

## Supplementary Information

### Structure-Activity Relationship Study of ProxPhos Chemosensors for the Detection of Proximal Phosphorylation and Other Phosphate Species

#### Supplementary Information

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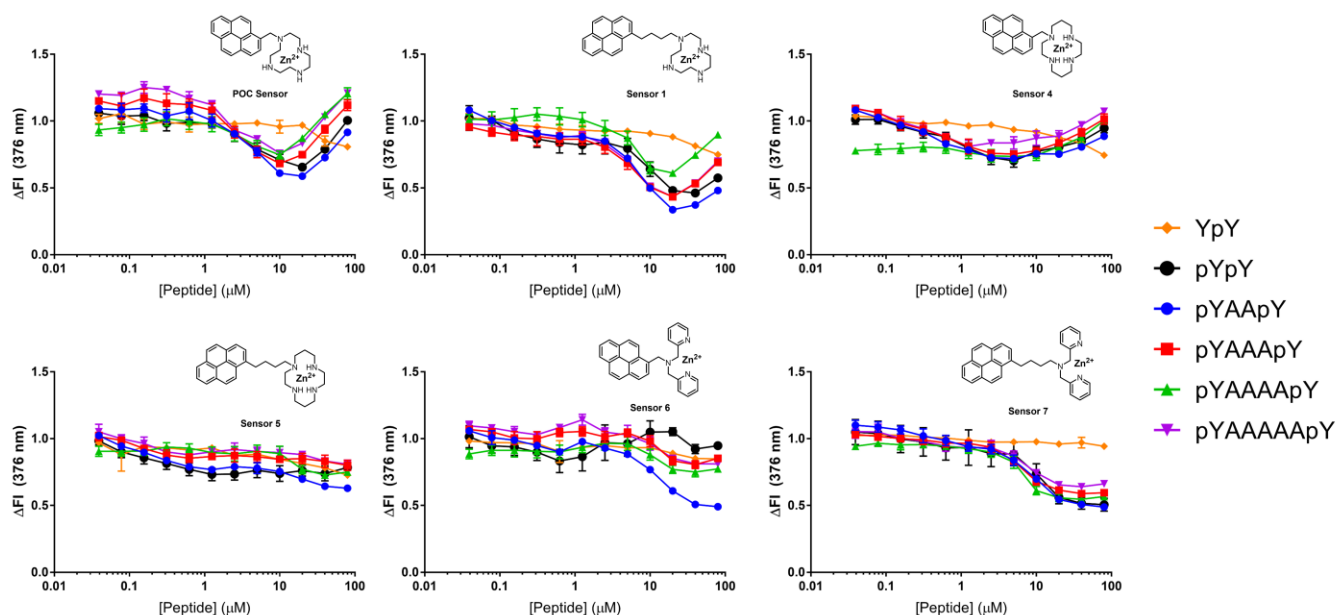
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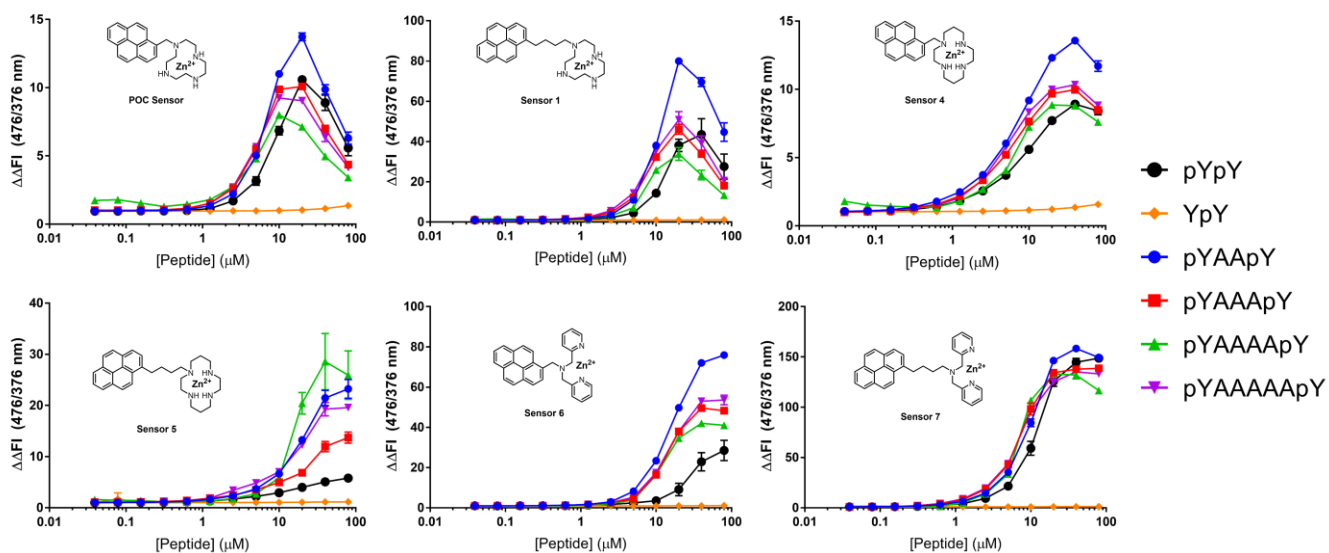
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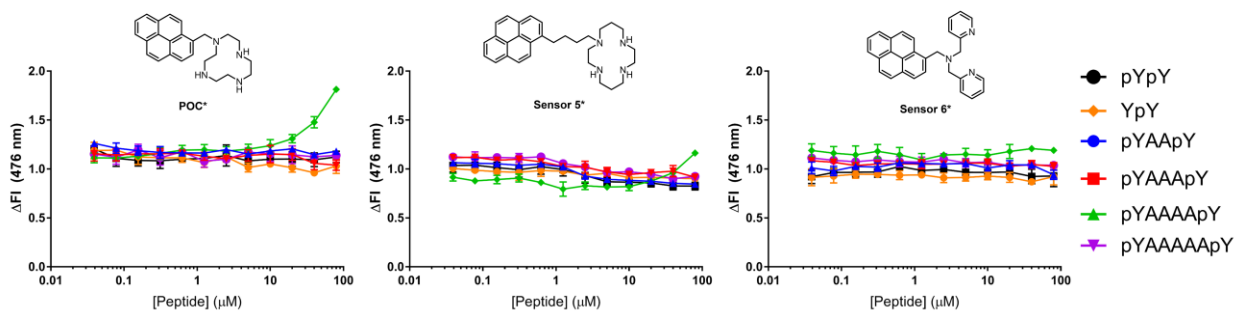


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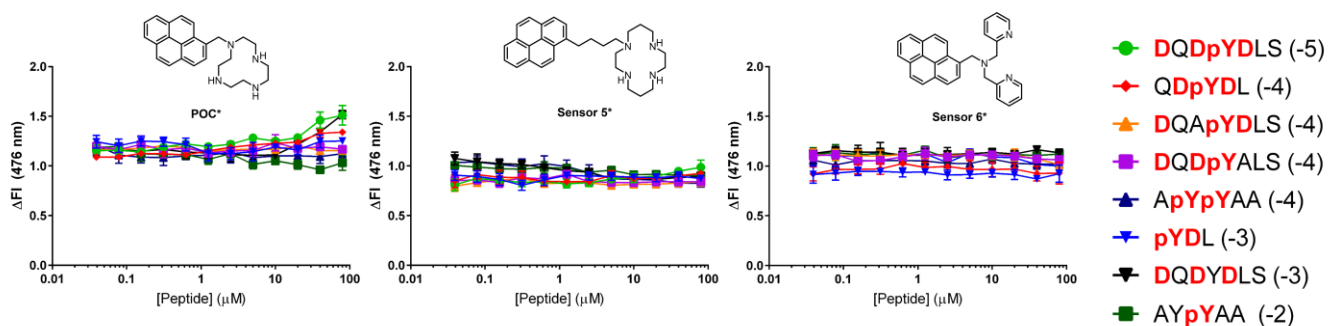


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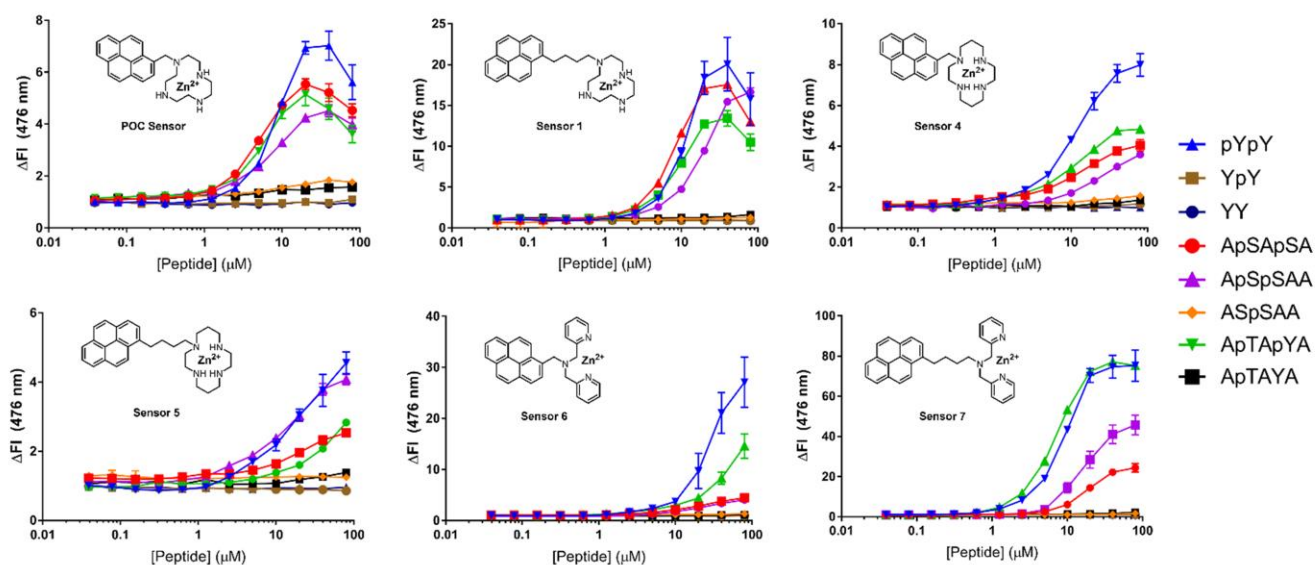


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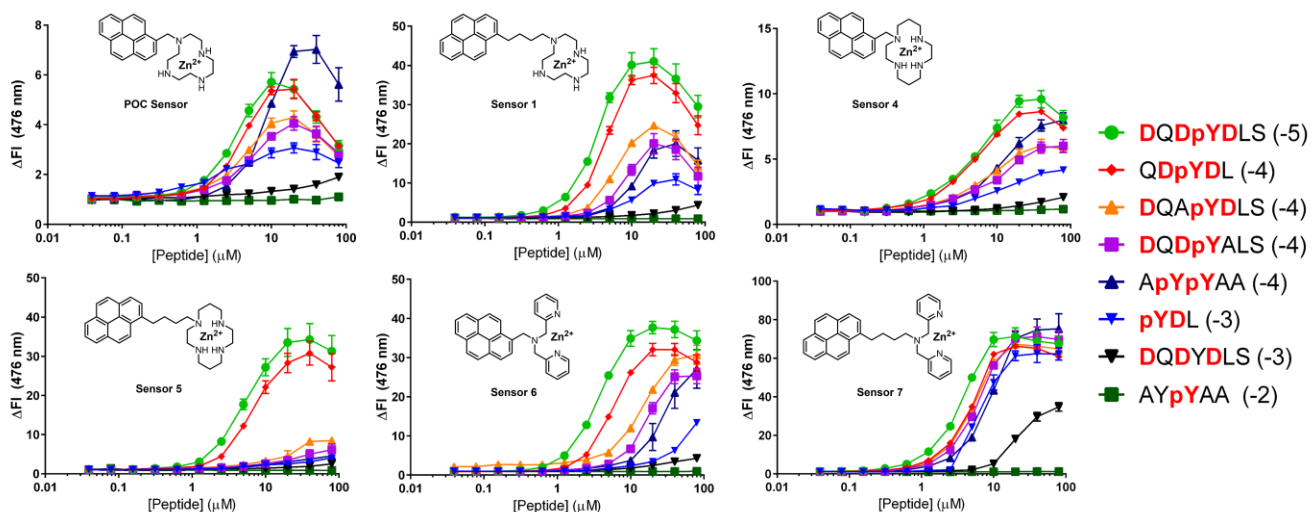
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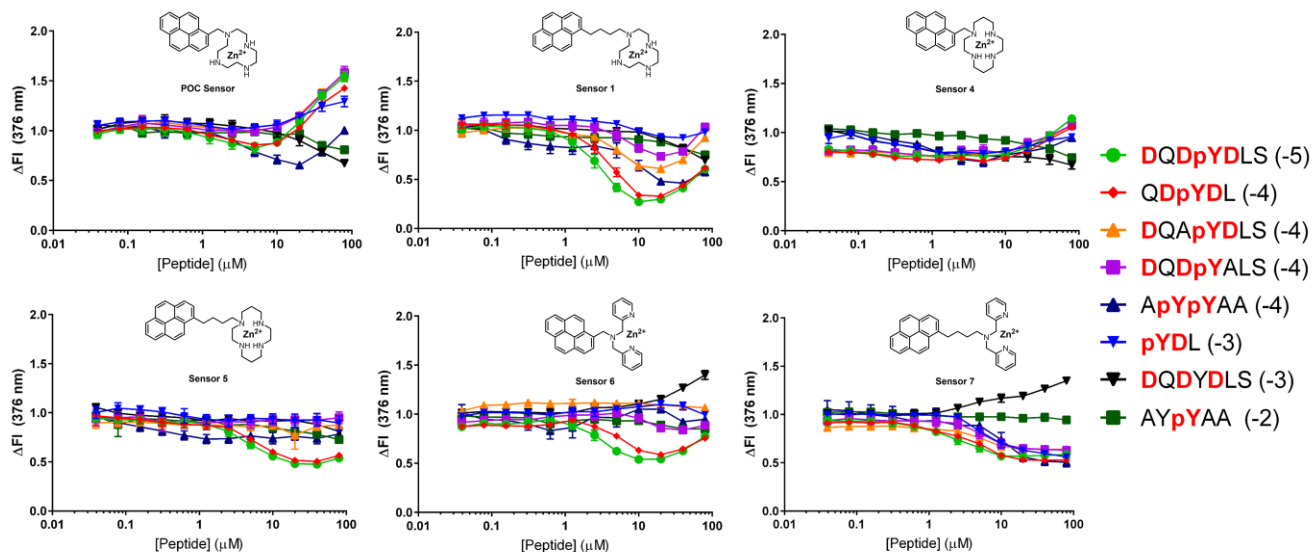
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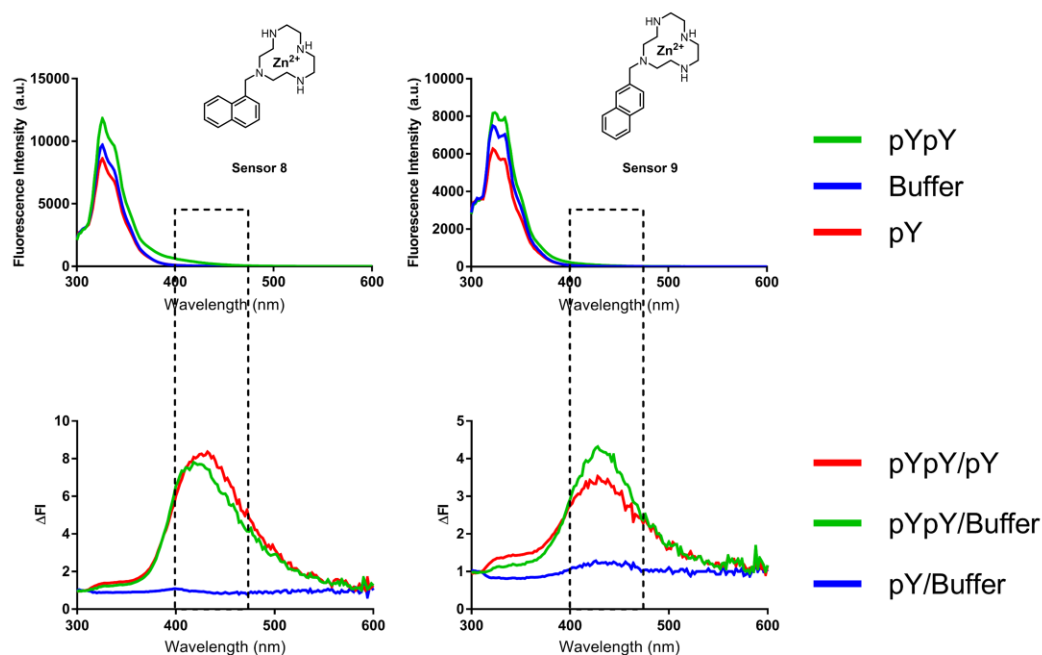


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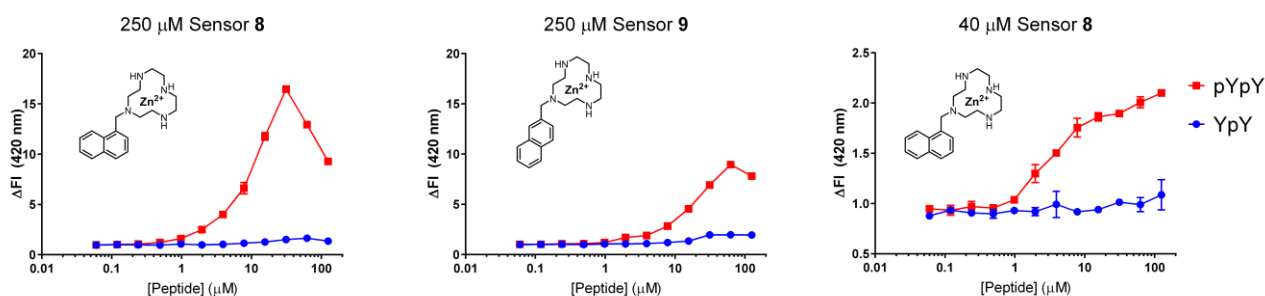


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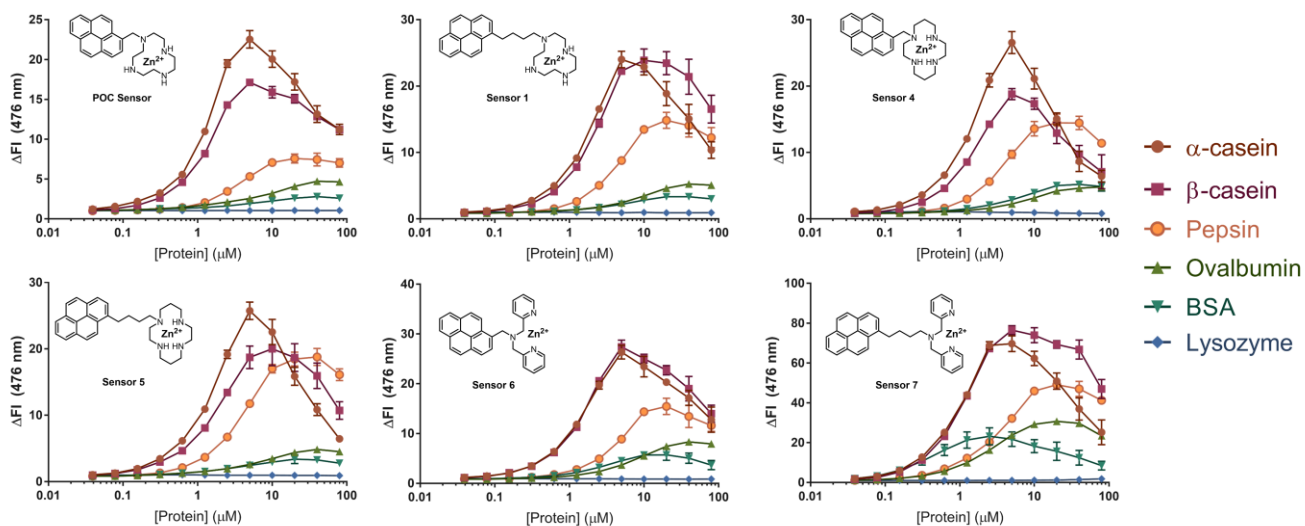
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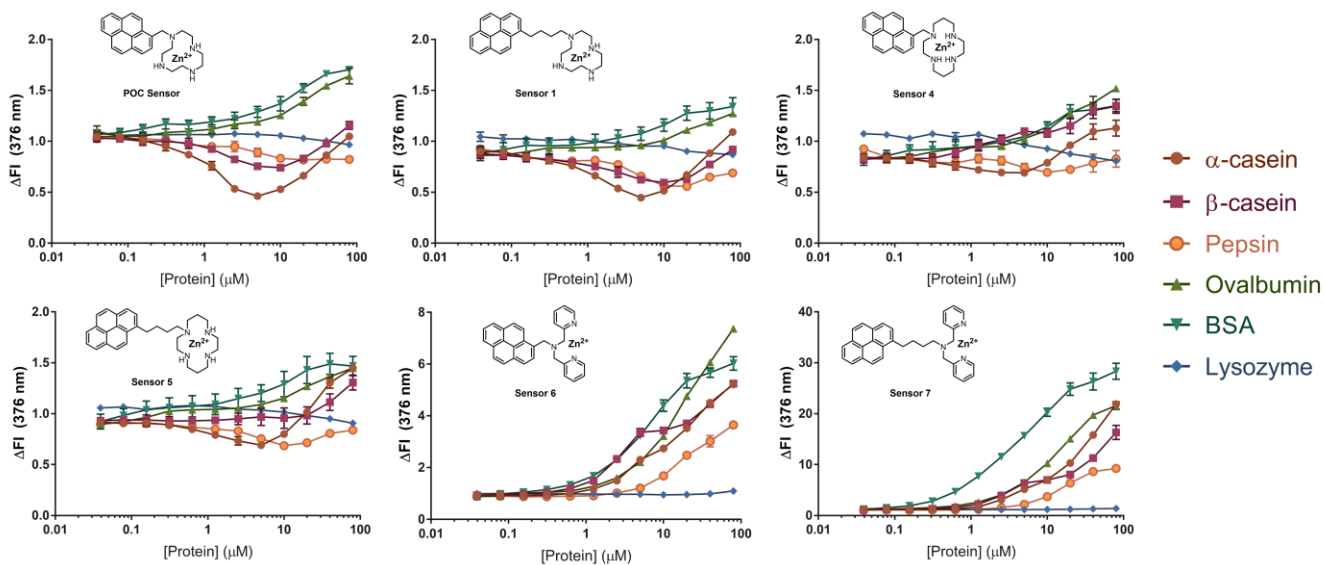
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**Figure S10.** Fluorescence titrations ( $\Delta FI_{\text{exc}}$ , 476 nm) of sensors with model proteins (pH 7.5, 50 mM HEPES, 20% DMSO, 25 mM NaCl; 40  $\mu\text{M}$  sensor,  $\lambda_{\text{ex}} = 350$  nm)

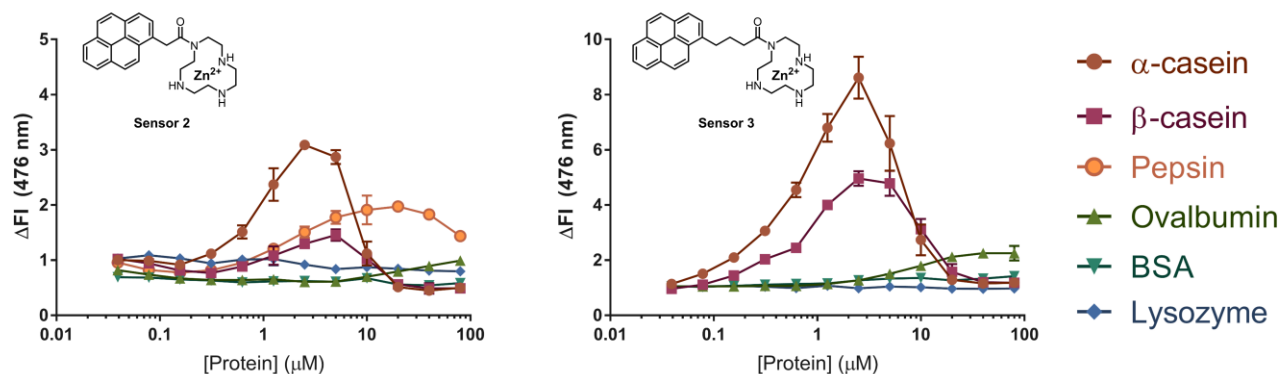


**Figure S11.** Fluorescence titrations ( $\Delta FI_{\text{mon}}$ , 376 nm) of sensors with model proteins (pH 7.5, 50 mM HEPES, 20% DMSO, 25 mM NaCl; 40  $\mu\text{M}$  sensor,  $\lambda_{\text{ex}} = 350$  nm)

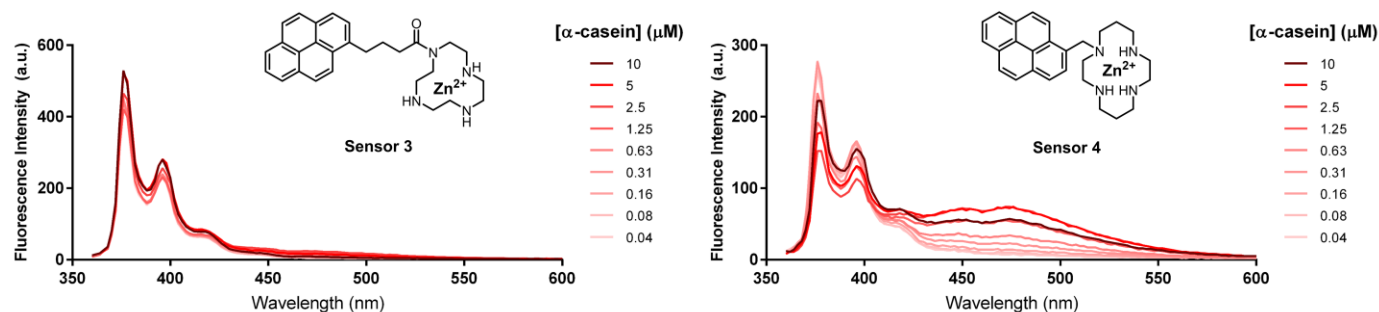




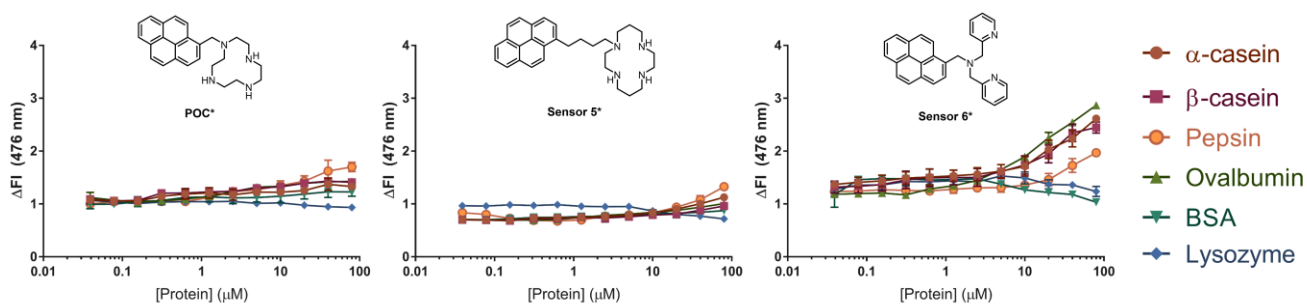
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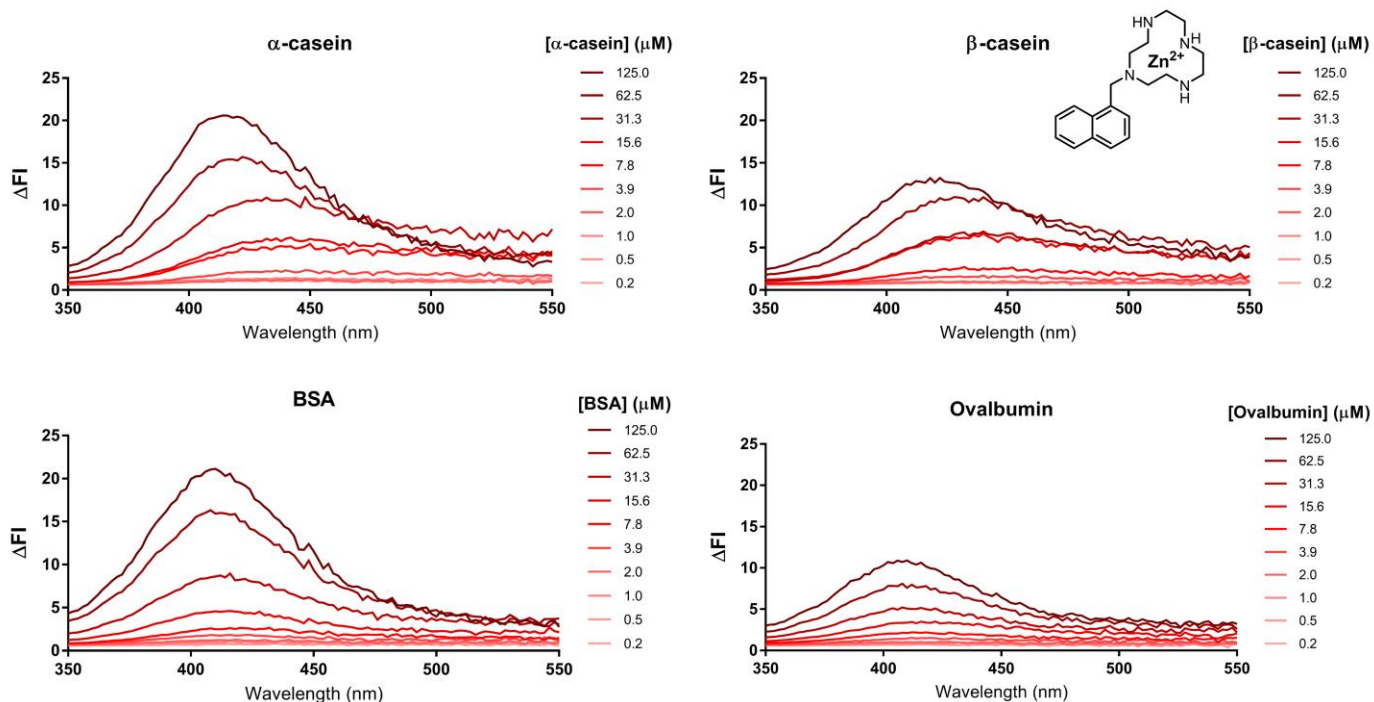
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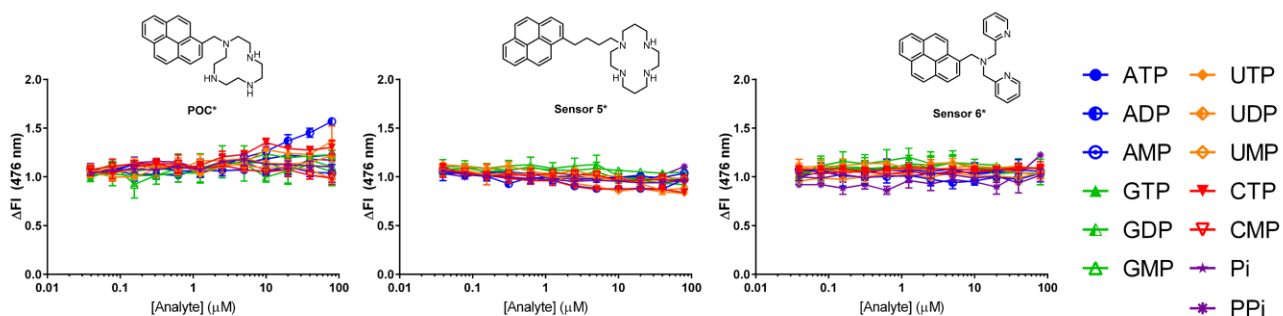
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**Figure S15.** Fluorescence emission spectra ( $\Delta FI$ ) of 1-naphthyl derivative sensor **8** from 350 nm to 550 nm in response to model proteins (50 mM HEPES, pH 7.5, 20% DMSO, 25 mM NaCl; 250  $\mu M$  sensor,  $\lambda_{ex} = 290$  nm)

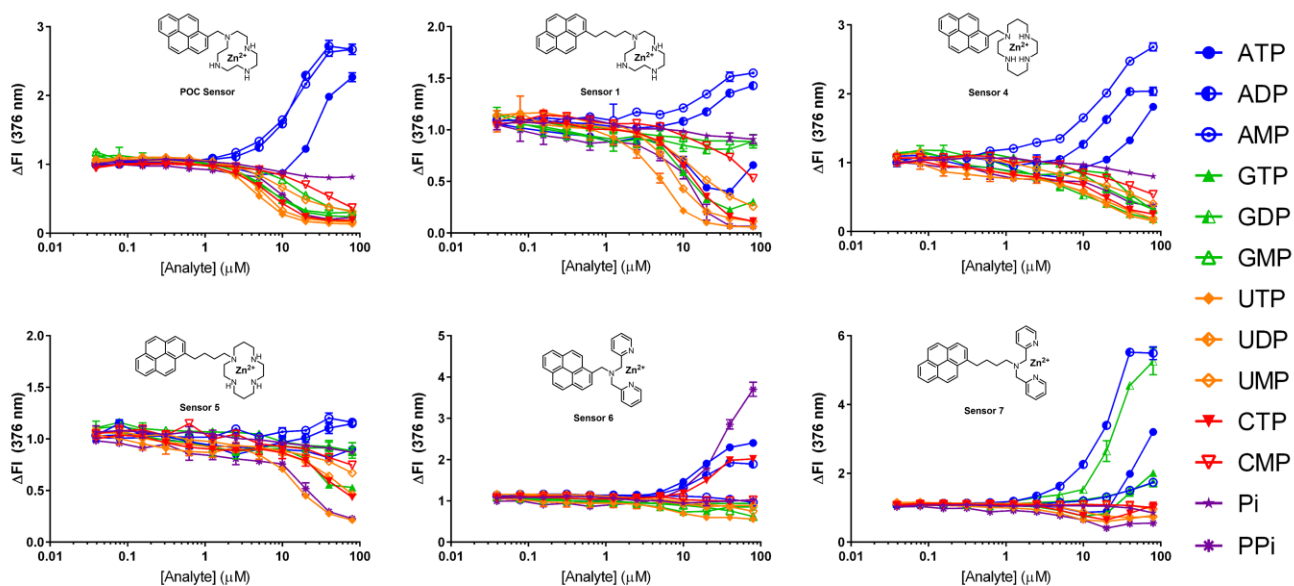


**Figure S16.** Fluorescence titrations ( $\Delta FI_{exc}$ , 476 nm) of unmetalated sensors (**POC\***, **5\*** and **6\***, no  $Zn^{2+}$ ) with nucleotides, inorganic phosphate and pyrophosphate (pH 7.5, 50 mM HEPES, 20% DMSO, 25 mM NaCl; 40  $\mu M$  sensor,  $\lambda_{ex} = 350$  nm).

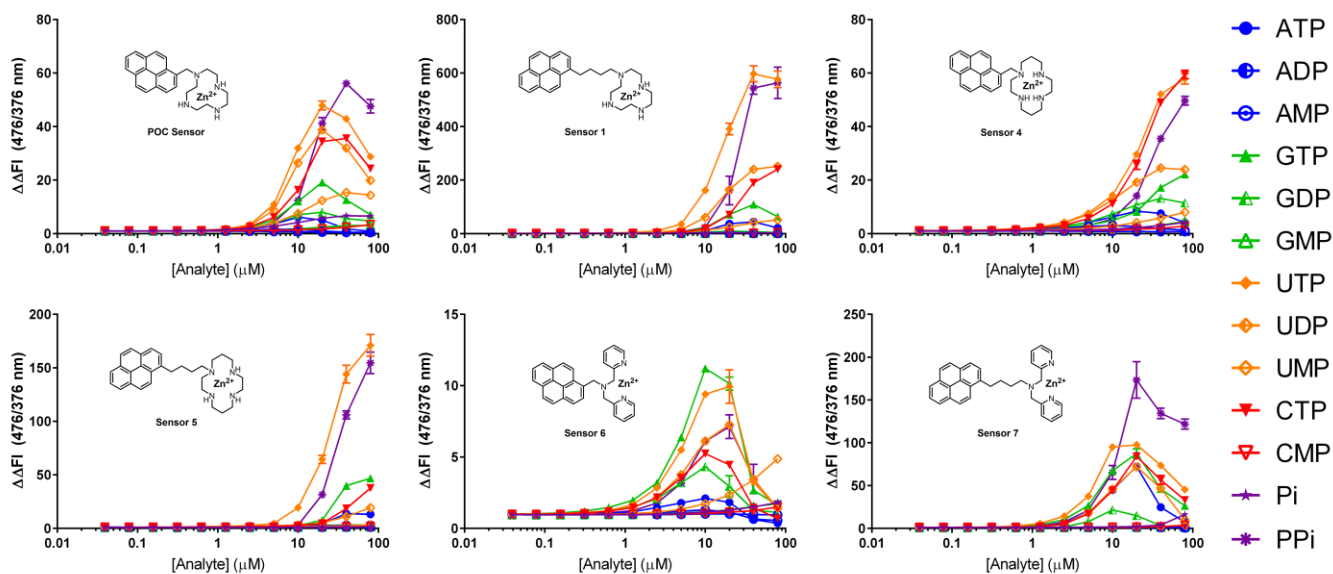


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**Figure S17.** Fluorescence titrations ( $\Delta F I_{\text{mon}}$ , 376 nm) of sensors with nucleotides, inorganic phosphate and pyrophosphate (pH 7.5, 50 mM HEPES, 10% DMSO; 40  $\mu\text{M}$  sensor,  $\lambda_{\text{ex}} = 350$  nm)

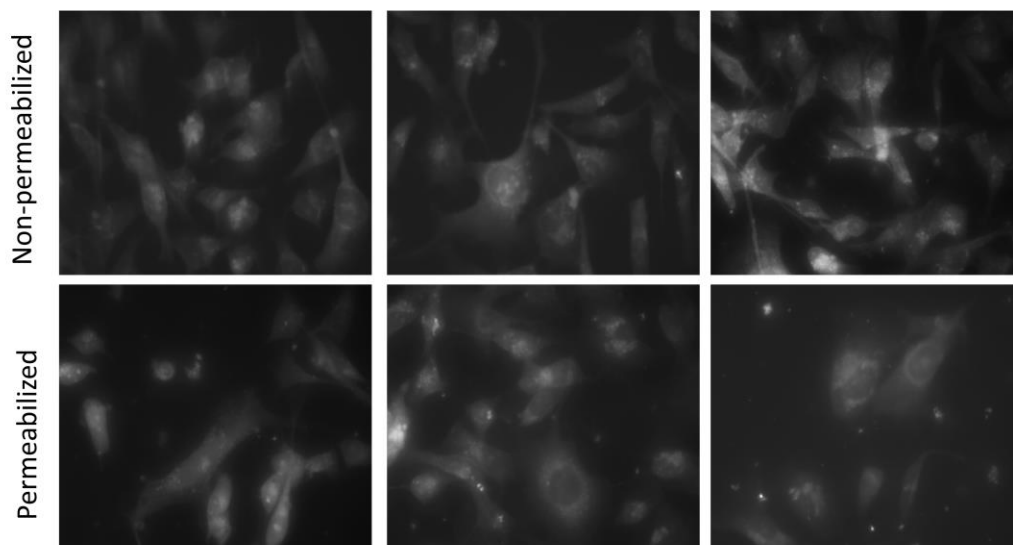


**Figure S18.** Fluorescence titrations ( $\Delta\Delta F I$ , 476/376 nm) of sensors with nucleotides, inorganic phosphate and pyrophosphate (pH 7.5, 50 mM HEPES, 10% DMSO; 40  $\mu\text{M}$  sensor,  $\lambda_{\text{ex}} = 350$  nm)



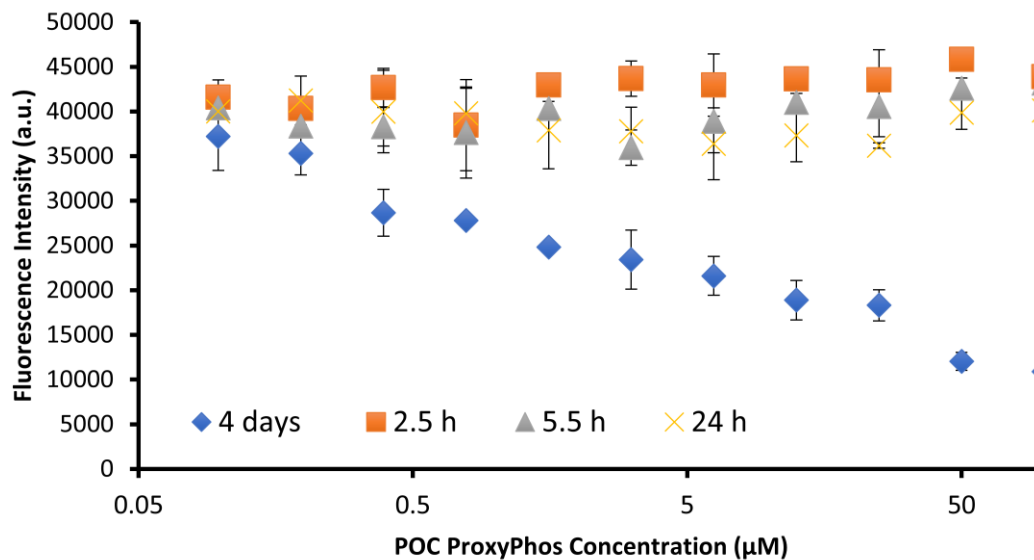
**Figure S19. ProxyPhos staining of permeabilized and non-permeabilized cells.**

Fixed MDA-MB-231 cells were stained with 40  $\mu\text{M}$  of the POC ProxyPhos sensor (see methods for details) with or without permeabilization prior to staining. Fluorescent images were acquired using a camera connected to an epi-fluorescent microscope using blue emission filters. As can be seen, permeabilization of cells is not required for efficient staining by the POC sensor.



**Figure S20. POC ProxyPhos cytotoxicity.**

Viability of MRC-9 cells in presence of 100  $\mu\text{M}$  to 200 nM the POC sensor following incubation for 2.5, 5.5 and 24 h and 4 days, as assessed by CellTiter Blue assay (see methods for details). The point at the left is without any sensor. Higher fluorescence reading corresponds to higher cell viability (error bars represent +/- s.d. n = 3).

**Supplementary Note 1.** List of full peptide sequences

**pYpY** = Ac-ApYpYAA-NH<sub>2</sub>

**YpY** = Ac-AYpYAA-NH<sub>2</sub>

**YY** = Ac-AYYAA-NH<sub>2</sub>

**pSpS** = Ac-ApSpSAA-NH<sub>2</sub>

**SpS** = Ac-ASpSAA-NH<sub>2</sub>

**pSApS** = Ac-ApSApSAA-NH<sub>2</sub>

**pTAY** = Ac-ApTAYAA-NH<sub>2</sub>

**pTApY** = Ac-ApTApYAA-NH<sub>2</sub>

**pYAApY** = Ac-ApYAApYA-NH<sub>2</sub>

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**pYAAApY** = Ac-pYAAApY-NH<sub>2</sub>

**pYAAAApY** = Ac-pYAAAApY-NH<sub>2</sub>

**pYAAAAApY** = Ac-pYAAAAApY-NH<sub>2</sub>

**pYDL** = NH<sub>2</sub>-pYDL-COOH

**QDpYDL** = NH<sub>2</sub>-QDpYDL-COOH

**DQDpYDLS** = NH<sub>2</sub>-DQDpYDLS-COOH

**DQDpYALS** = NH<sub>2</sub>-DQDpYALS-COOH

**DQApYDLS** = NH<sub>2</sub>-DQApYDLS-COOH

**DQDYDLS** = NH<sub>2</sub>-DQDYDLS-COOH

**Supplementary Table 1.** List of DpYD motifs found in human proteins

Gene	Protein	Accession	MW	Start	End	Modification Sites
ACTN3	ACTN3	UP:Q08043	103,241	435	437	Y436-p
AHNAK	AHNAK	UP:Q09666	629,101	1468	1470	Y1469-p
AMPH	amphiphysin	UP:P49418	76,257	144	146	Y145-p
ANK1	ANK1	UP:P16157	206,265	1072	1074	Y1073-p
APLP2	APLP2	UP:Q06481	86,956	232	234	Y233-p
APOBEC3A	APOBEC3A	UP:P31941	23,012	131	133	Y132-p
APOBEC3B	APOBEC3B	UP:Q9UH17	45,924	314	316	Y315-p
ARFGAP1	ARF GAP1	UP:Q8N6T3	44,668	92	94	Y93-p
ARRB2	ARRB2	UP:P32121	46,106	403	405	Y404-p
ASCC3	ASCC3	UP:Q8N3C0	251,460	1908	1910	Y1909-p
ATXN2L	ataxin-2L	UP:Q8WWM7	113,374	240	242	Y241-p
BIN1	BIN1	UP:O00499	64,699	149	151	Y150-p
BIN2	BIN2	UP:Q9UBW5	61,874	148	150	Y149-p
BLM	BLM	UP:P54132	159,000	295	297	Y296-p
CACNG5	CACNG5	UP:Q9UF02	30,903	266	268	Y267-p
CACTIN	CACTIN	UP:Q8WUQ7	88,702	553	555	Y554-p
CALU	CALU	UP:O43852	37,107	46	48	Y47-p
CALU	CALU	UP:O43852	37,107	262	264	Y263-p
NEDD9	Cas-L	UP:Q14511	92,861	240	242	Y241-p
NEDD9	Cas-L	UP:Q14511	92,861	628	630	Y629-p

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CBLB	Cbl-b	UP:Q13191	109,450	801	803	Y802-p
CBLB	Cbl-b	UP:Q13191	109,450	888	890	Y889-p
CCNB2	CCNB2	UP:O95067	45,282	297	299	Y298-p
LY9	CD229	UP:Q9HBG7	72,139	582	584	Y583-p
CD5	CD5	UP:P06127	54,578	486	488	Y487-p
CD6	CD6	UP:P30203	71,801	661	663	Y662-p
CDC42	CDC42	UP:P60953	21,259	63	65	Y64-p
CDC42	CDC42 iso1	UP:P60953-1	21,311	63	65	Y64-p
CDH2	CDH2	UP:P19022	99,809	784	786	Y785-p
CDH2	CDH2	UP:P19022	99,809	883	885	Y884-p
CDH3	CDH3	UP:P22223	91,418	712	714	Y713-p
CDH4	CDH4	UP:P55283	100,281	794	796	Y795-p
ARAP1	CENTD2	UP:Q96P48	162,192	230	232	Y231-p
CEP104	CEP104	UP:O60308	104,448	265	267	Y266-p
CHD1	CHD-1	UP:O14646	196,688	224	226	Y225-p
CSNK2A1	CK2A1	UP:P68400	45,144	130	132	Y131-p
CLASP2	CLASP2	UP:O75122	141,133	926	928	Y927-p
CLDN5	Claudin-5	UP:O00501	23,147	211	213	Y212-p
CLCN7	CLCN7	UP:P51798	88,679	98	100	Y99-p
CSNK2A3	CSNK2A3	UP:Q8NEV1	45,220	130	132	Y131-p
DAGLB	DAGLBETA	UP:Q8NCG7	73,732	317	319	Y318-p
CCAR2	DBC-1	UP:Q8N163	102,902	197	199	Y198-p
DDX3X	DDX3	UP:O00571	73,243	103	105	Y104-p
DDX39A	DDX39	UP:O00148	49,130	12	14	Y13-p
DDX3Y	DDX3Y	UP:O15523	73,154	102	104	Y103-p
DENND4A	DENND4A	UP:Q7Z401	209,244	546	548	Y547-p
DSP	Desmoplakin	UP:P15924	331,774	1138	1140	Y1139-p
DGKH	DGKH	UP:Q86XP1	134,866	655	657	Y656-p
DMRT3	DMRT3	UP:Q9NQL9	51,199	455	457	Y456-p
DNAH17	DNAH17	UP:Q9UFH2	511,787	1118	1120	Y1119-p
DNAH5	DNAH5	UP:Q8TE73	529,021	4258	4260	Y4259-p
DNAJC25	DNAJC25	UP:Q9H1X3	42,404	119	121	Y120-p
DROSHA	DROSHA	UP:Q9NRR4	159,316	268	270	Y269-p
DYSF	DYSF	UP:O75923	237,295	1649	1651	Y1650-p
MAPRE1	EB1	UP:Q15691	29,999	123	125	Y124-p
EIF3L	eIF3S6IP	UP:Q9Y262	66,727	22	24	Y23-p
EIF4B	EIF4B	UP:P23588	69,151	265	267	Y266-p
ENKUR	ENKUR	UP:Q8TC29	29,454	171	173	Y172-p
FAM129B	FAM129B	UP:Q96TA1	84,138	481	483	Y482-p
FBXL4	FBXL4	UP:Q9UKA2	70,097	466	468	Y467-p

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FLG	FLG	UP:P20930	435,170	3983	3985	Y3984-p
FOSB	FosB	UP:P53539	35,928	8	10	Y9-p
FREM1	FREM1	UP:Q5H8C1	244,154	162	164	Y163-p
FRMD4A	FRMD4A	UP:Q9P2Q2	115,458	600	602	Y601-p
GIT1	GIT1	UP:Q9Y2X7	84,341	382	384	Y383-p
GIT1	GIT1 iso3	UP:Q9Y2X7-3	85,446	391	393	Y392-p
GIT2	GIT2	UP:Q14161	84,543	591	593	Y592-p
GSTM5	GSTM5	UP:P46439	25,675	40	42	Y41-p
ST13	HIP	UP:P50502	41,332	212	214	Y213-p
HIVEP2	HIVEP2	UP:P31629	269,053	637	639	Y638-p
HNRNPC	hnRNP C1/C2	UP:P07910	33,670	125	127	Y126-p
HNRNPC	hnRNP C1/C2 iso2	UP:P07910-2	32,338	112	114	Y113-p
HNRNPC	hnRNP C1/C2 iso4	UP:P07910-4	27,822	112	114	Y113-p
HNRNPK	hnRNP K	UP:P61978	50,976	279	281	Y280-p
HNRNPUL2	HNRPUL2	UP:Q1KMD3	85,105	697	699	Y698-p
HPGD	HPGD	UP:P15428	28,977	255	257	Y256-p
MAP4K1	HPK1	UP:Q92918	91,296	380	382	Y381-p
CLNS1A	ICLN	UP:P54105	26,215	146	148	Y147-p
ING4	ING4 iso4	UP:Q9UNL4-4	28,089	120	122	Y121-p
ING4	ING4 iso5	UP:Q9UNL4-5	28,218	120	122	Y121-p
BAIAP2L1	IRTKS	UP:Q9UHR4	56,883	273	275	Y274-p
ITGB4	ITGB4	UP:P16144	202,167	1342	1344	Y1343-p
ITGB4	ITGB4 iso2	UP:P16144-2	195,013	1342	1344	Y1343-p
SLC12A4	KCC1	UP:Q9UP95	120,650	16	18	Y17-p
KNOP1	KNOP1	UP:Q1ED39	51,589	419	421	Y420-p
KPNB1	KPNB1	UP:Q14974	97,170	751	753	Y752-p
IPO5	KPNB3	UP:O00410	123,630	819	821	Y820-p
MYCL	L-Myc	UP:P12524	40,327	2	4	Y3-p
LTV1	LTV1	UP:Q96GA3	54,855	65	67	Y66-p
MAD1L1	MAD1L1	UP:Q9Y6D9	83,067	534	536	Y535-p
MAP1B	MAP1B	UP:P46821	270,634	1886	1888	Y1887-p
MAP3K15	MAP3K15	UP:Q6ZN16	147,437	297	299	Y298-p
MAPKAPK3	MAPKAPK3	UP:Q16644	42,987	345	347	Y346-p
MATR3	matrin 3	UP:P43243	94,623	201	203	Y202-p
MAP3K2	MEKK2	UP:Q9Y2U5	69,741	249	251	Y250-p
MELK	MELK	UP:Q14680	74,642	3	5	Y4-p
MDN1	midasin	UP:Q9NU22	632,820	4857	4859	Y4858-p
MIER3	MIER3	UP:Q7Z3K6	61,437	32	34	Y33-p



## Supplementary Information

MRC1	MRC1	UP:P22897	166,012	199	201	Y200-p
MTX1	MTX1	UP:Q13505	51,477	231	233	Y232-p
MYC	Myc	UP:P01106	48,804	15	17	Y16-p
MYO6	MYO6	UP:Q9UM54	149,691	1145	1147	Y1146-p
MYOF	myoferlin	UP:Q9NZM1	234,709	1624	1626	Y1625-p
NADK	NADK	UP:O95544	49,228	167	169	Y168-p
NAP1L1	NAP1L1	UP:P55209	45,374	376	378	Y377-p
NCBP2	NCBP2	UP:P52298	18,001	141	143	Y142-p
NEB	NEB	UP:P20929	772,914	3741	3743	Y3742-p
N4BP2	Nedd4-BP2	UP:Q86UW6	198,801	1618	1620	Y1619-p
NIFK	NIFK	UP:Q9BYG3	34,222	182	184	Y183-p
NIPBL	NIPBL	UP:Q6KC79	316,051	1749	1751	Y1750-p
TENM1	ODZ1	UP:Q9UKZ4	305,011	2377	2379	Y2378-p
TENM1	ODZ1 var1	UP:Q9UKZ4_ VAR_N2389Y	305,034	2377	2379	Y2378-p
BCAR1	P130Cas	UP:P56945	93,372	663	665	Y664-p
PCDH18	PCDH18	UP:Q9HCL0	126,149	884	886	Y885-p
PDE4B	PDE4B	UP:Q07343	83,343	141	143	Y142-p
PDE4D	PDE4D	UP:Q08499	91,115	198	200	Y199-p
P4HB	PDIA1	UP:P07237	57,116	267	269	Y268-p
PDIA6	PDIA6	UP:Q15084	48,121	251	253	Y252-p
PEX5	PEX5	UP:P50542	70,865	311	313	Y312-p
PPARGC1B	PGC-1 beta	UP:Q86YN6	113,222	989	991	Y990-p
PHACTR4	PHACTR4	UP:Q8IZ21	78,211	655	657	Y656-p
PHB	PHB	UP:P35232	29,804	113	115	Y114-p
PHF14	PHF14	UP:O94880	100,053	205	207	Y206-p
PHKA1	PHKA1	UP:P46020	137,312	647	649	Y648-p
PIK3C2A	PIK3C2A	UP:O00443	190,680	72	74	Y73-p
PIK3C2B	PIK3C2B	UP:O00750	184,768	227	229	Y228-p
PKP3	plakophilin 3	UP:Q9Y446	87,082	175	177	Y176-p
PLD1	PLD1	UP:Q13393	124,184	41	43	Y42-p
PPHLN1	PPHLN1	UP:Q8NEY8	52,737	60	62	Y61-p
PQBP1	PQBP1	UP:O60828	30,472	32	34	Y33-p
PQBP1	PQBP1 iso3	UP:O60828-3	26,158	32	34	Y33-p
PRPF8	PRPF8	UP:Q6P2Q9	273,600	1670	1672	Y1671-p
PSMD4	PSMD4	UP:P55036	40,737	325	327	Y326-p
PTPRCAP	PTPRCAP	UP:Q14761	21,196	114	116	Y115-p
RAB27A	RAB27A	UP:P51159	24,868	5	7	Y6-p
RAB27B	RAB27B	UP:O00194	24,608	5	7	Y6-p
RAB35	RAB35	UP:Q15286	23,025	4	6	Y5-p

Supplementary Information

RAC1	RAC1	UP:P63000	21,450	63	65	Y64-p
RAC1	RAC1 iso2	UP:P63000-2	23,467	63	65	Y64-p
RAC2	RAC2	UP:P15153	21,429	63	65	Y64-p
RAC3	RAC3	UP:P60763	21,379	63	65	Y64-p
FAM208A	RAP140	UP:Q9UK61	189,032	675	677	Y676-p
RBM10	RBM10	UP:P98175	103,533	56	58	Y57-p
RBM10	RBM10 iso2	UP:P98175-2	103,433	56	58	Y57-p
RBM27	RBM27	UP:Q9P2N5	118,718	145	147	Y146-p
RBM5	RBM5	UP:P52756	92,154	56	58	Y57-p
RBM8A	RBM8A	UP:Q9Y5S9	19,889	53	55	Y54-p
RCN1	RCN1	UP:Q15293	38,890	277	279	Y278-p
RHOA	RHOA	UP:P61586	21,768	65	67	Y66-p
RHOB	RHOB	UP:P62745	22,123	65	67	Y66-p
RHOC	RHOC	UP:P08134	22,006	65	67	Y66-p
RHOF	RHOF	UP:Q9HBH0	23,625	79	81	Y80-p
RHOQ	RHOQ	UP:P17081	22,659	69	71	Y70-p
RLF	RLF	UP:Q13129	217,953	513	515	Y514-p
ROCK2	ROCK2	UP:O75116	160,900	91	93	Y92-p
RPL23A	RPL23A	UP:P62750	17,695	143	145	Y144-p
RPL3	RPL3	UP:P39023	46,109	306	308	Y307-p
RRAS	RRas	UP:P10301	23,480	57	59	Y58-p
RRAS2	RRas2	UP:P62070	23,400	42	44	Y43-p
RUVBL2	RUVBL2	UP:Q9Y230	51,157	214	216	Y215-p
EXOC2	Sec5	UP:Q96KP1	104,066	298	300	Y299-p
SRSF1	SF2	UP:Q07955	27,745	78	80	Y79-p
SF3B3	SF3B3	UP:Q15393	135,577	1040	1042	Y1041-p
SCAF4	SFRS15	UP:O95104	125,869	266	268	Y267-p
SH3BGRL	SH3BGRL	UP:O75368	12,774	78	80	Y79-p
SH3GL3	SH3GL3	UP:Q99963	39,285	167	169	Y168-p
FYB	SLAP-130	UP:O15117	85,387	570	572	Y571-p
SLU7	SLU7	UP:O95391	68,387	162	164	Y163-p
SASH3	SLY	UP:O75995	41,595	315	317	Y316-p
NAPG	SNAP-gamma	UP:Q99747	34,746	30	32	Y31-p
SQSTM1	SQSTM1	UP:Q13501	47,687	147	149	Y148-p
SRRT	SRRT	UP:Q9BXP5	100,666	57	59	Y58-p
SRSF10	SRSF10	UP:O75494	31,301	112	114	Y113-p
SRSF11	SRSF11	UP:Q05519	53,542	424	426	Y425-p
SYCP2	SYCP2	UP:Q9BX26	175,639	1129	1131	Y1130-p
SYDE1	SYDE1	UP:Q6ZW31	79,793	674	676	Y675-p

## Supplementary Information

TAF9	TAFII31	UP:Q16594	28,974	260	262	Y261-p
TIRAP	TIRAP	UP:P58753	23,883	85	87	Y86-p
TTN	Titin	UP:Q8WZ42	3,816,030	33002	33004	Y33003-p
TRA2A	TRA2A	UP:Q13595	32,689	251	253	Y252-p
TRIM9	TRIM9	UP:Q9C026	79,177	59	61	Y60-p
TRPC4	TRPC4	UP:Q9UBN4	112,101	958	960	Y959-p
TXNRD1	TRXR1	UP:Q16881	70,906	162	164	Y163-p
TXNRD2	TXNRD2	UP:Q9NNW7	56,507	39	41	Y40-p
UGGT1	UGCGL1	UP:Q9NYU2	177,190	1515	1517	Y1516-p
SCIMP	UNQ5783	UP:Q6UWF3	16,618	130	132	Y131-p
WDR48	WDR48	UP:Q8TAF3	76,210	337	339	Y338-p
YTHDC1	YT521	UP:Q96MU7	84,700	263	265	Y264-p
YTHDC1	YT521	UP:Q96MU7	84,700	539	541	Y540-p
YTHDC1	YT521	UP:Q96MU7	84,700	655	657	Y656-p
YLPM1	ZAP3	UP:P49750	219,985	1532	1534	Y1533-p
YLPM1	ZAP3	UP:P49750	219,985	1891	1893	Y1892-p
ZC3H18	ZC3H18	UP:Q86VM9	106,378	129	131	Y130-p
ZC3H4	ZC3H4	UP:Q9UPT8	140,257	216	218	Y217-p
ZC3H6	ZC3H6	UP:P61129	131,670	105	107	Y106-p
ZC3H8	ZC3H8	UP:Q8N5P1	33,576	72	74	Y73-p
ZG16B	ZG16B	UP:Q96DA0	22,739	69	71	Y70-p
ZMAT2	ZMAT2	UP:Q96NC0	23,612	139	141	Y140-p
ZNRF3	ZNRF3	UP:Q9ULT6	100,574	593	595	Y594-p
TJP2	ZO2	UP:Q9UDY2	133,958	425	427	Y426-p
TJP2	ZO2 iso2	UP:Q9UDY2-2	117,758	425	427	Y426-p

## Synthetic Methods

### General: Synthesis and Characterization

All reagents and solvents were purchased from Sigma–Aldrich. Silica gel chromatography was performed with Silica Gel 60 (particle size 40–63  $\mu\text{m}$ ) obtained from EMD. Thin layer chromatography (TLC) plates were obtained from EMD. Care was taken to minimize exposure of compounds to light during synthesis, storage and testing. Molecular sieves were activated by heating to 125  $^{\circ}\text{C}$  under vacuum overnight. NMR spectra were recorded on a Bruker Avance III spectrometer at 23  $^{\circ}\text{C}$ , operating at 400 MHz for  $^1\text{H}$  NMR and 100 MHz for  $^{13}\text{C}$  NMR spectroscopy in either  $\text{CDCl}_3$ ,  $\text{CD}_3\text{CN}$  or  $\text{CD}_3\text{OD}$ . Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) referenced to residual isotopic solvent. Coupling constants (J) are reported in Hertz (Hz). High

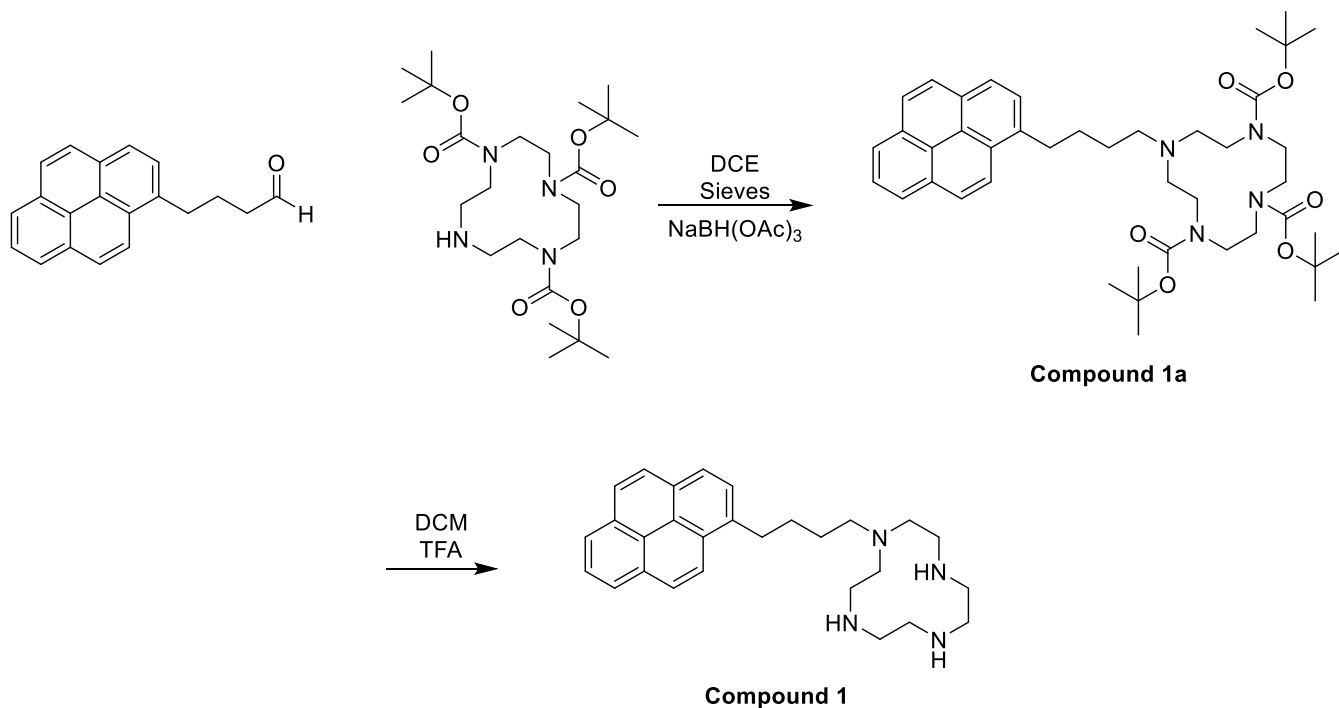
## Supplementary Information

Resolution Mass Spectrometry (HRMS) was performed on an AB/Sciex QStar mass spectrometer with an ESI source, MS/MS and accurate mass capabilities, associated with an Agilent 1100 capillary LC system. Low Resolution Mass Spectrometry (LRMS) was performed on a Waters Micromass ZQ model MM1. Purifications by prep-HPLC were performed using Atlantis Prep T3 10  $\mu\text{m}$  C18 (2) 250 x 19 mm column run at 20 mL/min (preparative) using gradient mixtures of water with 0.1% TFA and 10:1 acetonitrile/water with 0.1% TFA. The crude mixture was injected as a solution 4:1 0.1% TFA in water / acetonitrile. Analysis by rpHPLC was performed using a Phenomex Luna 5  $\mu\text{m}$  C18 (2) 150 x 4.60 mm column run at 1.2 mL/min (analytical) using gradient mixtures of 0.1% TFA in water and acetonitrile. Condition (A) started with 0.1% TFA water with a gradient going to 100% acetonitrile over 30 min, followed by 5 min at 100% acetonitrile. Condition (B) started with 0.1% TFA in water with a gradient going to 100% acetonitrile over 50 min, followed by 5 min at 100% acetonitrile. All final metalated compounds and unmetalated controls were lyophilized from water/acetonitrile after purification by chromatography prior to testing.

The POC sensor was synthesized by methods described previously.<sup>1</sup>

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<sup>1</sup> D. Kraskouskaya, M. Bancarz, H. S. Soor, J. E. Gardiner and P. T. Gunning, *J. Am. Chem. Soc.*, 2014, **136**, 1234–1237.

**Synthesis of compound 1****Compound 1a****tri-*tert*-butyl 10-(4-(pyren-1-yl)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate**

To a solution of 4-(pyren-1-yl)butanal (96 mg, 0.35 mmol) in 10mL 1,2-dichloroethane (DCE), Boc<sub>3</sub>Cyclen (167mg, 0.35 mmol) was added and stirred together with 4Å molecular sieves for 2 h under N<sub>2</sub> atmosphere. To this solution sodium triacetoxyborohydride (90 mg, 0.42 mmol) was added and the reaction mixture was allowed to stir at ambient temperature over 24 h under N<sub>2</sub> atmosphere. Subsequently, the reaction mixture was extracted with ethyl acetate (EtOAc) and washed three times with sodium bicarbonate. The extract was purified by flash chromatography with ethyl acetate/hexanes (1:1) to give tri-*tert*-butyl 10-(4-(pyren-1-yl)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (compound **1a**) as a white solid (212 mg, 83%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.27 (d, *J* = 9.3 Hz, 1H), 8.17 (dd, *J* = 7.6, 4.1 Hz, 2H), 8.11 (d, *J* = 8.5 Hz, 2H), 8.03 (s, 2H), 8.00 (t, *J* = 8 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 3.63–3.12 (m, 12H), 2.77–2.49 (m, 6H), 2.17 (s, 1H), 2.06 (s, 1H), 1.90–1.78 (m, 2H), 1.71–1.60 (m, 2H), 1.50 (s, 9H), 1.47 (s, 18H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 136.6, 131.4, 130.9, 129.8, 128.6, 127.5, 127.2, 127.2, 126.6, 125.8, 125.1, 125.0, 124.9, 124.7, 123.3, 79.4, 79.2, 60.4, 55.0,

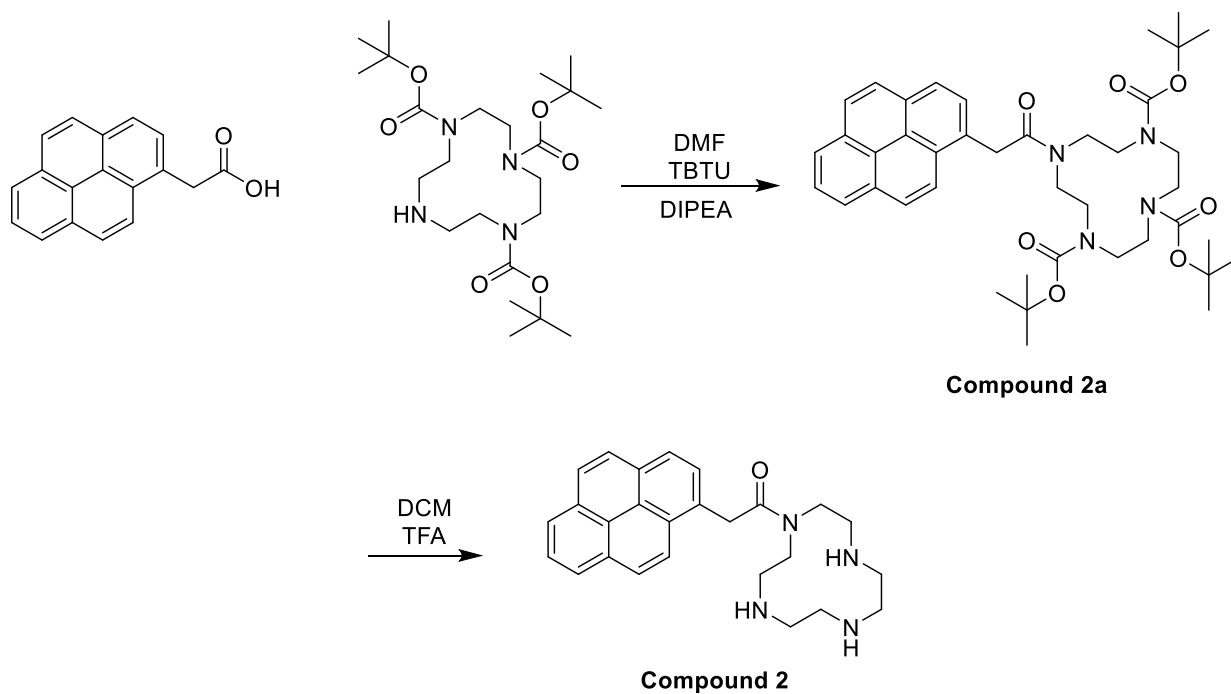
## Supplementary Information

53.7, 52.6, 50.0, 48.0, 33.5, 29.9, 29.7, 28.7, 21.0, 14.2; LRMS (ESI+)  $m/z$  calc'd for  $C_{43}H_{60}N_4O_6Na$  ( $[M+Na]^+$ ) 751.44, found 751.57.

### Compound 1

#### 1-(4-(pyren-1-yl)butyl)-1,4,7,10-tetraazacyclododecane

To a solution of compound **1a** (100 mg, 0.14 mmol) in 15 mL DCM, 1 mL TFA was added. The reaction mixture was stirred at rt. The progress of the reaction was monitored using MS. The reaction mixture was concentrated down *in vacuo* and the TFA was azeotroped off *in vacuo* with MeOH. The crude product was taken up in MeOH and passed through a column packed with Amberlite IRN-78. The solvent was evaporated *in vacuo*. The mixture was then purified by preparative HPLC. The product was again passed through a column packed with Amberlite IRN-78 to give 1-(4-(pyren-1-yl)butyl)-1,4,7,10-tetraazacyclododecane (compound **1**) as an oil (54 mg, 90%);  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.27 (d,  $J = 9.3$  Hz, 1H), 8.16-8.11 (m, 2H), 8.10-8.06 (m, 2H), 8.03-7.94 (m, 3H), 7.85 (d,  $J = 7.8$  Hz, 1H), 3.33 (t,  $J = 7.8$  Hz, 2H), 2.69-2.64 (m, 4H), 2.61-2.56 (m, 4H), 2.53-2.44 (m, 10H), 1.87 (quint,  $J = 7.8$  Hz, 2H), 1.66 (quint,  $J = 7.5$  Hz, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  136.9, 131.3, 130.8, 129.6, 128.4, 127.4, 127.1, 126.9, 126.3, 125.6, 124.91, 124.88, 124.65, 124.63, 124.5, 123.4, 54.4, 51.5, 47.0, 45.8, 45.2, 33.4, 29.7, 27.5; LRMS (ESI+)  $m/z$  calc'd for  $C_{28}H_{36}N_4$   $[M + H]^+$  428.29, found 429.17; HRMS, (ESI+)  $m/z$  calc'd for  $C_{28}H_{37}N_4$   $[M + H]^+$  429.3013, found 429.3021; rpHPLC  $t_R$ : condition (A) 12.949 min., condition (B) 20.751 min., purity 99.6% and 99.2% respectively.

**Synthesis of compound 2****Compound 2a****tri-tert-butyl 10-(2-(pyren-1-yl)acetyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate**

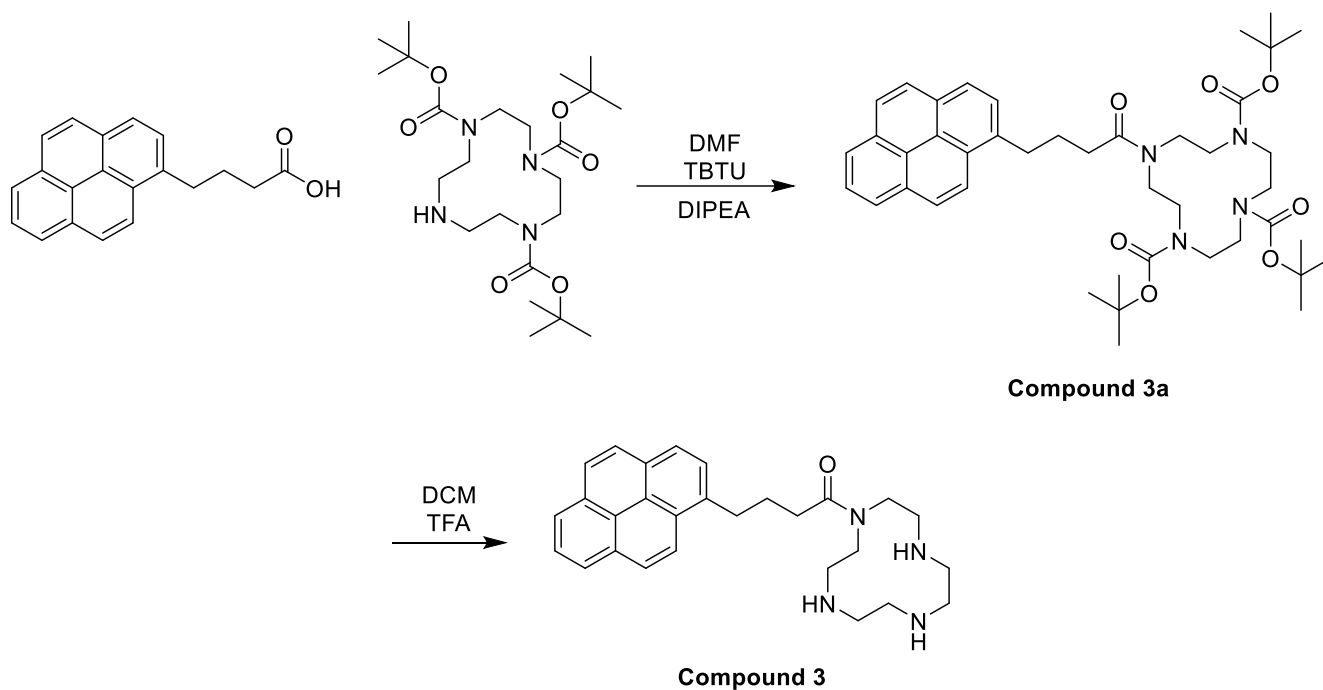
To a solution of 1-Pyreneacetic acid (100 mg, 0.38 mmol) in 3.8mL DMF was added Boc<sub>3</sub>Cyclen (179 mg, 0.38 mmol) and TBTU (297 mg, 0.77 mmol) and the reaction mixture was stirred for 20 min. DIPEA (196 μL, 1.14 mmol) was then added to this reaction mixture and stirred at rt for 16 h. Subsequently, this was extracted using sodium bicarbonate. The extract was purified by flash chromatography with 30-40% ethyl acetate/hexanes to give tri-tert-butyl 10-(2-(pyren-1-yl)acetyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (compound **2a**) (217 mg, 80%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.25 (d, *J* = 8.9 Hz, 1H), 8.19 – 8.11 (m, 4H), 8.03 (s, 2H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 4.43 (s, 2H), 3.87 – 3.23 (m, 16H), 1.56 -1.43 (m, 27H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.2, 155.6, 131.3, 130.9, 130.6, 129.7, 129.0, 127.7, 127.5, 127.4, 127.1, 125.9, 125.1, 124.9, 124.9, 124.8, 123.5, 80.5, 80.3, 60.4, 51.5, 50.6, 50.1, 49.90, 49.7, 28.5, 21.1, 14.2; LRMS (ESI<sup>+</sup>) *m/z* calc'd for C<sub>41</sub>H<sub>54</sub>N<sub>4</sub>O<sub>7</sub>Na [M+Na]<sup>+</sup> 737.39, found 737.53.

## Compound 2

### 2-(pyren-1-yl)-1-(1,4,7,10-tetraazacyclododecan-1-yl)ethanone

To a solution of compound **2a** (106 mg, 0.15 mmol) in 10 mL DCM, 5 mL TFA was added. The reaction mixture was stirred at rt. The progress of the reaction was monitored using MS. The reaction mixture was concentrated down *in vacuo* and the TFA was azeotroped off *in vacuo* with MeOH. The crude product was taken up in MeOH and passed through a column packed with Amberlite IRN-78. The solvent was evaporated *in vacuo*. The mixture was then purified by preparative HPLC. The product was again passed through a column packed with Amberlite IRN-78. This was lyophilized with water/ACN to give 1-(1,4,7,10-tetraazacyclododecan-1-yl)-2-(pyren-1-yl)ethan-1-one (compound **2**) as an off white powder (45 mg, 72%); mp 75-79 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.23 (d, *J* = 9.2 Hz, 1H), 8.18-8.06 (m, 4H), 8.04-7.94 (m, 3H), 7.84 (d, *J* = 7.8 Hz, 1H), 4.47 (s, 2H), 3.60-3.54 (br, 4H), 3.39 (s, 1H), 2.88 (s, 1H), 2.84 (s, 1H), 2.79-2.72 (m, 4H), 2.72-2.65 (m, 2H), 2.62-2.52 (m, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.0, 131.3, 130.8, 130.5, 129.8, 129.5, 127.8, 127.7, 127.4, 127.1, 125.9, 125.2, 125.0, 124.8, 123.4, 50.3, 48.8, 48.5, 47.7, 47.3, 47.1, 46.5, 43.7, 39.5, 29.7; LRMS (ESI+) *m/z* calc'd for C<sub>26</sub>H<sub>31</sub>N<sub>4</sub>O [M + H]<sup>+</sup> 415.25, found 416.27; HRMS (ESI+) *m/z* calc'd for C<sub>26</sub>H<sub>31</sub>N<sub>4</sub>O [M + H]<sup>+</sup> 415.2498, found 415.2502; rpHPLC *t*<sub>R</sub>: condition (A) 12.241 min., condition (B) 17.924 min., purity 100.0% and 97.887% respectively.



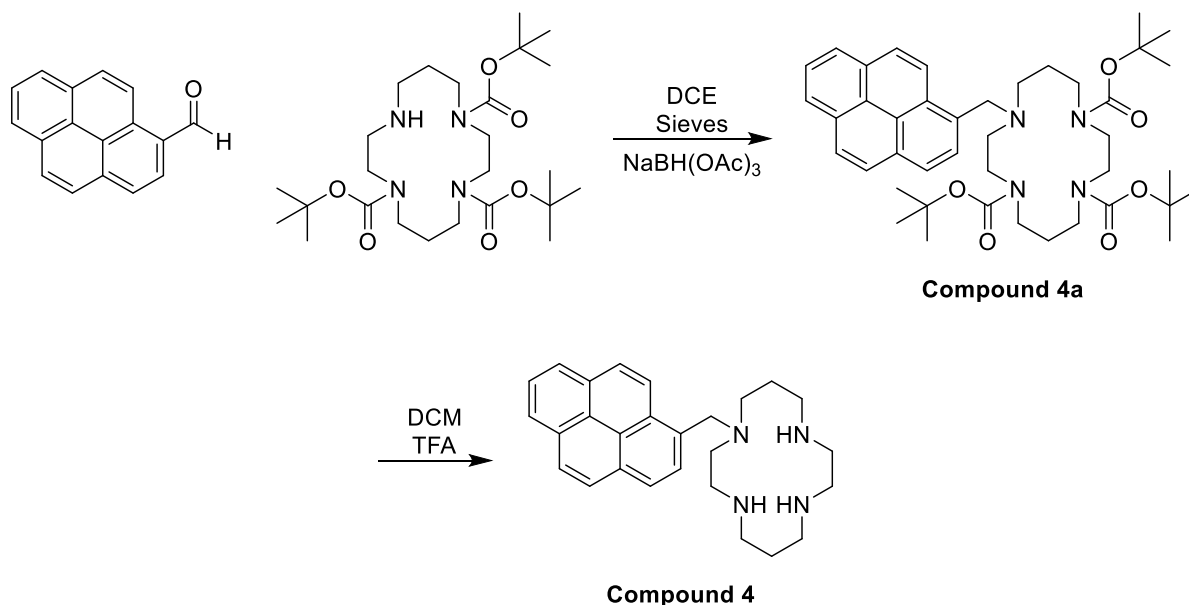
**Synthesis of compound 3****Compound 3a****tri-tert-butyl 10-(4-(pyren-1-yl)butanoyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate**

To a solution of 1-Pyrenebutyric acid (48.8 mg, 0.169 mmol) in 1 mL DMF was added Boc<sub>3</sub>Cyclen (80 mg, 0.169 mmol) and TBTU (87 mg, 0.271 mmol) and the reaction mixture was stirred for 30 min at rt. DIPEA (103 μL, 0.592 mmol) was then added to this reaction mixture and stirred at rt for 16 h. Subsequently, the mixture was diluted with water and extracted with dichloromethane. The combined organic phases were dried over sodium sulfate and concentrated. The crude product was purified by flash chromatography with 30% ethyl acetate/hexanes to give tri-tert-butyl 10-(4-(pyren-1-yl)butanoyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (compound **3a**) (93 mg, 74%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.35 (d, J = 9.2 Hz, 1H), 8.19–8.09 (m, 4H), 8.01 (s, 2H), 7.98 (t, J = 7.6 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 3.80–3.19 (m, 16H), 2.50 (bs, 2H), 2.24 (p, J = 7.0 Hz, 2H), 2.05 (s, 2H), 1.48 (s, 9H), 1.42 (s, 18H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.1, 155.4, 136.1, 131.4, 130.9, 129.9, 128.8, 127.5, 127.3, 127.2, 126.6, 125.8, 125.1, 125.0, 124.8, 124.8, 124.7, 123.5, 80.3, 80.3, 80.1, 60.4, 51.4, 49.7, 33.1, 28.5, 28.4, 27.1, 21.0, 14.2; LRMS (ESI<sup>+</sup>) m/z calc'd for C<sub>43</sub>H<sub>58</sub>N<sub>4</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup> 751.42, found 751.42.

### Compound 3

#### 4-(pyren-1-yl)-1-(1,4,7,10-tetraazacyclododecan-1-yl)butan-1-one

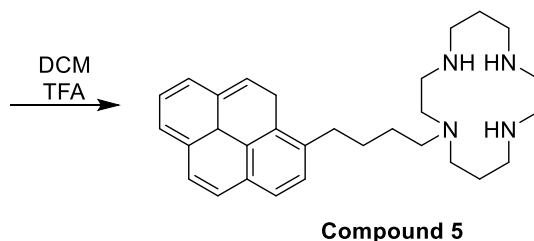
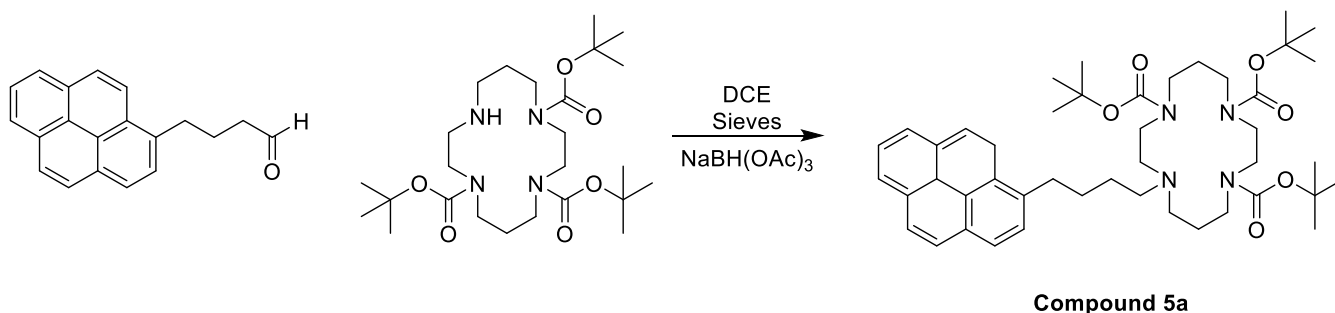
To a solution of compound **3a** (102 mg, 0.14 mmol) in 10 mL DCM, 5 mL TFA was added. The reaction mixture was stirred at rt. The progress of the reaction was monitored using MS. The reaction mixture was concentrated down *in vacuo* and the TFA was azeotroped off *in vacuo* with MeOH. The crude product was taken up in MeOH and passed through a column packed with Amberlite IRN-78. The solvent was evaporated *in vacuo*. The mixture was then purified by preparative HPLC. The product was again passed through a column packed with Amberlite IRN-78 to give 1-(1,4,7,10-tetraazacyclododecan-1-yl)-4-(pyren-1-yl)butan-1-one (compound **3**) (49 mg, 79%); mp 83-86 °C; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 8.31 (d, *J* = 9.3 Hz, 1H), 8.15-8.10 (m, 2H), 8.09-8.04 (m, 2H), 8.01-7.91 (m, 3H), 7.84 (d, *J* = 7.8 Hz, 1H), 3.60-3.53 (m, 4H), 3.36-3.29 (m, 2H), 3.10 (br, 10H), 3.00 (br, 2H), 2.53 (t, *J* = 7.3 Hz, 2H), 2.08 (quint, *J* = 7.7 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 175.3, 136.1, 131.4, 130.9, 129.9, 128.6, 127.2, 127.1, 127.0, 126.3, 125.7, 124.8, 124.7, 124.6, 124.6, 124.5, 123.2, 48.3, 47.3, 46.7, 44.8, 43.9, 32.3, 32.0, 26.8; LRMS (ESI+) *m/z* calc'd for C<sub>28</sub>H<sub>35</sub>N<sub>4</sub>O [M + H]<sup>+</sup> 443.28, found 444.22; HRMS (ESI+) *m/z* calc'd for C<sub>28</sub>H<sub>35</sub>N<sub>4</sub>O [M + H]<sup>+</sup> 443.2811, found 443.2815; rpHPLC *t<sub>R</sub>*: condition (A) 13.382 min., condition (B) 19.843 min., purity 100.0% and 99.8% respectively.

**Synthesis of compound 4****Compound 4a****tri-*tert*-butyl 11-(pyren-1-ylmethyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate**

To a solution of 1-pyrenecarboxaldehyde (35 mg, 0.15 mmol) in 1 mL DCE, Boc<sub>3</sub>Cyclam (50 mg, 0.10 mmol) was added and stirred together with 4 Å molecular sieves for 2 h under N<sub>2</sub> atmosphere. To this solution sodium triacetoxyborohydride (42 mg, 0.2 mmol) was added and the reaction mixture was allowed to stir at ambient temperature over 24 h under N<sub>2</sub> atmosphere. Subsequently, the reaction mixture was diluted with sodium bicarbonate and extracted with DCM. The extract was purified by flash chromatography with 30% ethyl acetate/hexanes to give tri-*tert*-butyl 11-(pyren-1-ylmethyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (compound **4a**) (63 mg, 88%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.47 (d, J = 9.3 Hz, 1H), 8.21 – 7.93 (m, 8H), 4.22 (s, 2H), 3.34 (s, 10H), 3.13 (s, 2H), 2.78 (s, 2H), 2.50 (s, 2H), 1.88 (s, 2H), 1.76 – 1.66 (m, 2H), 1.51 – 1.17 (m, 27H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 155.55, 132.64, 131.33, 130.87, 130.77, 129.71, 128.41, 127.47, 127.10, 125.83, 125.00, 124.83, 124.50, 124.06, 79.67, 79.44, 58.59, 54.24, 53.43, 47.81, 47.07, 29.72, 28.50, 28.46, 28.31; LRMS (ESI<sup>+</sup>) *m/z* calc'd for C<sub>42</sub>H<sub>58</sub>N<sub>4</sub>O<sub>6</sub>Na [M + Na]<sup>+</sup> 737.42, found 737.65.

**Compound 4****1-(pyren-1-ylmethyl)-1,4,8,11-tetraazacyclotetradecane**

To a solution of compound **4a** (108 mg, 0.15 mmol) in 10 mL DCM, 5 mL TFA was added. The reaction mixture was stirred at rt. The progress of the reaction was monitored using MS. The reaction mixture was concentrated down *in vacuo* and the TFA was azeotroped off *in vacuo* with MeOH. The crude product was taken up in MeOH and passed through a column packed with Amberlite IRN-78. The solvent was evaporated *in vacuo*. The mixture was then purified by preparative HPLC. The product was again passed through a column packed with Amberlite IRN-78 to give 1-(pyren-1-ylmethyl)-1,4,8,11-tetraazacyclotetradecane (compound **4**) (45 mg, 72%); mp 101-104 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.47 (d, *J* = 9.3 Hz, 1H), 8.18-8.05 (m, 5H), 8.02 (s, 2H), 7.97 (t, *J* = 7.6 Hz, 1H), 4.19 (s, 2H), 2.90-2.79 (m, 6H), 2.78-2.74 (m, 2H), 2.70-2.64 (m, 6H), 2.62-2.57 (m, 2H), 2.56-2.48 (m, 4H), 1.87 (quint, *J* = 5.3 Hz, 2H), 1.60 (quint, *J* = 5.3 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 132.8, 131.2, 130.6, 130.4, 129.5, 128.0, 127.3, 127.0, 126.9, 125.7, 124.9, 124.8, 124.74, 124.72, 124.4, 123.5, 56.8, 54.8, 54.1, 50.3, 48.9, 48.6, 48.3, 47.5, 47.4, 27.9, 26.4; LRMS (ESI+) *m/z* calc'd for C<sub>27</sub>H<sub>35</sub>N<sub>4</sub> [M + H]<sup>+</sup> 415.29, found 415.20; HRMS (ESI+) *m/z* calc'd for C<sub>27</sub>H<sub>35</sub>N<sub>4</sub> [M + H]<sup>+</sup> 415.2862, found 415.2854; rpHPLC *t<sub>R</sub>*: condition (A) 12.499 min., condition (B) 19.601 min., purity 99.8% and 96.1% respectively.

**Synthesis of compound 5**

## Compound 5a

### tri-*tert*-butyl 11-(4-(pyren-1-yl)butyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate

To a solution of 4-(pyren-1-yl)butanal (37 mg, 0.14 mmol) in 0.9 mL DCE, Boc<sub>3</sub>Cyclam (45 mg, 0.09 mmol) was added and stirred together with 4Å molecular sieves for 2 h under N<sub>2</sub> atmosphere. To this solution sodium triacetoxyborohydride (38 mg, 0.18 mmol) was added and the reaction mixture was allowed to stir at ambient temperature over 24 h under N<sub>2</sub> atmosphere. Subsequently, the reaction mixture was diluted with sodium bicarbonate and extracted with DCM. The extract was purified by flash chromatography with 35% ethyl acetate/hexanes to give tri-*tert*-butyl 11-(4-(pyren-1-yl)butyl)-1,4,8,11-tetraazacyclotetradecane-1,4,8-tricarboxylate (compound **5a**) (43 mg, 63%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.29 (d, *J* = 9.3 Hz, 1H), 8.21 – 7.98 (m, 7H), 7.88 (d, *J* = 7.8 Hz, 1H), 3.46 – 3.09 (m, 14H), 2.59 (s, 2H), 2.50 – 2.33 (m, 4H), 1.93 – 1.74 (m, 4H), 1.73 – 1.58 (m, 4H), 1.53 – 1.41 (m, 27H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 155.69, 136.80, 131.44, 130.91, 129.80, 128.58, 127.52, 127.24, 127.20, 126.57, 125.81, 125.11, 125.05, 124.86, 124.81, 124.67, 123.40, 79.55, 79.36, 55.42, 48.69, 48.55, 47.30, 46.90, 46.62, 45.69, 33.54, 29.85, 29.72, 28.56, 28.50, 26.79; LRMS (ESI+) *m/z* calc'd for C<sub>45</sub>H<sub>65</sub>N<sub>4</sub>O<sub>6</sub> [M + H]<sup>+</sup> 757.49, found 757.69.

## Compound 5

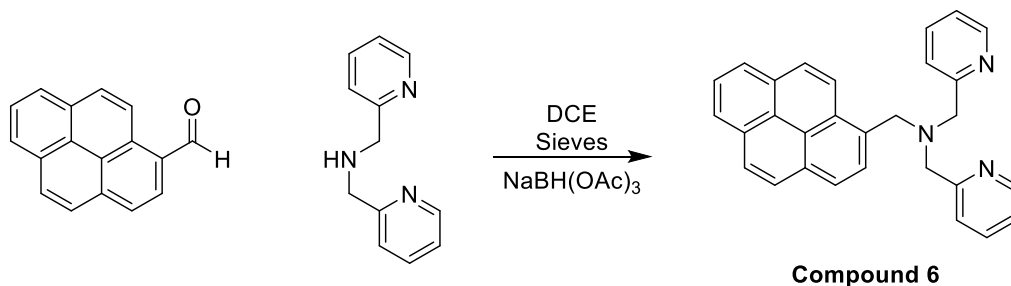
### 1-(4-(pyren-1-yl)butyl)-1,4,8,11-tetraazacyclotetradecane

To a solution compound **5a** (79 mg, 0.1 mmol) in 15 mL DCM, 1mL TFA was added. The reaction mixture was stirred at rt. The progress of the reaction was monitored using MS. The reaction mixture was concentrated down *in vacuo* and the TFA was azeotroped off *in vacuo* with MeOH. The crude product was taken up in MeOH and passed through a column packed with Amberlite IRN-78. The solvent was evaporated *in vacuo*. The mixture was then purified by preparative HPLC. The product was again passed through a column packed with Amberlite IRN-78 to give 1-(4-(pyren-1-yl)butyl)-1,4,8,11-tetraazacyclotetradecane (compound **5**) (38 mg, 80%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.30 (d, *J* = 9.3 Hz, 1H), 8.17-8.08 (m, 4H), 8.04-7.95 (m, 3H), 7.88 (d, *J* = 7.8 Hz, 1H), 3.35 (t, *J* = 7.5 Hz, 2H), 2.62 (t, *J* = 5.3 Hz, 2H), 2.59-2.54 (m, 4H), 2.51-2.47 (m, 2H), 2.46-2.41 (m, 4H), 2.40-2.33 (m, 4H), 2.24-2.20 (m, 2H), 1.85 (quint, *J* = 8.1Hz, 2H), 1.73-1.66 (m, 2H), 1.65-1.59 (m, 2H), 1.59-1.51 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 137.1, 131.3, 130.8, 129.6, 128.4, 127.3, 127.2, 127.1, 126.4, 125.6, 124.9,

## Supplementary Information

124.67, 124.65, 124.5, 123.4, 54.6, 54.2, 52.6, 51.2, 49.8, 49.3, 48.5, 47.7, 47.6, 33.4, 30.0, 28.6, 26.4, 26.1; LRMS (ESI+)  $m/z$  calc'd for  $C_{30}H_{40}N_4$   $[M + H]^+$  456.33, found 457.32. rpHPLC  $t_R$ : condition (A) 13.550 min., condition (B) 21.547 min., purity 99.0% and 98.9% respectively.

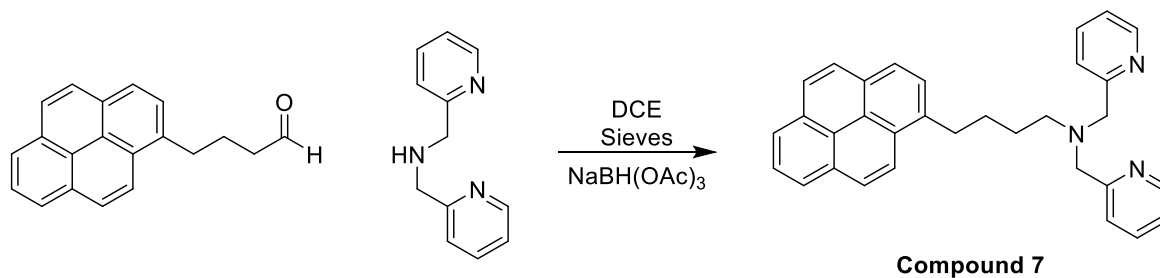
### Synthesis of compound 6



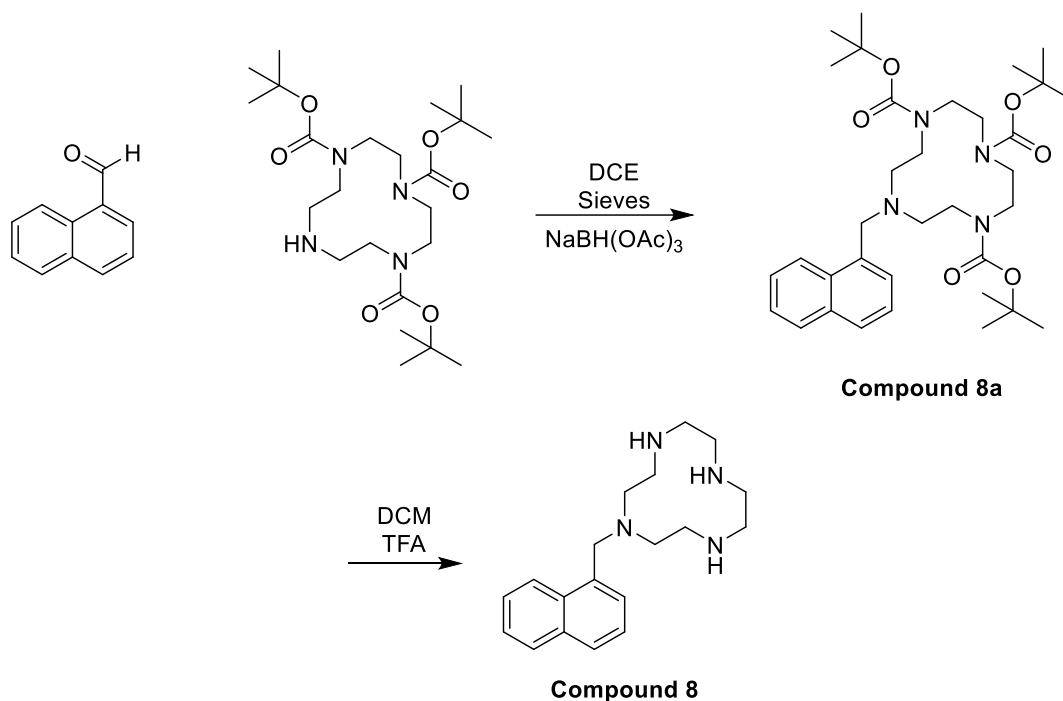
### Compound 6

#### 1-(pyren-1-yl)-*N,N*-bis(pyridin-2-ylmethyl)methanamine

To a solution of 1-Pyrenealdehyde (200 mg, 0.87 mmol) in 4.35 mL DCE, Di-(2-picolyl)amine (DPA) (137.4  $\mu$ L, 0.87 mmol) was added along with sodium triacetoxyborohydride (553.2 mg, 2.61 mmol). To this reaction mixture, 4 Å molecular sieves were added. The mixture was left to stir at rt overnight. The mixture was extracted with DCM/sodium bicarbonate. The extract was purified by flash chromatography (96% DCM, 3.5% MeOH, 0.5%  $NH_4OH$ ) to give 1-(pyren-1-yl)-*N,N*-bis(pyridin-2-ylmethyl)methanamine (compound 6) (291 mg, 81%); mp 108-112 °C;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.53 (d,  $J = 4.7$  Hz, 2H), 8.40 (d,  $J = 9.3$  Hz, 1H), 8.20-8.06 (m, 5H), 8.05-7.97 (m, 3H), 7.61 (t,  $J = 7.6$  Hz, 2H), 7.49 (d,  $J = 7.8$  Hz, 2H), 7.12 (t,  $J = 6.3$  Hz, 2H), 4.47 (s, 2H), 3.99 (s, 4H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  148.5, 136.5, 131.1, 130.73, 130.67, 129.8, 128.3, 127.3, 127.1, 127.0, 125.7, 124.90, 124.86, 124.6, 124.4, 123.9, 123.4, 122.0, 77.1, 60.1, 57.0; LRMS (ESI+)  $m/z$  calc'd for  $C_{29}H_{24}N_3$   $[M + H]^+$  414.20, found 414.24; HRMS (ESI+)  $m/z$  calc'd for  $C_{29}H_{24}N_3$   $[M + H]^+$  414.1970, found 414.1981; rpHPLC  $t_R$ : condition (A) 13.531 min., condition (B) 19.731 min., purity 98.8% and 97.5% respectively.

**Synthesis of compound 7****Compound 7****4-(pyren-1-yl)-*N,N*-bis(pyridin-2-ylmethyl)butan-1-amine**

To a solution of 4-(pyren-1-yl)butanal (143 mg, 0.526 mmol) in 2.6 mL DCE, DPA (95  $\mu$ L, 0.526 mmol) was added along with sodium triacetoxyborohydride (334 mg, 1.578 mmol). To this reaction mixture, 4 Å molecular sieves were added. The mixture was left to stir at rt overnight. The mixture was extracted with DCM/sodium bicarbonate. The extract was purified by flash chromatography (96% DCM, 3.5% MeOH, 0.5%  $\text{NH}_4\text{OH}$ ) to give 4-(pyren-1-yl)-*N,N*-bis(pyridin-2-ylmethyl)butan-1-amine (compound **7**) (189 mg, 79%); mp 61-62 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.52 (d,  $J = 4.8$  Hz, 2H), 8.19 (d,  $J = 9.5$  Hz, 1H), 8.15-8.10 (m, 2H), 8.07-8.02 (m, 2H), 8.01-7.94 (m, 3H), 7.76 (d,  $J = 7.8$  Hz, 1H), 7.53 (td,  $J = 7.8$  Hz, 1.3 Hz, 2H), 7.46 (d,  $J = 7.8$  Hz, 2H), 7.06 (t,  $J = 6.2$  Hz, 2H), 3.83 (s, 4H), 3.24 (t,  $J = 7.4$  Hz, 2H), 2.65 (t,  $J = 7.1$  Hz, 2H), 1.84 (quint,  $J = 7.5$  Hz, 2H), 1.71 (quint,  $J = 7.5$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  159.9, 148.8, 136.7, 136.2, 131.3, 130.8, 129.6, 128.4, 127.4, 127.1, 127.0, 126.4, 125.6, 124.94, 124.91, 124.7, 124.6, 124.5, 123.3, 122.8, 121.7, 60.5, 54.2, 33.1, 29.3, 27.0; LRMS (ESI+)  $m/z$  calc'd for  $\text{C}_{32}\text{H}_{30}\text{N}_3$   $[\text{M} + \text{H}]^+$  456.24, found 456.31; HRMS (ESI+)  $m/z$  calc'd for  $\text{C}_{32}\text{H}_{30}\text{N}_3$   $[\text{M} + \text{H}]^+$  456.2440, found 456.2445; rpHPLC  $t_R$ : condition (A) 17.561 min., condition (B) 26.221 min., purity 98.8% and 98.9% respectively.

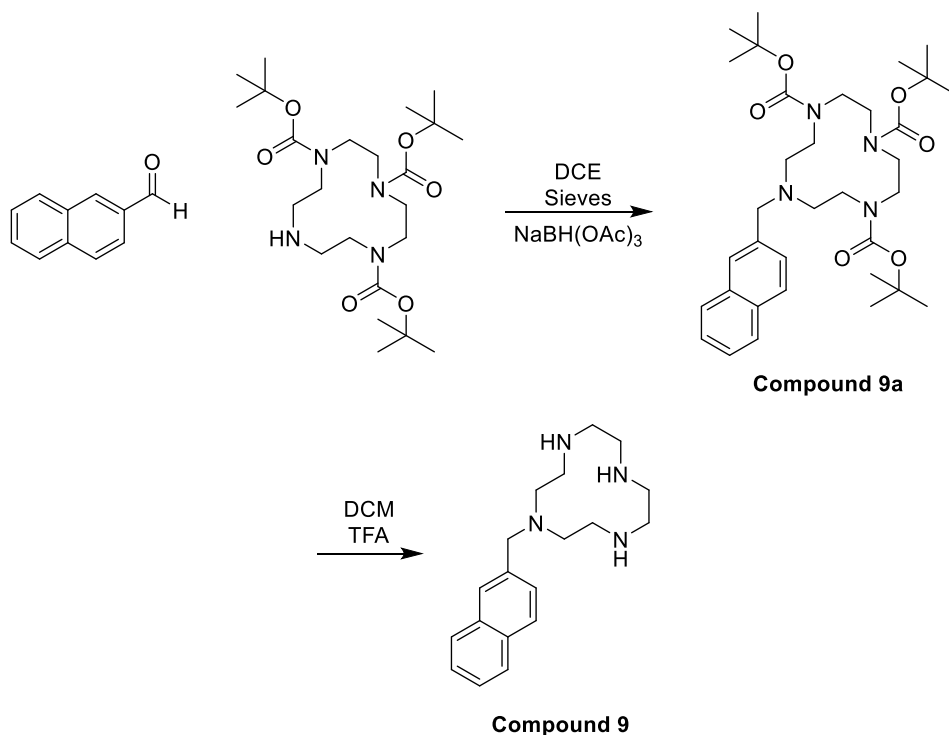
**Synthesis of compound 8****Compound 8a****tri-tert-butyl 10-(naphthalen-1-ylmethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate**

To a solution of Boc<sub>3</sub>Cyclen (100 mg, 0.21 mmol) in 2mL DCE, 1-Naphthaldehyde (29  $\mu$ L, 0.31 mmol) was added. To this reaction mixture, 4 Å molecular sieves were added. The reaction was left to stir for 2 h, after which sodium triacetoxyborohydride was added (66 mg, 0.31 mmol) and the reaction was allowed to stir for 24 h. Subsequently, the mixture was extracted with DCM/sodium bicarbonate. The organic extract was purified by flash chromatography with 30% ethyl acetate/hexanes to give the tri-tert-butyl 10-(naphthalen-1-ylmethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate as a white solid (compound **8a**) (109 mg, 85%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.19 (d,  $J$  = 6.8 Hz, 1H), 7.82 (d,  $J$  = 8.3 Hz, 1H), 7.75 (d,  $J$  = 8.2 Hz, 1H), 7.51 – 7.42 (m, 3H), 7.39 (t,  $J$  = 7.5 Hz, 1H), 4.12 (s, 2H), 3.57 – 2.60 (m, 16H), 1.49 – 1.29 (m, 27H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  156.30, 155.79, 155.45, 134.02, 133.73, 132.58, 128.57, 128.08, 125.91, 125.64, 125.24, 124.28, 57.11, 49.55, 48.42, 48.13, 28.67, 28.44; LRMS (ESI+)  $m/z$  calc'd for C<sub>34</sub>H<sub>53</sub>N<sub>4</sub>O<sub>6</sub> [M + H]<sup>+</sup> 613.40, found 613.39.



**Compound 8****1-(naphthalen-1-ylmethyl)-1,4,7,10-tetraazacyclododecane**

To a solution of compound **8a** (105 mg, 0.17 mmol) in 10 mL DCM, 5 mL TFA was added. The reaction mixture was stirred at rt. The progress of the reaction was monitored using MS. The reaction mixture was concentrated down *in vacuo* and the TFA was azeotroped off *in vacuo* with MeOH. The crude product was taken up in MeOH and passed through a column packed with Amberlite IRN-78. The solvent was evaporated *in vacuo*. The mixture was then purified by preparative HPLC. The product was again passed through a column packed with Amberlite IRN-78 to give 1-(naphthalen-1-ylmethyl)-1,4,7,10-tetraazacyclododecane as an oil (compound **8**) (45mg, 89%);  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.92 (d,  $J = 8.5$  Hz, 1H), 7.90 – 7.86 (m, 2H), 7.85 (s, 1H), 7.54 – 7.48 (m, 3H), 4.02 (s, 2H), 3.29 – 3.12 (m, 8H), 3.04 – 2.87 (m, 8H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  134.3, 132.0, 131.6, 129.0, 128.9, 128.6, 126.6, 125.8, 125.3, 122.5, 54.7, 48.8, 44.2, 42.1, 41.8; LRMS (ESI+)  $m/z$  calc'd for  $\text{C}_{19}\text{H}_{28}\text{N}_4$   $[\text{M} + \text{H}]^+$  312.23, found 313.23; HRMS, (ESI+)  $m/z$  calc'd for  $\text{C}_{19}\text{H}_{29}\text{N}_4$   $[\text{M} + \text{H}]^+$  313.2387, found 313.2380; rpHPLC  $t_{\text{R}}$ : condition (A) 8.675 min., condition (B) 11.866 min.

**Synthesis of compound 9**

## Compound 9a

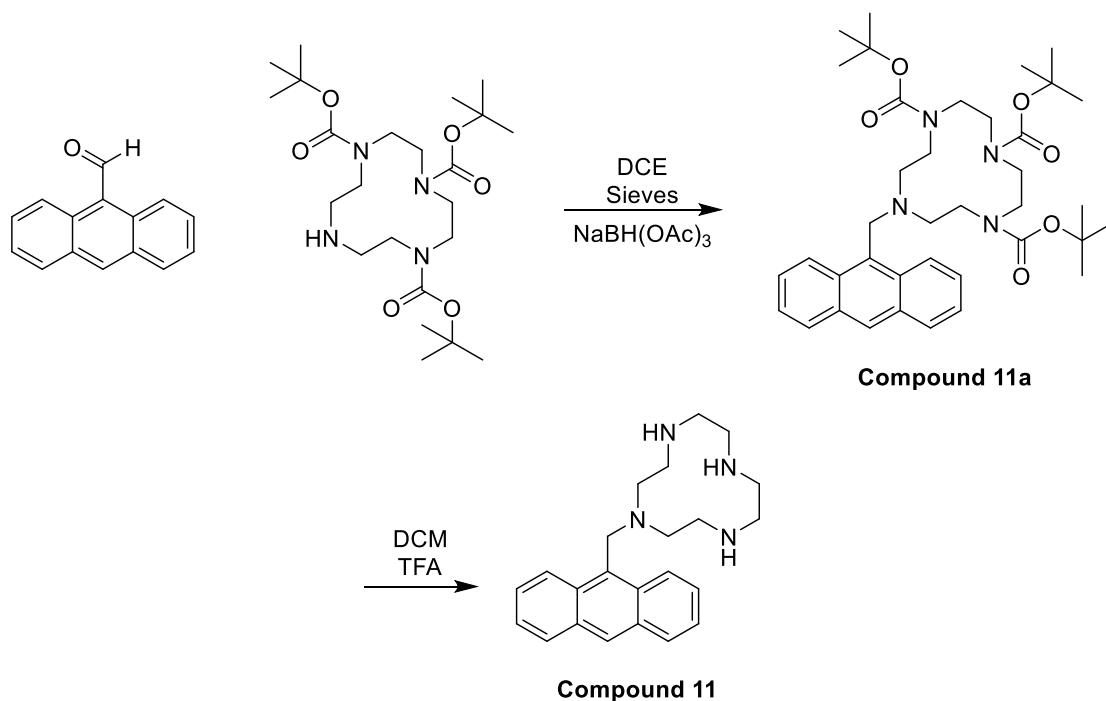
### tri-*tert*-butyl 10-(naphthalen-2-ylmethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate

To a solution of Boc<sub>3</sub>Cyclen (300 mg, 0.64 mmol) in 5 mL DCE, 2-Naphthaldehyde (112 mg, 0.72 mmol) was added. To this reaction mixture, 4 Å molecular sieves were added. The reaction was left to stir for 2 h, after which sodium triacetoxyborohydride was added (270 mg, 1.28 mmol). Subsequently, the mixture was extracted with DCM/sodium bicarbonate. The organic extract was purified by flash chromatography with 30% ethyl acetate/hexanes to give the tri-*tert*-butyl 10-(naphthalen-2-ylmethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate as an oil (compound **9a**) (314 mg, 80%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.76 – 7.68 (m, 3H), 7.62 (s, 1H), 7.42 – 7.33 (m, 3H), 3.82 (s, 2H), 3.68-3.11 (m, 16H), 2.65 (s, 3H), 1.44 – 1.32 (m, 27H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 156.16, 155.75, 155.38, 134.65, 133.27, 132.71, 128.89, 128.43, 127.75, 127.58, 126.02, 125.73, 79.46, 79.35, 57.72, 56.18, 55.17, 49.90, 48.29, 47.74, 28.71, 28.45; LRMS (ESI<sup>+</sup>) m/z calc'd for C<sub>34</sub>H<sub>52</sub>N<sub>4</sub>O<sub>6</sub> [M + H]<sup>+</sup> 613.40, found 613.86;

## Compound 9

### 1-(naphthalen-2-ylmethyl)-1,4,7,10-tetraazacyclododecane

To a solution of compound **9a** (101 mg, 0.16 mmol) in 10 mL DCM, 5 mL TFA was added. The reaction mixture was stirred at rt. The progress of the reaction was monitored using MS. The reaction mixture was concentrated down *in vacuo* and the TFA was azeotroped off *in vacuo* with MeOH. The crude product was taken up in MeOH and passed through a column packed with Amberlite IRN-78. The solvent was evaporated *in vacuo*. The mixture was then purified by preparative HPLC. The product was again passed through a column packed with Amberlite IRN-78 to give 1-(naphthalen-2-ylmethyl)-1,4,7,10-tetraazacyclododecane as an oil (compound **9**) (73 mg, 75%); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 7.92 (d, *J* = 8.5 Hz, 1H), 7.90 – 7.86 (m, 2H), 7.86 - 7.84 (br, 1H), 7.54 – 7.49 (m, 3H), 4.02 (s, 2H), 3.27 – 3.10 (m, 8H), 3.05 – 2.86 (m, 8H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 133.4, 133.1, 132.4, 128.9, 128.4, 127.5, 127.3, 126.9, 126.2, 126.1, 57.0, 47.8, 44.4, 42.0 41.8; LRMS (ESI<sup>+</sup>) m/z calc'd for C<sub>19</sub>H<sub>28</sub>N<sub>4</sub> [M + H]<sup>+</sup> 312.23, found 313.27; HRMS, (ESI<sup>+</sup>) m/z calc'd for C<sub>19</sub>H<sub>29</sub>N<sub>4</sub> [M + H]<sup>+</sup> 313.2387, found 313.2395; rpHPLC t<sub>R</sub>: condition (A) 9.530 min., condition (B) 13.277 min., purity 99.5% and 98.6% respectively.

**Synthesis of compound 10****Compound 10a****tri-tert-butyl 10-(anthracen-9-ylmethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate**

To a solution of Boc<sub>3</sub>Cyclen (150 mg, 0.32 mmol) in 3.2 mL DCE, 9-Anthracenecarboxaldehyde (204 mg, 0.99 mmol) was added. To the reaction mixture, 4 Å molecular sieves were added. The mixture was stirred at rt for 3 h, after which sodium triacetoxyborohydride (271 mg, 1.3 mmol) was added. The reaction was left to stir at rt overnight. Upon reaction completion, the crude mixture was filtered through a coarse porosity sintered glass funnel and the filtrate quenched with water. The aqueous phase was extracted thrice with DCM and the combined organic phase washed with brine. The crude material was purified *via* flash chromatography employing a 5%-40% gradient of ethyl acetate in hexanes to give the tri-tert-butyl 10-(anthracen-9-ylmethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (compound **11a**) as a yellow powder (160 mg, 75%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.47 (d, *J* = 8.8 Hz, 2H), 8.38 (s, 1H), 7.96 (d, *J* = 8.3 Hz, 2H), 7.51 – 7.40 (m, 4H), 4.67 (s, 2H), 3.43 – 2.80 (m, 16H), 1.45 – 1.28 (m, 27H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 155.7, 155.3, 131.23, 131.20, 128.9, 127.7, 125.7, 125.0, 124.8, 79.1, 60.0, 52.2, 49.1, 47.8, 28.5, 28.3; LRMS (ESI<sup>+</sup>) *m/z* calc'd for C<sub>38</sub>H<sub>54</sub>N<sub>4</sub>O<sub>6</sub> [M + H]<sup>+</sup> 663.41, found [M + H]<sup>+</sup> 663.40, found [M + Na]<sup>+</sup> 685.47.

## Compound 10

### 1-(anthracen-9-ylmethyl)-1,4,7,10-tetraazacyclododecane

To a solution of compound **11a** (120 mg, 0.19 mmol) in 3 mL DCM was added 1 mL TFA. The reaction mixture was stirred at -10°C and the progress of the reaction was monitored using HPLC. Upon completion, the crude mixture was concentrated down *in vacuo* using MeOH to azeotrope off TFA. The crude mixture was then purified by preparative HPLC to afford 1-(anthracen-9-ylmethyl)-1,4,7,10-tetraazacyclododecane (compound **11**) as a slightly brown oil that solidified upon standing; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN) δ 7.23 (s, 1H), 6.98 (d, *J* = 9.1 Hz, 2H), 6.75 (d, 8.6 Hz, 2H), 6.30 (t, *J* = 7.3 Hz, 2H), 6.17 (t, *J* = 7.6 Hz, 2H), 3.47 (s, 2H), 1.77 – 1.71 (m, 4H), 1.68 – 1.60 (m, 8H), 1.59 – 1.50 (m, 4H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>CN) δ 130.1, 129.4, 128.1, 127.5, 125.6, 125.0, 123.5, 121.5, 48.5, 47.7, 42.4, 40.4, 40.2; LRMS (ESI+) *m/z* calc'd for C<sub>23</sub>H<sub>30</sub>N<sub>4</sub> [M + H]<sup>+</sup> 362.25, found 363.34; HRMS, (ESI+) *m/z* calc'd for C<sub>23</sub>H<sub>31</sub>N<sub>4</sub> [M + H]<sup>+</sup> 363.2543, found 363.2533; rpHPLC *t<sub>R</sub>*: condition (A) 10.287 min., condition (B) 14.659 min., purity 99.1% and 99.0% respectively.

## Peptide Synthesis

The synthesis of the peptides was achieved using 9-fluorenylmethyl carbamate (Fmoc) solid phase peptide synthesis (Fmoc-SPPS) strategy on the respective resins.

### Peptide 1, Ac-pYAAAAPY-NH<sub>2</sub>

Initial Fmoc deprotection of Rink amide MBHA resin (0.200 g, 0.136 mmol, resin capacity 0.678 mmol g<sup>-1</sup>) was carried out by treatment with piperidine in dimethylformamide (DMF) (1:4, v/v; 2 × 5 min). The resin was drained, then washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). A solution of Fmoc protected amino acid (4 equiv. relative to resin capacity), diisopropylcarbodiimide (DIC) (4 equiv. relative to peptide) and Oxyma (8 equiv. relative to peptide) in DMF (final concentration of 0.1 M) was pre-activated for 4 min. After 4 min of pre-activation, the mixture was added to the resin. Next, the resin was agitated for 2 h at room temperature, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). All Fmoc-protected amino acids were coupled using DIC/Oxyma with the exception of Fmoc-Tyr(HPO<sub>3</sub>Bzl)-OH (4 equiv. relative to resin capacity), which was coupled using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium

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hexafluorophosphate (HBTU) (4 equiv. relative to peptide) and *N,N*-diisopropylethylamine (DIPEA) (12 equiv. relative to peptide) in DMF for 2 h at room temperature. After the removal of the Fmoc protecting group of the final amino acid to be coupled, the resin was treated with acetic anhydride/pyridine (1:4, v/v, 3 × 3 mL × 4 min), followed by washing with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). The resin was washed with CH<sub>2</sub>Cl<sub>2</sub> (5 × 4 mL) and dried *in vacuo*. An acidic cocktail containing trifluoroacetic acid (TFA) TFA/*i*Pr<sub>3</sub>SiH/H<sub>2</sub>O (95:2.5:2.5, v/v/v, 5 mL) was then added to the resin which was gently agitated for 120 min. The resin was subsequently filtered and washed with TFA (4 × 4 mL). The acidic cleavage solution and acid washes were concentrated under reduced pressure to ~1–3 mL. The crude peptide was then purified by preparative reversed-phase high performance liquid chromatography RP-HPLC (0 to 100% MeCN with 0.1% TFA over 60 min). The appropriate fractions were lyophilised, affording the peptide as a white solid (*t*<sub>R</sub> = 6.6 min; λ = 214 nm; 40.6 mg, 36%); Calculated Mass [M+H]<sup>+</sup>: 830.7, [M + Na]<sup>+</sup>: 852.7, Mass Found (ESI) [M+H]<sup>+</sup>: 830.3, [M + Na]<sup>+</sup>: 852.2.

### Peptide 2, Ac-ApTAYA-NH<sub>2</sub>

Initial Fmoc deprotection of Rink amide MBHA resin (0.200 g, 0.136 mmol, resin capacity 0.678 mmol g<sup>-1</sup>) was carried out by treatment with piperidine in dimethylformamide (DMF) (1:4, v/v; 2 × 5 min). The resin was drained, then washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). A solution of Fmoc protected amino acid (4 equiv. relative to resin capacity), diisopropylcarbodiimide (DIC) (4 equiv. relative to peptide) and Oxyma (8 equiv. relative to peptide) in DMF (final concentration of 0.1 M) was pre-activated for 4 min. After 4 min of pre-activation, the mixture was added to the resin. Next, the resin was agitated for 2 h at room temperature, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). All Fmoc-protected amino acids were coupled using DIC/Oxyma with the exception of Fmoc-Thr(HPO<sub>3</sub>Bzl)-OH (4 equiv. relative to resin capacity), which was coupled using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (4 equiv. relative to peptide) and *N,N*-diisopropylethylamine (DIPEA) (12 equiv. relative to peptide) in DMF for 2 h at room temperature. After the removal of the Fmoc protecting group of the final amino acid to be coupled, the resin was treated with acetic anhydride/pyridine (1:4, v/v, 3 × 3 mL × 4 min), followed by washing with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). The resin was washed with CH<sub>2</sub>Cl<sub>2</sub> (5 × 4 mL) and dried *in vacuo*. An acidic cocktail containing trifluoroacetic acid (TFA) TFA/*i*Pr<sub>3</sub>SiH/H<sub>2</sub>O (95:2.5:2.5, v/v/v, 5 mL) was then added to the resin which

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was gently agitated for 120 min. The resin was subsequently filtered and washed with TFA ( $4 \times 4$  mL). The acidic cleavage solution and acid washes were concentrated under reduced pressure to  $\sim 1\text{--}3$  mL. The crude peptide was then purified by preparative reversed-phase high performance liquid chromatography RP-HPLC (0 to 100% MeCN with 0.1% TFA over 60 min). The appropriate fractions were lyophilised, affording the peptide as a white solid ( $t_R = 7.4$  min;  $\lambda = 214$  nm; 34.4 mg, 41%); Calculated Mass  $[M+H]^+$ : 617.6, Mass Found (ESI)  $[M+H]^+$ : 617.2.

### Peptide 3, Ac-ApSpSAA-NH<sub>2</sub>

Initial Fmoc deprotection of Rink amide MBHA resin (0.200 g, 0.136 mmol, resin capacity 0.678 mmol  $\text{g}^{-1}$ ) was carried out by treatment with piperidine in dimethylformamide (DMF) (1:4, v/v;  $2 \times 5$  min). The resin was drained, then washed with DMF ( $5 \times 3$  mL),  $\text{CH}_2\text{Cl}_2$  ( $5 \times 3$  mL) and DMF ( $5 \times 3$  mL). A solution of Fmoc protected amino acid (4 equiv. relative to resin capacity), diisopropylcarbodiimide (DIC) (4 equiv. relative to peptide) and Oxyma (8 equiv. relative to peptide) in DMF (final concentration of 0.1 M) was pre-activated for 4 min. After 4 min of pre-activation, the mixture was added to the resin. Next, the resin was agitated for 2 h at room temperature, the resin was washed with DMF ( $5 \times 3$  mL),  $\text{CH}_2\text{Cl}_2$  ( $5 \times 3$  mL) and DMF ( $5 \times 3$  mL). All Fmoc-protected amino acids were coupled using DIC/Oxyma with the exception of Fmoc-Ser(HPO<sub>3</sub>Bzl)-OH (4 equiv. relative to resin capacity), which was coupled using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (4 equiv. relative to peptide) and *N,N*-diisopropylethylamine (DIPEA) (12 equiv. relative to peptide) in DMF for 2 h at room temperature. After the removal of the Fmoc protecting group of the final amino acid to be coupled, the resin was treated with acetic anhydride/pyridine (1:4, v/v,  $3 \times 3$  mL  $\times 4$  min), followed by washing with DMF ( $5 \times 3$  mL),  $\text{CH}_2\text{Cl}_2$  ( $5 \times 3$  mL) and DMF ( $5 \times 3$  mL). The resin was washed with  $\text{CH}_2\text{Cl}_2$  ( $5 \times 4$  mL) and dried *in vacuo*. An acidic cocktail containing trifluoroacetic acid (TFA) TFA/*i*Pr<sub>3</sub>SiH/H<sub>2</sub>O (95:2.5:2.5, v/v/v, 5 mL) was then added to the resin which was gently agitated for 120 min. The resin was subsequently filtered and washed with TFA ( $4 \times 4$  mL). The acidic cleavage solution and acid washes were concentrated under reduced pressure to  $\sim 1\text{--}3$  mL. The crude peptide was then purified by preparative reversed-phase high performance liquid chromatography RP-HPLC (0 to 100% MeCN with 0.1% TFA over 60 min). The appropriate fractions were lyophilised, affording the peptide as a white solid ( $t_R = 10.5$  min;  $\lambda = 214$  nm; 32.2 mg, 39%); Calculated Mass  $[M-H]^-$ : 605.4, Mass Found (ESI)  $[M-H]^-$ : 605.1.

### Peptide 4, H<sub>2</sub>N-pYDL-OH

Initial Fmoc deprotection of Fmoc-Leu Wang resin (0.340 g, 0.194 mmol, resin capacity 0.570 mmol g<sup>-1</sup>) was carried out by treatment with piperidine in dimethylformamide (DMF) (1:4, v/v; 2 × 5 min). The resin was drained, then washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). A solution of Fmoc protected amino acid (4 equiv. relative to resin capacity), diisopropylcarbodiimide (DIC) (4 equiv. relative to peptide) and Oxyma (8 equiv. relative to peptide) in DMF (final concentration of 0.1 M) was pre-activated for 4 min. After 4 min of pre-activation, the mixture was added to the resin. Next, the resin was agitated for 2 h at room temperature, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). All Fmoc-protected amino acids were coupled using DIC/Oxyma with the exception of Fmoc-Tyr(HPO<sub>3</sub>Bzl)-OH (4 equiv. relative to resin capacity), which was coupled using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (4 equiv. relative to peptide) and *N,N*-diisopropylethylamine (DIPEA) (12 equiv. relative to peptide) in DMF for 2 h at room temperature. After the removal of the Fmoc protecting group of the final amino acid to be coupled, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). The resin was then washed with CH<sub>2</sub>Cl<sub>2</sub> (5 × 4 mL) and dried *in vacuo*. An acidic cocktail containing trifluoroacetic acid (TFA) TFA/*i*Pr<sub>3</sub>SiH/H<sub>2</sub>O (95:2.5:2.5, v/v/v, 5mL) was then added to the resin which was gently agitated for 120 min. The resin was subsequently filtered and washed with TFA (4 × 4 mL). The acidic cleavage solution and acid washes were concentrated under reduced pressure to ~1–3 mL. The crude peptide was then purified by preparative reversed-phase high performance liquid chromatography RP-HPLC (0 to 100% MeCN with 0.1% TFA over 60 min). The appropriate fractions were lyophilised, affording the peptide as a white solid (*t*<sub>R</sub> = 8.6 min; λ = 214 nm; 43.7 mg, 46%); Calculated Mass [M+H]<sup>+</sup>: 490.4, Mass Found (ESI) [M+H]<sup>+</sup>: 490.2.

### Peptide 5, H<sub>2</sub>N-QDpYDL-OH

Initial Fmoc deprotection of Fmoc-Leu Wang resin (0.300 g, 0.171 mmol, resin capacity 0.570 mmol g<sup>-1</sup>) was carried out by treatment with piperidine in dimethylformamide (DMF) (1:4, v/v; 2 × 5 min). The resin was drained, then washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). A solution of Fmoc protected amino acid (4 equiv. relative to resin capacity), diisopropylcarbodiimide (DIC) (4 equiv. relative to peptide) and Oxyma (8 equiv. relative to peptide) in DMF (final concentration of 0.1 M) was pre-activated for 4 min. After 4 min of pre-activation, the mixture was

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added to the resin. Next, the resin was agitated for 2 h at room temperature, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). All Fmoc-protected amino acids were coupled using DIC/Oxyma with the exception of Fmoc-Tyr(HPO<sub>3</sub>Bzl)-OH (4 equiv. relative to resin capacity), which was coupled using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (4 equiv. relative to peptide) and *N,N*-diisopropylethylamine (DIPEA) (12 equiv. relative to peptide) in DMF for 2 h at room temperature. After the removal of the Fmoc protecting group of the final amino acid to be coupled, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). The resin was then washed with CH<sub>2</sub>Cl<sub>2</sub> (5 × 4 mL) and dried *in vacuo*. An acidic cocktail containing trifluoroacetic acid (TFA) TFA/*i*Pr<sub>3</sub>SiH/H<sub>2</sub>O (95:2.5:2.5, v/v/v, 5mL) was then added to the resin which was gently agitated for 120 min. The resin was subsequently filtered and washed with TFA (4 × 4 mL). The acidic cleavage solution and acid washes were concentrated under reduced pressure to ~1–3 mL. The crude peptide was then purified by preparative reversed-phase high performance liquid chromatography RP-HPLC (0 to 100% MeCN with 0.1% TFA over 60 min). The appropriate fractions were lyophilised, affording the peptide as a white solid (*t*<sub>R</sub> = 9.5 min; λ = 214 nm; 41.3 mg, 33%); Calculated Mass [M+H]<sup>+</sup>: 733.6, Mass Found (ESI) [M+H]<sup>+</sup>: 733.3.

### Peptide 6, H<sub>2</sub>N- DQDYDLS-OH

Initial Fmoc deprotection of Fmoc-Ser(*t*Bu) Wang resin (0.301 g, 0.177 mmol, resin capacity 0.589 mmol g<sup>-1</sup>) was carried out by treatment with piperidine in dimethylformamide (DMF) (1:4, v/v; 2 × 5 min). The resin was drained, then washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). A solution of Fmoc protected amino acid (4 equiv. relative to resin capacity), diisopropylcarbodiimide (DIC) (4 equiv. relative to peptide) and Oxyma (8 equiv. relative to peptide) in DMF (final concentration of 0.1 M) was pre-activated for 4 min. After 4 min of pre-activation, the mixture was added to the resin. Next, the resin was agitated for 2 h at room temperature, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). All Fmoc-protected amino acids were coupled using DIC/Oxyma in DMF for 2 h at room temperature. After the removal of the Fmoc protecting group of the final amino acid to be coupled, the resin was washed with DMF (5 × 3 mL), CH<sub>2</sub>Cl<sub>2</sub> (5 × 3 mL) and DMF (5 × 3 mL). The resin was then washed with CH<sub>2</sub>Cl<sub>2</sub> (5 × 4 mL) and dried *in vacuo*. An acidic cocktail containing trifluoroacetic acid (TFA) TFA/*i*Pr<sub>3</sub>SiH/H<sub>2</sub>O (95:2.5:2.5, v/v/v, 5mL) was then added to the resin which was gently agitated for 120 min. The resin was subsequently filtered and washed with TFA (4 × 4 mL). The acidic cleavage solution and acid washes



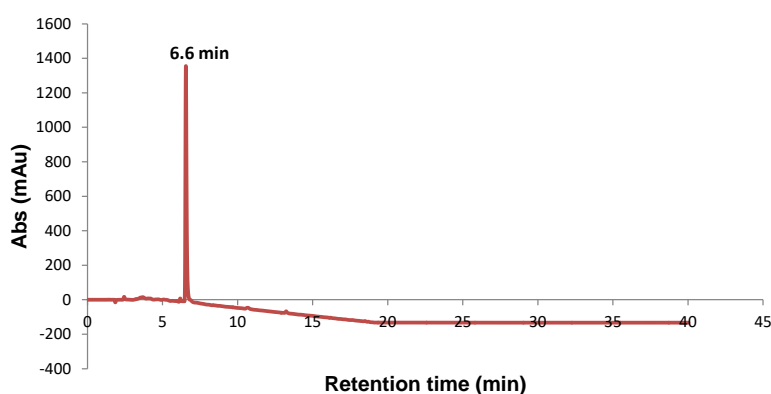
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were concentrated under reduced pressure to ~1–3 mL. The crude peptide was then purified by preparative reversed-phase high performance liquid chromatography RP-HPLC (0 to 100% MeCN with 0.1% TFA over 60 min). The appropriate fractions were lyophilised, affording the peptide as a white solid ( $t_R = 9.2$  min;  $\lambda = 214$  nm; 46.9 mg, 31%); Calculated Mass  $[M+H]^+$ : 855.8, Mass Found (ESI)  $[M+H]^+$ : 855.3.

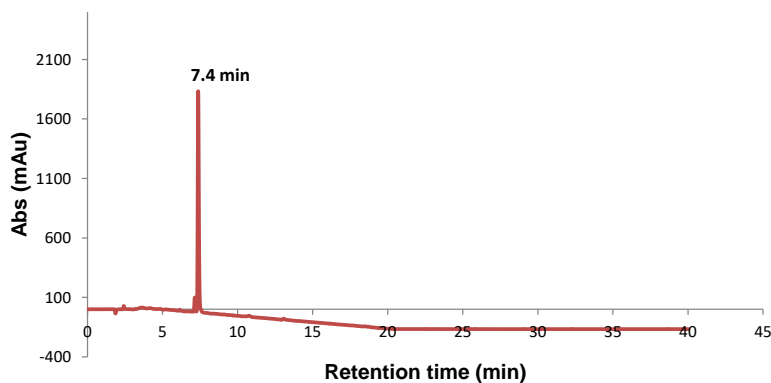
### Analytical HPLC Traces of Peptides

Method: 0% to 100% MeCN (with 0.1% TFA) over 40 min;  $\lambda = 214$  nm.

#### Peptide 1, Ac-pYAAAAPY-NH<sub>2</sub>

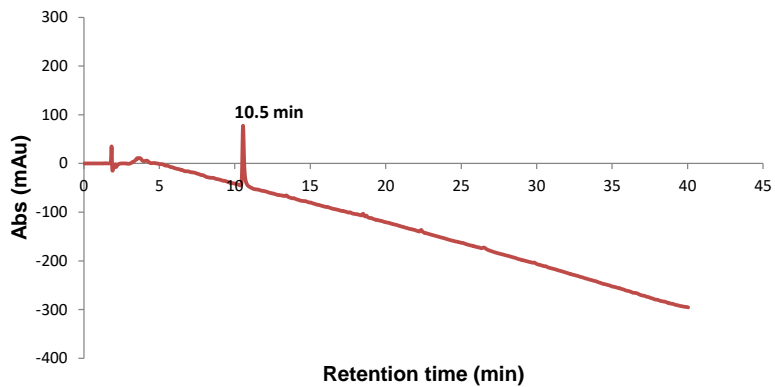


#### Peptide 2, Ac-APTAYA-NH<sub>2</sub>

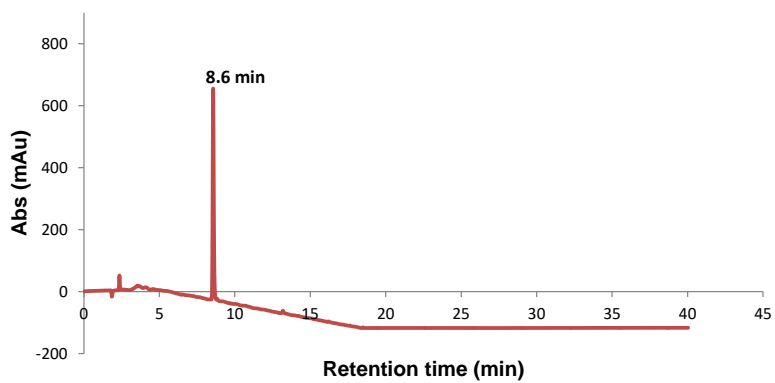


#### Peptide 3, Ac-APSpSAA-NH<sub>2</sub>

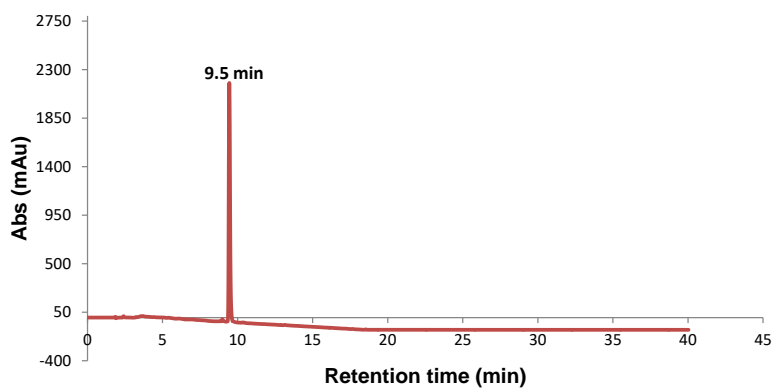
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## Peptide 4, H<sub>2</sub>N-pYDL-OH



## Peptide 5, H<sub>2</sub>N-QDpYDL-OH



## Peptide 6, H<sub>2</sub>N-DQDYDLS-OH

# Supplementary Information

