Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Magnetic Peptide Nucleic Acids for DNA targeting

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General Materials and Methods

All reagents were obtained from commercial suppliers and used without further purification. Dry DMF and dry methanol over molecular sieves were obtained from Fluka. THF was dried over sodium/benzophenone. Compounds **1** and **5** were prepared according to the literature.[1, 2] Unless otherwise specified, all of the reactions were performed in an inert atmosphere under dry conditions. Nanoparticles were obtained from commercial suppliers (Alpha Aesar) and dried under vacuum at 120 °C for 2 h and then at rt for 6 h prior to their use.

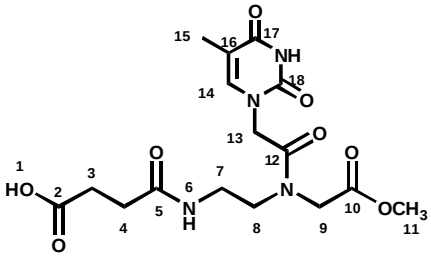
Vials ALLTECH of 1.5 mL, 4 mL, 8 mL, 25 ml and 50 mL with frits of PTFE were used as reactor for solid phase synthesis. Automated solid phase syntheses were performed with peptide synthesizer "ABI 433A" of Applera Italia, according to Applied Biosystems ABI 433A Peptide Synthesis 3 mL Reaction Vessel User's Manual for the MBHA (4-methylbenzhydrylamine hydrochloride salt) resin. The software for peptide synthesis was Synassist 2.0, installed on the PC unit linked to the synthesizer.

The IR spectra were recorded on a Perkin-Elmer FT-IR 1725X1. ^{1}H and ^{13}C NMR were recorded on Bruker AC200, AC300 and AMX300 instruments, and the chemical shifts (δ) are reported in parts per million relative to solvent peak. Melting points were obtained with a Büchi Melting Point B-540. Mass spectra were recorded using a Thermo Finnigan LCQ Advantage; high-resolution mass spectra were recorded using a Bruker Daltonics ICR-FTMS APEX II.

HPLC spectra of PNA monomers and oligomers were obtained with a HPLC AGILENT 1100 Series, using an analytical column DISCOVERY® BIO WIDE PORE C18 (25 cm x 4.6 mm, 5 μm) and a semi-preparative column DISCOVERY® BIO WIDE PORE C18 (25 cm x 10 mm, 10 μm). MALDI-TOF spectra were recorded with a Bruker Daltonics Microflex. UV spectra were recorded by using a Jasco V-520 UV/Vis spectrophotometer in a range of λ from 190 nm to 600 nm. HRMAS NMR experiments were carried out on a Bruker BioSpin FT-NMR Avance 500 equipped with a 11.7 T superconducting ultrashield magnet. MAS experiments were performed on MPNA (monomers), as DMSO dispersion, at spinning rates of up to 15 kHz (15 kHz maximum MAS rotation available) using a 50 μL zirconia rotor. ICP-OES measurements were carried out by means of a Thermo instrument (model Iris Intrepid) equipped with a low noise CID detector and an echelle spectrometer. The data were acquired and elaborated using TEVA software. The analytical data produced as concerns Fe concentration are given with a precision of 5%. T_2 relaxation times were acquired using a 0.47 T Bruker Minispec mq20 system (Ettlingen, Germany) working with ^{1}H at 20 MHz magnetic field, T =

313 K, with the following parameters: CMPG pulse sequence, 1000 echoes with a 20 ms echo time and 2 s repetition time. Samples were introduced using 10 mm NMR tubes pre-warmed and sonicated in a S15H Elmasonic apparatus (Elma, Singen, Germany). Melting temperature of PNA-DNA hybrid was obtained using a Peltier Perkin Elmer Lambda2S spectrophotometer linked to a Peltier PTP6, heating/cooling temperature of 0.5 °C/sec, recording absorbance values at 260 nm every 12 sec.

Synthesis of monomer 2. A mixture of succinic anhydride (1.46 g, 14.6 mmol) and DIPEA (2.5 mL, 14.6 mmol) was added to a solution of **5** (600 mg, 1.46 mmol) in dry DMF (20 mL) at 0 °C. The reaction mixture was warmed at r.t. and stirred overnight. Then, the solvent was evaporated at reduced pressure and the crude product was precipitated from MeOH and recovered by filtration to afford **2** (539 mg, 93%) as white solid: mp 206 °C.



2

^{1}H-NMR ($DMSO_d$, 300 MHz, mix of rotamers): δ, ppm 1.76 (s, 3H, CH_3 **15**); 2.28 – 2.44 (m, 4H, CH_2 **3** and **4**); 3.15 – 3.42 (m, 4H, CH_2 **7** and **8**); 3.64 (ma) – 3.73 (mi) (d, 3H, CH_3 **11**); 4.08 (ma) – 4.32 (mi) (d, 2H, CH_2 **9**); 4.47 (mi) – 4.65 (ma) (d, 2H, CH_2 **13**); 7.28 (mi) – 7.34 (ma) (d, 1H, CH **14**); 11.25 (s, 1H, OH **1**).

^{13}C-NMR ($DMSO_d$, 50 MHz): δ, ppm 17.4 (CH_3 **15**); 34.4 (CH_2 **3**); 35.3 (CH_2 **4**); 42.4 (CH_2 **7**); 52.2 (CH_2 **8**); 52.9 (CH_2 **13**); 53.2 (CH_2 **9**); 57.2 (CH_3 **11**); 113.5 (C_q **16**); 147.7 (CH **14**); 156.5 (C_q **18**); 169 (C_q **19**); 172.9 (C_q **12**); 175.1 (C_q **10**); 177.2 (C_q **5**); 179.4 (C_q **2**).

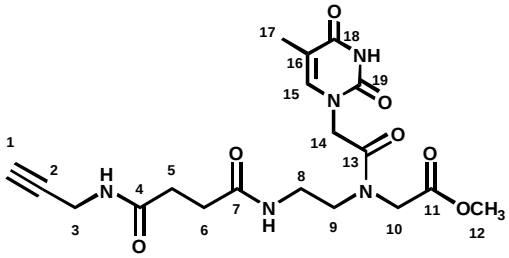
HR-ESI MS: *m/z* 399.1514 (M^++H). (Calculated for $C_{22}H_{39}N_5O_9Si_1$ + H 399.1510); *m/z* (M^++Na) 421.1337 (Calculated for $C_{22}H_{39}N_5O_9Si_1$ + Na 421.1329); *m/z* (M^+-H) 397.1355 (Calculated for $C_{22}H_{39}N_5O_9Si_1$ - H 397.1364).

Elemental analysis: found: C 48.25; H 5.93; N 12.72. Calc. for $C_{16}H_{22}N_4O_9$: C 48.24; H 5.57; N 14.06 %.

FT-IR (nujol): ν_{max}/cm^{-1} 3316, 1741, 1698, 1663, 1462, 1376.

Synthesis of monomer 3. EDC.HCl (216 mg, 1.13 mmol) and propargylamine (78.5 μL, 14.6 mmol) were added to a solution of compound **2** (150 mg, 0.37 mmol) in dry DMF (20 mL). The mixture was stirred at rt overnight. The solvent was removed in vacuum and the crude purified by

column chromatography on silica gel (ethyl acetate/MeOH 8:2) yielding **3** (126 mg, 77%) as white solid, mp 65 °C.



3

^{1}H-NMR ($DMSO_d$, 200 MHz, mix of rotamers): δ, ppm 1.74 (s, 3H, CH_3 **17**); 2.27 – 2.33 (m, 4H, CH_2 **5** and **6**); 2.33 – 2.49 (m, 1H, CH **1**); 3.06 – 3.30 (m, 4H, CH_2 **8** and **9**); 3.62 (ma) – 3.70 (mi) (d, 3H, CH_3 **12**); 3.80 – 3.82 (m, 2H, CH_2 **3**); 4.05 (ma) – 4.35 (mi) (d, 2H, CH_2 **10**); 4.45 (mi) – 4.63 (ma) (d, 2H, CH_2 **14**); 7.28 (mi) – 7.35 (ma) (d, 1H, CH **15**).

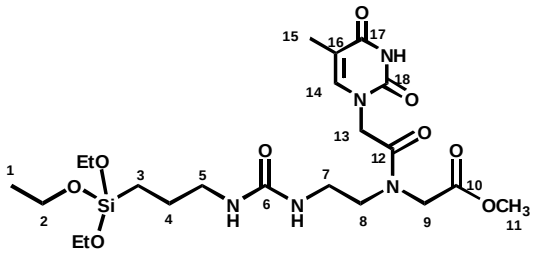
^{13}C-NMR ($DMSO_d$, 75 MHz): δ, ppm 11.7 (CH_3 **17**); 27.7 (CH_2 **3**); 30.0 (CH_2 **5**); 30.1 (CH_2 **6**); 39.2 (CH_2 **8**); 46.6 (CH_2 **9**); 47.3 (CH_2 **10**); 47.6 (CH_2 **14**); 52.1 (CH_3 **12**); 72.7 (C_q **2**); 107.9 (C_q **16**); 142.1 (CH **15**); 150.9 (C_q **19**); 164.2 (C_q **18**); 167.3 (C_q **13**); 169.4 (C_q **11**); 171.0 (C_q **7**); 171.8 (C_q **4**).

HR-ESI MS: (+c) *m/z* 435.1637 (M^+ +Na), (calculated for $C_{19}H_{25}N_5O_7$ + Na: 435.1646).

Elemental analysis: found: C 51.77; H 5.95; N 15.18. Calc. for $C_{19}H_{25}N_5O_7$: C 52.41; H 5.79; N 16.08 %.

FT-IR (KBr): ν_{max}/cm^{-1} 3300, 2826, 1744, 1664, 1536, 1214.

Synthesis of monomer 4. DIPEA (176 μL, 0.97 mmol) and (3-isocyanatopropyl)triethoxysilane (180 μL, 0.73 mmol) were added to a solution of **5** (200 mg, 0.49 mmol) in dry THF (4 mL), and the mixture stirred at rt for 6 h The solvent was removed in vacuum and the crude product was purified by column chromatography on silica gel (ethyl acetate/MeOH 8:2) yielding **4** (220 mg, 80%) as white solid: mp 95 °C.



4

^{1}H-NMR ($CDCl_3$, 300 MHz, mix of rotamers): δ, ppm 0.70 – 0.77 (m, 2H, CH_2 **3**); 1.20 – 1.25 (m, 9H, CH_3 **1**); 1.56 – 1.59 (m, 2 H, CH_2 **4**); 1.91 (s, 3H, CH_3 **15**); 3.12 – 3.22 (m, 2H, CH_2 **7**); 3.41 (m, 2H, CH_2 **5**); 3.59 (m, 2H, CH_2 **8**); 3.79 (s, 3H, CH_3 **11**); 3.83 – 3.94 (m, 8H, CH_2 **2**); 4.20 (ma) – 4.40 (mi) (s, 2H, CH_2 **9**); 4.55 (mi) -4.70 (ma) (s, 2H, CH_2 **13**); 7.36 (s, 1H, CH **14**).

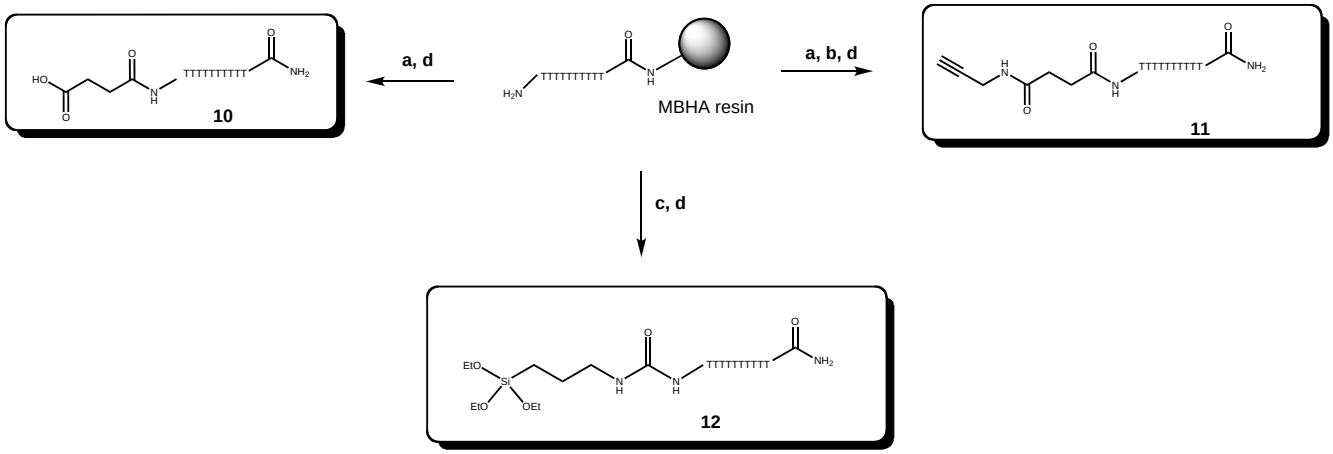
^{13}C-NMR ($CDCl_3$, 75 MHz): δ, ppm 8.93 (CH_2 **3**); 13.44 (CH_3 **15**); 19.56 (CH_3 **1**); 23.70 (CH_2 **4**); 36.99 (CH_2 **5**); 39.53 (CH_2 **7**); 45.35 (CH_2 **8**); 50.0 (CH_2 **9**); 50.14 (CH_2 **13**); 53.83 (CH_3 **11**); 59.73 (CH_2 **2**); 112.38 (C_q **16**); 142.90 (CH **14**); 153.13 (C_q **17**); 160.19 (C_q **6**); 166.10 (C_q **18**); 169.08 (C_q **12**); 171.63 (C_q **10**).

HR-ESI MS: (+c) *m/z* 568.2418, (M^++Na), (calculated for $C_{22}H_{39}N_5O_9Si$ + Na: 568.2409).

Elemental analysis: found: C 42.98; H 6.39; N 12.54. Calc. for $C_{22}H_{39}N_5O_9Si$: C 48.43; H 7.20; N 12.83 %.

FT-IR (nujol): ν_{max}/cm^{-1} 3380, 1746, 1670, 1460, 1376, 1122.

Synthesis of decamers 10-12



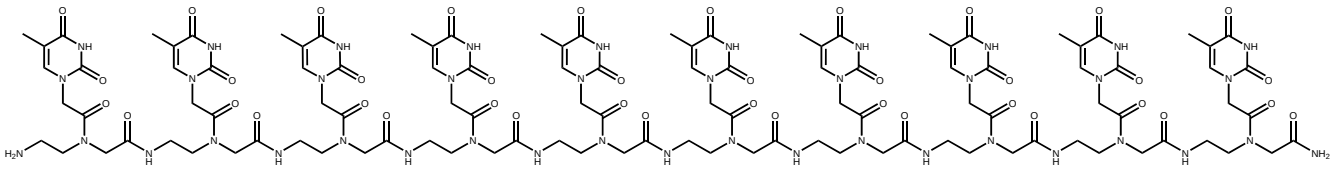
Scheme S1. Synthesis of functionalized decamers **10**, **11** and **12**. a) Succinic anhydride, NMP, DIPEA, 2 h, rt ; b) propargylamine, DIPEA, HATU, NMP, 2 h, rt c) (3-isocyanatopropyl)triethoxysilane, NMP, 2 h, rt ; d) cleavage: TFA/TFMSA/thioanisole/*m*-cresol 6:2:1:1, 1 h, rt.

Loading of *aeg*-(T)PNA-COOH 1 on MBHA resin . The MBHA resin (1.56 g) was washed with CH_2Cl_2 (2 × 5 mL) and activated by treatment with 5% DIPEA in CH_2Cl_2 for 3 min; and then washed with CH_2Cl_2 (2 × 5 mL). In a vial, DIPEA (95 μL, 0.6 mmol) was added to a solution of *aeg*-(T)PNA-COOH monomer **1** (115 mg, 0.3 mmol) in NMP (2.5 mL); then, a solution of HBTU (114 mg, 0.3 mmol) in NMP (2.5 mL) was added. The mixture was then added to the resin and stirred at rt for 1 h. The resin was washed with NMP (10 × 5 mL). A solution of Ac_2O/Py/NMP 1:2:2 (5 mL) was added to the resin (capping of unreacted amino groups) and left under stirring at rt for 1 h. After this time, the resin was washed with DMF (5 mL), CH_2Cl_2 (4 × 5 mL), 5% DIPEA in CH_2Cl_2 (2 × 5 mL) and CH_2Cl_2 (4 × 5 mL); and finally dried in vacuum to afford the MBHA resin downloaded with **1**

Preparation of the resin-supported homo-thymine *aeg*PNA decamer. Automated solid phase synthesis was performed on an ABI 433A peptide synthesizer in a reactor of 3 mL on a 20 μM scale using Boc strategy. The resin was swollen with CH_2Cl_2, the Boc group of the loaded monomer was removed by treatment with TFA/*m*-cresol (95 : 5), the resin was rinsed with CH_2Cl_2 and DIPEA 1.6M in NMP. The monomers were loaded into cartridges as NMP solutions, activated with HBTU 0.38M in NMP and transferred to the resin. After each coupling step (30 min) the resin was washed with NMP

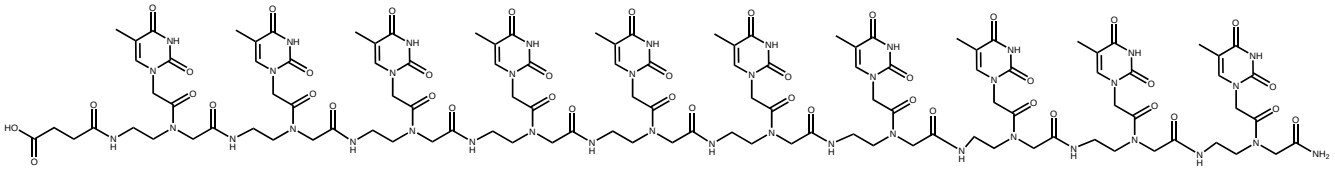
and then treated with Ac_2O/Py/NMP 1:25:25 capping solution twice for 3 min. The cycle was repeated for each base. After the last coupling the resin was washed several times with NMP and CH_2Cl_2, and finally dried under nitrogen yielding **NH_2-TTTTTTTTTT-MBHA resin** (141 mg).

Cleavage. 100 mg of resin was washed with TFA (2 × 200 μL) and then stirred for 1 h with TFA/TFMSA/thioanisole/*m*-cresol 6:2:1:1 (500 μL). The reaction mixture was filtered and the resin washed with TFA (4 × 200 μL). The filtrate was concentrated under nitrogen flow, and Et_2O (5 mL) was added to precipitate PNA as a white solid. Centrifugation of the slurry gave the product which was washed with Et_2O (8 × 5 mL) and dried to afford the decamer **NH_2-TTTTTTTTTT-$CONH_2$** as a white solid (39 mg).



The product was characterized by reverse phase HPLC and MALDI-TOF: *m/z* 2679.41 (M^+); 2700.60 (M^+ + Na); 2717.80 (M^+ + K).

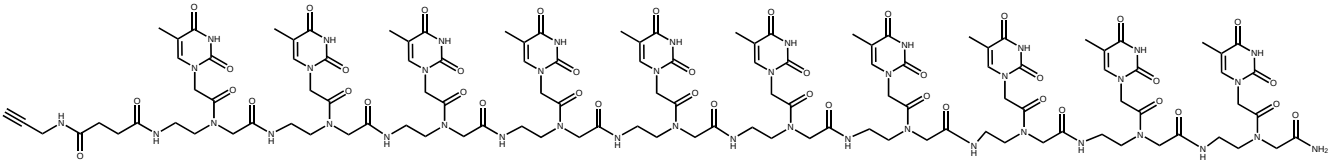
Synthesis of the homo thymine decamer 10. The **NH_2-TTTTTTTTTT-MBHA resin** (102 mg, 20 μmol) was swolled with CH_2Cl_2 (3 mL) for 1 h, then washed with CH_2Cl_2 (2 × 3 mL), activated with DIPEA 5% in CH_2Cl_2 (3 mL) for 3 min and then washed again with CH_2Cl_2 (2 × 3 mL). A solution of succinic anhydride (41 mg, 0.41 mmol) in NMP (1 mL) was added to the resin; the resulting mixture was stirred at rt for 2 h. After this time, the solution was filtered and the resin washed with NMP (10 × 3 mL). The cleavage of PNA decamer **10** from the resin was done as described above. The crude decamer was purified by reverse phase HPLC (solvent A: H_2O with 0.1% TFA; solvent B: CH_3CN with 0.1% TFA; elution gradient: from 100% of A to 100% of B in 50 min) to afford **10** (45 mg) as white solid.



10

The purified product was characterized by reverse phase HPLC and MALDI-TOF *m/z* 2778.2 (M^+) (calculated for $C_{114}H_{148}N_{41}O_{43}$ 2778.06).

Synthesis of the homo-thymine decamer 11. The **NH_2-TTTTTTTTTT-MBHA resin** (121 mg, 24 μmol) was swolled with CH_2Cl_2 (3 mL) for 1 h, then washed with CH_2Cl_2 (2 × 3 mL), activated with DIPEA 5% in CH_2Cl_2 (3 mL) for 3 min and then washed again with CH_2Cl_2 (2 × 3 mL). A solution of succinic anhydride (48 mg, 0.48 mmol) in NMP (1 mL) was added to the resin; the resulting mixture was stirred at rt for 2 h. After this time, the solution was filtered and the resin washed with NMP (10 × 3 mL). A solution of HATU (18 mg, 48 μmol) and DIPEA (8 μL, 48 μmol) in NMP (1 mL) was then added to the resin and, after 2 min, a solution of propargyl-amine (56 mg, 0.48 mmol) in NMP (1 mL) was also added. The mixture was stirred at rt for 2 h. At the end of the reaction, the solution was filtered and the resin washed with NMP (10 × 3 mL) and CH_2Cl_2 (5 × 3 mL). The cleavage of PNA decamer **11** from the resin was done as described above. The crude decamer was purified by reverse phase HPLC (solvent A: H_2O with 0.1% TFA; solvent B: CH_3CN with 0.1% TFA; elution gradient: from 100% of A to 100% of B in 50 min) to afford **11** (30 mg) as white solid.

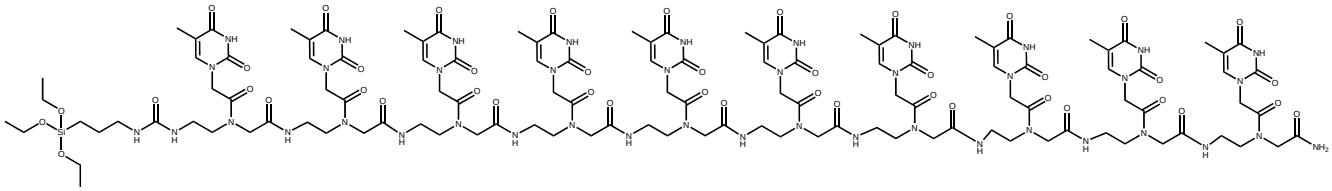


11

The product was characterized with reverse phase HPLC and Maldi TOF: *m/z* (M+H) 2817.1(calculated for $C_{117}H_{151}N_{42}O_{42}$ 2817.10).

Synthesis of the homo-thymine decamer 12. The **NH_2-TTTTTTTTTT-MBHA resin** (121 mg, 24 μmol) was stirred with CH_2Cl_2 (3 mL) for 1 h. Then the homo-thymine PNA decamer was cleaved from the resin and the reaction with 3-(triethoxysilyl)propyl isocyanate performed in solution. Therefore, the resin was washed with TFA (2 × 200 μL); then stirred for 1 h with TFA/TFMSA/thioanisole/*m*-cresol 6:2:1:1 (500 μL) and filtered. The resin was washed with TFA (4 × 200 μL) and the filtrate was evaporated under nitrogen flow. Et_2O (7 mL) was added to precipitate PNA as a white solid which was recovered by centrifugation. The product was washed again with Et_2O (8 × 7 mL), dried under nitrogen flow and then in vacuum for several hours to afford **NH_2-TTTTTTTTTT-$CONH_2$**.

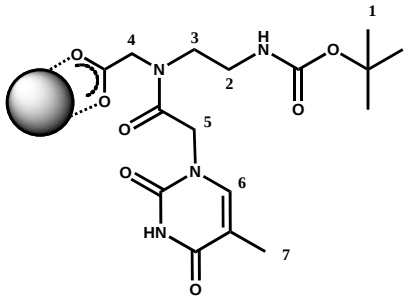
To a solution of decamer **NH_2-TTTTTTTTTT-$CONH_2$** (30 mg, 0.0108 mmol) in dry DMF (2 mL), DIPEA (17.6 μL, 0.097 mmol) and 3-(triethoxysilyl)propyl isocyanate (18.0 μL, 0.073 mmol) were added and the mixture stirred for 2 h at 80 °C. The solvent was removed under vacuum and the crude product was purified with washing with Et_2O affording **12** (31 mg, quantitative yield) as white solid.



The product was characterized with MALDI-TOF: *m/z* 2881.8 (M^+–OEt) (calculated for $C_{118}H_{159}N_{42}O_{43}Si$ 2882.14).

Supporting of PNA monomers 6-9 to maghemite nanoparticles

Synthesis of 6. A solution of monomer **1** (30 mg, 0.075 mmol) in dry toluene (1.5 mL) was added to a suspension of dry NPs (30 mg) in dry toluene (1.5 mL) previously sonicated at 60 °C for 30min; the mixture was stirred, under sonication, for 4 h at 60 °C. After this time, the reaction mixture was centrifuged at 3000 turns/min for 15 min and the solvent was removed. The solid residue was washed with toluene (1 × 5 mL) and MeOH (2 × 5 mL); functionalized NPs were eventually dried in vacuum for several hours, to afford **6** (30 mg) as brown solid.



6

[1]HR-MAS (D_2O, 8 KHz): δ, ppm 1.38 (ma) (s, 9H, **1**); 1.84 (s, 3H, **7**); 3.15 – 3.47 (m, 4H, **2** e **3**); 3.90 (ma) – 3.99 (mi) (s, 2H, **4**); 4.60 (m, 2H, **5**); 7.33 (s, 1H, **6**).

FT-IR (in KBr)**:** 3429, 1632, 1457, 1384, 1094, 889 cm^{-1}.

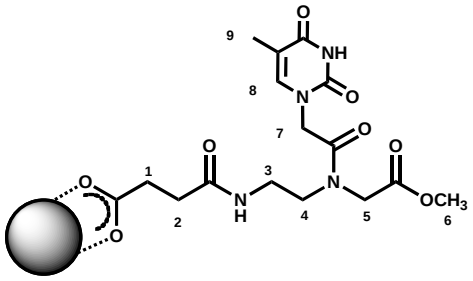
UV-vis (in H_2O): λ_{max}: 260 nm; 280 nm (PNA); 400 nm (NP).

Elemental analysis: Calcd (%) for $C_{16}H_{24}O_7N_4$ found: C 15.36, H 4.48, N 1.92. (O 7.68 calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 49.3, Fe (%) found = 47.9. O of maghemite γ-Fe_2O_3 = 20.60 calculated by difference.

Synthesis of 7. A solution of monomer **2** (30 mg, 0.075 mmol) in dry toluene (1.5 mL) was added to a suspension of dry NPs (30 mg) in dry toluene (1.5 mL) previously sonicated at 60 °C for 30 min; the mixture was stirred, under sonication, for 4 h at 60 °C. After this time, the reaction mixture was centrifuged at 3000 turns/min for 15 min and the solvent was removed. The solid residue was

washed with toluene (1 × 5 mL) and MeOH (2 × 5 mL); functionalized NPs were eventually dried in vacuum for several hours, to afford **7** (30 mg) as brown solid



7

[1]HR-MAS ($DMSO_d$, 8 KHz): δ = 1.76 (ma) – 1.84 (mi) (d, 3H, CH_3 **9**); 2.29 – 2.44 (m, 4H, CH_2 **1** e **2**); 3.17 – 3.42 (m, 4H, CH_2 **3** e **4**); 3.60 (ma) – 3.74 (mi) (d, 3H, CH_3 **6**); 4.08 (ma) – 4.32 (mi) (d, 2H, CH_2 **5**); 4.48 (mi) – 4.65 (ma) (d, 2H, CH_2, **7**); 7.28 (mi) – 7.34 (ma) (d, 1H, CH, **8**).

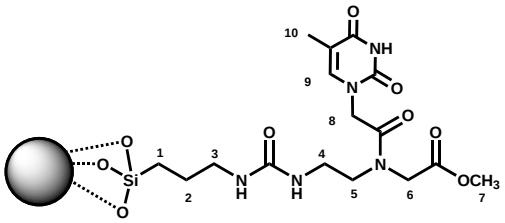
FT-IR (in KBr): 3317, 1742, 1699, 1663, 1556, 1476, 1213 cm^{-1}

UV-vis (in H_2O): λ_{max}: 260 nm; 280 nm (PNA); 400 nm (NP).

Elemental analysis C, H, N: Calcd (%) for $C_{16}H_{21}O_8N_4$ found: C 27.19, H 3.08, N 7.67. (O 17.53 calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 32.4, Fe (%) found = 31.11. O of maghemite γ-Fe_2O_3 = 13.42 calculated by difference.

Synthesis of 8. A solution of **4** (64 mg, 0.12 mmol) in dry toluene (5 mL) was added to a suspension of dry NPs (32 mg) in dry toluene (5 mL), previously sonicated at 60 °C for 30 min; the mixture was stirred, under sonication, for 4 h at 60 °C. At the end of reaction, *n*-hexane (1 mL) was added and the slurry centrifuged at 3000 turns/min for 15 min. The solid residue was washed with toluene (2 × 10 mL) and absolute EtOH (1 × 10 mL); functionalized NPs were eventually dried in vacuum for several hours, to afford **8** (30 mg) as brown solid.



8

[1]HR-MAS (D_2O presat, 3 KHz): δ = 0.55 – 0.56 (m, 2H, CH_2 **1**); 1.48 (m, 2H, CH_2 **2**); 1.81 – 1.83 (d, 3H, CH_3 **10**); 3.00 – 3.05 (m, 2H, CH_2 **5**); 3.21 – 3.33 (m, 2H, CH_2 **3**); 3.45 – 3.51 (m, 2H, CH_2 **4**); 3.59 – 3.75 (d, 3H, CH_3 **7**); 4.11 (ma) – 4.31 (mi) (d, 2H, CH_2 **6**); 4.56 (mi) – 4.70 (ma) (d, 2H, CH_2, **8**); 7.25 (s, 1H, CH, **9**).

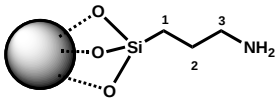
FT-IR (in KBr pill): 3400, 1750, 1630, 1550, 1440, 1380, 1210, 1108 cm^{-1}.

UV-vis (in H_2O): λ_{max}: 260 nm; 280 nm (PNA); 400 nm (NP).

Elemental analysis C, H, N: Calcd (%) for $C_{16}H_{24}N_5O_9Si$ found: C 10.47, H 1.53, N 2.29. O 4.71 and Si 0.91, calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 59, Fe (%) found = 56.68. O of maghemite γ-Fe_2O_3 = 23.41 calculated by difference.

Synthesis of APTES-NPs (MNP-NH_2). In a dry flask, a solution of 3-amino-propyl-thriethoxy-silane (600 mg, 2.71 mmol) in dry toluene (5 mL) was added to a suspension of NPs (300 mg) in dry toluene (5 mL); previously kept under sonication at 60 °C for 1 hour. The mixture was stirred, under sonication, for 4 h at 60 °C. Then, *n*-hexane (1 mL) was added and the NPs centrifuged at 3000 turns/min for 15 min. The solid residue was washed with toluene (2 × 5 mL) and MeOH (2 × 5 mL); functionalized NPs were eventually dried in vacuum for several hours, to afford APTES-NPs (30 mg) as brown fine solid.



[1]HR-MAS (D_2O presat, 3 KHz): δ = 0.55 – 0.61 (m, 2H, CH_2 **1**); 1.64 – 1.70 (m, 2H, CH_2 **2**); 2.86 – 2.96 (m, 2H, CH_2 **3**).

FT-IR (KBr pill)**:** 3390, 1550, 1490, 1110, 1002 cm^{-1}.

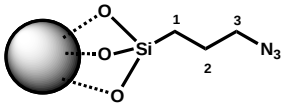
Elemental analysis C, H, N: Calcd (%) for $C_3H_8O_3NSi$ found: C 2.81, H 1.05, N 0.82. (O 2.81 and Si 1.64 calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 66.9, Fe (%) found = 65; O of maghemite γ-Fe_2O_3 = 25.87 calculated by difference.

Synthesis of TfN_3. To a solution of NaN_3 (1.76 g, 27.41 mmol) in water (4 mL) at 3 °C, CH_2Cl_2 (7 mL) was added to form a biphasic system. While maintaining the temperature at 3 °C, triflic anhydride (0.9 mL, 5.42 mmol) was added and the mixture stirred for 3 h and 30 min. After this time, the two phases were separated, the aqueous one extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic phases dried over Na_2SO_4. The CH_2Cl_2 solution of TfN_3 was used as such for the synthesis of azide-NPs.

FT-IR (CH_2Cl_2 solution): 2153 cm^{-1}.

Synthesis of MNP-N_3. A CH_2Cl_2 solution of TfN_3 (5 mL), water (660 μL), Et_3N (132 μL) and $CuSO_4$ (6 mg dissolved in 60 μL of water) were added to a suspension of dry APTES-NPs (100 mg, loading = 0.075 mmol/mg) in CH_2Cl_2 (660 μL) and MeOH (660 μL), previously kept under sonication for 30min The mixture was stirred, under sonication, at rt for 4 h. Functionalized NPs were then centrifuged at 3000 turns/min for 15 min. The solid residue was washed with water (1 × 5 mL), MeOH (1 × 5 mL) and CH_2Cl_2 (3 × 5 mL). NPs were then dried in vacuum for several hours to afford MNP-N_3 (95 mg) as brown solid.



MNP-N_3

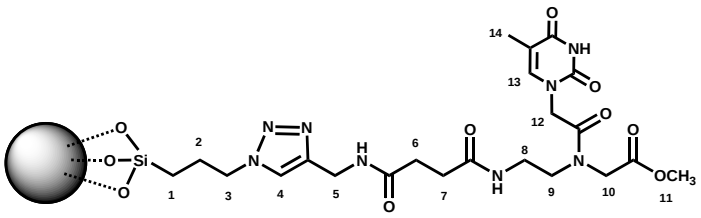
^{1}HR-MAS (D_2O presat, 4 KHz): δ = 0.64 – 0.67 (m, 2H, CH_2 **1**); 1.63 – 1.73 (m, 2H, CH_2 **2**); 2.94 – 2.97 (m, 2H, CH_2 **3**).

FT-IR (in KBr)**:** 3390, 2094, 1623, 1441, 1107 cm^{-1}.

Elemental analysis C, H, N: Calcd (%) for $C_3H_6O_9N_3Si$ found: C 2.45, H 0.80, N 1.38. (O 1.57 and Si 0.92 calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 66.6, Fe (%) found = 63.51. O of maghemite γ-Fe_2O_3 = 29.37 calculated by difference.

Synthesis of 9. $Na^{(+)}$-ascorbate (3 mg), $CuSO_4$ (6 mg dissolved in 60 μL of water) and **3** (40 mg, 92 μmol) were added to a suspension of dry MNP-N_3 (20 mg, 15 μmol) in water (2 mL), previously kept under sonication for 30 min. The mixture was stirred under sonication, for 3 h at 60 °C. Functionalized NPs were centrifuged at 3000 turns/min for 15 min. The solid residue was washed with H_2O (2 × 5 mL) and MeOH (2 × 5 mL). NPs were finally dried in vacuum for several hours, to afford **9** (22 mg) as brown solid.



9

^{1}HR-MAS (D_2O presat, 4 KHz): δ = 1.23 – 1.31 (m, 2H, CH_2 **1**); 1.85 – 1.89 (m, 3H, CH_3 **14**); 2.45 – 2.54 (m, 4H, CH_2 **8** e **9**); 2.45 – 2.54 (m, 4H, CH_2 **6** e **7**); 3.69 – 3.79 (m, 3H, CH_3 **11**); 3.87 – 3.89 (m, 2H, CH_2 **5**); 4.13 (ma) – 4.46 (mi) (m, 2H, CH_2 **10**); 7.3-7-40 (m, 2H, CH **13** and **4**).

FT-IR (in KBr): 3400, 1640, 1353, 1105 cm^{-1}.

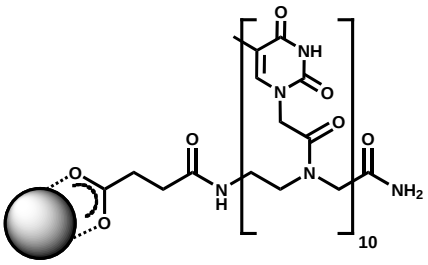
UV-vis (in H_2O): λ_{max}: 260 nm; 280 nm (PNA); 400 nm (NP).

Elemental analysis C, H, N: Calcd (%) for $C_{22}H_{31}O_{10}N_8Si$ found: C 5.88, H 1.16, N 1.44.; O 2.05 and Si 0.36, calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 65, Fe (%) found = 61.13. O of maghemite γ-Fe_2O_3 = 27.98 calculated by difference.

Supporting of PNA decamers 10-12 to maghemite nanoparticles

Synthesis of 13. A suspension of decamer **10** (11 mg, 0.075 mmol) in dry toluene (1 mL) was added to a suspension of dry NPs (7 mg) in dry toluene (5 mL), previously sonicated at 60 °C for 1 h. The mixture was stirred, under sonication, for 4 h at 40 °C. Functionalized NPs were centrifuged at 3000 turns/min for 15 min. The solid residue was washed with toluene (1 × 5 mL) and MeOH (3 × 5 mL). NPs were finally dried in vacuum for several hours, to afford **13** (7 mg) as brown fine solid.



13

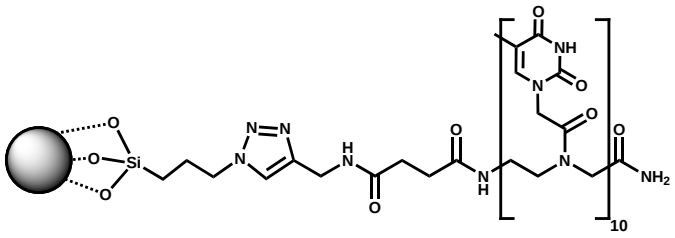
FT-IR (in KBr): 3299, 1557, 1659, 1663, 1556, 1476, 1227 cm^{-1}.

UV-vis (in H_2O): λ_{max}: 260 nm; 280 nm (PNA); 400 nm (NP).

Elemental analysis C, H, N: Calcd (%) for $C_{114}H_{146}O_{43}N_{41}$ found: C 18.83, H 3.26, N 6.63. (O 7.94 calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 47.8, Fe (%) found = 45.24. O of maghemite γ-Fe_2O_3 = 18.10 calculated by difference.

Synthesis of 14. A solution of **11** (8 mg, 29 μmol) in water (4 mL) was added to a suspension of dry MNP-N_3 (8 mg, 6 μmol) in water (500 μL) previously kept under sonication at 60 °C for 1 h. Then, $Na^{(+)}$ ascorbate (2 mg) and $CuSO_4$ (2 mg dissolved in 20 μL of water) were added to the mixture which was stirred, under sonication, for 4 h at 40 °C. At the end of reaction, NPs were centrifuged at 3000 turns/min for 15 min. The solid residue was washed with water (3 × 5 mL). NPs were finally dried in vacuum for several hours, to afford **14** (10 mg) as brown solid.



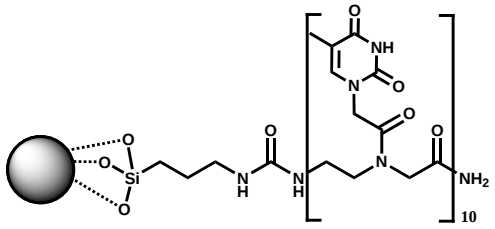
FT-IR (in KBr)**:** 3334, 2919, 2094, 1633, 1556, 1109, 1065 cm^{-1}.

UV-vis (in H_2O): λ_{max}: 260 nm; 280 nm (PNA); 400 nm (NP).

Elemental analysis C, H, N: Calcd (%) for $C_{120}H_{156}O_{45}N_{45}$ found: C 9.52, H 1.87, N 1.50. (O 1.71 and Si 0.05, calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 65, Fe (%) found = 59 O of maghemite γ-Fe_2O_3 = 26.35 calculated by difference.

Synthesis of 15: A suspension of **12** (10 mg, 0.0034 mmol) in dry toluene (5 mL) was added to a suspension of NPs (10 mg) in dry toluene (3 mL), previously sonicated at 60 °C for 30 min. The mixture was stirred, always under sonication, for 4 h at 60 °C. Then, *n*-hexane (1 mL) was added and the NPs were centrifuged at 3000 turns/min for 15 min. The solid residue was washed with toluene (2 × 10 mL) and absolute EtOH (1 × 10 mL). NPs were finally dried in vacuum for several hours, to afford **15** (11 mg) as brown solid.



FT-IR (in KBr): 3400, 1750, 1630, 1550, 1440, 1380, 1210, 1108 cm^{-1}.

UV-vis (in H_2O): λ_{max}: 260 nm; 280 nm (PNA); 400 nm (NP).

Elemental analysis C, H, N: Calcd (%) for $C_{114}H_{149}O_{44}N_{42}Si$ found: C 24.34; H 3.67; N 8.92; (O 10.65 and Si 0.40, calculated on molar % of N).

ICP-OES analysis Fe: Fe theoretical (%) (calculated on molar base) = 59, Fe (%) found = 35.30 O of γ-Fe_2O_3 = 16.64 calculated by difference.

FTIR - MPNA

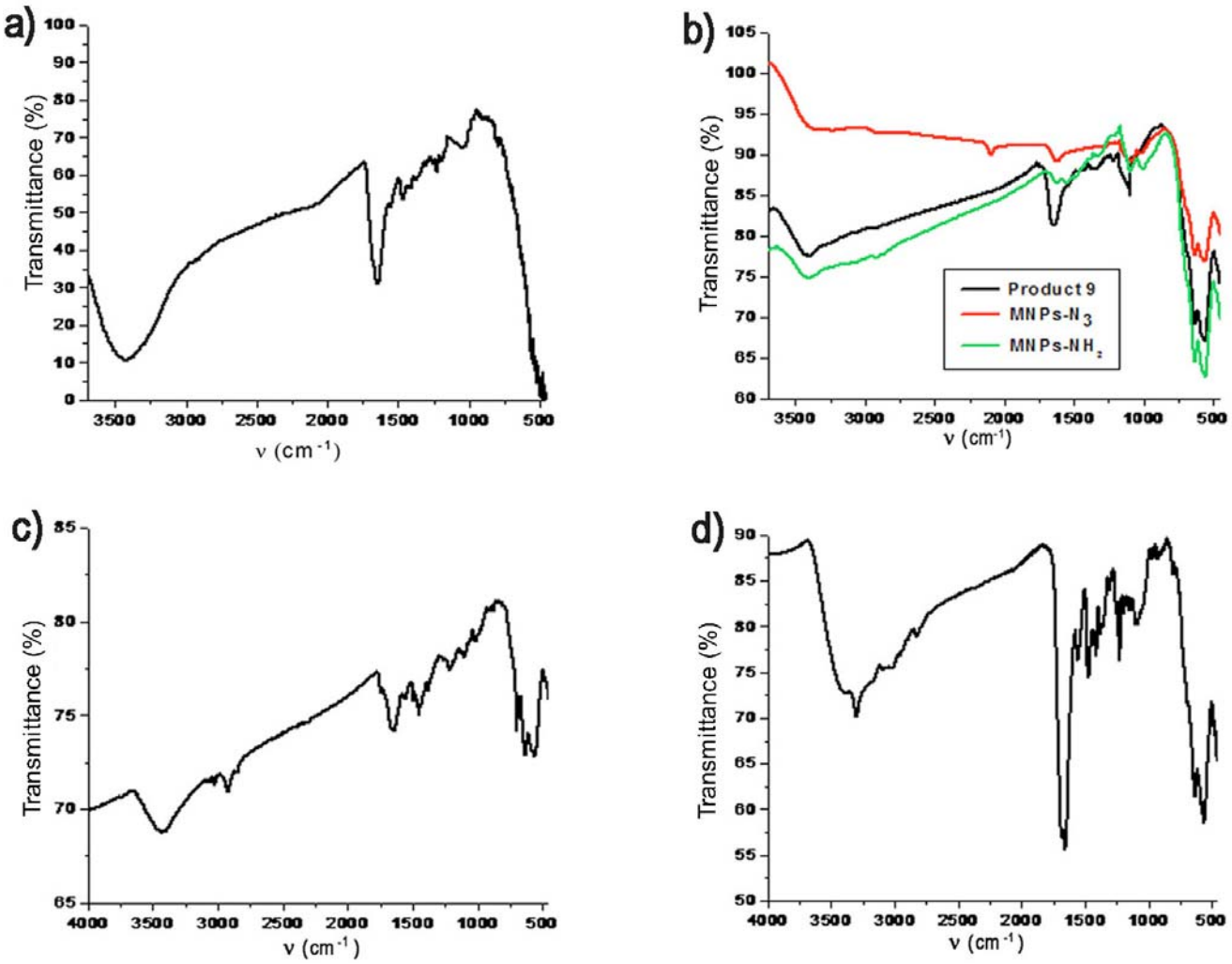


Fig. S1. FT-IR spectra of a) **15**, b) green MNP-NH_2, red MNP-N_3, product **9**, c) **7**, d) **13**.

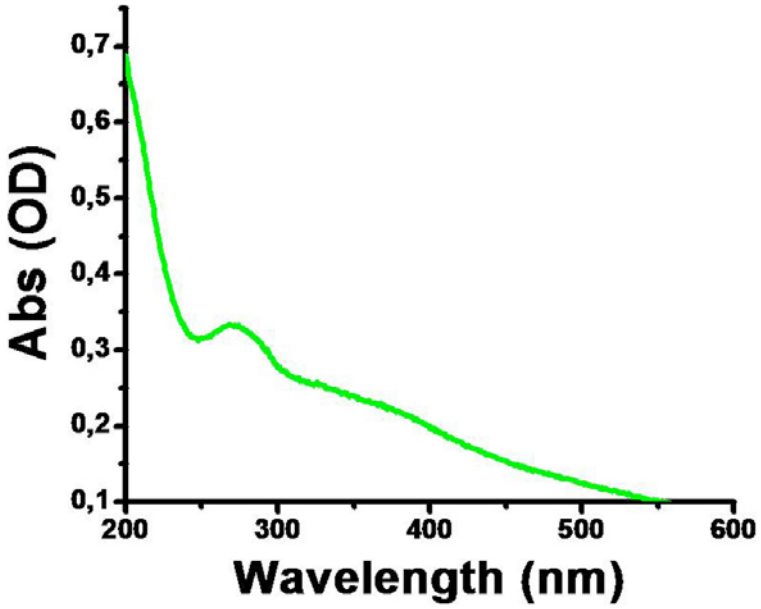


Fig. S2. UV-Vis spectra of **13**.

T_2 measurements

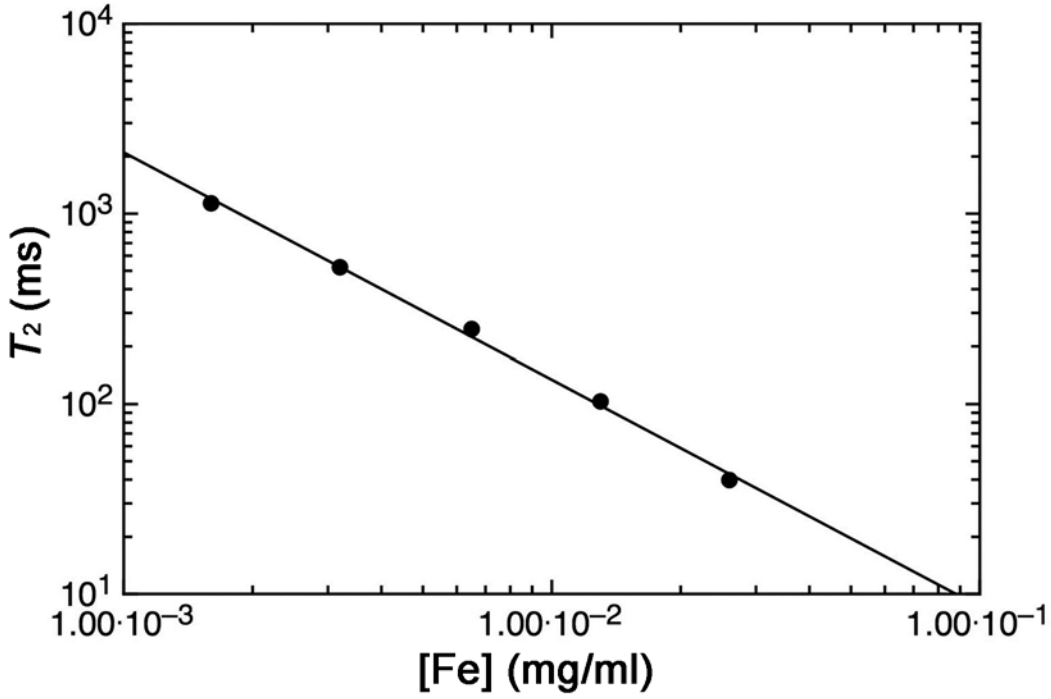


Fig. S3. Relaxation time (T_2) vs. iron concentration of MPNA **13**.

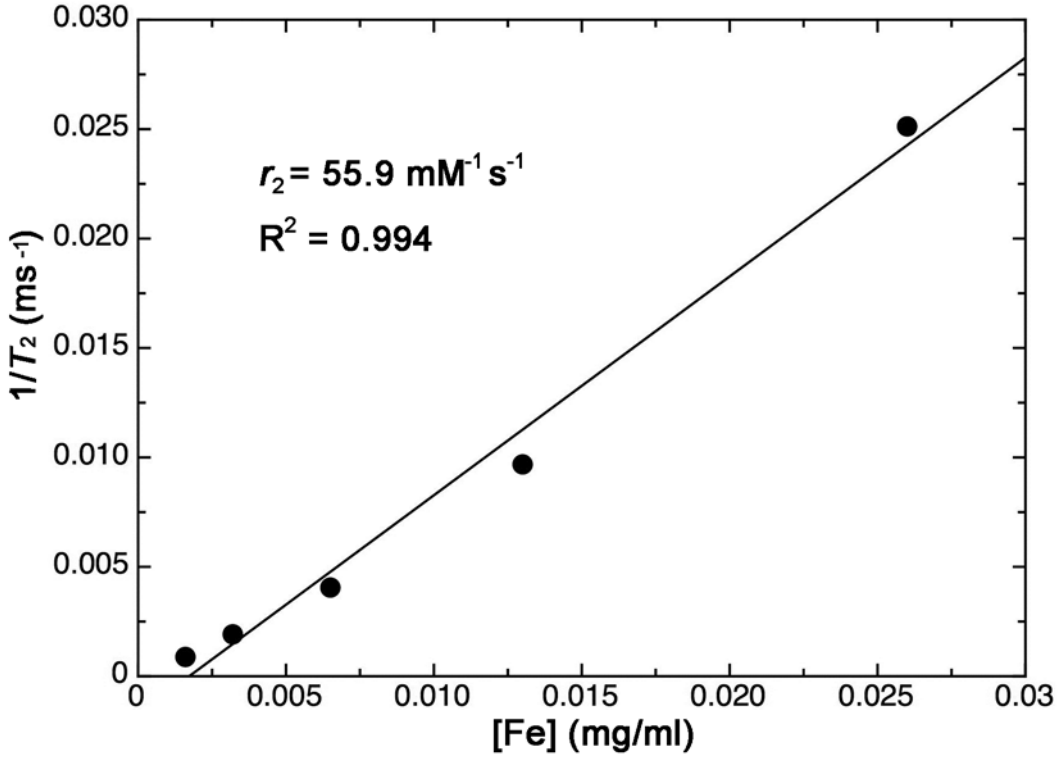


Fig. S4. $1/T_2$ vs. iron concentration of MPNA **13**.

Measurement of proton transverse relaxation times (T_2)

Proton relaxation time T_2 experiments were performed at 313 K in a 0.47 T Bruker Minispec mq20 relaxometer. Before T_2 measurements, PNA-bearing nanoparticle (**13**) samples (13 mg/mL iron) were transferred into the tube, kept 5 min at room temperature before the addition of DNA solutions (12.8 mg/mL in 1:1 H_2O/phosphate buffer, pH 7.4). Subsequently, the mixture was incubated and the temperature progressively increased with a constant increment rate of 0.5 °C/min up to a final temperature of 90 °C. Then, the mixture was kept at 90 °C for 5 min, followed by slow cooling to room temperature. Then the tubes were pre-warmed at 313 K for 10 min for thermal equilibration and T_2 values were acquired on the samples at this stage. Relaxivity was determined as the slope of a $1/T_2$ plot as a function of iron concentration expressed in μg/mL.

UV-Melting

Binding affinity of the MPNA **13** with complementary homo-adenine ssDNA decamer was evaluated. Samples were prepared by mixing **13** and complementary ssDNA in equimolar concentration (2.5 μM) in 1:1 H_2O/phosphate buffer solution (pH 7.0, 100 mM NaCl, EDTA 0.1 mM). Hybridization was done by heating the solution in a sand bath at 90 °C for 5 min; the solution was then slowly cooled to 4 °C. The resulting solution was subjected to UV measurements at 260 nm from 20 °C to 90 °C using a PerkinElmer Lambda2S spectrometer equipped with a PerkinElmer PTP-6 Peltier temperature controller, by increasing the temperature at a rate of 0.5 °C/min. The obtained UV absorbances, collected at 260 nm every 12 sec, were plotted to prepare the melting curve.

A control experiment for T_m was also performed by incubating **13** under the same conditions as described above but in absence of DNA. In this case we did not obtain the classical melting curve with the characteristic flex and no significant variation of the absorbance was observed during the whole experiment.

HR-Mas ^{1}H-NMR:

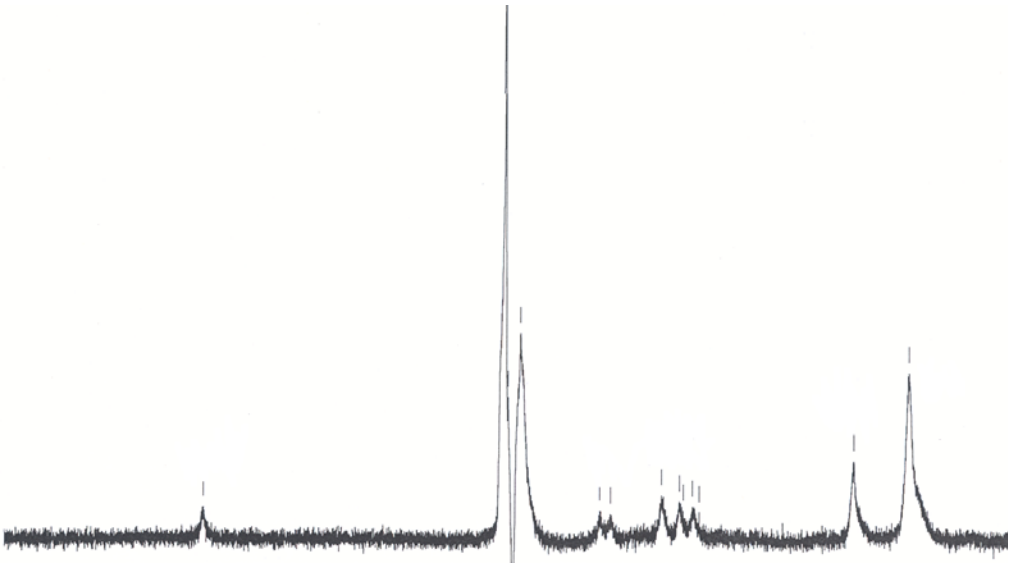


Fig. S5. HR-Mas ^{1}H-NMR of **6**

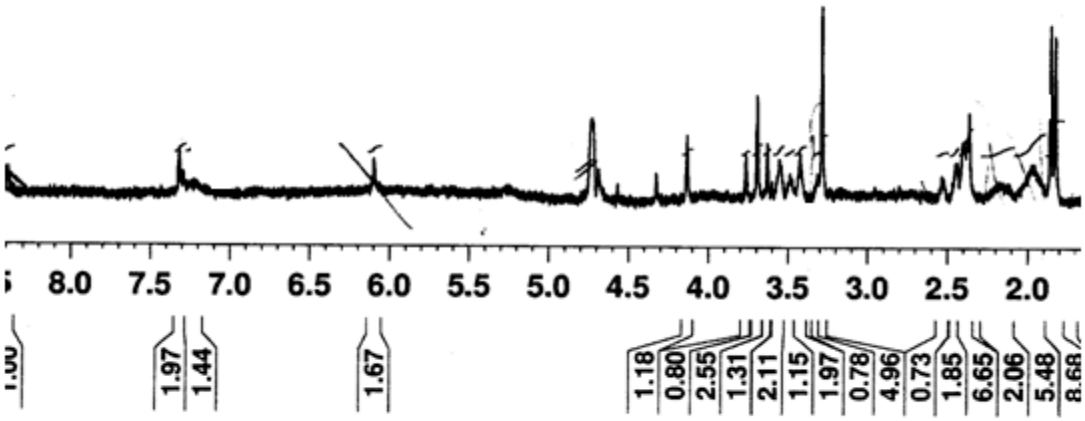


Fig. S6. HR-Mas ^{1}H-NMR of **7**

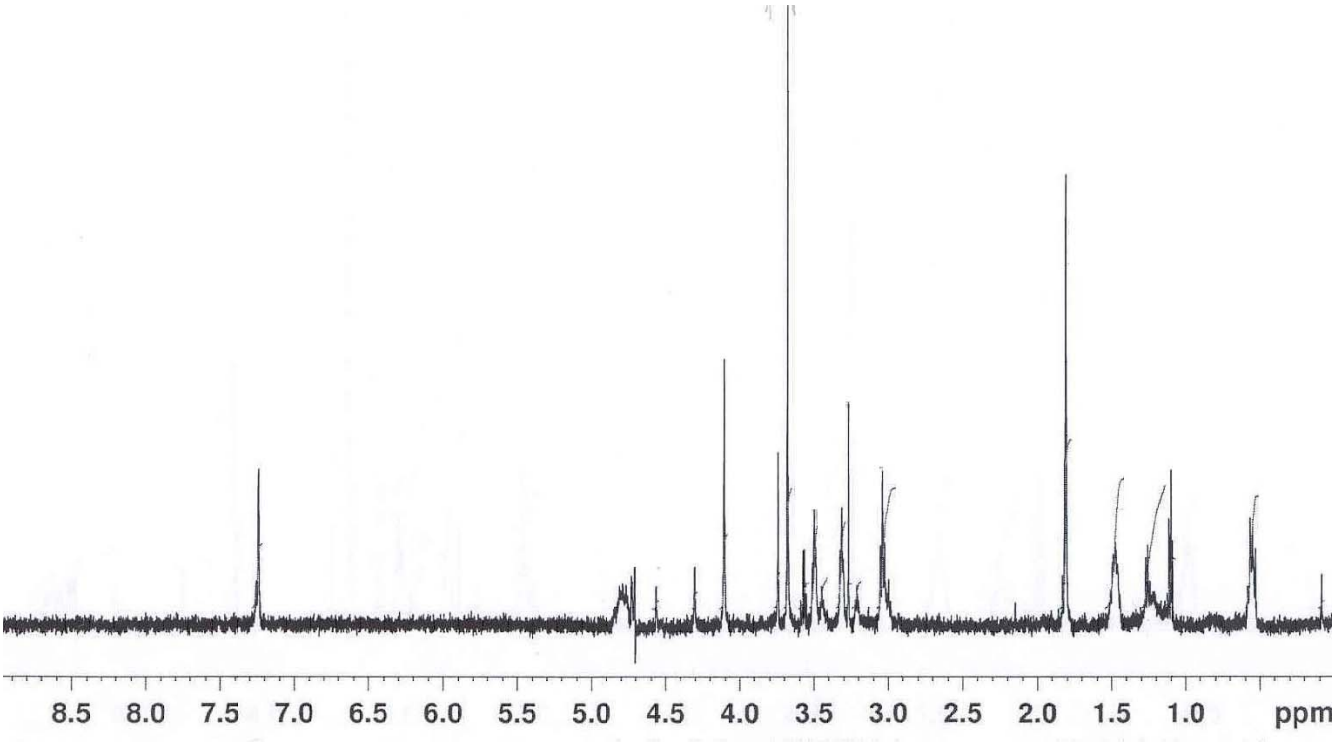


Fig. S7. HR-Mas ^{1}H-NMR of **8**

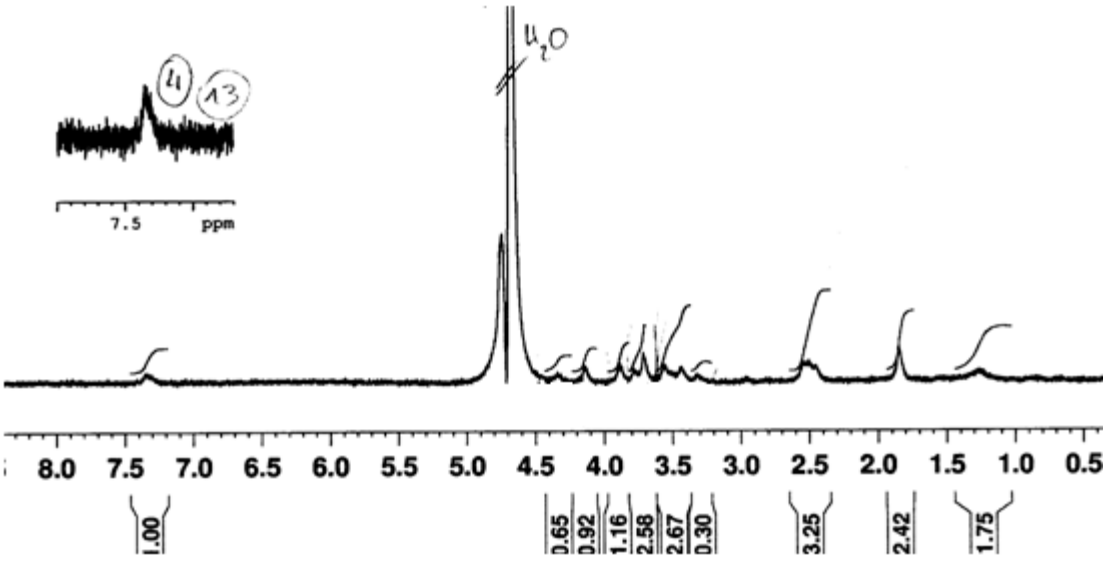


Fig. S8. HR-Mas ^{1}H-NMR of **9**

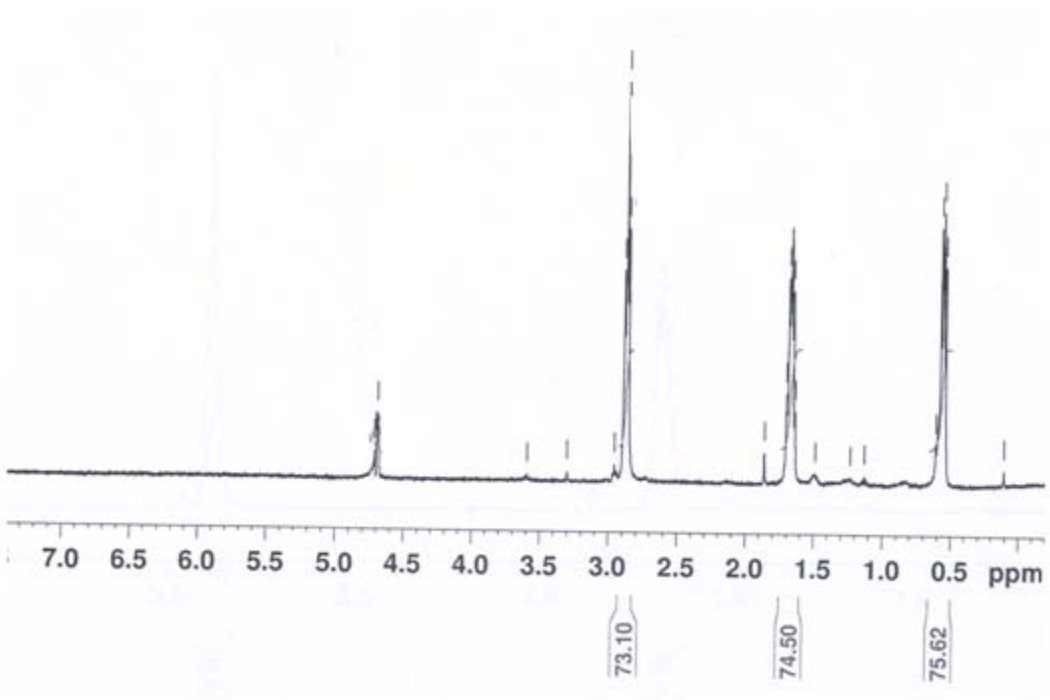


Fig. S9. HR-Mas ^{1}H-NMR of **APTES-NPs**

MALDI spectra and HPLC traces of 10, 11 and 12.

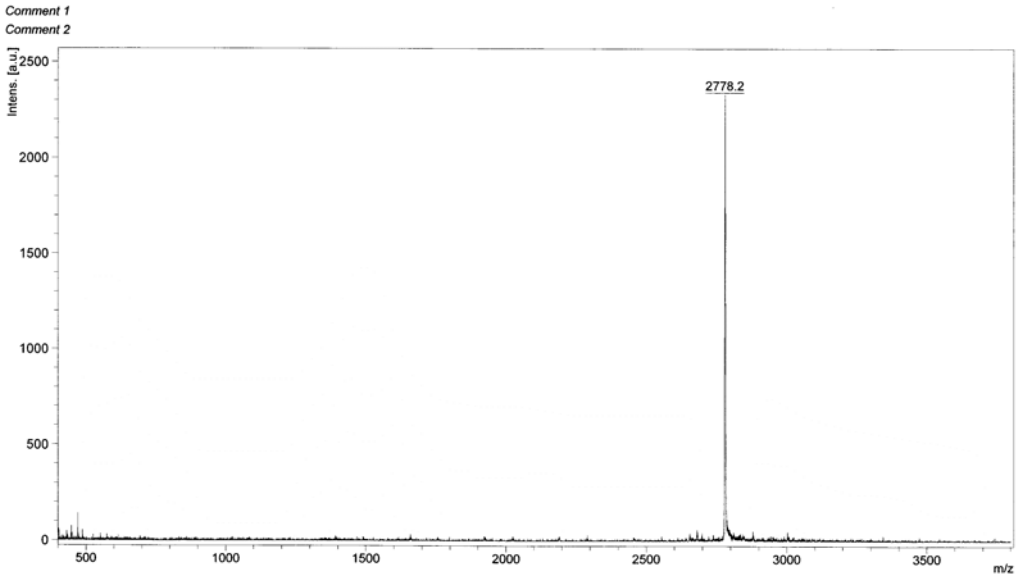


Fig. S10. MALDI spectrum of **10**: *m/z* 2778.2 (M^{+}) (calculated for $C_{114}H_{148}N_{41}O_{43}$: 2778.06).

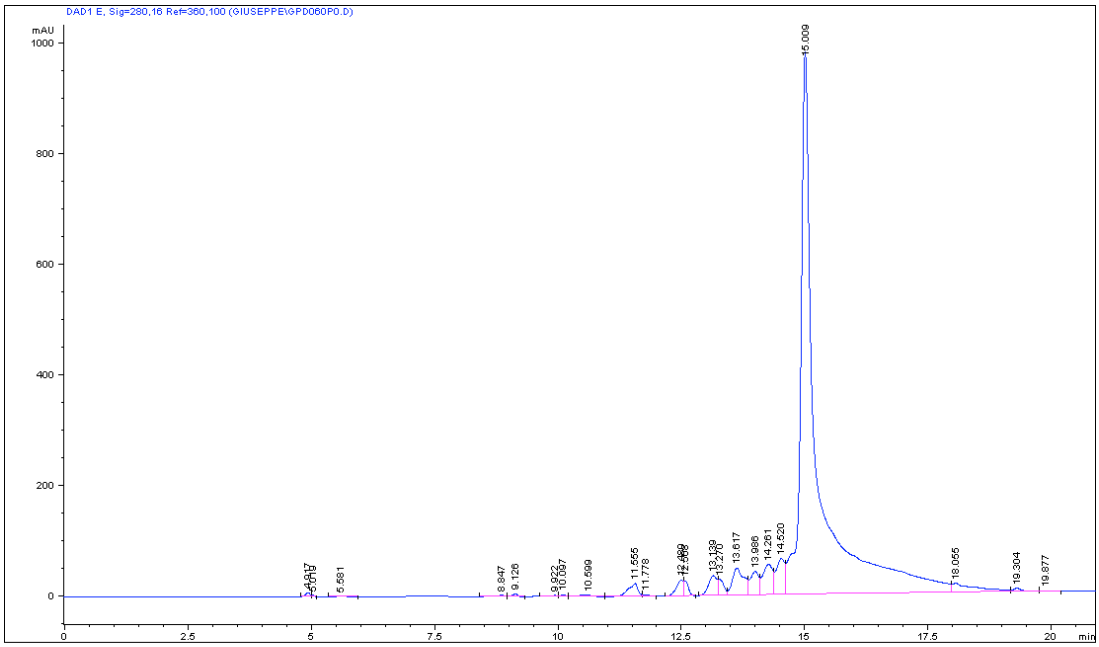


Fig. S11. HPLC trace of **10**: retention time 15.0 min.

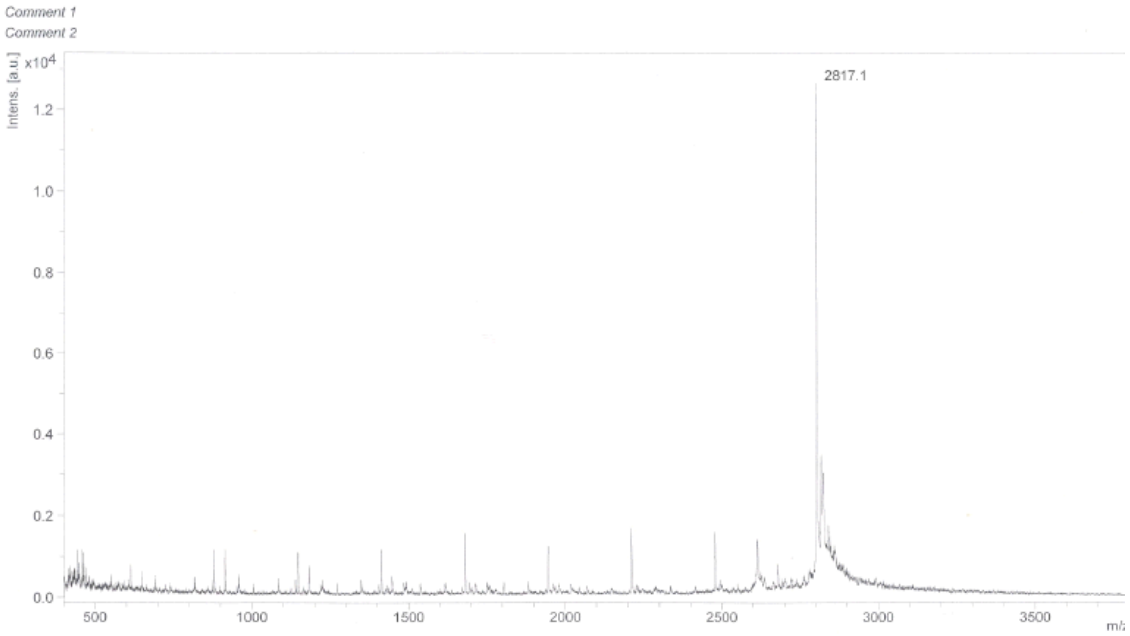


Fig. S12. MALDI spectrum of **11**: *m/z* 2817.1 (M^+ +H). (calculated for $C_{117}H_{151}N_{42}O_{42}$: 2817.10).

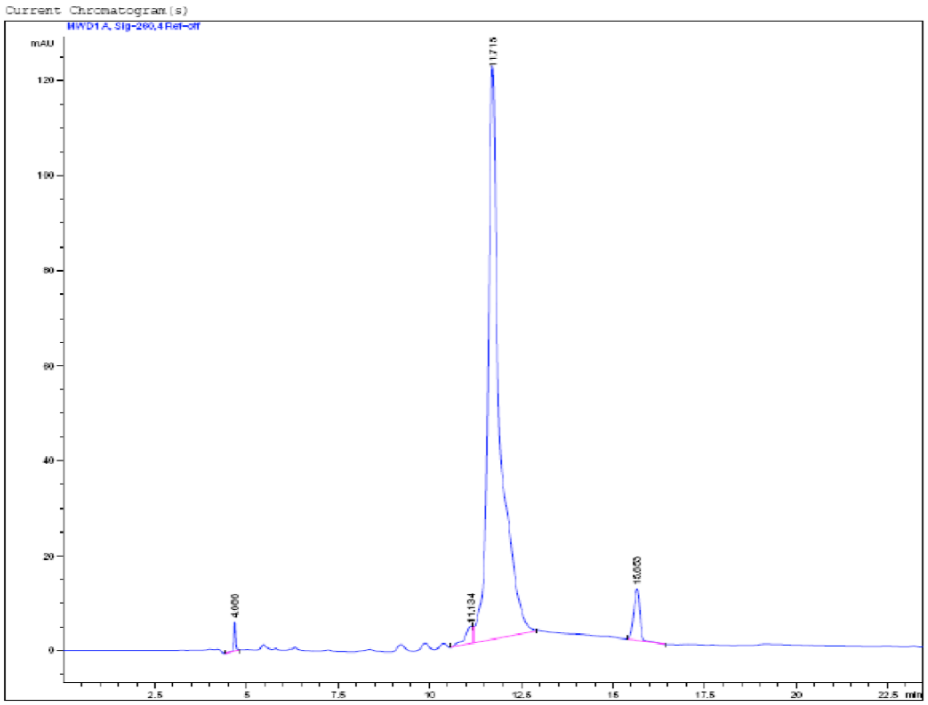


Fig. S13. HPLC trace of **11**: retention time 11.7 min.

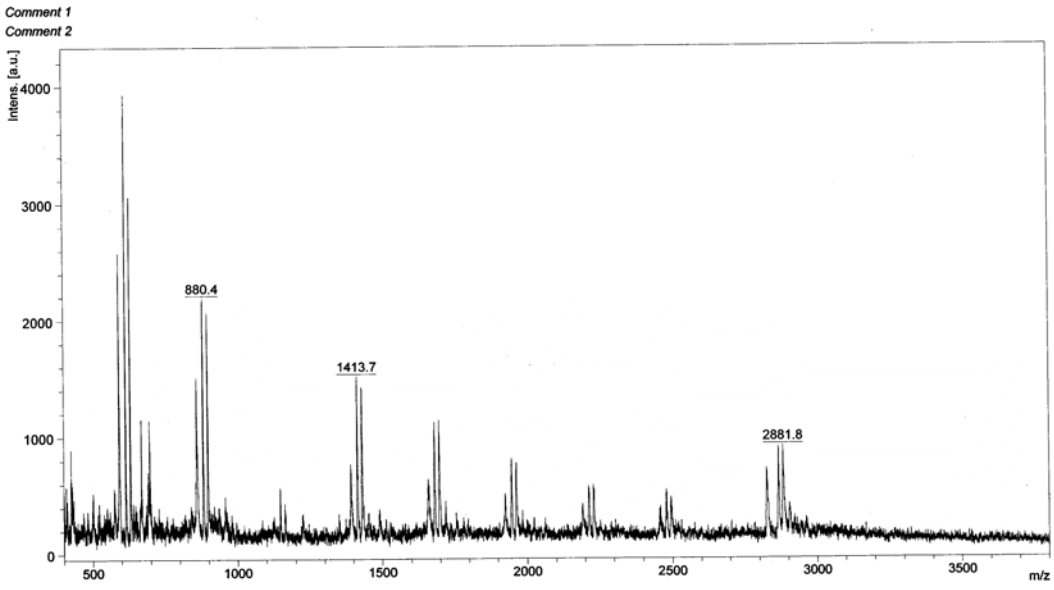


Fig. S14. MALDI spectrum of **12**: *m/z* 2881.8 [M^+ +H-OEt). (calculated for $C_{118}H_{160}N_{42}O_{43}Si$: 2882.14).

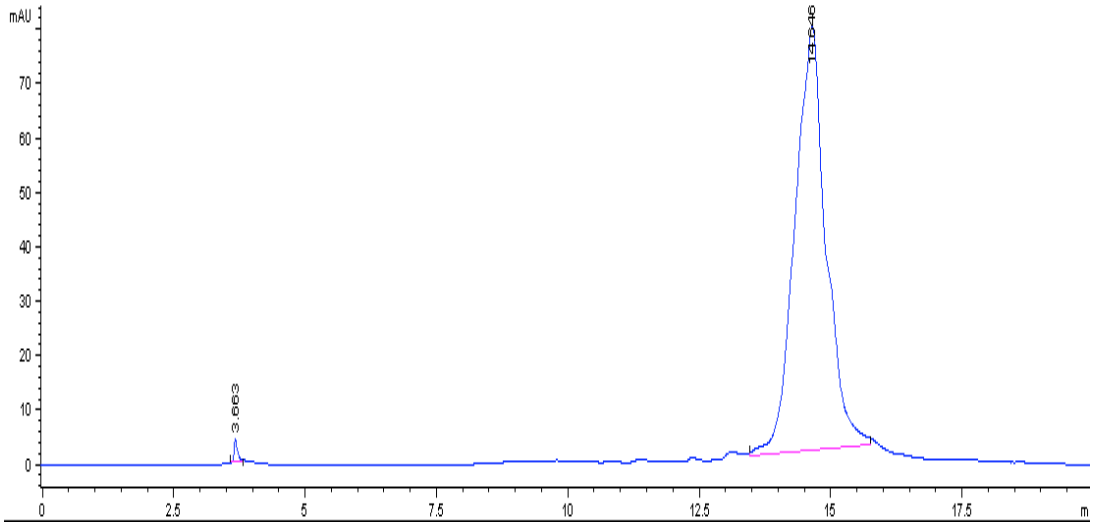


Fig. S15. HPLC trace of **12**: retention time 14.6 min.

Bibliography

1 K. L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H. F. Hansen, T. Vulpius, K. H. Petersen, R. H. Berg, P. E. Nielsen and O. Buchardt, *J. Org. Chem.*, 1994, **59**, 5767.

2 S. A. Thomson, J. A. Josey, R. Cadilla, M. D. Gaul, C. F. Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe and S. A. Noble, *Tetrahedron*, 1995, **51**, 6179.