Integrated Synthesis of Nucleotide and Nucleosides Directed by Amino Acids

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1. Materials and Methods

Reagents

All solvents used were HPLC grade, 99.9% (VWR) and used as supplied. Glycine, arginine, glutamic acid, threonine, methionine, phenylalanine, tryptophan, D-ribose 5′-phosphate disodium salt hydrate, adenosine 5′-triphosphate disodium salt hydrate, D-(−)-ribose, adenosine 5′-monophosphate disodium salt, adenosine, guanine and thymine were purchased from Sigma Aldrich. Cytosine was purchased from Tokyo Chemical Industry UK Ltd.

2. Synthesis protocols and apparatus

2.1. Protocol

A typical synthesis experiment involves:

1. 0.1 M solutions were prepared using HPLC grade water for all starting materials. However, in the case of thymine and guanine, 1 M NaOH had to be added to dissolve the solid. When making adenine and cytosine solutions, 1 M HCl was added.

2. In a 7 mL glass reaction vessel the below reagents were added: 1000 µL of 0.1 M glycine, 1000 µL of 0.1 M 5′-phosphate D-ribose and 1000 µL of 0.1 M adenine. The pH was adjusted to the desired value using acid (HCl) or base (NaOH) and finally the total volume was taken to 4 mL using the corresponding amount of HPLC water.

3. Then, the hot plate (equipped with Drysyn hotplate inserts) was pre-heated at 90° C.

4. Glass reaction vessels were placed in the corresponding Drysyn hotplate inserts. Lids with three integrated holes were placed on each vial which facilitated the evaporation during the drying step (See Figure S1).

Figure S1. *Experimental set-up and detailed picture of vial lid.*

5. The vials were kept at 90° C for a given time, in order to evaporate the solution to complete dryness. Once a cycle was finished, the vials were taken out of the heating plate; otherwise 4 mL of HPLC water was added (depending if the reaction was run for 1 cycle or more).

6. Once finished, products were collected for analysis by adding 8 mL of HPLC water to the reaction vial. Then, 1.5 mL sample was filtered using nylon syringe filters (cut off=0.22 µm). 500 µl of the extracted sample were mixed with 500 µl of HPLC water for RP-HPLC-MS analysis.

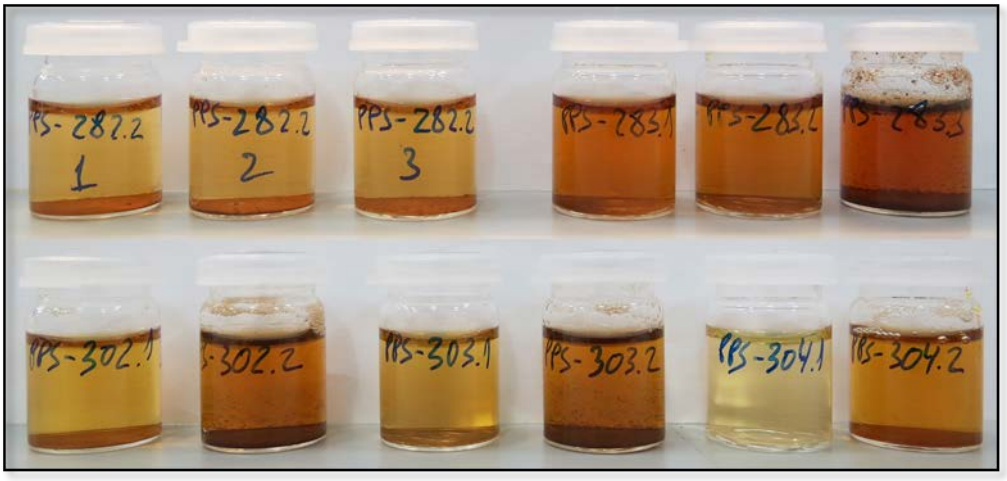


Figure S2. *Typical samples after reaction and water addition.*

2.2. Apparatus

Two contact hotplates (both IKA brand) fitted with DrySyn heat-transfer blocks were used in parallel to perform an array of experiments. Both heating plates had a feedback temperature controller inserted into the DrySyn block, which allows for more precise and stable temperature control over the reaction.

Reversed-phase HPLC-MS. Reversed-phase-HPLC-MS was performed with an Agilent 1200 series instrument fitted with an Agilent Poroshell 120 EC-C18 (4.6 x 50 mm, 2.7 μm) column. Samples were injected in 10 μL aliquots and eluted with a linear gradient mixture of solvents A (water w/ 0.1% v/v formic acid) and B (100% acetonitrile w/ 0.1% v/v formic acid) over 21 mins as follows: 0 min – 100% A; 3 min – 100% A; 13 min – 100% B; 15 min – 100% B; 18 min – 100% A. The column was maintained at 30° C. The MS apparatus was a Bruker MaXis Impact instrument, calibrated for the 50 – 1200 Da range using sodium formate solution. The eluent stream was introduced directly into the source (no splitting) following the DAD detector, at a dry gas temperature of 200° C. The ion polarity for all MS scans recorded was positive, with the voltage of the capillary tip set at 4800 V, end plate offset at −500 V, funnel 1 RF at 400 Vpp and funnel 2 RF at 400 Vpp, hexapole RF at 100 Vpp, ion energy 5.0 eV, collision energy at 5 eV, collision cell RF at 200 Vpp, transfer time at 100.0 μs, and the pre-pulse storage time at 1.0 μs. In MS/MS experiments CID energies were optimised according to products (typically between 20 and 30 eV).

General MS control and data processing. All data were acquired using the Bruker MaXis Impact instrument, controlled by the Compass software suite; where chromatography analysis was added, this process was controlled by accompanying Bruker Hystar software (running Agilent ICF for instrument interface). Where extracted ion chromatograms are presented, they were extracted from the raw data using Compass Data Analysis.

Generation of BPC/vBPC comparison data. Observed base peak chromatogram (BPC) data for RP-HPLC-MS runs was compared with "virtual" BPC (vBPC) traces, constructed from a combination of all the observed extracted ion chromatograms (EIC) corresponding to the members of a list of possible oligomer products (*vide infra*). This was accomplished in the following steps (all operations in R using a custom script): (a) Acquisition of RP-HPLC-MS data for a set of glycosylation products. (b) Conversion of the raw data files from Bruker proprietary format to the .mzXML format, using the MSConvert software. (c) Extraction of BPC data for the RP-HPLC run using the xcms library, running under R. (d) Generation of a combinatorial list of all the oligomeric products of the condensation reaction between adenine and glycine molecules, glycine polymers and/or purination products. (e) Extraction of EIC data for each member of the mass list using xcms under R. (f) Generation of a csv document based on the highest intensity observed in the EIC of any member of the mass list.

3. Comparing methods for MS data analysis

MS data showed in the SI was analysed using a script, which automatically extracts the maximum intensity observed on the correspondent extracted ion chromatogram (EIC) (see "generation of BPC/vBPC comparison data" in section 2.2), instead of analysing the MS data using the integration of the area below the EICs.

In order to certify that the data extracted using both methods shows the same trend, results from several reactions were treated with both methods. As a result, the same trend was observed (see Figures S3 and S4), so automated extraction of maximum EIC intensities was used for the rest of the data.

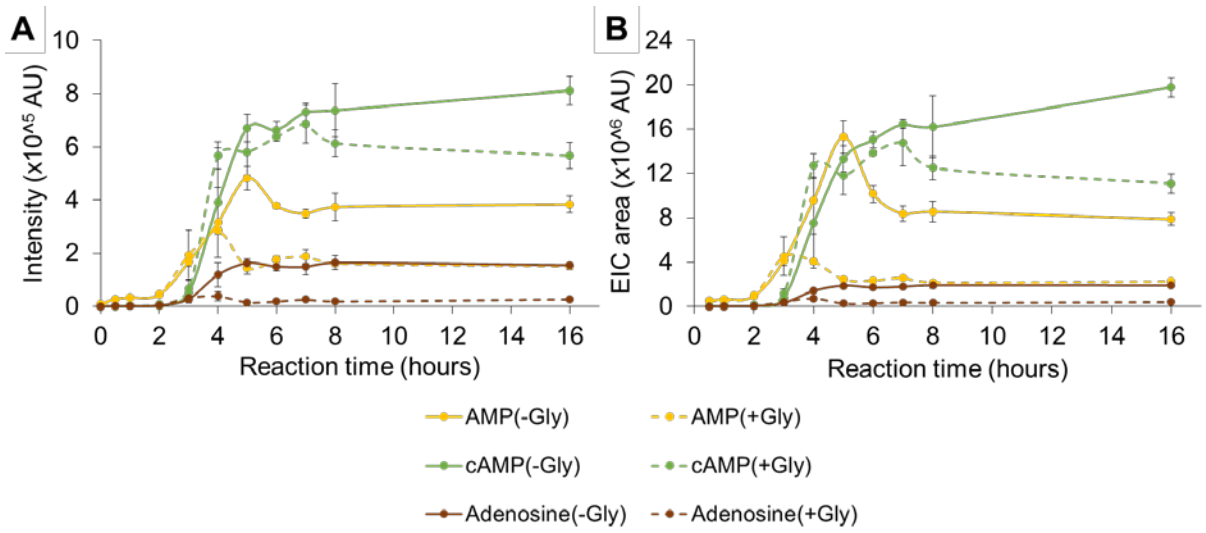


Figure S3. Comparison of two methods for the analysis of MS data. Time dependency study for formation of adenine glycosylation products in the reaction of 25 mM P-ribose + 25 mM adenine with vs without 25 mM glycine. *A) Extracting the maximum intensity observed in the correspondent EIC. B) Integrating the area below the correspondent EIC. Adenosine monophosphate = AMP (m/z=348.0683±0.01). Cyclic adenosine monophosphate = cAMP (m/z=330.0597±0.01). Adenosine (m/z=268.1040±0.01). All reactions were heated for 16 hours at 90˚C and pH=2.5 (HCl).*

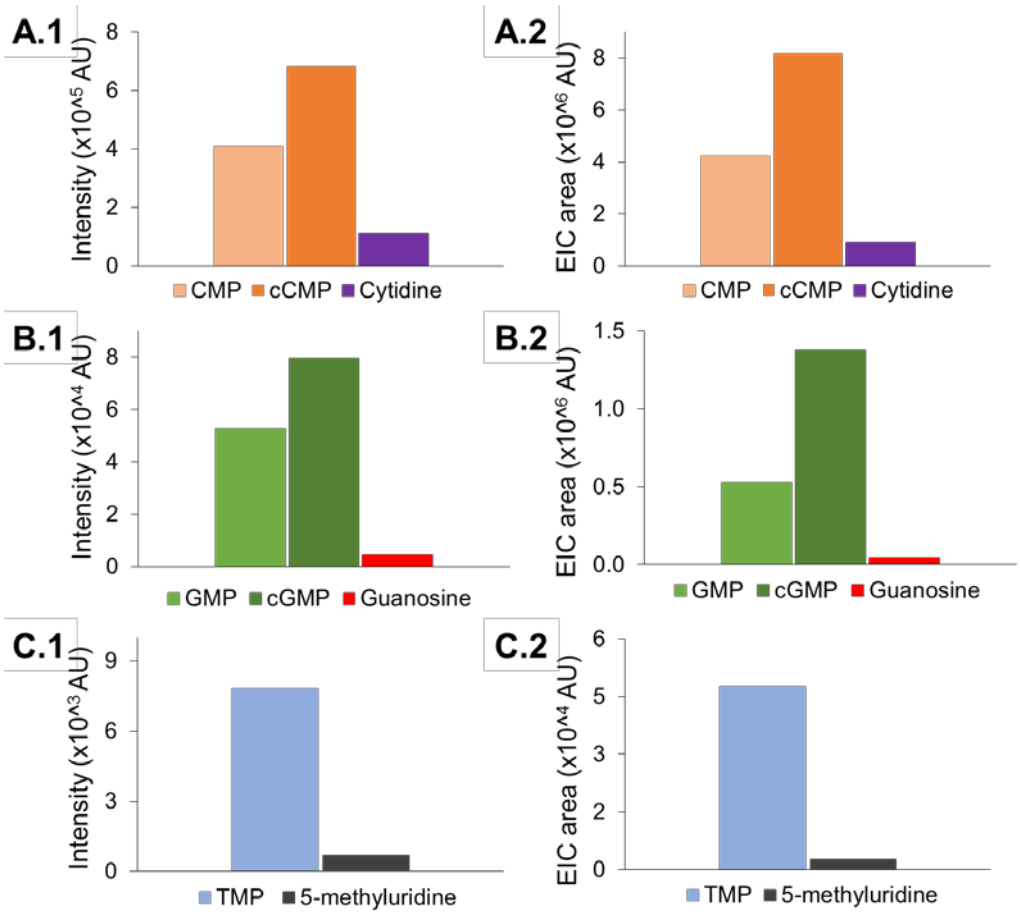


Figure S4. Comparison of two methods for the analysis of MS data. *A1-C1) Extracting the maximum intensity observed in the correspondent EIC. A2-C2) Integrating the area below the correspondent EIC. Cytidine monophosphate = CMP (m/z=324.0591±0.01). Cyclic cytidine monophosphate = cCMP (m/z=306.0485±0.01). Cytidine (m/z=244.0927±0.01). Guanosine monophosphate = GMP (m/z=364.0652±0.01). Cyclic guanosine monophosphate = cGMP (m/z=346.0547±0.01). Guanosine (m/z=284.0989±0.01). 5-methyluridine monophosphate = TMP (m/z=339.0587± 0.01). 5-methyluridine (m/z=243.0975±0.01). All reactions were heated for 5 hours at 90˚ C and pH=2.5 (HCl) including: A. (1-2) 25 mM cytosine + 25 mM P-ribose B (1-2) 25 mM guanine + 25 mM P-ribose C (1-2) 25 mM thymine + 25 mM P-ribose.*

4. Results for adenine glycosylation products

4.1. Canonical adenine nucleotide and nucleoside standards

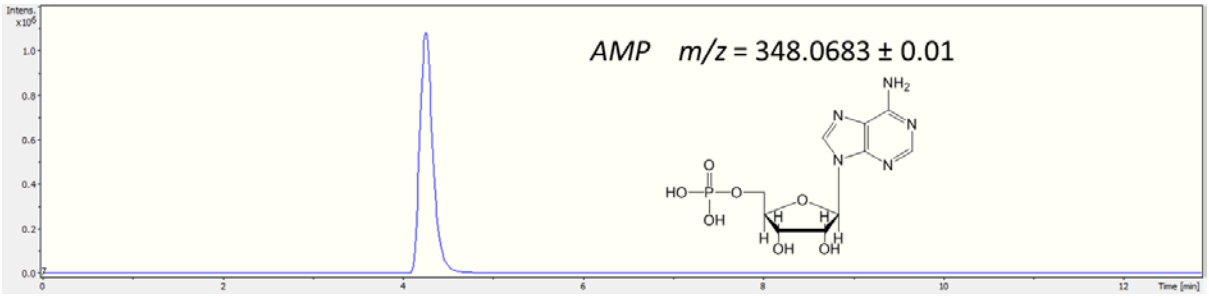


Figure S5. *EICs of adenosine 5´ -monophosphate disodium salt standard 5x10^{-4} M (m/z=348.0683±0.01) analysed with RP-HPLC-ESI-MS-MS.*

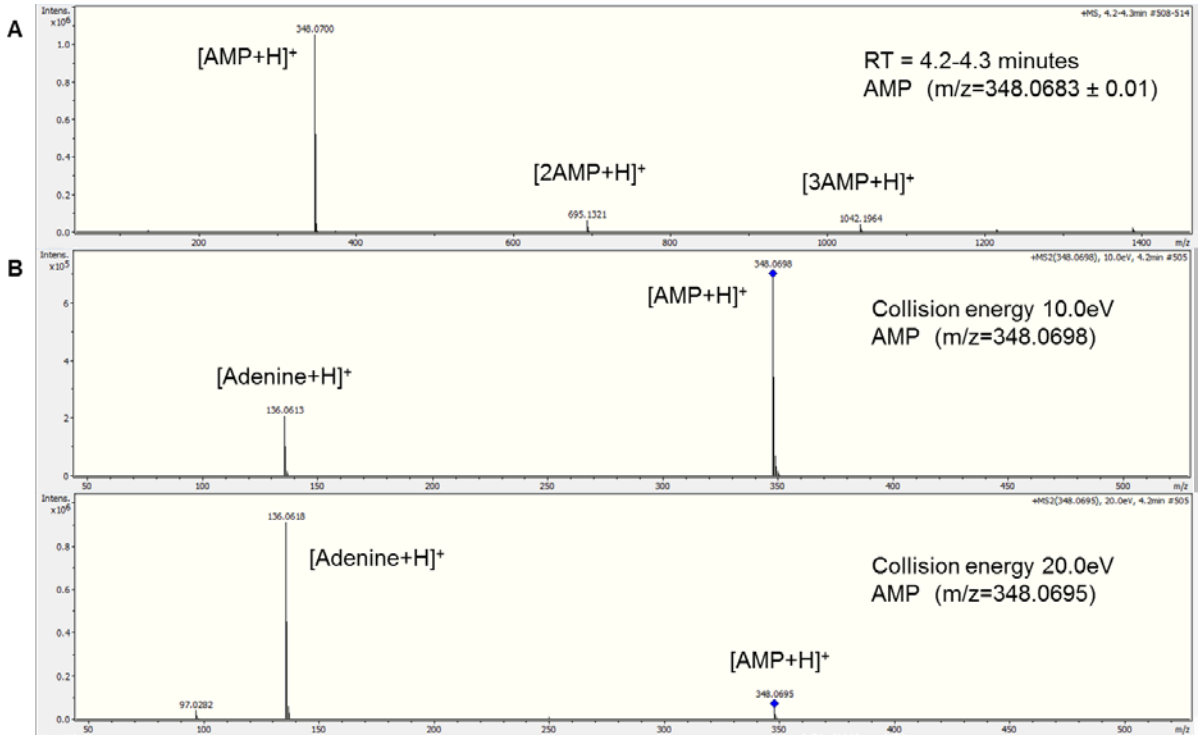


Figure S6. *A) Mass distribution corresponding to the peak at a retention time of 4.2-4.3 minutes in the EIC of AMP standard (showed in Fig. S5). B) MS-MS data for the fragmentation of AMP mass at two different collision energies.*

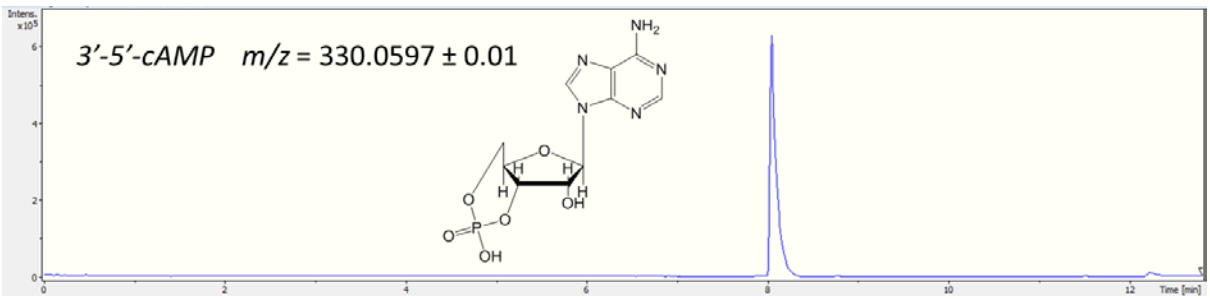


Figure S7*. EICs of adenosine 3′,5′-cyclic monophosphate sodium salt standard 1x10^{-4} M (m/z=330.0597±0.01) analysed with RP-HPLC-ESI-MS-MS.*

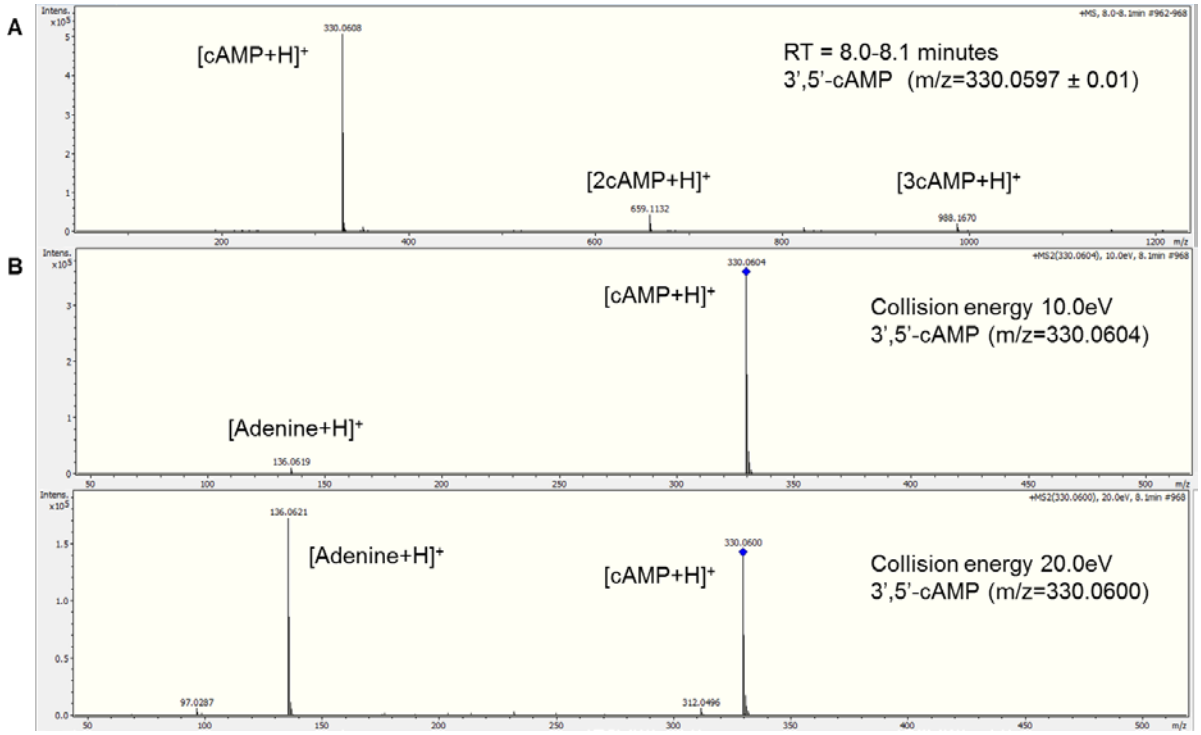


Figure S8*. A) Mass distribution corresponding to the peak at a retention time of 8.0-8.1 minutes in the EIC of 3′,5′-cAMP standard (showed in Fig. S7). B) MS-MS data for the fragmentation of 3′,5′-cAMP mass at two different collision energies.*

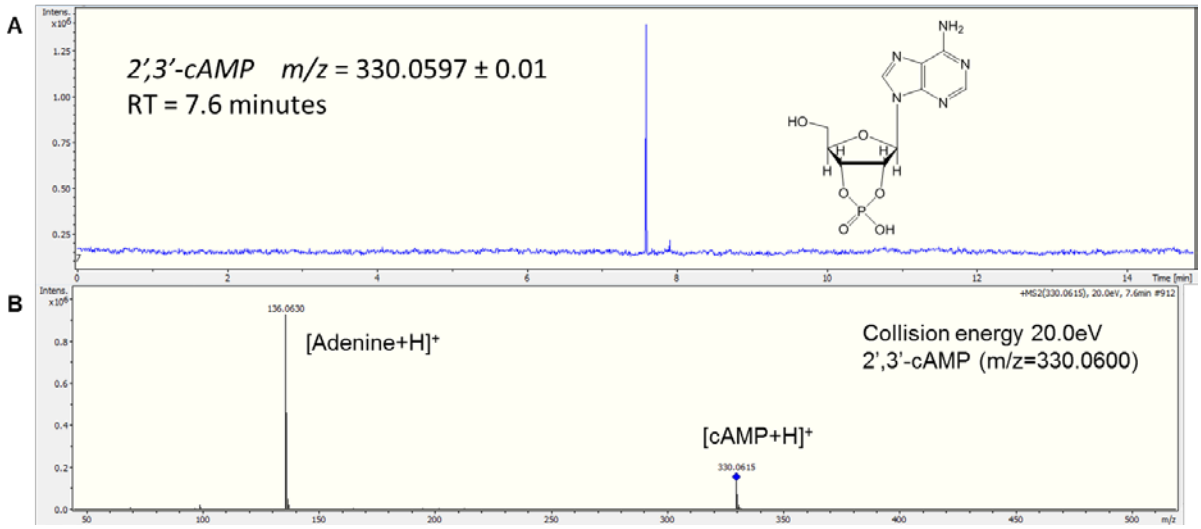


Figure S9. *A) Total Ion Current corresponding to the peak at a retention time of 7.6 minutes of 2´ ,3´ -cAMP standard. B) MS-MS data for the fragmentation of 2´ ,3´ -cAMP mass at 20 eV.*

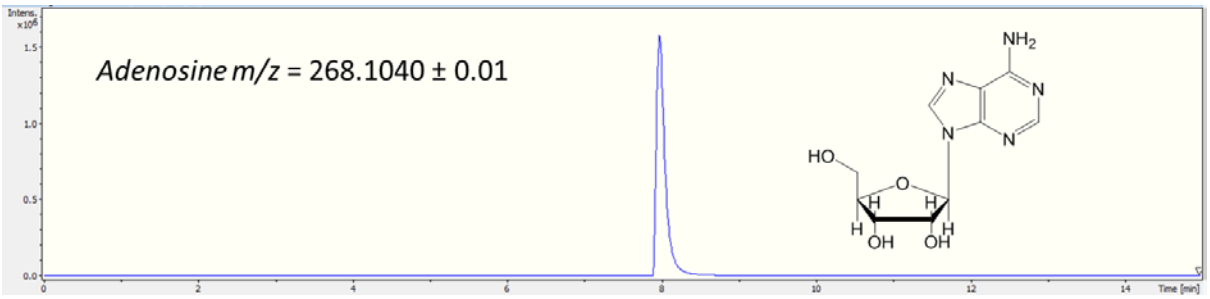


Figure S10. *Extracted Ion Chromatograms of adenosine standard 5x10^{-4} M (m/z=268.1040±0.01) analysed with RP-HPLC-ESI-MS-MS.*

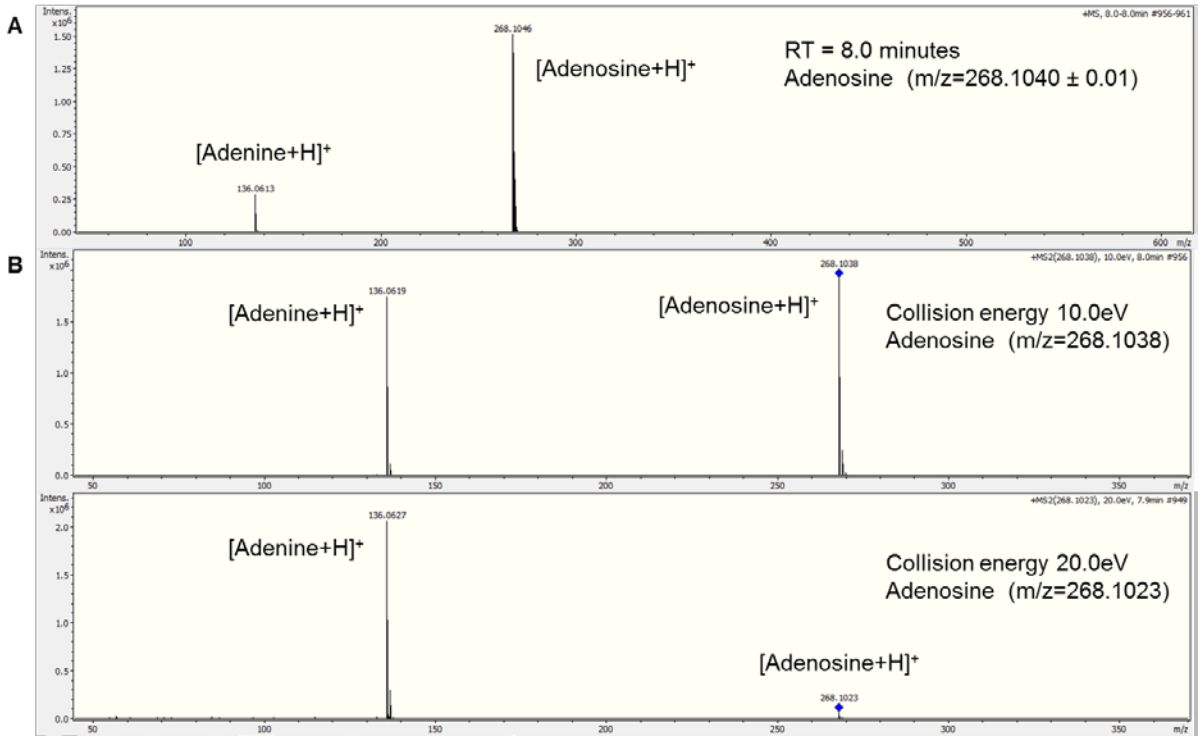


Figure S11. *A) Mass distribution corresponding to the peak at a retention time of 8.0 minutes in the EIC of adenosine standard (showed in Fig. S10). B) MS-MS data for the fragmentation of adenosine mass at two different collision energies.*

4.2. Possible structures for adenine glycosylation products

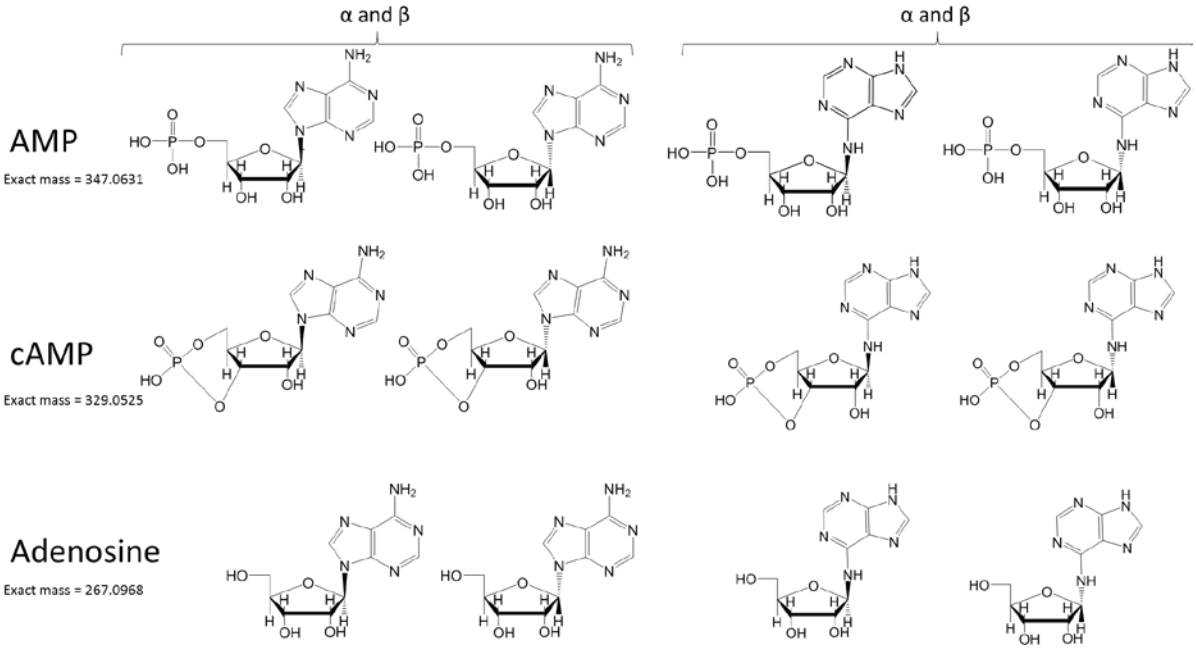
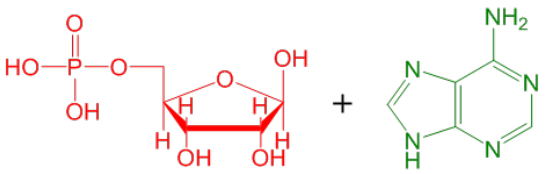


Figure S12. Proposed structures for adenine glycosylation products. *AMP isomers, Cyclic-AMP isomers and Adenosine isomers.*

4.3. Reaction of P-ribose + Adenine



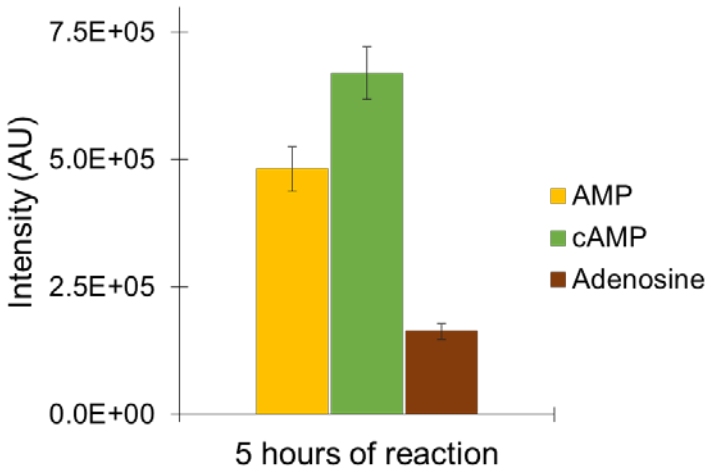


Figure S13. *Formation of glycosylation products when 25 mM D-ribose 5´ -phosphate + 25 mM adenine were heated at 90˚C for 5 hours at pH 2.5.*

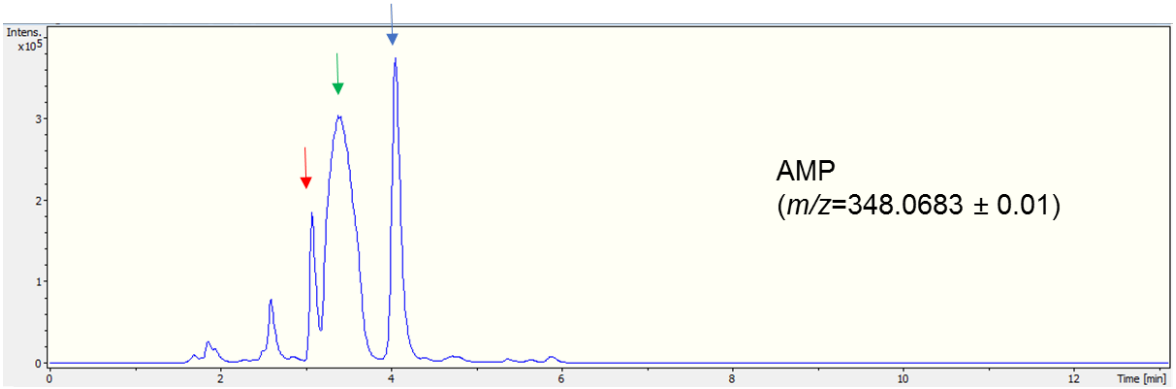


Figure S14. *EIC of adenosine 5´ -monophosphate when 25 mM D-ribose 5´ -phosphate + 25 mM adenine were used as starting materials. From data shown in Fig. S13.*

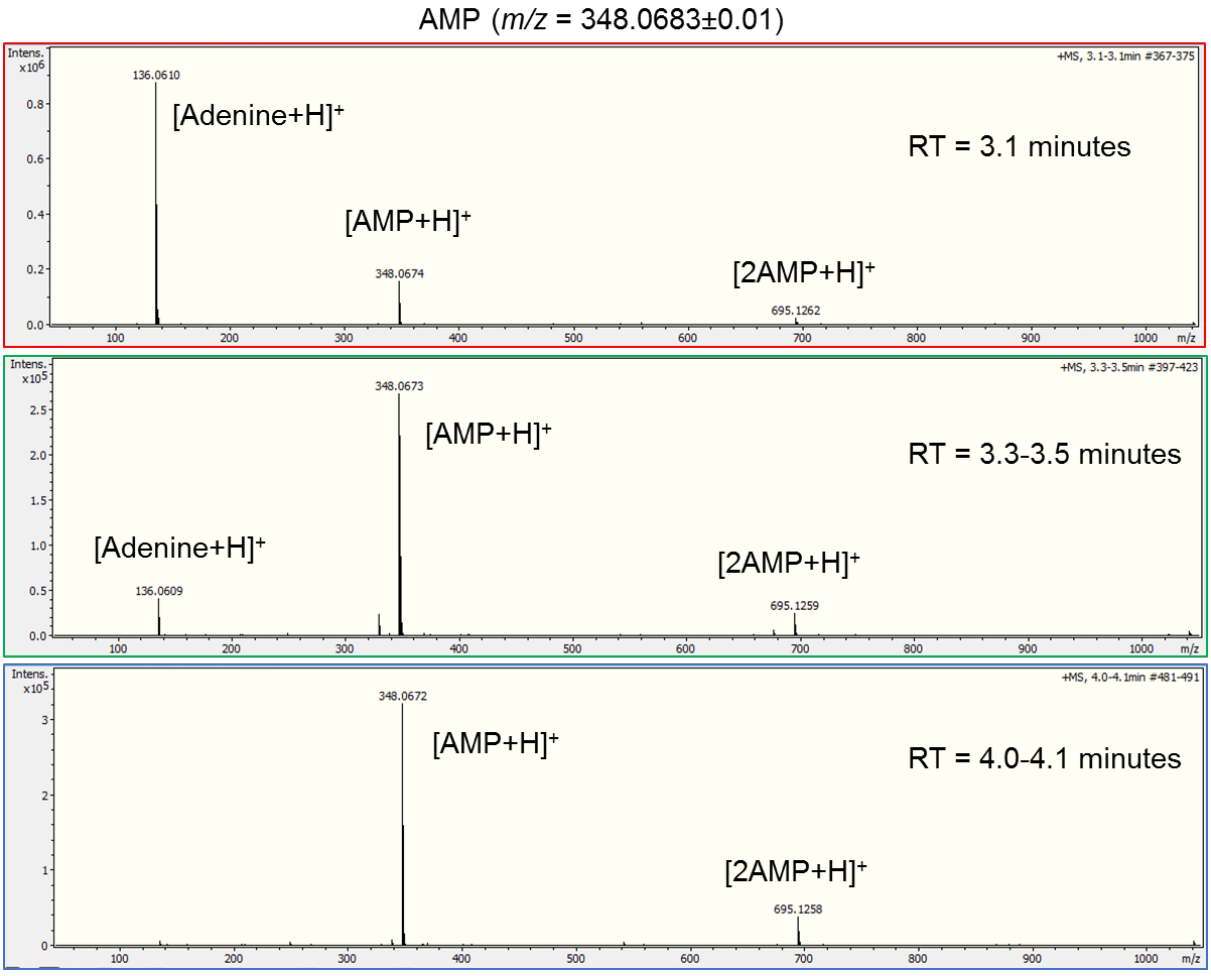


Figure S15. *Mass corresponding to adenosine 5'-monophosphate showed in the EIC in Figure S14. Chromatographic retention time in minutes = RT. MS/MS analysis in Figure S20.*

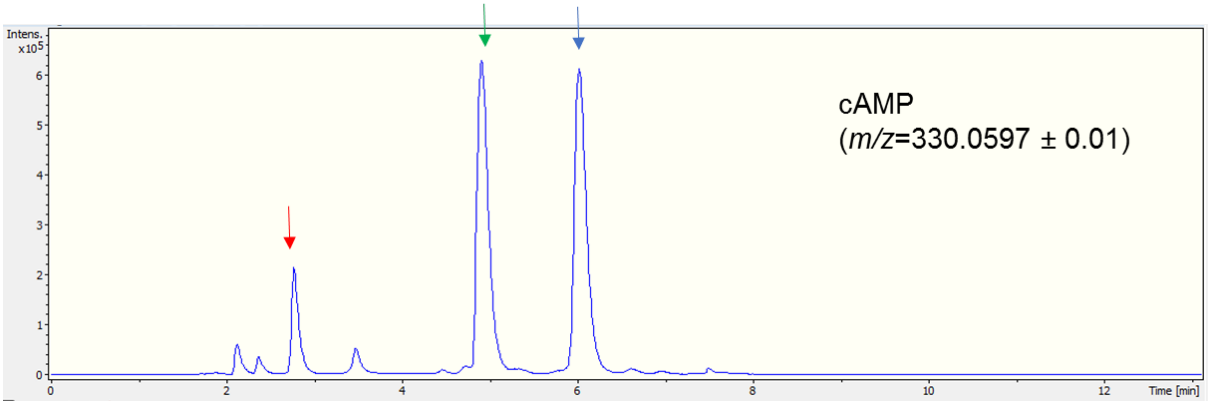


Figure S16. *Extracted Ion Chromatograms of cyclic adenosine 5'-monophosphate when 25 mM D-ribose 5´-phosphate + 25 mM adenine were used as starting materials. From data shown in Fig. S13.*

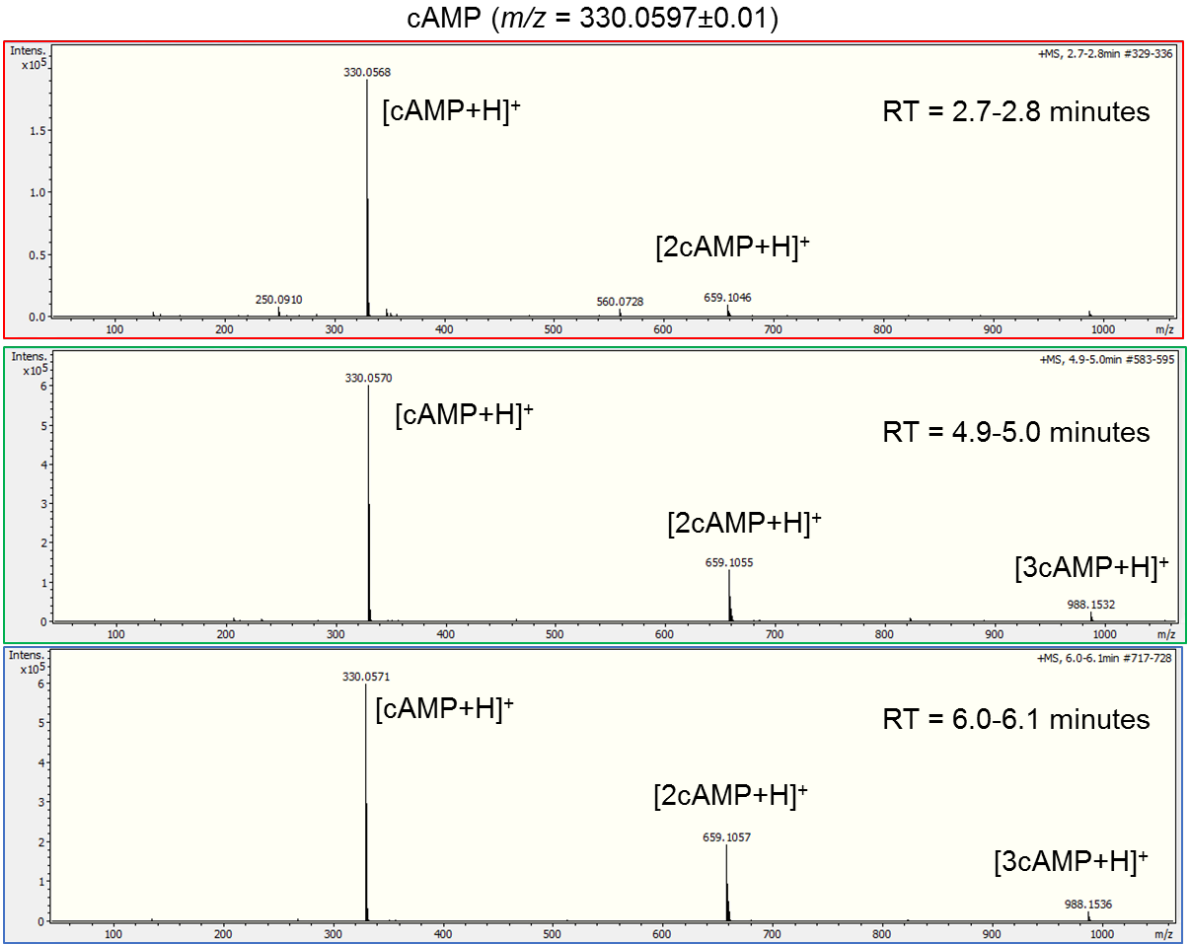


Figure S17: *Mass corresponding to cyclic adenosine 5´-monophosphate showed in the Extracted Ion Chromatograms on Figure S16. Chromatographic retention time in minutes = RT. MS/MS analysis in Figure S21.*

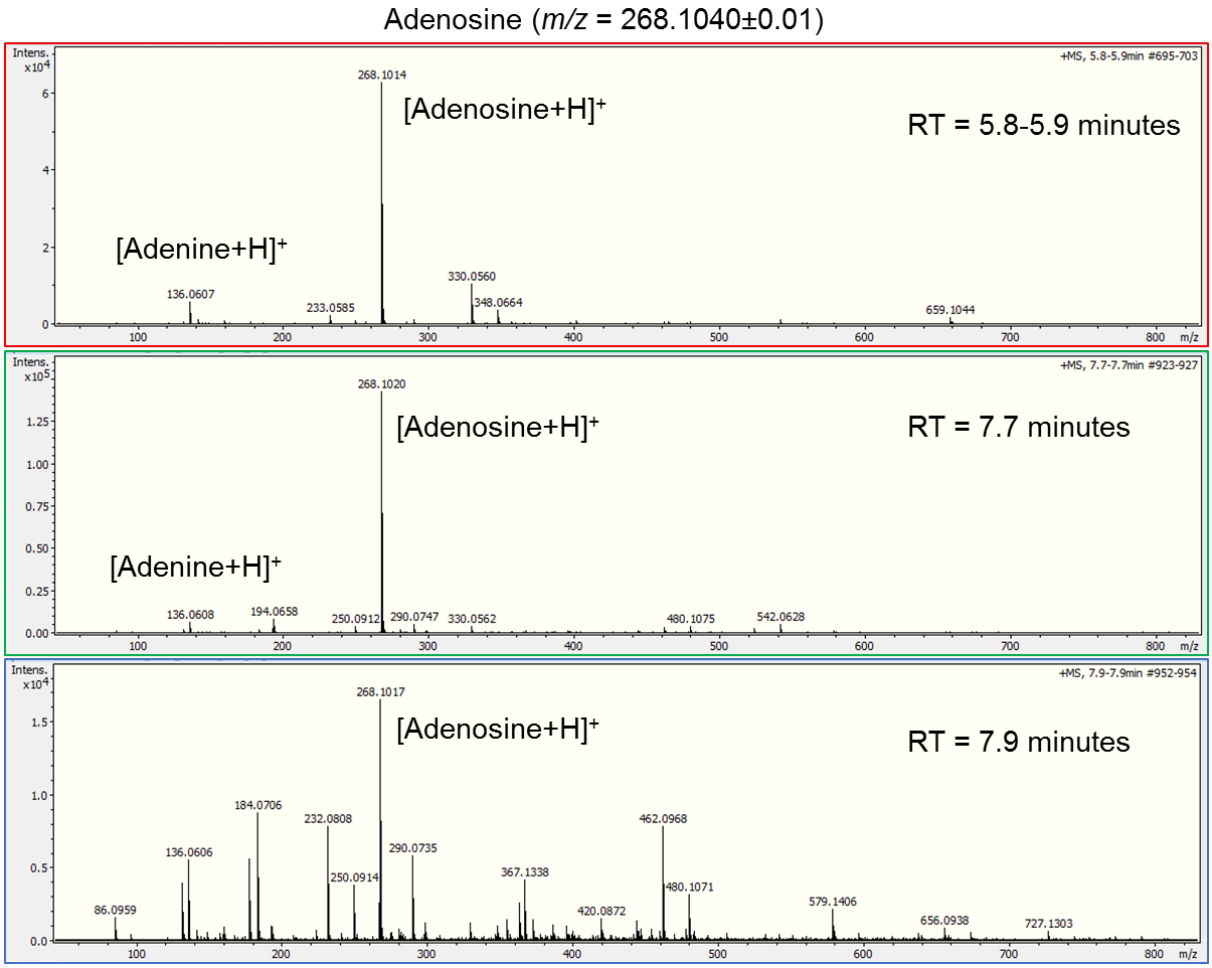


Figure S18. *EICs of adenosine when 25 mM D-ribose 5´ -phosphate + 25 mM adenine were used as starting materials. From data shown in Fig. S13.*

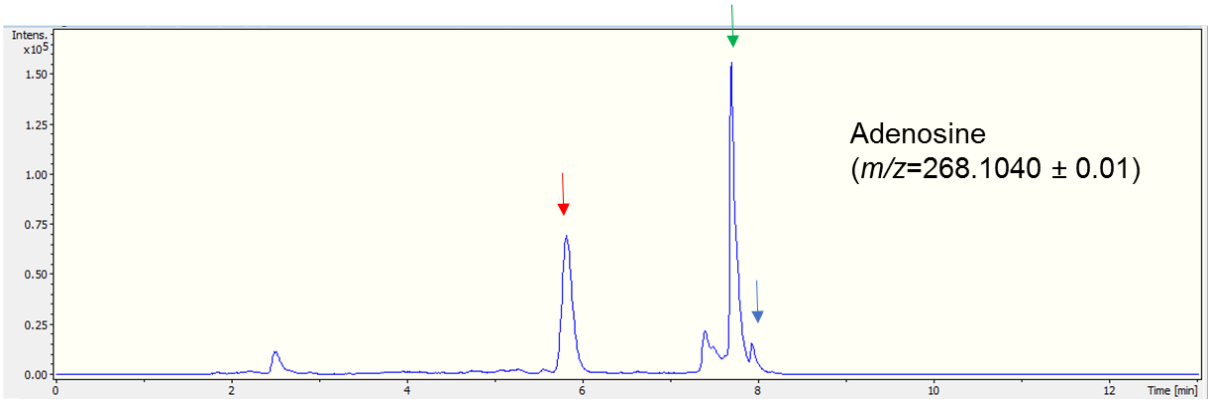


Figure S19. *Mass corresponding to adenosine showed in the EICs on Figure S18. Chromatographic retention time in minutes = RT. MS/MS analysis in Figure S22.*

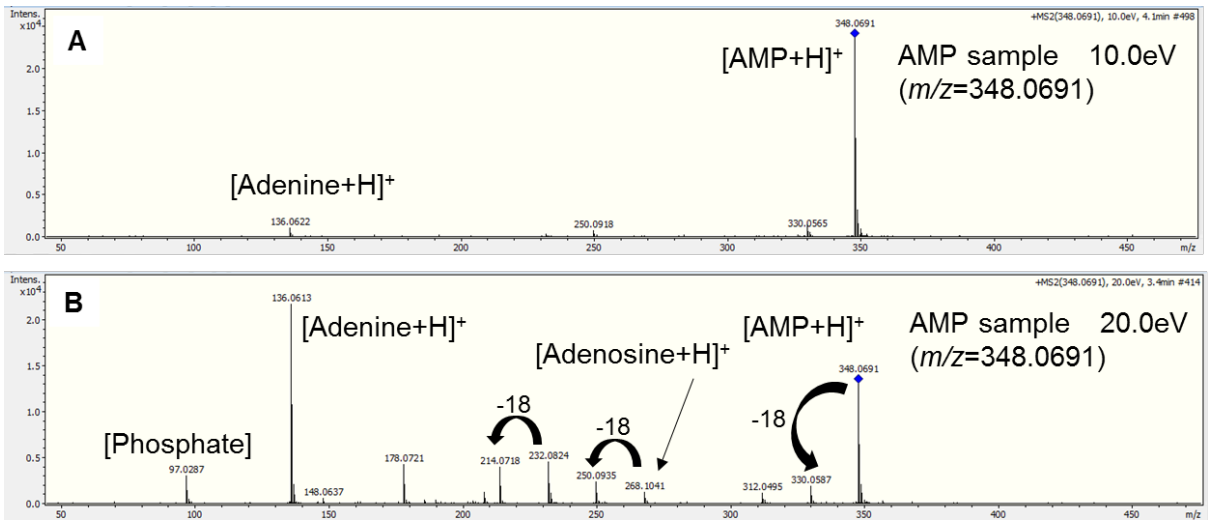


Figure S20. *MS-MS data for the fragmentation of adenosine 5´ -monophosphate (AMP) mass (peak at RT = 4.1 minutes) in the sample showed in Figure S14 at two different collision energies A) 10.0 eV B) 20.0 eV.*

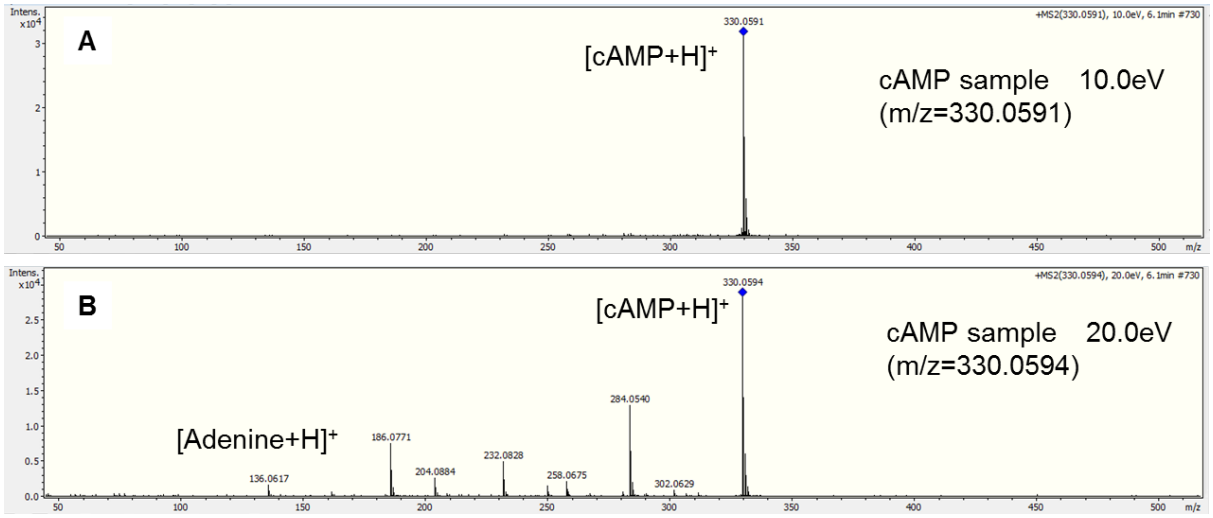


Figure S21: *MS-MS data for the fragmentation of cyclic adenosine 5'-monophosphate (peak at RT = 6.1minutes) in the sample showed in Figure S16 at two different collision energies A) 10.0 eV B) 20.0 eV.*

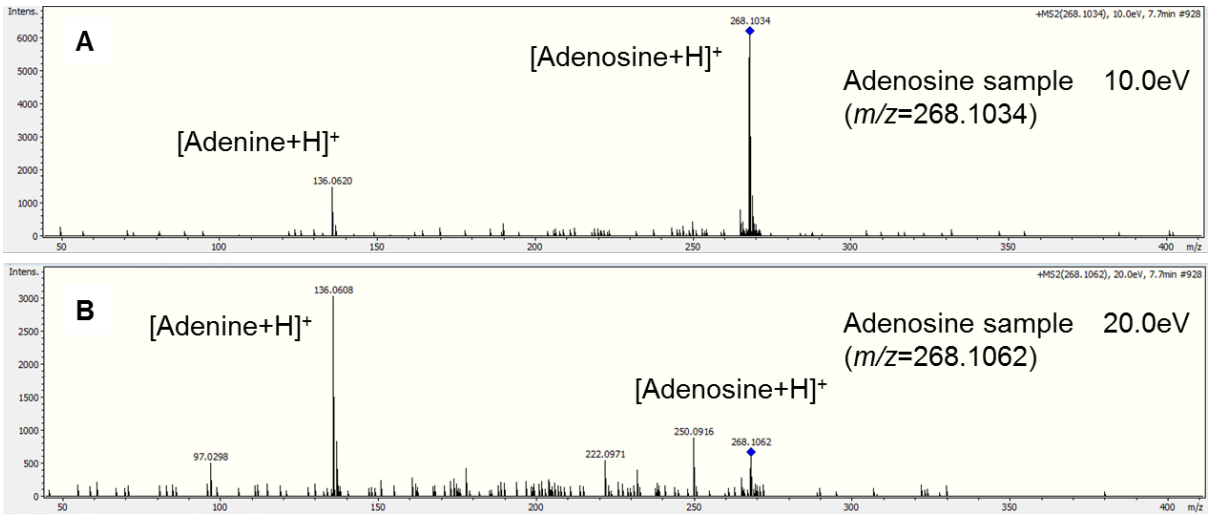


Figure S22: *MS-MS data for the fragmentation of adenosine mass (peak at RT = 7.7 minutes) in the sample showed in Figure S18 at two different collision energies A) 10.0 eV B) 20.0 eV.*

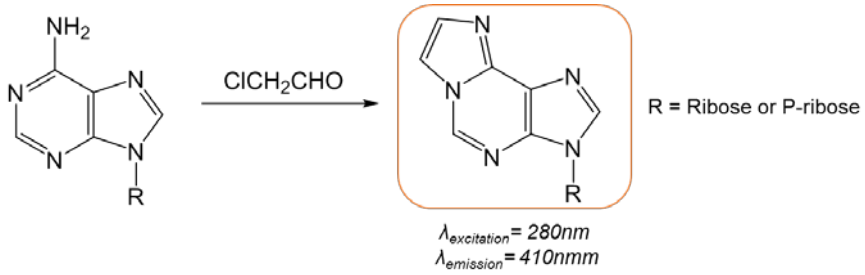


Figure S23. *Formation of 1,N6-etheno-derivatives ($\lambda_{excitation}$= 280 nm; $\lambda_{emission}$= 410 nm) from adenine nucleotide and nucleoside by incubating adenylate nucleotides with chloroactealdehyde (60 minutes at 60˚C, pH=4.5).*

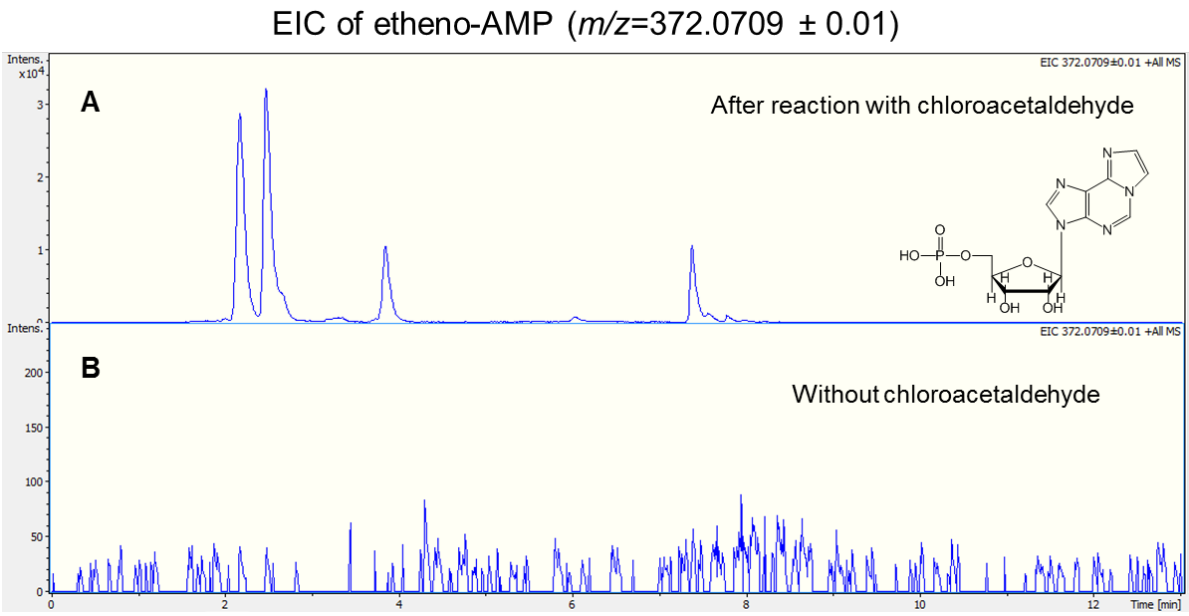


Figure S24. *EICs of the etheno-AMP derivative (m/z=372.0709±0.01) after the reaction of 25 mM D-ribose 5´ -phosphate + 25 mM adenine heated at 90˚ C for 5 hours and pH 2.5 A) After chloroacetaldehyde derivatisation B) Without chloroacetaldehyde derivatisation.*

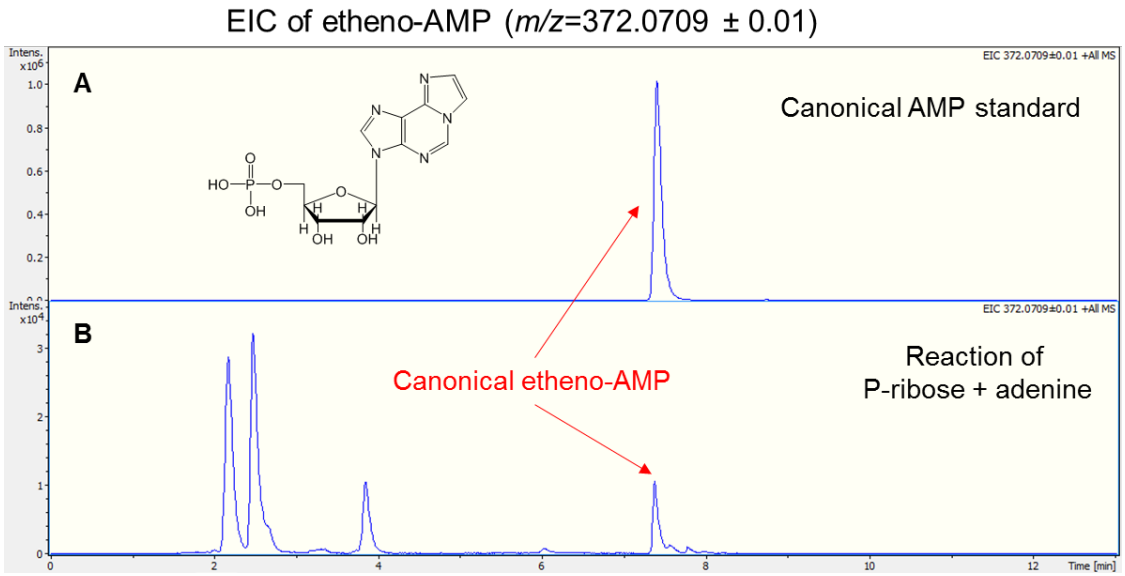


Figure S25. *EICs of the etheno-AMP derivative (m/z=372.0709±0.01) after chloroacetaldehye derivatisation of A) Canonical adenosine 5´ -monophosphate B) After the reaction of 25 mM D-ribose 5´ -phosphate + 25 mM adenine heated at 90˚ C for 5 hours and pH 2.5.*

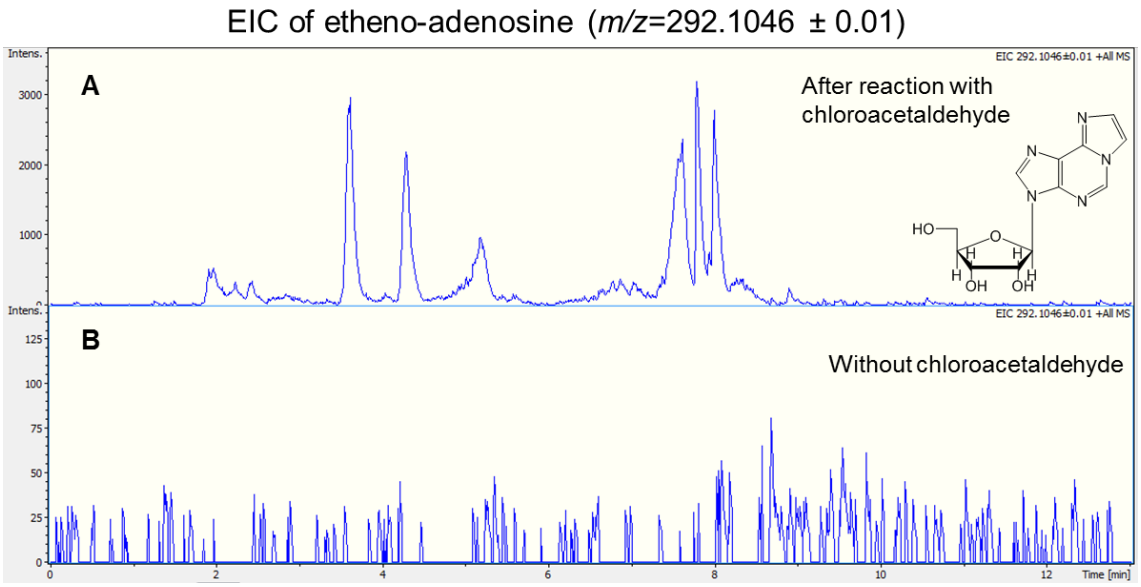


Figure S26: *Extracted Ion Chromatograms of the etheno-adenosine derivative (m/z=292.1046±0.01) after the reaction of 25 mM D-ribose 5´ -phosphate + 25 mM adenine heated at 90˚C for 5 hours and pH 2.5 A) After chloroacetaldehyde derivatisation B) Without chloroacetaldehyde derivatisation.*

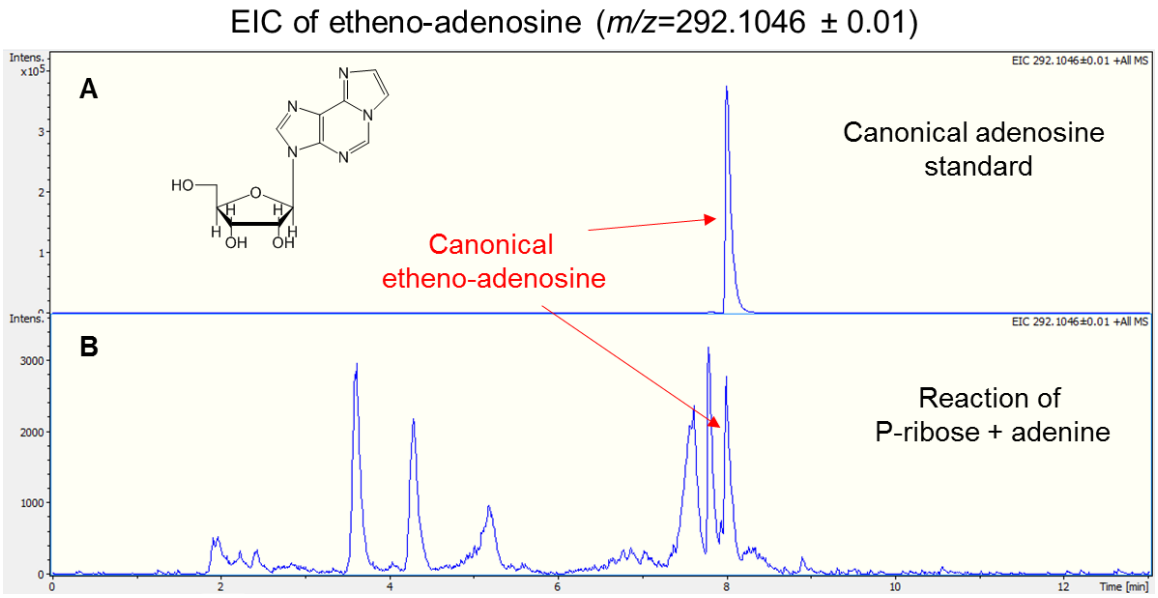


Figure S27. *EICs of the etheno-adenosine derivative (m/z=292.1046±0.01) after chloroacetaldehye derivatisation of A) Canonical adenosine 5´ -monophosphate B) After the reaction of 25 mM D-ribose 5´ -phosphate + 25 mM Adenine heated at 90˚C for 5 hours and pH 2.5.*

4.4. Reaction of P-ribose + Adenine + Glycine

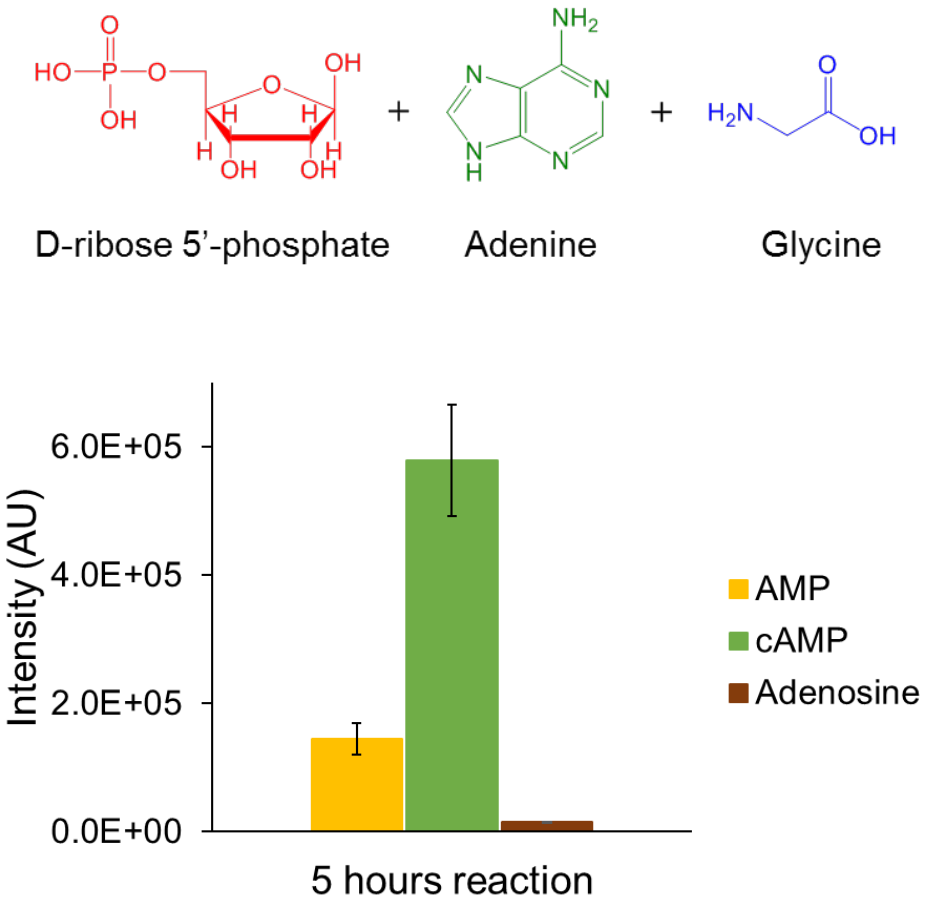


Figure S28. *Formation of glycosylation products when 25 mM D-ribose 5´ -phosphate + 25 mM adenine + 25 mM glycine were heated at 90˚C for 5 hours at pH 2.5.*

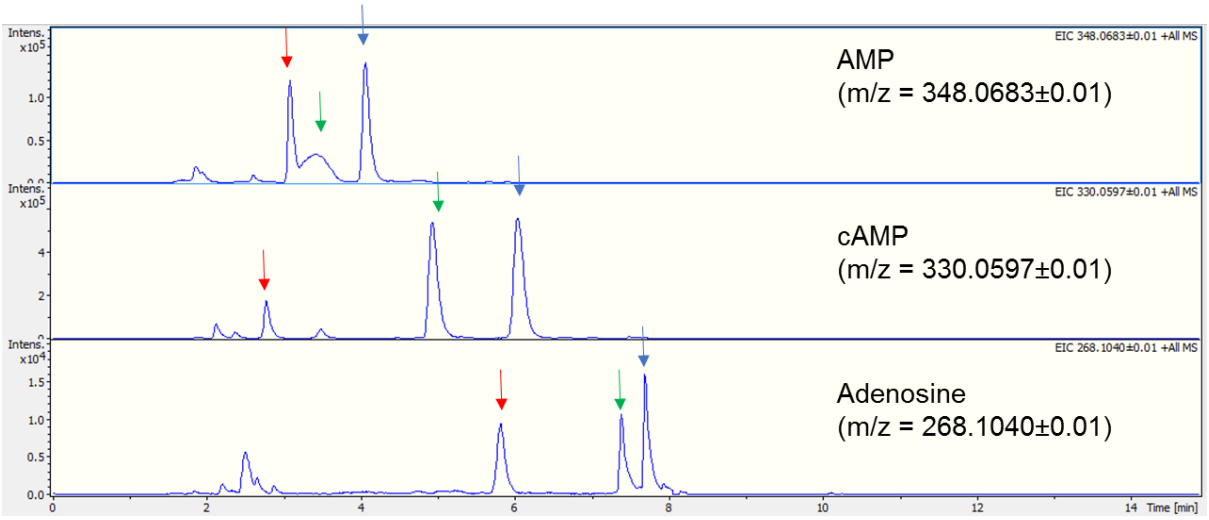


Figure S29. *EICs of adenine nucleotide and nucleoside products when 25 mM D-ribose 5´ -phosphate + 25 mM adenine + 25 mM glycine were heated at 90˚C for 5 hours (pH=2.5).*

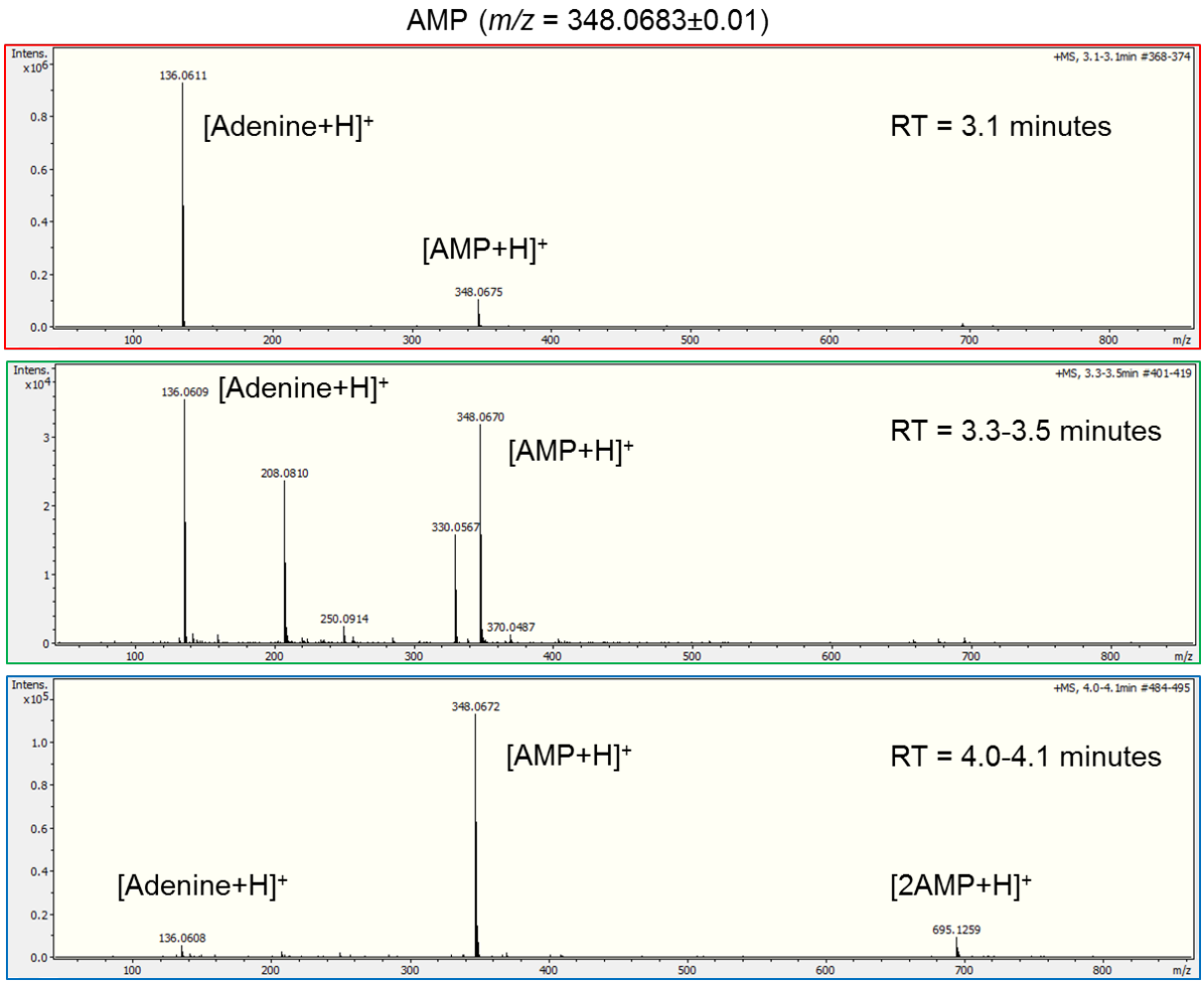


Figure S30. *Mass corresponding to adenosine 5′ -monophosphate showed in the EICs in Figure S29.*

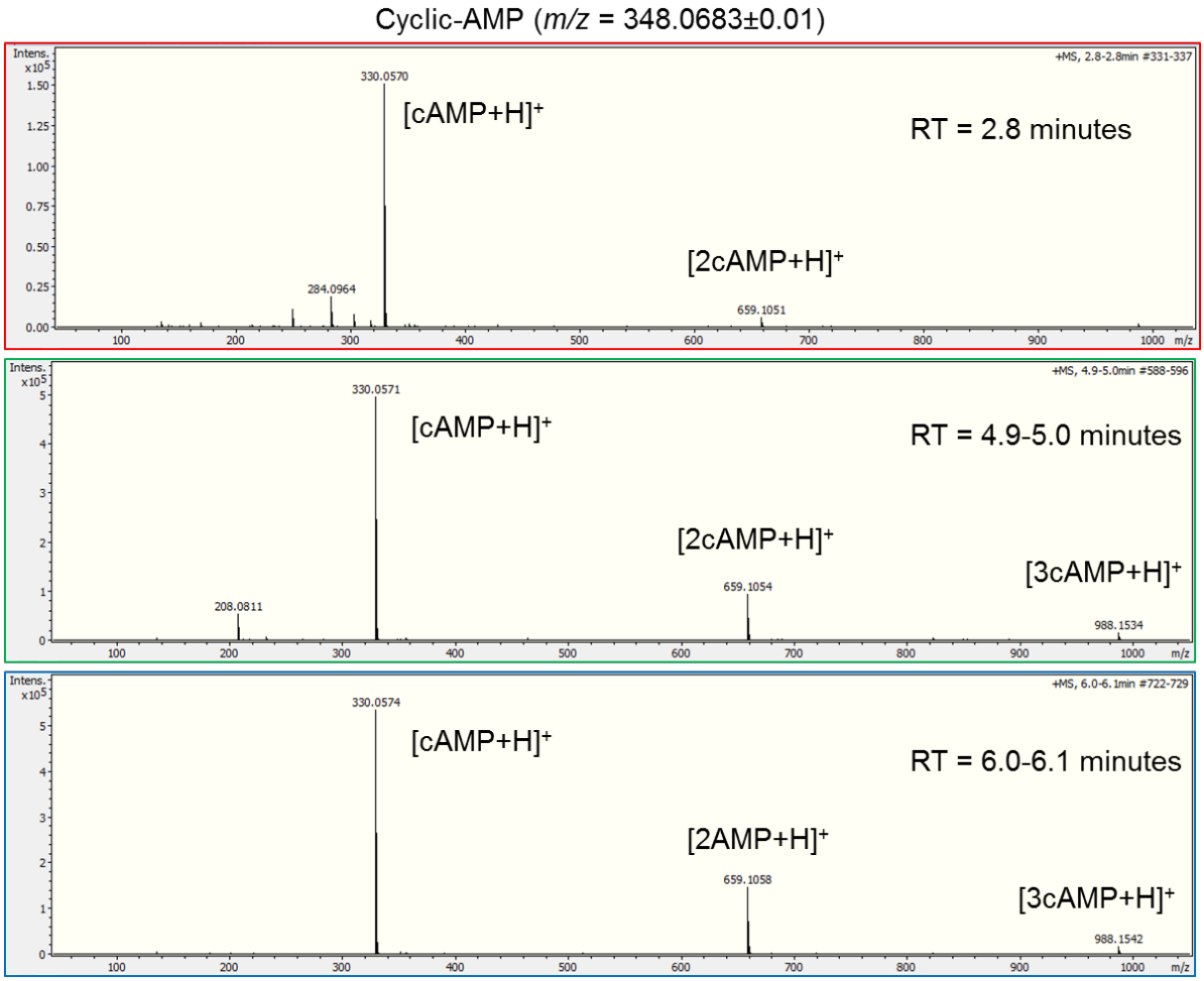


Figure S31. *Mass corresponding to cyclic adenosine 5′ -monophosphate showed in the EICs in Figure S29.*

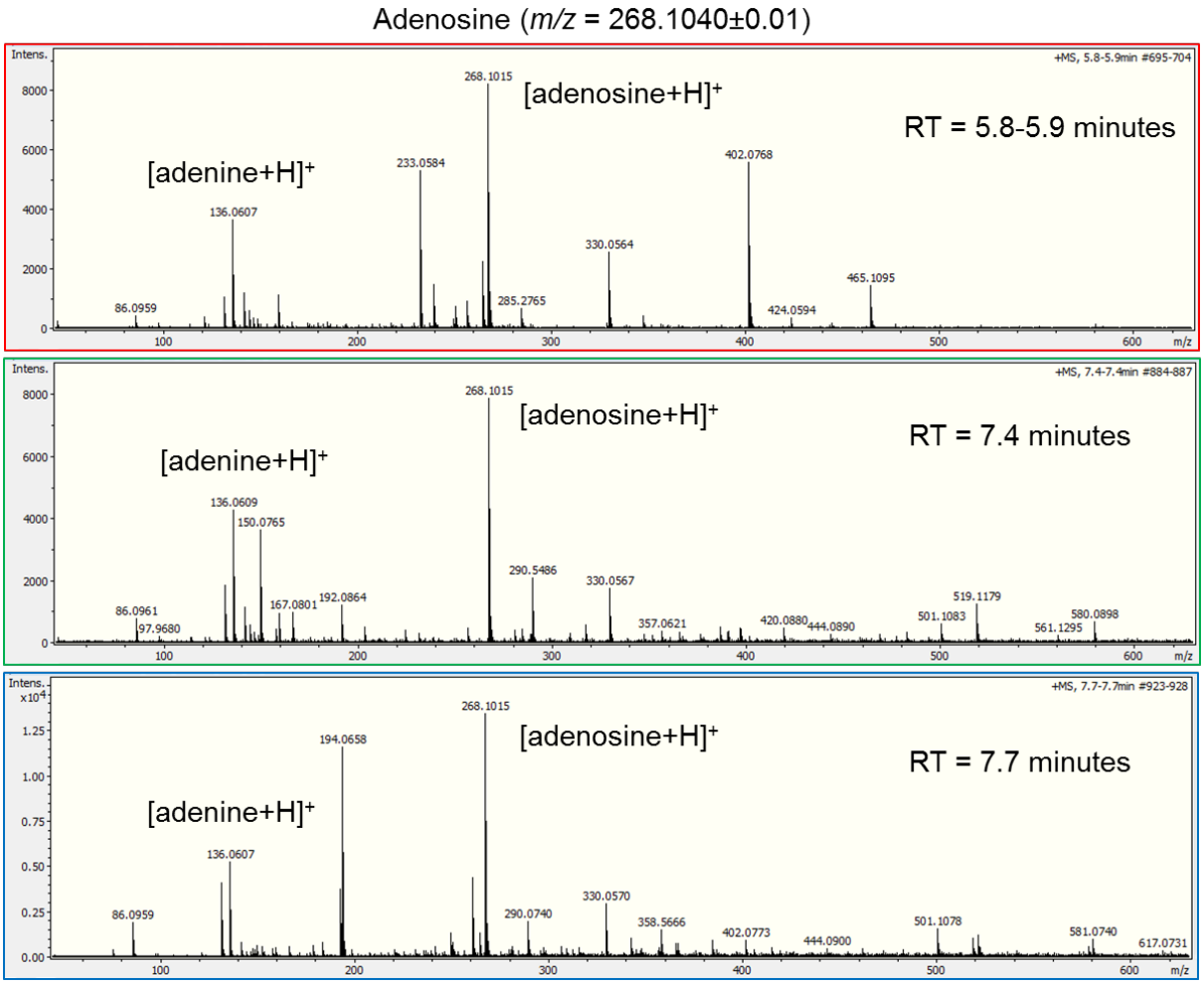
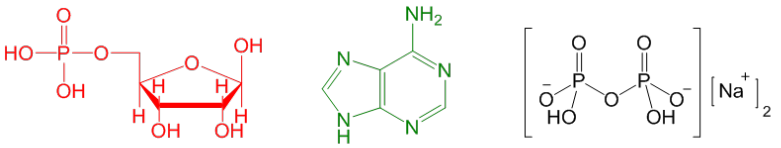
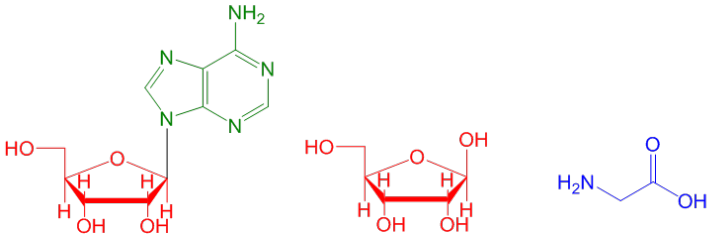


Figure S32. *Mass corresponding to adenosine showed in the EICs in Figure S29.*

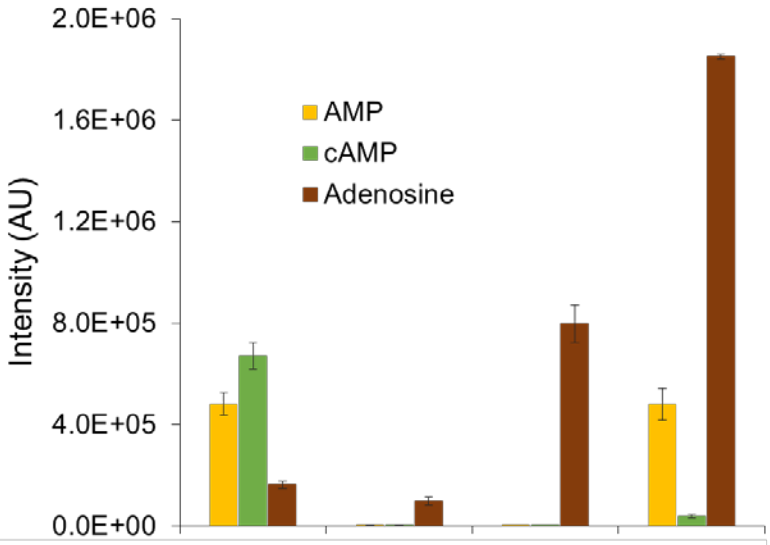
4.5. Control reactions with adenine nucleotide/nucleoside building blocks



D-ribose 5'-phosphate Adenine Sodium pyrophosphate



Adenosine D-Ribose Glycine



Reaction #	1A	2A	3A	4A
Glycine	+	+	+	+
Ribose	-	+	+	-
Adenine	+	+	+	-
P-ribose	+	-	-	-
PPi	-	+	-	+
Adenosine	-	-	-	+

Figure S33. *Summary for adenine nucleotide formation. Study of reactions (heated at 90˚ C for 5 hours) of nucleotide building blocks at 25 mM starting material concentration. All samples were analysed by RP-HPLC-MS.*

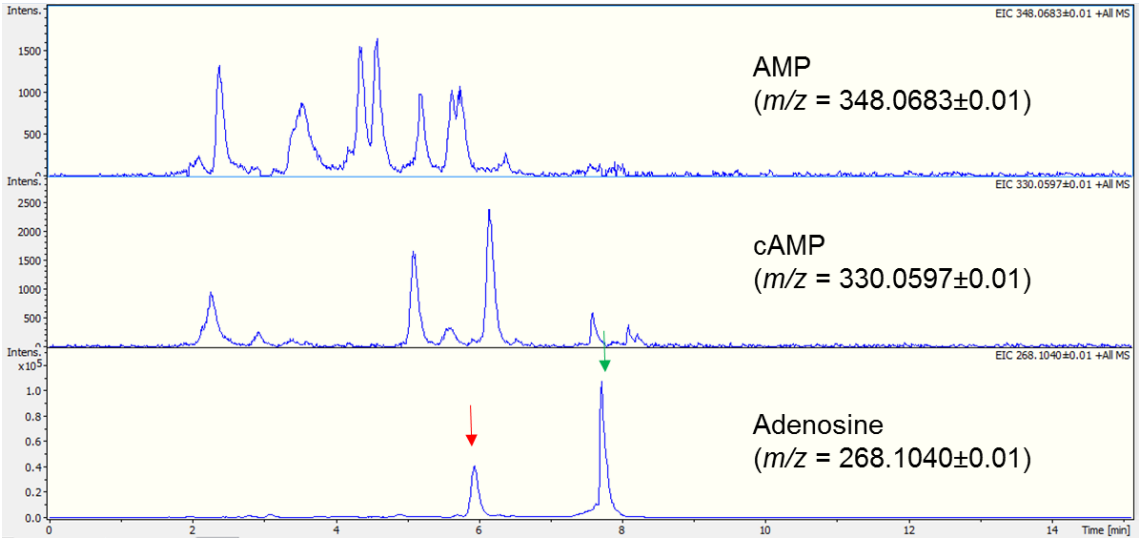


Figure S34. *EICs for the formation of glycosylation products after the reaction of 25 mM glycine + 25 mM adenine + 25 mM ribose + 25 mM pyrophosphate at 90˚C for 5 hours (pH 2.5) (reaction 2A in Figure S33).*

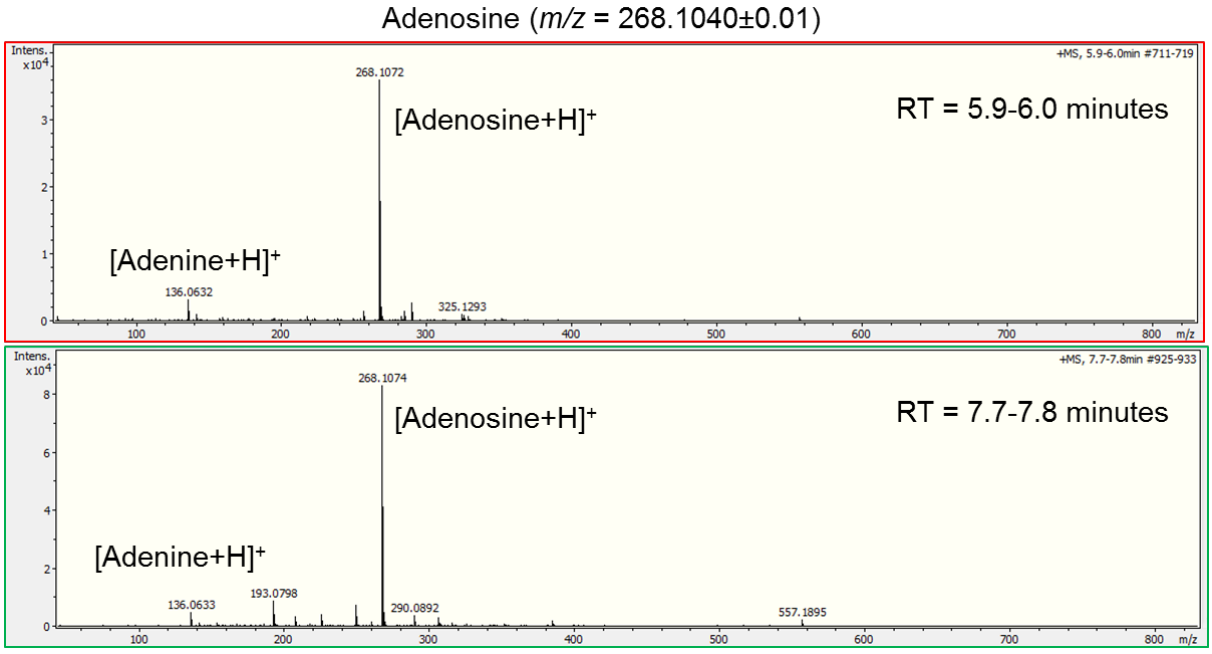


Figure S35. *Extracted masses corresponding to the adenosine showed in the EICs in Figure S34.*

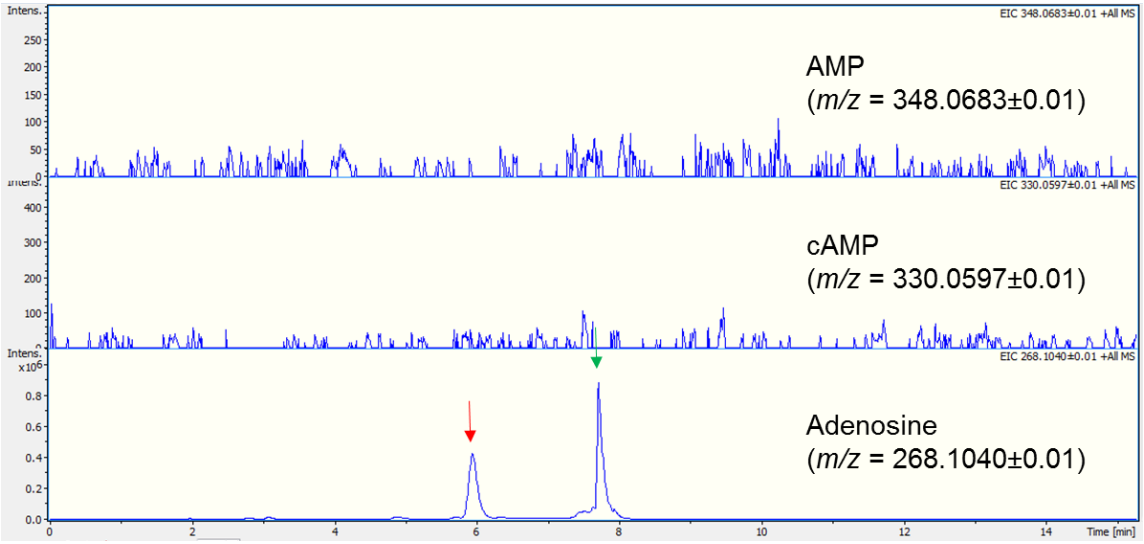


Figure S36. *EICs for the formation of glycosylation products after the reaction of 25 mM glycine + 25 mM adenine + 25 mM ribose at 90˚C for 5 hours (pH 2.5) (reaction 3A in Figure S33).*

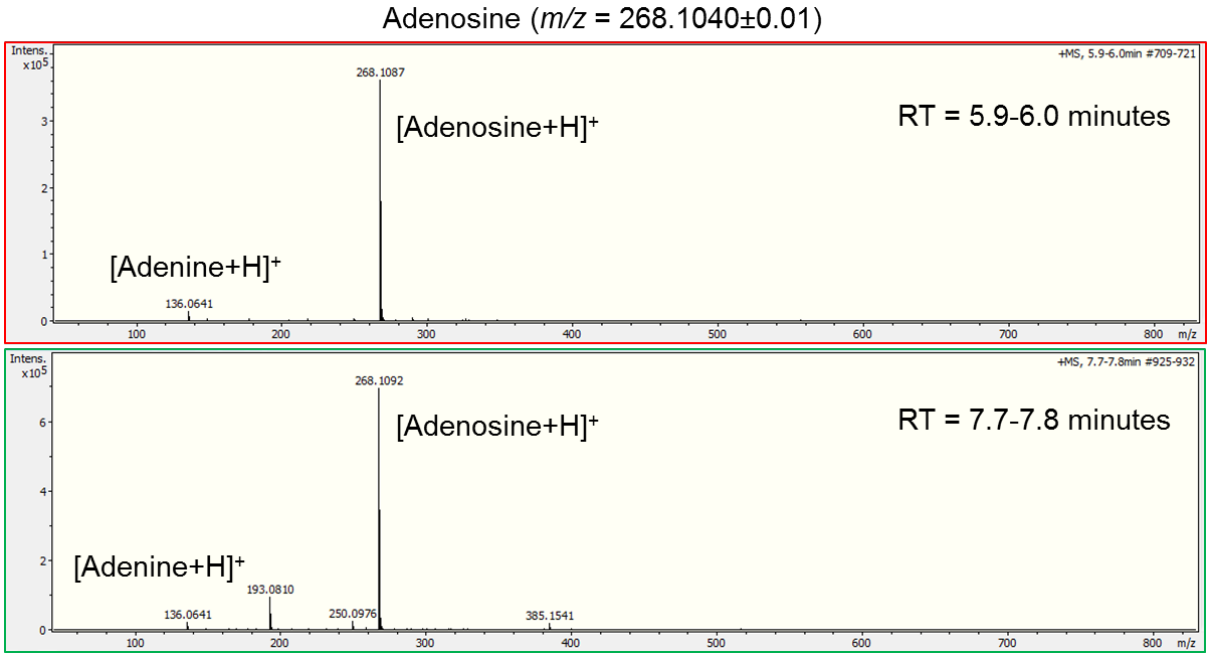


Figure S37. *Extracted masses corresponding to the adenosine showed in the EICs in Figure S36.*

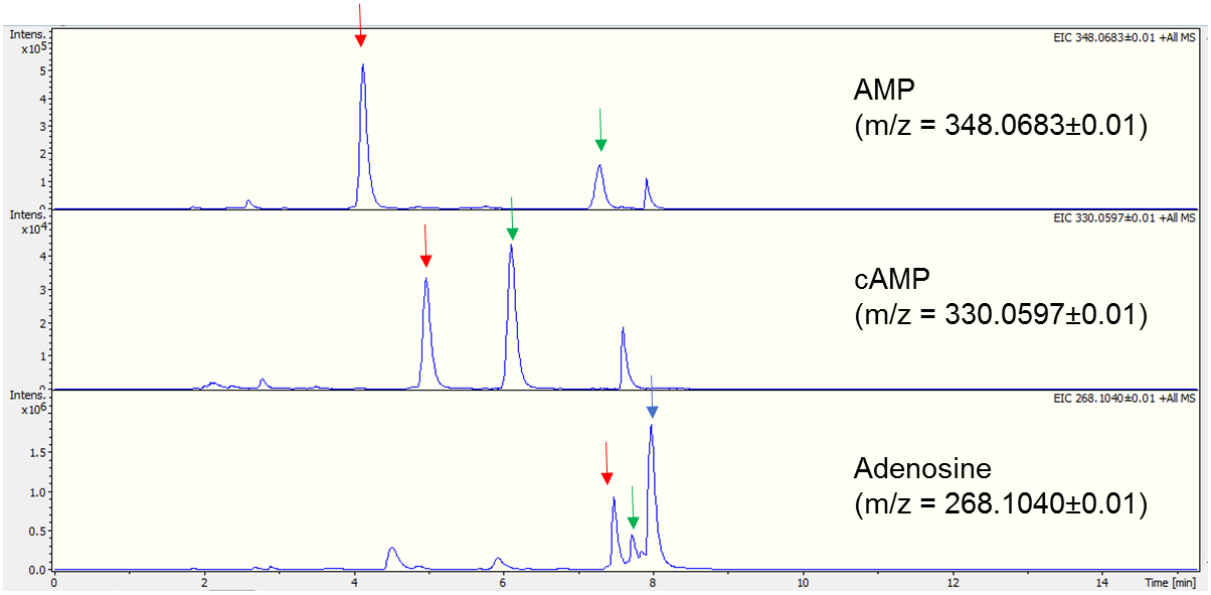


Figure S38. *EICs for the formation of glycosylation products after the reaction of 25 mM glycine + 25 mM adenosine + 25 mM pyrophosphate at 90˚C for 5 hours (pH 2.5) (reaction 3A in Figure S33).*

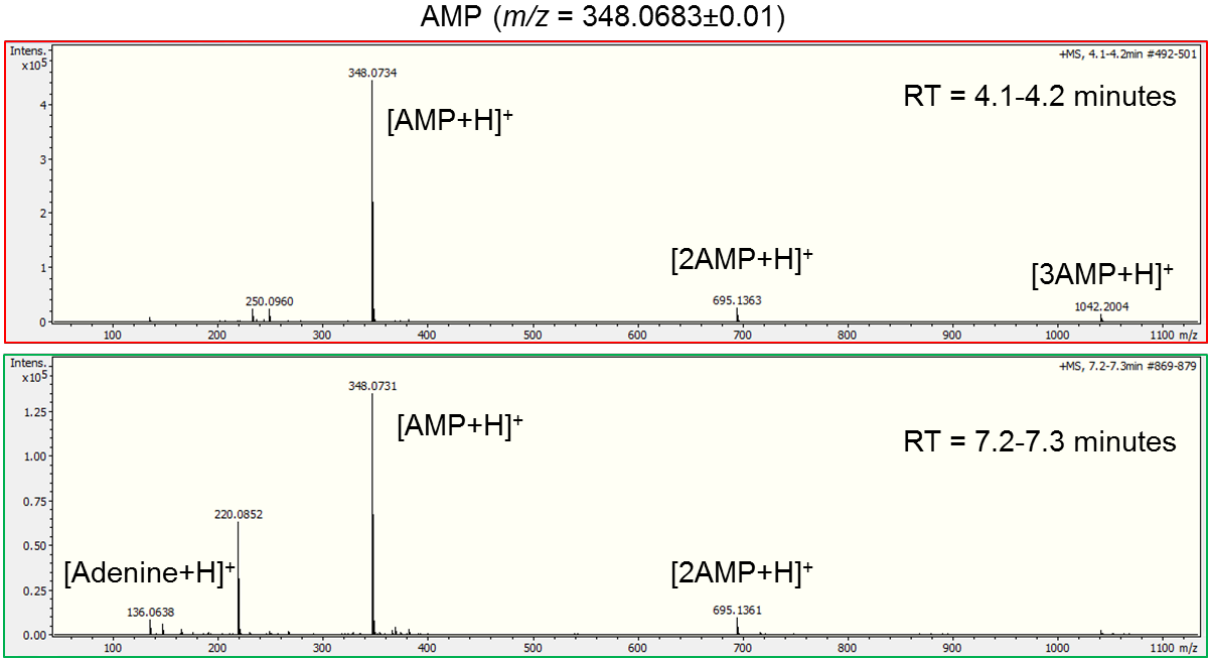


Figure S39. *Extracted masses corresponding to the adenosine monophosphate showed in the EICs in Figure S38.*

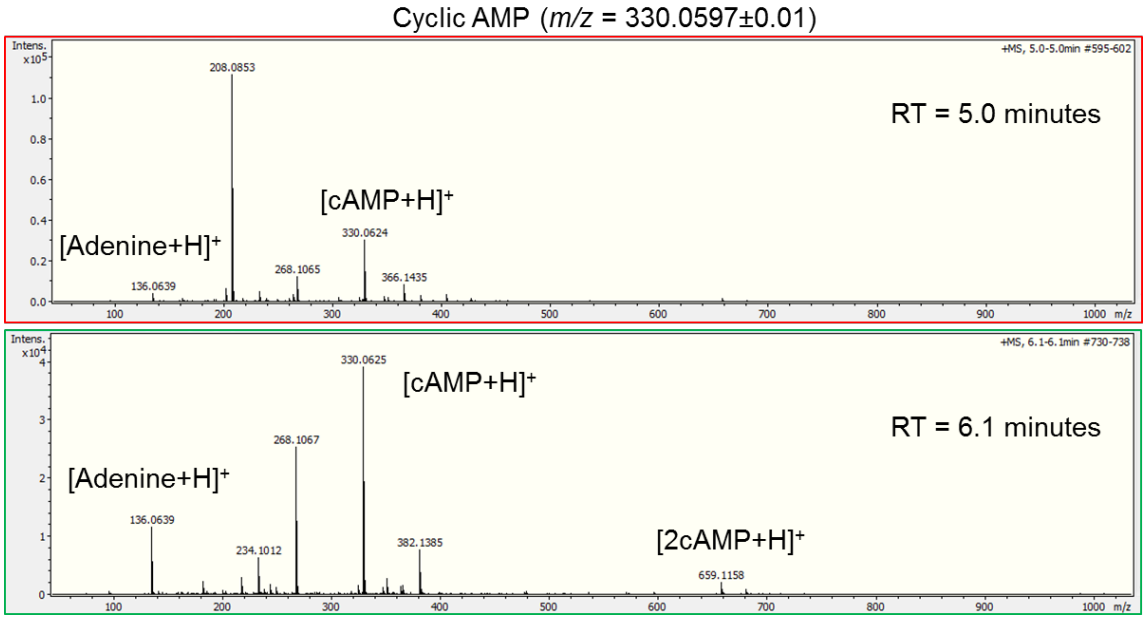


Figure S40. *Extracted masses corresponding to the cyclic adenosine monophosphate showed in the EICs in Figure S38.*

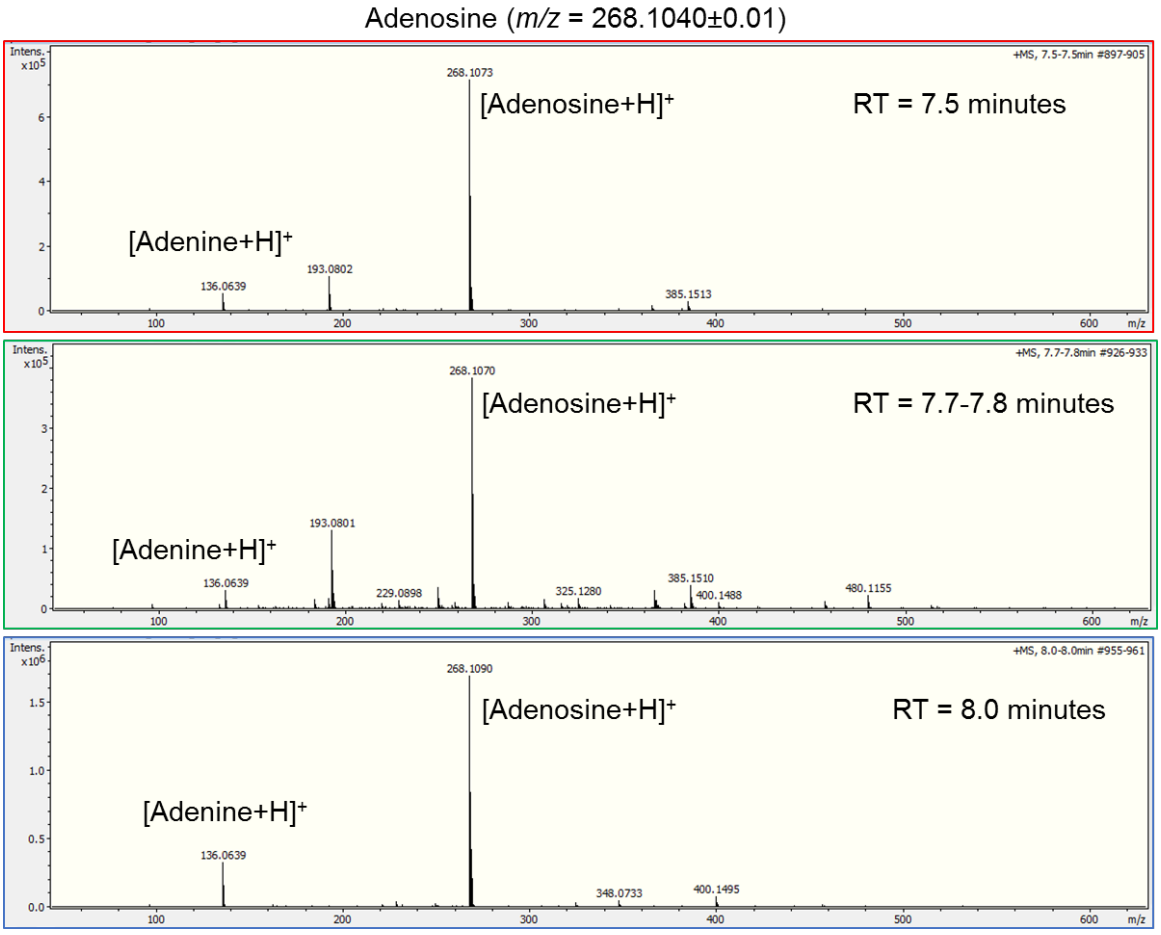


Figure S41. *Extracted masses corresponding to the adenosine showed in the EICs in Figure S38.*

5. Results for guanine/cytosine/thymine glycosylation products

5.1. Canonical nucleotide and nucleoside standards

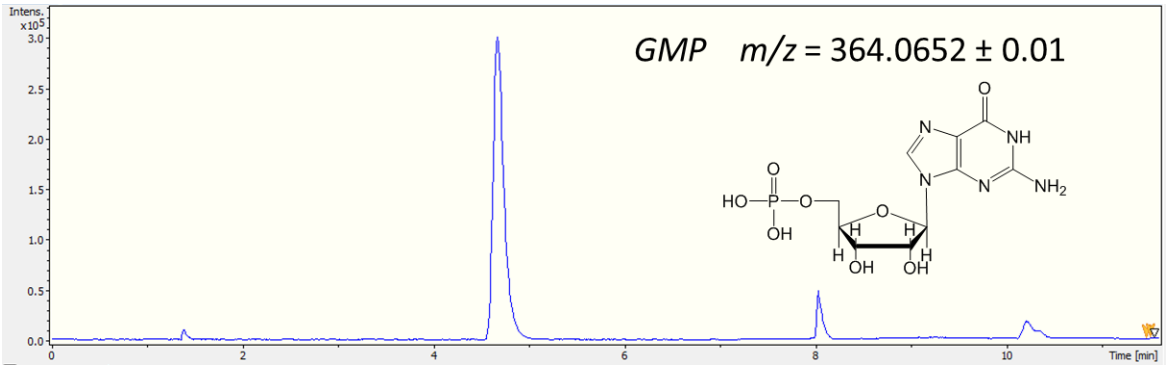


Figure S42. *EICs of guanosine 5´ -monophosphate disodium salt standard 5x10^{-4} M (m/z=364.0652±0.01) analysed with RP-HPLC-ESI-MS-MS.*

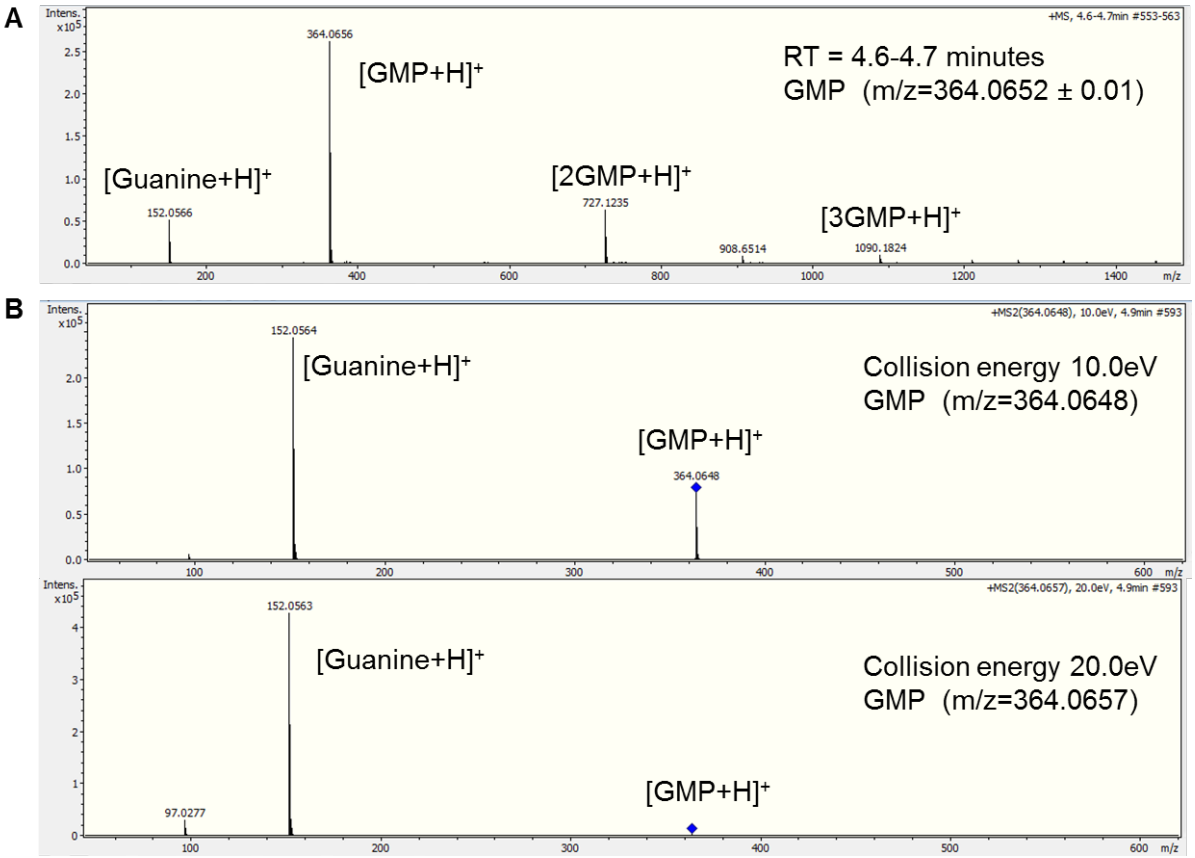


Figure S43. *A) Mass distribution corresponding to the peak at a retention time of 4.6-4.7 minutes in the EIC of GMP standard (showed in Fig. S42). B) MS-MS data for the fragmentation of GMP mass at two different collision energies.*

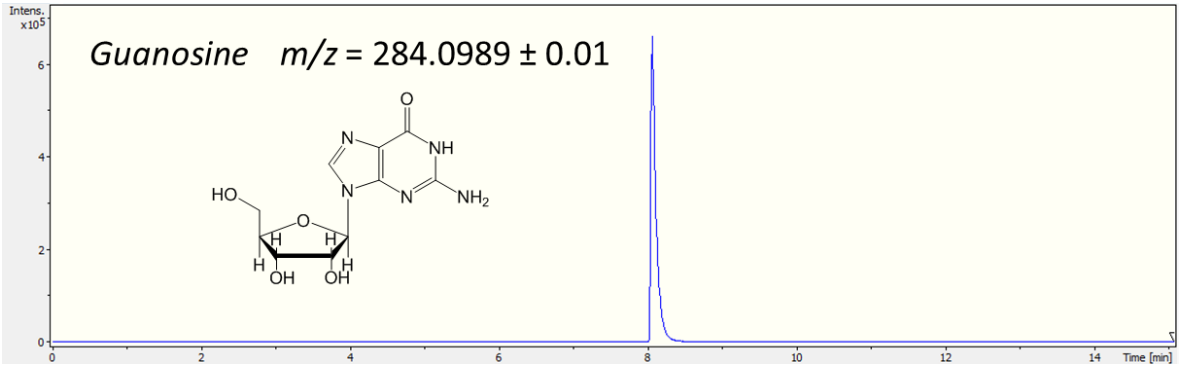


Figure S44. *EICs of guanosine standard 5x10^{-4} M (m/z= 364.0652 ±0.01) analysed with RP-HPLC-ESI-MS-MS.*

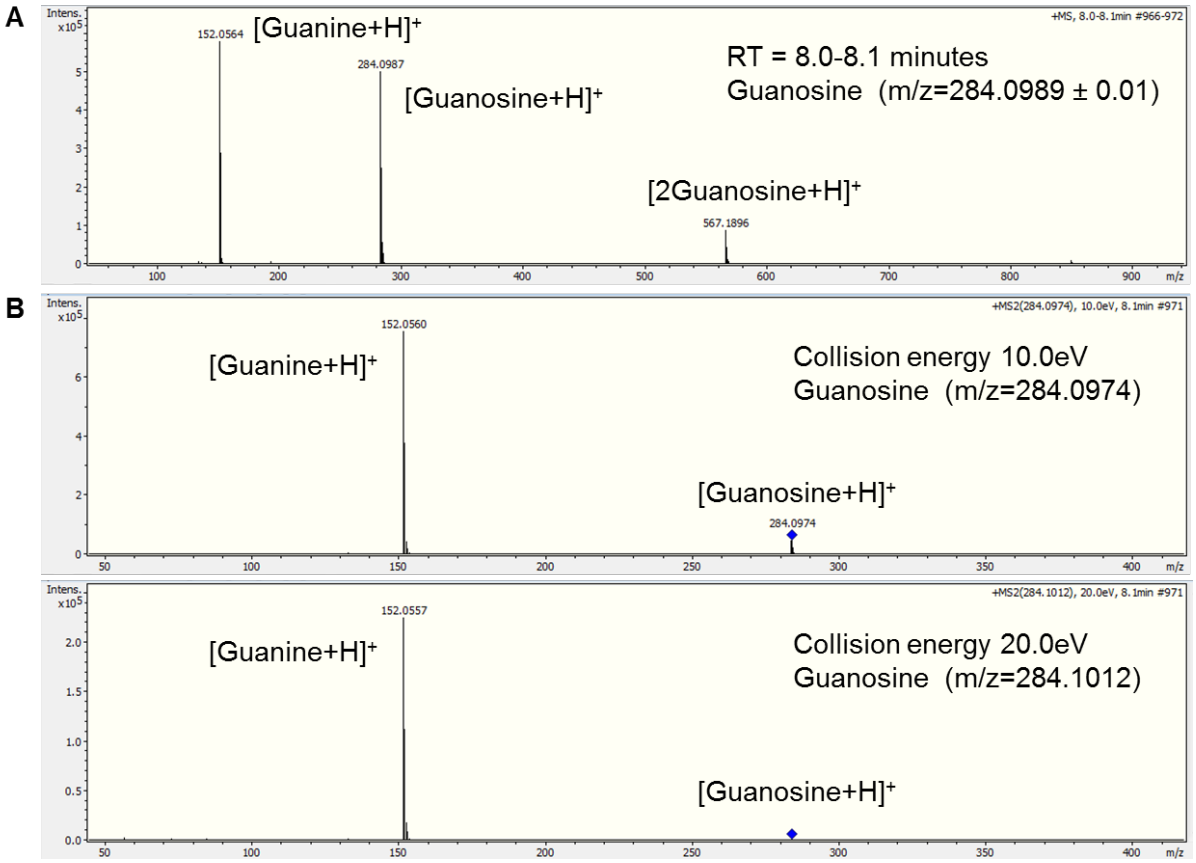


Figure S45. *A) Mass distribution corresponding to the peak at a retention time of 8.0-8.1 minutes in the EIC of guanosine standard (showed in Fig. S44). B) MS-MS data for the fragmentation of guanosine mass at two different collision energies.*

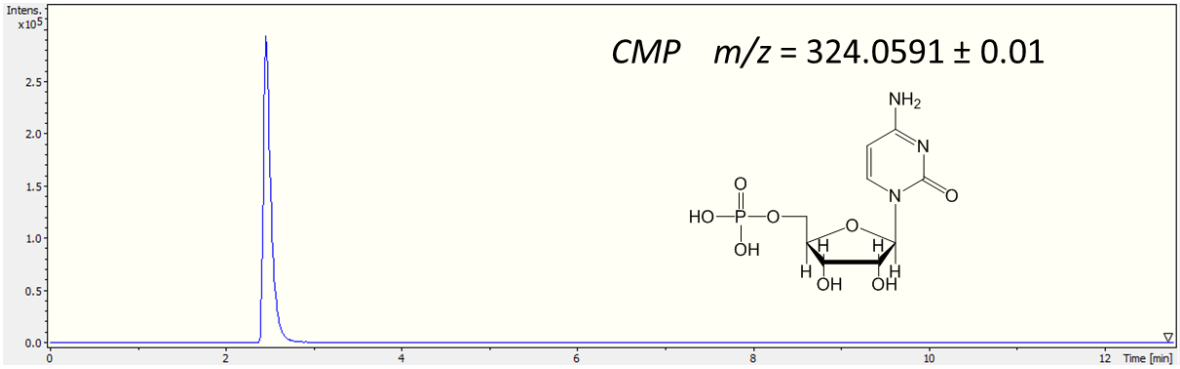


Figure S46. *EICs of CMP standard 1x10^{-4} M (m/z=364.0652±0.01) analysed with RP-HPLC-ESI-MS-MS.*

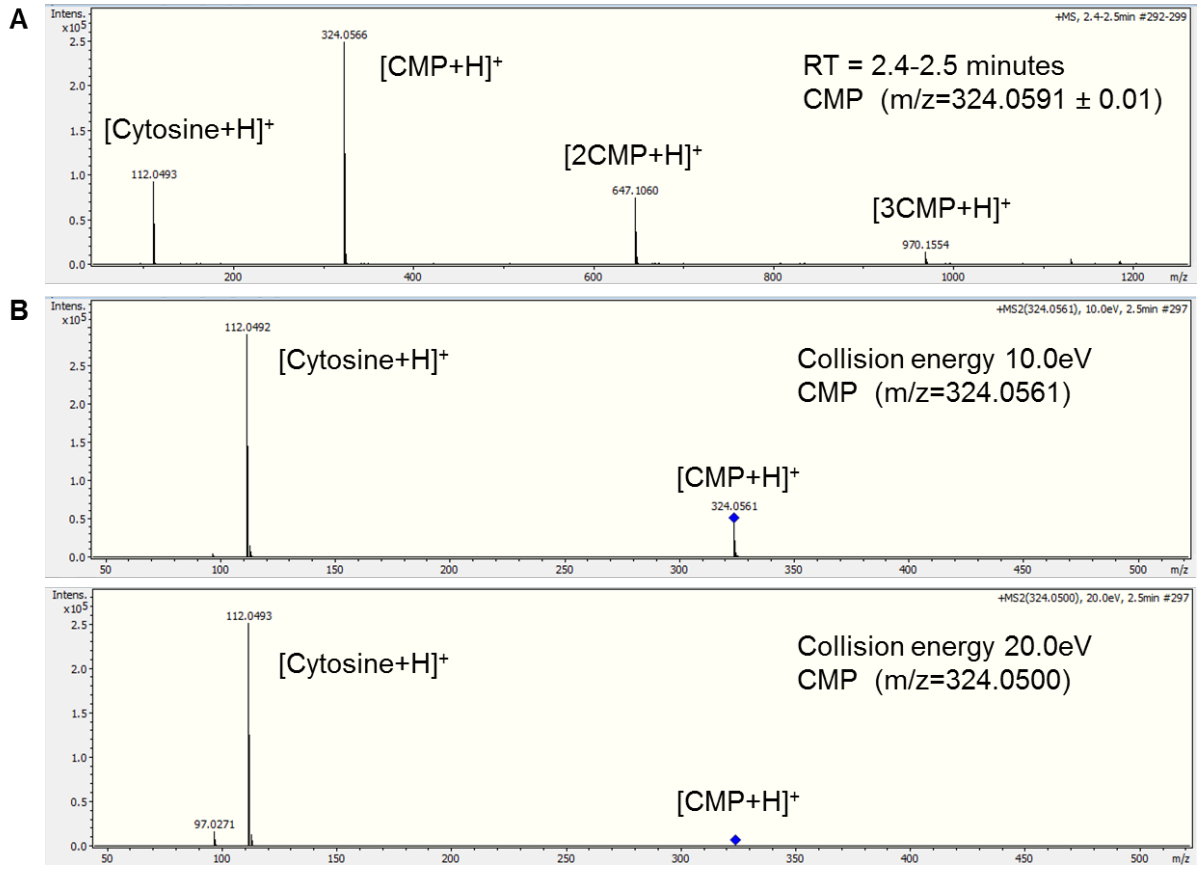


Figure S47. *A) Mass distribution corresponding to the peak at a retention time of 2.4-2.5 minutes in the EIC of CMP standard (showed in Fig. S46). B) MS-MS data for the fragmentation of CMP mass at two different collision energies.*

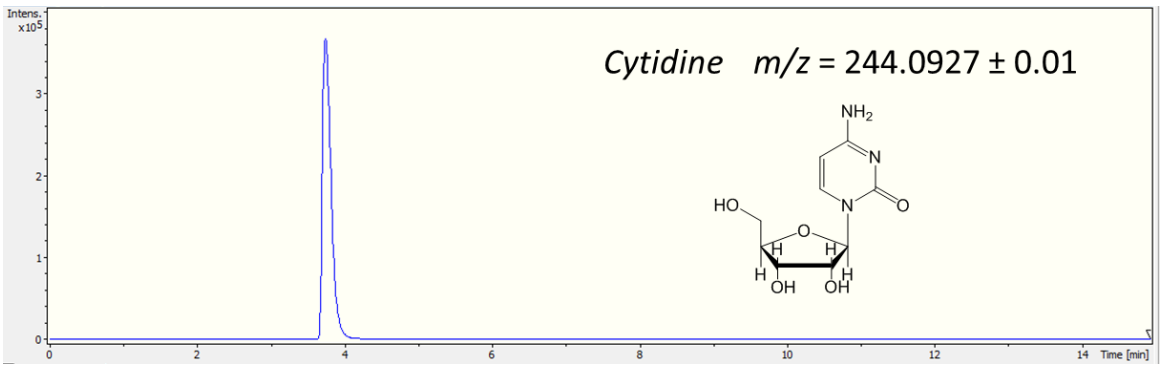


Figure S48. *EICs of cytidine standard 1x10^{-4} M (m/z=364.0652±0.01) analysed with RP-HPLC-ESI-MS-MS.*

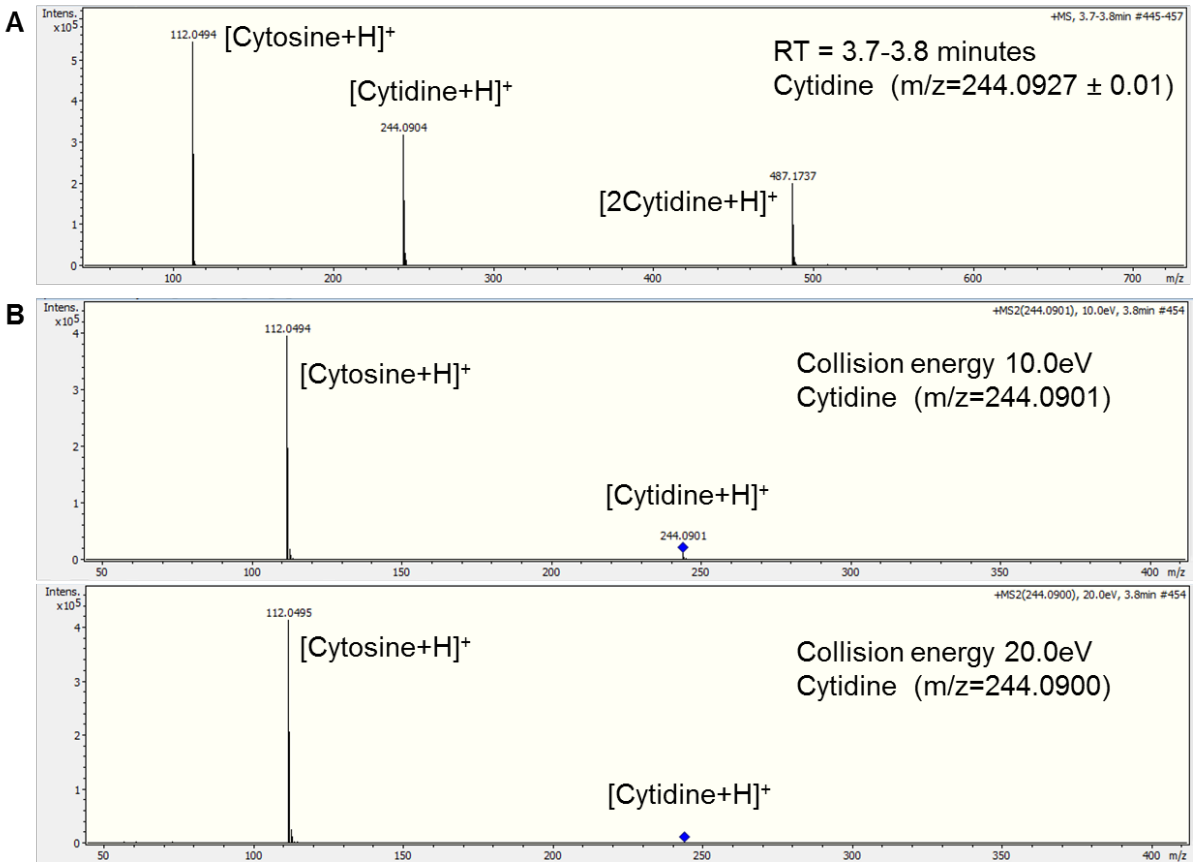


Figure S49. *A) Mass distribution corresponding to the peak at a retention time of 3.7-3.8 minutes in the EIC of cytidine standard (showed in Fig. S48). B) MS-MS data for the fragmentation of cytidine mass at two different collision energies.*

5.2. Possible structures for guanine and cytosine glycosylation products

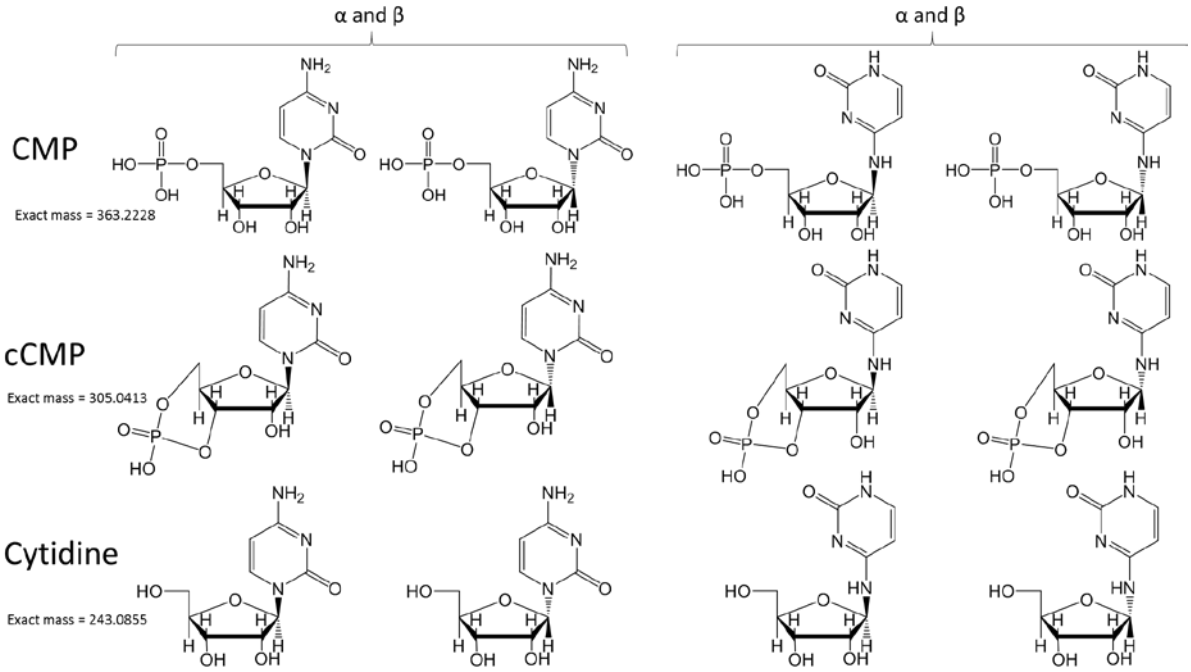


Figure S50. Proposed structures for cytosine glycosylation products. *CMP isomers, Cyclic-CMP isomers and Cytidine isomers.*

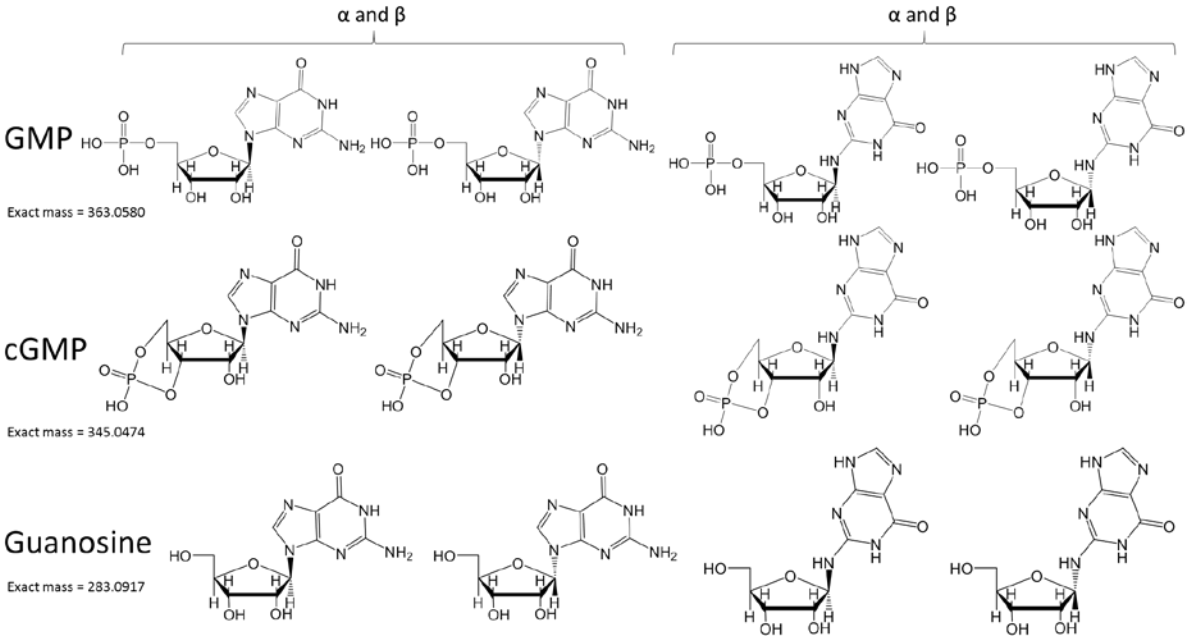


Figure S51. Proposed structures for guanine glycosylation products. *GMP isomers, Cyclic-GMP isomers and Guanosine isomers.*

5.3. Reaction of cytosine/guanine/thymine + P-ribose

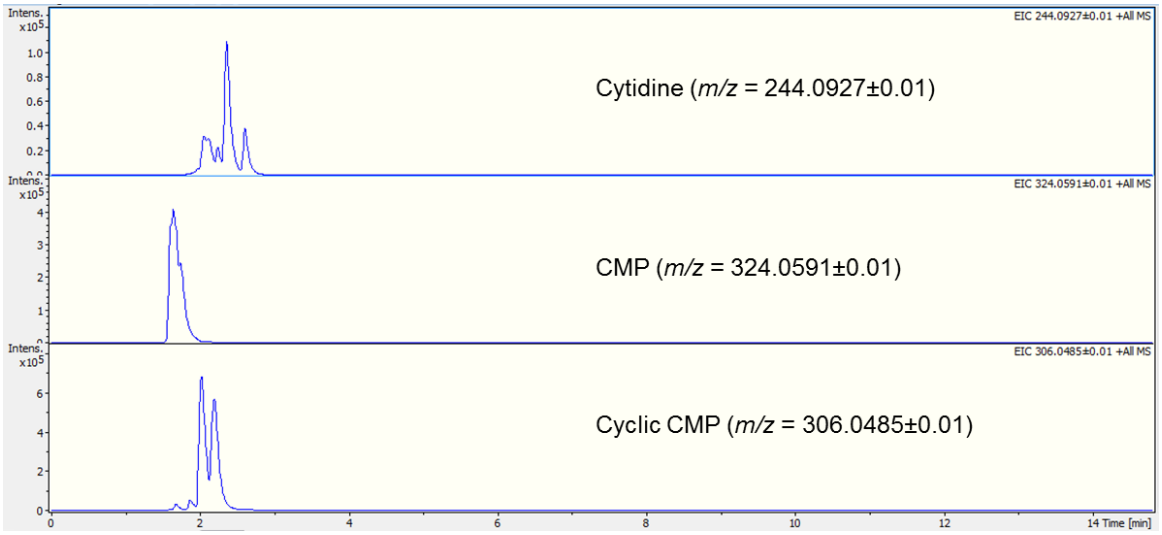


Figure S52. *EICs of cytosine glycosylation products after the reaction of 25 mM P-ribose + 25 mM cytosine for 5 hours at 90˚ C (pH 2.5).*

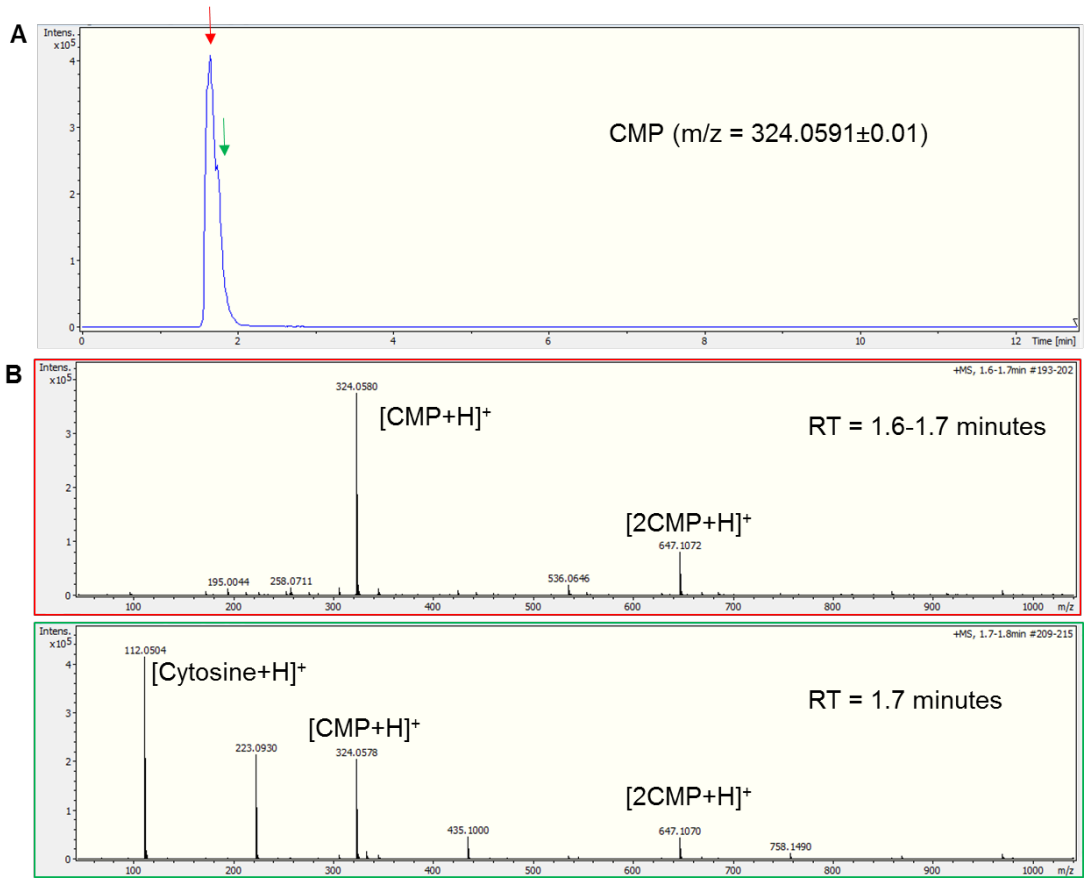


Figure S53. *A) EICs of CMP mass (m/z=324.0591±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

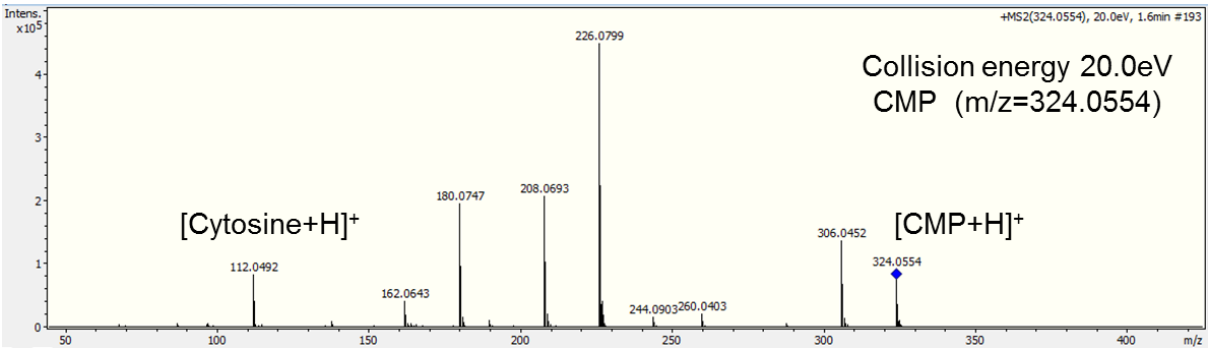


Figure S54. *MS-MS data for the fragmentation of CMP mass at a collision energy of 20.0 eV, corresponding to the most intense peak observed in the EIC shown in Figure 53.A.*

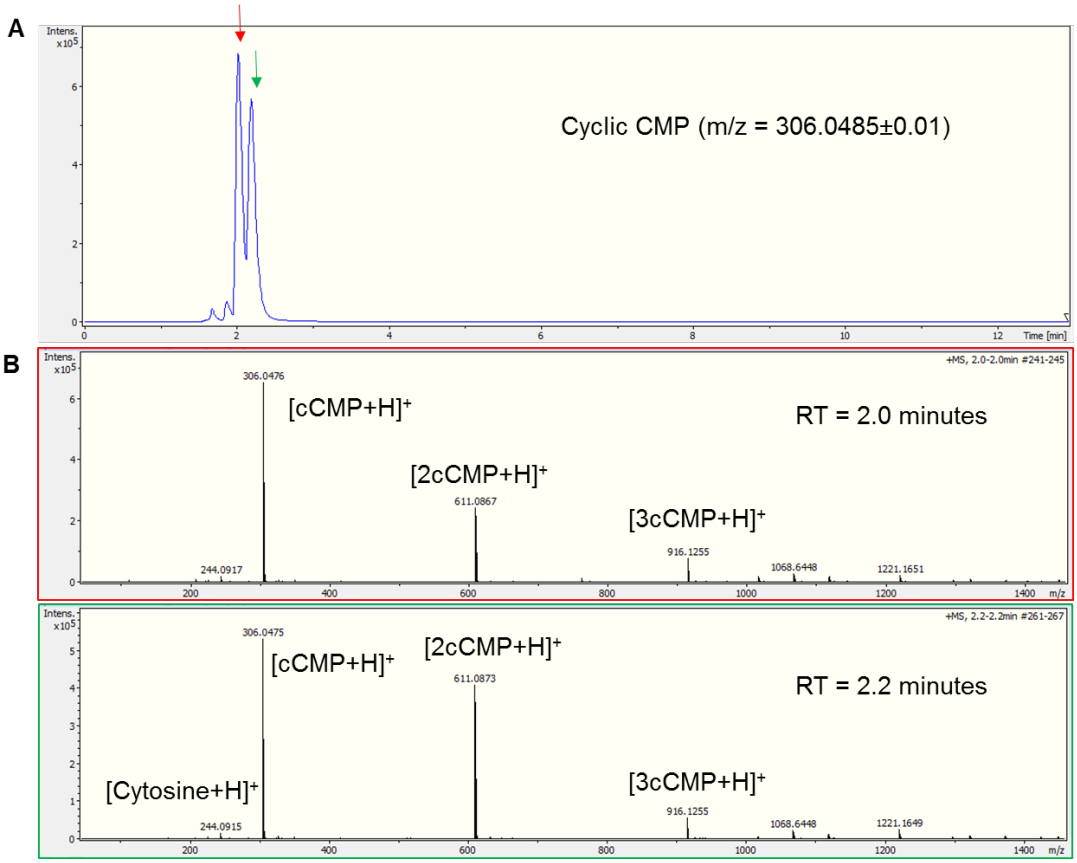


Figure S55. *A) EICs of cyclic CMP mass (m/z=306.0485±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

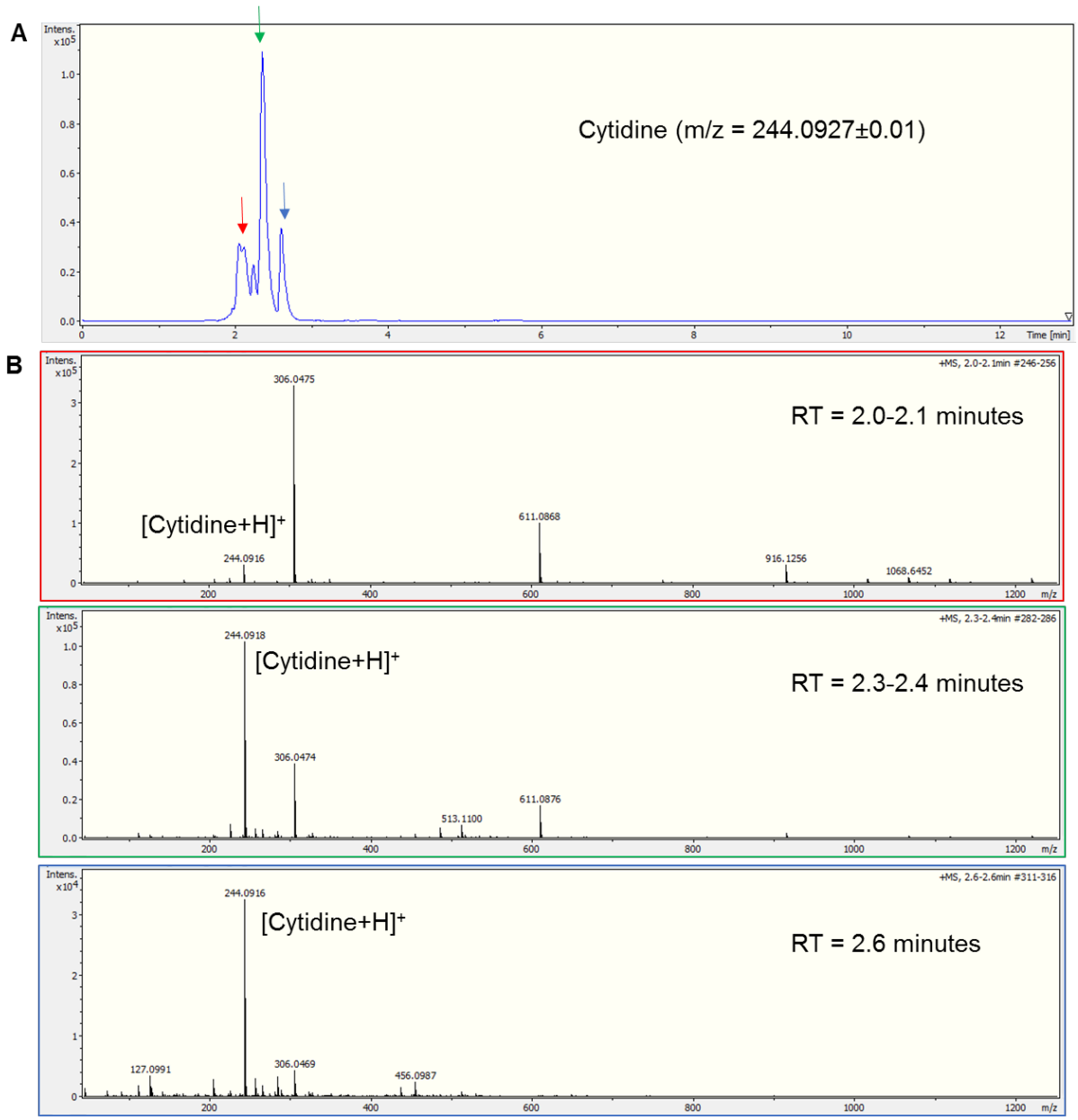


Figure S56. *A) EIC of cytidine mass (m/z=244.0927±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

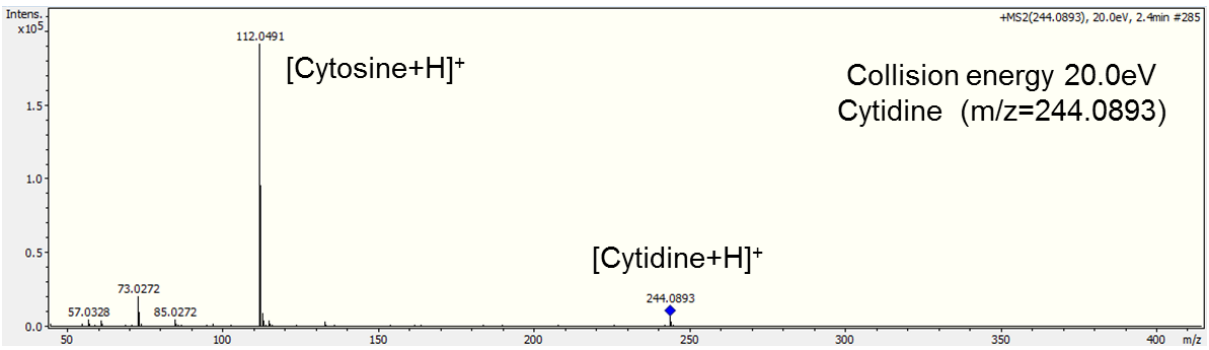


Figure S57. *MS-MS data for the fragmentation of cytidine mass at a collision energy of 20.0 eV, corresponding to the most intense peak observed in the EIC shown in Figure 56.A.*

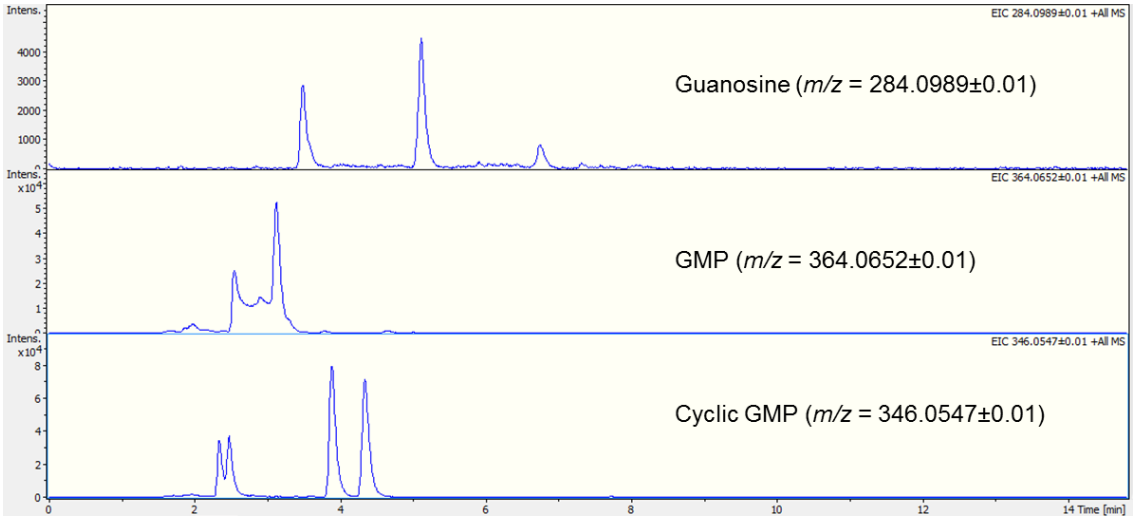


Figure S58. *EICs of guanine glycosylation products after the reaction of 25 mM P-ribose + 25 mM guanine for 5 hours at 90˚C (pH 2.5).*

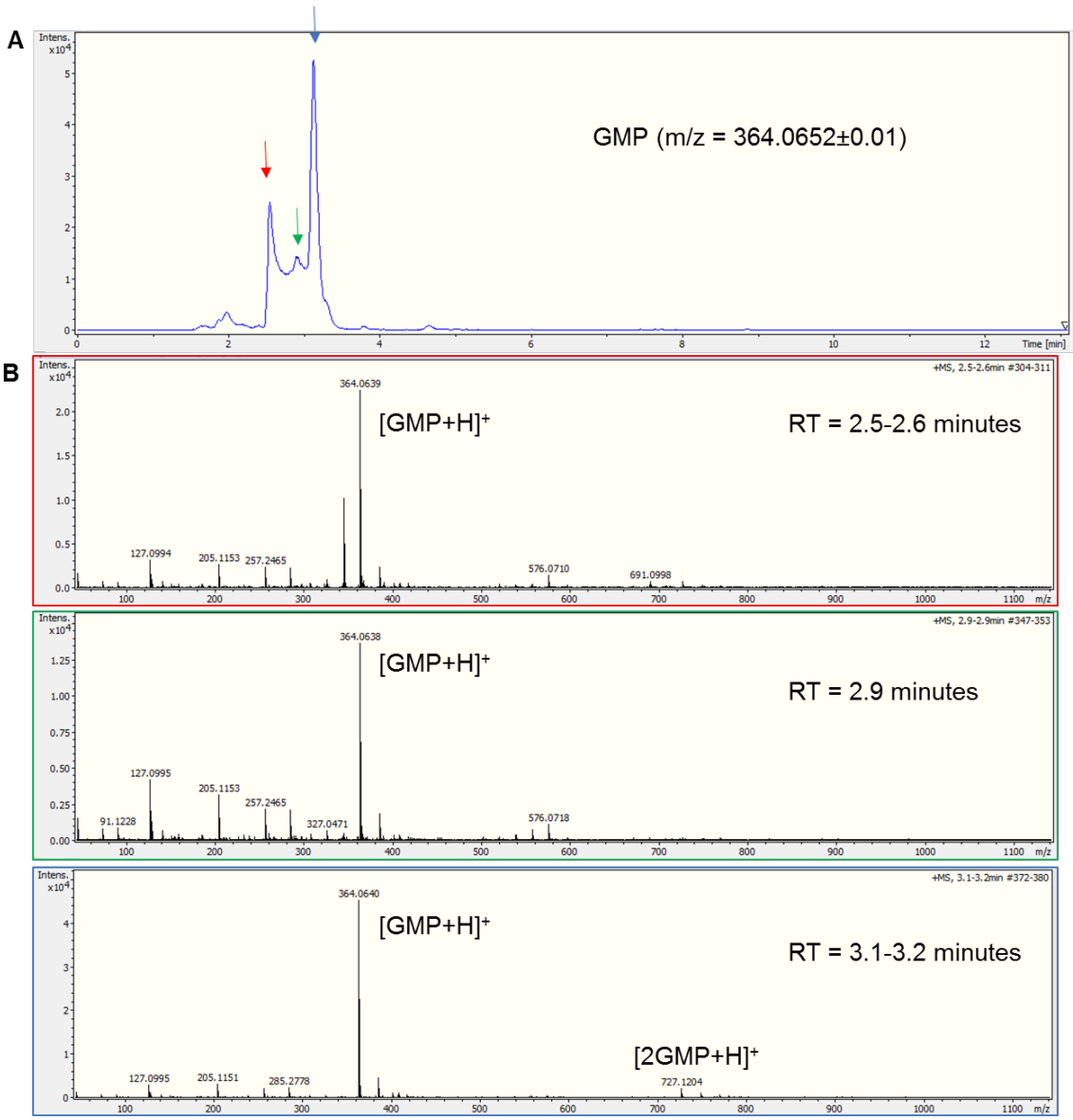


Figure S59. *A) EIC of GMP mass (m/z=364.0652±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

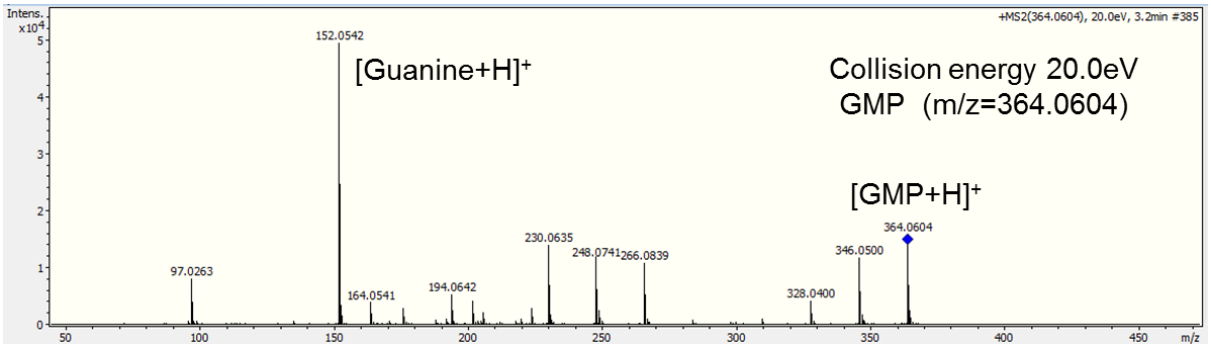


Figure S60. *MS-MS data for the fragmentation of GMP mass at a collision energy of 20.0 eV, corresponding to the most intense peak observed in the EIC shown in Figure 59.A.*

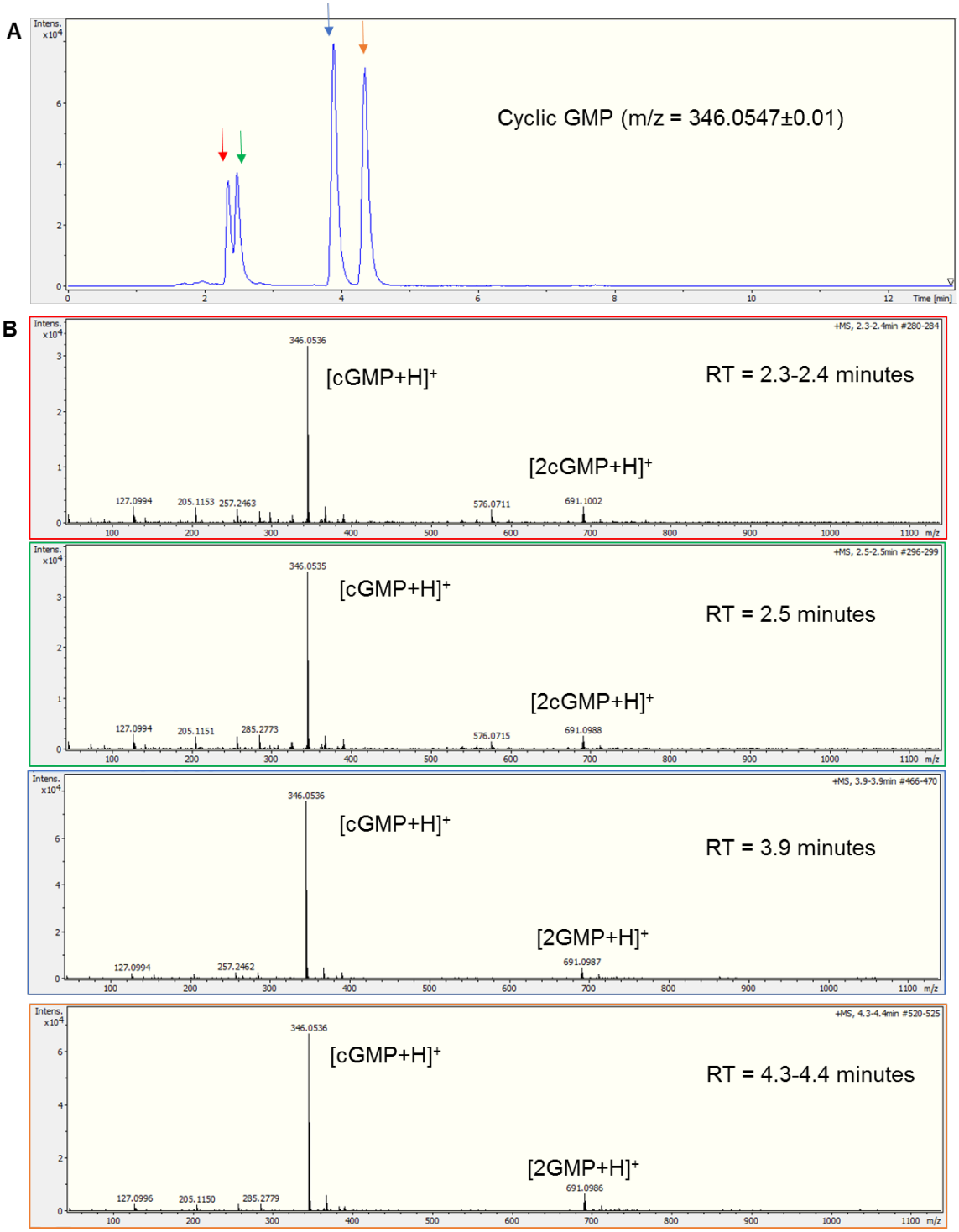


Figure S61. *A) EIC of cyclic GMP mass (m/z=346.0547±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

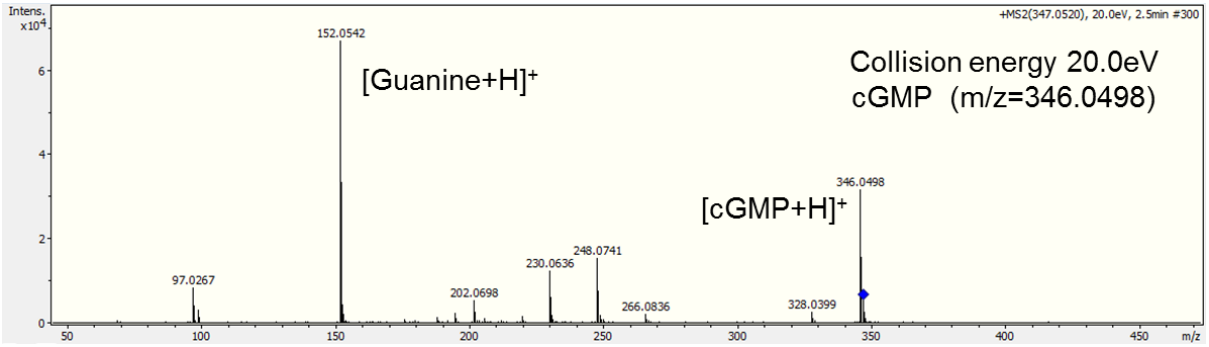


Figure S62. *MS-MS data for the fragmentation of cyclic GMP mass at a collision energy of 20.0 eV, corresponding to the most intense peak observed in the EIC shown in Figure 61.A.*

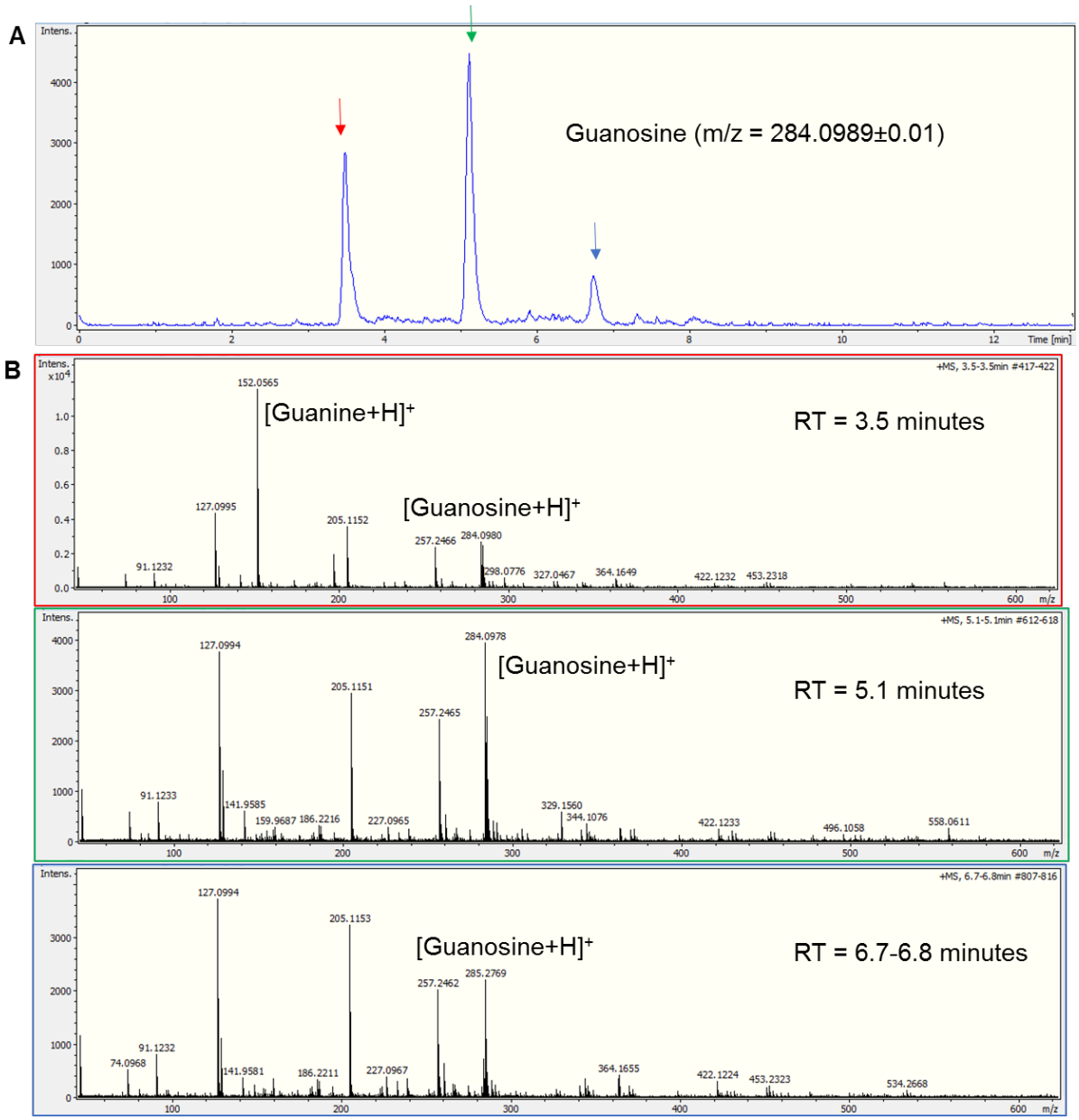


Figure S63. *A) EIC of guanosine mass (m/z=284.0989±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

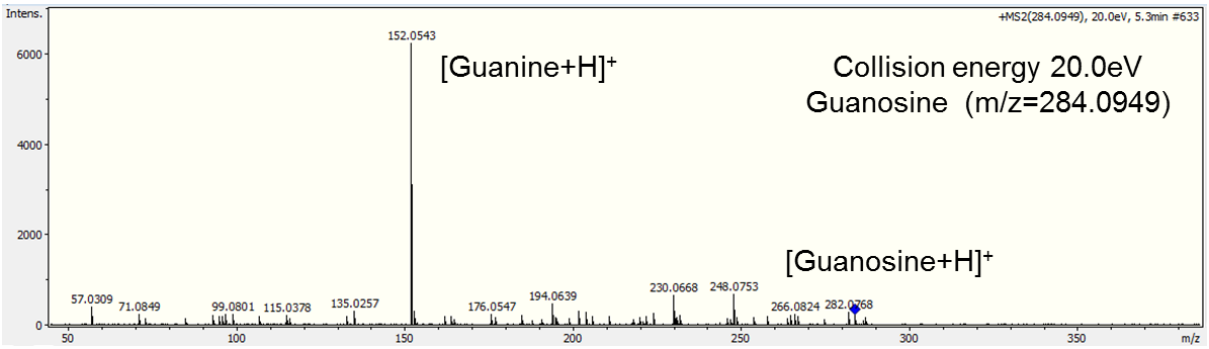


Figure S64. *MS-MS data for the fragmentation of guanosine mass at a collision energy of 20.0 eV, corresponding to the most intense peak observed in the EIC shown in Figure 63.A.*

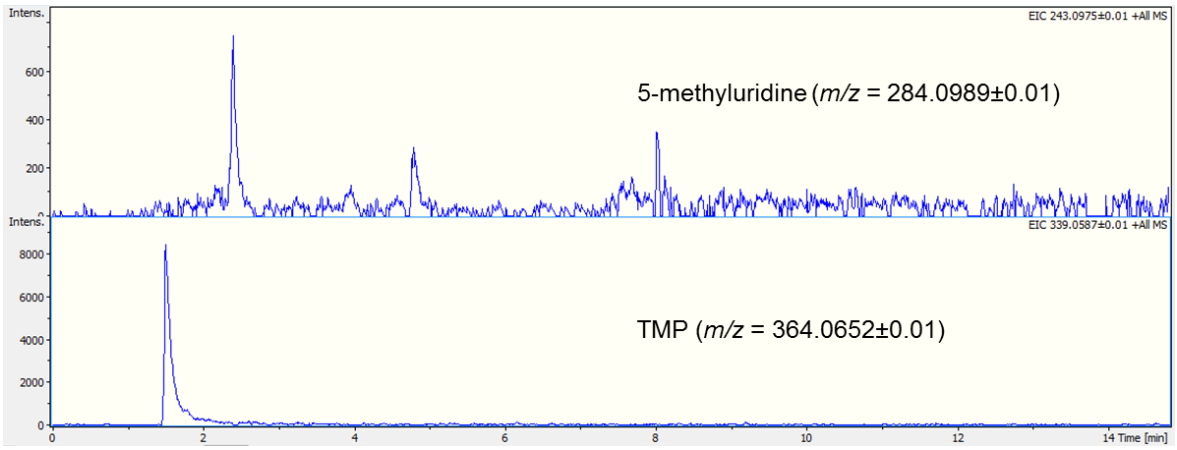


Figure S65. *EICs of thymine glycosylation products after the reaction of 25 mM P-ribose + 25 mM thymine for 5 hours at 90˚C (pH 2.5).*

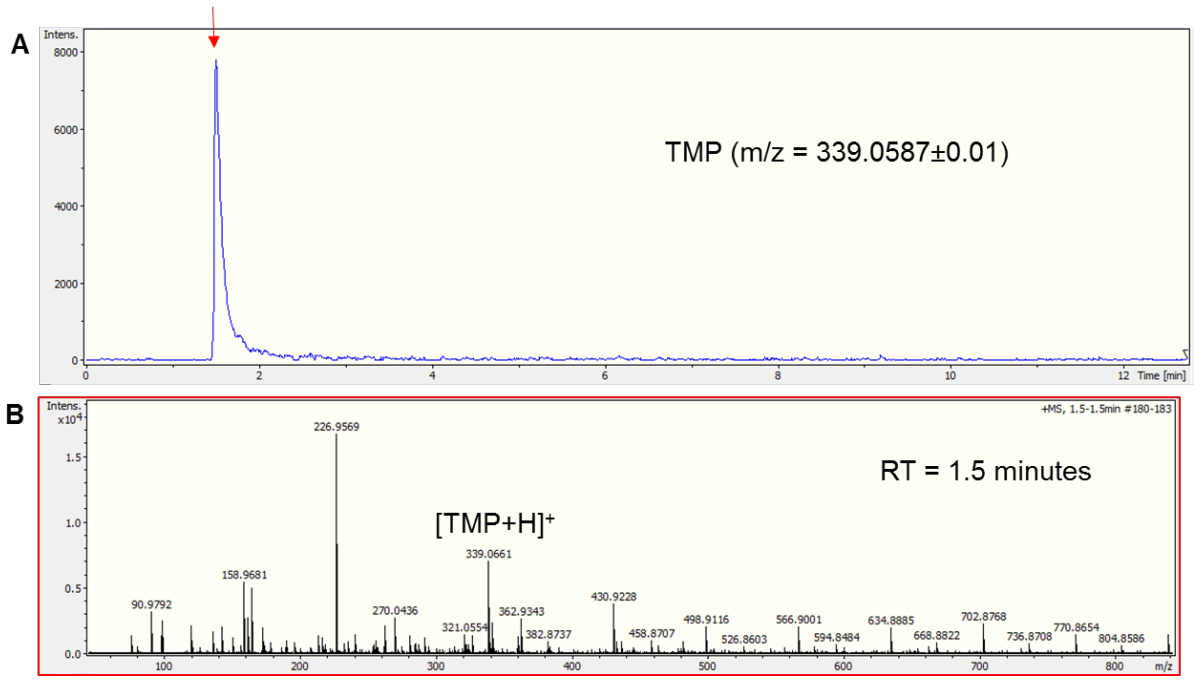


Figure S66. *A) EIC of TMP mass (m/z=339.0587±0.01) after the reaction of 25 mM P-ribose + 25 mM thymine at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

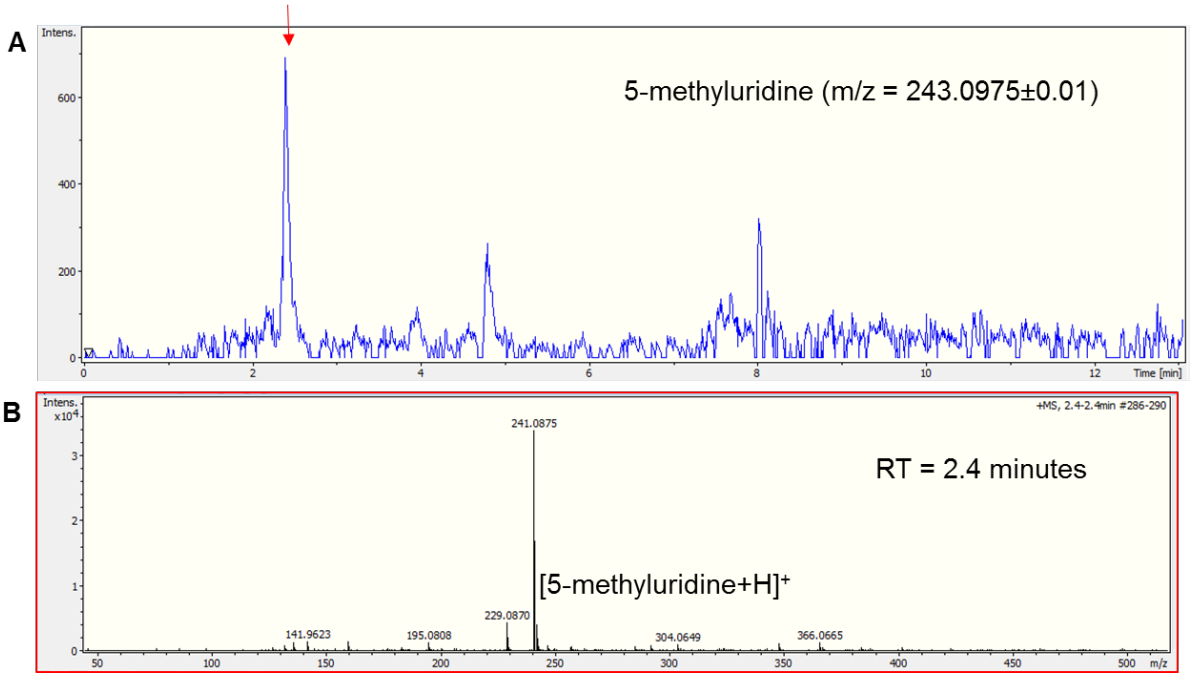


Figure S67. *A) EIC of 5-methyluridine mass (m/z=243.0975±0.01) after the reaction of 25 mM P-ribose + 25 mM thymine at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

5.4. Simultaneous reaction of two or three nucleobases + P-ribose

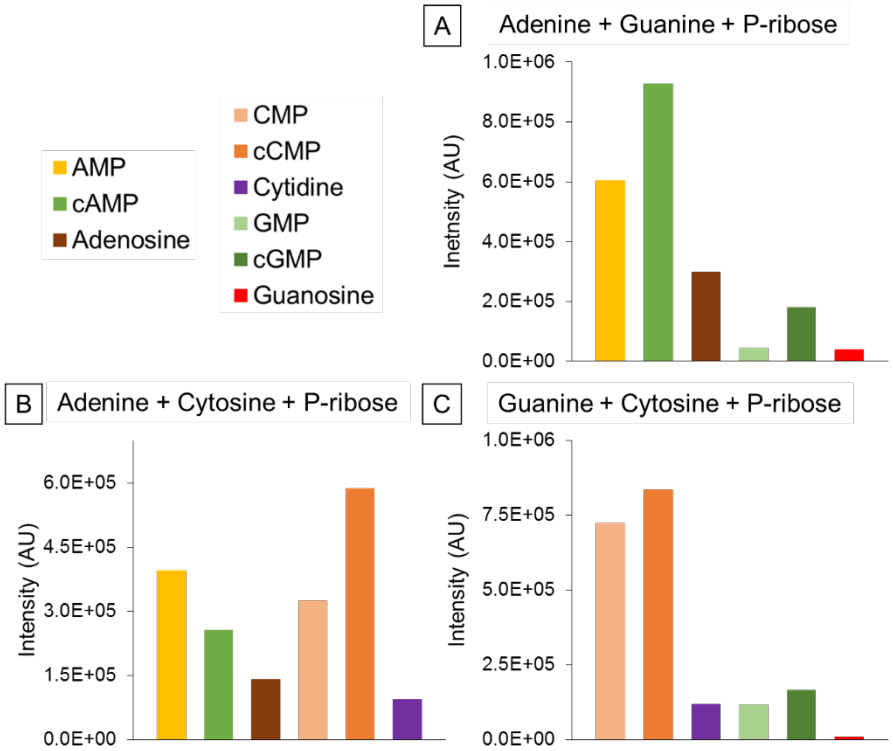


Figure S68. *Formation of glycosylation products when 25 mM D-ribose 5´ -phosphate was heated at 90˚C for 5 hours (pH 2.5) in the presence of (A) 25 mM guanine + 25 mM adenine (B) 25 mM cytosine + 25 mM adenine (C) 25 mM cytosine + 25 mM guanine.*

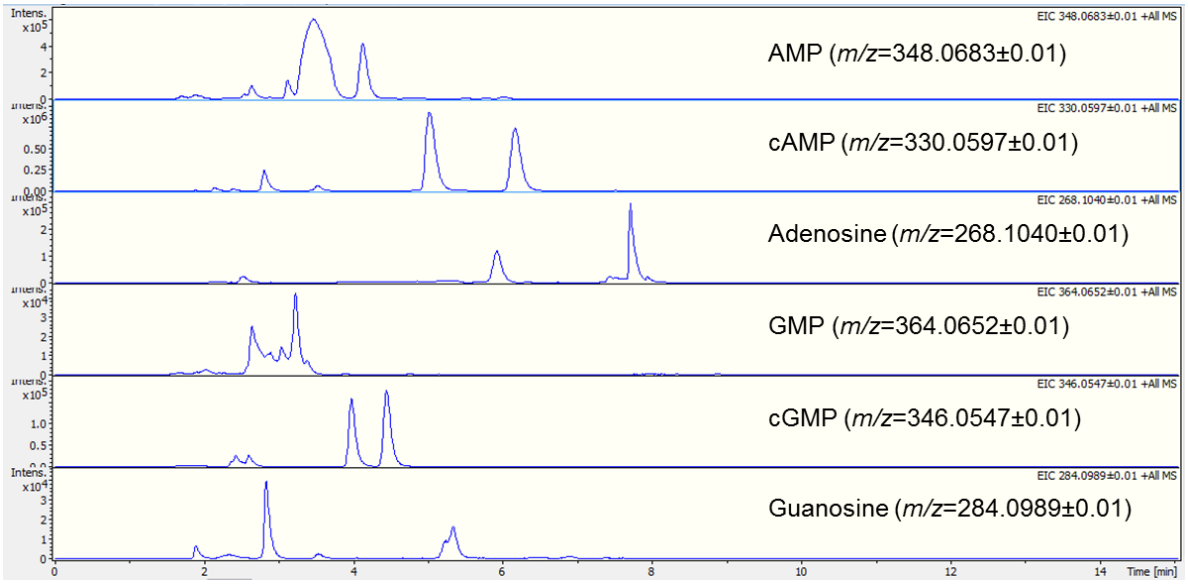


Figure S69. *EICs of adenine and guanine glycosylation products after the reaction of 25 mM P-ribose + 25 mM adenine + 25 mM guanine for 5 hours at 90˚C (pH 2.5).*

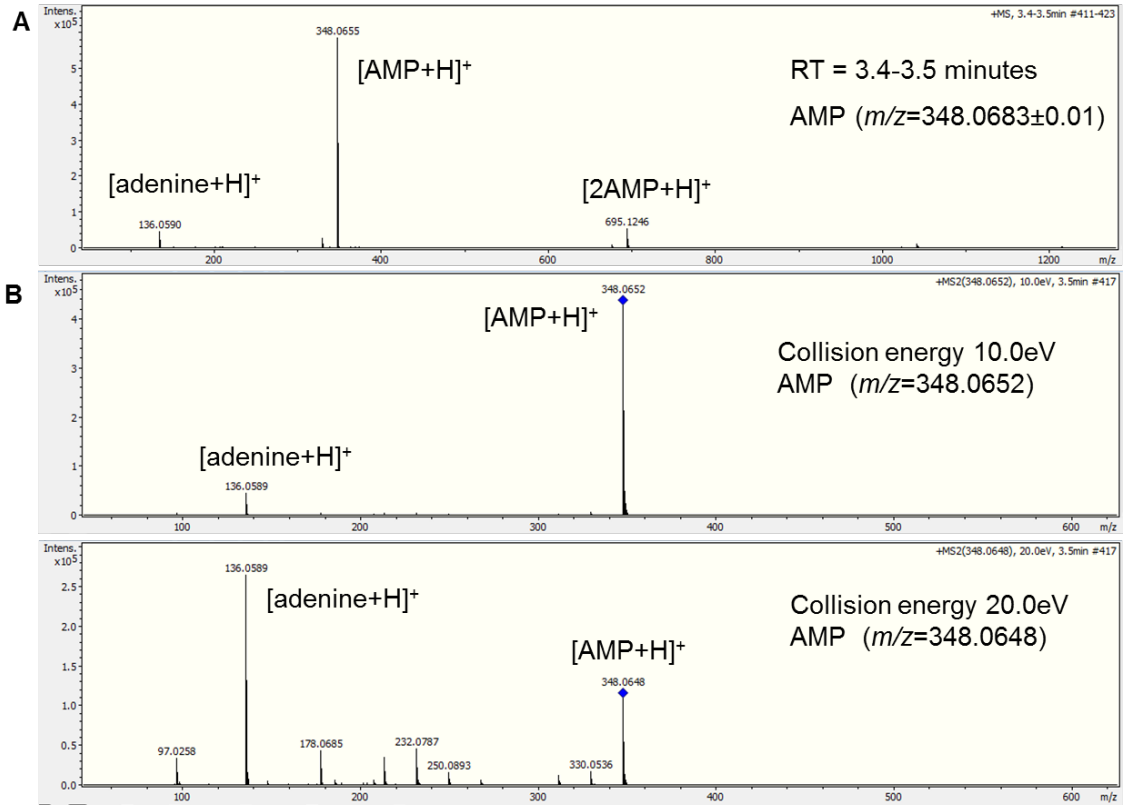


Figure S70. *A) Mass distribution on the most intense peak of AMP EICs in the reaction of 25 mM adenine + 25 mM guanine + 25 mM P-ribose from Figure S64. B) AMP MS-MS data at two different collision energies.*

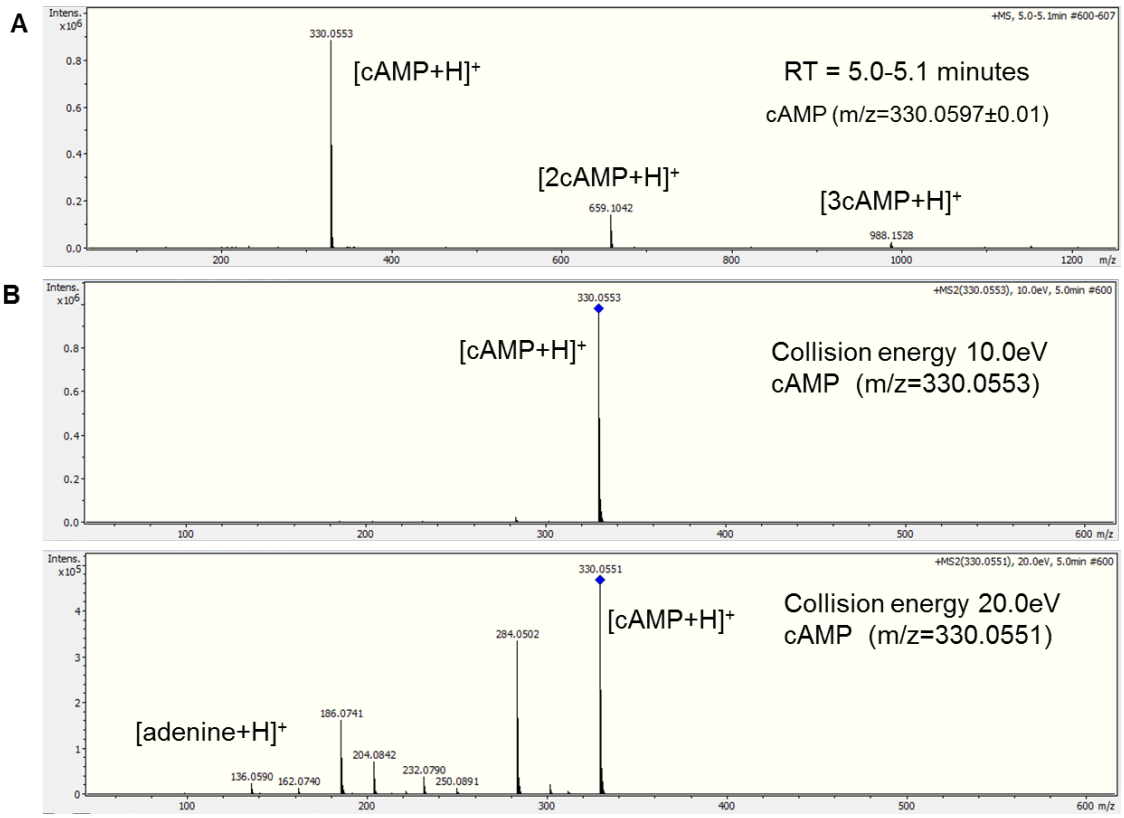


Figure S71. *A) Mass distribution on the most intense peak of cyclic AMP EICs in the reaction of 25 mM adenine + 25 mM guanine + 25 mM P-ribose from figure S64. B) Cyclic AMP MS-MS data at two different collision energies.*

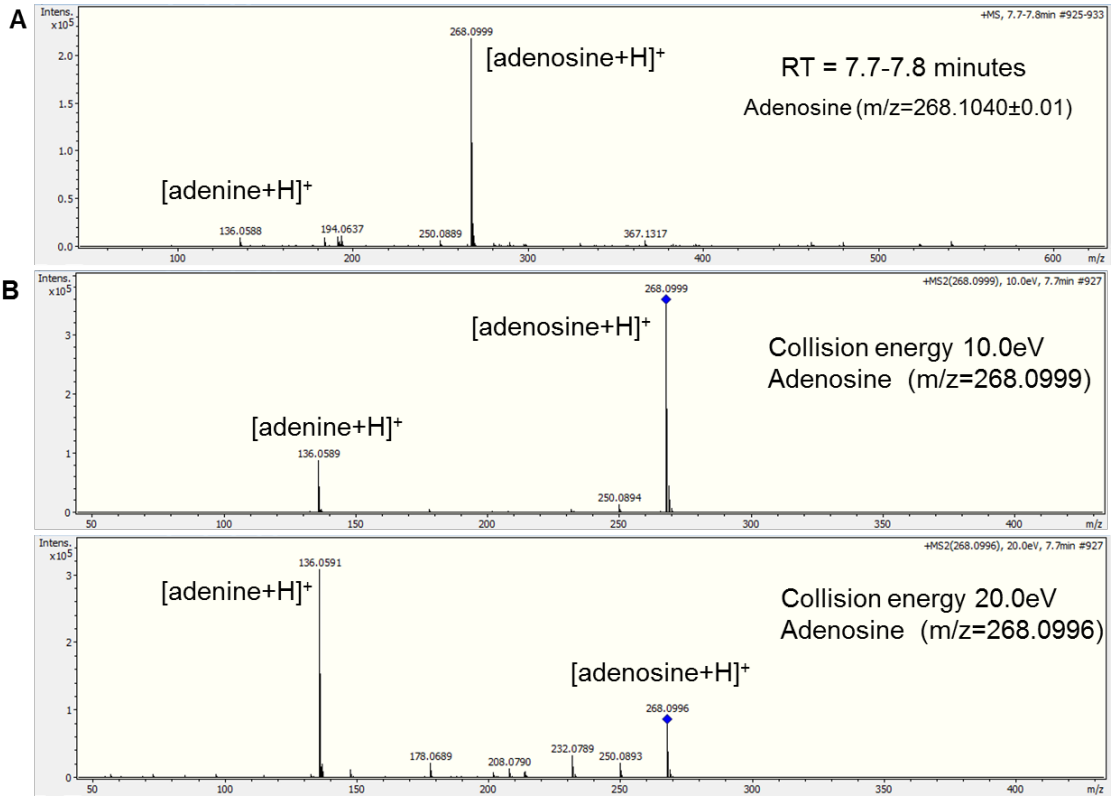


Figure S72. *A) Mass distribution on the most intense peak of adenosine extracted ion chromatograms in the reaction of 25 mM adenine + 25 mM guanine + 25 mM P-ribose from Figure S64. B) Adenosine MS-MS data at two different collision energies.*

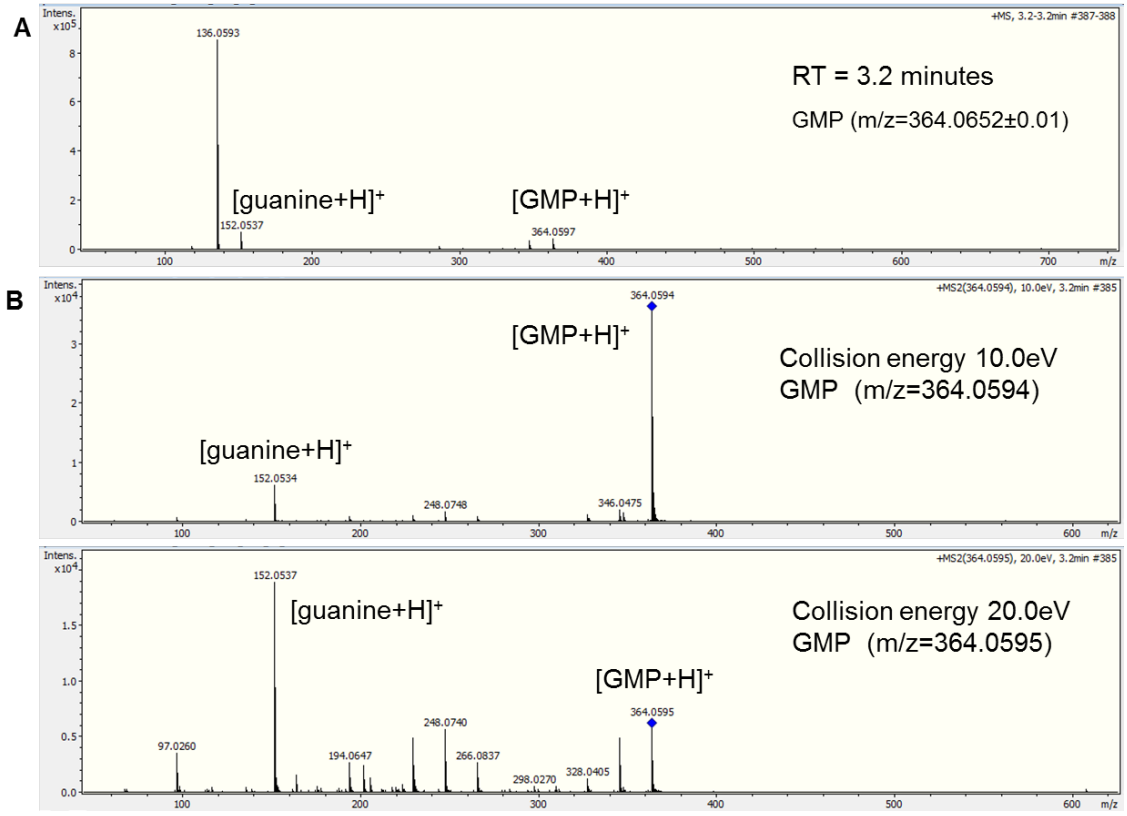


Figure S73. *A) Mass distribution on the most intense peak of GMP EICs in the reaction of 25 mM adenine + 25 mM guanine + 25 mM P-ribose from Figure S64. B) GMP MS-MS data at two different collision energies.*

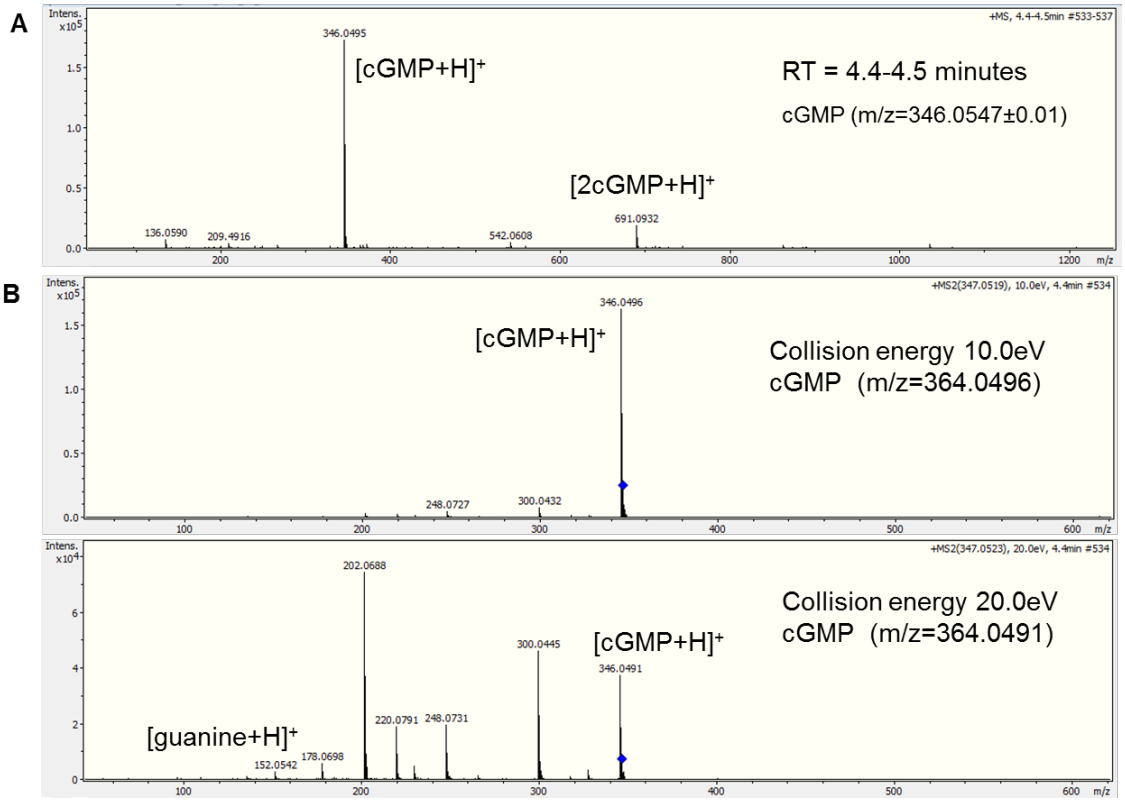


Figure S74. *A) Mass distribution on the most intense peak of cyclic GMP EICs in the reaction of 25 mM adenine + 25 mM guanine + 25 mM P-ribose from Figure S64. B) Cyclic GMP MS-MS data at two different collision energies.*

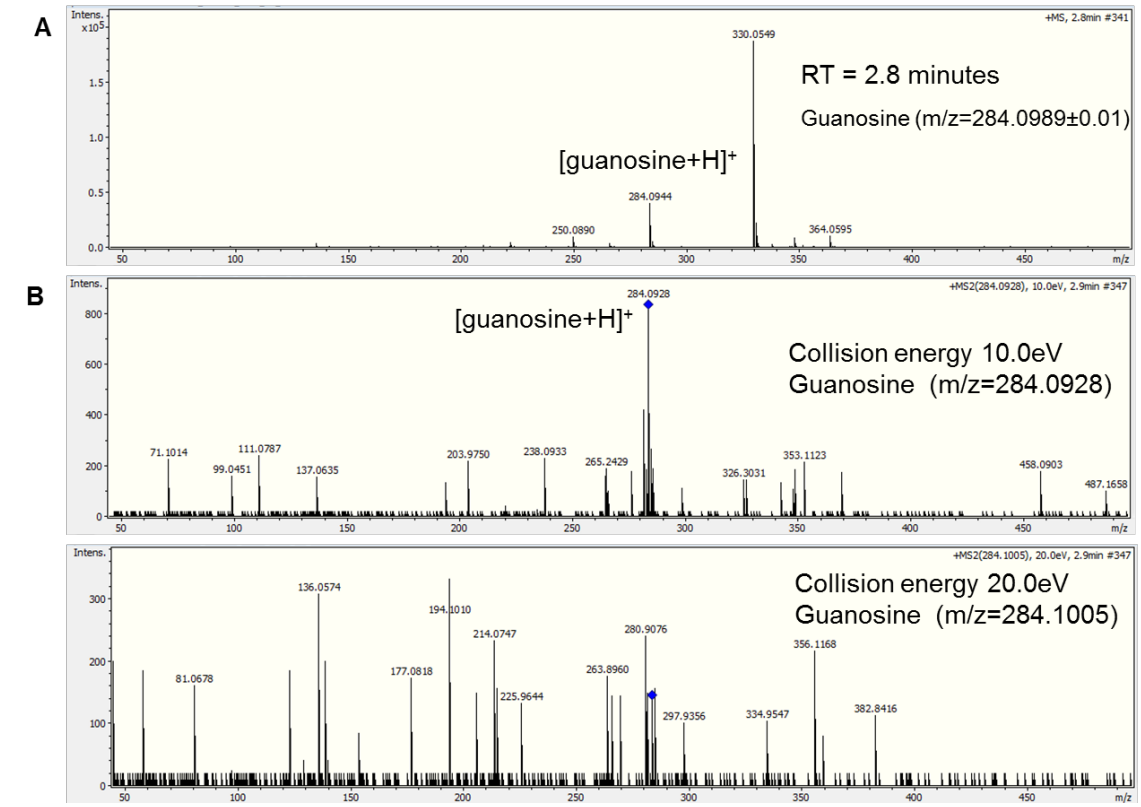


Figure S75. *A) Mass distribution on the most intense peak of guanosine EICs in the reaction of 25 mM adenine + 25 mM guanine + 25 mM P-ribose from Figure S64. B) Guanosine MS-MS data at two different collision energies.*

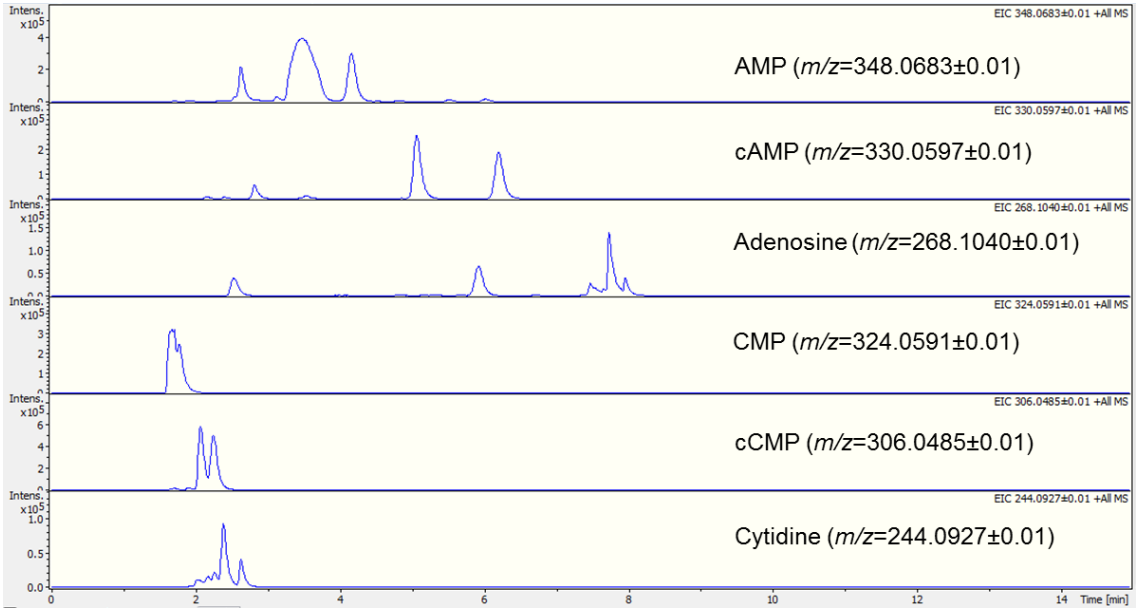


Figure S76. *EICs of adenine and guanine glycosylation products after the reaction of 25 mM P-ribose + 25 mM adenine + 25 mM cytosine for 5 hours at 90˚C (pH 2.5).*

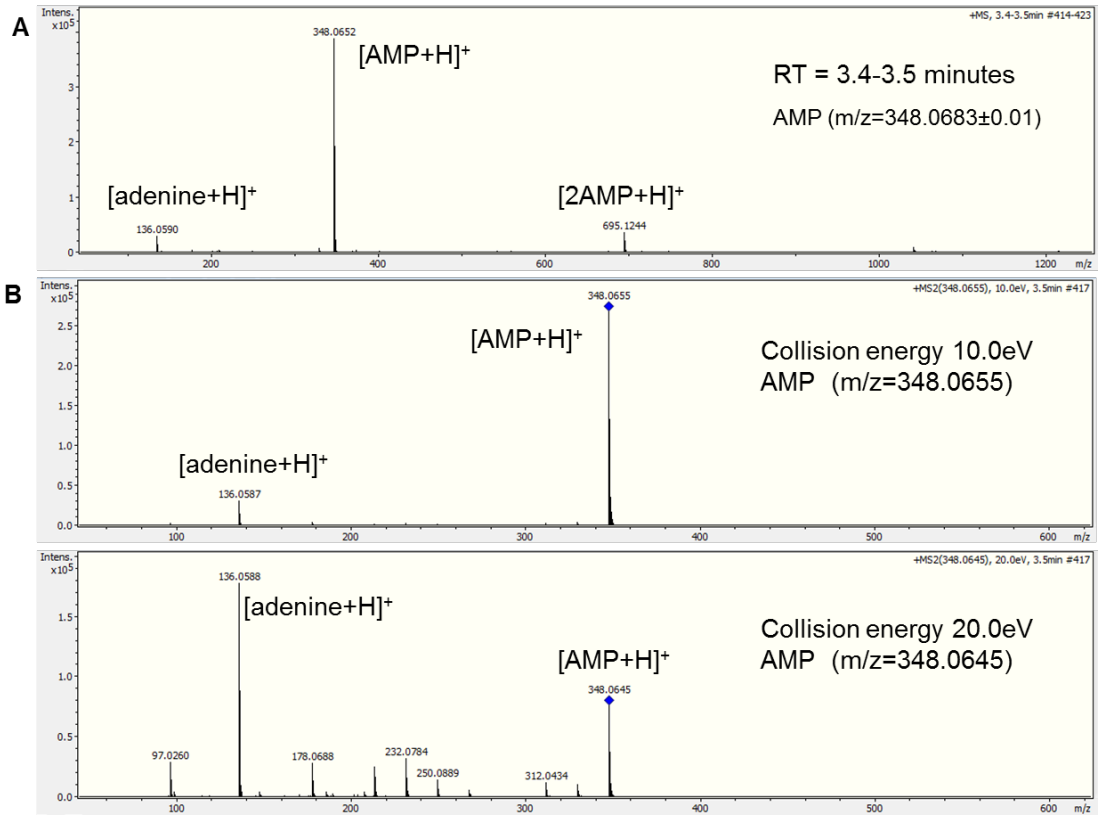


Figure S77. *A) Mass distribution on the most intense peak of AMP EICs in the reaction of 25 mM adenine + 25 mM cytosine + 25 mM P-ribose from Figure S71. B) AMP MS-MS data at two different collision energies.*

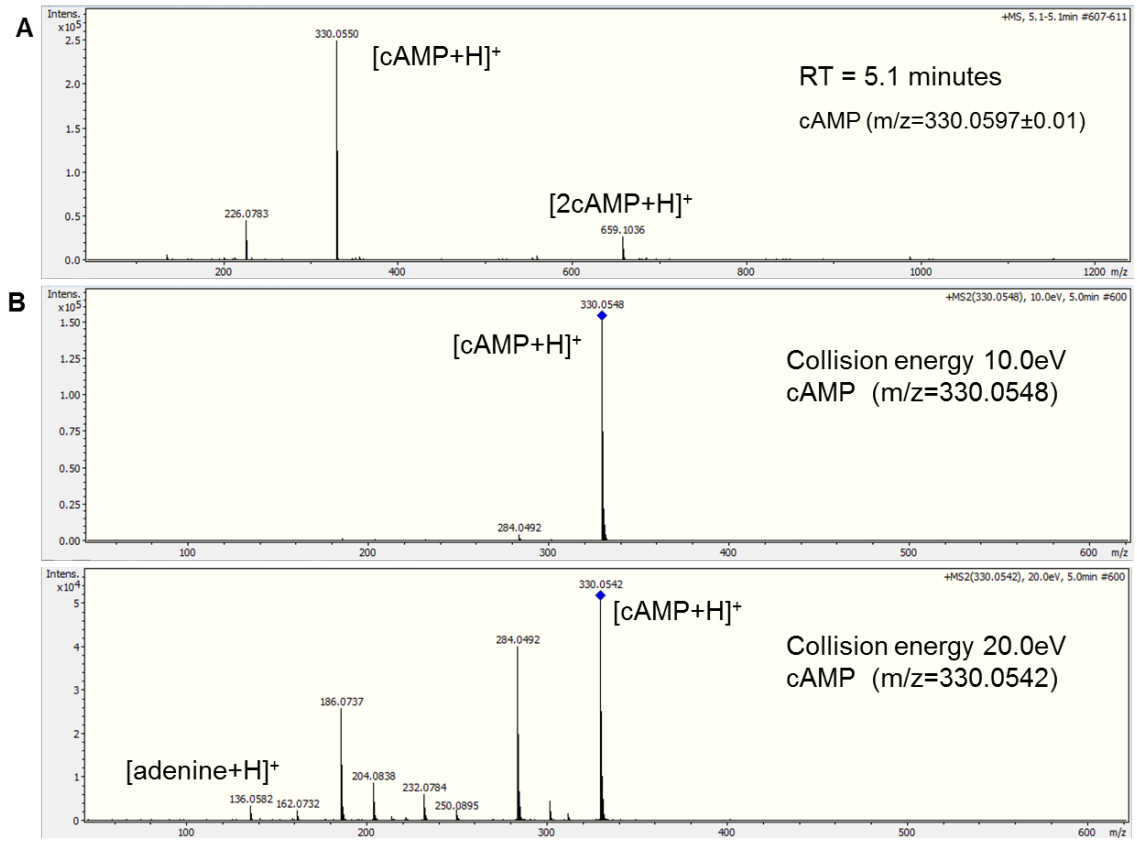


Figure S78. *A) Mass distribution on the most intense peak of cyclic AMP EICs in the reaction of 25 mM adenine + 25 mM cytosine + 25 mM P-ribose from Figure S71. B) Cyclic AMP MS-MS data at two different collision energies.*

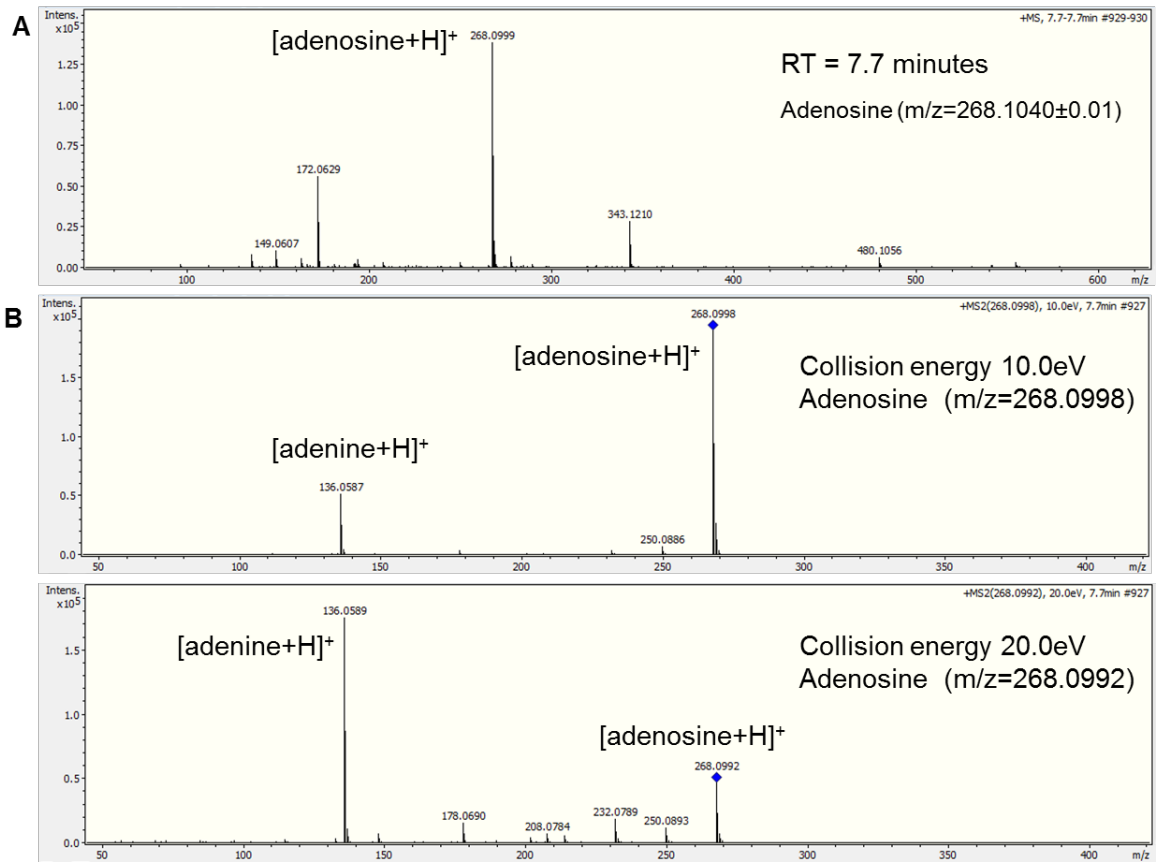


Figure S79. *A) Mass distribution on the most intense peak of adenosine EICs in the reaction of 25 mM adenine + 25 mM cytosine + 25 mM P-ribose from Figure S71. B) Adenosine MS-MS data at two different collision energies.*

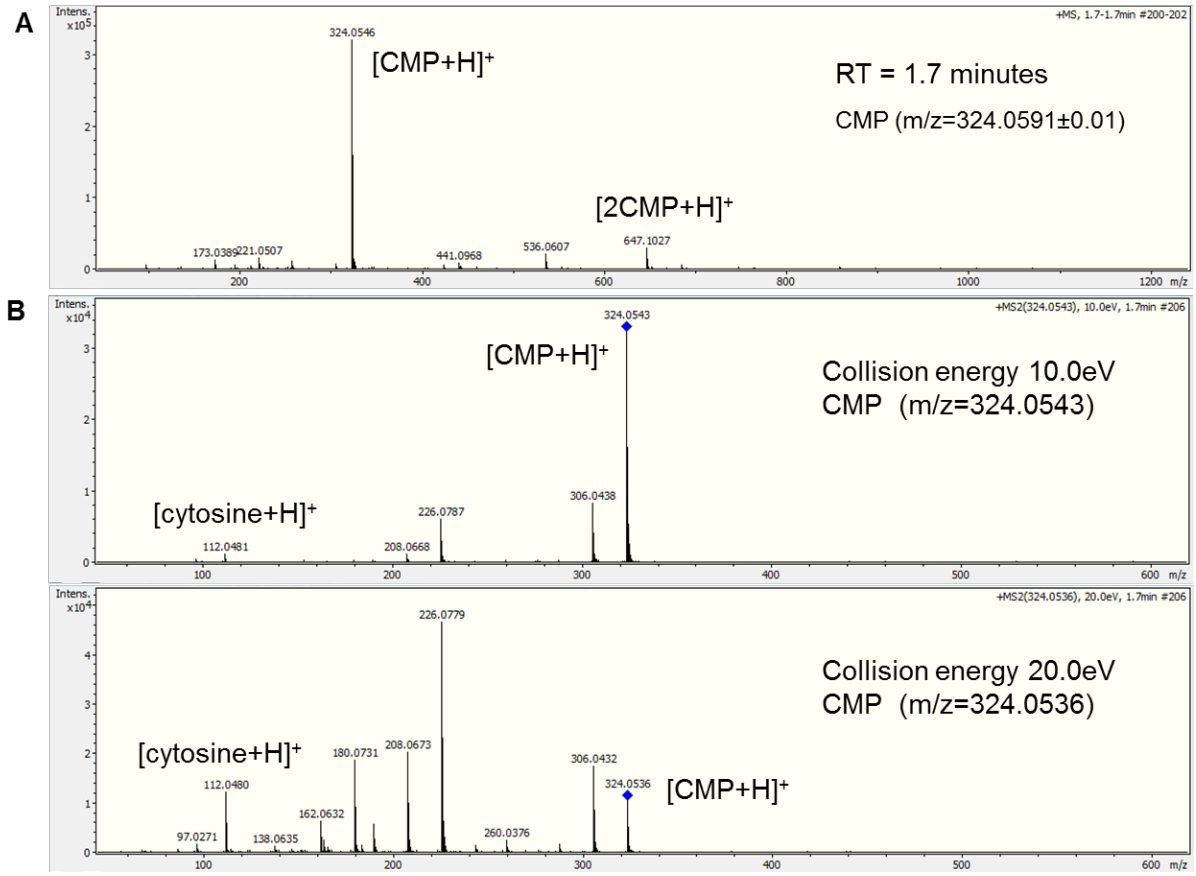


Figure S80. *A) Mass distribution on the most intense peak of CMP EICs in the reaction of 25 mM adenine + 25 mM cytosine + 25 mM P-ribose from Figure S71. B) CMP MS-MS data at two different collision energies.*

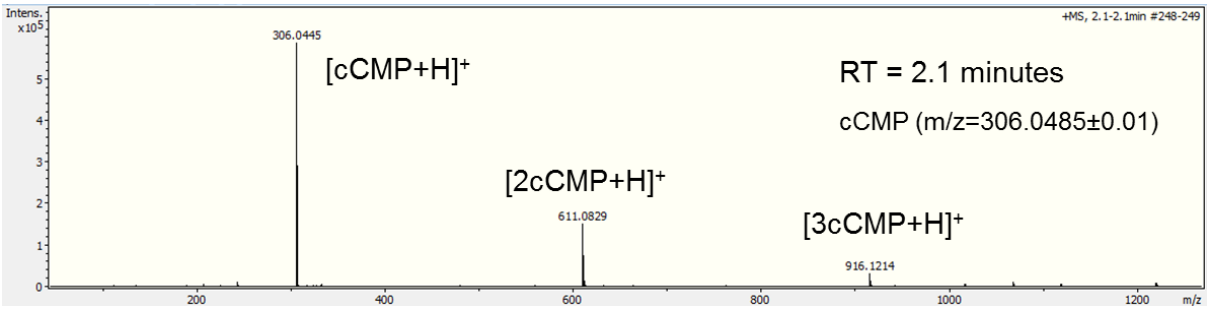


Figure S81. *Mass distribution on the most intense peak of cyclic CMP EICs in the reaction of 25 mM adenine + 25 mM cytosine + 25 mM P-ribose from Figure S71.*

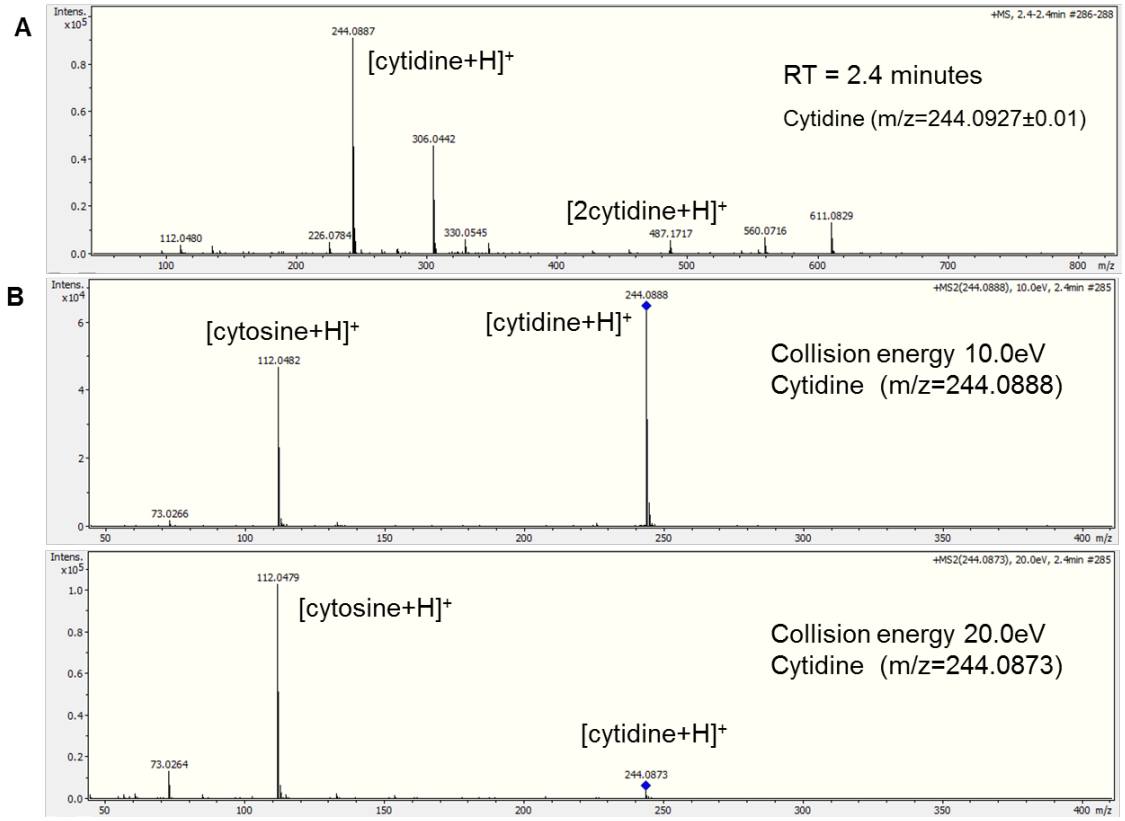


Figure S82. *A) Mass distribution on the most intense peak of cytidine EICs in the reaction of 25 mM adenine + 25 mM cytosine + 25 mM P-ribose from Figure S71. B) Cytidine data at two different collision energies.*

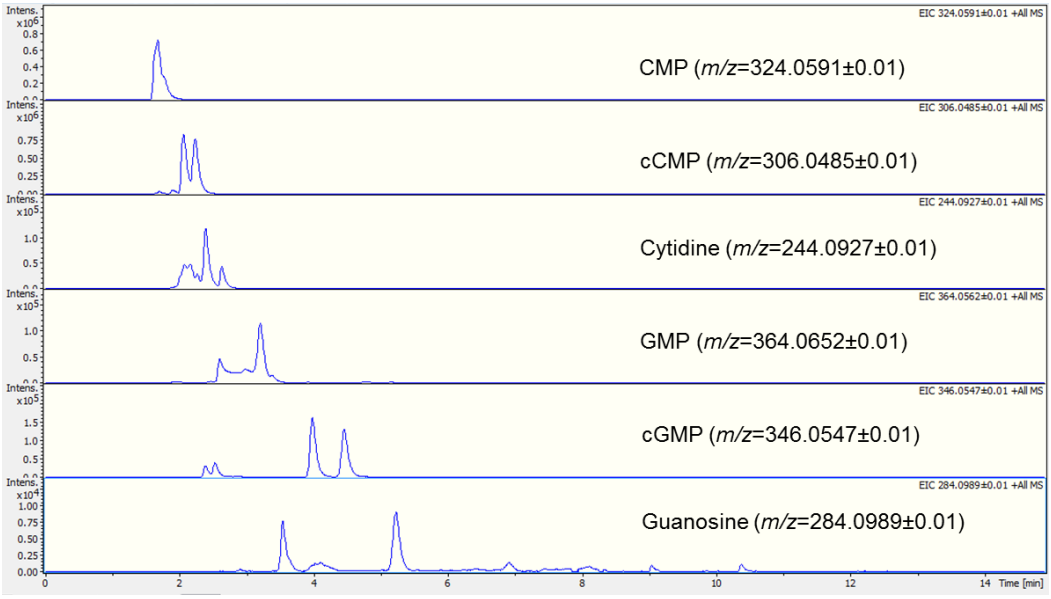


Figure S83. *EICs of adenine and guanine glycosylation products after the reaction of 25 mM P-ribose + 25 mM cytosine + 25 mM guanine for 5 hours at 90˚C (pH 2.5).*

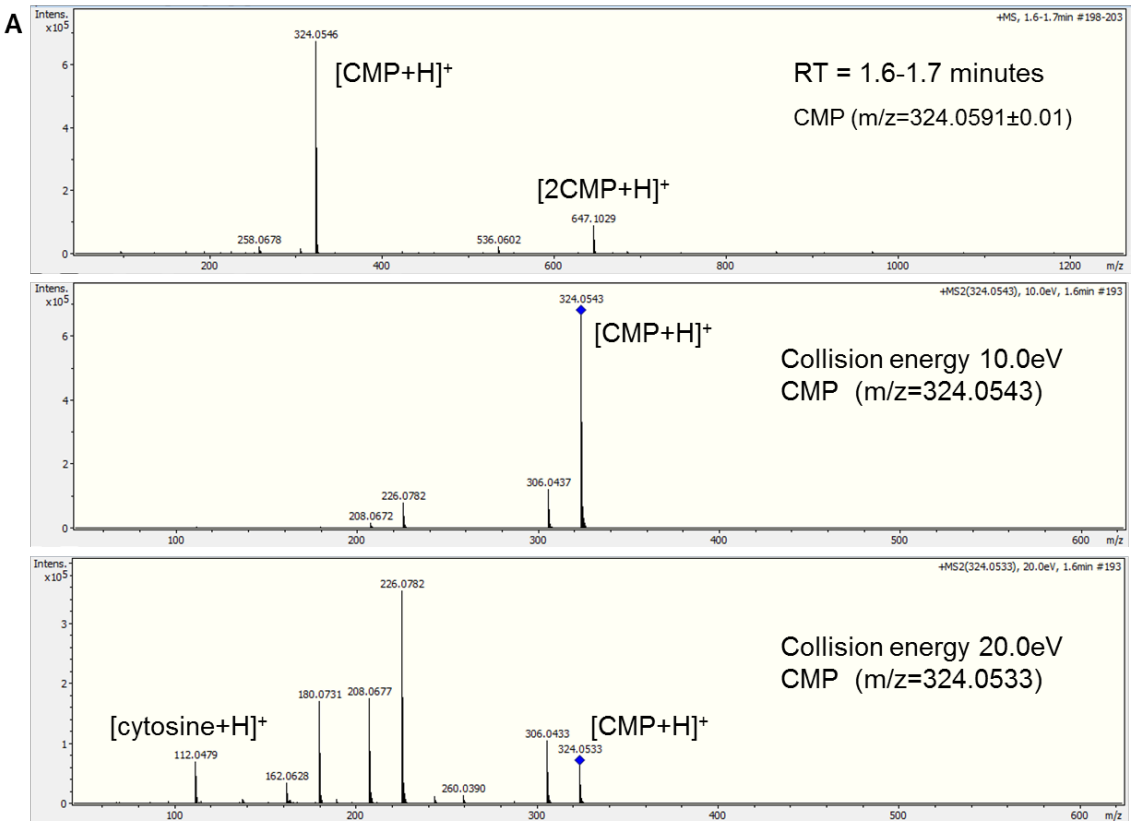


Figure S84. *A) Mass distribution on the most intense peak of CMP EICs in the reaction of 25 mM guanine + 25 mM cytosine + 25 mM P-ribose from Figure S78. B) CMP data at two different collision energies.*

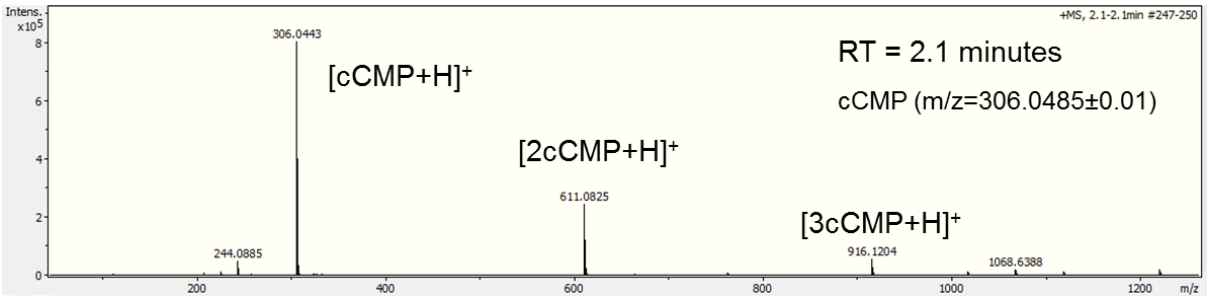


Figure S85. *Mass distribution on the most intense peak of cyclic CMP EICs in the reaction of 25 mM guanine + 25 mM cytosine + 25 mM P-ribose from Figure S73.*

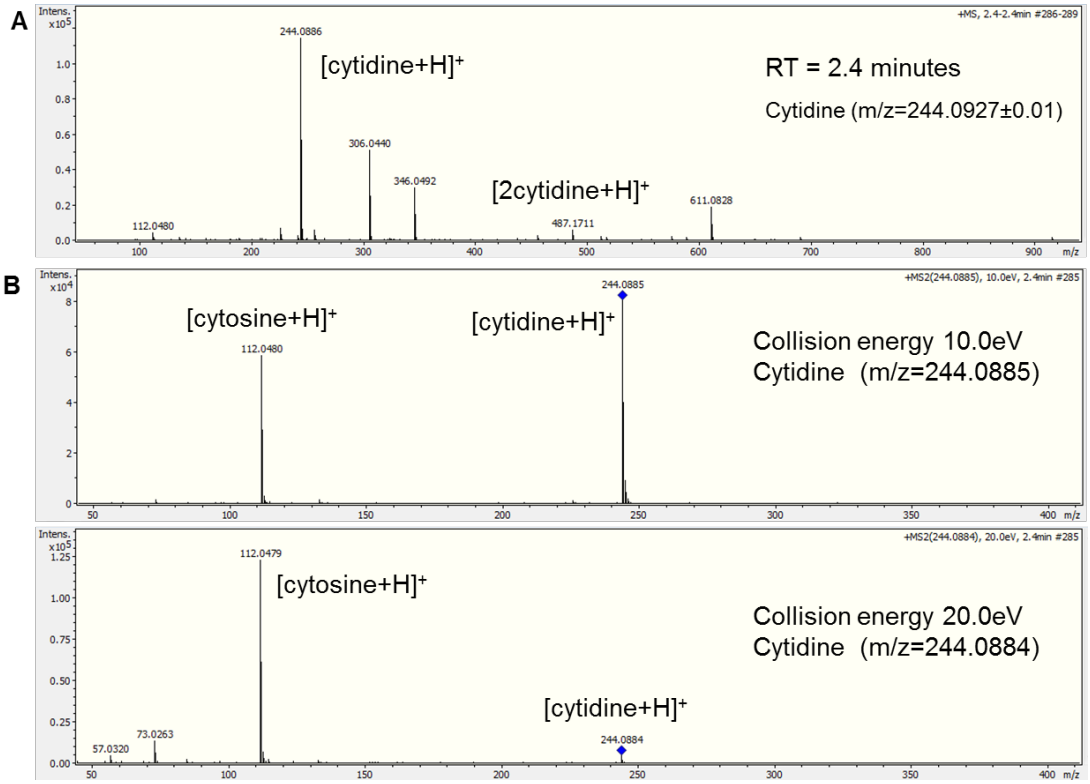


Figure S86. *A) Mass distribution on the most intense peak of cytidine EICs in the reaction of 25 mM guanine+ 25 mM cytosine + 25mM P-ribose from Figure S78. B) Cytidine data at two different collision energies.*

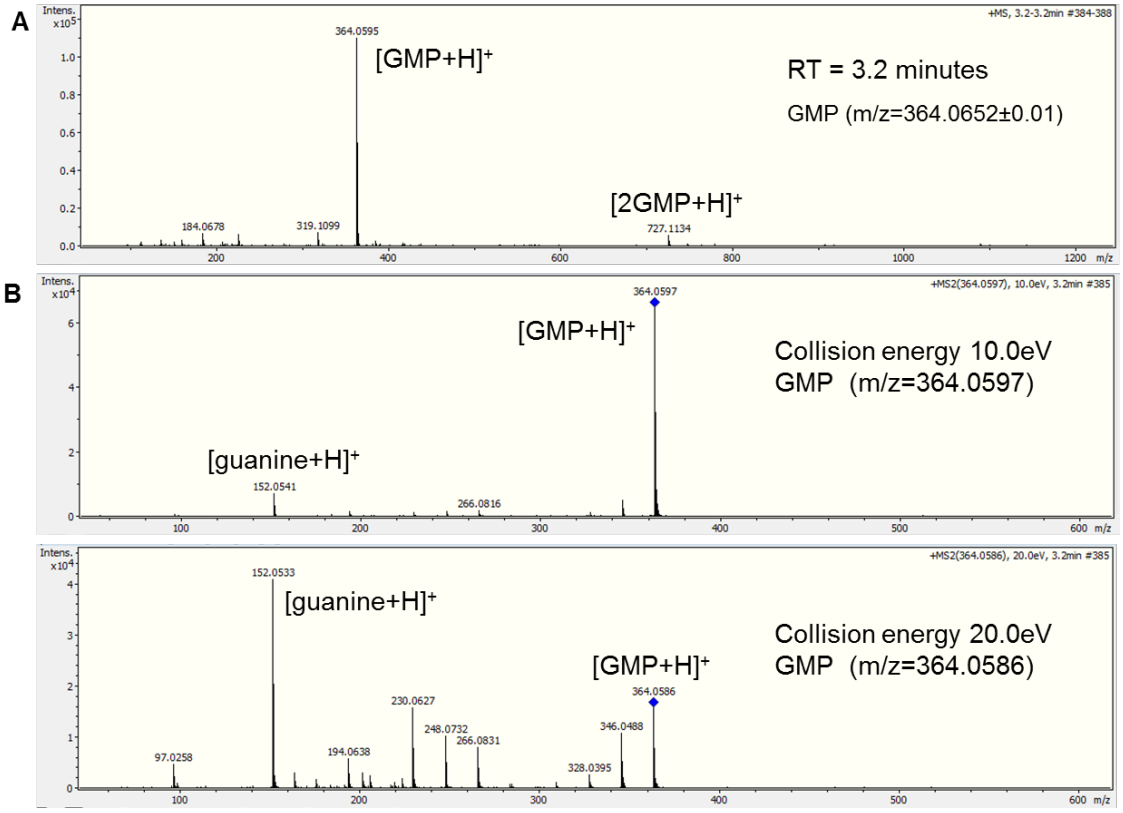


Figure S87. *A)Mass distribution on the most intense peak of GMP EICs in the reaction of 25 mM guanine + 25 mM cytosine + 25 mM P-ribose from Figure S78. B) GMP data at two different collision energies.*

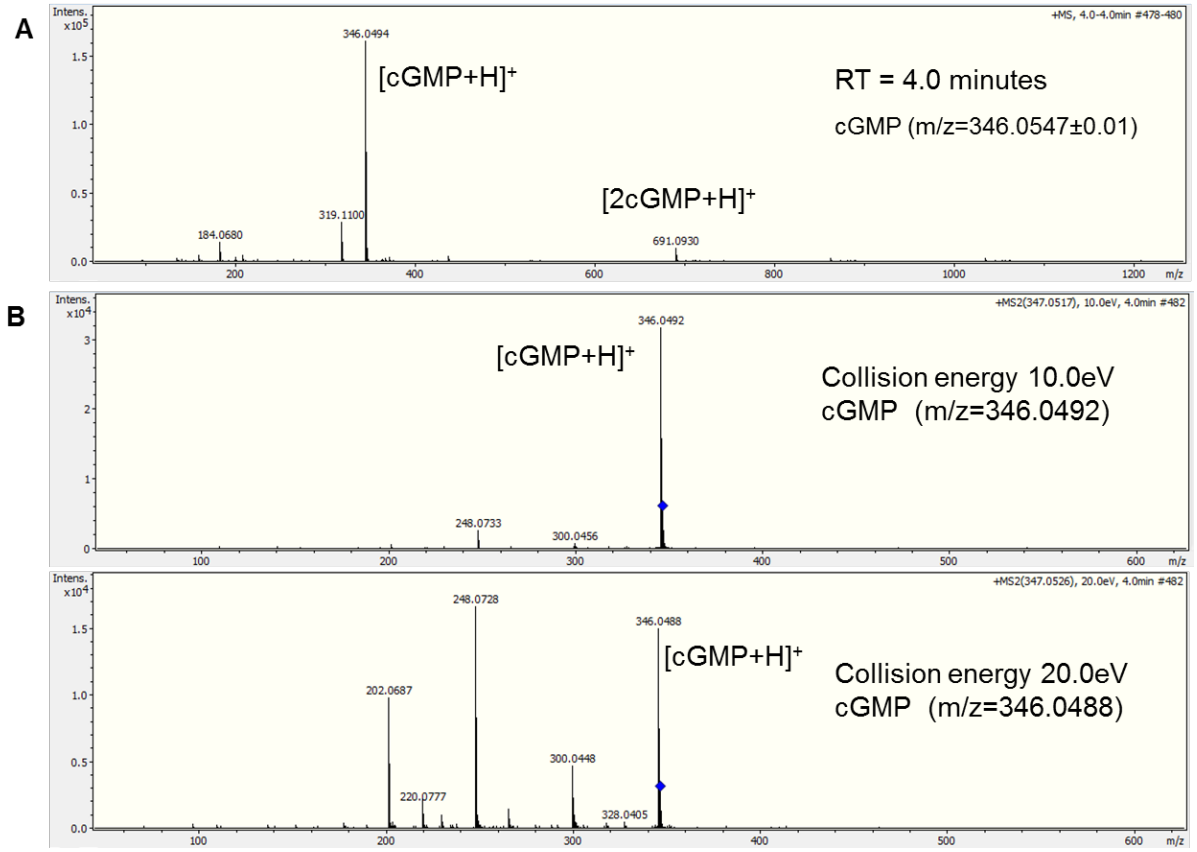


Figure S88. *A) Mass distribution on the most intense peak of cyclic GMP EICs in the reaction of 25 mM guanine + 25 mM cytosine + 25 mM P-ribose from figure S78. B) Cyclic GMP data at two different collision energies.*

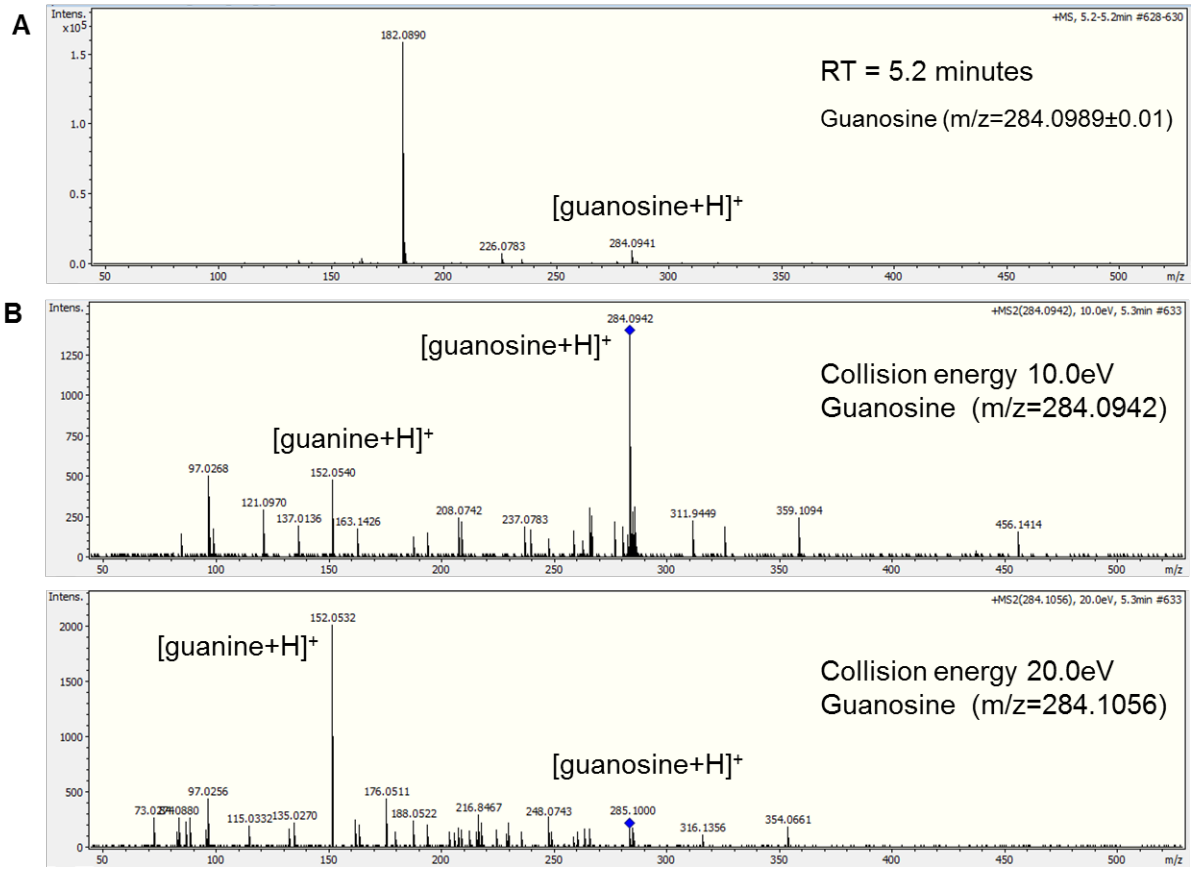
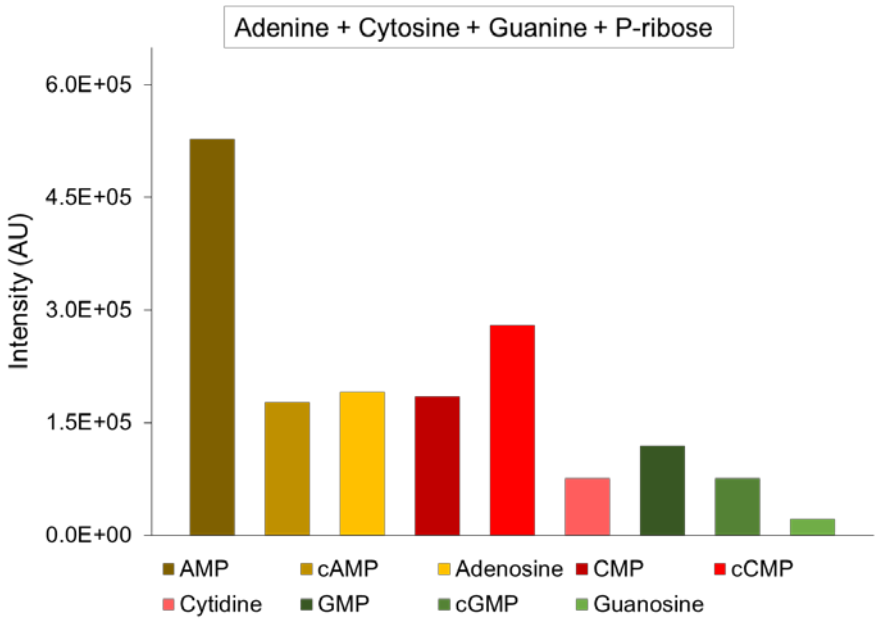


Figure S89. *A) Mass distribution on the most intense peak of guanosine EICs in the reaction of 25 mM guanine + 25 mM cytosine + 25 mM P-ribose from figure S78. B) Guanosine data at two different collision energies.*

Figure S90. *Formation of glycosylation products when 25 mM D-ribose 5´ -phosphate was heated at 90˚C for 5 hours (pH 2.5) in the presence of 25 mM guanine + 25 mM adenine + 25 mM cytosine.*



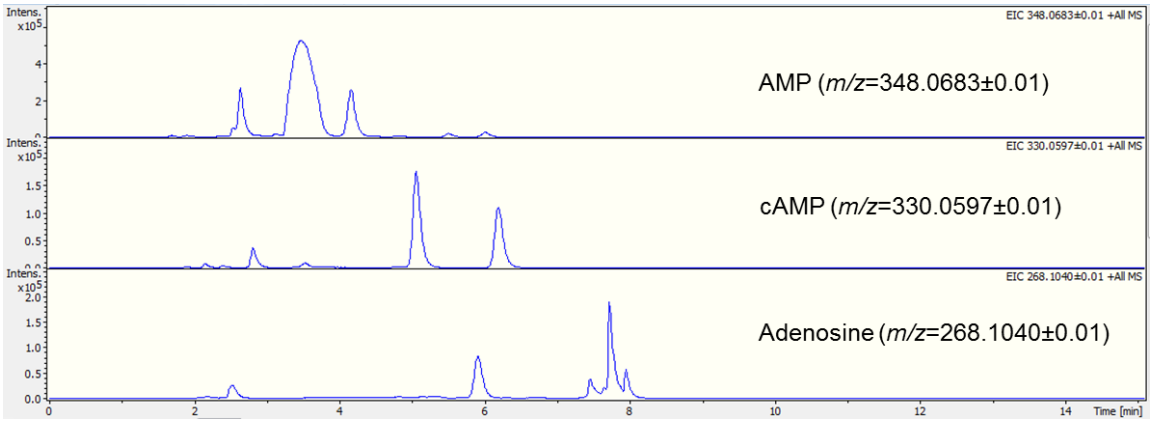


Figure S91. *Formation of glycosylation products when 25 mM D-ribose 5´ -phosphate was heated at 90˚C for 5 hours (pH 2.5) in the presence of 25 mM guanine + 25mM adenine + 25mM cytosine.*

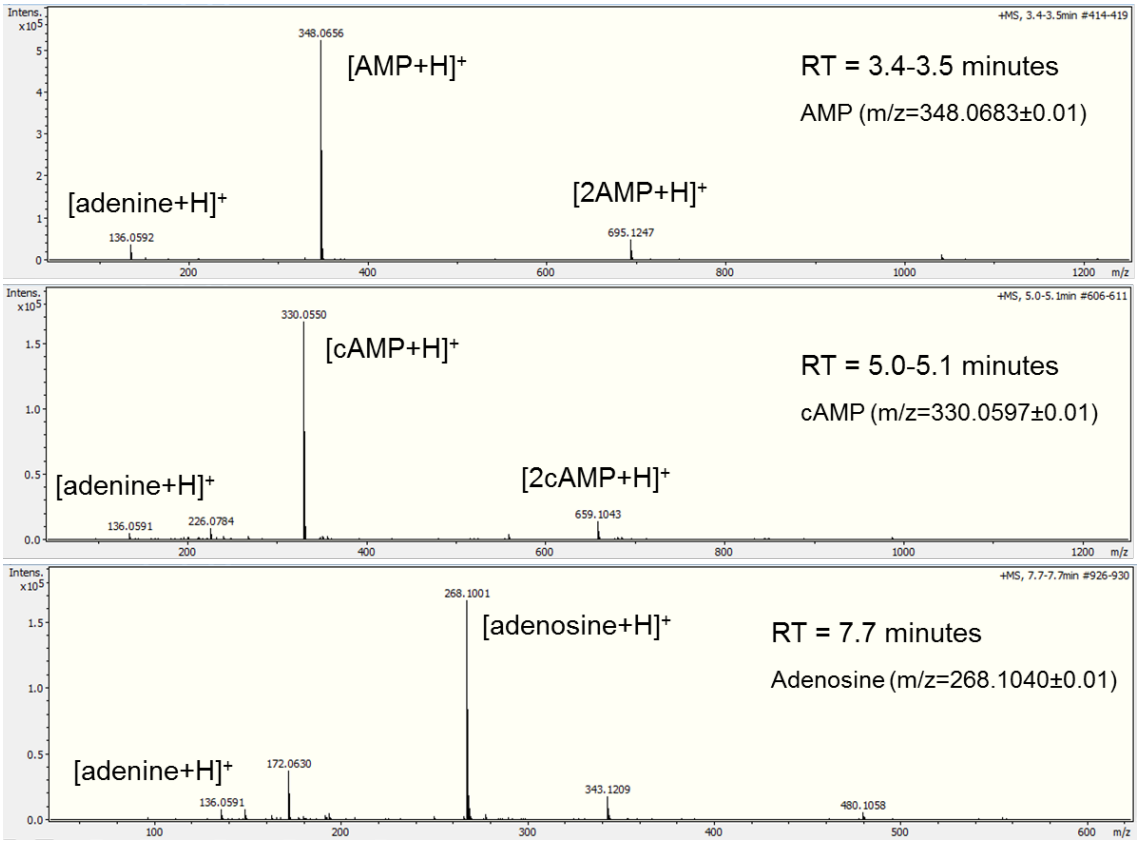


Figure S92. *Mass distribution on the most intense peak of the EICs for adenine glycosylation products in the reaction of 25 mM adenine + 25 mM guanine + 25 mM cytosine + 25 mM P-ribose from Figure S86.*

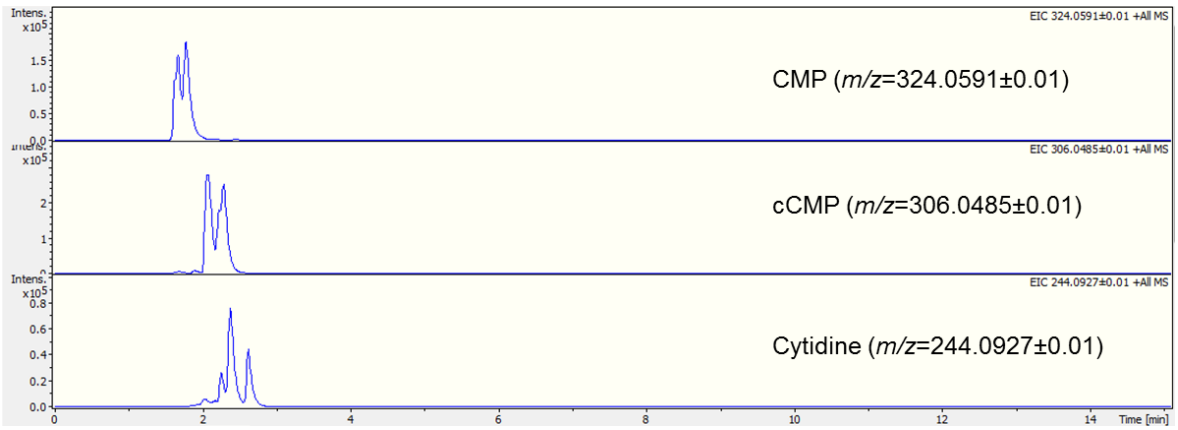


Figure S93. *EICs of cytidine glycosylation produces after the reaction of 25 mM P-ribose + 25 mM cytosine + 25 mM guanine + 25 mM adenine for 5 hours at 90˚C (pH 2.5).*

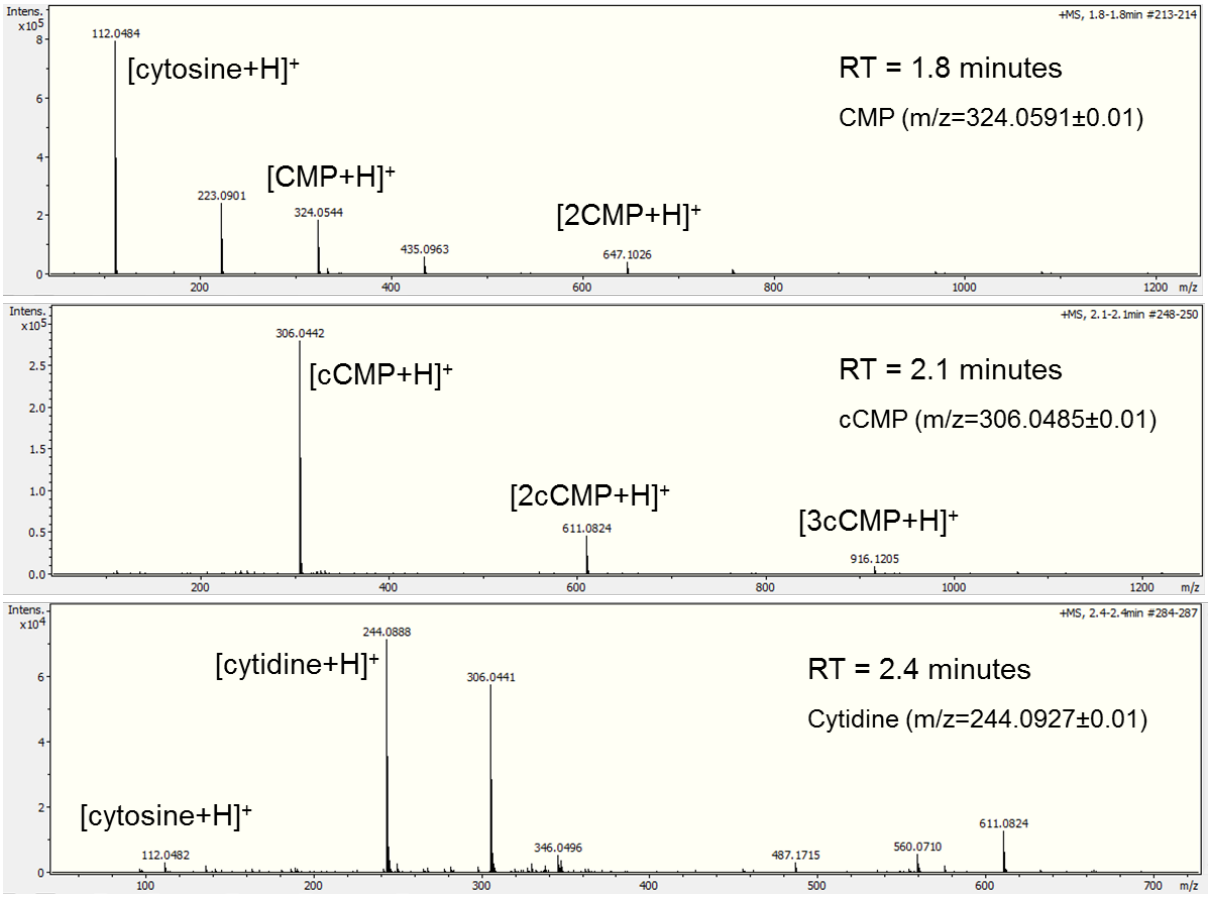


Figure S94. *Mass distribution on the most intense peak of the EICs for cytosine glycosylation products in the reaction of 25 mM adenine + 25mM guanine + 25mM cytosine + 25 mM P-ribose from Figure S88.*

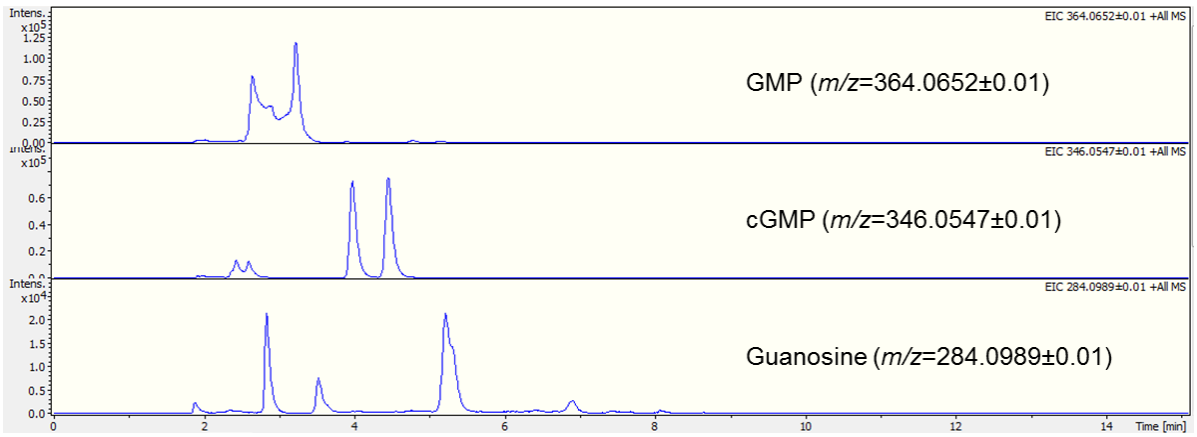


Figure S95. *EICs of guanine glycosylation produces after the reaction of 25 mM P-ribose + 25 mM cytosine + 25 mM guanine + 25 mM adenine for 5 hours at 90˚C (pH 2.5).*

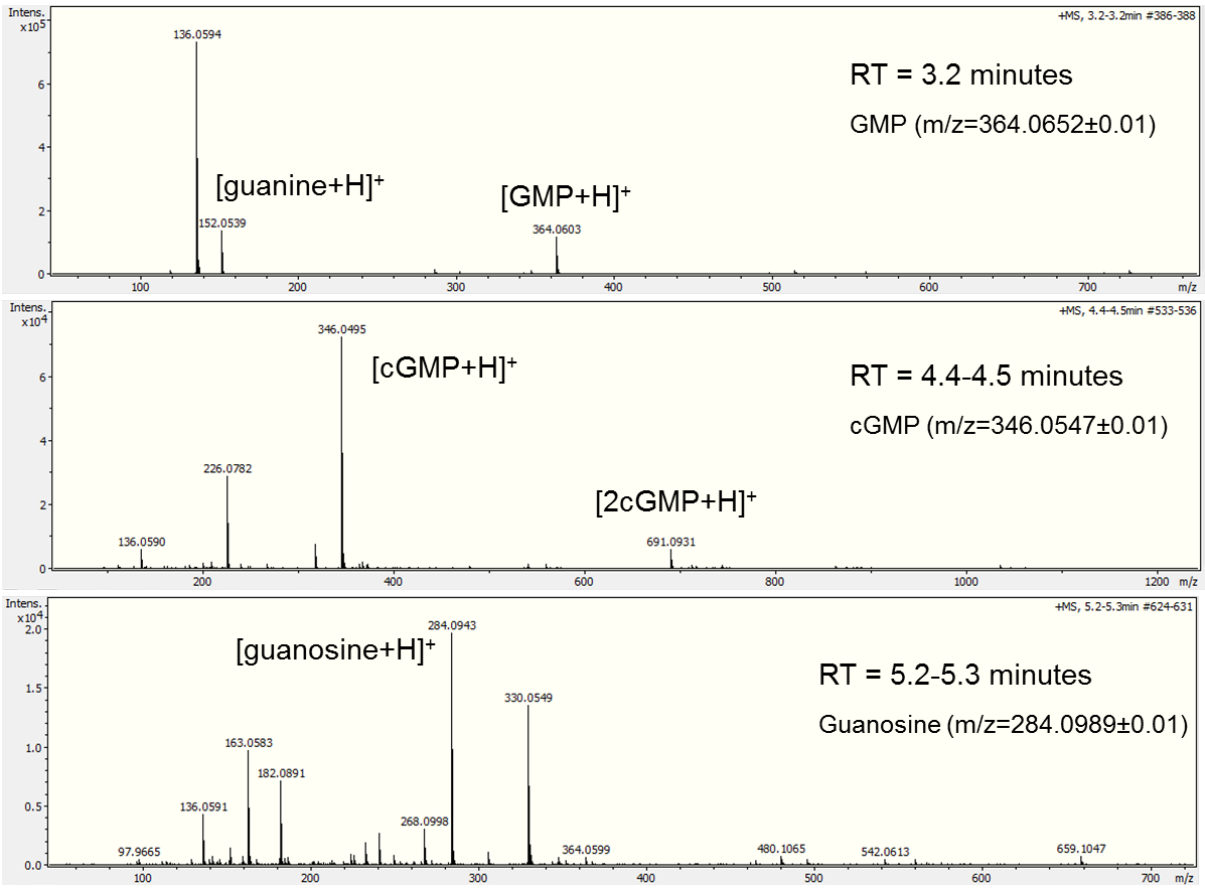


Figure S96. *Mass distribution on the most intense peak of the EICs for guanine glycosylation products in the reaction of 25 mM adenine + 25mM guanine+ 25 mM cytosine + 25 mM P-ribose from Figure S90.*

6. Nucleobase exchange

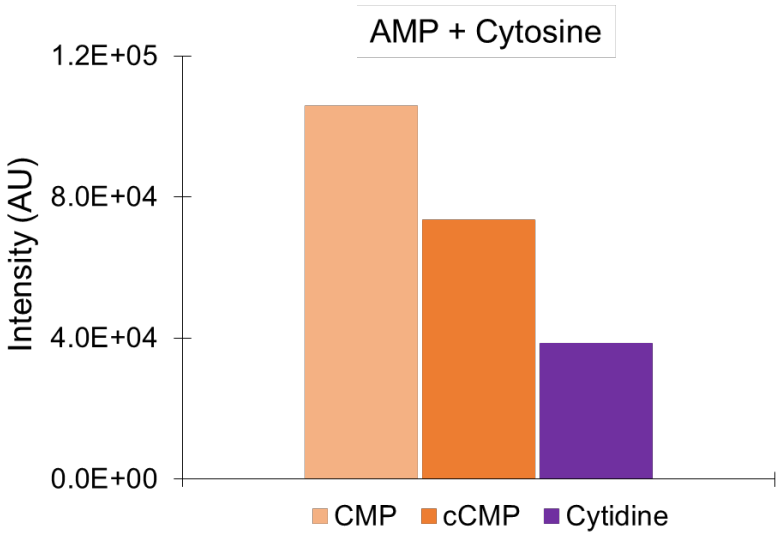


Figure S97. *Formation of cytosine glycosylation products when 25 mM adenosine 5´ -monophosphate was heated at 90˚C for 5 hours (pH 2.5) in the presence of 25 mM cytosine.*

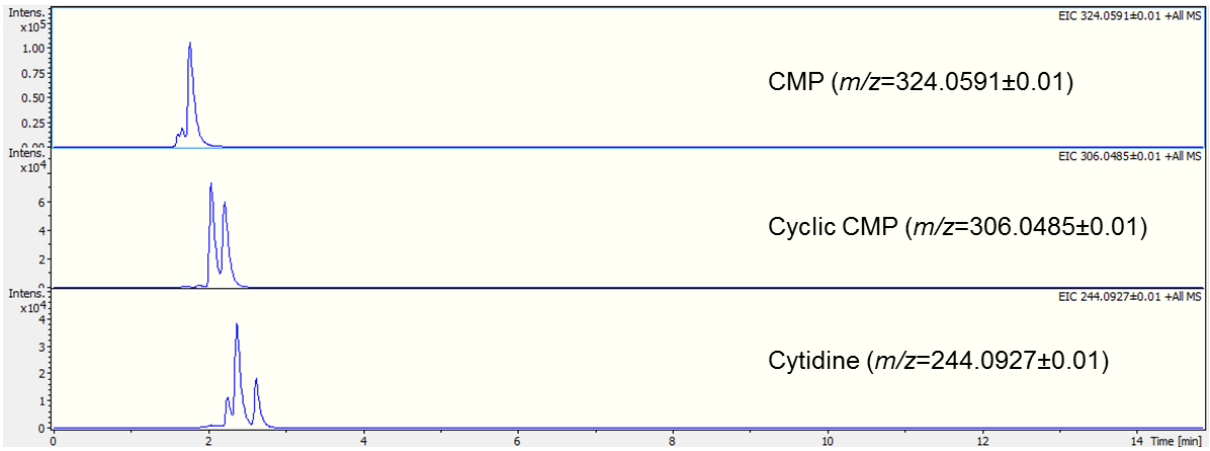


Figure S98. *EICs of cytosine glycosylation products after the dehydration reaction at 90˚C for 5 hours (pH 2.5) of 25 mM adenosine 5´ -monophosphate with 25 mM cytosine (from Fig. S92).*

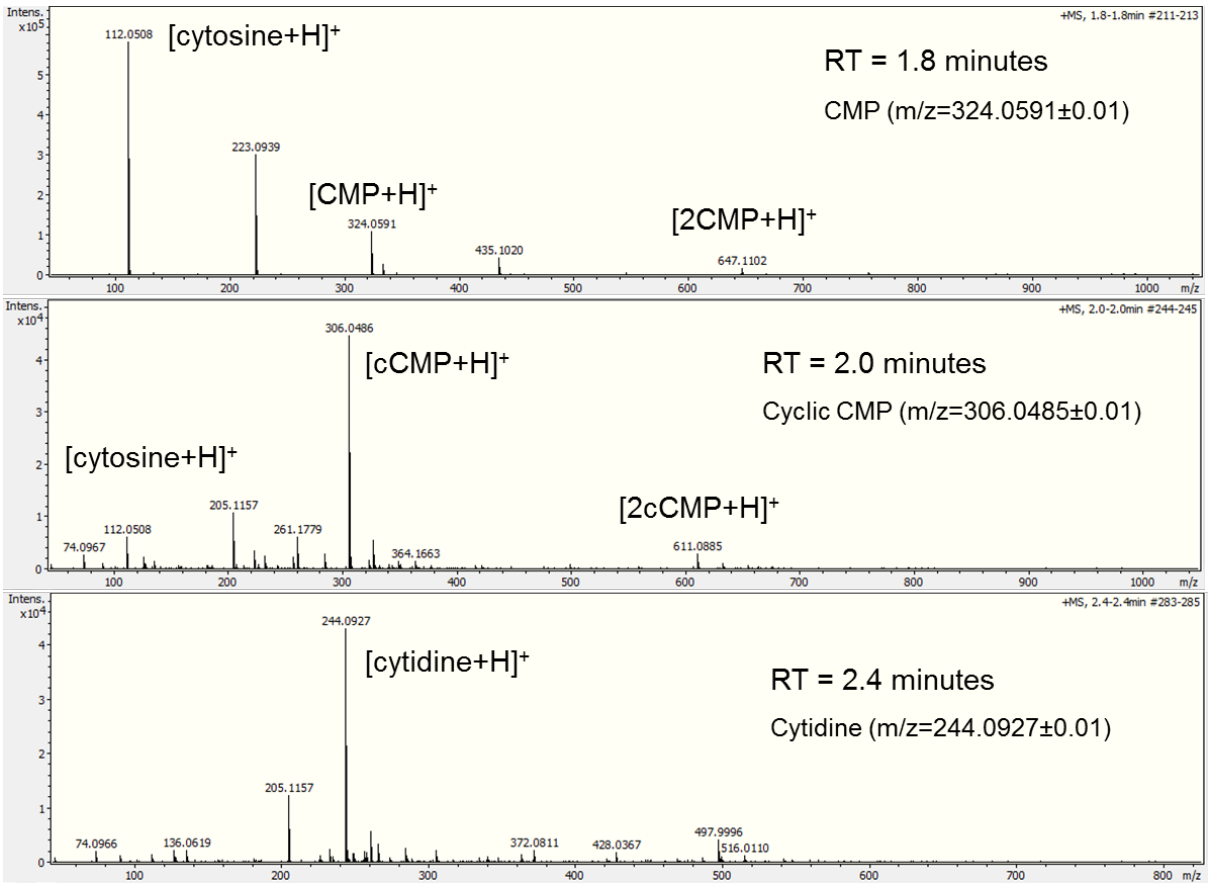


Figure S99. *Mass distribution on the most intense peak of the EICs for cytosine glycosylation products in the reaction of 25 mM AMP + 25 mM cytosine; showed in Figure S93.*

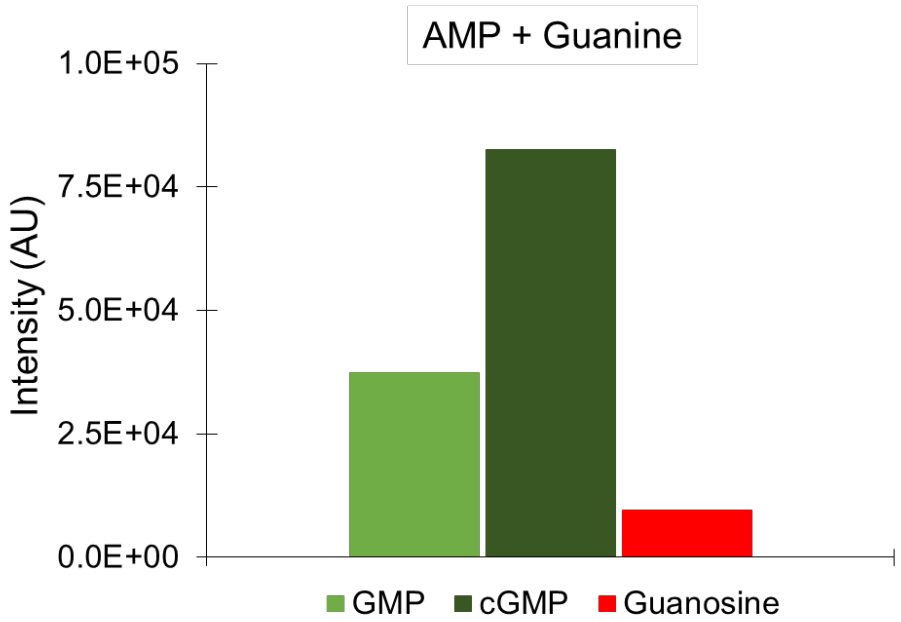


Figure S100. *Formation of guanine glycosylation products when 25 mM adenosine 5′ -monophosphate was heated at 90˚C for 5 hours (pH 2.5) in the presence of 25 mM guanine.*

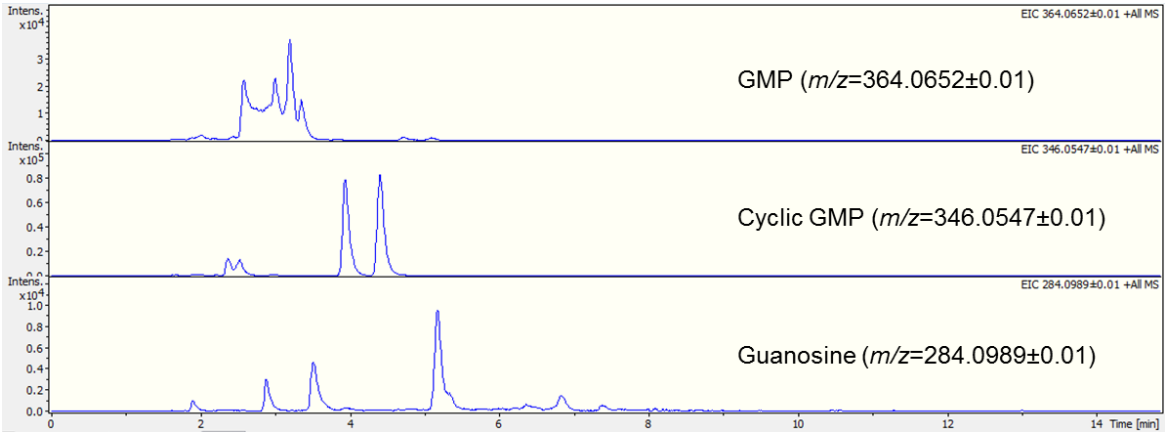


Figure S101. *Mass distribution on the most intense peak of the EICs for cytosine glycosylation products in the reaction of 25 mM AMP + 25 mM cytosine (from Fig. S95).*

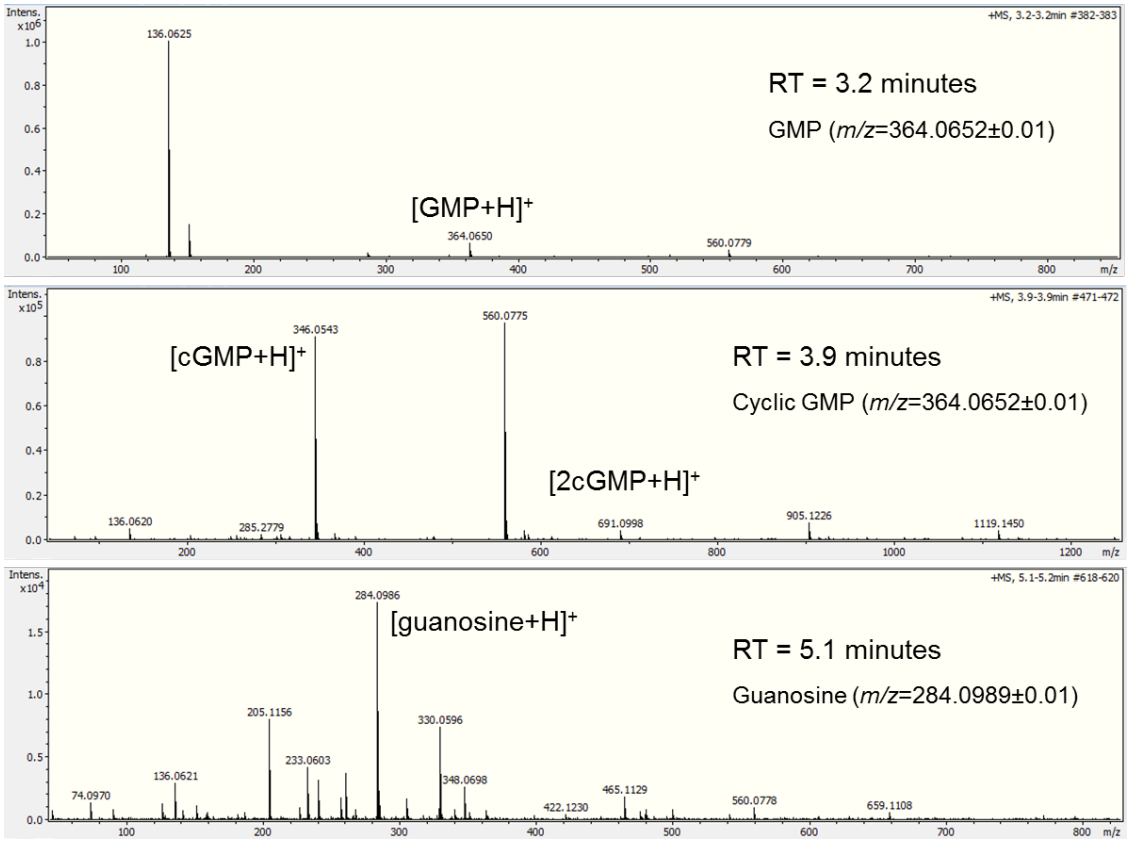


Figure S102. *Mass distribution on the most intense peak of the EICs for guanine glycosylation products in the reaction of 25 mM AMP + 25 mM guanine; showed in Figure S96.*

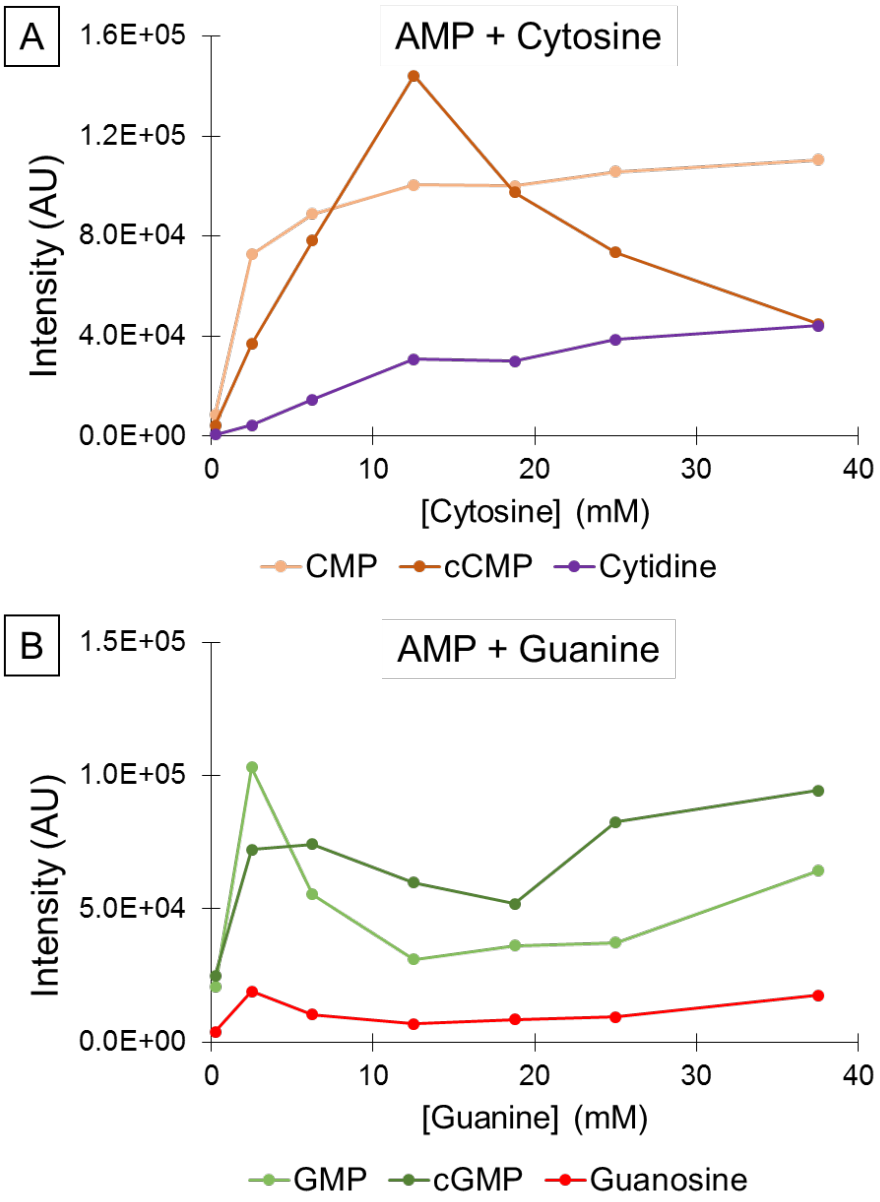


Figure S103. *Formation of cytosine and guanine glycosylation products when 25 mM adenosine 5´-monophosphate was heated at 90˚ C for 5 hours (pH 2.5) in the presence of A) Different concentrations of cytosine B) Different concentrations of guanine.*

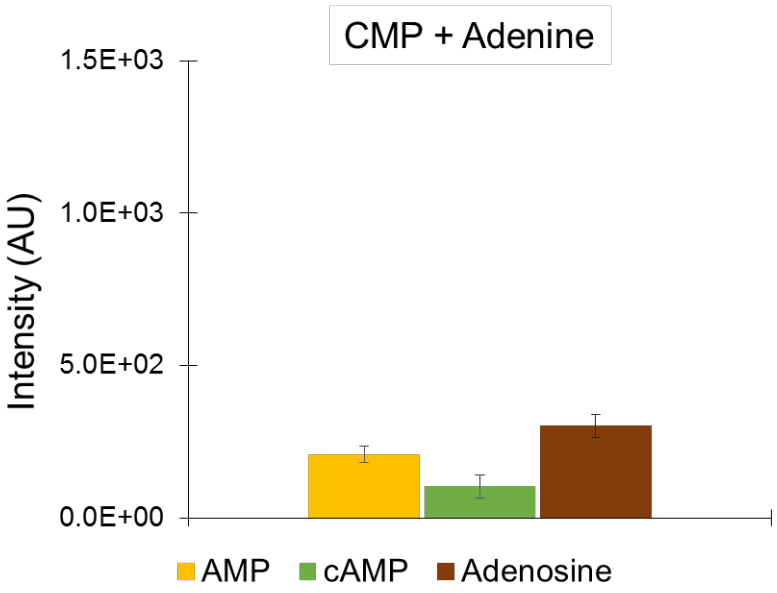


Figure S104. *Formation of adenine glycosylation products when 25 mM cytidine 5′ -monophosphate + 25 mM adenine were heated at 90˚C for 5 hours (pH 2.5).*

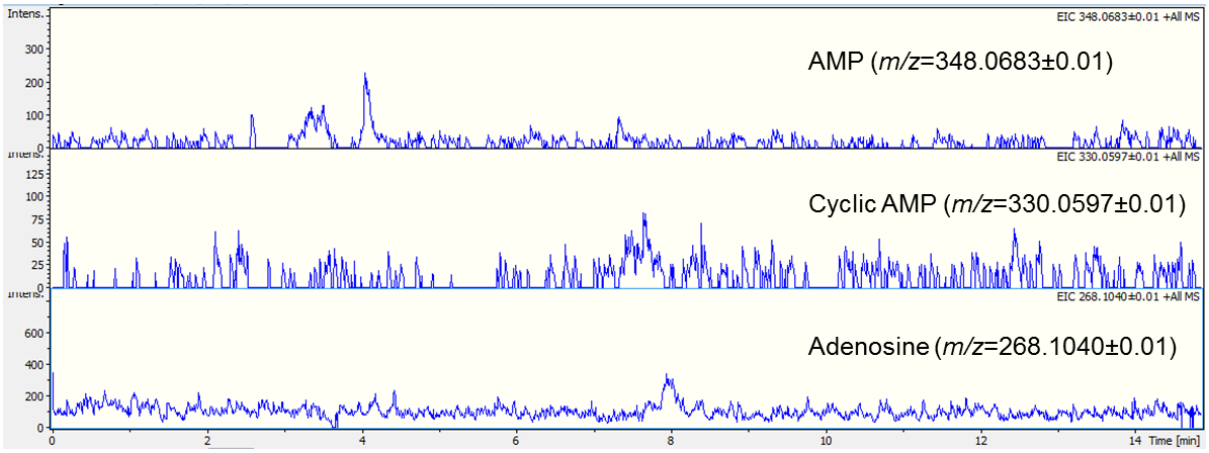


Figure S105. *Extracted Ion Chromatogram of adenine glycosylation products after the dehydration reaction of 25 mM cytidine 5′ -monophosphate + 25 mM adenine at 90˚C for 5 hours (pH 2.5).*

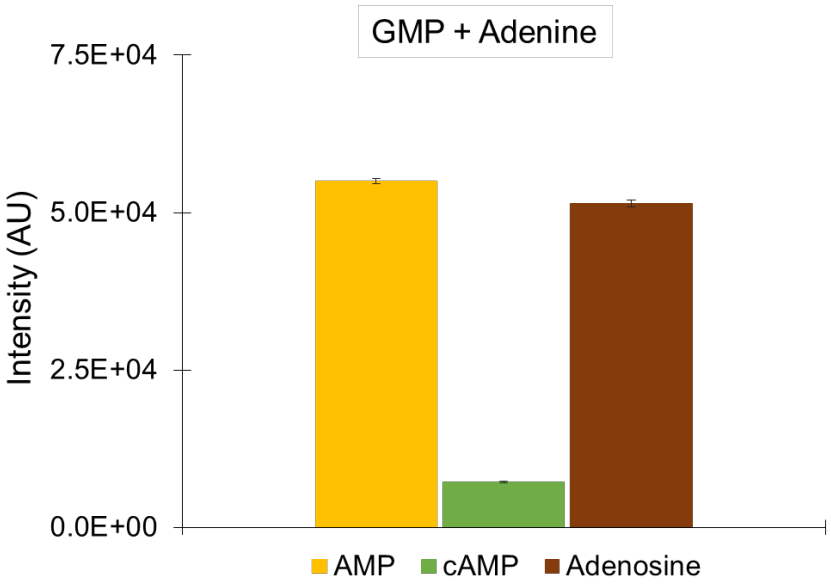


Figure S106. *Formation of adenine glycosylation products when 25 mM guanosine 5´ -monophsopahte + 25 mM adenine were heated at 90˚C for 5 hours (pH 2.5).*

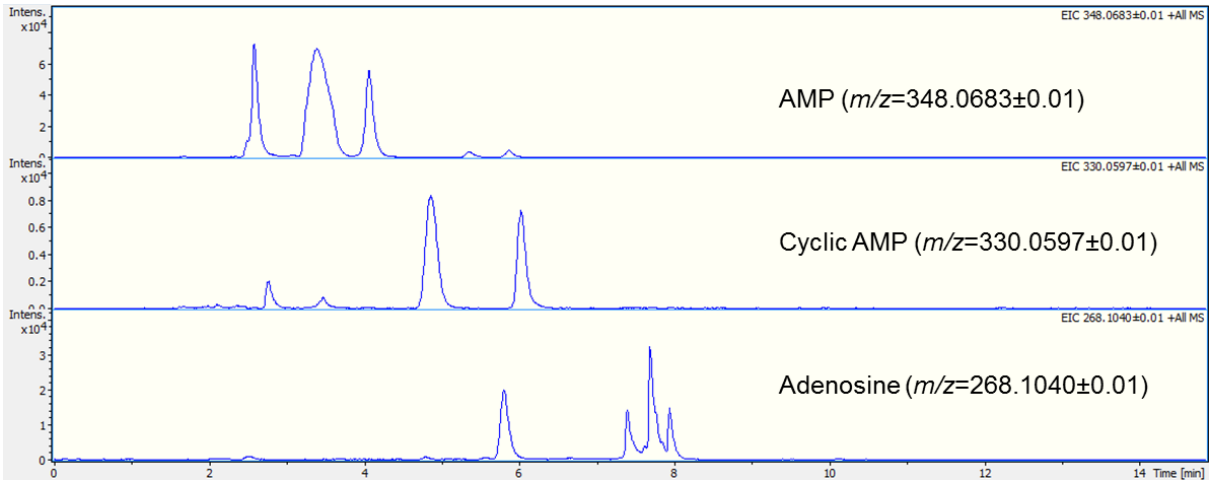


Figure S107. *EICs of adenine glycosylation products after the dehydration reaction of 25 mM guanosine 5´ -monophosphate + 25 mM adenine at 90˚C for 5 hours (pH 2.5).*

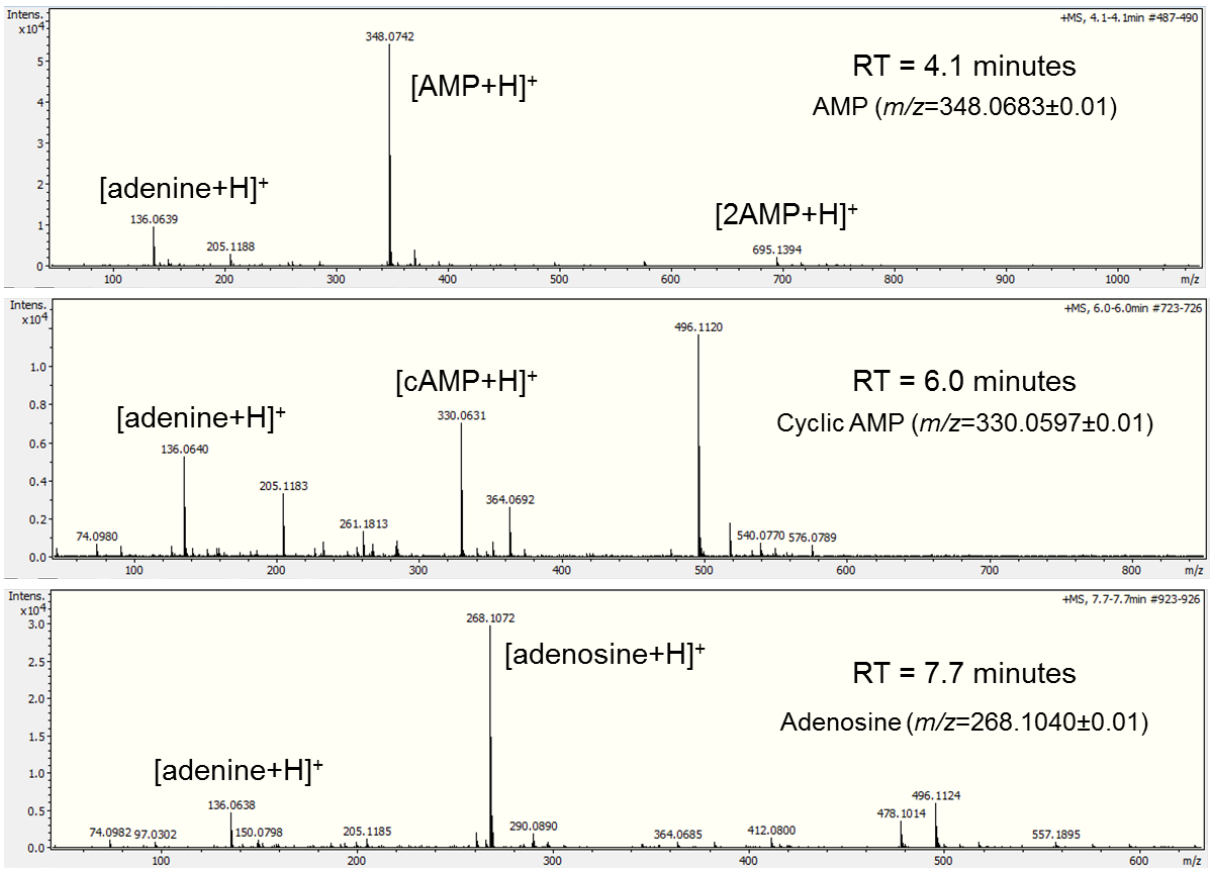


Figure S108. *Mass distribution on the most intense peak of the EICs for adenine glycosylation products in the reaction of 25 mM guanosine 5′ -monophosphate + 25 mM adenine at 90° C for 5 hours (pH 2.5).*

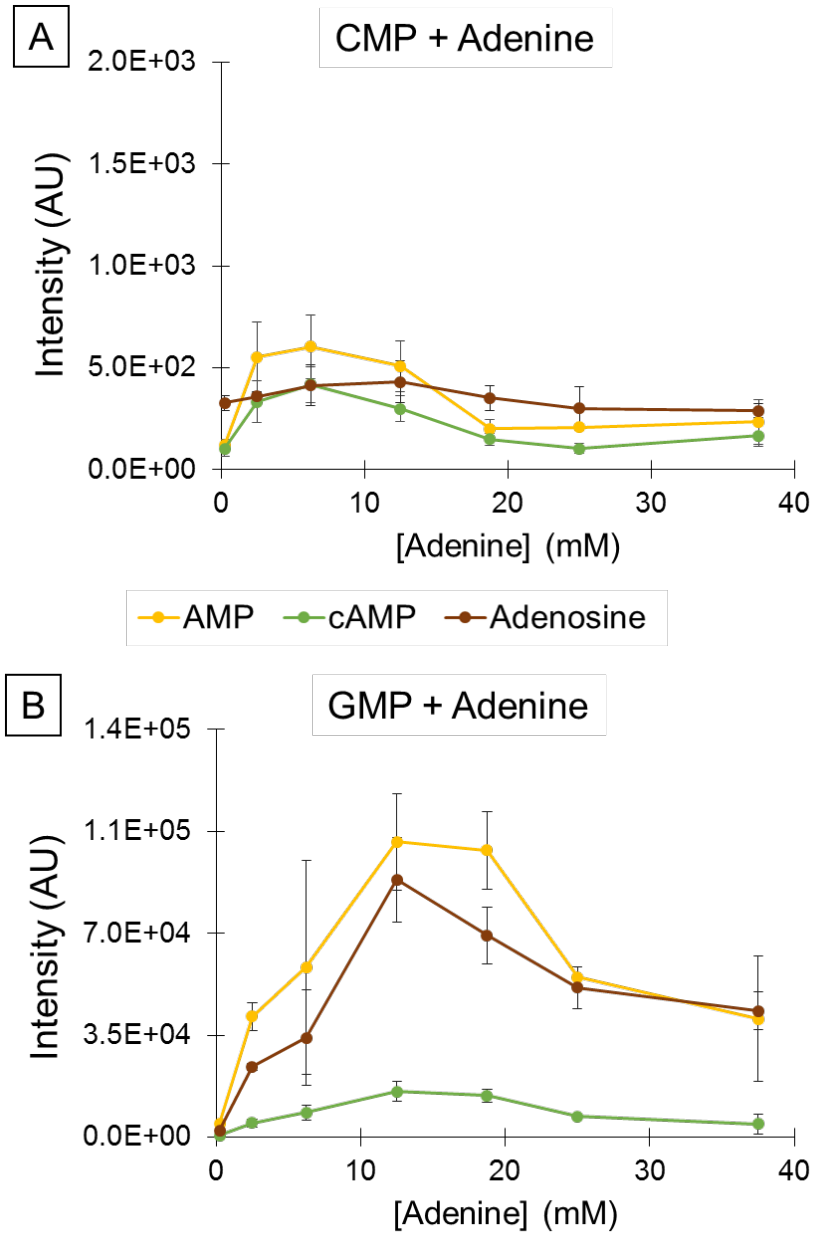


Figure S109. *Formation of adenine glycosylation products when A) 25 mM cytidine 5′ -monophosphate B) 25 mM guanosine 5′ -monophosphate were heated in the presence of increasing concentrations of adenine at 90˚C for 5 hours (pH 2.5).*

7. Amino acids effect on isomer distribution of glycosylation products

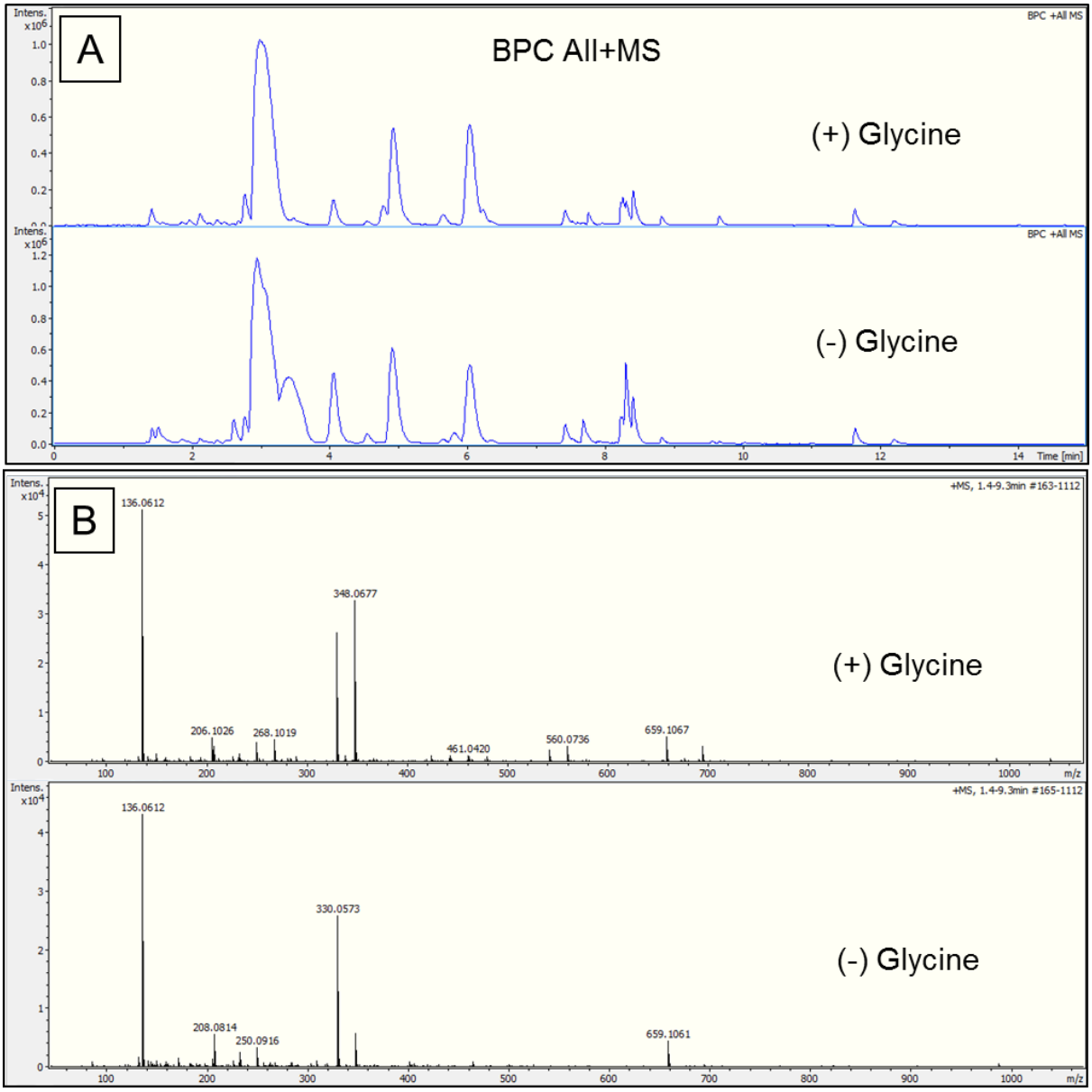


Figure S110. *A) Base Peak Chromatogram (BPC) for All+MS comparing the reaction of 25 mM adenine + 25 mM P-ribose with and without 25 mM glycine B) Mass distribution correspondent to the BPC All+MS, within a retention time of 1.3-9.0 minutes, comparing the reaction of 25 mM P-ribose + 25 mM adenine with 25 mM glycine + 25 mM P-ribose+ 25 mM adenine. All the reactions were heated at 90˚C for 5 hours (pH 2.5).*

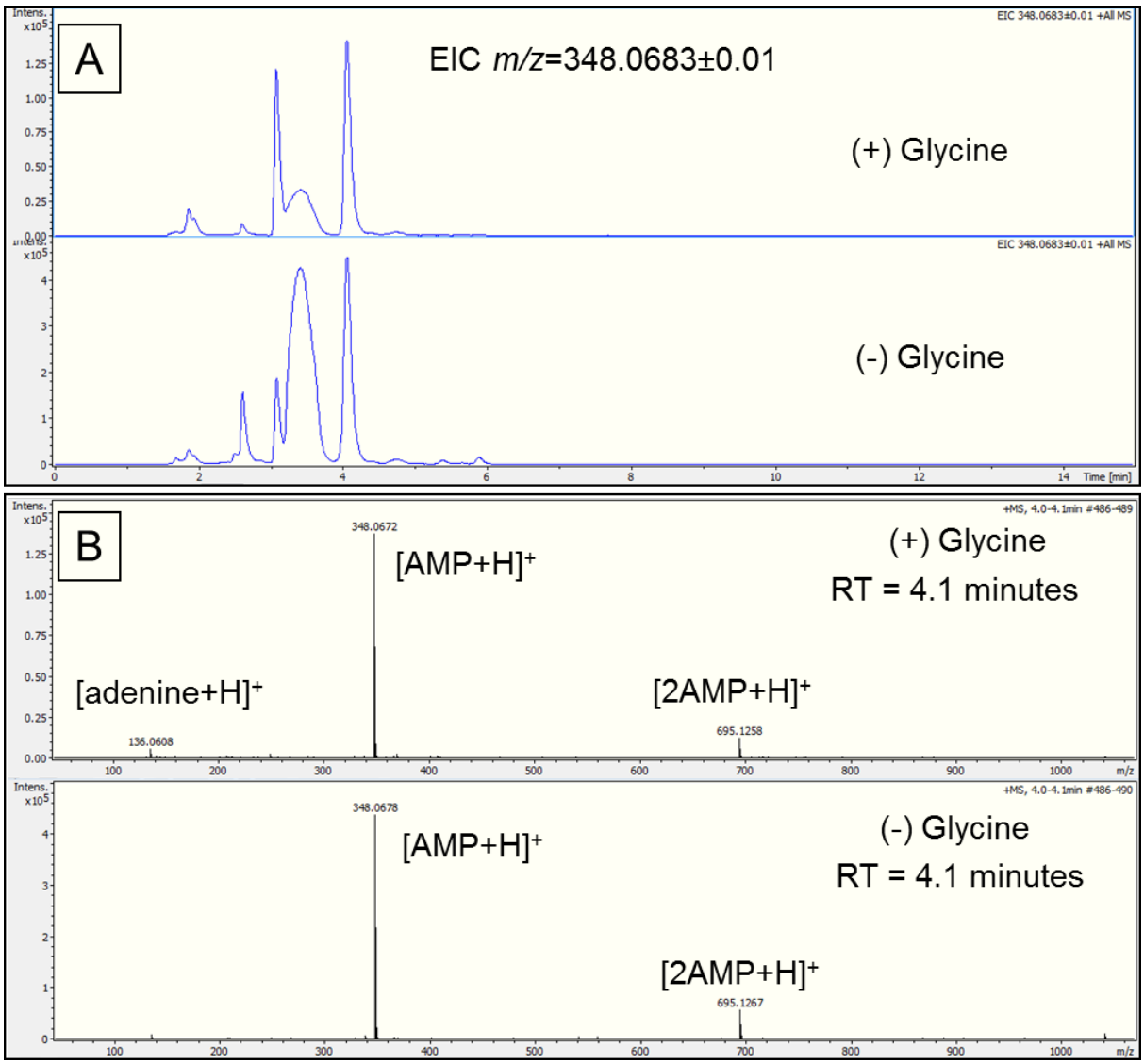


Figure S111. *A) EICs for adenosine monophosphate mass (m/z=348.0683±0.01). B) Mass distribution on the most intense peak of the EICs ; comparing the reaction of 25 mM adenine + 25 mM P-ribose with and without 25 mM glycine. All the reactions were heated at 90˚ C for 5 hours (pH 2.5).*

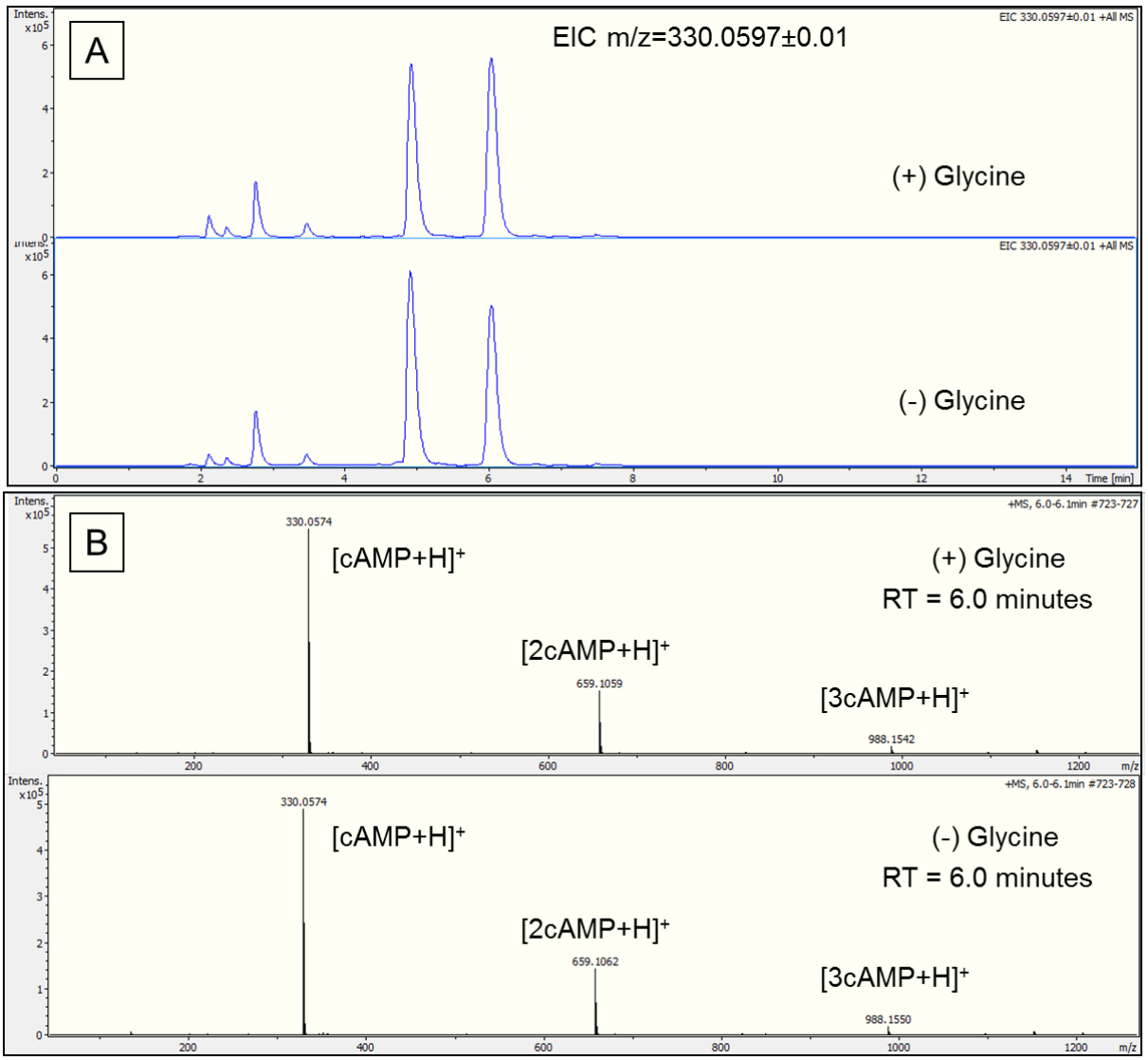


Figure S112. *A) EICs for cyclic adenosine monophosphate mass (m/z=330.0597±0.01). B) Mass distribution on the most intense peak of the EICs ; comparing the reaction of 25 mM adenine + 25 mM P-ribose with and without 25 mM glycine. All the reactions were heated at 90˚C for 5 hours (pH 2.5).*

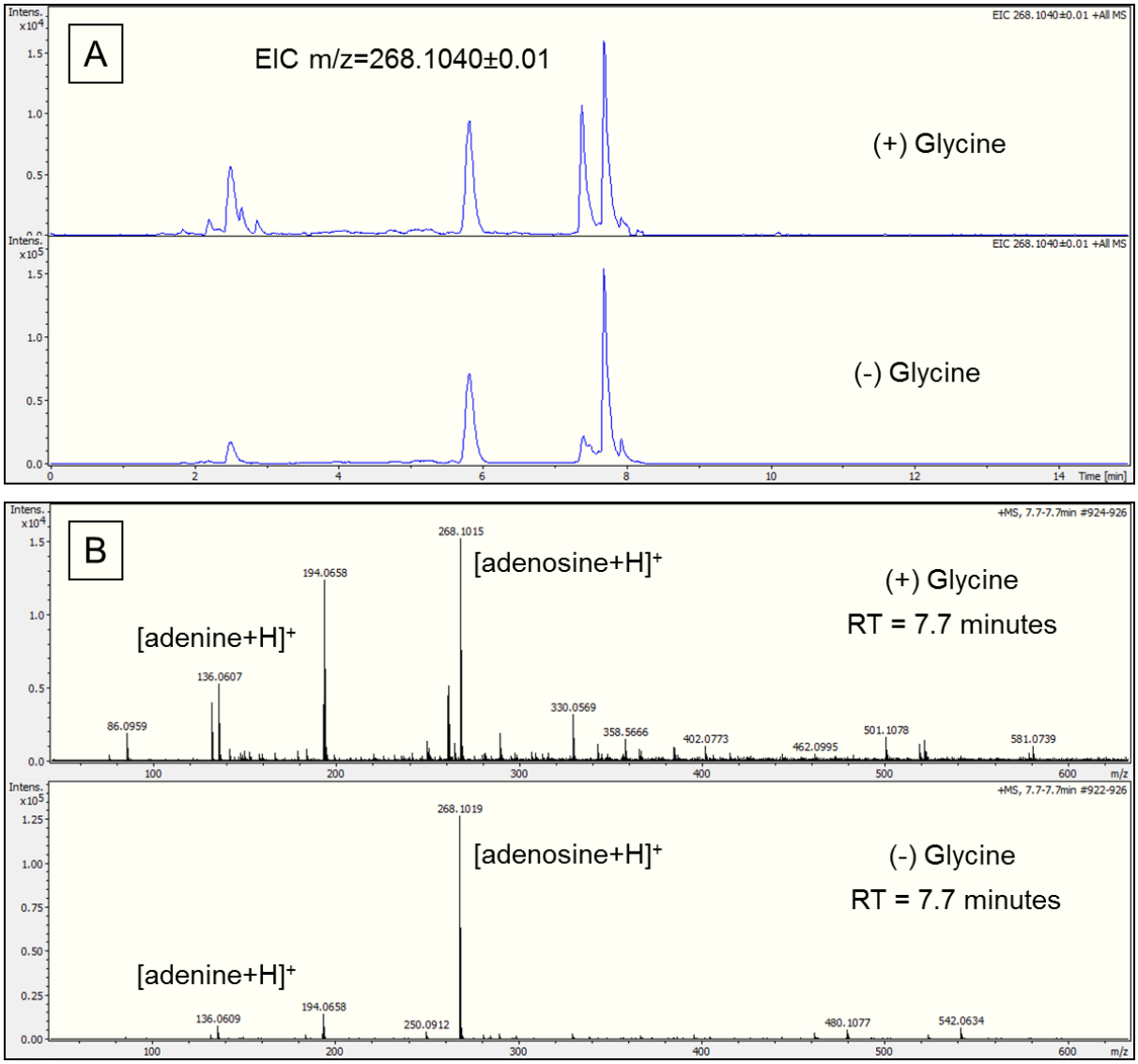


Figure S113. *A) EICs for adenosine mass (m/z=268.1040±0.01). B) Mass distribution on the most intense peak of the EICs ; comparing the reaction of 25 mM adenine + 25 mM P-ribose with and without 25 mM glycine. All the reactions were heated at 90˚C for 5 hours (pH 2.5).*

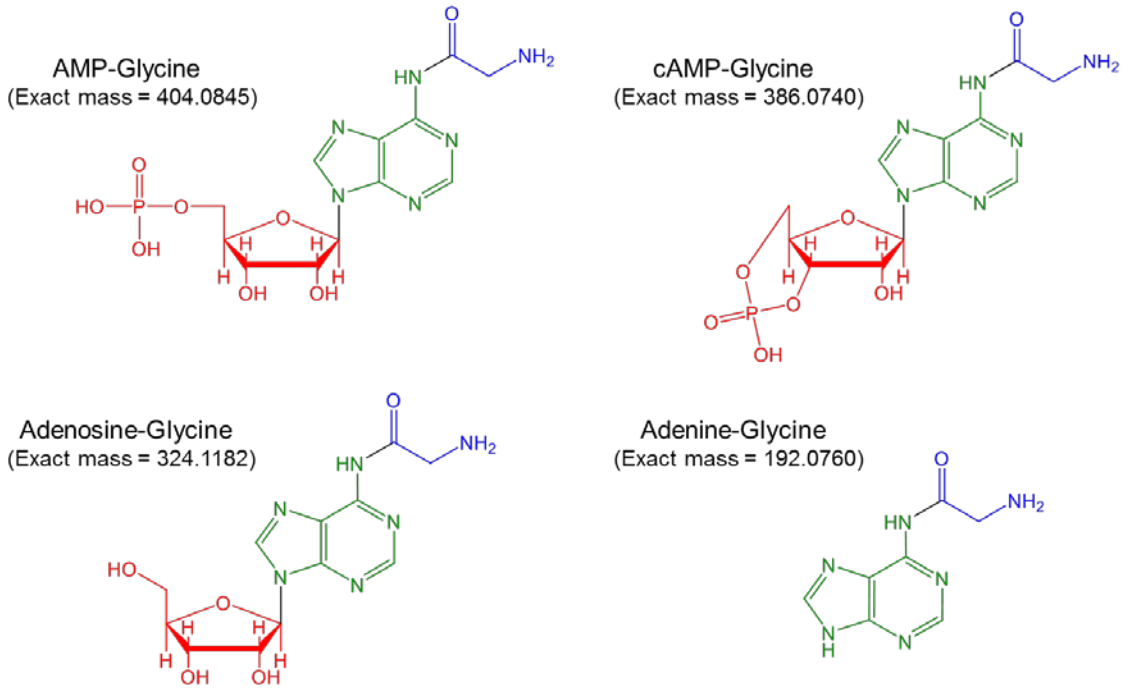


Figure S114. *Structure and exact mass of possible side products during the dehydration reaction of P-ribose + adenine in the presence of glycine.*

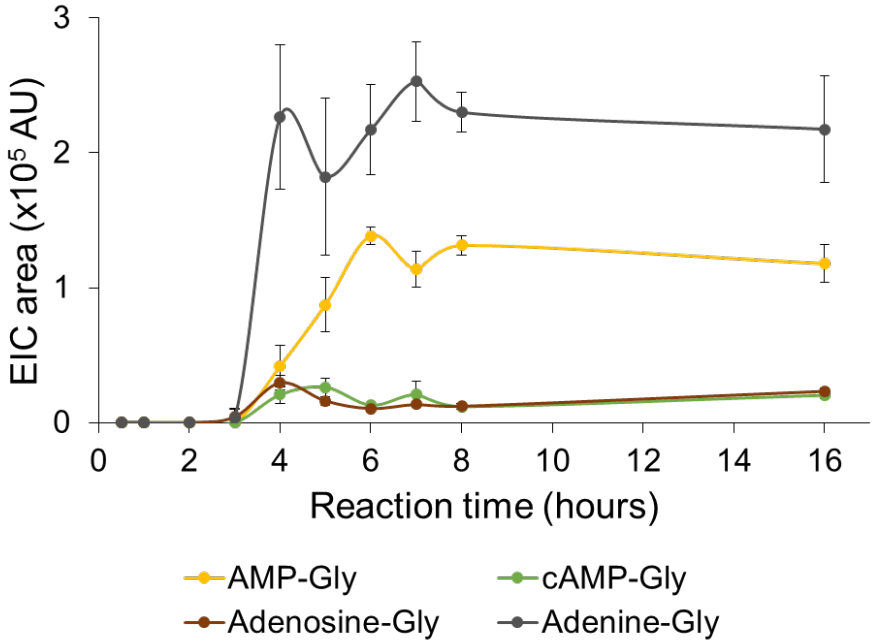


Figure S115. *Formation of side products AMP-Gly (m/z= 405.0918±0.01); cAMP-Gly (m/z= 387.0813±0.01); adenosine-Gly (m/z= 325.1255±0.01); adenine-Gly (m/z= 193.0832±0.01); when 25 mM D-ribose 5´ -phosphate + 25 mM adenine + 25 mM glycine were heated at 90˚C for 16 hours (pH = 2.5).*

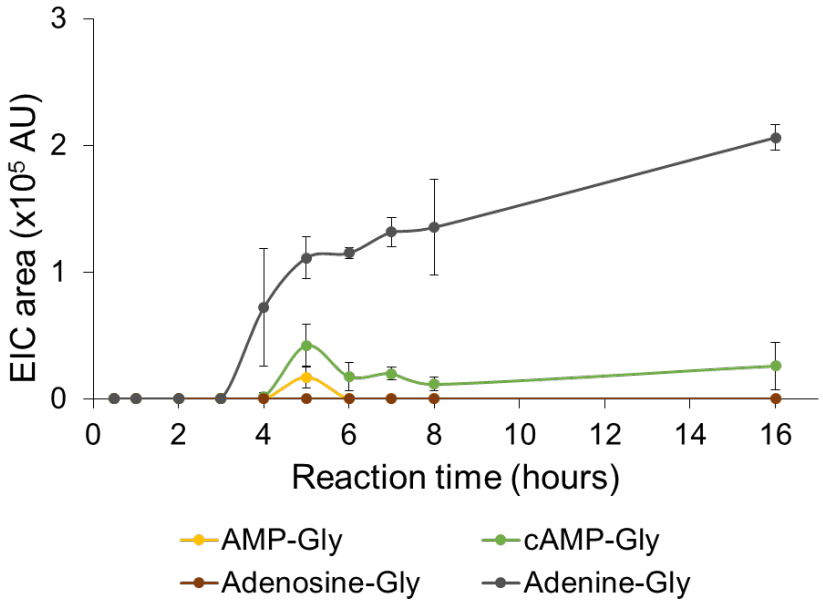


Figure S116. *Formation of side products AMP-Gly (m/z= 405.0918±0.01); cAMP-Gly (m/z= 387.0813±0.01); adenosine-Gly (m/z= 325.1255±0.01); adenine-Gly (m/z= 193.0832±0.01); when 25 mM D-ribose 5′ -phosphate + 25 mM adenine were heated at 90˚C for 16 hours (pH = 2.5).*

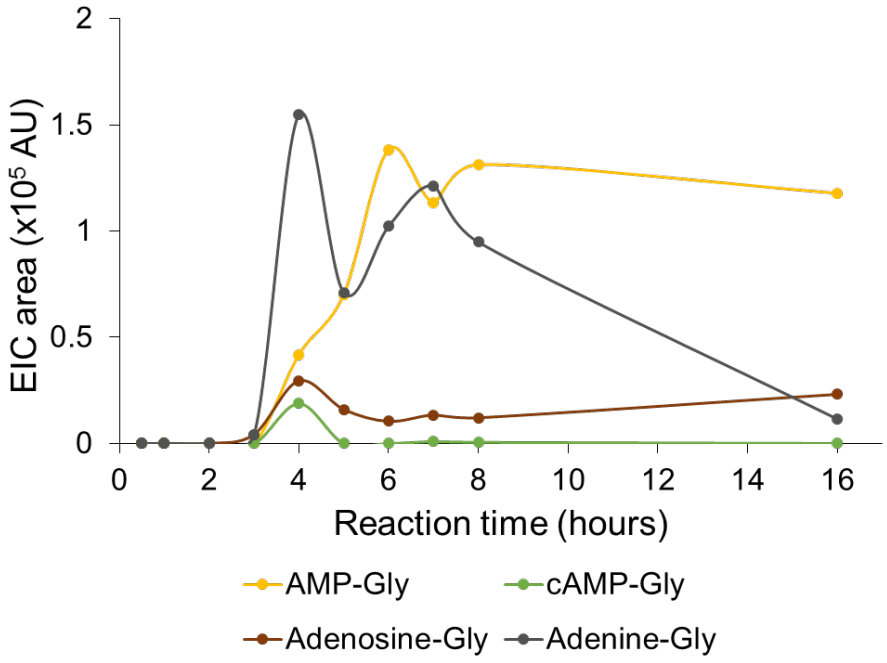


Figure S117. *Formation of side products AMP-Gly (m/z= 405.0918±0.01); cAMP-Gly (m/z= 387.0813±0.01); adenosine-Gly (m/z= 325.1255±0.01); adenine-Gly (m/z= 193.0832±0.01); when the EIC areas extracted from the reaction of 25 mM D-ribose 5′ -phosphate + 25 mM adenine were subtracted from the reaction EIC areas extracted from the reaction of 25 mM D-ribose 5′ -phosphate + 25 mM adenine + 25 mM glycine.*

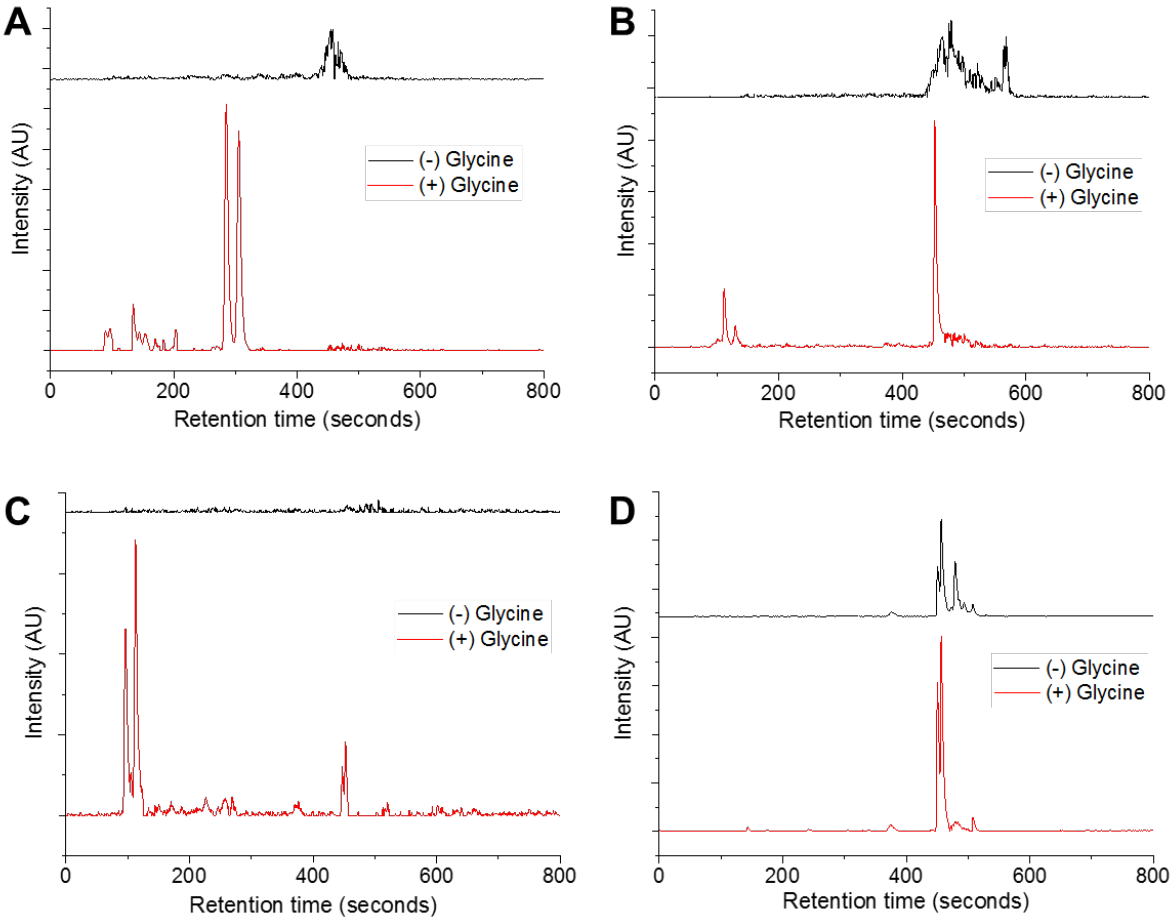


Figure S118. *EICs of A) AMP-Glycine (m/z= 405.0918±0.01); B) cAMP-Glycine (m/z= 387.0813±0.01); C) adenosine-Glycine (m/z= 325.1255±0.01); D) adenine-Glycine (m/z= 193.0832±0.01) after the reaction of P-ribose + adenine (black) vs reaction of P-ribose + adenine + glycine; both heated at 90˚C for 5 hours (pH= 2.5).*

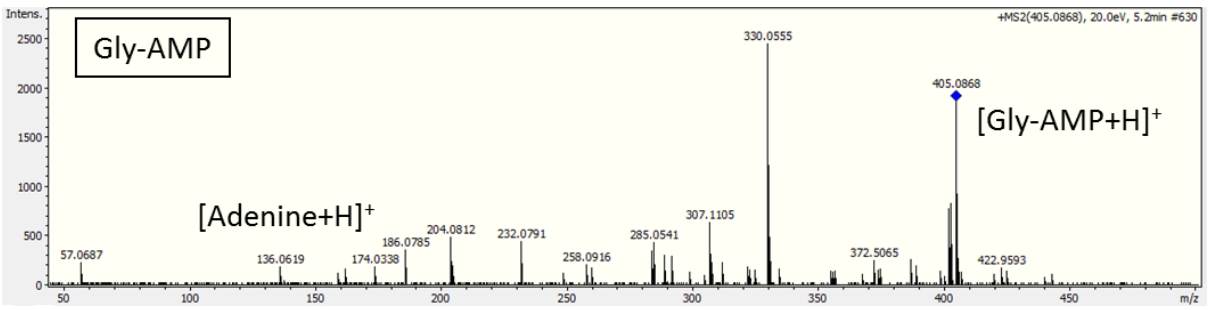


Figure S119. *MS-MS data for the fragmentation Glycine-AMP mass (m/z=405.0918±0.01) at a collision energy of 20.0 eV.*

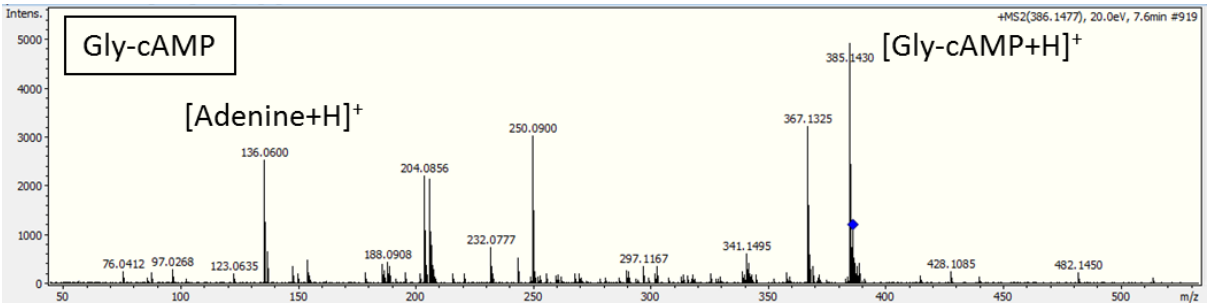


Figure S120. *MS-MS data for the fragmentation Glycine-cAMP mass (m/z=387.0813±0.01) at a collision energy of 20.0 eV.*

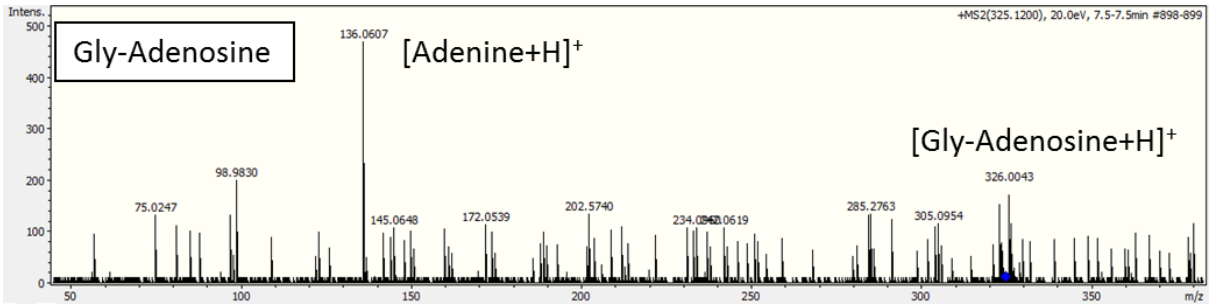


Figure S121. *MS-MS data for the fragmentation Glycine-Adenosine mass (m/z=325.1255±0.01) at a collision energy of 20.0 eV.*

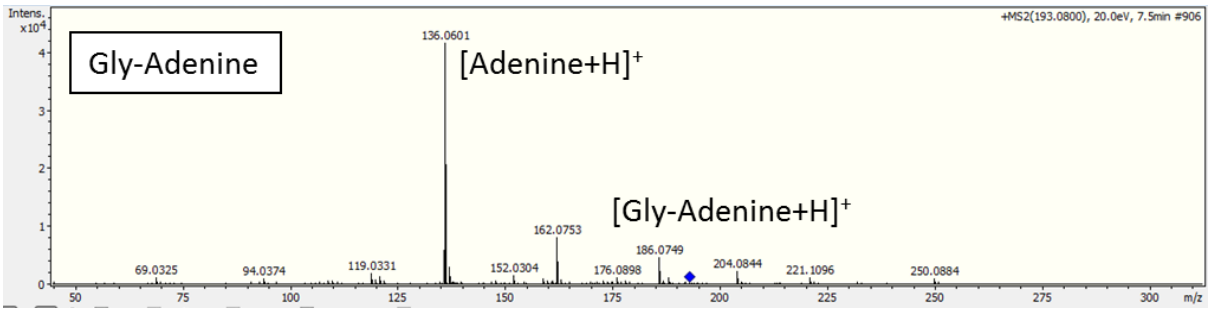


Figure S122. *MS-MS data for the fragmentation glycine-adenine mass (m/z=193.0832±0.01) at a collision energy of 20.0 eV.*

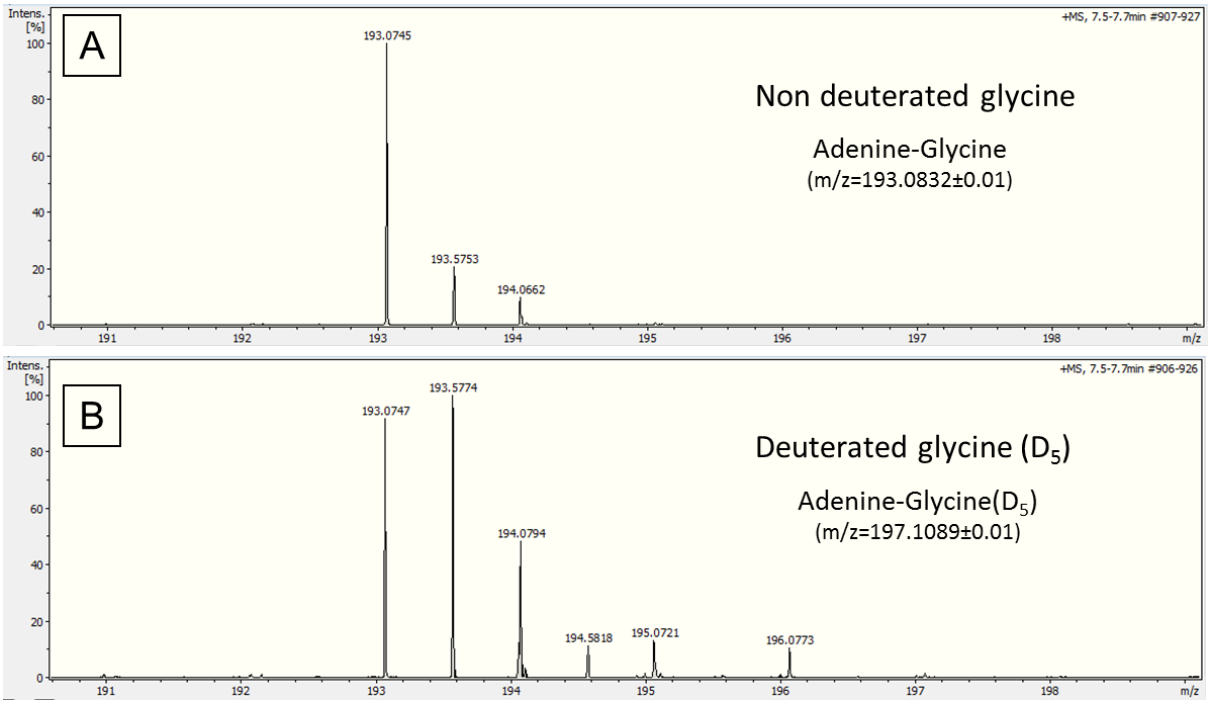


Figure S123, *Isotopic distribution for the mass of adenine-glycine when 25 mM P-ribose + 25 mM adenine was reacted with A) 25 mM glycine B) 25 mM deuterated glycine (Gly-D_5).*

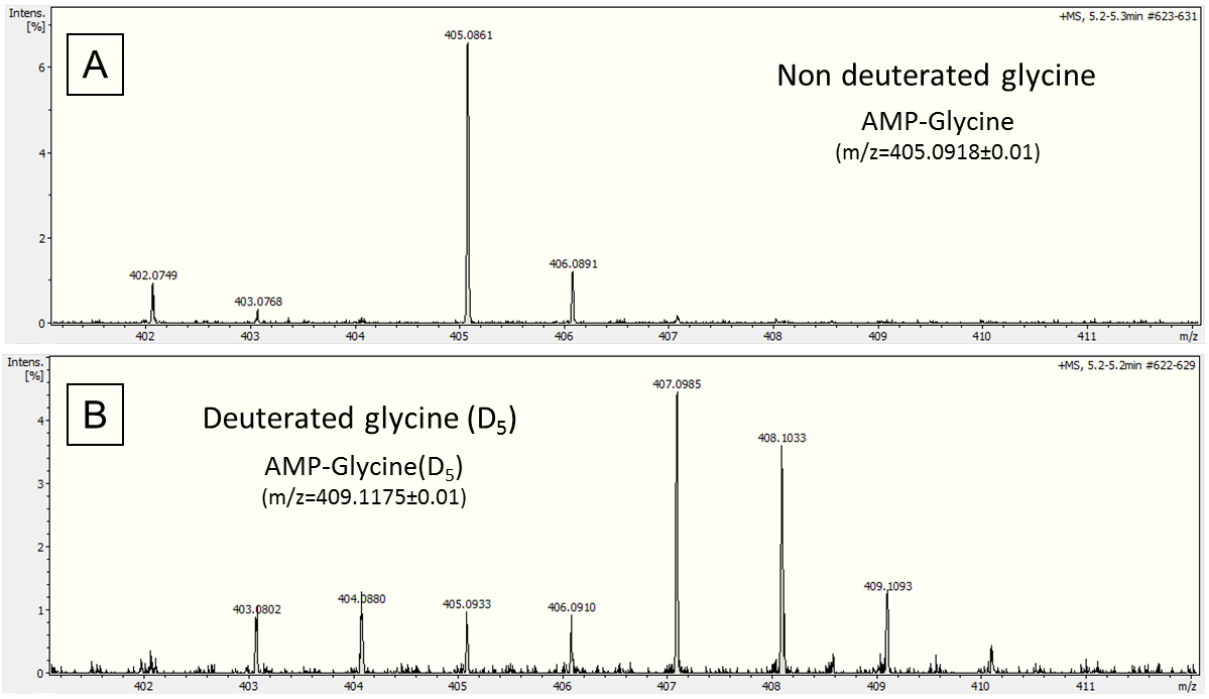


Figure S124. *Isotopic distribution for the mass of AMP-Glycine when 25 mM P-ribose + 25 mM adenine was reacted with A) 25 mM glycine B) 25 mM deuterated glycine (Gly-D_5).*

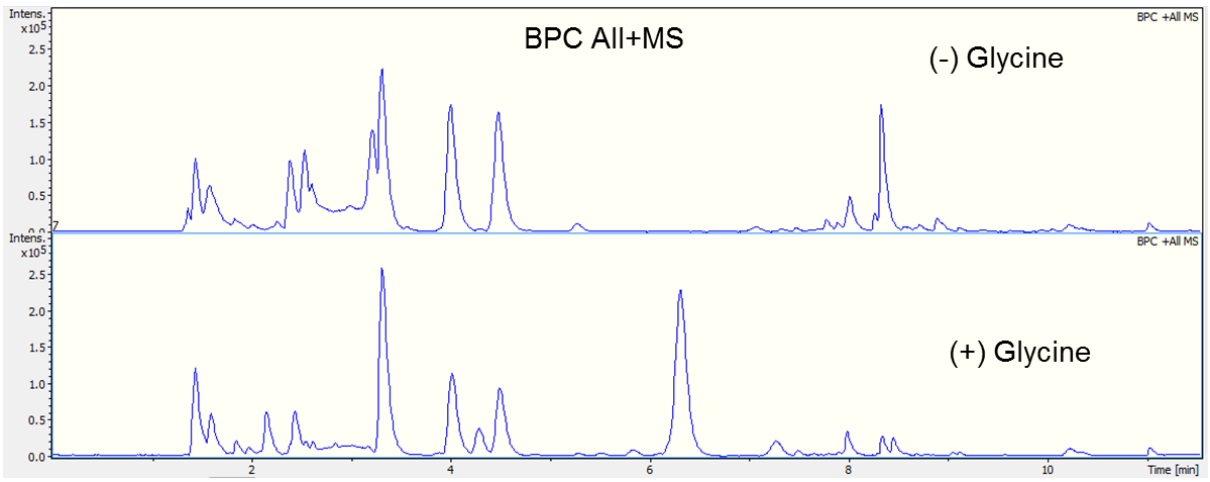


Figure S125. *BPC for All+MS comparing the de-hydration reaction of 25 mM guanine + 25 mM P-ribose with and without 25 mM glycine heated at 90˚C for 5 hours (pH = 2.5).*

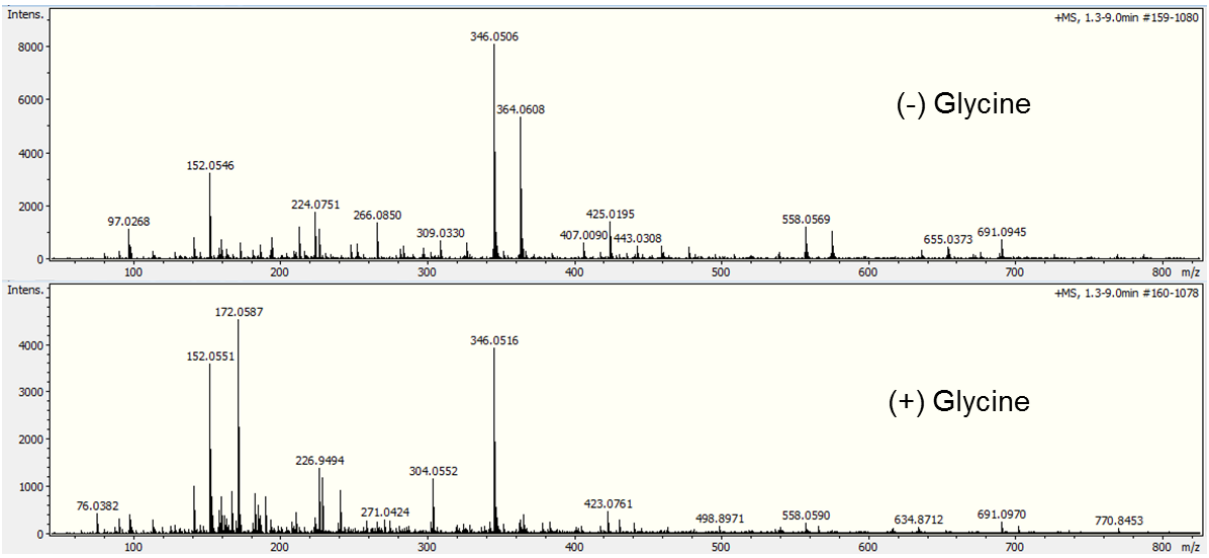


Figure S126. *Mass distribution correspondent to the BPC All+MS (Fig. S114), within a retention time of 1.3-9.0 minutes, comparing the reaction of 25 mM P-ribose + 25 mM guanine with 25 mM glycine + 25 mM P-ribose + 25 mM guanine. All the reactions were heated at 90˚C for different time (pH 2.5).*

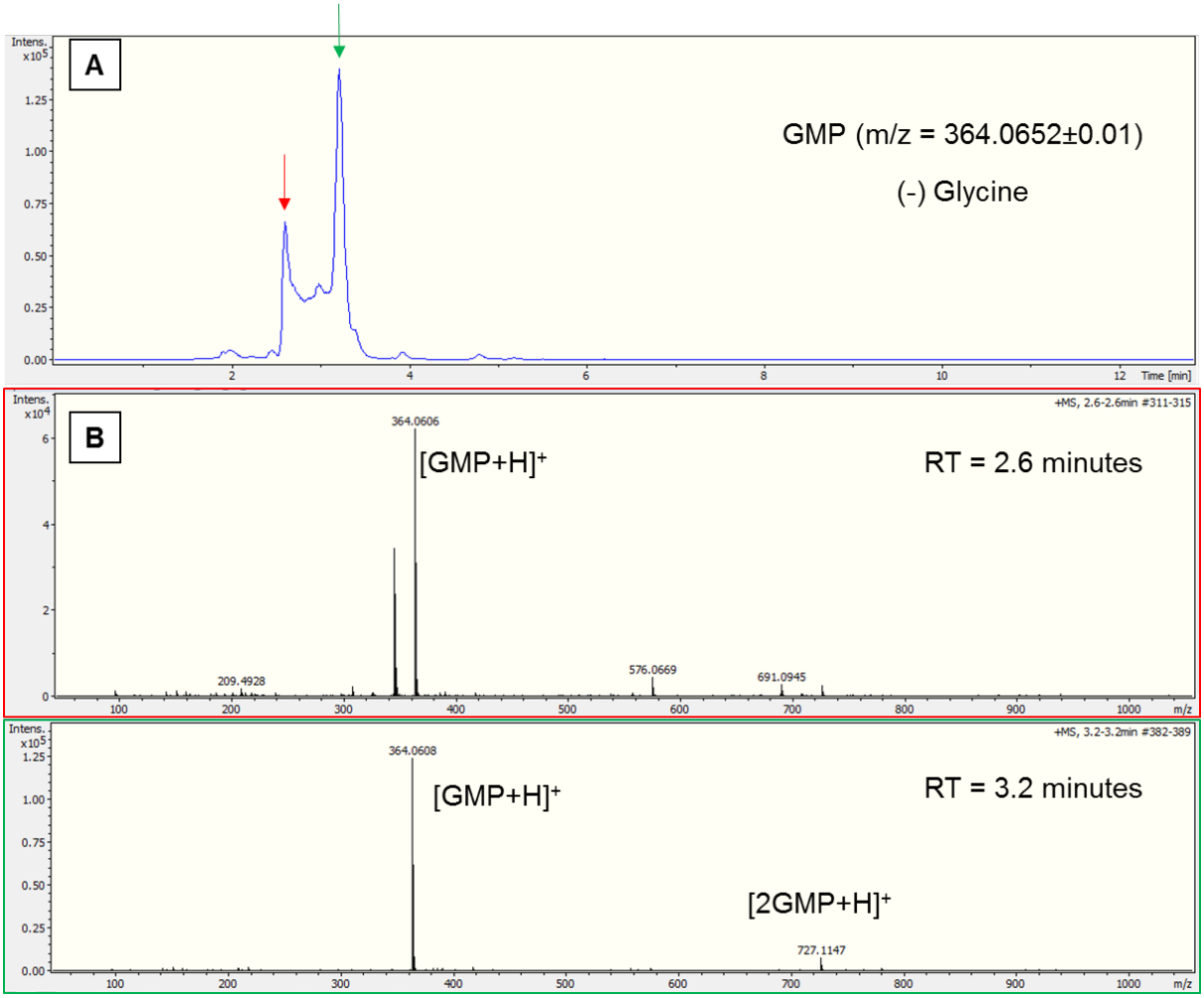


Figure S127. *A) EIC of GMP mass (m/z=364.0652±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine without 25 mM glycine, at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

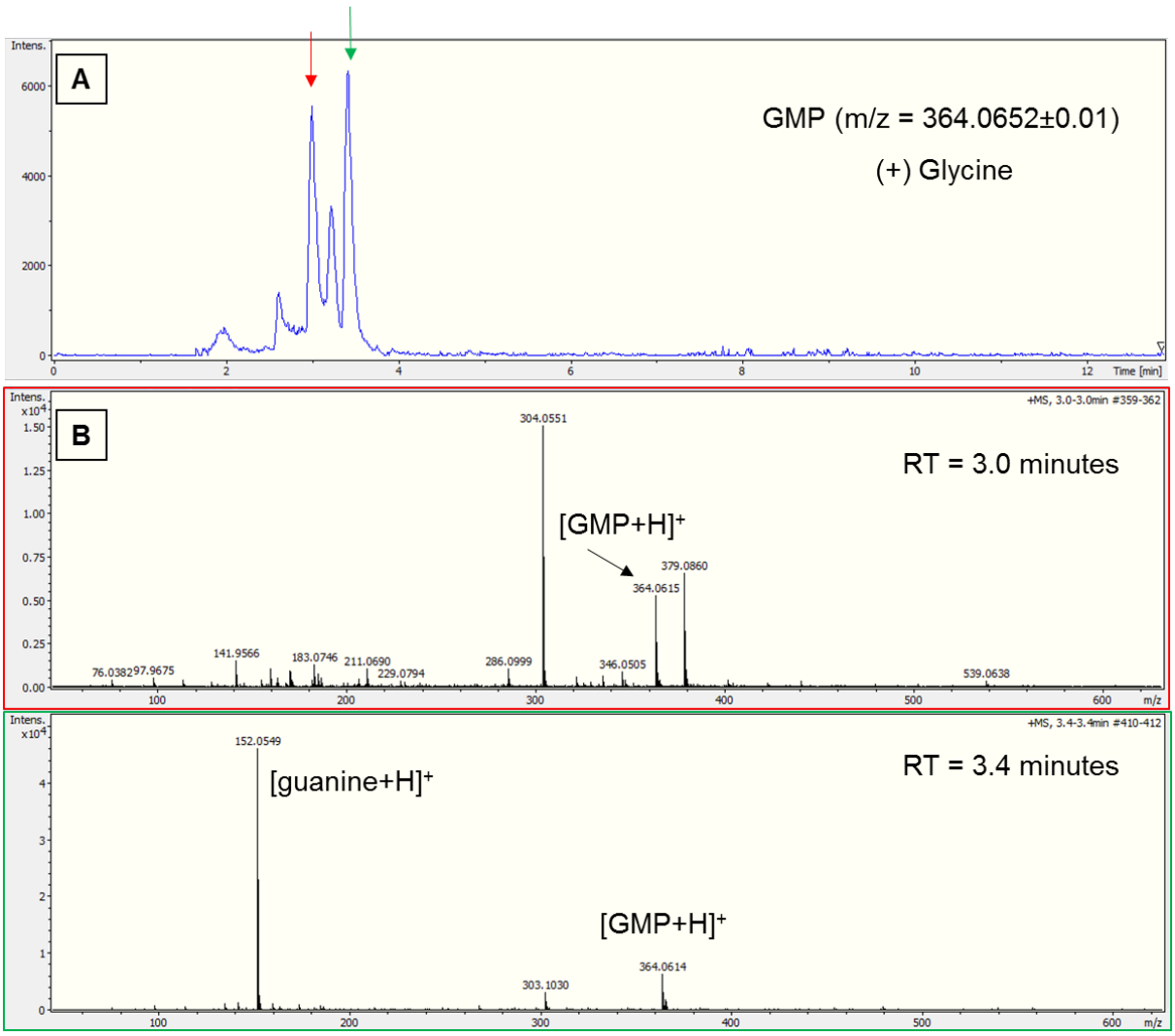


Figure S128. *A) EIC of GMP mass (m/z=364.0652±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine, with and without 25 mM glycine, at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

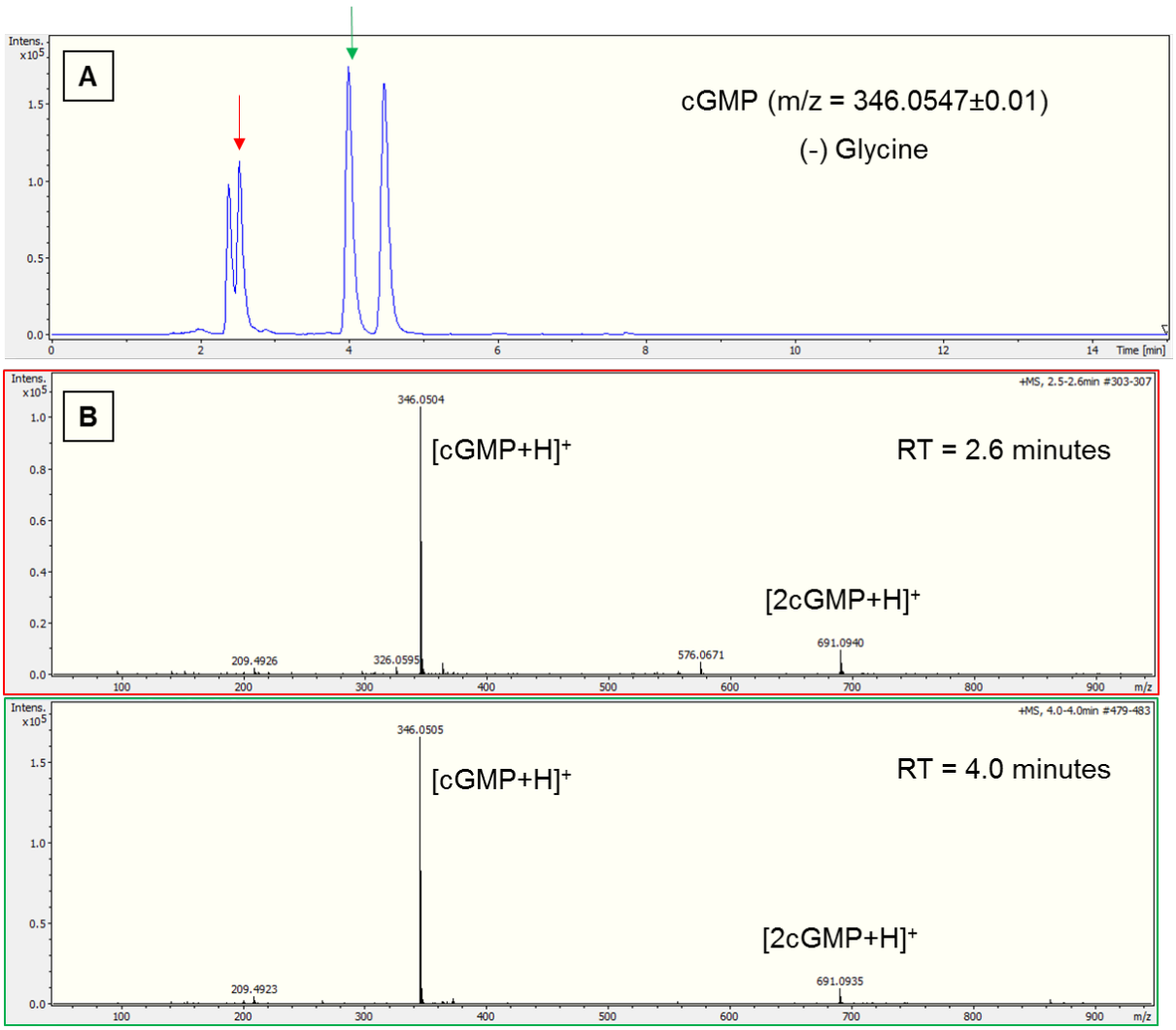


Figure S129. *A) EICs of cyclic GMP mass (m/z=346.0547±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine without 25 mM glycine, at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

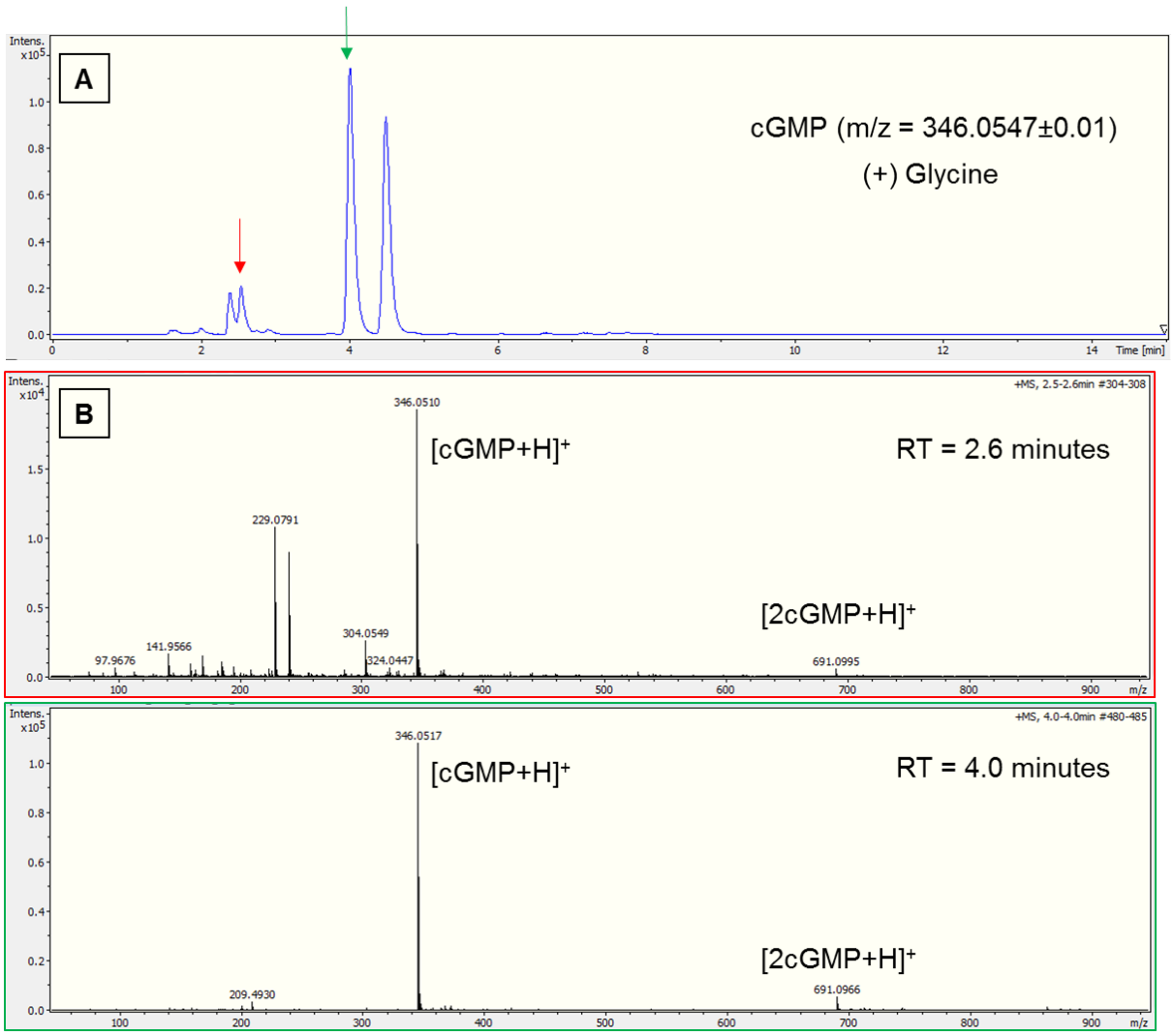


Figure S130. *A) EICs of cyclic GMP mass (m/z=346.0547±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine with 25 mM glycine, at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

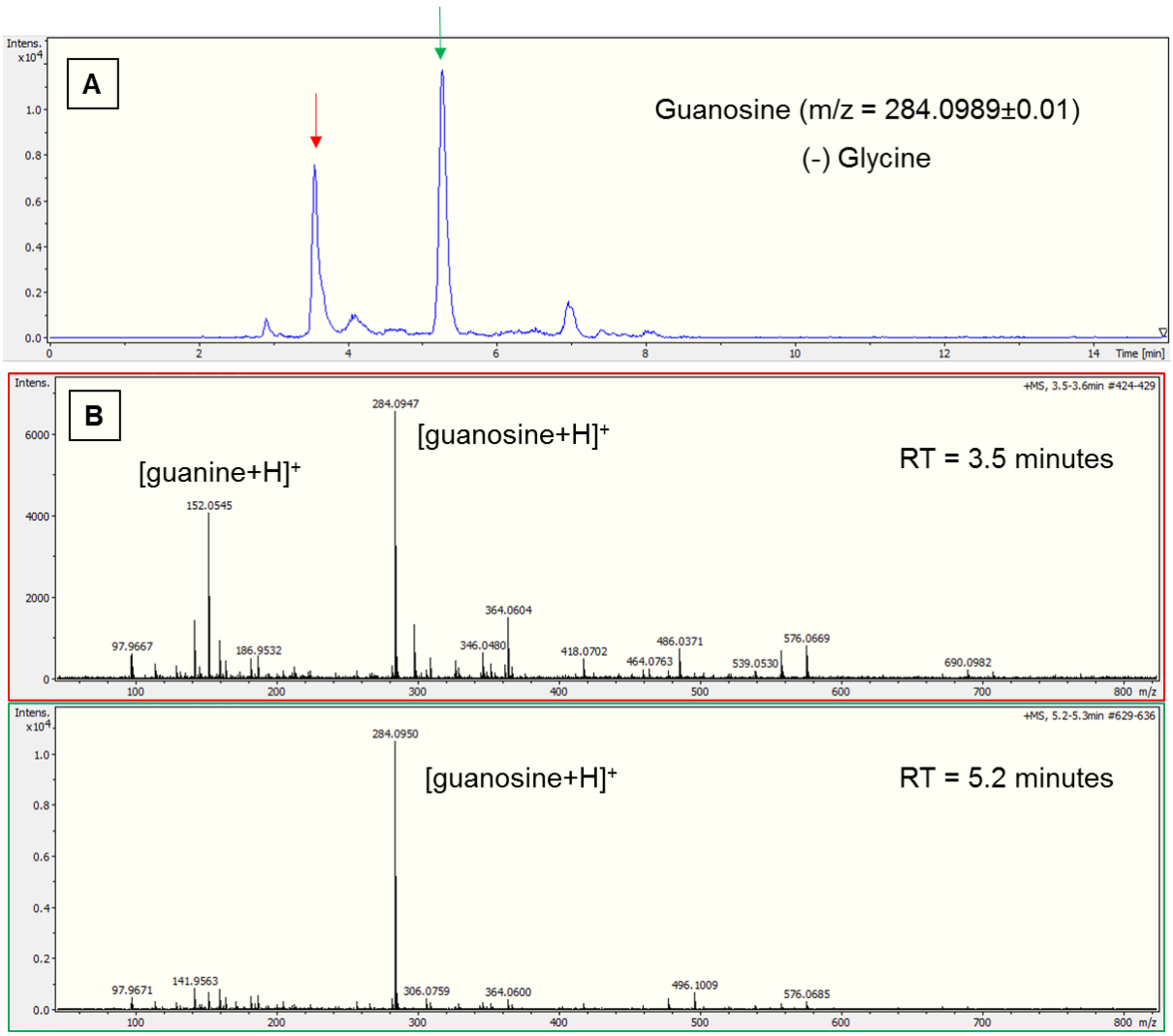


Figure S131. *A) EIC of guanosine mass (m/z=284.0989±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine without 25 mM glycine, at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

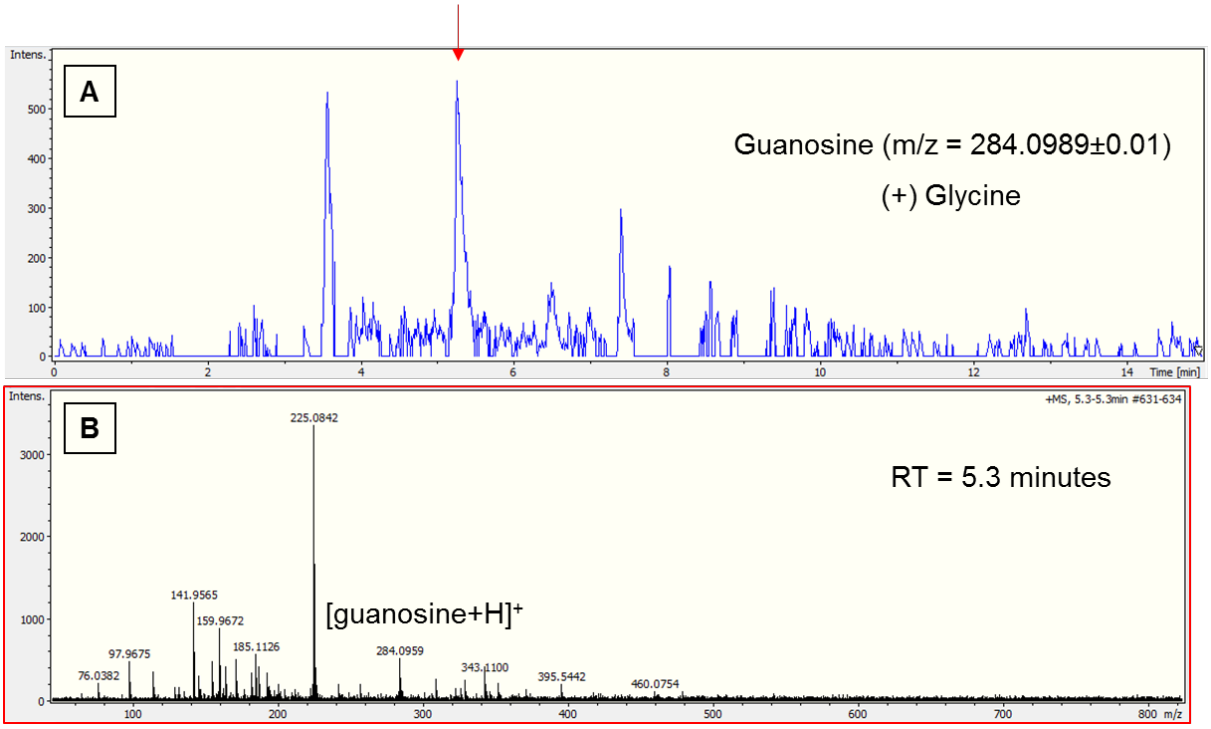


Figure S132. *A) EIC of guanosine mass (m/z=284.0989±0.01) after the reaction of 25 mM P-ribose + 25 mM guanine with 25 mM glycine, at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peaks showed in the EIC show in section A.*

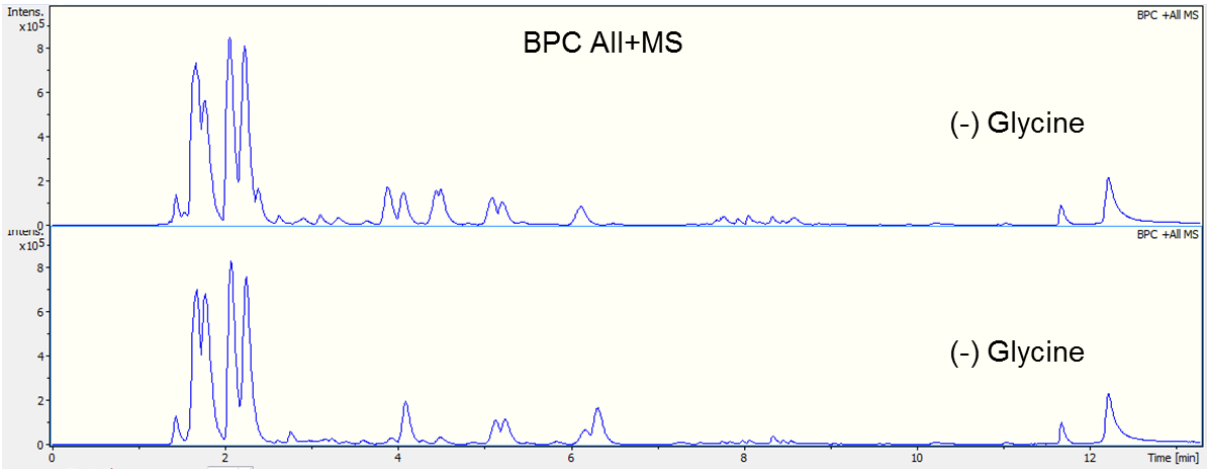


Figure S133. *BPC for All+MS comparing the dehydration reaction of 25 mM cytosine + 25 mM P-ribose with and without 25 mM glycine. Both reactions were heated at 90˚ C for 5 hours (pH = 2.5).*

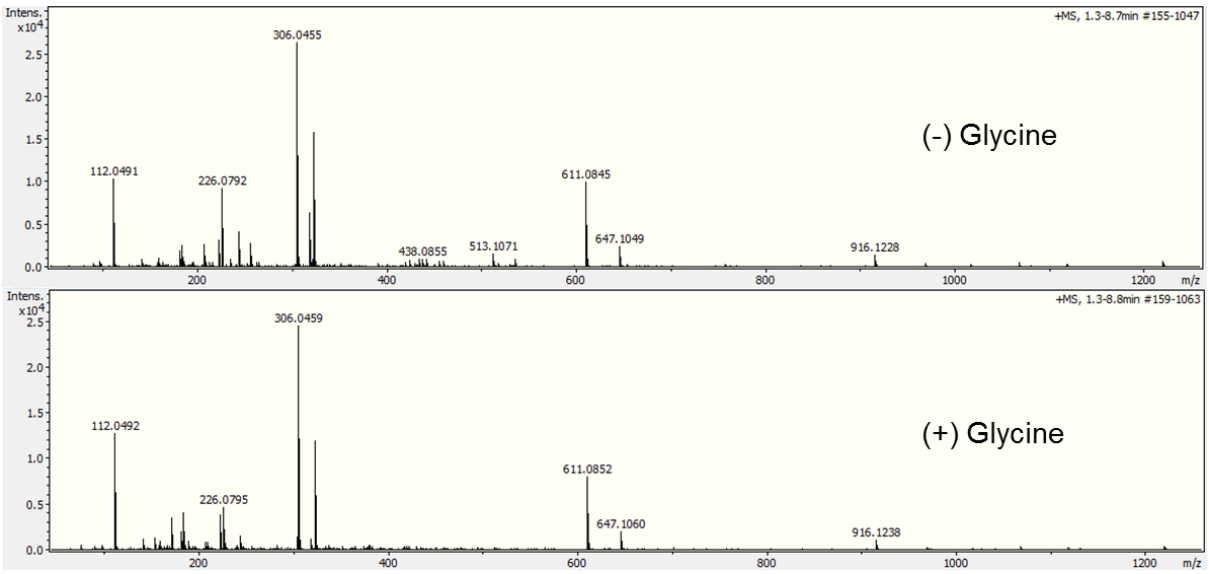


Figure S134. *Mass distribution correspondent to the BPC All+MS (Fig. S122), within a retention time of 1.3-8.7 minutes, comparing the reaction of 25 mM P-ribose + 25 mM cytosine vs 25 mM glycine + 25 mM P-ribose+ 25 mM cytosine. All the reactions were heated at 90˚C for 5 hours (pH = 2.5).*

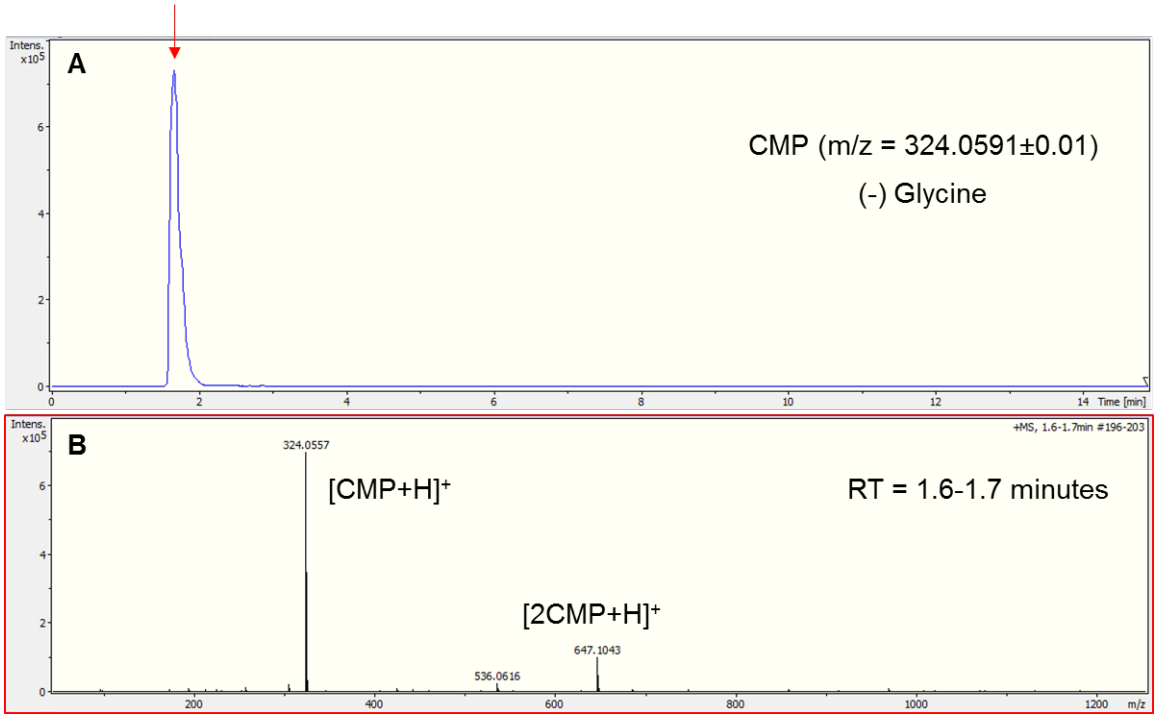


Figure S135. *A) EIC of CMP mass (m/z=324.0591±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine without 25 mM glycine, at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peak showed in the EIC show in section A.*

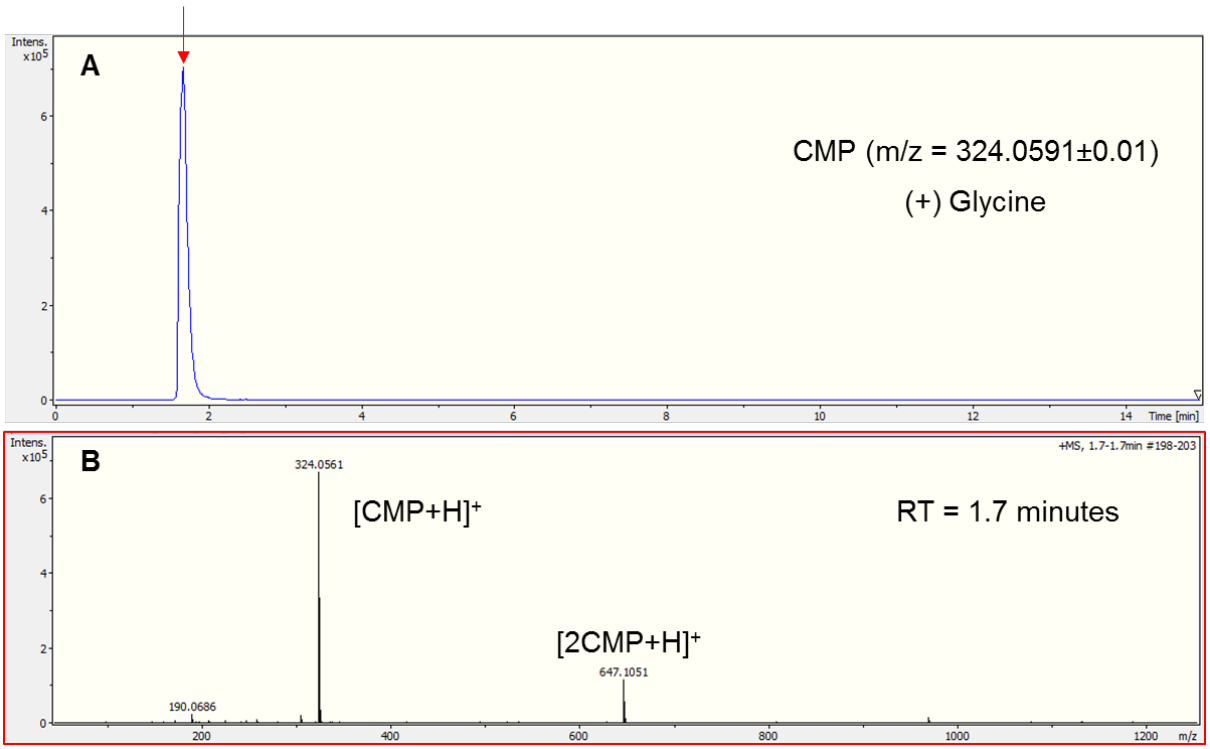


Figure S136. *A EIC of CMP mass (m/z=324.0591±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine with 25 mM glycine, at 90˚C for 5 hours (pH 2.5) B) Mass distribution in each of the peak showed in the EIC show in section A.*

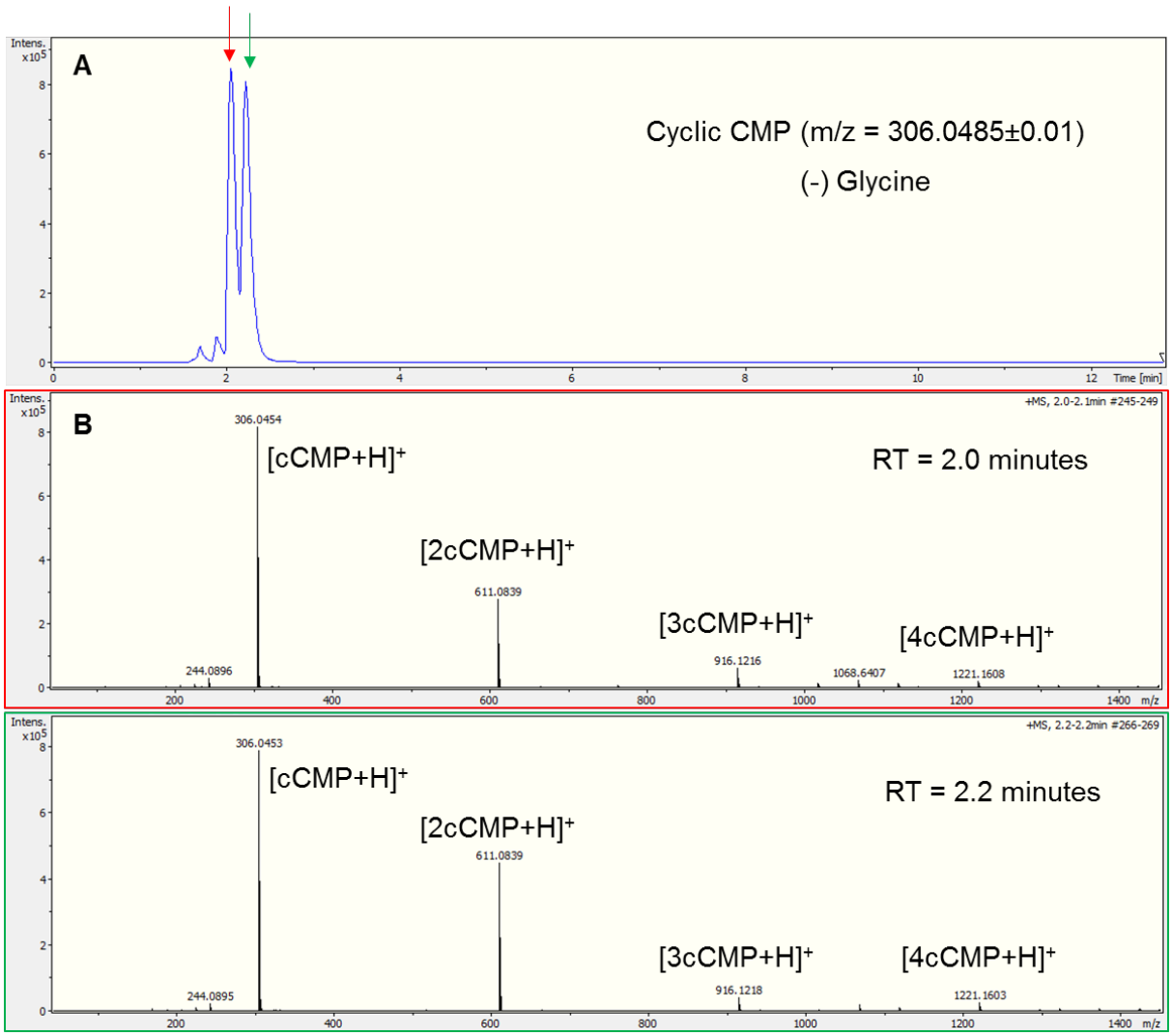


Figure S137. *A) EIC of cyclic CMP mass (m/z=306.0485±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine without 25 mM glycine, at 90˚C for 5 hours (pH 2.5). B) Mass distribution in each of the peak showed in the EIC show in section A.*

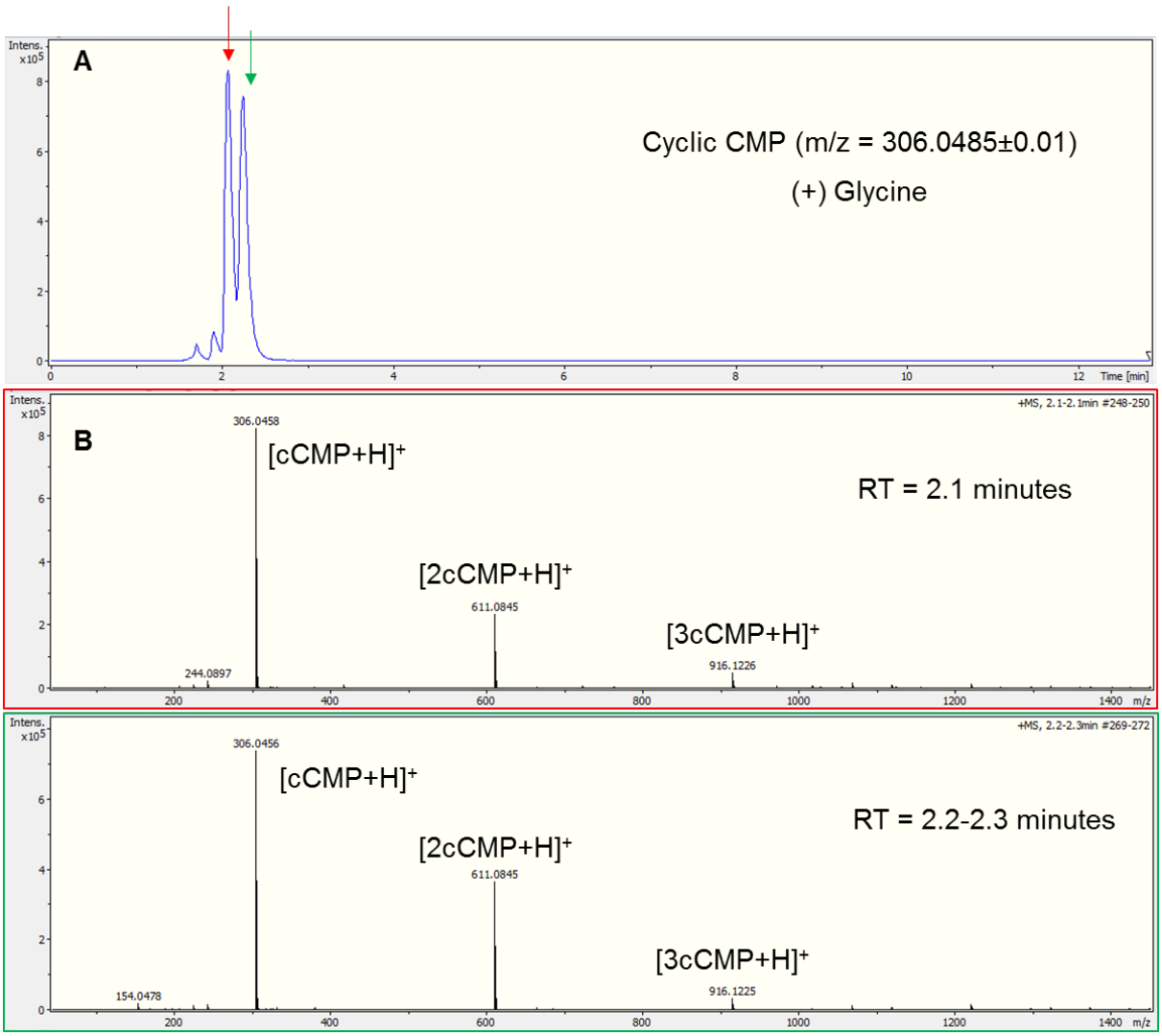


Figure S138. *A) EIC of cyclic CMP mass (m/z=306.0485±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine with 25 mM glycine, at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peak showed in the EIC show in section A.*

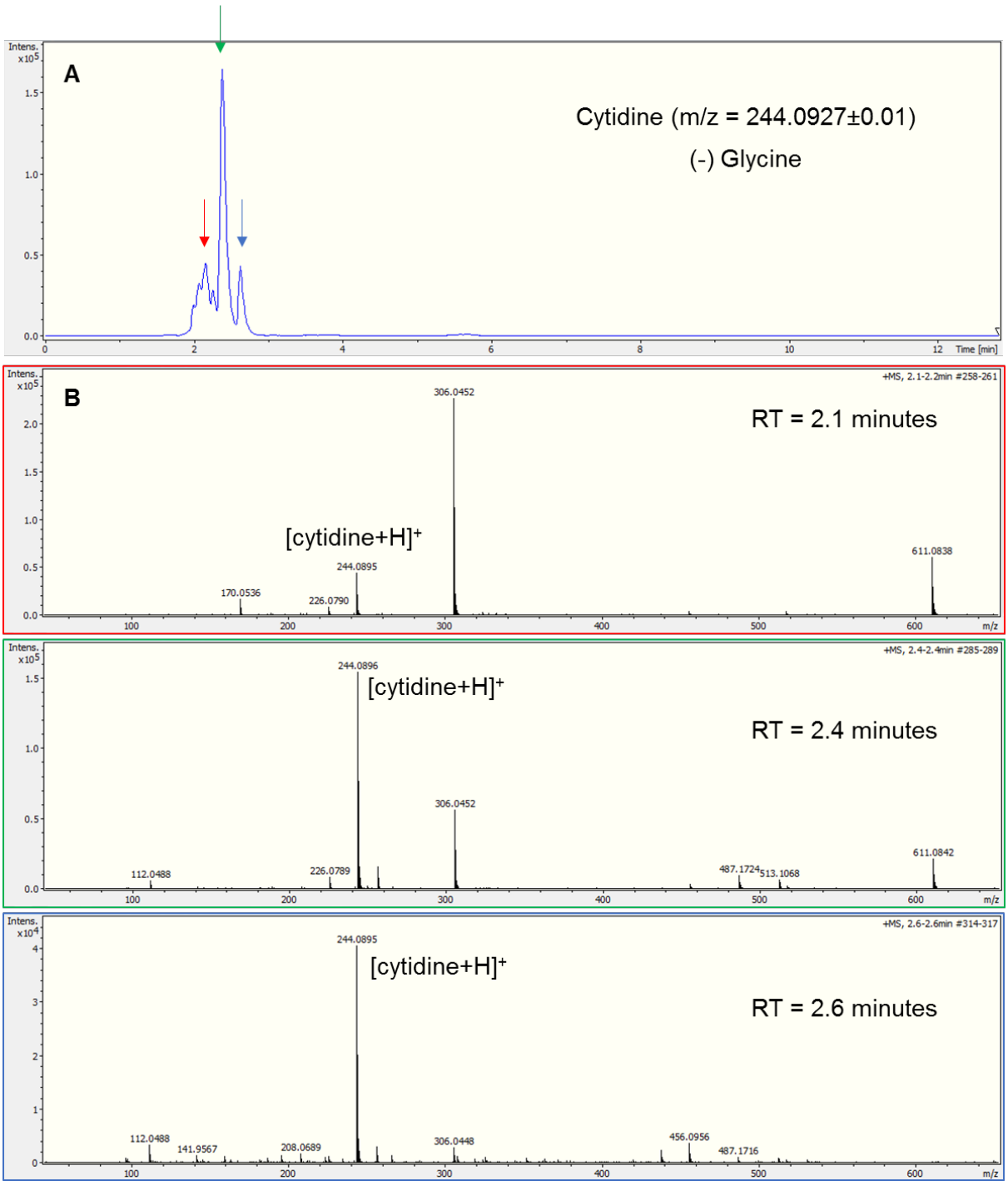


Figure S139. *A) EIC of cytidine mass (m/z=244.0927±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine without 25 mM glycine, at 90˚C for 5 hours (pH 2.5) B) Mass distribution in each of the peak showed in the EIC show in section A.*

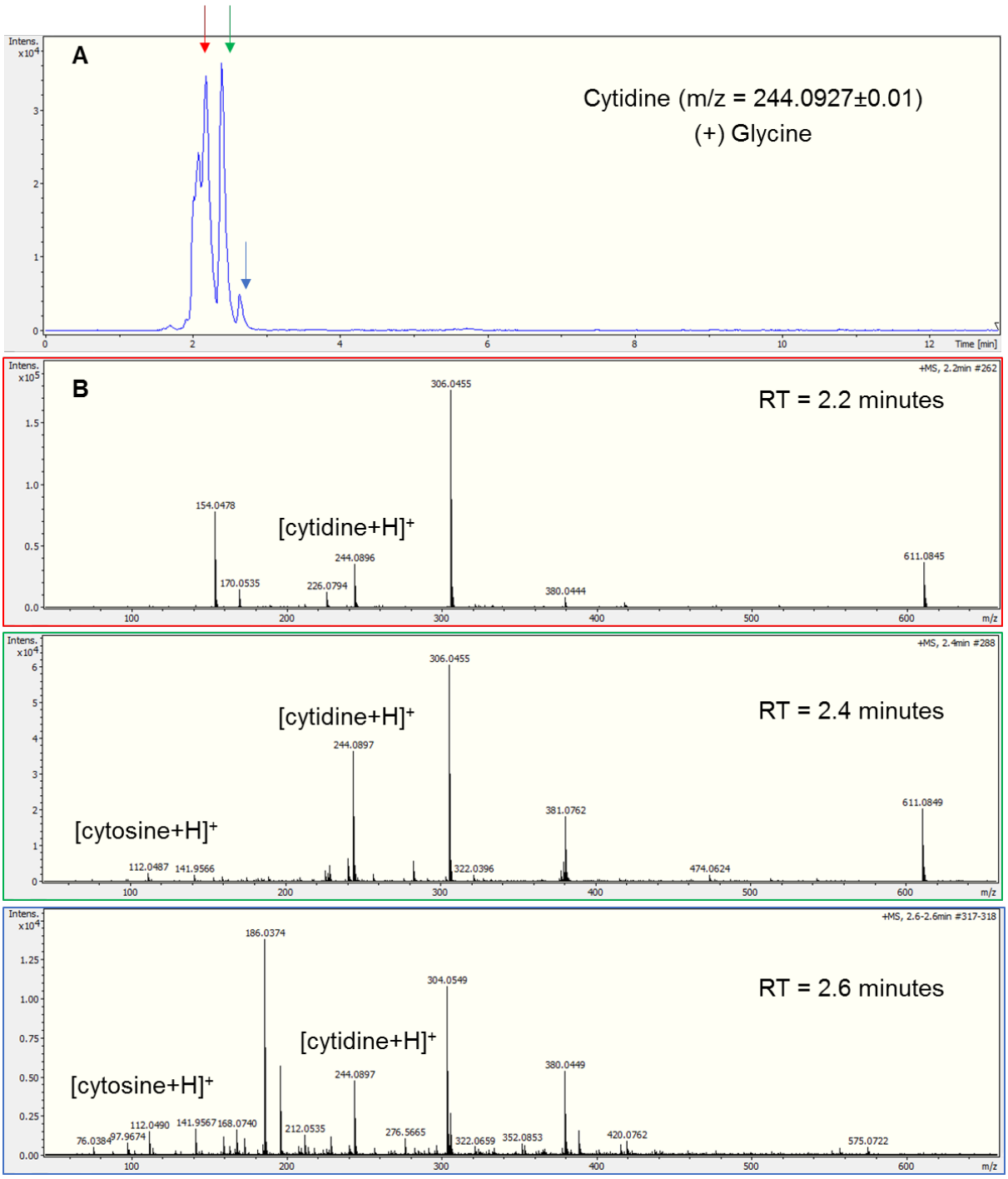


Figure S140. *A) EICof cytidine mass (m/z=244.0927±0.01) after the reaction of 25 mM P-ribose + 25 mM cytosine with 25 mM glycine, at 90˚ C for 5 hours (pH 2.5). B) Mass distribution in each of the peak showed in the EIC show in section A.*

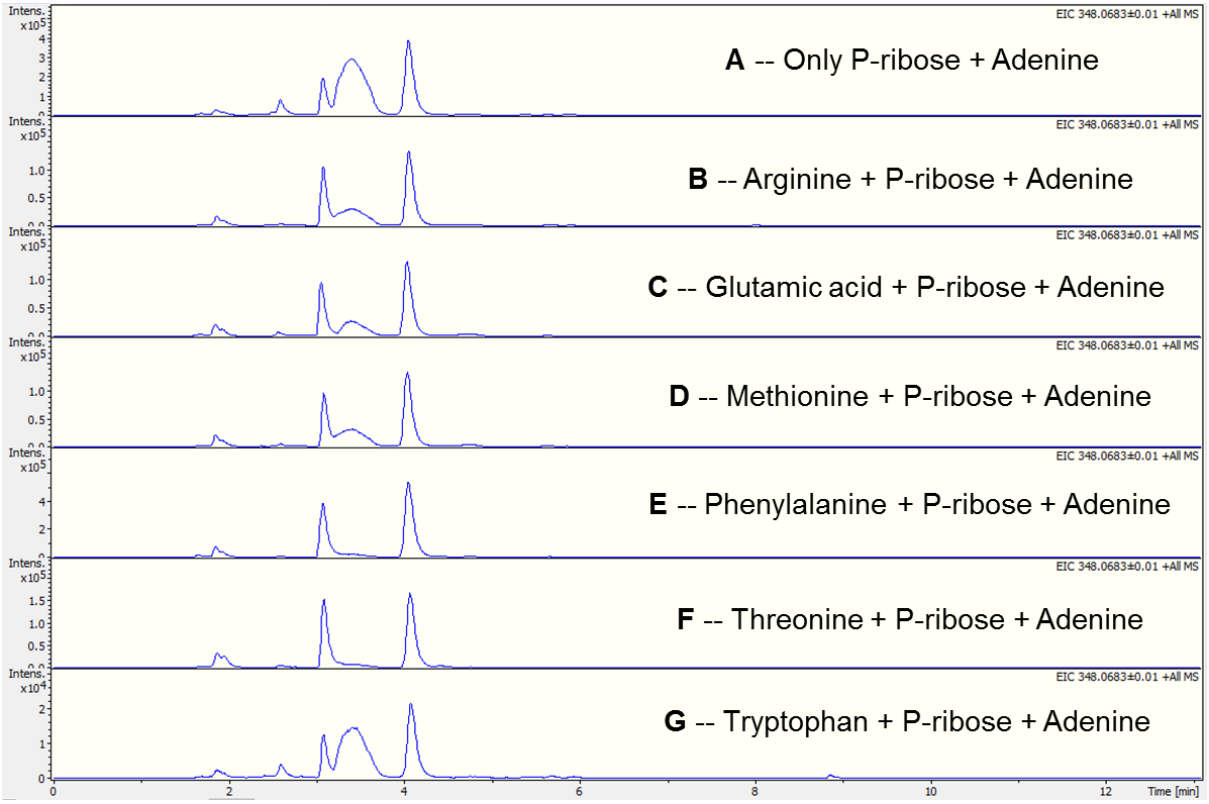


Figure S141. *EICs for adenosine monophosphate mass (m/z=348.0683±0.01) comparing the reaction of 25 mM adenine + 25 mM P-ribose (reaction A) with the reactions of 25 mM adenine + 25 mM P-ribose including different amino acids. B) 25 mM arginine C) 25 mM glutamic acid D) 25 mM methionine E) 25 mM phenylalanine F) 25 mM threonine G) 25 mM tryptophan. All the reactions were heated at 90˚ C for 5 hours (pH = 2.5).*

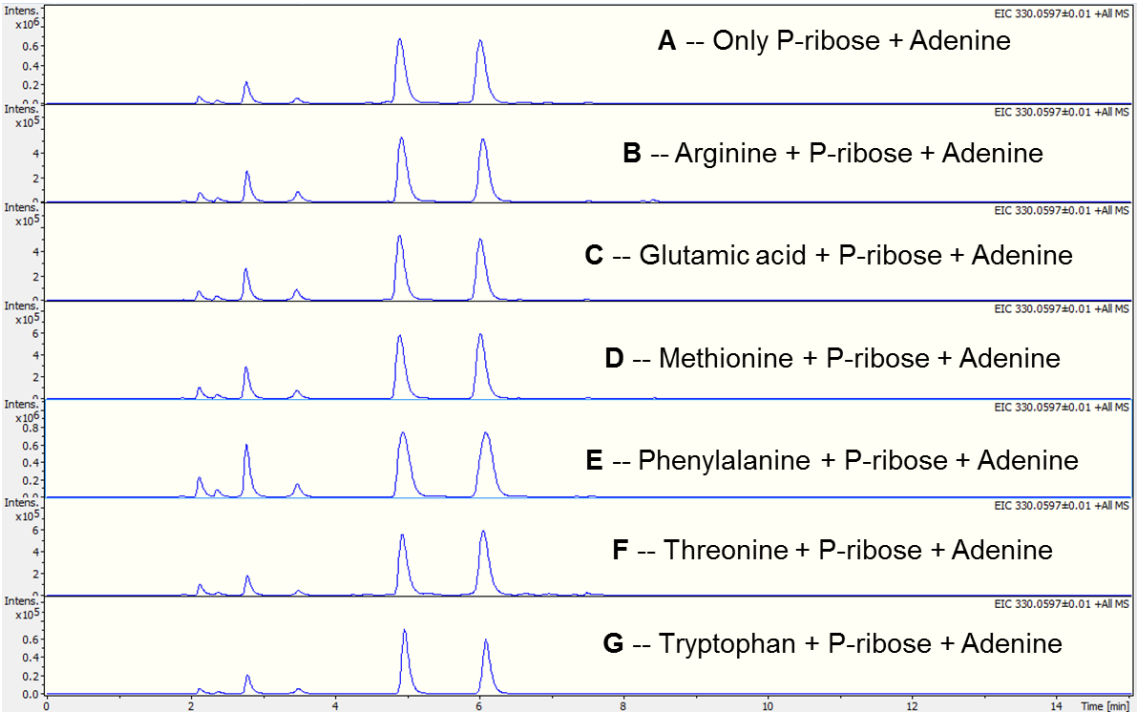


Figure S142. *EICs for cyclic AMP mass (m/z=330.0597±0.01) comparing the reaction of 25 mM adenine + 25 mM P-ribose (reaction A) with the reactions of 25 mM adenine + 25 mM P-ribose including different amino acids. B) 25 mM arginine C) 25 mM glutamic acid D) 25 mM methionine E) 25 mM phenylalanine F) 25 mM threonine G) 25 mM tryptophan. All the reactions were heated at 90˚C for 5 hours (pH = 2.5).*

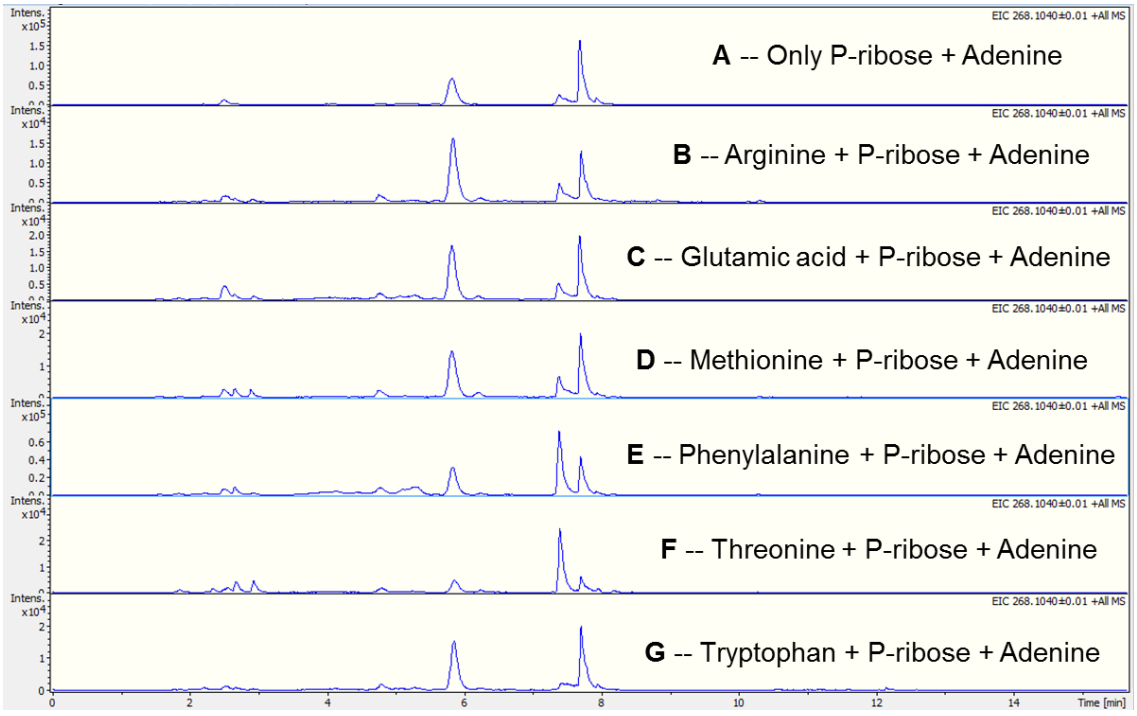


Figure S143. *EICs for adenosine mass (m/z=268.1040±0.01) comparing the reaction of 25 mM adenine + 25 mM P-ribose (reaction A) with the reactions of 25 mM adenine + 25 mM P-ribose including different amino acids. B) 25 mM arginine C) 25 mM glutamic acid D) 25 mM methionine E) 25 mM phenylalanine F) 25 mM threonine G) 25 mM tryptophan. All the reactions were heated at 90˚C for 5 hours (pH = 2.5).*

8. Dendrogram / Statistical analysis

The R function *hclust()* from "stats" package based on average linkage for eleven different samples was employed for implementation of hierarchical clustering. The *dist()* function from the same package was used to compute and return the auto-distance/similarity matrix between rows of data frame (Base Peak Chromatogram extorted from MS data of each sample).

9. Semi-quantitative yields

The semi-quantitative yields were calculated using the integrated are below the Extracted Ion Chromatogram.

$$\%\ \text{glycosylation products} = \frac{100 \times (Add\ all\ EIC\ intregrals\ of\ glycosylation\ products\ )}{(Add\ all\ EIC\ integrals\ of\ all\ compounds\ with\ adenine)}$$

Where:

-EIC integrals: area below the extracted ion chromatogram for each of the masses of the cited compounds.

-All compounds with adenine include: AMP, 2AMP, cAMP, 2cAMP, adenosine, adenine, Gly-Adenine, Gly-Adenosine, Gly-AMP and Gly-cAMP.

-Glycosylation products include: AMP, cAMP and adenosine.

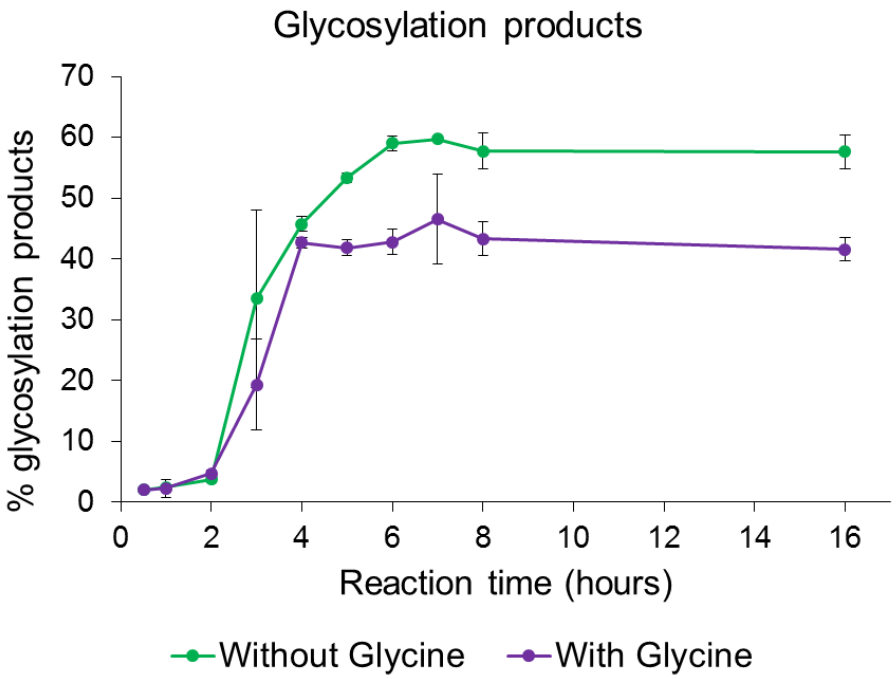


Figure S144. *Time dependent representation of the semi-quantitative yields for the formation of glycosylation products (AMP, cyclic-AMP and adenosine) after the reaction of 25 mM adenine + 25 mM P-ribose (in green) and the reaction of 25 mM glycine + 25 mM adenine + 25 mM P-ribose (in purple) at 90˚C, pH 2.5 (HCl) and at pre-set time intervals.*

$$\% \text{ glycine hybrids} = \frac{100 \times (Add\ all\ EIC\ intregrals\ of\ glycine\ hybrids)}{(Add\ all\ EIC\ integrals\ of\ all\ compounds\ with\ adenine)}$$

Where:

-EIC integrals: area below the extracted ion chromatogram for each of the masses of the cited compounds.

-All compounds with adenine include: AMP, 2AMP, cAMP, 2cAMP, adenosine, adenine, Gly-Adenine, Gly-Adenosine, Gly-AMP and Gly-cAMP.

-Glycine hybrids include: Gly-Adenine, Gly-Adenosine, Gly-AMP and Gly-cAMP.

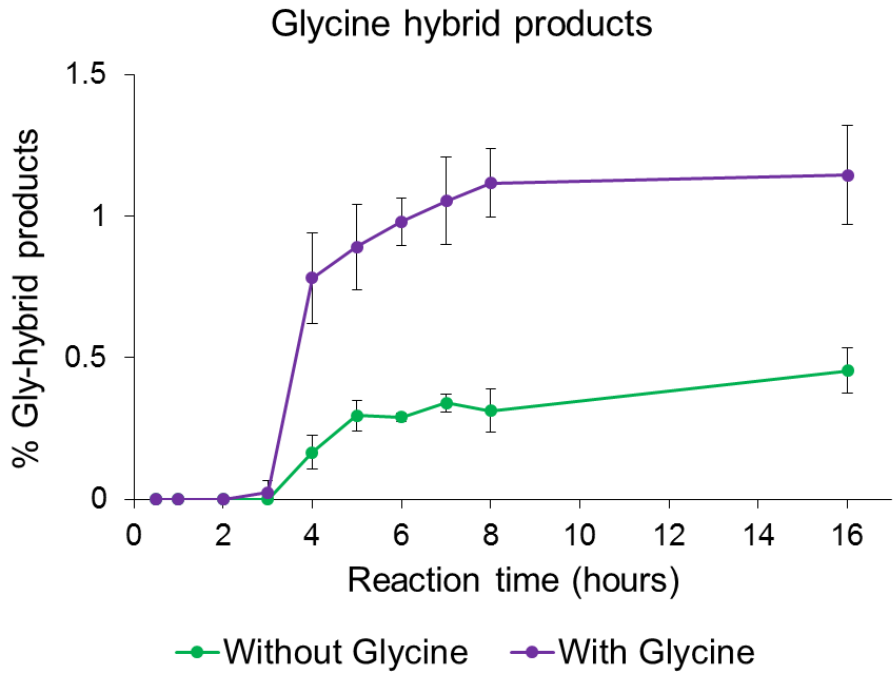


Figure S145. *Time dependent representation of the semi-quantitative yields for the formation of glycosylation products (AMP, cyclic-AMP and adenosine) after the reaction of 25 mM adenine + 25 mM P-ribose (in green) and the reaction of 25 mM glycine + 25 mM adenine + 25 mM P-ribose (in purple) at 90˚C, pH 2.5 (HCl) and at pre-set time intervals.*

% cytosine glycosylation products =

$$\frac{Sum\ areas\ below\ (CMP + cCMP + Cytidine)\ EIC}{Sum\ areas\ below\ (AMP + cAMP + Adenosine + Adenine + CMP + cCMP + Cytidine + Cytosine)\ EIC} \times 100$$

% guanine glycosylation products =

$$\frac{Sum\ areas\ of\ (GMP + cGMP + Guanine)\ EIC}{Sum\ areas\ below\ (AMP + cAMP + Adenosine + Adenine + GMP + cGMP + Guanosine + Guanine)\ EIC} \times 100$$

% conversion of cytosine into cytosine glycosylation products =

$$\frac{Sum\ areas\ below\ (CMP + cCMP + Cytidine)\ EIC}{Sum\ areas\ below\ (CMP + cCMP + Cytidine + Cytosine)\ EIC} \times 100$$

% conversion of guanine into guanine glycosylation products =

$$\frac{Sum\ areas\ below\ (GMP + cGMP + Guanosine)\ EIC}{Sum\ areas\ below\ (GMP + cGMP + Guanosine + Guanine)\ EIC} \times 100$$

	% cytosine/ guanine glycosylation products	**% conversion of cytosine/guanine into cytosine / guanine glycosylation products**
AMP + Guanine	3.85%	65.15%
AMP + Cytosine	3.47%	26.89%

Figure S146. Table showing the semi-quantitative yields for the formation of glycosylation products (AMP, cyclic-AMP and adenosine) after the reaction of 25 mM adenine + 25 mM P-ribose (in green) and the reaction of 25 mM glycine + 25 mM adenine + 25 mM P-ribose (in purple) at 90˚ C, pH 2.5 (HCl) and at pre-set time intervals.

10. Isomeric distribution of AMP

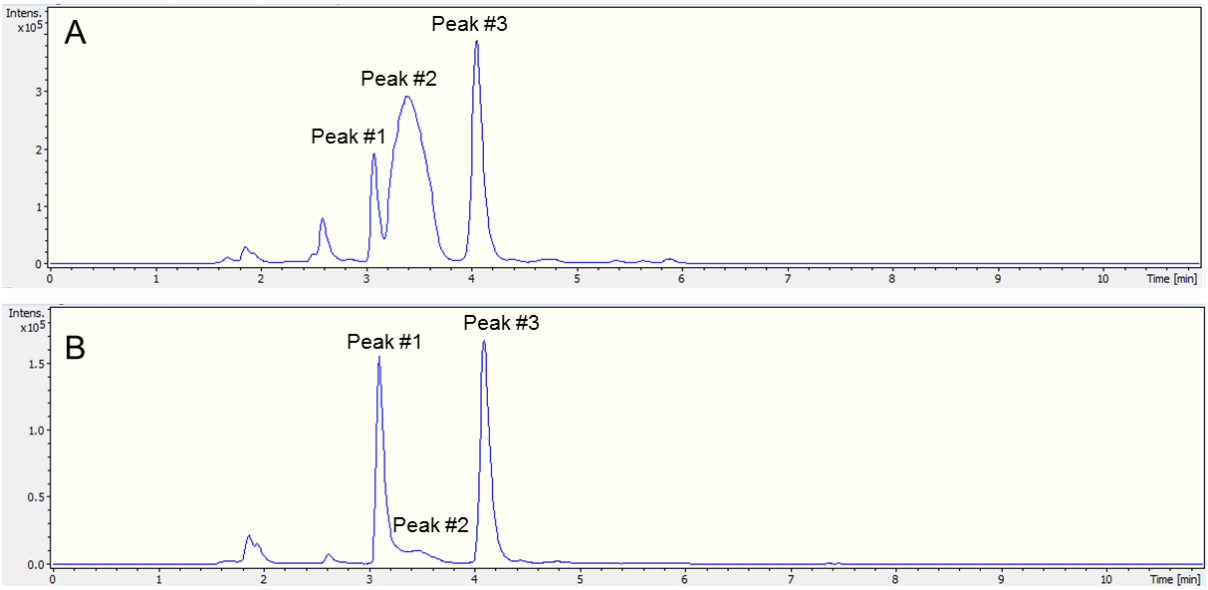


Figure S147. *EICs for AMP mass (m/z=348.0683±0.01) comparing the reaction of 25 mM adenine + 25 mM P-ribose (reaction A) with the reaction of 25 mM glycine + 25 mM adenine + 25 mM P-ribose (reaction B). All the reactions were heated at 90˚C for 6 hours (pH = 2.5).*

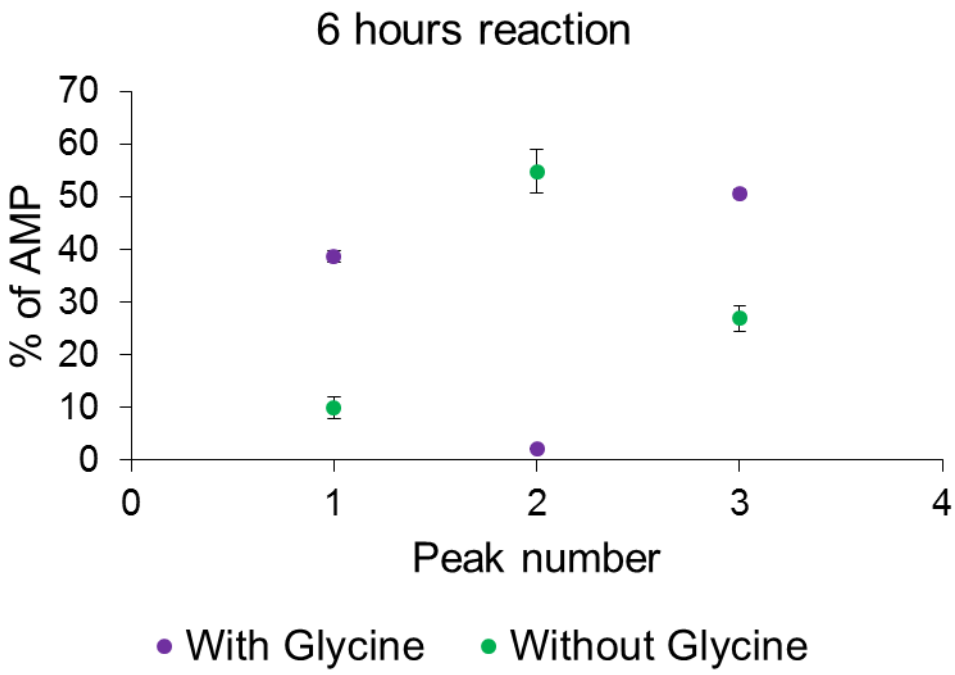


Figure S148. *Relative percentage of AMP on each of the isomers represented and number in Figure S146.*