Tetrahedron, 1967, Vol. 23, pp. 1297 to 1304. Pergamon Press Ltd. Printed in Northern Ireland

4β-CHLOROETHYLAMINOPYRIMIDINES AND THE FORMATION OF IMIDAZOLIDINO[1.2-c]PYRIMIDINES ON ACID TREATMENT OF 4-BIS-β-HYDROXYETHYLAMINO-PYRIMIDINES

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(*Received* 28 *June* 1966; *accepted for publication* 2 *August* 1966)

Abstract—During syntheses aimed at obtaining 4-β-chloroethylamino and 4-bis-β-chloroethylamino pyrimidines, it has been found that treatment of *2-chloro-4-bis-β-hydroxyethylaminopyrimidine* with hydrochloric acid or of *4-bis-β-hydroxyethylamino-2-mercaptopyrimidine* with chloroacetic acid and hydrochloric acid yields *1-β-hydroxyethyl-5-oxo-imidazolidino*[1.2-c]*pyrimidine*. Further, 2-chloro-4-bis-β-hydroxyethylamino pyrimidine and *2-benzylmercapto-4-bis-β-hydroxyethylaminopyrimidine* yield *1-β-chloroethyl-5-oxo-imidazolidino*[1.2-c]*pyrimidine* on treatment with thionyl chloride followed by exposure to moisture.

IN CONTINUATION of our studies on pyrimidines of possible therapeutic interest,[1,2] the syntheses of 4-bis-β-chloroethylamino and 4-β-chloroethylamino pyrimidines were undertaken. It is well known that nitrogen mustards and similar alkylating agents are inhibitors of tumour growth and are mutagenic agents.[3] It is also likely that these effects are a consequence of the alkylation, by such agents, of the nitrogen atom at position 7 of the guanine residues in DNA.[4,5] In view of the fact that guanine and cytosine residues are often proximally situated in biological systems, it seemed of interest to use cytosine and similar molecules as carriers of the alkylating function. The syntheses of 4-bis-β-chloroethylamino-2-hydroxypyrimidine and 4-β-chloroethylamino-2-hydroxypyrimidines were, therefore, investigated.

The compounds obtained in the present study are shown in Chart 1. Treatment of cytosine with ethylene oxide yielded predominantly *2-hydroxy-4-β-hydroxyethylaminopyrimidine* (I), which on treatment with thionyl chloride was converted to *4-β-chloroethylamino-2-hydroxypyrimidine* (II).

With a view to obtaining the 4-bis-β-chloroethylamino derivative, both 2,4-dichloropyrimidine and 2,4-dimercaptopyrimidine were treated with diethanolamine. It has been found in the case of 2,4-dimercaptopyrimidine that the reaction with amines leads exclusively to the 4-amino-2-mercaptopyrimidines.[6] As the products from both the above reactions could be converted to IV, it was clear that the reaction with 2,4-dichloropyrimidine had also involved substitution at position 4. The products obtained were therefore 2-chloro-4-bis-β-hydroxyethylaminopyrimidine (III) and 4-bis-β-hydroexyethylamino-2-mercaptopyrimidine (VII), These two compounds were treated with hydrochloric acid and with chloroacetic acid and hydrochloric acid respectively to replace the functional groups at position 2 by hydroxyl. The

[1] K. L. Nagpal and M. M. Dhar, *Indian J. Chem.* **3**, 126 (1965).
[2] K. L. Nagpal, K. L. Agarwal and M. M. Dhar, *Indian J. Chem.* **3**, 356 (1965).
[3] W. C. J. Ross, *Biological Alkylating Agents* p. 64. Butterworth, London (1962).
[4] P. D. Lawley and C. A. Wallick, *Chem. & Ind.* 633 (1957).
[5] B. Reiner and S. Zamenhof, *J. Biol. Chem.* **228**, 475 (1957).
[6] P. B. Russel, G. B. Elion, E. A. Falco and G. H. Hitching, *J. Amer. Chem. Soc.* **71**, 2279 (1949).

compound so obtained, however, was not the expected 2-hydroxy-4-bis-β-hydroxyethylaminopyrimidine. The evidence leading to a imidazolidino[1.2-c]pyrimidine structure for this compound (IV) is discussed below.

The compound IV analysed for $C_8H_{12}ClN_3O_2$. It was a hydrochloride, which on treatment with an ion-exchange resin yielded a free base V, $C_8H_{11}N_3O_2$. Its UV spectra (Table 1) was of the 4-amino-2-hydroxypyrimidine type but it had a p*Ka* of 6·84 (Table 2). The p*Ka* of cytosine and 2-hydroxy-4-β-hydroxyethylaminopyrimidine (I) under similar conditions was found to be 4·60 and 4·64 respectively. The *I*R spectra[7] of IV had a strong 2-oxo-pyrimidine C=O stretching peak at 1715 cm^{-1}, the pyrimidine ring absorption at 1645 cm^{-1} and O—H and C—O stretching peaks at 3225 and 1053 cm^{-1}. A strong peak which was absent in the spectra of III and VII, was present at 1230 cm^{-1}. It was, therefore, clear that this compound had been formed by the expected introduction of a OH function at position 2 and also the loss of a molecule of water from the rest of the molecule. The presence of an alcoholic OH (IR absorption) did not favour the morpholino structure XI. Nevertheless, this compound XI was synthesized from the known 2-mercapto-4-morpholinopyrimidine (IX)[5] by conversion to the 2-benzylmercapto compound X and subsequent hydrolysis with hydrochloric acid. Compound XI was clearly different from IV. Its IR spectrum had no OH bands and the strong absorption at 1230 cm^{-1}, which was present in the spectra of IV was absent. The C=O stretching absorption at 1720 cm^{-1} was present and a strong peak at 1125 cm^{-1} could be assigned to the ether function.

The NMR spectra of some of the compounds shown in Chart 1 have been determined. From these spectra, it may be generalized that —O—CH_2— (cyclic) and —$\overset{+}{N}$H—CH_2— as in the hydrochloride of the morpholino compounds are indistinguishable and give rise to a single peak at 6·08–6·11 τ. Also that HO—CH_2— and —N—CH_2—, as in β-hydroxyethylamino chains, give rise to signals at 6·16–6·20 τ are also not distinguishable. The two protons at positions 5 and 6 of the pyrimidine ring give rise to two doublets around 2·1 and 3·6, τ (J, 8·0 c/s).

The NMR spectrum of compound IV has two doublets at 2·11 and 3·65 τ (J, 8·0 c/s) due to the protons at positions 5 and 6 of the pyrimidine ring. The position 5 of the pyrimidine ring is therefore not involved in the formation of an additional ring. The eight methylene protons give rise to three sets of peaks at 6·2, 6·05 and 5·74 τ. Integration of these peaks indicates that the peaks at 6·2 and 6·05 τ are due to two protons each and the peaks at 5·74 τ due to four protons. The imidazolidino[1.2-c]pyrimidine structure IV is consistent with the IR spectra of this compound and by analogy with the other NMR spectra, the peaks at 6·2 and 6·05 τ could be assigned to the α and β methylenes respectively of the β-hydroxyethyl chain. It is also not unreasonable that the four imidazolidine protons show up similarly and give rise to the peaks around 5·74 τ.

The mass spectrum of the free base V has a strong molecular ion peak at *m/e*, 181. The base peak is at m/e 150 and two strong peaks are at *m/e* 137 and 108. This spectrum is consistent with an imidazolidino[1.2-c]pyrimidine structure for V as fragments with *m/e* 150, 137 and 108 can be readily derived from V (Chart 2).

The formation of IV from the 2-hydroxy compound XII may be rationalized as

[7] The IR spectra of 2,4 substituted pyrimidines will be discussed more fully elsewhere.

CHART 1

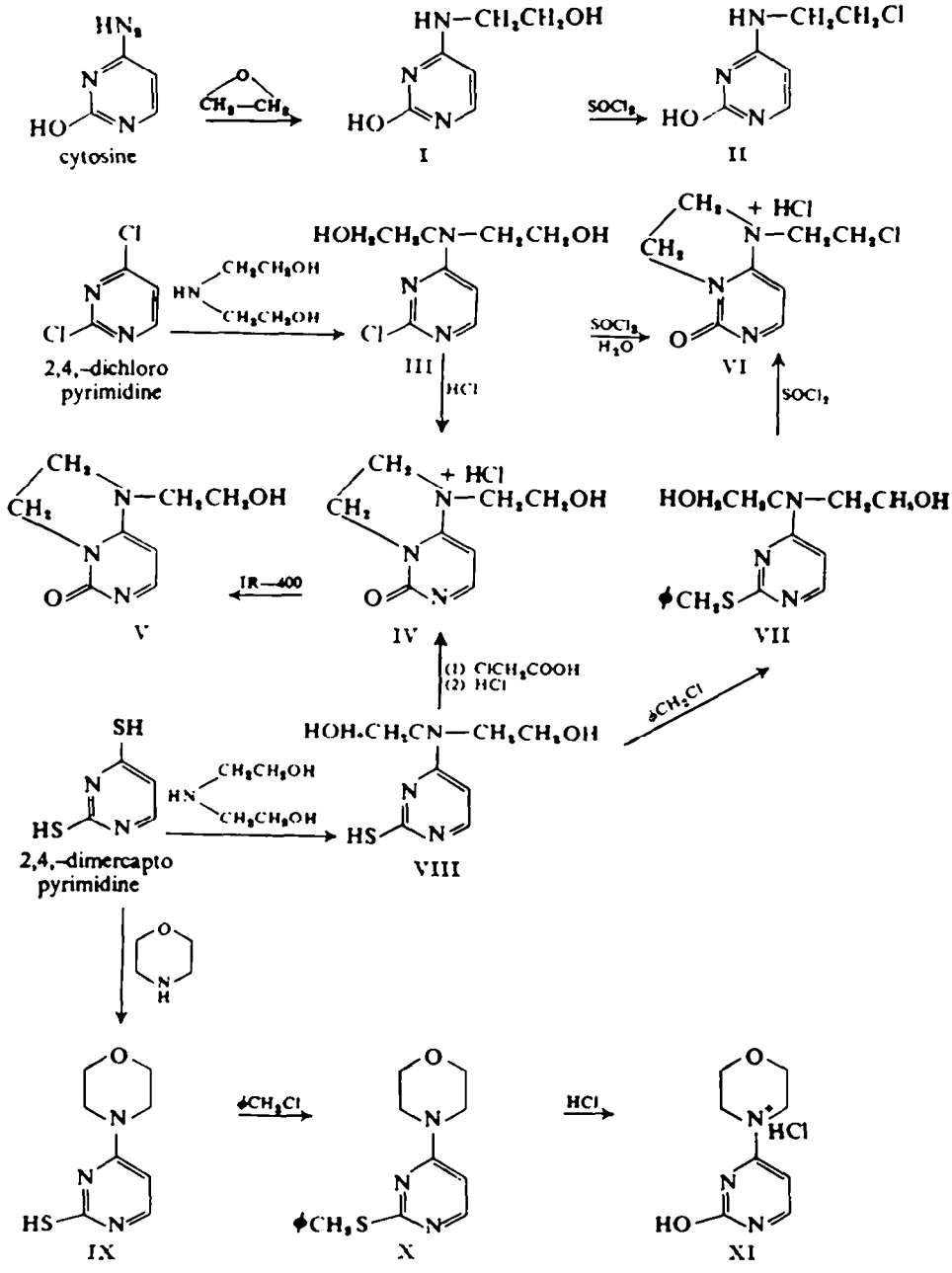
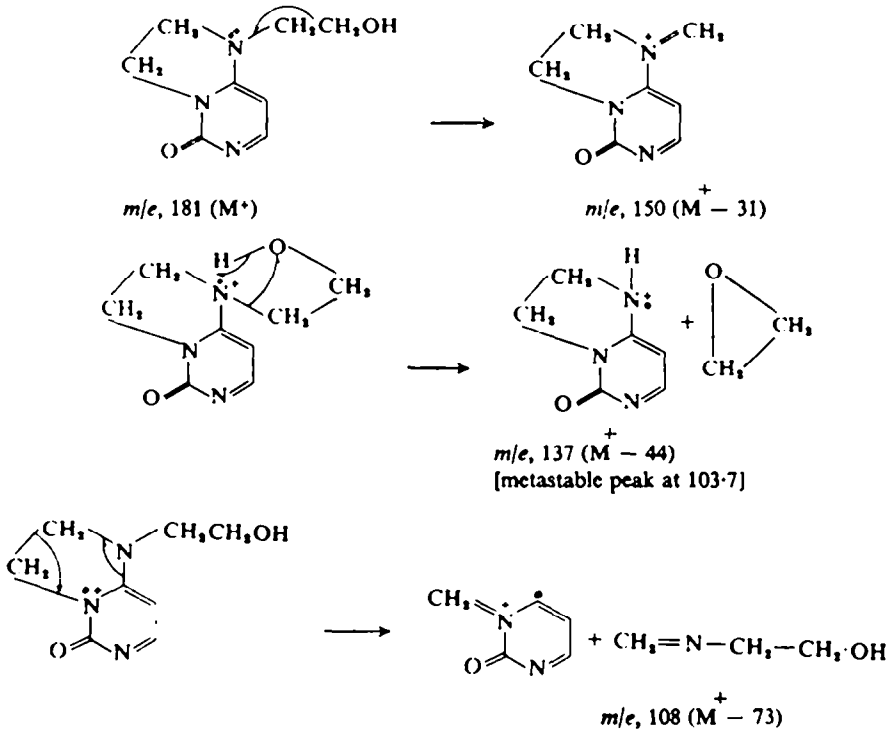

cytosine
I
II
2,4,-dichloro pyrimidine
III
VI
V
IV
VII
VIII
2,4,-dimercapto pyrimidine
IX
X
XI

CHART 2



shown in Chart 3. Protonation of XII could lead to the quarternary intermediate XV by attack by the pyrimidine ring nitrogen or alternatively via the ammonium ion XIV.

The treatment of 2-chloro-4-bis-β-hydroxyethylaminopyrimidine (III) and of 2-benzylmercapto-4-bis-β-hydroxyethylaminopyrimidine (VIII) with thionyl chloride yielded in each case, extremely hygroscopic products which reacted readily with atmospheric moisture or moisture present in the solvent to yield 1-chloroethyl-5-oxo-imidazolidino[1.2-c]pyrimidine hydrochloride (VI). The assignment of this structure follows from the analytical data, the IR and NMR spectra of this compound. Its IR spectra is completely similar to that of 1-hydroxyethyl-5-oxo-imidazolidino[1.2-c]-pyrimidine hydrochloride (IV) except for the absence of the O—H (3225 cm^{-1}) and C—O (1053 cm^{-1}) stretching absorptions. Its NMR spectra is also similar to that of IV in having two doublets at 2·09 and 3·55 τ (J, 8·0 c/s) and in having a four proton signal at 5·74 τ. The other four methylene protons, those of the β-chloroethyl chain, give a single peak at 6·05 τ. The β-CH_2 is, as might be expected, slightly more deshielded than in the β-hydroxyethyl compounds.

The hygroscopic compounds, formed on treatment with thionyl chloride, presumably have the structure XVII (X = Cl or ϕCH_2S) (Chart 4). Subsequent reaction with water leads to the liberation of hydrochloric acid and benzyl mercaptan in the cases of the 2-chloro and 2-mercapto compounds respectively. The identity of the liberated benzyl mercaptan has been confirmed by the formation of a 2,4-dinitrophenyl thioether on reaction with 1-fluoro-2,4-dinitrobenzene.

CHART 3

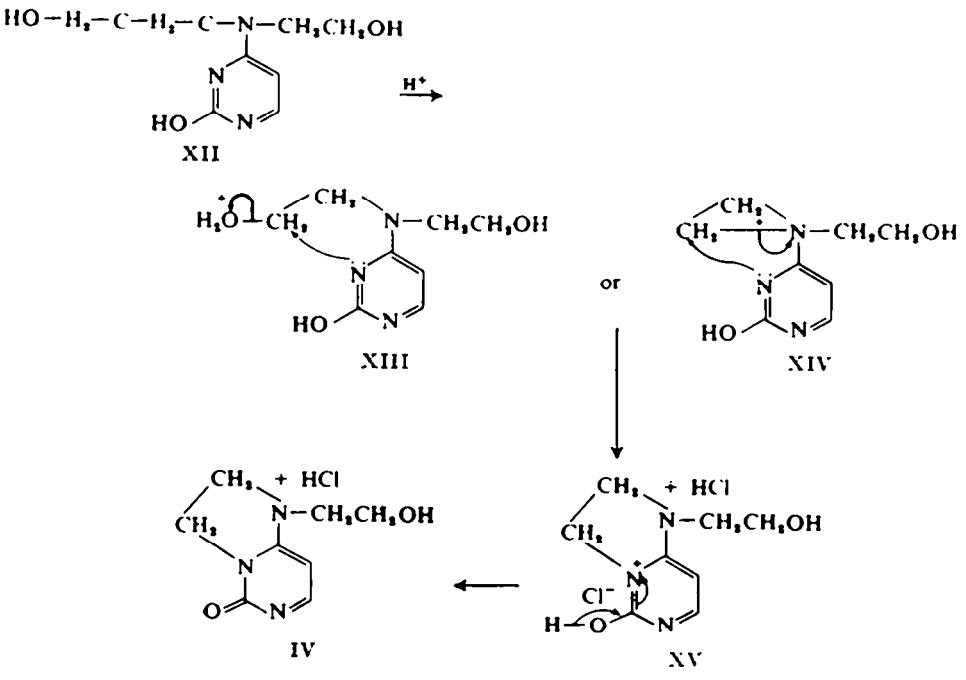


CHART 4

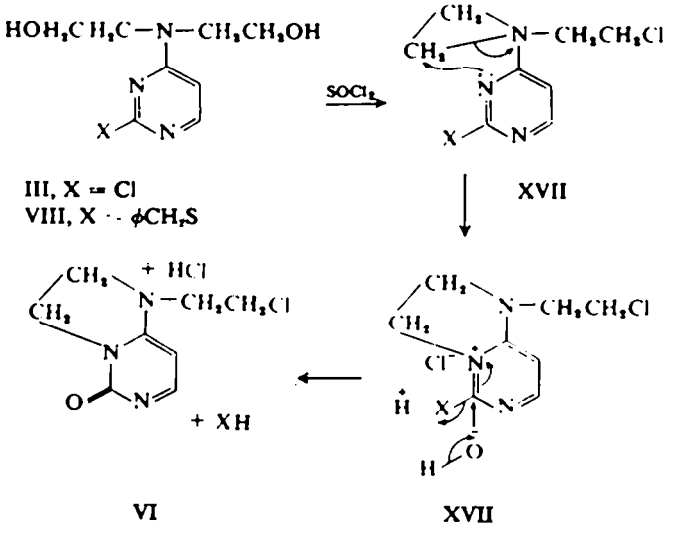


TABLE 1.—UV ABSORPTION SPECTRA DATA

Compound	λ_{max} mμ (Log ε)		
	pH 2·0	6·24	10·0
Cytosine	276 (3·93)	267 (3·73)	283 (3·83)
2 Hydroxy 4-β-hydroxyethylamino pyrimidine (I)	284 (4·08)	274 (3·90)	273 (3·92)
4-β-Chloroethylamino-2-hydroxy pyrimidine (II)	280 (4·10)	272 (3·99)	272 (3·95)
2-Chloro-4-bis-β-hydroxy-ethylamino-pyrimidine (III)	—	252 (4·01) 286 (3·55)*	—
1-β-Hydroxyethyl-5-oxo-imidazolidino[1.2-c]-pyrimidine (IV)	290 (4·10)	290 (3·99)	306 (4·09)
1-β-Chloroethyl-5-oxo-imidazolidino[1.2-c]-pyrimidine (VI)	288 (4·10)	294 (4·05)	305 (4·16)
4-Bis-β-hydroxyethylamino-2-mercapto-pyrimidine (VII)	278 (4·36)	271 (4·49)	249 (4·40) 304 (3·85)*
2-Benzylmercapto-4-bis-β-hydroxyethylamino-pyrimidine (VIII)	257 (4·43)	—	—
2-Mercapto-4-morpholinopyrimidine (IX)	244 (4·08) 278 (4·42)	270 (4·53)	—
2-Benzylmercapto-4-morpholinopyrimidine (X)	259 (4·43)	—	—
2-Hydroxy-4-morpholinopyrimidine (XI)	288 (4·20)	277 (4·10)	289 (4·08)

* Shoulder

TABLE 2.—p*Ka* DATA

Compound	p*Ka*
Cytosine	4·60 (Lit, 4·60[a])
1-β-Hydroxyethyl-5-oxo-imidazolidino-[1.2-c] pyrimidine (IV)	6·84
2-Hydroxy-4-β-hydroxyethyl-pyrimidine (I)	4·64
2 Chloro-4-bis-β-hydroxyethylamino-pyrimidine (III)	3·70

EXPERIMENTAL

IR spectra in KBr and UV spectra of all the compounds shown in Chart 1 were routinely determined.[a] UV maxima are recorded in Table 1. p*Ka*'s were determined from potentiometric titrations with a glass electrode of 0·001M solns in water and are listed in Table 2. NMR spectra were determined in D_2O on a Varians A-60 instrument at the Research Laboratories of Lepitit Milan and are summarised in Table 3. Mps are uncorrected.

2-Hydroxy-4-β-hydroxyethylaminopyrimidine (I)

A soln of cytosine (0·5 g, 4·5 mmoles) in DMF (50 ml) containing ethylene oxide (0·5 g, 11·3 mmoles) was mechanically shaken in a stoppered bottle at 60–70° for 40 hr. The soln was evaporated to dryness under reduced press and two lots of xylene (2 × 35 ml) were added and evaporated. The residual solid was extracted thrice with chf (3 × 30 ml) and the insoluble material

[a] The compounds reported are being tested for anti-viral activity. Results of these tests will be reported by Dr. O. P. Babbar.

[a] P. A. Levene, L. W. Bass and H. S. Simms, *J. Biol. Chem.* 70, 229 (1926).

TABLE 3.—DATA FROM NMR SPECTRA

Compound	Methylene proton signals*		
	Chemical shift (τ)	No. of protons	Shape
2-Hydroxy-4-morpholinopyrimidine hydrochloride (XI)	6·11	8	Single peak
2-Chloro-4-bis-β-hydroxy-ethylamino-pyrimidine (III)	6·20	8	Three peaks, centre peak considerably larger than other two.
4-Bis-β-hydroxyethylamino-2-mercapto-pyrimidine (VII)	6·16	8	Single peak
1-β-Hydroxyethyl-5-oxo-imidazolidino-[1.2-c]-pyrimidine hydrochloride (IV)	5·74	4	Five peaks, centre peak very much larger than others.
	6·05	2	Broad single peak
	6·2	2	Complex pattern with two prominent peaks.
1-β-Chloroethyl-5-oxo-imidazolidino-[1.2-c] pyrimidine hydrochloride (VI)	5·74	4	Five peaks, centre peak very much larger than others.
	6·05	4	Single peak

* In every spectrum two methine protons showed up as two doublets around 2, 2 ± 0·15 and 3·55 ± 0·10τ (J, 8 c/s).

crystallized from 95% EtOH, yield, 0·423 g (61%), m.p. 234–236°. (Found: C, 46·77; H, 6·13; N, 26·88. $C_6H_9N_3O_3$ requires: C, 46·46; H, 5·80; N, 27·10%.)

4-β-Chloroethylamino-2-hydroxypyrimidine hydrochloride (II)

Compound I (0·350 g) was suspended in a soln of THF (25 ml) containing $SOCl_2$ (1·6 ml) and the mixture refluxed with stirring for 48 hr. At the end of this period, the solvent and excess $SOCl_2$ were removed under reduced press. The residue on crystallization from EtOH–ether gave the product (0·342 g (74%)), m.p. 223° (softens at 197°). For elemental analysis a sample was recrystallized from dry MeOH. (Found: C, 34·48; H, 4·40; N, 19·67. $C_6H_8Cl_2N_3O$ requires: C, 34·29; H, 4·26; N, 20·00%.)

2-Chloro-4-bis-β-hydroxyethylaminopyrimidine (III)

Diethanolamine (5·56 g, 0·053 mole) in EtOH (60 ml) was added dropwise to an ice-cooled and stirred soln of 2,4-dichloropyrimidine (8 g, 0·053 mole) and Et_3N (5·35 g, 0·053 mole) in EtOH (100 ml). After the addition was complete (ca. 110 min), the soln was left at room temp overnight. EtOH was then removed under reduced press and 4 portions of benzene (50 ml) were added to the residue and evaporated. The residue so obtained was repeatedly extracted with ether and crystallized from AcOEt, yield, 6·52 g (56%), m.p. 109–110°. (Found: C, 44·53; H, 5·80; N, 19·27; Cl, 16·78. $C_8H_{12}ClN_3O_2$ requires: C, 44·13; H, 5·51; N, 19·31: Cl, 16·32%.)

4-Bis-β-hydroxyethylamino-2-mercaptopyrimidine (VII)

A mixture of 2,4-dimercaptopyrimidine (2 g, 0·013 mole) and diethanolamine (4 g, 0·038 mole) was heated on a steam bath for 48 hr. The brown gum so obtained was taken up in EtOH (25 ml) and the soln scratched and left overnight at room temp. The separated solid was filtered, washed with ice-cold EtOH and recrystallized from hot EtOH, yield, 1·13 g (40%), m.p. 182–184°. (Found: C, 45·01; H, 6·19; N, 19·85. $C_8H_{13}N_3O_2S$ requires: C, 44·65; H, 6·04; N, 19·53%.)

1-β-Hydroxyethyl-5-oxo-imidazolidino[1.2-c]pyrimidine hydrochloride (IV)

(a) *From 2-chloro-4-bis-β-hydroxyethylamino pyrimidine.* A soln of 2-chloro-4-bis-β-hydroxy-ethylaminopyrimidine (1 g,) in conc. HCl (4 ml) was heated on a steam bath for 18 hr. The acid was

evaporated off on the steam bath in an open dish and 5 portions of water (5 × 15 ml) were successively added to the residue and evaporated to dryness. Recrystallization of the resulting solid from EtOH afforded 0·63 g (63%) of the hydrochloride m.p. 244–246°. (Found: C, 44·39; H, 5·66; N, 19·09; Cl, 15·89. $C_8H_{12}ClN_3O_3$ requires: C, 44·13; H, 5·51; N, 19·31; Cl, 16·32%.)

(b) *From 4-bis-β-hydroxyethylamino-2-mercaptopyrimidine.* A soln of 4-bis-β-hydroxyethylamino-2-mercapto pyrimidine (0·29 g, 1·3 mmoles) and chloroacetic acid (0·13 g, 1·3 mmoles) in water (7 ml) was refluxed for 80 min. Conc. HCl (2·3 ml) was added and the refluxing continued for a further 2 hr. The soln was then evaporated to dryness and the resulting sticky solid triturated with ether to yield a solid. Recrystallization from EtOH yielded the hydrochloride (0·148 g, 50%) m.p. and mixed m.p. with product obtained by method (a), 244–246°. IR spectra of both products were superimposable. (Found: C, 44·50; H, 5·39; N, 19·07. $C_8H_{12}ClN_3O_3$ requires: C, 44·13; H, 5·51; N, 19·31%.)

1-β-Hydroxyethyl-5-oxo-imidazolidino[1.2-c]*pyrimidine* (V)

A soln of the hydrochloride IV in water was treated with an excess of Amberlite IR 400 (OH^- form). The soln of the free base so obtained was evaporated to dryness under reduced press and the residue crystallized from MeOH–AcOEt, m.p. 200–201°. (Found: C, 52·76; H, 6·40; N, 23·31. $C_8H_{11}N_3O_3$ requires: C, 53·03; H, 6·06; N, 23·20%.)

2-Benzylmercapto-4-bis-β-hydroxyethylaminopyrimidine (VIII)

4-Bis-β-hydroxyethylamino-2-mercaptopyrimidine (1·13 g, 5·2 mmoles) was dissolved in water (10 ml) containing NaOH (0·4 g) and a soln of benzyl chloride (0·72 g, 5·3 mmoles) in EtOH (25 ml) added portionwise with stirring and the mixture heated at 60–70° for 2 hr. Most of the EtOH was removed under reduced press and the aqueous soln kept in a refrigerator overnight. The separated solid was filtered and crystallized from acetone–hexane, yield 1·37 g (86%), m.p. 104–106°. (Found: C, 58·91; H, 6·34; N, 14·12. $C_{15}H_{19}N_3O_3S$ requires: C, 59·02; H, 6·23; N, 13·77%.)

2-Benzylmercapto-4-morpholinopyrimidine (X)

This pyrimidine was prepared in 82% yield by treating 2-mercapto-4-morpholinopyrimidine[4] with benzyl chloride as described for the preparation of VIII, m.p. 117–118°. (Found: C, 63·03; H, 6·26; N, 14·64. $C_{15}H_{17}N_3OS$ requires: C, 62·72; H, 5·92; N, 14·63%.)

2-Hydroxy-4-morpholinopyrimidine hydrochloride (XI)

2-Benzylmercapto-4-morpholinopyrimidine (0·322 g) was added to conc. HCl (2·5 ml) and the mixture gently refluxed for 5 hr. The acid soln was then diluted with water, extracted with 3 portions of ether and evaporated to dryness. Crystallization of the residue from MeOH–AcOEt gave the hydrochloride (0·16 g (66%)), m.p. 240–241°. Mixed m.p. with IV 228–233°.

An analytical sample was obtained by recrystallization from abs EtOH m.p. 241–242°. (Found: C, 43·87; H, 5·40; N, 19·12. $C_8H_{13}ClN_3O_3$ requires: C, 44·13; H, 5·51; N, 19·32%.)

1-β-Chloroethyl-5-oxo-imidazolidino[1.2-c]*pyrimidine hydrochloride* (VI)

(a) *From 2-chloro-4-bis-β-hydroxyethylamino pyrimidine.* A soln of 2-chloro-4-bis-β-hydroxyethylaminopyrimidine (0·3 g) and $SOCl_2$ (0·8 ml) in chf (25 ml) was allowed to stand at room temp for 24 hr. The solvent and excess $SOCl_2$ were removed under reduced press (water pump). The hygroscopic solid thus obtained was crystallized twice from MeOH–AcOEt to give the hydrochloride, yield 0·19 g (62%), m.p. 224–225°. (Found: C, 41·10; H, 5·10; N, 17·96. $C_8H_{11}Cl_2N_3O$ requires: C, 40·68; H, 4·65; N, 17·79%.)

(b) *From 2-benzylmercapto-4-bis-β-hydroxyethylaminopyrimidine.* A soln of 2 benzylmercapto-4-bis-β-hydroxyethylaminopyrimidine (0·5 g) and $SOCl_2$ (1 ml) in chf (25 ml) was allowed to stand at room temp for 24 hr and worked up as in (a), yield 0·228 g (65%), m.p. and mixed m.p. with product from 2-chloro-4-bis-β-hydroxyethylaminopyrimidine, 224–225°. IR spectra of both products were superimposable. (Found: C, 41·02; H, 5·02; N, 17·79. $C_8H_{11}Cl_2N_3O$ requires: C, 40·68; H, 4·65; N, 17·79%.)

Acknowledgements—We express our gratitude to Dr. E. Testa for NMR spectra, to Dr. R. S. Kapil for the mass spectrum, to Mr. J. Saran and his associates for microanalysis and to Drs. Nitya Anand and S. P. Popli for helpful discussions.