Artificial DNA: PNA & XNA 3:2, 31–44; April/May/June 2012; © 2012 Landes Bioscience

Helix control in polymers
Case of peptide nucleic acids (PNAs)

Filbert Totsingan,[1,*] Vipul Jain[2,3] and Mark M. Green[2]

[1]Department of Chemistry; New York University; New York, NY USA; [2]Herman F. Mark Polymer Research Institute; Polytechnic Institute of New York University; Brooklyn, NY USA; [3]Dow Electronic Materials; Marlborough, MA USA

Keywords: helix control, chiral amplification, cooperativity, helical polymers, PNA

The helix is a critical conformation exhibited by biological macromolecules and plays a key role in fundamental biological processes. Biological helical polymers exist in a single helical sense arising from the chiral effect of their primary units—for example, DNA and proteins adopt predominantly a right-handed helix conformation in response to the asymmetric conformational propensity of D-sugars and L-amino acids, respectively. In using these homochiral systems, nature blocks our observations of some fascinating aspects of the cooperativity in helical systems, although when useful for a specific purpose, "wrong" enantiomers may be incorporated in specific places. In synthetic helical systems, on the contrary, incorporation of non-racemic chirality is an additional burden, and the findings discussed in this review show that this burden may be considerably alleviated by taking advantage of the amplification of chirality, in which small chiral influences lead to large consequences. Peptide nucleic acid (PNA), which is a non-chiral synthetic DNA mimic, shows a cooperative response to a small chiral effect induced by a chiral amino acid, which is limited, however, due to the highly flexible nature of this oligomeric chimera. The lack of internal stereochemical bias is an important factor which makes PNA an ideal system to understand some cooperative features that are not directly accessible from DNA.

Helix Control in Biological Systems (Nucleic Acids and Proteins): Overwhelming Chirality

The helix is a widely encountered conformational state found in biological molecules, peptides, DNA, RNA and viruses as well as in a multitude of synthetic macromolecules. In nature these structures are formed using building blocks of one mirror form with the consequence that the helices formed are of a single mirror-image sense.[1] Most biological polymers adopt helical conformations which are clearly seen in the polynucleotide duplexes and the α-helices formed by peptides and portions of protein structures. The presence of stabilizing soft interactions in such biological systems gives rise to a barrier for inversion of helix handedness,[2-4] which is usually not overcome. Many internal and external factors have an effect on the handedness of the helix and have been an interesting topic for scientists who study the origin of chirality and the evolution of biological molecules.[5]

In DNA and RNA (**Fig. 1A**), one helical sense prevails because each monomer along the chain is forced to maintain the same chirality. One limitation of this chiral constraint is the lack of ability to vary the overall chirality of the polymer and through this, the helical handedness and thus the chiral optical properties. Control of chiral properties would yield helical systems in which the helical sense is a variable.

In the situation of amino acid-derived biomolecules such as peptides (**Fig. 1B**), the chiral stereocenter is internal to each of the many monomeric units and therefore is overwhelming in control of helical sense. However, biological systems can occasionally turn to mirror-related amino acids to accomplish specific structural objectives.[6] The biological synthetic mechanisms at work block the use of "wrong" enantiomer amino acids in the formation of proteins, requiring isomerizing enzymes to introduce these mirror isomers on the completed protein.[6-8] In vitro, however, methods of synthesis of peptides offer no impediment to the placement of mirror-image-related amino acids. Work in this area has discovered the helical changes, following from enantiomerically mixed monomer units, on the conformational properties of peptides derived from α-helical-forming amino acids.[9,10] Biologically interesting polymers other than peptides from natural amino acids have been synthesized from mixed enantiomers.[11-13]

Helical Polymers in which Helical Sense is a Variable: Seminal Input from the Italian School

In using homochiral systems, nature blocks our observation of certain interesting aspects of the cooperativity of helical systems, a cooperativity which can lead to large chiral amplification effects. This can act to alleviate the burden of synthesizing enantiomerically pure building blocks for these synthetic systems, and this can be seen most clearly in the simplest version of the nylons, the polyisocyanates, which are stiff helical polymers with a high degree of cooperativity along the polymer chains (**Fig. 2**).[14] This cooperativity arises from infrequent helical reversals which separate long blocks of opposing helical senses, forcing many units of the chain to take the same helical sense. In this way the chiral bias on each unit of the chain is amplified, with the resulting relative proportions of right- and left-handed segments determined experimentally by measuring the chiral optical properties of the polymers.

*Correspondence to: Filbert Totsingan; Email: ft437@nyu.edu
Submitted: 02/08/12; Revised: 04/20/12; Accepted: 04/30/12
http://dx.doi.org/10.4161/adna.20572

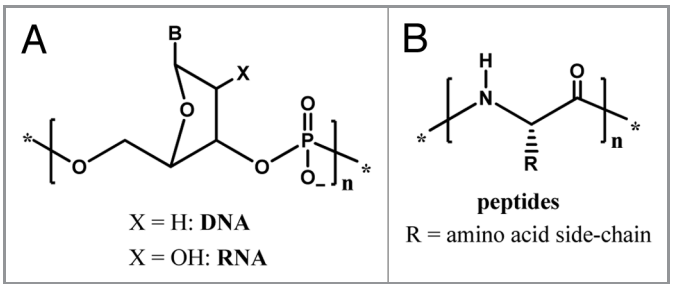

Figure 1. Schematic structures of DNA, RNA (A) and peptides (B).

Questions concerning helical polymers with variable sense were first addressed by Italian researchers in Pisa under the supervision of Piero Pino in the 1960s who began to synthesize stereoregular isotactic vinyl polymers with chiral, non-racemic pendant groups [i.e., from (S)- and (R)-3-methyl-1-pentene] and discovered the absence of a linear relationship between the optical activities of such polymers in solution and the configurational enantiomeric characteristics of the monomer units. This nonlinear relationship was difficult to understand except as arising from the chiral units affecting some aspect of the chain conformation. Although the measurements were made in solution rather than with the crystal, the results pointed to the presence of a helical conformation. A full understanding, however, was prevented by the absence of an accessible chromophore, therefore limiting a direct observation of the chiral optical properties by circular dichroism (CD). Moreover, the magnitudes of the cooperative effects were limited by the flexibility of the polymer since helical reversal states were relatively easily accessible. Such structural defects limited the cooperativity, which is responsible for the nonlinear effects.

To gain further insight into the chiral optical properties of isotactic vinyl polymers, Pino and coworkers in Pisa used the Ziegler-Natta catalysts to produce polymers with high stereoregularity from chiral monomers of 1-alkenes. It was well-known from previous work that increasing isotacticity leads to increased crystallinity and therefore to decreased solubility, so that samples could be fractioned based on their isotacticity by dissolution in increasingly high boiling solvents at high temperatures. All fractions of the optically active polymers produced in this way showed increased optical activity compared with the starting monomer units (**Table 1**).[15,16] However, as isoctaticity increased, as evidenced by decreasing solubility, so did the temperature-dependence of the optical activity.

According to the data reported in **Table 1**, Pino hypothesized that helical conformations can exist above the melting point and in dilute solution. Although such conformations could be experimentally evidenced in the crystals of isotactic vinyl polymers, there had been no experimental means to directly address the question in solution. This issue was taken up by Allegra and coworkers in Milan using force field calculations. They found that helical conformations were important in isolated chains,[17,18] therefore strongly supporting Pino's hypothesis.

If the isotactic polymer chain exists as a series of left-handed and right-handed segments separated by helical reversals, the data in **Table 1** can be understood. The bias per unit from the chiral pendant groups casting the left- and right-handed helical senses into a diastereomeric relationship must be multiplied by the number of units with the same helical sense, and the chiral optical properties arise not from the units but rather from the helical conformation. Later study performed by Pino and coworkers suggested the possibility of roughly estimating the population of the proposed helical reversals and even their possible effect on the overall polymer properties.[19] At the time of initial work, direct experimental evidence was sought through observation by CD of the helical chromophore, but this could not be probed because of its inaccessible wavelength. To further seek other avenues to support their hypothesis, Pino and coworkers synthesized an isotactic copolymer of styrene with a large excess of (R)-3,7-dimethyl-1-octene.[20] The result of their findings is reported in **Figure 3**. The large increase in the CD signal of the aromatic chromophore in the copolymer compared with the model compound was suggested as arising from an extended conformation of the polymer, which could only reasonably be helical. The

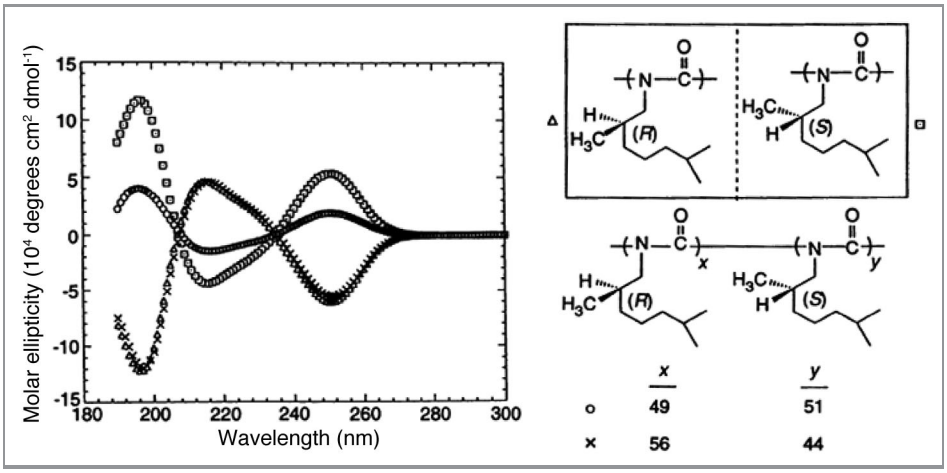

Figure 2. Circular dichroism spectra in hexane solution at room temperature of the homopolymers and copolymers of (R)- and (S)-2,6-dimethylheptyl isocyanates plotted as molar ellipticity against wavelength. Reprinted with permission from reference 14.

Table 1. Physical properties of poly-(S)-3-methyl-1-pentene fractions having different stereoregularity

Fraction	Sample A[l] Catalyst: Al(*i* – $C_4H_9)_3$/$TiCl_4$					Sample B[i] Catalyst: Al(*i* – $C_4H_9)_3$/$TiCl_4$				
	%	$[M]_D$[15] (°)	$[\eta]$[b] (dl/g)	m.p. (°C)	$\Delta[M]_D/\Delta T$[a]	%	$[M]_D$[15c,h] (°)	$[\eta]$[b] (dl/g)	m.p. (°C)	$\Delta[M]_D/\Delta T$[a]
Acetone sol.	6.3	+29.4	[d]	n.d.	-0.08	2.4	+75.8	n.d.	n.d.	n.d.
Acetone insol. diethyl ether sol.	2.6	+96.4	0.08	65–75[e]	-0.23	4.8	+127	0.13	93–96[f]	n.d.
Diethyl ether insol. isoctane sol.	0.9	+120	0.10	135–140[e]	-0.26	1.5	+146	0.13	187–193[f]	-0.31
Isoctane insol. benzene sol.	0.4	+158	0.11	175–180[e]	-0.34	0.5	+157	n.d.	200–210[e]	-0.39
Benzene insol. decalin sol.	2.0	+161[m]	0.50	228–232[e]	-0.36	1.7	+158[m]	0.60	200–210[e]	-0.40
Residue	87.8	n.d.	n.d.	271–273[e]	n.d.	89.1	n.d	n.d.	265–275[e]	n.d.

Reprinted with permission from reference 16. Original data reprinted with permission from reference 15. [a]In tetralin solution; [b]determined in tetralin at 120°C; [c]in toluene solution; [d]molecular weight determined cryosc. in benzene: 1200 ± 100; [e]determined by a Kofler m.p. apparatus; [f]determined by X-ray method; [g]determined by the capillary method; [h]referred to one monomeric unit; [i]monomer optical purity: 91%; [l]monomer optical purity: 89%; [m]± 10%.

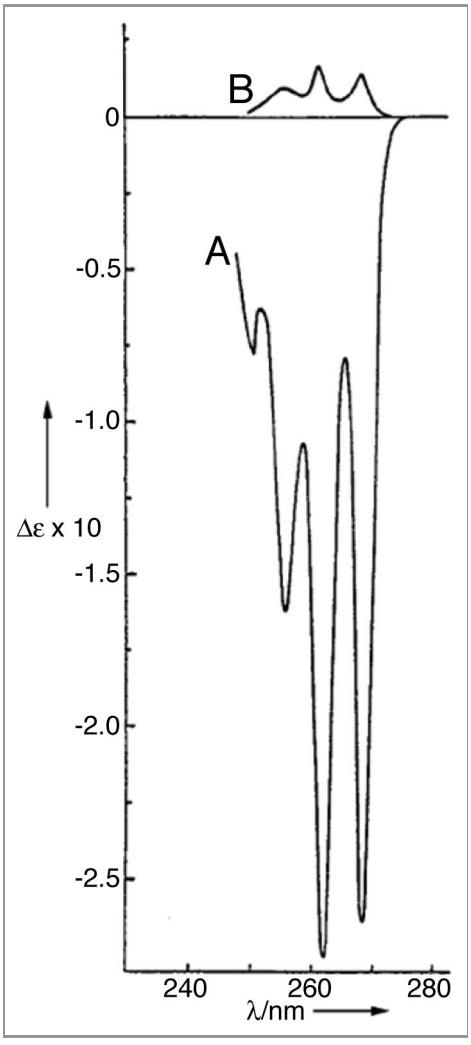

Figure 3. CD spectra at 25°C of the diethyl ether extractable fraction of the styrene-(R)-3,7-dimethyl-1-octene copolymer (A) and of (3S,9S)-3,9-dimethyl-6-phenylundecane (B) in chloroform; De is based on one unit of styrene. Reprinted with permission from reference 20.

experiment was further extended to isotactic copolymers derived from monomeric units offering conflicting information to the helical sense of the chain. A series of copolymers of enantiomers from three structurally different 1-alkenes [(S)-5-methyl-1-heptene; (S)-4-methyl-1-hexene and (R)-3,7-dimethyl-1-octene] were then synthesized and their optical activities were measured.[21] The results are reported in **Figure 4**.

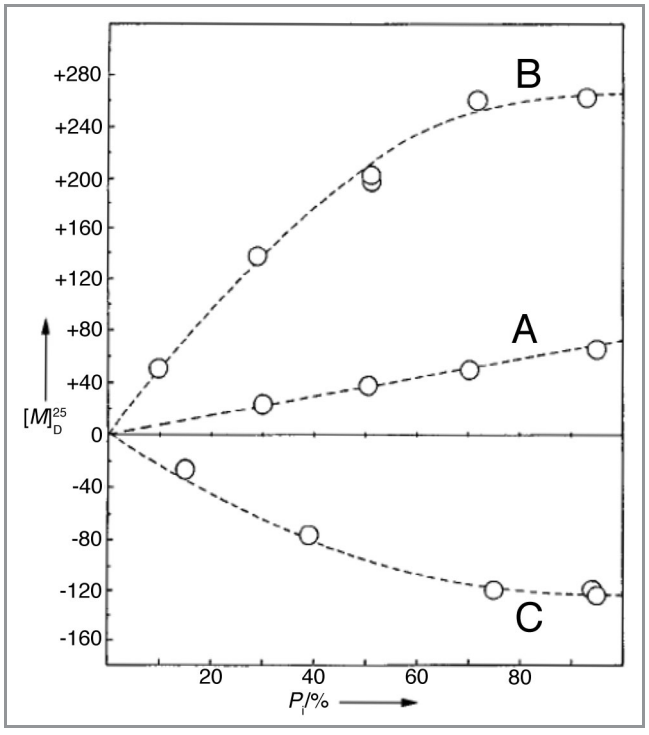

Figure 4. Molar rotator power ($[M]_D^{25}$), referred to one monomeric unit, in hydrocarbon solution for the unfractionated methanol-insoluble polymers obtained from samples having different optical purity (P_i) of (S)-5-methyl-1-heptene (A), (S)-4-methyl-1-hexene (B), and (R)-3,7-dimethyl-1-octene (C). Reprinted with permission from reference 21.

Alternative studies to confirm the helical hypothesis were also performed by the Italian researchers by synthesizing copolymers of enantiomerically pure units randomly dispersed among achiral units and by measuring the optical activity using mixtures of homopolymers as a control. A linear response of the optical activity to the proportion of the chiral units was observed only in the mixture of homopolymers. In the copolymer of the chiral and achiral units, 50% of the chiral unit was enough to yield the full optical activity.[22]

However, in the seminal works performed in Italy, cooperative effects were limited by the flexible nature of vinyl polymers. Clearly, these chiral, nonlinear relationships could be further amplified using helical polymers with a lower probability of helical reversals, that is, stiff polymers. This is the subject of the next section.

Helix Control in Stiff Helical Polymers

Dynamic helical systems: e.g., polyisocyanates and poly(phenylacetylene) derivatives. As in the isotactic vinyl polymers discussed above, other helical polymers such as polyisocyanates, which are synthesized from achiral units, allow equal populations of both helical senses, which are separated by helical reversals. Such reversals are likely to cause a change in the direction of the polymer chain, that is, an effect on the persistence length of the chain. What is the critical chain length that allows these changes to occur? Focusing on this question and others concerning the chiral amplification in polyisocyanates, which are archetypical stiff helical polymer, Green and coworkers first synthesized and measured the optical activities of homopolymers of 1- and 2-deuterio-n-hexyl isocyanates (**Fig. 5**).[14,16]

Poly(n-hexyl isocyanate) has no preference for one helical sense and the concept of introducing a single stereospecific deuterium substituent in the side chain of every unit arose from the desire to cast the mirror helical states into a diastereomeric relationship without changing the torsional characteristics of the backbone.[23] The poly(1-deuterio-n-hexyl isocyanate) and poly(2-deuterio-n-hexyl isocyanate) exhibited large $[\alpha]_D$ values of opposite sign compared with the precursor monomer (see **Fig. 5**; -444° vs. +0.65° and +302° vs. -0.43°, respectively). Therefore the optical activity of the polymer could not be arising only from the deuterated stereocenter. **Figure 5** shows a large CD signal for the helical chromophore of the two polymers, which is direct evidence proving an excess of one helical sense in these polymers and demonstrating a large chiral amplification. The per unit bias favoring one helical sense arising from deuterium substitution must be very small, while the CD intensities are consistent with a large excess of the preferred helical sense.

In chains of a stiff helical polymer that are short enough there can be expected no disruption of handedness, no helical reversal. In such chains the helical sense excess, as measured by the CD intensity, should track with chain length, that is, with degree of polymerization. As the chain length increases, the helical reversal energy allows the possibility of reversals and the CD intensity should level off and become independent of degree of polymerization, but should decrease greatly with increasing temperatures.[24,25] These expectations are precisely observed, and in combination with theoretical calculations[24] have led to determination of the helical reversal energy in the range of 4,000 cal/mole, which at ambient temperature corresponds to one reversal

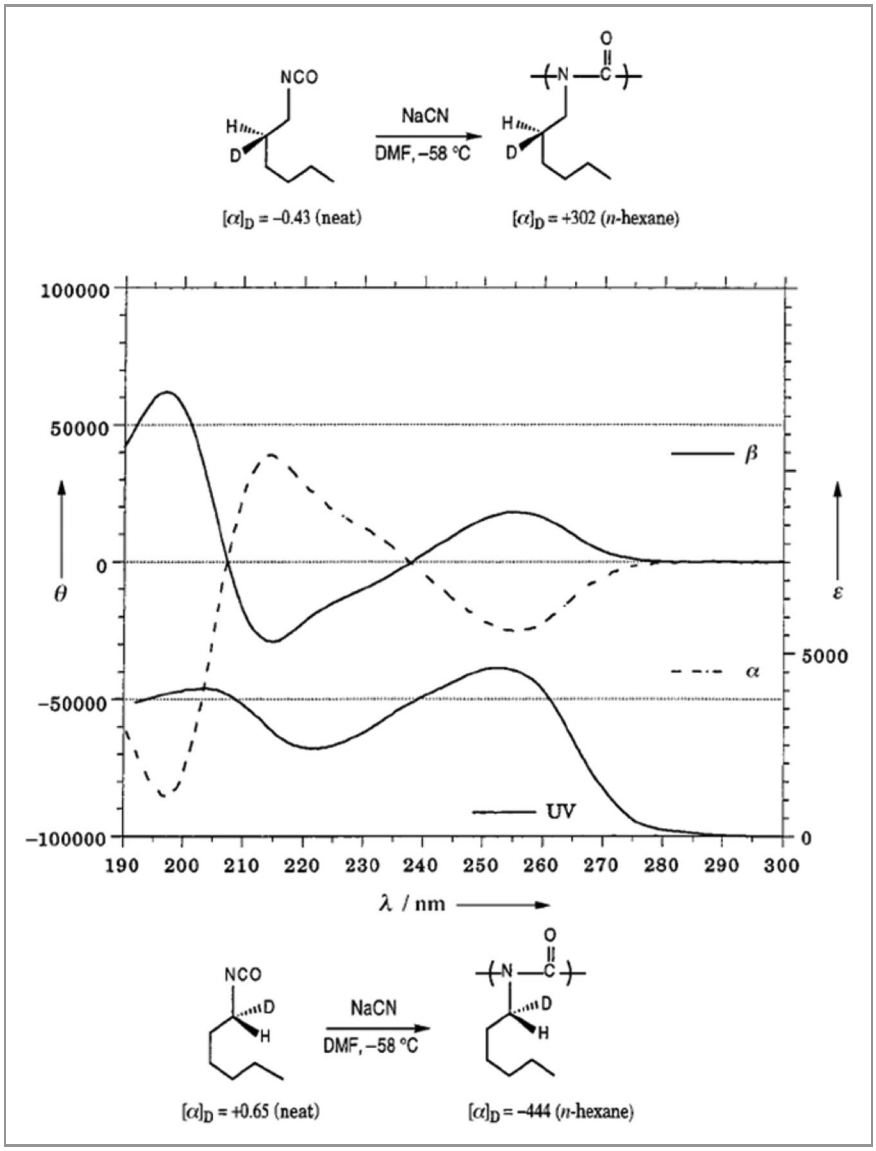

Figure 5. CD and UV spectra of poly(1-deuterio-n-hexyl isocyanate) (α) and poly(2-deuterio-n-hexyl isocyanate) (β). Also shown are the structures and changes in optical activities from the monomers into these polymers. Reprinted with permission from reference 16.

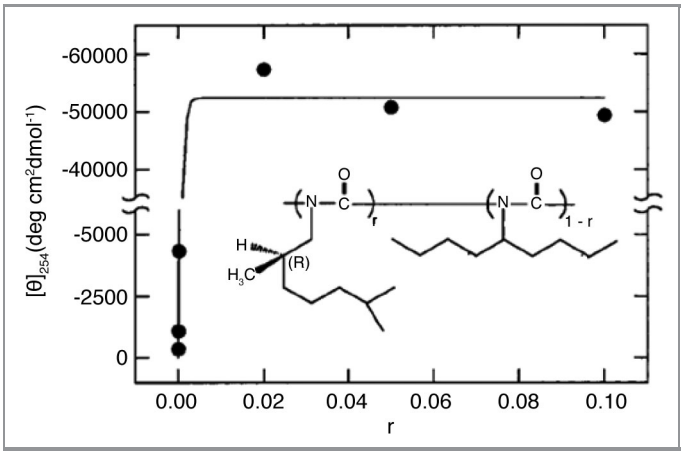

Figure 6. Molar ellipticity at 254 nm of achiral/chiral copolymer as function of the fraction of the chiral units (r). Reprinted with permission from reference 27.

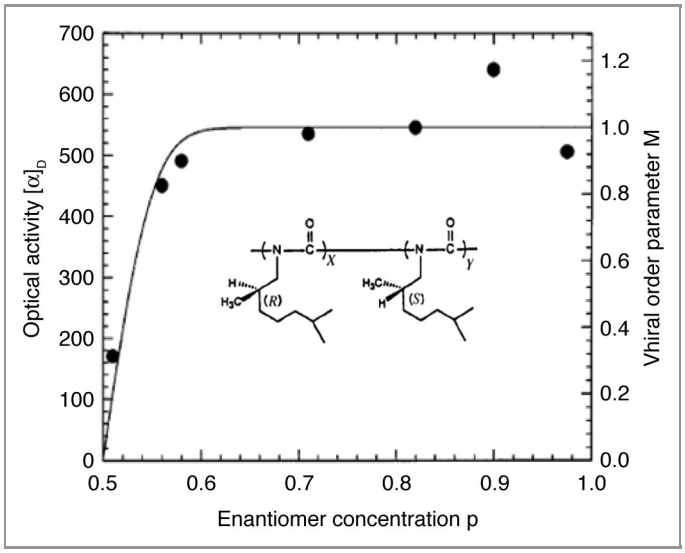

Figure 7. Optical activity of (R)/(S) copolymer as function of fraction of (S)-enantiomer [p = S/(R+S)]. Reprinted with permission from reference 28. Also shown is the structure of (R)/(S) copolymer.

approximately every 800 units along the chain. Knowledge of this energy solved a problem of understanding the source of the limit to the persistence length in the nylon-1 which had perplexed physicists interested in these problems.[26]

Amplification of the minute chiral information per monomeric unit, as shown in **Figure 5**, demonstrates the power of cooperativity in these helical polymers which can be expressed in a different way as reported in **Figure 6**.[27] In this case, incorporation of few chiral units which have preference for one helical sense in a polymer with a large number of achiral monomers (e.g., one chiral unit among many hundred achiral units) causes the entire polymer to adopt one helical sense so that additional chiral units have no significant effect on the CD intensity. This is termed the "sergeant and soldiers experiment."[25]

Another experiment measuring the cooperativity of a helical polymer is possible, in which enantiomeric chiral units with propensities favoring opposite helical senses compete in controlling the handedness of the polymer (**Fig. 7**).[28-30] Unlike the "sergeant and soldiers experiment," or the deuterium substituted experiment discussed above the helical reversal probability is dependent not solely on the helical reversal energy but also on the nature of the competition between the enantiomeric units. As shown in the experiment below (**Fig. 7**), a small enantiomeric excess can control the entire polymer chain, which may be compared with the "majority rule" situation in a democracy.[25,29,30]

Following the initial work on the polyisocyanates discussed above, chiral amplification phenomena have been found in other covalent polymers also in supramolecular helical systems involving non-covalent assemblies of molecular components.[31-40] For example, poly(phenylacetylene) derivatives bearing achiral functional side groups have been synthesized. The polymers possess a stereoregular cis-transoidal structure. Excess single-handed helicity of the main chain can be induced for the polymers by the interaction with chiral molecules.[41-43] In early studies performed by Yashima et al.,[41] the concept of memory of macromolecular helicity in poly[(4-carboxyphenyl)acethylene] (**Fig. 8**) was reported. This highly dynamic helical polymer possesses a large number of helical units separated by helical reversals and has therefore no preference for one helical sense. However, interaction of the polymer carboxyl group with optically actives amines causes induction of macromolecular helicity, therefore resulting in optical activity (**Fig. 8**). Apparently, the highly cooperative interactions in the pendant groups give rise to a large excess of one helical sense arising from complexation with the chiral guests, which considerably alters the population of left- and right-handed helices of the polymer. The complex of the polymer with optically active amines shows a characteristic CD signal which depends on the configuration of the chiral amine (**Fig. 8B**). Remarkably, the induced macromolecular helicity remains even after substitution of the chiral inducers by achiral ones (**Fig. 8**).

The CD absorptions in the example discussed above are not based on the chiral amine but on the excess helicity of the main chain of polymer as clearly understood from the wavelength range. These results have been interpreted to mean that poly[(4-carboxyphenyl)acetylene] originally having a rather irregular twist of the adjacent double bonds around a single bond may be transformed into the helical conformation with an excess screw sense by the interaction with the chiral amines (**Fig. 9**). Poly (phenylacetylene) derivatives having a chiral side chain have also been reported to show interesting helical effects that depend on side chain chirality[44] as have polysilanes.[35,45]

Non-dynamic helical systems: poly(trityl methacrylate) and related polymers. Vinyl polymers without the capacity to undergo helical reversals can be prepared from methacrylates with a bulky side group (e.g., trityl) by isotactic anionic or radical polymerization.[46,47] This type of polymer (**Fig. 10**) was first anionically asymmetrically synthesized from trityl methacrylate using a complex of n-butyl lithium.[48]

Asymmetric anionic polymerization is performed using a complex of an organolithium with a chiral ligand (ligand control) or using a chiral organolithium initiator (initiator control)

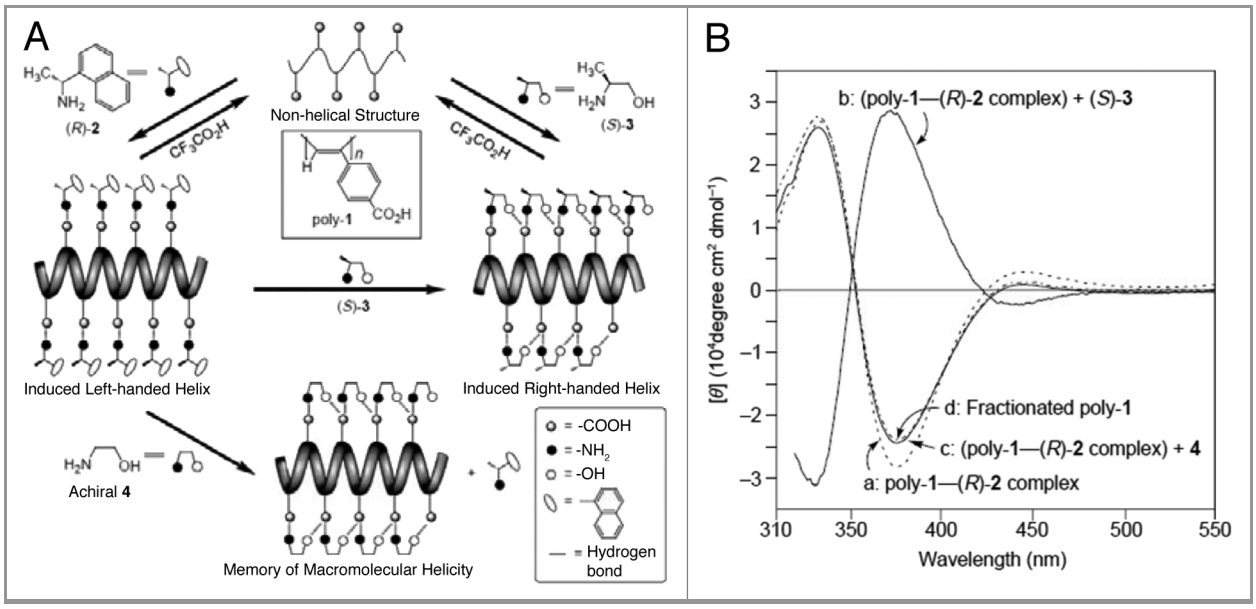

Figure 8. Schematic representation (A) of induced macromolecular helicity the memory of macromolecular helicity in (poly[(4-carboxyphenyl)acethylene]; poly-1) and CD changes at 374 nm (B) in the presence of amines. Reprinted with permission from reference 41.

(**Fig. 11**).[48,49] Selection of the helix-sense occurs on the basis of the chirality of the ligand or the initiator.

Although a chiral side group was necessary in inducing a helical conformation with an excess helical sense in solution for stereoregular vinyl polymers, the synthesis of helical poly(trityl methacrylate) is achieved from the achiral (prochiral) vinyl monomer with the chiral information arising from the initiator. A nearly completely isotactic configuration and a single-handed helical conformation of the main chain, which is stabilized by steric repulsion of the bulky side groups, is obtained, which shows high optical activity based on the conformation.[48] The helical conformation is lost when the trityl group is removed from the polymer chain. Thus, the poly (methacrylate) derived from the poly(trityl methacrylate) after removal of the trityl group shows only a small optical activity based on the configurational chirality of the stereocenters in the vicinity of the chain ends.[50] Helical copolymers of trityl methacrylate with other monomers have also been synthesized and their chiral optical properties have been studied.[51]

Other poly(trityl methacrylate) derivatives have parallel characteristics. For example, monomers having the pyridyl group in the side chain,[52-54] were polymerized and were designed with the idea that the ester linkage should be more stable to methanolysis than that of poly(trityl methacrylate). Helix control in these polymers was achieved via anionic polymerization as for trityl methacrylate (**Fig. 11**)[49] and also by radical polymerization using chiral entities as chain transfer agents or initiators (**Fig. 12**).[49] In radical polymerization of bulky methacrylate derivatives, the helix control is governed by the chirality of the monomer itself or an additive.

Figure 13 shows the relationship between enantiomeric excess (ee) of the monomer and the optical activity of the derived polymer obtained by radical polymerization of phenyl-2-pyridyl-*o*-tolylmethyl methacrylate.[55] A nonlinear dependence indicates that the optical activity of the polymer is not based only on the side chain chirality. Furthermore, the chirality of a one-handed helical part induced by the major enantiomer (in excess) units can overcome the opposing chiral induction by the minor enantiomer

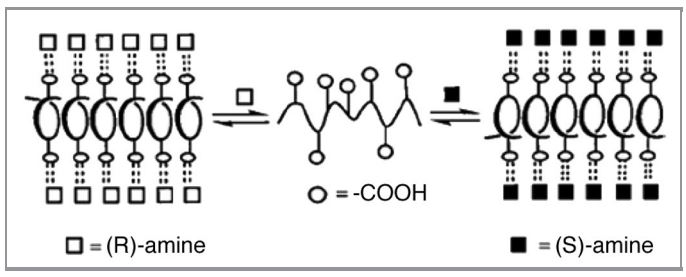

Figure 9. Helix control in poly(phenylacetylene) derivatives through interactions with chiral amines.

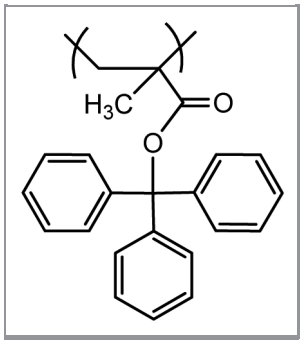

Figure 10. Structure of poly(trityl methacrylate):poly(TrMA).

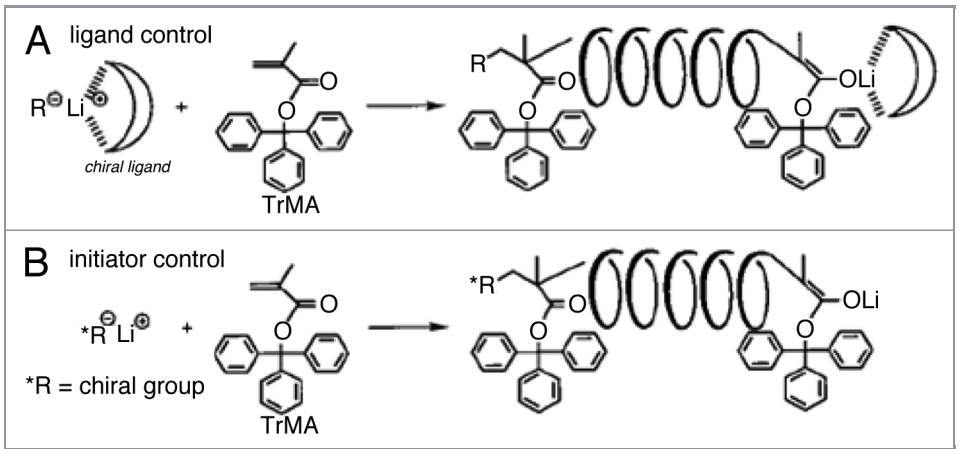

Figure 11. Helix control in asymmetric anionic polymerization by a chiral ligand (A) or a chiral organolithium initiator (B). Reprinted with permission from reference 49.

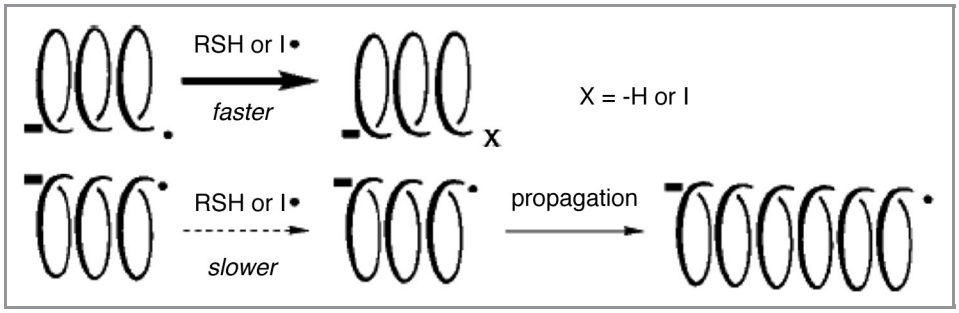

Figure 12. Helix control in radical polymerization using optically active thiols as initiator or chain transfer agent. Reprinted with permission from reference 49.

units. In other words, once a one-handed helical radical comes under the influence of the major enantiomer, an entering minor enantiomer becomes a part of the one-handed helix whose direction may be unfavorable to the chiral nature of the minor enantiomer, that is, the majority rule.[29,30]

Helix Control in Peptide Nucleic Acids (PNAs)

Helix induction in single-stranded PNAs: helical preorganization. Peptide nucleic acids (PNAs)[56] are DNA analogs in which the sugar-phosphate backbone has been replaced by an N-(2-aminoethyl) glycine unit covalently linked to the nucleobase (**Fig. 14**).

Using the original N-(2-aminoethylglycine) unit as starting point, several modifications have been introduced into the backbone[57,58] based on the "preorganization" concept, i.e., the ability to adopt a conformation which is more suitable for DNA binding. In particular, incorporation of a stereocenter at the γ-position results in stabilization of PNA-DNA duplex by ~3°C per chiral unit. The basis of this enhanced stabilization can be understood from structural data. Superposition of PNA strands from a crystal structure of γ-PNA:DNA duplex with methyl group at the γ-position,[59] to the solution structure of a single-stranded (CT) γ-PNA dimer[60] shows structural similarities of the γ-PNA strands

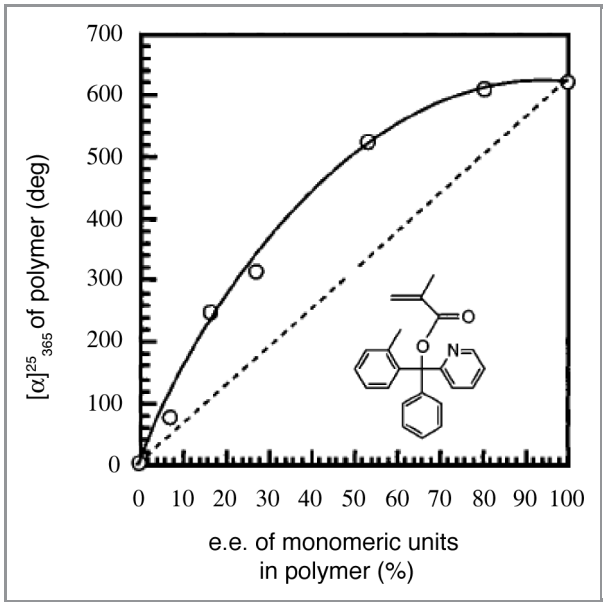

Figure 13. Optical activity of the polymer derived from radical polymerization of phenyl-2-pyridyl-*o*-tolylmethyl methacrylate as function of enantiomeric excess of the monomer. Reprinted with permission from reference 55. Also shown is the structure of the monomer.

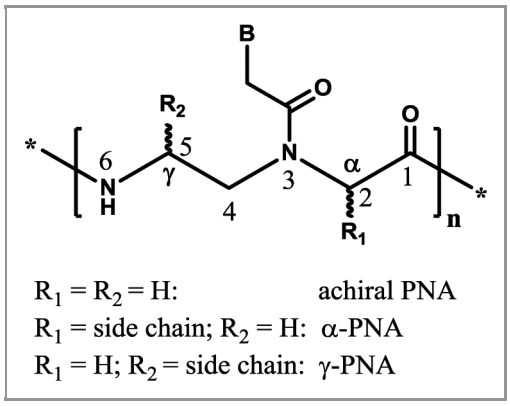

Figure 14. Chemical structures of achiral and acyclic chiral PNAs.

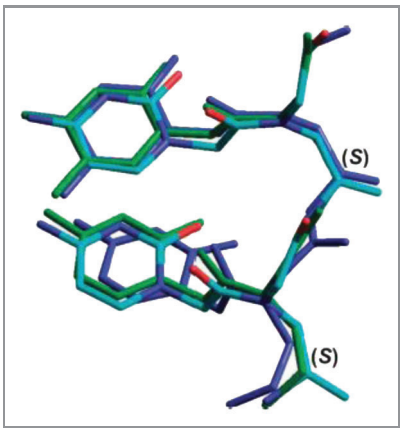

Figure 15. Comparison of PNA strands from crystal structures of γ-PNA:DNA (cyan)[59] and chiral box α-PNA:DNA (green)[61] with the NMR structure of single-stranded (CT) γ-PNA dimer (blue).[60] Reprinted with permission from reference 59.

to one another in backbone conformation, nucleobase orientation and carboxymethylene bridge projection (**Fig. 15**). In both the single-strand and the duplex forms, the (S) methyl group at the γ-position is in a *trans* configuration with the carboxymethylene bridge, which, in turn, is in *trans* with the backbone carbonyl oxygen within the same unit. The striking similarities between the two structures, in both the unbound and the bound states, suggest that the molecular interactions that direct the folding of the γ-PNA:DNA duplex also direct the folding of the individual γ-PNA single strands.

To elucidate whether conformational preorganization of γ-PNA occurs as a result of base stacking or is intrinsic to the individual building blocks, a series of γ-modified Boc-protected PNA monomers containing amino acids with increasing side-chains [glycine (unmodified), L-alanine, L-valine and L-isoleucine] (**Fig. 16A**) was investigated.[59] Solution structures obtained by ^{1}H NMR revealed that glycine and alanine monomers do not have a preferred helical conformation, while valine and isoleucine monomers show ^{3}J-coupling constants consistent with a right-handed conformation (**Fig. 16B**), presumably due to the large isopropyl and *sec*-butyl side-chain of valine and isoleucine, respectively, which cause restricted rotation around the C2-C3 bond. The fact that the alanine monomers do not exhibit a preferred conformation as individual units but that they do when covalently linked together into an oligomer suggests that base stacking between adjacent units play an essential role in determining the helical sense as well as in stabilizing the helical conformation.

Standard single stranded PNA is not chiral and tends to fold into complex globular structures,[62] probably due to the conformational collapse of the hydrophobic nucleobases. However, insertion of a stereocenter either at the α- or at the γ-position (**Fig. 14**) can preorganize the PNA strand,[59,60,63] by shifting the preference toward a left- or right-handed helical conformation, according to the configuration of the stereocenter. For example, a γ-backbone modification by L-amino acids such as L-isoleucine[59] or L-serine[60] can transform the randomly folded peptide nucleic acid into a conformationally preorganized right-handed helix,

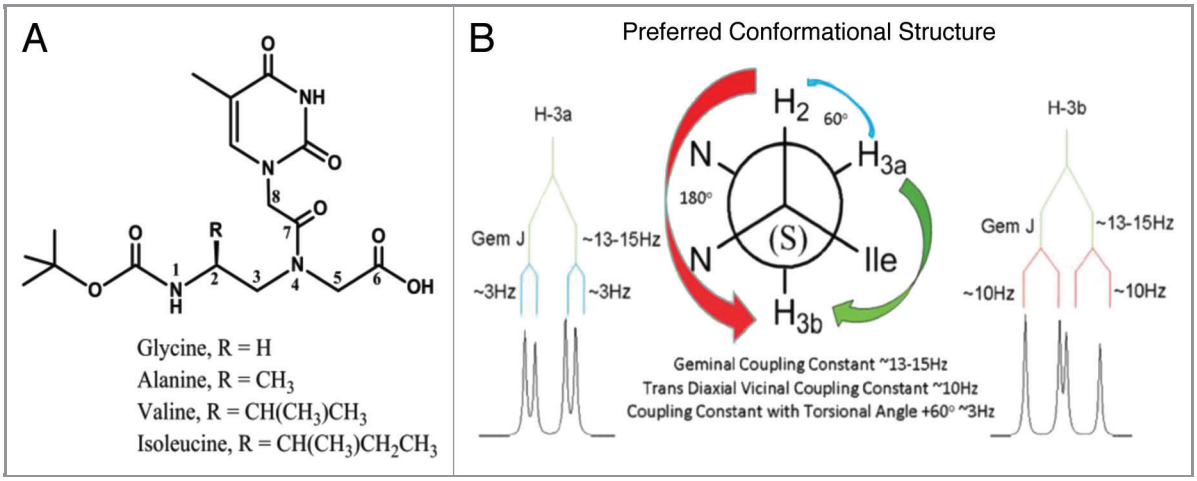

Figure 16. (A) Structures of Boc-protected unmodified and γ-modified PNA thymine monomers (R, amino acid side-chain). (B) Newman projection showing the preferred conformation around the C2-C3 bond for (S)-isoleucine. ^{1}H NMR splitting patterns showed estimated J-coupling constants according to the Karplus relationship, consistent with a right-handed helix. Reprinted with permission from reference 59.

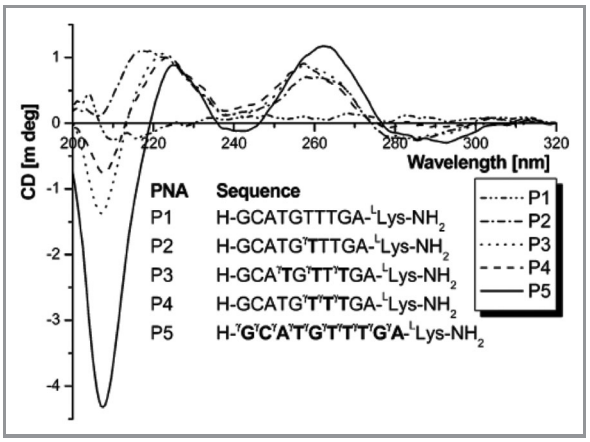

Figure 17. CD spectra of single-stranded achiral PNA (P1) and L-serine γ-PNAs (P2-P5). Reprinted with permission from reference 60.

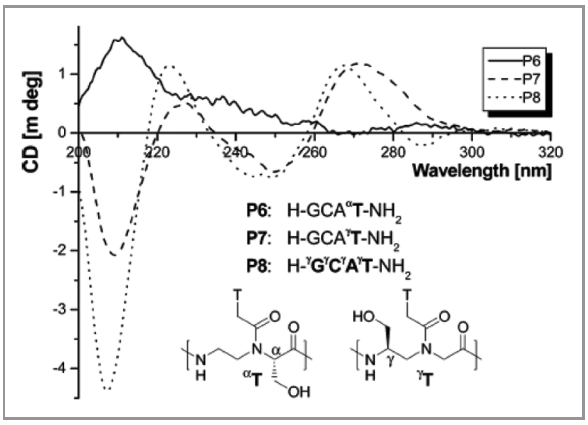

Figure 19. CD spectra of single-stranded L-serine α-PNA (P6) and L-serine γ-PNAs (P7 and P8). Reprinted with permission from reference 60.

as reported by Ly and coworkers. **Figure 17** compares the CD spectra of a series of PNAs with increasing number of γ-modified monomers with that of the corresponding achiral PNA, the latter, of course, unable to exhibit a CD band in the nucleobase absorption region (220–300 nm) even if there were base stacking (P1, **Fig. 17**). However, γ-PNAs (P2-P5, **Fig. 17**) exhibit CD signals with biphasic exciton coupling patterns characteristic of right-handed helices.[64] The CD signal of γ-PNAs gradually decreases with increasing temperatures and becomes similar to that of achiral PNA at high temperatures. Furthermore, upon cooling, the CD signal returns to the original spectral pattern indicating that the observed exciton coupling occurs as a result of molecular preorganization rather than an intermolecular interaction. The results of this study[60] were interpreted as showing that the determining factor for helical induction in γ-PNAs appears to be sterically driven and stabilized by base stacking [for stereochemical model obtained from a monomer in the crystal structure, showing the effect of substituents derived from D- or L-amino acids either on the C2 (α) or on the C5 (γ) carbon of the monomers see ref. 57].

Conformational preorganized single-stranded PNAs have also been reported by Sforza and coworkers.[63] They reported that modification at the α-position induces in single-stranded PNA a helical conformation which exhibits a CD profile similar to that of a PNA-PNA double-helix (see **Fig. 18**), although the CD signals of the α-PNAs reported were weak compared with those of γ-PNAs studied by Ly et al. A direct comparison between α- and γ-PNA is seen in **Figure 19**, which shows that γ-position has a larger chiral effect than the α-position (P6 vs P7, **Fig. 19**). Interestingly, the fact that the CD shape and intensity in the nucleobase absorption region is not dependent on the number of the chiral units (P7 vs. P8, **Fig. 19**), suggests that a single chiral unit may be sufficient to preorganize the entire oligomer into a single favored helical conformation, therefore following the "sergeant and soldiers" concept developed by Green and coworkers to explain the chiral amplification in polyisocyanates.[16,25]

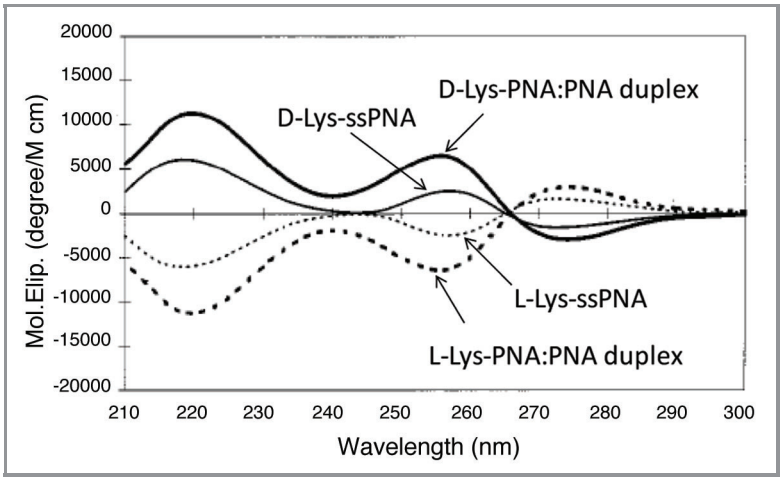

Figure 18. CD spectra of single-stranded α-(L/D)-Lys-PNAs and the corresponding PNA:PNA duplexes with the complementary achiral PNA strand. Reprinted with permission from reference 63.

Helix induction in double-stranded PNAs. The PNA:PNA duplex is expected to form a mixture of equally populated right-handed and left-handed helices.[65] Driven by questions of induction and amplification of chirality in these non-natural DNA-like molecules, Nielsen and coworkers investigated the influence of a C-terminal amino acid on the helical sense of PNA:PNA duplexes.[66] Attachment of a lysine to the C-terminal base of one of the two PNA strands leads to a preferential helical sense for the PNA:PNA double helix (**Fig. 20**) and helices induced by L- and D-lysine were found to be exactly of opposite helical sense as expected and as shown by the CD spectra in the nucleobase absorption region (**Fig. 21**).[66] In this experiment, the initial stereochemical bias is propagated through the PNA:PNA duplex by cooperative interactions throughout the helical conformation, with a propagation of chirality via the "sergeant and soldiers" type.[17,26]

Choice of the proper base sequence was also found to be fundamental in inducing the preferred helicity

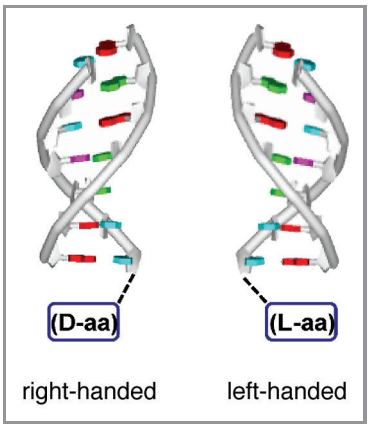

Figure 20. Helical sense preference induced in PNA:PNA duplex by a C-terminal chiral amino acid. D- and L-amino acid attached to the C-terminus of one PNA strand induce helices with opposite helical sense.

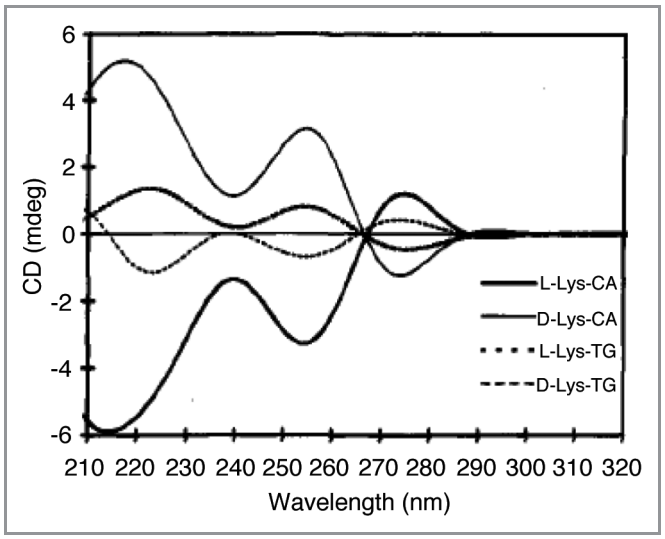

Figure 21. CD spectra of PNA:PNA duplexes with L- and D-lysine linked to the C-terminus of one PNA strand: H-AGTGATCTAC-(L/D-Lys)-NH_2 and H-GTAGATCAGT-(L/D-Lys)-NH_2. Reprinted with permission from reference 66.

in the PNA:PNA duplex, stabilized by the C-terminal amino acid. It was found that the base closest to the terminal amino acid must be either a guanine or a cytosine to give a well-defined CD pattern, indicative of the preferred helical sense. Substitution of lysine with other amino acids gave the same CD spectral pattern, demonstrating that the intrinsic chirality of the amino acid is not of major importance in affecting the coupling between the transition moments of the nucleobases as a result of their helical stacking.

The effect of a terminal L-lysine in a series of PNA:PNA duplexes with increasing chain lengths led to the conclusion that the chiral influence of lysine extends only as far as the tenth base pair, at which point a loss of stereochemical bias was supposed to occur, leading to constant CD signals on a per monomer unit basis.[66] This result was interpreted as suggesting a limit of the cooperative properties of PNA:PNA duplexes: after 10 base pairs the chiral bias of base stacking interactions is attenuated to such an extent as allowing a reversal of the double-helical sense.

To further understand the problem of chiral amplification in PNA:PNA duplexes, we extended the studies of the effect of terminal amino acids on the PNA double-helix by varying the appending various amino acids and using longer oligomers[67] than previously investigated by Nielsen and coworkers.[66] Circular dichroism, UV melting, NMR as well as temperature-dependence of CD signals showed that the terminal amino acid has an influence not only on the helical sense of the double helix, but also on the conformational properties of the double helix. The terminal amino acid affects the duplex structure distal to its point of attachment as a result of an extended range of conformational states in solution with a population which depends on both the amino acid and the oligomer length of the duplex. A simple model illustrating this fact for a terminal L-lysine is reported in **Figure 22**.

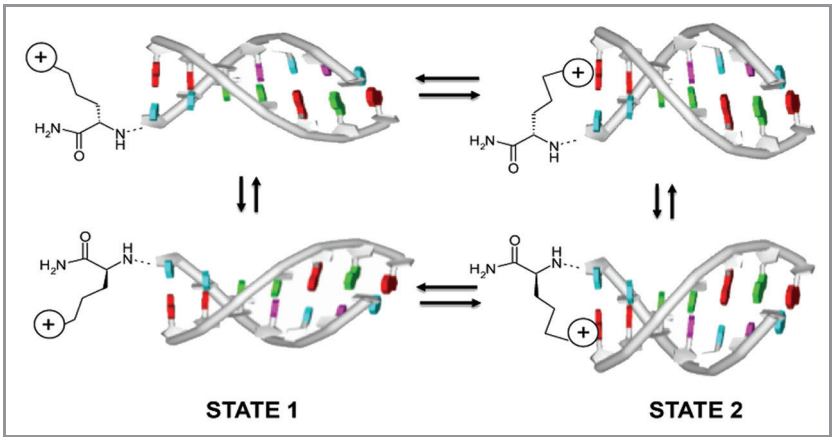

Figure 22. Schematic representation of multiple conformational states model. Only two states are shown for simplicity. The terminal L-lysine can stabilize one of the two handedness and one of the two states by interacting with the adjacent base pair. Reprinted with permission from reference 67.

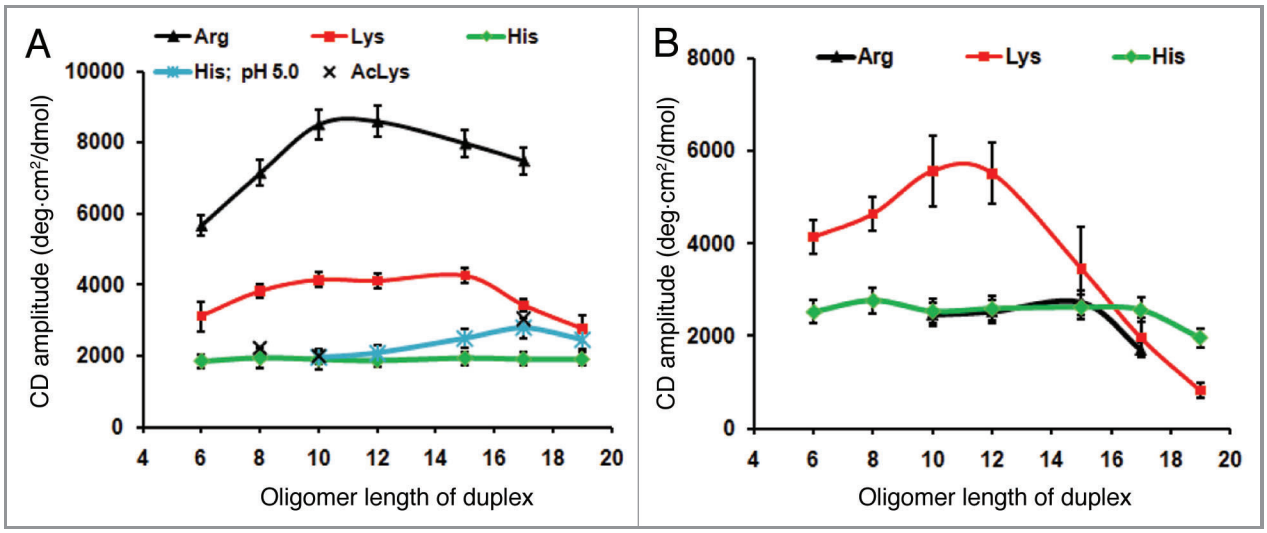

Figure 23. Optical activities of PNA:PNA duplexes in water (A) and in glycerol/water (9:1) mixture (B), as function of the terminal amino acid and the oligomer chain length. Reprinted with permission from reference 67.

The optical activities as function of the terminal amino acids and the chain lengths are shown in **Figure 23** for two different solvents (water and glycerol/water). The length dependence of the optical activity from different amino acids in particular showed complex behavior, consistent with a structural model in which an ensemble of PNA duplex conformations differ as a function of the terminal amino acid, suggesting that the PNA duplex itself is intrinsically conformationally heterogeneous, in contrast to the solid state where a unique helical conformation is the situation as observed by X-ray crystallography.[68] Attachment of an amino acid may stabilize and restrict this manifold to a smaller set, but the system remains multistate in nature. These results support and extend conclusions gained from a crystallographic study of partially self-complementary PNA showing that several types of H-bonding schemes can occur in PNA duplex.[69]

Helix control in PNA:PNA duplexes bearing one or more chiral units within the oligomer chain have also been investigated.[63,70-72] Early studies performed by Sforza and coworkers[63] showed that incorporation into the PNA strand of one chiral unit of either L- or D-lysine-based PNA monomer modified at the α-position (α-PNAs) induces in the PNA:PNA duplex a preferred helical sense. In this case, the exciton coupling patterns in the nucleobase absorption region were opposite when comparing αD-Lys-PNA:PNA and αL-Lys-PNA:PNA duplexes (**Fig. 18**).[63] As shown in **Figure 18**, the CD spectra of the PNA:PNA duplexes exhibit a higher intensity, but were quite similar in shape to the spectra of single-stranded PNAs suggesting that the preferred handedness was already induced in the single strand, and that this preference was enhanced in the duplex, therefore supporting the concept of "preorganization" in single-stranded PNAs discussed above. Interestingly, the shape and intensity of the PNA:PNA duplexes containing three lysine monomers distributed along the chain was similar to those of the PNA:PNA duplexes bearing only one lysine monomer in the middle of the strand (**Table 2**), suggesting that one chiral unit was enough to induce the helical sense preference, consistent with the "sergeant and soldiers" concept.[17,26]

Replacement of lysine with other amino acids was also studied and the optical activities at 256 nm of various PNA duplexes are shown in **Table 2**. The helical sense preference of the PNA:PNA duplex with three α-L-Asp-based monomer units distributed along the chain was identical to that of the duplex with three α-L-Lys-based monomer units, and the helical sense preference of the PNA:PNA duplex with three α-D-Glu-based monomer units was identical to that of the duplex with three α-L-Lys-based monomer units. Since two groups of opposite charges showed the same helical sense, it can be deduced that the interaction involved is not a specific ion-dipole interaction or H-bonding, but rather a solvation effect of the polar groups, which are likely to be shifted away from the PNA backbone because of water solvation. The induced conformational changes are transmitted to the nearest base, thus modifying the stacking interactions and affecting the helical preference.

The effect of substitution in either the 5-position (γ) or both the 2- (α) and 5-position on the helical sense preference of PNA:PNA duplexes has also been reported.[70] **Figure 24** shows the CD spectra of PNA:PNA duplexes bearing one chiral (L/D)-lysine-based monomers at either the 2-position, 5-position or both.

Table 2. Molar ellipticity at 256 nm of PNA:PNA duplexes bearing one or three α-substituted chiral units, reprinted with permission from reference 63

	Molar ellipticity at 256 nm	
Duplex	$[\theta]_D$	$[\theta]_L$
α-Lys (C-term)	-500	+500
α-Lys (Middle)	+6,500	-6,500
3 α-Lys	+7,500	-7,500
3 α-L-Asp	-	-10,800
3 α-D-Glu	+11,200	-

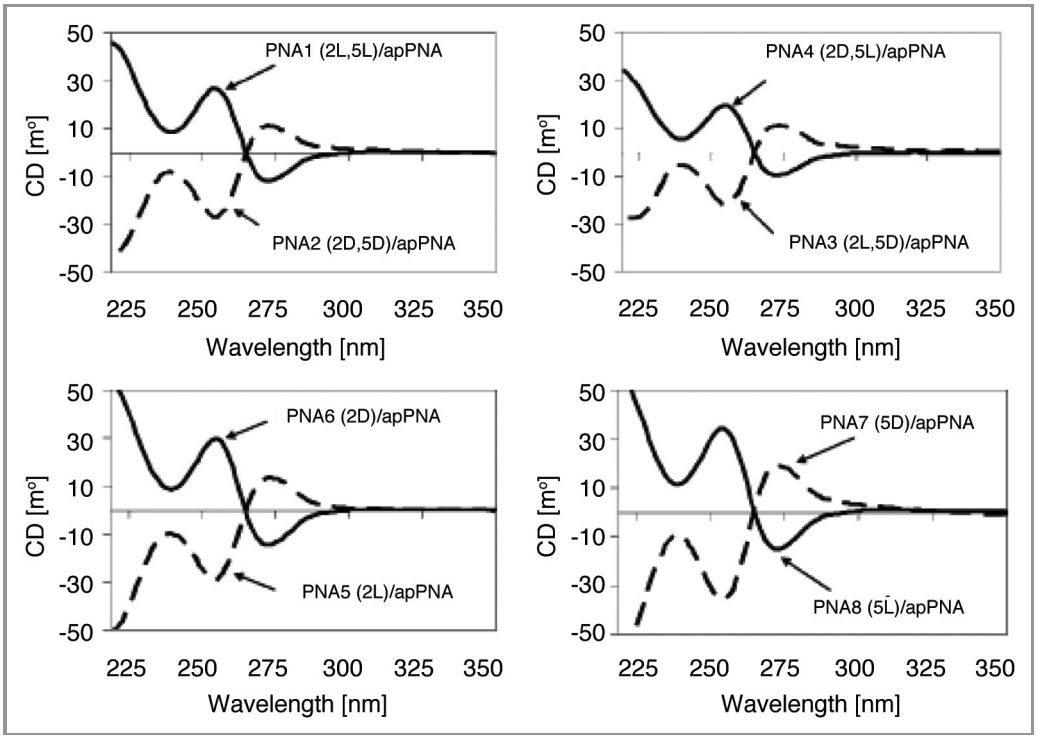

Figure 24. CD spectra of PNA:PNA duplexes bearing a chiral lysine-based monomer with one or two stereogenic centers at 2- (α) or 5- (γ) position. Reprinted with permission from reference 70.

In the 2-position (α), the D-configuration favors right-handed helices, while the L-configuration favors left-handed helices, which is consistent with the results reported in **Figure 18** (see the discussion above). Quite interestingly, the opposite is true if the stereogenic center is placed in the 5-position (γ): the D-enantiomer favors left-handed helices, while the L-enantiomer favors right-handed helices. When two stereogenic centers were simultaneously present, two different situations arose: (1) (2L,5D) as well as (2D,5L) leads to "chiral accordance" since both stereocenters induce the same preferential handedness; (2) (2L,5L) and also (2D,5D) give rise to a "chiral conflict,"[73] by inducing opposite helical handedness. The helical sense of the derived PNA:PNA duplexes was similar to that of the duplexes with only the 5-substitution demonstrating that the 5-position appears to be prevalent in inducing the preferred helical conformation and overwhelms the effect of the chiral induction exerted by the stereogenic center at the 2-position.

This behavior may be rationalized according to the following considerations. The preferential helical senses are hypothesized to arise from the attempt to minimize the intra-strand steric clashes due to the amino acid side chains.[70,72] When a PNA with a "chiral conflict" adopts a helical conformation that is either right-handed or left-handed, this helical arrangement should be sterically unfavorable for one of its two "conflicting" amino acid side chains; therefore, one or more bonds must rotate in order to minimize the clashes, eventually inducing a modification in the preferred PNA conformation. Interestingly, based on the chiral conflict concept encountered in the polyisocyanates, temperature could play an interesting chiral optical role in these chirally conflicted PNA-PNA duplexes.[73]

Acknowledgments

The present review is being written in honor of Prof Rosangela Marchelli for her leading contributions in the field of DNA recognition by peptide nucleic acids (PNAs).

References

1. Cantor CR, Schimmel PR. The conformation of biological macromolecules. Biophysical Chemistry, Part I. New York: W. H. Freeman and Co, 1980.
2. Ellison MJ, Kelleher RJ, 3rd, Wang AH-J, Habener JF, Rich A. Sequence-dependent energetics of the B-Z transition in supercoiled DNA containing nonalternating purine-pyrimidine sequences. Proc Natl Acad Sci U S A 1985; 82:8320-4; PMID:3866225; http://dx.doi.org/10.1073/pnas.82.24.8320
3. Tobias DJ, Brooks CL, III. Thermodynamics and mechanism of α-helix iniatiation in alanine and valine peptides. Biochem 1991; 30:6059-70; http://dx.doi.org/10.1021/bi00238a033
4. Ho PS, Ellison MJ, Quigley GJ, Rich A. A computer aided thermodynamic approach for predicting the formation of Z-DNA in naturally occurring sequences. EMBO J 1986; 5:2737-44; PMID:3780676
5. Green MM, Jain V. Homochirality in life: two equal runners, one tripped. Orig Life Evol Biosph 2010; 40:111-8; PMID:19911302; http://dx.doi.org/10.1007/s11084-009-9180-7
6. Kreil G. D-amino acids in animal peptides. Annu Rev Biochem 1997; 66:337-45; PMID:9242910; http://dx.doi.org/10.1146/annurev.biochem.66.1.337
7. Fujii N, Momose Y, Ishii N, Takita M, Akaboshi M, Kodama M. The mechanisms of simultaneous stereoinversion, racemization, and isomerization at specific aspartyl residues of aged lens proteins. Mech Ageing Dev 1999; 107:347-58; PMID:10360687; http://dx.doi.org/10.1016/S0047-6374(98)00129-8

8. Lorenzi GP, Jackle H, Tomasic L, Rizzo V, Pedone C. Nature and relative stability of monomeric and dimeric species of the D,L-alternating octapeptide Boc-(L-Val-D-Val)$_4$-OMe in cyclohexane or chloroform solution. J Am Chem Soc 1982; 104:1728-33; http://dx.doi.org/10.1021/ja00370a047
9. Lotz B, Colonna-Cesari F, Heitz F, Spach G. A family of double helices of alternating poly(γ-benzyl-D-L-glutamate), a stereochemical model for gramicidin A. J Mol Biol 1976; 106:915-42; PMID:62054; http://dx.doi.org/10.1016/0022-2836(76)90343-0
10. Paolillo L, Temussi P, Trivellone E, Bradbury EM, Crane-Robinson C. Conformational studies of random DL copolypeptides in solution using high-resolution nucleic magnetic resonance. Macromolecules 1973; 6:831-8; http://dx.doi.org/10.1021/ma60036a010
11. Haque TS, Little JC, Gellman SH. Mirror image reverse turns promote β-hairpin formation. J Am Chem Soc 1994; 116:4105-6; http://dx.doi.org/10.1021/ja00088a067
12. Gulik-Krzywicki T, Fouquey C, Lehn JM. Electron microscopic study of supramolecular liquid crystalline polymers formed by molecular-recognition-directed self-assembly from complementary chiral components. Proc Natl Acad Sci U S A 1993; 90:163-7; PMID:11607345; http://dx.doi.org/10.1073/pnas.90.1.163
13. Khazanovich N, Granja JR, McRee DE, Milligan RA, Ghadri MR. Nanoscale tubular ensembles with specified internal diameters. Design of self-assembled nanotube with a 13-Å pore. J Am Chem Soc 1994; 116:6011-2; http://dx.doi.org/10.1021/ja00092a079
14. Green MM, Peterson NC, Sato T, Teramoto A, Cook R, Lifson S. A helical polymer with a cooperative response to chiral information. Science 1995; 268:1860-6; PMID:17797527; http://dx.doi.org/10.1126/science.268.5219.1860
15. Pino P, Lorenzi GP. Optically active vinyl polymers. II. Optical activity of isotactic and block polymers of optically active a-olefins in dilute hydrocarbon solution. J Am Chem Soc 1960; 82:4745-7; http://dx.doi.org/10.1021/ja01502a077
16. Green MM, Park J-W, Sato T, Teramoto A, Lifson S, Selinger RLB, et al. The macromolecular route of chiral amplification. Angew Chem Int Ed Engl 1999; 38:3138-54; PMID:10556885; http://dx.doi.org/10.1002/(SICI)1521-3773(19991102)38:21<3138::AID-ANIE3138>3.0.CO;2-C
17. Allegra G, Corradini P, Ganis P. A model of the chain conformation of an isotactic vinyl polymer having optically active side groups. Makromol Chem 1966; 90:60-5; http://dx.doi.org/10.1002/macp.1966.020900106
18. Farina M.. The stereochemistry of linear macromolecules. Top Stereochem 1987; 17:1-111.
19. Neuensch-wander P, Pino P. Optically active vinyl polymers. XXVI. The unperturbed dimensions of poly (S)-4-methyl-1-hexene and poly-4-methyl-1-pentene. Eur Polym J 1983; 19:1075-9; http://dx.doi.org/10.1016/0014-3057(83)90075-7
20. Pino P, Carlini C, Chiellini E, Ciardelli F, Salvadori P. Dissymmetric perturbed aromatic chromophore in stereoregular copolymers of (R)-3,7-dimethyl-1-octene with styrene. J Am Chem Soc 1968; 90:5025-7; http://dx.doi.org/10.1021/ja01020a047
21. Pino P, Ciardelli F, Montagnoli G, Pieroni O. The relation between monomer optical purity and polymer rotatory power in some linear poly-α-olefins. J Polym Sci Polym Lett 1967; 5:307-11; http://dx.doi.org/10.1002/pol.1967.110050406
22. Carlini C, Ciardelli F, Pino P. Optical activity and conformation in solution of stereoregular co-polymers of (S)-4-methyl-1-hexene with 4-methyl-1-pentene. Makromol Chem 1968; 119:244-8; http://dx.doi.org/10.1002/macp.1968.021190128
23. Green MM, Andreola C, Munoz B, Reidy MP, Zero K. Macromolecular stereochemistry: a cooperative deuterium isotope effect leading to a large optical rotation. J Am Chem Soc 1988; 110:4063-5; http://dx.doi.org/10.1021/ja00220a070
24. Gu H, Nakamura Y, Sato T, Teramoto A, Green MM, Andreola C, et al. Molecular-weight dependence of the optical rotation of poly((R)-2-deuterio-n-hexyl isocyanate). Macromolecules 1995; 28:1016-24; http://dx.doi.org/10.1021/ma00108a032
25. Jain V, Cheon K-S, Tang K, Jha S, Green MM. Chiral cooperativity in helical polymers. Isr J Chem 2011; 51:1067-74; http://dx.doi.org/10.1002/ijch.201100050
26. Mansfield ML. Broken wormlike chain model of semiflexible polymers. Macromolecules 1986; 19:854-9; http://dx.doi.org/10.1021/ma00157a064
27. Jha SK, Cheon K-S, Green MM, Selinger JV. Chiral optical properties of a helical polymer synthesized from near racemic chiral monomers highly diluted with achiral monomers. J Am Chem Soc 1999; 121:1665-73; http://dx.doi.org/10.1021/ja983202s
28. Selinger JV, Selinger RLB. Theory of chiral order in random copolymer. Phys Rev Lett 1996; 76:58-61.
29. Green MM, Garetz BA, Munoz B, Chang H, Hoke S, Cooks RG. Majority rules in the copolymerization of mirror image isomers. J Am Chem Soc 1995; 117:4181-2; http://dx.doi.org/; 10.1021/ja00119a039
30. Cheon K-S, Selinger JV, Green MM. Counterintuitive influence of microscopic chirality on helical order in polymers, (Birthday Issue for Kurt Mislow). J Phys Org Chem 2004; 17:719-23.
31. Yashima E, Maeda K. Chirality-responsive helical polymers. Macromolecules 2008; 41:3-12; http://dx.doi.org/10.1021/ma071453s
32. Yashima E, Maeda K, Iida H, Furusho Y, Nagai K. Helical polymers: synthesis, structures, and functions. Chem Rev 2009; 109:6102-211; PMID:19905011; http://dx.doi.org/10.1021/cr900162q
33. Pijper D, Feringa BL. Control of dynamic helicity at the macro- and supramolecular level. Soft Matter 2008; 4:1349-72; http://dx.doi.org/10.1039/b801886c
34. Nakano T, Okamoto Y. Synthetic helical polymers: conformation and function. Chem Rev 2001; 101:4013-38; PMID:11740925; http://dx.doi.org/10.1021/cr0000978
35. Fujiki M, Koe JR, Terao K, Sato T, Teramoto A, Watanabe J. Optically active polysilanes. Ten years of progress and new polymer twist for nanoscience and nanotechnology. Polym J 2003; 35:297-344; http://dx.doi.org/10.1295/polymj.35.297
36. Palmans ARA, Meijer EW. Amplification of chirality in dynamic supramolecular aggregates. Angew Chem Int Ed Engl 2007; 46:8948-68; PMID:17935098; http://dx.doi.org/10.1002/anie.200701285
37. Masuda M, Jonkheijm P, Sijbesma RP, Meijer EW. Photoinitiated polymerization of columnar stacks of self-assembled trialkyl-1,3,5-benzenetricarboxamide derivatives. J Am Chem Soc 2003; 125:15935-40; PMID:14677985; http://dx.doi.org/10.1021/ja037927u
38. Kim H-J, Lim Y-B, Lee M. Self-assembly of supramolecular polymers into tunable helical structures. J. Polym. Sci. Polym Chem 2008; 46:1925-35; http://dx.doi.org/10.1002/pola.22569
39. Delsuc N, Massip S, Léger J-M, Kauffmann B, Huc I. Relative helix-helix conformations in branched aromatic oligoamide foldamers. J Am Chem Soc 2011; 133:3165-72; PMID:21306159; http://dx.doi.org/10.1021/ja110677a
40. Jin W, Fukushima T, Niki M, Kosaka A, Ishii N, Aida T. Self-assembled graphitic nanotubes with one-handed helical arrays of a chiral amphiphilic molecular graphene. Proc Natl Acad Sci U S A 2005; 102:10801-6; PMID:16043721; http://dx.doi.org/10.1073/pnas.0500852102
41. Yashima E, Maeda K, Okamoto Y. Memory of macromolecular helicity assisted by interaction with achiral small molecules. Nature 1999; 399:449-51; http://dx.doi.org/10.1038/20900
42. Yashima E, Matsushima T, Okamoto Y. Chirality assignment od amines and amino alcohols based on circular dichroism induced by helix formation of stereoregular poly(4-carboxyphenylacetylene) through acid-base complexation. J Am Chem Soc 1997; 119:6345-59; http://dx.doi.org/10.1021/ja964470y
43. Saito MA, Maeda K, Onouchi H, Yashima E. Synthesis and macromolecular helicity induction of a stereoregular polyacetylene bearing a carboxy group with natural amino acids in water. Macromolecules 2000; 33:4616-8; http://dx.doi.org/10.1021/ma000484j
44. Yashima E, Maeda Y, Okamoto Y. Helix-Helix transition of optically active poly((1R,2S)-N-(4-ethynylbenzyl) norephedrine) induced by diastereomeric acid-base complexation using chiral stimuli. J Am Chem Soc 1998; 120:8895-6; http://dx.doi.org/10.1021/ja9818349
45. Fujiki M, Nakashima H, Toyoda S, Koe JR. Chirality in the polysilanes. Top Stereochem 2003; 24:209-80; http://dx.doi.org/10.1002/0471471895.ch4
46. Okamoto Y, Nakano T. Asymmetric polymerization. Chem Rev 1994; 94:349-72; http://dx.doi.org/10.1021/cr00026a004
47. Nakano T, Shikisai Y, Okamoto Y. Helix-sense-selective free radical polymerization of 1-phenyldibenzosuberyl methacrylate. Polym J 1996; 28:51-60; http://dx.doi.org/10.1295/polymj.28.51
48. Okamoto Y, Suzuki K, Ohta K, Hatada K, Yuki H. Optically active poly(triphenylmethyl methacrylate) with one-handed helical conformation. J Am Chem Soc 1979; 101:4763-5; http://dx.doi.org/10.1021/ja00510a072
49. Nakano T, Okamoto Y. Synthetic helical polymers: conformation and function. Chem Rev 2001; 101:4013-38; PMID:11740925; http://dx.doi.org/10.1021/cr0000978
50. Nakano T, Hidaka Y, Okamoto Y. Asymmetric polymerization of 9-phenylfluoren-9-yl methacrylate leading to a polymer with main-chain configurational chirality. Synthesis of optically active poly(methyl methacrylate). Polym J 1998; 30:596-600; http://dx.doi.org/10.1295/polymj.30.596
51. Ren C, Chen C, Xi F. Helix-sense-selective copolymerization of phenyl[bis(2-pyridyl)] methyl methacrylate and diphenyl(2-pyridyl)methyl methacrylate and triphenylmethyl methacrylate with chiral anionic initiators. J. Polym. Sci. Polym Chem 1998; 36:2127-33; http://dx.doi.org/10.1002/(SICI)1099-0518(19980915)36:12<2127::AID-POLA19>3.0.CO;2-8
52. Nakano T, Taniguchi K, Okamoto Y. Asymmetric polymerization of diphenyl-3-pyridylmethyl methacrylate leading to optically active polymer with helical conformation and chiral recognition ability of the polymer. Polym J 1997; 29:540-4; http://dx.doi.org/10.1295/polymj.29.540

53. Ren C, Chen F, Xi F, Nakano T, Okamoto Y. Helix-sense-selective polymerization of phenyl[bis(2-pyridyl)] methyl methacrylate and chiral recognition ability of the polymer. J. Polym. Sci. Polym Chem 1993; 31: 2721-8; http://dx.doi.org/10.1002/pola.1993.080311107
54. Okamoto Y, Mohri H, Hatada K. Highly helix-sense-selective polymerization of diphenyl-2-pyridylmethyl methacrylate. Chem Lett 1988; 11:1879-82; http://dx.doi.org/10.1246/cl.1988.1879
55. Okamoto Y, Nishikawa M, Nakato T, Yashima E, Hatada K. Induction od a single-handed conformation through radical polymerization of optically active phenyl-2-pyridyl-o-tolylmethyl methacrylate. Macromolecules 1995; 28:5135-8; http://dx.doi.org/10.1021/ma00118a048
56. Nielsen PE, Egholm M, Berg RH, Buchardt O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science 1991; 254:1497-500; PMID:1962210; http://dx.doi.org/10.1126/science.1962210
57. Corradini R, Sforza S, Tedeschi T, Totsingan F, Manicardi A, Marchelli R. Peptide nucleic acids with a structurally biased backbone. Updated review and emerging challenges. Curr Top Med Chem 2011; 11:1535-54; PMID:21510833; http://dx.doi.org/10.2174/156802611795860979
58. Ganesh KN, Nielsen PE. Peptide nucleic acids: analogs and derivatives. Curr Org Chem 2000; 4:931-43; http://dx.doi.org/10.2174/1385272003375969
59. Yeh JI, Shivachev B, Rapireddy S, Crawford MJ, Gil RR, Du S, et al. Crystal structure of chiral gammaPNA with complementary DNA strand: insights into the stability and specificity of recognition and conformational preorganization. J Am Chem Soc 2010; 132: 10717-27; PMID:20681704; http://dx.doi.org/10.1021/ja907225d
60. Dragulescu-Andrasi A, Rapireddy S, Frezza BM, Gayathri C, Gil RR, Ly DH. A simple γ-backbone modification preorganizes peptide nucleic acid into a helical structure. J Am Chem Soc 2006; 128:10258-67; PMID:16881656; http://dx.doi.org/10.1021/ja0625576
61. Menchise V, De Simone G, Tedeschi T, Corradini R, Sforza S, Marchelli R, et al. Insights into peptide nucleic acid (PNA) structural features: the crystal structure of a D-lysine-based chiral PNA-DNA duplex. Proc Natl Acad Sci U S A 2003; 100:12021-6; PMID:14512516; http://dx.doi.org/10.1073/pnas.2034746100
62. Tackett AJ, Corey DR, Raney KD. Non-Watson-Crick interactions between PNA and DNA inhibit the ATPase activity of bacteriophage T4 Dda helicase. Nucleic Acids Res 2002; 30:950-7; PMID:11842106; http://dx.doi.org/10.1093/nar/30.4.950
63. Sforza S, Haaima G, Marchelli R, Nielsen PE. Chiral peptide nucleic acids (PNAs): Helix handedness and DNA recognition. Eur J Org Chem 1999; 197-204; http://dx.doi.org/10.1002/(SICI)1099-0690(199901)1999:1<197::AID-EJOC197>3.0.CO;2-N
64. Johnson WC. In Circular Dichroism: Principles and Applications; Wiley-VCH (New York) 2000.
65. Wittung P, Nielsen PE, Buchardt O, Egholm M, Nordén B. DNA-like double helix formed by peptide nucleic acid. Nature 1994; 368:561-3; PMID:8139692; http://dx.doi.org/10.1038/368561a0
66. Wittung P, Eriksson M, Lyng R, Nielsen PE, Norden B. Induced chirality in PNA-PNA duplexes. J Am Chem Soc 1995; 117:10167-73; http://dx.doi.org/10.1021/ja00146a001
67. Totsingan F, Jain V, Bracken WC, Faccini A, Tedeschi T, Marchelli R, et al. Conformational heterogeneity in PNA:PNA duplexes. Macromolecules 2010; 43:2692-703; http://dx.doi.org/10.1021/ma902797f
68. Rasmussen H, Kastrup JS, Nielsen JN, Nielsen JM, Nielsen PE. Crystal structure of a peptide nucleic acid (PNA) duplex at 1.7 A resolution. Nat Struct Biol 1997; 4:98-101; PMID:9033585; http://dx.doi.org/10.1038/nsb0297-98
69. Petersson B, Nielsen BB, Rasmussen H, Larsen IK, Gajhede M, Nielsen PE, et al. Crystal structure of a partly self-complementary peptide nucleic acid (PNA) oligomer showing a duplex-triplex network. J Am Chem Soc 2005; 127:1424-30; PMID:15686374; http://dx.doi.org/10.1021/ja0458726
70. Sforza S, Tedeschi T, Corradini R, Marchelli R. Induction of helical handedness and DNA binding properties of peptide nucleic acids (PNAs) with two stereogenic centers. Eur J Org Chem 2007; 5879-85; http://dx.doi.org/10.1002/ejoc.200700644
71. Bezer S, Rapireddy S, Skorik YA, Ly DH, Achim C. Coordination-driven inversion of handedness in ligand-modified PNA. Inorg Chem 2011; 50:11929-37; PMID:22059624; http://dx.doi.org/10.1021/ic200855p
72. Tedeschi T, Sforza S, Dossena A, Corradini R, Marchelli R. Lysine-based peptide nucleic acids (PNAs) with strong chiral constraint: control of helix handedness and DNA binding by chirality. Chirality 2005; 17(Suppl):S196-204; PMID:15952136; http://dx.doi.org/10.1002/chir.20128
73. Tang K, Green MM, Cheon KS, Selinger JV, Garetz BA. Chiral conflict. The effect of temperature on the helical sense of a polymer controlled by the competition between structurally different enantiomers: from dilute solution to the lyotropic liquid crystal state. J Am Chem Soc 2003; 125:7313-23; PMID:12797806; http://dx.doi.org/10.1021/ja030065c