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# About a Formamide-Based Origin of Informational Polymers: Syntheses of Nucleobases and Favourable Thermodynamic Niches for Early Polymers

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**Abstract** Formamide $NH_2CHO$ chemistry provides a unitary frame into which several pieces of the origin-of-life puzzle may be adjusted. Synthetic processes were uncovered which, starting from formamide and prebiotically easily available common catalysts, yield all the necessary nucleic bases precursors, including acyclonucleosides. Formamide allows phosphorylations and trans-phosphorylations, favours the micellar aggregation of surfactants and, most importantly, determines conditions in which the formation of nucleic polymers is thermodynamically favoured. In the detected conditions, the phosphoester bonds are more stable in the polymeric than in the monomeric form, thus allowing formation and survival of informational nucleic polymers.

**Keywords** formamide · nucleic bases · acyclonucleosides · informational polymers · thermodynamic niches

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## Introduction

This text is the report of the communications by R. Saladino and E. Di Mauro, presented here under the same title for reasons of coherence and continuity. Life is a robust phenomenon and had an early start on planet Earth. Thus, the process that led to accumulation and replication of polymeric information must have been correspondingly robust. One consequently sound assumption is that a single unitary chemical frame should have provided all (or the majority of) the necessary building blocks. Ideally, the early abiotic synthetic processes should have been based on easily available and widespread catalysts. In addition, the robust chemistry involved should have allowed not only the very first syntheses but should have facilitated as many as possible of the following steps leading to self protecting self-replicating structures. In this logic, formamide appears to be a good candidate.

## Syntheses from Formamide

To date, the simple gaseous molecule hydrogen cyanide HCN had the role of absolute protagonist in the prebiotic chemistry of nucleic acids and of their components. The success of this molecule derives from the classic Urey and Miller's experiments, in which HCN participated with ammonia and aldehydes to a Strecker condensation yielding amino acids (Miller 1953; Miller and Urey 1959; Miller et al. 1976).

The role of HCN in the prebiotic synthesis of nucleobases was futher established, under hot or cold conditions, both in its monomeric, condensed (aminomaleonitrile AMN and diaminomaleonitrile DMAN) and polymeric "alzulmin" forms (for a recent review: Saladino et al. 2005a). These studies uncovered efficient syntheses of purine nucleobases. However, no examples were reported of pyrimidine nucleobases syntheses. Thus, a prebiotic one-carbon precursor different from HCN is required for the synthesis of cytosine, thymine and uracil. In any plausible prebiotic scenario for the heterotropic origin of life on primitive Earth, the fate of HCN was to be adsorbed by the hydrosphere. After its adsorption, HCN could have been polymerised to purines or hydrolysed to formamide $NH_2CHO$ and ammonium formate $HCOO^-NH4^+$, depending on its local concentration. Little efforts have been so far devoted to study the role of $NH_2CHO$ and $HCOO^-NH_4^+$ as co-protagonists in the origin of nucleic acids. Pioneering studies have shown that the simple purine scaffold can be synthesized from neat $NH_2CHO$ at 160–190°C, while adenine becomes the major reaction product in the presence of HCN (Okamoto et al. 1972). However, purines were recovered in low yield, while pyrimidines were not detected at all. Does the lack of attention for $NH_2CHO$ as prebiotic precursor justify the low yield of purines and the total absence of pyrimidines? Does a special key exist opening a way to pyrimidine nucleobases from $NH_2CHO$? This special key does exist, its lock being the catalysis by minerals and metal oxides largely diffused on the surface of our planet. A simple carbonate (calcium carbonate) significantly increases the yield of purine when used as a catalyst in the thermal condensation of $NH_2CHO$. In addition, adenine, 4(3H)-pyrimidinone and cytosine are efficiently synthesized when the condensation is performed in the presence of silica, alumina, kaolin and a Y type zeolite, the selectivity of the reaction depending on the catalyst used in the transformation (Saladino et al. 2001). The prebiotic synthesis of cytosine from a one-carbon precursor as simple as $NH_2CHO$ is straightforward and opens a novel prebiotic pathway to purine and pyrimidine nucleobases simply by changing the catalyst used in the condensation. A few examples: uracil, cytosine,

hypoxantine (a bioisoster of guanine), 4-aminoimidazole-5-carboxamide (AICA) and 4-formylaminoimidazole-5-carboxamide (fAICA) are obtained (in addition to purine and adenine) by heating $NH_2CHO$ in the presence of the montmorillonites K-10, K-30, KSF and of aluminum-pillared clays, (compounds differing for pore size distribution, surface area, acidity and cation exchange capability – Saladino et al. 2004b). It is noteworthy that the ribosyl-5′-monophosphate derivatives of AICA, fAICA and hypoxantine are the main intermediates in the actual biosynthesis of inosine 5′-monophosphate (IMP), the precursor of adenosine 5′-monophosphate (AMP) through the action of a formyltransferase and IMP-cyclohydrolase. Thus, in agreement with the Eschenmoser definition, the extant biosynthesis of IMP might be an example of a chemiomimetic process of its pristine chemical origin. Additional properties of the $NH_2CHO$ condensation protocol were detected performing the reaction in the presence of titanium dioxide $TiO_2$, a catalyst able to partially decompose $NH_2CHO$ to formaldehyde, the most important prebiotic precursor of sugars, as discovered by Butlerow 150 years ago. In fact, when $NH_2CHO$ was heated at 160°C in the presence of catalytic amounts of $TiO_2$, a large panel of nucleobase derivatives was obtained, including thymine, uracil, 5-hydroxymethyl uracil 5-HMU and three purine acyclonucleosides, in which an alicyclic sugar side-chain is linked to the purine heterocycle at N-9 atom (Saladino et al. 2003). In both cases formaldehyde played a relevant role in the mechanism of the reaction. As an example: thymine was synthesized by addition of formaldehyde to the C(5)–C(6) double bond of newly formed uracil to give 5-HMU, which in turn loses, after esterification with formic acid (produced from ammonium formate), carbon dioxide yielding thymine. Again, the ribosyl-5′-monophosphate derivatives of uracil and 5′-HMU are the main intermediates in the actual biosynthesis of thymidine 5′-monophosphate. In this biosynthesis formaldehyde is transferred from methylenetetrahydrofolate to uridine 5′-monophosphate by action of a thymidilate synthase. The mixture of $NH_2CHO$ and formaldehyde is also responsible for the formation of purine acyclonucleosides, probably by a formose-like condensation of an activated formaldehyde on the exocyclic formyl moiety of newly synthesized formylpurine and adenine derivatives. This reaction provides a solution to the long-standing problem of the lack of reactivity between nucleobases and ribose or 2′-deoxyribose in the prebiotic synthesis of nucleosides. Extraterrestrial minerals also show interesting catalytic properties in the condensation of $NH_2CHO$ to nucleobases. A large panel of pyrimidine nucleobases (including uracil, cytosine, 4(3H)-pyrimidinone and 5,6-dihydrouracil) were in fact selectively synthesized from hot formamide in the presence of cosmic dust analogues CDAs of terrestrial olivine, fayalite and fosferite. Noteworthy, CDAs were more reactive than the corresponding terrestrial minerals, the selectivity of the reaction being finely tuned by the elemental composition of the catalyst. The yield of pyrimidines increased by increasing the amount of iron in the mineral (Saladino et al. 2005a). For a recent review on the role of formamide on the prebiotic chemistry of nucleic acids see: Saladino et al. 2004a.

## A Model for Ur-Polymerizations

Let us start from the classical, far-sighted words by Darwin "... we could conceive in some warm little pond, with all sort of ammonia and phosphoric salts, light, heat, electricity, etc,..." (Darwin 1888). Let us also assume in the pond the presence of formamide. Figure 1 simplistically depicts in *a* and *b* this reductionist scenario. Formamide may be prebiotically formed in several ways (summarized in Saladino et al. 2004a, 2005a), the most direct and possibly prebiotically relevant being the hydrolysis of HCN (Figure 1c). One of the

important properties of formamide in this context is the wide range of temperatures in which it keeps its liquid state: from 4 to 210°C. Depending on the environmental presence of water and on the temperature it may thus easily form completely anhydrous or variously concentrated aqueous solutions.

Let us also imagine that these liquids fill crevices in rocks made of any of the catalysts reported in the preceding Section (or of a combination thereof), possibly of clays and/or

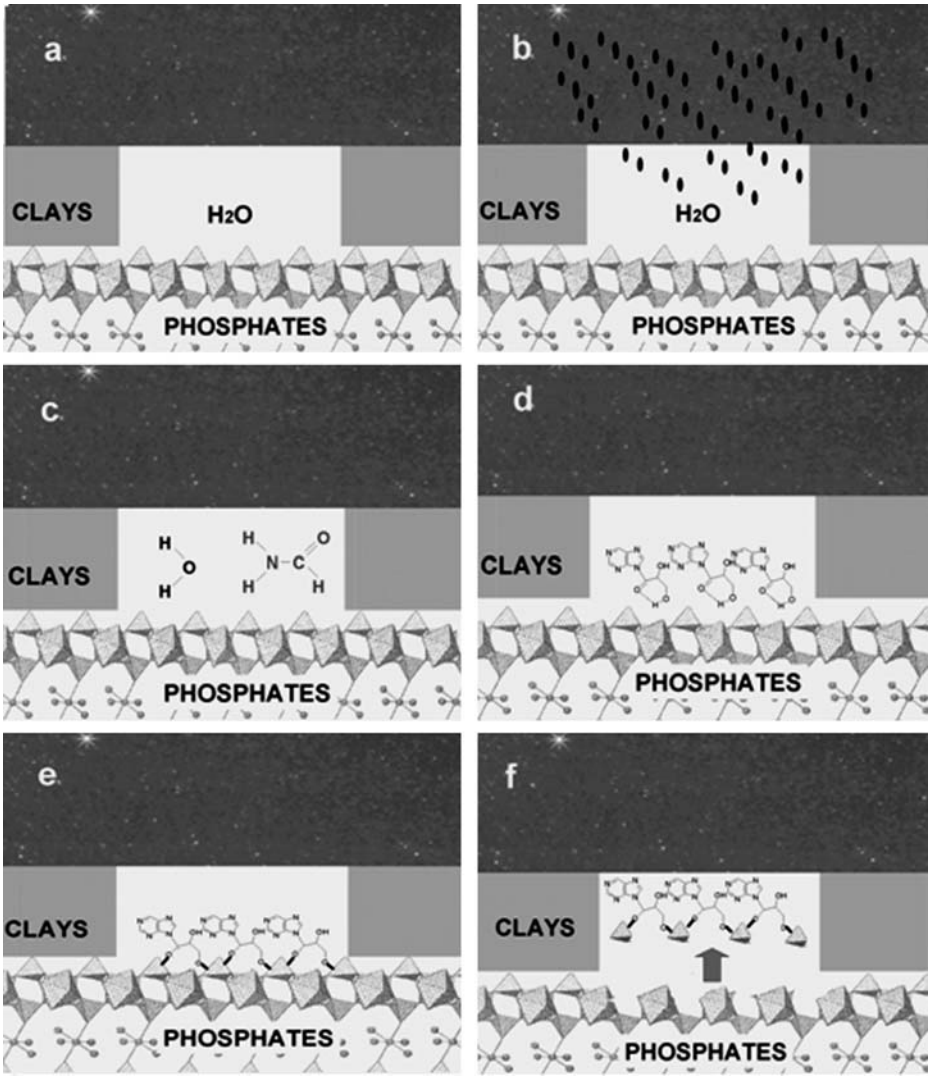


**Figure 1** A minimalist view of ur-polymerizations. In a crevice whose walls are made of one (or more) of the catalysts described in "Syntheses from Formamide" and of a phosphate-rich surface, filled with water (**a**), the addition of HCN (**b**) might have led by hydrolysis to the formation of formamide (**c**). The synthesis of nucleic bases and of acyclonucleosides (**d**) might have followed. We have observed that acyclonucleosides are actively synthesized in the presents of $TiO_2$ (not indicated in the cartoon for simplicity). The resulting compounds might have reacted with the phosphate surfaces (**e**), polymerized by formamide-mediated trans-phosphorylation and eventually released (**f**) into the solution.

olivines of terrestrial and/or sidereal origin. At moderately hot temperatures (in our experimental settings between 90° and 160°C, Saladino et al. 2001, 2003, 2004b, 2005b) this pristine little pond would quickly be enriched by nucleic bases and, if $TiO_2$ was also present, by sugar chains growing onto them (Saladino et al. 2003). How widespread might have been the photochemistry at the basis of this latter process has not been thoroughly studied; however, it can be confidently assumed that additional catalysts might have also been able to carry out similar synthetic processes.

In hot formamide nucleic bases are rapidly degraded (Saladino et al. 1996) and the synthesis/degradation cycle would remain totally sterile, in spite of the robustness of the formamide-based synthetic reactions. A way-out from such a futile cycle could have been found if two conditions were met: an equally robust and simple polymerization process and the existence of a set of conditions resulting in a thermodynamic niche in which the survival of the polymer is favoured.

We have not (yet) studied the role of formamide in the abiotic polymerization process. Its plausibility and possible robustness are hinted to by the pioneering observations by Schoffstall and co-authors who described the facile and high-yield phosphorylation and trans-phosphorylation processes of nucleic bases by formamide and various inorganic phosphates (Schoffstall 1976, Schoffstall et al. 1982, Schoffstall and Laing 1985, Schoffstall and Mahone 1988). We have confirmed their observations and found that the yields of de novo phosphorylated nucleosides may be quite high and position selective (to be detailed elsewhere).

## Thermodynamic Niches

The steps to life following the so far hypothetical chain-phosphorylation of cyclic and/or acyclic nucleosides obtained in the presence of phosphates and made possible by formamide meets two major difficulties. The first has been clearly pointed out by K. Van Holde (1980) and relies on the consideration that condensation reactions are not thermodynamically spontaneous in dilute aqueous solution or even at moderate water activities. The solution (besides the complex enzymatic activation processes present in extant living systems) is provided by the evasion from the unfavourable Gibbs free-energy charge ($\Delta G^{\circ\prime}$) by reducing the water activity. This requires that the ur-polymerizations of oligonucleotides (or, for that matter, of any other type of polymer) occurred in highly dehydrating conditions. That is: on a phosphate or phosphate-rich template surface in formamide and/or in dry conditions. Figure 1 depicts this process in *d* (formation of nucleosides) and *e*. This latter panel hints to the chain-wise trans-phosphorylation of nucleosides using as phosphate donor, in the presence of formamide, the surface of clays onto which phosphates had previously deposited or directly onto the surface of phosphate minerals. In panel *f* the neo-formed polymer leaves its template mineral cradle and passes in solution.

No matter how hypothetical, this scheme relies on chemically (Schoffstall 1976, Schoffstall et al. 1982, Schoffstall and Laing 1985, Schoffstall and Mahone 1988) and thermodynamically (Van Holde 1980) sound assumptions.

Let us further imagine that water slips into the dry (Van Holde 1980) phosphate-clay crevice in which the nucleic bases have formed (Saladino et al. 2001, 2003, 2004b, Saladino et al. 2005b) and have been (trans)-phosphorylated (Schoffstall 1976, Schoffstall et al. 1982, Schoffstall and Laing 1985, Schoffstall and Mahone 1988), thus binding one to the other. The question is: how long will the polymers survive in the water solution in

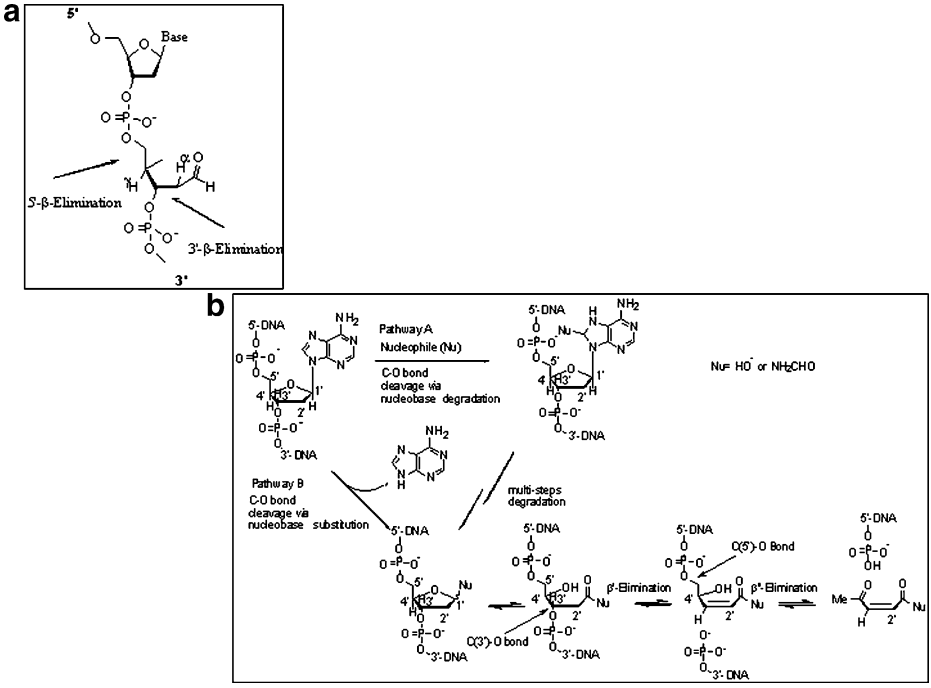


**Figure 2** The degradation pathway of the deoxy polymeric chain. **a** Degradation or removal of the nucleic base leads to the destabilization of the sugar ring, followed by β-elimination (at 3′ or at 5′) (Negri et al. 1996; Saladino et al. 2004b, 2005a). **b** The first part of the hydrolysis may occur via nucleobase degradation (pathway A) or nucleobase substitution (pathway B). The two pathways have different rates and different preferential physico–chemical optimal conditions (Saladino et al. 2005c).

which they dissolve and in which they are supposed to endure for a while long enough to replicate and start their evolutionary endeavour? Thus, the half-life values of the phosphoester bonds were analyzed in detail both in deoxyribo nucleosides and nucleotides, and in the DNA chain (Saladino et al. 2005c) in a partial panel of conditions similar to those in which all the processes mentioned above do occur: temperatures up to 110°C and formamide content in water between 0 and 100%. Figure 2 describes the degradation pathways of the deoxy polymer chain, the Hydrolysis via Nucleobase Substitution (HNS) pathway prevailing in water, the Hydrolysis via Nucleobase Degradation (HND) pathway prevailing in formamide. These degradations pathways of the phosphoester bonds are described in detail in Saladino et al. 2005c. Figure 3 compares the half-lives of the 5′-phosphoester bond in the monomer 5′-dAMP with that of the average half-life of the same bond in DNA (determined as in Saladino et al. 2005c). A defined set of conditions results from these determinations in which the stability of these bonds is higher for the polymeric form than for the monomeric one.

Figure 4 summarizes these conditions in form of a phase diagram: the polymer is favoured at high temperature and high formamide. A similar analysis is currently being performed for the RNA system.

The high temperature favouring the polymer over the monomer fits well with the scenarios depicting biological evolutionary correlations: the forms of life arising at the temperatures characterizing early Earth were supposedly thermophilic or hyperthermo-

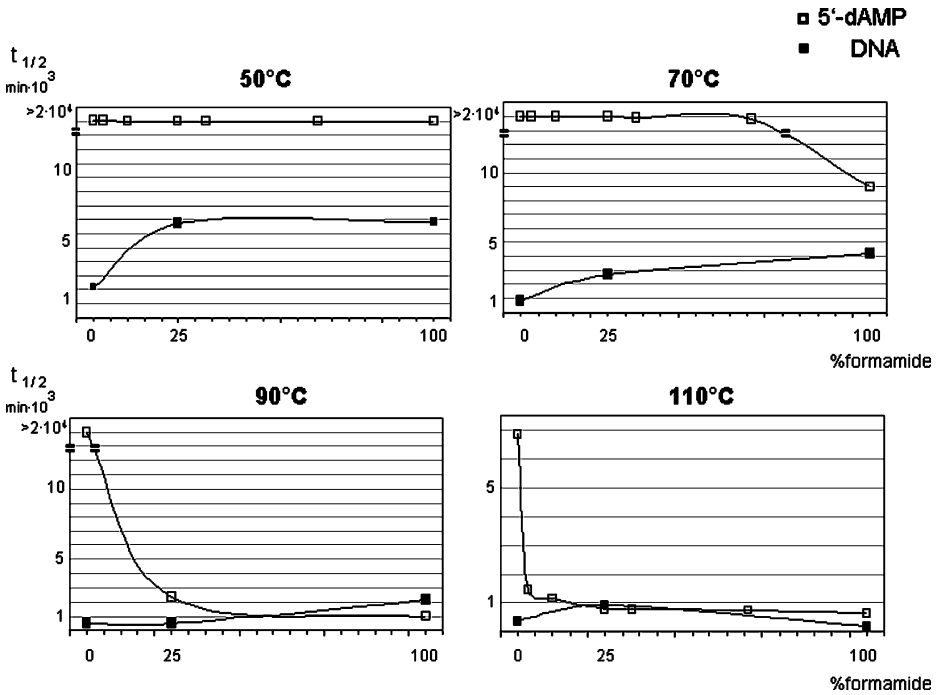


**Figure 3** Comparison of the half-life of the 5′-phosphoester bond in the monomer 5'-dAMP with that of the same bond in DNA. The half-lives, determined as detailed in Saladino et al. 2005c, were calculated at the temperatures of 50, 70, 90 and 110°C, as indicated, for 5′-dAMP (*open symbols*) and for a deoxy 46mer (Saladino et al. 2005c) (*filled symbols*). Note that the ordinate of the 110°C panel is at different scale.

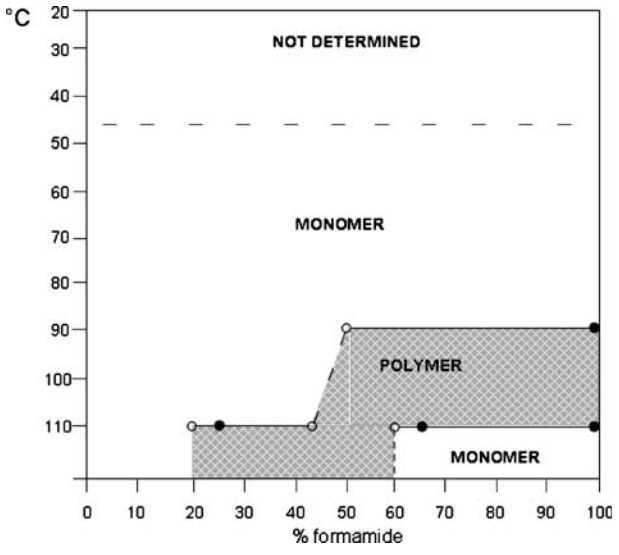


**Figure 4** The physico–chemical conditions in which the 5′-phosphoester bonds of deoxy nucleotides are more stable in the polymeric than in the monomeric form are indicated by two type of symbols: filled symbols: the actual experimental points. Open symbols: the cross-over points of the curves in Figure 3 (data from the set of experiments shown in Figure 3). The shaded area delimits the conditions in which the 5′-phosphoester bonds are more stable in the polymer.

phylic (Wiegel and Adams 1998; Forterre et al. 1995). The evolutionary tree had thermophilic roots (Schwartzman and Lineweaver 2004; and references therein).

The exploitment of the set of conditions (here dubbed "thermodynamic niche") favouring the survival of the phosphoester bond when embedded in a polymer is considered the initial Darwinian property necessary to allow accumulation and evolution of chemical ur-genetic information.

The polymer-vs-monomer advantage identified is small, and polymers long enough to code information would not survive for long in the bare scenario outlined here. Nevertheless, once this physico-chemical protective principle had been established, other positive selection factors might have come into play and exerted an evolutionary positive function. Among experimentally testable possibilities: did the aminoacids that were firstly observed in the pioneering syntheses described by Miller 1953; Miller and Urey 1959; Miller et al. 1976, reviewed in Delaye and Lazcano 2005) exert a protective role? Did strand (self-)complementarities contribute to increase polymer stability? Did the mineral catalytic surfaces provide additional physical and chemical protection? Do other physical thermodynamic niches exist (i.e., at lower temperatures) favouring survival?

Irrespectively of how close the set of observations and hypotheses reported is to the process that actually occurred, formamide chemistry provides a testable scenario and a complete line of pre-genetic possibilities, from the hydrolysis of HCN up to the favoured formation of micelles (Akter and Alawi 2003; Shirota and Segawa 2004).

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