Amino Acids (2012) 42:2331–2341
DOI 10.1007/s00726-011-0975-2

ORIGINAL ARTICLE

Activation of carboxyl group with cyanate: peptide bond formation from dicarboxylic acids

Grégoire Danger · Solenne Charlot · Laurent Boiteau · Robert Pascal

Received: 4 May 2011 / Accepted: 29 June 2011 / Published online: 16 July 2011

Abstract The reaction of cyanate with C-terminal carboxyl groups of peptides in aqueous solution was considered as a potential pathway for the abiotic formation of peptide bonds under the condition of the primitive Earth. The catalytic effect of dicarboxylic acids on cyanate hydrolysis was definitely attributed to intramolecular nucleophilic catalysis by the observation of the ^{1}H-NMR signal of succinic anhydride when reacting succinic acid with KOCN in aqueous solution (pH 2.2–5.5). The formation of amide bonds was noticed when adding amino acids or amino acid derivatives into the solution. The reaction of *N*-acyl aspartic acid derivatives was observed to proceed similarly and the scope of the cyanate-promoted reaction was analyzed from the standpoint of prebiotic peptide formation. The role of cyanate in activating peptide C-terminus constitutes a proof of principle that intramolecular reactions of adducts of peptides C-terminal carboxyl groups with activating agents represent a pathway for peptide activation in aqueous solution, the relevance of which is discussed in connexion with the issue of the emergence of homochirality.

Keywords Prebiotic chemistry · Peptide · Activation · Intramolecular catalysis · Neighbouring group assistance

Introduction

How could the oligomers or polymers of amino acids or nucleotides required for the emergence of biochemical systems be formed? This issue is far from obvious, and it is one of the more important questions that prebiotic chemistry tries to answer. The condensation of amino acids is not highly thermodynamically unfavourable, especially in the pH range from 4 to 8, where short oligopeptides can be formed from monomers at high concentrations (40–100 mM) (Imai et al. 1999; Kawamura et al. 2005; Aubrey et al. 2009). However, for this pH range, sluggish kinetics are usually observed, and therefore, high temperatures, catalysts or activating agents are needed to detect amino acid condensation (Brack 2007).

Numerous strategies have been developed to promote peptide bond formation under conditions devised to reproduce a prebiotic environment. Physical means have thus been proposed as for example the effect of a lower water activity in concentrated salt solutions (together with copper catalysts) inducing an increase in peptide stability (Rode 1999; Rode et al. 1999; Plankensteiner et al. 2005). A similar behaviour is observed in presence of clays or silica surfaces (Lambert 2008), by submitting an amino acid mixtures to alternations of wet and dry phases (Bujdák and Rode 1999). Chemical means have also been proposed through the use of activated amino acid derivatives. *N*-Phosphoryl amino acids have indeed been shown to yield

Electronic supplementary material The online version of this article (doi:10.1007/s00726-011-0975-2) contains supplementary material, which is available to authorized users.

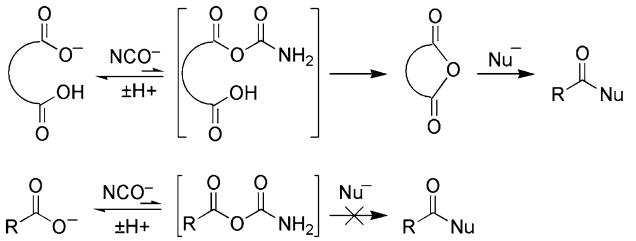
G. Danger (✉)
Spectrométries et Dynamique Moléculaire, Physique des Interactions Ioniques et Moléculaires Université de Provence, Université Aix-Marseille I, Centre Saint-Jérôme, Case 252, 13397 Marseille, France
e-mail: gregoire.danger@univ-provence.fr

S. Charlot · L. Boiteau · R. Pascal (✉)
Institut des Biomolécules Max Mousseron, UMR 5247, CNRS-Université de Montpellier 1, Université de Montpellier 2, Place Eugène Bataillon CC 17006, 34095 Montpellier Cedex 5, France
e-mail: rpascal@univ-montp2.fr

peptides through a conversion into a cyclic phosphorane behaving as an activated carboxylic acid derivative (Li et al. 1992). During the last decades, *N*-carboxyanhydrides of amino acids (NCAs) have taken an increasing part as prebiotically relevant activated amino acids (Pascal et al. 2005). They easily polymerize into oligopeptides in aqueous solutions (Kricheldorf 2006) and different pathways have been proposed involving their formation as intermediates (Pascal et al. 2005). They are produced by cyclization of activated amino acids (active ester, thioesters…) in the presence of carbonate buffers (Brack 1982). This route is the result of the equilibrium formation of a carbamate adduct in bicarbonate buffers, which cyclizes into NCA. A similar reaction has been observed when an α-amino thiocarboxylic acid is reacted in presence of an oxidizing agent in carbonate buffers (Maurel and Orgel 2000). Activating agents have also been proposed to result in peptide formation through NCAs. Carbonyl sulfide promotes peptide formation (mostly in the presence of oxidizing or alkylating agents) via a thiocarbamate intermediate (Leman et al. 2004). Isocyanate can also promote NCA formation in aqueous media, though its reaction with an amino acid mainly leads to an *N*-carbamoylamino acid (CAA) (Taillades et al. 2001), which can be converted into NCA under an oxidative (NO/O_2) treatment in a dry phase (Collet et al. 1996; Lagrille et al. 2009). But NCAs can be also formed directly in aqueous solution from the breakdown of the CAA urea group by abstraction of NH_3 leading to the isocyanate intermediate, which cyclizes into NCA (Danger et al. 2006).

Some of these processes can eventually lead to amino acid/peptide cycles in which polypeptides are continuously formed through steady or intermittent activating processes and then hydrolyzed back into amino acids over geological timescales (Huber and Wächtershäuser 1998; Huber et al. 2003; Commeyras et al. 2004, 2005). Scenarios involving that kind of peptide protometabolism have thus been proposed to give rise to selection of given properties (Huber and Wächtershäuser 1998; Huber et al. 2003; Commeyras et al. 2004, 2005), as, for example, chirality (Plasson et al. 2004; Danger et al. 2010) as a result of the supply of energy by chemical vectors (Boiteau and Pascal 2011). As the above-mentioned scenarios are based on peptide elongation by addition of activated α-amino acids, the direction of growth is from C-terminus to the N-terminus, which may raise difficulties related to side-reactions of the amino group as for instance that of cyanate, which can induce the formation of an hydantoin ring at the N-terminal position of oligopeptides (Danger et al. 2006), which prevents any further elongation. To overcome this drawback, alternative pathways to this N-terminal elongation have to be found. An obvious alternative worthy to investigate consists in activating the carboxyl group of the C-terminal residue to give rise to a C-terminal elongation rather than an N-terminal one. Among the numerous carboxyl-activating agents of current use in peptide chemistry (Benoiton 2006), only compounds that could be present in primitive environments have to be used in the context of prebiotic chemistry. As far as we know, cyanate (Danger et al. 2006), cyanamide (Lohrmann 1972; Brack 2007) and its isomer carbodiimide (Duvernay et al. 2004) are likely to have been available in these primitive environments or delivered through exogenous sources. These compounds are known to induce the activation of carboxyl groups (Stark 1965a, b; Cavadore and Previero 1969; Brack 2007). Since cyanate was the major energy vector in the previously studied scenario involving N-terminus elongation (Danger et al. 2006), we first investigated its ability to activate carboxyl groups in peptides. Pioneering studies carried out by Stark have shown that the reaction of cyanate with γ-aminobutyric and δ-aminovaleric acids does not only yield a urea by carbamoylation but can also promote a cyclization reaction into lactams (Stark 1965a, b). Lactam formation was thought to be the result of the reactions of a carboxylic acid-carbamic acid mixed anhydride intermediate (called "carbamylcarboxylate" in the original work). To the best of our knowledge, the ability of cyanate to activate carboxyl groups, seems to be limited to the occurrence of favourably positioned nucleophiles (Stark 1965a, b). But, it has independently been demonstrated that various divalent anions including phosphate, carbonate, succinate, and phthalate behave as catalysts for the hydrolysis of cyanate in aqueous solution (Vogels et al. 1970). These reactions are thought to involve a nucleophilic adduct (e.g. carbamoyl phosphate) that undergoes hydrolysis faster than cyanate. With dicarboxylic acids an adduct similar to carbamoyl phosphate may be formed but the nature of the subsequent reaction responsible for catalysis remains unclear. It may undergo either a subsequent breakdown through intramolecular general base catalysis as initially proposed (Vogels et al. 1970) to account for the kinetic analysis of dicarboxylic acids reactions or intramolecular nucleophilic catalysis. However, the comparison of the data published for the kinetic effects of fumaric acid (no significant catalysis) to that for the *cis*-isomer maleic acid (higher by a factor >240) as well as a similar difference between isophthalic and phthalic acids, strongly suggest the occurrence of an intramolecular nucleophilic reaction (Scheme 1) and the formation of the corresponding maleic and phthalic anhydrides. The later possibility is likely to facilitate the reaction of other nucleophiles and may find some applications in amide or peptide bond formation.

We report here the results of a study undertaken to check the possibility that cyanate-promoted intramolecular



Scheme 1 Intramolecular formation of carboxylic acid anhydrides as a way to trap the unstable carbamic acid mixed anhydride formed by carboxylic acid addition to cyanate

reactions of carboxyl groups can lead to peptide bonds in aqueous solution at pH values close to neutrality. Evidence will be provided that a cyclic anhydride intermediate is formed by neighbouring group assistance in dicarboxylic acids, and that this intermediate can be used to bring about amide or peptide bond formation starting from simple models including succinic and *N*-acyl aspartic acids (Scheme 1). By contrast, in the absence of carboxylate neighbouring group, no further reaction of the carbamic acid mixed anhydride intermediate will be shown to take place (Scheme 1). General consequences of these observations on the possibility that a C-terminal peptide elongation may have played a role in prebiotic peptide formation will be discussed.

Results and discussion

Reaction of succinic acid with cyanate

We first reassessed the reaction of succinic acid with cyanate in aqueous solution to determine if the catalytic effect reported in the literature (Vogels et al. 1970) is the consequence of intramolecular nucleophilic assistance or general base catalysis. To this aim 10 mM succinic acid was reacted in 200 mM acetate buffers in D_2O (pD 5.5) and in the presence of 20 mM KOCN (Fig. 1B). A singlet corresponding to the four identical protons of succinic anhydride **3** (δ 2.97 ppm) was clearly identified by ^{1}H-NMR (Fig. 1B). Therefore, based on these first results, we can conclude that cyanate activates a carboxyl group of succinic acid whereas the second one quickly traps the mixed anhydride to bring about the formation of succinic anhydride (Scheme 2). In a control experiment succinic anhydride was allowed to react in 200 mM acetate buffers in D_2O (pD 5.5) under the same conditions but in the absence of KOCN (Fig. 1A). The signal corresponding to succinic anhydride (δ 2.97 ppm) rapidly vanishes. After 10 min, the anhydride is hydrolyzed to a 45% extent into succinic acid (**1**, δ 2.42 ppm) corresponding to the behaviour of anhydride **3** formed as an intermediate in the previous experiment. The evolution of the amount of succinic anhydride formed by reaction of cyanate was monitored by ^{1}H-NMR in formate and acetate buffers at different pD values (Fig. 2). The anhydride **3** transiently accumulates in solution and its maximum concentration is observed at pD 3.5. The occurrence of an optimal pH for anhydride formation may be related to the complex kinetic behaviour dependent on different factors: (*i*) the pK_a of cyanic acid [pK_a = 3.8 (Taillades et al. 2001)] determining the ratio of the reactive neutral form of cyanate and hence its rate of hydrolysis, (*ii*) the neutral, mono- or di-charged state of succinic acid governing the rates of its reaction with nucleophiles, and (*iii*) the rates of hydrolysis of succinic anhydride likely to be pH-dependent, at least to a certain extent in this pH range. Anyway, this behaviour is consistent with the formation of succinic anhydride as an intermediate in the reaction of cyanate with succinic acid over a wide pH range, even though its formation is likely to become hardly detectable above pH 5.5.

Having established that the anhydride **3** is transiently formed in the reaction of succinic acid with cyanate, we investigated its ability to acylate α-amino acid derivatives in aqueous media. The detection of small amounts of succinamic acid **4**, probably formed by the reaction of NH_3 (produced by cyanate hydrolysis) with anhydride **3** as a product of the experiment supported this hypothesis (data not shown). An amino acid derivative should act in the same way. When 10 mM succinic acid was reacted with

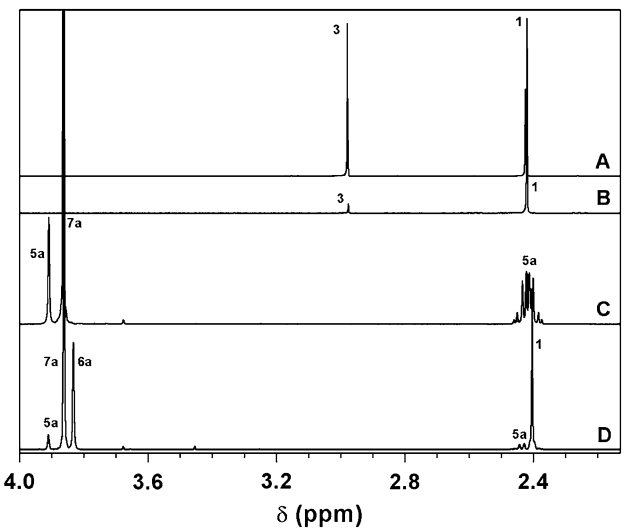
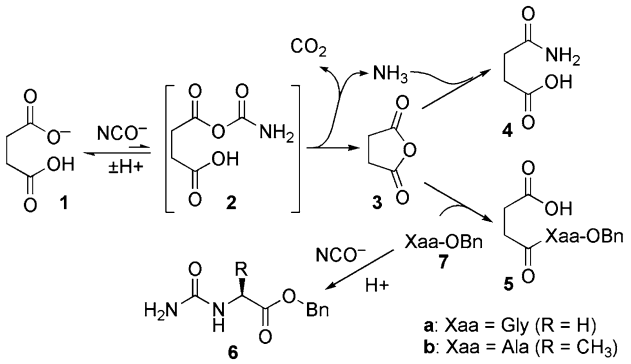


Fig. 1 ^{1}H NMR spectra of **A** the hydrolysis of 10 mM succinic anhydride in a 200 mM acetate buffer (D_2O, pD 5.5), after 10 min of incubation; **B** the reaction between 10 mM succinic acid and 20 mM KOCN after 30 min; **C** the reaction of 10 mM succinic anhydride and 100 mM Gly-OBn after 20 min; **D** the reaction of 10 mM succinic acid and 100 mM Gly-OBn promoted by 20 mM KOCN after 48 h of incubation. *Numbers* refer to chemical structures presented in Scheme 2. For further details on experimental and analytical conditions, see the "Experimental section"



Scheme 2 General scheme for the reactions of succinic acid with cyanate and with amino acid benzyl esters in aqueous solution. Reactions occurring when succinic acid reacts in presence of cyanate with or without amino acid

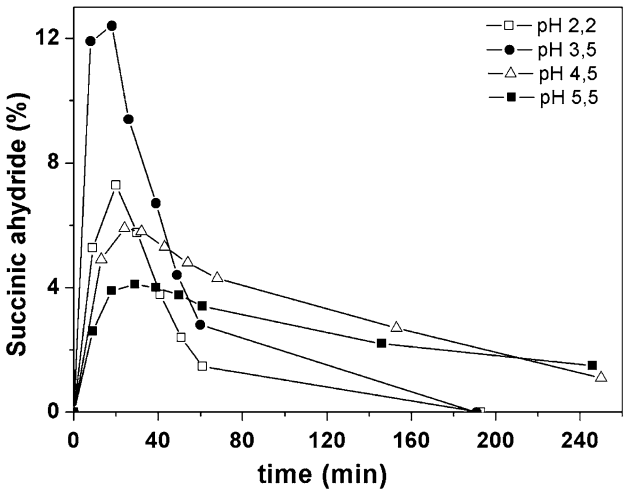


Fig. 2 The formation of succinic anhydride (ratio relative to the initial amount of succinic acid) from the reaction of 10 mM succinic acid with KOCN (20 mM). Buffers are 200 mM of formate for pD 2.2 (*open square*) and 3.5 (*closed circle*), or 200 mM of acetate for pD 4.5 (*open triangle*) or 5.5 (*closed square*) in D_2O. Data were obtained from direct ^{1}H NMR integrations. For further details on experimental and analytical conditions, see the "Experimental section"

20 mM KOCN and 100 mM glycine benzyl ester (Gly-OBn, Fig. 1D, **7a**, δ 3.86 ppm), the formation of a glycine-containing product was observed by NMR (Fig. 1D, **5a**, δ 3.90 and 2.41 ppm). The reaction mixture was complicated by the formation of *N*-carbamoylglycine benzyl ester **6a** (δ 3.83 ppm) as expected from the usual reactivity of cyanate in presence of α-amino acid esters or α-amino acids (Stark 1965a, b; Taillades et al. 2001). In a control reaction, 100 mM Gly-OBn (δ ^{1}H 3.86 ppm) was reacted with succinic anhydride (10 mM) in an acetate buffer (Fig. 1C) confirming the nature of the glycine adduct characterized by a singlet at 3.90 ppm and a multiplet at 2.41 ppm revealing the dissymmetric structure of the succinamic acid derivative **5a**.

To confirm these results, the same reactions were performed in buffers with different pH values (formate pH 3.5, acetate pH 5.5 or phosphate pH 6.5) with two different α-amino acid esters. Analyses were performed by capillary electrophoresis. Table 1 displays the yield of (20 mM) cyanate-promoted amide formation from 10 mM succinic acid and 100 mM glycine benzyl ester (Gly-OBn) or alanine benzyl ester (Ala-OBn). For both amino esters the higher yields of amide products **5a** and **5b** are obtained at pH 5.5 (acetate buffer). The effect of pH and the nature of the buffer is likely to be the result of three different processes, namely the formation of the mixed anhydride **2** [faster from cyanic acid, $pK_a \approx 3.8$ (Stark 1965a, b; Taillades et al. 2001)], the aminolysis of succinic anhydride (faster at high pH) and the competing hydrolysis of cyanate, fast at low pH or subject to catalysis by phosphate (Vogels et al. 1970) through the formation of a carbamoyl phosphate adduct (Jones and Lipmann 1960; Miller and Parris 1964).

Reactivity of *N*-protected aspartic acid in the presence of cyanate

Although succinic acid has been detected in meteorites, as well as 44 other dicarboxylic acids (Pizzarello and Huang 2002), the cyanate-promoted carboxyl group activation identified here remains limited to amide bond formation. However, aspartic acid has also been detected in the soluble phase fraction of meteorite organic extracts (Cronin et al. 1980; Cronin and Pizzarello 1983) as well as in the Miller experiment (Miller 1953). Since aspartic acid presents a structure similar to that of succinic acid, a possibility of elongation of peptides containing a C-terminal aspartic acid can be considered in the presence of cyanate.

The reaction was carried out from a mixture of 10 mM *N*-acetylaspartic acid **8a** and 100 mM glycine in the presence of 20 mM cyanate in a 200 mM phosphate buffer (pD 6.5) at 25°C (Fig. 3D). Two main products (Fig. 3D, **11a** or **12a**, δ 4.38 and 4.54 ppm) and a minor side-product (Fig. 3D, **14**, δ 4.62 ppm) were observed in the medium corresponding to the two isomers **11a** and **12a** in addition to the unchanged reactant **8a** (δ 4.29 ppm, Scheme 3). This behaviour is consistent with the reaction of the anhydride **10a**, able to yield two different adducts upon aminolysis. The nature of the isomeric products **11a** and **12a** (Scheme 3) was confirmed by monitoring the reaction of anhydride **10a** (10 mM) and glycine (100 mM) without addition of cyanate by ^{1}H NMR spectrometry (Fig. 3C).

Two main products were also obtained by the reaction of benzoyl aspartic acid **8b** under conditions similar to that of Fig. 3D. They were analyzed by HPLC and identified by HPLC-MS (ESI, m/z 295.2, $[M + H]^+$) as the two isomers **11b** and **12b** (Scheme 3), and were characterized by HPLC

Table 1 Yields of formation of succinamic acids **5a** and **5b** after 48 h reactions of 10 mM succinic acid with 20 mM KOCN and 100 mM Gly-OBn or Ala-OBn in buffered aqueous solution (pH 3.5: 200 mM formate; pH 5.5: 200 mM acetate; pH 6.5: 200 mM phosphate)

pH	3.5	5.5	6.5
Succinyl-Gly-OBn **5a**–yield%	6.4	13.4	8.1
Succinyl-Ala-OBn **5b**–yield%	9.0	27.1	18.0

Yields assessed by capillary electrophoresis after calibration with corresponding standards. For further details on experimental and analytical conditions, see the "Experimental section"

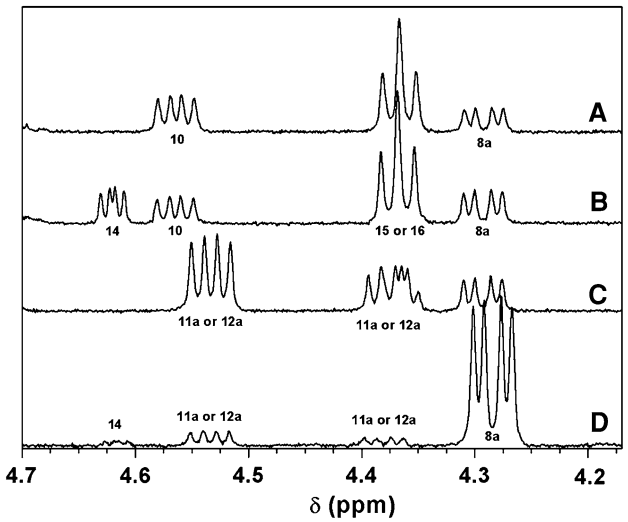
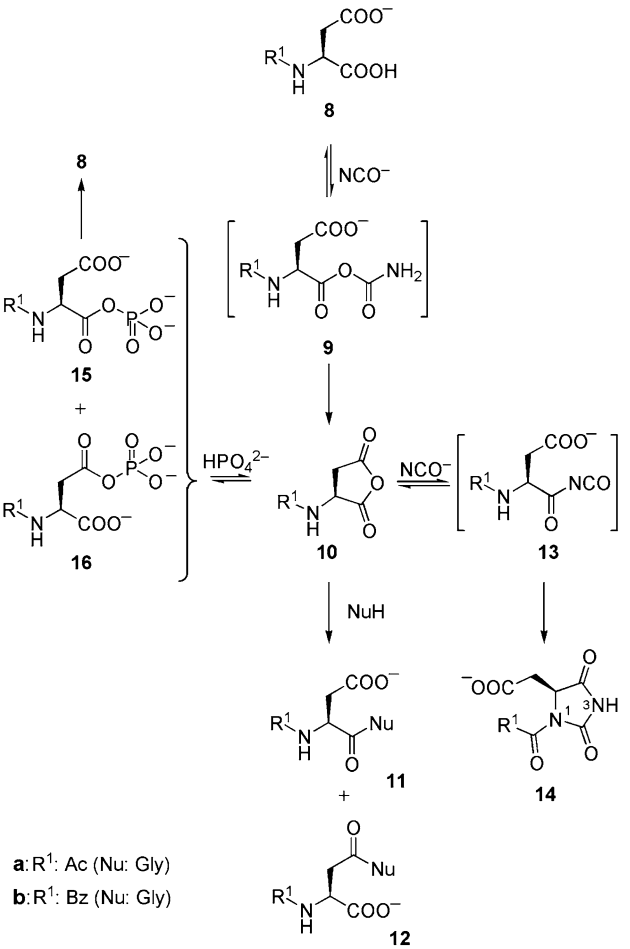


Fig. 3 The reaction of *N*-acetylaspartic acid **8a** and anhydride **10a** monitored by ^{1}H NMR in 200 mM phosphate buffers (D_2O, pD 6.5). **A** The hydrolysis of 10 mM anhydride **10a** after 10 min of incubation; **B** the reaction between 10 mM anhydride **10a** and 20 mM KOCN after 5 min; **C** the reaction of 10 mM anhydride **10a** and 100 mM Gly-OH after 40 min; **D** the reaction of 10 mM acid **8a** with 20 mM KOCN and 100 mM Gly-OH after 7 days of incubation. *Numbers* refer to chemical structures presented in Scheme 3. For further details on experimental and analytical conditions, see the "Experimental section"



Scheme 3 Reactions of *N*-acylaspartic acids **8a** and **8b** or anhydrides **10a** and **10b** with cyanate and nucleophiles

elution profiles identical to the products of the reaction of anhydride **10b** with glycine (see Supporting Information).

The side-product obtained from cyanate and the acid **8a** exhibiting a ^{1}H-NMR signal at 4.62 ppm (Fig. 3D, **14**) was not observed when reacting the anhydride **10a** with glycine, but turned out to result from the reaction of **10a** with cyanate (Fig. 3B). A similar reaction has been reported to yield the acylhydantoin **14a** through the intramolecular reaction of cyanate adduct **13a** (Stark 1965a, b). The reaction starting from anhydride **10b** and cyanate was performed at a larger scale in order to isolate the side-product. It was purified by preparative HPLC and characterized by the presence of a molecular ion (m/z 261.1, [M-H]$^-$) in the ESI-MS (Table 2) and a signal at $\delta = 4.91$ ppm (t, $J_1 = J_2 = 3.9$ Hz) for the H^{α} in the 1H-NMR spectrum ($(CD_3)_2SO$), which is consistent with the absence of hydrogen bound to nitrogen 1 in the cycle of hydantoin **14b**. Both the formation of hydantoin **14** and a mixture of aspartyl and isoaspartyl peptides **11** and **12** are consistent with the activation of acyl aspartic acid as a cyclic anhydride though it has not been observed by monitoring the reaction by NMR. An additional intermediate was observed during the reaction of *N*-acetylaspartic anhydride in a 200 mM phosphate buffer (pD 6.5) at 25°C. The corresponding ^{1}H NMR spectra (Fig. 3A) show the signals corresponding to the H^{α} of anhydride **10a** at 4.55 ppm, that of *N*-acetylaspartic acid **8**a at 4.29 ppm and another signal at 4.35 ppm. The acid is formed by hydrolysis of anhydride **10a**. The signal at 4.35 ppm disappears rapidly and is attributed to the formation of a phosphate mixed anhydride (**15a** or **16a**) since it is not detected in acetate buffer (pD 5.5). This behaviour is consistent with the published data showing that a mixed anhydride is reversibly formed by

Table 2 Data from HPLC-MS analyses of the reaction between *N*-benzoylaspartic acid, cyanate and glycine in 200 mM phosphate buffer at pH 6.5

Compounds		**8b**	**11b/12b**	**14b**
Retention time/min		9,4	8,6	14.7
m/z	$M + H^+$	238.1	295.2	
	$M–H^+$			261.1

Numbers refer to chemical structures presented in Scheme 3. For further details on experimental and analytical conditions, see the "Experimental section"

reaction of phosphate with cyclic anhydrides (Higuchi et al. 1967; Biron and Pascal 2004; Leman et al. 2006).

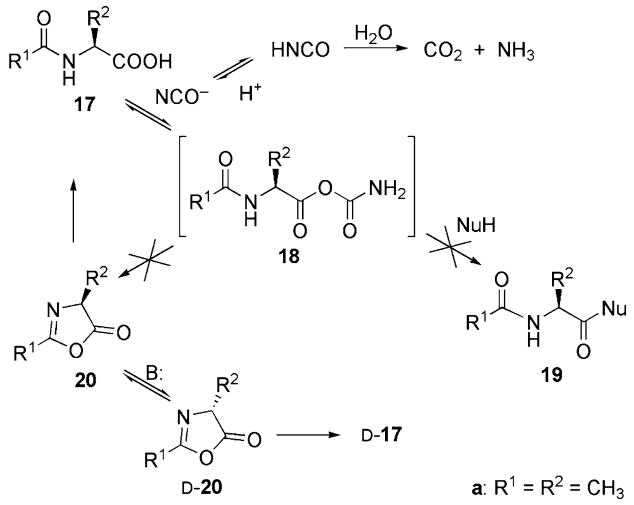
Reactivity of *N*-acetylalanine in the presence of cyanate

The cyanate-promoted reactions of both succinic acid and *N*-acyl-aspartic reported here suggest that a neighbouring carboxyl group is needed to any subsequent reaction through the formation of a cyclic anhydride intermediate responsible for the formation of amides or peptides from these reactants. In the absence of two conveniently positioned carboxyl groups no peptide bond should then be formed in the presence of cyanate. To confirm this assumption, we used *N*-acyl amino acids **17** (Scheme 4) devoid of neighbouring reacting groups that mimic the C-terminal end of an oligopeptide. To this aim, the reactivity of 20 mM cyanate was checked using *N*-acetylalanine (**17a**, Scheme 4) dissolved in acetate buffers (200 mM in D_2O, pD 5.5, 25°C) in the presence of excess amino acid (100 mM glycine) or without added reagent. No peptide formation could be detected by monitoring the reaction with ^1H NMR in D_2O. Moreover, no signal corresponding to a deuterium/proton exchange at the C^{α} carbon could be detected though the reaction of cyanate was performed in D_2O and though any activation would be likely to be accompanied by epimerization (and exchange in D_2O), as usually observed when activating acylated amino acids or C-terminal peptide segments (Benoiton et al. 1992; Benoiton 2006). This result indicates that the mixed anhydride **18a** does not undergo a cyclization into the 5(4H)-oxazolone **20a** that is likely to be chiraly unstable. Similar results were obtained at 50°C. Both the absence of peptide bond formation and of epimerization demonstrate that the reaction of cyanate comes to an end with the formation of the carbamate mixed anhydride and that, in the absence of conveniently positioned nucleophile, the fate of the mixed anhydride is to expel the carboxylate group and to revert to cyanate that is hydrolyzed (or reacted with other nucleophiles) in aqueous solution before that any reaction of the mixed anhydride could be detected (Scheme 4).

Intramolecular reaction of activated carboxyl groups

The reaction of carboxyl groups with activating agents leads to the reversible formation of unstable adducts such as the O-acylisourea intermediate formed from carbodiimide activation (Ibrahim and Williams 1980; Williams and Ibrahim 1981; Valeur and Bradley 2009). In peptide chemistry, the O-acylisourea addition intermediate is usually not observed since it is quickly rearranged into condensation products or 5(4H)-oxazolones (Benoiton and Chen 1981). A similar intermediate has been proposed to account for the formation of 5-, 6- and 7-membered lactams when the corresponding γ-, δ- and ε-amino acids were reacted with cyanate (Stark 1965a, b). This description is consistent with the general schemes proposed for the reactions of cyanate with *N*-acyl-aspartic acid derivatives (Scheme 3) and *N*-acyl-α-amino acids lacking neighbouring reacting groups (Scheme 4). The possibility of trapping the mixed anhydride intermediates **9**, needed for the acylation reaction to proceed, is brought about by the efficiency of the nucleophilic assistance by the neighbouring carboxylic acid consistent with the high rates found for many intramolecular reactions (Kirby 1980), which has been accounted for by the loss of entropy associated with bimolecular reactions (Page and Jencks 1971). This assistance results in the occurrence of a reaction with nucleophiles that would otherwise be inefficient. This may be considered as a new example illustrating the validity of the concept of *overactivation by cyclization,* which has been introduced to account for the behaviour of α-amino acid *N*-carboxyanhydrides produced by a variety of reaction pathways from intermediates that are much less reactive with nucleophiles (Pascal et al. 2005). This chemical principle allows the formation of highly reactive cyclic intermediates having a potential higher than their precursors simply because they take advantage of an entropic stabilization (the proximity of reacting functional groups stabilize cyclic intermediates that would not have been formed by intermolecular processes). The cyanate-promoted reactions of dicarboxylic acids studied here demonstrates that the instable mixed anhydride formed at levels that remain neither detectable by analytical tools (^1H-NMR) nor by their reactions with nucleophiles, can be converted into substantial yields of a cyclic anhydride having a high reactivity with nucleophiles. As a result, the effectiveness of cyanate as activating agent for carboxyl groups is revealed, which allows the reactant flow to be driven towards amide bond formation. This process is a new example of the role of the fast rates of intramolecular catalysis in prebiotic chemistry as an important factor that is able of selecting reaction pathways of polyfunctional reactants (Pascal 2003).

The reactions of *N*-acyl aspartic acid reported in this work constitute a proof of principle that intramolecular

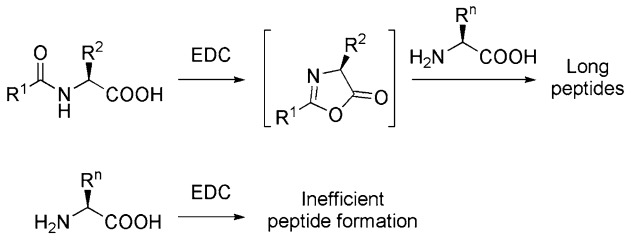


Scheme 4 The absence of cyanate-promoted reactions of *N*-acylamino acids without nucleophilic neighbouring groups

reactions can be helpful in generating activated intermediates by activation of the C-terminus of peptides. A generalization of this principle to peptide containing no additional nucleophilic groups would need activating agents more powerful than cyanate from which no further reaction could be observed. Carbodiimides have been reported to promote the formation of peptides from aqueous solutions of amino acids (Ranganathan and Singh 1990; Brack 2007; Kawamura et al. 2009). *N*-Ethyl-*N*′-dimethylaminopropyl carbodiimide (EDC) turned out to be especially efficient provided that *N*-acylaminoacids were added to initiate polymerization (Cavadore and Previero 1969), which has been proposed as a model for the prebiotic formation of peptides. Though the following analysis has not been developed in the original report, this observation strongly suggests that 5(4H)-oxazolone are the actual aminoacylating species and then that overactivation by cyclization also accounts for the observed reactivity of α-amino acid residues in peptides without specific neighbouring groups (Scheme 5). Substitutes for EDC, which was not likely to be present on the early Earth [namely, cyanamide (Lohrmann 1972; Brack 2007), cyanoacetylene (Orgel 2002), or isocyanides (Mullen and Sutherland 2007)] or photochemical processes (Hagan 2010) may have led to C-terminus activation. Therefore 5(4H)-oxazolones may be reasonably conceived as potential prebiotic intermediates.

Epimerization is not a drawback for prebiotic peptide formation

The chemical synthesis of peptides is usually performed in a direction that is the opposite of that used by cells in the ribosomal synthesis. This choice is guided by the need of obtaining low levels of epimerization in peptides. Since the



Scheme 5 5(4H)-oxazolone as postulated intermediates proposed to account for the EDC-promoted peptide formation in aqueous solution (pH 4–5) (Cavadore and Previero 1969)

chiral integrity of peptide chains is essential to their proper folding and activity, the issue of epimerization of peptide chains during the activation of peptide segments has received much attention in the literature (Benoiton 2006). Numerous strategies have been proposed to suppress the formation of 5(4H)-oxazolones during the activation of *N*-acylamino acids and then to limit epimerization (Miyazawa et al. 1989; van den Nest et al. 2001). As far as the synthesis of random prebiotic peptides is concerned, the occurrence of epimerization at the C-terminal residue during segment activation may not be considered as a drawback starting from enantiomeric mixtures of amino acids. On the contrary, any possibility of conversion of racemic mixtures into homochiral compounds requires a possibility of reversal of configuration at chiral centres or at least that the non-selected configuration be removed by chemical or physical means. A model leading to the amplification of chirality through the epimerization of the N-terminal residues has been proposed to render the racemic state dynamically unstable in pools of amino acids subjected to a activation/polymerization/epimerization/depolymerization (APED) process (Plasson et al. 2004). It can thus be conceived that the activation of peptide segments and the subsequent transient formation of the enantiomerically instable 5(4H)-oxazolone may constitute an alternative to the APED model by its ability to promote epimerization at the C-terminal residue of peptide segments and that *the influence of the configuration at the vicinal residue* may lead to a reproduction of chirality in the polymeric chain that is worth to be experimentally investigated. Then, studies aimed at discovering prebiotic pathways of formation of peptides proceeding from the N-terminus to the C-terminus, as the cyanate-promoted pathway reported here, should not be discarded on the ground that this leads to high levels of epimerization. Although data obtained from peptide chemistry showed that when *N*-acylaspartic acids are activated, the reaction can lead to oxazolone and anhydride (Barker 1953), we observed no epimerization during cyanate-mediated activation, which is consistent with the incapacity of this pathway to lead to 5(4H)-oxazolones.

Conclusion

This work was devoted to investigate the reactivity of cyanate in activating the C-terminus of peptide segments in aqueous solution. Cyanate was confirmed to be capable of activating carboxyl groups, probably under the form of a mixed anhydride addition intermediate, which can undergo a conversion into anhydrides by reaction of conveniently positioned carboxyl groups. The mixed anhydride turned out to be insufficiently stable to promote the condensation of amino acids in the absence of neighbouring group. However, it is proposed that a similar strategy of neighbouring group assistance may be practicable in the general case by using activating agents more powerful than cyanate and capable of ensuring the formation of a 5(4H)-oxazolone intermediate. An example of the use of oxazolones to amplify the enantiomeric excesses of α,α-dialkylated amino acids on the early Earth (Crisma et al. 2004) has already been published. Though this strategy is inapplicable to the usual synthesis of peptides because of the chiral instability of oxazolones bearing a hydrogen at C^{α} position, the corresponding pathways deserve to be more deeply understood for their potency to give rise to the amplification of symmetrical imbalance in a prebiotic world.

Experimental section

Disopropylethylamine 99+%, ethyl acetate 99+%, sodium sulfate, succinic acid, succinic anhydride, succinamic acid, potassium cyanate, sodium acetate, sodium phosphate monobasic, sodium phosphate dibasic, dichloromethane (DCM) and sodium chloride were purchased from Acros Organics (Noisy le Grand, France). Sodium hydroxide was from VWR (Paris, France). Acetic anhydride, benzoyl chloride, sodium hydrogen sulfate, magnesium sulfate and glycine were obtained from Aldrich (Lyon, France). Aspartic acid was obtained from Degussa (Germany). D,L-alanine benzyl ester, *N*-acetyl-aspartic acid and glycine benzyl ester were purchased from Bachem. Acetonitrile (HPLC grade) was obtained from Carlo Erba. Deuterium oxide and dimethylsulfoxyde-d_6 (DMSO) were purchased from Eurisotop. All chemicals were used as received unless otherwise stated. Pure water (18 MΩ) was produced using a MilliQ apparatus (Millipore). ^{1}H NMR spectra were recorded on Bruker Avance 300 (300 MHz) or Avance 400 (400 MHz) spectrometers.

Synthesis of starting materials and standards

N-benzoyl aspartic acid ***8b***. Aspartic acid (5 g, 37.6 mmol) was suspended in water (15 mL), then $NaHCO_3$ (15.9 g, 150.4 mmol, 4 eq) was added under vigorous stirring followed by dioxan (20 mL). The mixture was cooled to 0°C, and benzoyl chloride (4.8 mL, 41.4 mmol, 1.1 eq) was added dropwise. The mixture was stirred for 1 h at 0°C and left at r.t. overnight. The reaction mixture was then poured in water (50 mL) and acidified to pH 2 by addition of 10 N HCl. The mixture was washed three times with DCM (50 mL); the aqueous layer was extracted three times with ethyl acetate (50 mL). The collected organic layers were washed with water, dried over $MgSO_4$ then concentrated in vacuo. The residue was recrystallized from water then dried in vacuo to afford 4.55 g (19.2 mmol, yield 51 %) of *N*-benzoyl aspartic acid as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) ppm: 2.74 (1 H, dd, $J_1 = 16.4$ Hz, $J_2 = 8.0$ Hz: H_β), 2.89 (1 H, dd, $J_1 = 16.4$ Hz, $J_2 = 5.8$ Hz: $H_{\beta'}$), 4.79 (1 H, ddd, $J_1 = J_2 = 8$ Hz, $J_3 = 6$ Hz: H_α), 7.49–7.59 (3 H, m: Ar-H), 7.89 (2 H, d, $J = 6.0$ Hz: Ar-H), 8.79 (1 H, d, $J = 7.9$ Hz: NH).

N-benzoyl aspartic anhydride ***10b***. *N*-benzoyl aspartic acid **8b** (2.92 g, 12.3 mmol) was suspended in acetic anhydride (15 mL) in a 50-mL, magnetically stirred flask. The suspension was dissolved by heating to 80–100°C under stirring for 5 min; the mixture was then allowed to cool to r.t. under stirring. The white precipitate was collected by filtration, rinsed with cold, dry ether, then dried in vacuo, to afford 1.23 g (5.6 mmol, yield 46%) of *N*-benzoyl aspartic anhydride as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) ppm: 3.06 (1 H, dd, $J_1 = 18.6$ Hz, $J_2 = 5.8$ Hz: H_β), 3.35 (1 H, dd, $J_1 = 18.6$ Hz, $J_2 = 10.0$ Hz: $H_{\beta'}$), 4.85 (1 H, ddd, $J_1 = 10$ Hz, $J_2 = 7$ Hz, $J_3 = 6$ Hz: H_α), 7.51–7.64 (3 H, m: Ar-H), 7.87 (2 H, d, $J = 7.0$ Hz: Ar-H), 9.52 (1 H, d, $J = 7.1$ Hz: NH).

*Synthesis of Gly-OBn adduct of succinic acid (****5a****, capillary electrophoresis standard)*. Glycine benzylester hydrochloride (200 mg, 0.99 mmol) were dissolved in dichloromethane (10 mL) with diisopropylethylamine (1.1 mmol). Succinic anhydride (100 mg, 1.00 mmol) was added in portions at room temperature. After 20 h, the solvent was removed under vacuum. The resulting oil was dissolved in ethyl acetate (10 mL) and extracted twice with 20 mL of 1 M $NaHSO_4$ and twice with saturated NaCl (20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to obtain a white solid. This solid is then recrystallized from ethyl acetate. ^{1}H NMR, *400 MHz* (*DMSO-d_6, δ in ppm*): 12.3 (1 H, s, –COOH); 8.35 (1 H_1, t, $J_{1\alpha} = 5.7$ Hz, –NH–); 7.37 (5 H, m, ArH,); 5.12 (2 H, s, $–CH_2–$); 3.88 (2 H_α, d, $J_{a1} = 5.7$ Hz, $–CH_2–$); 2.40 (4 H, m, $–(CH_2)_2–$). ^{13}C NMR, *(DMSO- d_6, δ in ppm):* 173.87 (HO(O<u>C</u>)CH_2–); 172.58 ($–CH_2$(O<u>C</u>)NH–); 170.64 ($–CH_2$(O<u>C</u>)O–); 128.38 (Ar); 66.45 (–(OC)<u>C</u>H_2Ar); 40.64 ($–NH\underline{C}H_2$(CO)–); 30.00 (HO(CO)<u>C</u>H_2CH_2–); 29.03 ($–CH_2\underline{C}H_2$(CO)–). ^{1}H NMR, *400 MHz (D_2O pD 3.50, δ in ppm):* 7.35 (5 H, m, ArH,); 5.13 (2 H, s, $–CH_2–$); 3.94 (2 H, s, $–CH_2–$); 2.53 (4 H, m, $–(CH_2)_2–$). HRMS m/z (ESI-ToF) 266.1025 (calcd: $[M + H]^+ = 266.1023$ for $C_{13}H_{16}NO_5$).

*Synthesis of Ala-OBn adduct on succinic acid (**5b**, capillary electrophoresis standard)* Same procedure as for Gly-Obz adduct **5a** above, with 200 mg of Ala-Obz ($9.27.10^{-4}$ mol). ^{1}H NMR, *400 MHz (DMSO- d_6, δ in ppm):* 12.07 (1 H, s, –COOH); 8.33 (1 H_1, d, $J_{1\alpha} = 6.9$ Hz, –NH–); 7.36 (5 H, m, ArH,); 5.11 (2 H, d, $J = 1.9$ Hz, $–CH_2–$); 4.29 (2 H, m, $J_{\alpha 1} = 6.9$ Hz, $J_{\alpha\beta} = 7.2$ Hz, –CH–); 2.39 (4 H, m, $–(CH_2)_2–$); 1.28 (4 H, d, $J_{\beta\alpha} = 7.2$ Hz, $–CH_3$). ^{13}C NMR, *(DMSO- d_6, δ in ppm):* 173.71 ($HO(O\underline{C})CH_2–$); 172.43 ($–CH(CH_3)(O\underline{C})O–$); 171.28 ($–CH_2(O\underline{C})NH–$); 127.82 (Ar); 65.38 ($–(OC)\underline{C}H_2Ar$); 47.69 ($–NH\underline{C}H(CH_3)(CO)–$); 29.61 ($HO(CO)\underline{C}H_2CH_2–$); 28.84 ($–CH_2\underline{C}H_2(CO)–$); 16.66 ($–CH(\underline{C}H_3)–$). ^{1}H NMR, *400 MHz ($D_2O$ pD 3.50, δ in ppm):* 7.35 (5 H, m, ArH,); 5.13 (2 H, s, $–CH_2–$); 4.31 (2 H, q, $J_{\alpha\beta} = 7.3$ Hz, –CH–); 2.50 (4 H, m, $–(CH_2)_2–$); 1.31 (3 H, d, $J_{\alpha\beta} = 7.3$ Hz, $–CH_3$). HRMS m/z (ESI-ToF) 302.1001 (calcd: $[M + Na]^+ = 302.0999$ for $C_{14}H_{17}NO_5Na$).

Investigation of reactivity

For reactions monitored by NMR spectroscopy, the buffers (200 mM) were prepared in D_2O by dissolving either: formic acid (15 µL) in D_2O (1985 µL) (pD 2.23), or acetic acid (30 µL) and NaOH (12.4 mg) in D_2O (3970 µL) (pD 3.5), or NaOH (6.4 mg) and formic acid (23 µL) in D_2O (1977 µL) (pD 4.5), or NaOH (28 mg) and acetic acid (46 µL) in D_2O (3954 µL) (pD 5.5), or NaH_2PO_4,H_2O (91.8 mg) and Na_2HPO_4 (18.4 mg) in D_2O (4000 µL) (pD 6.5).

For reactions analyzed by capillary electrophoresis (CE) and/or High Performance Liquid Chromatography (HPLC), the buffers (200 mM) were prepared by dissolving, either NaOH (317.5 mg) and formic acid (750 µL) (pH 3.50), or NaOH (700 mg) and acetic acid (1.150 mL) (pH 5.50), or NaH_2PO_4 (1.998 g, 16.65 mmol) and Na_2HPO_4 (0.457 g, 3.21 mmol) (pH 6.50) in 100 mL of pure water.

Reaction samples for NMR analysis and capillary electrophoresis analysis Solutions including 10^{-2} M succinic acid or 10^{-2} M *N*-acetyl aspartic acid with or without 2.10^{-2} M KOCN and 0.1 M amino acid derivatives were prepared in either 200 mM formate, acetate or phosphate buffer (1 mL), depending of the desired pH. The corresponding solutions were stored at room temperature (22°C) in test tubes for CE analysis or directly in NMR tubes for NMR analysis.

*For reactions of Bz-Asp **8b** and anhydride **10b** in presence of NaOCN*, the stock phosphate buffer (200 mM, pH 7.13) was prepared by dissolving $NaH_2PO_4.H_2O$ (920.4 mg, 6.67 mmol) and Na_2HPO_4 (1892 mg, 13.33 mmol) in water (100 mL).

*Reactivity of Bz-Asp-anhydride **10b** in the presence of cyanate* In a 25-mL flask fitted with a glass stopper and a magnetic stirrer, Bz-Asp-anhydride (10.9 mg, 50 µmol) was dissolved in 3 mL of phosphate buffer. NaOCN (6.5 mg, 100 µmol, 2 eq) was dissolved separately in 2 mL phosphate buffer then added to the reaction mixture, which was then stirred at rt for 3 days. Aliquots of the reaction mixture were diluted to 1/10 in 1% aq TFA then analysed by HPLC or HPLC/MS, eluent: water/acetonitrile (+0.1 % TFA) gradient 95:5 to 70:30 over 25 min (t_r of *N*-acyl hydantoin **14b**: 12 min), m/z (LC-MS, ESI-ToF negative mode) 261.1 (calcd: $[M–H]^- = 261.06$ for $C_{10}H_9N_2O_5$). The above reaction was repeated at a larger scale with: Bz-Asp-anhydride (218 mg, 1 mmol) and NaOCN (131 mg, 2 mmol) in phosphate buffer (100 mL) in a 250 mL flask. The crude reaction mixture was acidified pH 3.5 with 1 M $NaHSO_4$, separated by preparative HPLC then freeze-dried to afford *N*-acyl hydantoin **14b**. ^{1}H NMR (DMSO-d_6, 300 MHz) ppm: 2.97 (m, 2 H: H_β), 4.91 (t, 1 H, $J_1 = J_2 = 3.9$ Hz: H_α), 7.43–7.59 (m, 5 H: C_6H_5), 7.9 (s, 1 H: imido NH); other exchangeable H signals coalesced with H_2O signal at 3.39 ppm.

*Reactivity of Bz-Asp **10b** with Gly and cyanate* In a 25-mL flask fitted with a glass stopper and a magnetic stirrer, Bz-Asp (11.85 mg, 50 µmol) and Gly (37.9 mg, 5100 µmol, 10 eq) were dissolved in 3 mL of phosphate buffer. NaOCN (6.6 mg, 100 µmol, 2 eq) was dissolved separately in 2 mL phosphate buffer then added to the reaction mixture, which was then stirred at r.t. for 3 days. Aliquots of the reaction mixture were diluted to 1/10 in 1% aq TFA then analyzed by HPLC or HPLC/MS, eluent: water/acetonitrile (+0.1 % TFA) gradient 95:5 to 70:30 over 25 min. *Control reaction:* Bz-Asp-anhydride **10b** (10.7 mg, 50 µmol) was reacted with Gly (7.5 mg, 100 µmol, 2 eq) in 5 mL phosphate buffer for 24 h, giving a mixture of Bz-Asp(OH)-OH **8b**, Bz-Asp(OH)-Gly-OH **11b** and Bz-Asp(Gly-OH)-OH **12b**. Analysis by HPLC (the two Gly adducts **11b/12b** were not individually identified).

Conditions of analyses

NMR-monitored reactions were directly carried out in 5-mm NMR tubes (D_2O solution), and analyzed by pulsed 1H NMR spectroscopy (32 scans) recorded at 400 MHz.

HPLC analyses were carried out on a Waters Alliance system including a Waters 2690 Separation Module, a Waters 996 Photodiode Array Detector, using a Thermo Hypersil C18 column (50 × 2.1 mm). Eluent (degassed online): water (+0.1% TFA) /acetonitrile (+0.1 % TFA), linear gradient from 95:5 to 70:30 over 25 min; flow rate 0.2 mL min^{-1}; separations carried out at room temperature (25°C). Reaction aliquots were diluted 10 times in 1% aqueous TFA to standardize their pH prior to HPLC injection.

Preparative HPLC separations (of *N*-benzoyl-hydantoin compound **14b**) were carried out on an Armen Instruments

Spot System flash-chromatography apparatus, using a Grace Reveleris™ C18 40 g column (Grace Davison Discovery Sciences, USA), with the same eluent and elution gradient scheme as in analytical HPLC (online UV-visible absorbance detection at $\lambda = 254$ nm).

HPLC-MS analyses were carried out using the same Thermo Hypersil C18 column (50 × 2.1 mm) and same eluent gradient and elution conditions as above, on a Waters Alliance system including a Waters 2790 separation module, a Waters 996 Photodiode Array Detector, coupled to a Micromass Q-TOF (Waters) mass spectrometer, using electrospray ionization mode (either positive or negative mode; capillary voltage: 3 kV; cone voltage: 30 V).

Capillary electrophoresis experiments were performed using an automated 3D-CE instrument (Agilent technologies system, Waldbronn, Germany) equipped with a diode array detector. Samples volumes were introduced hydrodynamically (17 mbar, 3 s). The temperature of the capillary cassette was maintained constant at 25 °C. Data were collected at 214 nm. Separation capillaries were prepared from bare silica tubing purchased from Composite Metal Services (Worcester, UK). Capillary dimensions were 33.5 cm (20 cm to the detector) × i.d. 50 μm. A background electrolyte (BGE) made of Tris-phosphate buffer pH 7.25 (100 mM phosphoric acid and 190 mM TRIS; 2.30 g of Tris and 659 μL of phosphoric acid dissolved in 100 mL of pure water) was used. New capillaries were conditioned by performing the following washes: 1 M NaOH for 15 min (20 psi), 0.1 M NaOH for 15 min (930 mbar), water for 1 min (930 mbar), BGE for 2 min (930 mbar). Finally, a +15 kV voltage was applied for 10 min in BGE. Between runs, the capillary was flushed for 5 min with NaOH 1 N, 5 min with NaOH 0.1 N, 2 min with pure water and 2 min with BGE (930 mbar).

Acknowledgments The authors thank the European research action COST CM0703 "Systems Chemistry" and the CNRS interdisciplinary program "Environnements Planétaires et Origines de la Vie" (EPOV).

References

Aubrey AD, Cleaves HJ, Bada JL (2009) An evaluation of the critical parameters for abiotic peptide synthesis in submarine hydrothermal systems. Orig Life Evol Biosph 39:109–126

Barker CC (1953) The dehydration and racemisation of N-acyl-L-aspartic acids by acetic anhydride. J Chem Soc 453–456

Benoiton NL (2006) Chemistry of peptide synthesis. CRC Press, Taylor & Francis, Boca Raton

Benoiton NL, Chen FMF (1981) 2-Alkyl-5(4H)-oxazolones from N-alkoxycarbonylamino acids and their implication in carbodiimide-mediated reactions in peptide synthesis. Can J Chem 59:384–389

Benoiton NL, Lee YC, Steinauer R, Chen FMF (1992) Studies on sensitivity to racemization of activated residues in couplings of N-benzyloxycarbonyldipeptides. Int J Pept Protein Res 40:559–566

Biron JP, Pascal R (2004) Amino acid N-Carboxyanhydrides: activated peptide monomers behaving as phosphate-activating agents in aqueous solution. J Am Chem Soc 126:9198–9199

Boiteau L, Pascal R (2011) Energy sources, self-organization, and the origin of life. Orig Life Evol Biosph 41:23–33

Brack A (1982) Aqueous polymerization of L-amino acid active esters in bicarbonate solution via Leuch anhydrides. Biosystems 15:201–207

Brack A (2007) From interstellar amino acids to prebiotic catalytic peptides: a review. Chem Biodivers 4:665–679

Bujdák J, Rode BM (1999) Silica, alumina and clay catalyzed peptide bond formation: enhanced efficiency of alumina catalyst. Orig Life Evol Biosph 29:451–461

Cavadore JC, Previero A (1969) Polycondensation of free amino acids in aqueous solution with a soluble carbodiimide. Bull Soc Chim Biol 51:1245–1253

Collet H, Bied C, Mion L, Taillades J, Commeyras A (1996) A new simple and quantitative synthesis of α-aminoacid-N-carboxyanhydrides (oxazolidines-2, 5-dione). Tetrahedron Lett 37:9043–9046

Commeyras A, Boiteau L, Vandenabeele-Trambouze O, Selsis F (2005) Peptide emergence, evolution and selection on the primitive Earth. I. Convergent formation of N-carbamoyl amino acids rather than free α-amino acids? In: Gargaud M, Barbier B, Martin H, Reisse J (eds) Lectures in astrobiology-part II: from prebiotic chemistry to the origins of life on Earth. Springer, Berlin, pp 531–554

Commeyras A, Taillades J, Collet H, Boiteau L, Vandenabeele-trambouze O, Pascal R, Rousset A, Garrel L, Rossi JC, Biron JP, Lagrille O, Plasson R, Souaid E, Danger G, Selsis F, Dobrijevic M, Martin H (2004) Dynamic co-evolution of peptides and chemical energetics, a gateway to the emergence of homochirality and the catalytic activity of peptides. Orig Life Evol Biosph 34:35–55

Crisma M, Moretto A, Formaggio F, Kaptein B, Broxterman Q, Toniolo C (2004) Meteoritic Cα-methylated α-amino acids and the homochirality of life: searching for a link. Angew Chem Int Ed 43:6695–6699

Cronin JR, Gandy WE, Pizzarello S (1980) Amino acids of the murchison meteorite. In: Hare PE, Hoering TC, King K Jr (eds) Biogeochemistry of amino acids. Wiley, New York, pp 153–168

Cronin JR, Pizzarello S (1983) Amino acids in meteorites. Adv Space Res 3(9):5–18

Danger G, Boiteau L, Cottet H, Pascal R (2006) The peptide formation mediated by cyanate revisited. N-carboxyanhydrides as accessible intermediates in the decomposition of N-carbamoylaminoacids. J Am Chem Soc 128:7412–7413

Danger G, Plasson R, Pascal R (2010) An experimental investigation of the evolution of chirality in a potential dynamic peptide system: N-terminal epimerization and degradation into diketopiperazine. Astrobiology. 10:651–662

Duvernay F, Chiavassa T, Borget F, Aycard JP (2004) Experimental study of water-ice catalyzed thermal isomerization of cyanamide into carbodiimide: implication for prebiotic chemistry. J Am Chem Soc 126:7772–7773

Hagan WJ (2010) Uracil-catalyzed synthesis of acetyl phosphate: a photochemical driver for protometabolism. ChemBioChem 11:383–387

Higuchi T, Flynn GL, Shah AC (1967) Reversible formation and hydrolysis of phthaloyl and succinyl monophosphates in aqueous solution. J Am Chem Soc 89:616–622

Huber C, Eisenreich W, Hecht S, Wächtershäuser G (2003) A possible primordial peptide cycle. Science 301:938–940

Huber C, Wächtershäuser G (1998) Peptides by activation of amino acids with CO on (Ni, Fe)S surfaces: implications for the origin of life. Science 281:670–672

Ibrahim IT, Williams A (1980) Concerted general acid catalysis in the reaction of acetate ion with water soluble carbodiimide. J Chem Soc Chem Commun 25–27

Imai EI, Honda H, Hatori K, Matsuno K (1999) Autocatalytic synthesis of oligoglycine in a simulated submarine hydrothermal system. Orig Life Evol Biosph 29:249–259

Jones ME, Lipmann F (1960) Chemical and enzymatic synthesis of carbamyl phosphate. Proc Natl Acad Sci USA 46:1194–1205

Kawamura K, Nishi T, Sakiyama T (2005) Consecutive elongation of alanine oligopeptides at the second time range under hydrothermal conditions using a microflow reactor system. J Am Chem Soc 127:522–523

Kawamura K, Takeya H, Kushibe T (2009) Effect of condensation agents and minerals for oligopeptide formation under mild and hydrothermal conditions in related to chemical evolution of proteins. Adv Space Res 44:267–275

Kirby AJ (1980) Effective molarities for intramolecular reactions. Adv Phys Org Chem 17:183–278

Kricheldorf HR (2006) Polypeptides and 100 years of chemistry of α-amino acid N-carboxyanhydrides. Angew Chem Int Ed 45:5752–5784

Lagrille O, Danger G, Boiteau L et al (2009) Process improvement in amino acid N-carboxyanhydride synthesis by N-carbamoyl amino acid nitrosation. Amino Acids. 36:341–347

Lambert JF (2008) Adsorption and polymerization of amino acids on mineral surfaces: a review. Orig Life Evol Biosph 38:211–242

Leman L, Orgel L, Ghadiri MR (2004) Carbonyl sulfide–mediated prebiotic formation of peptides. Science 306:283–286

Leman LJ, Orgel LE, Ghadiri MR (2006) Amino acid dependent formation of phosphate anhydrides in water mediated by carbonyl sulfide. J Am Chem Soc 128:20–21

Li Y, Yin Y, Zhao Y (1992) Phosphoryl group participation leads to peptide formation from N-phosphorylamino acids. Int J Pept Protein Res 39:375–381

Lohrmann R (1972) Formation of urea and guanidine by irradiation of ammonium cyanide. J Mol Evol 1:263–269

Maurel MC, Orgel L (2000) Oligomerization of α-thioglutamic acid. Orig Life Evol Biosph 30:423–430

Miller SL (1953) A production of amino acids under possible primitive Earth conditions. Science 117:528–529

Miller SL, Parris M (1964) Synthesis of pyrophosphate under primitive Earth conditions. Nature 204:1248–1250

Miyazawa T, Donkai T, Yamada T, Kuwata S (1989) Racemization suppression by copper (II) chloride in peptide synthesis by the mixed anhydride and related methods. Chem Lett 2125–2128

Mullen LB, Sutherland JD (2007) Simultaneous nucleotide activation and synthesis of amino acid amides by a potentially prebiotic multi-component reaction. Angew Chem Int Ed 46:8063–8066

Orgel LE (2002) Is cyanoacetylene prebiotic? Orig Life Evol Biosph 32:279–281

Page MI, Jencks WP (1971) Entropic contribution to rate accelerations in enzymic and intramolecular reactions and the chelate effect. Proc Natl Acad Sci USA 68:1678–1683

Pascal R (2003) Catalysis through induced intramolecularity: what can be learned by mimicking enzymes with carbonyl compounds that covalently bind substrates? Eur J Org Chem 1813–1824

Pascal R, Boiteau L, Commeyras A (2005) From the prebiotic synthesis of α-amino acids towards a primitive translation apparatus for the synthesis of peptides. Top Curr Chem 259:69–122

Pizzarello S, Huang YS (2002) Molecular and isotopic analyses of Tagish Lake alkyl dicarboxylic acids. Meteorit Planet Sci 37:687–696

Plankensteiner K, Reiner H, Rode B (2005) Prebiotic chemistry the amino acid and peptide world. Curr Org Chem 9:1107–1114

Plasson R, Bersini H, Commeyras A (2004) Recycling Frank: spontaneous emergence of homochirality in noncatalytic systems. Proc Natl Acad Sci USA 101:16733–16738

Ranganathan D, Singh GP (1990) The demonstration of selective peptide bond formation in clear aqueous solutions. J Chem Soc Chem Commun 142–143

Rode BM (1999) Peptides and the origin of life. Peptides 20:773–786

Rode BM, Son HL, Suwannachot Y, Bujdak J (1999) The combination of salt induced peptide formation reaction and clay catalysis: a way to higher peptides under primitive Earth conditions. Orig Life Evol Biosph 29:273–286

Stark GR (1965a) Reactions of Cyanate with functional groups of proteins. III. Reactions with amino and carboxyl groups. Biochemistry 4:1030–1036

Stark GR (1965b) Reactions of cyanate with functional groups of proteins. IV. Inertness of aliphatic hydroxyl groups. Formation of carbamyl- and acylhydantoins. Biochemistry 4:2363–2367

Taillades J, Boiteau L, Beuzelin I, Lagrille O, Biron JP, Vayaboury W, Vandenabeele-Trambouze O, Giani O, Commeyras A (2001) A pH-dependent cyanate reactivity model: application to preparative N-carbamoylation of amino acids. J Chem Soc Perkin 1 1247–1254

Valeur E, Bradley M (2009) Amide bond formation: beyond the myth of coupling reagents. Chem Soc Rev 38:606–631

van den Nest W, Yuval S, Albericio F (2001) Cu(OBt)2 and Cu(OAt)2, copper-based racemization suppressors ready for use in fully automated solid-phase peptide synthesis. J Pept Sci 7:115–120

Vogels GD, Uffink L, Van der Drift C (1970) Cyanate decomposition catalyzed by certain divalent anions. Recl Trav Chim Pays-Bas 89:500–508

Williams A, Ibrahim IT (1981) Carbodiimide chemistry: recent advances. Chem Rev 81:589–636