

SUPPLEMENTARY DATA

Animal Hen1 2'-O-methyltransferases as tools for 3'-terminal functionalization and labelling of single-stranded RNAs

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SUPPLEMENTARY METHODS

Cloning of plasmids carrying recombinant proteins

pGEX-DmHen1 coding N-terminal GST-tag containing DmHen1 protein was a kind gift from Mikiko C. Siomi (Keio University School of Medicine, Tokyo, Japan). This plasmid was used as a template for GST-DmHen1 Δ C amplification by PCR with DmHen1 Δ C-Fw and DmHen1 Δ C-Rv. The resulting PCR product contained GST sequence joined to the first 281 aa of DmHen1 followed by the stop codon and XhoI restriction site. DmHen1 Δ C's coding sequence replaced full length protein in the original plasmid by cloning it with MunI (an internal restriction endonuclease) and XhoI (*Thermo Scientific*) resulting in pGEX-DmHen1 Δ C.

HsHEN1 gene was cloned from human genomic DNA using the unique method of exon assembly. Shortly, each of the six exons from 2nd to 7th of HsHEN1 was amplified by PCR primers HsHEN1-E2-7-Fw and HsHEN1-E2-7-Rv which were partly complementary to the following exon. Amplified exons were joined into one sequence through PCR reaction with terminal primers HsHEN1-E2-Fw and HsHEN1-E7-Rv. The first exon (of only 21 nt) was obtained through annealing of HsHEN1-E1-Fw and HsHEN1-E1-Rv, which contained a 3' part of BamHI recognition sequence at 5' end, exon 1 in the middle, and part of exon 2 at its 3' end with three last nucleotides being the 5' half of HincII recognition sequence. This dsRNA was ligated to HincII (*Thermo Scientific*) restricted exons 2-7, resulting in full HsHEN1 coding sequence. After XhoI restriction, the recognition sequence of which was added to exon 7, HsHEN1 coding sequence replaced DmHen1 in pGEX-DmHen1 plasmid digested with BamHI and XhoI restriction endonucleases generating pGEX-HsHEN1. Primers used for plasmids are listed in Supplementary Table S3.

Expression and purification of recombinant proteins

GST tagged Hen1 proteins were expressed in *Escherichia coli* BL21(DE3)RIL strain (*Invitrogen*). Cultures were grown at 37°C till OD reached 0.6 at which point proteins' expression was induced with 0.1 mM IPTG (*Thermo Scientific*). After overnight growth at 16°C cells were harvested and lysed by sonication in 50 mM phosphate buffer (pH 7.6) containing 10% sucrose, 250 mM NaCl, 1 mM EDTA, 0.1% (v/v) Triton X-100, 1 μ M pepstatin and 0.5 mM PMSF supplemented with Complete EDTA-free protease inhibitor cocktail tablets (*Roche*). Insoluble fraction was removed by centrifugation for 20 min at 45000 x g and the soluble one was filtered through 0.45 μ m Millex-HV PVDF filter (*Merck Millipore*).

In order to avoid nuclease contamination two-step purification scheme was applied. As a first step affinity chromatography was used as soluble filtered lysate was loaded onto equilibrated 5 ml GSTrap HP column (*GE Healthcare*; the used Equilibration buffer (pH 7.3) contained 140 mM NaCl, 10 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄ and 1 mM EDTA). GST tagged proteins were eluted with 50 mM Tris-HCl (pH 8.0), 10 mM GSH and 1 mM DTT. Then fractions that were enriched in the proteins of interest were further loaded on the 5 ml anion exchange column HiTrap Q HP (*GE Healthcare*) washed with 50 mM Tris-HCl (pH 8.0). Hen1 proteins were eluted with 1 M NaCl and dialyzed against 25 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM DTT and 0.1% Triton X-100 at first without and later with 50% glycerol, collected and stored at -20°C.

Preparation of templates for *in vitro* transcription

For *in vitro* transcription of 43 nt and longer RNA substrates PCR-amplified DNA was used as a template, which itself was amplified from pUC19-T7II-siR43/60 or 80-HDV plasmid with T7II-Fw and HDV-Rv primers. These plasmids were constructed using pTZ19-siR43/60/80. pTZ19-siR43 was a result of ligation of siR-DNA-43-1/2 with PstI and HindIII (*Thermo Scientific*) digested vector. pTZ19-siR60 and siR80 was generated after siR-DNA-60-1/2 and siR-DNA-80-1/2, respectively, were ligated with MunI and HindIII (*Thermo Scientific*) restricted pTZ19-siR43. As initial *in vitro* transcription experiments resulted in heterogeneous transcripts, T7 class III promoter was changed to class II promoter and HDV ribozyme at the 3' end of each sequence was added. This was done through three steps of PCR amplification: first, sequences of T7 II promoter, respective RNA and 5' of HDV ribozyme were amplified using T7II-Fw and siR-HDV-Rv primers and respective plasmid as a template, second, HDV ribozyme was obtained after PCR from partially complementary HDV-Fw and HDV-Rv primers (1), third, PCR products from first and second steps were mixed and amplified with terminal primers, namely T7II-Fw and HDV-Rv. DNA fragments generated in the last step were ligated with pUC19/SmaI (*Thermo Scientific*) resulting in pUC19-T7II-siR43/60 or 80-HDV.

All primers used for preparation of longer RNA substrates are listed in Supplementary Table S4.

RNA modification and analysis on denaturing polyacrylamide gel

RNA modification reactions were performed in 20 µl of reaction volume in the presence of 0.02-0.2 µM ³²P-labelled RNA (³²P-RNA), 1-2 µM protein, 0.1 mM AdoMet **1**/Ado-6-amine **2**/Ado-6-azide **3** or 0.05 mM Ado-13-biotin **4** in the Reaction buffer containing 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5% glycerol, 10 mM Co²⁺ in the form of CoCl₂ salt, 0.2 mM DTT, 0.1 mg/ml BSA and 0.04 u/µl RiboLock RNase inhibitor (*Fisher Scientific*) and incubated for 30 min at 37°C. When different metal cofactors were used, Co²⁺ was replaced by corresponding chloride salts (hexaamincobalt trichloride for Co³⁺), except for nickel(II) sulphate. When 3-6 µM of Ado-14-Cy3 **5** was applied as a cofactor, RNA was modified for 1 h in Cy3 reaction buffer composed of 0.1 M Tris-HCl (pH 7.0), 0.2 mM DTT, 0.1 mg/ml BSA, 10 mM Co²⁺ and 50 µM tryptophan. In all cases Hen1 proteins were diluted up to required concentration in the Protein dilution buffer (10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mg/ml BSA, 0.1% Triton X-100, 2 mM DTT, 50% glycerol and 0.02 u/µl RiboLock). Reactions were quenched with equal volume of 2 mg/ml Proteinase K (*Thermo Scientific*) in Quenching buffer (20 mM Tris-HCl (pH 7.5), 10 mM NaCl, 0.5 mM EDTA and 0.5% SDS) by heating at 55°C for 20 min. To monitor the reaction products of AdoMet **1**, Ado-6-amine **2** and Ado-6-azide **3** modification sodium periodate treatment and β-elimination was applied following RNA precipitation. Shortly, RNA was dissolved in 17 µl 60 mM Borate

buffer (pH 8.6) and 3 μ l of 0.2 M sodium periodate was added. After 15 min of incubation in the dark at room temperature reactions were stopped with 3 μ l of 67% glycerol. Following further incubation in the dark for 10 min RNA was precipitated and dissolved in 20 μ l 60 mM Borate buffer (pH 9.6) and incubated for 1.5 h at 45°C. For polyacrylamide (PAA) gel analysis RNA was mixed with 2 x RNA loading buffer (*Fisher Scientific*), heated for 5 min at 85°C and resolved on 10-13% denaturing PAA gel with 7 M urea. Phosphor imaging plates (*Fujifilm*) were exposed to radioactive gels and later scanned with FLA-5100 Image Reader (*Fujifilm*). Results were analysed using MultiGauge v.3.0 software (*Fujifilm*).

HPLC-MS analysis of modified nucleosides

1 μ M of methyltransferase was used to modify 2 μ M of miR173 in the presence of 0.1 mM of AdoMet **1**/Ado-6-amine **2**/Ado-6-azide **3** or 0.05 mM Ado-13-biotin **4** in the Reaction buffer or 6 μ M of Ado-14-Cy3 **5** in the Cy3 reaction buffer. After 1.5 h of incubation at 37°C, RNA was extracted with chloroform, precipitated and re-suspended in P1 buffer (10 mM NaOAc, 1mM Zn(OAc)₂, pH 6.5) for degradation to nucleotides with 0.01 u/ μ l of P1 nuclease (*Sigma-Aldrich*). After 2 h of incubation at 50°C, followed the overnight dephosphorylation to nucleosides with 0.02 u/ μ l of FastAP phosphatase at 37°C. Enzymes were inactivated by heating at 70°C for 15 min and precipitated by centrifugation at 20,000 x g for 40 min at 4 °C. The collected supernatant was loaded on Discovery SH C18 column (*Supelco*) for analyses using 1290 Infinity HPLC System (*Agilent Technologies*) and eluted with linear gradient from 20 mM ammonium formate (pH 3.5) to 80% methanol. Mass spectra of modification products were acquired on an Agilent Q-TOF 6520 mass analyser in a positive ionization mode and assessed with Agilent MassHunter Workstation software.

Electrophoretic mobility shift assay (EMSA)

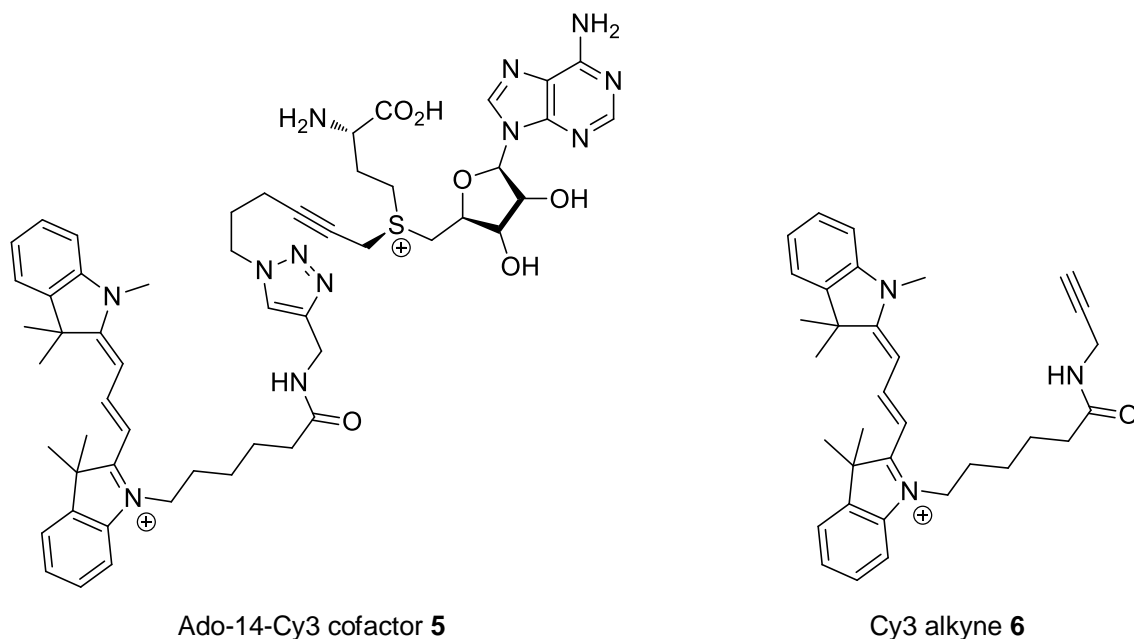
0.2 μ M of ³²P-labelled miR173 was incubated with no protein (control) or with 1 μ M of DmHen1 or HsHEN1 for 30 min at 37°C in Binding buffer (10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mg/ml BSA and 5% glycerol) containing 0.1 mM of AdoHcy without or with 1, 5 or 10 mM metal cofactor in the form of chloride salt. Potential RNA-protein complexes were analysed on native 8% PAA gel in 0.5 x TB buffer (pH 7.5). The gel was dried and exposed to phosphor imaging plate. After scanning with FLA-5100 Image Reader (*Fujifilm*) results were analysed using MultiGauge v.3.0 software (*Fujifilm*).

Synthesis of Ado-14-Cy3 cofactor

(S-Adenosyl-S-6-{4-[N-6-(3,3-dimethyl-2-[(1E,3E)-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-enyl]-3H-indolium-1-yl)hexanoyl]aminomethyltriazol-1-yl}hex-2-yn-1-yl-L-homocysteine bisformate)

Reaction was monitored performing HPLC/MS experiments, HRMS data were obtained using Agilent 6230 TOF mass spectrometer (ESI). The purification of target cofactor was performed with preparative reversed-phase HPLC (column - Agilent Prep-C18, dimensions: 30x150, 10 μ m, PN 413910-302). The yield of cofactor was determined according to the data of spectrometer NanoDrop ND-1000 UV/VIS.

To a stirred solution of enantiomerically pure Ado-6-azide **3** (1.1 mg, 0.0021 mmol) in ammonium formate buffer (0.3 mL, pH 3.5) Cy3 alkyne **6** (5 mg, 0.0073 mmol) dissolved in DMF (30 μ l), CuSO₄ x 5H₂O (0.50 mg, 0.0021 mmol) and L-ascorbate (0.82 mg, 0.0042 mmol) were added. The mixture was left to stir overnight.



After that, water was added to the reaction mixture. The resulting solution was washed with CH_2Cl_2 (5×5 ml). The aqueous layer was evaporated under reduced pressure. The residue was flushed through ion-exchange column (Dowex 1 Å~ 8, 50-100 mesh, anion resin, eluent – 20 mmol/L ammonium formate buffer (pH 3.5)). Due to a low solubility of target cofactor in eluent preparative reversed-phase HPLC was used three times for purification of target cofactor eluting at a flow rate of 30 ml/min using 20 mmol/L ammonium formate buffer (pH = 3.5) as eluent A and 80% methanol in water as eluent B (the gradient of eluent is shown in table below).

T, min	0	2	10	18	22	24	25	33
A, %	50	50	40	20	0	0	50	50
B, %	50	50	60	80	100	100	50	50

Target cofactor was collected in a fraction from 15.9 min till 17.0 min. The solvent was evaporated under reduced pressure and then lyophilized to afford target product **5** as a pink solid. The concentration of cofactor in solution was determined by UV absorption (Cy3 extinction – $\epsilon_{550} = 95744 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ was used).

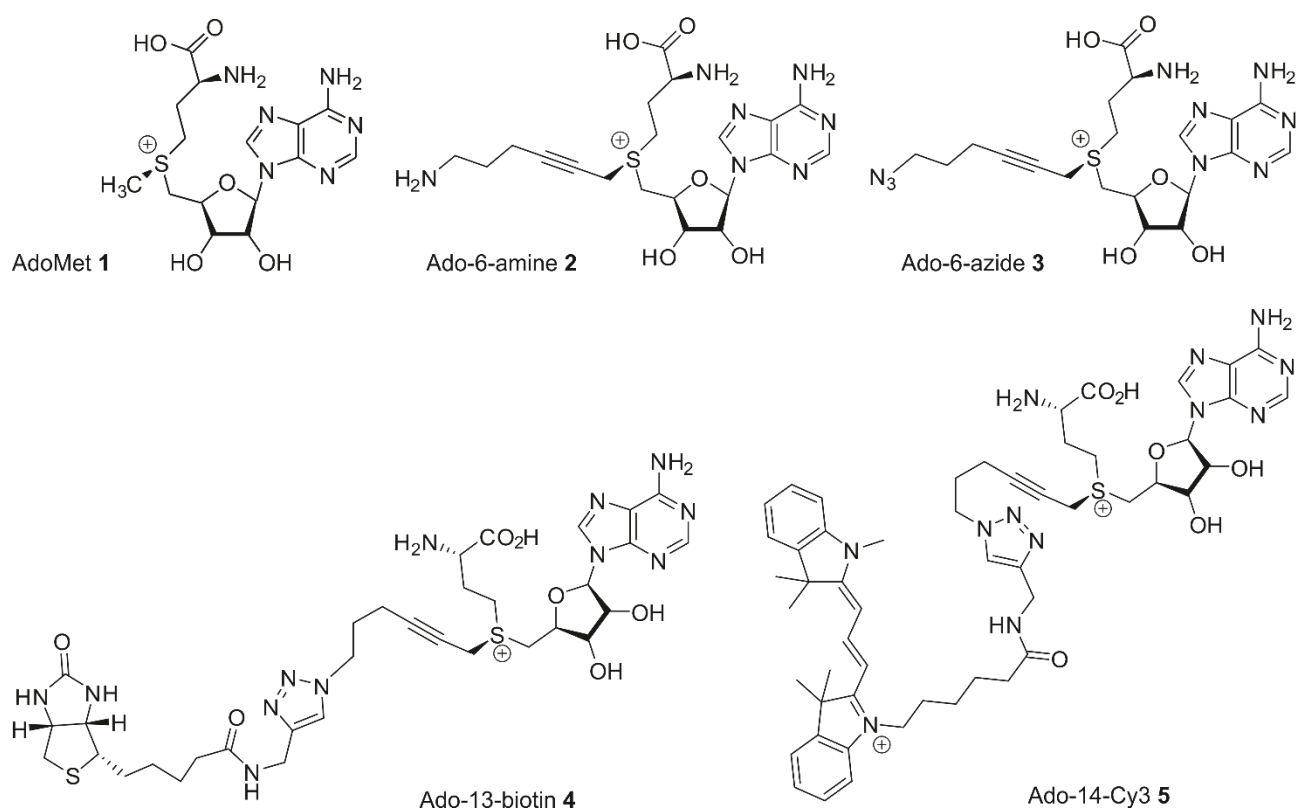
Yield: 3.94 μg (0.18%)

HRMS calc. for $[\text{M}^{2+}] \text{C}_{33}\text{H}_{47}\text{N}_{12}\text{O}_7\text{S}_2$, m/z: 500.2547; found: 500.2540.

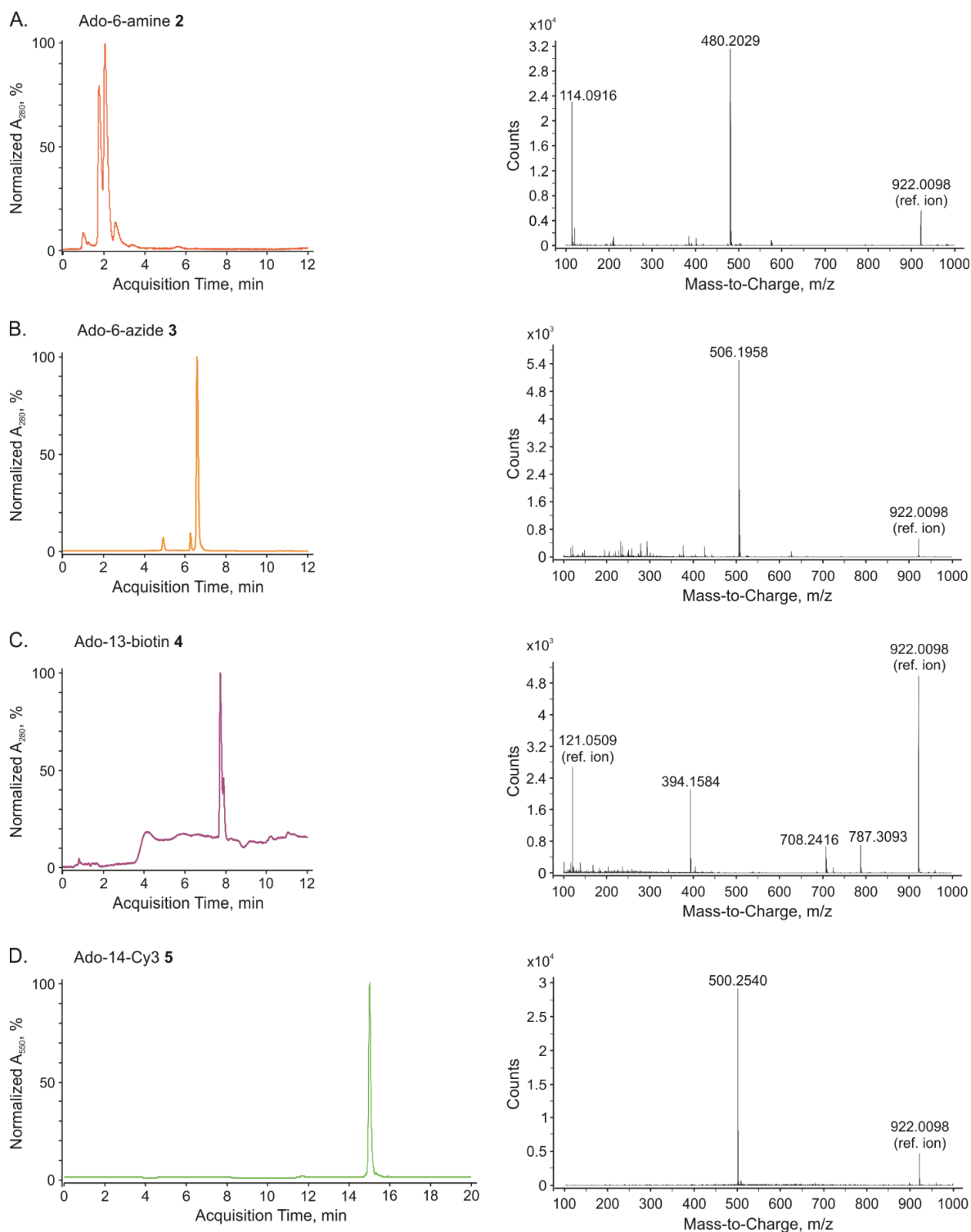
FRET analysis on polyacrylamide gel

For visualization of siR23-Cy3/DNA-2Cy5 FRET pair in PAA gel, siR23 was modified with Cy3 as described above and siR23- CH_3 was prepared as a control. Three pairs of RNA/DNA hybrids were annealed: siR23- CH_3 /DNA, siR23-Cy3/DNA and siR23-Cy3/DNA-2Cy5, mixed with 66.6% glycerol in 7:3 ratio and fractionated on 12% native PAA gel in 0.5 x TB buffer (pH 7.5). The gel was scanned with FLA-5100 Image Reader (*Fujifilm*) using 532 nm laser for Cy3 detection at 570 nm with LPG filter and for FRET signal registration at 662 nm with LPRF filter. The obtained data were analysed using MultiGauge v.3.0 software (*Fujifilm*).

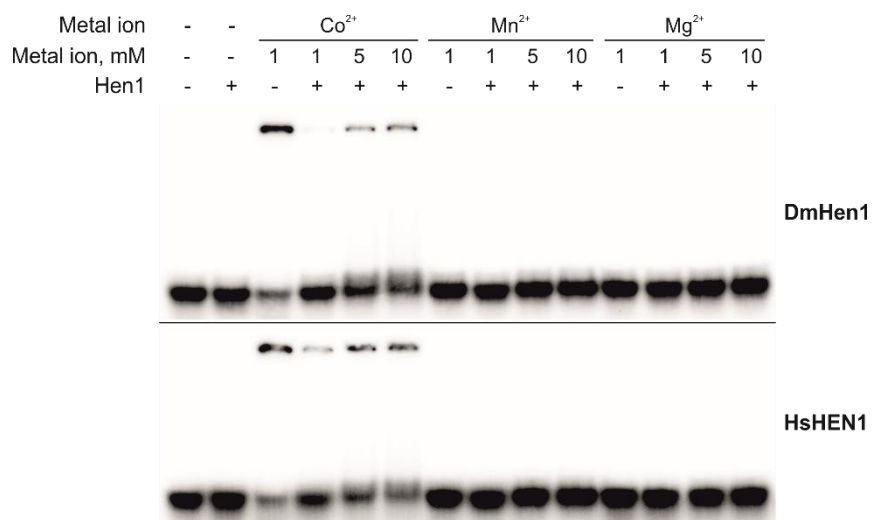
SUPPLEMENTARY FIGURES



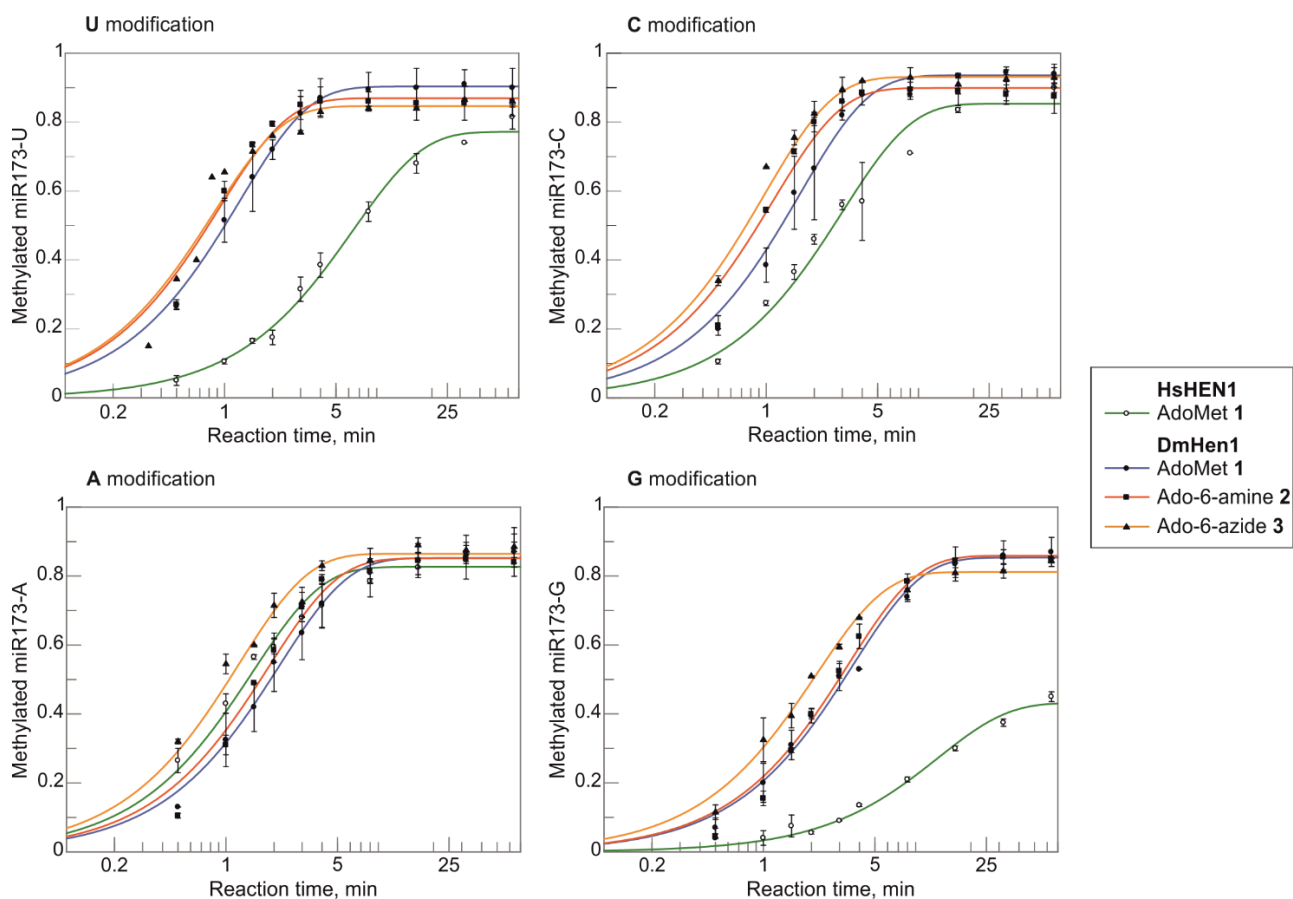
Supplementary Figure S1. Chemical structures of AdoMet and its synthetic analogues used in this study.



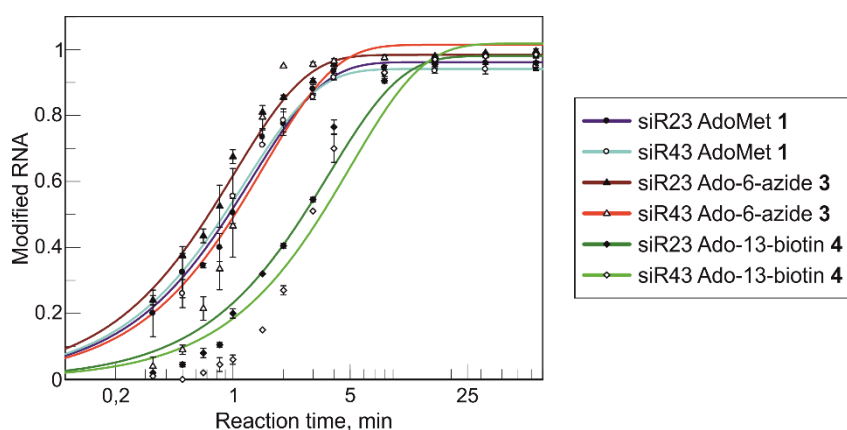
Supplementary Figure S2. Characterization of synthetic cofactors (*S,R*-diastereomers at sulfonium centre) by HPLC and ESI-MS. For Ado-14-Cy3 the absorption at 550 nm, which is indicative of Cy3 fluorophore, was registered. Presented cofactors are characterized by single or double charged molecular ion species in MS spectra: **(A)** Ado-6-amine, $C_{20}H_{30}N_7O_5S^+$ (theoretical 480.2024 $[M^+]$; observed 480.2029); other peak can be attributed to cofactor hydrolysis product 6-aminohex-2-yn-1-ol, $C_6H_{11}NO$ (theoretical 114.0913 $[M+H]^+$; observed 114.0916), **(B)** Ado-6-azide, $C_{20}H_{28}N_9O_5S^+$ (theoretical 506.1927 $[M^+]$; observed 506.1958); **(C)** Ado-13-biotin, $C_{33}H_{47}N_{12}O_7S_2^+$ (theoretical 394.1600 $[M^+H]^2+$; observed 394.1584 and theoretical 787,3127 $[M^+]$; observed 787.3093); other peak can be attributed to cofactor fragmentation product – compound without amino acid moiety, $C_{29}H_{39}N_{11}O_5S_2$ (theoretical 708.2469 $[M+Na]^+$; observed 708.2416); **(D)** Ado-14-Cy3, $C_{53}H_{68}N_{12}O_6S^{2+}$ (theoretical 500.2547 $[M^{2+}]$, observed 500.2540).



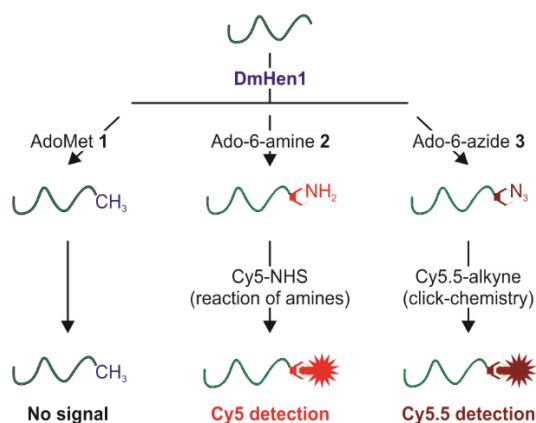
Supplementary Figure S3. piRNA methyltransferases do not form stable complexes with substrate RNAs. 1 μ M of DmHen1 or HsHEN1 was incubated with 0.2 μ M of ³²P-labelled miR173 and 0.1 mM of AdoHcy for 30 min at 37°C. RNA was separated on 8% native PAA gel. All salts used were chlorides.



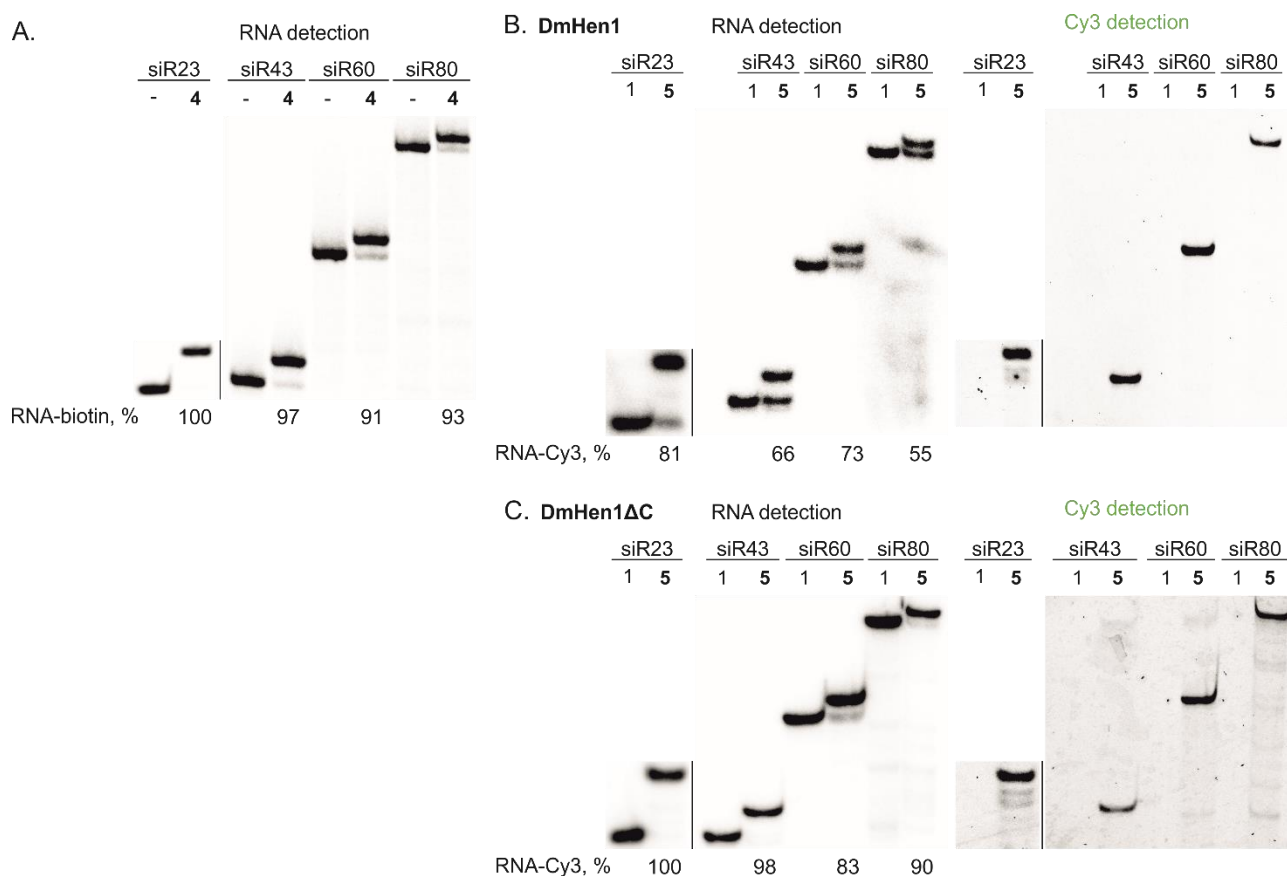
Supplementary Figure S4. Alkyl group transfer rate (k_{chem} , min^{-1}) analysis of different terminal nucleosides in the presence of AdoMet 1 or synthetic cofactor analogues. Reactions were performed at 37°C for 0-60 min using 0.2 μM of ^{32}P -labelled miR173 with different 3' terminal nucleosides, 0.1 mM of AdoMet 1 or its synthetic analogues, 10 mM of Co^{2+} and 2 μM of HsHEN1 or DmHen1. Results are mean of two experiments. Calculated k_{chem} values, which were acquired by using first order kinetics and fitting single-exponential equation to experimental data from at least two replicates, are listed in Table 1.



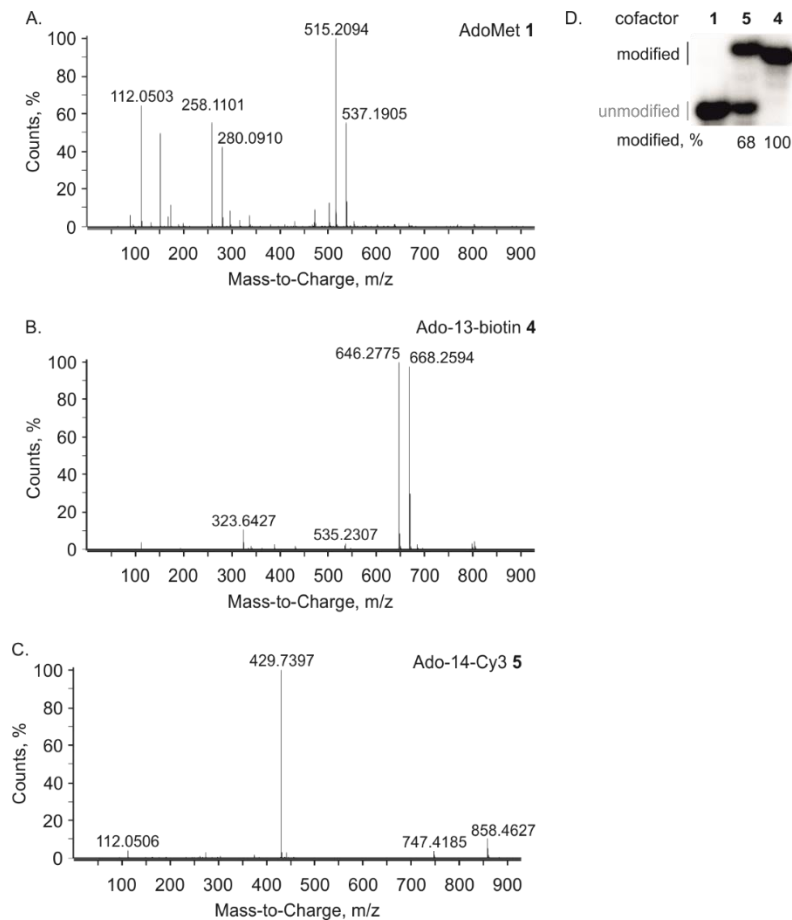
Supplementary Figure S5. DmHen1 modifies RNA substrates of different length with identical 3' terminal sequences at similar rate in the presence of AdoMet 1 or its synthetic analogues. Reactions were performed as described in Supplementary Figure S4 with the exception that 0.05 mM of Ado-13-biotin 4 was used instead. Results are mean of two experiments. k_{chem} values are listed in Table 2.



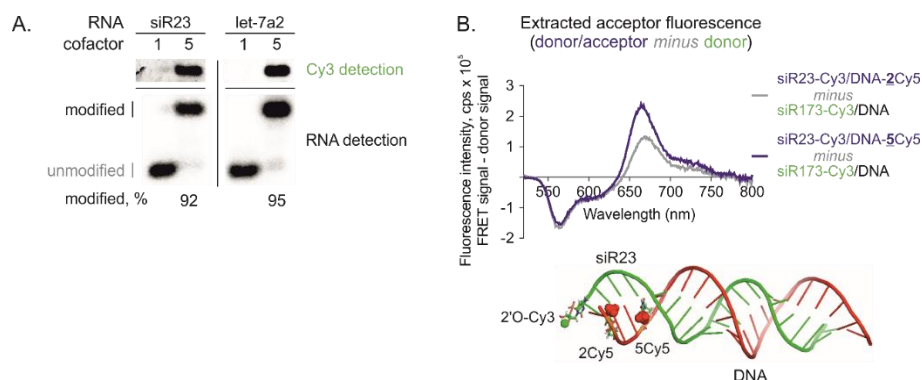
Supplementary Figure S6. Scheme of attachment of required fluorophore, Cy5 or Cy5.5, using two-step labelling approach for RNA functionalization. Experimental results are presented in Figure 5B.



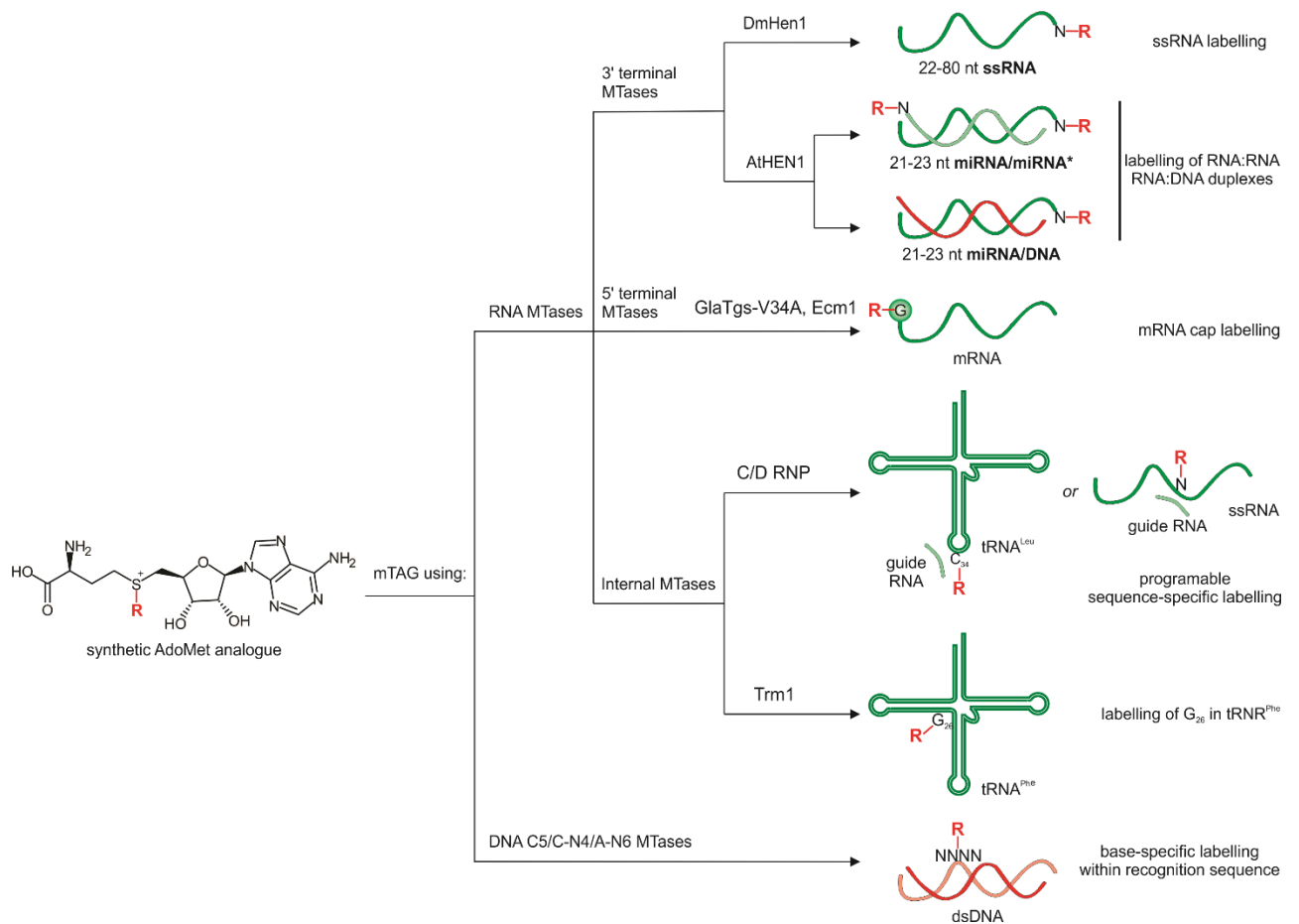
Supplementary Figure S7. One-step labelling of long ssRNAs with bulky side chains of synthetic cofactor analogues – Ado-13-biotin **4** (A) or Ado-14-Cy3 **5** (B and C using DmHen1 or DmHen1ΔC, respectively). While the portion of modified ³²P-RNA is seen by the lower mobility of respective bands (A, B and C, left), Cy3 labelling is also detected using 532 nm laser (B and C right). Modification reactions were performed with 1 μM of DmHen1 or DmHen1ΔC, 0.1 μM of ³²P-labeled RNA and 50 μM of Ado-13-biotin **4**, 6 μM of Ado-14-Cy3 **5** or AdoMet **1** as a control for 30 min at 37°C.



Supplementary Figure S8. ESI-MS analysis of cytidine alkylated with bulky side chains of synthetic AdoMet analogues. 2 μM of miR173 was modified using 1 μM of DmHen1 in the presence of 100 μM of AdoMet **1** (A), 50 μM of Ado-13-biotin **4** (B) or 6 μM of Ado-14-Cy3 **5** (C), digested with P1 nuclease and dephosphorylated to nucleosides. Mass-to-charge ratios of modified 3' terminal cytidine derivatives are indicated in the spectrum and assigned to particular compounds in Supplementary Table S2. D. Denaturing PAA gel of modified RNA samples analysed by ESI-MS.



Supplementary Figure S9. FRET-based detection of specific ssRNA labelled with Cy3 using single-step approach. A. let-7a2 and siR23 are efficiently labelled with Cy3. B. Comparison of extracted acceptor fluorescence of DNA-2Cy5 and DNA-5Cy5 by FRET. In the presence of DNA-5Cy5 higher FRET signal is registered, even though 5th position for Cy5 attachment holds no apparent advantage as can be seen from siR23/DNA heteroduplex structure modelled using 3D-Nus Web server (2). Green sphere – 2'-O atom of ribose, to which Cy3 moiety is transferred by DmHen1; red spheres – methyl groups of thymine, which are replaced by Cy5 in DNA-2Cy5 and DNA-5Cy5 oligonucleotides at second and at fifth position, respectively.



Supplementary Figure S10. Existing applications of mTAG for native nucleic acid labelling. Up to date six types of MTases have been applied for transfer of activated groups from synthetic AdoMet 1 analogues to DNA (red) or RNA (green) substrates resulting in modified base (GlaTgs, Ecm1 Trm1 or DNA MTases) or pentose sugar (DmHen1, AtHEN1, C/D RNP). MTases and first publications regarding each application: DmHen1 – *Drosophila melanogaster* Hen1, current publication; AtHEN1 – *Arabidopsis thaliana* HEN1 (4, 5); GlaTgs-V34A – variant of *Gardia lambia* trimethylguanosine synthase (6); Ecm1 – *Encephalitozoon cuniculi* cap methyltransferase (7); C/D RNP – *Pyrococcusabyssi* C/D RNP (8); Trm1 – *Pyrococcusfuriosus*tRNA MTase (9); DNA MTases – cytosine-5, cytosine-N4, adenine-N6 MTases (10).

SUPPLEMENTARY TABLES

Supplementary Table S1. List of 2'-O alkylated cytidine derivatives identified through ESI-MS analysis (Figure 2 C) with their formulas, theoretical and observed m/z values outlined.

Modifies nucleoside	Derivative	Formula	Theoretical m/z	Observed m/z
C-CH ₃	[cytosine + H] ⁺	[C ₄ H ₆ N ₃ O] ⁺	112.0505	112.0507
	[cytidine-CH ₃ + H] ⁺	[C ₁₀ H ₁₆ N ₃ O ₅] ⁺	258.1084	258.1075
	[cytidine-CH ₃ + Na] ⁺	[C ₁₀ H ₁₅ N ₃ O ₅ Na] ⁺	280.0904	280.0896
	[2cytidine-CH ₃ + H] ⁺	[C ₂₀ H ₃₁ N ₆ O ₁₀] ⁺	515.2096	515.2069
	[2cytidine-CH ₃ + Na] ⁺	[C ₂₀ H ₃₀ N ₆ O ₁₀ Na] ⁺	537.1916	537.1894
C-6-amine	[cytosine + H] ⁺	[C ₄ H ₆ N ₃ O] ⁺	112.0505	112.0508
	[ribose-6-amine] ⁺	[C ₁₁ H ₁₈ NO ₄] ⁺	228.1230	228.1223
	[cytidine-6-amine + H] ⁺	[C ₁₅ H ₂₃ N ₄ O ₅] ⁺	339.1663	339.1661
	[cytidine-6-amine + Na] ⁺	[C ₁₅ H ₂₂ N ₄ O ₅ Na] ⁺	361.1482	361.1466
C-6-azide	[cytosine + H] ⁺	[C ₄ H ₆ N ₃ O] ⁺	112.0505	112.0507
	[cytidine-6-azide + H] ⁺	[C ₁₅ H ₂₁ N ₆ O ₅] ⁺	365.1568	365.1569
	[cytidine-6-azide + Na] ⁺	[C ₁₅ H ₂₀ N ₆ O ₅ Na] ⁺	387.1387	387.1383
	[2cytidine-6-azide + Na] ⁺	[C ₃₀ H ₄₀ N ₁₂ O ₁₀ Na] ⁺	751.1883	751.2881

Supplementary Table S2. List of 2'-O alkylated cytidine derivatives identified in ESI-MS spectra (Supplementary Figure S8) with their formulas, theoretical and observed m/z values outlined.

Modifies nucleoside	Derivative	Formula	Theoretical m/z	Observed m/z
C-CH ₃	[cytosine + H] ⁺	[C ₄ H ₆ N ₃ O] ⁺	112.0505	112.0503
	[cytidine-CH ₃ + H] ⁺	[C ₁₀ H ₁₆ N ₃ O ₅] ⁺	258.1084	258.1101
	[cytidine-CH ₃ + Na] ⁺	[C ₁₀ H ₁₅ N ₃ O ₅ Na] ⁺	280.0904	280.0910
	[2cytidine-CH ₃ + H] ⁺	[C ₂₀ H ₃₁ N ₆ O ₁₀] ⁺	515.2096	515.2094
	[2cytidine-CH ₃ + Na] ⁺	[C ₂₀ H ₃₀ N ₆ O ₁₀ Na] ⁺	537.1916	537.1905
C-13-biotin	[cytidine-13-biotin + 2H] ²⁺	[C ₂₈ H ₄₁ N ₉ O ₇ S] ²⁺	323.6420	323.6427
	[ribose-13-biotin] ⁺	[C ₂₃ C ⁺ H ₃₅ N ₆ O ₆ S]	535.2333	535.2307
	[cytidine-13-biotin + H] ⁺	[C ₂₈ H ₄₀ N ₉ O ₇ S] ⁺	646.2766	646.2775
	[cytidine-13-biotin + Na] ⁺	[C ₂₈ H ₃₉ N ₉ O ₇ SNa] ⁺	668.2585	668.2594
C-14-Cy3	[cytosine + H] ⁺	[C ₄ H ₆ N ₃ O] ⁺	112.0505	112.0506
	[cytidine-14-Cy3 ⁺ + H] ⁺	[C ₄₈ H ₆₁ N ₈ N ⁺ O ₆] ⁺	429.7367	429.7397
	[c-ribose-14-Cy3 ⁺]	[C ₄₄ H ₅₅ N ₅ N ⁺ O ₅]	747.4228	747.4185
	[cytidine-14-Cy3 ⁺]	[C ₄₈ H ₆₀ N ₈ N ⁺ O ₆]	858.4661	858.4627

Supplementary Table S3. The list of forward (Fw) and reverse (Rv) primers used for cloning of plasmids carrying recombinant proteins. Primers used for pGEX-HsHEN1 construction are labelled with letter “E” and a number for respective exon. Restriction endonucleases’ recognition sequences (or parts of them) are underlined, STOP codon is **bolded** and sequences complementary to neighbouring exons are *in italic*.

Construction of pGEX-DmHen1ΔC		
Primer	Sequence 5’-3’	Description
DmHen1ΔC-Fw	TTGCGCCGACATCATAACGG	Forward primer for GST-DmHen1ΔC amplification complementary to the backbone of the plasmid upstream of the gene.
DmHen1ΔC-Rv	CACTTCCTCGAGG GAT CTTTTCTTCTCGGTGCG	Reverse primer for GST-DmHen1ΔC amplification with STOP codon instead of 282nd aa, XhoI restriction site and 6 additional nucleotides.
Construction of pGEX-HsHEN1		
Primer	Sequence 5’-3’	Description
HsHEN1-E1-Fw	<u>GATCCCCATGGAAGAAATAATCTACAG</u> <i>TGCAGT</i> <i>AGTGTGGTT</i>	Forward primer for exon 1, composed of 3’ part of BamHI recognition sequence, two additional nucleotides, exon 1 and part of exon 2.
HsHEN1-E1-Rv	<i>AACCACACTACTGCACTGTAGATTATTTTCTTCCA</i> <i>TGGG</i>	Reverse primer for exon 1, complementary to the forward one, but containing only one nucleotide of BamHI recognition sequence.
HsHEN1-E2-Fw	TGCAGTAGTGTGGTTGACGGT	Forward primer for exon 2 amplification.
HsHEN1-E2-Rv	<i>AGGTCTGCAACCTTCTTAGGCTCATGTTGATCCA</i>	Reverse primer for exon 2 amplification with a portion complementary to exon 3.
HsHEN1-E3-Fw	<i>GCCTAAGAAGGTTGCAGACCTGGGATGTGGT</i>	Forward primer for exon 3 amplification with a portion complementary to exon 2.
HsHEN1-E3-Rv	<i>CTAACGAATCCCCTCTCCATCGTAATTTATCCT</i>	Reverse primer for exon 3 amplification with a portion complementary to exon 4.
HsHEN1-E4-Fw	<i>GATGGAGAGGGGATTCGTTAGCTCCTTTCTG</i>	Forward primer for exon 4 amplification with a portion complementary to exon 3.
HsHEN1-E4-Rv	<i>ATGTTCTATTAATTCAATACACGTTATCAAG</i>	Reverse primer for exon 4 amplification with a portion complementary to exon 5.
HsHEN1-E5-Fw	<i>GTATTGAATTAATAGAACATTTGGATTCAGGT</i>	Forward primer for exon 5 amplification with a portion complementary to exon 4.
HsHEN1-E5-Rv	<i>CATATAAGCCCAGGTCTGAACTCCATTCTGG</i>	Reverse primer for exon 5 amplification with a portion complementary to exon 6.
HsHEN1-E6-Fw	<i>TCAGACCTGGGCTTTATATGTGGCAAATCG</i>	Forward primer for exon 6 amplification with a portion complementary to exon 5.

HsHEN1-E6-Rv	<i>GTGGTAAAAACAGCTTTATAAACATGCTGATCA</i>	Reverse primer for exon 6 amplification with a portion complementary to exon 7.
HsHEN1-E7-Fw	<i>GTTTATAAAGCTGTTTTTACCACCTCATACCCA</i>	Forward primer for exon 7 amplification with a portion complementary to exon 6.
HsHEN1-E7-Rv	<u>CTCGAGTCAA</u> AACTCAA <u>ACTGTT</u> CATC	Reverse primer for exon 7 amplification with XhoI recognition sequence.

Supplementary Table S4. The list of primes used for cloning of sequences encompassing T7 class II promoter, RNA and HVD ribozyme. Forward and reverse primers contain “Fw” and “Rv” at the end of their names, respectively. Restriction endonucleases’ recognition sequences (or parts of them) are underlined and T7 class II promoter is **in bold**.

Prime	Sequence 5'-3'	Description
siR-DNA-43-1	<u>GCACGTGATTCTCTCTGCAAGCGTTAACAATTG</u> <u>ATCGA</u>	Oligo with partial siR43 sequence and two nucleotides of PstI and HindIII recognition sequences at its 5' and 3' ends, respectively.
siR-DNA-43-2	<u>AGCTTCGATCAATTGTTAACGCTTGCAGAGAGA</u> <u>ATCACGTGCTGCA</u>	Oligo complementary to siR-DNA-43-1 with parts of HindIII and PstI recognition sequences at its 5' and 3' ends, respectively.
siR-DNA-60-1	<u>AATTGGCATTCTAATCTTAATCTTCAA</u>	Oligo with partial siR60 sequence and parts of MunI and HindIII recognition sequences at its 5' and 3' ends, respectively.
siR-DNA-60-2	<u>AGCTTTGAAGATTAAGATTAGAATGCC</u>	Oligo complementary to siR-DNA-60-1 with parts of HindIII and MunI recognition sequences at its 5' and 3' ends respectively.
siR-DNA-80-1	<u>AATTGGCATTCTAATCTTAATCGGTACTACGTCT</u> <u>TGTGTACATGGTA</u>	Oligo with partial siR80 sequence and parts of MunI and HindIII recognition sequences at its 5' and 3' ends, respectively.
siR-DNA-80-2	<u>AGCTTACCATGTACACAAGACGTAGTACCGATT</u> <u>AAGATTAGAATGCC</u>	Oligo complementary to siR-DNA-60-1 with parts of HindIII and MunI recognition sequences at its 5' and 3' ends respectively.
T7II-Fw	CAGTAATACGACTCACTATTAGGAAAGC	Primer containing T7 class II sequence and part of 5' sequence of longer RNAs'.
siR-HDV-Rv	GCCATGCCGACCCGTGATTCTCTCTGCAAGCG TTAAC	Primer with partial complementarity to 3' sequence of longer RNAs' and to 5' sequence of HDV ribozyme.
HDV-Fw	GGGTCGGCATGGCATCTCCACCTCCTCGCGGT CCGACCTGGGCTA	5' half of HDV ribozyme, partially overlapping with HDV-Rv.
HDV-Rv	CTTCTCCCTTAGCCTACCGAAGTAGCCCAGGTC GGACCGCGAGGA	3' half of HDV ribozyme, partially overlapping with HDV-Fw.

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