bonate was also added to neutralize the *p*-tolylsulfonic acid; yield, 46%, m.p. 124.5–126°; $\lambda_{max(\mu)}^{Nujol}$ 3.13–3.18 (OH); 5.72 (ester C=O); 8.32, 8.50, 8.60 (ester C—O—C); 9.34, 9.48 (C—OH); 13.2 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.73) in System A.[9]

Anal. Calcd. for $C_{15}H_{23}NO_4 \cdot HCl$: C, 56.7; H, 7.57; Cl, 11.2. Found: C, 56.6; H, 7.70; Cl, 11.3.

4-{o-[Bis(2-chloroethyl)amino]phenyl}butyric acid (XV). Chlorination of methyl 4-{*o*-[bis(2-hydroxyethyl)amino]-phenyl}butyrate hydrochloride was accomplished using phosphorus oxychloride in the same manner as in the preparation of IX; yield 64% of tan, light-sensitive crystals, m.p. 30–30.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.72 (acidic OH); 5.81 (carboxyl C=O); 6.24, 6.68 (aryl); 13.3 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.34) in System A.[9]

Anal. Calcd. for $C_{14}H_{19}Cl_2NO_2$: C, 55.3; H, 6.25; Cl, 23.3. Found: C, 55.5; H, 6.50; Cl, 22.9.

When methyl 4-{*o*-[bis(2-hydroxyethyl)amino]phenyl}-butyrate hydrochloride was chlorinated with thionyl chloride in refluxing chloroform for 30 min., a 52% yield of an oil was obtained that had the infrared absorption spectrum expected for methyl 4-{*o*-[bis(2-chloroethyl)amino]phenyl}-butyrate. This crude material was refluxed in concentrated hydrochloric acid for 30 min. to hydrolyze the ester, yielding, after crystallization from petroleum ether, 18% of XV, m.p. 29.5–30.5°.

4-(m-Aminophenyl)butyric acid hydrochloride. Reduction of the ketone group of 3-(*m*-aminobenzoyl)propionic acid[11] by the Huang-Minlon modified Wolff-Kishner reduction gave the desired product in 0–13% yields as white crystals, m.p. 155–157°; $\lambda_{max(\mu)}^{Nujol}$ 5.82 (carboxyl C=O), absence of ketone at 5.92. The compound traveled as a single spot (R_f 0.69) in System A.[9]

Anal. Calcd. for $C_{10}H_{11}NO_2 \cdot HCl$: C, 55.7; H, 6.54; Cl, 16.4; N, 6.50. Found: C, 55.7; H, 6.69; Cl, 16.4; N, 6.37.

Other methods, such as hydrogenation of 3-(*m*-nitrobenzoyl)propionic acid as its sodium salt at 90–100° in the presence of Raney Nickel or as its hydrochloride in the presence of palladium-charcoal, Clemmenson reduction, or hydrogenolysis of the ethylenethioketal, were no better.

Acknowledgment. The authors wish to thank Dr. Peter Lim for interpretation of the infrared spectra and his staff for the spectrophotometry and paper chromatography. They also thank Mr. O. P. Crews, Jr., and his staff for large-scale preparation of intermediates.

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] LI. Synthesis of 2-Amino-9-(5′-deoxy-β-D-ribofuranosyl)-9-H-purine-6-thiol

ELMER J. REIST, PHILLIP A. HART, LEON GOODMAN, AND B. R. BAKER

Received September 6, 1960

The synthesis of 2-amino-9-(5′-deoxy-β-D-ribofuranosyl)-9-H-purine-6-thiol (III) from 2′,3′-*O*-isopropylideneguanosine (IV) in seven steps is described. The intermediate 2′,3′-*O*-isopropylidene-5′-*O*-(*p*-tolylsulfonyl)guanosine (V) showed much less tendency toward cyclonucleoside formation than did the corresponding adenosine derivative. Thus, displacement of the tosylate of V by mercaptide gave 61% of recrystallized 5′-*S*-ethyl-2′,3′-*O*-isopropylidene-5′-thioguanosine (IX). Deacetonation followed by desulfurization yielded 5′-deoxyguanosine (XI), which was acetylated, then thiated and deacetylated to give the title compound (III).

Thioguanine[2], an analog of guanine, is a potent inhibitor of certain animal tumors[3] and of human leukemia[4]; in addition it is synergistic with azaserine.[5,6] Thioguanine is rapidly converted to its ribonucleotide and partially incorporated into the nucleic acid of thioguanine-sensitive neoplasms.[7] More recently, thioguanosine has been synthesized and evaluated as an antitumor agent.[8]

As part of the continuing study in this institute on the mechanism of action of thioguanine, LePage has given a preliminary report on the metabolism and antitumor effects[9] of 9-methylthioguanine,[10] where conversion to a nucleotide does not take place. The enzymic interconversion of purines at the free base level or at the nucleotides level is well established[11]; in contrast, little is known about interconversion of purines at the nucleoside level. As metabolism of thioguanine at the nucleo-

(1) This program is carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, *cf.* B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, *J. Med. Pharm. Chem.*, **2**, 633 (1960).

(2) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **77**, 1676 (1955).

(3) D. A Clarke, F. S. Philips, S. S. Sternberg, and C. C. Stock, *Ann. N. Y. Acad. Sci.*, **60**, 235 (1954).

(4) S. Farber, R. Toch, E. M. Sears, and D. Pinkel in J. P. Greenstein and A. Haddow, *Adv. Cancer Research*, **4**, 42 (1956), Academic Press Inc., New York, N. Y.

(5) A. C. Sartorelli and G. A. LePage, *Cancer Research*, **18**, 938 (1958).

(6) G. S. Tarnowski and C. C. Stock, *Cancer Research*, **17**, 1033 (1957).

(7) A. C. Sartorelli, G. A. LePage, and E. C. Moore, *Cancer Research*, **18**, 1232 (1958).

(8) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).

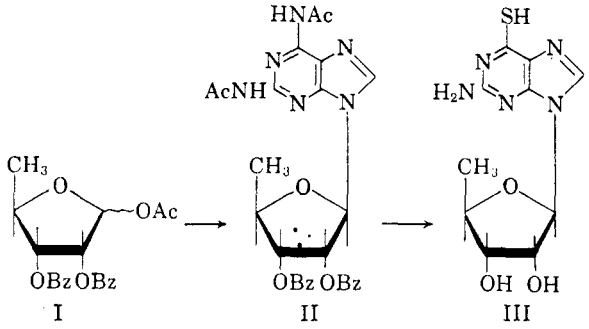
(9) Private communication from Dr. G. A. LePage to the Cancer Chemotherapy National Service Center.

(10) H. C. Koppel and R. K. Robins, *J. Am. Chem. Soc.*, **80**, 2751 (1958).

(11) G. Schmidt in *The Nucleic Acids*, Vol. I, E. Chargaff and J. N. Davidson, Academic Press, New York, p. 555.

side level is nevertheless possible, it must be considered. LePage[12] has suggested that 2-amino-9-(5′-deoxy-β-D-ribofuranosyl)-9-H-purine-6-thiol (III),[13] as it cannot be phosphorylated to a 5′-phosphate, may enter or block metabolic pathways at the nucleoside level. The synthesis of III for biological study is the subject of this paper.

There are two obvious approaches to the synthesis of 5′-deoxythioguanosine (III). One of them involves the condensation of the blocked 5-deoxyribofuranose (I)[14] with an appropriate purine such as 2,6-diacetamidopurine to give the 2,6-diamino nucleoside (II), which by suitable known transformations[15] could be convertible to 5′-deoxythioguanosine (III). The second approach starts with the commercially available 2′,3′-*O*-isopropylideneguanosine (IV) and by the sequence of reactions (IV→XII→III) could also yield 5′-deoxythioguanosine (III). The second sequence involves techniques which would be useful



I II III

in other problems in these laboratories so it was considered the method of choice.

The intermolecular displacement of a 5′-*O*-leaving group of a purine nucleoside by a nucleophile (such reactions as V→VI and V→IX) has as a competing side reaction, the intramolecular displacement of this same 5′-*O*-leaving group by the heterocyclic portion of the nucleoside to give, presumably, a 3,5′-cyclonucleoside such as VIII. The ease of formation of such cyclonucleosides has been reported to be a function of the basicity of the heterocyclic ring.[16] Thus, the heterocyclic ring system of adenosine is sufficiently basic that the tosylation of C'_5 of XV resulted in a mixture of covalent XVII and ionic XIX tosylate,[16] whereas inosine (XVI) with its less basic heterocyclic system could be tosylated to the covalent tosylate (XVIII) with no difficulty.[17] This difference is also illustrated in the formation in high yield of the 5′-*S*-methyl-5′-thioinosine (XXII) from isopropylideneinosine (XVI) *via* its tosylate[18,19] compared with the low yields obtained in the same sequence with adenosine.[18–20] Similarly, displacement of the adenosine 5′-tosylate (XVII) by iodide gave exclusively the cyclic iodide (XX).[16] Unfortunately, the product of the displacement of the inosine 5′-tosylate (XVIII) by iodide was not fully characterized.[17] If this tendency toward cyclization is a function of the basic character of the heterocyclic moiety, it would follow that the guanosine series would be more resistant to cyclization than the adenosine series and probably less resistant to cyclization than inosine nucleosides. In this respect it is interesting to note that Khorana, *et al.*[21] reported that 2′,3′-*O*-isopropylidene guanosine 5′-*O*-[di(*p*-nitrophenylphosphate)] (XXIII) was converted to the cyclonucleoside (XXIV) when it was heated in refluxing acetonitrile or treated with sodium benzoxide in benzyl alcohol.

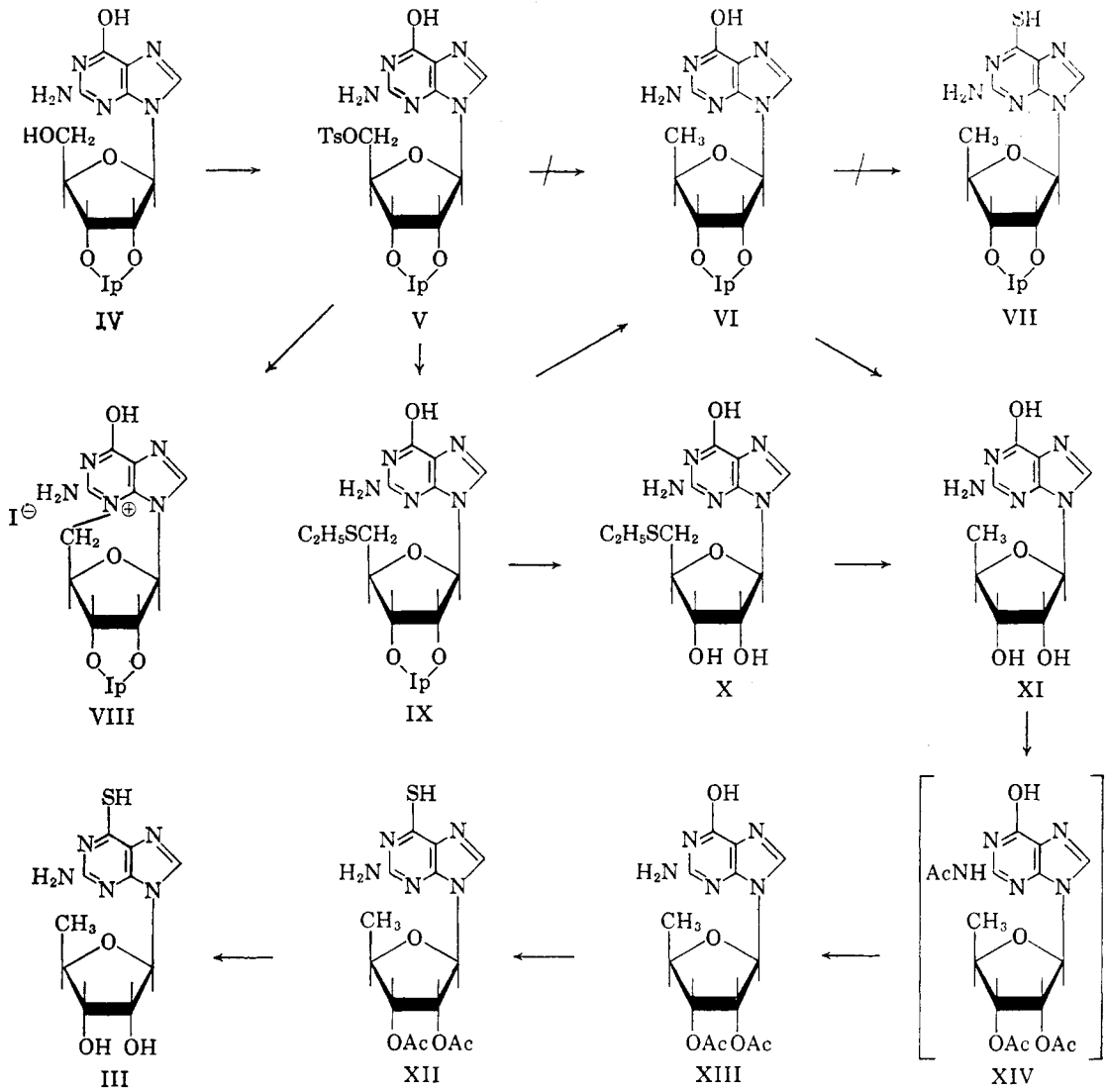
Tosylation of 2′,3′-*O*-isopropylideneguanosine (IV) gave a 73% yield of covalent tosylate (V) with no evidence for the cyclonucleoside which would be analogous to VIII. Treatment of the tosylate (V) with sodium iodide in acetonylacetone gave the cyclic iodide (VIII) exclusively with no evidence for the covalent iodide. Khorana, *et al.*[21] pointed out the possibility of the cyclization of C'_5 with the 2-amino group although they, too, tentatively assumed a 3–5′-ring structure for the cycloguanosine.

An attempted lithium aluminum hydride reduction of the tosylate (V) did not give the 5′-deoxyguanosine derivative (VI). As the ultraviolet spectrum of the crude product of the hydride reduction suggested that reduction of the purine ring had occurred, this approach was not further investigated.

Treatment of the tosylate (V) with sodium ethylmercaptide in alcohol at room temperature or below gave a 70% yield of 5′-*S*-ethyl-2′,3′-*O*-isopropylidene-5′-thioguanosine (IX).

Hydrolysis of the isopropylidene thioether (IX) with refluxing 3% aqueous acetic acid gave a 70% yield of 5′-*S*-ethyl-5′-thioguanosine (X). Desulfurization of X could be effected in 80% crude yield to 5′-deoxyguanosine (XI) by the use of 15 weight equivalents of Davison sponge nickel[22] under a hydrogen atmosphere at 100°. The use of this catalyst for the desulfurization proved to be much superior to the Raney Nickel C[23] used previously

(12) Private communication from Dr. G. A. LePage.
(13) Hereafter referred to as 5′-deoxythioguanosine.
(14) H. M. Kissman and B. R. Baker, *J. Am. Chem. Soc.*, **79**, 5534 (1957).
(15) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).
(16) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).
(17) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **111**, 313 (1935).
(18) J. Baddiley, *J. Chem. Soc.*, 1348 (1951).
(19) K. Satok and K. Makino, *Nature*, **167**, 238 (1951).
(20) F. Weygand and O. Trauth, *Ber.*, **84**, 633 (1951).
(21) R. W. Chambers, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3747 (1957).
(22) Sponge nickel catalyst, Davison Chemical Co., Cincinnati 29, Ohio.
(23) C. D. Hurd and B. Rudner, *J. Am. Chem. Soc.*, **73**, 5157 (1951).

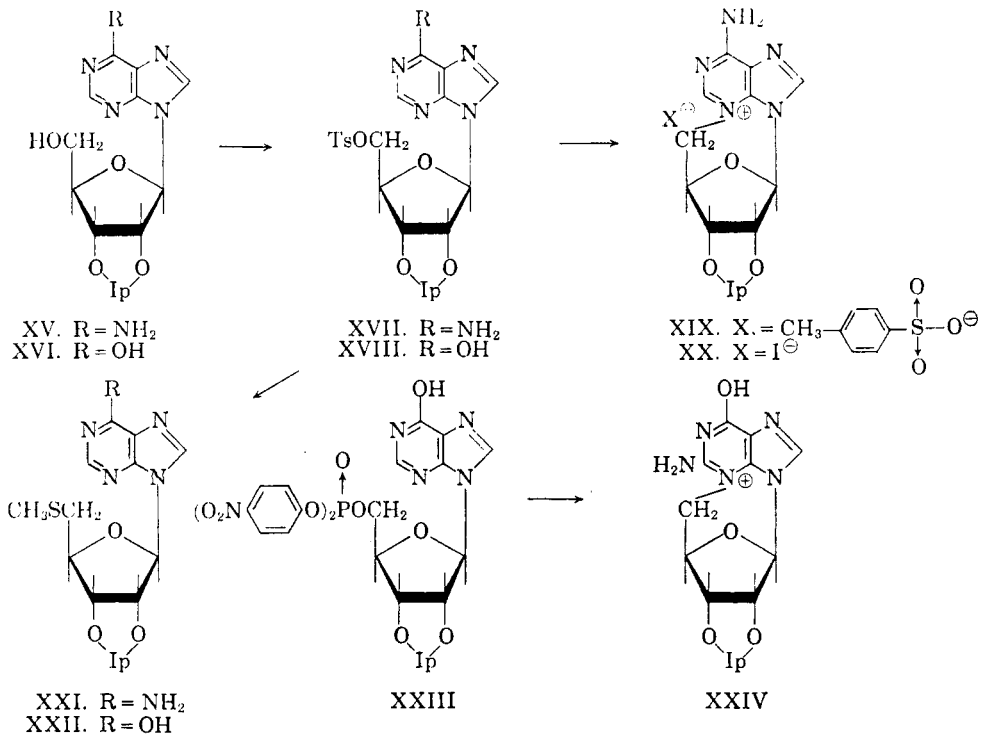


for the desulfurization of ethylthio nucleosides,[24] both from the standpoint of yield and convenience. Raney Nickel C when used to desulfurize X frequently failed to effect complete desulfurization. In addition, the losses of nucleoside due to absorption on the catalyst were always higher with Raney Nickel C.

5′-Deoxyguanosine (XI) was also prepared by desulfurization of the isopropylidene ethylthio nucleoside (IX) to give 5′-deoxy-2′,3′-*O*-isopropylideneguanosine (VI) in 81% crude yield; deacetonation of VI with aqueous acetic acid gave XI in 36% yield. This latter route to 5′-deoxyguanosine (XI) *via* VI is somewhat less satisfactory than the previously described route *via* X. This appears to be due mainly to the marked ease of hydrolysis of 5′-deoxyguanosine (XI) to guanine by dilute acetic acid. It was found necessary to use much greater care during the hydrolysis of the acetonyl group of the deoxyguanosine (VI) than for that of the ethylthioguanosine (IX).

The difference in reactivity between guanosine and 5′-deoxyguanosine (XI) is striking. Thus guanosine when treated with excess benzoyl chloride in pyridine at 65° gave an 80% yield of 2′,3′5′-tri-*O*-benzoylguanosine;[8] thus, less than 20% benzoylation of the purine molecule could have taken place. When 5′-deoxyguanosine (XI) was benzoylated, however, a sirupy product was obtained that had an infrared spectrum in which the guanine pattern at 3–3.5 μ and 5.7–6.3 μ was altered and which had strong evidence for *N*-benzoylation in addition to the expected *O*-benzoylation. Khorana, *et al.*[21] commented on the inertness of the 5′-hydroxyl of guanosine to phosphorylation and suggested that this inertness might be due to hydrogen bonding between the 5′-hydroxyl and 2-amino group in guanosine. It would appear on the basis of the above experiments that this hydrogen bonding of the 2-amino group with the 5′-hydroxyl or 5′-benzoate deactivates the 2-

(24) C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, 81, 3967 (1959).



XV. R = NH_2
XVI. R = OH

XVII. R = NH_2
XVIII. R = OH

XIX. X = CH_3—C$_6$H$_4$—SO_3^-
XX. X = I^-

XXI. R = NH_2
XXII. R = OH

XXIII

XXIV

amino group as well. Thus when the hydrogen bonding is eliminated as in 5′-deoxyguanosine (XI), *N*-benzoylation can easily take place and does.

Acetylation of 5′-deoxyguanosine (XI) also gave a crude product which apparently contained *N*-acetyl in addition to the desired di-*O*-acetate and is assigned the structure XIV. Recrystallization of this *N*,*O*-triacetate (XIV) from water caused the complete disappearance of *N*-acetate bands in the infrared together with the reappearance of the guanine pattern at 3.0–3.5 and 5.7–6.3μ; this product then had an analysis agreeing well for 2′,3′-di-*O*-acetyl-5′-deoxyguanosine (XIII).

Thiation of the 5′-deoxyguanosine diacetate (XIII) using the procedure described by Fox, *et al.*,[8] for the thiation of 2′,3′,5′-tri-*O*-benzoylguanosine gave a 55% yield of crude 2′,3′-di-*O*-acetyl-5′-deoxythioguanosine (XII). The crude XII was deacetylated directly using methanolic ammonia to give crystalline 5′-deoxythioguanosine (III) in 32% yield from XIII.

An alternative method for the synthesis of III by the thiation of 5′-deoxy-2′,3′-*O*-isopropylideneguanosine (VI) to 5′-deoxy-2′,3′-*O*-isopropylidenethioguanosine (VII) was unsuccessful. The ultraviolet spectrum of the crude thiation product of VI contained the thioguanine pattern. Paper chromatography indicated the presence of free thioguanine, however, and no VII could be isolated. Subsequent deacetonation in an effort to obtain III gave no crystalline material, and the paper chromatograms showed no spots corresponding to deoxythioguanosine (III).

EXPERIMENTAL[25]

2′,3′-O-Isopropylidene-5′-O-(p-tolysulfonyl)guanosine (V). To a solution of 7.5 g. (39.4 mmoles) of *p*-tolysulfonyl chloride in 75 ml. of freshly distilled dry pyridine and 150 ml. of dry benzene was added 5.0 g. (15.5 mmoles) of 2′,3′-*O*-isopropylideneguanosine (IV).[27] The reaction mixture was stirred vigorously at room temperature overnight protected from moisture, then the thick, gel-like material was filtered and the filter cake was suspended in 150 ml. of saturated aqueous sodium bicarbonate. To this suspension was added 60 ml. of ethanol. The solid material was thoroughly stirred in the medium, then filtered, and the filter cake was washed with water, ethanol and finally ether to give 5.4 g. (73%) of the white, powdery tosylate (V), m.p. 283–284° dec.; $[\alpha]_D^{30}$ +36° (1% in *N*,*N*-dimethylformamide). The infrared spectrum was identical with that of the analytical sample.

The analytical sample, m.p. 288–289° dec. obtained from a previous tosylation by recrystallization of the crude tosylate (V) from acetonitrile had $\lambda_{max\ (\mu)}^{Nujol}$ 2.95, 3.03, 3.19, 5.87, 6.08, 6.23 (guanine pattern); 8.50 (OSO_2).

(25) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Standard Polarimeter Model D attachment to the Beckman DU spectrophotometer calibrated with sucrose solutions. Paper chromatograms were run with water-saturated butyl alcohol (solvent A) and 5% aqueous disodium phosphate (solvent B) by the descending technique on Whatman No. 1 paper. The spots were located by visual examination with an ultraviolet lamp, and where applicable by a bromine spray for thioether.[26] Adenine was used as a standard and spot locations were expressed as R_{Ad} units with adenine at 1.00.

(26) E. J. Reist, P. A. Hart, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 5176 (1959).

(27) California Corporation for Biochemical Research, 3625 Medford St., Los Angeles 63, Calif.

Anal. Calcd. for $C_{20}H_{23}N_5O_7S$: C, 50.4; H, 4.86; S, 6.70. Found: C, 50.8; H, 5.04; S, 6.28.

2',3'-O-Isopropylidene-8,5'-cycloguanosine iodide (VIII). To a suspension of 1.0 g. (2.1 mmoles) of 2',3'-*O*-isopropylidene-5'-*O*-(*p*-tolysulfonyl)guanosine (V) in 30 ml. of acetonylacetone was added 0.37 g. (2.5 mmoles) of sodium iodide and the mixture was heated with stirring at 100–110° for 4 hr. The reaction mixture was cooled and filtered. The filter cake was slurried in water and filtered to give 0.78 g. (87%) of a violet solid, m.p. >300°.

Anal. Calcd. for $C_{13}H_{16}IN_5O_4$: I, 29.3. Found: I, 28.1.

This material was readily soluble in hot water and contained ionic iodide as shown by an immediate silver nitrate test. The infrared spectrum of the compound showed no absorption assignable to covalent sulfonate at 8.5 μ or ionic sulfonate at 9.8 μ.

The analytical sample m.p. >300°, was obtained by recrystallization from hot water, and had $\lambda_{max\,(m\mu)}^{pH\,1}$ 2.29 (ϵ 18,300), shoulder at 260 (ϵ 12,100); $\lambda_{max\,(m\mu)}^{pH\,7}$ 220 (ϵ 37,800), 266 (ϵ 11,200); $\lambda_{max\,(m\mu)}^{pH\,13}$ 223 (ϵ 35,300).

Anal. Calcd. for $C_{13}H_{10}IN_5O_4$: C, 36.1; H, 3.72; N, 16.2. Found: C, 36.4; H, 3.93; N, 16.0.

5'-S-Ethyl-2',3'-O-isopropylidene-5'-thioguanosine (IX). A solution of 28.6 g. (0.53 mole) of sodium methoxide and 44 g. (0.71 mole) of ethyl mercaptan in 1300 ml. of absolute ethanol was cooled to 15° and 22 g. (0.46 mole) of 2',3'-*O*-isopropylidene-5'-*O*-(*p*-tolylsulfonyl)guanosine (V) was added. The mixture was stirred in an ice bath for 2 hr., then at room temperature for an additional 2.5 hr.

The reaction mixture was then concentrated *in vacuo* to a slurry which was dissolved in 1 l. of water. The aqueous solution was carefully neutralized to *p*H 7 with acetic acid, causing the precipitation of the product. The mixture was cooled to 5°, then filtered and the precipitate was washed with water and dried to give 11.8 g. (70%) of crude product (IX), m.p. 233–295°.

Recrystallization of 9.0 g. of the above crude product from 500 ml. of 95% ethanol gave 7.8 g. (61%) of an off-white solid in two crops, m.p. 301–303° dec.; $[\alpha]_D^{28}$ 0.0° (1% in *N,N*-dimethylformamide) with an infrared spectrum identical with that of the analytical sample.

Recrystallization of a previous preparation from ethanol gave the analytical sample, m.p. 299–301° dec.; $\lambda_{max\,(\mu)}^{Nujol}$ 2.92, 3.03, 3.14, 5.82, 6.07, 6.23 (guanine pattern), no OSO_2 at 8.5; $\lambda_{max\,(m\mu)}^{pH\,1}$ 257 (ϵ 11,500) shoulder at 280 (ϵ 7,290).

Anal. Calcd. for $C_{15}H_{21}N_5O_4S$: C, 49.2; H, 5.76; S, 8.72. Found: C, 49.4; H, 6.09; S, 8.56.

5'-Deoxy-2',3'-O-isopropylideneguanosine (VI). To a suspension of 80 g. of Davison sponge nickel[22] in 350 ml. of 2-methoxyethanol was added 5.0 g. (13.6 mmoles) of 5'-*S*-ethyl-2',3'-*O*-isopropylidene-5'-thioguanosine (IX). The mixture was maintained under 1 atm. of hydrogen and stirred vigorously in an oil bath regulated at 100° for 16 hr. The hot mixture was filtered and the filter cake was washed with several portions of hot ethanol. The combined filtrate and washings were concentrated to dryness *in vacuo* to yield 3.4 g. (81%) of a pale yellow solid that was essentially free of starting material as shown by paper chromatography.[28]

The crude product was purified by heating at reflux with 90 ml. of 95% ethanol, then cooling and filtering. The resultant product, 1.6 g. (38%), was chromatographically homogeneous and did not melt below 300°.

Recrystallization from 95% ethanol gave the analytical sample, m.p. >300°; $[\alpha]_D^{27}$ −16.0° (1% in *N,N*-dimethylformamide); $\lambda_{max\,(\mu)}^{Nujol}$ 2.89, 3.02, 3.14, 5.80, 6.08, 6.23 (guanine pattern); 8.25, 8.53 (isopropylidene); 9.24, 9.40 (C—O—C).

Anal. Calcd. for $C_{13}H_{17}N_5O_4$: C, 50.8; H, 5.57; N, 22.8. Found: C, 50.7; H, 5.83; N, 23.1.

5'-S-Ethyl-5'-thioguanosine (X). A suspension of 8.8 g. of 5'-*S*-ethyl-2',3'-*O*-isopropylidene-5'-thioguanosine (IX) in 300 ml. of 3% aqueous acetic acid was stirred at reflux temperature for 2.5 hr., by which time solution was complete. Cooling the hot solution in an ice bath at 0° caused the product (X) to separate as white crystals, 7.7 g. (99%), m.p. 245–247°.

The crude product was recrystallized from 250 ml. of 20% aqueous pyridine to give 5.4 g. (70%) of white crystals, m.p. 248–250°, which were homogeneous on paper chromatography with R_{Ad} 1.2 in solvent A and 1.6 in solvent B.[25]

The analytical sample from a previous preparation had m.p. 248–250° dec.; $[\alpha]_D^{20}$ −10.0° (0.9% in *N,N*-dimethylformamide); $\lambda_{max\,(m\mu)}^{pH\,1}$ 257 (ϵ 13,000), shoulder at 280 (ϵ 8,800); $\lambda_{max\,(m\mu)}^{pH\,13}$ 265 (ϵ 12,600). The infrared spectrum showed no absorption assignable to the isopropylidene group at 8.25 and 8.53 μ.

Anal. Calcd. for $C_{12}H_{17}N_5O_4S$: C, 44.1; H, 5.23; N, 21.4; S, 9.80. Found: C, 43.9; H, 5.40; N, 21.6; S, 9.62.

5'-Deoxyguanosine (XI). *A. From 5'-S-ethyl-5'-thioguanosine* (X). Desulfurization of 5.0 g. of 5'-*S*-ethyl-5'-thioguanosine (X) using the procedure described above for the preparation of 5'-deoxy-2',3'-*O*-isopropylideneguanosine (VI) gave an 80% yield of crude XI. Recrystallization from about 25 ml. of water gave 1.8 g. (44%) of material, m.p. 226–228°, which was homogeneous on paper chromatography in solvents A and B.[25]

The analytical sample from a previous run had m.p. 226–228°; $[\alpha]_D^{28}$ −21° (1% in *N,N*-dimethylformamide); $\lambda_{max\,(\mu)}^{Nujol}$ 2.92, 3.03 (OH); 3.13, 5.80, 6.12, 6.25 (guanine pattern); $\lambda_{max\,(m\mu)}^{pH\,1}$ 256 (ϵ 11,300), 280 (ϵ 7620); $\lambda_{max\,(m\mu)}^{pH\,13}$ 264 (ϵ 10,800); R_{Ad} 0.34 in solvent A and 1.84 in solvent B.

Anal. Calcd. for $C_{10}H_{13}N_5O_4 \cdot {}^1/_2H_2O$: C, 43.5; H, 5.15; N, 25.3. Found: C, 43.4; H, 5.29; N, 25.4.

B. From 5'-deoxy-2',3'-O-isopropylideneguanosine (VI). Treatment of 100 mg. of 5'-deoxy-2',3'-*O*-isopropylideneguanosine (VI) with 4 ml. of refluxing 2% aqueous acetic acid for 45 min. resulted in complete solution. The reaction was then concentrated to dryness *in vacuo* to give 86 mg. of crude product which was homogeneous on paper chromatography in solvents A and B[25] and corresponded with authentic 5'-deoxyguanosine (XI). Recrystallization from 5 ml. of water gave 31 mg. (36%) of product, m.p. 213–216°. Repeated recrystallization failed to raise the melting point significantly; however, the infrared spectrum was essentially identical with that of 5'-deoxyguanosine (XI) prepared by method A. A mixed melting point with 5'-deoxyguanosine (XI) from method A gave no depression.

2',3'-Di-O-acetyl-5'-deoxyguanosine (XIII). To a mixture of 0.30 g. (1.1 mmoles) of 5'-deoxyguanosine (XI) in 6 ml. of dry pyridine was added 0.58 ml. (6.2 mmoles) of acetic anhydride. The reaction was heated at 75° with stirring for 1.5 hr., then stirred at room temperature for 16 hr. The excess acetic anhydride was destroyed by the addition of 2 ml. of ethanol.

The mixture was concentrated to dryness *in vacuo* to give 460 mg. of off-white solid (presumably triacetate XIV), m.p. 252–255°; $\lambda_{max(\mu)}^{Nujol}$ 2.93, 3.04, 3.19, 5.85, 6.10, 6.25 (relative intensities changed from guanine pattern); 5.70 (*O*-acetate C=O); shoulder at 5.90 (*N*-acetate C=O); 6.48 (amide II).

Anal. Calcd. for $C_{16}H_{19}N_5O_7$ (triacetate): C, 48.8; H, 4.88; N, 17.8. Calcd. for $C_{14}H_{17}N_5O_6$ (diacetate): C, 47.9; H, 4.88; N, 19.9. Found: C, 48.5; H, 5.25; N, 19.0.

Recrystallization of the crude triacetate (XIV) from water gave 130 mg. (32%) of the diacetate (XIII), m.p. 262.5–

(28) Paper chromatograms of the blocked nucleoside (VI and IX) in solvents A and B gave variable R_{Ad} values. The progress of the desulfurization could be followed satisfactorily by paper chromatography if starting material and reaction product were examined simultaneously. Thus in one desulfurization, starting material (IX) had R_{Ad} 1.71 in solvent A while the product (VI) had R_{Ad} 1.54. A second desulfurization chromatogram gave R_{Ad} values of 1.84 and 1.70 for IX and VI, respectively.

264°; $[\alpha]_D^{27}$ −26.0° (0.32% in 95% ethanol); $\lambda_{max(\mu)}^{Nujol}$ 2.93, 3.03, 3.14, 5.82, 6.12, 6.25 (guanine pattern); 5.70 (*O*-acetate), no *N*-acetate at 5.90 or 6.48; $\lambda_{max(m\mu)}^{pH\ 1}$ 257 (ε 12,400), 280 (ε 8,700); R_{Ad} 1.42, in solvent A.[25]

Anal. Calcd. for $C_{14}H_{17}N_5O_6 \cdot {}^1/_2H_2O$: C, 46.7; H, 5.01; N, 19.4. Found: C, 46.4; H, 5.00; N, 19.0.

A subsequent acetylation of 1.8 g. of 5′-deoxyguanosine (XI) gave after one recrystallization 1.9 g. (78%) of diacetate (XIII), m.p. 258–260°, that had an infrared spectrum very similar to that of the analytical sample and had identical paper chromatographic behavior.

2-Amino-9-(5′-deoxy-β-D-ribofuranosyl)-9-H-purine-6-thiol (III). A mixture of 550 mg. (1.53 mmoles) of 2′,3′-di-*O*-acetyl-5′-deoxyguanosine (XIII) and 1.25 g. (5.6 mmoles) of phosphorus pentasulfide was added to 33 ml. of dry pyridine. The reaction was heated to reflux and 82 λ of water were added dropwise from a microburette, giving a cloudy solution which was heated at reflux for 8 hr. with stirring under nitrogen atmosphere. After the addition of 50 ml. of water the mixture was heated on a steam bath for 2 min., then cooled and adjusted to *p*H 6 with saturated aqueous sodium bicarbonate.

Extraction of the aqueous solution with 20 ml. of chloroform caused the separation of a precipitate which remained suspended in the chloroform layer. The chloroform layer containing the precipitate was drawn off and the aqueous layer was extracted further with four additional 20-ml. portions of chloroform. The combined chloroform extracts and solid were concentrated to dryness *in vacuo* to yield 340 mg. of a red-brown solid (XII). Trituration of the crude blocked nucleoside (XII) with 10 ml. of chloroform gave 270 mg. (47%) of an off-white product (XII) m.p. 230–236°. Continuous extraction of the aqueous phase above for 6 hr. with chloroform gave an additional 47 mg. (total yield 55%) of white solid, m.p. 230–235°; $\lambda_{max(\mu)}^{Nujol}$ 5.71 (acetate C=O) 8.34 (>C=S); $\lambda_{max(m\mu)}^{pH\ 1}$ 344 (ε 18,700); R_{Ad} 1.62 in solvent A[25] with trace components at R_{Ad} 0.0 and 0.25.

A mixture of 270 mg. of the crude diacetate (XII) and 10 ml. of methanol was saturated with ammonia at 5–10°, causing complete solution. After 16 hr. at 5°, a small amount of insoluble residue was removed by filtration and the filtrate was concentrated to dryness *in vacuo* to give 225 mg. of brown solid which had $\lambda_{max(m\mu)}^{pH\ 13}$ 318 (ε 15,100). Pure 5′-deoxythioguanosine (III) had $\lambda_{max(m\mu)}^{pH\ 13}$ 318 (ε 19,000). Thus, crude III above contained 80% of III (thioguanyl moiety). The crude nucleoside (III) showed a major component at R_{Ad} 0.61 and 1.55 in solvents A and B,[25] respectively, along with minor amounts of contaminants.

Recrystallization from 25 ml. of water gave 115 mg. (32% based on XIII) of material, m.p. 241–246° dec. Further recrystallizations from water gave the analytical sample, m.p. 252–252.5° dec.; $[\alpha]_D^{28}$ −62° (0.5% in *N,N*-dimethylformamide); $\lambda_{max(m\mu)}^{Nujol}$ 3.01, 3.07, 3.16, 6.08, 6.23, 6.28 (thioguanine pattern); 8.31 (C=S); $\lambda_{max(m\mu)}^{pH\ 1}$ 264 (ε 7,700), 344 (ε 21,300); $\lambda_{max(m\mu)}^{pH\ 13}$ 252 (ε 12,900), 318 (ε 19,000)[29]; R_{Ad} 0.61 in solvent A and 1.55 in solvent B.

Anal. Calcd. for $C_{10}H_{13}N_5O_3S$: C, 42.4; H, 4.62; N, 24.7; S, 11.2. Found: C, 42.3; H, 5.51; N, 24.6; S, 11.3.

A second crop of 35 mg., m.p. 243–247° dec., was obtained on concentration of the mother liquors. The paper chromatograms of the second crop were essentially identical with that of the analytical sample with R_{Ad} 0.68 in solvent A and R_{Ad} 1.47 in solvent B.

Acknowledgment. The authors are indebted to Dr. Peter Lim and staff for the chromatograms, optical rotations, and interpretation of the infrared spectra, and to Mr. O. P. Crews, Jr., and his staff for the large-scale preparations of intermediates.

MENLO PARK, CALIF.

(29) Fox, *et al.*[8] reported $\lambda_{max(m\mu)}^{pH\ 4-6}$ 257 (ε 8820), 342 (ε 24,800); $\lambda_{max(m\mu)}^{pH\ 10-12}$ 252 (ε 14,700), 319 (ε 21,000) for thioguanosine.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CINCINNATI]

Substituted γ-Lactones. VI.[1] Synthesis of Certain *p*-Substituted α-Benzylidene- and α-Benzyl-γ-butyrolactones as Potential Anticancer Compounds

HANS ZIMMER[2] AND RALPH E. DEBRUNNER[3]

Received July 18, 1960

A series of *N*-substituted glycines and precursors has been prepared. These compounds are derivatives of α-(4-aminobenzylidene)- and α-(4-aminobenzyl)-γ-butyrolactone. They can be obtained best from the corresponding α-(4-aminobenzylidene)- or α-(4-aminobenzyl)-γ-butyrolactones. The chemistry of these compounds and their preparation is discussed.

In our investigation of substituted γ-butyrolactones we became interested in the chemistry and pharmacology of amines derived from α-benzylidene-γ-butyrolactone and α-benzyl-γ-butyrolactone, because it recently has been shown that certain unnatural amino acids and derivatives thereof have interesting properties as cancer chemotherapeutica.[4] It also has been known for sometime that various compounds structurally related to, but not identical with, essential amino acids exhibit antimetabolic character.[5] Zimmer and Rothe[6] have prepared α-(4-dimethylaminobenzyl-

(1) Paper V in this series: H. Zimmer, J. Rothe, and J. Holbert, *J. Org. Chem.*, **25**, 1234 (1960).

(2) Presented before the Division of Medicinal Chemistry, ACS Meeting, Cleveland, Ohio, April 11, 1960.

(3) Taken in part from Ph.D. thesis of R. E. DeBrunner, University of Cincinnati (1960), 1957–58, Procter and Gamble fellow, 1959, Chattanooga Medicine Company fellow.

(4) F. Bergel, J. M. Johnson, and J. A. Stock, *Chem. & Ind. (London)*, 1489 (1959).