Supporting Information

Convergent Synthesis of Digitally-encoded Poly(alkoxyamine amide)s

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Content:

A. Materials and methods.

A.1. Materials. 4-Amino-2,2,6,6-tetramethylpiperidine-1-oxyl (4-amino TEMPO, TCI, 97%), tris(2-dimethylaminoethyl)amine (Me_6TREN, Alfa Aesar, >99%), trifluoroacetic acid (Sigma-Aldrich,99%), *N*,*N*-dicyclohexylcarbodiimide (Alfa Aesar, 99%), piperidine (Sigma-Aldrich, 99%), potassium carbonate (Prolabo, 99%), *N*-ethyldiisopropylamine (DIPEA, Alfa Aesar, 99%), dichloromethane (DCM, Carlo Erba, 99.9%), tetrahydrofuran (THF, Aldrich, 99%, stabilized with BHT), anhydrous dimethyl sulfoxide (DMSO, Aldrich, >99.6%), O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU, Iris Biotech, 99%) and 4-(Dimethylamino)pyridine (DMAP, Sigma Aldrich, 99%) were used as received. Copper-(I)-bromide (Sigma-Aldrich, 98%) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried. 2-Bromopropionic anhydride (**a-0**) and 2-bromoisobutyryl anhydride (**a-1**) were synthesized as previously reported.[1, 2] Fmoc-Gly-Wang resin (**R_wG**; 0.79 mmol/g loading) and Wang resin (**R_w**; 0.70 mmol/g loading) were purchased from Novabiochem/Merck. The microwave reactor used for synthesis is a Monowave 300 from Anton Paar.

A.2. Synthesis of the sequence-coded dyads. The sequence-coded dyads **0**T**0**, **0**T**1**, **1**T**0** and **1**T**1** were synthesized by iterative synthesis on a classical Wang resin **R_w**. The paragraphs below describes the main steps used in the synthesis.

A.2.1. Attachment of the first coded motif to the resin R_w by esterification. It is recommended to swell the resin with DCM prior to the reaction. The anhydride (5 Eq. of either **a-0** or **a-1**), HBTU (1.326 g, 5 Eq.) and DMAP (0.427 g, 5 Eq.) were added to a glass vial containing **R_w** (1.0 g, 0.7 mmol, 1Eq.). To the reaction mixture, 12 mL of THF were added and stirred for 90 min in a microwave reactor (~10W, 40°C). After completion, the reaction mixture was transferred to a fritted funnel and the beads were washed with THF to remove excess reagents.

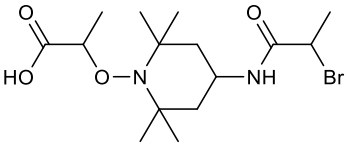
A.2.2. Attachment of the T spacer to the sequence. Amino-TEMPO (0.72 g, 6 Eq.) and Me_6TREN (0.7 mL, 3.3 Eq.) were dissolved in 12 mL of anhydrous DMSO and were placed into a fritted funnel containing bromide-functionalized resin beads. The funnel was sealed with a septum and the reaction mixture was purged with argon for 15 minutes. Then, CuBr (0.35 g, 3.5 Eq.) was rapidly added. The mixture was shaken for 35 min under inert atmosphere. After completion, the solution was drained out from the funnel and the beads were washed with THF.

A.2.3. Attachment of the second coded motif by anhydride-amine coupling. A mixture of an anhydride (5Eq. of either **a-0** or **a-1**) and a base (18 Eq., DIPEA in case of **a-0** and K_2CO_3 for **a-1**) was added to a fritted funnel for solid-phase synthesis containing amino-functionalized

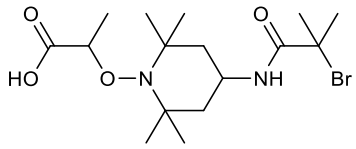
resin beads. The use of DIPEA is recommended when **a-0** is used because it was observed that K_2CO_3 forms an inhomogeneous gel with this anhydride in THF. 12 mL of THF was added and the mixture was shaken for 60 min on a mechanical shaker. After completion, the solution was drained out from the fritted funnel. When K_2CO_3 was used, the beads were washed with MeOH-H_2O (1:1) and afterwards with THF. When DIPEA was used, washing was only done with THF.

A.2.4. Cleavage of the dyads from the resin. Cleavage of the dyads from the resin was performed in TFA/DCM solution (1/1) for 2 h. After reaction, the solution was filtered, concentrated and precipitated in diethyl ether. The crude mixture was diluted in THF and small amounts of insoluble resin fragments were separated by filtration. The filtrate was concentrated by the removal of solvents and TFA. Finally the oligomers were isolated either as crystalline solid (**1**T**1**) or as precipitates from diethylether (for **0**T**0**, **0**T**1**, and **1**T**0**).

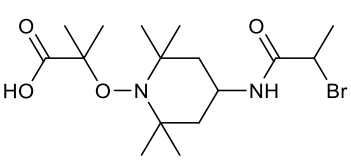
A.2.5. Characterization of the dyads.



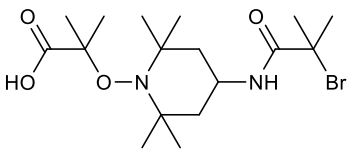
Dyad 0T0. 183 mg, 69.2 %. ^{1}H NMR: ($CDCl_3$, δ, ppm) 4.64 (o, 1H, CH_3**CH**BrCO-); 4.44 (o,1H, HO_2C**CH**CH_3ON-), 4.39 (m,1H, -CH_2**CH**NHCO-); 1.96 (m, 2H, -$C(CH_3)_2$**CH_2**CH-); 1.85 (s, 3H, BrCH**CH_3**CO-); 1.75 (s, 3H, HO_2CCH**CH_3**ON-); 1.54 (m, 2H, -$C(CH_3)_2$**CH_2**CH-); 1.31-1.43 (s, 12H, -ONC**$(CH_3)_2$**CH_2-). ^{13}C NMR: ($CDCl_3$, δ, ppm) 175.06, 169.77, 67.92, 64.37, 64.23, 43.5, 40.33, 30.87, 30.52, 21.12, 17.29. HR-ESI-MS: (*m/z*): $[M+H]^+$ calculated from $C_{15}H_{28}N_2O_4Br^+$: 379.1227, found 379.1227.



Dyad 0T1. 184.1 mg, 67.1 %. ^{1}H NMR: ($CDCl_3$, δ, ppm) 6.65 (t, 1H, –**NH**$COC(CH_3)_2Br$); 4.59 (o, 1H, HO_2C**CH**CH_3ON-); 4.19 (m,1H, -CH_2**CH**NHCO-), 2.02 (m, 2H, -$C(CH_3)_2$**CH_2**CH-); 1.94 (s, 6H, -C**$(CH_3)_2$**Br); 1.71 (m, 2H, -$C(CH_3)_2$**CH_2**CH-); 1.62 (s, 3H, HO_2CCH**CH_3**ON-); 1.51-1.27 (s, 12H, -ONC**$(CH_3)_2$**CH_2-). ^{13}C NMR: ($CDCl_3$, δ, ppm) 174.38, 171.79, 62.85, 62.63, 61.74, 44.36, 41.18, 32.13, 30.55, 21.12, 17.15. HR-ESI-MS: (*m/z*): $[M+H]^+$ calculated from $C_{16}H_{30}N_2O_4Br^+$: 393.1383, found 393.1380.



Dyad 1T0. 195.1 mg, 71.4 %. ^{1}H NMR: ($CDCl_3$, δ, ppm) 8.0 (t, 1H, –**NH**$COCHCH_3Br$); 4.46 (o,1H, CH_3**CH**BrCO-), 4.28 (m, 1H, -CH_2**CH**NHCO-); 2.20-1.88 (m, 4H, -$C(CH_3)_2$**CH_2**CH); 1.75 (d, 3H, -BrCH**CH_3**CO-); 1.64 (s, 6H, HO_2CC**$(CH_3)_2$**ON-); 1.35 (s, 12H, -ONC**$(CH_3)_2$**CH_2-). ^{13}C NMR: ($CDCl_3$, δ, ppm) 178.04, 169.78, 82.60, 63.76, 43.70, 39.99, 31.02, 28.35, 21.93, 21.16. HR-ESI-MS: (*m/z*): $[M+H]^+$ calculated from $C_{16}H_{30}N_2O_4Br^+$: 393.1383, found 393.1381.



Dyad 1T1. 247 mg, yield = 87 %. ^{1}H NMR: (CDCl3, δ, ppm) 6.86 (t, 1H, –**NH**COC(CH$_3$)$_2$Br); 4.20 (m,1H, -CH$_2$**CH**NHCO-), 2.1 (m, 2H, -C(CH$_3$)$_2$**CH$_2$**CH-); 2.01 (s, 6H, -C**(CH$_3$)$_2$**Br); 1.79 (m, 2H, -C(CH$_3$)$_2$**CH$_2$**CH-); 1.63 (s, 6H, HO$_2$CC**(CH$_3$)$_2$**ON-); 1.34 (s, 12H, -ONC**(CH$_3$)$_2$**CH$_2$-). ^{13}C NMR: (CDCl3, δ, ppm) 176.61, 171.81, 82.26, 62.68, 61.77, 44.70, 40.98, 32.14, 31.44, 28.32, 21.21. HR-ESI-MS: (*m/z*): $[M+H]^+$ calculated from $C_{17}H_{32}N_2O_4Br^+$: 407.1540, found 407.1539.

A.3. Synthesis of the modified resin. The solid support containing short amino-functional sequences **R$_w$G1**T**1**T was synthesized by 4-steps iterative synthesis on a glycine-loaded Wang resin **R$_w$G**. As described in previous publications,[2, 3] the coupling steps A.3.2. and A.3.3. were used successively until the desired tetramer was reached.

A.3.1. Deprotection of the resin RwG. 0.1 g of **R$_w$G** (0.079 mmol, 1 Eq., loading 0.79 mmol/g) was placed in a fritted plastic funnel. The resin beads were swollen by gentle shaking in DCM for 0.5h. Next, the Fmoc was removed by treatment with piperidine/DCM (2 mL/2 mL) for 10 minutes. The deprotection step was repeated a second time to ensure complete removal of the Fmoc protecting groups from the resin beads. A Kaiser test made on few resin beads to confirm the presence of deprotected primary amine groups.

A.3.2. General procedure for anhydride-amine coupling. A mixture of anhydride **a-1** (5 Eq.) and K_2CO_3 (18 Eq.) was added to a fritted funnel for solid-phase synthesis containing amino-functionalized resin beads. 4 mL of THF were added and the mixture was shaken for 50 min on a mechanical shaker. After completion, the solution was drained out from the fritted funnel. The beads were washed with MeOH-H_2O (1:1) and afterwards with THF.

A.3.3. General procedure for radical-radical coupling. Amino-TEMPO (0.08 g, 6 Eq.) and Me_6TREN (0.078 mL, 3.3 Eq.) were dissolved in 4 mL of anhydrous DMSO and were placed into a fritted funnel containing bromide-functionalized resin beads. The funnel was sealed with a rubber septum and the reaction mixture was purged with argon for about 15 minutes. Then, CuBr (0.034 g, 3 Eq.) was rapidly added. The mixture was shaken for 25 min under inert atmosphere. After reaction, the solution was drained out from the funnel and the beads were washed several times with THF.

A.4. Synthesis of poly(alkoxyamine amide)s by dyad ligation. Sequence-coded poly(alkoxyamine amide)s were synthesized by successive ligation of dyads on the modified resin **RwG1**T**1**T as shown in Fig. 1b of the main manuscript. In this approach, steps A.4.1. (dyad ligation) and A.4.2. (coupling of an amino-TEMPO spacer) are performed successively a certain number of times until a poly(alkoxyamine amide)s with desired length and sequence is formed.

A.4.1. General procedure for dyad ligation by carboxylic acid/amine coupling. A dyad (4 Eq. of **0**T**0**, **0**T**1**, **1**T**0** or **1**T**1**), HBTU (0.175 mmol, 5 Eq.) along with DIPEA (0.525 mmol, 15 Eq.) were dissolved in 5 mL of anhydrous DMF and kept for two minutes. Then the reaction mixture was transferred into a glass vial containing amine-functionalized resin beads **RwG1**T**1**T (0.035 mmol, 1 Eq.). The oligomer coupling reaction was performed in a microwave reactor. Typically, the reactions were carried out by the irradiation of 5 to 10 W microwave power at 40 ºC for about 120 minutes (180 minutes for the preparation of 15^{th} and 19^{th} mer from 12^{th} mer and 16^{th} mer respectively). After completion, the reaction mixture was transferred to a fritted funnel and the beads were washed several times with THF to remove excess reagent.

A.4.2. General procedure for the attachment of the spacer T by radical-radical coupling. Amino-TEMPO (0.08 g, 12 Eq.) and Me_6TREN (0.078 mL, 6.6 Eq.) were dissolved in 4 mL of anhydrous DMSO and were placed into a glass vial containing bromide-functionalized resin beads. The vial was sealed with a rubber septum and the reaction mixture was purged with argon for about 15 minutes. Then, CuBr (0.034 g, 6 Eq.) was rapidly added. The mixture was stirred for 90 min in a microwave reactor. Typically, the reaction mixture was irradiated with 5-10 W of microwave power at 40 ºC for about 90 minutes. After completion, the reaction mixture was transferred to a fritted funnel and the beads were washed several times with THF.

A.4.3. Cleavage of the polymers from the resin. Cleavage of the poly(alkoxyamine amide)s from the resin was performed in TFA/DCM solution (1/1) for 2 h. After reaction, the solution was filtered, concentrated and precipitated in cold diethyl ether. The crude mixture was diluted in THF and small amounts of insoluble resin fragments were separated by filtration. The filtrate was concentrated and the oligomers were isolated from cold diethylether precipitation. Example of final yield: (For 19^{th} mer) = 31%.

B. Measurements and analysis.

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a BrukerAvance 400 MHz spectrometers equipped with Ultrashield magnets. Molecular weights and molecular weight distributions were determined using a SEC system equipped with a Shimadzu RiD-10A refractive index detector and five PLgel 10µ Mixed-B columns. The mobile phase was THF with a flow rate of 1 $mL \cdot min^{-1}$ using a Shimadzu LC20AD pump. Toluene was used as internal reference. The molecular weight calibration was based on sixteen narrow molecular weight linear polystyrene standards from Polymer Laboratories. High resolution MS and MS/MS experiments were performed using a QStar Elite mass spectrometer (Applied Biosystems SCIEX, Concord, ON, Canada) equipped with an electrospray ionization (ESI) source operated in the positive mode. The capillary voltage was set at +5500 V and the cone voltage at +75 V. In this hybrid instrument, ions were measured using an orthogonal acceleration time-of-flight (oa-TOF) mass analyzer. In the MS mode, accurate mass measurements were performed using reference ions from a poly(propylene glycol) or a poly(ethylene glycol) internal standard. In the MS/MS mode, a quadrupole was used for selection of precursor ions to be further submitted to collision-induced dissociation (CID) in a collision cell. The precursor ion was used as the reference for accurate measurements of product ions in MS/MS spectra. In this instrument, air was used as the nebulizing gas (10 psi) while nitrogen was used as the curtain gas (20 psi) as well as the collision gas. Instrument control, data acquisition and data processing of all experiments were achieved using Analyst software (QS 2.0) provided by Applied Biosystems. Oligomer solutions were prepared in methanol supplemented with ammonium acetate (3 mM) and introduced in the ionization source using a syringe pump (flow rate: 5 $\mu L\ min^{-1}$).

C. Additional Figures.

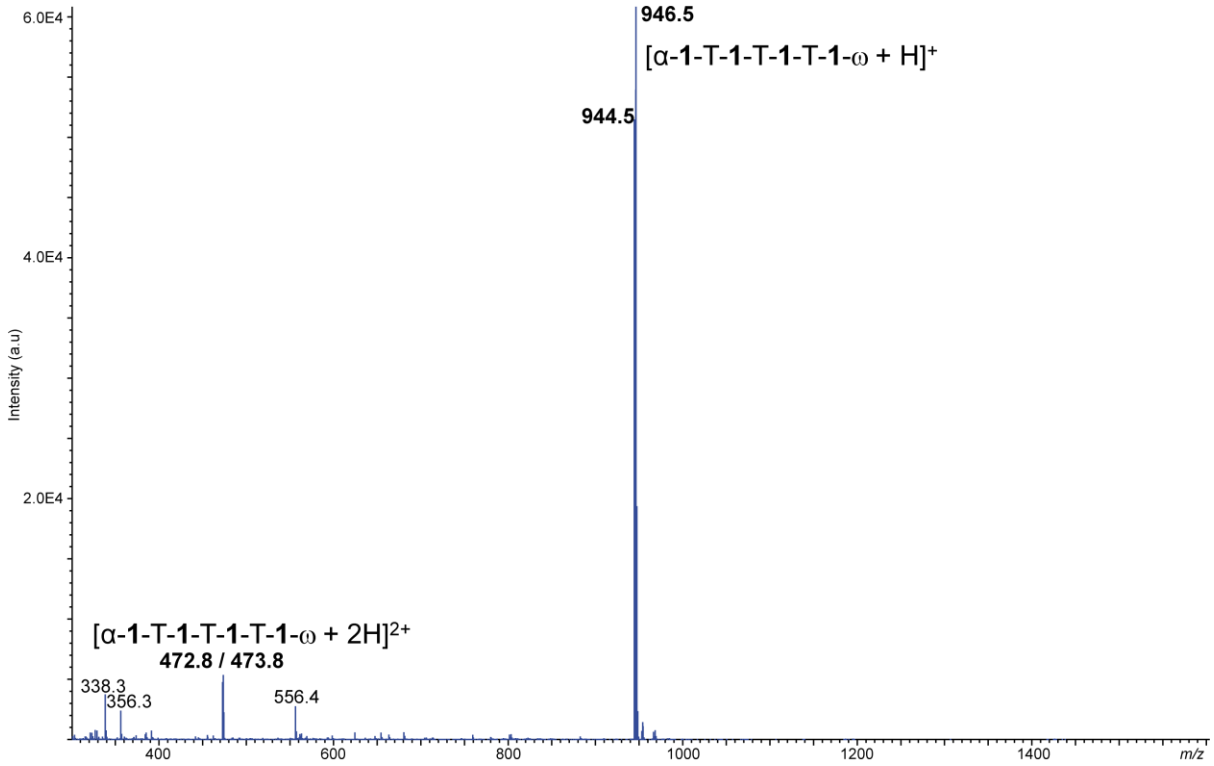


Fig. S1. ESI mass spectrum of the α-[**1**-T]$_3$-**1**-ω sample.

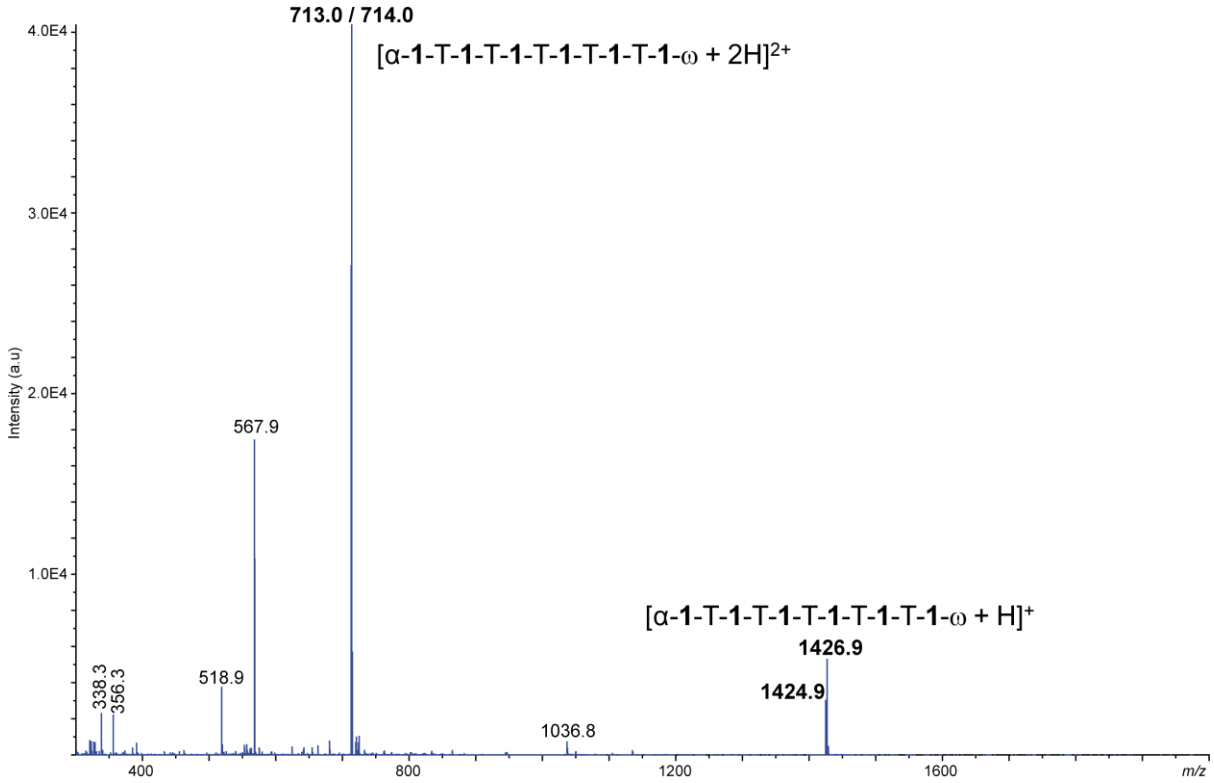


Fig. S2. ESI mass spectrum of the α-[**1**-T]$_5$-**1**-ω sample.

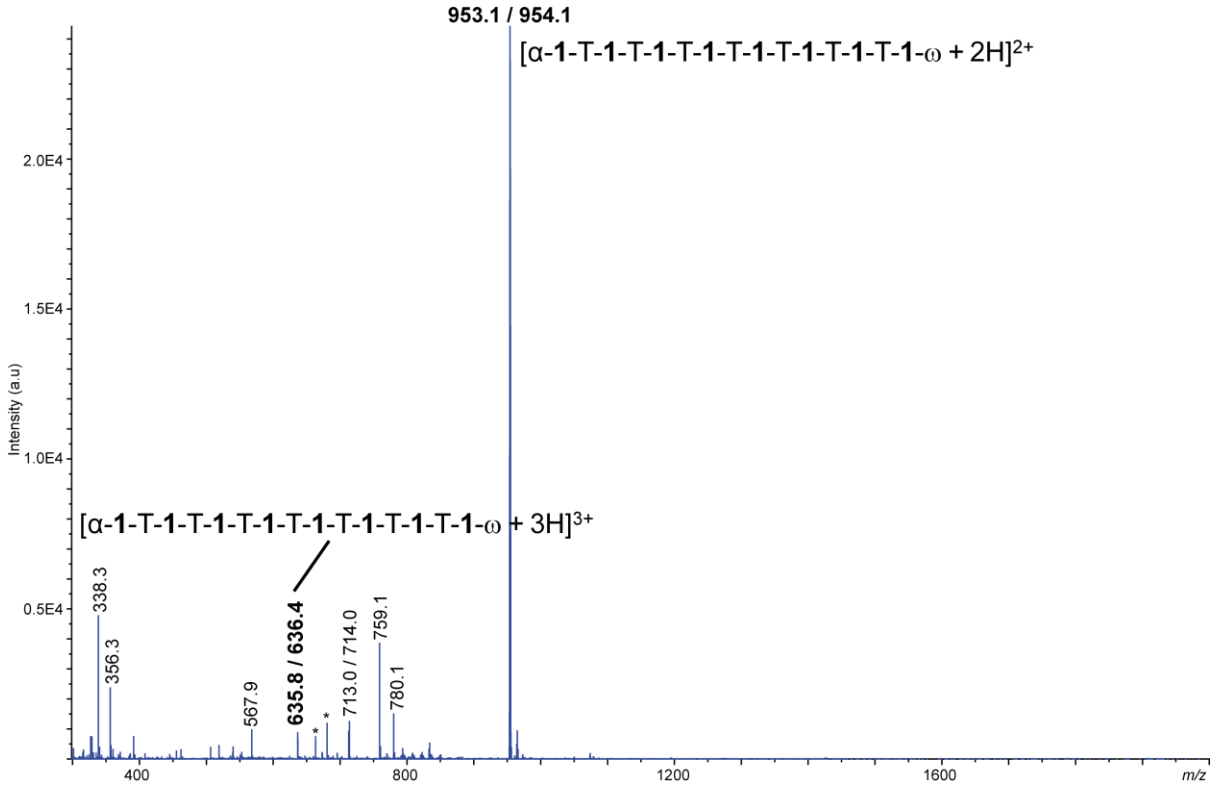


Fig. S3. ESI mass spectrum of the α-[**1**-T]$_7$-**1**-ω sample.

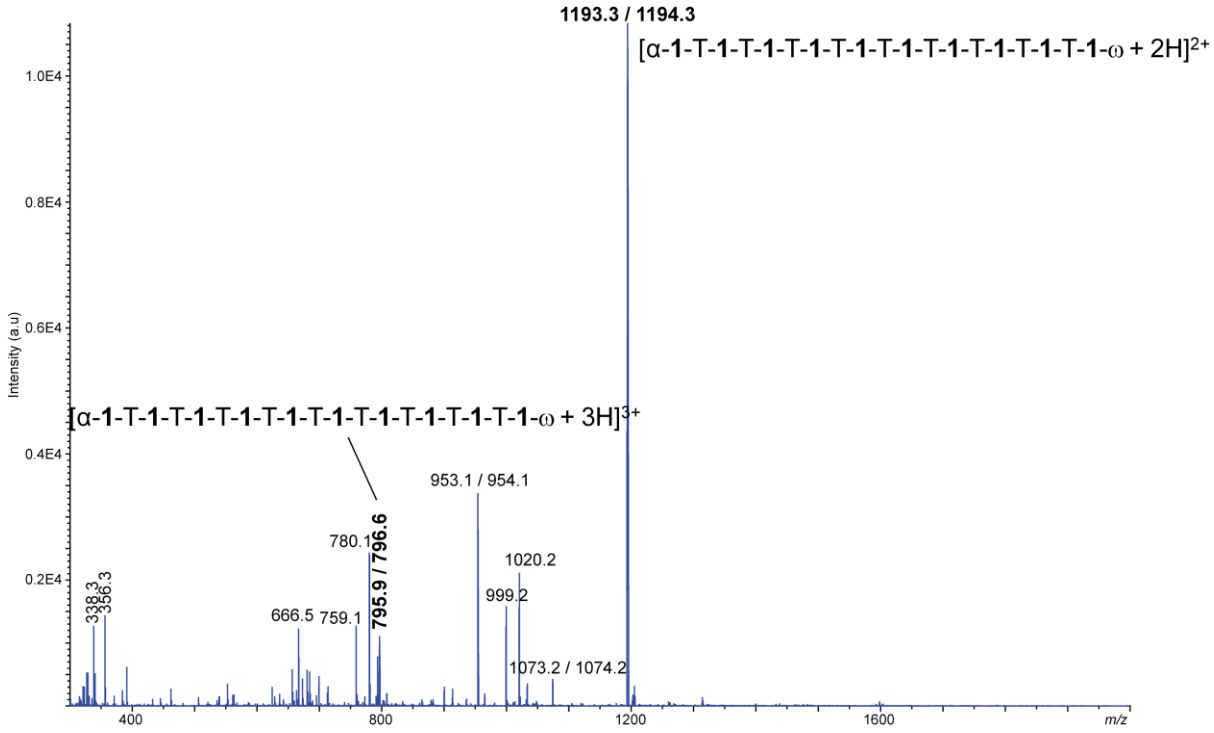


Fig. S4. ESI mass spectrum of the α-[**1**-T]$_9$-**1**-ω sample.

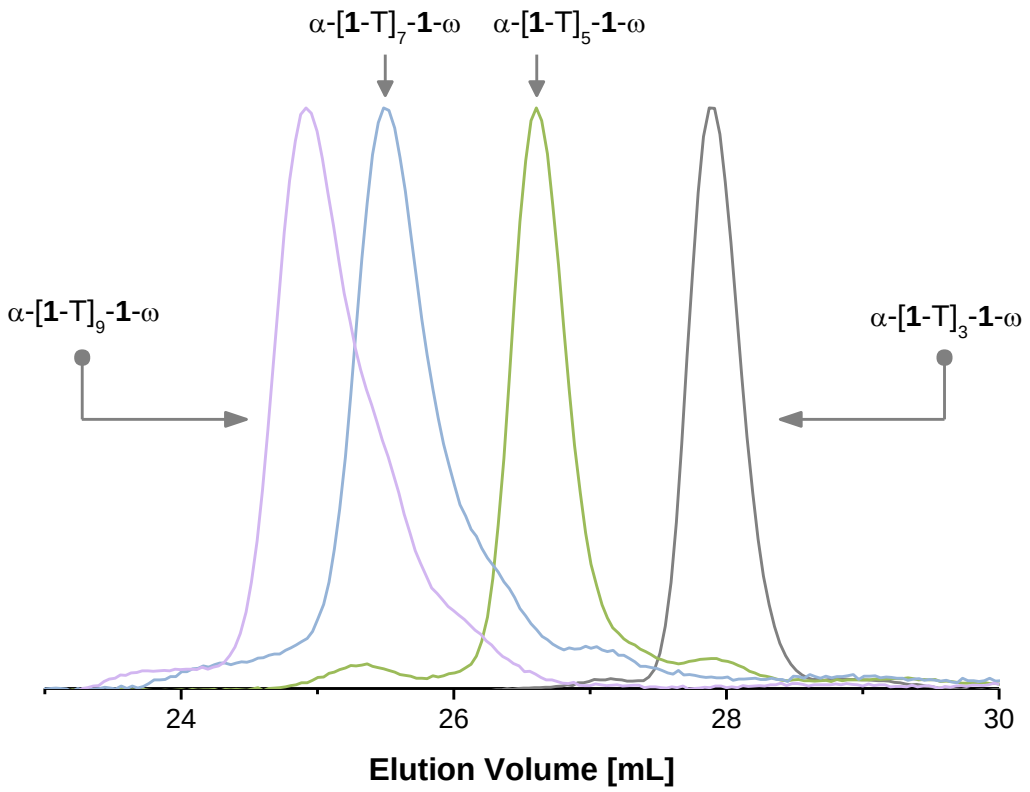


Fig. S5. SEC chromatograms recorded in THF for the successive samples α-[**1**-T]$_3$-**1**-ω (grey), α-[**1**-T]$_5$-**1**-ω (green), α-[**1**-T]$_7$-**1**-ω (blue) and α-[**1**-T]$_9$-**1**-ω (purple).

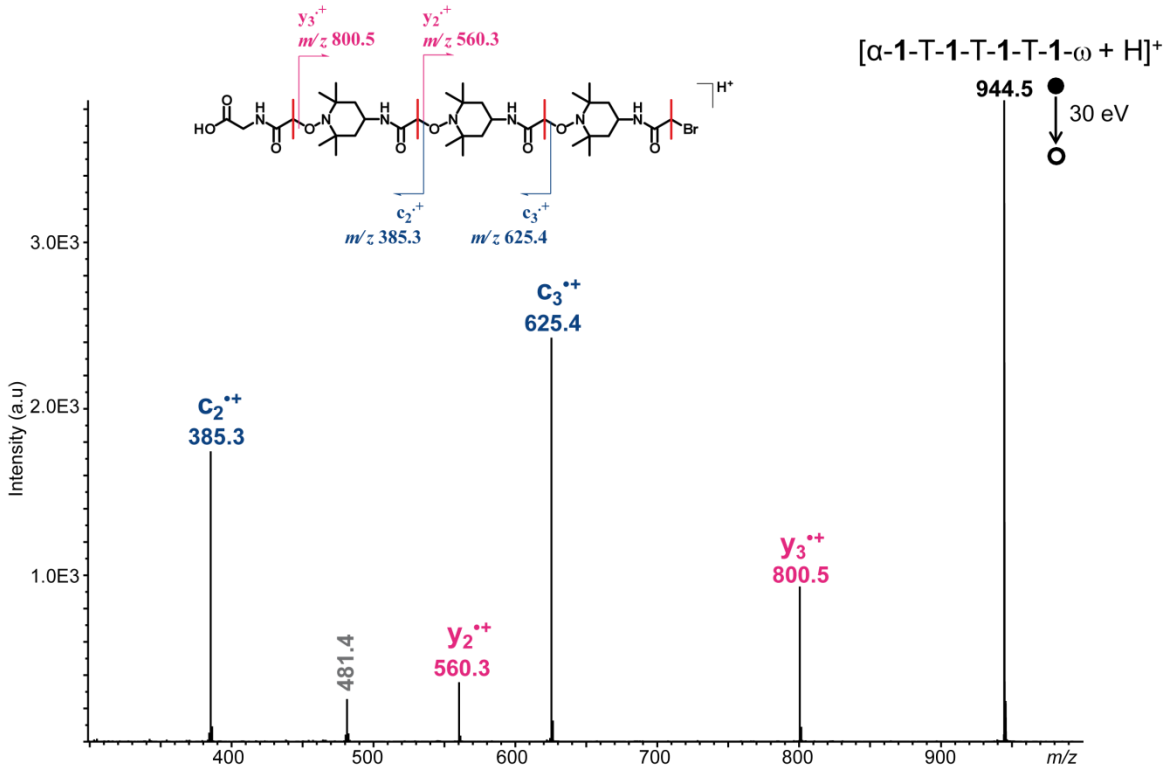


Fig. S6. ESI-MS/MS spectrum obtained after collisional activation (30 eV) of the *m/z* 944.5 precursor ion [α-[**1**-T]$_3$-**1**-ω +H]$^+$, with ω = ^{79}Br. Grey *m/z* values correspond to protonated cyclic (**1**T)$_n$ internal fragments. Inset: dissociation scheme of the precursor ion.

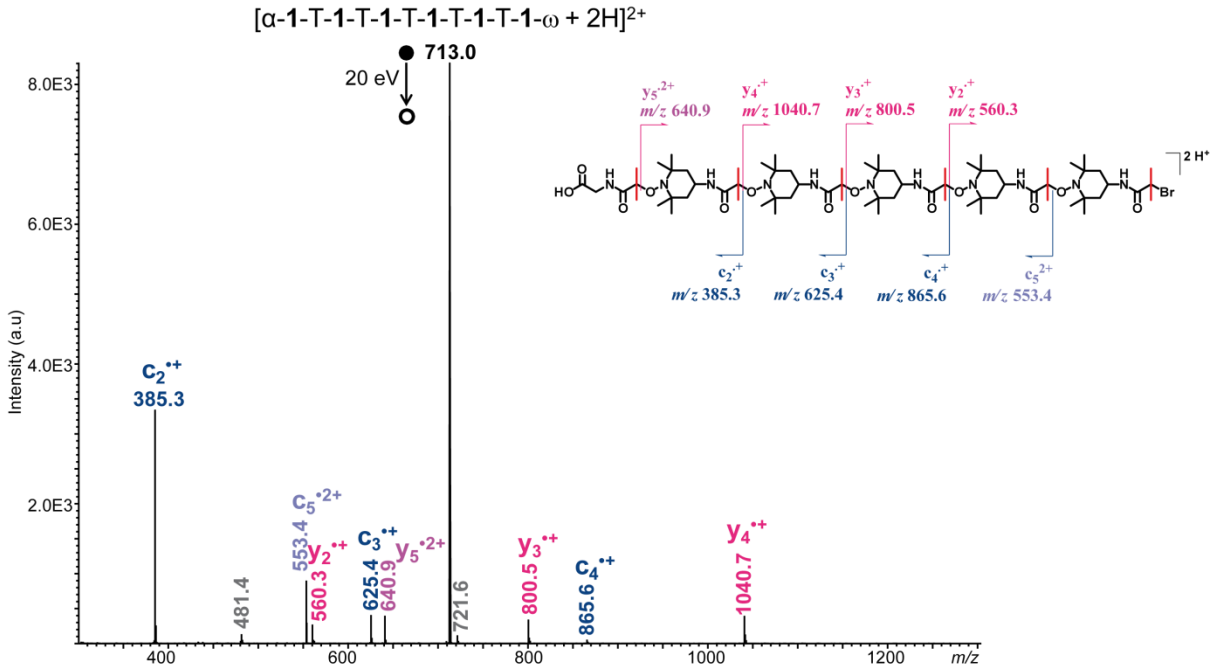


Fig. S7. ESI-MS/MS spectrum obtained after collisional activation (20 eV) of the *m/z* 713.0 precursor ion [α-[**1**-T]$_5$-**1**-ω +2H]$^{2+}$, with ω = ^{79}Br. Grey *m/z* values correspond to protonated cyclic (**1**T)$_n$ internal fragments. Inset: dissociation scheme of the precursor ion.

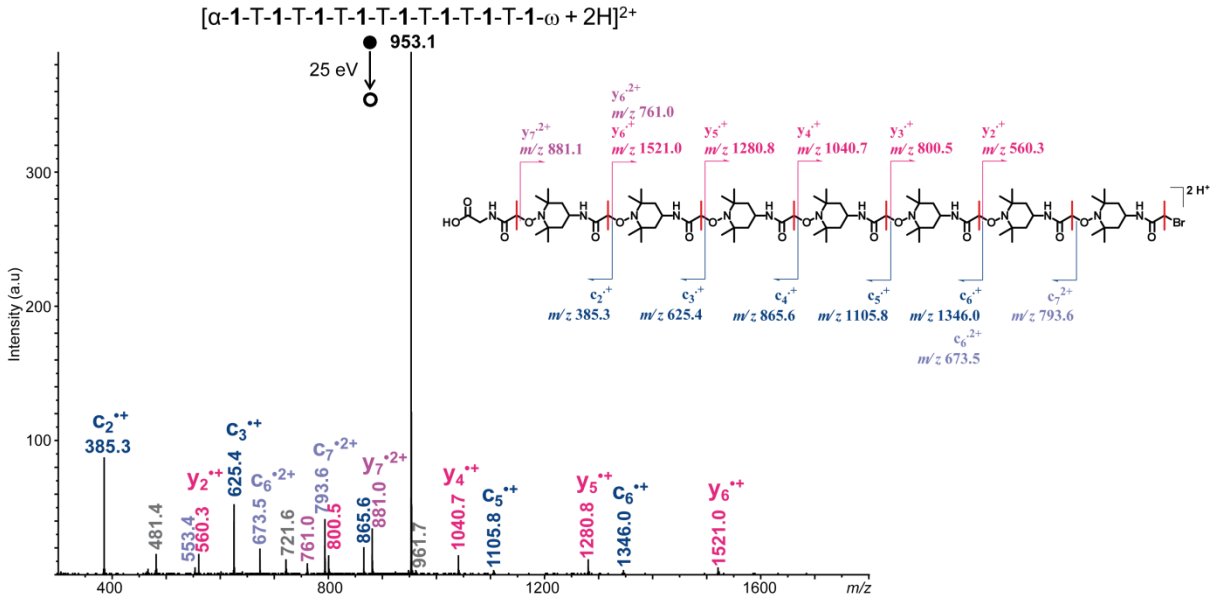


Fig. S8. ESI-MS/MS spectrum obtained after collisional activation (25 eV) of the *m/z* 953.1 precursor ion [α-[**1**-T]$_7$-**1**-ω +2H]$^{2+}$, with ω = ^{79}Br. Grey *m/z* values correspond to protonated cyclic (**1**T)$_n$ internal fragments. Inset: dissociation scheme of the precursor ion.

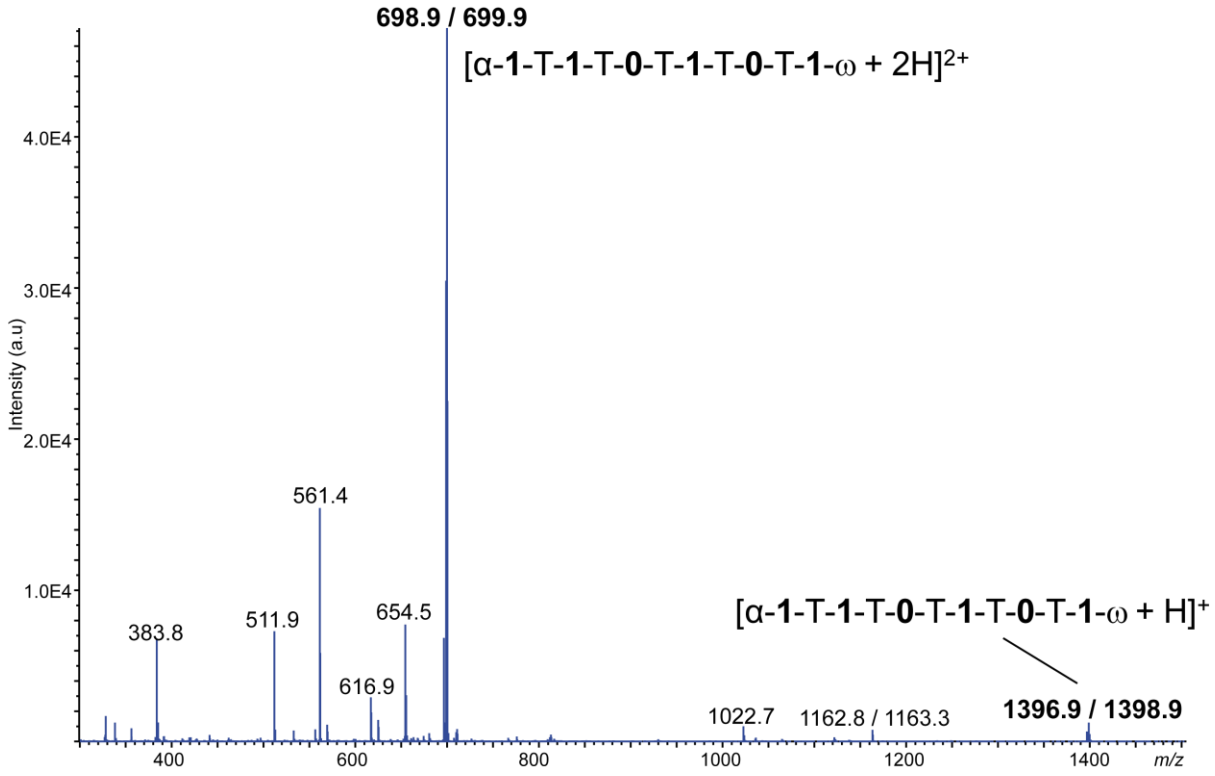


Fig. S9. ESI mass spectrum of the α-(**1**-T-**1**-T)-(**0**-T-**1**)-T-(**0**-T-**1**)-ω sample (Table 1, Entry 1).

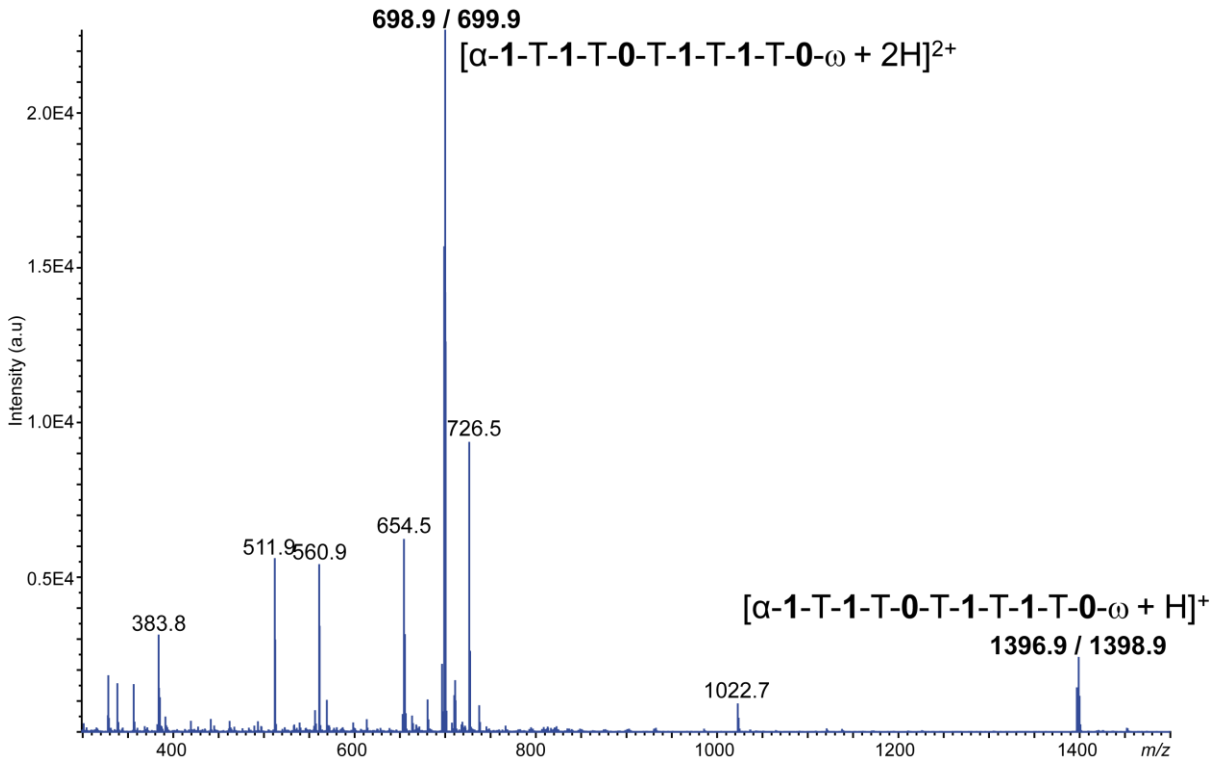


Fig. S10. ESI mass spectrum of α-(**1**-T-**1**-T)-(**0**-T-**1**)-T-(**1**-T-**0**)-ω (Table 1, Entry 2).

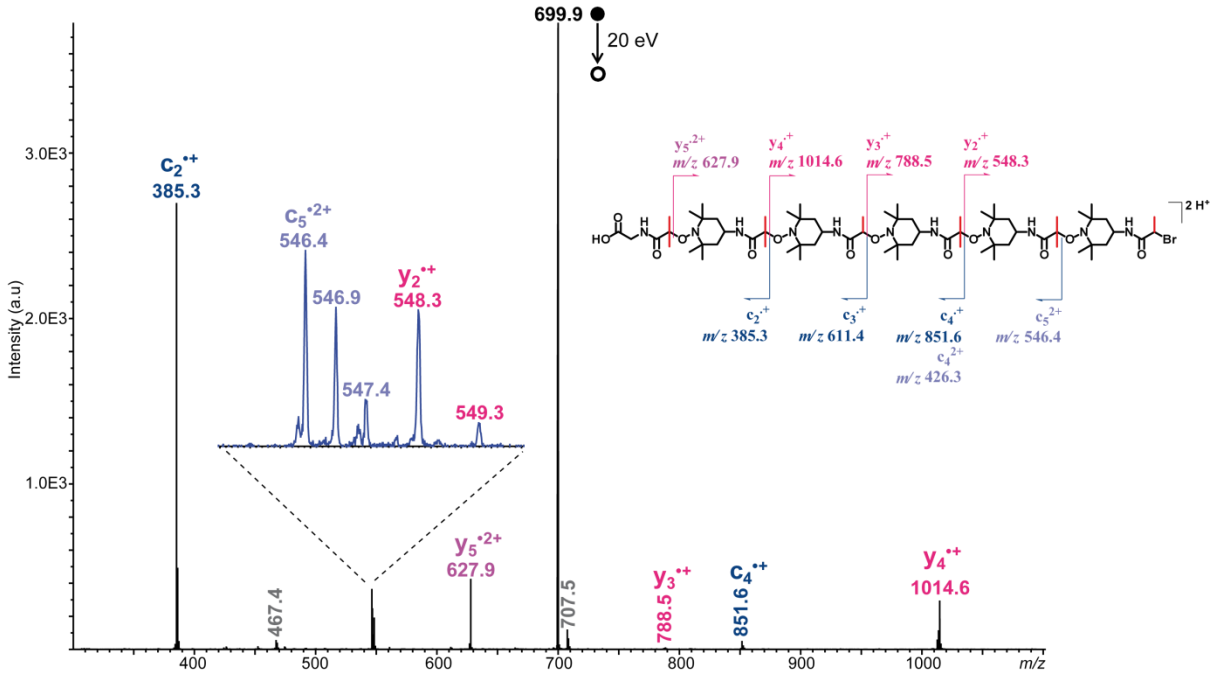


Fig. S11. ESI-MS/MS spectrum obtained after collisional activation (20 eV) of the *m/z* 699.9 precursor ion [α-(**1**-T-**1**-T)-(**0**-T-**1**)-T-(**1**-T-**0**)-ω +2H]$^{2+}$ (Table 1, Entry 2), with ω = ^{81}Br. Grey *m/z* values correspond to protonated cyclic (**1**T)$_n$ internal fragments. Inset: dissociation scheme of the precursor ion.

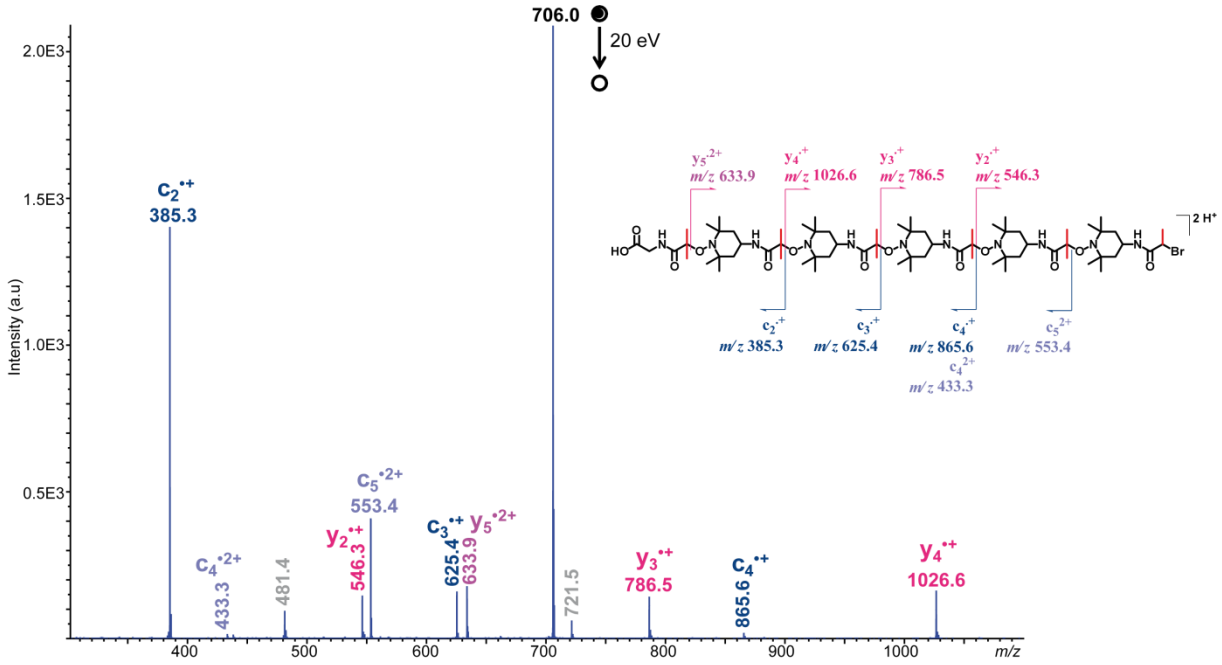


Fig. S12. ESI-MS/MS spectrum obtained after collisional activation (20 eV) of the *m/z* 706.0 precursor ion [α-**1**-T-**1**-T-**1**-T-**1**-T-**1**-T-**0**-ω +2H]$^{2+}$ (Table 1, Entry 3). Grey *m/z* values correspond to protonated cyclic (**1**T)$_n$(**0**T)$_m$ internal fragments. Inset: dissociation scheme of the precursor ion.

D. References.

1. E. Östmark, S. Harrisson, K. L. Wooley and E. E. Malmström, *Biomacromolecules*, 2007, **8**, 1138-1148.
2. R. K. Roy, A. Meszynska, C. Laure, L. Charles, C. Verchin and J.-F. Lutz, *Nat Commun*, 2015, **6**, 7237.
3. L. Charles, C. Laure, J.-F. Lutz and R. K. Roy, *Macromolecules*, 2015, **48**, 4319-4328.