Supporting Information

Cyclic peptidomimetics as inhibitor for miR-155 biogenesis

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Supplementary Figures

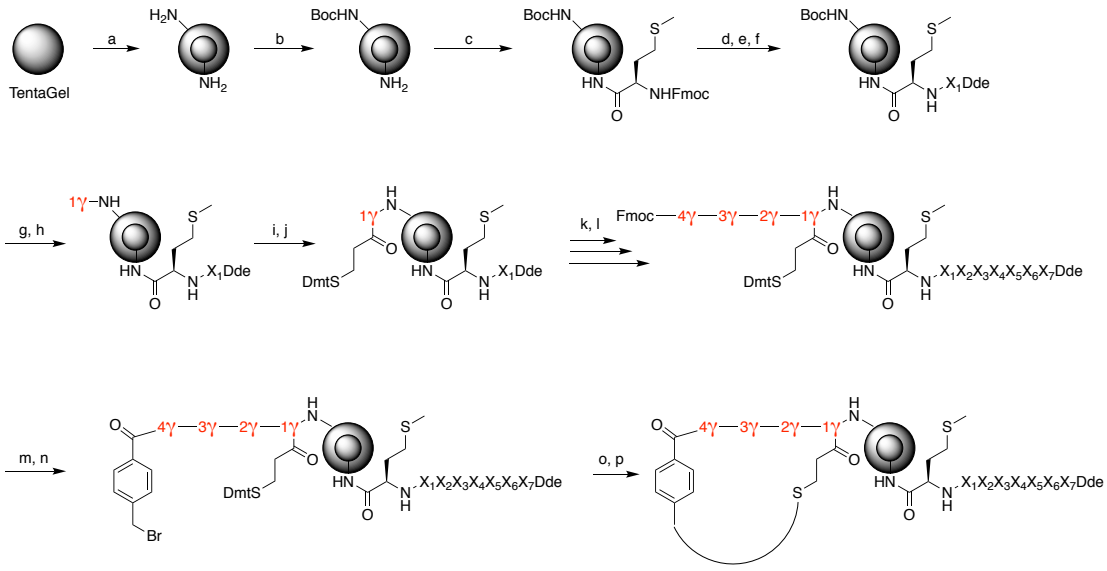


Figure S1. Design and synthesis route for thioether-bridged cyclic γ-AApeptide library. (a) Soak in water for overnight; (b) $(Boc)_2O$ (0.5 equiv.) in DCM/ether; (c) Fmoc-Met-OH/HOBt/DIC (2:4:4 equiv.) in DMF; (d) 20% piperidine in DMF; (e) split into 5 portions equally; (f) Dde protected amino acids/PyBOP/NEM (2:6:6) in DMF; (g) TFA/triisopropylsilane/H_2O/Thioanisole (v:v:v:v 94:2:2:2); (h) Fmoc protected γ-AApeptide/HOBt/DIC (2:4:4 equiv.) in DMF; (i) $Pd(PPh_3)_4$ (0.007 mmol) and $Me_2NH{\bullet}BH_3$ (0.42 mmol) in DCM; (j) Dmt protected mercaptopropionic acid/HOBt/DIC (2:4:4 equiv) in DMF; (k) 0.180 mmolof $NH_2OH{\bullet}HCl$ and (0.135 mmol) of imidazole in 5 mL NMP, diluted with DCM; (l) split-and-pool synthesis, repeated the previous steps; (m) 20% piperidine in DMF; (n) 4-(bromomethyl)benzoyl chloride/DIPEA (2:4 equiv) in DCM; (o) TFA/triisopropylsilane/DCM (2:2:96; v/v/v); (p) $(NH_4)_2CO_3$ (10 equiv.) in 1:1 (v/v) DMF/H_2O.

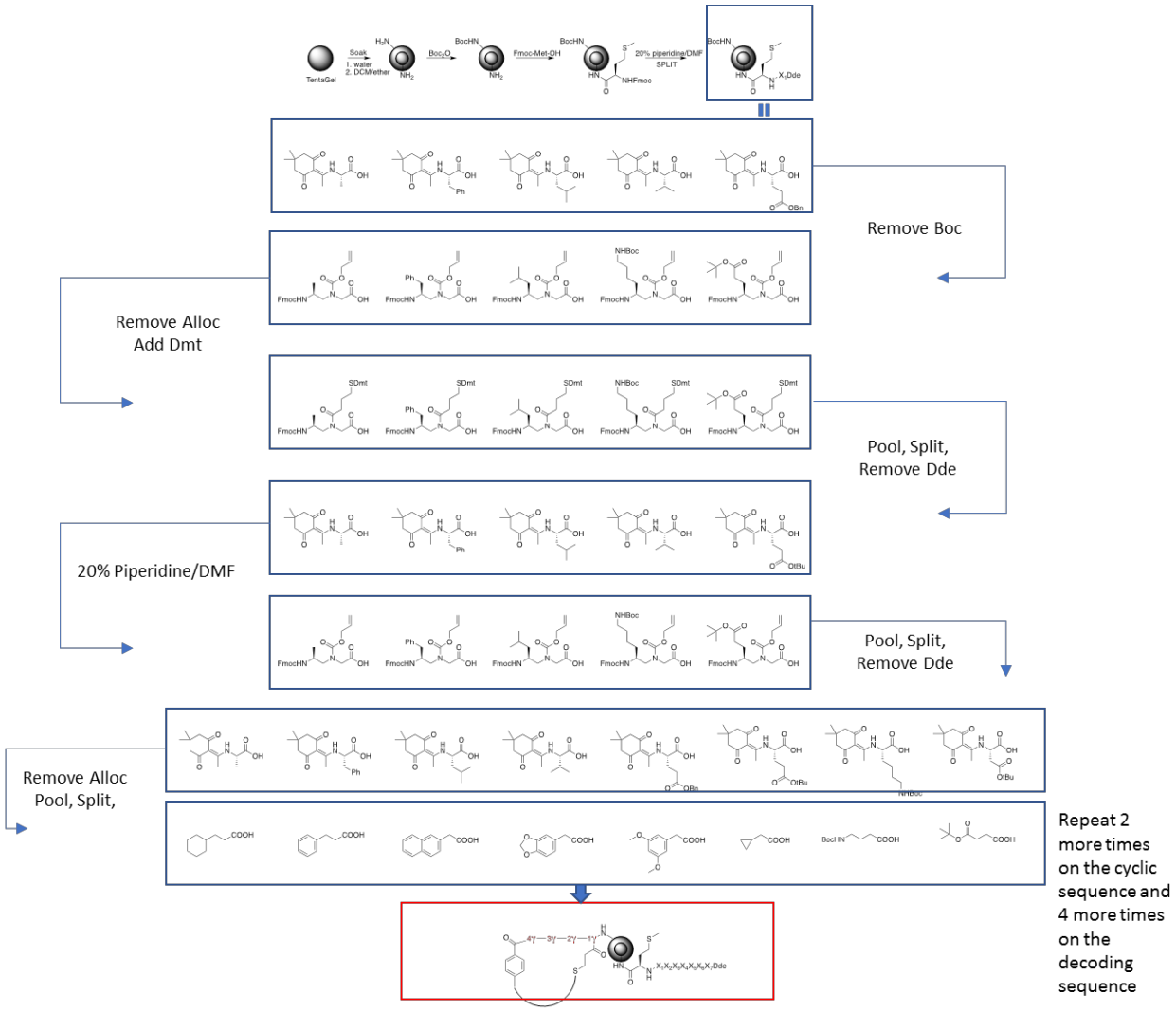


Figure S2. Structures of the building blocks used for the library preparation and the general synthetic scheme.

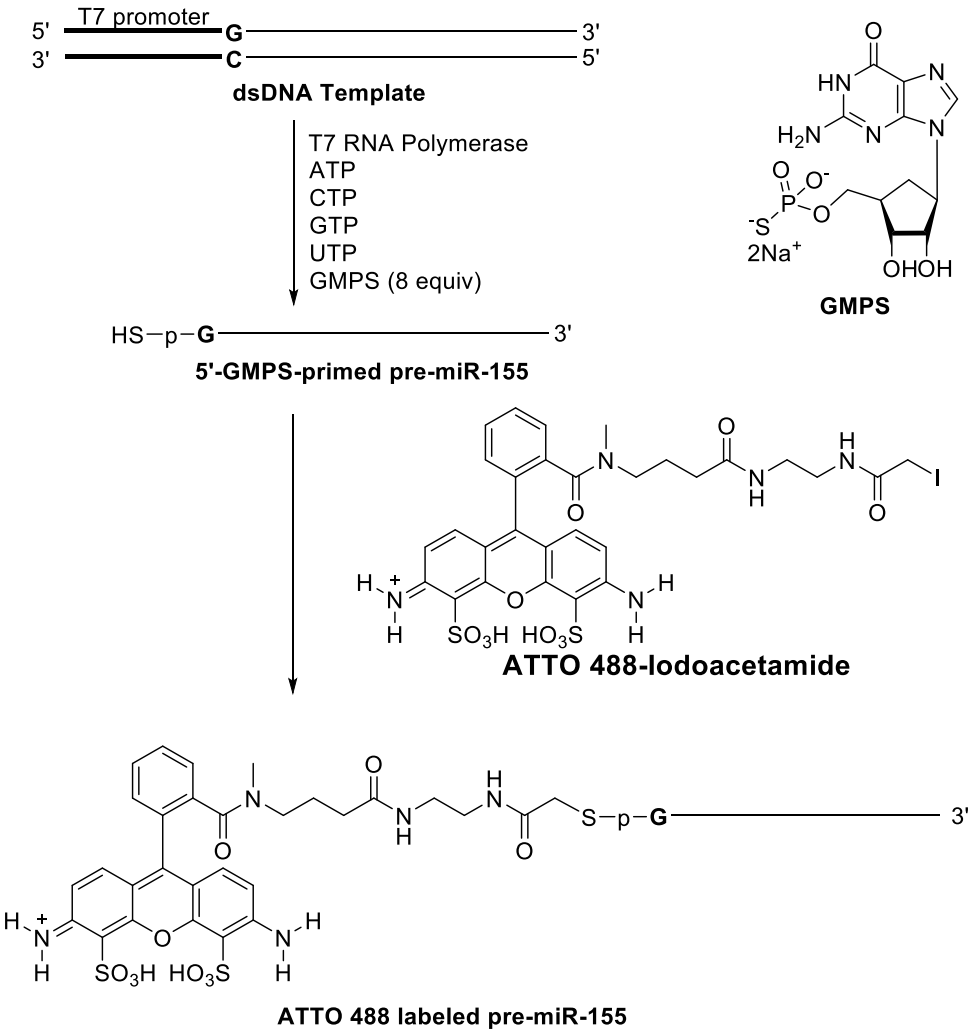


Figure S3. Preparation of fluorophore labeled pre-miR-155.

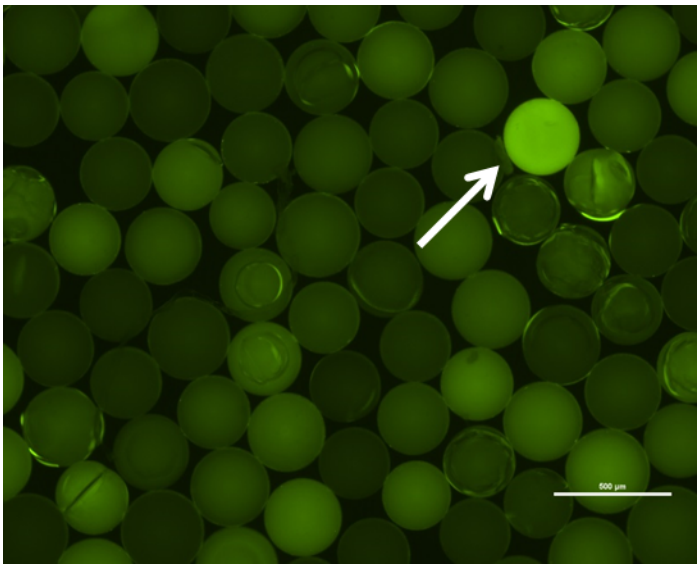


Figure S4. Fluorescence microscope image of the hit bead.

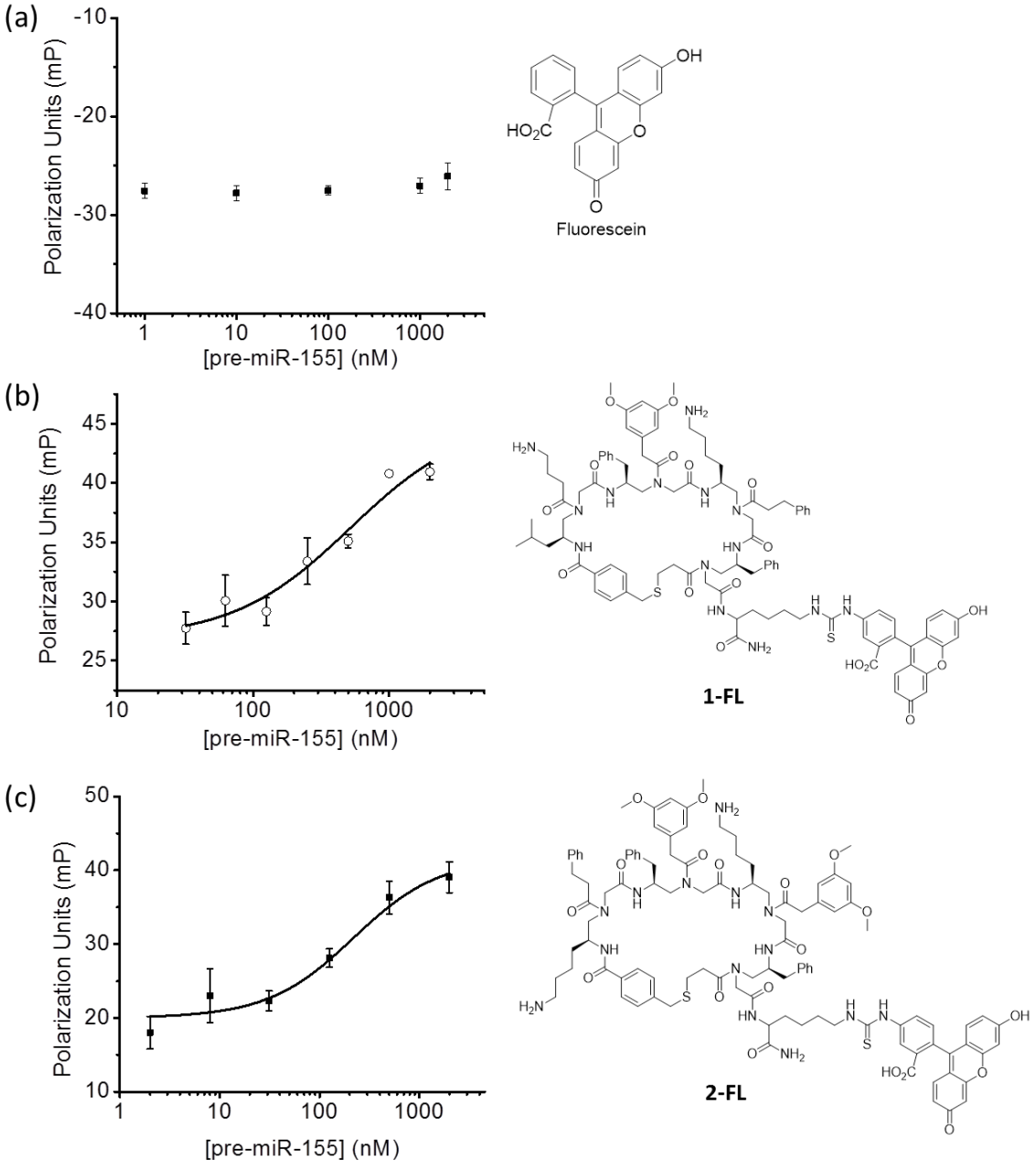


Figure S5. Fluorescence polarization analysis of fluorescein (a), **1-Fl** (b) and **2-Fl** (c) in the presence of different concentrations of pre-miR-155. The error bars represent the standard error of mean (N = 3).

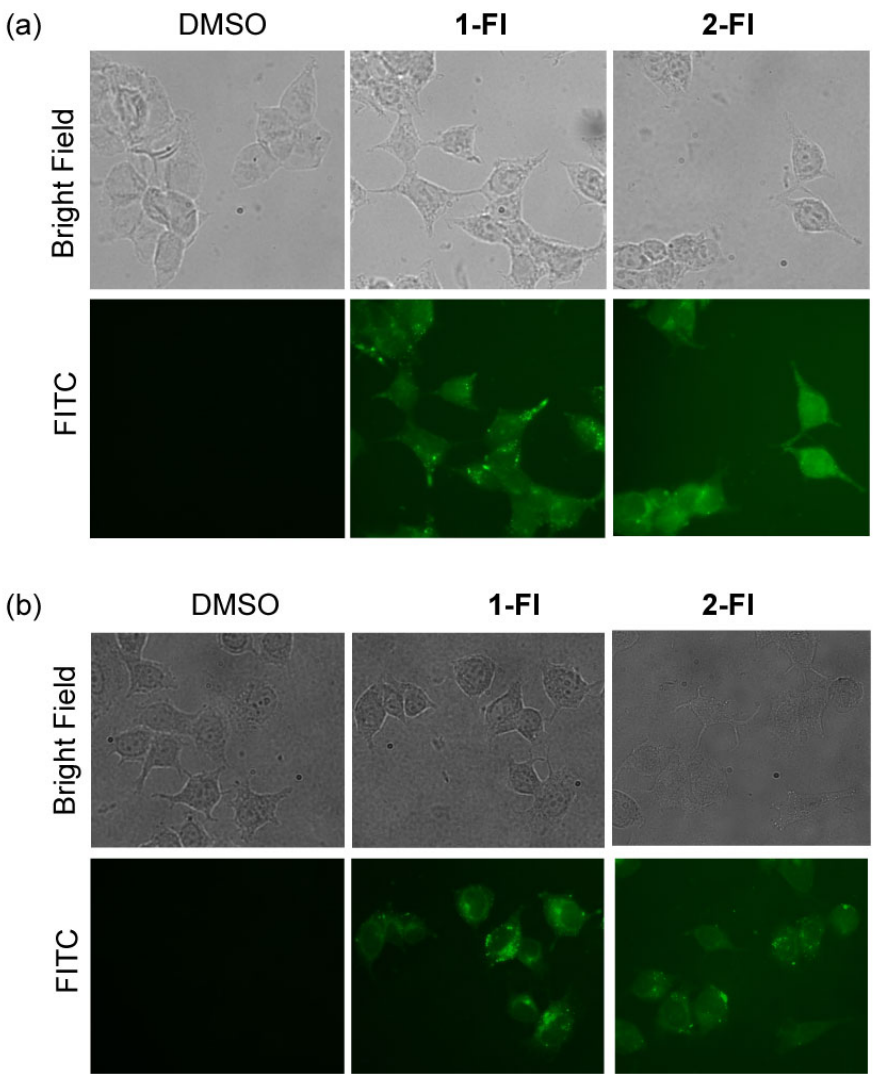


Figure S6. Fluorescence microscope imaging of HEK293T (a) and MCF-7 (b) cells treated with DMSO, **1-Fl** or **2-FL**.

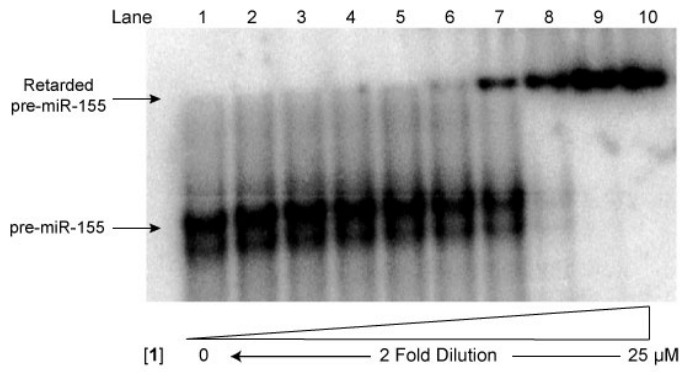


Figure S7. The electrophoretic mobility of pre-miR-155 in the presence of compound **1**.

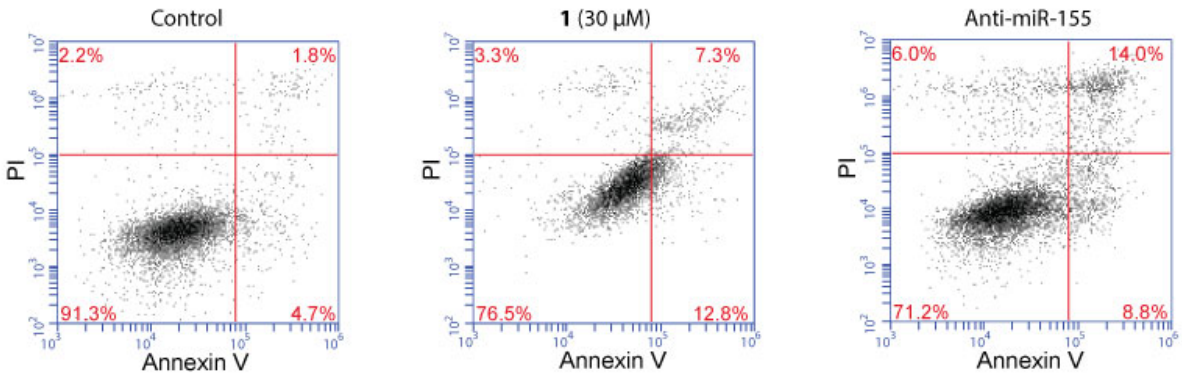


Figure S8. Flow cytometry of MCF-7 cells treated with different compounds and then stained with fluorescein-Annexin V and PI.

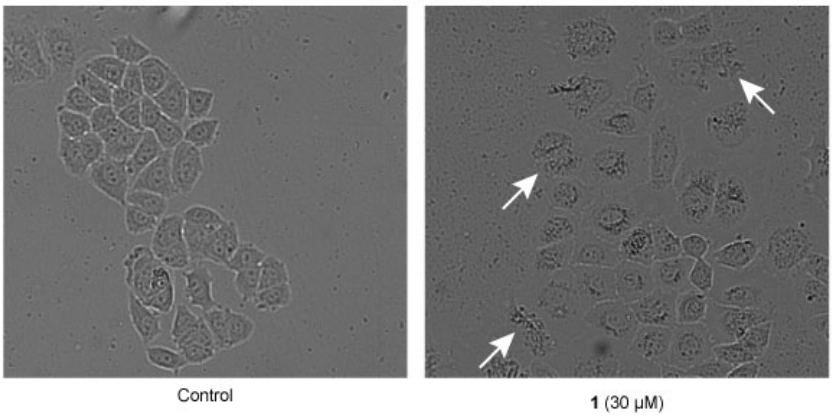


Figure S9. Morphological changes of MCF-7 cells after 4 day treatment. DMSO was used as a control. Arrows indicate the apoptotic cells showing blebbing.

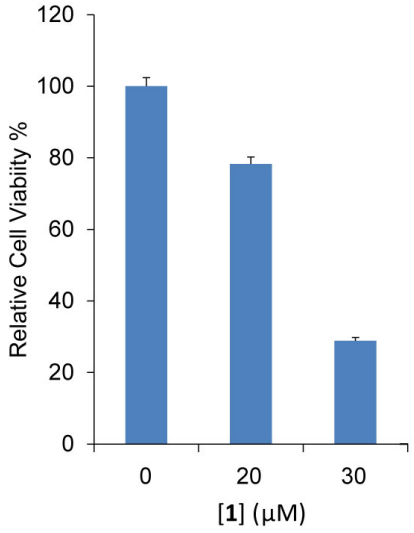


Figure S10. Viability analysis for MCF-7 cells treated with **1**. The error bars represent the standard error of mean (N = 3).

Supplementary Table

Table S1. Primers used for RT-qPCR and pre-miR-155 preparation.

Assay	Forward	Reverse
pre-miR-155 *in vitro* transcription	GAAATTAATACGACTCACTATAGGCTGTTAAT GCTAATCGTGATAGGGGTTTTTGCCTCCAACTG	CTGTTAATGCTAATATGTAGGAGTCAGTTG GAGGCAAAAACCCCTA
FOXO3A qPCR	TGACACAGTCGGACCCCTTG	GTTCTGATTGACCAAACTTCCCT
GAPDH qPCR	GGTGGTCTCCTCTGACTTCAACA	GTTGCTGTAGCCAAATTCGTTGT
pre-miR-155 qPCR	CTGTTAATGCTAATCGTGATAGGG	TGTAGGAGTCAGTTGGAGGC

Experimental Details

Library Synthesis

As shown in Figure S1 & S2, the cyclized combinatorial libraries were prepared based on five different γ-AApeptide building blocks and eight different side chains. Based on the structural nature of γ-AApeptides, four chiral side chains came from the side chains of five different N-Alloc protected γ-AApeptide building blocks. Other three side chains were introduced by acylating the secondary amino group with eight different acyl chlorides or carboxylic acids after the deprotection of alloc group. As a result, the theoretical diversity of the library was expected to be 320 000, and 960 000 beads were used for the preparation of the library by using the split and pool method.

The general preparation protocol was similar with the work reported before,[1] but with eight different side chains being chosen in the current library to increase the diversity visibly. Firstly, the TentaGel beads (200-250 um; 1.5 nmol/bead) were soaked in DI water for overnight, then Boc protecting group was attached to the outer surface by exposing the beads in DCM/Et2O(1:1(v/v)) containing 0.5 equiv of di-tert-butyl decarbonate (Boc_2O). The inner layer should still contain free amino group since the beads remain in the water. After wash with DMF, the interior of the beads were coupled with Met. which facilitated the coding peptide cleavage aftertreatment with cyanogen bromide (CNBr). Next, then Fmoc protection group was removed and reacted with five different Dde protected amino acid respectively after the beads were split into five equal aliquots. These amino acids were used to code the first γ-AApeptide building block on the outer layer. Next, TFA was used to remove the out layer Boc protection group, followed by the coupling of five Alloc protected γ-AApeptide building blocks. Subsequently, in the presence of $Pd(PPh_3)_4$ and $Me_2NH\cdot BH_3$, the Alloc protection group was removed efficiently, followed by the attachment of the Dmt protected 3-mercaptopropanoic acid on the secondary amino group of the γ-AApeptide building blocks. The Dde deprotection group was removed by $NH_2OH\cdot HCl$ and imidazole

under mild condition, and the beads were pooled and split into five aliquots one more time. After that the second set of the Dde protected amino acids was added to introduce the coding tag for the second γ- AApeptide building block on the outer layer of the. Subsequently, the third and fourth γ- AApeptide building blocks were also introduced to the sequence by repeating the first step for 3 times. During the last step, the N-terminus of γ-AApeptides on the outer layer was capped with the 4-(bromomethyl)benzoyl chloride, then the Dmt group on the thiol linker was removed with 2% TFA in DCM. The macrocyclization was achieved by the SN2 reaction between thiol and bromomethyl moieties in the presence of the ammonium carbonate ($(NH_4)_2CO_3$). In the end, all the side chain protecting groupss were removed with 94% TFA, 2% triisopropylsilane, 2% water, and 2% thioanisole (v:v:v:v). After the accomplishment of the cyclic γ-AApeptides library, the quality of the library was examined by picking out 10 beads randomly, which were treated with CNBr and followed by MALDI-MS/MS analysis. 9 out 10 selected beads showed unambiguous MS/MS fragmentation patterns based on the decoding information, indicating the quality of the library was high.

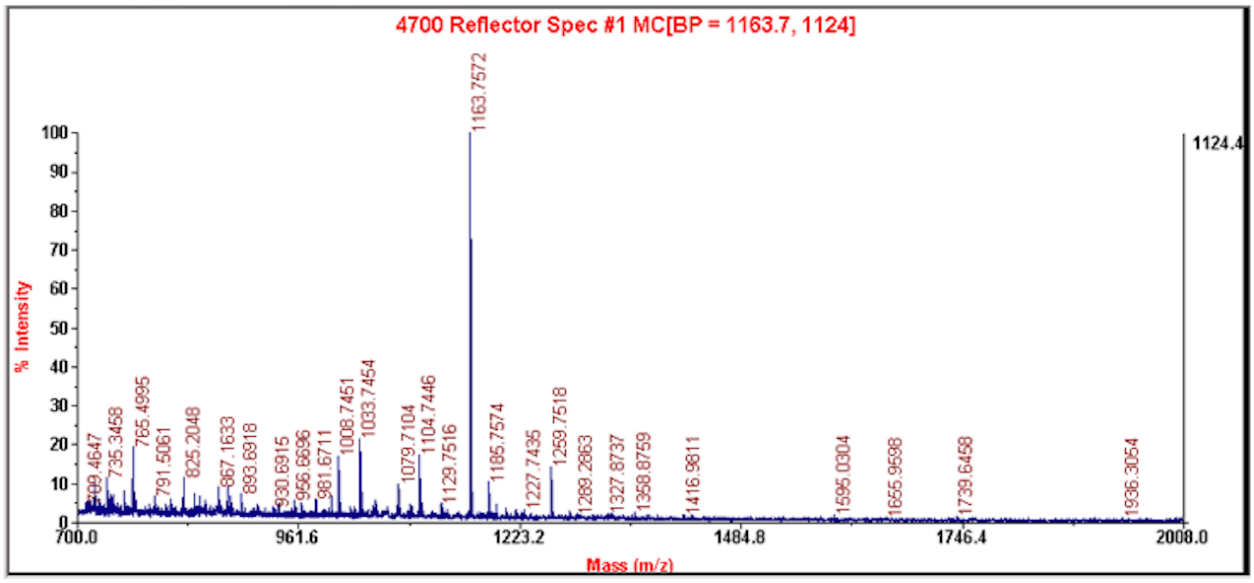
*Synthesis of the **1-Fl** and **2-Fl***

The synthesis of the FITC-labeled hits was conducted on the Rink amide resin. After the Fmoc protecting group of the Rink amide was removed, the Fmoc-Lys(Dde)-OH was first attached. Next, the desired building blocks which were determined by MALDI MS/MS were subsequently coupled to the resin under standard peptide coupling conditions. After the cyclization was achieved, the Dde group of the Fmoc-Lys(Dde)-OH was removed, then the exposed amine was coupled with 5,6-carboxyfluorescein (2 equiv.) and DIPEA (6 equiv.) in DMF by shaking the reaction for 12 h at room temperature. The FITC labeled cyclic γ-peptide was treated with 1:1 (v/v) DCM/TFA containing 2% triisopropylsilane. The crude product was purified by the Waters HPLC system with flow rate of 1 mL/min for analytic module and 16 mL/min for preparative module with a linear gradient from 5% to 100% (CH_3CN in water) in 40 min.

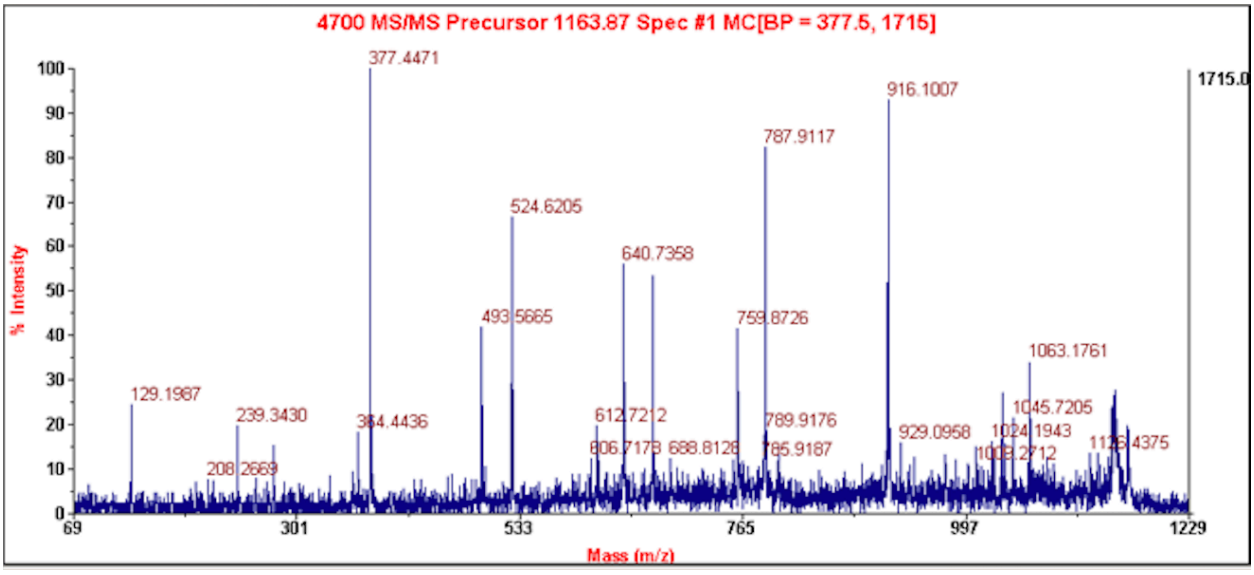
References:

[1] Y. Shi, S. Challa, P. Sang, F. She, C. Li, G. M. Gray, A. Nimmagadda, P. Teng, T. Odom, Y. Wang, A. van der Vaart, Q. Li, J. Cai, *J. Med. Chem.* **2017**, *60*, 9290-9298.

MALDI-Mass of the selected hits



MALDI-MS/MS of the selected hits



The determination of decoding sequences

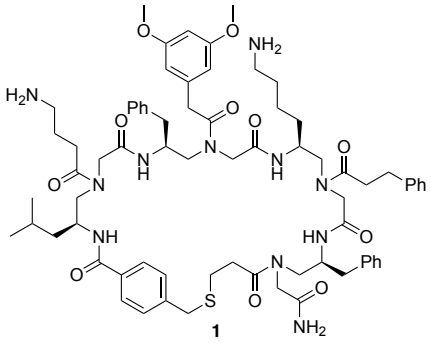
H* stands for the homoserine lactone.

Compound 1 Dde-Val-Leu-Asp-Phe-Phe-Lys-Phe-H*

MS/MS Fragment: 377-493-640-787-916-1063

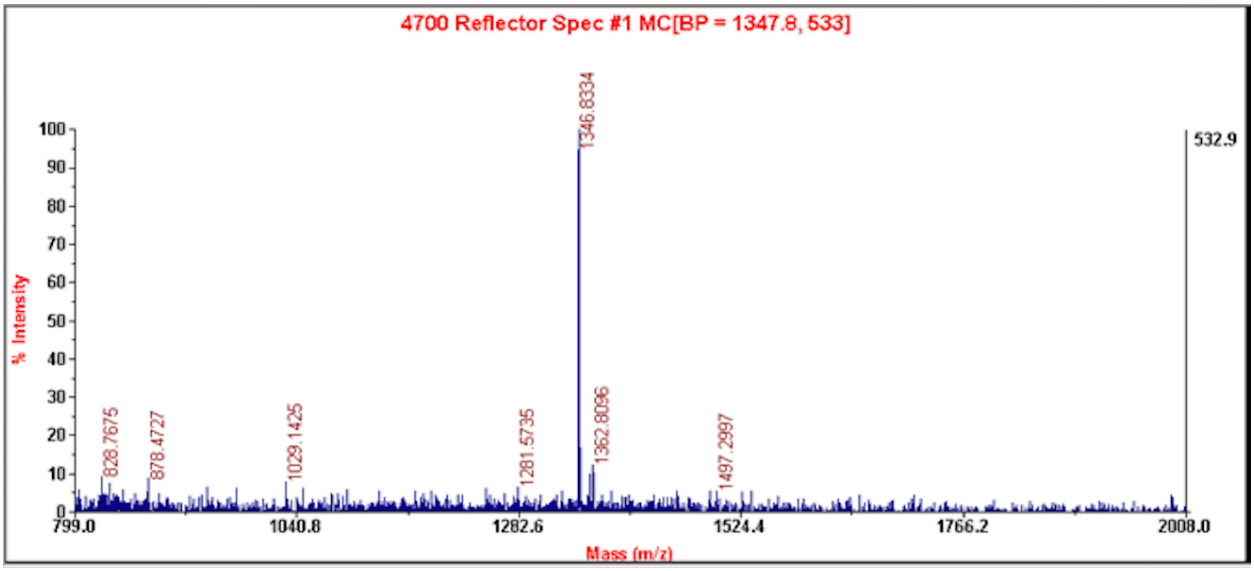
Stand For: 264 (Dde-Val), 377 (147, Leu), 492 (113, Asp), 639 (113, Phe), 786 (147, Phe), 914 (128, Lys), 1061 (147, Phe)

MALDI-Mass spectra of 1

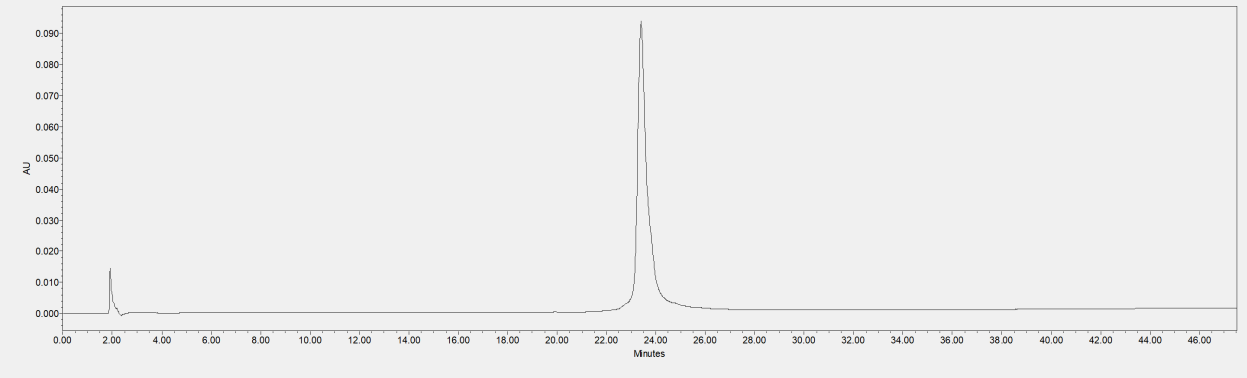


1

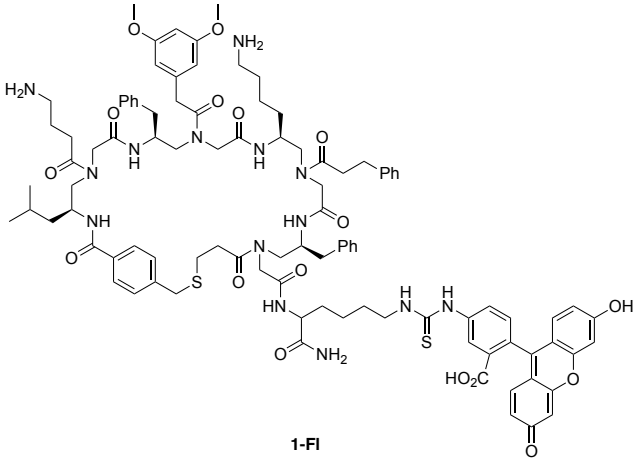
Exact Mass: 1323.71
Molecular Weight: 1324.69



HPLC Trace of 1



MALDI-Mass spectra of 1-Fl



1-Fl

Exact Mass: 1840.84
Molecular Weight: 1842.25