

Supporting Information

Benzoselenadiazole-based responsive long-lifetime photoluminescence probes for protein kinases

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1. Materials and methods

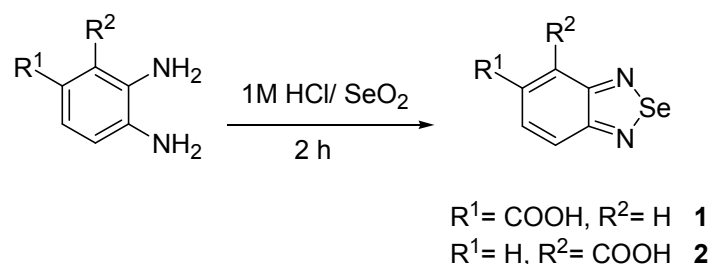
The chemicals and solvents were purchased from Rathburn, Sigma-Aldrich and Scharlau, and used without further purification. Fmoc Rink-amide MBHA resin and Fmoc-protected amino acids were purchased from Iris Biotech. Fluorescent dye PromoFluor-647 NHS ester was purchased from PromoKine.

^1H and ^{13}C NMR spectra were taken on Bruker AC 200P and Bruker 400 MHz spectrometers. High resolution mass spectra of all synthesized compounds were measured on Thermo Electron LTQ Orbitrap mass spectrometer. Thermo Scientific NanoDrop 2000c was used for measuring UV-VIS spectra and quantification of the products. Purification of peptide conjugates was performed with Shimadzu LC Solution (Prominence) system with manual injector and a diode array (SPD M20A) detector. Separation was achieved with a Gemini C18 5 μm column (250 \times 4.6 mm i.d., Phenomenex) protected by a 5 μm Gemini C18 4 \times 2.0 mm guard column.

Kinases:

Pim-1 kinase,¹ PKAc (bovine full length)² and CK2 α (amino acids 1-335)³ were expressed and purified as described previously.

2. Synthesis of ARC-compounds



Scheme S1. Synthesis of 2,1,3-benzoselenadiazole-5-carboxylic acid (**1**) and 2,1,3-benzoselenadiazole-4-carboxylic acid (**2**).

Synthesis of 2,1,3-benzoselenadiazole-5-carboxylic acid (**1**)

3,4-Diamino-benzoic acid 151 mg (1 mmol) was dissolved in 3.5 ml of 1M HCl and heated to 80 °C, thereafter 222 mg (2 mmol) of selenium dioxide in 1.5 ml of water was added. The mixture was stirred for 2 h and the brown precipitate was separated, washed with water and dried to get the compound **1** (95%).

^1H NMR (200 MHz, DMSO_{d_6}) δ 7.89-8.01 (2H, m), 8.43 (1H, s), 13.40 (1H, br).

^{13}C NMR (50 MHz, DMSO_{d_6}) δ 123.1, 125.3, 127.8, 131.2, 159.1, 160.6, 166.7.

ESI-HRMS m/z calcd for $\text{C}_7\text{H}_4\text{N}_2\text{O}_2\text{Se}$ $[\text{M}+\text{H}]^+$ 228.95108, found 228.95103.

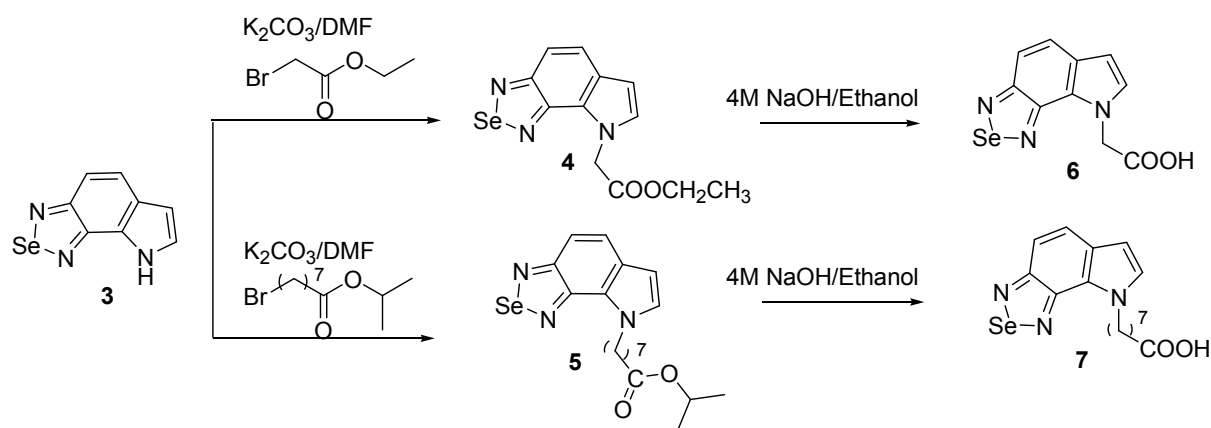
Synthesis of 2,1,3-benzoselenadiazole-4-carboxylic acid (**2**)

Synthesis of **2** was performed by the procedure described for **1**, starting from 2,3-diaminobenzoic acid. Yield 97%.

^1H NMR (200 MHz, DMSO_{d_6}) δ 7.65 (1H, dd, $J = 9.0$ Hz and 6.8 Hz), 8.07-8.14 (2H, m), 13.2 (1H, br).

^{13}C NMR (50 MHz, DMSO_{d_6}) δ 125.6, 127.4, 128.3, 132.3, 156.4, 160.1, 165.8.

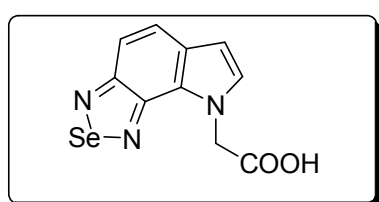
ESI-HRMS m/z calcd for $\text{C}_7\text{H}_4\text{N}_2\text{O}_2\text{Se}$ $[\text{M}+\text{H}]^+$ 228.95108, found 228.95087.



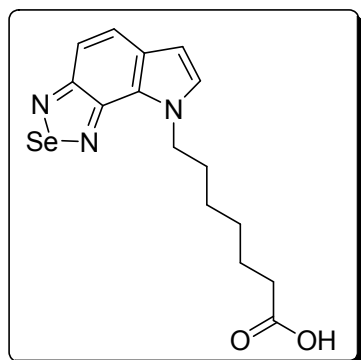
Scheme S2. Synthesis of 1,2,5-selenadiazolo[3,4-g]indol-yl-acetic acid (**6**) and 1,2,5-Selenadiazolo[3,4-g]indol-yl-octanoic acid (**7**)

Compound **3** (1 eq) and K_2CO_3 (1.5 eq) in 4 ml of DMF were stirred at room temperature for 10 min, then ethyl bromoacetate or isopropyl ester of 8-bromooctanoic acid (1.2 eq) was added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched by the addition of water. The mixture was partitioned between EtOAc and brine. The organic layer was dried over Na_2SO_4 , filtered and concentrated. The esters (**4** and **5**) were treated with 4 M NaOH/ethanol at 60 °C. After 3 h the reaction mixture was neutralized with 1 M HCl, then partitioned between EtOAc and brine. The organic layer was dried over Na_2SO_4 , concentrated and purified by silica gel column chromatography to yield acids **6** (65%) and **7** (47%).

1,2,5-Selenadiazolo[3,4-g]indol-yl-acetic acid (**6**):



1H NMR (200 MHz, $DMSO_{d6}$) δ 5.48 (2H, s), 6.57 (1H, d, $J = 2.8$ Hz), 7.35 (1H, d, $J = 9.2$ Hz), 7.42 (1H, d, $J = 2.8$ Hz), 7.74 (1H, d, $J = 9.2$ Hz), 13.03 (1H, br). ^{13}C NMR (50 MHz, $DMSO_{d6}$) δ 50.0, 104.1, 115.6, 125.0, 125.9, 126.8, 129.4, 150.8, 159.8, 170.2. HRMS m/z calcd monoisotopic mass for $C_{10}H_7N_3O_2Se$ 280.97035, found: 280.97037.



1,2,5-Selenadiazolo[3,4-g]indol-yl-octanoic acid (**7**):

1H NMR (400 MHz, $CDCl_3$) δ 1.37 (6H, m), 1.61 (2H, quin, $J = 7.2$ Hz), 1.94 (2H, quin, $J = 7.2$ Hz), 2.32 (2H, t, $J = 7.2$ Hz), 4.71 (2H, t, $J = 7.2$ Hz), 6.49 (1H, d, $J = 2.8$ Hz), 7.10 (1H, d, $J = 2.8$ Hz), 7.37 (1H, d, $J = 9.2$ Hz), 7.65 (1H, d, $J = 9.2$ Hz). ^{13}C

NMR (100 MHz, CDCl_3) δ 24.6, 26.4, 28.83, 28.87, 31.1, 33.7, 49.7, 103.9, 115.7, 125.71, 125.76, 127.1, 127.3, 151.1, 160.8, 178.1. HRMS m/z calcd monoisotopic mass for $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_2\text{Se}$ 365.06425, found: 365.06393.

Synthesis of peptide conjugates

Peptide fragments were prepared by using traditional Fmoc solid phase peptide synthesis on Rink amide MBHA resin. In general, protected amino acids (3 eq) were dissolved in DMF and activated with HBTU/HOBt (2.8 eq each) in the presence of *N*-methylmorpholine (9 eq). Coupling solutions were added to the resin and shaken for 1 h. The resin was washed 5 times with DMF. The completeness of each coupling step was monitored with Kaiser test. The *N*-terminal Fmoc group was removed with 20% piperidine solution in DMF (20 min) and the resin was washed 5 times with DMF.

A selenadiazole carboxylic acid (3 eq) was activated with HBTU/HOBt (2.8 eq each) in DMF in the presence of *N*-methylmorpholine (9 eq). Coupling solutions were added to the resin and shaken for 3 h. The resins were washed 5 times with each solvent (DMF, isopropanol, DCE) and dried. Finally the protection groups were removed and the conjugates cleaved from the resin by 2 h treatment with a mixture of trifluoroacetic acid, triisopropylsilane and water (90:5:5, by volume). The conjugates were purified with C18 reversed phase HPLC and lyophilized.

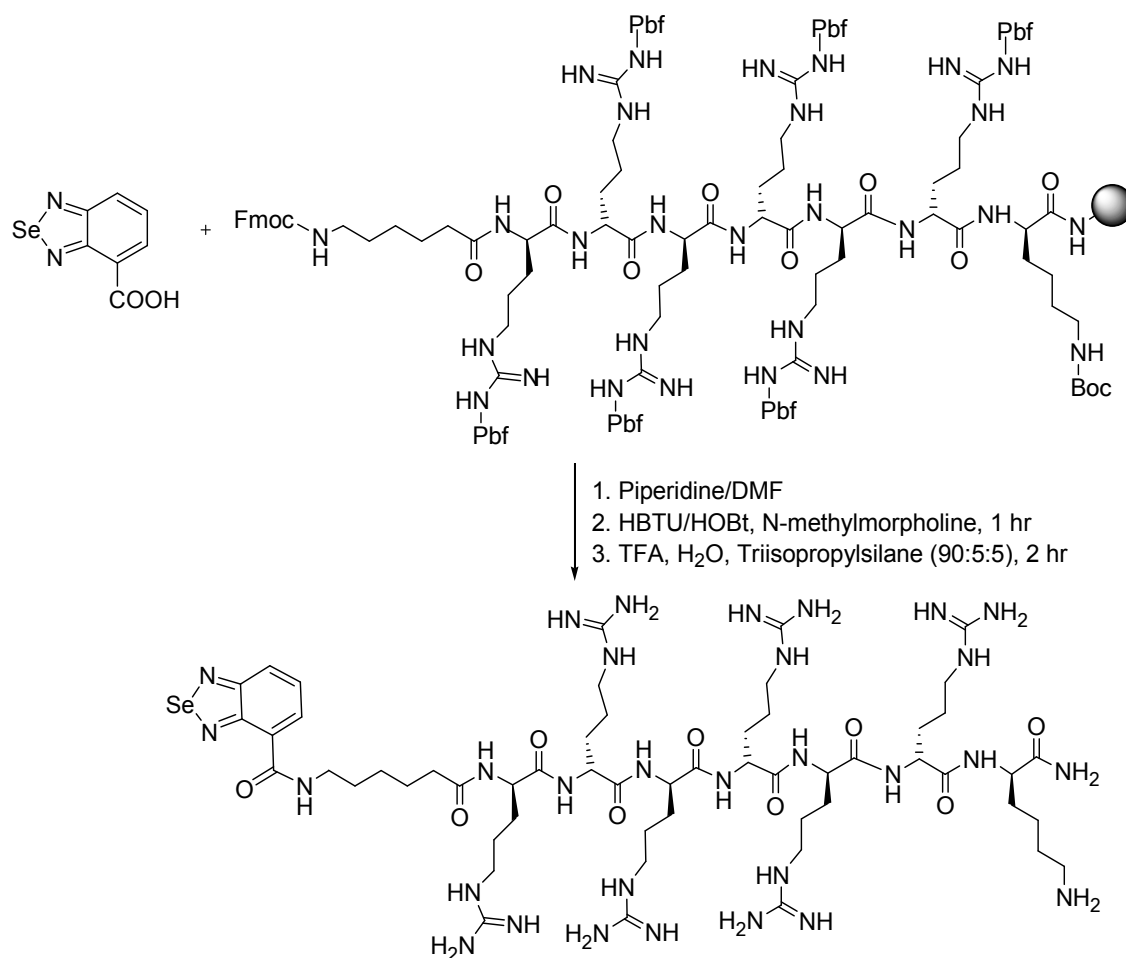
Labelling of peptide conjugates with the fluorescent dye PromoFlour-647

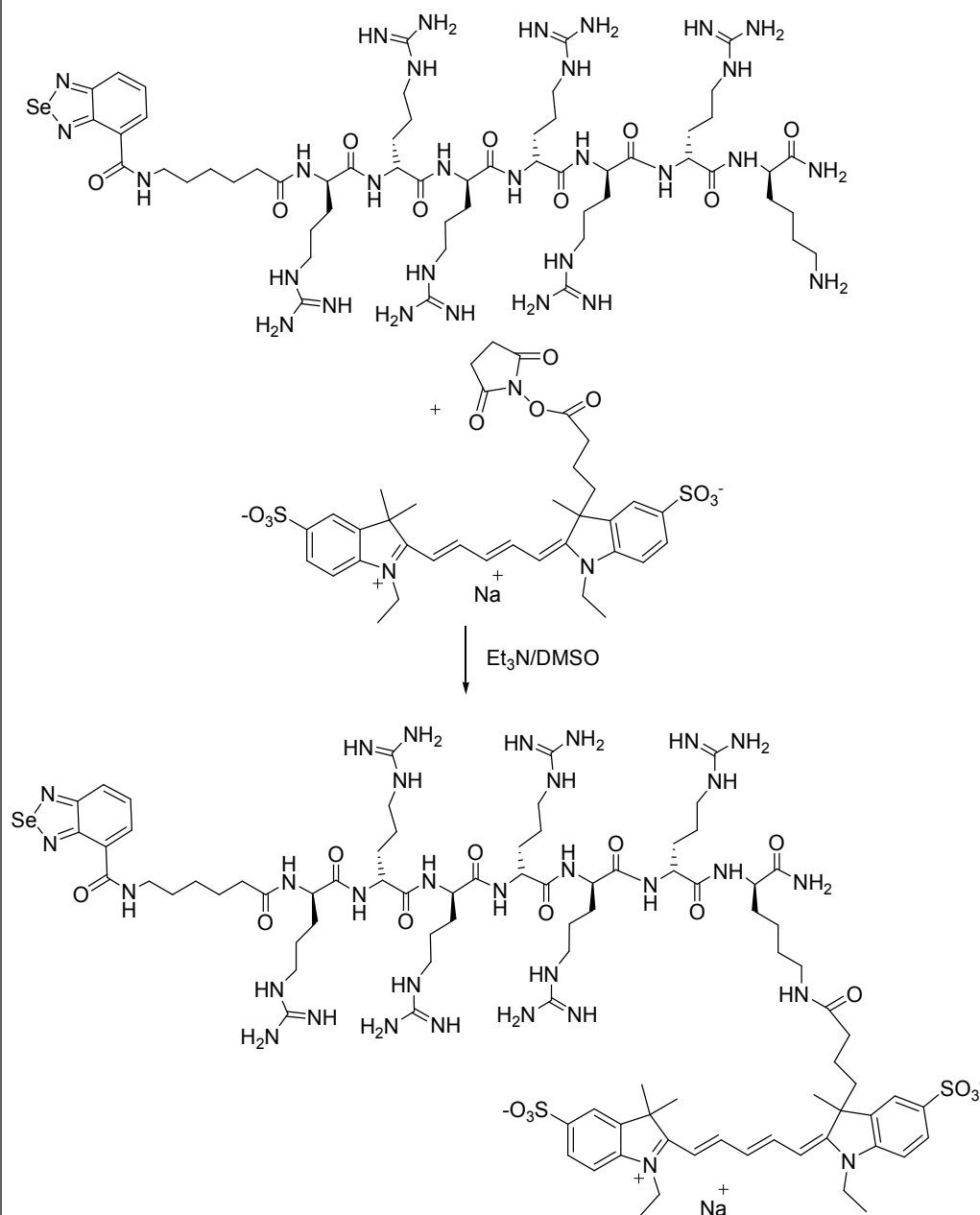
Peptide conjugate (ARC-1601, ARC-1608, ARC-3131 and ARC-3138) and NHS ester of PromoFlour-647 were dissolved in DMSO and Et_3N . After 3 h reaction the solvents were removed in vacuum and the products were purified by HPLC with C18 reverse phase column to yield ARC-1602, ARC-1609, ARC-3132 and ARC-3141, respectively.

Synthesis of ARC-3168

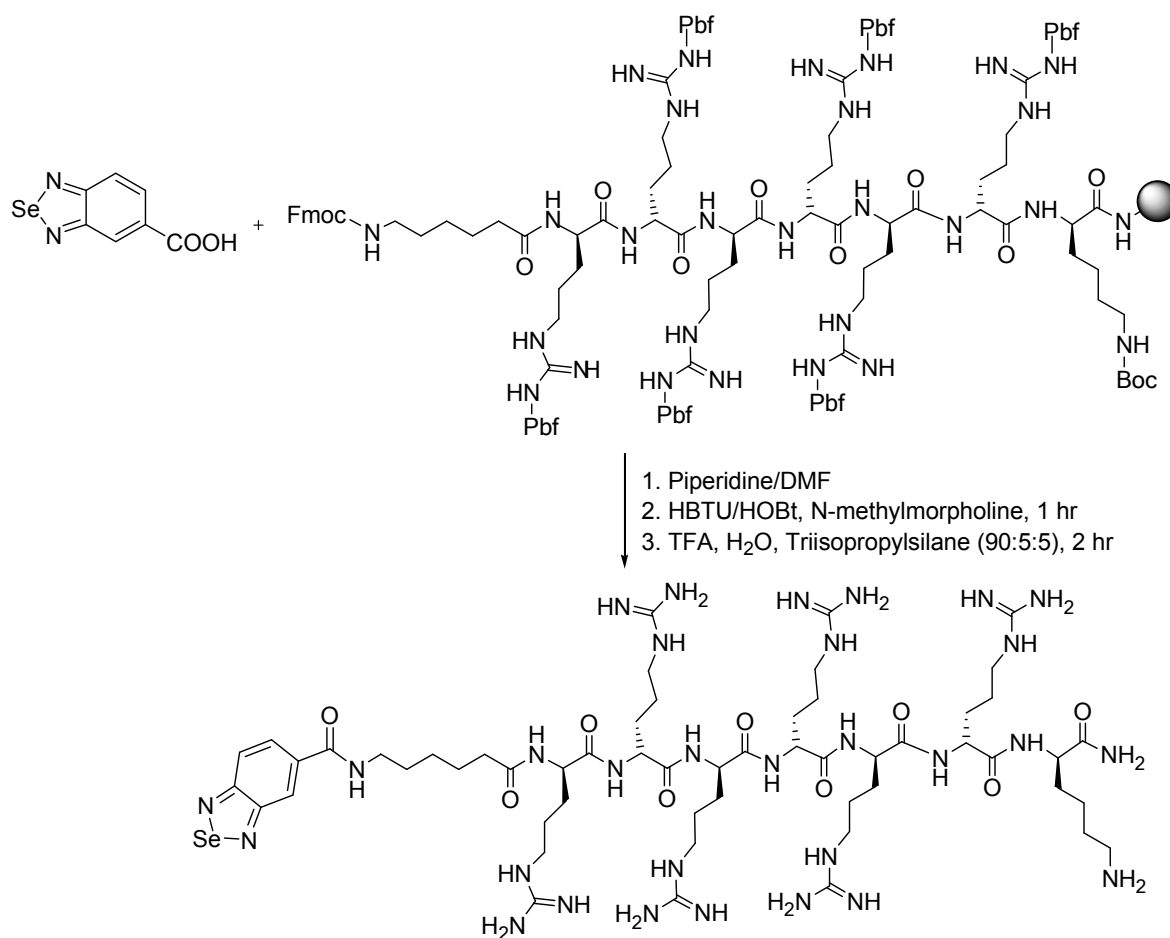
ARC-3138 (13 nmol) was treated with Ac_2O (2 eq) and Et_3N (1 μl) in DMF (50 μl) overnight. The solvent was removed in vacuum and the residue was purified by HPLC with C18 reverse phase column to yield ARC-3168.

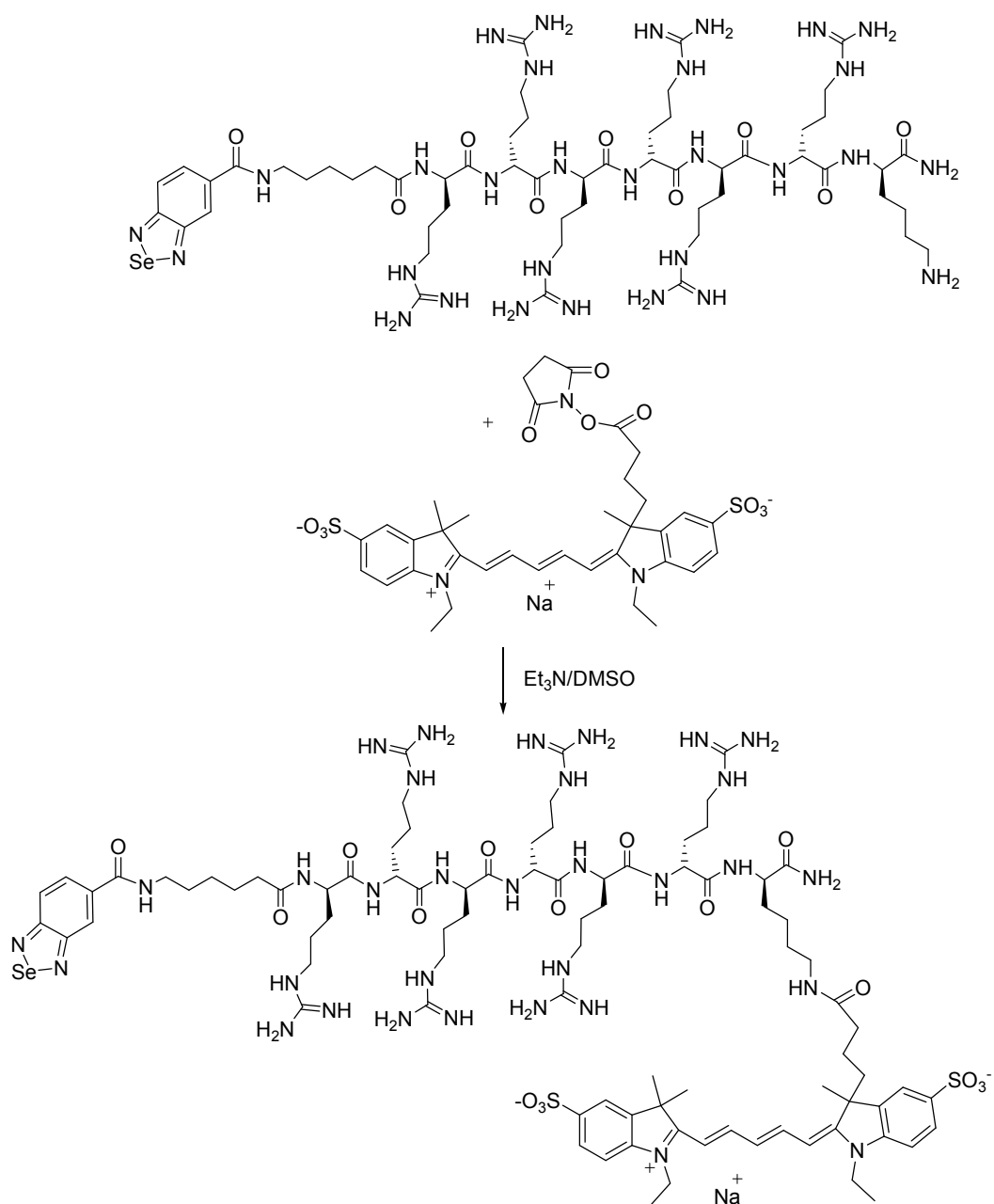
Synthesis of peptide conjugates : ARC-1601

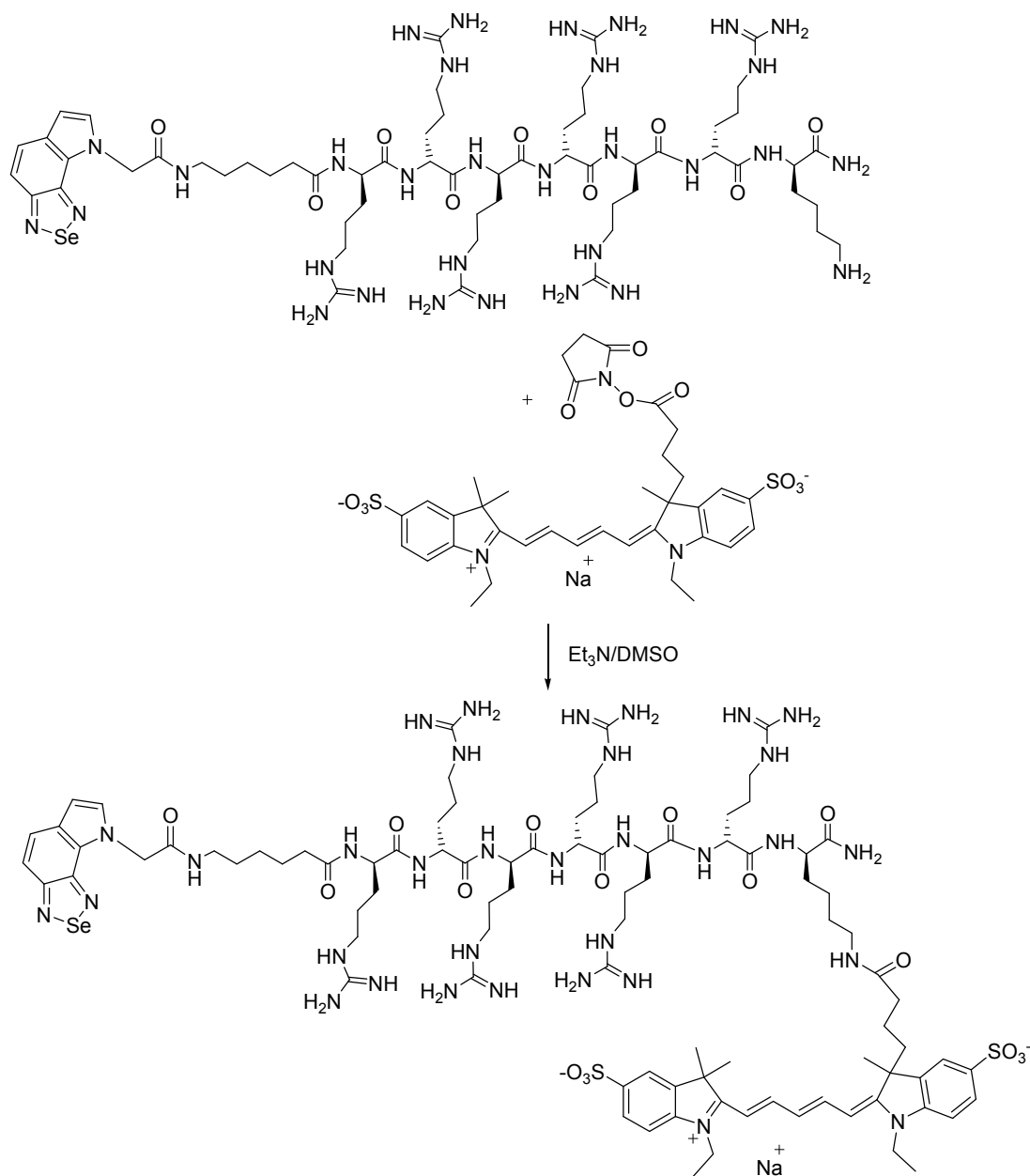


Synthesis of peptide conjugates : ARC-1602

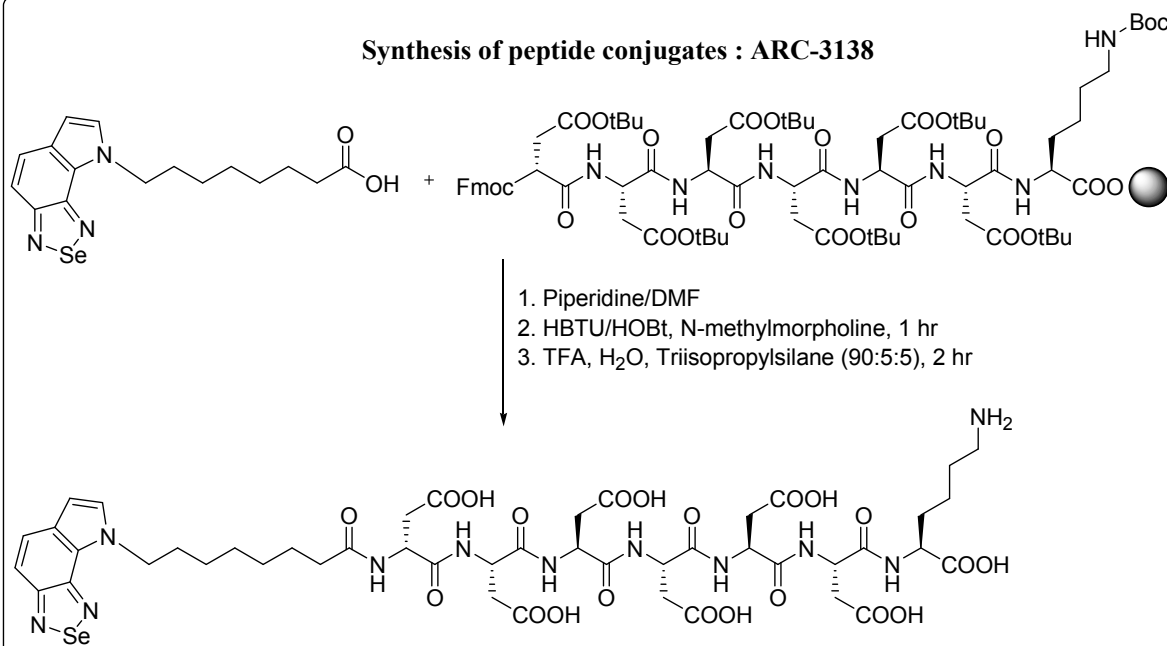
Synthesis of peptide conjugates : ARC-1608



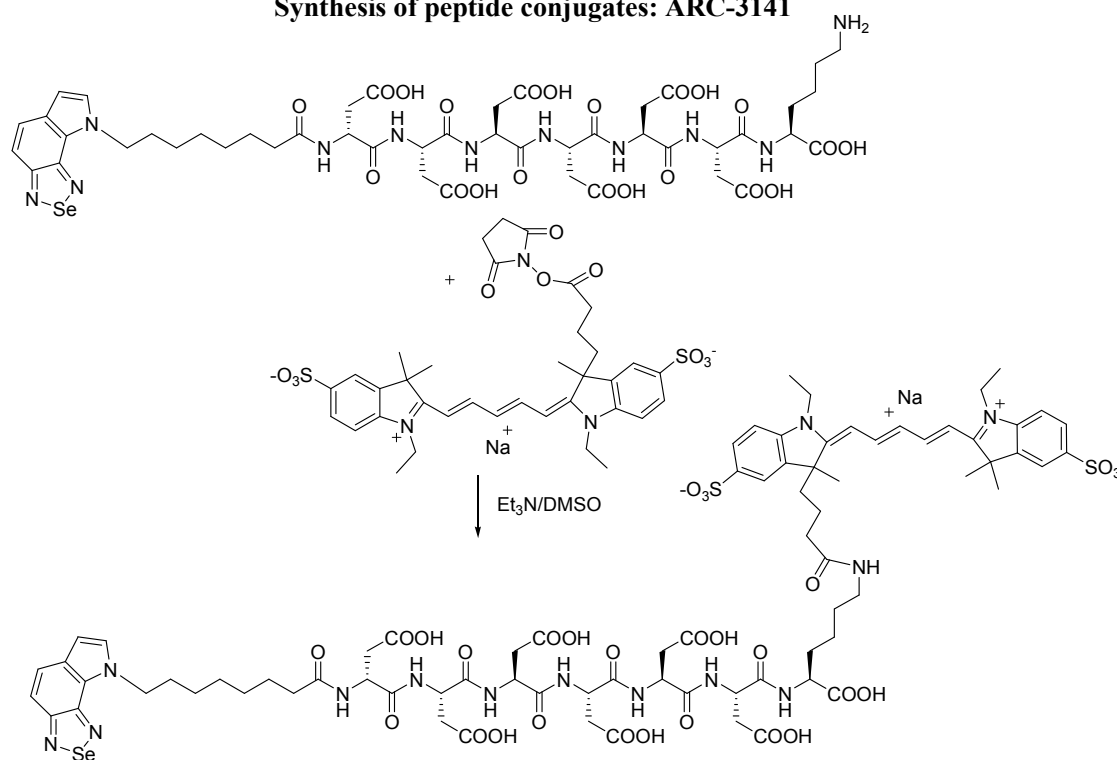
Synthesis of peptide conjugates : ARC-1609

Synthesis of peptide conjugates : ARC-3132

Synthesis of peptide conjugates : ARC-3138



Synthesis of peptide conjugates: ARC-3141



3. UV-Visible absorption spectra of compounds

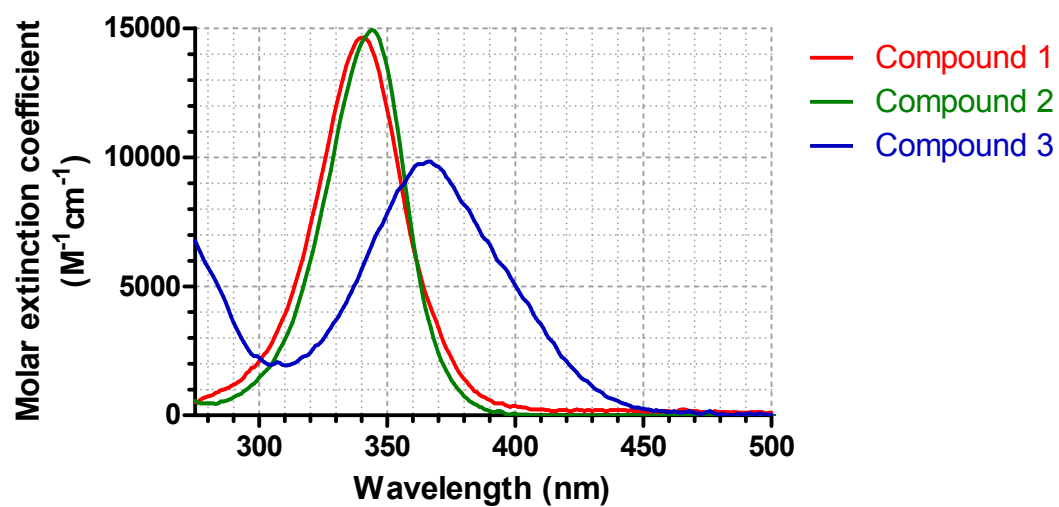
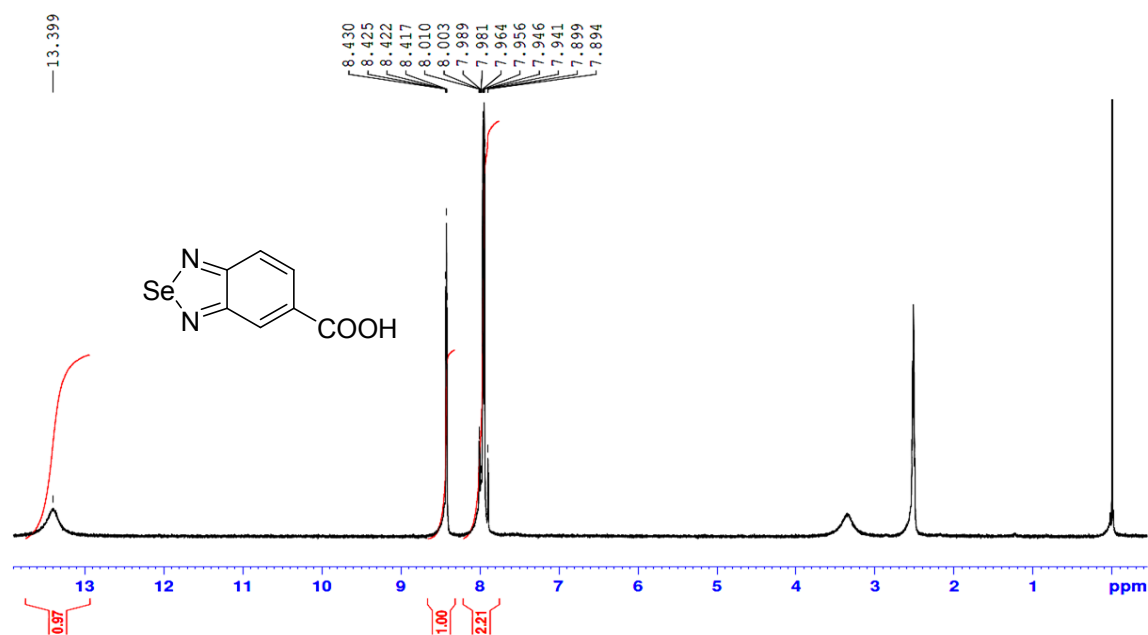
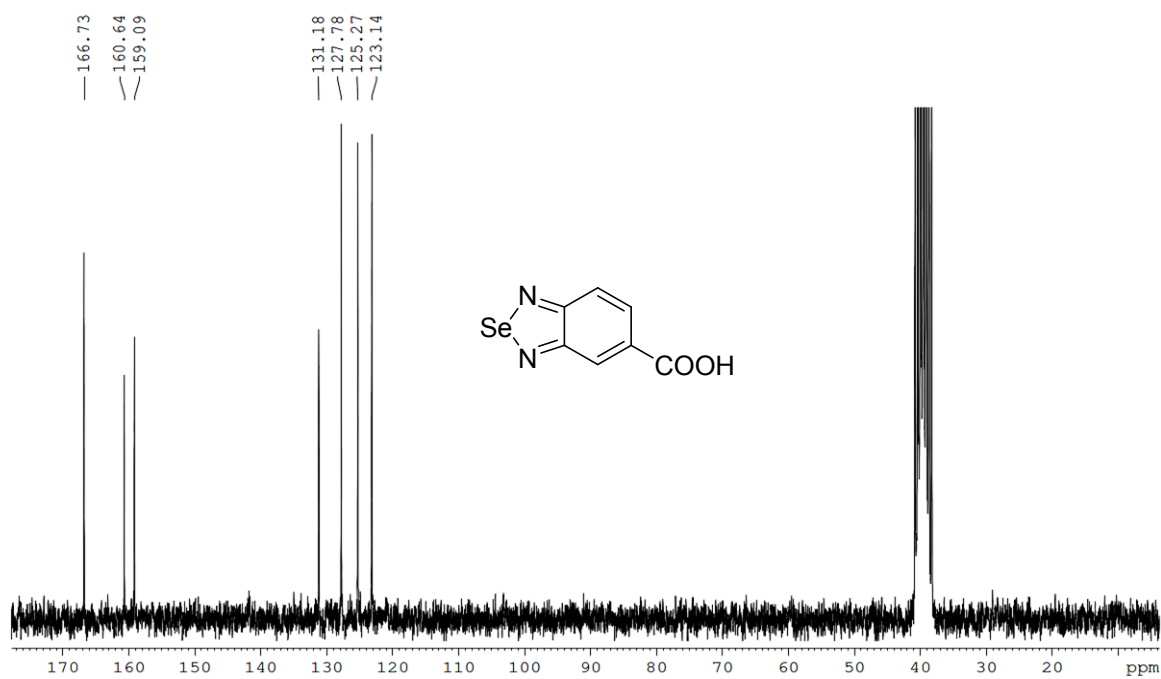


Figure S1. UV-Visible spectra of compounds 1, 2, 3 at 0.4 mM concentration (aqueous solution, pH 7.5).

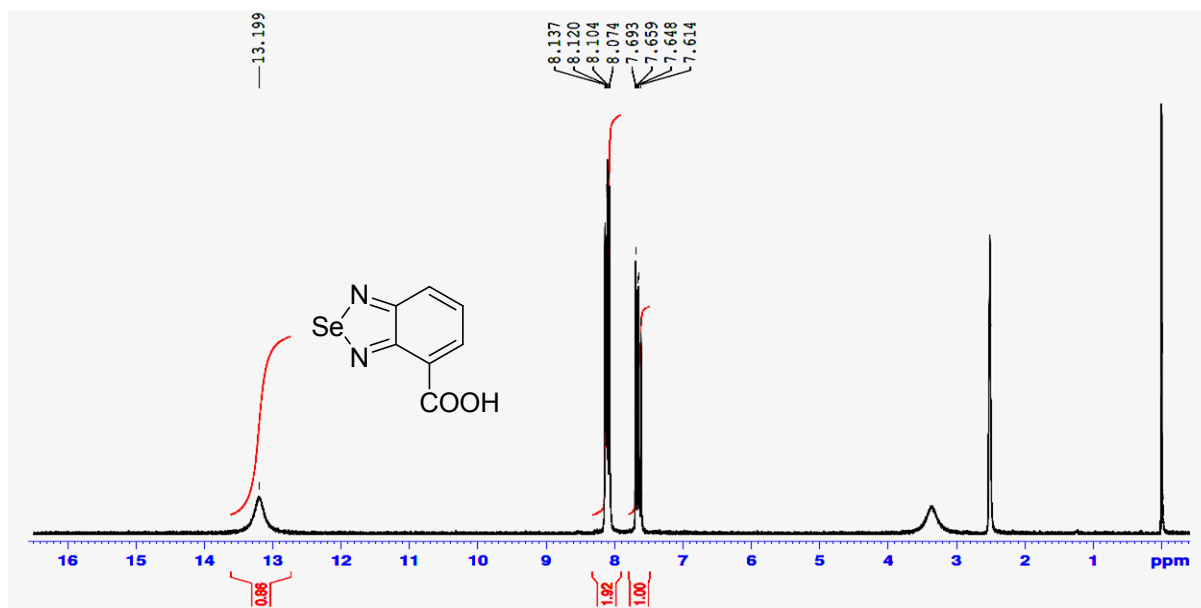
4. NMR Data



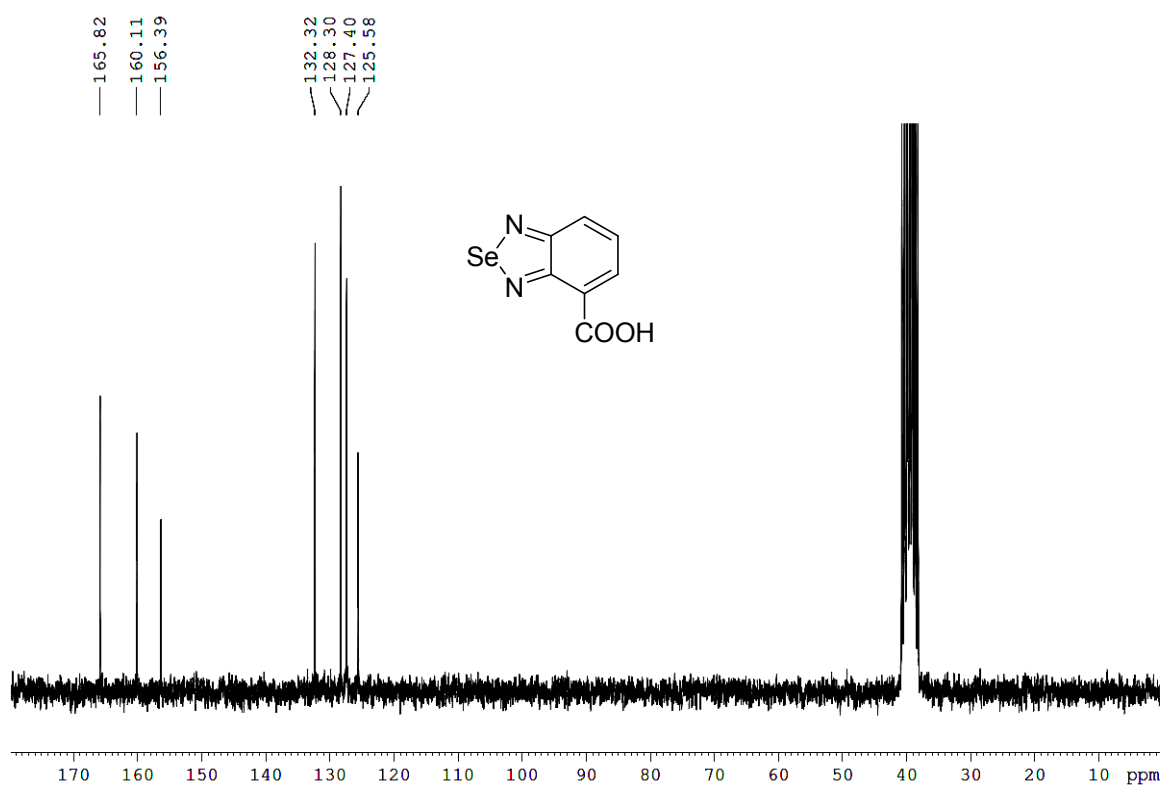
¹H NMR spectrum of compound **1**.



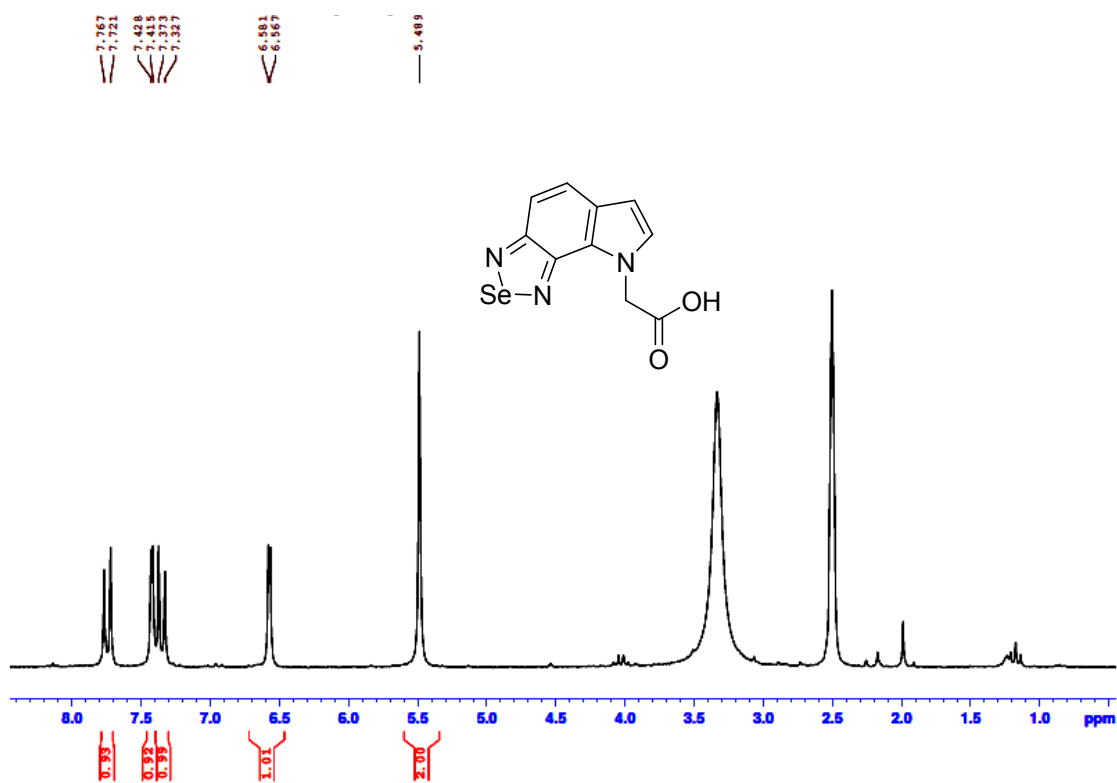
¹³C NMR spectrum of compound **1**.



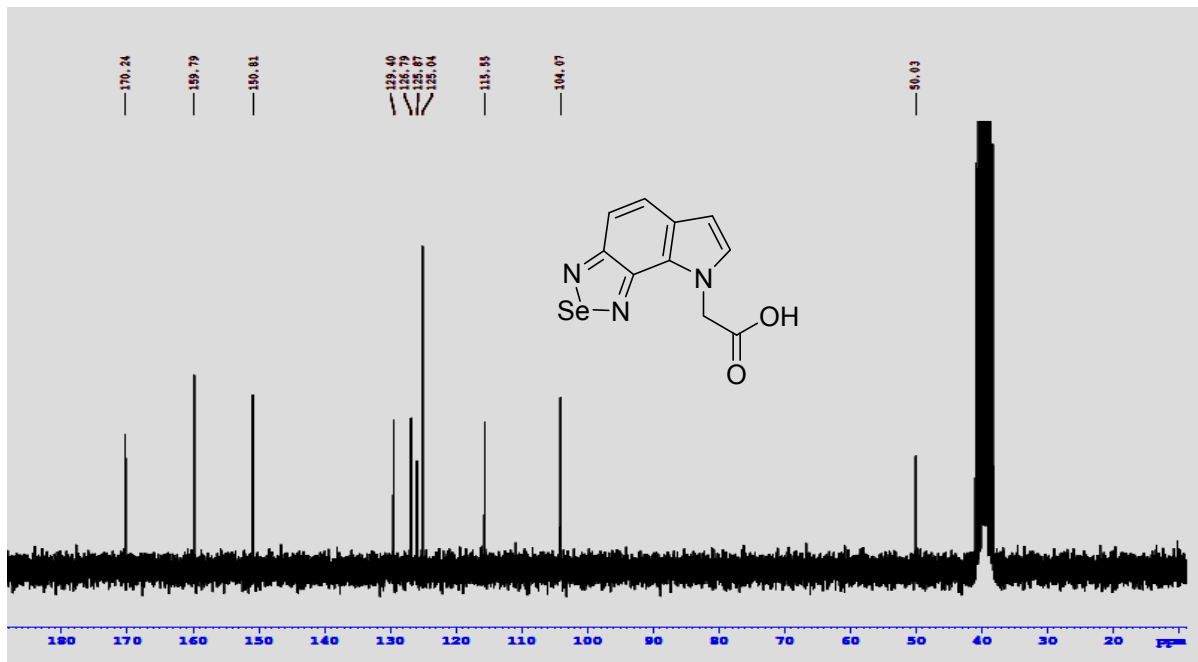
¹H NMR spectrum of compound **2**.



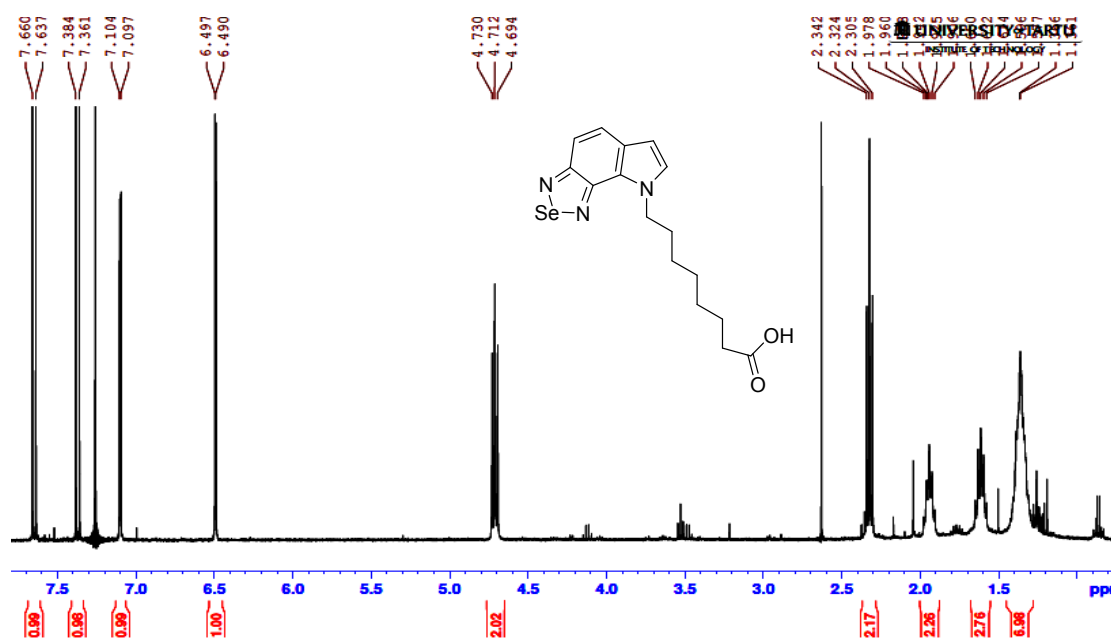
¹³C NMR spectrum of compound **2**.



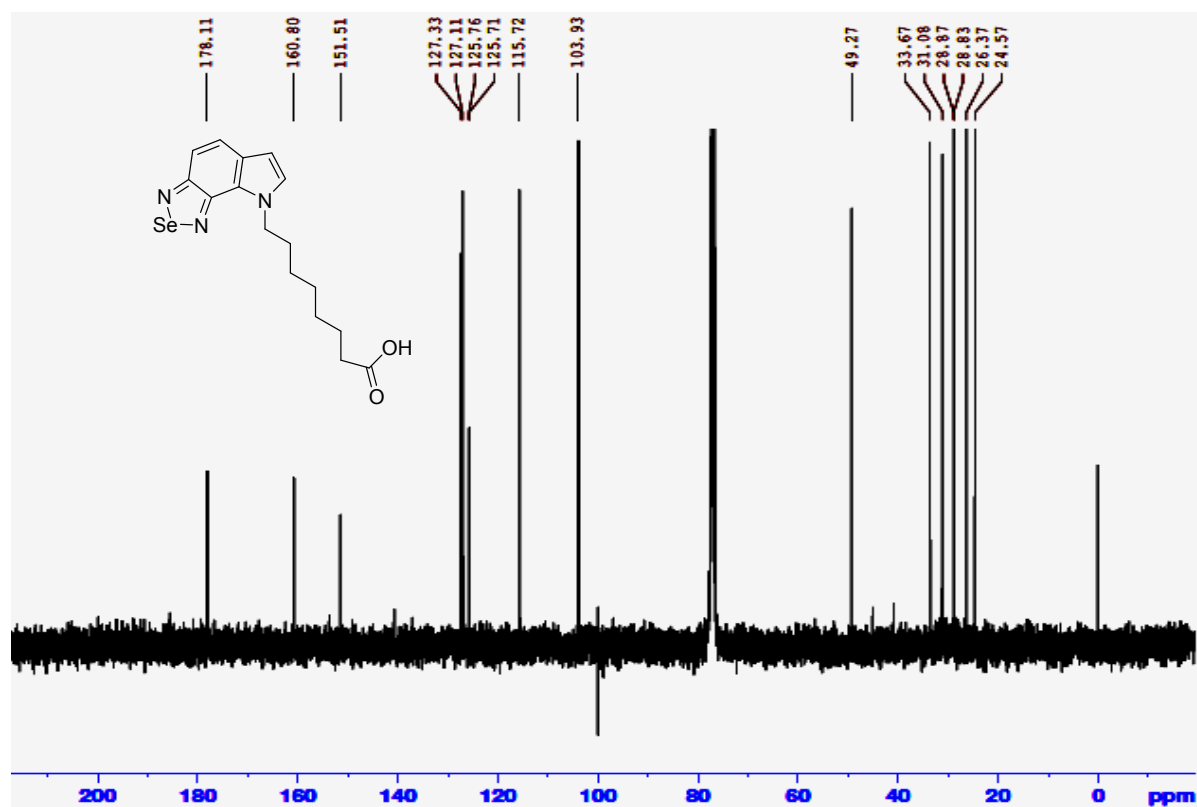
¹H NMR spectrum of compound 6.



¹³C NMR spectrum of compound 6.



¹H NMR spectrum of compound 7.



¹³C NMR spectrum of compound 7.

5. HPLC data for purified compounds

HPLC separation of the compounds

The mobile phase for gradient HPLC consisted of solution A (0.1% TFA) and solution B (0.1% TFA in ACN). The flow rate was 1 ml/min. Linear gradient and elution were started at 3 min.

Table S1.

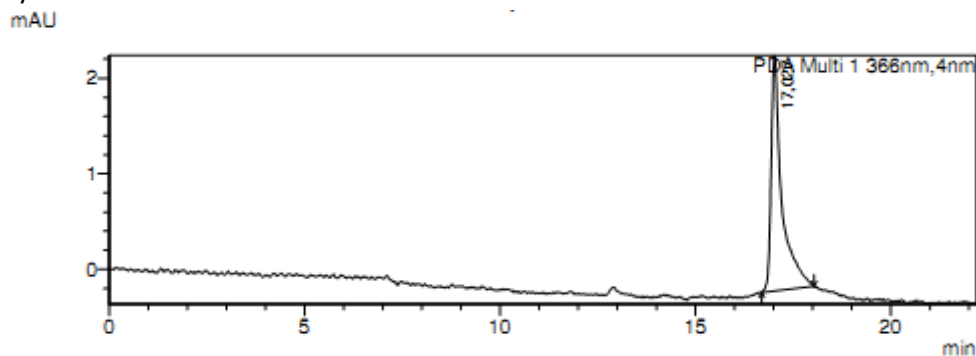
Compound code	Molecular formula	Gradient speed ACN%	Retention time, t_R (min)	Purity, area % of HPLC peak
ARC-3131	$C_{58}H_{103}N_{31}O_9Se$	5%-40%/30 min	14.0	100
ARC-3132	$C_{90}H_{139}N_{33}O_{16}S_2Se$	10%-60%/30min	13.7	100
ARC-3138	$C_{46}H_{61}N_{11}O_{21}Se$	5%-40%/30 min	27.7	100
ARC-3141	$C_{78}H_{97}N_{13}O_{28}S_2Se$	10%-60%/30min	23.3	100
ARC-3168	$C_{48}H_{63}N_{11}O_{22}Se$	10%-60%/30min	24.5	100
ARC-1601	$C_{55}H_{100}N_{30}O_9Se$	5%-40%/30 min	11.4	100
ARC-1602	$C_{87}H_{136}N_{32}O_{16}S_2Se$	10%-60%/30min	12.5	100
ARC-1608	$C_{55}H_{100}N_{30}O_9Se$	5%-40%/30 min	10.0	99.2
ARC-1609	$C_{87}H_{136}N_{32}O_{16}S_2Se$	5%-60%/30 min	13.8	100

Chromatograms

ARC-3131

Gradient 5%-40%ACN/30 min

Purity 100%



Peak Table

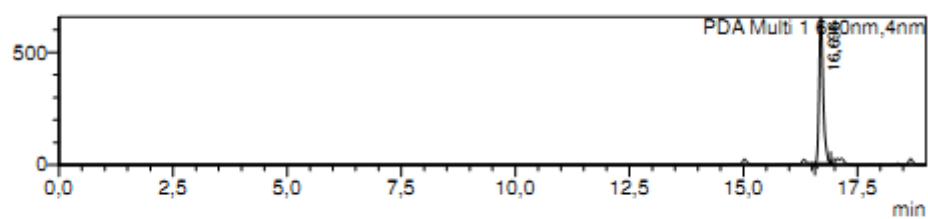
Peak#	Ret. Time	Area	Height	Height%	Area%
1	17.027	46569	2463	100,000	100,000
Total		46569	2463	100,000	100,000

ARC-3132

Gradient 10%-60%ACN/30 min

Purity 100%

mAU



Peak Table

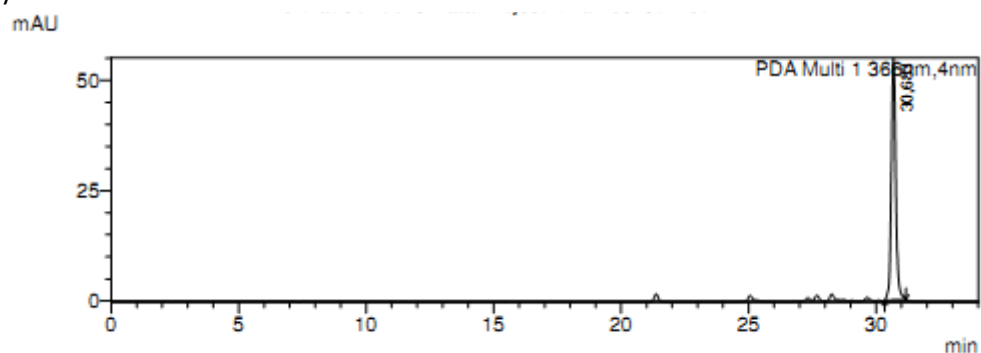
PDA Ch1 640nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	16,696	4093607	648925	100,000	100,000
Total		4093607	648925	100,000	100,000

ARC-3138

Gradient 5%-40%ACN/30 min

Purity 100%



Peak Table

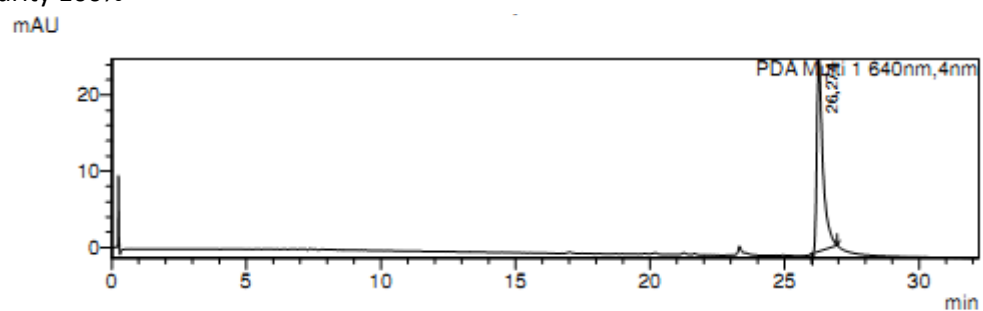
PDA Ch1 366nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	30.683	664693	54859	100,000	100,000
Total		664693	54859	100,000	100,000

ARC-3141

Gradient 10%-60%ACN/30 min

Purity 100%



Peak Table

PDA Ch1 640nm

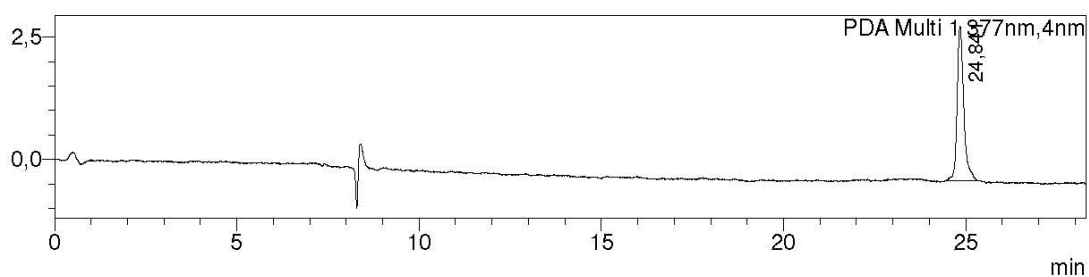
Peak#	Ret. Time	Area	Height	Height%	Area%
1	26.274	395992	25259	100,000	100,000
Total		395992	25259	100,000	100,000

ARC-3168

Gradient 10%-60% ACN/30 min

Purity 100%

mAU



Peak Table

PDA Ch1 377nm

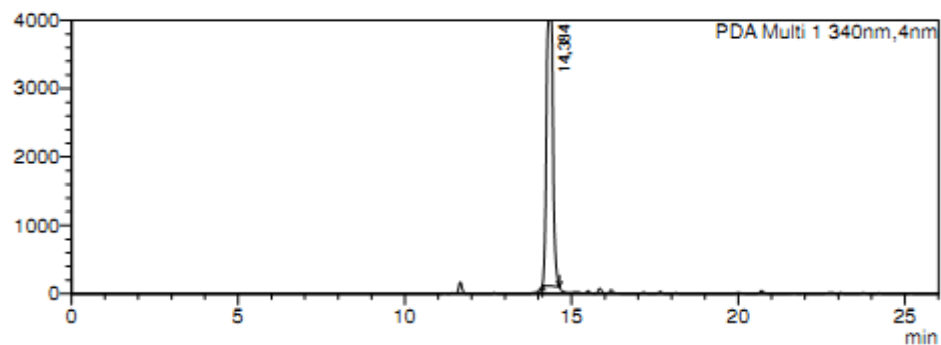
Peak#	Ret. Time	Area	Height	Height%	Area%
1	24,841	39623	3163	100,000	100,000
Total		39623	3163	100,000	100,000

ARC-1601

Gradient 5%-40%ACN/30 min

Purity 100%

mAU



Peak Table

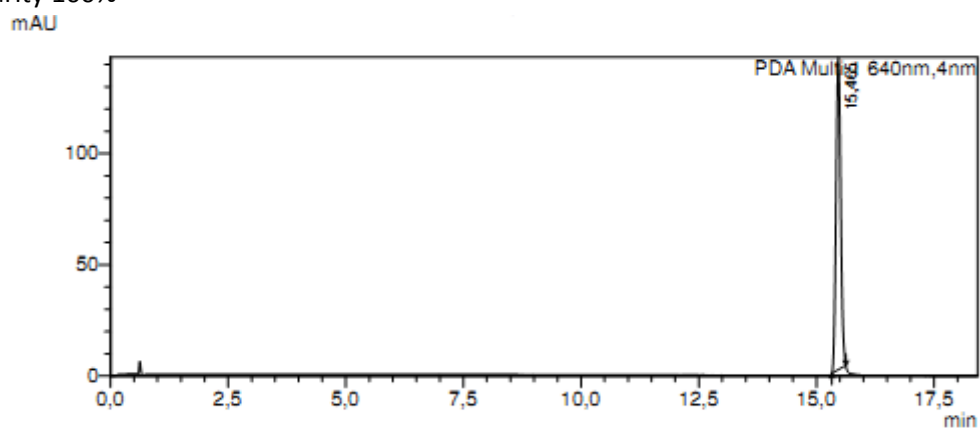
PDA Ch1 340nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	14,384	53501484	3887119	100,000	100,000
Total		53501484	3887119	100,000	100,000

ARC-1602

Gradient 10%-60%ACN/30 min

Purity 100%



Peak Table

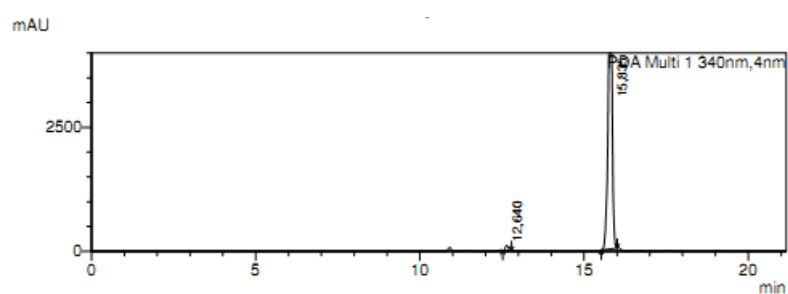
PDA Ch1 640nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	15.465	973453	140285	100,000	100,000
Total		973453	140285	100,000	100,000

ARC 1608

Gradient 5%-40%ACN/30 min

Purity 98.4%



Peak Table

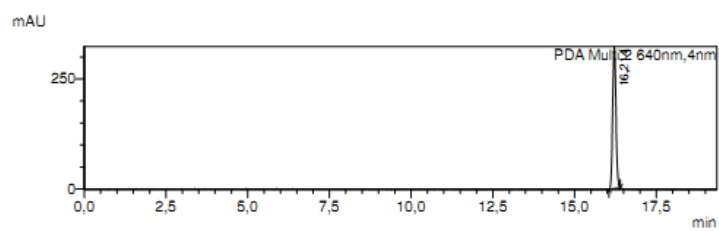
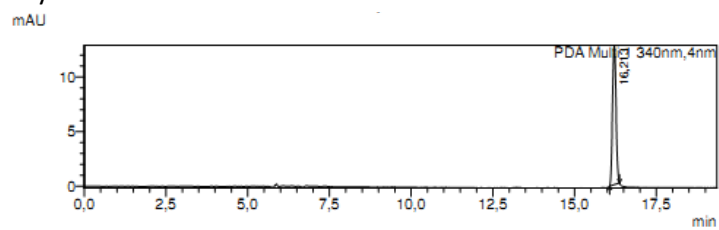
PDA Ch1 340nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	12.640	662759	104700	2,583	1,602
2	15.835	40714419	3948428	97,417	98,398
Total		41377177	4053128	100,000	100,000

ARC 1609

Gradient 10%-60%ACN/30 min

Purity 100%



Peak Table

PDA Ch1 340nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	16,213	95148	12733	100,000	100,000
Total		95148	12733	100,000	100,000

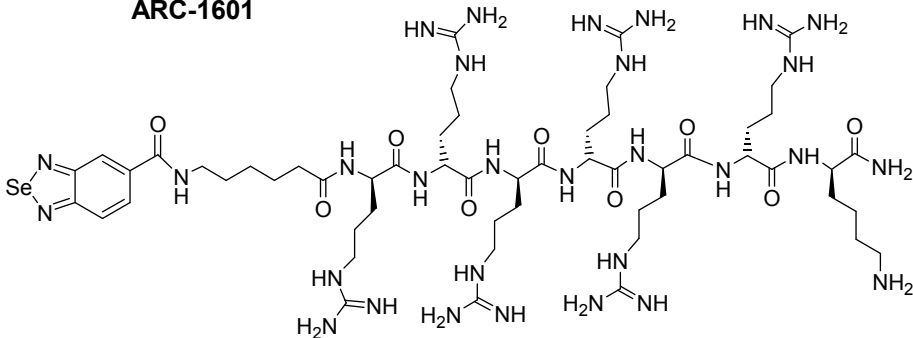
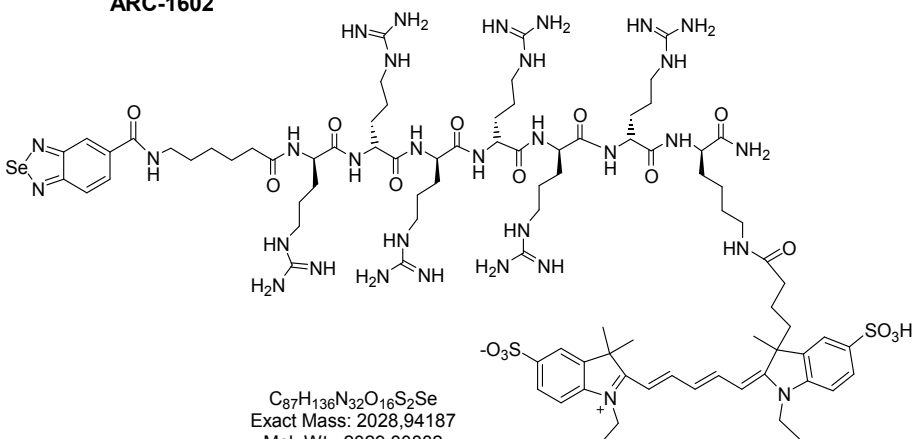
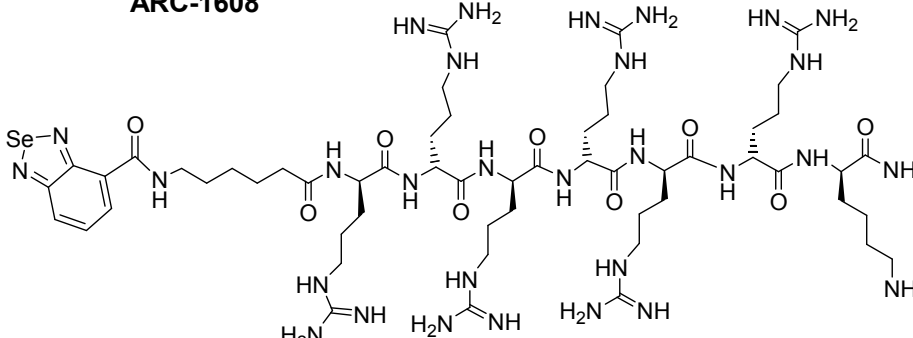
Peak Table

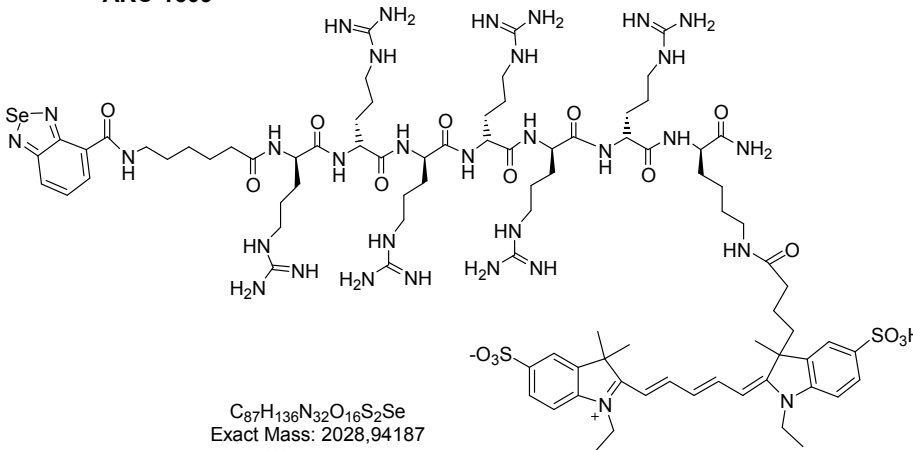
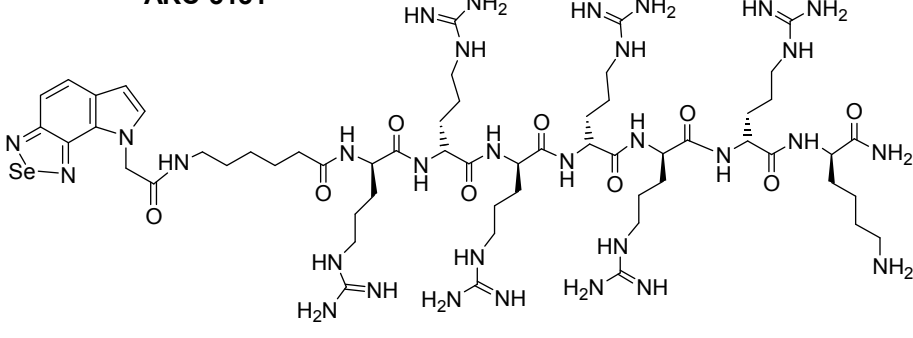
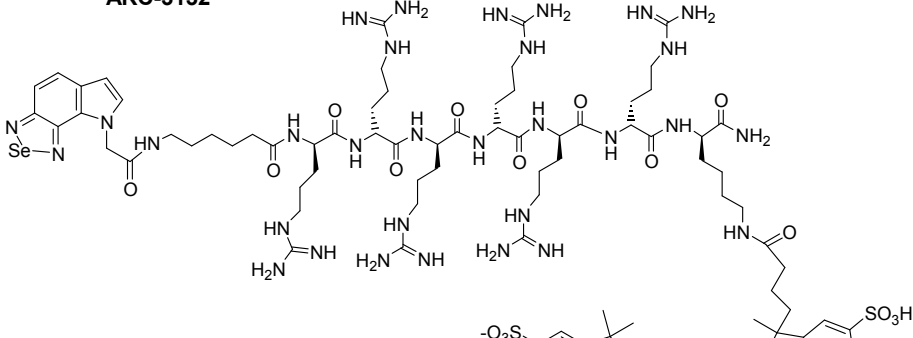
PDA Ch2 640nm

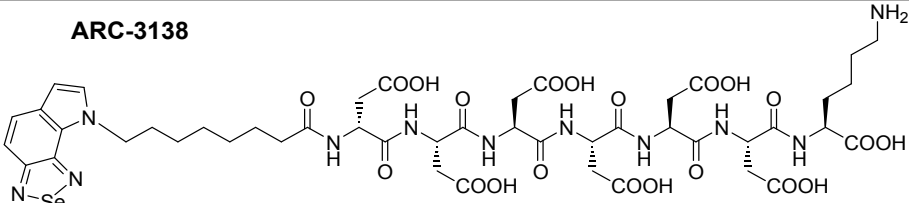
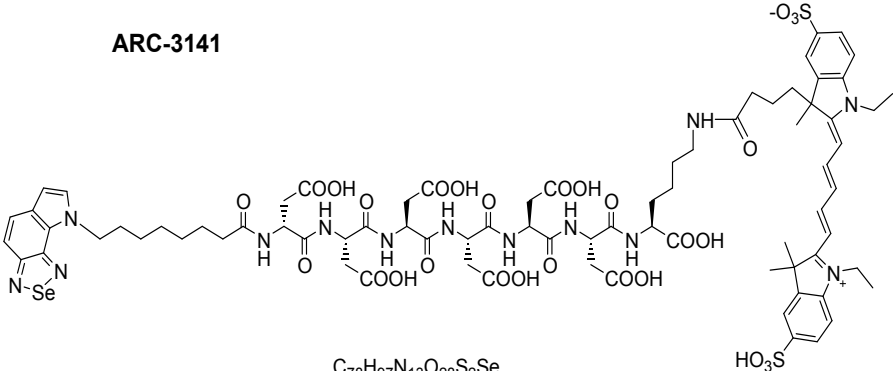
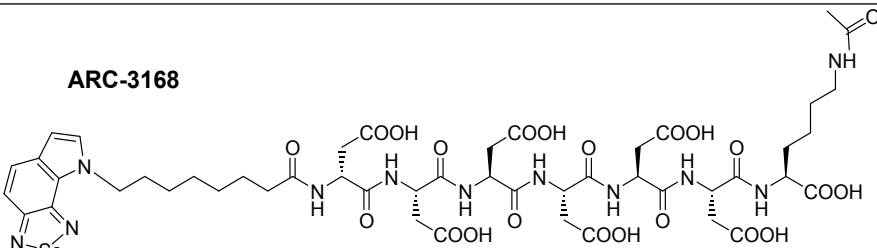
Peak#	Ret. Time	Area	Height	Height%	Area%
1	16,214	2258701	323167	100,000	100,000
Total		2258701	323167	100,000	100,000

6. Structures and HRMS data

Table S2. Compound codes, structures and HRMS data of compounds. Deconvoluted monoisotopic masses are presented

Code	Structure	HRMS found
ARC-1601	<p>ARC-1601</p>  <p> $C_{55}H_{100}N_{30}O_9Se$ Exact Mass: 1404,74547 Mol. Wt.: 1404,53810 </p>	1404.74524
ARC-1602	<p>ARC-1602</p>  <p> $C_{87}H_{136}N_{32}O_{16}S_2Se$ Exact Mass: 2028,94187 Mol. Wt.: 2029,30882 </p>	2028.94119
ARC-1608	<p>ARC-1608</p>  <p> $C_{55}H_{100}N_{30}O_9Se$ Exact Mass: 1404,74547 Mol. Wt.: 1404,53810 </p>	1404.74411

ARC-1609	<p>ARC-1609</p>  <p> $C_{87}H_{136}N_{32}O_{16}S_2Se$ Exact Mass: 2028,94187 Mol. Wt.: 2029,30882 </p>	2028.93739
ARC-3131	<p>ARC-3131</p>  <p> $C_{58}H_{103}N_{31}O_9Se$ Exact Mass: 1457,77202 Mol. Wt.: 1457,60072 </p>	1457.77195
ARC-3132	<p>ARC-3132</p>  <p> $C_{90}H_{139}N_{33}O_{16}S_2Se$ Exact Mass: 2081,96842 Mol. Wt.: 2082,37148 </p>	2081.97034

ARC-3138	<p>ARC-3138</p>  <p>$C_{46}H_{61}N_{11}O_{21}Se$ Exact Mass: 1183,32087 Mol. Wt.: 1182,99808</p>	1183.32244
ARC-3141	<p>ARC-3141</p>  <p>$C_{78}H_{97}N_{13}O_{28}S_2Se$ Exact Mass: 1807,51727 Mol. Wt.: 1807,76760</p>	1807.51603
ARC-3168	<p>ARC-3168</p>  <p>$C_{48}H_{63}N_{11}O_{22}Se$ Exact Mass: 1225,33144 Mol. Wt.: 1225,03476</p>	1225.33282

7. Selectivity data

Table S3. Residual activities (%) of PKs in the presence of ARC-3138 (1 μ M). Data of CK2 is presented in yellow.

Selectivity testing was performed on the commercial basis at the Division of Signal Transduction

Kinase	Residual activity	Kinase	Residual activity	Kinase	Residual activity
CK2	20	PRAK	87	PKCz	95
ERK8	53	p38d MAPK	87	STK33	95
Aurora B	60	CK1 γ 2	87	NUAK1	95
GSK3b	63	CHK1	87	SIK2	95
MLK1	65	SIK3	88	PKC γ	96
TTK	68	p38a MAPK	88	p38b MAPK	96
IGF-1R	69	MKK2	88	TESK1	96
ERK2	70	ERK1	89	OSR1	96
CDK2-Cyclin A	72	PKBb	89	CHK2	98
VEG-FR	72	MAPKAP-K3	89	p38g MAPK	98
EPH-B3	73	EPH-B1	89	EPH-A4	98
PLK1	74	TGFBR1	89	MAP4K5	98
MST4	74	TSSK1	89	RIPK2	98
TIE2	74	IKKb	89	IR	98
IKKe	75	MAP4K3	90	MARK1	99
MLK3	75	HIPK2	90	TAK1	99
BTk	76	PAK5	90	PKBa	99
PIM3	77	JNK3	90	PAK2	100
CLK2	77	TrkA	90	MNK1	100
JAK2	77	DAPK1	90	PAK4	100
BRK	77	IRAK4	90	MNK2	101
RSK1	78	MSK1	91	TTBK2	101
CSK	78	RSK2	91	MST2	101
TAO1	79	CAMK1	91	LKB1	101
ROCK 2	80	EPH-B2	91	BRSK2	101
MST3	80	EIF2AK3	91	CAMKKb	102
Src	81	NEK2a	91	Aurora A	102
SYK	81	PRK2	91	SmMLCK	103
PDK1	81	HER4	92	PDGFRA	103
PKD1	81	PIM2	92	HIPK3	104
SRPK1	81	AMPK (hum)	92	ULK2	105
DYRK2	82	HIPK1	92	IRAK1	105
FGF-R1	82	MARK3	92	IRR	106
MKK6	83	MINK1	92	PHK	106
PKCa	84	DYRK3	92	ABL	106
ERK5	84	TBK1	92	MAPKAP-K2	107
EPH-B4	84	BRSK1	93	PKA	108
JNK2	84	PIM1	93	ASK1	108
EPH-A2	84	MARK4	93	PAK6	108
GCK	84	NEK6	93	S6K1	109
MARK2	85	YES1	94	MPSK1	110
DYRK1A	85	TTBK1	94	MELK	111
MKK1	85	TLK1	94	MEKK1	111
CK1 δ	85	Lck	94	DDR2	117
ULK1	86	WNK1	94	CDK9-Cyclin TI	119
PINK	87	EF2K	95		
JNK1	87	SGK1	95		

Therapy, University of Dundee. In the assays ATP was used at a concentration close to the ATP K_m value of the kinase.

8. ARC-Lum binding assay

Binding assay with time-gated luminescence intensity detection

The binding curves were measured according to the protocol described previously.⁴ Briefly, all biochemical binding experiments were performed on black low-volume 384-well non-bonding-surface microplates (Corning #3676) on a PHERAstar platereader (BMG Labtech) with TRF optical module [$\lambda_{\text{ex}} = 337$ (50) nm, $\lambda_{\text{em}} = 675$ (50) nm] when using the time-resolved fluorescence measurement mode or with fluorescence anisotropy module [$\lambda_{\text{ex}} = 590$ (50) nm, $\lambda_{\text{em}} = 675$ (50) nm] when using the fluorescence anisotropy readout. The microplates were incubated at 30 °C for 20 min before each measurement.

To characterize the binding of luminescence probe ARC-1602, ARC-1609, ARC-3132, ARC-3141 to PKAc, Pim-1 and CK2 α , the concentration series of kinases (3-fold dilutions) were made in the assay buffer and the fixed concentration of luminescent probe was added to each well.

In TRF mode, ARC-Lum probes were excited with a flash of the xenon lamp at 337 (50) nm, followed by 50 μ s delay time and subsequent acquisition (150 μ s) of the luminescence signal at 675(50) nm. The data were fitted with the aid of GraphPad Prism software version 5.0 (GraphPad Software, Inc.) and K_D values were calculated using nonlinear regression analysis:

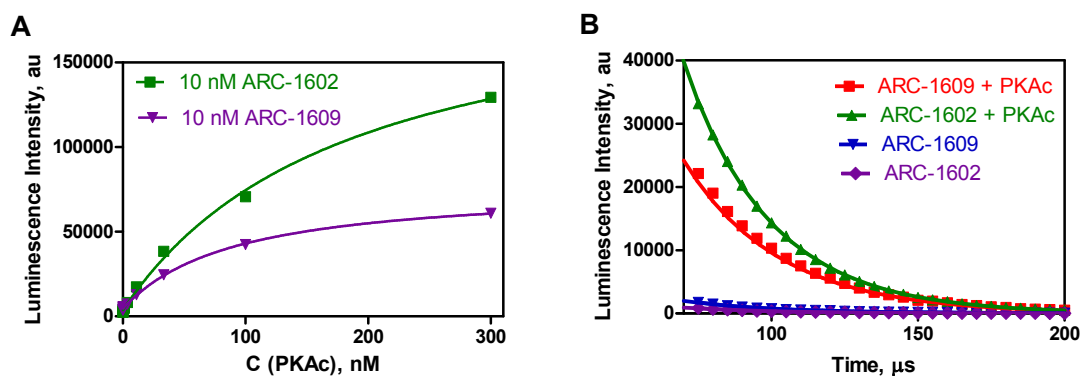
$$TGL = B + M \frac{[L_t + K_D + kE_0 - \sqrt{(L_t + K_D + kE_0)^2 - 4L_t kE_0}]}{2} \quad (\text{Eq.1})$$

where B is the background signal; M is the luminescence intensity of the PK/ARC-Lum complex; L_t is the total concentration of ARC-Lum; E_0 is the nominal concentration of the kinase; K_D is the dissociation constant between ARC-Lum and PK; k is the fraction of the active kinase.

Measurement of luminescence lifetimes

The luminescence lifetimes of complexes of ARC-probes with kinases were measured on a PHERAstar platereader using the luminescence decay mode. The complex of ARC-Lum(Fluo) probe with kinases PKAc, Pim1 or CK2 α was excited with a flash of the xenon lamp at 337 nm, and the luminescence decay was subsequently recorded. Luminescence lifetime was calculated from the decay curves by using exponential decay function with the Prism software. Because of long afterglow of xenon flash-lamps minimal delay time of 50 μ s could be used time-gated measurements.

Binding curve and decay curve: ARC-1602 and ARC-1609 with PKAc



Figur

e S2. (A) Titration of ARC-1602 or ARC-1609 (both at 10 nM total concentration) with PKAc [$\lambda_{\text{ex}} = 337$ (50) nm, $\lambda_{\text{em}} = 675$ (50) nm]. ARC-1609: $K_D = 152 \pm 46$ nM, ARC-1602: $K_D = 84 \pm 30$. (B) Decay curve of luminescence intensities of ARC-1602 and ARC-1609 in the presence or absence of PKAc [$\lambda_{\text{ex}} = 337$ (50) nm, $\lambda_{\text{em}} = 675$ (50) nm]. ARC-1602/PKAc: $\tau = 29 \pm 3$ μs, ARC-1609/PKAc: $\tau = 32 \pm 3$ μs.

Decay curve: ARC-3132 with PKAc

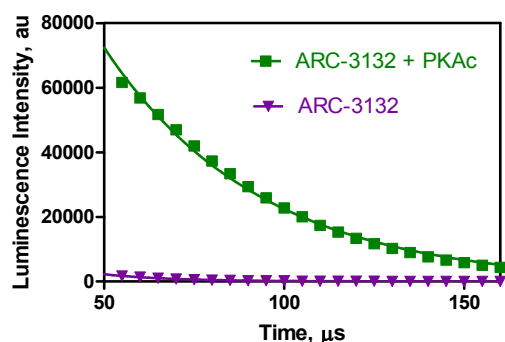


Figure S3. Decay curve of luminescence intensity of ARC-3132 (100 nM) in the presence or absence of PKAc (300 nM) [$\lambda_{\text{ex}} = 337$ (50) nm, $\lambda_{\text{em}} = 675$ (50) nm]. ARC-3132/PKAc: $\tau = 43 \pm 2$ μs.

Decay curve and binding curve: ARC-3141 with CK2 α

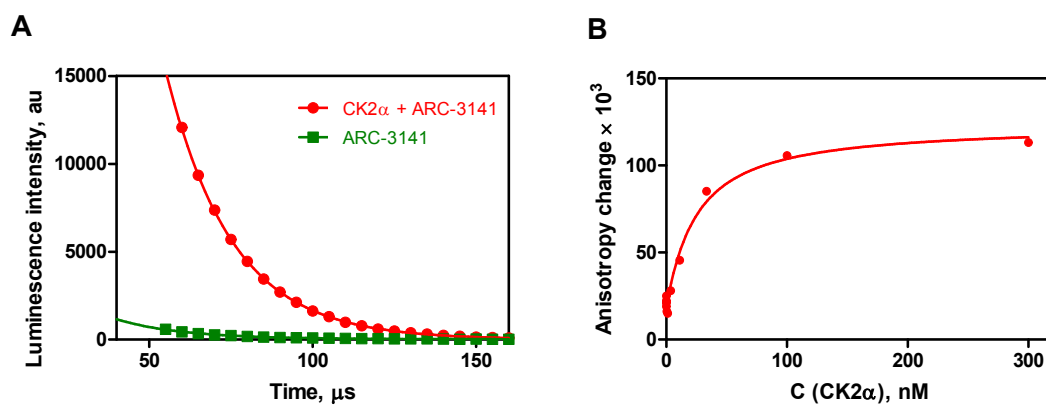


Figure S4. (A) Decay curve of luminescence intensities of ARC-3141 (30 nM) in the presence or absence of CK2 α^{1-335} (150 nM) [$\lambda_{\text{ex}} = 337(50)$ nm, $\lambda_{\text{em}} = 675(50)$ nm]. ARC-3141/CK2 α^{1-335} : $\tau = 20 \pm 2$ μ s. (B) Titration of CK2 α^{1-335} with 5 nM ARC-3141 detected by fluorescence anisotropy [$\lambda_{\text{ex}} = 590(50)$ nm, $\lambda_{\text{em}} = 675(50)$ nm].

Luminescence intensities: ARC-3138, ARC-3141 with CK2 α and ARC-3131, ARC-3132 with PKAc

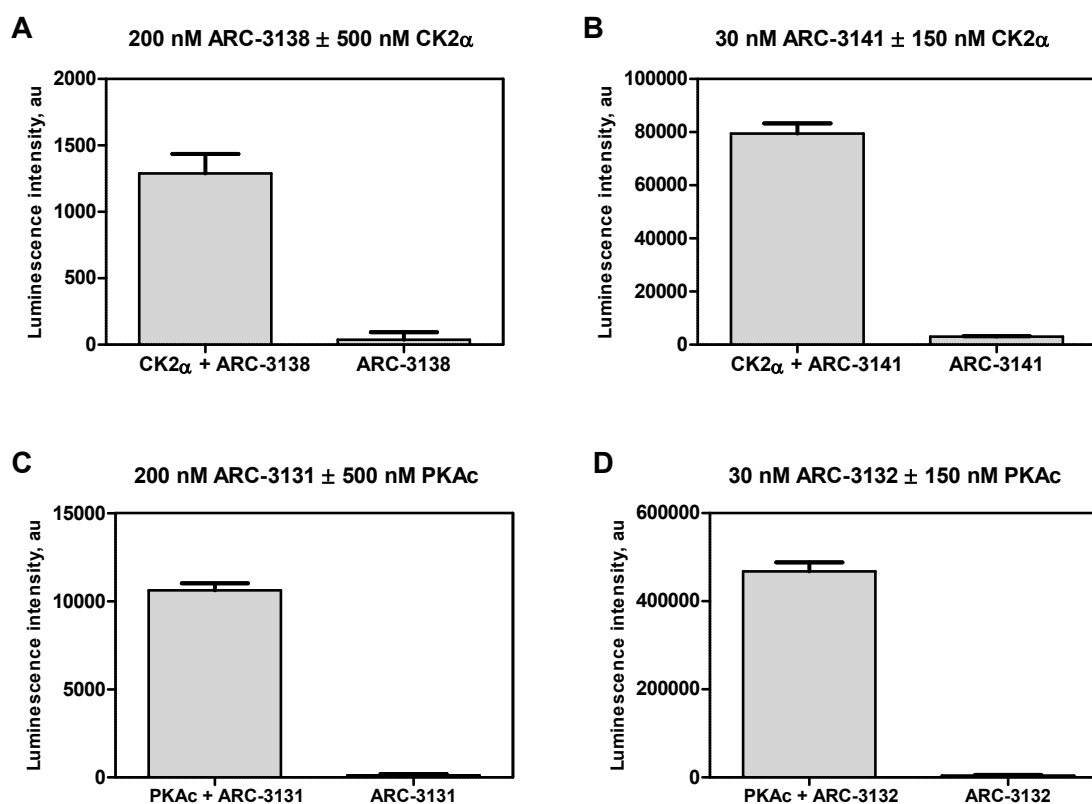


Figure S5. Luminescence intensities of compounds with and without fluorescent dyes, [$\lambda_{\text{ex}} = 337$ (50) nm, $\lambda_{\text{em}} = 675$ (50) nm, delay time 50 μs , acquisition time 150 μs , mean of three readings plotted with 95% confidence interval]. (A) ARC-3138 (200 nM) in the presence or absence of CK2 α^{1-335} (500 nM), (B) ARC-3141 (30 nM) in the presence or absence of CK2 α^{1-335} (150 nM), (C) ARC-3131 (200 nM) in the presence or absence of PKAc (500 nM), (D) ARC-3132 (30 nM) in the presence or absence of PKAc (150 nM).

9. Inhibition of CK2 α by ARC-3138 and ARC-3141

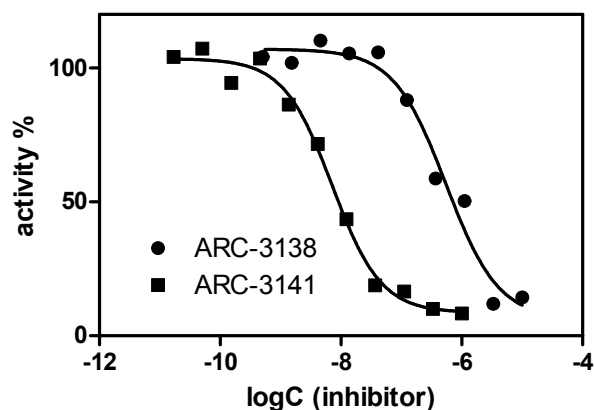


Figure S6. The inhibitory potencies of ARC-3138 and ARC-3141 were determined by TLC-based fluorometric phosphorylation assay as described previously⁵ at the following concentrations of the reaction components: 5-TAMRA-RADDSDDDDDD (30 μ M), ATP (100 μ M), Mg(OAc)₂ (10 mM), CK2 α^{1-335} (0.6 nM) and 3-fold dilutions of the inhibitors. ARC-3138: IC₅₀ = 600 \pm 100 nM; ARC-3141: IC₅₀ = 8 \pm 5 nM.

10. Displacement of ARC-3138 and ARC-3168 from the complex with CK2 α

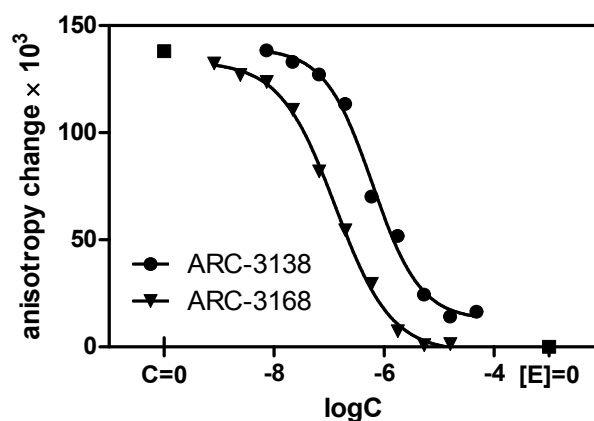


Figure S7. Displacement assay was carried out as described previously⁵ at the following concentrations of the components: fluorescent probe (2 nM), CK2 α^{1-335} (3 nM) and 3-fold dilutions of ARC-3138 or ARC-3168. ARC-3138: IC₅₀ = 600 nM (logIC₅₀ = -6.22 \pm 0.06), K_d = 82 \pm 22 nM; ARC-3168: IC₅₀ = 190 nM (logIC₅₀ = -6.73 \pm 0.07), K_d = 34 \pm 10 nM.

11. Comparison of novel selenadiazole containing probe ARC-3132 with previously reported thiophene (ARC-1182) and selenophene (ARC-1139) containing probes

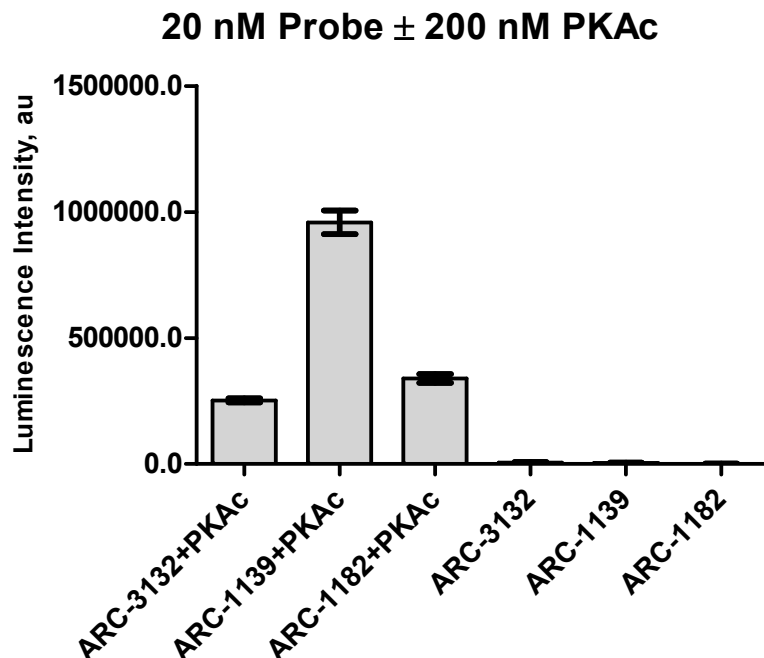


Figure S8. Luminescence intensities of compounds with and without fluorescent dyes [$\lambda_{\text{ex}} = 337$ (50) nm, $\lambda_{\text{em}} = 675$ (50) nm, delay time 50 μs , acquisition time 150 μs , mean of three readings plotted with 95% confidence interval]. ARC-3132, ARC-1139 and ARC-1182 (20 nM) in the presence or absence of PKAc (200 nM). All three probes are labelled with PromoFluor-647. It is important to note that current excitation wavelengths are ideal for ARC-1182 and ARC-1139, but not for ARC-3132.

12. References

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