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SIMILAR ^{31}P-NMR SIGNALS EMITTED BY IMIDAZOLE AND THE NUCLEOBASES PRESUMABLY PHOSPHORAMIDO-BONDED TO TRI(META)PHOSPHATES

BY

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ABSTRACT

Similar ^{31}P-NMR signals emitted by imidazole and the nucleobases presumably phosphoramido-bonded to tri(meta)phosphates. - The carbodiimide (EDC)-induced catalytic conversion of linear triphosphate to cyclic trimetaphosphate was spectrally signalized by ^{31}P NMR at –21.5 ppm. Further enhancement of this peak in the additional presence of either imidazole or adenine (also other nucleobases) was interpreted as resulting of the opening of the trimetaphosphate ring by their phosphoramidate bonding followed by polymerization to linear polybasephosphates also signalized at –21.5 ppm.

Key-words: RMN, Nucleobases, Imidazole, Tri(meta)phosphate.

INTRODUCTION

In attempting to answer the bioevolutionary question «RNA first?», we have proposed a deterministic scheme involving primal riboseless prenucleic polymers produced in the two following steps: (1) stereospecific coding of amino acids by doublets of nucleobases; (2) «freezing» of such doublets by straight phosphoramide bonding of the NH groups of the bases (N1 in pyrimidines/N9 in purines) on the P of one phosphate group in the incubated triphosphates, further polymerizing into polyphosphate chains (TURIAN, 1996-97).

First spectral evidence for such phosphoramidic nucleobasephosphate compounds has been obtained from their acid-sensitive, UV hypochromaticity (TURIAN & SCHOENENBERGER, 1997). Now, we have investigated their presumed production by ^{31}P-NMR in close comparison with the other nitrogenous, aromatic base, imidazole chosen because of its quality of phosphate (P) acceptor by phosphoramido (P-N)-bonding and then, as imidazolylphosphate, to act as phosphorylating catalyst (SHABAROVA, 1970; RABINOWITZ & HAMPAI, 1979) and as linker of nucleotides (GRYAZNOV & CHEN, 1994; ERTEM & FERRIS, 1996). It was expected that nucleobases which share the same type of aromatic ring as imidazole (N_9 in purines and N_1 in pyrimidines) would react similarly,

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by N-P bondings, in the presence of primary P donors such as tri(meta)phosphates. To improve the efficiency of those presumptive reactions, we have added the water-soluble condensing agent EDC, a derivative of carbodiimide, itself a tautomer of prebiotic cyanamide, and considered by SHABAROVA (1988) as «the only reagent capable of synthesizing phosphoramidate». Moreover, carbodiimide compounds may exert a dual role as they are also known to catalyze the dehydrating change from the linear triphosphate to the cyclic trimetaphosphate (BECK & ORGEL, 1965; KEELE & MILLER, 1995), a ring structure which could thus provide high energy bonds (7.0 Kcal.mol-1) favorable to drive the presumed phosphoramidations of both imidazole and nucleobases.

MATERIALS AND METHODS

Following chemicals were dissolved in 2 ml of the mineral water San Pellegrino (SP) (Milano, Italy) chosen for its richness in Ca^{2+} (208 mg/l) and Mg^{2+} (55.9 mg/l) cations and its moderate enrichment in HCO_3^- anions lowering its pH 7.7 at the source and which, thereby, could counteract the Na^+ ions liberated from the split phosphates: sodium triphosphate ($Na_5P_3O_{10}$ from Merck) 50 mM; nitrogenous bases: imidazole (I from Fluka), adenine (A), guanine (G), cytosine (C), uracil (U) all from Sigma, 5 mM; EDC (N-(dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride from Merck) 1 mM (20 µl from 100 mM aqueous solution kept at 4°C). Incubations were pursued for 2-5 days at 22-23°C in pyrex tubes mildly shaken (40 alternatives/min).

Application of our enrichment technique to putative organophosphates (TURIAN & SCHOENENBERGER, 1997) involved, in the morning of the last day of incubation, addition of ethanol ad 50% to precipitate non-reacted triphosphate then eliminated by centrifugation at 4000 t/min (Sorvall SS34). Ethanol from the supernatant was further partially evaporated under vacuum back to 2 ml volume in the tubes. In the afternoon, measurements of 0.5 ml of the concentrated supernatants added with 0.1 ml of D_2O were recorded by ^{31}P-NMR on a Brucker AC200F QNP spectrometer at a frequency of 81.01 MHz.

RESULTS AND DISCUSSION

In first assays, the spectrum of triphosphate gave in solution a 6 line spectrum. One single line at –6.5 ppm comes from the pyrophosphate (PPi) and two peaks from the triphosphate (tP), a doublet at around –5.5 ppm from the "outer" phosphorus nuclei and a triplet at around –19.5 ppm from the "central" phosphorus. Only a low peak was detected at –21.5 ppm in the presence of either imidazole or adenine. It was only in the additional presence of EDC as condensing agent that this low peak increased at –21.5 ppm (Fig. 1a) and was even enhanced in the additional presence of imidazole or adenine (Fig. 1b). Such a peak at –21.5 ppm corresponds to the signal of trimetaphosphates (Table in VAN WAZER *et al.*, 1956) as checked with a saline solution of Na trimetaphosphate (25 mM, tmP from Merck) and of other ring or linear oligo- to polyphosphates according to GILLIES *et al.* (1982).

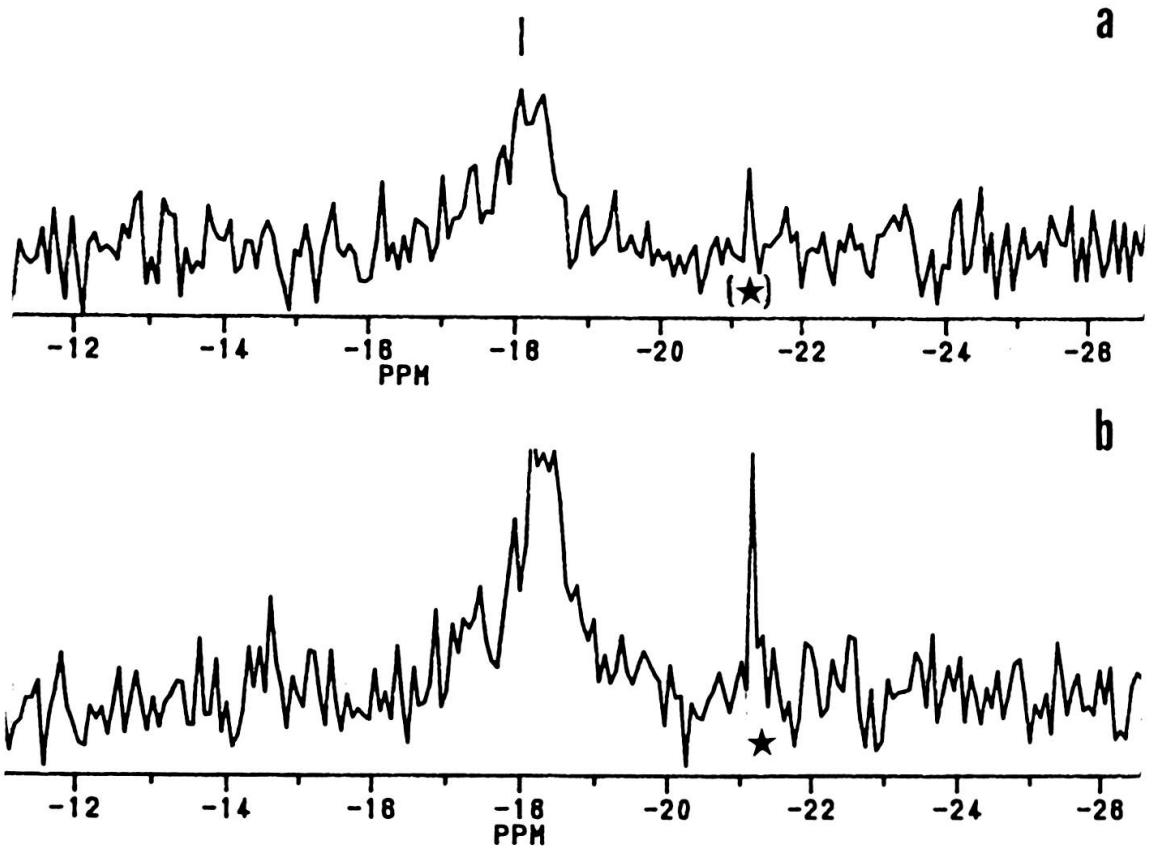

FIG. 1.

Comparative signals at –21.5 ppm (★) of ^{31}P-NMR spectra in the following mixtures: (a) TriP + adenine; (b) TriP+ EDC + adenine, incubated for 3 days in saline solution (SP) at final pH 7.8-8.0.

After organophosphate enrichment in the ethanolic supernatant, the peak at –21.5 ppm was still low in either imidazole or nucleobases alone with triphosphate (Fig. 2a). It was only significantly increased in triphosphate enriched with EDC (Fig. 2b), thereby suggesting partial conversion of triphosphate into its cyclic, metaform. Interestingly, imidazole could further promote this EDC-induced trimetaphosphate formation as shown by the significant enhancement of the peak at -21.5 ppm with either imidazole (Fig. 2c) or imidazole + adenine (Fig. 2d). Adenine, alone (Fig. 3b) or enriched in another nucleobase such as uracil (no cumulative effect, see Fig. 3c) - as also individually assayed (results not shown) - similarly enhanced the EDC signal (Fig. 3a), strongly suggesting that they were also phosphoramido-linked to trimetaphosphate as is imidazole known to become a phosphoimidazolide or imidazolylphosphate. It should be further noted that the peaks at –21.5 ppm, both trimetaP and P-N linkages of either imidazole or nucleobases, were stable around pH 7.0-8.0 but unstable and «corroded» after acidification by HCl 0.1 N to average p*Kas* of these bases (pH 4.0).

These preliminary results lead us to hypothesize that the raise of the peak at -21.5 ppm occurred by a self-enhanced process insuring continuous catalytic replenishment by EDC of the trimetaphosphate ring continuously split by the phosphoramidate (P-N)

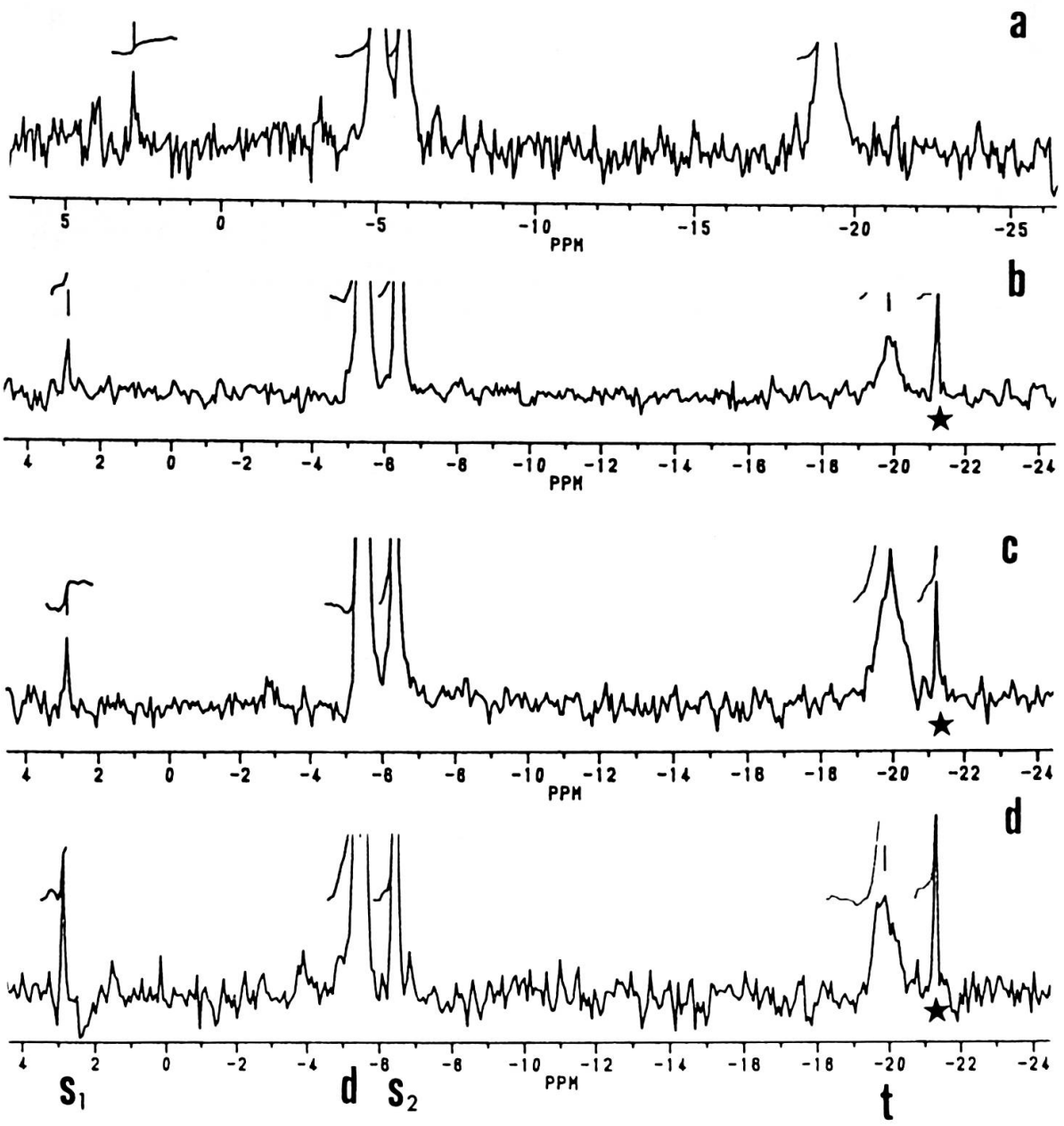

FIG. 2.

Comparative signals at -21.5 ppm (*) of ^{31}P-NMR spectra in the following mixtures incubated as in Fig. 1 but then extracted in the 50 % ethanolic supernatant: (a) TriP + imidazole; (b) TriP + EDC; (c) TriP + imidazole + EDC; (d) TriP + imidazole + adenine + EDC. Other signals: singulets of Pi (S_1) and PPi (S_2), doublet (d) + triplet (t) of residual triP.

of either nucleobases or imidazole (Fig. 4). Such newly unraveled driving role of trimetaphosphate as non enzymatic phosphorylating agent of nucleobases modifies our first proposed of mechanism of P-N bonding by anhydrization on a linear triphosphate (TURIAN & SCHOENENBERGER, 1997) as following: the more trimetaphosphate-EDC induced rings woud be split by their N-P bonding with imidazole or any of the nucleobases and probably further polymerized to linear polybasephosphates, the more

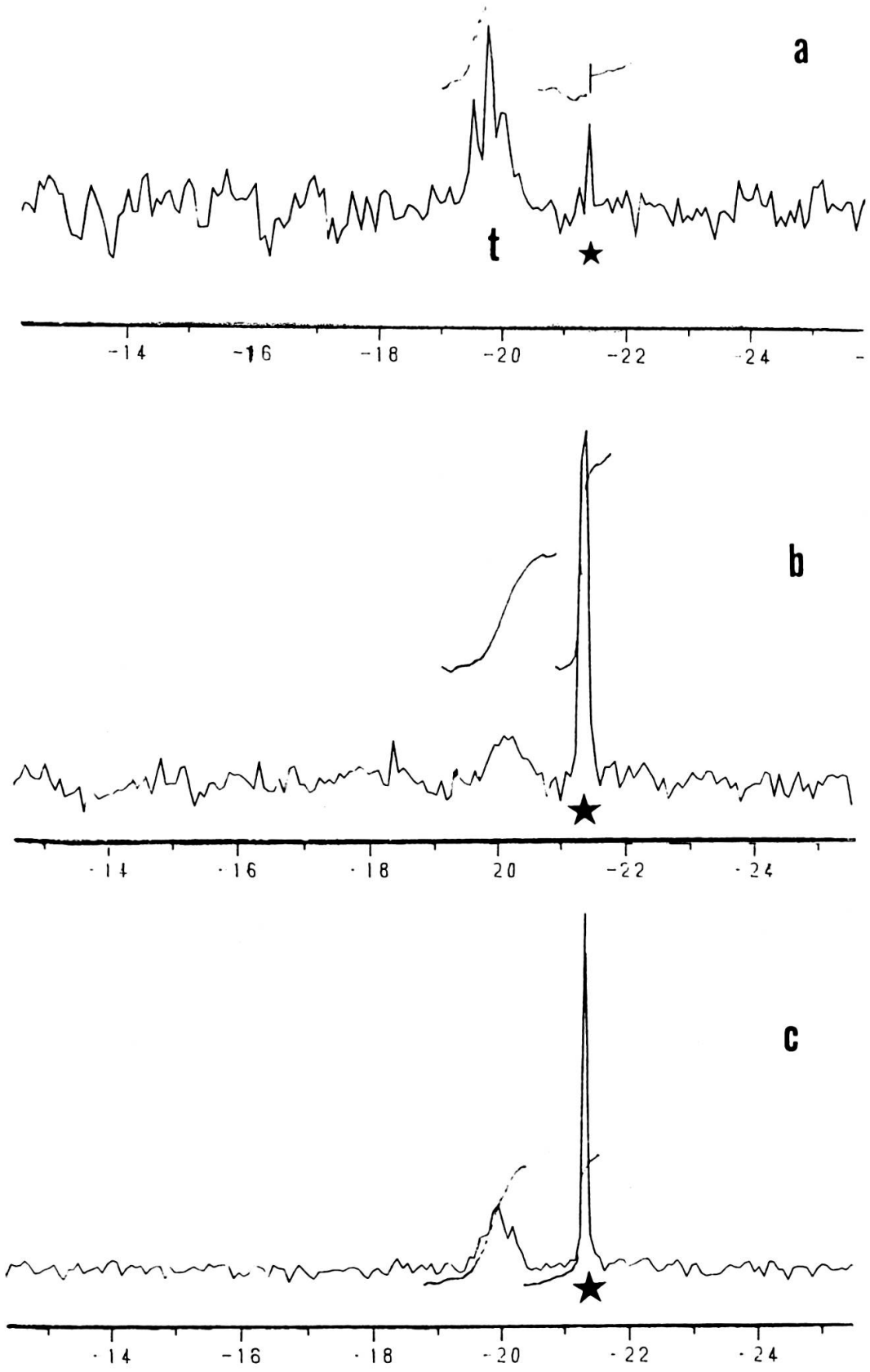

FIG. 3.
Idem to Fig. 2 but with: (a) TriP(t) + EDC; (b) TriP + EDC + adenine; (c) TriP + EDC + adenine + uracil. Peaks signalized at –21.5 ppm (★/★).

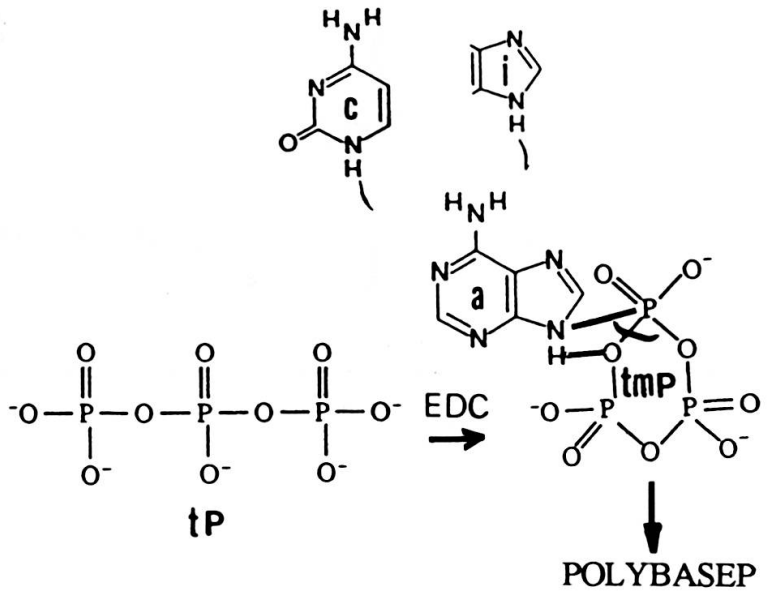

FIG. 4.

Proposed model attempting to explain the paradoxal increased NMR signal at –21.5 ppm after the opening of the ring of trimetaphosphate (tmP) (induced by EDC from linear triphosphate (tP)), by phosphoramidate P-N bonding of either imidazole (i) or nucleobases such as adenine (a) or cytosine (c) by a self-entrained mechanism involving: (1) sink effect (→) of the polymerization of freed linear basetriphosphates into a polybaseP contributing to the signal at –21.5 ppm; (2) retro-induced replenishment (→) of the trimetaP –21.5 ppm peak from the linear tP pool.

linear triphosphate would be cyclized by EDC into additional trimetaphosphate. Such self-entrainment mechanism, with the progressive appearance of oligo-polyphosphates (see p. 190) would lead to the highering of the –21.5 ppm signal which would thus be the consequence of processes of phosphoramido-bondings of either imidazole or nucleobases on the energized phosphate (-O-P-O-) groups. By extension, a further polymerization of basetriphosphates into polybasephosphates (Fig. 4), possibly as basediphosphate units after splicing of one Pi, noticeably increased in such conditions (S_1 in Fig. 2 c,d) and their lining as adenilylphosphates, cytosinylphosphates, etc. along polyphosphate chains, eventually further stabilized as Ca salts, would suggest a primordial mechanism of «freezing» of the stereospecifically-controlled base encodings of amino acids on prebiotic peptides (TURIAN, 1998).

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RÉSUMÉ

SIGNALISATION SIMILAIRE PAR ^{31}P RMN DE L'IMIDAZOLE ET DES NUCLÉOBASES PRÉSUMÉMENT LIÉES PAR PHOSPHORAMIDE AUX TRI(MÉTA)PHOSPHATES

La conversion catalytiquement induite par la carbodiimide (EDC) du triphosphate linéaire en trimétaphosphate cyclique a été observée par ^{31}P-RMN à –21.5 ppm. Un accroissement de ce pic en présence supplémentaire soit d'imidazole soit d'adénine (ou d'autres nucléobases) a été interprétée comme due à l'ouverture de l'anneau trimétaphosphate par suite de la liaison phosphoramidate de ces bases, suivie de leur polymérisation en polybasephosphates linéaires aussi signalisés à –21.5 ppm.

Mots-clés: RMN, Nucléobases, Imidazol, Tri(méta)phosphate.

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