8-Phosphorus substituted isosteres of purine and deazapurines

Tasneem A. Khwaja and Hema Pande

Department of Pathology and USC Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90033, USA

Received 8 June 1979

ABSTRACT

Synthesis of 8-phosphorus substituted isosteres of purine [pyrimidino(4,5-d)-1,3,2-diazaphosphole], 1-deazapurine [pyridino(2,3-d)-1,3,2-diazaphosphole] and 3-deazapurine [pyridino(4,5-d)-1,3,2-diazaphosphole] has been achieved by the reaction of equimolar amounts of triphenylphosphite and 4,5-diaminopyrimidine, 2,3-diaminopyridine and 3,4-diaminopyridine, respectively. These compounds hydrolyzed (cleavage of the phosphorus-nitrogen bonds) in aqueous solutions to provide the corresponding diaminopyrimidines. These three new basic ring systems constitute the first reported synthesis of purines in which ring carbon atom is substituted with a phosphorus atom. 8-Phosphorus substituted purine at a concentration of $4 \times 10^{-4}$M caused a 50% inhibition in the growth of leukemia L1210 cells in culture. The biochemical rationale for the synthesis of these compounds is discussed.

INTRODUCTION

8-Phosphorus substituted purine isosteres should present extremely interesting adenine and guanine analogues. We have studied molecular models which show that such a modification should not cause any major conformational or steric change in the purine ring system. Thus, there is a considerable probability that like 8-aza-guanine these analogues would be metabolized to the corresponding 5'-phosphates and incorporated into cellular nucleic acids (1). The instability of P-N bond of 8-phosphorus substituted analogues of adenosine 5'-diphosphate (ADP) and adenosine 5'-triphosphate (ATP) in aqueous solution may provide interference in cellular energy transfer reactions mediated via ADP or ATP. Similarly, incorporation of 8-phosphorus substituted adenine and guanine analogues into cellular nucleic acids and consequent P-N bond cleavage should provide undesirable depurination of the nucleic acids (due to opening of diazaphosphole ring) and this interference in nucleic acid synthesis should result in the inhibition of the cell growth. The synthetic procedure could be applied for the synthesis of $^{32}$P-labelled adenine/guanine isosteres which may incorporate into cellular nucleic acids. Hydrolytic depurination of such nucleic acids could be utilized for studying their sequence.
Studies on the synthesis of phosphorus substituted purine derivatives have been reported by Dannley and Wagner (2). They reported the preparation of dihydro-1,3,2-benzodiazaphosphole-2-oxides (I, R=R' = CH) by refluxing an addition product of phenylphosphonic dichloride with o-diamines in bromobenzene. Under similar conditions, a corresponding pyridine derivative (I, R=CH and R'=N) was also prepared but attempts to synthesize the corresponding pyrimidine derivative (I, R=R'=N) were unsuccessful (3). Lister and Timmis (4) have reported the condensation of 4,5-diaminopyrimidines with phenylphosphonodiamidate to provide a number of 3- and 7-substituted pyrimidine diazaphosphole derivatives (II). None of these compounds (II) showed any biological activity which may be due to the lack of their structural analogy with the parent purine.

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\text{(I)}
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\[
\text{(II)}
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Malavaud et al (5) have recently described synthesis of polymeric N-alkyl benzodiazaphospholes (III) by the reaction of tris(dimethylamino)phosphine with 2-(N-alkyl)-1-aminobenzane. At high temperature in solutions these compounds (6) were found to exist in monomeric form (IV).

\[
\text{(III)}
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\[
\text{(IV)}
\]
METHODS AND MATERIALS

Elemental analysis was performed by Galbraith Laboratories, Inc., Knoxville, Tennessee. The ultraviolet spectra were determined on a Cary 118C spectrophotometer. The infra-red spectra were taken on a Beckman Model 4210 spectrophotometer. The mass spectral data was obtained on a MS 9 AEI mass spectrometer. NMR data was obtained on a Varian EM-390 instrument.

Synthesis of 1,3-dihydro-2-chloro,1,3,2-pyrimidino(4,5-d)diazaphosphole (VI). A solution of phosphorus trichloride (0.63 g, 4.58 mM) in 5 ml bromobenzene was slowly added to a heated (110°) suspension of 4,5-diaminopyrimidine (V, 0.50 g, 4.54 mM) in 15 ml of bromobenzene. The resulting solution was kept under reflux for 18 hours in the absence of moisture. The precipitated product VI was carefully washed with dry bromobenzene and hexane, filtered and dried under vacuum. Yield, 0.32 g, 40.3%, m.p. 198°. NMR (DMSO-d_6): T 1.48 (S,2H, exchangeable with D_2O, N-H), 1.8(S,1H, Ar-H), 2.46(S,1H, Ar-H). Analysis: C_{12}H_{12}N_4PCl. 0.75 H_2O requires C, 25.53; H, 2.93; N 29.79; Cl, 18.28 and P, 16.48%. Found: C, 26.16; H, 3.19; N, 30.67; Cl, 17.76 and P, 16.03%.

Synthesis of pyrimidino(4,5-d)-1,3,2-diazaphosphole (VII). A mixture of 4,5-diaminopyrimidine (V, 0.40 g, 3.63 mM) and triphenylphosphite (1.14 g, 3.67 mM) was heated (bath temperature 150-180°) under vacuum (1 mm) in a flask connected with a distillation set up. During 4-5 hours period 0.92 g (10.45 mM) of phenol distilled over. The residue was titurated with hot xylene (3 X 10 ml) to obtain crystalline product VII. The product was filtered and washed with dry chloroform (3 X 10 ml) and ethanol (3 X 10 ml) and dried to obtain 0.26 g (52%) of VII, m.p. 360°. Analysis: C_{14}H_{14}N_4P. 2.5 H_2O requires C, 26.23; H, 4.40; N, 30.59 and P, 16.9%. Found: C, 26.35; H, 4.45; N, 29.09 and P, 16.83%.

Synthesis of pyridino(2,3-d)-1,3,2-diazaphosphole (X). A stirring mixture of 2,3-diaminopyridine (0.40 g, 3.67 mM) and triphenylphosphite (1.16 g, 3.67 mM) was heated under reduced pressure (1 mm) in a distillation apparatus for 5 hours at 150-180° until no more phenol distilled over. The residue containing the product X was worked up as described for the synthesis of compound VII, yield 0.26 g (52%), m.p. 360°. Analysis: C_{15}H_{15}N_4P. 3 H_2O requires C, 31.41; H, 5.27; N, 21.98 and P, 16.23%. Found: C, 31.91; H, 5.39; N, 20.93 and P, 16.73%.

Synthesis of pyridino(4,5-d)-1,3,2-diazaphosphole (XI). 4,5-Diaminopyridine (0.40 g, 3.67 mM) was heated with 1.16 g (3.67 mM) of triphenylphosphite (bath temperature 150-180°) under reduced pressure. During 4 hours 0.86 g of phenol distilled
The product was isolated as described for the synthesis of compound VII, yield 0.28 g (55.6%), m.p. 196°. Analysis: C_9H_4N_3P; 3H_2O requires C, 31.41; H, 5.27; N, 21.98 and P, 16.23%. Found: C, 30.77; H, 5.13; N, 21.80 and P, 16.82%.

Studies on hydrolysis of compounds VII, X and XI. Each compound (1 mg) was suspended in 0.5 ml of water, or HC1 (pH 1), or NaOH (pH 11), or phosphate buffer (0.04 M, pH 7). The stoppered suspensions were kept at 37° in a shaker bath. After fixed intervals of time aliquots of the solution were spotted and analyzed by thin layer chromatography (cellulose coated plates) in n-butanol-water (84:16) system. The ultraviolet absorbing spots corresponding to the starting materials and the products were scrapped, eluted and the quantity of each component was calculated by optical density measurements at their absorption maxima in water. The half-life of each compound was calculated from the slope of the curve obtained by plotting the percent decomposition of each as a function of time.

Studies on the effect of compounds on the growth of leukemia L1210 in culture. Mouse leukemia L1210 was obtained from Associated Biomedic Systems, Inc., Buffalo, New York and are grown in suspension culture in RPMI-1640 media containing 10% fetal calf serum. These cells have a doubling time of 12 hours. For drug screening logarithmically growing cells (1 X 10^5 cells per ml) were exposed to a series of drug dilutions (10^{-6} M to 10^{-3} M) for 48 hours in a CO_2 incubator maintained at 37°. The growth of the cells was followed by counting cell number in a Coulter Counter. Compound VII at concentrations of 4 X 10^{-4} M and 1 X 10^{-3} M caused a 50% and 100%, respectively, inhibition of the growth of leukemia L1210 in culture. Compounds X and XI were inactive at 1 X 10^{-3} M concentration.

RESULTS AND DISCUSSION
We have used two approaches for the synthesis of 8-phosphorus substituted purine isosteres. 4,5-Diaminopyrimidine (V) was refluxed with phosphorus trichloride (7) to provide the corresponding phosphorochloridite (VI). Attempted dehydrohalogenation of VI was not successful. This may be due to the generation of hydrogen chloride during the reaction which should form a phosphinium salt with the product (VII) and thus catalyze the decomposition of the diazaphosphole ring system.
Interestingly when phosphorus trichloride was replaced by triphenylphosphite, the reaction went smoothly with the elimination of three equivalents of phenol and the residue was crystallized to obtain pyrimidino(4,5-d)-1,3,2-diazaphosphole (VII) in 52% yield.

The structure of VII was assigned on the basis of following. It gave correct elemental analysis. Mass spectral analysis showed highest molecular ion peak at 138 mass unit which corresponds to the molecular weight of the proposed structure VII. Field desorption mass spectrometry of VII did not give any signals.
corresponding to higher molecular weight species showing (c.f. reference 5) that the compound existed in a single monomeric form (table 1). The ultraviolet spectrum of VII corresponded to a N-substituted diaminopyrimidine and was different than the starting 4,5-diaminopyrimidine (V, table 1). The infrared spectrum of VII showed a strong band at 1210 cm\(^{-1}\) due to P=N double bond and a moderate stretching vibration at 775 cm\(^{-1}\) due to the presence of P-N single bond (8).

### TABLE 1

Some characteristics of 8-phosphorus substituted purine isomers.

<table>
<thead>
<tr>
<th>Product</th>
<th>IR (P=N)</th>
<th>UV ((\lambda_{\text{max}}^\text{H}_2\text{O}))</th>
<th>Mass Fragmentation m/e</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>1210 cm(^{-1})</td>
<td>285 nm ((\varepsilon_{\text{max}}^\text{7,500}))</td>
<td>138 (M(^+)), 110, 83, 66, 56, 44, 28</td>
</tr>
<tr>
<td>X</td>
<td>1185 cm(^{-1})</td>
<td>248, 316 nm ((\varepsilon_{\text{max}}^\text{4132, 6688}))</td>
<td>137 (M(^+)), 109, 105, 91, 82, 65, 55, 44, 28</td>
</tr>
<tr>
<td>XI</td>
<td>1167 cm(^{-1})</td>
<td>223, 273 nm ((\varepsilon_{\text{max}}^\text{11,900, 7130}))</td>
<td>137 (M(^+)), 109, 82, 81, 65, 55, 44, 28</td>
</tr>
</tbody>
</table>

Compound VII rapidly dissolved in aqueous hydrochloric acid (rapid quaternization of the dicoordinated phosphorus atom) and decomposed to release 4,5-diaminopyrimidine (V, table 2).

\[
\text{VII} + 3\text{H}_2\text{O} \rightarrow \text{IV} + \text{H}_3\text{PO}_3
\]

(VII)
Table 2

Rates of hydrolysis ($t_{0.5}$) of 8-Phosphorus substituted purine isosteres

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conditions of hydrolysis</th>
<th>HCl (pH 1)</th>
<th>NaOH (pH 11)</th>
<th>H₂O</th>
<th>Phosphate buffer pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>5 min.</td>
<td>30 min.</td>
<td>24 hr.</td>
<td>11 hr.</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>10 min.</td>
<td>1 hr.</td>
<td>24 hr.</td>
<td>8 hr.</td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>10 min.</td>
<td>2.5 hr.</td>
<td>10 hr.</td>
<td>24 hr.</td>
<td></td>
</tr>
</tbody>
</table>

Pyridino(2,3-d)-1,3,2-diazaphosphole (X) and pyridino(4,5-d)-1,3,2-diazaphosphole (XI) were synthesized by the reaction of triphenylphosphite with appropriate diaminopyridines as isosteres of 1-deazapurine and 3-deazapurine, respectively. Studies on the synthesis of benzo-1,3,2-diazaphosphole (IV, R=H) will be described elsewhere.

Our studies on the effect of the new purine analogues on the growth of murine leukemia L1210 showed that only 8-phosphorus substituted purine isostere (VII) was biologically active whereas 1-deazapurine (X) and 3-deazapurine (XI) isosteres were completely inactive. The modest activity of VII is due to the structure VII itself as 4,5-diaminopyrimidine (V) (the product of hydrolytic cleavage of VII) at 1 X 10⁻³ M concentration did not show any effect on the growth of L1210 leukemia in culture. We are currently developing methods for the synthesis of 8-phosphorus substituted adenine and guanine, isosteres in order to test the hypothesis presented here (9,10).
ACKNOWLEDGEMENTS

The research was supported by a National Cancer Institute, PHS Grant CA 14089-03 and CA 25715-01. We are thankful to Mrs. Louise Momparler and Ms. Jan Varven for excellent technical assistance during leukemia L1210 assays. We are also thankful to Dr. H. Boettger, JPL Laboratories, Pasadena, California for providing mass spectrum analysis.

REFERENCES

3. We have synthesized 1,3-dihydro-2-phenyl-pyrimidine(4,5-d)-1,3,2-diazaphosphole-2-oxide (I, R=R'=N and R''=R'''=H) by adding equimolar amounts of phenyl phosphonic dichloride to a heated (120°) solution of 4,5-diaminopyrimidine (V) in dry bromobenzene. In this way HCl gas generated during the reaction was immediately eliminated. The structure of the compound was confirmed by its PMR and mass spectrum analysis, m.p. 218°.
6. Pilgram, K. and Krote, K. (1963) Tetrahedron, 19, 137-141 have described a synthesis of monomeric N-phenyl benzodiazaphosphole. However, Malavaud et al (5) by mass spectrometric analysis showed that the compound was a mixture of monomeric, dimeric, trimeric and higher forms.
9. We are also developing methods for the synthesis of 2-phosphorus substituted adenine and guanine isosteres.
10. The present work in part was presented at 'Pacific Conference on Chemistry and Spectroscopy' held in San Francisco, California during Sept. 27-29, 1978.