Amino Alcohol Phosphorylation

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Formation of Potentially Prebiotic Amphiphiles by Reaction of β-Hydroxy-*n*-alkylamines with Cyclotriphosphate**

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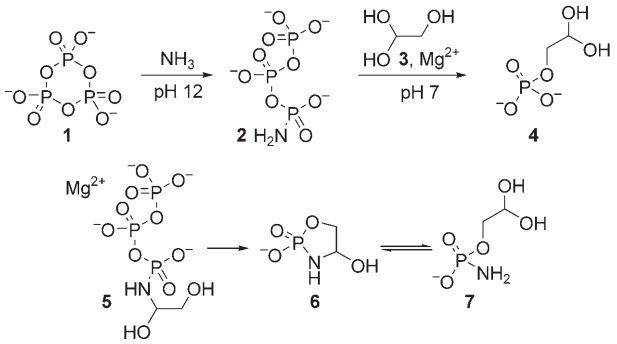
Compartmentalization is thought to be crucial to the development of evolvable genetic systems in the origins of life.[1–3] A wide range of surfactant assemblies can form spontaneously depending on conditions and the type of amphiphile, but only bilayer structures such as vesicles are suitable for genetic compartmentalization. Although double-chain amphiphiles generally form vesicles more readily than single-chain amphiphiles, the latter do form vesicles and other bilayer structures in special cases.[3] A number of studies, for example, have shown that long-chain carboxylic acid products of the Fischer–Tropsch reaction form vesicles at a pH approximately equal to the pK_a of the acid in the bilayer.[4–7] However, the nucleic acid compartmentalization properties of these assemblies are not ideal, and they are unstable to the ionic conditions necessary for RNA folding and catalysis.[8] Although these instability problems can be partly overcome by the addition of glycerol esters,[8] we sought to find alternative prebiotic amphiphiles that might have better vesicle-forming properties.

Because we envisage the formation of double-chain amphiphiles, by a process of constitutional self-assembly, to be more difficult than the formation of single-chain amphiphiles, we first focused on the single-chain species. In particular, we were attracted to a chemical scenario in which cationic amphiphiles undergo partial conversion to anionic amphiphiles since mixtures of oppositely charged single-chain amphiphiles are known to spontaneously form "catanionic" vesicles.[3,9] The presence of phosphate in the lipids of contemporary biochemistry further steered our search for alternative amphiphiles. We recognized that if a singly charged cationic amphiphile could be phosphorylated, then, depending on the chemistry and pH, the product might be anionic due to the ability of a phosphate group to be doubly negatively charged. β-Hydroxy-*n*-alkylammonium salts seemed to us to be suitable cationic amphiphiles in this regard, both with respect to their possible prebiotic availability, and because of a potentially predisposed phosphorylation with cyclotriphosphate.

On the issue of prebiotic availability, β-hydroxy-*n*-alkylamines are the potential reduction products of cyanohydrins, which themselves could arise from the reaction of hydrogen cyanide and alkanals. Medium- and long-chain alkanals are found in the oxygenate fraction of Fischer–Tropsch reaction products.[10]

As regards the phosphorylation of β-hydroxy-*n*-alkylamines, we took our cue from the work of Quimby and Flautt,[11] and Eschenmoser and co-workers (Scheme 1).[12] On treatment with ammonia at pH 12, Quimby and Flautt showed that cyclotriphosphate **1**[13] is converted into amidotriphosphate **2**.[11] Eschenmoser and co-workers showed that at near neutral pH in the presence of Mg^{2+} ions and **2**, glycolaldehyde hydrate **3** undergoes smooth conversion to its monophosphate **4**.[12] The reaction is thought to proceed by reversible formation of the hemiaminal **5**, intramolecular phosphorylation to give the phosphoramidate **6**, and subsequent hydrolysis of **6**, or the open-chain form **7**. A high pH is necessary for the conversion of **1** to **2** in the first stage of this two-step process in order that ammonia is predominantly in its free base form. However, for the second stage a high pH would be deleterious for two reasons. Firstly the Mg^{2+} ions—needed to coordinate to the pyrophosphate moiety of **5** rendering it a good leaving group—would be removed from solution by formation of the insoluble hydroxide salt. Secondly, the hydrolysis of **6**/**7** requires that the nitrogen be



Scheme 1. Two-step process for the phosphorylation of glycolaldehyde hydrate **3** by cyclotriphosphate **1**.[11,12] Initial ammonolysis of **1** to **2** allows the reversible tethering of a phosphorylating agent to **3** giving **5**. Intramolecular phosphorylation of **5** assisted by divalent metal ions followed by hydrolytic removal of the tethering attachment from **6** or **7** then gives the monophosphate product **4**.

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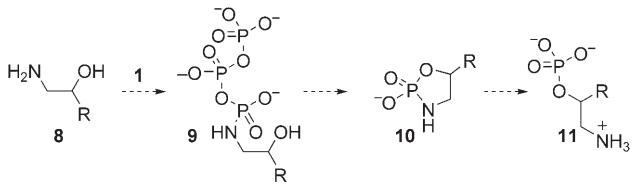
protonated, and related phosphoramidates are only very slowly hydrolyzed at pH > 8.[14]

In the case of long-chain β-hydroxy-*n*-alkylamines **8**, we hoped to be able to demonstrate similar phosphorylation chemistry using **1** (Scheme 2), but in a single reaction at one



Scheme 2. Potential phosphorylation of β-hydroxy-*n*-alkylamines **8** by cyclotriphosphate **1**.

pH, in the absence of Mg^{2+} ions, and with the key steps controlled by chain-length effects. At a pH at which **8** is at least partially available in free base form, reaction with **1** to give an *N*-triphosphate **9** was anticipated.[15] In the absence of effects due to the long alkyl chain, this species was not expected to undergo intramolecular attack of the OH group to give the phosphoramidate **10**, because of the poor leaving-group ability of pyrophosphate when it is unprotonated or not coordinated to a divalent metal ion. However, if the alkyl chain was sufficiently long for **9** to be incorporated into some sort of surfactant assembly, we hoped that the unfavorability of negative charge repulsion between hydrophobically associated molecules of **9** would increase the degree of protonation of the pyrophosphate moiety of **9**, and consequently increase its leaving-group ability. If cyclization to the phosphoramidate were then to occur, we again hoped that incorporation into, or retention in, a surfactant assembly would increase the degree of N-protonation of **10** such that hydrolysis to the *O*-monophosphate **11** would occur.

To test the foregoing predictions we used conventional synthesis[16] to prepare a series of β-hydroxy-*n*-alkylamines **8** ($R = C_2H_5$, C_4H_9, C_6H_{13}, C_8H_{17}) by reduction of the corresponding azidoalcohols which, in turn, were easily accessible from the terminal epoxides (see the Supporting Information). We then studied the reaction of **8** with cyclotriphosphate **1** under a variety of conditions. It was found that reaction with **1** was insignificant at pH < 10, but at pH 10 reaction occurred over a number of days. For the short-chain compounds ($R = C_2H_5$, C_4H_9), **8** was converted to the *N*-triphosphate **9** (Table 1). The structure of **9** followed from ^1H and ^{31}P NMR analysis,[15] in particular the ^1H-coupled ^{31}P NMR spectrum showed a doublet of apparent triplets ($\delta = 0.0$ ppm $J_{P,P} = 19.8$ Hz, $J_{H,P} = 8.5$ Hz, $J_{H,P} = 7.9$ Hz for **9** ($R = C_4H_9$)) for Pα which collapsed to a doublet ($J_{P,P} = 19.8$ Hz) in the ^1H-decoupled spectrum (Figure 1). In the ^1H NMR spectrum, signals for H–C1 and H–C2 of **9** were shifted upfield relative to the corresponding signals for **8** (see Figure S1 in the Supporting Information). For the longest-chain compound ($R = C_8H_{17}$), **8** was converted in moderate yield to a single species which had NMR spectroscopic data consistent with the *O*-monophosphate **11** (Table 1). No *N*-triphosphate **9** was apparent as evidenced by the lack of a signal at $\delta \approx 0$ in the

Table 1: Yields [%] of the *N*-triphosphate **9**, *O*-monophosphate **11**, and residual starting material observed in the reactions of β-hydroxy-*n*-alkylamines **8** with cyclotriphosphate **1** after 6 days.[a]

$RCH(OH)CH_2NH_2$ **8**	**9**	**11**	Residual **8**
$R = C_2H_5$	19	0	81
$R = C_4H_9$	19	0	81
$R = C_6H_{13}$	10	20	70
$R = C_8H_{17}$	0	40 (55[b])	60 (45[b])

[a] A solution (or suspension) of **8** (100 mM) and **1** (250 mM) in D_2O was adjusted to pD 10 and maintained at 26 °C. NMR spectra were recorded periodically (samples were filtered when necessary). The pD was readjusted to 10 daily by addition of NaOD solution. For full experimental details see the Supporting Information. [b] The yield of **11** ($R = C_8H_{17}$) increased significantly after 4 weeks; (overall) yields of the shorter-chain products were unchanged.

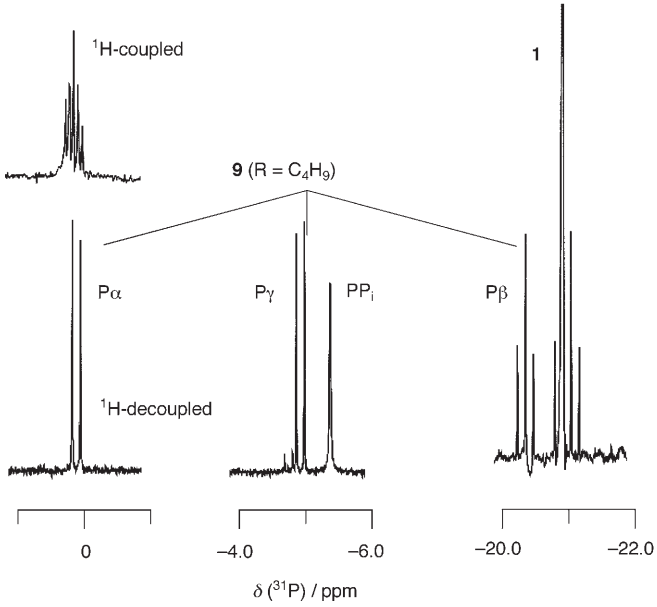


Figure 1. ^{31}P NMR analysis of the product from the reaction of **8** ($R = C_4H_9$) with **1**. Selected regions of the ^1H-decoupled spectrum (bottom) and ^1H-coupled spectrum (top) are shown.

^{31}P NMR spectrum (Figure 2). A key factor in the assignment of the *O*-monophosphate **11** was the observation of a doublet signal in the ^1H-coupled ^{31}P NMR spectrum at $\delta = 4.0$ ($J_{H,P} = 8.5$ Hz) which collapsed to a singlet in the ^1H-decoupled spectrum (Figure 2). To verify this assignment, we synthesized an authentic sample of **11** ($R = C_8H_{17}$) and used it to spike the ^1H NMR sample of the reaction products of **8** ($R = C_8H_{17}$) and **1** (see Figure S2 in the Supporting Information). The spiking experiment confirmed the assignment. The second-longest-chain compound tested **8** ($R = C_6H_{13}$) was converted to a mixture of **9** and **11** (Table 1, see Figures S3 and S4 in the Supporting Information), and over a prolonged period of time, the amount of **11** was seen to increase while the amount of **9** decreased.

The phosphorylation chemistry uncovered in this investigation is noteworthy for a number of reasons. Owing to the long-chain effects, significantly different products form from long-chain and short-chain β-hydroxy-*n*-alkylamines **8**. The

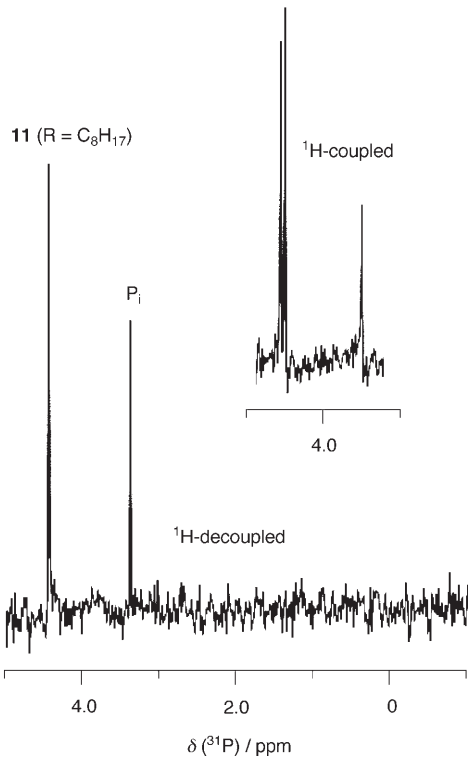


Figure 2. ^{31}P NMR analysis of the product from the reaction of **8** (R = C_8H_{17}) with **1**. Selected region of the ^{1}H-decoupled spectrum (bottom) and ^{1}H-coupled spectrum (top) are shown.

formation of *O*-monophosphates **11** from the long-chain compounds in a single reaction at one pH value and in the absence of divalent metal ions is remarkable[17] especially given the results of Quimby and Flautt,[11] and Eschenmoser and co-workers.[12] Although we do not observe phosphoramidates **10**, it would seem most reasonable to invoke them as intermediates in the conversion of **8** to **11** especially given the results of Eschenmoser and co-workers. It would thus appear that they are rapidly hydrolyzed at pH 10 even though related phosphoramidates are hydrolytically inert at pH > 8.[14] The long-chain effects on the closure of **9** to **10** and the subsequent hydrolysis of **10** to **11** mean that our goal of partially converting a cationic amphiphile to an anionic amphiphile is potentially possible depending on the pK_a values of the various species in surfactant assemblies. Whether the resultant mixtures of **8** and **11** will form bilayer structures—especially catanionic vesicles—remains to be determined. The dynamic nature of the surfactant assemblies formed as the reaction progresses also remains to be investigated. These studies will likely be complex, but with the covalent chemistry established, the elucidation of the noncovalent chemistry now becomes a worthwhile target.

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[1] J. W. Szostak, D. P. Bartel, P. L. Luisi, *Nature* **2001**, *409*, 387.
[2] I. A. Chen, K. Salehi-Ashtiani, J. W. Szostak, *J. Am. Chem. Soc.* **2005**, *127*, 13213.
[3] P. Walde, *Origins Life Evol. Biosphere* **2006**, *36*, 109.
[4] J. M. Gebicki, M. Hicks, *Nature* **1973**, *243*, 232.
[5] P. Walde, R. Wick, M. Fresta, A. Mangone, P. L. Luisi, *J. Am. Chem. Soc.* **1994**, *116*, 11649.
[6] D. W. Deamer, *Nature* **1985**, *317*, 792.
[7] T. M. McCollom, G. Ritter, B. R. T. Simoneit, *Origins Life Evol. Biosphere* **1999**, *29*, 153.
[8] P. A. Monnard, C. L. Apel, A. Kanavarioti, D. W. Deamer, *Astrobiology* **2002**, *2*, 139.
[9] E. W. Kaler, A. K. Murthy, B. E. Rodriguez, J. A. N. Zasadzinski, *Science* **1989**, *245*, 1371.
[10] R. B. Anderson, *The Fischer-Tropsch Synthesis*, Academic Press, Orlando, **1984**.
[11] O. T. Quimby, T. J. Flautt, *Z. Anorg. Allg. Chem.* **1958**, *296*, 220.
[12] R. Krishnamurthy, G. Arrhenius, A. Eschenmoser, *Origins Life Evol. Biosphere* **1999**, *29*, 333.
[13] For discussion of the prebiotic availability of phosphate and polyphosphates including cyclotriphosphate see: A. W. Schwartz, *Philos. Trans. R. Soc. London Ser. B* **2006**, *361*, 1743; Y. Yamagata, H. Watanabe, M. Saitoh, T. Namba, *Nature* **1991**, *352*, 516.
[14] R. A. Lazarus, P. A. Benkovic, S. J. Benkovic, *J. Chem. Soc. Perkin Trans. 2* **1980**, 373.
[15] M. Tsuhako, C. Sueyoshi, T. Miyajima, S. Ohashi, H. Nariai, I. Motooka, *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3091.
[16] M. E. Dyen, D. Swern, *J. Org. Chem.* **1968**, *33*, 379.
[17] In the case of the short-chain compounds, we even sought to convert *N*-triphosphate **9** to *O*-monophosphate **11** by lowering the pD from 10 to 7, but this instead caused reversion of **9** to β-hydroxy-*n*-alkylamine **8**.[15]

