A Convenient Method for the Synthesis of 2-Fluoroadenosine[1]

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2-Fluoroadenosine (**7**)[2] is readily metabolized to its 5′-mono-, 5′-di-, and 5′-triphosphates.[3] Its triphosphate inhibits DNA-dependent syntheses of RNA and polyadenylate.[3] 2-Fluoroadenosine is a poor substrate for adenosine deaminase[4-7] but an excellent substrate for adenosine phosphorylase[5,8] and adenosine kinase[9,10] and, probably as its 5′-monophosphate, inhibits *de novo* purine nucleotide synthesis by negative pseudofeedback.[11] This feedback inhibition, or incorporation into RNA,[3] is probably responsible for its high cytotoxicity,[2] its high toxicity to rodents,[12] and its broad-spectrum antibacterial activity.[13,14] 2-Fluoroadenosine (**7**) is an inhibitor of ribonucleoside metabolism[15] and of blood-platelet aggregation.[16] It has a synergistic effect on the antimicrobial action of actinobolin[17] and is inhibitory to cell lines resistant to 2-fluoroadenine.[18] The broad and high level biological activity of 2-fluoroadenosine (**7**) has made its ready accessibility for further study desirable.

We have now developed a new, more convenient synthesis of this material (Scheme I). 2,6-Diazido-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (**2**), prepared in high yield by the reaction of 9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (**1**)[19] with sodium azide,[20] was facilely reduced to 2,6-diamino-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (**3**) using hydrogen and palladium-on-charcoal catalyst. Diazotrization of **3** in 48% fluoroboric acid with sodium nitrite at −10° gave three products: (2′,3′,5′-tri-O-acetyl crotonoside (**4**, 35%), 2′,3′,5′-tri-O-acetyl-2-fluoroadenosine (**5**, 8%), and 9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-2,6-difluoropurine (**6**, 21%).[21] Compounds **5** and **6** could be separated from **4** by extraction with benzene and diethyl ether. Initially **5** and **6** were then separated and identified by spectral data and elemental analyses. For the preparation of **7**, it was not necessary to separate **5** and **6**. The purified mixture was treated with ethanolic ammonia at 4° for 3 days to effect removal of the acetyl groups and, in the case of **6**, concomitant replacement of the 6-fluoro group. This step proceeded almost quantitatively and thus the total yield of **7** from **3** was 20%.

The procedure described above not only gives two and one-half times the yield (*ca.* 8%) of **7** given by the original procedure,[2] but also permits a fast, facile isolation of the desired products from the Schiemann reaction to replace the laborious column procedures necessary for the isolation of 2-fluoroadenosine directly from this reaction.

Experimental Section

The melting points reported were determined on a Kofler Heizbank and are corrected. The ultraviolet spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer, whereas the infrared spectra were determined in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer. The optical rotations were determined in the solvents specified with a Rudolph Model 80 polarimeter. Silica gel H (Brinkmann) was used for most of the chromatographic separations. Spots were detected with ultraviolet light after spraying the plates with Ultraphor WT highly concentrated (BASF Colors & Chemicals, Inc., Charlotte, N. C.).

9-(2′,3′,5′-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-diazidopurine (2).—A sodium azide solution (5.0 g, 76 mmoles in 20 ml of water) was added to a warm solution of 9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (**1**, 17 g, 38 mmoles) in ethanol (300 ml) and the resulting reaction mixture was refluxed for 1 hr. The inorganic salts that precipitated were removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in benzene (200 ml) and the resulting mixture concentrated *in vacuo* to remove residual ethanol and water. The resulting dry benzene solution was filtered through dry Celite and the filtrate was evaporated to dryness *in vacuo* to give **2** as a glass. Thin layer chromatography using anhydrous ether as the eluent indicated that the amorphous product contained only trace impurities and was suitable for use as an intermediate: $\tilde{\nu}_{max}$ (cm^{-1}) 3100, 2940 (CH); 2160, 2130 (N≡N); 1745 (ester C=O); 1595, 1570 (C=C, C=N); 1240, 1220, 1090, 1040 (C-O-C).

2,6-Diamino-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (3).—5% Palladium-on-charcoal catalyst (3.5 g) was added to a solution of 2,6-diazido-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-purine (**2**, 17.5 g, 38 mmoles) in absolute ethanol (1 l.) and the mixture was hydrogenated at atmospheric pressure for 6 hr. The hydrogen atmosphere was removed and replaced with fresh hydrogen after 30 min, 1 hr, and 2 hr. After hydrogenation was complete, the catalyst was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in ethyl acetate (30 ml) and the solution was filtered through dry Celite. The filtrate was evaporated to dryness to give **3** as a glass: yield, 10.9 g (72%). Thin layer chromatography using chloroform–methanol (95:5) as the eluent indicated the product was sufficiently pure for use as an intermediate. Furthermore, the absence of azide bands in the infrared spectrum of the reduction product indicated essentially complete reduction. Positive identification of **3** was accomplished by deacetylation in methanolic ammonia at 5° to give 2,6-diamino-9-β-D-ribofuranosylpurine identified by a comparison of its ultraviolet and infrared spectra and of its chromatographic travel on silica gel in three solvent systems with those of an authentic sample.

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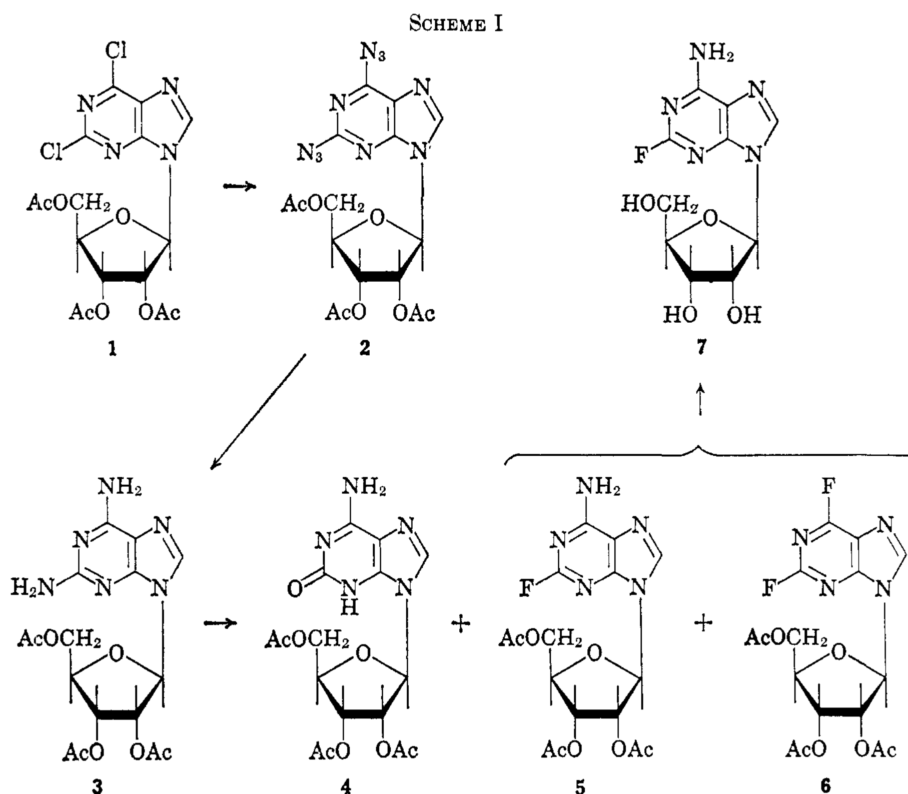
SCHEME I



When the reduction was carried out in a small volume of ethanol, 2′,3′,5′-tri-O-acetyl-2-azidoadenosine (mp 230°) (identified by conversion into 2-azidoadenosine and a comparison of its spectra with those from an authentic sample[22]) precipitated from the reaction mixture preventing complete reduction. This incomplete reduction is a matter of insolubility only, since complete reduction to **3** was achieved when a sample of 2′,3′,5′-tri-O-acetyl-2-azidoadenosine (2.4 g) was dissolved in ethanol (1 l.) and hydrogenated at atmospheric conditions.

2′,3′,5′-Tri-O-acetyl-2-fluoroadenosine (5) and 9-(2′,3′,5′-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-difluoropurine (6).—A solution of 9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-2,6-diaminopurine (**3**, 10.9 g, 27 mmoles) in 48% fluoroboric acid (145 ml) was cooled to −20° and stirred continuously during the dropwise addition of a sodium nitrite solution (3.3 g, 49 mmoles in 7 ml water). After completion of the nitrite addition (10 min), the reaction mixture was stirred for an additional 20 min at −10°. Chloroform (150 ml) was added to the reaction mixture (150 ml), which was stirred vigorously for 10 min at −10° before it was cooled to −20°. The resulting emulsion was neutralized with 50% sodium hydroxide solution (*ca.* 45 ml) to pH 5, not allowing the temperature to exceed −10°. After the neutralization was complete, the chloroform layer was separated from the aqueous salt solution and the aqueous layer was extracted with three 100-ml portions of chloroform. The combined chloroform extracts were washed with cold water several times before they were dried over $MgSO_4$ and then evaporated to dryness *in vacuo*. The resulting residue was triturated with benzene (250 ml) and the insoluble solid was collected by filtration and set aside. The filtrate was evaporated to dryness and the residue was triturated with two 100-ml portions of anhydrous ether. The insoluble solid was removed by filtration and the filtrate was evaporated to dryness to give crude **6**. The ether-insoluble solid was dissolved in chloroform (10 ml) and allowed to stand until crystallization was complete. The crystals were collected by filtration, washed with ethyl acetate, and dried *in vacuo* to give the crude **5**. The chloroform filtrate and ethyl acetate wash solution were combined and evaporated to dryness. The residue was combined with the benzene-insoluble solid and the mixture was magnetically stirred with three 400-ml portions of anhydrous ether for 1 hr at room temperature before decantation. The combined ether extracts were evaporated to dryness to give crude **5** and **6**. The ether-insoluble solid (5.13 g) was identified as impure 2′,3′,5′-tri-O-acetyl crotonoside (**4**) by its ultraviolet spectrum and by deacetylation by treatment with methanolic ammonia at 5° to give crotonoside, identified by a comparison of its ultraviolet and infrared spectra and its chromatographic behavior on silica gel in three solvent systems with that of an authentic sample.

The fractions of crude **5** and **6** were combined and triturated with two 25-ml portions of anhydrous ether. The insoluble solid was collected by filtration, washed with fresh ether, triturated on the funnel with two 10-ml portions of ethyl acetate, and dried *in vacuo* to give essentially pure **5**: yield, 875 mg (8%), mp 198°. Thin layer chromatography using ethyl acetate as the eluent indicated **6** was the only significant contaminant. Evaporation of the ether filtrate to dryness gave essentially pure **6**: yield, 2.37 g (21%).

Analytically pure samples of each of the fluoropurine ribonucleosides were obtained as follows.

Purified 2′,3′,5′-tri-O-acetyl-2-fluoroadenosine (**5**, 1.3 g) was recrystallized from ethyl acetate (80 ml) to give the analytically pure material: yield, 990 mg; mp 204°; $[\alpha]^{25}D$ −28.6 ± 0.3° (1.00 g/100 ml of $CHCl_3$); λ_{max} [mμ (ε × 10^{-3})] pH 1—261 (14.2), 267 (sh); pH 7—261 (14.9), 267 (sh); pH 13—262 (15.4), 268 (sh); $\tilde{\nu}_{max}$ (cm^{-1}) 3345, 3290, 3145, 3130, 2980, 2925 (NH and CH); 1755, 1740, 1730 (C=O); 1665 (NH); 1610, 1585 (C=C, C=N); 1220, 1095, 1055, 1020 (C-O-C).

Anal. Calcd for $C_{16}H_{18}FN_5O_7$: C, 46.72; H, 4.41; N, 17.03. Found: C, 46.70; H, 4.43; N, 16.95.

9-(2′,3′,5′-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-difluoropurine (**6**, 394 mg) was purified by thin layer chromatography using chloroform–ethyl acetate (1:1) as the eluent. The analytically pure material was isolated as a gum containing 0.5 mole of ethyl acetate: yield, 283 mg; $[\alpha]^{25}D$ 0 (1.00 g/100 ml of $CHCl_3$). The presence of ethyl acetate in the product was confirmed by gas chromatography: λ_{max} [mμ (ε × 10^{-3})] pH 1—233 (3.9), 253.5 (7.1); pH 7—233 (4.0), 253.5 (7.3); pH 13—243 (sh), 255 (11.3), 262 (sh); $\tilde{\nu}_{max}$ (cm^{-1}) 1745 (C=O); 1625, 1585 (C=C, C=N); 1220, 1090, 1035, 1010 (C-O-C).

Anal. Calcd for $C_{16}H_{16}F_2N_4O_7 \cdot 0.5C_4H_8O_2$: C, 47.20; H, 4.38; N, 12.23. Found: C, 47.56; H, 4.19; N, 12.44.

2-Fluoroadenosine (7).—A mixture of 2′,3′,5′-tri-O-acetyl-2-fluoroadenosine (**5**, 875 mg, 2.1 mmoles) and 9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-2,6-difluoropurine (**6**, 2.37 g, 5.7 mmoles) in absolute ethanol (500 ml) was saturated at 5° with dry ammonia. After complete solution was effected, the reaction was refrigerated for 3 days before it was evaporated to dryness. The residue was triturated with chloroform and the insoluble solid was collected by filtration, washed with chloro-

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form, and dried *in vacuo* to give the crude product. Recrystallization of the crude product from ethanol (100 ml) gave the essentially pure material in three crops: yield, 1.53 g (71%). Spectral data indicated the product was 98% pure. Thin layer chromatography using chloroform-methanol (9:1) as the eluent showed a trace impurity which was removed by recrystallization from ethanol.

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Opening of Ring B of Lanosterol by Beckmann Fission of a γ-Keto Oxime Tosylate

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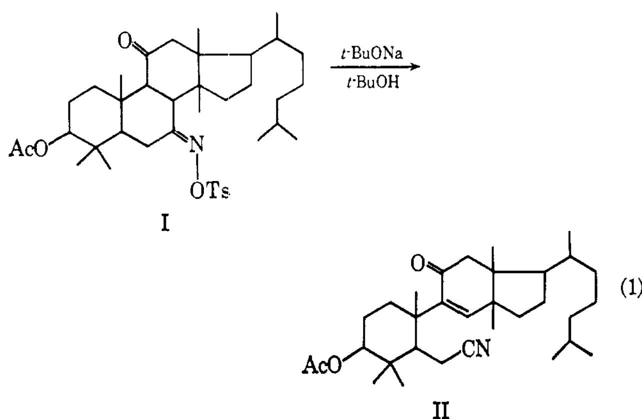
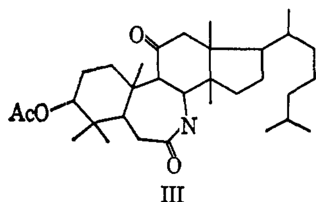
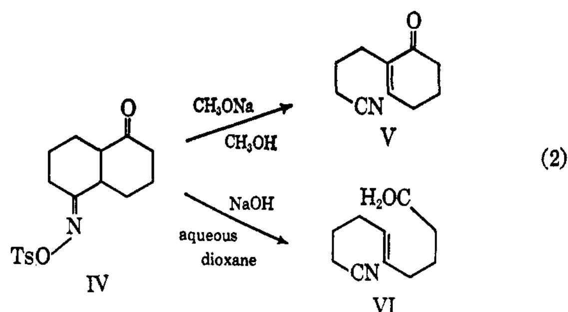
Application of a second-order Beckmann rearrangement to a γ-keto oxime, such as that derived from a 7,11-dione derivative of a steroid nucleus, provides a potentially convenient way of opening ring B. In particular, treatment of 3-acetoxylanostane-7,11-dione 7-oxime tosylate with sodium *t*-butoxide in *t*-butyl alcohol (I) has been found to lead to the anticipated reaction with generation of the nitrile (II) (eq 1). The



parent oxime of (I) on treatment with phosphorus pentachloride gives the expected lactam (III).[2,3]



A similar ring-opening fragmentation reaction was observed by Grob[4] in the conversion of the γ-keto oxime (IV) to the unsaturated ketonitrile (V) by treatment with sodium methoxide; treatment with 1 *N* sodium hydroxide in aqueous dioxane led to an unsaturated nitrile acid, presumed to be VI (eq 2).



(2)

The γ-keto oxime tosylate, I, was prepared by refluxing the 7,11-dione in hydroxylamine hydrochloride with pyridine in alcohol, the 11-keto function being much less reactive than the carbonyl group at C-7 by virtue of steric hinderance from the angular methyl groups at C-10 and C-13. Treatment of the resulting oxime with sodium hydride in ether followed by toluenesulfonyl chloride yielded the oxime tosylate (I).

The infrared spectrum in chloroform of the product obtained from (I) by treatment with sodium *t*-butoxide in *t*-butyl alcohol showed bands at ν_{max} 2245 cm^{-1} for the nitrile, 1725 for the ester, 1658 for the unsaturated ketone, and 1601 for the carbon-carbon double bond. The ultraviolet spectrum had λ_{max} 246 mμ (log ε 3.83). These spectral observations thus confirm the structure of the product as II.

Experimental Section

3-Acetoxylanostane-7,11-dione.—The procedure of Ruzicka[5] was used without modification, 3-acetoxylanostene being oxidized with chromic acid to 3-acetoxylanost-8-ene-7,11-dione in 58% yield and the double bond being reduced with zinc dust in glacial acetic acid to give the product in 81% yield, mp 221-223° (lit.[6] mp 222-224°).

3-Acetoxylanostane-7,11-dione 7-Oxime.—The procedure was essentially that of Falco, *et al.*[3] 3-Acetoxylanostane-7,11-dione (488 mg, 1.0 mmole) was dissolved in absolute ethanol (30 ml) and hydroxylamine hydrochloride (1.0 g, 15 mmoles) and pyridine (12 ml) were added. The resulting solution was refluxed for 6 hr, neutralized with dilute sulfuric acid, and extracted with benzene. Chromatographic separation on alumina and crystallization from ethanol gave 383 mg of white needles of the monoxime, mp 211-213° (lit.[3] mp 213-214°).

3-Acetoxylanostane-7,11-dione 7-Oxime Tosylate.—A solution of 3-acetoxylanostane-7,11-dione 7-oxime (100 mg, 0.2 mmole) in 10 ml of dry ether was stirred and cooled in ice while 15 mg (0.30 mmole) of a 50% suspension of sodium hydride in mineral oil was added. The solution was then refluxed under nitrogen with vigorous stirring for 24 hr. A solution of *p*-toluenesulfonyl chloride (36 mg, 0.19 mmole) in 10 ml of dry ether was added dropwise. The ether suspension was stirred for 3 hr at room temperature and the ether solution drawn up through a piece of cotton into a capillary pipette. Great care was taken to exclude air and moisture. The resulting clear solution was evaporated under reduced pressure in rotary evaporator. The white needles thus obtained were dissolved in a small amount of carbon tetrachloride and ligroin was added. In this way 52 mg (70%) of white needles of the tosylate were obtained, mp 243-246°.

Anal. Calcd for $C_{39}H_{59}O_6SN$: C, 70.00; H, 8.83; S, 4.79; N, 2.09. Found: C, 69.77; H, 8.91; S, 4.84; N, 1.83.

Nitrile II.—To a solution of 3-acetoxylanostane-7,11-dione 7-oxime tosylate (35 mg, 0.052 mmole) in 10 ml of *t*-butyl alcohol was added dropwise 5 ml of a sodium *t*-butoxide suspension made

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