SUPPORTING INFORMATION FOR:

A Designed Small Molecule Inhibitor of a Non-Coding RNA Sensitizes HER2 Negative Cancers to Herceptin

Matthew G. Costales, Dominic G. Hoch, Daniel Abegg, Jessica L. Childs-Disney, Sai Pradeep Velagapudi, Alexander Adibekian, and Matthew D. Disney*

Department of Chemistry,

The Scripps Research Institute,

130 Scripps Way, Jupiter, FL 33458

*Email: Disney@scripps.edu

SUPPLEMENTARY TABLES & FIGURES

Table S1. Binding affinities of compounds to nucleic acids (n=4). K_d is indicated in μM. Data are presented as mean ± s.e.m.

Compound	Nucleic Acid					
	RNA 1 (Drosha 885)	**RNA 2 (Drosha 515)**	**RNA 3 (adjacent to Drosha 515)**	**RNA 4 (Drosha and adjacent 515)**	**tRNA**	**DNA Hairpin**
1	9.0 ± 1.0	12 ± 2.1	7.5 ± 0.7	15 ± 1.9	>100	>100
2	>100	>100	>100	0.06 ± 0.01	>100	>100
1a	-	-	**-**	18 ± 0.56		-
1b	-	-	**-**	20 ± 2.4		-

Table S2. Pathway analysis from RNA-Seq differential expression data between untreated and **2**-treated MCF-7 cells after 24 h, as determined by Ingenuity Pathway Analysis (Qiagen). Only adjusted p-values are displayed. On-target pathways are highlighted in gray.

Upstream Analysis		
Upstream Regulator	**Molecule Type**	**p-value of overlap**
E2F1	Transcription regulator	8.08E-04
SP1	Transcription regulator	2.46E-03
TOP1	Enzyme	3.95E-03
KLF5	Transcription regulator	3.95E-03
RHOA	Enzyme	3.95E-03
BHLHE40	Transcription regulator	7.88E-03
SRA1	Transcription regulator	1.18E-02
TGFB1	Growth factor	1.52E-02
CDKN1A	Kinase	1.57E-02
PPP5C	Phosphatase	1.57E-02
ESR1	Ligand-dependent nuclear receptor	1.78E-02
HEXIM1	Transcription regulator	2.35E-02
SP3	Transcription regulator	2.35E-02
ERBB2	Kinase	2.68E-02
NAMPT	Cytokine	2.73E-02
SMC3	Other	3.12E-02
RAD21	Transcription regulator	3.12E-02
PARP1	Enzyme	3.88E-02

Diseases and Functions		
Disease or Function Annotation	**Molecules**	**p-value**
Apoptosis of breast cancer cell lines (Inhibition)	CYR61, DDIT4, DUSP1, DUSP4	5.93E-03
Cell proliferation of breast cancer cell lines (Activation)	CYR61, GDF15, ID1, IRS2	1.59E-02

Table S3. Primers used for RT-qPCR. Universal reverse primer from Qiagen was used for each miRNA unless a specific reverse primer is indicated.

Primer	Sequence (5' – 3')	Primer	Sequence (5' – 3')
pri-miR-515 F	AGGCGTGAGCCACCGCG	hsa-miR-23b-3p	ATCACATTGCCAGGGATTACC
pri-miR-515 R	GCACGCTCTGCAAATGTGTTA	hsa-miR-23b-5p	TGGGTTCCTGGCATGCTGATTT
hsa-miR-124	TAAGGCACGCGGTGAATGCC	hsa-miR-24-3p	TGGCTCAGTTCAGCAGGAACAG
hsa-miR-506	TAAGGCACCCTTCTGAGTAGA	hsa-miR-25-3p	CATTGCACTTGTCTCGGTCTGA
hsa-miR-206	TGGAATGTAAGGAAGTGTGTGG	hsa-miR-25-5p	AGGCGGAGACTTGGGCAATTG
hsa-miR-495	AAACAAACATGGTGCACTTCTT	hsa-miR-26a-5p	TTCAAGTAATCCAGGATAGGCT
hsa-miR-411	TAGTAGACCGTATAGCGTACG	hsa-miR-26b-5p	TTCAAGTAATTCAGGATAGGT
hsa-miR-876-5p	TGGATTTCTTTGTGAATCACCA	hsa-miR-27a-3p	TTCACAGTGGCTAAGTTCCGC
hsa-miR-28	CACTAGATTGTGAGCTCCTGGA	hsa-miR-27a-5p	AGGGCTTAGCTGCTTGTGAGCA
hsa-miR-708	AGCTTACAATCTAGCTGGGAA	hsa-miR-27b-3p	TTCACAGTGGCTAAGTTCTGC
hsa-miR-659	CTTGGTTCAGGGAGGGTCCCCA	hsa-miR-27b-5p	AGAGCTTAGCTGATTGGTGAAC
hsa-miR-608	AGGGGTGGTGTTGGGACAGCTCCGT	hsa-miR-28-3p	CACTAGATTGTGAGCTCCTGGA
hsa-miR-1229	GGGCTCTCACCACTGCCCTCCCACAGAA	hsa-miR-28-5p	AAGGAGCTCACAGTCTATTGAG
hsa-miR-330-3p	GCAAAGCACACGGCCTGCAGAGA	hsa-miR-296-3p	GAGGGTTGGGTGGAGGCTCTCC
hsa-miR-525-5p	CTCCAGAGGGATGCACTTTCT	hsa-miR-298	AGCAGAAGCAGGGAGGTTCTCCCA
hsa-miR-1207-3p	TCAGCTGGCCCTCATTTC	hsa-miR-299-5p	TGGTTTACCGTCCCACATACAT
hsa-miR-1290	TGGATTTTTGGATCAGGGA	hsa-miR-29a-3p	TAGCACCATCTGAAATCGGTTA
hsa-miR-668	TGTCACTCGGCTCGGCCCACTAC	hsa-miR-29a-5p	ACTGATTTCTTTTGGTGTTCAG
hsa-miR-1291	TGGCCCTGACTGAAGACCAGCAGT	hsa-miR-29b-3p	TAGCACCATTTGAAATCAGTGTT
hsa-miR-940	AAGGCAGGGCCCCCGCTCCCC	hsa-miR-29b-2-5p	CTGGTTTCACATGGTGGCTTAG
hsa-miR-342-5p	AGGGGTGCTATCTGTGATTGA	hsa-miR-29c-3p	TAGCACCATTTGAAATCGGTTA
hsa-miR-1184	CCTGCAGCGACTTGATGGCTTCCAA	hsa-miR-300	TATACAAGGGCAGACTCTCTCT
hsa-miR-296-5p	AGGGCCCCCCCTCAATCCTGT	hsa-miR-301a-3p	CAGTGCAATAGTATTGTCAAAGC
RNU6	ACACGCAAATTCGTGAAGCGTTC	hsa-miR-302a-3p	TAAGTGCTTCCATGTTTTGGTGA
hsa-let-7a-5p	TGAGGTAGTAGGTTGTATAGTT	hsa-miR-302a-5p	ACTTAAACGTGGATGTACTTGCT
hsa-let-7b-5p	TGAGGTAGTAGGTTGTGTGGTT	hsa-miR-302b-3p	TAAGTGCTTCCATGTTTTAGTAG
hsa-let-7c-5p	TGAGGTAGTAGGTTGTATGGTT	hsa-miR-302b-5p	ACTTTAACATGGAAGTGCTTTC
hsa-let-7d-5p	AGAGGTAGTAGGTTGCATAGTT	hsa-miR-302c-3p	TAAGTGCTTCCATGTTTCAGTGG
hsa-let-7d-3p	CTATACGACCTGCTGCCTTTCT	hsa-miR-30a-5p	TGTAAACATCCTCGACTGGAAG
hsa-let-7e-5p	TGAGGTAGGAGGTTGTATAGTT	hsa-miR-30b-5p	TGTAAACATCCTACACTCAGCT
hsa-let-7f-5p	TGAGGTAGTAGATTGTATAGTT	hsa-miR-30c-5p	TGTAAACATCCTACACTCTCAGC
hsa-let-7g-5p	TGAGGTAGTAGTTTGTACAGTT	hsa-miR-30c-1-3p	CTGGGAGAGGGTTGTTTACTCC
hsa-let-7g-3p	CTGTACAGGCCACTGCCTTGC	hsa-miR-30d-5p	TGTAAACATCCCCGACTGGAAG
hsa-let-7i-5p	TGAGGTAGTAGTTTGTGCTGTT	hsa-miR-30d-3p	CTTTCAGTCAGATGTTTGCTGC
hsa-miR-1-3p	TGGAATGTAAAGAAGTATGTAT	hsa-miR-30e-5p	TGTAAACATCCTTGACTGGAAG
hsa-miR-100-5p	AACCCGTAGATCCGAACTTGTG	hsa-miR-30e-3p	CTTTCAGTCGGATGTTTACAGC
hsa-miR-101-3p	TACAGTACTGTGATAACTGAA	hsa-miR-31-5p	AGGCAAGATGCTGGCATAGCT
hsa-miR-101-5p	CAGTTATCACAGTGCTGATGCT	hsa-miR-31-3p	TGCTATGCCAACATATTGCCAT
hsa-miR-103a-3p	AGCAGCATTGTACAGGGCTATGA	hsa-miR-32-5p	TATTGCACATTACTAAGTTGCA

hsa-miR-105-5p	TCAAATGCTCAGACTCCTGTGGT	hsa-miR-32-3p	CAATTTAGTGTGTGTGATATTT
hsa-miR-106a-3p	CTGCAATGTAAGCACTTCTTAC	hsa-miR-320a	AAAAGCTGGGTTGAGAGGGCGA
hsa-miR-106b-5p	TAAAGTGCTGACAGTGCAGAT	hsa-miR-320b	AAAAGCTGGGTTGAGAGGGCAA
hsa-miR-106b-3p	CCGCACTGTGGGTACTTGCTGC	hsa-miR-323b-5p	AGGTTGTCCGTGGTGAGTTCGCA
hsa-miR-107	AGCAGCATTGTACAGGGCTATCA	hsa-miR-324-3p	ACTGCCCCAGGTGCTGCTGG
hsa-miR-10a-5p	TACCCTGTAGATCCGAATTTGTG	hsa-miR-324-5p	CGCATCCCCTAGGGCATTGGTGT
hsa-miR-10a-3p	CAAATTCGTATCTAGGGGAATA	hsa-miR-325	CCTAGTAGGTGTCCAGTAAGTGT
hsa-miR-10b-5p	TACCCTGTAGAACCGAATTTGTG	hsa-miR-326	CCTCTGGGCCCTTCCTCCAG
hsa-miR-10b-3p	ACAGATTCGATTCTAGGGGAAT	hsa-miR-328-3p	CTGGCCCTCTCTGCCCTTCCGT
hsa-miR-1180-3p	TTTCCGGCTCGCGTGGGTGTGT	hsa-miR-330-3p	GCAAAGCACACGGCCTGCAGAGA
hsa-miR-1207-5p	TGGCAGGGAGGCTGGGAGGGG	hsa-miR-331-5p	CTAGGTATGGTCCCAGGGATCC
hsa-miR-122-5p	TGGAGTGTGACAATGGTGTTTG	hsa-miR-335-5p	TCAAGAGCAATAACGAAAAATGT
hsa-miR-122-3p	AACGCCATTATCACACTAAATA	hsa-miR-335-3p	TTTTTCATTATTGCTCCTGACC
hsa-miR-1224-3p	CCCCACCTCCTCTCTCCTCAG	hsa-miR-337-5p	GAACGGCTTCATACAGGAGTT
hsa-miR-1224-5p	GTGAGGACTCGGGAGGTGG	hsa-miR-338-3p	TCCAGCATCAGTGATTTTGTTG
hsa-miR-124-3p	TAAGGCACGCGGTGAATGCC	hsa-miR-338-5p	AACAATATCCTGGTGCTGAGTG
hsa-miR-124-5p	CGTGTTCACAGCGGACCTTGAT	hsa-miR-339-3p	TGAGCGCCTCGACGACAGAGCCG
hsa-miR-125a-3p	ACAGGTGAGGTTCTTGGGAGCC	hsa-miR-33a-5p	GTGCATTGTAGTTGCATTGCA
hsa-miR-125a-5p	TCCCTGAGACCCTTTAACCTGTGA	hsa-miR-33a-3p	CAATGTTTCCACAGTGCATCAC
hsa-miR-125b-5p	TCCCTGAGACCCTAACTTGTGA	hsa-miR-33b-5p	GTGCATTGCTGTTGCATTGC
hsa-miR-126-3p	TCGTACCGTGAGTAATAATGCG	hsa-miR-340-5p	TTATAAAGCAATGAGACTGATT
hsa-miR-126-5p	CATTATTACTTTTGGTACGCG	hsa-miR-340-3p	TCCGTCTCAGTTACTTTATAGC
hsa-miR-1265	CAGGATGTGGTCAAGTGTTGTT	hsa-miR-342-3p	TCTCACACAGAAATCGCACCCGT
hsa-miR-127-3p	TCGGATCCGTCTGAGCTTGGCT	hsa-miR-342-5p	AGGGGTGCTATCTGTGATTGA
hsa-miR-127-5p	CTGAAGCTCAGAGGGCTCTGAT	hsa-miR-345-5p	GCTGACTCCTAGTCCAGGGCTC
hsa-miR-128-3p	TCACAGTGAACCGGTCTCTTT	hsa-miR-346	TGTCTGCCCGCATGCCTGCCTCT
hsa-miR-1284	TCTATACAGACCCTGGCTTTTC	hsa-miR-34a-5p	TGGCAGTGTCTTAGCTGGTTGT
hsa-miR-129-1-3p	AAGCCCTTACCCCAAAAAGTAT	hsa-miR-34a-3p	CAATCAGCAAGTATACTGCCCT
hsa-miR-1290	TGGATTTTTGGATCAGGGA	hsa-miR-34b-3p	CAATCACTAACTCCACTGCCAT
hsa-miR-129-2-3p	AAGCCCTTACCCCAAAAAGCAT	hsa-miR-34b-5p	TAGGCAGTGTCATTAGCTGATTG
hsa-miR-129-5p	CTTTTTGCGGTCTGGGCTTGC	hsa-miR-34c-3p	AATCACTAACCACACGGCCAGG
hsa-miR-130a-3p	CAGTGCAATGTTAAAAGGGCAT	hsa-miR-34c-5p	AGGCAGTGTAGTTAGCTGATTGC
hsa-miR-130b-3p	CAGTGCAATGATGAAAGGGCAT	hsa-miR-361-5p	TTATCAGAATCTCCAGGGGTAC
hsa-miR-132-3p	TAACAGTCTACAGCCATGGTCG	hsa-miR-363-3p	AATTGCACGGTATCCATCTGTA
hsa-miR-132-5p	ACCGTGGCTTTCGATTGTTACT	hsa-miR-363-5p	CGGGTGGATCACGATGCAATTT
hsa-miR-1324	CCAGACAGAATTCTATGCACTTTC	hsa-miR-365a-3p	TAATGCCCCTAAAAATCCTTAT
hsa-miR-133a-3p	TTTGGTCCCCTTCAACCAGCTG	hsa-miR-370-3p	GCCTGCTGGGGTGGAACCTGGT
hsa-miR-133b	TTTGGTCCCCTTCAACCAGCTA	hsa-miR-372-3p	AAAGTGCTGCGACATTTGAGCGT
hsa-miR-134-5p	TGTGACTGGTTGACCAGAGGGG	hsa-miR-373-3p	GAAGTGCTTCGATTTTGGGGTGT
hsa-miR-135a-5p	TATGGCTTTTTATTCCTATGTGA	hsa-miR-373-5p	ACTCAAAATGGGGGCGCTTTCC
hsa-miR-135b-5p	TATGGCTTTTCATTCCTATGTGA	hsa-miR-374a-5p	TTATAATACAACCTGATAAGTG

hsa-miR-136-5p	ACTCCATTTGTTTTGATGATGGA	hsa-miR-374b-5p	ATATAATACAACCTGCTAAGTG
hsa-miR-137	TTATTGCTTAAGAATACGCGTAG	hsa-miR-375	TTTGTTCGTTCGGCTCGCGTGA
hsa-miR-138-5p	AGCTGGTGTTGTGAATCAGGCCG	hsa-miR-376a-3p	ATCATAGAGGAAAATCCACGT
hsa-miR-139-3p	TGGAGACGCGGCCCTGTTGGAGT	hsa-miR-376b-3p	ATCATAGAGGAAAATCCATGTT
hsa-miR-139-5p	TCTACAGTGCACGTGTCTCCAGT	hsa-miR-376c-3p	AACATAGAGGAAATTCCACGT
hsa-miR-140-3p	TACCACAGGGTAGAACCACGG	hsa-miR-377-3p	ATCACACAAAGGCAACTTTTGT
hsa-miR-140-5p	CAGTGGTTTTACCCTATGGTAG	hsa-miR-377-5p	AGAGGTTGCCCTTGGTGAATTC
hsa-miR-141-3p	TAACACTGTCTGGTAAAGATGG	hsa-miR-378a-3p	ACTGGACTTGGAGTCAGAAGGC
hsa-miR-142-3p	TGTAGTGTTTCCTACTTTATGGA	hsa-miR-378a-5p	CTCCTGACTCCAGGTCCTGTGT
hsa-miR-142-5p	CATAAAGTAGAAAGCACTACT	hsa-miR-379-5p	TGGTAGACTATGGAACGTAGG
hsa-miR-143-3p	TGAGATGAAGCACTGTAGCTC	hsa-miR-381-3p	TATACAAGGGCAAGCTCTCTGT
hsa-miR-143-5p	GGTGCAGTGCTGCATCTCTGGT	hsa-miR-382-5p	GAAGTTGTTCGTGGTGGATTCG
hsa-miR-144-3p	TACAGTATAGATGATGTACT	hsa-miR-383-5p	AGATCAGAAGGTGATTGTGGCT
hsa-miR-144-5p	GGATATCATCATATACTGTAAG	hsa-miR-409-3p	GAATGTTGCTCGGTGAACCCCT
hsa-miR-145-5p	GTCCAGTTTTCCCAGGAATCCCT	hsa-miR-410-3p	AATATAACACAGATGGCCTGT
hsa-miR-145-3p	GGATTCCTGGAAATACTGTTCT	hsa-miR-411-5p	TAGTAGACCGTATAGCGTACG
hsa-miR-146a-5p	TGAGAACTGAATTCCATGGGTT	hsa-miR-421	ATCAACAGACATTAATTGGGCGC
hsa-miR-146b-5p	TGAGAACTGAATTCCATAGGCT	hsa-miR-423-3p	AGCTCGGTCTGAGGCCCCTCAGT
hsa-miR-147a	GTGTGTGGAAATGCTTCTGC	hsa-miR-423-5p	TGAGGGGCAGAGAGCGAGACTTT
hsa-miR-148a-3p	TCAGTGCACTACAGAACTTTGT	hsa-miR-424-5p	CAGCAGCAATTCATGTTTTGAA
hsa-miR-148b-3p	TCAGTGCATCACAGAACTTTGT	hsa-miR-424-3p	CAAAACGTGAGGCGCTGCTAT
hsa-miR-149-5p	TCTGGCTCCGTGTCTTCACTCCC	hsa-miR-425-5p	AATGACACGATCACTCCCGTTGA
hsa-miR-150-5p	TCTCCCAACCCTTGTACCAGTG	hsa-miR-425-3p	ATCGGGAATGTCGTGTCCGCCC
hsa-miR-151a-3p	CTAGACTGAAGCTCCTTGAGG	hsa-miR-4258	CCCCGCCACCGCCTTGG
hsa-miR-151a-5p	TCGAGGAGCTCACAGTCTAGT	hsa-miR-429	TAATACTGTCTGGTAAAACCGT
hsa-miR-152-3p	TCAGTGCATGACAGAACTTGG	hsa-miR-431-5p	TGTCTTGCAGGCCGTCATGCA
hsa-miR-153-3p	TTGCATAGTCACAAAAGTGATC	hsa-miR-432-5p	TCTTGGAGTAGGTCATTGGGTGG
hsa-miR-154-5p	TAGGTTATCCGTGTTGCCTTCG	hsa-miR-433-3p	ATCATGATGGGCTCCTCGGTGT
hsa-miR-155-5p	TTAATGCTAATCGTGATAGGGGT	hsa-miR-449a	TGGCAGTGTATTGTTAGCTGGT
hsa-miR-155-3p	CTCCTACATATTAGCATTAACA	hsa-miR-451a	AAACCGTTACCATTACTGAGTT
hsa-miR-15a-5p	TAGCAGCACATAATGGTTTGTG	hsa-miR-454-3p	TAGTGCAATATTGCTTATAGGGT
hsa-miR-15b-5p	TAGCAGCACATCATGGTTTACA	hsa-miR-455-5p	TATGTGCCTTTGGACTACATCG
hsa-miR-15b-3p	CGAATCATTATTTGCTGCTCTA	hsa-miR-483-5p	AAGACGGGAGGAAAGAAGGGAG
hsa-miR-16-5p	TAGCAGCACGTAAATATTGGCG	hsa-miR-484	TCAGGCTCAGTCCCCTCCCGAT
hsa-miR-17-5p	CAAAGTGCTTACAGTGCAGGTAG	hsa-miR-485-5p	AGAGGCTGGCCGTGATGAATTC
hsa-miR-181a-5p	AACATTCAACGCTGTCGGTGAGT	hsa-miR-486-3p	CGGGGCAGCTCAGTACAGGAT
hsa-miR-181a-3p	ACCATCGACCGTTGATTGTACC	hsa-miR-486-5p	TCCTGTACTGAGCTGCCCCGAG
hsa-miR-181b-5p	AACATTCATTGCTGTCGGTGGGT	hsa-miR-488-3p	TTGAAAGGCTATTTCTTGGTC
hsa-miR-181c-5p	AACATTCAACCTGTCGGTGAGT	hsa-miR-489-3p	GTGACATCACATATACGGCAGC
hsa-miR-181c-3p	AACCATCGACCGTTGAGTGGAC	hsa-miR-490-3p	CAACCTGGAGGACTCCATGCTG
hsa-miR-181d-5p	AACATTCATTGTTGTCGGTGGGT	hsa-miR-491-5p	AGTGGGGAACCCTTCCATGAGG

hsa-miR-182-5p	TTTGGCAATGGTAGAACTCACACT	hsa-miR-493-3p	TGAAGGTCTACTGTGTGCCAGG
hsa-miR-182-3p	TGGTTCTAGACTTGCCAACTA	hsa-miR-495-3p	AAACAAACATGGTGCACTTCTT
hsa-miR-183-5p	TATGGCACTGGTAGAATTCACT	hsa-miR-497-5p	CAGCAGCACACTGTGGTTTGT
hsa-miR-183-3p	GTGAATTACCGAAGGGCCATAA	hsa-miR-498	TTTCAAGCCAGGGGGCGTTTTTC
hsa-miR-184	TGGACGGAGAACTGATAAGGGT	hsa-miR-499a-3p	AACATCACAGCAAGTCTGTGCT
hsa-miR-185-5p	TGGAGAGAAAGGCAGTTCCTGA	hsa-miR-499a-5p	TTAAGACTTGCAGTGATGTTT
hsa-miR-186-5p	CAAAGAATTCTCCTTTTGGGCT	hsa-miR-500a-5p	TAATCCTTGCTACCTGGGTGAGA
hsa-miR-186-3p	GCCCAAAGGTGAATTTTTTGGG	hsa-miR-504-5p	AGACCCTGGTCTGCACTCTATC
hsa-miR-187-3p	TCGTGTCTTGTGTTGCAGCCGG	hsa-miR-505-3p	CGTCAACACTTGCTGGTTTCCT
hsa-miR-187-5p	GGCTACAACACAGGACCCGGGC	hsa-miR-506-3p	TAAGGCACCCTTCTGAGTAGA
hsa-miR-18a-5p	TAAGGTGCATCTAGTGCAGATAG	hsa-miR-508-5p	TACTCCAGAGGGCGTCACTCATG
hsa-miR-18a-3p	ACTGCCCTAAGTGCTCCTTCTGG	hsa-miR-509-3p	TGATTGGTACGTCTGTGGGTAG
hsa-miR-18b-5p	TAAGGTGCATCTAGTGCAGTTAG	hsa-miR-511-5p	GTGTCTTTTGCTCTGCAGTCA
hsa-miR-18b-3p	TGCCCTAAATGCCCCTTCTGGC	hsa-miR-512-5p	CACTCAGCCTTGAGGGCACTTTC
hsa-miR-190a-5p	TGATATGTTTGATATATTAGGT	hsa-miR-513a-5p	TTCACAGGGAGGTGTCAT
hsa-miR-1908-5p	CGGCGGGGACGGCGATTGGTC	hsa-miR-514a-3p	ATTGACACTTCTGTGAGTAGA
hsa-miR-191-5p	CAACGGAATCCCAAAAGCAGCTG	hsa-miR-517a-3p	ATCGTGCATCCCTTTAGAGTGT
hsa-miR-191-3p	GCTGCGCTTGGATTTCGTCCCC	hsa-miR-518b	CAAAGCGCTCCCCTTTAGAGGT
hsa-miR-1914-3p	GGAGGGGTCCCGCACTGGGAGG	hsa-miR-519d-3p	CAAAGTGCCTCCCTTTAGAGTG
hsa-miR-192-5p	CTGACCTATGAATTGACAGCC	hsa-miR-520c-3p	AAAGTGCTTCCTTTTAGAGGGT
hsa-miR-192-3p	CTGCCAATTCCATAGGTCACAG	hsa-miR-520g-3p	ACAAAGTGCTTCCCTTTAGAGTGT
hsa-miR-193a-3p	AACTGGCCTACAAAGTCCCAGT	hsa-miR-522-3p	AAAATGGTTCCCTTTAGAGTGT
hsa-miR-193a-5p	TGGGTCTTTGCGGGCGAGATGA	hsa-miR-524-5p	CTACAAAGGGAAGCACTTTCTC
hsa-miR-193b-3p	AACTGGCCCTCAAAGTCCCGCT	hsa-miR-532-5p	CATGCCTTGAGTGTAGGACCGT
hsa-miR-193b-5p	CGGGGTTTTGAGGGCGAGATGA	hsa-miR-539-5p	GGAGAAATTATCCTTGGTGTGT
hsa-miR-194-5p	TGTAACAGCAACTCCATGTGGA	hsa-miR-542-3p	TGTGACAGATTGATAACTGAAA
hsa-miR-195-5p	TAGCAGCACAGAAATATTGGC	hsa-miR-542-5p	TCGGGGATCATCATGTCACGAGA
hsa-miR-195-3p	CCAATATTGGCTGTGCTGCTCC	hsa-miR-549a	TGACAACTATGGATGAGCTCT
hsa-miR-196a-5p	TAGGTAGTTTCATGTTGTTGGG	hsa-miR-551b-3p	GCGACCCATACTTGGTTTCAG
hsa-miR-196a-3p	CGGCAACAAGAAACTGCCTGAG	hsa-miR-567	AGTATGTTCTTCCAGGACAGAAC
hsa-miR-196b-5p	TAGGTAGTTTCCTGTTGTTGGG	hsa-miR-570-3p	CGAAAACAGCAATTACCTTTGC
hsa-miR-197-3p	TTCACCACCTTCTCCACCCAGC	hsa-miR-574-3p	CACGCTCATGCACACACCCACA
hsa-miR-199a-3p	ACAGTAGTCTGCACATTGGTTA	hsa-miR-575	GAGCCAGTTGGACAGGAGC
hsa-miR-199a-5p	CCCAGTGTTCAGACTACCTGTTC	hsa-miR-580-3p	TTGAGAATGATGAATCATTAGG
hsa-miR-199b-5p	CCCAGTGTTTAGACTATCTGTTC	hsa-miR-581	TCTTGTGTTCTCTAGATCAGT
hsa-miR-19a-3p	TGTGCAAATCTATGCAAAACTGA	hsa-miR-583	CAAAGAGGAAGGTCCCATTAC
hsa-miR-19a-5p	AGTTTTGCATAGTTGCACTACA	hsa-miR-588	TTGGCCACAATGGGTTAGAAC
hsa-miR-19b-3p	TGTGCAAATCCATGCAAAACTGA	hsa-miR-589-3p	TCAGAACAAATGCCGGTTCCCAGA
hsa-miR-200a-3p	TAACACTGTCTGGTAACGATGT	hsa-miR-600	ACTTACAGACAAGAGCCTTGCTC
hsa-miR-200a-5p	CATCTTACCGGACAGTGCTGGA	hsa-miR-605-5p	TAAATCCCATGGTGCCTTCTCCT
hsa-miR-200b-3p	TAATACTGCCTGGTAATGATGA	hsa-miR-606	AAACTACTGAAAATCAAAGAT

hsa-miR-200c-3p	TAATACTGCCGGGTAATGATGGA	hsa-miR-608	AGGGGTGGTGTTGGGACAGCTCCGT
hsa-miR-200c-5p	CGTCTTACCCAGCAGTGTTTGG	hsa-miR-622	ACAGTCTGCTGAGGTTGGAGC
hsa-miR-202-3p	AGAGGTATAGGGCATGGGAA	hsa-miR-626	AGCTGTCTGAAAATGTCTT
hsa-miR-203a-3p	GTGAAATGTTTAGGACCACTAG	hsa-miR-639	ATCGCTGCGGTTGCGAGCGCTGT
hsa-miR-204-5p	TTCCCTTTGTCATCCTATGCCT	hsa-miR-643	ACTTGTATGCTAGCTCAGGTAG
hsa-miR-205-5p	TCCTTCATTCCACCGGAGTCTG	hsa-miR-649	AAACCTGTGTTGTTCAAGAGTC
hsa-miR-205-3p	GATTTCAGTGGAGTGAAGTTC	hsa-miR-652-3p	AATGGCGCCACTAGGGTTGTG
hsa-miR-206	TGGAATGTAAGGAAGTGTGTGG	hsa-miR-661	TGCCTGGGTCTCTGGCCTGCGCGT
hsa-miR-208a-3p	ATAAGACGAGCAAAAAGCTTGT	hsa-miR-7-5p	TGGAAGACTAGTGATTTTGTTGT
hsa-miR-208b-3p	ATAAGACGAACAAAAGGTTTGT	hsa-miR-708-5p	AAGGAGCTTACAATCTAGCTGGG
hsa-miR-20a-5p	TAAAGTGCTTATAGTGCAGGTAG	hsa-miR-708-3p	CAACTAGACTGTGAGCTTCTAG
hsa-miR-20a-3p	ACTGCATTATGAGCACTTAAAG	hsa-miR-515-5p	TTCTCCAAAAGAAAGCACTTTCTG
hsa-miR-20b-5p	CAAAGTGCTCATAGTGCAGGTAG	hsa-miR-744-5p	TGCGGGGCTAGGGCTAACAGCA
hsa-miR-20b-3p	ACTGTAGTATGGGCACTTCCAG	hsa-miR-765	TGGAGGAGAAGGAAGGTGATG
hsa-miR-21-5p	TAGCTTATCAGACTGATGTTGA	hsa-miR-770-5p	TCCAGTACCACGTGTCAGGGCCA
hsa-miR-21-3p	CAACACCAGTCGATGGGCTGT	hsa-miR-802	CAGTAACAAAGATTCATCCTTGT
hsa-miR-210-3p	CTGTGCGTGTGACAGCGGCTGA	hsa-miR-885-5p	TCCATTACACTACCCTGCCTCT
hsa-miR-211-5p	TTCCCTTTGTCATCCTTCGCCT	hsa-miR-888-5p	TACTCAAAAAGCTGTCAGTCA
hsa-miR-212-3p	TAACAGTCTCCAGTCACGGCC	hsa-miR-9-5p	TCTTTGGTTATCTAGCTGTATGA
hsa-miR-214-3p	ACAGCAGGCACAGACAGGCAGT	hsa-miR-9-3p	ATAAAGCTAGATAACCGAAAGT
hsa-miR-215-5p	ATGACCTATGAATTGACAGAC	hsa-miR-920	GGGGAGCTGTGGAAGCAGTA
hsa-miR-216a-5p	TAATCTCAGCTGGCAACTGTGA	hsa-miR-924	AGAGTCTTGTGATGTCTTGC
hsa-miR-217	TACTGCATCAGGAACTGATTGGA	hsa-miR-92a-3p	TATTGCACTTGTCCCGGCCTGT
hsa-miR-218-5p	TTGTGCTTGATCTAACCATGT	hsa-miR-92a-1-5p	AGGTTGGGATCGGTTGCAATGCT
hsa-miR-218-1-3p	ATGGTTCCGTCAAGCACCATGG	hsa-miR-92b-3p	TATTGCACTCGTCCCGGCCTCC
hsa-miR-219a-5p	TGATTGTCCAAACGCAATTCT	hsa-miR-92b-5p	AGGGACGGGACGCGGTGCAGTG
hsa-miR-22-3p	AAGCTGCCAGTTGAAGAACTGT	hsa-miR-93-5p	CAAAGTGCTGTTCGTGCAGGTAG
hsa-miR-22-5p	AGTTCTTCAGTGGCAAGCTTTA	hsa-miR-93-3p	ACTGCTGAGCTAGCACTTCCCG
hsa-miR-221-3p	AGCTACATTGTCTGCTGGGTTTC	hsa-miR-95-3p	TTCAACGGGTATTTATTGAGCA
hsa-miR-221-5p	ACCTGGCATACAATGTAGATTT	hsa-miR-96-5p	TTTGGCACTAGCACATTTTTGCT
hsa-miR-222-3p	AGCTACATCTGGCTACTGGGT	hsa-miR-98-5p	TGAGGTAGTAAGTTGTATTGTT
hsa-miR-222-5p	CTCAGTAGCCAGTGTAGATCCT	hsa-miR-99a-5p	AACCCGTAGATCCGATCTTGTG
hsa-miR-223-3p	TGTCAGTTTGTCAAATACCCCA	hsa-miR-99a-3p	CAAGCTCGCTTCTATGGGTCTG
hsa-miR-223-5p	CGTGTATTTGACAAGCTGAGTT	hsa-miR-99b-5p	CACCCGTAGAACCGACCTTGCG
hsa-miR-224-5p	CAAGTCACTAGTGGTTCCGTT	hsa-mir-525-5p	CTCCAGAGGGATGCACTTTCT
hsa-miR-224-3p	AAAATGGTGCCCTAGTGACTACA	SNORD48	CCCCAGGTAACTCTTGAGTGT
hsa-miR-23a-3p	ATCACATTGCCAGGGATTTCC	SNORD47	TAATGATATCACTGTAAAACC
hsa-miR-23a-5p	GGGGTTCCTGGGGATGGGATTT	SNORD44	ATGCTGACTGAACATGAAGGTC
18S F	GTAACCCGTTGAACCCCATT	18S R	CCATCCAATCGGTAGTAGCG
SK1 F	TATGAATGCCCCTACTTGGTATATG	SK1 R	GCCTCGCTAACCATCAATTCC
SK2 F	GACAGAACGACAGAACCATGC	SK2 R	CAGTCTGGCCGATCAAGGAG

FZD5 F	TGTGGGATGAAGTGGATGAA	FZD5 R	AGGGACAGCAGAGGTGAGAA
Aromatase F	ACGGAAGGTCCTGTGCTCG	Aromatase R	GTATCGGGTTCAGCATTTCCA
GCM1 F	CTGAAGGGGAGCACAGAGAC	GCM1 R	TAGAGCTTCATGGGGTCCAC
HER2 F	GGGAAGAATGGGGTCGTCAAA	HER2 R	CTCCTCCCTGGGGTGTCAAGT
hsa-miR-515-3p	GAGTGCCTTCTTTTGGAGCGTT		

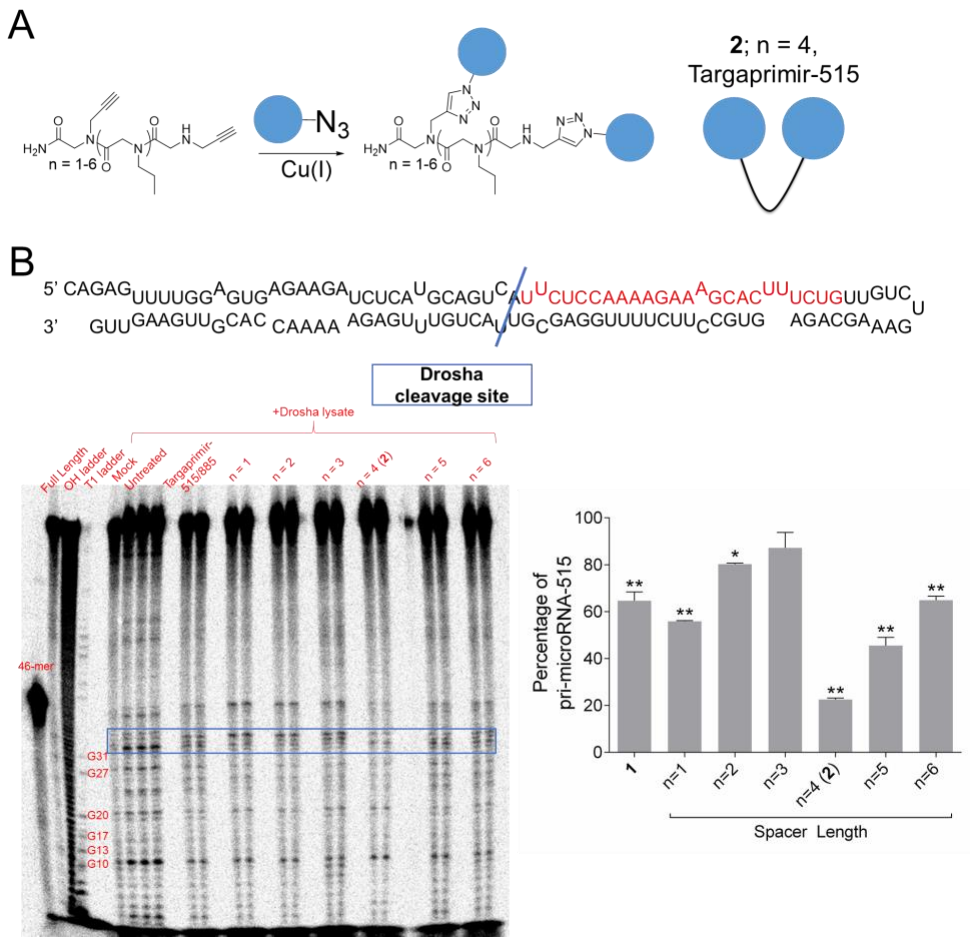


Figure S1: Inhibition of Drosha processing of pri-miR-515 by library of dimer compounds. (**A**) Structures of the library of multivalent (dimeric) compounds that were designed to target the miR-515 hairpin precursor selectively. Compound **2** is Targaprimir-515, which has four propylamine spacers between RNA binding modules. (**B**) Secondary structure of the *in vitro* transcribed pri-miR-515 product; red text indicates mature miR-515-5p product while the blue line indicates the Drosha cleavage site. Representative gel image of the inhibition of pri-miR-515 processing by Drosha with the addition of Targaprimir-515/885 (**1**), n = 1-6 Dimers, n = 4; Targaprimir-515 (**2**) after 30 min of incubation at room temperature. Addition of cell lysate overexpressing Drosha to pri-miR-515 results in the formation of the Drosha product bands (blue box). Gel also includes "46-mer", an RNA marker that is 46 nucleotides in length, untreated full length pri-miR-515, "T1 ladder" generated by digestion with RNase T1, which cleaves after guanine residues, "OH ladder" generated by base hydrolysis, and "Mock" cell lysate not transfected with Drosha. Bottom right, quantification of the protection from Drosha cleavage with compound addition. Data represent mean ± s.e.m. (n≥3). *p<0.05, **p<0.01 compared to untreated samples (normalized to 100%), as determined by a two-tailed Student t-test.

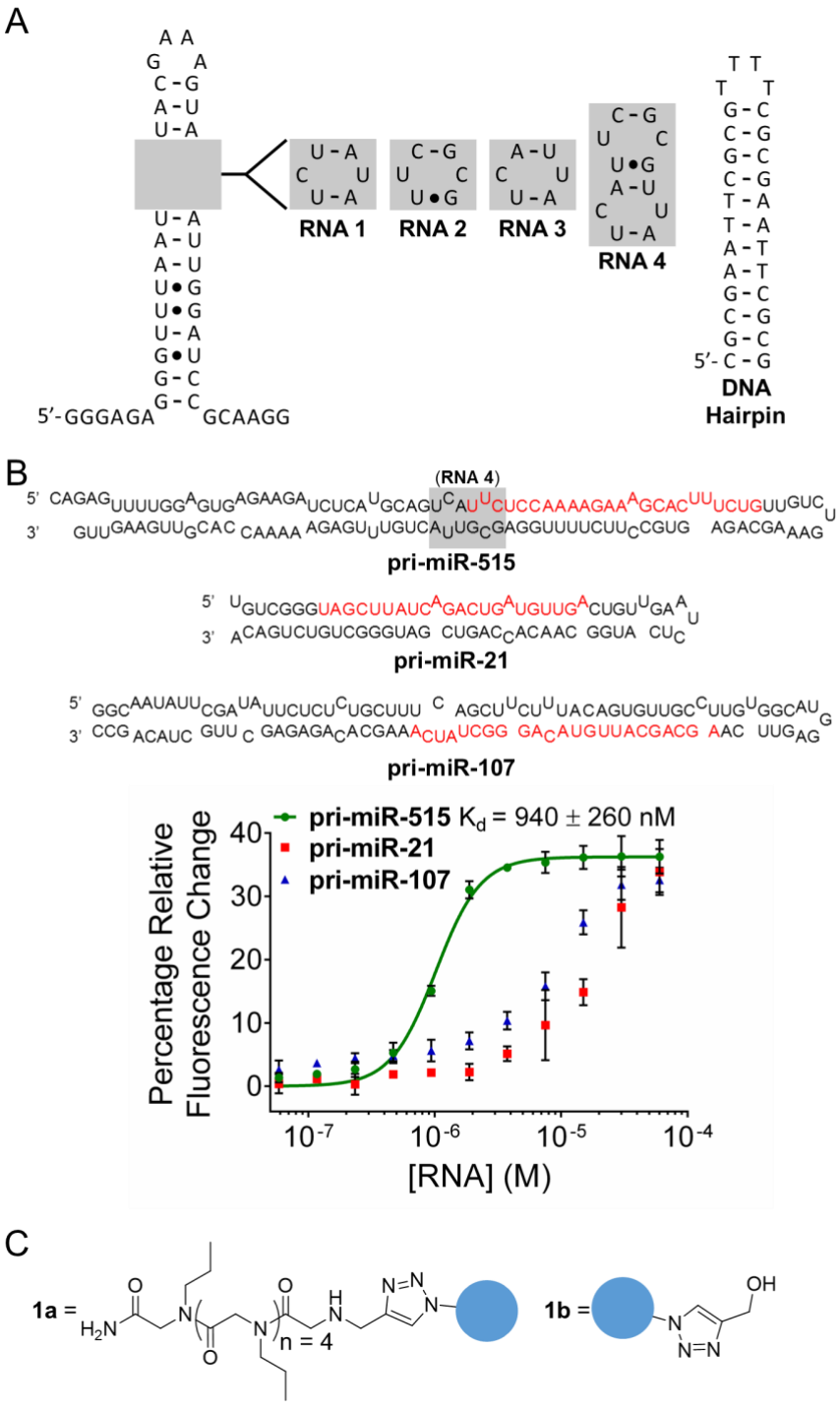


Figure S2: Nucleic acids and compounds used for assessing binding affinity and selectivity. (**A**) Nucleic acid secondary structures used for binding affinity measurements

after 30 min of compound incubation at room temperature. **RNA 1** displays the motif found in the Drosha site of pri-miR-885, while **RNA 2** displays the motif found in the Drosha site of pri-miR-515. **RNA 3** displays the adjacent **1**-binding site in pri-miR-515 and **RNA 4** contains both the Drosha site and the adjacent **1**-binding site in pri-miR-515. The DNA binding capacity of compounds was measured using **DNA Hairpin**. (**B**) Secondary structures of the primary transcripts of miR-515, miR-21, and miR-107 (pri-miR-515, pri-miR-21, and pri-miR-107, respectively) used for binding affinity measurements after 30 min of compound incubation at room temperature. Red text highlights the more abundant mature miRNA strand of each miRNA. The Drosha site of pri-miR-515 is highlighted in gray, the same motif represented by **RNA 4**. Pri-miR-515 contains 1×1 nucleotide internal loops in its Drosha site and a base paired region in its Dicer site. Pri-miR-21 contains a base paired region in its Drosha site and a 5' bulge in its Dicer site. Pri-miR-107 contains a 1×3 nucleotide internal loops in its Drosha site and a 5' bulge in its Dicer site. Representative binding isotherms of **2** to pri-miR-515, pri-miR-21, and pri-miR-107 (bottom). (**C**) Structure of **1a**, a peptoid derivative of **1**, and **1b**, a triazole derivative of **1**.

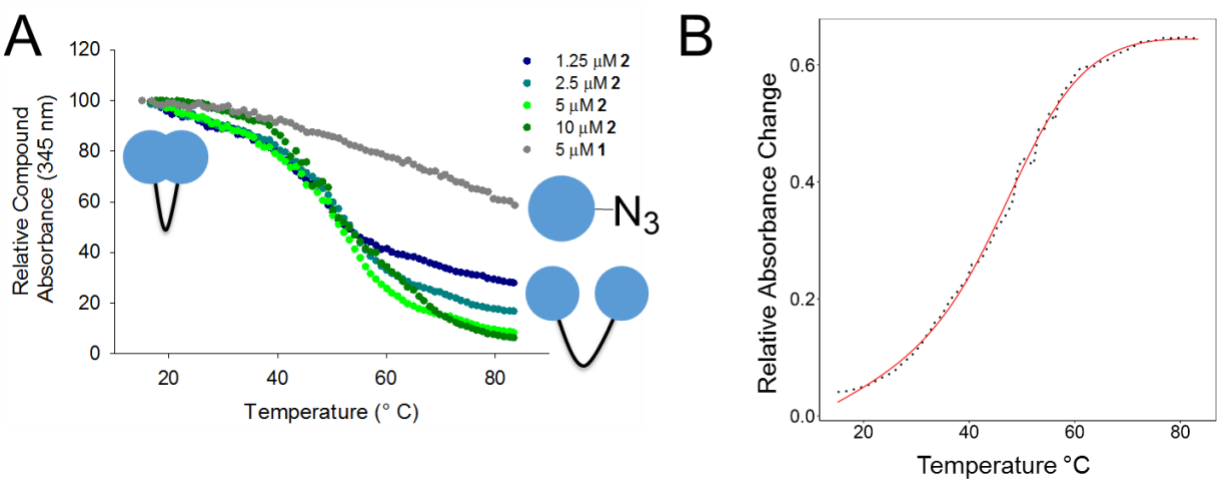


Figure S3: Evaluation of intramolecular structure through thermal melting analysis. (**A**) Representative melting temperature curves of **1** and **2** with concentration independent hyperchromicity changes observed with **2**. (**B**) Representative curve fit of relative absorbance change of compound at 345 nm to calculate thermodynamic parameters.

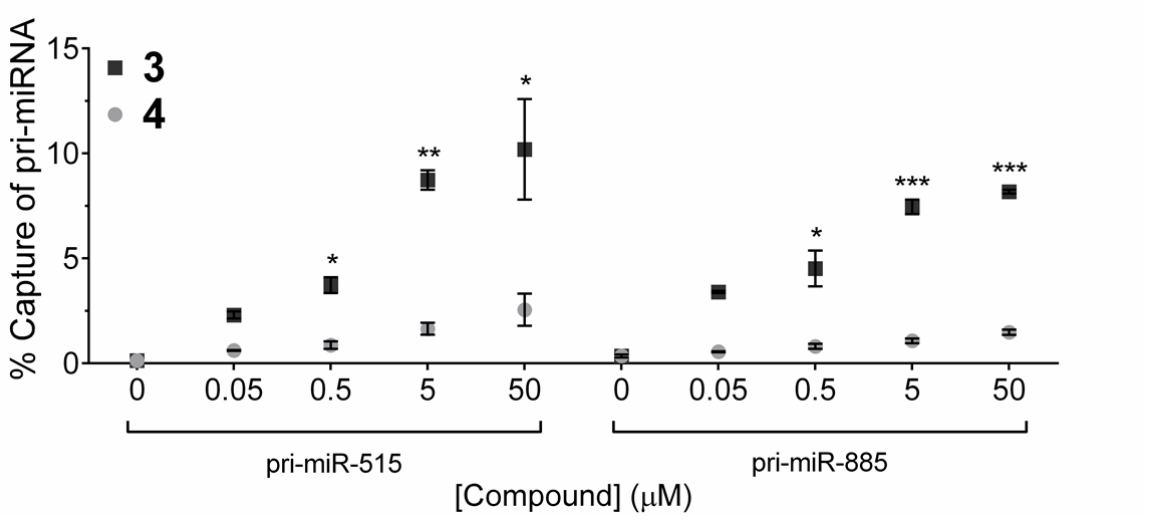


Figure S4: Validating *in vitro* pri-miR-515 target engagement by Chemical Cross-Linking and Isolation by Pull Down (Chem-CLIP). *In vitro* validation of **3** pulling down pri-miR-515 and pri-miR-885 selectively over **4** (lacks RNA-binding module) after 16 h of incubation at room temperature. Data represent mean ± s.e.m. (n≥4). *p<0.05, **p<0.01, ***p<0.001 compared to percent capture of RNA by control compound **4**, as determined by a two-tailed Student t-test.

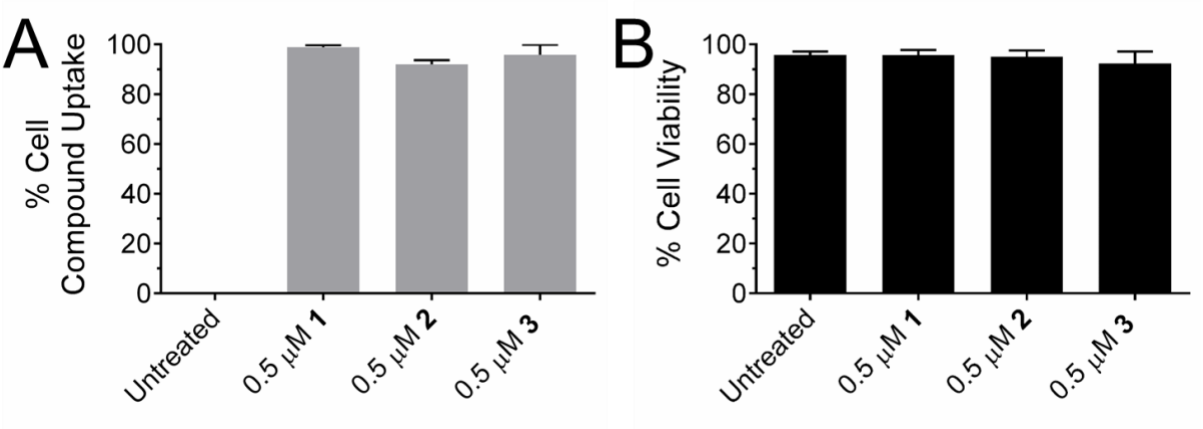


Figure S5: Compound uptake and viability of treated cells. (**A**) MCF-7 cells were treated with 0.5 μM of **1**, **2**, or **3** and stained with propidium iodide (PI). Cells were then analyzed by flow cytometry for compound uptake [positive for compound signal (ex: 345, em: 460] and (**B**) for viability (negative for PI stain) after 24 h of treatment.

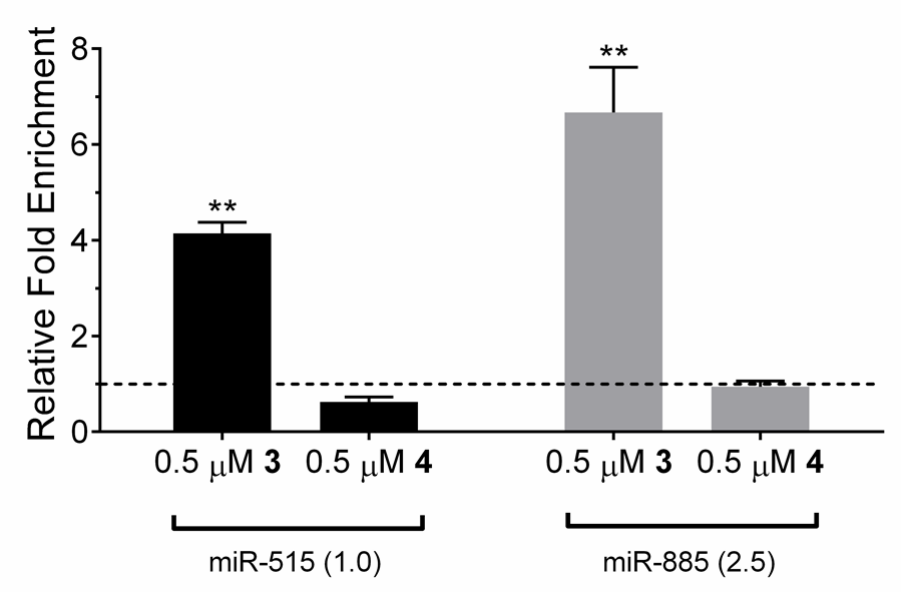


Figure S6: Validating pri-miR-515 target engagement by Chemical Cross-Linking and Isolation by Pull Down (Chem-CLIP) in MCF-7 cells. MCF-7 cells were treated with **3**, Targaprimir-515/885-CA-Biotin, or **4**, control CA-Biotin, the compound lacking an RNA-binding module for 48 h. Enrichment of miR-515 and miR-885 was measured by qRT-PCR. Relative expression levels of miRNAs are shown in parentheses. Above the dotted line indicates enrichment levels of the measured miRNA in the pulled down fraction versus the levels of the miRNA before pulldown. Data represent mean ± s.e.m. (n≥3). **p<0.01 compared to Chem-CLIP samples before pulldown, as determined by a two-tailed Student t-test.

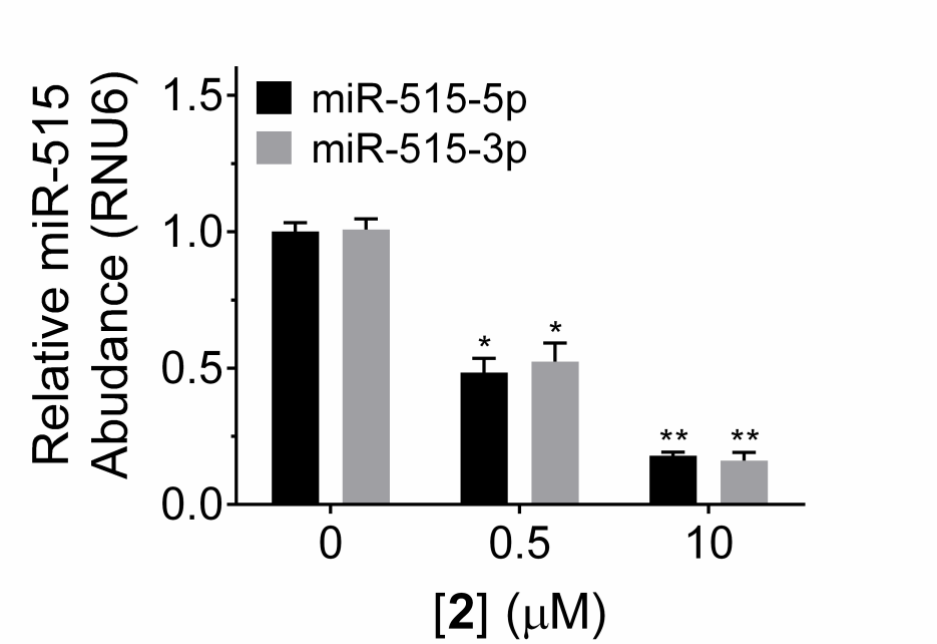


Figure S7: Inhibition of miR-515-5p and miR-515-3p. RT-qPCR analysis of mature miR-515-5p and miR-515-3p levels when treated with **2** for 48 h. By affecting pri-miR-515 at the level of biogenesis, treatment of **2** in MCF-7 cells decreases both the 5p and 3p mature miR-515 strands.

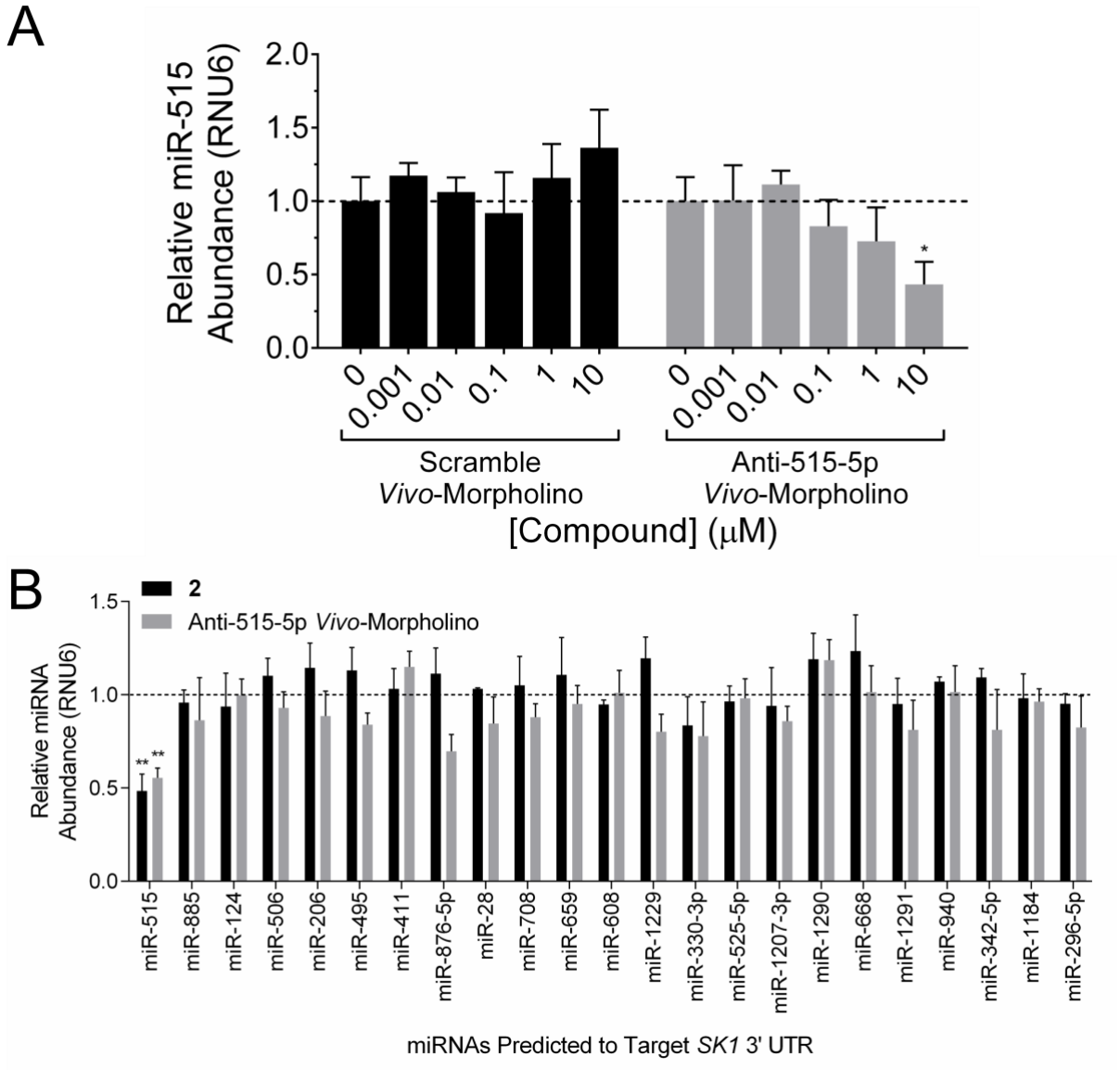
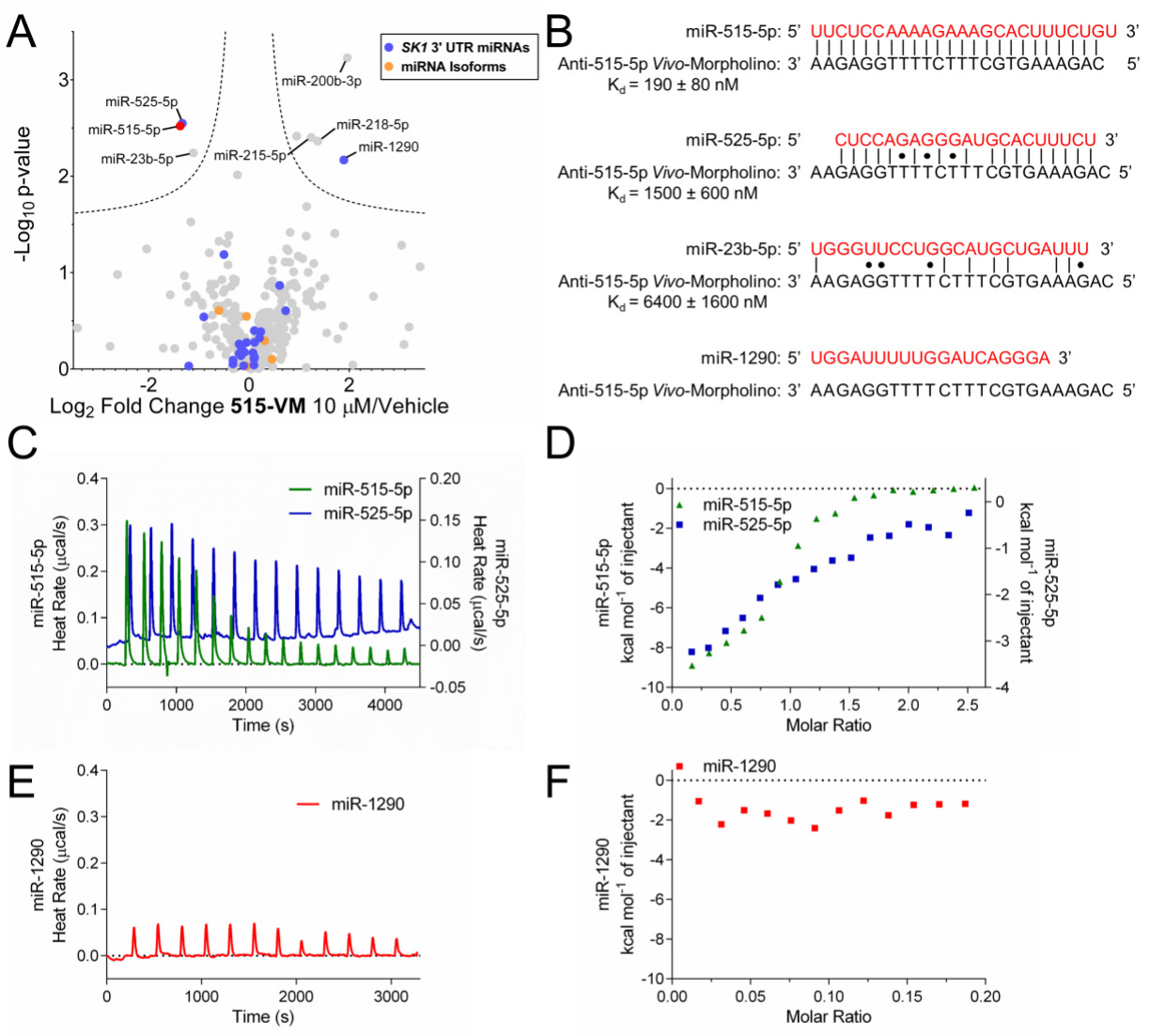


Figure S8: Evaluation of antisense oligonucleotide for reduction of miR-515 levels in MCF-7 cells and selectivity against miRNAs predicted to target the *SK1* 3' UTR. (**A**) Effect of *vivo*-morpholino modified antisense oligonucleotide for miR-515-5p (Anti-515-5p *Vivo*-Morpholino) on miR-515 levels as determined by RT-qPCR after 48 h of treatment. Data represent mean ± s.d. Dotted line represents levels of miR-515 in untreated samples. (**B**) RT-qPCR analysis of miRNAs predicted to interact with the *SK1* 3' UTR upon treatment with 0.5 μM **2** and 10 μM antisense oligonucleotide targeting miR-515-5p (Anti-515-5p *Vivo*-Morpholino™) after 48 h of treatment. Dotted line represents levels of miRNA in untreated samples. *p<0.05, **p<0.01 compared to untreated samples, as determined by a two-tailed Student t-test.



G

Oligonucleotide	N	K_d (nM)	ΔG_{298} (kcal/mol)	ΔH (kcal/mol)	ΔS (e.u.)
miR-515-5p	0.90 ± 0.030	190 ± 80	-9.3 ± 0.50	-39 ± 8.3	-0.10 ± 0.024
miR-525-5p	1.0 ± 0.13	1500 ± 580	-8.4 ± 0.20	-20 ± 7.2	-0.038 ± 0.014
miR-23b-5p	0.55 ± 0.14	6400 ± 1600	-7.1 ± 1.8	-15 ± 3.8	-0.029 ± 0.0072
Anti-515-3p *Vivo*-Morpholino	0.80 ± 0.30	1300 ± 570	-8.1 ± 2.0	-13 ± 3.6	-0.019 ± 0.048

Figure S9. Specificity of Anti-515-5p *Vivo*-Morpholino™ for various miRNAs as determined by isothermal titration calorimetry (ITC). (**A**) Volcano plot representing fold change and p-value of untreated samples compared to samples treated with 10 µM of Anti-515-5p *Vivo*-Morpholino™ (**515-VM**) after 48 h of treatment. Data represent mean; Dotted lines represent a false discovery rate of 1% and an S0 of 0.1; p-values were determined by a two-tailed Student t-test when compared to untreated samples. Red

point, effect on miR-515; Blue points, miRs predicted by TargetScan to bind to the SK1 3'UTR; Orange points, RNA isoforms to miR-515 hairpin precursor; and, Grey points all other expressed miRs in MCF7 cells. (**B**) Potential pairing formed between the miRNA targets and Anti-515-5p *Vivo*-Morpholino™. The dissociation constants were measured by ITC. (**C**) Representative ITC raw thermal data and (**D**) plotted isotherms of miR-515-5p and miR-525-5p with Anti-515-5p *Vivo*-Morpholino™. (**E**) Representative raw thermal data and (**F**) plotted isotherm of non-complementary oligonucleotide miR-1290 with Anti-515-5p *Vivo*-Morpholino™. (**G**) Calculated parameters from curve fitting of the isotherms. "N" refers to number of molecules complexed with one molecule of Anti-515-5p *Vivo*-Morpholino.

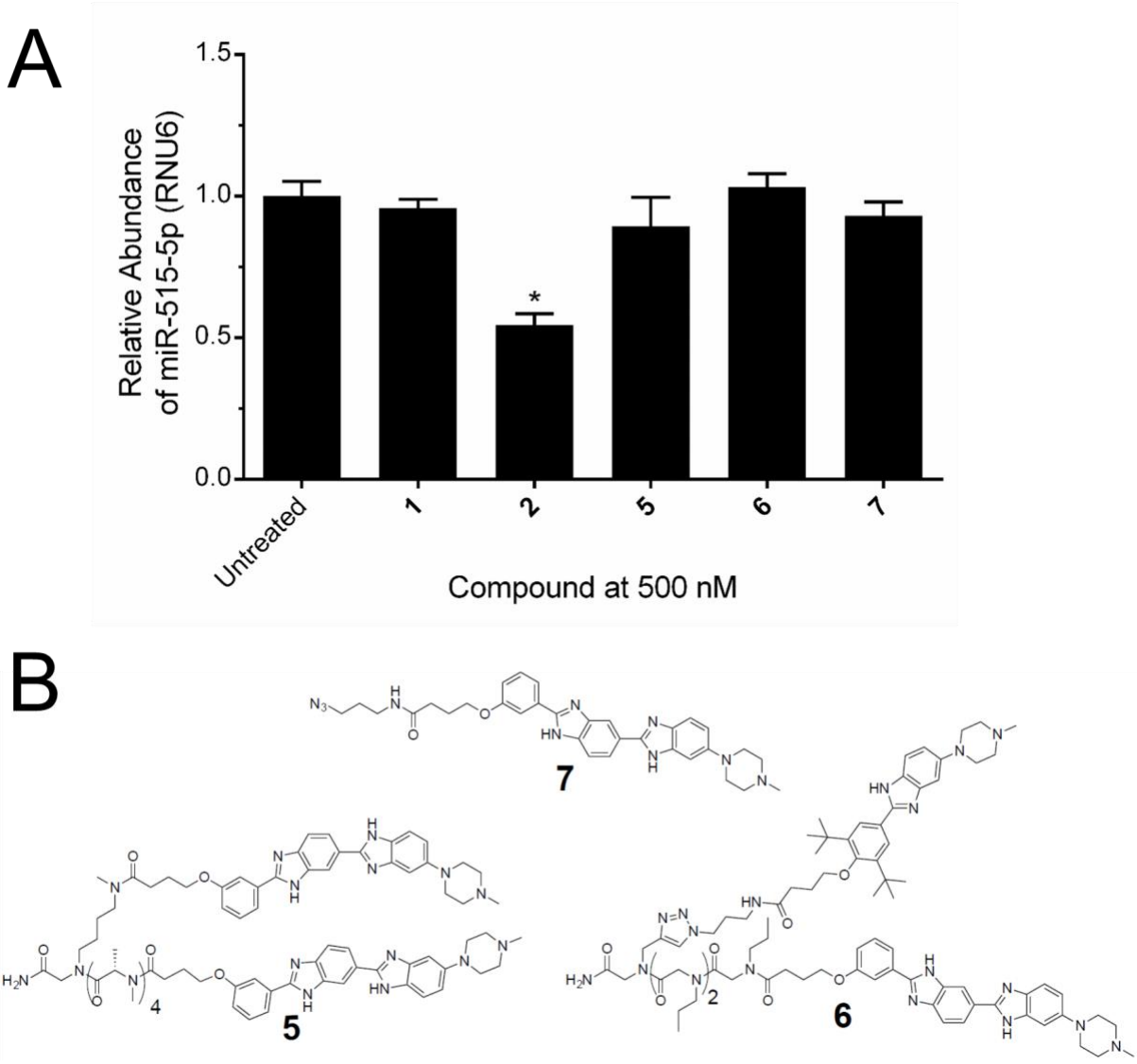


Figure S10: Studying the effect of other RNA-targeting compounds on mature miR-515-5p levels in MCF-7 cells. (**A**) Assessment of the effect of different RNA-targeting small molecules on the levels of miR-515 in MCF-7 cells at 500 nM after 48 h of treatment. Data represent mean ± s.e.m. (n≥3). *p<0.05, compared to untreated samples, as determined by a two-tailed Student t-test. (**B**) The structures of various small molecules identified by Inforna to target other RNAs.

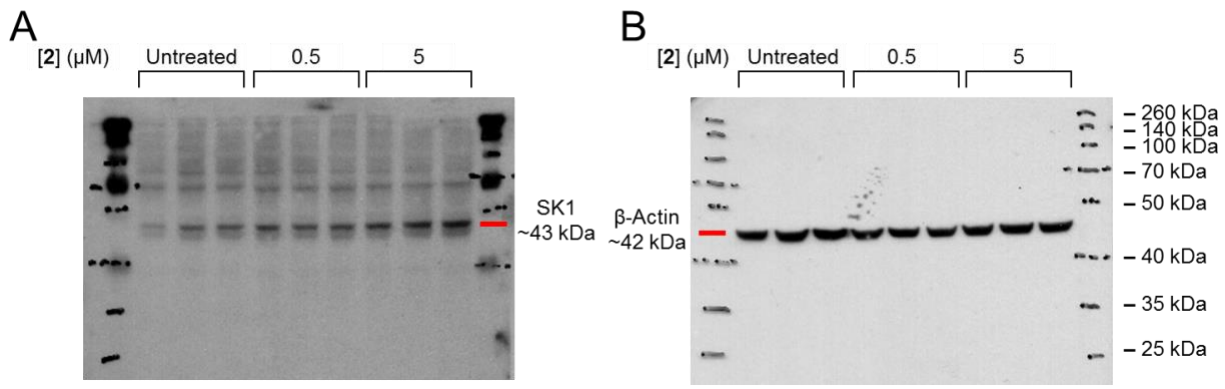


Figure S11: Western blot analysis of MCF-7 cells treated with 2. (**A**) Representative western blot image of total protein from MCF-7 cells treated with or without **2** for 48 h and stained with SK1 primary antibody. (**B**) The blot was then stripped and reblotted with a β-actin primary antibody.

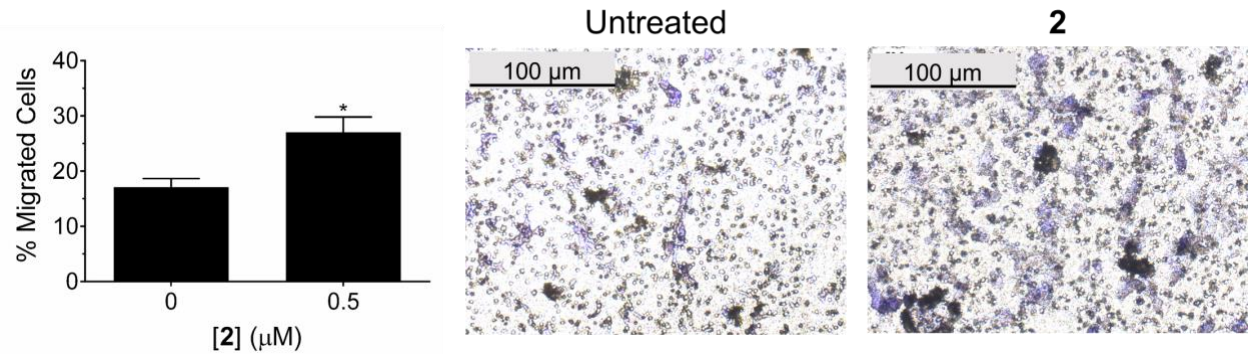
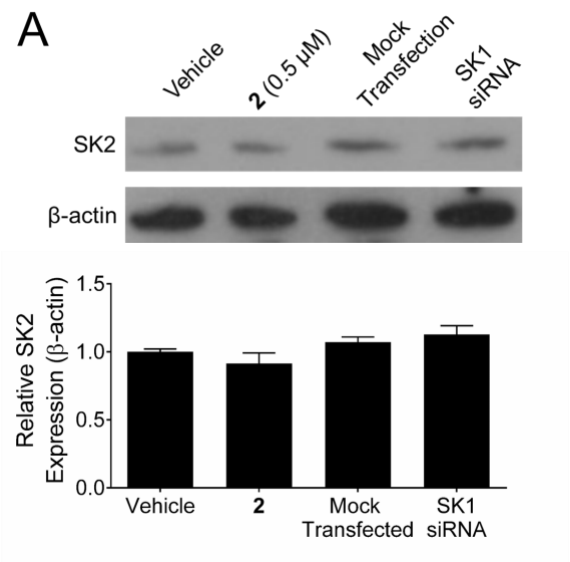


Figure S12: S1P-mediated migratory phenotype in MDA-MB-231 cells. Treatment of MDA-MB-231 cells with 0.5 μM of **2** caused an increase in migration after 16 h, suggesting miR-515 de-repression of SK1 can affect S1P second messenger levels, resulting in a migratory phenotype in another breast cancer cell line. Data represent mean ± s.e.m. Data represent mean ± s.e.m. (n≥3, 4 fields of view). *$p<0.05$ compared to untreated samples, as determined by a two-tailed Student t-test.



B

MCF-7 Basal Ct values		
18S	**SK1**	**SK2**
15 ± 0.25	26 ± 0.45	*N.M.
*N.M. indicates Ct > 32		

Figure S13: Expression of sphingosine kinase 2 (SK2) with 2 treatment and SK1 siRNA transfection. (**A**) Top, representative Western blot for SK2 and β-actin for total protein from MCF-7 cells treated vehicle or with **2** (0.5 μM) after 48 h and for total protein from MCF-7 cells that were mock transfected or transfected with an SK1 siRNA using Lipofectamine RNAiMAX after 48 h. Bottom, quantification of SK2 protein from MCF-7 cells. (**B**) RT-qPCR analysis was performed on total RNA from untreated MCF-7 cells (MCF-7 Basal) using primers to measure 18S, SK1, and SK2. 18S and SK1 had measurable cycle threshold values (Ct) below 32, while the Ct value for SK2 was not measurable (N.M.). Data represent mean ± s.e.m. (n≥3).

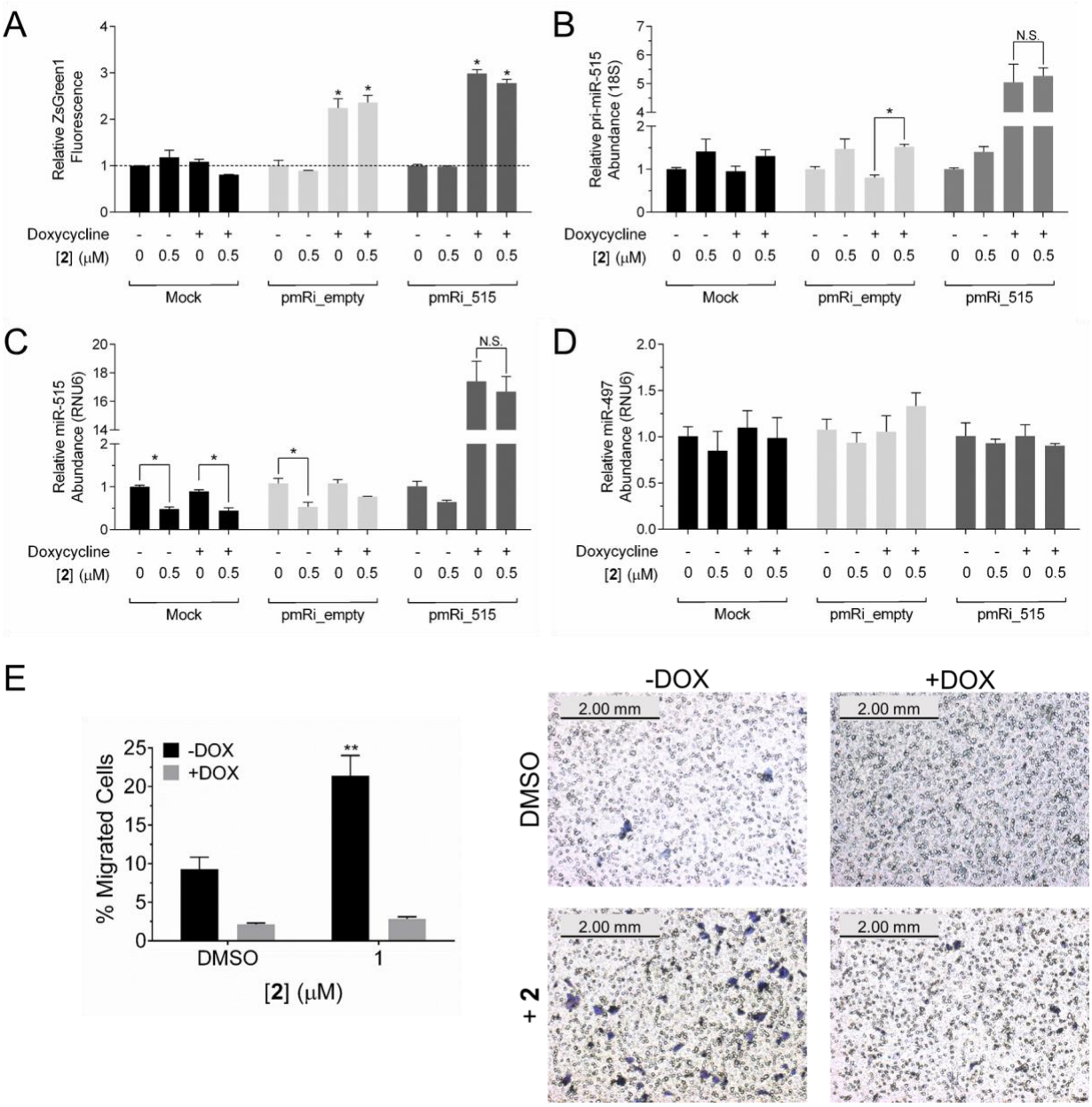


Figure S14: Induction of miR-515 ablates 2-mediated inhibition of miR-515 and migration. (**A**) Increase in ZsGreen1 fluorescence indicates that protein levels in both the empty inducible vector (pmRi-empty) and the pri-miR-515 inducible plasmid (pmRi_515) are responsive to doxycycline induction after 48 h. (**B**) Treatment of **2** for 48 h significantly boosts pri-miR-515 levels in pmRi_empty transfected cells, but compound effect is ablated upon pri-miR-515 induction. (**C**) Similarly, mature miR-515 levels are not significantly affected upon induction, while **2** treatment significantly inhibits miR-515 in a pmRi_empty background. (**D**) miR-497 levels remained unchanged with and without doxycycline treatment and **2** treatment, suggesting specificity of miR-515 induction and selective binding of **2** to miR-515 in cells. *p<0.05, compared to untreated samples,

unless specified otherwise, as determined by a two-tailed Student t-test. (**E**) Increased migration of MCF-7 after 16 h without induction (-DOX) by **2** treatment is ablated upon induction of miR-515 (+DOX). *p<0.05, **p<0.01 compared to non-induced samples, as determined by a two-tailed Student t-test.

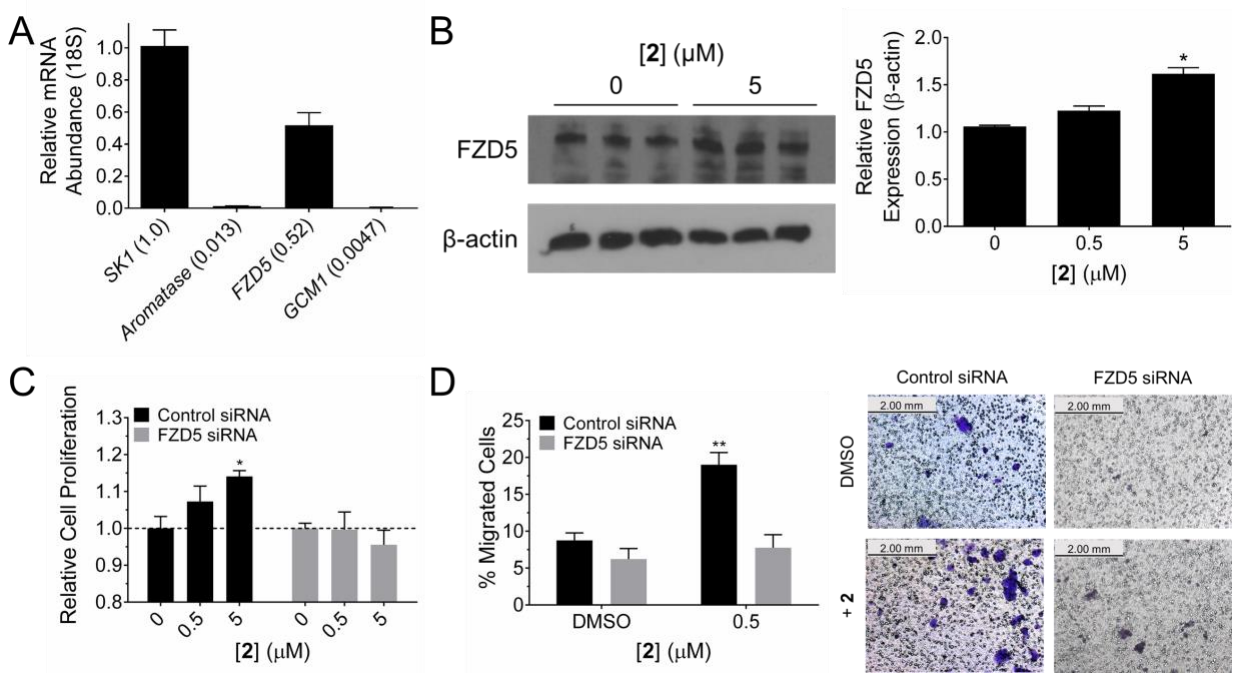


Figure S15: De-repression of FZD5 by 2 increases proliferation and migration in MCF7 cells. (**A**) SK1, Aromatase, FZD5, and GCM1 mRNA levels endogenously observed in MCF7 cells. Aromatase, FZD5, and GCM1 are previously reported targets of miR-515-5p. (**B**) Western blot analysis of FZD5 de-repression upon treatment with **2** for 48 h. (**C**) Increased proliferation of **2**-treated MCF7 cells, as measured by CellTiter Glo after 24 h. FZD5 siRNA ablates cellular proliferation observed upon **2** treatment. (**D**) Quantification and representative images of cell migration after 16 h with **2** treatment and with FZD5 siRNA knockout. Enhanced migration of crystal violet stained MCF-7 cells is observed, indicating phenotypic modification through an FZD5-mediated pathway. Data represent mean ± s.e.m. (n≥3, 4 fields of view). *p<0.05, **p<0.01, compared to untreated samples, as determined by a two-tailed Student t-test.

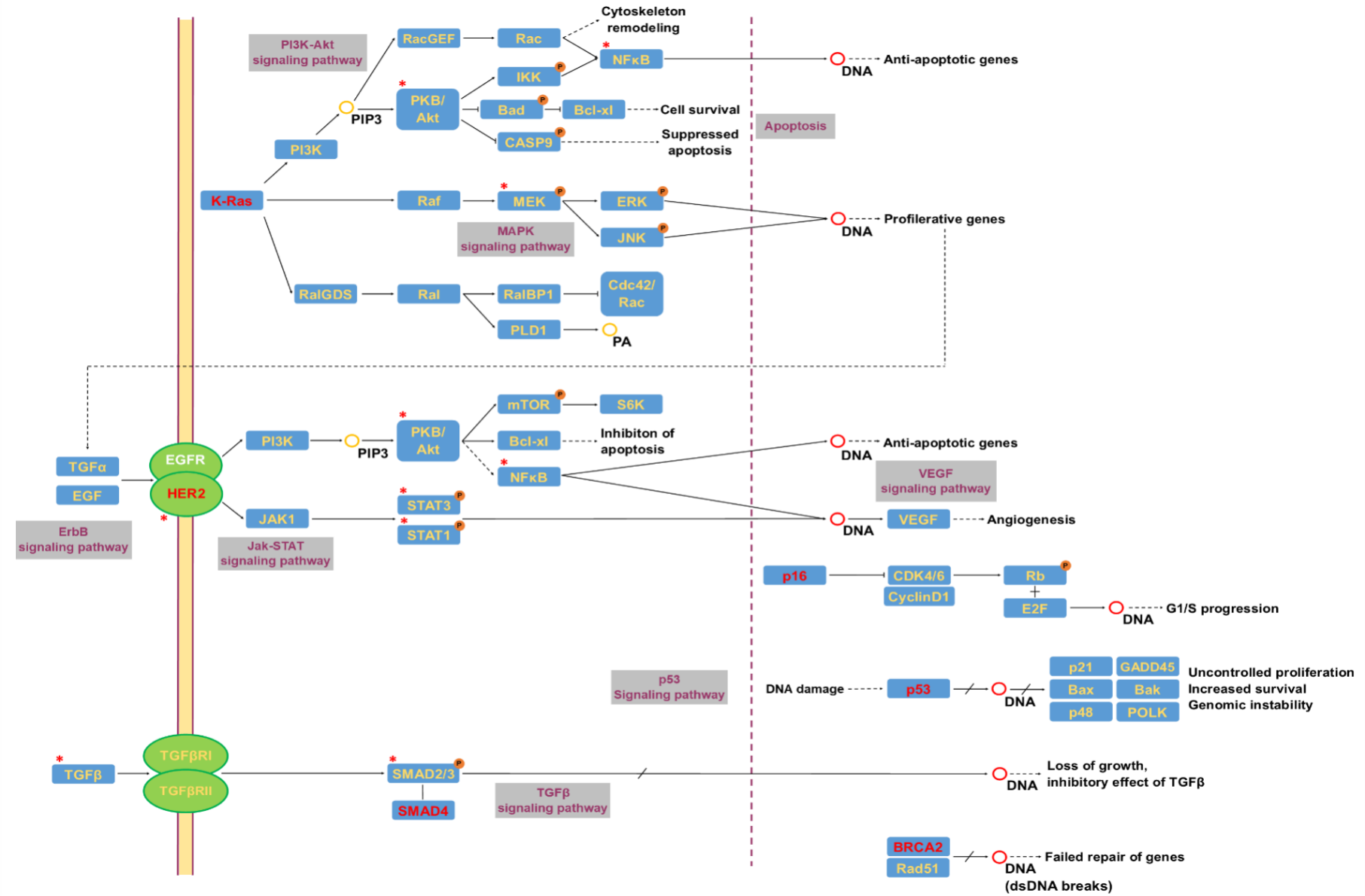


Figure S16: Upregulated pathways upon 48 h treatment of MCF-7 cells with 1 μM 2. Proteins marked with a red asterisk indicate identified upregulated proteins. Proteins in red font are tumor suppressors or oncogenes. Pathway analysis was performed using proteins with 50% increased expression via the online tool DAVID 6.8. Scheme adapted from KEGG (www.kegg.jp).

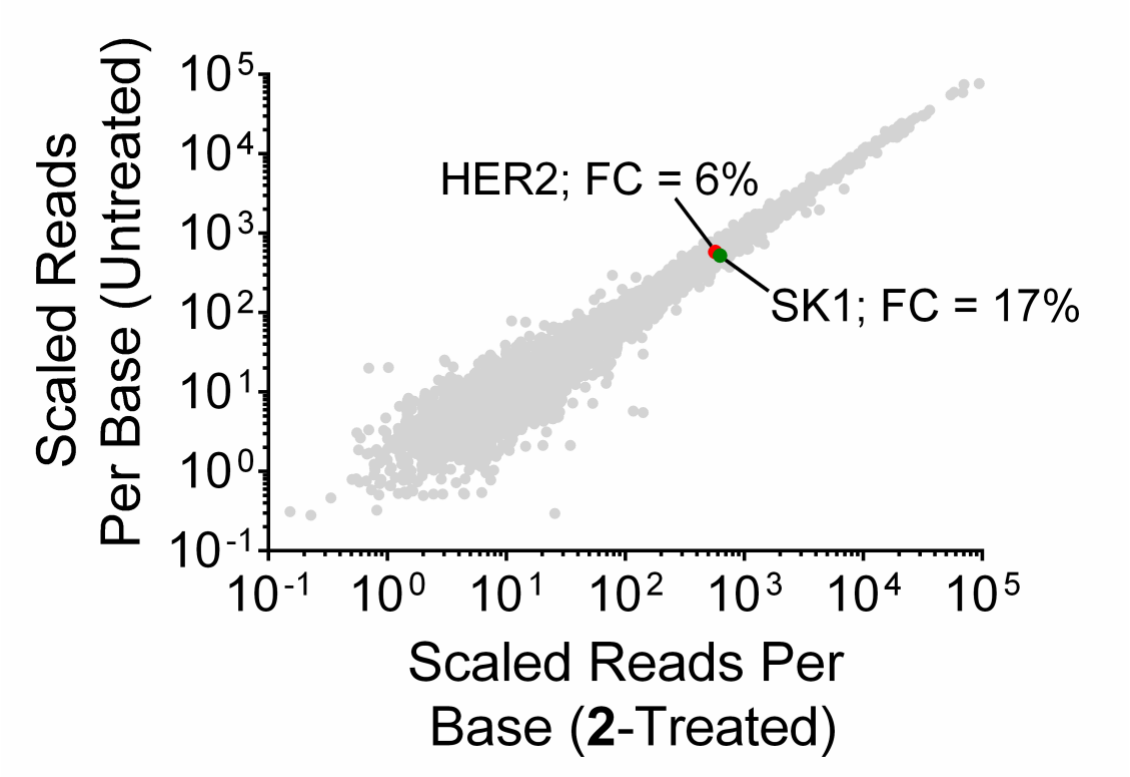


Figure S17: Differential gene expression analysis in MCF-7 cells with 2 treatment after 24 h. RNA-Seq was performed on total RNA from untreated and **2**-treated MCF-7 cells after 24 h of treatment. Differential gene expression between the samples was plotted as a scatter plot of scaled reads per base of genes in untreated samples (y-axis) and scaled reads per base of genes in **2**-treated samples (x-axis). 17992/18044 genes were not significantly affected (adjusted p-value < 0.05), demonstrating that compound **2** has limited off-target effects. Red and green dots represent HER2 and SK1 expression levels, respectively. Fold change (FC) of HER2 and SK1 after 24 h of treatment increased by 6% and 17%, respectively, from the untreated to the **2**-treated samples.

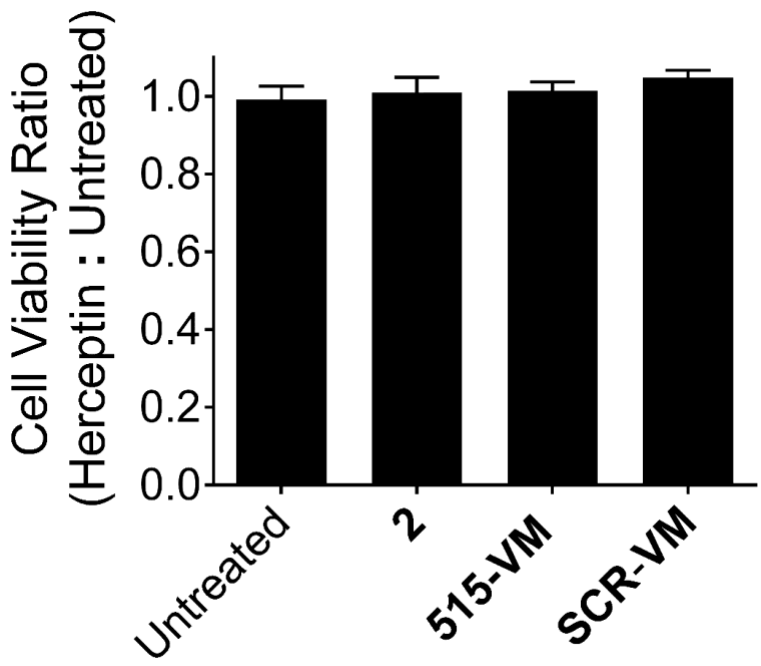


Figure S18: MCF-10A cells that do not express miR-515 are unaffected by Herceptin with 2 treatment. MCF-10A healthy breast cells that do not express measurable levels of miR-515 are not sensitive to Herceptin after treatment with **2** for 24 h, Anti-515-5p *Vivo*-Morpholino™ (**515-VM**), or a scrambled control *Vivo*-Morpholino™ (**SCR-VM**), followed by Herceptin treatment for 48 h.

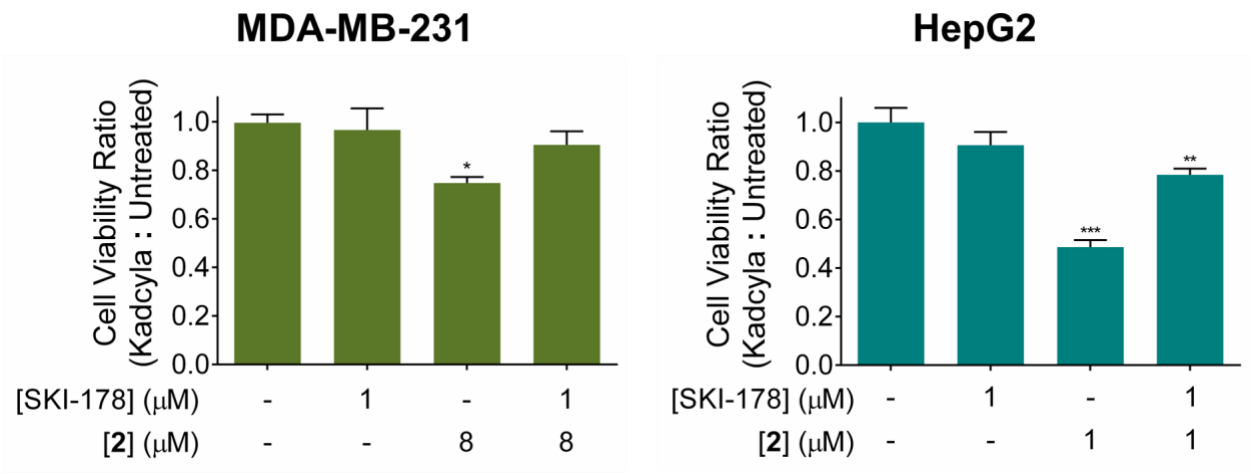


Figure S19: MDA-MB-231 and HepG2 cells pre-treated with 2 are affected by Kadcyla via a SK1-dependent mechanism. Application of 8 μM or 1 μM of **2** for 24 h, followed by 48 h of treatment of Herceptin renders the triple negative breast cancer cell line, MDA-MB-231, or the hepatocellular carcinonoma cell line, HepG2, sensitive to the HER2-targeting antibody drug conjugate, Kadcyla (200 nM), respectively. Treatment with SK1 chemical inhibitor, SKI-178 (1 μM) decreases the sensitivity of both cell lines to Kadcyla, indicating that the effect on these cells is dependent on SK1. *p<0.05, **p<0.01, ***p<0.001 compared to untreated samples, as determined by a two-tailed Student t-test.

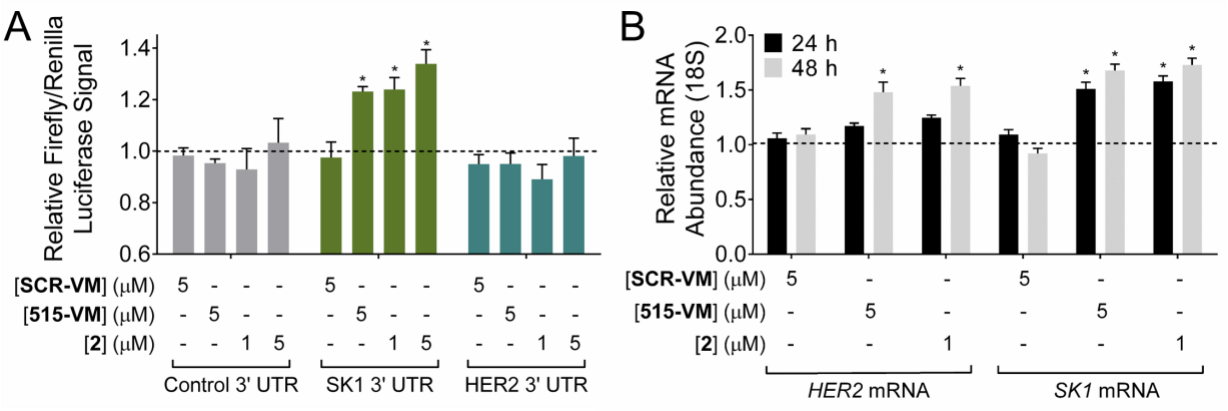


Figure S20: HER2 is not directly affected by miR-515-5p. (**A**) Luciferase reporters of SK1 and HER2 containing the TargetScan predicted 3' UTR targeting sites of miR-515-5p were treated with **2** for 48 h. De-repression of SK1 luciferase signal upon **2** treatment indicates direct targeting of miR-515-5p to SK1. In contrast, the HER2 luciferase does not change, suggesting that miR-515-5p does not interact with HER2. (**B**) *SK1* and *HER2* mRNA levels measured after 24 h and 48 h of **2** treatment. *HER2* mRNA levels only increase after 48 h of treatment, suggesting it comes downstream of increased *SK1* mRNA levels. *p<0.05, compared to untreated samples, as determined by a two-tailed Student t-test.

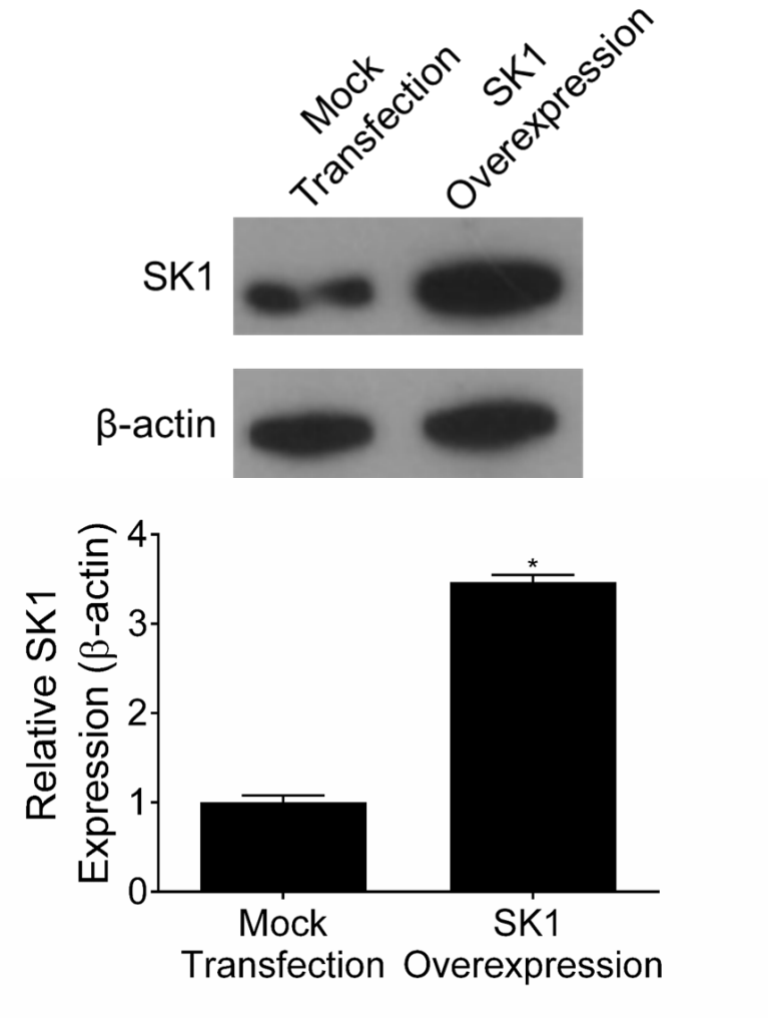


Figure S21: Plasmid overexpression of SK1. A 3-fold increase in SK1 protein levels was observed by Western blotting after transfection of MCF-7 cells with a SK1 overexpression plasmid for 48 h.

SUPPLEMENTARY METHODS

PCR amplification & in vitro transcription: The DNA templates for the miR-515 primary transcript RNA (pri-miR-515) (5' – CAGAGTTTTGGAGTGAGAAGATCTC ATGCAGTCATTCTCCAAAAGAAAGCACTTTCTGTTGTCTGAAAGCAGAGTGCCTTC TTTTGGAGCGTTACTGTTTGAGAAAAACCACGTTGAAGTTGATG) was purchased from Eurofins MWG Operon and used without further purification. This template was PCR amplified in 1× PCR Buffer (10 mM Tris, pH 9.0, 50 mM KCl, and 0.1% (v/v) Triton X-100), 2 µM forward primer (5'- TAATACGACTCACTATAGGCCAACAAAA AACCCAGAGTTTTGGAG), 2 µM reverse primer (5'- CCAAGATCAG CATCAACTTCAAC), 4.25 mM $MgCl_2$, 330 µM dNTPs, and 1 µL of Taq DNA polymerase in a 600 µL reaction. PCR cycling conditions were initial denaturing at 95 °C for 90 s, followed by 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 60 s for 25 cycles. The same procedure was followed for the miR-21 primary transcript RNA (pri-miR-21) (5' – GTCGGGTAGCTTATCAGACTGATGTTGACTGTTGAATCTCATGGCAACACCA GTCGATGGGCTGTCTGAC) using the following as forward (5' – TAATACGACTCACTATAGGTCGGGTAGCTTATC) and reverse (5' – GTCAGACAGCCCATCGAC) primers, and the miR-107 primary transcript RNA (pri-miR-107) (5' – GCAATATTCGATATTCTCTCTGCTTTCAGCTTCTTTACAGTGTTG CCTTGTGGCATGGAGTTCAAGCAGCATTGTACAGGGCTATCAAAGCACAGAGAGC TTGCTACAGCC) using the following as forward (5' – TAATACGACTCACTATAGGCAA TATTCGATATTC) and reverse (5' – GGCTGTAGCAAGCTCTCTG) primers.

The DNA templates for the RNA cassette displaying the pri-miR-885 Drosha site (**RNA 1**) (5’ – GGGAGAGGGTTTAATTCTTACGAAAGTAATAATTGGATCCGCAAGG), the RNA cassette displaying the **1**-binding site adjacent to the pri-miR-515 Drosha site (**RNA 2**) (5’ – GGGAGAGGGTTTAATTTCTACGAAAGTAGCGATTGGATCCGCAAGG), the RNA cassette displaying the pri-miR-515 Drosha site (**RNA 3**) (5’ – GGGAGAGGG TTTAATTCATACGAAAGTATTAATTGGATCCGCAAGG), and the RNA cassette containing the Drosha site and the adjacent **1**-binding site in pri-miR-515 (**RNA 4**) (5’ – GGGAGAGGGTTTAATTCATTCTACGAAAGTAGCGTTAATTGGATCCGCAAGG) were purchased from Eurofins MWG Operon and used without further purification. These templates were PCR amplified in 1× PCR Buffer (10 mM Tris, pH 9.0, 50 mM KCl, and 0.1% (v/v) Triton X-100), 2 µM cassette forward primer (5’- GGCCGAATTCTAATACGA CTCACTATAGGGAGAGGGTTTAAT), 2 µM cassette reverse primer (5’- CCTTGCGG ATCCAAT), 4.25 mM $MgCl_2$, 330 µM dNTPs, and 1 µL of Taq DNA polymerase in a 600 µL reaction. PCR cycling conditions were initial denaturing at 95 °C for 90 s, followed by 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s for 25 cycles.

RNA was *in vitro* transcribed by T7 RNA polymerase using 300 µL of the PCR product in 1× Transcription Buffer (40 mM Tris-HCl, pH 8.1, 1 mM spermidine, 0.001% (v/v) Triton X-100 and 10 mM DTT) with 2.25 mM of each rNTP and 5 mM $MgCl_2$ at 37 °C for 18 h. The RNA was then purified on a denaturing 15% polyacrylamide gel and isolated as previously described [1]. RNA concentration was determined by UV absorbance at 260 nm at 90 °C using a Beckman Coulter DU800 UV-Vis spectrophotometer with a Peltier temperature controlling unit. Extinction coefficients were

calculated using the Oligo Extinction Coefficient Calculator (https://www.scripps.edu/california/research/dna-protein-research/forms/biopolymercalc2.html).

Determination of Compound Binding Affinities. Dissociation constants for the binding of nucleic acids (RNA was *in vitro* transcribed from DNA templates ordered from Eurofins MWG Operon; DNA hairpin was ordered from Eurofins MWG Operon and used without further purification) to compounds were determined using an in solution, fluorescence-based binding assay. Nucleic acid was folded in 1× Binding Buffer (8 mM Na_2HPO_4, 190 mM NaCl, 1 mM EDTA, and 40 µg/mL BSA) by heating at 60 °C for 5 min and then cooled to room temperature. Compounds were added to a final concentration of 0.5 µM. Next, 1:2 serial dilutions (from 100 to 0.098 µM) of RNA were performed in 1× Binding Buffer supplemented with 0.5 µM of compound. Solutions were incubated for 30 min and then transferred to Corning non-binding surface half area 96-well black plates. Fluorescence intensity (Ex: 345 nm, Em: 460 nm) was then measured on a Molecular Devices SpectraMax M5 plate reader. Change in fluorescence intensity was fit as a function of RNA concentration with equation 1 (Eq 1):

$$I = I_0 + 0.5\Delta\varepsilon([\mathrm{FL}]_0 - (([\mathrm{FL}]_0 + [\mathrm{RNA}]_0\mathrm{K_t})^2 - 4[\mathrm{FL}]_0[\mathrm{RNA}]_0)^{0.5}) \quad \text{(Eq 1)}$$

where I and I_0 are the observed fluorescence intensity in the presence and absence of nucleic acid, respectively, $\Delta\varepsilon$ is the difference between the fluorescence intensity in the absence and in the presence of infinite nucleic acid concentration, $[\mathrm{FL}]_0$ and $[\mathrm{RNA}]_0$ are the concentrations of compound and nucleic acid, respectively, and K_d is the dissociation constant.

***In vitro* Drosha processing inhibition assay:** Pri-miR-515 was 5'-end labeled with [γ-^{32}P] ATP and T4 polynucleotide kinase as previously described [1]. The RNA was folded by heating at 95 °C for 30 s and cooled to room temperature for 10 min. RNA was

then supplemented with 6.4 mM $MgCl_2$ and incubated with compounds for 15 min at room temperature. A 1 µL aliquot of Drosha-cmyc lysate, prepared as previously described [1], or mock transfected cell lysate was added and the samples were incubated for an additional 30 min at 37 °C. Reactions were stopped by adding in 2× Gel Loading Buffer (8 M urea, 20 mM EDTA, 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol). To generate T1 and hydrolysis sequencing markers, pri-miR-515 was incubated with RNase T1 (3 U/µL) in T1 Buffer (25 mM sodium citrate, pH 5, 7 M urea, and 1 mM EDTA) for 20 min at room temperature. An RNA hydrolysis ladder was generated by incubating RNA in 1× RNA Hydrolysis Buffer (50 mM $NaHCO_3$, 1 mM EDTA, pH 9.4) at 95 °C for 1.5 min. Cleavage products were resolved on a denaturing 15% polyacrylamide gel, which was imaged using a Molecular Dynamics Typhoon phosphorimager and quantified with Bio-Rad's QuantityOne software.

Melting Temperature Experiments: Thermodynamic parameters of compounds were measured in 1× PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, KH_2PO_4) using a Coulter DU800 UV-Vis spectrophotometer with a Peltier temperature controlling unit by heating from 15 to 90 °C at a rate of 1 °C/min. Dilutions of compounds were suspended in 1× PBS. The absorbance change versus temperature was analyzed using MeltWin [2].

Isothermal Titration Calorimetry: ITC experiments were performed using a Low Volume Nano ITC (TA Instruments). Mature miRNA strands of miR-515-5p, miR-525-5p, miR-23b-5p (GE Healthcare Dharmacon, Inc.), Anti-515-3p *Vivo*-Morpholino™, and Anti-515-5p *Vivo*-Morpholino™ (Gene Tools, LLC) were buffer exchanged with 10 mM sodium

chloride and 90 mM sodium phosphate buffer, pH 7.4 using Amicon Ultra Centrifugal Filters Utracel -3K (EMD Millipore) according to the manufacturer's protocol. Titrations consisted of 2.5 µL injections with 300 s injection intervals of Anti-515-5p *Vivo*-Morpholino™ oligonucleotide into mature miRNA or Anti-515-3p *Vivo*-Morpholino™ in the sample cell at 25°C with a mixing speed of 200 rpm. Mature miRNA and Anti-515-3p *Vivo*-Morpholino™ were used at concentrations of 5–10 µM and Anti-515-5p *Vivo*-Morpholino™ was used at concentrations of 60–70 µM. The data were integrated to generate curves in which the areas under the injection peaks were plotted against the molar ratio of Anti-515-5p *Vivo*-Morpholino™ to mature miRNA or Anti-515-3p *Vivo*-Morpholino™. Analysis of the data was performed using NanoAnalyze Data Analysis, Version 2.3.2. software provided by the manufacturer according to the independent site model.

Doxycycline inducible pri-miR-515 Plasmid: The MiR-X Inducible miRNA System was purchased from Takara Bio USA, Inc. A pri-miR-515 DNA template (5' – CAGAGTTTTGGAGTGAGAAGATCTCATGCAGTCATTCTCCAAAAGAAAGCACTTTC TGTTGTCTGAAAGCAGAGTGCCTTCTTTTGGAGCGTTACTGTTTGAGAAAAACCAC GTTGAAGTTGATG; Eurofins MWG Operon) was PCR amplified with a forward primer (5' – CGGGATCCCATTTTAATCTGGTAAGGTAAATTCCAACAAAAAACCCAGAG TTTTGGAGTG; Integrated DNA Technologies) extending 45 bases with a BamHI restriction enzyme site and a reverse primer (5' – ACGTTGAAGTTGATGCTGATCTTG GTAACACATTTGCAGAGCGTGCTTATCATCTAGAGC; Integrated DNA Technologies) extending 45 bases with a XBaI restriction enzyme site as described above in the **PCR**

amplification method. This pmRi-ZsGreen1 Vector (Takara Bio USA, Inc.) was cut with BamHI and XBaI in NEBuffer 3.1 (New England BioLabs, Inc) at 37 °C for 1 h. The digested DNA was then purified on a 1% agarose gel in 1× TBE and the DNA was extracted using a QIAquick Gel Extraction Kit (Qiagen), per the manufacturer's protocol. The purified, double digested pmRi-ZsGreen1 vector DNA was then resuspended in 1× T4 DNA Ligase Buffer (New England BioLabs, Inc) and ligated with the PCR amplified pri-miR-515 sequence in a 1:1 ratio with T4 DNA Ligase (New England BioLabs, Inc) at room temperature for 3 h. After heat inactivation at 65 °C for 10 min, the ligation reaction was chilled on ice and then 1 µL was transformed into competent cells (5-alpha Competent E. coli, High Efficiency; New England BioLabs, Inc.). Colonies were grown on LB ampicillin agar plates and incubated overnight at 37 °C. Selected colonies were isolated using a Zyppy Plasmid Miniprep Kit (Zymo Research). Plasmid sequences containing the intact pri-miR-515 insert were confirmed by Sanger Sequencing by Genewiz using the SV40pA reverse primer.

Inducible pri-miR-515 System Assays: Confirmation of induction by ZsGreen1 fluorescence was performed in MCF-7 cells plated in a black walled, cell culture treated 96-well plate (3904; Corning). The MCF-7 cells were grown to 70% confluency and transfected with a 1:1 ratio of pTet-On Advanced (Takara Bio USA, Inc) with pmRI-ZsGreen1 vector without an insert (pmRi_empty) or with the pmRi-ZsGreen1 vector with the pri-miR-515 insert (pmRi_515) using Lipofectamine 2000, per the manufacturer's protocol. After 5 h, fresh media with or without doxycycline (Fisher BioReagents) at 1 µg/mL and with or without **2** at 0.5 µM was added to the cells. After 24 h of incubation,

cells were washed with 1× PBS and lysed with 1× Lysis Buffer (100 mM potassium phosphate buffer, pH 7.8, 0.2% Tween 20) for 10 min at room temperature. ZsGreen1 fluorescence (Ex: 493 nm, Em: 505 nm) was read using a Molecular Devices SpectraMax M5 plate reader. For qPCR analysis, a similar procedure was performed in 24-well cell culture plates, followed by RNA Extraction using a Zymo Quick-RNA MiniPrep kit. Migration assays were performed as described in the main text, however, MCF-7 cells were batch transfected with pmRi_515 as described above and incubated with or without 1 µg/mL of doxycycline overnight, before seeding into Hanging Cell Culture inserts for migration.

3' UTR Luciferase Reporter Assays: MCF-7 cells were grown in 48-well plates to ~60% confluency in complete growth medium. The cells were transiently transfected with 250 ng of a firefly/Renilla Duo Luciferase reporter vector (pEZX-MT06) encoding the 3' untranslated region (UTR) of SK1 (HmiT062347-MT06; GeneCopoeia Inc), HER2 (HmiT070373-MT06; GeneCopoeia Inc), or a control (CmiT000001-MT06; GeneCopoeia Inc) downstream of firefly luciferase, with a Renilla luciferase reporter cloned into the same vector using Lipofectamine 2000 per the manufacturer's protocol. At 5 h post-transfection, compounds were added in complete growth medium, and the cells were incubated for 48 h. Luciferase assays were completed using the Luc-Pair Duo-Luciferase Assay Kit 2.0 (LF002; GeneCopoeia Inc) per the manufacturer's protocol.

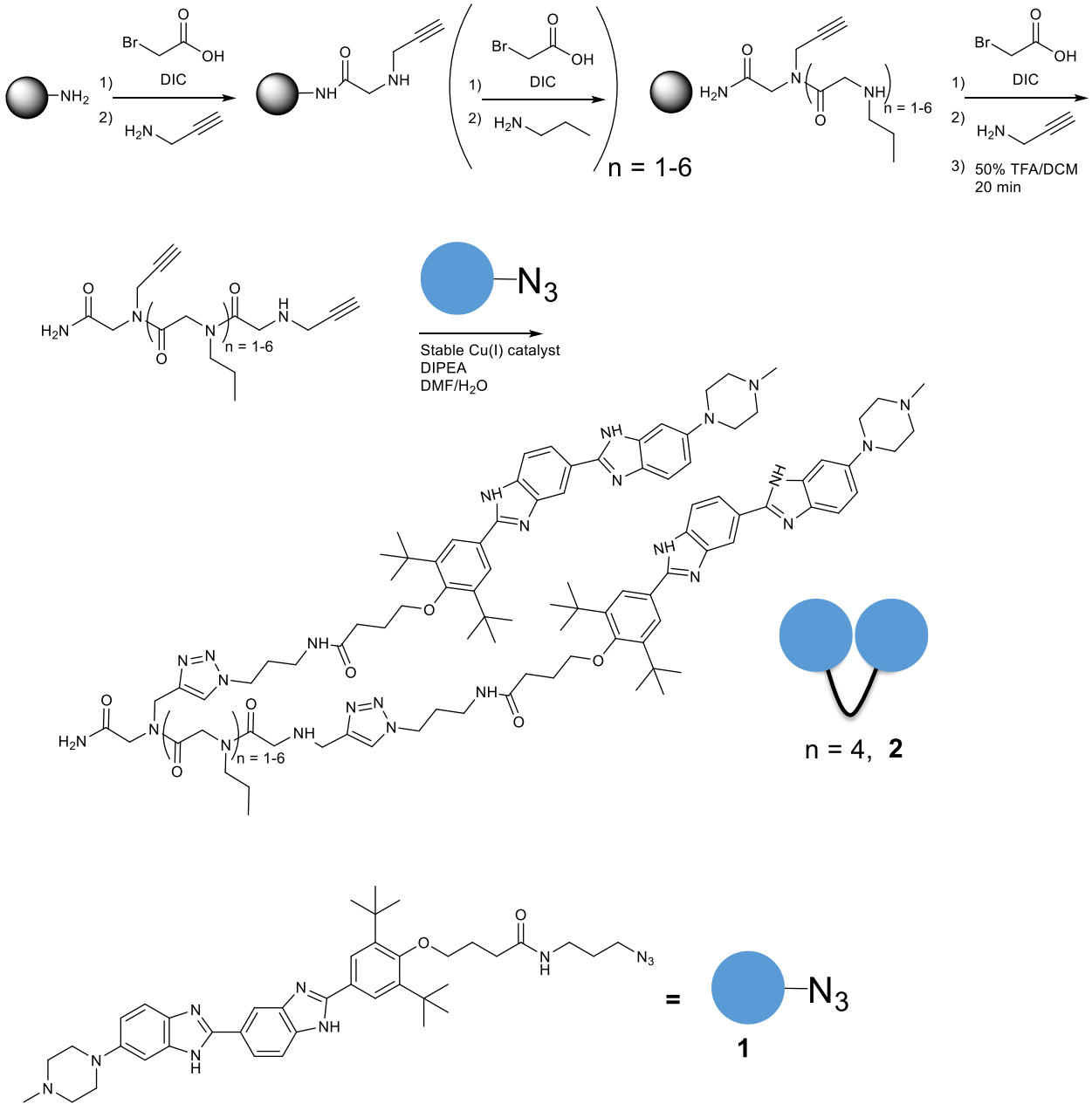
General Synthetic Methods

Synthesis. Fmoc-Rink amide resin (loading = 0.6 mmol/g), 1-hydroxy-7-azabenzotriazole (HOAt), and *N*, *N*'-diisopropylcarbodiimide (DIC) were purchased from Advanced ChemTech and used without further purification. Anhydrous *N*, *N*-dimethyl-formamide (DMF) was purchased from EMD and used without further purification. Piperidine, trifluoroacetic acid (TFA), *N, N*-diisopropylethylamine (DIEA), 2-bromoacetic acid, acetic anhydride were purchased from Sigma-Aldrich and used without further purification. 1-[Bis(dimethylamino)-methylene-]1H-1,2,3-trizaolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) was purchased from Oakwood Products and used without further purification. Propargylamine was purchased from Combi-Blocks and used without further purification. Propyl amine and propargyl alcohol were purchased from Acros Organics and used without further purification. D-biotin was purchased from Chem Impex and used without further purification. Chlorambucil (CA) was purchased from MP Biomedicals and used without further purification. Sphingosine Kinase 1 Inhibitor, SKI-178 was purchased from (567732, Calbiochem) and used without further purification. Microwave reactions were carried out using a Biotage Initiator+ SP Wave microwave reactor. Stable copper (I) catalyst [3], Targaprimir-515/885 [4] (**1**), and *N*-(2-aminoethyl)biotinamide [5] were synthesized as previously described.

Compound purification and analysis. Compounds were purified by preparative HPLC using a Waters 1525 Binary HPLC pump, a Waters 2487 dual absorbance detector system monitoring absorbance at 220 and 254 nm, and a Waters Sunfire C_{18} OBD 5 µm 19 × 150 mm column. A gradient of 20-100% methanol or acetonitrile in water containing 0.1% TFA over 60 min was used for small molecule purification. Purity was monitored by analytical HPLC using a Waters 1525 Binary HPLC pump, a Waters 2487 dual absorbance detector system monitoring absorbance at 220 and 254 nm, and a Waters Symmetry C_{18} 5 µm 4.6 × 150 mm column. A gradient of 0-100% methanol or acetonitrile in water containing 0.1% TFA over 60 min was used for small molecule purity analysis. Mass spectrometry was performed using an Applied Biosystems MALDI-TOF/TOF Analyzer 4800 Plus with an α-cyano-4-hydroxycinnamic acid matrix.

Synthetic Methods and Schemes for Small Molecules

Synthesis of 1 Dimers (n = 4, 2; Targaprimir-515).



Synthesis of ***1 dimers, n=1-6 (n=4, 2; Targaprimir-515)****.* Rink amide resin (1 g, 0.6 mmol) was swollen in DCM for 5 min and then with DMF for 5 min. The resin was de-protected with a solution of 20% piperidine in DMF (5 mL, 2 × 20 min) and then washed

with DMF (3 × 5 mL). The resin was then treated with a solution of bromoacetic acid (0.412 g, 3 mmol) and DIC (0.464 mL, 3 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and treated twice with a solution of propargylamine (170 µL, 2.65 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power.

The resin was washed with DMF (3 × 5 mL) and reacted with a solution of bromoacetic acid (442 mg, 3.18 mmol) and DIC (224 µL, 1.43 mmol) in DMF (10 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. Then, the resin was washed with DMF (3 × 5 mL) and treated twice with a solution of propylamine (261 µL, 3.18 mmol) in DMF (10 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. After that, the resin was washed with DMF (3 × 5 mL) and reacted with a solution of bromoacetic acid (442 mg, 3.18 mmol) and DIC (224 µL, 1.43 mmol) in DMF (10 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. This cycle was repeated 1 to 6 times to add 1 to 6 propylamine spacing units as appropriate.

The resin was washed with DMF (3 × 5 mL) and treated twice with a solution of propargylamine (170 µL, 2.65 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and DCM (3 × 5) and then treated with 50% TFA/DCM (5 mL) for 20 min. The solution was concentrated *in vacuo* and azeotroped with toluene three times. The resulting oil was purified via preparative HPLC as described in the **General Methods**.

The resulting alkyne compound was treated with a solution of **1** (1.52 mg, 2.15 µmol), stable Cu(I) catalyst (0.43 mg, 0.72 µmol) and DIPEA (100 µL, 0.57 mmol) in 4 mL of DMF in a microwave vial at 110 °C for 2 h in a Biotage Initiator+ SP Wave microwave reactor. The resulting mixture was concentrated *in vacuo* and purified via preparative HPLC as described in the **General Methods**.

Isolated 37.2 nmol of **n=1 Dimer** from a 60 µmol scale reaction for a 0.062% total yield. Compound **n=1 Dimer** calculated mass ($C_{95}H_{126}N_{24}O_7$): 1716.0563 (M+H), 1738.0139

(M+Na), and 1753.9878 (M+K); found 1716.0563 (M+H), 1738.0217 (M+Na), and 1754.0035 (M+K); t_R = 19 min. Analytical HPLC run with acetonitrile in water with 0.1% TFA gradient.

Isolated 17.2 nmol of **n=2 Dimer** from a 60 µmol scale reaction for a 0.029% total yield. Compound **n=2 Dimer** calculated mass ($C_{100}H_{135}N_{25}O_8$): 1815.1004 (M+H) and 1837.0823 (M+Na); found 1815.0576 (M+H) and 1837.0264 (M+Na); t_R = 31 min. Analytical HPLC run with 0-100% acetonitrile in water with 0.1% TFA gradient.

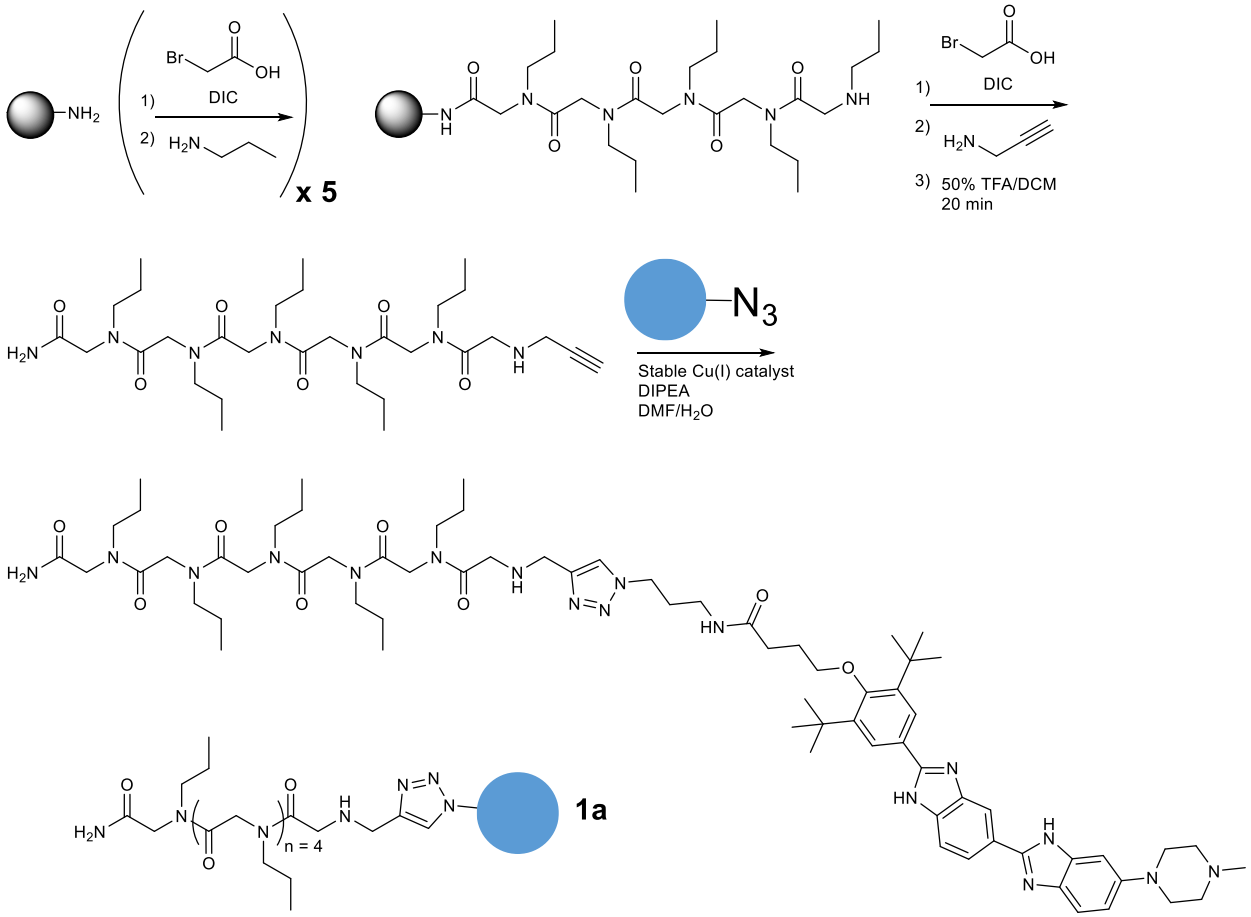
Isolated 37.2 nmol of **n=3 Dimer** from a 60 µmol scale reaction for a 0.033% total yield. Compound **n=3 Dimer** calculated mass ($C_{105}H_{144}N_{26}O_9$): 1914.1688 (M+H), 1936.1507 (M+Na), and 1952.1247 (M+K); found 1914.1986 (M+H), 1936.1824 (M+Na), and 1952.0435 (M+K); t_R = 38 min. Analytical HPLC run with 0-100% acetonitrile in water with 0.1% TFA gradient.

Isolated 710 nmol of **n=4 Dimer** (**2**) from a 600 µmol scale reaction for a 0.12% total yield. **2** calculated mass ($C_{110}H_{153}N_{27}O_{10}$): 2013.2372 (M+H), 2035.2191 (M+Na), and 2051.1931 (M+K); found: 2013.4368 (M+H), 2035.4155 (M+Na), and 2051.3867 (M+K); t_R = 49 min. Analytical HPLC run with 0-100% methanol in water with 0.1% TFA gradient.

Isolated 15.2 nmol of **n=5 Dimer** from a 60 µmol scale reaction for a 0.025% total yield. Compound **n=5 Dimer** calculated mass ($C_{115}H_{162}N_{28}O_{11}$): 2112.3056 (M+H), 2134.2876 (M+Na), and 2150.2615 (M+K); found 2112.2147 (M+H), 2134.2074 (M+Na), and 2150.1672 (M+K); t_R = 33 min. Analytical HPLC run with 0-100% acetonitrile in water with 0.1% TFA gradient.

Isolated 22.1 nmol of **n=6 Dimer** from a 60 µmol scale reaction for a 0.037% total yield. Compound **n=6 Dimer** calculated mass ($C_{120}H_{171}N_{29}O_{12}$): 2211.3740 (M+H) and 2233.3560 (M+Na); found 2211.4622 (M+H) and 2234.4590 (M+Na); t_R = 47 min. Analytical HPLC run with 0-100% methanol in water with 0.1% TFA gradient.

Synthesis of 1a (Targaprimir-515/885 appended to peptoid linker).

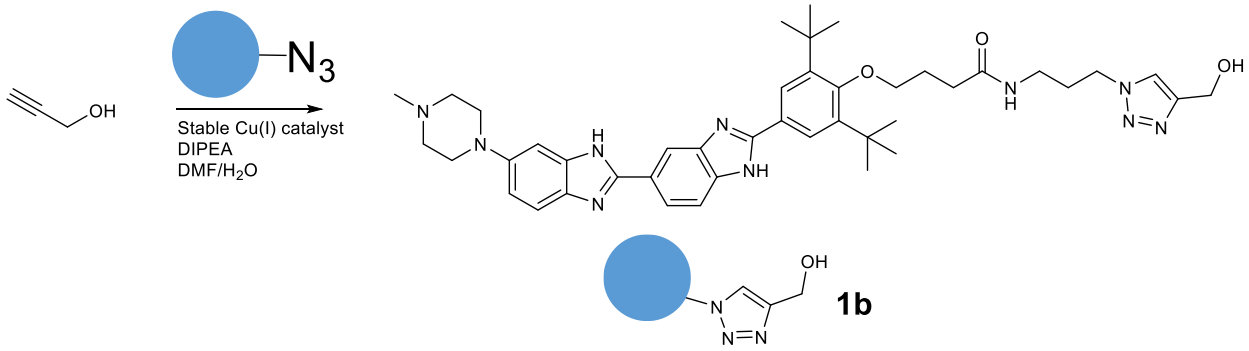


*Synthesis of **1a**.* Rink amide resin (1 g, 0.6 mmol) was swollen in DCM for 5 min and then with DMF for 5 min. The resin was de-protected with a solution of 20% piperidine in DMF (5 mL, 2 × 20 min) and then washed with DMF (3 × 5 mL). The resin was then treated with a solution of bromoacetic acid (0.412 g, 3 mmol) and DIC (0.464 mL, 3 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and treated twice with a solution of propylamine (261 µL, 3.18 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and reacted with a solution of bromoacetic acid (442 mg, 3.18 mmol) and DIC (224 µL, 1.43 mmol) in DMF (10 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. This cycle was repeated 5 times to add a total of 5 propylamine spacing units.

The resin was washed with DMF (3 × 5 mL) and treated twice with a solution of propargylamine (170 µL, 2.65 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF and DCM and then treated with 50% TFA/DCM (5 mL) for 20 min. The solution was concentrated *in vacuo* and azeotroped with toluene three times. The resulting oil was purified via preparative HPLC as described in the **General Methods**.

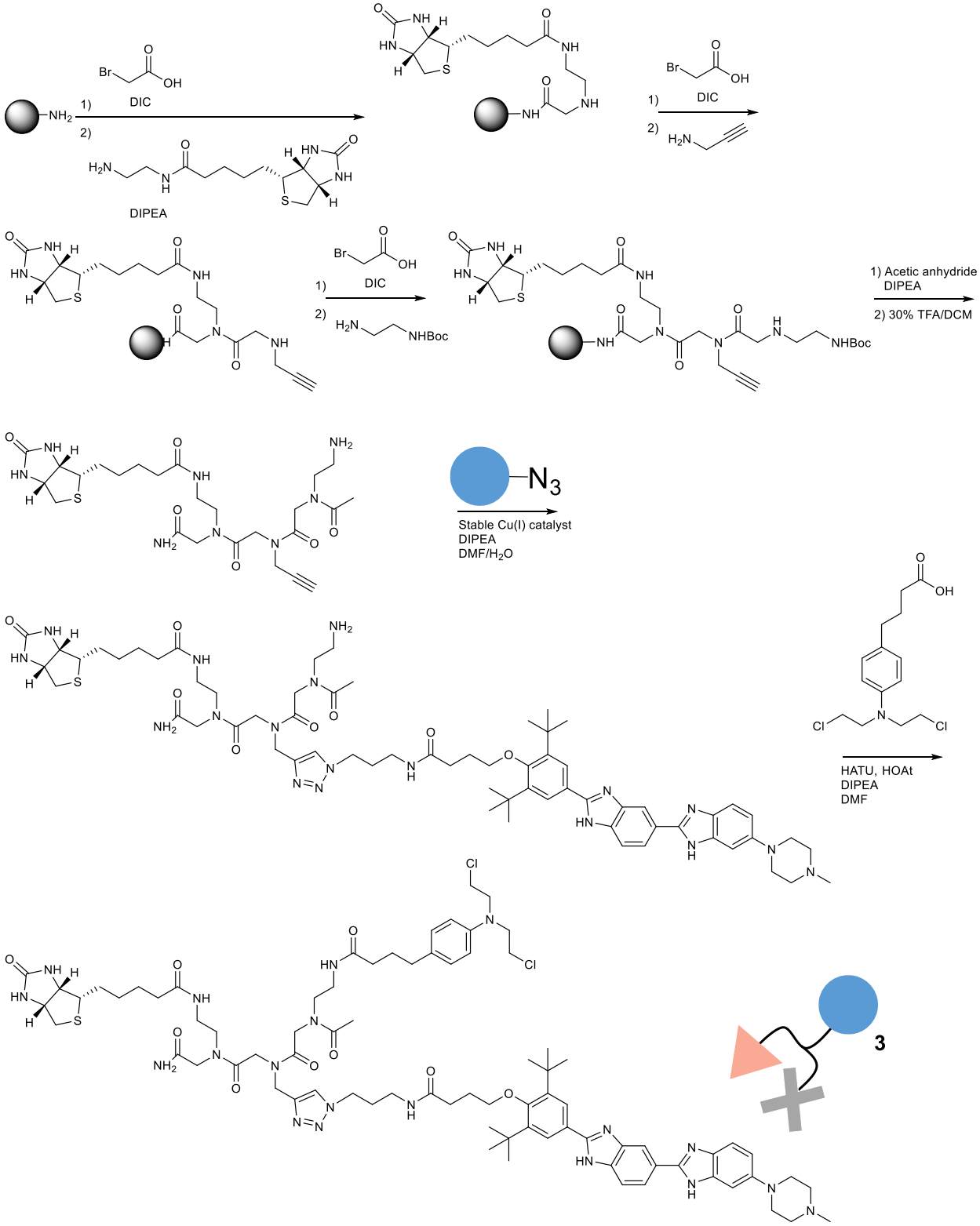
The resulting alkyne compound (0.00607 g, 0.01 mmol) was treated with a solution of **1** (0.00705 g, 0.01 mmol), stable Cu(I) catalyst (0.0018 g, 0.003 mmol) and DIPEA (150 µL, 1.18 mmol) in 2 mL of DMF/water in a microwave vial at 110 °C for 30 min in a Biotage Initiator+ SP Wave microwave reactor. The resulting mixture was concentrated *in vacuo* and purified via preparative HPLC as described in the **General Methods**. Isolated 1.83 µmol of **1a** for an 18.3% yield. **1a** calculated mass ($C_{70}H_{105}N_{17}O_8$): 1312.8410 (M+H); found: 1312.9287 (M+H); t_R = 40 min. Analytical HPLC traces were acquired using a gradient of 0-100% methanol in water containing 0.1% TFA.

Synthesis of 1b (Targaprimir-515/885 appended to triazole).



*Synthesis of **1b**.* Propargyl alcohol (0.00224 g, 0.04 mmol) was treated with a solution of **1** (0.0282 g, 0.04 mmol), stable Cu(I) catalyst (0.0072 g, 0.012 mmol) and DIPEA (150 µL, 1.18 mmol) in 2 mL of DMF/water in a microwave vial at 110 °C for 30 min in a Biotage Initiator+ SP Wave microwave reactor. The resulting mixture was concentrated *in vacuo* and purified via preparative HPLC as described in the **General Methods**. Isolated 27.2 µmol of **1b** for a 68% yield. **1b** calculated mass ($C_{43}H_{56}N_{10}O_3$): 761.4615 (M+H); found: 761.5650 (M+H); t_R = 39 min. Analytical HPLC traces were acquired using a gradient of 0-100% methanol in water containing 0.1% TFA.

Synthesis of 3 (Targaprimir-515/885-CA-Biotin).

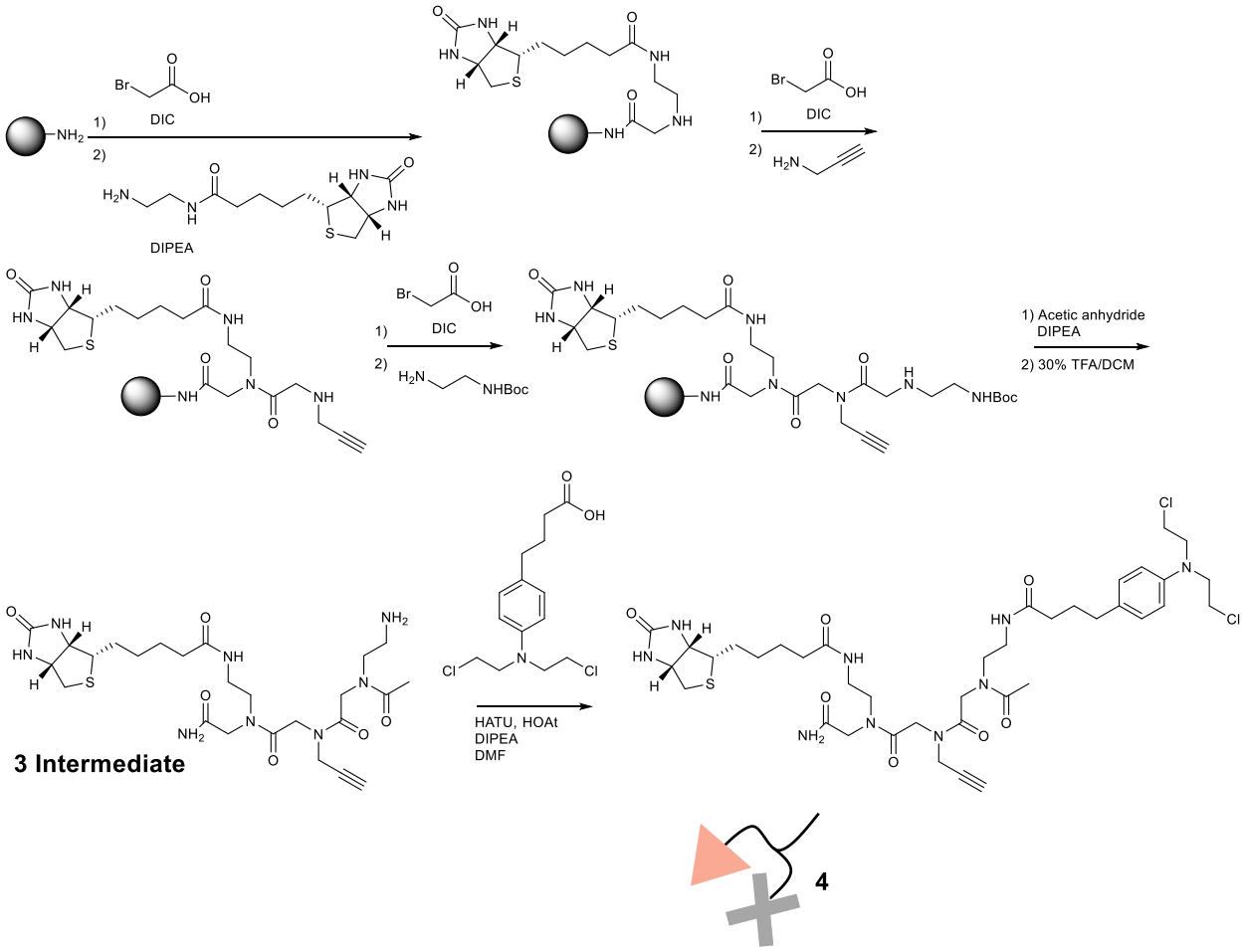


*Synthesis of **3**.* Rink amide resin (1 g, 0.6 mmol) was swollen in DCM for 5 min and then with DMF for 5 min. The resin was de-protected with a solution of 20% piperidine in DMF (5 mL, 2 × 20 min) and then washed with DMF (3 × 5 mL). The resin was then treated with a solution of bromoacetic acid (0.412 g, 3 mmol) and DIC (0.464 mL, 3 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and treated twice with a solution of *N*-(2-aminoethyl)biotinamide (0.2 g, 0.737 mmol) and DIPEA (400 µL, 2.31 mmol) in DMF (4 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) then treated with a solution of bromoacetic acid (0.412 g, 3 mmol) and DIC (0.464 mL, 3 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and treated twice with a solution of propargylamine (450 µL, 6.31 mmol) in DMF (4 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and treated with a solution of bromoacetic acid (0.412 g, 3 mmol) and DIC (0.464 mL, 3 mmol) in DMF (5 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and treated twice with a solution of *N*-Boc-ethylenediamine (0.48 g, 0.300 mmol) in DMF (4 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF (3 × 5 mL) and treated with a solution of acetic anhydride (152 µL, 1.592 mmol) and DIPEA (400 µL, 2.31 mmol) in DMF (4 mL) via microwave irradiation (3 × 15 s) using a 700 W microwave set to 10% power. The resin was washed with DMF and DCM and then treated with 30% TFA/DCM (5 mL) for 20 min.

The solution was concentrated *in vacuo* and azeotroped with toluene three times. The resulting oil was purified via preparative HPLC. The resulting alkyne amine intermediate, **3 intermediate**, compound was treated with a solution of **1** (4.56 mg, 3.21 µmol), stable Cu(I) catalyst (0.99 mg, 1.65 µmol) and DIPEA (50 µL, 0.287 mmol) in 2 mL of DMSO at 60 °C overnight. The resulting mixture was concentrated *in vacuo* and purified via preparative HPLC as described in the **General Methods**.

The amine compound (1.9 mg, 1.47 µmol) was reacted with a solution of chlorambucil (3.49 mg, 1.65 µmol), HOAt (0.51 mg, 3.71 µmol), HATU (1.5 mg, 3.94 µmol) and DIPEA (5 µL, 28.7 µmol) in 500 µL of DMF at room temperature overnight. The resulting mixture was concentrated *in vacuo* and purified via preparative HPLC as described in the **General Methods**. Isolated 6.54 µmol of **3** for a 1.1% total yield. **3** calculated mass ($C_{79}H_{109}Cl_2N_{19}O_9S$): 1570.7832 (M+H); found: 1572.6862 (M+H, isotopic distribution of Cl); t_R = 39 min. Analytical HPLC traces were acquired using a gradient of 0-100% acetonitrile in water containing 0.1% TFA.

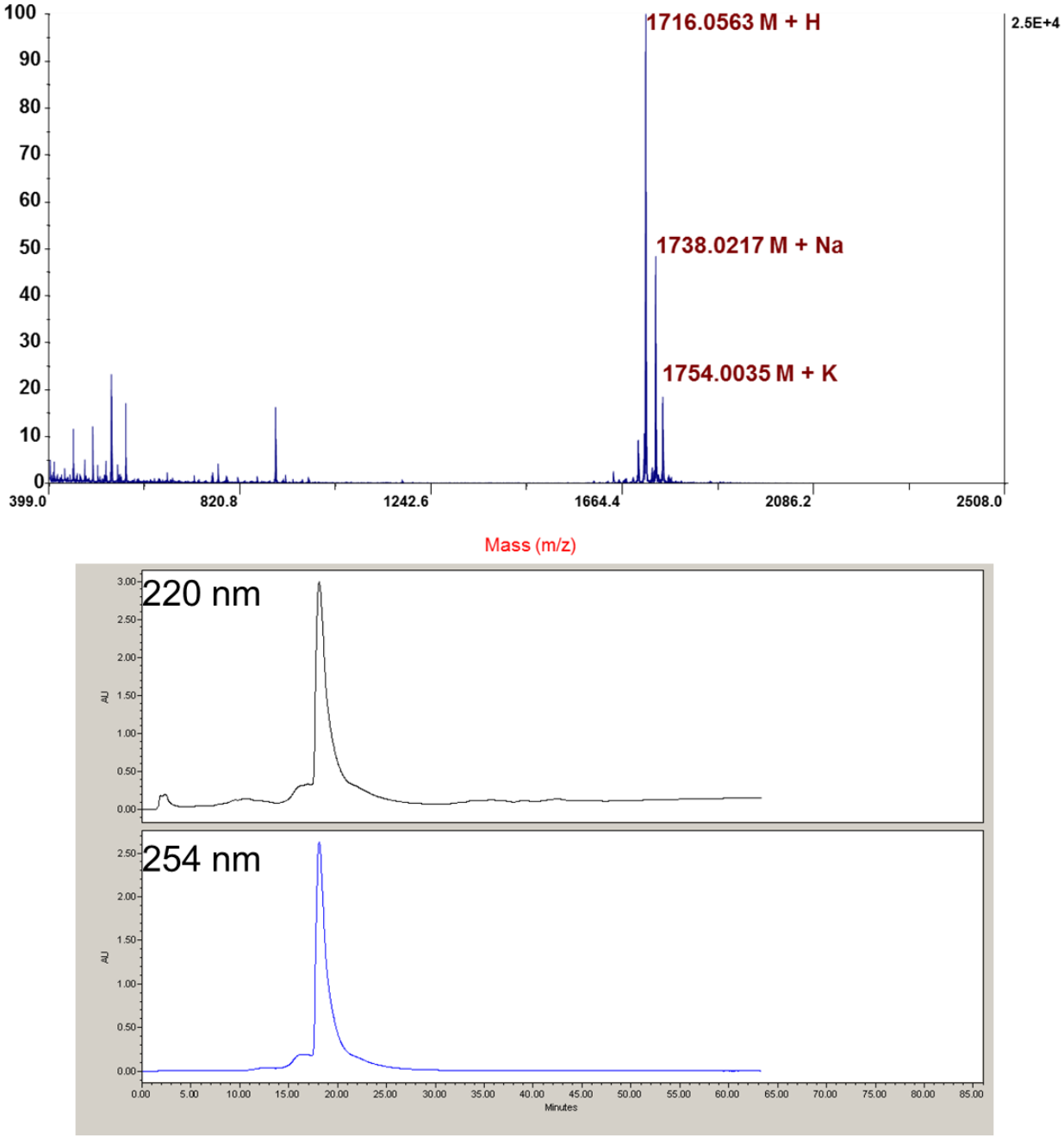
Synthesis of 4 (Control CA-Biotin).



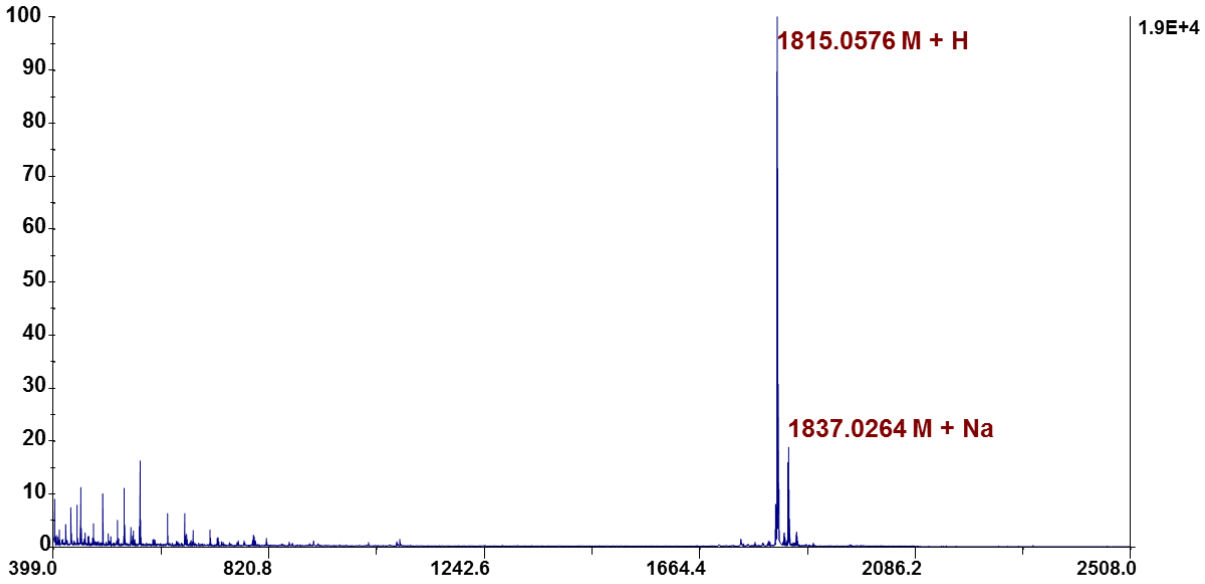
*Synthesis of **4**.* Compound **4** was synthesized as described above for compound **3**, except the **3 intermediate** (6.0 mg, 10.76 µmol) was directly reacted with a solution of chlorambucil (3.6 mg, 11.8 µmol), HOAt (1.6 mg, 11.8 µmol), HATU (4.6 mg, 12.1 µmol) and DIPEA (8.4 µL, 48.4 µmol) in 500 µL of DMF at room temperature overnight. The resulting mixture was concentrated *in vacuo* and purified via preparative HPLC as described above in the **General Methods**. Isolated 7.15 µmol of **4** for a 66% yield. **4** calculated mass ($C_{39}H_{57}Cl_2N_9O_7S$): 888.3376 (M+Na); found: 888.3744 (M+Na); t_R = 28 min. Analytical HPLC traces were acquired using a gradient of 0-100% acetonitrile in water containing 0.1% TFA.

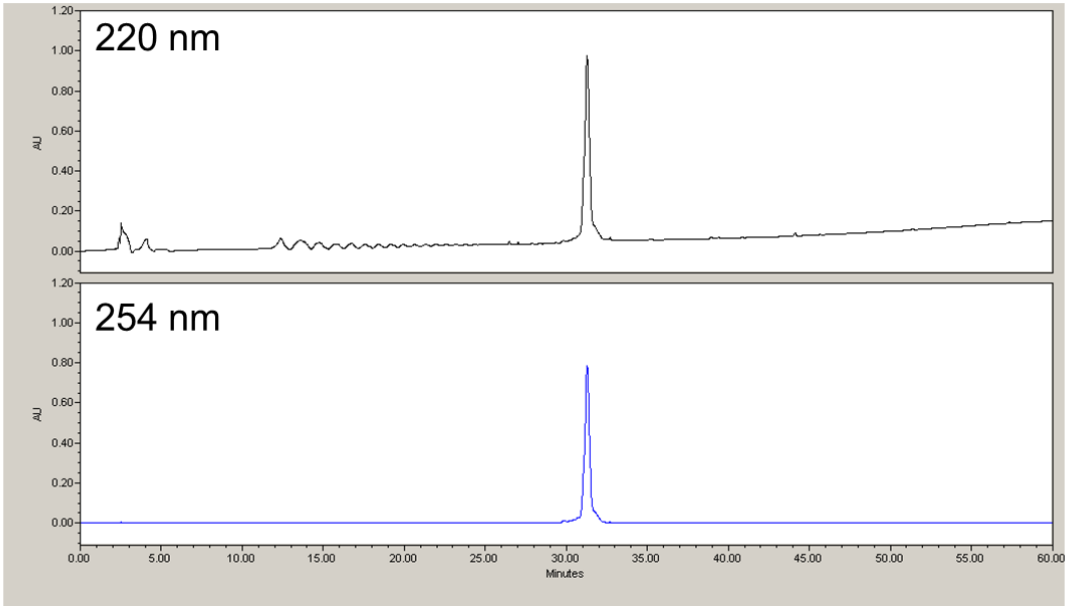
Characterization of Small Molecules

n=1 Dimer

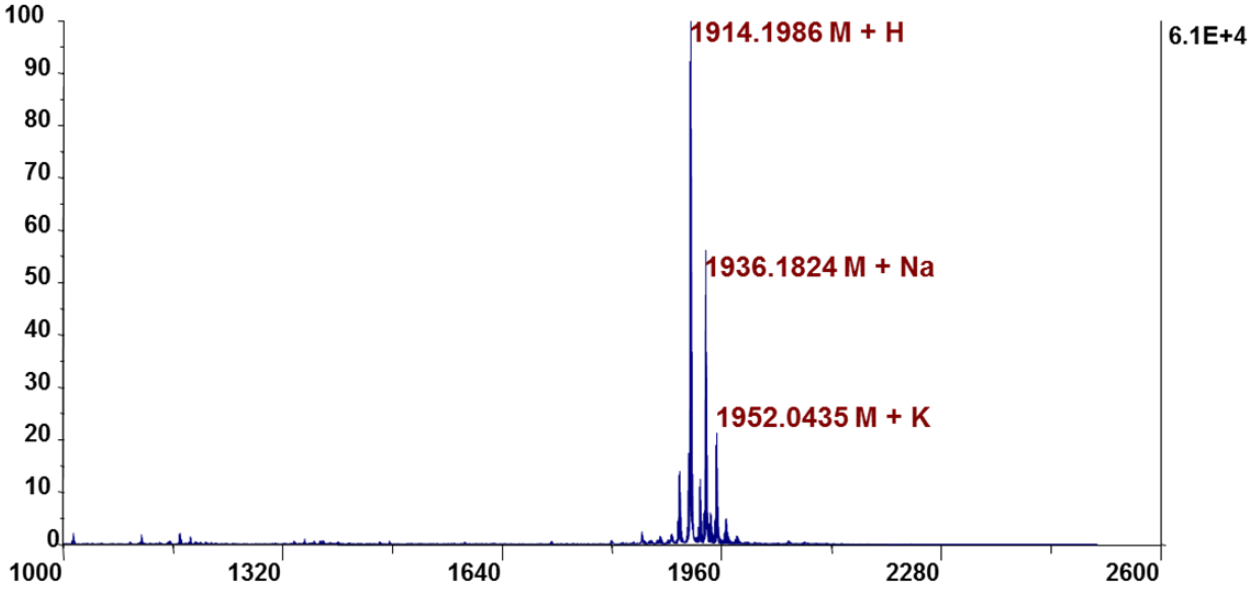


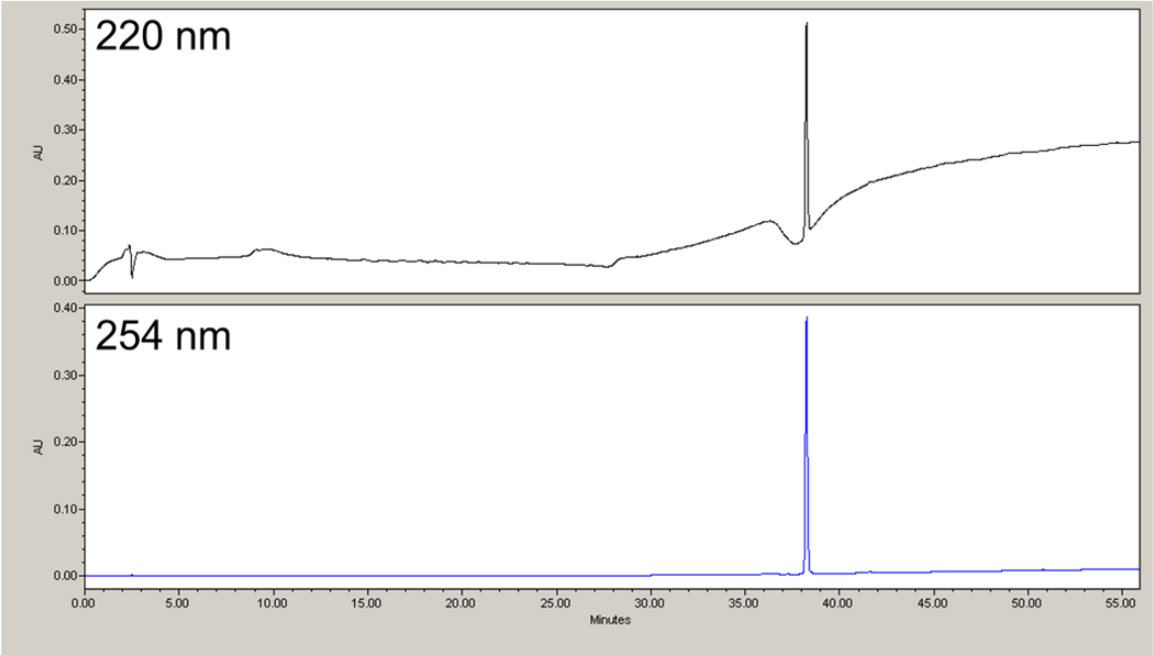
n=2 Dimer


1815.0576 M + H
1837.0264 M + Na
1.9E+4
100
90
80
70
60
50
40
30
20
10
0
399.0
820.8
1242.6
1664.4
2086.2
2508.0
Mass (m/z)

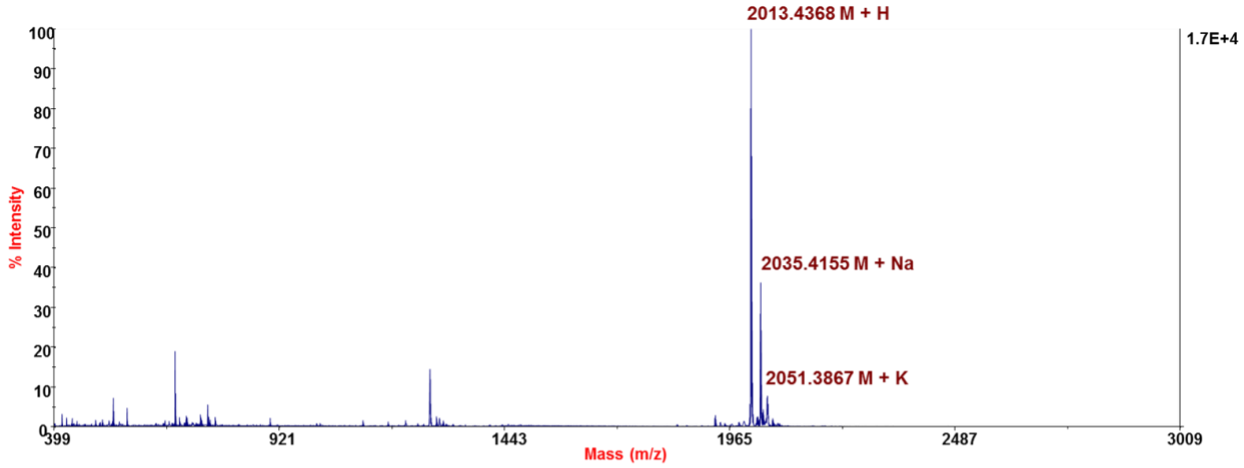

220 nm
254 nm
AU
Minutes
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00

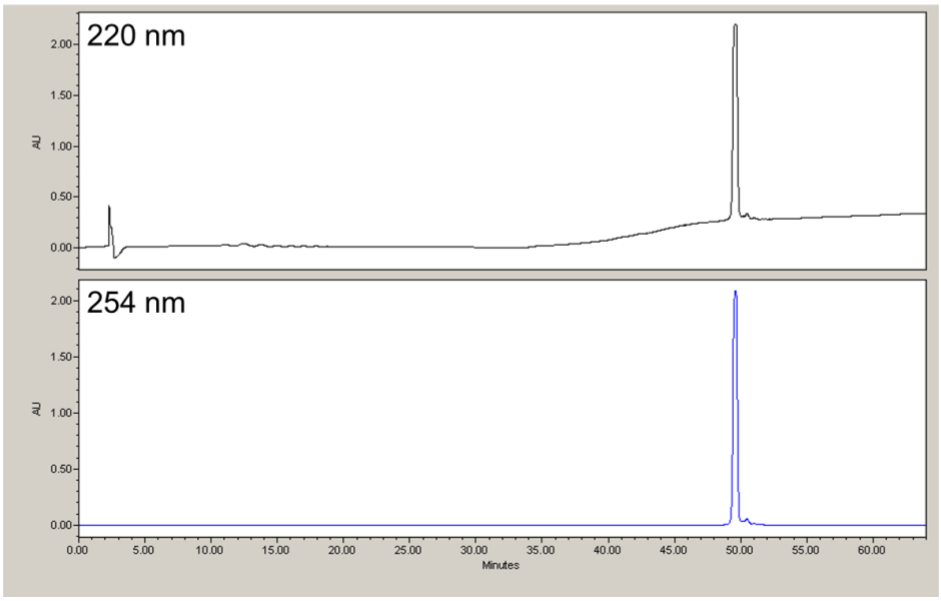
n=3 Dimer


1914.1986 M + H
6.1E+4
1936.1824 M + Na
1952.0435 M + K
100
90
80
70
60
50
40
30
20
10
0
1000
1320
1640
1960
2280
2600
Mass (m/z)

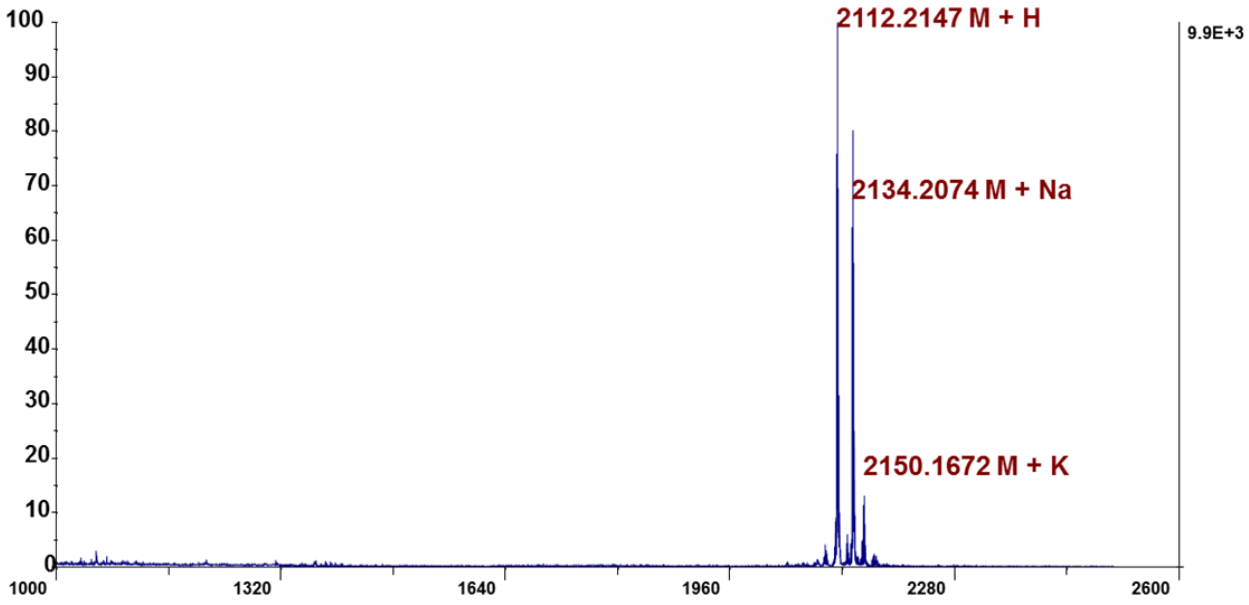

220 nm
254 nm
AU
Minutes
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00

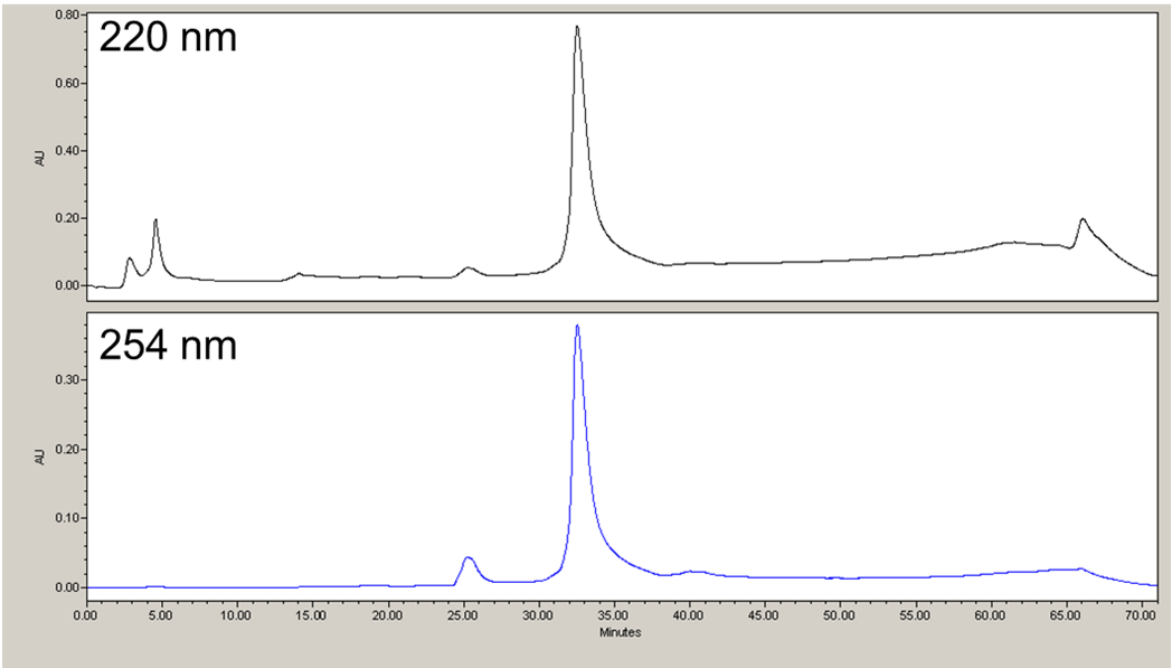
n=4 Dimer; **2**, Targaprimir-515



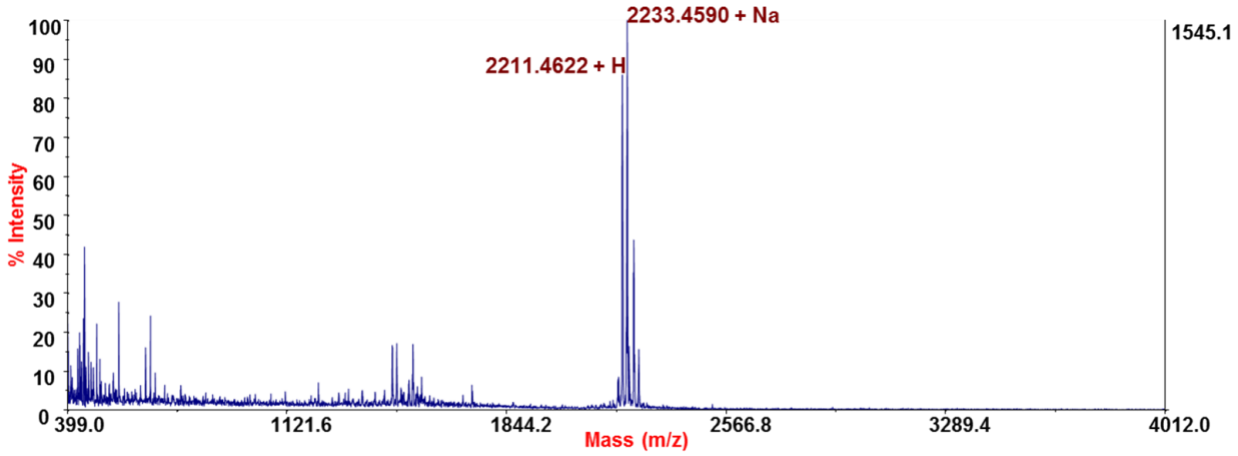


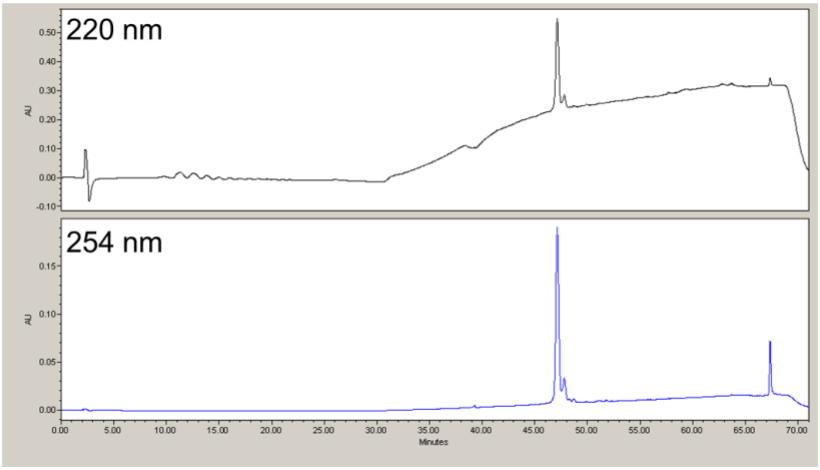
n=5 Dimer


2112.2147 M + H
2134.2074 M + Na
2150.1672 M + K
9.9E+3
100
90
80
70
60
50
40
30
20
10
0
1000
1320
1640
1960
2280
2600
Mass (m/z)

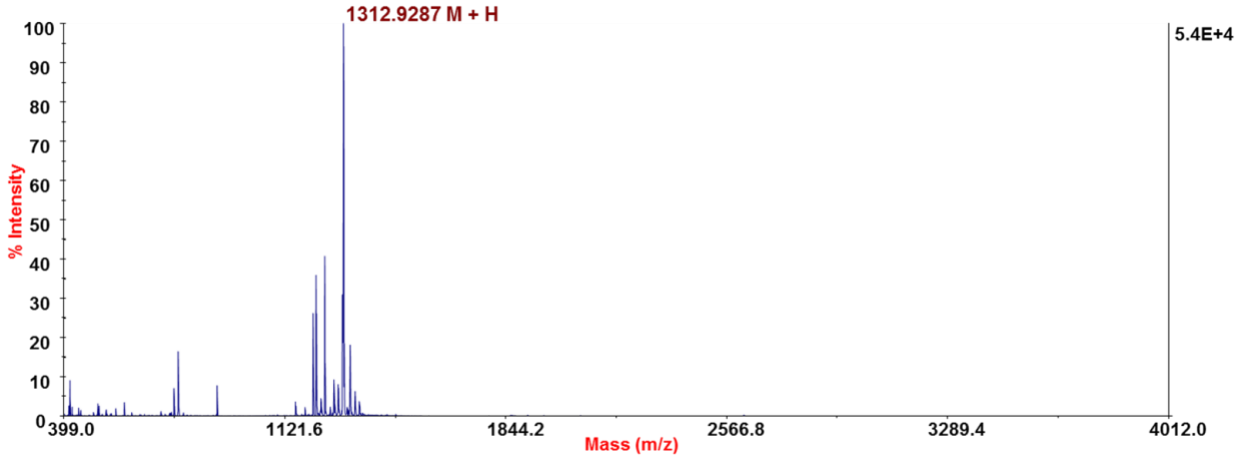

220 nm
254 nm
AU
Minutes
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
65.00
70.00

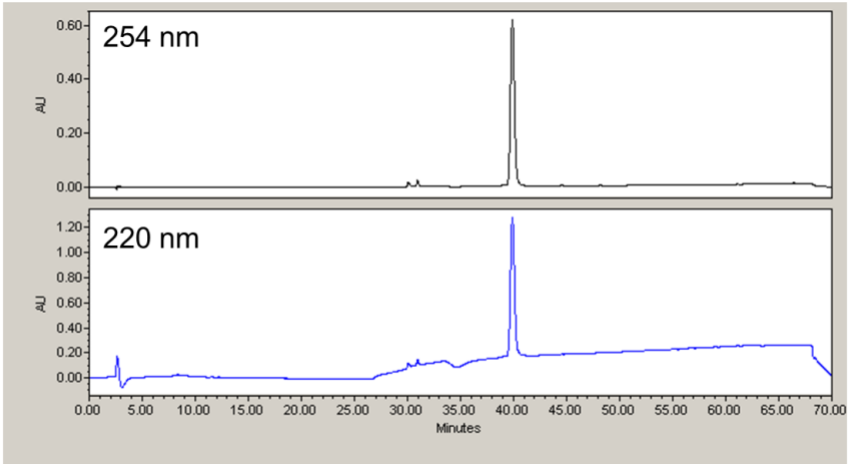
n=6 Dimer


2233.4590 + Na
2211.4622 + H
1545.1
% Intensity
Mass (m/z)
399.0
1121.6
1844.2
2566.8
3289.4
4012.0

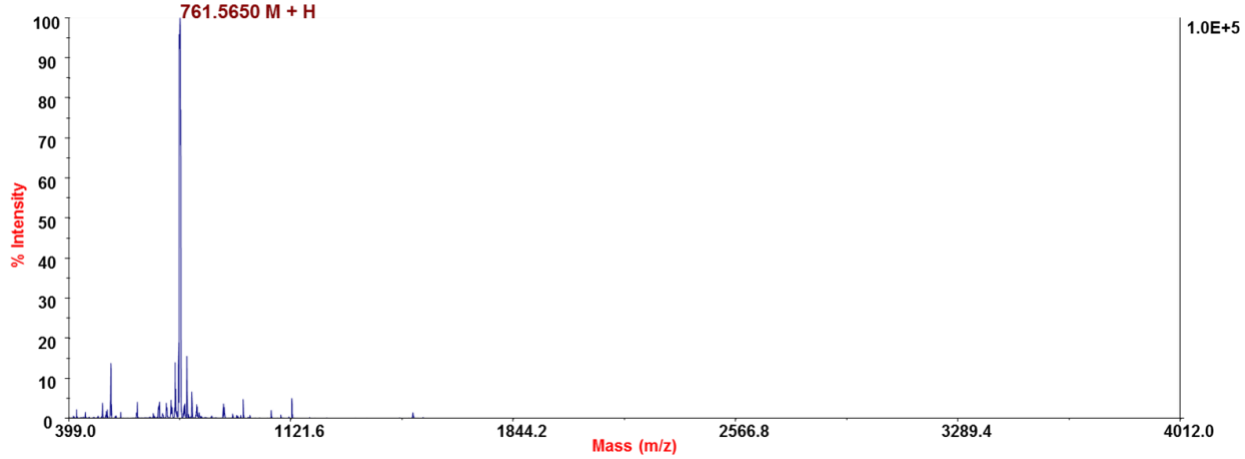

220 nm
254 nm
AU
Minutes

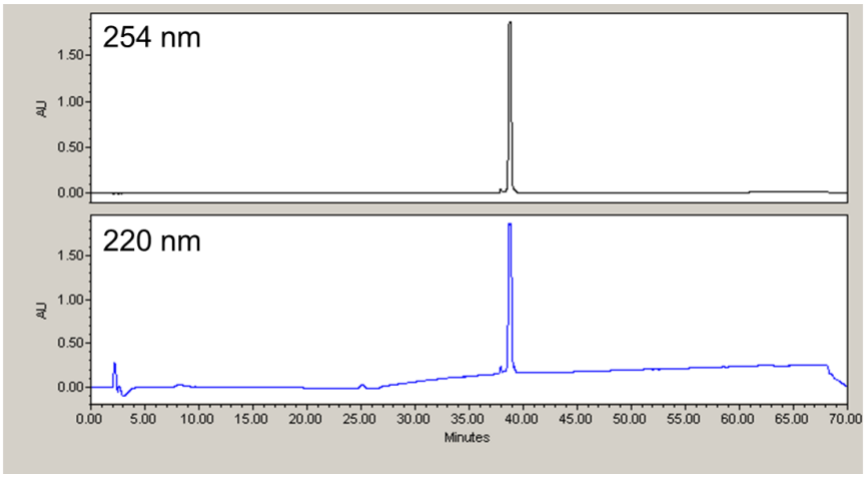
1a (Targaprimir-515/885 appended to peptoid linker)



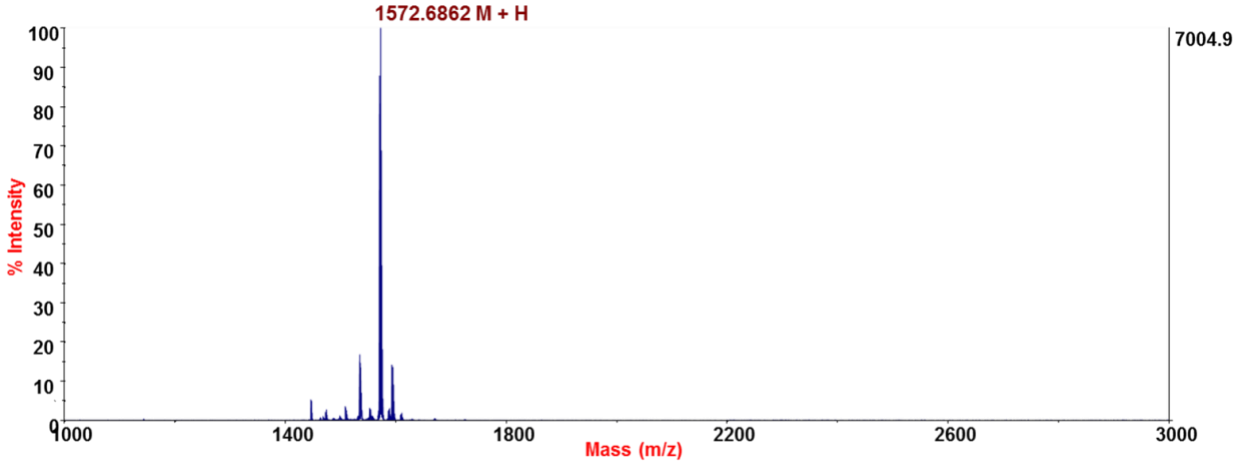


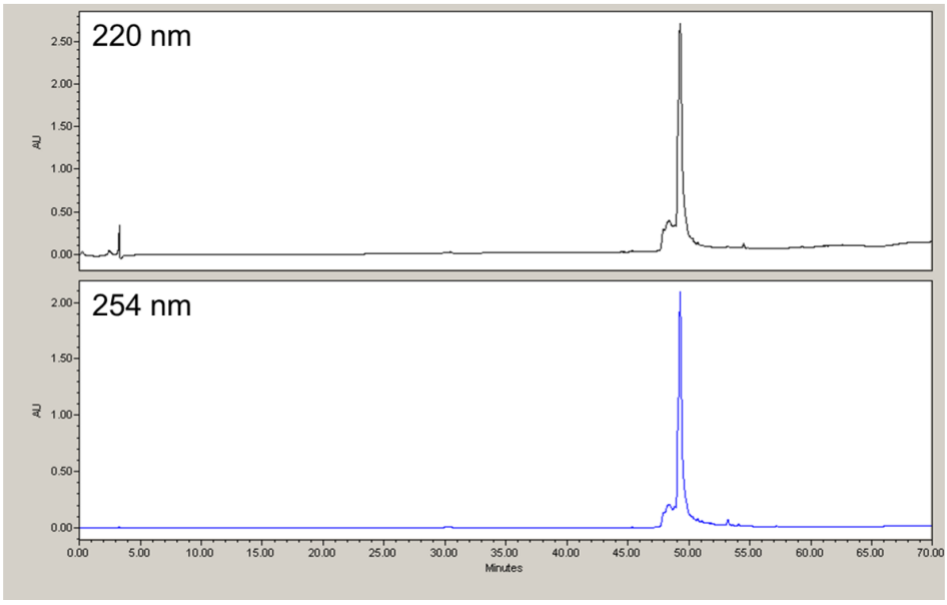
1b (Taragprimir-515/885 appended to triazole)



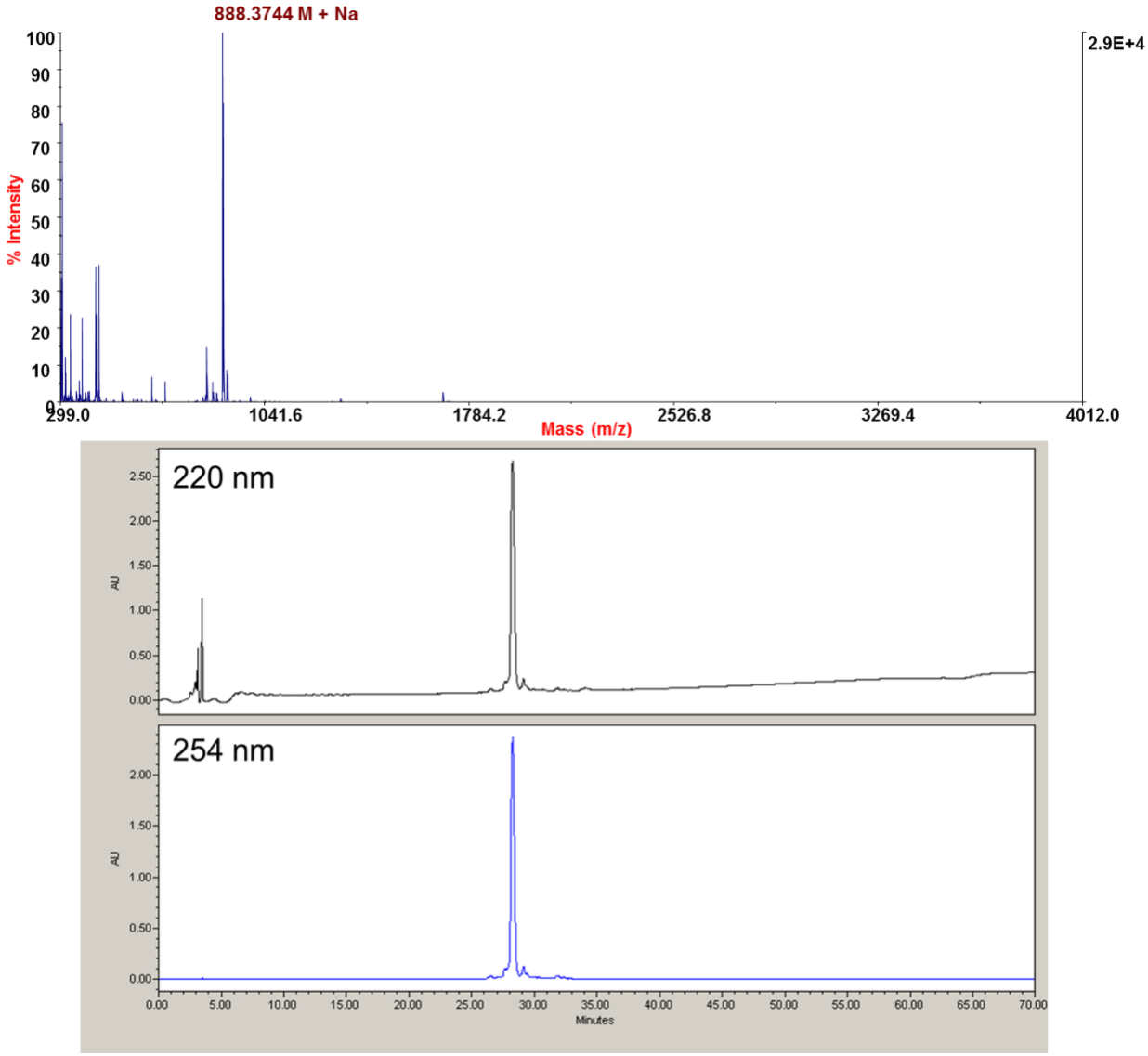


3 (Targaprimir-515/885-CA-Biotin)





4 (Control CA-Biotin)



SUPPLEMENTARY REFERENCES

(1) Velagapudi, S. P.; Gallo, S. M.; Disney, M. D. *Nat. Chem. Biol.* **2014**, *10*, 291.
(2) McDowell, J. A.; Turner, D. H. *Biochemistry* **1996**, *35*, 14077.
(3) Özçubukçu, S.; Ozkal, E.; Jimeno, C.; Pericàs, M. A. *Org. Lett.* **2009**, *11*, 4680.
(4) Velagapudi, S. P.; Seedhouse, S. J.; French, J.; Disney, M. D. *J. Am. Chem. Soc.* **2011**, *133*, 10111.
(5) Yamada, M.; Harada, K.; Maeda, Y.; Hasegawa, T. *New J. Chem.* **2013**, *37*, 3762.