

*Supporting Information*

**Amphiphilic 3'-Peptidyl-RNA Conjugates**

Silvia Terenzi, Ewa Biała, Nhat Quang Nguyen-Trung, Peter Strazewski\*

*Institute of Organic Chemistry*

*University Basel*

*Switzerland*

	<b>Page</b>
<b>1. General Information</b>	<b>2</b>
<b>2. Preparation of the solid support</b>	<b>2</b>
<b>3. General procedure for the synthesis of the conjugates</b>	<b>4</b>
<b>3.1. Synthesis of the peptidic moieties of the conjugates</b>	<b>4</b>
<b>3.2. Synthesis of the oligoribonucleotidic moieties of the conjugates</b>	<b>5</b>
<b>4. Cleavage / Deprotection / Work up</b>	<b>5</b>
<b>5. Purification</b>	<b>6</b>
<i>Strong Anion Exchange</i>	<b>6</b>
<i>Reversed Phase Desalting</i>	<b>7</b>
<i>Reversed Phase Chromatography</i>	<b>7</b>
<b>6. Mass spectrometry</b>	<b>10</b>
<b>7. CD spectroscopy</b>	<b>10</b>
<b>8. Denaturation profiles and thermodynamic analysis</b>	<b>13</b>
<b>9. Particle size distributions in suspension (DLS) and on a glass surface (AFM)</b>	<b>14</b>
<b>10. References</b>	<b>16</b>

---

\* Send correspondence to [peter.strazewski@unibas.ch](mailto:peter.strazewski@unibas.ch)

## 1. General Information

BOC/FMOC-amino acids, reagents and solvents, all of the highest quality available, were obtained from *Fluka Chemie AG* (Switzerland) unless otherwise mentioned. They were used as purchased. HATU and HBTU were obtained from *Novabiochem* (Switzerland). DEPBT was prepared as described.<sup>[1]</sup> Pd(PPh<sub>3</sub>)<sub>4</sub> was obtained from *Strem Chemicals* (France). Solid phase peptide syntheses were carried out manually using a Teflon syringe equipped with a polyethylene filter (*MultiSynTech*, Germany). Oligoribonucleotide syntheses were carried out on an Applied Biosystems DNA/RNA Synthesizer, model 392. The solid support, aminomethyl polystyrene 50% crosslinked with divinylbenzene, was obtained from ABI (360865 C, Lot: 9609225 GB, capacity of loading: 28 μmol NH<sub>2</sub>/g resin). The monomers and the reagents for the oligoribonucleotide synthesis were obtained from *Glen Research*.

The purification of the conjugates was performed with a Shimadzu LC-7A HPLC system (high pressure gradient mixing, max. total flow rate 20 ml/min, 2 ml loop) coupled with a Kontron-Instruments UV spectrophotometer, model 432. The chromatograms were recorded on a Shimadzu Integrator, model C-R3A. Some purifications were performed with a Waters 600 system equipped with a pump model 511 and a photodiode array detector 991. The spectra were processed using the software Millennium<sup>®</sup> V. 2.01 from Waters. Anion-exchange HPLC was carried out using a Nucleogen<sup>®</sup> DEAE 500-7 *Macherey-Nagel* column (125 x 8 mm) maintained at 50 °C; gradients of sodium chloride (0-400 mM) in a buffer of 20 mM Na/K-phosphate (pH 7.0) with a flow rate of 2 ml/min and detection at 260 nm were used for the elution of the products. Reversed-phase HPLC was carried out using a Eurospher<sup>®</sup> 100/5 RP<sub>18</sub> (*Macherey-Nagel*, 8 x 250 mm), a Nucleosil<sup>®</sup> 500-5 C<sub>18</sub>-PPN (*Macherey-Nagel*, 4.6 x 250 mm) and a Spherisorb<sup>®</sup> S5X C<sub>18</sub> (4.6 x 250 mm); gradients of acetonitrile (0-40%) in 0.1 M ammonium acetate buffer pH ~ 6.5 with a flow rate of 2 ml/min (Eurospher column) or 1 ml/min (Nucleosil and Spherisorb columns) were used.

## 2. Preparation of the solid support

Aminomethyl polystyrene solid support (1.5 g, 42 μmol NH<sub>2</sub>) was placed in a Teflon syringe. Succinic anhydride (210 mg, 2.1 mmol) and DMAP (15.4 mg, 0.126 mmol) were dissolved in dry pyridine (6 ml) and poured onto the polymer support. The reaction was allowed to proceed overnight at room temperature under shaking. At the end of the reaction, the solution was pushed out of the syringe and the solid support was carefully rinsed with pyridine (10 ml x 4) and CH<sub>2</sub>Cl<sub>2</sub>

(10 ml x 4) followed by drying under reduced pressure. The quantitative ninhydrin test<sup>[2]</sup> allowed to check for the completeness of the reaction (1.5  $\mu\text{mol/g}$  of residual  $\text{NH}_2$  groups, 98.2% substitution).

Capping: Any residual reactive amino group was then treated with equal volumes (3 ml of each) of capping solutions (Cap. A: 1 M acetic anhydride, 1 M pyridine in DMF, Cap. B: 1 M *N*-methylimidazole (NMM) in DMF) for 5 minutes, washed thoroughly with DMF (10 ml x 5) and dried in vacuo.

1,6-diaminohexane was added to the succinylated polymer acting as spacer between the resin surface and the point of the conjugate assembly. The carboxylic groups on the polymer were activated with oxalyl chloride (2 ml, 16.2 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (4 ml) for 2 h. The resin was then washed with dry  $\text{CH}_2\text{Cl}_2$  under argon atmosphere and dried under high vacuum overnight. 1,6-Diaminohexane (941 mg, 8.1 mmol) and DMAP (98 mg, 0.8 mmol) were dissolved in  $\text{CH}_2\text{Cl}_2$  (5 ml) and added to the solid support. The reaction was allowed to proceed for 4 h at the end of which a quantitative ninhydrin test was performed (25.8  $\mu\text{mol/g}$ , 97.3% substitution).

BOC-sarcosine was then coupled as base-stable linker between the spacer and the building block adenosine.<sup>[3]</sup> BOC-sarcosine (640 mg, 3.38 mmol) and HBTU (1.28 g, 3.38 mmol) were dissolved in dry DMF (5 ml), NMM (750  $\mu\text{l}$ , 6.76 mmol) was added and the mixture was shaken for 15 min. The coupling solution was then added to the solid support and reacted for 1 h 30 min. The same procedure was repeated, followed by a capping step (see above). The BOC group was then removed by treating the resin twice (8 + 3 min) with a mixture of TFA/ $\text{CH}_2\text{Cl}_2$  (95:5, 6 ml). A neutralization step with triethylamine/DMF (1:4) for 1 min followed the removal of the BOC group.

Coupling of the building block **1** to the derivatised solid support was the last step before the conjugate synthesis. Building block **1** (31 mg, 28  $\mu\text{mol}$ ) and HATU (10.6 mg, 28  $\mu\text{mol}$ ) were dissolved in dry DMF (2 ml). NMM (6  $\mu\text{l}$ , 56  $\mu\text{mol}$ ) was added and the mixture was shaken for 15 min before being added to the syringe containing the solid support (batch 1: 580 mg,  $\sim 15$   $\mu\text{mol}$ ). After 20 h the solution was filtered out and used for another portion of resin (batch 2: 300 mg). A capping step (5 min) followed the coupling of the adenosine monomer **1** on the resin.

The final loadings, as determined by the quantitative ninhydrin test, were 11.4  $\mu\text{mol/g}$  (batch 1) and 10.6  $\mu\text{mol/g}$  (batch 2). Only 2 equivalents of building block were used because a loading within the range of 6-10  $\mu\text{mol/g}$  was desired. According to Warras and coworkers,<sup>[4]</sup> a

low level of resin substitution ( $\sim 7 \mu\text{mol/g}$ ) would allow the synthesis of long polyalanine based peptides (18-21 amino acids) in good yields. Low substitution hamper the aggregation between the growing peptide chains ( $\beta$ -sheet formation) responsible for the drop of the coupling yields. Since we planned to synthesize conjugates with peptidic moieties based on alanine sequences, we tried to obtain a solid support with a low loading.

### 3. General procedure for the synthesis of the conjugates

#### 3.1 Synthesis of the peptidic moieties of the conjugates

The peptidic moieties of the conjugates were synthesized prior the RNA synthesis following a stepwise, Fmoc-based solid support oligopeptide synthesis. They were synthesized on batches of resin **2** ( $\sim 120 \text{ mg}$ , loading  $\sim 11 \mu\text{mol/g}$ ) prepared as described above. The quality of the syntheses was monitored by measuring the Fmoc-release solution at 300 nm (10 % v/v in DMF against pure DMF) after every coupling and by the quantitative ninhydrin test<sup>[2]</sup> every four couplings. The allyl group was used as side-chain protection for glutamic acid.

The following coupling cycles were used for the synthesis of **4a**, **4b** and **4f**: a) Fmoc-deprotection: treatment with 2% piperidine, 2% DBU in DMF for 8 min followed by a second deprotection step of 5 min; b) Amino acid coupling : Fmoc-amino acid (200 eq.), DEPBT (400 eq.), NMM (400 eq.) dissolved in dry DMF to a final concentration of 1-1.2 M and preactivated for 30 min. The coupling mixture (deep yellow), added to the solid support, was shaken for 1 h and the reaction repeated a second time. After the coupling of the 7<sup>th</sup> alanine, difficulties in the release of the Fmoc group indicated beginning aggregation of the peptide chains on the resin. The coupling times were then prolonged to 2 h till the end of the synthesis; c) Capping procedure: 2 min treatment using an equivalent volume of Cap. A and Cap. B solutions.

The quantitative ninhydrin test gave an estimation of the peptide synthesis yields: **4a**, 80%; **4b**, 70%; **4f**, 55%. After the RNA synthesis and the purification, conjugates **4b** and **4f** showed the presence of side products containing 1 or 2 amino acids more. The side products probably resulted from overactivation due to the high concentration of amino acid used leading to Fmoc-alanylation of the Fmoc-carbamate nitrogen (both in solution and on solid support).<sup>[5]</sup> A lower concentration of Fmoc-amino acid was therefore used for the other peptide syntheses. Conditions of synthesis used for **4c**, **4d**, **4e**: a) Fmoc-deprotection: as before; b) amino acid coupling: Fmoc-amino acid (50-60 eq.), DEPBT (100-120 eq.), NMM (100-120 eq.) dissolved in DMF to a final concentration of 0.3-0.4 M and preactivated for 30 min. Coupling time: as

before. c) Capping: as before. Using less activated amino acid led to lower overall yields but less or no side product resulting from overactivation was observed. Peptide yields: **4c** (50%), **4d** (36%), **4e** (24%).

### 3.2 Synthesis of the oligoribonucleotidic moieties of the conjugates

Immediately after the coupling of the last amino acid, a part of the peptidylated solid support was transferred to a '1  $\mu$ mole-reaction column' for ABI DNA/RNA Synthesizers (70 mg maximum capacity for aminomethyl polystyrene based resin). 35 mg ( $\sim$ 0.4  $\mu$ mol) for **4a**, **4b**, **4f** and 70 mg ( $\sim$ 0.7  $\mu$ mol) for **4c**, **4d**, **4e** of the resin were used for the RNA synthesis. The following 2'-*O*-*tert*-butyldimethylsilyl ribonucleoside  $\beta$ -cyanoethyl phosphoramidites were used: A<sup>Pac</sup>, G<sup>iPrPac</sup>, C<sup>Ac</sup>, U. The reagents used were: 0.25 M 5-ethylthio-1*H*-tetrazole/CH<sub>3</sub>CN (activator), 3% Cl<sub>3</sub>CCOOH/CH<sub>2</sub>Cl<sub>2</sub> (deprotection), Ac<sub>2</sub>O/lutidine/THF (Cap A), NMM/THF (Cap B); 0.02 M I<sub>2</sub>/H<sub>2</sub>O/pyridine/THF (oxidation), 40% aq. CH<sub>3</sub>NH<sub>2</sub>/33% ethanolic CH<sub>3</sub>NH<sub>2</sub> (1:1) (final cleavage and deprotection).

Coupling yields per step were monitored by automated conductivity integration. Coupling cycles: the standard 1  $\mu$ mol-RNA coupling cycle (ABI, version 2.01) was modified into a 'couple-cap-ox-cap' cycle: coupling (WAIT 420 sec), 1<sup>st</sup> capping (WAIT 5 sec), oxidation (WAIT 45 sec), thorough CH<sub>3</sub>CN/Ar wash (2 x), 2<sup>nd</sup> identical capping, another CH<sub>3</sub>CN/Ar wash (2 x), deprotection. Average stepwise yields ( $\sim$ 97.5%). The overall RNA yields varied from 43% to 67%.

For the conjugates **4c**, **4d**, **4e** containing glutamic acid residues a Pd(0) treatment followed the end of the synthesis to remove the allyl side chain protecting group. The deprotection was accomplished under Ar atmosphere with Pd(PPh<sub>3</sub>)<sub>4</sub> (15 mg,  $\sim$ 20 eq) and PhSiH<sub>3</sub> (2 mg, 24 eq.) as an allyl group scavenger in CH<sub>2</sub>Cl<sub>2</sub> (1 ml, 2 x 15 min). After pushing out of the column the Pd solution, the resin was extensively washed with CH<sub>2</sub>Cl<sub>2</sub>. The polymer material was finally washed with ammonium *N,N*-diethyldithiocarbamate (0.5% w/w) in DMF to ensure the removal of any contaminating palladium residue.

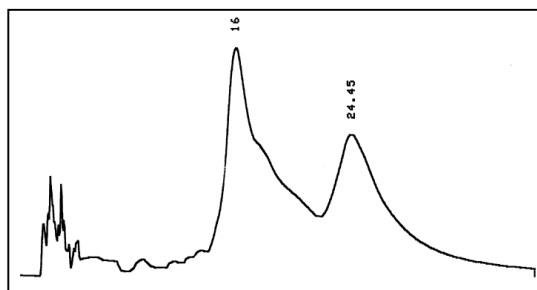
### 4. Cleavage / Deprotection / Work up

After treatment of the solid support on the synthesizer with CH<sub>3</sub>NH<sub>2</sub> at room temperature for 2 h (see above), the collected liquid ( $\sim$ 1.5 ml) was immediately evaporated in a SpeedVac.

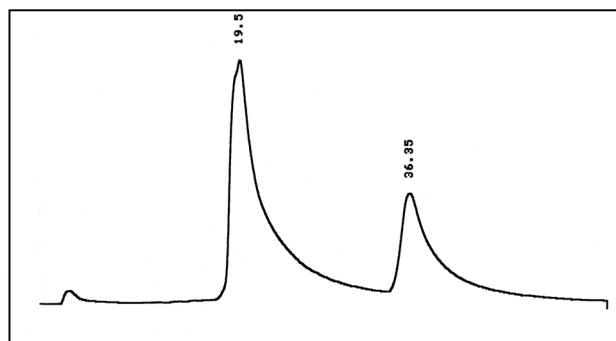
Desilylation: the crude product (persilylated RNA) from 0.7  $\mu\text{mol}$  (0.4  $\mu\text{mol}$ ) syntheses was treated with 200  $\mu\text{l}$  (150  $\mu\text{l}$ ) neat  $\text{Et}_3\text{N}\cdot 3\text{HF}$  and 60  $\mu\text{l}$  (40  $\mu\text{l}$ ) DMF, heated and vortexed at 65  $^\circ\text{C}$ , and quenched at RT after 1.5 h with 30  $\mu\text{l}$  (20  $\mu\text{l}$ ) sterile water. The crude deprotected RNA was precipitated by adding 2 ml (1 ml) *n*-butanol. After 2 h at  $-20$   $^\circ\text{C}$ , the precipitate was spun down and the pellets dissolved in 1 ml buffer A for strong anion exchange HPLC. For some conjugates (**4b**, **4f** in particular), it was not possible to redissolve all the precipitate, leading to a loss of material. Neither 6 M guanidinium hydrochloride (pH 8) and nor a detergent (Triton-X) were able to redissolve the insoluble precipitate. Only pure TFA could dissolve this material, therefore it was not possible to purify it by HPLC. Crude material: **4a**: 20 O.D.; **4b**: 52 O.D.; **4c**: 55 O.D.; **4d**: 70 O.D.; **4e**: 72 O.D.; **4f**: 28 O.D. (optical density at 260 nm in 1.0 ml).

## 5. Purification

**Strong Anion Exchange** : After heating the RNA solution in a closed Eppendorf vial for 1 min at 100  $^\circ\text{C}$ , 500 ml crude material were injected (still warm) onto the SAX HPLC column. Buffer A : 20 mM Na/K phosphate, pH 7.0 ; buffer B : 0.6 N NaCl in buffer A. Gradient used for **4a**, **4b**, **4f**: 0-65% B in 10 min / 8 min isocratic section at 65% B / 65-100% B in 3 min / stay at 100% for 20 min. Gradient used for **4c**, **4d**, **4e**: 0-20% B in 6 min / 20-60% B in 7 min/ 15 min isocratic section at 65% B / 65-100% B in 2 min / stay at 100% for 20 min. For all the conjugates the product eluted as two main peaks, the first one at around 60-65% buffer B and the second one at 100% B. For the conjugates with a more hydrophobic peptide, the ratio between the two peaks is almost 1:1, while for the conjugates with some glutamic acid residues the second peak is smaller (Fig. S1A, S1B).



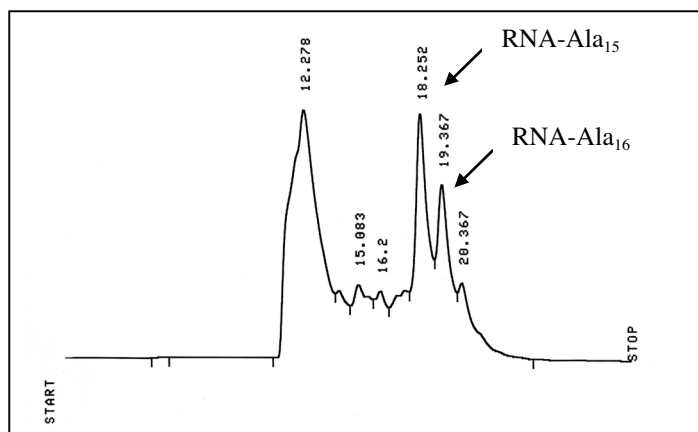
**Figure S1A:** SAX-HPLC profile of crude **4f**.



**Figure S1B:** SAX-HPLC profile of crude **4d**.

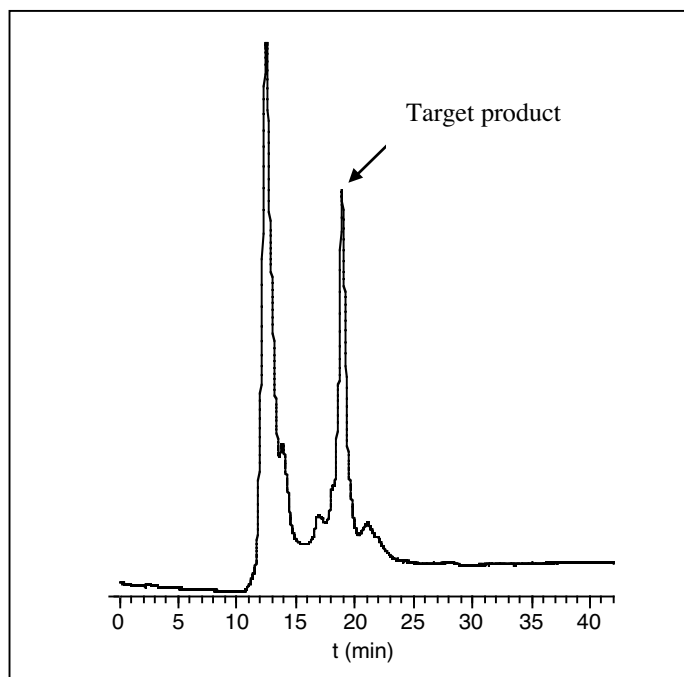
**Reversed Phase Desalting:** The central part of each of the two peaks was collected (~10-15 ml) and injected into a  $C_{18}$  reverse phase HPLC column (8 x 250 mm, ODS, buffer A: nanopure water, flow rate 2 ml/min) to be desalted. Multiple injections of 1.5 ml volume were necessary to load all the product onto the column. After all the conjugate had been adsorbed, the excess salts were washed off the column with water (up to 6 ml/min no conjugate desorption was observed). The product was eluted by a step gradient to 90%  $CH_3CN/H_2O$  (= 100% B, 3 ml/min) into a ~4 ml fraction. The fraction was subsequently concentrated on a Rotavap system under reduced pressure to a volume of ~1 ml (heating bath 40 °C, interior of the flask silanized with ~2%  $(CH_3)_2SiCl_2/CCl_4$ ). Concentration using a SpeedVac caused the precipitation of a part of the product that was impossible to redissolve afterwards.

**Reversed Phase Chromatography:** The two concentrated and desalted fractions from SAX-HPLC were then analyzed and purified (when possible) by RP-HPLC. Buffer A : 0.1 M aq. NaOAc; buffer B :  $CH_3CN$ /buffer A (9 :1). Gradient used: 0-60% B in 60 min (linear). For all the conjugates, the target product proved to be present only in the first peak of the SAX injection. RP-HPLC profiles of all of the conjugates revealed the presence of a very broad peak eluting in front of all the other peaks ( $t_R \sim 10-15$  min). This peak is predominant for the conjugates containing very hydrophobic peptides leading to the impossibility to isolate a single species (Fig. S2).



**Figure S2:** RP-HPLC of SAX-purified **4b**.

The presence of glutamic acid residues along the peptidic chain could not eliminate the appearance of this peak (Fig. S3).

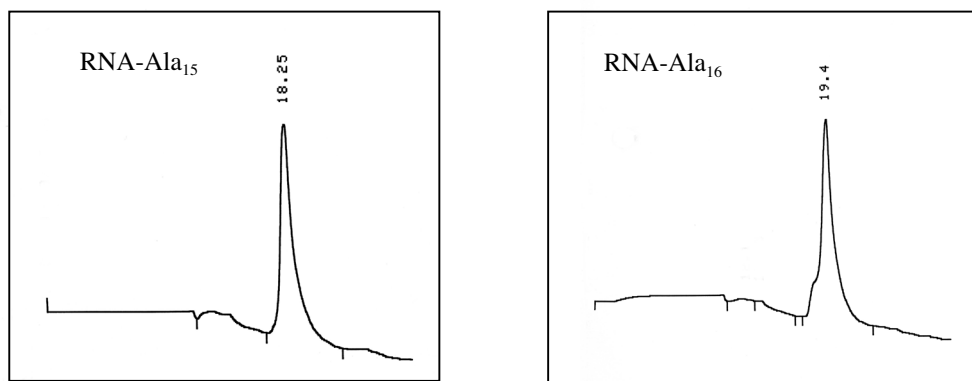


**Figure S3:** RP-HPLC of SAX-purified **4e**.

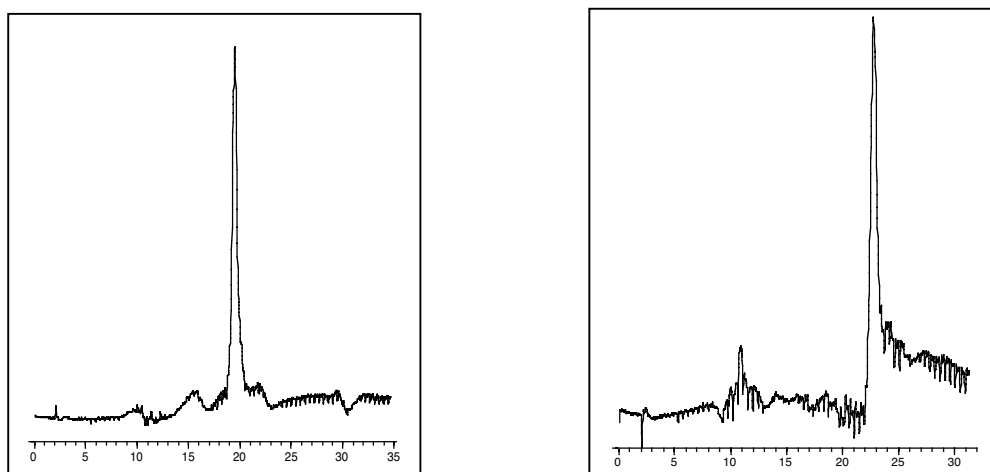
For all the compounds this broad peak resulted in poorly ionizable material that could not be analyzed by MALDI-ToF. We hypothesize that the first broad peak, present in all the species, is formed by the aggregation of some molecules of the conjugates. The molecules are formed by a hydrophilic, charged part (RNA) and a hydrophobic one (the peptide) giving our construct an

amphiphilic character that might lead to the formation of micelles or other aggregates masking the hydrophobic part, hence, shortening the retention time of this material on RP-HPLC.

The presence of these ‘aggregates’ lowered the yield of the final, purified material. The fractions collected from RP-HPLC purification were desalted (as described above) and, before desorption of the product from the column, the  $K^+/Na^+$  counterions were exchanged with  $NH_4^+$  ions by injecting 1.5 ml 0.25 M  $NH_4OAc$  (sterile-filtered). The excess ammonium salt was washed off with water (6 ml/min) and the desalted conjugate ( $NH_4^+$  form) was eluted within 4-5 ml as described above. This fraction was concentrated and analyzed by MALDI-ToF. 1-4 O.D. were obtained as purified material. Aqueous stock solutions of desalted material were directly used for CD spectroscopy and thermal denaturing measurements and could be stored for over 12 months in RNase-free Eppendorf tubes (Ambion) at 4 °C without degradation (do not freeze!).



**Fig S4:** RP-HPLC of SAX- and RP-purified conjugate **4b**.



**Fig S5:** RP-HPLC of SAX- and RP-purified conjugates **4e** and **4d**, respectively.

## 6. Mass spectrometry

MALDI-ToF mass spectra were acquired on a Bruker Reflex III spectrometer in negative ion, linear mode. A mixture of 2,4,6-trihydroxy acetophenone (0.3 M in ethanol), diammonium citrate (0.1 M in water), and CH<sub>3</sub>CN (5:2:3) was used as matrix.<sup>[6]</sup> 1 µl of the matrix were mixed with 1 µl of the product (~3 O.D.) and 0.3 µl of this solution were deposited on the plate and air dried. Results : **4a** [pRNA-(Ala)<sub>8</sub>: C<sub>232</sub>H<sub>303</sub>N<sub>91</sub>O<sub>163</sub>P<sub>22</sub>] [M-H]<sup>-</sup>: calcd 7654.8, found 7649.8; **4b** [RNA-(Ala)<sub>15</sub>: C<sub>253</sub>H<sub>337</sub>N<sub>98</sub>O<sub>167</sub>P<sub>21</sub>] [M-H]<sup>-</sup>: calcd 8072.4, found 8072.2 ; **4b** [RNA-(Ala)<sub>16</sub>: C<sub>256</sub>H<sub>342</sub>N<sub>99</sub>O<sub>168</sub>P<sub>21</sub>] [M-H]<sup>-</sup>: calcd 8143.5, found 8139.9; **4c** [RNA-(Ala)<sub>10</sub>Glu: C<sub>243</sub>H<sub>319</sub>N<sub>94</sub>O<sub>165</sub>P<sub>21</sub>] [M-H]<sup>-</sup>: calcd 7846.1, found 7839.8; **4d** [RNA-(Ala)<sub>18</sub>(Glu)<sub>2</sub>pGlu: C<sub>277</sub>H<sub>371</sub>N<sub>104</sub>O<sub>178</sub>P<sub>21</sub>] [M-H]<sup>-</sup>: calcd 8654.9, found 8654.0 ; **4e** [RNA-(Ala)<sub>7</sub>Glu(Ala)<sub>7</sub>Glu(Ala)<sub>4</sub> (Glu)<sub>2</sub>: C<sub>282</sub>H<sub>380</sub>N<sub>105</sub>O<sub>182</sub>P<sub>21</sub>] [M-H]<sup>-</sup>: calcd 8802.1, found 8819.5 ([M+NH<sub>4</sub>]<sup>+</sup>); **4f** [RNA-(Ala)<sub>20</sub>: C<sub>268</sub>H<sub>361</sub>N<sub>103</sub>O<sub>172</sub>P<sub>21</sub>]/[RNA-(Ala)<sub>21</sub>: C<sub>271</sub>H<sub>367</sub>N<sub>104</sub>O<sub>173</sub>P<sub>21</sub>]/[RNA-(Ala)<sub>22</sub>: C<sub>274</sub>H<sub>371</sub>N<sub>105</sub>O<sub>174</sub>P<sub>21</sub>] [M-H]<sup>-</sup>: calcd 8427.8/8498.9/8569.9, found 8429.9/8501.6/8571.8 (approximately 43:37:20).

## 7. CD spectroscopy

CD measurements were performed in 100 mM NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.5 (NaOH) using an Aviv Model 62 A DS instrument equipped with a thermoelectric temperature controller. Spectra were registered at 0° (Fig. 2 in the publication, also Fig. S7), 25° and 60 °C (Figs. S6, S7) using a 1 cm-3 ml Teflon-stoppered quartz cuvette with 2 ml solution volume (1.75 ml of buffer and 0.25 ml of conjugate stock solution : ~ 2.4 µM conjugate solution, A<sub>260nm,25°C</sub> ≈ 0.35). The region scanned was 200-350 nm with 0.5 nm wavelength increments. Spectra are the average of 3 scans, baseline corrected, normalized with respect to the number of nucleotide residues and to concentration ( $\lambda_{\max, \text{RNA}}$  at 260 nm and  $\epsilon_{260, \text{calcd}}=145 \cdot 100 \text{ M}^{-1} \text{ cm}^{-1}$ ) and smoothed. The difference CD spectra in Figure S8 focus on the Cotton effect due to the peptidic region (200-240 nm) at 0° C, but were additionally normalised for the number of amino acids per conjugate.

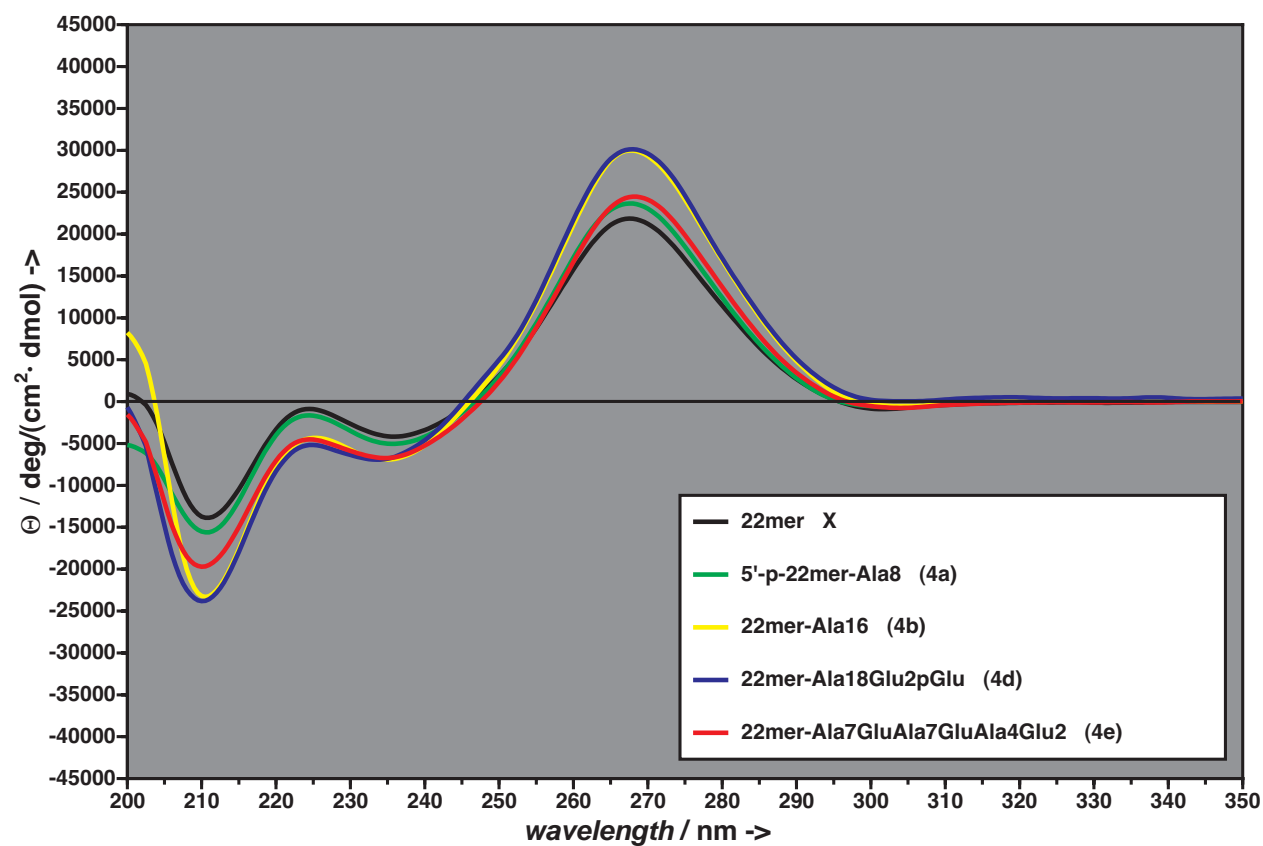
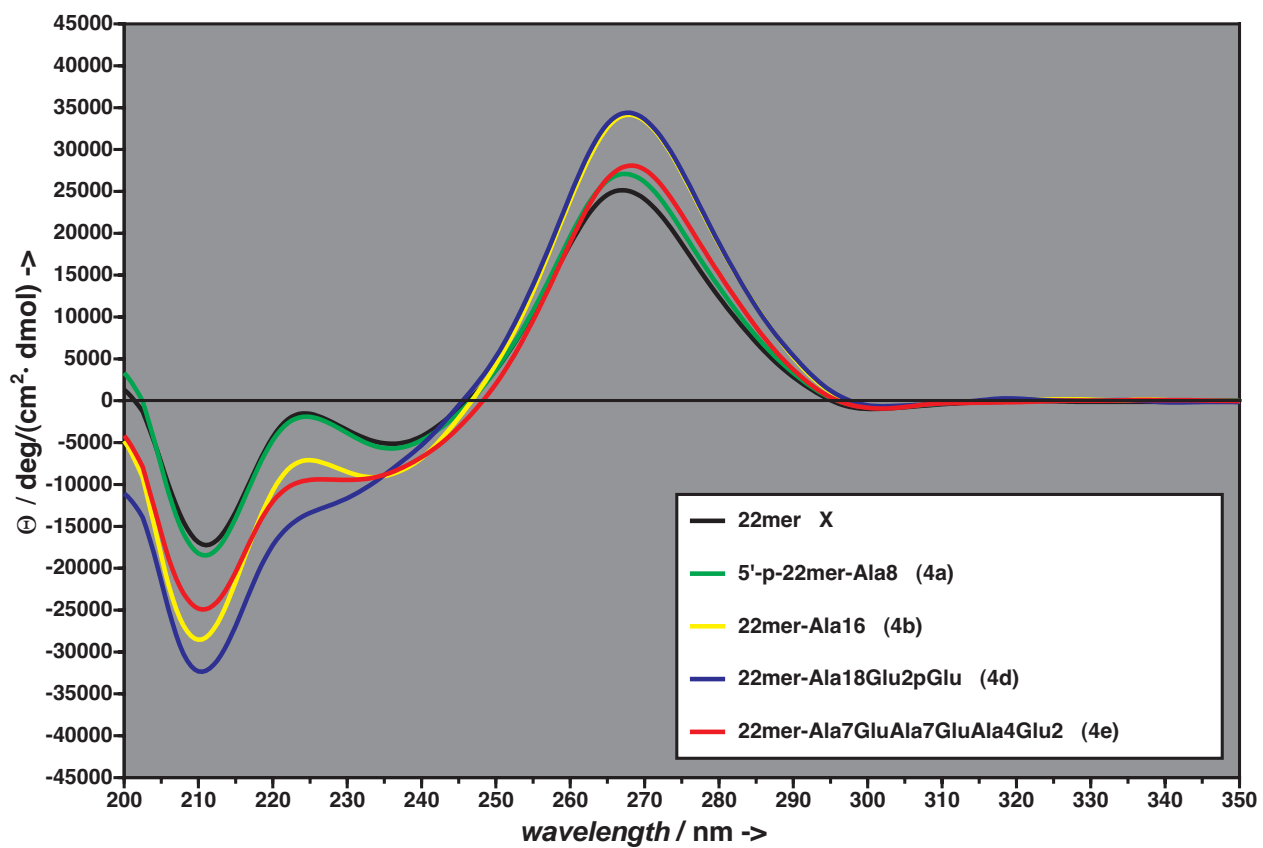


Figure S6: CD spectra of 4a,b,d,e,f and the unpeptidylated RNA hairpin (x) at 25 and 60° C.

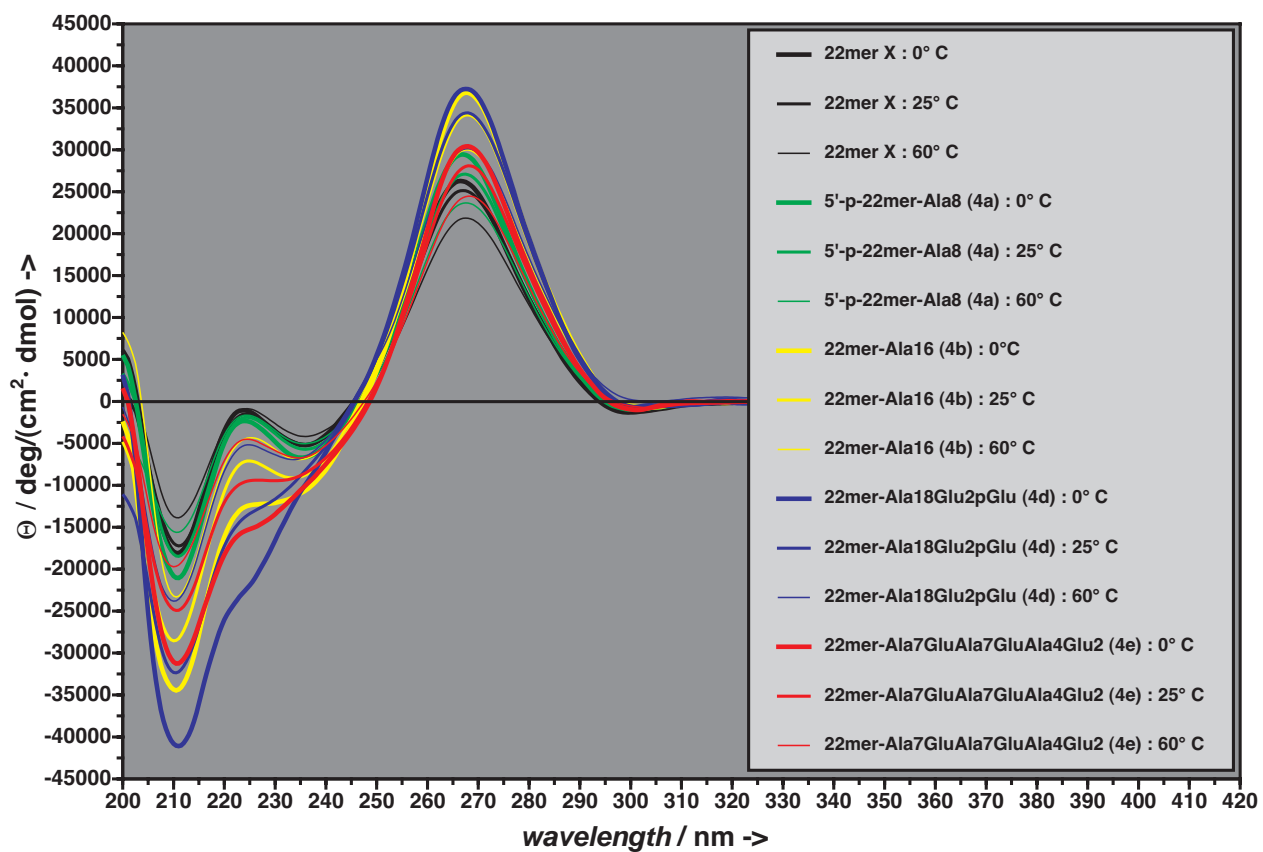


Figure S7: All CD spectra at 0, 25, and 60° C overlaid.

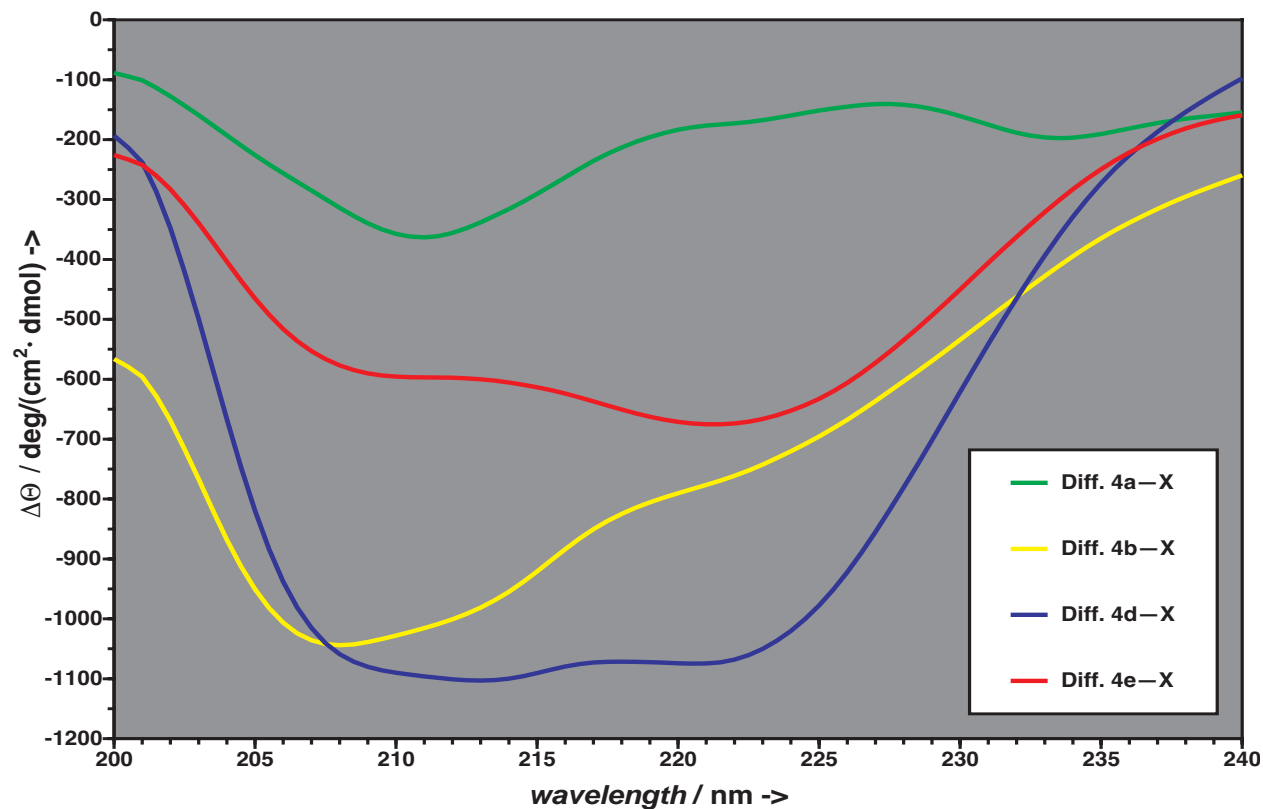
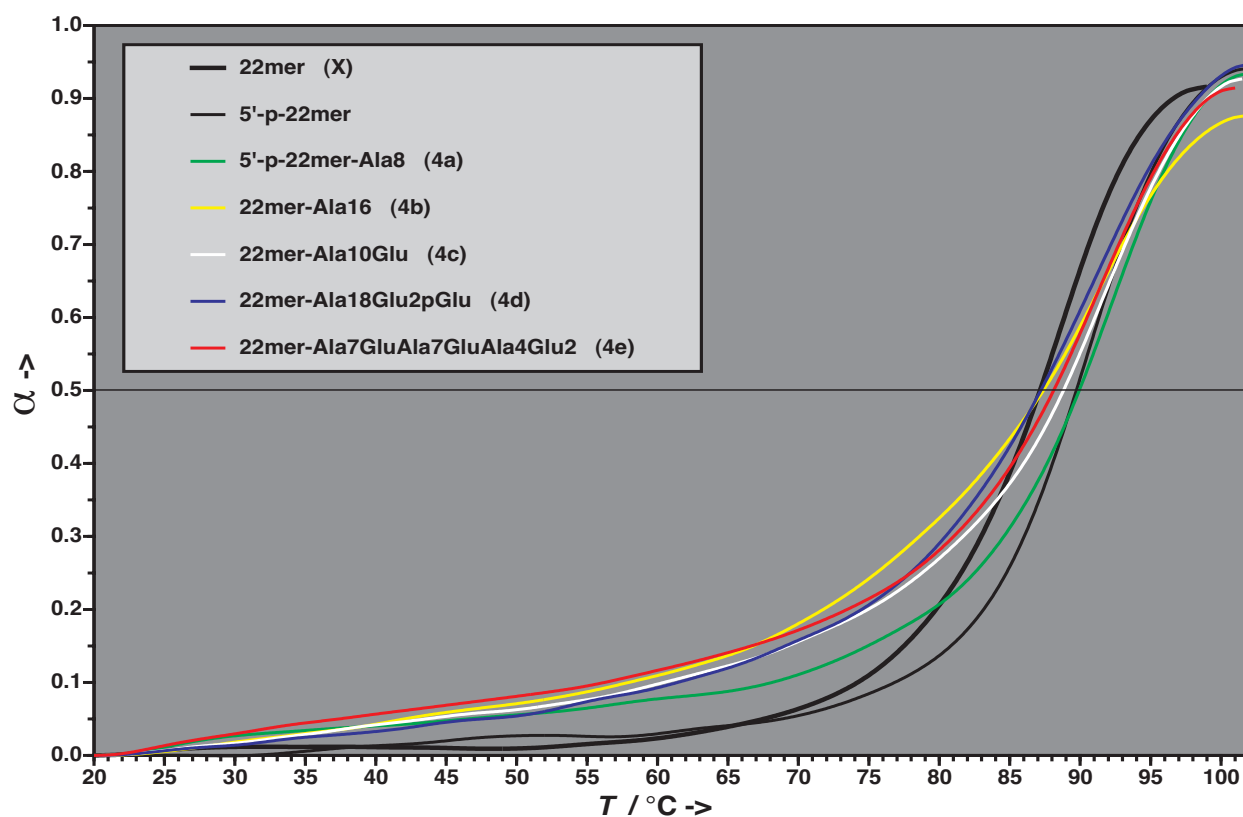


Figure S8: Difference CD spectra at 0° C normalised for the number of amino acids.

## 8. Denaturation profiles and thermodynamic analysis

Melting experiments were carried out on a Perkin-Elmer Lambda Bio 40 spectrophotometer equipped with a heating-cooling block thermocontroller PTP-6. The data were collected using the software UV WinLab<sup>®</sup> (for Lambda) and WinTemp<sup>®</sup> (for PTP-6). The melting profiles were registered using 1 cm/1 ml Teflon-stoppered quartz cuvette using as a buffer a solution of 100 mM NaCl/10 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.5 (NaOH). 1 ml of conjugate solution (1.0 ml buffer + few  $\mu$ l conjugate stock solution) were instantaneously pre-heated to 100 °C for 2 minutes and cooled down slowly before measuring. The absorbance at 260 nm was recorded every 0.1 ° between 20 and 102 °C with a heating rate of 1.0 °/min. For stability reasons all profiles were measured in the 'up' mode and every sequence was analyzed at least three times.

Thermal denaturation profiles of the following compounds were obtained at 260 nm (only RNA denaturation observed): The parent RNA hairpin (X), the RNA hairpin bearing a 5'-phosphate, a 3'-alanine, or both, and the conjugates **4a,b,c,d,e**, all at pH 7.5 (10 mM phosphate) and 0.1 M ionic strength (NaCl). The denaturation profiles are depicted in Figure S9.



**Figure S9:** Normalised  $A_{260}$  thermal denaturation profiles between 20 and 102° C. Note the shallower RNA hairpin transitions of the conjugates, in comparison to the RNA-only hairpins.

The ‘optimized temperature-window fitting’ method developed by us, which takes into account only the main transition of the RNA hairpin above  $\sim 85^\circ\text{C}$ , was used to abstract the van’t Hoff thermodynamics from the denaturation profiles (Table). The fitting procedure is described in detail in the Supporting Information to ref. [7].

**Table.** Thermodynamic parameters of RNA hairpin formation. Buffer solutions measured at  $\sim 0.3\ \mu\text{M}$  strand concentration ( $A_{260\text{nm},100^\circ\text{C}} \approx 0.04$ ), however, the profile of the RNA alone was shown to be concentration-independent.<sup>[7]</sup>

Conjugate	$T_m$ [ $^\circ\text{C}$ ]	$\Delta H^\circ$ [kcal/mol]	$\Delta S^\circ$ [cal/(mol·K)]	$\Delta G^\circ_{25^\circ\text{C}}$ [kcal/mol]
5'-RNA-3'-OH (x)	87.6	$-64.0 \pm 2.7$	$-177.4 \pm 7.8$	$-11.1 \pm 0.8$
<b>4a</b>	89.3	$-69.2 \pm 1.8$	$-190.9 \pm 4.8$	$-12.3 \pm 0.3$
<b>4b</b>	87.7	$-65.9 \pm 1.3$	$-182.8 \pm 3.3$	$-11.5 \pm 0.3$
<b>4c</b>	88.8	$-56.0 \pm 1.4$	$-154.8 \pm 3.9$	$-9.9 \pm 0.2$
<b>4d</b>	88.3	$-58.6 \pm 1.9$	$-162.0 \pm 5.4$	$-10.2 \pm 0.3$
<b>4e</b>	88.7	$-56.3 \pm 0.5$	$-155.6 \pm 1.4$	$-9.9 \pm 0.1$

Not shown in the Table are the  $T_m$  values of the other controls : A positive charge (3'-terminal  $\alpha$ -ammonium, as in the 3'-alanyl-amino-3'-deoxyoligoribonucleotide of the same primary sequence as x) does not influence the stability of the hairpin ( $T_m = 87.9^\circ\text{C}$  versus  $87.6^\circ\text{C}$ ) whereas a 5'-terminal negative charge stabilizes the structure somewhat ( $T_m = 89.9^\circ\text{C}$ , see also Table entry **4a**). When both groups are present an even larger stabilization is observed ( $T_m = 91.2^\circ\text{C}$ ), perhaps due to an interaction between the two terminal charges. External bivalent cations ( $\text{Mg}^{++}$  up to 1 mM) do not influence the stability of the hairpin. Hence, despite of the shallower transitions that suggest a departure from strict unimolecularity, no major influence on the *stability* of the hairpins was observed in any of the 3'-peptidyl-RNA conjugates ( $T_m = 87.7 - 88.8^\circ\text{C}$ ).

## 9. Particle size distributions in suspension (DLS) and on a glass surface (AFM).

Before depositing an aqueous solution of conjugate **4f** (7 O.D.) on a glass plate for an AFM scan, it was submitted to a laser light scattering experiment at an angle of  $90^\circ$ . The corresponding correlation function analysis, which calculates the size distribution of suspended particles, is depicted in Figure S10.

The AFM scanning image of conjugate **4f** (Figure 4 in the publication), was analysed with respect to the size and shape of the nanovesicles. In Figure S11 the histograms of particle diameters, heights and height/diameter ratios of 50 representative surface-deposited vesicles are shown.

RNApeptide S5-  
jerome  
File data from C:\PCS\JEROME\JEROME.sz2 Record 967  
S4700

Size(nm)	Intensity	Volume	Number
54.5	0.0	0.0	0.0
64.6	0.0	0.1	0.1
76.6	0.3	0.4	0.4
90.8	1.0	1.1	1.1
107.7	1.9	1.9	1.9
127.6	2.6	2.6	2.6
151.3	3.2	3.3	3.3
179.3	4.0	4.1	4.1
212.6	5.3	5.5	5.5
252.0	7.2	7.3	7.3
298.7	9.6	9.5	9.5
354.1	11.8	11.6	11.6
419.7	13.1	12.8	12.8
497.6	13.2	12.8	12.8
589.8	11.5	11.2	11.2
699.2	8.5	8.4	8.4
828.8	4.9	5.0	5.0
982.5	1.7	2.1	2.1
1164.6	0.1	0.5	0.5
1380.5	0.0	0.0	0.0
1636.5	0.0	0.0	0.0
1939.9	0.0	0.0	0.0
2299.6	0.0	0.0	0.0
2726.0	0.0	0.0	0.0

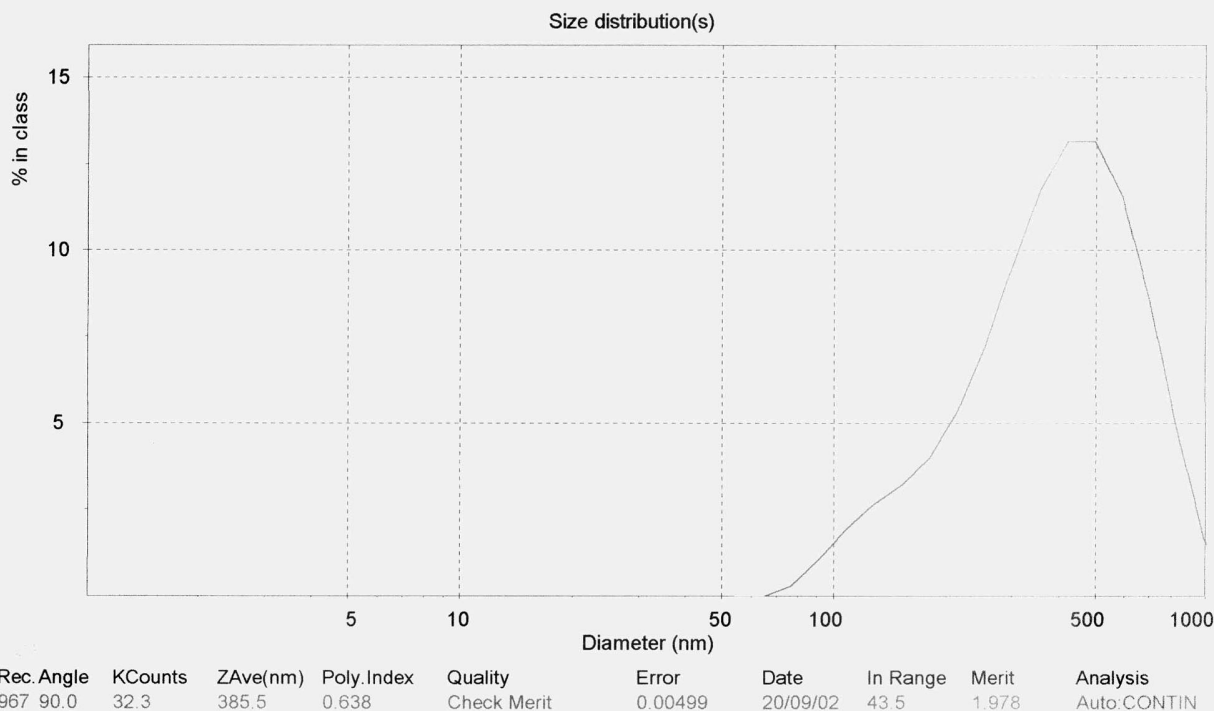
Peak Analysis by intensity			
Peak	Area	Mean	Width
1	100.0	425.3	529.5

Peak Analysis by volume			
Peak	Area	Mean	Width
1	100.0	428.3	542.6

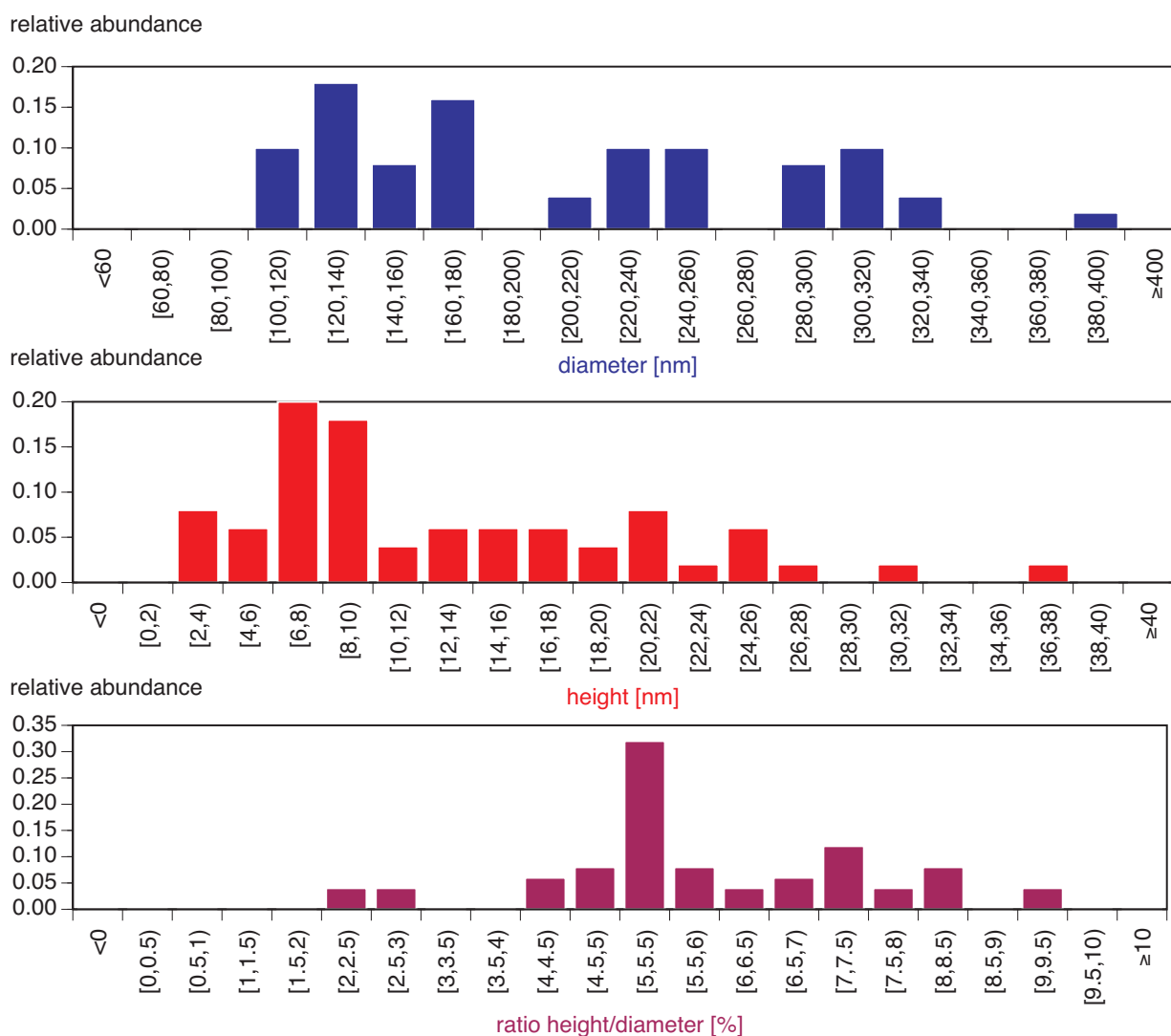
  

Peak Analysis by number			
Peak	Area	Mean	Width
1	100.0	428.3	542.6



Malvern Instruments Ltd, Malvern UK +44 1684 892456

**Figure S10.** Correlation function analysis of the particle size distribution of an aqueous solution of **4f** as determined by laser light scattering at an angle of 90°.



**Figure S11.** Vesicle diameter and height distribution after depositing and air-drying an aqueous solution of conjugate **4f** on a glass surface, as determined by AFM. The analysis of the scanning image was performed with 50 representative vesicles.

## 10. References

- [1] H. Li, X. Jiang, Y.-H. Ye, C. Fan, T. Romoff, M. Goodman, *Org.Lett.* **1999**, *1*, 91.
- [2] V. K. Sarin, S. B. Kent, J. P. Tam, R. B. Merrifield, *Anal. Biochem.* **1981**, *117*, 147.
- [3] T. Brown, C. E. Pritchard, G. Turner, S. A. Salisbury, *Chem.Comm.* **1989**, 891.
- [4] R. Warras, J.-M. Wieruszkeski, C. Boutillon, G. Lippens, *J. Am. Chem. Soc.* **2000**, *122*, 1789.
- [5] M. Bodanszky, J. Martinez, *Synthesis*, **1981**, *5*, 333.
- [6] I. Schwope, C. F. Bleczinski, C. Richert, *J. Org. Chem.* **1999**, *64*, 4749.
- [7] E. Biała, P. Strazewski, *J. Am. Chem. Soc.* **2002**, *124*, 3540.