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The paper is dedicated to Professor Bogdan Liberek on the 50th anniversary of his scientific work

Review

Peptide nucleic acids and their structural modifications*°

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Peptide (polyamide) analogues of nucleic acids (PNAs) make very promising groups of natural nucleic acid (NA) ligands and show many other interesting properties. Two types of these analogues may be highlighted as particularly interesting: the first, containing a polyamide with alternating peptide/pseudopeptide bonds as its backbone, consisting of *N*-(aminoalkyl)amino-acid units (type I), with nucleobases attached to the backbone nitrogen with the carboxyalkyl linker; and the second, containing a backbone consisting of amino-acid residues carrying the nucleobases in their side chains (type II). So far, these two groups have been studied most intensively. The paper describes main groups of peptide nucleic acids, as well as various other amino acid-derived nucleobase monomers or their oligomers, which were either studied in order to determine their hybridisation to nucleic acids, or only discussed with respect to their potential usefulness in the oligomerisation and nucleic acids binding.

Amino-acid-derivatives of nucleobases were detected in Nature, e.g. the first isolated 3-(N^1-uracilyl)alanine (Willardiine) from *Acacia Willardiana* Rose (Gmelin, 1959). These com-

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Abbreviations: B, natural or modified nucleobase; ds, double-stranded; NA, natural nucleic acids (DNA or RNA); PNA, peptide (polyamide) nucleic acid; ss, single-stranded.

pounds were the source of the idea to use nucleobases attached to polypeptides or other polyamides for the recognition of natural nucleic acids (NA). In addition, the natural nucleopeptides and nucleoamino acids serve as a potential primordial genetic material (Miller, 1997), and a set of their components was obtained in conditions similar to those probably prevalent in the primitive Earth's biosphere (Nelson & Miller, 1996).

The first synthetic/hybridisation studies on the analogues were carried out in the late 1960s. Since then, numerous amino acid- or polyamide-derived structures carrying natural or modified nucleobases have been suggested, some have been oligomerised or synthesised from submonomeric parts, and some have been intensively studied.

Peptide (or more generally, polyamide) nucleic acids (PNAs) represent a rather abundant group of NA analogues, in which a polyamide or peptide backbone replaces the phosphodiester pentose backbone of DNA or RNA (Liberek, 1995). The name "PNA" does not include analogues in which amide bond serves only as a part of internucleoside linker. Depending on the manner of the attachment of the basic nucleobase to polyamide backbone, two main groups of polyamide nucleic acids may be discerned:

Type **I**. PNAs containing polyamide with alternating peptide/pseudopeptide bonds as a backbone, consisting of *N*-(aminoalkyl)amino-acid units, to which secondary nitrogen nucleobases are attached with the carboxyalkyl linker (Figs. 1, 2, and 3)

Type **II**. PNAs containing a backbone consisting of amino-acid residues carrying the nucleobases in their side chains, and which may contain other elements (Fig. 4).

To date these two groups have been analyzed most intensively. The paper describes these two main groups of peptide nucleic acids, as well as various other amino acid-derived nucleobase monomers or their oligomers, which were studied in order to determine their hybridisation to nucleic acids, or only introduced in view of their potential usefulness in the oligomerisation and nucleic acids binding.

TYPE I PNAs

The most widely known PNAs, based on *N*-(aminoethyl)glycine backbone (Fig. 2.1), were designed and synthesised in 1991 by a group of Dutch chemists: Peter E. Nielsen, Michael Egholm, Rolf H. Berg and Ole Buchardt (Nielsen *et al.*, 1991). These are NA analogues of numerous applications and seem to have the most interesting properties (for review, see: Żekanowski, 1995; 1996; Hyrup & Nielsen, 1996; Corey, 1997; Dueholm & Nielsen, 1997; Knudsen & Nielsen, 1997; Nielsen & Haaima, 1997; Nielsen, 1996a; 1997; 1998; Uhlmann *et al.*, 1998; Koch *et al.*, 1997; Greiger *et al.*, 1998). These compounds were primarily designed for sequence-specific binding of homopyrimidine sequences in double-stranded homopurine/homopyrimidine DNA in Hoogsteen manner, in the main groove of DNA (Nielsen *et al.*, 1991). However, the generous reality frequently surpasses the scope of human imagination, and oligomers apparently have properties one has never expected. PNA molecules highly efficiently and sequence-specifically bind to the complementary (according to Watson- Crick or Hoogsteen rules) strand of DNA, RNA or PNA oligomers (Egholm *et al.*, 1993). They hybridise in the antiparallel orientation better than in the parallel one, with respectively the amino end of oligomer reflecting 3′ and carboxy reflecting 5′ end of the nucleic acid. The thermal stability of the PNA-forming duplexes decreases in the following order: PNA-PNA > PNA-DNA > DNA-DNA. Complexes of homopyrimidine PNA oligomers with homopurine nucleic acids show unusually high thermal stability. PNAs of this type prove to be better ligands of DNA or RNA than native nucleic acids and show the ability to displace the pyrimidine strand of homopurine/homopyrimidine dsDNA and to form duplexes PNA-DNA or triplexes PNA*-

DNA–PNA (Demidov *et al.*, 1995; 1997; Wittung *et al.*, 1996; Kuhn *et al.*, 1999). In these structures, the homopyrimidine DNA strand becomes extruded as a single stranded loop named D- or P-loop, respectively (Eriksson & Nielsen, 1996b). In addition, PNA oligomers can also form PNA*-DNA–DNA triplexes (Wittung *et al.*, 1997a) and pure PNA triplexes (Wittung *et al.*, 1997b).

PNA as conjugates with various, so-called "transfer" molecules effectively penetrates into cells and could be active under *in vivo* conditions (Pardridge *et al.*, 1995; Scarfi *et al.*, 1997; Pooga *et al.*, 1998; Aldrian-Herrada *et al.*, 1998a). Due to their hybridisation properties, synthetic flexibility (Dueholm *et al.*, 1994a; Christensen *et al.*, 1995; Nielsen, 1996b; Dueholm & Nielsen, 1997; Aldrian-Herrada *et al.*, 1998b), and very high chemical stability, also in body fluids (Demidov *et al.*, 1994), PNA oligomers have numerous applications as tools in molecular biology and molecular diagnostics, and as – although so far only experimental – therapeutic agents in antisense and antigene strategies (Knudsen & Nielsen, 1997). PNA inspires also the hope in the possibility of its various interesting applications in technology, especially as electrochemical biosensors (Paleček *et al.*, 1998) and in optical data storage (Berg *et al.*, 1996).

The structures of various PNA oligomer complexes were studied by means of X-ray crystallography (Betts *et al.*, 1995; Rasmussen *et al.*, 1997), nuclear magnetic resonance (Brown *et al.*, 1994; Leijon *et al.*, 1994; Eriksson & Nielsen, 1996a), linear and circular dichroism spectroscopy (e.g. Egholm *et al.*, 1993; Kim *et al.*, 1993), and by means of molecular dynamics methods (e.g. Almarsson *et al.*, 1993; Almarsson & Bruice, 1993; Chen *et al.*, 1994; Torres & Bruice, 1996; Sen & Nilsson, 1998; Shields *et al.*, 1998). The structures show that PNA molecules can mimic the structure of a nucleic acid partner very well (Egholm *et al.*, 1993); the PNA–RNA duplex is clearly similar to A-form (Brown *et al.*, 1994), and the PNA–DNA one to both B- and A-form (Leijon *et al.*, 1994; Eriksson & Nielsen, 1996a,b) (Fig. 5). Yet the PNA–PNA duplex (Rasmussen *et al.*, 1997) (Fig. 6), and the evidently smaller PNA_2:DNA triplex (Betts *et al.*, 1995) (Fig. 7), have helical structures described as P-form significantly different from other known nucleic acid structures. P-form has a relatively big diameter (28 Å) and consists of large pitches, composed of 18 bases. The crystal structure of the retro-inverso PNA analogue (Fig. 2.7) has also been determined (Krotz *et al.*, 1998).

Monomers of the type **I** PNA are built up of four conventional parts: aminoalkyl (Fig. 1a),

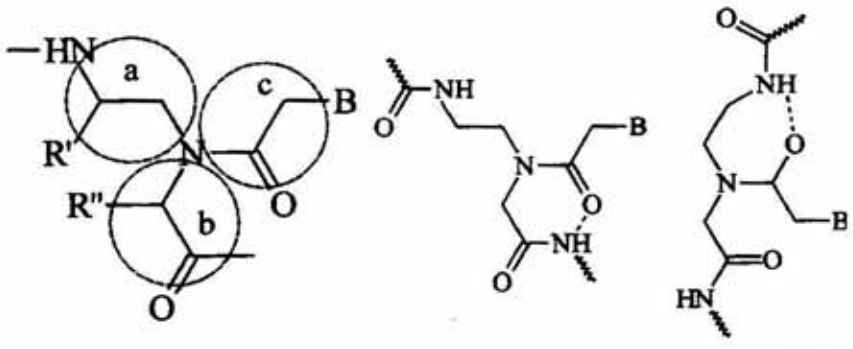

Figure 1. Left: structure of unmodified (R′ = R″ = H) and chirally modified (R′ or R″ ≠ H) *N*-(aminoethyl)-glycine-based PNA monomers.

Conventional parts of the type **I** PNA monomers: aminoalkyl (a), amino acid (b), linker between nucleobase and backbone (c), and nucleobase (B). For details on R″ studied, see Table 2.

Right: schemes of interresidue and intraresidue hydrogen bonds in PNA type I oligomers.

amino acid (Fig. 1b), linker between nucleobase and backbone (mainly carboxyalkyl, the most frequently carboxymethyl, Fig. 1c), and nucleobase. Similar monomers – structures on the border-line between PNAs' type **I** and **II** – are those derived from L- or D-ornithine residues (Fig. 2.10) (Lioy & Kessler, 1996; Petersen *et al.*, 1996; van der Laan *et al.*, 1998) as the backbone and those derived from β-aminoalanine monomers interlinked by glycine spacer (Fig. 3.4) (Fuji *et al.*, 1997), but their tendency to hybridise with NA is rather mediocre. Two other interesting analogues of PNA have been studied: the first with a reversed amide bond (Fig. 3.1) (Lagriffoull *et al.*, 1994), obtained from two structurally different, "diamine" and "diacid" monomers, and the second, with amide bond reversed and then with carboxyl and secondary amine groups replaced (retro-inverso PNA, Fig. 2.7) (Krotz *et al.*, 1995a,b; 1998). The PNA type **I** structure modifications studied in order to determine their hybridisation potency with NA are shown in Fig. 2 (for hybridisation data, see Tables 1 and 2). Other variants of modified PNA type **I** monomer structures have also been reported and seem to be potentially useful in the synthesis of oligomers; they are shown in Fig. 3.

The extensive studies on monomers' structure/hybridisation activity relationships (see e.g. data included in Tables 1 and 2) show that, if oligomers are to retain a strong hybridisation potential, the possibility of forming of both intraresidue and interresidue hydrogen bonds in oligomers (see Fig. 1) is necessary and, therefore, the presence of properly localised amide group in the linker between nucleobase and backbone is essential (Almarsson *et al.*, 1993; Almarsson & Bruice, 1993; Hyrup *et al.*, 1993; 1994; 1996; Torres & Bruice, 1996). However, fulfilment of this condition alone is not suffi-

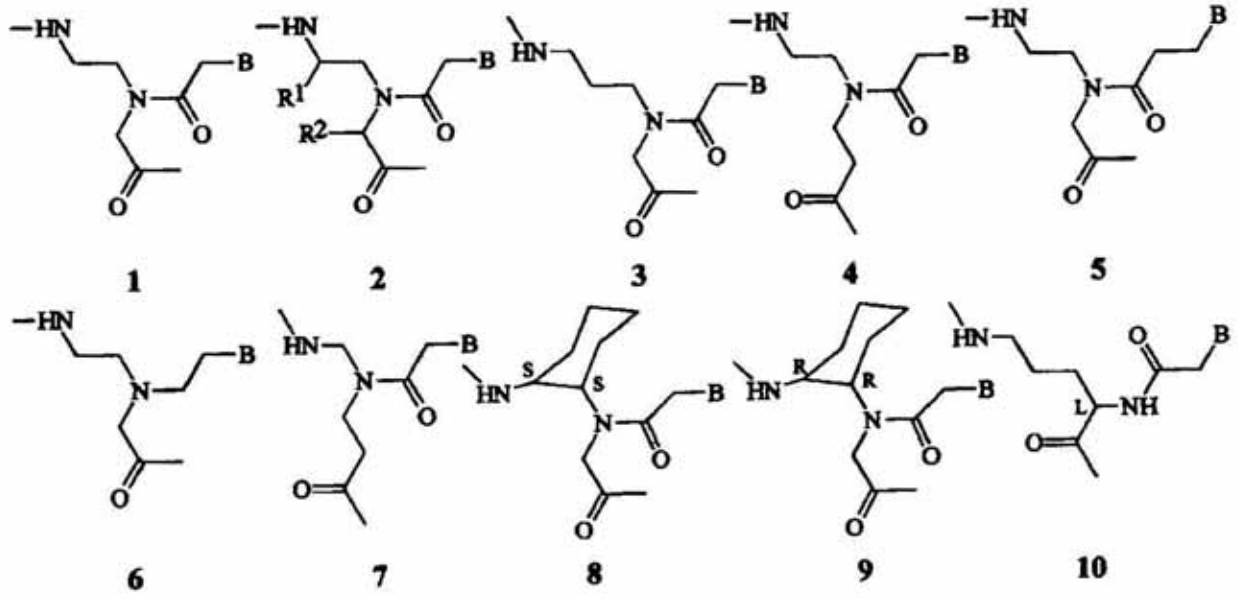

Figure 2. PNA type I structure modifications studied in order to determine their hybridisation potency with DNA or RNA.

For hybridisation data, see Table 1. **2**, R^1 and R^2: for details, see original papers. References: **1** (Nielsen *et al.*, 1991; Dueholm *et al.*, 1994a; and numerous others); **2** (Dueholm *et al.*, 1994b; Kosynkina *et al.*, 1994; Haaima *et al.*, 1996; Püschl *et al.*, 1998; Sforza *et al.*, 1999; Stammers & Burk, 1999; Falkiewicz *et al.*, 1999a,b); **3** (Hyrup *et al.*, 1993; 1994); **4** (Hyrup *et al.*, 1993; 1994; Krotz *et al.*, 1998); **5** (Hyrup *et al.*, 1994); **6** (Hyrup *et al.*, 1996); **7** (Krotz *et al.*, 1995a; 1998); **8** and **9** (Lagrifoull *et al.*, 1997); **10** (Lioy & Kessler, 1996; Petersen *et al.*, 1996; van der Laan *et al.*, 1998).

cient for formation of stabile hybrids, as shown in the case of the backbone built from alternating β-aminoalanine and glycine, despite the theoretical possibility of latter to form interresidue and intraresidue hydrogen bonds (Fuji *et al.*, 1997). Even in the case of the application of a positively-charged monomer, without amide in the linker part – as "ethylene-linker" (Fig. 2.6), the PNA oligomer's tendency to hybridise is significantly decreased as compared to the use of unmodified monomer (Hyrup *et al.*, 1996) (see Table 1).

The poor results of duplex formation obtained when *retro-inverso* PNA oligomers are used emphasise that, in addition to the monomer parts' length and the possibility of hydrogen bonds formation, other structural properties of the oligomers have to be present as well. A simple inversion of intraresidue amide bond, as could be found in the *retro-inverso* analogue, results in a considerably decreased DNA mimicking properties of the PNA oligomers (Krotz *et al.*, 1995b; 1998). It is possible that other, probably more subtle requirements, for example those related to dipole-dipole interactions or the pattern of oligomers' hydration, are involved (Nielsen & Haaima, 1997). Efforts to modify the length of monomer parts, both in the case of the backbone and the linker, caused a reduction of the hybridisation potency (Hyrup *et al.*, 1993; 1994).

Preliminary data suggest that the sterically allowed constriction of the PNA backbone may improve its tendency to hybridise with NA, but this strongly depends on the stereochemistry of the residue used. For example, the PNAs based partially on L-4-*trans*-amino proline (Fig. 3.3) (Jordan *et al.*, 1997a,b) or all *S,S*-cyclohexyl-derived (Fig. 2.8) (Lagriffoull *et al.*, 1997) aminoethyl part of classical PNA backbones have a tendency to hybridise with NA similar to that of the unmodified PNA. In a sharp contrast, the hybridisation of PNA with fully *R,R*-cyclohexyl-derived (Fig. 2.9) or partially L-4-*cis*-aminoproline, D-4-*trans*-aminoproline backbones significantly decreases the binding properties as compared to the unmodified achiral PNA. However, inclusion of even a single 4-aminoproline into a PNA sequence, either at the N-terminus or in the interior, leads to stabilisation of the PNA-DNA hybrid, as studied by circular dichroism spectroscopy (Gangamani *et*

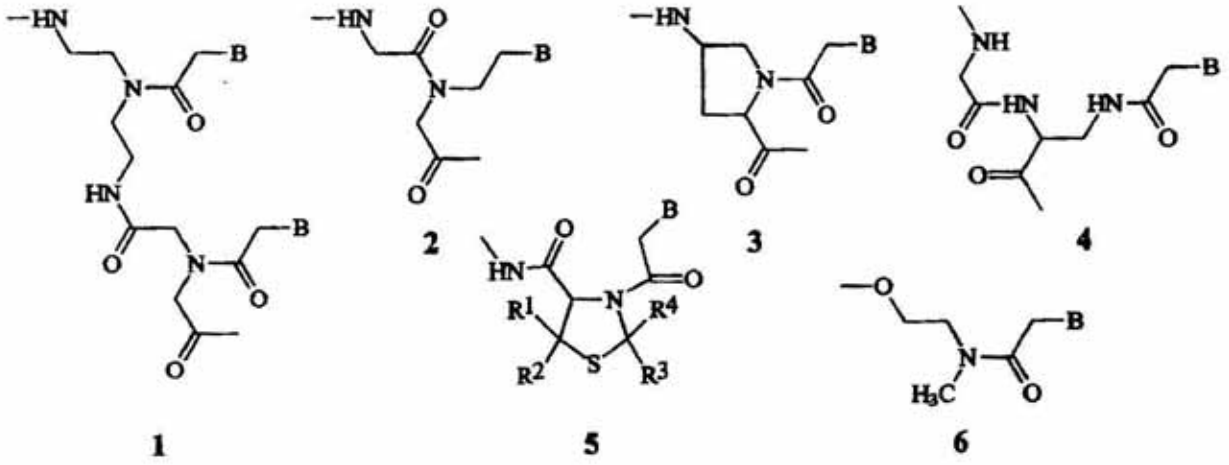

Figure 3. Variants of modified PNA type I monomer structures reported and potentially useful in the synthesis of oligomers.

For details on stereochemistry of **5**: R^1-R^4, see original papers. References: **1** (Lagrifoulle *et al.*, 1994); **2** (Almarsson *et al.*, 1993); **3** (Jordan *et al.*, 1997a; Gangamani *et al.*, 1999); **4** (Fuji *et al.*, 1997); **5** (Groger *et al.*, 1996); **6** (Martinez *et al.*, 1997).

al., 1999). Thermodynamic calculations of enthalpic and entropic contributions to the hybridisation energy show that, in the case of *S,S*-cyclohexyl-derived PNAs, upon hybridisation a significant reduction of entropy loss is accompanied by a decrease in enthalpic gain, therefore it may be presumed that the constraining of backbone flexibility leads to a low

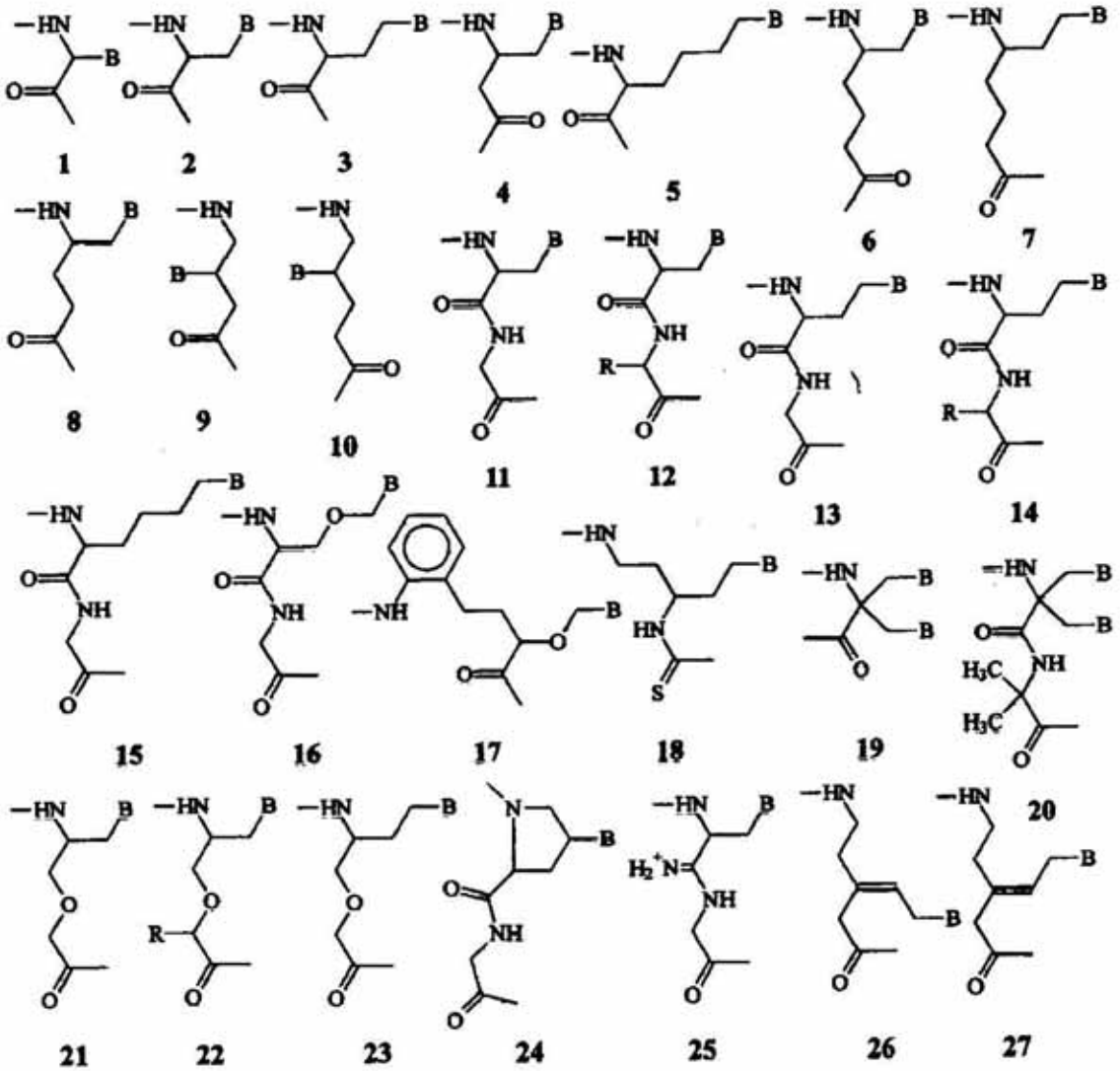

Figure 4. Selected PNA type II monomer structures introduced and potentially useful in the synthesis of oligomers.

For details on stereochemistry and R, see original papers, R ≠ H. References: **1** (Shvachkin, 1979; Kingsbury *et al.*, 1984); **2** (Nollet & Pandit, 1969a; Doel *et al.*, 1969; 1974; Buttrey *et al.*, 1975; Shvachkin, 1979; Ceulemans *et al.*, 1995; Diederichsen, 1996); **3** (Nollet *et al.*, 1969; Nollet & Pandit, 1969b; Lenzi *et al.*, 1995a,b; Shah *et al.*, 1996a,b; Diederichsen & Schmitt, 1996; Howarth & Wakelin, 1997); **4** (Weller *et al.*, 1991; Diederichsen & Schmitt, 1998a,b); **5** (De Koning & Pandit, 1971); **6** (Huang *et al.*, 1991); **7** (Savithri *et al.*, 1996); **8** (Huang *et al.*, 1991); **9** (Weller *et al.*, 1991); **10** (Weller *et al.*, 1991); **11** (Tyaglov *et al.*, 1987; Weller *et al.*, 1991); **12** (Doel *et al.*, 1974; Shvachkin, 1979; Ceulemans *et al.*, 1995); **13** (Howarth & Wakelin, 1997; Yamazaki *et al.*, 1997); **14** (Yamazaki *et al.*, 1997); **15** (De Koning & Pandit, 1971); **16** (Garner & Yoo, 1993); **17** (Tsantrizos *et al.*, 1997); **18** (Dallaire & Arya, 1998); **19** and **20** (Azumaya *et al.*, 1995); **21** and **22** (Altmann *et al.*, 1997); **23** (Altmann *et al.*, 1997; Kuwahara *et al.*, 1999); **24** (Jordan *et al.*, 1997a; Lowe & Vilaivan, 1997a,b); **25** (Lohse *et al.*, 1996); **26** and **27** (Cantin *et al.*, 1997).

Table 1. Changes in the melting temperature Δt_m/°C per 1 monomer of modified *T* unit incorporated into the sequence H-GTAGA*T*CACT-(*C-terminal group*) and hybridised with complementary ssDNA or ssRNA decamer in antiparallel orientation, as compared to T unmodified *N*-(aminoethyl)glycine based PNA.

Backbone	Linker	C-terminal group	Δt_m/°C (DNA)[a]	Δt_m/°C (RNA)[a]	References	Figure
-NH(CH_2)$_2$NHCH_2CO-	-CH_2CO-	NH_2	0	0		2.1
-NH(CH_2)$_2$-L-Leu-	-CH_2CO-	NH_2	-5	-5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Leu-	-CH_2CO-	NH_2	-2		Sforza *et al.*, 1999	2.2
-NH(CH_2)$_2$-L-Val-	-CH_2CO-	NH_2	-8.5	-7.5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Phe-	-CH_2CO-	NH_2	-8.5	-7.5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Tyr-	-CH_2CO-	NH_2	-8	-6.5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Trp-	-CH_2CO-	NH_2	-8	-7	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-His-	-CH_2CO-	NH_2	-8	-6.5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Thr-	-CH_2CO-	NH_2	-6.5	-6.5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Gln-	-CH_2CO-	NH_2	-7	-5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Arg-	-CH_2CO-	NH_2	-5	-5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Lys-	-CH_2CO-	NH_2	-4.5	-5.5	Püschl *et al.*, 1998	2.2
-NH(CH_2)$_2$-L-Lys-	-CH_2CO-	NH_2	-2		Sforza *et al.*, 1999	2.2
-NH(CH_2)$_2$-D-Lys-	-CH_2CO-	NH_2	+2		Sforza *et al.*, 1999	2.2
-NH(CH_2)$_3$NHCH_2CO-	-CH_2CO-	LysNH_2	-8.0		Hyrup *et al.*, 1994	2.3
-NH(CH_2)$_3$NHCH_2CO-	-CH_2CO-	NH_2		-6.5	Nielsen & Haaima, 1997	2.3
-NH(CH_2)$_2$NH(CH_2)$_2$CO-	-CH_2CO-	NH_2	-5.0[b]	-6.5[b]	Krotz *et al.*, 1998	2.4
-NH(CH_2)$_2$NH(CH_2)$_2$CO-	-CH_2CO-	LysNH_2	-10		Hyrup *et al.*, 1994	2.4
-NH(CH_2)$_2$NH(CH_2)$_2$CO-	-CH_2CO-	NH_2		-7.5	Nielsen & Haaima, 1997	2.4
-NH(CH_2)$_2$NHCH_2CO-	-(CH_2)$_2$CO-	LysNH_2	-21		Hyrup *et al.*, 1994	2.5
-NH(CH_2)$_2$NHCH_2CO-	-(CH_2)$_2$CO-	NH_2	-20	-16	Nielsen & Haaima, 1997	2.5
-NH(CH_2)$_2$NHCH_2CO-	-CH_2CH_2-	LysNH_2	-24	-18	Hyrup *et al.*, 1996	2.6
-NHCH_2NH(CH_2)$_2$CO-	-CH_2CO-	NH_2	-8.5[b]	-7.5[b]	Krotz *et al.*, 1998	2.7
-NH((*S,S*)-cyclohexyl)-NHCH_2CO-	-CH_2CO-	NH_2	+0.5[b]	+0.5[b]	Lagrifoulle *et al.*, 1997	2.8
-NH((*S,S*)-cyclohexyl)-NHCH_2CO-	-CH_2CO-	LysNH_2	-1.3[c]	-0.5[c]	Lagrifoulle *et al.*, 1997	2.8
-NH((*R,R*)-cyclohexyl)-NHCH_2CO-	-CH_2CO-	LysNH_2	-7.2[c]	-7.5[c]	Lagrifoulle *et al.*, 1997	2.9
-NH(CH_2)$_3$CH(NH_2)CO-	-CH_2CO-	NH_2		-8	Petersen *et al.*, 1996	2.10
-NH(CH_2)$_3$CH(NH_2)CO-	-CH_2CO-	NH_2	-14		Nielsen & Haaima, 1997	2.10

[a]Measurements performed in 10 mM Na-phosphate buffer, 100 mM NaCl, 0.1 mM EDTA, pH = 7.0; [b]tested using 15-mer, sequence: H-TGTACG*T*CACAACTA-NH_2; [c]tested using 10-mer, sequence: H-G*T*AGA*T*CAC*T*-LysNH_2

energy conformation different from the conformation preferred for the formation of a stable PNA–NA complex (Nielsen, 1998).

Four stereoisomers of 4-aminoproline coupled with nucleic acid bases in the side chain and their two dimers were obtained and oligomerised by Gangamani *et al.* (1996; 1999) (Fig. 3.3). The circular dichroism spectra of dimers indicated different base stacking depending on the stereochemistry of monomers.

An other possible modification of the monomers' structures is the introduction of chirality into backbones (Fig. 1 and 2.2). So far two ideas have been put forward. The first was the chiralisation of the "amino-acid" part of the backbone, realised either by using reductive amination of amino-acid ester by *N*-protected glycinal (Dueholm *et al.*, 1994b; Haaima *et al.*, 1996; Püschl *et al.*, 1998; Sforza *et al.*, 1999) or by catalytic hydrogenation of enamido esters

Table 2. Changes in the melting temperature Δt_m/°C per 1 modified monomer for PNA decamer containing three chirally modified *T* units, incorporated into the sequence H-G*T*AGA*T*CAC*T*-(*C-terminal group*) and hybridised with complementary ssDNA or ssRNA decamer in antiparallel orientation, as compared to T unmodified *N*-(aminoethyl)glycine based PNA.

R', R'' = H (Fig. 1)	Configuration	C-terminal group	Δt_m/°C (DNA)[a]	Δt_m/°C (RNA)[a]	References
CH_3	L	NH_2	-1.5		Dueholm *et al.*, 1994b
CH_3	L	NH_2	-1.3	-1.7	Püschl *et al.*, 1998
CH_3	D	NH_2	-0.3		Dueholm *et al.*, 1994b
iso-Bu	L	NH_2	-1.8	-2.3	Püschl *et al.*, 1998
iso-Bu	L	NH_2	-1.3		Sforza *et al.*, 1999
sec-Bu	L	$LysNH_2$	-2.6	-3.0	Haaima *et al.*, 1996
CH_2OH	L	$LysNH_2$	-1.0	-1.0	Haaima *et al.*, 1996
CH_2OH	D	$LysNH_2$	-0.6	-1.0	Haaima *et al.*, 1996
CH_2COOH	L	NH_2	-3.3		Haaima *et al.*, 1996
CH_2CH_2COOH	D	NH_2	-2.3		Haaima *et al.*, 1996
$CH_2CH_2CH_2CH_2NH_2$	L	$LysNH_2$	-1.0	-1.3	Haaima *et al.*, 1996
$CH_2CH_2CH_2CH_2NH_2$	L	$LysNH_2$	-1.2	-2.2	Püschl *et al.*, 1998
$CH_2CH_2CH_2CH_2NH_2$	L	NH_2	0		Sforza *et al.*, 1999
$CH_2CH_2CH_2CH_2NH_2$	D	$LysNH_2$	+1.0	0	Haaima *et al.*, 1996
$CH_2CH_2CH_2CH_2NH_2$	D	NH_2	+1.3		Sforza *et al.*, 1999
4-imidazolyl	L	$LysNH_2$	-2.8	-2.7	Püschl *et al.*, 1998

[a]Measurements performed in 10 mM Na-phosphate buffer, 100 mM NaCl, 0.1 mM EDTA, pH = 7.0.

(Stammers & Burk, 1999). The second was the chiralisation of the aminoalkyl backbone part, accomplished by applying reductive amination of glycine esters by N-protected amino aldehydes (Kosynkina *et al.*, 1994; Falkiewicz *et al.*, 1999a,b). The optical purity of monomers obtained by reductive amination may be not rewarding, mainly due to the optical instability of N-protected amino aldehydes. Recently, a new method of PNA monomers synthesis has been proposed (Wiśniewski *et al.*, 1997; Falkiewicz *et al.*, 1998; 1999c) which makes it possible to obtain both types of chirally modified monomers in an optically pure state, using the Mitsunobu reaction (Wiśniewski *et al.*, 1998). The properties of chiral PNA monomers incorporated into PNA oligomers have become modified as compared to those of *N*-(aminoethyl)glycine based units (see Tables 1 and 2). Oligomers with chiral backbone retain strong hybridisation properties, and three-dimensional structures of PNA-nucleic acid complexes show that various substituents at glycine α-position might be accommodated without a serious damaging effect. The changes in hybridisation properties are dependent in part on the configuration of chiral unit, on the dimension and chemical properties of the substituent (Table 2). The sterically large substituents are only slightly less well-tolerated than the smaller ones. In general, the incorporation of residues of D-configuration in amino-acid part of monomers results in formation of oligomers with better hybridisation properties than those of L-configuration. For the right-handed helix, the "L-substituents" are positioned to interact with the dsDNA major groove, while the "D-substituents" are directed towards the solution (Sforza *et al.*, 1999). The hydrophilic and positively charged substituents are preferred (Dueholm *et al.*, 1994b; Haaima *et al.*, 1996), and negatively-charged units have a diminished potency to hybridise with NA (Haaima *et al.*, 1996). Interestingly, the incorporation of chiral monomers enhances the sequence selectivity of oligomers during hybridisation, with the maximum for D-Glu and D-Lys (Haaima *et al.*, 1996). In addition to the hybridisation properties, modified oligomers may be significantly more

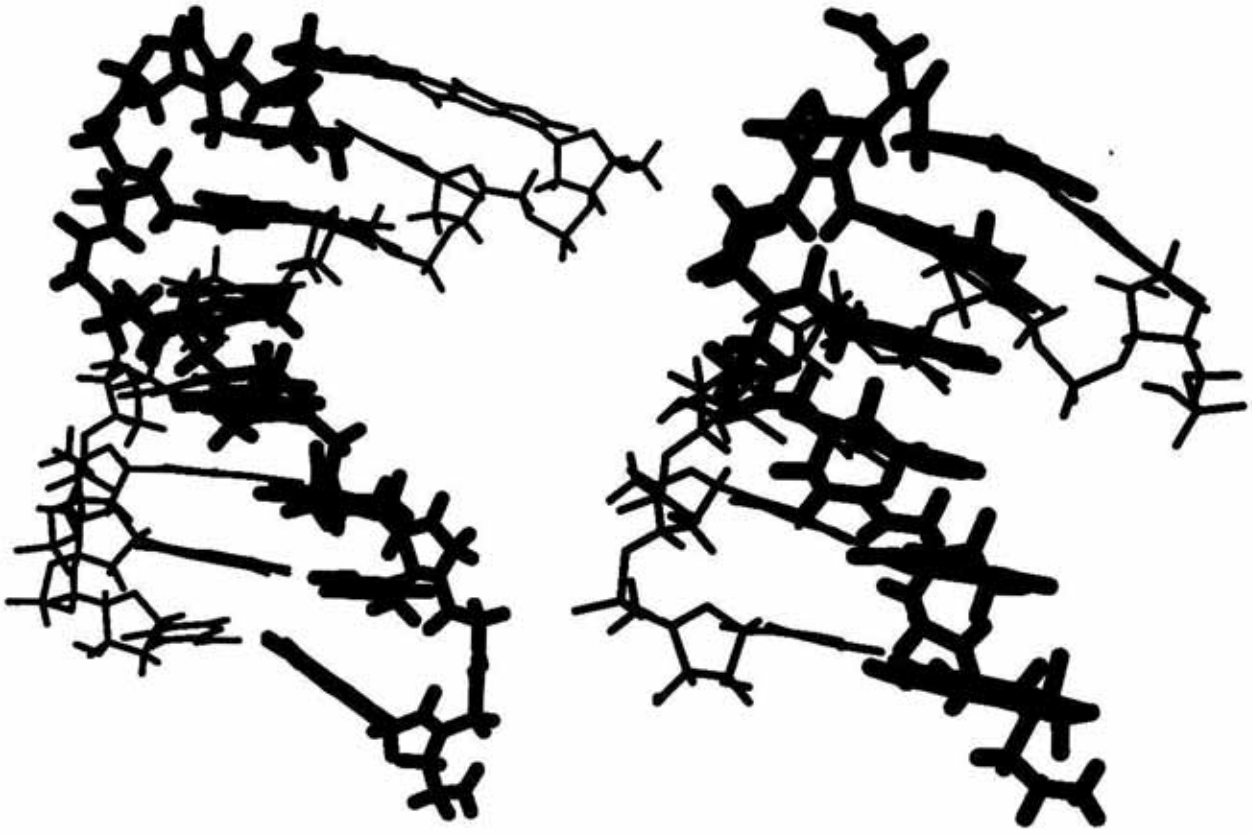

Figure 5. Comparison of the PNA–NA duplexes' structures, determined by NMR.

Left: PNA–DNA duplex (structure determined by Eriksson & Nielsen (1996a), Protein Data Bank code 1PDT); right: PNA–RNA duplex (structure determined using NMR by Brown *et al.* (1994), Protein Data Bank code 176D). PNA chains are thickened. Figures were generated using RasMol v. 2.6 software.

readily soluble and have a higher tendency to permeate into cells. For example, the introduction of only two D-Lys based monomers into a PNA dodekamer sequence enhances its solubility five fold (Haaima *et al.*, 1996). Functional groups of amino-acid side chains might be the sites for an easy attachment of other ligands to PNA monomers/oligomers.

Other interesting NA analogues are based on the peptoide backbone (Fig. 3.2). However, the oligomers synthesised by Almarsson *et al.* (1993) showed no interaction with a complementary DNA strand.

TYPE II PNAs

The syntheses of various PNA analogues derived from natural and unnatural amino acids were developed, sometimes repeatedly (Fig. 4), but only a small part of the compounds synthesised were studied in hybridisation experiments.

The first natural amino-acid conjugates carrying the nucleobases in side chains have been prepared since late 1960s (Nollet *et al.*, 1969; Nollet & Pandit, 1969a,b), but studies on their hybridisation with natural NA did not show very positive results. De Konig and Pandit (1971) prepared nucleopeptides derived from [-HNCH($(CH_2)_4$B)CO-] or [-HNCH((CH_2)B)CO-NHCHRCO-]monomers, where B is a nucleobase, and found no significant interaction of this type uridine oligomer with polyadenylic acid. Doel *et al.* (1969; 1974) and Buttrey *et al.* (1975) prepared oligomers of L-, D-, and D,L-β-(thymin-1-yl)alanine and found no significant interaction between them and poly-

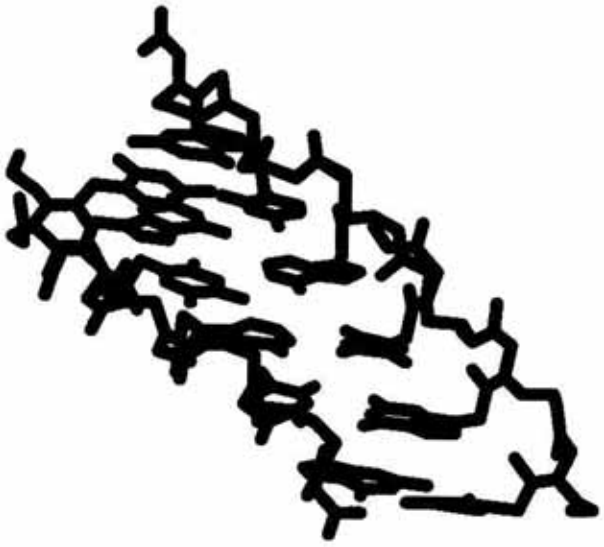

Figure 6. Structure of the PNA–PNA duplex, determined using X-ray crystallography by Rasmussen *et al.* (1997; Protein Data Bank code 1PUP). Figure was generated using RasMol v. 2.6 software.

adenylic acid, but the same monomers interspaced by valine formed stable complexes with complementary sequences (Tyaglov *et al.*, 1987). A series of other modifications were also proposed (Fig. 4).

Monomers and oligomers derived from γ-substituted-α-aminobutyric acid (Fig. 4.3) were prepared by Lenzi *et al.* (1995a,b), Umemiya *et al.*, (1995; 1996), Shah *et al.* (1996a,b), Yamazaki *et al.* (1997), and Howarth & Wakelin (1997). When the uridylic acid analogues were interspaced with glycine (Fig. 4.13), L-, D-, or *N*-methyl-L-serine (Fig. 4.14), they showed no hypochromic effect on mixing with poly(A) or poly(dA) (Umemiya *et al.*, 1995; 1996), but the adenylic analogues interspaced with glycine (Fig. 4.13), serine, threonine or tyrosine (Fig. 4.14) showed strong affinity to poly(dT) and poly(U) (Umemiya *et al.*, 1995; Yamazaki *et al.*, 1997). The hypochromic effect and CD spectra of these complexes pointing to formation of triplexes more stable than those formed between phosphodiester Ade-Ade dinucleotide and complementary polynucleotides. When the γ-substituted-α-aminobutyric acid monomers were interspaced with glycine (Fig. 4.13), the oligomers obtained showed a very interesting tendency to specific complexation of Cu(II) ions and this binding was realised exclusively by peptide backbone nitrogens, but nucleobases interacted with each other very efficiently stabilising the metal-peptide bond (Szyrwiel *et al.*, 1998). These chiral PNAs exhibited self-recognition similar to that of NA duplexes (Lenzi *et al.*, 1995b).

Compounds derived from monomers composed of thymine derivatives: 4-substituted 2*S*,4*S*-proline or 2*S*,4*R*-proline, interspaced with glycine (Fig. 4.24), were developed and incorporated into various oligomers, but their hybridisation properties seem to be imperfect (Jordan *et al.*, 1997a,b).

Tsantrizos *et al.* (1997) synthesised a potential aromatic PNA analogue, with thymine monomer based on *S*-2-hydroxy-4-(2-aminophenyl)butanoic acid backbone (Fig. 4.17). The monomers were oligomerised up to tetramer, which showed favourable base stacking interactions when investigated by UV and ^{1}H-NMR.

A series of very interesting studies on α-alanyl, α-homoalanyl and β-homoalanyl PNA analogues (Fig. 4.2, 4.3, and 4.4, respectively) were published by Diederichsen (1996; 1997a,b; 1998a,b) and Diederichsen & Schmitt (1996; 1998a,b). Alanyl and homoalanyl PNAs with alternating backbone configuration (D- *versus* L-alanine) along each strand form linear and fully extended band complexes with a base pair distance of about 3.5 Å and, interestingly, higher stability of Ade-Ade pairing compared with Ade-Thy (Diederichsen, 1996; 1997b). The complexes may be stabilised by addition of intercalators at the abasic site (Diederichsen, 1997a). β-Homoalanyl oligomers tend to exist as double strands and prefer the extended β-sheet-like backbone conformation, with uniformly aligned nucleobases (Diederichsen & Schmitt, 1998a,b).

A positively-charged PNA analogue carrying various nucleobases, e.g. as L-oligo-nucleodipeptamidinium salt (Fig. 4.25), was obtained by Lohse *et al.* (1996), but did not show any ten-

Figure 7. Structure of two PNA chains in the PNA_2–DNA triplex, determined using X-ray crystallography by Betts *et al.* (1995; Protein Data Bank code 1PNN). Figure was generated using RasMol v. 2.6 software.

dency to interact by base pairing with complementary ligands.

Altmann *et al.* (1997) prepared PNA monomers derived from serine and homoserine coupled by the ether linker with glycine or alanine derivative (Fig. 4.21, 4.22, and 4.23), which were able to bind sequence-specifically to RNA (Garcia-Echeverria *et al.*, 1997).

OTHER BACKBONE MODIFICATIONS

Various other modified monomers or oligomers derived from amino acids or polyamides were synthesised, but no DNA or RNA binding studies have been reported for the majority of these compounds, and the interbase and base-to-backbone bonding topologies of all of them differ from those of nucleic acids (selected structures are shown on Fig. 8).

Very intensively studied were the interesting analogues of PNA with *N*-(2-hydroxyethyl)aminomethylphosphonic acid (Fig. 8.7) or *N*-(2-aminoethyl)aminomethylphosphonic acid (Fig. 8.6) backbone (named: PPNA, PGNA, PAGNA, or PHONA) (van der Laan *et al.*, 1996; Peyman *et al.*, 1996; 1997; 1998; Efimov *et al.*, 1996; 1998a, b). They were oligomerised as homooligomers (Efimov *et al.*, 1996; Peyman *et al.*, 1996; van der Laan *et al.*, 1996), and incorporated into those of PNA or DNA oligomers (Peyman *et al.*, 1997; 1998; Efimov *et al.*, 1998a, b); the chimeric DNA–PHONA–PNA oligomer showed binding properties comparable to those of PNA (Peyman *et al.*, 1998). Interestingly, the PNA oligomer with deoxynucleotide attached *via* *N*-(2-aminoethyl)-aminomethylphosphoglycine monomer to its carboxy terminus is recognized as a substrate by various DNA polymerases (Lutz *et al.*, 1999).

The second notable group comprises nucleosides and oligonucleotides derived from all stereoisomers of 3- (Fig. 8.1) or 4-hydroxy-*N*-acetylprolinol (Fig. 8.2) as sugar substitute, synthesised, respectively, from N-protected L-serine or D- or L-4-hydroxyproline (Ceulemans *et al.*, 1997a,b; 1998). The derived oligo-

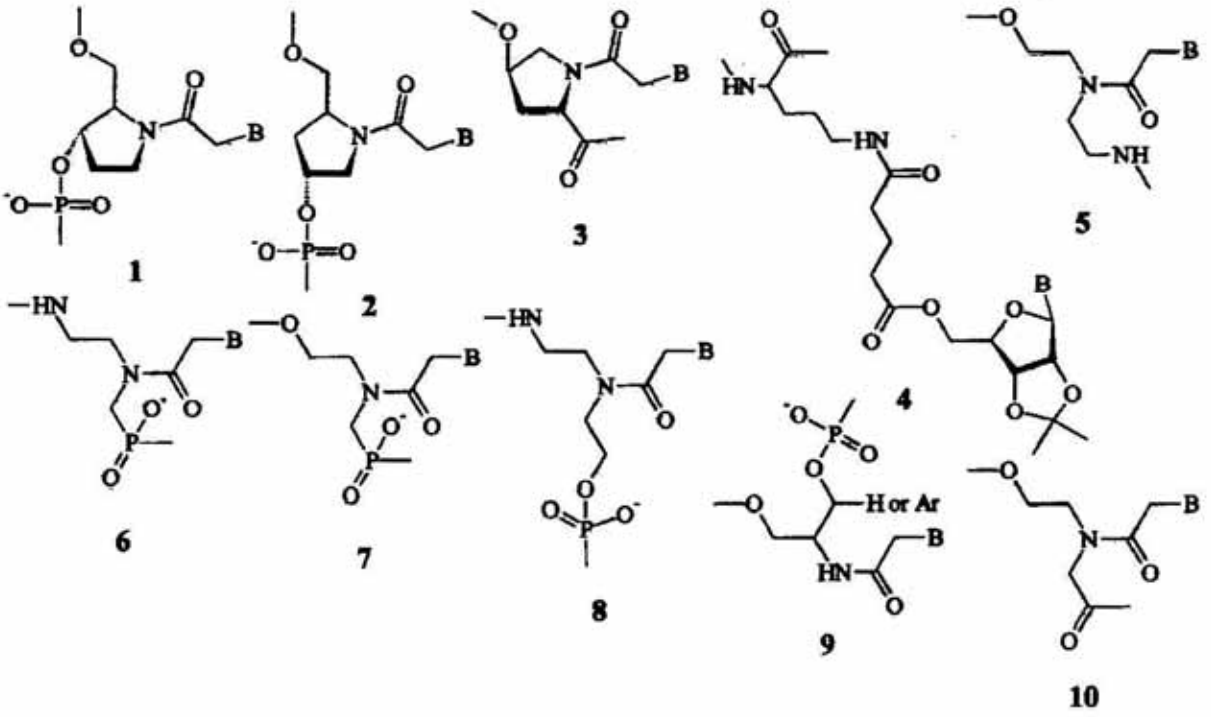

Figure 8. Selected other monomers derived from amino acids.

References: **1** (Ceulemans *et al.*, 1997b; 1998); **2** (Ceulemans *et al.*, 1997a; 1998); **3** (Verheijen *et al.*, 1999); **4** (Takemoto & Inaki, 1988); **5** (Wenninger & Seliger, 1997a,b); **6** (Efimov *et al.*, 1996; 1998a, b; Peyman *et al.*, 1997; 1998); **7** (Peyman *et al.*, 1996; 1997; Efimov *et al.*, 1996; 1998a, b; van der Laan *et al.*, 1996; Kehler *et al.*, 1998); **8** (Lutz *et al.*, 1999); **9** (Rana *et al.*, 1997); **10** (Wenninger & Seliger, 1997a,b)

nucleotides are able to form stable complexes with NA and the system has a strong preference for isochiral interaction.

Chimerical DNA–PNA-derived amino alcohol dimer blocks (Fig. 8.5 and 8.10), developed by Wenninger and Seliger (1997a,b), also draw much interest. They might be incorporated into DNA oligomers with only a slight decrease in binding capacity, and they are stable as far as exonucleolytic degradation is concerned.

MODIFIED NUCLEOBASES ATTACHED TO PNAs

A set of a few other than natural nucleobases was used for the formation of PNA monomers and then oligomers. 2,6-Diaminopurine used in the place of adenine significantly enhances the PNA tendency to hybridise due to the possibility of its pairing with thymine through three hydrogen bonds (Haaima *et al.*, 1997). Pseudoisocytosine was used for a more efficient formation of triplex structures due to its applicability to bind guanine by Hoogsteen bonds (Nielsen *et al.*, 1994; Egholm *et al.*, 1995; Kuhn *et al.*, 1998). In contrast, incorporation of N^4-benzoylcytosine into PNA oligomers inhibits formation of the triplex structures, without any severe interference with Watson–Crick hydrogen bonding (Christensen *et al.*, 1998). The PNA monomer with nucleobase replaced by 3-oxo-2,3-dihydropyridazine, connected to the PNA backbone *via* a β-alanine linker from the 6-position, was designed specifically for the recognition of thymine in triple-helix structures (Eldrup *et al.*, 1997). Also 5-thio-substituted thymine and N^3-(methyl)thymine PNA dimers were synthesised for studying photochemical behaviour and crystal structure of PNA–PNA duplex containing thiothymine (Clivio *et al.*, 1997; 1998). Inter-

estingly, with regard to photochemistry, none of the dimers reflected the photochemical behaviour known from dinucleotide studies, and the results of the study suggested that in solution these dimers adopt a conformation reminiscent of A-type DNA (Clivio *et al.*, 1998). A series of interesting studies on NA duplexes intercalation, photoinduced cleavage and strand invasion by PNA oligomers with internally linked antraquinone was published by Armitage *et al.* (1997a,b; 1998). Pyreneacetic acid was used in place of a nucleobase for obtaining PNA monomers for the synthesis of potential intercalators (Challa & Woski, 1999), and 2-aminopurine for the synthesis of fluorescent PNA analogues (Gangamani *et al.*, 1997). Another base introduced in PNA oligomers was 6-thioguanine (Hansen *et al.*, 1999) which caused a decrease in PNA-DNA duplex melting temperature and a characteristic shift in wavelength and absorbance as a result of hybridisation.

CONCLUSIONS

So far various modifications of the PNA monomers and oligomers have been obtained. Some of them seem to offer hope for future applications and need further studies. Due to the versatility of PNA chemistry, the PNA structure may still be improved, while many other nucleobases designed for specific purposes may be attached to the PNA backbone, as well as to the chemically modified natural NA and other NA analogues.

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