Sephadex A-25 (HCO_3^-) (Pharmacia, Uppsala, Sweden), Dowex 50X8 or Amberlite IRC 50 (H^+-form, 100–200 mesh) on a 30 × 2·5 cm column. The chromatographic procedures were followed continuously using a Uvicord instrument (LKB, Uppsala, Sweden). For the R_F and capacity factor values see Table VI. NMR spectra were measured on Varian XL-200 or Tesla BS-497 (100 MHz) instruments at 295 K in hexadeuteriodimethyl sulfoxide or deuteriochloroform (99·8% deuterium; Aldrich, USA) with tetramethylsilane as internal standard. The chemical shifts (ppm) and coupling constants (Hz) are given in Tables I–III. Ultraviolet spectra (Table IV) were taken in water or methanol on a Specord UV-VIS spectrometer (Carl Zeiss, Jena, GDR). Mass spectra were measured on an AEI MS 902 instrument (ion source temperature 120°C, electron energy 70 eV, direct inlet). The elemental composition measurements were done at resolution 10 000.

Silylation of 3-Amino-4-ethoxycarbonylpyrazole (*IV*)

A stirred mixture of compound *IV* (4 g; 26 mmol; ref.[14]), hexamethyldisilazane (35 ml) and ammonium sulfate (20 mg) was refluxed for 7 h under exclusion of moisture. After concentration at 40°C/2 kPa, the residue was distilled *in vacuo*, yielding 7·55 g (97%) of *VI*, b.p. 120–122°C/13 Pa.

Silylation of 3,5-Diamino-4-ethoxycarbonylpyrazole (*V*)

A mixture of compound *V* (4·25 g; 25 mmol; ref.[15]) hexamethyldisilazane (35 ml) and ammonium sulfate (20 mg) was processed as described for compound *VI*, affording 7·67 g (70%) of *VII*, b.p. 148–152°C/13 Pa.

1-(β-D-Ribofuranosyl)-3-amino-4-ethoxycarbonylpyrazole (*VIIIa*)
and 1-(β-D-Ribofuranosyl)-3-amino-4-methoxycarbonylpyrazole (*VIIIb*)

A) Compound *VI* (5·02 g; 16 mmol) was added to a solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride[28] (20 mmol) in acetonitrile (100 ml). After standing in a stoppered flask at room temperature for 5 days, the mixture was taken down *in vacuo*, the residue dissolved in chloroform (200 ml), washed with water (3 × 50 ml), dried over magnesium sulfate and taken down *in vacuo*. The residue was dissolved in 0·1 mol l^{-1} methanolic sodium methoxide (300 ml) and set aside at room temperature overnight. The mixture was neutralized with Dowex 50X8 (H^+-form), made alkaline with triethylamine and the suspension was filtered. The filtrate was evaporated *in vacuo*, the residue was dissolved in water (100 ml) and extracted with ether (3 ×

TABLE VI
Chromatography and electrophoresis data

Formula	k^a		R_F		$E_{Up}{}^b$
	S2	S3	S4	S5	
VIIIa	6·52	—	—	—	—
VIIIb	2·20	—	—	—	—
VIIIc	0·85[c]	—	—	0·60	0·62
Xa	4·58	—	—	—	—
Xb	0·03	—	—	0·55	0·16
XIIa	—	3·46	0·40	0·88	—
XIIb	—	0·32	—	0·67	0·58
XIIIa	—	4·50	0·46	—	—
XIIIb	—	2·45	0·20	0·75	—
XIVb	—	0·30	—	0·54	0·12
XV	—	—	—	0·40	0·65
XVI	—	—	—	0·35	0·14
XVIIIa	—	1·88	0·44	0·90	—
XVIIIb	—	0·43	—	—	—
XIXa	—	2·95	0·52	—	—
XIXb	—	0·18	—	0·65	0·58
XXa	—	0·92	0·23	0·81	—
XXb	—	0·20	—	0·57	0·18

[a] $k = (t_R - t_0)/t_0$, t_R retention time, t_0 hold-up time; [b] electrophoretic mobility related to uridine 3′-phosphate; [c] in S1.

25 ml). The aqueous phase was concentrated *in vacuo* to about 20 ml and applied on a column of Dowex 50X8 (H^+-form; 100 ml). Elution with water afforded a mixture of compounds *VIIIa* and *VIIIb* which, after evaporation, were separated on a column of Separon SIX C 18 (200 × 4 mm) in the system S1 (compound *VIIIb*) and then in S2 (compound *VIIIa*). The fractions containing pure compounds were desalted on a 50 ml column of Dowex 50X8 (H^+-form; elution with water) and taken down *in vacuo*, yielding 1·0 g (22%) of ethyl ester *VIIIa* and 1·0 g (23%) of methyl ester *VIIIb* (for characterization see Table IV—VI) as amorphous colourless foams. Mass spectrum (*m/z*): *VIIIa*: 287 (M^+), 257 (M − CH_2O), 256 (M − CH_2OH), 242 (M − C_2H_5O), 184 (B + 30), 168 (B + CH_2), 155 (BH), 110 (BH − C_2H_5O). *VIIIb:* 273 (M^+), 243 (M − CH_2O), 242 (M − CH_2OH), 170 (B + 30), 154 (BCH_2), 151 (BH), 110 (BH − OCH_3), 109 (BH − CH_3OH).

B) The reaction of compound *VI* (23·5 mmol) with the halogenose (20 mmol) was performed as described under *A*. After washing the chloroform solution with water and evaporation of the solvent, the residue was allowed to stand overnight with 0·1 mol l^{-1} sodium ethoxide in ethanol, neutralized with Dowex 50X8 and filtered. The filtrate was taken down *in vacuo*, the residue dissolved in water (100 ml) and the solution extracted with ether (3 × 25 ml). The aqueous layer was concentrated *in vacuo* and applied on a column of octadecyl-silica gel, pre-equilibrated with water. The column was washed with water (3 ml/min) until the principal UV-absorbing product began to be eluted, and then dioxane–water (1 : 4) was used as eluant. The product fractions were combined, the solvents were evaporated *in vacuo* and the residue in a small amount of water was applied on a column of Amberlite IRC 50 (H^+-form; 100 ml). Elution with water afforded pure (HPLC) product *VIIIa* which was again chromatographed on octadecyl-silica gel under the above-described conditions (3 ml/min) in water–ethanol (99 : 1). The obtained *VIIIa* (3·7 g; 64%) was identical with the product obtained according to *A*.

1-(β-D-Ribofuranosyl)-3-aminopyrazole-4-carboxylic Acid (*VIIIc*)

A solution of compound *VIIIb* (0·77 g; 2·8 mmol) in 0·4 mol l^{-1} sodium hydroxide (4 ml) was set aside at room temperature overnight and neutralized by addition of Dowex 50X8 (H^+-form). The suspension was applied on a column (50 ml) of the same ion exchanging resin. Elution with water afforded a UV-absorbing eluate which, after evaporation *in vacuo*, was dried by codistillation with ethanol. The residue, on dissolving in ethanol and precipitation with ether gave amorphous *VIIIc* (0·72 g; 99%) (see Table IV—VI). Mass spectrum (*m/z*): 215 (M − CO_2), 197 (215 − H_2O), 184 (215 − CH_2OH), 112 (B + 30 − CO_2), 83 (BH − CO_2).

1-(β-D-Ribofuranosyl)-3,5-diamino-4-ethoxycarbonylpyrazole (*Xa*)

A) Compound *VII* (5·02 g; 16 mmol) was added to a solution of the halogenose (20 mmol) in acetonitrile (100 ml). After standing in a stoppered flask for 5 days at room temperature, the mixture was taken down *in vacuo* and the residue dissolved in chloroform (200 ml). The solution was washed with water (3 × 50 ml) and dried over magnesium sulfate. The solvent was evaporated, the residue allowed to stand with 0·1 mol l^{-1} methanolic sodium methoxide (300 ml) at room temperature overnight and neutralized with Dowex 50X8 (H^+-form). The suspension was made alkaline with triethylamine, filtered and the resin was washed with methanol. The filtrate was evaporated *in vacuo* and the residue dissolved in water and extracted with ether (3 × 25 ml). The aqueous phase was concentrated *in vacuo* to a small volume and applied on a column of Dowex 50X8 (H^+-form; 50 ml). After washing with water to drop of conductivity and UV absorption, the material was eluted with dilute (1 : 10) aqueous ammonia and the UV-absorbing eluate was collected and taken down *in vacuo*. The residue was dissolved in a small

amount of water, made alkaline (pH 8—9) with ammonia, applied on a column of Dowex 1X2 (acetate; 50 ml) and eluted with water. The UV-absorbing eluate was evaporated, the residue codistilled with ethanol and precipitated with ether from methanol (2 ml). The product was dried *in vacuo*; yield 2·92 g (60%) of *Xa*, homogeneous according to HPLC. For analysis, NMR and UV spectra and other data see Tables I—VI. Mass spectrum (*m/z*): 302 (M^+), 271 (M − CH_2OH), 257 (M − C_2H_5O), 170 (BH), 124 (B − C_2H_5OH).

B) The mixture, prepared from compound *VII* (20 mmol) and the halogenose (20 mmol) according to *A*, after evaporation of chloroform was heated with 30% methanolic ammonia (100 ml) to 100°C for 12 h in an autoclave. After evaporation *in vacuo*, the residue was dissolved in water (100 ml), extracted with chloroform (5 × 20 ml) and the aqueous phase was concentrated *in vacuo* to about 20 ml. This solution was applied on a column of Dowex 50X8 (H^+-form) and processed as described under *A*, affording 3·22 g (53%) of compound *Xa*, homogeneous on HPLC and identical with the product prepared according to *A*.

1-(β-D-Ribofuranosyl)-3,5-diaminopyrazole-4-carboxylic Acid (*Xb*)

A solution of compound *Xa* (0·61 g; 2 mmol) in 1 mmol l^{-1} sodium hydroxide (25 ml) was set aside at room temperature overnight, neutralized with Dowex 50X8 (H^+-form) and the suspension was poured onto a column of the same ion-exchanging resin (50 ml). The column was washed with water to loss of UV-absorption and then with dilute (1 : 10) ammonia. The UV-absorbing ammonia fraction was taken down, the residue dissolved in water (10 ml) and applied on a column of Sephadex A-25 (HCO_3^-; 100 ml). After washing the column with water, the material was eluted with a gradient (0—0·2 mol l^{-1}) of triethylammonium hydrogen carbonate, pH 7·5 (0·5 l each). The product-containing fractions were combined and the solvent evaporated *in vacuo*. The residue was codistilled with methanol to remove the buffer and chromatographed on a column of octadecyl-silica gel in water. The UV-absorbing eluate was taken down, the residue codistilled with ethanol *in vacuo* and precipitated from ethanol (1 ml) with ether, yielding 0·32 g (58%) of compound *Xb*, homogeneous according to HPLC (Table VI).

Attempted Ammonolysis of Ester *VIIIa*

A) Compound *VIIIa* (0·2 g) was heated with 30% methanolic ammonia (30 ml) in an autoclave to 140°C for 16 h. HPLC analysis in systems S1 and S2 showed that the reaction mixture contained only the unchanged starting *VIIIa*.

B) To a solution of compound *VIIIa* (0·14 g; 0·5 mmol) in 30% methanolic ammonia (50 ml) was added 1 mol l^{-1} sodium methoxide (60 µl) and the mixture was heated to 140°C for 12 h. After evaporation of the solvent, HPLC analysis showed the residue to be the starting *VIIIa* with only traces (<1%) of *VIIIc*.

C) 1,8-Diazabicyclo[5.4.0]undec-7-ene (0·5 ml) was added to a solution of compound *VIIIa* (0·57 g; 2 mmol) in 30% methanolic ammonia (60 ml) and the mixture was heated to 120°C for 7 h. After evaporation *in vacuo*, the residue was analyzed by HPLC in the systems S1 and S2; it contained 61% of the starting *VIIIa*, 24% of *VIIIc*, and 13% of an unknown product (k = 1·87 in S1).

1-(*RS*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-5-amino-4-
-ethoxycarbonylpyrazole (*XIIIa*) and 1-(*RS*)-(2,2-Dimethyl-
-1,3-dioxolan-4-yl)methyl-3-amino-4-ethoxycarbonylpyrazole (*XIIa*)

A) 4-Chloromethyl-2,2-dimethyl-1,3-dioxolane (*XI*; 6 ml) was added dropwise at 100°C to a stirred suspension of compound *IV* (4·65 g; 30 mmol) and potassium carbonate (9 g) in di-

methylformamide (75 ml). After stirring and refluxing at 140°C for 7 h (calcium chloride protective tube), potassium carbonate (4·5 g) and compound *XI* (3 ml) were added and heating was continued for 9 h. The hot mixture was filtered and the solvent was evaporated at 70°C/13 Pa. The dry residue was brieffly boiled with chloroform (200 ml), filtered through Celite which was then washed with chloroform, and the solvent was evaporated. The residue was chromatographed on a column of silica gel (350 ml) in chloroform, affording 3·9 g (48%) of oily *XIIIa* and 2·9 g (37%) of *XIIa*, m.p. 99°C (ethyl acetate–light petroleum). Mass spectrum (*m/z*) (the same for *XIIa* and *XIIIa*): 269 (M^+), 254 (M − CH_3), 224 (M − OC_2H_5), 211 (M − CH_3COCH_3), 168 (B + CH_2), 155 (BH).

B) Sodium hydride (10 mmol) was added to a solution of compound *IV* (10 mmol) in dimethylformamide (30 ml) and the mixture was stirred at room temperature to homogeneity (30 min). Compound *XI* (10·5 mmol) was added, the mixture was heated to 100°C for 8 h under exclusion of moisture and processed as described under *A*, yielding 1·34 g (49%) of *XIIIa* and 1·33 g (49%) of *XIIa*, m.p. 99°C.

1-(*RS*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-3,5-diamino-4-ethoxycarbonylpyrazole (*XIIIb*)

A) Compound *XI* (6 ml) was added dropwise at 100°C to a stirred mixture of compound *V* (5·1 g; 30 mmol), potassium carbonate (9 g) and dimethylformamide (75 ml). After refluxing for 7 h with stirring (bath temperature 150°C, calcium chloride tube), further potassium carbonate (4·5 g) and compound *XI* (3 ml) were added and heating was continued for another 9 h. The subsequent work-up procedure was the same as described for the preparation of *XIIa* and *XIIIa*. After chromatography on silica gel, the product was crystallized from ethyl acetate and light petroleum to give 3·65 g (43%) of *XIIIb*, m.p. 86°C. Analytical and other data are given in Tables I—VI. Mass spectrum (*m/z*): 284 (M^+), 269 (M − CH_3), 239 (M − OC_2H_5), 183 (B + CH_2), 170 (BH), 137 (B + CH_2 − C_2H_5OH), 124 (BH − C_2H_5OH; base peak).

B) Sodium hydride (10 mmol) was added to a solution of compound *V* (10 mmol) in dimethylformamide (30 ml). When the mixture became homogeneous (stirring for 30 min at room temperature), compound *XI* (10·5 mmol) was added. After heating to 100°C for 8 h (calcium chloride tube), the mixture was processed as described under *A*, affording 2·2 g (77%) of *XIIIb*, m p. 86°C.

1-(*RS*)-(2,3-Dihydroxypropyl)-3-aminopyrazole-4-carboxylic Acid (*XV*)

A solution of compound *XIIa* (0·8 g; 3 mmol) in 1 mol l^{-1} sodium hydroxide (25 ml) was heated to 100°C and the reaction was monitored by HPLC in S3. After 7 h the conversion was almost quantitative. The mixture was cooled, neutralized with Dowex 50X8 (H^+-form), filtered and taken down. The residue (compound *XIVa*) was set aside overnight with 0·25 mol l^{-1} sulfuric acid (25 ml) at 37°C and accurately neutralized with barium hydroxide solution. The mixture was warmed to 80°C and after 20 min filtered through Celite which was then washed with hot water (100 ml). After evaporation *in vacuo*, the residue was chromatographed on a column of octadecyl-silica gel (90 ml) in water. The UV-absorbing eluate was taken down and chromatographed on Dowex 50X8 (Li^+-form; 10 ml). The UV-absorbing aqueous eluate on evaporation and precipitation with ether from methanol afforded 0·60 g of compound *XV* (as the Li^+ salt), homogeneous in S2 and S5. The free acid *XV* was obtained by chromatography on a column of Amberlite IRC 50 (H^+-form; 25 ml) in water; the product was precipitated from ethanol with ether, yield 90% (based on the lithium salt). Mass spectrum (*m/z*): 157 (M^+ − CO_2).

1-(*RS*)-(2,3-Dihydroxypropyl)-3,5-diaminopyrazole-4-carboxylic Acid (*XVI*)

A solution of compound *XIIIb* (1·0 g; 3·5 mmol) in 1 mol l^{-1} sodium hydroxide (30 ml) was heated to 100°C till the reaction was complete (6 h, determined in S3). The mixture was processed analogously as described for compound *XV* (the intermediate *XIVb* was hydrolyzed with 25 ml of 0·25 mol l^{-1} sulfuric acid at 70°C for 4 h) and the barium salt of compound *XVI* was chromatographed on a column of Dowex 50X8 (H^+-form; 20 ml). After washing the column with water to drop of UV-absorption, the product was eluted with dilute (1 : 10) aqueous ammonia.The UV-absorbing eluate was taken down *in vacuo*, the residue was dissolved in water (5 ml), applied on a column of octadecyl-silica gel (90 ml) and the product was eluted with water. Water was evaporated and the dry residue was precipitated with ether from methanol to give 0·60 g (79%) of compound *XVI*, homogeneous in S2 and S5. Mass spectrum (*m/z*): 172 ($M^+ - CO_2$).

5-*p*-Toluenesulfonyloxy-1,3-dioxane (*XVIIb*)

A mixture of glycerol (300 g; 3·26 mol), paraformaldehyde (100 g), *p*-toluenesulfonic acid monohydrate (5 g) and benzene (500 ml) was refluxed with azeotropic removal of water. After no more water had separated (8 h), the mixture was cooled, made alkaline with triethylamine, washed with water (2 × 100 ml) and dried over magnesium sulfate. Benzene was evaporated *in vacuo* and the residue distilled to give 160 g (49%) of mixture of 5-hydroxy-1,3-dioxane (*XVIIa*) and 4-hydroxymethyl-1,3-dioxolane, b.p. 98°C/2 kPa. The mixture (104 g; 1 mol) was dissolved in pyridine (500 ml) and *p*-toluenesulfonyl chloride (209·5 g; 1·1 mol) in pyridine (500 ml) was added during 1 h with stirring and cooling with ice. After stirring for 6 h in ice, the mixture was allowed to stand at room temperature overnight and concentrated *in vacuo* to about 400 ml. The residue was diluted with benzene (1 500 ml), washed with water (3 × 200 ml), dried over magnesium sulfate, filtered and the solvent was removed *in vacuo*. The residue was codistilled with toluene (3 × 200 ml) and mixed with ether (1 litre). The suspension was stirred for 1 h at 0°C, the product was filtered, washed with ether, dried and crystallized from ethyl acetate (ether added to turbidity) in a refrigerator overnight. The needles of *XVIIb*, m.p. 93°C, were filtered, washed with ether and dried; yield 168 g (65%). For $C_{11}H_{14}O_5S$ (258·3) calculated: 51·15% C, 5·46% H, 12·41% S; found: 51·49% C, 5·50% H, 12·05% S. ^{1}H NMR spectrum (deuteriochloroform): 2·45 (s, 3 H) CH_3; 3·77 (dd, 2 H) + 3·98 (dd, 2 H) ($J_{CH,CH} = 3·5$, $J_{CH,CH'} = 5·7$, $J_{gem} = 14·0$) OCH_2C; 4·46 (m, 1 H) CH—OTs; 4·77 (2 d, 2 H, $J_{gem} = 7·0$) O—CH_2—O; 7·36 (d, 2 H) + 7·82 (d, 2 H) arom. protons.

1-(1,3-Dioxan-5-yl)-3-amino-4-ethoxycarbonylpyrazole (*XVIIIa*)
and 1-(1,3-Dioxan-5-yl)-5-amino-4-ethoxycarbonylpyrazole (*XIXa*)

Sodium hydride (0·48 g; 20 mmol) was added to a solution of compound *IV* (3·1 g; 20 mmol) in dimethylformamide (60 ml). After stirring for 30 min under exclusion of moisture, compound *XVIIb* (6 g; 23 mmol) was added and the mixture was stirred at 100°C for 8 h under exclusion of moisture. The solvent was evaporated *in vacuo* and the residue extracted with boiling chloroform (200 ml). The extract was stripped of the solvent and chromatographed on a column of silica gel (300 ml) in chloroform, affording 1·36 g (28%) of compound *XVIIIa*, m.p. 53—54°C (ethyl acetate–light petroleum) and 1·40 g (29%) of compound *XIXa*, m.p. 75—76°C. Analytical and other data for both compounds are given in Tables I—VI. Mass spectra (identical for both isomers), *m/z*: 241 (M^+), 196 ($M - OC_2H_5$), 155 (base peak), 109 ($BH - C_2H_5OH$).

1-(1,3-Dioxan-5-yl)-3,5-diamino-4-ethoxycarbonylpyrazole (*XXa*)

Compound *V* (3·4 g; 20 mmol) was treated with sodium hydride and compound *XVIIb*, and the

mixture was worked up in the same manner as described for compound *XVIIIa* and *XIXa*. Chromatography on silica gel and crystallization from ethyl acetate–light petroleum afforded 1·32 g (26%) of compound *XXa*, m.p. 161°C. Mass spectrum (*m/z*): 256 (M^+), 211 (M − C_2H_5O), 170 (BH; base peak), 124 (BH − C_2H_5OH).

1-(1,3-Dioxan-5-yl)-3-aminopyrazole-4-carboxylic Acid (*XVIIIb*)

A mixture of compound *XVIIIa* (1·2 g; 5 mmol) and 0·7 mol l^{-1} sodium hydroxide (40 ml) in 50% aqueous methanol was kept at 37°C for 48 h and then neutralized by addition of Dowex 50X8 (H^+-form). After filtration and evaporation, the residue was warmed with 0·25 mol l^{-1} sulfuric acid (30 ml) to 37°C for 48 h, made alkaline with ammonia, taken down *in vacuo* and the residue was applied on a column of Dowex 1X2 (acetate; 100 ml). The column was washed with water to drop of UV absorption and conductivity and then with a linear gradient (à 500 ml) of 0—0·5 mol l^{-1} acetic acid. The UV-absorbing fraction was taken down, the residue codistilled with ethanol and crystallized from 70% aqueous ethanol (ether added to turbidity), affording 0·45 g (42%) of compound *XVIIIb*, m.p. 213°C. Mass spectrum (*m/z*): 213 (M^+), $C_8H_{11}N_3O_4$, 153 (B − $CH_2CH_2^+$, $C_6H_7N_3O_2$), 135 (153 − H_2O), 127 (BH), 109 (BH − H_2O).

1-(1,3-Dioxan-5-yl)-3,5-diaminopyrazole-4-carboxylic Acid (*XXb*)

A mixture of compound *XXa* (0·75 g; 2·9 mmol) and 0·7 mol l^{-1} sodium hydroxide in 50% aqueous methanol (40 ml) was processed similarly as described for compound *XVIIIb*. The product was eluted from Dowex already with 0·02 mol l^{-1} acetic acid and, after evaporation and codistillation with ehanol, precipitated from ethanol (5 ml) with ether (100 ml); yield 0·30 g (45%) of amorphous *XXb*, not melting below 260°C. Mass spectrum (*m/z*): 184 (M − CO_2; $C_7H_{12}N_4O_2$), 124 (B + CH_2CH_2 − CO_2, $C_5H_8N_4$).

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Note added in proof: In formulae *VI* and *VII*, substituent R should be attached in the free position 5 of the given structure. In formulae *XIIIa*—*XIVb*, an NH_2 group should be added into the position 5.