Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary information

A subnanomolar fluorescent probe for protein kinase CK2 interaction studies

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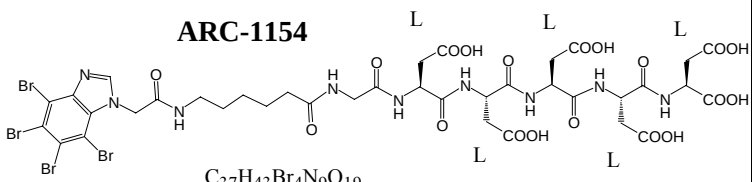
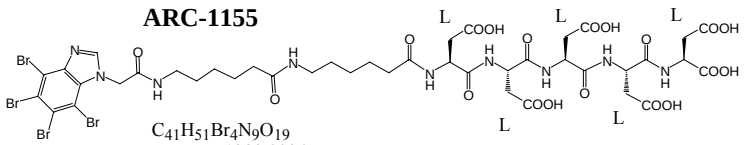
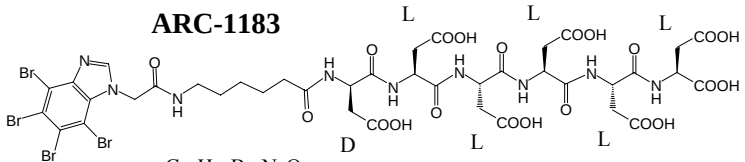
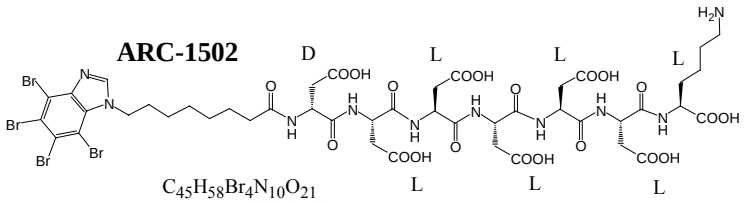
Contents

HPLC-MS data

HPLC retention times and purity of the compounds.

Purification of the compounds was performed with Schimadzu LC Solution (Prominence) system by using manual injector and a diode array (SPD M20A) detector. Separation was achieved with a Gemini C18 5 µ column (250×4.6 mm i.d Phenomenex) protected by a 5 µ Gemini C18 4×2.0 mm guard column. Mobile phase A: 0.1 % TFA, mobile phase B: 0.1% TFA in ACN and a flow of 1 ml/min were employed. Linear gradient elution was started at 3 min (injection time was also at 3 min) with 10% to 30% B. The speed of the gradient is specified in the table.

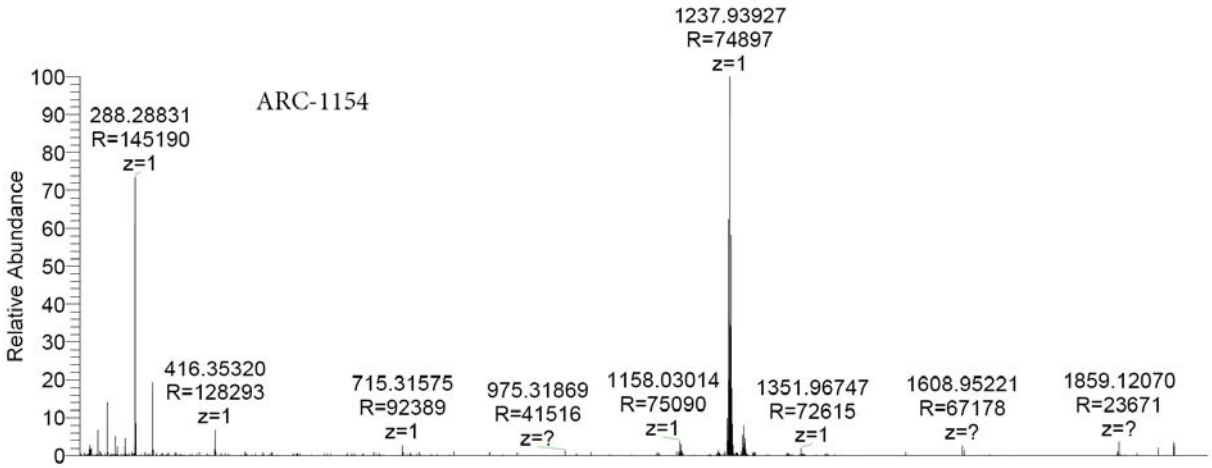
Table S1

Code, structure, molecular formula, exact monoisotopic mass and MW. Letters L or D note configurations of amino acids.	Gradient	R_t, min	Purity	ESI-MS
ARC-1154  $C_{37}H_{43}Br_4N_9O_{19}$ Exact Mass: 1232,94087 Mol. Wt.: 1237,40258	15%B-90%B/30min	13.5	100%	1238
ARC-1155  $C_{41}H_{51}Br_4N_9O_{19}$ Exact Mass: 1289,00347 Mol. Wt.: 1293,50890	30%B-90%B/30 min	8.6	95.7%.	1294
ARC-1183  $C_{39}H_{45}Br_4N_9O_{21}$ Exact Mass: 1290,94635 Mol. Wt.: 1295,43866	10%B-90%B/30 min	13.7	100,0%	1296
ARC-1502  $C_{45}H_{58}Br_4N_{10}O_{21}$ Exact Mass: 1390,05115 Mol. Wt.: 1394,61282	15%B-90%B/30 min	14.5	100%	1395

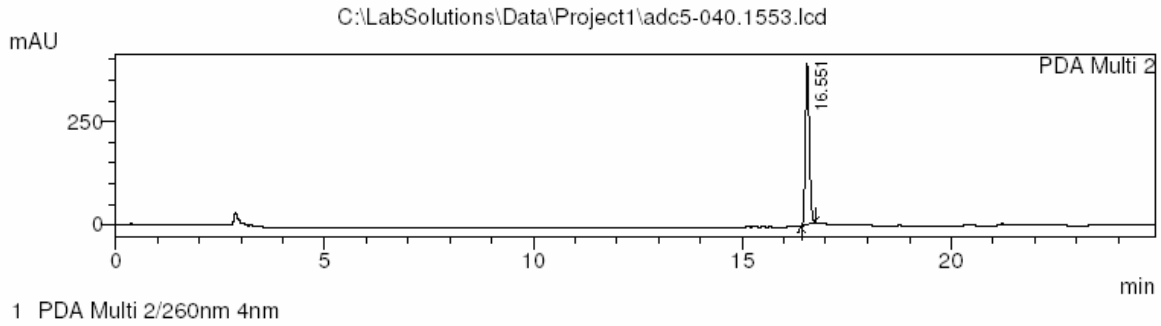
ARC-1504 $C_{77}H_{94}Br_4N_{12}O_{28}S_2$ Exact Mass: 2014,24754 Mol. Wt.: 2019,38234	30%B-60%B/30min	18.0	100%	$[M+H]^{2+}$ 1010
5-Tamra-RADDSDDDDD $C_{65}H_{79}N_{15}O_{30}$ Exact Mass: 1549,51173 Mol. Wt.: 1550,40586	10%B-60%B/30min	12.8	100,0%	$[M+H]^{2+}$ 776
5-Tamra-RADDS(p)DDDDD $C_{65}H_{80}N_{15}O_{33}P$ Exact Mass: 1629,47807 Mol. Wt.: 1630,38576	10%B-60%B/30min	13.5		$[M+H]^{2+}$ 816

HRMS spectra and HPLC chromatograms

ARC-1154



ARC-1154
TBBI-Ac-Ahx-Gly Asp5
Gradient 15-90/30 min
Rt 13.6 min
Purity 100%



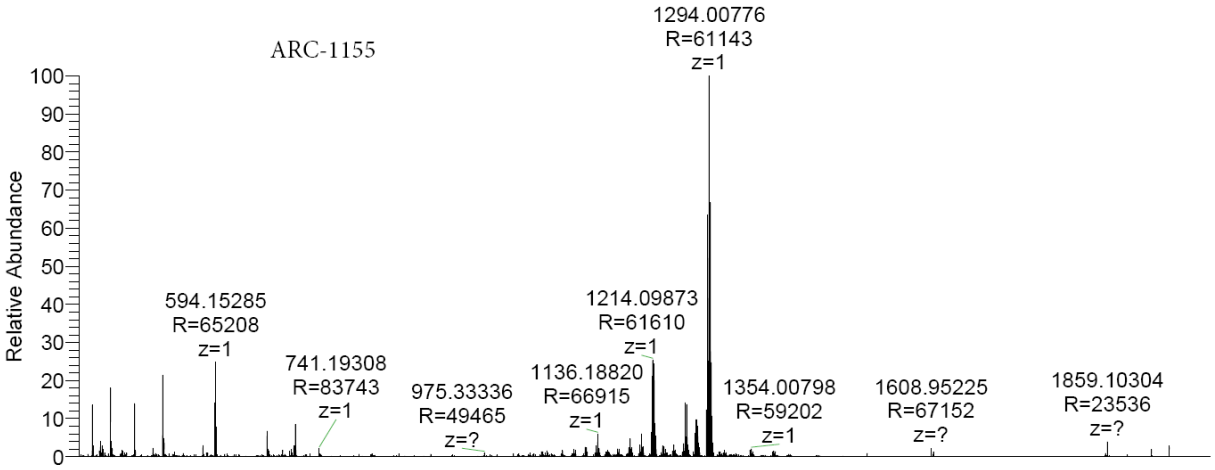
1 PDA Multi 2/260nm 4nm

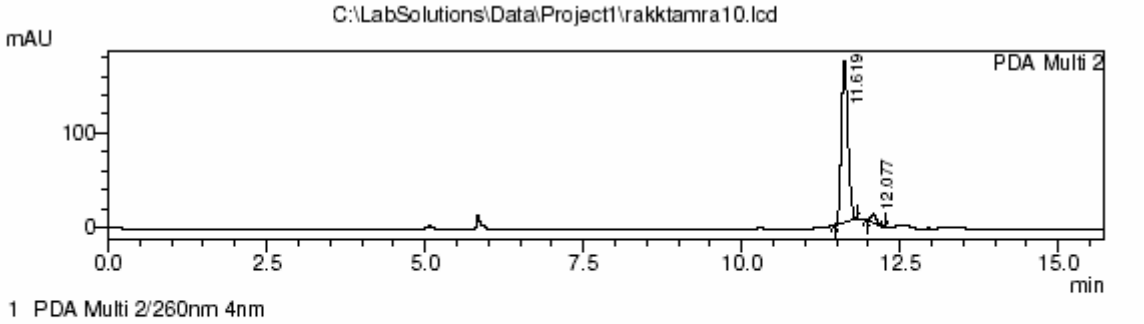
PeakTable

PDA Ch2 260nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	16.551	2470348	391481	100.000	100.000
Total		2470348	391481	100.000	100.000

ARC-1155





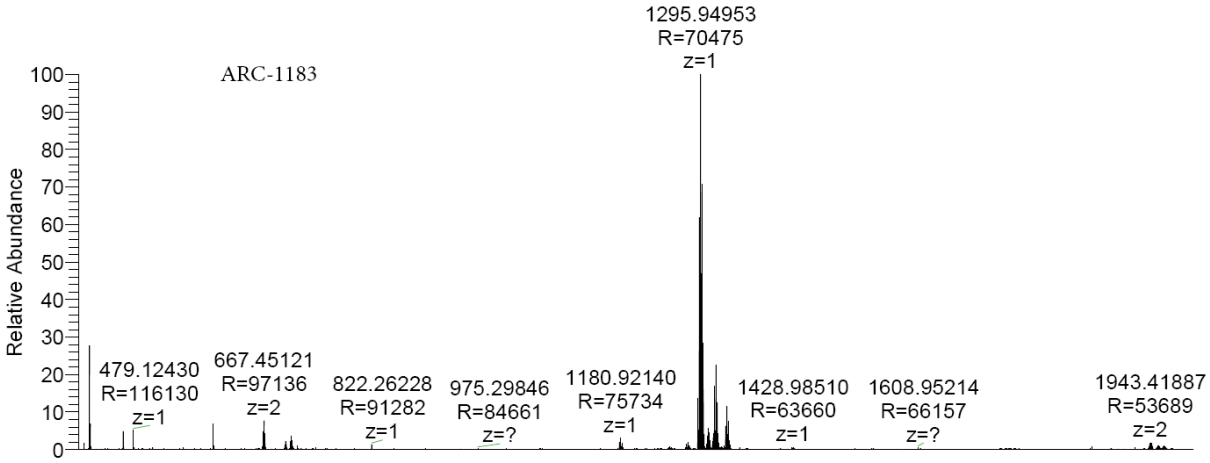
1 PDA Multi 2/260nm 4nm

PeakTable

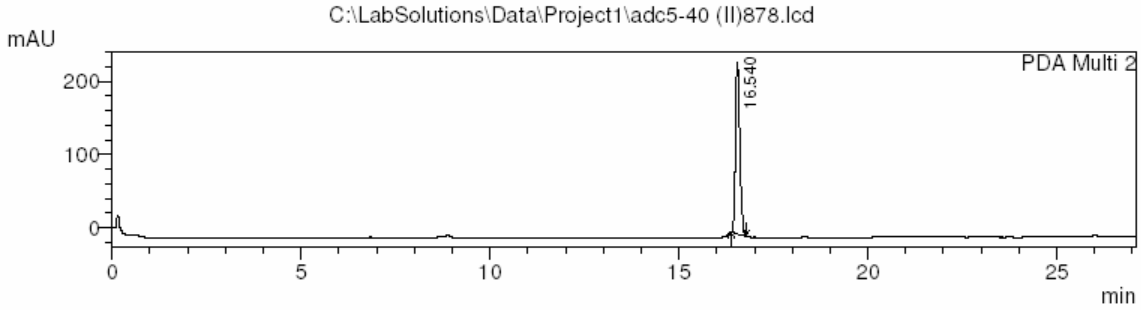
PDA Ch2 260nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	11.619	1224807	171916	95.726	95.109
2	12.077	54682	8841	4.274	4.891
Total		1279489	180757	100.000	100.000

ARC-1183



ARC 1183
TBBI-Ac-Ahx-dAsp-Asp5
Gradient 10-50/30 min
Rt 13.7 min
Purity 100%



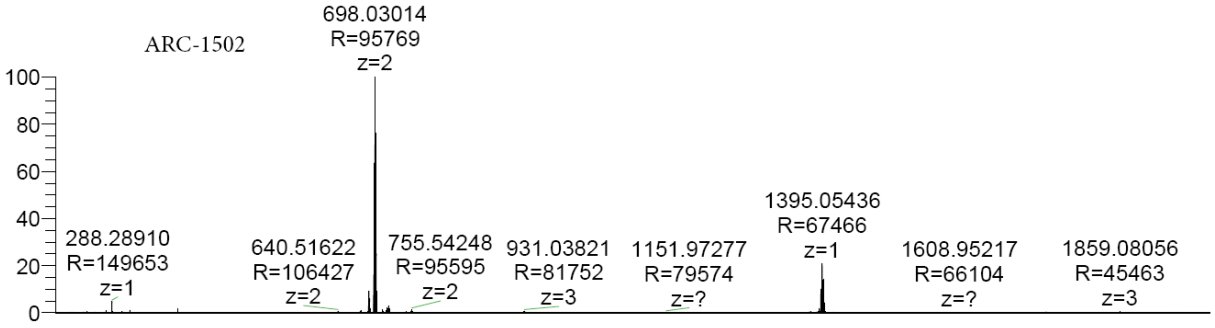
1 PDA Multi 2/260nm 4nm

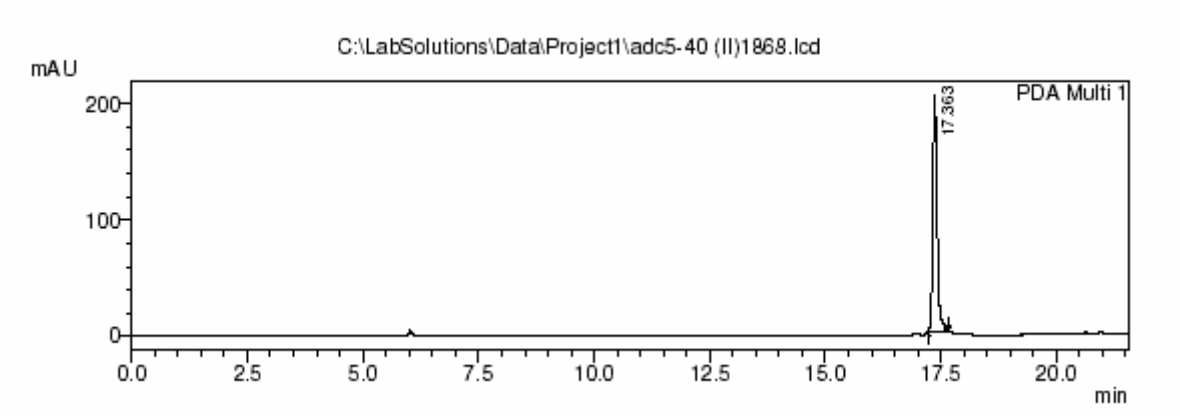
PeakTable

PDA Ch2 260nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	16.540	1865736	234930	100.000	100.000
Total		1865736	234930	100.000	100.000

ARC-1502



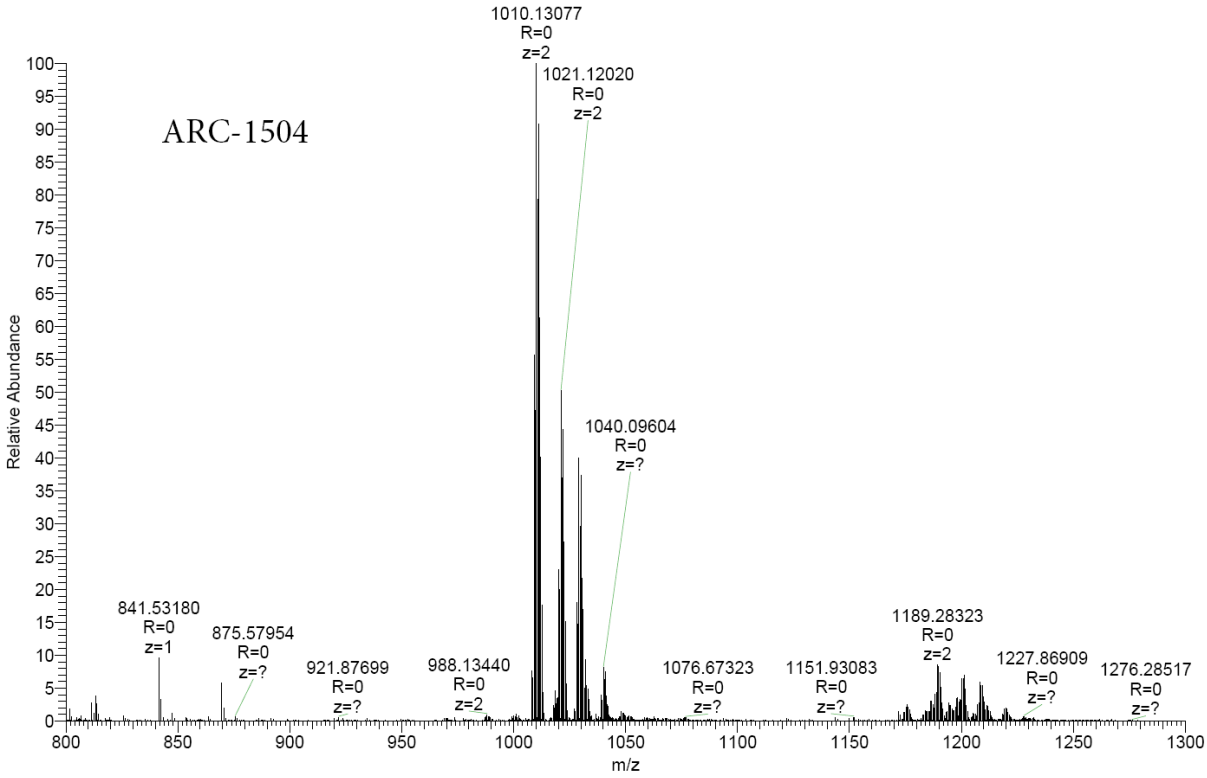


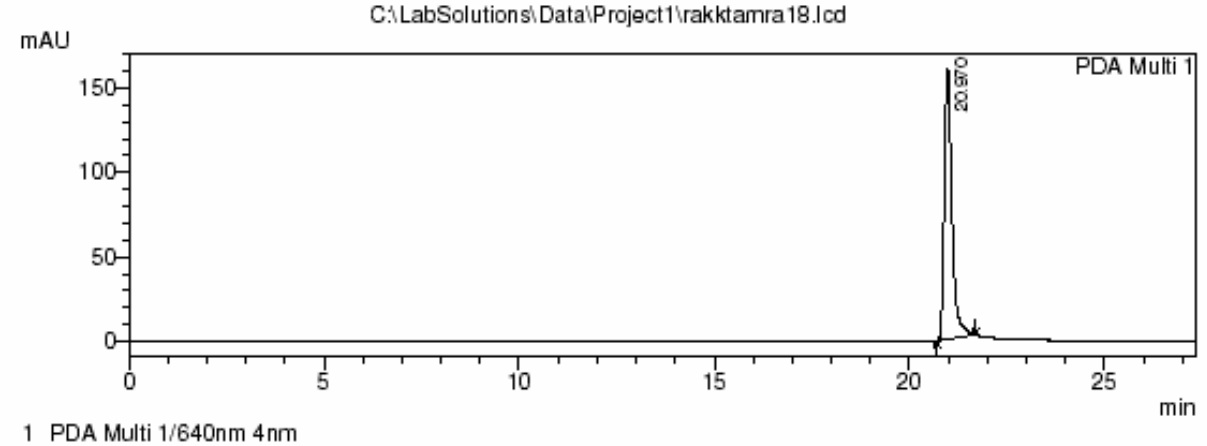
PeakTable

PDA Ch1 260nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	17.363	1294233	203635	100.000	100.000
Total		1294233	203635	100.000	100.000

ARC-1504





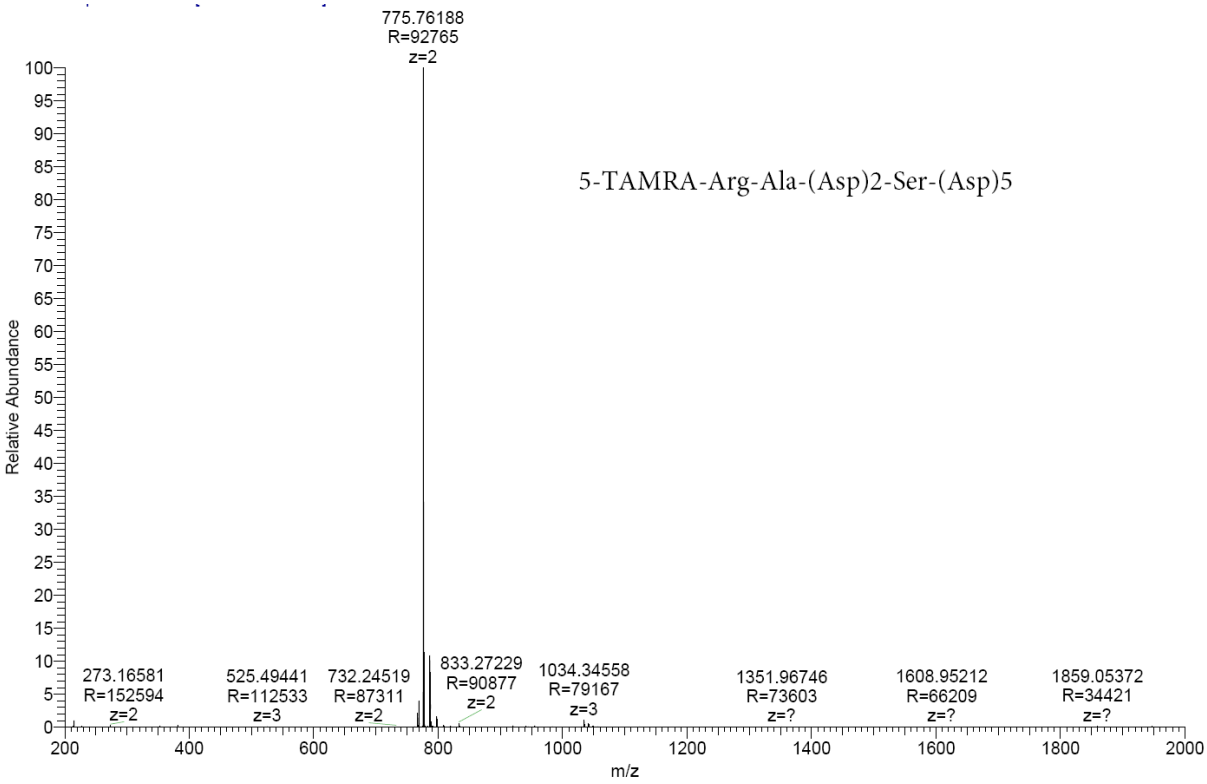
1 PDA Multi 1/640nm 4nm

PeakTable

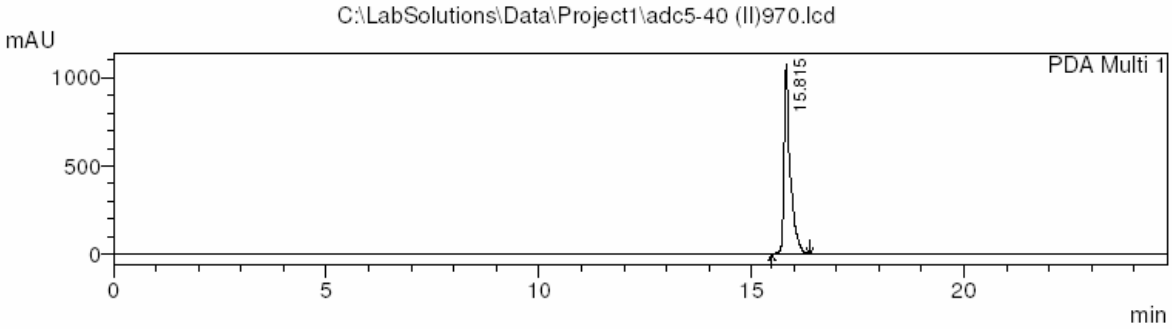
PDA Ch1 640nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	20.970	2171086	160224	100.000	100.000
Total		2171086	160224	100.000	100.000

5-Tamra-RADDSDDDDD



5-Tamra-RADDSDDDD
Gradient 10-60/30min
Rt 12.8 min
Purity 100%



1 PDA Multi 1/550nm 4nm

PeakTable

PDA Ch1 550nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	15.815	10769154	1077464	100.000	100.000
Total		10769154	1077464	100.000	100.000

5-Tamra-RADDS(p)DDDDD

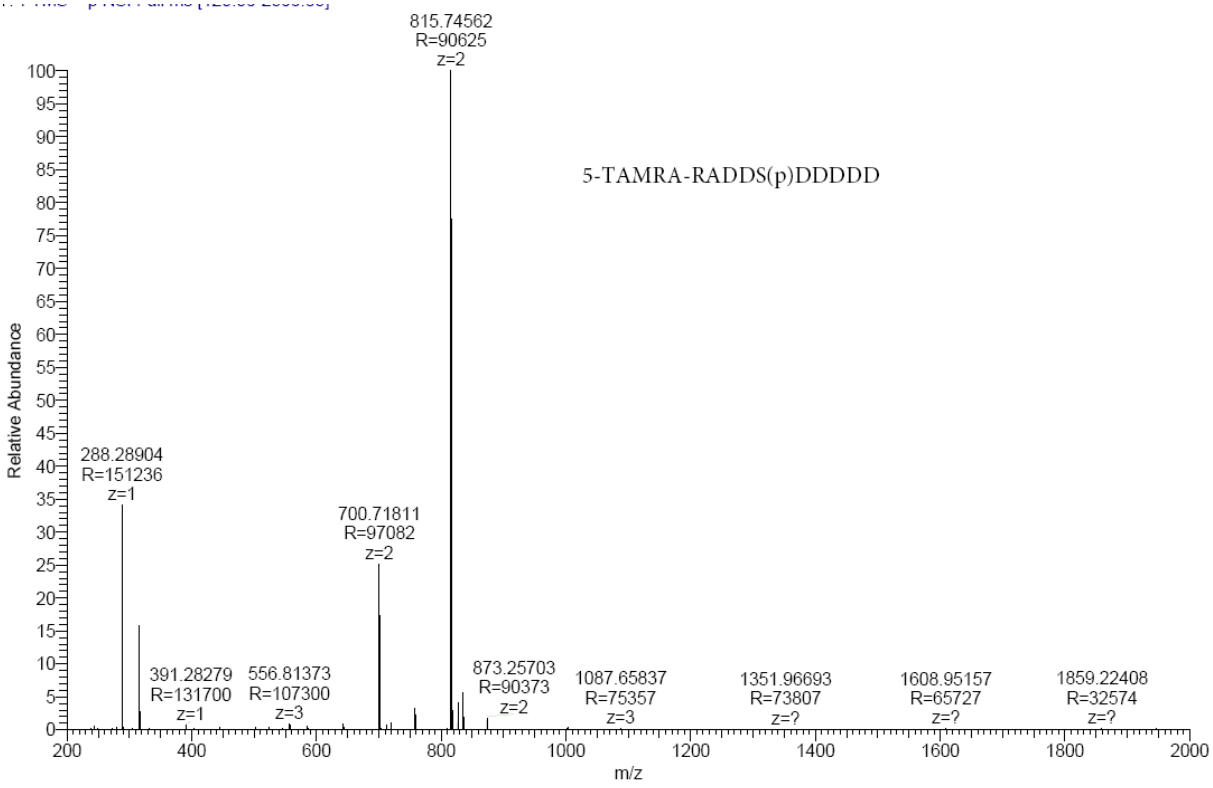


Table S2 Residual activities of 140 kinases in the presence of **ARC-1502** (1 μM final concentration). Kinases correspond to sequences of human origin unless specified. The selectivity panel was conducted on commercial basis by International Centre for Kinase Profiling (University of Dundee) using radiometric filter-binding assay. The ATP concentrations are at or below the calculated K_m value for that kinase except when denoted with asterisk. Residual activities are expressed as a percentage of the control without inhibitor (means of duplicate determinations).

kinase	Activity % at 1 μM ARC-1502	ATP (μM)
CK2α	1 (±0)	5
DYRK2	17 (±1)	50
PLK1	18 (±3)	5
CLK2	24 (±2)	5
ERK8	25 (±0)	5
DYRK3	30 (±2)	5
DYRK1a	36 (±3)	50
HIPK2	36 (±1)	5
GSK3ß	46 (±7)	5
CK1δ	49 (±5)	20
TTK	52 (±21)	20
IRR	55 (±20)	5
IKKß	55 (±6)	5
Src	57 (±3)	50
NUAK1	58 (±13)	20
PIM3	59 (±3)	20
SRPK1	61 (±11)	50
Lck (mouse)	62 (±4)	50
TAK1	62 (±3)	5
S6K1	62 (±10)	20
PIM1	62 (±10)	20
CDK2-Cyclin A2	64 (±2)	20
CDK9-Cyclin T1	65 (±19)	50
MARK2	66 (±3)	20
JAK2	66 (±3)	5
MKK2	66 (±0)	5
HIPK1	68 (±9)	20
AMPK (rat)	69 (±1)	50
MAPKAP-K3	69 (±20)	20
IR	70 (±7)	20
RSK2	70 (±1)	50
CK1γ2	71 (±5)	20
PAK4	71 (±3)	5
eIF2AK3	71 (±1)	5
PKBß	72 (±5)	50
PKD1	72 (±7)	50
MARK3	73 (±11)	5
PKBα	73 (±22)	5
SIK2	74 (±7)	50
PRAK	74 (±5)	20
SIK3	74 (±3)	50
HIPK3	75 (±0)	20
MARK4	75 (±8)	50
BRSK2	75 (±4)	50
VEGFR1	76 (±4)	20
AMPK	76 (±2)	20
PDGFRA	77 (±4)	5
EPH-A2	80 (±5)	50
EF2K	80 (±8)	5
BTK	80 (±4)	50
PAK5	81 (±10)	20
TESK1	81 (±4)	50
MKK6	82 (±2)	50*
MARK1	83 (±6)	20
DAPK1	84 (±11)	5
IKKε	84 (±7)	50
TAO1	84 (±8)	20
JNK3α1	85 (±11)	20
PDK1	86 (±20)	20
TSSK1	86 (±11)	20
PAK6	86 (±11)	20
MAP4K3	86 (±17)	20
WNK1	88 (±1)	20
RSK1	88 (±0)	50
MNK2α	89 (±22)	50
MAPKAP-K2	89 (±11)	20

IRAK1	89 (±2)	5
MLK3	89 (±3)	20
TTBK1	90 (±2)	5
ROCK 2 (rat)	91 (±5)	20
CaMKKß	91 (±6)	20
MELK	92 (±1)	50
CSK	92 (±5)	20
ERK2	92 (±6)	20
PRK2	92 (±4)	5
PIM2	92 (±0)	5
TBK1	93 (±9)	50
p38δ MAPK	93 (±6)	5
NEK2A	94 (±7)	50
PKCα	94 (±10)	20
MEKK1	95 (±13)	50
BRSK1	95 (±3)	20
TTBK2	95 (±6)	5
GCK	95 (±13)	20
smMLCK	96 (±1)	50
MPSK1	96 (±2)	50*
STK33	96 (±4)	50*
LKB1	96 (±15)	20
p38γ MAPK	97 (±10)	5
ZAP70	97 (±4)	5
EPH-B3	98 (±10)	20
TGFBR1	98 (±4)	20
ERK5	98 (±8)	50
MNK1	99 (±11)	50
MST2	99 (±7)	20
SYK	100 (±3)	20
MST3	100 (±3)	20
PKA	100 (±2)	20
Aurora B	100 (±19)	20
MKK1	101 (±9)	5
ABL1	101 (±26)	5
CHK2	101 (±6)	20
PAK2	102 (±4)	20
EPH-B1	102 (±11)	20
MSK1	102 (±8)	20

JNK1α1	103 (±8)	20
YES1	103 (±21)	20
HER4	103 (±5)	5
EPH-B4	104 (±9)	50
PhKγ1	105 (±8)	50
PKCζ	105 (±7)	5
TIE2	105 (±13)	20
JNK2α2	105 (±5)	20
CaMK1α	105 (±3)	50
PKCγ	106 (±8)	20
TLK1	107 (±3)	5
DDR2	109 (±6)	5
FGF-R1	110 (±7)	20
ERK1	110 (±9)	5
ULK2	110 (±2)	5
TrkA	111 (±15)	20
ULK1	111 (±25)	20
MLK1	112 (±7)	20
BRK	113 (±8)	20
EPH-B2	113 (±11)	20
MST4	114 (±20)	20
ASK1	114 (±34)	50*
p38α MAPK	116 (±11)	50
IGF-1R	116 (±16)	5
EPH-A4	117 (±5)	50
IRAK4	118 (±16)	20
Aurora A	118 (±0)	5
RIPK2	120 (±3)	20
NEK6	121 (±9)	50
p38ß MAPK	122 (±1)	20
SGK1	127 (±0)	20
OSR1	130 (±50)	5
MAP4K5	132 (±14)	20
CHK1	134 (±14)	20
MINK1	139 (±18)	50

Table S3 X-ray diffraction data and refinement statistics of the CK2α$^{1-335}$/ ARC-1154 co-crystal structure (4FBX)

Diffraction data	
Space group	$P4_32_12$
Cell dimensions [Å]	72.114, 72.114, 135.056
Resolution [Å]	32.25 – 2.33 (2.46 – 2.33)[#]
Unique reflections	15925
R_{sym} [%]	9.5 (64.0)[#]
I/σI (last shell)	18.8 (2.0)[#]
Completeness [%]	99.9 (99.8)[#]
Redundancy	8.0 (4.0)[#]
Wilson B-factor [Å^2]	38.8
Structure refinement and validation	
Resolution [Å]	31.81 – 2.33
No. of refl. in working set/test set	14944 / 924
R_{work} / R_{free} [%]	19.9 / 24.9
Composition of asymm. unit	
protomers	1
missing (disordered) res	Met1, Gly335
ATP-site ligands	1 ARC-1154 molecule
further ligands	5 chloride ions
water molecules	122
Ramachandran plot quality[##]	
favoured	295 (97.3%)
allowed	7 (2.1%)
outliers	1 (0.6%)
R.m.s. deviations	
bond lengths [Å]	0.003
bond angles [deg]	0.677

[#]Values in brackets refer to the highest res. shell
[##]according to MolProbity[1] embedded in PHENIX[2]

References:

1 V.B. Chen, W.B. Arendall 3rd, J.J. Headd, D.A. Keedy, R.M. Immormino, G.J. Kapral, L.W. Murray, J.S. Richardson and D.C. Richardson, *Acta Crystallogr D Biol. Crystallogr.*, 2010, **66**, 12–21.

2 P.D. Adams, P.V. Afonine, G. Bunkoczi, V.B. Chen, I.W. Davis, N. Echols, J.J. Headd, L.W. Hung, G.J. Kapral, R.W. Grosse-Kunstleve, A.J. McCoy, N.W. Moriarty, R. Oeffner, R.J. Read, D.C. Richardson, J.S. Richardson, T.C. Terwilliger and P.H. Zwart, *Acta Crystallogr.*, 2010, D66, 213−221.

UV-spectrum of 8-(4,5,6,7-tetrabromo-1H-benzimidazol-1-yl)octanoic acid.