H_2O layer was acidified with AcOH to precipitate yellow crystals of IV, mp above 300°. Yield, almost theoretical. Recrystallization of this compound was difficult and accordingly it was converted to the 2-ethylthio derivative (V) without purification as mentioned below.

Method B: A solution of 0.36 g of III in 15 ml of EtOH containing 0.26 g of KSH was heated at 60° for 2 hr. After removal of the solvent, a small amount of H_2O was added, treated with charcoal, and acidified with AcOH to yield 0.3 g of IV.

5-Chloro-2-ethylthiothiazolo(5,4-*d*)pyrimidine (V)——From IV: To a solution of 0.2 g of IV (crude) in 6 ml of MeOH containing 0.05 g of KOH was added 0.12 g of EtBr and the mixture was refluxed for 20 min. Removal of the solvent left an oily residue which was solidified soon. It was recrystallized from petr. ether–MeOH to give 0.2 g of V as colorless needles, mp 85—88°. *Anal.* Calcd. for $C_7H_6N_3ClS_2$: C, 36.21; H, 2.59; N, 18.10. Found: C, 36.28; H, 2.31; N, 18.20.

From III: To a solution of EtSNa (prepared from 0.14 g of Na, 0.5 g of EtSH and 10 ml of EtOH) was added a solution of 1.2 g of III in 30 ml of EtOH and the reaction mixture was refluxed for 20 min. After evaporation of the solvent, a small amount of H_2O was added, the separated crystals were recrystallized from petr. ether–MeOH to colorless needles. Yield, 1.1 g. This product was identical with V prepared from IV.

5-Ethylamino-2-ethylthiothiazolo(5,4-*d*)pyrimidine (VIb)——To 5 ml of MeOH containing 0.07 g of NaOH was added 0.14 g of $EtNH_2HCl$ and after shaking for a few minutes, 0.2 g of V was added to this solution. The reaction mixture was heated at 100° for 5 hr in a sealed tube. MeOH was distilled off in vacuum and addition of a small amount of H_2O to the residue gave 0.23 g of the crystalline product of VIb. Recrystallization from MeOH gave colorless prisms, mp 119—121°. *Anal.* Calcd. for $C_9H_{12}N_4S_2$: C, 45.00; H, 5.00. Found: C, 44.90; H, 5.10. All the other 5-alkylamino-2-ethylthio derivatives in Table II were prepared by the same method.

5-Chloro-2-ethylaminothiazolo(5,4-*d*)pyrimidine (VIIa)——A mixture of two moles of $EtNH_2$ (0.45 g) and 2 g of III in 60 ml of EtOH was refluxed for 4 hr. The reaction mixture was treated similarly as described under the reaction of VIb with $EtNH_2$. The product was recrystallized from benzene to give colorless scales of VIIa, mp 159°. Yield, 1.9 g. *Anal.* Calcd. for $C_7H_7N_4ClS$: N, 26.05. Found: N, 26.16. Other 2-alkylamino derivatives, shown in Table III, were prepared by essentially the same method.

2-Ethylamino-5-ethylthiothiazolo(5,4-*d*)pyrimidine (VIIIb)——A solution of 1 g of VIIa in 10 ml of EtOH was added to a solution of EtSNa (prepared from 0.11 g of Na, 0.5 g of EtSH and 10 ml of EtOH) and the mixture was heated under reflux for 2 hr. After removal of the solvent, a small amount of H_2O was added to the residue and the separated crystalline solid was collected. Recrystallization from benzene–petr. benzin gave colorless scales, mp 128°. *Anal.* Calcd. for $C_9H_{12}N_4S_2$: N, 23.33. Found: N, 23.42. All the other 2-alkylamino-5-alkylthiothiazolo(5,4-*d*)pyrimidines prepared from 2-alkylamino-5-chlorothiazolopyrimidines were listed in Table IV.

[Chem. Pharm. Bull. 16(4) 745—750 (1968)]

UDC 547.857.4.07 : 615.778

Studies on Pyrimidine Derivatives. IX.[1] Synthesis of Some Alkylamino- and Alkylthio-, Thiazolo(5,4-*d*)pyrimidines. (2)

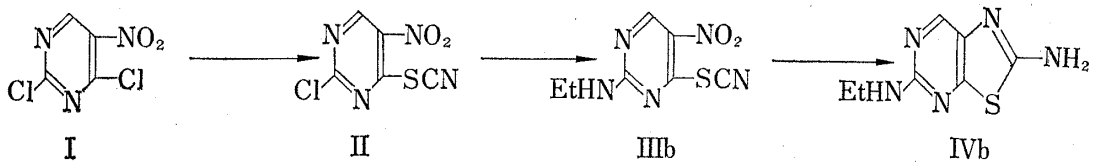
Sumi Sugiura, Eiji Suzuki, Takio Naito, and Shoji Inoue[2]

Faculty of Pharmacy, Meijo University[2]

(Received September 28, 1967)

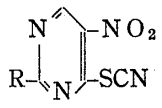
Since 2-amino-5-anilinothiazolo(5,4-*d*)pyrimidine has shown some anticancer activities as reported in the foregoing paper,[3] we have prepared several 5-alkylamino-2-amino derivatives and related compounds in the hope of finding more beneficial change in their activities. Synthesis of these derivatives was carried out by the same procedure as in the case of 2-amino-

1) Part VIII: *Chem. Pharm. Bull.* (Tokyo), **16**, 741 (1968).
2) Location: *Yagotourayama, Tenpaku-cho, Showa-ku, Nagoya.*
3) S. Sugiura, E. Suzuki, T. Naito, and S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **16**, 741 (1968).

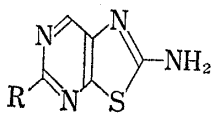


I II IIIb IVb

Chart 1

Table I.  IIIa—i

No.	Substituents R	Crude yield (%)	Appearance	Recrystn. solv.	mp (decomp.) (°C)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
IIIa	CH_3NH	97	pale yellow prism	EtOH	191.5—192	$C_6H_5O_2N_5S$	34.12	2.37	34.31	2.78
IIIb	C_2H_5NH	88	pale yellow prism	benzene	166—167	$C_7H_7O_2N_5S$	37.33	3.11	37.73	3.23
IIIc	C_3H_7NH	80	pale yellow prism	benzene	134—135	$C_8H_9O_2N_5S$	40.17	3.77	40.77	3.94
IIId	C_4H_9NH	85	pale yellow prism	benzene	103—104	$C_9H_{11}O_2N_5S$	42.69	4.35	42.88	4.76
IIIe	$C_6H_5CH_2NH$	95	pale yellow prism	benzene	165	$C_{12}H_9O_2N_5S$	50.17	3.14	50.48	3.28
IIIf	p-$CH_3C_6H_4NH$	88	red yellow prism	MeOH	213—214	$C_{12}H_9O_2N_5S$	50.17	3.14	50.27	3.46
IIIg	p-$CH_3OC_6H_4NH$	99	red yellow prism	benzene	189—191	$C_{12}H_9O_3N_5S$	47.52	2.97	48.12	3.29
IIIh	C_6H_5NHNH	63	pale yellow prism	EtOH	(158)	$C_{11}H_8O_2N_6S$	45.83	2.78	45.91	2.79
IIIi	$C_6H_5N(CH_3)$	91	yellow scale	MeOH	152—153	$C_{12}H_9O_2N_5S$	50.17	3.14	50.51	3.54

Table II.  IVa—i

No.	Substituents R	Crude yield (%)	Appearance	Recrystn. solv.	mp (°C)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
IVa	CH_3NH	76	colorless amorphous solid	H_2O or benzene–MeOH	251	$C_6H_7N_5S$	39.75	3.87	39.93	4.17
IVb	C_2H_5NH	100	colorless amorphous solid	acetone	214—215	$C_7H_9N_5S$	43.08	4.62	43.02	4.61
IVc	C_3H_7NH	77	colorless amorphous solid	20% EtOH or AcOEt	189—190	$C_8H_{11}N_5S$	45.93	5.26	46.15	5.43
IVd	C_4H_9NH	91	colorless amorphous solid	benzene or AcOEt	187	$C_9H_{13}N_5S$	48.43	5.83	48.68	5.85
IVe	$C_6H_5CH_2NH$	98	colorless amorphous solid	EtOH	207	$C_{12}H_{11}N_5S$	56.03	4.28	55.79	4.38
IVf	p-$CH_3C_6H_4NH$	98	colorless amorphous solid	MeOH	255—256	$C_{12}H_{11}N_5S$	56.03	4.28	56.23	4.27
IVg	p-$CH_3OC_6H_4NH$	94	colorless amorphous solid	H_2O–MeOH	222	$C_{12}H_{11}ON_5S$	52.75	4.03	53.25	4.25
IVh	$C_6H_5N(CH_3)$	91	colorless amorphous solid	60% MeOH	188—189	$C_{12}H_{11}N_5S$	56.03	4.28	56.02	4.53
IVi	p-ClC_6H_4NH	77	colorless amorphous solid	EtOH	254	$C_{11}H_8N_5ClS$	47.48	2.88	47.91	2.99

5-anilinothiazolo(5,4-*d*)pyrimidine from the corresponding 5-nitro-4-thiocyanato derivative, as reported in Part II[4] of this series, which may be considered to be the best method for the preparation of the 2-aminothiazolo(5,4-*d*)pyrimidines. As an example of the above method, the synthesis of ethylamino derivative (IVb) was shown as follows:

A benzene solution of 2-chloro-5-nitro-4-thiocyanatopyrimidine (II) was treated with two moles of ethylamine at 0° to give in good yield, 2-ethylamino-5-nitro-4-thiocyanatopyrimidine (IIIb), which was subsequently cyclized to 2-amino-5-ethylaminothiazolo(5,4-*d*)pyrimidine (IVb)by reduction with iron powder and acetic acid. Compounds obtained by the same treatment of II with several amines were shown in Table I, and their reduced derivatives were listed in Table II.

2-Alkylamino-5-amino-4-mercaptopyrimidine derivatives (Va—d), shown in Table III, were obtained by heating of the corresponding 2-aminothiazolo(5,4-*d*)pyrimidines (IVa—d) at 100° with 15% sodium hydroxide for more than ten hours. Refluxing of Va—d with ethyl orthoformate (or formic acid) or potassium methylxanthate in methanol gave the corresponding 5-alkylamino- and 5-alkylamino-2-mercapto-thiazolo(5,4-*d*)pyrimidine respectively as shown in Chart 2, and compounds obtained were listed in Table IV.

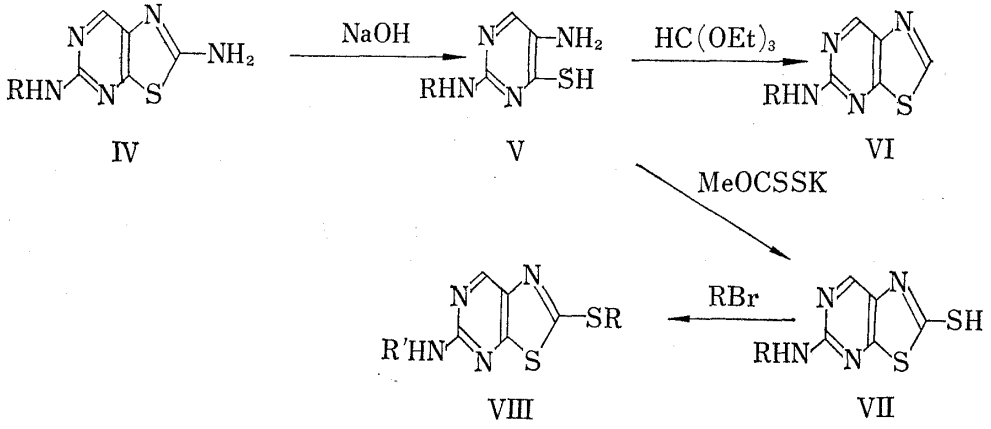
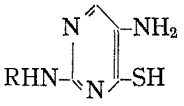


Chart 2

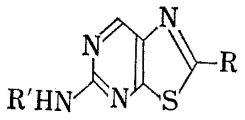
TABLE III.  Va—d

No.	Substituents R	Crude yield (%)	Appearance	Recrystn. solv.	mp (°C)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
Va	C_2H_5	98	yellow scale	benzene–MeOH	156	$C_6H_{10}N_4S$	42.35	5.88	42.67	6.21
Vb	$C_6H_5CH_2$	96	yellow scale	AcOEt	157—158	$C_{11}H_{12}N_4S$	56.90	5.17	57.11	5.34
Vc	*p*-ClC_6H_4	73	yellow amorphous solid	reprecipitation	216	$C_{10}H_9N_4ClS$	47.43	3.56	47.36	3.71
Vd	H	95	yellow needle	H_2O	235	$C_4H_6O_4N$	33.80	4.26	33.53	4.33[4]

Finally, 2-mercapto derivatives in Table IV were converted by the usual method to the 2-alkylthio derivatives (Table V), which were submitted to their antibacterial screening tests.

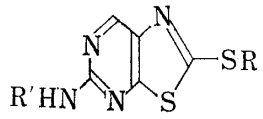
Some results on the antibacterial screening tests obtained up to the present time were shown in Table VI. Further results and details concerning antibacterial and anticancer activities will be reported in the near future.

4) T. Naito and S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 338 (1958).

Table IV.  Ⅵa—d, Ⅶa—d

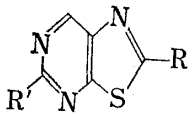
No.	Substituents R	Substituents R′	Reagent	Crude yield (%)	Appearance
Ⅵa	H	C_2H_5	$HC(OC_2H_5)_3$	86	pale yellow needle
Ⅵb	H	$C_6H_5CH_2$	$HC(OC_2H_5)_3$	86	colorless needle
Ⅵc	H	*p*-ClC_6H_4	$HC(OC_2H_5)_3$	98	colorless needle
Ⅵd	H	H	HCOOH	55	colorless prism
Ⅶa	SH	C_2H_5	CH_3OCSSK	92	yellow amorphous solid
Ⅶb	SH	$C_6H_5CH_2$	CH_3OCSSK	96	yellow amorphous solid
Ⅶc	SH	*p*-ClC_6H_4	CH_3OCSSK	85	yellow amorphous solid
Ⅶd	SH	H	CH_3OCSSK	81	yellow needle

No.	Recrystn. solv.	mp (deomp.) (°C)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
Ⅵa	benzene–petr. benzin	136—137	$C_7H_8N_4S$	46.67	4.44	47.24	4.11
Ⅵb	benzene–ligroin	124—125	$C_{12}H_{10}N_4S$	59.50	4.13	59.77	4.28
Ⅵc	benzene	178—179	$C_{11}H_7N_4ClS$	50.19	2.66	50.56	2.65
Ⅵd	EtOH	248—249	$C_5H_4N_4S$	39.48	2.65	39.49	2.87[4]
Ⅶa	reprecipitation	(240—241)	$C_7H_8N_4S_2$	39.62	3.77	39.66	3.51
Ⅶb	reprecipitation	(229—232)	$C_{12}H_{10}N_4S_2$	52.55	3.65	52.25	3.94
Ⅶc	reprecipitation	>260	$C_{11}H_7N_4ClS_2$	44.75	2.37	45.12	2.58
Ⅶd	EtOH	>300	$C_5H_4N_4S_2$	32.62	2.19	32.84	2.40[4]

Table V.  Ⅷa—k

No.	Substituents R	Substituents R′	Crude yield (%)	Appearance	Recrystn. solv.	mp (°C)	Fourmla	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
Ⅷa	C_2H_5	C_2H_5	95	colorless needle	MeOH	119—121	$C_9H_{12}N_4S_2$	45.00	5.00	44.90	5.10
Ⅷb	C_2H_5	$C_6H_5CH_2$	91	colorless scale	MeOH	125—126	$C_{14}H_{14}N_4S_2$	55.63	4.64	54.92	4.78
Ⅷc	CH_3	H	78	colorless needle	benzene	190	$C_6H_6N_4S_2$	36.36	3.03	36.22	3.06
Ⅷd	C_3H_5	H	98	colorless pillar	benzene	145	$C_8H_8N_4S_2$	42.86	3.57	42.79	3.79
Ⅷe	C_3H_7	H	97	colorless scale	benzene	147—148	$C_8H_{10}N_4S_2$	42.48	4.42	42.62	4.60
Ⅷf	*iso*-C_3H_7	H	85	colorless scale	benzene	173	$C_8H_{10}N_4S_2$	42.48	4.42	42.47	4.36
Ⅷg	C_4H_9	H	88	colorless scale	benzene	161	$C_9H_{12}N_4S_2$	45.00	5.00	44.87	5.09
Ⅷh	C_5H_{11}	H	78	colorless scale	benzene	157	$C_{10}H_{14}N_4S_2$	47.24	5.51	47.18	5.40
Ⅷi	C_6H_{13}	H	43	colorless scale	benzene	141	$C_{11}H_{16}N_4S_2$	49.25	5.97	49.36	6.09
Ⅷj	C_8H_{17}	H	44	colorless scale	benzene	152	$C_{13}H_{20}N_4S_2$	52.70	6.76	52.80	6.59
Ⅷk	$C_{10}H_{21}$	H	44	colorless scale	benzene	151—152	$C_{15}H_{24}N_4S_2$	55.38	7.38	55.29	7.46

Table VI.



Substituents R	Substituents R′	Organism	Days incub.	γ/ml causing Complete inhibition	γ/ml causing Partial inhibition	Standard control Compound	Standard control γ/ml compl.
Cl	Cl	*M. tuber.*[a]	7	20	—	INH[e]	0.024
SH	Cl	*Strep.*[b]	1	10	5	CM[f]	0.78
SH	$NHCH_2C_6H_5$	*Strep.*	1	>20	20	CM	0.78
SH	$NHCH_2C_6H_5$	*Staph.*[c]	1	20	—	CM	3.13
SH	*p*-NHC_6H_4Cl	*Strep.*	1	20	10	CM	0.78
SC_2H_5	NH-*iso*-C_3H_7	*cholerae*[d2]	1	50	—	CM	0.5
						SM[g]	20
SC_3H_5	NH_2	*cholerae*[d1]	1	30	—	CM	0.5
						SM	20
SC_3H_7	NH_2	*cholerae*[d2]	1	10	—	CM	0.5
						SM	20
S-*iso*-C_3H_7	NH_2	*cholerae*[d1,2]	1	50	—	CM	0.5
						SM	20
NHC_2H_5	SC_2H_5	*Strep.*	1	10	—	CM	0.78
NHC_2H_5	SC_2H_5	*Staph.*	1	10	—	CM	3.13
NHC_3H_5	SC_2H_5	*M. tuber.*	7	20	—	INH	0.024
NHC_4H_9	SCH_3	*cholerae*[d1]	1	50	—	CM	0.5
						SM	20
NHC_4H_9	SC_3H_7	*cholerae*[d1]	1	50	—	CM	0.5
						SM	20
NHC_4H_9	SC_4H_9	*cholerae*[d1,2]	1	50	—	CM	0.5
						SM	20

The screening tests against *a*), *M. tuberculosis* H 37 RV; *b*), *Strep. pyogenes* C 203, and *c*), *Staph. aureus* UC 76 were done at Parke, Davis Research Division and against d^1) *V. cholerae* Inaba type and d^2) *V. cholerae* Ogawa type were done by Prof. T. Ogawa and his assistants at Nagoya City University. *e*) Isonicotinic acid hydrazide; *f*) Chloramphenicol; *g*) Dihydrostreptomycin.

Experimental[5]

2-Ethylamino-5-nitro-4-thiocyanatopyrimidine (IIIb)——To a solution of 2 g of II in 20 ml of benzene, a solution of $EtNH_2$ (prepared from 1.51 g of $EtNH_2HCl$ and 0.42 g of Na in 20 ml of EtOH) was added dropwise at 5—10° with stirring. After stirring for 10 min, the deposited crystals were collected by filtration, and the filtrate was concentrated to one-fifth of the original volume. Upon cooling, the separated second crop of crystals was combined, washed with H_2O and dried. Recrystallization from benzene gave IIIb as colorless needles, mp 166—167°. Yield, 1.92 g. *Anal.* Calcd. for $C_7H_7O_2N_5S$: C, 37.33; H, 3.11. Found: C, 37.73; H, 3.42. All the other 2-alkylamino derivatives in Table I were prepared by the same method.

2-Amino-5-ethylaminothiazolo(5,4-*d*)pyrimidine (IVb)——A mixture of 6.4 g of IIIb, 6 g of Fe powder and 120 ml of AcOH was stirred at 60° for 2 hr. The reaction mixture was filtered and the filtrate was concentrated to almost dryness. After addition of H_2O to the residue, the mixture was extracted with AcOEt. It was washed with dil. NaOH, dried over Na_2SO_4, and evaporated. Recrystallization of crude IVb from acetone gave 4.4 g of colorless amorphous solid, mp 214—215°. *Anal.* Calcd. for $C_7H_9N_5S$: C, 43.08; H, 4.62. Found: C, 43.02; H, 4.61. All the other 2-aminothiazolopyrimidines, shown in Table II, were prepared by essentially the same method.

5-Amino-2-ethylamino-4-mercaptopyrimidine (Va)——A mixture of 1.5 g of IVb and 15 ml of 15% NaOH was heated at 120—130° for 15 hr. After cool, the reaction mixture was carefully acidified with AcOH and extracted with AcOEt. The extract was dried over Na_2SO_4. Removal of the solvent left 1.3 g of IVb, which was recrystallized from benzene–MeOH to give yellow scales, mp 156° (This product was unstable). *Anal.* Calcd. for $C_6H_{10}N_4S$: C, 42.35; H, 5.88. Found: C, 42.67; H, 6.21. All the other 5-amino-4-mercaptopyrimidines in Table III were prepared by the same method.

5) All melting points are uncorrected.

5-Ethylaminothiazolo(5,4-*d*)pyrimidine (VIa)——A solution of 0.7 g of Va in 20 ml of ethyl orthoformate was refluxed for 2 hr. The reaction mixture was evaporated to almost dryness in vacuum and the residue was treated with 5% NaOH, and the insoluble crystalline product was taken up in benzene. Removal of the solvent provided 0.64 g of VIa, mp 136—137°. Purification data for this compound and the similarly prepared **5**-benzylamino- (VIb) and *p*-chloroanilino- (VIc)derivatives were shown in Table IV.

5-Ethylamino-2-mercaptothiazolo(5,4-*d*)pyrimidine (VIIa)——To a solution of MeOCSSK (prepared from 3.3 g of KOH, 3.5 g of CS_2 and 120 ml of MeOH) was added 5 g of Va and the mixture refluxed for 18 hr. After cooling, the reaction mixture was treated with charcoal and filtered, and the filtrate was concentrated to almost dryness. To the residue was added H_2O and the solution was acidified with AcOH to give yellow precipitate. Reprecipitation from 2% NH_4OH and AcOH gave yellow amorphous solid. mp 240—241° (decomp.). *Anal.* Calcd. for $C_7H_8N_4S_2$: C, 39.62: H, 3.77. Found: C, 39.66; H, 3.51. The other 2-mercaptothiazolopyrimidines, shown in Table IV, were prepared by the same method.

5-Amino- or 5-Alkylamino-2-alkylthiothiazolo(5,4-*d*)pyrimidines (VIIIa—k)——The potassium salt of 2-mercaptothiazolopyrimidines and a slight excess of alkyl halide in EtOH was heated for a short time. The solvent was removed, a small amount of H_2O was added to the residue, and the separated crystals were collected. All 2-alkylthio derivatives thus prepared were listed in Table V.

[Chem. Pharm. Bull. 16(4) 750—755 (1968)]

UDC 547.857.2.07

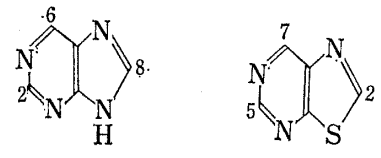
Studies on Pyrimidine Derivatives. X[1]

Eiji Suzuki, Sumi Sugiura, Takio Naito, and Shoji Inoue

Faculty of Pharmacy, Meijo University[2]

(Received September 28, 1967)

Present study was undertaken to examine the relation between the order of reactivity to nucleophilic substitutions of chlorine atoms in 2,5,7-trichlorothiazolo(5,4-*d*)pyrimidine (II) and the proton chemical shifts of thiazolo(5,4-*d*)pyrimidine nucleus.



purine thiazolo(5,4-*d*)pyrimidine.

Chart 1

It is usually considered that electron density on each of the carbon atoms in the aromatic nucleus is roughly parallel to the chemical shift of the protons attached to the carbon atom, and ease of the nucleophilic aromatic substitutions is also in relation to the electon density of the carbon atom. In the case of purine, such a parallel relation could be observed; thus, the order of chemical shifts is H_6–H_2–H_8,[3,4] and the order of reactivity to nucleophilic substitutions of chlorine atoms[5] in 2,6,8-trichloropurine by means of diethylamine and sodium ethoxide was C_6–C_2–C_8. In the thiazolo(5,4-*d*)pyrimidine (XVI), a sulfur analog of purine, the same relation would be expected, but in fact no such simple relation could be observed (Table I and Fig. 1).

Assignments of signals to the protons in thiazolo(5,4-*d*)pyrimidine (XVI) can be easily done by inspection of the signal shapes (Fig. 1). Thus, the sharpest peak at 569.0 cps is assigned to the proton at C_2 and the broadest peak at 553.0 cps to that at C_5, since the proton at C_5

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3) S. Matsuura and T. Goto, *Tetrahedron Letters*, **1499** (1963); *J. Chem. Soc.*, **1965**, 623.
4) ^{13}C chemical shifts of purine were also determined; the order is H_2–H_8–H_6. R.J. Pugmire, D.M. Grant, R.K. Robins, and G.W. Rhodes, *J. Am. Chem. Soc.*, **87**, 2225 (1965).
5) R.K. Robins and B.E. Christensan, *J. Am. Chem. Soc.*, **74**, 3624 (1952).