

## Supporting Information

### Construction of anti-parallel G-quadruplexes through sequential templated click†

Romarc Bonnet,<sup>‡</sup> Thomas Lavergne,<sup>‡\*</sup> Béatrice Gennaro, Nicolas Spinelli and Eric Defrancq<sup>\*</sup>

*Département de Chimie Moléculaire, Université Grenoble Alpes, CNRS UMR 5250, Grenoble, France. E-mail: thomas.lavergne@ujf-grenoble.fr, eric.defrancq@ujf-grenoble.fr.*

Abbreviations: .....	2
General details:.....	2
Peptide Synthesis: .....	3
Oligonucleotide and conjugates synthesis and characterisations: .....	7
Oligonucleotide Synthesis and characterisation:.....	7
Oxime ligations .....	9
CuAAC reactions .....	12
Methoxyamine treatment.....	17
Circular Dichroism Studies .....	21
NMR Experiments.....	24
Bibliography.....	26

## Abbreviations:

AELC: Anion Exchange Liquid Chromatography; CD: Circular Dichroism; CuAAC: Copper Catalysed Alkyne-Azide Cycloaddition; DIEA: Diisopropylethylamine; DMF: Dimethylformamide; DMT: Dimethoxytrityl ether; EDTA: Ethylenediaminetetraacetic acid; ESI-MS: Electrospray Ionisation Mass Spectrometry; Fmoc: Fluorenylmethyloxycarbonyl; MALDI: Matrix Assisted Laser Desorption Ionisation; NMR: Nuclear Magnetic Resonance; PyBOP: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; RP-HPLC: Reverse-Phase High Performance Liquid Chromatography; TFA: Trifluoroacetic Acid; THPTA: Tris-(hydroxypropyltriazolylmethyl)amine; TIS: Triisopropylsilane; TNBS: 2,4,6-trinitrobenzenesulfonic acid; TRIS: 2-Amino-2-hydroxymethyl-propane-1,3-diol; UV: Ultra Violet.

## General details:

ESI mass spectra were performed on an Esquire 3000 spectrometer from Bruker or on an Acquity UPLC/MS system from Waters equipped with a SQ Detector 2. NMR spectra were obtained with an Avance III Bruker spectrometer. MALDI-ToF mass spectra were performed on an Autoflex Bruker using hydropiccolinic acid (HPA, 45 mg; ammonium citrate 4 mg in 500  $\mu$ L H<sub>2</sub>O/CH<sub>3</sub>CN) as matrix. Peptides were analysed in positive mode and oligonucleotide and conjugates in negative mode. All solvents and reagents used were of highest purity commercially available.

# Peptide Synthesis:

## General details for peptide synthesis:

The course of reactions were monitored on RP-HPLC on Waters HPLC system on a Nucleosil C18 column (Macherey Nagel, 300 Å, 125x 3 mm, 5 µm) with UV monitoring at 214 nm and 250 nm using 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 in 20 minutes.

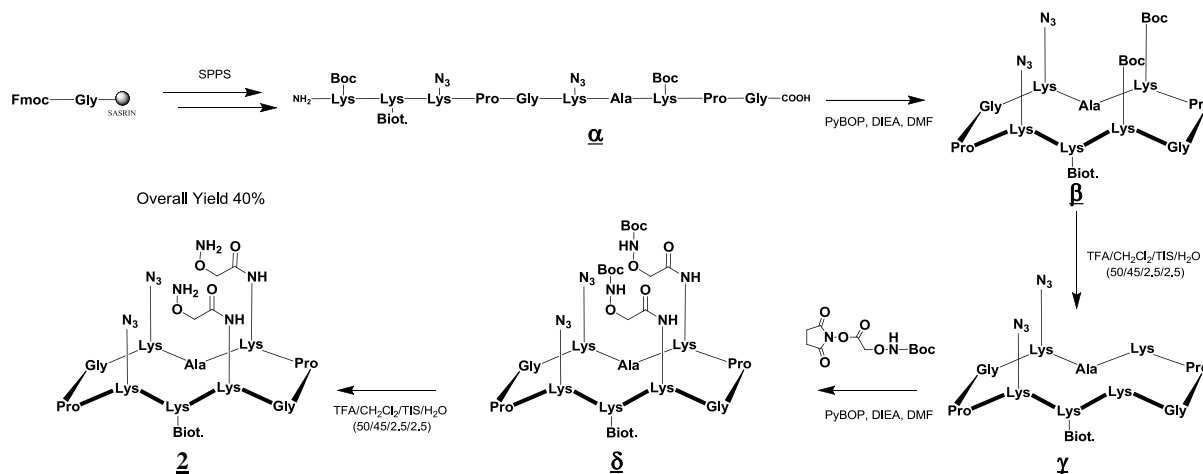
Purifications were performed on a Waters RP-HPLC on a Nucleosil C18 (Macherey Nagel, 300 Å, 250x 21mm, 7 µm ) with UV monitoring at 214 nm and 250 nm using 22 mL/ min flow linear gradient from 95% solvent A and 5% solvent B to 100% solvent B in 30 min.

## Synthesis of peptide scaffold 1

1 has been synthesized according to previously reported procedures.<sup>1</sup>

## Synthesis and characterization of peptide scaffold 2

2 has been synthesized according to Scheme S1.



Scheme S1. Synthesis of scaffold 2

**a. Linear peptide  $\alpha$ :**

Peptide  $\alpha$  was synthesized using Fmoc-tBu protocol using Fmoc-Gly-SASRIN<sup>®</sup> (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 and Fmoc-Ala-OH Fmoc-Pro-OH and Fmoc-Lys(Boc)-OH were commercially available. Fmoc-aminonorleucine was obtained using the reported protocol.<sup>2</sup> The following protocol was used for each amino acid coupling:

Fmoc protecting group was removed using three washing (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) of DMF (10 mL) with of PyBOP (2 eq) as activator. pH was adjusted to 8-9 with DIEA. The completion of the coupling reaction was analysed using TNBS test after washing the resin with DMF (2x 10 mL) and dichloromethane (10 mL). Deprotection and coupling reactions were performed toward supported  $\alpha$ .

The resin was treated with a 1% Trifluoroacetic acid in dichloromethane solution (10x 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. The crude product was used without any further purification. The yield was considered as quantitative.  $t_r$ = 13.6 min.

ESI MS(+) m/z calcd for C<sub>67</sub>H<sub>113</sub>N<sub>21</sub>O<sub>17</sub>S: 1516.8; found: 1517.8 [M+H]<sup>+</sup>.

**b. Cyclic Peptide  $\beta$ :**

Peptide  $\alpha$  (0.5 mmol, 760 mg) was dissolved in DMF (500 mL) and PyBOP (2 eq; 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. The crude product was used without any further purification. The yield was considered as quantitative.  $t_r$ = 12.6 min.

ESI MS(+) m/z calcd for C<sub>67</sub>H<sub>111</sub>N<sub>21</sub>O<sub>16</sub>S: 1498.8; found: 1499.0 [M+H]<sup>+</sup>.

**c. N-free peptide  $\gamma$ :**

Peptide  $\beta$  (0.5 mmol; 750 mg) was treated with a TFA/DCM/H<sub>2</sub>O/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. The crude product was used without any further purification.  $t_r = 9.6$  min

ESI MS(+) m/z calcd for C<sub>57</sub>H<sub>95</sub>N<sub>21</sub>O<sub>12</sub>S: 1298.6; found: 1299.8 [M+H]<sup>+</sup>.

**d. Protected aminoxy peptide  $\delta$ :**

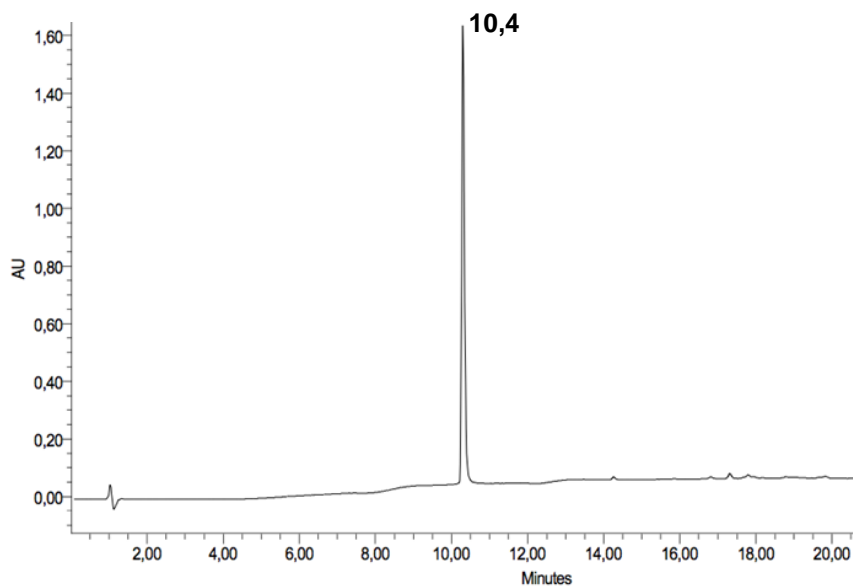
Peptide  $\gamma$  (0.5 mmol; 650 mg) was dissolved in anhydrous DMF (100 mL). *t*-Boc-aminoxyacetic Acid N-Hydroxysuccinimide Ester<sup>3</sup> (2.5 eq; 1.25 mmol; 360 mg) was then added. pH was adjusted to 8-9 with DIEA. The solution was stirred at room temperature and monitored by RP-HPLC until the completion of the reaction (2h). The solvent was evaporated and the peptide was precipitated with ether. The crude product was used without any further purification.  $t_r = 12.6$  min.

ESI MS(+) m/z calcd for C<sub>71</sub>H<sub>117</sub>N<sub>23</sub>O<sub>20</sub>S: 1645.0; found: 1646.0 [M+H]<sup>+</sup>

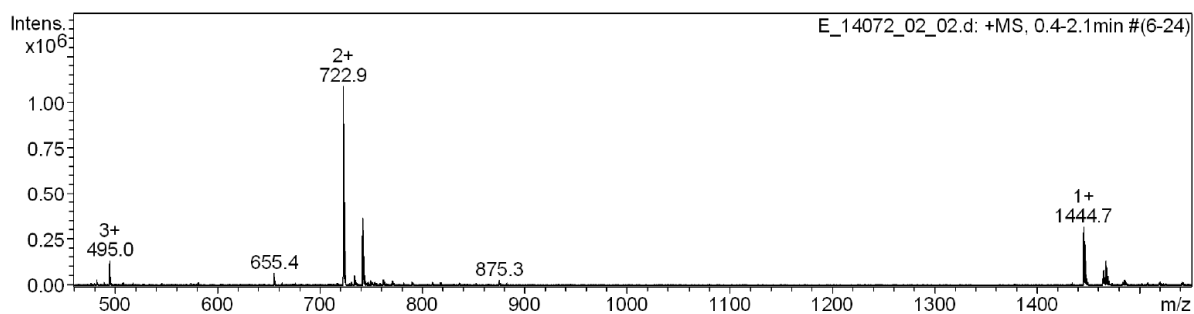
**e. Peptide scaffold  $\underline{2}$ :**

Peptide  $\delta$  (12  $\mu$ mol; 20 mg) was dissolved in a TFA/DCM/H<sub>2</sub>O/TIS (50/45/2.5/2.5) solution (10 mL) and the reaction was stirred one hour at room temperature. The peptide was precipitated with ether. The product was then purified on RP-HPLC and freeze dried into a white powder. (4.8  $\mu$ mol; 7 mg; yield: 40%).  $t_r = 10.4$  min.

ESI MS(+) m/z calcd for C<sub>61</sub>H<sub>101</sub>N<sub>23</sub>O<sub>16</sub>S: 1444.7; found: 1444.7 [M+H]<sup>+</sup>.



**Figure S1:** RP-HPLC chromatogram of purified compound 2



**Figure S2:** ESI mass spectrum of compound 2

## Oligonucleotide and conjugates synthesis and characterisations:

### General details:

RP-HPLC analyses were performed on a Waters HPLC system using C18 Nucleosil column (Macherey-Nagel, 250x 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' in 30 minutes.

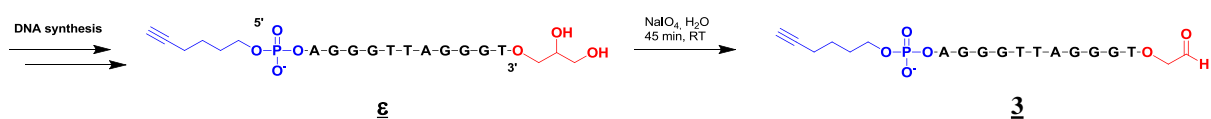
The RP-HPLC purifications of oligonucleotides were performed on a Gilson system with Nucleosil C-18 column (Macherey-Nagel 250 mm x 10 mm, 100 Å, 7 µm) using 4 mL/min flow linear gradients with solvent A' and B' with UV-monitoring at 260 nm and 280 nm.

Desalting of oligonucleotide was performed on size exclusion cartridge NAP 25 from GE Healthcare using the recommended protocol.

AELC analyses were performed on a Dionex column (DNAPac PA-100, 9 x 250 mm) at 75°C with monitoring at 260 nm and 280 nm with linear gradient of solvent A'' (Tris buffer 25 mM pH 7 with 5% acetonitrile) and solvent B'' (Tris buffer 25 mM, 0,4 M LiClO<sub>4</sub> with 5% acetonitrile).

Quantification of oligonucleotides is performed at 260 nm using CARY 400 Scan UV-Visible Spectrometer (Absorbance are estimated according to the nearest neighbour model).

### Oligonucleotide Synthesis and characterisation:



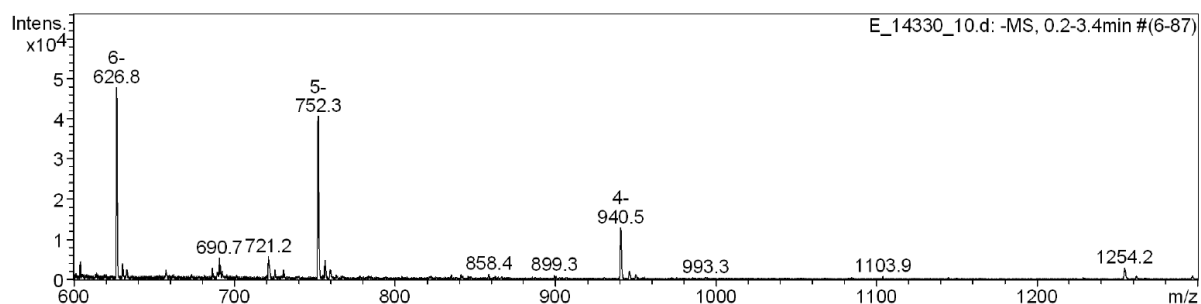
Scheme 2. Synthesis of 3'-aldehyde oligonucleotide **3**

#### 1. 3'-diol-5'-alkyne oligonucleotide **2**

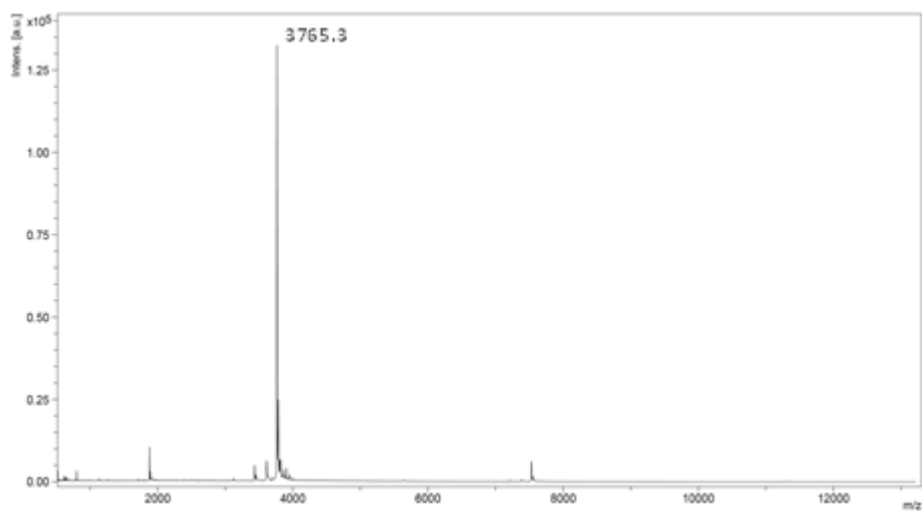
Oligonucleotide **2** was obtained from automated synthesis on a 3'-glyceryl CPG resin at 1 µmol scale using a 3400 DNA synthesizer from Applied Biosystems. The last coupling was carried using commercially available 5' hexynyl (β-cyanoethyl) phosphoramidite (GlenResearch). After synthesis, cyanoethyl protecting groups were removed using 20% piperidine in acetonitrile. Cleavage from the resin and deprotection was performed in 28% NH<sub>4</sub>OH for 16h at 55°C. The product was purified on RP-HPLC with a gradient from 0% to

30% solvent B' in solvent A' for 20 min. (721 nmol, yield: 72%,  $\epsilon_{260\text{ nm}} = 114800\text{ M}^{-1}\cdot\text{cm}^{-1}$ ).  
 $t_r = 14.7\text{ min}$ .

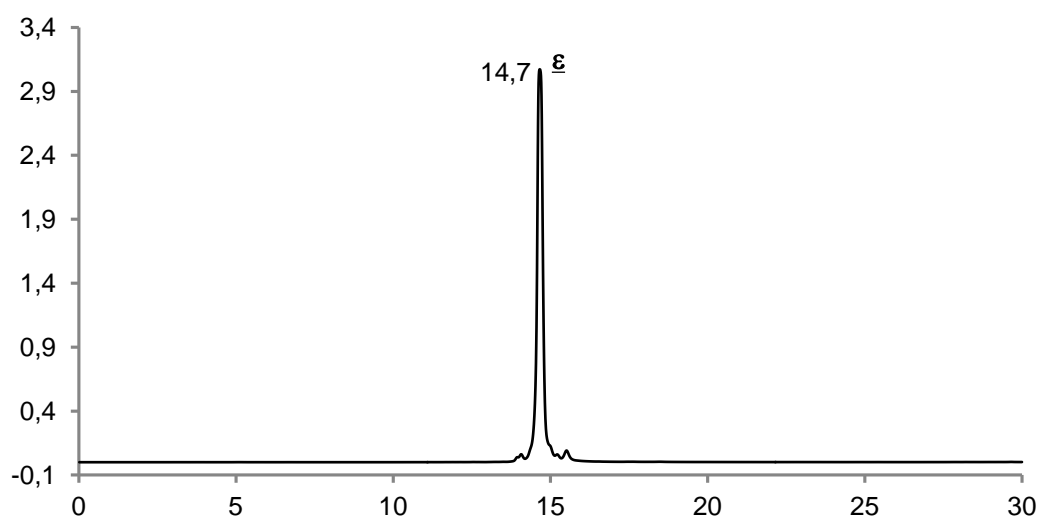
ESI MS(-) m/z calcd for  $\text{C}_{119}\text{H}_{152}\text{N}_{46}\text{O}_{73}\text{P}_{12}$ : 3766.4, found: 3766.6 (ESI)



**Figure S3:** ESI mass spectrum of compound  $\epsilon$



**Figure S4:** MALDI mass spectrum of compound  $\epsilon$



**Figure S5:** RP-HPLC chromatogram of purified compound  $\epsilon$  ( $\lambda_{\text{abs}}=260\text{ nm}$ ).



## 2. 3' aldehyde-5'alkyne oligonucleotide **3**:

Sodium metaperiodate (20 eq; 4.4  $\mu\text{mol}$ ; 942  $\mu\text{g}$ ) was added to a solution of oligonucleotide **12** (1 eq; 220 nmol) in water (220  $\mu\text{L}$ ). The reaction was stirred for 1h at room temperature in dark conditions. The product was then desalted on NAP 25 and the fractions were collected to obtain the crude product (UV-monitored at 260 nm). The oxidation was considered quantitative and the crude containing oligonucleotide **3** was used in the next step without further purification.

## Oxime ligations

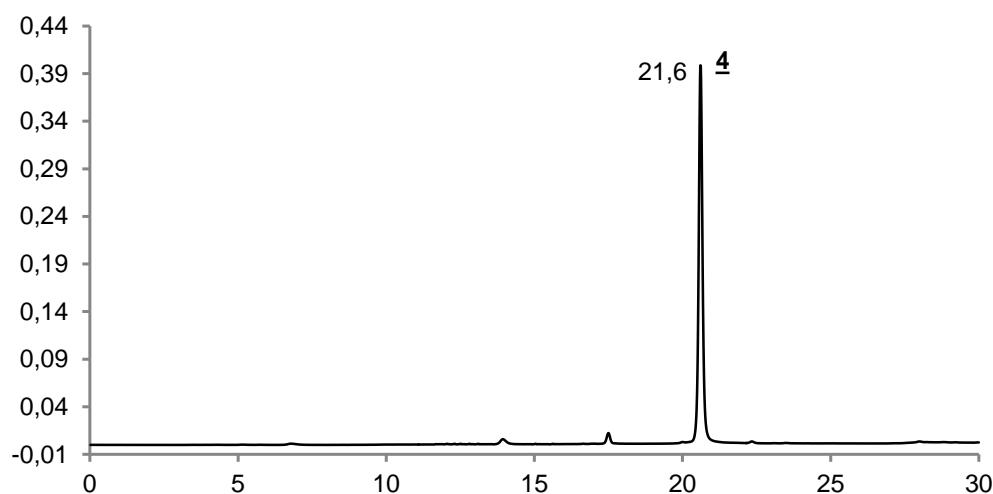
### General procedure

Aldehyde oligonucleotide **3** (1.2 eq by oxyamine function) was dissolved in 0.4 M ammonium acetate buffer (pH 4.5, concentration  $10^{-3}$  M) and free aminoxy peptide was added. The solution was stirred at 50°C for 30 min then the crude was purified on RP-HPLC with a gradient from 0% to 45% solvent B' in solvent A' for 20 min.

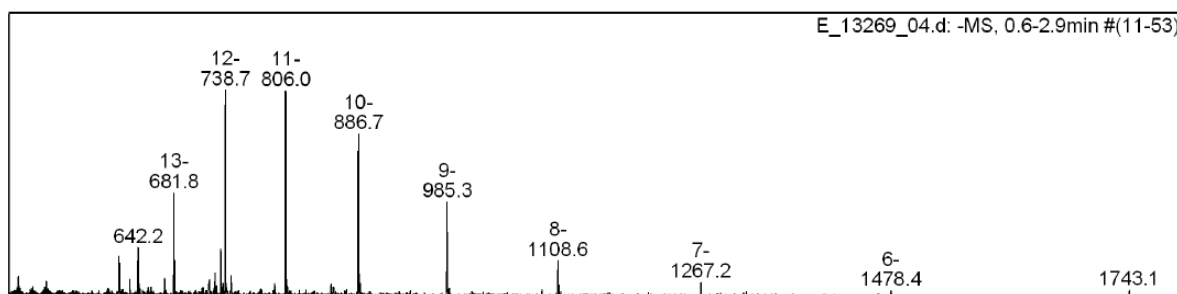
### 1. Synthesis and characterization of **4**

The oxime ligation was carried out with aldehyde (480 nmol) and free aminoxy peptide **2** (200 nmol) according general procedure. After RP-HPLC purification, the conjugate **4** was freeze-dried. Quantification was performed by UV-spectrometry (128 nmol, yield: 64%,  $\epsilon_{260\text{ nm}} = 229600\text{ M}^{-1}\cdot\text{cm}^{-1}$ ).tr = 21.6 min

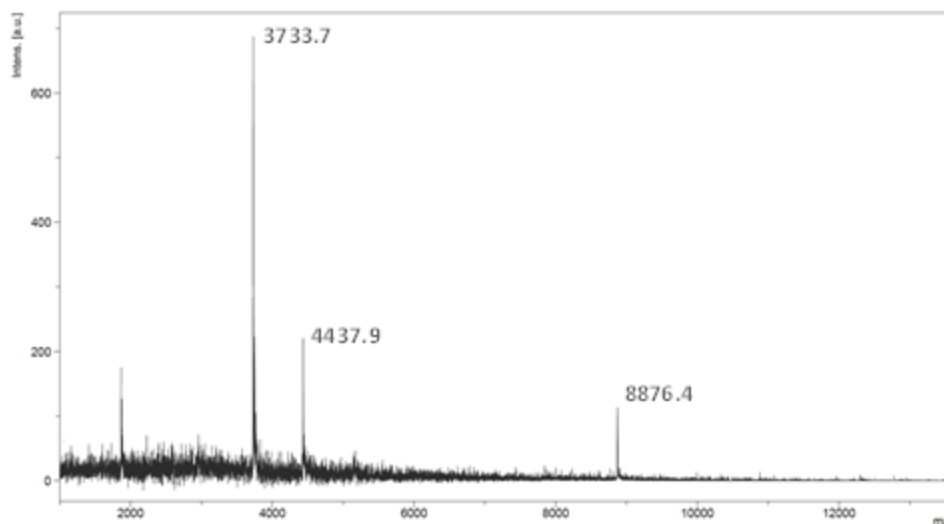
ESI MS(-) m/z calcd for  $\text{C}_{297}\text{H}_{393}\text{N}_{115}\text{O}_{158}\text{P}_{24}\text{S}$ : 8877.4, found: 8877.1 (ESI)



**Figure S6:** RP-HPLC chromatogram of crude compound **4** ( $\lambda_{\text{abs}}=260\text{ nm}$ ).



**Figure S7:** ESI mass spectrum of compound **4**

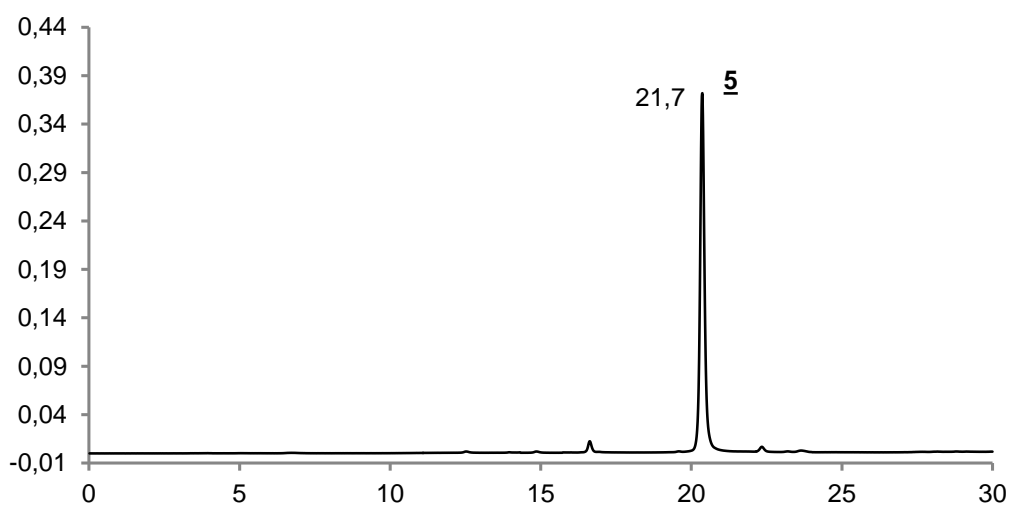


**Figure S8:** MALDI mass spectrum of compound **4**

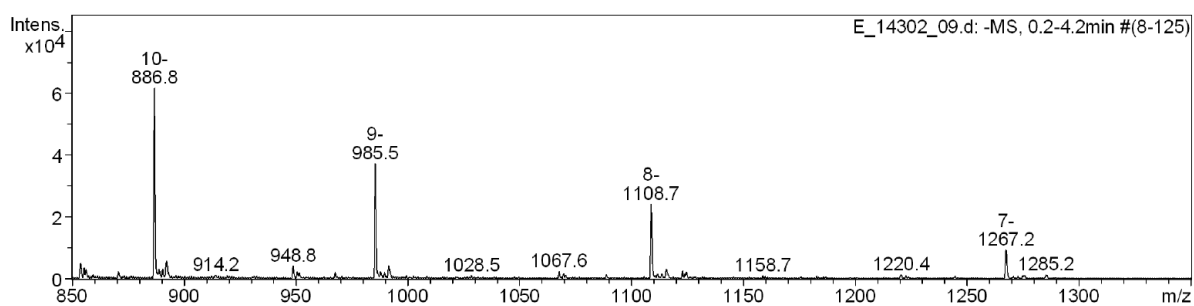
## 2. Synthesis and characterization of **5**

The oxime ligation was carried out with aldehyde **3** (240 nmol) and free aminooxypeptide **1** (100 nmol) according general procedure. After RP-HPLC purification, the conjugate was freeze-dried. Quantification was performed by UV-spectrometry (69 nmol, yield: 69%,  $\epsilon_{260\text{ nm}} = 229600\text{ M}^{-1}\cdot\text{cm}^{-1}$ ).tr = 21.7 min.

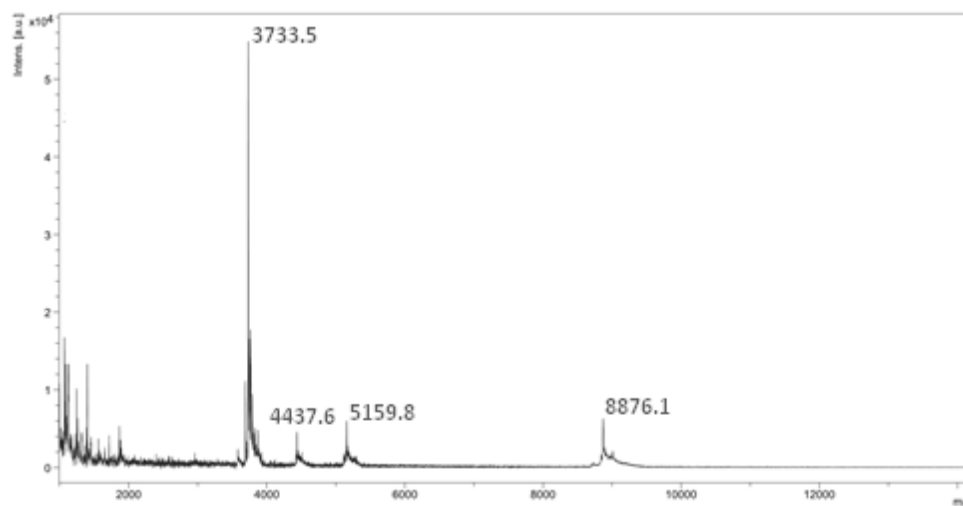
ESI MS(-) m/z calcd for  $\text{C}_{297}\text{H}_{393}\text{N}_{115}\text{O}_{158}\text{P}_{24}\text{S}$ : 8877.4, found: 8878.0 (ESI)



**Figure S9:** RP-HPLC chromatogram of crude compound 5 ( $\lambda_{\text{abs}}=260$  nm).

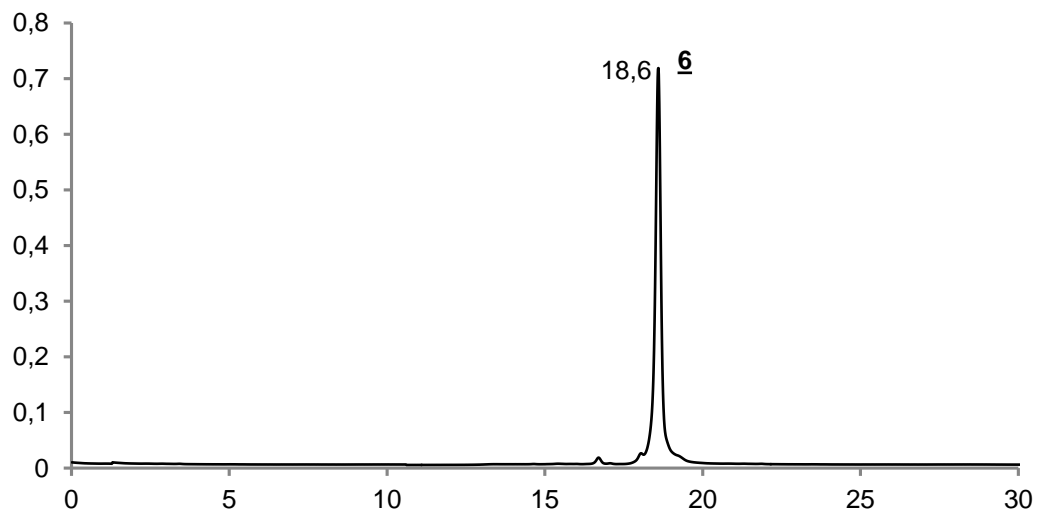


**Figure S10:** ESI mass spectrum of compound 5

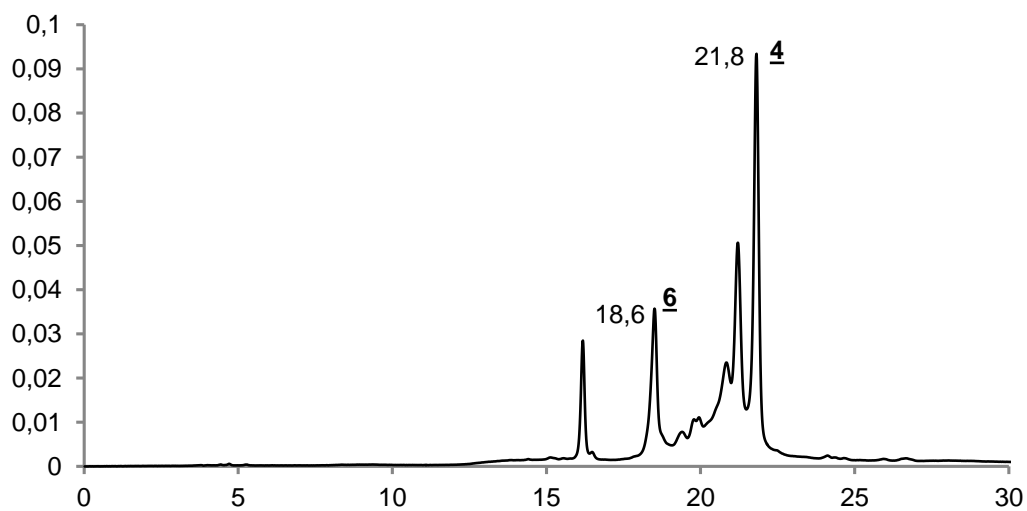


**Figure S11:** MALDI mass spectrum of compound 5

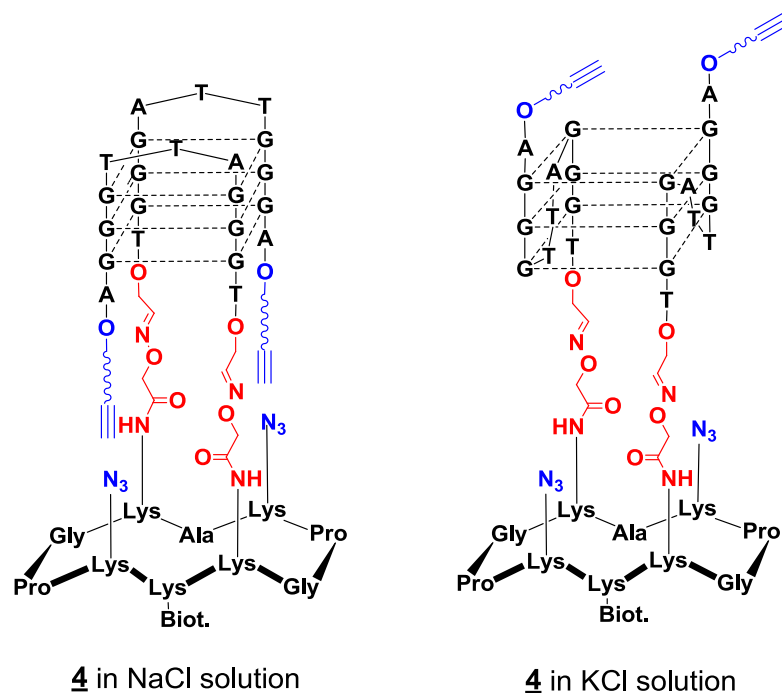
## CuAAC reactions



**Figure S13:** RP-HPLC chromatogram of crude CuAAC reaction on 4 in the presence of 100 mM NaCl



**Figure S14:** RP-HPLC chromatogram of crude CuAAC reaction on 4 in the presence of 100 mM KCl



**Figure S15:** Expected structuration of **4** in the presence of either 100 mM NaCl or 100 mM KCl solution

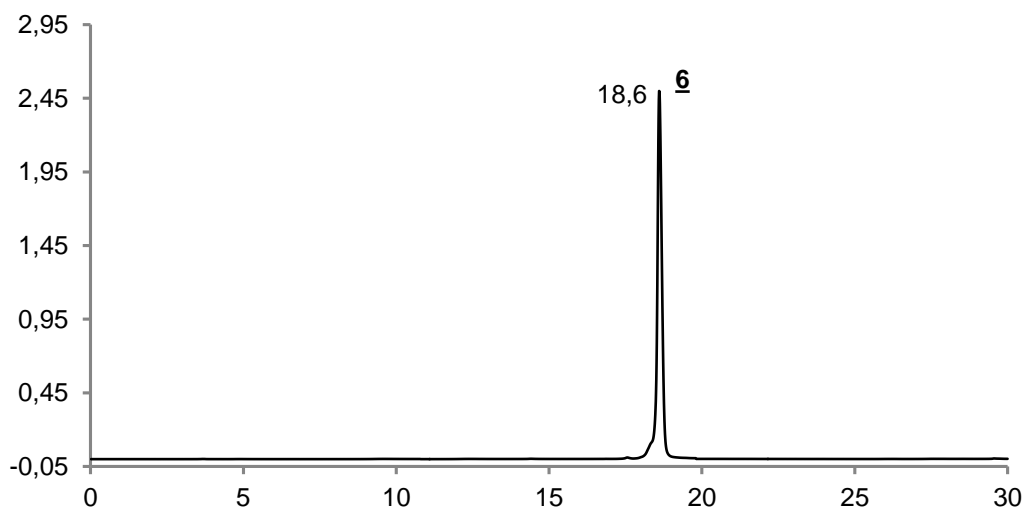
### General procedure

A solution of **4** or **5** (1 eq) at 100  $\mu$ M in 100 mM HEPES buffer (pH 7.4,) and 100 mM NaCl was heated at 90°C for 5 min and slowly cooled to r.t. for 2h. To this solution was added CuSO<sub>4</sub> (2 eq by azido function), THPTA (5 eq by azido function) and sodium ascorbate (10 eq by azido function). The reaction was stirred at room temperature for 3h and quenched with 0.5M EDTA solution (50 eq by azido function).

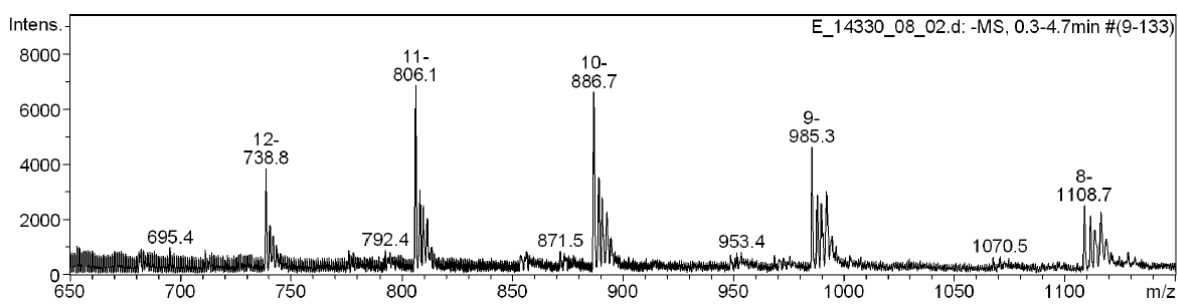
#### 1. Synthesis and characterization of **6**

CuAAc protocol was applied to conjugate **4** (100 nmol) and the resulting solution was desalted by SEC. The product was purified on RP-HPLC with a gradient from 0% to 45% solvent B' in solvent A' for 20 min. (62 nmol, yield: 62%,  $\epsilon_{260\text{ nm}} = 229600\text{ M}^{-1}\cdot\text{cm}^{-1}$ ). tr = 18,6 min.

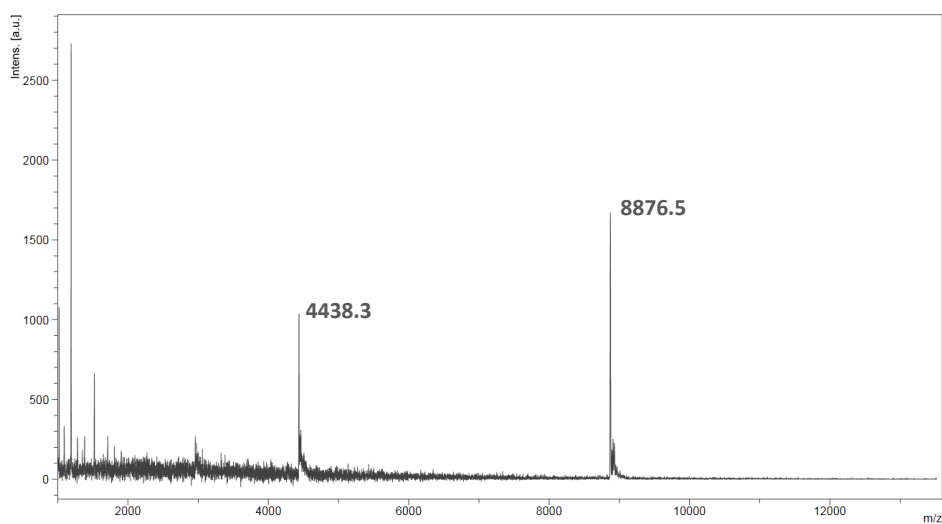
ESI MS(-) m/z calcd for C<sub>297</sub>H<sub>393</sub>N<sub>115</sub>O<sub>158</sub>P<sub>24</sub>S: 8877.4, found: 8877.6 (ESI)



**Figure S16:** RP-HPLC chromatogram of compound 6 ( $\lambda_{\text{abs}}=260$  nm)



**Figure S17:** ESI mass spectrum of compound 6

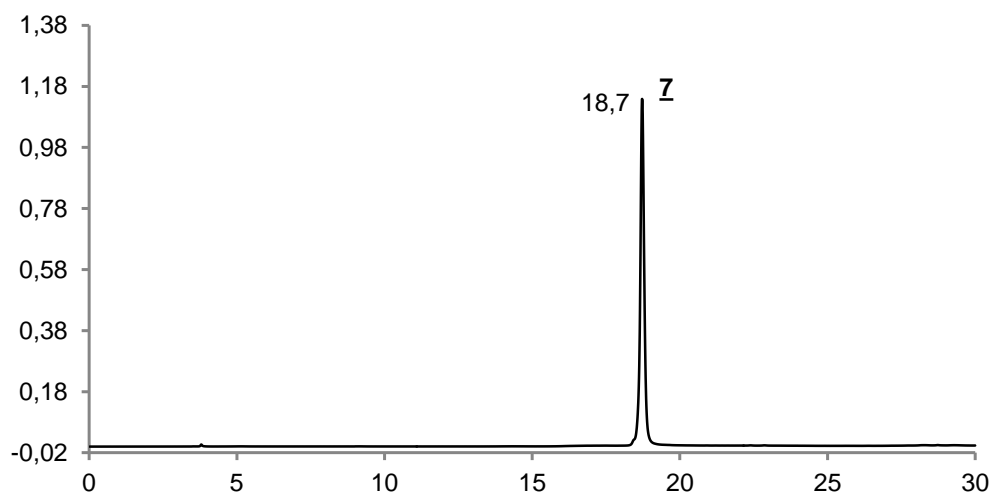


**Figure S18:** MALDI mass spectrum of compound 6

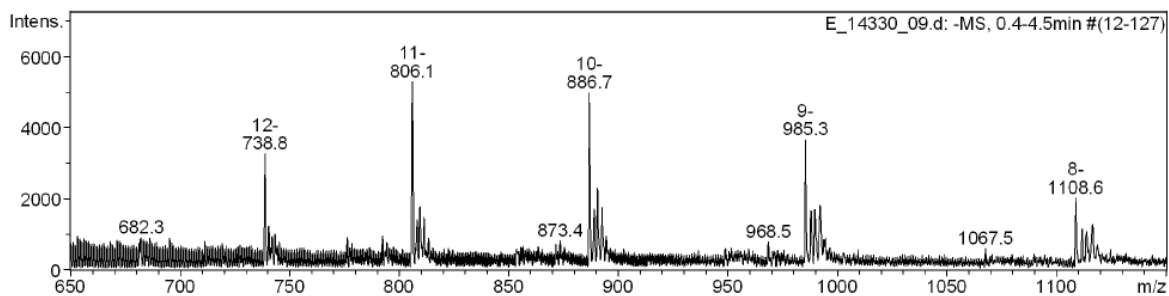
## 2. Synthesis and characterization of 7

CuAAc protocol was applied to conjugate 5 (100 nmol) and the resulting solution was desalted by SEC. The product was purified on RP-HPLC with a gradient from 0% to 45% solvent B' in solvent A' for 20 min. (60 nmol, yield: 60%,  $\epsilon_{260\text{ nm}} = 229600\text{ M}^{-1}\cdot\text{cm}^{-1}$ ).  $t_r = 18.7$  min.

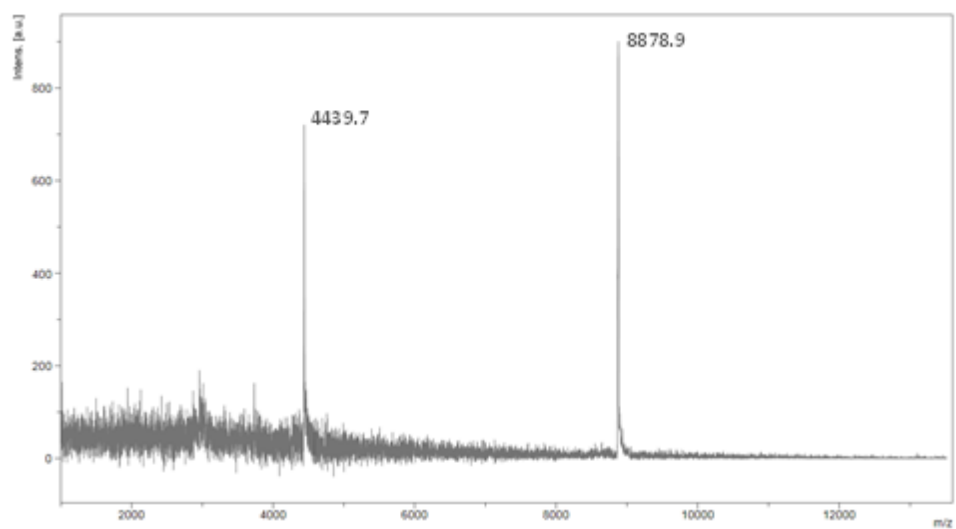
ESI MS(-)  $m/z$  calcd for  $\text{C}_{297}\text{H}_{393}\text{N}_{115}\text{O}_{158}\text{P}_{24}\text{S}$ : 8877.4, found: 8877.7 (ESI)



**Figure S19:** RP-HPLC chromatogram of crude compound 7 ( $\lambda_{\text{abs}}=260\text{ nm}$ )



**Figure S20:** ESI mass spectrum of compound 7



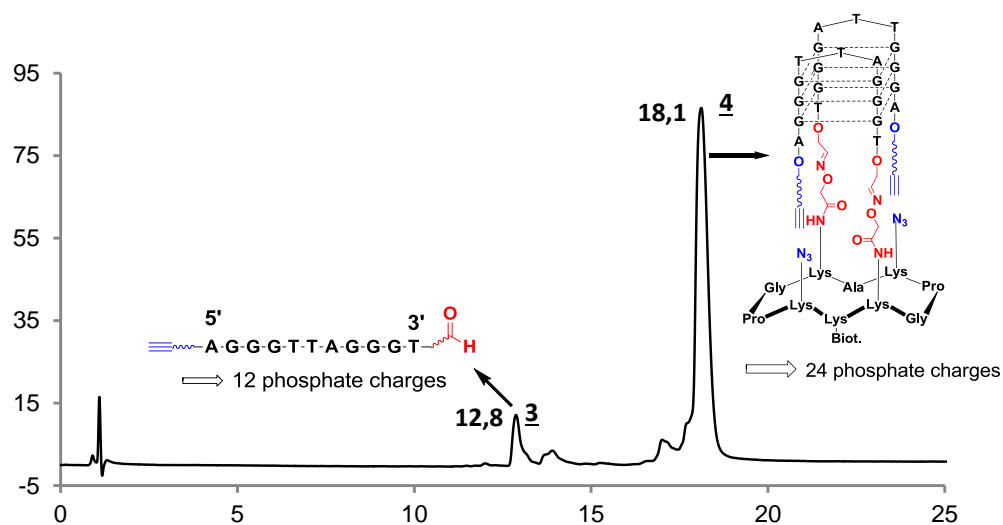
**Figure S21:** MALDI mass spectrum of compound **7**



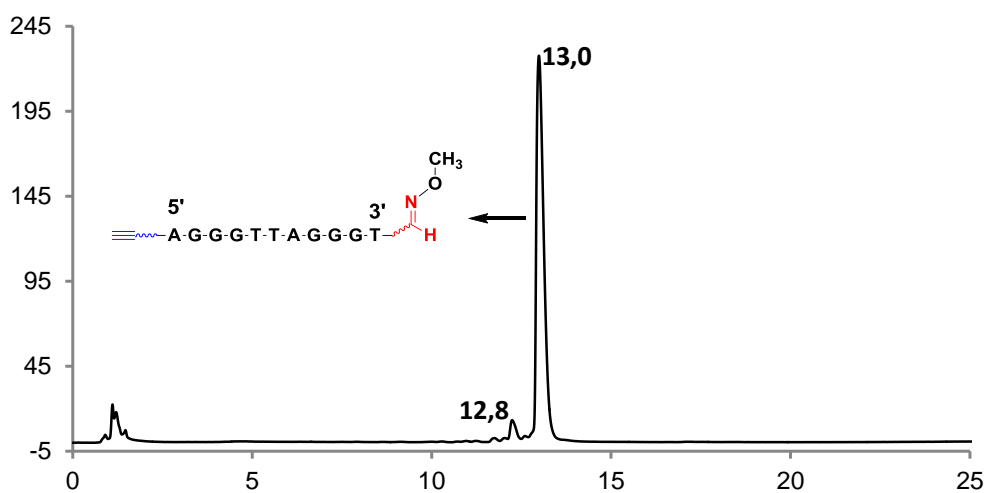
# Methoxyamine treatment

## General procedure

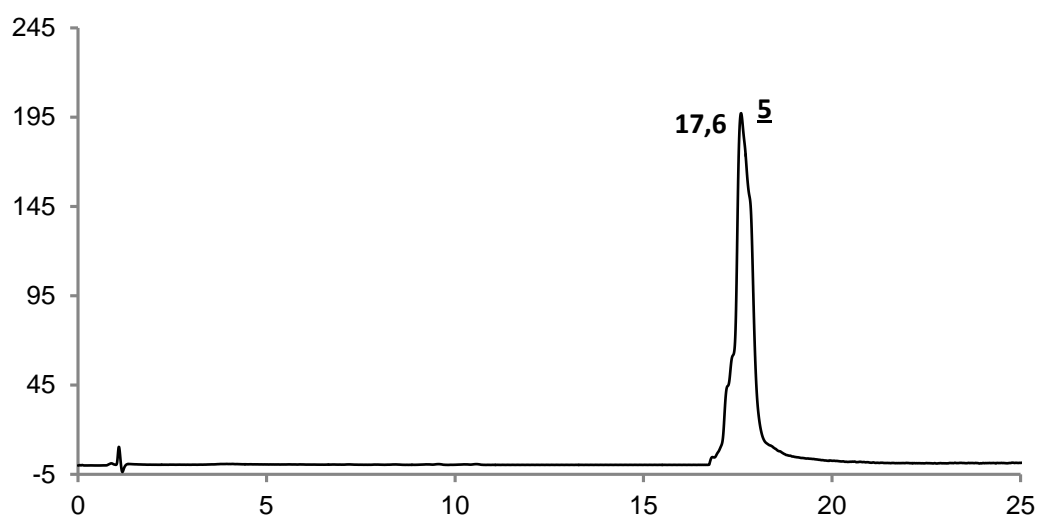
To a solution of crude material **4**, **5**, **6** or **7** (from desalted oxime ligations or CuAAC reactions) (1 eq, 2 nmol) in 0.4 M ammonium acetate buffer (pH 4.5, 25  $\mu$ L) was added 500 eq of methoxyamine (1 mmol). The reaction mixture was heated to 50°C for 1h and the crude was analysed by AELC at 75°C.



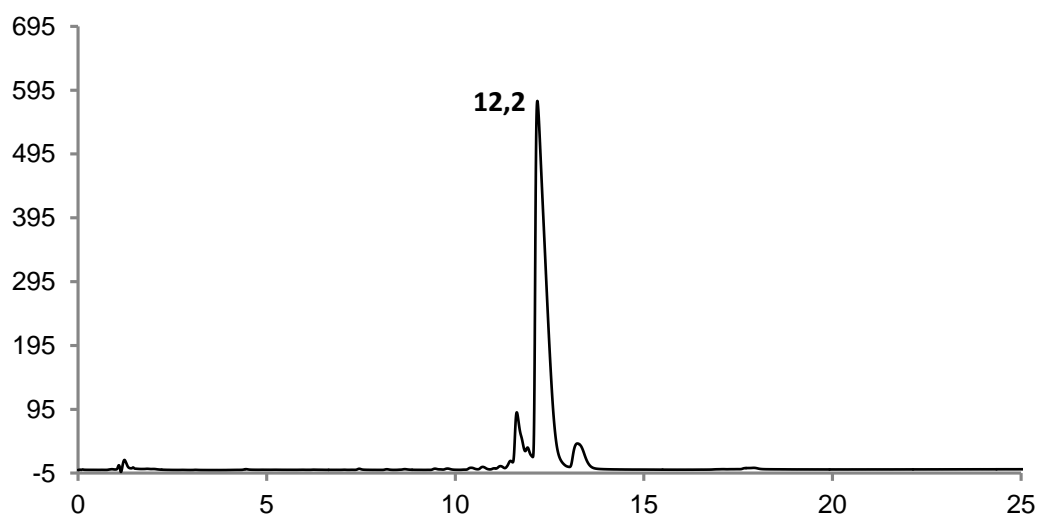
**Figure S22:** AELC chromatogram of crude **4** after oxime ligation



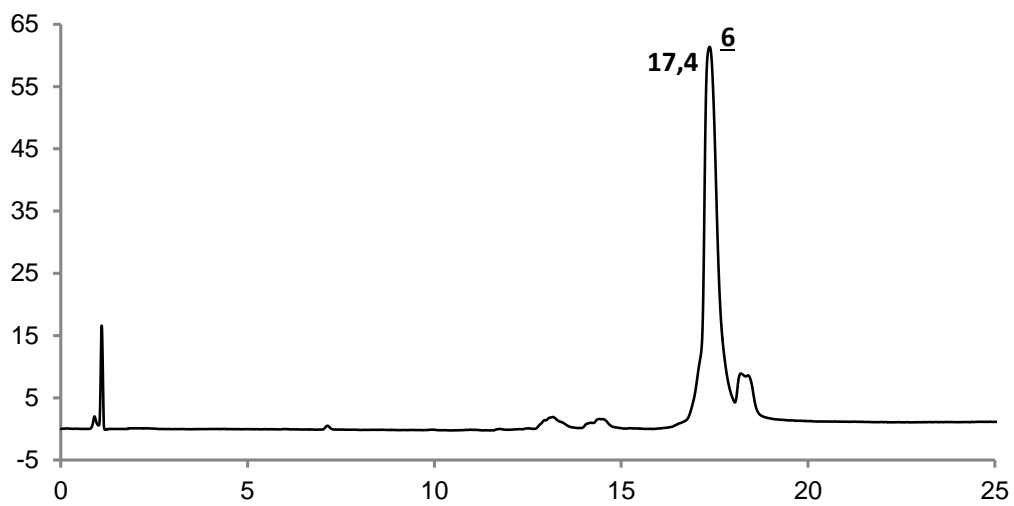
**Figure S23:** AELC chromatogram of crude **4** treated with MeONH<sub>2</sub>



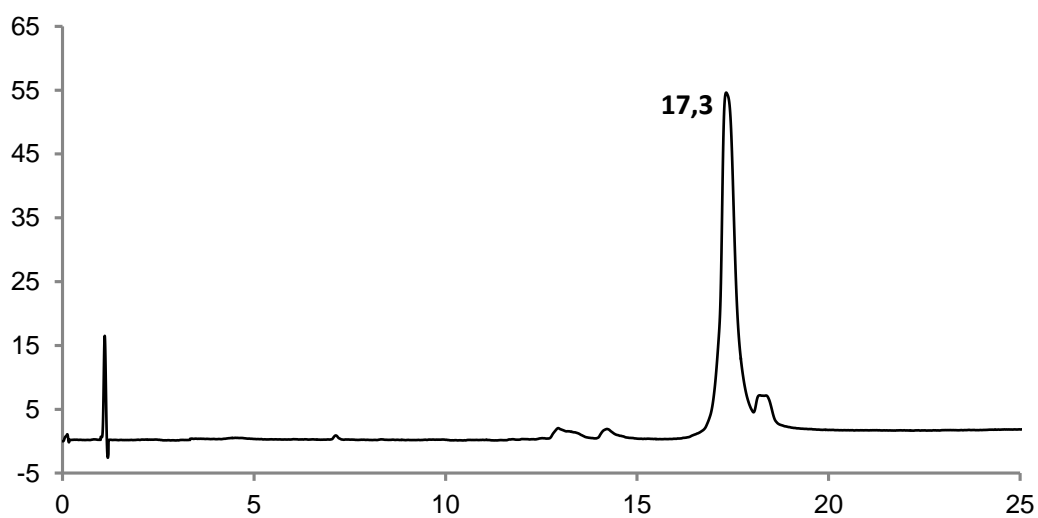
**Figure S24:** AELC chromatogram of crude 5 after oxime ligation



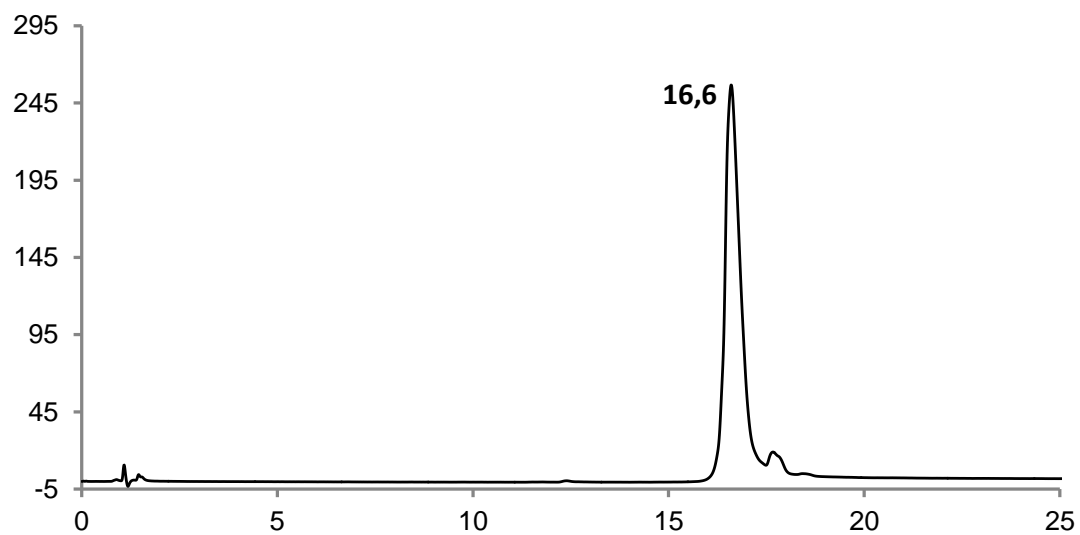
**Figure S25:** AELC chromatogram of crude 5 treated with MeONH<sub>2</sub>



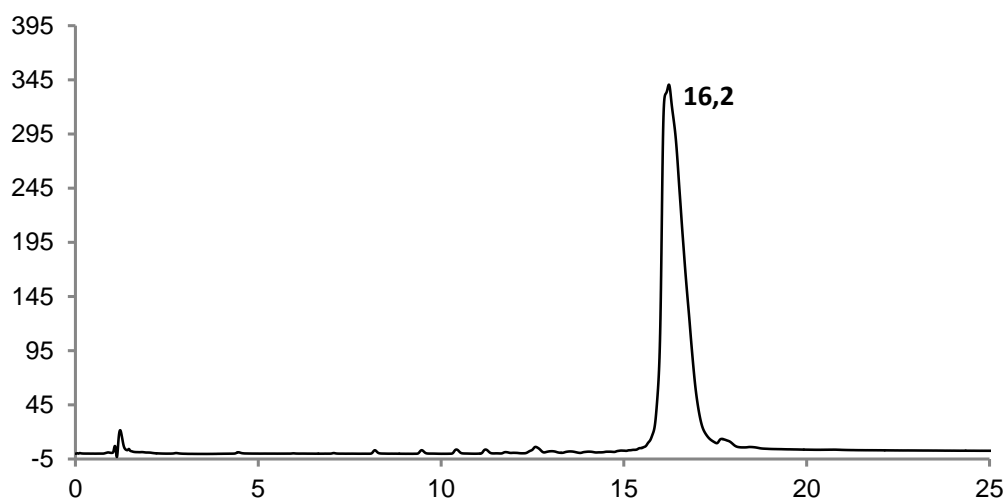
**Figure S26:** AELC chromatogram of crude 6 after CuAAC reaction



**Figure S27:** AELC chromatogram of crude 6 treated with MeONH<sub>2</sub>



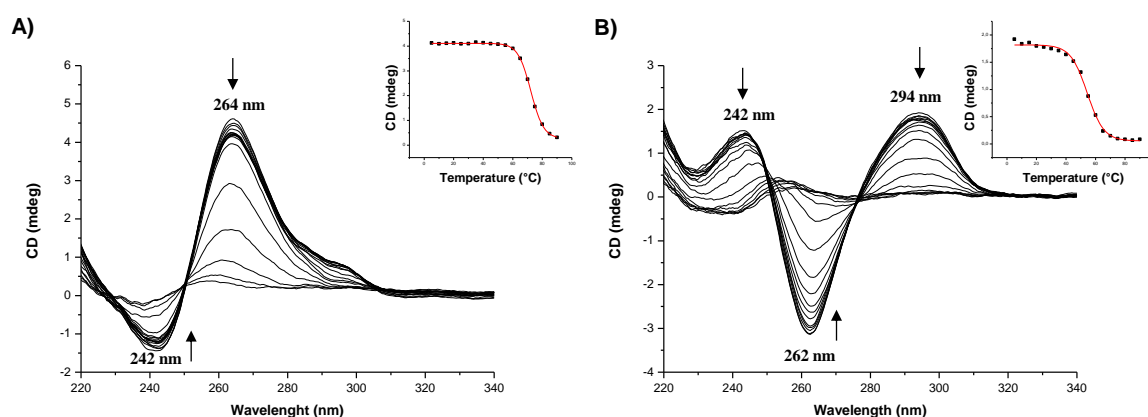
**Figure S28:** AELC chromatogram of crude 7 after CuAAC reaction



**Figure S29:** AELC chromatogram of crude 7 treated with MeONH<sub>2</sub>

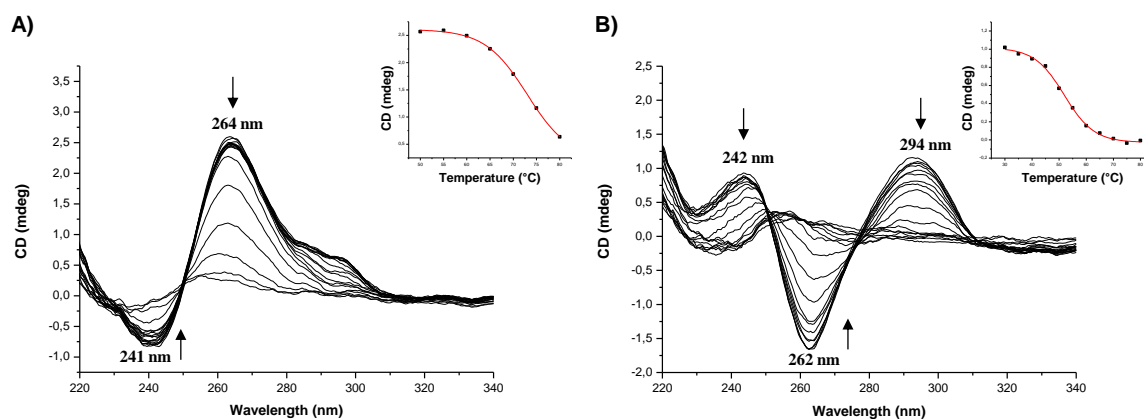
## Circular Dichroism Studies

For each compounds, circular dichroism studies were performed after having firstly desalted the product. A step of annealing was applied, heating the sampler at 90°C for 5 min in buffer (Tris 10 mM pH 7.4 with 100 mM NaCl or 100 mM KCl) and cooling it over 2h to room temperature. Analyses were recorded on a Jasco J-810 spectropolarimeter using 1 cm length quartz cuvette. Spectra were recorded at 20°C or every 5°C in a range of 5 to 90°C with a wavelength range of 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<sup>-1</sup> scanning speed. Blank spectra of buffer were subtracted for each measure. Melting temperatures were obtained using Boltzmann fit on Origin software. Each curve fit was only accepted with a  $r_{\text{value}} > 0.99$ .

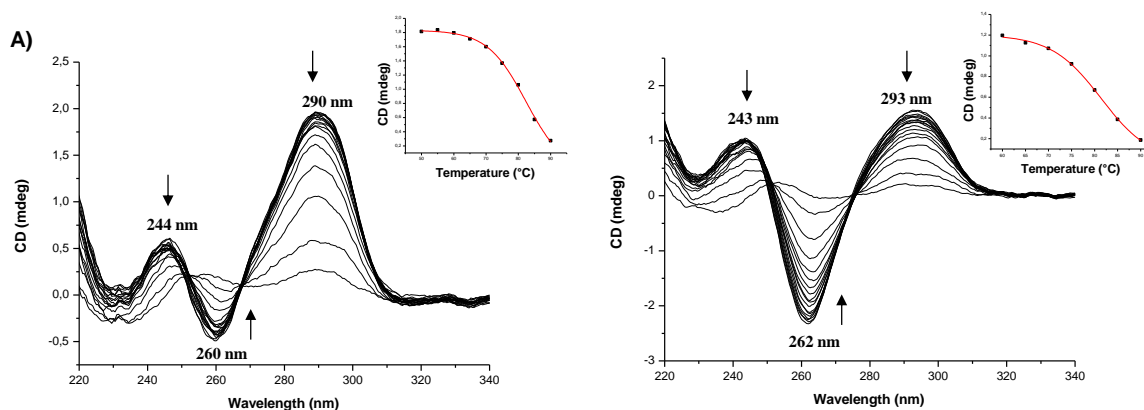


**Figure S30:** CD analyses of **4** (5  $\mu\text{M}$  in 10 mM Tris buffer pH 7.4 with A: 100 mM KCl or B: 100 mM NaCl). Superposition of CD spectra with CD melting curve in the corner at 264 nm (A) or 294 nm (B) in the corner ( $\blacksquare$ : experimental results; curve: Boltzmann fit).

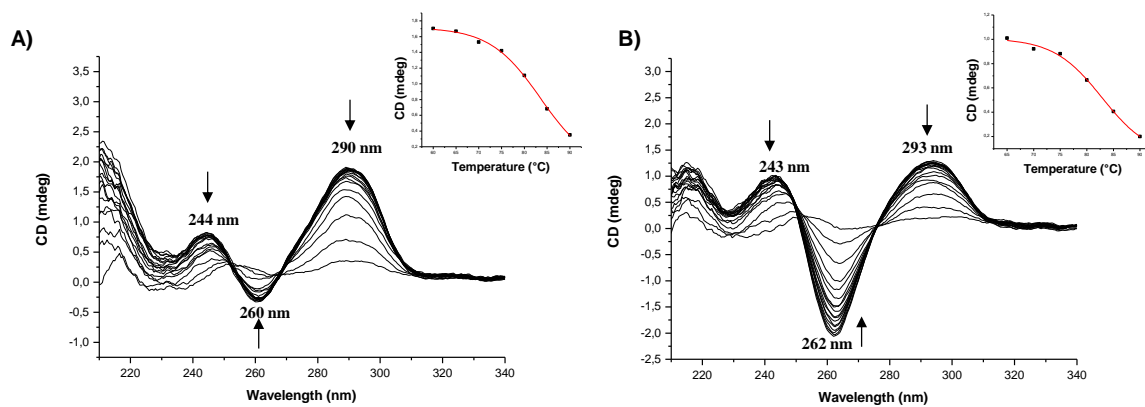
Arrows indicate the signal evolution during denaturation from 5°C to 80°C.



**Figure S31:** CD analyses of **5** (5  $\mu$ M in 10 mM Tris buffer pH 7.4 with A: 100 mM KCl or B: 100 mM NaCl). Superposition of CD spectra with CD melting curve in the corner at 264 nm (A) or 294 nm (B) in the corner ( $\blacksquare$ : experimental results; curve: Boltzmann fit). Arrows indicate the signal evolution during denaturation from 5°C to 80°C.



**Figure S32:** CD analyses of **6** (5  $\mu$ M in 10 mM Tris buffer pH 7.4 with A: 100 mM KCl or B: 100 mM NaCl). Superposition of CD spectra with CD melting curve in the corner at 290 nm (A) or 293 nm (B) in the corner ( $\blacksquare$ : experimental results; curve: Boltzmann fit). Arrows indicate the signal evolution during denaturation from 5°C to 90°C.

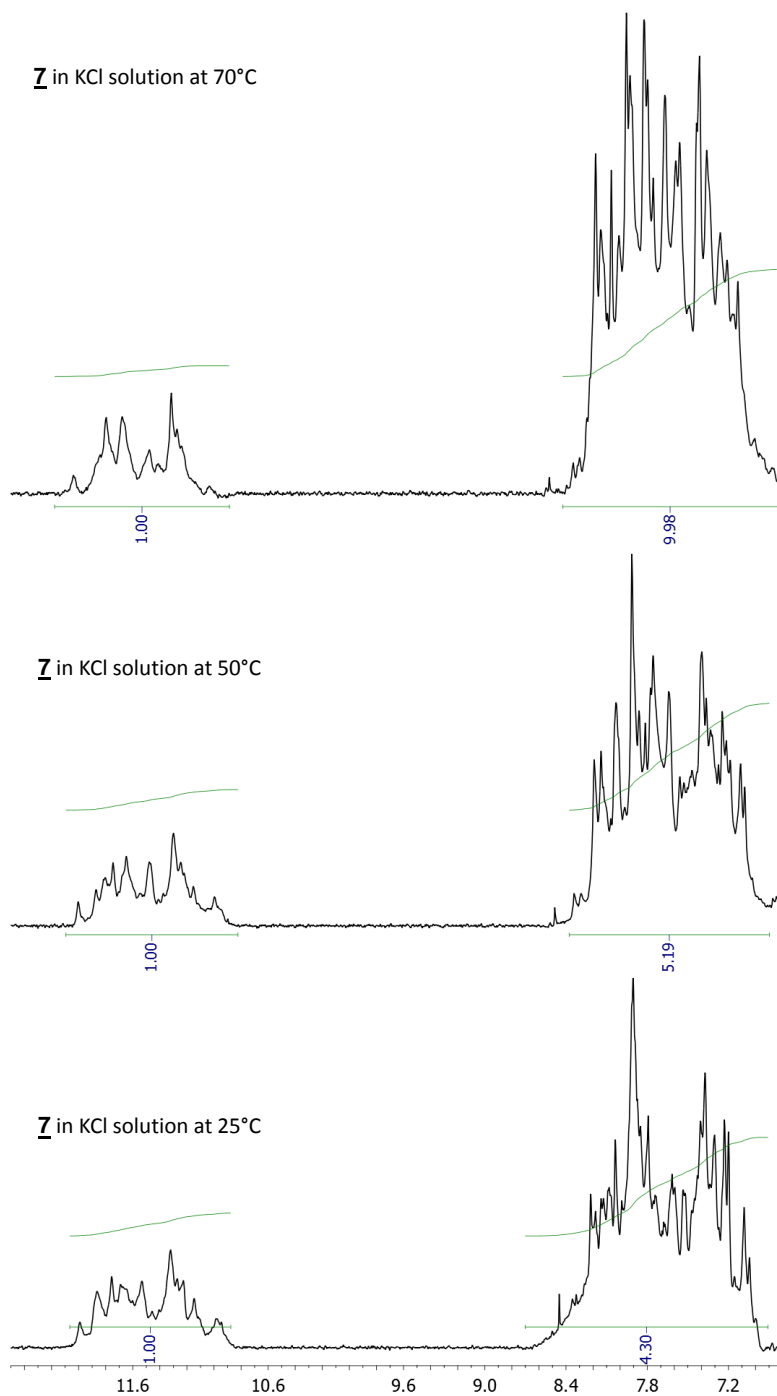


**Figure S33:** CD analyses of 7 (5  $\mu$ M in 10 mM Tris buffer pH 7.4 with A: 100 mM KCl or B: 100 mM NaCl). Superposition of CD spectra with CD melting curve in the corner at 290 nm (A) or 293 nm (B) in the corner ( $\blacksquare$ : experimental results; curve: Boltzmann fit).

Arrows indicate the signal evolution during denaturation from 5°C to 90°C.

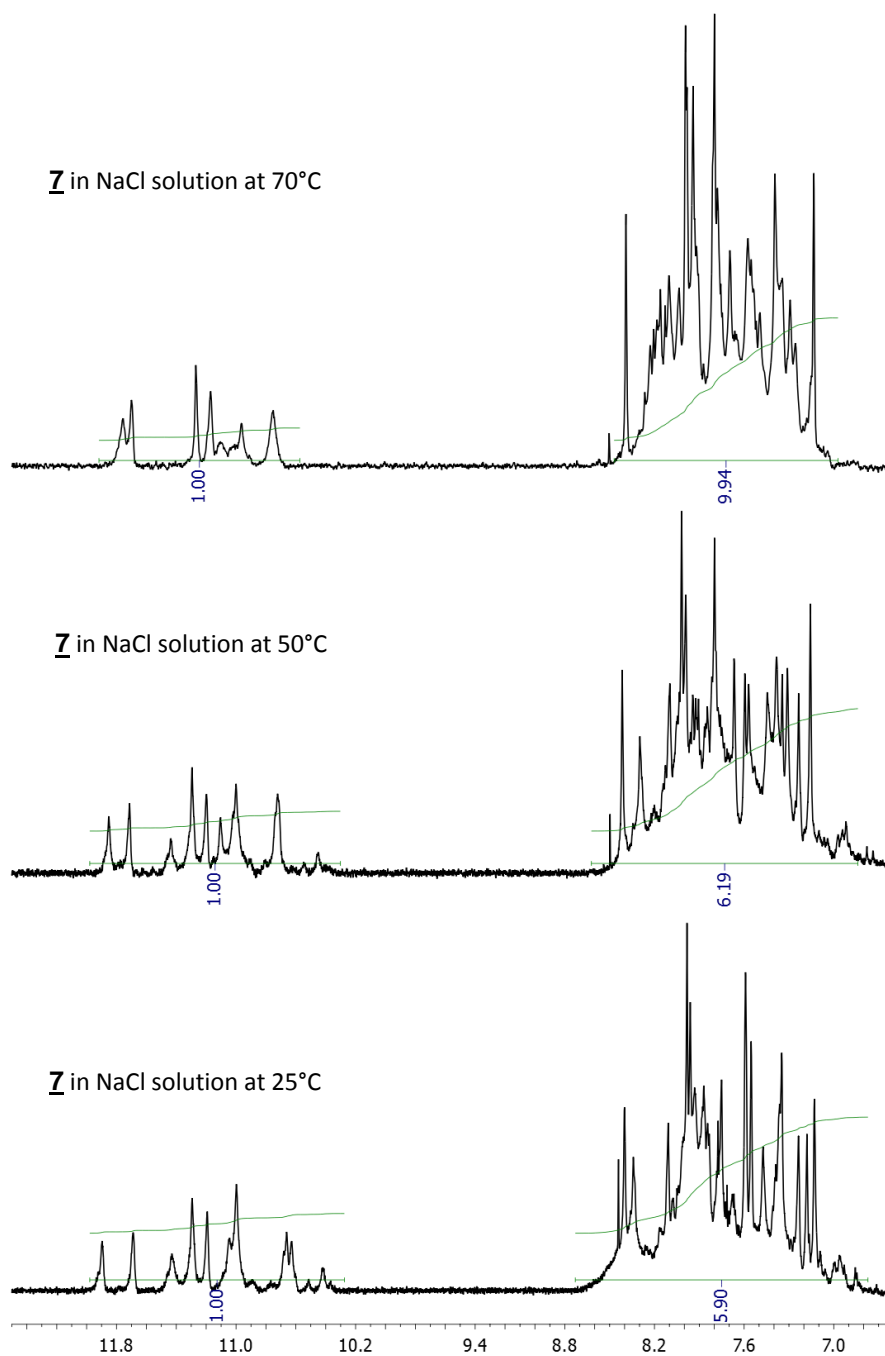
## NMR Experiments

NMR spectra of **7** at 130  $\mu\text{M}$  were obtained in phosphate buffer (10 mM) and in the presence of 10%  $\text{D}_2\text{O}$  and either 100 mM concentration of NaCl or 100 mM KCl. Spectra were obtained after 5h of accumulations at each temperature.

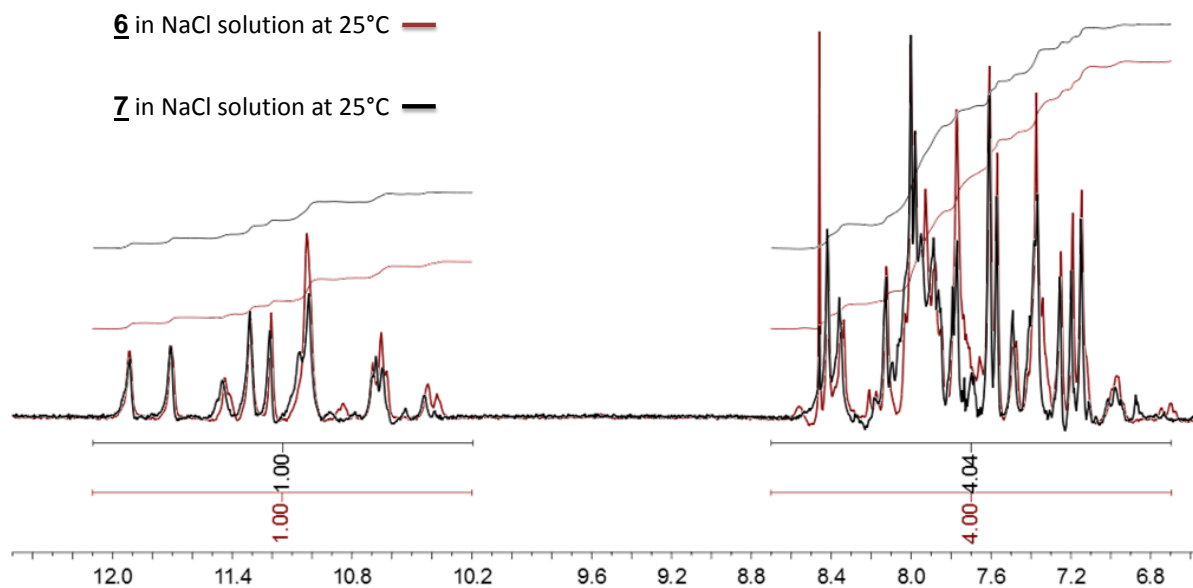


**Figure S34:** 12.5-7 ppm region of 1D  $^1\text{H}$  NMR of **7** at 25, 50 and 70°C in the presence of 100 mM KCl





**Figure S35:** 12.5-7 ppm region of 1D  $^1\text{H}$  NMR of **7** at 25, 50 and 70°C in the presence of 100 mM NaCl



**Figure S36:** Overlay of the 12,5-6,5 ppm region of  $^1\text{H}$ -NMR of **6** and **7** at 25°C in the presence of 100 mM NaCl solution.

## Bibliography

1. R. Bonnet, P. Murat, N. Spinelli and E. Defrancq, *Chem. Commun.*, 2012, **48**, 5992-5994.
2. E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797-3800.
3. S. Foillard, M. O. Rasmussen, J. Razkin, D. Boturnyn and P. Dumy, *J. Org. Chem.*, 2008, **73**, 983-991.