Foldamers

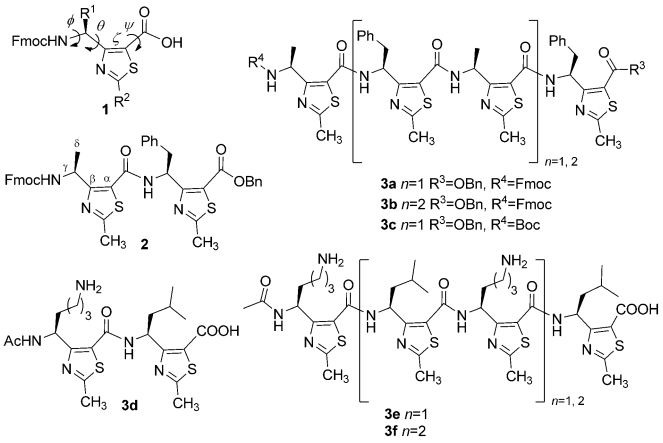
DOI: 10.1002/ange.201302106

Helical Oligomers of Thiazole-Based γ-Amino Acids: Synthesis and Structural Studies**

*Loïc Mathieu, Baptiste Legrand, Cheng Deng, Lubomir Vezenkov, Emmanuel Wenger, Claude Didierjean, Muriel Amblard, Marie-Christine Averlant-Petit, Nicolas Masurier, Vincent Lisowski, Jean Martinez, and Ludovic T. Maillard**

Peptides and proteins are molecular devices that can adopt specific folded and organized structures for performing diverse functions in living systems. The formation of such tertiary and quaternary structures arises from the assembly of stable secondary structures such as helices, sheets, and turns. The ability of synthetic oligomers of β- and γ-amino acids to adopt protein-like secondary structures is of great interest to develop helical mimics with functional properties.[1] Incorporation of constrained cyclic building blocks reducing the backbone flexibility has permitted to improve the stability of the folding and to substantially enlarge the helical foldamer realms. According to the results obtained with β-peptides,[2] γ-amino acids were expected to be valuable to design new scaffolds.[3] Nevertheless, to date, only few γ-subunits are available owing to the difficulty to access stereochemically pure species, and only few γ-oligomer secondary structures have been described.[4] The first helical fold of γ-peptides, independently reported by the research groups of Hanessian[5] and Seebach,[6] was a helix stabilized by 14-membered pseudo-rings. Sheets,[7] ribbons,[8] or helices[9] stabilized by C_7 or C_9 hydrogen-bonded pseudocycles were obtained later by using constrained cyclic γ-amino acid building blocks. Additionally, while the conformational control is crucial for biological function, the access to foldamers bearing a wide variety of side chains and being soluble under physiological conditions is essential to purchase biological applications. However, the combination of these three criteria remains challenging.[10]

In this context, we report herein the design of 4-amino-(methyl)-1,3-thiazole-5-carboxylic acids (ATCs) **1** as new γ-building blocks (Scheme 1). These highly constrained monomers were built around a thiazole ring to limit conformational flexibility around 0° about the $^{\alpha}C$–$^{\beta}C$ bond. We proposed a fast and robust synthetic pathway for the formation of



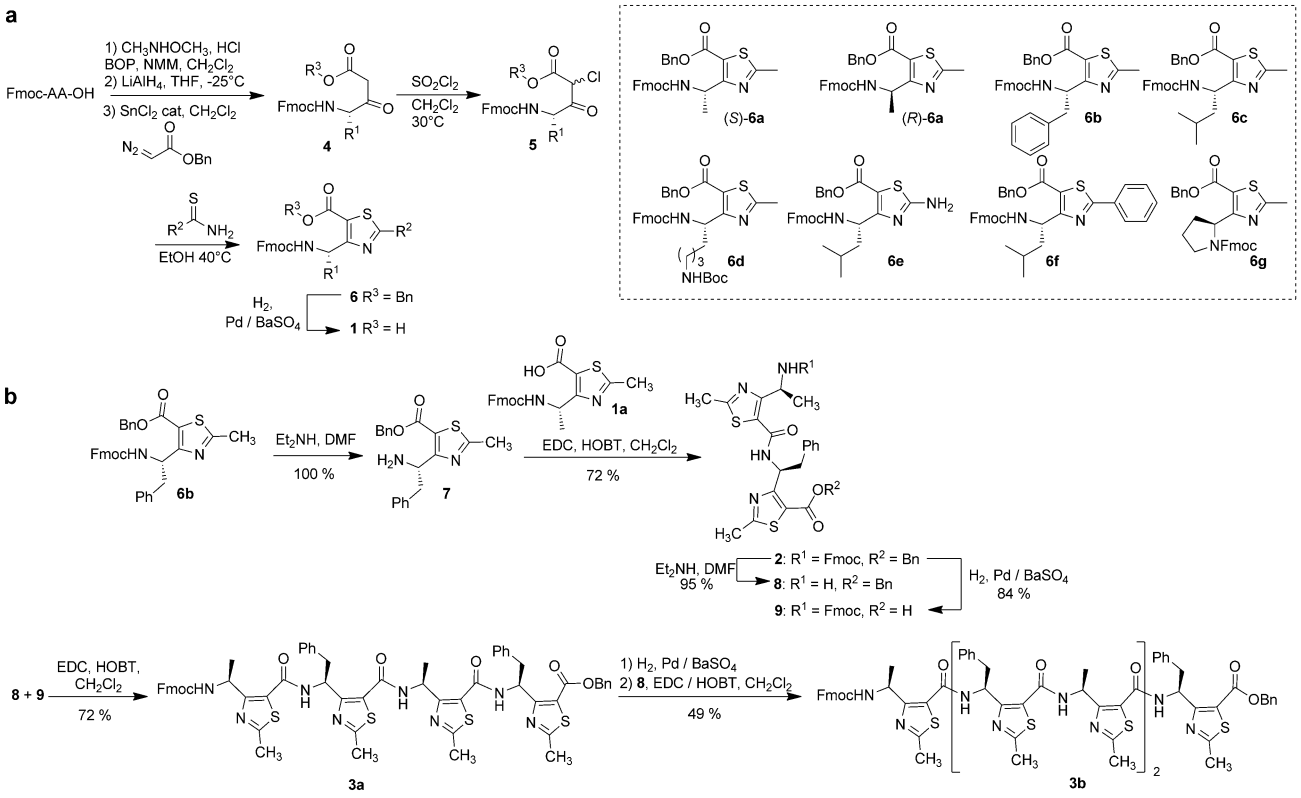
Scheme 1. Thiazole-based γ-amino acid building blocks and oligomeric derivatives. Fmoc = 9-fluorenylmethoxycarbonyl, Boc = *tert*-butoxycarbonyl.

ATCs, thereby giving opportunity of a straightforward modulation of the two substitution positions while controlling the stereochemistry at the γ-carbon atom. We characterized the structures of various ATC oligomers in solution by CD and NMR spectroscopy and in the solid state by X-ray crystallography. Significantly, the ATC sequences adopted a well-defined 9-helix structure in the solid state and in aprotic and protic organic solvents as well as in aqueous solution.

Our synthetic approach to access ATCs is described in Scheme 2. We started from *N*-Fmoc-protected α-amino acids to access benzyl β-ketoesters **4**. Although the cross-Claisen condensation is a well-known reaction to access β-ketoesters, successful results were only achieved when sterically non-hindered esters like ethyl or benzyl acetate were used.[11] Additionally, the required basic conditions are usually not compatible with Fmoc protection.[11a] Thus, we turned our attention toward another straightforward reaction: the Lewis

[*] L. Mathieu,[+] Dr. B. Legrand,[+] Dr. L. Vezenkov, Dr. M. Amblard, Dr. N. Masurier, Prof. V. Lisowski, Prof. J. Martinez, Dr. L. T. Maillard
Institut des Biomolécules Max Mousseron, UMR 5247, CNRS
Universités Montpellier I et II
UFR des Sciences Pharmaceutiques et Biologiques
15 Avenue Charles Flahault, 34093 Montpellier Cedex 5 (France)
E-mail: ludovic.maillard@univ-montp1.fr
C. Deng, Dr. M.-C. Averlant-Petit
Laboratoire de Chimie-Physique Macromoléculaire
LCPM-UMR 7568 CNRS Université de Lorraine
1 rue Grandville, 54001 Nancy Cedex 1 (France)
E. Wenger, Dr. C. Didierjean
Laboratoire de Cristallographie, Résonance Magnétique et Modélisation, UMR 7063 CNRS Université de Lorraine
Boulevard des Aiguillettes, 54506 Vandoeuvre-lès-Nancy Cedex (France)
[+] These authors contributed equally to the work.

[**] We thank the IBMM, the CNRS, and the University of Montpellier 1 for financial support and the University of Lorraine for NMR and XRD facilities.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201302106.



Scheme 2. Synthesis of a) *N*-Fmoc protected γ-amino acids and b) oligomers **2**, **3a**, **3b**. AA = α-amino acid; BOP = benzotriazol-1-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate, NMM = *N*-methylmorpholine, EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, HOBT = 1-hydroxybenzotriazole.

acid catalyzed C–H insertion of a diazoacetate on aldehydes. The *N*-Fmoc-protected α-amino acids were converted into Weinreb amides. Reduction with $LiAlH_4$ led to the corresponding aldehydes, which were promptly condensed with benzyl diazoacetate in CH_2Cl_2 in the presence of a catalytic amount of tin(II) chloride to yield the orthogonally protected β-ketoesters **4**. Effective monohalogenations were performed with sulfuryl chloride,[12] then the α-chloro-β-ketoesters **5** were engaged without any further purification in a Hantzsch cyclization with a thioamide or thiourea to lead to the thiazole-based γ-amino benzylesters **6**. Because racemization is a major issue when aminoaldehyde intermediates are used, the enantiomeric excess was ascertained by determination of the optical rotation of both enantiomers (*S*)-**6a** ($[\alpha]_D^{20} = +4.9°$ ($c = 1.95$, $CHCl_3$)) and (*R*)-**6a** ($[\alpha]_D^{20} = -4.9°$ ($c = 2.08$, $CHCl_3$)) and by using HPLC on a chiral stationary phase (see Figure S11 in the Supporting Information). The rate of racemization was 2% when the synthesis was performed on a 1 g scale (37% yield on the overall sequence). Starting from a set of N-protected amino acids and thioamides, the synthetic pathway provided a highly versatile and flexible method for the introduction of different substitution patterns either on the γ-carbon atom of the backbone or at position 2 of the thiazole core (Scheme 2a). Ab initio calculations from Hofmann and co-workers[13] suggested that oligomers of *Z*-vinylogous γ-amino acids would favor two stable helical conformers with seven- and nine-membered hydrogen-bonded pseudocycles. On the other hand and to our knowledge, Mann and Kessler reported the unique example of (hetero)aromatic-based γ-amino acids in which the ring is an oxazole.[14] Included into a small peptide, such a building block acts as a C_9 turn inducer.

Four γ-peptides, **2**, **3a**, **3b**, and **3c** of different lengths (2, 4, and 6 monomers) were first synthesized through an Fmoc/OBn strategy (Scheme 2b). The peptides exhibited a high solubility in chloroform but were poorly soluble in polar solvents. Hence, assuming that the introduction of lateral chains containing amine groups could greatly improve the solubility in polar solvents, we synthesized γ-peptides **3d**, **3e**, and **3f** from building blocks **6c** and **6d** (see the Supporting Information).

NMR-spectroscopic analyses of **2**, **3a**, and **3b** were conducted in $CDCl_3$. The water-soluble compounds **3d**, **3e**, and **3f** were studied in CD_3OH and H_2O/D_2O (9:1, pH 6.5). In all cases, the NMR signals were well dispersed and nearly all ^1H, ^{13}C and ^{15}N resonances could be assigned by combining ^{15}N-HSQC and ^{13}C-HSQC at ^{15}N and ^{13}C natural abundance (Tables S1–S18 in the Supporting Information). The sequential assignment was based on the strong NH(*i*)/$^{\gamma}$CH(*i*−1) NOE correlations on the ROESY/NOESY spectra. In addition, characteristic weak NH*i*/$^{\delta}$CH(*i*−1) and $^{\gamma}$CH(*i*)/$^{\delta}$CH(*i*+1) sequential NOE connectivities were observed along the backbones. Similar NOE sets were obtained for dimers **2** and **3d**, for tetramers **3a** and **3e**, and for hexamers **3b** and **3f** (Figure 1, Tables S20–22). The 14-helical fold typical for γ-peptides could be excluded at this stage, since no

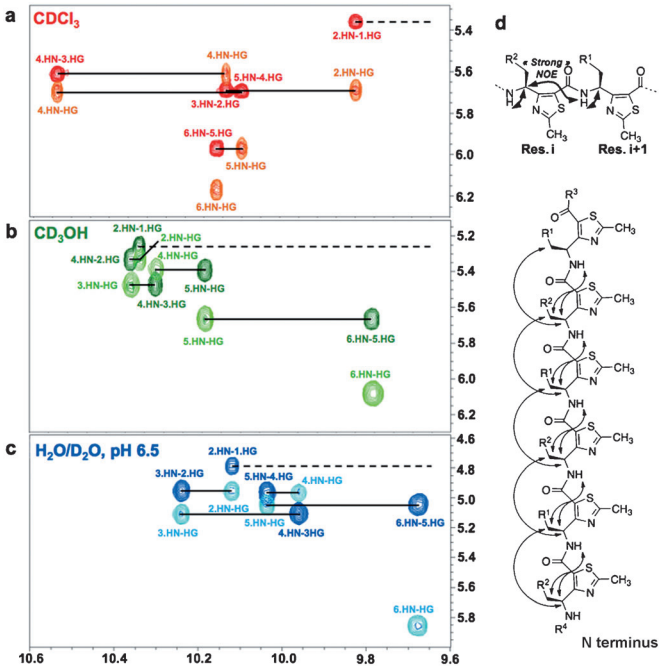


Figure 1. Superimposition of the NH/$^{\gamma}$CH region of the TOCSY (in dark) and ROESY (in light) spectra of a) **3b** recorded in $CDCl_3$, b,c) **3f** recorded in CD_3OH and H_2O/D_2O, pH 6.5 at 2–10 mM. Typical strong sequential NOE correlations of HN(i)/$^{\gamma}$CH($i-1$) are annotated. ^{1}H chemical shifts on both axes are given in ppm. HG corresponds to γCH. d) Typical interresidue NOE pattern along the **3b** and the **3f** hexamers.

typical (i, $i+2$), (i, $i+3$) medium-range NOEs were detected.[15] Whatever the length of the oligomers and the nature of the solvent, all the amide protons exhibited remarkable downfield chemical shifts, for example, from 9.18 ppm for dimer **2** (Table S1) to 10.67 ppm for hexamer **3b** in $CDCl_3$ (Table S5). As a comparison, Kunwar and co-workers previously described a 9-helix for a γ-peptide oligomer in which amide resonances were found between 6.92 and 7.60 ppm in $CDCl_3$.[9a] This marked unshielding of the amide protons could be attributed to a highly stable hydrogen-bonding pattern and/or to a ring-current effect of the thiazole heterocycle. In addition, ^3J(NH,$^{\gamma}$CH) values smaller than 6 Hz (5.4 ± 0.4 Hz) were typical of ϕ values around −60° (Table S19). Taken together, these data were strong evidence of a well-organized system in solution for all compounds whatever the solvent considered.

The ATC monomers were parameterized using the Antechamber package[16] starting from the X-ray crystal structure of **6b**, which exhibited an extended conformation with dihedral angles $\phi = -126°$, $\theta = 158°$, $\zeta = -4°$ and $\psi = 180°$ (Figure S6 and Table S25). NOEs were used as restraints for calculations on the structure in solution determined by NMR spectroscopy by using a simulated annealing protocol with AMBER 10.[17] The solution structures of **2**, **3a**, and **3b** in $CDCl_3$ were solved by using 39, 87, and 124 unambiguous restraint distances, respectively. Figure S5 depicts a superimposition of the 20 lowest-energy structures calculated for each compound. The root-mean-square deviation (RMSD) values for the backbone are 0.11 Å, 0.48 Å, 0.63 Å, respectively, when the capping groups are omitted. The tetramer **3a** and the hexamer **3b** exhibited tight right-handed 9-helix structures stabilized by C=O(i)···HN(i+2) hydrogen bonds. Notably only two residues were necessary to initiate the folding into C_9 hydrogen-bonded pseudocycles as observed on dimer **2**. The hydrogen-bonding patterns were homogenous along the entire sequences in a forward direction, from N to C. The average values of the dihedral angles for the ATC helix were $\phi = -76 \pm 16°$, $\theta = 128 \pm 12°$, $\zeta = -6 \pm 5°$, and $\psi = -28 \pm 6°$ (Table 1). Only small distortions were observed on the ψ angles of the C-terminal residues of each oligomer.

Table 1: Average backbone torsion angles for γ-peptides adopting 9-helical fold. The ψ angles of the last residue of the NMR and XRD structures were omitted.

	ϕ	θ	ζ	ψ
Balaram's oligomers[9b]	109°	−63°	−77°	88°
Kunwar's oligomers[9a]	125°	−68°	−65°	97°
Predicted *Z*-vinylogous γ-oligomers[13]	−79.8°	122.8°	0.1°	−46.5°
ATC oligomers				
Average NMR values:				
2, **3a**, **3b**	−76 ± 16°	128 ± 12°	−6 ± 5°	−28 ± 6°
3d, **3e**, **3f**	−72 ± 33°	114 ± 29°	−5 ± 6°	−23 ± 11°
XRD structure of **3c**	−78 ± 3°	127 ± 14°	0 ± 3°	−41 ± 4°

Importantly, we were able to crystallize the tetramer **3c** by solvent diffusion of diisopropyl ether into a solution of the oligomer in toluene. Low-resolution X-ray diffraction data were sufficient to show that ATC γ-peptides adopt the same helical structure in solid state and solution (Figure 2a,b). All amide groups of the molecule form inter- or intrahelix hydrogen bonds (Figure S7). The typical average dihedral angles were comparable to those measured in NMR-spectroscopic experiments ($\phi = -78 \pm 3°$, $\theta = 127 \pm 14°$, $\zeta = 0 \pm 3°$ and $\psi = -41 \pm 4°$, Table 1) with a notable deviation of the C-terminal ψ torsion angle ($\psi = -165°$), which allowed the packing of the molecules into infinite chains in the crystal. Simulations of Hofmann and co-workers predicted that *Z*-vinylogous γ-oligomers could adopt a helical fold with nine-membered hydrogen-bonded pseudocycles with close torsion angles ($\phi = -79.8°$, $\theta = 122.8°$, $\zeta = 0.1°$, $\psi = -46.5°$).[13] By comparison, the C_9 hydrogen-bonded helices reported by the research groups of Balaram and Kunwar have values of $\phi = 109°$, $\theta = -63°$, $\zeta = -77°$, $\psi = 88°$ and $\phi = 125°$, $\theta = -68°$, $\zeta = -65°$, $\psi = 97°$, respectively.[9] Considering the global shape of the ATC oligomers, the edifice appeared to be close to a C_3-symmetric helix with three residues to achieve a complete rotation with a pitch of 11.8 Å. This helix had a rise-per-residue of 3.9 Å and exhibited six substitution patterns per turn, which were distributed around a 60° angle all along the axis (Figure 2c). We then solved the NMR solution structures of the compounds **3d**, **3e**, and **3f** in water (pH 6.5) by using 12, 49, and 72 constraints, respectively, with the generalized Born solvation model[18] in AMBER (Figure 3a and S5, Table 1). As expected, since similar NOE sets were obtained for the water-soluble series (Tables S20–22), comparable

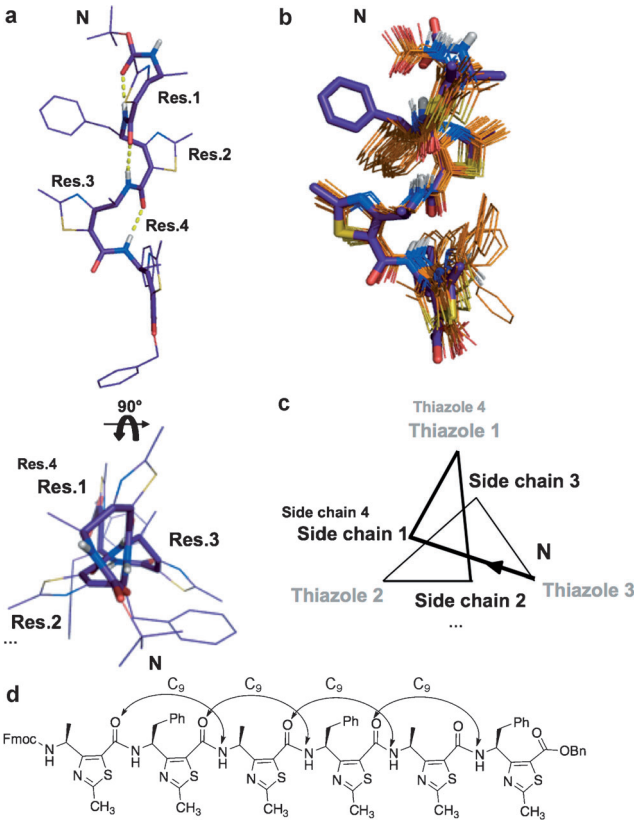


Figure 2. a) Orthogonal and axial views of the crystal structure of the tetramer **3c**.[19] b) Superimposition of the 20 lowest-energy NMR structures of **3a** (in orange) with the XRD structure of **3c** (in purple). Nonamide protons and capping groups are omitted for clarity. c) Axial projection of the ATC oligomers. d) Characteristic hydrogen-bond network of oligomer **3b**.

structures were calculated showing that the ATC oligomers remarkably conserved their unique fold even in water.

The far-UV CD spectra for **3d**, **3e**, **3f** were then recorded in methanol and water (pH 6.5) between 190 and 300 nm (Figure 3b). The CD signatures of the hexamer **3f** shared similar global shapes in methanol and in water. We observed two minima at 195 and 231 nm and 195 and 226 nm in methanol and water, respectively, and a strong maximum around 263 nm. The two minima were assigned to the π–π* and n–π* transitions of the amide chromophore, respectively, while the maximum could be attributed to the thiazole core. The classical blue shift observed for the n–π* transition in aqueous solution is certainly a result of stronger solute–solvent hydrogen-bond interactions and/or the higher water polarizability. The first NV1 π–π* transition minimum was weaker in water, but no significant wavelenght shift was detected. Interestingly, the intensity of the maximum dramatically increased with the oligomer size from two to six residues to reach a molar ellipticity value per residue of 82700 deg cm^2 dmol^{-1} in methanol and 74000 deg cm^2 dmol^{-1} in water for **3f**, thus suggesting an increased stability with length. Some differences were observed for tetramer **3e**, since a third weak minimum appeared at 211 nm, and for dimer **3d** for which the first minimum was red-shifted at 200–205 nm in all solvents.

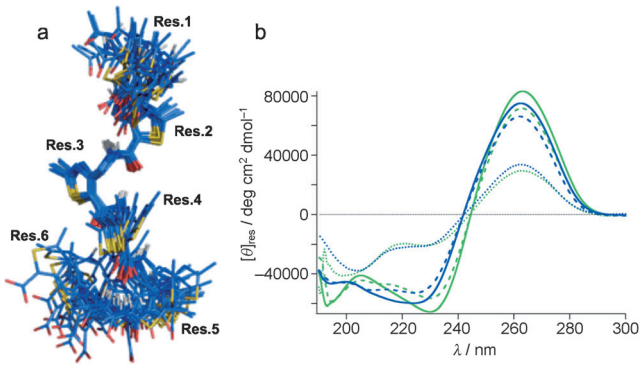


Figure 3. a) Overlay of the 20 lowest-energy structures of hexamer **3f** calculated from NMR data in water, pH 6.5. Lateral chains are omitted for clarity. b) CD spectra of **3d** (••••), **3e** (-----), and **3f** (——) at 200 μM in MeOH (green) and water, pH 6.5 (blue). $[\theta]_{res}$ = molar ellipticity per residue.

This study thus presents the design of a new family of ring-constrained γ-amino acids and the identification of a novel right-handed 9-helix structure in γ-peptides. These features resulted from the planar conformation of the $^{\gamma}$C-$^{\beta}$C-$^{\alpha}$C-C(O) torsion angle imposed by the thiazole heterocycle. Moreover, we demonstrate that ATC oligomers display a high stability in organic and in aqueous media, which is of particular importance for applications in medicinal chemistry. The fast and highly robust synthetic pathway ensures the access to a wide diversity of enantiopure ATCs, thereby giving a real opportunity to straightforwardly modulate the physicochemical properties of the oligomers and to drive molecular recognition.

Received: March 13, 2013
Published online: April 25, 2013

Keywords: conformation analysis · foldamers · helical structures · peptidomimetics · γ-peptides

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[19] CCDC-922304 (**3c**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.