Department of Medicinal Chemistry
School of Pharmacy, University of Kansas

The Synthesis of 2-(2'-Deoxy-D-ribofuranosyl)-6-trifluoromethyl-*as*-triazine-3,5-dione, (5-Trifluoromethyl-6-aza-2'-deoxyuridine) (1)

Mathias P. Mertes, Souheil E. Saheb, and Duane Miller

Sir:

Reports of the synthesis (2) and biological activity (3) of the fluorinated pyrimidines 5-difluoromethyluracil (I), 5-trifluoromethyluracil (IIa) and the deoxyriboside (IIb) have appeared recently. The strong inhibition of the enzyme thymidylate synthetase noted for the deoxyribonucleotide (IIc) is not unexpected in view of the similarity to the natural substrate deoxyuridylic acid. Coupled with the activity of the azapyrimidine nucleosides on several enzymes (4) it was of interest to evaluate the effects of a 5-trifluoromethyl-6-aza analog.

6-Trifluoromethyl-*as*-triazine-3,5-(2*H*,4*H*)-dione (III, 5-trifluoromethyl-6-azauracil) (5) was converted to the acetyl derivative (IV) (m.p. 118) by refluxing with acetic anhydride (6). To avoid formation of the nucleoside bond at N_4 it was protected by alklyation with diphenyldiazomethane (7) in dioxane (8) to give 2-acetyl-4-diphenylmethyl-6-trifluoromethyl-*as*-triazine-3,5-(2*H*,4*H*)-dione (V) as a solid melting at 85° after purification on silica, λ max (5% sodium hydroxide solution) 308 mμ, (*Anal.* Calcd. for $C_{19}H_{14}F_3N_3O_3$: C, 58.61; H, 3.58; F, 14.65; N, 10.79. Found: C, 58.47; H, 3.73; F, 14.33; N, 10.51). The mercury salt of 4-diphenylmethyl-6-trifluoromethyl-*as*-triazine-3,5-(2*H*,4*H*)-dione (VI) was synthesized by treating a basic solution of V with two molar equivalents of mercuric chloride in 25% ethanol (*Anal.* Calcd. for $(C_{17}H_{11}F_3N_3O_2)_2Hg$: C, 45.72; H, 2.48. Found: C, 45.96; H, 2.62).

3,5-Di-*O*-toluyl-D-2'-deoxyribofuranosyl chloride (9) (VII) was added to a toluene solution of VI which after purification on alumina (Wohlm-Grade III) gave the protected deoxynucleoside (VIII) as a pale glass. The integration and assignments in the n.m.r. verified structure VIII. Methanolysis with a catalytic quantity of sodium followed by neutralization with Dowex 50W (H form) and chromatography on silica gave 4-diphenylmethyl-2-(2'-deoxy-D-ribofuranosyl)-*as*-triazine-3,5-(2*H*,4*H*)-dione (IX) melting over the range 47-60°. (*Anal.* Calcd. for $C_{22}H_{20}F_3N_3O_5 \cdot CH_3OH$: C, 55.75; H, 4.88; F, 11.50; N, 8.48. Found: C, 56.05; H, 5.23; F, 11.52; N, 8.78) λ max (*p*H 1) (HCl), 273 mμ. The diphenylmethyl derivative (IX) was reduced in a hydrogen atmosphere using 10% palladium on carbon to give after purification on a silica column a quantitative yield of diphenylmethane (identified by n.m.r.) and the product (X) isolated as the mixed calcium and sodium salt. Quantitative spectrographic analysis showed variations of the metal salt ranging up to 10% calcium and 2% sodium. Neutralization was effected by washing an aqueous solution of the metal salt of X through a Dowex 50W (H form) column and subsequent drying from methanol to give (±)-2-(2'-deoxy-D-ribofuranosyl)-6-trifluoromethyl-*as*-triazine-3,5-(2*H*,4*H*)-dione (X, 5-trifluoromethyl-6-aza-deoxyuridine) (*Anal.* Calcd. for $C_9H_{10}F_3N_3O_5 \cdot CH_3OH$: C, 36.48; H, 4.28; N, 12.76. Found (two samples): C, 36.83, 36.12; H, 3.86, 4.18; N, 13.22).

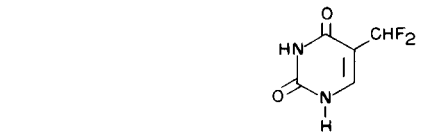
A partial separation of the anomers of the diphenylmethane derivative (IX) was accomplished on a silica column. The first fraction (dextrorotatory) was reduced with 10% palladium on carbon and purified on silica to give the (+) anomer (Xa), $[\alpha]_D^{24°}$ = +76° (2.34 g., CH_3OH), λ max (*p*H 1.0) 269 mμ (ε molar = 5,000); (*p*H 12.4) 264 mμ (ε molar = 5,000).

The levorotatory anomer (IXb) was collected as the second fraction, reduced with 10% palladium on carbon and chromatographed with methanol to give Xb, $[\alpha]_D^{24°}$ = -36° (2.41 g., CH_3OH), λ max (*p*H 1.0) 269 mμ (ε molar = 4,900); (*p*H 12.4) 264 mμ (ε molar = 4,900). The n.m.r. spectra (deuterium oxide) of IX and X are in agreement with the structures. Compound Xb integrates for the following assignments in the sugar portion of the molecule: H_1', 6.6 δ; H_2', 2.5 δ; H_3', 4.5 δ; H_4', 4.0 δ; and H_5', 3.7 δ. The presence of methanol in compounds IX and X was confirmed by n.m.r.

Assignment of structure to the anomers has not been attempted. Many exceptions to Hudson's Isorotation rules have been reported thus prohibiting the structural assignment based solely on optical rotation (10). Although n.m.r. has been an effective tool in distinguishing α and β anomers (11) no conclusions could be made at this time for the configuration of Xa or Xb due to poor resolution of the H_1 proton peaks.

The *in vitro* activity of Xa and Xb was examined using dihydrofolate reductase (chicken liver) and thymidylate synthetase (*E. coli*). The assays employed were those reported by Matthews and Huennekens (12) for dihydrofolate reductase and Wahba and Friedkin (13) for thymidylate synthetase. No inhibition of the enzymes was observed at the concentrations tested (Table I).

We wish to acknowledge helpful discussion of this problem with Dr. Morris Friedkin and his associates and the assistance of Mrs. William Riggs in the enzyme studies carried out in our laboratories.



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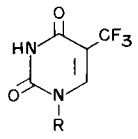
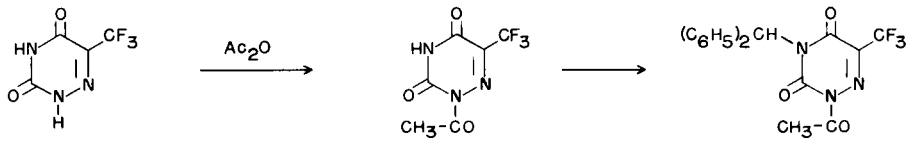


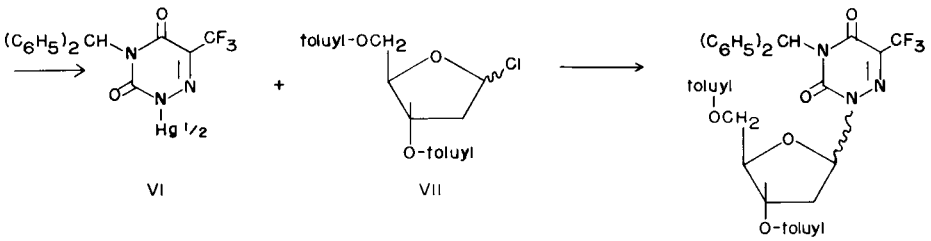
IIa, R = H

IIb, R = 2'-deoxyribose

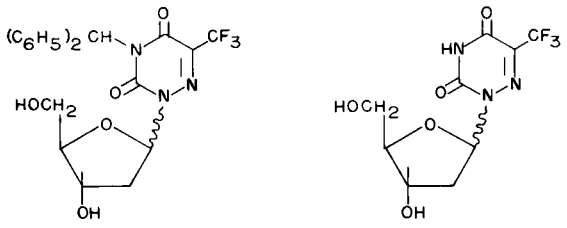
IIc, R = 2'-deoxyribose-5'-monophosphate



III IV V



VI VII VIII



IX a, dextrorotatory

IX b, levorotatory

X a, $[\alpha]_D$ + 76°

X b, $[\alpha]_D$ - 36°

TABLE I

Substrate and Inhibitor Concentration in Enzyme Assay Mixtures

	Dihydrofolate Reductase	Thymidylate Synthetase
Dihydrofolic acid	3.7×10^{-4}M	
Deoxyuridine-5'-phosphate		4×10^{-5}M
Xa	5.7×10^{-4}M	6.8×10^{-4}M
Xb	5.7×10^{-4}M	6.8×10^{-4}M

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Lawrence, Kansas 66045