Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for:

Supramolecular Protection from the Enzymatic Tyrosine Phosphorylation in a Polypeptide

Enrico Faggi,[a] Yolanda Pérez,[b] Santiago V. Luis,*[c] and Ignacio Alfonso*[a]

[a] Department of Biological Chemistry and Molecular Modeling and [b]NMR facility, Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Jordi Girona, 18-26, E-08034 Barcelona (Spain), Fax: (+34)932045904, E-mail: ignacio.alfonso@iqac.csic.es

[c] Department of Inorganic and Organic Chemistry, ESTCE, Universitat Jaume I, 12004, Castellón, Spain, Avda. Sos Baynat, s/n, E-12071 Castellón (Spain), Fax: (+34)964728214, E-mail: luiss@uji.es

Table of contents:

1. General procedures:

Synthesis and Purification: Reagents and solvents were purchased from commercial suppliers (Aldrich, Fluka, Manchester Organics, Iris Biotech) and were used without further purification. Compounds **CySer** and **CyThr** were prepared as previously described.[1] Preparative reverse phase purifications were performed on an Isolera Biotage instrument (KP-C18-HS, CH_3CN and water with 0.1% TFA). Analytical RP-HPLC was performed with a Hewlett Packard Series 1100 (UV detector 1315A) modular system using a reverse-phase Kromasil 100 C8 (15 x 0.46 cm, 5 µm) column. CH_3CN-H_2O mixtures containing 0.1% TFA at 1 mL/min were used as mobile phase and monitoring wavelengths were set at 220 and 254 nm.

NMR spectroscopy: The NMR spectroscopic experiments were carried out at 25 °C on VNMRS-400 and Inova-500 spectrometers (Agilent Technologies, 500 and 400 MHz for ^{1}H and 125 and 100 MHz for ^{13}C NMR). The chemical shifts are reported in ppm using trimethylsilane (TMS) as a reference. Data were processed with the software programs VnmrJ3.2 (Agilent Technologies, Santa Clara, CA) and/or MNova (Mestrelab Research, Santiago de Compostela, Spain). For the T1ρ relaxation filtered experiments, we used a proton pulse sequence with spin-lock filter and water suppression through excitation sculpting (PROTON_ES, Chempack Library, Agilent),[2] with a spin-lock pulse of 50 ms at 2.7 kHz.

Mass spectrometry: High resolution mass spectra (HRMS) were performed on Acquity UPLC System and a LCT PremierTM XE Benchtop orthogonal acceleration time-of-flight (oa-TOF) (Waters Corporation, Milford, MA) equipped with an electrospray ionization source.

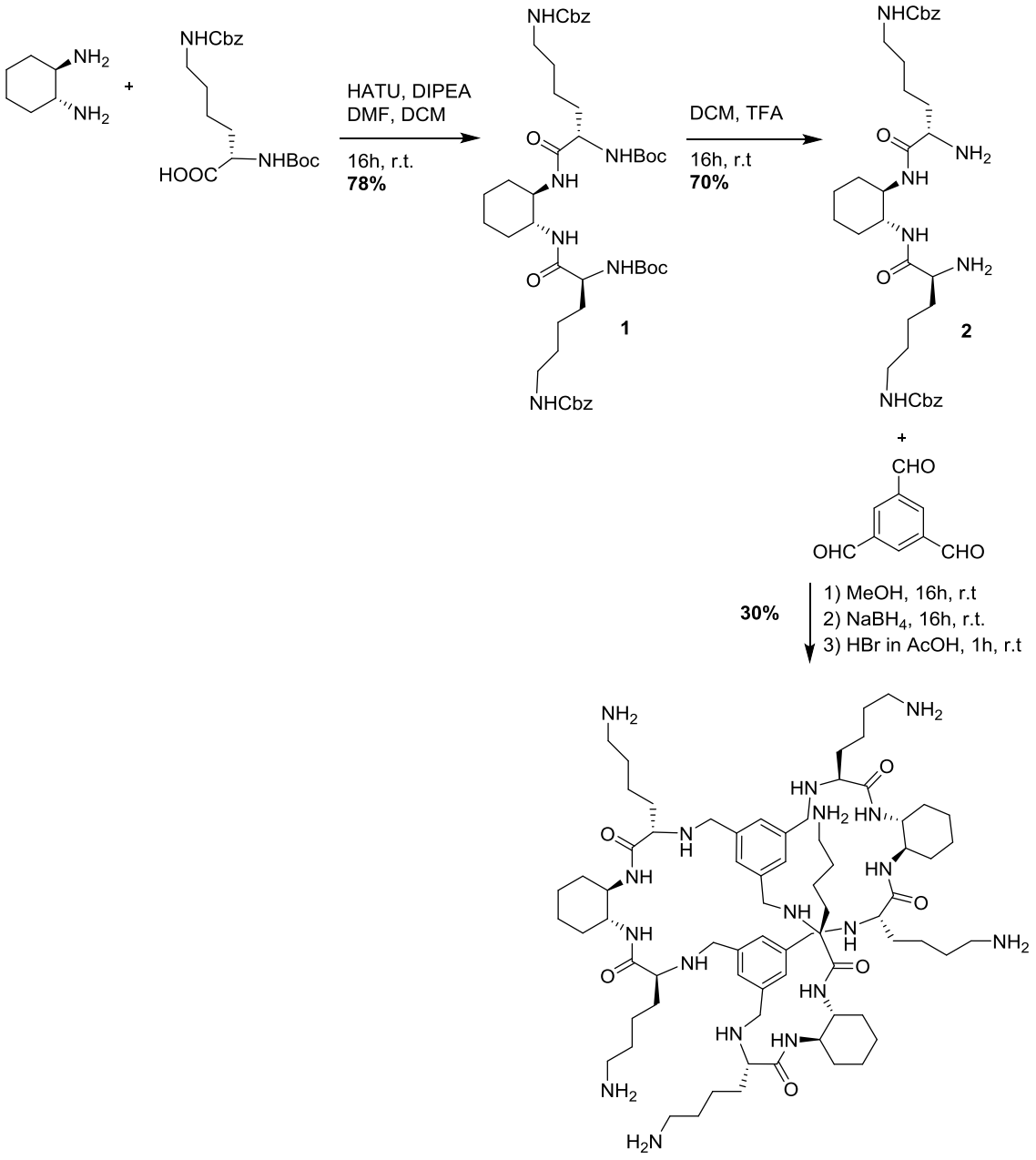
Kinase activity study: the Universal Tyrosine Kinase Assay Kit was purchased from Takara Bio Inc. (Otsu, Shiga, Japan). Absorbance of the 96-wells microplate was read on a SpectraMax M5 instrument.

Fluorescence spectroscopy: fluorescence emission spectra were collected on a SpectraMax M5 instrument using 10 mm path length cuvettes, excitation bandwidth: 9 nm, emission bandwidth: 15 nm, light source: Xenon flash lamp (1 joule/flash), emission read every 1 nm. All the fluorescence experiments were performed at 25 °C and specific measuring details and fitting procedures are given in the corresponding section for each titration example.

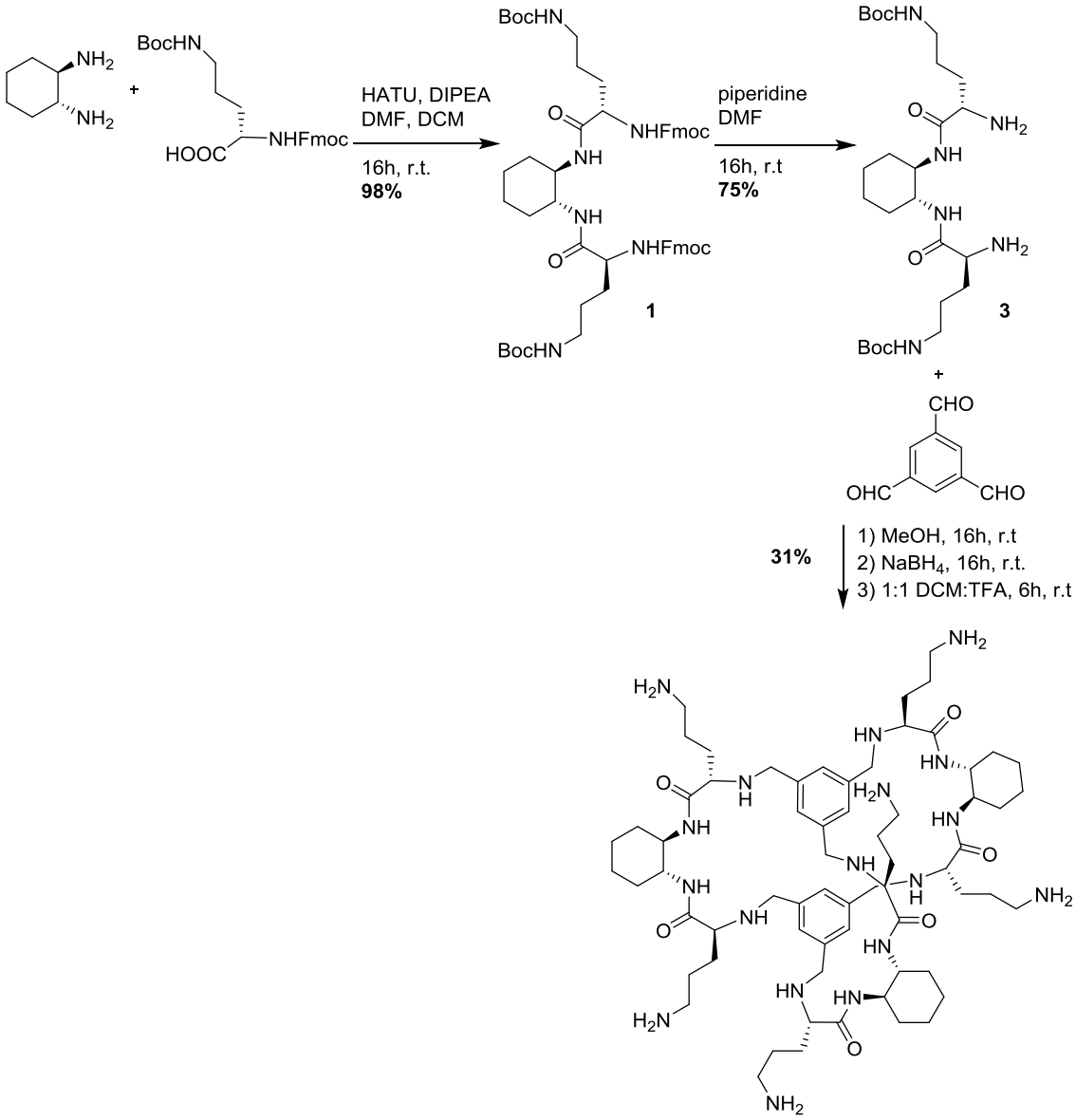
Circular Dichroism: Spectra were recorded with a JASCO J-815 spectropolarimeter at RT. The normalized spectra were obtained by transforming the data in the molar circular-dichroic absorption ($\Delta\varepsilon$, $cm^2 \cdot mmol^{-1}$), using the formula: $\Delta\varepsilon = \theta / (32980 \cdot C \cdot l)$ where θ is the measured ellipticity (in mdeg), C in the molar concentration and l is the path-length (in cm).

[1] a) E. Faggi, A. Moure, M. Bolte, C. Vicent, S. V. Luis, I. Alfonso *J. Org. Chem*. **2014**, *79*, 4590;
b) E. Faggi, C. Vicent, S. V. Luis, I. Alfonso *Org. Biomol. Chem*. **2015**, *13*, 11721-11731.
[2] Hwang, T. and Shaka, J. *J. Magn. Reson.* **1995**, 112, 275-279.

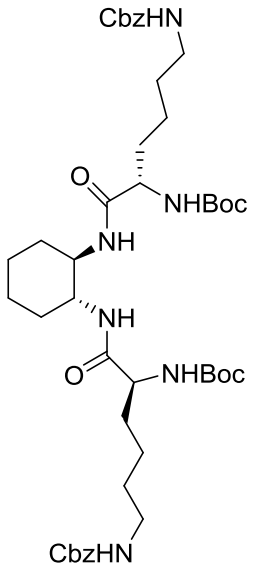
2. Synthesis and characterization of the pseudopeptidic cages



Scheme S1. Synthetic route to **CyLys**



Scheme S2. Synthetic route to **CyOrn**



Boc-Lys(Cbz)-OH (1.70 g, 4.49 mmol) was dissolved in dry DMF (6 ml) and dry DCM (6 ml). 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronoium hexafluorphosphate (HATU, 1.63 g, 4.28 mmol) and *N*,*N*-Diisopropylethylamine (DIPEA, 3.0 ml, 17.1 mmol) were added over the solution. The reaction mixture was cooled to 0 °C. A solution of the dihydrochloride salt of (*R,R*)-1,2-diaminocyclohexane (400 mg, 2.14 mmol) in dry DMF (6 ml) was added over the mixture. The solution was allowed to warm to room temperature for 16 hours, after which complete conversion of starting material was observed by TLC. The mixture was diluted with water and extracted with AcOEt (3 x 50 ml). Combined organic fractions were washed with aqueous LiCl (5% w/w), dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography using hexane: AcOEt as eluent (from 30% to 90% AcOEt) to give 1.40 g of **1** (1.67 mmol, 78% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.29 (m, 10H), 6.66 (bs, 2H), 5.43 (bs, 2H), 5.09 (s, 4H), 4.94 (bs, 2H), 3.98-3.93 (m, 2H), 3.61 (bs, 2H), 3.17 (bs, 4H), 1.99 (d, *J* = 10.6 Hz, 2H), 1.86-1.78 (m, 2H), 1.72 (d, *J* = 7.3 Hz, 2H), 1.62-1.47 (m, 6H), 1.43 (s, 18H), 1.38-1.18 (m, 8H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 156.8, 156.3, 136.7, 128.6, 128.2, 128.2, 80.4, 66.8, 54.5, 54.1, 40.5, 32.2, 31.2, 29.6, 28.5, 24.7, 22.8.

HRMS (ESI-TOF) *m/z* [**1** + H]$^+$ Calcd for $C_{44}H_{67}N_6O_{10}$ 839.4919, found 839.4994.

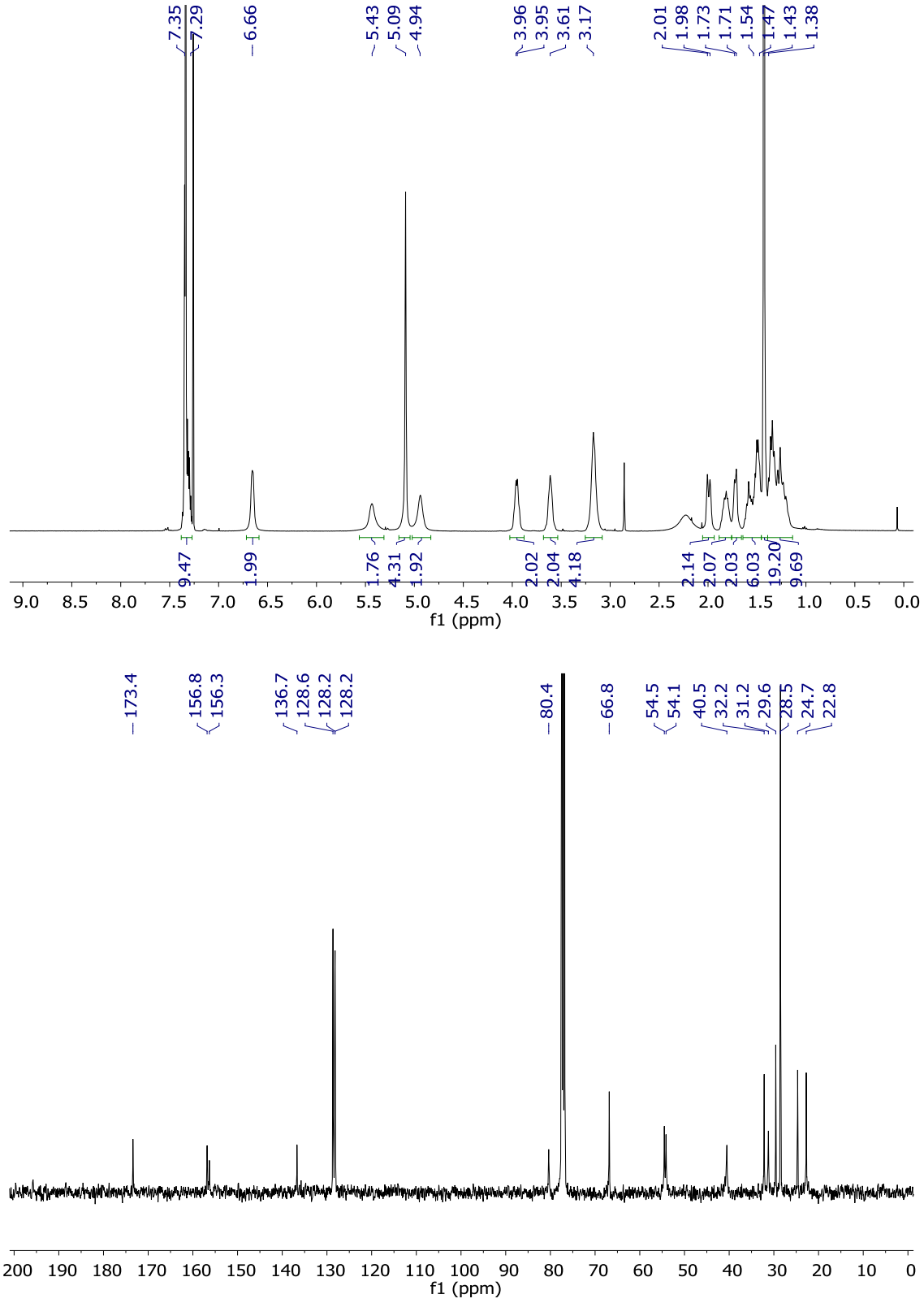


Figure S1. ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (101 MHz, $CDCl_3$) spectra of **1**

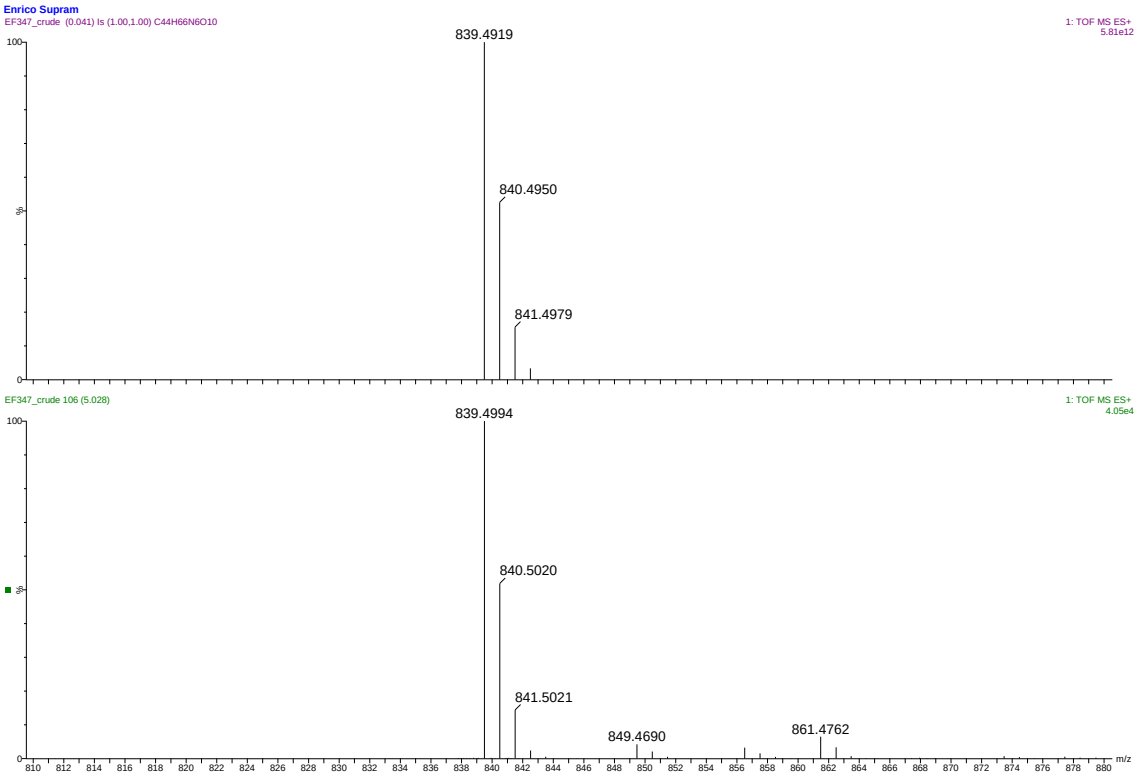
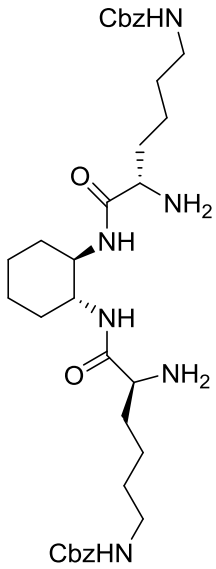


Figure S2. HRMS (ESI+) experimental spectrum of **1** (bottom) and simulated spectrum for $[C_{44}H_{67}N_6O_{10}]^+$ (top)



1 (1.62 g, 1.93 mmol) was dissolved in dry DCM (20 ml) and trifluoroacetic acid (5.0 ml) was added dropwise. The solution was stirred at room temperature during 16 hours and then the solvents were evaporated at reduced pressure, affording a pale yellow oil. It was washed several times with diethyl ether and dried. It was treated with 1M aqueous NaOH, affording a white sòlid that was washed with more NaOH. Wet solid was taken in chloroform; the aqueous layer was discarded while the organic layer was dried over $MgSO_4$ and concentrated to dryness. Compound **2** was obtained as a white solid (860 mg, 1.32 mmol, 70% yield).

^{1}H NMR (400 MHz, CD_3OD) δ 7.34-7.27 (m, 10H), 5.05 (s, 4H), 3.65-3.58 (m, 2H), 3.18 (t, *J* = 6.7 Hz, 2H), 3.11 (t, *J* = 6.8 Hz, 4H), 1.96-1.90 (m, 2H), 1.75-1.69 (m, 2H), 1.67-1.60 (m, 2H), 1.53-1.46 (m, 6H), 1.39-1.26 (m, 8H).

^{13}C NMR (101 MHz, CD_3OD) δ 177.4, 158.9, 138.5, 129.4, 128.9, 128.7, 67.3, 56.1, 54.0, 41.5, 36.2, 33.2, 30.7, 25.8, 24.0.

HRMS (ESI-TOF) *m/z* [**2** + H]$^+$ Calcd for $C_{34}H_{51}N_6O_6$ 639.3870, found 639.3879.

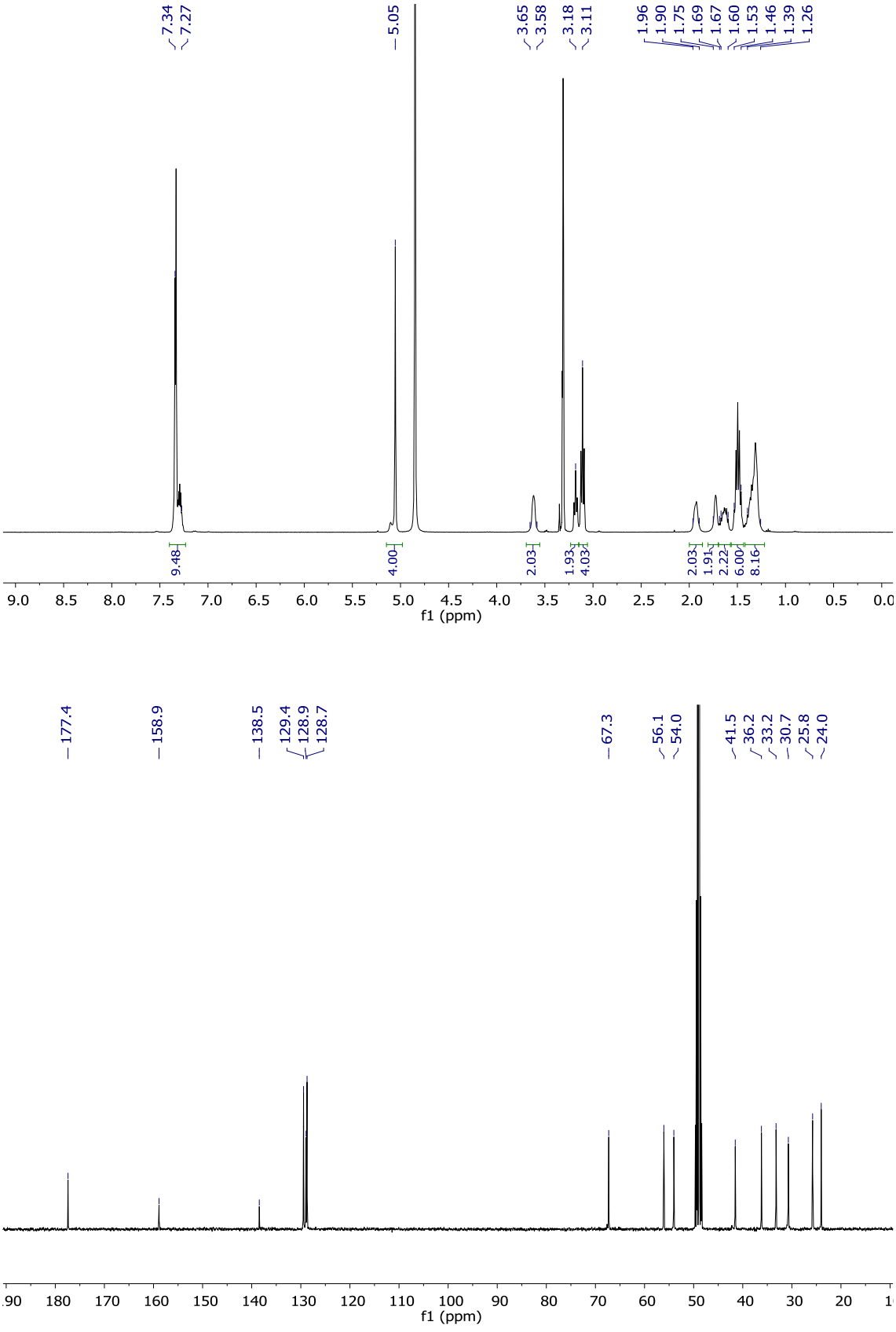


Figure S3. ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (101 MHz, CD_3OD) spectra of **2**

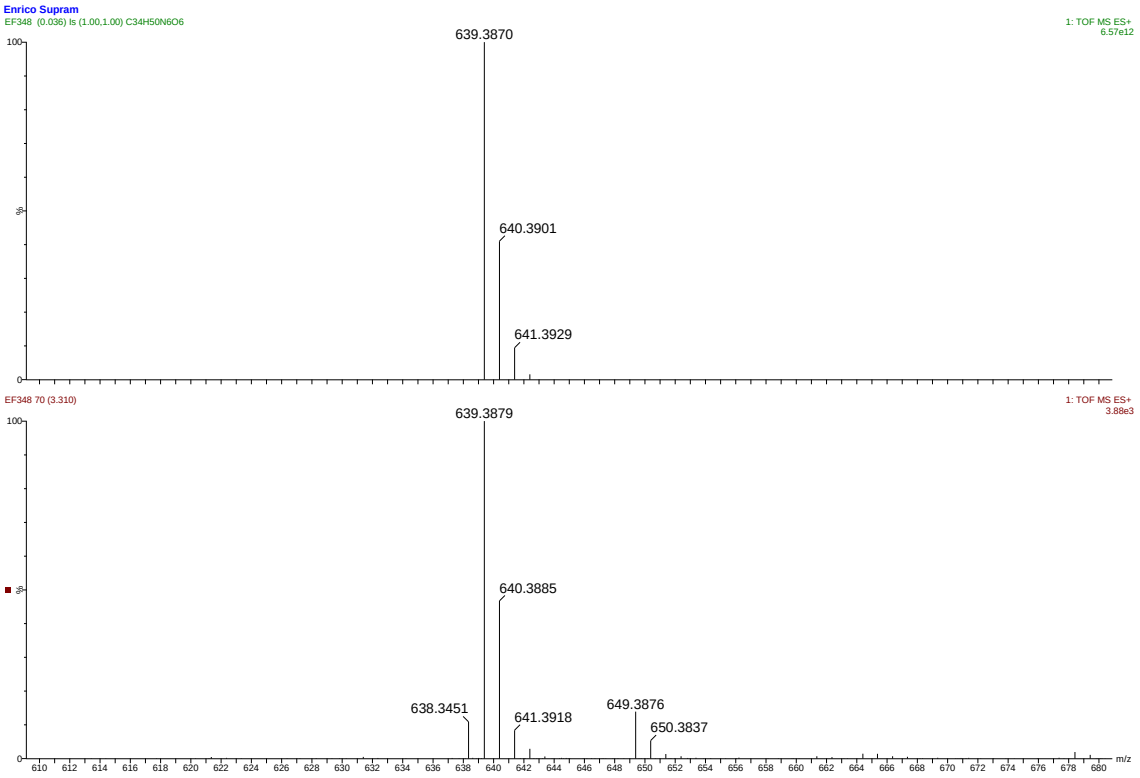
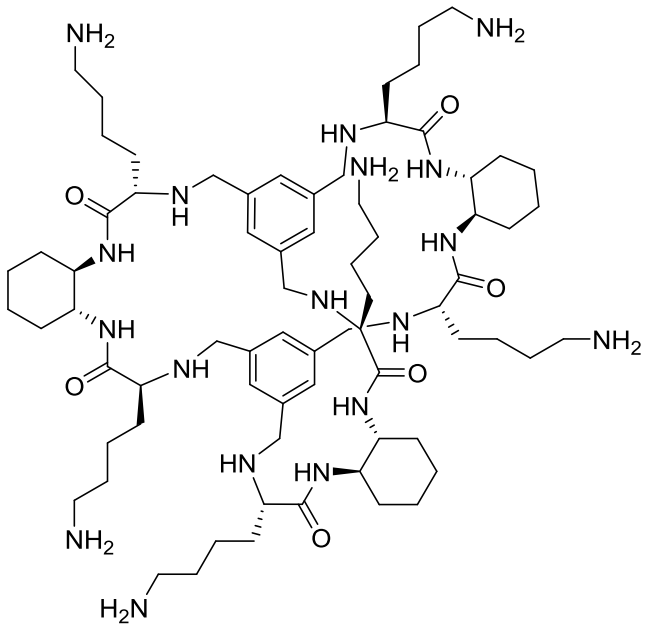


Figure S4. HRMS (ESI+) experimental spectrum of **2** (bottom) and simulated spectrum for $[C_{34}H_{51}N_6O_6]^+$ (top)

CyLys



A solution of benzene-1,3,5-tricarbaldehyde (152 mg, 0.94 mmol) in methanol (15 ml) was added over a solution of **2** (900 mg, 1.41 mmol) in methanol (10 ml). The mixture was stirred at room temperature during 20 hours. Then, $NaBH_4$ (260 mg, 6.84 mmol) was carefully added and the mixture was allowed to react for 16 hours. The mixture was concentrated to half volume and concentrated HCl (*ca.* 2 ml) was added. After 2 hours the mixture was evaporated to dryness. In an ice bath, 33% HBr in AcOH was added (8 ml); the ice bath was removed and the reaction mixture was stirred at room temperature during 1 hour. Excess diethyl ether (*ca.* 60 ml) was poured over the mix, affording a white solid. Crude product was purified using reversed-phase flash chromatography (eluant: 1% to 20% MeCN in water; 0.1% TFA in both solvents). The trifluoroacetate ammonium salt was transformed into the free-base amine using an ion-exchange resin, affording **CyLys** as a white solid (188 mg, 30% yield).

^{1}H NMR (400 MHz, D_2O+DCl) δ 7.31 (s, 6H), 4.00-3.96 (m, 12H), 3.81-3.79 (m, 6H), 3.71 (d, *J* = 12.4 Hz, 12H), 2.88 (t, *J* = 5.3, 4.1 Hz, 6H), 1.91-1.80 (m, 12H), 1.64-1.59 (m, 12H), 1.34-1.23 (m, 30H).

^{13}C NMR (101 MHz, D_2O+DCl) δ 169.4, 132.7, 132.3, 61.9, 52.6, 49.6, 38.8, 32.6, 30.2, 26.3, 23.8, 21.4.

HRMS (ESI-TOF) *m/z* [**CyLys** + H]$^+$ Calcd for $C_{72}H_{127}N_{18}O_6$ 1340.0186, found 1340.0201.

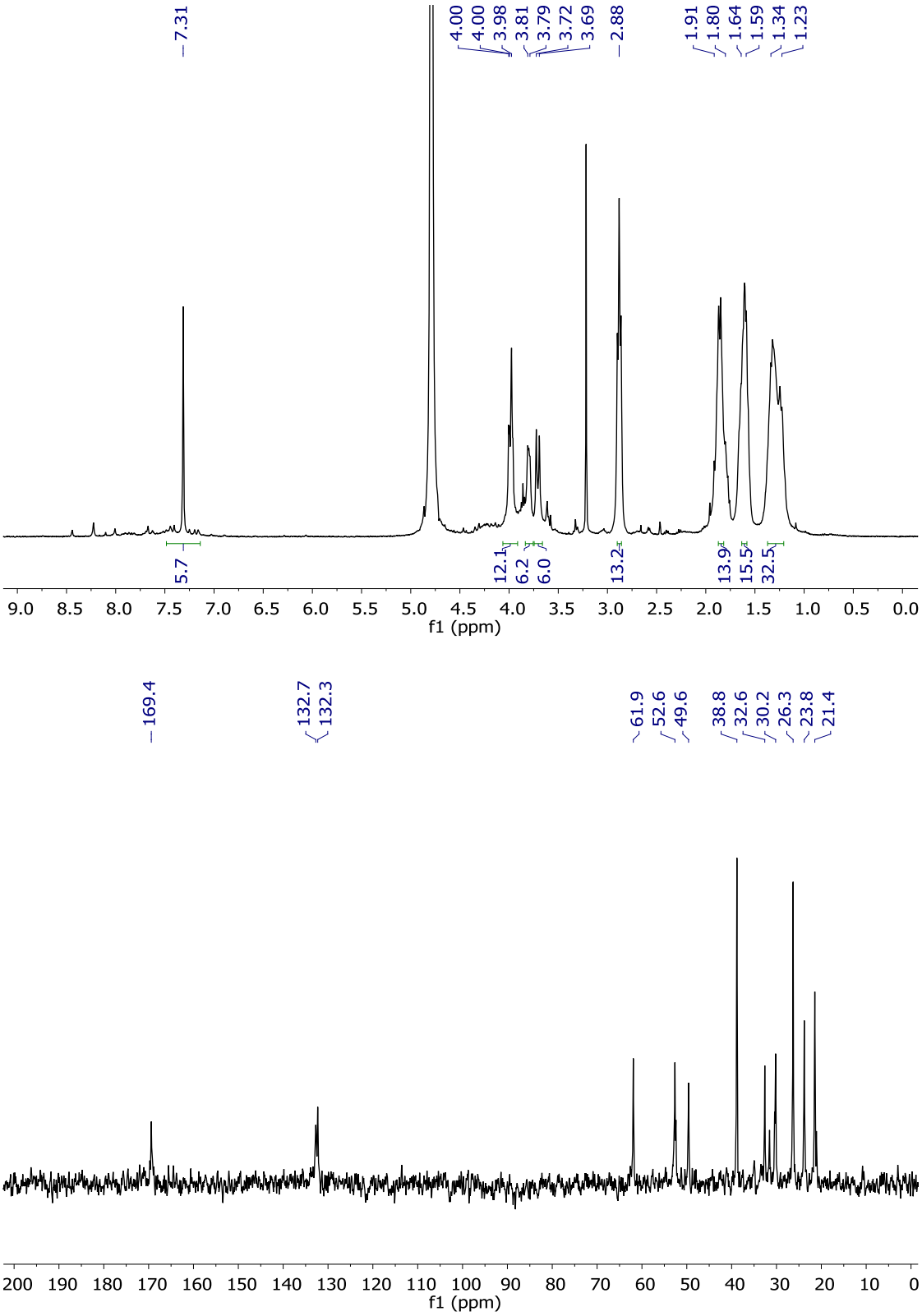


Figure S5. ^{1}H-NMR (400 MHz, D_2O+DCl) and ^{13}C-NMR (101 MHz, D_2O+DCl) spectra of **CyLys**

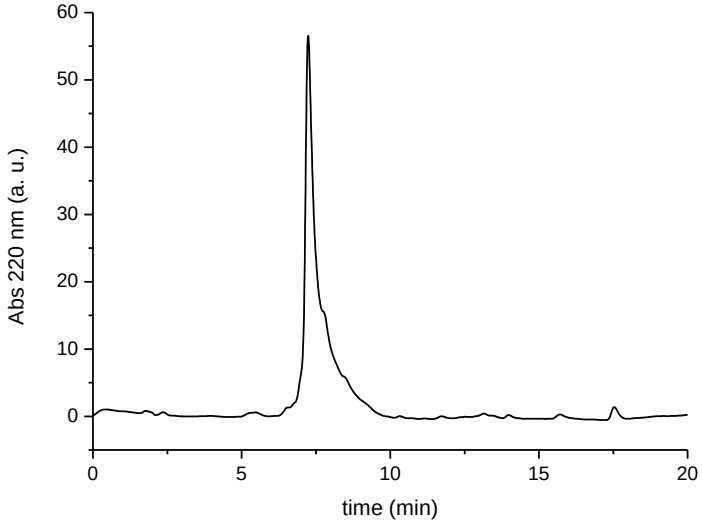


Figure S6. HPLC chromatogram of **CyLys** (5% to 100% MeCN in water, linear gradient during 20 minutes, with 0.1% TFA in both solvents). Retention time: 7.24 minutes

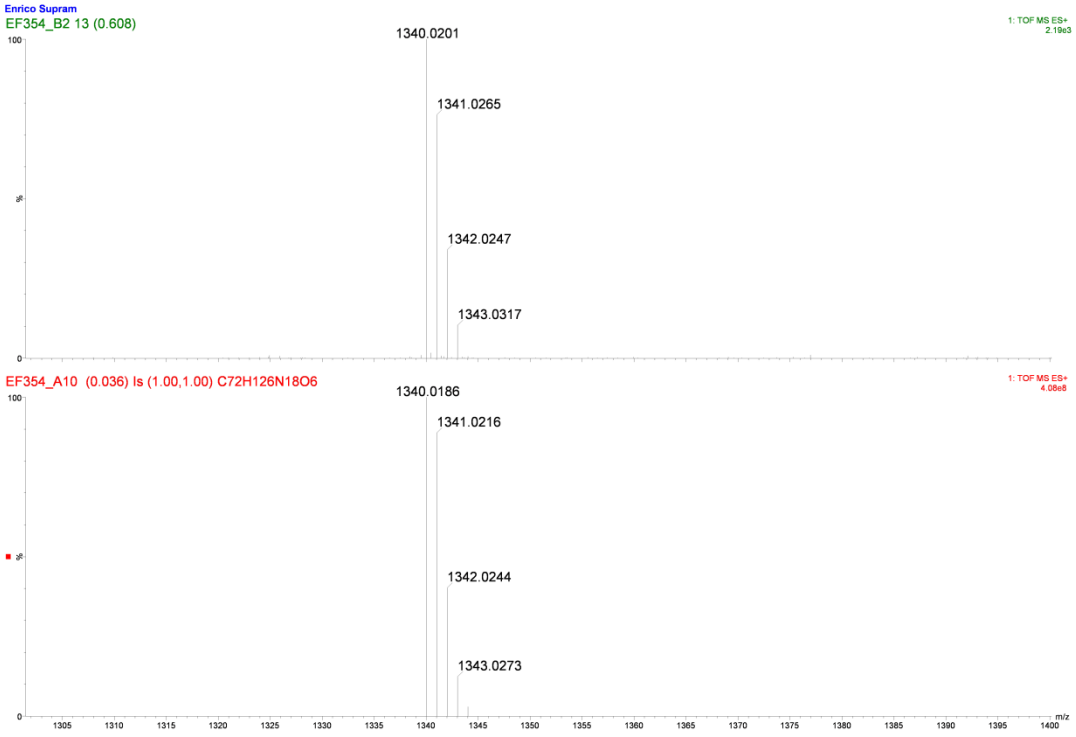
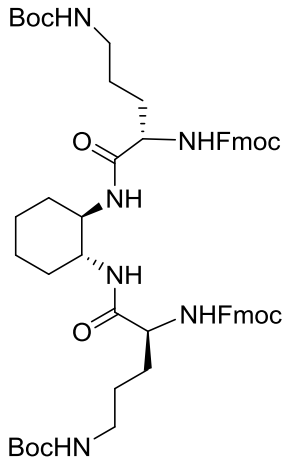


Figure S7. HRMS (ESI+) spectrum of **CyLys** (top); simulated spectrum for $[C_{72}H_{127}N_{18}O_6]^+$ (bottom)



Fmoc-Lys(Boc)-OH (3.06 g, 6.73 mmol) was dissolved in dry DMF (10 ml) and dry DCM (5 ml). 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronoium hexafluorphosphate (HATU, 2.56 g, 6.73 mmol) and *N*,*N*-Diisopropylethylamine (DIPEA, 3.36 ml, 19.3 mmol) were added over the solution. The reaction mixture was cooled to 0 °C. A solution of the dihydrochloride salt of (*R,R*)-1,2-diaminocyclohexane (600 mg, 3.21 mmol) in dry DMF (12 ml) was added over the mixture. The solution was allowed to warm to room temperature for 16 hours, after which complete conversion of starting material was observed by TLC. The mixture was diluted with water and extracted with AcOEt (3 x 50 ml). Combined organic fractions were washed with aqueous LiCl (5% w/w), dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography using DCM: MeOH as eluent (from 4% to 10% MeOH) to give 3.10 g of **3** (3.14 mmol, 98% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (t, *J* = 8.5 Hz, 4H), 7.49 (bs, 4H), 7.36-7.18 (m, 8H), 6.73 (bs, 2H), 5.94 (d, *J* = 5.7 Hz, 2H), 4.80 (bs, 2H), 4.34-4.04 (m, 8H), 3.66 (bs, 2H), 3.11 (bs, 4H), 2.01 (d, *J* = 8.6 Hz, 2H), 1.87 (bs, 2H), 1.71-1.26 (m, 12H), 1.42 (s, 18H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 156.8, 156.4, 143.8, 141.3, 127.8, 127.2, 125.1, 120.0, 79.4, 67.3, 54.6, 53.8, 47.1, 40.0, 32.3, 29.4, 28.6, 26.4, 24.7.

HRMS (ESI-TOF) *m/z* [**3** + H]$^+$ Calcd for $C_{56}H_{71}N_6O_{10}$ 987.5232, found 987.5267.

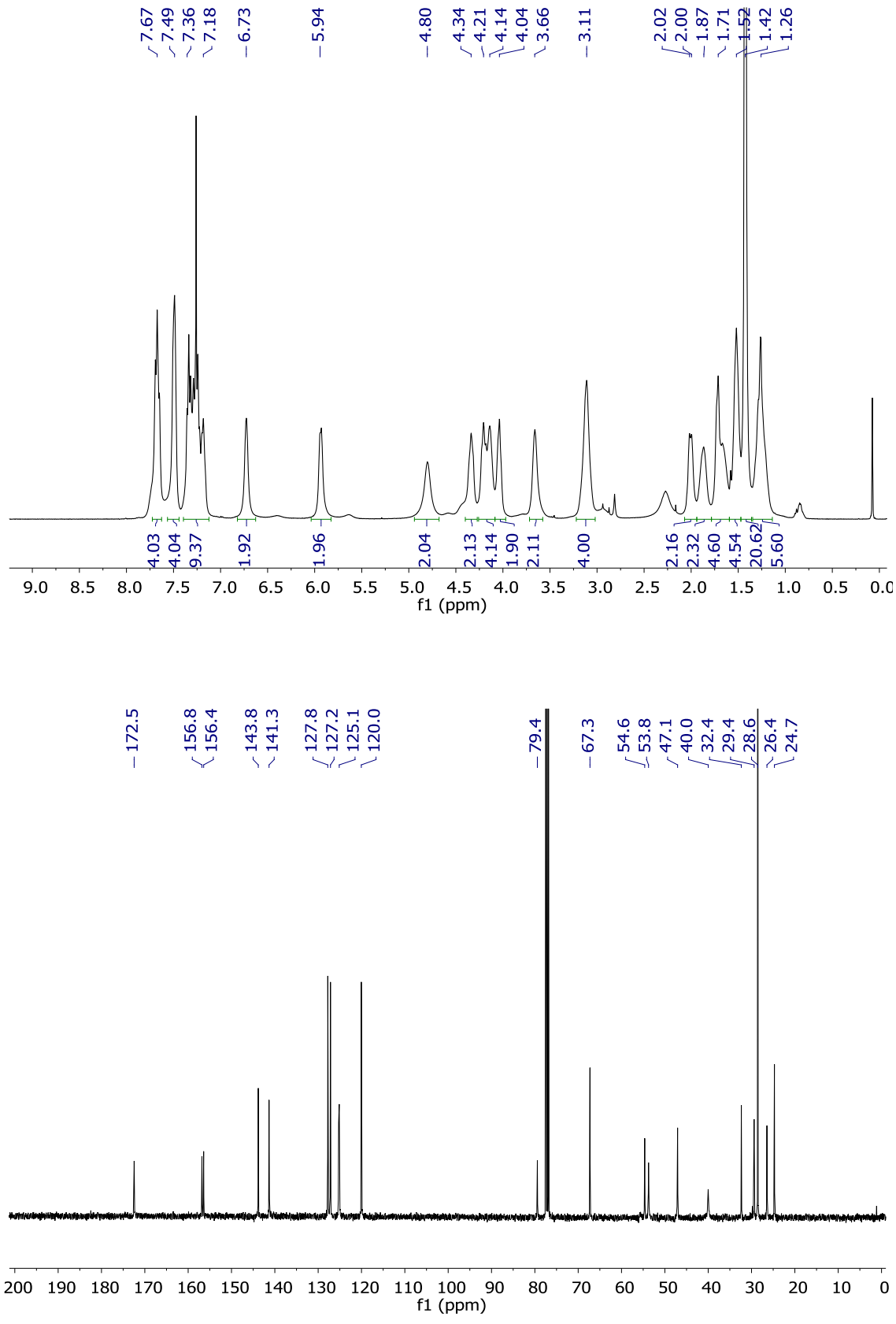


Figure S8. ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (101 MHz, CD_3OD) spectra of **3**

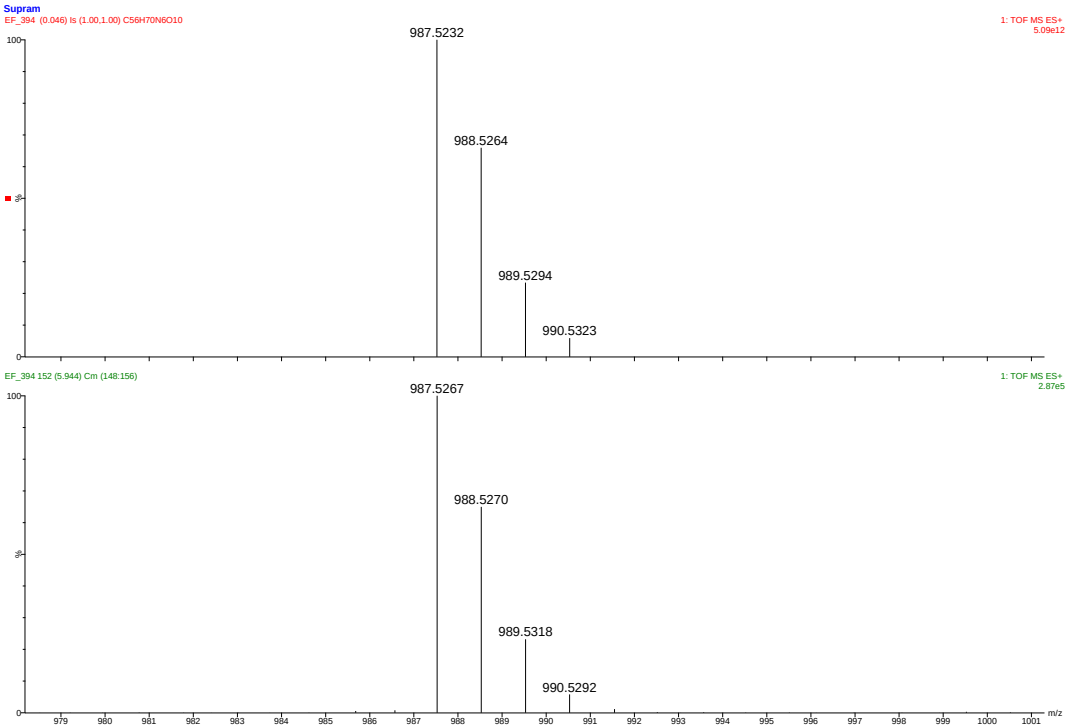
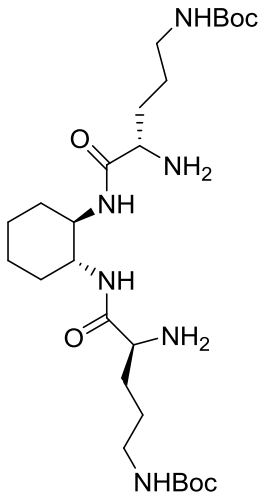


Figure S9. HRMS (ESI+) spectrum of **3** (bottom); simulated spectrum for $[C_{56}H_{71}N_6O_{10}]^+$ (top)



3 (3.10 g, 3.14 mmol) was dissolved in 15 ml of 20% Piperidine in DMF. After several minutes the product precipitated as a white solid but the mixture was allowed to react for 16h until complete conversion of starting material was observed by TLC. Excess diethyl ether was added over the reaction mixture and the product was filtered off and washed with additional diethyl ether. **4** was obtained as a white solid (1.30 g, 75% yield).

^{1}H NMR (400 MHz, CD_3OD) δ 3.63 (bs, 2H), 3.23 (bs, 2H), 3.03 (bs, 4H), 1.94 (bs, 2H), 1.77 (bs, 2H), 1.64-1.49 (m, 8H), 1.43 (s, 18H), 1.34 (bs, 4H).

^{13}C NMR (101 MHz, CD_3OD+$CDCl_3$) δ 176.7, 158.3, 79.8, 55.6, 54.0, 40.9, 33.5, 33.1, 28.8, 27.1, 25.7.

HRMS (ESI-TOF) *m/z* [**4** + H]$^+$ Calcd for $C_{26}H_{51}N_6O_6$ 543.3870, found 543.3900.

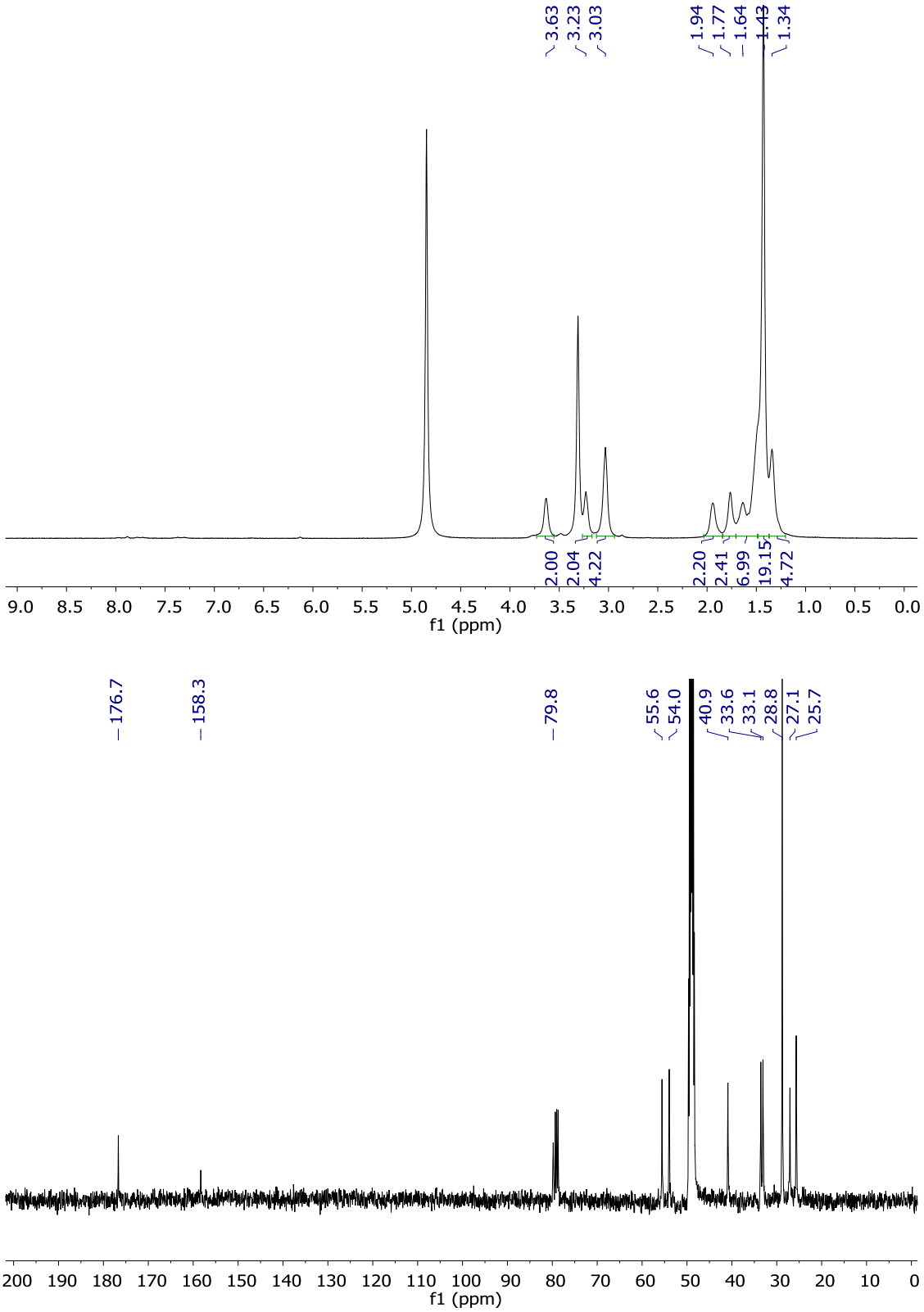


Figure S10. ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (101 MHz, CD_3OD+$CDCl_3$) spectra of **4**

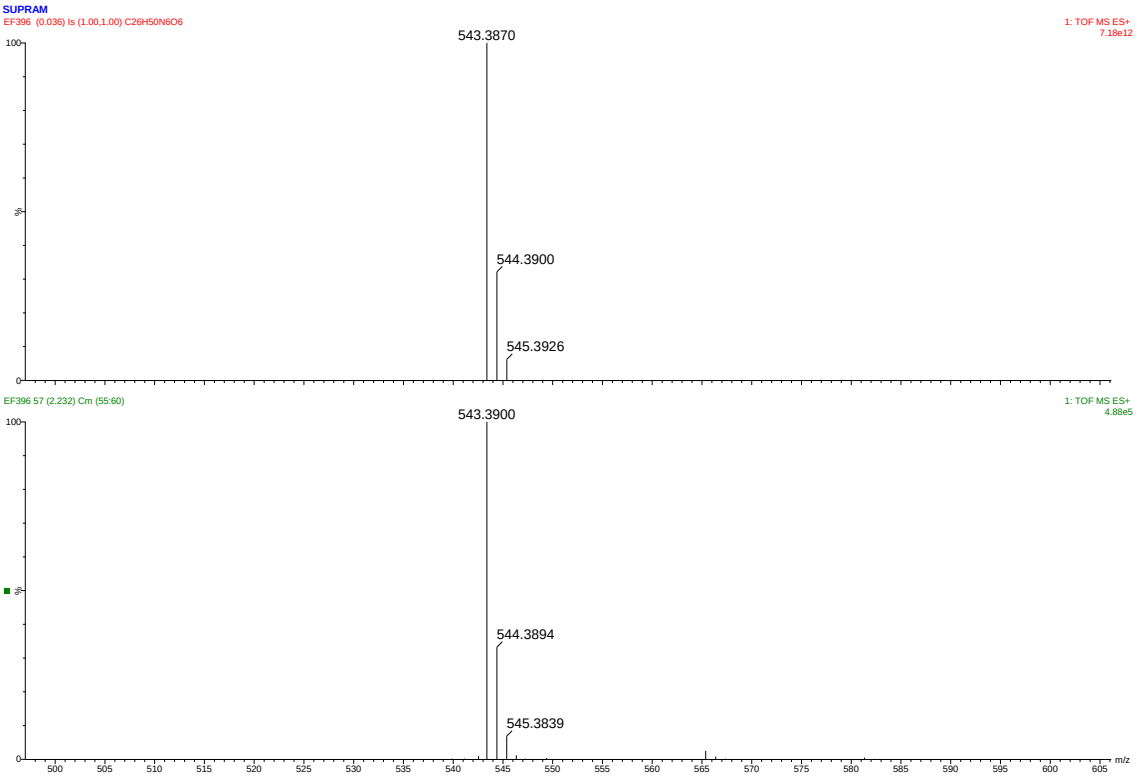
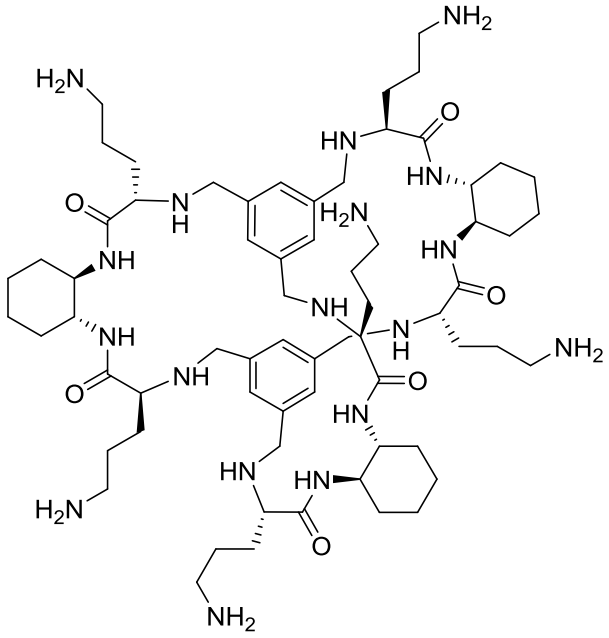


Figure S11. HRMS (ESI+) spectrum of **4** (bottom); simulated spectrum for $[C_{26}H_{51}N_6O_6]^+$ (top)

CyOrn



A solution of benzene-1,3,5-tricarbaldehyde (324 mg, 1.60 mmol) in CH_3OH (30 ml) and DCM (15 ml) was added over a solution of **4** (1.30 g, 2.39 mmol) in CH_3OH (30 ml). The mixture was stirred at room temperature during 16 hours. Then, $NaBH_4$ (375 mg, 9.60 mmol) was carefully added and the mixture was allowed to react for 16 hours. The mixture was concentrated to dryness affording a yellow solid that was dissolved in a 1 : 1 DCM : TFA mixture (*ca.* 20 ml). Solution was stirred during 6 hours at room temperature. With an air flow, solvents were evaporated to little volume and excess diethyl ether (*ca.* 60 ml) was poured over the mix, affording a brown solid. Crude product was purified using reversed-phase flash chromatography (eluant: 0% to 15% MeCN in water). The obtained free-base amine was washed several times with diethyl ether and dried, affording **CyOrn** as a white solid (310 mg, 0.247 mmol, 31% yield).

^{1}H NMR (400 MHz, D_2O+DCl) δ 7.44 (s, 6H), 4.14 (t, *J* = , 6.8 Hz, 6H), 4.12 (d, *J* = 12.6 Hz, 6H), 3.94 (dd, *J* = 5.3, 4.1 Hz, 6H), 3.85 (d, *J* = 12.3 Hz, 6H), 3.05 (t, *J* = 6.8 Hz, 12H), 2.04-2.00 (m, 16H), 1.81-1.70 (m, 16H), 1.49-1.36 (m, 16H).

^{13}C NMR (101 MHz, D_2O+DCl) δ 167.0, 133.3, 131.5, 60.8, 52.5, 48.8, 38.6, 32.4, 27.1, 23.7, 22.3.

HRMS (ESI-TOF) *m/z* [**CyOrn** + H]$^+$ Calcd for $C_{66}H_{115}N_{18}O_6$ 1255.9247, found 1255.9443.

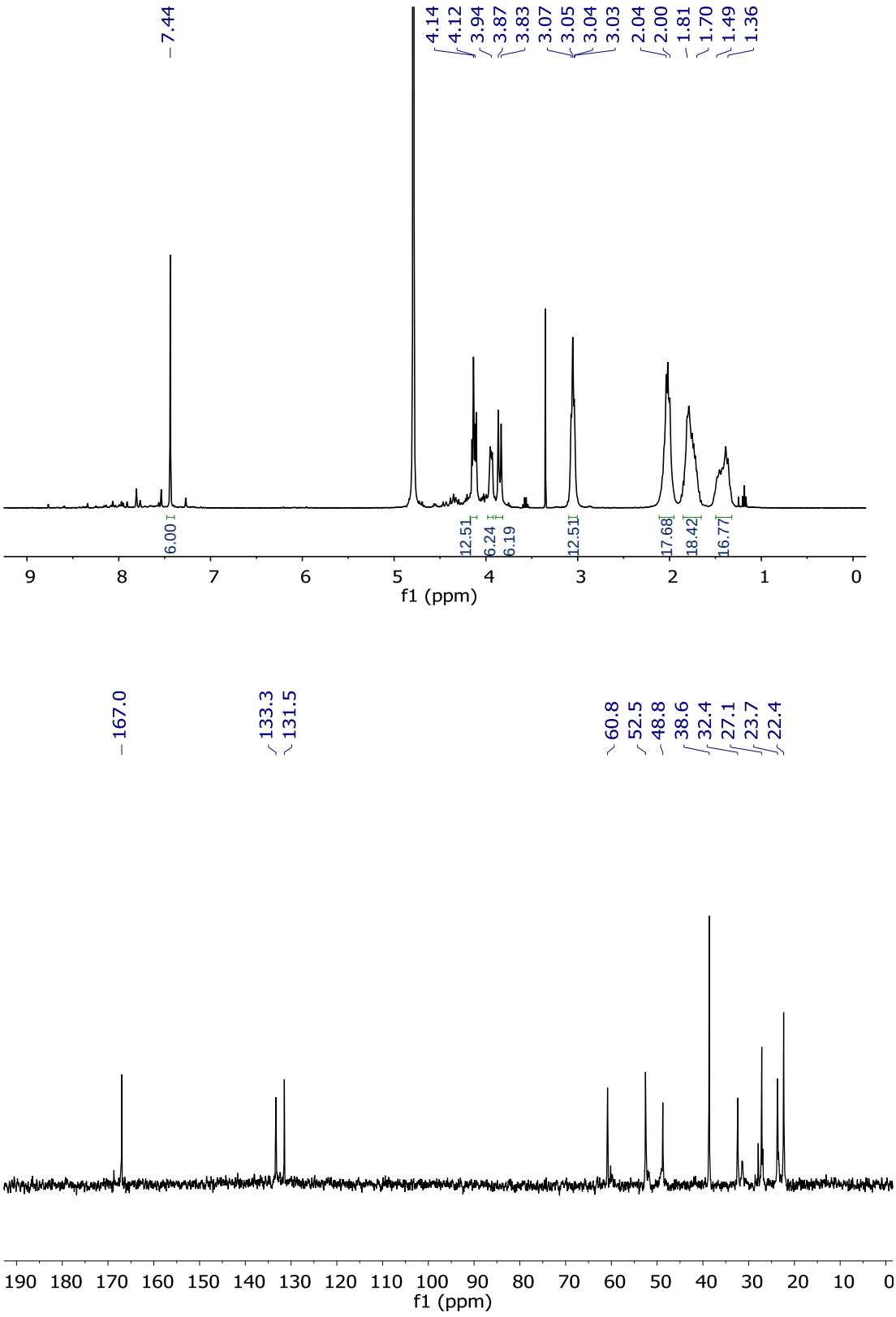


Figure S12. ^{1}H-NMR (400 MHz, D_2O+DCl) and ^{13}C-NMR (101 MHz, D_2O+DCl) spectra of **CyOrn**

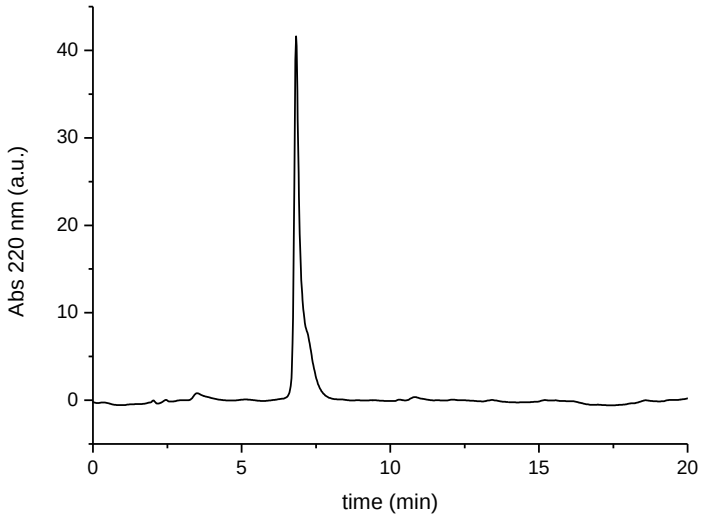


Figure S13. HPLC chromatogram of **CyOrn** (5% to 100% MeCN in water, linear gradient during 20 minutes, with 0.1% TFA in both solvents). Retention time: 6.83 minutes

Figure S14. HRMS (ESI+) spectrum of **CyOrn** (top); simulated spectrum for $[C_{66}H_{115}N_{18}O_6]^+$ (bottom)

Acid-base pH-metric titration of Orn and Lys cages (starting as the corresponding HCl salts, [cage] ~0.5 mM in 150 mM NaCl, titration with 0.1 M NaOH)

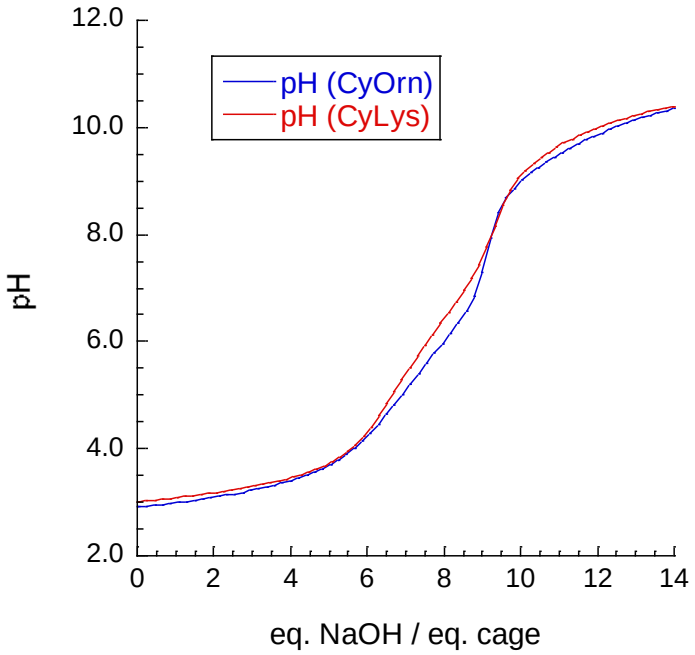


Figure S15. Superposition of the pH-metric titration curves for CyOrn (blue) and CyLys (red).

We started at acidic pH with the fully protonated cages as the HCl salts (with a 20% excess of HCl) and added 0.1 M NaOH as the titrant (with a precision burette automatic system). The titration curve with **CyLys** is above the one with **CyOrn**. This means that upon the addition of the same amount of NaOH, **CyLys** renders a slightly higher pH (**CyLys** is therefore more basic). In other words, in the pH 6-9 range where the curves differ more, **CyLys** has an overall higher protonation degree than **CyOrn**, since the Lys cage reaches those pH values with a slightly lower number of equivalents of NaOH. Therefore, although we were not able to get reliable pKa values, we can confirm that under our experimental conditions **CyLys** is more basic than **CyOrn**. The different basicity of the cages can be correlated to the trend in the successive protonation of polyamines (see, for instance Bencini et al. *Coord. Chem. Rev.* **1999**, 188, 97–156). The pKa values of isolated Orn and Lys are very similar, but when several residues are covalently connected, the pKa values are mutually dependent since the stepwise protonation of a given amine nitrogen will depend on the number and disposition of the other amine groups, already protonated in the molecule. As a rule of thumb, following purely electrostatic reasoning, the closer proximity of positive charges will make the successive protonation of free amines less favorable. Therefore, the longer is the distance between protonable sites, the less important will be the electrostatic repulsion between positive charges. Within this rationale, the cage made of Lys amino acids will be able to set the corresponding protonated nitrogen atoms at a longer distance than the Orn cage. Accordingly, the stepwise protonation of **CyLys** would be more favorable than for **CyOrn**.

3. Characterization of polyE$_4$Y

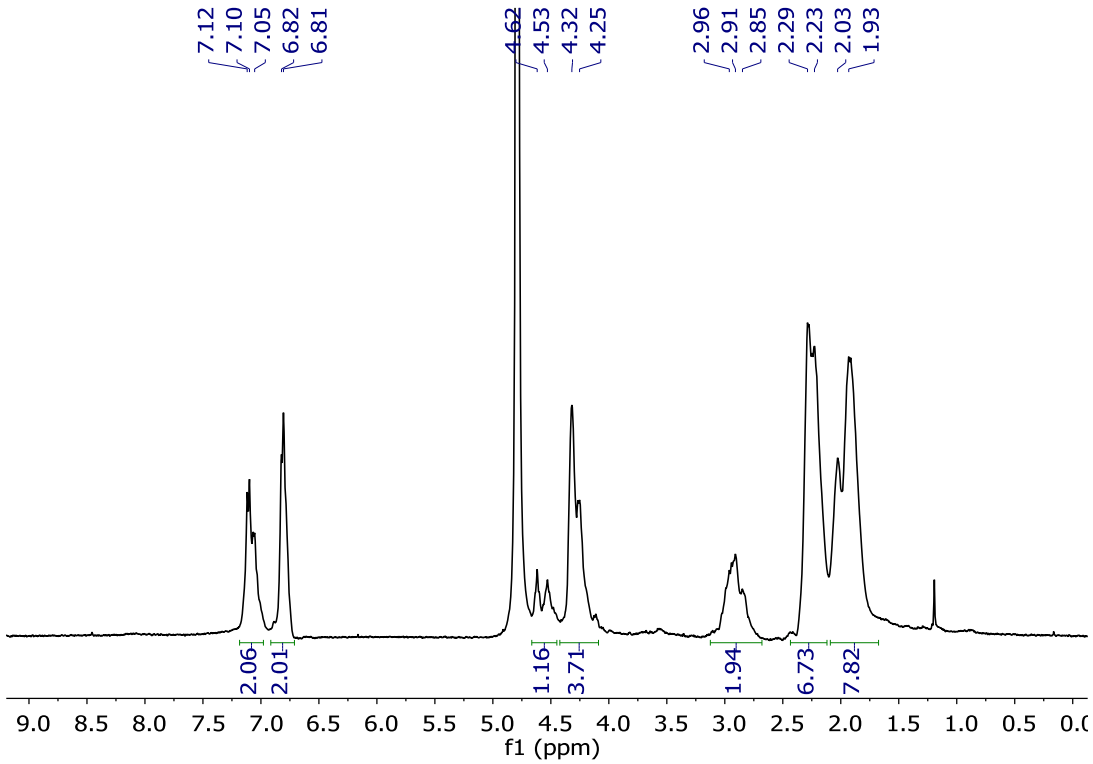


Figure S16. ^{1}H-NMR (400 MHz, D_2O) spectrum of **polyE$_4$Y** (*ca.* 4 mM, tetrasodium salt)

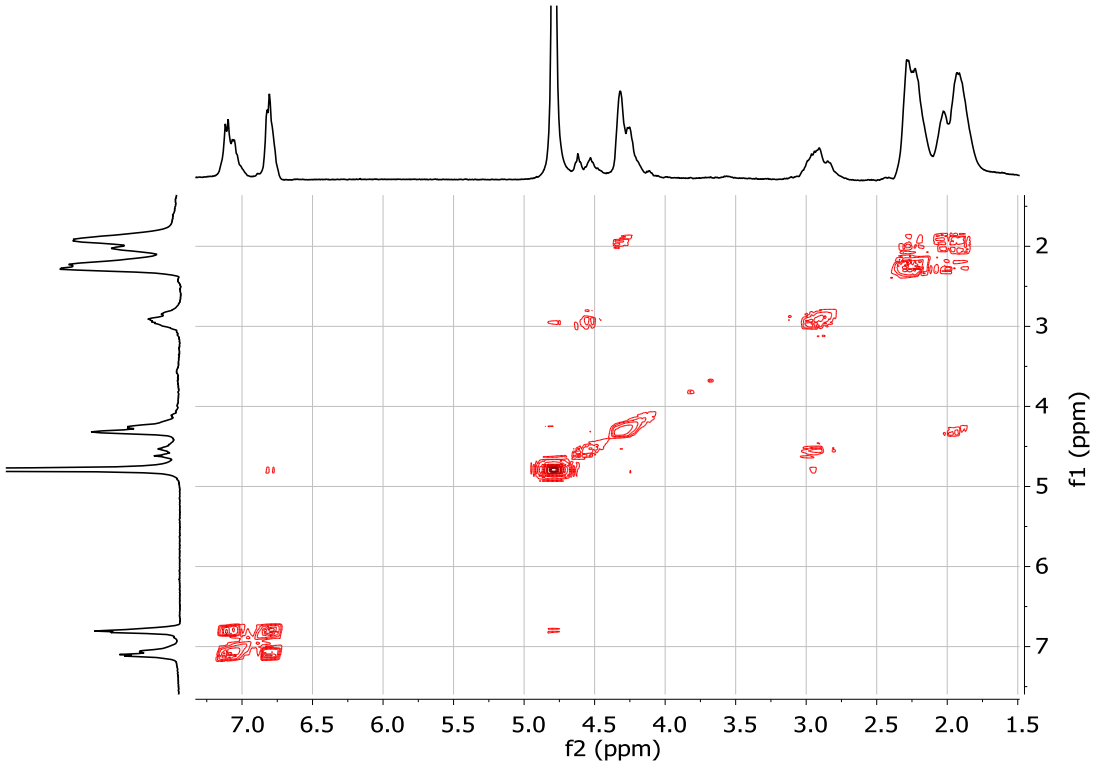


Figure S17. H-H COSY spectrum (400 MHz, D_2O) of **polyE$_4$Y** (tetrasodium salt, *ca.* 4 mM)

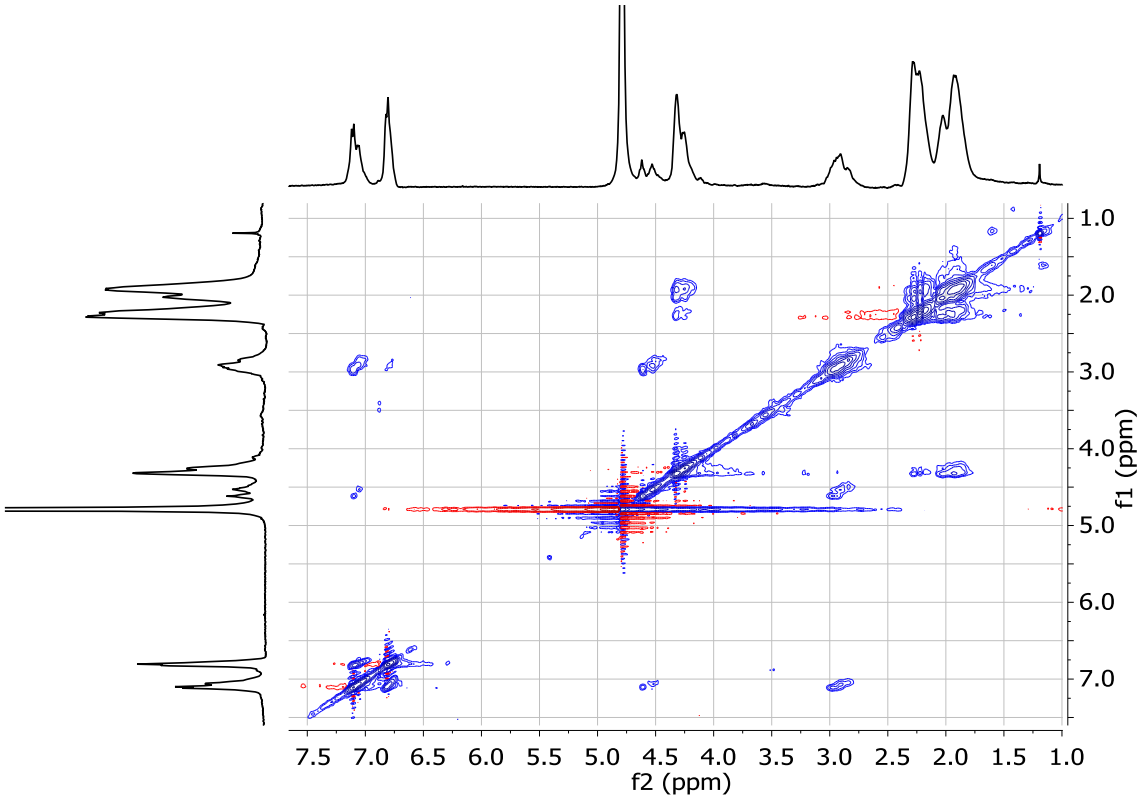


Figure S18. NOESY spectrum (400 MHz, D_2O) of **polyE$_4$Y** (tetrasodium salt, *ca.* 4 mM)

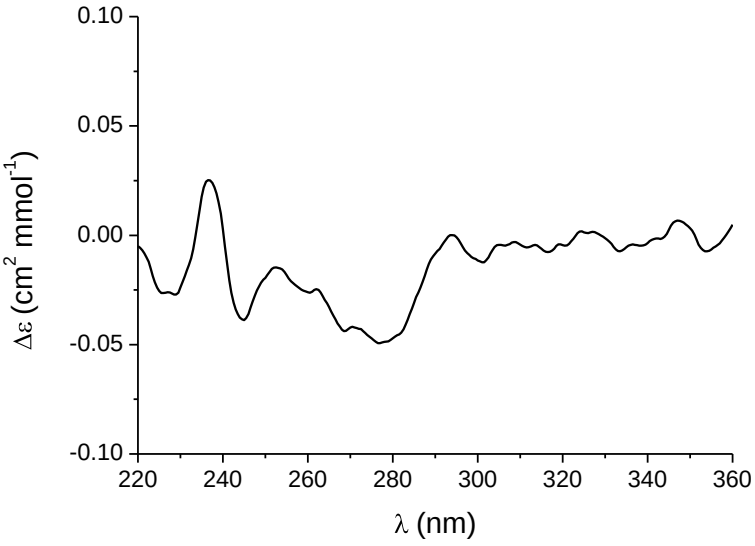


Figure S19. Circular dichroism spectrum of **polyE$_4$Y** (ca. 0.5 mM in 60 mM tris buffer, pH 7.4)

4. Fluorescence Spectroscopy

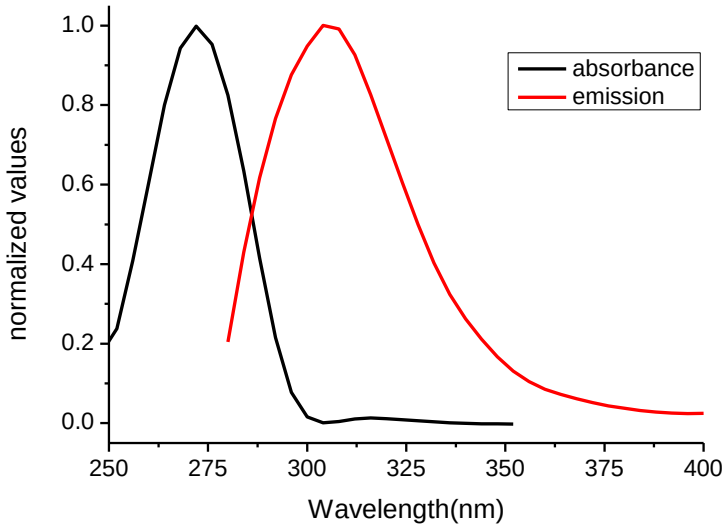


Figure S20. Normalized absorption and emission spectra of **polyE$_4$Y** in buffered water (50 mM TRIS, pH = 7.3)

Fluorescence titrations.

The binding constants were calculated applying equation S1 to the emission band that appears after addition of the cage to the peptide.

$$\Delta F = F - F0 = \left(\frac{\Delta Fmax}{[P]}\right) \times \frac{([P] + [C] + K_D) - \sqrt{([P] + [C] + K_D)^2 - 4[P][C]}}{2}$$

Equation S1. [*P*] and [*C*] are the total concentrations of Peptide and Cage, respectively. *F* is the fluorescence of the system with a given *C* concentration. *F0* is the fluorescence of the system when *C* = 0. *ΔFmax* and K_D are the fitting parametres.

Titration of polyE$_4$Y *vs*. CyOrn

A $2.0 \cdot 10^{-4}$ M solution of **polyE$_4$Y** in buffered water (50 mM TRIS, pH = 7.3) is titrated with a $4 \cdot 10^{-3}$ M solution of **CyOrn** that contains **polyE$_4$Y** ([**polyE$_4$Y**] = $2.0 \cdot 10^{-4}$ M).

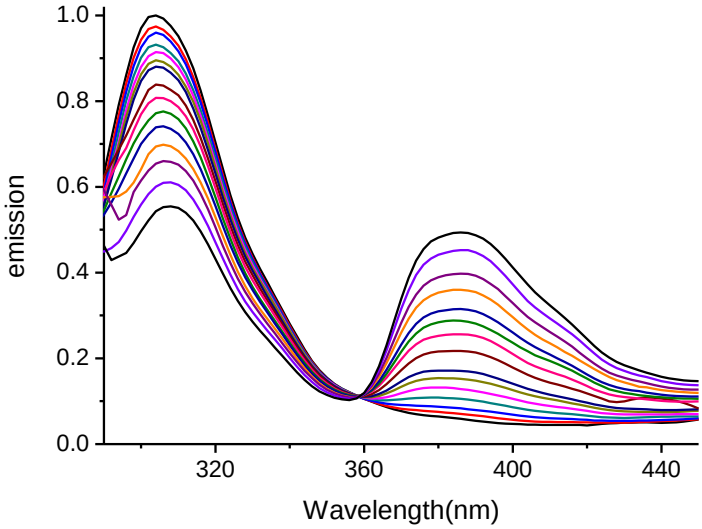


Figure S21. Normalized emission spectra of **polyE$_4$Y** in buffered water (50 mM TRIS, pH 7.3); in absence and in presence of different amounts of **CyOrn** ([**CyOrn**] = 0–$1.33 \cdot 10^{-3}$ M). [**polyE$_4$Y**] = $2 \cdot 10^{-4}$ M; λexc = 276 nm

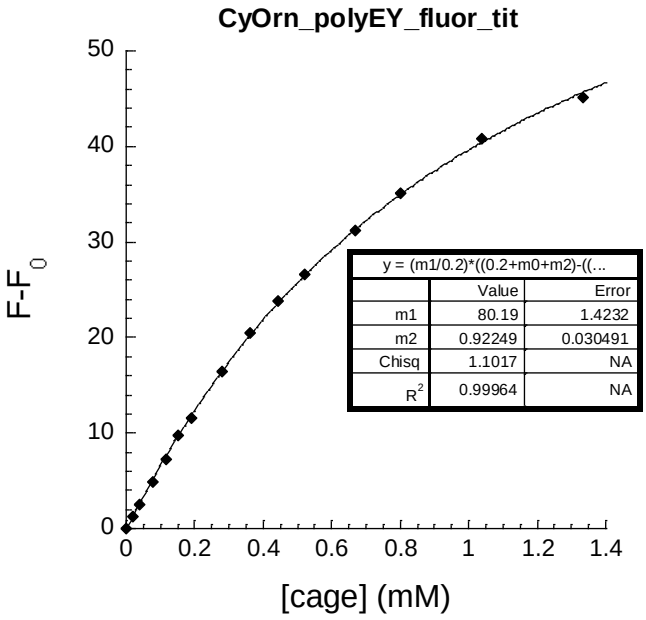


Figure S22. Fitting of the titration of **polyE$_4$Y** (0.2 mM) with **CyOrn** at pH 7.3

Titration of AcEYENH$_2$ *vs*. CyOrn

A 2.0·10^{-4} M solution of **AcEYENH$_2$** in buffered water (50 mM TRIS, pH = 7.3) is titrated with a 4·10^{-3} M solution of **CyOrn** that contains **AcEYENH$_2$** ([**AcEYENH$_2$**] = 2.0·10^{-4} M).

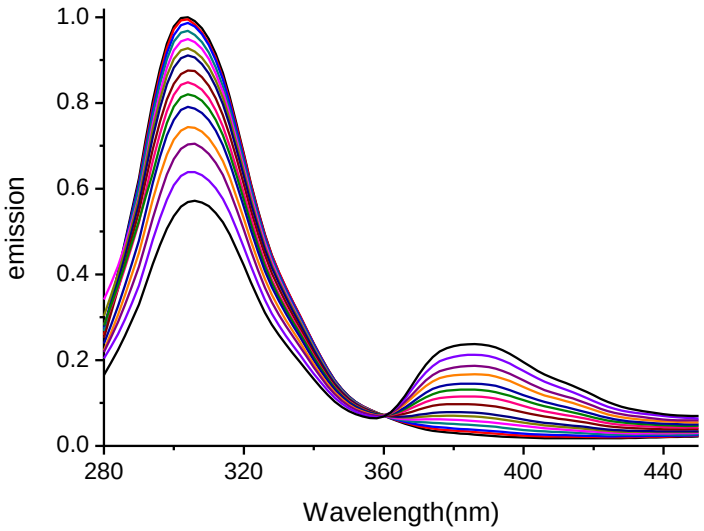


Figure S23. Normalized emission spectra of **AcEYENH$_2$** in buffered water (50 mM TRIS, pH 7.3); in absence and in presence of different amounts of **CyOrn** ([**CyOrn**] = 0–1.33·10^{-3} M). [**AcEYENH$_2$**] = 2·10^{-4} M; λexc = 276 nm

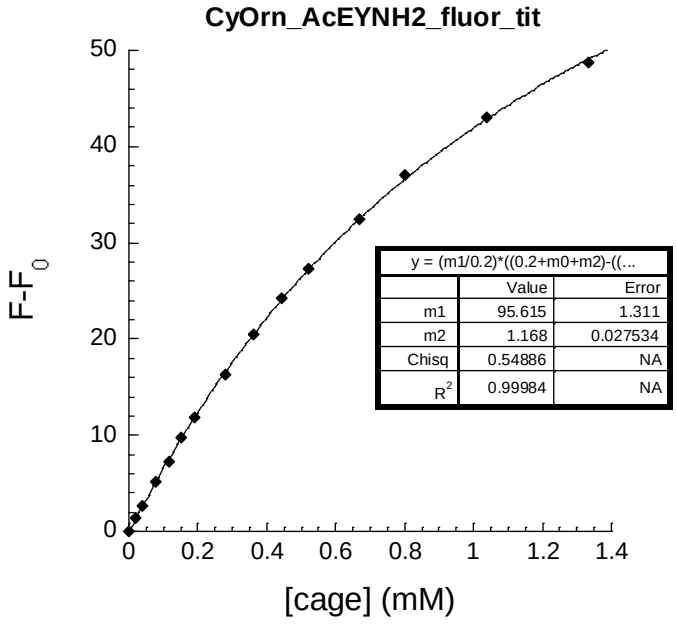


Figure S24. Fitting of the titration of **AcEYNH$_2$** (0.2 mM) *vs.* **CyOrn** at pH 7.3

Titration of polyE$_4$Y *vs*. CyLys pH 7.3

A 4.0·10^{-5} M solution of **polyE$_4$Y** in buffered water (50 mM TRIS, pH = 7.3) is titrated with a 8·10^{-4} M solution of **CyLys** that contains **polyE$_4$Y** ([**polyE$_4$Y**] = 4.0·10^{-5} M).

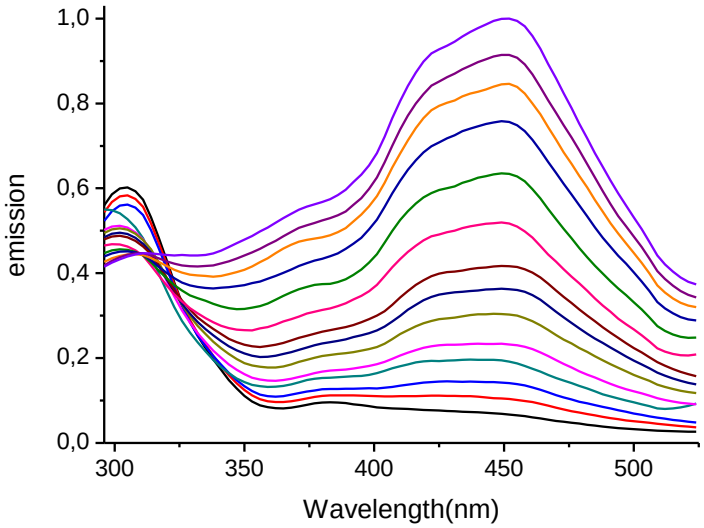


Figure S25. Normalized emission spectra of **polyE$_4$Y** in buffered water (50 mM TRIS, pH 7.3); in absence and in presence of different amounts of **CyLys** ([**CyLys**] = 0–0.329·10^{-3} M). [**polyE$_4$Y**] = 4·10^{-5} M; λexc = 276 nm

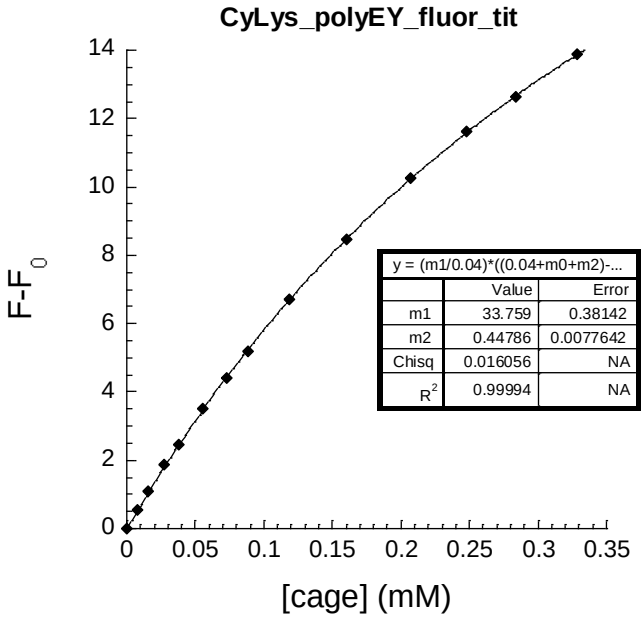


Figure S26. Fitting of the titration of **polyE$_4$Y** (0.04 mM) *vs.* **CyLys** at pH 7.3

Titration of AcEYENH$_2$ *vs*. CyLys

A 4.0·10^{-5} M solution of **AcEYENH$_2$** in buffered water (50 mM TRIS, pH = 7.3) is titrated with a 8·10^{-4} M solution of **CyLys** that contains **AcEYENH$_2$** ([**AcEYENH$_2$**] = 4.0·10^{-5} M).

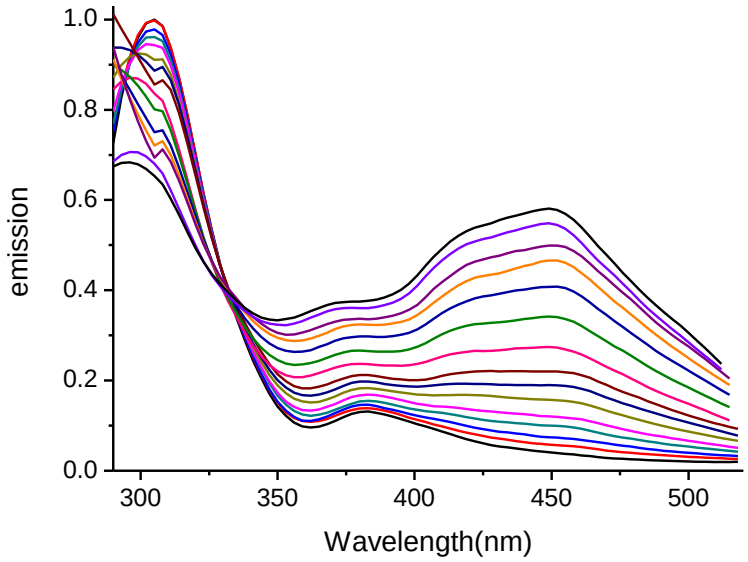


Figure S27. Normalized emission spectra of **AcEYENH$_2$** in buffered water (50 mM TRIS, pH 7.3); in absence and in presence of different amounts of **CyLys** ([**CyLys**] = 0–0.367·10^{-3} M). [**AcEYENH$_2$**] = 4·10^{-5} M; λexc = 276 nm.

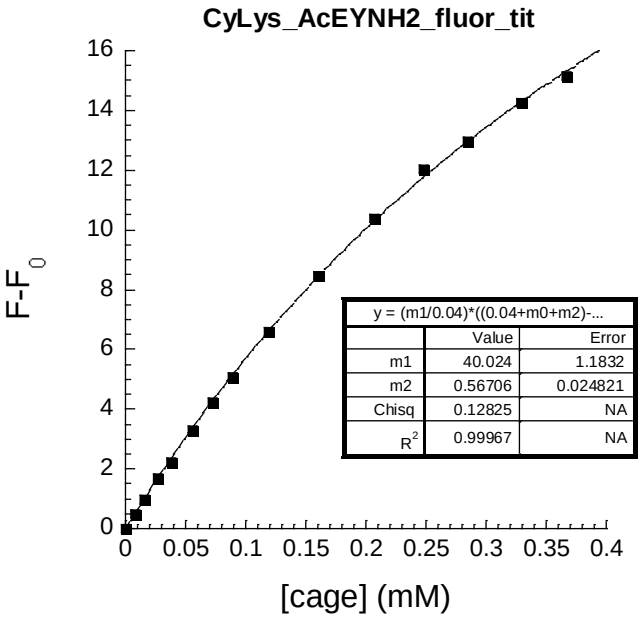


Figure S28. Fitting of the titration of **AcEYENH$_2$** (0.04 mM) *vs.* **CyLys** at pH 7.3

Titration of polyE$_4$Y *vs*. CyLys pH 5.3

A 4.0·10^{-5} M solution of **polyE$_4$Y** in buffered water (50 mM acetate, pH = 5.3) is titrated with a 8·10^{-4} M solution of **CyLys** that contains **polyE$_4$Y** ([**polyE$_4$Y**] = 4.0·10^{-5} M).

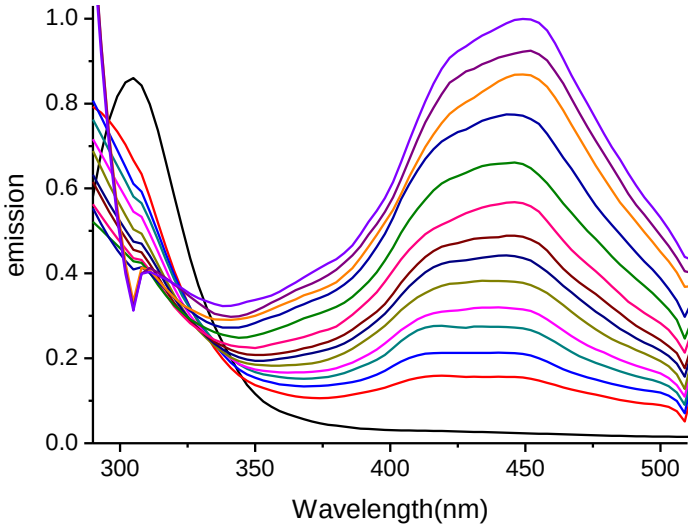


Figure S29. Normalized emission spectra of **polyE$_4$Y** in buffered water (50 mM acetate, pH 5.3); in absence and in presence of different amounts of **CyLys** ([**CyLys**] = 0–0.329·10^{-3} M). [**polyE$_4$Y**] = 4·10^{-5} M; λexc = 276 nm.

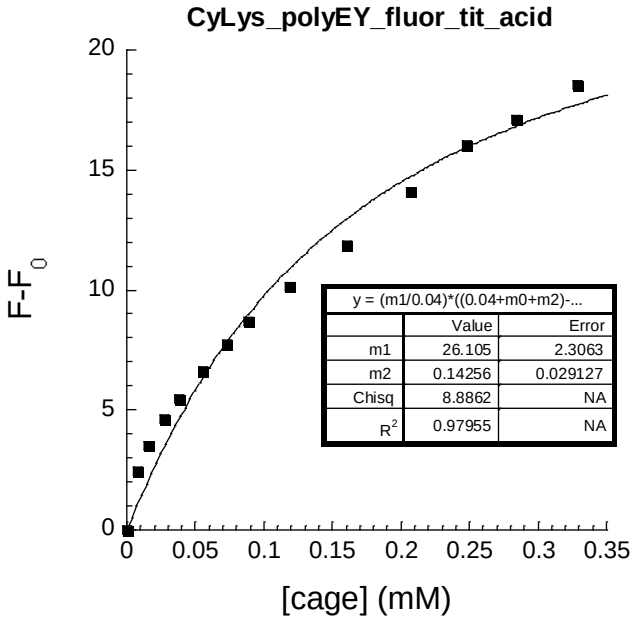


Figure S30. Fitting of the titration of **polyE$_4$Y** (0.04 mM) *vs.* **CyLys** at pH 5.3

Titration of polyE$_4$Y *vs*. CyLys pH 8.7

A 4.0·10^{-5} M solution of **polyE$_4$Y** in buffered water (50 mM TRIS, pH = 8.7) is titrated with a 8·10^{-4} M solution of **CyLys** that contains **polyE$_4$Y** ([**polyE$_4$Y**] = 4.0·10^{-5} M).

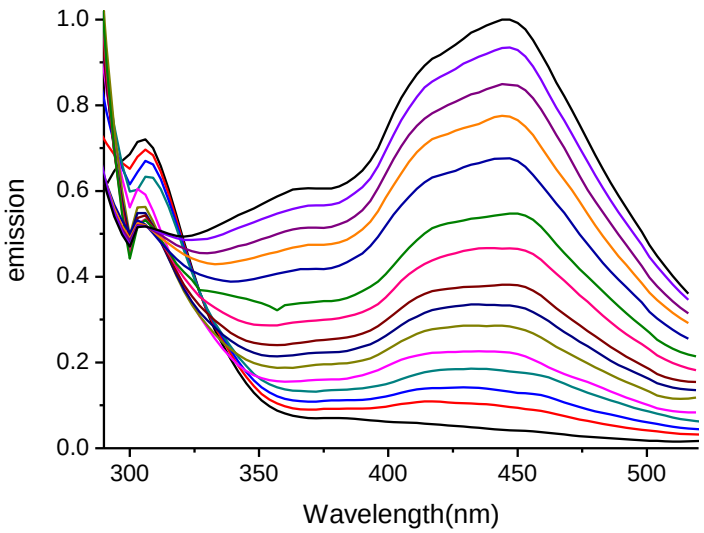


Figure S31. Normalized emission spectra of **polyE$_4$Y** in buffered water (50 mM TRIS, pH 8.7); in absence and in presence of different amounts of **CyLys** ([**CyLys**] = 0–0.367·10^{-3} M). [**polyE$_4$Y**] = 4·10^{-5} M; λexc = 276 nm.

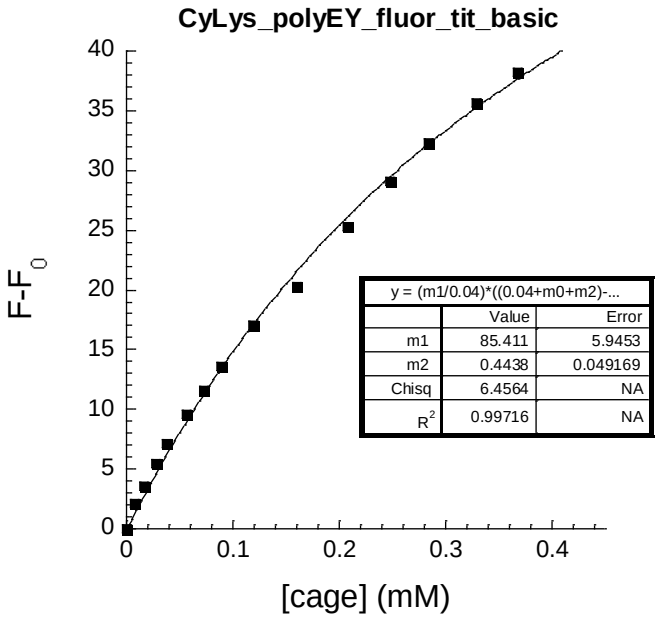


Figure S32. Fitting of the titration of **polyE$_4$Y** (0.04 mM) *vs.* **CyLys** at pH 8.7

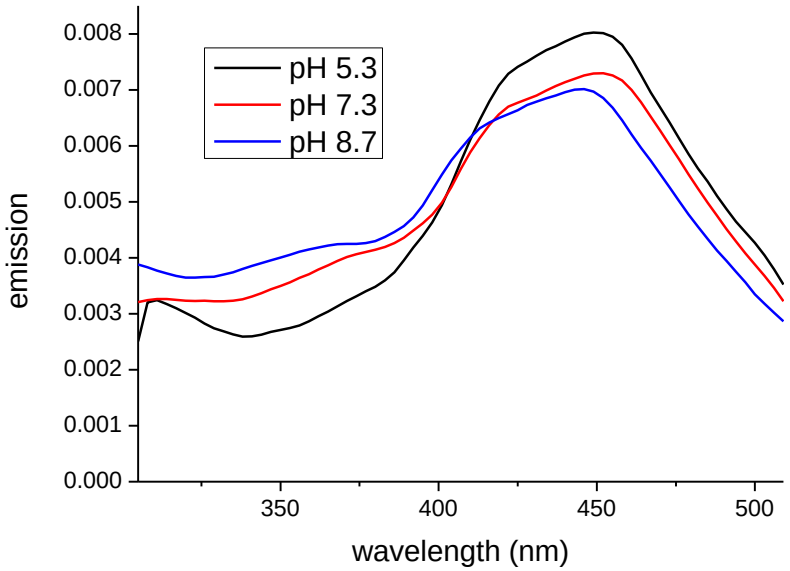


Figure S33. Emission spectra of **polyE$_4$Y** ($4 \cdot 10^{-5}$ M) in presence of **CyLys** ($3.29 \cdot 10^{-4}$ M) at different pHs. Spectra were normalized by their area in the 305-510 nm range.

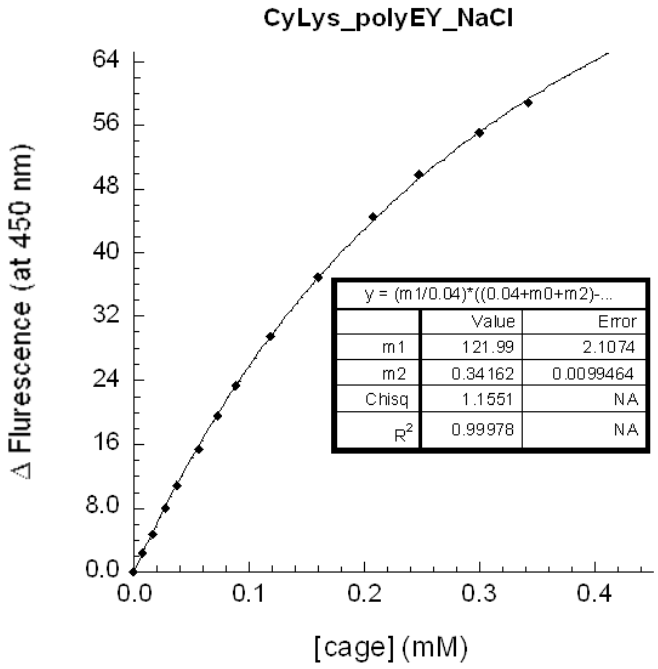


Figure S34. Fitting of the titration of **polyE$_4$Y** (0.04 mM) *vs.* **CyLys** at pH 7.3 in the presence of 150 mM of NaCl.

Titration of AcYEEINH$_2$ *vs*. CyLys

A 4.0·10^{-5} M solution of **AcYEEINH$_2$** in buffered water (60 mM TRIS, pH = 7.4) is titrated with a 8·10^{-4} M solution of **CyLys** that contains **AcYEEINH$_2$** ([**AcYEEINH$_2$**] = 4.0·10^{-5} M).

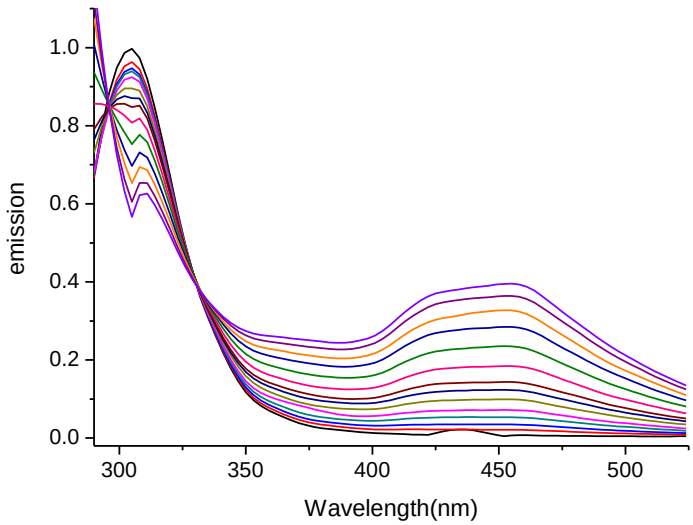


Figure S35. Normalized emission spectra of **Ac-YEEI-NH$_2$** in buffered water (60 mM TRIS, pH 7.4); in absence and in presence of different amounts of **CyLys** ([**CyLys**] = 0–0.343·10^{-3} M). [**Ac-YEEI-NH$_2$**] = 4·10^{-5} M; λexc = 276 nm.

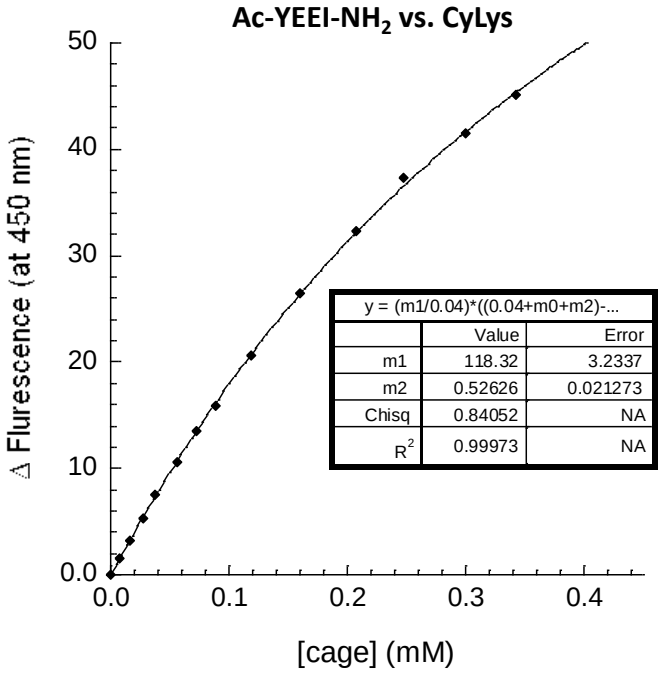


Figure S36. Fitting of the titration of **Ac-YEEI-NH$_2$** (0.04 mM) *vs.* **CyLys** at pH 7.3

Titration of AcEEEIYEEFDNH$_2$ *vs*. CyLys

A 4.0·10^{-5} M solution of **AcEEEIYEEFDNH$_2$** in buffered water (60 mM TRIS, pH = 7.4) is titrated with a 8·10^{-4} M solution of **CyLys** that contains **AcEEEIYEEFDNH$_2$** ([**AcEEEIYEEFDNH$_2$**] = 4.0·10^{-5} M).

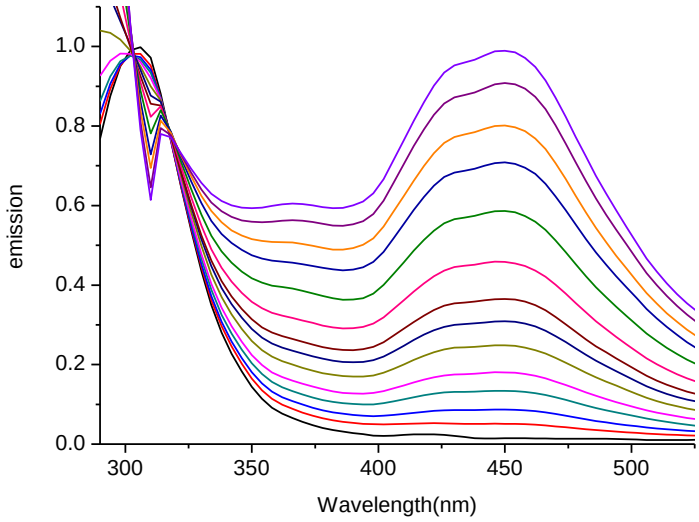


Figure S37. Normalized emission spectra of **AcEEEIYEEFDNH$_2$** in buffered water (60 mM TRIS, pH 7.4); in absence and in presence of different amounts of **CyLys** ([**CyLys**] = 0–0.343·10^{-3} M). [**AcEEEIYEEFDNH$_2$**] = 4·10^{-5} M; λexc = 276 nm.

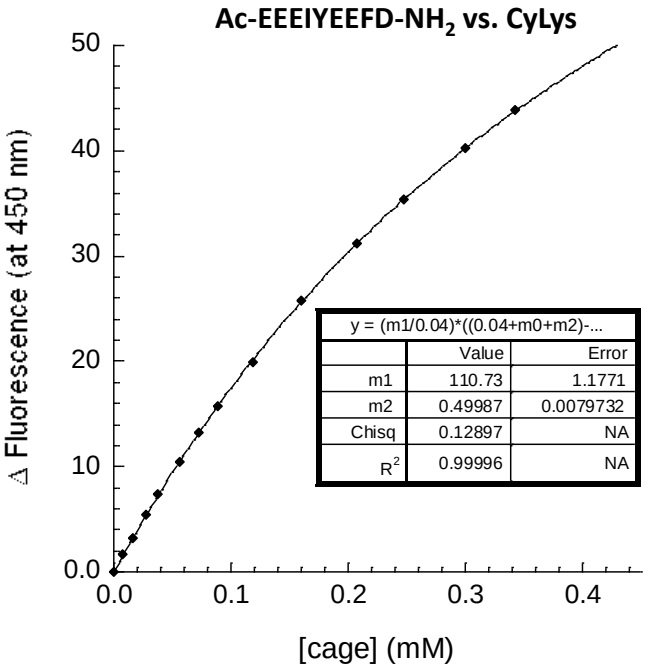


Figure S38. Fitting of the titration of **Ac-EEEIYEEFD-NH$_2$** (0.04 mM) *vs.* **CyLys** at pH 7.3.

5. ^{1}H NMR titration procedure and data

The titrations were performed with the cage receptors as free amines. Stock solutions of the cages were prepared by weighting the corresponding amount of the receptor and dissolving it in buffered D_2O until reaching a final concentration of 0.4 mM.

For the preparation of buffered D_2O: 75 mM tris-d_{11} solution was obtained by dissolving 29.7 mg of tris(hydroxymethyl-d_3)amino-d_2-methane in 3.0 ml of D_2O; pD was adjusted to 7.40 by addition of small amounts of DCl, until the pH reading was 7.00. The following equation was taken into account:

pD = pH* + 0.40 (where pH* is the pH reading of D_2O solution).[3]

In both titrations stock solutions of the titrant containing 3.0 mM polyE_4Y (sodium salt, MW = 5000-20000; concentration calculated on the basis of the molecular weight of the repeating unit: 785.5) were prepared by dissolving polyE_4Y sodium salt in the stock solution of the corresponding cage, thus maintaining the concentration of the cage constant during the titration experiment. The stock solution of the cage was introduced in a NMR tube and the ^{1}H NMR spectrum (500 MHz, 298 K) was acquired, then volumes of the stock solution of the titrant were added and the ^{1}H NMR spectrum recorded after each addition. 12 spectra were acquired.
Sequence used: water-ES excitation sculpting (DPFGSE)
t1rho pulse duration: 50.0 ms; power = 43.0
512 scans; gain = 60.

Different signals shifted upon addition of polyE_4Y, and their shifts were fitted to a logistic model using OriginPro 8.0 software.

Equation used for the the logistic fit:

$$y = ((A_1-A_2)/1+ (x-x_0)^p) + A_2$$

where:
A_1 = initial value
A_2 = final value
x_0 = center (inflection point)
p = power ($\approx K_{ass} = 1/K_d$)

[3] a) E. W. Baumann *Anal. Chem*. **1966**, *38*, 1255; b) A. K. Covington, M. Paabo, R. A. Robinson, R. G. Bates *Anal. Chem*. **1968**, *40*, 700.

Titration of CyLys *vs.* polyE$_4$Y

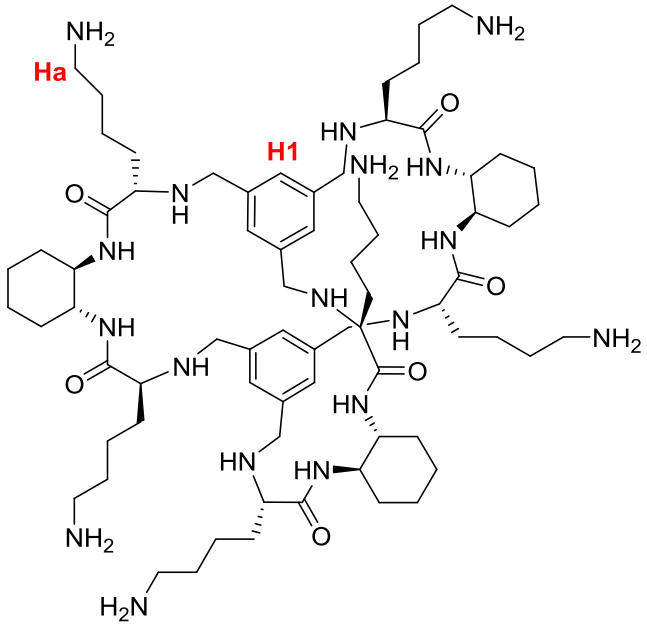


Figure S39: Structure of **CyLys** with the arbitrary lettering used to indicate protons

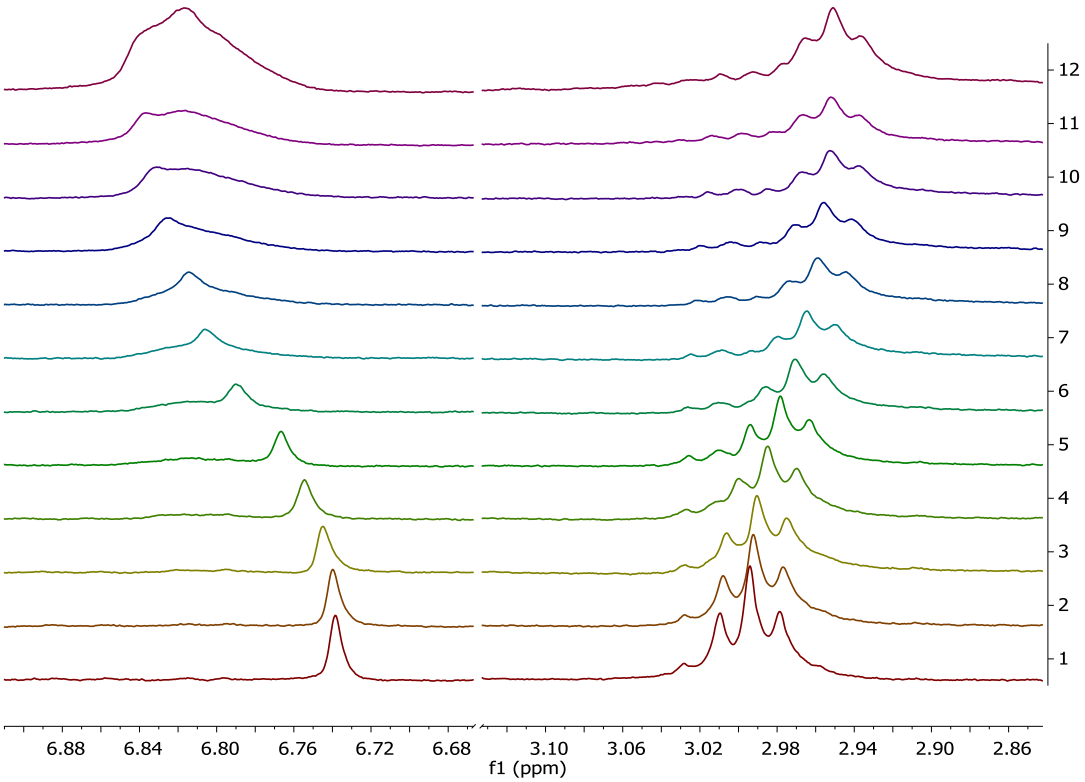


Figure S40. Selected ranges of the ^{1}H-NMR spectra of the titration **CyLys** *vs.* **polyE$_4$Y**. From bottom to top, [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the MW of the repeating unit), while [**CyLys**] is kept constant (0.40 mM)

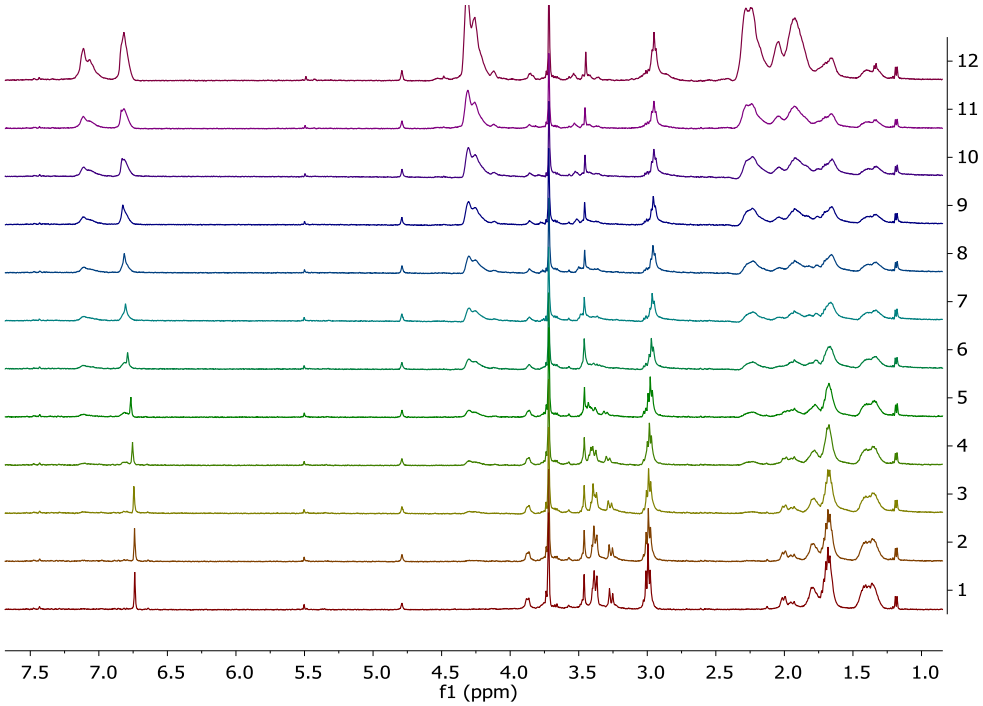


Figure S41. Stacked spectra of the titration **CyLys** *vs.* **polyE$_4$Y**. From bottom to top [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the monomer MW), while [**CyLys**] is kept constant (0.40 mM)

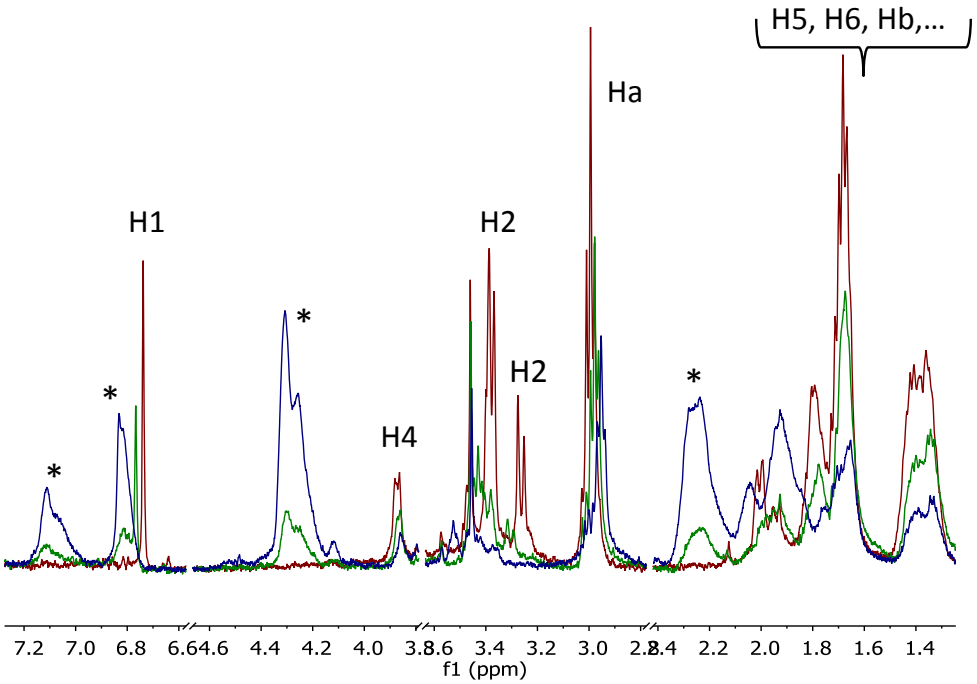


Figure S42. Superimposed partial T1ρ-filtered ^{1}H NMR spectra of **CyLys** (D_2O, 75 mM TRIS buffer, pD 7.4) alone (red) and with increasing amounts of **polyE$_4$Y** (green, blue). Signals marked with an asterisk correspond to the **polyE$_4$Y** molecule.

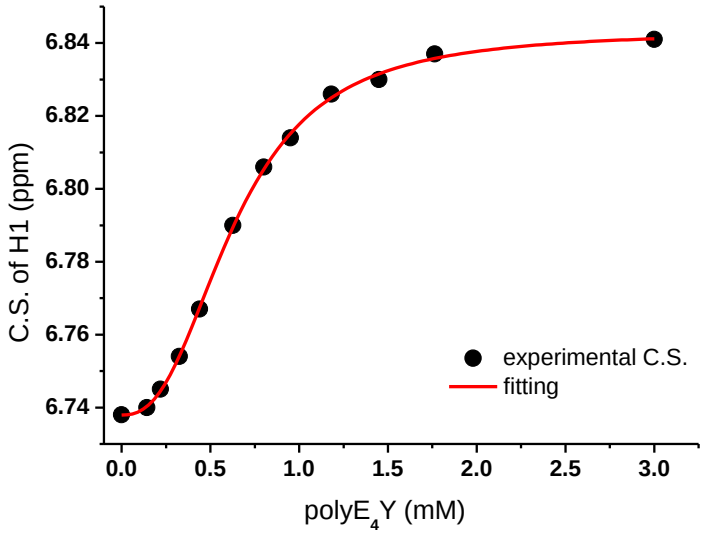


Figure S43. Chemical shift of the H1 proton signal as a function of the concentration of **polyE$_4$Y** (calculated according to the MW of the repeating unit, black circles) and logistic fitting (red line)

Fitting details:

Model: Logistic
Equation: y = A2 + (A1-A2)/(1 + (x/x0)^p)
Reduced Chi-Sqr: 9.29429E-7
Adj. R-Square: 0.99942

	Value	Standard Error
A1	6.73796	7.05094E-4
A2	6.84323	9.67627E-4
x0	0.63733	0.00822
p	2.5308	0.07477

Titration of CyOrn *vs.* polyE$_4$Y

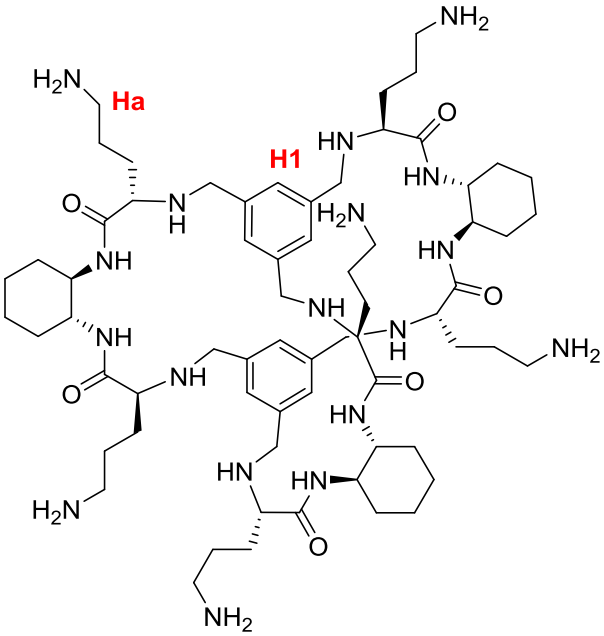


Figure S44. Structure of **CyOrn** with the arbitrary lettering used to indicate protons.

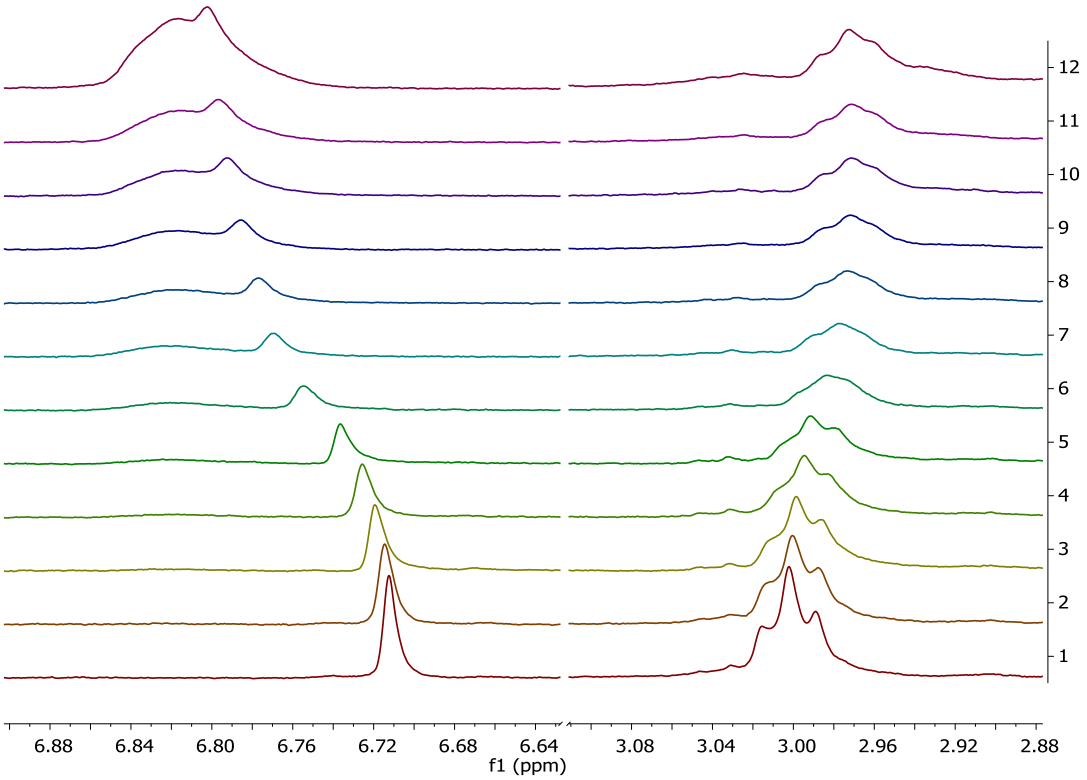


Figure S45. Selected ranges of the ^{1}H-NMR spectra of the titration **CyOrn** *vs.* **polyE$_4$Y**. From bottom to top [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the MW of the repeating unit), while [**CyOrn**] is kept constant (0.40 mM)

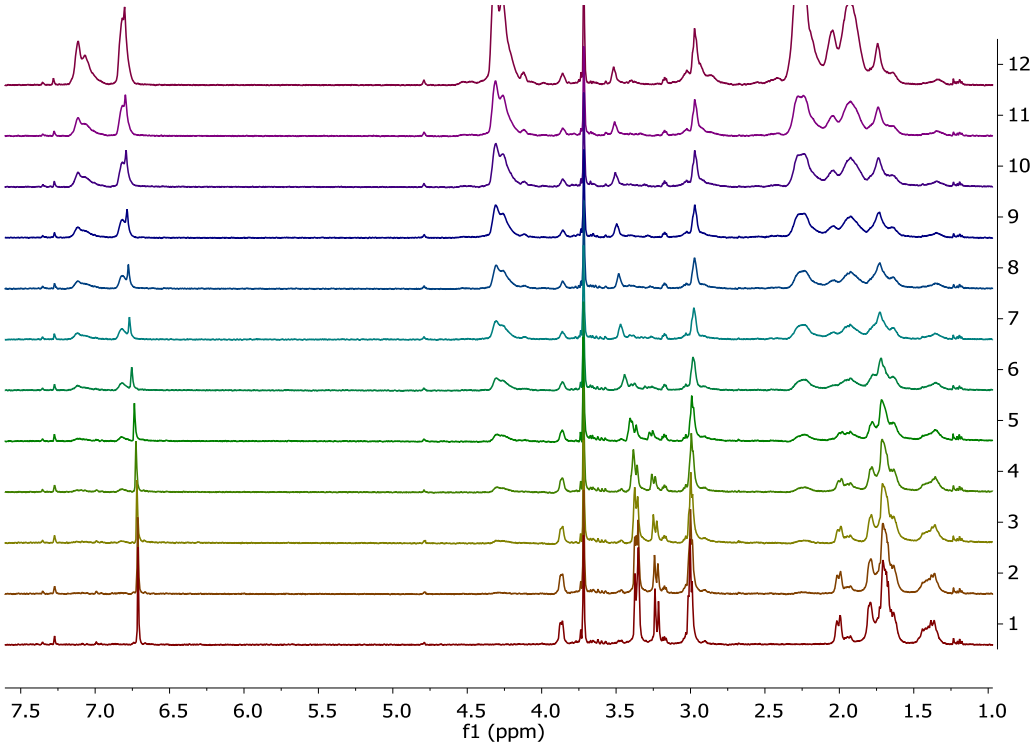


Figure S46. Stacked spectra of the titration **CyOrn** *vs.* **polyE$_4$Y**. From bottom to top [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the monomer MW), while [**CyOrn**] is kept constant (0.40 mM).

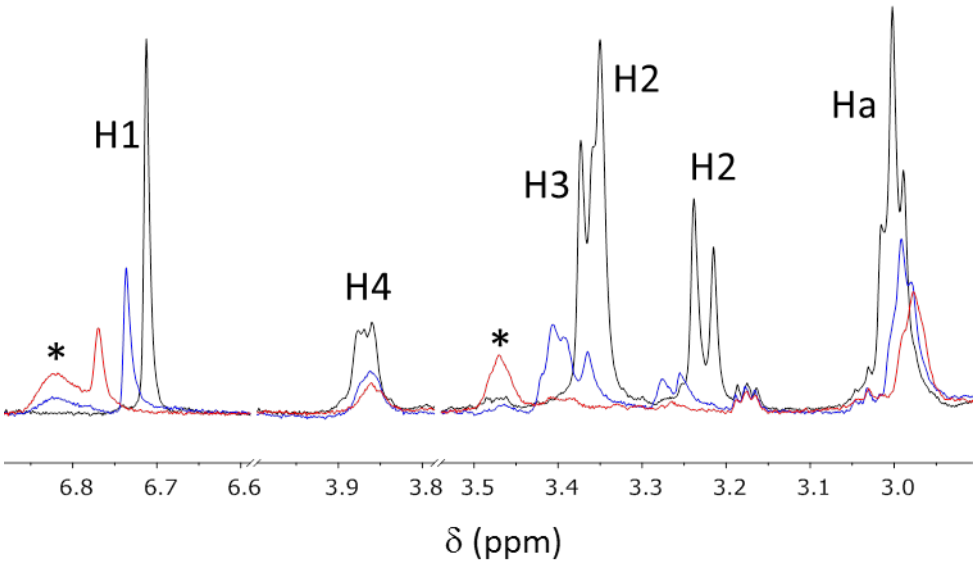


Figure S47. Superimposed partial T1ρ-filtered ^{1}H NMR spectra of **CyOrn** (D_2O, 75 mM TRIS buffer, pD 7.4) alone (black) and with increasing amounts of **polyE$_4$Y** (blue, red). Signals marked with an asterisk correspond to the **polyE$_4$Y** molecule

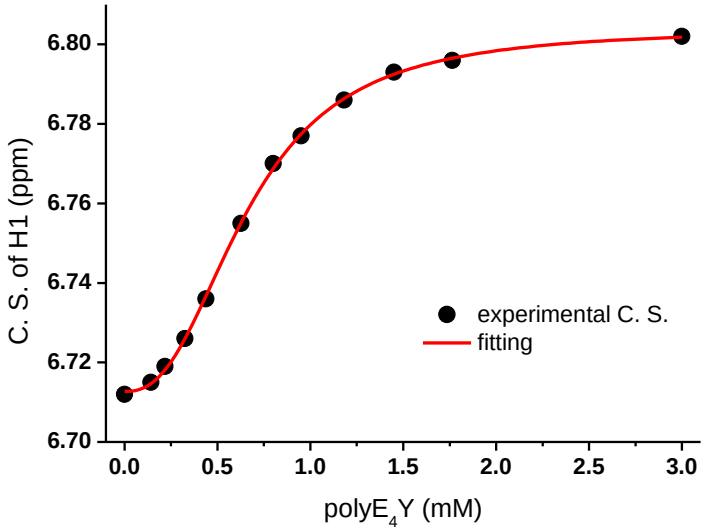


Figure S48. Chemical shift of the H1 proton signal as a function of the concentration of **polyE$_4$Y** (calculated according to the MW of the repeating unit, black circles) and logistic fitting (red line).

Fitting details:

Model: Logistic
Equation: y = A2 + (A1-A2)/(1 + (x/x0)^p)
Reduced Chi-Sqr: 5.50484E-7
Adj. R-Square: 0.99953

	Value	Standard Error
A1	6.71272	5.40486E-4
A2	6.80392	7.87419E-4
x0	0.66302	0.00781
p	2.48214	0.06609

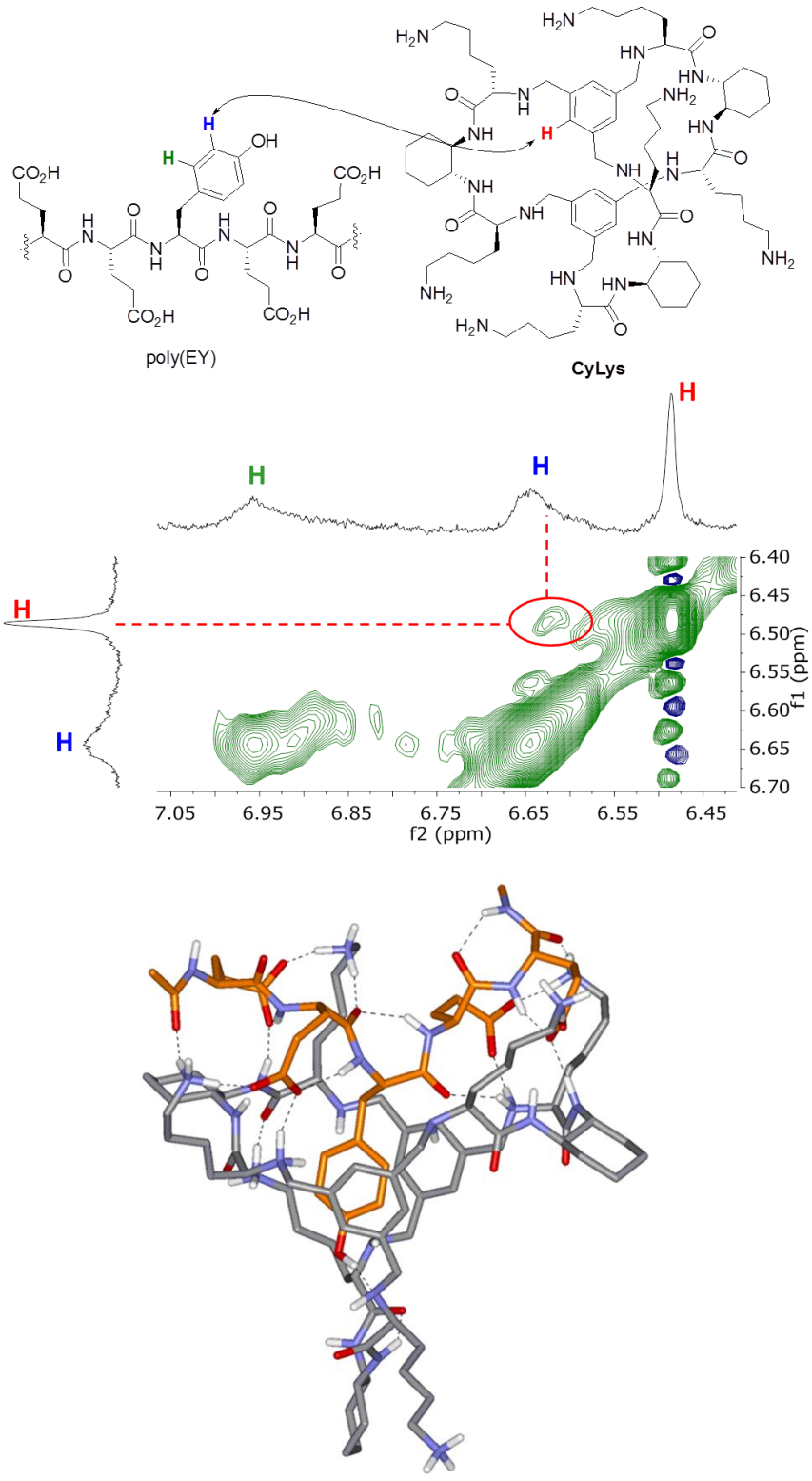


Figure S49. Selected area of the NOESY spectrum of **polyE$_4$Y** : **CyLys** 1 : 1 mixture in buffered D_2O and proposed model for the interaction between the EYE epitope and the **CyLys** cage

6. Binding studies of polyE4Y with CySer/CyThr

Fluorescence titration of polyE$_4$Y *vs*. CySer

A 2.0·10^{-4} M solution of **polyE$_4$Y** in buffered water (50 mM TRIS, pH = 7.3) is titrated with a 4·10^{-3} M solution of **CySer** that contains **polyE$_4$Y** ([**polyE$_4$Y**] = 2.0·10^{-4} M).

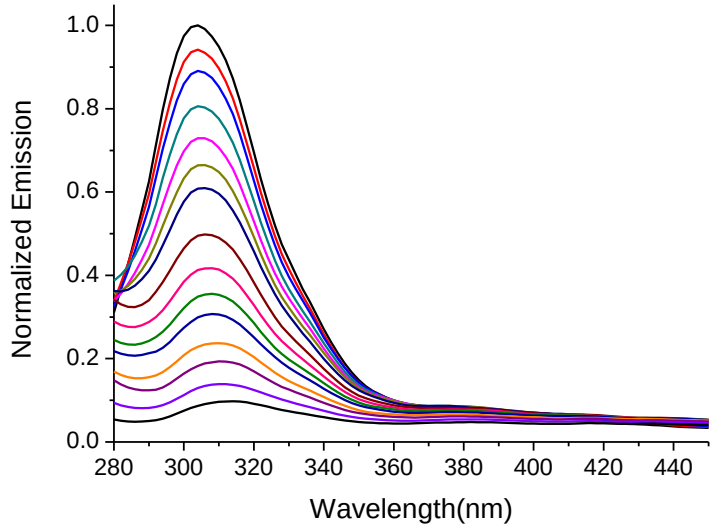


Figure S50. Normalized emission spectra of **polyE$_4$Y** in buffered water (50 mM TRIS, pH 7.3); in absence and in presence of different amounts of **CySer** ([**CySer**] = 0–1.33·10^{-3} M). [**polyE$_4$Y**] = 2·10^{-4} M; λexc = 276 nm. The experiment did not show the exciplex emission band.

Fluorescence titration of polyE$_4$Y *vs*. CyThr

A 2.0·10^{-4} M solution of **polyE$_4$Y** in buffered water (50 mM TRIS, pH = 7.3) is titrated with a 4·10^{-3} M solution of **CyThr** that contains **polyE$_4$Y** ([**polyE$_4$Y**] = 2.0·10^{-4} M).

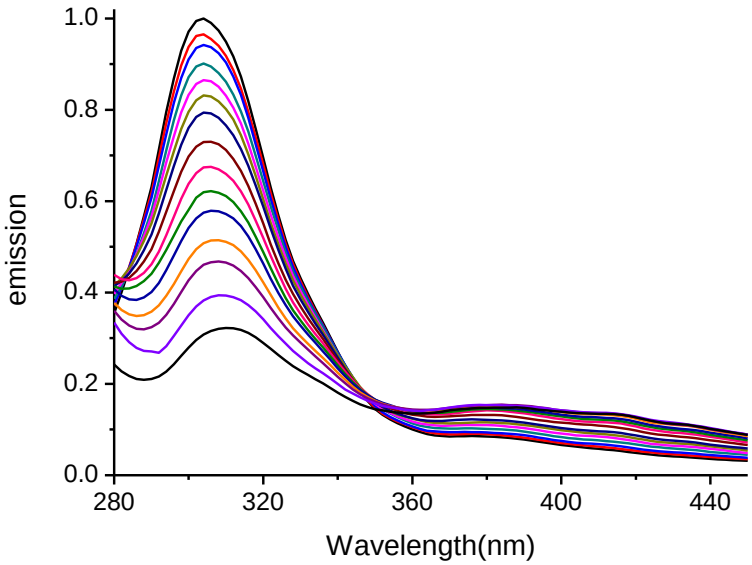


Figure S51. Normalized emission spectra of **polyE$_4$Y** in buffered water (50 mM TRIS, pH 7.3); in absence and in presence of different amounts of **CyThr** ([**CyThr**] = 0–1.33·10^{-3} M). [**polyE$_4$Y**] = 2·10^{-4} M; λexc = 276 nm. The experiment showed a very weak exciplex emission band.

^{1}H-NMR Titration of CySer *vs.* polyE$_4$Y

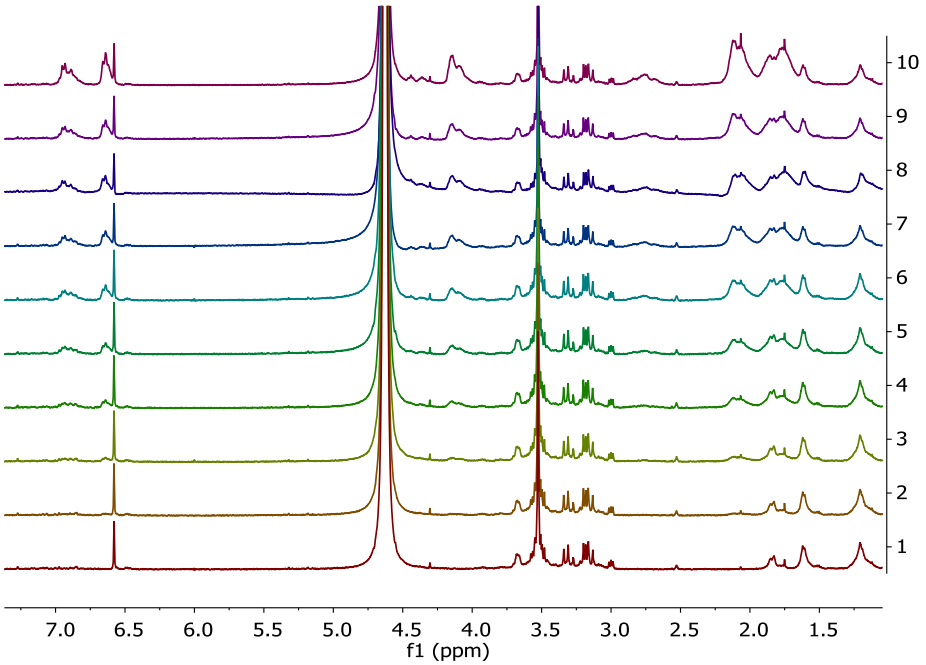


Figure S52. Stacked spectra of the titration **CySer** *vs.* **polyE$_4$Y**. From bottom to top [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the monomer MW), while [**CySer**] is kept constant (0.40 mM)

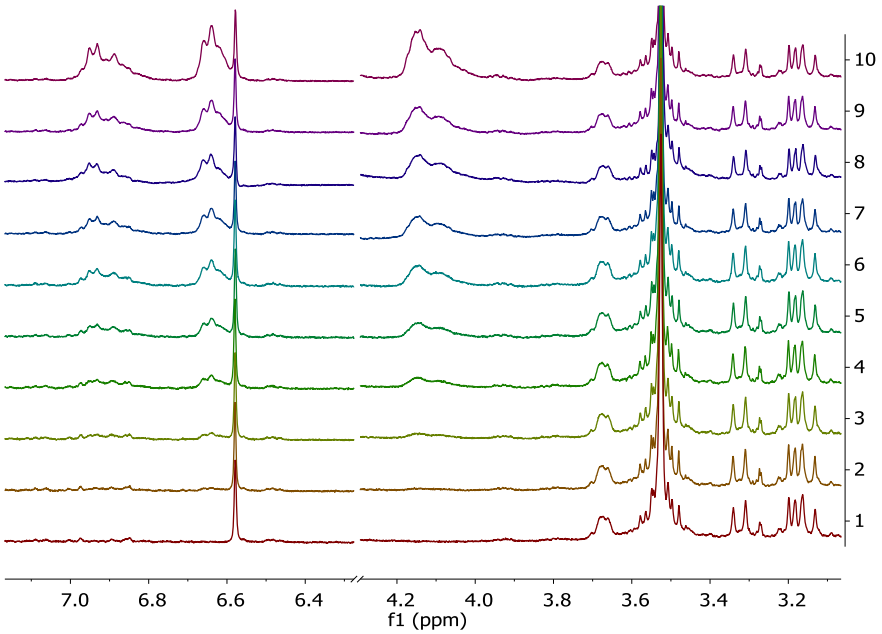


Figure S53. Selected ranges of the ^{1}H-NMR spectra of the titration **CySer** *vs.* **polyE$_4$Y**. From bottom to top [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the MW of the repeating unit), while [**CySer**] is kept constant (0.40 mM). No changes were observed in the chemical shifts of the cage during the titration.

^{1}H-NMR Titration of CyThr *vs.* polyE$_4$Y

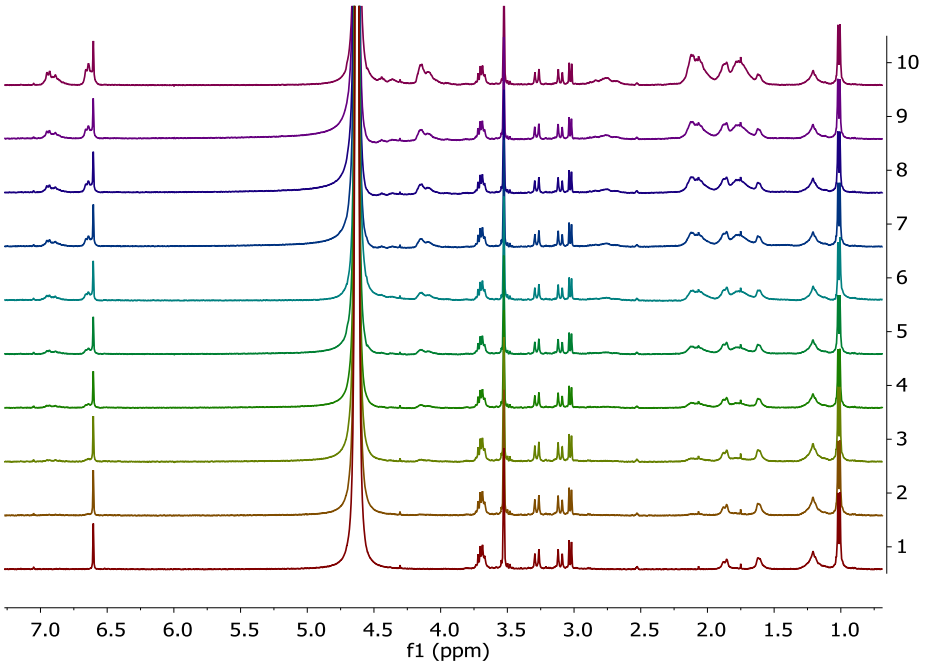


Figure S54. Stacked spectra of the titration **CyThr** *vs.* **polyE$_4$Y**. From bottom to top [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the monomer MW), while [**CyThr**] is kept constant (0.40 mM)

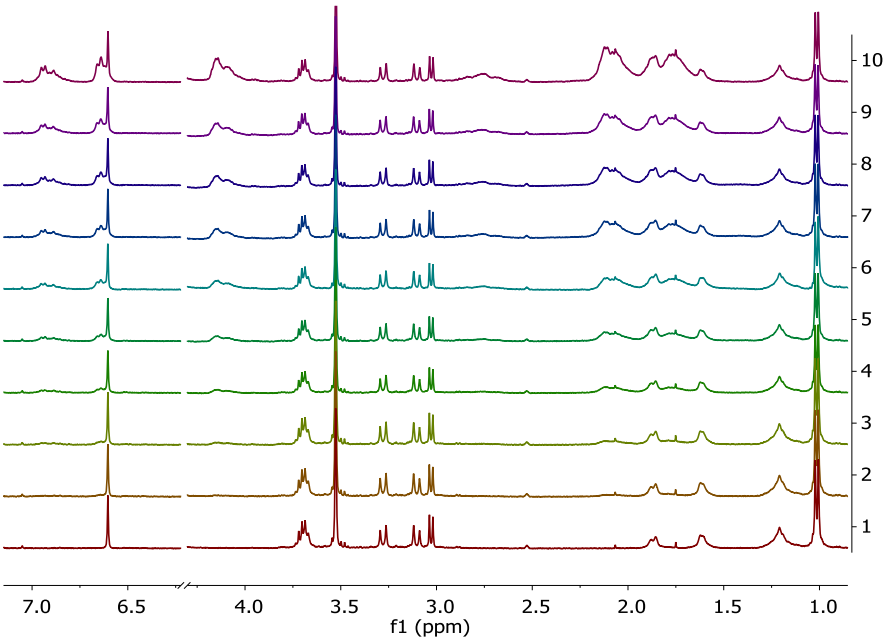


Figure S55. Selected ranges of the ^{1}H-NMR spectra of the titration **CyThr** *vs.* **polyE$_4$Y**. From bottom to top [**polyE$_4$Y**] increases from 0 to 3.00 mM (calculated according to the MW of the repeating unit), while [**CyThr**] is kept constant (0.40 mM) No changes were observed in the chemical shifts of the cage during the titration.

7. Kinase Activity Assay

Experiments were carried out using the commercially available Takara Universal Tyrosine Kinase Assay Kit.[4] The kit was developed to determine the activity of tyrosine kinase proteins (present, for example, in cell extracts). The kit consists of a microplate with 96-wells; the surface of the wells is functionalized with a tyrosine-containing polypeptide (**polyE$_4$Y**). In the standard experiment, a control kinase (in some wells) and cell extracts (in other wells) are added to the microplate together with ATP. Phosphorylation of tyrosine takes place. A specific antibody is added to all wells, and coordinates to the phosphorylated tyrosine residues. This antibody is conjugated to a peroxidase (POD) that is capable to oxidize a substrate (3,3',5,5'-tetramethylbenzidine, TMBZ), converting it into a strongly colored derivative. After a final acidification, the absorbance due to this derivative is read. Absorbance values can be directly correlated to the activity of the kinase proteins.

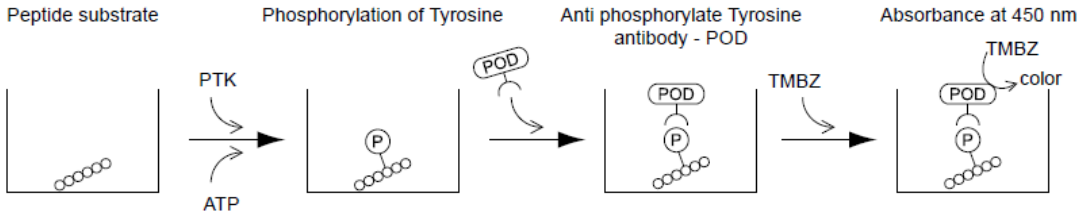


Figure S56. Scheme of the chain of reactions taking place in the tyrosine kinase activity assay (standard experiment)

In the modified experiment developed for this study, receptors tested for protection from the kinase activity (**CyLys**, **CyOrn**, **CySer** and **CyThr**) were added to the wells containing **polyE$_4$Y** shortly before the addition of PTK and ATP, as shown in Figure S57. Beside this modification, the experimental protocol provided by the manufacturer of the assay was scrupulously followed. The PTK used for the experiments was the one provided by the manufacturer as PTK control kinase, which is a cell extract enzymatic system based on the c-Src kinase. The activity of the enzyme is based on the activity of recombinant c-Src. One unit (U) of the enzyme is defined as the amount needed to incorporate 1 pmol of phosphate into the substrate (KVEKIGEGTYGVVYK: 6 - 20 residue of p34cdc2) in 1 min. For all the measurements, the phosphorylation degree was compared with the one obtained in the absence of any cage, for the same batch of the PTK control enzyme. The data displayed in the figure of the main text are the average of two independent experiments, each one in three replicated measurements, with error bars showing the standard deviations.

[4] www.**takara**.co.kr/file/manual/pdf/MK410_e.pdf

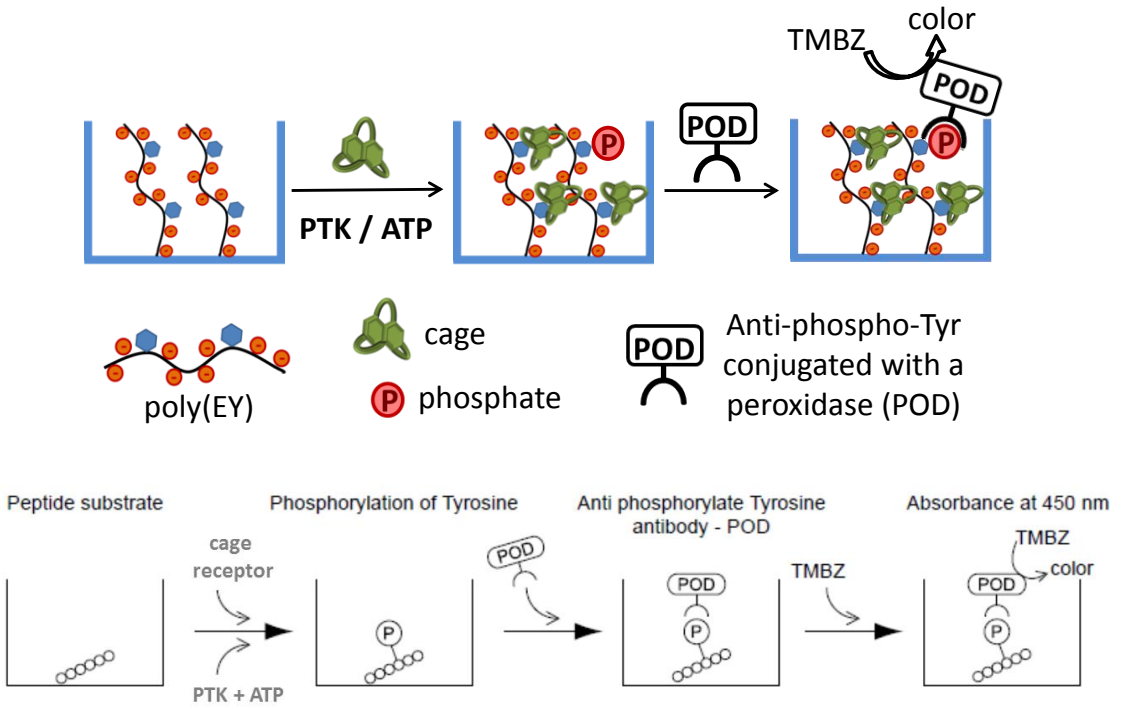


Figure S57. Cartoon representation and scheme of the chain of reactions taking place in the tyrosine kinase activity assay (modified experiment)

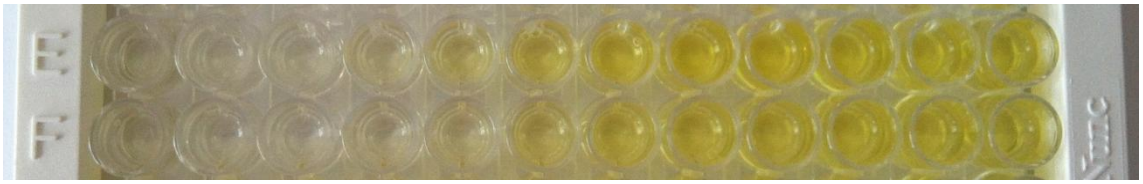


Figure S58. Wells of the 96-wells microplate containing **CyLys** at different concentrations, at the end of the chain of reactions described in Figure S50.