Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Photoactivatable microRNA probe for identification of microRNA targets and light-controlled suppression of microRNA target expression

Lei Chen, Yu Sun, Jinbo Li* and Yan Zhang,*

State Key Laboratory of Analytical Chemistry for Life Sciences, Jiangsu Key Laboratory of Advanced Organic Materials, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China.

*Emails: jinboli@nju.edu.cn; njuzy@nju.edu.cn

Contents

Materials and Methods

1. General materials and methods

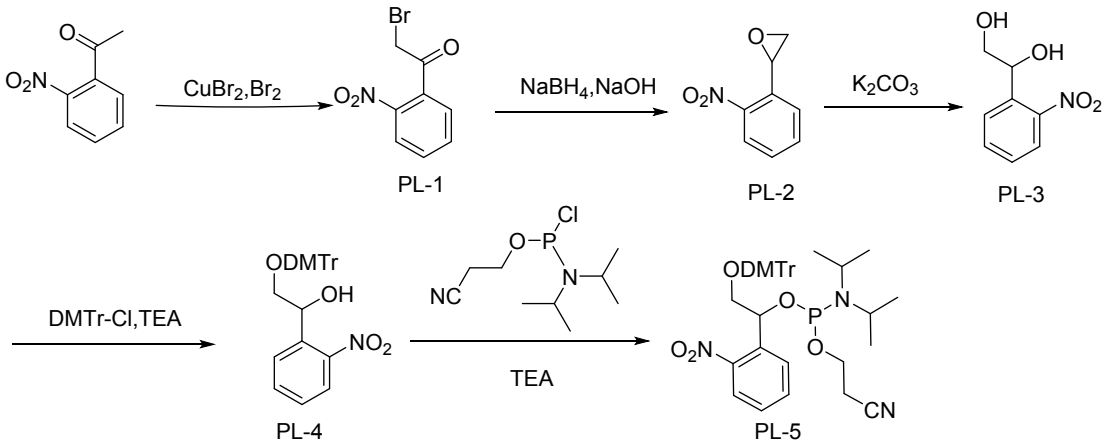
All chemicals and solvents were purchased from J&K chemicals or Sigma-Aldrich. High glucose Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penincilin/streptomycin, Lipofectamine 2000 were purchased from Life Technologies. Luciferase assay kits were purchased from Promega. Luciferase and green fluorescent protein reporter genes were ordered from Genescript. All aqueous solutions were treated by diethypyrocarbonate (DEPC) before use.

^{1}H NMR and ^{13}C NMR spectra were obtained on a 400 MHz Bruker AVANCE III–400 spectrometer. Chemical shifts are reported in δ (ppm) relative to the solvent residual peak. Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). MS and HRMS were done on a SHIMADZULCMS-2020 and Agilent 6550 iFunnel Q-TOF LC/MS. HPLC was carried out on Thermo UltiMate 3000 with CH_3CN/H_2O (0.1 M CH_3COONH^+) as eluents. Fluorescence images were taken under confocal microscope (Leica).

An 8 W hand-held 365 nm UV lamp was used as the light source. When using light irradiation to trigger the photolysis reaction, the distance from lamp to samples was ~1-2 cm and the corresponding irradiation intensity was ~10 mW/cm^2.

Sequence of 3'-UTR of SIRT1 that is inserted in the 3'-UTR of luciferase or GFP: 5'-CAATAAAAAGGGTTTGAAATATAGCTGTTCTTTATGCATAAAACACCCAGCTAGGAC CATTACTGCCAGAGAAAAAAATCGTATTGAATGGCCATTTCCCTACTTA-3'.

2. Synthesis and characterization of the photo-labile linker



The photo-labile linker PL-5 was synthesized according to the method reported by Tang *et al*.[1]

Synthesis of PL-1

To a mixture of compound 2-nitroacetophenone (6.72 g, 40 mmol) and $CuBr_2$ (17.88 g, 80 mmol) in ethyl acetate (45 mL) and chloroform (45 mL), several drops of liquid bromine were slowly added to activate the reaction. The reaction mixture was stirred at 80 ^oC for 8 h, and was then cooled down to room temperature. The precipitate was filtered out, and the filtrate was washed with saturated brine and dried over anhydrous Na_2SO_4. The solvent was removed by vacuum, and the mixture was purified by silica gel column chromatography to give the product PL-1 (5.33 g, 55%).^{1}H NMR (400 MHz, DMSO) δ 8.21 (dd, J = 8.3, 0.8 Hz, 1H), 7.91 (td, J = 7.5, 1.0 Hz, 1H), 7.83 - 7.80 (m, 1H), 7.77 (dd, J = 7.5, 1.3 Hz, 1H), 4.84 (s, 2H).

Synthesis of PL-2

To a solution of compound PL-1 (3.66 g, 15 mmol) dissolved in 1,2-dioxane (25 mL) and placed in ice bath, $NaBH_4$ (1.71 g, 45 mmol) in methanol (16 mL) was slowly added. The reaction mixture was slowly warmed up to room temperature and stirred until compound PL-1 disappeared as monitored by TLC. Then aqueous NaOH solution (10%) was added to the reaction at 0 ^oC and the solution was stirred for 0.5 hour at room temperature. The reaction mixture was extracted with ethyl ether for three times. The combined mixture was dried over anhydrous Na_2SO_4. The solution was then filtrated and concentrated. The mixture was then purified by silica gel column chromatography to give compound PL-2 (1.68 g, 67.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.2 Hz, 1 H), 7.63 (t, J = 7.5 Hz,1H), 7.56 (d, J = 7.8 Hz, 1H), 7.44 (t, J = 7.5 Hz ,1 H), 4.43 (dd, J = 4.2, 2.6 Hz, 1H), 3.25 (dd, J = 5.5, 4.5 Hz, 1H), 2.63 (dd, J = 5.6, 2.6 Hz, 1H).

Synthesis of PL-3

To a solution of compound PL-2 (0.99 g, 6 mmol) in 1,2-dioxane (8 mL), 10% aqueous K_2CO_3 solution (25 mL) was added. The solution was refluxed for about 18 h. The solution was then cooled down to room temperature, and hydrochloric acid was added to adjust the pH value to 5. The mixture was extracted by ethyl ether for three times. The combined solutions were then dried over anhydrous Na_2SO_4 and were concentrated in vacuo after filtration. The mixture was purified by silica gel column chromatography to give compound PL-3 (0.9 g, 82%).^{1}H NMR (400 MHz, DMSO) δ 7.85 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.70 (t, J = 7.6Hz, 1H), 7.49 (t, J = 7.0 Hz, 1H), 5.63(d, J = 4.6 Hz, 1 H), 5.10 – 5.05 (m, 1 H), 4.92-4.89 (m, 1H), 3.49-3.44 (m,2H).

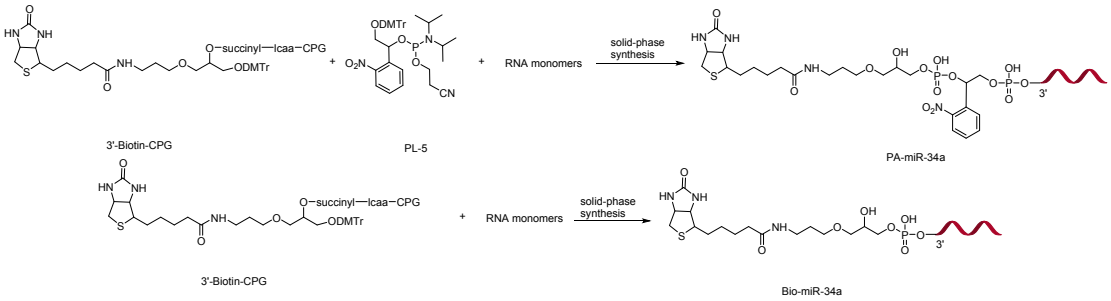
Synthesis of PL-4

To a solution of compound PL-3 (0.549 g, 3.0 mmol) in dichloromethane (5 mL) and triethylamine (1.25 mL), 4,4′-dimethoxytriphenylmethyl chloride (1.016 g, 3.0 mmol) in dichloromethane (5 mL) was added dropwise. The mixture was stirred for 2 hours. After concentration in vacuo, the mixture was purified by silica gel column chromatography to give compound PL-4 (0.94 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 8.2 Hz, 1 H), 7.77 (d, J = 7.9 Hz,1H), 7.58 (t, J = 7.6, 1 H), 7.40 - 7.38 (m, 3H), 7.31-7.26 (m, 6 H), 7.22 - 7.20 (m, 1H), 6.83 - 6.79 (m, 4H), 5.53 (dd, J = 7.4, 3.8 Hz, 1H), 3.77 (s, 6H), 3.62 (dd, J = 9.4, 3.5 Hz, 1H), 3.17 (dd, J = 9.4, 7.4Hz, 2H).

Synthesis of PL-5

Compound PL-4 (0.37 g, 1 mmol) and triethylamine (250 μL) were dissolved in anhydrous CH_2Cl_2 (3 mL) in dry flask. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 g, 1.5 mmol) in anhydrous CH_2Cl_2 (1 mL) was then injected to the flask under dry nitrogen. The resulted solution was stirred at room temperature for 1 h and then was concentrated in vacuo. The mixture was purified by silica gel column chromatography to give compound PL-5 (240 mg, 35%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 8.2 Hz, 1 H), 7.81 (d, J = 8.9 Hz,1H), 7.57 (t, J = 7.6 Hz, 1 H), 7.41 - 7.37 (m, 3H), 7.29 - 7.26 (m, 6 H), 7.21 - 7.18 (m, 1H), 6.81 - 6.78 (m, 4H), 5.80 - 5.77 (m, 1H), 3.88 - 3.85 (m, 1H), 3.78 (m, 6H), 3.48 - 3.41 (m, 2H), 3.28 - 3.24 (m, 1H), 2.60 - 2.46 (m, 2H), 1.15 (d, J = 6.8 Hz, 6H), 0.84 (d, J = 6.8 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.

3. Preparation of PA-miR-34a



All the oligonucleotides including nc, PA-nc, miR-34a, PA-miR-34a and Bio-miR-34a were synthesized on a K&A H-8 DNA/RNA synthesizer. miR-34a: 5'-UGGCAGUGUCUUAGCUGGUUGU-3'; miR-34a*: 5'-CAAUCAGCAAGUAUACUGCCCU-3'; nc: 5'-UUCUCCGAACGUGUCACGUUU-3'; nc*: 5'AAGAGGCUUGCACAGUGCAAA-3'. 3'-Biotin-CPG-1000 was used to synthesize PA-nc, PA-miR-34a or Bio-miR-34a, which contain biotin at their 3'-ends. PL-5 was directly used as the monomer on the DNA/RNA synthesizer. After cleavage from resin and further deprotection, these oligonucleotides were purified with HPLC. After lyophilization, the oligonucleotides were dissolved in DEPC-treated H_2O and their concentrations were determined by using NanoDrop 2000 (Thermo Scientific). The miRNA mimics were characterized with MS. nc: calculated, 6588 $[M+H]^+$; found, 6588; miR-34a: calculated, 7030 $[M+H]^+$; found, 7028; PA-miR-34a: calculated, 7712 $[M+H]^+$; found, 7711; Bio-miR-34a: calculated, 7467 $[M+H]^+$; found, 7466; PA-nc: calculated, 7271 $[M+H]^+$; found, 7270; Liberated miR-34a: calculated, 7256 $[M+H]^+$; found, 7256.

4. Gel shift assay

Gel mobility shift assays were conducted to investigate the mobility of PA-miR-34a before and after light irradiation. PA-miR-34a was dissolved in PBS with a final concentration of 50 μM, followed by irradiation with an hand-held 8 W 365 nm lamp (10 mW/cm^2) for 0-2 min. The reaction mixtures were then analyzed on TBE (15%) gel, which was stained with SYBR Green. The gel picture was taken by Geldoc using SYBR green filter.

5. Cell culture

HeLa cells were cultured in high-glucose DMEM containing 10% fetal bovine serum and 1% penicillin/streptomycin and maintained in 5% CO_2 at 37 °C.

6. Pull down assay

HeLa cells were seeded in 10-cm dish. After reaching ~70% confluency, HeLa cells were transfected with nc (100 nM), PA-miR-34a (100 nM), PA-nc (100 nM) or Bio-miR-34a (100 nM) using Lipofectamine 2000 according to manufacturer's protocol. At 4 h post transfection, medium were replaced with fresh culture medium. At 12 h post transfection, cells were collected, resuspended in 150 μl of PBS buffer (with 50 U/mL RNase inhibitor), and lysed by repeated freeze-thaw using liquid nitrogen. After centrifugation, the supernatant was collected. The cell lysate was incubated with streptavidin magnetic beads at 25 ^{o}C for 2 h. After washing with the wash/binding buffer (0.5 M NaCl, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 50 U/mL RNase inhibitor) four times and cold low-salt buffer (0.15 M NaCl, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 50 U/mL RNase inhibitor) one time, biotinylated species were eluted from the magnetic beads with elution buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 50 U/mL RNase inhibitor). The eluted samples were then subjected to reverse transcription and quantified by qPCR on Applied Biosystem 7300 Real-time PCR system for analyzing the enrichment of miRNA or mRNAs. GAPDH was used as the internal control for quantification of miRNA targets.

7. Luciferase assay

HeLa cells were seeded on 24-well plates and transfected the following day with 0.5 μg of luciferase reporter plasmids and 0.5 μg of β-galactosidase expressing plasmids by using Lipofectamine 2000 according to manufacturer's protocol. β-Galactosidase was used as the internal control. At the same time, nc (30 nM), miR-34a (30 nM), PA-miR-34a (30 nM), PA-nc (30 nM) or Bio-miR-34a (30 nM) were co-transfected by using Lipofectamine 2000 according to manufacturer's protocol. At 4 h post transfection, medium were replaced with fresh culture

medium. HeLa cells were then kept in dark or immediately irradiated using an 8 W hand-held 365 nm lamp for 0 - 5 min, or irradiated at the time point of 4, 10, 16, 28, 40, 46 h after transfection. At 48 h post transfection, luciferase signals were then measured according to manufacturer's instruction. β-Galactosidase expression was detected by using β-Galactosidase assay kit (Beyotime, China) according to manufacturer's instruction. Briefly, activity of β-Galactosidase was indicated by the enzymatic cleavage of *o*-Nitrophenyl β-D-galactopyranoside (ONPG) into colored *o*-Nitrophenyl, which can be detected by measuring absorbance at ~420 nm. Luciferase signals were then normalized by using β-Galactosidase activity data.

8. GFP assay

1×10^5 HeLa cells were seeded in 35-mm glass-bottom tissue culture dishes and transfected the following day with 1 μg/mL of GFP reporter genes by using Lipofectamine 2000 according to manufacturer's protocol. At the same time, nc (30 nM), miR-34a (30 nM), PA-miR-34a (30 nM), PA-nc (30 nM) or Bio-miR-34a (30 nM) were co-transfected by using Lipofectamine 2000 according to manufacturer's protocol. At 4 h post transfection, medium were replaced with fresh culture medium. HeLa cells were then kept in dark and immediately irradiated using an 8 W hand-held 365 nm lamp for 2 min, or HeLa cells were irradiated for 2 min by using a photomask to shield a part of cells from irradiation. After further culture for 44 h, HeLa cells were first incubated with DRAQ5 to stain the nucleus according to the manufacturer's protocol. The images of HeLa cells were then taken under confocal microscope by using filters corresponding to GFP or DRAQ5.

9. Western blotting

HeLa cells were seeded in 60-mm dish. After reaching ~70% confluency, HeLa cells were transfected with nc (100 nM), miR-34a (100 nM), PA-miR-34a (100 nM), PA-nc (100 nM) or Bio-miR-34a (100 nM) using Lipofectamine 2000 according to manufacturer's protocol. At 4 h post transfection, medium were replaced with fresh culture medium. HeLa cells transfected with

PA-miR-34a were then kept in dark or immediately irradiated using an 8 W hand-held 365 nm lamp for 2 min. After further culture for 44 h, cell lysates were extracted using RIPA lysis buffer (150 mM NaCl, 10 mM Tris, pH 7.5, 1% NP40, 1% deoxycholate, 0.1% SDS, 1 × protease inhibitor cocktail). Protein concentration was determined using BCA protein assay kit. A total of 30 μg of protein was denatured in 1 × SDS loading buffer and resolved by 10% SDS-PAGE, followed by transferring to PVDF membrane and block in 5% nonfat milk in TBST. The PVDF membrane was then stained with primary antibodies for SIRT1 and GAPDH. After washing, the membrane was further incubated with the appropriate secondary antibody and finally visualized using ECL reagents.

10. MTT assay

HeLa cells were seeded in 96-well plates at a concentration of 5,000 cells per well. HeLa cells were transfected with nc (0-200 nM), miR-34a (0-200 nM), or PA-miR-34a (0-200 nM) using Lipofectamine 2000 according to manufacturer's protocol. At 4 h post transfection, medium were replaced with fresh culture medium. HeLa cells transfected with PA-miR-34a were then kept in dark or immediately irradiated using an 8 W hand-held 365 nm lamp for 2 min. The cells were further cultured for 44 h. For each well, 20 uL of MTT solution (final concentration: 1 mg/ml) was added. Then, 150 uL DMSO was added after incubation for 4 h, and absorbance at 490 nm was measured to indicate the cell viability.

11. Flow cytometry analysis of cell apoptosis

HeLa cells were cultured in 6-well plates. When reaching ~80% confluency, HeLa cells were transfected with nc (100 nM), miR-34a (100 nM), PA-miR-34a (100 nM), PA-nc (100 nM) or Bio-miR-34a (100 nM) using Lipofectamine 2000 according to manufacturer's protocol. At 4 h post transfection, medium were replaced with fresh culture medium. HeLa cells transfected with PA-miR-34a were then kept in dark or immediately irradiated using an 8 W hand-held 365 nm lamp for 2 min. The cells were further cultured for 44 h and harvested for apoptosis analysis. The

apoptotic cells were identified through flow cytometry by using an Annexin V-FITC/PI staining kit according to manufacturer's protocol.

Reference

[1]. Y. Ji, J. Yang, L. Wu, L. Yu and X. Tang, *Angew. Chem. Int. Ed.*, 2016, 55, 2152-2156

Supporting Figures

Figure S1. Gel shift analysis of PA-miR-34a after light irradiation.

PA-miR-34a was dissolved in PBS with a final concentration of 50 μM, followed by irradiation with an 8 W hand-held 365 nm lamp for 0, 0.5, 1, or 2 min. The reaction mixtures were then analyzed by electrophoresis. MiRNA was stained with SYBR green.

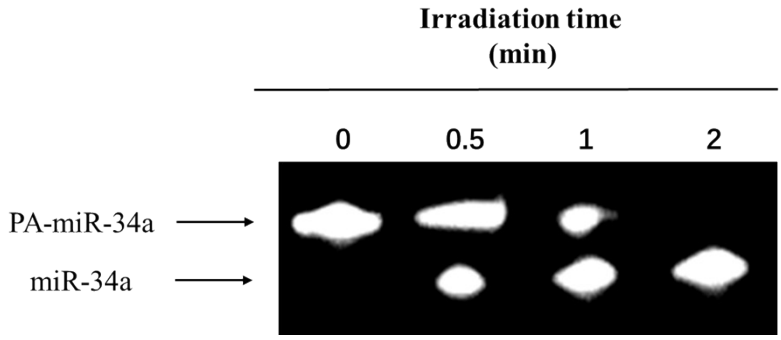


Figure S2. Stability of PA-miR-34a in dark or under ambient light.

PA-miR-34a was dissolved in PBS with a final concentration of 50 μM. After stay in dark or under ambient light for 48 h, PA-miR-34a was analyzed by HPLC.

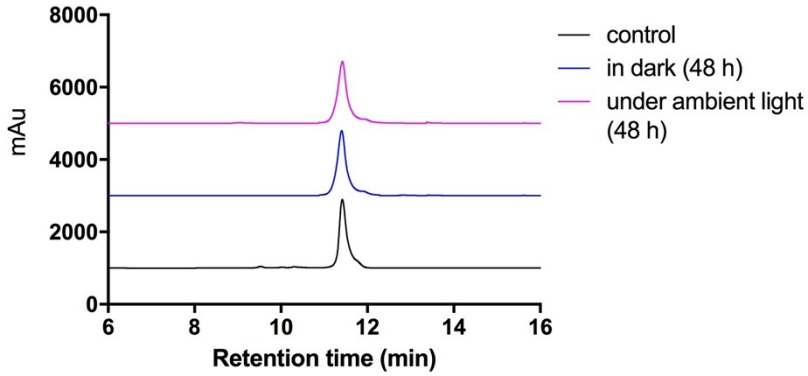


Figure S3. Intracellular function of liberated miR-34a.

HeLa cells were co-transfected with luciferase reporter gene (0.5 μg) and nc (30 nM), or miR-34a (30 nM), or liberated miR-34a (30 nM). Luciferase signals were measured after HeLa cells were

further cultured for 48 hours. Data are shown as mean ± SEM (n=3).

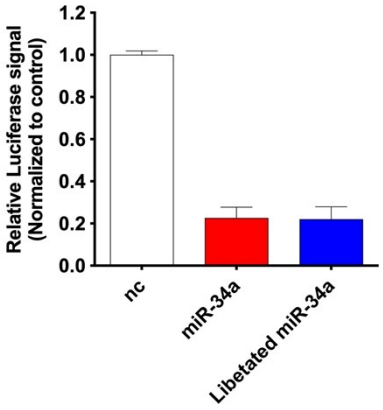


Figure S4. The activity of PA-miR-34a in repressing luciferase expression without or with irradiation.

HeLa cells were co-transfected with luciferase reporter gene (0.5 μg) and nc (30 nM), or miR-34a (30 nM), or PA-miR-34a (30 nM). At 4 h post transfection, HeLa cells were irradiated with an 8 W hand-held 365 nm lamp for 0, 0.5, 1, 2, 3 or 5 min. Luciferase signals were measured after HeLa cells were further cultured for 48 hours. Data are shown as mean ± SEM (n=3).

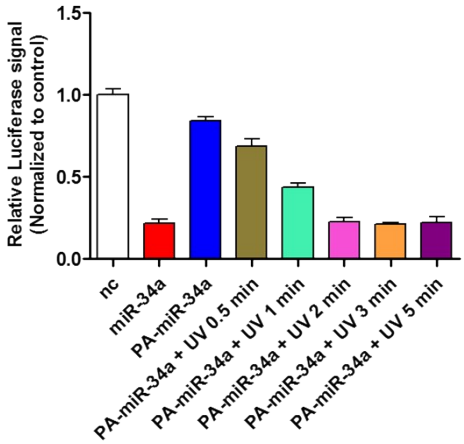


Figure S5. Effect of light irradiation on viability of HeLa cells.

HeLa cells were irradiated with an 8 W hand-held 365 nm lamp for 1 or 2 minutes. After further culture for 48 hours, cell viability was evaluated by MTT. Data are shown as mean ± SEM (n=3).

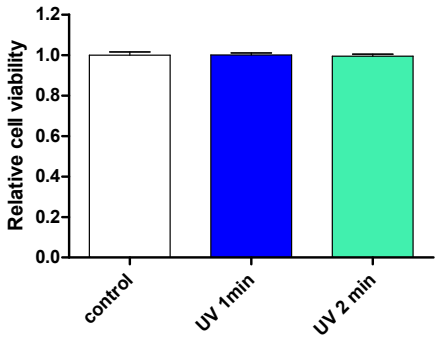


Figure S6. Activities of PA-miR-34a in repressing GFP expression.

HeLa cells were co-transfected with GFP reporter gene (1 μg/mL) and nc (30 nM), miR-34a (30 nM), PA-nc (30 nM) or PA-miR-34a (30 nM). At 4 h post transfection, HeLa cells were irradiated with an 8 W hand-held 365 nm lamp for 2 minutes. After further culture for 44 h, fluorescence images of HeLa cells were taken under confocal microscope. DRAQ5 was used to stain the nucleus. Scale bar: 100 μm.

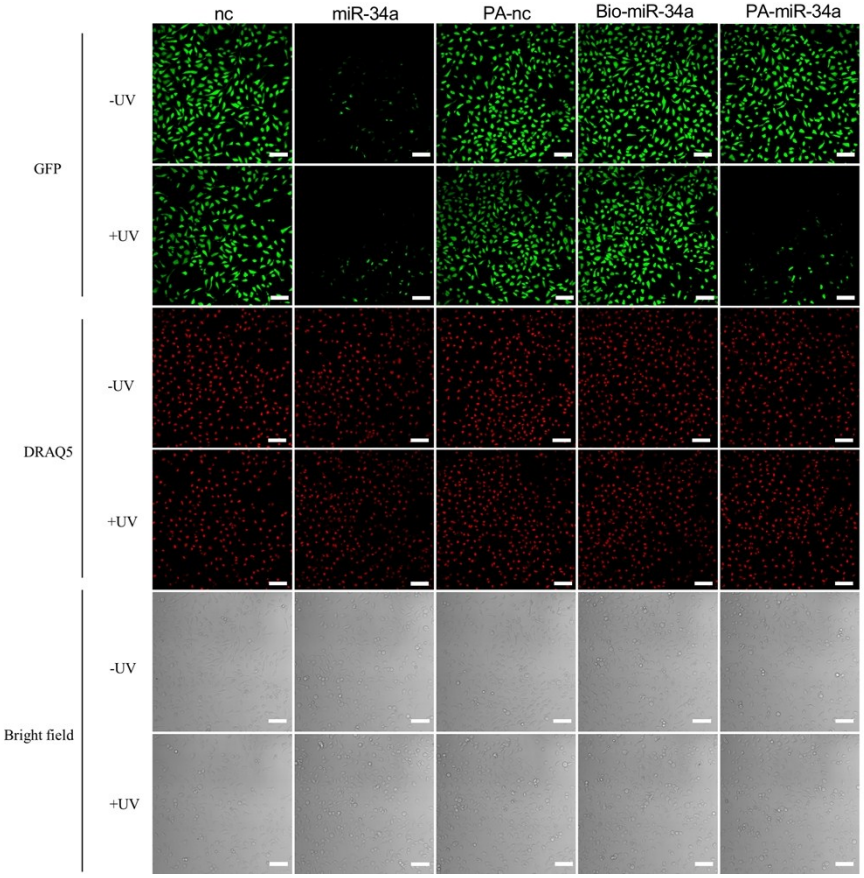
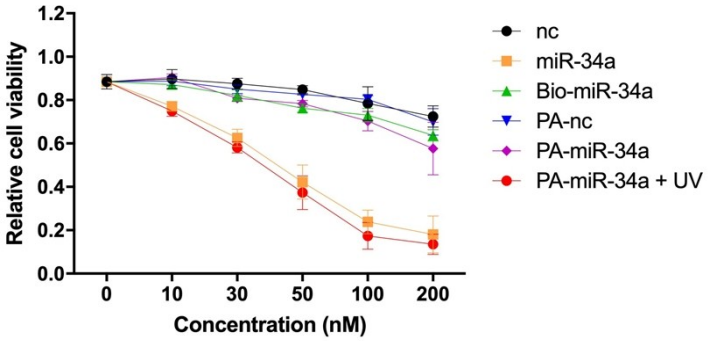


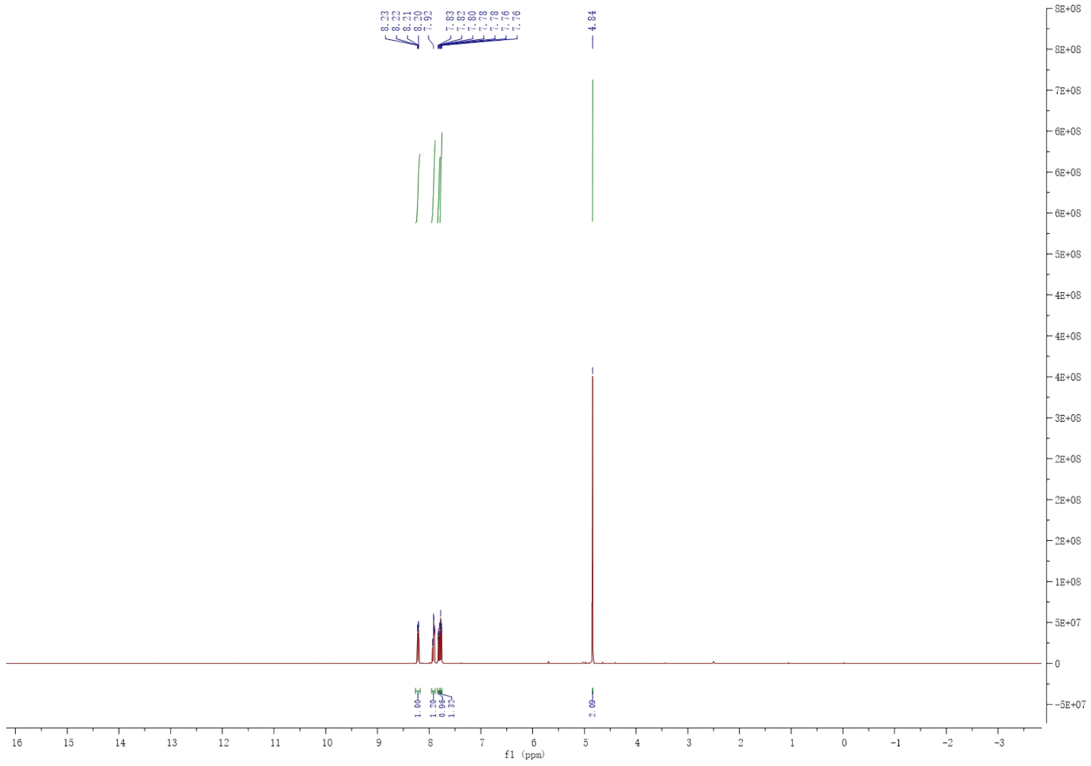
Figure S7. Cell viability of HeLa cells after transfection with PA-miR-34a.

HeLa cells were transfected with nc (0-200 nM), miR-34a (0-200 nM), Bio-miR-34a (0-200 nM), or PA-miR-34a (0-200 nM). Cells transfected with PA-miR-34a were subject to UV irradiation for 2 min at 4 h post transfection. After further culture for 44 h, HeLa cells were analyzed by MTT. Data are shown as mean ± SEM (n=3). $^{*}P < 0.05$.

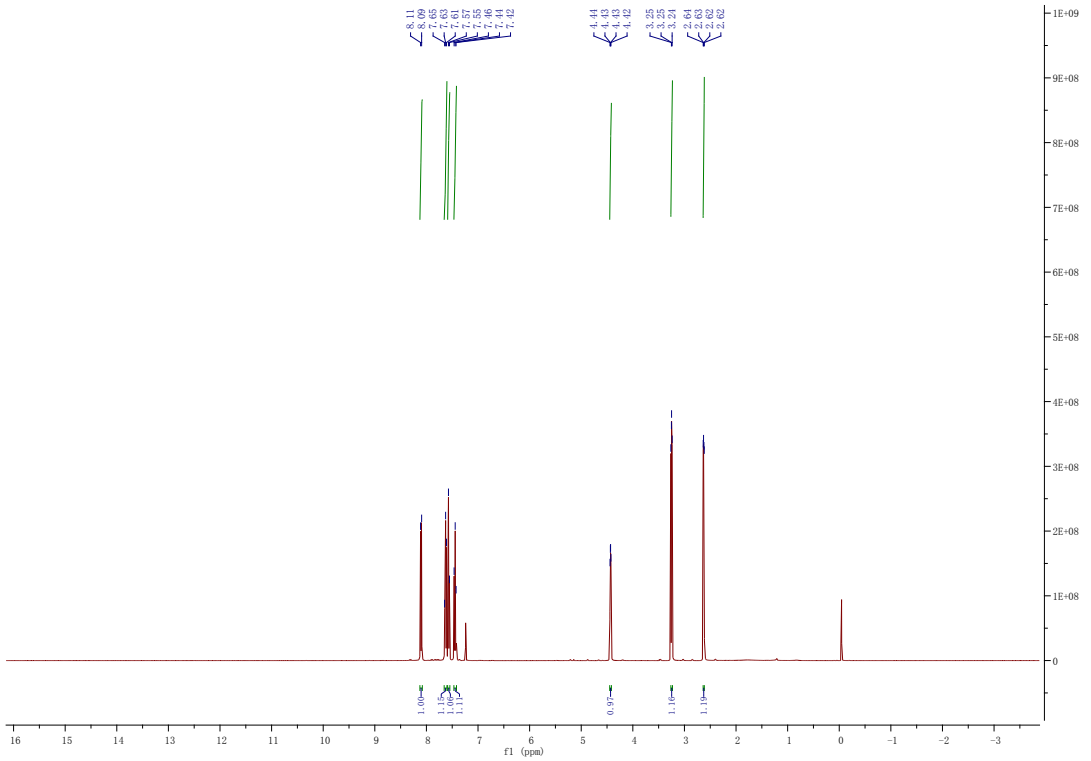


NMR Spectra

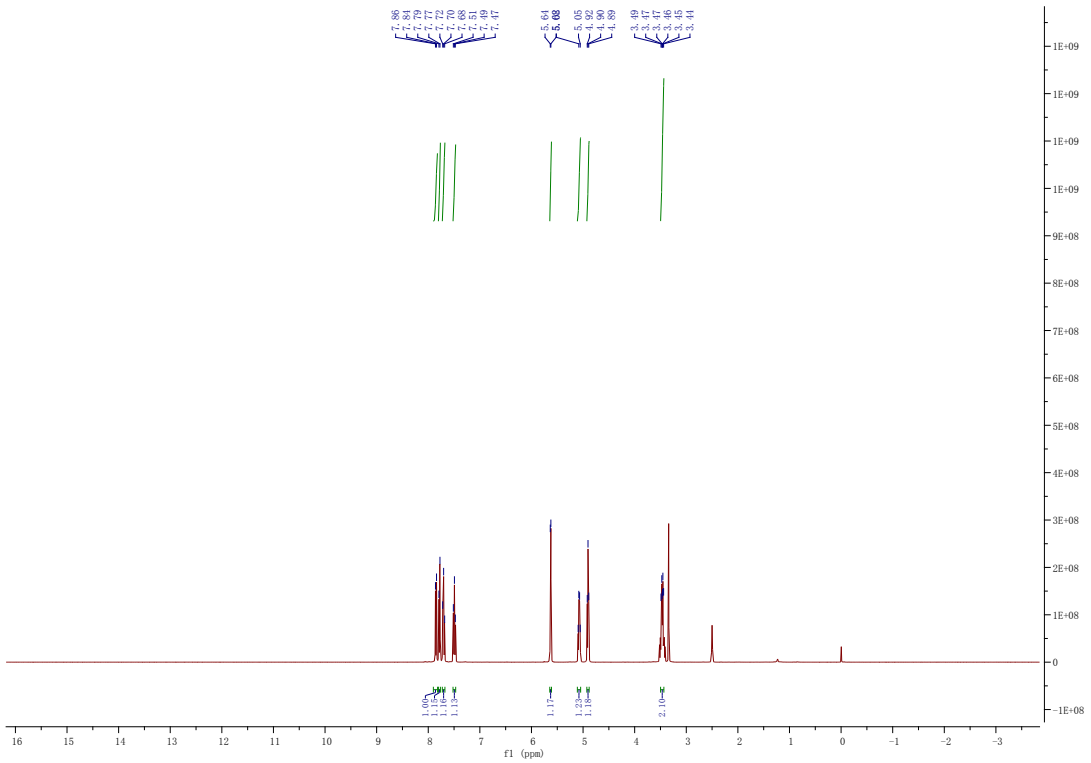
^{1}H NMR of PL-1



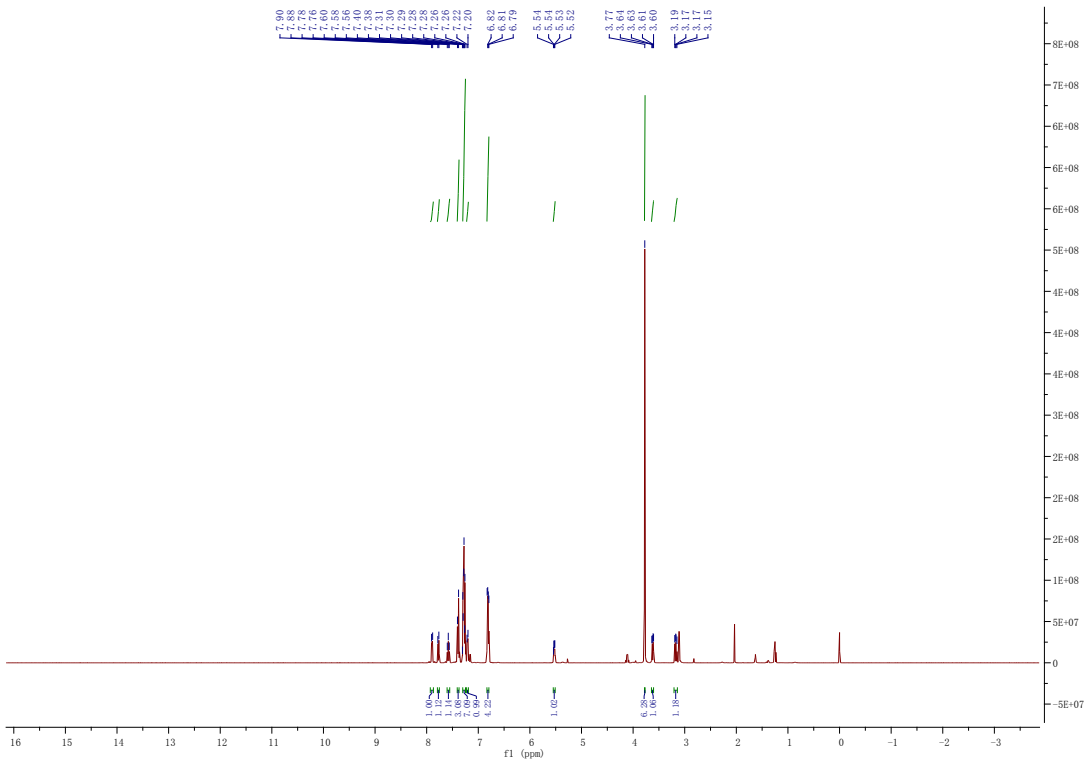
^{1}H NMR of PL-2



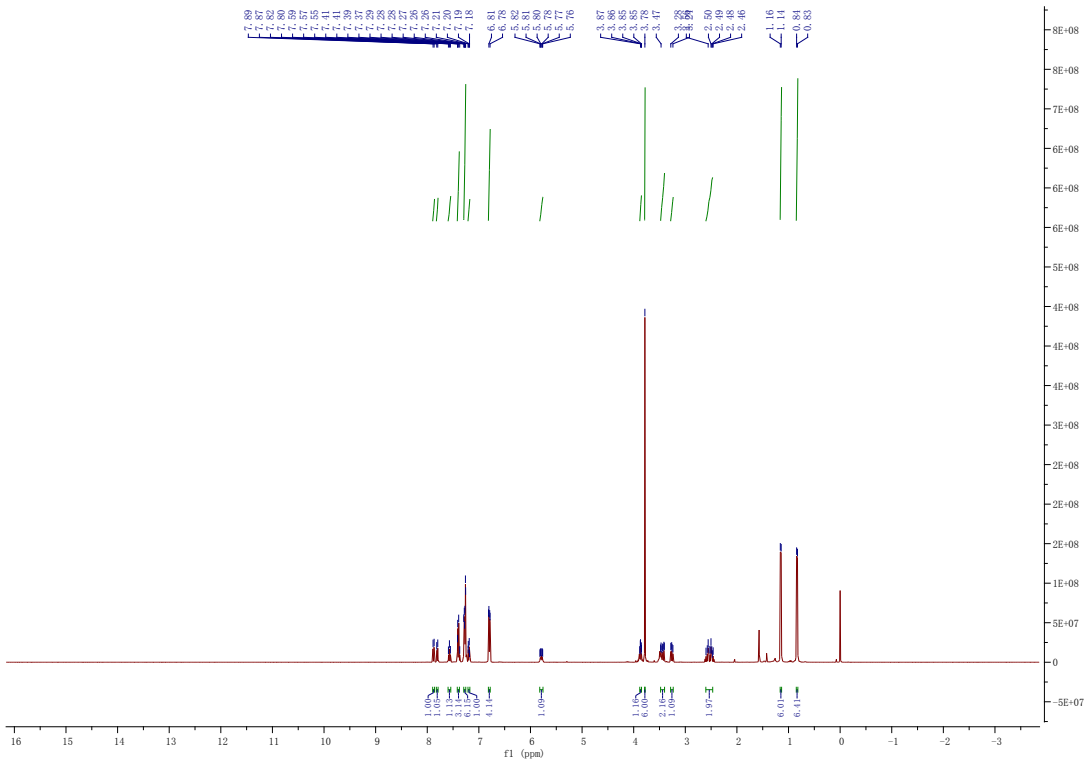
^{1}H NMR of PL-3



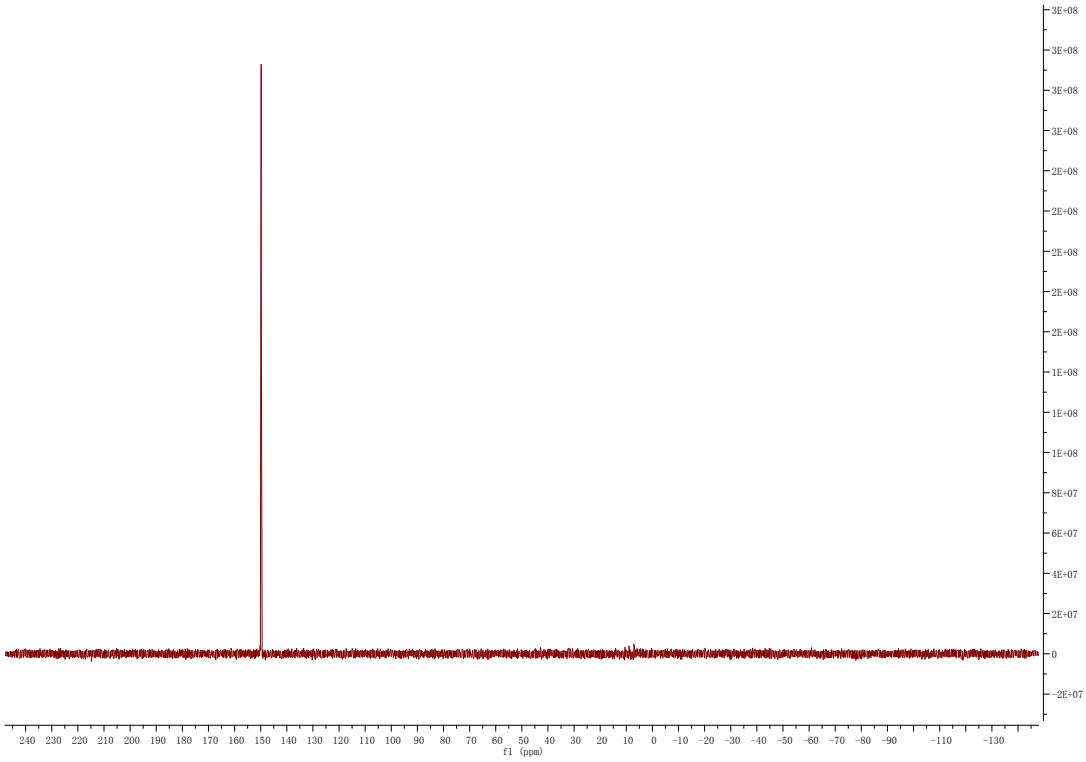
^{1}H NMR of PL-4



^{1}H NMR of PL-5

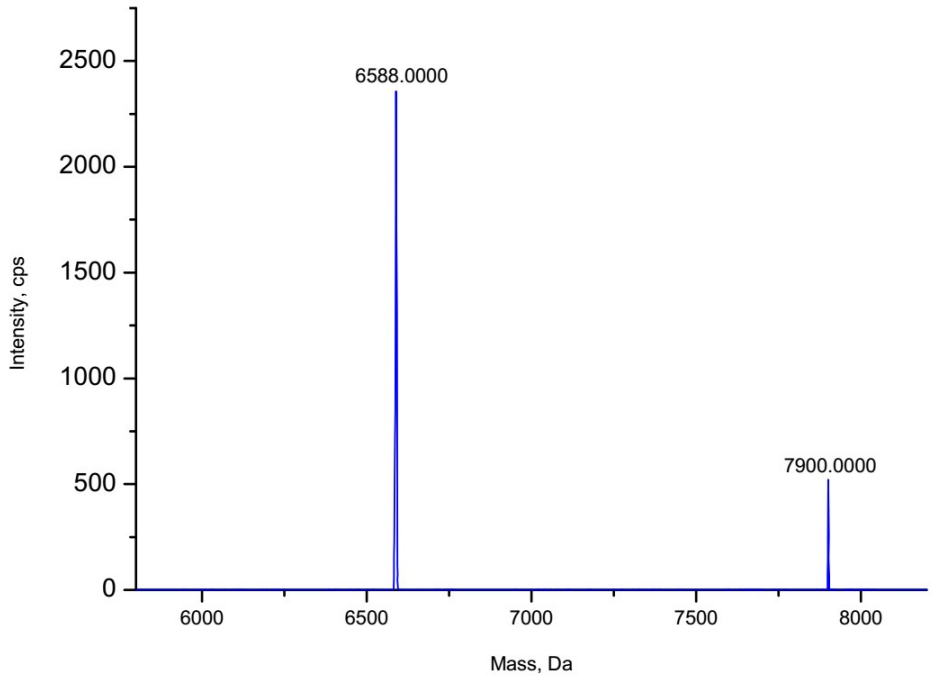


^{31}P NMR of PL-5

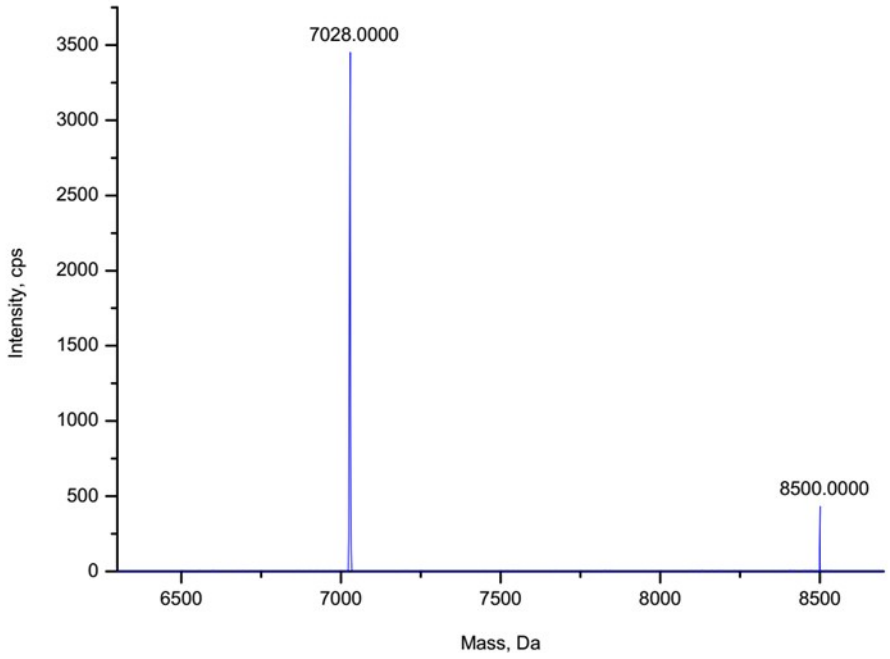

3E+08
3E+08
3E+08
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -110 -130
f1 (ppm)

MS Spectra

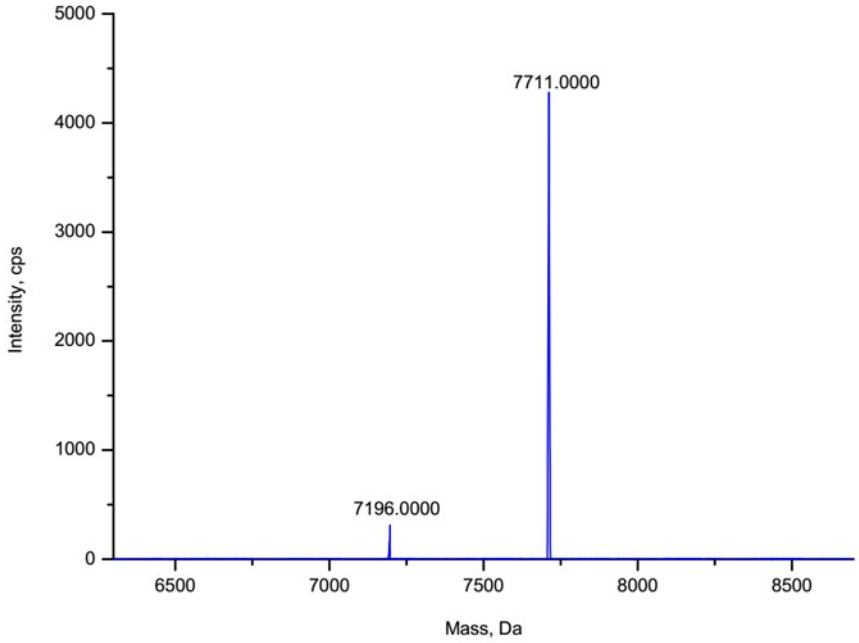
nc



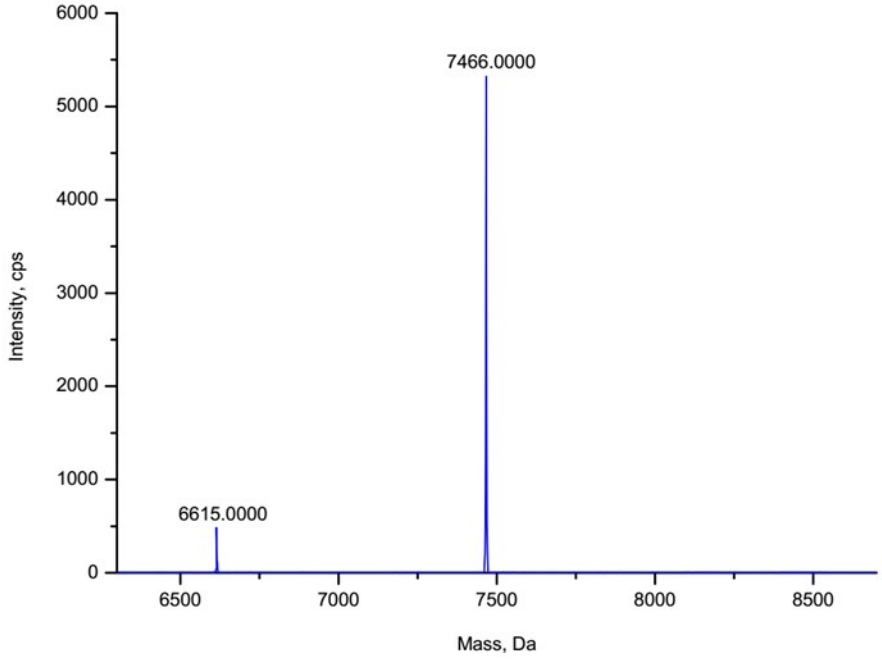
miR-34a



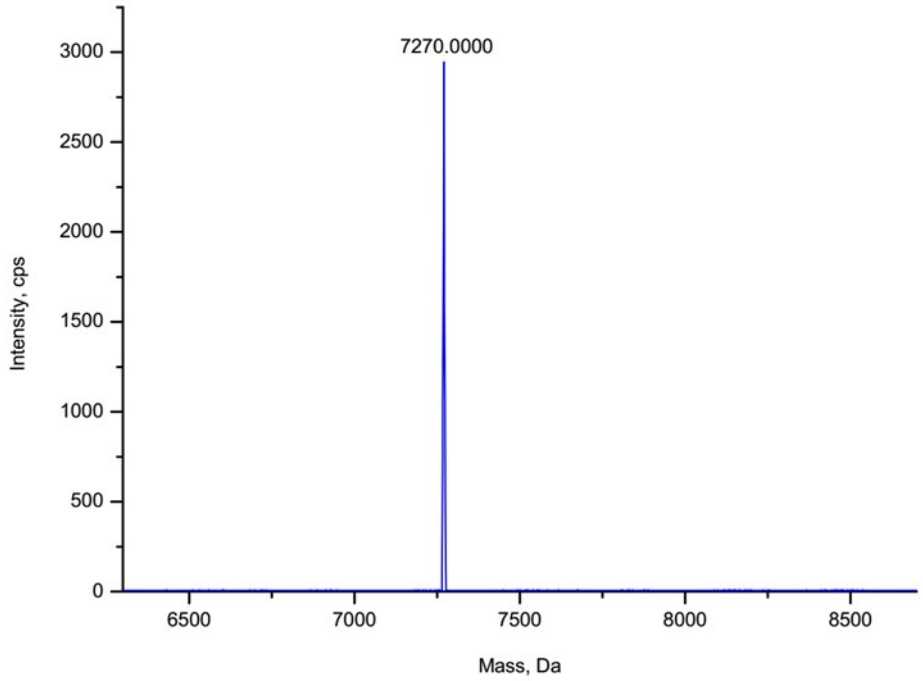
PA-miR-34a



Bio-miR-34a



PA-nc



Liberated miR-34a