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Research paper

Development of linear β-turn inducers containing peptides as arc mimetics with DNA topological and sequence selectivity

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ABSTRACT

In general, biological macromolecules such as proteins interact with the major groove of the ds-DNA via hydrogen bonds formation, thus blocking the site access of TFs to specific DNA sequences. Considering that the primary sequence of arc repressor responsible for DNA binding is well-characterized as well as the 3D-conformational requisites for its optimal interactions with the specific DNA base-pairs, a series of well-tailored arc analogues could be designed using computational molecular tools and available structural data. These novel molecular entities have been synthesized following ultrasound assisted-solid phase peptide synthesis (US-SPPS), characterized by NMR experiments and screened for TAGA box selectivity on DNA oligomers using a battery of DNA displacement assays. Data obtained show a clear tendency of peptide **ACAS_4** to assume a 3-D β-sheet like structure responsible of the interaction with DNA major groove and to bind selectively to the consensus sequence of DNA. For the best of our knowledge this is the first report on a β-sheet arc mimetic endowed with topological and sequence selectivity for the TAGA box of DNA.

1. Introduction

The design of peptides with a β-sheet like structure as arc mimetic is an appealing goal in the field of drug design considering its novelty and specificity related to the site-selective DNA binding. This aspect is fundamental because most of the proteins and their synthetic analogues described till now are characterized by alpha-helix binding motif joining both minor and major grooves for DNA recognition [1]. Arc repressor belongs to the TFs superfamily called ribbon-helix-helix (RHH), which is extremely prevalent in bacteria, while they are absent in eukaryotes. The archaeo-viral TFs share similarities with both their bacterial and eukaryotic counterparts and the eukaryote-like transcription system can be regulated by bacterial-like regulators [2].

While the structural characterization of TFs is underway, their functional characterization *in vivo* is limited by the genetic tractability of viruses, which renders the research's evolution slow and cumbersome [3]. The majority of TFs encoded by archaeal viruses are repressors and many of them are subjected to autoregulation, like arc repressor in Salmonella bacteriophage P22 [4].

Salmonella systemic infections claim thousands of lives worldwide even today; infection by Salmonella Typhimurium results in a self-limiting gastroenteritis in humans that frequently can contaminate meat products. Many temperate phage induction occurs following DNA damage, which induces the bacterial SOS system, leading to repressor

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inactivation by cleavage [5,6]. Bacteriophage P22 is one of the best studied model systems for viral assembly and infections. In 1985 Sauer et al. cloned and purified arc and mnt genes onto plasmids that overproduce arc protein; N-terminal sequences and amino acid compositions were determined together with its binding site on DNA [7,8]These data, in combination with previously determined gene sequences, establish the complete protein sequence of 53 residues for arc isolated from Salmonella phage P22. During lytic growth of P22, arc binds as dimer to its operator site on DNA major groove sequence called TAGA box in a site-specific manner, and represses its own synthesis as well as that of antirepressor. The mature P22 and the tail machine have been recently studied by cryo-electron microscopy (cryo-EM), thus providing new insights into P22 infection initiation on *Salmonella enterica* serovar Typhimurium [4,9].

Diverse crystal structures of free arc protein, its DNA complex and biochemical data are reported in literature (*e.g.* 1PAR, 1ARR, 1ARQ), revealing a specific DNA operator-site recognition by a well-defined β-sheet motif [9,10].

Though the primary sequence of arc protein responsible for DNA binding is known together with the 3D-conformational requisites necessary for its optimal interactions with the specific base-pairs into the major groove of the DNA, a series of well-tailored arc analogues could be designed using computational molecular tools and available structural data. Some of them have been already developed and characterized by us [11,12], they have been also screened for TAGA box selectivity on DNA1/2 oligomers using a first battery of DNA assays, however we were not able to reach a selective binding to a specific DNA-sequence [11,12].

The preparation of such molecular entities as peptides of various geometry, length and molecular weight requires manual solid-phase peptide synthesis (SPPS), purification and structural characterization by NMR experiments. In this work novel arc peptidomimetics have been synthesized (*e.g.* **ACAS_1**–**8**) by ultrasound assisted-solid phase peptide synthesis (US-SPPS) using a greener protocol, a series of binding assays on diverse DNA strains have been conducted with the aim to identify those sequences able to interact efficiently and selectively with the target TAGA-box into the major groove of the DNA.

2. Materials and methods

2.1. Chemicals

Ethydium bromide (EB) dye was purchased from Sigma Aldrich (Merck Group). EB was dissolved as a concentrated stock solution (~5 mg/mL) in H_2O, and serial dilutions were made from this stock. Concentrations of the dye were determined in H_2O using the following extinction coefficients supplied by Molecular Probes: EB = 5.5×10^3 M^{-1} cm^{-1} (546 nm). DNA1 (5′-GCGAGTAGAGCTTTTTGCTCTACTCGC-3′) and DNA2 (5′-GCGAGCACAGCTTTTTGCTGTGCTCGC-3′) oligomers were provided from Biomers (Germany) and used without further purification. DNAs were dissolved in 10 mM Tris-HCl buffer (pH 7.4) and then annealed to allow the formation of their corresponding hairpin structures. Peptides **ACAS_1**–**8** were prepared by a well-established US-SPPS protocol, with some modifications (see Supplementary Material for experimental details) [13,14]. They were analyzed by analytical RP-HPLC and ESI-MS to assess their purity and to confirm their chemical identity (Supplementary Material, Tables S1 and S2).

2.2. Circular dichroism (CD)

CD spectra were carried out on a Jasco-715 coupled with a thermostat Nestlab RTE-11, using an acquisition range: 250-190 nm; bandwidth: 2.0 nm; resolution: 0.2 nm; accumulation: 3 scans; sensitivity: 10 mdeg; response time: 0.25 s, speed: 100 nm/min. CD measurements were made in a 1 mm cell at 20 °C. Peptides **ACAS_1**–**8** (80 μM) were dissolved in water solution or 10 mM phosphate buffer (pH 7.5) containing 100 mM of NaCl as indicated. The experiments with DNAs were carried out on a Jasco J-815 spectropolarimeter equipped with a PTC-423S/15 Peltier temperature controller. The oligonucleotides (DNA1 and DNA2) were prepared at a concentration of 20 μM in 10 mM Tris-HCl buffer (pH 7.4), and their concentration verified by measuring the UV absorption at 90 °C, ensuring denaturation and separation into single strands. This minimizes interference from secondary structures (like hairpins or double-stranded regions), which can skew the absorption measurement. Concentrations were determined using the appropriate molar extinction coefficient values ε (λ = 260 nm), calculated as previously described [15]. DNA samples were annealed before CD measurements, as described elsewhere [16] CD spectra of DNA/-peptide mixtures were obtained in 10 mM Tris-HCl buffer (pH 7.4) by adding 4 mol equiv of each peptide (**ACAS_1**–**8**) with respect to the oligonucleotide. Spectra of DNA molecules in the absence and presence of peptides were recorded at 20 and 100 °C in the wavelength range of 200–320 nm using a scan rate of 100 nm/min, with a 0.5 s response time and 1 nm bandwidth. CD melting experiments were carried out in the 20–100 °C temperature range at 1 °C/min heating rate by following CD signal changes at the wavelengths of the minimum CD intensity (256 nm). CD melting experiments were performed in the absence and presence of the peptides (4 mol equiv) added to the folded DNA structures. The apparent melting temperatures (T_m) were determined from a curve fit using Origin 7.0 software, and the ΔT_m values calculated as the difference in the T_m values of the DNA structures in the presence and absence of the peptide [17]. All experiments were performed in triplicate, and the reported values are the average of the three measurements.

2.3. NMR spectroscopy

The samples for NMR spectroscopy were prepared by dissolving the appropriate amount of peptide **ACAS_3**,**4**,**7**,**8** in 0.18 mL of H_2O and 0.02 mL of ^2H_2O (pH 5.5), to obtain a concentration 0.1 mM in peptide solution. NMR spectra were recorded on a Varian INOVA 700 MHz spectrometer equipped with a z-gradient 5 mm triple-resonance probe head. All the spectra were recorded at a temperature of 25 °C. One-dimensional (1D) NMR spectra were recorded in the Fourier mode with quadrature detection. The water signal was suppressed by gradient echo [18]. 2D DQF-COSY [19–21], TOCSY [22], and NOESY [23] spectra were recorded in the phase-sensitive mode using the method from States [24]. Data block sizes were 2048 addresses in t2 and 512 equidistant t1 values. Before Fourier transformation, the time domain data matrices were multiplied by shifted $\sin^2$ functions in both dimensions. A mixing time of 80 ms was used for the TOCSY experiments. NOESY experiments were run with a mixing time of 100 ms. The qualitative and quantitative analyses of DQF-COSY, TOCSY, and NOESY spectra, were obtained using the interactive program package XEASY [25]. Due to signal broadening, a complete ^1H NMR chemical shift assignments were effectively achieved only for peptide **ACAS_7** according to the Wüthrich [21] procedure via the usual systematic application of DQF-COSY, TOCSY, and NOESY experiments with the support of the XEASY software package (Supplementary Material, Table S3).

As for the NMR study of the interaction between selected peptides and DNA1/2, oligonucleotides were prepared at a concentration of 20 μM in 10 mM Tris-HCl buffer (pH 7.4) and annealed before NMR measurements. NMR spectra of DNA/peptide mixtures were recorded in the same buffer by adding 0, 0.5, 1, 2, 8, and 10 mol equiv of the peptide with respect to the oligonucleotide. For the K_D calculation, the experimental points were fitted by a nonlinear regression method, using the Origin 7.0 software.

2.4. Ethidium bromide displacement assay

Ethidium bromide (EB) displacement spectra were obtained with a Jobin-Yvon Fluoromax-3 (DataMax 2.20) coupled to a Wavelength Electronics LFI-3751 temperature controller, using the following settings: increment: 1.0 nm; integration time: 0.1 s; excitation slit width:

4.0 nm; emission slit width: 6.0 nm at 20 °C. In the case of ethidium bromide, the excitation wavelength applied was 540 nm and the emission spectra were acquired from 565 to 650 nm. For each assay 1 μM of pre-folded DNA1 or DNA2 targets and 4.5 μM of EB were used, and the corresponding DNA/EB mixtures were stirred at room temperature for 1 h before use. Next, each peptide (**ACAS_1**–**8**) was added to the corresponding DNA/dye solution to a final concentration of 15 μM. Stock solutions of peptides were 2 mM in ultra-pure H_2O.

3. Results and discussion

3.1. Design and synthesis

We have recently described a series of peptides showing a β-hairpin structure which was designed to mimic the β-sheet region of the arc repressor interacting with the DNA. In particular, we have found a peptide with β-sheet like structure able to interact efficiently with the major grove of DNA, thus we assumed this peptide (namely, peptide **6**, Table 1) as the *lead compound* for further structural modifications [11].

Such linear peptide presents the Gly-Asn (GN) dipeptide, which is a type II' β-turn inducer that was found to be optimal for DNA interaction. GN dipeptide has been retained in all of the new sequences. Peptide **6** contained the Q1, N3, R5, Q8, N10, R12 as DNA interacting residues and the W2,4,9,11 forming the so-called Trp-zipper structure aimed at stabilizing the β-hairpin [26]. Firstly, we inverted the Q and R residue positions as suggested by the comparison of the arc dimer β-sheet (Fig. 1A) with the model structure of peptide **6** (Fig. 1B) [11] obtaining peptides **ACAS_1, 2** (Table 1). Then, considering the non-interacting face of the peptides we both left the $Phe^{2,9}$ and $Leu^{4,11}$, residues of the arc dimer (**ACAS_1**, **2**) or replaced them with Trp's to obtain the Trp-zipper (**ACAS_3**, **4**). 3D models of peptides **ACAS_1**–**4** are reported in Fig. 1C. Furthermore, to achieve the correct overlapping with the protein β-sheet also regarding the backbone direction we added Tyr and Thr at the positions i and i+3 of the β-turn, respectively.

As shown in Fig. 1D, the last modification allowed the positioning of both the side chains and the backbone corresponding to those of the β-sheet of the arc dimer.

The Tyr and Thr residues were chosen to enhance the β-hairpin stability, in fact, they are the most common residues found in the indicated positions in proteins for a type II' β-turn [27]. The addition of the two residues, combined with the aforementioned Trp-zipper mutation, gave peptides **ACAS_5**–**8** (Table 1).

Since these peptides could undergo to unspecific ionic interactions with DNA, an acetyl capping has been alternately applied on the N-terminal amino acid (**ACAS_2**, **4**, **6**, **8**) and all the original sequences have been prepared as *C*-terminal amides (**ACAS_1**–**8**).

Regarding the synthetic protocol applied to the preparation of titled peptides, we performed a well-planned US-SPPS in order to promote a fast recovery of the crude compound involving also, a greener approach to the coupling reactions on resin (see Supplementary Material).

In particular, we choose the Cyrene solvent for washing and coupling reactions on vessel; Cyrene is a dipolar, safe for end-of-life solvent with aprotic behavior. It's an alternative to common solvents by REACH as substances of very high concern (SVHC), *e.g.* *N*-Methylpyrrolidone (NMP) and *N*,*N*-Dimethylformamide (DMF) [28]. It was proved to be able to completely solubilize coupling reactants with a good thermal stability on US bath reactions. Pure peptides have been obtained in good overall yields and high purity >90 % following RP-HPLC tandem MS purification and analytical RP-HPLC for purity checking.

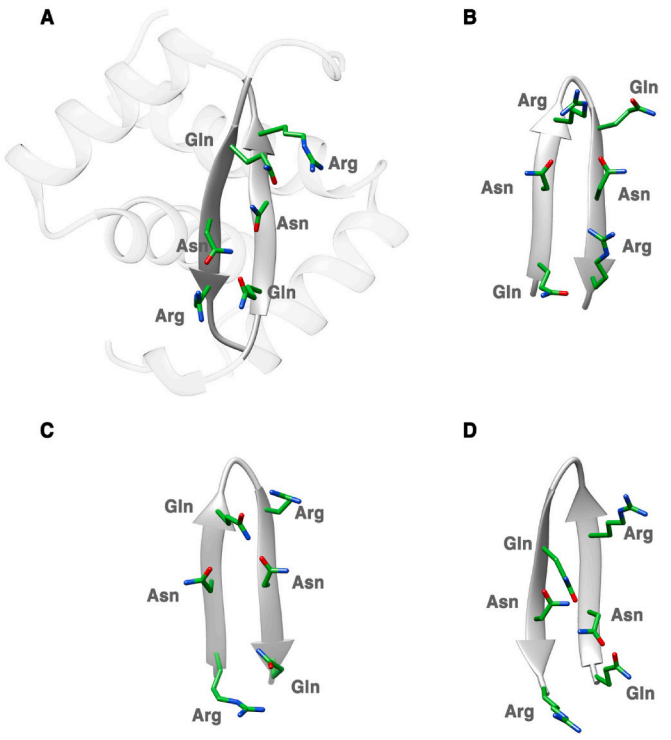

Fig. 1. Comparison of protein arc dimer conformation (pdb code 1PAR) [9] (**A**), with models of peptide **6** (**B**), peptides **ACAS_1**–**4** (**C**), and peptides **ACAS_5**–**8** (**D**). Strand directions are indicated with arrows. For sake of clarity, only the side chains of DNA interacting residues are shown (color code: carbon, green; oxygen, red; nitrogen, blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Amino acid sequences of the novel synthetic peptides.

	COMPOUND	SEQUENCE
Lead compound	**6**[a]	Q-W-N-W-R-G-N-Q-W-N-W-R-NH_2
Novel peptides	**ACAS_1**	R-F-N-L-Q-G-N-R-F-N-L-Q-NH_2
	ACAS_2	Ac-R-F-N-L-Q-G-N-R-F-N-L-Q-NH_2
	ACAS_3	R-W-N-W-Q-G-N-R-W-N-W-Q-NH_2
	ACAS_4	Ac-R-W-N-W-Q-G-N-R-W-N-W-Q-NH_2
	ACAS_5	Q-F-N-L-R-Y-G-N-T-Q-F-N-L-R-NH_2
	ACAS_6	Ac-Q-F-N-L-R-Y-G-N-T-Q-F-N-L-R-NH_2
	ACAS_7	Q-W-N-W-R-Y-G-N-T-Q-W-N-W-R-NH_2
	ACAS_8	Ac-Q-W-N-W-R-Y-G-N-T-Q-W-N-W-R-NH_2

[a]*Lead compound* described in a previous work [11], sequence in violet. In black are shown the conserved residues of the arc repressor. In red is shown the β-turn inducer. In green are shown mutated Trp residues. In blue, the acetyl-capping of the N-terminus.

3.2. Conformational analysis

CD spectra of all peptides were acquired in water to establish their intrinsic conformational tendencies (Fig. 2). Many of them, peptides **ACAS_1**,**2,5,6**, don't have a well-defined structure as indicated by the minimum at about 200 nm, **ACAS_3**, **4** CD spectra in water strictly resemble those of the trp-zipper peptides with intense exciton-coupled bands at 215 and 229 nm, indicating interaction between the aromatic chromophores [26]. The shoulder at about 200 nm indicates a certain degree of flexibility of the two peptides. In peptides **ACAS_7** and **ACAS_8**, the same exciton coupled bands are also present in the CD spectra but their intensities are relatively weak compared to the minimum at 200 nm pointing to a prevalence of disordered conformations. Peptides **ACAS_3,4** composed of 12 amino acids, contain Trp residues in position 2,4,9,11 as for peptide **6**, while in **ACAS_1,2,5,6** this residue is completely absent; peptides **ACAS_7,8** composed by 14 residues, also possess four Trp. Considering that two strands are joined by the same β-turn inducer in all of them, we can suppose that this feature is not enough to force the structure in the β-hairpin conformation; the incorporation of the four Trp residues in peptides **ACAS_3,4,7,8** favor the rearrangement in the desired 3-D conformation which is well-defined in linear structures of 12 amino acids *e.g.* peptide **6**. The insertion of two residues Y and T in peptides **ACAS_7,8** (14 residues linear sequences) probably induces different solvent/peptide-peptide interactions which disfavor their ability to assume the β-hairpin conformation.

Starting from CD results, NMR analysis was performed on peptides **ACAS_3,4,7**, and **8** in water solution. Due to peptide aggregation at concentration as low as 100 μM, proton spectra of peptides **ACAS_4,8** showed broad lines, with the worst situation observed for **ACAS_8** which did not allow for any proton signal assignments (Fig. S1, Supplementary Material). Also **ACAS_3** showed different broad signals which hampered the assignment, while peptide **ACAS_7** proton signals could be fully assigned (Table S3, Supplementary Material). However, NMR diagnostic parameters of **ACAS_7**, specifically $^{3}J_{\alpha N}$ coupling constants, Hα chemical shift deviations, and the absence of medium- and long-range NOEs, all indicate the prevalence of random conformation in accordance with its CD spectrum.

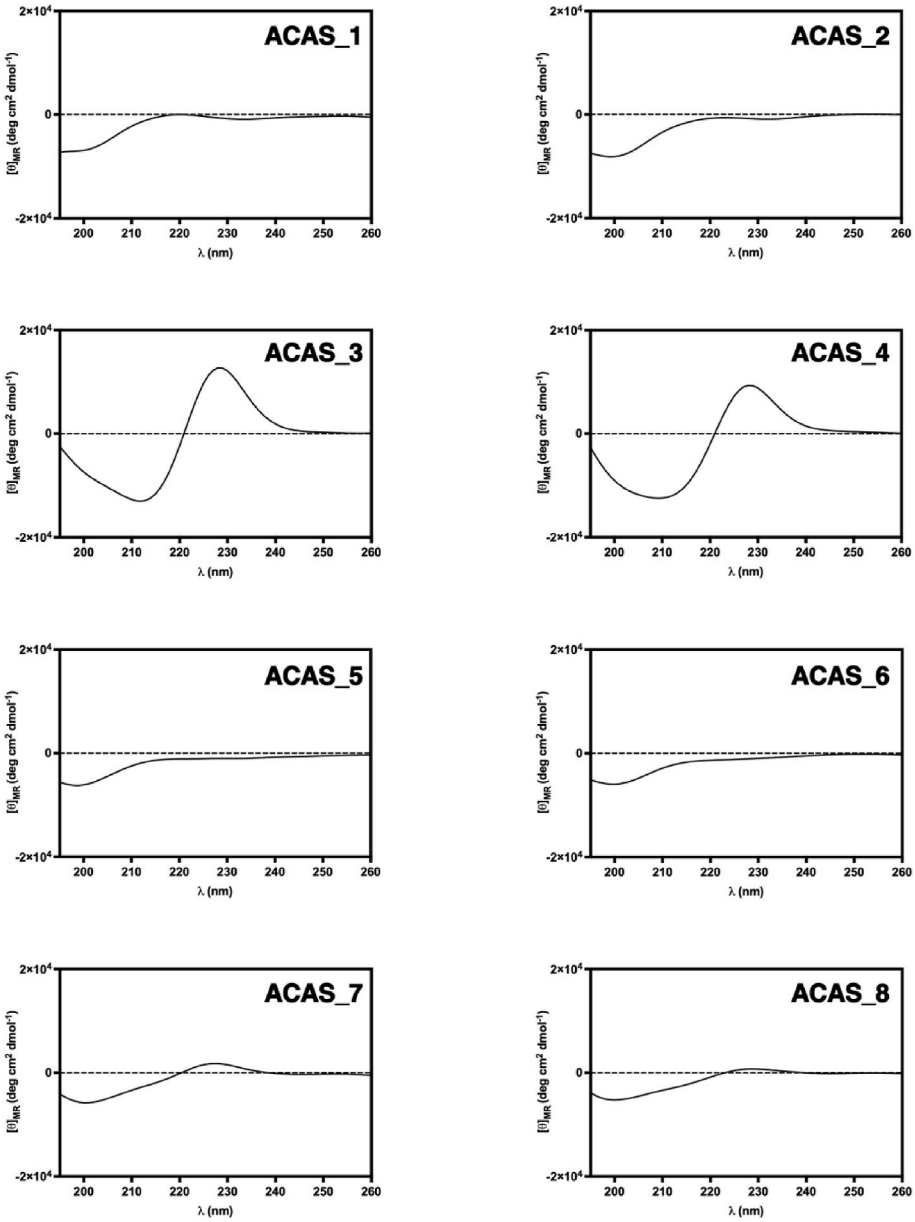

Fig. 2. CD spectra of peptides **ACAS_1**–**8** in water solution.

3.3. Interaction of β-hairpin-forming peptides with DNA oligomers

All of our novel peptides were studied to investigate their ability to interact with DNA, using both the ds-oligonucleotide DNA1 containing the consensus target TAGA (ds-DNA1: 5′-GCGAG TAGA GC TTTTT GC TCTA CTCGC-3′) and DNA2 with a double mutation in the consensus sequence (ds-DNA2: 5′-GCGAG CACA GC TTTTT GC TGTG CTCGC-3′).

Ethidium bromide fluorescent probes was considered in a displacement assay [29]. The fluorescence intensity of the dyes increases remarkably upon binding to DNA, while a marked quenching of the fluorescence is expected if the peptide competes for the same binding site of the dye. In the case of EB displacement from DNA1, a slight but significant effect was observed for peptides **ACAS_4** and **8** (Fig. 3) especially for the first one and, notably, this effect was completely absent on DNA2. This interesting result deserved further investigation, thus selected peptides/DNA interactions were examined by probe independent methods.

3.4. CD and melting point studies on DNA and selected peptides

In light of the peptides ability to form β-hairpin deduced by their CD spectra and/or to modulate the fluorescence emission of EB fluorescence dye, **ACAS_3,4,7** and **8** were further studied. First, peptides alone were investigated in Tris buffer (pH 7.4, 80 μM, Fig. 4, first column). As in water solution, peptides **ACAS_7** and **ACAS_8** were mostly random at all the tested concentrations, as well as peptide **ACAS_3** showed the characteristic dichroic shape of a trp-zipper peptide (see above). Differently from its behavior in water, in Tris buffer peptide **ACAS_4** showed a different shape of the CD spectrum clearly indicating that it is almost unstructured in this buffer.

Then, it was investigated whether peptide addition changes the CD spectrum of the DNAs (considering the lack of absorbance at 280 nm for these peptides) and their effects on the thermal stabilization of DNA. Interestingly, only peptide **ACAS_4** induced significant changes in the spectrum of DNA1 and, to a lesser extent, of DNA2, causing a decrease in the intensity of the dichroic band around 280 nm (Fig. 4, second and fourth columns, respectively). Moreover, the comparison of the spectra of the peptide alone and in the presence of DNA reveals some differences in the region ranging from 200 to 240 nm. Indeed, the appearance of a minimum at 225 nm in the **ACAS_4**/DNA1 CD spectrum suggests that the peptide underwent a conformational change upon binding of the DNA.

As for the effect of peptides on the melting temperatures of DNAs, it was investigated only for DNA1. For DNA2, this analysis was not possible due to the lack of a sigmoidal shape in its melting curve (Supplementary Material, Fig. S2). Interestingly, peptide **ACAS_4** caused a significant increase of the T_m (4.4 °C, Fig. 4 third column) of DNA1, indicating a slight but significant stabilization of the DNA1 structure [30]. Other peptides gave less intense and/or negative variations, the last are mostly indicative of non-specific interactions with the DNA.

3.5. NMR investigation of the peptide ACAS_4/DNA interaction

After the interesting Tm's results, interaction of peptide **ACAS_4** with both DNA's was investigated by solution NMR. In particular, both DNA1 and DNA2 were titrated with **ACAS_4**. Upon titration, **ACAS_4** caused selective shifts of a few DNA signals: the signal at 8.20 ppm assignable at the H-8 of the guanines, the signals at 7.18 ppm 7.14 ppm attributable to the H-6 protons of thymine and cytosine residues (Fig. 5A and B). Very interestingly, all these protons are located and directed towards the

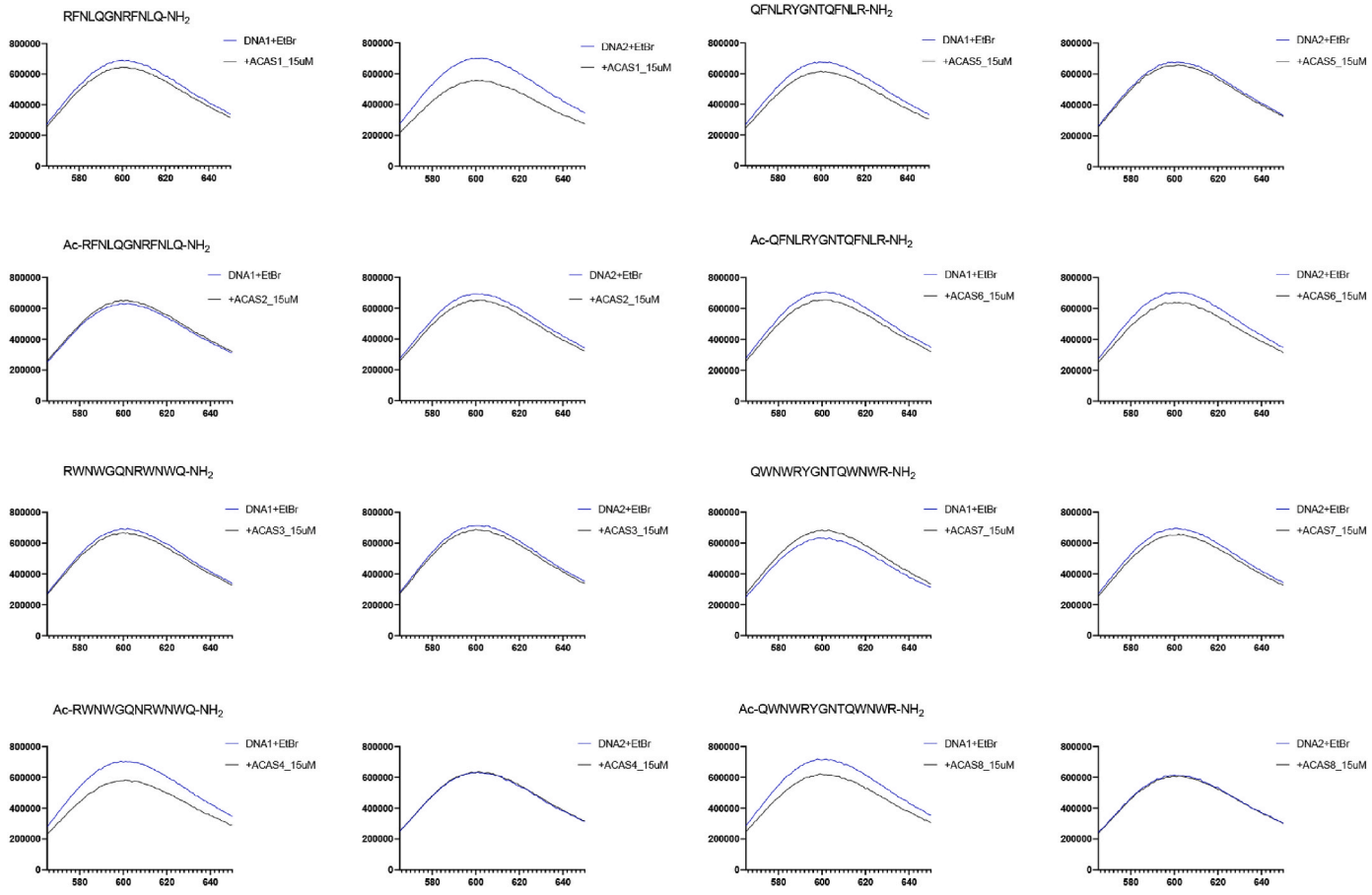

Fig. 3. Displacement of ethidium bromide (EB) from DNA1 and DNA2 by the investigated peptides.

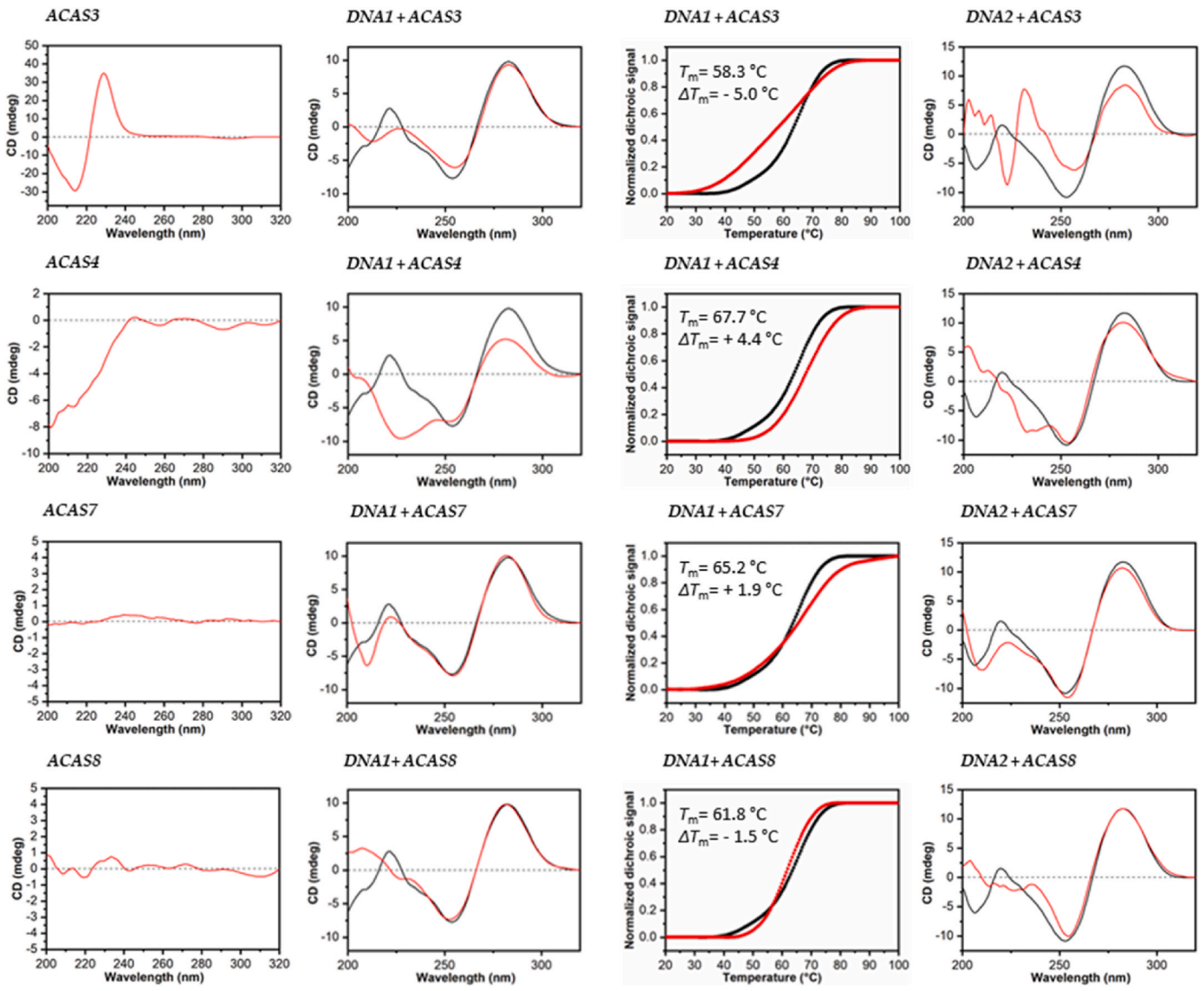

Fig. 4. (*Column 1*) CD spectra of peptides **ACAS_3,4,7,8** in Tris-HCl solution (pH 7.4); (*Column 2*) CD spectra of DNA1 in the absence (black) and presence (red) of 4 mol equiv. of each peptide; (*Column 3*) CD melting curves of DNA1 in the absence (black) and presence (red) of 4 mol equiv. of each peptide; (*Column 4*) CD spectra of DNA2 in the absence (black) and presence (red) of 4 mol equiv. of each peptide. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

major groove of a B-DNA which is a net indication that the peptide binds the same region occupied by the arc dimer β-sheet as illustrated in Fig. 6. Notably, the observed shifts are more intense in DNA1 than in DNA2 (Supplementary Material, Fig. S3) again pointing to some degree of selectivity of **ACAS_4** towards DNA1. Moreover, DNA imino protons were not affected by the binding demonstrating that the peptide does not perturb the DNA conformation (Supplementary Material, Fig. S4).

The titration of DNA1 with **ACAS_4** was also used for a quantitative evaluation of the binding affinity, yielding an affinity constant K_D of 21.7 μM (Fig. 5C). Interestingly, titration of DNA1 with **ACAS_8** by NMR resulted in a negligible shift of the oligonucleotide signals (Supplementary Material, Fig. S5) confirming the superiority of **ACAS_4** compared to the other peptide.

4. Conclusion

Conceiving peptides able to bind the specific DNA sequence is a challenging task at the borderline of medicinal chemistry and chemical biology. The majority of peptides binding DNA in nature are α-helix structures thus the design of β-sheet like peptides endowed with the same activity represents a step forward in the discovery of novel ligands with high affinity and selectivity for the target. In this work we have developed new linear β-hairpin peptides as mimetics of the β-sheet arc repressor aiming at interacting with DNA TAGA-box sequence; among them, peptide **ACAS_4** displays a β-sheet structure able to bind DNA consensus sequence with good selectivity into the major groove and towards the TAGA-box. This is particularly interesting because none of our previously described peptides possess the capacity to interact selectively with DNA1 strain. Although the peptide shows a high tendency to aggregate, its interesting properties make it a valuable *lead compound* in the discovery of novel agent able to interfere with the arc repressor activities.

CRediT authorship contribution statement

Azzurra Stefanucci: Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Federica Santoro:** Investigation, Conceptualization. **Sara D'Ingiullo:** Formal analysis, Data curation.

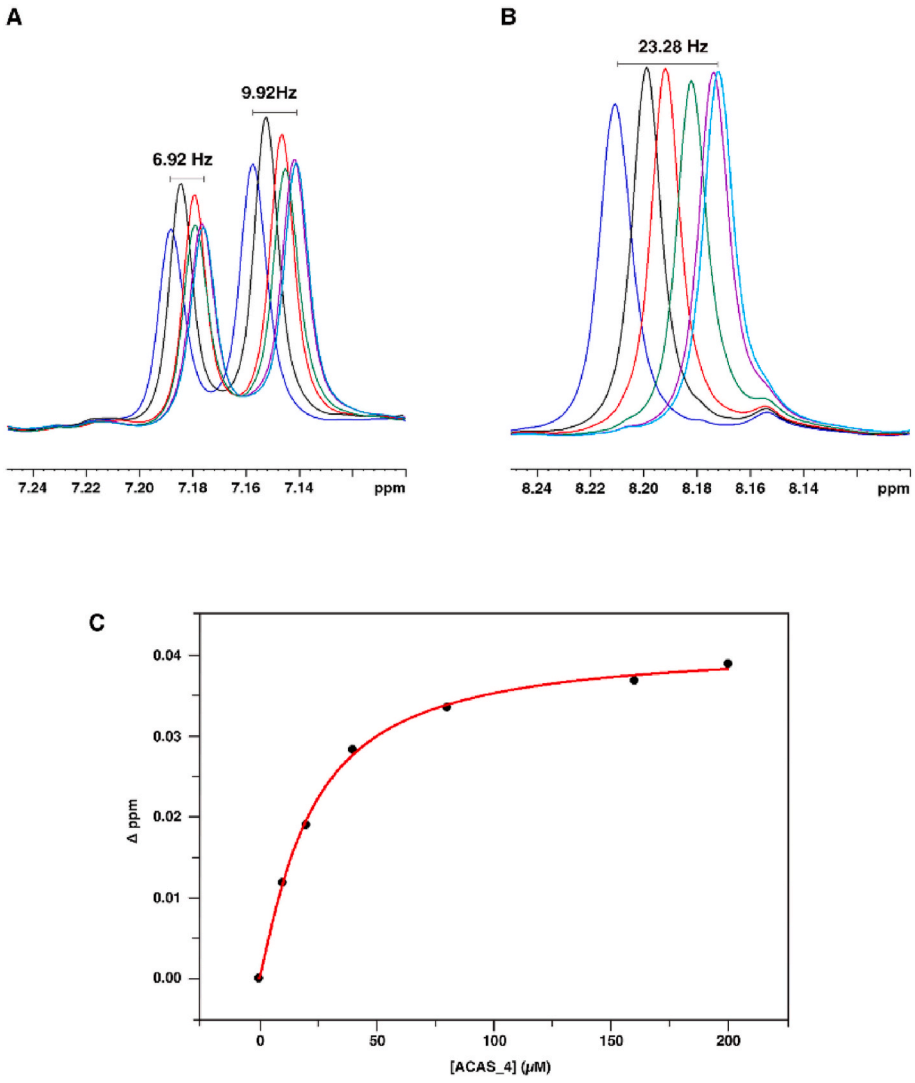

Fig. 5. DNA1 proton signal shifts upon titration with **ACAS_4**. (**A**) thymine and cytosine H6 signals; (**B**) guanine H8 signals in the absence (blue) and presence of 1/2 mol equiv. (black), of 1 mol equiv. (red), of 2 mol equiv. (green), 8 mol equiv. (violet), and of 10 mol equiv. (sky-blue) of **ACAS_4**. Shifts from 0 mol equiv. to 10 mol equiv. are reported in Hz. (**C**) Titration curve of DNA1 with **ACAS_4** obtained by tracking the shifts of the guanine H8 signal. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

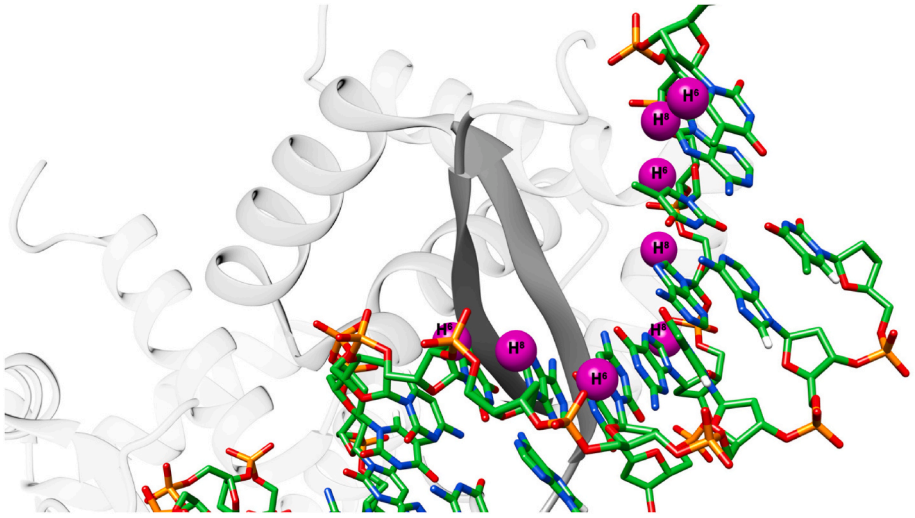

Fig. 6. Illustration of the DNA protons mainly involved in **ACAS_4** binding (evidenced as magenta balls) as determined by NMR titration depicted within the arc dimer complex.

Lorenza Marinaccio: Formal analysis, Data curation. **Eleonora Procino:** Formal analysis, Data curation. **Soraya Learte-Aymamí:** Methodology. **Jessica Rodriguez:** Methodology. **José Luis Mascareñas:** Methodology. **Jussara Amato:** Visualization, Software. **Valentina Arciuolo:** Visualization, Software. **Antonio Randazzo:** Visualization, Software. **Martina De Rosa:** Visualization, Software. **Diego Brancaccio:** Validation, Supervision, Funding acquisition. **Adriano Mollica:** Writing – original draft, Supervision. **Alfonso Carotenuto:** Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2025.117423.

Data availability

Data will be made available on request.

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