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Building Blocks for Polyamide Nucleic Acids: Facile Synthesis Using Potassium Fluoride Doped Natural Phosphate as Basic Catalyst

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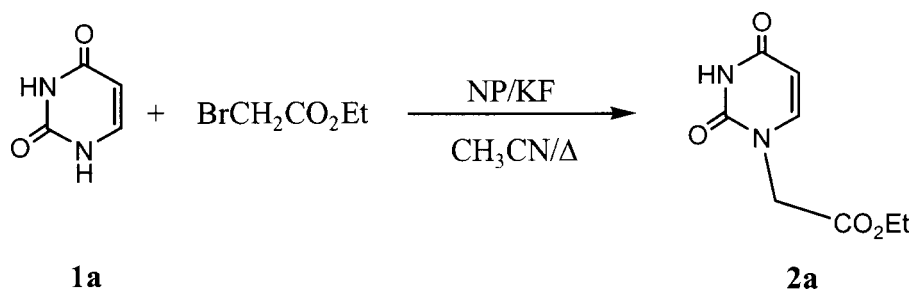
ABSTRACT

Potassium fluoride doped natural phosphate, inexpensive and environmentally friendly catalyst, is shown to be an efficient basic catalyst for the N1/N9 alkylation of different nucleobases as synthons for PNAs.

In 1991, Nielson et al.^[1] developed a new class of oligonucleotide analogues known as Polyamide (or Peptide) Nucleic Acids (PNAs) in which the entire sugar phosphate backbone has been replaced by a peptide-like backbone. These oligomers

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Scheme 1.

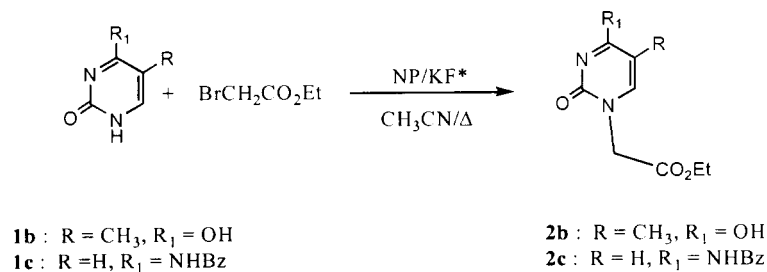
of nucleobase are derived from N-(2-aminoethyl) glycine which recognize and bind strongly to specific DNA or RNA sequences.^[2] These characteristics make them potentially and extremely useful as an antisense or antigene drug.^[3]

The application of inorganic solid acids as heterogeneous catalysts for organic synthesis is an area of intense research. Silica gel, alumina, montmorillonite, zeolite and natural phosphate have been shown to function as effective catalyst for liquid-phase organic transformations.^[4] The advantages of these heterogeneous catalysts over the homogeneous systems include stability, ease handling, lack of corrosion and other environmental hazards, and ease of recovery and regeneration.

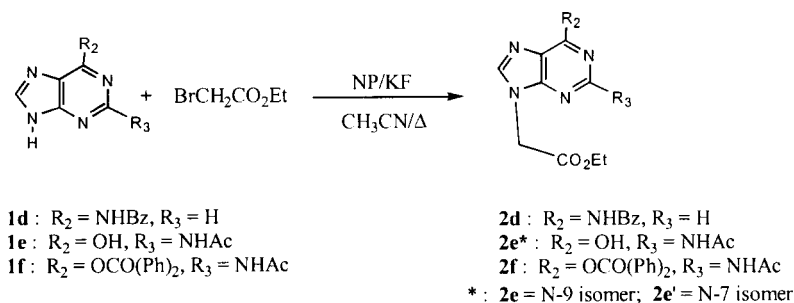
We have shown recently that natural phosphate is a new Lewis acids catalysts for 1,3-dipolar cycloaddition^[5] and for acyclonucleoside synthesis.^[6] In continuation of our program on the use of natural phosphate as catalyst and in the search of an alternative strategy which would open the way to a combination of PNA and oligonucleotides synthesis, we have developed a new and easy synthesis of the PNA precursor (ethyl acetate-nucleobase) using a cheap KF doped natural phosphate as a basic catalyst (Sch. 1, 2, 3).

The use of potassium fluoride on alumina (KF/Al₂O₃) as a base for functionalization of amide and N-alkylation, has been described in the literature.^[7] In order to assess influence of natural phosphate doped with KF as basic catalyst on the synthesis of ethyl acetate-nucleobase derivatives, a number of experiments were performed to optimize reaction conditions. Results of these studies, are summarized in Table 1.

Both KF and NP/KF (175/25) had a weak catalytic activity (entries 1 and 2). When the amount of NP/KF was increased (350/50), the reaction yield was tripled



Scheme 2.



Scheme 3.

Table 1. Catalyst influence on the N-alkylation of uracil.

Entry	Catalyst	Weight ratio (mg/mg) ^a	Time (h)	Yield (%) ^b
1	KF	50	10	28
2	NP/KF	175/25	2	15
3	NP/KF	350/50	1.5	50

^aThe amount of catalyst used in reactions with 100 mg of uracil.^bPurification by silica gel chromatography.

(entry 3). The reaction was monitored by thin layer chromatography, and it was stopped when the N1,N3-bisalkylated product appeared. This procedure appears to be regioselective and gives only the N1 isomer for uracil (Sch. 1).

To expand the scope and the synthetic utility of this reaction using NP/KF (350/50), we next examined the N-alkylation of other nucleobases under similar conditions (Sch. 2, 3 and Table 2).

The poor solubility of unprotected nucleobases (cytosine, adenine and guanine) excluded their use in most reactions. Introduction of a protecting group was necessary to increase the solubility of these bases.^[8,9]

Table 2. Alkylation of different heterocyclic bases.

Entry	Heterocyclic base	Time (h)	Yield (%) ^a
1	Uracil	1a 1.5	50
2	Thymine	1b 2	55
3	4- <i>N</i> -Benzoylcytosine	1c 1	60
4	4- <i>N</i> -Benzoyladenine	1d 4	50
5	2- <i>N</i> -Acetylguanine	1e 4	43 ^b
6	2- <i>N</i> -acetyl-6- <i>O</i> -(<i>N,N</i> -diphenylcarbamoyl)guanine	1f 3	70 ^c

^aPurification by silica gel chromatography.^bN9/N7: 60/40 yield ratio.^cN9/N7: 95/5 yield ratio.

These protected nucleobases were alkylated to give the desired N1-alkylated pyrimidines (entries 1–3) and N9-alkylated purines derivatives along or with other regioisomers (entries 4–6). It was reported that 6-*O*-(*N,N*-diphenylcarbamoyl) protected guanine **1f** has been reported to undergo alkylation with high regioselectivity to give in some cases 99/1 ratio in favour of the N9 regioisomer.^[9]

Interesting, we found that the 2-*N*-acetyl 6-*O*-(*N,N*-diphenylcarbamoyl)guanine **1f** (Sch. 3) when reacted with ethyl bromoacetate in the presence of NP/KF afforded a nearly 95/5 ratio of N9/N7 isomers in 70% yield (entry 6, Table 2).

All compounds were characterized fully by spectroscopic and elemental analyses, which were found to be in accordance with the proposed structures.^[8] In conclusion, we showed that our method, in general, provides the desired ethyl acetate-nucleobases in yields comparable to those reported in the literature using basic conditions.

On the other hand the use of KF doped natural phosphate as basic catalyst provides a significant new and effective method for the environmentally compatible and practical synthesis of these derivatives.

EXPERIMENTAL

The ¹H NMR spectra were recorded using a Bruker AC 250 MHz spectrometer DMSO-*d*₆ was used as a solvent and internal reference. Mass spectra (MS) were obtained with JEOL JMS DX 300 instrument using fast atomic bombardment (FAB⁺). Thin layer chromatography was performed on plates of kieselgel 60 F254 (Merck) and short-wave ultraviolet light (254 nm) was used to detect the UV-absorbing spots. Column chromatography separation was carried out on silica gel (0.063–0.2 mm Merck).

For the preparation of natural phosphate see Ref.^[4c]

Preparation of Doped Natural Phosphate

350 mg of natural phosphate and 50 mg of KF were mixed in 10 mL of water and evaporated to dryness and dried for 6 h at 150°C. The obtained solid residue was used as basic catalyst in alkylation reactions.

General Procedure

A typical experimental procedure is described for uracil. To a mixture of uracil (100 mg, 0.892 mmol) and ethyl bromoacetate (2 eq) in dry acetonitrile (12 mL) was added NP/KF (350 mg/50 mg). After stirring for 1.5 h at reflux, the mixture was filtered and the filtrate was concentrated in vacuo. Purification of the residue by flash column chromatography gave N-1 (ethyl acetate) uracil (80 mg, 50%) as a white precipitate.

1-(Ethoxycarbonylmethyl)uracil 2a. Yield: 50%, *R*_f = 0.62 (CHCl₃/MeOH) (90/10, v/v), ¹H NMR δ : 11.4 (s, 1H, NH-3); 7.65 (d, 1H, H-6, *J* = 7.8 Hz); 5.65 (d, 1H, H-5, *J* = 7.8 Hz); 4.55 (s, 2H, NCH₂); 4.17 (q, 2H, COOCH₂CH₃); 1.23 (t, 3H, COOCH₂CH₃). MS (FAB⁺, GT) *m/z* 199 [M + H]⁺.

1-(Ethoxycarbonylmethyl)thymine 2b. Yield: 55%, $R_f = 0.70$ ($\text{CHCl}_3/\text{MeOH}$) (90/10, v/v), ^1H NMR δ : 7.21 (s, 1H, H-6); 4.40 (s, 2H, NCH_2); 4.20 (q, 2H, $\text{COOCH}_2\text{CH}_3$); 1.90 (s, 3H, CH_3); 1.25 (t, 3H, $\text{COOCH}_2\text{CH}_3$), MS (FAB^+ , GT) m/z 213 $[\text{M} + \text{H}]^+$.

4-N-benzoyl-1-(Ethoxycarbonylmethyl)cytosine 2c. Yield: 60%, $R_f = 0.81$ ($\text{CHCl}_3/\text{MeOH}$) (90/10, v/v), ^1H NMR δ : 11.25 (s, 1H, NHBz); 8.12 (d, 2H, *o*-H benzoyl); 8.0–7.34 (m, 5H, H-5 and H-6; *m,p*-H Benzoyl); 4.64 (s, 2H, NCH_2); 4.15 (q, 2H, $\text{COOCH}_2\text{CH}_3$); 1.20 (t, 3H, $\text{COOCH}_2\text{CH}_3$), MS (FAB^+ , GT) Mass spectrum FAB^+ (GT) m/z 304 $[\text{M} + \text{H}]^+$.

6-N-(Benzoyl)-9-(Ethoxycarbonylmethyl)adenine 2d. Yield: 47%, $R_f = 0.51$ ($\text{CHCl}_3/\text{MeOH}$) (90/10, v/v), ^1H NMR δ : 11.24 (s, 1H, NHBz); 8.80 (s, 1H, H-2); 8.50 (s, 1H, H-8); 8.10 (d, 2H, *o*-H Benzoyl); 7.70–7.58 (m, 3H, *m,p*-H Benzoyl); 5.28 (s, 2H, NCH_2); 4.24 (q, 2H, $\text{COOCH}_2\text{CH}_3$); 1.27 (t, 3H, $\text{COOCH}_2\text{CH}_3$); MS (FAB^+ , GT) m/z 326 $[\text{M} + \text{H}]^+$.

2-N-(Acetyl)-9-(Ethoxycarbonylmethyl)guanine 2e. Yield: 25%, $R_f = 0.34$ ($\text{CHCl}_3/\text{MeOH}$) (90/10, v/v), ^1H NMR δ : 12.12 (s, 1H, NHAc); 11.61 (s, 1H, NH-3); 8.15 (s, 1H, H-8); 5.22 (s, 1H, NCH_2); 4.18 (q, 2H, $\text{COOCH}_2\text{CH}_3$); 2.51 (s, 3H, CH_3CO); 1.22 (t, 3H, $\text{COOCH}_2\text{CH}_3$); MS (FAB^+ , GT) m/z 280 $[\text{M} + \text{H}]^+$.

2-N-(Acetyl)-7-(Ethoxycarbonylmethyl)guanine 2e'. Yield: 18%, $R_f = 0.52$ ($\text{CHCl}_3/\text{MeOH}$) (90/10, v/v), ^1H NMR δ : 12.12 (s, 1H, NHAc); 11.62 (s, 1H, NH-3); 8.15 (s, 1H, H-8); 5.21 (s, 1H, NCH_2); 4.17 (q, 2H, $\text{COOCH}_2\text{CH}_3$); 2.20 (s, 3H, CH_3CO); 2.2 (t, 3H, $\text{COOCH}_2\text{CH}_3$); MS (FAB^+ , GT) m/z 280 $[\text{M} + \text{H}]^+$.

2-N-(Acetyl)-9-(Ethoxycarbonylmethyl)guanine 2g. Yield: 67%, $R_f = 0.80$ ($\text{CHCl}_3/\text{MeOH}$) (90/10, v/v), ^1H NMR δ : 10.80 (s, 1H, NH-3); 8.70 (s, 1H, H-8); 7.35–7.60 (m, 10H, phenyl); 5.26 (s, 1H, NCH_2); 4.20 (q, 2H, $\text{COOCH}_2\text{CH}_3$); 2.58 (s, 3H, CH_3CO); 1.26 (t, 3H, $\text{COOCH}_2\text{CH}_3$); MS (FAB^+ , GT) m/z 555 $[\text{M} + \text{H}]^+$.

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