ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA CCA

Croat. Chem. Acta **2019**, *92*(2), 249–258
Published online: October 12, 2019
DOI: 10.5562/cca3542

HKD 1926

The Phenanthridine-modified Tyrosine Dipeptide: Synthesis and Non-covalent Binding to DNA and RNA

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RECEIVED: July 1, 2019 ✭ REVISED: September 5, 2019 ✭ ACCEPTED: September 5, 2019

THIS PAPER IS DEDICATED TO PROFESSOR KATA MLINARIĆ-MAJERSKI ON THE OCCASION OF HER 70TH BIRTHDAY

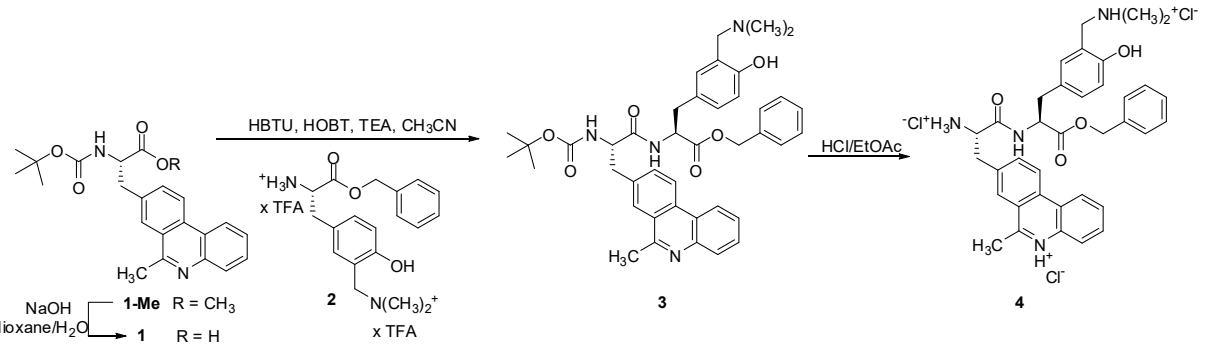
Abstract: Dipeptide **4** containing two unnatural amino acids, a modified tyrosine and a phenanthridine derivative, was synthesized. Binding of the dipeptide to a series of polynucleotides including ct-DNA, poly A - poly U, poly $(dAdT)_2$, poly dG - poly dC and poly $(dGdC)_2$ was investigated by thermal denaturation experiments, fluorescence spectroscopy and circular dichroism. Thermal denaturation experiments indicated that dipeptide **4** at pH 5.0, when phenanthridine is protonated, stabilizes ds-DNA, whereas it destabilizes ds-RNA. At pH 7.0, when the phenanthridine is not protonated, effects of **4** to the polynucleotide melting temperatures are negligible. At pH 5.0, dipeptide **4** stabilized DNA double helices, and the changes in the CD spectra suggest different modes of binding to ds-DNA, most likely the intercalation to poly dG- poly dC and non-specific binding in grooves of other DNA polynucleotides. At variance to ds-DNA, addition of **4** destabilized ds-RNA against thermal denaturation and CD results suggest that addition of **4** probably induced dissociation of ds-RNA into ss-RNA strands due to preferred binding to ss-RNA. Thus, **4** is among very rare small molecules that stabilize ds-DNA but destabilize ds-RNA. However, fluorescence titrations with all polynucleotides at both pH values gave similar binding affinity (log $K_a \approx 5$), indicating nonselective binding. Preliminary photochemical experiments suggest that dipeptide **4** reacts in the photochemical reaction, which affects polynucleotides chirality, presumably *via* quinone methide intermediates that alkylate DNA.

Keywords: non-covalent binding to polynucleotides, oligopeptides, quinone methide precursors.

INTRODUCTION

PEPTIDES have emerged as promising drug candidates,[1] although it is generally known that peptides are prone to intracellular enzymatic degradation. This problem can be circumvented by use of unnatural analogues modified by N-methylation,[2] cyclic peptides,[3] or oligopeptides containing noncanonical amino acids.[4] Thus, peptide based drug conjugates have recently been used for targeted delivery of toxic warheads to malignant tumor sites.[5] Furthermore, a special endeavor has been devoted to the understanding of the process of selective recognition of nucleobase sequences by oligopeptides leading to gene transcriptions.[6] Moreover, peptide based DNA/RNA intercalators have been discovered, which have potential to be developed into selective anticancer drugs or highly specific diagnostic tools.[7]

With the continuing interest in developing DNA/RNA targeting molecules I. Piantanida et al. prepared a series of phenanthridine derivatives[8] that were covalently linked by different alkyl spacers to one nucleobase[9] or to two nucleobases.[10] Investigation of noncovalent binding to different polynucleotides showed particularly interesting properties for phenanthridine derivatives tethered to adenine, which selectively recognized poly U.[9] On the other hand, incorporation of two nucleobases diminished antiproliferative activity.[10] Furthermore, phenanthridine, has recently been incorporated into an amino acid **1** (Scheme 1),[11] which was used in the synthesis of oligopeptides targeting nucleic acids.[11,12] In addition to non-covalent binding to polynucleotides, numerous anticancer drugs base their action on covalent modification of DNA, where cross-linking is particularly cytotoxic event



Scheme 1. Synthesis of dipeptide **4**.

leading to the cell death.[13] For example, anticancer antibiotic mitomycin exerts its antiproliferative action on metabolic formation of a reactive intermediate quinone methide (QM) that cross-links DNA.[14] Consequently, QMs have been intensively investigated reactive intermediates of phenol derivatives,[15] and their biological activity[16] has mainly been connected to the reactivity towards nucleosides[17] and alkylation of DNA.[18] Furthermore, S. Rokita et al. demonstrated reversible alkylation ability of QMs leading to "immortalization of QM" by DNA as a nucleophile,[19] whereas Freccero et al. reported ability of QMs to alkylate G4 regions of DNA.[20]

QMs are reactive intermediates that due to short lifetimes cannot be stored, they have to be prepared *in situ*. Photochemical methods offer much milder approach to QMs then the use of conventional synthetic methods, since photons are traceless reagents, and photoinitiated reactions allow for spatial and temporal control of the process, which is particularly important for biological systems.[21] The most common reactions to generate QMs in photochemical reactions are photodehydration[22] and photodeamination from the suitably substituted phenols.[23] An on-going interest is the photochemical generation of QMs from suitable precursors, and investigation of their biological effects.[24] Recently we incorporated QM precursor into tyrosine and showed that **2** (Scheme 1) remains photochemically reactive when incorporated in oligopeptide.[25] Herein we report the synthesis of dipeptide **4** (Scheme 1) containing unnatural amino acids **1** and **2**. The N-terminus contains phenanthridine amino acid **1**, which is anticipated to bind to polynucleotides by noncovalent interactions. On the other hand, the C-terminal amino acid is photochemically reactive tyrosine derivative **2** that is anticipated to deliver QM upon deamination and allow for covalent DNA modification. Covalent linking of QM precursors to DNA binding units is known to enhance reactivity of QMs with DNA.[26] Therefore, we investigated non-covalent binding of dipeptide **4** to different polynucleotides, by thermal denaturation experiments, fluorescence and CD spectroscopy. Understanding supramolecular interaction of this dipeptide is important for its potential application in DNA fluorescence labeling, or for the rational design of the next generation of DNA-targeting molecules.

RESULTS AND DISCUSSION

Synthesis

The synthesis of dipeptide **3** was based on the standard peptide coupling procedure where the N-site of **1** was protected by Boc and the C-terminus of **2** by Bn. The carboxylic functional group of **1** was activated by *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBT).[27] The dipeptide **3** was isolated in moderate yield, and by use of HCl/EtOAc it was transformed to triprotonated salt **4** (Scheme 1).

Non-covalent Binding to Polynucleotides

Studied compound was moderately soluble in aqueous solution and its UV-Vis spectra (see Figure S1 in the SI) corresponded well to previously studied analogues.[11,12]

All supramolecular studies were conducted in cacodylate buffered aqueous solution at pH 5.0 and 7.0, because two different prototropic forms of **4** are anticipated. Namely, the pK_a value of protonated phenanthridine is ≈ 6.0,[9] whereas the pK_a values for the phenolic OH and the trimethylamine moiety of amino acid **2** are 8.46 and 11.15, respectively.[23c] Therefore, at pH 7 compound **4** has 2+ net positive charge, while at pH 5 it has 3+ net positive charge. Since DNA/RNA is polyanion, such difference in charge could have pronounced effect on interactions of **4** with DNA/RNA.

Since many phenanthridine analogues bind to ds-DNA or ds-RNA by intercalation (e.g. ethidium bromide),[28] resulting in strong stabilization of double helices against thermal denaturation,[28,29] we investigated effects of **4** to thermal denaturation of ct-DNA (*calf thymus*-DNA), as well

Table 1. The ΔT_m values (°C)[a] of studied ds- polynucleotides upon addition of **4** at pH 5.0 or pH 7.0 (sodium cacodylate buffer, I = 0.05 mol dm^{-3}).

Polynucleotide	ΔT_m / °C	
	pH = 5.0	pH = 7.0
ct-DNA	3.4	0.3
poly A - poly U	−3.3 and −1.1[b]	−0.6 and 0.3[b]
poly $(dAdT)_2$	5.5	1.0

[a] Error in ΔT_m : ± 0.5°C; The melting temperature was determined from the inflection point of the dependence of absorbance on temperature.[30] r [**4**] / [polynucleotide] = 0.3.

[b] Biphasic transitions: the first transition at T_m = 44.3 °C is attributed to denaturation of poly A-poly U and the second transition at T_m = 67.1 °C is attributed to denaturation of poly AH$^+$-poly AH$^+$ since poly A at pH 5.0 is mostly protonated and forms ds-polynucleotide.[31]

as to synthetic AT-DNA sequence poly $(dAdT)_2$, and also poly A - poly U as a model for ds-RNA. Results compiled in Table 1 show that **4** at pH 5 induced only moderate stabilization effect to ds-DNAs, whereas the destabilization of ds-RNA was observed (see Figures S3–S5 in the SI) suggesting preferential binding of **4** to ss-RNA. At pH 7.0 the stabilization effects (ΔT_m) are less pronounced, in agreement with a deprotonation of the phenanthridine (pK_a ≈ 6.0[9]), demonstrating the importance of protonated phenanthridinium on the interaction with the polynucleotide.

To determine binding constants for the complexes of **4** with different polynucleotides including ct-DNA, poly A - poly U, poly $(dAdT)_2$, poly dG - poly dC, poly $(dGdC)_2$, taking

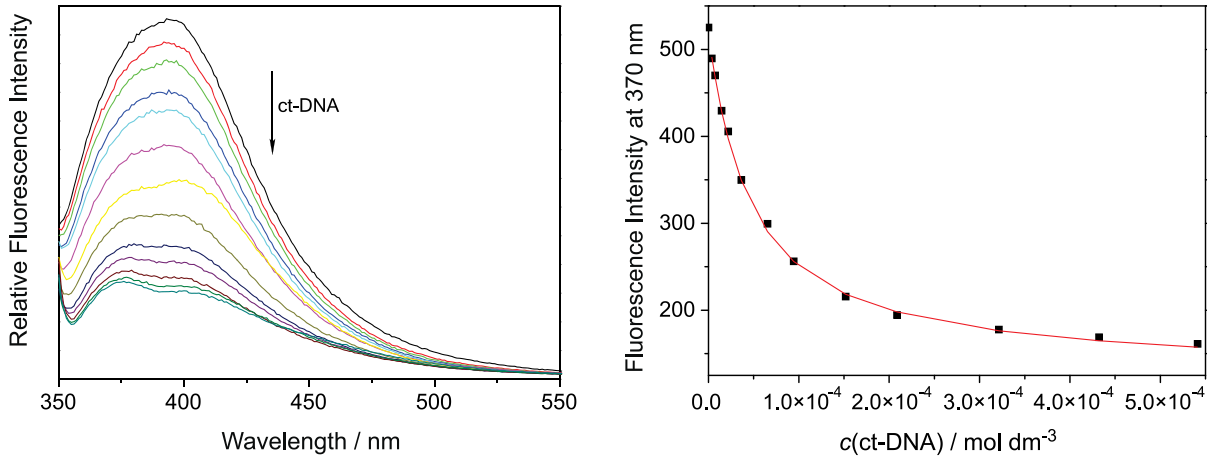


Figure 1. LEFT: Fluorimetric titration of **4** (c = 2.0×10^{-6} mol dm^{-3}, λ_{exc} = 330 nm) with *ct*-DNA in cacodylate buffer (I = 0.05 mol dm^{-3}, pH = 5.0, at 25 °C); RIGHT: dependence of the fluorescence intensity at 370 nm on the ct-DNA concentration; dots are experimental values and the red line is calculated non-linear fit according to the Scatchard model.[32]

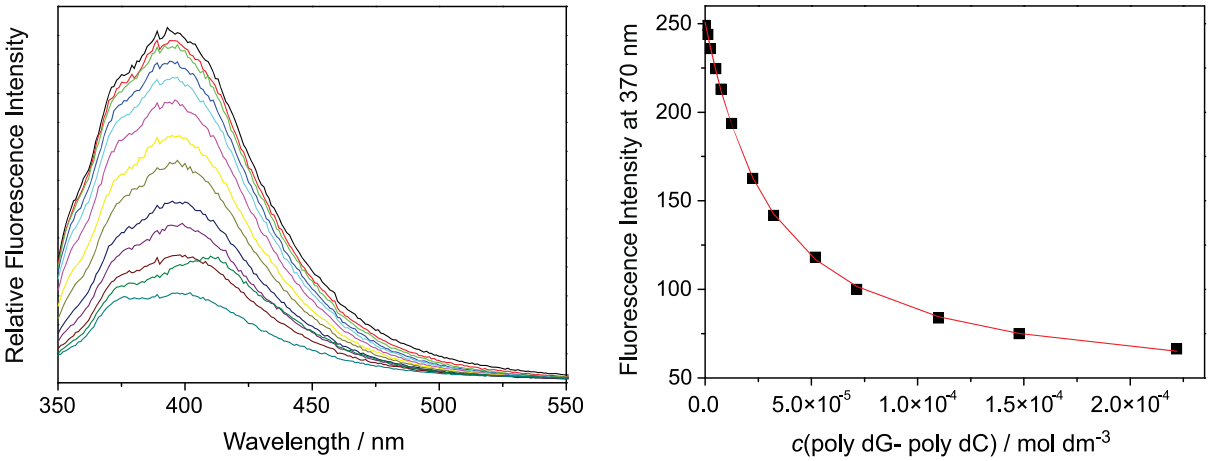


Figure 2. LEFT: Fluorimetric titration of **4** (c = 2.0×10^{-6} mol dm^{-3}, λ_{exc} = 330 nm) with poly dG – poly dC in cacodylate buffer (I = 0.05 mol dm^{-3}, pH = 5.0, at 25 °C); RIGHT: dependence of the fluorescence intensity at 370 nm on the poly dG – poly dC concentration; dots are experimental values and the red line is calculated non-linear fit according to the Scatchard model.[32]

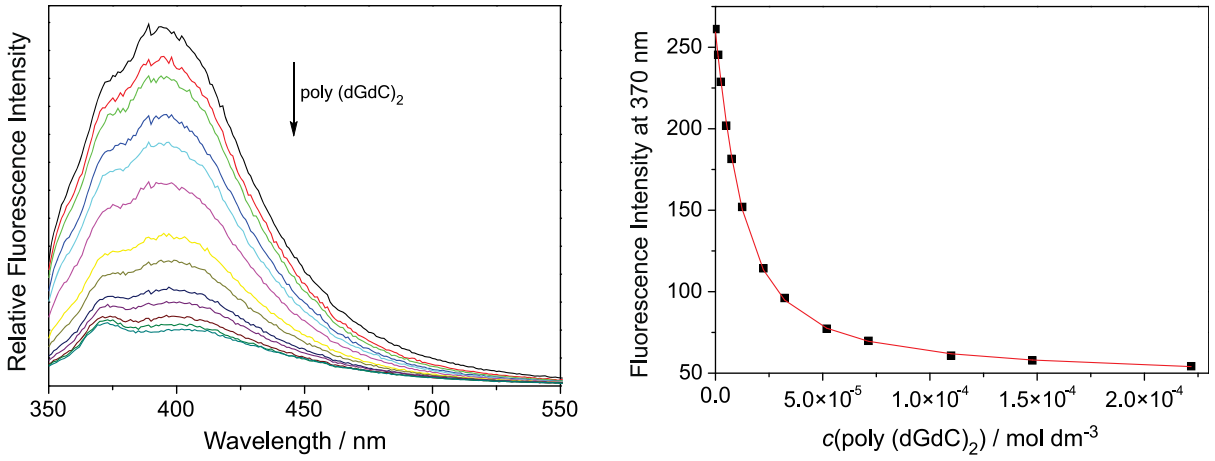


Figure 3. LEFT: Fluorimetric titration of **4** (c = 2.0×10^{-6} mol dm^{-3}, λ_{exc} = 330 nm) with poly (dGdC)$_2$ in cacodylate buffer (I = 0.05 mol dm^{-3}, pH = 5.0, at 25 °C); RIGHT: dependence of the fluorescence intensity at 370 nm on the poly (dGdC)$_2$ concentration; dots are experimental values and the red line is calculated non-linear fit according to the Scatchard model.[32]

advantage of intrinsic fluorescence of phenanthridine we performed fluorescence titrations. Similar to the thermal denaturation experiments, the titrations were conducted in cacodylate buffered aqueous solutions at two pH values, 5.0 and 7.0. In all titration experiments addition of ds-DNA/RNA to the solution of **4** resulted in fluorescence quenching. Some representative results obtained by fluorescence titrations are shown in Figures 1–4 (for other data see Figures S6–S11 in the SI). Processing of the titration data by Scatchard analysis[32] yielded binding constants (Table 2). It is interesting to note that similar values for the binding constants were observed (log K_a ≈5) for solutions regardless of the polynucleotide type and the solution pH. The fluorescence titration data indicate that dipeptide **4** nonselectively binds to polynucleotide chains, regardless of DNA or RNA type, with moderate binding constants and similar spectral responses.

Circular dichroism (CD) spectroscopy is a very valuable tool in the binding study of different small molecules to chiral macromolecules such as DNA, or peptides.[33] In particular, CD titrations can provide information on the binding mode of small molecules to

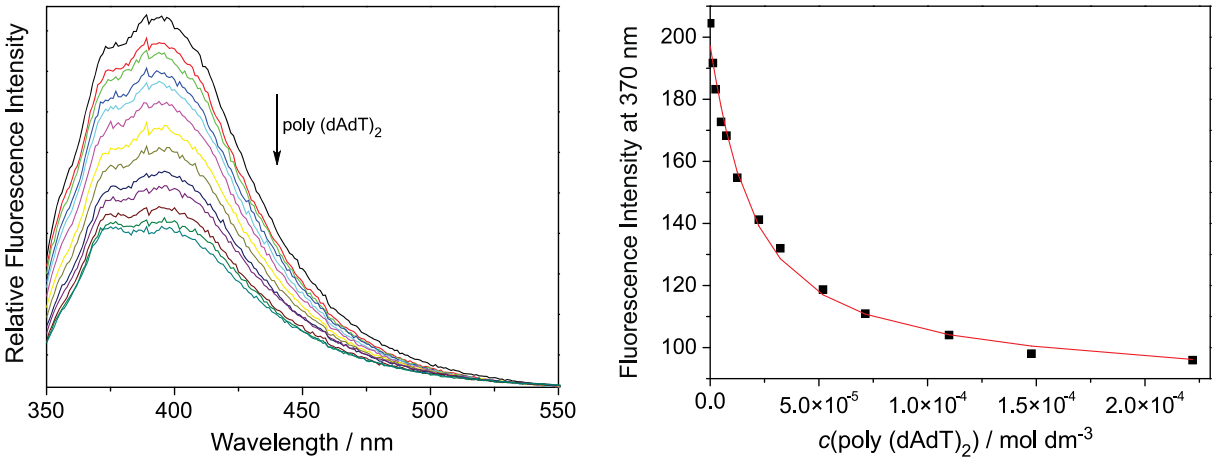


Figure 4. LEFT: Fluorimetric titration of **4** (c = 2.0×10^{-6} mol dm^{-3}, λ_{exc} = 330 nm) with poly (dAdT)$_2$ in cacodylate buffer (I = 0.05 mol dm^{-3}, pH = 5.0, at 25 °C); RIGHT: dependence of the fluorescence intensity at 370 nm on the poly (dAdT)$_2$ concentration; dots are experimental values and the red line is calculated non-linear fit according to the Scatchard model.[32]

Table 2. Binding constants (log K_a), ratio n [bound compound **4**]/[polynucleotide], and ratio of $(I/I_0)^{(b)}$, obtained from fluorescence titrations of **4** with different polynucleotides.[a]

	pH = 5.0		pH = 7.0	
	logK_a	I/I_0[b]	logK_a	I/I_0[b]
ct-DNA	5.1	0.3	4.9	0.3
poly A - poly U	5.2	0.4	5.0	0.6
poly dG - poly dC	5.3	0.2	5.1	0.2
poly (dGdC)$_2$	5.9	0.2	5.1	0.3
poly (dAdT)$_2$	5.1	0.3	5.9	0.5

[a] The titrations were performed in cacodylate buffered aqueous solutions (I = 0.05 mol dm^{-3}) at 25°C. The binding constants (log K_a) were obtained by nonlinear regression analysis of fluorescence data according to Scatchard equation.[32] In the fitting procedure ***n*** [bound compound **4**]/[polynucleotide] was kept constant at the value of 0.2, whereas the other parameters were freely adjustable.

[b] I_0 – starting fluorescence intensity of **4**; I – fluorescence intensity of **4**/polynucleotide complex calculated by Scatchard equation.

polynucleotide, with distinctive spectral differences for intercalators and groove binding derivatives.[34,35]

Although phenanthridine chromophore is not chiral, in compound **4** it is closely connected to the chiral center of amino acids, and in agreement with previously studied phenanthridine amino acids[11,12] showed positive CD band at 250 nm (Figure 5). Detailed comparison of CD spectra intensity revealed that phenanthridine amino acid **Phen-AA** (de-Boc **1**×2HCl, Scheme 1) had the weakest CD signal, whereas addition of glycine (Phen-AA-Gly) or tyrosine (**4**) increased the intensity due to somewhat better chiral organization. However, previously studied bis-phenanthridine or phenanthridine-thymine peptides showed bisignate CD spectra[11,12] characterized by distinctly coupled positive and negative CD bands attributed to the intensive intramolecular aromatic stacking interactions. Such a CD coupling was not observed for **4**, supporting dominant conformation in which the phenanthridine and tyrosine aromatic units do not stack with each other. Such a loose organization could be favorable for interactions of **4** with ds-DNA/RNA, since it leaves the phenanthridine unit free for interactions with polynucleotide, thus bringing the photoreactive QM precursor in the proximity to a DNA/RNA reaction site.

The titrations with **4** and different polynucleotides were conducted in cacodylate buffer (I = 0.05 mol dm^{-3}) at pH 5.0 or 7.0 in the range of concentration ratio ***r***[**4**]/[polynucleotide] = 0.1–0.7. Representative CD spectra are shown in Figures 6–8, whereas all CD data can be found in the SI (Figures S12–S14). In all cases, the addition of compound **4** to the solution of polynucleotide apparently decreased the negative CD signal of polynucleotide in the range 220–280 nm (Figure 6 and Figures S12–S14 in the SI), but this change was attributed to the positive CD signal for chiral peptide **4** in the 220–285 nm range, with a maximum at 245 nm (dotted black line in Figure 6), thus upon correction showing only negligible changes in the CD spectra of DNA/RNA. Such a behavior was observed for most polynucleotides at both pH values and suggested non-specific binding of the peptide in grooves along

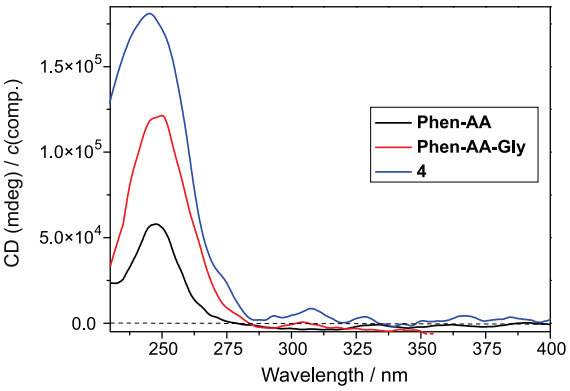


Figure 5. The CD spectra of **Phen-AA** (de-Boc **1**×2HCl),[12] **Phen-AA-Gly**[12] and **4** normalized for concentration at pH = 5.0 (buffer Na cacodylate, I = 0.05 M).

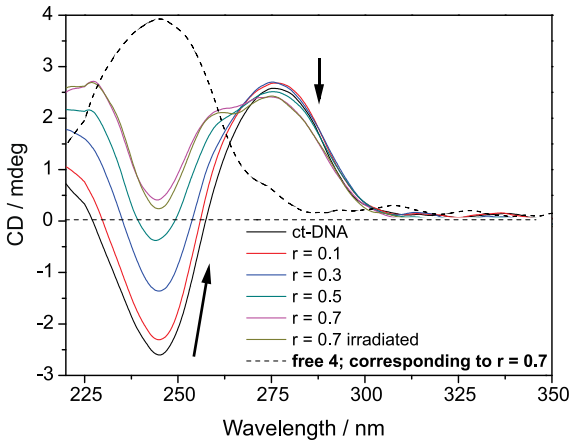


Figure 6. CD spectra of ct-DNA (c = 3.0×10^{-5} mol dm^{-3}) in cacodylate buffered aqueous solution (I = 0.05 mol dm^{-3}), at pH 5.0 in the presence of different ratio ***r***[**4**]/[ct-DNA]. Note the CD spectrum of free **4** (dotted line; conc. corresponding to r = 0.7).

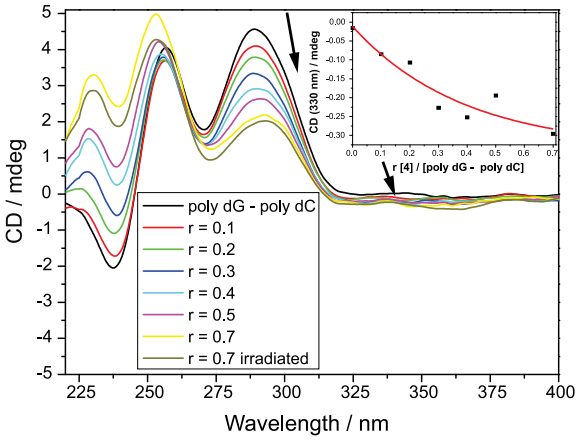


Figure 7. CD spectra of poly dG- poly dC (c = 3.0×10^{-5} mol dm^{-3}) in cacodylate buffered aqueous solution (I = 0.05 mol dm^{-3}, at pH 5.0) in the presence of different ratio r[**4**]/[poly dG- poly dC]; Inset: dependence of the induced CD signal at 330 nm on r[**4**]/[poly dG - poly dC].

ds-DNA/RNA with chromophores of **4** poorly oriented in respect to the polynucleotide chiral axis (thus no ICD bands).

However, several peculiar results were obtained. For poly dG - poly dC at pH 5 addition of **4** induced a pronounced decrease of the band at 289 nm, accompanied with a bathochromic shift of 5 nm (Figure 7). It should be stressed that this change is not due to the overlapping positive CD signal of **4**, which should cause increase of the CD band instead of the observed decrease. Detailed inspection of results showed a weak negative ICD band in 300–370 nm range (Figure 7, Inset), its intensity non-linearly increasing with the ratio r[**4**]/[poly dG - poly dC]. Both, decrease of the DNA CD band and the weak ICD band in 300–370 nm range are characteristic for the intercalation of phenanthridine into ds-polynucleotide.[34] Intriguingly, with alternating polynucleotide poly (dGdC)$_2$ at the same pH (Figure 8, left), the observed change < 300 nm was opposite (increase, attributed to the contribution of intrinsic CD of **4**), and no ICD bands were visible. The only difference between homo-poly dG - poly dC and alternating - poly (dGdC)$_2$ is distribution of amino groups of guanine within DNA minor groove, whereby in latter DNA amino groups sterically occupy both sides of the groove and strongly hinder insertion of small molecule.[31]

Further, a pronounced decrease of the band at 262 nm was also observed for poly A - poly U (Figure 8, right), accompanied by a hypsochromic shift of 2 nm, which also cannot be attributed to the positive CD band of **4**. However, in this case no ICD bands were observed in 300–370 nm range, suggesting that decrease of RNA CD bands is not due to intercalation but more likely due to disproportionation of ds-RNA into ss-RNA strands induced by ss-RNA-preferred binding of **4** (in agreement with destabilization of ds-RNA in thermal denaturation experiments, Table 1).

The dipeptide **4** contains modified tyrosine susceptible to the photoinduced deamination,[23,25] that gives rise to QMs, which again can alkylate DNA or RNA.[26] To preliminary test the photochemical reactivity for dipeptide **4** with DNA/RNA, dipeptide **4** was irradiated (λ=300 nm) in the presence of polynucleotides, followed by recording CD spectra (Figures 6–8, r = 0.7 irradiated). Since 300 nm irradiation is not absorbed by polynucleotide, the changes in the CD spectra indicate that photochemical reactions take place and affect the polynucleotide chirality. It is plausible that irradiation gives rise to the photo-induced alkylation of DNA,[26] but further experiments are needed for full characterization of photochemical products.

CONCLUSION

We synthesized dipeptide **4** containing two unnatural amino acids composed of modified and photochemically reactive tyrosine, and phenanthridine. Thermal denatu-

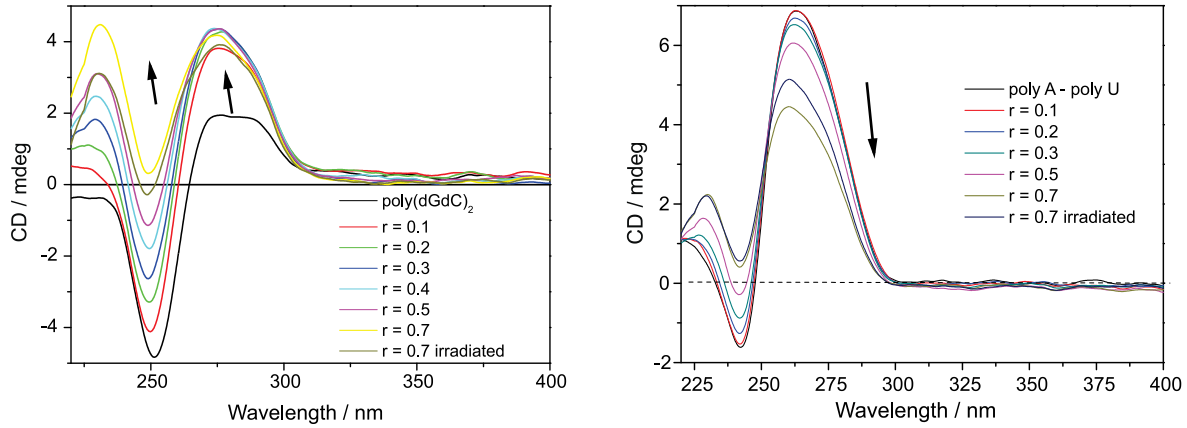


Figure 8. CD spectra of poly (dGdC)$_2$ (left) and poly A - poly U (right) (c = 3.0×10^{-5} mol dm^{-3}) in cacodylate buffered aqueous solution (I = 0.05 mol dm^{-3}) at pH 5.0 in the presence of different ratio r[**4**]/[polynucleotide].

ration experiments indicated different effects to polynucleotides at pH 5.0 and 7.0, depending on the phenanthridine moiety being protonated or not. At pH 5.0, dipeptide **4** thermally stabilized DNA double helices, and destabilized ds-RNA. However, fluorescence titrations with all polynucleotides at both pH values gave similar binding affinity (log K_a ≈ 5), indicating nonselective binding. Nevertheless, changes in the CD spectra suggest different modes of binding to polynucleotides, most likely the intercalation to poly dG- poly dC or non-specific binding to other DNA polynucleotides. On the other hand, binding of **4** probably induces dissociation of ds-RNA into ss-RNA strands due to preferred binding to ss-RNA. Thus, **4** is among very rare small molecules that stabilize ds-DNA but destabilize ds-RNA. Preliminary photochemical experiments aimed toward formation of reactive QMs and their reaction with DNA/RNA revealed structural changes in polynucleotide CD spectra, supporting further studies of photo-induced reactions of **4** and its analogues. Understanding and controlling binding modes of novel dipeptides, particularly photoreactive species, to different DNA or RNA sequences is essential for the rational design of next generation of peptidoids, either as potential drugs, or analytical reagents applicable in biology or medicine.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV- 300, or 600 MHz. The NMR spectra were taken in CD_3OD at rt using TMS as a reference. HRMS were obtained on an Applied Biosystems 4800 Plus MALDI TOF/TOF instrument (AB, Foster City, CA). Analytical thin layer chromatography was performed on Polygram® SILG/UV$_{254}$ (Machery-Nagel) plates. Chemicals for the synthesis were purchased from the usual suppliers, whereas solvents for the synthesis and chromatographic separations were purified by distillation, or used as received (p.a. grade). Silica gel (0.05–0.2 mm) was used for chromatographic purifications. Precursor molecules **1-Me**[11,36] and **2**[25] were prepared according to the published procedures. Methyl ester deprotection of **1-Me** and characterization of **1** is given in the SI.

Boc-Phen-Tyr[$CH_2N(CH_3)_2$]-OBn (3)

Prior to the reaction, amino acid **1**, TFA×**2** HBTU, and HOBT were dried over night in a desiccator over P_2O_5. A round bottom flask (50 mL) equipped with a septum and under N_2 atmosphere was charged with a solution of **1** (30 mg, 0.08 mmol), HBTU (30 mg, 0.08 mmol) and HOBT (10 mg, 0.08 mmol) in dry CH_3CN. By use of a syringe triethylamine (TEA) (40 µL, 0.32 mmol) was added and the reaction mixture was stirred 30 min. A solution of TFA×**2** (40 mg, 0.08 mmol) in dry CH_3CN was added dropvise. The reaction mixture was stirred at rt over night. The solvent was removed on a rotary evaporator and the oily residue chromatographed on a column of silica gel using CH_3OH/CH_2Cl_2 (20→100% CH_3OH) to afford the pure product (11 mg, 20 %) in the form of oil.

^{1}H NMR (CD_3OD, 600 MHz) δ/ppm: 8.63 (t, J = 8.4 Hz, 2H), 8.11 (s, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.79 (dd, J = 1.5, 8.6 Hz, 1H), 7.71 (t, J = 7.4 Hz, 1H), 7.65 (t, J = 8.4 Hz, 1H), 7.32−7.26 (m, 3H), 7.21−7.16 (m, 2H), 7.07−7.01 (m, 2H), 6.74 (d, J = 9.0, Hz, 1H), 4.95 (s, 2H), 4.68 (d, J = 7.0 Hz, 1H), 4.45 (dd, J = 5.8, 9.0 Hz, 1H), 3.98 (s, 2H), 3.28−3.22 (m, 1H), 3.08−3.01 (m, 1H), 3.01−2.96 (m, 3H), 2.93 (dd, J = 8.0, 14.0 Hz, 1H), 2.62 (s, 6H), 1.28 (s, 9H); ^{13}C NMR (CD_3OD, 75 MHz) δ/ppm: 160.6 (s, 1C), 144.0 (s, 1C), 138.6 (s, 1C), 138.5 (s, 1C), 134.0 (d, 1C), 132.7 (s, 1C), 131.3 (d, 1C), 130.8 (d, 1C), 130.7 (d, 1C), 129.8 (d, 1C), 129.5 (d, 1C), 129.34 (d, 1C), 129.29 (d, 1C), 129.1 (d, 1C), 128.5 (d, 1C), 128.1 (s, 1C), 128.0 (d, 1C), 127.9 (d, 1C), 127.0 (s, 1C), 123.7 (d, 1C), 123.4 (d, 1C), 116.6 (d, 1C), 67.8 (t, 1C), 62.1 (t, 1C), 56.9 (d, 1C), 55.5 (d, 1C), 44.6 (q, 2C), 37.7 (t, 1C), 28.6 (q, 3C); HRMS (MALDI-TOF) m/z $[M+H]^+$ calculated for $C_{41}H_{46}N_4O_6$ 691.3496; observed 691.3483.

HCl×Phen×HCl-Tyr[$CH_2N(CH_3)_2$×HCl]-OBn (4)

Dipeptide **3** Boc-Phen-Tyr[$CH_2N(CH_3)_2$]-OBn (6 mg, 0.01 mmol) was dissolved in HCl/ EtOAc (1 mL). The reaction mixture was stirred over night at rt. The solvent was removed on vacuum and the residue washed with ether to afford the pure product in the form of oil (7 mg, 99 %).

^{1}H NMR (CD_3OD, 300 MHz) δ/ppm: 9.10−8.86 (m, 2H), 8.76−8.59 (m, 1H), 8.24 (s, 2H), 8.02 (s, 2H), 7.41−7.05 (m, 7H), 6.88−6.74 (m, 1H), 5.08−4.91 (m, 2H), 4.75−4.64 (m, 1H), 4.35−4.16 (m, 2H), 3.76−3.43 (m, 2H), 3.20−2.93 (m, 2H), 2.81 (d, J = 9.0 Hz, 6H); ^{13}C NMR (CD_3OD, 75 MHz) δ/ppm: 169.3 (s, 1C), 163.1 (s, 1C), 156.8 (s, 1C), 140.1 (s, 1C), 136.8 (s, 1C), 135.2 (s, 1C), 134.5 (d, 1C), 134.0 (d, 1C), 133.9 (d, 1C), 133.7 (d, 1C), 133.6 (d, 1C), 132.6 (d, 1C), 131.9 (d, 1C), 131.1 (d, 1C), 129.6 (d, 1C), 129.4 (d, 1C), 129.2 (d, 1C), 129.0 (s, 1C), 125.7 (s, 1C), 125.33 (d, 1C), 125.29 (d, 1C), 124.9 (d, 1C), 117.6 (s, 1C), 116.6 (d, 1C), 68.0 (t, 1C), 58.0 (t, 1C), 55.9 (d, 1C), 55.4 (d, 1C), 43.3 (q, 2C), 38.3 (t, 1C), 37.4 (t, 1C), two carbon signals were not observed; HRMS (MALDI-TOF) m/z $[M+H]^+$ calculated for $C_{36}H_{40}N_4O_4$ 591.2971; observed 591.2982.

Polynucleotides

Polynucleotides were purchased as noted: poly A – poly U, poly $(dGdC)_2$, poly dG - poly dC, poly $(dAdT)_2$, calf thymus, ct-DNA (Sigma). Polynucleotides were dissolved in Na-cacodylate buffer, I=0.05 mol dm^{-3}, pH 7.0. The calf thymus ct-DNA was additionally sonicated and filtered through a 0.45 mm filter.[29] Polynucleotide concentration was determined spectroscopically as the concentration of phosphates.

Thermal Denaturation Experiments

A stock solution of **4** was prepared in mQ H_2O (c = 1.0×10^{-3} mol dm^{-3} or c = 1.43×10^{-3} mol dm^{-3}), whereas the stock solutions of polynucleotides were prepared in aqueous cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}) in the following concentrations: c(ct-DNA) = 1.46×10^{-2} mol dm^{-3}, c(poly A - poly U) = 5.0×10^{-3} mol dm^{-3}, c(poly (dAdT)$_2$) = 1.68×10^{-3} mol dm^{-3}. The solution of ct-DNA was sonicated and filtered (pores 0.45 μm) to assure narrow distribution of polynucleotide chain lengths. In the denaturation experiments, the polynucleotide solution was diluted in a quartz UV-vis cell (with the optical path of 1.0 cm) by cacodylate buffer to the concentration of c = 3.0×10^{-5} mol dm^{-3}, and the appropriate amount of the solution of **4** was added to reach the desired ratio r ([**4**]/[polynucleotide]) = 0.3. The dependence of the absorbance at 260 nm as a function of temperature was measured on a Cary 100 Bio (Agilent Varian) UV-vis spectrometer. The temperature was varied from 25 °C to 98 °C in intervals of 0.5 °C.[37,38] The denaturation temperature T_m values are the midpoints of the transition curves, determined from the maximum of the first derivative.[39] ΔT_m values were calculated by subtracting T_m of the free nucleic acid from that of the respective complex with ΔT_m values (Eq S1 in the SI) are the average of at least two independent measurements and the error in ΔT_m is ca. ± 0.5 °C.

Fluorescence Titrations

For the titration, solution of **4** was diluted in a fluorescence cell (3 mL) with cacodylate to reach the concentration of c = 2.0×10^{-6} mol dm^{-3}. Polynucleotide stock solutions were c = 5.0×10^{-3} mol dm^{-3}. The fluorescence spectra were measured on a Cary Eclipse (Agilent Technologies) at 25 °C. The samples were excited at 330 nm, and the emission was recorded in the range 350–600 nm. In the titration with ct-DNA and poly A - poly U, the excitation slit was set to the bandpass of 10 nm, and the emission slit to 20 nm, whereas in the titrations with other polynucleotides (poly (dAdT)$_2$, poly dG - poly dC and poly (dGdC)$_2$) both slits were set to the bandpass of 10 nm. Small aliquots of the solutions of polynucleotides were added to the solution of **4** and after an incubation time of 2 min, fluorescence spectra were taken. Data obtained by fluorescence titrations were processed by nonlinear regression analysis according to the Scatchard equations (eq. S2 in the SI).

Circular Dichroism Spectroscopy

Circular dichroism spectra were measured on a Jasco J-815 spectrometer in quartz cells with the optical path of 1 cm at 25 °C. The polynucleotide solutions in cuvette were of c = 3.0 × 10^{-5} mol dm^{-3} in cacodylate buffer (pH = 7.0 or 5.0 , I = 0.05 mol dm^{-3}). Aliquots of the solution of **4** in buffer (c = 1.0 × 10^{-3} mol dm^{-3}) were added into cuvette to reach the concentration ratio r[**4**]/[polynucleotide] = 0.1–0.7. The CD spectra were recorded in the wavelength range 220-600 nm with the scanning rate of 200 nm/min and with 2 accumulations.

Photochemistry

The samples containing **4** and polynucleotide in the ratio r = 0.7 were irradiated in a Luzchem reactor equipped with 8 lamps (1 lamp 8 W) with the output at 300 nm over 3 min. After the irradiation, CD spectra were measured.

Acknowledgment. These materials are based on work financed by the ESF project HR.3.2.01-0254, Croatian Science Foundation (HRZZ grant no. HRZZ IP-2014-09-6312 to NB and HrZZ IP-2013-1447 to IP).

Supplementary Information. Synthetic procedures for the preparation of intermediate 1, NMR spectra for 1, 3 and 4, data for thermal denaturation of polynucleotides, fluorescence titration data and CD spectroscopy. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3542.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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