Supporting Information - *Organic Letters*

Combinatorial Approach to Selective Multivalent Ion-Pairing in Mixed Aqueous-Organic Media Using Bead-Supported Libraries of Unnatural Polyamines.

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General Procedure.

FMOC-protected amino acids and *N*-acetyl amino acids were purchased from Novabiochem (La Jolla, California) or Advanced Chemtech (Louisville, Kentucky). Those FMOC amino acids not commercially available were prepared using the procedure of Lapatsanis *et. al.* [1]. All*N*-butyryl and those *N*-acetyl amino acids not commercially available were prepared using the procedure outlined in Bodansky and Bodansky [2]. Polystyrene trityl chloride resin (90 μm) was purchased from Rapp-Polymere (Tübingen, Germany). THF was dried by distillation over sodium/benzophenone; CH_2Cl_2, pyridine, and triethylamine over calcium hydride. Anhydrous DMF and NMP were obtained commercially from Aldrich. All glassware employed in solid-phase reactions were silanized by treatment with 20% TMSCl/toluene for >12 hours. All solid phase amide coupling reactions were done using HBTU and HOBt as coupling reagents. Amide coupling reactions performed for the library synthesis were done on an Argonaut Quest® semi-automated synthesizer. Another solid-phase reactions that did not require heating were performed in polypropylene reaction vessels. LC/MS analyses were done on a Hewlett-Packard/Agilent 1100 MSD.

Library Synthesis.

Preparation of 1,2-diaminodedacane trityl polystyrene resin. In a 100 mL silanized round bottom flask, 1,12-diaminodedacane (12.54 g, 62.58 mmol) was dissolved in 60 mL of dry CH_2Cl_2 (turbid solution). Chlorotrityl polystyrene resin (1.07 mmol/g according to the commercial supplier, 1.950 g, 2.086 mmol) was then added slowly to the stirring solution over an hour. After an additional hour of stirring 10 mL of methanol was added followed by stirring for another 20 minutes. The suspension was then transferred into a large polypropylene reaction vessel then rinsed with methanol (3×), CH_2Cl_2 (3×), 1:3 Et_3N/DMF (3×), methanol (3×) and then CH_2Cl_2 (5×). Drying under high vacuum for 16 hours led to 1.469 g of resin (Theoretical loading is 0.91 mmol/g.)

Ladder synthesis of the triamide library.

The resin was split into 14 portions (102 mg, 0.093 mmol each) into 5 mL reaction vessels. Into each vessel was added 0.7 mL of a 0.50 M solution containing a 9:1 mixture of FMOC amino acid (0.36 mmol) and *N*-acyl amino acid (0.04 mmol) in NMP. The suspensions were mixed for 10 minutes followed by the addition of 1.2 mL of a solution in NMP containing 0.30 M HBTU (0.35 mmol) and 0.30 M HOBt (0.35 mmol) into each vessel. After mixing for 1 minute the DIPEA (0.12 mL 0.7 mmol) was added. The suspensions were then mixed for 2 hours before they were drained and rinsed with NMP (3×) and CH_2Cl_2 (5×). Ninhydrin assays of all 14 portions were negative indicating no free amines. The resins, suspended in CH_2Cl_2, were then mixed thoroughly into a single polypropylene reaction vessel and dried under high vacuum for 16 hours. The average loading, using the average molecular mass of all amino acids that had been coupled was calculated to be 0.69 mmol/g. The resin was again split into 14 portions

(100 mg 0.069 mmol) into 5 mL PP-vessels. The FMOC protective groups were removed by two treatments with 1:4 piperidine in NMP (5 minutes, then 30 minutes) and then rinsed with NMP (5×). A similar amide coupling procedure as described above was employed except using 0.55 mL of a 0.50 M solution of the *N*-acyl amino acids (0.0276 mmol) in NMP, 0.92 mL of an NMP solution containing 0.30 M HBTU (0.245 mmol) and 0.30 M HOBt (0.245 mmol), and then DIPEA (96 µL, 0.552 mmol). Ninhydrin assays from each vessel were negative. The resin portions were then mixed using the method described above. The average loading was calculated to be 0.72 mmol/g.

Preparation of the tetraamide library. This was conducted in a similar manner as described for the triamide library, except involving a second amide coupling with the 9:1 mixtures of FMOC amino acid and *N*-acyl amino acid followed by sequence termination with the *N*-acyl amino acid.

Reduction of the tripeptide library. A portion of the tripeptide library (397.6 mg, approx. 0.286 mmol) was weighed into a 25 mL silanized round bottom flask. While under nitrogen atmosphere 1.0 M BH_3/THF (8.6 mL, 8.6 mmol) was added. The suspension was slowly stirred for 2 days at 65^{o}C. The resin was then transferred to a 10 mL polypropylene reaction vessel and rinsed with THF (4×). 1 mL of THF was added followed by 0.28 mL of DIPEA, 0.56 mL AcOH and a 1 mL solution of I_2 (1.11 g, 4.29 mmol) in THF. The suspension was vortexed for four hours until it was filtered and rinsed with THF (4×), 1:3 Et_3N/DMF (4×), methanol (4×), and CH_2Cl_2 (5×) and then dried over 16 hours under high vaccumm to give the triamine library. Average loading: 0.74 mmol/g.

Reduction of the tetraamide library. This was done in a similar manner as with the tripeptide library except the reaction was over 3 days.

Single bead resin cleavage and LCMS analysis.

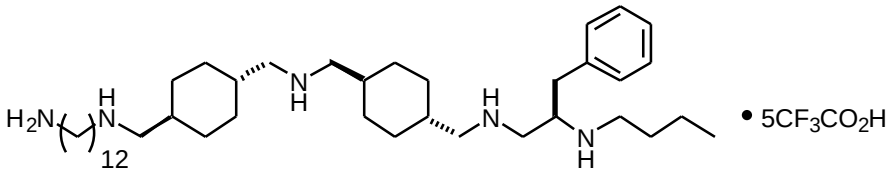
The dried beads were placed on glass dish and observed by a microscope. The beads were picked up using the tip of a 25 µL glass microsyringe containing 5 to 7.5 µL of a 5% TFA in CH_2Cl_2 and then transferred into a 200 µL glass injection vial insert. The TFA solution was injected into the vial insert removing the bead from the syringe tip and into the vial with the solution. The vial insert was placed inside an autosampler vial and then capped. After 15 minutes the cap was removed to allow the TFA solution to evaporate. Methanol (7.5 µL) was added to the vial and the solution injected into an LCMS. The identity of each polyamine sequence was determined by the mass differences between the each partially terminated sequence and the full sequence that eluted through the LC column. (LC conditions - column: Zorbax SB-C8 4.6×50 mm, 3.5 µm; eluent: 15 to 85 % acetonitrile (0.1 % TFA) in water (0.1 % TFA) over 5 minutes then maintained for 7 minutes at 0.7 mL/min; MSD conditions - capillary voltage: 3200 V; fragmentor voltage: 120 V; nebulizer pressure: 40 psig; gas temperature: 350^{O}C; drying gas flow: 10.0 L/min.)

Sample triamine and tetraamine library beads decoded by LCMS. Shown below, though figures S1 to S4, are examples of single resin beads decoded by LCMS. Each figure gives the chromatogram and the electrospray mass spectrum of the chromatographic peaks that are produced by the polyamines. Common impurities in

most samples are small amounts of unreduced material and ubiquitous impurities that cannot be identified.

Figure S1.

Mass spectrum shown is of LC peaks eluting between 5.1 and 6.3 minutes.



Amc^{R}-Amc^{R}-DPhe^{R}*n*Bu

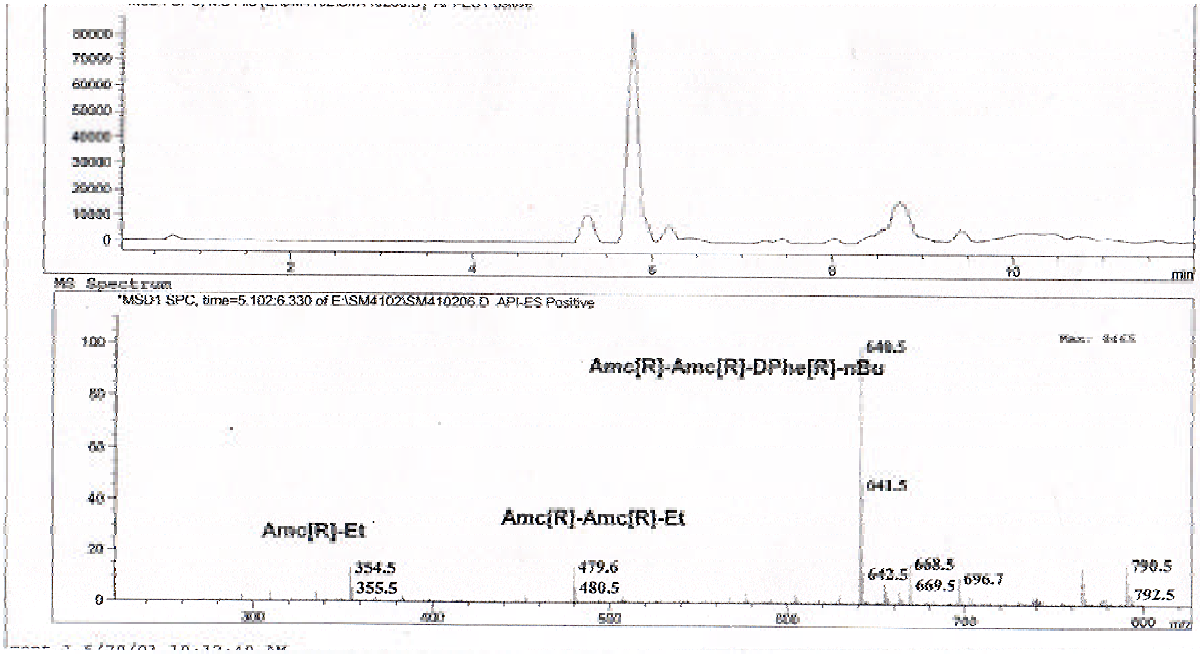
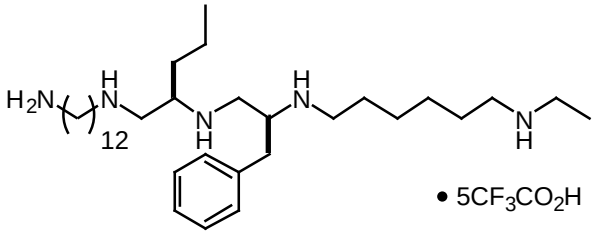


Figure S2.

Mass spectra shown are of LC peaks eluting between 5.5 and 5.8 minutes, which gives the first encoding sequence, and between 5.8 and 6.3 minutes, which shows the second encoding sequence and the full sequence.



DNva^{R}-LPhe^{R}-εAhx^{R}-Et

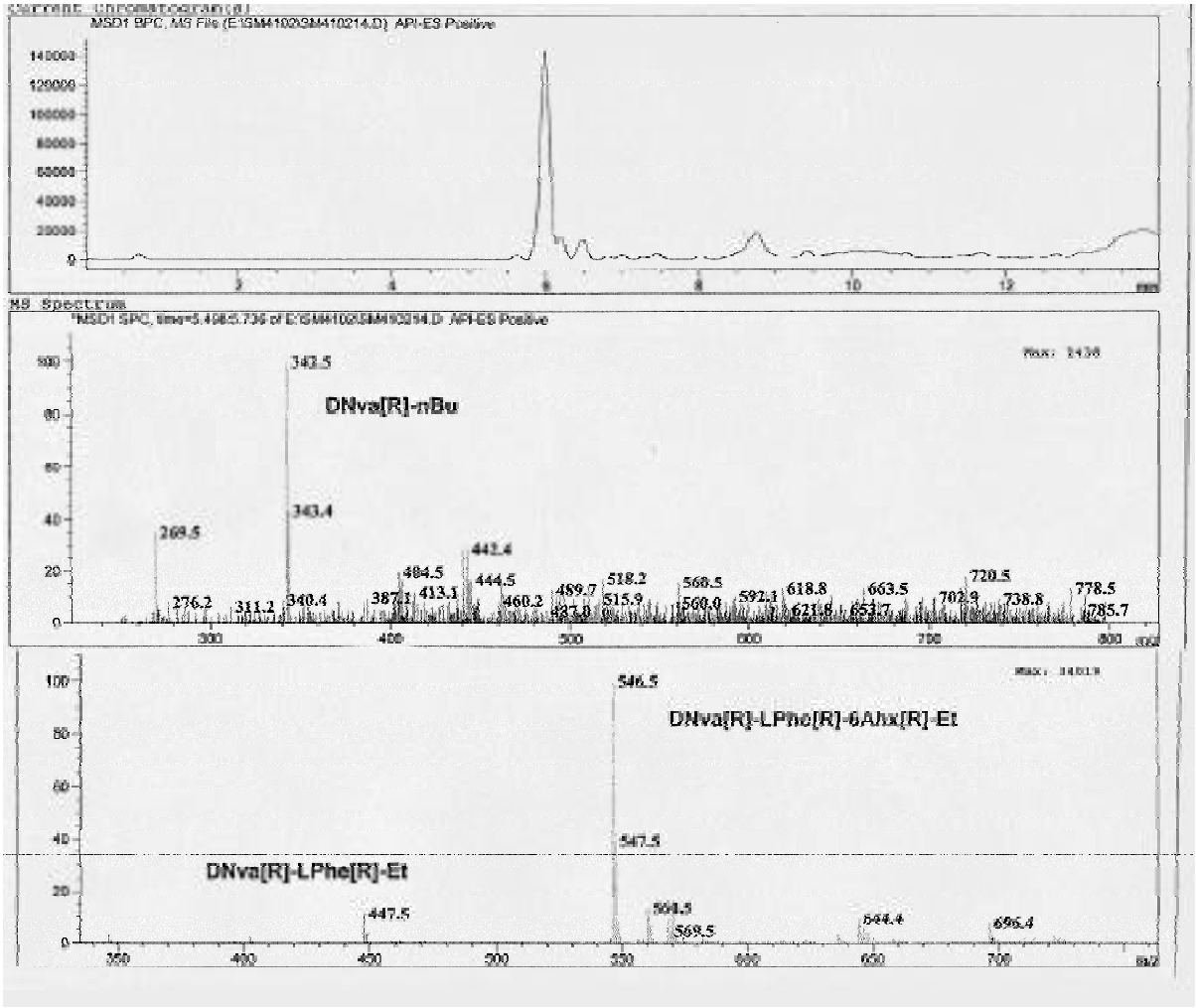
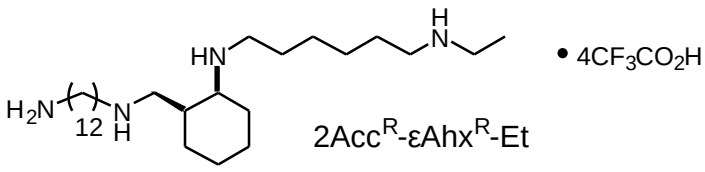


Figure S3.

Mass spectrum shown is of LC peaks co-eluting at 5.3 minutes.



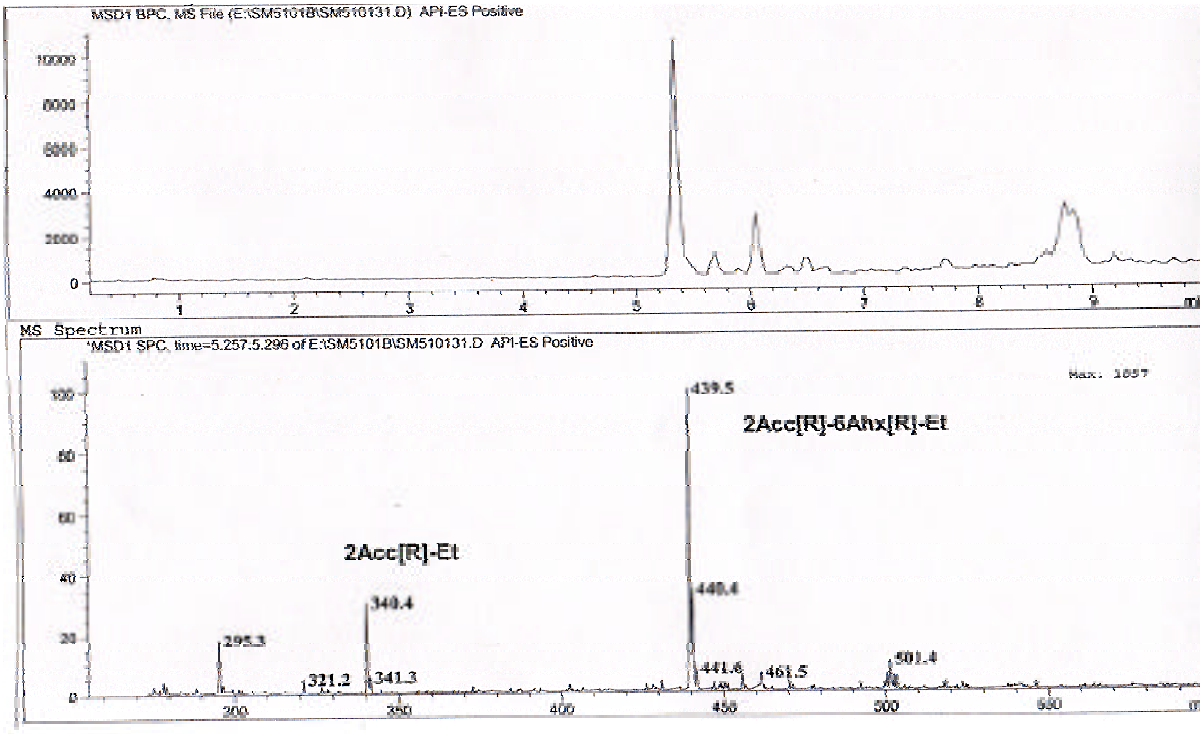
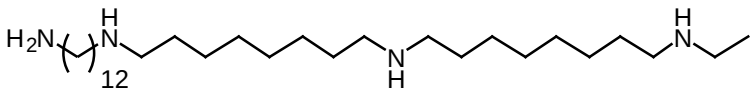
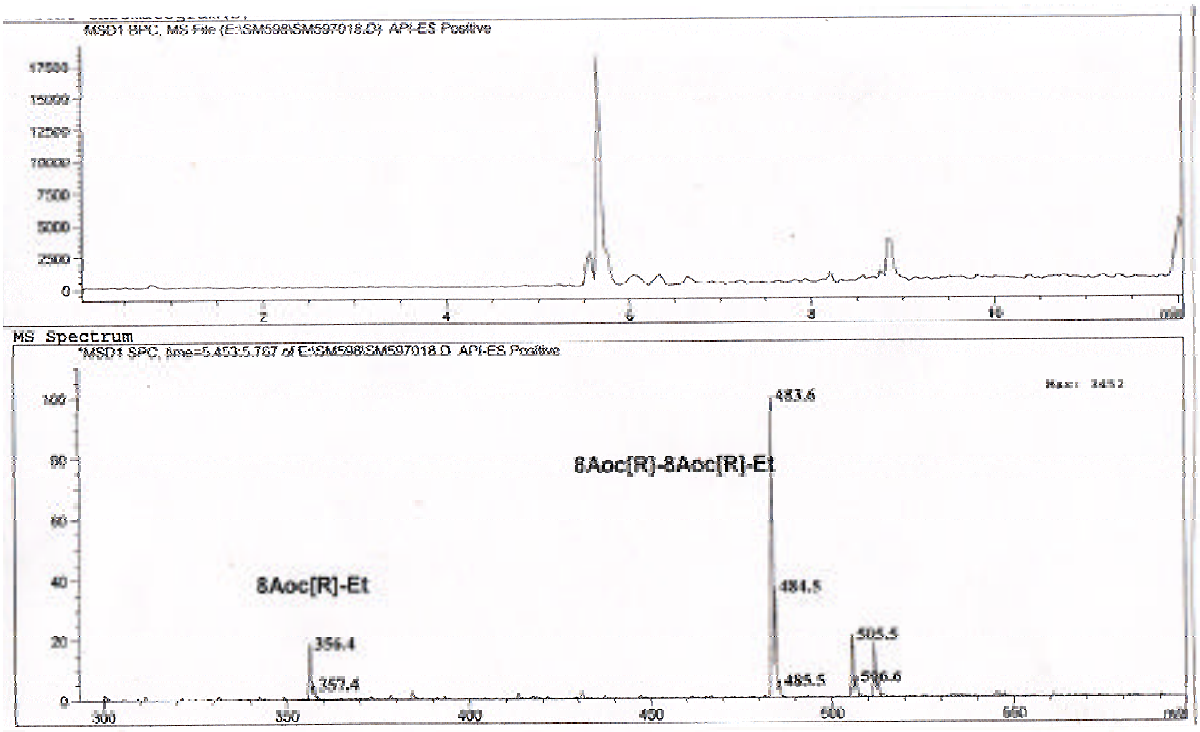


Figure S4.

Mass spectrum shown is of LC peaks eluting between 5.5 and 5.8 minutes.



8Aoc^{R}-8Aoc^{R}-Et • 4CF_3CO_2H



Screening of tetramine library against 1 and 2.

Approximately 3 mg of resin-bound triamine (~ 13000 beads) was added to 400 µL of a 50 mM solution of MES-TRIS (pH 7.0 in water) in 1:9 water and DMF (*solution A*) inside a 2.5 cm diameter glass dish. Resin clumps were broken up either physically or by extensive swirling of the dish. 12.8 µL of a 1.25 mM solution of the dye in *solution A* was then quickly added to the swirling suspension to make a final dye concentration in the screening dish of 37.5 µM. For screening in the presence of spermidine, 12.8 µL of a 10.5 mM solution of spermidine in *solution A* is first added and after 10 minutes of swirling is followed by the addition of 12.8 µL of a 1.25 mM solution of the dye in *solution A*. The final concentration of spermidine was 316 µM. In both cases the suspension was swirled for 1 hour during which time the dye solution turned colourless. While on a microscope the darkest beads were isolated with a thin glass capillary and transferred onto another glass dish where they were washed with 1:9 water in DMF (5×). The bead are then transferred into vial and analysed by LCMS using the method outlined above.

Screenings at pH 5.5 were done the same way as outlined above except in a solution containing 25 mM MES buffer (pH 5.5 in water) in 1:9 water/DMF (*solution B*).

Table S1. Approximately 3 mg of resin screened against 37.5 μM of **1** in 50 mM MES-TRIS buffer (pH 7.0 in water) in 1:9 water/DMF. 4 to 5 % of beads were dark red, 40% were clear, while the remaining were red to pink. 30 of the darkest beads were decoded and the following consensus sequences were found.

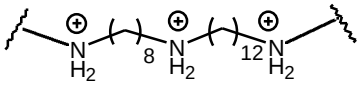
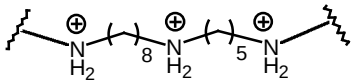
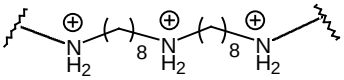
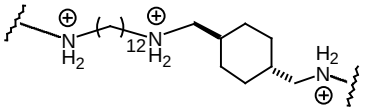
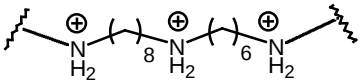
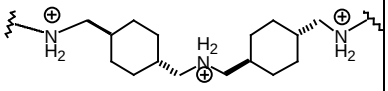
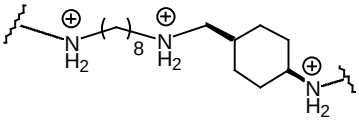
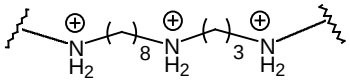
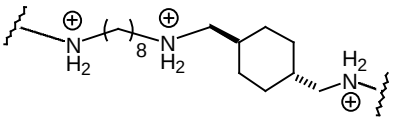
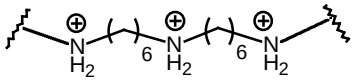
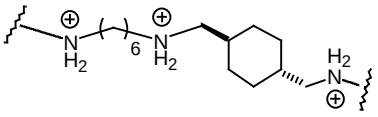
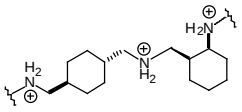
Residue pair	**Number of occurrences**	**Residue pair**	**Number of occurrences**
8Aoc^{R}, 12Abo^{R} 	4	8Aoc^{R}, γAbu^{R} 	2
8Aoc^{R}, 8Aoc^{R} 	4	12Abo^{R}, 4Acc^{R} 	2
8Aoc^{R}, εAhx^{R} 	3	Amc^{R}, Amc^{R} 	1
8Aoc^{R}, 4Acc^{R} 	3	8Aoc^{R}, βAla^{R} 	1
8Aoc^{R}, Amc^{R} 	3	εAhx^{R}, εAhx^{R} 	1
εAhx^{R}, Amc^{R} 	3	Amc^{R},2Acc^{R} 	1

Table S1 continued.

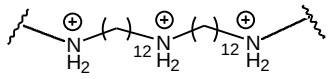
Residue pair	Number of occurrences	Residue pair	Number of occurrences
12Abo^{R}, 12Abo^{R} 	3		

Table S2. Approximately 3 mg of resin screened against 37.5 μM of **1** with 316 μM spermidine in 50 mM MES-TRIS buffer (pH 7.0 in water) in 1:9 water/DMF. 5 % of beads were dark red, the rest were clear or slightly pink. 26 of the darkest beads were decoded and the following consensus sequences were found.

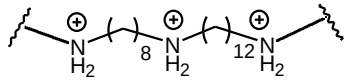
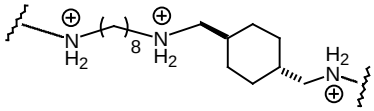
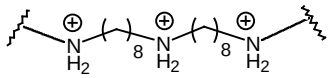
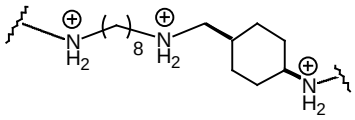
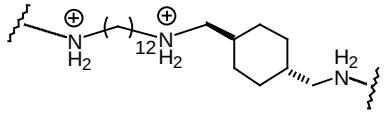
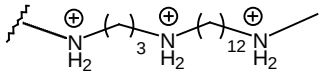
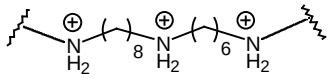
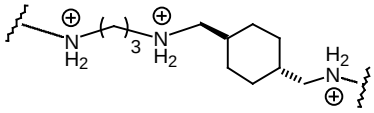
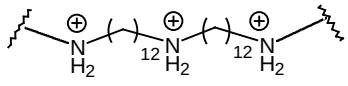
Residue pair	**Number of occurrences**	**Residue pair**	**Number of occurrences**
8Aoc^R, 12Abo^R 	5	8Aoc^R, Amc^R 	2
8Aoc^R, 8Aoc^R 	5	8Aoc^R, 4Acc^R 	2
12Abo^R, 4Acc^R 	4	βAla^R, 12Abo^R 	1
8Aoc^R, εAhx^R 	3	βAla^R,Amc^R 	1
12Abo^R, 12Abo^R 	3		

Table S3. Approximately 3 mg of resin screened against 37.6 μM of **1** with 322 μM spermidine in 25 mM MES buffer (pH 5.5 in water) in 1:9 water/DMF. Roughly 5 % of beads were dark red, the rest clear or slightly pink. 23 of the darkest beads were decoded and the following consensus sequences were found.

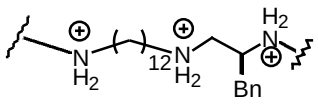
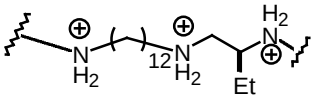
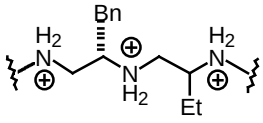
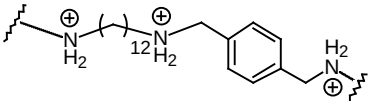
Residue pair	**Number of occurrences**	**Residue pair**	**Number of occurrences**
12Abo^{R}, 4Acc^{R}	6	12Abo^{R}, LPhe^{R} 	2
12Abo^{R}, 12Abo^{R}	5	εAhx^{R},εAhx^{R}	1
12Abo^{R}, 8Aoc^{R}	2	12Abo^{R}, LNva^{R} 	1
8Aoc^{R}, 4Acc^{R}	2	βAla^{R}, 12Abo^{R}	1
LPhe^{R}, D/LNva^{R} 	2	12Abo^{R}, Amb^{R} 	1

Table S4. Approximately 3 mg of resin screened against 37.5 μM of **2** with 316 μM spermidine in 50 mM MES-TRIS buffer (pH 7.0 in water) in 1:9 water/DMF. 12 % of beads were dark red, the rest clear or slightly pink. 39 of the darkest beads were decoded and the following consensus sequences were found.

Residue pair	**Number of occurrences**	**Residue pair**	**Number of occurrences**
8Aoc^{R}, εAhx^{R}	4	2Acc^{R}, βAla^{R}	1
8Aoc^{R}, 12Abo^{R}	4	8Aoc^{R}, LNva^{R}	1
8Aoc^{R}, 8Aoc^{R}	3	8Aoc^{R}, γAbu^{R}	1
εAhx^{R}, 4Acc^{R}	3	Amc^{R}, Amc^{R}	1
8Aoc^{R}, 2Acc^{R}	2	4Acc^{R}, γAbu^{R}	1
εAhx^{R}, Amc^{R}	2	12Abo^{R}, Amc^{R}	1
12Abo^{R}, 4Acc^{R}	2	12Abo^{R}, εAhx^{R}	1
2Acc^{R}, εAhx^{R}	2	Amc^{R}, Gly^{R}	1
εAhx^{R}, βAla^{R}	2	8Aoc^{R}, Amc^{R}	1
8Aoc^{R}, D/LPhe^{R}	2	εAhx^{R}, DNva^{R}	1
2Acc^{R}, Amc^{R}	1	8Aoc^{R}, 4Amb^{R}	1
8Aoc^{R}, 4Acc^{R}	1	βAla^{R}, γAbu^{R}	1

Table S5. Approximately 3 mg of resin screened against 37.6 μM of **2** with 322 μM spermidine in 25 mM MES buffer (pH 5.5 in water) in 1:9 water/DMF. Roughly 12 % of beads were dark red, the rest clear or slightly pink. 31 of the darkest beads were decoded and the following consensus sequences were found.

Residue pair	Number of occurrences	Residue pair	Number of occurrences
$12Abo^R$, $4Acc^R$	3	$2Acc^R$, $8Aoc^R$	1
$8Aoc^R$, $12Abo^R$	2	$4Acc^R$, $DNva^R$	1
$2Acc^R$, $2Acc^R$	2	$4Acc^R$, $DPhe^R$	1
$2Acc^R$, Amc^R	2	$12Abo^R$, $LNva^R$	1
$2Acc^R$,$12Abo^R$	2	$12Abo^R$, $12Abo^R$	1
εAhx^R, Amc^R	2	$12Abo^R$, Amc^R	1
εAhx^R, $DNva^R$,	2	Gly^R, $DPhe^R$	1
εAhx^R, Amb^R,	2	εAhx^R, $8Aoc^R$	1
εAhx^R, $2Acc^R$	1	$8Aoc^R$, $8Aoc^R$	1
εAhx^R, $4Acc^R$	1	$8Aoc^R$, Amc^R	1
$2Acc^R$, $4Acc^R$	1		