Indian Journal of Chemistry
Vol. 29B, January 1990, pp. 40-46

# Studies in nucleosides: Part XXV – Synthesis of alicyclic and cyclic nucleosides of 4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine and 4-aminopyrazolo[3,4-*d*]pyrimidine and their antileishmanial activity†

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Received 23 January 1989; accepted 10 May 1989

1-[(2-Hydroxy-1-hydroxymethylethoxy)methyl]-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**7**), 4-amino-1-[(2-hydroxy-1-hydroxymethylethoxy)methyl]pyrazolo[3,4-*d*]pyrimidine (**5**), 1-[(2-hydroxy-1-aminoethylethoxy)methyl]-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**12**), 1-[(2-hydroxyethoxy)methyl]-4(5*H*)-oxopyrazolo-[3,4-*d*]pyrimidine (**19**), 4-amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-*d*]pyrimidine (**17**), 4(5*H*)-oxo-1-(tetrahydrofuran-2-yl)pyrazolo[3,4-*d*]pyrimidine (**22**) and 4-amino-1-(tetrahydrofuran-2-yl)pyrazolo-[3,4-*d*]pyrimidine (**23**) have been synthesized and evaluated for their antileishmanial activity (*in vivo*) against the amastigotes of *Leishmania donovani* in hamsters. Compounds **7** and **18** show good activity (75% and 82% inhibition respectively); compounds **12**, **17** and **16** show low order of activity (15%, 25% and 16% inhibition respectively) and remaining compounds are found to be inactive at 25 mg/kg dose.

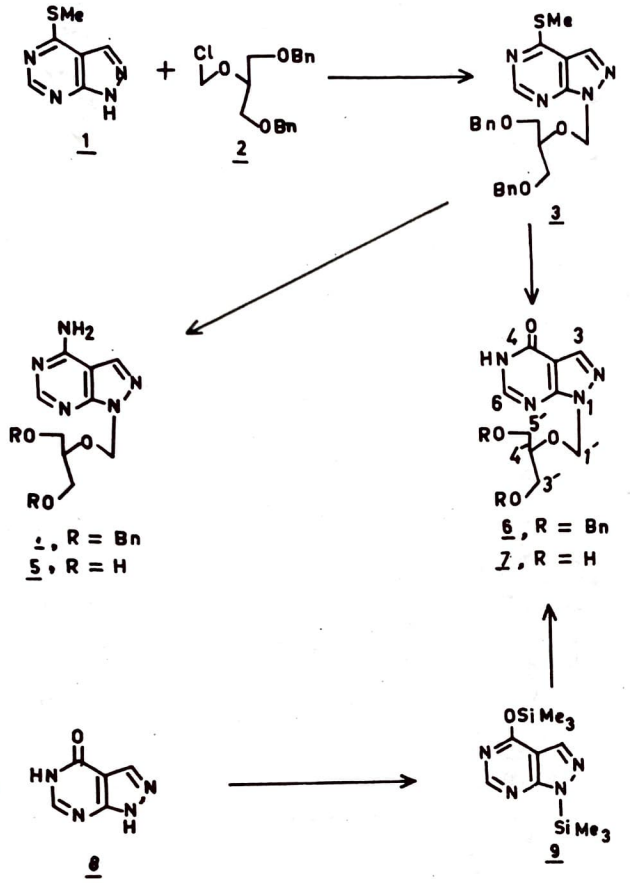
1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine- 4-one (allopurinol)[1] (**8**), an effective xanthine oxidase inhibitor[2], is in clinical use for controlling gout and related metabolic disorders[3]. The main metabolite of this drug in mammalian systems is oxipurinol, the 6-oxo derivative of allopurinol which acts an inhibitor of xanthine oxidase. Further, allopurinol-1-ribonucleotide like 6-azauridine-5′-phosphate[4] inhibits orotidylate decarboxylate[5] and, thus, is a potential antiviral and antitumor agent.

The impressive antiparasitic activity[6] of 1-β-D-riboside of 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (allopurinol-1-β-riboside) (**25**) and its nontoxicity in mammals[7] revived the interest in the nucleosides of 4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine. A number of ribonucleosides of allopurinol (**8**) have been synthesized and their xanthine oxidase inhibitory activity has been evaluated[8]. The N-1-riboside (**25**) being an excretion product of the patients using allopurinol for relief from gout was found to have no xanthine oxidase inhibitory activity[9] as expected, and thus it is considered to be a detoxication metabolite of allopurinol. The 1,5- and 2,5-bis-ribosides and N-2 and N-5 ribosides of allopurinol are found inactive as inhibitors of xanthine oxidase.

Several reports are available which suggest that 4-aminopyrazolo[3,4-*d*]pyrimidine nucleosides and related compounds may function as a substrate for anabolic[10-12] and catabolic enzymes[13,14]. Further, nonclassical nucleosides having tetrahydrofuranyl[15], (2-hydroxyethoxy)methyl[16], and [(2-hydroxy-1-hydroxymethyl ethoxy)methyl][17] moieties attached to purine and pyrimidine bases have been found very effective in modulating the biological activities of these nucleosides. The above reports prompted us to undertake the synthesis of the nonclassical nucleosides of 4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**8**) and 4-aminopyrazolo[3,4-*d*]pyrimidine and in the present paper we report the synthesis of 1-[(2-hydroxy-1-hydroxymethylethoxy)-methyl]-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**7**), 4-amino-1-[(2-hydroxy-1-hydroxymethylethoxy)methyl]pyrazolo[3,4-*d*]pyrimidine (**5**), 1-[(2-hydroxy-1-aminomethyl ethoxy)methyl]-4(5*H*)-oxopyrazolo-[3,4-*d*]-pyrimidine (**12**), 1-[(2-hydroxyethoxy)methyl]-4-(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**19**), 4-amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-*d*]pyrimidine (**17**), 4(5*H*)-oxo-1-(tetrahydrofuran-2-yl)pyrazolo-[3,4-*d*]pyrimidine (**22**) and 4-amino-1-(tetrahydrofuran-2-yl)pyrazolo[3,4-*d*]pyrimidine (**23**) and their antileishmanial activity.

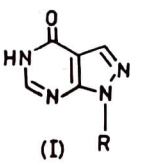
Condensation of 4-methylthiopyrazolo[3,4-*d*]pyrimidine[18] (**1**) (Scheme 1) with 1,3-dibenzyloxy-2-chloromethoxypropane (**2**) in the presence of $Et_3N$ gave 1-[(2-benzyloxy-1-benzyloxymethylethoxy)methyl]-4-methylthiopyrazolo[3,4-*d*]pyrimidine (**3**) in a good yield (70%). Treatment of **3** with sodium hydroxide afforded the 4(5*H*)-oxo derivative (**6**) (yield 30%). Hy-

† CDRI Communication No. 4448.

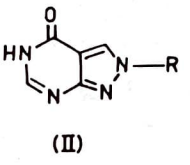


SCHEME 1

Table 1 – UV absorption maxima of substituted pyrazolo[3,4-*d*]pyrimidine derivatives



(I)



(II)

| Compd | R | $\lambda_{max}$ (nm) at | |
|---|---|---|---|
| | | *p*H 7 | *p*H 11 |
| | N-1 Isomers (I) | | |
| 7 | $-CH_2O-CH(CH_2OH)-CH_2OH$ | 251 | 270 |
| 12 | $-CH_2-O-CH(CH_2-NH_2)-CH_2OH$ | 250 | 270.6 |
| 19 | $-CH_2-O-CH_2-CH_2OH$ | 250.6 | 271 |
| 24 | –Me | 249 | 270 |
| 25 | –Ribose | 252 | 271 |
| 22 | –tetrahydrofuranyl | 252 | 269 |
| | N-2 Isomer (II) | | |
| 26 | –Me | 255 | 280 |
| 27 | –Ribose | 261 | 284 |

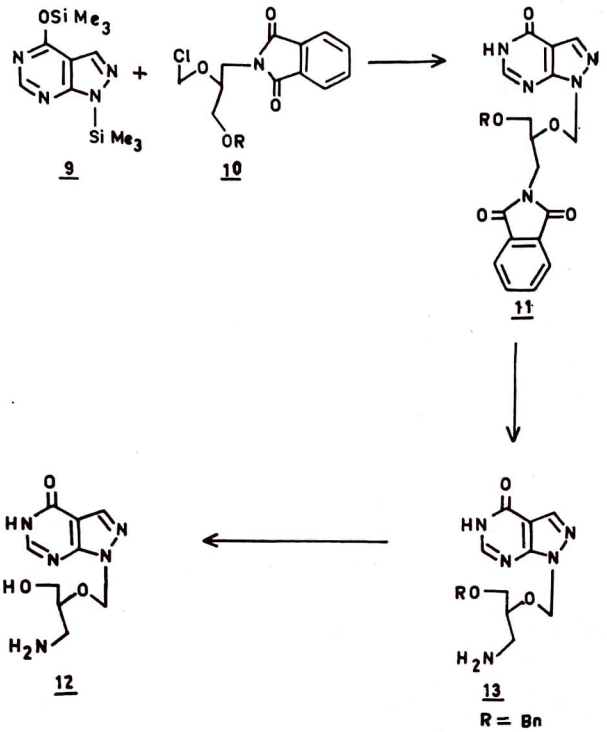
drogenolysis of **6** with $PdCl_2/H_2$ furnished the desired alicyclic nucleoside **7** (yield 35%). The nucleoside **7** was also prepared by an alternative route. Silylation of 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4-one (**8**) (Scheme 1) with hexamethyldisilazane (HMDS) in the presence of $(NH_4)_2SO_4$ gave the disilyl derivative (**9**) which on reaction with **2** gave **6** in a better yield (55%). Hydrogenolysis of **6** finally gave **7**.

Various ribonucleosides of allopurinol (**8**), the N-1 (**25**), N-2 (**27**) and N-5 isomers as well as the 1,5- and 2-5-bis-ribosylated derivatives have been prepared[8]. The site of ribosylation and β-configuration of these ribosides have been established on the basis of UV, PMR and $^{13}C$ NMR data. The bathochromic shift (Table 1) in UV maxima of allopurinol-2-riboside (**27**) at *p*H 7 and 11 as compared with the N-1-isomer (**25**) reflected the *ortho*-quinonoid distribution of electrons and is a useful method in deciding the site of ribosylation or alkylation. The UV absorption maxima (Table 1) of the nucleoside **7**, allopurinol-1-methyl (**24**) and allopurinol-1-riboside (**25**)[8] were almost identical. This confirmed N-1 to be the site of alkylation in nucleoside formation.

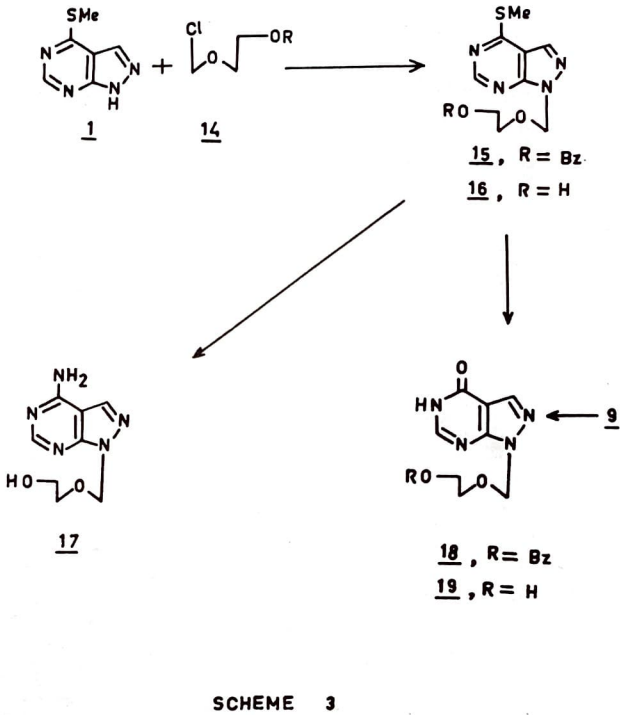
4-Amino-1-[(2-hydroxy-1-hydroxymethylethoxy)-methyl]pyrazolo[3,4-*d*]pyrimidine (**5**) (Scheme 1) was prepared from **3** as follows: Treatment of compound **3** with ammonia under pressure and at elevated temperature yielded **4** which on hydrogenolysis with $PdCl_2/H_2$ gave **5**.

Condensation of 1,4-bis(trimethylsilyl)allopurinol (**9**) (Scheme 2) with 1-benzyloxy-2-chloromethyloxy-3-phthaloylimidopropane[19] (**10**) in refluxing benzene gave 1-[(2-benzyloxy-1-(phthaloylimidomethylethoxy) methyl]-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**11**). Treatment of **11** with hydrazine hydrate removed the phthaloyl group and afforded 1-[(2-benzyloxy-1-aminomethylethoxy)methyl]-4(5*H*)-oxopyrazolo[3,4-*d*]-pyrimidine (**13**). Hydrogenolysis of **13** with $PdCl_2/H_2$ finally yielded 1-[(2-hydroxy-1-aminomethylethoxy)-methyl]-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**12**). The UV spectrum of **12** (Table 1) suggested that the alkylation had occurred specifically at N-1 of the heterocyclic moiety.

Condensation of 4-methylthiopyrazolo[3,4-*d*]pyrimidine (**1**) (Scheme 3) with benzoyloxyethoxymethyl-ene chloride (**14**) in the presence of $Et_3N$ gave 1-benzoyloxyethoxymethyl-4-methylthiopyrazolo[3,4-*d*]pyrimidine (**15**) in a good yield (60%). Treatment of **15** with methanolic ammonia at ambient temperature afforded 1-[(1-hydroxyethoxy)methyl]-4-methylthiopyrazolo[3,4-*d*]pyrimidine (**16**). Compound **16** when heated with aq. sodium hydroxide yielded the desired 1-hydroxyethoxymethyl-4(5*H*)-oxopyrazolo[3,4-*d*]-pyrimidine (**19**) in a poor yield (30%). The nucleoside



SCHEME 2

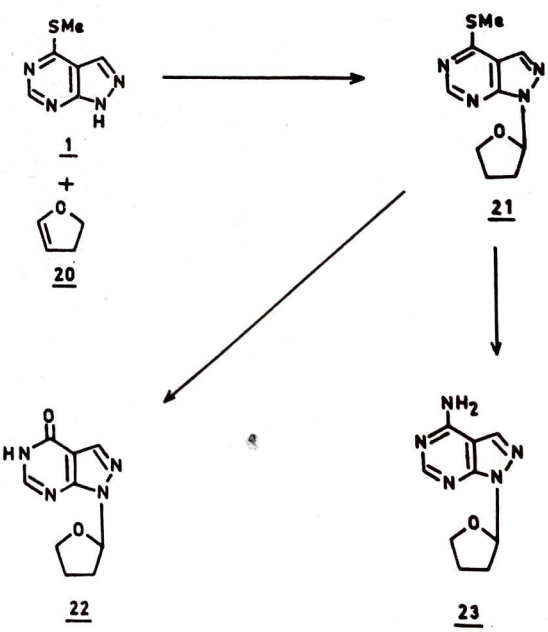


SCHEME 3

**19** was, therefore, prepared by an alternative route. Condensation of 1,4-bis(trimethylsilyl)allopurinol (**9**) (Scheme 3) with benzoyloxyethoxymethylene chloride (14) in refluxing benzene gave 1-[(2-benzoyloxyethoxy)methyl]-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (**18**) in a better yield (58%). Treatment of **18** with methanolic ammonia furnished finally the nucleoside **19** in a fairly good yield (60%). The UV spectrum of **19** (Table 1) suggested that the alkylation had occurred at N-1 in the heterocyclic moiety.

4-Amino-1-[(2-hydroxyethoxy)methyl]pyrazolo-[3,4-*d*]pyrimidine (**17**) (Scheme 3) was prepared from **16** by treatment with ammonia at elevated temperature.

Condensation of 4-methylthiopyrazolo[3,4-*d*]pyrimidine (**1**) (Scheme 4) with 2,3-dihydrofuran (**20**) in the presence of *p*-toluenesulphonic acid (PTSA) according to Robins's method[20] gave 4-methylthio-1-(tetrahydrofuran-2-yl)pyrazolo[3,4-*d*]pyrimidine (**21**) in 87% yield. Treatment of **21** with ammonia at elevated temperature afforded 4-amino-1-(tetrahydrofuran-2-yl)pyrazolo[3,4-*d*]pyrimidine (**23**) and reaction of **21** with sodium hydroxide furnished 4-(5*H*)-oxo-1-(tetrahydrofuran-2-yl)pyrazolo[3,4-*d*]pyrimidine (**22**). The UV spectrum of **22** was almost identical with that of allopurinol-1-methyl (**24**), thus confirming N-1 tobe the site of alkylation.



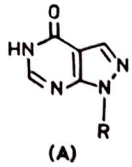
Scheme 4

*Antileishmanial activity*

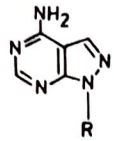
The *in vivo* antileishmanial activity of the nucleosides against amastigotes of *L. donovani* was determined in hamsters infected with Dd-8 strain according to the procedure described earlier[21]. Allopurinol (**8**) was used as a standard drug.

The activity of the compounds (series A) is given in Table 2. 1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (allopurinol) (**8**) exhibited 88% inhibition at 25 mg/kg dose on the 7th day. Substitution of (2-hydroxyethoxy)methyl function representing $C_1$-$C_4$-$C_5$ chain of ribose at N-1 of heterocyclic moiety (compound **19**)

Table 2 – Antileishmanial activity (*in vivo*) of the nucleosides (series **A** and **B**) at 25 mg/kg on 7th day, against amastigotes of *Leishmania donovani* in hamsters



(A)



(B)

| Compd | R | Inhibition (%) |
|---|---|---|
| | Series-A | |
| **8** | H | 88 |
| **19** | $-CH_2-O-CH_2-CH_2OH$ | 0 |
| **7** | $-CH_2-O-CH_2-CH_2OH$ <br> $\vert$ <br> $CH_2OH$ | 75 |
| **12** | $-CH_2-O-CH_2-CH_2OH$ <br> $\vert$ <br> $CH_2-NH_2$ | 15 |
| **18** | $-CH_2-O-CH_2-CH_2-O-\overset{\overset{O}{\Vert}}{C}-Ph$ | 82 |
| **22** | Tetrahydrofuran-2-yl | 0 |
| | Series-B | |
| **17** | $-CH_2-O-CH_2-CH_2OH$ | 25 |
| **5** | $-CH_2-O-CH-CH_2OH$ <br> $\vert$ <br> $CH_2-OH$ | 0 |
| **23** | Tetrahydrofuran-2-yl | 0 |

rendered the compound inactive. Introduction of [2-hydroxy-1-(hydroxymethyl)ethoxy] function representing $C_1$-$C_3$-$C_4$-$C_5$ of ribose at N-1 (compound **7**) considerably increased the activity (75% inhibition). The activity was reduced drastically when the hydroxy function of [2-hydroxy-1-(hydroxymethyl)ethoxy] group was replaced by an amino function as in **12**. The compound **22** became almost inactive when tetrahydrofuranyl moiety was introduced at N-1 of the heterocyclic moiety. The data, thus, suggested that not only the nature and chain length of glucone moiety at N-1 is critical for antileishmanial activity of the alicyclic nucleosides but also the nature of the functional groups present in it.

The antileishmanial activity of other types of nucleosides is recorded in Table 2. The activity of the nucleoside **17** was considerably decreased when (2-hydroxyethoxy)methyl function was introduced at N-1 of the heterocyclic moiety. However, when the hydroxy group in the nucleoside **17** was protected with benzoyloxy function, the corresponding protected nucleoside (**18**) exhibited a high order of activity. The compound **5** became inactive when an additional hydroxy function was introduced in the glycone moiety. Compound **23** in which a tetrahydrofuranyl function was introduced at N-1 was found devoid of activity. The antileishmanial activity in this type of alicyclic nucleosides, thus depends on the chain length and the nature of the functional groups. Further, the high order of activity of the blocked nucleoside (**18**) indirectly suggested that perhaps the compound is an inhibitor of some important enzyme involved in the purine salvage process of the parasite.

## Experimental Procedure

For experimental details see earlier paper in the series[22].

1-[(2-*Benzyloxy*-1-*benzyloxymethylethoxy*)*methyl*]-4-*methylthiopyrazolo*[3,4-*d*]*pyrimidine* (**3**)

A mixture of 4-methylthiopyrazolo[3,4-*d*]pyrimidine (**1**) (3g, 18 mmole), DMF (30 ml) and $Et_3N$ (15 ml) was stirred at ambient temperature. To it was added dropwise a solution of 1,3-dibenzyloxy-2-chloromethoxypropane[22] (**2**) (6 g, 18 mmole) in DMF (10 ml) and stirring continued for 14 hr. The excess of reagent and the solvent were removed under reduced pressure and the residue taken in EtOAc, washed with $H_2O$ (2 × 100 ml), dried ($Na_2SO_4$) and the solvent removed. The product thus obtained was chromatographed on $SiO_2$ column. Elution of the column with $CHCl_3$ gave **3** as an oil (3.9 g, yield 70%), MS: m/z 450 ($M^+$); PMR($CDCl_3$): δ 8.7 (s, 1H, H-6), 7.9 (s, 1H, H-3), 7.2 (m, 10H, 2 × Ph), 5.88 (s, 2H, H-1′), 4.4 and 4.3 (each s, 4H, 2 × $CH_2$Ph), 3.9 (m, 1H, H-4′), 3.6-3.3 (m, 4H, H-3′, H-5′), 2.6 (s, 3H, $SCH_3$).

1-[(2-*Benzyloxy*-1-*benzyloxymethylethoxy*)*methyl*]-4(5*H*)-*oxopyrazolo*[3,4-*d*]*pyrimidine* (**6**)

*Method* 1

Compound **3** (1.5 g, 3 mmole) in dioxan (30 ml) was refluxed with 20% aq. KOH (30 ml) for 12 hr. The resulting mixture was cooled, neutralised with AcOH, and the solvent removed under reduced pressure. The residue was taken in $CHCl_3$, washed with $H_2O$, dried ($Na_2SO_4$) and concentrated. The product, thus, obtained was chromatographed on $SiO_2$ column. Elution with $CHCl_3$ – MeOH (96:4, v/v) gave **6** as an oil, yield 0.4 g (30%); MS: m/z 420 ($M^+$); IR(neat): 1710 $cm^{-1}$ (C = O); (PMR $CDCl_3$): δ 8.4 (s, 1H, H-6), 8.1 (s, 1H, H-3), 7.2 (m, 10H, 2 × Ph), 5.75 (s, 2H, H-1′), 4.4 (bs, 4H, H-3′, H-5′), 3.7-3.4 (m, 1H, H-4′), 3.3 and 3.5 (each s, 4H, 2 × O – $CH_2$Ph) (Found: C, 65.7; H, 5.1; N, 13.3. $C_{23}H_{24}N_4O_4$ requires C, 65.8, H, 5.8; N, 13.5%).

*Method* 2

A mixture of pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one

(2g, 15 mmole), HMDS (8 ml), drytoluene (50 ml) and a catalytic amount of $(NH_4)_2SO_4$ was refluxed for 24 hr. The solvent and excess of reagent were removed under reduced pressure to give the trimethylsilyl derivative (**9**) which without purification was used in the next step. A solution of **9** and 1,3-dibenzyloxy-2-chloromethoxypropane (**2**) (6 g, 19 mmole) in dry benzene (100 ml) was refluxed for 12 hr, cooled, filtered, and the solvent removed. The residue was extracted with $CHCl_3$, washed successively with saturated aq. $NaHCO_3$ (2 × 100 ml), brine (2 × 100 ml) and $H_2O$, dried ($Na_2SO_4$) and the solvent removed. The product, thus, obtained was chromatographed on $SiO_2$ column. Elution of the column with $CHCl_3$ – MeOH (96:4, v/v) gave **6**, (1.1 g, yield 55%), as an oil. The product **6** prepared by this procedure was identical (co-TLC, superimposible IR) with the product obtained by Method 1.

1-[(2-*Hydroxy*-1-*hydroxymethylethoxy*)*methyl*]-4(5*H*)-*oxopyrazolo*[3,4-*d*]*pyrimidine* (**7**)

A mixture of compound **6** (0.4 g, 95 mmole), $PdCl_2$ (0.5 g) and MeOH (20ml) was shaken in $H_2$ atmosphere (45 lbs, pressure) for 14 hr and the catalyst filtered. The filtrate was neutralized with resin (IR-45, $OH^-$ form) and filtered. The solvent from filtrate was removed and the residue chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (80:20, v/v) afforded **7** (0.14 g, yield 35%), m.p. 162-63° (EtOH); MS: m/z 240 ($M^+$); IR(KBr): 1680 $cm^{-1}$ (C=O); UV(MeOH): 251, 206 nm; (NaOH): 270, 213 nm; (HCl): 250, 208 nm; PMR($CDCl_3$ + DMSO-$d_6$): δ 7.95 (s, 1H, H-6), 7.9 (s, 1H, H-3), 5.75 (s, 2H, H-1′), 3.55-3.7 (m, 1H, H-4′), 3.3-3.5 (m, 4H, H-3′, H-5′) (Found: C, 40.0; H, 5.0; N, 23.7. $C_9H_{12}N_4O_4$ requires C, 40.1; H, 5.2; N, 23.4%).

4-*Amino*-1-[(2-*benzyloxy*-1-*benzyloxymethylethoxy*)*methyl*]*pyrazolo*[3,4-*d*]*pyrimidine* (**4**)

Compound **3** (2.0 g, 4.6 mmole) and methanolic $NH_3$ (25 ml, MeOH saturated with $NH_3$ at 0°) was heated in a steel bomb at 110° for 14 hr. Solvent and excess of $NH_3$ were removed under reduced pressure. The product was chromatographed on $SiO_2$ column. Elution with $CHCl_3$ – MeOH (96:4) gave **4** as an oil yield 1 g (50%); MS: m/z 419 ($M^+$); PMR($CDCl_3$): δ 8.3 (s, 1H, H-6), 7.8 (s, 1H, H-3), 7.3 and 7.2 (each s, 5H, Ar – H), 5.82 (s, 2H, H-1′), 4.5-4.4 (each s, 2H, $-OCH_2$), 4.0 (m, 1H, H-4′), 3.7-3.3 (m, 4H, H-3′, H-5′) (Found: C, 66.0; H, 6.0; N, 16.5. $C_{23}H_{25}N_5O_3$ requires C, 65.8; H, 6.1; N, 16.8%).

4-*Amino*-1-[(2-*hydroxy*-1-*hydroxymethylethoxy*)*methyl*]*pyrazolo*[3,4-*d*]*pyrimidine* (**5**)

Compound **4** (0.8 g, 1.9 mmole) and $PdCl_2$ (100 mg) in MeOH (30 ml) were shaken in $H_2$ atmosphere for 20 hr. The resulting mixture was filtered, neutralized with resin (IR-45, $OH^-$ form) and concentrated. The product was chromatographed over $SiO_2$ column. Elution with MeOH – $CHCl_3$ (20:80) afforded **5** (0.3 g, yield 40%), m.p. 182° (EtOH); MS: m/z 239 ($M^+$); IR(KBr): 3100 $cm^{-1}$ (N – H); PMR($CDCl_3$ + DMSO-$d_6$): δ 8.1 (s, 1H, H-6), 7.5 (s, 1H, H-3), 5.72 (s, 2H, H-1′), 4.3 (m, 1H, H-4′), 3.1-3.5 (m, 4H, H-3′,5′) (Found: C, 45.6; H, 5.7; N, 29.3. $C_9H_{13}N_5O_3$ requires C, 45.2; H, 5.6; N, 28.7%).

1-[(2-*Benzyloxy*-1-*phthaloylimidomethylethoxy*)*methyl*]-4(5*H*)-*oxopyrazolo*[3,4-*d*]*pyrimidine* (**11**)

A mixture of trimethylsilyl derivative (**9**) (3 g, 20 mmole) and 1-benzyloxy-2-chloromethoxy-3-phthaloylimidopropane (**10**)[19] (9 g, 20 mmole) in dry benzene (100 ml) was stirred at ambient temperature for 2 hr and then refluxed for 14 hr. The resulting mixture was cooled, filtered and concentrated *in vacuo*. The residue was taken in $CHCl_3$, washed successively with aq. $NaHCO_3$ (2 × 150 ml), NaCl (2 × 100 ml) and $H_2O$, dried ($Na_2SO_4$) and the solvent removed. The product, thus, obtained was chromatographed on $SiO_2$ column. Elution with $CHCl_3$ – MeOH (98:2, v/v) gave **11** as an oil, yield 2.5 g (52%); MS: m/z 459 ($M^+$); PMR($CDCl_3$): δ 8.5 (s, 1H, H-6), 8.2 (s, 1H, H-3), 7.6-7.8 (bs, 5H, ArH), 7.3 (bs, 4H, ArH), 5.7 (s, 2H, H-1′), 4.45 (s, 2H, O – $CH_2$Ph), 4.0-4.2 (m, 1H, H-4′), 3.4-3.8 (m, 4H, H-3′, H-5′).

1-[(2-*Benzyloxy*-1-*aminomethylethoxy*)*methyl*]-4(5*H*)-*oxopyrazolo*[3,4-*d*]*pyrimidine* (**13**)

A mixture of **11** (2 g, 4 mmole), MeOH (60ml) and hydrazine hydrate (6 ml, 98%) was left at 0° for 12 hr. The solvent and excess of reagent from the resulting mixture were removed under reduced pressure. The residue was taken in $CHCl_3$ and filtered. The filtrate was concentrated *in vacuo*. The crude product, thus obtained, was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (95:5, v/v) gave **13** as an oil, yield 0.8 g (40%); MS: m/z 329 ($M^+$); IR(neat): 1710 $cm^{-1}$; PMR($CDCl_3$): δ 8.2 (s, 1H, H-6), 7.9 (s, 1H, H-3), 7.2 (s, 5H, ArH), 5.75 (s, 2H, H-1′), 4.2 (s, 2H, C$H_2$Ph), 3.9-3.6 (m, 1H, H-4′), 3.4-3.2 (m, 4H, H-3′, H-5′).

1-[(2-*Hydroxy*-1-*aminomethylethoxy*)*methyl*]-4(5*H*)-*oxopyrazolo*[3,4-*d*]*pyrimidine* (**12**)

A mixture of compound **13** (0.6 g, 1.8 mmole), MeOH (30 ml), and $PdCl_2$ (0.09 g) was shaken in $H_2$ atmosphere (45 lbs pressure) for 12 hr. The resulting mixture was filtered, the filtrate neutralised with resin (IR-45, $OH^-$ form) and the solvent removed. The product thus obtained was chromatographed over

$SiO_2$ column. Elution with $CHCl_3$ – MeOH (80:20, v/v) gave **12**, yield 0.2 g (30%), m.p. 202-4° (EtOH), MS: m/z 239 ($M^+$); UV(MeOH): 250, 206 nm; (NaOH): 272, 212 nm; (HCl): 250, 207 nm; IR(KBr): 1710 $cm^{-1}$; PMR($CDCl_3$ + DMSO-$d_6$): δ 8.0 (s, 1H, H-6), 7.8 (s, 1H, H-3), 5.8 (s, 2H, H-1'), 3.0-2.5 (m, 4H, H-3', H-5'),2.6-2.4 (m, 1H, H-4') (Found: C, 45.1; H, 5.6; N, 29.6. $C_9H_{13}N_5O_2$ requires C, 45.2; H, 5.4; N, 29.3%).

1-[(2-*Benzoyloxyethoxy*)*methyl*-4-*methylthio-pyrazolo*[3,4-*d*]*pyrimidine* (**15**)

A mixture of **1** (3.0 g, 18 mmole), $Et_3N$ (20 ml) and dry DMF (50 ml) was stirred at 10° to 15°. To it was added dropwise a solution of (2-benzoyloxyethoxy)methyl chloride (**14**[23] (6.0 g, 28 mmole) (prepared by passing dry HCl gas into a mixture of paraformaldehyde and 1-benzoyloxy-2-hydroxyethane in $CH_2Cl_2$ at 0° for 2 hr) and the mixture stirred at ambient temperature for 15 hr. The solvent and the excess reagent were removed under reduced pressure. The residue was extracted with $CHCl_3$, washed with $H_2O$, dried ($Na_2SO_4$) and concentrated *in vacuo*. The product thus obtained was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ gave **15** as an oil yield 2.2 g (60%); MS: m/z 344 ($M^+$); PMR($CDCl_3$): δ 8.65 (s, 1H, H-6), 7.7-8.1 (m, 3H, ArH adjacent to C=O, H-3), 7.1-7.5 (m, 3H, Ar*H*), 5.85 (s, 2H, H-1'), 4.1-4.4 (m, 2H, H-3'), 3.6-3.0 (m, 2H, H-4'), 2.6 (s, 3H, $SCH_3$).

1-[(2-*Hydroxyethoxy*)*methyl*]-4-*methylthiopyrazolo*-[3,4-*d*]*pyrimidine* (**16**)

A mixture of **15** (2 g, 6 mmole) and methanolic $NH_3$ (45 ml, MeOH saturated with $NH_3$ at 0°) was kept at ambient temperature for 24 hr. The solvent and the excess of reagent from the resulting mixture were removed and the residue was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (96:4, v/v) afforded **16** (1.1 g, yield 55%), m.p. 94-96° (EtOH); MS: m/z 240 ($M^+$); IR(KBr): 3336 (OH); PMR($CDCl_3$ + DMSO-$d_6$): δ 8.85 (s, 1H, H-6), 8.5 (s, 1H, H-3),5.8 (s, 2H, H-1'), 3.6 (bs, 4H, H-3', H-4'), 2.6 (s, 3H, $SCH_3$) (Found: C, 45.0; H, 5.0; N, 23.3. $C_9H_{12}N_4O_2S$ requires C, 45.1; H, 5.2; N,23.3%).

1-[(2-*Hydroxyethoxy*)*methyl*]-4(5*H*)-*oxopyrazolo*-[3,4-*d*]*pyrimidine* (**19**)

*Method I*

A mixture of **16** (0.5 g, 2 mmole) and aq. KOH (20%, 15 ml) in dioxan (30 ml) was refluxed for 12 hr, cooled, neutralised with AcOH and the solvents were removed under reduced pressure. The residue was taken in $CHCl_3$, washed with $H_2O$, dried ($Na_2SO_4$) and concentrated. The product thus obtained was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (90:10, v/v) gave, **19** yield 0.2 g (30%), m.p. 138-40° (EtOH); UV(MeOH): 250, 208 nm; (NaOH): 221, 215 nm; (HCl): 251, 213 nm; MS: m/z 210 ($M^+$); IR(KBr): 1710; PMR($CDCl_3$ + DMSO-$d_6$): 7.95 (s, 1H, H-6), 7.9 (s, 1H, H-3), 5.7 (s, 2H, H-1'), 3.5 (bs, 4H, H-3', H-4') (Found: C, 45.9; H, 5.0; N, 26.4. $C_8H_{10}N_4O_3$ requires C, 45.8; H, 4.9; N, 26.6%).

*Method II*

A mixture of **18** (0.8 g, 2.5 mmole) and methanolic $NH_3$ (25 ml, MeOH saturated with $NH_3$ at 0°) was kept at ambient temperature for 24 hr. The solvent and the excess of $NH_3$ were removed under reduced pressure. The product, thus obtained, was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (92:8, v/v) gave **19**, yield 0.5 g (60%), m.p. 138-40°. The compound **19** prepared by this procedure was found identical (co-TLC and superimposible IR) with the product obtained by method I.

1-[(2-*Benzoyloxyethoxy*)*methyl*]-4(5*H*)-*oxopyrazolo*-[3,4-*d*]*pyrimidine* (**18**)

A mixture of **9** (3 g, 20 mmole), 1-benzoyloxy-2-chloromethoxyethane (6 g, 28 mmole) and dry benzene (40 ml) was refluxed for 16 hr. The resulting mixture was cooled, filtered and the filtrate concentrated under reduced pressure. The residue was taken in $CHCl_3$, washed with aq. $NaHCO_3$, $H_2O$, dried ($Na_2SO_4$) and the solvent removed under reduced pressure. The product, thus obtained, was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (98:2, v/v) gave **18**, yield 1.7 g (58%), m.p. 118-19° (EtOH); MS: m/z 314 ($M^+$); PMR($CDCl_3$): δ 8.25 and 8.1 (each s, 2H, H-6, H-3), 8.0-7.8 (m, 2H, ArH adjacent to >C=O), 7.45-7.25 (m, 3H, ArH), 5.5 (s, 2H, H-1'), 4.5-4.3 (m, 2H, H-3'), 4.1-3.8 (m, 2H, H-4') (Found: C, 57.3; H, 48; N, 17.9. $C_{15}H_{14}N_4O_4$ requires C, 57.4; H, 4.5; N, 17.7%).

1-[(2-*Hydroxyethoxy*)*methyl*]-4-*aminooyrazolo*-[3,4-*d*]*pyrimidine* (**17**)

A mixture of **16** (0.5 g, 21 mmole) and methanolic $NH_3$ (20 ml, MeOH saturated with $NH_3$ at 0°) was heated in a steel bomb at 110° for 14 hr. The solvent and the excess of $NH_3$ were removed under reduced pressure. The product, thus obtained, was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (90:10, v/v) gave **17**, yield 0.3 g (50%), m.p. 153-54° (EtOH); MS: m/z 209 ($M^+$); PMR($CDCl_3$ + DMSO-$d_6$): δ 8.2 (s, 1H, H-6), 8.1 (s, 1H, H-3), 5.7 (s, 2H, H-1'), 3.6 (s, 4H, H-3', H-4') (Found: C, 45.9; H, 5.5; N, 33.5. $C_8H_{11}N_5O_2$ requires C, 45.8; H, 5.3; N, 33.7%).

*4-Methylthio-1-(tetrahydrofuran-2-yl)pyrazolo-[3,4-d]pyrimidine* (**21**)

A mixture of **1** (1 g, 6 mmole), 2,3-dihydrofuran (**20**, 1.2 g, 18 mmole), *p*-toluenesulphonic acid (PTSA) (0.1 g) and ethyl acetate (50 ml) was stirred at 50° for 12 hr. The resulting mixture was cooled, washed with aq. $K_2CO_3$ solution, $H_2O$ and dried ($Na_2SO_4$). Ethyl acetate was removed *in vacuo* and the product chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (99:1, v/v) gave **21**, yield 1.0 g (71.4%), m.p. 66°; UV(MeOH): 270 nm, MS: m/z 236 ($M^+$); PMR($CDCl_3$): δ 8.65 (s, 1H, H-6), 8.0 (s, 1H, H-3), 6.75 (m, 1H, H-1′), 4.1-3.6 (m, 2H, H-4′), 2.5 (s, 3H, $SCH_3$), 2.1-1.6 (m, 4H, H-2′ and H-3′) (Found: C, 50.5; H, 5.1; N, 24.1. $C_{10}H_{12}N_4OS$ requires C, 50.8; H, 5.0; N, 23.7%).

*4-Amino-1-(tetrahydrofuran-2-yl)pyrazolo-[3,4-d]pyrimidine* (**23**)

A mixture of **21** (1 g, 42 mmole) and methanolic $NH_3$ (50 ml, MeOH saturated with ammonia at 0°) was heated at 120° in a steel bomb for 12 hr. The solvent and excess of reagent were removed *in vacuo*. The product was chromatographed over $SiO_2$ column. Elution with $CHCl_3$ – MeOH (97:3 v/v) afforded **23**, yield 0.75 g (88%) m.p. 135°; MS: m/z 205 ($M^+$): PMR($CDCl_3$ + DMSO-$d_6$): δ 8.15 (s, 1H, H-6), 8.0 (s, 1H, H-3), 7.4-7.0 (bs, 2H, $NH_2$), 6.0-6.2 (m, 1H, H-1′), 4.3-3.4 (m, 2H, H-4′), 2.0-1.6 (m, 4H, H-2′ and H-3′) (Found: C, 52.9; H, 5.6; N, 34.0. $C_9H_{11}N_5O$ requires C, 52.6; H, 5.4; N, 34.1%).

*4(5H)-Oxo-1-(tetrahydrofuran-2-yl)pyrazolo-[3,4-d]pyrimidine* (**22**)

A mixture of **21** (1 g, 4.2 mmole) and 2*N*NaOH (50 ml) was stirred at 70° for 4 hr. The resulting mixture was cooled, extracted with EtOAc, washed with $H_2O$, dried ($Na_2SO_4$) and the solvent removed *in vacuo*. The product, thus obtained, was chromatographed over $SiO_2$ column. Elution of the column with $CHCl_3$ – MeOH (97:3, v/v) afforded **22**, yield 0.6 g (69%), m.p. 147°; MS: m/z 206 ($M^+$); PMR($CDCl_3$ + DMSO-$d_6$): δ 8.3 (s, 1H, H-6), 8.1 (s, 1H, H-3), 6.3-6.0 (m, 1H, H-1′), 4.3-3.8 (m, 2H, H-4′), 2.0-1.6 (m, 4H, H-2′ and H-3′) (Found: C, 52.3; H, 4.8; N, 27.0. $C_9H_{10}N_4O_2$ requires C, 52.4; H, 4.8; N, 27.1%).

**Acknowledgement**

Financial support from the office of Naval Research (Grant: N00014-82-9-0130) is gratefully acknowledged. Thanks are also due to RSIC staff, CDRI, Lucknow for providing spectral data.

**References**

1 Robins R K, *J Am chem Soc*, 78 (1956) 784; Schmidt P & Druey J, *Helv chim Acta*, 39 (1956) 986.
2 Elion G B, Callahan S W, Nathan H, Bieber S, Rundles R W & Hitchings G H, *Biochem Phrmacol*, 12 (1963) 85; Rundles R W, Wyngaarden J B, Hitchings G H, Elion G B & Silbermann H R, *Trans Assoc Am Physicians*, 76 (1963) 126.
3 Elion G B & Hand B, *Exp Pharmacol*, 51 (1978) 485 ff.
4 Handschuhmacher R E, *J biol Chem*, 235 (1960) 2917; Janku I, Krsiak M, Volicer L, Capek R, Smetana R & Novotny J, *Biochem Pharmacol*, 14 (1965) 1525; Saenger W & Suck D, *Nature*, 242 (1973) 610.
5 Fyfe J A, Miller R L & Krenitsky T A, *J biol Chem*, 248 (1973) 3801; Traut T W & Jones M E, *Biochem Pharmacol*, 26 (1977) 2291.
6 Nelson D J, Lafon S W, Tuttle J V, Miller W H, Miller R U, Krenitsky T A, Elion G B, Berens R L & Marr J J, *J biol Chem*, 254 (1979) 11544.
7 Krenitsky T A, Elion G B, Strelitz R A & Hitchings G H, *J biol Chem*, 242 (1967) 2675; Krenitsky T A, Neil S M, Elion G B & Hitchings G H, *Arch Biochem Biophys*, 150 (1972) 585.
8 Lichtenthaler F W & Cuny E, *Chem Ber*, 114 (1981) 1610; Bergmann F, Frank A & Neiman Z, *J chem Soc Perkin I*, (1979) 2795.
9 Sakai T, Ushio K, Ichimoto I & Omata S, *Agric biol Chem*, 38 (1974) 433.
10 Schnebli H P, Hill D L & Bennett (Jr) L L, *J Biol Chem*, 242 (1967) 1997.
11 Agarwal R P, Crabtree G W, Agarwal K C & Parks (Jr) R E & Townsend L B, *Proc Am Assoc Cancer Res*, 17 (1976) 214.
12 Henderson J F & Jungo I G, *Cancer Res*, 21 (1961) 118.
13 Bennett (Jr) L L, Allan P W, Smithers D & Vail M H, *Biochem Pharmacol*, 18 (1969) 725.
14 Hecht S M, Frye R B, Werner D, Fukui T & Hawrelak M H, *Biochemistry*, 15 (1976) 1005.
15 Sutcliffe E Y, Zee-Cheng K Y, Cheng C C & Robins R K, *J Med Pharm Chem*, 5 (1962) 588.
16 (a) Elion G B, Furman P A, Fyfe J A, de Miranda P, Beauchamp L & Schaeffer H J, *Proc Natl Acad Sci USA*, 74 (1977) 5716; (b) Schaeffer H J, Beauchamp L, de Miranda P, Elion G B, Bauer D J & Collins P, *Nature*, 272 (1978) 583.
17 (a) Drugs Fut, 13 (1988) 477, (b) Tucker P, Nordin M C, Silver M, Sobotka P, Robinson J & I'keef J P, *Clin Res*, 35 (1986) 860A.
18 Robins R K, *J Am chem Soc*, 78 (1956) 784.
19 Liu M C, Kuzmich S & Lin T S, *Tetrahedron Lett*, 25 (1984) 613.
20 Robins R K, *J Mednl Chem*, 7 (1964) 186.
21 Hasan A, Tripathi R P, Pratap R, Bhakuni D S, Pal R, Mishra A, Guru P Y & Katiyar J C, *Indian J Chem*, 28B (1989) 403.
22 Singh P K, Saluja S, Pratap R, George C X & Bhakuni D S, *Indian J Chem*, 25B (1986) 823.
23 Deo K, Avasthi K, Pratap R, Kar K & Bhakuni D S, *Indian J Chem*, 26B (1987) 963.