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 $C_{19}H_{26}N_6O_8S$: 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.

PEARL RIVER, NEW YORK

[CONTRIBUTION FROM THE MEDICINAL CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID COMPANY]

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

BY B. R. BAKER, J. P. JOSEPH AND R. E. SCHAUB

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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 non-basic 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-

(1) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 12 (1955), Paper IX of this series.

(2) B. R. Baker, R. E. Schaub and H. M. Kissman, *ibid*, **77**, 5911 (1955), paper XV of this series.

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

(4) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 967 (1948).

(5) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954), paper V of this series.

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

(7) H. Ing and R. Manske, *J. Chem. Soc.*, 2348 (1926).

creasing availability of compounds for study rather than on the basis of continuity as follows.

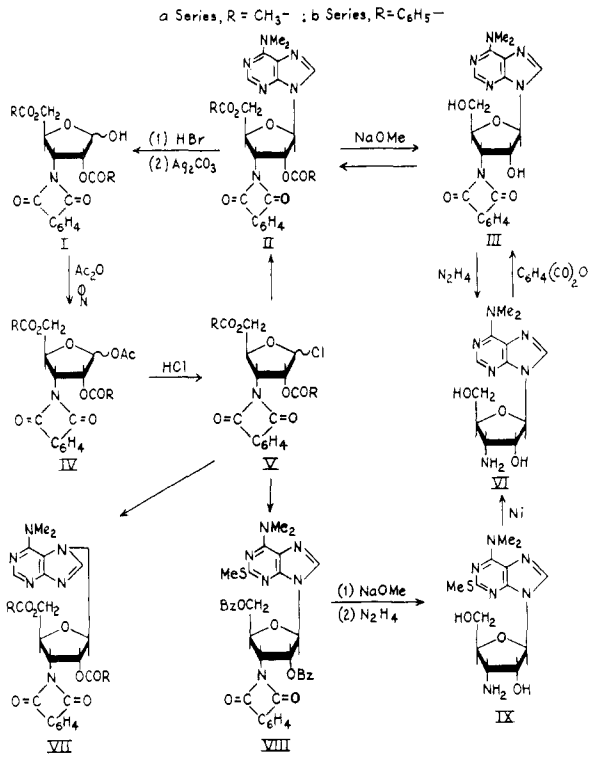
The first problem was to establish whether or not the blocking groups from an N-phthalyl-2′,5′-di-*O*-acyl aminonucleoside II could be removed without disruption of the nucleoside, since II would be the type of a blocked nucleoside obtained as an intermediate during coupling of a blocked halo sugar with a purine. Treatment of 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (VI),[6] "the aminonucleoside," with phthalic anhydride in boiling dimethylformamide gave a 92% yield of crystalline N-phthalyl aminonucleoside III. The latter on benzoylation with benzoyl chloride in pyridine at 100° gave the dibenzoate IIb, isolated as a crystalline hemi-hydrate in 95% yield. Similarly acetylation of III with acetic anhydride in pyridine at 100° gave the crystalline diacetate IIa in 95% yield. The preparation of IIa could be further simplified by reaction of the aminonucleoside VI with phthalic anhydride in pyridine to give a solution of a phthalamic acid which, by addition of acetic anhydride, was ring closed to the phthalimido derivative and *O*-acetylated simultaneously to form IIa in 97% over-all yield from VI.

Debenzoylation of the hydrate of the N-phthalyl di-*O*-benzoyl aminonucleoside IIb with 1 mole of methanolic sodium methoxide proceeded with concurrent ring opening of the phthalimido group to a sodium phthalamate derivative.[8] When the crude sodium phthalamate was refluxed in dimethylformamide containing 2 moles of acetic acid, ring closure back to the N-phthalyl aminonucleoside III took place in 51% over-all yield from IIb. This difficulty of ring opening of the phthalimido group was partially avoided by removal of water of crystallization by treatment with acetic anhydride prior to debenzoylation of IIb, the N-phthalyl aminonucleoside III being obtained directly in 65% yield. By cyclization of the sodium phthalamate in the mother liquor an additional 27% of III was obtained. Drying of IIb by distillation with toluene gave 48% of III directly and an additional 38% was obtained by refluxing in dimethylformamide.

Similarly, the N-phthalyl di-*O*-acetyl aminonucleoside IIa was catalytically deacetylated under as strictly anhydrous conditions as possible in methanol–chloroform containing 30 mole % of sodium methoxide to 65% of III. From the mother liquor could be isolated 13% of the corresponding crystalline phthalamic acid. If no effort was made to keep the reaction strictly anhydrous, 1 mole of water was gradually absorbed over 1.5 hours in boiling methanol since a total of slightly more than 1 mole of sodium methoxide was necessary to keep the reaction mixture basic. The intermediate phthalamate derivative was then recyclized by treatment with ethyl chlorocarbonate and triethylamine in dimethylformamide[5] giving III in 55% yield.

The aminonucleoside VI could be regenerated from its N-phthalyl derivative III in 85% yield by

(8) It has been well known for many years that the phthalimido group is rapidly opened to a sodium phthalamate with sodium hydroxide; *cf.* R. Andreasch, *Monatsh.*, **25**, 774 (1904); J. v. Braun, *Ber.*, **37**, 3586 (1904); S. Gabriel and J. Wiener, *ibid.*, **21**, 2670 (1888); S. Gabriel and T. Posner, *ibid.*, **27**, 1043 (1894); L. Reese, *Ann.*, **242**, 1 (1887).



the action of hydrazine[7] in methyl Cellosolve followed by elimination of the phthalyl group as phthalhydrazide by treatment with acetic acid.[6]

The second problem was cleavage of the blocked aminonucleoside II to a blocked aminosugar (I or IV) suitable for synthesis of other nucleosides. Attempted hydrolysis of 6-dimethylamino-9-(2′,5′-di-*O*-benzoyl-3′-phthalimido-3′-deoxy-β-D-ribofuranosyl)-purine (IIb) with the boiling two-phase system 2 *N* hydrochloric acid–ethylene dichloride, conditions successfully used with the corresponding 3′-acetamido nucleoside,[1] failed since most of the IIb was recovered unchanged and no reducing sugar was formed. This difficulty was surmounted by cleavage of the nucleoside with 30% hydrogen bromide in acetic acid, followed by hydrolysis of the resultant bromo sugar with water and silver carbonate,[9] 2,5-di-*O*-benzoyl-3-phthalimido-D-ribofuranose (Ib) being formed in 83% yield as a strongly reducing glass. Acetylation in pyridine gave a mixture of anomeric 1-*O*-acetates IVb, from which one anomer, presumably the α, was crystallized in 57% yield. Similarly, hydrogen bromide cleavage of IIa followed by treatment with silver carbonate and acetylation gave a 63% over-all yield of the crystalline anomer of 1,2,5-tri-*O*-acetyl-3-phthalimido-3-deoxy-D-ribofuranose (IVa).

Direct acid-catalyzed acetolysis of the N-phthalyl di-*O*-acetyl aminonucleoside IIa to the crystalline triacetate IVa, proceeded in an optimum yield of 82%,[10] the ratios of sulfuric acid, acetic acid and

(9) R. K. Ness, H. W. Diehl and H. G. Fletcher, THIS JOURNAL, **75**, 2624 (1953); **76**, 763 (1954), have used this sequence of reagents for conversion of methyl 2,3,5-tri-*O*-benzoylpentofuranosides to 2,3,5-tri-*O*-benzoylpentoses.

(10) About 1% yield of 1,1,2,4,5-pento-*O*-acetyl-3-phthalimido-3-deoxy-D-ribose m.p. 169°, was obtained as a by-product in this acetolysis reaction.

acetic anhydride[11] were varied. Similarly, acetolysis of the di-*O*-benzoyl nucleoside, IIb, to the crystalline anomer of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-ribofuranose (IVb) proceeded in 77% yield. Equilibration[11] of the anomeric 1-*O*-acetate in the mother liquor gave an additional 9% of IVb.

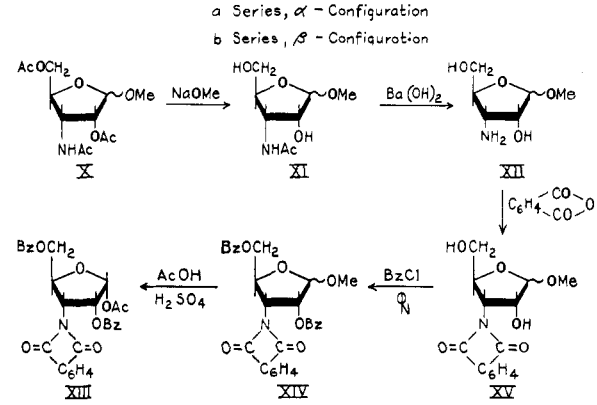
The third problem was resynthesis of nucleosides from the blocked 3-phthalimido-D-ribofuranoses, IV. As expected the phthalimido group did not form an insoluble hydrochloride with ethereal hydrogen chloride, the *O*-triacetate IVa being converted to a crude chloro sugar Va in 71–75% yield. This chloroaceto sugar condensed poorly with chloromercuri - 6 - dimethylaminopurine. The blocked nucleoside IIa could not be crystallized, but *O*-deacylation gave 5–10% over-all yields of III. Since 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride has been shown to give better yields of a nucleoside than 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride,[12] the use of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-ribofuranose (IVb) was investigated. Reaction of IVb with ethereal hydrogen chloride proceeded smoothly to the stable, crystalline ribofuranosyl chloride, Vb, m.p. 160–162°, in 98% yield, which had the β-configuration.[13] Condensation of the crystalline Vb with chloromercuri-6-dimethylaminopurine[14] in boiling xylene proceeded smoothly forming the crystalline blocked 9-β-nucleoside IIb in 47% yield, m.p. 128–130°. The latter gave no depression in m.p. when mixed with IIb (m.p. 130–132°) prepared from III.[15] However, the ultraviolet spectrum showed contamination of the resynthesized IIb with the 7-nucleoside VIIb since along with the major peak at 275 mμ there was a large inflection at 295 mμ.[16] From the relative molecular extinctions at 275 and 295 mμ,[14] it was estimated that the 9-nucleoside IIb was contaminated with 18–26% of the 7-nucleoside VIIb. These two nucleosides did not separate on recrystallization, but the pure 9-isomer could be isolated by partition chromatography on Celite[12] using the system water–methanol–ethyl acetate–petroleum ether (90–100°) in the ratio of 1:25:5:25. The 9-nucleoside IIb was eluted first and no attempt was made to isolate the 7-nucleoside VIIb. However, the process gave poor recoveries—due in part to the insolubility of the compounds in the solvent system—and was not considered practical.

Since chloromercuri-6-dimethylaminopurine gave a mixture of the 7- and 9-nucleoside, IIb and VIIb, the condensation of 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-ribofuranosyl chloride (Vb) with chloromercuri-2-methylmercapto-6-dimethylaminopurine was investigated since in previous work the latter had orientated an incoming sugar only to the 9-position.[1,5,12,14] The crude 2-methylmercapto-6-dimethylamino-9-(2′,5′-di-*O*-benzoyl-3′-phthalimido-3′-deoxy-β-D-ribofuranosyl)-purine (VIII) could not be obtained crystalline. Desulfurization with Raney nickel did not give the expected IIb. A test run with Raney nickel showed that the IIb was attacked by the reagent in some deep-seated manner since IIb could not be recovered even when the product was worked up with the assumption that either partial debenzoylation or ring opening to a phthalmic acid had taken place.

Debenzoylation and dephthalation of the crude blocked methylmercapto nucleoside gave 13% over-all yield of crystalline 2-methylmercapto-6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (IX) when the product was isolated by absorption on a carboxylic acid type resin.[2] Desulfurization afforded the aminonucleoside VI from puromycin in 32% yield, thus proving the β-configuration of IX.[15]

It is clear that for synthesis of aminonucleosides with a 6-dimethylaminopurine moiety the earlier method using the N-acetyl blocking group with titanium tetrachloride[1] is preferred. However, for synthesis of aminonucleosides from adenine[2] the N-phthalyl blocking group method is preferred. Thus it appears that the two methods can be complementary.

The fourth and final problem for completion of the use of the N-phthalyl blocking group was to prepare 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-ribofuranose (XIII) by total synthesis. Catalytic deacetylation of methyl 2,5-di-*O*-acetyl-3-acetamido-3-deoxy-β-D-ribofuranoside (Xb), previously synthesized from D-xylose,[17] gave crude XIb which was hydrolyzed with hot barium hydroxide to methyl 3-amino-3-deoxy-β-D-ribofuranoside (XIIb). The latter was not purified, but reacted directly with phthalic anhydride in dimethylforma-



(11) The use of these reagents for acetolysis of methyl glycoside acetates to fully acetylated glycosides has been described: *cf.* N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **63**, 1727 (1941); **65**, 740 (1943); B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954), paper III of this series.

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

(13) When this chloro sugar Vb in dioxane was allowed to react with 2 moles of water the initial specific rotation gradually changed from +75° to +121° over a period of 24 hours, thus showing the β-configuration of Vb; *cf.* H. G. Fletcher, Jr., and R. K. Ness, THIS JOURNAL, **76**, 760 (1954). In 18:7 dioxane–water the hydrolysis was complete in less time than that required to measure the initial rotation (three minutes), a specified rotation of +116° being observed which did not change further in 3 hours.

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

(15) This formation of a 1,2-*trans*-nucleoside IIb from a 1,2-*trans*-halosugar Vb verifies the previous prediction[9] that double Walden inversion should occur during nucleoside formation from a 1,2-*trans*-halosugar.

(16) 7-Glycosides of 6-dimethylaminopurine have λ_{max} 297 mμ whereas the corresponding 9-nucleosides have λ_{max} 275 mμ.[14]

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

mide to give the crystalline N-phthalyl derivative XVb in 49% over-all yield from Xb. Benzoylation gave the non-crystalline dibenzoate XIVb which was subjected to acetolysis with acetic anhydride–acetic acid–sulfuric acid forming crystalline 1-*O*-acetyl-3,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-ribofuranose (XIII), identical with the same compound IVb obtained by degradation of puromycin and in 61% over-all yield from XV. Similarly XIII could be obtained from methyl 2,5-di-*O*-acetyl-3-acetamido-3-deoxy-α-D-ribofuranoside (Xa) in about the same over-all yield as in the β-series.

Acknowledgment.—The authors are grateful to L. Brancone and staff for the microanalyses, to W. Fulmor and staff for the rotations and spectrophotometric data and to C. Pidacks for the partition chromatography.

Experimental

6-Dimethylamino-9-(3′-phthalimido-3′-deoxy-β-D-ribofuranosyl)-purine (III). (A).—A mixture of 5.0 g. of the aminonucleoside VI,[6] 2.8 g. of phthalic anhydride and 25 cc. of dimethylformamide was refluxed for 45 minutes, solution being complete at the b.p. The cooled solution was poured into 250 cc. of water with stirring, the product was collected on a filter and washed with water; yield 6.6 g. (92%), m.p. 274–275° dec. Recrystallization of a similar preparation from alcohol gave white crystals, m.p. 276–277° dec., $[\alpha]^{25}D$ −186° (2% in pyr.); λ_{max}^{nuj} 3.00, 3.07 μ (OH); 5.63, 5.82 μ (phthalyl C=O); 6.23 μ (C=N).

Anal. Calcd. for $C_{20}H_{20}N_6O_5$: C, 56.6; H, 4.72; N, 19.8. Found: C, 56.8; H, 4.84; N, 19.4.

(B).—A solution of 350 mg. of IIb hydrate and 0.66 cc. of 1 *N* methanolic sodium methoxide in 10 cc. of methanol was refluxed for 30 minutes, then evaporated to dryness *in vacuo*. The residual sodium phthalamate derivative was refluxed in 3 cc. of dimethylformamide containing 0.66 cc. of glacial acetic acid for 30 minutes. The cooled solution was poured into a stirred mixture of 10 cc. of benzene and 30 cc. of water to remove methyl benzoate. The product was collected by filtration and washed with water and benzene; yield 120 mg. (51%), m.p. and mixed m.p. with preparation A 274–276° dec.

(C).—A solution of 1.00 g. of IIb hydrate in 5 cc. of acetic anhydride was heated on the steam-bath for 1 hour, then evaporated to dryness *in vacuo*. The residue was thrice evaporated with 5-cc. portions of xylene to remove traces of acetic anhydride leaving anhydrous IIb as a glass. The residue was dissolved in 2.5 cc. of reagent chloroform and 7.5 cc. of reagent methanol. It was necessary to add 0.73 cc. (40 mole %) of 1 *N* methanolic sodium methoxide over a period of several hours to keep the *p*H > 10 when spotted on moist indicator paper. After 18 hours more in a stoppered flask, the product was collected on a filter and washed with methanol; yield 430 mg. (65%), m.p. and mixed m.p. with preparation A, 277–278° dec.

The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 3 cc. of dimethylformamide containing 0.04 cc. of acetic acid and refluxed for 30 minutes. The cooled solution was poured into 30 cc. of water and 10 cc. of benzene giving an additional 175 mg. (27%) of product, m.p. and mixed m.p. with preparation A, 277–278° dec.

If the IIb was dehydrated by refluxing a toluene solution under a constant water separator until anhydrous, the solvent removed and the debenzoylation carried out as above 40 mole % of sodium methoxide was required. From the debenzoylation solution, 48% of product was obtained. Refluxing the residue from the mother liquor in dimethylformamide as above gave an additional 38% of III.

(D).—To a solution of 508 mg. of IIa (dried 8 hours at 11° in high vacuum over P_2O_5) in 1.5 cc. of methylene chloride was added 3.5 cc. of reagent methanol and 0.30 cc. of 1 *N* methanolic sodium methoxide. The solution was allowed to stand in a stoppered flask for 3 hours. The product was collected on a filter and washed with methylene chloride: yield 280 mg. (64%), m.p. 267–269° dec. The filtrate was evaporated to dryness *in vacuo*. The residue readily dissolved in 2 cc. of water. The solution was clarified by filtration and acidified with acetic acid. After standing overnight the white crystals of the phthalamic acid, **6-dimethylamino-9-(3′-(*o*-carboxybenzamido)-3′-deoxy-β-D-ribofuranosyl)-purine** were collected; yield 57 mg. (13%), m.p. 180–182° (gas), resolidifies and remelts at 277–278° dec. (the m.p. of III).

This same phthalamic acid was obtained by solution of 300 mg. of III in 2 cc. of warm 1 *N* sodium hydroxide followed by acidification to *p*H 4 with acetic acid and standing overnight to complete crystallization; yield 238 mg., m.p. 182–184° (gas) followed by resolidification. Recrystallization from methanol gave a methanol solvate, m.p. 175–178° (gas) followed by resolidification.

Anal. Calcd. for $C_{20}H_{22}N_6O_6 \cdot CH_3OH$: C, 53.2; H, 5.52; N, 17.7. Found: C, 53.6; H, 5.59; N, 17.9.

This compound showed absorption in the infrared at 2.93, 3.03, 3.13 μ (OH, NH), 5.77 μ (COOH), 5.98, 6.42 μ (amide) and 6.20 μ (C=N).

Regeneration of 6-Dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (VI) from III.—A solution of 1.00 g. of III in 6.3 cc. of methyl Cellosolve and 0.126 cc. of 100% hydrazine hydrate was heated on a steam-bath for 1.5 hours. (After 5 minutes solution was nearly complete, then another solid began to separate.) Then 1.26 cc. of acetic acid in 6.3 cc. of methyl Cellosolve was added and heating was continued for another 10 minutes. The mixture was evaporated to dryness *in vacuo*. The residue was treated with 5 cc. of water. The insoluble phthalhydrazide (335 mg., 88%) was removed by filtration and washed with water. The combined filtrate and washings were evaporated to dryness *in vacuo*. A solution of the residue in 5 cc. of alcohol was basified with 0.63 cc. of triethylamine. After being chilled at 3° for 2 hours, the mixture was filtered and the product washed with cold alcohol; yield 590 mg. (85%), m.p. 215–216°, identical with an authentic sample of VI.

6-Dimethylamino-9-(2′,5′-di-*O*-acetyl-3′-phthalimido-3′-deoxy-β-D-ribofuranosyl)-purine (IIa). (A).—A solution of 11.1 g. of III in 57 cc. of reagent pyridine and 57 cc. of acetic anhydride was heated on a steam-bath for 1 hour, then poured into 600 cc. of ice-water. A gum separated which soon solidified when the mixture was stirred. The product was collected by filtration, washed well with water, then with alcohol; yield 12.5 g. (95%), m.p. 188–190°. Recrystallization from alcohol gave white crystals, m.p. 190–192°, $[\alpha]^{25}D$ −118° (1.7% in $CHCl_3$); λ_{max}^{KBr} 5.60, 5.78 μ (phthalyl C=O); 5.68, 8.22 μ (acetate); 6.25 μ (C=N).

Anal. Calcd. for $C_{24}H_{24}N_6O_7$: C, 56.8; H, 4.77; N, 16.6. Found: C, 56.5; H, 4.60; N, 17.0, 17.0.

(B).—A solution of 10 g. of VI and 5.5 g. of phthalic anhydride in 72 cc. of reagent pyridine was heated on a steam-bath for 15 minutes after the temperature reached 90°. Then 72 cc. of acetic anhydride was added and the heating continued for 1 hour longer. Work-up as in A gave 16.7 g. (97%) of product, m.p. 188–191°.

6-Dimethylamino-9-(2′,5′-di-*O*-benzoyl-3′-phthalimido-3′-deoxy-β-D-ribofuranosyl)-purine (IIb).—To a solution of 18.4 g. of III in 184 cc. of reagent pyridine was added 20.2 cc. of benzoyl chloride. The solution was heated on a steam-bath, protected from moisture for 2 hours, then poured into 1 l. of ice-water. The mixture was extracted with chloroform. The combined chloroform extracts, washed with excess aqueous sodium bicarbonate, then water, were dried with anhydrous magnesium sulfate. Evaporation to dryness *in vacuo* left a gum which was dissolved in 100 cc. of boiling 3A alcohol, then cooled to 0°. The product was collected on a filter and washed with cold 3A alcohol, then ether; yield 26.3 g. (95%), m.p. 130–132° (gas). Recrystallization of a similar preparation from 3A alcohol gave large white plates, m.p. 130–132° (gas), $[\alpha]D$ −77° (1.3% in pyr.); λ_{max}^{KBr} 2.75 μ (H_2O); 5.62, 5.83 μ (phthalyl C=O); 5.73, 7.85 μ (benzoate); 6.23 μ (C=N); λ_{max}^{alc} 274 mμ (ε 23,600); $\lambda_{max}^{0.1\ N\ HCl}$ 268 mμ (ε 22,200). That the compound had no alcohol of crystallization was shown by lack of alkoxyl content.

Anal. Calcd. for $C_{34}H_{28}N_6O_7 \cdot {}^1/_2H_2O$: C, 63.7; H, 4.56; N, 13.1. Found: C, 63.7, 63.8; H, 4.73, 4.75; N, 13.2; EtO, 0.0.

2,5-Di-O-acetyl-3-phthalimido-3-deoxy-D-ribofuranose (Ia).—To a solution of 6.0 g. of IIa in 18 cc. of methylene

chloride was added 30 cc. of 30% hydrogen bromide. The solution was stirred magnetically protected from moisture for 3.5 hours. After 15 minutes 6-dimethylaminopurine dihydrobromide began to crystallize. The reaction mixture was poured into 150 cc. of ice-water and extracted with 100 cc. of methylene chloride in several portions. Evaporation of the aqueous solution to dryness *in vacuo* and trituration of the residue with acetone gave 3.5 g. (90%) of 6-dimethylaminopurine dihydrobromide, m.p. 255–258° dec. (This aqueous wash to remove the purine is more convenient than the direct filtration from the acetic acid reaction mixture due to the gelatinous nature of the precipitate.)

The combined methylene chloride extracts were washed with ice-water, excess aqueous sodium bicarbonate and ice-water. To the organic solution was added 60 cc. of acetone, 0.6 cc. of water and 6.0 g. of silver carbonate. After being stirred for 30 minutes, the mixture was filtered through a pad of Norit and Celite and the filtrate evaporated to dryness *in vacuo;* yield 3.5 g. (81%) of a gum which gave a strong Benedict test in dilute alcohol and had $[\alpha]^{25}D$ 0.0 ± 0.3° in chloroform. Ultraviolet absorption analysis showed that less than 3% IIa was present. The compound had λ_{max}^{KBr} 2.92 μ (OH); 5.60, 5.78 μ (phthalyl C=O), 5.71, 8.10 μ (acetate).

Anal. Calcd. for $C_{17}H_{17}NO_8$: C, 56.2, H, 4.71, N, 3.86. Found: C, 55.7, H, 4.68, N, 3.58.

Similarly, **2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-ribofuranose** (Ib) was obtained from IIb in 83% yield as a colorless glass which gave a positive Benedict test and had λ_{max}^{KBr} 2.85 μ (OH); 5.60, 5.83 μ (phthalyl C=O); 5.76, 7.85 μ (benzoate). Ultraviolet analysis showed that less than 3% of IIb was present.

Anal. Calcd. for $C_{27}H_{21}NO_8$: C, 66.5; H, 4.34; N, 2.87. Found: C, 66.6; H, 4.36; N, 3.53.

1,2,5-Tri-*O*-acetyl-3-phthalimido-3-deoxy-D-ribofuranose (IVa) (A).—A solution of 1.30 g. of Ia in 6.5 cc. of reagent pyridine and 6.5 cc. of acetic anhydride was heated on a steam-bath for one hour, then poured into 65 cc. of ice-water. The mixture was extracted with methylene chloride (3 × 20 cc.). The combined extracts, washed with excess aqueous sodium bicarbonate, then water, were dried with anhydrous magnesium sulfate and evaporated to dryness *in vacuo*. The residual gum was dissolved in toluene and again evaporated *in vacuo* to remove pyridine. The semi-solid (1.40 g.) was heated to boiling with 5 cc. of absolute alcohol, then cooled. The product was collected on a filter and washed with cold absolute alcohol; yield 1.13 g. (78%), m.p. 140–142°. A similar preparation was recrystallized from absolute alcohol and formed white crystals, m.p. 140–142°, $[\alpha]^{25}D$ −30.5° (1.6% in $CHCl_3$); λ_{max}^{KBr} 5.62, 5.80 μ (phthalyl C=O); 5.73, 8.20 μ (acetate); no OH absorption in 3 μ region.

Anal. Calcd. for $C_{19}H_{19}NO_9$: C, 56.4; H, 4.74; N, 3.46; 3 OAc. Found: C, 56.4; H, 5.15; N, 3.52; OAc, 3.01.

(B).—To a solution of 16.2 g. of IIa in 143 cc. of acetic acid and 16 cc. of acetic anhydride was added 8.6 cc. of 96% sulfuric acid dropwise with cooling so that the temperature was 20–25°. After 17 hours in a stoppered flask, the solution deposited white crystals of **6-dimethylaminopurine bis-acid sulfate**. The solid was collected on a filter and washed with 30 cc. of acetic acid containing 5% acetic anhydride; yield 10.7 g. (92%), m.p. 210–215°. For analysis a sample was washed well with chloroform.

Anal. Calcd. for $C_7H_9N_5 \cdot 2H_2SO_4$: C, 23.4; H, 3.65; N, 19.5. Found: C, 23.7; H, 4.00; N, 19.2.

The acetic acid filtrate was poured into 1 l. of ice-water with stirring. The white solid was collected by filtration and washed with water. The solid was slurried with 400 cc. of 3% ice-cold aqueous sodium bicarbonate, collected on a filter and washed with water; yield 10.6 g. (82%), m.p. 133–135°, of IVa. A mixture with preparation A gave no depression in m.p.

After standing for 3 days the dilute acetic acid mother liquor deposited 150 mg. (0.9%) of white needles, m.p. 165–168°. Recrystallization from alcohol gave white crystals of **1,1,2,4,5-penta-*O*-acetyl-3-phthalimido-3-deoxy-D-ribose**, m.p. 167–169°, $[\alpha]^{25}D$ +5.3° (2% in $CHCl_3$); λ_{max}^{KBr} 5.61, 5.77 μ (phthalyl C=O); 5.68, 8.20 μ (acetate); no absorption in 3.0 μ OH region or 6.2 μ (C=N region).

Anal. Calcd. for $C_{23}H_{25}NO_{12}$: C, 54.3; H, 5.00; N, 2.76; 5 OAc. Found: C, 54.1; H, 5.47; N, 2.96; OAc, 5.01.

Methyl 3-Phthalimido-3-deoxy-β-D-ribofuranoside (XVb).—A solution of 980 mg. of Xb[17] in 13 cc. of methanol and 0.90 cc. of 1 *N* methanolic sodium methoxide was refluxed for 0.5 hour, then evaporated to dryness *in vacuo*. The residual glassy XIb was dissolved in 53 cc. of 0.5 *N* barium hydroxide and heated on a steam-bath under a condenser for 18 hours. The excess base was neutralized with solid carbon dioxide, the solution filtered through Celite, then evaporated to dryness *in vacuo* leaving XIIb as a low-melting solid (610 mg.), which contained no amide carbonyl in the infrared. This was dissolved in 10 cc. of dimethylformamide, 550 mg. of phthalic anhydride was added and the solution was refluxed for 45 minutes. Solvent was removed *in vacuo* and the residue crystallized by trituration with 15 cc. of water; yield 480 mg. (49%), m.p. 174–176°. Recrystallization from ethyl acetate afforded white crystals, m.p. 185–187°, $[\alpha]^{24}D$ +29° (0.3% in EtOH); λ_{max}^{KBr} 2.85 2.92 μ (OH); 5.61, 5.78 μ (CO of phthalimido).

Anal. Calcd. for $C_{14}H_{15}NO_6$: C, 57.4; H, 5.16; N, 4.78. Found: C, 57.1; H, 5.19; N, 4.84.

Similarly, hydrolysis of Xa[17] (1.99 g.) and treatment with phthalic anhydride gave a product soluble in water. Extraction of the aqueous solution with chloroform and evaporation of the dried extracts afforded 1.54 g. (77%) of methyl 3-phthalimido-3-deoxy-α-D-ribofuranoside (XVa) as a sirup which had λ_{max}^{KBr} 2.82, 2.90 μ (OH); 5.62, 5.80 μ (CO of phthalimido).

1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-ribofuranose (IVb). (A).—Acetylation of 5.4 g. of Ib in 27 cc. of acetic anhydride and 27 cc. of reagent pyridine as described for the acetylation of Ia gave 5.1 g. of crude product. Recrystallization from hot methanol by cooling to room temperature gave 2.5 g. (43% of product, m.p. 137–139°. Recrystallization of a similar preparation from absolute alcohol afforded white crystals, m.p. 138–140°, $[\alpha]^{25}D$ +122° (0.5% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{33}NO_9$: C, 65.9; H, 4.38; N, 2.67. Found: C, 65.6; H, 4.69; N, 2.56.

(B).—To a solution of 27.1 g. of IIb in 191 cc. of acetic acid and 21 cc. of acetic anhydride was added 11.4 cc. of 96% sulfuric acid dropwise with cooling at such a rate that the temperature was 20–25°. After 18 hours at room temperature in a stoppered flask the mixture was filtered from **6-dimethylaminopurine bis-acid sulfate** (100%) and the solid washed with chloroform. The filtrate was poured into 1 l. of ice-water and extracted with chloroform. The chloroform solution was washed with excess 3% aqueous sodium bicarbonate and water, then dried with anhydrous magnesium sulfate and evaporated to dryness *in vacuo*. Crystallization of the residue from 100 cc. of methanol gave 17.4 g. (77%) of IVb, m.p. 138–140°. A mixture with preparation A gave no depression in m.p.

The methanol filtrate was evaporated to dryness *in vacuo*. The residue (3.4 g.) was allowed to react with 24 cc. of acetic acid, 2.65 cc. of acetic anhydride and 1.43 cc. of 96% sulfuric acid as in the preceding paragraph. An additional 2.07 g. (total 86%) of IVb was obtained, m.p. and mixed m.p. with preparation A, 136–138°.

An eight hour reaction in the first cycle gave only $^5/_6$ the yield.

(C).—To a solution of 150 mg. of XVb in 2 cc. of reagent pyridine was added 0.15 cc. of benzoyl chloride. After being heated on the steam-bath under a condenser and protected from moisture for 2.5 hours, the solution was poured into 10 cc. of ice-water and extracted with chloroform. The combined extracts, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving 313 mg. of XIVb as a gum (contaminated with benzoic anhydride) which had no OH absorption in the infrared.

To a solution of 310 mg. of this crude XIVb in 2.3 cc. of acetic acid and 0.256 cc. of acetic anhydride was added 0.082 cc. of 96% sulfuric acid. After 18 hours in a stoppered flask at room temperature, the solution was diluted with water and worked up as in (B). Crystallization from 3 cc. of methanol gave 165 mg. (61% from XIb), m.p. and mixed m.p. with preparation B, 137–139°.

(D).—Benzoylation and acetolysis of XVa as in procedure C gave 43% of product, m.p. 126–132°. Recrystalli-

zation from methanol afforded white crystals, m.p. and mixed m.p. with preparation B, 136–138°.

2,5-Di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-ribofuranosyl Chloride (Vb).—To a solution of 2.75 g. of IVb in 60 cc. of anhydrous ether freshly saturated with hydrogen chloride at 0° was added 2.8 cc. of acetyl chloride. In about 5 minutes crystals of the product began to separate. After 3 days at −3° protected from moisture, the mixture was evaporated to residue *in vacuo* (bath 30°). The residue was twice suspended in dry benzene and the evaporation repeated leaving 2.58 g. (90%) of white crystals, m.p. 160–162° dec., $[\alpha]^{25}D$ +76.5° (1.1% in dioxane).

Anal. Calcd. for $C_{27}H_{20}ClNO_7$: C, 64.1; H, 4.00; N, 2.77; Cl, 7.03. Found: C, 64.3; H, 4.30; N, 2.89; Cl, 7.04.

This compound appeared to be quite stable in a stoppered container. When a solution of Vb in dioxane was treated with 2 moles of water, hydrolysis to Ib took place slowly giving the following specific rotations: +75.5° (3 min.), +75.5° (6 min.), +80° (75 min.), +90° (2 hr.), +104° (6 hr.), +120° (18 hr.), +121° (24 hr.), thus showing the β-configuration of Vb. The solvent system of Fletcher and Ness,[13] dioxane–water (18:7), gave very rapid hydrolysis: $[\alpha]^{25}D$ +116° (3 min.), +118° (3 hr.).

Similarly, treatment of IVa with ethereal hydrogen chloride gave crude **2,5-di-*O*-acetyl-3-phthalimido-3-deoxy-D-ribofuranosyl chloride (Va)** as a gum in quantitative yield which contained 71–75% of the theoretical chlorine. Material prepared in this fashion was used immediately in subsequent reactions since it decomposed with the evolution of hydrogen chloride on standing in a desiccator.

6-Dimethylamino-7- and 9-(2′,5′-di-*O*-benzoyl-3′-phthalimido-3′-deoxy-β-D-ribofuranosyl)-purine (VIIb and IIb) (Synthetic).—To a suspension of 2.3 g. of chloromercuri-6-dimethylaminopurine[14] and 0.9 g. of Celite in 250 cc. of xylene which had been dried by a distillation of about 80 cc. of xylene was added a solution of 2.58 g. of Vb in 50 cc. of hot xylene. The mixture was refluxed and stirred for 3 hours protected from moisture, then filtered hot. The filter cake was washed with chloroform. The combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was partitioned between 25 cc. of chloroform and 20 cc. of 30% aqueous potassium iodide. Washed with water and dried with magnesium sulfate, the chloroform solution was evaporated to dryness *in vacuo*. Crystallization of the residue (3.6 g.) from 50 cc.of 3A alcohol gave 1.55 g. (47%) of product, m.p. 128–130°. Recrystallization from 3A alcohol gave white crystals of unchanged m.p. which gave no depression in m.p. when mixed with IIb prepared from III. Ultraviolet analysis showed that this IIb was contaminated with 18–26% of the 7-nucleoside VIIb.

The solvent system water–methanol–ethyl acetate–petroleum ether (90–100°) in the ratio of 1:25:5:25 was used for partition chromatography in Celite.[12] Due to the insolubility of IIb in either phase large volumes were required; for the purification of 100 mg. of crude IIb, 220 g. of Celite mixed with 110 cc. of lower phase were employed. The sample was dissolved in 10 cc. of the lower phase, mixed with 20 g. of Celite and packed on top of the column. The column was developed with the upper phase, following the elution by ultraviolet absorption. The first peak eluted contained only the 9-nucleoside IIb. These pooled fractions were evaporated to dryness *in vacuo*. Trituration of the residue with methanol gave IIb, m.p. 127–129° (gas). The ultraviolet spectrum showed the compound was free from 7-nucleoside VIIb, but recovery was poor.

2-Methylmercapto-6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (IX).—Condensation of 3.8 g. of Vb with 4.0 g. of chloromercuri-2-methylmercapto-6-dimethylaminopurine[14] as described for the preparation of VIIb gave 5.19 g. of crude VIII as a glass. This material (4.9 g.) was dissolved in toluene and evaporated *in vacuo* to remove traces of water. The residue dissolved in 35 cc. of reagent methanol was treated with 2.17 cc. of 1 *N* methanolic sodium methoxide. After several hours in a closed flask the reaction mixture no longer had *p*H > 9 when spotted on moist indicator paper. The solution was treated with another 2.17 cc. of 1 *N* methanolic sodium methoxide and the *p*H remained > 9 after 24 hours. The solution was evaporated to dryness *in vacuo*. The residue was partitioned between 25 cc. each of chloroform and water. (Although the aqueous phase contained half of the solids, it did not contain appreciable nucleoside as shown by ultraviolet analysis.) The separated chloroform layer, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, was evaporated to dryness *in vacuo* leaving 1.8 g. of crude 2-methylmercapto-6-dimethylamino-9-(3′-phthalimido-3′-deoxy-β-D-ribofuranosyl)-purine as a tan amorphous solid with λ_{max}^{KBr} 2.95 μ (OH); 5.58, 5.77 μ (CO of phthalimido); 6.20 μ (C=N).

This compound (1.80 g.) was dissolved in 10 cc. of methyl Cellosolve, 0.185 cc. of 100% hydrazine hydrate was added and the solution heated on a steam-bath under a condenser for 1.5 hours. After the addition of 1.92 cc. of acetic acid in 10 cc. of methyl Cellosolve the solution was heated 10 minutes longer, then evaporated to dryness *in vacuo*. The residue was dissolved in 30 cc. of 10% acetic acid, filtered from phthalhydrazide (290 mg.), and the solution evaporated to dryness *in vacuo*. The residue was dissolved in 15 cc. of 50% methanol and absorbed on a column of IRC-50(H).[2] Elution with 2 *N* ammonium hydroxide in 50% methanol[2] and crystallization of the residue from 10 cc. of absolute ethanol gave 275 mg. (11% based on Vb) of product, m.p. 185–187° dec. A second crop raised the yield to 13%.

A similar preparation was recrystallized from absolute ethanol with the aid of Norit to give white crystals, m.p. 185–187° dec.; $[\alpha]^{24}D$ −2 ± 2° (1% in dimethylformamide); λ_{max}^{KBr} 2.87, 2.95, 3.01, 3.15 μ (OH and NH); 6.20 (C=N); 9.06, 9.38, 9.54, 9.74 (OH and C–O–C); λ_{max}^{alc} 247 mμ (ε 26,400), 286 mμ (ε 17,800); $\lambda_{max}^{0.1\ N\ HCl}$ 277 mμ (ε 16,200); $\lambda_{max}^{0.1\ N\ NaOH}$ 247 mμ (ε 22,600), 286 mμ (ε 17,700).

Anal. Calcd. for $C_{13}H_{20}N_6O_3S$: C, 45.9; H, 5.95; N, 24.7. Found: C, 46.3; H, 6.12; N, 24.4.

6-Dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (VI) from Desulfurization of IX.—To a solution of 92 mg. of IX in 10 cc. of 50% methyl Cellosolve was added about 0.6 g. of desulfurizing Raney nickel catalyst and the mixture was heated on the steam-bath under a condenser for 1 hour with occasional mixing. The desulfurization was complete at this time as determined by ultraviolet analyses on an aliquot from the reaction solution. After filtration through Celite, 25 cc. of water being used to wash the Celite–nickel cake, the combined filtrate and washings were evaporated to dryness *in vacuo* leaving 25 mg. (32%) of a white crystalline solid, m.p. 211–213°. A mixed m.p. with an authentic sample of VI[6] gave no depression. The infrared curves of authentic VI[6] and material obtained in above reaction were identical.

PEARL RIVER, N. Y.