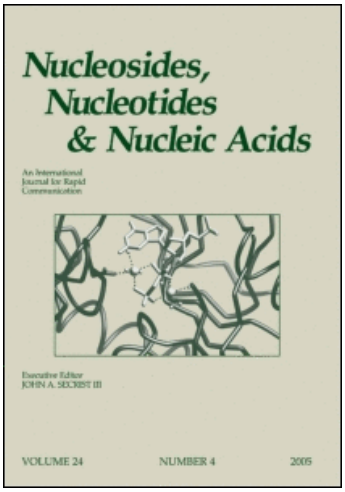

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Anomalously Coupled Nucleosides

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ANOMALOUSLY COUPLED NUCLEOSIDES

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Abstract. Polyphosphoric amide reagents are used to prepare purines which are coupled with unprotected 2-deoxy-D-ribose at C-3 of the carbohydrate using tributylammonium polyphosphates in chloroform. Phthalimide can be used instead of purines to produce N-protected 3-amino-2,3-dideoxypentoses.

In the synthesis of peptides, nucleosides, nucleotides, and other biologically important substances phosphates have often been used to simulate conditions under which evolution could occur.

The biosynthesis of proteins is assumed to proceed through amino-acyl adenylates formed from the enzymatic reaction of adenosine triphosphate (ATP) with the amino-acid. Therefore, it has been suggested that the earliest organisms may have used polyphosphates or pyrophosphates. In TABLE 1 are reported the results of Rabinowitz et al.[1] who observed that the best yield of diglycine was obtained with 0.1 M sodium trimetaphosphate at pH 7-8.

Concerning prebiotic synthesis of nucleotides a variety of experimental approaches have met with success. Nucelotides have been obtained with dry heating of nucleosides and phosphates. In aqueous solution very similar condensations have been achieved. The phosphorylation of uridine with ethylisocyanide as the condensing reagent was reported by Lohrmann and Orgil[2].

In order to achieve biologically important nucleosides and to avoid time-consuming routes with protected sugar derivatives, Schramm et al.[3] investigated the possibility of simply heating of a mixture of adenine, deoxyribose, and phenyl polyphosphate under acidic conditions at 50°C in DMF. Although they succeeded in employing unprotected sugars for the

TABLE 1. Phosphates in synthesis under prebiotic conditions.

Reactants	Product	Yield	Conditions
glycine + trimetaphosphate	diglycine	35%	pH 8, 70°C
uridine + orthophosphate + EtNC	uridine 5'-phosphate	3%	pH 7, 65°C
adenine + deoxyribose polyphosphate-ester	deoxy-adenosine	30%	pH 2, 50°C in DMF
N^6-substituted adenines + polyphosphates + deoxyribose	2,3-dideoxy-3-(9-adenyl)-D-threo pentopyranoses	14-73%	40°C, 7 days in $CHCl_3$
hypoxanthine + polyphosphoric amides	N^6-substituted adenines	21-83%	150°C, 24 h without solvent

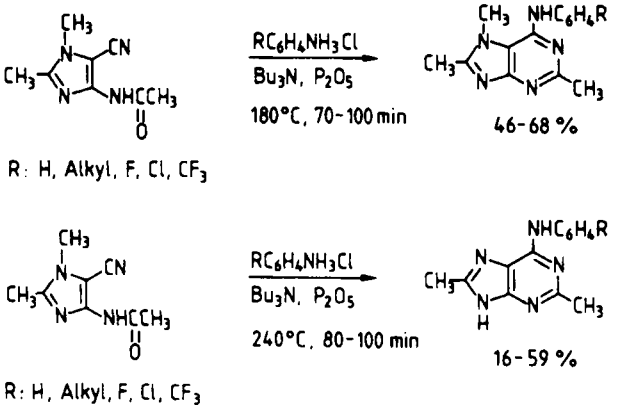
coupling reaction, their procedure was nevertheless time-consuming since a Dowex column was needed for isolation of all the isomers of which only α- and β-deoxyadenosine were well characterized.

In order to improve the nucleoside coupling synthesis of Schramm et al. we changed the reaction conditions into tributylammonium polyphosphates in chloroform, but the coupling of purine now took place in an anomalous fashion at C-3 of the carbohydrate[4]. This will be the main subject of this presentation. Also it will be shown how conditions corresponding to very vigorous conditions in the prebiotic world can be used for synthesis of purines. In TABLE 1 an example is given for pre-

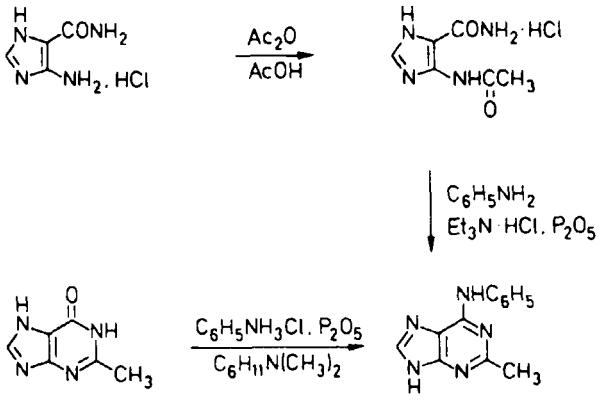
paration of N^6-substituted adenines using polyphosphoric amides as reagents at 150°C without any solvent[5]. We have also developed several new syntheses of purines from imidazole or pyrimidine derivatives using the polyphosphoric amide reagents.

We have prepared a series of 2,7,8-trimethyl-6-arylaminopurines by heating one equivalent of 4-acetylamino-1,2-dimethyl-1H-imidazole-5-carbonitrile with four equivalents of P_2O_5, tributylamine, and a primary amine hydrochloride at 180-190°C for 70-100 min. in 46-68% yield (SCHEME 1)[6]. A series of the corresponding N7-demethylated compounds was synthesized from the same starting materials in 16-59% yield simply by raising the oil bath temperature to 240°C. The latter compounds may prove useful as bases in synthetic nucleoside analogues. N,N-Dimethylcyclohexylamine (DMCA) could also be used as the tertiary amine instead of tributylamine, but this resulted in a side reaction with methylation of the demethylated compound at N9, probably because DMCA could act as an alkylating agent under the extreme reaction condition used. If alkylamine hydrochlorides were used instead of arylamine hydrochlorides, dealkylation took place at N6 of the primarily formed purine.

A mixture of commercially available triethylamine hydrochloride (TEA•HCl), phosphorus pentoxide, and an aniline was reacted with 5-acetylamino-1H-imidazole-4-carboxamide hydrochloride at 180°C for 18 h to give 2-methyl-N^6-aryladenines in 25-77% yields (SCHEME 2)[7]. The reaction can proceed via a hypoxanthine derivative or via a nitrile derivative corresponding to the starting material in SCHEME 1. Support for an



SCHEME 1

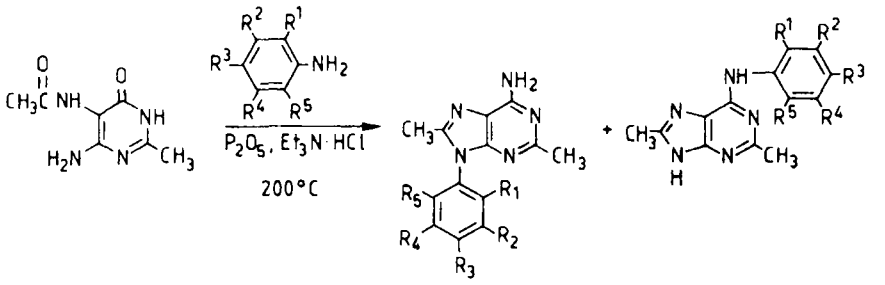


SCHEME 2

intermediate hypoxanthine derivative was found by reaction with N-methylaniline which afforded 2,N^6-dimethyl-N^6-phenyladenine.

In SCHEME 3 is shown the reaction of 5-acetamido-4-amino-2-methyl-pyrimidin-6(1H)-one[8] with two groups of arylamines: the first group includes di-ortho substituted anilines; The second group mono-ortho substituted anilines. Even for the extremely sterically hindered aniline with isopropyl groups in both ortho positions 19% yield was obtained of the 9-arylpurinamine. Using anilines with methyl or chlorine in both ortho positions to the amino group only the 9-arylpurinamines could be isolated. When the size of the ortho substituents was diminished by replacement with flourine atoms, a mixture of purines was obtained. Regarding the second group of amines it was shown that a small ortho substituent like methyl or flourine in the aniline molecule resulted in formation of the 6-arylaminopurines only. If the size of the ortho substituent was increased by using 2-aminobiphenyl, a mixture of purines was obtained. By observing the ratio of the purines as the reaction proceeded it was concluded that 6-arylaminopurines were formed by rearrangement of 9-arylpurinamines.

Recently, we have observed that an anomalous coupling of N^6-aryladenines with 2-deoxy-D-ribose could be completed in a reagent from phosphorus pentoxide, water, and tributylamine in chloroform (SCHEME 4)[4]. After 7 days at 40°C an anomeric mixture of 2,3-dideoxy-3-(9-adenyl)-D-threo-pentopyranoses precipitated in 14-73% yields. The α : β ratio was approximately 2 : 1.

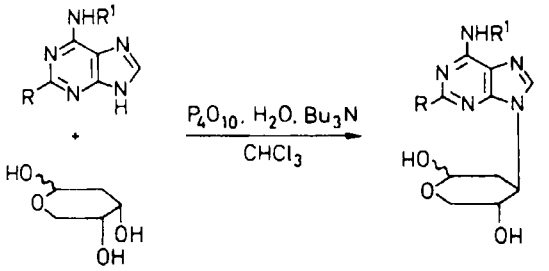


	R1	R2	R3	R4	R5	%	%
Diortho	$CH(CH_3)_2$				$CH(CH_3)_2$	19	
	CH_3				CH_3	35	
	Cl				Cl	55	
	F	F	F	F	F	45	19
Monoortho	C_6H_5					32	22
	CH_3						40
	F		F				50

SCHEME 3

Since the products could be isolated by simple precipitation, we investigated nearly all commercially available purines, but half of the purines investigated were insoluble in the reaction mixture. The remaining purines did react in the expected manner according to TLC, but only a few compounds precipitated. Solubilities of starting materials and products are decisive of a simple reaction procedure. If the products do not precipitate from the reaction mixture, chromatography is necessary since the anomalously coupled nucleosides decompose during aqueous workup. In order to overcome these problems one can modify the purine derivative, for example as in the following case. Adenine was acylated to give the N^6-isobutyryl derivative which, after the anomalous coupling reaction, was deprotected with ammonia in methanol.

Two reaction paths seem possible for the formation of the anomalously coupled product in SCHEME 4. The reaction can proceed via 2-deoxy-D-ribose phosphorylated on oxygen at C3 which is attacked by adenine in an S_N2 substitution reaction to give the pure threo isomer. Or

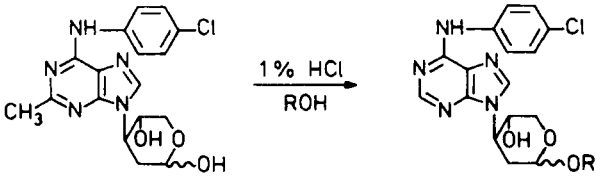


SCHEME 4

the reaction can proceed via the α,β-unsaturated aldehyde obtained by dehydration of the open chain form of 2-deoxy-D-ribose promoted by the reagent mixture. The final step is then a Michael addition type of reaction of adenine to the α,β-unsaturated aldehyde to give threo isomer. For some other purines it was possible to isolate both the threo and the erythreo form of the anomalously coupled nucleoside which means that the latter mechanism is more likely. Furthermore, this mechanism is supported by NMR investigations of the reaction mixture. ^{13}C-NMR showed from the start of the reaction peaks at 193.2 ppm corresponding to an unsaturated aldehyde and H-NMR improved the evidences for this interpretation with vinylic protons at 5.88 and 6.57 ppm and an aldehyde proton at 9.09 ppm with the expected coupling pattern of an α,β-unsaturated aldehyde.

From ^{31}P NMR (relative to 85% H_3PO_4) on a solution of the $P_4O_{10}/H_2O/Bu_3N$ reagent in chloroform we found that the reagent was composed of tributylammonium salts of trimetaphosphoric acid (22.6 ppm), pyrophosphoric acid (9.7 ppm), and phosphoric acid (-1.7 ppm) in a molar ratio of 0.5 : 4 : 10, along with some unidentified minor components. The chemical shift values found were in fair agreement with those reported considering the pH dependence of the shift values.

Since the reaction conditions for the anomalous coupling reaction in SCHEME 4 show some similarity with the nucleoside synthesis using polyphosphates as shown in TABLE 1, one could expect that normal nucleosides were also formed in our coupling reactions. The coupling reaction of N^6-benzoyladenine with 2-deoxy-D-ribose using $P_2O_5/NBu_3/H_2O$ in $CHCl_3$ as the coupling reagent was followed by ^{13}C-NMR, but the normal nucleoside could not be detected. When this was added to the reaction



R: CH_3, CH_2CH_3, $CH_2CH_2CH_3$

SCHEME 5

mixture, new peaks occurred in the ^{13}C-NMR spectrum showing that it was not already present.

The anomalously coupled nucleosides can undergo typical reactions for carbohydrates at the anomeric carbon and an example is shown in SCHEME 5 for preparation of alkyl glycosides. An anomeric mixture was obtained after heating at 80°C. After 3 days the pure α-anomer could be obtained.

A CAS online substructure search in early 1986 showed that quite a few examples are known with nucleobases (B) attached to the carbohydrate ring at other positions than C-1. However, for the furanose form no examples were known with coupling at C-3. For pyranose 19 structures could be retrieved, but only two 2-deoxy structures[9] were found (hexose structures in SCHEME 6). Great care must be undertaken in CAS online substructure search of carbohydrates because the search shown in FIG. 1 will not include carbohydrates abstracted in their chain form. The CAS online substructure search was therefore extended to the chain form of pentoses with nucleobases attached to C-3 and the report on adenine derivatives by Carbon[10] and the report on hypoxanthine derivatives by Nelsestuen[11] were found (SCHEME 6). In both cases the products could be obtained by heating deoxyribose and the purine derivative in water, but chromatography was needed in order to isolate the products.

In our laboratory it has been shown that phthalimide can undergo similar reactions with unprotected 2-deoxy-D-ribose to give N-protected 2,3-dideoxy-3-amino-D-pentoses (SCHEME 7). The erythreo isomer was isolated as a mixture of pyranose and furanose forms by chromatography whereas the threo isomer was a pure pyranose form. Quite a few phthali-

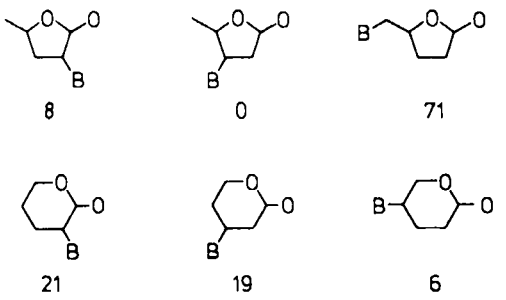
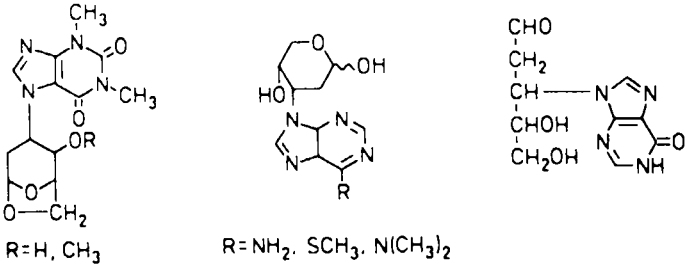
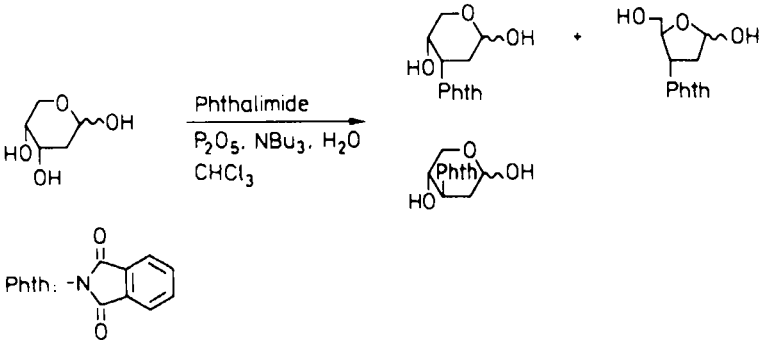


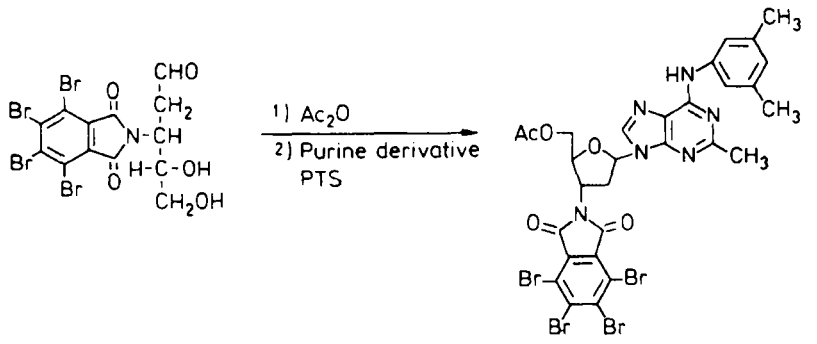
FIG. 1. CAS online substructure search.



SCHEME 6



SCHEME 7



SCHEME 8

mide derivatives were reacted in order to find a carbohydrate derivative that could be isolated by simple filtration but only tetrabromophthalimide gave a high yield of precipitated coupled product. This product will probably open up an easy route to 3-amino-2,3-deoxynucleosides as shown with one example from out laboratory in SCHEME 8.

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