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Menthols as Chiral Auxiliaries for Asymmetric Cycloadditive Oligomerization: Syntheses and Studies of β-Proline Hexamers

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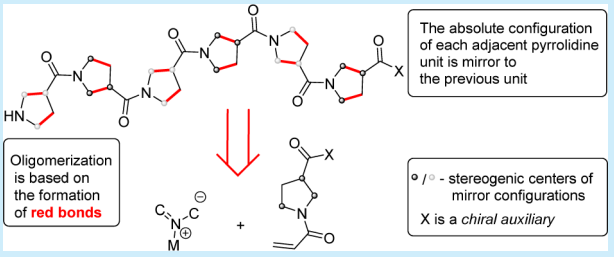
Supporting Information

ABSTRACT: To produce a novel class of structurally ordered poly-β-prolines, an emergent method for synthesizing chiral β-peptide molecular frameworks was developed based on 1,3-dipolar cycloaddition chemistry of azomethine ylides. Functionalized short β-peptides with up to six monomeric residues were efficiently synthesized in homochiral forms using a cycloadditive oligomerization approach. X-ray, NMR, and CD structural analyses of the novel β-peptides revealed secondary structure features that were generated primarily by *Z*/*E*-β-peptide bond isomerism. Anticancer *in cellulo* activity of the new β-peptides toward hormone-refractory prostate cancer cells was observed and was dependent on the absolute configuration of the stereogenic centers and the chain length of the β-proline oligomers.



Creation of artificial oligomers mimicking structure and functions of natural peptides is an emerging area in biomedicinal and material sciences. One of the most efficient strategies toward the functional unnatural oligomeric architectures and scaffolds involves addition of a methylene unit between carboxylic and amino functions of the peptide backbone that results in a diverse and large family of β-peptides.[1] Proline-rich regions in native proteins are characterized by the absence of fixed patterns of intra- or interchain hydrogen bonds that makes corresponding protein regions more flexible and favors protein−protein interactions.[2] Few types of β-proline oligomers have been developed, and different folding control factors have been established for this specific class of β-peptides.[3] Previously we have developed a protecting-group-free (PGF)[4] synthesis of short β-peptides consisting of pyrrolidine-3-carboxylic acid (3-PCA) or β-proline units that obtained well-defined, highly ordered functionalized oligomers in both racemic and enantiopure forms.[5] The developed cycloadditive oligomerization method[5] differs conceptually from traditional protecting-group-use (PGU) β-peptide synthesis methods[1,3] and is based on a 1,3-dipolar cycloaddition of azomethine ylide and acrylamide components.[6] Despite that various chiral auxiliaries including menthols,[7] lactates,[8] and optically active amines[9] were successfully used for diastereoselective synthesis of monomeric pyrrolidine products, it is us who applied acrylamides, derived from enantiomerically pure 3-PCA esters,[10] as dipolarophiles for the construction of chiral poly-β-proline framework.[5] Moreover, we observed that these unusual β-peptides[5] influenced the progression of tumor cell cycles and revealed potent anticancer activity in PC-3 cell tests.[11] In the present study, using L-menthol and D-menthol as an accessible chiral pool for asymmetrical induction, we synthesized for the first time both mirror sets of

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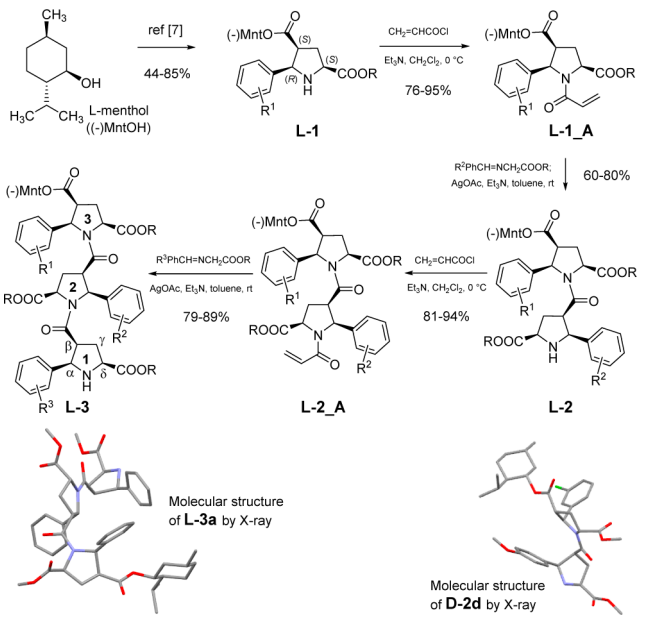
well-defined 3-PCA-oligomers with up to six pyrrolidine units and studied their structural and biological properties.

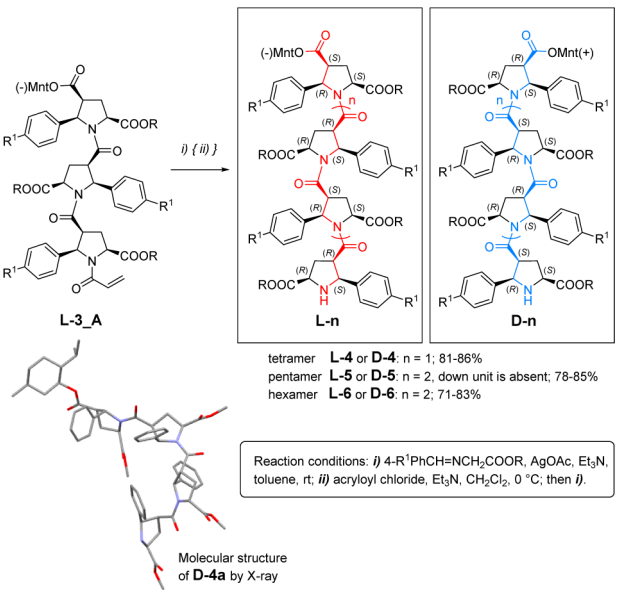
Four series of trimeric compounds with various substituents were synthesized from L-menthol (**a**: R = CH_3, $R^1 = R^2 = R^3$ = H. **b**: R = CH_3, $R^1 = R^2 = R^3$ = 4-Br. **c**: R = *t*-Bu, $R^1 = R^2 = R^3$ = H. **d**: R = CH_3, R^1 = 3-Cl, R^2 = 4-MeO; R^3 = H), and **a** and **d** sets of trimers were synthesized from D-menthol (Scheme 1). The

Scheme 1. PGF Synthesis of Homochiral 3-PCA Trimers (for the Example of the L-Menthol Set); the D-3 Trimers Were Synthesized by Analogous Transformations Starting from D-Menthol



starting monomers of **L-1** and **D-1** series were synthesized from L- and D-menthol, respectively, as reported by Grigg.[7a] The absolute configurations of the L-menthol-derived pyrrolidine monomers were determined by X-ray in the original Grigg's study,[7a] and we used these data to make structural assignments and establish the homochiral integrity of the **L-1** and **D-1** compounds. Acryloylation transformed the nonracemic, diastereomerically pure 5-arylpyrrolidine-2,4-dicarboxylates **L-1** and **D-1** to the chiral acrylamides **L-1_A** and **D-1_A** (Scheme 1). The last dipolarophiles contained complex chiral auxiliaries consisting of the chiral all-*cis*-trisubstituted pyrrolidine moiety and adjusted chiral menthol fragment that differs them from enantiopure 4-*tert*-butyl 2-methyl *N*-acryloyl-5-arylpyrrolidine-2,4-dicarboxylate with only three chiral centers in the initial research.[5,11] Fortunately, six stereogenic centers of olefins **L-1_A** and **D-1_A** took concerted action, and their reactions with iminoesters $R^2PhCH{=}NCH_2COOR$ in the presence of silver(I) acetate as a Lewis acid for ylide generation led to the regio- and stereospecific[5,11] formation of the second pyrrolidine ring (Scheme 1). Dimers **L-2** and **D-2** were isolated as individual diastereomers in homochiral form and characterized by NMR. Additional step of acryloylation of dimers **L-2** and **D-2** provided dipolarophiles **L-2_A** and **D-2_A** with nine stereogenic centers, and subsequent asymmetric cycloadditions led to alternating trimers **L-3** and **D-3**. The absolute configurations of compounds **D-2d** and **L-3a** were confirmed by single crystal X-ray analysis (Scheme 1, Supporting Information, Table S1, Figures S1–S3).[12] These data also supported the previously observed alternating stereocenter configurations of adjacent pyrrolidine units with each new oligomeric chain enlargement reaction.[5] Consequent acryloylation and cycloaddition procedures led to alternating oligomers **L-(4–6)** and **D-(4–6)** of **a–c** and **a** series, respectively (Scheme 2). It should be noted that in each case we

Scheme 2. PGF Synthesis of Homochiral Higher 3-PCA Oligomers



registered with NMR and chromatography methods only one product of the cycloaddition step indicating an excellent asymmetric induction of acrylamides **L-3_A**(**D-3_A**), **L-4_A**(**D-4_A**), and **L-5_A**(**D-5_A**) with 12, 15, and 18 well-defined stereogenic centers, respectively. The absolute configuration of tetramer **D-4a** was confirmed by single crystal X-ray analysis (Scheme 2, Supporting Information, Table S1, Figures S4–S8).[12] Two types of crystals grown from MeOH and EtOAc were obtained for compound **D-4a**,[12] representing a rare example of *pseudo*-isomorphism.[13]

The secondary structures of tetramer **D-4a**, pentamer **D-5a**, and hexamer **D-6a** in DMSO-d_6 solutions were studied by NMR. The configurations of the *β*-peptide bonds were determined by analyzing the NOE-resonance $H^{\alpha}(i)/H^{\beta}(i-1)$ distinguishing for a *Z*- or *cis*-peptide bond and the NOE-resonance $H^{\delta}(i+1)/H^{\beta}(i)$ distinguishing for an *E*- or *trans*-peptide bond (Figure 1).[5,11] The ^1H NMR signals of the dominant conformers of

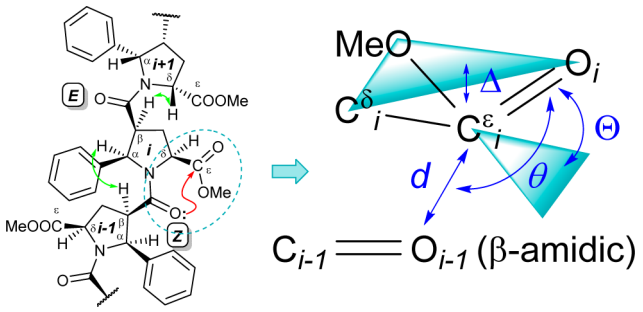


Figure 1. NOE interactions (green arrows) distinguishing between *E*-*β*- and *Z*-*β*-peptide bonds (left). An n → *π** interaction (red arrow, left) stabilizing the *Z*-*β*-peptide bond configuration and its structural parameters (right).

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Table 1. Conformational Parameters of the L-n and D-n Oligomers[a]

O_{i-1}	C^{ε}_{i}	OMe	O_i	C^{δ}_{i}	*d* (Å)	*θ* (deg)	Δ (Å)	Θ (deg)
				L-3a (CCDC 981128)				
O_1	C^{ε}_{2}	OMe	O_2	C^{δ}_{2}	2.656(2)	98.09(13)	0.034(2)	4.0
O_2	C^{ε}_{3}	OMe	O_3	C^{δ}_{3}	2.796(2)	94.62(13)	0.031(2)	3.7
				D-4a (CCDC 981129)				
O_1	C^{ε}_{2}	OMe	O_2	C^{δ}_{2}	2.775(8)	110.9(5)	0.048(7)	5.8
O_2	C^{ε}_{3}	OMe	O_3	C^{δ}_{3}	2.684(7)	99.3(4)	0.021(7)	2.6

[a]From X-ray diffraction analysis of the crystalline compounds. Parameters are defined in Figure 1.

oligomers **D-4a**, **D-5a**, and **D-6a** were assigned unambiguously using characteristic correlations observed in TOCSY and NOESY or ROESY experiments (Table S2). Compound **D-4a** preferentially exists as a *Z-Z-Z* conformer in DMSO-d_6 solution, in contrast to its solid-state conformation. The *Z-Z-E-Z* conformer of pentamer **D-5a** is more prevalent in DMSO-d_6 solution. Hexamer **D-6a** has a strong tendency to adopt the *Z-Z-E-Z-Z* configurations of the peptide bonds in both DMSO-d_6 and CD_2Cl_2 solutions. Under elevated temperatures, the ^{1}H signals of the **D-6a** *β*-peptide backbone shift to high field, but the *Z-Z-E-Z-Z* conformer dominates below 90 °C (Figure S9).

The strong preference for the *Z*-configuration of *β*-amide bonds in the **L-n** and **D-n** oligomers may originate from the interpenetration by the filled lone pair (n) of the carbonyl oxygen atoms of the *β*-amide group of the empty π^* orbitals of C^{ε} of the methoxycarbonyl groups (Figure 1). Similar $n \rightarrow \pi^*$ interactions contribute up to 0.7 kcal/mol of stabilizing energy per occurrence.[14] Experimental evidence of $n \rightarrow \pi^*$ interactions was obtained from the short contacts (*d*)[14] and pyramidalization of the C^{ε} acceptors toward the O_{i-1} donors (Δ, Θ),[14] which were detected by X-ray diffraction (Figure 1, Table 1). For all *Z*-peptide fragments in the trimer **L-3a** (*Z-Z*) and the tetramer **D-4a** (*Z-Z-E*), the *d*-distances were substantially shorter than 3.22 Å, which is the sum of the van der Waals radii of oxygen and carbon (Table 1). The distortion parameter Θ, which represents strong evidence of an attractive $n \rightarrow \pi^*$ interaction,[14] amounts to a substantial 2.6−5.8 degrees (Table 1).

Well-separated Cotton effect signals of the absorption peaks were observed in circular dichroism (CD) experiments for the **L-na** and **D-na** oligomers sets (Figure 2). Distinct differences were observed between oligomers containing even numbers (Figure 2a,c) or odd numbers (Figure 2b,d) of 3-PCA subunits. Strong positive absorption at 187−190 nm, zero-cross at 193−196 nm, and a negative Cotton effect at 198−201 nm were observed for the dimer, tetramer, and hexamer of the **D-na** set series (Figure 2a). The corresponding magnitudes for the trimer and pentamer of the **D-na** set series were shifted to 193−195 nm for the strong positive Cotton effect, 205−214 nm for the first zero-cross and 224−225 nm for the second one, and 217−220 nm for the negative absorption (Figure 2b). Odd **D-na** oligomers exhibited additional strong negative absorption at 183−185 nm and zero-cross at 186−190 nm (Figure 2b). The band positions and intensities in the CD spectra of the **L-na** oligomers were inverted relative to those described for the **D-na** compounds (Figure 2c,d). Another extraordinary feature of the studied oligomers was the monotonic decrease observed in the CD signal intensities when normalizing by the number of monomeric residues (Figure S10). This decrease was not observed in the CD spectra for the reported *β*-proline oligomers[3] and is apparently attributable to the mirror heterogeneity of the adjacent monomeric units. The CD signals of the **L-na** and **D-na** oligomers were characterized by increased intensities relative to the **L-1a** and **D-1a** monomers (Figure S11) as well as different signs and bands positions, indicating that secondary structure transition dipoles primarily contribute to the CD absorbance of the oligomers. The CD spectra of the **D-na** oligomers were fully matched to those of the original chiral 3-PCA oligomers,[5] indicating prevailing influence of the poly-*β*-proline backbone secondary structure on observed Cotton effects and diastereomeric purity of menthol oligomers (Figure S12). The conformational stability of the alternating *β*-peptide backbone under elevated temperatures observed by NMR was confirmed by the stability of the pentamer **D-5a** CD curves up to 80 °C (Figure S13). However, the **L-nc** oligomers, which possess peripheral *tert*-butyl substituents, exhibited different CD absorbance values in the 190−220 nm region (Figures S12 and S14).

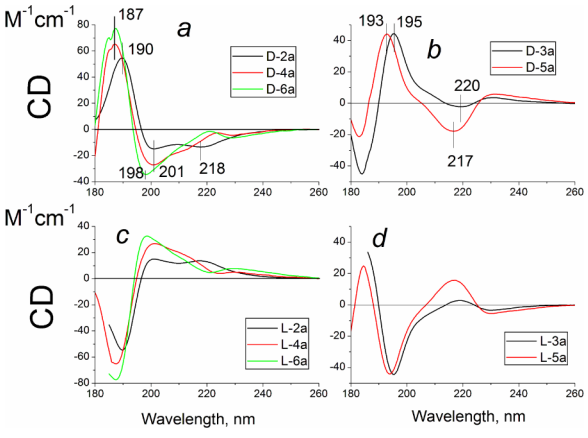

Figure 2. CD spectra of **D-na** and **L-na** oligomers in acetonitrile normalized to the compound concentration.

Chemotherapeutic treatment of hormone-refractory prostate cancer (HRPC) was of little value until the emergence of novel agents that target crucial tumor growth pathways.[15] We evaluated the effects of the synthesized homochiral 3-PCA oligomers on the proliferation of the HRPC cell line PC-3. In our previous work, where only one enantiomeric set of corresponding oligomers was available, racemic oligomeric acrylamides were the most active toward proliferation of PC-3 cells.[11] Anti-proliferative activity of several **L-n** and **D-n** compounds at low micromolar concentrations was observed with the trimer **D-3a** (GI_{50} 4.4 *μ*M) as the most active one (Table S3). In addition to measuring growth inhibition (GI_{50}), we determined the PC-3 cell cycle phase in which progress stopped (Table S3). Whether the absolute configuration of the 3-PCA oligomeric backbone determines antiproliferative activity remains unclear; we noticed that menthol residue at the C-end of *β*-peptides benefited the activity: the trimer (+)-**3c**[11] with the backbone identical to the trimer **D-3a** was inactive against PC-3 survival.

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To conclude, we have developed an asymmetric protecting-group-free method for the efficient synthesis of complex, well-defined β-proline oligomers utilizing the stereospecific cycloaddition of nonracemic homochiral acrylamides to azomethine ylides. For the first time we obtained homochiral alternating poly-β-prolines with up to six monomeric units in both enantiomeric forms. Several members of this novel β-peptide class display cell-cycle directed antiproliferative activity in HRPC cells that requires further investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03154.

Experimental procedures, NMR spectra of the synthesized compounds, X-ray data, and biological testing details (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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