Supporting information:

Click Modification of RNA at Adenosine: Structure and Reactivity of 7-Ethynyl- and 7-Triazolyl-8-aza-7-deazaadenosine in RNA

Kelly J. Phelps, José M. Ibarra-Soza, Kiet Tran, Andrew J. Fisher and Peter A. Beal*

Department of Chemistry, University of California, Davis, One Shields Ave, Davis, California (USA) 95616

Contents of Supporting Information:

Supplementary Figures

Supplementary Figure 1…….....S2

Supplementary Figure 2…….S2

Supplementary Figure 3…….S3

Supplementary Figure 4…….S3

Supplementary Information

Purification of RNAs…….S4

Sequences of oligonucleotides used……..S4

Inhibition of CuAAC reaction by duplex formation……..S4

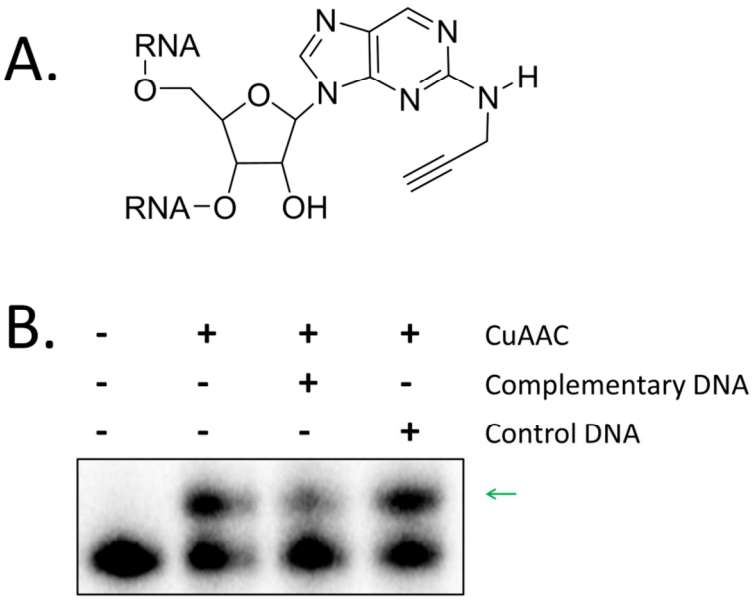
CuAAC reaction for 7-EAA- and 5-EU-containing RNAs in GluR B sequence……..S5

CuAAC reaction for 7-EAA- and 5-EU- containing RNAs with other azides…….S5

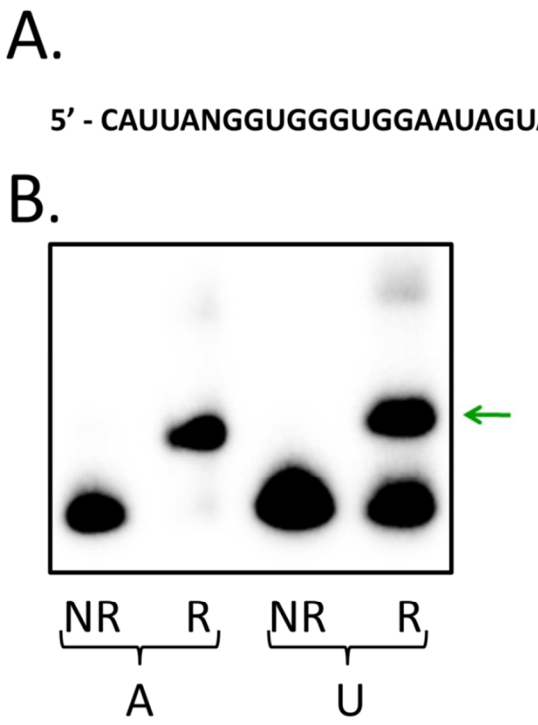
Supplementary Tables

Table 1……..S6

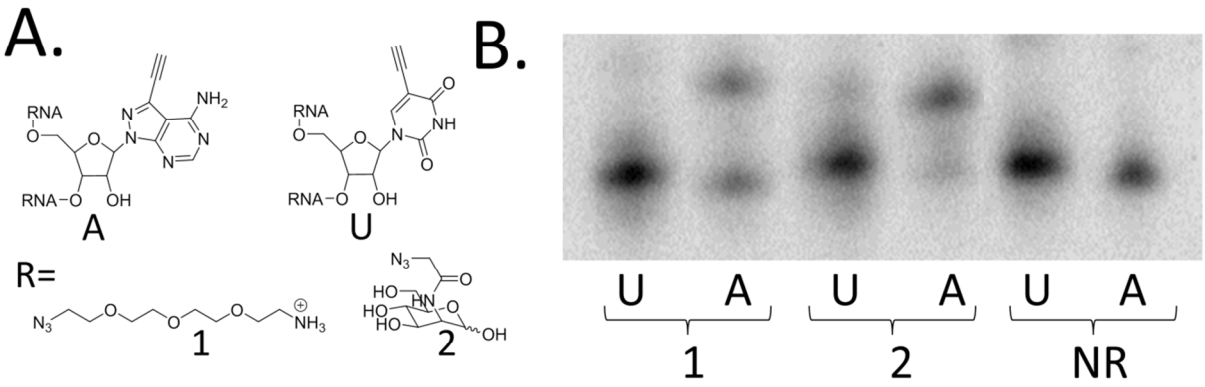
Supplementary Figure 1: Inhibition by a complementary DNA strand of CuAAC reaction involving a 21 nt 7-EAA-containing RNA and N-ethylpiperidine azide. Arrow indicates location of triazole product in gel.



Supplementary Figure 2: CuAAC reactions with N^2-propargyl-2-aminopurine RNA. **A.** Structure of the N^2-propargyl-2-aminopurine analog. **B.** Inhibition of CuAAC reaction involving a 21 nt N^2-propargyl-2-aminopurine containing RNA and N-ethylpiperidine azide by complementary or control DNA. Arrow indicates location of triazole product in gel.



Supplementary Figure 3: Comparison of CuAAC reaction for 7-EAA- and 5-EU-containing RNAs in GluR B pre-mRNA-derived sequence. **A.** Sequence of RNA (N = site of modification). **B.** Products for 5-EU- and 7-EAA-containing RNAs in reaction with a biotin triazole (A: 7-EAA, U: 5-EU, NR: no reaction control, R: reaction products after 7.75h). Arrow indicates location of triazole product in gel.



Supplementary Figure 4: Comparison of CuAAC reaction for 7-EAA- and 5-EU-containing RNAs with other azides. **A.** Structures of the modified ribonucleoside analogs (A: 7-EAA, U: 5-EU, 1: 11-azido-3,6,9-trioxaundecan-1-amine, 2: N-azidoacetyl-D-mannosamine). **B.** Products of 5-EU- and 7-EAA-containing RNA in reaction with 11-azido-3,6,9-trioxaundecan-1-amine (1) and N-azidoacetyl-D-mannosamine (2). NR refers to no reaction control.

Purification of RNAs. The 7-EAA containing phosphoramidite was synthesized as previously described (*1*). RNAs were synthesized as previously described (*2*). 7-EAA-containing 21 nt RNAs, the native 16 nt RNA, 7-EAA containing 16 nt RNA, the 7-EAA containing GluR B pre-mRNA and the GluR B pre-mRNA complement were purified as previously described with a 19% (w/v) polyacrylamide gel (*3*). The 21 nt complementary DNA, 21 nt control DNA and GluR B pre-mRNA primer were obtained in desalted form from the manufacturer. The 5-EU containing 21 nt RNA was purified on a 19% (w/v) denaturing polyacrylamide gel. Gel bands were visualized by UV shadowing (254 nm light, F254 TLC plate as backing) and extracted from the gel by crush and soak method using a solution containing 500 mM NH_4OAc and 0.1 mM EDTA (pH 8) overnight at 4 ^oC (*3*). The solution was then filtered through a Centrex filter (0.2 mm) to remove polyacrylamide particles, phenol-chloroform extracted and ethanol precipitated. Samples were then washed with 70% (v/v) ethanol and lyophilized to dryness. Oligoribonucleotides were redissolved in DEPC treated water and quantified by absorbance measurements at 260 nm. MALDI-MS values $[M+H]^+$ for RNAs are as follows: 7-EAA containing 21 nt RNA: calcd. 6767.9, obsd. 6768.7; native 16 nt RNA: calcd. 5079.7, obsd. 5079.5; 7-EAA containing 16 nt RNA: calcd. 5103.7, obsd. 5103.7; GluR B pre-mRNA: calcd. 8742.2, obsd. 8741.0; ESI-MS values for RNAs are as follows: 5-EU containing 21 nt RNA: calcd. 6766.9, obsd. 6767.0; GluR B pre-mRNA complement: calcd. 8487.1, obsd. 8487.1; 7-EAA containing GluR B pre-mRNA: calcd. 8765.2, obsd. 8763.7.

Sequences of oligonucleotides. 7-EAA containing 21 nt RNA: 5' - AUA GGA UUC NUA UUA GGA GAU - 3' where N is the 7-EAA containing analog; 5-EU containing 21 nt RNA: 5' - AUA GGA UUC AXA UUA GGA GAU - 3' where X is the 5-EU containing analog; 21 nt complementary DNA: 5’ - ATC TCC TAA TAT GAA TCC TAT – 3’; 21 nt control DNA: 5’ – ATA CAT ATC GTT ATC CTT ACA – 3’; native 16 nt RNA: 5’ – GCA GAC UUA AGU CUG C – 3’; 7-EAA containing 16 nt RNA: 5’ – GCA GNC UUA AGU CUG C - 3’; 7-EAA containing GluR B pre-mRNA: 5’ - CAU UAN GGU GGG UGG AAU AGU AUA ACA - 3’; GluR B pre-mRNA: 5’ - CAU UAA GGU GGG UGG AAU AGU AUA ACA - 3’; GluR B pre-mRNA complement: 5’ - UGU UAU AGU AUC CCA CCU ACC CUG AUG - 3’; GluR B pre-mRNA 21 nt primer: 5’ – TGT TAT ACT ATT CCA CCC ACC – 3’; GluR B pre-mRNA 18 nt primer: 5’ – TGT TAT ACT ATT CCA CCC – 3’.

Inhibition of CuAAC reaction by duplex formation. For the reactions containing 21 nt complementary DNA or 21 nt control DNA, ^{32}P labeled 7-EAA or N^2-propargyl-2-aminopurine containing 21 nt RNA was mixed with 21 nt complementary DNA or 21 nt control DNA so that the concentration of RNA was 50 μM and DNA was 80 μM. Samples were heated at 95 °C for 5 min and then allowed to slowly cool to 4 °C. Next, a solution of copper sulfate, sodium ascorbate, tris(3-hydroxypropyltriazolylmethyl)amine, and N-ethylpiperidine azide that was pre-equilibrated to 4 °C was added so that the final concentrations were as follows: copper sulfate at 0.4 mM, sodium ascorbate at 4 mM, tris(3-hydroxypropyltriazolylmethyl)amine at 4 mM, N-

ethylpiperidine azide at 0.2 mM, 2 mM Tris-HCl (pH 8), 25 μM RNA and 40 μM DNA. The reaction was allowed to proceed for 7 min at 4 °C before it was stopped by adding an equal volume of stop solution containing 80% formamide and 10 mM EDTA and freezing the sample in liquid nitrogen. Samples were thawed and resolved on a 19% denaturing polyacrylamide gel. Gels were dried and bands imaged using storage phosphor imaging plates. Each experiment was carried out in triplicate.

CuAAC reaction for 7-EAA- and 5-EU-containing RNAs in GluR B sequence. Reactions were carried out using the same procedure as the CuAAC reaction kinetics except the following: instead of using the N-ethylpiperidine azide at 0.2 mM, the biotin azide was used at a final concentration of 10 mM in 20% DMSO; there was no Tris-HCl in the reaction; the reaction was allowed to go to a single time point of 7.75 h; and samples were resolved on a 15% denaturing polyacrylamide gel.

CuAAC reaction for 7-EAA- and 5-EU- containing RNAs with other azides. For the click reactions with N-azidoacetyl-D-mannosamine and 11-azido-3,6,9-trioxaundecan-1-amine, reaction conditions were the same as with N-ethylpiperidine azide except the concentration of the azides varied (N-azidoacetyl-D-mannosamine at 2 mM and 11-azido-3,6,9-trioxaundecan-1-amine at 0.6 mM), there is no Tris-HCl in the reaction and reactions were allowed to proceed for 1 hour. Each experiment was carried out in duplicate.(*1*)

Table 1: Data Collection, Phasing, and Refinement Statistics

	Native	Ethyne	Triazole
Data Collection Statistics			
X-ray Source	SSRL BL7-1	SSRL BL7-1	SSRL BL7-1
Wavelength	1.148 Å	1.127 Å	1.127 Å
Resolution	1.96 (2.01-1.96) Å	1.85 (1.90-1.85) Å	1.70 (1.74-1.70) Å
Space Group	*C*2	*C*2	*C*2
Cell Parameters	*a* = 74.98 Å *b* = 43.08 Å *c* = 48.76 Å β = 120.98°	*a* = 74.80 Å *b* = 43.19 Å *c* = 48.79 Å β = 120.82°	*a* = 77.36 Å *b* = 44.38 Å *c* = 48.83 Å β = 121.97°
16mer strands / ASU	3	3	3
V_M (Å^3/Da); % solvent	2.01 (60.0)	2.09 (60.2)	2.19 (62.0)
No. of reflections	17,295 (1,278)	28,774 (1,191)	27,547 (1,696)
No. unique	9,115 (683)	10,615 (647)	14,671 (1,004)
R_{merge}[a] (%)	2.0 (31.0)	2.9 (33.0)	2.5 (31.4)
Mean (I) / σ(I)	17.06 (2.19)	18.04 (2.32)	16.42 (2.27)
Completeness (%)	93.4 (96.7)	91.3 (75.4)	93.7 (88.6)
Refinement Statistics			
Resolution (Å)	35.81-1.96	32.17-1.85	36.79-1.70
No. reflections in working set ($F \geq 0$)	8,672	10,100	13,931
R-factor[b] (%)	21.7	22.2	22.2
R-free[b] (%)	24.2	24.9	24.4
RMS Bond Lengths	0.011 Å	0.012 Å	0.012 Å
RMS Bond Angles	1.584°	1.394°	1.408°
Overall Mean B Values	38.9 Å^2	38.6 Å^2	19.6 Å^2
Asymmetric Unit Content			
Nonhydrogen atoms	1047	1055	1104
Water	22	29	78
PDB ID	4NFO	4NFP	4NFQ

[a] $R_{merge} = [\Sigma_h\Sigma_i|I_h - I_{hi}|/\Sigma_h\Sigma_i I_{hi}]$ where I_h is the mean of I_{hi} observations of reflection h. Numbers in parenthesis represent highest resolution shell.

[b] *R*-factor and $R_{free} = \Sigma||F_{obs}| - |F_{calc}||/\Sigma|F_{obs}| \times 100$ for 95% recorded data (*R*-factor) or 5% of data (*R*-free). Numbers in parenthesis represent highest resolution shell

References:

1. Ibarra-Soza, J. M., Morris, A. A., Jayalath, P., Peacock, H., Conrad, W. E., Donald, M. B., Kurth, M. J., and Beal, P. A. (2012) 7-Substituted 8-aza-7-deazaadenosines for modification of the siRNA major groove, *Org. Biomol. Chem. 10*, 6491-6497.

2. Pokharel, S., Jayalath, P., Maydanovych, O., Goodman, R. A., Wang, S. C., Tantillo, D. J., and Beal, P. A. (2009) Matching Active Site Structure to Substrate Analog for an RNA Editing Reaction, *J. Am. Chem. Soc. 131*, 11882-11891.
3. Mizrahi, R. A., Phelps, K. J., Ching, A. Y., and Beal, P. A. (2012) Nucleoside analog studies indicate mechanistic differences between RNA-editing adenosine deaminases, *Nucleic Acids Res. 40*, 9825-9835.