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Control of Azomethine Cycloaddition Stereochemistry by CF_3 Group: Structural Diversity of Fluorinated β-Proline Dimers

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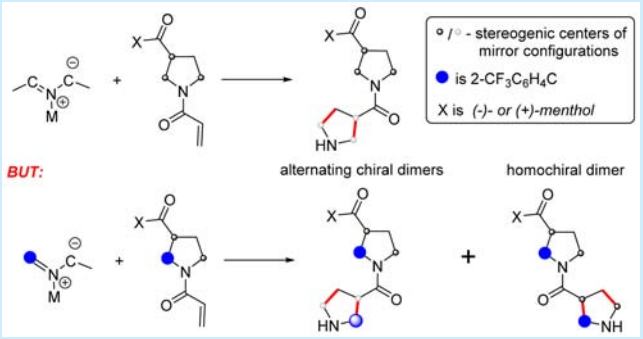
Supporting Information

ABSTRACT: β-Proline-functionalized dimers consisting of homochiral monomeric units were synthesized by a non-peptidic coupling method for the first time. The applied synthetic methodology is based on 1,3-dipolar cycloaddition chemistry of azomethine ylides and provides absolute control over the β-proline backbone stereogenic centers. An *o*-(trifluoromethyl)phenyl substituent contributes to appropriate stabilization of the definite acrylamide chiral *cis* conformation and to achieve the dipole reactivity that is not observed for aryl groups lacking strong electronegative character.



Patterns of consecutive proline residues are abundant for signal transduction peptides and recognized by several domain families known as proline-rich motif (PRM) binding domains.[1] Peptidic ligands that have proline-rich segments are characterized by backbone carbonyl groups freely available for protein−protein interactions (PPIs). PPIs mediated by PRMs induce many facets of immune responses,[2] cell-penetrating events,[3] neuroprotection,[4] and host−pathogen recognition.[5] Different types of protein surface mimetics are considered as privileged structures for potential therapeutic applications.[6] PRMs preferentially adopt the left-handed polyproline II (PPII) helical secondary structure and frequently occur in the eukaryotic proteome. Recently PPII helix formation was demonstrated for oligomers constituted of β-proline (pyrrolidine-3-carboxylic acid (3-PCA)) units.[7] The secondary structure of known β-proline oligomers is formed by appropriate folding[8] or complementary covalent fixation of monomeric units.[9] The diproline structural motif is a minimal core fragment that is able to provide a helical PPII geometry in the case of homochiral proline residues and a reverse-turn conformation in the case of linked heterochiral or alternating proline monomers.[6] Furthermore, a specially designed additional covalent bridge between two proline units allows the production of conformationally restricted mimetics of the native PRM ligand with increased stability and higher binding affinity toward the target protein.[10]

We previously developed a protecting-group-free (PGF) synthesis of short β-peptides consisting of 3-PCA units in both racemic and enantiopure forms (Scheme 1).[11,12] For the first time, alternating β-proline oligomers were obtained without the use of any monomeric building blocks.[11,12] Moreover, we observed that these unusual poly-β-prolines influenced the progression of tumor cell cycles and revealed potent anticancer activity in PC-3 cell tests.[12,13]

In the current work, we disclose the synthesis of homochiral β-proline dimers by the cycloadditive oligomerization approach[11] and reveal the conformational preferences of both homochiral and alternating fluorinated β-proline dimers in solution. Mechanistic insights into the observed stereo-

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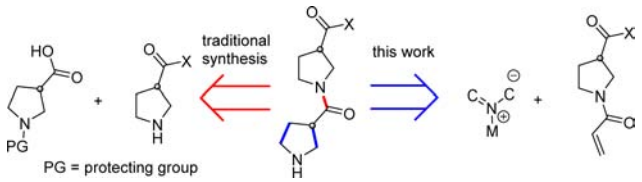
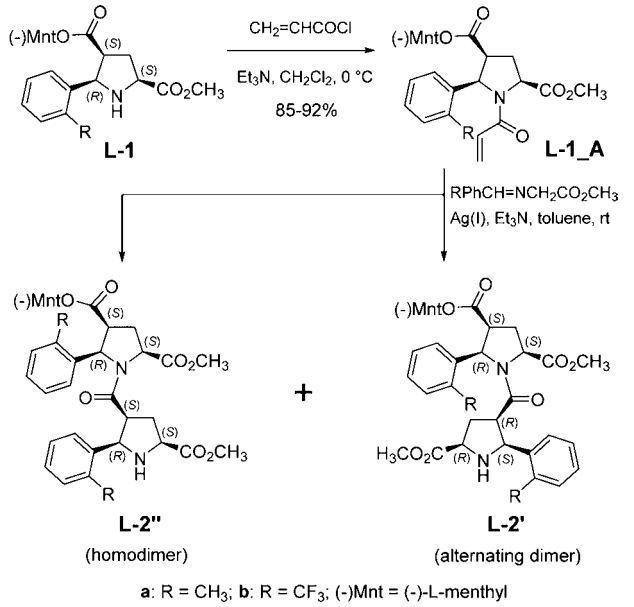
Scheme 1. Approaches to a Dimeric β-Proline Fragment by the Conventional Synthesis (Red Arrow and Newly Formed Bond) and by PGF Cycloadditive Oligomerization (Blue Arrow and Newly Formed Bonds)



selectivities of the studied azomethine ylide cycloadditions are also proposed.

Structural units of chiral 5-arylpyrrolidine-2,4-dicarboxylates **L-1**, synthesized from L-menthyl acrylate and iminoglycinates, constitute the novel β-peptide class by formal amide connection through the 4-carboxylic functionality of the monomer (Scheme 2).[12] When the process presented in

Scheme 2. Cycloadditive Dimerization of Chiral 5-Arylpyrrolidine-2,4-dicarboxylates



Scheme 2 was realized for *o*-CH_3-substituted and *o*-CF_3-substituted substrates, significantly different results were obtained. In the case of the reaction of methyl-substituted chiral acrylamide **L-1a_A** and imino ester *o*-$CH_3C_6H_4CH{=}NCH_2CO_2CH_3$, the single product corresponding to the alternating dimer **L-2a′** was detected in the reaction mixture by TLC and NMR and later isolated in individual form (Scheme 2 and Table 1). Structural assignments for the heterochiral dimer **L-2a′** were made using previous extensive NMR spectral data.[11−13] Application of trifluoromethyl substrates **L-1b_A** and *o*-$CF_3C_6H_4CH{=}NCH_2CO_2CH_3$ in the same processes afforded two different 3-PCA dimeric compounds that were never observed for other aryl substituents.[11−13] As a Lewis acid source we used equimolar quantities of silver(I) acetate,[14] as in the original cycloadditive oligomerization method,[11] or catalytic amounts of the preformed complex of AgOAc with Ph_3P (Scheme 2 and Table 1).

Table 1. Cycloadditions Leading to β-Proline Dimers

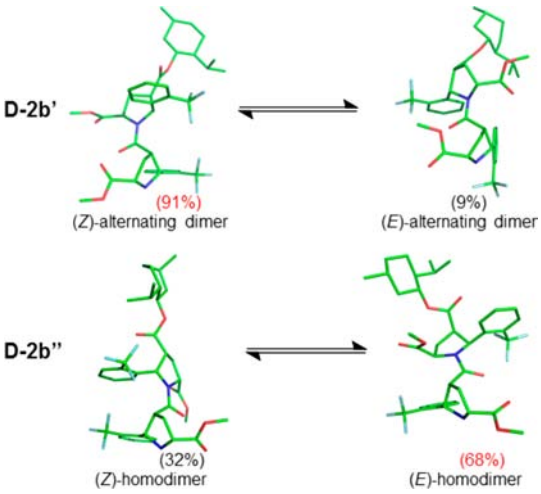
compound	cond[a]	yield [%]	compound	cond[a]	yield [%]
L-2a′	**A**	71	**L-2a″**	**A**	0
L-2a′	**B**	93	**L-2a″**	**B**	0
L-2b′	**A**	47	**L-2b″**	**A**	38
L-2b′	**B**	44	**L-2b″**	**B**	27
D-2b′	**A**	53	**D-2b″**	**A**	42

[a]Reaction conditions: **A**: AgOAc (1.5 equiv), Et_3N (1.5 equiv), toluene, rt. **B**: AgOAc·$2Ph_3P$ (0.1 equiv), Et_3N (0.1 equiv), toluene, rt.

Even though the AgOAc/Ph_3P/*i*-Pr_2NEt catalytic system was found to be efficient for azomethine ylide cycloadditions,[15] to the best of our knowledge, this is the first case of the isolation and synthetic application of the individual stoichiometric complex AgOAc·$2Ph_3P$ (see the Supporting Information). The equimolar and catalytic Ag(I) complex systems afforded matching yields and stereoselectivities of the discussed cycloadditions (Scheme 2 and Table 1). D-Menthol-derived fluorinated dimers **D-2b′** and **D-2b″** were obtained by the identical reaction sequence starting from D-menthyl acrylate and *o*-CF_3-iminoglycinate (Table 1).

Comprehensive structural attributions of fluorinated β-proline dimers **L-2b′** and **L-2b″** and their mirror isomers **D-2b′** and **D-2b″** were provided by NMR studies (Figures S1−S5 and Table S1). Analysis of the cross-peak volumes in $^1H{-}^1H$ ROESY spectra furnished interproton distance restraints for structural calculations carried out using the molecular dynamics method.[16] Calculated families of NMR structures of alternating **D-2b′** and homochiral **D-2b″** dimers revealed (*Z*/*E*)-β-peptide bond isomers (Scheme 3 and Figures S6 and S7).[17]

Scheme 3. Conformational Equilibria of Heterochiral and Homochiral Dimers D-2b′ and D-2b″ in $CDCl_3$ Solution



As with previously reported alternating β-proline dimers,[11−13] compound **D-2b′** predominantly exists as the *Z* conformer in solution. The state with the *E* configuration of the β-peptide bond is populated more than doubly for fluorinated β-proline homochiral dimer **D-2b″** in solution (Scheme 3 and Figures S2−S4). Strong positive ROESY cross-peaks between cognate signals from the *Z* and *E* isomers indicate the existence of the discussed conformational equilibria on the millisecond time scale (Scheme 3 and Figure S5).

A feasible explanation of the observed stereochemical cycloaddition results may include an *endo* interaction of *trans*

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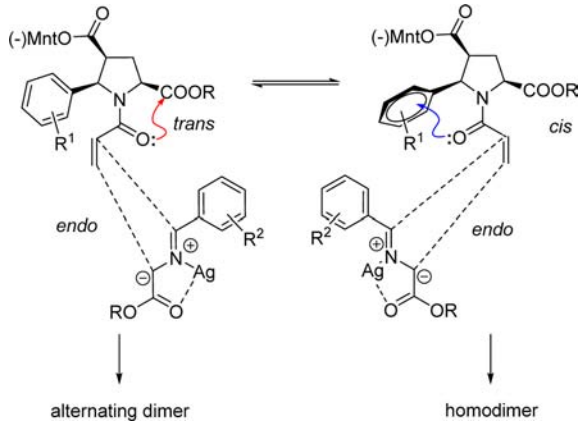
and *cis* isomers[18] of prolyl acrylamides **L-1_A** and ylides derived from iminoesters $R^2C_6H_4CH{=}NCH_2CO_2R$ (Scheme 4). *trans*-Acrylamides **L-1_A** are stabilized by the inter-

Scheme 4. Proposed Rationale for the Observed Stereoselectivity under Cycloadditive Dimerization



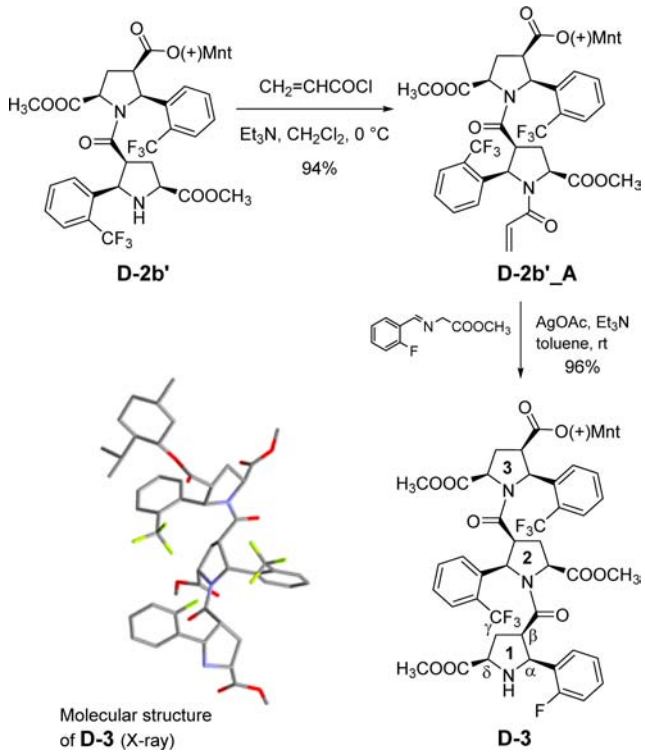
penetration by the filled lone pair (n) of the carbonyl oxygen atom of the acrylamide group into the empty π^* orbitals of the methoxycarbonyl C=O group (Scheme 4, left). This interaction mode is distinctive for prolyl peptide bonds and was used for regulation of the *cis*/*trans* conformer ratio.[19] The same oxygen lone pair can be delocalized by π^* orbitals of a proximal electron-deficient aromatic ring[20] that would settle the *cis* configuration of the **L-1_A** acrylamide group (Scheme 4, right). Apparently, the balance between the *trans* and *cis* forms of **L-1_A** depends on the stereoelectronic properties of the R^1 substituent, which in the case of a strong electron-withdrawing (EWD) effect (e.g., CF_3) increases the electrophilicity of the aromatic ring and favors the formation of the *cis* conformer of **L-1_A**. Subsequent cycloaddition of *cis*-acrylamide **L-1_A** with the corresponding azometine ylide produces the *β*-proline dimer with homogenic chirality of the monomeric units. This reaction route is not realized to a detectable extent in the event of R^1 substituents without or with poor electronegative abilities (H,[11−13] Br,[11−13] Cl,[12] OCH_3,[12] and CH_3 in the current work).

Experimental (NMR) and theoretical (DFT) studies of the introduced hypothesis were also performed. Acrylamides **L-1a_A** and **L-1b_A** were studied by NMR spectroscopy in toluene-d_8 solution, corresponding to the cycloaddition reaction medium (Figures S8 and S9 and Table S2). Both compounds exist in equilibria of *trans* and *cis* conformers with the former isomer predominating. The content of the minor *cis* isomer is 26% for trifluoromethylacrylamide **L-1b_A** (Figure S9) and only 10% for methylacrylamide **L-1a_A** (Figure S8). The increased stability of the *cis* isomer in fluorinated acrylamide **L-1b_A** was also demonstrated by DFT calculations at the B3LYP/6-31+g(d,p) level.[21] The differences in the energies of formation of the *trans* and *cis* isomers ($\Delta E = E_{trans} - E_{cis}$) constitute −5.4 kJ mol^{-1} in the case of acrylamide **L-1b_A**, −8.4 kJ mol^{-1} in the case of acrylamide **L-1a_A**, and −11.7 kJ mol^{-1} in the case of unsubstituted acrylamide (Figure S10).

Although the indicated n → π^* interactions have been reported for different model systems[19,20] we are the ones who observed the potential influence of these factors on the course of a concrete chemical transformation. Besides the influence on the dipolarophile state, the *o*-CF_3-substituent has to accelerate the rate of deprotonation of the corresponding azomethine and subsequent cycloaddition step relative to H or electron-donating substituents.[22] Fast trapping of the *trans* and *cis* conformers of chiral acrylamides such **L-1_A** with reactive *o*-CF_3-phenyl azomethine ylide enables the formation of the alternating and homochiral fluorinated *β*-proline dimers (Scheme 4).

Further oligomeric chain elongation was briefly studied for the obtained fluorinated *β*-proline dimers. Alternating dimer **D-2b′** was transformed into acrylamide **D-2b′_A**, and the latter was allowed to interact with the azomethine ylide generated from the imino ester *o*-$FC_6H_4CH{=}NCH_2CO_2CH_3$ (Scheme 5). Only the alternating trimer **D-3**, which was characterized by

Scheme 5. Synthesis of Alternating Trimer D-3



single-crystal X-ray analysis,[23] was obtained in high yield under the indicated conditions (Scheme 5, Figure S11, and Tables S3 and S4). These experimental results point at the earlier observed predominant formation of alternating poly-*β*-prolines[11−13] and a very susceptible influence of certain electronic and steric characteristics on the cycloadditive oligomerization results.

To conclude, important stereoelectronic factors controlling the outcome of the asymmetric 1,3-dipolar cycloaddition of azomethine ylides and chiral acrylamides have been determined, providing a novel approach to full chiral sequence control in the poly-*β*-proline family. Specially designed dipolarophiles containing structural motifs of chiral 5-arylpyrrolidine-2,4-dicarboxylate furnished, under succeeding PGF cycloaddition, well-defined functionalized nonracemic poly-*β*-prolines with alternating or homochiral units of the *β*-peptide chain.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02327.

Experimental procedures, NMR spectra of the synthesized compounds, and X-ray data (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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■ REFERENCES

(1) Ball, L. J.; Kühne, R.; Schneider-Mergener, J.; Oschkinat, H. *Angew. Chem.* **2005**, *117*, 2912−2930; *Angew. Chem., Int. Ed.* **2005**, *44*, 2852−2869.

(2) Srinivasan, M.; Dunker, A. K. *Int. J. Pept.* **2012**, *2012*, 634769.

(3) Pujals, S.; Giralt, E. *Adv. Drug Delivery Rev.* **2008**, *60*, 473−484.

(4) Gladkevich, A.; Bosker, F.; Korf, J.; Yenkoyan, K.; Vahradyan, H.; Aghajanov, M. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2007**, *31*, 1347−1355.

(5) Berisio, R.; Vitagliano, L. *Curr. Protein Pept. Sci.* **2012**, *13*, 855−865.

(6) Che, Y.; Brooks, B. R.; Marshall, G. R. *J. Comput.-Aided Mol. Des.* **2006**, *20*, 109−130.

(7) Caumes, C.; Delsuc, N.; Azza, R. B.; Correia, I.; Chemla, F.; Ferreira, F.; Carlier, L.; Luna, A. P.; Moumné, R.; Lequin, O.; Karoyan, P. *New J. Chem.* **2013**, *37*, 1312−1319.

(8) (a) Huck, B. R.; Langenhan, J. M.; Gellman, S. H. *Org. Lett.* **1999**, *1*, 1717−1720. (b) Kim, Y. J.; Kaiser, D. A.; Pollard, T. D.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2417−2419. (c) Huck, B. R.; Fisk, J. D.; Guzei, I. A.; Carlson, H. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2003**, *125*, 9035−9037.

(9) Schafmeister, C. E.; Brown, Z. Z.; Gupta, S. *Acc. Chem. Res.* **2008**, *41*, 1387−1398.

(10) Opitz, R.; Müller, M.; Reuter, C.; Barone, M.; Soicke, A.; Roske, Y.; Piotukh, K.; Huy, P.; Beerbaum, M.; Wiesner, B.; Beyermann, M.; Schmieder, P.; Freund, C.; Volkmer, R.; Oschkinat, H.; Schmalz, H. G.; Kühne, R. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 5011−5016.

(11) Kudryavtsev, K. V.; Ivantcova, P. M.; Churakov, A. V.; Wiedmann, S.; Luy, B.; Muhle-Goll, C.; Zefirov, N. S.; Bräse, S. *Angew. Chem.* **2013**, *125*, 12969−12973; *Angew. Chem., Int. Ed.* **2013**, *52*, 12736−12740.

(12) Kudryavtsev, K. V.; Ivantcova, P. M.; Muhle-Goll, C.; Churakov, A. V.; Sokolov, M. N.; Dyuba, A. V.; Arutyunyan, A. M.; Howard, J. A. K.; Yu, C. C.; Guh, J. H.; Zefirov, N. S.; Bräse, S. *Org. Lett.* **2015**, *17*, 6178−6181.

(13) Kudryavtsev, K. V.; Yu, C. C.; Ivantcova, P. M.; Polshakov, V. I.; Churakov, A. V.; Bräse, S.; Zefirov, N. S.; Guh, J. H. *Chem. - Asian J.* **2015**, *10*, 383−389.

(14) Chen, Q. A.; Wang, D. S.; Zhou, Y. G. *Chem. Commun.* **2010**, *46*, 4043−4051.

(15) Longmire, J. S.; Wang, B.; Zhang, X. *J. Am. Chem. Soc.* **2002**, *124*, 13400−13401.

(16) Brünger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1998**, *54*, 905−921.

(17) The families of NMR structures, conformational constraints used for calculations, and ^{1}H and ^{13}C chemical shifts have been deposited in the Biological Magnetic Resonance Data Bank database (http://www.bmrb.wisc.edu) under the following accession numbers: BMRB-21070 for (*E*)-**D-2b″**, BMRB-21071 for (*Z*)-**D-2b″**, BMRB-21072 for (*E*)-**D-2b′**, and BMRB-21073 for (*Z*)-**D-2b′**.

(18) Dugave, C.; Demange, L. *Chem. Rev.* **2003**, *103*, 2475−2532.

(19) Kuemin, M.; Nagel, Y. A.; Schweizer, S.; Monnard, F. W.; Ochsenfeld, C.; Wennemers, H. *Angew. Chem.* **2010**, *122*, 6468−6471; *Angew. Chem., Int. Ed.* **2010**, *49*, 6324−6327.

(20) Gorske, B. C.; Nelson, R. C.; Bowden, Z. S.; Kufe, T. A.; Childs, A. M. *J. Org. Chem.* **2013**, *78*, 11172−11183.

(21) The full reference is included in the Supporting Information.

(22) Barr, D. A.; Grigg, R.; Gunaratne, H. Q. N.; Kemp, J.; McMeekin, P.; Sridharan, V. *Tetrahedron* **1988**, *44*, 557−570.

(23) $C_{51}H_{56}F_7N_3O_{10}$, M = 1003.99, a = 12.5530(2) Å, b = 53.9500(16) Å, hexagonal $P6_1$, Z = 6, D_c = 1.359 g/cm^3, μ(Mo Kα) = 0.111, 108 255 total reflections, 5696 unique (R_{int} = 0.0565), 650 parameters, R_1 [$I > 2\sigma(I)$] = 0.0374, wR_2 (all data) = 0.0868. Crystallographic data (excluding structure factors) for compound **D-3** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-1474544. Copies of the data can be obtained free of charge on application to CCDC via www.ccdc.cam.ac.uk/data_request/cif.

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