Electronic Supplementary Material (ESI) for MedChemComm.


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## Targeting miRNA by Tunable Small Molecule Binders: Peptidic Aminosugar Mediated Interference in miR-21 Biogenesis Reverts Epithelial to Mesenchymal Transition

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**Supporting Information**

**Contents**

**General protocol for solid phase synthesis of peptidic kanamycin conjugates:**

1. Put 35.0 mg (6.3 μmol) Fmoc-PAL-PEG-PS Resin (loading capacity 0.18 mmol/g) into reaction cartridge, add 3 mL DCM for swelling, stir at a very low rate for overnight (cover the reaction cartridge with parafilm to avoid evaporation of DCM), then drain. Add 1 mL DMF, stir for ~2 min and drain. Repeat the last step (addition of DMF) for another 2 times.
2. Prepare 20 mL 25% piperidine in DMF solution and 20 mL capping solution (1:9 $Ac_2O$:DMF v/v).
3. Add 25% piperidine in DMF solution into cartridge, stir for 45 min then drain.
4. Wash resin with DMF (1 mL) four times (stir for ~1min each).
5. Mix 31.5 μmol modified base (5 equiv.) and 31.5 μmol HCTU (5 equiv.) in 200 μL NMP and 200 μL DMF in an eppendorf tube followed by addition of 11.0 μL DIPEA (63 umol, 10 equiv.) and vortex to make a clear transparent solution.
6. Add solution from step 5 into reaction cartridge, stir for 1 hour, then drain.
7. Wash resin with DMF (1 mL) four times (stir for ~1 min each).
8. Add 2 mL capping solution (1:9 $Ac_2O$:DMF v/v) into cartridge, stir for 10 min, then drain.
9. Wash resin with DMF (1 mL) four times (stir for ~1 min each).
10. Repeat step 3-9 (until the desired PNA chain length is attained).
11. Repeat step 3 and 4.
12. Mix 18.9 μmol (3 equiv.) Kanamycin acid and 31.5 μmol HCTU (5 equiv.) in 300 μL NMP in an eppendorf tube followed by addition of 11.0 μL DIPEA (63 μmol, 10 equiv.) and vortex to make a clear transparent solution.
13. Add solution from step 12 into reaction cartridge; Rinse the eppendorf tube with another 100 μL DMF and transfer it to the cartridge again, then stir for 24 hours, then drain.
14. Repeat step 12 and 13 and stir for another 24 h, then drain.
15. Repeat step 4.
16. Repeat step 4 with DCM.
17. Air dry the resin before cleavage.

Deprotection and cleavage of peptidic-Kanamycin conjugates from resin

1. Make cleavage solution (88% TFA, 5% phenol, 5% water and 2% TIPS).
2. Add 1.0 mL freshly prepared cleavage solution in cartridge, keep stirring for 2 hours (cover the reaction cartridge with parafilm to avoid evaporation).
3. Drain cleavage solution into a 20 mL glass vial.

4. Concentrate cleavage solution under nitrogen.
5. Add 10 mL cold diethyl ether to the vial and store at -20°C for precipitation of the desired product.
6. Wash three times with diethyl ether. Dissolve the compound in 1 mL water.
7. Freeze dry water solution overnight.

Store the final product at -10 °C protect from light and moisture.

**General experimental information**

Unless otherwise specified, chemicals were purchased from commercially available sources and used without further purification. NMR spectra were recorded on a Bruker Avance-500 spectrometer. MS (MALDI-TOF) spectra were recorded using a Bruker Daltonics/Miroflex MALDI-TOF spectrometer.

**Bacterial Screen**.

Initial screening of Kanamycin-peptide conjugates against exponential phase microbial cultures was performed at a 12.5 µM concentration of each compound performed in duplicate using 96-well polystyrene microplates along with the reference compound kanamycin. The same volume (10 µL) of sterile water with 1% DMSO was used for antibiotic addition was added to the broth (background control) and microbial culture (growth control). Plates were incubated in a humidified incubator at 37°C for 15-20 hours. The percent growth inhibition was calculated using the formula:

$$\%\ Growth\ Inhibition = 100 - 100 \times \frac{A_{compound} - A_{background}}{A_{\ control} - A_{background}}$$

MIC values for select kanamycin-peptide conjugate was determined for exponential phase bacterial cultures by the microdilution method in triplicate according to the Clinical Laboratory Standards protocol. Stock test compounds were prepared at ten times the final concentration in 10% dimethylsulfoxide (DMSO). For each dilution, a ten microliter aliquot of stock test compound was combined with 90 µl diluted bacterial suspension (~3 x $10^5$ cells per mL in cation-adjusted Mueller-Hinton broth). Final test compound concentrations from serial 2-fold dilutions ranged from 50 µM to 0.78 µM. Plates were incubated in a humidified incubator at 37°C for 15-20 h. After incubation with test compound growth was measured by absorbance at 595 nm using a TecanM100Pro plate reader. The percent growth inhibition at each concentration was averaged from triplicate assays calculated from the formula above.

**In-vitro screening**

Pre-miR21 was ordered from IDT (Coralville, IA) and had the following basen sequence; 5'UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGC UGUCUGACA-3'. The thermodynamically favored structure of pre-miR21 was created by Unafold software. [37]2, 2 All experiments were performed in 10 mM phosphate buffer supplemented with 25 mM KCl, 0.05 mM EDTA (pH 6.5). Pre-miR21 was annealed using heating block and solutions of pre-miR21 in 2X buffer were cooled from 75 °C to 25 °C at 1 °C/min rate.

**Stoichiometry of F-NEO binding to pre-miRNA21**

F-neo (100 nM) was titrated with increasing concentrations of pre-miR21 using 96-well plates in triplicate. Plates were scanned using 485 nm excitation wavelength and 525 nm emission wavelength. The emission signal was collected on TECAN M1000Pro plate-reader.

**$IC_{50}$ values of pre-miRNA21:F-NEO competitively titrated with kanamycin**

The pre-miR21:F-neo complex was titrated with kanamycin to determine the $IC_{50}$ value. $IC_{50}$ is the concentration of a compound at which the intensity of F-neo emission is half of the maximum value. Pre-miR21 at 12.5 nM was mixed with 100 nM F-neo. Kanamycin was serially diluted to achieve a concentration ranging from 50 □M to 12 nM after mixing with pre-miR21. $IC_{50}$ values were measured in duplicate.

**Single point screening of PA (peptidic-aminosugar) conjugates**

Screening of pre-miR21 against PA-Kanamycin (PA-Kan) conjugates was assessed using competition fluorescence assay using a single concentration of added compound. PA-Kan conjugates or kanamycin were added at 100 nM concentration to F-neo:pre-miR21 complex. Each plate contained duplicates of F-neo:pre-miR21 complex without added compound and F-neo:pre-miR21 complex with added 100 nM kanamycin as a reference compound. Pre-miR21 was mixed with 100 nM F-neo at 12.5 nM. The chosen ratio of F-neo:[pre-miR21] is based on binding stoichiometry. F-neo emission intensity was measured at λex = 485 nm / λem = 525 nm. For each PA kanamycin conjugate the percent binding relative to kanamycin was calculated using formula: (I-Ic)*100/(Ineo-Ic), where I is the emission intensity of the complex with added PA kanamycin conjugate, Ic is the emission intensity of the complex only, INEO is the intensity of the complex with added kanamycin.

**Cell culture, Treatments and Transfection**

All experiments were performed with the MCF7 breast cancer cell line which was purchased from European Collection of Cell Cultures (EACC). The cell line was maintained in DMEM

(Dulbecco's Modified Eagle's Medium) with 10% Foetal Bovine Serum (FBS) without antibiotic or anti-mycotic and was incubated in 5% $CO_2$ in a humidified incubator at 37 °C. For experiments involving qRT-PCR and Western blot the general protocol followed is described below.

1. The cells were seeded in 12-well plates at a density of $4x10^4$ cells per well and were incubated for 24 hours.
2. After 24 hours, the cells were treated with a library of peptidic-kanamycin (PA-Kan) conjugates and controls. Each treated set of PA-Kan conjugates had kanamycin sulphate, kanamycin TFA, streptomycin, antisense-microRNA21 (LNA) and untreated cells as controls. All the molecular controls were treated at similar concentrations of 5 µM and antisense-microRNA (LNA) was transfected at 50 nM concentration using Lipofectamine®2000.
3. The transfected cells were maintained in Opti-MEM™ (Modified Eagle's Media) which is a reduced serum media, for 4 hours after which the media was changed to complete DMEM (DMEM with FBS Supplement).
4. The plates were then incubated for 48 hrs after which the cells were harvested for their respective experimental procedures.

**RNA Isolation and cDNA preparation**

RNA was isolated from MCF-7 breast cancer cells treated with PA-Kan conjugate and positive and negative controls after 48 hours. Total RNA was isolated using TRIZOL® reagent (Ambion®) in the step wise protocol provided with it.

cDNA was prepared using the following method:

1. To avoid DNA contamination the extracted RNA was given DNase treatment using TURBO™ DNase (Ambion®) at a reaction of 37 °C for 30 minutes followed by inactivation of enzyme at 65 °C for 15 minutes.
2. Post DNase treatment, 400-500 ng of RNA was used to prepare cDNA. The RNA was polyadenylated by ATP using Poly A Polymerase and incubated at 37 °C for 30 minutes.
3. Reverse transcription was performed with oligo (DT) adaptor primer using Revert-aid reverse transcriptase enzyme (Thermo Fisher). Oligo (DT) adaptor was first added to reaction mixture and incubated at 60 °C for 5 minutes for annealing.
4. This step was followed by adding the reverse transcriptase reaction mixture and then incubating at 42 °C for one hour and then at 95 °C for 10 minutes.

**Real Time Quantitative Polymerase Chain Reaction (RT-qPCR)**

After the cDNA was prepared, real time qPCR was performed for all the treated samples and controls in triplicates. The reaction volume for each was 10 µL. The transcript level of mature

miR21 was determined using primers specific for miR21 having the sequence 5' TAGCTTATCAGACTGATGTTGA 3' and also an internal control transcript was evaluated for small nuclear RNA U6 using the sequence- 5' CGCAAGGATGACACGCAAATTC 3'. Transcripts were quantified using SYBR Green Master Mix: SYBR *Premix Ex Taq II* (Tli RNase H Plus) (from TaKaRa) in the instrument Light Cycler 480 (Roche). All the Ct values obtained for microRNA21 transcript levels were normalized to that of the small nuclear U6 transcript levels. The fold change analysis in the transcript levels of microRNA-21 for comparative analysis amongst all the treated samples and controls was done using the 2-ΔΔCt method.

**Western Blot**

**Western Blot**

To check the levels of PTEN and PDCD4 post treatment with the molecules and the controls, western blot was performed as explained in the following steps:

1. Protein lysates were prepared from the treated samples using Cell Lytic™ (Sigma) buffer post 48 hours of treatment. In each 12-well plate 50 µl of the lysis buffer was added along with 5 µl of Proteinase Inhibitor Cocktail (Sigma). The lysis reaction was incubated for 1 hour in a rocker at 4 °C.
2. After incubation, the lysates for each sample was collected and protein concentration was estimated using Pierce™ BCA Protein Assay Kit (ThermoFisher). From each sample 40µg of protein was loaded into the wells of SDS-PAGE.
3. After the gel was run, transfer of the proteins from the gel to PVDF membrane (GE Healthcare Life-Science) was carried out in Bio-Rad vertical gel Transfer Apparatus at 4 °C for 3 hours at 75 volts.
4. After the transfer procedure, the membrane was cut according to the respective protein size required and kept for blocking (with 5% BSA in TBST) at room temperature on a rocker for 5-6 hours.
5. Post blocking, the blots were incubated overnight with primary antibody, PDCD4 (from Abcam) at 1:1000 dilution, PTEN (from Abcam) at 1:1000 dilution and GAPDH (from Abcam) at 1:2000 dilutions on a rocker at 4 °C.
6. After incubation with the primary antibody blots were washed thrice for 15 minutes using 1X TBST and then incubated with secondary antibody with HRP conjugate for three hours.
7. Post incubation a similar washing step (as mentioned after primary antibody) was performed.
8. The blots were then developed using EMD Millipore™ Immobilon Western Chemiluminiscent HRP Substrate (ECL) in Syngene Gel doc instrument.

**Modeling of hsa-mir- 21**

We used our previously published structure of hsa-pre-mir-21 (PMID: 25824952). Briefly, the pre-miRNA was modeled using mc-fold | mc-sym pipeline. The structure was then minimized with all restraints removed, steepest descent minimization of 1000 step, followed by a conjugate gradient minimization of 1500 steps. The long-range cutoff for non-bonded interactions during the minimization was 8 Å.

**Small molecule preparation and docking**

The compounds were drawn using ChemDraw 8 software. The two-dimensional molecules were then converted to three-dimensional structures using OpenBabel (3). The energy minimization was performed using Maestro 9.8. Docking was performed using AutoDock 4.2.6. and MGLTools of The Scripps Research Institute. Hydrogen atoms and Kollman and Gasteiger partial charges were assigned to the ligands (compounds) with all torsions allowed during the docking. A grid box was built around the entire miRNA to allow the ligands to move freely and affinity maps of the miRNA (200 X 200 X 200 with random number generator seeded) were calculated using AutoGrid. Two hundred Lamarckian Genetic Algorithm (LGA) runs with 250000000 number of energy evaluations were performed. The docking results were ranked according to the lowest docked energy for the ligands. Molecular graphics and analyses were performed with the UCSF Chimera package.

**All atomistic molecular dynamics simulation**

To check for conformational stability, molecular dynamic simulation was done using GROMACS 4.6.1. All atomistic simulations were carried out using the CHARMM36 all-atom force field (November release) using periodic boundary condition. The starting docked models were solvated in a periodic box with TIP3 water model. Na+ ions were added to the solvent to neutralize electrical net charge of the system. Each system was then minimized for 50000 steps using a steepest decent algorithm. The NPT ensemble was used for production simulation. Systems were simulated at 310K, maintained separately for miRNA, docked molecule, water by a Berendsen thermostat with a time constant of 1 ps. Pressure coupling was done employing a Berendsen barostat using a 1 bar reference pressure and a time constant of 2 ps. Electrostatic interactions were calculated using the Particle Mesh Ewald (PME) summation. The CHARMM topology and parameters for the small molecules were generated using SwissParam (PMID: 21541964).

**Binding affinity studies**

The binding affinity for the pre-miR21 with PA-Kan conjugates was performed using technology from ForteBio called Octet® RED96 System. It is a high throughput machine/software where several analytes can be tested at once for binding affinity with a specific compound using its 8-channel plate system over a range of concentrations within a minimum amount of time. Analysis of data is also done in the Octet® software platform that provides reliable kinetic parameters. Biotinylated pre-miR21 was ordered from Integrated DNA Technologies and was

immobilized on the welled plates with streptavidin coating. The reaction volume for each well containing the adhered pre-miR21 and the analyte solution was 200 µl. The pre-miR21 was immobilized at a concentration of 25mg/ml. The molecules were tested over a concentration range of 8nM to 128nM. The buffer system used comprised of 10 mM HEPES, 50 mM NaCl and 0.05% Tween20 that was degassed and filtered before use. The regeneration buffer system used was 50mM NaOH, 1M NaCl. The association reaction time occurred over 5 minutes followed by dissociation period for 10 minutes.

**Proliferation Check Assay by Immunoflurescene using Ki67 marker**

Step wise detailed protocol for the experiment is as follows:

1. MCF7 breast cancer cells for slide preparation were seeded at a density of $8x10^4$ in a 6-well plate that contained Corning 22 mm sq. square cover slips in each well to which cells adhered.

2. After 24 hours incubation at 37 °C with 5% $CO_2$ in a humidified environment, cells were treated with PA-Kan conjugates that were identified as positive hits from the qPCR and Western blot experiments at a concentration of 5µM. Cells seeded to kanamycin-treated and untreated wells served as controls for the experiment.

3. After 48 hours, the cells were washed with 1x PBS after removing DMEM.

4. Cells on cover slips from culture wells were then fixed using fixation buffer (3% paraformaldehyde, 5 µM EGTA pH 8, 1 µM $MgCl_2$) for 7 minutes.

5. Post fixation, cells were washed twice with washing buffer (30 µM Glycine in PBS, 5 µM EGTA and 10 µM $MgCl_2$) and then permeabilized using permeabilization buffer (0.2% Triton X-100 in PBS, 5 µM EGTA, 10 µM $MgCl_2$). Cells were incubated with the permeabilization buffer for 7 minutes.

6. After permeabilization the same washing step was performed. The wells containing the cover slip adhered cells were treated with blocking buffer (0.5% BSA in PBS, 5 µM EGTA, 10 µM $MgCl_2$) for 30 minutes.

7. After blocking was performed the cells were incubated overnight with primary antibody Ki67 (Abcam) at a dilution of 1:1000.

8. Post incubation with primary antibody, cells were washed thrice for 5 minutes each using blocking buffer followed by incubation with secondary antibody Alexa Fluor 488 (Invitrogen) at 1:2000 dilution for 2 h.

9. After incubation with secondary antibody, cells were washed thrice for 5 minutes each using blocking buffer.

10. In each well 600 µl of 1X PBS was added. The cover slips were then each mounted on glass slides (Corning) with a drop of Prolong® Gold Antifade Mountant with DAPI (ThermoFisher) and then viewed and analysed with Nikon Eclipse Ti-ϵ Inverted Microscope system (Epi-TIRF module) under 100x objective focus.

**Cell Viability Assay**

For cell viability tests the MTT assay was performed in the following steps as explained.

1. Cells were seeded at a density of 8000 cells per well on a 96 well plate.
2. 24 hours post seeding, cells were treated with PA-kan conjugates and controls tested in triplicate over a concentration range of 0 µM, 2.5 µM, 5µM, 7.5 µM and 10 µM.
3. Cells were then incubated for 24 hours after which 20µl of media from each well was removed to add 20 µl of MTT (dimethyl thiazole diphenyl tetrazolium bromide) (Sigma-Aldrich) into each well with a concentration of 0.5 mg/ml. Cells were incubated with MTT for 4 hours in the 37 °C incubator.
4. After four hours the entire media and MTT was removed and the formation of violet crystals was visible at the bottom of the wells.
5. The violet crystals were then dissolved using 150 µl of DMSO (SIGMA).
6. Absorbance was recorded using TECAN plate reader at 570nM. Percentage of viable cells was then calculated according to the manufacturers recommendation.

**Epithelial to Messenchymal (EMT) Assays by Immunoflurescene**

Step wise detailed protocol for the experiment is as follows:

1. MCF7 breast cancer cells for slide preparation were seeded at a density of $8x10^4$ in a 6-well plate that contained Corning 22 mm sq. square cover slips in each well to which cells adhered.

2. After 24 hours incubation at 37 °C with 5% $CO_2$ in a humidified environment, cells were treated with PA-Kan conjugates at 5 µM concentration that served as the best hits from all the experiments performed. Untreated cells and cells treated with Kan-TFA served as the controls.

3. After 48 hours, the cells were washed with 1x PBS after removing DMEM.

4. Cells on cover slips from culture wells were then fixed using fixation buffer (3% paraformaldehyde, 5 µM EGTA pH 8, 1 µM $MgCl_2$) fand incubated for 7 minutes.

5. Post fixation, cells were washed twice with washing buffer (30 µM Glycine in PBS, 5 µM EGTA and 10 µM $MgCl_2$) and then permeabilized using permeabilization buffer (0.2% Triton X-100 in PBS, 5 µM EGTA, 10 µM $MgCl_2$). Cells were incubated with the permeabilization buffer for 7 minutes.

6. After permeabilization the same washing step was performed as before. The wells containing the cover slip adhered cells were treated with blocking buffer (0.5% BSA in PBS, 5 µM EGTA, 10 µM $MgCl_2$) for 30 minutes.

7. After blocking was performed the cells were incubated overnight with primary antibody. The markers used for EMT as primary antibodies were E-cadherin and Vimentin (from Cell Signalling Technology) which were used at a ratio of 1:500.

8. Post incubation with primary antibody, cells were washed thrice for 5 minutes each using blocking buffer followed by incubation with secondary antibody Alexa Fluor 488 (Invitrogen) at 1:1000 dilution for 2 h.

9. After incubation with secondary antibody, cells were washed thrice for 5 minutes each using blocking buffer.

10. In each well 600 µl of 1X PBS was added. The cover slips were then each mounted on glass slides (Corning) with a drop of Prolong® Gold Antifade Mountant with DAPI (ThermoFisher) and then viewed and analysed with Nikon Eclipse Ti-ϵ Inverted Microscope system (Epi-TIRF module) under 100x objective focus.

**Wound Healing Assay to detect Migration of cells**

For understanding the migratory potential of cells after treatment with the molecules, wound healing assay was performed as described in the following steps:

1. MCF-7 cells were densely seeded in a 24 well plate.
2. Upon confluency, using a 200ul pipette tip, a vertical wound was administered in all the wells. Caution must be taken for the uniformity of the wounds across all the wells.
3. Cells were then stringently washed with 1x PBS to clear off the non-adhered cells.
4. After washing, cells were then treated with the PA-Kan conjugate DPA 2089 and control Kanamycin-TFA at 5µM concentration and one well was left untreated.
5. Images were captured for the wound at Day 0 using Evos FL Imaging system at 10x magnification.
6. The plate was then kept at 37C humidified incubator with 5% CO2 for 48 hours.
7. Images were again captured 48 hours post treatment. Imaging was done using Evos FL Imaging system at 10x magnification.

**Characterization of peptidic-kanamycin conjugate library**

**DPA 1930 (KanaRR)** $^{1}$H NMR (500 MHz, $D_2O$) δ 5.58 (1H), 4.99 (1H), 4.18 (2H), 3.99 – 3.20 (17H), 3.10 (4H), 2.45 (5H), 1.90 (1H), 1.71 (4H), 1.54 (4H); $^{13}$C NMR (125 MHz, $D_2O$) δ 163.4, 163.1, 162.8, 162.6, 156.7, 100.8, 97.4, 83.6, 78.5, 73.3, 72.0, 71.0, 70.7, 68.8, 66.6, 53.5, 53.4, 53.0, 52.9, 52.3, 40.4, 40.3, 38.7, 29.8, 28.9, 28.1, 28.0, 27.9, 24.4, 24.3, 24.0; HRMS (ESI) *m/z* cacld for $C_{34}H_{65}N_{14}O_{14}$ $[M - H]^+$ 893.4805, found 893.4796; MS (MALDI-TOF) *m/z* calcd for $C_{34}H_{68}N_{14}O_{14}$ $[M-H]^+$ 895.5, found 895.7.

**DPA 1967 (KanaYD)** $^{1}$H NMR (500 MHz, $D_2O$) δ 7.03 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 8.3 Hz, 2H), 5.47 (s, 1H), 4.94 (s, 1H), 4.56 – 4.47 (m, 1H), 4.37 (m, 1H), 3.93 – 3.73 (m, 5H), 3.65 (m, 2H), 3.56 (m, 1H), 3.53 – 3.39 (m, 4H), 3.32 (m, 4H), 3.11 (m, 1H), 2.91 – 2.83 (m, 2H), 2.73 – 2.67 (m, 1H), 2.61 (m, 1H), 2.51 – 2.33 (m, 5H), 1.87 – 1.72 (m, 1H); $^{13}$C NMR (125 MHz, $D_2O$) δ 175.0, 174.6, 173.3, 163.1, 162.8, 154.5, 130.8, 130.6, 130.5, 128.0, 127.9, 100.7, 97.0, 83.6, 78.4, 73.0, 71.9, 70.9, 70.8, 70.7, 68.7, 68.1, 66.6, 55.5, 54.7, 49.8, 49.6, 47.9, 40.2, 38.6, 35.9, 35.7, 30.5, 30.3, 27.5; HRMS (ESI) *m/z* cacld for $C_{35}H_{55}N_8O_{17}$ $[M - H]^+$ 859.3685, found 859.3677; MS (MALDI-TOF) *m/z* calcd for $C_{35}H_{57}N_8O_{17}$ $[M+2H]^+$ 861.4, found 861.7.

**DPA 2042 (KanaRT)** $^{1}$H NMR (500 MHz, $D_2O$) δ 5.53 (s, 1H), 4.32 – 4.19 (m, 2H), 4.15 m, 1H), 3.98 – 3.73 (m, 5H), 3.73 – 3.53 (m, 3H), 3.53 – 3.22 (m, 8H), 3.10 (m, 3H), 2.45 (m, 5H), 1.81 (m, 2H), 1.67 (s, 1H), 1.55 (s, 2H), 1.09 (m, 3H); $^{13}$C NMR (125 MHz, $D_2O$) δ 163.4, 163.1, 162.8, 162.6, 156.7, 100.8, 97.2, 83.6, 78.4, 73.2, 70.7, 68.7, 68.1, 67.0, 66.9, 66.5, 58.6, 53.7, 53.5, 52.3, 49.9, 47.9, 40.4, 40.3, 38.7, 30.4, 29.8, 28.9, 27.9, 24.3, 18.7; HRMS (ESI) *m/z* cacld for $C_{32}H_{60}N_{11}O_{15}$ $[M - H]^+$ 838.4270, found 838.4258; MS (MALDI-TOF) *m/z* calcd for $C_{32}H_{62}N_{11}O_{15}$ $[M]^+$ 840.4, found 840.9.

**DPA 2084 (KanaRC)** $^{1}$H NMR (500 MHz, $D_2O$) δ 5.52 (d, J = 3.6 Hz, 1H), 4.97 (s, 1H), 4.12 (m, 1H), 3.93 – 3.72 (m, 5H), 3.66 (m, 2H), 3.55 (m, 1H), 3.53 – 3.40 (m, 4H), 3.39 (m, 1H), 3.31 (m, 4H),

3.10 (m, 4H), 2.49 (m, 4H), 2.42 (m, 1H), 1.91 – 1.80 (m, 1H), 1.74 (m, 1H), 1.69 – 1.61 (m, 1H), 1.55 (m, 2H); $^{13}$C NMR (125 MHz, $D_2O$) δ 163.4, 163.1, 162.8, 162.6, 156.7, 100.8, 97.3, 83.6, 78.5, 73.2, 71.9, 70.9, 70.8, 70.7, 68.7, 68.1, 66.6, 55.4, 54.7, 53.7, 49.9, 48.0, 40.4, 40.2, 38.7, 30.3, 30.2, 27.9, 25.1, 24.3, 23.9; HRMS(ESI) *m/z* cacld for $C_{31}H_{58}N_{11}O_{14}S$ $[M - H]^+$ 840.3885, found 840.3870; MS (MALDI-TOF) *m/z* calcd for $C_{31}H_{60}N_{11}O_{14}S$ $[M]^+$ 842.4, found 842.6.

**DPA 2089 (KanaFC)** $^1$H NMR (500 MHz, $D_2O$) δ 7.31 – 7.13 (m, 5H), 5.47 (s, 1H), 4.94 (s, 1H), 4.47 (m, 1H), 4.36 – 4.26 (m, 1H), 3.81 (m, 5H), 3.62 (m, 3H), 3.53 – 3.22 (m, 8H), 3.10 (m, 1H), 3.00 (m, 2H), 2.82 – 2.67 (m, 2H), 2.51 – 2.32 (m, 5H), 1.86 – 1.71 (m, 1H); $^{13}$C NMR (125 MHz, $D_2O$) δ 163.5, 163.1, 162.8, 162.5, 129.4, 129.2, 128.8, 127.3, 100.8, 97.1, 83.7, 78.6, 73.1, 72.0, 70.9, 70.6, 68.7, 68.1, 66.6, 55.3, 54.7, 50.0, 48.0, 40.2, 38.7, 36.9, 30.3, 27.5, 25.1, 24.0, 22.6; HRMS(ESI) *m/z* cacld for $C_{34}H_{55}N_8O_{14}S$ $[M - H]^+$ 831.3558, found 840.3531; MS (MALDI-TOF) *m/z* calcd for $C_{34}H_{57}N_8O_{14}S$ $[M+H]^+$ 833.4, found 833.8.

**DPA 2093 (KanaYC)** $^1$H NMR (500 MHz, $D_2O$) δ 7.04 (d, J = 6.9 Hz, 2H), 6.73 (d, J = 6.4 Hz, 2H), 5.47 (s, 1H), 4.94 (s, 1H), 4.41 (m, 1H), 4.30 (s, 1H), 3.81 (m, 5H), 3.62 (m, 3H), 3.52 – 3.39 (m, 4H), 3.39 – 3.24 (m, 4H), 3.12 (m, 1H), 2.89 (s, 2H), 2.74 (s, 2H), 2.42 (m, 5H), 1.81 (m, 1H); $^{13}$C NMR (125 MHz, $D_2O$) δ 163.2, 163.0, 162.8, 162.2, 154.5, 130.5, 129.3, 128.4, 128.0, 100.8, 97.5, 83.7, 77.6, 72.0, 70.9, 70.8, 68.7, 68.1, 66.7, 55.6, 55.3, 54.6, 49.9, 48.0, 40.3, 39.3, 30.3, 30.2, 27.6; HRMS(ESI) *m/z* cacld for $C_{34}H_{55}N_8O_{15}S$ $[M - H]^+$ 847.3508, found 847.3510; MS (MALDI-TOF) *m/z* calcd for $C_{34}H_{57}N_8O_{15}S$ $[M+H]^+$ 849.4, found 849.9.

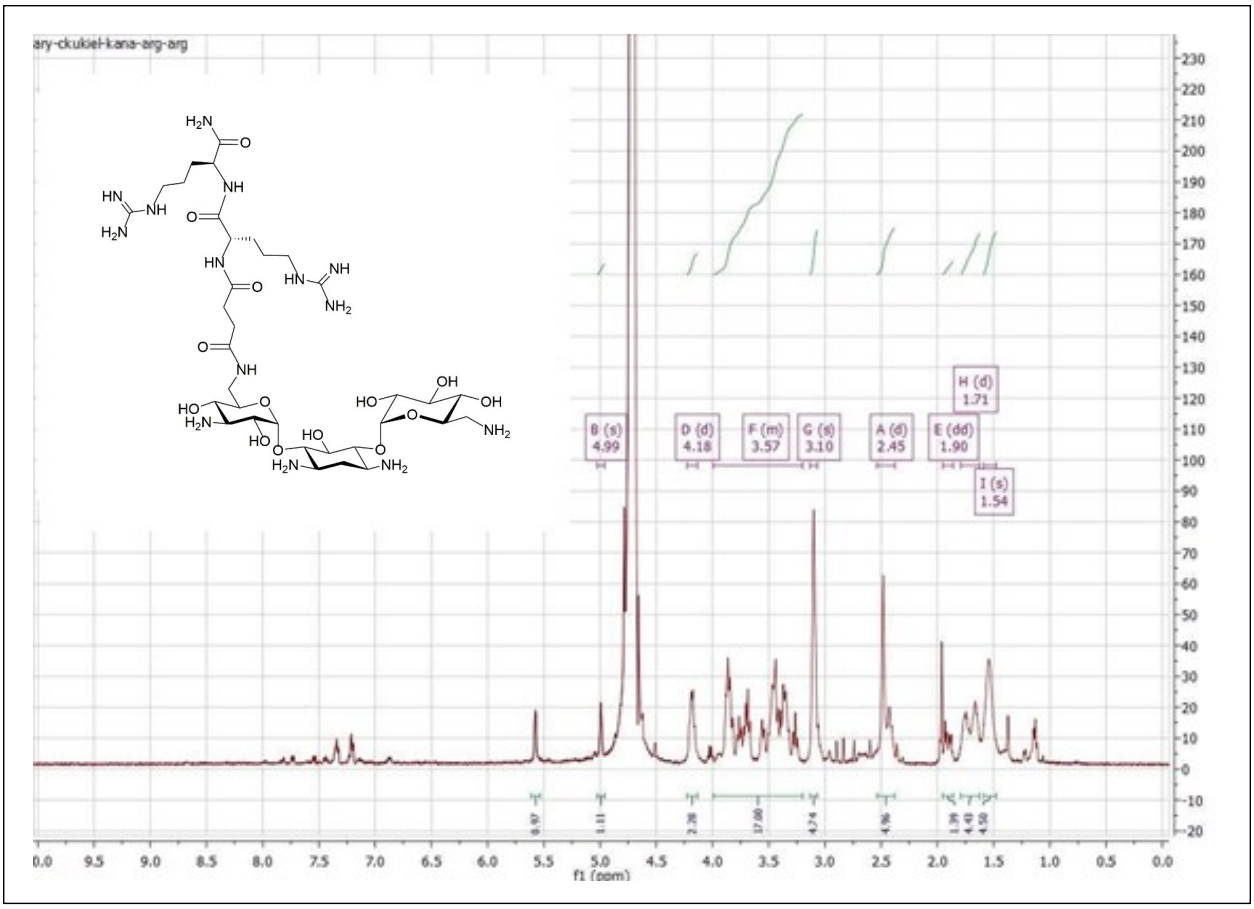


**Figure S1.** $^{1}H$ NMR spectrum of **DPA 1930 (KanaRR)** ($D_2O$, 500 MHz).

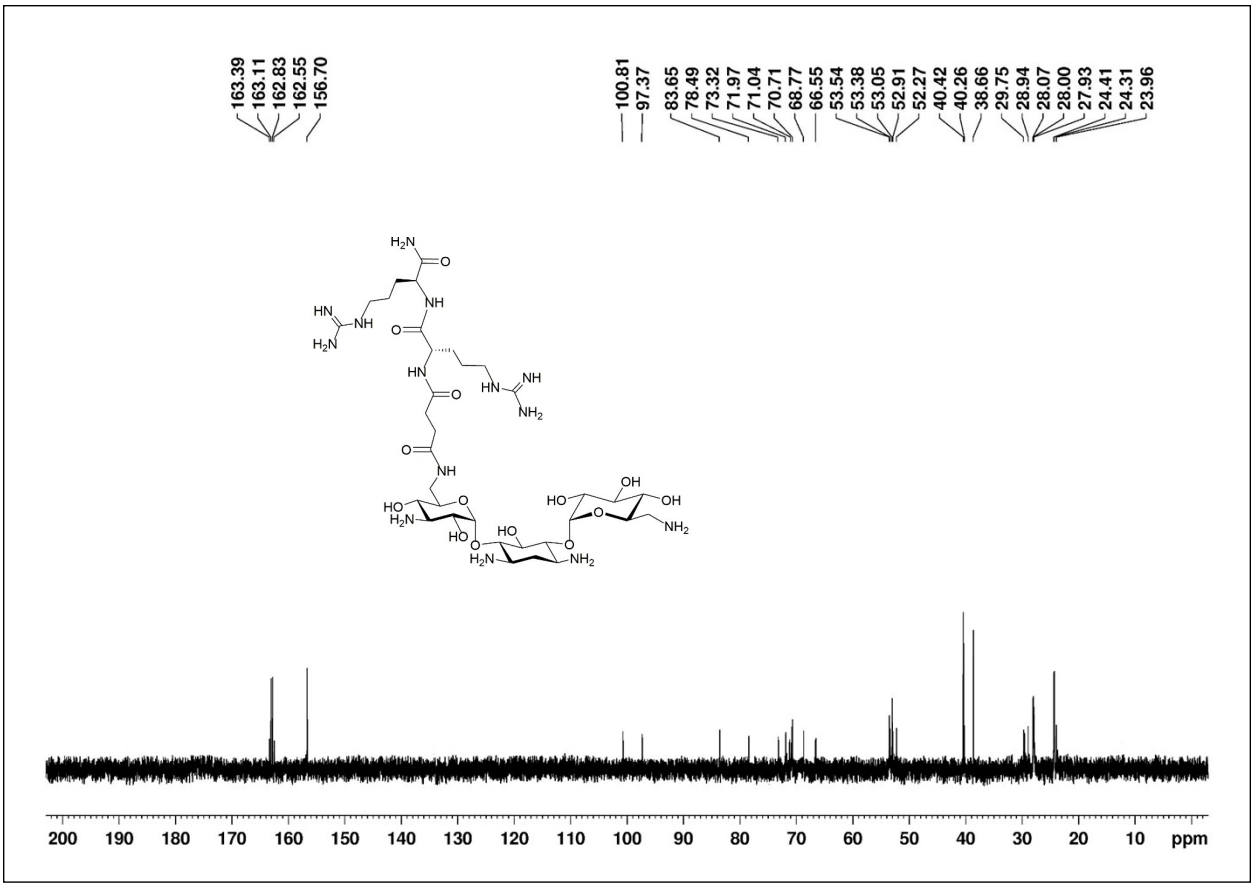


**Figure S2.** $^{13}C$ NMR spectrum of **DPA 1930 (KanaRR)** ($D_2O$, 125 MHz).

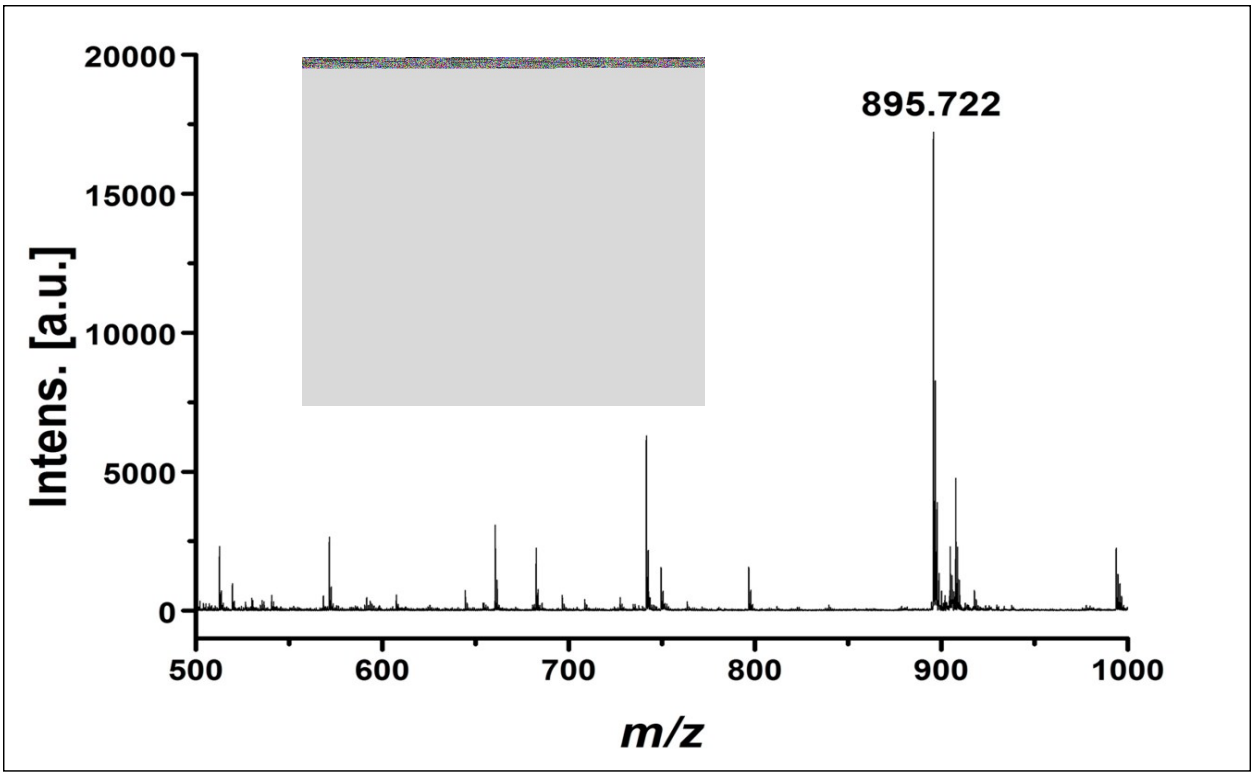


**Figure S3.** MALDI spectrum of **DPA 1930 (KanaRR)**.

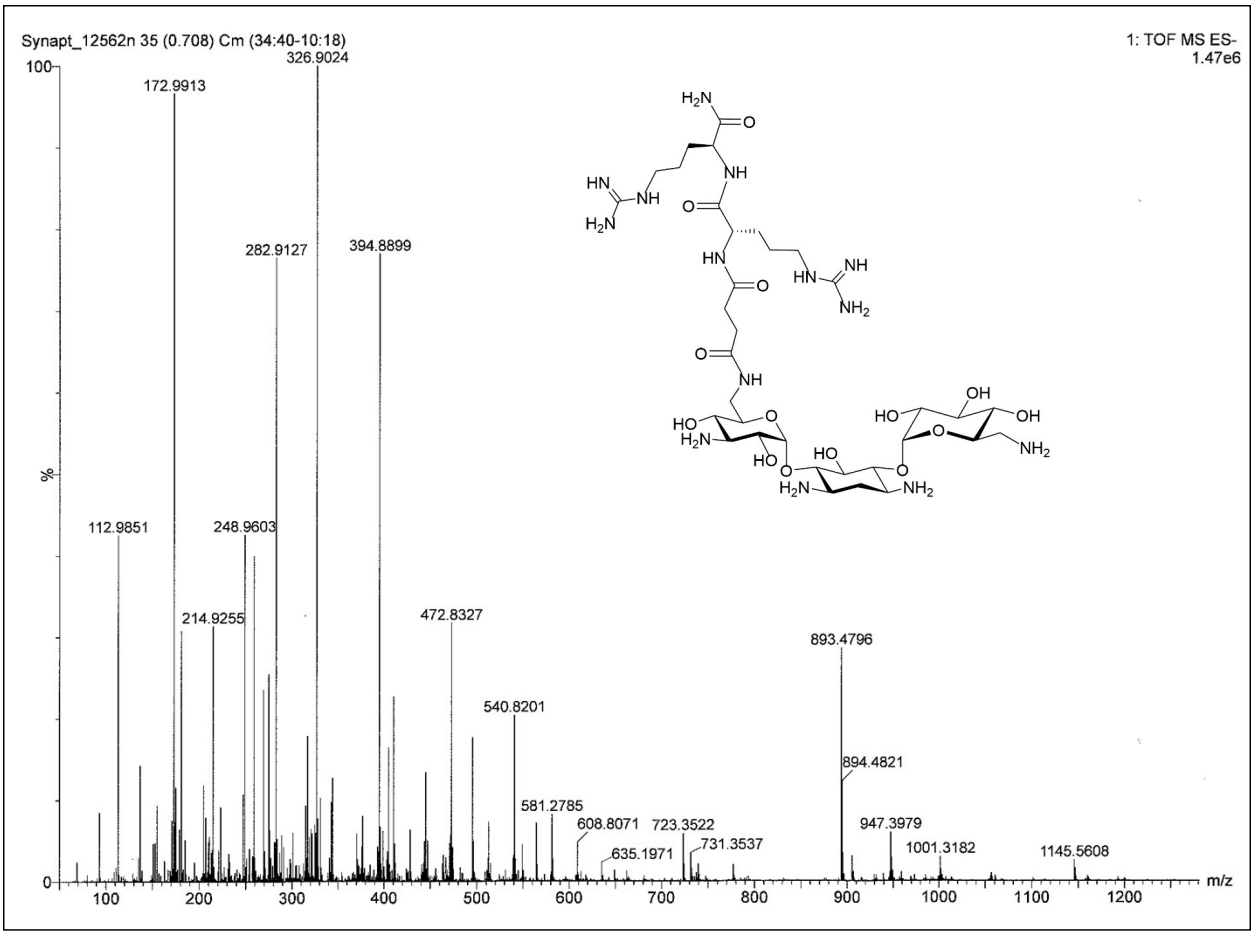


**Figure S4.** HRMS (ESI) spectrum of **DPA 1930 (KanaRR)**.

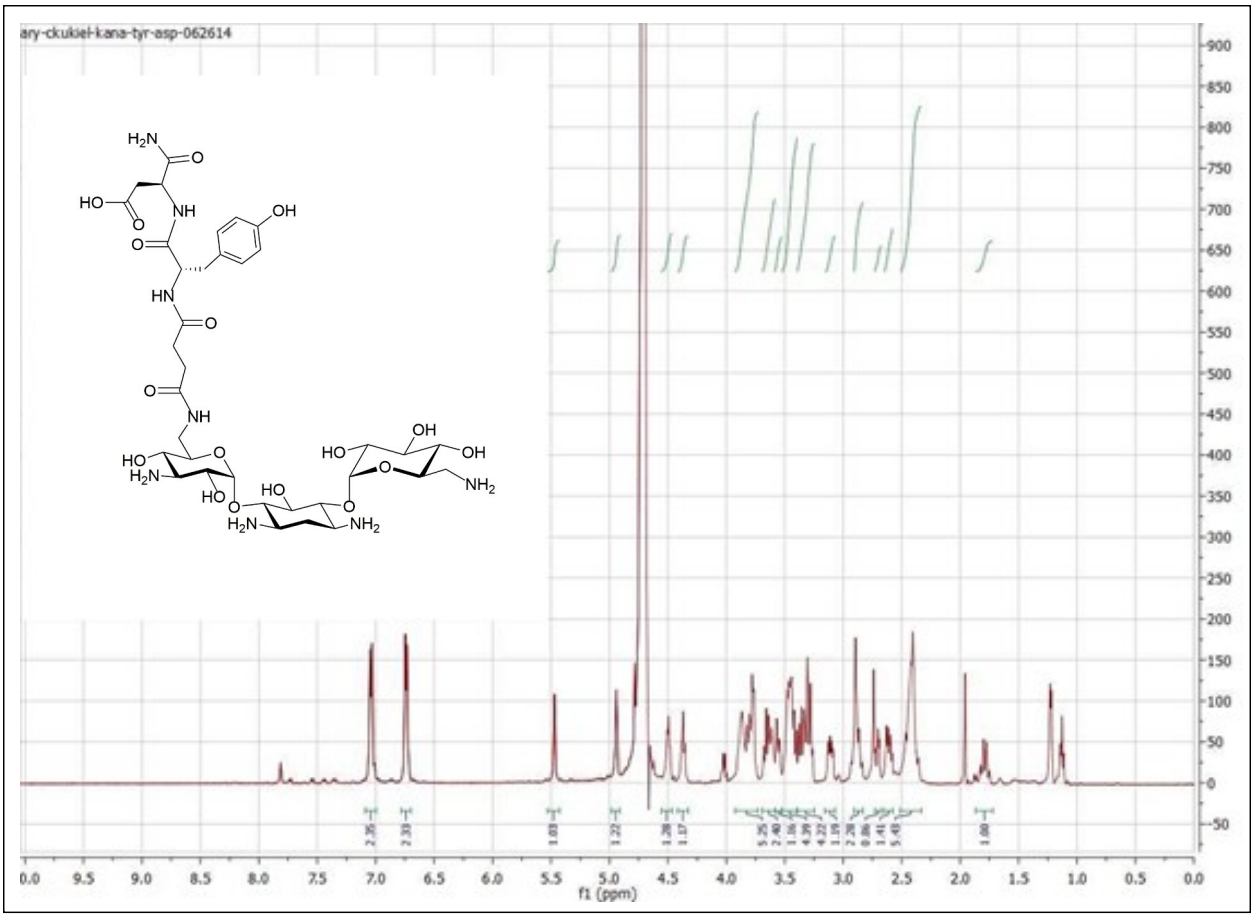


**Figure S5.** $^{1}H$ NMR spectrum of **DPA 1967 (KanaYD)** ($D_2O$, 500 MHz).

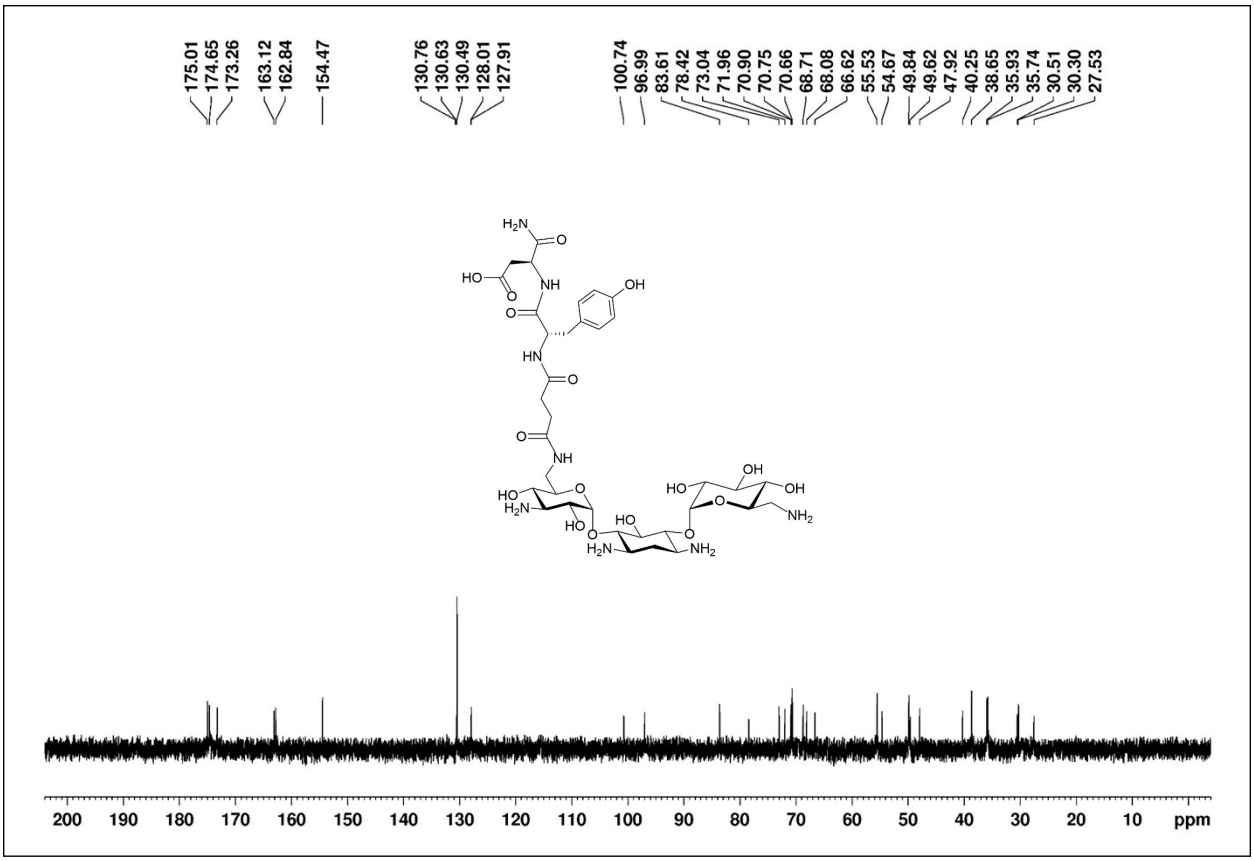


**Figure S6.** $^{13}C$ NMR spectrum of **DPA 1967 (KanaYD)** ($D_2O$, 125 MHz).

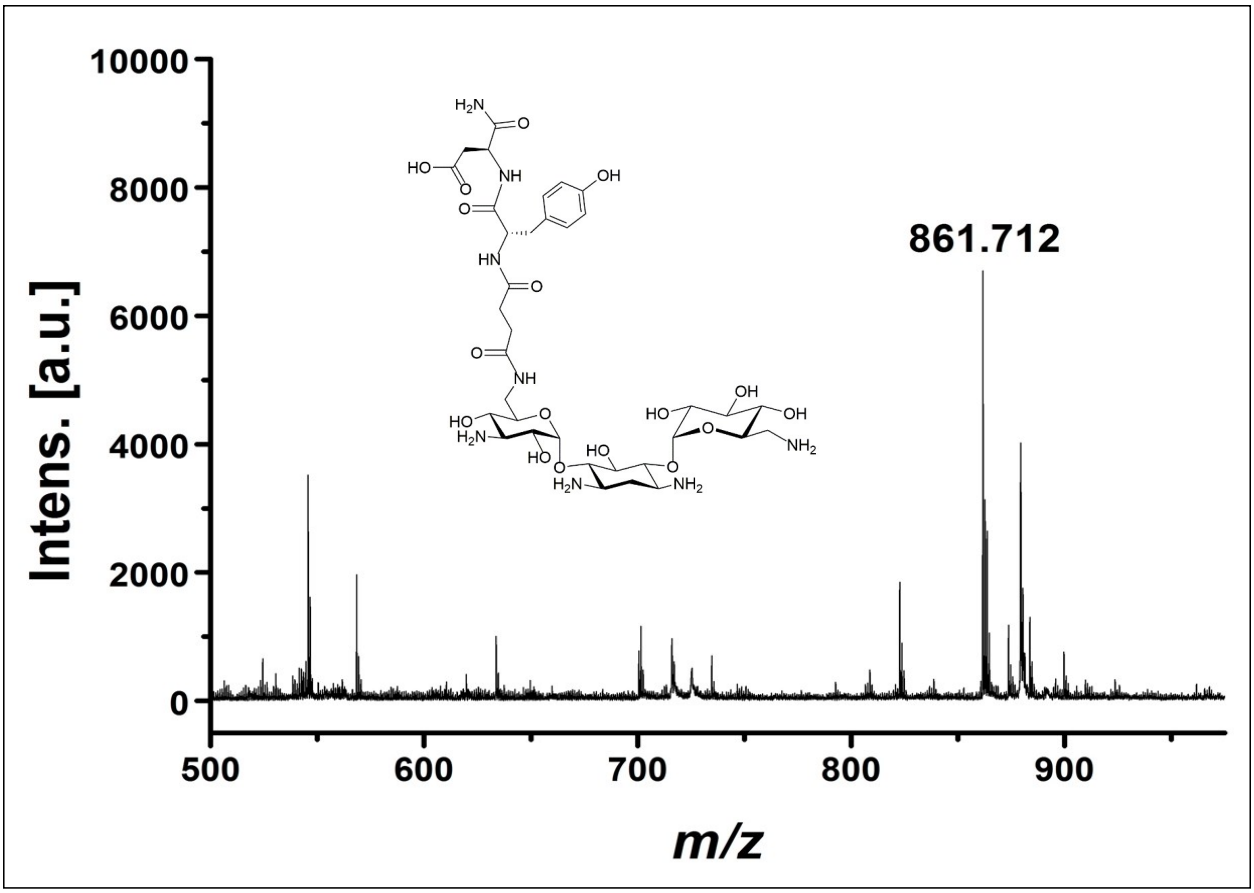


**Figure S7.** MALDI spectrum of **DPA 1967 (KanaYD).**

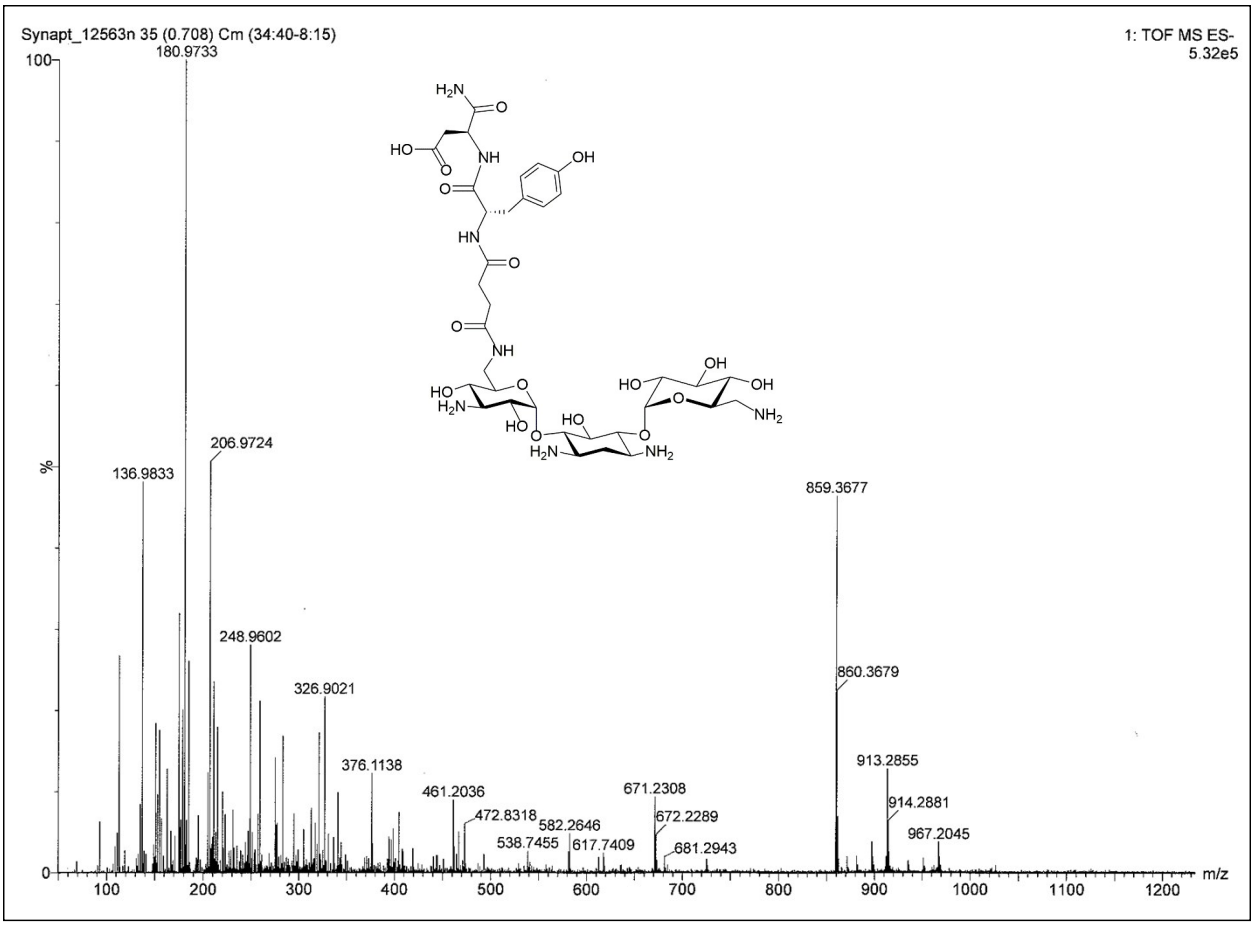


**Figure S8.** HRMS (ESI) spectrum of **DPA 1967 (KanaYD).**

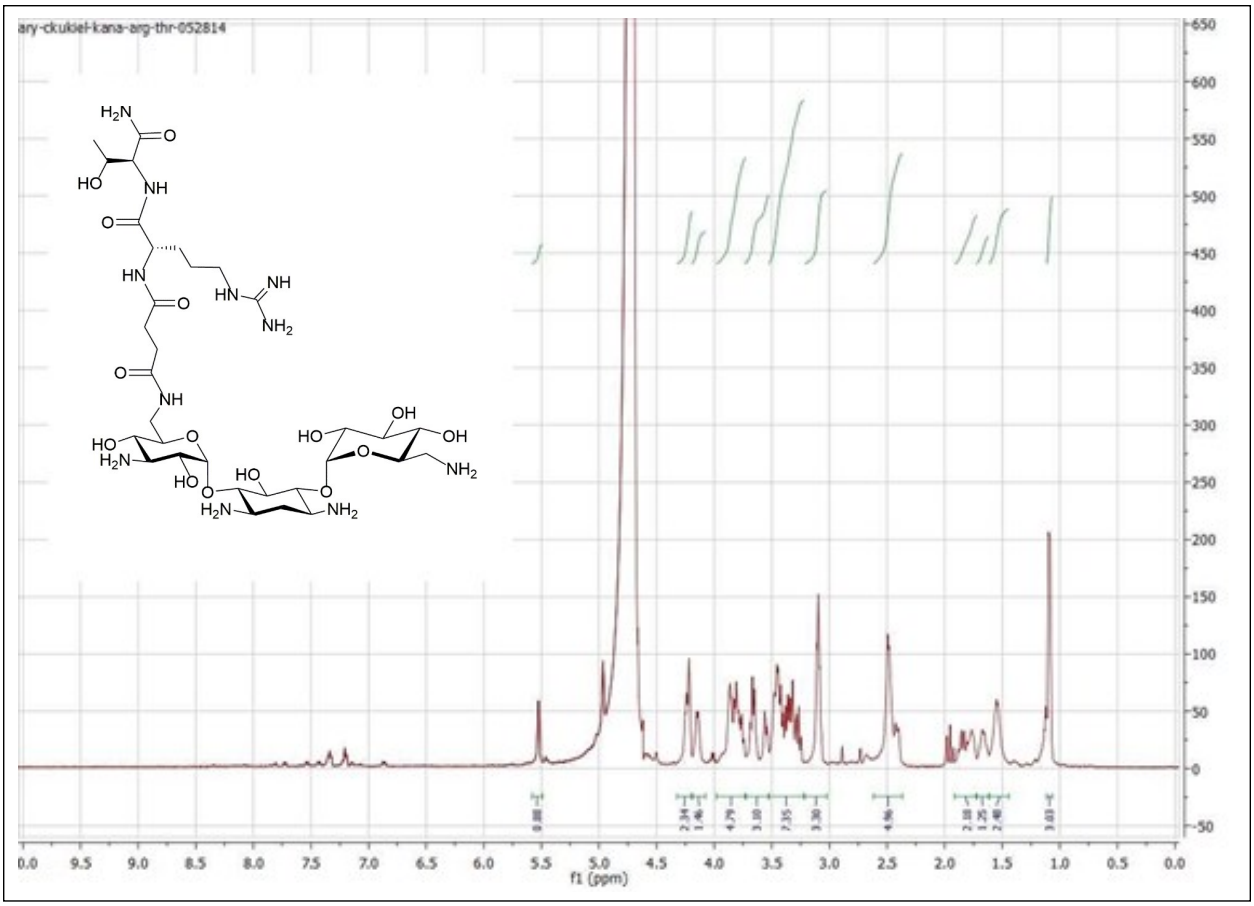


**Figure S9.** $^{1}H$ NMR spectrum of **DPA 2042 (KanaRT)** ($D_2O$, 500 MHz).

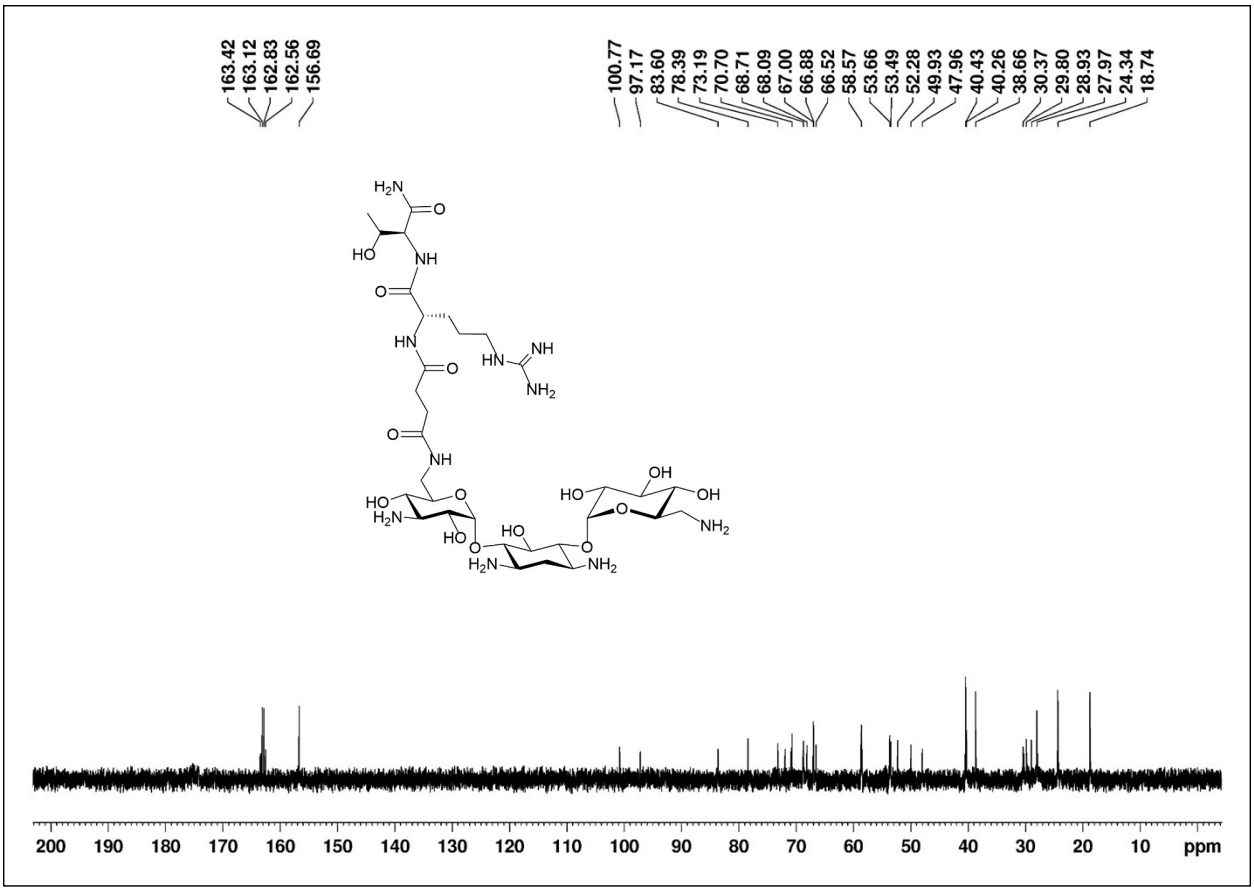


**Figure S10.** $^{13}C$ NMR spectrum of **DPA 2042 (KanaRT)** ($D_2O$, 125 MHz).

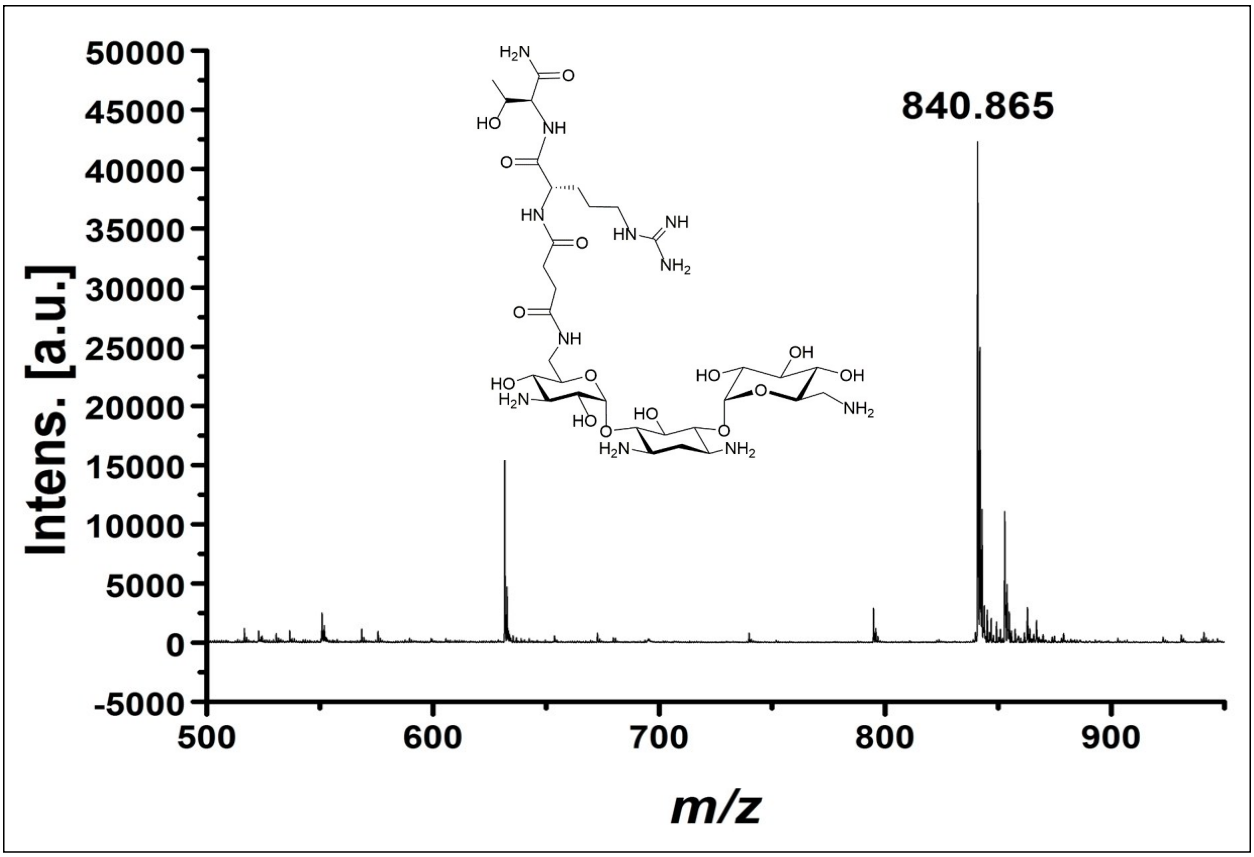


**Figure S11.** MALDI spectrum of **DPA 2042 (KanaRT).**

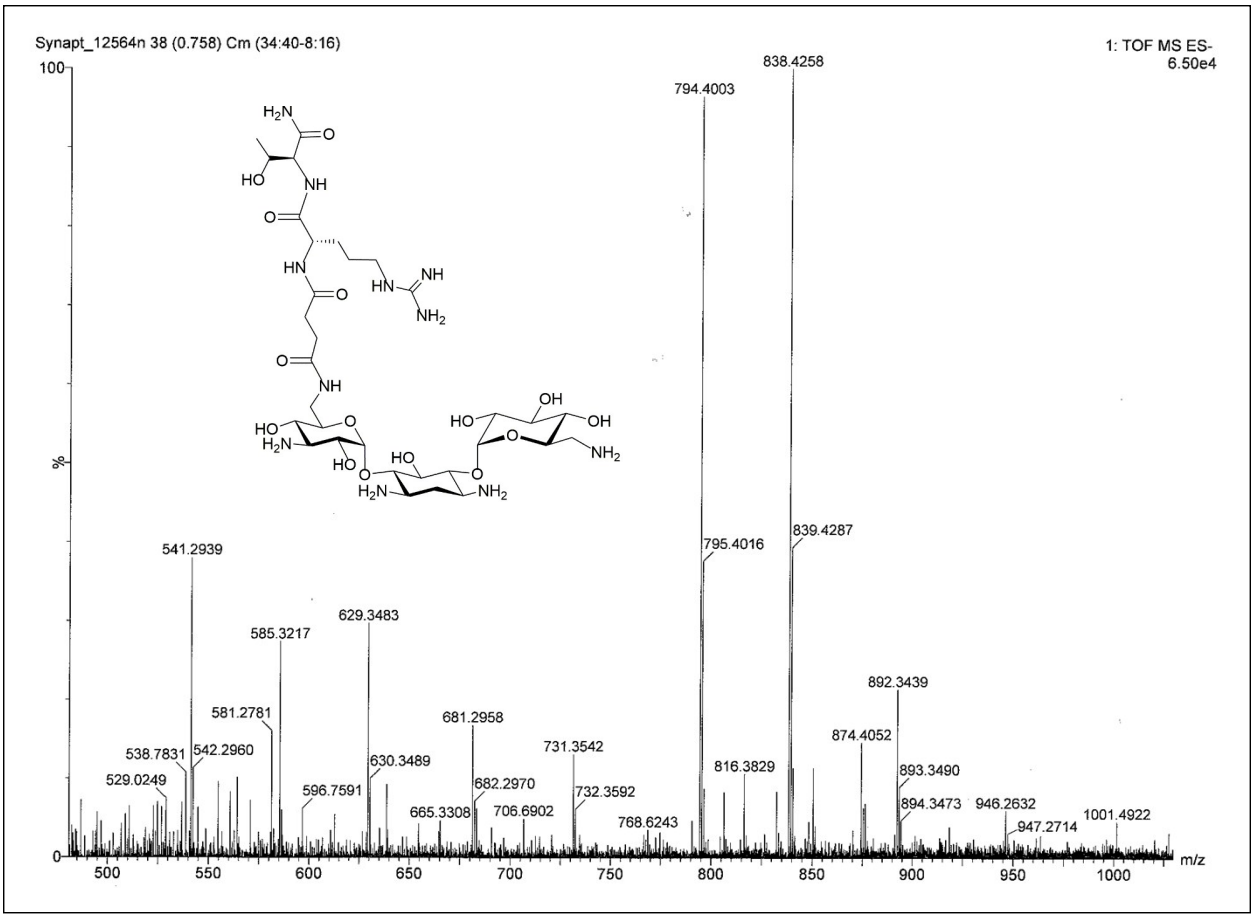


**Figure S12.** HRMS (ESI) spectrum of **DPA 2042 (KanaRT).**

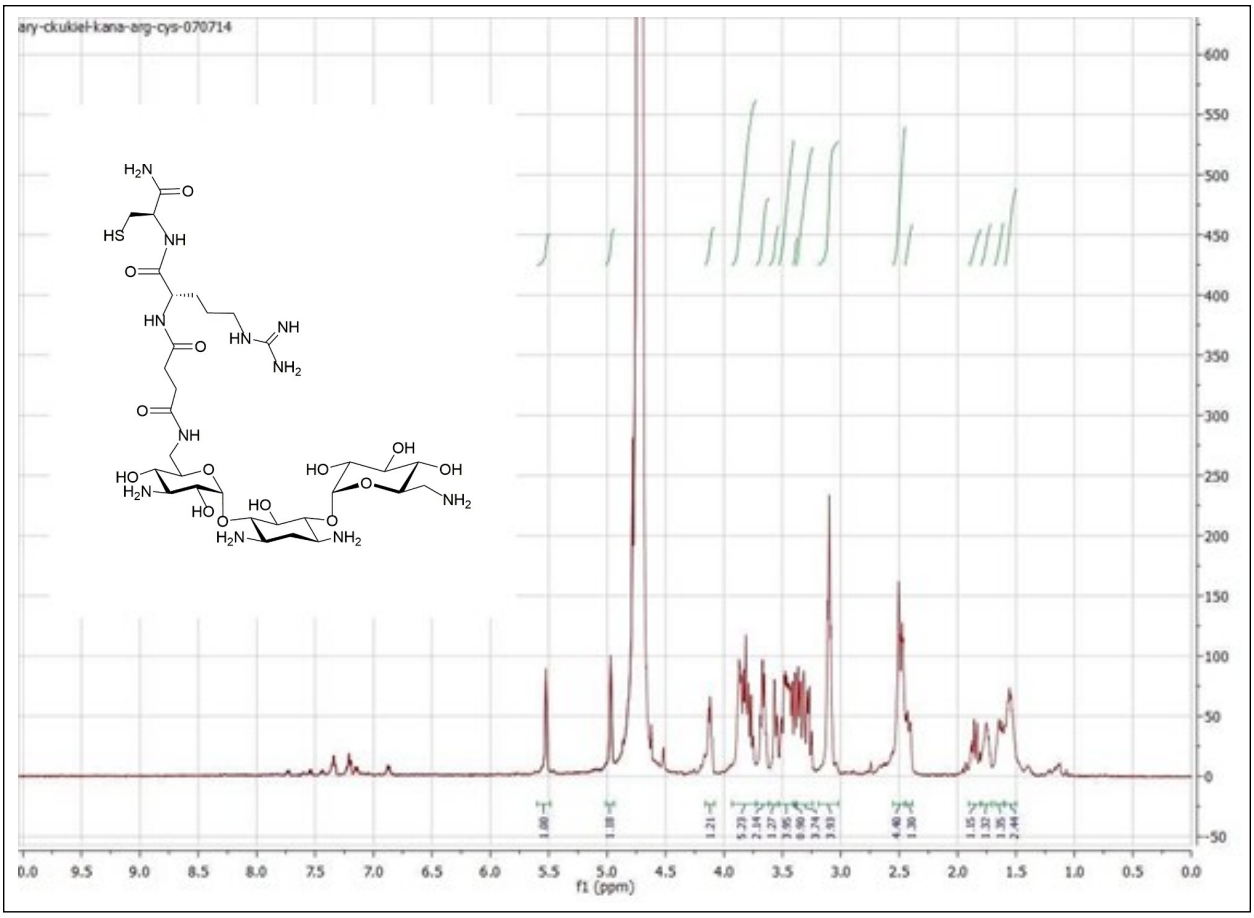


**Figure S13.** $^{1}$H NMR spectrum of **DPA 2084 (KanaRC)** ($D_2O$, 500 MHz).

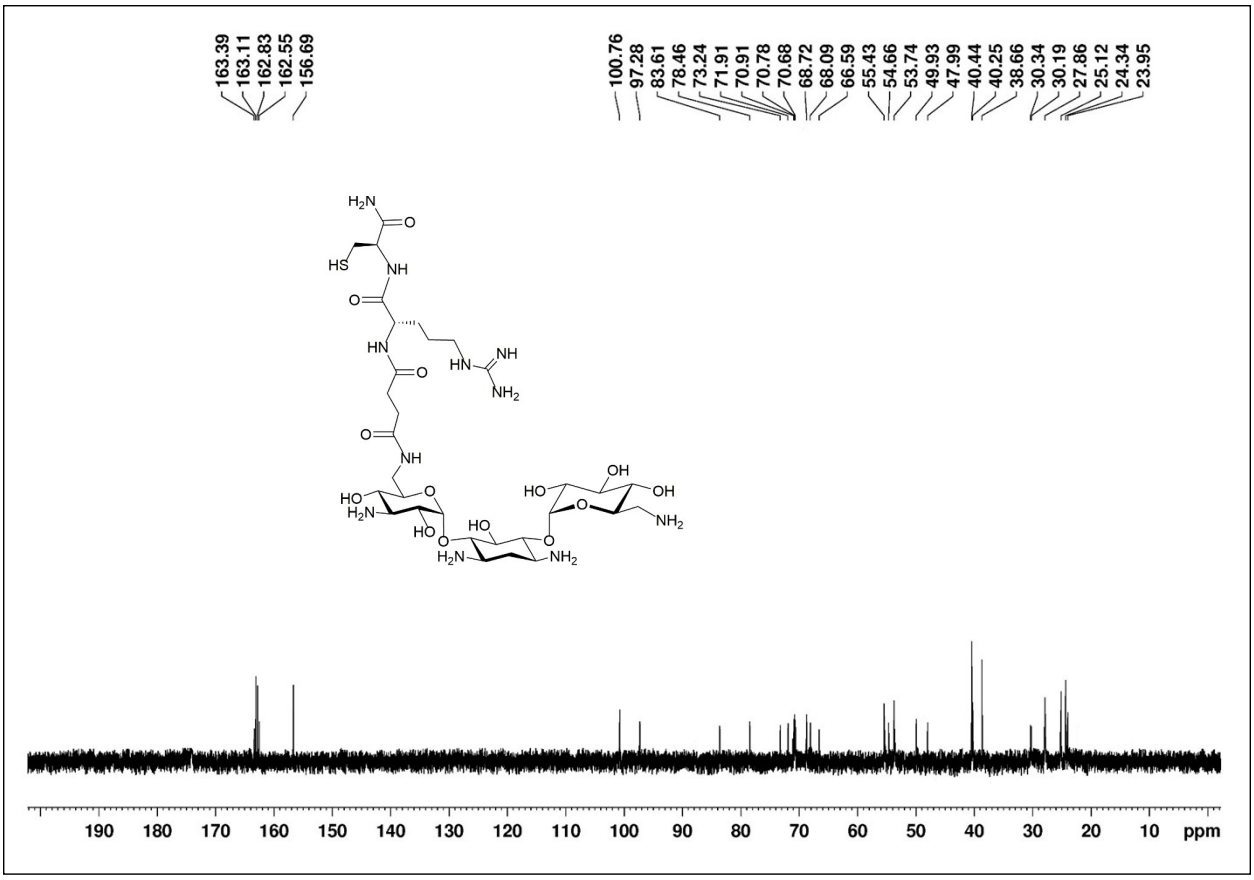


**Figure S14.** $^{13}C$ NMR spectrum of **DPA 2084 (KanaRC)** ($D_2O$, 125 MHz).

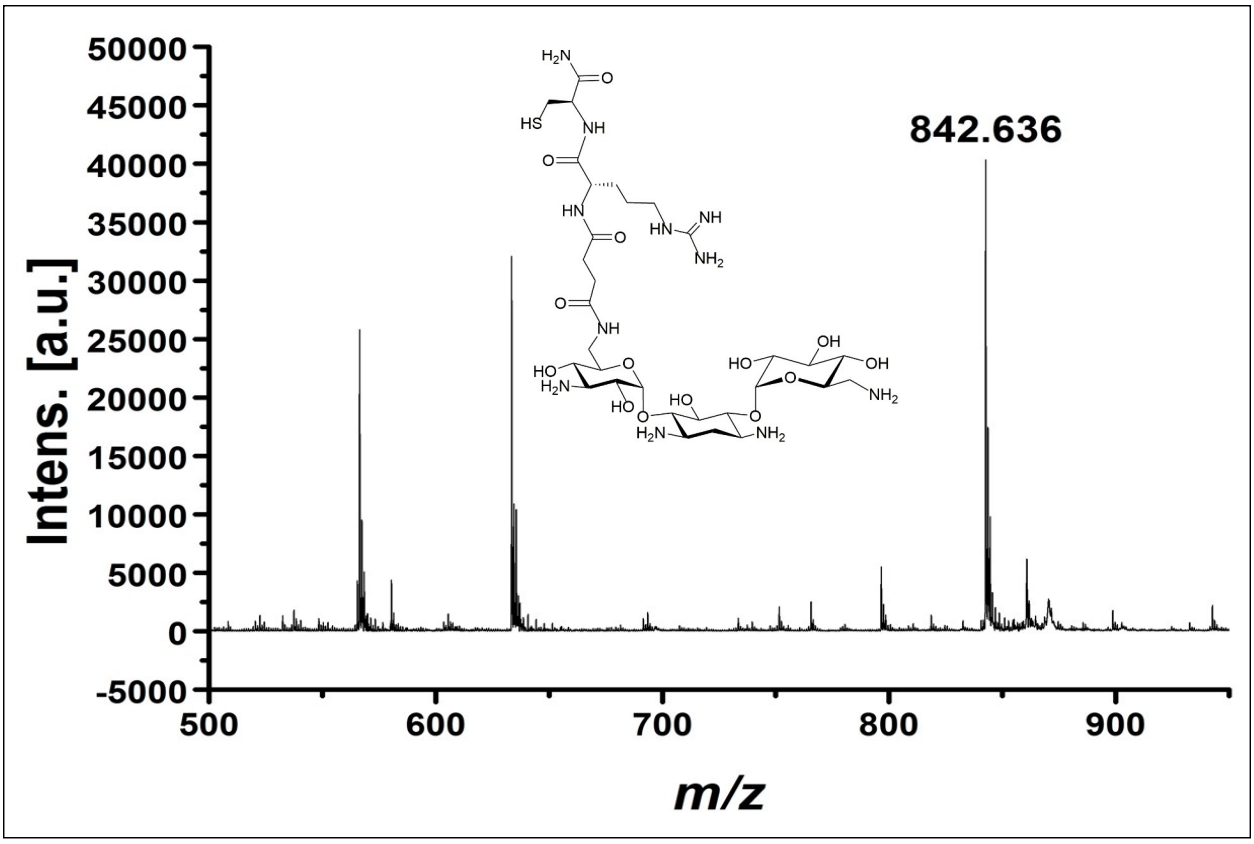


**Figure S15.** MALDI spectrum of **DPA 2084 (KanaRC).**

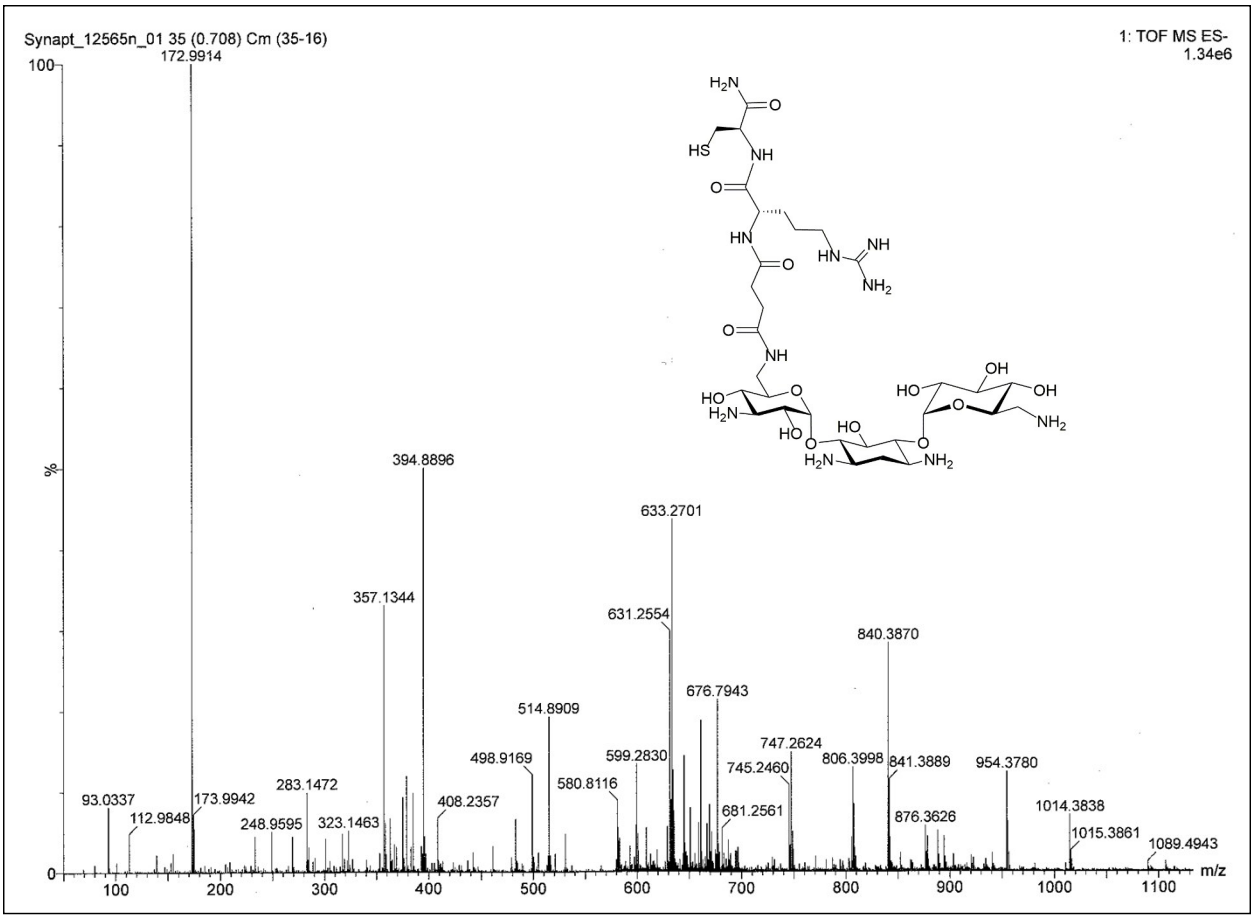


**Figure S16.** HRMS (ESI) spectrum of **DPA 2084 (KanaRC).**

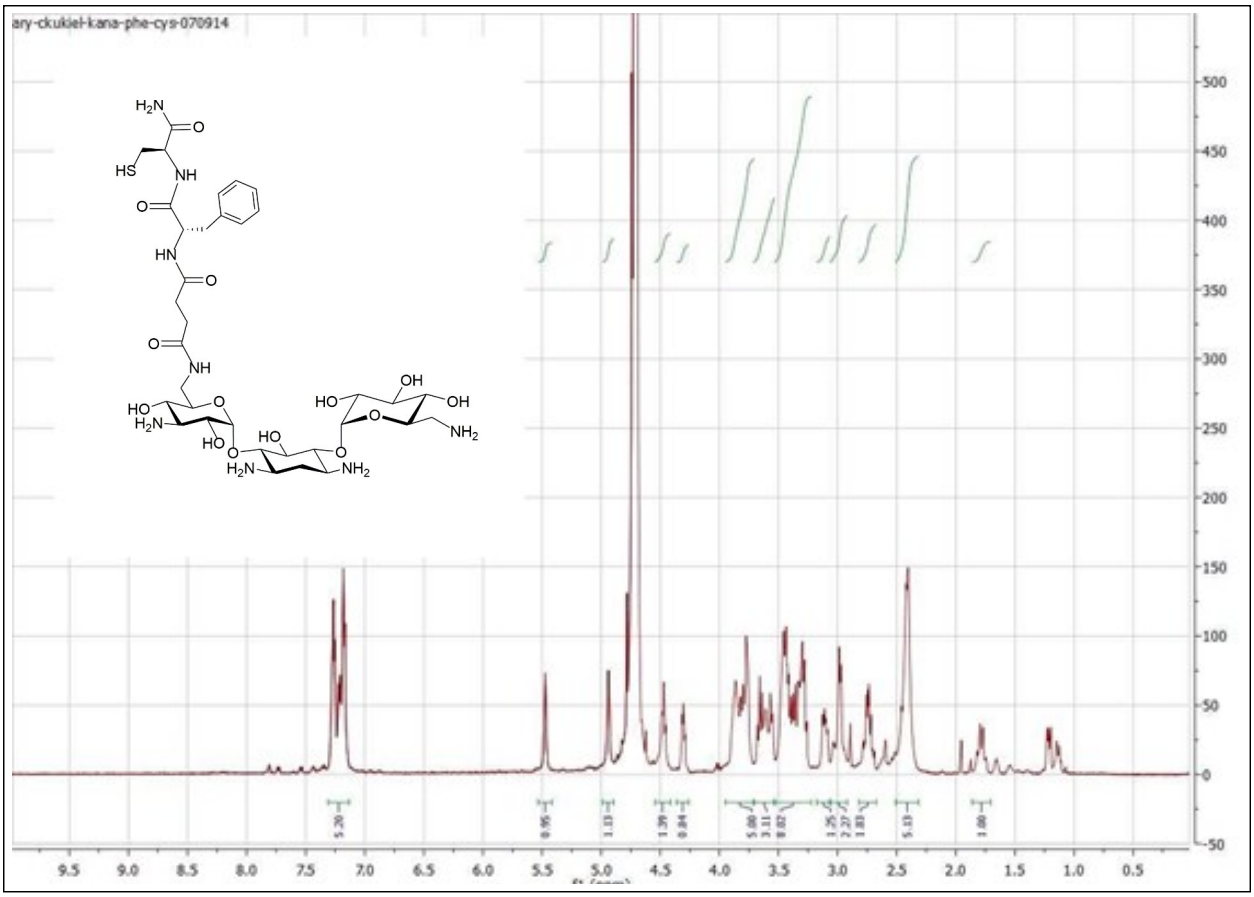


**Figure S17.** $^{1}H$ NMR spectrum of **DPA 2089 (KanaFC)** ($D_2O$, 500 MHz).

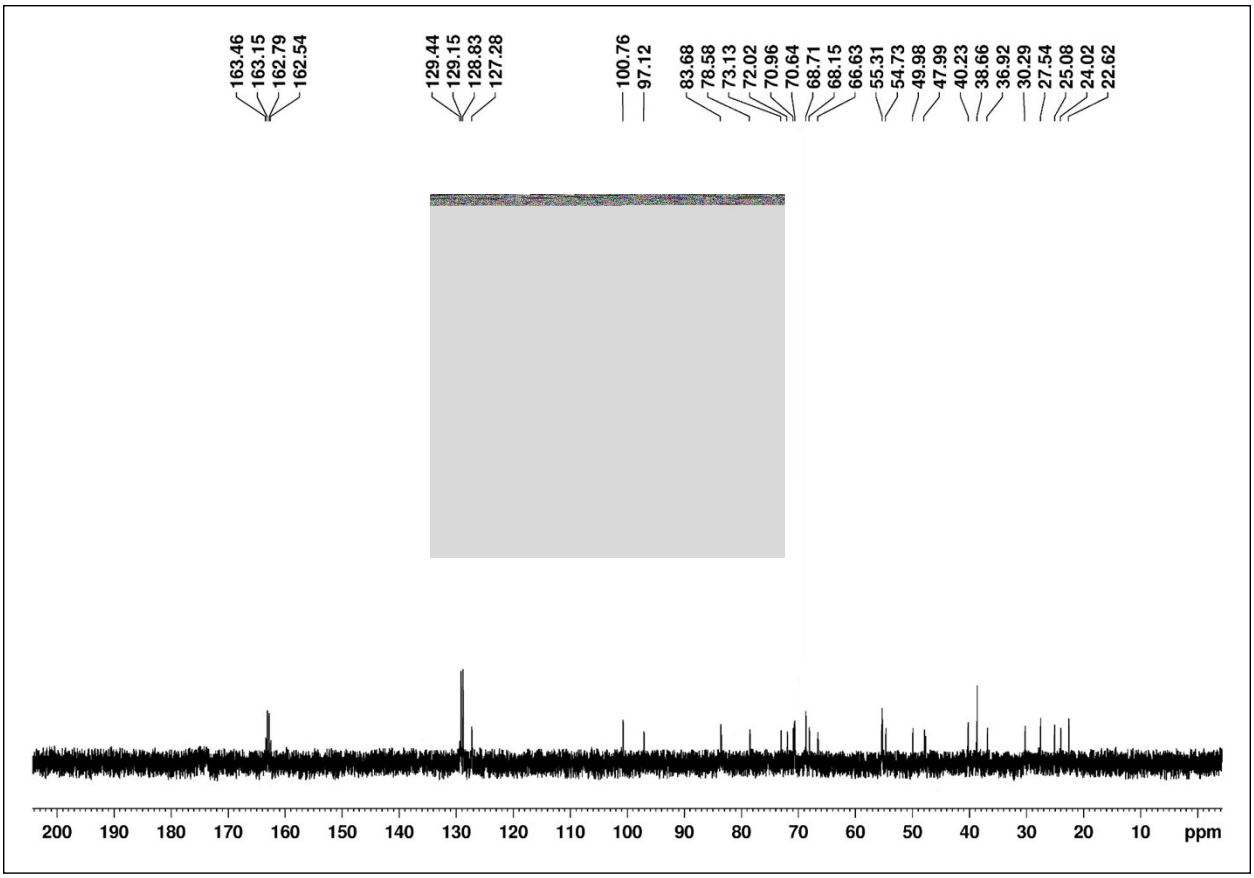


**Figure S18.** $^{13}C$ NMR spectrum of **DPA 2089 (KanaFC)** ($D_2O$, 125 MHz).

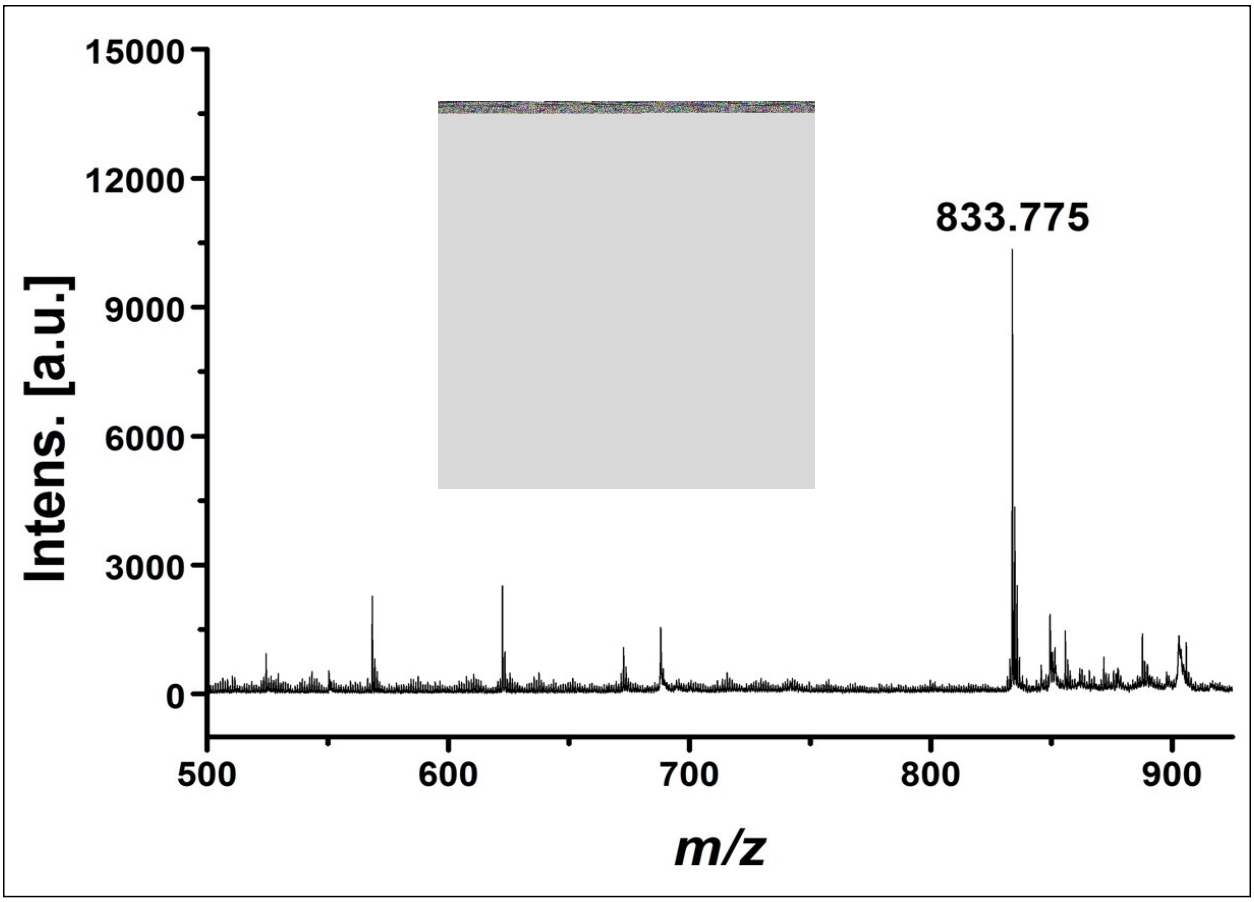


**Figure S19.** MALDI spectrum of **DPA 2089 (KanaFC).**

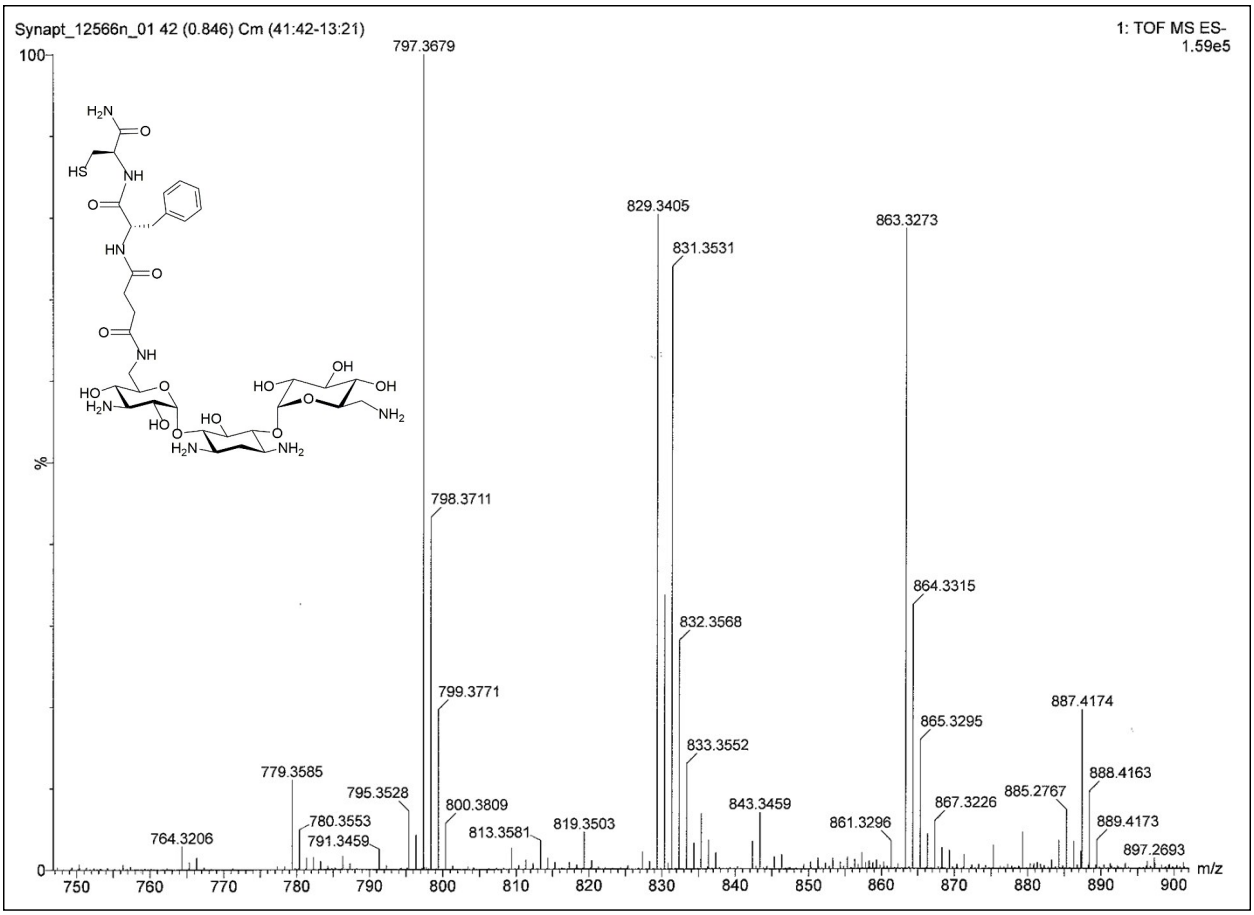


**Figure S20.** HRMS (ESI) spectrum of **DPA 2089 (KanaFC).**

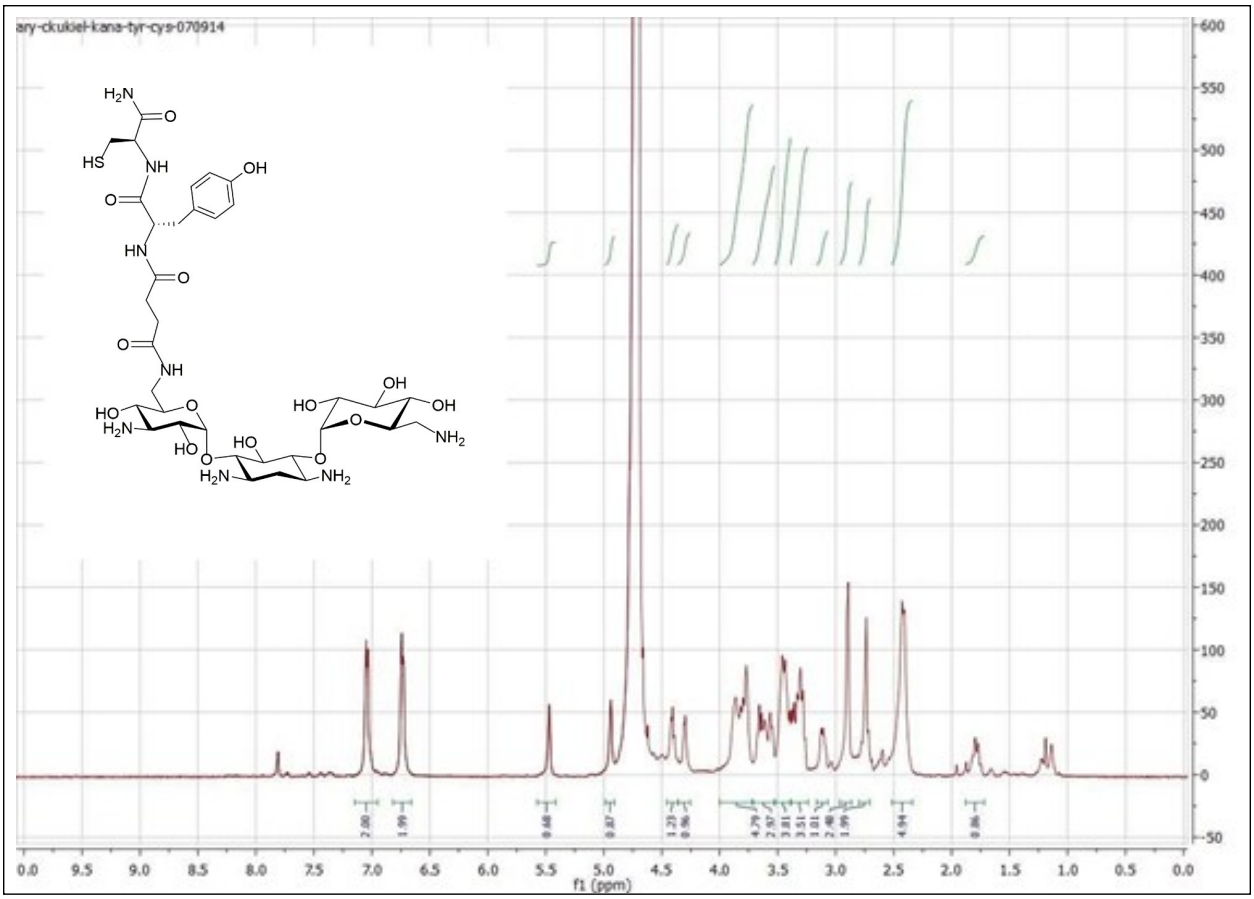


**Figure S21.** $^{1}$H NMR spectrum of **DPA 2093 (KanaYC)** ($D_2O$, 500 MHz).

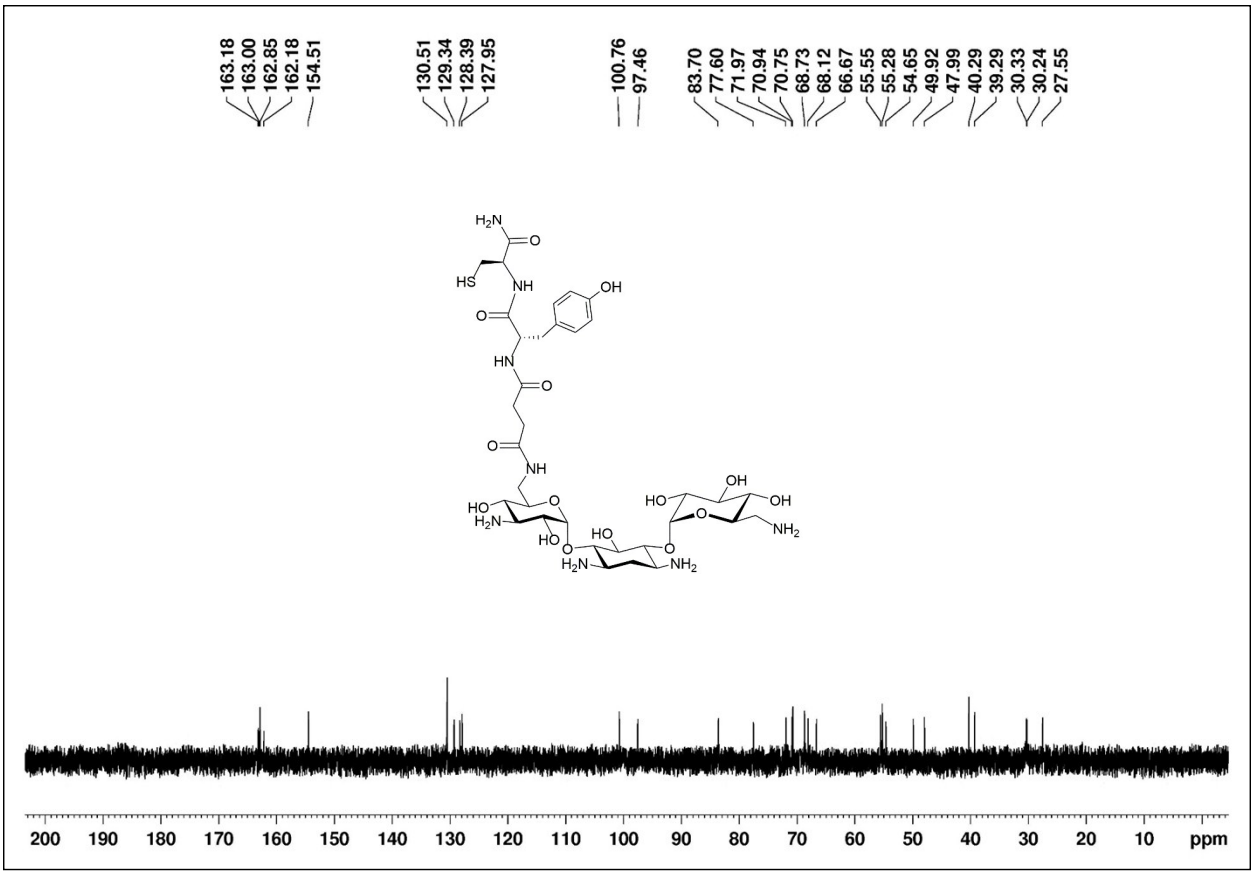


**Figure S22.** $^{13}C$ NMR spectrum of **DPA 2093 (KanaYC)** ($D_2O$, 125 MHz).

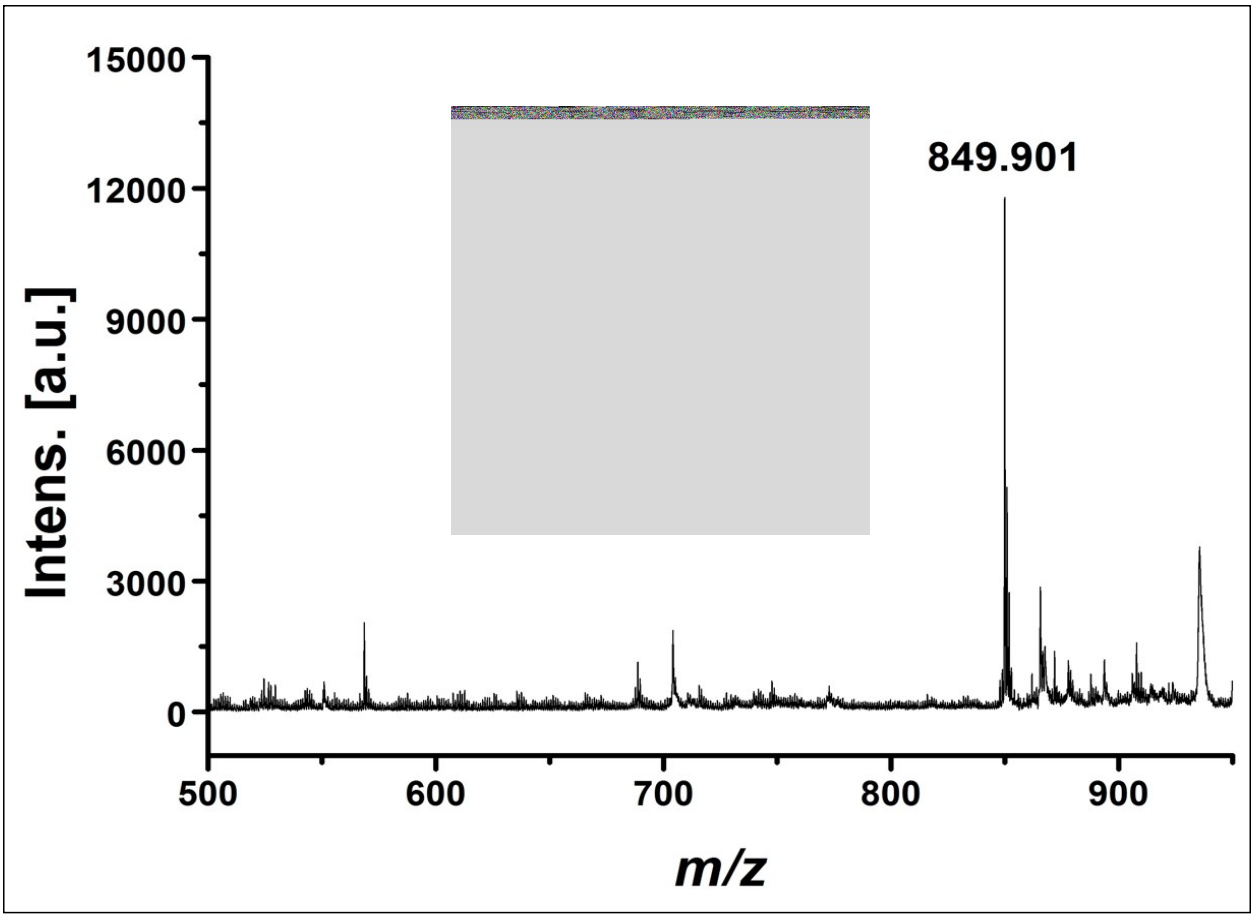


**Figure S23.** MALDI spectrum of **DPA 2093 (KanaYC).**

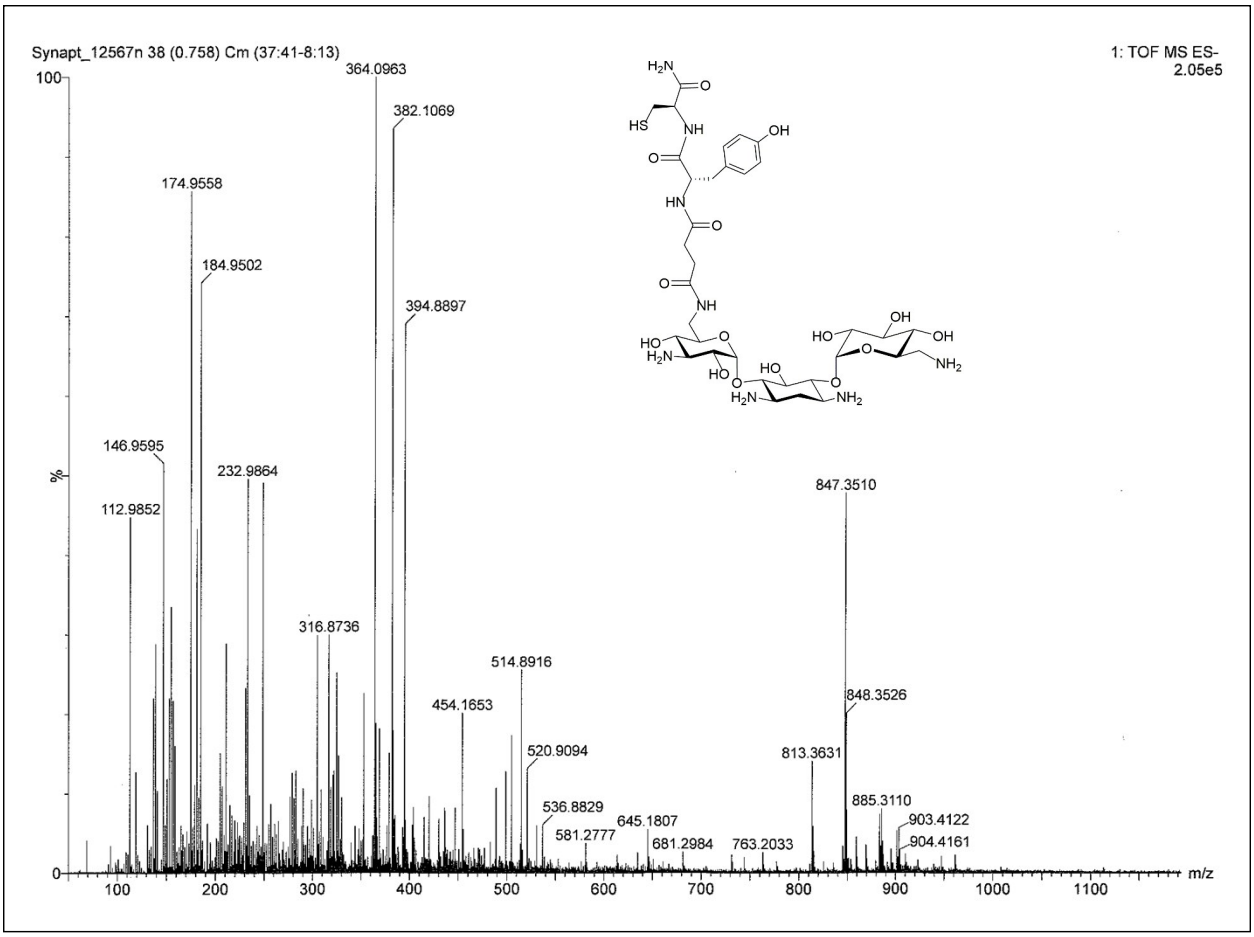


**Figure S24.** HRMS (ESI) spectrum of **DPA 2093 (KanaYC).**

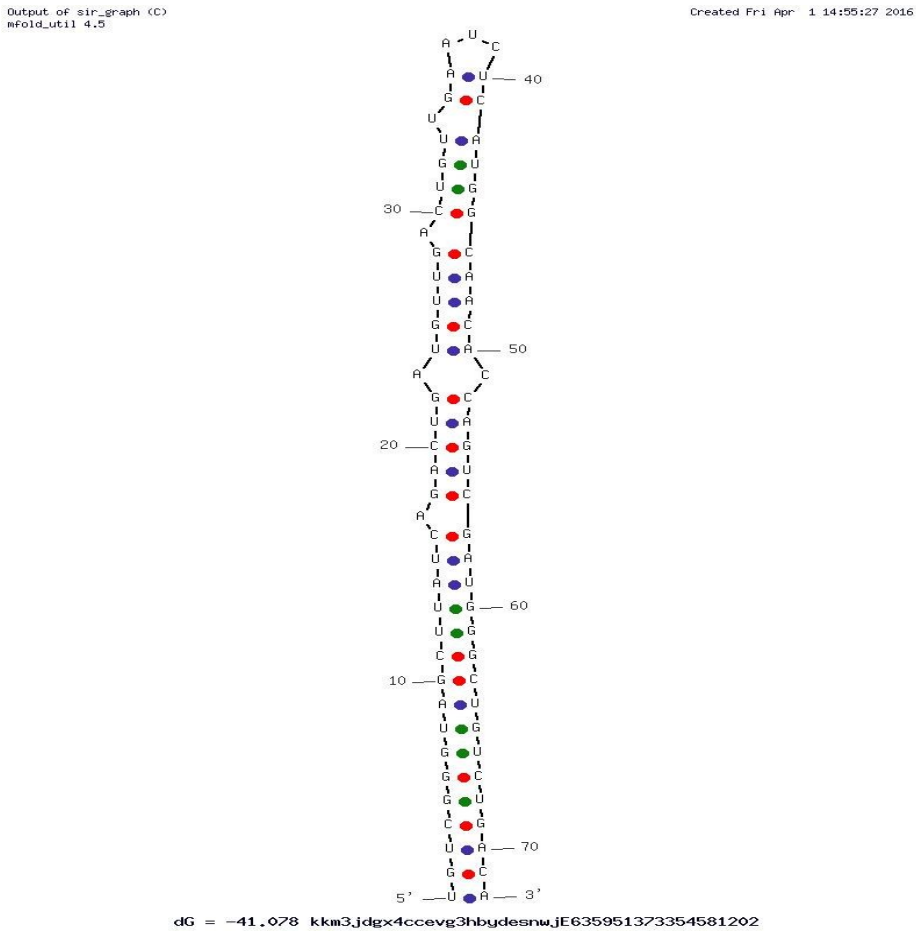


**Figure S25**. Image of the thermodynamically favored secondary structure of pre-miR21 created by Unafold software. Bulges are potential PA-Kan conjugate binding sites.

**Table S1: Screening results of selected PA-Kan conjugates with pre-miR21**

| PA-Kan Conjugate | Compound # | Percent Binding | Standard Deviation |
|---|---|---|---|
| | control | 0 | 0 |
| | KAN | 100 | 3 |
| Kan-RC | 2084 | 154 | 10 |
| Kan-RK | 2098 | 142 | 7 |
| Kan-FC | 2089 | 140 | 8 |
| Kan-RR | 1930 | 137 | 5 |
| Kan-HK | 2101 | 134 | 8 |
| Kan-FR | 1935 | 133 | 6 |
| Kan-YC | 2093 | 133 | 7 |
| Kan-RT | 2042 | 131 | 8 |
| Kan-RN | 1944 | 129 | 7 |
| Kan-RL | 1986 | 124 | 4 |
| Kan-YR | 1939 | 120 | 4 |
| Kan-RH | 1972 | 118 | 4 |
| Kan-YH | 1981 | 110 | 5 |
| Kan-RV | 2070 | 108 | 7 |
| Kan-R | 1901 | 107 | 5 |
| Kan-HR | 1933 | 106 | 9 |
| Kan-RY | 2056 | 106 | 3 |
| Kan-FK | 2103 | 106 | 9 |
| Kan-HβA | 1919 | 106 | 15 |

| | | | |
|---|---|---|---|
| Kan-HC | 2087 | 106 | 7 |
| Kan-RF | 2000 | 104 | 3 |
| Kan-FH | 1977 | 104 | 3 |
| Kan-HP | 2017 | 104 | 4 |
| Kan-RS | 2028 | 102 | 3 |
| Kan-HS | 2031 | 102 | 5 |
| Kan-βHA | 1971 | 101 | 5 |
| Kan-HT | 2045 | 94 | 3 |
| Kan-HF | 2003 | 91 | 3 |
| Kan-HY | 2059 | 89 | 3 |
| Kan-FL | 1991 | 86 | 3 |
| Kan-HV | 2073 | 82 | 4 |
| Kan-YK | 2107 | 82 | 4 |
| Kan-W | 1913 | 81 | 3 |
| Kan-RβA | 1916 | 80 | 6 |
| Kan-FN | 1949 | 79 | 3 |
| Kan-RD | 1958 | 78 | 2 |
| Kan-HN | 1947 | 78 | 2 |
| Kan-Y | 1910 | 72 | 2 |
| Kan-H | 1904 | 71 | 5 |
| Kan-FβA | 1921 | 69 | 2 |
| Kan-YF | 2009 | 66 | 2 |
| Kan-YN | 1953 | 62 | 2 |
| Kan-FV | 2075 | 60 | 3 |

| | | | |
|---|---|---|---|
| Kan-F | 1906 | 60 | 5 |
| Kan-FY | 2061 | 58 | 2 |
| Kan-YY | 2065 | 56 | 4 |
| Kan-HD | 1961 | 56 | 2 |
| Kan-YT | 2051 | 54 | 2 |
| Kan-FP | 2019 | 54 | 2 |
| Kan-YP | 2023 | 52 | 2 |
| Kan-YL | 1995 | 45 | 2 |
| Kan-YV | 2079 | 41 | 2 |
| Kan-FD | 1963 | 39 | 1 |
| Kan-FT | 2047 | 33 | 3 |
| Kan-YS | 2037 | 33 | 1 |
| Kan-FF | 2005 | 26 | 1 |
| Kan-FS- | 2033 | 12 | 1 |
| Kan-YD | 1967 | 5 | 0 |

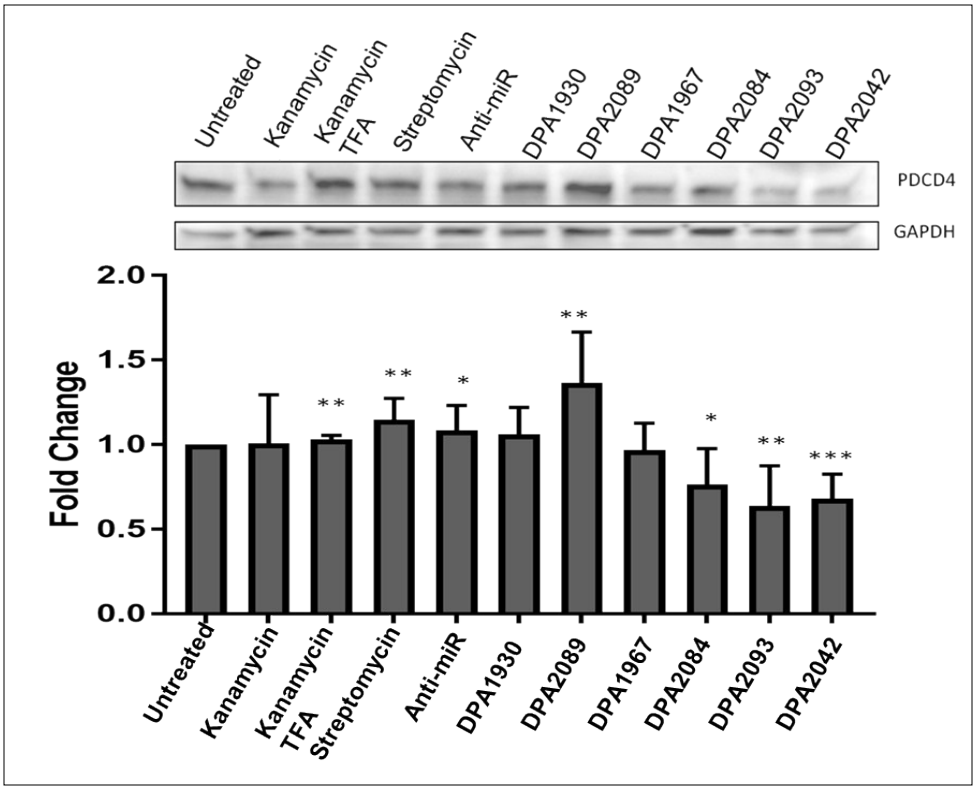


**Figure S26.** Fold change in the levels of PDCD4 (target protein for miR21) for cells treated with Kanamycin conjugates at 5µM concentration compared to the control samples chosen as measured by the quantitative analysis done for western blots performed. Hits for the molecules are shown in the figures with error bars representing ±S.D. *,P<=0.1; **,P<=0.05; ***,P<=0.005.(Student's T Test).

**Table S2. Estimated biophysical statistics for the best docked structure for the four molecules calculated using AutoDock. The number of hydrogen bonds shown here accounts for both intra (within the molecule) as well as inter (between molecule and pre-miRNA) interactions.**

| Molecule Name | Est. free energy of binding (kcal/mol) | Est. Inhibition Constant, Ki | Electrostatic Energy (kcal/mol) | # of H-bonds |
|---|---|---|---|---|
| **DPA1930** | -11.03 | 8.19 nM | -12.04 | 2 |
| **DPA2089** | -10.4 | 23.74 nM | -10.79 | 3 |
| **DPA2093** | -8.88 | 311.10 nM | -4.86 | 5 |
| **DPA1967** | -6.53 | 16.35 µM | -5.58 | 7 |

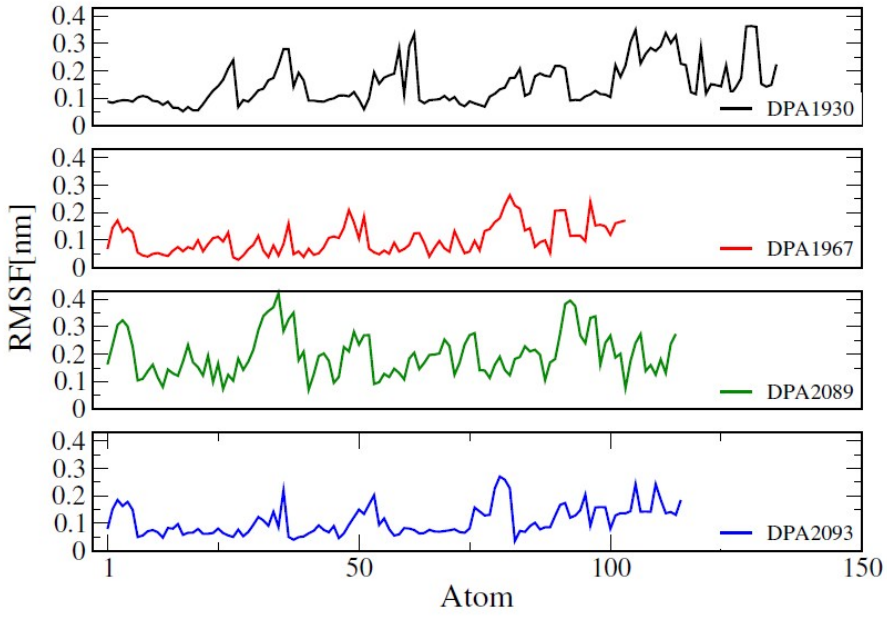


**Figure S27.** PA-Kan region imparts stable interaction. Root mean square fluctuation for each atom was calculated based upon the whole trajectory using gromacs tools.

**Table S3: Comparative $K_D$ values for PA-Kan conjugate hits by SPR**

| Serial No. | Molecule code | $K_D$ value |
|---|---|---|
| 1. | DPA 2089 | 4.36E-08 |
| 2. | DPA 1930 | 1.51E-08 |

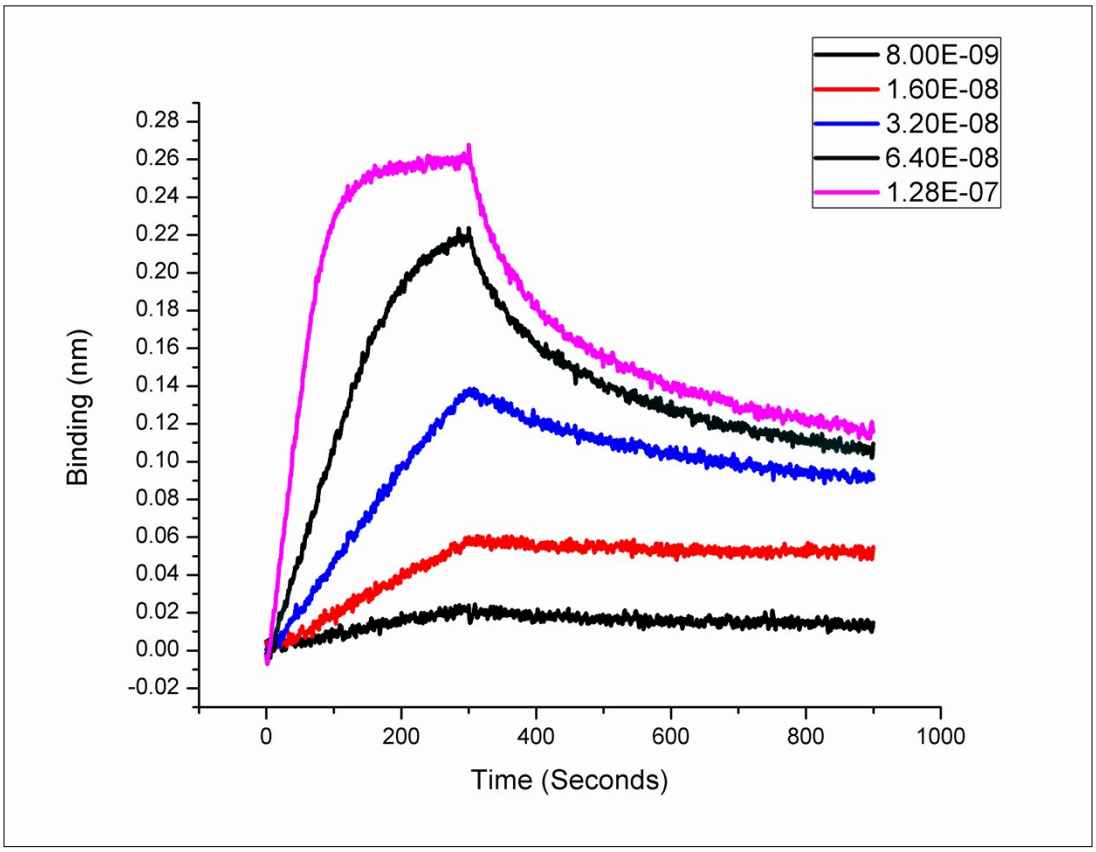


**Figure S28.** Association and dissociation pattern for Molecule 1930 over the concentration range of 8-128 nM.

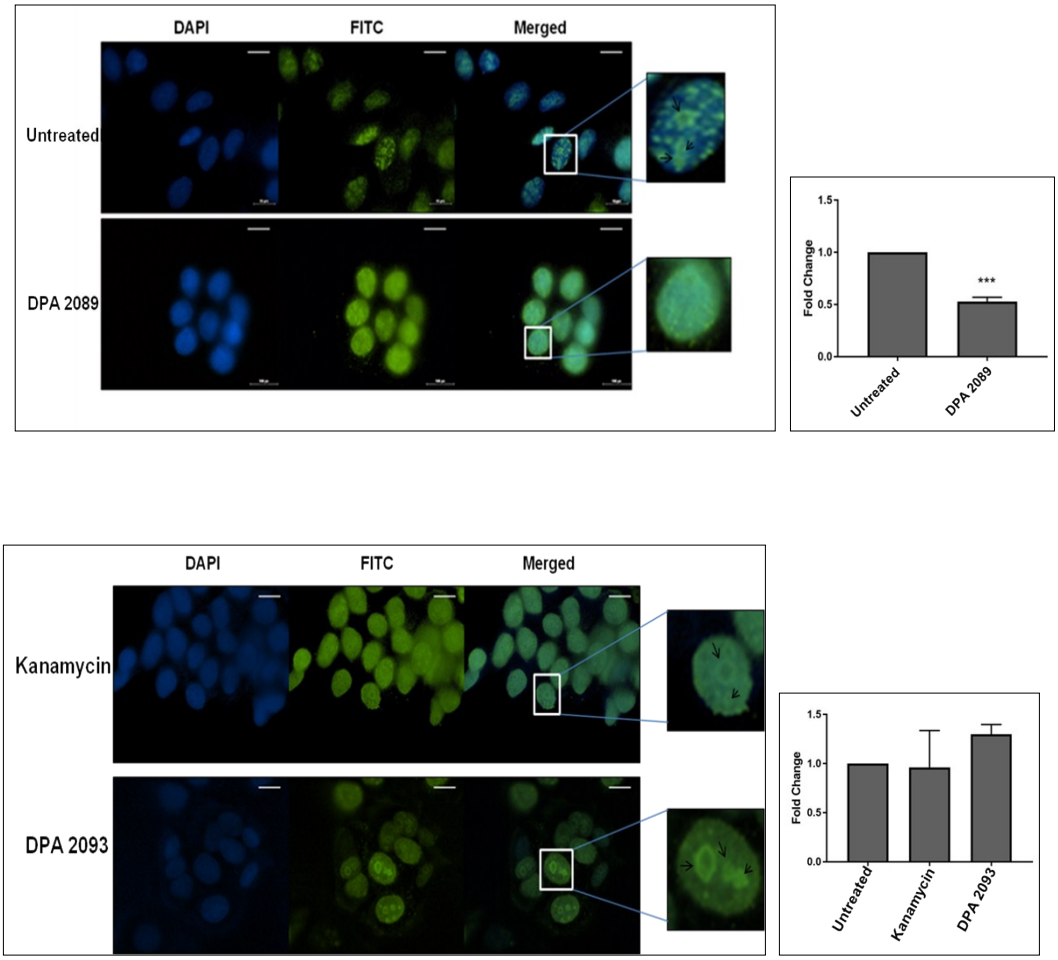


**Figure S29**. Images for Ki67 cell proliferation assay showing speckles within the cell nucleus as a proof for them in proliferative phase. The figure shows cells which are untreated (positive control), cells treated with Untreated control, Kanamycin sulphate, and with Molecule 2089 at 5µM concentration. The cells were viewed under 100x magnification and all the images are normalised to equal intensity. Magnified boxes from each image show the best focussed cell.

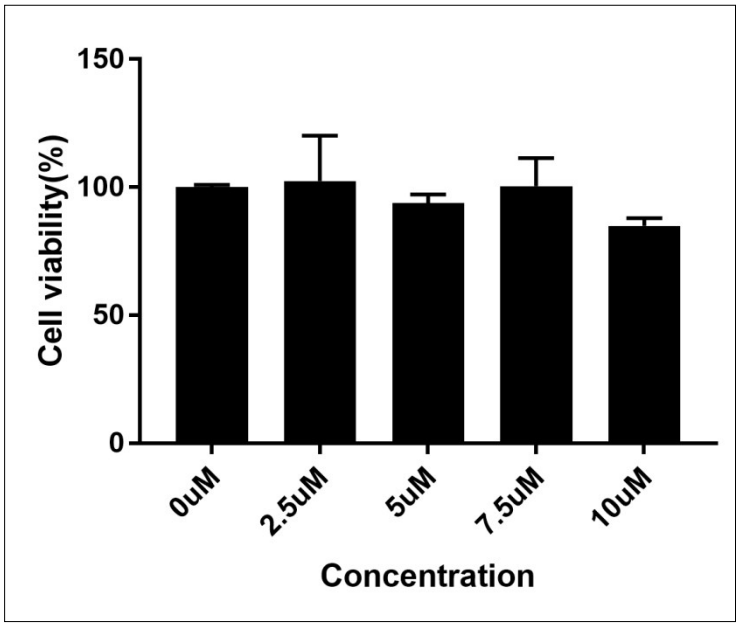


**Figure S30**. MTT results show 5 µM concentration to be optimum for DPA 2089 treatment without imposing any cellular toxicity. The results are correlated to three biological replicates and the error bars represent ±S.D.

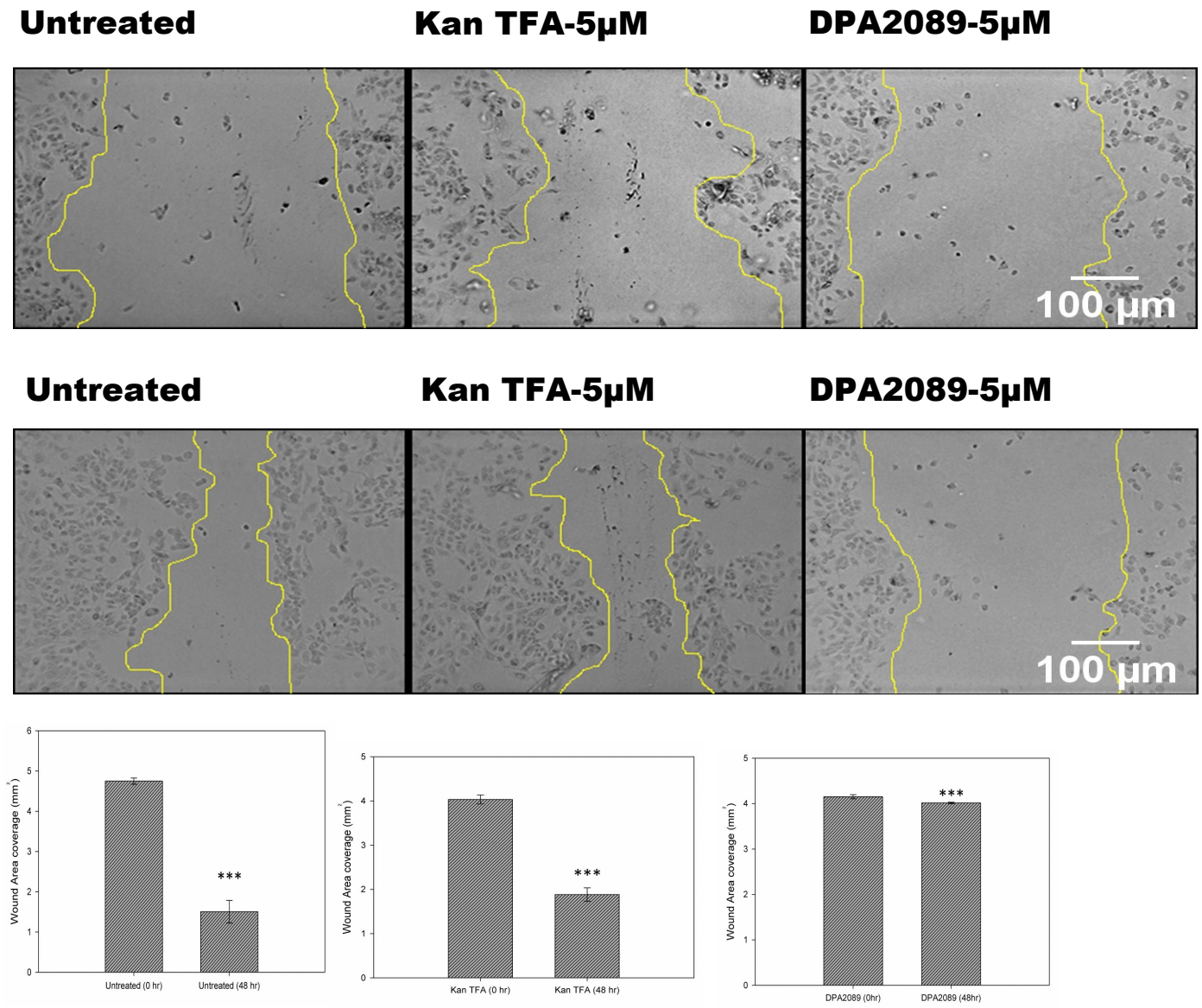


**Figure S31**. Wound healing assay to demonstrate the anti-migratory effect of DPA 2089 as compared to Untreated cells and Kanamycin TFA. Quantification of the images was done by ImageJ. The results are correlated to three biological replicates and the error bars represent ±S.D. *,P<=0.1; **,P<=0.05; ***,P<=0.005.(Student's T Test). Images were viewed under 10X resolution.

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