Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Fiore, W. Madanamoothoo, A. Berlioz-barbier, O. Maniti, A. Girard-Egrot, R. Buchet and P. Strazewski, *Org. Biomol. Chem.*, 2017, DOI: 10.1039/C7OB00708F.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C7OB00708F

Journal Name

ARTICLE

Giant vesicles from rehydrated crude mixtures containing unexpected mixtures of amphiphiles formed under plausibly prebiotic conditions

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Michele Fiore,[a,*] Warren Madanamoothoo,[a] Alexandra Berlioz-Barbier,[a] Ofelia Maniti,[a] Agnès Girard-Egrot,[a] René Buchet [a] and Peter Strazewski [a,*] †

Giant lipid vesicles resemble compartments of biological cells, mimicking them in their dimension, membrane structure and partly in their membrane composition. The spontanenous appearance of closed membranes composed of bilayers of self-assembling amphiphiles was likely a prerequisite for Darwinian competitive behavior to set in at the molecular level. Such compartments should be dynamic in their membrane composition (evolvable), sufficiently stable to harbor macromolecules (leak-free), yet semi-permeable for reactive small molecules to get across the membrane (stay away from chemical equilibrium). Here we describe bottom-up experiments simulating prebiotic environments that support the formation of simple amphiphilic molecules capable of self-assembling into vesicular objects on the micrometer scale. Long-chain alkyl phosphates, together with related amphiphilic compounds, were formed under simulated prebiotic phosphorylation conditions by using cyanamide, a recognized prebiotic chemical activator and precursor for several compound classes. Crude dry material of thus obtained prebiotic mixtures formed multilamellar giant vesicles once rehydrated at the appropriate pH and in the presence of plausibly prebiotic co-surfactants, as observed by optical microscopy. The size and the shape of lipid aggregates tentatively suggest that prebiotic lipid assemblies could encapsulate peptides or nucleic acids that could be formed under similar chemical prebiotic conditions. The formation of prebiotic amphiphiles was montiored by using TLC, IR, NMR and ESI-MS and UPLC-HRMS. In addition we provide a spectroscopic analysis of cyanamide under simulated prebiotic conditions in the presence of phosphate sources and specroscopic analysis of *O*-phosphorylethanolamine as a plausible precursor for phosphoethanolamine lipids.

Introduction

How primitive protocells gradually transformed into the three domains of life (*Archaea*, *Bacteria* and *Eukaryota*) is a challenging issue addressed in systems chemistry and synthetic biology. Life as we know it today has probably evolved from very simple chemical systems that assembled spontaneously under favorable conditions. [1] A crucial step for life's development has been the compartmentalization of the essential components required for replication, translation, and transcription, in lipidic or otherwise amphiphilic closed bilayer membranes.[2] Over the past 60 years, research in prebiotic chemistry has given several plausible explanations for the formation, by condensation, of important biomolecules such as amino acids and nucleotides. At first, a significant boost was given by the pioneering experiments of Stanley Miller,[3] later revisited by Jeffrey Bada & colleagues.[4] In the recent years, considerable progresses were made to find plausibly prebiotic conditions leading to the formation of pyrimidine and purine nucleotides on the primordial Earth. Different chemical approaches have been considered. John Sutherland & colleagues have shown that several important classes of biomolecules (2',3'-cyclic pyrimidine nucleotides, various–amino acids and glyceryl phosphate) may have hydrogen cyanide and formaldehyde as common chemical precursors. [5,6] Raffaele Saladino, Ernesto Di Mauro & colleagues obtained many prebiotically relevant compounds, including polyhydroxy alcohols, aldehydes, carboxylic acids, amino acids, urea, guanidine, nitriles, N-heterocycles and, most notably, pyrimidine nucleosides and purine nucleosides, from liquid formamide after irradiation with a proton beam (simulating the Solar wind) and catalyzed by various meteorite powders. [7] Hendrik Zipse, Thomas Carell & colleagues obtained several purine nucleosides from the double cyclization of formamido pyrimidines in the presence of ribose or lower sugars under aqueous conditions. [8] Most recently, Mattew Powner & colleagues demonstrated how pyrimidine nucleotides and 8-oxopurine nucleotides could be formed under similar prebiotic conditions.[9] Lipid amphiphiles can be generated under comparable chemical conditions.[10] The classic example is David Deamer's pioneering work on the simulated prebiotic synthesis of phosphatidic acids (PA), phosphatidyl glycerol

[a] *Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, Université de Lyon, Claude Bernard Lyon 1, 43 bdv du 11 novembre 1918, 69622 Villeurbanne Cedex, France*
**E-mail:* michele.fiore@univ-lyon1.fr, strazewski@univ-lyon1.fr
†Electronic Supplementary Information (ESI) available: NMR, ESI-MS in positive ion and negative ion modes, ESI-HRMS and UPLC-HRMS. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C7OB00708F

(PG), and phosphatidyl glycerophosphate (PGP) by condensation of glycerol, *n*-dodecylic acid (or aldehyde) in the presence of sodium dicyanamide and disodium hydrogen phosphate.[11] Recently, Damer and Deamer pointed out those fluctuating hydrothermal pools (FHPs) could be considered as plausibly prebiotic reactors for the synthesis of several key molecules for the development of life, including lipids, nucleic acids and peptides.[12] FHPs could be the receptacles of organic, moderately hydrophobic compounds that precipitated, fell into an FHP and accumulated, like a bathtub ring, around its borders at the fluctuating water-atmosphere interface. On the other hand, the role of single chain amphiphiles like mono-alkyl phosphates (APs) and fatty acids has enjoyed a growing interest ever since the pioneering studies of Hargreaves and Deamer.[13] Only few experiments were reported in the literature concerning the abiotic formation of AP precursors like *n*-alkanols, *n*-alkanoic acids, *n*-alkenes, *n*-alkanes and alkanones from simple organic compounds. UV irradiation of *n*-alkanes followed by the oxidation and reduction through radical intermediates generated *n*-alkanols (*n*-ROH).[14] Mixtures of cold condensed gases (CH_4, NH_3 and H_2O) produced aldo- and keto-compounds of medium length.[15] Fischer-Tropsch type reactions from the disproportion of formic or oxalic acid under simulated hydrothermal conditions produced long-alkyl chain amphiphilic material that was hydrated to give liposome-like objects.[16,17] Here we focus on APs as the driving amphiphiles for the growth of primitive vesicles. We reason that, given favourable geochemical conditions (phosphate source, *n*-alkanols, dehydrating agents), APs could have been possible early products originating from FHPs. In this work we asked the question whether crude 'prebiotic' phosphorylation mixtures containing APs would favour or disfavour vesiculation upon rehydration. In particular, in view of preliminary work done by others (*vide infra*), we wished to test the growth of giant vesicles from the rehydration of crude phosphorylation mixtures that contained naturally occurring co-surfactants, instead of externally added co-surfactants.

Prebiotic phosphorylation of *n*-alkanols to APs. While the phosphorylation of carbohydrates and nucleosides under simulated prebiotic conditions has been extensively studied,[18-20] only few examples have been reported on the phosphorylation of long chain alcohols.[21] Two mechanisms, not yet proven, can be considered for the phosphorylation of long chain alcohols. An associative reaction mechanism involving organocatalysis by urea,[22] in which a mixed carbamidic phosphoric anhydride could offer urea as good leaving group[20, 23-24] for the replacement with the *n*-alkanol and/or any other alcohol. Alternatively, and more in accord with studies on the degradation of urea in water,[25-28] a dissociative mechanism could explain the effective phosphorylation of alcohols with urea and phosphate at elevated temperatures, whereby urea eliminates ammonia to give isocyanic acid which is trapped by P_i to provide carbamoyl phosphate as the phosphorylating agent. Monnard & Sutherland phosphorylated 1-decanol with urea and P_i and identified by ESI-MS decyl phosphate, accompanied by its sodium salt, and didecyl pyrophosphate.[29] Furthermore, rehydrations at pH 2, 7 and 12 of the dry crude phosphorylation mixtures, in the absence and presence of added co-surfactants like 1-decanol, decyl amine and 1-decanoyl-*rac*-glycerol, were tested for the formation of vesicles. Vesiculation of pure decyl phosphate occurred only at pH 2, more complex mixtures grew vesicles at higher pH. The critical vesicle concentrations (CVC) were in the millimolar range (0.7–6.4 mM).

Cyanamide as plausibly prebiotic precursor of urea for the phosphorylation of *n*-alkanols. In our rationale we assumed that various alkanols, with even or odd number of carbons, can be phosphorylated under the same conditions as reported for the preparation of decylphosphate.[29] We wished to extend the procedure for the preparation of mono-alkyl phosphoryl ethanolamines (polar head PE), since no example for the preparation of these compounds was reported in the literature, but an obvious advantage for the growth of vesicles was to be expected from this type of zwitterionic polar headgroup. We selected undecan-1-ol (**1**) that may have existed on early Earth, formed under hydrothermal conditions by the reduction of the formic acid in the presence of iron.[30] As condensing agent we envisaged to use cyanamide (**2a**) and not only urea (**2b**). Cyanamide was extensively used as condensing agent for the formation, under simulated prebiotic conditions, of acylglycerols,[31] phosphatidic acids,[32] glycerophosphates[33] and phosphatidylcholines.[34] Cyanamide also promoted short peptide formation under different conditions.[4,35-37] Most recently, Richert & coll. tested cyanamide for the concomitant condensation of adenosine monophosphate (AMP) and some amino acids.[38,39] As source of inorganic orthophosphate P_i we used $NH_4H_2PO_4$, (**3a**) a plausible source of inorganic phosphates and used for similar purposes.[20,29] For the preparation of undecyl phosphoethanolamine (**5**) we selected 2-aminoethyl phosphate (**3b**) as plausibly prebiotic organophosphate source.[40] Concerning the geochemical origins and availability of soluble P_i and cyanamide on the early Earth, we refer to published reviews on these topics[21,41] and to the most recent developments in the field.[42,43]

Results

Control experiments in the absence of the undecan-1-ol (**1**) were carried out to monitor the stability-reactivity profile of the cyanamide (**2a**) and 2-aminoethyl phosphate (**3b**) under simulated prebiotic conditions. The experiments carried out on **2a** had a double purpose, as negative control and to optimize later phosphorylation reaction conditions. The experiments on **3b** were carried out to better understand the formation of some unexpected side products, *vide infra*. TLC, ^{1}H NMR, ^{13}C NMR and ESI-MS or UPLC-HRMS analyses followed all the experiments. ^{31}P NMR served to confirm the presence of phosphate species in prebiotic mixtures and control experiments.

Cyanamide under simulated prebiotic conditions.† We observed that a 0.1 mM solution of **2a** hydrolyses to urea (**2b**) in 24 hrs at pH 4.5 in the presence of 1 mM ammonium dihydrogen phosphate (**3a**) at 65, 80 and 100°C (Table S1). **^{13}C** NMR spectra in H_2O/D_2O 9:1 *v/v* showed that the chemical shift corresponding to that of **2a** (118.1 ppm) disappeared with the apparition of another signal at 163.2 ppm characteristic of the carbonyl of **2b**.

View Article Online
DOI: 10.1039/C7OB00708F

Journal Name

ARTICLE

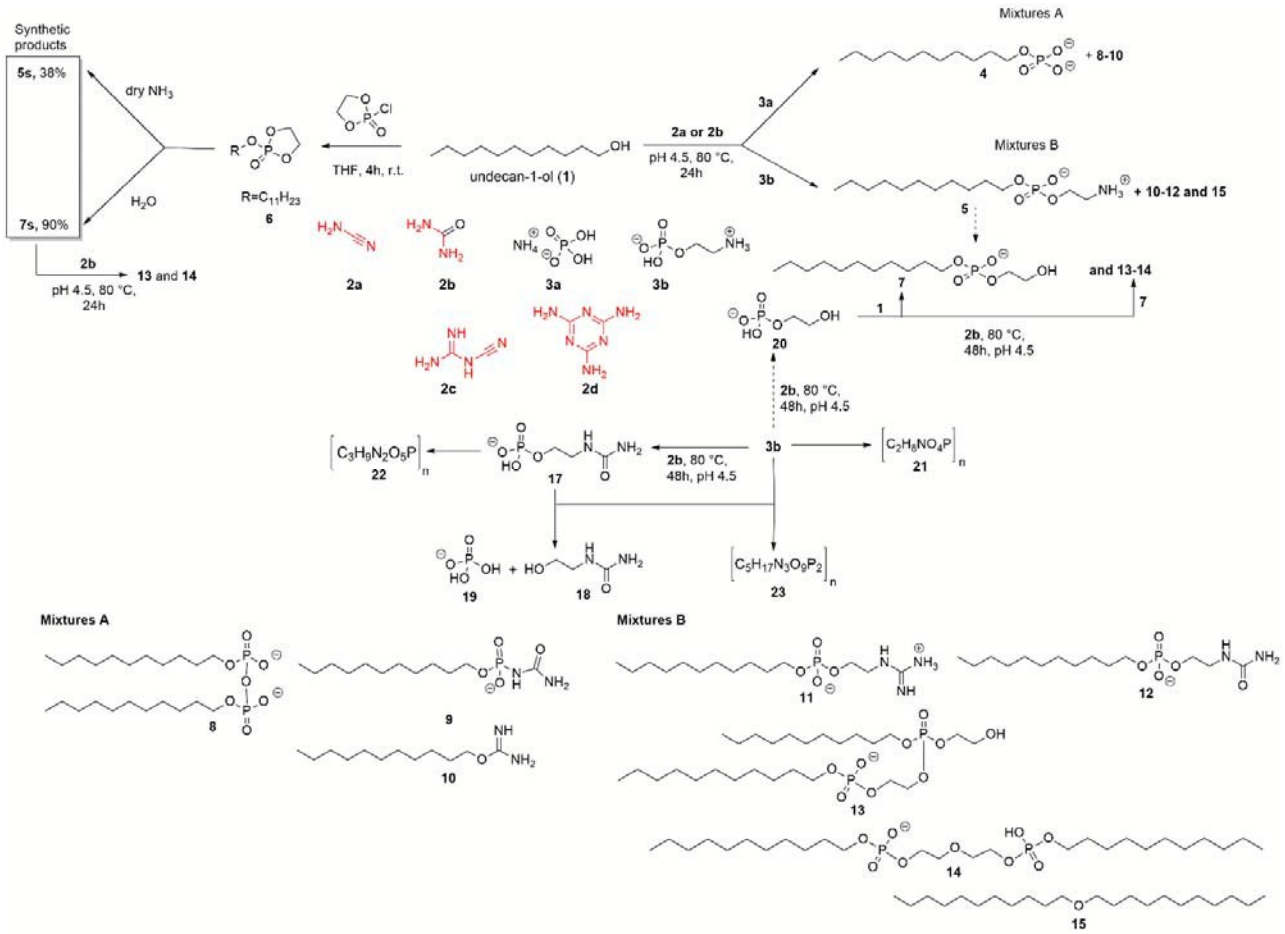

Figure 1. Structure of *n*-alkanol **1** and reagents used in the presented experiments. Condensing agents: cyanamide (**2a**) and urea (**2b**). Phosphorylating agents: ammonium dihydrogen phosphate (**3a**) and 2-aminoethyl phosphate (**3b**). Cyanoguanidine (**2c**) and melamine (**2d**), both in red, result from homologation of **2a**. Compounds **4**–**15** obtained using **2a** or **2b** under simulated prebiotic phosphorylation reactions occurred in the presence of added ammonium dihydrogen phosphate (**3a**) or 2-aminoethyl phosphate (**3b**). Products **17**–**20** were obtained by direct transformation of **3b** while **20**–**23** were the direct products of polymerization and co-polymerization of **3b** and **17**. The structures of **4**–**20** are consistent with the ESI-MS and/or UPLC-HRMS analyses of the crude mixtures and where possible, confirmed by NMR spectroscopy. Standards **5s** and **7s** were prepared from undecan-1-ol (**1**) *via* undecanol-2-oxo-1,3,2-dioxaphospholane (**6**). Condensation of **7s** in presence of **2b** produced both **13** and **14**.

^{31}P NMR showed after 6, 12, 24 and 48 hours no other chemical shift than the signal of **3a** at –0.17 ppm, typical of phosphates, irrespective of whether the sample was kept at ambient temperature or 80°C. When a sample containing 0.1 mmol **2a** was heated to 65, 80 or 100 °C under neat conditions or in neutral water (pH 7) to dryness, the ^{13}C NMR spectrum in H_2O/D_2O 9:1 *v/v* showed new signals at 120.1 and 166.0 ppm. While the starting material **2a** was still present (117.2 ppm), a signal at 163.2 ppm would match the chemical shift of **2b** (Figures S1 and S2). As a further control, all the samples analyzed in H_2O:D_2O were reanalyzed in DMSO-d_6 using as control compounds pure commercial samples of **2a**-**d** (Figure S3). ^{13}C NMR spectra of commercial cyanamide (**2a**), urea (**2b**), cyanoguanidine (**2c**) and melamine (**2d**) were recorded in DMSO-d_6 and compared to those of an analytical sample obtained from heating equimolar amounts of **2a** + **3a** to dryness. When the temperature was kept at 25 or 40 °C at neutral or acidic pH for 48 hours, **2a** did not react to give neither **2b**, nor **2c** or **2d**. In fact, when **2a** was hydrolyzed under acidic conditions at 65°C for 24 hours, **2c** was obtained as the minor product with residual **2a** and **2b**. No apparent reaction between **2b**

View Article Online
DOI: 10.1039/C7OB00708F

and **3a** occurred at pH 4.5 and 80°C (Figure S5). Melamine (**2d**) was produced in negligible amount, and was not detected in control experiment sample dissolved in DMSO-d_6 and it was detected by ESI-MS (*vide infra*). In spite of the elevated reaction temperatures (80-100°C), only the dimer **2c** and cyclic trimer **2d** but no higher oligomers of cyanamide were observed by direct ESI-MS analysis of the crude mixture. [44] To confirm NMR analysis, sample analyzed by ESI-MS showed that two derivatives of cyanamide were formed under the conditions used: cyanoguanidine (**2c**) and melamine (**2d**) with characteristic ionization peaks $[M+H]^+$ at *m/z* 85 and 127, respectively (Figure S4).

Table 1.

Entry	Added Compounds	Temp/time	Products	Vesicles
1	**1** + **2a** + **3a**	65°C/24h	**4** & **10**	–
2	**1** + **2a** + **3a**	80°C/24h	**4**, **8**–**9**	Yes*
3	**1** + **2b** + **3a**	100°C/24h	**4**, **8**–**9**	–
4	**1** + **2b** + **3a**	80°C/360h	mainly **4**	Yes§
5	**1** + **2a** + **3b**	100°C/24h	**11**–**15**	–
6	**1** + **2a** + **3b**	80°C/24h	**10** & **11**	–
7	**1** + **2b** + **3b**	80°C/72h	**13** & **14**	–
8‡	**1** + **2b** + **3b**	80°C/48h	**5**, **7**, **12**–**14**	Yes#
9‡	**7s** + **2b**	80°C/48h	**13** & **14**	–
10	**1** + **2a**	80°C/48h	**10**	–
11	**5s** + **2a**	80°C/48h	n.c.	–
12	**7s** + **2a**	80°C/48h	n.c.	–

Structures of compounds **4** – **15** are shown in figure 4.
‡ from UPLC – HRMS *Vesicles obtained only at pH 2.0 and pH 9.21 (50 mM Bicine); §Vesicles formed in the presence of 10% *w/w* synthetic co-surfactants **5s** or **7s** (*vide infra*), the sample had a poor tendency to vesiculate ; #Vesicles obtained at pH 7.8 (100mM Tris-HCl) and pH 9.10 (50mM Bicine). n.c = no conversion. Details reported in Supporting Information Table S2.†

Formation of APs under prebiotic conditions.† Alkanol **1** was mixed with the condensing agents, **2a** or **2b** and **3a** or **3b**, respectively. All reactions were carried out using molar ratios 1:10:10 for **1**/**2a-b**/**3a-b**. We will refer to "Mixture A" for those analytes obtained from the reaction with either **2a** or **2b** in the presence of **3a** (PA polar headgroups) and to "Mixture B" obtained from the reaction with **2a** or **2b** in the presence of **3b** (PE polar headgroups). The reaction conditions were optimized by testing different temperatures (T), reaction times (t) and reaction conditions. Two main conditions were tested. In the first case, a mixture suspended in water was let dry for a period of time between 24 and 48 hrs. The second tested reaction mixtures did not contain any added water, the neat reaction components were kept partly melted at the indicated temperatures (65, 80 and 100 °C) for 24 or 48 hrs. Neat reaction conditions required additional 24 hrs for a virtually complete consumption of the starting alkanol. In certain cases, the experiments were prolonged up to 2 weeks to check the stability of formed compounds. For all conditions, except in control experiments, the starting material undecan-1-ol (**1**, R_f = 0.90) disappeared with the concomitant appearance of more polar products (R_f = 0.62 and 0.40). The purification by conventional chromatography (preparative TLC, column chromatography) of the individual components of Mixtures A or B failed due to the tendency of the mixture's components to co-migrate in any of the examined eluent systems. The reaction conditions used and the corresponding results are summarized in Table 1

Analysis of crude Mixtures A and B. To monitor the formation of APs, crude reaction mixtures were submitted to infrared analysis. The anti-symmetrical phosphate vibration and the symmetrical phosphate vibrations appear in the region of 1300-900 cm^{-1} of IR spectra. The position of the phosphate bands is sensitive not only to the chemical nature of phosphate ester-bonds but also to their ionic environment such as divalent metal ions and pH. Such positions of phosphate bands may reveal mixtures of different phosphate esters, for example, distinguish monoesters from diesters. The IR spectra of crude Mixtures A (figure 2, upper spectra) are very similar and show three bands located at 1157, 1078 and 940 cm^{-1}, reminiscent of the three peaks of the **3a** (figure 2, bottom lane). An additional weak but distinct band at 989 cm^{-1} indicates the presence of another chemical specimen than **3a** in the crude mixture, which could be assigned to **4** on the basis of the ESI-MS analysis of the product of the reaction reported below. IR spectra of crude Mixtures B obtained from the reaction with **2a** or **2b** in the presence of **3b**, are quite similar. Minor differences, indicates distinct populations of chemical species (figure 2, middle spectra). The bands of **3b** located at 1187-83, 1078 and 1029 cm^{-1} (Figure 2, bottom spectrum) were also found in both IR spectra of crude mixture B, with an additional band at 978 cm^{-1} (figure 2 middle spectra) indicating another chemical specie than **3b** (figure 2, bottom lane).[45,46] The presence of undecyl phosphate (UnPA, **4**) in Mixture A was confirmed by direct ESI-MS analysis of the crude dry mixtures. Surprisingly, **4** was accompanied by a plethora of related amphiphilic compounds **8**–**10** most of which with higher molecular weight (figure 1). The structures of the side products **8**–**10** were tentatively deduced only from the analysis of ESI-MS spectra recorded in positive and negative ion modes. UnPA (**4**) was detected essentially in the negative ion mode at *m/z* 251 $[M–H]^–$. *Bis*-(undecyl) pyrophosphate (**8**) was observed as *m/z* 244 $[M+2H]^{2+}$, 255 $[M+Na+H]^{2+}$. Ureyl phosphoramidate **9** was detected as *m/z* 294 $[M+H]^+$, 317 $[M+Na]^+$ and 293 $[M–H]^–$. Undecyl isourea **10**, formally the adduct of **2a** on **1**, was observed as *m/z* 215 $[M+H]^+$ also in presence of **2b** (Table S2, entries 1-7 and figures S23–S27 and S28).† Mainly **4** was formed when the reaction of **1** was carried out not only at 65°C but, surprisingly, also at 80°C for 360 hrs. Unexpectedly, 2-aminoethyl undecyl phosphate (**5**) was not detected by direct ESI-MS in any of the crude Mixtures B, instead, MS signals attributed to compounds **10**–**15** were found. By direct ESI-MS at low resolution either compounds **11** or **12** were observed as *m/z* 338 and were impossible to distinguish from one another. Supposed (isomeric) **13** or **14** were detected as *m/z* 288 $[M+2H]^{2+}$ and ether **15** was observed only in presence of **2a** and was detected as *m/z* 348 as $[M+Na–H]^–$ (Table S2, entries 8-14 and figures

View Article Online
DOI: 10.1039/C7OB00708F

S29–S35).† The unexpected results obtained for Mixture B made us investigate more closely in two directions. First, we synthesized an analytical sample of 2-aminoethyl undecyl phosphate (**5s**) as a reference compound. Second, we analyzed Mixture B by NMR spectroscopy and UPLC-HRMS (Ultra Performance Liquid Chromatography coupled with High Resolution Mass Spectrometry) for a more complete analysis of the compounds formed under simulated prebiotic conditions. When reactions conditions required cyanamide (**2a**) in all ESI-MS spectra, the peaks of cyanoguanidine (**2c**) and melanine (**2d**) were found.

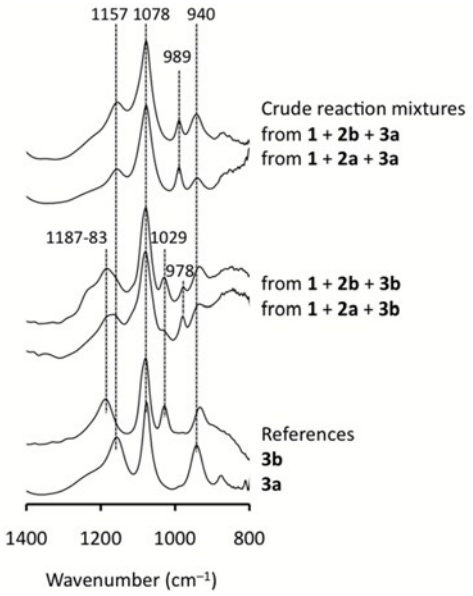

Figure 2. Infrared spectra of crude Mixtures A and B, respectively, containing different APs obtained under simulated prebiotic conditions. Top spectra: crude mixtures A obtained either with cyanamide (**2a**) or with urea (**2b**) in the presence of P_i (**3a**). Middle spectra: crude Mixtures B from the reaction with either **2a** or **2b** in the presence of 2-aminoethyl phosphate (**3b**). Bottom spectra: IR spectra of phosphates **3a** and **3b** used as phosphorylating agents. All the samples were prepared in 100 mM Tris buffer pH=8.1.

Synthesis of standard compounds for the analysis of Mixture B.† Synthetic 2-aminoethyl undecyl phosphate (**5s**) was prepared by reacting reasonably anhydrous ammonia with 2-(undecyloxy)-1,3,2-dioxaphospholane 2-oxide (**6**, figure 4, figures S7-11†)[47] as reported for the synthesis of analogues of phospholipids[48] and APs.[49] The synthesis of pure **5s** was more difficult than anticipated, owing to the formation of an another less polar compound that formed under the same reaction conditions and not easy to separate from **5s**. This side product was isolated pure and identified as 2-hydroxyethyl undecyl phosphate (**7s**). The chemical shifts of this compound were very similar to those of **5s** and, even though the structure was elucidated by mono- and bi-dimensional NMR spectroscopy, only high resolution mass analysis (HR-MS) could confirm that **7s** was the product obtained from ring opening of **6** by a molecule of residual water (figures S21-22).† To ascertain this hypothesis, we prepared directly **7s** from **6** with an excess of water (yield 90%, figures S12–S20)† accordingly to what was previously reported by de Jongh and de Kruijfft.[50]

NMR and HR-MS analysis of crude Mixture B. The NMR analysis of crude Mixture B performed in DMSO-d_6 or $CDCl_3$ yielded unsatisfactory results due to the overlapping of the aliphatic signals of the compounds present in the mixture (figures S37-44).† Only a ^{31}P NMR spiking experiment carried out in $CDCl_3$ with standard compounds **5s** and **7s** showed by comparison with the un-spiked original spectra that the major components of the mixture were **5** and **7** (figures S45-48).† UPLC–HRMS of the same Mixture B showed that **5** (R_T 10.2 minutes, *m/z* 294.1844 as $[M-H]^-$) was detected together with **7** (R_T 10.8 minutes, *m/z* 295.1685 as $[M-H]^-$) and **12** (R_T 10.3 minutes, *m/z* 337.1890 as $[M-H]^-$). These compounds were accompanied by other two compounds (R_T 17.4 and 18.0 min) both having *m/z* 573.3342 for $[M+H]^+$ and supposed structures **13** and **14** as shown in figure 4 (figure S49).† Part of dried Mixture B (20 mg) was purified by preparative TLC using the eluent $CHCl_3$/MeOH/H_2O 65:45:4 *v/v/v*. A single TLC band was collected, extracted and analyzed by UPLC-HRMS. Spiking with **5s** and **7s** confirmed by UPLC-HRMS the presence of only **5** and **7**. No traces of compounds **11**-**14** were could be found (figure S50).† To confirm that **13** and **14** were produced by the condensation of **7** under simulated prebiotic conditions, synthetic **7s** was heated at 80°C for 24 hrs in the presence of urea (**2b**) with a molar ratio 1:10 (Table 1, entry 9). A mixture of more polar compounds, inseparable by TLC, was obtained. UPLC-HRMS analysis confirmed the presence of the two compounds with same molecular formula $C_{26}H_{55}O_9P_2$ and for both *m/z* 573.3327 as $[M-H]^-$ with retention times 17.4 and 18.0 minutes and with complete disappearance of the starting material **7s** (R_T 10.8 minutes). The presence of these two distinct chemical compounds at R_T 17.4 and 18.0 minutes was confirmed by 1D and 2D NMR analysis and revealed a molar ratio o 2:1 (figures S52-S57). † In addition, **1** was heated under neat conditions for 48 hours in the presence of 10 equiv. dry **2a** to give **10** together with the starting material **1** (Table S2, entry 7 and figure S36).† Neither **11** nor **12** were obtained by direct condensation of **5s** or **7s** in presence of **2a** (or **2b**) under the conditions described above (Table 1, entries 11 and 12). At this point it was clear that, in Mixtures B, UnPE **5** was generated only as a minor component by direct condensation of **1** with **3b** in presence of **2a** or **2b**, and that the other detected compounds (**7**, **12**, **13** and **14**) were a direct product of **1** with **3b** to give ureido derivative **17** followed by **18**-**20** (figure 1) and the products of the direct condensation of **7** under the same simulated prebiotic conditions. To better understand the reactivity of **3b** under simulated prebiotic conditions, we set up a series of experiments monitored by NMR and UPLC–HRMS analysis.

Reactivity of 2-aminoethyl phosphate (3b) under prebiotic conditions. Compound **3b** was fully characterized by NMR spectroscopy and used as reference compound (figures S58-S62).† A 1M solution of **3b** (1 mmol) together with 1 mmol of urea (**2b**) was heated to dryness for 16 and 48 hrs and the crude mixtures were analyzed by NMR spectroscopy showing a plethora of signals. COSY showed the presence of four major compounds together with

View Article Online
DOI: 10.1039/C7OB00708F

several minor ones in the region between 4.5 and 2.5 ppm (figures S64 and S69).† ^{13}C and DEPT experiments confirmed the presence of methylenes only (–CH_2–) and several quaternary carbons (urea or ureyl groups at 163.0 and between 162.1 and 161.5 ppm, figures S65 and S70).† The main difference between the spectra registered after 16 and 48 hrs was the increase of the signals with respect to the residual **3b** (figures S63, S68 and S74).† Furthermore, ^{31}P NMR spectra showed that two different patterns of signals were present in the mixtures. Phosphates resonate in the region between 0.24 and –0.02 ppm and pyrophosphates, present only in the sample obtained after 48 hrs reactions, showed very small signals between –10.40 and –11.30 ppm. (figures S67, S73 and S75).† The presence of residual **3b** was confirmed by ^{13}C spiking experiment carried out on the mixture obtained after 48 of heating (figure 76).† Direct HR-MS analysis and UPLC-HRMS analysis confirmed that, together with **3b**, six major compounds were present in the mixtures (figures S77–83).† Undecyl(2-ureidoethyl) hydrogen phosphate (**17**) showed at *m/z* 185.0321 as $[M+H]^+$ (figure S84). † This compound was reported as being of high industrial interest and produced on a large scale under similar condensation conditions.[51] 1-(2-Hydroxyethyl)urea (**18**) was found at *m/z* 105.0659 as $[M+H]^+$ (figure S85)† together with monophosphate **19** at *m/z* 98.9841 as $[M+H]^+$ (figure S84).† 2-Hydroxyethyl dihydrogen phosphate (**20**) was not found by HR-MS but its presence is plausible in other mixtures that allowed forming products **7** by direct condensation of **20** with **1** in presence of **2a** or **2b** as hypothesized in figure 1, middle scheme. Three distinct polymers **21-23** were also found in the mixture as products of the direct polymerization and co-polymerization of **2b** and **17** (figures S83 and S84).

Formation of vesicles and lipid aggregates upon hydration. The crude (dry or wet) reaction mixtures A or B were hydrated using the gentle hydration method.[52] These suspensions, containing 1 to 5 mg of membranogenic compounds and co-surfactants, were hydrated with an adequate volume of hydration buffer (or unbuffered Milli-Q water) to a final concentration of 10 mg mL^{-1}. First trials were made on Mixtures A (Table 1, entry 2). These mixtures formed, in accordance with the results on crude decyl phosphate reported by Monnard & Sutherland [29] small supramolecular structures at pH 2 and surprisingly, aggregate lipids looking like vesicles (with 5µm diameter) at pH 9.21 (figures 3a–b). Hydration of Mixture B produced small vesicle-like structures when hydrated at pH 7.81 (not shown), while larger vesicle-like structures were formed at pH 9.21 (figures 3c). Mixture B had the tendency to form oil droplets that disappeared upon increasing the pH value. Decreasing the pH (upon hydration with unbuffered Milli-Q water) did not allow to observe objects larger than 0.5 µm diameter accompanied by an increasing number of oil droplets (data not shown). We also tried to vesiculate mixtures that did not contain **5** or **7** (Table 1, entries 5-7). These mixtures did not yield any vesicles or oil droplets and the film stayed as if not hydrated at any examined pH (data not shown). At this point, we decided to compare the microscopic images of re-hydrated crude prebiotic Mixtures A and B with those of "reconstituted" mixtures obtained from hydrating thin films of known amounts of synthetic products **1**, **5s** and **7s**. Compounds **1** and **5s** on their own, formed oil droplets when hydrated at, both, pH 7.81 and 9.21 as shown in figure 3d–e, whereas **7s** formed similar oil droplets at pH 7.81 and oil droplets accompanied by lipid aggregates or giant vesicles (GVs of ≤ 5µm diameter) at pH 9.21 (figure 5f). When **1** (4 % mol:mol) was added to **5s**, the hydrated mixed film produced lipid aggregates and GVs at pH 7.81 (5µm diameter); their size increased with increasing amounts of **1** (up to 10 % mol:mol) and higher pH (9.21, figures 3g-i). Dried thin films of **7s** containing 1 (10% mol:mol) gave small assemblies at pH 7.82 (≤ 2µm diameter, figure 5l) but when hydrated at pH 9.21 produced interesting huge (> 40 µm diameter) lipid aggregate looking like multilamellar and multivesicular GVs (figure 3m–n). Surprised by this result, we decided to investigate the capacity of **5s** and its analogue **7s** to act as co-surfactant in Mixture A that did contain UnPA (**4**) and side products 8-10 but had a poor tendency to vesiculate or to even form hydrated aggregates in unbuffered water.

Role of 1, 5s and 7s in the vesiculation of Mixture A. As a first experiment, we added to a 'prebiotic' Mixture A (10 mg mL^{-1}) small amounts of **1**, **5s** and **7s** in ratio 1:1:1 *w/w/w*. GVs (>10µm) appeared when the amount of co-surfactants reached 10% *w/w*. We used unbuffered Milli-Q water with a measured pH 7 for the hydration (figures 6a–c). In a second experiment, **1** (5% *w/w*) was added together with **5s** (5% *w/w*). Hydration at pH 7.0 (unbuffered Milli-Q water) gave neither GVs nor oil droplets (data not shown) but when the sample was hydrated at pH 7.8 (Tris-HCl) GVs (figure 6d) appeared together with worm-like objects and other lipid assemblies (not shown). A third experiment was carried out by adding to mixture A 10% *w/w* of **7s**. GVs and lipid aggregates very similar to those observed for added **5s**+**1** were obtained, but no addition of **1** was needed this time (figures 6e–f). These experiments demonstrate that an important role is played by **5s** and **7s** in the vesiculation of mixtures that require acidic or basic pH values, in order to vesiculate or to aggregate from those mixtures (Mixtures A and B) that had a poor tendency to form GVs or lipid aggregates at neutral pH (being in our experiments unbuffered Milli-Q water).

Discussion

A non negligible, if not crucial, aspect of the described results is that long-chain monoalkyl phosphatidic acids (*n*-ROPAs) and phosphatidylethanolamines (*n*-ROPEs) could both have been formed under the same prebiotic conditions. Our experimental findings showed that long-chain monoalkyl phosphates (UnPA, **4**) and monoalkyl phosphoethanolamines (UnPE, **5**) were formed under simulated prebiotic conditions by condensing undecan-1-ol (**1**) and ammonium phosphate (**3a**) or phosphorylethanolamine (**3b**) in the presence of cyanamide (**2a**) or urea (**2b**). The reaction conditions led to the formation of both products accompained by a plethora of amphiphiles shown to act as co-surfactants and possessing chemical structures close or related to those of **4** and **5.** The use of **2a** produced more amphiphilic side products than the same reactions promoted by **2b**. For Mixture B, the formation of UnPE (**5**) was accompanied by chemically related compounds that

View Article Online
DOI: 10.1039/C7OB00708F

results unexpected respect to those obtained for Mixture A. In fact, under prebiotic conditions, 2-aminoethyl phosphate (**3b**) reacts to give at least six compounds that, once formed, can condense with alkanol (**1**) yielding unexpected mixtures of compounds that can act as co-surfactants. Some of those, like **7**, **11** and **12** seem to be the direct product of the transformation of UnPE (**5**) under the prebiotic condensation reaction conditions carried out in presence of **2a** or **2b**. The latest results on the phosphorylation of glycerol with geochemically more plausible soluble phosphate sources in a urea-ammonium formate-formamide-water eutectic do suggest that **7** might have prebiotic roots as well.[41]

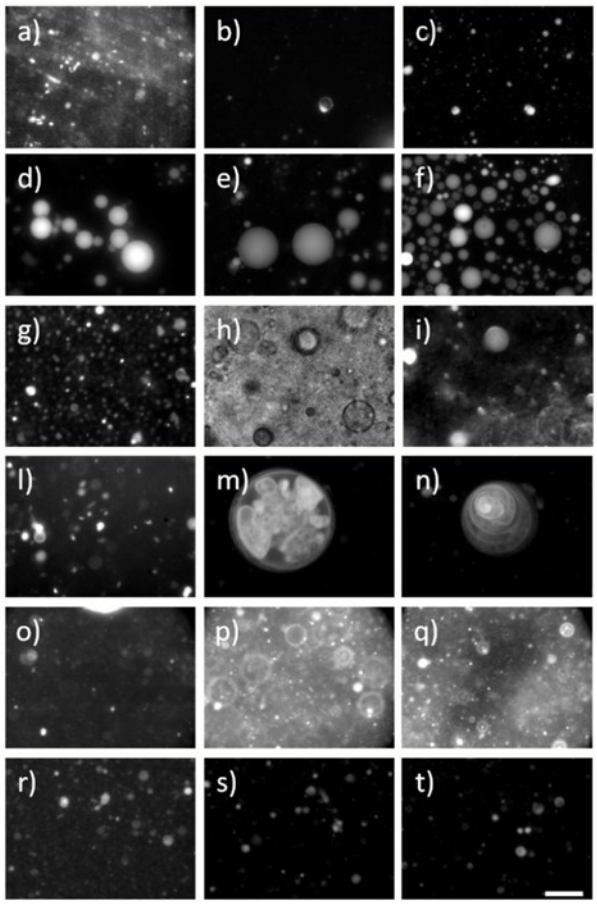

Figure. 3. Micrographs of giant vesicles and lipid aggregates obtained from the gentle hydration of crude reaction **Mixtures A** and **B** and GVs obtained from gently hydrating films of crude Mixture A enriched with of **1**, **5s** and **7s** alone or in combination. **a**) **Mixture A** at pH 2; **b**) **Mixture A** at pH 9.21; **c**) **Mixture B** at pH 9.21; **d**) **1** at pH 9.21 (Bicine 50mM); **e**) 5s pH 9.21 (Bicine 50mM); **f**) 7s at pH 9.21; **g**) **5s+1** (96:4%mol:mol) at pH 7.81 (Tris-HCl 100mM); **h-i**) **5s+1** (90:10%mol:mol) at pH 9.21 (Bicine 50 mM); **l**) **7s+1** (90:10%mol:mol) at pH 7.81 (Tris-Bicine). **m-n**) **7s+1** (90:10%mol:mol) at pH 9.21 (Bicine 50mM). Micrograph **h** was taken under bright field illumination. **o-q**) GVs or lipid aggregates obtained from gently hydrating **Mixture A** that contained mainly **4** enriched with added 10 %mol:mol:mol synthetic **5s**, **7s** and **1** (ratio 1:1:1) as co-sufactants. **r**) **Mixture A**+(**5s+1**) (90:10% w/w) pH 7 (unbuffered Milli-Q water); **s-t**) **Mixture A**+**7s** (90:10% w/w) pH 7 (unbuffered Milli-Q water) All samples were prepared at 10 mg mL^{-1}. All the samples prepared as 10mM in 3 mL of buffer and were stained with NileRed (1.2. mM, $\lambda_{[excit]}$=561 nm). Scale bar 20μm for all micrographs in this figure.

Concerning the formation of the other co-surfactants, their synthesis under prebiotic conditions was possible only for compound **10** (by condensation of **1** and **2a**) and for the products **13** and **14** (formed by condensation of **7s** in presence of **2b**). In particular, even if present only in close to negligible amounts, UnPE (**5**) was formed under simulated prebiotic conditions with a certain analogy to the biochemical route evolved for the biosynthesis of 1,2-diacyl-*sn*-glycero-3-phosphoethanolamines (PEs). In modern cells PEs are biosynthesized, among other known pathways, *via* the cytidine diphosphate-ethanolamine pathway (CDP-ethanolamine or "Kennedy" Pathway) using ethanolamine as the substrate.[52] Since the major function of PEs in modern biological membranes is to fusion and promote membrane curvature,[53] it is plausible that small amounts of UnPE (**5**) generated under prebiotic conditions might have played a role in promoting and forming membranes in first protocells, especially if the available membranogenic compounds were *n*-ROPAs that required narrow pH ranges for vesiculation.[54,55] Unfortunately, no paleontological evidence exists to confirm this hypothesis, since the oldest micro-fossils date back to 3.7 Gya.[56,57] Certain is that in our experiments the role of **5** and its unexpected analogue **7** (UnPOH, the major of the two compounds formed) acting as co-surfactants was demonstrated by adding small amounts of those compounds, together with starting material **1**, to crude Mixtures A, that on their own had a poor tendency to vesiculate. The GVs observed upon hydration of thin films showed that not only mixtures of **5** and **1** promoted vesiculation but that **7** played and unexpected role in GV formation in the absence of **1**. The size and the shape of generated GVs or lipid aggregates having not necessarily bilayer structures tentatively suggest that prebiotic APs assemblies could be able to encapsulate polymers such as peptides or nucleic acids that could be formed under similar prebiotic reaction conditions (time, temperature, pH, presence of condensing agents like urea-phosphate mixtures), which remains to be shown by experiment. It is plausible that one single chemical environment, such as a fluctuating hydrothermal pools (FHP), might have contained all the prebiotic reagents to form APs: mono-alkyl alkanols were in contact with sufficient amounts of chemicals acting as condensing agents (cyanamide, urea) and phosphorylating agents (organophosphates or phosphites).[58] To date, the first proto-cellular membranes are mostly assumed as being formed from hydrating either single-chain "fatty" (carboxylic) acids or double-chain phospholipids (glyceryl phosphate esters) rather than APs. APs do, however, unify two favourable properties that are not combined in either of the other amphiphiles. Thus, joining small pH-robust polar headgroups with comparingly short and easy-to-make hydrophobic single-chains can give rise to just the right truncated-cone geometry to produce curved bilayers needed for vesiculation and compartmentation. The facts that the found amphiphilic species **4–14** were formed at mild temperatures, i.e. conditions that are compatible with the prebiotic condensation of amino acids to peptides, indicate that these molecules might also be formed

View Article Online
DOI: 10.1039/C7OB00708F

together with nucleotides and nucleic acids. [59] Furthermore our findings confirm that cyanamide, being a plausible reservoir of urea,[21] is the precursor of related molecules, such as the dimer cyanoguanidine (**2c**) and its cyclic trimer melamine (**2d**) that could have played a crucial role in the prebiotic synthesis of biomolecules in the early stage of life. In particular **2c** is reported as efficient condensing agent for peptides[4] and as phosphorylation promotor of nucleosides and sugars at low pH through the presumed intermediacy of N-[O-(phosphatidyl)carbamoyl]guanidine.[23] Melamine instead can undergo the equivalent of Watson-Crick base pairing with barbituric acid once ribosylated and ribophosphorylated under simulated prebiotic conditions to give nucleoside- and nucleotide-like compounds that spontaneously assemble in non-covalent hexad-stacked supramolecular fibrous polymers.[60] Again, orthogonal reactions could occur in similar heterogeneous media allowing the formation of covalent fibrous assemblies needed to kick off the start for the emergence of the first replicating proto-cells and primitive self-replicating organisms.

Experimental

***General procedure for simulated prebiotic formation of amphiphiles*.** In a typical experiment, an Eppendorf tube or a glass test tube (volume 2 ml), was filled with 0.1 mmol of **1** (17.2 mg) and mixed with 10 equivalents (1mmol) of **2a** or **2b** (42 or 60 mg, respectively) in the presence of 10 equivalents (1mmol) of **3a** or **3b** (115 or 141 mg, respectively). The heterogeneous mixture was suspended in 0.9 mL of ultrapure water and 0.1 mL of ethanol were added in order to dissolve 1 in water and avoid the formation of giant oil droplets. The obtained suspension was vortexed (1 minute), sonicated (5 minutes) and the obtained clear solution was left heated without any cap until dryness of the water/ethanol mixture in a thermo-shaker apparatus set up at 80°C between 24 and 48 hrs. Reactions mixtures were monitored by analytical TLC using eluent A and eluent B. A variant of this procedure was to mix all the reactants and to let them melt at 80°C (neat conditions). For all reaction conditions, a solid yellowish residue was obtained. An aliquot was directly analysed by ESI-MS, UPLC-HRMS, IR and NMR (where possible).

Mixture B (selected signals) ^{1}H NMR (DMSO-d_6), mixtures of aliphatic compounds: δ_H = 0.83 (br*t, -CH$_3$*), 1.18-1.24 (br*s, -CH$_2$-*) 1.37 (*t, -CH$_2$-*), 3.34 (br*t, -CH$_2$-*); then 3.62 (br*t, -CH$_2$-*) correlates with 3.11 (br*t, -CH$_2$-*) as a -CH$_2$-CH$_2$- system; ^{13}C NMR (DMSO-d_6): δ_C = 14.3, 22.6, 26.1, 29.1, 29.6, 31.7, 32.9, 40.9 61.3, 64.1 (signals at 3.62 and 3.11 correlates with those at 64.1 and 40.9 accordingly with HSQC spectra). ^{31}PNMR ($CDCl_3$): δ_P = -0.07 and -0.05 ppm. HRMS (ESI): M^+ calcd. for $C_{13}H_{29}NO_4P$ $[M\text{-}H]^-$ 294.1844; found 294.1844; M^+ calcd. for $C_{13}H_{28}O_5P$ $[M\text{-}H]^-$ 294.1685; found 294.1685; M^+ calcd. for $C_{14}H_{30}N_2O_5P$ $[M\text{-}H]^-$ 337.1892; found 337.1890; M^+ calcd. for $C_{26}H_{55}O_9P_2$ $C_{26}H_{57}O_9P_2$ $[M+H]^+$ 574.3437; found 575.3474.

General procedure for hydrolysis of cyanamide in prebiotic conditions. In a typical experiment, an Eppendorf tube or a glass test tube (volume 2 ml), was filled with 1 mmol (42 mg) of cyanamide (**2a**) in the presence of 1 equivalent of **3a** or **3b** (115 or 141 mg, respectively). Samples were left heated (where required) without any cap until dryness of the water/ethanol mixture in a thermo-shaker apparatus set to 80°C for 48 hrs. Dried samples were dissolved in DMSO-d_6 or D_2O and then analyzed mixtures were compared to the commercial or synthetic standard solutions. **Cyanamide** (**2a**) ^{13}C NMR (DMSO-d_6): δ_C = 117.2; ^{13}C NMR (D_2O): δ_C = 118.1; **Urea** (**2b**) ^{13}C NMR (DMSO-d_6): δ_C = 159.3; ^{13}C NMR (D_2O): δ_C = 163.2; **Cyanoguanidine** (**2c**) ^{13}C NMR (DMSO-d_6): δ_C = 119.1, 163.4; ^{13}C NMR (D_2O): δ_C = 120.0, 163.3; **Melamine** (**2d**) ^{13}C NMR (DMSO-d_6): δ_C = 167.4; ^{13}C NMR (D_2O): δ_C = 166.0.

General procedure for transformation of 2-aminoethyl phosphate in prebiotic conditions. In a typical experiment, an Eppendorf tube or a glass test tube (volume 2 ml), was filled with 1 mmol (141 mg) of *2-aminoethyl phosphate (***3b***)* in the presence of 1 equivalent of **2b** (60 mg). Samples were left heated (where required) without any cap until dryness of the water/ethanol mixture in a thermo-shaker apparatus set to 80°C for 48 hrs. Dried samples were dissolved in D_2O and the analyzed mixtures were compared to the commercial or synthetic standard solutions. **Mixture of compounds** (selected signals) ^{1}H NMR (D_2O), mixtures of aliphatic compounds: δ_H = 2.95, 3.12 (**3b**), 3.18, 3.25, 3.56, 3.77, 3.81, 3.95 (**3b**), 4.42; ^{13}C NMR (D_2O): δ_C = 40.2 (**3b**, from spiking), 40.3, 40.7, 40.8, 41.4, 42.2, 57.8, 60.8, 61.4 (**3b**, from spiking), 61.6, 64.5, 64.6, 161.8, 161.9, 162.0, 163.2 (**2b**). ^{31}P NMR (D_2O): δ_P = 0.24 (**3b**, from spiking) -0.02, -10.40, -10.60, -11.20, -11.30 ppm.

***Amphiphile films*:** In a 5 ml round bottom flask, 1 ml of $CHCl_3$ containing the mixture of amphiphiles (10 mg mL^{-1} or 1 mM for 1 mL of buffer solution or unbuffered milli-Q water) was slowly evaporated in a rotary evaporator, in order to obtain a film as thin as possible. The film was further dried for 2 hours under vacuum (membrane pump) in order to avoid any presence of residual organic solvents. The solutions were hydrated by using the methodologies as reported below.

Gentle hydration: For the preparation of GVs a thin layer of crude amphiphile mixture obtained under simulated prebiotic conditions (10 mg mL^{-1}) was hydrated with unbuffered milli-Q water (pH 7.0) freshly prepared Tris-HCl (100 mM, pH 7.8), or Bicine 50mM, at pH 9.2 for 8-12 hours at 20°C. Once the hydration solution was added, the samples were vortexed for 5 seconds, then sonicated for further 10 seconds. To be able to observe GVs under the epifluorescence microscope, and clearly distinguish them from oil droplets or other assemblies, each suspension (20 to 40 μL) was stained with 2.0 μL of Nile red (NR) prepared as 1.2mM ethanol solution.

View Article Online
DOI: 10.1039/C7OB00708F

Summary, conclusion and future perspectives

Undecyl phosphate (**4**), 2-aminoethyl undecyl phosphate (**5**) and 2-hydroxyethyl undecyl phosphate (**7**) were formed from undecan-1-ol (**1**) under simulated prebiotic conditions described as being possible in fluctuating hydrothermal pools on an early Earth. The reaction conditions included the use of either cyanamide or urea and two different, plausibly prebiotic phosphate sources, soluble inorganic orthophosphate (P_i) and 2-aminoethyl phosphate. When cyanamide was used as condensing agent, the obtained mixtures were enriched with amphiphilic side products. Most of these side products turned out to act as co-surfactants in later experiments. Urea as the phosphorylation catalyst/mediator was very effective but gave less varied side products. A study using a combination of mass spectrometry and ^{13}C NMR spectroscopy showed that cyanamide partially hydrolyzed to urea under the applied reaction conditions, but the direct addition of cyanamide to hydroxyl or amino groups could occur as well. Dried samples of most of the simulated 'prebiotic' crude mixtures produced giant vesicles (GVs) or lipid aggregates upon rehydration at neutral and weakly basic pH values. The GVs were always accompanied by (non vesicular) oil droplets and other supramolecular assemblies. In particular, mixtures containing **5**, or where **5** was present as co-surfactant, GVs or lipid aggregates formed at close to neutral pH. A fully synthetic amphiphile found by serendipity and structural analogue of **5**, that might be a prebiotic product as well, was identified as 2-hydroxyethylundecyl phosphate (**7**). Compound **7** showed a tendency to promote vesiculation when present with **5** and **1** acting as co-surfactants. The presented results enlarge considerably the scope of vesiculation capacity of prebiotically plausible APs first described by Monnard & Sutherland.[28] The main conclusion here is that, for spontaneous vesiculation to occur from crude APs, any prebiotic reaction condition that favours the concomitant production of effective co-surfactants should be seen as favorable for the evolution of more complex prebiotic compartments. These findings suggest that further research have to be carrying out using mono-alkyl phosphates as amphiphiles for self-organized lipid structures. In the best of our knowledge no explorations were made to compare the stability of APs vesicles *versus* fatty acids ones, neither stabilization of the APs membranes in presence of metal ions. Furthermore, the use of APs in prebiotic protocells models, not yet done, can be used to see if GVs made of mixtures of APs are able to exchange encapsulated genetic material 'horizontally',[62] which is likely to have been the main evolutionary pathway for early life,[63] thus stimulate membrane growth and subsequent division.[64]

Acknowledgements

We thank Yoan Dougnon, Éléa Wallisky and Valentin Travers-Lesage for their valuable help in the preparation of GVs from prebiotic mixtures, and the members of the CO2-Glyco research group (ICBMS) for technical and scientific support. The support from the European Coordination of Science and Technology Action on "Emergence and Evolution of Complex Chemical Systems" (COST Action CM1304) is gratefully acknowledged.

Notes and references

1 C. R. Woese, O. Kandler, M.L. Wheelis, *Proc. Natl. Acad. Sci. USA.* 1990, **87**, 4576
2 J. Oró, A. Lazcano, A. *Adv. Space Res.* 1984, **12**, 167; N. Ichihashi, T. Yomo *Life* 2016, **6,** 26; doi:10.3390/life6030026
3 S. L. Miller, *Science* 1953, **117**, 528
4 E. T. Parker, M. Zhou, A. S. Burton, D. P. Glavin, J.P.; Dworkin, R. Krishnamurthy, F. M. Fernández, J. L. Bada, *Angew. Chem. Int. Ed.* 2014, **53**, 8132
5 J. D. Sutherland, *Angew. Chem. Int. Ed.* 2016, **55**, 104
6 B. H. Patel, C. Percivalle, D. J. Ritson, C. D. Duffy, J. D. Sutherland, *Nat. Chem.* 2015, **7**, 301
7 R. Saladino, E. Carota, G. Botta, M. Kapralov, G. N. Timoshenko, A. Y. Rozanov, E. Krasavin, E. Di Mauro, *Proc. Natl. Acad. Sci. USA* 2015, **112**, E2746-E2755
8 S. Becker, I. Thoma, A. Deutsch, T. Gehrke, P. Mayer, H. Zipse, T. Carell, *Science* 2016, **352**, 833
9 S. Stairs, A. Nikmal, D.-K. Bučar, S.-L. Zheng, J. W. Szostak, M. W. Powner, *Nature Commun.* 2017, doi:10.1038/nchem.2703
10 B. R. T. Simoneit, *Adv. Space Res.* 2003, **33**, 88
11 W. R. Hargreaves, S. J. Mulvhill, D. W. Deamer, *Nature* 1977, **266**, 78, 1977
12 B. Damer, D. W. Deamer, *Life* 2015, **5**, 872
13 W. R. Hargreaves, D. W. Deamer, *Biochemistry* 1978, **17**, 3759
14 A. E. Klein, N. Pilpel, *J. Chem. Soc. Faraday I* 1973, **55**,1729
15 C. I. Simionescu, F. Dénes, M. Totolin, *BioSystems* 1981, **13**, 149
16 T. M. McCollom, G. Ritter, B.R.T. Simoneit, *Orig. Life Evol. Biosph.* 1998, **29**, 153
17 T. M. McCollom, B. R. T. Simoneit, *Orig. Life Evol. Biosph.* 1999, **29**, 167
18 R. Lohrmann, L. E. Orgel, *Science* 1968, **161**, 64
19 R. Lohrmann, L. E. Orgel, *Science* 1970, **171**, 490
20 R. Lohrmann, L. E. Orgel, *Nature* 1973, **244**, 418
21 M. Fiore, P. Strazewski, *Life* 2016, **6**, 17
22 M. W. Powner, J. D. Sutherland, *Philos. Trans. R. Soc. B* 2011, **366**, 2870
23 G. Steinman, R. M. Lemmon, M. Calvin, *Proc. Natl. Acad. Sci. USA* 1964, **52**, 27
24 M. W. Powner, B. Gérland, J. D. Sutherland, *Nature* 2009, ***459**, 239*
25 K. Dimroth, R. Ploch, *Chem. Ber.* 1957, **90**, 801
26 W. H. R. Shaw, J. D. Bordeaux, *J. Am. Chem. Soc.* 1955, **77**, 4729
27 Danger, L. Boiteau, H. Cottet, R. Pascal, *J. Am. Chem. Soc* . **1**2006, **128**, 7412
28 B. P. Callahan, Y. Yuan, R. Wolfenden, *J. Am. Chem. Soc.* 2005, **127**, 10828
29 A. N. Albertsen, C.D. Duffy, J.D.; Sutherland, P-A. Monnard, *Astrobiology* 2014, **14**, 462
30 T. M. McCollom, J. S. Seewald, *Earth and Planetary Science Letters*, 2006, **243**, 74
31 J. Eichberg, E. Sherwood, D. E. Epps, J. Oró, *J. Mol. Evol*. 1977, **10**, 221
32 D. E. Epps, E. Sherwood, J. Eichberg, J. Oró, *J. Mol. Evol*. 1978, **11**, 279
33 D. E. Epps, D. W. Nooner, J. Eichberg, J. Oró, *J. Mol. Evol*. 1979, **14**, 235
34 M. Rao, J. Eichberg, J. Oró, *J. Mol. Evol*. 1982, **18**, 196
35 M. Halman, *Archives of Biochemistry and Biophysics* 1968, **128**, 808

View Article Online
DOI: 10.1039/C7OB00708F

36 J. Hulshof, C. Ponnamperuma, *Orig. Life* 1976, **7**, 197
37 J. R. Hawker J. Oró, *J. Mol. Evol*. 1981, **17**, 285
38 M. Jauker, H. Griesser, C. Richert, *Angew. Chem. Int. Ed*. 2015, **54**, 14559
39 M. Jauker, H. Griesser, C. Richert, *Angew. Chem. Int. Ed*. 2015, **54**, 14564
40 M. Gull, M. Zhou, F.M. Fernandez, M. A. Pasek, *J. Mol. Evol*. 2014, **78**, 109
41 M. Gull, *Challenges* 2014, **5**, 193
42 B. Burcar, M. Pasek, M. Gull, B. J. Cafferty, F. Velasco, N. V. Hud, C. Menor-Salván, *Angew. Chem. Int. Ed.* 2016, **55**, 13249
43 H.-J. Kim, Y. Furukawa, T. Kakegawa, A. Bita, R. Scorei, S. A. Benner. *Angew. Chem. Int. Ed.* 2016, **55**, 15816–15820.
44 V. A. Pankratov, A. E. Chesnokova, *Russian Chemical Reviews* 1989, **58**, 879
45 I. Estrela-Lopis, G. Brezesinski, H. Möhwald, *Biophys. J.* 2001, **80**, 749
46 L. D. Do, R. Buchet, S. Pikula, A. Abousalham, S. Mebarek. *Analytical Biochemistry* 2012, **430**, 32
47 F. M. Menger, F. M. Peresypkin, *J. Am. Chem. Soc.* 2001, **123**, 5614
48 F. S. Roodsari, D. Wu, G. S. Pum, J. Hajdu *J. Org. Chem.* 1999, **64**, 7727
49 H. H. J. de Jongh, J. A. Killian, B. de Kruijfft, *Biochemistry*, 1992, **31**, 1636
50 H. H. J. de Jongh, B. de Kruijfft, *Biochim. Biophys. Acta*, 1990, **1029**, 105
51 Monophosphoric acid esters of hydroxyalkylureas and their use in scale prevention By: Krueger, Friedrich; Schmidt, GuenterAssignee: Benckiser, Joh. A., G.m.b.H., Fed. Rep. Ger. Ger. Offen. (1976), 10 pp. CODEN:GWXXBX; DE2453037
52 K. Akashi, H. Miyata, H. Itoh, K. Kinosita, *Biophys J*. 1996, **71**, 3242
53 F. Gibellini, T.K. Smith, *IUBMB Life*, 2010, **62**, 414
54 J. E. Vance, T. Guergana *Biochimica et Biophysica Acta* 2012, **1831**, 543
55 P. Walde, M. Wessicken, U. Rädler, N. Berclaz, K. Condes-Friboes, P-L. Luisi *J. Phys. Chem. B.* 1997, **101**, 7390
56 K. Kurihahra, Y. Okura, M. Matsuo, T. Toyota, K. Suzuki, T. Sugawara, *Nat. Commun.* 2015, DOI: 10.1038/ncommns9352
57 A. Knoll, E. S. Barghoorn, *Science* 1977, **198**, 396.
58 A. P. Nutman, V. C. Bennett, C. R. L. Friend, M. J. Van Kranendonk, A. R. Chivas, A. R. *Nature* 2016 doi:10.1038/nature19355
59 T. P. Kee, D.E. Bryant, B. Herschy, K. E. R. Marriott, N.E. Cosgrove, M.A. Pasek, D.Z. Atlas, C.R. Cousins, *Life* 2015, **3**, 386
60 M. Fiore, P. Strazewski, *Angew. Chem. Int. Ed*. 2016, **55**, 13930
61 B. J. Cafferty, D.M. Fialho, J. Khanam, R. Krishnamurthy, N.V. Hud, *Nat. Commun*. 2016, **7**, 11328
62 K. Kurihahra, Y. Okura, M. Matsuo, T. Toyota, K. Suzuki, T. Sugawara, *Nat. Commun.* 2015, DOI: 10.1038/ncommns9352
63 C. Woese, *Proc. Natl. Acad. Sci. USA* 2002, **99**, 8742
64 M. D. Hardy, J. Yang, J. Selimkhanov, C. M. Cole, L. S. Tsimring, N. K. Devaraj *Proc. Natl. Acad. Sci. USA*. 2015, **112**, 818