Supplementary Information:
Click-click chemistry on a peptidic scaffold for the easy access to tetrameric DNA structures

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Abreviations:

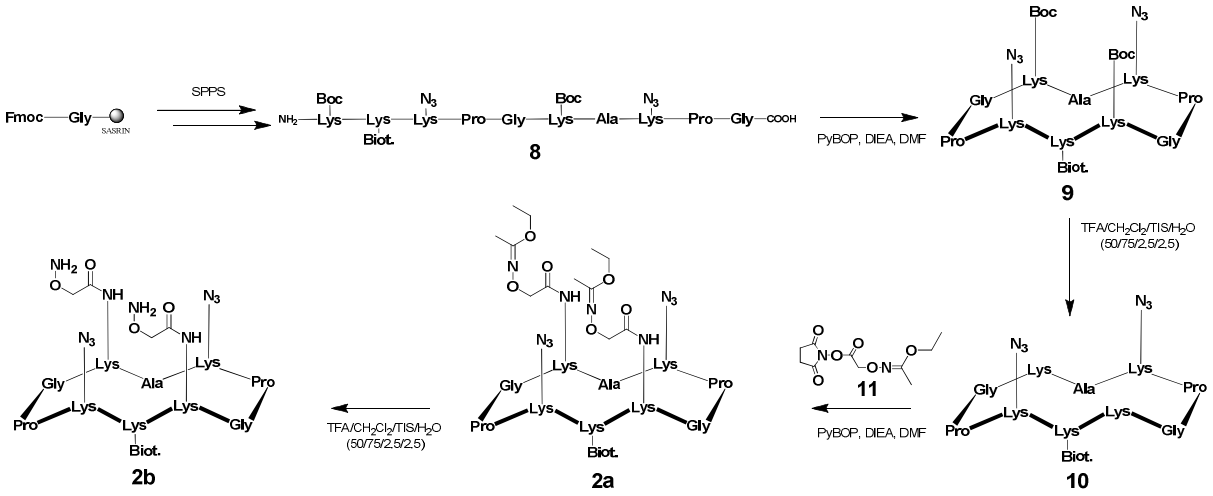
CD: Circular Dichroism, CuAAC: Copper Catalyzed Alkyne-Azide Cycloaddition, DCM: Dichloromethane, DIEA: Diisopropylethylamine, DMF: Dimethylformamide, DMT: Dimethoxytrityl, EDTA: Ethylenediaminetetraacetic acid, ESI -MS: Electrospray Ionisation Mass Spectrometry, Fmoc: Fluorenylmethyloxycarbonyl, HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, MALDI: Matrix Assisted Laser Desorption Ionisation, NMR: Nuclear Magnetic Resonance, PyBOP: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, RP-HPLC: Reverse-Phase High Performance Liquid Chromatography, SAX-HPLC: Strong Anion Exchange High Performance Liquid Chromatography, SEC: Size Exclusion chromatography, TFA: Trifluoroacetic Acid, THAP: 2,4,6-trihydroxyacetophenone, THPTA: Tris-(hydroxypropyltriazolylmethyl)amine, TIS: Triisopropylsilane, TNBS: 2,4,6-trinitrobenzenesulfonic acid, UV: Ultra Violet, ToF: Time of Flight.

General details:

ESI mass spectra were performed on an Esquire 3000 spectrometer from Bruker. Peptides were analyzed in positive mode and oligonucleotides and conjugates in negative mode. MALDI ToF mass spectrum of **1** was performed on Voyager DE mass spectrometer (Perseptive Biosystems) equipped with an N_2 laser (337 nm). Analyses were operated in THAP matrix in presence of ammonium citrate buffer after a treatment with DOWEX 50 W

X8 resin (ammonium form). All solvents and reagents used were of highest purity commercially available.

General procedure for peptide synthesis:



The course of reactions were monitored on RP-HPLC using a HPLC system on a Nucleosil C18 column (300 Å, 125 x 3 mm, 5 µm) with UV monitoring at 214 nm and 250 nm. A 1 mL/min flow linear gradient from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1 % TFA in acetonitrile/water: 9/1) to 100% B for 20 minutes was applied.

RP-HPLC purifications were performed on a Nucleosil C18 column (300 Å, 250 x 21mm, 7 µm) with UV monitoring at 214 nm and 250 nm using a 22 mL/min flow linear gradient from 95% solvent A and 5% solvent B to 100% solvent B for 30 min.

1. Linear peptide **8**

Peptide **8** was synthesized using the Fmoc-tBu protocol using Fmoc-Gly-SASRIN® (1 g, loading of 0.8 mmol/g) in a glass reaction vessel fitted with a sintered glass. Fmoc-Lys(biotin)-OH, Fmoc-Gly-OH, Fmoc-Ala-OH Fmoc-Pro-OH and Fmoc-Lys(Boc)-OH were commercially available. Fmoc-azidonorleucine was obtained using the reported protocol[1]. The following protocol was used for each amino acid coupling:

Fmoc protecting group was first removed using three washing (for 10, 5 and 5 min) with 20% piperidine in DMF (10 mL). The resin loading was monitored by quantification of free dibenzofulvene using UV absorbance at 299 nm. Each coupling reaction was operated using the classical protocol with amino acid (2 eq) in DMF (10mL) with PyBOP (2 eq) as activator. pH was adjusted to 8-9 with DIEA. The completion of the coupling reaction was analyzed using TNBS test after washing the resin with DMF (2 x 10 mL) and dichloromethane (10

[1] E. D. Goddard-Borger, and R. V. Stick, *Org. Lett.* 2007, **9**, 3797-3800.

mL). Deprotections and coupling reactions were performed until obtaining the supported NH_2- free peptide **8**.

The resin was washed with a 1% TFA in dichloromethane solution (10 x 20 mL) for cleaving the peptide from the resin. Each fraction was collected and neutralized with DIEA. The solution was evaporated under vacuum and the peptide was precipitated with ether to obtain a yellow powder. t_r= 13.6 min.

ESI MS(+) m/z calcd: 1517.8, found: 1517.8 $[M+H]^+$.

2. Cyclic peptide **9**

Peptide **8** (0.5 mmol, 760 mg) was dissolved in DMF (500 mL) and PyBOP (1 mmol, 512 mg) was added. The pH was adjusted to 8-9 using DIEA and the solution was stirred at room temperature until the complete peptide cyclisation (RP-HPLC monitoring). The solvent was evaporated under vacuum then the crude peptide was precipitated with ether to obtain a yellow powder. t_r= 12.6 min.

ESI MS(+) m/z calcd: 1499.8, found: 1499.0 $[M+H]^+$.

3. *N*-ε-free peptide **10**

Peptide **9** (0.5 mmol, 750 mg) was treated a TFA/DCM/H_2O/TIS (50/45/2.5/2.5) solution (100 mL) and stirred at room temperature (2h). The solvent was evaporated under vacuum and the crude peptide was precipitated with ether to obtain a yellow powder. t_r = 9.6 min

ESI MS(+) m/z calcd: 1299.6, found: 1299.8 $[M+H]^+$.

4. Protected aminooxy peptide **2a**

Peptide **10** (0.2 mmol, 260 mg) was dissolved in anhydrous DMF (100 mL). *N*-Hydroxysuccinimidyl 2-(1-ethoxy-ethylidenaminooxy)acetate[2] **11** (0.5 mmol, 130 mg) was then added. pH was adjusted to 8-9 with DIEA. The solution was stirred at room temperature and monitored by RP-HPLC until the completion of the reaction (2h). The solvent was evaporated and the peptide was precipitated with ether. The product was purified on RP-HPLC to obtain a white powder (82 mmol, 130 mg, yield: 41%). t_r = 13.3 min.

ESI MS(+) m/z calcd: 1585.9, found: 1586.1 $[M+H]^+$

[2] S. Foillard, M. O. Rasmussen, J. Razkin, D. Boturyn, and P. Dumy, *J. Org. Chem*., 2008, **73**, 983-991.

Figure S1: ESI mass spectrum of compound **2a** (ESI MS(+) m/z calcd: 1585.9)

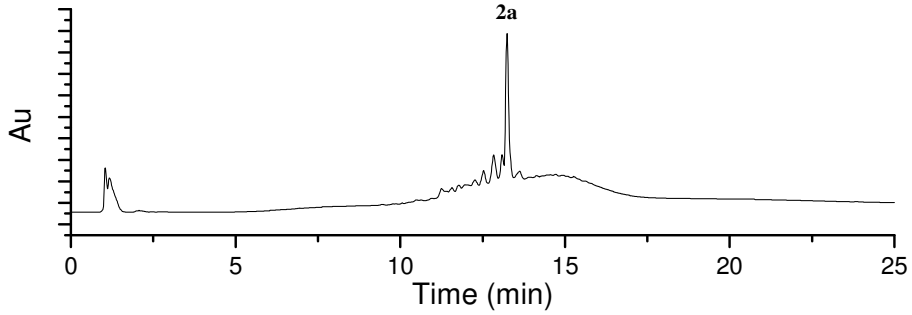


Figure S2: RP-HPLC chromatogram of crude compound **2a** (λ_{abs}=214nm).

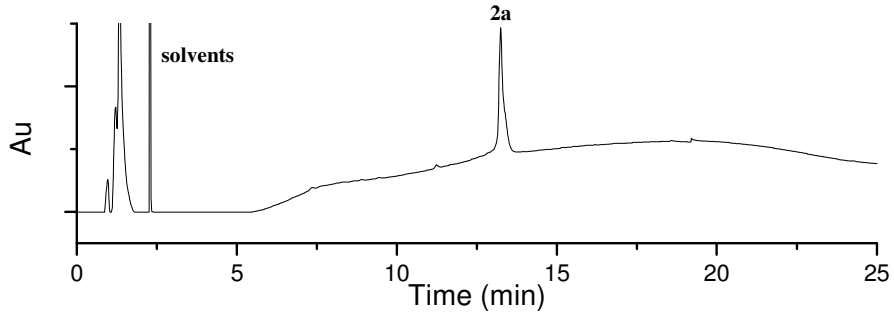


Figure S3: RP-HPLC chromatogram of compound **2a** (λ_{abs}=214 nm).

5. Aminooxy peptide **2b**

Peptide **2a** (0.1 mmol, 158 mg) was dissolved in a TFA/DCM/H_2O/TIS (50/45/2.5/2.5) solution (50 mL) and the reaction was stirred for one hour at room temperature. The peptide was next precipitated with ether and finally, it was purified on RP-HPLC and freeze dried. **2b** was obtained as a white powder. (0.042 mmol, 60 mg, yield: 42%). t_r = 9.4 min.

ESI MS(+) m/z calcd: 1445.7, found: 1445.9 $[M+H]^+$.

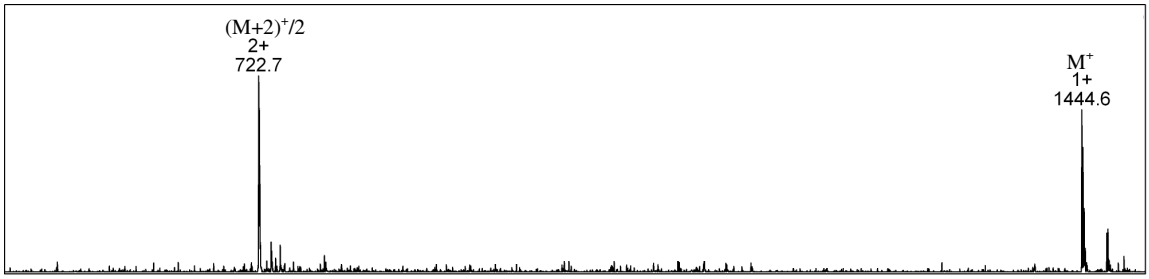


Figure S4: ESI mass spectrum of compound **2b** (ESI MS(+) m/z calcd: 1445.7)

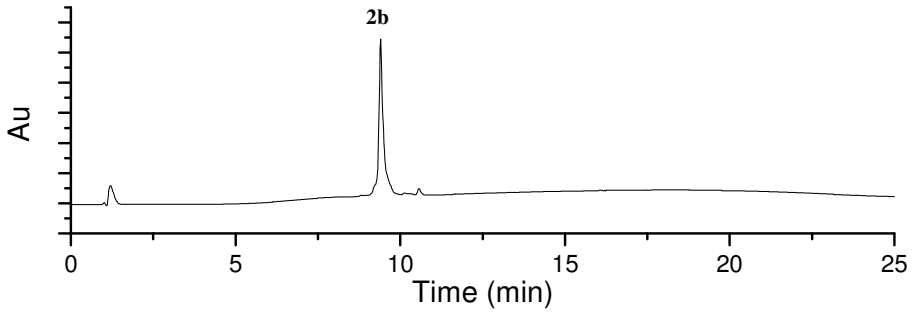


Figure S5: RP-HPLC chromatogram of purified compound **2b** (λ_{abs}=214 nm).

Oligonucleotides and conjugates synthesis:

Oligonucleotides were synthesized by β-cyanoethylphosphoramidite chemistry using a 3400 DNA synthesizer at 1 µmol scale.

RP-HPLC analysis was performed on a HPLC system using C18 Nucleosil column (Macherey-Nagel, 250 x 4.6 mm, 100 Å, 5 µm) with 1 mL/min flow linear gradients of solvent A' (50 mM triethylammonium acetate buffer with 5% acetonitrile) and solvent B'(acetonitrile with 5% water) with UV-monitoring at 260 nm and 280 nm. Gradients start from 100% solvent A' to 30% B' for 20 minutes.

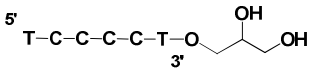
RP-HPLC purifications of oligonucleotides were performed on Nucleosil C-18 column (250 mm x 10 mm, 100 Å, 7µm) using 4 mL/min flow linear gradients with solvent A' and B' with UV-monitoring at 260 nm and 280 nm.

Strong Anion Exchange HPLC (SAX-HPLC) analyses and purifications were performed on a Nucleogel anion exchange column (SAX 50 x 4.6mm, 1000 Å, 8 µm) with monitoring at 260 nm and 280 nm. 1 mL/min flow linear gradient starts from 100% solvent A'' (Tris buffer 25 mM pH 8 with 10% methanol) to 60% solvent B'' (Tris buffer 25 mM, 1.5 M NaCl with 10% methanol) for 20 minutes.

Desalting of oligonucleotide was performed by SEC on NAP 25 cartridge using the recommended protocol.

Quantification of oligonucleotides was performed at 260 nm (ε= 45600 $L.mol^{-1}.cm^{-1}$, estimated according to the nearest neighbour model).

1. 3'-diol oligonucleotide **12**



Oligonucleotide **12** was obtained from automated synthesis on a 3'-glyceryl CPG resin at 1 µmol scale. After synthesis cyanoethyl protecting groups were removed using 20% piperidine in acetonitrile. Cleavage from the resin and deprotections was performed in 28% NH_4OH for 16h at 55°C. The product was purified on RP-HPLC with a gradient from 0% to 45% solvent B' in solvent A' for 20 min. 5'-DMT group was then removed using 80% aqueous acetic acid solution for 45 min at room temperature. After freeze-drying, the residue was diluted in water and washed 5 times with diethyl ether. (784 nmol, yield 78%). t_r = 13.7 min.

ESI MS (-): m/z calcd: 1856.3, found: 1855.0 $[M-H]^-$

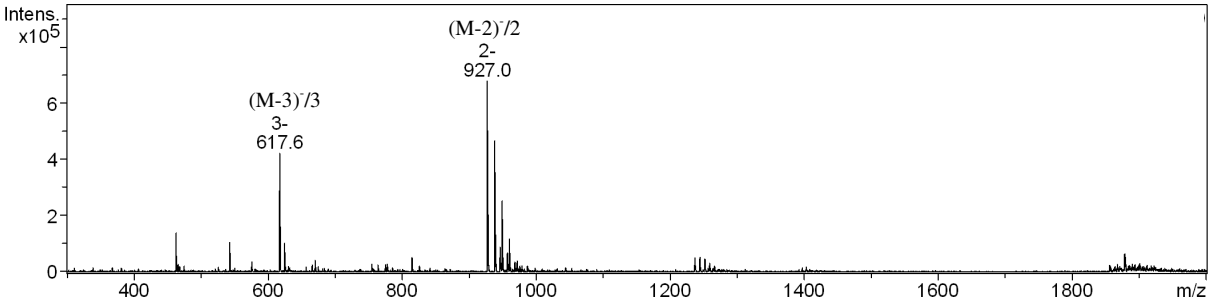


Figure S6: ESI mass spectrum of compound **12** (m/z calcd: 1856.3)

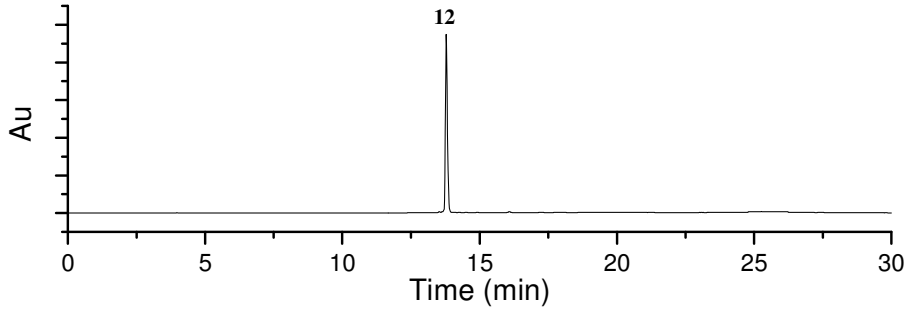
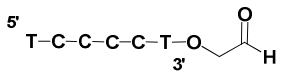


Figure S7: RP-HPLC chromatogram of purified compound **12** (λ_{abs}=260 nm).

2. 3'-aldehyde oligonucleotide **3**



Sodium metaperiodate (4 µmol, 860 µg) was added to a solution of oligonucleotide **12** (200 nmol) in water (200 µL). The mixture was stirred for 1h at room temperature in dark conditions. Excess of $NaIO_4$ was then removed by SEC. Crude oligonucleotide **3** was used in the next step without further purification. (172 nmol, yield 86%). t_r= 13.7 min.

ESI MS(-) m/z calcd: 1824.2, found: 1823.2 $[M-H]^-$.

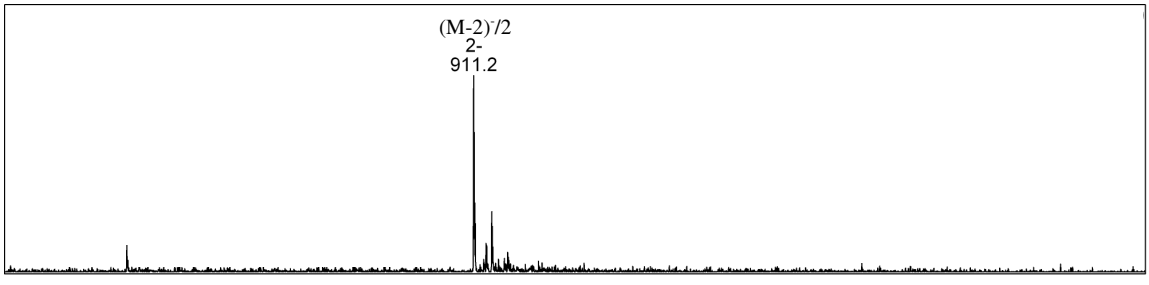


Figure S8: ESI mass spectrum of compound **3** (ESI MS(-) m/z calcd: 1824.2)

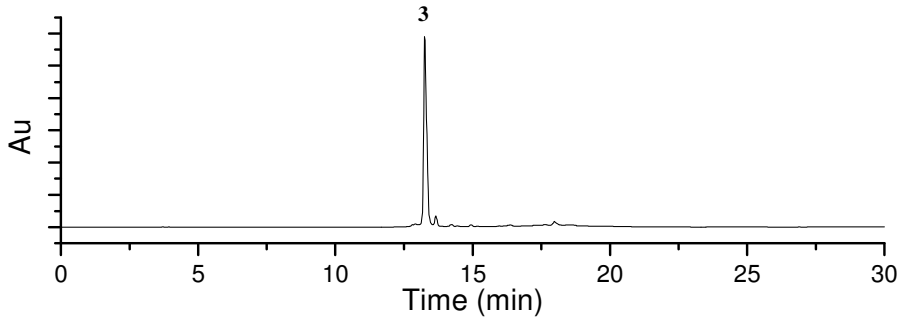
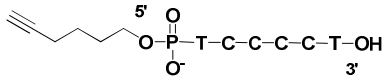


Figure S9: RP-HPLC chromatogram of crude compound **3** (λ_{abs}=260 nm).

3. 5'-alkyne oligonucleotide **4**



Oligonucleotide **4** was obtained from automated synthesis using commercially available 5'-hexynyl (β-cyanoethyl) phosphoramidite at 1µmol scale. Cleavage from the resin and subsequent deprotection were performed in 28% ammoniac solution for 16h at 55°C. The crude was purified on RP-HPLC with a gradient from 0% to 45% solvent B' in solvent A' for 20 min (851 nmol, yield 85%). t_r = 15.3 min.

ESI MS(-) m/z calcd: 1862.3, found: 1861.2 $[M-H]^-$.

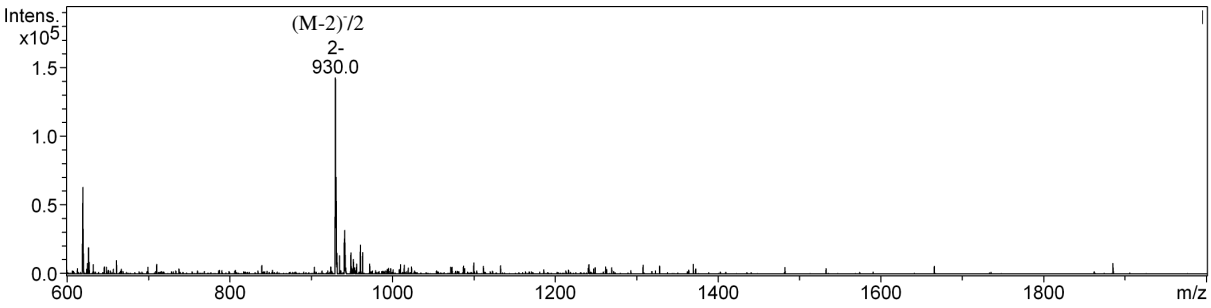


Figure S10: ESI mass spectrum of compound **4** (ESI MS(-) m/z calcd: 1862.3)

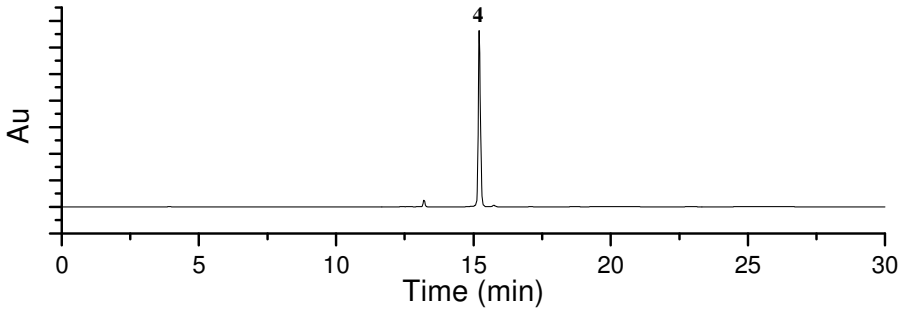
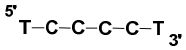


Figure S11: RP-HPLC chromatogram of purified compound **4** (λ_{abs}=260nm).

4. Oligonucleotide **7**



Oligonucleotide **7** was obtained from automated synthesis at 1 µmol scale. Cleavage from the resin and deprotections were performed in 28% ammoniac solution for 16h at 55°C. The product was purified on RP-HPLC with a gradient from 0% to 45% solvent B' in solvent A' for 20 min. 5'-DMT group was removed using 80% aqueous acetic acid solution for 45 min at room temperature. After freeze-drying, the residue was diluted in water and washed 5 times with diethyl ether. The oligonucleotide was desalted using NAP 25 cartridge. (579 nmol, yield 58%). t_r = 14.0 min.

ESI MS(-) m/z calcd: 1702.2, found: 1701.0 $[M-H]^-$.

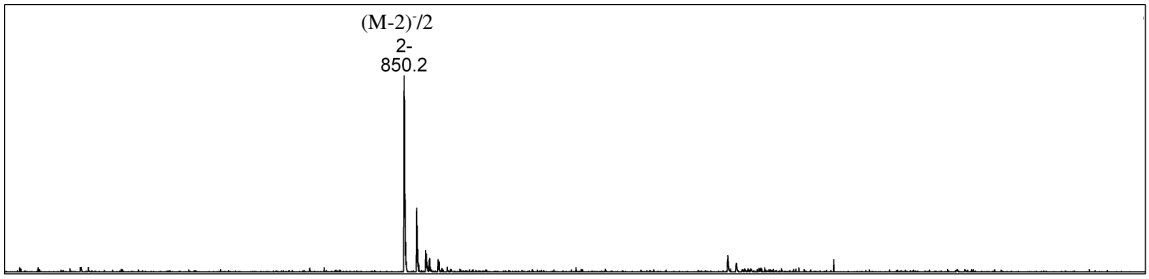


Figure S12: ESI mass spectrum of compound **7** (ESI MS(-) m/z calcd: 1702.2)

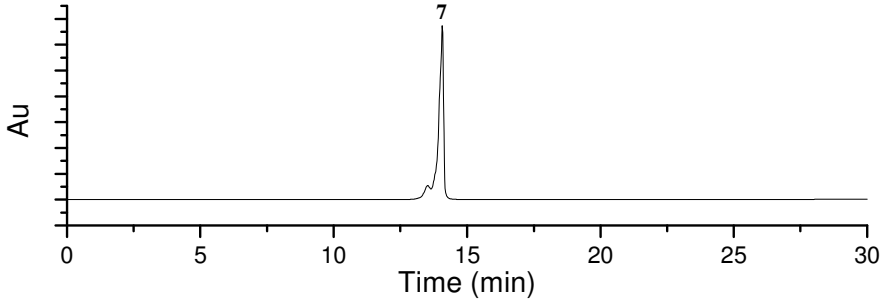


Figure S13: RP-HPLC chromatogram of compound **7** (λ_{abs}=260nm).

5. Conjugates synthesis.

a. Strategy 1 *(oxime ligation following by CuAAC reaction)*

*Oxime ligation (compound **5**:*

Oligonucleotide **3** (172 nmol) was dissolved in 0.4 M ammonium acetate buffer (pH 4.5, 200 µL) and aminooxy peptide **2b** (72 nmol, 103 µg) was added. The solution was stirred at 55°C

for 2h then the crude was purified on RP-HPLC with a gradient from 0% to 45% solvent B' in solvent A' for 20 min (42 nmol, yield: 59%). t_r = 19.8 min

ESI MS(-) m/z calcd: 5060.1, found: 5057.4 $[M-H]^-$.

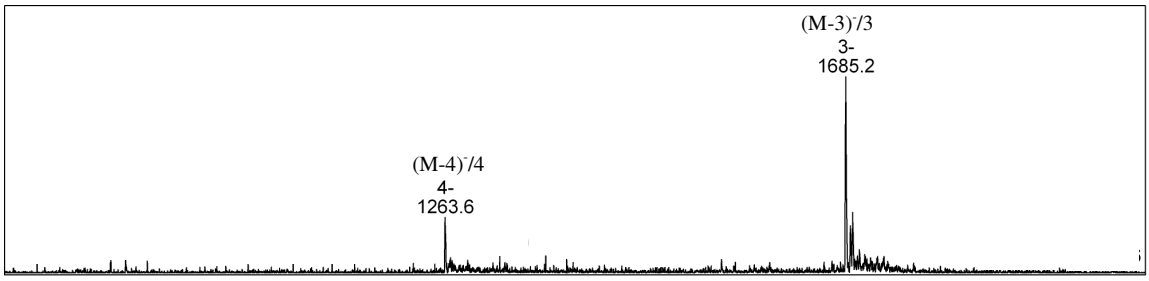


Figure S14: ESI mass spectrum of compound **5** (ESI MS(-) m/z calcd: 5060.1)

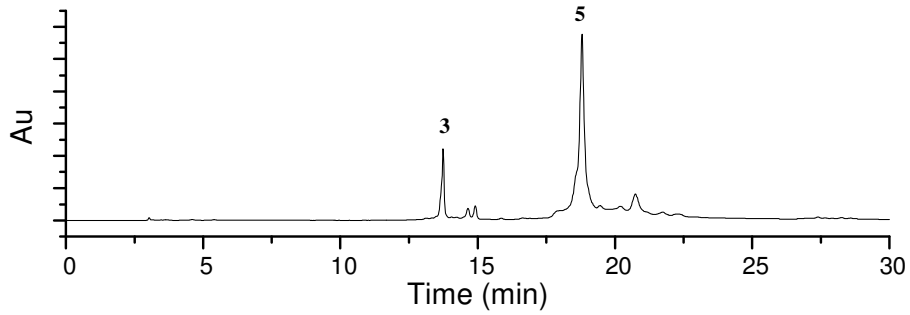


Figure S15: RP-HPLC chromatogram of crude conjugate **5** (λ_{abs}=260 nm).

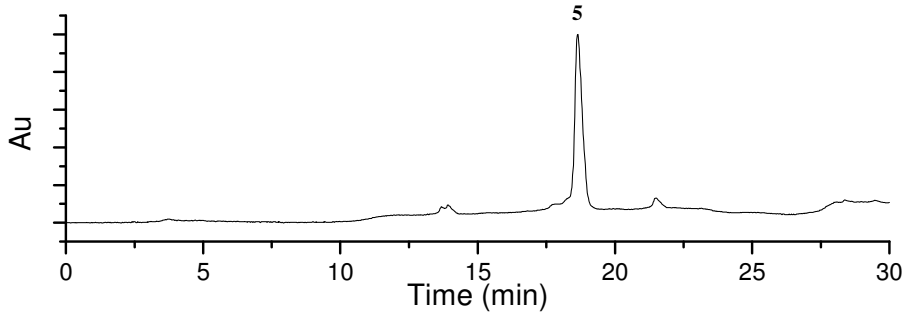


Figure S16: RP-HPLC chromatogram of conjugate **5** (λ_{abs}=260 nm).

*CuAAC reaction (compound **1**)*

To a solution of oligonucleotide **4** (70 nmol) and conjugate **5** (35 nmol) in 100 mM HEPES buffer (pH 7.4, 100 µL) solution was added $CuSO_4$ (88 nmol, 22 µg), THPTA (175 nmol, 70 µg) and sodium ascorbate (350 nmol, 70 µg). The reaction was stirred at room temperature for 2h. Excess of 0.5 M EDTA (pH 8) solution (200 µL) was added and after 30 min the solution was desalted by SEC. **1** was then purified by using SAX purification (16 nmol, yield 45%). t_r = 14.6 min

ESI MS(-) m/z calcd: 8786.6, found: 8788.1$[M-H]^-$.

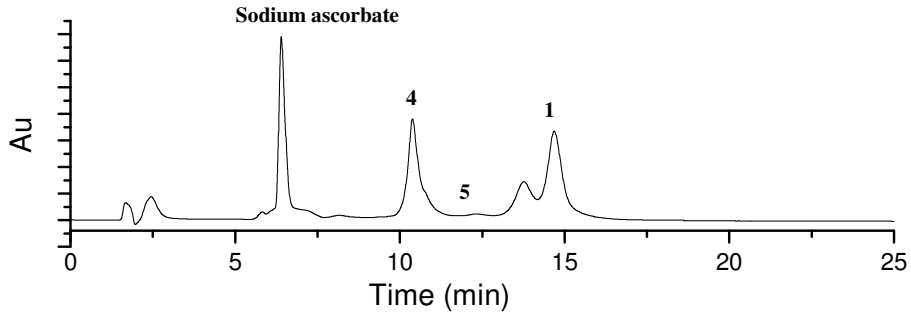


Figure S17: SAX-HPLC chromatogram of crude compound **1** obtained *via* the strategy 1 (λ_{abs}=260 nm).

b. Strategy 2 *(CuAAC reaction following by oxime ligation)*

*CuAAC reaction (compound **6**)*

To a solution of oligonucleotide **4** (150 nmol) and protected aminooxy peptide **2a** (75 nmol, 119 µg) in HEPES buffer (pH 7.4, 100µL) was added $CuSO_4$ (188 nmol, 46 µg), THPTA (375 nmol, 150 µg) and sodium ascorbate (750 nmol, 149 µg). The mixture was stirred at room temperature for 1h. 0.5 M EDTA (pH8) solution (200µL) was added and after 30 min the solution was desalted by SEC. **6** was purified on RP-HPLC with a gradient from 0% to 80% solvent B' in solvent A' for 30 min (47 nmol, yield 63%). t_r = 21.1 min.
ESI MS(-) m/z calcd: 5312.5, found: 5311.3 $[M-H]^-$.

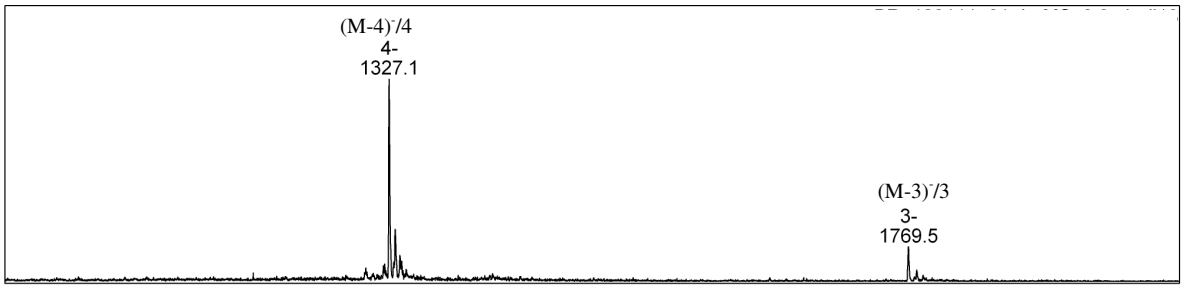


Figure S18: ESI mass spectrum of compound **6** (ESI MS(-) m/z calcd: 5312.5)

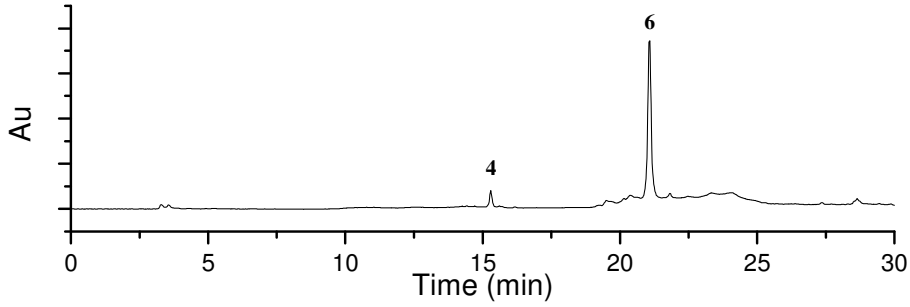


Figure S19: RP-HPLC chromatogram of crude conjugate **6** (λ_{abs}=260 nm).

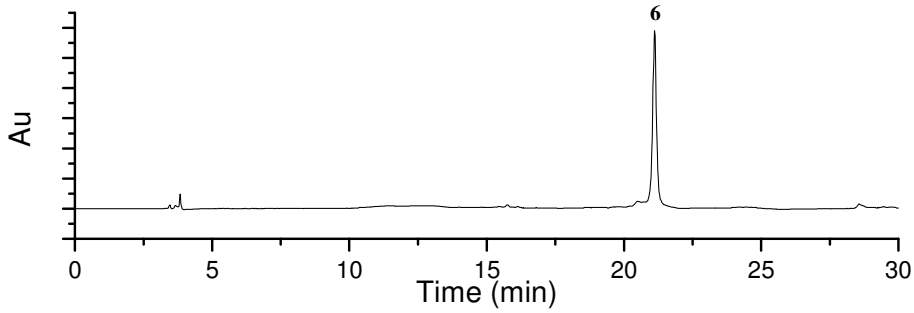


Figure S20: RP-HPLC chromatogram of purified conjugate **6** (λ_{abs}=260 nm).

*Oxime ligation (compound **1**):*

Conjugate **6** (40 nmol) was dissolved in 10% TFA aqueous solution (100 µL) and the solution was stirred for 30 minutes for oxyamine deprotection. The product was freeze dried. Oligonucleotide **3** (160 nmol) in 0.4 M ammonium acetate buffer (pH 4.5, 100 µL) was next added . The solution was stirred at 55°C for 2h and purified by SAX-HPLC (15 nmol, yield: 39%). t_r = 14.6 min.

ESI MS(-) m/z calcd.: 8786.6, found: 8788.1

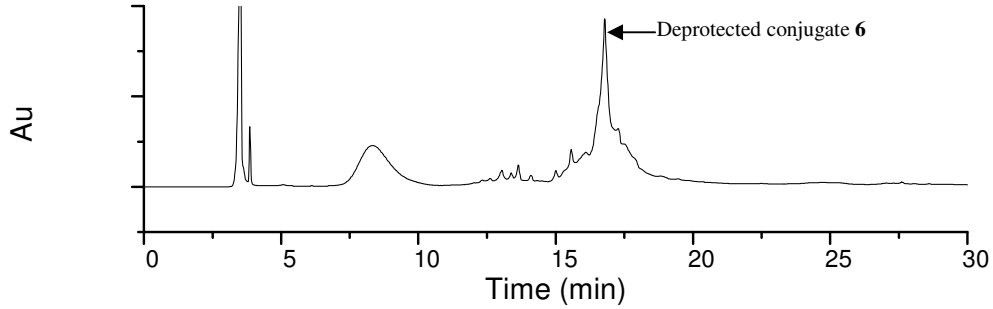


Figure S21: RP-HPLC chromatogram of deprotection of conjugate **6** (λ_{abs}=260 nm).

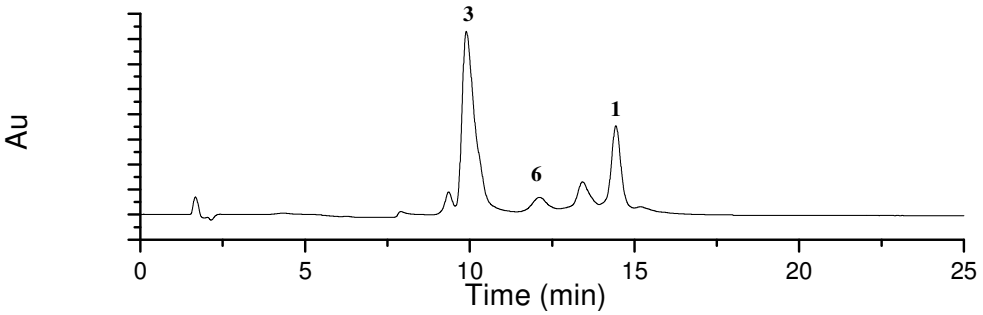


Figure S22: SAX-HPLC of crude compound **1** obtained *via* the strategy 2 (λ_{abs}=260 nm).

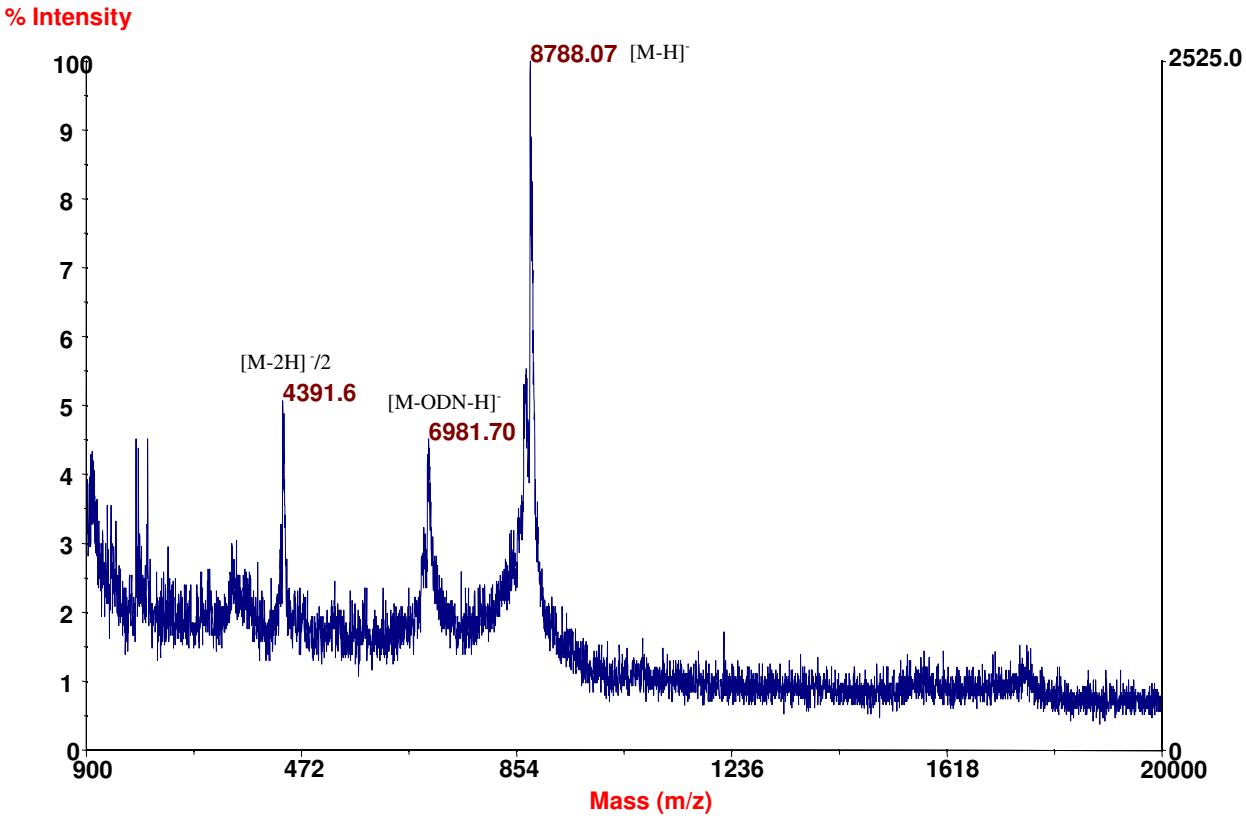


Figure S23: MALDI-ToF mass spectrum of conjugate **1** (m/z calcd: 8786.6).

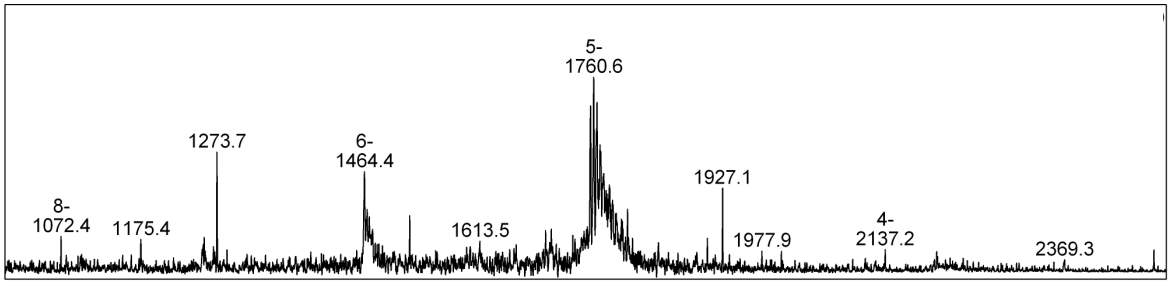


Figure S24: ESI mass spectrum of compound **1** (m/z calcd: 8787.6)

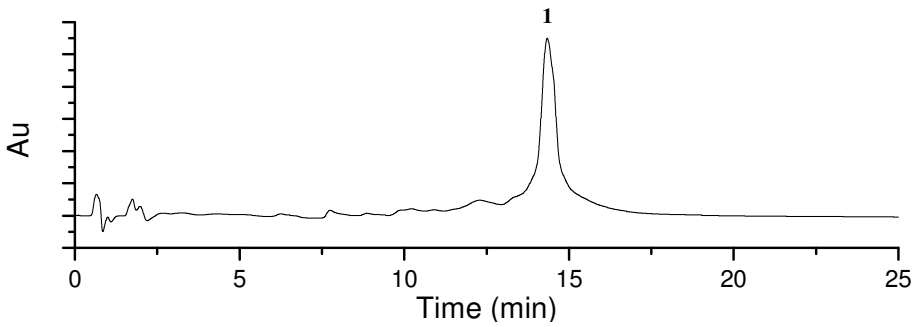


Figure S25: SAX-HPLC of purified compound **1** (λ_{abs}=260nm).

Circular Dichroism studies:

Compounds **1** was annealed by heating at 90°C for 5 min in PBS buffer (10 mM phosphate buffer containing 0.137 M KCl and 0.003 M NaCl, pH adjusted at pH 4,pH 5, pH 6,pH 7 and pH 8 by HCl or NaCl) and cooling slowly at room temperature. A control was carried with oligonucleotide **7** in the same conditions.

Spectra were recorded on a Jasco J-810 circular dichroism spectropolarimeter using 0.1 mm or 1 cm length quartz cuvette. Spectra were recorded every 5°C in a range from 5 to 80°C with a wavelength range from 200 to 340 nm (only 220 to 340 nm was shown). For each temperature, the spectrum was an average of three scans with a 0.5 s response time, a 1 nm data pitch, a 4 nm bandwidth and a 200 $nm.min^{-1}$ scanning speed. Blank spectra of buffer were subtracted for each measure. Measures were obtained with a concentration of 7.5 µM for **1** and with a concentration of 30 µM for **7**. Melting temperatures were obtained using Boltzmann fit on Origin software. Each curve fit was only accepted with a r_{value}>0.99.

pH	**7**	**1**
4	44°C	57.0°C
5	43°C	56°C
6	Nd.	47°C
7	Nd.	24°C

Table 1: Melting temperature of templated system **1** and intermolecular *i*-motif **7** depending of the pH.

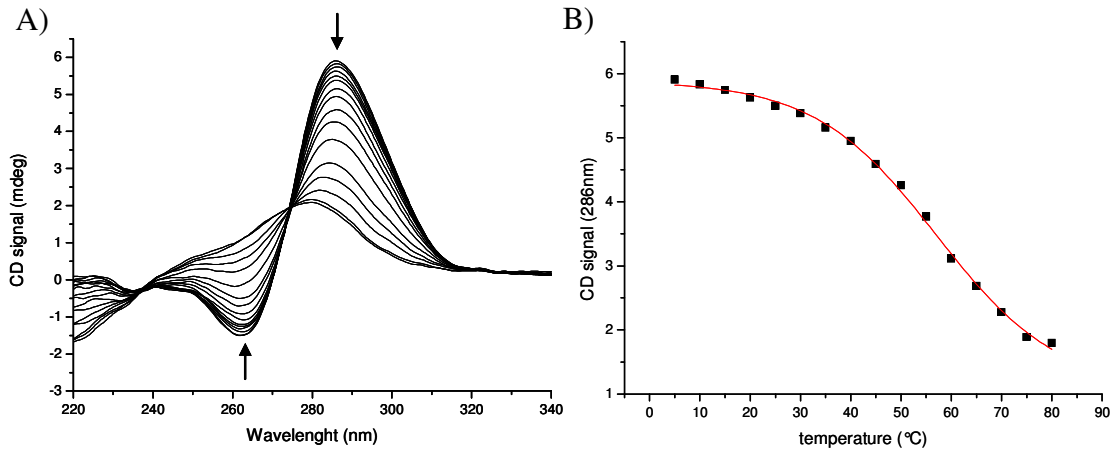


Figure S26: CD analyses of **1** at pH 4 from 5°C to 80°C (7.5 µM in PBS buffer): A) superposition of CD spectra. Arrows indicate the sense of the signal decrease. B) CD melting curve at 286 nm (▪: experimental results, curve: Boltzmann fit)

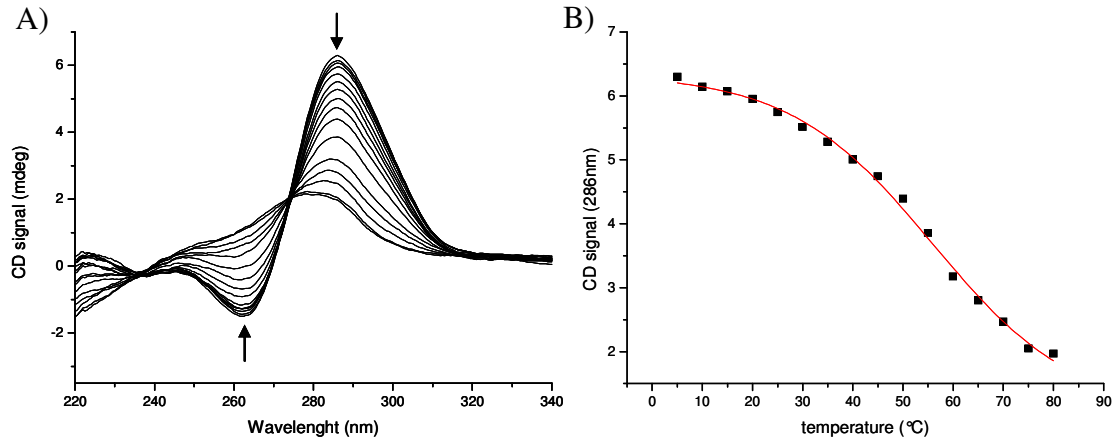


Figure S27: CD analyses of **1** at pH 5 from 5°C to 80°C (7.5 µM in PBS buffer): A) superposition of CD spectra. Arrows indicate the sense of the signal decrease. B) CD melting curve at 286 nm (▪: experimental results, curve: Boltzmann fit)

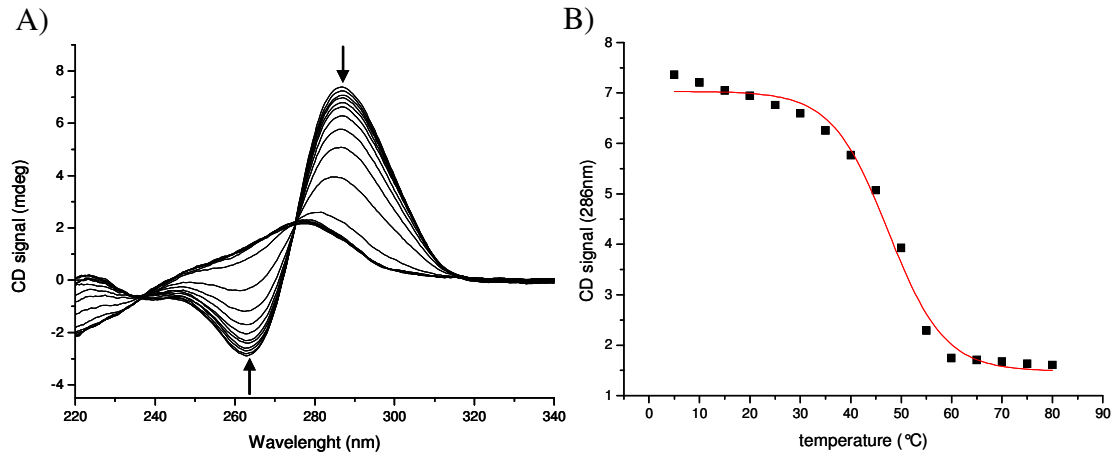


Figure S28: CD analyses of **1** at pH 6 from 5°C to 80°C (7.5 µM in PBS buffer): A) superposition of CD spectra. Arrows indicate the sense of the signal decrease. B) CD melting curve at 286 nm (▪: experimental results, curve: Boltzmann fit)

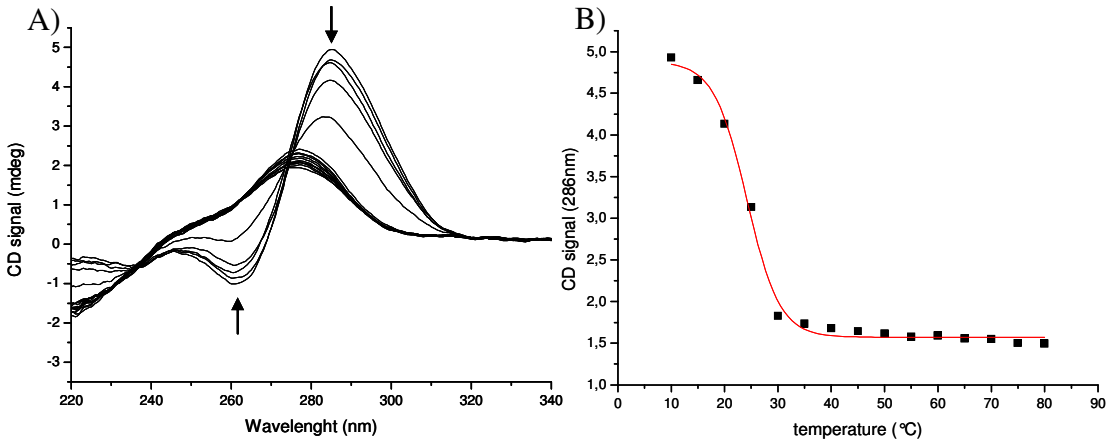


Figure S29: CD analyses of **1** at pH 7 from 5°C to 80°C (7.5 µM in PBS buffer): A) superposition of CD spectra. Arrows indicate the sense of the signal decrease. B) CD melting curve at 286 nm (▪: experimental results, curve: Boltzmann fit).

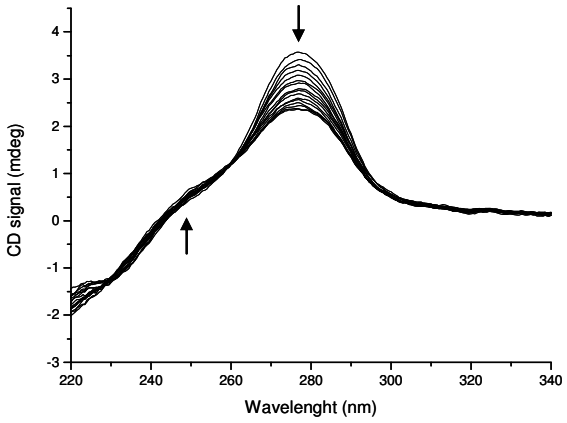


Figure S30: CD analyses of **1** at pH 8 from 5°C to 80°C (7.5 µM in PBS buffer). Superposition of CD spectra. Arrows indicate the sense of the signal decrease.

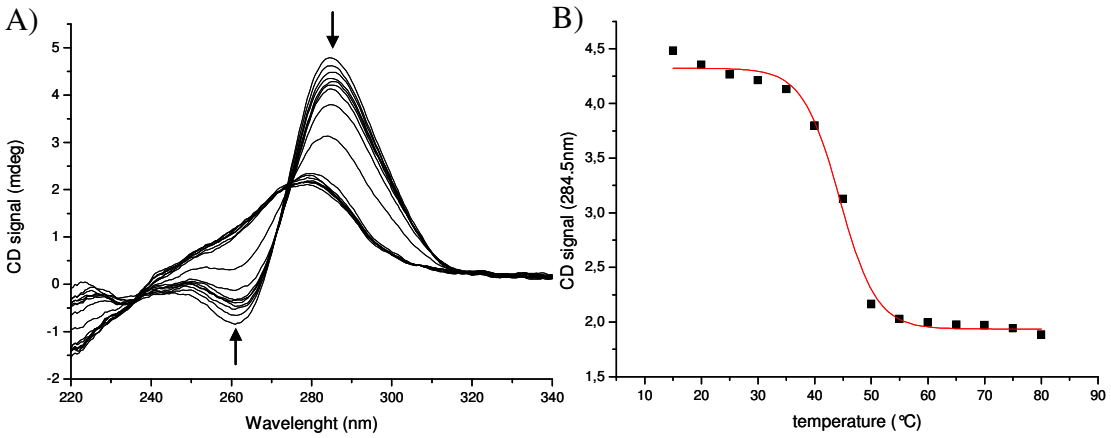


Figure S31: CD analyses of **7** at pH 4 from 5°C to 80°C (30 µM in PBS buffer): A) superposition of CD spectra. Arrows indicate the sense of the signal decrease. B) CD melting curve at 285 nm (▪: experimental results, curve: Boltzmann fit).

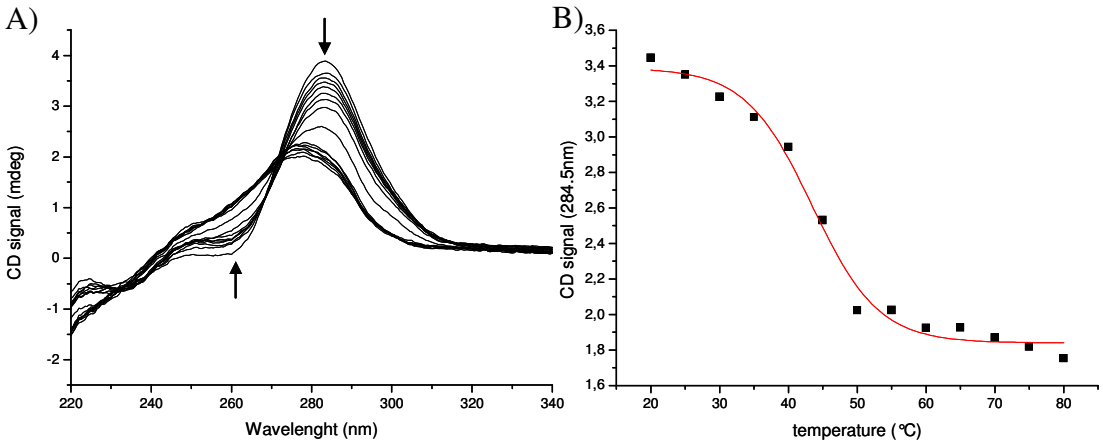


Figure S32: CD analyses of **7** at pH 5 from 5°C to 80°C (30 µM in PBS buffer): A) superposition of CD spectra. Arrows indicate the sense of the signal decrease. B) CD melting curve at 285 nm (▪: experimental results, curve: Boltzmann fit).

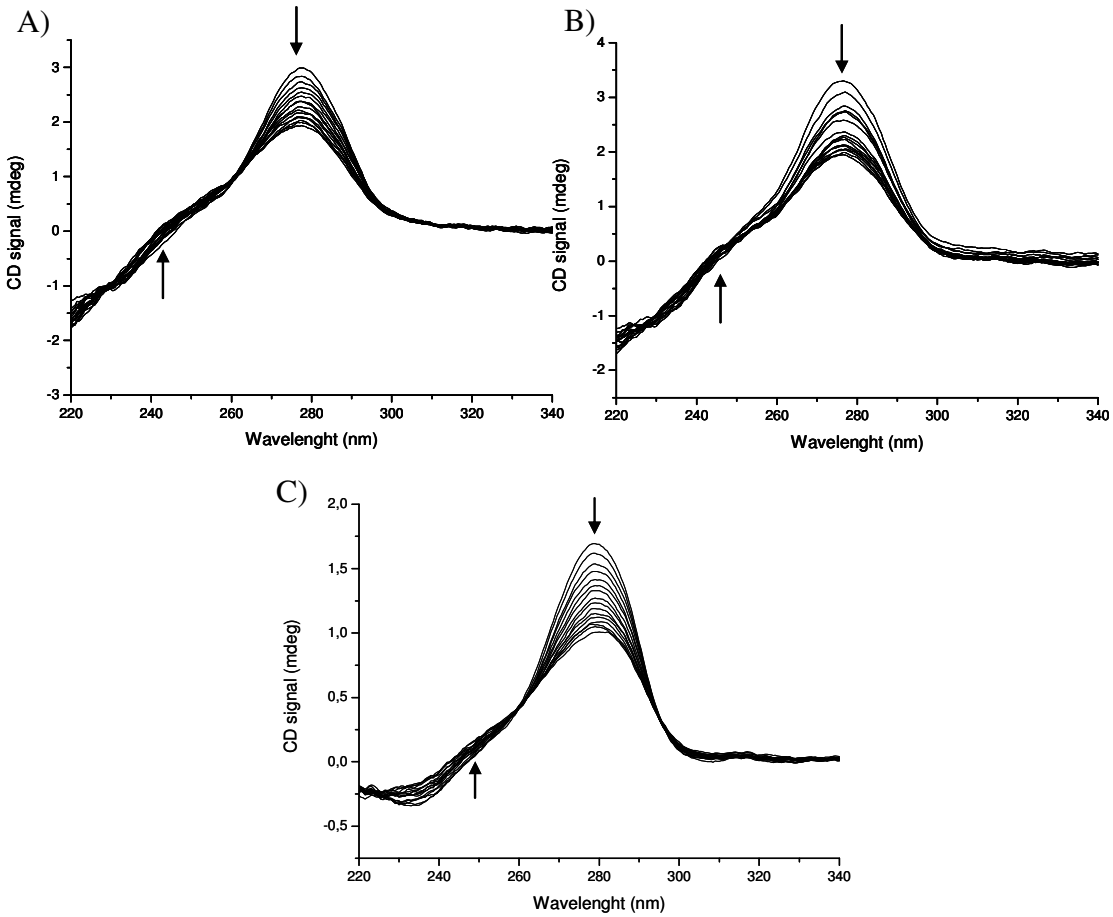


Figure S33: CD analyses of **7** at pH 6 (A), pH 7 (B) and pH 8 (C) from 5°C to 80°C (30 µM in PBS buffer). This is the superposition of CD spectra. Arrows indicate the sense of the signal decrease. Any melting curves can not be obtained at 285 nm.

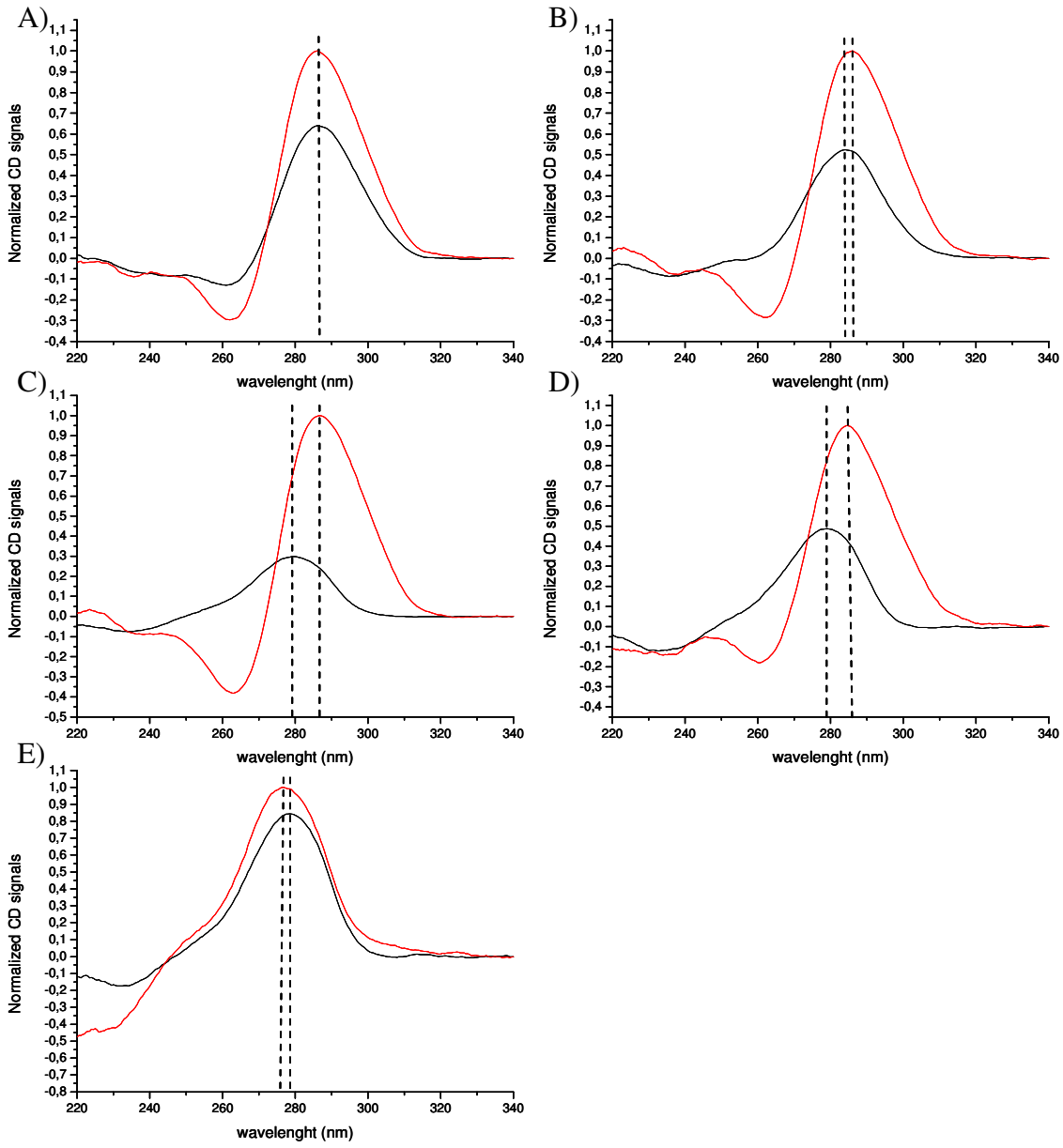


Figure S34: Comparison of normalized CD spectra at 5°C of **1** (7.5 µM in PBS buffer, in red) and **7** (30 µM in PBS buffer, in black): A) pH 4, B) pH 5, C) pH 6, D) pH 7, E) pH 8.

NMR Studies:

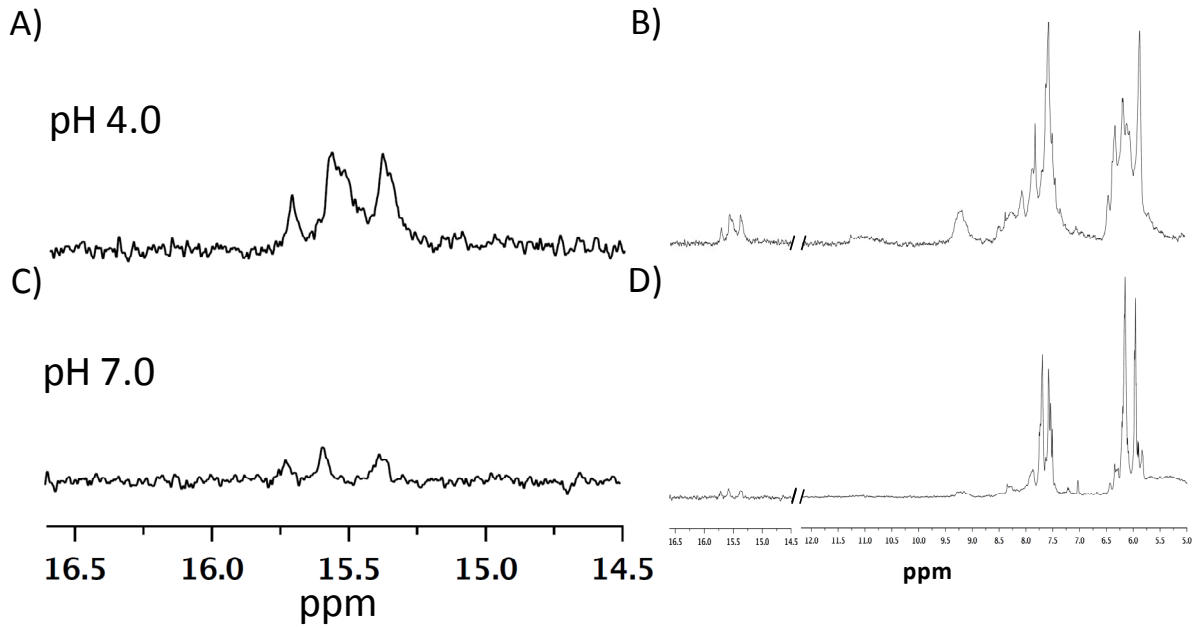


Figure S35: NMR spectra of **1** at pH 4 (A, B) and **7** (C,D): A) zoom of imino region, B) full spectrum, C) zoom of imino region, D) full spectrum. NMR spectra were recorded at 500 MHz in 10 mM PBS buffer containing 100 mM NaCl.