Tetrahedron Letters No.16, pp. 1759–1764, 1966. Pergamon Press Ltd.
Printed in Great Britain.

STEREOSELECTIVE SYNTHESIS OF THE ANOMERIC 5-MERCAPTO-2'-DEOXYURIDINES AND OF SOME OTHER α- AND β-DEOXYRIBONUCLEOSIDES*

Thomas J. Bardos, Michael P. Kotick, and Csaba Szantay

Department of Medicinal Chemistry, School of Pharmacy,
State University of New York at Buffalo, Buffalo, New York 14214

(Received 31 January 1966)

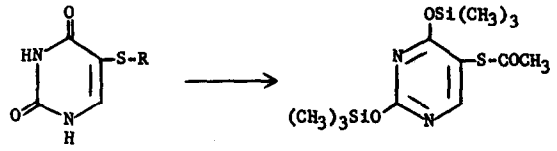
5-Mercaptouracil (I), an antimetabolite of thymine in L. leichmannii (1), potentiates the inhibitory effects of 5-fluorouracil and folic-antagonists against several transplanted tumors in rodents (2,3). The β-2'-deoxyribonucleoside of its disulfide was recently obtained by enzymatic method and was found to be a potent inhibitor in microbiological assay systems (4).

Our efforts directed at the chemical synthesis of 5-mercapto-2'-deoxyuridine (VI) via the chloromercuri-procedure (5) were unsuccessful. A new method for the synthesis of nucleosides was recently described by Nishimura, et.al. (6). These authors reported that bis-O-trimethylsilyl-derivatives of uracil, thymine and cytosine, on fusion with various acyl-halogeno sugars gave the corresponding N_1-glycosides with simultaneous removal of the trimethylsilyl group from the 2-O-position. We applied this method (which appears to be analogous to the Hilbert-Johnson synthesis (7) of nucleosides from 2-O-alkyl-pyrimidines), in various modifications to the synthesis of VI.

Compound I was refluxed with acetic anhydride in dry pyridine, to give II (71.5%). Recrystallization from methanol gave white needles; m.p.

* This investigation was supported by grant CA-06695 from the National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

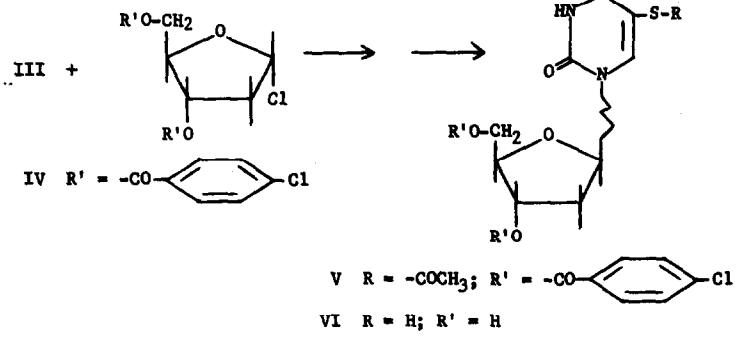
254-255.5°. Disappearance of the sharp infrared peak of I at 2550 cm^{-1} (S-H) indicated acetylation on the thiol. (Ultraviolet λ_{max}^{EtOH} 271 mμ (ε 5,950). Anal. Calcd.: C, 38.70; H, 3.25; N, 15.05; S, 17.22. Found: C, 39.01; H, 3.38; N, 14.80; S, 17.02). Treatment of this compound with



I R = H

II R = $-COCH_3$

III



IV R' = -CO-C6H4-Cl

V R = $-COCH_3$; R' = -CO-C6H4-Cl

VI R = H; R' = H

trimethylchlorosilane under refluxing in benzene, in the presence of triethylamine (6), gave, after separation of the triethylamine hydrochloride and concentration of the filtrate, a viscous oil which was purified by vacuum distillation (b.p. 110-111°/0.35 mm.). This solidified on prolonged standing at -5°, to give white, crystalline 5-acetylmercapto-2,4-O-bis(trimethylsilyl)uracil (III, 75.5%), m.p. 53-5°. Infrared ν_{max}^{film} 1715 (C=O), 1530-1570 (C=C and C=N of fully aromatic pyrimidine ring), 1250 ($Si(CH_3)_3$). (Anal. Calcd.: C, 43.60; H, 6.66. Found: C, 42.89; H, 6.38). This was reacted, under various conditions (see below), with equimolar amounts of the halogenose IV. The latter was

prepared according to Fox, et.al. (5) but further purified by recrystallization from CCl_4; m.p. 131° (reported: 118-120°). The reaction mixtures were subsequently treated with water, at room temperature, to remove the 4-O- (and any unreacted 2-O-) trimethylsilyl groups (6). The blocked nucleoside V was extracted with boiling toluene and the recovered pyrimidine II (from unreacted III) was separated by filtration. Yields of V, after recrystallization from toluene, were based (a) on moles of starting material (III and IV), and (b) on moles of converted (i.e., unrecovered) pyrimidine (Table I).

We found that the steric course of the coupling reaction was controlled in a decisive manner by the temperature at which it was conducted. When III and IV were mixed in boiling benzene and refluxed for 1-1/2 hrs. (Method A), or, when the two reactants were fused at 100-110° for 15-20 min. (Method B), only the β-anomer of V (m.p. 169-170°; $[\alpha]_D^{25}$ -53.9° [c 1.0, $CHCl_3$]; ultraviolet λ_{max}^{EtOH} 272 and 241 mμ [ε 10,820 and 36,610]; Anal. Calcd.: C, 51.72; H, 3.49; Cl, 12.22; N, 4.82; S, 5.50. Found: C, 52.15; H, 3.45; Cl, 12.54; N, 4.86; S, 5.72) could be isolated from the reaction mixture after hydrolysis. In contrast, when the reaction was conducted in benzene solution at 37°, for 90 hrs. or longer (Method C), only the α-anomer of V (m.p. 168-168.5°; $[\alpha]_D^{25}$ +53.3° [c 1.1, $CHCl_3$]; ultraviolet spectrum: same as β-V; Anal. Found: C, 52.09; H, 3.67; Cl, 12.40; N, 4.90; S, 5.63) was isolated. In each case, crystallization from the same solvent (toluene) yielded either the α or the β anomer. On mixing the two, sharp-melting anomers, the m.p. was depressed 15-20°. The relatively high yield of the pure anomer, based on the total amount of pyrimidine which took part in the coupling reaction (Table I (b)), indicated that the other anomer could have been formed only as a minor product (particularly, in Methods B and C).

TABLE I

Method	Yield (a)	Yield (b)	Anomer
A	29.5	54.0	β
B	37.5	87.4	β
C	11.8	70.3	α

(a) Yield of purified product (V) based on starting material;
(b) same, calculated on the basis of the amount of pyrimidine reacted.

Assignment of the anomeric configurations was based (a) on the n.m.r. spectra of the blocked nucleosides, α-V and β-V, in DMSO, which, in the nucleosidic proton (H_1') region, clearly showed the characteristic 4- and 3-peak patterns, respectively, of benzoylated α- and β-deoxyribonucleosides (8) (these spectral bands were, in fact, identical in spacing, relative size and shape with those of the corresponding anomers of 3',5'-di-O-p-chlorobenzoylthymidine, see below), and (b) on the biological activities of their respective deacylation products (α-VI and β-VI).

Deacylation of β-V with 1.5 equivalents of $NaOCH_3$ in methanol at room temperature, gave the biologically active β-anomer of VI (82.7%; m.p. 170°; $[\alpha]_D^{25}$ +26.4° [c 2.0, H_2O]; Anal. Calcd.: C, 41.53; H, 4.61; N, 10.76; S, 12.32. Found: C, 42.10; H, 4.66; N, 10.44; S, 11.74), while similar treatment of α-V gave the α-VI anomer (m.p. 183-5°; $[\alpha]_D^{25}$ +7.85° [c 2.0, H_2O]; Anal. Found: C, 41.70; H, 4.80; N, 10.58; S, 12.11). The two anomers have the same ultraviolet spectra: $\lambda_{max}^{pH\ 2}$ 284 mμ (ε 6400); $\lambda_{max}^{pH\ 8}$ 334 and 253.5 mμ (ε 5000 and 9400). A detailed study (9) of these and related compounds showed that this large bathochromic shift is due to ionization of the SH-group. When the latter was oxidized by allowing the solutions to stand in air, the spectra became identical with those of the enzymatically prepared β-VI disulfide (4) which showed no bathochromic shift in alkali, in agreement with other N_1-substituted uracil derivatives (10).

Biological activity was determined in the L. leichmannii assay (11) in which β-VI showed half-maximal inhibition at a concentration of 0.02 μg/ml., while α-VI was inactive at < 1.0 μg/ml.

The observed temperature-controlled stereoselectivity of the coupling reaction is not limited to the above case of nucleoside synthesis. When bis(trimethylsilyl)thymine (6) was reacted with IV according to "Method B", 26.2% yield (based on the starting materials) of 3',5'-di-O-p-chlorobenzoylthymidine (12) was obtained, together with 8.0% yield of the corresponding α-anomer, i.e., an α/β ratio of 0.3. When "Method C" was applied to the same reactants, the anomer-ratio obtained was α/β = 5.5. This is a much larger range of stereoselectivity than that achieved by Šorm and coworkers (12) in the analogous Hilbert-Johnson synthesis (at room temperature) of the same pair of anomers by varying the solvent (α/β = 1.9 - 3.6). Also in the reaction of bis(trimethylsilyl)-5-fluorouracil with IV, "Method B" yielded substantially the β, and "Method C" the α anomer of 3',5'-di-O-p-chlorobenzoyl-2'-deoxy-5-fluorouridine (details will be reported).

We found that the halogenose IV on standing at room temperature in benzene solution gradually undergoes a change in its optical rotation, from $[\alpha]_D^{25}$ +102° to +70.1° (c 1.12) within 6 hrs. Since our sharp-melting, crystalline preparation of IV is probably the pure α-anomer (13), we believe that the coupling reaction, in the case of Methods A and B, proceeds by S_N2 mechanism, with inversion of configuration. The mechanism of the reaction in the case of "Method C" is under investigation.

REFERENCES

1. T. J. Bardos, R. R. Herr, and T. J. Enkoji, J. Amer. Chem. Soc. 77, 960 (1955).

2. T. J. Bardos, A. Segaloff, and J. L. Ambrus, Nature 183, 612 (1959).

3. J. L. Ambrus, and A. Segaloff, Proc. Cancer Res. 3, 2 (1959).

4. T. J. Bardos, K. Baranski, and V. C. Czebotar, to be published.

5. J. J. Fox, N. C. Yung, I. Wempen, and M. Hoffer, J. Amer. Chem. Soc. 83, 4066 (1961).

6. T. Nishimura, B. Shimisu, I. Iwai, Chem. Pharm. Bull. (Japan) 11, 1470 (1963).

7. G. E. Hilbert and T. B. Johnson, J. Amer. Chem. Soc. 52, 2001 (1930).

8. R. U. Lemieux and M. Hoffer, Can. J. Chem. 39, 110 (1961).

9. T. J. Bardos and T. I. Kalman, to be published.

10. D. Shugar and J. J. Fox, Biochim. Biophys. Acta 9, 199 (1952).

11. T. J. Bardos, G. M. Levin, R. R. Herr, and H. L. Gordon, J. Amer. Chem. Soc. 77, 4279 (1955).

12. M. Prystaš and F. Šorm, Coll. Czech. Chem. Comm. 30, 2960 (1965).

13. A. K. Bhattacharya, R. K. Ness, and H. G. Fletcher, Jr., J. Org. Chem. 28, 428 (1963).