SYNTHETIC PURINE-PYRIMIDINE BASE PAIRS. N-(5-PYRIMIDINYL)PURINE-6-CARBOXAMIDES†

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N-(4-Amino-5-pyrimidinyl)purine-6-carboxamide (I) was synthesized by condensation of 4,5-diaminopyrimidine with 6-purinoyl chloride in pyridine or with 6-trichloromethylpurine in buffer citrate. Related purine-pyrimidine pairs of the form Pu-CONH-Py were prepared similarly. The spectral properties of the base pairs suggest a preferred conformation involving hydrogen bonding between the amide hydrogen and the purine N(7).

The phenomenon of base-pairing in double-stranded DNA is the first step to a "grammar of biology"[1]. Purine-pyrimidine pairs served as a focus of interest, both theoretically and experimentally.[2-4] Surprisingly, synthetic base pairs have received little attention. Interaction between adjacent bases in a nucleic acid strand contribute significantly to the structural stability of both single and double-stranded nucleic acids. Leonard and coworkers investigated a series of dinucleotide analogs in which the purine and pyrimidine

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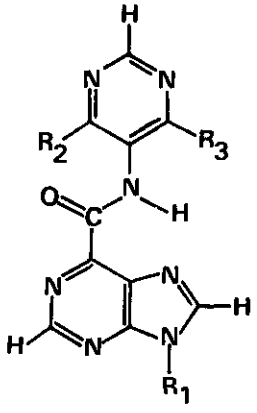
† Dedicated to Professor Tetsuo Nozoe illustrious master of organic chemistry and doyen of Japanese chemists — on the occasion of his seventy seventh birthday.



I. $R_1 = R_2(R_3) = H,\ R_3(R_2) = NH_2$

II. $R_1 = H,\ R_2 = R_3 = NH_2$

III. $R_1 = CH_3,\ R_2 = R_3 = NH_2$

IV. $R_1 = H,\ R_2 = R_3 = NHCH_3$

bases were connected by a polymethylene chain ($B-C_n-B'$).[5] These synthetic base pairs permitted a study of the interaction between the adjacent bases in the absence of complicating factors associated with hydrogen bonding and the usual carbohydrate and phosphodiester linkages. Pyrimidine derivatives of the toxic thioguanine showed anticancer activity.[6] We now report the synthesis and spectral properties of purine-pyrimidine model pairs of the general structure Pu—CONH—Py, which were prepared in an attempt to simulate a condition of permanent pairing between the bases. Such base pairs may serve as a basis for potential anticancer agents.

The preparation of N-(4-amino-5-pyrimidinyl)purine-6-carboxamide (I) illustrates the synthetic methods used. Method A. 6-Methylpurine was chlorinated with SO_2Cl_2 in CF_3CO_2H to 6-trichloromethylpurine;[7] ^1H NMR,

δ ($CDBr_3$): 8.63 (s, 1H) and 9.13 ppm (s, 1H). Condensation of the latter with 4,5-diaminopyrimidine in an aqueous-methanolic buffer citrate solution (pH 4) gave I. This method, first applied by Cohen and coworkers for the synthesis of N-(4,6-diamino-5-pyrimidinyl)purine-6-carboxamide (II),[8-9] suffers from the disadvantage of being relatively slow and of giving products contaminated with polymers derived from 6-purinoic acid. Method B. 6-Methylpurine was oxidized to 6-purinoic acid,[10] which was converted to 6-purinoyl chloride by PCl_5[11] or $SOCl_2$. Condensation of the acyl halide with 4,5-diaminopyrimidine in boiling pyridine afforded I. Purification by repeated triturations and recrystallizations from water and DMF gave I as an orange powder, mp >300° (dec). Mass spectrum, m/e 256 (M^+, rel. int. 72), 238 ($[M - H_2O]^+$, 27), 228(98), 211(22), 200(21), 137(70), 120(100), and 119(79). ^1H NMR (270 MHz), δ (DMSO - d_6): 6.89(s, 2H, NH_2), 8.10(s, 1H, Py), 8.30 (s, 1H, Py), 8.76(s, 1H, Pu), 9.11(s, 1H, Pu), and 10.52 ppm (s, 1H, NH amide). UV; λ_{max} (H_2O), nm, 231 and 292; λ_{max} (0.1 N NaOH), nm 280 and 342s; λ_{max} (0.1 N HCl) nm, 262s and 292. Compounds II, N-(4,6-diamino-5-pyrimidinyl)-9-methylpurine-6-carboxamide (III) and N-(4,6-bismethylamino-5-pyrimidinyl)-purine-6-carboxamide (IV) were prepared analogously. The purity of each base pair was verified by its high-field (270 MHz) ^1H NMR spectrum. The following spectral properties of the base pairs should be noted. (i) The amide proton absorbs at a very low field: δ(I) = 10.52 ppm. For comparison, in N-methylpurine-6-carboxamide, δ (NH amide) = 9.06 ppm. The fixation of the N(7) purine tautomer by the 9-methyl group shifts this absorption to a significantly lower field (δ(III) = 11.17 ppm), while the introduction of two

bulky substituents ortho the amide linkage leads to shifts in the opposite direction (δ(II) = 9.86 and δ(IV) = 9.68 ppm). (ii) Unlike base pairs previously reported,[8,9,12] the present compounds hardly displayed any novel features in the UV spectra, viz. an intense absorption in the 340-370 nm region. Such a band tends to appear only after keeping the base pair in solution for an extended period. (iii) The mass spectrum of each base pair is characterized by an abundant signal due to an $[M - H_2O]^+$ species, which is absent in N-methylpurine-6-carboxamide.

The following conformational aspects of the base pairs were considered: (a) The spatial orientation of the amide linkage vis-a-vis the planes of the Pu and the Py rings. (b) Hydrogen bonding between the 4-amino group of the Py and N(1) or N(7) of the Pu ring. (c) Hydrogen bonding between the amide hydrogen and N(7) of the Pu ring. (d) Trans versus cis configuration of the amide linkage. (e) Stacking between the Pu and the Py rings. Definite conclusions pertaining to the conformation of the synthetic base pairs cannot be reached at this stage. However, the evidence favors a preferred conformation with hydrogen bonding between the amide hydrogen and the N(7) of Pu rather than a conformation with hydrogen bonding between the ortho amino group of Py and N(7) (or N(1)) of Pu. The Pu - CONH - Py pair tends to remain planar but becomes sensitive to steric hindrance by two substituents ortho to the amide linkage.

A trans-configuration of the base-pair may explain a facile elimination of an H_2O molecule (by condensation of an ortho amino group and the carboxyl) leading to an 8-purinoyl-6-purine.[13]

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