Supplementary Material (ESI) for Chemical Communications

Supplementary information

First Prebiotic Generation of a Ribonucleotide from Adenine, D–Ribose and Trimetaphosphate

Graziano Baccolini, [a]* Carla Boga,[a] and Gabriele Micheletti[a]

[a]* Department of Organic Chemistry 'A. Mangini' ALMA MATER STUDIORUM– Università di Bologna, Viale del Risorgimento 4, 40136 Bologna Italy
Fax: (+)39 051 2093654
E-mail: baccolin@ms.fci.unibo.it

Content

1. General Methods

Starting reagents (adenine, D-ribose, and trisodium trimetaphosphate) are commercially available, as well as adenosine (9-β-D-Ribofuranosyladenine), adenosine-2'-phosphate hemihydrate (2'-Adenylic acid, 2'-AMP), adenosine-3'-monophosphate (3'-Adenylic acid, 3'-AMP), adenosine-5'-monophosphate (5'-AMP), and adenosine-3',5'-cyclic monophosphate (3',5'-AMP), used both as standard reagents for HPLC analyses and as comparison samples to identify the products formed during the reaction. Adenosine 2',3'-cyclic monophosphate (2',3'-cAMP) was obtained by acidification of an aqueous solution of its commercial sodium salt. NMR spectra were recorded on Varian Inova 300, Varian Mercury 400 or Varian Inova 600 MHz instruments. Chemical shifts are referenced to external 3-(trimethylsilyl)propionic acid for ^{1}H and in H_2O, and to external standard 85% H_3PO_4 for ^{31}P NMR. *J* values are given in Hz.

Analytical HPLC analysis was carried out with a Merck-Hitachi Hitachi L-4200 UV-Vis detector on a Supelcosil LC-18-DB 5 μm 250x4.6 mm column at 25 °C. Peaks were determined at 260 nm. HPLC separation was achieved using continuous gradient elution (Merck-Hitachi L-6200 Intelligent Pump). The elution conditions are reported in the caption of the respective chromatogram reported below; a 20 μL of the sample was injected.

Preparative HPLC analysis was carried out with a Waters 600 controller with Waters 2487 dual λ absorbance detector on a Supelcosil LC-18-DB 5 μm 250x21.2 mm column at 25 °C The elution was performed with a isocratic method: 100% H_2O (Waters 600 pump). Flow rate of mobile phase was 17 mL/min; a 430 μL of the sample was applied to the column.

The yields were determined by HPLC using calibration curves made with solutions at exact concentration of commercial AMPs.

2. Reaction between adenine, D–ribose and trisodium trimetaphosphate

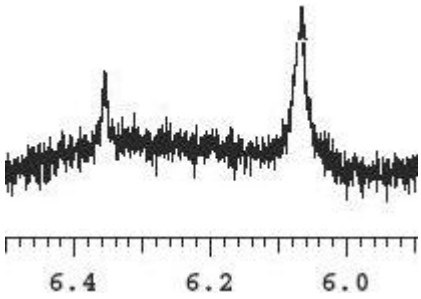
Case A: *Reactions in highly concentrated aqueous solution*

Trisodium salt of TMP (459 mg, 1.5 mmol), ribose (150 mg, 1.0 mmol) and adenine (135 mg, 1.0 mmol), were added in 10 mL of water in a 1.5:1:1 relative molar ratio (pH ~ 7.0). At this concentration value adenine, being much less soluble with respect to the two other reagents, remains partially undissolved. The initial pH (pH ~ 7.0) was that obtained after introduction of the three components in water. Variations of pH until the final value of 6.5 are due to the very slow hydrolysis of trimetaphosphate and at the course the reaction with formation of phosphoric acid which forms a salt with the remaining adenine. However, we did not restore the neutral pH. The

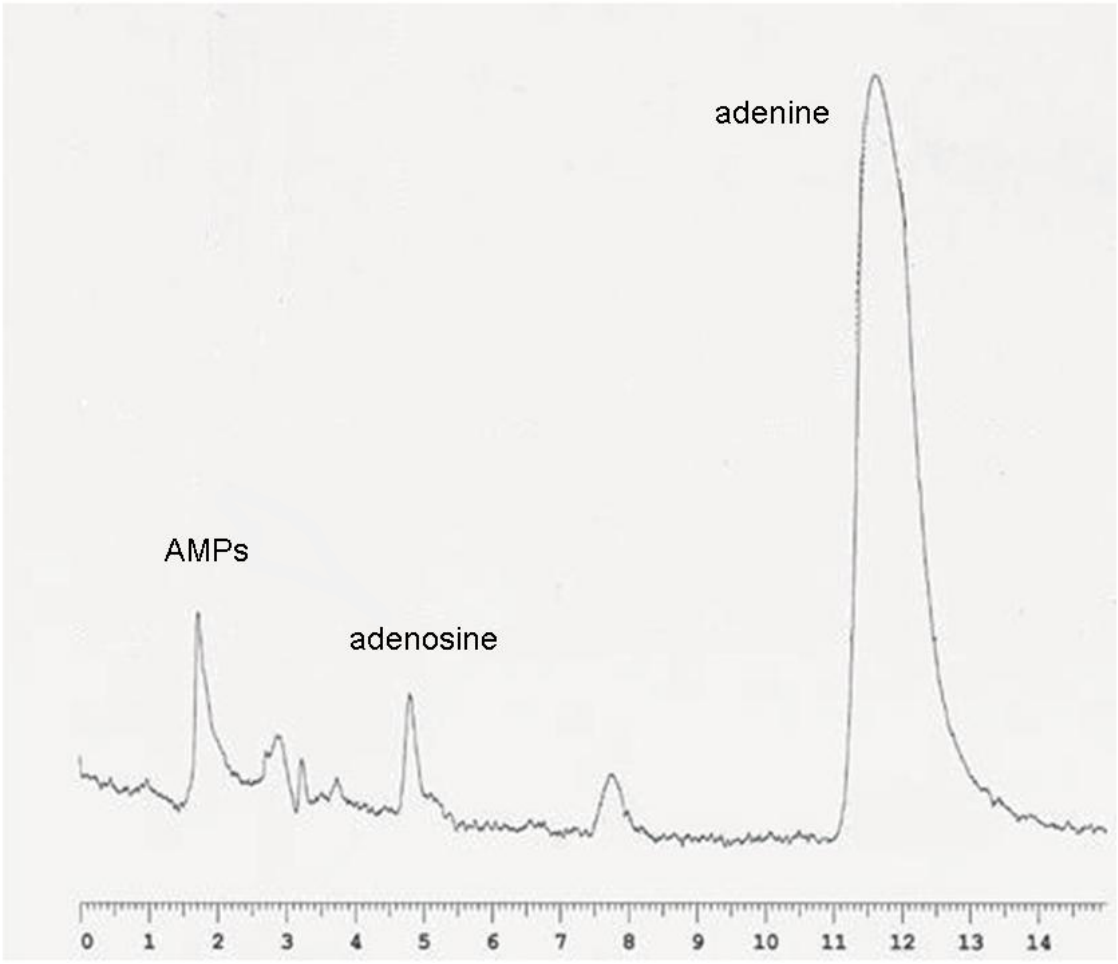
reaction course was monitored for 60 days through HPLC, ^{31}P NMR, and TLC. Yield in adenosine monophosphates: 10%.

Case B: ***Reactions in highly diluted aqueous solution***

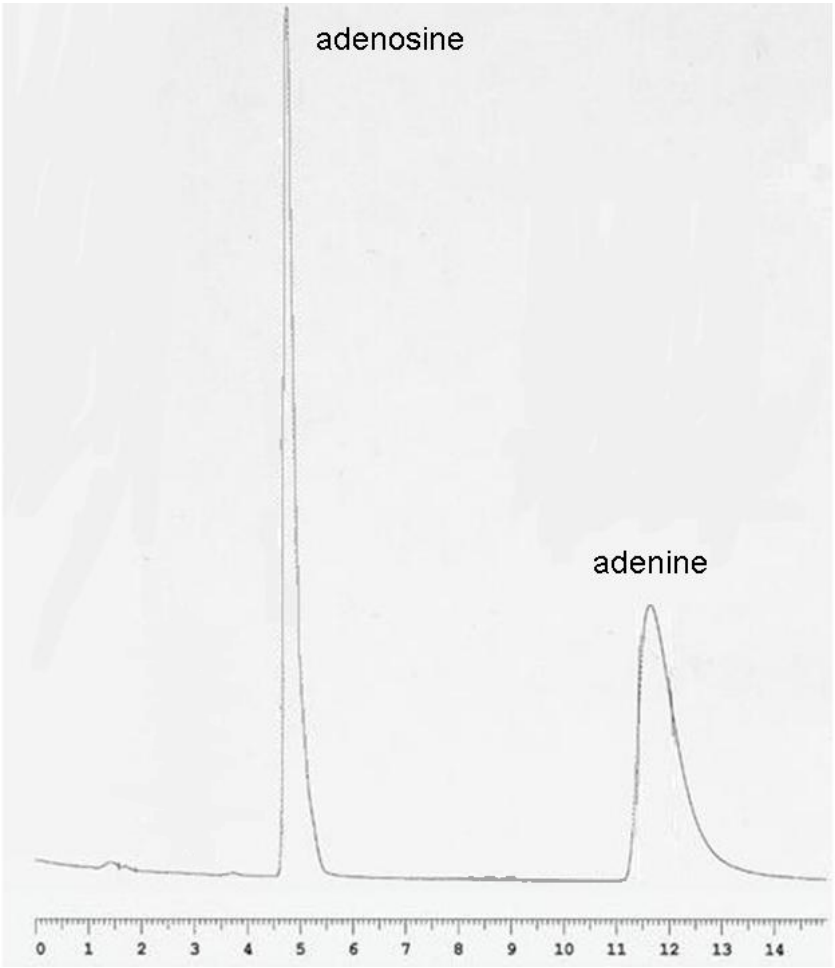
The reaction was carried out in water solution (pH ~ 7.0÷6.5) with high dilution of the three reagents (1.85×10^{-4} M). Adenine (50 mg, 0.37 mmol), D-ribose (55 mg, 0.37 mmol), and trisodium salt of TMP (168 mg, 0.55 mmol) were dissolved in 2 L of aseptic water, in a 1:1:1.5 relative molar ratio. Also in this case we did not adjust the final pH value (6.5). At this concentration value all the reagents were completely dissolved. The reaction course was followed by HPLC for 60 days and, after concentration, also by TLC and ^{31}P NMR spectroscopy. The final reaction mixture was separated by preparative HPLC column We have obtained pure 2'-AMP, 3'-AMP, and 2',3'-cAMP, which were analyzed by NMR spectroscopy. Yield in adenosine monophosphates: 35%.



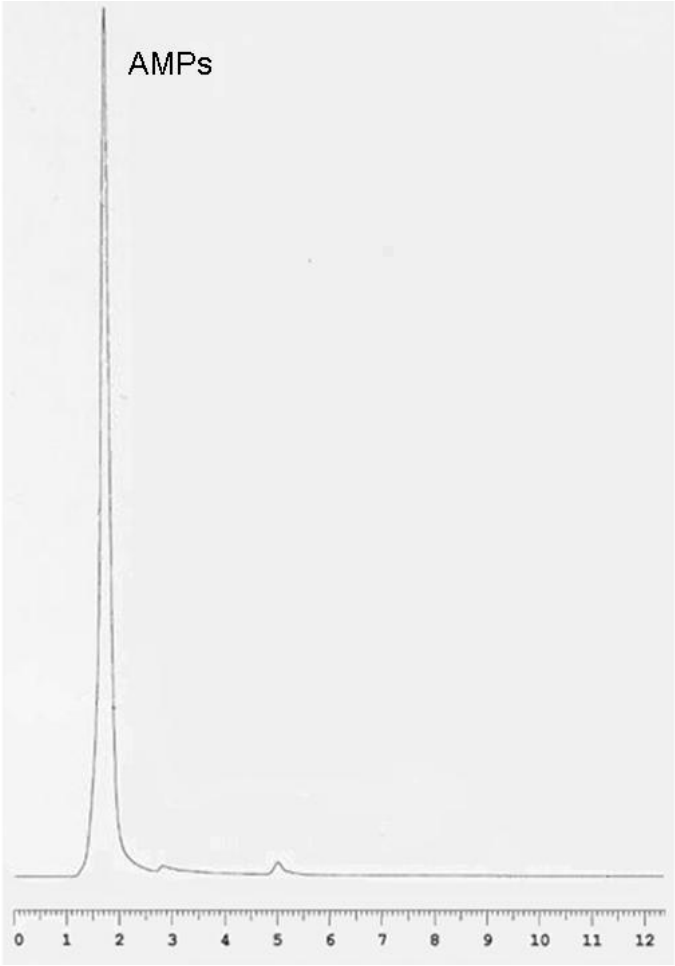
Expanded view (between 6.5 ppm and 5.9 ppm) of ^{1}H NMR spectrum in DMSO–d_6 of the reaction mixture (case B**)** after 5 days. The spectrum shows presence of two signals, corresponding to the anomeric proton of α–adenosine (δ = 6.35 ppm) and of β–adenosine (δ = 6.07 ppm) in about 1/6 relative molar ratio. The assignment of these signals was made by comparison with literature data[1].



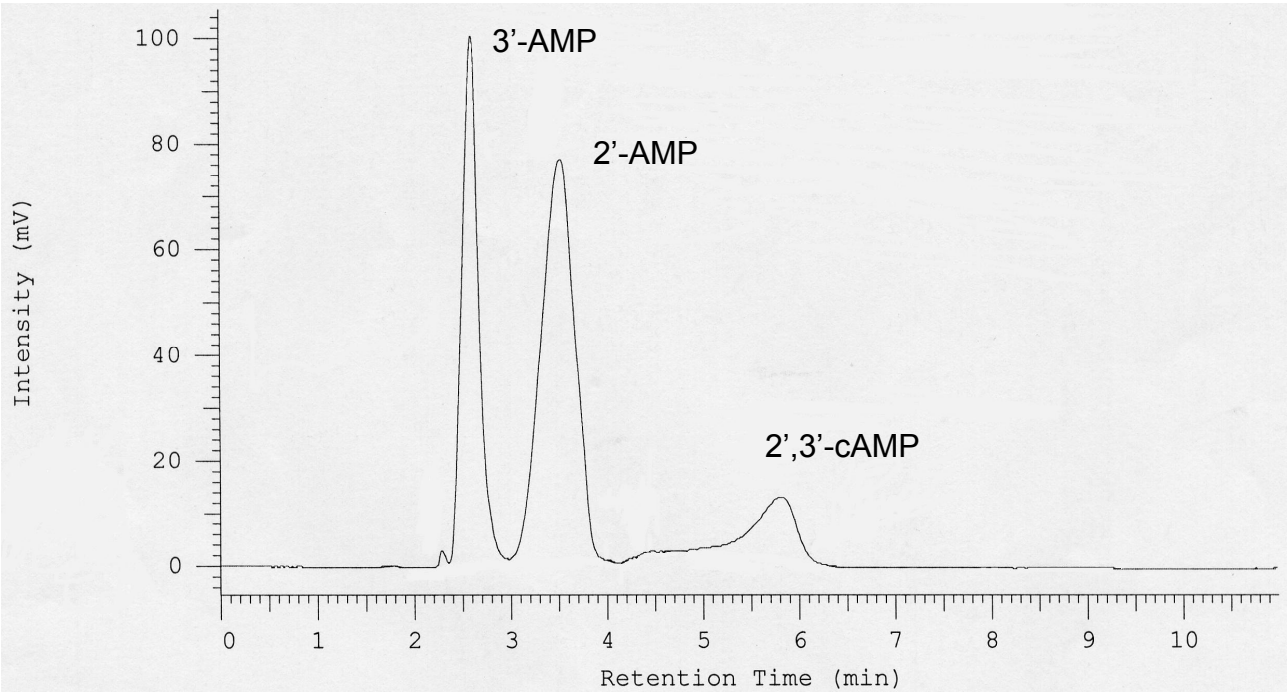
HPLC chromatogram of the reaction mixture (case B**)** after 5 days. HPLC conditions: CH_3CN 20% / H_2O 80%, flow rate: 1 mL/min. The assignment of the signals was made by comparison with commercial compounds, whose chromatograms are reported below.



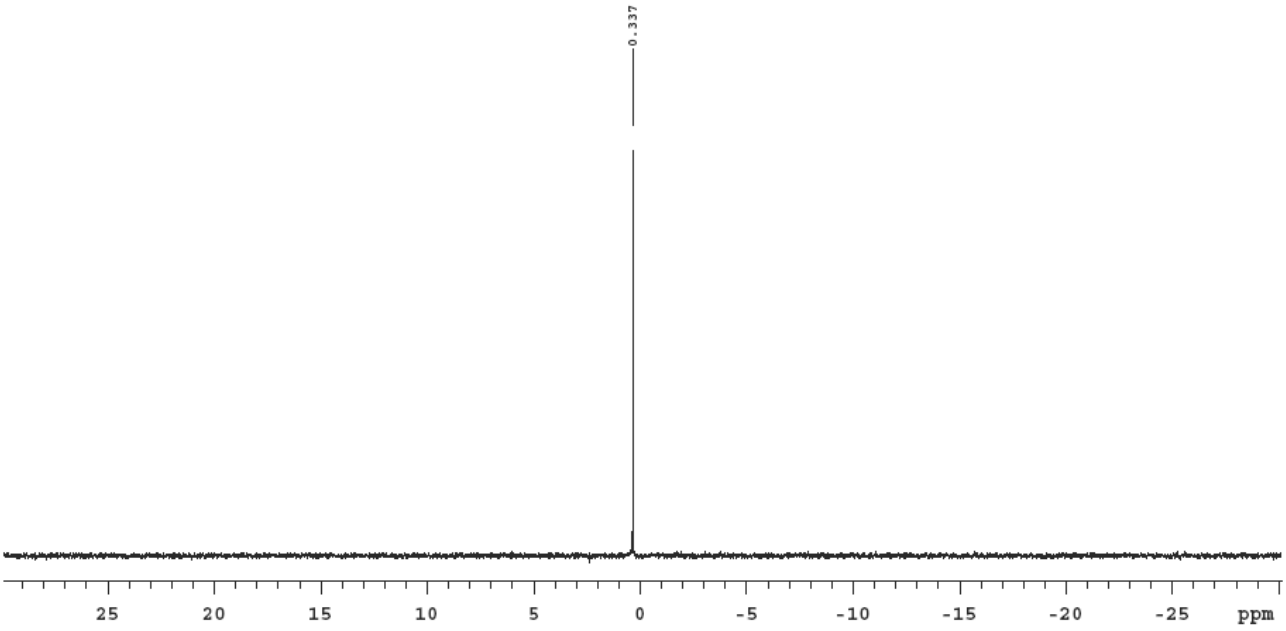
HPLC chromatogram of a mixture of commercial β–adenosine and adenine. HPLC conditions: CH_3CN 20% / H_2O 80%, flow rate: 1 mL/min.



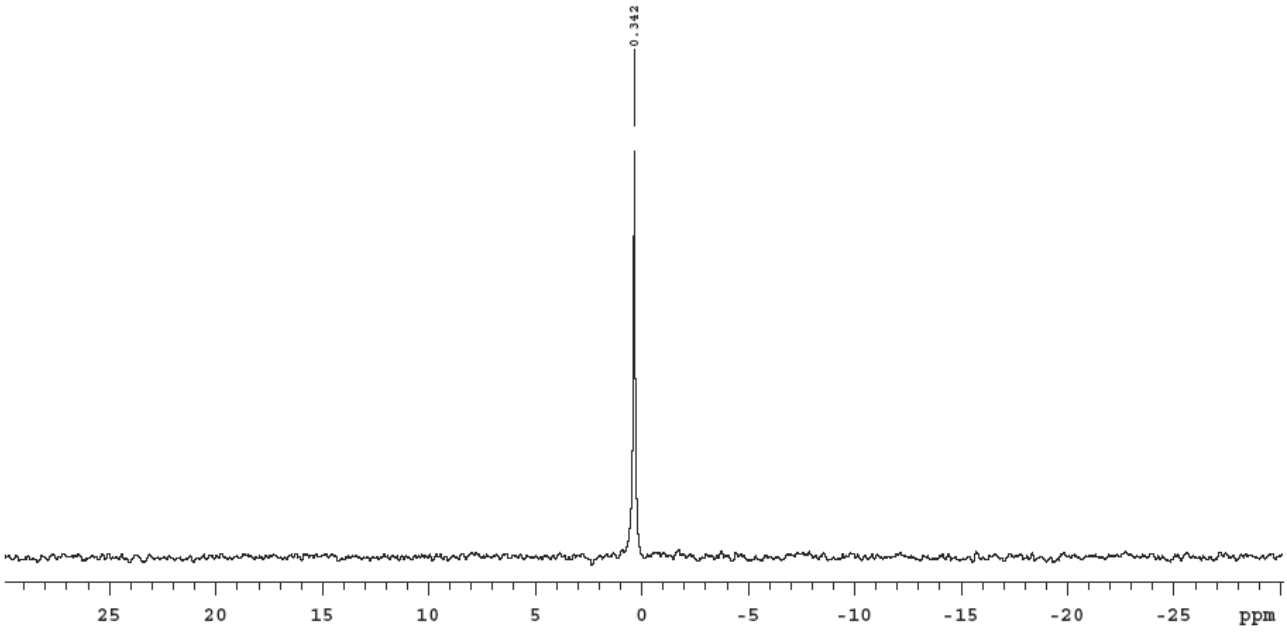
HPLC chromatogram of a mixture of commercial 2'-AMP, 3'-AMP, and 2',3'-cAMP. HPLC conditions: CH_3CN 20% / H_2O 80% flow 1 mL/min.



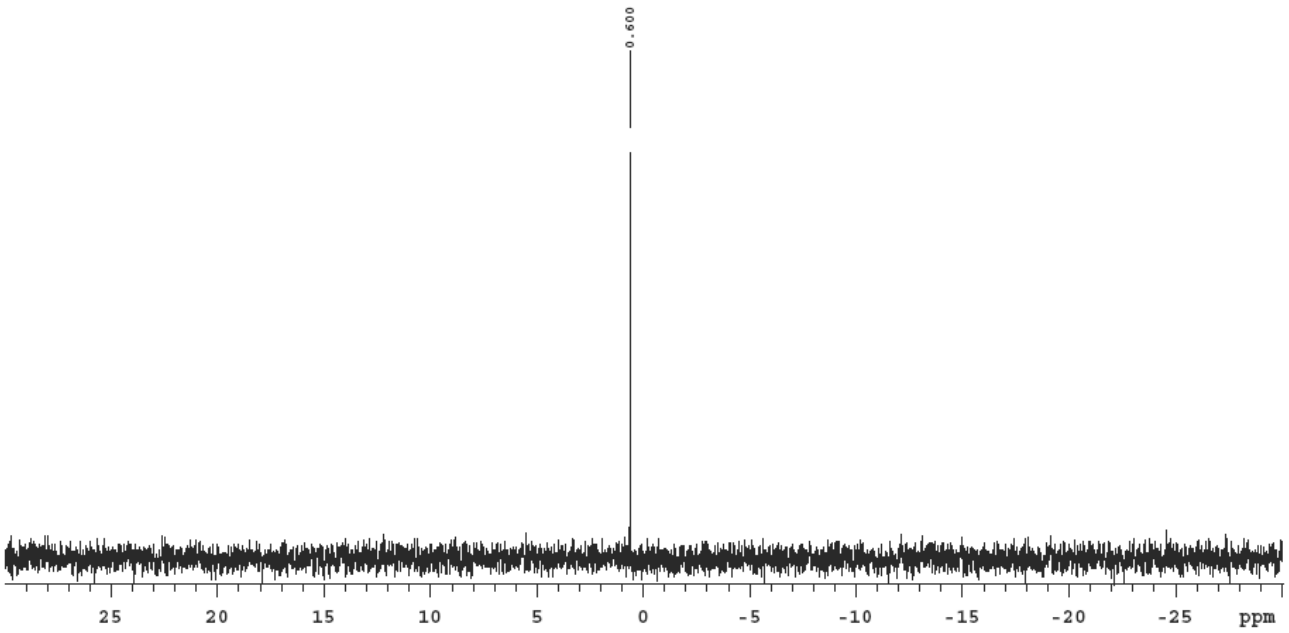
HPLC chromatogram of the reaction mixture prior of its separation by preparative HPLC. Preparative HPLC conditions: 100%H_2O, flow rate: 0.8 mL/min.



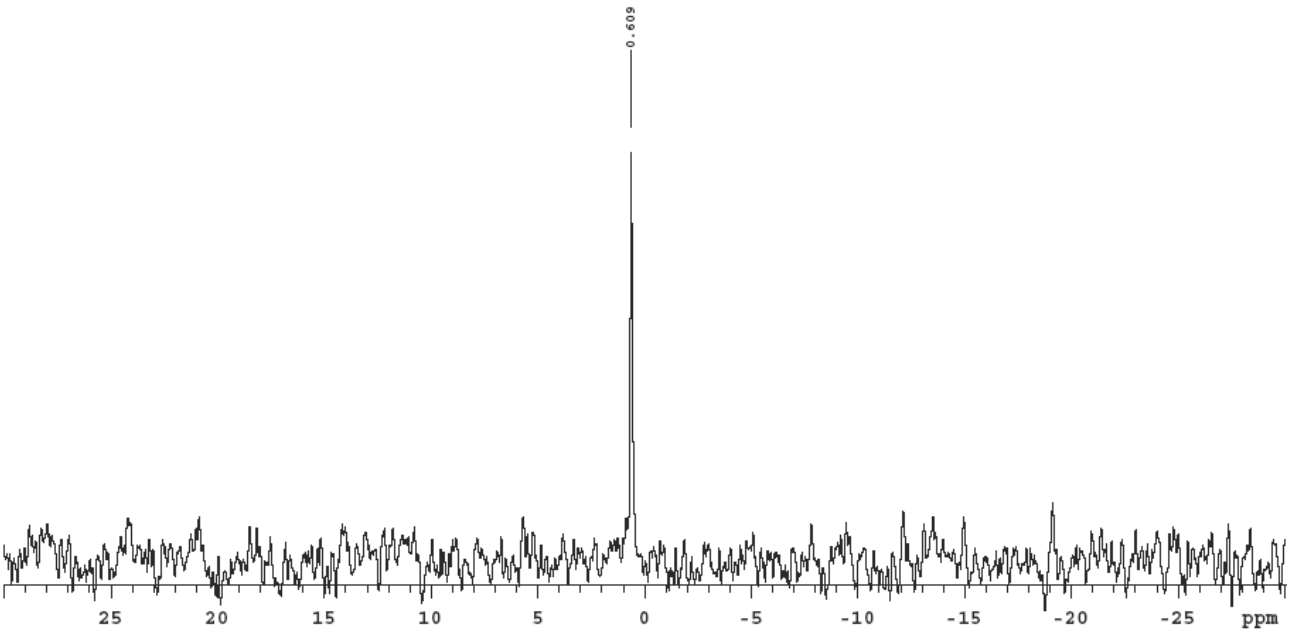
^{31}P NMR spectrum (D_2O) of commercial 2'–AMP.



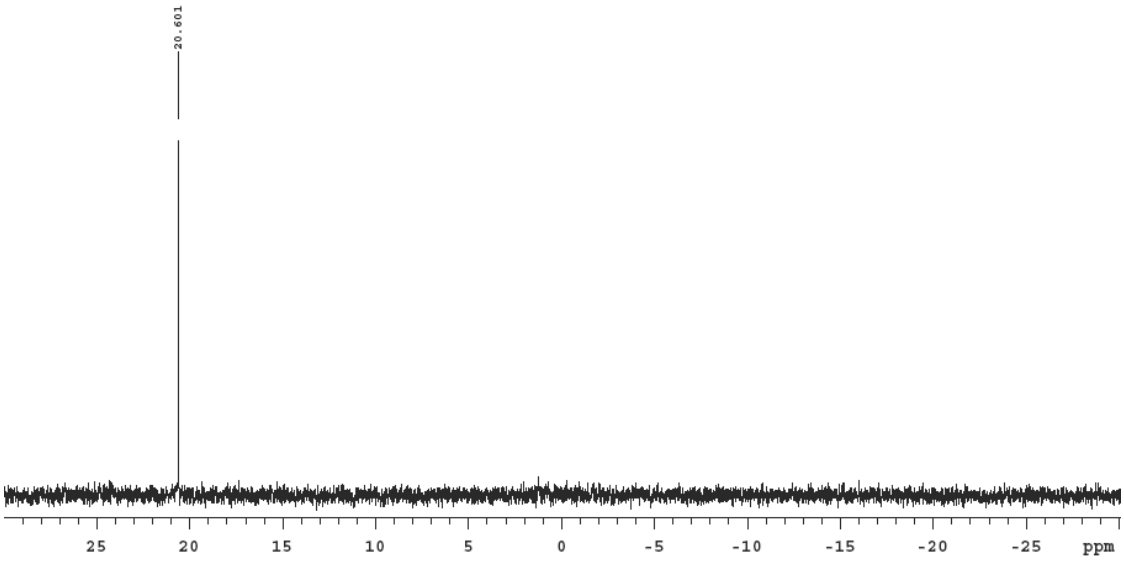
^{31}P NMR spectrum (D_2O) of 2'–AMP separated from the reaction mixture by preparative HPLC.



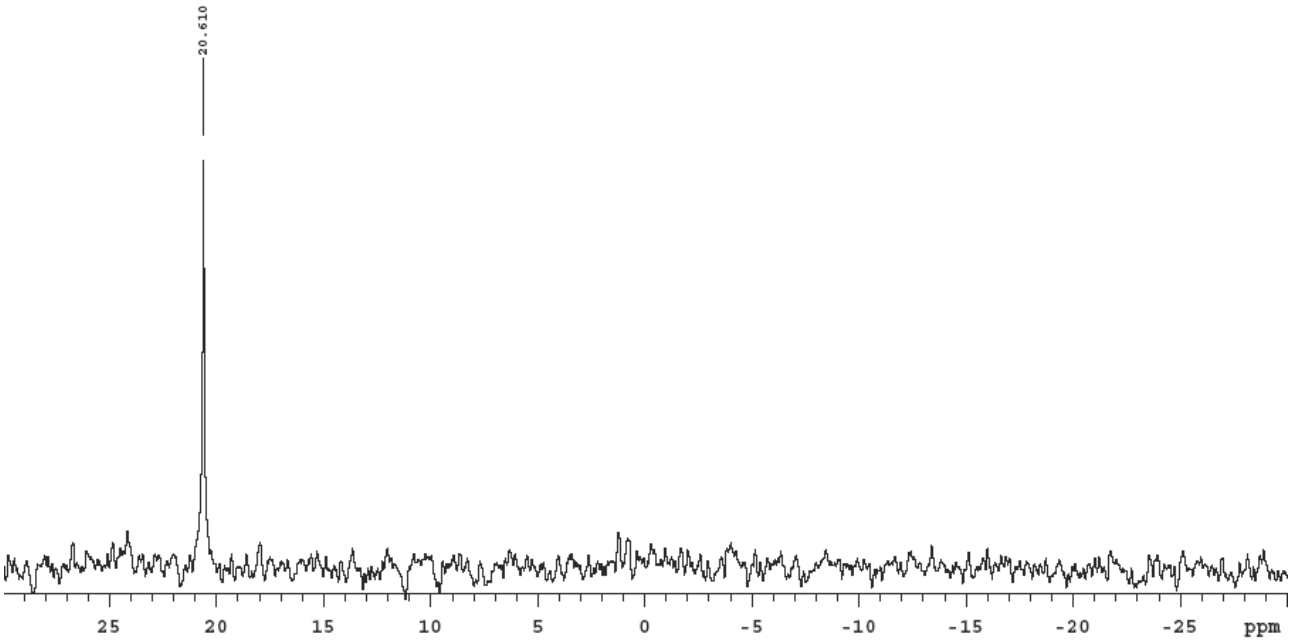
^{31}P NMR spectrum (D_2O) of commercial 3'–AMP.



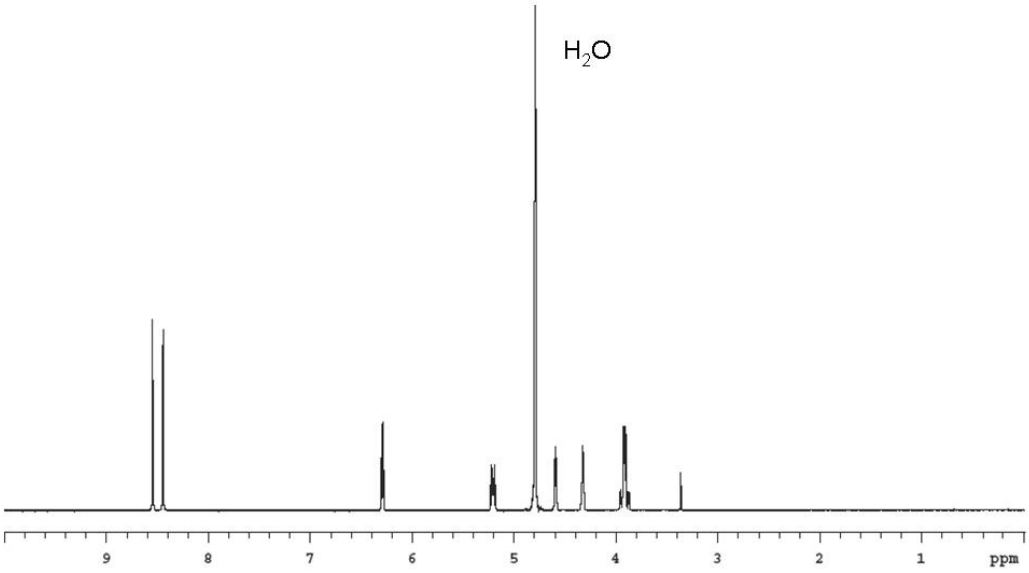
^{31}P NMR spectrum (D_2O) of 3'–AMP separated from the reaction mixture by preparative HPLC.



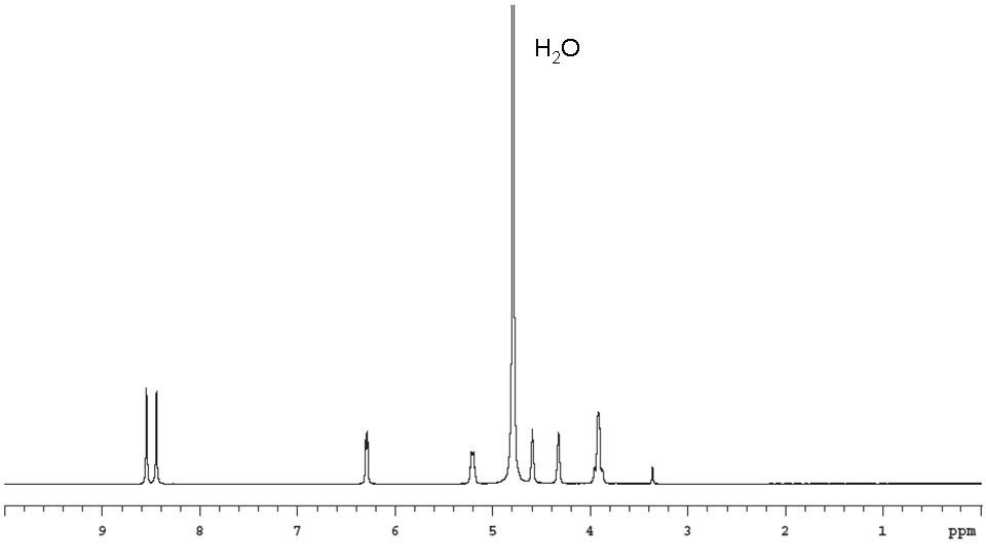
^{31}P NMR spectrum (D_2O) of commercial 2',3'–cAMP.



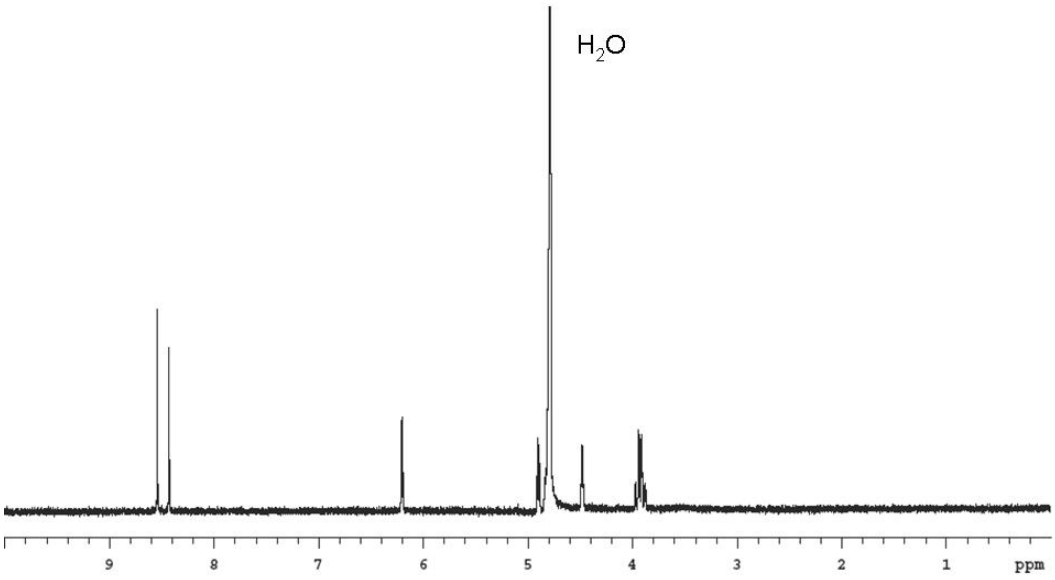
^{31}P NMR spectrum (D_2O) of 2',3'–cAMP separated from the reaction mixture by preparative HPLC.



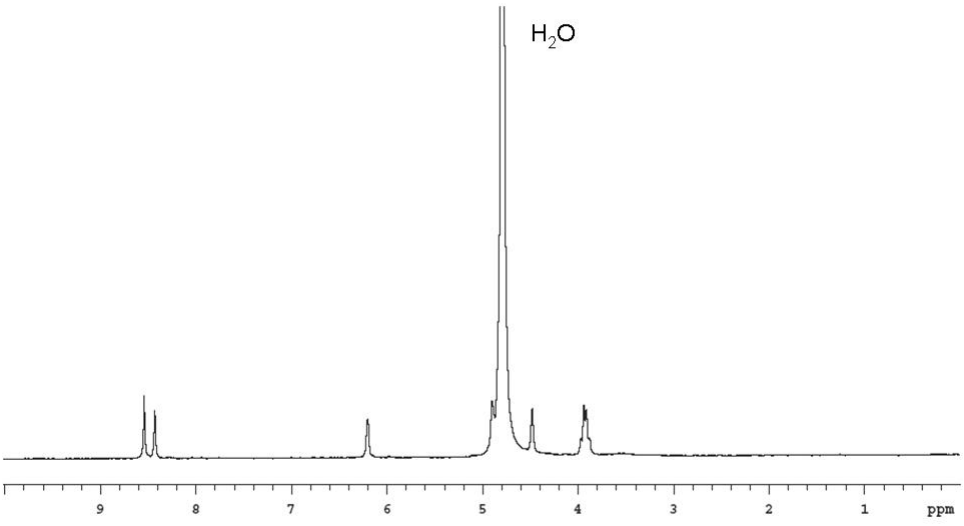
^{1}H NMR spectrum (D_2O) of commercial 2'–AMP.



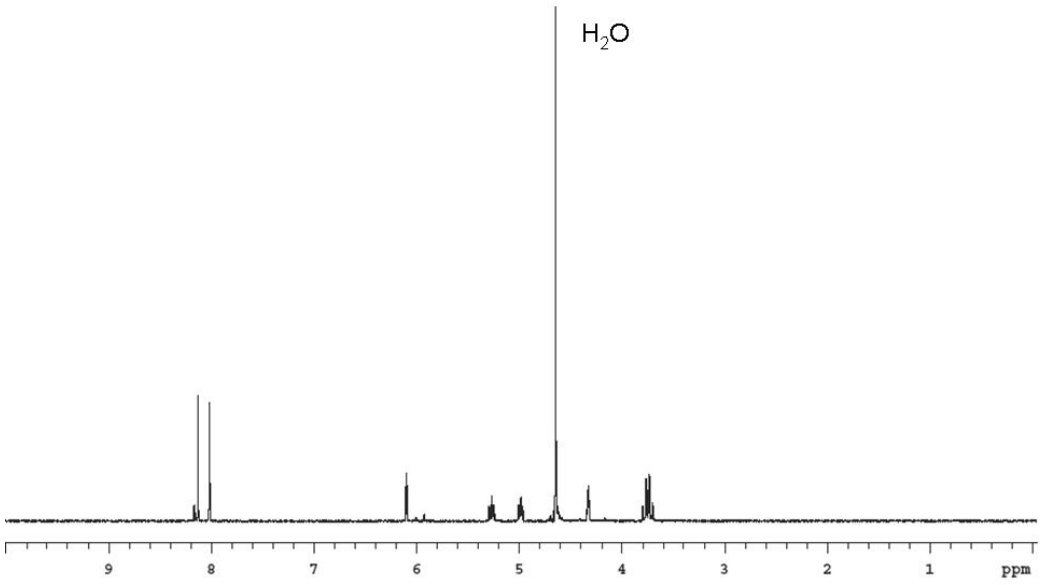
^{1}H NMR spectrum (D_2O) of 2'–AMP separated from the reaction mixture by preparative HPLC.



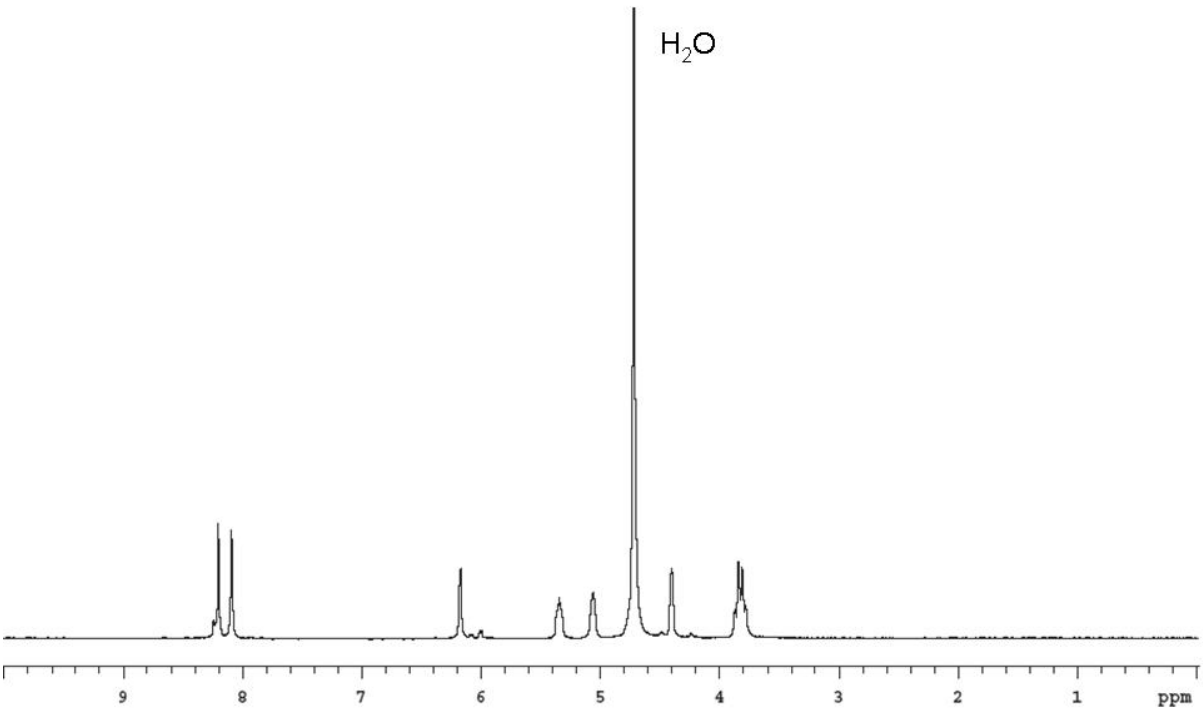
^{1}H NMR spectrum (D_2O) of commercial 3'–AMP.



^{1}H NMR spectrum (D_2O) of 3'–AMP separated from the reaction mixture by preparative HPLC.



^{1}H NMR spectrum (D_2O) of commercial 2',3'–cAMP.



^{1}H NMR spectrum (D_2O) of 2',3'–cAMP separated from the reaction mixture by preparative HPLC.

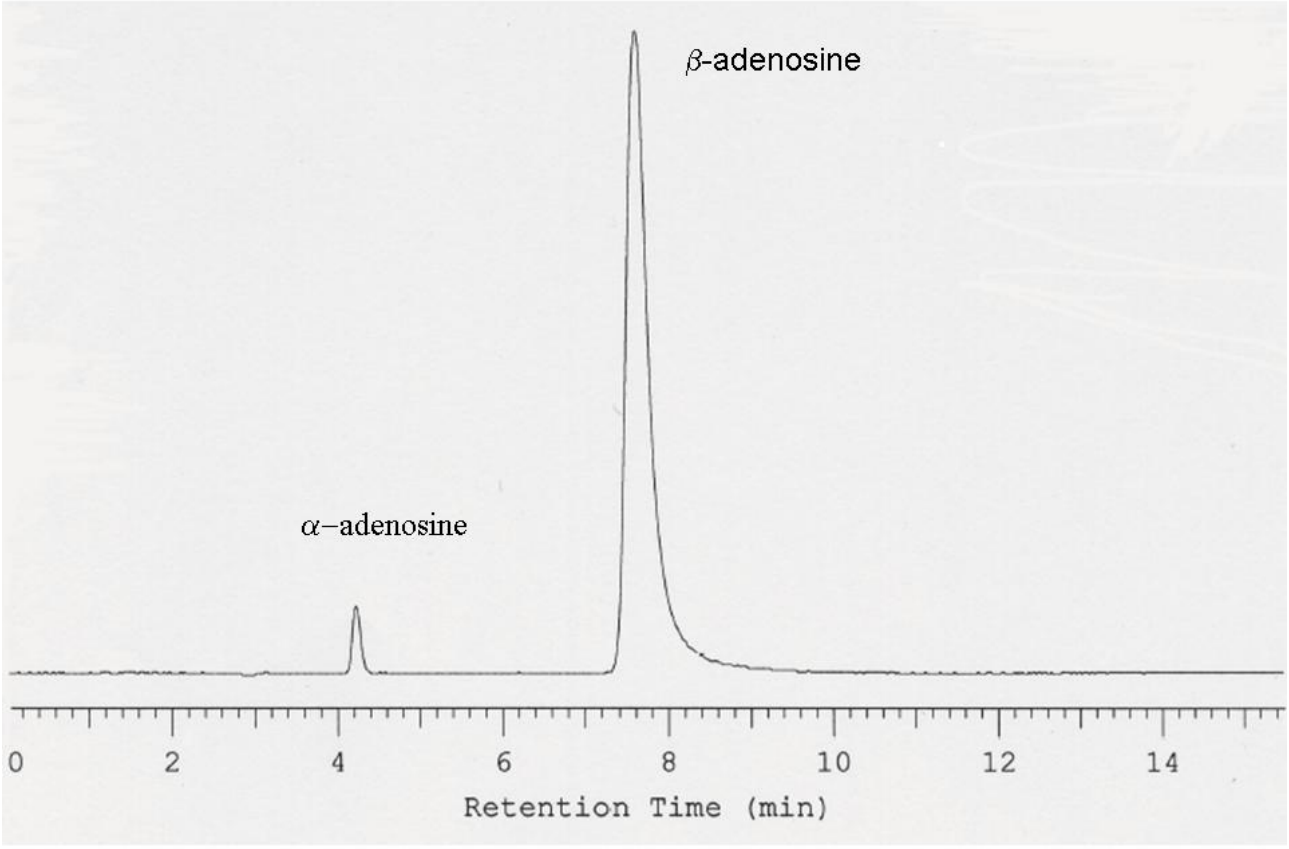
Case C: ***Reactions in highly diluted aqueous solution with an excess of adenine.***

The reaction was carried out in the same conditions of those described above (case B) but with an excess of adenine (60 mg, 0.44 mmol) (adenine/D-ribose/trisodium trimetaphosphate: 1.2/1/1.5 relative molar ratio). The reaction was monitored by HPLC and NMR spectroscopy. Yield in adenosine monophosphates: 43%.

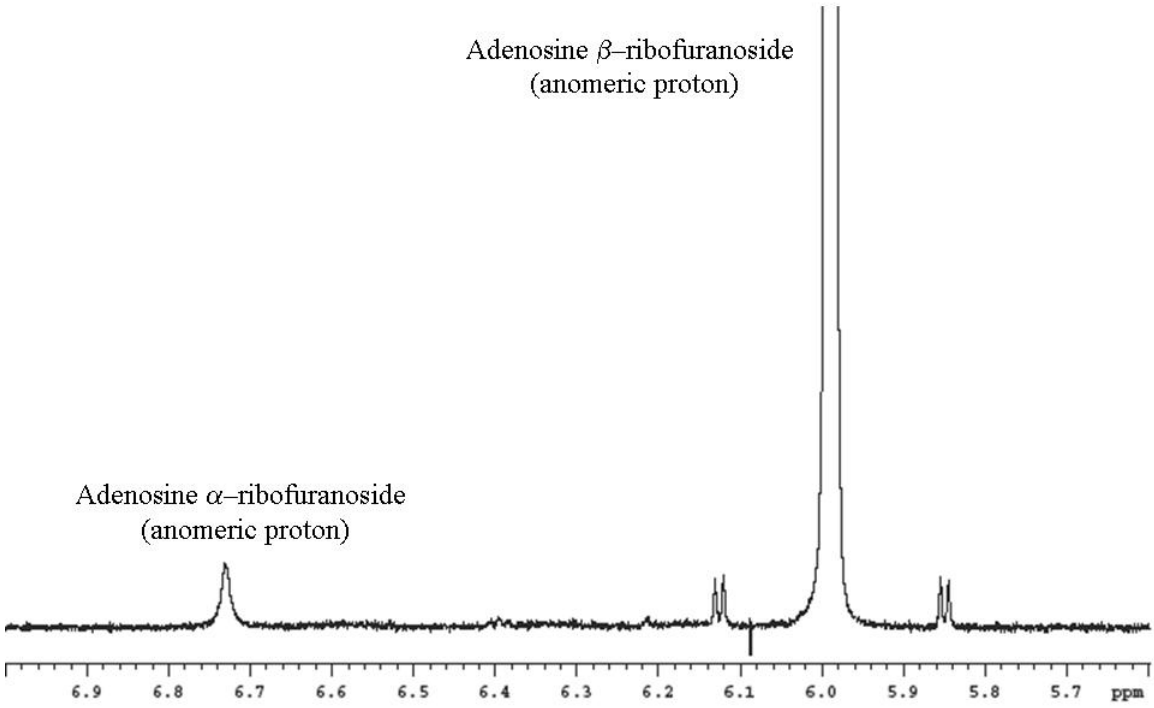
3. Isomerisation of commercial β–adenosine

Commercial adenosine (adenosine β-ribofuranoside, 10 mg, 0.037 mmol) was dissolved in D_2O (1.0 mL) at room temperature directly in a NMR spectroscopy tube. The reaction was monitored both through ^{1}H NMR spectroscopy and HPLC and, after about 30–35 days, we noted a partial isomerisation to adenosine α-ribofuranoside. Below we report the HPLC chromatogram and ^{1}H NMR spectrum of this reaction mixture.

It should be noted that in literature was reported[1] the isomerisation of adenosine β–ribofuranoside, but in that case the reaction was carried out at 150 °C obtaining all the four isomers (*i.e.* adenosine α-ribofuranoside, adenosine β-ribofuranoside, adenosine α-ribopyranoside and adenosine β-ribopyranoside) identified by ^{1}H NMR spectroscopy.



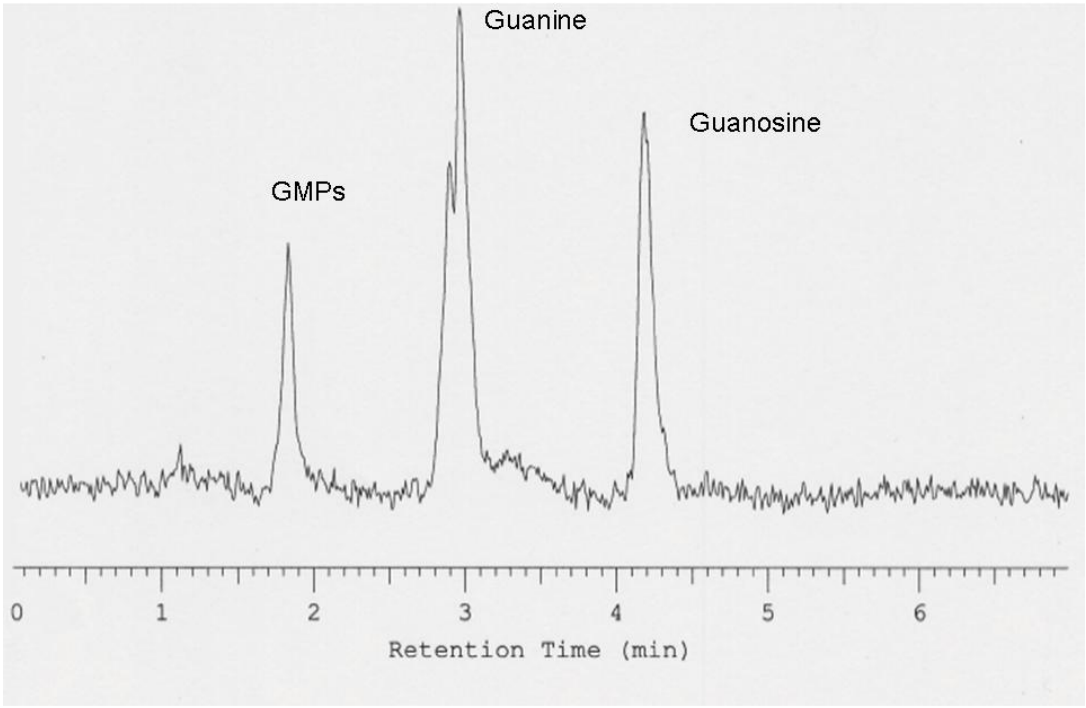
HPLC chromatogram showing the isomerisation of commercial β-adenosine in D_2O after 35 days. HPLC conditions: CH_3OH 20% / H_2O 80% flow rate: 1 mL/min.



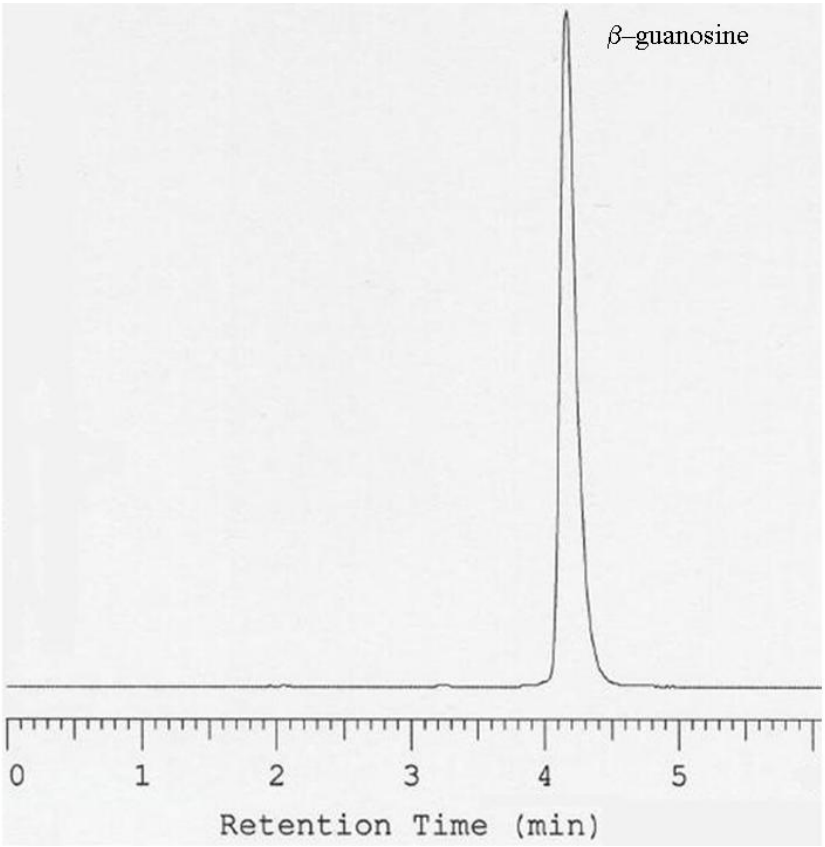
Expanded view of ^{1}H NMR spectrum of commercial β-adenosine showing the isomerisation of commercial β-adenosine in D_2O after 35 days.

4. Reaction between guanine, D–ribose and trisodium trimetaphosphate

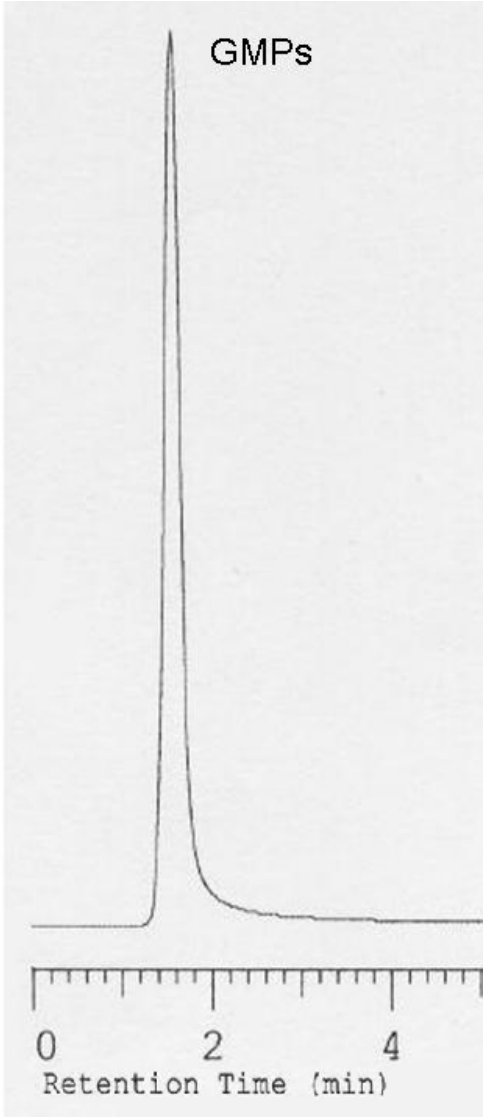
Preliminary results obtained carrying out the reaction between guanine (7 mg, 0.046 mmol), D–ribose (7 mg, 0.046 mmol), trisodium trimetaphosphate (14 mg, 0.046 mmol) in water (250 mL) and monitoring the reaction by HPLC (CH_3OH 20% / H_2O 80%, flow rate: 1 mL/min, 260 nm) showed, after 11 days, the presence of peaks of guanosine and of guanosine monophosphates (GMPs) as reported below in the HPLC chromatogram. In these conditions the peaks of GMPs are not separated but the observed peak corresponds to that obtained carrying out again a reaction, as described in literature,[2] between commercial β–guanosine (85 mg, 0.3 mmol) and trimetaphosphate (918 mg, 3 mmol) in aqueous NaOH (3 mL 1N, 3 mmol) in which the authors indicate formation of the guanosine monophosphates (2'–GMP and 3'–GMP). Guanosine was identify by comparison with authentic commercial sample of β–guanosine. The peak of GMPs was attributed by comparison with the retention time of commercial 5'-GMP, the only guanosine phosphate easily available and that of GMPs products derived from the phosphorylation[2] of commercial β–guanosine.



HPLC chromatogram of the reaction mixture between guanine, D–ribose, and trisodium trimetaphosphate after 11 days. HPLC conditions: CH_3OH 20% / H_2O 80% flow rate: 1 mL/min.



HPLC chromatogram of a commercial sample of β–guanosine. HPLC conditions: CH_3OH 20% / H_2O 80% flow rate: 1 mL/min.



HPLC chromatogram of the reaction between commercial β–guanosine and trisodium trimetaphosphate. HPLC conditions: CH_3OH 20% / H_2O 80% flow rate: 1 mL/min.

5. References

1 R. B. Stockbridge, G. K. Schroeder, R. Wolfenden, *Bioorg. Chem.* 2010, **38**, 224–228.

2 R. Saffhill, *J. Org. Chem.* 1970, **35**, 2881–2883.