2′-Deoxythioguanosine and Related Nucleosides[1]

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The conversion of 2-amino-6-chloropurine in two steps to the 2-acetamido derivative V is described. The condensation of a mercury derivative of V with a protected 2-deoxyribofuranosyl chloride VII afforded the blocked nucleosides, α- and β-VIII, whose structures were assigned on the basis of n.m.r. data. Deacylation and replacement of the 6-chlorines yielded the thiols, α-IX and β-IX (2′-deoxythioguanosine, which is of special interest for possible antitumor properties). The conversion of β-IX to 2′-deoxyguanosine provided a definitive chemical structure proof for β-IX.

Thioguanine (2-amino-6-mercaptopurine) and its riboside, thioguanosine,[3] have been studied as anticancer compounds, particularly against leukemia; they appear to be equally effective agents.[4] In his studies of the mechanism of action of thioguanine, LePage[5] has found a correlation between the tumor-inhibitory response and the incorporation of the drug into nucleic acids, probably specifically into the DNA. Resistance of tumors to thioguanine is a serious limitation in its use, and efforts have been made to circumvent the resistance. One possible mechanism of resistance lies in the inability of the tumor cells to reduce the ribonucleotide of thioguanine to its deoxyribonucleotide, which can then be incorporated into DNA.[6] To furnish thioguanine as the deoxyriboside might be a way of circumventing such a resistance mechanism; phosphorylation to the deoxyribonucleotide might then provide the desired form of the drug for incorporation. Thus the synthesis of 2′-deoxythioguanosine (β-IX) was of interest for investigation of this hypothesis. In a general sense deoxythioguanosine could be a therapeutically more useful form of thioguanine. Important therapeutic differences between the riboside and the 2′-deoxyriboside of a common heterocyclic base have been noted; the effectiveness of 5-iodo-2′-deoxyuridine against *herpes simplex* virus in contrast to the ineffectiveness of the riboside, 5-iodouridine, is an example.[7]

The general instability[8] of purine deoxyribosides (relative to ribosides or to pyrimidine nucleosides) suggested that the direct thiation[3] of 2′-deoxyguanosine would not provide a useful route to β-IX; indeed, studies in another laboratory are in accord with this view.[9] The most expedient method for preparing β-IX then seemed to us to be further application of the indirect method[10] used to prepare the related deoxyriboside of 6-mercaptopurine.

2-Amino-6-chloropurine (I) was the appropriate purine precursor to be condensed with an acylated chloro sugar (such as VII) by the mercuri procedure[11]; the final steps would be deacylation and replacement of the chlorine with the thiol group. When chloromercuri-2-amino-6-chloropurine was condensed with 2-deoxy-3,5-di-O-*p*-toluoyl-D-ribofuranosyl chloride (VII) in refluxing benzene, however, a stable N-glycosidic bond was formed at the free 2-amino group, as well as at N-9. If this condensation was carried out at room temperature in dimethyl sulfoxide,[12] the product appeared to consist of mono-9- and bis-2,9-furanoside in roughly equal amounts; when the product was deacylated with methanolic ammonia at 0°,[13] a crystalline material was isolated in 7% yield after fractional recrystallization which gave spectral and analytical data expected[14] for a 9-(2′-deoxyribofuranoside) of I. The anomeric nature of this material was not investigated, and it was concluded that use of unprotected I was impractical.

The useful derivative 2-acetamido-6-chloropurine (V) had to be prepared in two steps from I, since direct monoacetylation of I under a variety of conditions afforded only 2-amino-6-chloro-9-acetylpurine (II), identical with an authentic sample.[9] Polyacetylation[15] of I afforded 2-acetamido-6-chloro-9-acetylpurine (III) apparently mixed with 2-(N,N-diacetamido)-6-chloro-9-acetylpurine[16] (IV), after 7 hr. in refluxing acetic anhydride, or after 45 min. if phosphoric acid was used as catalyst.[17] The composition of mixtures of III and IV varied somewhat from run to run and was determined by elemental analyses. These materials were unstable to hot water or aqueous[15] or methanolic base, which generated the relatively stable 2-acetamido-6-chloropurine (V) in good yield. Under similar conditions the N-9-acetyl compound II was cleaved to I,

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(16) *Cf.* N,N-diacetylamino pyrimidines, D. J. Brown, "The Pyrimidines," Interscience Publishers, New York, N. Y., 1962, p. 395.

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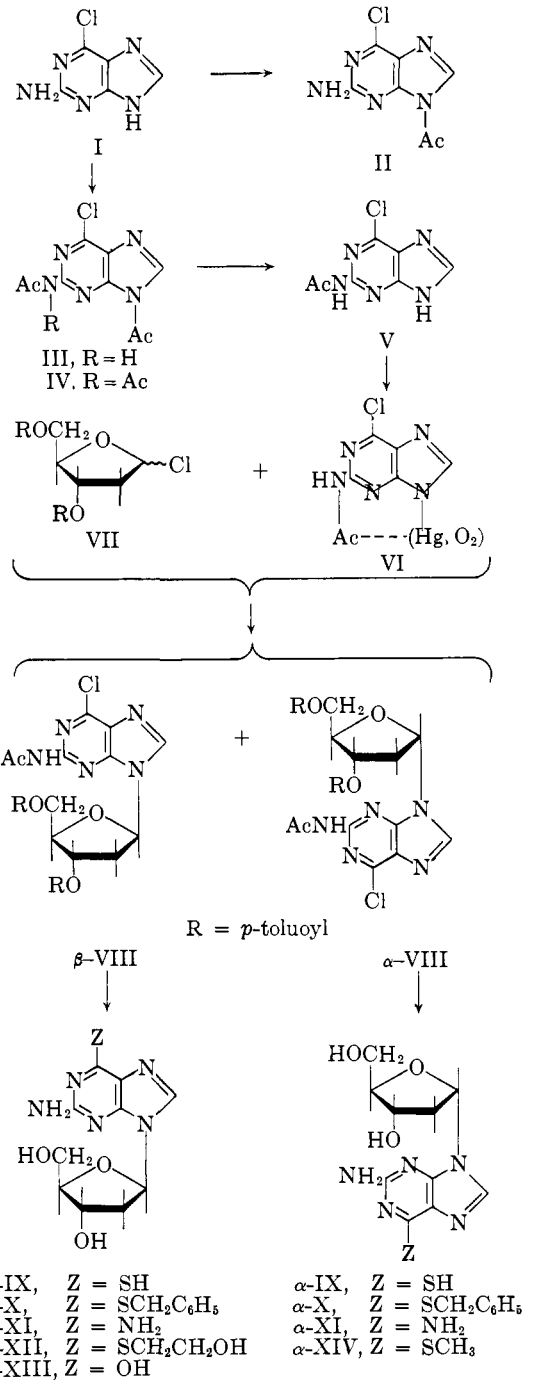
whereas V could be purified by reprecipitation from aqueous sodium hydroxide upon neutralization. Infrared carbonyl absorption for both II and V was at 5.80 μ, although the spectra were otherwise different.

Treatment of V and mercuric chloride in aqueous ethanol with sodium hydroxide,[13] designed to form the chloromercuri derivative, afforded instead a mercury derivative VI of undetermined structure. Elemental analyses, easily reproduced on material from separate experiments, indicated an empirical formula of $C_7H_{5\text{ or }6}ClHgN_5O_3$ (*i.e.*, HgO_2 added to V, with or without replacement of H). A permanent yellow end point[13] in the consumption of base was obtained only if 2 moles of mercuric chloride and 2 moles of base were used per mole of purine. Though with other ratios of reactants no permanent end point was attained and VI was often mixed with V, no other mercuri purine was ever detected. Absence of carbonyl absorption in the infrared spectrum of VI suggested some sort of interaction between the mercury atom and the acetyl group. It is extremely difficult to rationalize the low H/O ratio suggested in the empirical formula. Nevertheless, the substance VI reacted normally with the chloro sugar VII to form a nucleoside, and solution of VI in aqueous potassium iodide resulted in a pH of 10–11 and regenerated the 2-acetamide V upon neutralization.

In the reaction of the mercuri derivative VI with one molar equivalent of the chloro sugar VII in refluxing benzene, the yield of total protected deoxynucleoside (α- plus β-VIII) was 57%; the yield was not improved by use of two molar equivalents of VII, but rather decreased to less than one-half of this value. The anomers α- and β-VIII were easily separated by virtue of the ready crystallization of β-VIII from rather dilute chloroform solutions of the anomeric mixture and by virtue of the much greater solubility of α-VIII, which is recovered from the mother liquors as an amorphous glass. The nearly 1:1 proportion of anomers found was to be expected[18] from use of a 2-deoxy sugar. Homogeneous behavior of the amorphous α-VIII on an alumina chromatogram indicated its anomeric purity, since it was shown that a mixture of α- and β-VIII was resolved (β-VIII eluted first) under the same[19a] conditions. These anomers provide an exception[19b] to Hudson's rotation rules, as shown in Table I. Anomeric configurations were assigned by comparison of the n.m.r. spectra with those of the analogous anomeric pair lacking the 2-acetamido group,[10] which have been related to nucleosides (2′-deoxyadenosine and its α-anomer) of established configuration. The position and pattern of signals due to protons in the sugar moiety were identical for the two α-anomers (in deuteriochloroform), likewise for the β-anomers. Other pairs (α- and β-IX, X, XI) of anomeric nucleosides obtained from α- and β-VIII exhibited optical properties in accordance with Hudson's rules; the differences in molecular rotation (Table I) agree reasonably well and also agree with the differences found[10] for 6-substituted-purine 2′-deoxyribosides, though the different pairs were sometimes measured in different solvents.

(18) B. R. Baker in "The Chemistry and Biology of Purines," Ciba Foundation Symposium, Little, Brown, and Co., Boston, Mass., 1957, p. 120.

(19) (a) The chromatographic system was that described in ref. 10; (b) similar exceptions have been discussed in ref. 11, p. 340.



The 6-thiols (α- and β-IX) were prepared from α- and β-VIII, respectively, by replacement of the chlorines and removal of the toluoyl groups in one step with methanolic sodium methoxide saturated with hydrogen sulfide; the ultraviolet spectra closely resembled the spectrum of thioguanosine[3] and were distinct from that

TABLE I

2-AMINO-6-SUBSTITUTED-PURINE-α- AND β-DEOXYRIBOSIDES

Compounds	$[\alpha]_D$, deg.[a] α-	$[\alpha]_D$, deg.[a] β-	ΔM_D[b] ($\alpha - \beta$)
VIII[c]	−54.8	−28.7	−147
IX	+58.2	−32.0	+255
X	+48.1	−3.9	+194
XI	+58.8	−29.1	+199

[a] For solvents, see Experimental. [b] Values for a series[10] of 6-substituted-purine deoxyribosides ranged from +192 to +246. [c] An exception to Hudson's rules.

of 7-methylthioguanine.[20a] Hydrolytic conditions could not be found which permitted isolation of an intermediate between VIII and IX in which the chlorine was still present at C-6 but from which the tolnoyl groups had been cleaved. Attachment of the deoxyribosyl group at N-9 was verified by the identity of ultraviolet spectra of the S-benzyl derivatives (α- and β-X) with spectra of known 9-substituted-2-amino-6-benzylthiopurines,[20b] which are distinct from spectra of 7-alkyl[20c] isomers. Upon S-methylation of the thiols, only the α-anomer α-XIV could be isolated in a state of purity. Concurrent deacylation and amination of α- and β-VIII in methanolic ammonia afforded the 2,6-diaminopurine deoxynucleosides, α- and β-XI; the ultraviolet spectra agreed with the spectrum of the β-ribosyl analog.[21] Compound β-XI may also possess interesting anticancer properties.[22]

A definitive proof of the structure and configuration of β-IX by chemical means was attained through its conversion to 2′-deoxyguanosine β-XIII *via* the easily hydrolyzed S-(2-hydroxyethylthio) derivative β-XII. This also constitutes a second[23] chemical synthesis of β-XIII. Direct hydrolysis of β-VIII (*i.e.*, a labile deoxynucleoside) was not feasible, since 2-amino-6-chloropurine (I) is known[24] to resist hydrolysis to guanine in boiling alkali and at least 1 hr. is required for hydrolysis of I to guanine in boiling acid. On the other hand, β-XII, like the known 6-(2-hydroxyethylthio)purine,[25] was easily hydrolyzed in base at room temperature.

Biological Data.—Of the nucleosides synthesized in this work, β-IX is of prime interest for possible biological activity for reasons stated in the introductory paragraph. Initial biological experiments were designed (1) to determine if treatment with β-IX of mice bearing transplanted tumors permitted the incorporation of thioguanine into the DNA and (2) to determine briefly if actual tumor inhibition occurred on treatment with β-IX. Preliminary results are given in Tables II and III from data supplied by Dr. G. A. LePage and Mrs. I. G. Junga, who will present a detailed study elsewhere. We are indebted for the use of these results in advance of publication.

The data show that (1) the β-anomer (β-IX) does produce significant increase in incorporation of thioguanine into DNA over that obtained with α-IX of unnatural anomeric configuration used as control; (2) concomitant treatment with 9-methyl-6-thioguanine enhances this incorporation; (3) β-IX produces more incorporation into DNA than does the riboside of thioguanine; (4) β-IX produced better inhibition of tumor growth than α-IX used as control.

Table II

Incorporation of 6-Thioguanine into DNA and RNA of Transplanted Mammary Adenocarcinomas of C3H Mice[a,b]

Treatment	Incorporation of thioguanine, γ/g. DNA	RNA
β-IX + 9-methylthioguanine[c]	1.08 (0.84–1.55)	0.34 (0.12–0.99)
β-IX	0.73 (0.50–0.82)	0.40 (0.08–0.57)
α-IX[d]	0.33 (0.28–0.41)	0.34 (0.09–0.46)
Thioguanine riboside	0.38 (0.26–0.52)	0.58 (0.32–0.92)

[a] Groups of 14 mice were used except for the riboside where 5 mice were used. The animals were given 2 doses, equivalent to 8 mg./kg. of thioguanine at a 12 hr. interval and were sacrificed for analysis 2 hr. after the second dose. [b] The nucleosides were all labeled with C^{14} at C-8 of the base. [c] 9-Methyl-6-thioguanine has been found to be an inhibitor of nucleoside cleavage and was used to increase thioguanine incorporation. [d] *In vitro* studies showed that α-IX and β-IX were cleaved at equal rates in a transplanted C3H tumor.

Table III

Response of Mammary Adenocarcinomas in C3H Mice[a]

Treatment	Tumor weight, mg.
β-IX	303 (50–460)
α-IX[b]	807 (170–1230)
Control (untreated)	1015 (290–1150)

[a] Mice bearing tumor implants were selected into groups of ten on the basis of caliper measurements. The treated groups were treated once daily for 5 days at 13.5 mg./kg./day (equivalent to thioguanine at 8 mg./kg.); the animals were sacrificed on day 7 and the tumors weighed. [b] α-IX and β-IX were shown to have equal toxicity and the same toxicity as thioguanine riboside at an equimolar dose.

Experimental[26]

Paper chromatography was run by the descending technique on Whatman No. 1 paper. The solvent was 5% aqueous Na_2HPO_4, except where described for compounds III–V. Spots were detected visually under ultraviolet light and, for the nucleosides, with a cysteine-hydrochloric acid spray.[27] Adenine, R_f 0.34, was the standard of comparison.

2-Acetamido-6-chloropurine (V). (A) From 2-Amino-6-chloropurine (I).—Sixteen grams of I (0.094 mole) was dissolved with heating in 500 ml. of acetic anhydride containing 1.0 to 1.5 ml. of 85% phosphoric acid, and the solution was refluxed for 45 min. Removal of solvent *in vacuo* at 70° afforded a solid yellow residue, which was mixed with 600 g. of crushed ice to hydrolyze any remaining acetic anhydride. After 1 hr. at 0–5°, the crude mixture of III and IV was collected and washed with water, then with ether, until the odor of acetic acid was gone. The solid (19.8 g.) was digested in 1 l. of boiling toluene and the resultant solution, while hot, was filtered from 1.5 g. of brown solid. The filtrate was concentrated *in vacuo* to 400 ml. and chilled at 5° for 1 hr. The resultant precipitate, collected and washed with cold toluene, weighed 17.8 g., m.p. from 172–179° to 172–202° in different preparations. Paper chromatography in 5% Na_2HPO_4 revealed two spots, R_f 0.68 (attributed to III) and R_f 0.41 (same as V, apparently formed from III in the system), compared to one spot of R_f 0.26 for both I and II (also deacetylated in the system to I). The mixture, III and IV, appeared homogeneous in water-saturated 1-butanol, R_f 0.70 (attributed to III) *vs.* R_f 0.40 for I (both V, from the next step, and II were stable and indistinguishable in this system, R_f 0.30). Infrared spectra of the mixture showed three bands in the carbonyl region at 5.70, 5.80, and 5.85 μ which varied in intensity from run to run; of the two bands at 3.09 and 3.20 μ in the N–H region, the former varied in intensity and was absent from a sample which gave analytical data for IV.

Anal. Calcd. for $C_6H_6ClN_5O_3$: C, 44.7; H, 3.42; Cl, 12.0; O, 16.2. Found: C, 45.0; H, 3.30; Cl, 12.0; O, 15.9.

Partial deacylation to form V was carried out in 850 ml. of methanol containing 85 ml. of concentrated ammonium hy-

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(26) Melting points were observed on a Fisher–Johns apparatus and are corrected. Optical rotations were determined in 1% solutions (except where noted for β-XIII in water) and at 1-dm. path length. Infrared spectra were obtained in Nujol mull. 2-Amino-6-chloropurine was purchased from Cyclo Chemical Corp., Los Angeles 1, Calif.

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droxide. After 15 min. at room temperature, the solution was concentrated to form a white residual solid which was washed onto a filter with cold water. The solid (12.7 g., 64%) was pure enough for the next step, R_f as described. A sample for analysis was reprecipitated on neutralization with *M* hydrochloric acid of a solution in *M* sodium hydroxide; it did not melt below 280°; $\lambda_{max}^{0.1\ M\ NaOH}$ mμ (ε), 236 (20,100), 285 (8040).

Anal. Calcd. for $C_7H_6ClN_5O$: C, 39.7; H, 2.86; Cl, 16.8; N, 33.1; O, 7.56. Found: C, 39.9; H, 3.02; Cl, 16.6; N, 33.2; O, 7.74.

(B) From the Mercury Derivative VI.—A 500-mg. portion of VI was dissolved in 30% aqueous KI to form a solution of pH 10–11. Adjustment to pH 5 with acetic acid afforded 186 mg. (73%) of V as a white precipitate with properties identical to those recorded in A.

Mercury Derivative VI of V.—A solution of 28.65 g. (105.7 mmoles) of $HgCl_2$ in 1.5 l. of 50% aqueous ethanol was treated with pulverized V (11.16 g., 52.84 mmoles), and then with 105.7 ml. of *M* NaOH during *ca.* 1 hr., according to the procedure for chloromercuri-6-chloropurine.[13] The product (22.09 g., 101.5%) was mixed with 25.00 g. of Celite to aid filtration. On a 1-g. scale, the product was collected by centrifugation. The infrared spectrum disclosed loss of carbonyl absorption at 5.78 μ; loss of bands at 8.21 and 11.0 μ (s) and appearance of new bands at 8.28 and 10.53 μ (m) was also characteristic of the transformation.

Anal. Calcd. for $C_7H_5ClHgN_5O_3$: C, 19.0; H, 1.13; Cl, 8.00; Hg, 45.3; N, 15.8. Found for two separate preparations: C, 19.2, 19.0; H, 1.05, 1.26; Cl, 8.09, 7.92; Hg, –, 45.3; N, 15.8, 15.7.

2-Acetamido-6-chloro-9-(2-deoxy-3,5-di-O-*p*-toluoyl-β-D-ribofuranosyl)purine (β-VIII).—Using a previously described procedure,[10] 31.8 g. (0.0718 mmole calcd. as $C_{17}H_5ClHgN_5O_3$) of VI suspended in 1.9 l. of refluxing benzene was treated with 30.1 g. (0.0773 mole) of 2-deoxy-3,5-di-O-*p*-toluoyl-D-ribofuranosyl chloride[28] (VII). The chloroform solution of the crude product, which had been washed with aqueous KI, concentrated to 350 ml., and chilled overnight at 5°, afforded 12.75 g. (31.5%) of crystalline β-anomer, m.p. 180–187°. Recrystallization from chloroform (*ca.* 30 ml./g.) afforded 11.11 g. (27.4%), m.p. 189–190°, $[\alpha]^{24}D$ −27.2 ± 0.4° (chloroform). An infrared band at 13.52 μ (m), indicative of a chloroform solvate, was removed upon recrystallization from methanol (250 ml./g.) of a sample for analysis, m.p. unchanged; $[\alpha]^{24}D$ −28.7 ± 0.4° (chloroform); $\lambda_{max}^{CHCl_3}$ mμ (ε) 231 (48,900), 243 (sh, 38,800), 285 (10,400).

Anal. Calcd. for $C_{28}H_{26}N_3ClO_6$: C, 59.6; H, 4.65; Cl, 6.29; N, 12.4. Found: C, 59.7; H, 4.59; Cl, 6.14; N, 12.2.

2-Acetamido-6-chloro-9-(2-deoxy-3,5-di-O-*p*-toluoyl-α-D-ribofuranosyl)purine (α-VIII).—The mother liquor (350 ml. of chloroform, plus washings) remaining after initial separation of the crude (12.75 g.) β-VIII was diluted to 1 l. with ethyl ether. After standing at 3° overnight, a small amount of gum (to be discarded; some β-VIII was present) had separated, and the supernatant was decanted and diluted further with 2 l. of petroleum ether (b.p. 30–60°). Upon standing at 3° overnight, 10.53 g. (26.0%) of amorphous solid separated, $[\alpha]^{24}D$ −51.1 ± 0.9° (chloroform). Further purification (*ca.* 60% recovery) was achieved by reprecipitation of a sample (1 g.) from chloroform solution (20 ml., treated with decolorizing carbon) with petroleum ether (250 ml.); just as in the isolation procedure,[10] a benzene solution (30 ml.) of the precipitate was washed with aqueous KI, dried, and diluted with petroleum ether (500 ml.). The resultant amorphous precipitate, $[\alpha]^{24}D$ −54.8 ± 0.8° (chloroform), softened to a clear glass at *ca.* 92°; $\lambda_{max}^{CHCl_3}$ mμ (ε) 232.5 (49,800), 245 (sh, 36,900), 284 (10,800). Easy solubility in chloroform was in marked contrast to β-VIII.

Anal. Found: C, 60.1; H, 4.58; Cl, 5.87; N, 11.7.

2-Amino-9-(2-deoxy-β-D-ribofuranosyl)purine-6-thiol (β-IX).—A 5.08-g. (9.00 mmoles) portion of β-VIII was dissolved at reflux in 540 ml. of anhydrous methanolic H_2S,[29] and the hot solution was treated with 27.0 ml. of *M* methanolic NaHS[29,30] and refluxed for 2 hr. Following removal of the H_2S source and additional reflux for 15 min., 13.5 ml. of *M* methanolic $NaOCH_3$ was added, and the solution refluxed for 1 hr. In the isolation,[29] not ether but chloroform was used to wash the aqueous layer. The crystalline product weighed 2.24 g. (87.5%); $[\alpha]^{26}D$ −32.0 ± 0.5° (0.1 *M* sodium hydroxide); $\lambda_{max}^{0.1\ M\ NaOH}$ mμ (ε), 251 (13,600), 270 (6,870), 319 (19,900); $\lambda_{max}^{pH\ 7}$ mμ (ε), 208 (23,500), 225 (13,100), 258 (8,400), 341 (25,700); R_f 0.49 *vs.* 0.26 for thioguanine. The analytical sample (80% yield) was recrystallized from hot water (125 ml./g.); it decomposed on heating above 190°.

Anal. Calcd. for $C_{10}H_{13}N_5O_3S \cdot H_2O$: C, 39.9; H, 5.02; N, 23.2; S, 10.6. Found: C, 39.6; H, 5.11; N, 23.0; S, 10.6.

2-Amino-9-(2-deoxy-α-D-ribofuranosyl)purine-6-thiol (α-IX) was obtained by the same procedure from α-VIII in 76% yield, $[\alpha]^{25}D$ +58.2 ± 1.0° (0.1 *M* sodium hydroxide). After 1 recrystallization from hot water, the yield was 45%, $[\alpha]^{24}D$ +68.6 ± 1.0° (0.1 *M* sodium hydroxide); $\lambda_{max}^{0.1\ M\ NaOH}$ mμ (ε), 220 (14,800), 251 (14,000), 271 (6,930), 319.5 (20,200); $\lambda_{max}^{pH\ 7}$ mμ (ε), 209 (22,800), 227 (14,600), 258.5 (7,920), 341 (25,400); R_f 0.49. The compound decomposed on heating above 170°.

Anal. Found: C, 39.9; H, 4.83; N, 23.2; S, 10.8.

2-Amino-6-benzylthio-9-(2-deoxy-β-D-ribofuranosyl)purine (β-X) was obtained from β-IX and benzyl chloride in aqueous dioxane containing KOH.[31] The stirred reaction mixture, after 30 min. at room temperature, was seeded (seed obtained from a preliminary reaction, by evaporation) and stirred further for 3.5 hr. while the product crystallized. After standing 2 hr. at 3°, the product (60%) was collected; addition of water to the filtrate afforded a second crop (total yield 76%). Recrystallization from 50% aqueous methanol afforded 54%, m.p. 162–165°, $[\alpha]^{26}D$ −3.9 ± 0.1° (methanol); λ_{max}^{MeOH} mμ (ε), 218 (24,500), 246 (16,500), 312.5 (14,200); R_f 0.26.

Anal. Calcd. for $C_{17}H_{19}N_5O_3S$: C, 54.8; H, 5.13; N, 18.8; S, 8.59. Found: C, 54.4; H, 5.00; N, 18.4; S, 8.62.

2-Amino-6-benzylthio-9-(2-deoxy-α-D-ribofuranosyl)purine (α-X) was obtained by the same procedure from α-IX in 50% yield after recrystallization from acetonitrile, m.p. 163–166°, $[\alpha]D$ +48.1 ± 0.9° (methanol); λ_{max}^{MeOH} mμ (ε), 220 (24,100), 246 (16,500), 312.5 (13,900); R_f 0.26. A m.m.p. with β-X was 153–158°.

Anal. Found: C, 55.0; H, 5.09; N, 18.8; S, 8.68.

2-Amino-6-methylthio-9-(2-deoxy-α-D-ribofuranosyl)purine (α-XIV) was prepared from α-IX in aqueous NaOH solution with methyl iodide.[3] The product (78% yield) was purified by recrystallization from water (53% yield) rather than by treatment with boiling ethanol,[3] m.p. 104–107° with softening at 96°, $[\alpha]^{24}D$ +57.7 ± 0.8° (methanol); λ_{max}^{MeOH} mμ (ε), 220.5 (18,300), 245 (15,600), 310 (11,900); R_f 0.37.

Anal. Calcd. for $C_{11}H_{15}N_5O_3S \cdot 0.5H_2O$: C, 43.1; H, 5.20; N, 22.9; S, 10.5. Found: C, 42.6; H, 5.31; N, 22.8; S, 10.4.

2′-Deoxyguanosine (β-XIII).—2-Amino-6-(2-hydroxyethylthio)-9-(2-deoxy-β-D-ribofuranosyl)purine (β-XII) was prepared as a sirupy intermediate from β-IX, by the procedure for 6-(2-hydroxyethylthio)purine.[25] The isolation involved filtering the dimethylformamide reaction solution to remove inorganic solids and concentrating it *in vacuo*. A 0.2 *M* NaOH solution (10 ml.) of the sirup was stirred for 3 hr. at room temperature; a white solid separated but was discarded, since a paper chromatogram showed no trace of deoxyguanosine. The filtrate was neutralized with 50% acetic acid and concentrated to form a white solid residue which did contain deoxyguanosine. This was dissolved in hot water (70 ml./g.) and stored for several weeks at 5–20° to convert the resultant gel to a crystalline solid[23] (20% yield). Two further recrystallizations from water occurred with increasing ease, and the material exhibited physical properties identical with those of a sample of recrystallized natural 2′-deoxyguanosine when compared as follows[32]: $[\alpha]^{24}D$ −42.7 ± 0.7° *vs.* *−44.0 ± 0.7°* (*c* 0.3 in water); $[\alpha]^{24}D$ −39.2 ± 0.6° *vs.* *−40.1 ± 0.6°* (0.1 *M* sodium hydroxide); $\lambda_{max}^{pH\ 7}$ mμ (ε), 252.5 (14,100 *vs. 14,100*), $\lambda_{min}^{pH\ 7}$ mμ (ε) 222.5 (3590 *vs. 3850*), A_{280}^{280} 0.69 *vs. 0.69*, A_{260}^{250} 1.15 *vs. 1.16*; $\lambda_{max}^{0.1\ M\ NaOH}$ mμ (ε) 265 broad (11,300 *vs. 11,600*); R_f 0.60 *vs. 0.60*. X-Ray powder diffraction patterns were identical in spacing and intensity.

2,6-Diamino-9-(2-deoxy-α-D-ribofuranosyl)purine (α-XI).—Use of method A in ref. 10 with 400 mg. (0.709 mmole) of α-VIII in methanolic ammonia in a sealed bomb at 100° for 5 hr. afforded 245 mg. (112%) of crude product, R_f 0.26, containing a contaminating nucleoside of R_f 0.50 which was removed by two recrystallizations from ethanol–methanol (9/1). The amorphous solid (15%), m.p. 167° dec., was homogeneous, R_f 0.26; $[\alpha]^{21}D$

(28) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

(29) Method C in ref. 10.

(30) J. A. Johnson, Jr. and H. J. Thomas, *J. Am. Chem. Soc.*, **78**, 3863 (1956); H. J. Schaeffer and H. J. Thomas, *ibid.*, **80**, 4896 (1958).

(31) Ref. 20b, method A at bottom of p. 586.

(32) Values for the natural material are given second and the numbers italicized. Ultraviolet data agree with those of Venner[23]; extinctions are calculated for the monohydrate.

+58.8 ± 1.0° (water); $\lambda_{max}^{pH\ 7,\ 14}$ mμ (ε) 216 (25,000), 256 (8850), 280 (9,910).

Anal. Calcd. for $C_{10}H_{14}N_6O_3 \cdot 2H_2O$: C, 39.7; H, 6.00; N, 27.8. Found: C, 39.8; H, 5.25; N, 28.0.

2,6-Diamino-9-(2-deoxy-β-D-ribofuranosyl)purine (β-XI) was prepared by the same procedure and purified from a similar contaminant by recrystallization from methanol and from carbon tetrachloride (10% yield), m.p. 176° dec.; R_f 0.26; $[\alpha]^{21}D$ −29.1 ± 0.5° (water); $\lambda_{max}^{pH\ 7,\ 14}$ mμ (ε), 216 (24,500), 256 (8890), 280 (9910).

Anal. Found: C, 39.4; H, 4.94; N, 27.7.

Chloromercuri-2-amino-6-chloropurine was prepared by the procedure[13] for chloromercuri-6-chloropurine in 96% yield. The transformation was characterized by loss of infrared bands at 6.1 and 7.91 μ due to I and appearance of new bands at 6.2 (strong, broad) and 8.03 μ (medium, sharp).

Anal. Calcd. for $C_5H_3Cl_2HgN_5$: C, 14.8; H, 0.76; Cl, 17.5; N, 17.3. Found: C, 14.9; H, 1.28; Cl, 17.1; N, 16.7.

Nucleoside Preparations Attempted with Chloromercuri-2-amino-6-chloropurine.—Coupling of the chlorosugar VII with chloromercuri-2-amino-6-chloropurine in refluxing benzene by the usual[10] procedure afforded a sirupy nucleoside (23% yield) with strong ester bands in the infrared at 5.80, 7.85, and 9.05 μ, and bands at 6.19 (m) and 6.35 and 6.50 μ (w) due to the purine moiety; λ_{max}^{EtOH} mμ (ε), 227 (52,100), 241 (62,400), 310 (7,000). Elemental analyses after alumina chromatography indicated two sugar moieties were attached to the purine.

Anal. Calcd. for $C_{47}H_{49}ClN_8O_{16}$: C, 64.6; H, 5.07; Cl, 4.06; N, 8.01. Found: C, 63.3; H, 4.80; Cl, 3.92; N, 7.87.

A coupling product obtained from VII and chloromercuri-2-amino-6-chloropurine in dimethyl sulfoxide[12] upon diluting the reaction mixture with water and extracting with benzene was purified by the usual[10] procedure (45% yield). The infrared spectrum differed from that of the product prepared in refluxing benzene only in the prominence of the purine bands at 6.21 (s) and 6.40 μ (m). Elemental analyses suggested half the product contained two sugar groups and half contained one.

Anal. Calcd. for $C_{28}H_{29}ClN_8O_{13}$: Cl, 6.79; N, 13.4. Found: Cl, 5.50; N, 9.90.

Deacylation[13] of the dimethyl sulfoxide product afforded a sirup which crystallized slowly from ethanol (16% yield), and then was recrystallized from methanol (7%), m.p. 167–169° dec.; $\lambda_{max}^{pH\ 6}$ mμ (ε), 219 (23,900), 244 (5,620), 313 (6,690); $\lambda_{max}^{pH\ 14}$ mμ (ε), 246 (6,440), 309 (7,660).

Anal. Calcd. for $C_{19}H_{22}ClN_5O_5 \cdot H_2O$: C, 39.6; H, 4.65; Cl, 11.7; N, 23.1. Found: C, 39.9; H, 4.95; Cl, 11.1; N, 22.8.

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Pyrimidines. I. The Synthesis of 6-Fluorocytosine and Related Compounds[1,2]

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Syntheses of 6-fluorocytosine and 6-fluoroisocytosine from 2,4,6-trifluoropyrimidine and the preparation of a number of mono- and difluoropyrimidine intermediates are described. 5-Chlorocytosine and 5-chloroisocytosine were obtained from cytosine or isocytosine by use of N-chlorosuccinimide in acetic acid. The relative effects of a 5- and a 6-halogeno atom on the ultraviolet absorption spectra and apparent pK_a values of cytosine and isocytosine are presented.

The importance of 5-fluorouracil and certain other 5-fluorinated pyrimidines and their nucleosides as antitumor agents[3] prompted an investigation of analogous 6-fluorinated pyrimidine derivatives.

A considerable amount of information is available[4,5] concerning the attack of various nucleophilic reagents on 2,4,6-trichloropyrimidine leading to the formation of 6-chloro-2,4-disubstituted pyrimidine derivatives. Therefore, an ideal intermediate for the proposed syntheses of the 6-fluoropyrimidines was provided by the reported preparation of 2,4,6-trifluoropyrimidine[6,7] obtained by treatment of 2,4,6-trichloropyrimidine with silver fluoride.

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(2) A preliminary report of this work has appeared in the Abstracts of the 144th National Meeting of the American Chemical Society, Los Angeles, California, April, 1963, p. 26L.

(3) (a) For a partial list of published studies on the antitumor activity of 5-fluorinated pyrimidines and their nucleosides, see ref. 8–11 and 13 in the following paper; (b) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).

(4) E. Buttner, *Ber.*, **36**, 2227 (1903).

(5) W. Winkelmann, *J. Prakt. Chem.*, [2] **115**, 292 (1929).

(6) H. Schroeder, *J. Am. Chem. Soc.*, **82**, 4115 (1960).

(7) H. Schroeder, E. Kober, H. Ulrich, R. Rätz, H. Agahigian, and C. Grundmann, *J. Org. Chem.*, **27**, 2580 (1962).

In the preparation of monosubstituted derivatives of 2,4,6-trifluoropyrimidine, the possibility of obtaining mixtures of isomers must be considered. It has been reported[4] that the reaction of 2,4,6-trichloropyrimidine with ammonia gave a mixture of the 2- and 4-aminodichloropyrimidines in the relative proportions of 2:1, respectively. If an analogous mixture of isomers could be obtained by amination of the trifluoropyrimidine, then separation of isomers followed by controlled hydrolysis of *one* of the remaining fluorine atoms should lead to 6-fluorocytosine, 6-fluoroisocytosine, and, conceivably though less likely, by analogy with the relative activities of the 2-chloro *vs.* the 6-chloro analogs, 4-amino-2-fluoro-6-hydroxypyrimidine. Deamination of the former two isomers could be expected to yield 6-fluorouracil.

In our hands, treatment of 2,4,6-trifluoropyrimidine (I) with alcoholic ammonia (see Chart I) gave a crystalline material which at first seemed homogeneous. There appeared to be no marked solubility differentiation; the melting point was not meaningful since sublimation was heavy and complete over a range somewhat dependent on the rate of heating. Paper chromatography showed only a single spot in several systems.