Synthetic approaches to nucleopeptides containing all four nucleobases, and nucleic acid-binding studies on a mixed-sequence nucleo-oligolsyne†

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In this article we describe two solid-phase synthetic routes to obtain a nucleo-oligolsyne α-peptide containing all four natural nucleobases. The first one is based on the oligomerization of the nucleobase-containing monomers, easily synthesized as herein described. The second strategy has the advantage of avoiding the solution synthesis of the monomeric building blocks, leading to the final nucleopeptide by direct solid-phase couplings of the suitably protected nucleobases with the free amino groups on the growing peptide chain still anchored to the resin. Both strategies are general and can be applied to the synthesis of nucleopeptides having backbones formed by any other diamino acid moiety decorated with the four nucleobases. We also report the CD and UV studies on the hybridization properties of the obtained nucleopeptide, containing all four nucleobases on alternate lysines in the sequence, towards complementary DNA and RNA strands. The nucleo-oligolsyne with a mixed-base sequence did not prove to bind complementary DNA, but was able to recognize the complementary RNA forming a complex with a higher melting temperature than that of the corresponding RNA/RNA natural duplex and comparable with that of the analogous PNA/RNA system.

Our previous studies on the thymine-based oligolysine suggested that base-driven interactions have a major role in the nucleic acid-binding, with respect to the expected electrostatic interactions. Furthermore, CD titrations with both homoadenine DNA and RNA sequences gave a 1:1 base/base complex stoichiometry, on the contrary, upon replacing the undecorated lysine spacers between two adjacent thymine-functionalized residues with l-arginine residues, complexes characterised by a 2:1 T/A binding stoichiometry were observed.*

Nevertheless, these findings did not enable us to obtain detailed information on the nature of the complexes formed between the homothymine oligolysine and the homoadenine nucleic acids. To this purpose, specific hybridization experiments with mixed-sequence nucleopeptides were required to prove the ability of this oligomer to recognize complementary DNA or RNA sequences by sequence-specific hybridization, not aided by the possible formation of a ternary complex stabilized by Hoogsten-type recognition, as in the case of PNA triplexes typically formed when homopurine and homopyrimidine strands are mixed.*

In this context, we designed and realized an oligomer with an oligo-α-L-lysine backbone bearing all the four nucleobases, and here reported the CD and UV-binding experiments with complementary DNA and RNA strands. We also described two alternative synthetic approaches for the preparation of
nucleobase-bearing oligo-\(\alpha\)-lysines with mixed-base sequences, which could be easily extended to other nucleopeptides with the backbone based also on different amino acids. One of the synthetic routes is based on the solid-phase oligomerization of the nucleo-lysine monomers, ad hoc synthesized, coupled in alternate manner with respect to underivatized lysines. The second strategy involves the on-line assembly of both the oligolysine backbone and the suitably protected carboxymethyl-nucleobases directly on the solid support, thus avoiding some synthetic and chromatographic steps for the obtainment of the nucleoamino acid monomers.

**Results and discussion**

Nucleobase-amino acid conjugates, such as nucleoamino amides and nucleopeptides,\(^{10-15}\) are interesting molecular systems endowed with several biological properties including the ability to interact with nucleic acids. Among others, a very important biological target is poly[A], an important RNA target involved in several biological processes,\(^6\) whose ligands show high potential as therapeutics and are, thus, extensively investigated.\(^{17-20}\) Diamino acid-based nucleopeptides with homo- or heteronucleobase sequences are easily obtainable by solid-phase peptide synthesis using protected nucleoamino acid monomers (ad hoc synthesized being generally not commercially-available),\(^9\) or by first assembling the desired peptide backbone using e.g. Dde/Fmoc protected monomers, followed by final nucleobase decoration, after selective Fmoc removal, using a molar excess of the suitable nucleobase acetic acid.\(^{21}\) For both strategies, properly protected amino acids can be introduced, whose side-chain groups can be regenerated after the insertion of the bases, in order to have a spacer between two consecutive nucleobase-functionalized amino acids, as well as useful moieties such as the guanidinium or ammonium groups.

The synthesis of a mixed-sequence nucleopeptide is more complex than that of a homosequence oligomer requiring four different, non-commercially available monomers that have to be ad hoc synthesized.

**Synthesis of the Fmoc-protected nucleolysines and their use for the nucleopeptide synthesis**

In the present study we first realized the new A, C and G nucleolysines (see Fig. S1†) adopting the same synthetic strategy we previously used to obtain the thymine monomer (Scheme 1A). The four nucleoamino acids have been then used in the oligomerization of a mixed-sequence oligolysine by solid-phase peptide synthesis using PyBOP/DIEA as activating system (Scheme 1B).

The LC ESI-MS profiles for the nucleoamino acids and nucleopeptide 1, both well soluble in aqueous solutions, are showed in Fig. S2 (ESI†) and Fig. 1, respectively. However, the yield for this oligomerization was low (less than 5%) since the coupling of the A, C, G nucleoamino acids was more difficult than with unfunctionalized lysine and thymine nucleoaminoacid, probably due to the steric hindrance caused by the nucleobase protecting groups.

In summary, the above-described synthetic approach had two major disadvantages: (1) it required the synthesis and purification of all the four nucleolysines, and (2) afforded the desired nucleopeptide in low yield. For this reason, special efforts have been subsequently devoted to the design of a more convenient and less time-demanding strategy for the realization of the heteronucleobase-functionalised nucleopeptide (Fig. S3†). Thus, we developed a solid-phase synthetic procedure using as starting materials the carboxymethyl-nucleobases, protected with acid-labile groups on the aromatic amino groups, and two different protected lysine monomers, i.e. the commercially available Fmoc-Lys(Boc)-OH and Dde-Lys(Fmoc)-OH. The proper nucleobase sequence was obtained by an on-line solid-phase strategy. More in detail, first, the lysine monomer with the Boc semi-permanent protecting group on its side chain, was anchored onto the amino group of the Rink-amide resin (step a, Scheme 2); then, after Fmoc-removal, the coupling with the Dde-Lys(Fmoc)-OH was accomplished (step b, Scheme 2).

Successively, a rapid treatment with 2% DBU in DMF allowed the selective removal of the Fmoc group from the lysine side

![Scheme 1](image-url)  
**Scheme 1** Synthetic schemes for the preparation of (A) the nucleoamino acid monomers and (B) the nucleopeptide of mixed-base sequence 1.
chain in the presence of the Dde protection: the obtained free ε-amino group was then coupled with the desired carboxymethyl-nucleobase (step c, Scheme 2). Removal of Dde from the lysine ε-amino group was achieved by treating the resin with a 2% hydrazine solution in DMF in order to perform the nucleopeptide-chain elongation. Steps a, b and c were repeated until the nucleopeptide with the desired base sequence was obtained. Following this protocol, a dodecapeptide oligolysine containing six nucleobases (2, Scheme 2) was achieved with a 31% overall yield, in terms of the peptide backbone (as determined by UV measurements of the released Fmoc groups). Since the ε-efficiency of the single nucleobase insertion cannot be determined on-line during the solid-phase synthesis, two consecutive couplings were performed to assure the best yields. After deprotection and cleavage of nucleopeptide 2 from the solid support with concentrated TFA, precipitation in cold diethyl ether and lyophilisation of the redissolved crude, HPLC purification on a RP-C18 column allowed to recover about 0.9 μmol of the purified product, i.e. 10% of the initial functionalization. ESI-MS characterization confirmed the identity of the desired nucleopeptide, which was well soluble in water as expected from the initial design (see Fig. 2).

The synthetic strategy reported in Scheme 2 is general and can be adapted to obtain other nucleopeptides with mixed nucleobase sequences by using diamino acids (or other amino acids as spacers) with properly protected amino groups (possibly commercially available) and the protected carboxymethyl-nucleobases. In this way, tedious purification steps relative to the synthesis of the nucleoaminoacid monomers can be avoided.

CD and UV spectroscopic studies

Subsequently, we studied by CD and UV spectroscopies the interaction of 2 with complementary DNA and RNA single strands. To this purpose, a two-chamber quartz cell (tandem cell) was used with each of the two reservoirs containing separately the nucleopeptide and the nucleic acid (AAAT[U]GC, DNA or RNA) solution at pH 7.5 and 5 °C. First, the CD profile of the two separate solutions was recorded (Fig. 3A and S4† black lines).

Successively, we recorded the CD spectrum after mixing the two solutions, (Fig. 3A and S4† red lines). In case of DNA no appreciable change in the CD profile upon nucleopeptide addition was observed (Fig. S4A†), as it would have been expected in the case of binding with DNA. On the contrary,
changes in both shape and intensity (ΔCD267 = 0.88 mdeg) of the CD spectrum with respect to the sum spectrum were observed in case of the nucleopeptide–RNA binding assay, as showed in Fig. 3A. Analogously, also sum and mix UV spectra were different in this case (Fig. 3B), and more in detail, a hypochromic effect was observed upon mixing the two ligand solutions, proving that the interaction with the nucleopeptide involved both H-bonding and stacking effects between complementary nucleobases. On the contrary, sum and mix UV spectra in the case of the binding experiment with DNA were almost superimposable (data not shown) (Fig. 4).

Furthermore, UV thermal denaturation experiment on the nucleopeptide–RNA complex evidenced a sigmoid-like curve with a Tm of about 26 °C consistent with the nucleopeptide–RNA binding. Remarkably, this Tm is about 10 °C higher than the corresponding melting temperature expected for r(GCAUUU)-r(AAAUGC) and d(GCATTT)-r(AAAUGC) double helices, as estimated by using Oligo-Calculator software (http://biotools.nubic.northwestern.edu/OligoCalc.html). Instead, no cooperative transition was observed in the UV-monitored melting of the system nucleopeptide/DNA (Fig. S4B, ESI†). In addition, a standard PNA of the same sequence as the nucleo-oligolysine (i.e. NH2-gcattt-CONH2) was used as reference for the stability of the RNA complex: UV-melting experiments evidenced a Tm of 28 °C for the PNA/RNA system (Fig. S5 and ESI†), which is similar to that relative to the nucleopeptide/RNA complex.

Finally, we conducted an analogous binding study with a double-stranded DNA (see Fig. S6, ESI†). Upon nucleopeptide addition, the CD spectrum of the duplex was not altered, thus showing that neither strand displacement nor formation of Hoogsteen base pairs occurred. Furthermore, UV thermal denaturation studies showed no variation of its melting temperature (data not shown).

Overall, our findings suggest that: (i) nucleo-oligolysine is able to induce conformational changes upon binding the complementary RNA strand; (ii) the structure of the formed complex is stabilized by W–C base pairs that lead to a duplex structure with stability higher than the corresponding natural counterparts (RNA/RNA and DNA/RNA) and similar to the analogous PNA/RNA system; (iii) the heteronucleobase
oligolysine nucleopeptide does not alter the DNA CD bands in the here-studied conditions nor form complexes with DNA with cooperative transition upon UV-monitored melting. The latter result is apparently in contrast with our previous findings for the homothymine oligomer, in which the complex with the homoadeninic DNA was probably based on a more stable triple helix structure (involving both W-C and Hoogsteen base pairings). In any case, the ability to selectively bind RNA, discriminating complementary DNA sequences, is commonly regarded as a highly desirable feature for antisense applications. In fact, we suggest that our antisense nucleopeptide could prove beneficial in those cases in which selectivity in binding RNA and not DNA tracts is required.

**Experimental section**

**Abbreviations**

Ac2O (acetic anhydride); Bhoc (benzhydryloxycarbonyl); CD (Circular Dichroism); DBU (diazabicyclo[5.4.0]undec-7-ene); Dde [1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-ethyl]; DIEA (N,N-diisopropylethylamine); DMF (dimetilformamide); Fmoc (9-fluorenylemethoxycarbonyl); HATU ([7-azabenzotriazole-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate); N-Boc Gly (N-tert-butoxycarbonylglycine); NMP (4-methylpyrrolidone); NMR (Nuclear Magnetic Resonance); PyBop (benzotriazole-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate); TFA (trifluoroacetic acid); TIS (triisopropyl silane); TMP (2,4,6-trimethylpyridine).

**Chemicals**

Dde-L-Lys(Fmoc)-OH, Fmoc-L-Lys(Boc)-OH, HATU, PyBOP were purchased from Novabiochem. Anhydrosan DMF and NMP were purchased from LabScan. Piperidine was Biosolve. Anhydroscan DMF and NMP were purchased from Novabiochem. Anhydrosan DMF and NMP were purchased from LabScan. Piperidine was Biosolve. Solvents for HPLC chromatography and acetic anhydride were purchased from Reidel-de Haén. TFA and TMP and Rink-amide resin were from Fluka. RNA and DNA oligomers in single strand form of sequence AAATGC, were purchased from Biomers.

**Apparatus**

Samples were analysed and characterized by LC-MS on an MSQ mass spectrometer (ThermoElectron, Milan, Italy) equipped with an ESI source operating at 3 kV needle voltage and 320 °C, and with a complete Surveyor HPLC system, comprising an MS pump, an autosampler, and a PDA detector, by using a Phenomenex Jupiter Proteo column (4 μm, 90 Å, 4.6 × 150 mm). Gradient elution was performed by building up a gradient starting with buffer A (0.05% TFA in water) and applying buffer B (0.05% TFA in acetonitrile) with a flow rate of 0.8 ml min⁻¹ and monitoring at 260 nm.

MALDI-TOF mass spectrometric analysis of the reference PNA was performed on a TOF/TOF 5800 System in positive mode, using 2,5-dihydroxybenzoic acid (DHB) as the matrix. Analytical chromatograms were obtained on a Hewlett Packard/Agilent 1200 series HPLC, equipped with a diode array detector, by using a Phenomenex Jupiter C18 column (5 μm, 300 Å, 4.6 × 250 mm). Gradient elution was performed at 25 °C by building up a gradient starting with buffer A’ (0.1% TFA in water) and applying buffer B’ (0.1% TFA in acetonitrile) with a flow rate of 1.0 ml min⁻¹ and monitoring at 260 nm. Semi-preparative purifications were performed on a Hewlett Packard/Agilent 1100 series HPLC, equipped with a diode array detector, by using a Phenomenex Jupiter C18 column (10 μm, 300 Å, 10 × 250 mm). Gradient elution was performed at 25 °C by building up a gradient starting with buffer A’ (0.1% TFA in water) and applying buffer B’ (0.1% TFA in acetonitrile) with a flow rate of 4.0 ml min⁻¹ (monitoring at 260 nm). Samples were lyophilized in a FD4 Freeze Dryer (Heto Lab Equipment) for 16 h. Circular dichroism (CD) studies were conducted in analogy to other previous reports. The CD spectra were obtained at 5 °C on a Jasco J-715 spectropolarimeter with a Tandem Hellma quartz cell. UV spectra and UV thermal denaturation profiles were obtained on a UV-vis Jasco model V-550 spectrophotometer equipped with a Peltier ETC-505T temperature controller, using a Hellma quartz cell with a light path of 1 cm.

**Synthesis of Fmoc-protected nucleoamino acids**

The synthesis of the Fmoc-protected T monomer was performed according to our previous report, and by analogy with this procedure we here report also the preparation of the A, C and G nucleoamino acids using for the N-ε derivationation of the lysine moiety the commercially available nuclease acid acetates [B = A(Bhoc), T, C(p-tert-butyl benzoyl) or G(Bhoc)]. Indeed, after Boc deprotection of the Fmoc-Lys(Boc)-OH, the obtained Fmoc-protected diamin hexanoic acid (175 mg, 0.47 mmol) was dissolved in anhydrous NMP (2.0 ml), treated with DIEA (0.9 equiv., 83 μl, 0.42 mmol) and TFA (0.6 equiv., 42 μl, 0.28 mmol), and reacted with BCH2COOH (2.2 equiv., 1.03 mmol), previously preactivated by addition of HATU (2.0 equiv., 372 mg, 0.94 mmol) and DIEA (2.0 equiv., 175 μl, 0.94 mmol) and TMP (2.0 equiv., 140 μl, 0.94 mmol) in NMP (2 ml) for 2 min. After 1.5 h, the solvent was removed under vacuum and the crude was purified by RP-HPLC (RP C18 column, HPLC method: 25 to 70% of B over 25 min, fR ~28 min). Yields were higher than 80% for all four monomers.

A nucleolysine. LC-ESIMS (Fig. S2) m/z: 711.04 (found), 710.82 (expected for [C42H39N7O7 + Na]+); 755.66 (found), 754.83 (expected for [C42H39N7O7 + Na]+); 777.96 (found), 776.81 (expected for [C42H39N7O7 + Na]+).

G nucleolysine. LC-ESIMS (Fig. S2) m/z: 727.86 (found), 726.82 (expected for [C42H39N7O8 + CO2 + H]+); 770.41 (found), 769.66 (expected for [C42H39N7O8 + CO2 + H]+).

C nucleolysine. LC-ESIMS (Fig. S2) m/z: 681.88 (found), 680.79 (expected for [C18H41N2O2 + H]+); 702.74 (found), 702.77 (expected for [C18H41N2O2 + Na]+).
solid-phase synthesis of oligoynucleotides

Route 1. The cationic nucleopeptide 1 (NH2-Gly-TkTkGGkAaAaTkTk-KONH2) was assembled on Rink amide MBHA resin (0.54 mmol g\(^{-1}\), 3.7 mg) by a Fmoc synthetic strategy similarly to other literature examples,\(^{16-24}\) using the four Fmoc-protected nucleoamino acid monomers, prepared as described above, and classical lysine amino acid [Fmoc-Lys(Boc)-OH]. More in detail, after Fmoc removal by treatment with 25% piperidine in DMF at 30 min, a mixture of the nucleobase monomer (A, T, C or G, 150 \(\mu\)mol of a 40 mM solution in NMP, 6 \(\mu\)mol, 3 equiv.) and PyBOP (150 \(\mu\)mol of a 80 mM solution in NMP, 12 \(\mu\)mol, 6 equiv.) and DIEA (4 \(\mu\)l, 24 \(\mu\)mol, 12 equiv.), or alternatively a mixture of Fmoc-Lys(Boc)-OH (150 \(\mu\)mol of a 0.31 M solution in NMP, 46 \(\mu\)mol, 23 equiv.) with PyBOP (150 \(\mu\)mol of a 0.32 M solution in NMP, 48 \(\mu\)mol, 24 equiv.) and DIEA (16 \(\mu\)l, 96 \(\mu\)mol, 48 equiv.) was introduced in the reactor and stirred at room temperature. After 20 min the liquid phase was removed from the resin which was washed with DMF, and subsequently, the unretracted amino groups were capped by treatment with N-Boc glycine (45 \(\mu\)mol, 0.1 M solution)/PyBOP (48 \(\mu\)mol)/DIEA (16 \(\mu\)l, 96 \(\mu\)mol, 48 equiv.) in DMF for 15 min. The Fmoc group was removed by treatment with 2% DBU in NMP for 20 min. The above-described procedure for the sequential introduction of the underivatized lysines and nucleoamino acid residues was repeated 6 times. The cleavage from the resin and protection of the nucleopeptide was performed after final glycine insertion (Scheme 1) by treatment with 1.5 ml of TFA/m-cresol/TIS/H2O (69/16/9/6, \(v/v/v/v\)) solution for 120 min. After precipitation from cold diethyl ether, the sample redissolved in milliQ H2O, was purified by RP-HPLC using a linear gradient of 10% (for 5 min) to 30% B’ in A over 30 min: \(t_R = 5.1\) min. The purified product was dissolved in a known amount of milliQ H2O and quantified by UV absorption at 260 nm by using the molar extinction coefficients of the A, T, C, and G PNA monomers (\(e_{260}\)) = 57 800 \(M^{-1}\ \text{cm}^{-1}\)) 84 nmol of nucleopeptide 1 (4.2% yield) were obtained; the compound was \(\geq 95\%\) pure by HPLC analysis. ESI MS /z: 681.41 (found), 681.02 (expected for \([\text{C}_{113}\text{H}_{180}\text{N}_{44}\text{O}_{26} + 2\text{H}]^{2+}\)\); 907.41 (found), 907.69 (expected for \([\text{C}_{114}\text{H}_{182}\text{N}_{45}\text{O}_{26} + 3\text{H}]^{3+}\)\); 1361.07 (found), 1361.03 (expected for \([\text{C}_{110}\text{H}_{182}\text{N}_{45}\text{O}_{26} + 2\text{H}]^{2+}\)\).

Route 2. The cationic nucleopeptide 2 (NH2-GKCKAATkTkTk-KONH2) was assembled on Rink-amide MBHA resin using a Fmoc synthetic strategy. In detail, 19.8 mg of resin (10.7 \(\mu\)mol of amino groups; functionalization: 0.54 mmol g\(^{-1}\)) were treated with 25% piperidine in DMF for 30 min for the Fmoc removal, and then the coupling with a solution of Fmoc-Lys(Boc)-OH (35.1 mg, 74.9 \(\mu\)mol, 7 equiv.)/HATU (27.6 mg, 72.8 \(\mu\)mol, 6.8 equiv.)/DIEA (26.0 \(\mu\)l, 149.8 \(\mu\)mol, 14 equiv.) in DMF (0.5 ml), pre-activated separately for 2 min, was performed over 20 min at room temperature. After capping of the unreacted NH2 groups with a solution of Ac2O/lutidine/DMF (2/17/v/v/v) for 2 \(\times\) 10 min, Fmoc removal was accomplished with a 2% solution of DBU in DMF for 2 \(\times\) 10 min and monitored spectrophotometrically. Subsequently, the coupling with the lysine monomer Dde-Lys(Fmoc)-OH, followed by the capping step, was performed as previously described. Then, a rapid treatment with a 2% solution of DBU in DMF (3 \(\times\) 3 min, alternating washing steps) allowed the selective removal from the side chain of the Fmoc group which was quantified by UV measurements. Free amino groups on the resin were coupled with the desired nucleobase acetic acid [T-CH2COOH, A(Bhoc)-CH2COOH, C(Boc)-CH2COOH, G(Bhoc)-CH2COOH, 7 \(\text{eq.}\)], preactivated for 2 min with HATU (7 \(\text{eq.}\)/DIEA (14 \(\text{equiv.}\)) in DMF, for 20 min at room temperature: a double coupling was performed in all cases. Then acetylation of the unreacted side chain amino groups was performed with Ac2O/lutidine/DMF (2/17/v/v/v) for 2 \(\times\) 5 min. Removal of the Dde groups was performed with a 2% NH4H2SO4 solution in DMF for 3 \(\times\) 3 min. The above-described procedure for the sequential introduction of the two kinds of lysines followed by the nucleobase coupling was repeated 6 times, until the following protected oligomer attached to the resin was obtained: ResinNH2-[Lys(Boc)-Lys(T)]-[Lys(Boc)-Lys(T)]-[Lys(Boc)-Lys(T)]-[Lys(Boc)-Lys(A Bhoc)]-[Lys(Boc)-Lys(CBoc)]-[Lys(Boc)-Lys(GBhoc)]\_OH, (from C to N terminus). A 31% overall yield in terms of the peptide backbone was determined by UV measurement of the Fmoc groups released. The cleavage from the resin and deprotection of the nucleopeptide was performed by treatment with 1 ml of TFA/pHENOL/TIS/H2O (85/10/2.5/2.5, v/v/v/v) for 90 min, followed by another 1 h treatment with 0.5 ml of the same solution. After TFA evaporation under a \(N_2\) flow and precipitation from cold diethyl ether, the crude pellet was resuspended in milliQ H2O and lyophilised. Then the crude sample was dissolved in \(A^\prime\) solution and purified by RP-HPLC using a linear gradient of 5% (for 5 min) to 30% B’ in A over 30 min: \(t_R = 15.2\) min. UV quantification, performed by dissolving the purified product in a known amount of milliQ H2O and measuring the absorbance at 260 nm, gave 0.9 \(\mu\)mol of the nucleopeptide 2 (10% yield); the compound was \(\geq 95\%\) pure by HPLC analysis. ESI MS /z: 644.22 (found), 643.75 (expected for \([\text{C}_{113}\text{H}_{180}\text{N}_{44}\text{O}_{26} + 4\text{H}]^{4+}\)\); 858.37 (found), 858.00 (expected for \([\text{C}_{113}\text{H}_{180}\text{N}_{44}\text{O}_{26} + 3\text{H}]^{3+}\)\); 1286.89 (found), 1286.49 (expected for \([\text{C}_{113}\text{H}_{180}\text{N}_{44}\text{O}_{26} + 2\text{H}]^{2+}\)\).

PNA synthesis

Reference PNA (NH2-gcattt-CONH2) was obtained manually under standard conditions\(^{28-31}\) on a 2 \(\mu\)M scale. The identity of the product was checked by MALDI-TOF MS: /z: 1648.74 (found), 1648.63 (expected for \([\text{C}_{66}\text{H}_{86}\text{N}_{32}\text{O}_{20} + \text{H}]^{+}\)\); 1671.68 (found), 1670.61 (expected for \([\text{C}_{66}\text{H}_{86}\text{N}_{32}\text{O}_{20} + \text{Na}]^{+}\)\).

Conclusions

In the present work two solid-phase syntheses of cationic nucleopeptides based on l-lysine containing all the four bases were described. In one case, we first synthesized in solution all the four nucleoamino acid monomers by a simple route, and...
RNA complex provided a sigmoid-like melting curve with denaturation studies demonstrated that the nucleopeptide/nucleic acid. On the other side, we found that the studied i.e. the Fmoc-Lys(Boc)-OH and Dde-Lys(Fmoc)-OH and the selective removal of the Dde protecting group in the presence of Fmoc was employed for the introduction of the carboxymethyl-nucleobases. The nucleopeptides obtained with both the synthetic strategies were analysed by LC-ESI MS which confirmed the identity of the expected products. These were well soluble in aqueous solutions and did not show any tendency to self-aggregate. The here described synthetic routes for the realization of the nucleo-oligolysines can be easily applied to the synthesis of other nucleopeptides with mixed base sequences starting from any other diamino carboxylic acid to be functionalised with the nucleobases, and using in combination any other natural or synthetic amino acid, possibly charged (e.g. L-arginine), as spacer unit.

Furthermore, we studied the binding of the nucleo-oligolysine with mixed-base sequence towards complementary DNA and RNA strands by CD and UV spectroscopies. In the case of DNA we did not observe any significant change of the DNA CD spectrum induced by the nucleopeptide nor even in the case of DNA we did not observe any significant DNA and RNA strands by CD and UV spectroscopies. In the present study, but presenting also different DNA tendencies. The here described synthetic routes can be easily applied to the synthesis of other nucleopeptides with mixed base sequences starting from any other diamino carboxylic acid to be functionalised with the nucleobases, and using in combination any other natural or synthetic amino acid, possibly charged (e.g. L-arginine), as spacer unit.

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