Extended diethylglycine homopeptides formed by desulfurization of their tetrahydrothiopyran analogues

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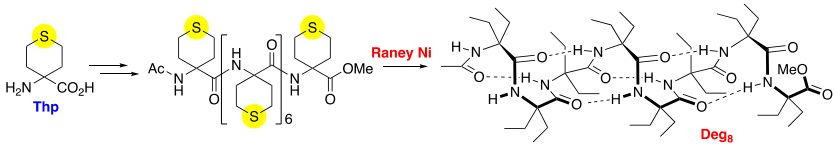
Supporting Information Placeholder



ABSTRACT: Diethylglycine (Deg) homopeptides adopt the rare 2.0_5-helical conformation, the longest three-dimensional structure that a peptide of a given sequence can adopt. Despite this unique conformational feature, Deg is rarely used in peptide design because of its poor reactivity. In the paper we show that reductive desulfurization of oligomers formed from more reactive tetrahydrothiopyran-containing precursors provides a practical way to build the longest Deg homopeptides so far made, and we detail some conformational studies of the Deg oligomers and their heterocyclic precursors.

Homopeptides **2** of diethylglycine (Deg) **1** (Figure 1a) have peculiar structural features, as they are able to adopt the rare, fully-extended conformation or 2.0_5-helix (Figure 1b). This motif is the longest 3D-structure that a peptide of a given sequence can adopt, with torsion angles: $\varphi = \psi = \omega = 180°$,[1] and is extremely rare in natural peptides. One of the few examples known is the $(Gly)_4$ sequence of the enzyme His-tRNA-synthetase.[2] The 2.0_5-helix is sensitive to external conditions and reversibly interconverts with the much shorter 3_{10}-helix on changing solvent polarity.[3] Deg peptides, being able to undergo such a conformational switch, may find application as molecular springs in peptide-based devices.[4]

Despite these unique features, Deg peptides are not widely exploited in peptide conformational design, mainly because of the low reactivity of even the most activated Deg derivatives, which hampers its coupling reactions. The longest Deg homopeptide synthesized so far is the hexapeptide Tfa-$(Deg)_6$-OEt.[5]

In this paper, we describe a new, generally applicable synthetic approach to Deg homopeptides that exploits the more readily coupled tetrahydrothiopyran-derived C^{α}-tetrasubstituted residue Thp (4-aminotetrahydrothiopyran-4-carboxylic acid, **3**) as a precursor to Deg, which may be revealed through desulfurization of **3**. Thp is a mimic of Met and has been employed in the design of Met-containing peptide analogues with improved biological activity/enzymatic stability.[6] Cyclic quaternary amino acids tend to be significantly more reactive than their acyclic homologues,[7] and the strategy of masking alkyl groups as rings by linking them through a sulfur atom is one that proved successful in classical diastereoselective aldol reactions. A temporary sulfur-containing ring was, for example, crucial to Woodward's seminal synthesis of erythromycin.[8]

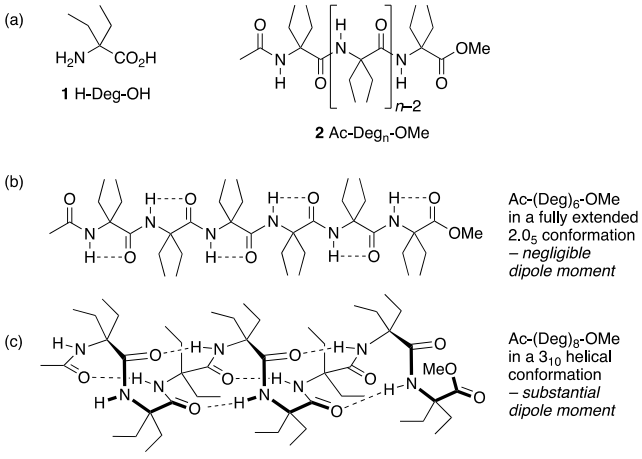


Figure 1. Diethylglycine (Deg) and its oligomers.

A supply of the α-amino acid Thp **3** was prepared using a modified Strecker reaction[9] (for details see Supporting Information, SI). Thp **3** was protected as its Fmoc derivative and activated towards coupling by conversion to its acid fluoride derivative Fmoc-Thp-F (Figure 2).[10]

The methyl ester of **3** was successively coupled to Fmoc-Thp-F in solution, allowing the synthesis of Thp homopeptides **4** with high yields (76% - 89%) for each coupling step (Figure

2). By fine tuning the excess of the acylating agent and reaction times, we could considerably improve both yield and purity even for the longer peptide sequences (yield >80% even for Fmoc-(Thp)$_n$-OMe, n = 7,8). Each coupling between Fmoc-Thp-F and H-(Thp)$_n$-OMe was achieved by stirring for 12 hours in anhydrous CH_2Cl_2. Simple purification by flash chromatography returned pure Thp-homopeptides **4**.

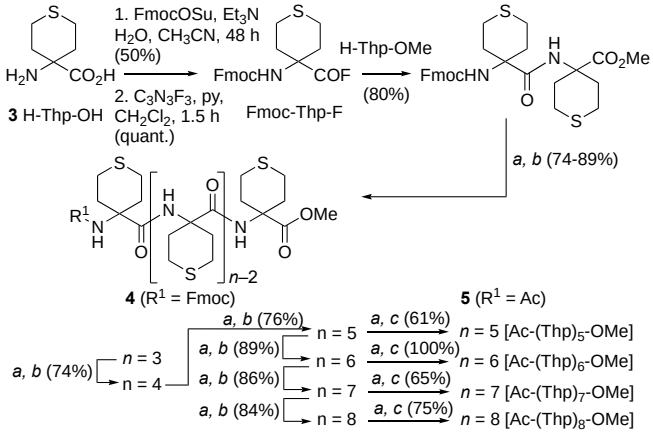


Figure 2. Synthesis of oligomers of Thp. Conditions: *a* 20% *v*/*v* Et_2NH in anhydr. CH_2Cl_2, room temp, 1 h; *b* Fmoc-Thp-F (1.1 equiv) in anhydr. CH_2Cl_2, room temp overnight; *c* 30% Ac_2O in anhydr. CH_2Cl_2, room temp, 1 h.

Thp homopeptides have never been synthesized before, so we carried out a detailed study of their conformational characteristics. It has been proposed[6] that Thp may induce the elusive γ-turn.[11] FTIR analysis in deuterochloroform (Figure 3) provided evidence that a γ-turn is indeed present in the shorter Thp homologues. Figure 3a shows a band centered at about 3290 cm^{-1} for Fmoc-(Thp)$_3$-OMe and 3315 cm^{-1} for Fmoc-(Thp)$_2$-OMe. A negligible dilution effect (see SI) shows that this band is due to an intramolecularly H-bonded NH, arising from a γ-turn C_7 *pseudo*-cycle between the NH of the second Thp residue with the Fmoc C=O. At the same time, the third Thp residue in Fmoc-(Thp)$_3$-OMe makes possible the formation of a β-turn (a sequence of which produces a 3_{10} helix). An i←i+3 H-bond between the NH of Thp[3] and the urethane C=O of the Fmoc protecting group is confirmed by a band centered at 3370 cm^{-1}.

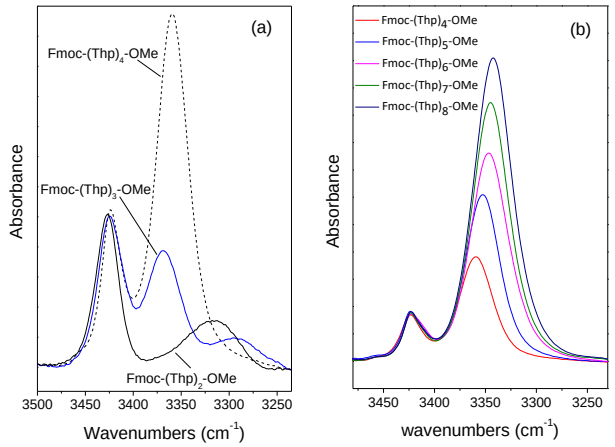


Figure 3. (a) Portion of the FTIR absorption spectra (NH stretching bands) of Fmoc-(Thp)$_n$-OMe (n = 2-4) in $CDCl_3$ (1 mM); (b) Amide A region of the FTIR absorption spectra of the Fmoc-(Thp)$_n$-OMe series (n= 4-8) in $CDCl_3$ (1 mM).

This rare γ-turn could not be detected for longer homopeptides, for which a 3_{10}-helical structure is preferably adopted (Figure 3b), indicating that the γ-turn (a sequence of which generates the 2.2_7-helix[12]) is stable only in short peptide sequences (n <4). The onset of a 3_{10}-helix for Fmoc-(Thp)$_n$-OMe (n= 4-8) peptides is confirmed by the negligible dilution effect (Figure S2, SI), the strong, red-shifted amide I band centered at about 1666 cm^{-1}, and the occurrence of the amide II band at around 1520 cm^{-1} (Figure S3, SI). More detailed information on the secondary structure of the Thp homooligopepeptides was provided by both FTIR absorption analysis and NMR studies of Ac-(Thp)$_n$-OMe **5**, obtained by deprotection and acetylation of **4** (Figure 2). The results pointed to a well developed 3_{10}-helical conformation[13] for all Ac-(Thp)$_n$-OMe (see SI).

Desulfurization of the tetrahydrothiopyran ring of Thp reveals two ethyl groups, allowing the synthesis of peptides containing the otherwise synthetically challenging diethylglycine residue. Treatment of Ac-(Thp)$_n$-OMe peptides (n = 6-8) with Raney Ni[14] converted the Thp-homooligopeptides straightforwardly to the corresponding Deg-peptides Ac-(Deg)$_n$-OMe (n = 6-8) in good yield and purity (Fig. 4).

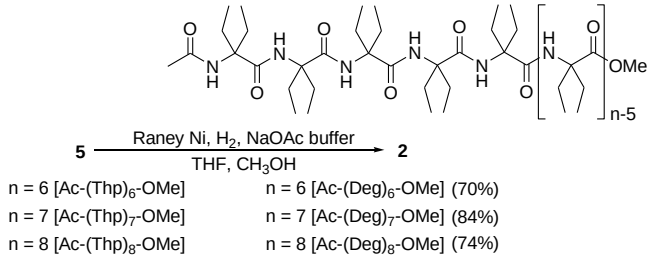


Figure 4. Synthesis of Ac-(Deg)$_n$-OMe by Raney Ni desulfurization of Ac-(Thp)$_n$-OMe.

As a result of the differences in their molecular dipole moments, Deg homopeptides undergo reversible conformational transitions between the 2.0_5- and 3_{10}-helix in response to a change in solvent polarity (Figure 1b,c). FTIR clearly distinguishes between a 2.0_5- and a 3_{10}-helix,[3] so in order to investigate this feature for these unprecedentedly long homopeptides, we acquired IR spectra in three different solvents ($CDCl_3$, cyclohexane-d_{12}, and CD_3CN).

(a) (b)

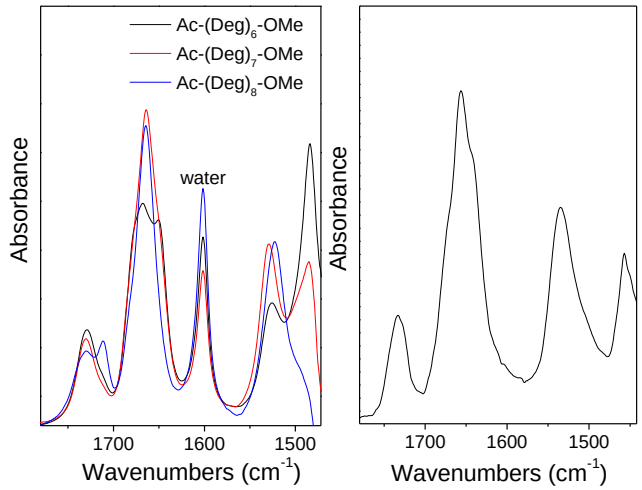


Figure 5. IR absorption spectra (Amide I and II region) of (a) Ac-$(Deg)_n$-OMe (n = 6-8) in $CDCl_3$ (peptide concentration: 1 mM) and (b) Ac-$(Deg)_7$-OMe in cyclohexane-d_6 (peptide concentration: 0.5 mM).

FTIR analysis on all peptides at both 0.1 and 1 mM concentrations revealed negligible spectral differences and thus ruled out peptide aggregation. Ac-$(Deg)_6$-OMe shows the typical IR absorption features of the fully extended conformation, namely a split amide I band ($\Delta\nu$ = 20 cm^{-1}). The amide II shifts to a frequency lower than 1500 cm^{-1} (about 1490 cm^{-1}) and is more intense than the amide I band (Figure 5).[15] Additionally, a less intense band at 1525 cm^{-1} may be tentatively ascribed to a small contribution from the 3_{10}-helical conformation arising from the relative destabilization of the 2.0_5-helix in longer oligomers of Ac-$(Deg)_n$-OMe.

The amide I and II bands of Ac-$(Deg)_7$-OMe in $CDCl_3$ solution (Figure 5) reveal the population of both 3_{10}- and 2.0_5-helical structures, with a broad amide I band and two equally intense amide II bands at the position typical of 3_{10}- (1525 cm^{-1}) and 2.0_5-helices (1490 cm^{-1}). This observation is further supported by the position and relative intensities of the bands in the amide A region, (Figure S6, SI). In a continuation of this trend, the IR absorption spectrum of Ac-$(Deg)_8$-OMe (Figure 5a) shows amide I and amide II bands characteristic only of a 3_{10}-helical structure. In particular, the (2.0_5-helix) amide II band at a frequency lower than 1500 cm^{-1} is completely absent.

This result suggests that the Deg_7 heptapeptide is already too long to adopt a fully extended 2.0_5 conformation, which apparently does not gain stability by increasing cooperativity. The same holds true for the 3_{10}-helix, which often disappears with peptide elongation in favour of the onset of an α-helix.[16] This is the first evidence of a length-dependent change in the conformational preferences of Deg homopeptides, with the 3_{10}-helix preferred over the fully extended conformation for longer 7- and 8-residue homologues.

Even in non-polar cyclohexane, which is expected to favour the greater number of intramolecular hydrogen bonds supported by a 2.0_5-helix, the amide I band of Ac-$(Deg)_7$-OMe revealed a mixture of 2.0_5- and 3_{10}-helix (Figure 5b), though with a greater contribution from the 'split' signal characteristic of the 2.0_5-helix. In the polar solvent CD_3CN (known to induce 3_{10}-helical conformation in Deg-peptides) the amide II band of all Ac-$(Deg)_n$-OMe peptides studied (n = 6-8) falls at about 1530 cm^{-1}, characteristic of helical structures (see Figure S7, SI). Ac-$(Deg)_6$-OMe thus reveals a conformational switch between a 2.0_5 helix in $CDCl_3$ and a 3_{10} helix in CD_3CN solutions.[17]

Further information on the secondary structure of Deg homopeptides was gained from 1D and 2D ^{1}H-NMR spectroscopy in $CDCl_3$, DMSO-d_6, and CD_3OH. NMR analysis on Ac-$(Deg)_6$-OMe in $CDCl_3$ solution in the presence of an increasing percentage of DMSO-d_6[18] caused no change on any of the NH proton chemical shifts, confirming the adoption of a fully extended conformation in $CDCl_3$ solution for Ac-$(Deg)_6$-OMe (Figure 6a). The same analysis carried out on Ac-$(Deg)_8$-OMe showed that two NHs are sensitive to the presence of the H-bond donor, as expected for a 3_{10}-helical conformation (Figure S8, SI). The 1D ^{1}H NMR spectrum of Ac-$(Deg)_7$-OMe in $CDCl_3$ solution at 23 °C was characterized by very broad signals which sharpened when acquired at 53 °C, presumably as a result of the exchange between the 3_{10}- and 2.0_5-helical structures revealed by FTIR. NOESY experiments in a more polar solvent CD_3OH (Figure 6b) revealed all sequential, NH-NH cross peaks (apart from the one between NH^6 and NH^7 falling into the diagonal). Such connectivity is possible only for 3_{10}- or α-helical peptides and is inconsistent with a 2.0_5-helix.[19]

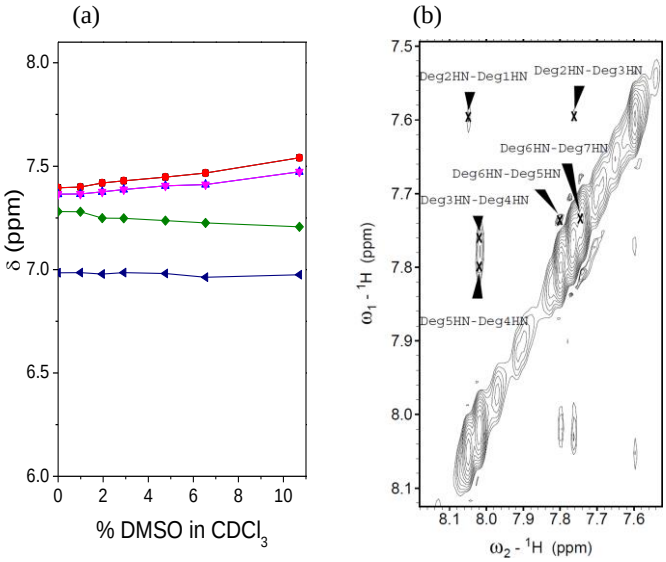


Figure 6 (a) Insensitivity of NH proton chemical shifts in the ^{1}H NMR spectra Ac-$(Deg)_6$-OMe as a function of the amount of DMSO-d_6 added to the $CDCl_3$ solution (*v*/*v*) (peptide concentration: 1 mM). (b) Amide region of the NOESY spectrum of Ac-$(Deg)_7$-OMe in CD_3OH solution (peptide concentration: 1 mM) showing NH(*i*)→NH(*i*+1) connectivities.

In summary, we report the synthesis of the homopeptide series Fmoc-$(Thp)_n$-OMe (*n* = 2-8) and Ac-$(Thp)_n$-OMe (*n* = 6-8). A conformational analysis by means of FTIR Absorption spectroscopy highlighted the presence of the elusive γ-turn conformation for short Thp homopeptides. Raney Ni reductive desulfurization of these Thp oligomers converted them into the corresponding Deg homopeptides Ac-$(Deg)_n$-OMe (*n* = 6-8) -

the longest Deg-homopeptides ever synthesized - with good yield and purity. This approach overcomes the usual challenge of coupling the highly unreactive Deg oligomers and paves the way to their synthesis on a solid support.

Ac-$(Deg)_n$-OMe oligomers adopt stable fully-extended conformations only for $n < 7$ in non-polar solvents. Such length-dependent stability may be explained considering that although for a single Deg residue the minimum energy conformation corresponds to a 2.0_5 helix, the 3_{10}-helical structure is less than 2 kcal/mol higher in energy.[3] Moreover, as the number of intramolecular H-bonds increases as a result of peptide elongation, the stabilization gained by the two additional intra-residue H-bonds in the 2.0_5 conformation becomes less important. We conclude that the longest homologue of the Ac-$(Deg)_n$-OMe series that can adopt a stable fully-extended 2.0_5 conformation is the hexapeptide, which thus represents the current limit for using peptide design to induce this maximally extended conformation. The fact that lengthening oligomers of Deg, and presumably also of other similar dialkylglycines, causes them to revert to a 3_{10} helical secondary structure has wider implications for the use of these residues in the design and synthesis of conformationally controllable and switchable helical foldamer structures,[20] particularly with regard to their potential membrane activity.[21] The use of Thp as a precursor to Deg has potential wider utility in peptide synthesis in solution and on solid phase, and opens opportunities for wider use of Deg as a conformational control element in the design of peptidomimetics.

Supporting Information

Experimental and spectroscopic data for all new compounds. Further spectroscopic data and conformational analysis for Thp and Deg peptides (PDF). The Supporting Information is available free of charge on the ACS Publications website.

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(10) (a) Fmoc-amino acid fluorides can be obtained from Fmoc-amino acids by treatment with cyanuric fluoride in the presence of pyridine and are both stable enough to isolate and sufficiently reactive to allow the formation of peptide bonds between bulky α-amino acids of extreme steric hindrance. See: Carpino, L.A. *J. Am. Chem. Soc.* **1990**, *112*, 9651 and Carpino, L. A. *J. Am. Chem. Soc*. **1993**, *115*, 4397. (b) An alternative approach employing the *in situ* formation of active esters [using HOAt/EDC] was also tried for the synthesis of the short peptides Fmoc-$(Thp)_n$-OMe ($n < 5$). Fmoc-$(Thp)_4$-OMe was prepared several times in order to compare these two methods, (see SI). Both yield and purity were better with Fmoc-Thp-F, and the reaction times were markedly shorter (12 h versus the three days needed with the activated ester), clearly demonstrating the superiority of the amino acid fluoride method.

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(15) A fully-extended conformation (2.0_5-helix) exhibits a broad band at about 3360 cm^{-1} in the Amide A region, because all its NHs are intramolecularly (intraresidue) H-bonded. 3_{10}-helical peptides exhibit a strong band at about 1666 cm^{-1} (amide I)[(a) Polese, A.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. *Chem. Eur. J.* **1996**, *2*, 1104], followed by a less intense band at about 1515-1530 cm^{-1} (amide II). In contrast, in 2.0_5-helical peptides the amide I absorption at 1660 cm^{-1} is split into two components [(b) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Barone, V.; Lelj, F.; Leplawy, M. T.; Kaczmarek, K.; Redlinski, A. *Biopolymers* **1988**, *27*, 373], and the amide II band, significantly more intense than the amide I, is found at wavenumbers lower than 1500 cm^{-1}[(c) Formaggio, F.; Crisma, M.; Ballano, G.; Peggion, C.; Venanzi, M.; Toniolo, C. *Org. Biomol. Chem.* **2012**, *10*, 2413]. For α-helical structures the amide I band falls typically at about 1659 cm^{-1}, while amide II is at about 1548 cm^{-1} [(d) Nevskaya, N.A.; Chirgadze, Y.N. *Biopolymers* **1976**, *15*, 637].

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