References and Notes

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Analogues of 8-Azainosine

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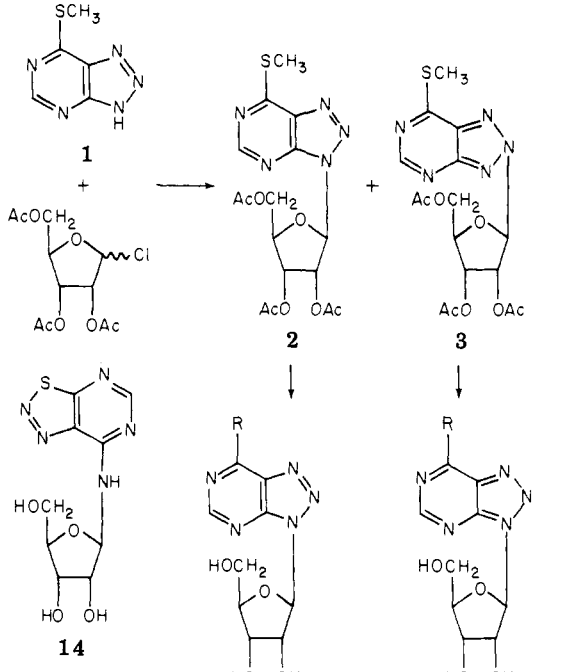
A convenient synthesis of 8-azapurine ribonucleosides substituted at the 6 position with thio, alkylthio, alkoxy, amino, and alkylamino groups is described. The reaction of 6-(methylthio)-8-azapurine (**1**) with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride in the presence of Linde AW-500 molecular sieve gave a 2:1 mixture of **2** and **3**, respectively. This mixture was rearranged by heating with molecular sieve in refluxing toluene to give a 6:1 mixture of **2** and **3**. Treatment of **2** or **3** with the appropriate nucleophiles at room temperature gave 6-substituted 8-azapurine ribonucleosides (7-substituted 2- or 3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidines) **4**–**13**. The thione **11** rearranges to *N*-β-D-ribofuranosyl[1,2,3]thiadiazolo[5,4-*d*]pyrimidin-7-amine (**14**) in the solid state or in solution. All of these compounds were cytotoxic to H.Ep. No. 2 cells in culture except the parent base, 8-aza-6-(methylthio)purine (**1**) and the 8-isomers (**3**, **12**, and **13**). Three of these compounds—8-azaadenosine (**4**), 8-aza-6-(methylthio)purine ribonucleoside (**5**), and 8-aza-6-(methoxy)purine ribonucleoside (**7**)—showed borderline activity in the leukemia L1210 system. The thiadiazolopyrimidine (**14**) showed activity at three dose levels.

The moderate anticancer activity of 8-azahypoxanthine against adenocarcinoma 755[1] is markedly enhanced by conversion of the azapurine to its ribonucleoside, 8-azainosine (3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one),[2] which is active not only against Ca 755 but also against leukemia L1210 and a strain of L1210 resistant to 6-mercaptopurine and 8-azahypoxanthine.[3] 8-Azaadenosine (3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine) and 6-(methylthio)purine ribonucleoside, substrates for adenosine kinase,[4] have also shown antileukemic activity in test systems resistant to the parent heterocycles.[5,6] These results prompted us to prepare a number of 8-azapurine ribonucleosides substituted with thio, alkylthio, alkoxy, and alkylamino groups at the 6 position in an effort to find other potentially useful anticancer agents.

8-Aza-6-(methylthio)purine [7-(methylthio)-1,2,3-triazolo[4,5-*d*]pyrimidine, **1**] was prepared in 75% yield by the procedure of Weiss et al.[7] by nitrosation of 4,5-diamino-6-(methylthio)pyrimidine.[8] The reaction of **1** with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride[9] in refluxing benzene containing Linde AW-500 molecular sieve gave after 21 h an 89% yield of a 2:1 mixture of 7-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,3-triazolo[4,5-*d*]pyrimidine and the 2-substituted isomer (**2** and **3**, respectively) as determined by ^{1}H NMR. A higher ratio of **2** to **3** (4:1) was formed in an earlier reaction, suggesting that **3** might rearrange to **2** with an increase in temperature or reaction time. The rearrangement of **3** to **2** was confirmed by refluxing a solution of **2** and **3** (2:1) in toluene containing molecular sieve for 4 days to give a 6:1 mixture of **2** and **3**. Additional details concerning this molecular sieve catalyzed rearrangement are described in a previous communication.[10] The mixture of **2** and **3** was separated by silica gel column chromatography to give a 61% overall yield of **2**, a 12% yield of **3**, and a 13% yield of a mixture of **2** and **3**. The high combined overall yield (86%) from **1** is proof that the final 6:1 ratio is a result of rearrangement rather than preferential decomposition of **3** from the initial 2:1 mixture. The structures of **2** and **3** were established by treatment with ammonia to give the known adenosine analogues, 3- and 2-β-D-ribofuranosyl-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amines[11,12] (**4** and **12**, respectively). Compounds **4** and **12** were identical (TLC, uv, and NMR) to the authentic samples. Further evidence for the β configuration of **3** is the small ^{1}H NMR coupling constant[13] ($J_{1',2'}$) of 2.8 Hz and the observation that only one anomer of **3** was formed, since, in the rare cases in which the formation of cis nucleosides from 2-acyloxy-1-halofuranoses has been observed, the trans nucleoside always predominates.

The 7-methylthio group of **2** and **3** was readily replaced at room temperature with a variety of nucleophiles to give the corresponding 7-substituted nucleosides **4**–**13**. During

the preparation of these compounds, a paper was published[12] which described a similar synthesis of several of these nucleosides (4, 7, 11, and 12) from the 2',3',5'-*O*-benzoyl analogues of 2 and 3. The *O*-acetyl intermediates 2 and 3 have proved in some cases to be a better source of nucleosides 4–13 because of the greater ease of hydrolysis of the acetyl groups. Treatment of 2 with excess MeSH in MeOH containing NaOMe gave a 50% yield of pure 7-(methylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (5). A similar reaction of 2 with EtSH in MeOH gave a 60% yield of the 7-(ethylthio) analogue 6. Treatment of 2 with NaOMe in MeOH resulted in clean displacement of the methylthio group to give an 85% yield of 7-methoxy-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (7). The ethoxy analogue 8 was prepared in 63% yield from 2 and NaOEt in EtOH. Pure *N*-butyl-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (9) was prepared by treatment of 2 with *n*-butylamine in MeOH and isolated in 51% yield by preparative TLC. Alkylation of potassium phthalimide with 3-methyl-2-butenyl chloride in DMF gave a 78% yield of *N*-(3-methyl-2-butenyl)phthalimide, which was treated with N_2H_4 to give, after fractional distillation, a 67% yield of 3-methyl-2-butenylamine. Treatment of 2 with this amine in MeOH gave a crude product which was purified by preparative TLC to give a 90% yield of *N*-(3-methyl-2-butenyl)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (10).



4, R = NH_2
5, R = SMe
6, R = SEt
7, R = OMe
8, R = OEt
9, R = NHBu
10, R = $NHCH_2$-CH=CMe_2
11, R = SH

12, R = NH_2
13, R = SCH_3

3,6-Dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo-[4,5-*d*]pyrimidine-7-thione (11) was prepared by reaction of 2 with NaSH in EtOH, followed by treatment with NaOMe. An aqueous solution of the Na salt of 11 was neutralized with dilute HCl to give a precipitate of 11 in 46% yield. This compound was identified by NMR and uv spectral comparisons with authentic 11[12] and 3,6-dihydro-3-benzyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione.[14]

Table I. Cytotoxicity, H.Ep. No. 2 Cells in Culture[a]

No.	ED_{50}, μM[b]	Degree of resistance[c] /MeMPR	/FA/FAR
1	>300		
2	0.5		
3	(7)		
4	0.8	2	2
5	0.02	2000	3000
6	0.1		300
7	0.3	6	6
8	0.1	20	
9	0.3		
10	<3		
11	1	90	40
12	>70		
13	(6)		
14	0.1		

[a] Human epidermoid carcinoma No. 2 cells, see ref 15. [b] The concentration required to inhibit the growth of treated cells to 50% of that of untreated controls. [c] The ratio of the ED_{50} in the resistant H.Ep. No. 2 cell line to the ED_{50} in the sensitive or wild H.Ep. No. 2 line; /MeMPR = resistant to 6-(methylthio)purine ribonucleoside, lacks adenosine kinase; /FA/FAR = resistant to 2-fluoroadenine and 2-fluoroadenosine, lacks adenine phosphoribosyltransferase and adenosine kinase.

Compound 11, as noted by Hutzenlaub et al.,[12] is unstable in aqueous solution, being quantitatively transformed (TLC) after several weeks into another product, which was presumed by Hutzenlaub et al. to be the disulfide of 11. We have found that this product is formed in good yield by heating 11 under N_2 at 138 °C for a short time. A ^1H NMR of this compound showed close coupling of $H_{1'}$ with an exchangeable proton which would be consistent with *N*-β-D-ribofuranosyl[1,2,3]thiadiazolo[5,4-*d*]pyrimidin-7-amine (14) resulting from rearrangement of 11. Addition of D_2O to the solution of 14 in Me_2SO-d_6 caused the multiplet for $H_{1'}$ to coalesce into a doublet with a coupling constant ($J_{1',2'}$) of 5.0 Hz. No evidence of a second anomer or a disulfide could be detected. Attempted iodine oxidation of 11 to the disulfide gave only 8-azainosine. A similar thermal rearrangement of 6-mercapto-8-azapurine to [1,2,3]thiadiazolo[5,4-*d*]-pyrimidin-7-amine (15) has been described by Albert.[14] The elemental analysis, mass ion of *m/e* 285, and a uv spectrum similar to 15 are further evidence for structure 14. Since conversion of 11 to 14 under the conditions of the cytotoxicity assay might be responsible for the activity of 11 (see below), this conversion in buffer at pH 7.3 was followed by high-pressure liquid chromatography (HPLC). At the end of 6 days, only 20% of 11 had rearranged to 14. Under the same conditions, no detectable amount of 14 was converted to 11.

The minor nucleoside 3 from the molecular sieve catalyzed rearrangement mixture was converted to 7-(methylthio)-2-β-D-ribofuranosyl-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (13) in addition to the previously described 12. The preparation of 13 in 41% yield from 3 and MeSH was carried out by the same procedure used for the synthesis of 5.

Biologic Evaluation. The compounds prepared in this study were evaluated for their ability to inhibit the growth of H.Ep. No. 2 cells in culture (Table I).[15] Only 8-aza-6-(methylthio)purine (1) and the 8-isomer (12) of 8-aza-adenosine were ineffective as inhibitors of growth of this mammalian cell line, although the ED_{50} of the other compounds varied from a low of 0.02 μM to a high of 7 μM. The most cytotoxic agent, 8-aza-6-(methylthio)purine

ribonucleoside (**5**), was 40 times as effective as 8-azaadenosine (**4**). The apparent cytotoxicities of the 8-isomers **3** and **13** can be explained by the contamination with 1–2% of the 9-isomers **2** and **5**. The presence of that small amount of **2** in **3** was established by HPLC. The triacetate (**2**) of **5** is only one twenty-fifth as active as **5**, presumably indicating some resistance to deacetylation under the conditions of these tests. The ED_{50} values of the 8-aza-9-ribosyl-6-substituted purines **6–9** are around 0.1–0.3 μM, which means they are highly cytotoxic nucleosides. Even 8-aza-6-thioinosine (**11**) is quite cytotoxic, its ED_{50} (1 μM) being only an order of magnitude (or less) greater than the others, whereas the parent base of **11**, 8-aza-6-thiopurine,[16] is not cytotoxic. The rearrangement product from **11**, *N*-β-D-ribofuranosyl[1,2,3]thiadiazolo[5,4-*d*]pyrimidin-7-amine (**14**), is also, surprisingly, quite cytotoxic but not cytotoxic enough to explain, at the level it was found in **11** (<4%, HPLC), the cytotoxicity of **11**. The base from which **14** derives, [1,2,3]thiadiazolo[5,4-*d*]pyrimidin-7-amine, shows no cytotoxicity at the highest level tested (ca. 50 μM).

All of the 8-aza-6-substituted purine ribonucleosides tested (**4–8, 11**) proved to be substrates for adenosine kinase,[4] and, presumably, this conversion to the nucleotide is essential to their activity, since, with a single exception, cell lines lacking adenosine kinase (H.Ep. No. 2 /MeMPR and /FA/FAR)[15] are resistant to these compounds, although the degree of resistance varies from compound to compound. The single exception, 8-azaadenosine (**4**), is readily deaminated by H.Ep. No. 2 cells to 8-azainosine, which is cytotoxic to cell lines lacking adenosine kinase. The 60-fold resistance of the H.Ep. No. 2 /MP/MeMPR cell line that lacks both adenosine kinase and hypoxanthine–guanine phosphoribosyltransferase to 8-azaadenosine indicates that the activity of 8-azaadenosine is due in part to its conversion to 8-azahypoxanthine (via 8-azainosine), since that cell line cannot convert the latter compound to its nucleotide. At the same time, the moderately low order of resistance is undoubtedly due to the direct phosphorylation of 8-azainosine to its nucleotide. The low order of resistance of H.Ep. No. 2 /MeMPR and /FA/FAR cells to the *O*-methyl (**7**) derivative of 8-azainosine is a result of its conversion to 8-azainosine by adenosine deaminase, and presumably this is also the explanation for the activity of **8**. The methylthio compound **5** is more resistant to the action of the deaminase.

All of these compounds were evaluated for their ability to inhibit leukemia L1210 in mice,[3] but only four showed reproducible activity. Compound **4** gave a 39% increase in lifespan, **5** a 23% increase, and **7** a 29% increase, indicating that these compounds are less effective in this test system than 6-mercaptopurine, 6-(methylthio)purine ribonucleoside, and 8-azainosine. Unexpectedly, the thiadiazolopyrimidine **14** was active at three dose levels giving increases in lifespan of 36, 28, and 25%.

Experimental Section

Melting points were determined on a Kofler Heizbank unless otherwise indicated. Absence of melting point data indicates an indefinite melting point. The ultraviolet absorption spectra were determined with a Cary Model 17 spectrophotometer. Each compound was dissolved in the solvent indicated in parentheses and diluted tenfold with 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH. The ^1H NMR spectra were determined in 3–7% w/v solutions in Me_2SO-d_6 unless otherwise indicated and with a Varian XL-100-15 spectrometer operating at 100 MHz or T-60A operating at 60 MHz (internal Me_4Si). The relative peak areas are given to the nearest whole number, and chemical shifts quoted in the case of multiplets are measured from the approximate center. The ^{13}C NMR spectra were determined in 6–11% w/v solutions in Me_2SO-d_6 with a Varian XL-100-15 spectrophotometer operating at 25.160 MHz and equipped with a Digilab FTS NMR-3 pulser and data system. Mass spectral data were taken with a Varian MAT 311A instrument equipped with a combination E1/F1/FD ion source. Merck 2-mm silica gel 60 F-254 preparative TLC plates (8 × 8 in.) were used for preparative TLC purifications. High-pressure liquid chromatography (HPLC) was carried out on an ALC-242 liquid chromatograph equipped with a uv detector (254 nm), an M-6000 pump, and a 30 cm × 4 mm (i.d.) column of μBondapak C_{18} (Waters Associates, Inc.). Compounds were eluted with water containing 5–40% acetonitrile. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

7-(Methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (2) and 7-(Methylthio)-2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (3). A stirred mixture of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride[9] (96.0 mmol), 7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine[7] (**1**) (15.8 g, 94.5 mmol), and Linde AW-500 molecular sieve (100 g) in anhydrous benzene (4.7 l.) was refluxed (Drierite-protected) for 1 h, treated with additional molecular sieve (100 g), and refluxed for 20 h. The solution was cooled, filtered, and evaporated in vacuo to a syrup (37.9 g) consisting of a 2:1 mixture (determined by ^1H NMR) of **2** and **3**. A solution of the mixture (21.5 g) in anhydrous toluene (500 ml) containing molecular sieve (10 g) was refluxed for 3 days, treated with additional molecular sieve (10 g), and refluxed for 3 additional days. Evaporation of the filtered solution gave 20.5 g of a 6:1 mixture of **2** and **3**. A solution of this mixture (9.0 g) in a minimum of $CHCl_3$ was applied to a 5 × 75 cm column of Mallinkrodt SilicAR TLC-7 (900 g),[17] prepared in $CHCl_3$. The column was developed with $CHCl_3$ until most of the faster moving **2** had been eluted. The eluting solvent was changed gradually to $CHCl_3$–MeOH (97:3) to remove **3**. Evaporation of the fractions in vacuo gave 6.25 g (61% overall yield) of **2**, 1.25 g (12%) of **3**, and 1.52 g (13%) of a mixture of **2** and **3**. Compound **2**: λ_{max} nm (ε × 10^{-3}) (EtOH) (0.1 N HCl and pH 7) 277 (11.5), 305 (15.2); ^1H NMR ($CDCl_3$) δ 2.07, 2.09, 2.15 (m, 9, H_{OAc}), 2.8 (s, 3, H_{SMe}), 6.61 (d, 1, $J_{1'2'}$ = 4.0 Hz, $H_{1'}$), 7.25 (s, $CHCl_3$). Anal. ($C_{16}H_{19}N_5O_7S$·0.1$CHCl_3$) C, H, N. Compound **3**: λ_{max} nm (ε × 10^{-3}) (EtOH) (pH 7) 221 (12.3), 264 (6.05), 271 (5.75), 321 (12.8); ^1H NMR ($CDCl_3$) δ 2.10, 2.13 (d, 9, OAc), 2.76 (s, 3, SCH_3), 6.50 (d, 1, $J_{1'2'}$ = 2.8 Hz, $H_{1'}$), 7.27 (s, $CHCl_3$), 8.91 (s, 1, H_5). Anal. ($C_{16}H_{19}N_5O_7S$·0.1$CHCl_3$) C, H, N.

3-β-D-Ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (4). A solution of **2** (437 mg, 1.00 mmol) in $CHCl_3$ (5 ml) was added to MeOH (50 ml) presaturated with NH_3. The solution was stirred for 90 h at 25 °C in a stoppered flask and evaporated to dryness in vacuo. The residue was triturated with $CHCl_3$, collected by filtration, dried in vacuo, and crystallized from hot water (6 ml) to give 240 mg (90% yield) of white crystalline product, mp 204–205 °C (Mel-Temp). Recrystallization from H_2O (4 ml) gave 220 mg (82%) of pure **4**: mp 205–207 °C; $[\alpha]^{40}D$ −84.5 ± 0.6° (*c* 0.52, H_2O): λ_{max} nm (ε × 10^{-3}) (H_2O) (0.1 N HCl) 262 (12.4), (pH 7) 209 (14.6), 278 (11.6), (0.1 N NaOH) 278 (11.6); ^1H NMR δ 6.19 (d, 1, $J_{1'2'}$ = 5.0 Hz, $H_{1'}$), 8.35 (s, 1, H_5); ^{13}C NMR δ (±0.02) 61.96 ($C_{5'}$), 70.81 ($C_{2'}$), 73.00 ($C_{3'}$), 86.29 ($C_{4'}$), 89.90 ($C_{1'}$), 124.21 (C_{7a}), 148.90 (C_{3a}), 156.30 (C_7), 156.84 (C_5). Anal. ($C_9H_{12}N_6O_4$) C, H, N.

7-(Methylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (5). A solution of NaOMe (81 mg, 1.50 mmol) in MeOH (60 ml) was saturated with MeSH, treated with a solution of **2** (874 mg, 2.00 mmol) in $CHCl_3$ (20 ml), allowed to stand in a stoppered flask for 66 h, diluted with H_2O (80 ml), and neutralized with Amberlite IR-120 H^+ ion-exchange resin. The aqueous layer was washed with $CHCl_3$ and evaporated to dryness in vacuo, and the residue was crystallized from EtOH and dried in vacuo (P_2O_5): yield 224 mg (50%); mp 150 °C; λ_{max} nm (ε × 10^{-3}) (EtOH) (0.1 N HCl) 226 (10.5), 304 (14.5), (pH 7) 226 (10.5), 303 (14.4); ^1H NMR δ 2.79 (s, 3, CH_3), 3.58 (m, 2, $H_{5'}$), 4.06 (m, 1, $H_{4'}$), 4.39 (m, 1, $H_{3'}$), 4.83 (m, 2, $O_{5'}H$, $H_{2'}$), 5.27 (d, 1, $O_{2'}H$), 5.59 (d, 1, $O_{3'}H$), 6.29 (d, 1, $J_{1'2'}$ = 5.0 Hz, $H_{1'}$), 8.97 (s, 1, H_5). Anal. ($C_{10}H_{13}N_5O_4S$) C, H, N.

7-(Ethylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (6). The title compound was prepared from **2** and

EtSH on the same scale and by the procedure described for **5**. The crude product was purified on two preparative TLC plates developed with $CHCl_3$–MeOH (9:1) and extracted with *i*-PrOH. Evaporation of the extract in vacuo gave a 60% yield of **6**: mp 42–46 °C (Mel-Temp); λ_{max} nm ($\epsilon \times 10^{-3}$) (H_2O) (0.1 N HCl) 227 (10.0), 305 (14.5), (pH 7) 227 (9.88), 305 (14.4); ^1H NMR δ 1.05 (d, 2-propanol), 1.43 (m, 3, CH_3), 6.27 (d, 1, $J_{1',2'}$ = 4.6 Hz, $H_{1'}$), 8.98 (s, 1, H_5). Anal. ($C_{11}H_{15}N_5O_4S \cdot 0.2H_2O \cdot 0.1C_3H_8O$) C, H, N.

7-Methoxy-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**7**). A solution of **2** (490 mg, 1.12 mmol) and NaOMe (47 mg, 0.87 mmol) in MeOH (15 ml) was stirred at 25 °C for 66 h, diluted with H_2O (15 ml), neutralized with Amberlite IR-20 H^+ ion-exchange resin, filtered, and evaporated to dryness in vacuo. The residue was crystallized from 20:1 H_2O–MeOH (5 ml) and dried in vacuo (P_2O_5): yield 240 mg (85%); mp 180 °C (Mettler FP1) (lit.[12] mp 181–182 °C); λ_{max} nm ($\epsilon \times 10^{-3}$) (EtOH) (0.1 N HCl) 252 (10.4), 305 (0.577), (pH 7) 252 (10.7), 305 (0.577), (0.1 N NaOH) 275 (9.47); ^1H NMR δ 4.24 (s, 3, CH_3), 6.3 (d, 1, $J_{1',2'}$ = 5.0 Hz, $H_{1'}$), 8.81 (s, 1, H_5). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

7-Ethoxy-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**8**). The blocked nucleoside **2** (500 mg, 1.15 mmol) was dissolved in a solution of NaOEt prepared from Na (20.4 mg, 0.888 mg-atom) and EtOH (10 ml) and allowed to stand for 18 h. The solution was diluted with H_2O (10 ml), neutralized with Amberlite IR-120 H^+ ion-exchange resin, and evaporated to dryness in vacuo. The product was purified on a preparative TLC plate developed with $CHCl_3$–MeOH (9:1) and the major band extracted with MeOH. The extract was evaporated to dryness and the residue dissolved in H_2O (6 ml), washed with $CHCl_3$ (2 × 2 ml), filtered through Celite, and lyophilized to a white powder: yield 225 mg (63%); mp 35–42 °C (Mel-Temp); λ_{max} nm ($\epsilon \times 10^{-3}$) (EtOH) (0.1 N HCl) 252 (10.6), (pH 7) 252 (11.2), (0.1 N NaOH) 267 (br) (7.95); ^1H NMR δ 1.49 (m, 3, CH_3), 6.31 (d, 1, $J_{1',2'}$ = 5.0 Hz, $H_{1'}$), 8.80 (s, 1, H_5). Anal. ($C_{11}H_{15}N_5O_5 \cdot 0.8H_2O$) C, H, N.

***N*-Butyl-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine** (**9**). A solution of **2** (437 mg, 1.00 mmol) and *n*-butylamine (2.00 ml, 20.0 mmol) in MeOH (5 ml) was stirred at 25 °C for 24 h and evaporated to a syrup in vacuo. A solution of the syrup in $CHCl_3$ was applied to two preparative TLC plates and developed with $CHCl_3$–MeOH (9:1). The product band was extracted with $CHCl_3$–MeOH (4:1) and the extract evaporated to dryness. The residue was purified by precipitation from a hot solution of *n*-butyl ether and crystallized from a small volume of $CHCl_3$ to give pure **9**: yield 165 mg (51%); mp 142 °C; λ_{max} nm ($\epsilon \times 10^{-3}$) (H_2O) (0.1 N HCl) 269 (14.9), (pH 7) 292 (14.3), (0.1 N NaOH) 292 (14.8); ^1H NMR δ 0.91 (m, 3, CH_3), 6.17 (d, 1, $J_{1',2'}$ = 5.2 Hz, $H_{1'}$), 8.39 (s, 1, H_5). Anal. ($C_{13}H_{20}N_6O_4$) C, H, N.

3-Methyl-2-butenylamine. A mixture of potassium phthalimide (104 g, 0.563 mol) and 3-methyl-2-butenyl chloride (55.0 g, 0.526 mol) in DMF (400 ml) was stirred at 65 °C for 5 h, cooled to 25 °C, diluted with $CHCl_3$ (800 ml), and poured into 2 l. of ice water. The $CHCl_3$ layer was washed with 2 N Na_2CO_3 and with H_2O, then dried over Na_2SO_4, and evaporated to a solid residue in vacuo. Recrystallization of the residue from MeOH gave 87.8 g (78%) of *N*-(3-methyl-2-butenyl)phthalimide, mp 100 °C (lit.[18] mp 100 °C). A mixture of the phthalimide (84.4 g, 0.392 mol), EtOH (231 ml), and 85% N_2H_4 (25.1 g, 0.427 mol) was refluxed for 1 h, cooled to 25 °C, treated with 6 N HCl (71.2 ml, 0.427 mol) and then H_2O (100 ml), and filtered. The precipitate was washed with H_2O (230 ml) and the combined filtrate and wash was evaporated to dryness in vacuo. The residue was stirred with 30% KOH (90 ml) and the mixture extracted with Et_2O (3 × 25 ml). The extract was dried over KOH and fractionally distilled through a 25-cm Vigreux column to give 22.5 g (67%) of the title amine: bp 107–108 °C (lit.[19] bp 105–108 °C), $n^{24.5}D$ 1.4415; ^1H NMR ($CDCl_3$) δ 1.04 (s, 2, NH_2), 1.63, 1.70 (d, 6, CH_3), 3.25 (d, 2, CH_2), 5.26 (m, 1, CH); ^{13}C NMR ($CDCl_3$) δ (±0.05) 17.64 ($C_{cis\text{-}Me}$), 25.59 ($C_{trans\text{-}Me}$), 39.81 (C_1), 126.56 (C_2), 132.70 (C_3).

***N*-(3-Methyl-2-butenyl)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine** (**10**). A solution of **2** (437 mg, 1.00 mmol), 3-methyl-2-butenylamine (2 ml), and MeOH (2 ml) was stirred for 19 h and evaporated to dryness in vacuo and the residue of crude product in a minimum of $CHCl_3$ was applied to two preparative TLC plates and developed with $CHCl_3$–MeOH (9:1). The major band was extracted with $CHCl_3$–MeOH (1:1) and the extract evaporated to dryness. A solution of the residue in $CHCl_3$ was filtered and evaporated to a foam under high vacuum: yield 217 mg (90%); λ_{max} nm ($\epsilon \times 10^{-3}$) (H_2O) (0.1 N HCl) 270 (13.5), (pH 7) 212 (16.2), 290 (13.4), (0.1 N NaOH) 290 (13.4); ^1H NMR δ 1.71 (d, 6, CMe_2), 6.18 (d, 1, $J_{1',2'}$ = 5.0 Hz, $H_{1'}$), 8.3 (s, $CHCl_3$), 8.40 (s, 1, H_5). Anal. ($C_{14}H_{20}N_6O_4 \cdot 0.13CHCl_3$) C, H, N.

3,6-Dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione (**11**). A solution of **2** (559 mg, 1.28 mmol) in EtOH (20 ml) containing powdered NaSH (1.0 g) was stirred in a stoppered flask for 64 h, filtered, and evaporated to dryness in vacuo. A solution of the residue and NaOMe (277 mg, 5.12 mmol) in EtOH (10 ml) was stirred for 18 h and evaporated to dryness. A solution of this residue in H_2O (7 ml) was filtered and adjusted to pH 7 with 1 N HCl. The mixture was refrigerated and the product collected, washed with cold H_2O, and dried in vacuo (P_2O_5): yield 187 mg (46%); mp 137 °C [resolidifies and melts at 192 °C (lit.[12] mp 128 °C)]; λ_{max} nm ($\epsilon \times 10^{-3}$) (EtOH) (0.1 N HCl) (unstable) 232, 328, (pH 7) (unstable) 233, 333, (0.1 N NaOH) 231 (14.7), 335 (17.2); ^1H NMR δ 6.12 (d, 1, $J_{1',2'}$ = 4.4 Hz, $H_{1'}$), 8.38 (s, 1, H_5).

2-β-D-Ribofuranosyl-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (**12**). A solution of **3** (63 mg, 0.144 mmol) in EtOH (25 ml), presaturated with NH_3 at 0 °C, was heated in a stainless steel bomb at 68 °C for 3 h and evaporated to dryness in vacuo. A solution of the residue in H_2O was washed with $CHCl_3$ and evaporated to give 35 mg of white solid, mp 196 °C (lit.[11] mp 194–196 °C). A uv spectrum and TLC of this compound were identical with those of an authentic sample.[11]

7-(Methylthio)-2-β-D-ribofuranosyl-2*H*-1,2,3-triazolo-[4,5-*d*]pyrimidine (**13**). The title compound, mp 138 °C (Mettler FP1), was prepared from **3** in 41% yield on the same scale and by the procedure described for **5**: λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 7) 218 (10.8), 2.64 (7.20), 272 (7.12), 3.20 (11.5); ^1H NMR δ 2.76 (s, 3, CH_3), 6.24 (d, $J_{1',2'}$ = 3.2 Hz, $H_{1'}$), 8.97 (s, 1, H_5). Anal. ($C_{10}H_{13}N_5O_4S$) C, H, N.

***N*-β-D-Ribofuranosyl[1,2,3]thiadiazolo[5,4-*d*]pyrimidin-7-amine** (**14**). A stoppered vial containing **11** (20 mg, 0.0629 mmol) under N_2 was heated in an oil bath at 138 °C for 30 min to give 18 mg (96% yield) of chromatographically homogeneous **14**: mp 189 °C; λ_{max} nm ($\epsilon \times 10^{-3}$) (EtOH) (0.1 N HCl) 215 (16.4), 244 (11.2), 267 (sh) (6.37), 272 (6.45), 306 (7.78), (pH 7) 216 (16.5), 244 (10.6), 267 (sh) (5.84), 272 (5.93), 310 (7.64); ^1H NMR δ 3.51 (m, 2, $H_{5'}$), 3.82 (m, 1, H_4), 4.13 (m, 2, $H_{2',3'}$), 4.79 (m, 1, $O_5'H$), 4.96 (d, 1, $O_3'H$), 5.14 d, 1, $O_2'H$), 5.97 (m, 1, $H_{1'}$), 8.68 (s, 1, H_5), 9.78 (d, 1, NH). Anal. ($C_9H_{11}N_5O_4S \cdot 0.7H_2O$) C, N; H: calcd, 4.20; found, 3.67.

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Synthesis and Some Pharmacological Properties of [4-Threonine,7-glycine]oxytocin, [1-(L-2-Hydroxy-3-mercaptopropanoic acid),4-threonine,7-glycine]oxytocin (Hydroxy[Thr^4,Gly^7]oxytocin), and [7-Glycine]oxytocin, Peptides with High Oxytocic–Antidiuretic Selectivity

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[4-Threonine,7-glycine]oxytocin and [1-(L-2-hydroxy-3-mercaptopropanoic acid),4-threonine,7-glycine]oxytocin (hydroxy [Thr^4,Gly^7]oxytocin) were synthesized by a combination of solid-phase and classical methods of peptide synthesis. A protected octapeptide was synthesized by the solid-phase method and following ammonolysis and purification 1 + 8 couplings in solution were employed to furnish the required key nonapeptide and acyl octapeptide intermediates, respectively. [7-Glycine]oxytocin was prepared from a sample of the protected nonapeptide intermediate used in the original synthesis of this peptide. [7-Glycine]oxytocin has an oxytocic potency (O) of 93 ± 4 units/mg and an antidiuretic potency (A) of 0.0056 ± 0.0003 units/mg. It has an O/A ratio of 16 000. [4-Threonine,7-glycine]oxytocin has an oxytocic potency of 166 ± 4 units/mg and an antidiuretic potency of 0.002 ± 0.0004 units/mg. Its O/A ratio is 83 000. Threonine substitution has thus brought about a substantial enhancement in oxytocic activity and a fivefold enhancement in O/A selectivity. Hydroxy[Thr^4,Gly^7]oxytocin has an oxytocic potency of 218 ± 8 units/mg and antidiuretic potency of 0.0040 ± 0.0005 units/mg. Its O/A ratio is thus 54 500. All three 7-glycine-substituted analogues exhibit a marked sensitivity to Mg^{2+} on the rat uterus assay system and in the presence of 0.5 mM Mg^{2+} had oxytocic potencies in the range of 900–1000 units/mg. Should these peptides exhibit enhanced oxytocic selectivity in humans, they might offer a greater margin of safety than oxytocin in those clinical situations in which the latter is currently employed.

In pursuing the goal of designing highly selective synthetic peptides derived from oxytocin and vasopressin, it has proved useful to combine within one molecule those structural changes which individually enhance a particular pharmacological activity in a selective manner.[1,2a] This approach led to the synthesis of the highly selective antidiuretic peptide [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP).[3] This compound has high antidiuretic potency (1230 units/mg; cf. arginine-vasopressin, 320 units/mg) and no pressor activity could be detected. The ratio of the antidiuretic/pressor (A/P) potencies thus approaches infinity.

It appeared worthwhile to utilize this approach in attempting to design an analogue of oxytocin with negligible vasopressin-like characteristics, i.e., a peptide exerting substantial oxytocic effects but neither antidiuretic nor pressor effects. Such a compound may have potential clinical use.

The literature shows that oxytocin has been modified in but two positions within the nonapeptide sequence with resulting enhancement of the ratio of oxytocic (O) to antidiuretic (A) potencies. [Gly^7]oxytocin was reported to exhibit a greatly enhanced oxytocic/antidiuretic (O/A) ratio relative to oxytocin (O/A 130).[4,5] The values for the O/A ratio of [Gly^7]oxytocin ranged from 7000 to 33 000, the discrepancies arising from differing estimates of oxytocic activities.[4,6,7] Although activity on the isolated rat uterus in the absence of Mg^{2+} was substantially less than that of oxytocin, the greater reduction in antidiuretic activity resulted in these high O/A ratios for [Gly^7]-oxytocin.

A number of 4-substituted analogues of oxytocin have enhanced O/A selectivity.[8–11] In most this stems from reduced antidiuretic activity rather than enhanced oxytocic activity. An exception is [Thr^4]oxytocin which shows both enhanced oxytocic activity and depressed antidiuretic activity, relative to those of oxytocin. We thus decided to incorporate threonine in the 4 position of [Gly^7]oxytocin in the hope of further enhancing its oxytocic activity and selectivity. We report here the synthesis and some pharmacological properties of the peptide [Thr^4,Gly^7]-oxytocin designed according to this rationale.[2b]

The discrepancy in the values reported from different laboratories for the oxytocic potency of [Gly^7]oxytocin[4,6,7]