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Racemic β -Sheets as Templates of Relevance to the Origin of Homochirality of Peptides: Lessons from Crystal Chemistry

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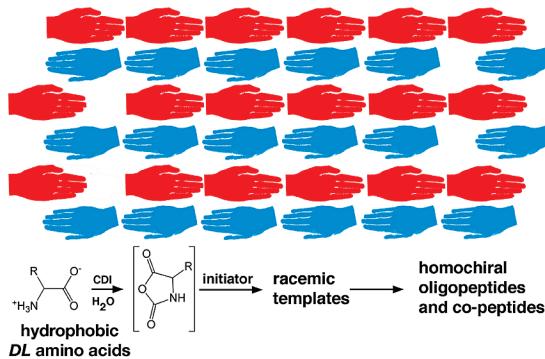
The origin of life is a historical event that has left no relevant fossils; therefore, it is unrealistic to reconstruct the chronology of its occurrence. Instead, by performing laboratory experiments under conditions that resemble the prebiotic world, one might validate feasible reaction pathways and reconstruct model systems of artificial life. Creating such life in a test tube should go a long way toward removing the shroud of mystery over how it began naturally.

The riddle of the appearance of natural proteins and nucleic acids—that is, biopolymers wholly consisting of homochiral subunits (L-amino acids and D-sugars, respectively)—from the unanimated racemic prebiotic world is still unsolved. There are two hypotheses concerning the sequence of their emergence: one maintains that long homochiral (isotactic) peptides must have been formed after the appearance of the first living systems, whereas the other presumes that such biopolymers preceded the primeval enzymes. The latter scenario necessitates, however, the operation of nonlinear synthetic routes, because the polymerization of racemates in ideal solutions yields chains composed of residues of either handedness. In this Account, we suggest applying lessons learned from crystal chemistry, in which molecules from isotropic media are converted into crystals with three-dimensional (3D) periodic order, to understand how the generation of homochiral peptides from racemic α -amino acids might be achieved, despite its seemingly overwhelming complexity.

We describe systems that include the self-assembly of activated α -amino acids either in two-dimensional (2D) or in 3D crystals, followed by a partial lattice-controlled polymerization at the crystal–aqueous solution interface. We also discuss the polymerization of mixtures of activated hydrophobic racemic α -amino acids in aqueous solutions, as initiated by primary amines or thiols. The distribution of the diastereomeric oligopeptides was analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and MS/MS with monomers enantioselectively tagged with deuterium. The reaction performed in aqueous solutions encompasses the following sequential steps: (i) formation of a library of short racemic peptides enriched with isotactic diastereoisomers during the early stages of the polymerization, and (ii) self-assembly of oligopeptides into racemic β -sheet colloidal-like aggregates that are delineated by enantiotopic sites or rims; these operate as templates (nuclei) for regio-enantioselective growth in the ensuing steps of chain elongation.

Desymmetrization of the racemic mixtures of peptides was achieved with enantiopure α -amino acid esters as initiators. The enantiomeric excess of the isotactic peptides, not including the initiator, varies with chain length, the result of a cross-enantiomeric impeding mechanism. Our results suggest a feasible scenario in which primitive homochiral peptides might have emerged early in the prebiotic world.

regio-enantioselection within racemic templates



1. Introduction

Origin of life cannot be discovered, it can be only re-invented.

Albert Eschenmoser¹

Possible routes and the chronology of when the long homochiral biopolymers, composed from residues of the same handedness, had emerged from the achiral or racemic prebiotic world has attracted, during the years, the interest of theoreticians and experimentalists alike.^{2–5} One scenario suggests that the homochiral peptides must have appeared prior to the appearance of the primeval enzymes.^{6,7} Polymerization of racemic α -amino acids, however, suffers from serious drawbacks as a result of a binomial distribution in the composition of the peptide chains comprising residues of either handedness. A way to override this problem is to take advantage of supramolecular architectures, such as α -helices^{8–10} or pleated β -sheets,^{11,12} formed during the polymerization and operating as templates for the emergence of isotactic peptides. Major limitations in the creation of the above templates are associated with the formation of isotactic chains composed of at least eight residues of the same chirality, a probability that according to a binomial distribution is as low as 1/2⁸ and therefore is difficult to materialize in a random polymerization of racemic monomers. Studies by Goldberg¹³ and Luisi^{14,15} have reported that during the polymerization of activated α -amino acids, the N-terminal residue of the growing oligopeptide chains exerts asymmetric induction to yield libraries of short oligopeptides, where the isotactic ones are expressed slightly beyond those anticipated from a random polymerization.

In our approach to the problem, we have applied lessons from our previous experience in the fields of solid-state chemistry,¹⁶ crystal growth,^{4,17} and the surface sciences.¹⁸ In all the experiments described in this Account, we used either racemic activated α -amino acids (described in sections 2, 3.1 and 3.2) or those activated *in situ* (described in sections 3.3 and 3.4) and performed the polymerization reactions in an aqueous environment. The diastereoisomeric composition of the oligopeptides was determined by MALDI-TOF MS and MALDI-TOF MS/MS, using enantioselectively deuterated samples. The two-pronged approach comprises: (i) the organization of the racemates into 2D or 3D crystalline architectures, prior to the polymerization reaction, followed by a lattice-controlled reaction^{19,20} and (ii) the polymerization of the racemates that starts in an isotropic aqueous solution but pursues under heterogeneous conditions as a result of the formation of

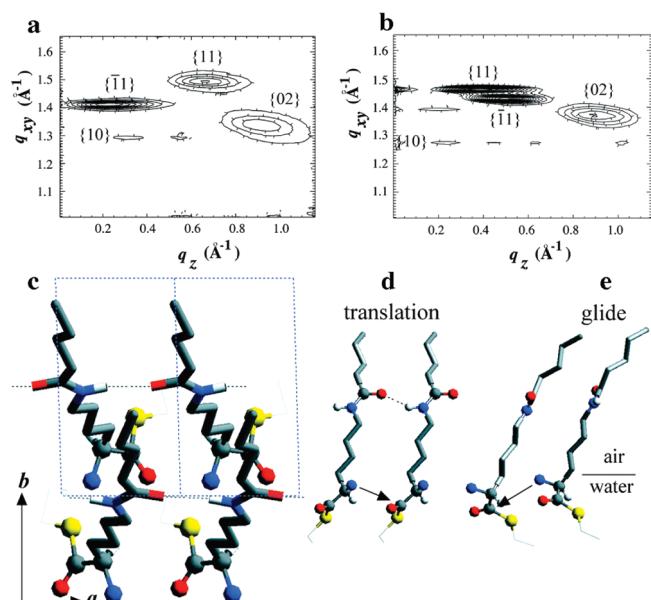


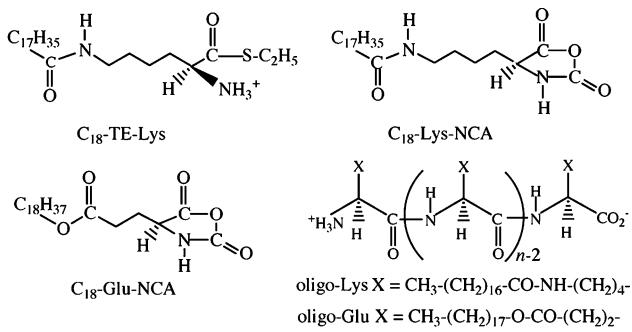
FIGURE 1. Two-dimensional GIXD patterns of (a) L- and (b) (DL)-C₁₈-TE-Lys crystallites on water, (c) 2D packing arrangement of (DL)-C₁₈-TE-Lys, and (d, e) pairs of molecules related by translation and by glide symmetry, respectively, viewed parallel to the water surface.

supramolecular aggregates that serve as templates in the propagation step of chain elongation.^{21–23}

2. Polymerization of Amphiphilic Activated Racemic Amino Acids at the Air/Water Interface^{19,24–27}

Amphiphilic racemates can self-assemble at the air/water interface into 2D crystallites comprising (i) racemic compounds, where the D and L enantiomers are packed in the same crystallite, (ii) 2D conglomerates due to spontaneous segregation of each enantiomer into separate domains and (iii) solid solutions. The crystalline structure of these architectures can be determined by grazing incidence X-ray diffraction (GIXD) at almost molecular level.^{18,28} We have taken advantage of such self-assembled architectures for the generation of homochiral peptides from racemic amphiphilic monomers in a crystalline environment.

A first example presents the polymerization of 2D racemic crystallites of N^ε-alkanoyl-lysine thio-ethyl ester (C₁₈-TE-Lys) at the air/water.^{19,25} The GIXD patterns of (DL)- and (L)-C₁₈-TE-Lys crystallites on water and the packing arrangement of racemate are shown in Figure 1. In the racemic crystallites, the two enantiomers are arranged in homochiral rows (Figure 1c). When the reaction was initiated with Ag⁺ ions, the oligomerization took place preferentially between molecules of the same handedness along the translation *a*-axis (Figure 1d), to yield isotactic diastereoisomers.



The homochiral hexapeptides were formed in excess, by a factor of 7.5–9 higher as compared with a binomial distribution, as determined by MALDI-TOF MS on samples where the C₁₈H₃₇ chains of one of the enantiomers were per-deuterated, Figure 2.

The racemic compositions of the homochiral oligopeptides could be desymmetrized by the insertion of small quantities of the corresponding esters of the amphiphilic enantiopure α -amino acids. Polymerization in monolayers of amphiphilic esters of α -amino acids at the air/water interface has been extensively studied by Fukuda et al.,²⁹ but our recent MALDI-TOF MS studies on the polymerization of such esters have shown that they yield only dipeptides.³⁰ These esters form 2D quasi-racemates by being incorporated enantioselectively in the rows of thio-esters of the same absolute configuration. Moreover, they operate not only as efficient initiators but also as chain terminators of the peptides of the same absolute configuration to yield shorter chains in comparison to the enantiomeric chains that grow unperturbed.²⁷

(D,L)-C₁₈-Glu-NCA also self-assembles as a 2D racemic crystal but with a packing arrangement different from that of C₁₈-TE-Lys, Figure 3.^{19,25}

The reaction in these crystallites takes place along the glide plane between heterochiral molecules to form syndiotactic oligopeptides in excess, Figure 4a. Nonracemic mixtures of 3:7 or 4:6 D,L-C₁₈-Glu-NCA on water (Figure 4b,c)²⁵ undergo a phase separation into racemic and enantiomorphous 2D domains. The polymerization products are rich in short heterochiral oligopeptides whereas the longer ones containing nine or ten residues are almost isotactic. Similar results were obtained for nonracemic C₁₈-TE-Lys within a phospholipid environment.²⁶

D,L-C₁₈-Lys-NCA undergoes spontaneous segregation into enantiomorphous 2D domains and the polymerization occurs preferentially within each crystallite.²⁴

3. Racemic β -Sheets as Intermediate Templates

3.1. Isotactic Oligopeptides Generated in Racemic Crystals of α -Amino Acid-NCA.^{20,31–33} In general, polymerization reactions of monomers in the crystalline state, occurring with reduction in density, yield atactic polymers as a result of the monomer crystal destruction during the reaction. However, the formation of syndiotactic polymers in the polymerization of C₁₈-Glu-NCA within the 2D crystallites at the air–water interface suggested the operation of a lattice-controlled mechanism that could also operate in 3D crystals. Therefore, we investigated the polymerization of (D,L)-PhenNCA^{20,31,32} and (D,L)-ValNCA³³ 3D crystals suspended in aqueous solutions. The packing arrangements of these crystals and their ability to undergo polymerization in hexane were reported previously by Kanazawa et al.^{34,35} We demonstrated by MALDI-TOF MS that the reaction is lattice-controlled using enantiomerically deuterium tagged samples (Figure 5a, b)^{20,33} or quasi-racemates of D-PheNCA/L-ThieNCA³² and determining the diastereoisomeric distribution of the oligomers formed in these crystals suspended in water. Figure 6 shows that the amount of the isotactic oligo-phenylalanine increases with peptide length departing up to 6 orders of magnitude from a random binomial distribution.

These results are explained by a self-assembly of the short isotactic oligopeptides into racemic β -sheets, antiparallel for oligo-phenylalanine and parallel for oligo-Val, where chains of opposite handedness are packed in alternating motifs, Figure 7a–c.²⁰ Once these β -sheets are formed they exert steric control in the formation of the long isotactic peptides.

The desymmetrization of the racemic mixtures of isotactic peptides was achieved by initiating the reaction with enantiopure methyl esters of α -amino acids. The enantiomeric excess (ee) of the isotactic tetrapeptides (not considering the residue of enantiopure initiator located at the C-terminus) was in favor of chains composed from residues of the same handedness as that of the initiator. However, beyond the tetramer, there is a reversal of the ee of the isotactic peptides, which increases with chain length, Figure 8.³²

The reversal in ee is in keeping with the formation of rippled antiparallel (ap) β -sheets since the initiator of say L-absolute configuration present at the C-terminus of the chains composed from D-residues should engender steric hindrance in the growth of the neighboring chains composed from L-residues. On the other hand, the same initiator attached to the L-chains integrates coherently in these chains and does not interfere with the regular growth of the adjacent D-chains, Fig-

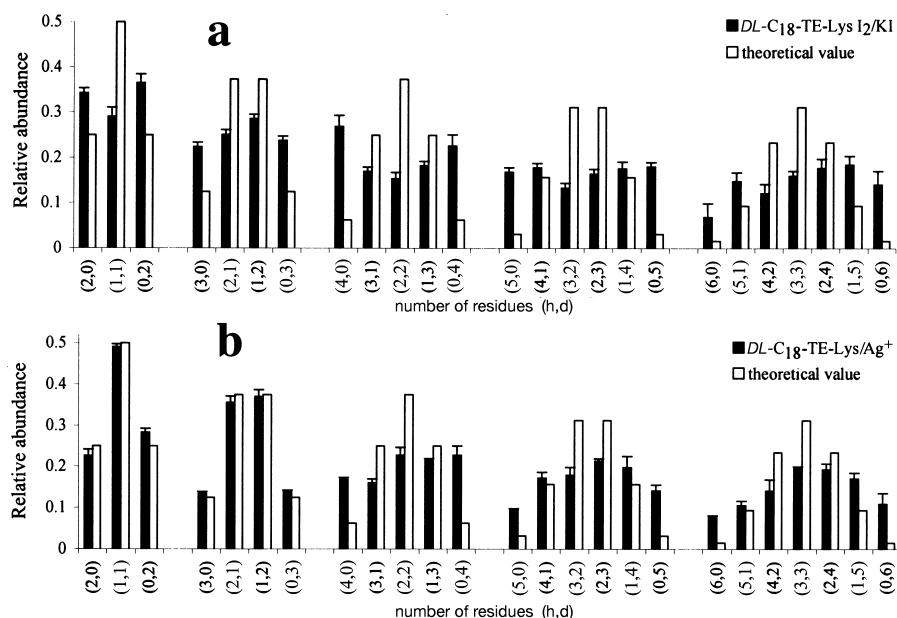


FIGURE 2. Histogram of the distribution of the diastereomeric oligopeptides obtained at the air–water interface from (DL)-C₁₈-TE-Lys with various catalysts, as analyzed by MALDI-TOF MS: (a) I₂/KI and (b) AgNO₃.

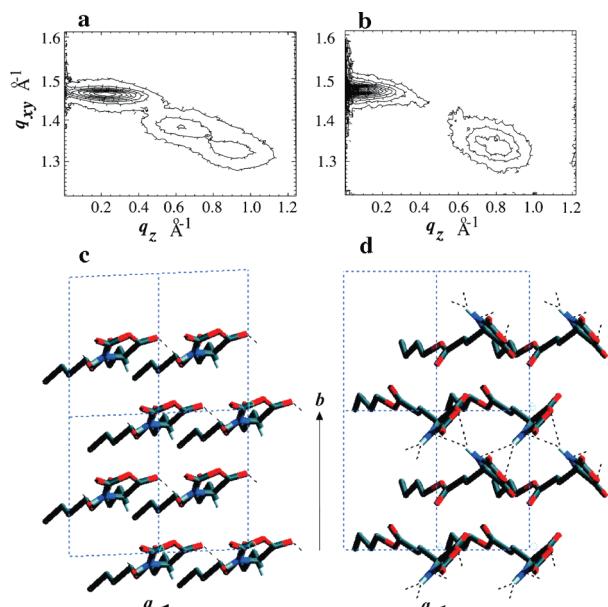


FIGURE 3. The GIXD patterns and the 2D packing arrangements of C₁₈-Glu-NCA on water: (a, c) enantiopure and (b, d) racemic.

ure 9. Such reversal in ee as function of length, due to enantiomeric cross-impediment, is unique for the antiparallel rippled β -sheets and has not been observed in the parallel β -sheets of oligo-valine formed in the (DL)-ValNCA crystals.³³

By contrast to the polymerization in (DL)-PheNCA and (DL)-ValNCA, in the crystals of (DL)-LeuNCA, there are two competitive reaction pathways, including one between molecules of opposite handedness that prevents the formation of the isotactic oligopeptides as the dominant diastereoisomers, Figure 5c.³³

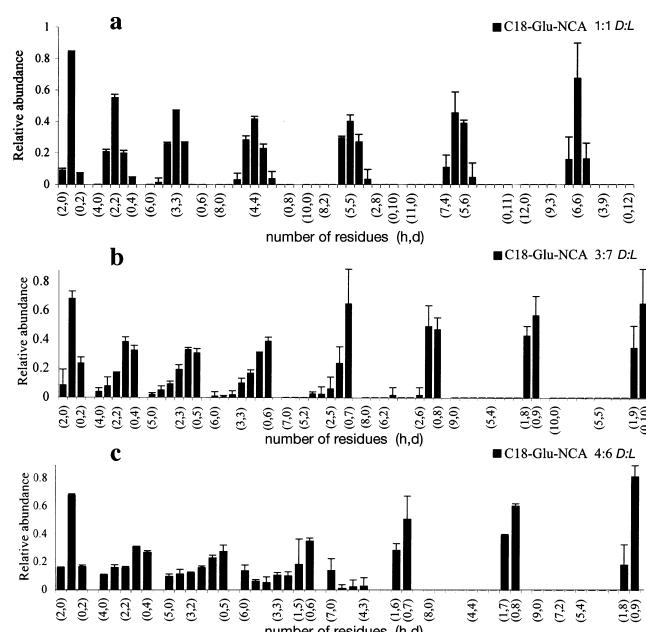


FIGURE 4. Histogram of diastereoisomeric distribution of the oligopeptides obtained at the air–water interface from C₁₈-Glu-NCA by MALDI-TOF MS analysis: (a) racemic; (b) 3:7 and (c) 4:6 D:L-C₁₈-Glu-NCA.

On the basis of the results from the reactions in the three crystals, we propose a three-step mechanism: (i) The reaction is initiated at the crystal/aqueous interface and the formation of short isotactic dimers to tetramers in excess is controlled by the unique packing arrangement of the crystal. (ii) Once a sufficient amount of such oligopeptides is formed, they self-assemble as racemic (rippled) antiparallel or parallel β -sheets, composed from alternating oligo L- and oligo D-chains, as dic-

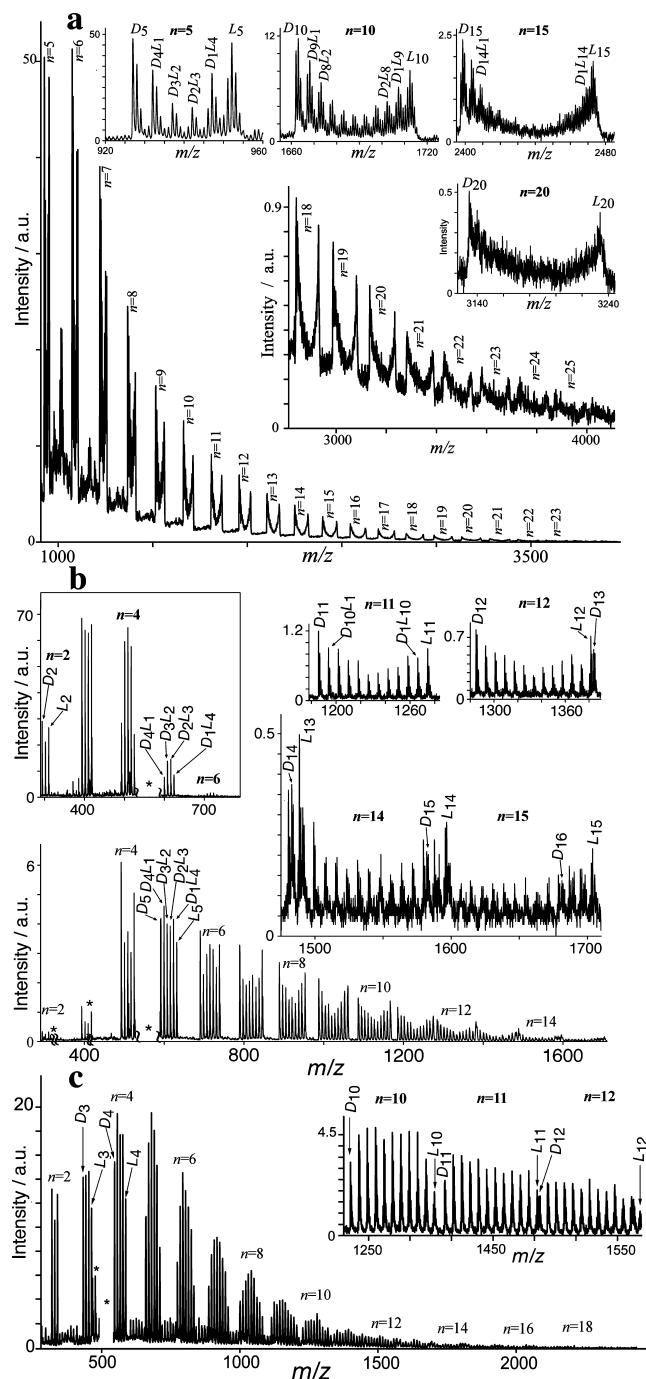


FIGURE 5. MALDI-TOF MS spectra of the water-insoluble oligopeptides (Na^+ cationized) obtained from the polymerization in crystals of (a) (DL)-PheNCA, (b) (DL)-ValNCA, and (c) (DL)-LeuNCA suspended in water containing *n*-butylamine initiator. The inset in panel b, left, shows the spectrum of the water-soluble fraction, and the other insets show expanded spectra of the diastereoisomers of various lengths, *n*. In contrast to (DL)-PheNCA and (DL)-ValNCA that yield primarily isotactic oligopeptides, those obtained from (DL)-LeuNCA were primarily heterochiral.

tated by the packing arrangement of the monomer crystal. (iii) Such sheets operate as regio-enantioselective templates in the ensuing stages of chain elongation.

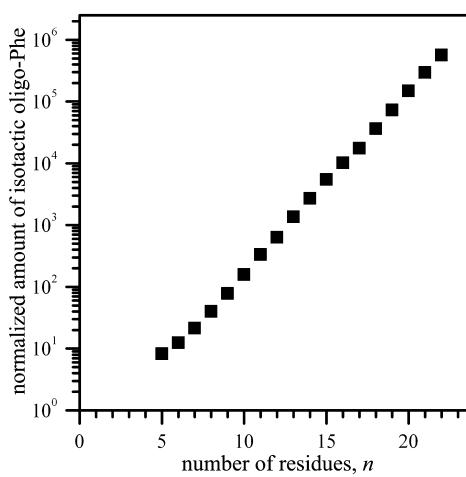


FIGURE 6. Plot of the normalized amount of isotactic oligo-phenylalanine of length *n* = 5–22, normalized to the values for a theoretical random process.

3.2. Isotactic Oligopeptides from the Polymerization of Racemic ValNCA or LeuNCA in Aqueous Solutions.^{21,22} The mechanism of the solid-state polymerization of racemic NCA crystals suggested that similar β -sheet templates might emerge also during the polymerization of such monomers in aqueous solutions and possibly induce the formation of isotactic peptides.²¹ Indeed, this expectation was experimentally fulfilled with the polymerization of DL-ValNCA and DL-LeuNCA in aqueous solutions. Colloidal β -sheets precipitated during the polymerization reactions, as confirmed by X-ray powder diffraction and FTIR measurements.²² The diastereoisomeric distribution of the oligopeptides depends upon the concentration of the initiator, as shown as histograms in Figure 10 for the oligo-valine products obtained with 25% or 5% *n*-butylamine initiator compared with the values calculated assuming a binomial distribution.²² The short peptides comprise all possible diastereoisomers of similar molar fractions, for both concentrations of the initiator. On the other hand, the quantity of the isotactic diastereoisomers beyond the heptamers (7–14-mers) increases with length, thus significantly departing from the binomial distribution. The enhancement of the isotactic peptides, Figure 11, has common features to that shown in Figure 6 for oligo-phenylalanine generated in the crystalline phase. For dodecapeptides, the enhancement of the isotactic peptides is 550 at 25% initiator and only 200 at 5%. However, for the peptides comprising 18 residues formed with 5% initiator, the enhancement increases up to \sim 7000.²²

These results suggest that the β -sheet templates are composed from libraries of racemic isotactic and atactic short oligopeptides, where the concentration of the isotactic ones is augmented with an increase of the concentration of the initiator. The NH_2 groups of the N-terminus residues, which are

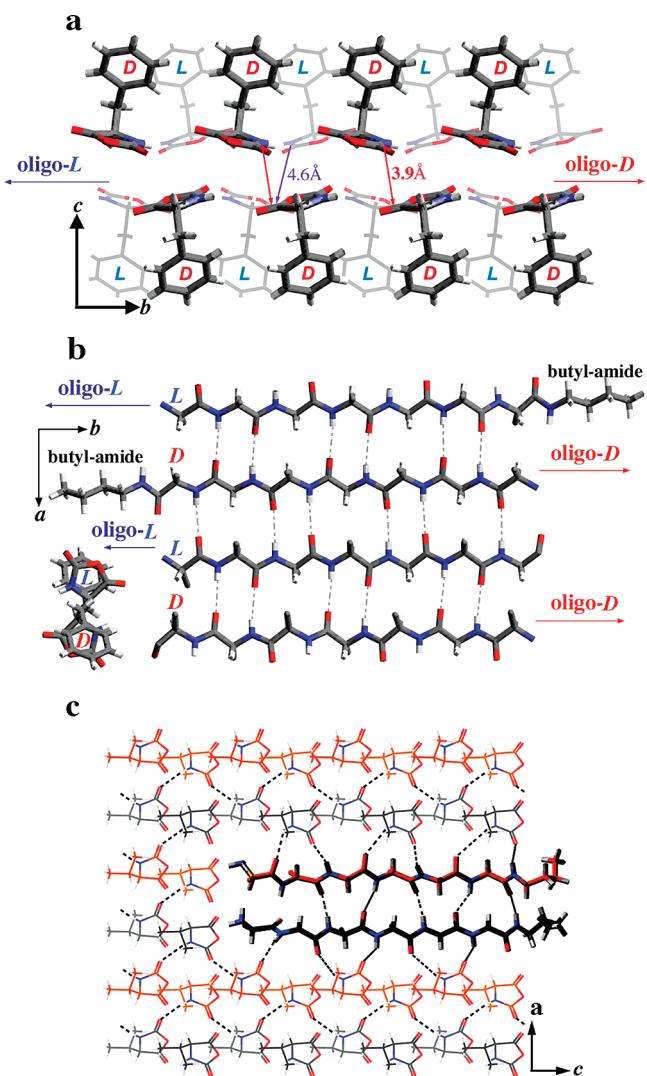


FIGURE 7. (a) Packing arrangement of (DL)-PheNCA crystal viewed along the a -axis, showing four rows of molecules in an enantio-polar arrangement. The polymerization of the D-molecules (in bold) occurs along the $+b$ direction to yield oligo-D chains and that of the L-molecules along the $-b$ direction to yield L-oligopeptides, as modeled in panel b. (c) Computer model of two adjacent isotactic D- and L-hexapeptides assembled into a racemic parallel β -sheet formed within the lattice of the (DL)-ValNCA crystal. For clarity, the C-atoms of the D-monomer molecules and those of the D-peptide are shown in orange color and the *i*-Pr side-chains of the two peptides are omitted.

exposed at the periphery of these templates, create homochiral sites that exert asymmetric induction in the ensuing process of chain elongation. According to this model, most of the heterochiral residues, when present, have been inserted in the early stages of the polymerization prior the formation of the template, as supported by MALDI-TOF MS/MS measurements.^{22,36} The stereoselective growth of the chains requires continued interactions of the β -sheets with the water-soluble short isotactic oligopeptides.

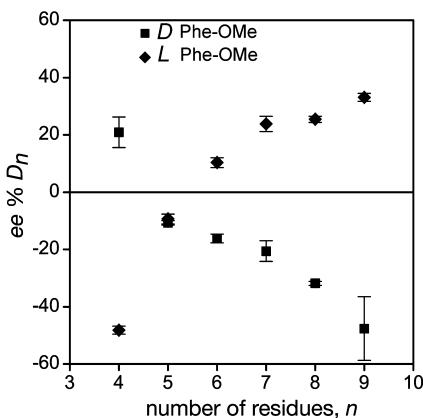


FIGURE 8. Plot of the ee % D_n of the isotactic oligo-phenylalanine of each length n obtained with initiator D-Phe-OMe (■) and L-Phe-OMe (◆) in hexane. The residue of the enantiopure initiator, located at the C-terminus of the chains, is not considered.

The predominance of the long isotactic oligopeptides obtained with 25% initiator could be rationalized by taking into account an increase in concentration of the short isotactic oligopeptides in the β -sheet templates. Such increase could be accounted for by the analysis of the water-soluble fraction indicating that the isotactic penta- and hexapeptides are less water-soluble in comparison to the heterochiral ones, Figure 12.

The composition of the racemic mixtures of the oligopeptides was desymmetrized by initiating the reaction with methyl esters of enantiopure α -amino acids. The MALDI-TOF MS analysis indicated that for both DL-ValNCA and DL-LeuNCA systems with 25% L- or D-initiator, the short isotactic oligopeptides were enriched with chains containing residues of the same handedness as that of the initiator, Figure 13. On the other hand, the ee of the longer isotactic chains and those that contain one residue of opposite handedness was reversed and increased as a function of length, Figure 13a,b. The single chirality of the initiator, located at the C-terminus of the chains, converts the two enantiotopic growing sites of the template into diastereotopic ones with different reactivity. This behavior of enantiomeric cross-impediment is similar to that obtained in the polymerization of the (DL)-PheNCA crystals (Figure 8) and is in keeping with the formation of racemic rippled antiparallel β -sheets as the major component of the templates (Figure 9).²² The rippled β -sheets might be more favored for kinetic and thermodynamic reasons. In kinetic terms, provided that the rippled and pleated sheets are of comparable stability, the rate of self-assembly of racemic β -sheet aggregates should depend upon the concentrations of the D- and L-chains, whereas the formation of the enantiomorphous pleated sheets should depend upon the concentration of only one of the enantiomers. Pauling and Corey³⁷ have

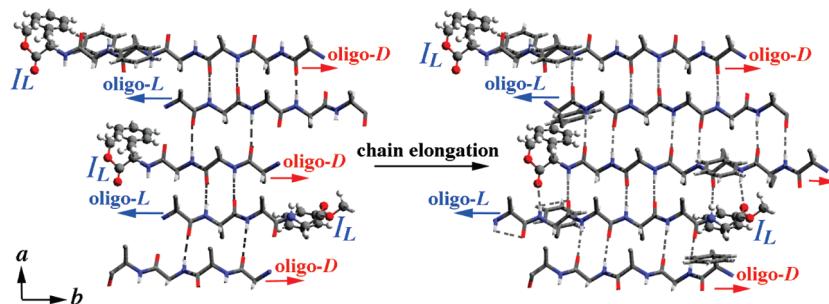


FIGURE 9. Proposed route for chain elongation via formation of racemic antiparallel (ap) β -sheets comprising alternating oligo-D and oligo-L chains, both with the residues of the L-Phe-OMe, I_L, initiator at their C-terminus, as modeled on the basis of the (DL)-PheNCA crystal structure, viewed down the c-axis. The arrows show the antiparallel direction of chain propagation of the growing NH₂ termini of the D- and L-chains.

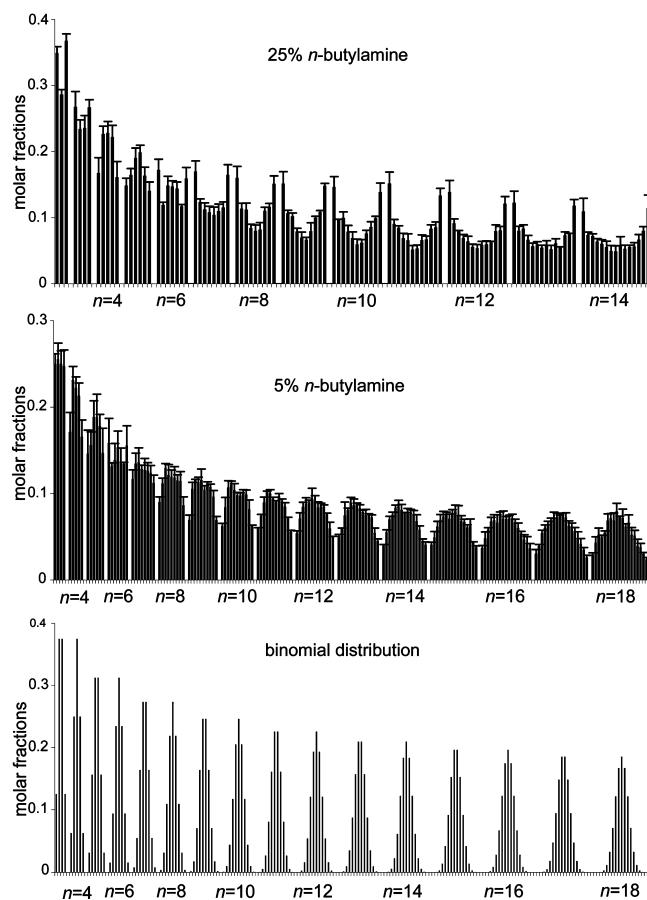


FIGURE 10. Histograms showing the distribution of the diastereoisomers of each length n obtained in the polymerization of DL-Val-NCA in water with n -butylamine initiator: (a) 25%; (b) 5% mol/mol. (c) Calculated values assuming a binomial distribution.

shown that rippled β -sheets are stable. The stability of the rippled and pleated β -sheets in water was studied recently using all-atom molecular dynamic simulations by Levy and Azia.³⁸ These simulations, which did not consider kinetic effects, indicate that rippled β -sheets are favored for oligo-Leu and oligo-phenylalanine but not for oligo-Val. Rippled-sheet motifs were observed in the crystal structure of the high molecular weight achiral polyglycine polymorph-I.³⁹

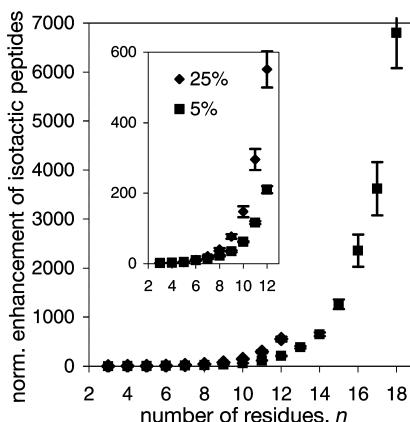


FIGURE 11. Plot of the normalized enhancement of the isotactic oligo-valine peptides of various lengths n obtained with 25% and 5% mol/mol of n -butylamine. The inset shows a magnified plot for $n = 2–7$.

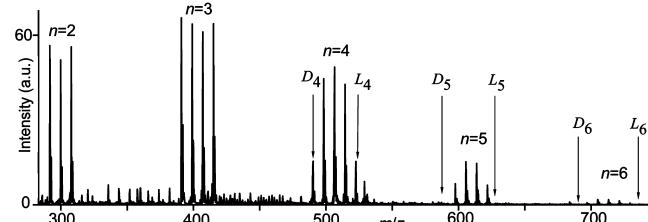


FIGURE 12. MALDI-TOF spectra of the water-soluble oligo-valine obtained from polymerization in water with 25% mol/mol n -butylamine initiator. Isotactic oligopeptides are labeled D_n and L_n .

As anticipated, for the less ordered templates, obtained with 10% and 5% initiator, the enantiomeric cross-impediment was less pronounced.^{40,41}

A schematic representation that might explain the regio-enantioselection applied by the homochiral rims of the rippled β -sheets is shown in Scheme 1. A NCA molecule, say of D-configuration, can react enantioselectively with the NH₂ group of a D-chain being assisted, in the transition state, by two hydrogen bonds, one of the to be reacted C=O carbonyl with an N–H of a neighboring L-chain and, the second between the N–H group of the NCA molecule and the C=O bond of the amide C-end group of a different neighboring

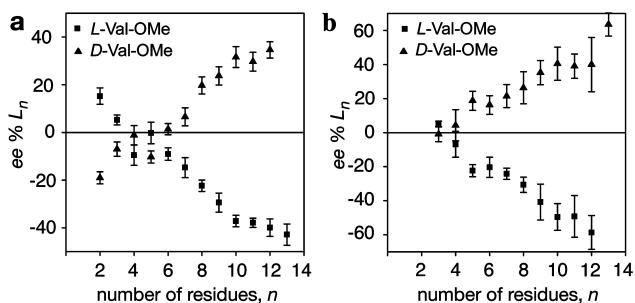
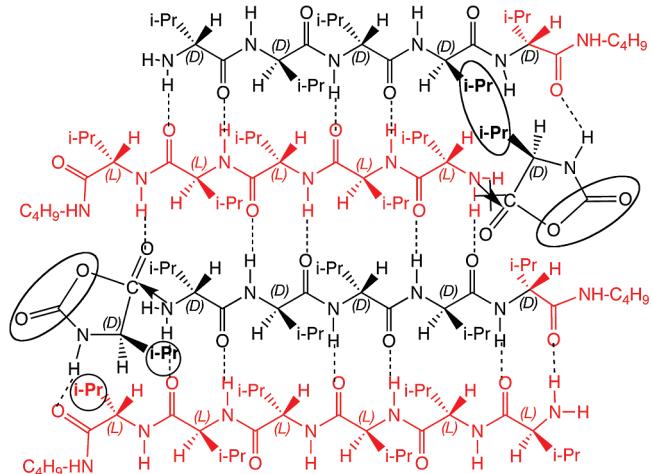


FIGURE 13. Plot of ee% L_n of oligopeptides of each length n obtained from the polymerization of (D,L)-Val-NCA with 25% L- or D-Val-OMe initiator: (a) isotactic; (b) those containing a one residue of opposite handedness. The residue of the enantio-pure initiator, located at the C-terminus of the chains, is not considered.

SCHEME 1. Schematic Representation of a Rippled ap β -sheet Template with D- or L-Monomer Molecules Val-NCA Interacting with the NH₂ Group at the N-Terminus of a Growing D-Chain (black) and L-Chain (red) Accounting for the Enantioselectivity of the Chain-Elongation Process



L-chain, Scheme 1. Such interactions will orient the NCA monomer molecule with the alkyl group away from the surface of the template and will bring the to be reacted C=O group in a proper orientation to form a new residue of the same absolute configuration as that of the growing peptide chain. Such transition state should reduce the energy of activation in comparison to that formed by the reaction of a NCA monomer molecule with an isolated peptide chain. At the same time, if a D-NCA molecule would approach the NH₂ reactive group of an L-chain, as needed for chain elongation, it will sense steric hindrance between its i-Pr group and the i-Pr group of an adjacent D-chain.²²

3.3. Homochiral Oligopeptides and Co-peptides²³ via the Ehler–Orgel Reaction. The Ehler–Orgel reaction^{42,43} on the polymerization of α -amino acids in aqueous solutions, activated *in situ* by solid 1,1'-carbonyldiimidazole (CDI), is considered a plausible model system for obtaining primeval pep-

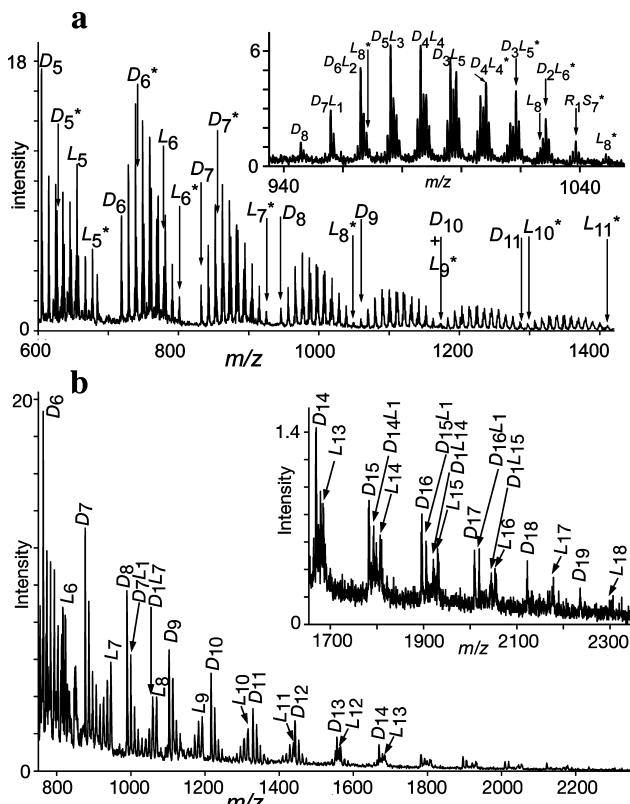


FIGURE 14. MALDI-TOF spectra of Na⁺ cationized oligopeptides obtained by polymerization in water of DL(*d*₁₀)-leucine (40 mM) activated with solid CDI: (a) without initiator; (b) initiated with 100% mol/mol CH₃–CH₂–SH. Note in panel a, signals corresponding to (M + 2Na – 1)⁺ cationized oligopeptides labeled with an asterisk. Note that insets in panel b show the region where the strongest signal D_n and L_n represent the long isotactic oligo-leucine.

tides. Luisi et al.^{14,15} reported the polymerization, via the above reaction, of racemic tryptophan, leucine, or isoleucine in buffered solutions to yield libraries of short oligopeptides in the range of 6–10 residues, where the isotactic peptides were formed as minor diastereomers, albeit in amounts larger than those predicted by a binomial distribution. The results were rationalized and simulated by a mathematical model⁴⁴ in terms of a kinetic Markov mechanism in which the homochiral residue at the N-terminus of the peptide exerts an asymmetric induction in the chain propagation. We observed similar results when the polymerization was performed in the absence of a buffer, Figure 14a.²³ By contrast, the β -sheet-like templates were formed when thiols,⁴⁵ primary amines, esters, or thio-esters of α -amino acids were used as initiators.²³ With such initiators, the formed isotactic oligopeptides are the dominant diastereoisomers, as shown by MALDI-TOF MS for the polymerization of DL-leucine initiated with ethyl-thiol⁴⁶ (Figure 14b). These results are very similar to those obtained in the polymerization of pristine DL-LeuNCA in aqueous solutions (section 3.2).

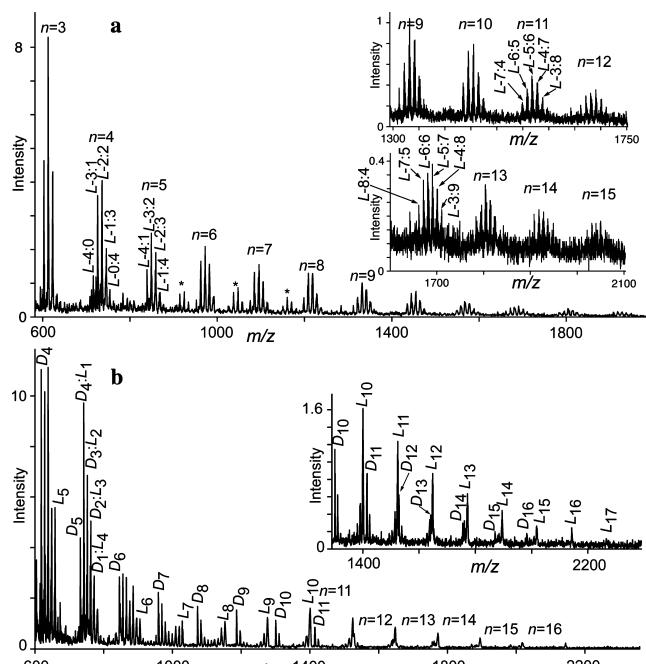


FIGURE 15. MALDI-TOF MS of $(M + Na)^+$ cationized oligopeptides (derivatized as $\text{CF}_3\text{-CO-}$ at the N-terminus) obtained from (a) L-isoleucine and L(d_{10})-leucine or (b) D-isoleucine + L(d_{10})-leucine, with 25% mol/mol L-Leu-OMe initiator.

In order to probe the feasibility to form isotactic co-peptides from mixtures of racemic α -amino acids, we studied whether the homochiral rims of the β -sheets display regio-enantioselection but not regio-chemoselection of different α -amino acids. Thus, for example, the polymerization of 1:1 mixtures of L-isoleucine with L(d_{10})-leucine resulted in the formation of random oligo-co-peptides containing L-isoleucine and L(d_{10})-leucine up to 16 detectable residues. Oligopeptides of a single component were not detected beyond pentamers, Figure 15a. By contrast, polymerization of D-isoleucine with L(d_{10})-leucine yielded, beyond the octamers, only mixtures of oligo-D-isoleucine and oligo-L(d_{10})-leucine isotactic chains, Figure 15b.

Similar results were obtained in the polymerization of D- or L-phenylalanine with L- N^1 -methyl-histidine (M 1 His) or L- N^3 -methyl-histidine (M 3 His). Oligopeptides up to 10 residues in both systems were formed. The polymerization of L-phenylalanine with L-M 1 His (Figure 16a) and -M 3 His, yielded oligo-L-phenylalanine, as well as co-peptides of the two L-components comprising up to six residues of L-M 1 His or -M 3 His, but not oligo-L-M 1 His or -M 3 His. On the other hand, when D-phenylalanine was polymerized with L-M 1 His or -M 3 His. The MALDI-TOF spectra (Figure 16b for M 1 His) demonstrate that only oligo-D-phenylalanine and sometimes those containing a single L-M 1 His or -M 3 His residue were formed.

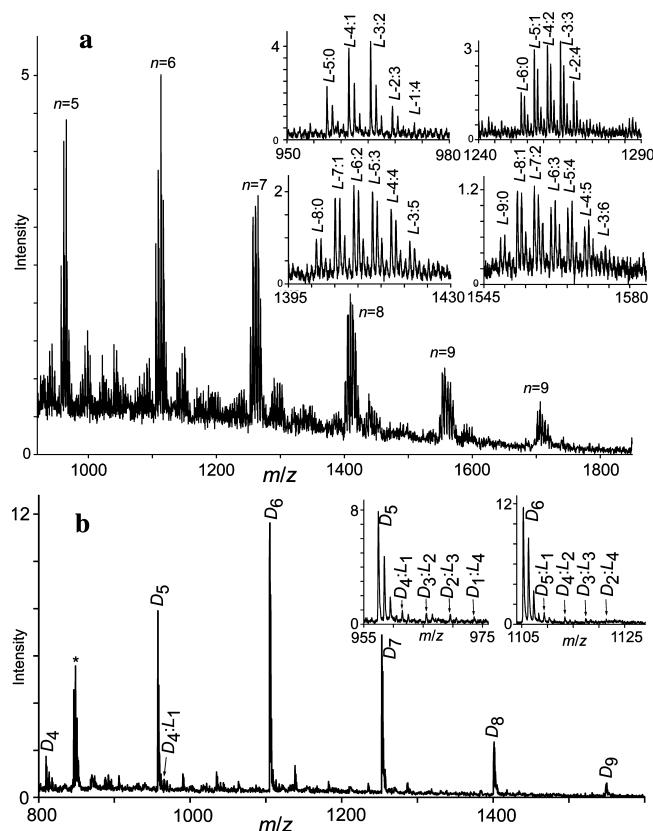


FIGURE 16. MALDI-TOF spectra of the $(M + Na)^+$ cationized oligopeptides (derivatized as $\text{CF}_3\text{-CO-}$ at the N-terminus) obtained from the polymerization in water, with 25% mol/mol glycine methyl ester initiator of (a) L-phenylalanine and L- N^1 -methyl-histidine (M 1 His) or (b) D-phenylalanine and L-M 1 His.

The polymerizations of mixtures of two to four racemic α -amino acids yielded complex libraries of peptides and co-peptides enriched with the isotactic diastereoisomers. The MALDI-TOF MS spectra of oligopeptides from DL-phenylalanine with either DL-tyrosine or DL-alanine or of mixtures of the three are presented in Figure 17.

The spectra in Figure 17a–c show, in addition to isotactic oligo-phenylalanine, the formation of isotactic co-peptides containing isotactic oligo-phenylalanine with either a single homochiral alanine or tyrosine or with both residues in chains up to a total of ten or seven residues, respectively, as the dominant diastereoisomers. Chains of isotactic oligo-alanine or oligo-tyrosine were not detected.

The polymerization route in the presence of the initiators is summarized in Scheme 2.

One should point out that in the polymerization via the Ehler–Orgel one-pot reaction using enantiopure α -amino acid ester as initiator, in variance to that of pristine DL-LeuNCA or DL-ValNCA (see section 3.2), a reversal and increase of the ee of the oligopeptide chains as function of length (Figure 13), confirming the formation of antiparal-

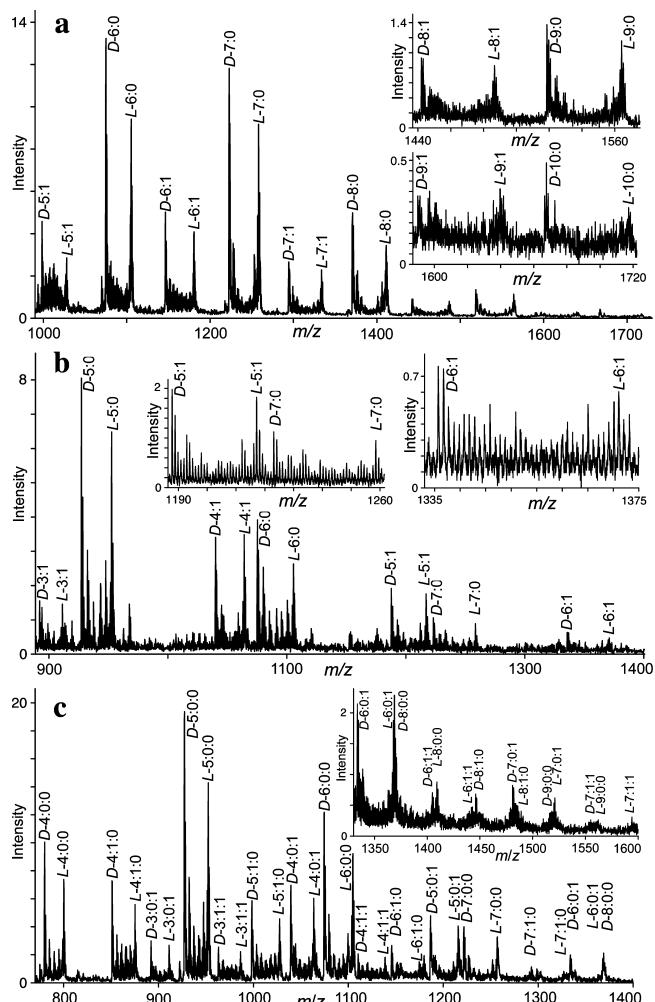


FIGURE 17. MALDI-TOF spectrum of the ($M + Na$)⁺ cationized co-peptides obtained in the polymerization with 25% mol/mol *n*-butylamine initiator of various binary and ternary mixtures of racemic α -amino acids: (a) $DL(d_5)$ -phenylalanine (20 mM) and $DL(d_4)$ -alanine (20 mM); (b) $DL(d_5)$ -phenylalanine (20 mM) and $DL(d_4)$ -tyrosine (6 mM); (c) $DL(d_5)$ -phenylalanine (20 mM), $DL(d_4)$ -alanine (20 mM), and $DL(d_4)$ -tyrosine (6 mM). Since tyrosine is scarcely water-soluble, a ratio of 3.3:1 DL -phenylalanine/ DL -tyrosine was used. The signals correspond to trifluoroacetyl derivatives and *O*-trifluoroacetyl-tyrosine residues.

lel racemic β -sheets, was not observed. A way to rationalize this result is to consider the significantly different kinetics of the two reactions, the first occurring in minutes and the second in hours. Such difference might imply that in the slow reaction the residue of the initiator, which is located at the C-terminus of the chains, populates conformations that do not exert cross-enantiomeric hindrance at the colloidal template/solution interface, where chain-elongation occurs. Nevertheless, on kinetic and thermodynamic grounds, the racemic β -sheets appear to be more probable than the pleated ones also in the one-pot reaction.

3.4. Racemic α -Amino Acid Thioesters as Initiators and Reactants in the Ehler–Orgel Reaction. Thio-esters of α -amino acids have been proposed by de Duve⁴⁷ as the possible monomers for the formation of the primeval peptides. Several syntheses of α -amino acids thio-esters (AATE) and their polymerization in the enantiomerically pure form under conditions that can be considered prebiotic have been reported.^{45,48–50} FeS and NiS, found near volcanic vents, have been shown to operate as efficient catalysts for the formation of organo-sulfur compounds including thiols and AATE.⁵¹

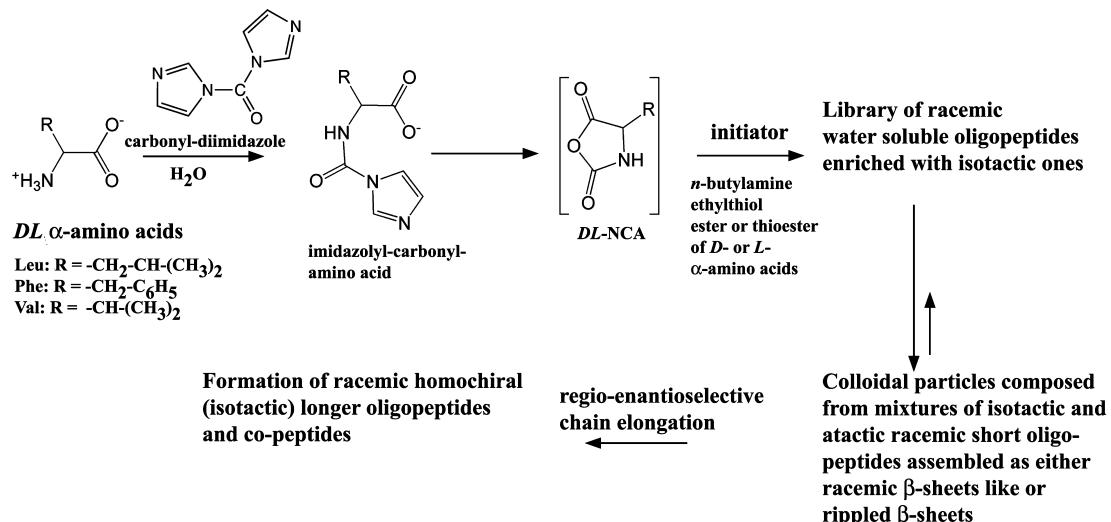
Polymerization of enatioselectively deuterated DL -leucine thio-esters (LeuTE) either in pure water or in the presence of CDI or imidazole/NaHCO₃ buffer, at pH of ~7.7, resulted in the formation of heterochiral peptides comprising up to 12 detectable residues and no isotactic peptides formed beyond octamers (spectrum not shown).⁴⁶ However, as shown in Figure 14, polymerization of the racemic α -amino acids in the Ehler–Orgel reaction but initiated with thiols resulted in the formation of long isotactic peptides. The reaction pathway is very complex comprising several steps. The thiol molecules can react with α -amino acid-NCA, generated by activation with CDI, to form the corresponding AATE that operates as an initiator of the reaction. Moreover, the AATE can also operate either as multimers by a direct insertion during the growth of the peptide chains⁴⁶ or by prior conversion back to NCA as mediated by bicarbonate ions formed during release of CO₂ in the decomposition of CDI. Independent support for a possible direct polymerization of AATE was provided also by that of dipeptide thio-esters that do not form the NCA⁴⁶ or by that of thio-esters of the amphiphilic α -amino acids¹⁹ at the air/water interface.

The dual function of the AATE was demonstrated by performing a polymerization reaction of mixtures of racemic leucine, ($DL(d_{10})$ -leucine, with L-enantiomer tagged with 10 deuterium atoms) activated *in situ* with solid CDI, with racemic Leute ($DL(d_3)$ -LeuTE, where L-LeuTE is tagged with 3 deuterium atoms).⁴⁶ This reaction yielded isotactic oligopeptides composed primarily from D-leucine residues (untagged) and co-peptides of L-leucine residues (labeled with d_3 and d_{10}), as shown in Figure 18a.

The method was also exploited for the generation of isotactic co-peptides in the polymerization of mixtures of CDI activated DL -valine with DL -LeuTE, Figure 18b.

The survival from hydrolysis of the thio-ester groups present at the C-terminus of the peptide chains provides a possible route for a chain elongation of the isotactic chains by chemical ligation.^{52–54}

SCHEME 2



4. Conclusions

The feasibility to generate isotactic peptides and co-peptides containing up to 25 residues of the same handedness start-

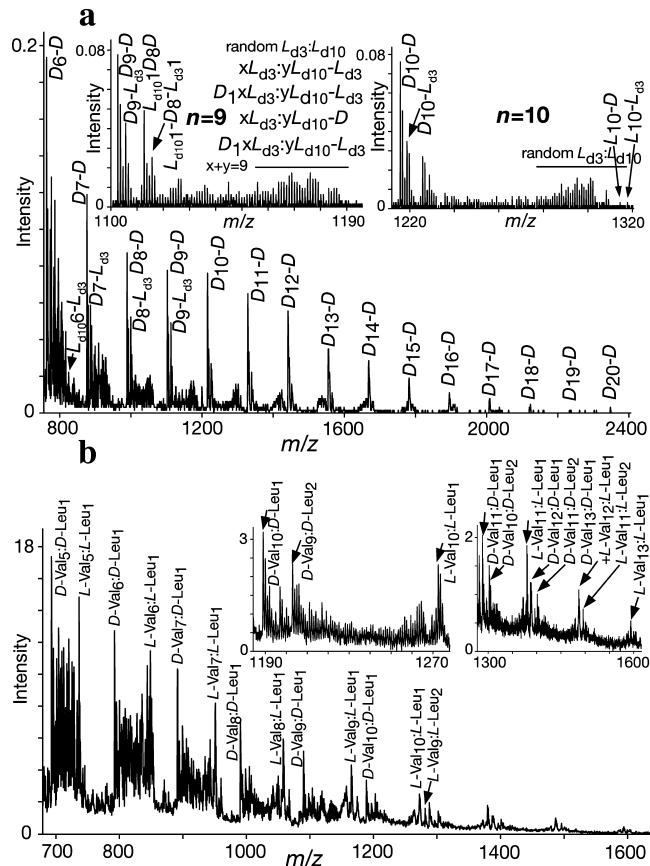


FIGURE 18. MALDI-TOF MS of Na^+ cationized oligopeptides obtained in the polymerization of (a) 4:1 mixture of activated $\text{DL}(d_{10})$ -leucine (40 mM)/ $\text{DL}(d_3)$ -LeuTE (10 mM), (b) 4:1 mixture of activated $\text{DL}(d_8)$ -valine (40 mM)/ $\text{DL}(d_3)$ -LeuTE (10 mM). Note that in panel a, the oligopeptides have a D-, L(d_3)-, or L(d_{10})-LeuTE residue at their C-terminus.

ing from activated racemic or nonracemic α -amino acids in various environments has been demonstrated.^{20–23} In some of the reactions, racemic β -sheet colloidal-like particles emerge and operate as stereoselective templates in the formation of the longer homochiral peptides and co-peptides. Such templates might have enjoyed a considerable enantioselective advantage in a prebiotic environment.⁶

A desymmetrization of the isotactic peptides into libraries of diastereoisomeric mixtures was demonstrated using enantiopure esters of α -amino acids as initiators. In the primeval world, such nonracemic α -amino acids could have been formed either by autocatalytic crystallization of racemates^{4,17} or from extraterrestrial sources.^{55–57}

Finally by applying these synthetic methods one may create homochiral peptides to be detected by combinatorial methods that could operate as primitive asymmetric catalysts that might have preceded the first enzymes.

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Dr. Gérard Bolbach did his Ph.D. with M. Cottin and is currently the head of the Mass Spectrometry and Proteomics Platform at P&M Curie University. His scientific interests comprise mechanism studies and applications of MALDI-TOF MS to bioanalytical proteomics.

Prof. Meir Lahav did his Ph.D. with G. M. J. Schmidt and, after postdoctoral studies at Harvard with P.D. Bartlett, joined the Weizmann Institute. His scientific interests comprise solid-state and surface chemistry, stereochemistry, and the emergence of homochirality on Earth. He received together with Prof. L. Leiserowitz The Prelog Medal for Stereochemistry from ETH Zurich and The G. Aminoff prize from the Swedish Academy of Science. In 2006, he was awarded the Chirality Medal instituted by the Italian Chemical Society.

FOOTNOTES

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