Tetrahedron Letters,Vol.24,No.25,pp 2571-2574,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

SYNTHESIS OF URACIL-5- AND ADENINE-8-PHOSPHONIC ACIDS

Tokumi Maruyama, Zenei Taira, Mitsuyo Horikawa, Yoshiko Sato and Mikio Honjo*

School of Pharmacy, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

Summary: Successive treatments of 5-bromo-2,4-dimethoxypyrimidine (I) with n-butyllithium and diethyl chlorophosphonate followed by dealkylation afforded uracil-5-phosphonic acid (V). Adenine-8-phosphonic acid (IX) was also prepared by a similar method, starting from 6-chloro-9-(tetrahydro-2-pyranyl)purine (VI).

A large variety of substituents has been introduced to the bases of nucleic acids,[1] some of which are useful in the treatment of certain types of cancer.[2] The C-phosphonate is one of the phosphorus-containing compounds of biological importance,[3] but there are no reports concerning the preparation of the C-phosphonate derivatives of the nucleobases with one exception, uracil-6-phosphonic acid. The synthesis thereof consists of cyclization of the linear phosphonate to the uracil ring.[4] We could successfully prepare a couple of phosphonates by introduction of the phosphono group to the nucleobases by substitution.

To a stirred solution of 5-bromo-2,4-dimethoxypyrimidine[5] (I) (2.86 g) in tetrahydrofuran (THF) (50 ml) was added dropwise, in the atmosphere of argon, 2.3 mM solution (II) (8.5 ml) of n-butyllithium in n-hexane.[6] The mixture was allowed to react for 1 h, to which was added a mixture of diethyl chlorophosphate (III) (5 ml) and THF (15 ml) at -78°C. After stirring for 30 min, the reaction mixture was successively treated with ammonium formate (10 ml) and concentrated aqueous ammonia (2 ml) in the usual manner. Evaporation of the organic solvent from the solution afforded a syrup, which was purified by silica gel column chromatography $\{CHCL_3$-AcOEt (4:1)$\}$ to yield a pale yellow oil (0.93 g, 26 %). The ^{1}H-NMR spectrum showed the absence of C-5 proton signal and the presence of signals due to methyl and methylen protons in the diethyl phosphonate group. The mass spectrum (MS) indicated a molecular ion peak $\{M^+$ (m/e) 276$\}$. The structure of the compound was thus established as 2,4-dimethoxypyrimidine-5-diethylphosphonate (IV).[7] In order to remove the alkyl groups,

chlorotrimethylsilane (3.6 ml) and sodium iodide (3.91 g)[8] were added to a solution of IV (900 mg) in acetonitrile (28 ml), and the mixture was stirred in room temparature for 3 h. Addition of methanol (10 ml) to the supernatant, followed by neutralization with concentrated aqueous ammonia afforded an amorphous solid. The solid was collected, dissolved in water. Treatment of the resulting aqueous solution with Amberlite IR 120B (H^+) yielded white needles (344 mg, 55 %), which showed a single UV absorbing spot ($M_{5'\text{-UMP}}$[9]=0.98) by paper electrophoresis (PEP) (0.0125 M phosphate buffer, pH 7.5, 22 V/cm). On the basis of the ^{1}H- and ^{13}C-NMR spectra (the absence of all alkyl signals) along with the elemental analysis, the product was assigned the uracil-5-phosphonic acid (V) structure.[10] To our best knowledge this is the first example of an introduction of the phosphono group to the nucleobase.

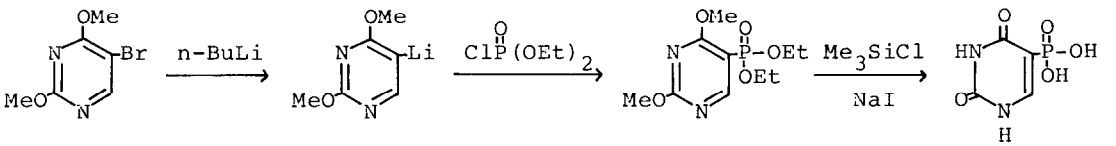

Chart 1

An analogous reaction of 6-chloro-9-(tetrahydro-2-pyranyl)purine[11] (6.95 g)(VI) with II (17.4 ml) and III (6.5 ml), followed by purification of the reaction product by silica gel column chromatography [$CHCl_3$-AcOEt (9:1)] gave a crude solid. Recryatallization of the solid from ether-n-hexane provided a slight yellow prisms (3.60 g, 33 %). The ^{1}H-NMR spectrum showed the absence of C-8 proton signal and the presence of signals due to diethyl protons. The MS gave molecular ion peaks [M^+ (m/e) 374, 376]. The product was thus assigned the 6-chloro-9-(tetrahydro-2-pyranyl)purine-8-diethyl phosphonate (VII) structure.[12]

The structure of VII was confirmed by X-ray analysis. The crystal data of the compound are a=10.142(2), b=17.552(5), c=9.830(2)Å and space group $P2_12_12_1$. The diffraction intensities with 2 up to 50° were collected on a Rigaku

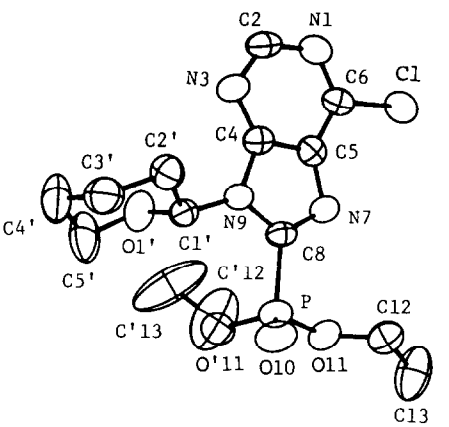

Fig. 1. ORTEP Drawing of the Compound (VII)

four-circle diffractometer with graphite-monochromated Mo-Kα radiation, by an ω scan mode. The structure was solved by the direct methods using the MULTAN program and refined by the block-diagonal least-squares calculation. The final R-value was 0.0409 for 1470 refrections ($I > 3\sigma_I$). For additional crystallographic details consult reference 13.

Successive treatments of VII (500 mg) with a saturated methanolic ammonia (20 ml) at 100°C for 24 h, and 80 % trifluoroacetic acid at room temperature for 30 min gave white prisms (201 mg, 62 %). The ^{1}H- and ^{13}C-NMR spectra showed the presence of signals due to monoethyl protons. The compound was proved to be adenine-8-monoethyl phosphonate (VIII).[14] Treatment of VIII (300 mg) with iodotrimethylsilane[8] followed by the usual work-up provided white needles (180mg, 68 %). The product had a relative mobility of $M_{5'\text{-AMP}}$[15]=1.15. The structure was established as adenine-8-phosphonic acid (IX) by the UV and ^{1}H-NMR spectrometries.[16]

Acknowledgement The work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, for which the authors' thanks are due.

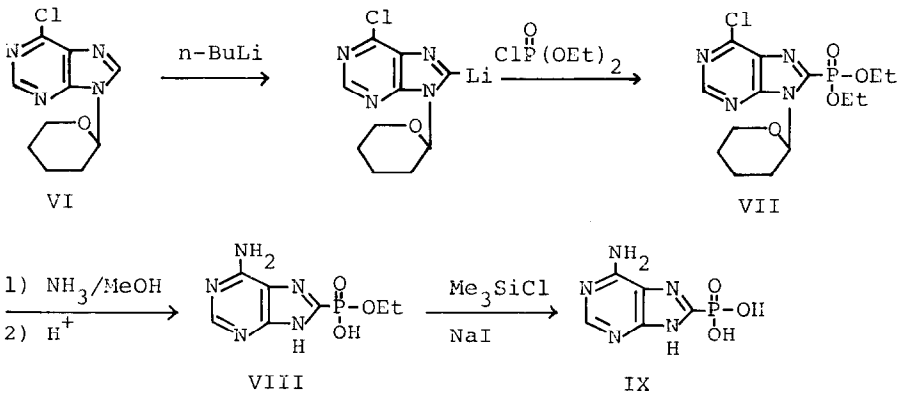

Chart 2

References and Footnotes

1) N. K. Kochetkov and E. I. Budovskii, ed., "Organic Chemistry of Nucleic Acids," Part B, Plenum Press, New York, 1972, p. 275.

2) M. J. Cline, "Cancer Chemotherapy," Vol. 1, W. B. Saunders Company, Philaderphia, 1971.

3) M. Horiguchi and M. Kandatsu, Nature, 184, 901 (1959); T. Hori, O. Itasaka, H. Inoue, and K. Yamada, J. Biochem., 56, 477 (1964); H. Shimizu, Y. Kakimoto, T. Nakajima, A. Kanazawa, and I. Sano, Nature, 207, 1197 (1965).

4) M. H. Maguire, R. K. Ralph, and G. Shaw, J. Chem. Soc.,Perkin I., 1958. 2299.

5) G. E. Hilbert and E. F.Jansen, J. Am. Chem. Soc., 56, 134 (1934).

6) B. W. Langley, J. Am. Chem. Soc., 78, 2136 (1956); N. J. Leonard and J. D. Bryant, J. Org. Chem., 37, 795 (1972); N. Cong-Danh, J. Beaucourt, and L. Pichat, Tetrahedron Letters, 1979, 2385; H. Tanaka, H. Hayakawa, and T. Miyasaka, Chem. Pharm. Bull., 29, 3565 (1981).

7) UV(MeOH): λmax 257nm, 300(sh). ^{1}H-NMR ($CDCl_3$): 8.66 (H6,d,1,J_{HCCP}=16Hz), 4.06 and 4.10 (two methoxy, 3 protons each), 3.9-4.5 ($-CH_2-$,m,4), 1.35 ($-CH_3$,t,6,J_{HCCP}=7Hz). MS m/e: 276 (M^+), 248 ($M^+-C_2H_4$), 231 ($M^+-OC_2H_5$).

8) R. B. Silverman, R. E. Radak, and N. P. Hecker, J. Org. Chem., 44, 4970 (1979); T. Morita, Y. Okamoto, and H. Sakurai, Tetrahedron letters, 1978, 2523.

9) Ratio of the migration distance of the sample to uridine-5'-phosphate.

10) Mp 290°C. Analysis Calcd. for $C_4H_5N_2O_6P\cdot 1/2H_2O$: C, 24.55; H, 2.78; N, 14.37. Found: C, 24.72; H, 2.93; N, 14.10. ^{1}H-NMR (D_2O): 7.93 (H6,d,1,J_{HCCP}=12.2Hz), ^{13}C-NMR (D_2O): 167.50 (C4,d,J_{CCP}=10.3Hz), 155.61 (C2,s), 152.07 (C6,d,J_{CCP}=15.4Hz), 107.28 (C5,d,J_{CP}=203.7Hz).

11) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis, and A. Jackson, J. Am. Chem. Soc., 83. 2574 (1961).

12) Mp 95-96°C. Analysis Calcd. for $C_{14}H_{20}N_4O_4ClP$: C, 45.01; H, 5.37; N, 14.91. Found: C, 44.93; H, 5.47; N, 14.81. UV(MeOH): λ max 270nm, 255(sh). ^{1}H-NMR ($CDCl_3$): 8.84 (H2,s,1), 6.15 (H1',q,1,$J_{1',2'a}$=2.1Hz,$J_{1',2'b}$=11), 4.1-4.6 ($-CH_2-$,m,4), 3.76 (H5'a,m,1), 3.11 (H5'b,m,1), 1.6-2.1 (H2',H3',and H4',m,6), 1.42 ($-CH_3$,q-like,6). MS m/e: 374, 376 (M^+), 291, 293 ($M^+-C_5H_9O$).

13) Final crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre.

14) Mp 222-226°C. UV(H_2O): λmax 269nm. ^{1}H-NMR (D_2O,NaOD): 8.14 (H2,s,1), 3.97 ($-CH_2-$,m,2, $J_{1',2'}=J_{HCOP}$=7.0Hz), 1.25 ($-CH_3$,t,3). ^{13}C-NMR (D_2O,NaOD): 162.52 (C4,d,J_{CNCP}=18.4Hz), 161.85 (C8,d,J_{CP}=200.8Hz), 156.34 (C6,s), 153.08 (C2,s), 124.36 (C5,d,J_{CNCP}=16.9Hz), 64.48 ($-CH_2-$,d,J_{COP}=5.2Hz), 18.93 ($-CH_3$,d,J_{CCOP}=5.9Hz).

15) Ratio of the migration distance of the sample to adenosine-5'-phosphate.

16) Mp>300°C. UV(0.05N HCl) λ max 268nm; UV(H_2O) λmax 269nm; UV(0.05N NaOH) λ max272-276nm. ^{1}H-NMR (D_2O,NaOD): 8.10 (H2,s,1).

(Received in Japan 26 February 1983)