Supporting Information to

Synthesis and sequencing of Informational Poly(amino phosphodiester)s

Ian Roszak,[a] Laurence Oswald,[a] Abdelaziz Al Ouahabi,[a] Annabelle Bertin,[a, b, c] Eline Laurent,[a] Olivier Felix,[a] Isaure Carvin-Sergent,[d] Laurence Charles[d] and Jean-François Lutz*[a]

[a] Université de Strasbourg, CNRS, Institut Charles Sadron UPR22, 23 rue du Loess, 67034 Strasbourg Cedex 2, France. [b] BAM Federal Institute for Materials Research and Testing, Unter den Eichen 87, 12205 Berlin, Germany. [c] Institute of Chemistry and Biochemistry − Organic Chemistry, Free University Berlin, Takustraße 3, 14195 Berlin, Germany. [d] Aix Marseille Université, CNRS, UMR 7273, Institute of Radical Chemistry, 13397, Marseille Cedex 20, France

List of Content

A Materials and Reagents

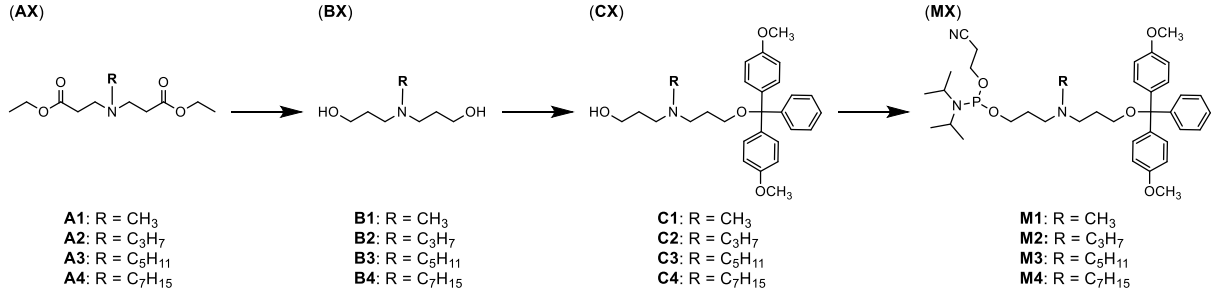
Amylamine (99%, Sigma-Aldrich), 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (97 %, ABCR), anhydrous dichloromethane (99.8 %, anh. DCM, cont. amylene as stabilizer, Sigma-Aldrich), *N*,*N*-diisopropylethylamine (>99.0 %, DIPEA, TCI), 4,4'-dimethoxytrityl chloride (99 %, DMT-Cl, ChemGenes), ethanol absolute (99.99%, Carlo Erba), ethyl acetate (EtOAc, Carlo Erba), ethyl acrylate (>99.0%, Sigma-Aldrich), heptylamine (99%, Sigma-Aldrich), hexane (Carlo Erba), lithium aluminum hydride solution (1.0 M in THF, Sigma-Aldrich), methanol (MeOH, Carlo Erba), methylamine solution (33 wt. % in absolute ethanol, Sigma-Aldrich), propylamine (>99.0%, Sigma-Aldrich), anhydrous pyridine (99.8 %, anh. pyridine, Sigma-Aldrich), anhydrous tetrahydrofuran (≥99.9%, inhibitor-free, anh. THF, Sigma-Aldrich), silica gel (high-purity grade, pore size 60 Å, 230 - 400 mesh, for flash chromatography, SiO_2, Sigma-Aldrich), sodium chloride (for brine solution, ESCO), anhydrous sodium sulfate (99.6 %, Na_2SO_4, VWR), and triethylamine (97 %, Et_3N, Acros Organics), were used as received without further purification.

Reagents for automated phosphoramidite synthesis: anhydrous methylamine (AMA, 98%, Sigma-Aldrich), ammonium hydroxide solution (28.0–30.0% NH_3, Sigma-Aldrich) were used as purchased. Anhydrous dichloromethane (DCM), pyridine, and acetonitrile were purchased from Sigma-Aldrich. All air-sensitive reactions have been carried out under argon atmosphere. Automated synthesis reagents, anhydrous acetonitrile (ACN, Glen Research), oxidizing solution (0.02 M I_2 in THF/H_2O/pyridine), deblocking mix (3% trichloroacetic acid in DCM), cap Mix A (THF/Ac_2O), activator (tetrazole in acetonitrile) were purchased from Glen Research. Nucleoside-functionalized controlled pore glass columns (T-lcaa-CPG), glen-pak DNA purification cartridge (10 nmole - 1.0 μmole) were also obtained from Glen Research. All the phosphoramidites were stored in the freezer at −18 °C.

B Experimental Procedures

B.1 Synthesis of phosphoramidite monomers **M1**-**M4**

The four phosphoramidite monomers **M1**, **M2**, **M3** and **M4** were prepared following a previously-reported procedure[1] and as shown in the scheme below.



Synthetic route used in this work for the preparation of **M1**, **M2**, **M3**, **M4**.

*B.1.1. Synthesis of **A1***. To ethyl acrylate (14.01 g, 0.140 mol, 2.0 eq.) under argon atmosphere was added dropwise at 0°C a methylamine solution 33 wt. % in absolute ethanol (6.57 g, 0.070 mmol, 1.0 eq.). After the addition, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was evaporated to dryness and kept 1 h under vacuum to remove the traces of starting materials. We obtain 15.30 g of **A1** (95%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, δ, ppm): 1.25 (t, 6H, $OCH_2\underline{CH_3}$), 2.24 (s, 3H, $N\underline{CH_3}$), 2.46 (t, 4H, $CO\underline{CH_2}$), 2.71 (t, 4H, $\underline{CH_2}N\underline{CH_2}$), 4.13 (q, 4H, $O\underline{CH_2}CH_3$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.2, 32.7, 41.8, 52.6, 60.4, 172.5.

*B.1.2. Synthesis of **B1***. To lithium aluminum hydride 1.0 M in THF (100 mL, 0.100 mol, 2.0 eq.) under argon atmosphere was added dropwise at 0°C a mixture of **A1** (11.88 g, 0.051 mol, 1.0 eq.) dissolved in 65 mL anh. THF. After the addition, the mixture was stirred 1 h at 0°C. Then it was allowed to warm to room temperature and stirred overnight. To hydrolyse the lithium aluminum hydride residues the mixture was cooled at 0°C. Slowly brine (8.9 mL), followed with a solution of NaOH 2.0 M in water (8.9 mL) and H_2O (23 mL) were added. The heterogeneous mixture was stirred 30 min at 0°C. The solid was removed by filtration and washed with 250 mL diethyl ether. The filtrate was evaporated to obtain 6.40 g of **B1** (85%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, δ, ppm): 1.72 (quint, 4H, $NCH_2\underline{CH_2}CH_2$), 2.25 (s, 3H, $N\underline{CH_3}$), 2.56 (t, 4H, $N\underline{CH_2}CH_2$), 3.74 (t, 4H, $HO\underline{CH_2}$), 4.11 (s br, 2H, $\underline{HO}CH_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 28.7, 42.0, 56.5, 62.4.

*B.1.3. Synthesis of **C1***. **B1** (6.40 g, 0.043 mol, 1.2 eq.) was coevaporated with 20 mL of anh. pyridine. Afterward, the diol was dissolved in 25 mL DIPEA and 120 mL anh. DCM under argon atmosphere. DMT-Cl (12.27 g, 0.036 mol, 1.0 eq.) was added in four portions each 45 min. After the four additions, the mixture was stirred at room temperature overnight. The reaction was stopped with the addition of 25 mL of methanol and the mixture was evaporated to dryness. The residue was mixed with 140 mL $NaHCO_3$ (sat., aq.) and was extracted with EtOAc (2 x 140 mL). The combined organic layers were washed with H_2O (1 x 160 mL), brine (1 x 160 mL), dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 70/30 to EtOAc / MeOH: 95/5 with 1% Et_3N) to obtain 9.93 g of **C1** (61%) as a yellowish oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{28}H_{36}NO_4^+$ 450.2639; found, 450.2634. ^{1}H NMR (400.13 MHz, $CDCl_3$, δ, ppm): 1.63-1.72 (m, 2H, $HOCH_2\underline{CH_2}CH_2$), 1.74-1.84 (m, 2H, $NCH_2\underline{CH_2}$), 2.24 (s, 3H, $N\underline{CH_3}$), 2.43-2.49 (m, 2H, $N\underline{CH_2}CH_2$), 2.58 (t, 2H, $HOCH_2CH_2\underline{CH_2}$), 3.08 (t, 2H, $\underline{CH_2}$ODMT), 3.76 (t, 2H, $HO\underline{CH_2}$), 3.79 (s, 6H, $Ar_{DMT}O\underline{CH_3}$), 6.82 (d, 4H, $Ar_{DMT}H$), 7.17-7.24 (m, 1H, $Ar_{DMT}H$), 7.26-7.34 (m, 6H, $Ar_{DMT}H$), 7.39-7.45 (m, 2H, $Ar_{DMT}H$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 27.9, 28.0, 42.2, 55.3, 55.6, 58.5, 61.8, 64.9, 85.9, 113.1, 126.8, 127.9, 128.3, 130.1, 136.6, 145.3, 158.5.

*B.1.4. Synthesis of **M1***. **C1** (9.90 g, 0.022 mol, 1.0 eq.) was dissolved in 43 mL anh. DCM. Then, DIPEA (15.34 mL, 0.088 mol, 4.0 eq.) was added. The solution was cooled to 0°C under argon atmosphere. 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (5.73 g, 0.024 mol, 1.1 eq.) dissolved in 8 mL anh. DCM was added at 0°C. The reaction mixture was stirred at 0°C for 30 min, then allowed to reach room temperature and stirred for 1 h. The reaction mixture was extracted with 43 mL $NaHCO_3$ (sat., aq.). The aqueous phase was washed with 50 mL DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 70/30 to EtOAc 100% with 1% Et_3N) to obtain 12.6 g of **M1** (88%) as a colorless oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{37}H_{53}N_3O_5P^+$ 650.3717;

found, 650.3723. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 1.11-1.22 (m, 12H, N(CH($\underline{CH_3}$)$_2$)$_2$), 1.70-1.82 (m, 4H, $\underline{CH_2}CH_2NCH_2\underline{CH_2}$), 2.20 (s, 3H, N$\underline{CH_3}$), 2.36-2.48 (m, 4H, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 2.57-2.64 (m, 2H, $CH_2\underline{CH_2}C{\equiv}N$), 3.07 (t, 2H, $\underline{CH_2}$ODMT), 3.51-3.71 (m, 4H, $\underline{CH_2}$OP, $\underline{CH_2}CH_2C{\equiv}N$), 3.72-3.89 (m, 2H, N($\underline{CH}(CH_3)_2)_2$), 3.79 (s, 6H, $Ar_{DMT}O\underline{CH_3}$), 6.81 (d, 4H, $Ar_{DMT}H$), 7.16-7.22 (m, 1H, $Ar_{DMT}H$), 7.26-7.34 (m, 6H, $Ar_{DMT}H$), 7.40-7.45 (m, 2H, $Ar_{DMT}H$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 20.4, 24.7, 28.1, 29.2, 43.0, 43.1, 54.5, 55.0, 55.3, 58.4, 62.0, 62.2, 85.9, 113.1, 117.8, 126.7, 127.8, 128.3, 130.1, 136.7, 145.4, 158.4. ^{31}P NMR (161.96 MHz, $CDCl_3$, δ, ppm): 147.4.

*B.1.5. Synthesis of **A2***. To a stirred solution of ethyl acrylate (19.22 g, 0.192 mol, 2.4 eq.) in 34 mL ethanol absolute at 0°C under argon atmosphere was added dropwise propargylamine (6.58 mL, 0.080 mol, 1.0 eq.). After the addition, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was well evaporated to dryness. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 1/1 with 1% Et_3N) to obtain 16.23 g of **A2** (78%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.85 (t, 3H, $NCH_2CH_2\underline{CH_3}$), 1.25 (t, 6H, $OCH_2\underline{CH_3}$), 1.37-1.50 (m, 2H, $NCH_2\underline{CH_2}CH_3$), 2.33-2.39 (m, 2H, $N\underline{CH_2}CH_2CH_3$), 2.42 (t, 4H, $CO\underline{CH_2}$), 2.76 (t, 4H, $\underline{CH_2}N\underline{CH_2}$), 4.12 (q, 4H, $O\underline{CH_2}CH_3$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 11.8, 14.3, 20.5, 32.8, 49.4, 55.8, 60.3, 172.8.

*B.1.6. Synthesis of **B2***. To lithium aluminum hydride 1.0 M in THF (115 mL, 0.115 mol, 2.0 eq.) under argon atmosphere was added dropwise at 0°C a mixture of **A2** (15.00 g, 0.058 mol, 1.0 eq.) dissolved in 65 mL anh. THF. After the addition, the mixture was stirred 1 h at 0°C. Then it was allowed to warm to room temperature and stirred overnight. To hydrolyze the lithium aluminum hydride residues the mixture was cooled at 0°C. Slowly brine (9.2 mL), followed with a solution of NaOH 2.0 M in water (9.2 mL) and H_2O (25 mL) were added. The heterogeneous mixture was stirred 30 min at 0°C. The solid was removed by filtration and washed with 250 mL diethyl ether. The filtrate was evaporated to obtain 9.67 g of **B2** (95%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.90 (t, 3H, $NCH_2CH_2\underline{CH_3}$), 1.45-1.58 (m, 2H, $NCH_2\underline{CH_2}CH_3$), 1.72 (quint, 4H, $NCH_2\underline{CH_2}CH_2$), 2.33-2.41 (m, 2H, $N\underline{CH_2}CH_2CH_3$), 2.61 (t, 4H, $N\underline{CH_2}CH_2CH_2$), 3.74 (t, 4H, $NCH_2CH_2\underline{CH_2}$), 4.20 (s br, 2H, $\underline{HO}CH_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 11.8, 19.8, 28.6, 52.8, 56.0, 62.4.

*B.1.7. Synthesis of **C2***. **B2** (9.67 g, 0.055 mol, 1.2 eq.) was coevaporated with 20 mL of anh. pyridine. Afterward, the diol was dissolved in 32 mL DIPEA and 150 mL anh. DCM under argon atmosphere. DMT-Cl (15.57 g, 0.046 mol, 1.0 eq.) was added in four portions each 45 min. After the four additions, the mixture was stirred at room temperature overnight. The reaction was stopped with the addition of 27 mL of methanol and the mixture was evaporated to dryness. The residue was mixed with 180 mL $NaHCO_3$ (sat., aq.) and was extracted with EtOAc (2 x 180 mL). The combined organic layers were washed with H_2O (1 x 200 mL), brine (1 x 200 mL), dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 50/50 to 100% EtOAc with 1% Et_3N) to obtain 12.74 g of **C2** (58%) as a colorless oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{30}H_{40}NO_4^+$ 478.2952 found, 478.2959. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.88 (t, 3H, $NCH_2CH_2\underline{CH_3}$), 1.41-1.54 (m, 2H, $NCH_2\underline{CH_2}CH_3$), 1.61-1.70 (m, 2H, $HOCH_2\underline{CH_2}$), 1,73-1.83 (m, 2H, $NCH_2\underline{CH_2}CH_2$), 2.33-2.41 (m, 2H, $N\underline{CH_2}CH_2CH_3$), 2.49-2.57 (m, 2H, $N\underline{CH_2}CH_2CH_2$), 2.63 (t, 2H, $HOCH_2CH_2\underline{CH_2}$), 3.08 (t, 2H, $\underline{CH_2}$ODMT), 3.77 (t, 2H, $HO\underline{CH_2}$), 3.79 (s, 6H, $Ar_{DMT}O\underline{CH_3}$), 6.82 (d, 4H, $Ar_{DMT}H$), 7.17-7.23 (m, 1H, $Ar_{DMT}H$), 7.26-7.34 (m, 6H, $Ar_{DMT}H$), 7.39-7.45 (m, 2H, $Ar_{DMT}H$). ^{13}C NMR (100.62 MHz,

$CDCl_3$, δ, ppm): 11.9, 20.2, 27.4, 27.9, 51.2, 55.3, 56.2, 61.8, 64.9, 85.9, 113.1, 126.7, 127.8, 128.2, 130.1, 136.6, 145.3, 158.4.

*B.1.8. Synthesis of **M2***. **C2** (12.74 g, 0.027 mol, 1.0 eq.) was dissolved in 50 mL anh. DCM. Then, DIPEA (18.58 mL, 0.107 mol, 4.0 eq.) was added. The solution was cooled to 0°C under argon atmosphere. 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (6.94 g, 0.029 mol, 1.1 eq.) dissolved in 15 mL anh. DCM was added at 0°C. The reaction mixture was stirred at 0°C for 30 min, then allowed to reach room temperature and stirred for 1 h. The reaction mixture was extracted with 50 mL $NaHCO_3$ (sat., aq.). The aqueous phase was washed with 40 mL DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 50/50 with 1% Et_3N) to obtain 15.76 g of **M2** (87%) as a colorless oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{39}H_{57}N_3O_5P^+$ 678.4030; found, 678.4035. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.83 (t, 3H, $NCH_2CH_2\underline{CH_3}$), 1.11-1.22 (m, 12H, $N(CH(\underline{CH_3})_2)_2$), 1.35-1.46 (m, 2H, $NCH_2\underline{CH_2}CH_3$), 1.65-1.78 (m, 4H, $\underline{CH_2}CH_2NCH_2\underline{CH_2}$), 2.30-2.37 (m, 2H, $N\underline{CH_2}CH_2CH_3$), 2.44-2.53 (m, 4H, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 2.57-2.63 (m, 2H, $CH_2\underline{CH_2}C{\equiv}N$), 3.06 (t, 2H, $\underline{CH_2}$ODMT), 3.51-3.71 (m, 4H, $\underline{CH_2}$OP, $\underline{CH_2}CH_2C{\equiv}N$), 3.72-3.89 (m, 2H, $N(\underline{CH}(CH_3)_2)_2$), 3.79 (s, 6H, $Ar_{DMT}O\underline{CH_3}$), 6.82 (d, 4H, $Ar_{DMT}H$), 7.16-7.23 (m, 1H, $Ar_{DMT}H$), 7.26-7.34 (m, 6H, $Ar_{DMT}H$), 7.40-7.45 (m, 2H, $Ar_{DMT}H$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 12.0, 20.4, 24.7, 27.8, 29.0 43.1, 50.7, 51.2, 55.3, 56.2, 58.4, 62.0, 62.3, 85.8, 113.0, 117.8, 126.7, 127.8, 128.3, 130.1, 136.7, 145.5, 158.4. ^{31}P NMR (161.96 MHz, $CDCl_3$, δ, ppm): 147.3.

*B.1.9. Synthesis of **A3***. To a stirred solution of ethyl acrylate (16.92 g, 0.169 mol, 2.4 eq.) in 30 mL ethanol absolute at 0°C under argon atmosphere was added dropwise amylamine (8.11 mL, 0.070 mol, 1.0 eq.). After the addition, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was well evaporated to dryness. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 3/7 with 1% Et_3N) to obtain 14.40 g of **A3** (72%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.88 (t, 3H, $NCH_2(CH_2)_2CH_2\underline{CH_3}$), 1.25 (t, 6H, $OCH_2\underline{CH_3}$), 1.16-1.35 (m, 4H, $NCH_2\underline{(CH_2)_2}CH_2CH_3$), 1.35-1.47 (m, 2H, $NCH_2(CH_2)_2\underline{CH_2}CH_3$), 2.34-2.46 (m, 6H, $N\underline{CH_2}(CH_2)_2CH_2CH_3$, $CO\underline{CH_2}$), 2.76 (t, 4H, $\underline{CH_2}N\underline{CH_2}$), 4.12 (q, 4H, $O\underline{CH_2}CH_3$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.1, 14.2, 22.6, 26.9, 29.6, 32.8, 49.3, 53.8, 60.3, 172.7.

*B.1.10. Synthesis of **B3***. To lithium aluminum hydride 1.0 M in THF (100 mL, 0.100 mol, 2.0 eq.) under argon atmosphere was added dropwise at 0°C a mixture of **A3** (14.40 g, 0.050 mol, 1.0 eq.) dissolved in 60 mL anh. THF. After the addition, the mixture was stirred 1 h at 0°C. Then it was allowed to warm to room temperature and stirred overnight. To hydrolyse the lithium aluminum hydride residues the mixture was cooled at 0°C. Slowly brine (8.9 mL), followed with a solution of NaOH 2.0 M in water (8.9 mL) and H_2O (23 mL) were added. The heterogeneous mixture was stirred 30 min at 0°C. The solid was removed by filtration and washed with 250 mL diethyl ether. The filtrate was evaporated to obtain 10.01 g of **B3** (98%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.89 (t, 3H, $NCH_2(CH_2)_2CH_2\underline{CH_3}$), 1.18-1.36 (m, 4H, $NCH_2\underline{(CH_2)_2}CH_2CH_3$), 1.43-1.53 (m, 2H, $NCH_2(CH_2)_2\underline{CH_2}CH_3$), 1.71 (quint, 4H, $NCH_2\underline{CH_2}CH_2$), 2.35-2.43 (m, 2H, $N\underline{CH_2}(CH_2)_2CH_2CH_3$), 2.60 (t, 4H, $N\underline{CH_2}CH_2CH_2$), 3.73 (t, 4H, $NCH_2CH_2\underline{CH_2}$), 4.24 (s br, 2H, $\underline{HO}CH_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.1, 22.7, 26.4, 28.6, 29.7, 52.9, 54.1, 62.6.

*B.1.11. Synthesis of **C3***. B3 (10.00 g, 0.049 mol, 1.2 eq.) was coevaporated with 20 mL of anh. pyridine. Afterward, the diol was dissolved in 28 mL DIPEA and 135 mL anh. DCM under argon atmosphere. DMT-Cl (13.89 g, 0.041 mol, 1.0 eq.) was added in four portions each 45 min. After the four additions, the mixture was stirred at room temperature overnight. The reaction was stopped with the addition of 28 mL of methanol and the mixture was evaporated to dryness. The residue was mixed with 160 mL $NaHCO_3$ (sat., aq.) and was extracted with EtOAc (2 x 160 mL). The combined organic layers were washed with H_2O (1 x 180 mL), brine (1 x 180 mL), dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 50/50 to 100% EtOAc with 1% Et_3N) to obtain 11.08 g of C3 (53%) as a yellowish oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{32}H_{44}NO_4^+$ 506.3265 found, 506.3270. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.90 (t, 3H, $NCH_2(CH_2)_2CH_2\underline{CH_3}$), 1.18-1.37 (m, 4H, $NCH_2\underline{(CH_2)_2}CH_2CH_3$), 1.40-1.51 (m, 2H, $NCH_2(CH_2)_2\underline{CH_2}CH_3$), 1.61-1.70 (m, 2H, $HOCH_2\underline{CH_2}$), 1,71-1.83 (m, 2H, $NCH_2\underline{CH_2}CH_2$), 2.34-2.44 (m, 2H, $N\underline{CH_2}(CH_2)_2CH_2CH_3$), 2.49-2.57 (m, 2H, $N\underline{CH_2}CH_2CH_2$), 2.63 (t, 2H, $HOCH_2CH_2\underline{CH_2}$), 3.08 (t, 2H, $\underline{CH_2}$ODMT), 3.77 (t, 2H, $HO\underline{CH_2}$), 3.79 (s, 6H, $Ar_{DMT}O\underline{CH_3}$), 6.82 (d, 4H, $Ar_{DMT}H$), 7.17-7.23 (m, 1H, $Ar_{DMT}H$), 7.26-7.34 (m, 6H, $Ar_{DMT}H$), 7.39-7.45 (m, 2H, $Ar_{DMT}H$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.2, 22.8, 26.7, 27.4, 27.9, 29.7, 51.2, 54.3, 55.3, 61.8, 64.9, 85.9, 113.1, 126.7, 127.8, 128.2, 130.1, 136.6, 145.3, 158.4.

*B.1.12. Synthesis of **M3***. **C3** (11.05 g, 0.022 mol, 1.0 eq.) was dissolved in 42 mL anh. DCM. Then, DIPEA (15.22 mL, 0.087 mol, 4.0 eq.) was added. The solution was cooled to 0°C under argon atmosphere. 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (5.68 g, 0.024 mol, 1.1 eq.) dissolved in 10 mL anh. DCM was added at 0°C. The reaction mixture was stirred at 0°C for 30 min, then allowed to reach room temperature and stirred for 1 h. The reaction mixture was extracted with 42 mL $NaHCO_3$ (sat., aq.). The aqueous phase was washed with 40 mL DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 50/50 with 1% Et_3N) to obtain 13.42 g of **M3** (87%) as a yellowish oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{41}H_{61}N_3O_5P^+$ 706.4343; found, 706.4348. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.88 (t, 3H, $NCH_2(CH_2)_2CH_2\underline{CH_3}$), 1.12-1.20 (m, 12H, $N(CH(\underline{CH_3})_2)_2$), 1.18-1.34 (m, 4H, $NCH_2\underline{(CH_2)_2}CH_2CH_3$), 1.34-1.45 (m, 2H, $NCH_2(CH_2)_2\underline{CH_2}CH_3$), 1.66-1.79 (m, 4H, $\underline{CH_2}CH_2NCH_2\underline{CH_2}$), 2.31-2.41 (m, 2H, $N\underline{CH_2}(CH_2)_2CH_2CH_3$), 2.44-2.55 (m, 4H, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 2.57-2.63 (m, 2H, $CH_2\underline{CH_2}C{\equiv}N$), 3.06 (t, 2H, $\underline{CH_2}$ODMT), 3.51-3.71 (m, 4H, $\underline{CH_2}$OP, $\underline{CH_2}CH_2C{\equiv}N$), 3.72-3.88 (m, 2H, $N(\underline{CH}(CH_3)_2)_2$), 3.79 (s, 6H, $Ar_{DMT}O\underline{CH_3}$), 6.81 (d, 4H, $Ar_{DMT}H$), 7.16-7.23 (m, 1H, $Ar_{DMT}H$), 7.26-7.34 (m, 6H, $Ar_{DMT}H$), 7.40-7.45 (m, 2H, $Ar_{DMT}H$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.3, 20.4, 22.8, 24.7, 27.0, 27.8, 29.0, 29.9, 43.1, 50.8, 51.2, 54.3, 55.3, 58.4, 62.1, 62.3, 85.8, 113.1, 117.8, 126.7, 127.8, 128.3, 130.1, 136.8, 145.5, 158.4. ^{31}P NMR (161.96 MHz, $CDCl_3$, δ, ppm): 147.3.

*B.1.13. Synthesis of **A4***. To a stirred solution of ethyl acrylate (16.82 g, 0.168 mol, 2.4 eq.) in 30 mL ethanol absolute at 0°C under argon atmosphere was added dropwise heptylamine (10.38 mL, 0.070 mol, 1.0 eq.). After the addition, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was well evaporated to dryness. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 2/8 with 1% Et_3N) to obtain 15.57 g of **A4** (71%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.88 (t, 3H, $NCH_2(CH_2)_4CH_2\underline{CH_3}$), 1.25 (t, 6H, $OCH_2\underline{CH_3}$), 1.16-1.35 (m, 8H, $NCH_2\underline{(CH_2)_4}CH_2CH_3$), 1.35-1.47 (m, 2H, $NCH_2(CH_2)_4\underline{CH_2}CH_3$), 2.34-2.47 (m, 6H, $N\underline{CH_2}(CH_2)_4CH_2CH_3$, $CO\underline{CH_2}$), 2.76 (t, 4H, $\underline{CH_2}N\underline{CH_2}$), 4.12 (q,

4H, O$\underline{CH_2}CH_3$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.2, 14.3, 22.7, 27.3, 27.4, 29.3, 32.0, 32.8, 49.4, 53.9, 60.4, 172.8.

*B.1.14. Synthesis of **B4**.* To lithium aluminum hydride 1.0 M in THF (100 mL, 0.100 mol, 2.0 eq.) under argon atmosphere was added dropwise at 0°C a mixture of **A4** (15.57 g, 0.049 mol, 1.0 eq.) dissolved in 60 mL anh. THF. After the addition, the mixture was stirred 1 h at 0°C. Then it was allowed to warm to room temperature and stirred overnight. To hydrolyse the lithium aluminum hydride residues the mixture was cooled at 0°C. Slowly brine (8.9 mL), followed with a solution of NaOH 2.0 M in water (8.9 mL) and H_2O (23 mL) were added. The heterogeneous mixture was stirred 30 min at 0°C. The solid was removed by filtration and washed with 250 mL diethyl ether. The filtrate was evaporated to obtain 11.30 g of **B4** (99%) as a colorless oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.88 (t, 3H, $NCH_2(CH_2)_4CH_2\underline{CH_3}$), 1.18-1.35 (m, 8H, $NCH_2\underline{(CH_2)_4}CH_2CH_3$), 1.43-1.53 (m, 2H, $NCH_2(CH_2)_4\underline{CH_2}CH_3$), 1.72 (quint, 4H, $NCH_2\underline{CH_2}CH_2$), 2.35-2.43 (m, 2H, $N\underline{CH_2}(CH_2)_4CH_2CH_3$), 2.61 (t, 4H, $N\underline{CH_2}CH_2CH_2$), 3.74 (t, 4H, $NCH_2CH_2\underline{CH_2}$), 4.17 (s br, 2H, $\underline{HO}CH_2$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.1, 22.7, 26.7, 27.5, 28.6, 29.3, 31.9, 52.9, 54.2, 62.5.

*B.1.15. Synthesis of **C4**.* **B4** (11.20 g, 0.048 mol, 1.2 eq.) was coevaporated with 20 mL of anh. pyridine. Afterward, the diol was dissolved in 28 mL DIPEA and 135 mL anh. DCM under argon atmosphere. DMT-Cl (13.66 g, 0.040 mol, 1.0 eq.) was added in four portions each 45 min. After the four additions, the mixture was stirred at room temperature overnight. The reaction was stopped with the addition of 25 mL of methanol and the mixture was evaporated to dryness. The residue was mixed with 160 mL $NaHCO_3$ (sat., aq.) and was extracted with EtOAc (2 x 160 mL). The combined organic layers were washed with H_2O (1 x 180 mL), brine (1 x 180 mL), dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 50/50 to 100% EtOAc with 1% Et_3N) to obtain 13.62 g of **C4** (63%) as a yellowish oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{34}H_{48}NO_4^+$ 534.3578 found, 534.3570. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.88 (t, 3H, $NCH_2(CH_2)_4CH_2\underline{CH_3}$), 1.18-1.37 (m, 8H, $NCH_2\underline{(CH_2)_4}CH_2CH_3$), 1.40-1.51 (m, 2H, $NCH_2(CH_2)_4\underline{CH_2}CH_3$), 1.61-1.70 (m, 2H, $HOCH_2\underline{CH_2}$), 1,71-1.83 (m, 2H, $NCH_2\underline{CH_2}CH_2$), 2.34-2.44 (m, 2H, $N\underline{CH_2}(CH_2)_4CH_2CH_3$), 2.49-2.58 (m, 2H, $N\underline{CH_2}CH_2CH_2$), 2.63 (t, 2H, $HOCH_2CH_2\underline{CH_2}$), 3.08 (t, 2H, $\underline{CH_2}$ODMT), 3.77 (t, 2H, $HO\underline{CH_2}$), 3.79 (s, 6H, $Ar_{DMT}O\underline{CH_3}$), 6.82 (d, 4H, $Ar_{DMT}H$), 7.17-7.23 (m, 1H, $Ar_{DMT}H$), 7.26-7.34 (m, 6H, $Ar_{DMT}H$), 7.39-7.45 (m, 2H, $Ar_{DMT}H$). ^{13}C NMR (100.62 MHz, $CDCl_3$, δ, ppm): 14.2, 22.8, 27.1, 27.4, 27.6, 27.9, 29.4, 32.0, 51.3, 54.3, 55.3, 61.8, 64.9, 85.9, 113.1, 126.7, 127.8, 128.3, 130.1, 136.6, 145.4, 158.5.

*B.1.16. Synthesis of **M4**.* **C4** (5.90 g, 0.011 mol, 1.0 eq.) was dissolved in 21 mL anh. DCM. Then, DIPEA (7.70 mL, 0.044 mol, 4.0 eq.) was added. The solution was cooled to 0°C under argon atmosphere. 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (2.88 g, 0.012 mol, 1.1 eq.) dissolved in 7 mL anh. DCM was added at 0°C. The reaction mixture was stirred at 0°C for 30 min, then allowed to reach room temperature and stirred for 1 h. The reaction mixture was extracted with 21 mL $NaHCO_3$ (sat., aq.). The aqueous phase was washed with 25 mL DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography on silica gel (EtOAc / hexane: 50/50 with 1% Et_3N) to obtain 7.94 g of **M4** (98%) as a colorless oil. HRMS *m/z*: $[M+H]^+$ calculated for $C_{43}H_{65}N_3O_5P^+$ 734.4656; found, 734.4655. ^{1}H NMR (400.13 MHz, $CDCl_3$, *δ*, ppm): 0.88 (t, 3H, $NCH_2(CH_2)_4CH_2\underline{CH_3}$), 1.12-1.20 (m, 12H,

N(CH(CH$_3$)$_2$)$_2$), 1.20-1.34 (m, 8H, NCH$_2$(CH$_2$)$_4$CH$_2$CH$_3$), 1.34-1.45 (m, 2H, NCH$_2$(CH$_2$)$_4$CH$_2$CH$_3$), 1.66-1.79 (m, 4H, CH$_2$CH$_2$NCH$_2$CH$_2$), 2.31-2.41 (m, 2H, NCH$_2$(CH$_2$)$_4$CH$_2$CH$_3$), 2.44-2.55 (m, 4H, CH$_2$CH$_2$NCH$_2$CH$_2$), 2.57-2.63 (m, 2H, CH$_2$CH$_2$C≡N), 3.06 (t, 2H, CH$_2$ODMT), 3.51-3.71 (m, 4H, CH$_2$OP, CH$_2$CH$_2$C≡N), 3.72-3.88 (m, 2H, N(CH(CH$_3$)$_2$)$_2$), 3.79 (s, 6H, Ar$_{DMT}$OCH$_3$), 6.81 (d, 4H, Ar$_{DMT}$H), 7.16-7.23 (m, 1H, Ar$_{DMT}$H), 7.26-7.34 (m, 6H, Ar$_{DMT}$H), 7.40-7.45 (m, 2H, Ar$_{DMT}$H). ^{13}C NMR (100.62 MHz, CDCl$_3$, δ, ppm): 14.2, 20.4, 22.8, 24.7, 27.3, 27.8, 29.0, 29.5, 32.0, 43.1, 50.8, 51.2, 54.3, 55.3, 58.4, 62.0, 62.3, 85.8, 113.1, 117.8, 126.7, 127.8, 128.3, 130.1, 136.8, 145.5, 158.4. ^{31}P NMR (161.96 MHz, CDCl$_3$, δ, ppm): 147.3.

B.2 Synthesis of polymers **H1**-**H4** and **P1**-**P8** by automated phosphoramidite chemistry.

All polymers were synthesized under argon in rigorously dry conditions by automated solid-phase phosphoramidite method on an Expedite DNA synthesizer (Perseptive Biosystem 8900). Phosphoramidite monomers were charged to the bottles of synthesizer as 0.05 M solutions in anhydrous ACN. Syntheses were carried out in the DMT-on mode, in which the terminal DMT protective group is not cleaved. Thymidine loaded dT-CPG 1000 columns (1 μmol in cartridge, Glen Research) were used as solid supports on the Expedite instrument. The crude products were cleaved by 1:1 mixture of MeNH$_2$ (40% in water) and NH$_3$ (30% in water) for 30 minutes and purified using reverse-phase C18 columns (Glen-Pak, DNA purification cartridge, Glen Research). This procedure permits to separate the DMT-terminated targeted structures from the truncated sequences deactivated by the capping reaction.[2] Then, the terminal DMT moiety of the desired sequence-coded polymers was removed directly on the Glen-pak column and washed out by solvent elution.

C Measurements and Analysis

C.1 Nuclear Magnetic Resonance (NMR)

All NMR were recorded on a Bruker Avance 400 spectrometer equipped with an Ultrashield magnet. Chemical shifts (δ) are reported in parts per million (ppm) against solvent residual signal (^{1}H NMR, CDCl$_3$: δ = 7.26 ppm; ^{13}C NMR, CDCl$_3$: δ = 77.16 ppm). ^{1}H NMR spectra were recorded at 400.13 MHz, ^{13}C NMR spectra at 100.62 MHz and ^{31}P NMR at 161.96 MHz. The NMR solvent deuterated chloroform (99,8 %, chloroform-d1) was purchased from Aldrich.

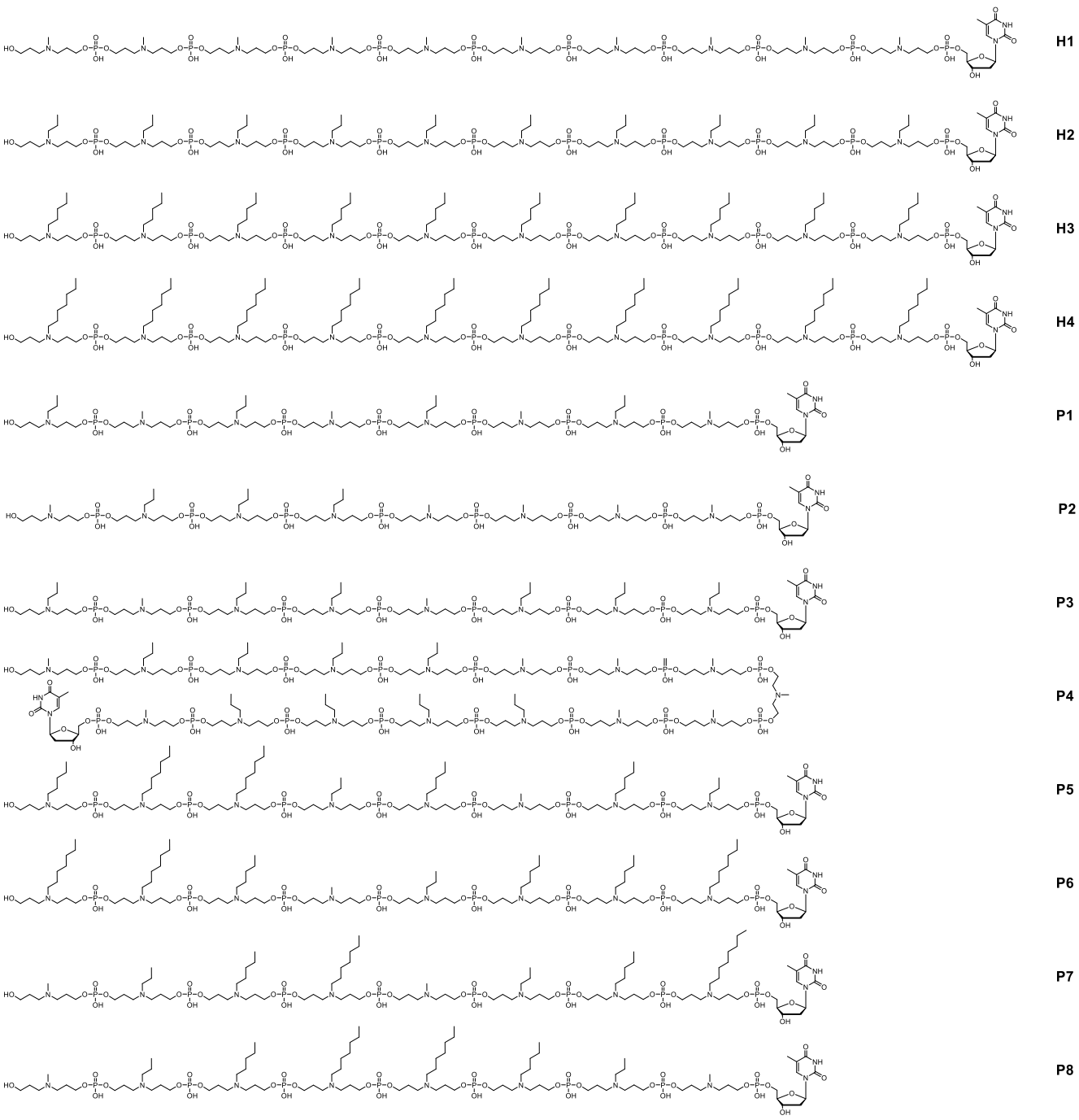
C.2 High Performance Liquide Chromatography (HPLC)

Anion exchange HPLC was used to verify the purity of the polymers on a 1220 Infinity II LC Analytical HPLC System from Agilent technology. The device was equipped with a column for oligonucleotides PA100 Thermo Scientific™ Dionex™ DNAPac™ (13 μm pores, 4 x 250 mm) and a UV detector (260 and 280 nm). Phase A was prepared with 10% ACN and 20% of 2 M NH$_3$ in MilliQ water and Phase B with 2.5 M NaCl in MilliQ water. Elution was performed by applying a linear gradient of Phase B (from 5 to 30%) over 25 min at a flow rate of 1 mL/min.

C.3 High Resolution Mass Spectrometry (HRMS)

MS experiments were performed using a QStar Elite mass spectrometer (Applied Biosystems SCIEX, Concord, ON, Canada), a QTOF equipped with an electrospray ionization (ESI) source operated in the positive mode (capillary voltage: +5500 V; cone voltage: +75 V). Air was used as the nebulizing gas (10 psi) and nitrogen as the curtain gas (20 psi). Dry samples were dissolved in 300 µL H_2O/ACN (50/50, v/v), further diluted (1/10 to 1/100) in a methanolic solution of ammonium acetate (3 mM) and introduced in the ESI source with a syringe pump at a 10 µL min^{-1} flow rate. In the MS mode, ions were accurately mass measured after internal calibration of the orthogonal acceleration time-of-flight (oa-TOF) mass analyzer, using two cationic adducts of PMMA to bracket the targeted analyte *m/z* value. MS/MS experiments were carried out with collision induced dissociation (CID), where precursor ions were selected in a quadrupole mass analyzer, injected into the collision cell filled with nitrogen, and product ions were measured in the oa-TOF. Instrument control, data acquisition and data processing of all experiments were achieved using Analyst software (QS 2.0) provided by Applied Biosystems SCIEX.

D Supplementary Figures



Scheme S1. Molecular structures of homopolymers **H1**-**H4** and copolymers **P1**-**P8**.

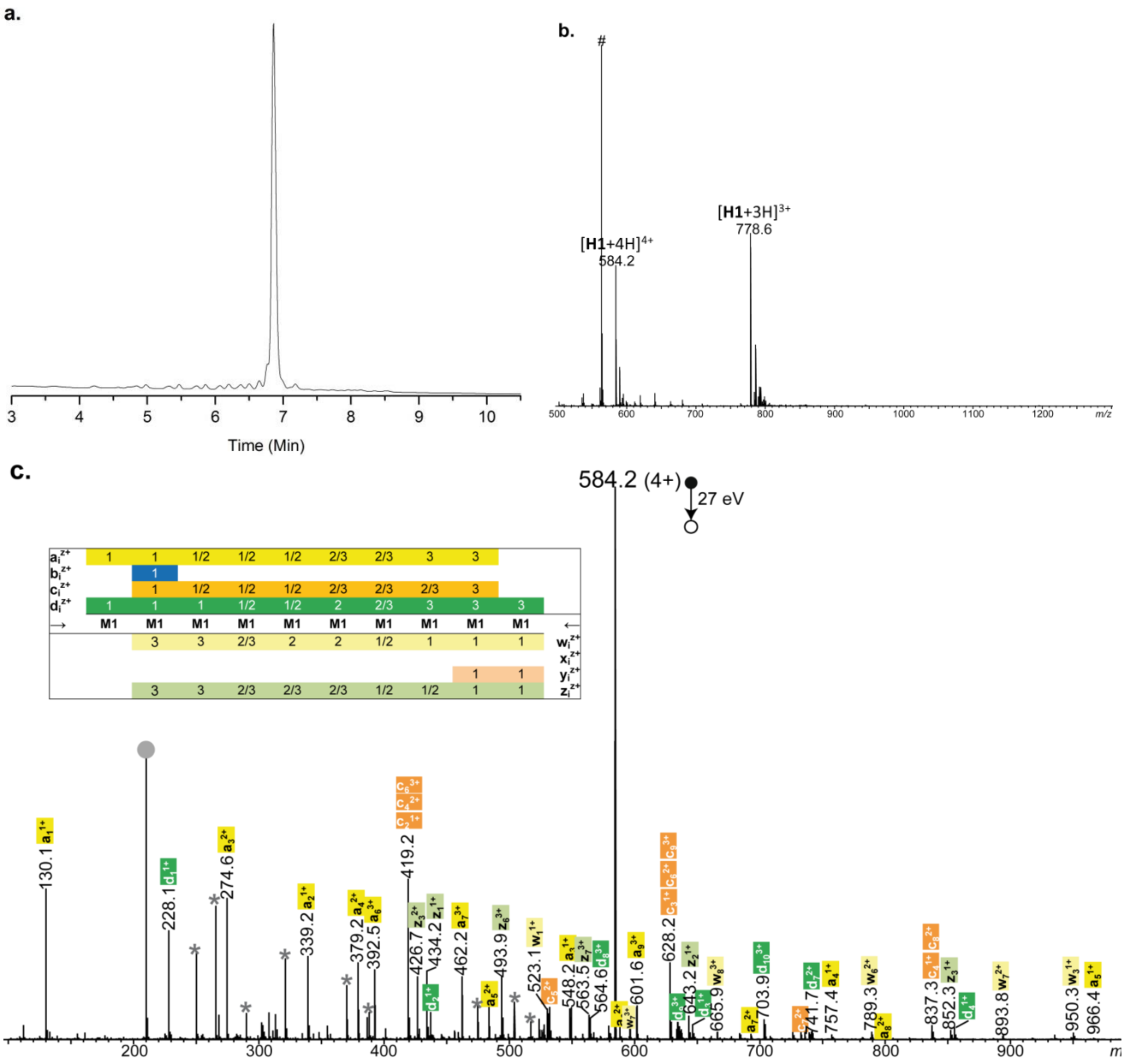


Figure S1. (**a**) HPLC analysis of homopolymer **H1**. (**b**) Mass spectrometry analysis of **H1**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **H1** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circle designates internal fragments corresponding to the protonated coded unit **M1** at *m/z* 210.1.

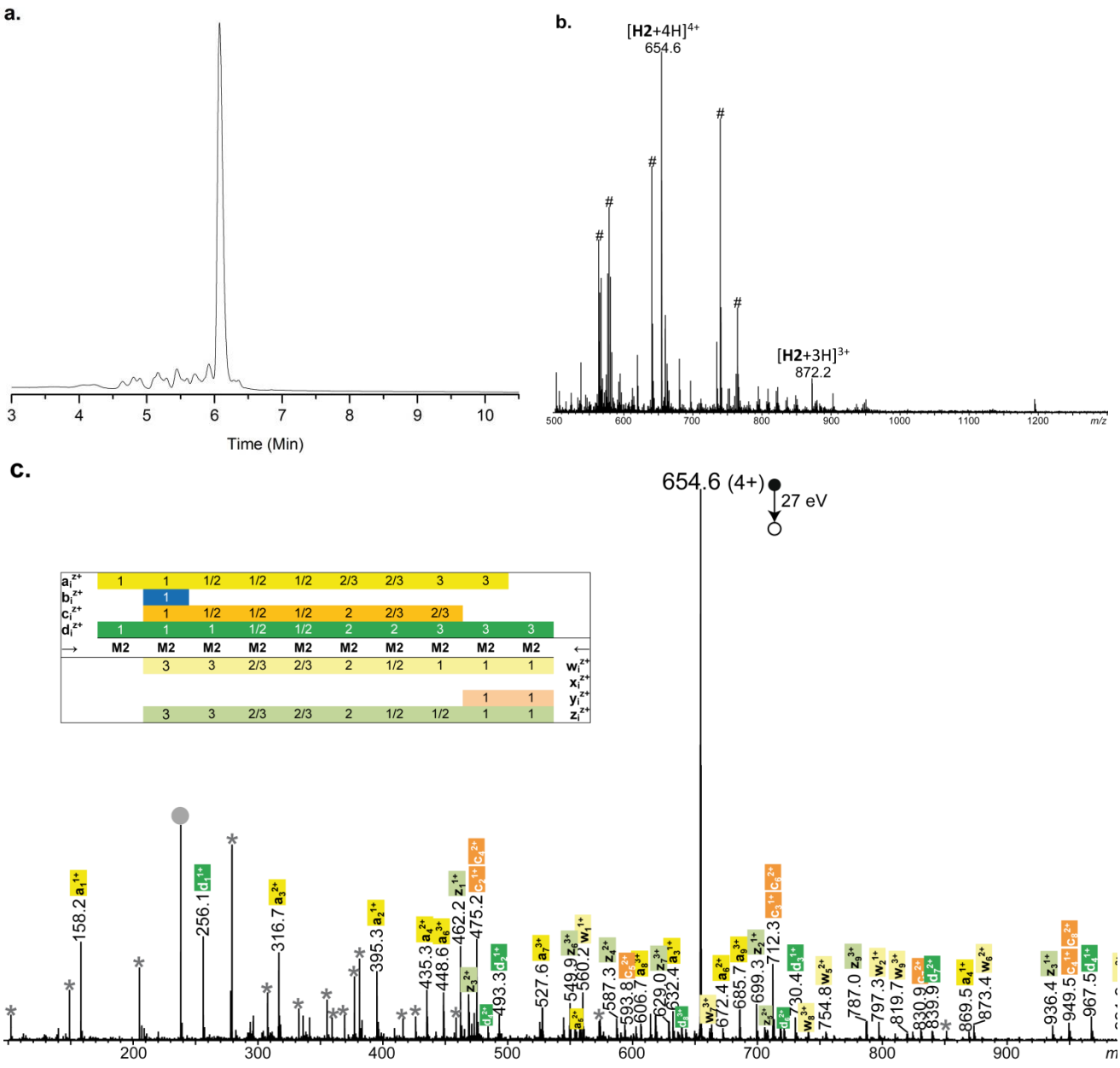


Figure S2. (**a**) HPLC analysis of homopolymer **H2**. (**b**) Mass spectrometry analysis of **H2**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **H2** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circle designates internal fragments corresponding to the protonated coded unit **M2** at *m/z* 238.1.

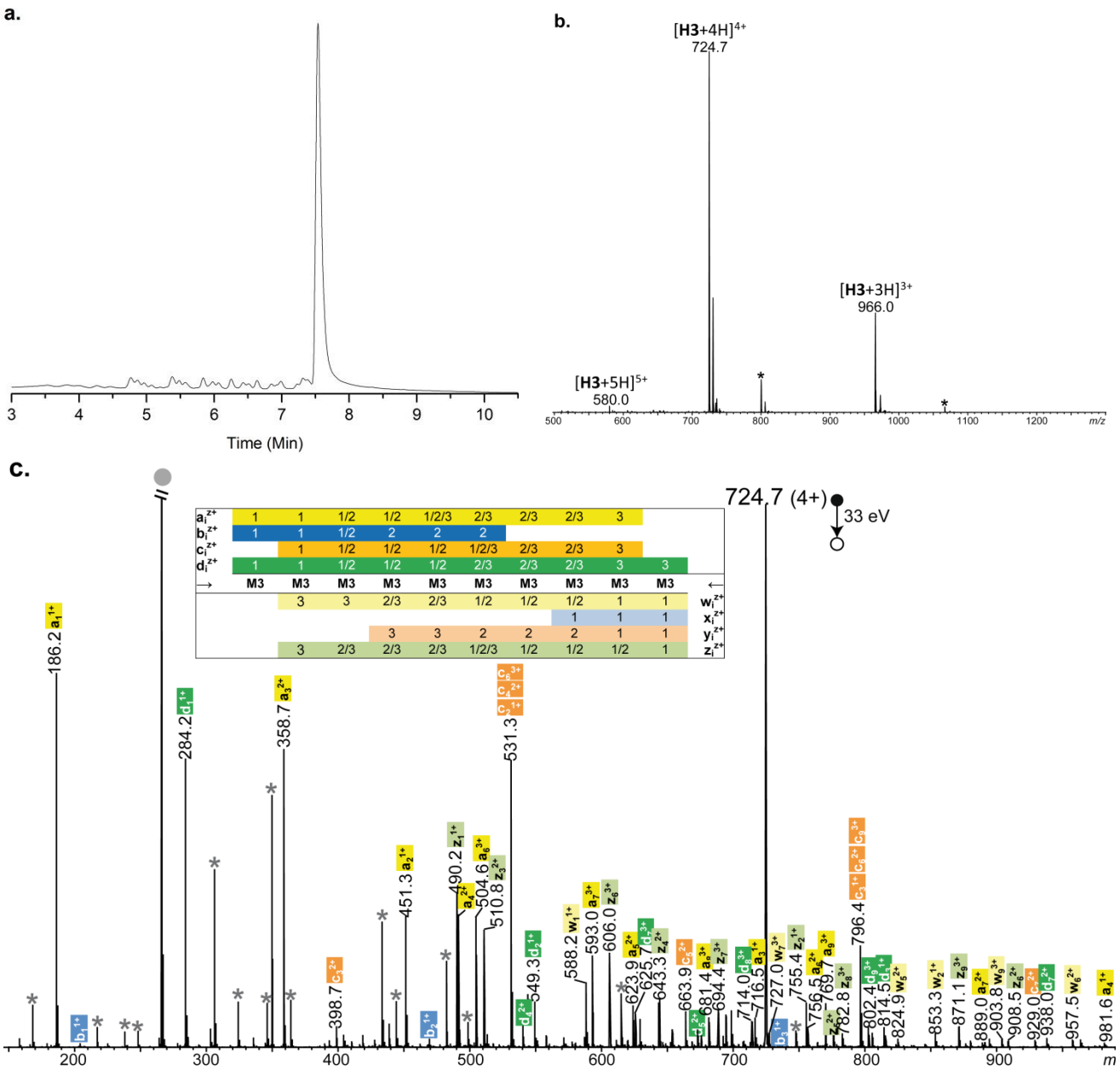


Figure S3. (**a**) HPLC analysis of homopolymer **H3**. (**b**) Mass spectrometry analysis of **H3**. Black asterisks indicate traces of DMT-protected polymer. (**c**) Tandem mass spectrometry analysis of **H3** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circle designates internal fragments corresponding to the protonated coded unit **M3** at *m/z* 266.1.

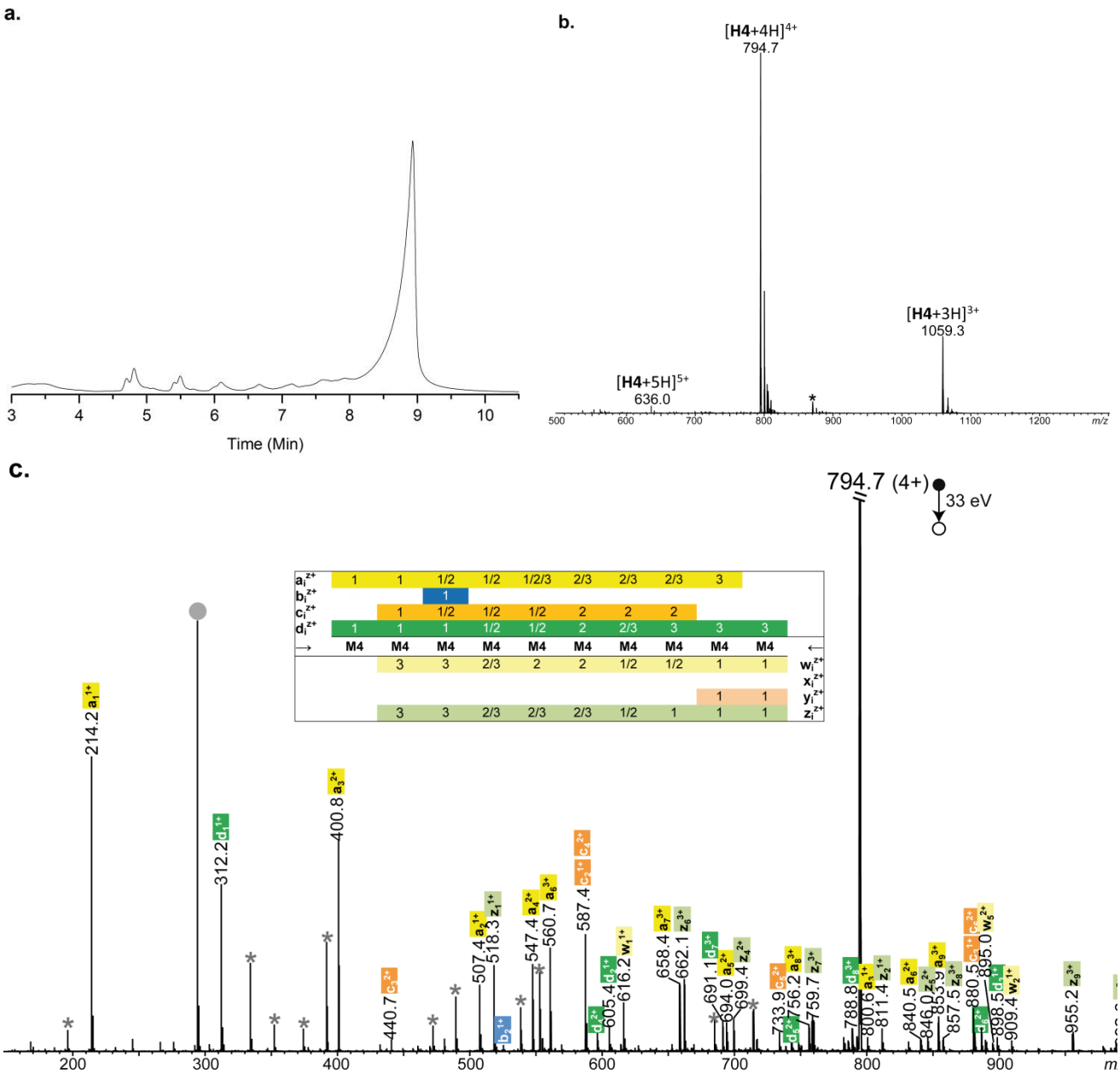


Figure S4. (**a**) HPLC analysis of homopolymer **H4**. (**b**) Mass spectrometry analysis of **H4**. Black asterisks indicate traces of DMT-protected polymer. (**c**) Tandem mass spectrometry analysis of **H4** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circle designates internal fragments corresponding to the protonated coded unit **M4** at *m/z* 294.2.

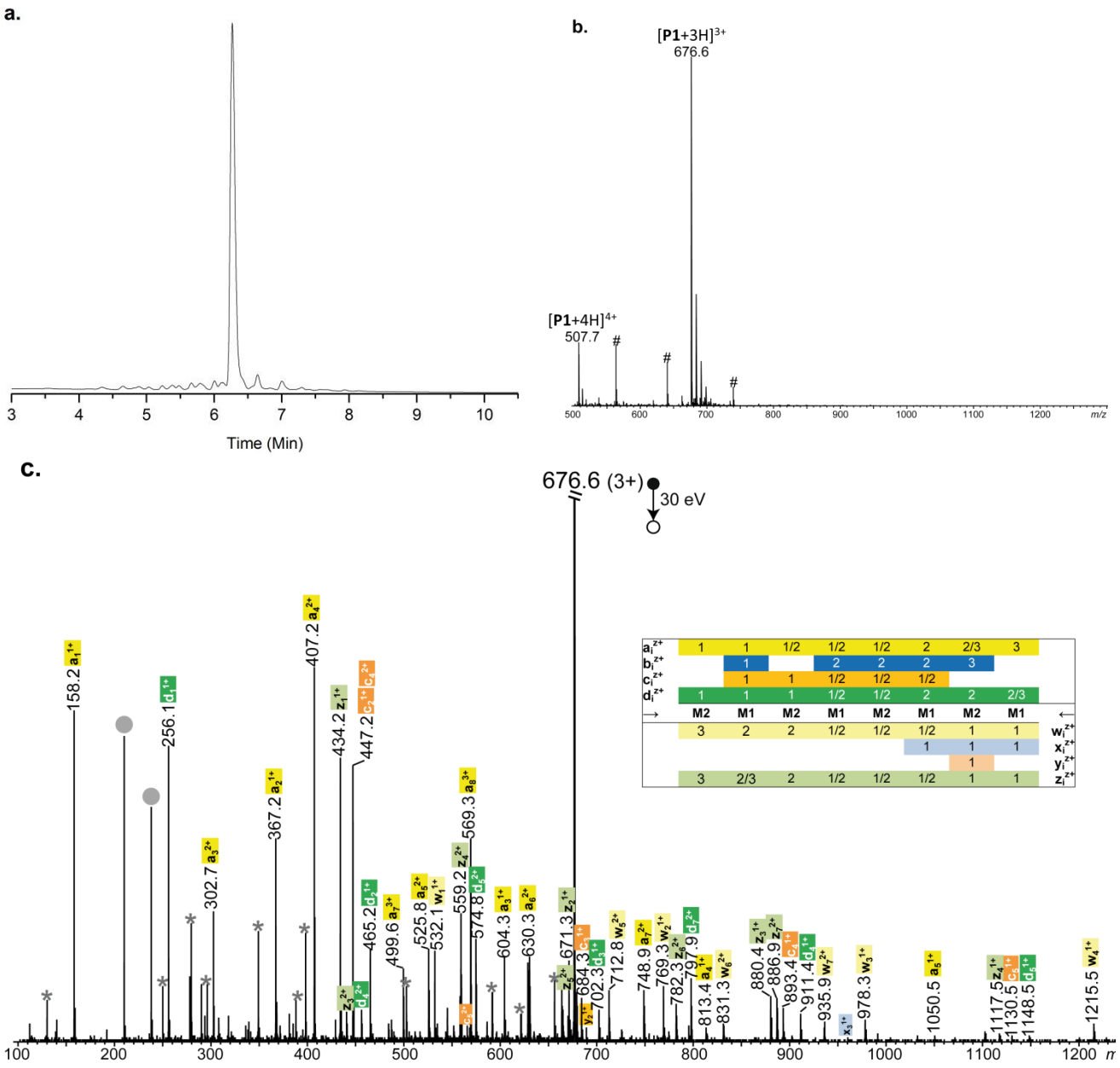


Figure S5. (**a**) HPLC analysis of copolymer **P1**. (**b**) Mass spectrometry analysis of **P1**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **P1** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circles designate internal fragments corresponding to the protonated coded units **M1** at *m/z* 210.1 and **M2** at *m/z* 238.1.

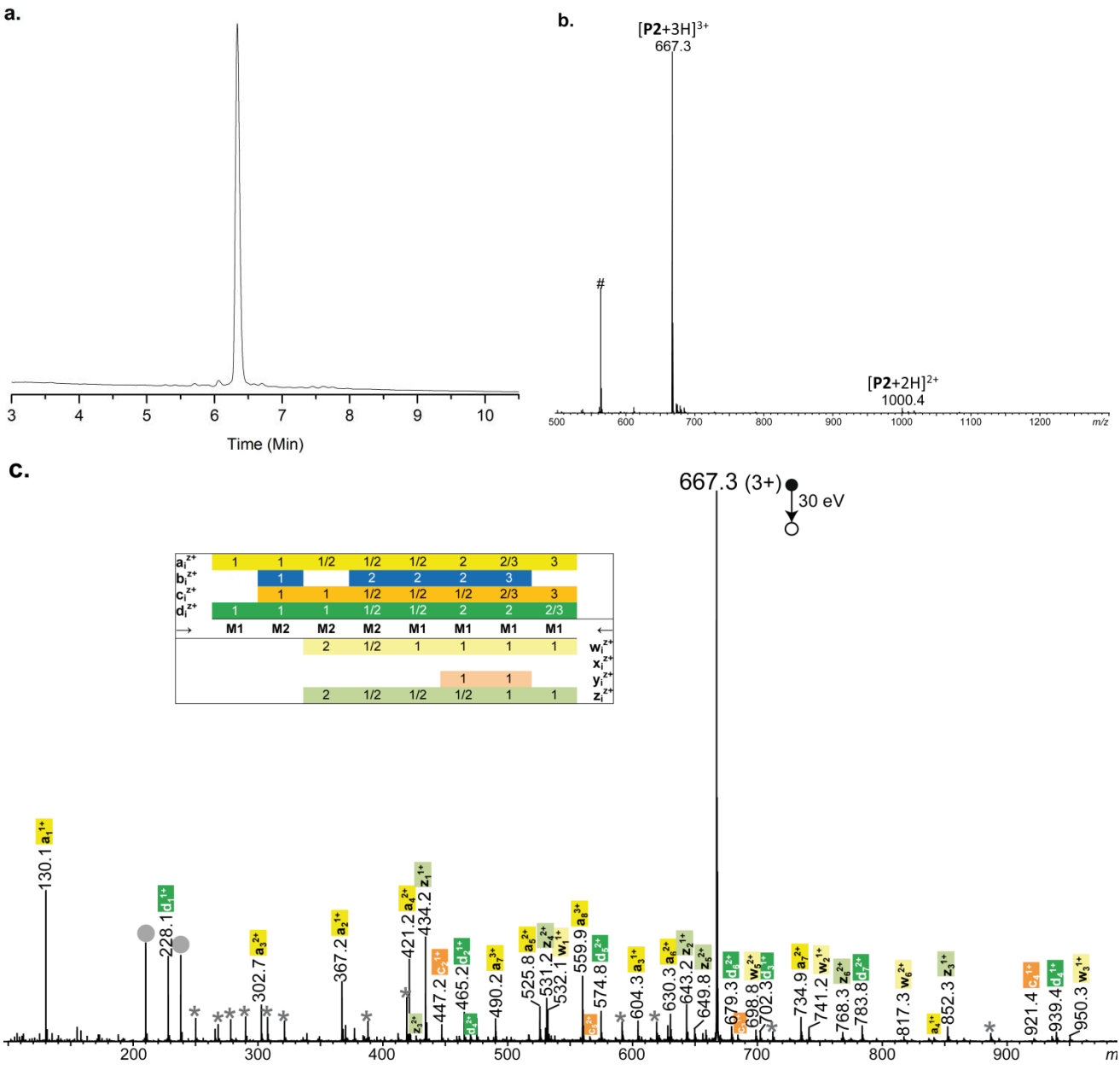


Figure S6. (**a**) HPLC analysis of copolymer **P2**. (**b**) Mass spectrometry analysis of **P2**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **P2** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circles designate internal fragments corresponding to the protonated coded units **M1** at *m/z* 210.1 and **M2** at *m/z* 238.1.

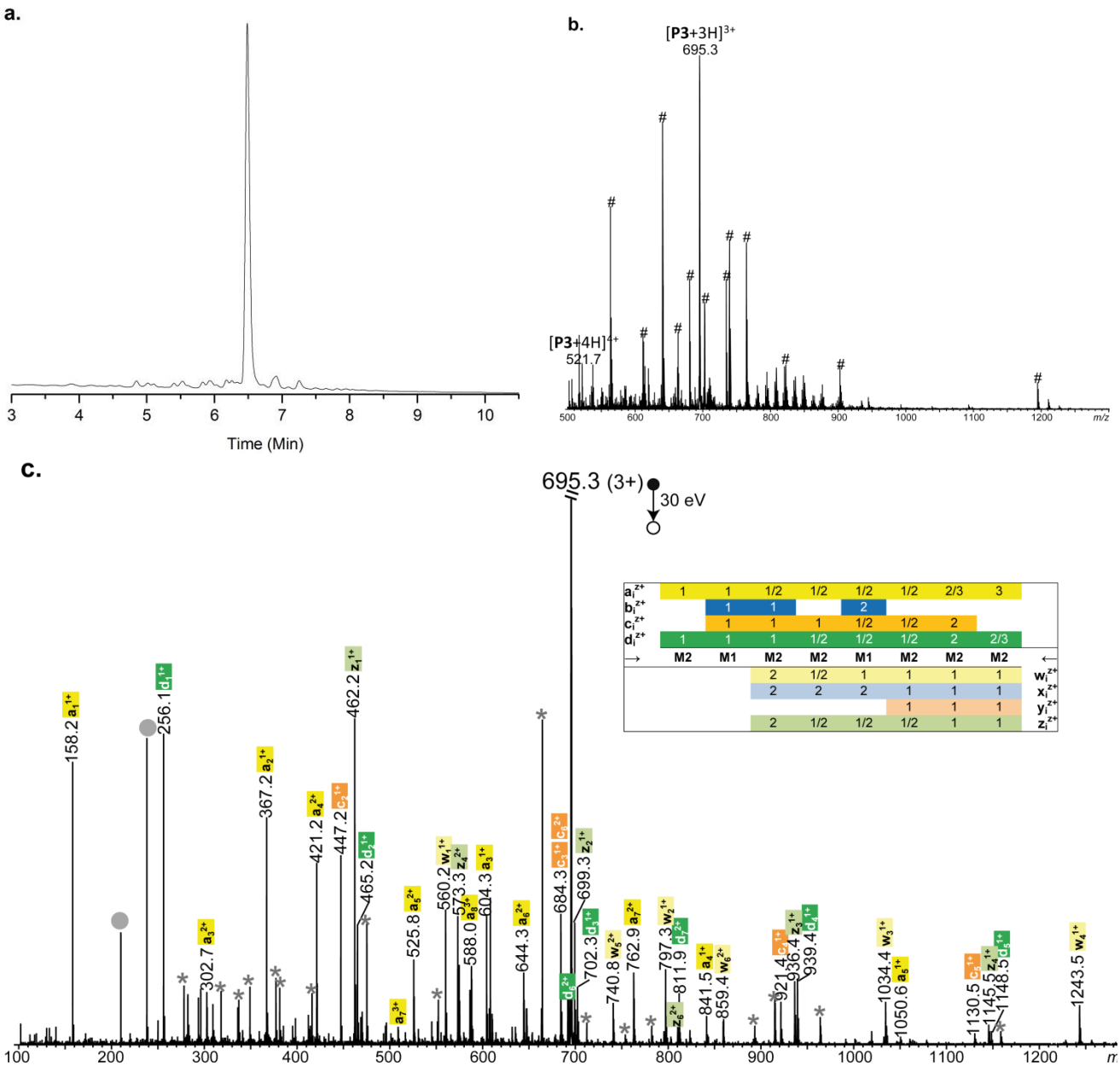


Figure S7. (**a**) HPLC analysis of copolymer **P3**. (**b**) Mass spectrometry analysis of **P3**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **P3** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circles designate internal fragments corresponding to the protonated coded units **M1** at *m/z* 210.1 and **M2** at *m/z* 238.1.

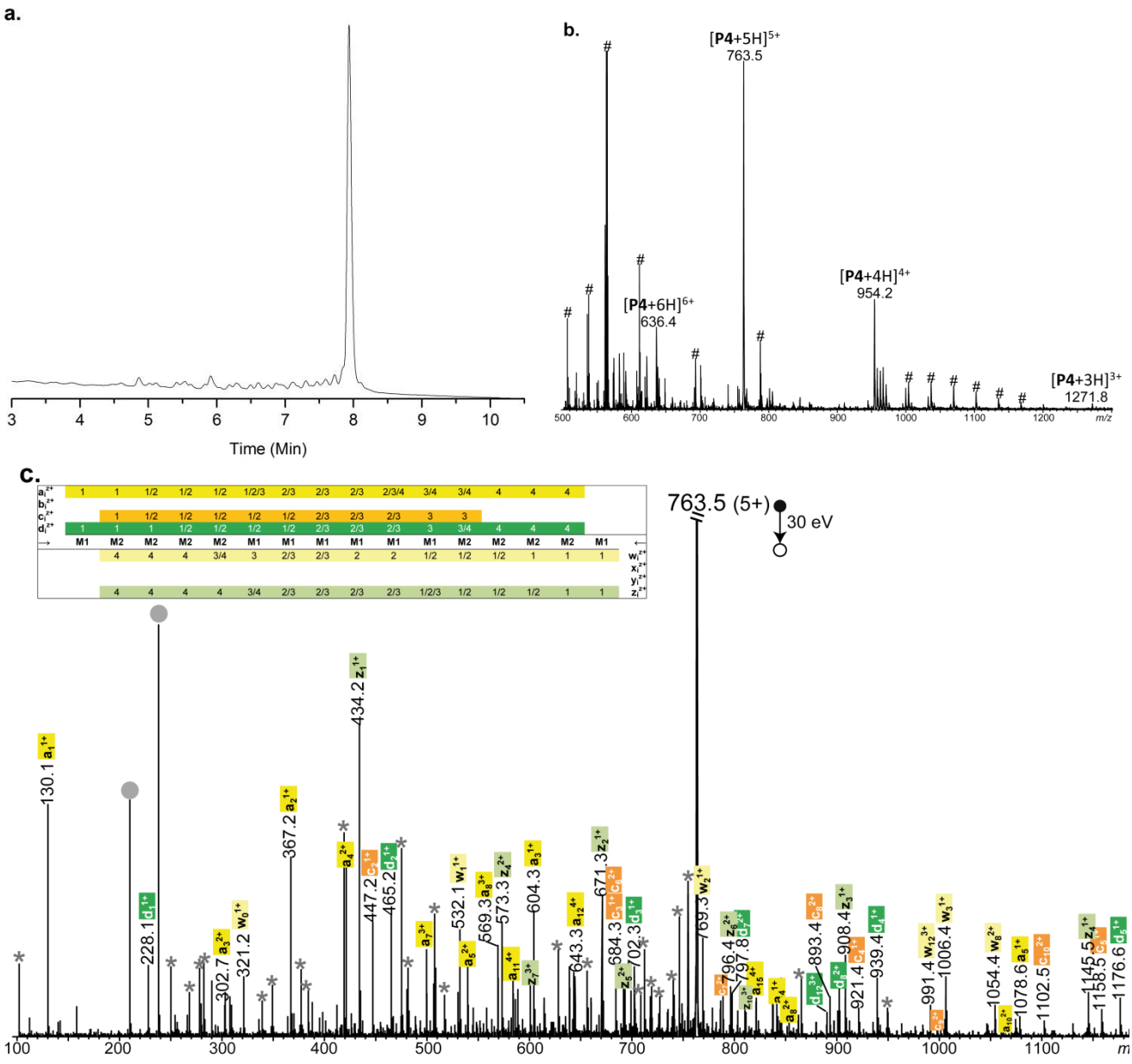


Figure S8. (**a**) HPLC analysis of copolymer **P4**. (**b**) Mass spectrometry analysis of **P4**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **P4** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circles designate internal fragments corresponding to the protonated coded units **M1** at *m/z* 210.1 and **M2** at *m/z* 238.1.

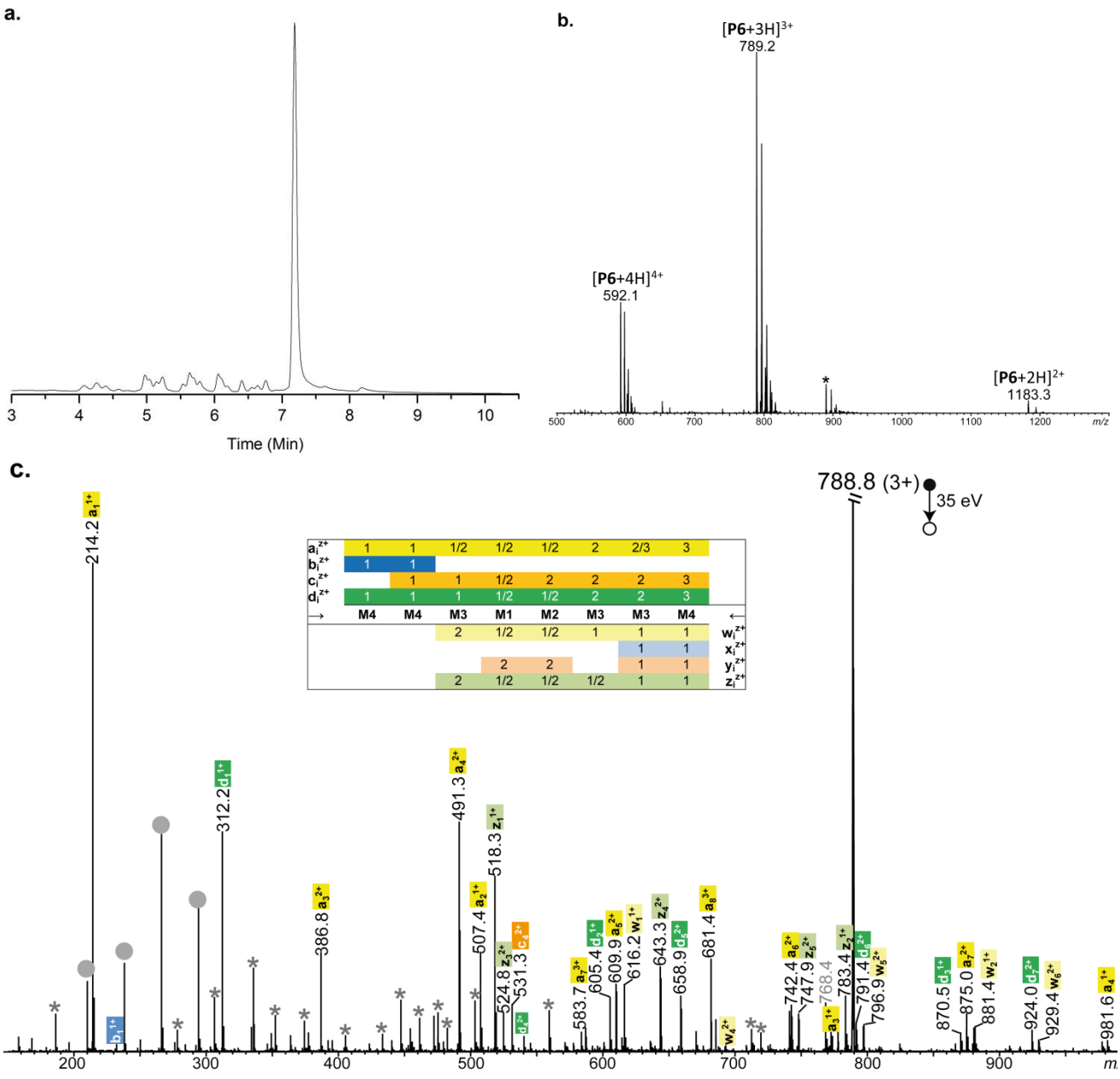


Figure S9. (**a**) HPLC analysis of copolymer **P6**. (**b**) Mass spectrometry analysis of **P6**. Black asterisks indicate traces of DMT-protected polymer. (**c**) Tandem mass spectrometry analysis of **P6** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circles designate internal fragments corresponding to the protonated coded units **M1** at *m/z* 210.1, **M2** at *m/z* 238.1, **M3** at *m/z* 266.1 and **M4** at *m/z* 294.2.

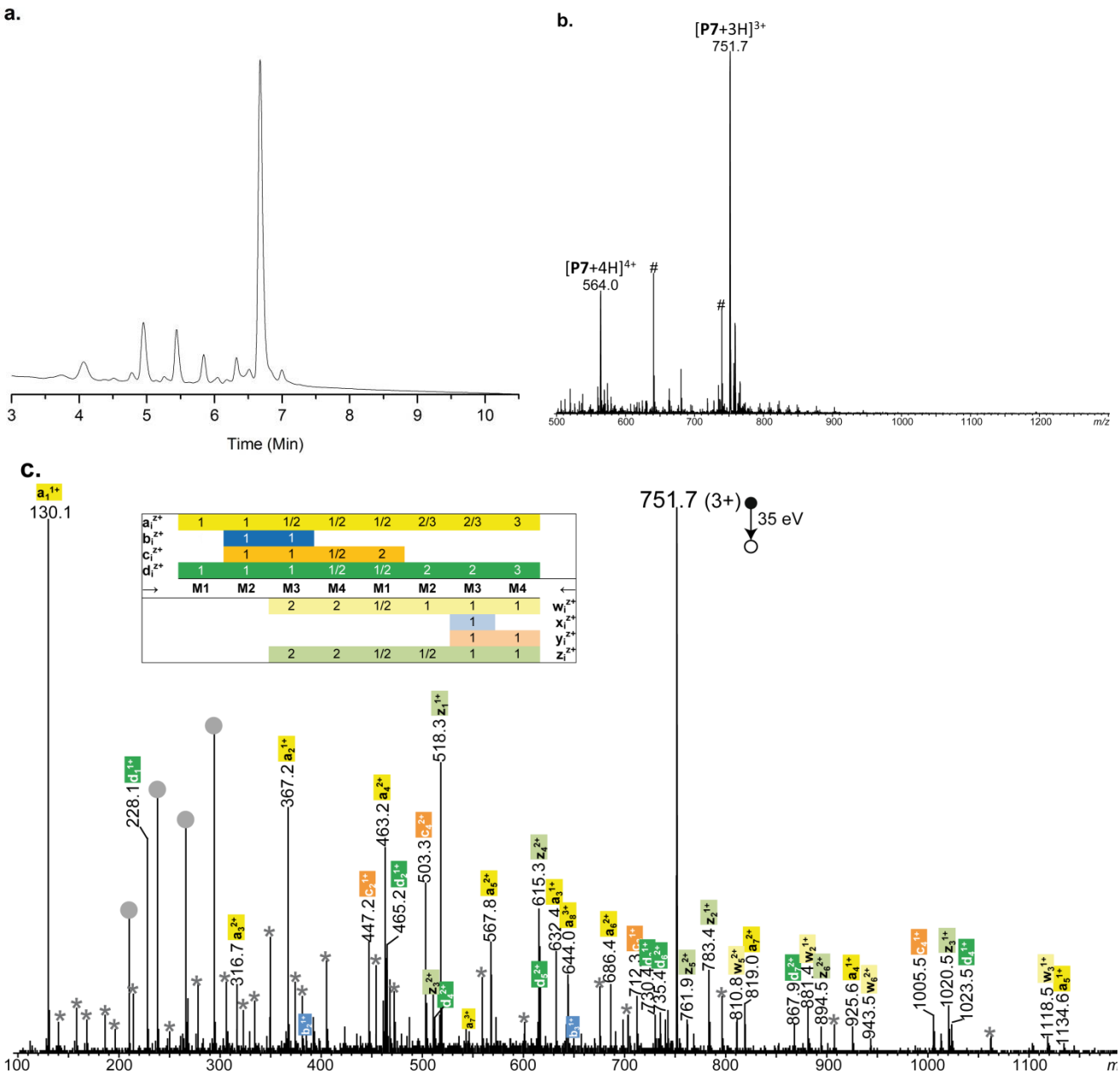


Figure S10. (**a**) HPLC analysis of copolymer **P7**. (**b**) Mass spectrometry analysis of **P7**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **P7** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circles designate internal fragments corresponding to the protonated coded units **M1** at *m/z* 210.1, **M2** at *m/z* 238.1, **M3** at *m/z* 266.1 and **M4** at *m/z* 294.2.

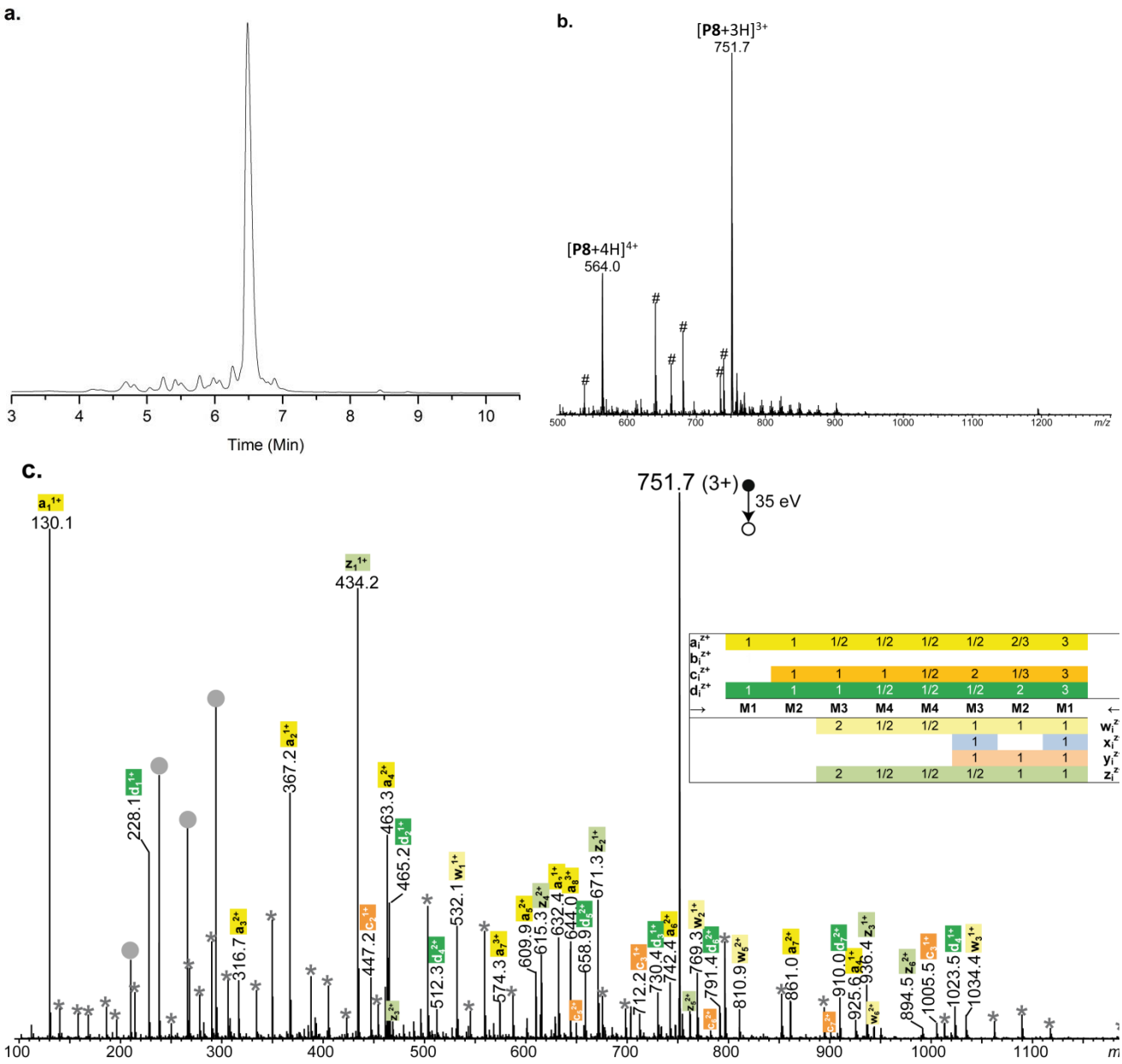


Figure S11. (**a**) HPLC analysis of copolymer **P8**. (**b**) Mass spectrometry analysis of **P8**. # designates signals from the chemical background. (**c**) Tandem mass spectrometry analysis of **P8** with sequence coverage table in inset. Grey asterisks denote internal fragments. The full grey circles designate internal fragments corresponding to the protonated coded units **M1** at *m/z* 210.1, **M2** at *m/z* 238.1, **M3** at *m/z* 266.1 and **M4** at *m/z* 294.2.

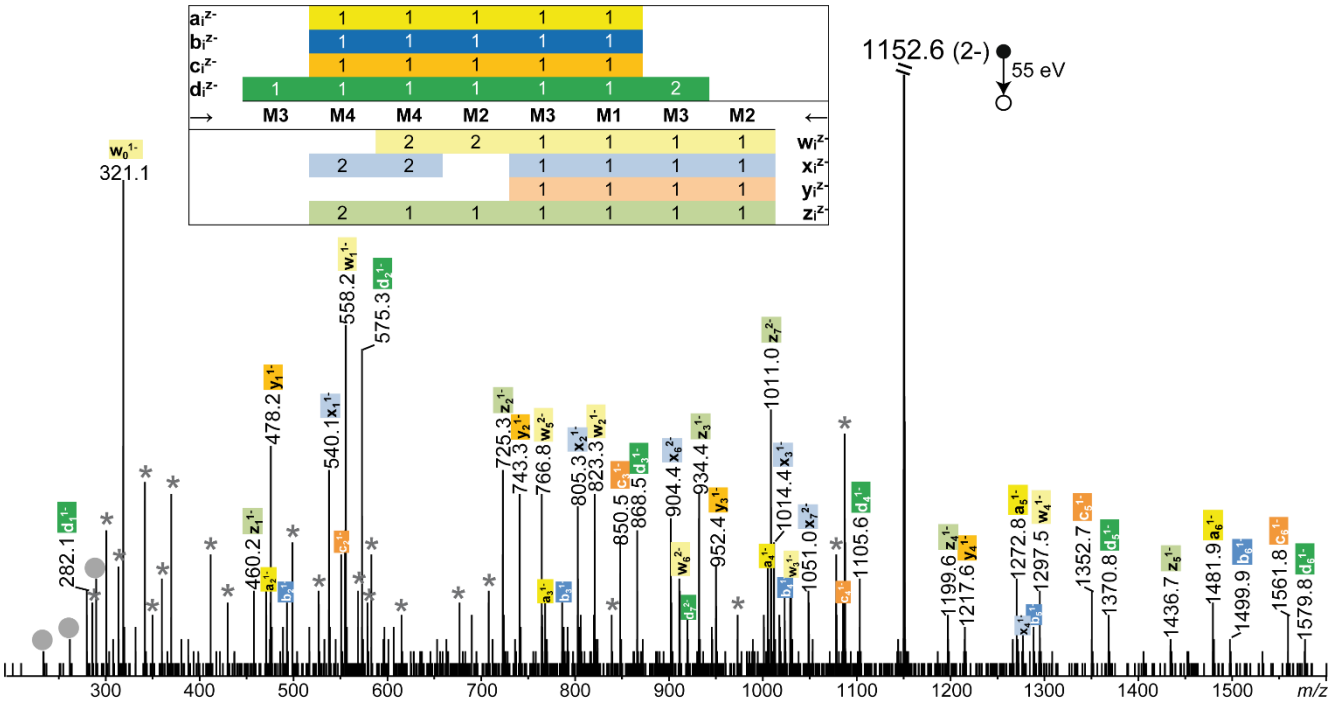


Figure S12. Tandem mass spectrometry analysis of **P5** in the negative ion mode, with sequence coverage table in inset. As compared to data obtained in the positive mode for [**P5** + 3H]$^{3+}$ (Figure 1c), activation of the [**P5** – 2H]$^{2-}$ deprotonated species leads to the formation of up to eight fragment series, which highly contributes to signal dilution and jeopardizes ion detection. For example, deprotonated coded units are usually released as internal fragments (designated by full grey circles); yet, in contrast to **M2** (*m/z* 237.1), **M3** (*m/z* 264.1) and **M4** (*m/z* 292.2), deprotonated **M1** expected at *m/z* 208.1 is not observed although present in the sequence of **P5**. Grey asterisks denote other internal fragments.

E References

[1] A. Al Ouahabi, L. Charles, J.-F. Lutz, *J. Am. Chem. Soc.*, **2015**,*137*, *5629–5635*.

[2] A. Al Ouahabi, M. Kotera, L. Charles, J.-F. Lutz, *ACS Macro Lett.* **2015**, *4*, 1077–1080.