Chapter 8

Chiral Acyclic PNA Modifications: Synthesis and Properties

A.M. Varizhuk*,†, A.V. Dezhenkov‡ and Yu. G. Kirillova*,‡

**Laboratory of Artificial Antibodies, Institute for Physical-Chemical Medicine, Moscow, Russia; †Laboratory of Stereochemistry of Enzymatic Reactions, Engelhardt Institute of Molecular Biology, Moscow, Russia; ‡Institute of Fine Chemical Technologies, Moscow Technological University, Moscow, Russia*

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INTRODUCTION

Intense studies in the field of nucleic acids (NAs), especially the development of antisense and antigen technologies [1–3], require new molecular tools that can interact with NAs and regulate their metabolism. Among such tools, peptide

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FIGURE 8.1 **Structure of chiral PNAs.**

nucleic acids (PNAs, Fig. 8.1), also termed PNA mimics, are of particular interest. PNAs were discovered in 1991 by a Danish group headed by Nielsen [4]. PNAs are chimeric molecules containing purine or pyrimidine heterocyclic bases [5] attached to a backbone composed of amino acids [6].

Nucleic bases enable recognition of target ssDNA and single-stranded RNA (ssRNA) sequences by PNAs [7–9]. In addition to binding with single-stranded NAs (ssNAs), PNAs are capable of displacing one DNA strand from double-stranded DNA (dsDNA) [10–12]. A polyamide *N*-(2-aminoethyl)glycine (aeg)-PNA backbone represents oligomerized units (Fig. 8.1) containing reduced amide bonds (the repeated unit is the aeg fragment). Nucleic bases are attached via carboxymethyl linkers. Such a structure renders aeg-PNAs resistant to nucleases and proteases [13,14]. PNAs are incapable of activating RNase H and act by causing steric hindrance in transcription and translation processes [15]. The aeg-PNAs have proven to be efficient molecular biology instruments with a great number of in vitro applications in biosensor technologies and analytical chemistry [16–18]. One particularly important field of aeg-PNA application, which has emerged recently, is investigation of noncanonical DNA structures [19].

Detailed studies have shown that along with the above-mentioned advantages, PNAs have certain significant drawbacks. These drawbacks include low water solubility, which limits in vivo applications of PNAs [20]; low bioavailability [21], which necessitates development of PNA carriers for intracellular delivery [22,23]; and the propensity of long PNAs (more than 20 bp) for self-aggregation, which results in decreased selectivity of binding with NAs due to nonspecific interactions [24].

Acyclic chiral PNAs containing various side chain functional groups appear to be a promising solution to the above-mentioned problems [25]. The usage of natural products, *trans*-4-hydroxy-L-proline or pyroglutamic acid, as initial

structural templates allowed researchers to obtain chiral cyclic PNAs containing two chiral centers in a monomer unit [26,27]. The presence of a stereogenic center in a PNA molecule increases structural regularity by determining the spatial preorganization of PNA, which in turn has an impact on its hybridization properties, that is, affinity toward complementary oligonucleotide (ON) sequences and sensitivity to single-nucleotide mismatches [28,29]. Functional groups in backbone side chains may also impart desired properties to PNAs, for example, they may improve their cellular uptake [30,31] and enable attachment of reporter groups [32,33] or introduction of thiol groups that can be used for subsequent ligations [34,35], etc. Thus, chiral PNAs are important objects of biochemical research and attractive molecular tools that meet all of the key requirements of modern biotechnologies. The problems of chiral PNA application in medicine, molecular biology and nanotechnology, and, to some extent, chiral PNA synthesis are reviewed in Refs. [25,36,37].

The current review is focused on classical and contemporary methods of synthesis and properties of chiral acyclic PNAs – typical challenging structures based on natural products, amino acids. We pay particular attention to thorough systematization of strategies for chiral PNA monomer synthesis and to assessment of the enantiomeric purity (EP) of the monomers. Considering that PNAs are artificial biopolymers, we also review solid-phase oligomerization strategies. The design of PNA oligomers containing chiral monomers is discussed and comparative analysis of the properties of such oligomers (mostly the isosequential ones) is presented.

SYNTHESIS OF PNA MONOMERS

Synthesis of classical and modified PNA oligomers is usually carried out according to known solid-phase protocols for peptides – *tert*-butyloxycarbonyl (Boc)/benzyloxycarbonyl (Cbz) [38], 9-fluorenylmethoxycarbonyl (Fmoc)/Boc [39,40], or Fmoc/benzhydryloxycarbonyl (Bhoc) [40] (Fig. 8.2).

In the synthesis of classical aeg-PNAs, nucleic bases function as side radicals in the polyamide chain. In the case of an extra functional group in the side chain moiety of an amino acid residue incorporated into the pseudopeptide backbone, an extra protecting group (PG) and a modification of the solid-phase synthesis protocol are required. The major steps of the synthesis include: (1) assembly of the pseudopeptide backbone; (2) attachment of the heterocyclic base via the carboxymethyl linker; and (3) removal of the C-terminal backbone PG. The pseudopeptide backbone monomer is usually obtained by conjugating two amino acid precursors. Basic methods of monomer synthesis are reductive *N*-alkylation, leading to α-Boc and γ-Boc monomers; Mitsunobu alkylation with subsequent removal of the sulfamide group, leading to α-Boc and γ-Boc monomers; reductive *N*-alkylation, leading to γ-Fmoc monomers; and alkylation of the modified ethylenediamine residues with chloro (bromo) acetates, leading to γ-Boc or β-Fmoc monomers. The methods are discussed in detail in the following sections.

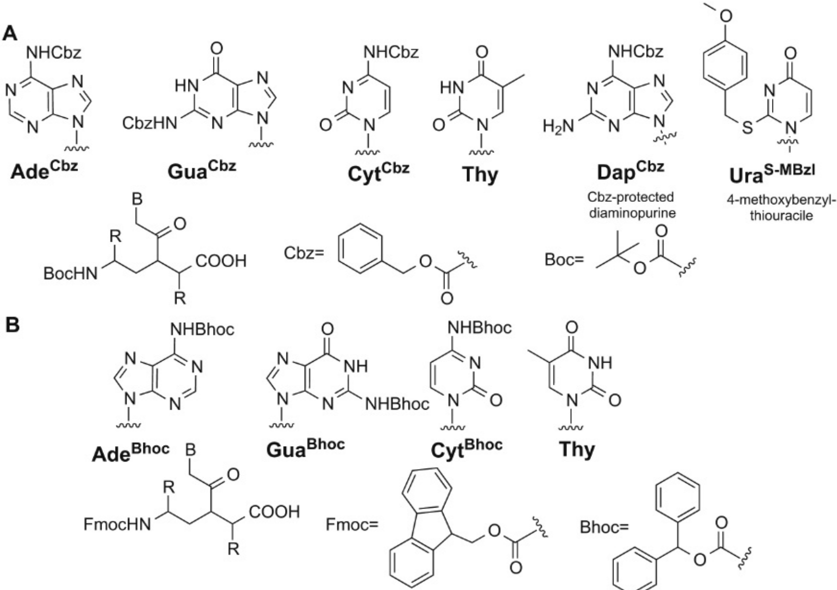


FIGURE 8.2 Protecting group strategies, commonly used in the synthesis of chiral PNA oligomers. (A) Boc/Cbz-strategy; (B) Fmoc/Bhoc-strategy.

Synthesis of α- and γ-PNA Monomers for Boc-Protocol Oligomerization via Reductive *N*-Alkylation

A major advantage of the α-modification of aeg-PNAs is relative simplicity of monomer synthesis. The monomers can be obtained by reductive *N*-alkylation, a standard method of aeg-backbone synthesis. The typical synthesis of α-PNA monomers is shown in Scheme 8.1. The aldehyde component, *N*-Boc-amino acetaldehyde, is commonly obtained in two steps from 1,2-aminopropandiol. PGs (R^1) of the amino acid component typically used in the pseudopeptide synthesis are listed in Table 8.1.

SCHEME 8.1 Synthesis of α-PNA monomers for Boc-protocol oligomerization via reductive N-alkylation.

TABLE 8.1 Protecting Groups and Overall Yields in the Syntheses of Chiral α-Monomers via Reductive *N*-Alkylation for the Boc-Protocol

No.	Start amino acid (α-configuration)	R^1	R^2	B	Overall yield (%)	References
1 **2**	L-Ala (α-*S*) D-Ala (α-*R*)	$-CH_3$	Me *sec*-^{i}Bu Bzl	Thy		[41] [41]
3	D-Glu (α-*R*)	$-(CH_2)_2COOBzl$	All	Thy	46	[42][a]
10	L-Val (α-*S*)	$-CH(CH_3)_2$	Bzl	Thy	72	[6][b]
9 **20** **21**	D-Lys (α-*R*)	$-(CH_2)_4NHCbz$	All	Thy Cyt^{Cbz} Ade^{Cbz}	26 25 9	[43]
8 **22** **23**	L-Lys (α-*S*)	$-(CH_2)_4NHCbz$	All	Thy Cyt^{Cbz} Ade^{Cbz}		[43]
17a	L-Arg (α-*S*)	$-(CH_2)_3NH\text{-}C(NH)\text{-}NHTos$	All	Thy		[44]
17(a–d) **24(a–d)**	L-Arg (α-*S*) D-Arg (α-*R*)	$-(CH_2)_3NH\text{-}C(NH)\text{-}NHTos$	All	Thy Cyt^{Cbz} Ade^{Cbz} Gua^{Cbz}	20 21 23 17	[45]
24e **24f**	D-Arg (α-*R*) D-Arg (α-*R*)	$-(CH_2)_3NH\text{-}C(NH)\text{-}NHTos$	All	$Ura^{S\text{-}MBzl}$ Dap^{Cbz}	21 22	[45]
25	L-Glu (α-*S*)	$-(CH_2)_2COOBzl$	All	Thy	21	[46]

(Continued)

TABLE 8.1 Protecting groups and overall yields in the syntheses of chiral α-monomers *via* reductive *N*-alkylation for the Boc-protocol (*cont.*)

No.	Start amino acid (α-configuration)	R^1	R^2	B	Overall yield (%)	References
11 **26**	L-Leu (α-*S*) D-Leu (α-*R*)	$-CH_2CH(CH_3)_2$	Bzl	Thy	19 10	[47]
27	α-*S*		Me	Thy	31	[48]
28	L-Asn (α-*S*)	$-CH_2CONH_2$ transformed into 2-(*N*-Cbz)-amino-methyl	Me	$ClCH_2COCl$/TEA, and then Thy, K_2CO_3, DMF	~12	[49]
29	D-Asn (α-*R*)	$-CH_2CONH_2$ transformed into 2-(*N*-Cbz)-amino-methyl	Me	$ClCH_2COCl$/TEA, and then Thy, K_2CO_3, DMF	12.4	[49]

[a] *In Ref. [41], thymine monomers* **4–9**, *based on* L-*Ile (α-S),* D-*Ser (α-R),* L-*Ser (α-S),* L-*Asp (α-S),* L-*Lys (α-S), and* D-*Lys (α-R), were also obtained in overall yields of 49%, 41%, 44%, 42%, 45%, and 42%, respectively.*

[b] *In Ref. [6], thymine monomers* **11–19**, *based on* L-*Leu (α-S),* L-*Phe (α-S),* L-*Asn (α-S),* L-*Gln (α-S),* L-*Trp (α-S),* L-*Thr (α-S),* L-*Arg (α-S),* L-*His (α-S), and* L-*Tyr (α-S), were obtained in overall yields 39%, 71%, 48%, 35%, 26%, 53%, 40%, 51%, and 16%, respectively.*

In the case of the Boc N-terminal PG, the C-terminal backbone PGs (R^2) are usually methyl (Me), ethyl (Et), benzyl (Bzl), or allyl (All). The former group can be removed by alkaline hydrolysis [LiOH, H_2O–tetrahydrofuran (THF) 1:1, or K_2CO_3, 3:1 MeOH/H_2O or $Ba(OH)_2$, H_2O]. However, it has been shown that under such conditions, partial racemization at the α-chiral center is possible (see the section "Enantiomeric Purity Tests for Chiral PNA Monomers"). For this reason, Bzl C-terminal backbone PG is now used more commonly (standard removal conditions: H_2/10% Pd/C), as it ensures a higher stability of the monomers with respect to stereochemistry. However, the Bzl PG is applicable only to thymine (Thy) monomers. The All PG is regarded as universal (applicable to all monomers), but its removal requires a relatively expensive palladium complex $[Pd(PPh_3)_4]^0$, and the synthesis is usually carried out in THF in the presence of morpholine.

Reductive *N*-alkylation is typically preformed with $NaBH_3CN$ and AcOH in MeOH. [Only in a single case were alternative conditions used (H_2, 5 atm Pd/C, MeOH, 67% yield) to avoid the reduction of a keto group in an asymmetric side chain moiety [48].] Subsequent condensation of a carboxymethyl heterocycle with the pseudopeptide is usually carried out in the presence of dicyclohexylcarbodiimide (DCC), 3-hydroxy-1,2,3-benzotriazine-4(3H)-one (DhbtOH), and diisopropylethylamine (DIEA) in dimethylformamide (DMF). In our recent work on L-Glu-based monomers [46], isobutyl chloroformate (IBCF), *N*-methyl morpholine (NMM), and trimethylamine (TEA) (20 min, −20°C, 3 eq., 78% yield) were used because the L-Glu-based backbone is prone to cyclization [50]. Monomer yields generally vary from moderate (16%) to good (75%) relative to the initial pseudopeptide.

Published works on the synthesis of chiral α-PNA monomers are mainly focused on preparation of Thy monomers and their incorporation into oligomers. Properties of oligomers obtained in this manner have been widely studied. To date, only two research groups reported on monomers containing heterocycles other than Thy. The first group, Sforza et al. [43], obtained the Thy, Cyt^{Cbz}, and Ade^{Cbz} monomers from D- or L-Lys. The second group, Ly and coworkers [45], obtained D- and L-Arg-based monomers with native nucleobases (Cyt^{Cbz}, Ade^{Cbz}, Gua^{Cbz}), as well with artificial heterocycles (Cbz-protected diaminopurine and 4-methoxybenzyl-thiouracile; see Fig. 8.2).

Some of the α-monomers were modified with carbohydrate moieties for the development of PNA intracellular transport systems [51]. Once again, only Thy derivatives were described. Initial N-terminal-Boc-C-terminal-All-hydroxy-(O^tBu) monomers were obtained by reductive alkylation of amines **30**, **31** with Boc-aminoacetaldehyde. Subsequent transformation of the pseudopeptide backbone and replacement of the PGs lead to corresponding N-terminal-Fmoc-C-terminal-All monomers **32** (L-Ser-based) and **33** (L-Thr-based). These compounds were used to obtain carbohydrate-bearing monomers **34** and **35**, in which the carbohydrate moieties were attached to the side chain via *O*-glycosidic bonds (Scheme 8.2).

Additional examples of this type of monomer are shown in Scheme 8.3. Monomers derived from L-Lys were synthesized following a similar scheme, that

SCHEME 8.2 Synthesis of α-PNA monomers based on L-Ser and L-Thr with carbohydrate moieties in the side residues.

is, by *N*-alkylation of the amino acid component with *N*-Boc-acetaldehyde, and the carbohydrate moieties were attached via either *N*-alkyl (**36A–C**) or *N*-acyl (**37A–C**) bonds.

Monomers **34**, **35**, **36(A–C)**, and **37(A–C)** were introduced into PNA oligomers, and the latter were tested for their ability to penetrate cellular membranes.

Mitra and Ganesh [49] described the synthesis of L- and D-Asn-based aminomethyl (Am) PNA monomers **28** and **29** (Scheme 8.4). L- and D-Boc-Asn (**38**, **39**) were converted into orthogonal protected *N*-Cbz-Am derivatives **40** and **41**. After the removal of the Boc- PG, the derivatives were subjected to *N*-alkylation

SCHEME 8.3 Synthesis of α-PNA monomers based on L-Lys with carbohydrate moieties in the side residues.

SCHEME 8.4 Synthesis of aminomethyl α-PNA monomers based on L- and D-Asn.

with Boc-aminoacetaldehyde to produce secondary amines **42** and **43**. The heterocyclic base was introduced by an alternative method, which consisted of acylation of amines **42**, **43** with chloroacetyl chloride and subsequent alkylation of Thy. This leads to the corresponding *R*- and *S*-monomers **28** and **29**.

The synthetic strategy described earlier (reductive *N*-alkylation) can be employed to introduce a chiral center not only into the glycine part (C-α) but also into the aminoethyl (C-γ) part of the aeg backbone (Scheme 8.5). In that case, the amino acid that constitutes the aminoethyl part of the pseudopeptide is termed "start amino acid" (Scheme 8.5; Table 8.2).

In most cases, monomer synthesis included the following steps:

- preparation of an aldehyde component; typical method: reduction of the Weinreb amide [56];
- reductive *N*-alkylation of glycine Et ester (hydroxychlorides and tosylates were preliminarily neutralized with a tertiary amine);
- condensation of the secondary amine thus obtained with carboxymethyl derivatives of heterocycles.

The products of the latter reaction were converted into target protected monomers by the removal of the C-terminal PG. PGs of the amino acid components (R^1, R^2) typically used in the pseudopeptide synthesis are listed in Table 8.2.

In 1994, Kosynkina et al. [52] reported the synthesis of a γ-PNA Thy monomer from L-Ala. A number of Thy monomers based on other amino acids (L-Leu, L-Phe, L-Tyr, L-Thr, L-Ile, L-Pro, L-Ser, L-Trp, and L-Val) [53,54] were obtained by a Polish group. In those works, Bzl moiety was chosen as a PG for

SCHEME 8.5 Synthesis of γ-PNA monomers for Boc-protocol oligomerization via reductive N-alkylation.

TABLE 8.2 Protecting Groups and Overall Yields in the Synthesis of Chiral γ-Monomers via Reductive *N*-Alkylation

No.	Start amino acid (γ-configuration)	R^1	R^2	B	Overall yield	References
44a	L-Ala (γ-*S*)	$–CH_3$	Me	Thy	62%	[52]
45	L-Ile (γ-*S*)	$–CH(CH_3)CH_2CH_3$	Me	Thy	53%	[53][a,b]
47a **47b** **47c** **47d**	L-Ser (γ-*S*)	$–CH_2OBzl$	Et	Thy Cyt^{Cbz} Ade^{Cbz} $Gua^{O6\text{-}Bzl}$	40% Nr[c] Nr[c] Nr[c]	[54]
44a **44b** **44c** **44d**	L-Ala (γ-*S*)	$–CH_3$	Et	Thy Cyt^{Cbz} Ade^{Cbz} $Gua^{O6\text{-}Bzl}$	Nr[c] Nr[c] Nr[c] Nr[c]	[55]
54	L-Cys (γ-*S*)	$–CH_2STrt$	Me	Ade^{Cbz}	5%	[35]
55	L-Cys (γ-*S*)	$–CH_2S$-2-MBzl	Et azidoglycine ethylester	Thy	38%	[34]
56a **56b**	L-Lys (γ-*S*)	$–(CH_2)_4NHFmoc$	Bzl	Thy Cyt^{Cbz}	13% 4%	[32]

MBzl, methoxy-benzyl.
[a]*In Ref. [53], thymine monomers* **46–49** *based on* L*-Pro (γ-S),* L*-Ser (γ-S),* L*-Trp (γ-S), and* L*-Val (γ-S) were also obtained in overall yields of 46%, 48%, 43%, and 51%, respectively.*
[b]*In Ref. [54], thymine monomers* **48–53** *based on* L*-Leu (γ-S),* L*-Phe (γ-S),* L*-Tyr (γ-S), and* L*-Thr (γ-S) are described. The yields were not reported.*
[c]*Nr, not reported.*

hydroxyl-bearing amino acids because it is commonly used in the Boc-protocol synthesis of peptides and α-PNAs.

Ly and coworkers [55] described the synthesis of γ-PNA monomers from L-Ala and L-Ser. Derivatives of all nucleic bases were obtained. Subsequently, synthesis of γ-PNA monomers from L-Cys [34,35] and L-Lys by reductive *N*-alkylation was also reported [32].

As in the case of α-PNAs, $NaBH_3CN$ in the presence of AcOH [32,34,35, 52–55] in methanol was used for reductive *N*-alkylation. In the case of azidoglycine Et ester alkylation [34], it was first activated with Me_3P in THF and then reduced with $NaBH_3CN$. On reduction of the neutralized glycine Et ester and the azidoglycine Et ester, the yields of target compounds were comparable. In the synthesis of a pseudopeptide based on an ε-Fmoc-protected L-Lys derivative, the reduction was performed with $NaHB(OAc)_3$ in CH_2Cl_2 in the presence of DIEA [32], and the yield was relatively low (30%). Carboxymethyl derivatives of the heterocycles were condensed with secondary amines under the following conditions: isobutyl chloroformate (IBCF), NMM, −20°C, DMF [52]; 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), TEA, scale 10 mmol, 42.5% [54]; 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide (EDC), DhbtOH, DIEA, DMF [34,54]; or DCC, DhbtOH, DMF, 50°C [55]. In the case of Et and Me esters, C-terminal PGs were removed by alkaline hydrolysis with NaOH [52,54], K_2CO_3 [53], or LiOH [34,35,55] with subsequent neutralization/acidation using acid or Dowex-H^+ [34]. The Bzl C-terminal backbone PG was removed by a conventional method (H_2, 10% Pd/C in methanol) [32]. In general, the reagents and PG strategies were basically similar to those used in the synthesis of α-PNA monomers for Boc-protocol oligomerization.

Synthesis of γ-PNA Monomers for Fmoc-Protocol Oligomerization via Reductive *N*-Alkylation

The preparation of γ-chiral PNA monomers for Fmoc-protocol oligomerization received considerable attention after the advantageous propensity of γ-PNAs for right-hand helix formation became apparent. The synthesis of such compounds (Scheme 8.6) required the development of distinct PG tactics, which depend on the functional groups of side residues (Table 8.3). The general PG strategy is very similar to that used in peptide synthesis.

In the case of Ser derivatives [57], hydroxyls were protected with *tert*-butyl (^{t}Bu) ether. In the case of L-Asp [58] and L-Glu [59] derivatives, ^{t}Bu ester protection was used for side-chain carboxyl groups. Predictably, side-chain amino groups were Boc-protected [60], and the thiol group [34] was initially blocked with a trityl PG, which was later (during the monomer synthesis) replaced with a ^{t}Bu residue. To this end, Thy monomers, a Cyt^{Bhoc} monomer derived from L-Lys and Bzl-protected [61–63] cytosine (Cyt), adenine (Ade), and *tert*-butyl-guanine γ-methylsulfate monomers have been obtained. However, only Thy monomers derived from L-Cys, L-Ser, and L-Asp have been subjected

SCHEME 8.6 Synthesis of γ-PNA monomers for Fmoc-protocol oligomerization via reductive N-alkylation.

to solid-phase oligomerization. Apart from the above-mentioned aspects, the synthesis of γ-monomers for solid-phase Fmoc-protocol oligomerization was similar to that for the Boc-protocol oligomerization. Conventional reagents, $NaBH_3CN$ in methanol in the presence of AcOH or $NaBH(OAc)_3$ in CH_2Cl_2 [60] also in the presence of AcOH, were used for reductive alkylation [58]. Chiral secondary amines were condensed with carboxymethyl heterocycles in the presence of EDC/4-dimethylaminopyridine (DMAP), DCC/DhbtOH in DMF, or *O*-(7-azabenzotriazol-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) [22]. C-terminal PGs were removed by alkaline hydrolysis (NaOH solution [57,59,60]) in the case of Me or Et esters. The Bzl C-terminal PG was removed by a conventional method (H_2, 10% Pd/C in methanol [58]), and the ^{t}Bu group was removed by treatment with trifluoroacetic acid (TFA) in CH_2Cl_2 [34]. To summarize, studies on γ-Fmoc monomers appear to be incomplete. Thus, obtaining a full set of the monomers with all natural heterocyclic bases and L-Cys, L-Ser, L-Asp, or L-Lys residues remains a challenging synthetic task.

Synthesis of the Precursors of α- and γ-PNA Monomers for Submonomer Oligomerization by Boc or Fmoc Protocols via Reductive *N*-Alkylation

The method of reductive alkylation has also been used to obtain precursors of PNA monomers for subsequent oligomerization via the submonomer strategy. After the synthesis of a pseudopeptide fragment, its secondary amino group was blocked with a PG, orthogonal to the N-terminal PG. It has been shown earlier that partial racemization of target compounds may occur during the synthesis of oligomers containing α-monomers based on L- or D-Lys [64]. To solve that problem, a "preventive" submonomer synthetic strategy [65] has been developed. This strategy ensures high optical purity of target chiral PNA oligomers.

TABLE 8.3 Synthesis of Chiral γ-Monomers for Fmoc-Protocol Oligomerization via Reductive *N*-Alkylation

No.	Start amino acid (γ-configuration)	R^1	R^2	B	Overall yield	References
57	L-Cys (γ-*S*)	$-CH_2STrt$ (converted into $-CH_2S^tBu$)	^tBu	Thy	27%	[34]
58	L-Ser (γ-*S*)	$-CH_2O^tBu$ (converted into $-CH_2OH$, and then into $-CH_2OH-OSO_3^-$)	Me	Thy Ade^{Bzl} Cyt^{Bzl} Gua^{iBu}	Nr[a] 24% Nr[a] Nr[a]	[57]
59	L-Asp (γ-*S*)	$-CH_2COO^tBu$	Bzl	Thy	24%	[58]
60	L-Glu (γ-*S*)	$-(CH_2)_2COO^tBu$	Et	Thy	43%	[59]
61a **61b**	L-Lys (γ-*S*)	$-(CH_2)_4NHBoc$	Et	Thy Cyt^{Bhoc}	52% Nr[a]	[60]

^{i}Bu, *iso*-butyl.
[a] *Nr, not reported.*

SCHEME 8.7 Synthesis of the precursors of α- and γ-PNA monomers for submonomer oligomerization by Boc- or Fmoc-protocols via reductive N-alkylation.

To date, synthesis of α- and γ-substituted, as well as α,γ-disubstituted, PNA submonomers based on L-Lys, D-Lys [65–67], L-Arg, and D-Arg [66] has been reported (Scheme 8.7; Table 8.4). The submonomers were primarily designated for subsequent oligomerization by Boc protocol. A D-Lys-based α-monomer for Fmoc-protocol oligomerization has also been described.

Reductive alkylation was performed by a conventional method, that is, using $NaBH_3CN$ in methanol in the presence of AcOH, after the removal of the Me PG from the backbone C-terminus. The secondary amino group was protected with Fmoc after the preliminary silylation of the intermediate **74** [67].

It can be concluded that reductive *N*-alkylation can be employed for the preparation of various chiral PNA monomers and for various modifications of the PNA structure using α-aminoaldehydes and amino acid esters. This method enables the specific introduction of amino acid residues because $LiAlH_4$ and reductive amination do not affect chiral centers of amino acids. The disadvantage of the reductive amination method is instability of *N*-substituted α-aminoaldehydes, the key intermediates of the reductive amination reaction. In the case of Fmoc derivatives, these intermediates are particularly unstable. The yields of target compounds are not very high because the following side reactions may proceed on *N*-alkylation: aldol–crotone self-condensation of the aldehyde; dialkylation of the initial amine; cyclization of the neutralized glycine esters leading to diketopiperazine; and reduction of the aldehyde to the respective alcohol, which is conditioned by pH dependence of the effect of $NaBH_3CN$ (rapid reduction of an aldehyde occurs at pH 3–4, while at pH 7–8, the Schiff base is reduced more rapidly than the aldehyde) [68].

In view of the above-mentioned factors, a different approach to building the pseudopeptide backbone, based on Mitsunobu *N*-alkylation with subsequent removal of the sulfonamide residue, has become more common since early 2000s.

Monomer Synthesis via Mitsunobu Alkylation With Subsequent Removal of the Sulfonamide Residues, Leading to α-Boc and γ-Boc Monomers

The Mitsunobu reaction between reduced and *N*-(2-nitrobenzenesulfonyl)-(2-NBS) amino acid derivatives is an alternative method for the synthesis of pseudopeptide fragments of PNA monomers [69] (Scheme 8.8; Table 8.5). After

TABLE 8.4 Protecting Groups and Overall Yields in the Synthesis of Chiral α- or γ-Monosubstituted and α,γ-Disu bstituted Submonomers via Reductive *N*-Alkylation

No.	Amino acid, position in a pseudopeptide moiety (α, γ, or α and γ) and its configuration	R^1	R^2	R^3	R^4	R^5	Overall yield	References
62	L-Lys (α-*S*)	Boc	H	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	Me	Fmoc	Nr[a]	[65,66]
63	D-Lys (α-*R*)	Boc	H	$-(CH_2)_4NH(^{2\text{-}Cl}Cbz)$	Me	Fmoc	34%	[65,66]
64	L-Lys (γ-*S*)	Boc	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	H	Me	Fmoc	Nr[a]	[66]
65	D-Lys (γ-*R*)	Boc	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	H	Me	Fmoc	Nr[a]	[66]
66	L-Lys, (α-*S*, γ-*S*)	Boc	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	Me	Fmoc	Nr[a]	[66]
67	D-Lys, L-Lys (α-*R*, γ-*S*)	Boc	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	Me	Fmoc	Nr[a]	[66]
68	L-Lys, D-Lys (α-*S*, γ-*R*)	Boc	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	Me	Fmoc	Nr[a]	[66]
69	D-Lys, (α-*R*, γ-*R*)	Boc	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	$-(CH_2)_4NHCbz^{2\text{-}Cl}$	Me	Fmoc	Nr[a]	[66]
70	D-Lys (α-*R*)	Fmoc	$-(CH_2)_4NHBoc$	H	H	All	48%	[67]
71	D-Arg (α-*R*)	Boc	$-(CH_2)_3NH$-$C(NH_2)$-NHTos	H	Me	Fmoc	Nr[a]	[66]
72	L-Arg (γ-*S*)	Boc	H	$-(CH_2)_3NH$-$C(NH_2)$-NHTos	Me	Fmoc	Nr[a]	[66]
73	D-Arg, L-Arg (α-*R*, γ-*S*)	Boc	$-(CH_2)_3NH$-$C(NH_2)$-NHTos	$-(CH_2)_3NH$-$C(NH_2)$-NHTos	Me	Fmoc	13%	[66]

[a] *Nr, not reported.*

SCHEME 8.8 Synthesis of γ-PNA monomers for Boc-protocol oligomerization via N-alkylation by Mitsunobu condensation.

the Mitsunobu condensation, sulfobenzyl group is removed, and the resulting secondary amines are condensed with carboxymethyl derivatives of heterocycles. Subsequent removal of C-terminal PGs yields the target monomers.

This method was first described by a Polish research group. In 2001 Falkiewicz et al. [70] conducted PNA monomer synthesis by a Mitsunobu reaction between the 2-NBS-amino acid ester and the *N*-Boc-protected β-aminoalcohol. Initial protected β-amino alcohols of various structures can be obtained easily from their amino acid precursors [72–74]. Similarly to reductive *N*-alkylation, Mitsunobu alkylation yields products of high optical purity. Five chiral PNA monomers were obtained from Gly, L-Ala, L-Val, L-Leu, and L-Phe derivatives. The Mitsunobu reaction between the acid component, Me 2-(2-nitrobenzenesulfonylamido)acetate (2-NBS-Gly-OMe), and the alcohol component, *N*-Boc-aminoalcohol (Boc-Xaa-ol), was conducted in dry THF with triphenylphosphine and diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD). In a later study, Ly and coworkers used 2-(2,4-dinitrobenzenesulfonylamido)glycine derivatives [20,30], which gave fully protected pseudopeptides. The NBS groups were removed by treatment with PhSH and K_2CO_3 in CH_3CN at rt in the case of mononitro derivatives, and by treatment with *n*-$PrNH_2$ at rt in CH_2Cl_2 (81% yield) in the case of dinitro derivatives. Subsequent condensation with carboxymethyl heterocycles was performed using carbodiimides (EDC or DCC in the presence of DhbtOH). In our recent studies, we employed the mixed anhydride method ($ThyCH_2COOH$, isobutyl chloroformate (IBCF), NMM, 3 eq. in DMF) for obtaining Thy γ-monomers based on L-Glu and L-Asp [71] because of the propensity of the initial pseudopeptide for cyclization, which is similar to that of the α-isomer [50]. The Me/Et C-terminal-PG was removed by alkaline hydrolysis, and the All group was removed by treatment with $[Pd(PPh_3)_4]^0$ in the presence of morpholine.

The only example of the α-monomer synthesis by the Mitsunobu reaction is our work on the preparation of the L-Glu-based Thy monomer **25** (Scheme 8.9) [46]. The overall yield was relatively high (33%) compared to an alternative synthetic route based on reductive *N*-alkylation (see previous text) due to the decrease in the lifetime of the intermediate **79**, which is prone to cyclization **80**.

TABLE 8.5 Synthesis of Chiral γ-Monomers for Boc-Protocol Oligomerization via *N*-Alkylation by Mitsunobu Condensation

No.	Start amino acid[a]	R^1	R^2	R^3	B	Overall yield (%)	References
	Gly	—	Me	–H	Thy	53[b]	[70]
44a	L-Ala (γ-*S*)	$–CH_3$	Me	–H	Thy	51[b]	[70]
49	L-Val (γ-*S*)	$–CH(CH_3)_2$	Me	–H	Thy	48[b]	[70]
50	L-Leu (γ-*S*)	$–CH_2CH(CH_3)_2$	Me	–H	Thy	40[b]	[70]
51	L-Phe (γ-*S*)	$–CH_2Ph$	Me	–H	Thy	39[b]	[70]
75	L-Asp (γ-*S*)	$–CH_2COOBzl$	All	–H	Thy	18	[71]
76	L-Glu (γ-*S*)	$–CH_2CH_2COOBzl$	All	–H	Thy	19	[71]
77	L-Lys converted into homo-L-Arg (γ-*S*)	$CH_2CH_2CH_2NHC(NHCbz)NHCbz$	Et	NO_2	Thy Cyt^{Cbz} Ade^{Cbz} Gua^{Cbz}	33 36 31 32	[30]
78	L-Ser (γ-*S*)		Et	NO_2	Thy Cyt^{Cbz} Ade^{Cbz} Gua^{Cbz}	26 ~25–38	[20]
44a	L-Ala (γ-*S*)	$–CH_3$	Me	NO_2	Thy		[28]
49	L-Val (γ-*S*)	$–CH(CH_3)_2$	Me	NO_2	Thy	7	[28]
45	L-Ile (γ-*S*)	$–CH(CH_3)CH_2CH_3$	Me	NO_2	Thy	22	[28]
51	L-Phe (γ-*S*)	$–CH_2Ph$	Me	NO_2,	Thy	33	[28]

[a]*Amino acid used for preparation of the start amino alcohol in Scheme 8.8.*
[b]*Without the removal of the α-protecting group.*

SCHEME 8.9 Synthesis of thymine α-PNA monomer based on L-Glu by the Mitsunobu reaction.

It should be mentioned that the Mitsunobu method also has limitations, that is, it cannot be used for the preparation of monomers for Fmoc-protocol oligomerization because the Fmoc PG is unstable under the conditions of nitro-/dinitrobenzenesulfonyl group removal.

Synthesis of β- and γ-PNA Monomers for Fmoc- and Boc-Protocol Oligomerization via *N*-Alkylation of a Chiral Ethylene Diamine Derivative With Chloro(bromo)acetic Acid Derivatives

Synthesis of PNA monomers via alkylation of β- and γ-substituted ethylene diamine derivatives has been reported recently. This approach has been employed in early works on the preparation of aeg-PNA [75], but it has also proven to be efficient in the case of chiral PNAs [49,76–78] (Scheme 8.10).

The general strategy proposed by Ganesh and coworkers [49,76] was employed to obtain γ-*S*-monomers **81** (the Am derivative), **82** (the aminoethyl derivative), and **83** (the guanidinoethyl derivative). Compounds **41** and **84**, derived from L-Asp and L-Glu, respectively, were used in a four-step synthesis of the corresponding mono-Boc-(Cbz-aminoalkyl)ethylenediamines **87** and **88**. Alkylation of the latter compounds with ethylbromacetate led to secondary amines **89** and **90** (the pseudopeptide fragments), which were subsequently converted into the target monomers **81** and **82** by a method analogous to that used in the synthesis of α-(*R*)- and α-(*S*)-Am isomers **61** and **62**. Selective removal of the Cbz group from the protected monomer **94**, followed by treatment of the intermediate **95** with *N*,*N'*-bis-di-Cbz-*S*-Me-isothiourea (**96**), gave a fully protected aminoethylguanidine derivative (monomer **97**). Removal of the Et PG by alkaline hydrolysis gave target monomer **83**. Overall yields of **81**, **82**, and **83** were 15%, 14.7%, and 9.4%, respectively, relative to the initial intermediates **41** and **84**.

SCHEME 8.10 Synthesis of the aminomethyl-, aminoethyl- and guanidinoethyl-γ-PNA monomers via alkylation of ethylene diamine derivatives.

In another work, an analogous strategy was employed to obtain azido-group-bearing Thy monomers **98** and **99** [77] (Scheme 8.11).

The γ-*S*-azidomethyl PNA monomer (**98**) was synthesized from commercially available *N*-α-Boc-asparagine (**39**). The Hoffman rearrangement was used to convert the amide group in the side residue of **39** into a primary amine, and 3-(amino)-2-(*N*-Boc-amino)propionic acid **100**, obtained thus, was then transformed into the azido derivative **102**. Etherification of the carboxyl group

SCHEME 8.11 Synthesis of the azidobutyl-γ-PNA monomers via alkylation of ethylene diamine derivatives.

SCHEME 8.12 Synthesis of the methyl-β-PNA monomers via alkylation of ethylene diamine derivatives.

with subsequent reduction of the respective ether gave alcohol **106**. The latter was treated with mesyl chloride and then with potassium phthalimide. The phthalyl group was removed by hydrazinolysis, and the resulting amine was alkylated with bromoacetyl bromide to give pseudopeptide **110**. Monomer **98** was obtained from **110** following the previously developed method (Schemes 8.4 and 8.10). The overall yield of **98** was 7.7%. The similar synthetic route was used to obtain the γ-*S*-azidobutyl PNA monomer (**99**) from *N*-α-Boc-lysine (**101**). The overall yield of **99** was 19%.

One more example of methyldiamine alkylation reported in Ref. [78] is presented in Scheme 8.12. Treatment of chiral diamine **116** with 2 mol equivalents of Cbz-OPh (**117**) in EtOH overnight lead to a mixture of isomers **118** and **119** in a 92:8 ratio with an overall yield of 76%. The mixture of the isomers was alkylated with 4 mol equivalents of bromoacetic Me ester, which led to dialkyl derivatives **120** and **121** and a monoalkyl derivative **122**. The target amine could be easily separated from the resulting mixture. The secondary amine **122** was obtained in a yield of 77% (84% from **116**) as a pure isomer. Subsequent condensation with thymine-1-yl-acetic acid proceeded in quantitative yield. Alkaline hydrolysis of the condensation product gave β-(*S*) Cbz monomer **123**. At the final stage of the synthesis, the *N*-Cbz-terminal PG was replaced with Fmoc by catalytic hydration and subsequent treatment with 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide (FmocOSu) (79% yield for three steps). The overall yield of the β-(*S*)-monomer **124** was 50%. The other enantiomer, β-(*R*) (**125**), was obtained analogously from (*R*)-1,2-diaminopropane. These monomers (**124** and **125**) were later used for the synthesis of PNA oligomers.

The strategies of monomer synthesis presented provide a diversity of chiral PNA monomer composition. Thus far, most of the monomers have been developed for Boc-protocol oligomerization. However, it appears that chiral monomers for Fmoc-protocol oligomerization will also be developed in the near future.

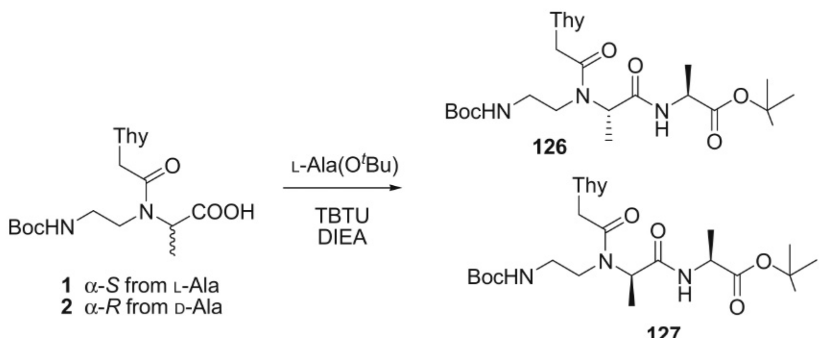
ESTIMATION OF THE ENANTIOMERIC PURITY OF PNA MONOMERS AND OLIGOMERS

It has been shown earlier that the configuration of chiral centers in amino acid residues used for the synthesis of chiral PNA monomers and oligomers determines the properties of the latter (the direction of the helix twist, thermostability of the PNA complexes with NAs, etc.) [25]. Therefore, the stereochemical homogeneity of chiral PNAs must be verified prior to the investigation of their hybridization properties. In this regard, the development of reproducible methods for estimating and controlling the EP of PNA monomers and oligomers is of utmost importance. In the chemistry of chiral PNA, both direct EP estimation methods and the indirect methods which require preliminary monomer/oligomer derivatization are used. These methods are reviewed in the following subsections.

Enantiomeric Purity Tests for Chiral PNA Monomers

The development of PNA EP control methods began with testing α-derivatives because they were the first chiral PNA monomers that became available [41]. Dueholm et al. reported a method based on diastereomer synthesis for alanine-derived α-PNA monomers. L-Ala-based (**1**) and D-Ala-based (**2**) monomers were reacted with L-alanine ^{t}Bu ester in the presence of an activator (TBTU) to give diastereomers **126** and **127** (Scheme 8.13). Their separation by reverse-phase high-performance liquid chromatography (HPLC) showed that optical purity of diastereomer **127** was 94%, while in the case of diastereomer **126** (the L-Ala derivative) no impurity was detected. It should be noted that the reaction conditions influenced the optical purity of the diastereomers. The best results were obtained on the addition of DIEA to the reaction mixture, while the usage of DhbtOH or the absence of a base catalyst led to substantial racemization. This experimental finding was later employed in the solid-phase synthesis of oligomers with Ala-based monomers.

Nielsen and coworkers [42] employed a similar approach to estimate EP of monomers based on L-Glu (**25**), L-Lys (**6**), and L-Ser (**8**). For this, diastereomers



SCHEME 8.13 Synthesis of C-terminal diastereomers composed of L-alanine *tert*-butyl ester and α-PNA monomers.

SCHEME 8.14 Synthesis of C-terminal diastereomers composed of L- or (L, D)-Ile and α-PNA monomers based on L-Glu (25), L-Lys (6) or L-Ser (8).

128–130a, b were obtained by reacting the target monomers with L-Ile and (L, D)-Ile (Scheme 8.14). Separation of the diastereomer mixture derived from racemic isoleucine, and HPLC analysis of diastereomers **128–130a, b** under optimized conditions, demonstrated that the optical purity of the diastereomers was >90%.

For γ-monomers of guanidinium peptide nucleic acids (GPNAs), Ly and coworkers [30] employed the indirect EP control method based on ^{19}F nuclear magnetic resonance (NMR) spectroscopy. N-terminal diastereomers were obtained via condensation of the monomers with a Mosher reagent α-methoxy-α-(trifluoromethyl)phenylacetyl chloride **131** (Scheme 8.15). Analysis of ^{19}F-NMR spectral data revealed that EP of the diastereomers **132** was >99%. An analogous strategy was used for mini-polyethylene glycol (PEG)–PNA monomers **78**, and the EP test results were similar to those obtained for GPNAs [20]. The same method was also reported in Ref. [49].

In the past several years, a number of direct chromatographic methods for estimating EP of PNA monomers have been described. The direct approach

SCHEME 8.15 Synthesis of N-terminal diastereomers via gamma-monomer condensation with a Mosher reagent (131).

60 γ-*S* from L-Glu
134 γ-*R* from D-Glu
(A)

61a γ-*S* from L-Lys
135 γ-*R* from D-Lys
(B)

FIGURE 8.3 Fmoc monomers of γ-PNAs based on glutamic acid (A) and lysine (B), respectively (**60**, **61a**).

to testing EP involves synthesis of both enantiomers of a particular monomer (from L- and D-amino acids), selection of an optimal system for their separation on a column with a chiral sorbent, and EP estimation for the target monomer in a selected system. A Korean group headed by Lee employed the direct method for determining the EP of PNA monomers. Enantiomeric forms of Glu-based PNA monomers (**60** and **134**, Fig. 8.3A) were separated on Chiralpak IB stationary phase with immobilized polycarbohydrates. HPLC separation of the racemic mixture and subsequent chromatographic analysis of the target L-Glu-based Thy monomer revealed that the EP of the target monomer was >99.9% [59]. Similar results were obtained in the case of L-Lys-based γ-PNA monomers (**61a** and **135**, Fig. 8.3B) [60].

Thus, γ-PNA monomers demonstrate generally high EP in both direct and indirect tests, while in the case of α-PNA monomers, the EP determined by indirect methods is ~90%, which may be due to partial monomer racemization on derivatization.

Enantiomeric Purity Tests for Chiral PNA Oligomers

Corradini et al. [79] have shown that acid hydrolysis of PNAs followed by treatment of *N*-aminoethylamino acids with trifluoroacetic anhydride (TFAA), which gives volatile trifluoroacetylated piperazine-2-ones **136** (Scheme 8.16), can be used for estimating the EP of chiral PNAs. Chiral gas–liquid chromatography (GLC) separation of the resulting PNA fragments on a Chirasil-Val column revealed the presence of the undesired enantiomer (5.5%) [80].

Use of the above-mentioned method in the synthesis of PNA oligomers containing the D-Lys-based α-“chiral box” enabled the development of a

SCHEME 8.16 Synthesis of chiral trifluoroacetylated piperasin-2-ones.

submonomer synthetic approach, which ensures acceptable stereochemical purity of α-modified PNA oligomers.

To summarize, there are several approaches to control the EP of chiral PNA monomers and related oligomers. The direct EP estimation method is the most accurate. In the indirect methods, N-terminal derivatization should be used for α-monomers to avoid racemization, while in the case of γ-monomers both N-terminal derivatization and C-terminal derivatization are applicable.

SYNTHESIS OF CHIRAL PNA OLIGOMERS

The majority of methods for chiral PNA oligomer synthesis proposed thus far are based on the solid-phase strategy [81] and the usage of modern condensing reagents. However, universal methods and standard conditions applicable to various types of PNAs are yet to be developed. The most popular PG strategies are Boc/Cbz [38] and Fmoc/Bhoc [40] ones (Fig. 8.2).

Solid-phase synthesis of chiral PNAs usually includes the following steps: loading of polymer support, condensation with the first monomer, capping of the unreacted amino groups, deblocking of the N-terminal amino group, condensation with the second monomer (the latter two steps are repeated until the desired PNA length is achieved), and oligomer cleavage from the polymer support. General protocols of oligomer synthesis are rather similar for different types of chiral PNAs. A comparison of the two basic PG strategies is presented in Table 8.6.

To date, a number of Boc protocols for the synthesis of various oligomers [20,30], both achiral with point chiral monomer inclusions and fully chiral, with a full set of canonical nucleic bases have been reported. In Fmoc-protocol studies [34,57,58], introduction of only Thy chiral monomers into PNAs has

TABLE 8.6 Comparison of Boc and Fmoc Strategies for Chiral PNA Synthesis

	Polymer support	Conditions of oligomer cleavage from the polymer support	References
Boc strategy	MBHA	HF; “low/high” TfOH method	[42]
		m-Cresol/thioanisole/TfOH/TFA	[20,30]
		TfOH/TFA/TES	[82]
		TfOH/TIS/TFA	[34]
Fmoc strategy	TentaGel (rink amide linker)	TFA/TIS/water	[34]
	Fmoc-PAL-PEG-PS	TFA/*m*-cresol	[57]
	NovaSyn TGR R	TFA/TIS/water	[58]

Fmoc-PAL-PEG-PS, [5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy) valeric acid]–polyethylene glycol–polystyrene resin; MBHA, (4-methylbenzhydrylamine) resin.

SCHEME 8.17 Synthesis of PNA oligomers by submonomer approach.

been described. A fivefold excess of the monomer was used in the respective condensation step in the case of aeg-PNAs. However, the results of recent studies on chiral PNA synthesis suggest that a threefold monomer excess is adequate for efficient condensation [37,83], which is of significant importance considering the high cost and labor requirement of chiral PNA monomer preparation.

An alternative, submonomer approach [37,65] has been proposed for obtaining Lys-based chiral α-PNAs. This approach includes preliminary synthesis of monomer precursors without heterocyclic bases (Scheme 8.17) and was first developed for aeg-PNAs [84]. The submonomer approach ensures high optical purity of target oligomers, which is crucial and particularly beneficial in the case of α-PNAs, considering their substantial racemization during standard solid-phase synthesis [64]. Within the scope of the submonomer approach, both Boc [65] and Fmoc protocols [66] have been described. The submonomer approach is currently also used for obtaining PNAs containing (α, γ)-disubstituted monomers [67,85,86].

The conditions for PNA removal from the polymer support (cleavage) significantly affect the overall efficiency of PNA synthesis, and optimization of those conditions may be a rather challenging tusk. We have studied the effects of various cleavage systems on the yield of L-Glu-based chiral α-PNAs, and the best results were obtained using trifluoromethanesulfonic acid (TfOH)/TFA/triethylsilane (TES) [82], while the conventional method for oligomer cleavage (the "low/high" method) led to complete degradation of target oligomers [42]. Analogously, use of the triisopropylsilane (TIS)-based system improved the oligomer synthesis efficiency in the case of PNAs with L-Glu-based γ-monomer inclusions [83]. In this regard, synthesis of chiral PNAs bearing side chain functional groups should be designed taking into account the positions of the side radicals in the PNA backbone and the possibility of PNA degradation at the final step of the solid-phase synthesis.

PROPERTIES OF CHIRAL PNAs

The prospects of new PNA modifications and their application primarily depend on their hybridization properties. The affinity of PNA for complementary NA fragments is usually studied by UV [circular dichroism (CD)] melting

experiments (T_m, melting temperature of a duplex or a triplex). Another important factor is selectivity, and the quantitative parameter characterizing the sensitivity toward mismatches in a complementary strand is ΔT_m (difference between the T_m values of a perfectly matched duplex and a mismatched one).

Structural analysis methods, for example, CD spectroscopy, are often employed to elucidate the geometry of ssPNAs and their complexes with ON complements.

Aeg-PNAs have demonstrated extremely high (perhaps even too high) hybridization affinity for NAs and remarkable selectivity ($\Delta T_m = 15°C$). Aeg-PNAs per se are not preorganized into helixes, but they easily form helixes in complexes with DNA or RNA. The formation of parallel duplexes with aeg-PNAs has been reported, but those duplexes are substantially less thermostable (by 10–15°C) than the antiparallel ones. As we have already mentioned, aeg-PNAs are poorly soluble compared with polyanionic ONs and their analogues due to the absence of a negative charge. Aeg-PNAs are prone to aggregation, and the stability of their complexes with NAs exhibits no dependence on the solution ionic strength. One more noteworthy property is the ability of homopyrimidine aeg-PNAs to invade DNA:DNA duplexes and displace one of the DNA strands, which results in the formation of PNA:DNA duplexes. Double-stranded aeg-PNAs can also invade DNA:DNA duplexes and displace one of the strands, which results in PNA:PNA:DNA triplexes [13].

To date, many PNA oligomers containing chiral acyclic monomer residues have been described. Oligomers with all-chiral monomers throughout the chain are rare. Only a few chiral residues are usually introduced into the oligomer chain. The few studies of fully modified chiral PNAs are reported in Refs. [25,36,37].

Although various PNA sequences have been used in hybridization studies, the decamer sequence proposed by Nielsen and coworkers [13] [R^1-GTAGATCACT-R^2 (**149**), where R^1=H or L-Lys, and R^2=NH_2 or L-Lys] is particularly popular. For such decamers, a significant number of PNA modifications have been described, CD spectra have been obtained, and T_m (ΔT_m) values have been determined. The N-terminal and C-terminal substituents (R^1 and R^2) can be different and have no effect on PNA affinity and selectivity. In many PNA decamers, only one or a few more chiral monomers have been incorporated, but even in those cases the effects of monomer inclusions could be evaluated by comparing the properties of the resulting PNAs with those of the initial aeg-PNA. Decamers containing exclusively chiral monomers and even chiral monomers with modified heterocyclic bases have also been described.

Systematic studies of the impact of the configuration (*R*- or *S*-) of α-chiral monomers on PNA properties have been reported by Nielsen and coworkers, Haaima et al., and Sforza et al. [6,42,43]. The researchers showed that a particular spatial organization of the monomers is required for efficient PNA binding with complementary ONs. CD data suggest that the PNAs R^1-GT$_x$AGAT$_x$CACT$_x$-R^2 derived from D-amino acids adopt the right-handed helix geometry, whereas

L-amino acid derivatives adopt the left-handed helix geometry. Initially, chiral α-PNAs derived from D-Ala, D-Asp, and D-Lys exhibited the best affinities for DNA. PNAs bearing nonpolar groups in the amino acid side residues (Val, Trp, and Phe derivatives) were inferior to Ala, Ser, Lys, and Arg derivatives in terms of affinity. Thus, steric hindrance may cause some destabilization of α-PNA:DNA duplexes. Later, it was shown that the presence of a chiral box composed of three monomers in the α-*R*-configuration in the middle of the oligomer increases the structural rigidity, which is favorable for α-PNA:DNA thermostability in terms of entropy; however, in general, the duplex was less stable than its aeg-PNA:DNA analogue due to steric hindrance. Negatively charged PNAs generally formed less stable duplexes with DNA than did positively charged PNAs. The authors attributed this fact to strong interactions (repulsion) between the carboxyl groups in PNA side residues and the negatively charged phosphate residues in DNA [6,42,43]. However, selectivity studies revealed that the sensitivity of the R^1-$GT_xAGAT_xCACT_x$-R^2 oligomer containing D-Glu-based Thy monomers to mismatches in a DNA complement was similar to the sensitivity of the D-Lys-based oligomer ($\Delta T_m = -20$°C for the D-Glu derivative and −19°C for the D-Lys derivative). The authors concluded that affinity of α-PNAs toward DNA complements is determined by several factors: electrostatic interactions, steric hindrance, and the configuration of the initial α-monomers.

The studies of PNAs containing D- and L-Lys-based monomers were continued by Corradini and coworkers [85,86], who employed synthetic approaches that guarantee the maintenance of high optical purity [65]. It was shown that the presence of a "chiral box" (three chiral monomers in succession) in R^1-$GTAGA_xT_xC_xACT$-R^2 PNA ensures exceptional specificity for the antiparallel DNA orientation and sensitivity to mismatches. The crystal structure of the α-PNA:DNA duplex in which three successive D-Lys-based monomers (the chiral box) were positioned in the middle of the PNA chain was investigated by X-ray diffraction (XRD) measurements [87]. It was shown that the D-configuration allows the lysine side chain to occupy the optimal position for a right-handed helix, whereas the L-configuration would result in substantial intrachain steric hindrance.

In the early 2000s, Ly and coworkers reported the development of L- and D-Arg-based chiral α-PNAs with improved bioavailability [44,45,88]. The inclusion of propyl-guanidine residues into PNAs significantly facilitated their intracellular transport. The hybridization properties of D-Arg-based α-PNAs were comparable to those of aeg-PNAs, whereas L-Arg-based α-PNAs were unable to form stable duplexes with ONs. [Data for H-GCATGTTTGA-R^1 (R_1 = H or L-Lys) are summarized in Table 8.7.]

The selectivity of GPNA8 **145** (H-G^D**C**A^D**T**G^D**T**T^D**T**G^DA-NH_2) binding to NA was also assessed and was comparable to that of the initial aeg-PNA (ΔT_m ranging from −11 to −18°C) H-GCATGTTTGA-^{L}Lys-NH_2 **137**.

The studies of L-Ser- and L-Ala-based γ-*S*-PNAs reported in Ref. [55] are of major interest. CD data suggest that γ-*S*-configuration of the monomer

TABLE 8.7 Hybridization Properties of α-PNAs Based on L- or D-Arg

No.	Structure	Code[a]	Sequence	T_m (°C)	
				DNA [45]	RNA [88]
137		PNA1	H-GCATGTTTGA-^{L}Lys-NH$_2$	43	53
138		GPNA1	H-GCATG^{L}**T**TTGA-NH$_2$	39	45
139		GPNA2	H-GCATG^{L}**T**^{L}**T**TGA-NH$_2$	37	38
140		GPNA3	H-GCATG^{L}**T**^{L}**T**^{L}**T**GA-NH$_2$	35	31
141		GPNA4	H-GCATG^{D}**T**TTGA-NH$_2$	41	52
142		GPNA5	H-GCATG^{D}**T**^{D}**T**TGA-NH$_2$	39	49
143		GPNA6	H-GCATG^{D}**T**^{D}**T**^{D}**T**GA-NH$_2$	38	45
144		GPNA7	H-GCA^{D}**T**G^{D}**T**T^{D}**T**GA-NH$_2$	47	51
145		GPNA8	H-G^{D}**C**A^{D}**T**G^{D}**T**T^{D}**T**G^{D}A-NH$_2$	50	53 (30 parallel)

[a] *The codes of the oligomers were taken from the original papers [45,88].*

FIGURE 8.4 Chemical structure of model dimer 146.

induces oligomer preorganization into a right-handed helix. The right-handed geometry was additionally confirmed by 2D NMR experiments on model dimer **146** (Fig. 8.4).

The hybridization characteristics obtained for γ-*S*-PNA oligomers confirmed high affinity and selectivity toward complementary ONs (Table 8.8).

As evident from Table 8.8, the inclusion of greater numbers of chiral monomers in the PNA oligomer leads to a higher T_m of the γ-PNA:DNA (RNA) duplex. Each monomer unit increases the T_m on average by ~2°C in the case of PNA:RNA duplexes and by ~3°C in the case of the PNA:DNA duplexes; these changes indicate the high affinity of γ-PNAs toward DNA. High selectivity (superior to that of aeg-PNAs) was demonstrated for L-Ser-based γ-*S*-PNAs with respect to both DNA (ΔT_m from −16 to −19°C) and RNA (ΔT_m from −12 to −18°C). Importantly, the hybridization properties of oligomer P3 (alternating chiral and achiral monomers in the middle of the chain) and oligomer P4 (three chiral monomers in succession) turned out to be very similar.

In a more recent work, homo-L-Arg-based γ-*S*-PNAs of the same sequence (H-GCATGTTTGA-R^1) were studied [30]. The obtained T_m (Table 8.9) and ΔT_m values were also high.

Analogously to the data presented in Table 8.8, the T_m of the duplex increased with the addition of each chiral monomer unit in a PNA oligomer structure.

TABLE 8.8 Hybridization Properties of L-Ser-Based γ-*S*-PNAs [55]

No.	Structure	Code[a]	Sequence	T_m (°C) DNA	T_m (°C) RNA
137		P1[a]	H-GCATGTTTGA-^{L}Lys-NH$_2$	44	54
147		P2	H-GCATG$^{\gamma}$**T**TTGA-^{L}Lys-NH$_2$	48	57
148		P3	H-GCA$^{\gamma}$**T**G$^{\gamma}$**T**T$^{\gamma}$**T**GA-^{L}Lys-NH$_2$	53	60
149		P4	H-GCATG$^{\gamma}$**T**$^{\gamma}$**T**$^{\gamma}$**T**GA-^{L}Lys-NH$_2$	53	59
150		P5	H-$^{\gamma}$**G**$^{\gamma}$**C**$^{\gamma}$**A**$^{\gamma}$**T**$^{\gamma}$**G**$^{\gamma}$**T**$^{\gamma}$**T**$^{\gamma}$**T**$^{\gamma}$**G**$^{\gamma}$**A**-^{L}Lys-NH$_2$	63	64

[a]*The codes of the oligomers were taken from the original paper [55].*

TABLE 8.9 Hybridization Properties of Homo-L-Arg-Based γ-*S*-PNAs [30]

No.	Structure	Code[a]	Sequence	DNA, T_m (°C)
137		PNA1	H-GCATGTTTGA-^{L}Lys-NH_2	43
151		$^{\gamma}$GPNA1	H-GCATG**T**TTGA-NH_2	45
152		$^{\gamma}$GPNA2	H-GCATG**TT**TGA-NH_2	47
153		$^{\gamma}$GPNA3	H-GCATG**TTT**GA-NH_2	49
154		$^{\gamma}$GPNA4	H-GCA**T**G**T**T**T**GA-NH_2	51
155		$^{\gamma}$GPNA5	H-G**C**A**T**G**T**T**T**G**A**-NH_2	59

[a]*The codes of the oligomers were taken from the original paper [30].*

Interestingly, the T_m of the duplex formed by DNA and $^{\gamma}$GPNA4 oligomer **154** (contains alternating chiral and achiral monomers in the middle of the chain) was higher than that of the duplex formed by DNA and $^{\gamma}$GPNA3 oligomer **153** (contains three chiral monomers in succession). For $^{\gamma}$GPNA5 oligomer **155**, preliminary studies of the selectivity toward RNA and DNA complements were conducted, and the results suggest that the selectivity is close to that of aeg-PNAs (ΔT_m from −11 to −18°C) and D-Arg-based α-PNAs. In the same work, efficient penetration of homo-L-Arg-based γ-*S*-PNAs through membranes was demonstrated using HeLa cell lines.

To investigate the role of steric factors in γ-*S*-PNA hybridization with NAs, oligomers containing L-Ala-, L-Ile-, L-Phe-, and L-Val-based monomers were synthesized and analyzed (Table 8.10).

The substitution of a single aeg-Thy monomer for the γ-*S*-monomer in the middle of a decamer had no effect on the T_m of the PNA:DNA duplex. Only introduction of three chiral monomers led to noticeable T_m changes (PNA6 vs. PNA8 and PNA7 vs. PNA 9). L-Val derivatives resulted in somewhat lower T_m values than those of L-Phe derivatives, which can be explained by steric hindrance similarly to the case of α-PNAs. Apart from the remarkable hybridization affinity, oligomers described in Ref. [28] exhibited high hybridization selectivity.

γ-*S*-Oligomers also turned out to be capable of intruding into DNA:DNA duplexes and displacing one DNA strand to yield PNA:DNA duplexes. Such heteroduplexes were stabilized by acridine residues [89]. Acridine was included because prior studies showed that it causes significant stabilization of DNA duplexes on intercalation due to efficient stacking with heterocyclic bases [90–92].

TABLE 8.10 Hybridization Properties of γ-*S*-PNAs Based on L-Ala, L-Ile, L-Phe, and L-Val [28]

No.	Structure	Code[a]	Sequence	DNA, T_m (°C)
156		PNA2 (Ala)	H-GCATG**T**TTGA-^{L}Lys-NH_2	51
157		PNA4 (Ile)	H-GCATG**T**TTGA-^{L}Lys-NH_2	51
158		PNA5 (Phe)	H-GCATG**T**TTGA-^{L}Lys-NH_2	51
159		PNA6 (Phe 3alt)	H-GCA**T**G**T**T**T**GA-^{L}Lys-NH_2	58
160		PNA7 (Phe 3con)	H-GCATG**TTT**GA-^{L}Lys-NH_2	60
161		PNA3 (Val)	H-GCATG**T**TTGA-^{L}Lys-NH_2	51
162		PNA8 (Val 3alt)	H-GCA**T**G**T**T**T**GA-^{L}Lys-NH_2	55
163		PNA9 (Val 3con)	H-GCATG**TTT**GA-^{L}Lys-NH_2	55

[a]*The codes of the oligomers were taken from the original paper [28].*

Alternatively, stabilization can be achieved by increasing the ssPNA length [29] or by incorporating a modified Cyt residue (Fig. 8.5), which can form five H-bonds with an opposing guanine (Gua), in the PNA chain [93,94].

Recent XRD studies of the γ-*S*-PNA:DNA duplex revealed that although γ-*S*-PNAs remain rather flexible, they easily adopt the beneficial P-helical conformation on hybridization with complementary DNA [95].

Another recent paper in this field was focused on mini-PEG–PNAs [20]. Unlike positively charged NA mimics, PNAs carrying PEG moieties in the γ-positions of the PNA backbone were not prone to nonspecific interaction with NAs, and high hybridization selectivity was retained (Tables 8.11 and 8.12).

Along with selective hybridization, γ-*S*-mini-PEG–PNAs demonstrated advantageous properties, such as good solubility in aqueous solutions, right helical preorganization, and ability to displace ssDNA from dsDNA (although the

FIGURE 8.5 H-bonding in (A) C–G and modified (B) C(X)–G base pairs.

TABLE 8.11 Hybridization Properties of γ-*S*-Mini-PEG–PNAs [20]

No.	Structure	Code[a]	Sequence	T_m (°C) DNA	T_m (°C) RNA
137		PNA1	H-GCATGTTTGA-NH_2	45	55
164		PNA2	H-GCATG**T**TTGA-NH_2	49	58
165		PNA3	H-GCA**T**G**T**T**T**GA-NH_2	54	62
166		PNA4	H-G**C**A**T**G**T**T**T**GA-NH_2	57	64
167		PNA5	H-**GCATGTTTGA**-NH_2	68	68

[a]*The codes of oligomers were taken from the original paper [20].*

TABLE 8.12 Mismatch Discrimination for γ-*S*-Mini-PEG–PNAs (**167**) [20]

X-T	T_m, °C (ΔT_m, °C) PNA1 (**137**):DNA[a]	PNA5 (**167**):DNA	PNA1 (**147**):RNA[b]	PNA5 (**167**):RNA
A-T	45	68	55	68
C<>T	31 (−14)[c]	47 (−21)	37 (−18)	48 (−20)
G<>T	31 (−14)	48 (−20)	44 (−11)	52 (−16)
T(U)<>T	35 (−10)	51 (−17)	40 (−15)	48 (−20)

[a]*PNA1: H-GCATGTTTGA-^{L}Lys-NH_2; PNA5: H-GCATGTTTGA-^{L}Lys-NH_2; DNA: 3′-CGTACAXACT-5′, X=A, C, G, T; RNA: 3′-CGUACAXACU-5′, X=A, C, G, U.*
[b]*Data for the PNA1–DNA and PNA1–RNA mismatched duplexes were taken from Ref. [55].*
[c]*T_m difference between the perfect and mismatched duplexes (ΔT_m) is given in parentheses.*

FIGURE 8.6 Structures of the achiral and chiral (types I, II, and III) PNAs. *R*, amino acid side chain; *B*, nucleobase.

latter occurred only in low-ionic-strength solutions, which limits the application of γ-*S*-mini-PEG–PNAs under physiological conditions) [23].

Several studies performed in parallel to the works of Ly et al. also confirmed the advantages of the γ-*S*-configuration of acyclic monomers. Tedeschi et al. [85] synthesized a series of R^1-GTAGATCACT-R^2 oligomers (Fig. 8.6; Table 8.13). As evident from the data in Table 8.13, the oligomer containing a single L-Lys-based γ-*S*-monomer formed a more stable duplex (T_m=56°C) than that of the oligomer containing a chiral box, that is, three adjacent monomers with α-*R*-configuration (T_m=43°C), whereas the oligomer with a consistent α-*R*-γ-*S*-configuration formed the most stable duplex.

TABLE 8.13 Melting Temperatures of Antiparallel PNA:DNA duplexes [85]

No.	PNA type	PNA[a]	T_m, PNA:DNA (°C)[b]
168	I	H-GTAGAT$_{(\alpha\text{-}S\text{-ab})}$CACT-$NH_2$	47
169	I	H-GTAGAT$_{(\alpha\text{-}R\text{-ab})}$CACT-$NH_2$	52
170	II	H-GTAGAT$_{(\alpha\text{-}R\text{-ab},\gamma\text{-}R\text{-ab})}$CACT-$NH_2$	52
171	II	H-GTAGAT$_{(\alpha\text{-}S\text{-ab},\gamma\text{-}S\text{-ab})}$CACT-$NH_2$	31
172	II	H-GTAGAT$_{(\alpha\text{-}R\text{-ab},\gamma\text{-}S\text{-ab})}$CACT-$NH_2$	57
173	II	H-GTAGAT$_{(\alpha\text{-}S\text{-ab},\gamma\text{-}R\text{-ab})}$CACT-$NH_2$	<15
174	III	H-GTAGAT$_{(\gamma\text{-}S\text{-ab})}$CACT-$NH_2$	56
175	III	H-GTAGAT$_{(\gamma\text{-}R\text{-ab})}$CACT-$NH_2$	32
176	Achiral	H-GTAGATCACT-NH_2	50[c]
177	"L-Chiral box"	H-GTAGA$_{(\alpha\text{-}S\text{-ab})}T_{(\alpha\text{-}S\text{-ab})}C_{(\alpha\text{-}S\text{-ab})}$ACT-$NH_2$	30[d]
178	"D-Chiral box"	H-GTAGA$_{(\alpha\text{-}R\text{-ab})}T_{(\alpha\text{-}R\text{-ab})}C_{(\alpha\text{-}R\text{-ab})}$ACT-$NH_2$	43[e]

[a]*ab, aminobutyl, side chain of Lys residue.*
[b]*T_m values were determined as the minimum of the first derivative of the CD melting curves at 260nm [80].*
[c]*Data for the PNA1–DNA duplexes were taken from Refs. [85,86].*
[d]*Data for the PNA1–DNA duplexes were taken from Ref. [96].*
[e]*Data for the PNA1–DNA duplexes were taken from Ref. [43].*

FIGURE 8.7 Chemical structure of oligomer 179.

Later, the same group reported oligomer **179**, which contained the amino acid residues of the Pro–Lys–Lys–Lys–Arg–Lys–Val peptide [nuclear localization signal (NLS)] in the side chains (Fig. 8.7) [97].

The respective monomers were obtained from L-Lys, L-Val, and L-Arg. Receptor-dependent transport of oligomer **179** through membranes, similar to NLS transport, was demonstrated.

Another study in this field was devoted to the synthesis of two R^1-GTAGATCACT-R^2 oligomers (**180**, **181**) based on L- and D-Arg (Fig. 8.8) [67].

The first oligomer (**180**) contained a monomer with a consistent -[γ-*S*-α-*R*-]-configuration of the chiral centers. In the other oligomer (**181**), the chiral centers adjacent to the disubstituted monomer were also stereochemically consistent (α-*R*-[γ-*S*-α-*R*-]-γ-*S*-); such a construct was termed "extended chiral box." The hybridization properties of the oligomers are summarized in Table 8.14. Both oligomers exhibited high affinity for DNA and RNA. The oligomer with the extended chiral box demonstrated high sensitivity to mismatches in RNA (ΔT_m was 37°C). Thus, these oligomers may be promising molecular tools for single-nucleotide polymorphism (SNP) detection.

Comparative studies of molecular recognition and the analysis of the respective stereochemical requirements were reported by Mitra and Ganesh [49] and Sugiyama and Kittaka [36]. The former group described Am oligomers containing Thy monomers with α-*R*-, α-*S*-, and γ-*S*-configurations (Fig. 8.9). An investigation of

FIGURE 8.8 Chemical structure of oligomers 180 (one α-*R*-γ-*S*-Arg monomer) and 181 (adjacent α-*R*-Arg, α-*R*-γ-*S*-Arg, and γ-*S*-Arg monomers).

TABLE 8.14 Melting Temperatures of the PNA:DNA and PNA:RNA Antiparallel Fully Matched/Mismatched Duplexes [67]

PNA[a]	T_m (°C)		ΔT_m (°C)	T_m (°C)		ΔT_m (°C)
	PNA/DNA full match	PNA/DNA mismatch		PNA/RNA full match	PNA/RNA mismatch	
H-GTAGAT$_{(\alpha\text{-}R\text{-ab},\gamma\text{-}S\text{-ab})}$CACT-$NH_2$ **172**	57[b]	35[c]	22	—	—	—
H-GTAGAT$_{(\alpha\text{-}R\text{-gp},\gamma\text{-}S\text{-gp})}$CACT-$NH_2$ **180**	59[b]	36[c]	23	60[d]	36[e]	24
H-GTAGA$_{(\alpha\text{-}R\text{-gp})}T_{(\alpha R\text{-gp},\gamma\text{-}S\text{-gp})}C_{(\gamma\text{-}S\text{-gp})}$ACT-$NH_2$ **181**	58[b]	35[c]	23	60[d]	23[e]	37

Conditions: phosphate buffer (pH 7); 5 μm concentration of each strand.
[a]*gp, guanidinopropyl (side chain of Arg residue).*
[b]*Fully matched ON: 5′-AGTGATCTAC-3′.*
[c]*Mismatched ON: 5′-AGTGCTCTAC-3′.*
[d]*Fully matched ON: 5′-AGUGAUCUAC-3′.*
[e]*Mismatched ON: 5′-AGUGCUCUAC-3′.*

FIGURE 8.9 Structures of aminomethyl PNAs [46].

the properties of Am PNA revealed that the efficiency of complementary binding decreases in the following order: γ-*S*- > α-*R*- ≫ 2(α)-*S*-derivatives.

Sugiyama and Kittaka [36] investigated how the configuration of a chiral center in the β-position (Fig. 8.10) of the pseudopeptide backbone affected the binding between PNA and NAs, and the researchers demonstrated unmistakable stereochemical selectivity. The hybridization affinities of the oligomers containing Thy monomers with the β-*S*-Me configuration were close to those of aeg-PNAs, whereas isomeric oligomers, containing β-*R*-Me monomers, were incapable of forming stable duplexes with ONs.

The introduction of a negative charge into the γ-position of the backbone via sulfate groups (Fig. 8.11A) [57] or carboxyl groups (Fig. 8.11B) [58] was reported to facilitate PNA binding with ONs, presumably due to the preorganization of such charged PNA oligomers.

Oligopyrimidine methylsulfate PNAs formed stable $(PNA)_2DNA$ triplexes with complementary DNAs. Those modified PNAs also exhibited antigene activity against the *ErbB2* gene [57].

Heemstra and coworkers [58] performed comparative studies of a series of R^1-$GT_xAGAT_xCACT_x$-R^2 oligomers containing Thy γ-*S*-monomers based on

FIGURE 8.10 Structures of β-methyl-PNAs [70].

FIGURE 8.11 Fragments of methylsulfate PNAs (A) and carboxymethyl PNAs (B).

L-Asp and L-Lys. Increasing the solution ionic strength was shown to stabilize duplexes with negatively charged PNA oligomers and destabilize duplexes with the positively charged oligomers. Nielsen's buffer [100 mM NaCl, 10 mM phosphate, and 0.1 mM ethylenediaminetetraacetic acid (EDTA); pH 7.0] was used in most of the hybridization experiments discussed thus far, and a similar buffer (10 mM sodium phosphate, 100 mM NaCl, and 0.1 mM EDTA; pH 7.4) was used in the works of Ly et al. Comparative hybridization studies of γ-*S*-positively charged and γ-*S*-negatively charged oligomers were performed in a different buffer: 3 mM PNA, 3 mM DNA, and 10 mM sodium phosphate (pH 7.2) with varying concentrations of NaCl (0 M, 50 mM, 100 mM, 250 mM, 500 mM, and 1 M).

The RNA affinity of γ-carboxymethyl PNAs was greater than the affinities of aeg-PNAs and positively charged PNAs under physiological pH conditions [54]. Analysis of their sensitivity to mismatches in DNA, which was performed on γ-Me oligomers with three negatively charged L-Asp-based monomer residues, revealed that these oligomers are inferior to aeg-PNA analogues and L-Lys-based positively charged PNAs. However, their sensitivity to mismatches in RNA was significantly higher [98] than the sensitivities of aeg-PNAs and positively charged PNAs. Taken together, the results reported in Refs. [57,58] argue against a key role of PNA charge in the efficient binding to NAs.

A number of papers reported the formation of parallel chiral PNA:DNA duplexes. Similarly to the case of aeg-PNAs, these duplexes were substantially less stable than the antiparallel ones.

To summarize, the configuration of chiral centers and their positions in PNA oligomers, rather than the charge and volume of the side residues, appear to play the major role in the accurate and selective recognition of ON targets. The preorganization of ssPNAs is determined by the positions of the chiral centers in the pseudopeptide fragment, the number of chiral monomers, and their arrangement. The following chiral PNA constructs have been described to date:

- oligomer composed of alternating achiral monomers and chiral monomers with the γ-*S*- and/or α-*R*-configuration [20,30];
- oligomer composed of alternating pairs of achiral monomers and chiral monomers with the γ-*S*- and/or α-*R*-configuration [43,57,58];
- oligomer containing a chiral box (three or more monomers with the γ-*S*- and/or α-*R*-configurations) in the middle of the chain [30,42,43,45,55,85,86,88];
- oligomer containing an extended chiral box, that is, three adjacent monomers with consistent stereochemistry (-α-*R*-[α-*R*-γ-*S*]-5-γ-*S*-), in the middle of the chain [67];
- oligomer containing an expanded chiral box, composed of alternating three-monomer boxes with the γ-*S*-configuration and aeg monomers [20,28,30,45,88];
- oligomer composed of chiral monomers exclusively [20,29,44,45,55,89,93,94].

As follows from the data in the literature, three to five chiral monomers in a decamer with "correct" stereochemistry are sufficient to ensure the desired hybridization properties. However, alternating chiral monomers with aeg monomers reduces the rigidity of PNA, which may also turn out to be beneficial.

In general, a more flexible molecule (aeg-PNA with a "compliant" backbone) adopts the geometry of the more rigid molecule (DNA or RNA, whose rigidity is caused by chiral centers). The flexible strand winds around the rigid one and adjusts its spatial parameters (helix pitch) to fit the complementary strand (the "rigid spring"/"soft wire" model). Once chiral monomers are incorporated into the PNA strand, the rigidity increases; in the case of incorrect stereochemistry, which causes geometrical incompatibility (poor fitting), complementary binding may be disrupted and selectivity may be lost. Affinity and selectivity might also be lost if the PNA strand is preorganized per se, and conformational adjustments are impossible.

APPLICATIONS OF CHIRAL PNAs

Similar to aeg-PNA, chiral PNAs are used in microarrays for SNP detection, and they are more promising than aeg analogues due to a higher hybridization affinity for NAs and due to their improved mismatch discrimination.

Tedeschi et al. reported the application of PNAs with a chiral box composed of three α-(D-Arg) monomers [99]. A PNA microarray platform containing seven such oligomers was developed for genotyping of seven tomato sorts, differing in fruit shapes. It was shown that five of the seven sorts can be identified using the chiral-PNA microarray platform. The efficiency of identification did not depend on the distance between the fluorescent label and the PNA, while in the case of DNA-based arrays, that distance was an important factor. A similar technology has proven to be efficient for detecting uncommon additives in olive oil [100].

PNAs containing Arg residues have been reported to penetrate easily through cellular membranes [25] and regulate NA metabolism in various cell lines, and thus show great promise potential as antigene and antisense agents [101,102].

Armitage and coworkers used miniprobes composed of 6 or 12 γ-mini-PEG–PNA monomers for the analysis of telomeres [103]. Fluorescence in situ hybridization (FISH) was performed with U2OS osteosarcoma and Jurkat T-lymphocyte cell lines. The results suggest that the probe containing six PNA residues formed a stable duplex with DNA in the U2OS cell line, while in Jurkat T-lymphocytes, the chiral PNA 12-mer appeared more efficient. The miniprobes stained chromosomal DNA at the mitosis stage of the cell cycle. This example illustrates applicability of chiral PNAs in diagnostics.

The high sensitivity of chiral γ-mini-PEG–PNAs to mismatches in ON complements have been used for genotyping closely related human immunodeficiency virus (HIV) subtypes. The usage of a solid-phase nanopore platform in combination with γ-PNAs allowed discrimination between the two HIV subtypes [104].

Other recently reported PNA applications include PNA-based mimics of cyclic peptide antibiotics, in which H-bonds between nucleic base pairs substitute for the disulfide bridge between cysteine residues. Ly and coworkers reported the synthesis of a γ-PNA/peptide hybrid. Cyclization of the hybrid gave a mimic of the peptide antibiotic rhesus theta defensin-1M (RTD-1M), which exhibited biological activity comparable with that of RTD-1M [105].

Another undoubtedly promising application is related to structural (conformational) studies of NAs. Chiral PNAs can be used for investigating noncanonical NA architectures, such as G-quadruplexes [106–108]. For instance, G-rich γ-*S*-PNAs were reported to form hybrid quadruplexes with homologous RNA and DNA, and the impact of the chiral modification on the equilibrium between PNA:DNA or PNA:RNA heteroduplexes and heteroquadruplexes was assessed [109].

Thus, chirality does not only improve PNA affinity toward NA (which is the key factor in the development of genotyping probes) but also open up opportunities for some new technological applications, which were not possible in the case of aeg-PNA. High-level intracellular uptake of Arg-based PNAs suggests that such compounds can be used in the development of new molecular tools for medicinal chemistry and in gene therapy. Although none of the therapeutics currently present at the pharmaceutical market contains chiral PNA as a reactant, it is likely that chiral PNA technologies will play a significant role in the treatment of various diseases in the near future.

CONCLUSIONS

Several strategies for synthesizing PNA monomers and oligomers have been developed. Although the Boc protocol is currently used most often, it is likely that studies on the Fmoc-protocol synthesis of complex chiral PNA oligomers bearing functional groups will be reported shortly. One important finding in the field of chiral PNAs is that a particular architecture and stereochemistry ensure efficient and selective hybridization with complementary NA fragments. Preorganization, which is required for efficient hybridization, may be achieved by introducing particular chiral monomers into the PNA oligomer structure. The monomers must contain chiral centers in the *S*-configuration in the γ-position of the pseudopeptide backbone. α-*R*-Monomers are less advantageous. However, a combination of γ-*S*- and α-*R*-substituents in a single monomer or introduction of extended chiral boxes (eg, a box of three monomers with consistent -α-*R*-[α-*R*-γ-*S*]-γ-*S*- stereochemistry) into PNA oligomers may be beneficial. Oligomers containing three or more boxes composed of alternating aeg monomers and chiral monomers with the γ-*S*-configuration of the pseudopeptide backbone have also demonstrated rather efficient hybridization. It should be taken into account that γ-*S*-derivatives can be obtained with high optical purity and are more stable than α-derivatives in terms of configuration (ie, less prone to racemization during the terminal stages of the synthesis and during solid-phase oligomerization). Thus, γ-derivatives appear to be generally superior to α-isomers with respect to constructing NA mimics with a predetermined structure.

In view of the above-mentioned regularities, it would be reasonable to develop general principles of PNA oligomer design. A specific arrangement of chiral and achiral monomers could yield PNAs with a desired hybridization affinity toward a particular ON target. Functional groups in chiral monomer side residues may impart additional favorable properties to PNAs, for example, improved solubility and cell permeability. Moreover, these groups allow ionic interactions with carrier molecules and can be used for the fluorescence labeling of PNAs, among other benefits.

In conclusion, further progress and more detailed studies of chiral PNAs are expected in the near future. Recent findings open up opportunities for new applications of such PNAs in molecular biology, biomedicine, or nanotechnology.

ABBREVIATIONS

Ab aminobutyl-
Ade adenine
Aeg *N*-(2-aminoethyl)glycine
Am aminomethyl-
Bhoc benzhydryloxycarbonyl-
Boc *tert*-butyloxycarbonyl-
Bzl benzyl-
Cbz benzyloxycarbonyl-
CD circular dichroism
Cyt cytosine
Dap 2,6-diaminopurine
DCC dicyclohexylcarbodiimide
DCM dichloromethane
Dde 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl
DEAD diethyl azodicarboxylate
DhbtOH 3-hydroxy-1,2,3-benzotriazine-4(3H)-one
DIAD diisopropyl azodicarboxylate
DIEA diisopropylethylamine
DMAP 4-dimethylaminopyridine
DMF dimethylformamide
EDC 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide
EDTA ethylenediaminetetraacetic acid
EP enantiomeric purity
Et ethyl-
FISH fluorescence in situ hybridization
Fmoc 9-fluorenylmethoxycarbonyl-
Fmoc-PAL-PEG-PS [5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy) valeric acid]–polyethylene glycol–polystyrene resin
FmocOSu 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide
GLC gas–liquid chromatography
Gp guanidinopropyl-
GPNA guanidinium peptide nucleic acid

Gua	guanine
HATU	*O*-(7-azabenzotriazol-yl)-1,1,3,3-tetramethyl uronium hexafluoro-phosphate
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
IBCF	isobutyl chloroformate
^{i}Bu	*iso*-butyl-
MBHA	(4-methylbenzhydrylamine) resin
MBzl	methoxy-benzyl-
Me	methyl-
Ms	methanesulfonyl-
NA	nucleic acid
2-NBS	*N*-(2-nitrobenzenesulfonyl)-
NLS	nuclear localization signal
NMM	*N*-methyl morpholine
NMR	nuclear magnetic resonance
Nr	not reported
ON	oligonucleotide
PEG	polyethylene glycol
PG	protecting group
PNAs	peptide nucleic acids (polyamide nucleic acids)
RTD-1M	rhesus theta defensin-1M
SNP	single-nucleotide polymorphism
TBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluorob-orate
^{t}Bu	*tert*-butyl-
TEA	trimethylamine
TES	triethylsilane
Tf	triflyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
Thy	thymine
TIS	triisopropylsilane
Tos	4-toluenesulfonyl
Ura	uracil
XRD	X-ray diffraction

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REFERENCES

[1] D.M. Barrett, N. Singh, D.L. Porter, S.A. Grupp, C.H. June, Annu. Rev. Med. 65 (2014) 333–347.

[2] N. Dias, C.A. Stein, Mol. Cancer Ther. 1 (2002) 347–355.

[3] K.L. Warfield, R.G. Panchal, M.J. Aman, S. Bavari, Curr. Opin. Mol. Ther. 8 (2006) 93–103.
[4] P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt, Science 254 (1991) 1497–1500.
[5] K.L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H.F. Hansen, T. Vulpius, K.H. Petersen, R.H. Berg, P.E. Nielsen, O. Buchardt, J. Org. Chem. 59 (1994) 5767–5773.
[6] A. Puschl, S. Sforza, G. Haaima, O. Dahl, P.E. Nielsen, Tetrahedron Lett. 39 (1998) 4707–4710.
[7] M. Egholm, P.E. Nielsen, O. Buchardt, R.H. Berg, J. Am. Chem. Soc. 114 (1992) 9677–9678.
[8] M. Egholm, O. Buchardt, P.E. Nielsen, R.H. Berg, J. Am. Chem. Soc. 114 (1992) 1895–1897.
[9] J.S. Kastrup, M. Pilgaard, F.S. Jørgensen, P.E. Nielsen, H. Rasmussen, FEBS Lett. 363 (1995) 115–117.
[10] D.Y. Cherny, B.P. Belotserkovskii, M.D. Frank-Kamenetskii, M. Egholm, O. Buchardt, R.H. Berg, P.E. Nielsen, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 1667–1670.
[11] P.E. Nielsen, L. Christensen, J. Am. Chem. Soc. 118 (1996) 2287–2288.
[12] J. Lohse, O. Dahl, P.E. Nielsen, PNAS 96 (1999) 11804–11808.
[13] P. Wittung, P.E. Nielsen, O. Buchardt, M. Egholm, B. Nordén, Nature 368 (1994) 561–563.
[14] M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S.M. Freier, D.A. Driver, R.H. Berg, S.K. Kim, B. Nordén, P.E. Nielsen, Nature 365 (1993) 566–568.
[15] R. Juliano, J. Bauman, H. Kang, X. Ming, Mol. Pharm. 6 (2009) 686–695.
[16] A. Ray, B. Nordén, FASEB J. 14 (2000) 1041–1060.
[17] P.E. Nielsen, Chem. Biodivers. 7 (2010) 786–804.
[18] C. Achim, B.A. Armitage, D.H. Ly, J.W. Schneider, Wiley Encyclopedia of Chemical Biology, John Wiley & Sons, (2008), pp. 1–10.
[19] I.Y. Krishnan-Ghosh, E. Stephens, S. Balasubramanian, J. Am. Chem. Soc. 126 (2004) 5944–5945.
[20] B. Sahu, I. Sacui, S. Rapireddy, K.J. Zanotti, R. Bahal, B.A. Armitage, D.H. Ly, J. Org. Chem. 76 (2011) 5614–5627.
[21] P. Wittung, J. Kajanus, K. Edwards, P.E. Nielsen, B. Nordén, B.G. Malmström, FEBS Lett. 365 (1995) 27–29.
[22] N. Bendifallah, F.W. Rasmussen, V. Zachar, P. Ebbesen, P.E. Nielsen, U. Koppelhus, Bioconjug. Chem. 17 (2006) 750–758.
[23] Y.M. Bae, M.H. Kim, G.S. Yu, B.H. Um, H.K. Park, H. Lee, K.T. Lee, Y.D. Suhe, J.S. Choi, J. Control. Release 175 (2014) 54–62.
[24] R. Bahal, B. Sahu, S. Rapireddy, C.-M. Lee, D.H. Ly, ChemBioChem 13 (2012) 56–60.
[25] R. Corradini, S. Sforza, T. Tedeschi, F. Totsingan, A. Manicardi, R. Marchelli, Curr. Top. Med. Chem. 11 (2011) 1535–1554.
[26] V.A. Kumar, Eur. J. Org. Chem. 2002 (2002) 2021–2032.
[27] P. Merino, R. Matute, Chemical Synthesis of Nucleoside Analogues, John Wiley & Sons (2013), p. 847–880.
[28] M.J. Crawford, S. Rapireddy, R. Bahal, I. Sacui, D.H. Ly, J. Nucleic Acids 2011 (2011) 1–10.
[29] G. He, S. Rapireddy, R. Bahal, B. Sahu, D.H. Ly, J. Am. Chem. Soc. 131 (2009) 12088–12090.
[30] B. Sahu, V. Chenna, K.L. Lathrop, S.M. Thomas, G. Zon, K.J. Livak, D.H. Ly, J. Org. Chem. 74 (2009) 1509–1516.
[31] T. Shiraishi, R. Hamzavi, P.E. Nielsen, Nucleic Acids Res. 36 (2008) 4424–4432.
[32] E.A. Englund, D.H. Appella, Org. Lett. 7 (2005) 3465–3467.
[33] E.A. Englund, D.H. Appella, Angew. Chem. Int. Ed. 46 (2007) 1414–1418.
[34] M.C. de Koning, L. Petersen, J.J. Weterings, M. Overhand, G.A. van der Marel, D.V. Filippov, Tetrahedron 62 (2006) 3248–3258.

[35] C. Dose, O. Seitz, Org. Lett. 7 (2005) 4365–4368.
[36] T. Sugiyama, A. Kittaka, Molecules 18 (2013) 287–310.
[37] P.E. Nielsen, D.H. Appella (Eds.), Peptide Nucleic Acids Methods and Protocols, second ed., Humana Press, New York, (2014).
[38] L. Christensen, R. Fitzpatrick, B. Gildea, K.H. Petersen, H.F. Hansen, T. Koch, M. Egholm, O. Buchardt, P.E. Nielsen, J. Coull, R.H. Berg, J. Pept. Sci. 3 (1995) 175–183.
[39] A. Porcheddu, G. Giacomelli, I. Piredda, M. Carta, G. Nieddu, Eur. J. Org. Chem. 2008 (2008) 5786–5797.
[40] S. Pothukanuri, Z. Pianowski, N. Winssinger, Eur. J. Org. Chem. 2008 (2008) 3141–3148.
[41] K.L. Dueholm, K.H. Petersen, D.K. Jensen, M. Egholm, P.E. Nielsen, O. Buchardt, Bioorg. Med. Chem. Lett. 4 (1994) 1077–1080.
[42] G. Haaima, A. Lohse, O. Buchardt, P.E. Nielsen, Angew. Chem. Int. Ed. 98 (1996) 1939–1941.
[43] S. Sforza, R. Corradini, S. Ghirardi, A. Dossena, R. Marchelli, Eur. J. Org. Chem. 2000 (2000) 2905–2913.
[44] P. Zhou, M. Wang, L. Du, G.W. Fisher, A. Waggoner, D.H. Ly, J. Am. Chem. Soc. 125 (2003) 6878–6879.
[45] P. Zhou, A. Dragulescu-Andrasi, B. Bhattacharya, H. O'Keefe, P. Vatta, J.J. Hyldig-Nielsen, D.H. Ly, Bioorg. Med. Chem. Lett. 16 (2006) 4931–4935.
[46] D.I. Prokhorov, Yu.G. Kirillova, N.P. Boyarskaya, A.N. Tevyashova, O.V. Esipova, E.N. Zvonkova, V.I. Shvets, Pharm. Chem. J. (Rus.) 39 (2005) 323–328.
[47] I. Dilek, M. Madrid, R. Singh, C.P. Urrea, B.A. Armitage, J. Am. Chem. Soc. 127 (2005) 3339–3345.
[48] G.P. Aguado, F. Rúa, V. Branchadell, P.E. Nielsen, R.M. Ortuño, Tetrahedron Asymmetry 17 (2006) 2499–2503.
[49] R. Mitra, K.N. Ganesh, J. Org. Chem. 77 (2012) 5696–5704.
[50] N.P. Boyarskaya, D.I. Prokhorov, Y.G. Kirillova, E.N. Zvonkova, V.I. Shvets, Tetrahedron Lett. 46 (2005) 7359–7362.
[51] R. Hamzavi, F. Dolle, B. Tavitian, O. Dahl, P.E. Nielsen, Bioconjug. Chem. 14 (2003) 941–954.
[52] L. Kosynkina, W. Wang, T.C. Liang, Tetrahedron Lett. 35 (1994) 5173–5176.
[53] B. Falkiewicz, W. Wiśniowski, A.S. Kołodziejczyk, K. Wiśniewski, Nucleosides Nucleotides Nucleic Acids 20 (2001) 1393–1397.
[54] B. Falkiewicz, K. Kowalska, A.S. Kolodziejczyk, K. Wiśniewski, L. Lankiewicz, Nucleosides Nucleotides 18 (1999) 353–361.
[55] A. Dragulescu-Andrasi, S. Rapireddy, B.M. Frezza, C. Gayathri, R.R. Gil, D.H. Ly, J. Am. Chem. Soc. 128 (2006) 10258–10267.
[56] J.A. Fehrentz, B. Castro, Synthesis 1983 (1983) 676–678.
[57] C. Avitabile, L. Moggio, G. Malgieri, D. Capasso, S. Di Gaetano, M. Saviano, C. Pedone, A. Romanelli, PLoS One 7 (2012) 1–10.
[58] N. Tilani, S. De Costa, J.M. Heemstra, PLoS One 8 (2013) 1–8.
[59] H. Huang, G.H. Joe, S.R. Choi, S.N. Kim, Y.T. Kim, C.S. Pak, J.H. Hong, W. Lee, Bull. Korean Chem. Soc. 31 (2010) 2054–2056.
[60] H. Huang, G.H. Joe, S.R. Choi, S.N. Kim, Y.T. Kim, H.S. Pak, S.K. Kim, J.H. Hong, H.-K. Han, J. Kang, W. Lee, Arch. Pharm. Res. 35 (2012) 517–522.
[61] P.J. Finn, N.J. Gibson, R. Fallon, A. Hamilton, T. Brown, Nucleic Acids Res. 24 (1996) 3357–3363.
[62] G. Aldrian-Herrada, A. Rabie, R. Wintersteiger, J. Brugidou, J. Pept. Sci. 4 (1998) 266–281.
[63] Z. Timár, L. Kovács, G. Kovács, Z. Schmél, J. Chem. Soc. Perkin Trans. 1 (2000) 19–26.

[64] T. Tedeschi, R. Corradini, R. Marchellia, A. Pushl, P.E. Nielsen, Tetrahedron Asymmetry 13 (2002) 1629–1636.
[65] S. Sforza, T. Tedeschi, R. Corradini, D. Ciavardelli, A. Dossena, R. Marchelli, Eur. J. Org. Chem. 2003 (2003) 1056–1063.
[66] T. Tedeschi, S. Sforza, F. Maffei, R. Corradini, R. Marchelli, Tetrahedron Lett. 49 (2008) 4958–4961.
[67] A. Calabretta, T. Tedeschi, G. Di Cola, R. Corradini, S. Sforza, R. Marchelli, Mol. Biosyst. 5 (2009) 1323–1330.
[68] A.F. Abdel-Magid, K.G. Carson, B.D. Harris, C.A. Maryanoff, R.D. Shah, J. Org. Chem. 61 (1996) 3849–3862.
[69] O. Mitsunobu, Synthesis 1 (1981) 1–28.
[70] B. Falkiewicz, A.S. Kołodziejczyk, B. Liberek, K. Wiśniewski, Tetrahedron 57 (2001) 7909–7917.
[71] N.P. Boyarskaya, Yu.G. Kirillova, E.A. Stotland, D.I. Prokhorov, E.N. Zvonkova, V.I. Shvets, Dokl. Chem. 408 (2006) 57–60.
[72] G. Kokotos, Synthesis 22 (1990) 299–301.
[73] M. Rodriguez, M. Linares, S. Doulut, A. Heitz, J. Martinez, Tetrahedron Lett. 32 (1991) 923–926.
[74] Yu.G. Kirillova, A.V. Baranov, D.I. Prokhorov, O.V. Esipova, V.I. Shvets, Russ. J. Org. Chem. 45 (2009) 1315–1317.
[75] P.C. Meltzer, A.Y. Lian, P. Matsudaira, J. Org. Chem. 60 (1995) 4305–4308.
[76] D.R. Jain, V.L. Anandi, M. Lahiri, K.N. Ganesh, J. Org. Chem 79 (2014) 9567–9577.
[77] D.R. Jain, K.N. Ganesh, J. Org. Chem. 79 (2014) 6708–6714.
[78] T. Sugiyama, Y. Imamura, Y. Demizu, M. Kurihara, M. Takano, A. Kittaka, Bioorg. Med. Chem. Lett. 21 (2011) 7317–7320.
[79] R. Corradini, G. Di Silvestro, S. Sforza, G. Palla, A. Dossena, P.E. Nielsen, R. Marchelli, Tetrahedron Asymmetry 10 (1999) 2063–2066.
[80] S. Sforza, G. Galaverna, A. Dossena, R. Corradini, Chirality 14 (2002) 591–598.
[81] J.P. Tam, W.F. Heath, R.B. Merrifeld, J. Am. Chem. Soc. 108 (1986) 5242–5251.
[82] Yu.G. Kirillova, M.V. Tankevich, D.I. Prokhorov, V.I. Shvets, Russ. J. Org. Chem. 49 (2013) 1768–1776.
[83] A.V. Dezhenkov, M.V. Tankevich, E.D. Nikolskaya, I.P. Smirnov, G.E. Pozmogova, V.I. Shvets, Yu.G. Kirillova, Mend. Comm. 25 (2015) 47–48.
[84] L.S. Richter, R.N. Zuckermann, Bioorg. Med. Chem. Lett. 5 (1995) 1159–1162.
[85] T. Tedeschi, S. Sforza, R. Corradini, R. Marchelli, Tetrahedron Lett. 46 (2005) 8395–8399.
[86] S. Sforza, T. Tedeschi, R. Corradini, R. Marchelli, Eur. J. Org. Chem. 2007 (2007) 5879–5885.
[87] V. Menchise, G. De Simone, T. Tedeschi, R. Corradini, S. Sforza, R. Marchelli, D. Capasso, M. Saviano, C. Pedone, Proc. Nat. Acad. Sci. U. S. A. 100 (2003) 12021–12026.
[88] A. Dragulescu-Andrasi, P. Zhou, G. He, D.H. Ly, Chem. Commun. 2005 (2005) 244–246.
[89] S. Rapireddy, G. He, S. Roy, B.A. Armitage, D.H. Ly, J. Am. Chem. Soc. 129 (2007) 15596–15600.
[90] E.J. Fechter, P.B. Dervan, J. Am. Chem. Soc. 125 (2003) 8476–8485.
[91] E.J. Fechter, B. Olenyuk, P.B. Dervan, Angew. Chem. Int. Ed. 43 (2004) 3591–3594.
[92] T. Bentin, P.E. Nielsen, J. Am. Chem. Soc. 125 (2003) 6378–6379.
[93] V. Chenna, S. Rapireddy, B. Sahu, C. Ausin, E. Pedroso, D.H. Ly, ChemBioChem 9 (2008) 2388–2391.
[94] S. Rapireddy, R. Bahal, D.H. Ly, Biochemistry 50 (2011) 3913–3918.

[95] J.I. Yeh, B. Shivachev, S. Rapireddy, M.J. Crawford, R.R. Gil, S. Du, M. Madrid, D.H. Ly, J. Am. Chem. Soc. 132 (2010) 10717–10727.

[96] T. Tedeschi, S. Sforza, R. Corradini, A. Dossena, R. Marchelli, Chirality 17 (2005) S196–S204.

[97] S. Sforza, T. Tedeschi, A. Calabretta, R. Corradini, C. Camerin, R. Tonelli, A. Pession, R. Marchelli, Eur. J. Org. Chem. 2010 (2010) 2441–2444.

[98] N. Tilani, S. De Costa, J.M. Heemstra, Bioorg. Med. Chem. Lett. 24 (2014) 2360–2363.

[99] T. Tedeschi, A. Calabretta, M. Bencivenni, A. Manicardi, G. Corrado, M. Caramante, R. Corradini, R. Rao, S. Sforza, R. Marchelli, Mol. Biosyst. 7 (2011) 1902–1907.

[100] S. Rossi, A. Calabretta, T. Tedeschi, S. Sforza, S. Arcioni, L. Baldoni, R. Corradini, R. Marchelli, Artif. DNA PNA XNA 3 (2) (2012) 63–72.

[101] E. Delgado, R. Bahal, J. Yang, J.M. Lee, D.H. Ly, S.P.S. Monga, Curr. Cancer Drug Targets 13 (2013) 867–878.

[102] S.M. Thomas, D. Ly, Cancer Res. 73 (Suppl.) (2013) (abstract no. 3301) doi:10.1158/1538-7445.AM2013-3301.

[103] H.H. Pham, C.T. Murphy, G. Sureshkumar, D.H. Ly, P.L. Opresko, B.A. Armitage, Org. Biomol. Chem. 12 (2014) 7345–7354.

[104] A. Singer, S. Rapireddy, D.H. Ly, A. Meller, Nano Lett. 12 (2012) 1722–1728.

[105] S. Rapireddy, L. Nhon, R.E. Meehan, J. Franks, D.B. Stolz, D. Tran, M.E. Selsted, D.H. Ly, J. Am. Chem. Soc. 134 (2012) 4041–4044.

[106] D.-L. Ma, H.-Z. He, K.-H. Leung, H.-J. Zhong, D.S.-H. Chan, C.H. Leung, Chem. Soc. Rev. 42 (2013) 3427–3440.

[107] K. Usui, A. Okada, K. Kobayashia, N. Sugimoto, Org. Biomol. Chem. 13 (2015) 2022–2025.

[108] I.G. Panyutin, M.I. Onyshchenko, E.A. Englund, D.H. Appella, R.D. Neumann, Curr. Pharm. Des. 18 (2012) 1984–1991.

[109] S. Lusvarghi, C.T. Murphy, S. Roy, F.A. Tanious, I. Sacui, W.D. Wilson, D.H. Ly, B.A. Armitage, J. Am. Chem. Soc. 131 (2009) 18415–18424.