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Puromycin. Synthetic Studies. XIII. Synthesis of 6-Dimethylamino-9-(3′-amino-3′-deoxy-β-D-arabinofuranosyl)-purine

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The title compound, the 2′-epimer of the biologically active aminonucleoside, I, from puromycin has been synthesized by a stereochemically controlled sequence *via* the key intermediates, 2-methylmercapto-6-dimethylamino-9-β-D-xylofuranosylpurine (V) and 2-methylmercapto-6-dimethylamino-9-(2′,3′-anhydro-β-D-lyxofuranosyl)-purine (IX). The title compound has been converted to the aminonucleoside triacetate XV, thus completing an alternate total synthesis of puromycin from D-xylose.

The partial synthesis[1] of the biologically active aminonucleoside, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (I), from the antibiotic puromycin and the total synthesis[2,3] of I from D-xylose have been previously described. The synthesis of the α-anomer[4] of I by a stereochemically controlled sequence has shown that the β-configuration is necessary for biological activity. Another structural variant of the aminonucleoside I is the 2′-epimer, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-arabinofuranosyl)-purine (X). This compound also has been synthesized by a stereochemically controlled sequence and is the subject of this paper.

6-Dimethylamino-9-(3′-amino-3′-deoxy-β-D-arabinofuranosyl)-purine (X) cannot be synthesized directly from a blocked 3-amino-3-deoxy-D-arabinose since the latter forms an α-nucleoside[4] with C_1-C_2 *trans*-configuration. This result was predicted from the rule[5] that the nucleoside obtained by condensing an acylated halo sugar with a heavy metal salt of a purine will have a C_1-C_2-*trans*-configuration regardless of the initial configuration of the acylated halo sugar. Thus, in order to synthesize the 3′-aminoarabinosyl β-nucleoside X it would be necessary to start with a blocked sugar furanose having the D-configuration at C_2. The 3-hydroxyl must be *trans* to the 2-hydroxyl in order to allow further inversions at C_2 and C_3, *i.e.*, inversion of the 2-group to L and the 3-group to D with introduction of an amino group at C_3. A sugar which satisfies these requirements is D-xylofuranose 2,3,5-tri-*O*-benzoate (II), readily obtainable from α-D-xylofuranose tetra-*O*-benzoate.[6]

When 2,3,5-tri-*O*-benzoyl-D-xylofuranose (II)[6] was allowed to react with ether saturated with hydrogen chloride for 8–15 days at −3° in the presence of acetyl chloride to remove the water formed in the reaction, 2,3,5-tri-*O*-benzoyl-D-xylofuranosyl chloride (III) was formed in quantitative yield as a sirupy mixture of α- and β-anomers. Condensation with chloromercuri-2-methylmercapto-6-dimethylaminopurine[8] in boiling xylene for 75 minutes gave an 87% crude yield of the nucleoside benzoate VI as a glass which by ultraviolet analysis had a maximum purity of 75%. Catalytic debenzoylation with methanolic sodium methoxide gave 2-methylmercapto-6-dimethylamino-9-β-D-xylofuranosylpurine (V) as a glass in 60% over-all yield from II, correcting for a purity of 87% according to ultraviolet analysis. This compound crystallized poorly and consequently the glassy product was found preferable for subsequent reactions.[7]

Methyl D-xylofuranoside has been shown to react with acetone and sulfuric acid in the presence of copper sulfate to give methyl 3,5-*O*-isopropylidene-D-xylofuranoside, thus effectively blocking all groups except the 2-hydroxyl.[2] It also has been shown that acetone and sulfuric acid can convert 1-β-D-ribofuranosyl-5,6-dimethylbenzimidazole to its 2′,3′-*O*-isopropylidene derivative.[10] The latter procedure with other ribonucleosides proceeded[11] in low yields which were subsequently increased by adding anhydrous copper sulfate prior to the addition of the sulfuric acid. The copper sulfate served the dual purpose of absorbing the water formed in the reaction mixture and keeping the sulfate salt of the purine derivative in suspension.[11] These results would then allow the prediction that 2-methylmercapto-6-dimethylamino-9-β-D-xylofuranosylpurine (V), when treated with acetone and

(1) B. R. Baker, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 1 (1955), paper VII of this series.

(2) B. R. Baker, R. E. Schaub and J. H. Williams, *ibid.*, **77**, 7 (1955), paper VIII of this series.

(3) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, *ibid.*, **77**, 12 (1955), paper IX of this series.

(4) B. R. Baker and R. E. Schaub, *ibid.*, **77**, 2396 (1955), paper XII of this series.

(5) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **18**, 1786 (1954), paper V of this series. For an exception to the rule see B. R. Baker, R. E. Schaub and H. M. Kissman, THIS JOURNAL, **77**, 5911 (1955), paper XV of this series.

(6) H. G. Fletcher, Jr., *ibid.*, **75**, 2624 (1953), has synthesized α-D-xylofuranose tetra-*O*-benzoate *via* methyl D-xylofuranoside to give an over-all yield of 23% based on D-xylose along with 9.7% of β-anomer. In the experimental his procedure has been somewhat modified. In addition a new synthesis of α-D-xylofuranose tetra-*O*-benzoate is described giving an over-all yield of 20.4% based on D-xylose and proceeding *via* the following derivatives of D-xylose: 1,2:3,5-di-*O*-isopropylidene → 1,2-*O*-isopropylidene → 1,2-*O*-isopropylidene-3,5-di-*O*-benzoyl → 3,5-di-*O*-benzoyl.

(7) Raney nickel desulfurization of V gave 6-methylamino-9-β-D-xylofuranosylpurine, also obtained as a glass with the correct ultraviolet and infrared spectra. Condensation of 2,3,5-tri-*O*-benzoyl-D-xylofuranosyl bromide directly with 6-dimethylaminopurine gave a poor yield of a mixture of 7- and 9-xylosides from which the 7-nucleoside could be crystallized. 6-Dimethylaminopurine mercuric chloride has been shown previously to orientate α-bromoacetoglucose to the 7-position[8] and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride to the 9-position.[9] The statement made earlier,[8] that the orientation of an incoming sugar by a purine appeared to be independent of the sugar moiety was based on the examples then available in the literature. From the data obtained in the puromycin series it is clear that both the purine and the sugar can influence the orientation.

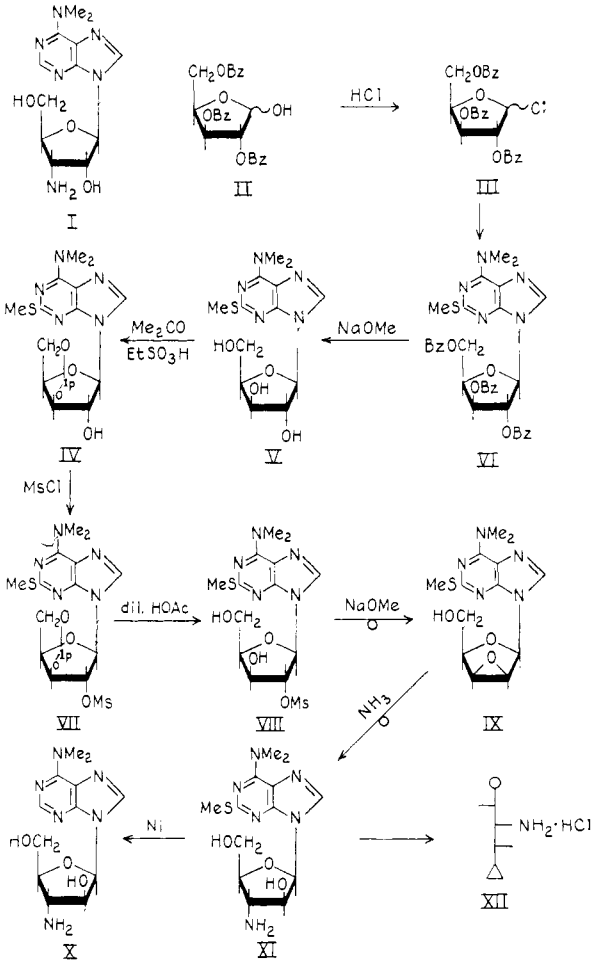
(8) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954), paper IV of this series.

(9) H. M. Kissman, C. Pidacks and B. R. Baker, THIS JOURNAL, **77**, 18 (1955), paper XI of this series.

(10) F. W. Holly, C. H. Shunk, E. W. Peel, J. J. Cahill, J. B. Lavigne and K. Folkers, *ibid.*, **74**, 4521 (1952).

(11) H. M. Kissman and B. R. Baker, unpublished results.

sulfuric acid in the presence of copper sulfate, should form the 3′,5′-*O*-isopropylidine derivative IV. Under these conditions IV was formed in variable yields of 49–66% crude and 27–40% crystalline. The low recovery on crystallization was subsequently shown to be due to the poor crystallizing powers of IV. When ethanesulfonic acid was substituted for the sulfuric acid in order to form a more acetone-soluble salt, the crude yield of IV was increased to 90%. When the crude isopropylidene derivative IV, prepared by the ethanesulfonic acid method, was treated with methanesulfonyl chloride in pyridine for 3 days at room temperature, the crystalline 2′-mesyl derivative VII was obtained in 72% yield. Mesylation of crystalline IV gave an 88% yield of VII under the same conditions, thus indicating that the crude isopropylidene derivative IV was about 90% pure.



Treatment of VII with 70% acetic acid,[2] rather than mineral acid which might cleave the glycosidic linkage, selectively removed the isopropylidene group to form the 2′-mesyl derivative VIII in 89% yield, m.p. 174–175°. When the latter was treated with excess methanolic sodium methoxide at the b.p., the mesyl group was rapidly eliminated by attack of the adjacent 3′-hydroxyl group with Walden inversion to form the 2′,3′-anhydrolyxoside IX, m.p. 212° in 88% yield.

Opening of the oxirane ring of IX with ammonia might be expected to give either a 2′-amino-2′-deoxy-D-xylo nucleoside or the isomeric 3′-amino-3′-deoxy-D-arabino nucleoside XI, depending upon whether the attack of ammonia, with Walden inversion, was at C_2' or C_3', respectively. It could be anticipated that the major product should be the 3′-amino-3′-deoxyarabino nucleoside XI since both α- and β-methyl 2,3-anhydro-D-lyxofuranoside gave only the 3-amino-3-deoxy-D-arabinoside.[2] When IX was treated with saturated methanolic ammonia at 100° for 2 hr., a 79% of crystalline aminonucleoside was obtained. That this product had the expected structure of a 3′-amino-3′-deoxy-D-arabino nucleoside XI was proved by hydrolysis with hydrochloric acid to 3-amino-3-deoxy-D-arabinose hydrochloride (XII), identical with an authentic sample.[2] The formation of XI proves unequivocally that V reacted with acetone to form a 3′,5′-*O*-isopropylidene derivative IV and that the further intermediates VII, VIII and IX had the assigned structures, otherwise XI could not be formed by this sequence.

Desulfurization of XI gave the desired 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-arabinofuranosyl)-purine (X) in 43% yield. The desulfurization was readily followed by the change in the ratio of ultraviolet absorption at 250 and 275 mμ.[9]

If the 2′-hydroxyl of XI could be inverted, desulfurization should then give 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (I), "the aminonucleoside" from puromycin. This sequence would complete an alternate[2,3] synthesis of puromycin from D-xylose, and has indeed proved successful in the following manner.

Treatment of the aminoarabinoside XI with acetic anhydride in 50% aqueous acetic acid gave the crystalline N-acetyl derivative XIII in 88% yield. Since preferential mesylation of the 2′-hydroxyl over the 5′-hydroxyl is not feasible, the acetaminonucleoside XIII was treated with triphenylmethyl chloride in pyridine at 51° which selectively blocked the 5′-hydroxyl.[12] Reaction with methanesulfonyl chloride in pyridine at 51° then gave the 2′-mesyl nucleoside XIV as a glass in 89% yield for the two steps. The trityl group was removed selectively by heating in dilute acetic acid at 100° giving the 2′-mesyl nucleoside XVII as a glass[13] in 75% yield. That a mesyl group was present in the product was clearly shown by an absorption band at 8.33 μ in the infrared. Reaction of the 2′-mesyl nucleoside XVII with sodium acetate in boiling methyl cellosolve containing 5% water caused displacement of the 2′-mesyl group by the neighboring 3′-acetamido group with Walden inversion *via* an oxazoline.[2,14] The oxazoline ring was

(12) (a) For the selective action of triphenylmethyl chloride in the carbohydrate field, *cf.* B. Helferich in "Advances in Carbohydrate Chemistry," Academic Press, Inc., New York, N. Y., 1948, Vol. 3, pp. 79. (b) Mesylation of both the 3′- and 5′-hydroxyls cannot be used, as in the case of inversion of C_2 in an α-nucleoside,[4] since the 5′-carbon of a β-nucleoside bearing a mesyl group will readily quaternize with the 3-nitrogen of the purine ring; *cf.* B. R. Baker and J. P. Joseph, THIS JOURNAL, **77**, 15 (1955), paper X of this series.

(13) J. Davoll, B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 2232 (1951), have used this procedure to block the 5′-position of a theophylline nucleoside prior to mesylation.

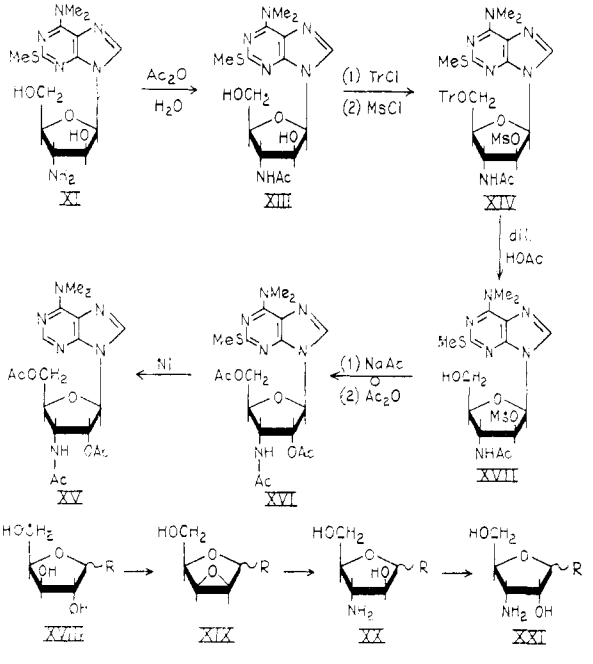
(14) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954), paper III of this series.

opened by the water present and the riboside XVI was isolated by acetylation in 79% yield from XVII. Desulfurization[3] with Raney nickel afforded crystalline 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine *O,N*-triacetate (XV) in 24% yield, thus giving unequivocal proof of the β-configuration of all of the intermediate nucleosides. The last mentioned yield is not as low as it appears since no crystalline intermediate products after XIII could be isolated. Only the over-all yield of 12.7% from XIII and the average yield as of 76% for each of the six steps are significant.

Since the aminonucleoside triacetate XV has previously been converted to puromycin,[1] this sequence of reactions provides an alternate synthesis of puromycin from D-xylose.

The stereochemically controlled syntheses of aminonucleosides described in this and previous papers can be summarized as follows.

There are four key compounds in the conversion of a D-xylofuranoside XVIII to a 3-amino-3-deoxy-D-ribofuranoside XXI, namely, XVIII–XXI.[2] The configuration of the aminonucleoside is controlled by the stage of this sequence at which the purine is introduced by application of the rule that a C_1-C_2-*trans*-nucleoside will be obtained.[5] Nucleoside formation from a blocked form of the XXb gives an *α-arabino* nucleoside XXc which, by inversion of the 2′-hydroxyl, can be converted to an *α-ribo* nucleoside XXIc.[4] Nucleoside formation from a blocked form of the xylofuranoside XVIIIb gives a *β-xylo* nucleoside XVIIIc which can be converted, *via* a 2′,3′-anhydro-*β-lyxo* nucleoside XIXc, to a *β-arabino* nucleoside XXc. The latter, by inversion of the 2′-hydroxyl group, forms the *β-ribo* nucleoside XXIc, the same compound obtained by condensing the blocked amino ribofuranoside XXIb with a purine.[3]



From these observations it follows that a blocked 3-amino-3-deoxy-D-xylofuranose should give a 3′-amino-3′-deoxy-β-D-*xylo* nucleoside, which, in turn, by inversion of the 2′-hydroxyl group, could be converted to a β-D-*lyxo* nucleoside. This completion of stereochemically controlled syntheses of all four possible 2′,3′-epimers of the biologically active aminonucleoside I will be the endeavor of future work.

Experimental[15]

α,D-Xylofuranose Tetra-*O*-benzoate. (A).—This compound was prepared by the sequence of Fletcher[6] with the following modifications. The reaction conditions for the preparation of 2,3,5-tri-*O*-benzoyl-D-xylose were the same as described for 2,3,5-tri-*O*-benzoyl-D-ribose[9] except that the methanol–hydrogen chloride reaction with D-xylose was allowed to proceed for 5 hr. This short reaction time avoids the formation of methyl D-xylopyranoside[16] which eventually must be disposed of as 2,3,4-tri-*O*-benzoyl-D-xylose under Fletcher's conditions. The over-all yield of α-anomer from D-xylose was 26%, m.p. 155–157°, compared to Fletcher's over-all yield of 23% of α-anomer and 9.7% of β-anomer.

(B).—1,2:3,5-Di-*O*-isopropylidene-D-xylofuranose was prepared by a combination of the better features of two methods[17,18] described in the literature. To 2 l. of reagent acetone in a 3-l. flask were added in order 10 cc. of 96% sulfuric acid, 200 g. of anhydrous copper sulfate and 100 g. of D-xylose. The mixture was stirred briskly protected from moisture for 25 hr. at room temperature, then filtered. The combined filtrate and washings were made basic by the rapid addition of 32 cc. of 15 *N* ammonium hydroxide. The filtered solution was evaporated to dryness *in vacuo* and the residue was distilled; yield 120 g. (84%) of a nearly colorless oil, b.p. 90–92° (0.2 mm.), m.p. 39–41°. Levene and Raymond[17] have recorded a b.p. of 85–87° (0.5 mm.) and m.p. 44–45°.

The above 1,2:3,5-di-*O*-isopropylidene-D-xylofuranose (219 g.) was warmed until molten, then cooled to room temperature to a sirup. Then 1100 cc. of 0.2% hydrochloric acid was added and the mixture shaken for 25 minutes. When about half the sirup had dissolved, the remainder crystallized. The solution was filtered from unreacted starting material (about 27 g.), then neutralized with sodium bicarbonate to *p*H 7–8. The 27 g. was retreated as above. The combined, neutralized solutions were evaporated to dryness *in vacuo*. The residue was dissolved in 500 cc. of chloroform. The solution, filtered from inorganic salts and dried with magnesium sulfate, was evaporated to dryness *in vacuo* leaving 178 g. (98%) of 1,2-*O*-isopropylidene-D-xylofuranose as a yellow viscous oil which gave a negative Benedict test. This oil was used without further purification, since distillation, as recommended by Levene and Raymond,[17] led to considerable decomposition in large runs. The procedure of hydrolysis recommended by Levene and Raymond,[17] employing stronger acid, led to the formation of considerable free D-xylose as shown by the strong Benedict test given by the product.

To a solution of 49.6 g. of crude 1,2-*O*-isopropylidene-D-xylofuranose in 248 cc. of reagent pyridine and 124 cc. of chloroform cooled in an ice-bath was added 71 cc. of benzoyl chloride in portions at such a rate that the temperature was 18–25° (10 minutes). The mixture was allowed to stand at room temperature protected from moisture for 22 hr., then it was poured into 1 l. of ice-water. The oil was collected by two 150-cc. extractions with chloroform. Washed with excess aqueous sodium bicarbonate, dried with magnesium sulfate and clarified with Norit, the combined extracts were evaporated to dryness *in vacuo* leaving 110 g. (106%) 1,2-*O*-

(15) Rotations and spectrophotometric data were performed by W. Fulmor and staff and microanalysis by L. Brancone and staff. Large scale preparations of 2-methylmercapto-6-dimethylaminopurine mercuric chloride and α-D-xylofuranose tetra-*O*-benzoate were run by W. McEwen and J. Poletto.

(16) P. A. Levene, A. L. Raymond and R. T. Dillon, *J. Biol. Chem.*, **95**, 699 (1932), have shown that the maximum yield of methyl D-xylofuranoside is obtained in 5 hr. Their data also show that the reaction time of 7 days recommended by Fletcher[6] gives about 50% each of furanoside and pyranoside.

(17) P. A. Levene and A. L. Raymond, *ibid.*, **102**, 317 (1933).

(18) O. Svanberg and K. Sjöberg, *Ber.*, **56**, 864 (1923).

isopropylidene-3,5-di-*O*-benzoyl-D-xylofuranose as an oil which could not be crystallized and was contaminated with ethyl benzoate.

To a solution of 25 g. of the preceding oil in 125 cc. of acetic acid was added 62.5 cc. of 12 *N* hydrochloric acid. After 30 minutes, the solution was diluted with 500 cc. of water and extracted with two 100-cc. portions of chloroform. The combined extracts, washed cautiously with excess aqueous sodium bicarbonate, were dried with magnesium sulfate, clarified with Norit and evaporated to dryness *in vacuo*; yield 15.0 g. (67%) of 3,5-di-*O*-benzoyl-D-xylose as an oil which did not crystallize. This compound darkens with gradual decomposition on standing and should be used within 1 day of preparation in the next step.

To a solution of 10.5 g. of the preceding compound in 175 cc. of chloroform was added 5.7 cc. of reagent pyridine and 9.1 cc. of benzoyl chloride. After 18 hr. in a stoppered flask the solution was washed with excess aqueous sodium bicarbonate, then dried with magnesium sulfate. Evaporation to dryness *in vacuo* left 19.5 g. of a semi-solid which was recrystallized by solution in 39 cc. of hot ethyl acetate and addition of 39 cc. of alcohol followed by chilling; yield 6.7 g. (35%) of α-D-xylofuranose tetra-*O*-benzoate, m.p. 154–158°, suitable for the next step. Recrystallization from ethyl acetate gave white crystals of constant m.p. 160.5–161°, $[\alpha]^{24}D$ + 162° (2.2% in $CHCl_3$). Fletcher has recorded $[\alpha]D$ +170° ($CHCl_3$) and m.p. 165–166°.

Anal. Calcd. for $C_{33}H_{26}O_9$: C, 70.0; H, 4.63. Found: C, 70.3; H, 4.88.

The over-all yield from D-xylose was 20.4%. Procedure A is considered the method of choice since the over-all yields were not only higher, but more consistent than in method B.

2-Methylmercapto-6-dimethylamino-9-β-D-xylofuranosylpurine (V).—The crude II[6] (32.6 g.) from 40 g. of α-D-xylofuranose tetra-*O*-benzoate was dissolved in 32.6 cc. of acetyl chloride with warming. To this solution cooled in an ice-bath was added 300 cc. of dry ether freshly saturated with hydrogen chloride at 0°. The solution was allowed to stand at −3° for 15 days[19] protected from moisture, then it was evaporated to dryness *in vacuo* (bath 50°). The evaporation was repeated with three 75-cc. portions of toluene. The residual chloro sugar III was dissolved in 100 cc. of xylene and added to a hot, stirred mixture of 34.2 g. of Celite (diatomaceous earth), 31.3 g. of chloromercuri-2-methylmercapto-6-dimethylaminopurine[8] and 700 cc. of xylene which had previously been freed of traces of water by distillation of 75 cc. of solvent. After being refluxed with stirring for 75 minutes, the hot mixture was filtered and the filter cake washed with three 65-cc. portions of hot toluene. The combined filtrate and washings were evaporated to dryness *in vacuo*. The filter cake was further washed with five 50-cc. portions of hot chloroform. The preceding residue from the xylene solution was dissolved in the combined chloroform solutions. After being washed with 300 cc. of 30% aqueous potassium iodide, then with water, the chloroform solution was dried with magnesium sulfate and clarified with Norit. Evaporation to dryness *in vacuo* left 40 g. (87%) from α-D-xylofuranose tetra-*O*-benzoate) of VI, which had a maximum purity of 75% since it had ε13,600 at 282 mμ in the ultraviolet.

To a hot mixture of the preceding 40 g. of VI and 700 cc. of methanol was added 12.6 cc. of 1 *N* methanolic sodium methoxide. The solution remained *p*H > 10[20] after a 35-minute reflux, solution being complete in 10 minutes. After being neutralized with 0.5 cc. of glacial acetic acid, the solution was evaporated to dryness *in vacuo*. Distillation of three 100-cc. portions of water *in vacuo* from the residue removed methyl benzoate. The residue was evaporated with some absolute alcohol to remove traces of water, then extracted with 100 cc. of boiling chloroform. The solution, filtered from inorganic salts, was further dried with magnesium sulfate and clarified with Norit. Evaporation to dryness *in vacuo* left 16.7 g. (69%) of tan glass with λ_{max}^{alc} 248 (ε 21,400), 287 mμ (ε 14,700). The ultraviolet absorption shows a purity of 87% thus giving an over-all yield of V from α-D-xylofuranose tetra-*O*-benzoate of 60%. This material was used for further work without further purification.

In a pilot run where the hydrogen chloride–ether reaction was allowed to proceed for 5.5 days, the over-all yield of V was 34% of product with λ_{max}^{alc} 248 (ε 24,000), 288 mμ (ε 16,500). The ultraviolet data shows a purity of 97%.

Anal. Calcd. for $C_{13}H_{19}N_5O_4S$: C, 45.8; H, 5.61; N, 20.6. Found: C, 46.2; H, 5.95; N, 20.7.

This compound could be obtained as nearly white crystals, m.p. 184–185°, from absolute alcohol–heptane, but it crystallized poorly; λ_{max}^{KBr} 2.95 μ (OH); 6.19 μ (C=N); 9.10, 9.46 (OH and C–O–C).

Anal. Found: C, 46.1; H, 5.68.

6-Dimethylamino-7-(and 9)-β-D-xylofuranosylpurine. (A).—A warm solution of 3.0 g. of α-D-xylofuranose tetra-*O*-benzoate in 6 cc. of ethylene dichloride was quickly cooled to about 30° and treated with 15 cc. of 30% hydrogen bromide in acetic acid.[6] After 30 minutes in a stoppered flask the solution was evaporated to a sirup *in vacuo* (bath 60–70°). The evaporation was repeated with two 9-cc. portions of xylene to remove traces of acetic acid. The residual crude 2,3,5-tri-*O*-benzoyl-D-xylofuranosyl bromide was condensed with 2.1 g. of chloromercuri-6-dimethyl aminopurine[8] and debenzoylated as described for V. The aqueous solution remaining after removal of methyl benzoate by distillation *in vacuo* was continuously extracted with chloroform for 18 hr. when extraction of solids was complete. The chloroform solution was evaporated to dryness *in vacuo*. Crystallization from acetone gave 42 mg. (2.7%) of 6-dimethylamino-7-β-D-xylofuranosylpurine, m.p. 196–199° dec. Recrystallization from methanol afforded white crystals, m.p. 214–215° dec., $\lambda_{max}^{pH\ 1}$ 293 mμ (ε 20,700), $\lambda_{max}^{pH\ 7.14}$ 298 mμ (ε 16,500); λ_{max}^{nujol} 2.98 μ (OH), 6.14 μ (C=N), 9.20, 9.41 and 9.69 μ (OH and C–O–C).

Anal. Calcd. for $C_{12}H_{12}N_6O_4$: N, 23.4. Found: N, 23.4.

Evaporation of the mother liquor from the 42 mg. left 400 mg. of a glass which had the ultraviolet spectra expected for a 9-glycoside of 6-dimethylaminopurine,[8] but had a purity of only 33% indicating a yield of 8.4%. This material was not further purified.

(B).—To a solution of 1.00 g. of V (87% pure) in 100 cc. of absolute alcohol was added about 12 g. of desulfurizing Raney nickel.[3] The solution was refluxed for 1 hr. when desulfurization was complete as shown by a ratio[9] of 3.4 for the ultraviolet absorption at 275/250 mμ compared to a ratio of 0.69 at the start. The hot solution was filtered through Celite and the filter cake washed with 50 cc. of boiling alcohol. The combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was dissolved in 3 cc. of acetone and clarified by filtration through Celite. Evaporation to dryness *in vacuo* left 232 mg. (27%) of 6-dimethylamino-9-β-D-xylofuranosylpurine as a glass with λ_{max}^{KBr} 2.97 μ (OH); 6.19 μ (C=N), 9.21, 9.47 μ (OH and C–O–C); λ_{max}^{alc} 274 mμ (ε 15,800). The ultraviolet data shows a purity of 85%. Acceptable analytical values could not be obtained for this compound although the nitrogen value shows a purity of 92%.

Anal. Calcd. for $C_{12}H_{17}N_5O_4$: C, 48.8; H, 5.80; N, 23.7. Found: C, 49.3; H, 6.41; N, 21.8.

2-Methylmercapto-6-dimethylamino-9-(3',5'-*O*-isopropylidene-β-D-xylofuranosyl)-purine (IV). (A).—To a solution of 260 mg. of V in 12 cc. of acetone was added 1.0 g. of anhydrous copper sulfate followed by a cold solution of 0.2 cc. of 96% sulfuric acid in 2 cc. of acetone. The latter addition caused the copper sulfate to become slightly gummy. The mixture was shaken for 2 hr., the lumps changing to a fine powder in about 20 minutes. The filtered solution was poured into 40 cc. of 5% aqueous sodium carbonate and extracted with chloroform (5 × 10 cc.). The combined extracts were washed with 70 cc. of water, dried with magnesium sulfate and evaporated to dryness *in vacuo* leaving 190 mg. (66%) of crude product. Crystallization from ethyl acetate–heptane gave 115 mg. (40%) of white crystals, m.p. 138–139° (gas). Further recrystallization from the same solvents did not change the m.p.; λ_{max}^{KBr} 2.92 μ (OH), 6.23 μ (C=N), 9.05, 9.23, 9.52, 9.74 μ (C–O–C and OH); $[\alpha]^{25}D$ −27° (1.9% in $CHCl_3$).

(19) In other runs 8–15 days gave the same over-all yield of V, but 3 days reaction gave considerably less yield of V.

(20) If the solution does not remain with *p*H > 10 when spotted on moist indicator paper, additional 6.3-cc. portions of 1 *N* sodium methoxide should be added until the solution remains with *p*H >10 for 25 minutes at the b.p.

Anal. Calcd. for $C_{16}H_{23}N_5O_4S$: C, 50.4; H, 6.07; N, 18.4. Found: C, 50.2; H, 6.29; N, 18.6.

This procedure gave variable yields of 49–66% crude and 27–40% crystalline.

(B).—To a solution of 9.6 g. of V in 440 cc. of reagent acetone was added 37 g. of anhydrous copper sulfate. Then with stirring a solution of 22.6 cc. of ethanesulfonic acid in 192 cc. of reagent acetone was added dropwise at a rapid rate (no heat was evolved). During the first part of the addition a sludge formed which soon disintegrated to a fine powder. The mixture was stirred an additional 20 minutes, filtered and the solids washed with acetone (2 × 200 cc.). The combined filtrate and washings were added to 1480 cc. of ice-cold 5% aqueous sodium carbonate, then extracted with chloroform (3 × 175 cc.). The combined extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving 9.6 g. (90%) of crude IV suitable for the next step.

In a similar preparation only about 1/3 of the product could be crystallized. Much better over-all yields of crystalline VII were obtained if the crude product IV was used for next step without further purification.

2-Methylmercapto-6-dimethylamino-9-(2′-*O*-mesyl-3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)-purine (VII). (A).—To an ice-cold solution of 100 mg. of crystalline IV in 1 cc. of reagent pyridine was added 0.036 cc. of methanesulfonyl chloride. The solution was allowed to stand in a stoppered flask for 65 hr. Dilution with 7 cc. of water immediately precipitated a crystalline solid which was collected and washed with water; yield 106 mg. (88%), m.p. 187–190° with previous shrinking. Recrystallization from absolute alcohol afforded white crystals, m.p. 204–205°, $[\alpha]^{25}D$ −25° (1.5% in $CHCl_3$).

Anal. Calcd. for $C_{17}H_{25}N_5O_6S_2$: C, 44.4; H, 5.49; N, 15.3. Found: C, 44.6; H, 5.64; N, 15.5.

Shorter reaction times gave lower yields, for example, 68% in 1 day.

(B).—To a solution of 9.6 g. of crude IV (prepared by method B) in 96 cc. of reagent pyridine was added 2.93 cc. of methanesulfonyl chloride dropwise with ice-cooling. After 65 hr. in a stoppered flask, the solution was poured into 600 cc. of water. The buff-colored crystals were collected on a filter and washed with water; yield 9.25 g. (80% or 72% based on V), m.p. 196–199°. The over-all yields of VII from V in other runs were 68–72%.

2-Methylmercapto-6-dimethylamino-9-(2′-*O*-mesyl-β-D-xylofuranosyl)-purine (VIII).—A mixture of 9.25 g. of VII and 93 cc. of 70% aqueous acetic acid was stirred at 50–52° for 4.5 hours, solution being complete in 2.5 hr. The solution was evaporated to dryness *in vacuo* leaving a sirup. The residue was dissolved in toluene by the addition of sufficient absolute alcohol to cause solution, then evaporated to dryness *in vacuo*. This evaporation was repeated twice more leaving a semi-solid which solidified completely when triturated with 30 cc. of hot toluene. The product was collected by filtration and washed with toluene; yield 7.5 g. (89%), m.p. 171–172°. Recrystallization from ethyl acetate–heptane gave white crystals, m.p. 174–175°, $[\alpha]^{25}D$ −26.5° (1% in $CHCl_3$).

Anal. Calcd. for $C_{14}H_{21}N_5O_6S_2$: C, 40.1; H, 5.05; N, 16.7. Found: C, 39.8; H, 5.35; N, 16.4.

2-Methylmercapto-6-dimethylamino-9-(2′,3′-anhydro-β-D-lyxofuranosyl)-purine (IX).—To a hot solution of 6.95 g. of VIII in 60 cc. of methanol was added a solution of 1.35 g. of sodium methoxide (Matheson) in 13 cc. of methanol. The solution was refluxed and stirred for 10 minutes, crystals separating at the b.p. The cooled mixture was filtered. The solids were washed with 10 cc. of methanol, then several times with water to remove sodium methanesulfonate leaving 4.6 g. (86%) of product, m.p. 211–212°. The combined filtrate and methanol wash were evaporated to dryness *in vacuo*. Trituration of the residue with water afforded an additional 125 mg. (total 88%) of product, m.p. 208–210°. Recrystallization of a sample of the main fraction from absolute alcohol gave white crystals of unchanged m.p.; $[\alpha]^{24}D$ −43° (2% in pyridine); λ_{max}^{nujol} 2.99 μ (OH); 6.15, 6.24 μ (C=N); 9.07, 9.32, 9.40 (OH and C–O–C).

Anal. Calcd. for $C_{13}H_{17}N_5O_3S$: C, 48.3; H, 5.30; N, 21.7. Found: C, 48.5; H, 5.62; N, 21.4.

2-Methylmercapto-6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-arabinofuranosyl)-purine (XI).—A mixture of 1.00 g. of IX and 40 cc. of methanol saturated with ammonia at 0° was heated in a steel-bomb at 100° for 2 hr. Evaporation of the solution to dryness *in vacuo* and heating the residue with 15 cc. of water gave 825 mg. (79%) of product, m.p. 191–192°.

In a pilot run where the reaction time was 4 hours the yield dropped to 58%, m.p. 188–189°. Recrystallization from water gave white crystals, m.p. 193–195°, $[\alpha]^{25}D$ −3.8° (1.5% in pyridine); λ_{max}^{KBr} 2.92 μ (OH), 3.07 μ (NH), 6.26 μ (C=N), 9.07, 9.50 μ (OH and C–O–C).

Anal. Calcd. for $C_{13}H_{20}N_6O_3S$: C, 45.9; H, 5.92; N, 24.7. Found: C, 45.5; H, 5.91; N, 24.5.

A reaction time of 17 hr. apparently caused complete breakdown of the nucleoside since no XI could be isolated.

3-Amino-D-arabinose Hydrochloride (XII).—A solution of 100 mg. of XI in 3 cc. of 1% hydrochloric acid and 0.06 cc. of 12 *N* hydrochloric acid was refluxed for 3 hr., then evaporated to dryness *in vacuo* (bath 50°). The residue was dissolved in 2 cc. of water, filtered from 13 mg. of 2-methylmercapto-6-dimethylaminopurine, and again evaporated to dryness as before. The gummy residue was covered with about 1 cc. of glacial acetic acid and then 2 drops of water were added causing solution. The solution soon deposited white crystals, m.p. 164° dec., which gave a positive Benedict test and a negative ninhydrin test in 3% sodium hydroxide. The infrared red spectrum and rotation were identical with an authentic sample.[2]

6-Dimethylamino-9-(3′-amino-3′-deoxy-β-D-arabinofuranosyl)-purine (X).—To a solution of 770 mg. of XI in 90 cc. of methyl Cellosolve was added 1 teaspoon of desulfurizing Raney nickel.[3] The mixture was stirred on the steam-bath for 1 hr., then processed according to the procedure described earlier in the experimental for desulfurization of V. Evaporation of the solution *in vacuo* left 339 mg. of a glass. Crystallization from ethyl acetate–absolute alcohol–heptane gave white crystals, m.p. 112–114°; yield 157 mg. (24%). Drying at 110° in high vacuum raised the m.p. to 116–118°; $\lambda_{max}^{alc.}$ 274 mμ (ε17,800), indicating 95% purity; λ_{max}^{KBr} 3.00, 3.05, 3.07 μ (OH, NH), 6.20 μ (C=N), 9.25, 9.56 μ (OH and C–O–C). The nitrogen analysis also showed 95% purity.

Anal. Calcd. for $C_{12}H_{18}N_6O_3$: C, 49.0; H, 6.17; N, 28.6. Found: C, 49.3; H, 6.45; N, 27.2.

2-Methylmercapto-6-dimethylamino-9-(3′-acetamido-3′-deoxy-β-D-arabinofuranosyl)-purine (XIII).—To a solution of 800 mg. of XI in 4 cc. of 50% acetic acid was added 0.33 cc. of acetic anhydride. After standing for 8 minutes, the solution was evaporated to dryness *in vacuo*. Trituration of the glassy residue with 5 cc. of hot ethyl acetate caused crystallization. The cooled mixture was filtered and the product washed with cold ethyl acetate; yield 793 mg. (88%), m.p. 191–193°.

In a pilot run the yield was 79% (88 mg.), m.p. 191–192°. A mixture with XI depressed the m.p. to 160–163°. Recrystallization from hot ethyl acetate by the addition of sufficient absolute alcohol to cause solution followed by cooling gave white crystals, m.p. 193–195°, $[\alpha]^{25}D$ +13° (2.3% in pyridine); λ_{max}^{KBr} 2.99, 3.16 μ (OH, NH), 6.02, 6.42 μ (amide), 6.25 μ (C=N), 9.25, 9.53, 9.72 μ (OH and C–O–C).

Anal. Calcd. for $C_{15}H_{22}N_6O_4S$: C, 47.1; H, 5.80; N, 22.0. Found: C, 47.5; H, 5.93; N, 21.6.

When the concentration of XI was reduced to 25 cc./g. the reaction proceeded poorly to give a mixture of XI and XIII, presumably by competitive hydrolysis of the acetic anhydride in higher dilution.

2-Methylmercapto-6-dimethylamino-9-(2′-*O*-mesyl-3′-acetamido-3′-deoxy-5′-*O*-trityl-β-D-arabinofuranosyl)-purine (XIV).—A solution of 793 mg. of XIII and 638 mg. of triphenylmethyl chloride (10% excess) in 4 cc. of reagent pyridine was heated in a bath at 51° in a flask protected by a drying tube for 72 hr. The cooled solution was diluted with 15 cc. of chloroform, then 30 cc. of water. Solid sodium bicarbonate was added until the mixture was slightly basic. The separated organic phase was dried with magnesium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in toluene and the evaporation repeated leaving 1.33 g. (102%) of product as a glass. This 5′-trityl derivative of XIII is contaminated with about 13% triphenylcarbinol as shown by the 11.9% nitrogen content.

To a solution of 1.12 g. of the preceding compound in 11.2 cc. of reagent pyridine was added 0.34 cc. of methanesul-

fonyl chloride. The solution was heated in a bath at 51° in a flask protected by a drying tube for 22 hr. The solution was diluted with 55 cc. of water and extracted with chloroform (3 × 20 cc.). The combined extracts, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness *in vacuo*. The residue was dissolved in 10 cc. of toluene and the evaporation repeated to remove pyridine; yield 1.09 g. (87%) of a glass which contained some triphenylcarbinol.

2-Methylmercapto-6-dimethylamino-9-(2′-*O*-mesyl-3′-acetamido-3′-deoxy-β-D-arabinofuranosyl)-purine (XVII).—A mixture of 790 mg. of XIV and 15.8 cc. of 80% acetic acid was heated for 22 minutes on the steam-bath, solution being complete in 2 minutes. The solution was diluted with 100 cc. of hot water and extracted with two 100-cc. portions of hot heptane to remove triphenylcarbinol. The aqueous solution was filtered from a small amount of solid, then evaporated to dryness *in vacuo* leaving 390 mg. (75%) of a glass with λ_{max}^{KBr} 3.0 μ (OH, NH), 6.00, 6.50 μ (amide), 6.23 μ (C=N), 8.33 μ (sulfonate) and no appreciable absorption at 14.3 μ (monosubstituted phenyl from the triphenylmethyl group). The compound was not pure.

2-Methylmercapto-6-dimethylamino-9-(2′,5′-di-*O*-acetyl-3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (XVI).—A mixture of 388 mg. of XVII, 282 mg. of anhydrous sodium acetate and 4 cc. of methyl Cellosolve containing 5% water was refluxed for 23 hours, then evaporated to dryness *in vacuo*. The residue was heated on the steam-bath with 4 cc. of pyridine and 4 cc. of acetic anhydride for 1 hr. The mixture, dilute with 25 cc. of water, was extracted with chloroform (4 × 15 cc.). The combined extracts were dried with magnesium sulfate and evaporated to dryness *in vacuo* leaving 280 mg. (71%) of product as a glass.

In a pilot run the yield was 68% (80 mg.). This compound had λ_{max}^{KBr} 3.02 μ (NH), 5.71, 8.17 μ (O-acetate), 5.98, 6.52 μ (amide), 6.25 μ (C=N) and no sulfonate absorption at 8.33 μ. The compound is probably contaminated with some triacetate of XI.

Anal. Calcd. for $C_{19}H_{26}N_6O_6S$: C, 48.9; H, 5.62; N, 18.0. Found: C, 48.6; H, 5.79; N, 17.7.

6-Dimethylamino-9-(2′,5′-di-*O*-acetyl-3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (XV).—To a solution of 280 mg. of XVI in 50 cc. of methyl Cellosolve was added about 1.5 g. of desulfurizing Raney nickel.[3] The mixture was stirred on the steam-bath for 1 hour, then filtered hot through Celite using hot methyl Cellosolve for washing. Evaporation to dryness *in vacuo* left 135 mg. of a glass. Crystallization from ethyl acetate gave 64 mg. (24%) of white crystals, m.p. 182–183°. Recrystallization from ethyl acetate afforded white crystals, m.p. 188–189°. The product was identical with authentic XV[1] as shown by mixed m.p. and infrared spectra.

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Puromycin. Synthetic Studies. XIV. Use of the N-Phthalyl Blocking Group for Synthesis of Aminonucleosides

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The preparation of 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-ribofuranosyl chloride (Vb) by proper degradation of the antibiotic puromycin and by total synthesis from D-xylose is described. The advantage and disadvantages of this phthalimido sugar compared to the corresponding acetamido sugar for the synthesis of biologically active aminonucleosides are shown.

One of the key steps in the total synthesis of puromycin[1] is the conversion of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranose to 2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranosyl chloride–titanium chloride complex, which is condensed, without isolation, with chloromercuri-2-methylmercapto-6-dimethylaminopurine to form the desired nucleoside. Although this method was satisfactory for synthesis of nucleosides from 2-methylmercapto-6-dimethylaminopurine, sometimes in other cases undesirable anomerization, isomerization or decomposition products were also formed. For example, this method with chloromercuri-6-benzamidopurine gave the expected 9-β-nucleoside, but also produced the corresponding α-nucleoside.[2] Reaction of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-acetamino-3-deoxy-D-arabinofuranose with titanium tetrachloride followed by condensation with 2-methylmercapto-6-dimethylaminopurine gave, after desulfurization and debenzoylation, not only the expected 6-dimethylamino-9-(3′-acetamino-3′-deoxy-α-D-arabinofuranosyl)-purine, but some of the corresponding α-D-ribofuranosyl-purine.[3]

To avoid these difficulties it would be necessary to prepare the chloroacyl aminosugar by a method not involving titanium tetrachloride. The usual method for preparation of chloroacyl furanosides, namely, hydrogen chloride in ether, failed with the N-acetyl blocking group due to the weakly basic properties of the acetamido group.[1] By use of the N-phthalyl blocking group, the resultant nonbasic phthalimido group should allow preparation of a chlorosugar such as V by use of the elegant ether–hydrogen chloride method,[4] thus, completely avoiding the use of titanium tetrachloride.

Another objection to the N-acetyl blocking group is that it can be base hydrolyzed to the amine only when the acetamido group is adjacent to a *cis*-hydroxyl.[5,6] This difficulty is again surmounted by the N-phthalyl blocking group which can be removed selectively by hydrazine[7] to form aminonucleosides.

In order to establish the usefulness of the N-phthalyl blocking group for synthesis of aminonucleosides, four distinct problems could be envisioned. These were investigated in order of de-

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