An Improved Procedure for the Preparation of 9-β-D-Arabinofuranosyl-2-fluoroadenine

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An improved procedure for the preparation of 9-β-D-arabinofuranosyl-2-fluoroadenine (**11**) utilizing the readily available 2,4,5,6-tetraaminopyrimidine (**1**) is described; the overall yield based on the arabinosugar was quadrupled.

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9-β-D-Arabinofuranosyladenine (ara-A) is a potent antiviral agent (1), but it has shown little or no activity against experimental animal cancers such as leukemia L1210 or P388 (2), a not surprising result in view of the fact that ara-A is a poor substrate for nucleoside kinases (3,4) that anabolize it to its active form, the triphosphate (5), and a good substrate for adenosine deaminase (6), which catabolizes it to the inactive 9-β-D-arabinofuranosylhypoxanthine (2,7). Administration of ara-A on its optimal schedule in combination with 2′-deoxycoformycin (8), an effective *in vivo* inhibitor of adenosine deaminase (9), converts this essentially inactive compound to a highly active one, comparable to 1-β-D-arabinofuranosylcytosine (ara-C), a useful clinical agent (10) [in contrast, the therapeutic effectiveness of ara-C is not enhanced by administration with tetrahydrouridine, an *in vivo* effective inhibitor of cytidine deaminase (11)]. Chemotherapeutic activity and inhibition of DNA synthesis can be correlated with increased levels of the ara-A triphosphate in leukemia cells in mice treated with the adenosine deaminase inhibitors in combination with ara-A (12).

An equally valid and chemotherapeutically simpler approach to the enhancement of the activity of ara-A is the design of an analog that is not a substrate for adenosine deaminase, but in all other ways is equivalent to ara-A. The observation that 2-fluoroadenosine (13) is not a substrate for adenosine deaminase (6) led to the synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A, **11**) (14). Although the result of an initial chemotherapeutic experiment, using a single dose of drug, was negative (14), an appreciation of the optimal dose schedule for ara-A plus 2′-deoxycoformycin (8) led to the resynthesis of F-ara-A and its evaluation on a more optimal schedule. The results were most encouraging (15) in that the activity on the more optimal schedule was comparable to that of ara-A plus 2′-deoxycoformycin. The need for an ample supply of drug to determine its optimal schedule and to study its biochemistry, pharmacology, and toxicology led us to reinvestigate the procedure for its synthesis. The published procedure (14) involves five steps starting with 2,6-dichloropurine, which is difficult to prepare by any of the literature procedures. To circumvent this problem, we investigated the preparation of 2,6-diacetamidopurine (**3**) (16) and its reaction with 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride and bromide in a number of ways. The reaction of the chlorosugar gave a better yield of purer product than the bromosugar. Reaction of the chlorosugar with 2,6-diacetamidopurine (**3**) followed by deacetylation to give **5** was superior to this sequence with 2,6-dibenzamidopurine. The trimethylsilyl derivative of 2,6-diacetamidopurine was prepared and allowed to react with the chlorosugar in the presence of tin(IV) chloride (17). Although a good crude yield of nucleosidic material was obtained, it turned out to be a mixture, primarily of α- and β-anomers (2α:1β), which was not satisfactory. The best procedure for the preparation of 2-amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)adenine (**5**) that we have found is the reaction of 2,6-diacetamidopurine (**3**) with the chlorosugar in ethylene chloride in the presence of Linde 4A molecular sieve followed by deacetylation with methanolic sodium methoxide. This procedure, which is described in the Experimental, differs somewhat in detail from that described in a patent (18) and apparently gives a higher yield of product (19) that can be used directly without recrystallization. The acetylation of 2-aminoadenine (**2**) with acetic anhydride in pyridine gave a higher yield of purer product than the acetylation in acetic anhydride alone (16). No triacetyl purine was formed, and it was unnecessary to recrystallize the material for use in the sugar condensation reaction.

Because of the insolubility of **5** in 48% fluoboric acid, the original diazotization reaction to give **6** was carried out as a two-phase reaction in a mixture of the acid and chloroform. In addition to the mechanical difficulties it caused, this reaction was found, by hplc, to give a significant amount of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-chloroadenine (**7**) by reaction of the purine diazonium salt with the chloroform (20). Formation of the chloro nucleoside **7** reduced the yield of **6** by consuming some of the diazonium intermediate and by complicating the purification procedure, because it (**7**) is not readily separable from **6**. To circumvent these problems, the reaction was carried out in a homogeneous mixture of tetrahydrofuran and 48% fluoboric acid in which the nucleoside **5** is soluble. Formation of the chloro nucleoside was, of course, eliminated and the yield of **6** initially isolated somewhat improved. Hplc analyses,

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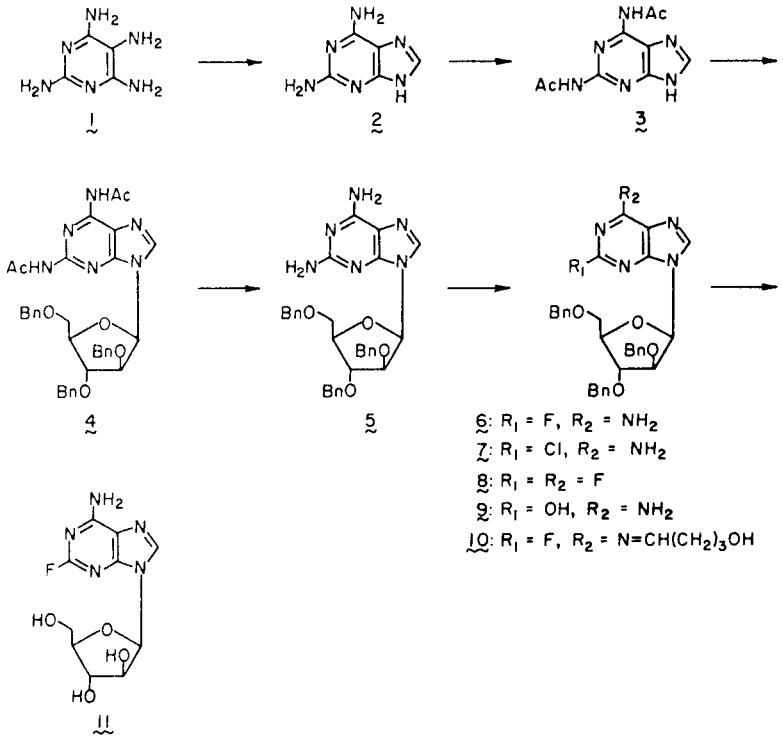

however, indicated the presence in the reaction mixture, in addition to the desired product **6** (41%), of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2,6-difluoropurine (**8**, 3%), 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-hydroxyadenine (**9**, 26%), and a new compound (30%). Treatment of the mixture with methanolic ammonia raised the amount of **6** to 53% of the total, eliminated the difluoro compound, reduced the amount of the unidentified compound to 22%, and did not affect the amount of the 2-hydroxy nucleoside. These results showed that part of the unidentified compound was converted to **6** by ammonia. Refluxing the unknown in hydrochloric acid converted essentially all of it to **6**. Some of this material was isolated by chromatography on a thick plate and subjected to spectral analyses. The uv maximum (*p*H 7) was shifted from 261 (shoulder at 272) to 280 (shoulder at 272) indicating (along with conversion to **6**, see above) attachment of a group to the amino group of **6**. The molecular ion was 625, and elemental analyses and nmr spectroscopy (^{1}H and ^{13}C) agreed with proposed structure 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-*N*-(4-hydroxybutylidene)adenine (**10**). Since 4-hydroxybutyraldehyde has been identified as an oxidation product of tetrahydrofuran (21), **10** probably results from the oxidation of tetrahydrofuran under the conditions of the reaction. Its formation is of minor consequence in any event, since it is readily converted to the desired **6**.

Finally, removal of the *O*-beznyl groups of **6** by any reductive procedure [catalytic hydrogenolysis, sodium in liquid ammonia (14)] always results in some defluorination, giving a mixture of F-ara-A (**11**) and ara-A that is difficult to separate. The use of boron trichloride in methylene chloride (22) gives yields approaching quantitative with no defluorination.

Thus, a number of difficulties and drawbacks of the original procedure for the preparation of F-ara-A (**11**) have been eliminated and the overall yield raised from 4.7% to 17.5% (based on starting sugar). Furthermore, the procedure utilizes the inexpensive and readily available tetraaminopyrimidine (**1**) instead of 2,6-dichloropurine.

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. All solvents were dried over Linde 4A molecular sieve, and samples were normally dried *in vacuo* over phosphorus pentoxide at room temperature (unless otherwise stated) for 16 hours. Analtech precoated (250 μ) silica gel G(F) plates developed in chloroform-methanol (ratio specified for each compound) were used for the tlc analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated ammonium sulfate. Melting points were determined with a Mel-Temp apparatus and are uncorrected. The uv absorption spectra were determined in 0.1 *N* hydrochloric acid (*p*H 1), *p*H 7 phosphate buffer, and 0.1 *N* sodium hydroxide (*p*H 13) with a Cary 17 spectrophotometer; the maxima are reported in nm ($\epsilon \times 10^{-3}$). The nmr spectra were determined with a Varian XL-100-15 spectrometer in deuteriodimethylsulfoxide with tetramethylsilane as an internal reference: chemical shifts (δ in ppm) quoted in the case of multiplets are measured from the approximate center. Mass spectral data were obtained with a Varian MAT 311A instrument equipped with a combination EI/FI/FD ion source. The hplc analyses were carried out with a Waters Associates ALC-242 chromatograph with an M-6000 pump

and equipped with a μBondapak C_{18} column (¼" x 30 cm) using the solvent systems specified. Quantitation was achieved by integration of the peaks.

2-Aminoadenine (2).

This compound was prepared from tetraaminopyrimidine (409 g.) by the method of Robins (23); yield, 372 g. (56%).

2,6-Diacetamidopurine (3).

A solution of 2-aminoadenine (2, 83.6 g., 557 mmoles) in a mixture of pyridine (1250 ml.) and acetic anhydride (168 ml.) was refluxed for 3 hours. On cooling overnight, the solution deposited a solid which was collected by filtration and washed with pyridine, ethanol, and then ether before it was dried overnight. This material was stirred with 1000 ml. of saturated sodium bicarbonate for 35 minutes, diluted with 1000 ml. of water and stirred for 10 minutes, collected by filtration, and washed with water until all the bicarbonate was removed. The solid was dried overnight over phosphorus pentoxide *in vacuo* at room temperature and then at 100°. The yield was 87 g. (67%), m.p. 293-295° (darkens at 230°) [lit. (16) 296-300°]; tlc homogeneous (3:1, 9:1).

2-Amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)adenine (5).

Dry hydrogen chloride gas was bubbled into a solution of 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-arabinofuranose (121 g., 212 mmoles) in methylene chloride (1.33 l.) at -10-0° for 2.5 hours. The precipitated *p*-nitrobenzoic acid was removed by filtration and washed with methylene chloride. The methylene chloride solution was evaporated *in vacuo*. The residue, dissolved in 1 l. of ethylene chloride, was added to a mixture of 2,6-diacetamidopurine (50.0 g., 213 mmoles) and molecular sieve (Linde 4A, 630 g.) in 6 l. of ethylene chloride. This mixture was refluxed until all the chlorosugar was consumed (tlc) (5 days). The mixture was then stirred with Celite and filtered. The solid was washed with chloroform, and the combined filtrates were evaporated to dryness *in vacuo*. The residue was dissolved in benzene and filtered (to remove unreacted 2,6-diacetamidopurine). The filtrate was again evaporated to dryness *in vacuo* and the residue dissolved in 700 ml. of 1 *N* methanolic sodium methoxide. After a 3-hour reflux period, the solution was chilled, neutralized with acetic acid, and refrigerated overnight. The solid that precipitated was removed by filtration, washed with methanol, and dried *in vacuo;* yield 47 g. (40%), m.p. 160-163° [lit. (18) 161-162°]; tlc homogeneous (19:1).

9-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (6).

To a solution of 2-amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)adenine (5, 15.0 g., 27.1 mmoles) in a mixture of 500 ml. of tetrahydrofuran and 1 l. of 48% fluoboric acid at -10° in a 4-l. beaker equipped with a mechanical stirrer was added dropwise in 20 minutes a saturated aqueous solution of sodium nitrite (7.5 g., 108 mmoles). The addition of sodium nitrite was repeated three times after which none of the 2-aminoadenine remained (tlc). The fluoboric acid was neutralized to *p*H 6 with 50% sodium hydroxide keeping the temperature between -40 and 0°. The solution was extracted four times with chloroform (300 ml., 250 ml., and two 200-ml. portions). The chloroform extract was washed with saturated sodium chloride solution and then dried over magnesium sulfate before it was evaporated to dryness *in vacuo*. The residual oil was dissolved in a liter of ethanolic ammonia (saturated at 0°), and the solution was allowed to stand for 4 days at 4° before it was evaporated to dryness. The residue was recrystallized from ethanol; yield 5.5 g. Additional material was recovered from the filtrate by recrystallization, and the final filtrate was evaporated to dryness, diluted with 50 ml. of 1 *N* hydrochloric acid, stirred on a steam bath for 30 minutes, cooled, and the aqueous portion decanted. A chloroform solution of the residue was washed with saturated sodium bicarbonate, then water, and dried over magnesium sulfate before evaporation *in vacuo*. The residue was recrystallized from ethanol; total yield of 6, 7.2 g. (48%), m.p. 155-157°. This material, which was essentially homogeneous according to tlc (19:1) and hplc [2 aqueous pentanesulfonic acid (0.005 *M*):3 acetonitrile-3% acetic acid], was used in the next step without further purification.

In a separate run the final filtrate was subjected to chromatography on a thick plate (1 cyclohexane:1 ethyl acetate), and the fastest traveling band (R_f *ca.* 0.65) was eluted with ethyl acetate. The uv spectrum of this material (10) at *p*H 7 showed a maximum at 280 nm and a shoulder at 272. The m/e was 625; nmr: 2.1 (m, $-CH_2CH_2-$), 3.65 (m, $2H_5'$), 3.9 (m, H_4' and CH_2OH), 4.3 and 4.6 (2m, H_2', H_3', $3C_6H_5CH_2$), 6.3 (m, H_1', $CH{=}N$, OH), 7-7.4 (m, $3C_6H_5$), 8.1 (H_8). On addition of deuterium oxide, the OH absorption at *ca.* 6.3 disappeared. The clearly defined doublet (6.38) had a coupling constant ($J_{1'2'}$) of 4. From the ^{13}C spectrum the carbons of the aliphatic side chain could be detected.

Anal. Calcd. for $C_{35}H_{36}FN_5O_5$: C, 67.19; H, 5.80; N, 11.19. Found: C, 67.00; H, 5.95; N, 10.94.

9-β-D-Arabinofuranosyl-2-fluoroadenine (11).

Boron trichloride gas was bubbled in methylene chloride (610 ml.) at 0° in a 2-l., 3-necked flask equipped with a thermometer, magnetic stirrer, and addition funnel and the stirred solution then cooled to -72° (dry ice-acetone) before the dropwise addition (1 hour) of a cold solution of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (6, 7.0 g., 12.5 mmoles) in methylene chloride (70 ml.). After a total reaction time of 2.75 hours, the cooling bath was removed and the solvent and gas removed *in vacuo*. The residue was dissolved in cold methylene chloride (40 ml.) and the solution evaporated to dryness (6 times) or until a solid white residue is obtained, before adding 450 ml. of cold 5% sodium bicarbonate followed by solid sodium bicarbonate to adjust the *p*H to 6-7. The mixture was diluted with ethanol, heated to boiling, treated with charcoal, filtered through Celite, and then allowed to stand at room temperature overnight. It was then chilled and the solid collected by filtration, washed with cold water and then ether, and dried *in vacuo;* yield 3.23 g. (91%), m.p. 259-260°; homogeneous according to tlc (4:1) and hplc (23 water:2 acetonitrile); nmr: 3.7 (m, H_4' and $2H_5'$), 4.15 (m, H_2' and H_3'), 5.1 (t, 5'-OH), 5.55 and 5.65 (2d, 2'-OH and 3'-OH), 6.14 (d, $J_{1'2'}$ 3 Hz, H_1'), 7.8 (broad s, NH_2), 8.2 (s, H_8); uv: *p*H 1, 262 (13.2); *p*H 7, 13, 261 (15.3).

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