LINEAR HEPTAPEPTIDES CONTAINING DNA-INTERCALATORS. SYNTHESIS AND INTERACTION WITH DNA

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Five peptides of general structure X-Ser-Pro-Thr-Ser-Pro-Ser-Y (X = Tyr, quinoxaline-2-carbonyl, acridine-9-carbonyl, Y = Tyr, (quinoxalin-2-yl)amino) were prepared using standard solid-phase peptide synthesis technique. Their interaction with DNA (calf thymus DNA and plasmids pUC9, pΔNS and pGEMEX) was studied using UV and CD spectroscopy, sedimentation analysis and agarose gel electrophoresis after treatment with topoisomerase I. In contrast to earlier findings (Suzuki M.: *Nature* **1990**, *344*, 562) intercalation into DNA structure has not been proved for any compound studied.

Key words: Intercalation; Peptides; DNA, Topoisomerase I; UV spectroscopy; CD spectroscopy; Sedimentation analysis; Acridines; Quinoxalines.

The concept of intercalation was first introduced by Lerman[1] to explain the reversible noncovalent binding to double-helical DNA of compounds with planar, aromatic (most often heteroaromatic) extended chromophores. The essence of intercalation is the insertion of such a chromophore between adjacent base pairs of DNA, thereby extending and stabilising the double helix; base-pair separation at an intercalation site thus increases[2–6]. In general, intercalating compounds have a polarizable, electron-deficient chromophore. A formal positive charge is common, located either on the central chromophore or on attached substituent groups. The presence of a substituent moiety appears to be a necessary precondition for antitumor action, though not for intercalate binding. Considerable variation in the

nature of the substituent is possible, ranging from uncharged cyclic polypeptides to protonated amino group(s).

In addition to monointercalating compounds, there exists also a class of natural biologically active bisintercalators (echinomycin and triostin) having a cyclic peptide unit with two quinoxaline intercalating units[4,6,11,12]. It is well documented by fifteen drug-DNA fragment X-ray structures[13–23]. These compounds are characterised by an octadepsipeptide ring, bearing two quinoxaline chromophores. Echinomycin and triostin families differ in the structure of the thioacetal crosslink in the peptide unit. Both these antibiotics bind to DNA by intercalation and show strong preference for the CpG unit. Synthetic analogues of these compounds, TANDEM and CysMeTANDEM which lack four and two *N*-methyl groups, respectively, bind selectively to ApT (TANDEM) or TpA (CysMeTANDEM) units. It is therefore clear that slight changes in the intercalators can lead to very important differences in their binding.

Peptide parts of the above mentioned compounds are relatively complex bridged structures. No wonder that attempts have been made to alter their structure. It was reported that a peptide with two aminoacridine moieties attached to both its sides does intercalate into DNA with at least 140-fold enhancement of affinity compared to 9-aminoacridine[24]. On the basis of solution conformation of Ser-Pro-Arg-Lys-Ser-Pro-Arg-Lys a model for the interaction for the peptide with DNA was proposed, in which the peptide mimics the hydrogen bonding of netropsin[25]. Rather than forming distinct hydrogen bonds to the AT base pairs, it was suggested that the AT preference may arise by recognition of structural characteristics such as the narrow minor groove of DNA. In contrast to these results, NMR study of this peptide interaction with AT-rich oligonucleotides showed only nonspecific interaction[26]. This study indentified the Arg-Pro-Arg-Gly-Arg sequence as the key fragment that determines the sequence preference for AT sites. In common with previous examples of minor groove binders, Ser-Pro-Thr-Ser-Pro-Ser-Tyr, the heptad repeat in the largest subunit of RNA polymerase II with a additional tyrosine added to the C-terminus has also been shown to interact with DNA. Fluorescence quenching and sedimentation velocity data showed that the peptide binds to DNA exclusively by bisintercalation of the two terminal tyrosine aromatic rings[27]. A model peptide Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr was also found to be partially structured in aqueous solution (by 600 MHz NMR). The solution conformation of the peptide appears to have crucial role in determining the interaction of the peptide with DNA (ref.[28]). Chemoenzymatic synthesis and incorporation of 3-(quinoxalin-2-yl)-L-alanine into TANDEM β-turn peptide motif have been

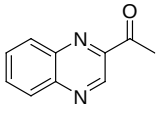
reported[29]. Finally Harding *et al.*[30] has recently proved that peptides of general structure X-Ser-Pro-Ser-Thr-Ser-Pro-Z which are unique DNA-binding class that differ from previously characterised Ser-Pro-X-Y motifs. It was also proved that their overall binding constant to DNA is low.

Consequently, we have decided to base our design on combination of this type of linear peptide structure with quinoxaline and other heterocyclic units on one or both ends. This resulted in the series of compounds **1**–**5**. In this communication we will describe their synthesis and their interaction with nucleic acids using various methods.

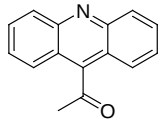
X-Ser-Pro-Thr-Ser-Pro-Ser-Y
1, X = H-Tyr; Y = -Tyr-OH
2, X = Qui-CO-; Y = -Tyr-OH
3, X = Acr-CO-; Y = -Tyr-OH
4, X = H-Tyr-; Y = -NH-Qui
5, X = Qui-CO-; Y = -NH-Qui

A-Ser(*t*-Bu)-Pro-Thr(*t*-Bu)-Ser(*t*-Bu)-Pro-OH
6, A = Qui-CO-
7, A = Fmoc-Tyr(*t*-Bu)

Boc-Ser-NH-Qui **8**



Qui-CO-



Acr-CO-

RESULTS

Peptide Synthesis

All peptides were synthesised manually in hypodermic syringes with Teflon frit, on a resin using standard solid-phase peptide synthesis techniques[31]. Each coupling step was monitored by ninhydrin reaction.

*Interaction of Peptides **1**–**5** with DNA*

UV and CD Spectroscopy

UV spectra of peptides **1**–**5** are shown in Figs 1 and 2. In the case of **1**, the most intense band is in the region below 200 nm, the band with maximum at about 274.5 nm probably corresponding to the tyrosine residue. All samples except **1** have bands in the region 240–260 nm which is the range of strong absorption of DNA. The peptides **2**–**5** also have absorption bands

outside the range of DNA absorption which is favorable for binding studies. Peptide **3** exhibits UV bands in the region around 360 nm corresponding to the acridine residue, whereas peptides **2**, **4** and **5** absorb in the region around 320 nm corresponding to the quinoxaline residue.

CD spectra of peptides **1**–**5** are shown in Figs 3 and 4. All ligands are chiral and show CD bands in the region of DNA absorption. Peptide **1** exhibits a negative maximum at about 210 nm, a positive maximum at 229.5 nm and a negative maximum at 237.5 nm. CD spectrum of peptide **3** shows intensive negative maxima in near 210 and 252 nm and a very weak band at about 360 nm. Peptide **2** exhibits a negative band at about 250 nm, peptide **4** a positive band at 248 nm and the peptide **5** a positive band at 245 nm and a negative band at 258 nm. The bands outside the DNA region found for **3**, **4** and **5** are weak.

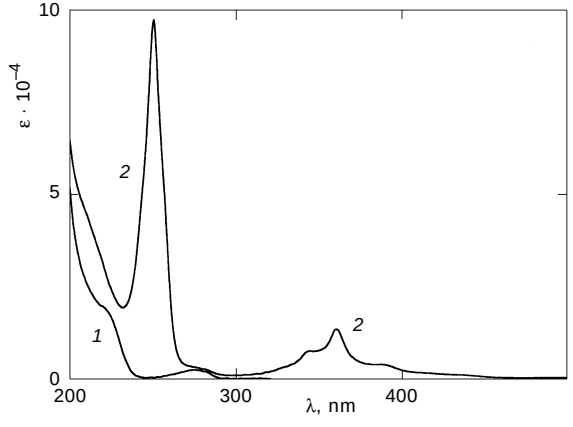


FIG. 1
UV spectrum of **1** (*1*) and **3** (*2*) in 0.01 M NaCl, 0.2 mM Na_2HPO_4, 0.05 mM Na_2EDTA (pH 6.3)

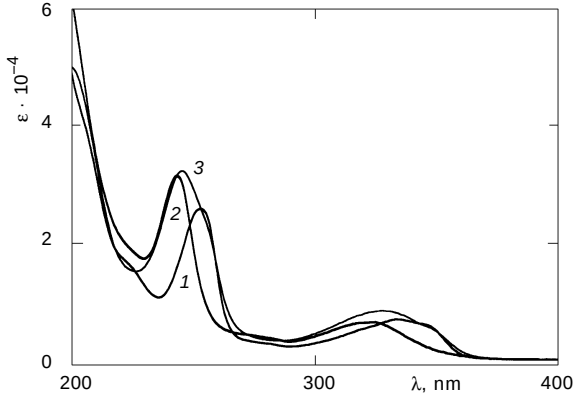


FIG. 2
UV spectrum of **4** (*1*), **2** (*2*) and **5** (*3*) in 0.01 M NaCl, 0.2 mM Na_2HPO_4, 0.05 mM Na_2EDTA (pH 6.3)

The interaction of ligands **1**–**5** with DNA was measured using both UV and CD, spectroscopy. The UV spectra of the mixture of DNA and **1** or **3** are almost identical to the sum of the spectra of both components. Similar results were also obtained with other ligands. CD measurements of the interaction of DNA and **5** are shown in Fig. 5, those using other components are similar. As in the case of the UV spectra, the difference between the CD spectra of the mixtures of ligands and DNA and the sum of the spectra of its components were within the limit of experimental error. The melting experiments which were made with **1** and **3** showed no or very small (within the limits of experimental error) influence of the added ligands on the melting transition of DNA.

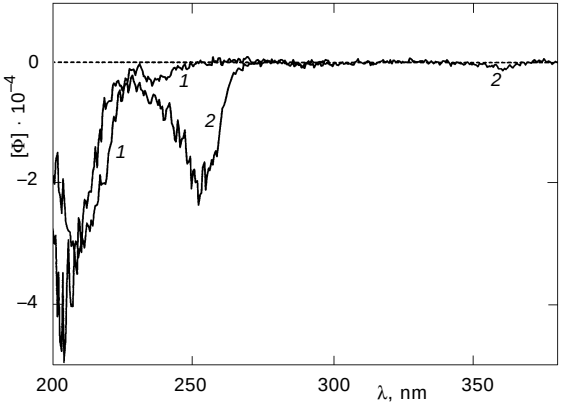


FIG. 3
CD spectrum of **1** (*1*) and **3** (*2*) in 0.01 M NaCl, 0.2 mM Na_2HPO_4, 0.05 mM Na_2EDTA (pH 6.3)

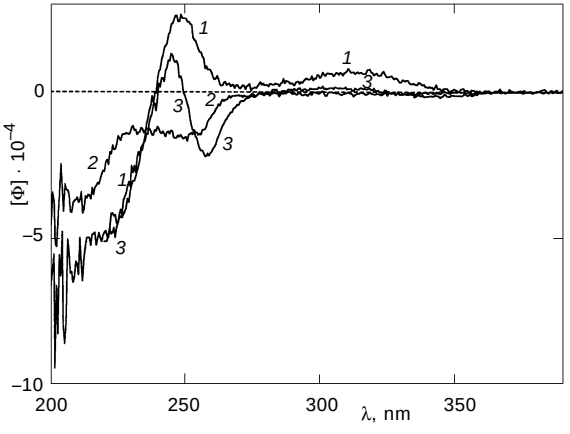


FIG. 4
CD spectra of **2** (*1*), **4** (*2*) and **5** (*3*); 0.01 M NaCl, 0.2 mM Na_2HPO_4, 0.05 mM Na_2EDTA (pH 6.3)

Gel Electrophoresis after Relaxation with Topoisomerase I

The interaction of DNA with all components was also examined by the agarose gel electrophoresis of plasmid DNA (pUC9 with 1, **2** and **3** and pGEMEX with **4** and **5**) relaxed with topoisomerase I in the presence of increasing amount of the ligand. The experiments were carried out in TOPO buffer (10 mM Tris pH 7.6, 5 mM $MgCl_2$, 0.1 M KCl, 0.5 mM 1,4-dithiothreitol – DTT). The results indicate that the electrophoretic mobility of plasmid DNA is not affected by the presence of ligands during the relaxation with topoisomerase I. Therefore, they do not support the hypothesis that the ligands are bound by intercalation.

With **1** and **3**, the experiments were also made in a solution of lower ionic strength (TOPO buffer without KCl). These results were similar to those obtained at higher ionic strengths.

The interaction of DNA with **1** and **3** was studied using both superhelical and relaxed plasmid. With ligands bound by intercalation, the results should be the same in both cases. This was the case for **1**; for **3**, however, the results depended on the fact whether the superhelical or relaxed plasmid was used in the experiment. For superhelical plasmid, electrophoresis showed the presence of relaxed DNA at low **3**/DNA ratios; for a mixture of superhelical and fully relaxed plasmid, at high **3**/DNA ratios. We suppose that the reason for this behavior is the inhibition of topoisomerase I in the presence of high amounts of **3**. The results obtained with relaxed plasmid showed no effect of **3** on the superhelicity of DNA. Therefore, it can be concluded that peptide **3** is not bound by intercalation to DNA.

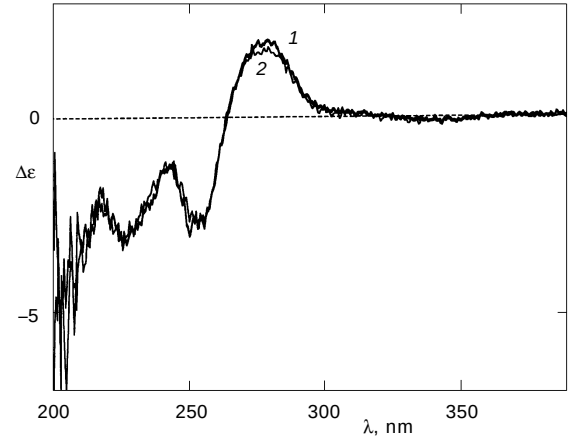


FIG. 5
CD spectra of a mixture of DNA and **5** (*1*) and the sum of the spectra of DNA and **5** (*2*); 0.01 M NaCl, 0.2 mM Na_2HPO_4, 0.05 mM Na_2EDTA, mol **5**/DNA bp = 0.5 (pH 6.3)

Sedimentation Analysis

The analysis was made with compounds **1** and **3**. Table I shows the sedimentation coefficients of the plasmid pΔNS in the presence of increasing amounts of peptide **1**. The values of the sedimentation coefficients differ within the limits of experimental error. These results show that peptide **1** does not unwind the plasmid DNA and, therefore, it does not intercalate into DNA. Table II shows the results obtained for peptide **3**. The range of **3**/DNA ratios is lower since **3** has a high absorption coefficient at 260 nm and the detection of higher amounts of **3** is beyond the potential of the absorption optics. However, Table II indicates that the sedimentation coefficient of plasmid DNA is not affected by the added **3**.

EXPERIMENTAL

Abbreviations: AcOH, acetic acid; AcrCO, acridine-9-carbonyl; Boc, *tert*-butoxycarbonyl; BOP, [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate; *t*-Bu, *tert*-butyl; Dcb, 2,6-dichlorobenzyl; DCM, dichloromethane; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DIC, *N*,*N*′-diisopropylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DMF, dimethylformamide; EDT, ethane-1,2-dithiol; FAB MS, fast-atom bombardment mass spectrometry; Fmoc, (fluoren-9-yl)methoxycarbonyl; HOBt, 1-hydroxybenzotriazole; MeOH, methanol; QuiCO, quinoxalyl-2-carbonyl; RP HPLC, reverse-phase high-performance liquid chromatography; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; Trt, trityl. Amino acid

TABLE I
The influence of the added ligand **1** on the sedimentation coefficient of the plasmid pΔNS (0.01 M phosphate, pH 7)

Ligand/DNA (mol **1**/bp)	0	2.74	5.36	7.94	10.44
$S_{20.w}$	25.4	24.2	24.9	24.8	25.1

TABLE II
The influence of the added ligand **3** on the sedimentation coefficient of the plasmid pΔNS (0.15 M NaCl + 0.015 M sodium citrate)

Ligand/DNA (mol **3**/bp)	0	0.72	1.45	2.87	4.80	7.26
$S_{20.w}$	23.4	22.8	22.0	23.2	22.5	23.1

symbols denote the L-configuration. Abbreviations for amino acids and nomenclature of peptides follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature [*Eur. J. Biochem*. **1984**, *138*, 9].

General

All melting points were measured on a Boetius melting point apparatus and are uncorrected. Ultraviolet spectra at ambient temperature were obtained on a spectrophotometer PU 8800 UV/VIS connected to a PC-XT computer, using the cell length 0.1 or 1.0 cm. The samples were dissolved in phosphate buffer (0.2 mM Na_2HPO_4, 0.01 M NaCl, 0.05 mM Na_2EDTA). Mass spectra were acquired with a ZAB-EQ (VG Analytical) spectrometer (EI: 70 eV; FAB: Xe, 8 kV, glycerol–thioglycerol matrix). HPLC analyses were performed on a Spectra-Physics instrument with C18 Vydac 218TP54 column (5 μm, 250 × 4.6 mm) and a 0.05% TFA–water–0.05% TFA–methanol gradient. Purification of products was performed on the same instrument with C18 Vydac 218TP510 column (10 μm, 250 × 10 mm) and in the same solvent system. Samples for amino acid analysis were hydrolysed in 6 M HCl at 110 °C for 20 h and analysed on a Biochrom 20 (Pharmacia, Manchester, U.K.). CD spectra were measured on a Jobin–Yvon Dichrographe Mark V using software Dichrosoft Version A written by one of the authors (P. M.). The cell length was 0.1, 0.2 or 0.5 cm. Sedimentation analysis was carried out in a Beckman ultracentrifuge, Model E at 44 000 rpm using UV absorption optics at 260 nm.

2-Chlorotrityl chloride resin and Fmoc amino acid derivatives were purchased from Calbiochem–Novabiochem AG (Laufelfingen, Switzerland), Boc-amino acid derivatives from Senn Chemicals AG (Dielsdorf, Switzerland). Solvents for peptide synthesis and DIC, HOBt and DIEA were obtained from Fluka (Buchs, Switzerland). DMF was vacuum-distilled prior to use.

Topoisomerase I from wheat germs was isolated using the modified[32] method of Maniatis[33]. Calf thymus DNA was isolated by the method of Kay[34] and co-workers. Plasmids pUC9 and pΔNS and pGEMEX were isolated by the modified alkali method of Birnboim and Doly[35]. Concentration of DNA was determined from the absorbance values at 260 nm using the average value of ε_{260} = 6 600. The absorbance was measured on a spectrophotometer PU 8800UV/VIS in 0.1 cm cells. Melting curves at wavelength 260 nm were measured on a CARY 219 spectrophotometer in thermostatted 1 cm cells. Temperature was being increased at a rate of about 1 °C/min using a circulating water bath. The DNA concentration was 20–25 μg/ml.

Relaxation of plasmid DNA with topoisomerase I in the presence of ligands. The plasmid pUC9 or pGEMEX (0.665 μg, dissolved in the TOPO buffer (10 mM Tris pH 7.6, 5 mM $MgCl_2$, 0.1 M KCl, 0.5 mM DTT)) was relaxed by topoisomerase I in the presence of different concentrations of the studied ligand for 30 min at 37 °C. The volume of the mixture was 20 μl. The reaction was stopped by an addition of sodium dodecyl sulfate (0.5% concentration) and proteinase K (0.5 mg/ml). After 30 min at 37 °C, 5 μl of the mixture of 1.5 M sodium acetate and glycogen (5 mg/ml) was added and DNA was precipitated with two volumes of ethanol. Then the solution was incubated 20 min at –20 °C and centrifuged for 5 min at 14 000 rpm. The pellet was dissolved in a mixture of 15% Ficoll and 0.25% Bromophenol Blue, and used for electrophoresis.

Agarose gel electrophoresis. 1% Agarose gels were made in the TEB buffer (89 mM Tris pH 8.2, 89 mM boric acid, 2.5 mM EDTA). The electrophoresis was run at 2.5 V/cm for 16 h. The gels were stained with ethidium bromide and photographed under UV light.

Synthesis of Peptides **1**–**3**

Peptides **1**, **2** and **3** were prepared *via* *N*-α-Boc strategy using a Merrifield chloromethyl resin (1.3 mmol Cl/g). The amino acids side chains were protected as follows: *O*-*t*-Bu (Ser, Thr), *O*-Dcb (Tyr). First amino acid, Boc-Tyr(Dcb)-OH, was attached to the resin the by caesium salt method[36] in DMF (degree of substitution 0.62 mmol Tyr/g, by amino acid analysis). The couplings were carried out with DIC/HOBt in DCM. The peptides attached to the resin were deprotected and cleaved from dried resin with anhydrous HF at 0 °C for 1 h in the presence of EDT as a scavenger. The crude peptides were obtained after evaporation of HF by precipitation with dry diethyl ether. The precipitate and resin were filtered off, washed with ether, air-dried and the peptide was separated from the resin by dissolution in 80% aqueous AcOH and filtration. The filtrate was diluted with water, lyophilised and the crude peptide was purified by RP HPLC.

H-Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-OH (**1**) was prepared from 635 mg (0.39 mmol) of Boc-Tyr(Dcb)O-resin in 82% (288 mg) yield and 92% HPLC purity. FAB MS, *m/z* (rel. int., %): 901.5 (M + 1, 100), 737.4 (8), 651.4 (14). Amino acid analysis: 1.0 Thr, 3.1 Ser, 2.1 Pro, 2.2 Tyr.

QuiCO-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-OH (**2**) was prepared from 165 mg (0.10 mmol) of Boc-Tyr(Dcb)O-resin analogously to peptide **1** with the exception that in the last coupling step was used quinoxaline-2-carboxylic acid, instead of Boc-Tyr(Dcb)-OH. The isolated yield of peptide **2** was 68% (61 mg), HPLC purity was 90%. FAB MS, *m/z* (rel. int., %): 895.2 (M + 1, 45), 651.2 (12), 366.1 (100). Amino acid analysis: 1.0 Thr, 2.6 Ser, 2.2 Pro, 1.0 Tyr.

AcrCO-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-OH (**3**) was prepared from 135 mg (0.08 mmol) of Boc-Tyr(Dcb)O-resin analogously to peptide **1** with the exception that in the last coupling step was used acridine-9-carboxylic acid, instead of Boc-Tyr(Dcb)-OH. The isolated yield of peptide **3** was 79% (60 mg), HPLC purity was 94%. FAB MS, *m/z* (rel. int., %): 943.3 (M + 1, 100), 898.7 (10), 738.0 (22), 651.3 (16). Amino acid analysis: 1.0 Thr, 3.3 Ser, 2.1 Pro, 1.1 Tyr.

Synthesis of Peptides **4** and **5**

Starting peptides **6** and **7** were prepared *via* *N*-α-Fmoc strategy using 2-chlorotrityl chloride resin (1.4 mmol Cl/g). The amino acid side chains were protected as *tert*-butyl ethers (Ser, Thr, Tyr). Acylation of the resin by the first amino acid, Fmoc-Pro-OH, in DMF and DIEA afforded substitution of 0.31 mmol Pro/g (by amino acid analysis). Amino acid couplings were carried out with BOP/DIEA (1 : 2 equivalents) in DMF. The peptides attached to the resin were *N*-deprotected with 20% piperidine in DMF and then cleaved off from the dry resin with a mixture of AcOH–TFE–DCM 1 : 1 : 8 at room temperature for 30 min. The resin was filtered off and the filtrate was concentrated *in vacuo*. The residue was diluted with 5% AcOH and lyophilised. Crude peptides were used in the next step without any purification.

QuiCO-Ser(t-Bu)-Pro-Thr(t-Bu)-Ser(t-Bu)-Pro-OH (**6**) was prepared from 300 mg (0.09 mmol) of Fmoc-Pro-O-resin. The isolated yield of peptide **6** was 74% (63 mg), HPLC purity was 89%. FAB MS, *m/z* (rel. int., %): 813.9 (M + 2, 100), 755.9 (50), 656.8 (17), 511.5 (40). Amino acid analysis: 1.0 Thr, 2.0 Ser, 2.0 Pro.

Fmoc-Tyr(t-Bu)-Ser(t-Bu)-Pro-Thr(t-Bu)-Ser(t-Bu)-Pro-OH (**7**) was prepared from 500 mg (0.16 mmol) of Fmoc-Pro-O-resin. The isolated yield of peptide **7** was 87% (148 mg), HPLC purity was 84%. FAB MS, *m/z* (rel. int., %): 1097.7 (M + 1, 92), 875.6 (47), 656.4 (100). Amino acid analysis: 1.0 Tyr, 1.1 Thr, 2.1 Ser, 2.2 Pro.

Boc-Ser-NHQui (**8**)

To a solution of Boc-Ser-OH (1.50 g, 7.3 mmol) in DCM–DMF (4 : 1, 25 ml) were added 2 M solution of HOBt in DMF (4 ml) and 1 M solution of DCC in DCM at 0 °C. Separated *N,N′*-dicyclohexylurea was filtered off and to the filtrate were added solutions of quinoxalin-2-amine (1.10 g, 7.3 mmol) in DMF (20 ml) and 4-methylmorpholine (0.5 ml). After 12 h, the solvents were evaporated, the residue was dissolved in *tert*-butyl methyl ether (250 ml) and the solution was extracted with 10% aqueous citric acid (7 × 60 ml), 10% aqueous sodium hydrogencarbonate (3 × 60 ml), brine (40 ml) and dried with magnesium sulfate. Ether was evaporated and the residue crystallised from AcOEt–hexane. The yield of light-brown crystals was 0.75 g (31%), m.p. 134–137 °C. For $C_{16}H_{20}N_4O_4$ (332.4) calculated: 57.825% C, 6.07% H, 16.86% N; found: 58,03% C, 6.20% H, 16.08% N. FAB MS, *m/z* (rel. int., %): 333.1 (M + 1, 30), 277.0 (30), 259.0 (10), 146.0 (100).

Peptides **4** and **5**

Amide **8** (33 mg, 0.1 mmol) was treated with a 55% TFA solution in DCM for 30 min. The solvents were evaporated, the residue was dissolved in DCM, again evaporated and then triturated with dry diethyl ether (2 × 2 ml). Ether was decanted, the residue was dissolved in DMF (5 ml) and 4-methylmorpholine (0.2 ml), and added to a solution of amide **6** or **7** (0.05 mmol) and BOP (44 mg, 0.1 mmol) in DMF (5 ml). After 6 h at room temperature, the solvent was evaporated and the residue was treated with a TFA–water (95 : 5) mixture for 2 h. The solvents were evaporated and the residue was triturated with dry ether (2 × 2 ml). The insoluble residue was dissolved in AcOH (0.7 ml) and purified by preparative HPLC. Pooled fractions containing the desired peptide were evaporated and lyophilised from 5% aqueous AcOH.

H-Tyr-Ser-Pro-Thr-Ser-Pro-Ser-NHQui (**4**) was prepared in 57% yield and 90% HPLC purity. FAB MS, *m/z* (rel. int., %): 865.7 (M + 1, 93), 556.5 (100). Amino acid analysis: 1.0 Tyr, 1.2 Thr, 3.4 Ser, 2.3 Pro.

QuiCO-Ser-Pro-Thr-Ser-Pro-Ser-NHQui (**5**) was prepared in 24% yield and 92% HPLC purity. FAB MS, *m/z* (rel. int., %): 858.7 (M + 1, 100). Amino acid analysis: 1.0 Thr, 2.9 Ser, 2.1 Pro.

DISCUSSION

The NMR study of the peptide **1** (ref.[28]) has shown that unlike most short peptides which are in the random conformation, this peptide shows the presence of a significant amount of the β-turn. CD spectrum of **1** does not correspond either to any known peptide conformation (random, α-helix or β-sheet) or to any of the published spectra of β-turn. Since NMR spectroscopy shows the presence of at least two types of conformation (random coil and β-turn), it is possible that the obtained spectrum is a sum of the spectra of these components. CD spectra of other peptides contain relatively intense bands corresponding to acridine or quinoxaline moieties and therefore these spectra are not suitable for determining conformation of the peptide part.

Spectroscopic methods can give an information about the interaction of the ligands with DNA but the possibilities of using these methods depend on the spectral properties of the ligands. It was shown in the literature[37] that the intercalation usually causes a shift of the UV bands to higher wavelengths and a decrease in the molar absorption coefficient. The intercalation also causes changes in the CD spectra[38]. The interaction of all peptide compounds with calf thymus DNA was studied by UV and CD spectroscopy. With the exception of **1**, the peptides show a UV band(s) at wavelengths outside the region of the DNA absorption. The interaction was studied in these wavelength regions and, with **1** and **3**, also at lower wavelengths where DNA shows an absorption. In all cases, the UV spectra of mixtures of the ligands and DNA are equal to the sum of the spectra of its components. From these results, we can conclude that the binding of the ligands to DNA is weak (if any) and that there is no intercalation. With **1** and **3**, we also measured the effect of the ligands on melting of the DNA. No changes in the T_m values were observed.

All peptide ligands are chiral and exhibit CD spectra. For measuring the interaction, this is a disadvantage because both free and bound forms of the ligand contribute to the final spectrum. We use CD spectroscopy for measuring the interaction of all peptide compounds. In all cases, the differences between the CD spectra of mixtures of both components and the sum of the spectra of these components were within the limits of experimental error. These results are in agreement with the results of UV spectroscopy and indicate weak (if any) interaction and no intercalation.

The ability of peptides to unwind the superhelical DNA was studied by gel electrophoresis of plasmid DNA after treatment with topoisomerase I. In no case, unwinding which would indicate the binding by intercalation was found.

The hypothesis that peptide **1** binds to the DNA by intercalation is based on the results of Suzuki[27]. He studied this compound by sedimentation analysis and found the unwinding of the superhelical DNA on the interaction. We tried to reproduce his results but without success. We have measured the sedimentation coefficient of the plasmid pΔNS in the presence of various concentrations of **1** under the conditions described by Suzuki but no change in the sedimentation coefficient was found. The experiment with peptide **3** was made at two ionic strengths, in 0.01 M phosphate buffer (pH 7) and SSC (0.15 M NaCl, 0.015 M sodium citrate). At both ionic strengths, no indication of unwinding of the superhelical DNA was found. The paper of Suzuki indicates an intercalation but the changes in sedimentation coefficients were found at very high peptide/DNA ratios, which does

not indicate strong binding. Moreover, the sedimentation measurements of Suzuki were made at very low ionic strengths where the measurement of sedimentation coefficients is not recommended due to a bad reproducibility. Our results with peptides **1**–**5** do not support the hypothesis that these ligands bind to DNA by intercalation and are in agreement only with a weak binding. In recent paper of Harding and co-workers[30] compounds derived from peptide **1** where both tyrosine residues were substituted by aromatic residues. One of their compounds also contained quinoxaline like our compounds but only on the amino terminus of the peptide while on the carboxy terminus, tyrosine was replaced by quinoline. None of their compounds was identical with any of our compounds. Using LD, CD, footprinting and NMR methods, they detected a weak nonspecific binding of the compounds to DNA. These results are in agreement with our results obtained with different compounds derived from the same peptide **1**. On the basis of the LD results, Harding *et al.*[30] concluded that the binding may be of the intercalation type. However, the binding was weak and the amount of peptides bound to DNA under the conditions of the LD experiment was very low. We did not try to interpret slight differences in the CD and UV spectra which were within the limits of experimental error assuming that such low level of intercalation binding as reported by Harding *et al.*[30] cannot be probably detected by our methods.

In general, the lack of positive charge on the chromophore(s) is probably a major factor that reduces favorable intercalative binding, although the neutral chromophores are present in the natural antitumor antibiotics as echinomycin and luzopeptin. However, the depsipeptide ring that links the two chromophores in these drugs serves an important role in preorganising the drugs for bisintercalation and in stabilising the drug–DNA complex through contacts in minor groove. The peptide linker used by us and others[30] is apparently considerably more flexible than the depsipeptide linker present in natural bisintercalator. It clearly does not stabilise the DNA–peptide complex in the same way as DNA–drug complex.

Therefore, it can be concluded that the compounds derived from peptide **1** by replacing tyrosine residue by aromatic moieties are not strong intercalators similar to natural bisintercalators.

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