Acta Biochimica Polonica

Vol. 45 No. 1/1998

41–57

QUARTERLY

Synthetic analogues of netropsin and distamycin — synthesis of a new pyridine and carbocyclic analogues of the pyrrolecarboxamide antitumour antibiotics*

Danuta Bartulewicz[1]✉, Krzysztof Bielawski[2], Agnieszka Markowska[1], Krzysztof Zwierz[3], Anna Pućkowska[1] and Andrzej Różański[1]

[1]*Department of Organic Chemistry,* [2]*Department of Drug Technology,* [3]*Department of Pharmaceutical Biochemistry, Medical Academy, A. Mickiewicza 2, 15-230 Białystok, Poland*

Key words: distamycin, netropsin, molecular modelling, drug design

A new series of pyridine-containing analogues III–XXII of distamycin A and netropsin was investigated by the molecular mechanics technique and molecular modelling. A pyridine analogue of netropsin (VII) is described, the first compound based on molecular studies, and two carbocyclic analogues of distamycin A with an N-terminal chloro- or bromoacetyl group (VIa, VIIa) were synthesized, as well as carbocyclic analogues of netropsin (VIIIb, Xb), potential carriers of alkylating elements.

The potential use of VIa, VII, VIIa, VIIIb and Xb as carriers to place into the minor groove of DNA chemical groups capable of modifying DNA, is discussed.

The rapidly increasing body of knowledge in molecular biology and the observation that most anticancer agents are able to bind DNA [1] have directed the interest of medicinal chemists towards investigation of the mechanism of the anticancer action and synthesis of novel, potentially useful, DNA-binding compounds. These compounds may be immediately taken advantage of for development of new pharmaceutical agents [2, 3], artificial restriction enzymes [4], as well as DNA probes [5]. In recent years nonintercalative groove-binding drugs have become objects of increasing interest as potential antineoplastic agents [3, 6, 7]. Netropsin and distamycin are among the earliest studied, and have become the paradigms for this class of compounds. The sequence-selective DNA-binding netropsin and distamycin were conjugated to various functional groups so as to deliver the drugs directly to the DNA target, thereby reducing their non-specific toxicity [6]. For example, analogues of distamycin containing four pyrrole rings, in combination with other cytotoxic agents, were less toxic than netropsin and distamycin [8]. A distamycin A derivative, tallmustin (FCE

*Presented as a poster at the 6th International Symposium on Molecular Aspects of Chemotherapy, July, 1997, Gdańsk, Poland.

✉To whom correspondence should be addressed.

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; TMS, tetramethylsilane.

24517), bearing at the N-terminus a benzoyl mustard moiety instead of the formyl group, has been found to display potent cytotoxic activity on human and murine tumour cell lines [9]. It is of particular interest to develop ligands with GC base sequence selectivity because most of the known DNA minor-groove binding compounds are AT-sequence-specific. This interest stems from the observation that regions of high GC content are commonly found in genomes in mammals, including humans, and that a functional role for GC-rich sequences is suggested by their frequent occurrence in genes associated with proliferation, among them in a number of oncogenes [2, 10].

Most minor groove DNA-binding drugs, such as distamycin, netropsin, Hoechst 33258, and DAPI (4′, 6-diamidino-2-phenylindole) bind with significant preference ($10-10^2$ times) to AT-rich sequences, and the preferred binding sites contain a run of at least four AT [6, 7]. From early X-ray studies on the binding of netropsin to an AT-rich DNA sequence a model based on specific hydrogen bonding between the amide protons of netropsin and the hydrogen-acceptor sites in AT minor groove was developed, in which the amide protons of netropsin form bifurcated hydrogen bonds with the adenine-N3 and thymine-O2 of two adjacent AT base pairs [11]. The hydrogen bonding pattern in the minor groove allows to discriminate between AT and GC base pairs. The GC base pair presents two hydrogen-acceptor (guanine-N3 and cytosine-O2) and one hydrogen-donor site (guanine-C2-NH_2) while at AT base pairs only two hydrogen-acceptor sites (adenine-N3 and thymine-O2) are available [7, 12]. The expected GC recognition should involve a specific hydrogen bond between a heteroatom of the ligand and the 2-aminogroups of guanine bases protruding into the minor groove. Wade & Dervan [13] reported that replacement of a terminal *N*-methylpyrrolecarboxamide unit of distamycin with pyridine-2-carboxamide affords a new DNA groove-binding molecule, pyridine-2-carboxamide-netropsin (Fig. 1), that accepts mixed (GC)-(AT) base pairs in preference to pure AT stretches of DNA.

The same approach was successfully used in the lexitropsin series where pyrrole was substituted by imidazole and in the Hoechst 33258 series where a benzimidazole was replaced by a pyridoimidazole [6, 7, 14]. These results offer hope for the design of more gen-

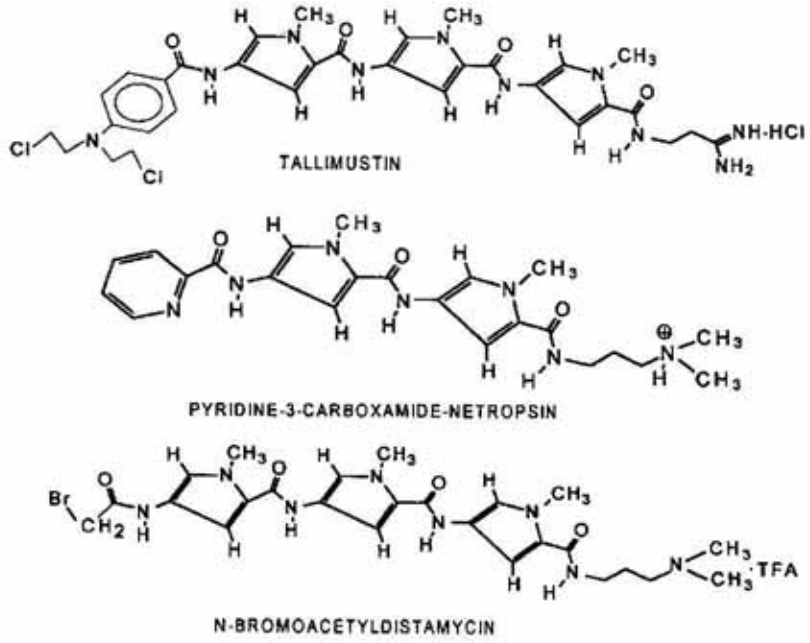


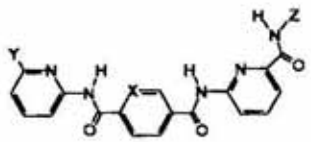
Figure 1. The structures of some analogues of distamycin A and netropsin.

eral sequence-specific DNA recognising molecules. However, a fuller understanding of all the forces involved in the recognition process is needed to reach the point at which it would be possible to rationally and reliably design sequence-specific DNA binding molecules.

The substitution of heterocyclic rings by carbocyclic rings [15] yields lexitropsins which in comparison with distamycin show reduced affinity to A–T pairs, increased affinity to G–C pairs [16] as well as lower toxicity, and increased antibacterial, antiviral and antitumor activity [17]. The addition to lexitropsins of compounds of known antitumor or antiviral activity allows to modify their selectivity and toxicity.

Of the alkylating groups widely used in the synthesis of compounds with antitumor activity, such as ethyleneimino, haloacetyl or epoxide groups, or moieties of nitrogen and sulphur mustards, in syntheses of lexitropsin mainly bromoacetyl, chloroacetyl and mustard groups are used, for example, tallimustin (FCE 24517; Fig. 1) is presently undergoing clinical trials [18].

An analogous compound characterised by a similar mechanism of action is *N*-bromoacetyldistamycin (Fig. 1) [19]. This compound has an *N*-bromoacetyl group instead of the formyl group, and a 3-dimethylaminopropylamino group instead of the amidine group. *N*-Bromoacetyldistamycin retains the A–T preference of distamycin and can selectively alkylate adenine-N3 sites in the minor groove. From the point of view of interactions with DNA, alkylating lexitropsins act by alkylating purine bases. Their alkylating properties concern the production, in a weakly acidic environment, of carbocations by splitting off strongly electronegative groups (Br^-) or open-



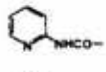
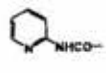
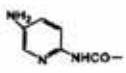
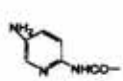
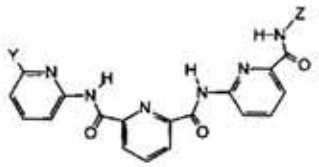
Compound	X	Y	Z
III	N	H	$CH_2CH_2CH_2N(CH_3)_2$
IV	C	H	$CH_2CH_2CH_2N(CH_3)_2$
V	N	H	$CH_2CH_2N(CH_3)_2$
VI	C	H	$CH_2CH_2N(CH_3)_2$
VII	N	$CONHCH_2CH_2CH_2N(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$
VIII	C	$CONHCH_2CH_2CH_2N(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$
IX	N		
X	C		
XI	N		
XII	C		

Figure 2a. The structure of pyridine analogues of distamycin A.



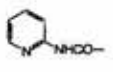
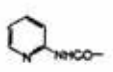
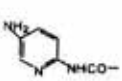
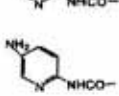
Compound	X	Y	Z
XIII	N	H	$CH_2CH_2CH_2N(CH_3)_2$
XIV	C	H	$CH_2CH_2CH_2N(CH_3)_2$
XV	N	$CONHCH_2CH_2N(CH_3)_2$	$CH_2CH_2N(CH_3)_2$
XVI	C	$CONHCH_2CH_2N(CH_3)_2$	$CH_2CH_2N(CH_3)_2$
XVII	N	$CONHCH_2CH_2CH_2N(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$
XVIII	C	$CONHCH_2CH_2CH_2N(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$
XIX	N		
XX	C		
XXI	N		
XXII	C		

Figure 2b. The structure of pyridine analogues of distamycin A.

ing three-member rings (ethyleneimine, epoxide). Carbocations react with N7 of guanine or N3 of adenine, blocking the processes of replication and transcription of nucleic acids.

In attempting to elucidate which structural elements are important in ligand-DNA sequence recognition, we have focused on a group of pyridine-containing distamycin analogues. The behaviour of a series of pyridine-containing analogues **III–XXII** of distamycin A (Fig. 2) was examined by force field and semi-empirical quantum mechanics calculations in order to predict their behaviour with respect to isohelicity and closeness of fit within the minor groove of DNA. We report here the synthesis of the first compound, which was synthesized on the basis of this concept.

Netropsin and distamycin, and their analogues are excellent carriers of alkylating elements. In this work we introduced lexitropsins with an introduced N-terminal chloro- or bromoacetyl group to a carbocyclic derivative of distamycin containing two aromatic rings.

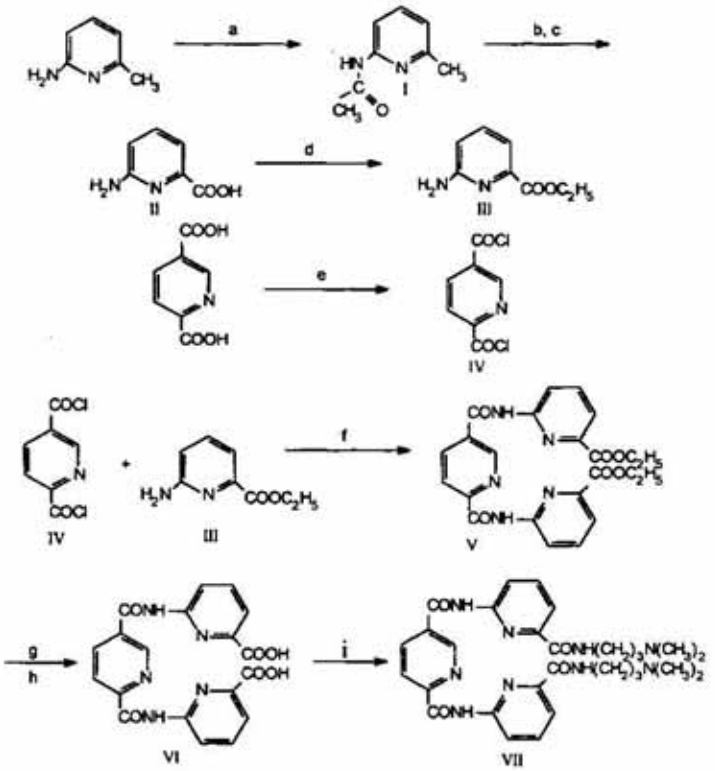
The synthesis of carbocyclic analogues of netropsin in which *N*-methylpyrrole rings were replaced by 1,3-disubstituted benzene rings was accomplished in eight steps starting from 4-hydroxyazobenzene-3-carboxylic acid.

Chemical formulas of distamycin and netropsin analogues are shown in Figs. 3, 4 and 5.

MATERIALS AND METHODS

Part I: Molecular modelling

Computational analysis and restrained molecular modelling were performed on a 486DX2-66 Personal Computer using the program HyperChem (version 3.0) [20]. Structures **I–XXII** were built within HyperChem and minimized with MM+ (molecular model-



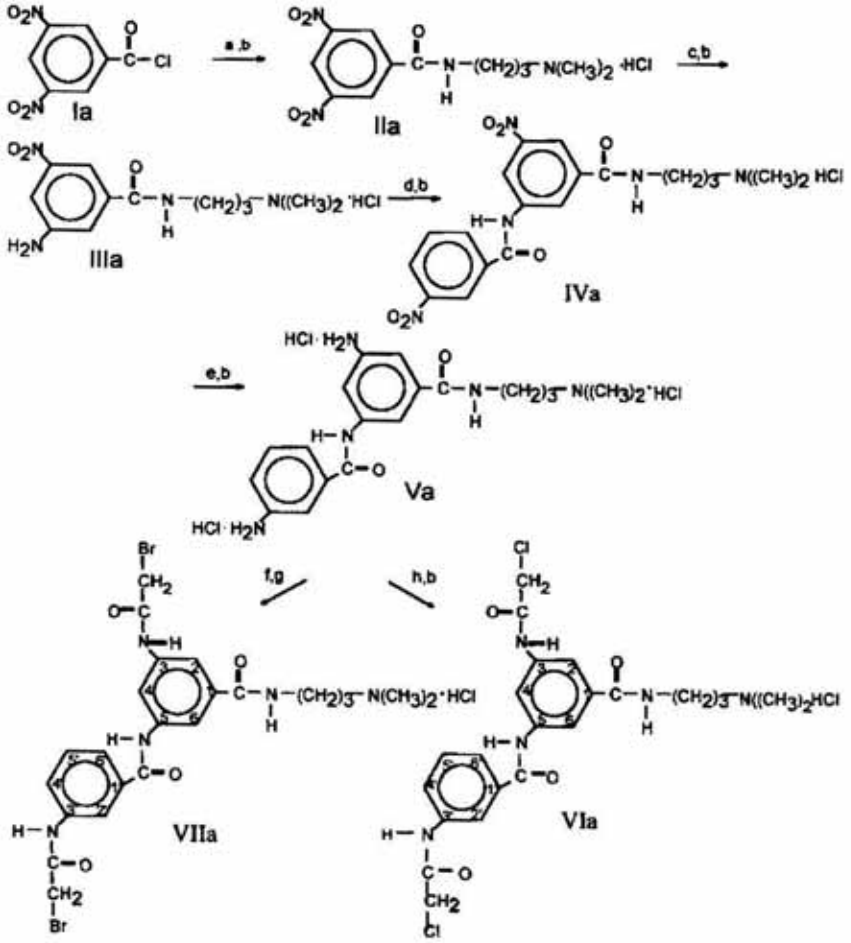
REAGENTS AND CONDITIONS: (a) $(CH_3CO)_2O$; (b) $KMnO_4$, H_2O; (c) HCl; (d) C_2H_5OH, HCl; (e) $SOCl_2$, C_6H_6, DMF; (f) CH_2Cl_2; (g) NaOH, H_2O, C_2H_5OH; (h) CH_3COOH; (i) $(CH_3)_2N(CH_2)_3NH_2$, carbonyldiimidazole, DMF

Figure 3. Synthesis of pyridine analogue of distamycin A – VII.

ling) force field, *in vacuo* conditions, to provide reasonable standard geometry. The HyperChem force field MM+ uses a 1993 parameter set for MM2 [21]. Molecules were minimized when there was a minimum energy charge of less than 0.08 kJ/mol per iteration [20]. The conjugate gradient method was used for minimization. The lowest-energy conformers thus obtained were submitted to AM1 calculations to optimize their geometry and determine atomic charge distributions. Molecules were deemed to be minimized when the gradient fell to less than 0.08 kJ/mol. Two typical nonintercalative groove-binding compounds, distamycin and pyridine-2-carboxamidenetropsin, were also investigated and employed as template for molecular superimposition.

The model of the B-DNA d(CGCAGCTTTGCG) duplex was built with HyperChem 3.0 which allows generation of structures by specifying generalized helical parameters. The structure generated in this way served as starting geometry for molecular mechanics minimization using Amber force field [22]. The electrostatic term was calculated using a distance-dependent dielectric constant to mimic solvent effects. After 800 cycles of energy minimization by the method of conjugate gradient, the potential energy was decreased to reasonable values without significant distortion of the initial structure.

A combined translational/rotational operation was used to insert the ligand at a symmetric location into the minor groove of the B-DNA d(CGCAGCTTTGCG) duplex. The naturally curved shape of the ligand facilitated a



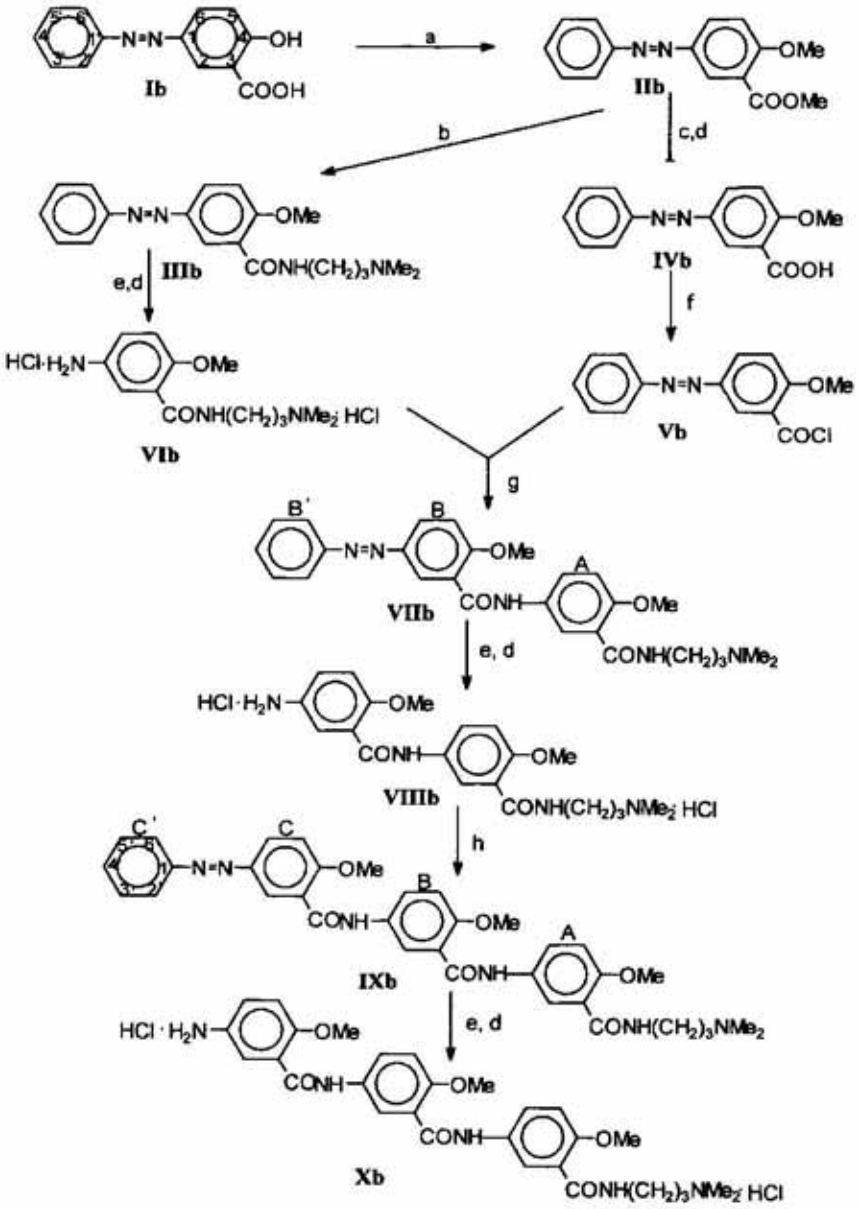
(a) $H_2N(CH_2)_3N(CH_3)_2$; (b) HCl/H_2O; (c) NaHS; (d) 3-nitrobenzoyl chloride pyridine, DMAP; (e) H_2/Pt; (f) $BrCH_2COBr$; (g) HBr/H_2O; (h) $ClCH_2COCl$

Figure 4. Synthesis of carbocyclic analogues of distamycin A – VIa and VIIa.

comfortable fit of the ligand in the DNA groove. The terminal dimethylamine nitrogen of the (dimethylamino)propyl tail of **III** was modelled as fully protonated. This model was subsequently optimized so that there were no incorrect contacts between the DNA and the ligand. Energy minimization was performed using the force field MM+. The cut-off distance for non-bonded interactions was set at 12 Å with a switching distance of 2 Å. Solvent effects were simulated the use of a distance-dependent dielectric of the form $\varepsilon = R$. The energy of the complex was minimized using conjugate gradient. Complex was deemed to be minimized when the gradient fell to less than 0.1 kJ/mol.

Part II: Synthesis of analogues of distamycin A and netropsin

Compounds **VIa, VII, VIIa, VIIIb** and **Xb**, pyridine and carbocyclic analogues of dista-



(a) CH_3J, K_2CO_3, cyclohexanone; (b) $H_2N(CH_2)_3NMe_2$; (c) NaOH, MeOH-H_2O; (d) HC
(e) H_2/Pt, EtOH; (f) $SOCl_2$, C_6H_6; (g) Et_3N, CH_2Cl_2; (h) 5, DMAP, pyridine;

Figure 5. Synthesis of carbocyclic analogues of netropsin — VIIIb and Xb.

mycin A and netropsin were synthesized as described below. The structures of the prepared compounds were confirmed by analyses of their IR, ^{1}H- and ^{13}C NMR spectra. Melting points were determined on Büchi 535 melting-point apparatus and are uncorrected. The IR absorption spectra were recorded using a Specord 75 IR spectrophotometer (C. Zeiss, Jena) and only the main sharply defined peaks are reported. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Bruker AC 200 F spectrometer, using TMS (tetramethylsilane) as an internal standard. Chemical shifts are expressed in δ value (p.p.m.) and coupling constants are given in J (Hertz). Multiplicity of resonance peaks is indicated as singlet (s), doublet (d), triplet (t), quartet (q), broad singlet (bs), and multiplet (m).

Thin-layer chromatograms were prepared on precoated plates (Merck, Silica gel 60F-254). Solvent systems (all proportions by volume):

Compound **VII**: (A), pyridine/ethyl acetate/acetic acid/water, 5:5:1:3; (B), butanol/pyridine/water, 6:4:3; (C), solvent (A)/solvent (B), 1:1; (D), ethyl acetate/methanol/water, 3:2:2.

Compounds **VIa** and **VIIa**: (A), methanol/acetone/xylene/25% ammonia, 7:1:1:1.

Compounds **VIIIb** and **Xb**: (A), pyridine/ethyl acetate/acetic acid/water, 5:5:1:3; (B), butanol/pyridine/water, 6:4:3; (C), solvent (A)/solvent (B), 1:1; (D), ethyl acetate/methanol/water, 3:1:1. All compounds were visualized with short-wave ultraviolet light.

Silica gel 60 (230–400 mesh ASTM) was used for column chromatography.

Chromatographic methods and careful analysis of NMR spectra ascertained the structures and purity of all the compounds. Location of the ^{1}H NMR and ^{13}C NMR signals and IR absorption bands were compared with those given in the literature [23, 24].

Synthesis of pyridine analogue of distamycin A – VII

Preparation of compound **VII** (*N,N'*-bis[6-(*N*-3-dimethylaminopropyl)carbamoylpyridin-2-yl]pyridine-2,5-dicarboxamide) was satisfactorily achieved by standard chemical transformations according to the reaction sequence shown in Fig. 3.

The 6-aminopyridine-2-carboxylic acid **II** was obtained after the acetylation and oxidation the former. Treatment of **II** with ethanol gave the ethyl ester **III**.

The second key substrate, diacid dichloride **IV**, was prepared by reaction of pyridine-2,5-dicarboxylic acid with thionyl chloride in refluxing benzene with a drop dimethylformamide.

Condensation of diacid dichloride **IV** and ethyl ester **III** in dry methylene chloride at 0°C afforded *N,N'*-bis(6-ethoxycarbonyl-2-pyridyl)pyridine-2,5-dicarboxamide **V**. Transformation of ester **V** into the desired diacid **VI** was achieved by hydrolysis with 2 M NaOH with ethanol at 80°C for 1 hour. The aminolysis of the above diacid **VI** with 3-dimethylaminopropylamine in the present 1,1'-carbonyldiimidazole gave the amine **VII** (m.p. 221–223°C (as dihydrochloride); IR (nujol, cm^{-1}): 3300 (NH); 1690 (CO); 1550 (CONH); 1520, 1420, 990, 770 (Py); 1363 (CH_3); 1132 (C-O); **^{1}H NMR** (DMSO-d_6, δ): 1.94 (m, 2H, CH_2); 2.51 (t, 2H, $C\underline{H}_2N(CH_3)_2$); 3.07 (t, 2H, $CONHC\underline{H}_2$); 3.39 (s, 6H, $N(CH_3)_2$); 8.13 (d, 1H, C_3-H); 8.42 (d, 1H, C_4-H); 9.25 (s, 1H, C_6-H); 8.28 (d, 2H, $C_{12'}$-H, $C_{12''}$-H); 8.05 (d, 2H, $C_{13'}$-H, $C_{13''}$-H); 7.86 (d, 2H, $C_{14'}$-H, $C_{14''}$-H); **^{13}C NMR** (DMSO-d_6, δ): 150.70 (C2); 121.90 (C3); 138.07 (C4); 129.31 (C5); 149.07 (C6); 161.23, 161.05 (C7', C7''); 151.64, 150.15 (C9', C9'); 146.00, 146.15 (C11', C11''); 121.00, 121.5 (C12', C12''); 138.99, 139.28 (X13', C13'); 119.10, 120.70 (C14', C14''); 163.95, 164.12'(C15', C15''); 36.72, 36.44

(C17', C17''); 24.86, 24.99 (C18', C18''); 55.24, 55.46 (C19', C19''); 42.90 ($N(CH_3)_2$).

Synthesis of carbocyclic analogues of distamycin A – VIa and VIIa

The starting material for the synthesis was 3,5-dinitrobenzoyl chloride (**Ia**). The transformation of this compound to desired products (**VIa** and **VIIa**) is outlined in Fig. 4.

Compound **Ia** was condensed with 3-dimethylaminopropylamine to obtain amide **IIa**. Using reduction with sodium hydrogen sulfide, one of the two nitro groups was reduced to amino, to yield derivative **IIIa**. The aromatic amine was reacted with 3-nitrobenzoyl chloride, to give dipeptide **IVa**. Compound **Va** was obtained by catalytic reduction of dipeptide **IVa** with hydrogen. The reduction product was reacted with chloroacetyl chloride or bromoacetyl bromide in acetonitrile, and two new *N*-chloro- and *N*-bromoacetyl derivatives were obtained: **VIa** (*N*-(3-dimethylamino)-propyl-3-chloroacetamido-5-[(3-chloroacetamido)-benzamido]-benzene-1-carboxamide; {**IR**, film, cm^{-1}: 623 (C-Cl), 796 (Ar-H), 928 (Ar-H), 1310 (Ar-N), 1776 (C=O), 2500 (C-H), 3040 (N-H); **^{1}H NMR**, CD_3OD:CD_3COCD_3 (1:1): 1.22 (m, 2H, C-CH_2-C), 1.43 (s, 6H, $N(CH_3)_2$), 3.23 (m, 2H, CH_2NMe_2), 3.71 (m, 2H, $CONHCH_2$), 4.18 (s, 4H, CH_2Cl), 7.00–8.00 (m, 7H, Ar-H), 9.45 (bs, 1H, CONH), 10.26 (bs, 2H, CONH); **^{13}C NMR**, CD_3OD:CD_3COCD_3 (1:1), δ: 41.69 (C-CH_2-C), 43.11 ($CONHCH_2$), 54.90 ($N(CH_3)_2$), 55.14 (CH_2NMe_2), 65.40 (CH_2Cl), 68.20 (CH_2Cl), 126.80, 129.20, 129.80 (C2, C4, C6, C2', C4', C5', C6'), 144.20, 144.40 (C3, C3'), 132.10, 132.20, 132.80 (C1, C1', C5), 166.80 (CONH), 167.00 (CONH), 168.69 (CONH)}; and **VIIa** (*N*-(3-dimethylamino)-propyl-3-bromoacetamido-5-[(3-bromoacetamido)-benzamido]-benzene-1-carboxamide) {**IR**, film, cm^{-1}: 590 (C-Br), 720 (C-H), 796 (Ar-H), 928 (Ar-H), 1320 (Ar-H), 1670 (C=O), 2820 (C-H), 3400 (N-H); **^{1}H NMR**, CD_3OD: CD_3COCD_3 (1:1): 1.89 (m, 2H, C-CH_2-C), 2.77 (s, 6H, $N(CH_3)_2$), 3.13 (m, 2H, CH_2NMe_2), 3.35 (m, 2H, $CONHCH_2$), 4.11 (s, 4H, CH_2Br), 7.00–8.50 (m, 7H, Ar-H), 9.63 (bs, 1H, CONH), 10.60 (bs, 2H, 2×CONH); **^{13}C NMR**, CD_3OD:CD_3COCD_3 (1:1): 41.89 (C-CH_2-C), 42.11 ($CONHCH_2$), 53.21 ($N(CH_3)_2$), 54.12 (CH_2NMe_2), 60.13 (CH_2Br), 62.81 (CH_2Br), 126.00, 128.30, 130.20, (C2, C4, C6, C2', C4', C5', C6'), 144.40, 146.00 (C3, C3'), 166.60 (CONH), 168.12 (CONH), 169.57 (CONH)}. Due to their instability and easy oxidation, the primary aromatic amines (**IIIa** and **Va**) were converted to hydrochlorides.

Synthesis of carbocyclic analogues of netropsin – VIIb and Xb

The required key starting material 4-hydroxyazobenzene-3-carboxylic acid **I** was prepared by the method described by Polaczkowa [25]. The convergent route of synthesis followed by us is outlined in Fig. 5.

Treatment of **Ib** with methyl iodide in refluxing cyclohexanone gave the methoxy methyl ester **IIb**. Aminolysis of ester **IIb** with 3-dimethylaminopropylamine gave the appropriate azoamide **IIIb** in good yield. The nitrogen protecting azo group was removed by catalytic hydrogenation at room temperature and at atmospheric pressure to yield the desired amine **VIb**. An examination of several systems that could be used in catalytic hydrogenation of azo group revealed the chloroplatinic acid/sodium borohydride system to give the best results.

The second key substrate, acid chloride **Vb**, was prepared starting from methyl 4-methoxyazobenzene-3-carboxylate (**IIb**). The ester **IIb** was hydrolyzed with 0.5 M NaOH in a 1:1 methanol/water mixture to give acid **IVb**. Transformation of acid **IVb** into the desired acid chloride **Vb** was achieved by treatment of the acid with thionyl chloride in refluxing benzene.

Condensation of acid chloride **Vb** and amine **VIb** in the presence of triethylamine in methylene chloride at room temperature gave the

dipeptide **VIIb**. Catalytic reduction of the azo group of **VIIb** to the amine derivative **VIIIb** (5-(5-amino-2-methoxybenzamido)-*N*-[3-(dimethylamino)propyl]-2-methoxy-benzene-1-carboxamide dihydrochloride) {m.p. 224–226°C; **IR** (nujol, cm^{-1}): 3360 (NH); 2910 ($ArOCH_3$); 2593 (CHN); 1653 (CONH); 1298 (NR_3); 1260 ($ArOCH_3$); **^{1}H NMR** (DMSO, δ): 2.07 (m, 2H, CH_2); 2.91 (s, 6H, $N(CH_3)_2$); 3.23 (t, 2H, $C\underline{H}_2N(CH_3)_2$); 3.53 (t, 2H, $CONHC\underline{H}_2$); 3.98 (s, 6H, OCH_3); 7.22–8.49 (m, 6H, Ar_A-H Ar_B-H); 10.55 (s, 1H, CONH); 10.74 (s, 1H, CONH); **^{13}C NMR** (DMSO, δ): 23.90 (CH_2); 36.31 ($CONHC\underline{H}_2$); 41.78 ($N(CH_3)_2$); 54.13 ($\underline{C}H_2N(CH_3)_2$); 56.08 ($OCH_3$); 56.33 ($OCH_3$); 112.20 ($C_{3A}$); 112,32 ($C_{3B}$); 121.42 ($C_{1A}$ C_{1B}); 123.52 (C_{6A}), 125.59 (C_{4A}); 126.79 (C_{6B}); 127.17 (C_{4B}); 130.24 (C_{5A}); 140.95 (C_{5B}); 156.12 (C_{2A}); 159.95 (C_{2B}); 164.01 (CONH); 170.21 (CONH)} proceeded satisfactorily, under the reaction conditions similar to those described in connection with the synthesis of **VIb**. Condensation of acid chloride **Vb** and amine **VIIIb** in the presence of a catalytic amount of 4-(dimethylamino)pyridine gave the tripeptide **IXb** in good yield. Catalytic hydrogenation of **IXb** gave the desired amine tripeptide **Xb** (5-[5-(5-amino-2-methoxybenzamido)-2-methoxybenzamido]-*N*-[3-(dimethylamino)propyl]-2-methoxybenzene-1-carboxamide dihydrochloride) {m.p. 222–224°C; **IR** (nujol, cm^{-1}): 3387 (NH); 2800 ($ArOCH_3$); 2580 (CHN); 1647 (CONH); 1502 (ArNH); 1347 (NR_3); 1293 ($ArOCH_3$); **^{1}H NMR** (DMSO, δ): 1.94 (m, 2H, CH_2); 2.72 (s, 6H, $N(CH_3)_2$); 3.05 (t, 2H, $C\underline{H}_2N(CH_3)_2$); 3.35 (t, 2H, $CONHC\underline{H}_2$); 3.83 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 7.01–8.43 (m, 9H, Ar_A-H Ar_B-H Ar_C-H); 10.15 (s, 2H, CONH); **^{13}C NMR** (DMSO, δ): 24.04 (CH_2); 36.32 ($CONHC\underline{H}_2$); 41.83 ($N(CH_3)_2$); 54.22 ($\underline{C}H_2N(CH_3)_2$); 56.15 ($OCH_3$); 56.18 ($OCH_3$); 56.49 ($OCH_3$); 112.23 ($C_{3A}$); 112.30 ($C_{3B}$); 113.45 ($C_{3C}$); 118.21 ($C_{1A}$ C_{1B}); 120.50 (C_{1C}); 121.26 (C_{6A}); 122.12 (C_{6B}); 123.03 (C_{4A}); 123.32 (C_{4B}); 123.66 (C_{6C}); 124.78 (C_{4C}); 128.32 (C_{5A}); 132.09 (C_{5B}); 136.09 (C_{5C}); 150.48 (C_{2A}); 152.54 (C_{2B}); 153.06 (C_{2C}); 163.96 (CONH); 163.98 (CONH); 165.07 (CONH)}. The amine developed a dark colour on exposure to atmosphere. Due to its inherent instability and difficulty in storing this compound, the hydrochloride salt of the amine was prepared.

RESULTS AND DISCUSSION

Part I

Computer-assisted molecular modelling was used to determine structural analogies between pyridine-2-carboxamide-netropsin and compounds **III–XXII** by way of the molecular modelling software HyperChem 30, as described in Methods. First, the input geometries were generated and initially minimized with the MM+ force field. The lowest-energy conformers thus obtained were submitted to AM1 calculations to optimize their geometry and determine atomic charge distributions. Two typical nonintercalative groove-binding compounds, distamycin (**I**) and pyridine-2-carboxamide-netropsin (**II**), were also investigated and used as templates for molecular superimposition.

Stevens [26] reported that the most potent agent, a minor groove binder, has a distance between the hydrogen bond donor sites of 9.2 Å, which corresponds to the distance between the two amide bonds in the low-energy conformers of **I–VI** and **XII–XXII**. The MM+ energy-minimized conformations of the compounds show that **III–VI** and **XIII–XXII** molecules adopt a crescent-shaped conformation (Fig. 6) and it is reasonable to suppose that they match the convex surface of the minor groove of DNA.

For the sake of clarity, only four compounds from the whole data, **III**, **XI**, **XVII** and **XXI**, are presented on the assumption that the examination of their conformational space might provide information valid for other closely related analogues. As an example,

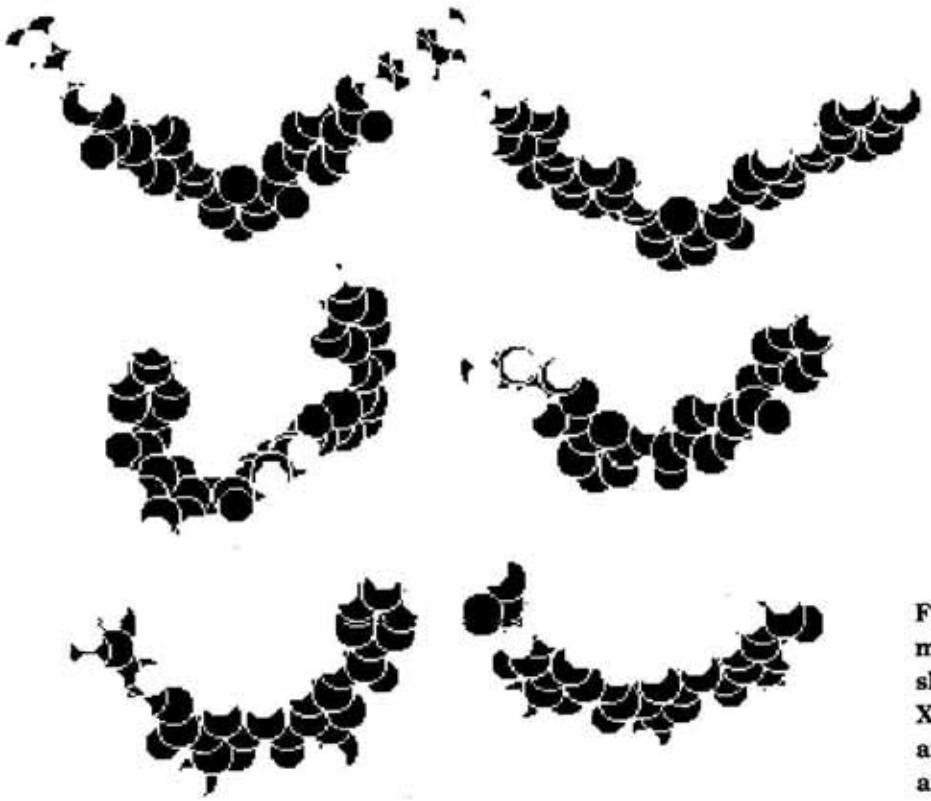


Figure 6. Energy minimized conformations showing curvature of XVII and XXI (top), XI and III (middle) and I and II (bottom).

compound **III** has most of the conformational features in common with compounds **IV–VI**. The minimized conformation of **III** had superimposed on that of pyridine-2-carboxamide-netropsin (Fig. 7) fits well the structure of the latter. The two structures have almost equal steric dimension, and about 90% of them could be overlaid. The nitrogen atom of the terminal pyridine ring of **III** is located in a position corresponding to that of the pyridine nitrogen of pyridine-2-carboxamide-netropsin.

On the contrary, it seems too difficult to fit properly to the minor groove the analogues **XIII–XXII** because of their increased curvature. If the terminal amido NH groups are positioned for hydrogen bonding, then the central amido NH groups would be positioned too far for hydrogen bonding to the DNA [11, 7]. These studies clearly show also that the length of three pyridine units is optimal for this class of compounds, and a further increase in the number of heterocycles to target for a longer DNA sequence is not feasible due to the phasing problem [6] which arises because of the lack of dimensional correspondence between oligopeptides and oligonucleotides.

As a further attempt to demonstrate structural similarities between **III** and **II**, we considered their atomic charges. In the case of **II**, the amide hydrogen atoms carried charges of 0.254e, 0.235e, and 0.220e. A charge of −0.140e was found for nitrogen atom of the pyridine ring. The corresponding values in **III**

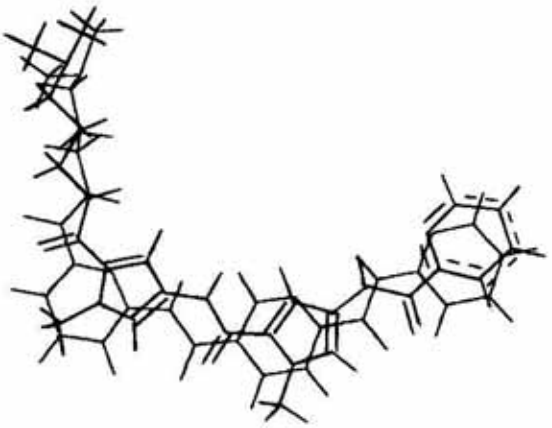


Figure 7. Superimposition of compound III (green) and pyridine-2-carboxamidenetropsin (red).

were found to be, respectively, 0.261e, 0.238e, 0.229e, and −0.185e, showing a similar electron distribution in the two structures.

In order to explore to what extent hydrogen-bonding plays a role in determining the binding preferences of **III** (Fig. 8), we have generated a molecular model for the 1:1 complex of **III** with a B-DNA dodecamer d(CGCAGCTTTGCG)·d(GCGTCGAAACGC).

The energy-minimized structure of the ligand-DNA complex is shown in Fig. 9.

The compound lies deep within the narrow groove, inserted snugly between the sugar-phosphate walls and is slightly displaced from the centre of the dodecamer. Selected distances between DNA and atoms of compound **III** are listed in Table 1. Amide NH(2) and NH(6) groups of **III** form hydrogen bonds to the O2 atom of T7 and N3 atom of A16 at distances of 3.31 Å and 2.92 Å, respectively. Hydrogen bond distances between N2 of **III** and N3 of A18 and between N4 of **III** and N3 of A17 became longer than 3.5 Å after refinement. These interactions are weak, although they have the right geometry for being part of three-centered H-bond systems. The nitrogen atom of the terminal pyridine ring of **III** lies opposite the amino group of G19, and the cationic *N,N*-dimethylammonium group is displaced away from the floor of the minor groove, not interacting with the bases. The molecular modelling indicates that the pyridine nitrogen (N1) of **III** participates in a hydrogen bond with one amino proton of G19. The compound **III** covers pairs C6·G19, T7·A18, T8·A17, and T9·A16. The protonated terminal dimethylamine nitrogen of the (dimethylamino)propyl tail is adjacent to a negatively charged phosphodiester linkage.

The three pyridine rings of **III** are slightly twisted so that each ring can be parallel to the enclosing walls of the DNA minor groove. It is possible that the pyridine rings were turned by the calculated van der Waals contacts with the walls of the groove, in the absence of planarity restraints around the amide–pyridine bond, reflecting resonance delocalization.

Table 1. Hydrogen bond distances in the complex d(CGCAGCTTTGCG)·d(GCGTCGAAACGC)·III

Compound III atom	DNA atom	Distance (Å)
N1	C2-NH2 (G19)	2.55, 3.70
N2	N3 (A18)	3.72
N2	O2 (T7)	3.31
N4	N3 (A17)	4.16
N6	N3 (A16)	2.91

From the viewpoint of either valence bond or molecular orbital theory, one would have expected these three aromatic rings to form a single delocalized electronic system, with coplanar rings. In long molecules this tendency toward coplanarity could be opposed by the effect exerted by the walls of the minor groove, which themselves are twisted as the groove snakes up and around the helix cylinder. The actual geometry of any given bond between rings would represent a balance or trade off between delocalization flattening and groove-wall twisting. The binding of **III** within the minor groove has a relatively minor influence on the DNA structure, in view of the fact that in B-DNA the minor groove is intrinsically wider in GC regions than AT [12]. The minor groove opens up in the vicinity of the drug by 0.05–0.10 nm. Local helix parameters such as twist, roll, rise and propeller twists are nearly unchanged. Undoubtedly more data is needed to further clarify the relative importance of

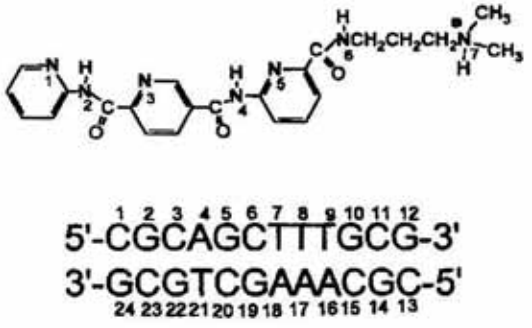


Figure 8. Structures and numbering schemes for compound III and the dodecadeoxyribonucleotide.

these factors. An accurate definition of the optimal binding site for **III** by molecular modelling alone has been hampered by the fact that DNA fragment used in the model contains a limited number of binding sites. Various dodecamer sequences even though they exhibit a similar general trend, show much dissimilarity in the detail [12]. It is obvious that the interaction model presented here must be validated by accurate physical measurements.

In addition to hydrogen-bonding interactions, another predominant force in stabilising the drug-DNA complex is provided by the van der Waals contacts between compound **III** backbone atoms and the O4′ atoms of the sugars and other atoms of the sugar-phosphate backbone forming the walls of the narrow minor groove [6, 7, 27]. More recent experimental work and theoretical calculations have suggested that the electrostatic attraction of the DNA for a cationic drug analogue may overcome the purely steric effects, and prevent a compound from exhibiting its intrinsic GC preference [7, 12, 27]. Greater sequence selectivity was observed when one of the two cationic ends of a netropsin analogue was made uncharged [6, 28]. However, changing the amidine tail of distamycin to a (dimethylamino)propyl group and the formamide to hydrogen cause a decrease in binding to DNA [29]. Compound **III** is monocationic at the physiological pH values, therefore its selectivity for 5′-AAAG-3′ sequence may derive rather from the particular geometry of such sequences than from electrostatic considerations. The DNA flexibility will also be an important feature to consider. Because of the inherently lower flexibility of GC-rich regions, the design of GC recognition elements will require extremely accurate matching of groove and ligand surfaces, whereas a much cruder match may suffice for AT recognition [12]. All of these findings reveal a complex interaction behaviour that is still far from being completely understood even though X-ray and NMR techniques have provided a wealth of structural data on some of complexes of DNA and minor groove-binding drugs [11, 12, 28,

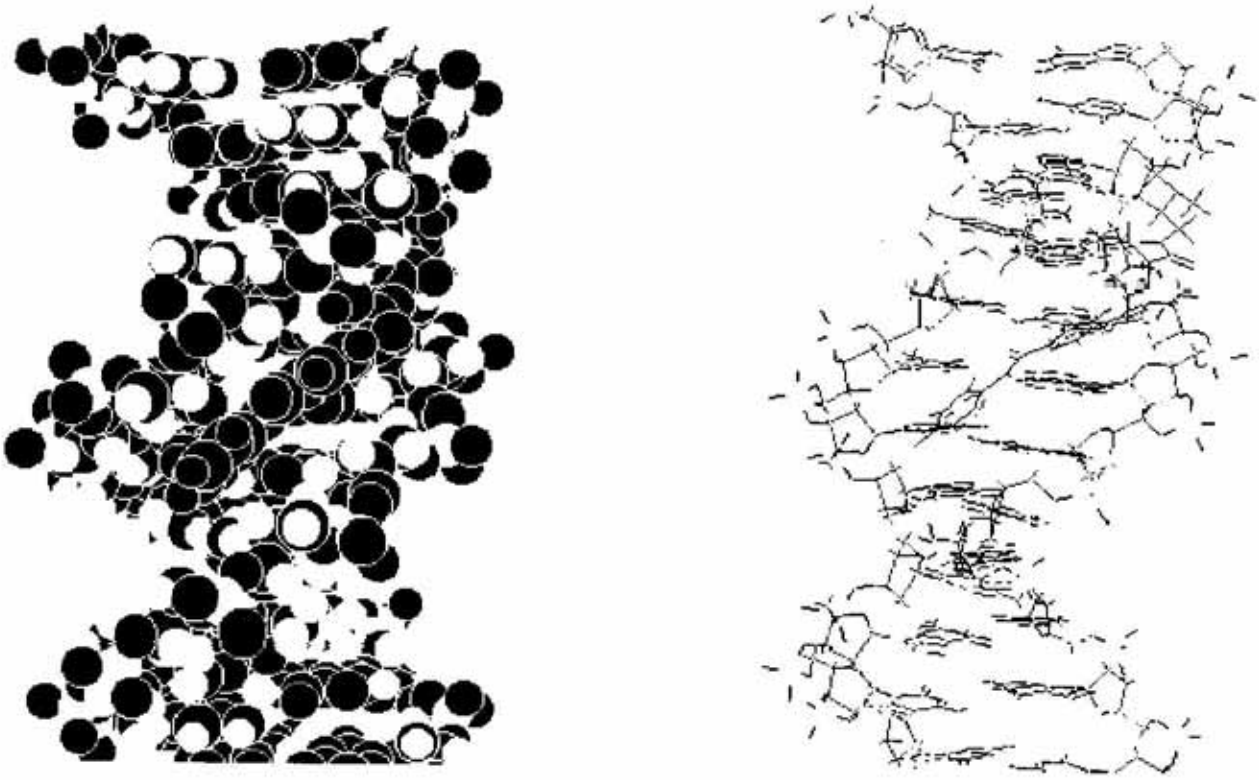


Figure 9. Diagram of the molecular model of the complex of compound III with d(CGCAGCTTTGCG) · d(GCGTCGAAACGC) obtained by energy minimization.

30]. Computational chemistry tools, on the other hand, have also been used in an attempt to assess the relative importance of the considerable number of forces and conformational variables involved in the binding of this family of compounds to DNA [6, 31–33]. It seems that the groove-binding drug design is more complex that anticipated, and that all three effects – groove width, steric repulsion, and electrostatic forces – must be taken into account in order to create a family of true sequence-reading drug molecules.

We feel that the approach described in this paper may provide molecular frameworks that may act as a source of inspiration for further drug design. It seems that future studies should be directed towards synthesis and biological testing of compounds **III–VI**. Work in this area is in progress in our laboratory.

Part II

1. On the basis of molecular modelling, it seems that the structure of the tripyridine peptide might be a useful starting framework for synthesis of selective DNA minor groove binding molecules. Although some data point to a positive correlation between DNA binding and antitumour activity of compound, it is usually accepted that DNA binding is a necessary, but not sufficient condition for antitumour activity. Our design is based on the assumption that an ideal antitumour agent should consist of two essential parts of compound: (i) a recognition unit which serves as a carrier and (ii) a functional moiety capable of modifying DNA. Our rationale for considering **VII** primarily as a vector for the delivery of DNA interactive agents stems from the available literature data. Recent work on the targeting of nitrogen mustard alkylating agents to DNA by the use of DNA minor groove-binding ligands has shown that this strategy can greatly enhance both cytotoxicity *in vitro* and antitumour activity *in vivo* of the mustard moiety, when compared with untargeted mustards of similar reactivity [9, 18, 34]. Such targeting can also significantly alter the pattern of DNA alkylation by the mustard. For example, tallimustine (FCE 24517) fails to produce some of the typical lesions commonly associated with classical alkylating agents such as alkylation of guanine N7 in the major groove of DNA. Preliminary evidence suggests a similar mode of action to that seen for CC-1065 (an extremely cytotoxic antitumour agent, a *Streptomyces zelensis* fermentation product [8]) with alkylation of adenine N3 in the minor groove of DNA [9, 35]. If it is assumed that the antitumour activity of tallimustine derives from the relatively low number of highly sequence-specific sites of adenine alkylation in the DNA of cancer cells, it may be postulated that the damage occurs in those genes that are particularly relevant for neoplastic growth. A variety of DNA-cleaving compounds have been covalently attached to distamycin in an effort to increase the affinity of these compounds for DNA, to give enhanced DNA cleavage. Such complexes include the intensely studied distamycin and penta-*N*-methylpyrrolecarboxamide Fe^{++}/EDTA complexes [7, 36]. More recently, other DNA-cleaving compounds, including enediynes and other radical-forming moieties, have been successfully linked to these peptides, altering their ability to damage DNA [37, 38]. In view of the above results, it becomes clear that the activity of **VIIa** can be modulated/improved by side chain modifications, such as replacement of one dimethylaminopropyl group by a DNA-cleaving moiety.

Initial design of these linkers involved trial and error and chemical intuition, but for a systematic, computer-based design of linker groups one must know the precise positioning of monomers on the DNA duplex. Work along these lines is currently conducted in our laboratories.

2. Products **VIa** and **VIIa**, in contrast to haloacetyldistamycins, are compounds containing, like biologically active difunctional nitrogen mustards, bis-epoxides (Epodyl) or aziridines (Thiotepa), two alkylating groups.

Electrophilic, haloacetyl groups are separated from each other by 14 atom chains, which increases the possibility of simultaneous alkylation of both strands of B-DNA. Assuming that compounds **VIa** and **VIIa** bind in the DNA minor groove in an identical manner like bromoacetyldistamycin or chloroacetyldistamycin, the N-terminal group Br-CH_2-CO-(Cl-CH_2-CO-)- should bind covalently to N3 of adenine. The haloacetyl side group, situated outside the minor groove, may alkylate other adjacent purine bases, both in the same or the other strand of the double helix. The above reaction mode of these compounds with DNA is possible after they have assumed conformation "A", in which the haloacetyl side group is situated outside the minor groove of B-DNA, whilst the three -CO-NH- groups of the main strand of a described compound bind with hydrogen bonds to N3 of adenine or O2 of thymine. However, an alternative reaction mode of compounds **VIa** and **VIIa** with DNA is possible, in which both haloacetyl groups are situated inside the minor groove (conformation "B"). The two conformations are illustrated in Fig. 10.

In the second instance, both haloacetyl groups can react with the adjacent purine bases, whilst the dimethylamine group may also make contact with the phosphate backbone. In both conformations it is possible for the three -CO-NH- groups to participate in the formation of hydrogen bonds. In conformation "B", compounds **VIa** and **VIIa** have forms such as derivatives of *m*-phenylenediamine with a structure similar to that of lexitropsin, so-called "BIGBEN" compounds [39], also displaying the ability to bind to DNA.

3. The compounds **VIIIb** and **Xb** possess a dimethylamino group in place of the amidinium moiety normally present in netropsin. The synthesis of C-terminus-modified analogues, in which a dimethylamino moiety is substituted for the parent amidine group, provides a number of advantages. First, the compounds containing a modified terminus are chemically stable, and thus the synthetic

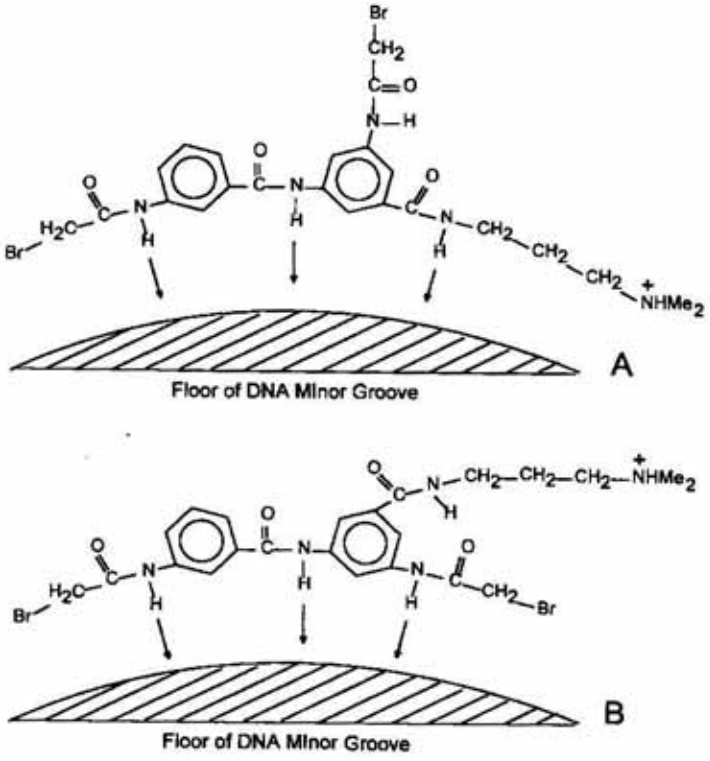


Figure 10. Alternative conformations (A, B) of compound VIIa.

The arrows indicate the NH-groups able to participate in the formation of hydrogen bonds with DNA.

methodology is readily adaptable to preparation of further analogues. Second, they are not hygroscopic and are easy to handle. Third, the dimethylamino group is uncharged, and thus column chromatography or recrystallization can readily purify products and intermediates. Finally, with a pK_a about 9.3, this moiety would be protonated at physiological pH of 7.4 to provide favourable electrostatic attraction to the negative electrostatic charge of the DNA. The synthesis and purification of the benzene analogues of distamycin have been reported by Rao *et al.* [40].

The compounds **VIIIb** and **Xb** have the requisite charged end groups and a number of potential hydrogen-bonding loci equal to that of distamycin. The backbone structures of pyridine and benzene analogues of distamycin suggest the possibility of interactions of these compounds with DNA. The decrease in curvatures of **X** in comparison with distamycin is a major difference between these ligands. The hydrophobic convex edge of bound **X** faces away from the hydrogen-binding region and thus the replacement of *N*-methyl pyrrole by benzene should not appreciably disturb the total binding interaction. It has been established that the replacement of the *N*-methyl substituent of distamycin by *N*-propyl or even *N*-isoamyl does not prevent binding to B-DNA [41]. Thus, three methoxy groups on the convex face of **VIIIb** and **Xb** also might not prevent binding to DNA.

DNA minor groove binding ligands such as distamycin and netropsin can act as suitable carriers for the alkylating functional groups, and the resulting compounds can act as very effective antitumour agents. We believe that the carbocyclic analogues of netropsin, **VIIIb** and **Xb** containing a terminal free amine group can be used as vectors for delivery of the DNA interacting agents, thereby producing new compounds that might be more effective in the treatment of cancer. Work in this area is in progress in our laboratory.

Determination of the biological properties of compounds **VIa**, **VII**, **VIIa**, **VIIIb** and **Xb** will be the subject of a separate publication.

REFERENCES

1. Gniazdowski, M. (1985) Molekularne mechanizmy hamowania syntezy RNA. Inhibitory oddziaływujące z DNA. *Post. Biochem.* **31**, 53–86 (in Polish).
2. Hartley, J.A., Lown, J.W., Mattes, W.B. & Kohn, K.W. (1988) DNA sequence specificity of antitumor agents: Oncogenes as possible targets for cancer therapy. *Acta Oncol.* **27**, 503–506.
3. Hurley, L.H. (1989) DNA and associated targets for drug design. *J. Med. Chem.* **32**, 2027–2033.
4. Dervan, P.B. (1986) Design of sequence-specific DNA binding molecules. *Science* **232**, 464–471.
5. Bains, W. (1989) Disease, DNA and diagnosis. *New Scientist* **11**, 48–51.
6. Lown, J.W. (1992) Lexitropsins in antiviral drug development. *Antiviral. Res.* **17**, 179–196.
7. Nelsen, P.E. (1991) Sequence-selective DNA recognition by synthetic ligands. *Bioconjugate Chem.* **2**, 1–12.
8. Cory, M. (1995) DNA/minor-groove binding compounds as antitumor agents; in *Cancer Chemotherapeutic Agents* (Foye, W.O., ed.) pp. 320–324, Am. Chem. Soc., Washington.
9. Fontana, M., Lestingi, M., Mondello, Ch., Braghetti, A., Montecucco, A. & Ciarrocchi, G. (1992) DNA binding properties of FCE24517, an electrophilic distamycin analogue. *Anti-Cancer Drug Design* **7**, 131–141.
10. Mattes, W.B., Hartley, J.A., Kohn, K.W. & Matheson, D.W. (1988) GC-rich regions in genomes as targets for DNA alkylation. *Carcinogenesis.* **9**, 2065–2072.

11. Kopka, M.L., Yoon, C., Goodsell, D., Pjura, P. & Dickerson, R.E. (1985) The molecular origin of DNA-drug specificity of netropsin and distamycin. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1376–1380.

12. Neidle, S., Pearl, L.H. & Skelly, J.V. (1987) DNA structure and perturbation by drug binding. *Biochem. J.* **243**, 1–13.

13. Wade, W. & Dervan, P.B. (1987) Alteration of the sequence specificity of distamycin on DNA by replacement of an *N*-methylpyrrolecarboxamide with pyridine-2-carboxamide. *J. Am. Chem. Soc.* **109**, 1574–1575.

14. Lown, J.W. (1988) Lexitropsins: Rational design of DNA sequence reading agents as novel anti-cancer agents and potential cellular probes. *Anti-Cancer Drug Design* **3**, 25–40.

15. Rajagopalan, M., Rao, K.E., Ayyer, J. & Sasisekharan, V. (1987) Synthesis of a distamycin analogue: Tris(*m*-benzamido) compound. *Indian J. Chem.* **26B**, 1021–1024.

16. Dasgupta, D., Rajagopalan, M. & Sasisekharan, V. (1986) DNA-binding characteristics of a synthetic analogue of distamycin. *Biochem. Biophys. Res. Commun.* **140**, 626–631.

17. Rao, K.E. & Sasisekharan, V. (1990) Synthesis of distamycin and netropsin analogues: Part III. Biologically active analogues of tris(*m*-benzamido) compound. *Indian J. Chem.* **29B**, 508–513.

18. Broggini, M., Coley, H.M., Mongelli, N., Pesenti, E., Wyatt, M.D., Hartley, J.H. & Dincalci, M. (1995) DNA sequence-specific adenine alkylation by the novel antitumor drug tallimustine (FCE 245 17), a benzoyl nitrogen mustard derivative of distamycin. *Nucleic Acids Res.* **23**, 81–87.

19. Baker, B.F. & Dervan, P.B. (1989) Sequence-specific cleavage of DNA by *N*-bromoacetyldistamycin. Product and kinetic analyses. *J. Am. Chem. Soc.* **111**, 2700–2712.

20. HyperChem, version 3.0. Autodesk Inc., 2320 Marinship Way, Sausalito, CA 94965.

21. Aped, P. & Allinger, N.L. (1992) A molecular mechanics study of cyclopropanes within the MM2 and MM3 force fields. *J. Am. Chem. Soc.* **114**, 1–16.

22. Weiner, S.J., Kollman, P.A., Nguyen, D.T. & Case, D.A. (1986) An all atom force field for simulations of proteins and nucleic acids. *J. Comput. Chem.* **7**, 230–252.

23. Kazicyna, L.A. & Kupletska, N.B. (1974) Metody spektroskopowe wyznaczania struktury związków organicznych. PWN, Warszawa (in Polish).

24. Szafran, M. & Dega-Szafran, Z. (1988) Określanie struktury związków organicznych metodami spektroskopowymi. Tablice i ćwiczenia. PWN, Warszawa (in Polish).

25. Polaczkowa, W. (1954) in *Preparatyka organiczna*, pp. 589–591, PWN, Warszawa (in Polish).

26. Stevens, M. (1989) AIDS drugs act to disrupt nucleic acids. *New Scientist* **11**, 37–42.

27. Zakrzewska, K., Lavery, R. & Pullmann, B. (1984) Theoretical studies of the selective binding to DNA of two nonintercalating ligands: Netropsin and SN 18071. *Nucleic Acids Res.* **11**, 8825–8839.

28. Dwyer, T.J., Geierstanger, B.H., Mrksich, M., Dervan, P.B. & Wemmer, D.E. (1993) Structural analysis of covalent peptide dimers, bis(pyridine-2-carboxamidonetropsin)$(CH_2)_{3-6}$, in complex with 5′-TGACT-3′ sites by two-dimensional NMR. *J. Am. Chem. Soc.* **115**, 9900–9906.

29. Bialer, M., Yagen, B., Mechoulam, R. & Becker, Y. (1979) Structure-activity relationships of pyrrole amidine antiviral antibiotics. 1. Modifications of the alkylamidine side chain. *J. Med. Chem.* **22**, 1296–1302.

30. Pelton, J.G. & Wemmer, D.E. (1990) Binding modes of distamycin A with d[CGCAAATTTGCG]$_2$ determined by two-dimensional NMR. *J. Am. Chem. Soc.* **112**, 1393–1399.

31. Grootenhuis, P.D.J., Kollman, P.A., Seibel, G.L., DesJarlais, R.L. & Kuntz, I.D. (1990) Computerised selection of potential DNA binding compounds. *Anti-Cancer Drug Design* **5**, 237–242.

32. Goodsell, D. & Dickerson, E. (1986) Isohelical analysis of DNA groove-binding drugs. *J. Med. Chem.* **29**, 727–733.

33. Rao, K.E., Zimmermann, J. & Lown, J.W. (1991) Sequence-selective DNA binding by bis-*N*-methylpyrrole dipeptides: An analysis by MPE footprinting and force field calculations. *J. Org. Chem.* **56**, 786–797.

34. Lee, H.H., Boyd, M., Gravatt, G.L. & Denny, W.A. (1991) Pyrazole analogues of the bispyrrolecarboxamide anti-tumour antibiotics: Synthesis, DNA binding and anti-tumour properties. *Anti-Cancer Drug Design* **6**, 501–517.

35. Boger, D.L. & Johnson, D.S. (1995) CC-1065 and the duocarmycins: Unraveling the keys to a new class of naturally derived DNA alkylating agents. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3642–3649.

36. Taylor, J.S., Schultz, P.G. & Dervan, P.B. (1984) DNA affinity cleaving: Sequence specific cleavage of DNA by distamycin-EDTA-Fe(II) and EDTA-distamycin-Fe(II). *Tetrahedron* **40**, 457–464.

37. Bailly, C., D'Hooghe, M.C., Lantoine, D., Fournier, C., Hecquet, B., Fosse, P., Saucier, J.M., Colson, P., Houssier, C. & Henichart, J.P. (1992) Biological activity and molecular interaction of a netropsin-acridine hybrid ligand with chromatin and topoisomerase II. *Biochem. Pharmacol.* **43**, 457–466.

38. Semmelhack, M.F. & Gallagher, J.J. (1994) The effect on DNA cleavage potency of tethering a simple cyclic enediyne to a netropsin analogue. *J. Org. Chem.* **59**, 4357–4359.

39. Watts, C.R., Kerwin, S.M., Kenyon, G.L., Kunts, I.D. & Kallick, D.A. (1995) Rationally designed *N,N'*-bis[(*N*-*p*-guanidinobenzyl-*N*-methyl)aminocarbonyl]-1,3-diaminobenzene, "BIGBEN", binds to the minor groove of $d(CGCGAATTCGCG)_2$ as determined by two-dimensional nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc.* **117**, 9941–9950.

40. Rao, K.E., Bathini, Y. & Lown, J.W. (1990) Synthesis of novel thiazole-containing DNA minor groove binding oligopeptides related to the antibiotic distamycin. *J. Org. Chem.* **55**, 728–737.

41. Grochowskij, S.L., Żuze, A.L. & Gottich, B.P. (1982) Ligandy obładajuszcze srodstwom k opredelennym posledowatelnostjam par osnowanij DNK. VII. Sintez analogow distamicina A, sostojaszczich iz dwuch i trech *N*-izoamiłpirrołkarboksamidnych fragmentow, i analoga, soderżajuszczego metiłamidnye swjazi w molekule. *Bioorg. Chim.* **8**, 1070–1076 (in Russian).