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Supplementary Information for

Site-specific covalent labeling of large RNAs with nanoparticles empowered by expanded genetic alphabet transcription

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This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.2005217117

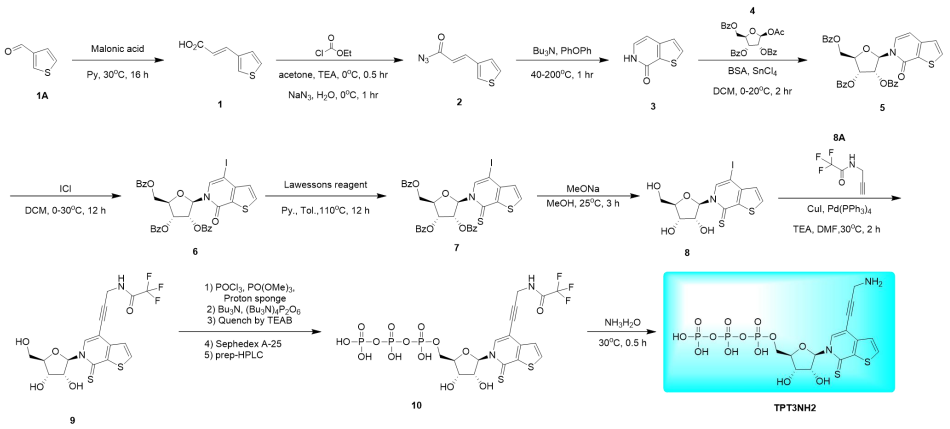
Supplementary Information Text

Synthetic procedures and characterizations of rTPT3^{A}

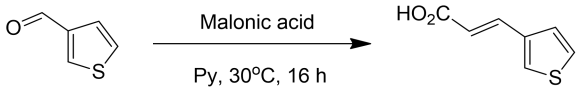
General

All solvents and reagents were purchased commercially and used without further purification. For synthetic procedures, all reactions were carried out in oven-dried glassware under an inert atmosphere. Solvents were distilled and/or dried over 4 Å molecular sieves. NMR spectra were recorded on an AVANCE III 1 BAY 400 MHz Bruker NMR spectrometer and the chemical shifts were reported relative to the deuterated NMR solvent used [^{1}H-NMR: CDCl3 (7.26 ppm), DMSO-d6 (2.50 ppm); ^{13}C-NMR: CDCl3 (77.16 ppm), DMSO-d6 (39.52 ppm)]. Mass spectra were recorded on an Agilent 1200 + G6110A.

Synthetic schemes and procedures



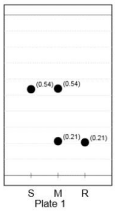
General procedure for preparation of compound 1



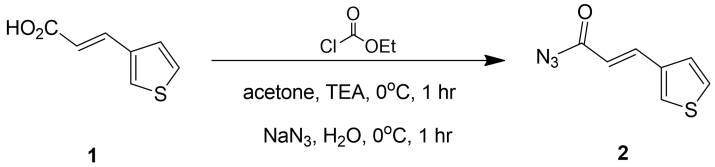
To a solution of **1A** (300.0 g, 2.6 mol, 243.9 mL, 1.00 *eq*) in Py (2000 mL) was added Malonic acid (389.7 g, 3.7 mol, 389.7 mL, 1.40 *eq*). The mixture was stirred at 30 °C for 16 hrs. TLC (petroleum ether/ethyl acetate **=** 1/1, R_f = 0.21) indicated that **1A** was consumed completely and one new spot formed. The reaction was clean according to TLC.

Added 12 M HCl adjust pH 2-3, then the solid precipitation, filtered and washed with water, collect filter cake. The filter cake was soluble in EtOAc (5000 mL), added dry Na_2SO_4. The organic phase was concentrated under reduced pressure to give a residue. Compound **1** (300 g, crude) was obtained as a light white solid.

TLC: petroleum ether/ethyl acetate **=** 1/1, R_f = 0.21



General procedure for preparation of compound 2



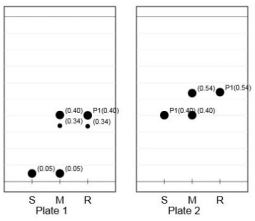
1 **2**

Add acetone (2000 mL) and TEA (236.3 g, 2.3 mol, 324.9 mL, 1.20 *eq*) and compound **1** (300.0 g, 1.9 mol, 1.0 *eq*) to a flask. Add ethyl carbonochloridate (232.3 g, 2.1 mol, 203.7 mL, 1.10 *eq*) dropwise at 0 °C to the mixture. Stir the mixture at 0 °C for 1 hr. TLC (plate 1: petroleum ether/ethyl acetate = 5/1, R_f (material) = 0.05, R_f (product) = 0.40) indicated material was consumed completely. Add NaN_3 (151.8 g, 2.3 mol, 1.20 *eq*) in H_2O (750 mL) dropwise at 0 °C to the mixture. Stir the mixture at 0 °C for 1 hr. TLC (plate 2: petroleum ether/ethyl acetate = 5/1, R_f = 0.54) indicated material was consumed completely and one new spot formed. Add H_2O (1.5 L) to the mixture and filtered and check water phase to pH > 9. The filtered cake wash with H_2O (500 mL) and filtered. The filtered cake was dissolved by EtOAc (1.0 L). Wash by brine (1.0 L x 2) and dry with Na_2SO_4. Concentrate until remained 1.0 L EtOAc. Add Ph_2O (600 mL) and concentrated to get a solution of product

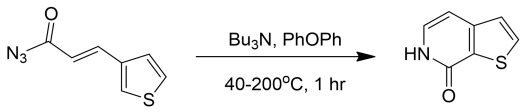
in Ph_2O. The crude compound **2** (348.0 g, crude) with white solid in Ph_2O was used into the next step without further purification.

TLC (plate 1: petroleum ether/ethyl acetate = 5/1, R_f (material) = 0.05, R_f (product) = 0.40)

TLC (plate 2: petroleum ether/ethyl acetate = 5/1, R_f (material) = 0.4, R_f (product) = 0.54)



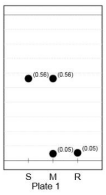
General procedure for preparation of compound 3



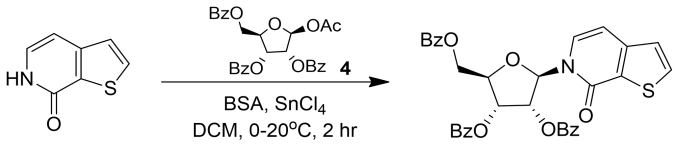
2 **3**

Add Ph_2O (0.9 L) and Bu_3N (239.8 g, 1.7 mol, 317.2 mL, 3.00 *eq*) to a flask at 150 °C. A heat solution (40-50 °C) of compound **2** (100.0 g, 558.0 mmol, 1.00 *eq*) in Ph_2O (300 mL) was added to the flask at 200 °C. Stir the mixture at 200 °C for 1 hr. TLC (petroleum ether/ethyl acetate **=** 5/1, R_f = 0.05) indicated compound **2** was consumed completely and one new spot formed. The reaction was clean according to TLC. The mixture was cooled to 20 °C and diluted with petroleum ether (10 L). All batches were combined together, the resulting solid was collected by filtration. Combine the two reactions to give a crude product. The filter cake was washed with hexane (5000 mL). The solid obtained was dried under vacuum to give the product. Compound **3** (200.0 g, 1.3 mol, 79.0% yield) was obtained as a yellow solid.

TLC: petroleum ether/ethyl acetate **=** 5/1, R_f = 0.05



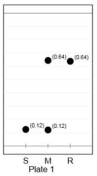
General procedure for preparation of compound 5



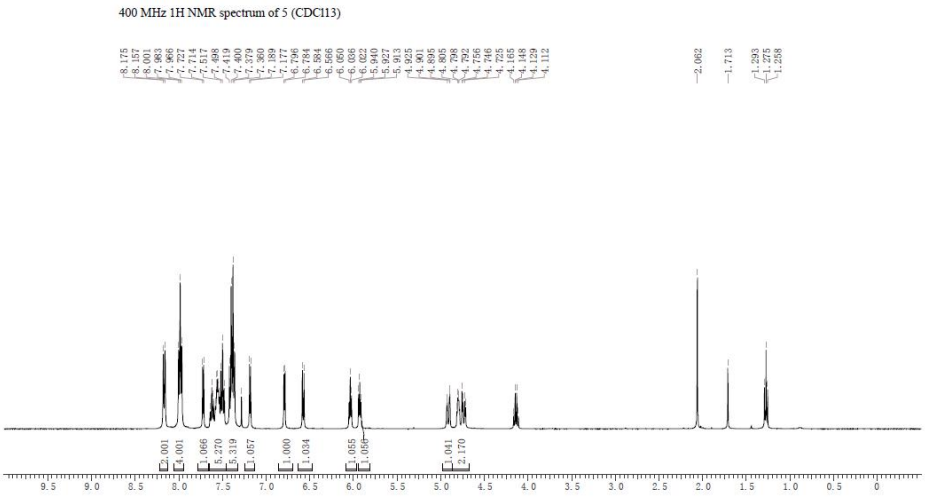
3 **5**

Added compound **3** (150 g, 992.16 mmol, 1.00 *eq*) and DCM (2500 mL) to a 5 L flask. Then add BSA (242 g, 1.2 mol, 294.3 mL, 1.20 *eq*) to the flask under N_2. Stir the mixture for 0.5 hr. Add **4** (550.6 g, 1.10 mol, 1.10 *eq*), then added $SnCl_4$ (310.2 g, 1.2 mol, 139.1 mL, 1.20 *eq*) drop wise to the mixture at 0 °C. Stir the mixture at 20 °C for 1.5 hrs. TLC (petroleum ether/ethyl acetate **=** 2/1, R_f = 0.64) indicated compound **3** was consumed completely and one new spot formed. The reaction was clean according to TLC. Added sat.aq. $NaHCO_3$ (3000 mL) to the mixture and adjust pH 7, then filtered. The filtrate was extracted by DCM (2000 mL x 2). Wash the organic phase with brine (1.5 L) and dry with Na_2SO_4, concentrate the organic phase to give crude product. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate **=** 5/1 to 2/1). Compound **5** (950.0 g, 1.52 mol, 76.4% yield, 95.0% purity) was obtained as a white solid.

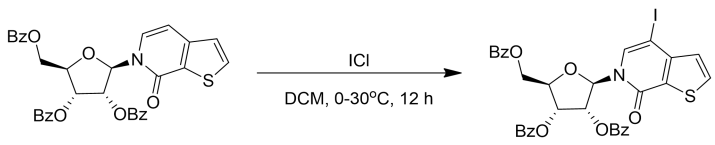
TLC: petroleum ether/ethyl acetate **=** 2/1, R_f = 0.64



^{1}H NMR: 400 MHz, $CDCl_3$. δ ppm 8.17 (d, *J* = 7.2 Hz, 2H), 7.98 (t, *J* = 7.0 Hz, 4H), 7.72 (d, *J* = 5.2 Hz, 1H), 7.45-7.66 (m, 5H), 7.39 (q, *J* = 7.6 Hz, 5H), 7.18 (d, *J* = 5.2 Hz, 1H), 6.79 (d, *J* = 4.8 Hz, 1H), 6.57 (d, *J* = 7.4 Hz, 1H), 6.04 (t, *J* = 5.8 Hz, 1H), 5.88-5.97 (m, 1H), 4.91 (dd, *J*=12.0, 2.8 Hz, 1H), 4.77-4.84 (m, 1H), 4.69-4.76 (m, 1H).



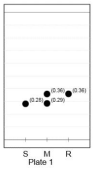
General procedure for preparation of compound 6



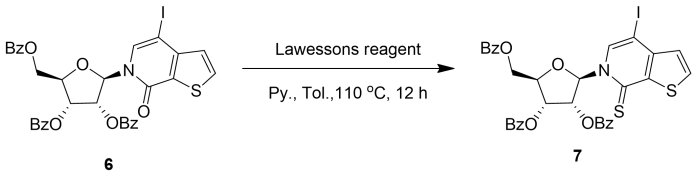
5 **6**

To a solution of compound **5** (340.0 g, 570.8 mmol, 1.00 *eq*)) in DCM (2000 mL) was added ICl (185.4 g, 1.10 mol, 58.3 mL, 2.00 *eq*) at 0 °C. The mixture was stirred at 30 °C for 12 h under exclusion of light. TLC (petroleum ether/ethyl acetate **=** 3/1, R_f = 0.36) indicated compound **5** was consumed completely and one new spot formed. The reaction was clean according to TLC. The reaction mixture was quenched by addition $Na_2S_2O_3$ (1500 mL) at 20 °C, and then diluted with DCM (2000 mL x 2). The organic layers were concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate **=** 5/1 to 3/1). Compound **6** (200 g, 249.5 mmol, 43.7% yield, 90.0% purity) was obtained as a white solid.

TLC: petroleum ether/ethyl acetate **=** 3/1, R_f = 0.36



General procedure for preparation of compound 7

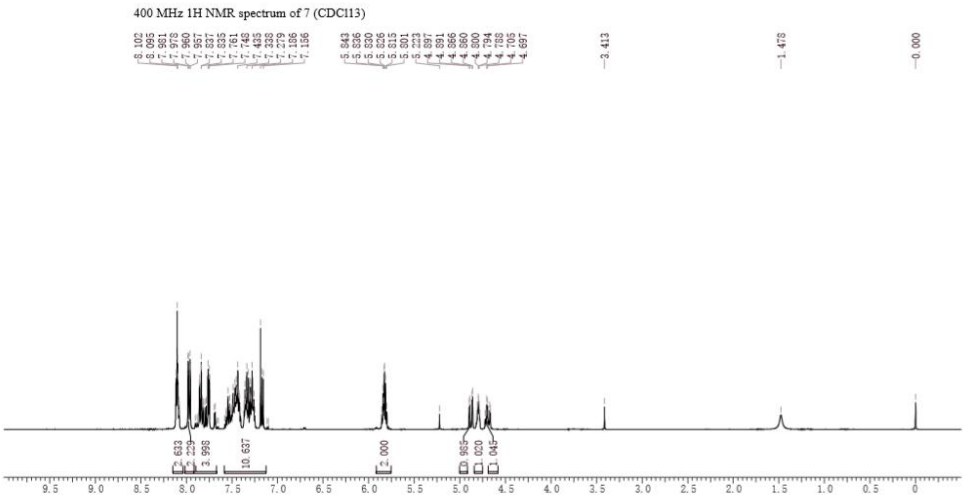


6 **7**

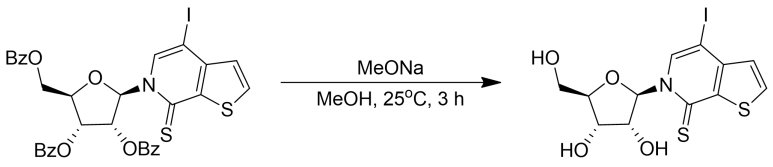
To a solution of compound **6** (140.0 g, 194.04 mmol, 1.00 *eq*) in toluene (1200 mL) was added 2, 4-bis (4-methoxyphenyl)-2, 4-dithioxo-1, 3, 2, 4-dithiadiphosphetane (117.7 g, 291.1 mmol, 1.50 *eq*) and Py (15.4 g, 194.0 mmol, 15.7 mL, 1.00 *eq*). The mixture was stirred at 110 °C for 12 h. TLC (petroleum ether/ethyl acetate **=** 3/1, R_f = 0.39) indicated compound **6** was consumed completely and one new spot formed. The reaction was clean according to TLC. The reaction mixture was concentrated under reduced pressure to give a residue, the residue was diluted with DCM (1500 mL) and extracted with H_2O (1000 mL x 2). The organic layers were concentrated under reduced pressure to give a residue, added 500 mL MeOH stirred for 2 h, filtered and get the cake. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate **=** 10/1 to 5/1). Compound **7** (100.0 g, crude) was obtained as a yellow solid.

TLC: petroleum ether/ethyl acetate **=** 3/1, R_f = 0.39

^{1}H NMR: 400 MHz, $CDCl_3$. δ ppm 8.07 - 8.13 (m, 3H), 7.97 (dd, *J* = 8.4, 1.2 Hz, 2H), 7.71-7.91 (m, 4H), 7.39-7.59 (m, 5H), 7.23-7.38 (m, 5H), 7.16 (d, *J* = 5.4 Hz, 1H), 5.78-5.87 (m, 2H), 4.88 (dd, *J* = 12.6, 2.4 Hz, 1H), 4.80 (td, *J* = 5.2, 2.6 Hz, 1H), 4.64-4.74 (m, 1H).



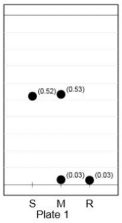
General procedure for preparation of compound 8



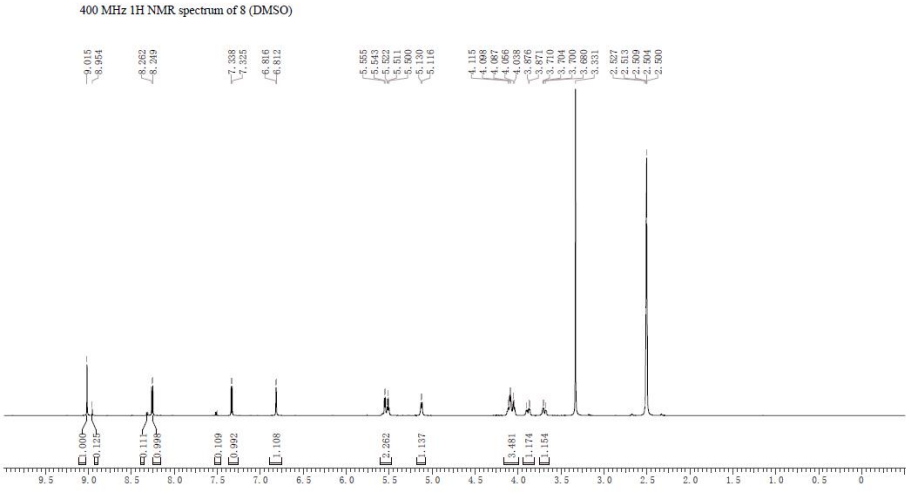
7 **8**

To a solution of compound **7** (100.0 g, 135.6 mmol, 1.00 *eq*) in MeOH (500 mL) and DCM (500 mL) was added NaOMe (3.66 g, 67.79 mmol, 0.500 *eq*). The mixture was stirred at 25 °C for 3 hrs. TLC indicated compound **7** was consumed completely and one new spot formed. The reaction was clean according to TLC. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH **=** 100/1 to 10/1). Compound **8** (40.0 g, 75.3 mmol, 55.5% yield, 80.0% purity) was obtained as a yellow solid.

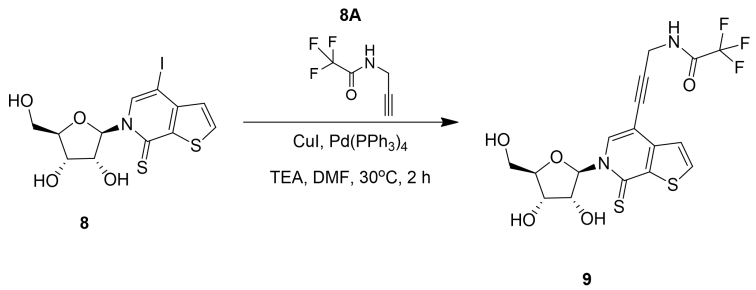
TLC: petroleum ether/ethyl acetate **=** 3/1, R_f = 0.03



^{1}H NMR: ET27024-50-P1a1, 400 MHz, DMSO. δ ppm 9.01 (s, 1H), 8.26 (d, *J* = 5.4 Hz, 1H), 7.33 (d, *J* = 5.4 Hz, 1H), 6.81 (d, *J* = 1.50 Hz, 1H), 5.46-5.66 (m, 2H), 5.12 (d, *J* = 5.6 Hz, 1 H), 4.00-4.22 (m, 4H), 3.88 (m, 1H), 3.60-3.76 (m, 1H).



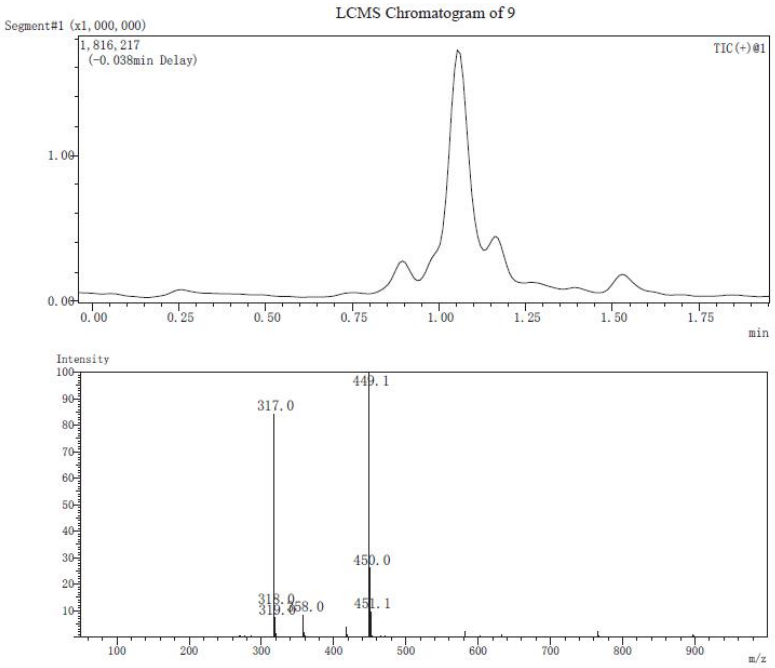
General procedure for preparation of compound 9



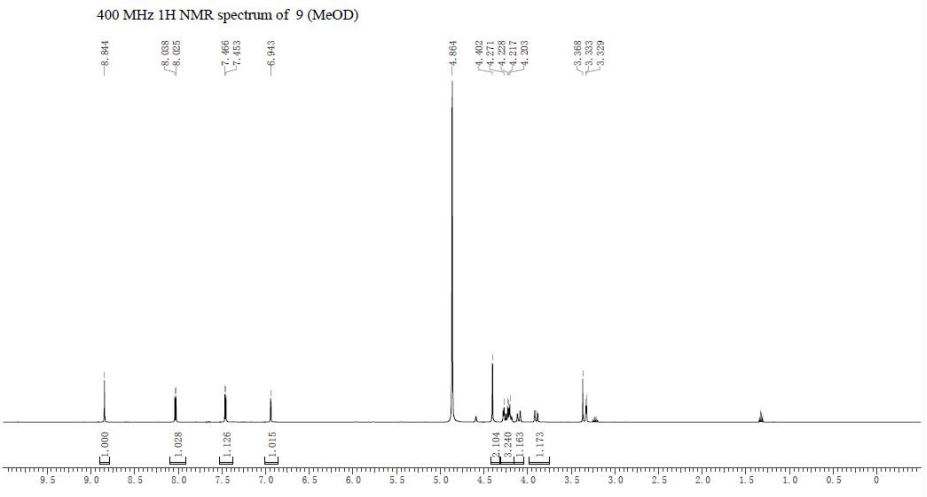
To a solution of compound **8** (9.0 g, 21.2 mmol, 1.00 *eq*) in DMF (150 mL) was added $Pd(PPh_3)_4$ (2.5 g, 2.10 mmol, 0.100 *eq*) and CuI (806.1 mg, 4.20 mmol, 0.200 *eq*) and TEA (3.20 g, 31.8 mmol, 4.4 mL, 1.50 *eq*), then added **8A** (4.8 g, 31.7 mmol, 1.50 *eq*). The mixture was stirred at 30 °C for 2 h. LC-MS showed compound **8** was consumed completely

and one main peak with desired m/z was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH = 20/1). Compound **9** (9.0 g, 18.1 mmol, 85.4% yield, 90.0% purity) was obtained as a yellow solid.

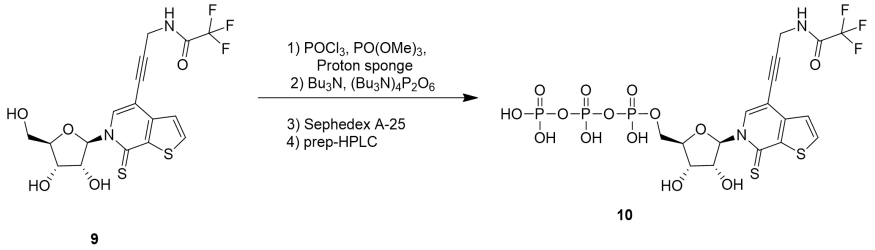
LCMS: (product: RT = 1.054 min, MS cal: 448.4, $[M+1]^+$ = 449.1);



^{1}H NMR: 400 MHz, MeOD. δ ppm 8.84 (s, 1H), 8.03 (d, *J* = 5.4 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 6.94 (d, *J* = 1.4 Hz, 1H), 4.40 (s, 2H), 4.15-4.32 (m, 3H), 4.10 (dd, *J* = 12.6, 2.0 Hz, 1 H), 3.90 (dd, *J* = 12.4, 2.2 Hz, 1H).



General procedure for preparation of compound 10

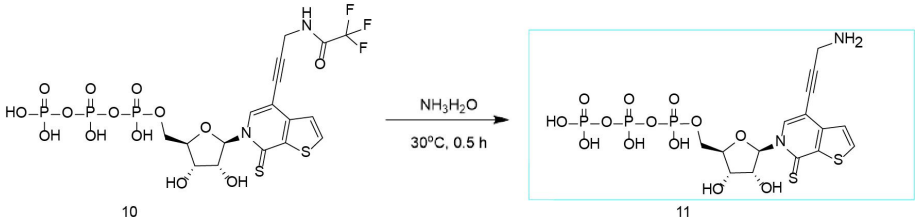


9 10

To a solution of compound **9** (2.0 g, 4.5 mmol, 1.0 *eq*) and proton sponge (955.8 mg, 4.46 mmol, 1.00 *eq*) in $PO(OMe)_3$ (20 mL) was added $POCl_3$ (1.00 g, 6.7 mmol, 621.7 uL, 1.50 *eq*). The mixture was stirred at 0 °C for 2 h. LC-MS showed compound **9** was consumed completely and one main peak with desired m/z was detected. The crude product (7.60 g, crude) with yellow oil was used into the next step without further purification.

To a solution of crude product (2.50 g, 4.42 mmol, 1.00 *eq*) in $PO(OMe)_3$ (20 mL) was added N, N - dibutylbutan -1-amine; phosphono dihydrogen phosphate (12.1 g, 22.1 mmol, 5.00 *eq*) and N, N- dibutylbutan-1-amine (4.90 g, 26.5 mmol, 6.3 mL, 6.00 *eq*). The mixture was stirred at 0 °C for 2 h. LC-MS showed material was consumed completely and one peak with desired m/z was detected. Added 1M TEAB to adjust pH7, the reaction mixture was purified by a DEAE Sephadex column (GE Healthcare) with an elution gradient of 0 to 1.0 M TEAB, evaporated to obtain a yellow oil, the crude product **10** (8.0 g, crude) was used into the next step without further purification.

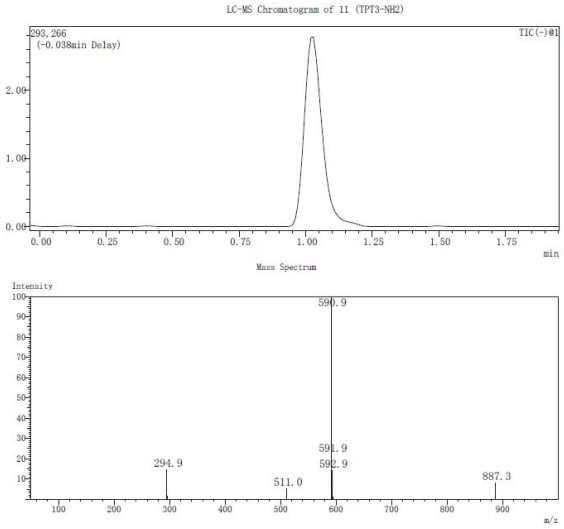
General procedure for preparation of compound 11(TPT3^A)



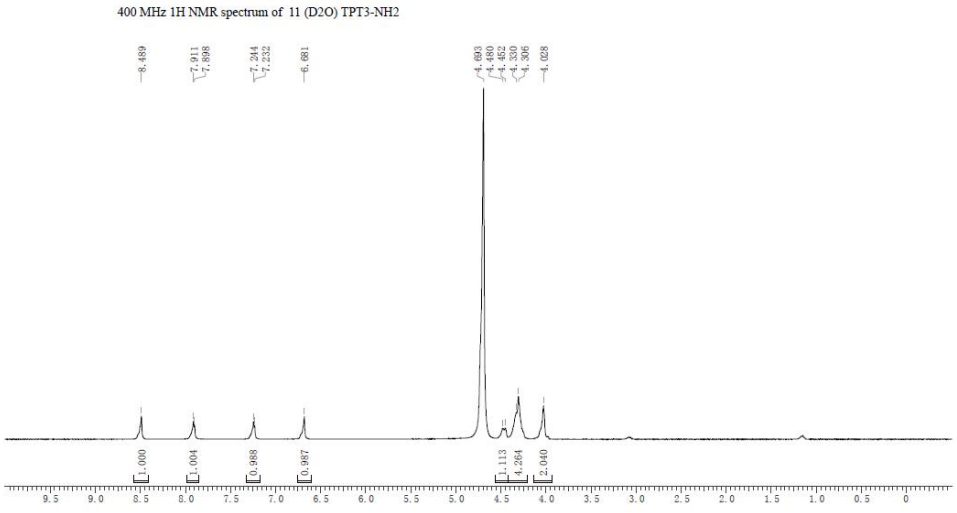
10 11

To a solution of compound **10** (5.0 g, 7.3 mmol, 1.00 *eq*) in $NH_3.H_2O$ (5.0 mL) and H_2O (5.0 mL), then stirred at 30 °C for 0.5 h. LC-MS showed compound **10** was consumed completely and one main peak with desired m/z was detected. The mixture of reaction was purified by prep-HPLC (neutral condition, column: Agela Dura Shell C18 250 x 70mm x 10um; mobile phase: [water (10mM NH_4HCO_3)-ACN]; B%: 1%-12%, 22 min). **TPT3-NH2 (TPT3^A)** (0.85 g, 1.4 mmol, 18.7% yield, 95.0% purity) was obtained as a yellow solid.

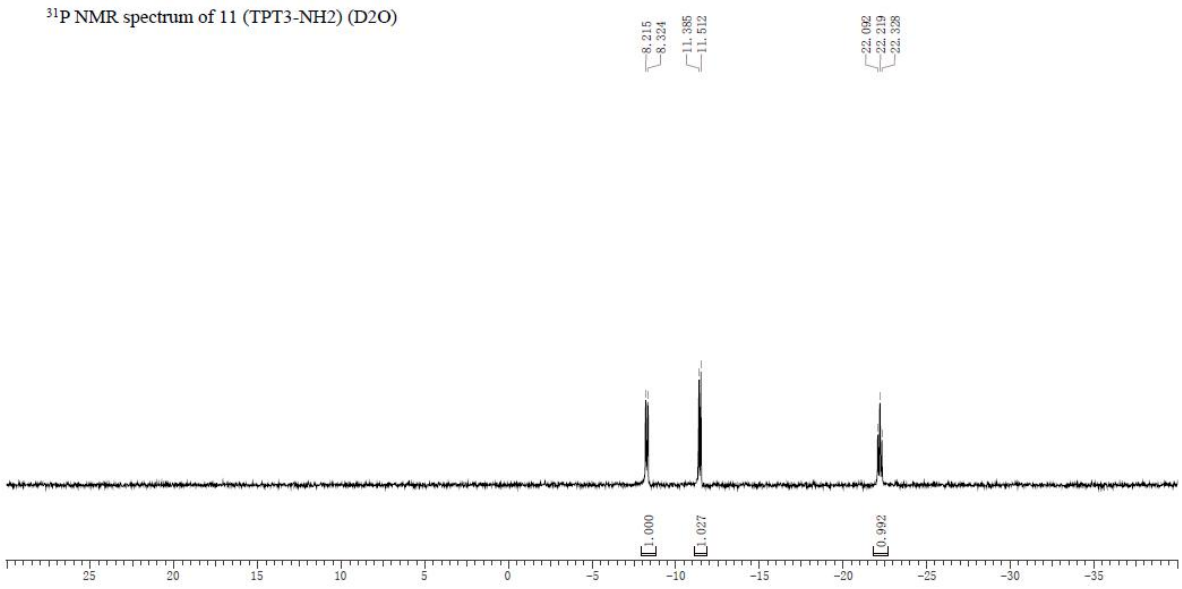
LCMS: (product: RT = 1.014 min);



^{1}H NMR: 400 MHz, D_2O. δ ppm 8.49 (s, 1H), 7.90 (m, 1H), 7.24 (m, 1H), 6.68 (s, 1H), 4.47 (m, 1H), 4.20-4.39 (m, 4H), 4.03 (s, 2H).



^{31}P NMR: 400 MHz, D_2O. δ ppm -8.27 (d, *J* = 17.6 Hz, 1P) -11.45 (d, *J* = 20.6 Hz, 1P) -22.21 (t, *J* = 19.0 Hz, 1P).



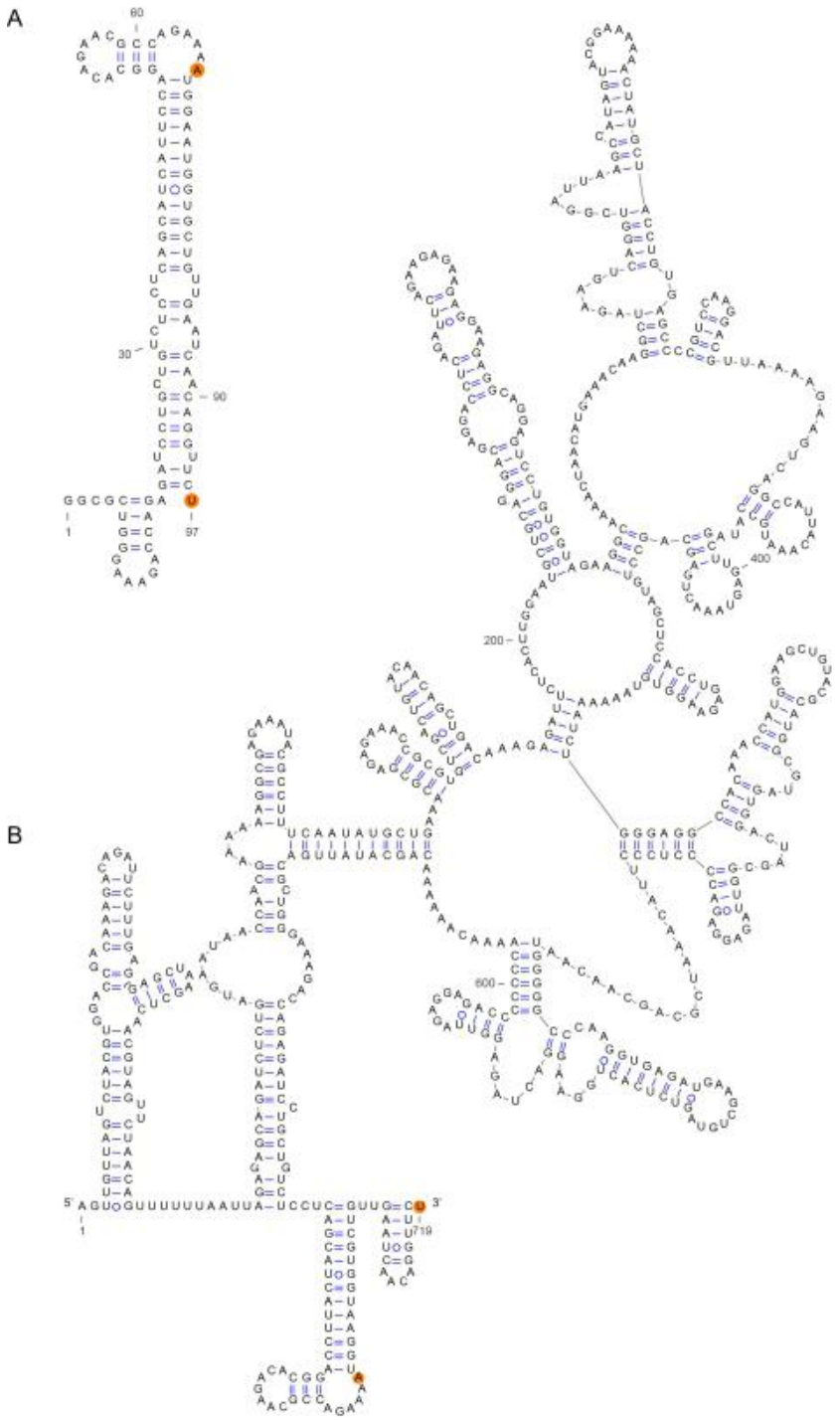


Fig. S1. (**A-B**) Secondary structures of the 97-nt 3'SL (**A**) and 719-nt DENV-mini (**B**) RNAs used in this study. The secondary structure of DENV-mini is the same as in literature (1).

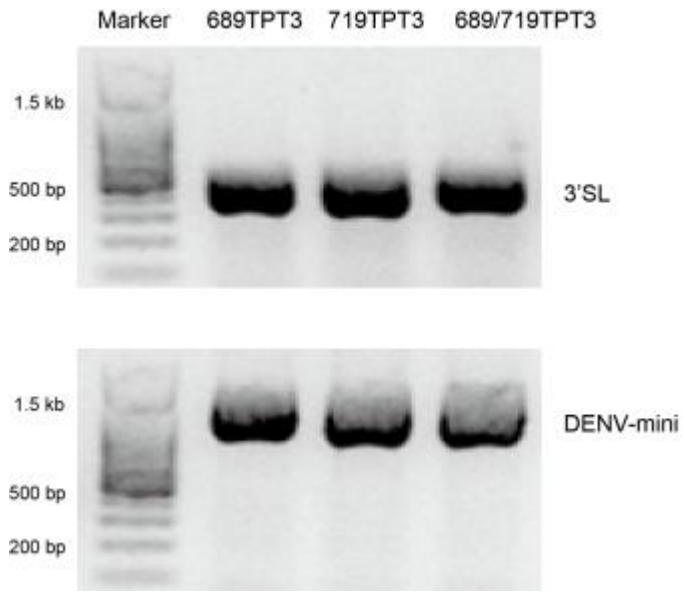


Fig. S2. 1% Agarose gel analysis of the 689-single modified, 719-single modified, and 689/719-double modified PCR products coding for 3'SL (504 bp, top) and DENV-mini (1128 bp, down).

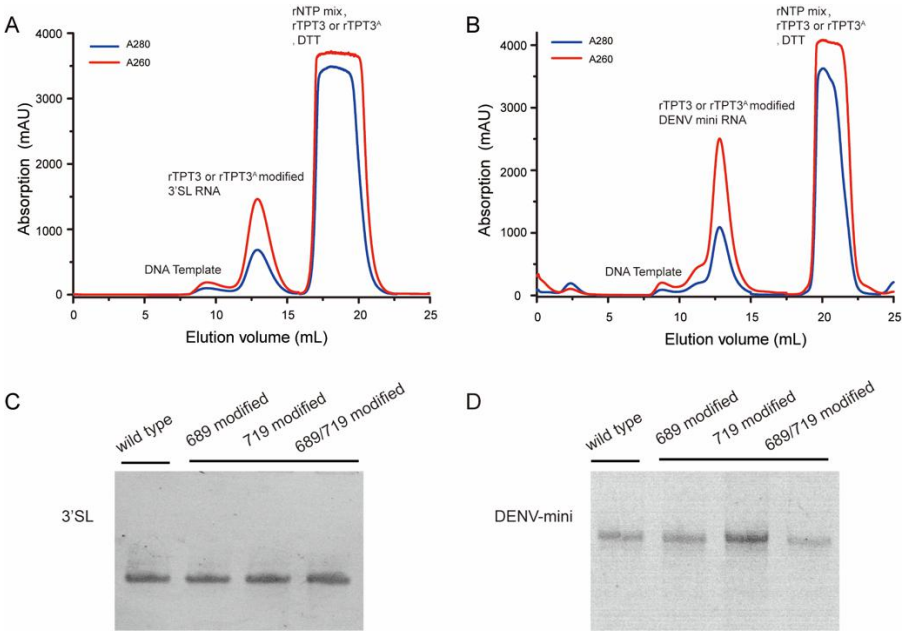


Fig. S3. Non-denaturing purification of TPT3 or TPT3^{A} modified RNAs. (**A-B**) Direct purification of TPT3 or TPT3^{A} modified 3'SL (**A**) or DENV-mini (**B**) RNAs from *in vitro* transcription reaction mixtures by size-exclusion chromatography using Superdex G75 (for 3'SL) or G200 (for DENV-mini RNA) columns. The DNA template, rTPT3 or rTPT3^{A} modified 3'SL (**A**) or DENV mini (**B**) RNAs, and the excess rNTPs, rTPT3^{A} or rTPT3 and DTT are well separated. (**C-D**) 10% (C, for 3'SL) and 5% (D, for DENV-mini) native PAGE gel analysis of purified TPT3-modified (singly modified at sites of 689, 719, or doubly modified at sites of both 689 and 719) RNAs. The unmodified wild type 3'SL (C) and DENV-mini (D) RNAs are included for comparison. .

Table S1. The DNA sequences of the total gene synthesized plasmids coding for full-length 3'SL and DENV-mini RNAs from DENV2.

Plasmids	Primary sequence
3'SL	AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGT ATCACGAGGCCCTTTCGTTGTAAAACGACGGCCAGTCCGTCTCTATCCG GTCTCGATCCGCAGTCTCTTGGCACAGGTAATACGACTCACTATAGGCGC TGGGAAAGACCAGAGATCCTGCTGTCTCCTCAGCATCATTCCAGGCACA GAACGCCAGAAAATGGAATGGTGCTGTTGAATCAACAGGTTCTACCGTG CGAGTTACTGCCAACCGAGACCCAACCGAGACGGGTCATAGCTGTTTCC AGTGTGCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCT GCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTACCCACA GAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAA AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCT CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGG CGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCT CCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTC CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGT AGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGC ACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCG TCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC ACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGT ATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCT CTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGC AAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGAT CTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGG ATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGA CAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATT TCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACG GGAGGGCTTACCATCTGGCCCCAGTGCTGCAATAATACCGCGGGACCCA CGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGG CCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATT AATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCG CAACGTTGTTGCCATCGCTACAGGCATCGTGGTGTCACGCTCGTCGTTT GGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATG ATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCG TTGTCAGAAGTAAGTTGGCCGCCGTGTTATCACTCATGGTTATGGCAGCA CTACATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACT GGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGA GTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGA ACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTC AAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCAC CCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCA AAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGA AATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAG GGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAG
DENV-mini	AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACC TGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGT ATCACGAGGCCCTTTCGTTGTAAAACGACGGCCAGTCGAACCACGCAAT GCGTCTCGATCCGCAGTGTCTTGCGTCTCTTAATACGACTCACTATAAGTT GTTAGTCTACGTGGACCGACAAAGACAGATTCTTTGAGGGAGCTAAGCT CAACGTAGTTCTAACAGTTTTTTAATTAGAGAGCAGATCTCTGATGAATAA CCAACGAAAAAAGGCGAGAAATACGCCTTTCAATATGCTGAAACGCGAGA GAAACCGCGTGTCGACTGTACAACAGCTGACAAAGAGATTCTCACTTGG AATGCTGCAGGGACGAGGACCTCAGATTCAGAAGAGAAGAGGAAGAGG CAGGAGTCCTGTGGTAGAAGGCAAAACTAACATGAAACAAGGCTAGAAG

	TCAGGTCGGATTAAGCCATAGTACGGAAAAAACTATGCTACCTGTGAGCC CCGTCCAAGGACGTTAAAAGAAGTCAGGCCATTACAAATGCCATAGCTTG AGTAAACTGAGCAGCCTGTAGCTCCACCTGAGAAGGTGTAAAAAATCTG GGAGGCCACAAACCATGGAAGCTGTACGCATGGCGTAGTGGACTAGCG GTTAGAGGAGACCCCTCCCTTACAAATCGCAGCAACAATGGGGGCCCAA GGTGAGATGAAGCTGTAGTCTCACTGGAAGGACTAGAGGTTAGAGGAGA CCCCCCCAAAACAAAAAACAGCATATTGACGCTGGGAAAGACCAGAGAT CCTGCTGTCTCCTCAGCATCATTCCAGGCACAGAACGCCAGAAAATGGA ATGGTGCTGTTGAATCAACAGGTTCTAGAGACGGAGTCACTGCCAACCG AGACGGTCATAGCTGTTTCCTGTGTGCCGCTTCCTCGCTCACTGACTCG CTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAG GCGGTAATACGGTTACCCACAGAATCAGGGGATAACGCAGGAAAGAACAT GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTT GCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAAT CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACC AGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCT GCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCG CTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCG CTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTG CGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACT TATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTAC ACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGT AGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAG GATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGG AACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATC TTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTAT ATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACC TATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGT CGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCT GCAATAATACCGCGGGACCCACGCTCACCGGCTCCAGATTTATCAGCAAT AAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTT ATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTA GTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATCGCTACAGGCATC GTGGTATCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCA ACGATCAAGGCGAGTTACATGATCCCCCATGTTGCGCAAAAAAGCGGTTA GCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCCGTGTT ATCACTCATGGTTATGGCAGCACTACATAATTCTCTTACTGTCATGCCATC CGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAG AATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGA TAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACG TTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT CGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCA CCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAA GGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCA ATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTT GAATGTATTTAG

[a]: The DNA sequences coding for the 3'SL (97 nts) and DENV-mini (719 nts) RNAs are colored in red;

[b]: The T7 promoter is colored in green;

[c]: The common upstream sequence targeted by pMVF primer is colored in blue.

Table S2. The ssDNA primers used for dsDNA template preparations by PCR.

Primers	Sequence	Application
pMVF	5'-GTAACCCACTCGTGCACCCAACTG-3'	Forward primer
689R	5'-AGAACCTGTTGATTCAACAGCACCATTCCA(NaM)TTTCTGGCGTTCTG-3'	Reverse primer for T_{689}NaM modification
719R	5'-(NaM)GAACCTGTTGATTCAACAGCACCATTC-3'	Reverse primer for A_{719}NaM modification
689/719R	5'-(NaM)GAACCTGTTGATTCAACAGCACCATTCCA(NaM)TTTCTGGCGTTCTGTG-3'	Reverse primer for T_{689}NaM /A_{719}NaM modification
3'SLR	5'-AGACCCATGGATTTCCCCACACCGG-3'	Reverse primer for native 3'SL and DENV-mini RNAs

Table S3. Specific PCR conditions for amplification of the UBP-modified dsDNA templates.

Product DNA	3'SL			DENV-mini		
	A_{689}NaM	T_{719}NaM	A_{689}NaM/ T_{719}NaM	A_{689}NaM	T_{719}NaM	A_{689}NaM/ T_{719}NaM
2×PCR mix	25 μL					
Template	4 ng/μL	4 ng/μL	6 ng/μL	8 ng/μL	8 ng/μL	10 ng/μL
dTPT3-TP	1 mM	1 mM	1.4 mM	1 mM	1 mM	1.4 mM
Forward primer	0.8 μM (pMVF)		1 μM (pMVF)	0.8 μM (pMVF)		1 μM (pMVF)
Reverse primer	0.8 μM (689R)	0.8 μM (719R)	1 μM (689/719R)	0.8 μM (689R)	0.8 μM (719R)	1 μM (689/719R)
dd H_2O	To 50 μL					

Table S4. Typical conditions for *in vitro* transcription of 3'SL and DENV-mini RNAs containing UBP.

Product RNA	3'SL			DENV-mini		
	A_{689}TPT3	T_{719}TPT3	A_{689}TPT3/ T_{719}TPT3	A_{689}TPT3	T_{719}TPT3	A_{689}TPT3/ T_{719}TPT3
10 ×Transcription buffer	10 μL					
$MgCl_2$	20 mM			30 mM		
T7 RNA polymerase	200 ng/μL					
NaM-modified DNA template	4 μM	4 μM	6 μM	4 μM	4 μM	6 μM
rNTP mix	4 mM each					
rTPT3 or $rTPT3^A$	0.8 mM		1 mM	0.8 mM		1 mM
DTT	10 mM					
DEPC-H_2O	To 100 μL					

Table S5. Overall structural parameters for native, UBP-modified and Nanogold-conjugated 3'SL and DENV-mini RNAs were calculated using similar procedures as described before (2).

Sample	R_g[a](Å)	R_g[b](Å)	D_{max} (Å)	MW[c](kDa)	MW[d](kDa)
3'SL WT	35.3±0.6	38.3±0.6	129	32,100	30,229
3'SL 689-TPT3	35.8±0.2	37.6±0.5	127	32,300	31,898
3'SL 719-TPT3	35.7±0.9	37.6±0.5	127	32,300	30,910
3'SL 689/719-TPT3	35.0±0.6	38.6±1.2	130	32,500	30,058
3'SL 689 Single-Nanogold	35.2±1.2	37.3±1.2	135	47,500	n/a
3'SL 719 Single-Nanogold	36.3±0.5	40.7±1.2	150	47,500	n/a
3'SL Double-Nanogold	39.4±0.5	41.2±1.1	156	62,500	n/a
DENV-mini WT	84.1±0.2	89.5±2.0	309	237,270	193,173
DENV-mini 689-TPT3	83.3±0.6	90.2±2.5	310	237,470	197,528
DENV-mini 719-TPT3	86.0±0.4	91.7±1.7	311	237,470	210,785
DENV-mini 689/719-TPT3	84.3±0.7	90.5±1.1	310	237,670	205,469
DENV-mini 689 Single-Nanogold	88.2±0.5	91.0±1.8	313	252,670	n/a
DENV-mini 719 Single-Nanogold	87.2±0.6	89.8±1.9	310	252,670	n/a
DENV-mini Double- Nanogold	89.9±0.9	91.5±1.4	316	267,670	n/a
1.4 nm Nanogold	8.1±0.03	8.1±0.06	18	15,000	n/a

[a] Derived from Guinier fitting;

[b] derived from GNOM analysis (3);

[c] MW: molecular weight predicted from sequences;

[d] MW: molecular weight calculated based on the power law of volume of correlation (4); n/a: not applicable.

Table S6. The coupling efficiencies for Nanogold-conjugated 3'SL and DENV-mini RNAs.

Sample	A_{260}	A_{420}	Con_{Gold}/Con_{RNA}	Labeling efficiency (%)
3'SL 689 Single-Nanogold	2.537	0.287	0.836	83.6
3'SL 719 Single-Nanogold	2.602	0.305	0.864	86.4
3'SL Double- Nanogold	2.061	0.332	1.750	87.5
DENV-mini 689 Single-Nanogold	7.536	0.119	0.738	73.8
DENV-mini 719 Single-Nanogold	6.942	0.101	0.791	79.1
DENV-mini Double- Nanogold	6.063	0.225	1.615	80.8

Table S7. SAXS data collection parameters and softwares employed for data analysis are similar as described before (2).

Data Collection Parameters	
Facilities and parameters	Settings and values
Beam line	12ID-B (APS, ANL)
Wavelength (Å)	0.8857
Detector	Pilatus 1M (SAXS)
q range ($Å^{-1}$)	0.005-0.89
Exposure time (s)	3 (XSI)-30 (SAXS)
Concentration range (mg/ml)	0.25-1 for unlabeled, 1-7 for Nanogold-labeled
Temperature (K)	298
Software Employed	
Primary Data Processing	Matlab/PRIMUS (5)
P(r) Function	GNOM (3)

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