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HCN AND CHEMICAL EVOLUTION: THE POSSIBLE ROLE OF CYANO COMPOUNDS IN PREBIOTIC SYNTHESIS

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INTRODUCTION

"Chemical evolution", as Calvin christened the discipline,[1] refers to the study of the chemical processes that preceded the appearance of life on the primitive earth. Implicit in this definition is the paradigm that was formulated first by Oparin:[2] the origin of life was a gradual process marked by successive stages of development—the abiotic synthesis of organic molecules, the formation of molecular aggregates with a primitive metabolism, the evolution into organisms with a biochemical apparatus resembling that which exists today. Oparin recommended that, in order to make the problem experimentally accessible, the study of the origin of life should focus on one stage at a time; research in chemical evolution largely has followed Oparin's experimental philosophy.

Over the past thirty years, considerable success has been achieved in the first stage outlined by Oparin, that is, the synthesis of organic compounds under prebiotic conditions. The search for plausible pathways to biomolecules, particularly polynucleotides and polypeptides, remains very active. This review will consider some of the accomplishments based on the use of cyano compounds as precursors: an impressive array of biological compounds, including amino acids, purines and pyrimidines, has been synthesized from HCN oligomers and other cyanide derivatives.

Before turning to the subject of cyanide chemistry, it is appropriate to define what is meant by the term "prebiotic conditions". Unfortunately, there is no direct evidence of what the

environment of the primitive earth was like. The composition of the atmosphere remains an area of debate, particularly with regard to the oxidation state of carbon and nitrogen, but one point should be emphasized: there was essentially no free oxygen on the primordial planet.[3] The absence of molecular oxygen and hence the lack of an ozone shield suggest that the solar intensity of ultraviolet radiation at the surface of the earth was significantly greater than at present, resulting in a higher rate of photochemical synthesis and photochemical decomposition of compounds However, many of the physical conditions of the primitive earth during prebiotic synthesis were probably not significantly different from those that exist today; in particular, oceans and other bodies of water were subject to similar buffering processes, resulting in a pH that presumably was close to that of the modern oceans (pH 8.0–8.5) or at least not far from neutrality.[3b] Since liquid water probably was required either as a solvent or as a medium for the redistribution of products and reactants, the limiting temperature range for prebiotic synthesis would appear to be 0–100°; however, the instability of complex organic molecules (e.g. sugars and amino acids) at temperatures near 100° suggests that the lower end of this temperature scale was a more likely regime for organic synthesis. Higher temperature zones that occurred in more restricted locales (such as submarine hot springs) have been suggested as possible sites for the origin of life,[4a] but many researchers have preferred to focus on systems that were perhaps more abundant, such as lagoons and tidal pools. A critical problem with this approach has been the probable lack of a sufficiently high concentration of reactants; one possible solution to this dilemma is the suggestion that reactants were concentrated by adsorption onto solid surfaces such as those of clay minerals. The catalytic properties of clay minerals therefore have been an important focus of research; some workers have suggested an even more radical role for clays in the origin of life.[4b]

The challenge to those who study the origin of life is to develop a self-consistent model in which a variety of biochemicals, capable of undergoing further evolution, may be formed under similar conditions and from common precursors. Nowhere is this pleasing unity more evident than in the chemistry of hydrogen cyanide.

SYNTHESIS AND DISTRIBUTION OF HCN

The occurrence of HCN on the primitive earth is supported by its facile formation under a variety of conditions[5–14] (Table 1). Since current theory suggests that the primitive earth contained limited amounts of reduced carbon,[15,16] it is significant that HCN may be synthesized from many diverse mixtures of gases, including $CO–N_2–H_2$ and $CO_2–N_2–H_2O$.[11] However, recent calculations suggest that the production of HCN by lightning would be strongly dependent on the

Table 1. Gas phase synthesis of cyano compounds

Gas Mixture	Energy Source	Products	Ref
CH_4-NH_3-H_2-H_2O	Spark Discharge	HCN	(5)
CH_4-N_2	Spark Discharge	HCN, HC≡C-CN	(6)
CO-N_2-H_2	Spark Discharge	HCN	(7)
CO_2-N_2-H_2	Spark Discharge	NCO^-	(8)
N_2-C_2H_2	Spark Discharge	HCN, NC-CN	(9)
CH_4-NH_3	High Frequency Electrical Discharge	HCN	(10)
CO_2-N_2-H_2	Shock Waves	HCN	(11)
CO_2-N_2-H_2O	Shock Waves	HCN	(11)
CH_4-N_2	Shock Waves	HCN	(11)
CH_4-NH_3	Shock Waves	HCN	(11)
CO_2-N_2	Shock Waves	·CN	(11)
CH_4-NH_3-H_2-H_2O	5 MeV e^- Bombardment	HCN	(12)
CH_4-NH_3	UV	HCN	(13a)
CO-NH_3	UV	NH_4CNO, HCN	(13b)
CH_4-NH_3	Heat (1200°C)	HCN	(14)

carbon/oxygen ratio and would be strongly disfavored in an atmosphere that contained carbon mainly in the form of CO_2.[16] Other cyano compounds that are synthesized in some of these reactions include cyanoacetylene[6], cyanogen[9] and cyanate.[8,13]

An extraterrestrial perspective on the distribution of HCN has been gained from observations of the outer planets of the solar system. The Voyager I spacecraft, for example, detected HCN, cyanoacetylene and cyanogen in the atmosphere of Titan, the largest moon of Saturn.[17] HCN has been detected in the atmosphere of Jupiter using ground-based infrared spectroscopy.[18] Another environment is that of comets, where CN^+, HCN and CH_3CN have been found.[19] Finally, cyanide derivatives have been observed in the interstellar medium: these include HCN,[20] cyanoacetylene[21] and cyanamide.[22] It seems clear that cyano compounds are widely distributed in the galaxy and probably were present on the primitive Earth.

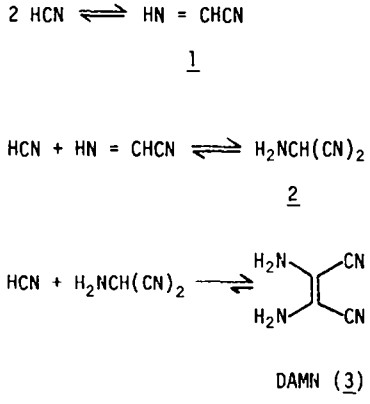


Fig. 1.

SELF-CONDENSATION OF HCN

Mechanistic studies

The initial steps in the oligomerization of HCN have been studied (Fig. 1). The tetramer, diaminomaleonitrile (DAMN, 3), is readily formed at room temperature in aqueous solutions of 0.1–1.0 M HCN.[23] At concentrations of less than 0.01 M HCN, the dominant reaction is hydrolysis of HCN to formamide and subsequently to formic acid.[23] DAMN is the lowest oligomer that is isolable from aqueous solution, and its formation can be assayed readily by its characteristic UV absorption ($\lambda_{max} = 296$ nm, $\epsilon = 13500$).[23]

The postulated stepwise condensation of cyanide to form DAMN is supported by the formation of the maleonitrile derivatives (**5a–d**) from N-alkyliminoacetonitriles (**4a–d**) in aqueous cyanide; no dimerization of the substituted iminoacetonitriles was observed (Fig. 2).[24] The suggestion by Matthews *et al.*[25] that the HCN dimer is a direct precursor to DAMN and higher oligomers therefore does not appear tenable.

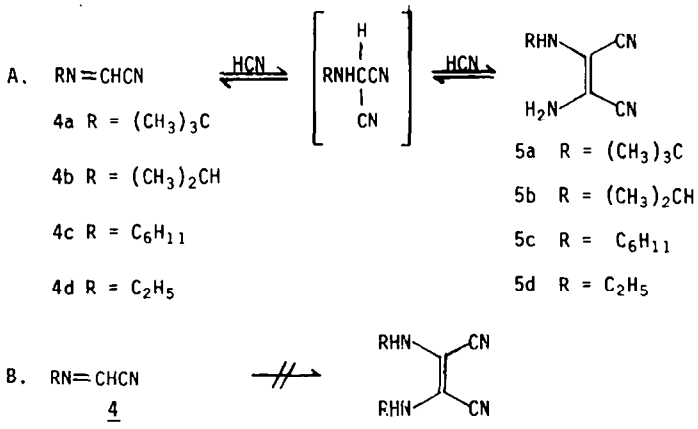


Fig. 2.

Table 2. Catalysts for tetramerization of HCN

Acceleration	Little or No Effect	Deceleration
Br_2	I^- Cl^- CNO^-	HS^-
I_2	HCO_3^- $H_2PO_4^-$	HSO_3^-
**Cu^{2+}	SO_4^{--}	
	Mg^{++} Ni^{++} Fe^{++}	Co^{++}
$(CN)_2$	Ca^{++} Al^{+++} Ag^+	Mn^{++}
	$NaVO_3$	V_2O_5
	CH_3NH_2 $(CH_3CH_2)_3N$ NaN_3	

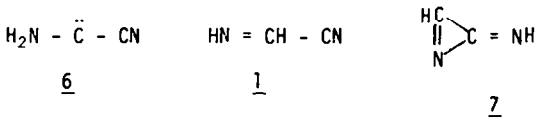
*Adapted from refs. (23) and (27)
**Promotes the formation of $(CN)_2$

DAMN is formed essentially irreversibly as shown by the lack of exchange of ^{13}C-labelled HCN with DAMN.[26] The rate of tetramerization at room temperature is optimal at pH 9.2, the pK_a of HCN, and may be catalyzed by various reagents (Table 2).[27] Schwartz and Goverde recently reported that the addition of formaldehyde, acetaldehyde or acetone can accelerate the formation of diaminomaleonitrile; however, the mechanism of this process is unclear.[28]

Dimers of HCN

Some controversy has arisen over the proposed structure of the dimer of HCN (Fig. 3). Since

Dimers of HCN



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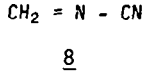

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Fig. 3.

the steady state concentration of the dimer in an oligomerizing cyanide solution is very low, it has never been isolated from solution and there are no spectroscopic data that bear on its structure. At one time, a diradical structure, aminocyanocarbene (**6**),[25,29] was suggested, but there was never any direct evidence for this species. On the contrary, several different sets of quantum mechanical calculations have shown that iminoacetonitrile (**1**) is the most stable (lowest energy) conformation of the dimer.[30-33] Yang *et al.*[34] have proposed azacyclopropenylidene (**7**) as the most stable form, based on INDO calculations, but Moffat[35] has pointed out that such a calculation overestimates the stability of the three-membered ring: geometry optimized *ab initio* molecular orbital calculations indicate that iminoacetonitrile is the most stable dimer.[35]

Another dimer of HCN (N-cyanoformamine, **8**) can be prepared by the pyrolysis of dimethylcyanamide or by allowing liquid HCN to stand at 277K for 2 years.[36] The surprising stability of this dimer indicates it is not the same as the dimeric species formed in the base-catalyzed oligomerization of HCN.

HCN Trimer: aminomalononitrile

As in the case of the dimer, the steady state concentration of the trimer in an oligomerizing solution is too low to permit isolation and characterization.[23] Although the trimer may be

synthesized and stored as the tosylate salt, aminomalononitrile itself is an oil that rapidly oligomerizes to form a dark brown precipitate.[37] Aminomalononitrile reacts rapidly with HCN to form DAMN; a 0.5×10^{-4} M solution of aminomalononitrile has a half-life of one minute in 1.0 M HCN (pH 9.2, 25°).[23]

Diaminomaleonitrile

The structure of DAMN (3) has been investigated by vibrational spectroscopy; specifically, the presence of a band at 1620 cm^{-1} in both the IR and Raman spectra was interpreted as evidence of the *cis*-configuration.[38] This is consistent with the dipole moment of 7.8 D, measured in dioxane.[39] X-ray diffraction analysis of the crystal structure has confirmed that the molecule has the *cis*-configuration, but has also shown that the molecule is non-planar in the crystalline state: one aminocarbocyano unit is twisted by 6° relative to the other.[40] More recently, molecular orbital (Iterative Extended Huckel) calculations have demonstrated that for the isolated molecule, the completely planar cis configuration has the lowest energy.[41]

Diiminosuccinonitrile

DAMN, in the presence of oxidizing agents such as MnO_2,[42] PbO_2,[42] Fe^{3+} salts or clay minerals,[43] is known to undergo oxidation to diiminosuccinonitrile (DISN, 9) (Fig. 4). The reverse

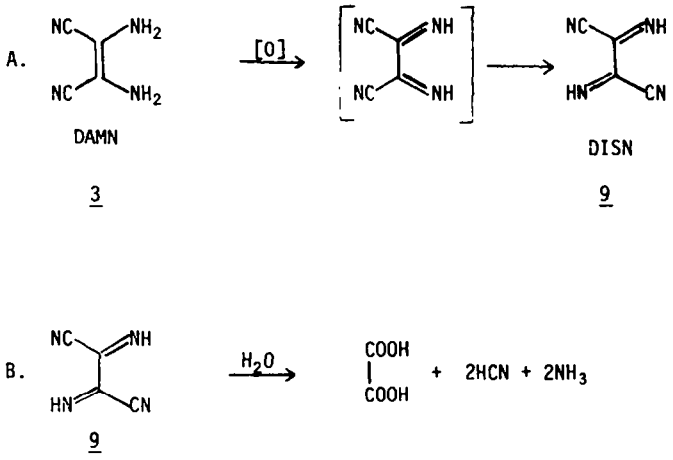


Fig. 4.

reaction, the reduction of DISN to DAMN, may be effected by reducing agents such as $NaBH_4$ or hydroquinone.[44] DISN is a reactive species that undergoes hydrolysis to yield oxalic acid and HCN;[43] in the presence of aqueous ammonia, urea and oxalic acid are the main solvolysis products.[45] DISN may be involved in the formation of higher oligomers (Section 3F).

The s-trans configuration of DISN (Fig. 2) is suggested by the low dipole moment of 1.59 D (dioxane).[44] Vibrational analysis of the IR and Raman spectra indicates that the s-trans isomer is the only species present in the solid or in acetonitrile solution.[46] Nevertheless, the s-cis isomer has been shown to exist in Pt^{2+} and Co^{3+} chelates.[47,48]

Higher oligomers: $(HCN)_{n>4}$

The structure and the mechanism of formation of higher oligomers of HCN is much less clear. Subsequent oligomerization steps appear to involve a series of internal redox reactions, inferred from the detection of oxidation products (oxalic acid and urea) as well as reduction products (e.g. 2, 3-diaminosuccinic acid) in the oligomer hydrolysate.[26] It was suggested that the DAMN-DISN interconversion could be mediating these processes:[45] the detection of urea and oxalic acid, the products of the ammonical hydrolysis of DISN (Section 3E), is consistent with this postulate. Diaminosuccinic acid must be formed by the reduction and hydrolysis of DAMN or derivatives of DAMN.[26] It has been shown that the oxidation products noted above are obtained even when oxygen is rigorously excluded from the oligomerizing solutions.[26]

The elucidation of the structure of the HCN oligomers is complicated by the heterogenous nature of the material, and only limited progress has been made in this area.[49] A 0.1 M solution of HCN, adjusted to pH 9.2 by the addition of ammonia, yields after six to twelve months at room temperature a mixture of yellow soluble and black insoluble products. Ferris and coworkers have partially characterized the soluble fraction by ^{13}C-NMR, differential thermal analysis, and pyrolysis GC–MS.[49] Functional group analysis of the acidic and amphoteric fraction (obtained by ion-exchange chromatography of the oligomers) indicated the presence of carboxylic acid, carboxamide or other groupings with the same oxidation state. Schaefer and coworkers[50] have recently applied the technique of cross-polarization magic angle spinning to obtain the ^{15}N-NMR of the HCN oligomers in the solid state. The ^{15}N-NMR spectra also indicate the presence of amide nitrogens, but there is no conclusive evidence that these amides are peptide linkages.

Matthews has proposed the idea that HCN condenses to give heteropolypeptides directly,[51,52] but there is no direct evidence to support this hypothesis.[53] The infrared data are ambiguous and, while a positive Biuret test has been cited as evidence for peptide linkages,[54] this assay is misleading when applied to HCN oligomers.[49]

Draganic *et al.*[55] have obtained some evidence that peptide linkages may be a minor constituent of HCN oligomers formed by the radiolysis of ammonium cyanide solutions with a ^{60}Co source. They report that enzymatic degradation of the oligomers by pronase releases approximately 10% of the quantity of amino acids that are obtained by hydrolytic degradation in acid. It is apparent from the data of Draganic[55] that, if peptidic amino acids are present in these cyanide oligomers, then they can account for no more than 1–3% of the total composition.

At present it is clear that the higher oligomers of HCN contain a complex variety of functional units. This complexity is apparent in the diverse mixture of biological molecules that are obtained upon hydrolysis of the oligomers. The hydrolytic products are treated in detail in the sections that follow.

AMINO ACIDS

The first evidence for a possible role for HCN in the prebiotic synthesis of amino acids came from Miller's observation that HCN and aldehydes were primary products in the electric discharge experiments with CH_4–NH_3–H_2–H_2O mixtures.[5] On this basis, he proposed that amino acids were formed by a Strecker-type condensation (Fig. 5).

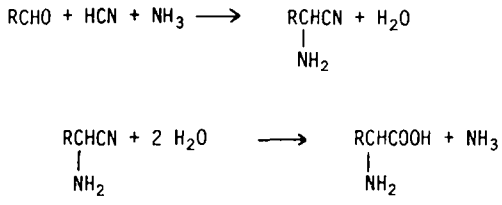


Fig. 5.

Since Miller's early experiments, an impressive number of amino acid syntheses have been accomplished using a variety of gas mixtures subjected to energy sources such as heat, UV light, shock waves, gamma radiation and electric discharges. These results have been reviewed elsewhere.[56,57] Although HCN has been identified as a primary product in many of these experiments and is probably involved in the formation of organic products,[7,19,58,59] the present discussion of amino acid syntheses will be restricted to those studies which directly employ HCN or its oligomers as starting materials (Table 3). It should be noted that in all these syntheses, the amino acids are obtained as racemic mixtures of D- and L-isomers; no-one has yet devised a synthesis of chiral amino acids under prebiotic conditions.

Oro first investigated the possible role of aqueous HCN in amino acid formation by heating 2.2 M HCN in ammoniacal solution for 25 days at 70°, after which he identified alanine, glycine and aspartic acid by paper chromatography.[60] The mechanism was not investigated, but it was suggested that the trimer or tetramer of HCN might be involved in the synthesis. Sanchez *et al.*[23] obtained similar results when aminomalononitrile (2) or DAMN itself was used as the starting

Table 3. Amino acids from oligomerization and hydrolysis of HCN

Starting Compound	Major Amino Acids Reported	Method(s) of Identification	Ref.
HCN (2.2 M)	Glycine, Alanine, Aspartic Acid	Chromatography	(60)
HCN	Glycine, Alanine, Aspartic Acid, Serine, β-Alanine, α,β-Diaminopropionic Acid, Glutamic Acid, Leucine, Isoleucine	Amino Acid Analyzer	(62)
HCN (0.1 M)	Glycine, Alanine, Aspartic Acid, β-Alanine, α-Aminoisobutyric Acid, Guanidine Acetic Acid, Diaminosuccinic Acid	GC, GC/MS	(67)
HCN (0.1 M)*	Glycine, Alanine, Aspartic Acid, β-Alanine, α-Amino-n-butyric Acid, Sarcosine	Amino Acid Analyzer, GC/MS	(69)
HCN (0.001 M)**	Glycine, Serine, Alanine, Aspartic Acid	Amino Acid Analyzer	(7)
$NH_2CH(CN)_2$ (>0.01 M) (2)	Glycine, Alanine, Aspartic Acid	Chromatography	(23)
$NH_2CH(CN)_2$ (Neat) (2)	Glycine, Alanine, Aspartic Acid, Glutamic Acid, Lysine, Arginine, Leucine, Valine	Amino Acid Analyzer	(37)
DAMN (3) (>0.01 M)	Glycine, Alanine, Aspartic Acid, Serine	Chromatography	(23)
DAMN (3)	Glycine, Alanine, Aspartic Acid, Serine, Glutamic Acid, Lysine, Threonine, Valine, Histidine, Leucine, Isoleucine, Arginine	Amino Acid Analyzer	(61)

*Subjected to γ-radiation from a 60°C source

**Irradiated at 254 nm

material. Glycine was the major product and, in the case of aminomalononitrile, a small amount of asparagine was detected.

Further studies on the formation of amino acids from aminomalononitrile[37] and DAMN[61] were conducted by Matthews and coworkers, who suggested that up to 13 amino acids could be obtained from the hydrolysate of the oligomers formed from these monomers. These results correspond to those reported by Lowe *et al.*[62] who claimed that up to 70 ninhydrin-positive substances could be obtained from the hydrolysis of NH_4CN-derived oligomers. However, the identifications of Matthews[37,61] and Lowe *et al.*[62] were based on the chromatographic behavior of a bulk sample that was analyzed with an amino acid analyzer; the identity of the chromatographic peaks is therefore complicated by components with very similar R_f values (such as isoleucine and isoasparagine[63,64]). For example, when Miller and Friedman performed a preliminary separation of an HCN oligomer hydrolysate on an ion-exchange column prior to analysis with an amino acid analyzer, they found only trace amounts (10^{-6}%) of leucine and isoleucine,[65] compounds which had been reported in significantly higher yield (0.05%) by Lowe *et al.*[62]

Ferris and coworkers have applied the technique of GC–MS to unambiguously identify the amino acids released upon hydrolysis of HCN oligomers.[66,67] They have shown that glycine, aspartic acid and diaminosuccinic acid are the major amino acids obtained from the acidic and amphoteric fractions of the oligomers (separated by ion-exchange chromatography), while smaller smounts of alanine, isoleucine, β-alanine, guanidineacetic acid and α-aminobutyric acid were also identified. Glutamic acid was obtained in low yield from the neutral fraction of the oligomers.

As noted in the previous section, the radiolysis of HCN or NH_4CN also yields oligomers. Draganic and coworkers have studied the amino acids formed by the acid hydrolysis of oligomers from the ^{60}Co-radioloysis of HCN.[68,69] Using GC–MS, they identified glycine, alanine, β-alanine, aspartic acid and sarcosine. However, while gamma radiation was undoubtedly present on the primitive Earth as a consequence of radioactive decay, the bulk of this energy would probably have been absorbed by rocks in the crust of the Earth and hence would not have been available for the synthesis of organic compounds.[70]

Other routes to amino acids from cyanide derivatives have been explored. Friedman and Miller[65] observed the formation of valine (**10**) in 0.3% yield (based on acetone) from ammonium cyanide (1.3 M) and acetone (0.09 M), and the formation of isoleucine (**11**) in 0.01% yield from ammonium cyanide (1.3 M) and methyl ethyl ketone (0.09 M). A mechanism was proposed in which aminoacetonitrile adds to the carbonyl carbon (Fig. 6). Apparently a high concentration of

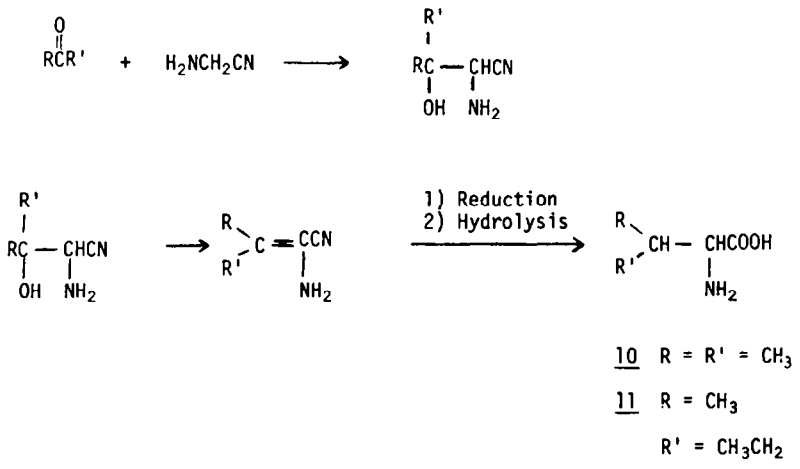


Fig. 6.

cyanide is required to generate a sufficient steady state concentration of aminoacetonitrile; thus this synthesis may be feasible only under conditions in which cyanide could be concentrated, such as in an HCN eutectic (see below).

In a similar vein, Thanassi has suggested that aminomalonitrile (**2**) could add to appropriate electrophiles to form amino acids.[71] Aminomalononitrile reacts with acrylonitrile to give glutamic acid (**12**) and with acetaldehyde to give threonine (**13**). Glycine was also obtained in each case, presumably from the hydrolysis of the aminomalononitrile. The mechanism for the threonine and glutamic acid syntheses was proposed on the basis of the first step occurring in neutral solution and the subsequent hydrolysis occurring in acid (Fig. 7). One obvious drawback here is that

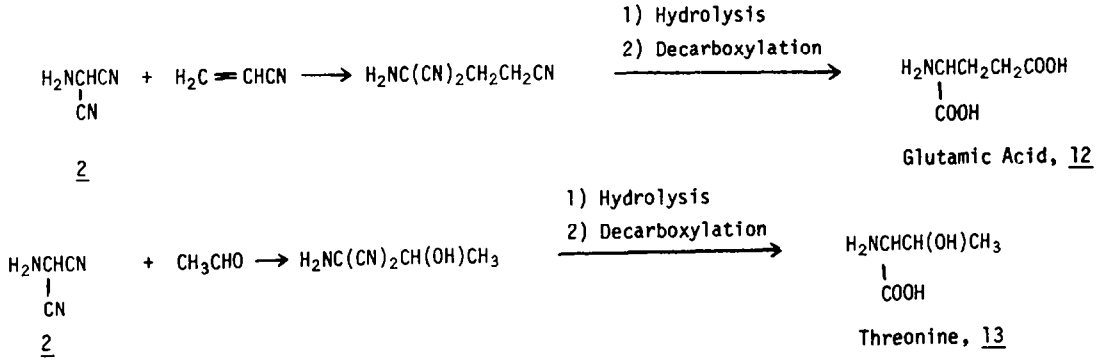


Fig. 7.

aminomalononitrile is present in very low concentration in an oligomerizing cyanide solution. Thanassi has shown that if a large excess of cyanide is present (i.e. a concentration greater than that of the electrophile), then DAMN is the only addition product. Thus, while the reagents employed in these syntheses may be prepared under prebiotic conditions, their steady state concentrations on the primitive earth would have been very low.

It is clear that some of the common amino acids can be synthesized from HCN under fairly mild conditions. These results are summarized in Table 3. The only real obstacle is the relatively high concentration of cyanide (0.01 M or greater in the absence of UV radiation) that is necessary in order to achieve oligomerization. The volatile nature of HCN eliminates the possibility of concentration by evaporation of a pond or tidal pool. The most effective means of concentration

would seem to be freezing: Orgel and coworkers have noted that if an aqueous solution of HCN is cooled to −23.4°, a eutectic phase is formed which contains 74.5 mole% HCN (25 M).[23] In this concentrated system, HCN oligomerizes quite rapidly as evidenced by the formation of the amber color of the oligomers; after 8 days, a 30.5% yield of DAMN was obtained.[23] (No DAMN was observed in control solutions that were kept at room temperature or 4°). Schwartz has recently observed the formation of adenine (see Section 5) from a frozen solution of HCN and formaldehyde.[72] Prebiotic synthesis thus may be imagined to occur in a pond in which a winter of freezing and oligomerization is followed by a summer of warming, hydrolysis and photolysis, providing a source of amino acids and other biomolecules (see below).[23] Since eutectic formation requires complete freezing, a condition that would be fulfilled only by the shallowest pools or under the most severe conditions, glaciers may have been a more favorable site for HCN oligomerization on the primitive earth.[72] The characteristics of eutectic systems, particularly with regard to higher salt concentrations, merit further investigation in the context of chemical evolution.

PURINES AND IMIDAZOLES

Thermal syntheses

The first experiments in the prebiotic synthesis of purines from cyanide were performed by Oro, who reported the synthesis of adenine (**14**), when 1–15 M aqueous ammonium cyanide was heated at 70° for several days and subsequently hydrolyzed in 6N HCl.[73,74] This observation was confirmed by Lowe *et al.*,[62] who also identified the purine hypoxanthine (**15**) in the reaction mixture after hydrolysis. Ferris and coworkers have measured the formation of adenine under milder conditions than were employed by Oro or Lowe and coworkers: a 0.1 M solution of HCN, adjusted to pH 9.2 with ammonia and kept at room temperature in the dark for 4–12 months, gave a 0.03–0.04% yield of adenine after hydrolysis of the oligomeric products.[67] In the same study, the authors were unable to detect adenine before acid hydrolysis, an observation which suggests that the purine is formed as a substituted derivative (see below) or is chemically associated with higher HCN oligomers. Small amounts of adenine were detected by Oro before acid hydrolysis, but the conditions (13 days at 70° in 3N ammonium hydroxide) of the reaction were such as to permit appreciable alkaline hydrolysis;[73] Ferris *et al.* have reported the detection of adenine after the hydrolysis of HCN oligomers in mildly alkaline (pH 8.5) solution.

Oro originally proposed that the formation of adenine proceeded by the reaction of the HCN trimer with formamidine.[74] Two findings mitigate against that proposal. First, the hydrolysis of formamidine is very rapid (half life of 3 days at pH 8.5 and 30°); hence it would be present in only trace amounts in HCN solutions.[23] Second, the relative rate of reaction of cyanide with HCN trimer is much greater than the rate of reaction of formamidine with the trimer.[23] These studies demonstrate that the conditions required for the formation of the trimer will result in its rapid conversion to the tetramer by reaction with cyanide. Consequently the tetramer or compounds derived from it must be precursors to adenine (Fig. 8).

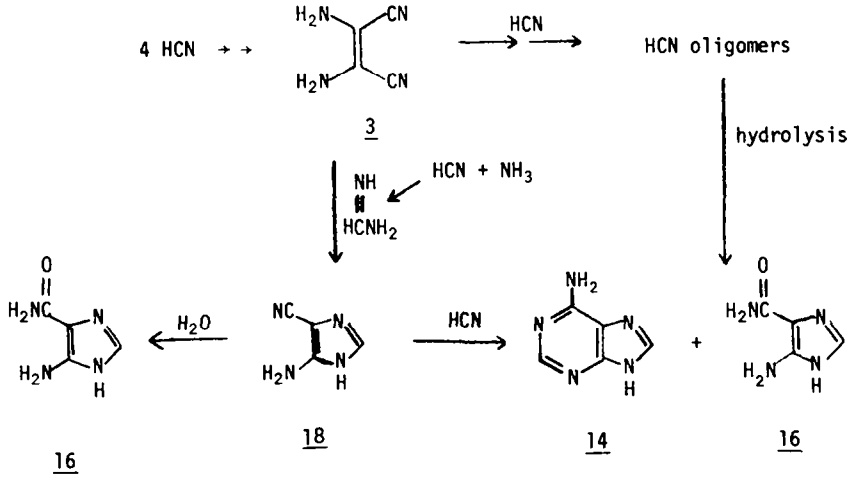


Fig. 8.

In Oro's original work he obtained 4-aminoimidazole-5-carboxamide (AICA, **16**), and 4-aminoimidazole-5-carboxamidine (AICAI, **17**) as reaction products.[74] These were observed when very concentrated solutions of HCN (1–15 M) were studied and are probably formed by the reaction of ammonium formate,[75] formamide and ammonia[75], or formamidine[23] with diaminomaleonitrile; known reactions under anhydrous conditions. The last reaction, DAMN with formamidine, gives 4-aminoimidazole-5-carbonitrile (AICN, **18**) as a primary product; adenine and AICA (**16**) are formed only after longer reaction times.[23] Recently it has been shown that the reaction of formamidine and DAMN in non-aqueous solvents yields a pentamer of HCN, N-aminomethylidene-diaminomaleonitrile (**19**), which cyclizes to give AICN (**18**) and AICAI (**17**); adenine may be synthesized by reaction of (**19**) with formamidine (Fig. 9).[76] The steady state

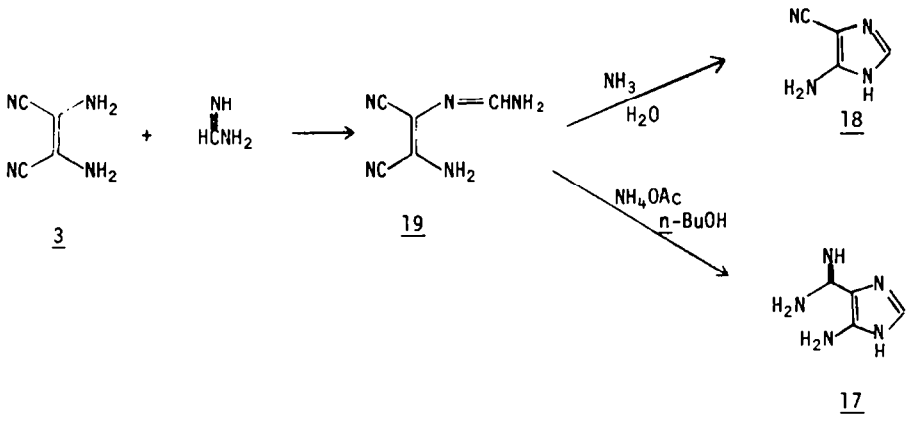


Fig. 9

concentrations of the formic acid derivatives are probably sufficiently high in 11 M NH_4CN so that there will be appreciable reaction of them with DAMN. Adenine (**11**) and AICA (**13**) are not formed in the reaction of 0.1 M NH_4CN, but they are released on hydrolysis of the HCN oligomers formed in these solutions.[67] This finding indicates that there are at least two different pathways for the formation of these heterocycles; one directly from DAMN and the other from the HCN oligomers formed from DAMN (Fig. 8).

Recently Schwartz has proposed a pathway to adenine from HCN that does not involve AICN or AICA as an intermediate.[77] The postulated mechanism shown in Fig. 10 was based on the

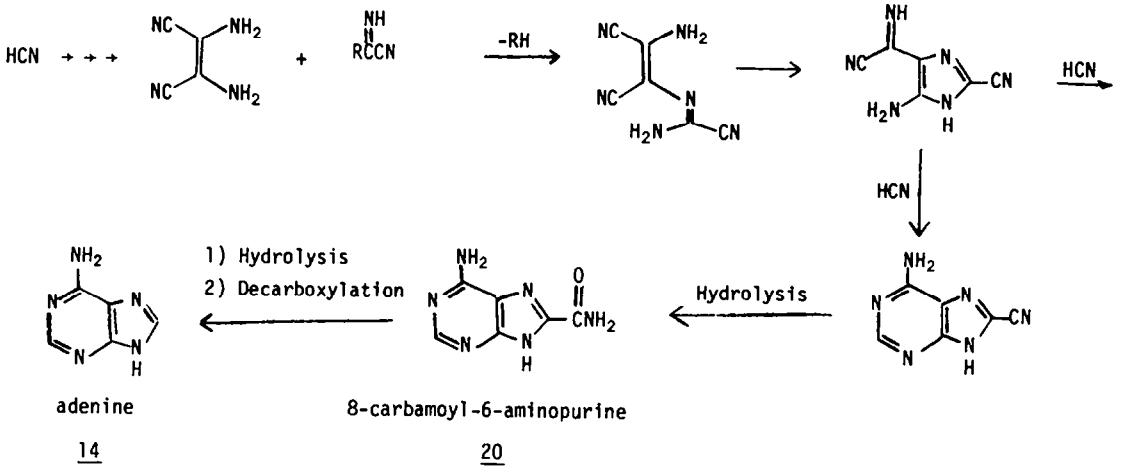


Fig. 10.

isolation of 8-carbamoyl-6-aminopurine (**20**), which readily hydrolyzes to adenine.[78] An attractive feature of this approach is that the precursor is the HCN tetramer, a compound that is stable and is present in appreciable concentration in an oligomerizing solution of HCN.[23]

The imidazoles, AICA (**16**) and AICN (**18**), have been shown to be useful precursors for the chemical synthesis of a variety of purines. Sanchez *et al.*[79] have shown that AICN reacts with HCN to give adenine (**14**) and hypoxanthine (**15**) in 7 and 1% yields, respectively. Guanine (**21**) and xanthine (**22**) are obtained in 5–10% yields from AICN and urea (Fig. 11). Guanine can also be

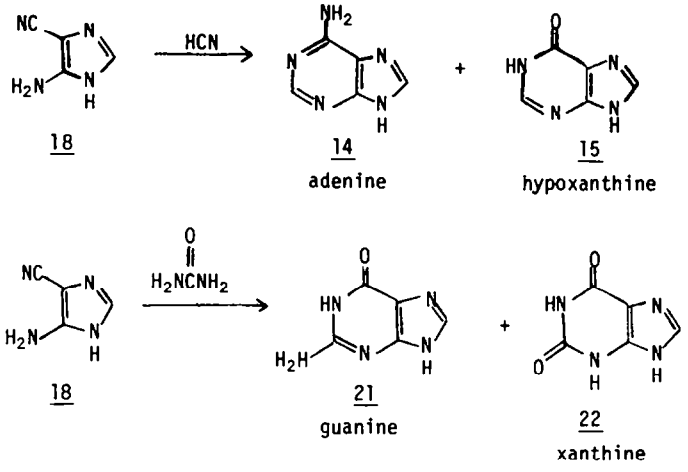


Fig. 11.

synthesized in 20% yield from AICA and potassium cyanate. Since one possible prebiotic source of cyanate might have been via the hydrolysis of cyanogen, the reaction of AICA was carried out in an ammoniacal solution of cyanogen: guanine was produced in up to 43% yield.[79]

Photochemical syntheses

Since AICN is a versatile precursor to purines, alternative approaches to its synthesis under prebiotic conditions have been examined. Ferris and Orgel found that AICN could be synthesized photochemically in 80% yield from diaminomaleonitrile in aqueous solution at room temperature by irradiating at 350 nm.[80] Close to quantitative yields were obtained when the solutions were carefully degassed to remove molecular oxygen.[81] The photochemical rearrangement appears to be quite general for primary and secondary aminomalononitriles,and it has been exploited in a variety of syntheses of substituted imidazoles (*vide infra*).

The proposed mechanism for the photochemical formation of AICN is shown in Fig. 12. The

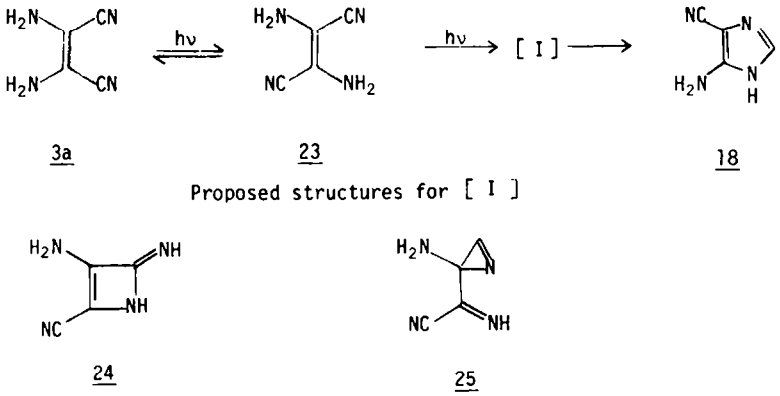


Fig. 12.

reaction involves at least two excited state processes: photoisomerization of diaminomaleonitrile to diaminofumaronitrile (**23**) ($\Phi = 0.05$),[82] and photochemical cyclization of the diaminofumaronitrile to AICN.[23,83] The suggestion that the reaction might involve an aminocyanocarbene intermediate[84] is not supported by studies of substituted enaminonitriles.[83] The nature of the intermediate in the cyclization of diaminofumaronitrile remains uncertain;[82] experimental studies indicate an azetine (**24**),[83] while recent theoretical calculations support azirine (**25**).[85,86]

PYRIMIDINES

The first prebiotic pyrimidine synthesis based on a cyano compound was the synthesis of cytosine (**26**) from cyanoacetylene and cyanate (Fig. 13).[87] Since cytosine is readily hydrolyzed to

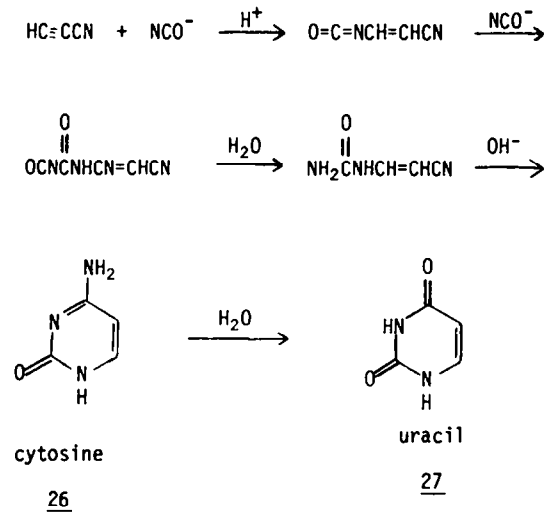


Fig. 13.

uracil (**27**), this synthesis also provides a pathway to that pyrimidine. The reactants are plausibly prebiotic, since they are produced in electric discharge experiments (Table 1); alternatively, cyanoacetylene may be obtained by heating HCN and acetylene,[88] while cyanate may also be obtained by the hydrolysis of cyanogen[89] (formed from HCN by electric discharge, UV radiation or oxidation). However, the rapid hydrolysis of cyanoacetylene and cyanate would have limited their possible concentrations in a primitive earth environment. Thus this synthesis is not completely satisfying from a prebiotic standpoint. Another prebiotic pyrimidine synthesis utilizes cyanoacetaldehyde, the first hydrolysis product of cyanoacetylene, as the starting material: 2,4-diaminopyrimidine (**28**) was obtained in yields of 1–3% by the reaction of cyanoacetaldehyde and guanidine, while the subsequent hydrolysis of the diaminopyrimidine gives uracil (**27**) and a small amount of cytosine (**26**) (Fig. 14).[90]

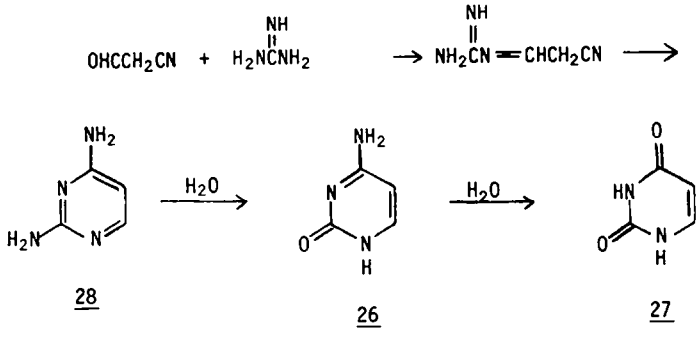


Fig. 14.

A novel route to uracil (**27**) proposed by Schwartz and Chittenden[91] involves the formation of dihydrouracil (**29**) from β-alanine and urea, both of which are products of the hydrolysis of HCN oligomers.[67] The dihydrouracil undergoes photochemical dehydrogenation to yield uracil; various catalysts, notably montmorillonite clays, enhance the dehydrogenation step (Fig. 15).

Perhaps the most plausible route to pyrimidines is from the oligomerization of HCN (Fig. 16).[67]

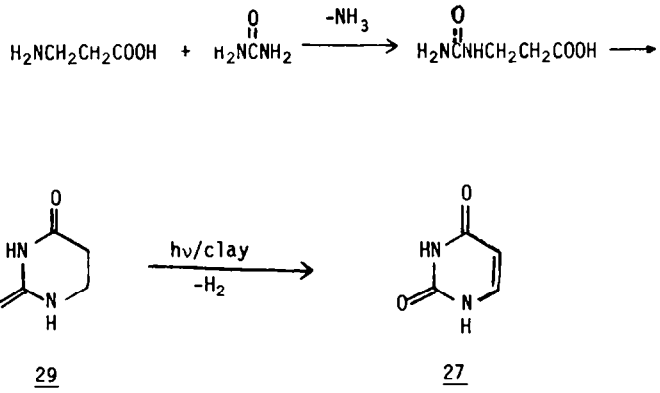


Fig. 15.

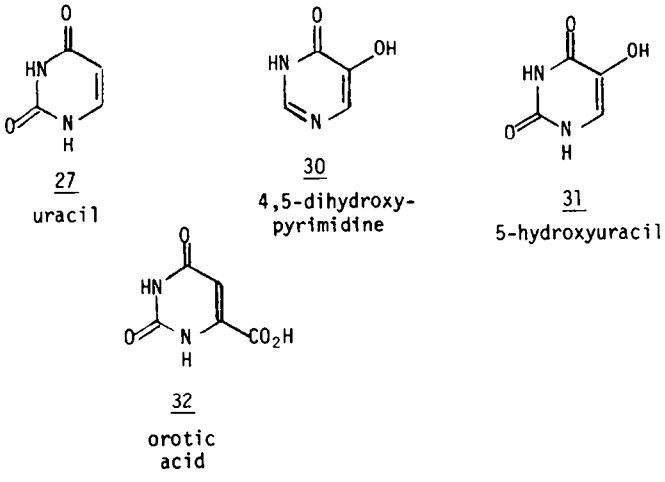


Fig. 16.

Uracil has recently been obtained as a minor product from the acid hydrolysis of HCN oligomers.[92] Several substituted pyrimidines also have been identified in the acid hydrolysate: 4, 5-dihydroxypyrimidine (**30**) and 5-hydroxyuracil (**31**).[67] Orotic acid (**32**) is obtained in 0.009% yield by hydrolysis of the oligomers at 100° and pH 8.5, and this pyrimidine readily undergoes a photochemical decarboxylation to give uracil.[93] The nucleoside orotidine (**33**) and the nucleotide orotidine-5′-phosphate (**34**) are also converted to the corresponding uracil derivatives (**35**, **36**) by ultraviolet light (Fig. 17).[94] These reactions are equivalent to the contemporary biotic pathway for the conversion of orotidine 5′-phosphate to uridine-5′-phosphate. Horowitz has argued that biotic pathways reflect the availability of precursors on the primitive Earth.[95] There is a striking similarity between some of the proposed prebiotic pathways and contemporary biosynthetic pathways. An extension of the Horowitz hypothesis is the proposal that the primitive biotic pathways were initially derived from the prebiotic routes to the same biomolecules. The primitive life that survived

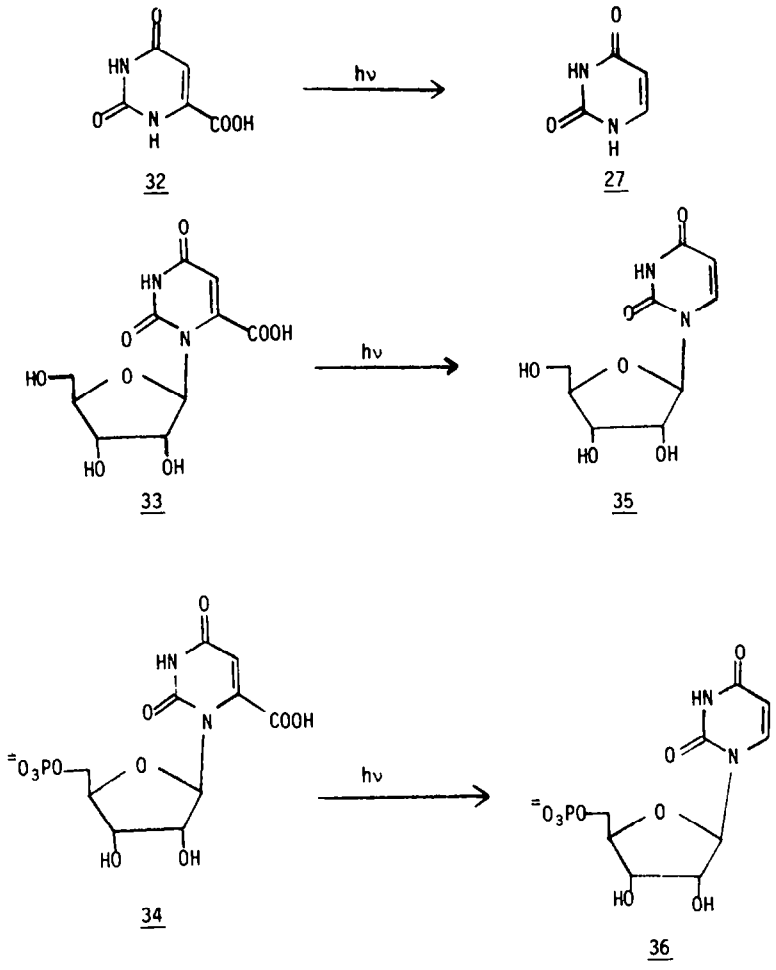
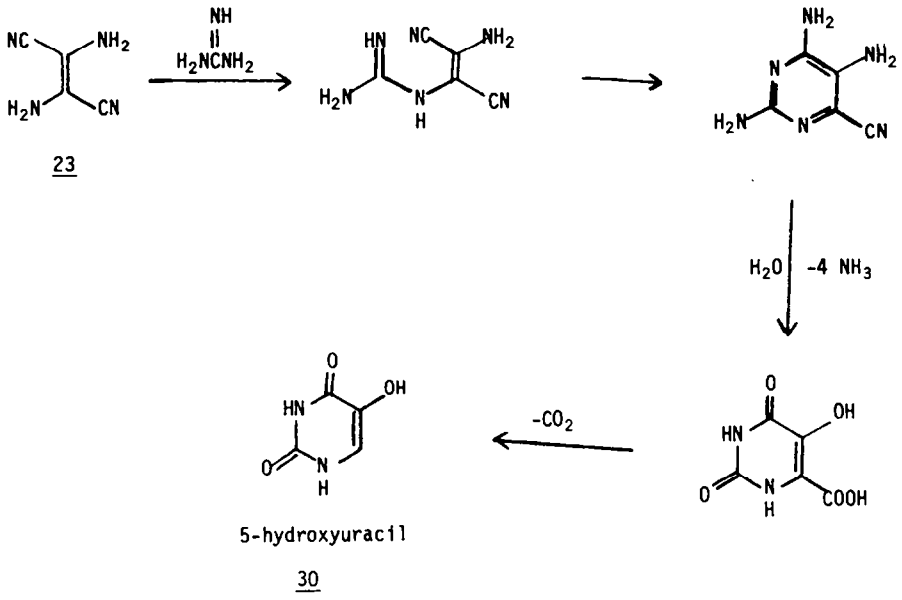


Fig. 17.

did so because it evolved catalysts which enhanced the efficiency of prebiotic syntheses when the supply of preformed biomolecules was depleted.[93]

A scheme which illustrates a plausible pathway for the formation of 5-hydroxyuracil (**31**) from diaminofumaronitrile (**23**) is shown in Fig. 18. A similar reaction pathway can be written for the synthesis of orotic acid (**32**) and 5-hydroxyuracil (**31**).[67] It should be noted that these schemes illustrate simplified postulates for the formation of these heterocyclic ring systems. The actual reaction pathways are more complex because these compounds are released by the hydrolysis of HCN oligomers. Diaminofumaronitrile is a plausible starting material for the synthesis of the pyrimidine ring system. Since there is a mobile equilibrium between diaminofumaronitrile (**23**) and diaminomaleonitrile (**3**), this proposal is consistent with the previously outlined condensation reactions of HCN.[23] It is also consistent with the proposal that the tetramer of HCN (*cis* or *trans* isomer) is the key intermediate in the formation of HCN oligomers and the biomolecules from the hydrolysis of these oligomers. It is significant that purines, pyrimidines and amino acids, the principal nitrogen-containing building blocks for the formation of nucleic acids and proteins, may be obtained by the hydrolysis of HCN oligomers. This finding greatly strengthens the case for HCN as an important starting material for chemical evolution. It is not necessary to postulate a variety of starting materials reacting under different conditions to form the requisite biochemicals, but rather one can propose a unified synthesis starting from HCN.



23

5-hydroxyuracil

30

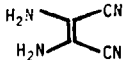
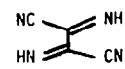
Fig. 18.

POSSIBLE ROLE OF CYANO COMPOUNDS AS CONDENSING AGENTS

A distinct challenge faced by chemists is to find a plausible chemical process by which monomers (e.g. amino acids) may be linked together in a primitive earth environment. Since such condensations are thermodynamically disfavored in aqueous solution, a number of systems cited below involve reactions in the dry state; such processes simulate the evaporation of a pond or shallow tidal pool. Nevertheless, the search continues for a prebiotic compound capable of effecting the formation of oligopeptides and oligonucleotides in solution. One problem is that condensing agents themselves are often unstable in aqueous systems; hence, one look for a prebiotic reagent that could be continuously generated and/or introduced into the reaction system.

A number of condensing agents that are directly or indirectly based on cyanide have been proposed (Table 4). Prebiotic condensing agents effective in peptide formation have been

Table 4. Cyano compounds as condensing agents

Compound	Types of Condensation Investigated	Reference
H_2N-CN (Cyanamide, 38)	Phosphorylation Peptide formation Nucleotide condensation Lipid formation	97, 102-105 106-108 109-114 115-117
H_2N-C(=NH)-NH-CN (Cyanoguanidine, 39)	Phosphorylation Peptide formation	102, 118 119
$^-$O-C≡N (Cyanate)	Phosphorylation Peptide formation	74, 121 122
NC-CN (Cyanogen)	Phosphorylation	105, 124
HC≡C-CN (Cyanoacetylene)	Phosphorylation	127, 128
 (DAMN, 3)	Peptide formation	129
 (DISN, 9)	Phosphorylation	132

reviewed.[96] Although attempts have been made to utilize HCN itself as a condensing agent, these have not been successful.[97] However, as will be discussed below, oligomers of HCN may have had a role in prebiotic condensation reactions.

Carbodiimide (**37**) and its derivatives, while among the most thoroughly studied of condensing agents in synthetic chemistry, unfortunately have not been synthesized under prebiotic conditions. Dicyclohexylcarbodiimide and 1-ethyl-3-(dimethylaminopropyl)-carbodiimide hydrochloride (which has the property of being water soluble) have been extensively used in the preparation of oligopeptides.[98–100] Nevertheless, a tautomer of carbodiimide, cyanamide (**38**), may be a more plausible prebiotic molecule, and it is with this reagent that our discussion of condensing agents begins.

Cyanamide

Cyanamide can be formed under prebiotic conditions from ammonium cyanide by a photochemical reaction,[101] and it has been detected in interstellar space.[22] As Table 5 indicates, cyanamide has been employed in a wide variety of potentially prebiotic condensations, including phosphorylation,[97,102–105] peptide formation,[106–108] nucleotide condensation[109–114] and lipid synthesis.[115–117]

A possible scheme for the formation of a peptide is shown in Fig. 19. The mechanism of the

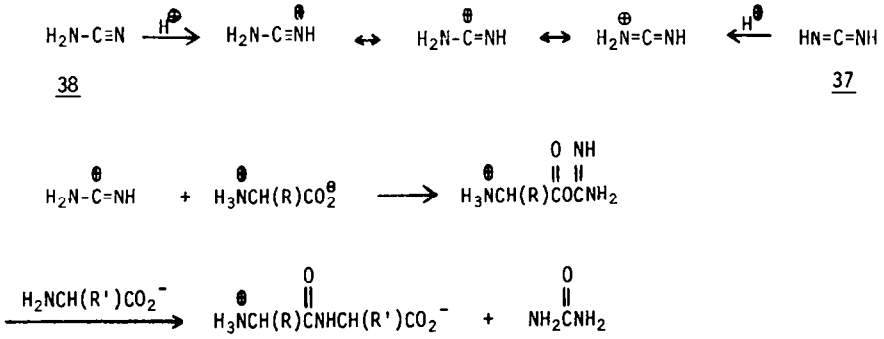


Fig. 19.

cyanamide-mediated condensation is probably similar to that which has been suggested for the carbodiimide-mediated reaction.[99,100] In fact, protonation of either cyanamide (**38**) or carbodiimide (**37**) yields the same intermediate (Fig. 19); protonation is required to activate cyanamide toward nucleophilic attack by the carboxylic group. The reaction therefore proceeds most efficiently under acidic (pH 2) conditions, although measurable yields of condensation products can be observed in neutral solution.[97] Oro has also shown that the addition of certain salts (such as $MgCl_2$ or NaH_2PO_4 and NH_4Cl) can markedly increase the yield of product.[108,112]

It will be noted from the reaction in Fig. 19 that the activated amino acid may also react with the unprotected carboxylate group of the second amino acid to give a "wrong" product, the acid anhydride. Such lack of selectivity can often by a serious problem in condensation reactions. An example from Table 5 is the cyanamide-mediated condensation of 5′-TMP, which yields the 5′, 5′-dinucleotide pyrophosphate rather than 3′, 5′-dinucleotide as the major product.[111,112]

Cyanamide undergoes self-addition to form a dimer, cyanoguanidine (**39**), which may also function as a condensing agent in phosphorylation[102,118] and peptide formation[119] (Table 6). Cyanoguanidine has been formed by electron irradiation of a mixture of methane, ammonia and water.[120] Calvin has observed that cyanoguanidine promotes the formation of ribose-5-phosphate from ribose and orthophosphate, although he has given no experimental details.[118] Cyanoguanidine was reported to be slightly more effective than cyanamide in the phosphorylation of glucose under acidic (pH = 2) conditions.[102] It should be noted that Halmann, studying the cyanamide-mediated condensation of glycine, has reported that 12% of a 0.05 M cyanamide solution (pH = 6.5) is converted to cyanoguanidine after 5 days at 60°.[106] The possible role of cyanoguanidine in condensation processes that employ cyanamide as the starting material merits further investigation.

Table 5. Cyanamide-mediated condensations

Starting Material(s)	Product(s)	Special Conditions	Reference
Uridine (0.1 M), phosphate (1 M)	5'-UMP (2.0% yield)	1 M $NC-NH_2$; 30 days at 65°C (pH = 7.0)	97
Uridine, Hydroxylapatite, Ammonium oxalate	UMP (21% yield)	10-fold excess $NCNH_2$; Evaporated to dryness and heated for 7 days at 90°C	103
Glucose (10^{-3}M), Phosphate (10^{-2}M)	Glucose-6-phosphate (1.5% yield)	10^{-2}M $NC-NH_2$; 19 hrs at 25°C (pH = 2)	102
D-Ribose (0.1 M), Orthophosphate (0.1 M)	β-Ribofuranose-1-phosphate (8% yield)	0.25 M $NC-NH_2$; 72 hrs at 65°C (pH = 7)	105
Glycerol Phosphate	Glycerophosphate (30% yield)	10-fold excess $NC-NH_2$; Evaporated to dryness and heated for 16 h at 85°C	104
Glycine (0.04 - 0.08 M)	Diglycine (3-5%)	0.04-0.18 M $NC-NH_2$; 7 days at 36°C (pH 2-3)	106
Glycine	Oligoglycines (5% yield) up to tetramer	ATP and AICA (16) added as catalysts; Mixtures (initial pH = 3) evaporated at 90°C for 24 h	107
Isoleucine	Dipeptide only (17% yield)	"	107
Phenylalanine	Oligopeptides (66% yield up to tetramer)	"	107
Leucine	Dileucine (11% yield), Trileucine (7% yield)	$MgCl_2$ added as catalyst; Reaction mixture (initial pH = 7) evaporated at 80°C for 24 hours	108
5'-dTMP (0.25 M)	Oligonucleotides (1% yield) up to pentamer	1.0 M $NC-NH_2$; heated at 85-90°C for 24 h (pH = 7) Montmorillonite catalyst	109
5'-dTMP, 5'-dTTP	Oligonucleotides (32% yield) up to tetramer	AICA (16) added as catalyst, dTMP/dTTP = 10^4. Reaction mixture evaporated at 90°C for 40 h.	110
5'-dTMP	Oligonucleotides (69-86% yield)	Evaporated at 90-95°C for 14-18 h (starting pH = 3)	111
5'dTMP	Cyclic dinucleotide and other products (total yield >70%)	NH_4Cl added as catalyst. Evaporated at 90°C overnight (starting pH = 7)	112
5'-dTMP	Dideoxythymidine-5'-pyrophosphate (58% yield)	AICA (16) added as catalyst. Reaction mixtures evaporated at 60°C for 18 hrs.	113
5'-dTMP, 5'-dTTP	Oligonucleotides up to heptamer	AICA (16) and NH_4Cl added. Reaction mixture evaporated at 60°C for 18 h.	114
Glycerol (limiting reagent), Ammonium palmitate	Monopalmitoylglycerol (49.6%), Dipalmitoylglycerol (35.7%), Tripalmitoylglycerol (17.6%)	Imidazole added. Reaction mixture evaporated and heated at 85°C for 16 hrs	115
sn-Glycero-1(3)-phosphate (limiting reagent), Ammonium palmitate	Monopalmitoylglycerophosphate (54.9% yield) Dipalmitoylglycerophosphate (35.4% yield)	Imidazole added. Reaction mixture evaporated and heated at 85°C for 16 hrs	116
Disodium phosphatidate (limiting reagent), Choline chloride	Phosphatidylcholine (15% yield)	HCl added. Reaction mixture evaporated and heated at 80°C for 7 h	117

Table 6. Condensation reactions mediated by cyanoguanidine (dicyandiamide)

Starting Material(s)	Product(s)	Special Conditions	Reference
Adenosine (10^{-4} M), Orthophosphate (10^{-2} M)	5'-AMP	10^{-2} M Cyanoguanidine; Reacted at 25°C for 4.5 h (pH =2)	102
Orthophosphate (10^{-2} M)	Pyrophosphate	10^{-2} M Cyanoguanidine; Reacted at 25°C for 10 h (pH = 2)	102
Glucose (10^{-3} M), Orthophosphate (10^{-2} M)	Glucose-6-phosphate	10^{-2} M Cyanoguanidine; Reacted at 25°C for 19 h	102
Alanine (10^{-2} M)	Alanylalanine (1.2% yield)	10^{-2} M Cyanoguanidine, 10^{-2} M HCl added	119

Cyanate

As was noted in Section 2, cyanate has been synthesized in gas phase reactions (Table 1). In particular, a solution of 0.012 M cyanate has been produced by electrical discharges in a mixture of N_2, CO_2 and H_2 over 0.2 M $NaHCO_3$.[8] Substitution of 0.2 M phosphate for $NaHCO_3$ in the same experiment leads to the formation of carbamoyl phosphate (**40**) (Fig. 20), an important phos-

carbamoyl phosphate (40) | (41) | cyanovinyl phosphate (42)

Fig. 20.

phorylating agent in contemporary biochemistry. Carbamoyl phosphate (**40**) has also been suggested as an intermediate in the cyanate-mediated formation of pyrophosphate from orthophosphate on an apatite mineral.[121] Cyanate has been reported to effect the phosphorylation of uridine, although the yield was lower than that obtained with other condensing agents such as cyanamide and cyanogen.[97] Cyanate has also been reported to augment the yield of diglycine obtained by heating glycine and apatite.[122]

Cyanogen

Cyanogen, which is produced in the gas phase in electrical discharge experiments (Table 1) and in solution as a secondary product of the photooxidation of ferrocyanide in the presence of cyanide ion,[123] has been employed in a number of prebiotic model phosphorylation reactions. As shown in Table 7, D-ribose, D-glucose and D-fructose have been phosphorylated to

Table 7.

Starting Material(s)	Product(s)	Special Conditions	Reference
Uridine (0.01 M), KH_2PO_4 (0.5 M)	5'-UMP (2.5% yield)	Cyanogen conc. not given, Reacted at 25°C for 24 h (pH = 7)	97
D-Glucose (2 M), Potassium phosphate (2 M)	D-Glucopyranose-1-phosphate (16% yield, mainly α-anomer)	1 M Cyanogen - Reacted at 25°C for 24 h (pH 7).	124
D-Fructose (0.2 M), Orthophosphate (0.2 M)	α-D-Fructopyranose-2-phosphate	Reaction carried out at 25°C for 1 h (pH 9)	125
D-Ribose (0.1-0.2 M), Orthophosphate (0.1-0.2 M)	α-D-Ribofuranose-1-phosphate (10-20% yield)	0.02-0.44 M Cyanogen - Reacted at 25°C (pH 7-9)	105

D-ribose-1-phosphate,[105] D-glucose-1-phosphate[124] and D-fructose-2-phosphate,[125] respectively. Interestingly, cyanogen results in phosphorylation exclusively at the hemiacetal function. The mechanism has been studied by Halmann,[124] who noted that in ^{18}O-labelled H_2O, the label is rapidly incorporated into the orthophosphate. Since direct exchange of oxygen is negligible in the absence of cyanogen, this result was interpreted as evidence of an activated phosphate adduct (**41**) (Fig. 20) which may subsequently undergo hydrolysis. It was suggested that the preference for the hemiacetal hydroxyl group was due to the greater acidity of this group. A transition state was thus proposed that involved proton transfer to the cyanogen adduct (Fig. 21).[124]

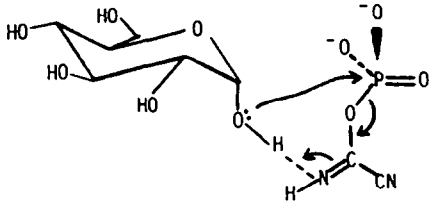


Fig. 21.

In addition to the glucose-1-phosphate, Degani and Halmann also obtained evidence for a phosphorylated disaccharide, formed from glucose, orthophosphate and cyanogen, but its structure was not characterized.[124] Cyanogen has been reported to be an effective phosphorylating agent for uridine, giving a 2.5% yield of 5′-UMP at a pH of 6.[97] However, this condensing agent was unsuccessful in bringing about the template-directed synthesis of oligonucleotides.[126]

Cyanoacetylene

As noted in Section 2, cyanoacetylene is obtained from electrical discharge experiments with methane and nitrogen, and it has been observed in extraterrestrial environments. Its role in phosphorylation reactions has been investigated by Ferris and coworkers.[127,128] Cyanoacetylene reacts with orthophosphate to form an adduct (cyanovinylphosphate, **42**) (Fig. 20), which can be isolated as a barium salt.[127] 5′-UMP was obtained in 3.9% yield by heating 0.75 M sodium cyanovinylphosphate and 1.6 M uridine at 60°; pyrophosphate can also be obtained in 2–4% yield from inorganic phosphate using cyanovinylphosphate as the condensing agent.

Diaminomaleonitrile and Diiminosuccinonitrile

As was discussed in Sections 3 and 4, DAMN (**3**) is a highly plausible prebiotic molecule, since it is one of the primary products from the thermal oligomerization of HCN. In a degassed 0.1 M HCN solution (25°, pH 9.2), DAMN reaches a steady-state concentration corresponding to a 1% yield.[26] However, there has been only one report of DAMN as a condensing agent. Chang, Flores and Ponnamperuma[129] observed the formation of diglycine in 3.1% yield from a solution of 0.5 M glycine and 0.025 M DAMN that was maintained at 94° and pH 6 for 24h. The yield was still 1.2% when the concentrations were reduced to 0.001 M DAMN and 0.01 M glycine. Unlike the previously described condensations, which gave the highest yields under acidic (pH 2) conditions, the DAMN-mediated formation of diglycine gave optimal results in alkaline solution, a condition that is more consistent with current models of a prebiotic ocean.[130] A mechanism was proposed whereby the amino acid displaces a cyanide ion to form the activated acid ester (**43**) (Fig. 22).

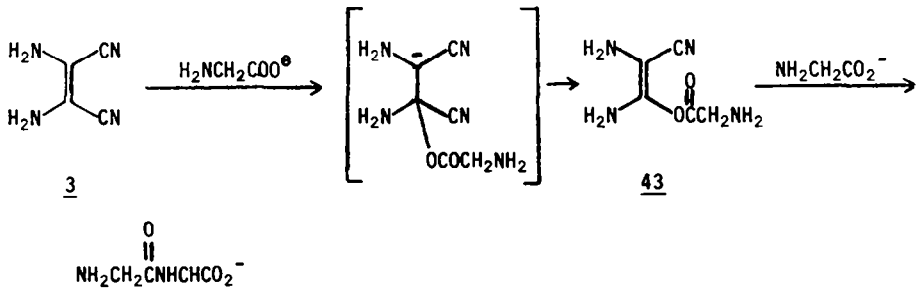


Fig. 22.

However, DAMN has not been applied successfully to any other condensation; the attempted phosphorylation of uridine using DAMN was unsuccessful.[97] The inability to detect oligoglycines higher than the dimer in the experiments of Chang *et al.*[129] suggests that amino acids and DAMN alone could not have provided a source of oligopeptides on the primitive earth.

The low yield of condensation products from DAMN-glycine suggests that DAMN is not sufficiently reactive to form activated intermediates. By contrast, diiminosuccinonitrile (DISN, **9**), which is readily formed by the oxidation of DAMN (Section 3E), reacts with a variety of nucleophiles to displace cyanide.[131] Although the oxidation of DAMN is easily effected by various metal salts or clay minerals,[43] the reaction is also effected by oxygen.[26] Since no attempt was made to exclude air in the experiments of Chang *et al.*,[129] the reported DAMN-mediated formation of diglycine may actually have proceeded through the partial oxidation of DAMN to DISN during the course of the reaction.

Ferris *et al.* have reported the first experiments with DISN as a condensing agent in prebiotic systems. Phosphorylation of uridine in an aqueous solution of orthophosphate proceeded with low efficiency to give small amounts of 2′(3′)- and 5′- UMP.[132] DISN was more effective in the cyclization of 2′(3′)-UMP to cyclic 2′, 3′-UMP (**44**). Interestingly, this reaction gave the highest yields in the range of pH 6-7, and the reaction was catalyzed by the divalent metals Zn^{2+}, Pb^{2+}, Mn^{2+} and Mg^{2+}. Yields of up to 42% were obtained at 60°. These yields in slightly acidic media are comparable to the yields of uridine-2′, 3′-cyclic phosphate from 2′(3′)-UMP and cyanamide.[97] The cyclization reaction is assumed to proceed by way of a DISN-phosphate adduct (**45**) (Fig. 23A).[132]

In the same study,[132] it was noted that the reaction between 5′-UMP and DISN failed to give oligonucleotides, but produced other nucleotide derivatives. An investigation of the chemistry of uridine and DISN showed that when the reaction was carried out at 2°, the main product was the carbamate ester of 2, 2′-anhydrouridine (**46**) (Fig. 23B). Minor products included the 2′, 3′-cyclic carbonate of uridine (**47**), 2, 2′-anhydrouridine (**48**) and arabinosyluridine (**49**). A mechanism was proposed that involves nucleophilic attack by the 2′-hydroxyl (the more acidic of the hydroxyl groups[133]) on DISN, followed by cyclization to the imino carbonate (Fig. 13B). This is the first reported prebiotic synthesis of an anhydropyrimidine nucleoside, a species that has been suggested as a precursor to oligonucleotides on the primitive earth.[134]

Further work is required to determine the efficacy of DISN in prebiotic condensation reactions, particularly in the formation of biopolymers. Current work is being directed toward nucleotide condensations on clay surfaces. With regard to possible phosphorylation reactions, it is intriguing to note that a major hydrolysis product of DISN is oxalate,[26] which may have had a role in releasing phosphate from apatite minerals by complexing calcium.[103] The fact that DISN is generated under the same chemical conditions as amino acids, purines and pyrimidines suggests that DISN could indeed have played a role in the synthesis of polynucleotides and polypeptides.

APPLICATIONS OF HCN CHEMISTRY TO ORGANIC SYNTHESIS

The synthetic concepts that have been developed in the course of prebiotic studies have been applied successfully to the preparative synthesis of a variety of biochemicals and their analogs. For example, the utility of aminomalononitrile (**2**) as a synthetic precursor has recently been reviewed.[135] By removing the constraint of an aqueous solvent, the yield of a desired product from a "prebiotic" reaction in many cases can be increased dramatically. Although a complete discussion of these synthetic applications is beyond the scope of the present review, a few examples will serve to illustrate the versatility of HCN derivatives in the preparation of diverse classes of compounds.

Diaminomaleonitrile

Many variations on the synthesis of DAMN from HCN have been developed.[136] By working in DMSO and adding certain catalysts, it is possible to obtain very high yields of DAMN: e.g. in the presence of Et_3Al, a yield of 96% may be achieved with 98% purity.[136b] The oligomerization of HCN has now been employed in a commercial preparation of DAMN.[137]

Imidazoles and Purines

The formation of adenine from HCN has been developed into a practical synthesis. Heating a solution of HCN in liquid ammonia gave adenine in 22% yield; 4, 5-dicyanoimidazole was also

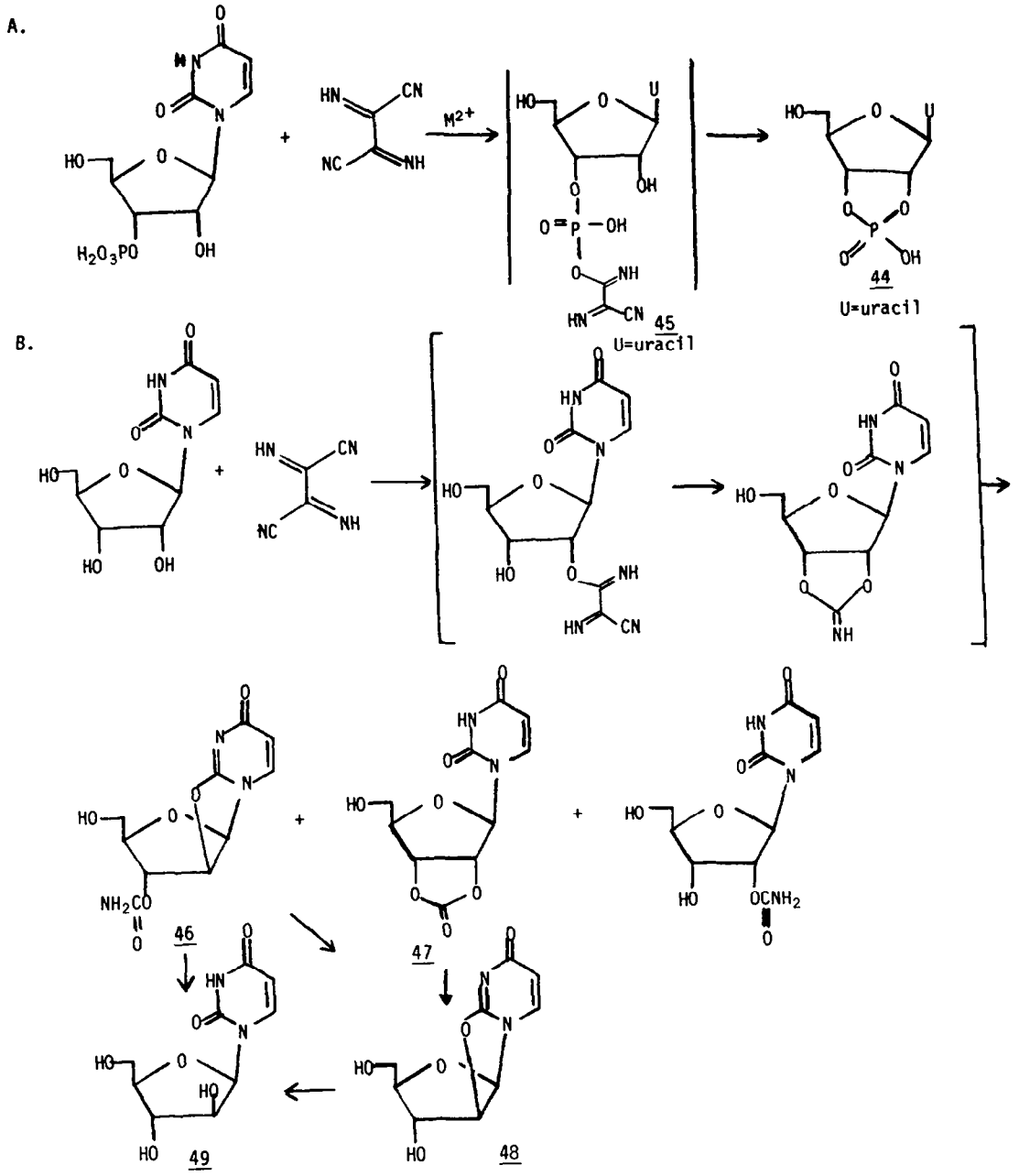


Fig. 23.

obtained in 21% yield.[138] The same products are obtained in 9 and 20% yields, respectively, by heating DAMN with ammonium formate at 140°.[75a] A 68% yield of adenine is achieved when DAMN is heated at 135° with formamide and NH_3.[75b]

The trimer of HCN, aminomalononitrile, has also been employed in a variety of heterocycle syntheses.[135] Although aminomalononitrile itself is unstable and rapidly oligomerizes (Section 3C), the compound can easily be stored as its p-toluenesulfonate salt.[139,140] When the reaction between aminomalononitrile and formamidine is carried out in ethanol and methoxyethanol (Fig. 24A), adenine is produced in 24% yield.[141] Acid anhydrides react with aminomalononitrile to form 5-amino-4-cyano-oxazoles (**50**), which can react with formamidine to give oxopurines, such as 7-aminooxazole-(5, 4-d)-pyrimidine (**51**) (Fig. 24B).[141]

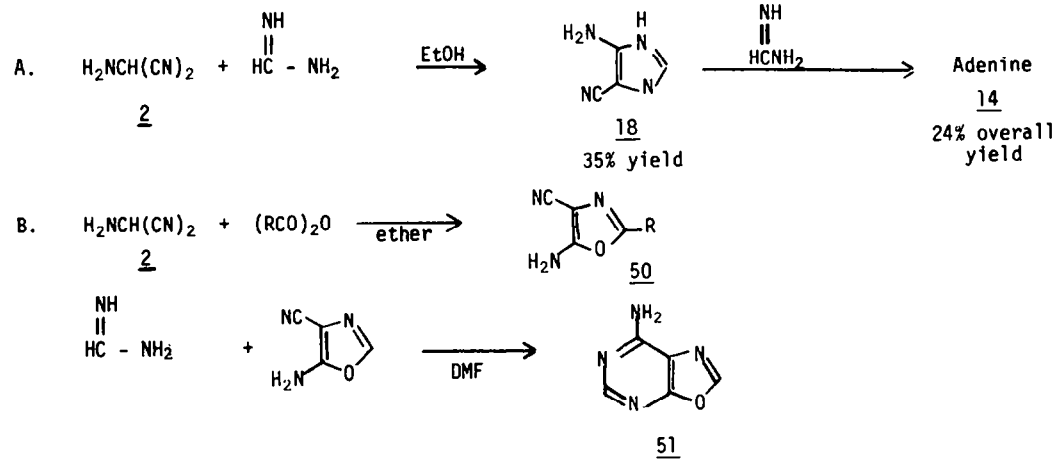


Fig. 24.

Pyrimidines and Pteridines

Pyrimidines, such as **52**, are formed from DAMN by the reaction with cyanoformimidates,[142] a reaction suggestive of the synthesis of pyrimidines by the hydrolysis of HCN oligomers (Fig. 25).

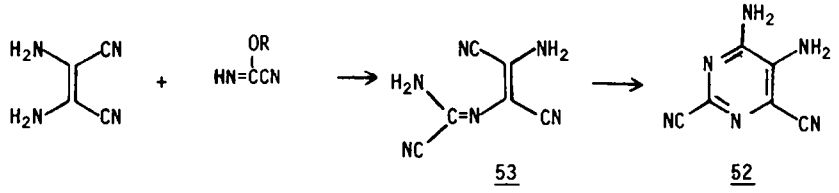


Fig. 25.

As was noted in Section 4, the cyanoformidine deriviative of DAMN (the cis isomer of **53**) has been postulated as an intermediate in the prebiotic synthesis of adenine (Fig. 20).

Aminomalononitrile has been found to be a versatile precursor to a variety of pteridines and pterins.[135,143] Among the many applications that have been developed is the synthesis of an isomerically pure sample of Cyprino-Pourpre B (**54**), one of the pteridine constituents of the scales and skin of fresh water fishes.[144] The synthesis involves a condensation of aminomalononitrile with dioximinoacetone as the first step (Fig. 26). A similar approach to the synthesis of pteridines that are analogs of methotrexate was noted recently.[143]

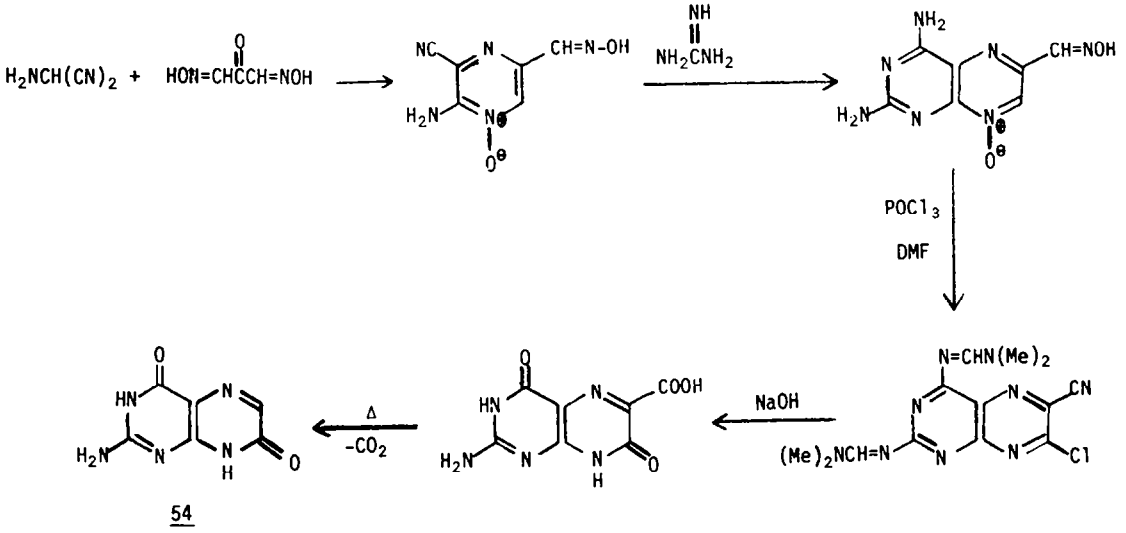


Fig. 26.

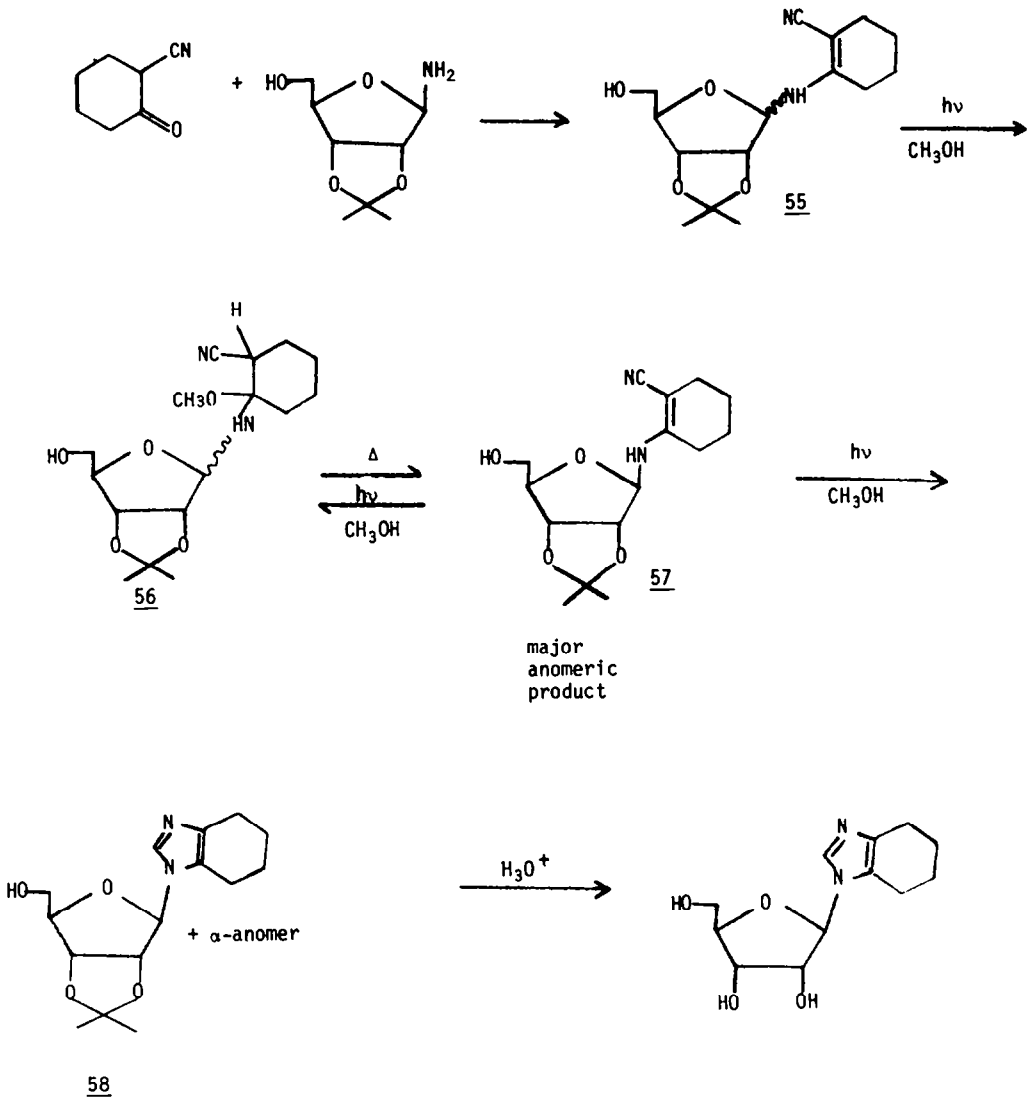


Fig. 27.

Nucleosides and their analogs

The photochemical rearrangement of enaminonitriles that had been noted in the synthesis of adenine (Section 5B) has been applied successfully to the synthesis of novel amidazole nucleosides (Fig. 27). Irradiation of an anomeric mixture of N-(2,3-O-isopropylidene-1-D-ribofuranosyl)-1-amino-2-cyano-cyclohexene (**55**) resulted in the rapid addition of methanol to give an adduct (**56**) which slowly eliminates the methanol thermally to give the β-anomer as the major product.[145] The photochemical cyclization to the imidazole (**58**) is a less efficient process than the methanol addition; thus the net result of the photolysis is the conversion of **55**, in which the α-anomer predominates, to **58** in which the β-anomer predominates.

DAMN has been employed in the thermal synthesis of several nucleoside analogs.[146,147] The outline in Fig. 28 illustrates an efficient route to C-nucleosides (**59**); the corresponding arabinofuranosylimidazoles can be obtained by starting with D-glucose or D-mannose in place of D-ribose (**60**).[147]

In a variation of the Shaw aminoimidazole synthesis,[148] aminomalononitrile has been employed

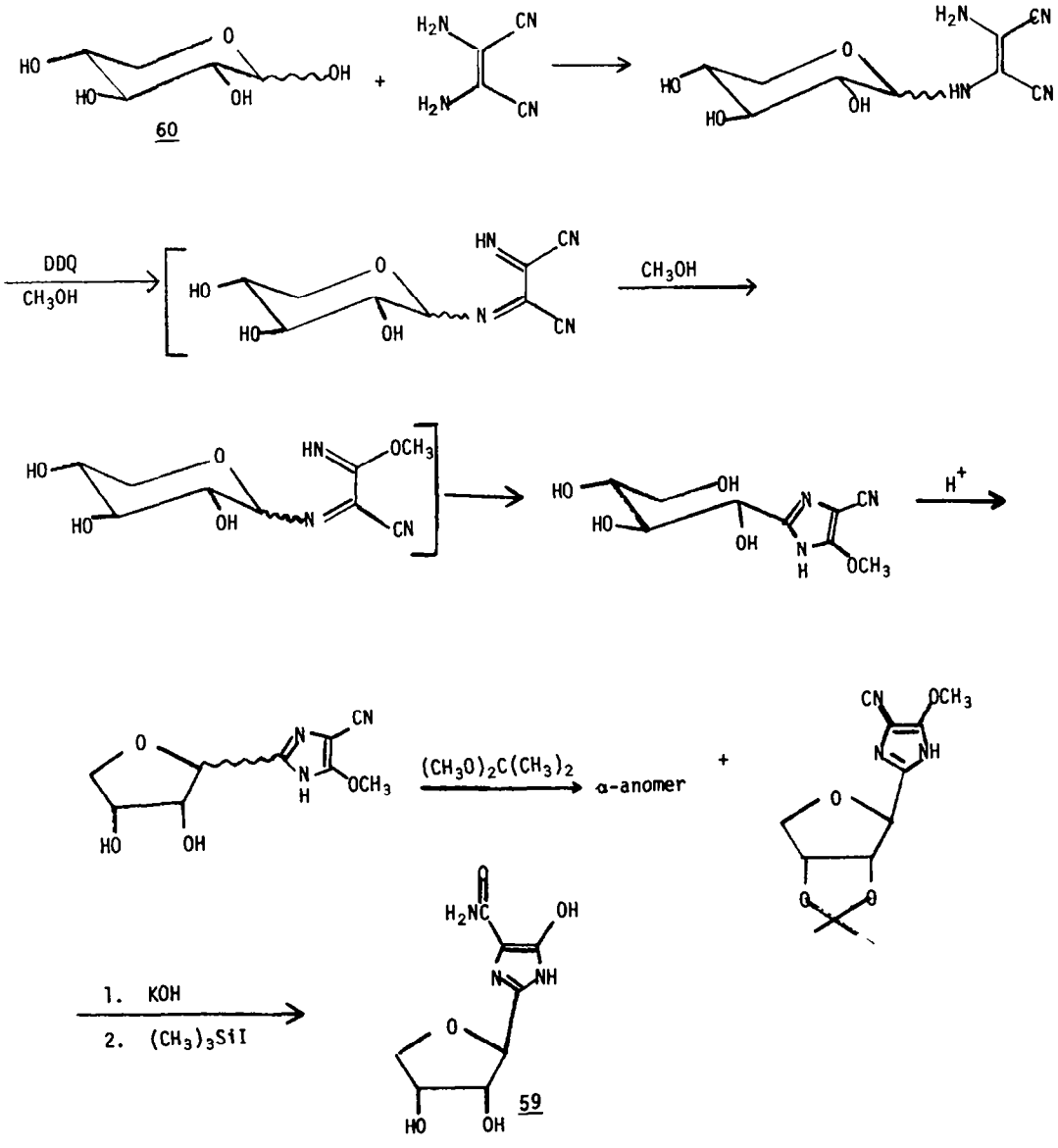


Fig. 28.

in a novel synthesis of the C-nucleoside analog (**61**) of adenosine (Fig. 29).[149] A similar synthesis of **61** from the reaction of aminomalononitrile with methyl α-D-ribofuranosyl-1-carboximidate has recently been reported.[150]

Cyanamide and cyanoacetylene have been used in a novel synthesis of the antitumor agent β-arabinosylcytosine (**62**) (Fig. 30). Cyanamide reacts with D-arabinose (**63**) to produce the aminooxazoline derivative (**64**), which reacts with cyanoacetylene to give the nucleoside in high yield.[151] Using D-ribose as the sugar, the α-anomer of cytidine could be synthesized; irradiation with 254 nm light resulted in a partial conversion to β-cytidine.

Bisanil dyes

DAMN condenses with aromatic aldehydes to give the bisanil compounds shown in Fig. 31.[152] These highly colored compounds range from blue to yellow, and have been used to dye polyester fibers.

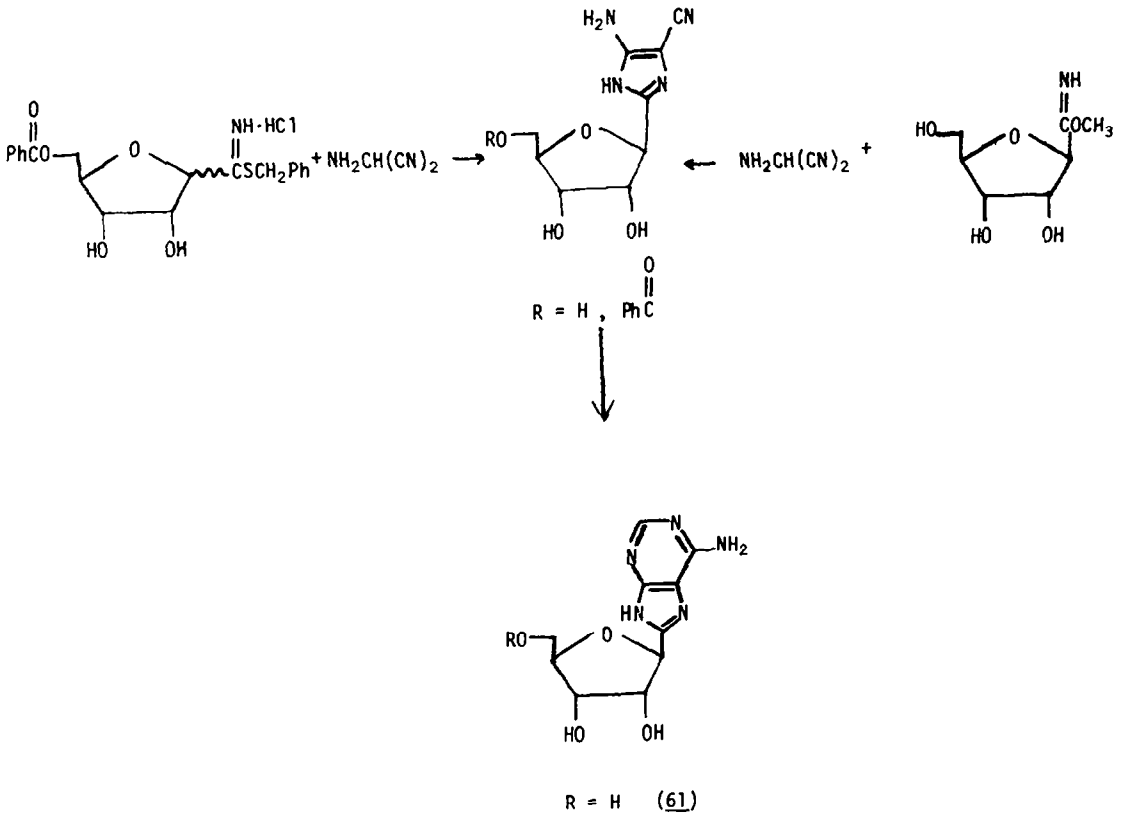

PhCO
NH·HCl
CSCH2Ph
NH2CH(CN)2
RO
HO
OH
H2N
CN
HN
NH
COCH3
R = H , PhC
NH2
R = H (61)

Fig. 29.

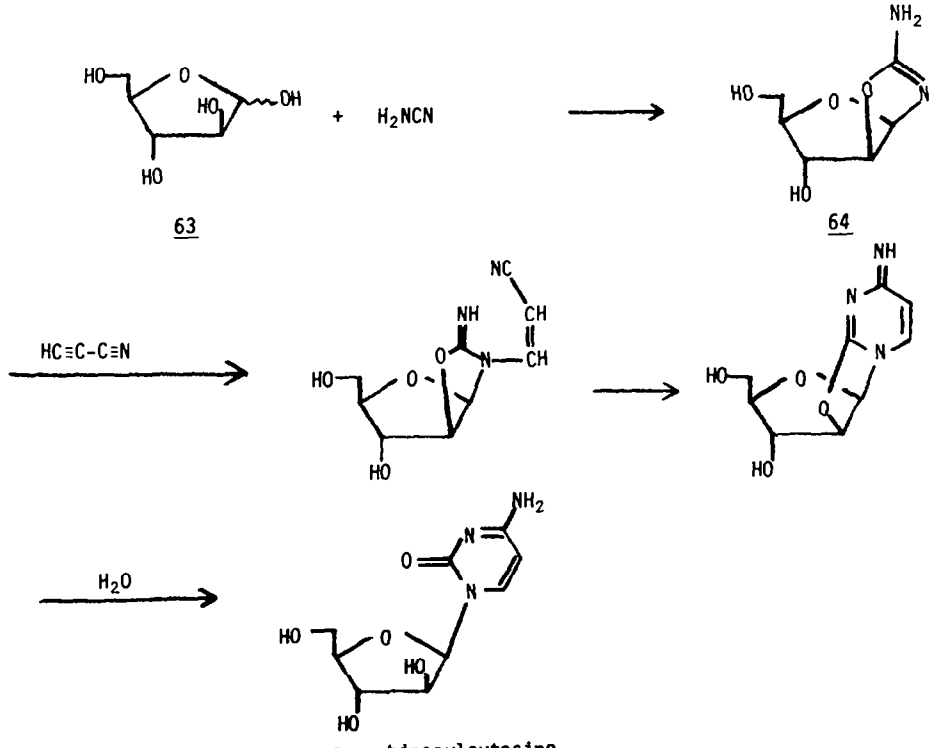

HO
OH
H2NCN
NH2
63
64
HC≡C-C≡N
NC
CH
NH
β-arabinosylcytosine
62

Fig. 30.

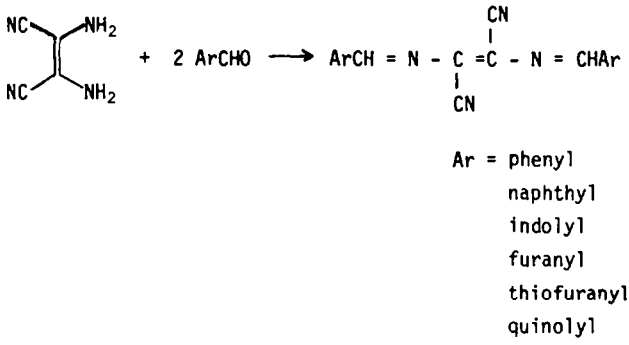


Fig. 31.

CONCLUSIONS AND FUTURE DIRECTIONS

It has been shown that a large variety of biological molecules can be synthesized using HCN as the only starting material. One need not be alarmed by the inability to synthesize the entire spectrum of biomolecules associated with contemporary organisms, since it is likely that early life could have existed with much less. Clearly, more than HCN alone is required: one needs a source of phosphate and a source of ribose before one can even consider preparing a nucleotide from the products of HCN oligomers. It nevertheless is encouraging that mildly alkaline solutions of cyanide can provide such an abundance of biomolecules.

Current work in our laboratory is directed toward the prebiotic formation of oligopeptides and oligonucleotides using cyano compounds as condensing agents. We hope to develop plausible prebiotic pathways to oligopeptides and oligonucleotides using cyanide-based condensing agents formed in the presence of inorganic and organic templates. If it is possible to demonstrate that HCN derivatives effect the condensation of monomers to oligomers, then there will be a continuum of prebiotic chemistry extending from monomer formation to polymer synthesis in which HCN has a central role.

This review has summarized some of the achievements in the synthesis of biomolecules using HCN and other cyano compounds as starting materials. The study of cyanide chemistry represents but one approach toward prebiotic synthesis, and it should be apparent from the foregoing discussion that many gaps remain in our understanding of the events that preceded the appearance of life on the earth. Indeed, chemical evolution as an experimental science is still relatively young, and its paradigms may yet be extensively revised or entirely replaced. The apparent successes that have been achieved in the study of the origins of life should not be a dissuasion from seeking new models for comparison. Nevertheless, further studies in the laboratory and future investigations of other planetary bodies will provide a surer foundation upon which to build our understanding of the prebiotic chemistry of the Earth.

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