Self-Assembly of Peptide-Based Diblock Oligomers

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ABSTRACT: The synthesis and supramolecular organization of a novel class of rod−coil type diblock oligomers will be discussed. The diblock oligomers consist of a rodlike α-helical oligopeptide segment that is conjugated to an oligo(styrene) coil. In comparison with most of the rod−coil type oligomers that have been investigated so far, these peptide-based diblock oligomers possess some unique features: (i) the conformation of the oligopeptide rod segment can be reversibly manipulated under the action of appropriate external stimuli, and (ii) the self-assembly of these molecules is (also) driven by directed hydrogen-bonding interactions. The diblock oligomers are prepared by ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride using a primary amine-terminated oligo(styrene) as the initiator. The diblock oligomers form thermotropic liquid-crystalline phases, whose supramolecular organization depends both on the relative block-lengths and on the conformation of the peptide segment.

Introduction

In contrast to coil−coil diblock copolymers,[1] the self-assembly of rod−coil diblock copolymers is directed not only by the microphase separation of the blocks but also by the tendency of the rigid segments to form anisotropic liquid-crystalline domains. This competition process can lead to morphologies that are distinctly different from those that are commonly observed for conventional coil−coil diblock copolymers.[2] Furthermore, the stiffness asymmetry in rod−coil diblock copolymers results in an increase in the Flory−Huggins χ parameter in comparison with coil−coil diblock copolymers. An important consequence of the enhanced Flory−Huggins χ parameter is that rod−coil diblock copolymers might already self-assemble into phase-separated structures at relatively small degrees of polymerization (N),[3] which could allow access to phase-separated structures with length scales that are not attainable with traditional coil−coil diblock copolymers. Whereas phase separation of coil−coil diblock *copolymers* typically results in morphologies with domain sizes between 50 and 1000 Å, rod−coil diblock *oligomers* have the potential to self-assemble into phase-separated structures with characteristic lengths of several nanometers. Rod−coil diblock oligomers, thus, represent an attractive class of building blocks for the preparation of self-assembled nanostructured materials.

Most of the rod−coil diblock oligomers that have been described so far are composed of a rigid segment, which is typically derived from a traditional mesogen.[4] The rigid segment usually drives the self-assembly of the molecules via nonspecific $\pi-\pi$ interactions and has only restricted conformational freedom. Thus, the rigid segment retains its rodlike character under virtually all circumstances. In contrast to these well-investigated systems, this contribution reports the synthesis and properties of a novel class of rod−coil type diblock oligomers that possess a larger degree of conformational freedom and whose self-assembly is (also) promoted by directed hydrogen-bonding interactions. The rod−coil oligomers that will be discussed consist of an α-helical oligopeptide as the rod portion, which is attached to a flexible coil. In contrast to traditional rigid mesogens, an α-helical peptide has more conformational freedom and might be reversibly transformed from a rigid rod into for example a random coil under the appropriate conditions of temperature, solvent, pH, or ion strength. A second feature that distinguishes such peptide-based diblock oligomers is the possibility for intermolecular hydrogen-bonding interactions between neighboring rod segments. Such hydrogen-bonding interactions could allow a directed self-assembly of the rod−coil oligomers.

The rod segment of the diblock oligomers presented in this contribution is composed of γ-benzyl-L-glutamate (Bn-Glu). The α-helical secondary structure of this peptide enforces a rodlike structure, which is responsible for the thermotropic and lyotropic liquid-crystalline properties of poly(γ-benzyl-L-glutamate).[5] Styrene was chosen for the synthesis of the coil part of the diblock oligomers. High molecular weight block copolymers comprised of a poly(γ-benzyl-L-glutamate) rod connected to either a poly(styrene) or a poly(butadiene) coil have been extensively studied by Gallot et al.[6] These materials were found to form lamellar mesophases, both in bulk and in concentrated solutions. This contribution, in contrast, focuses on unprecedented low-molecular-weight (styrene)-*b*-(γ-benzyl-L-glutamate) diblock oligomers, which are typically comprised of 10−20 units γ-benzyl-L-glutamate and approximately 10 repeat units of styrene. As was already discussed above, the enhanced Flory−Huggins χ parameter of rod−coil type diblock architectures could allow phase separation at smaller degrees of polymerization and give rise to morphologies and length scales different from the lamellar structures know for high-molecular-weight (styrene)-*b*-(γ-benzyl-L-glutamate) diblock copolymers.[6] By variations of the block length ratio, the self-assembly and supramolecular organization of the peptide-based

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diblock oligomers might be manipulated and eventually used to direct the formation of nanostructured peptide materials. In addition, the conformational flexibility of the peptide rod could provide the molecular basis for the development of stimuli-responsive materials, whose supramolecular organization and properties might be reversibly manipulated by means of small changes in environmental parameters.

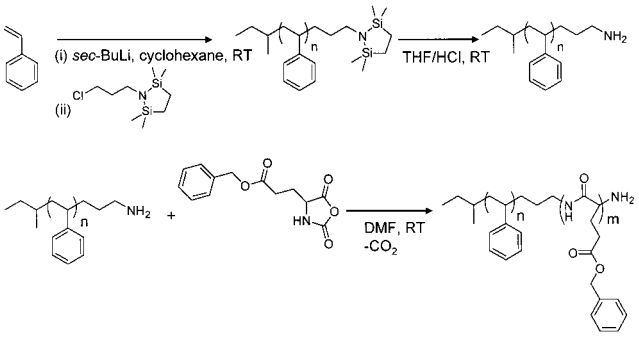
Experimental Part

Materials. Ethyl acetate (EtAc) was dried over molecular sieves (4 Å). *n*-Hexylamine and 1-(3-chloropropyl)-2,2,5,5-tetramethyl-1-aza-2,5,-disilacyclopentane were distilled from CaH_2 under reduced pressure and stored under argon atmosphere. *N,N′*-Dimethylformamide (DMF) was distilled from CaH_2 under reduced pressure and subsequently stored over molecular sieves (4 Å) under an argon atmosphere. γ-Benzyl-L-glutamate *N*-carboxyanhydride (Bn-Glu NCA) was prepared according to a literature procedure.[7] This method involves washing the chilled EtAc solution containing the crude NCA with water and aqueous $NaHCO_3$, which was found to be very effective in removing HCl and the hydrochloride of the starting α-amino acid.[7] In addition, the NCA was recrystallized at least two times from hexane/EtAc prior to polymerization. In our hands, this combination of aqueous workup and multiple recrystallizations resulted in NCA monomers that gave reproducible polymerization results with reasonable control of chain length. All other solvents and reagents were purchased from commercial suppliers and were used as received.

Physical and Analytical Methods. ^{1}H NMR spectra were recorded at room temperature on a Bruker Avance 250 spectrometer using the residual proton resonance of the deuterated solvent as the internal standard. GPC analysis was performed at 60 °C with a setup consisting of a Waters 510 pump and a series of three PSS−SDV columns (300 × 8 mm) with pore sizes of 500, 10^5, and 10^6 Å, respectively. A 0.1 M solution of LiBr in DMF was used as the mobile phase, and the elution of the samples was monitored using simultaneous UV and RI detection. Elution times were converted to molecular weights using a calibration curve that was constructed with narrow-polydispersity poly(styrene) standards. DSC experiments were carried out on a Perkin-Elmer DSC-7 with heating and cooling rates of 10 °C/min. A Leitz Laborlux optical microscope equipped with a CCD camera, a Mettler FP90 central processor, and a Mettler FP82 hot stage was used to analyze the anisotropic textures. FTIR spectra were recorded on a Nicolet 730 FTIR spectrometer under a nitrogen atmosphere. Samples were prepared by drop-casting a thin film from a diluted THF solution onto a piece of silicon wafer. Small-angle X-ray scattering was performed with Cu Kα radiation from an 18 kW rotating anode X-ray generator. A flat pyrolytic graphite (002) monochromator delivered a 0.5 × 0.5 mm^2 spot on the sample. The scattered radiation was collected on a 2D imaging plate system. The instrumental resolution in the reciprocal space was $\Delta q = 2.5$ mm^2 Å^{-1} fwhm (full-width at half maximum) in both vertical and horizontal directions. The measured q range was 0.01 Å$^{-1} < q < 1.6$ Å^{-1}.

Preparation of the Primary Amine-Terminated Oligo(styrene)$_{10}$ Coil ((PS)$_{10}$-NH$_2$). Styrene was oligomerized at room temperature under standard high-vacuum conditions in cyclohexane solution using *sec*-BuLi as the initiator. The oligomerization was quenched by the addition of a 5-fold molar excess of a THF-solution of 1-(3-chloropropyl)-2,2,5,5-tetramethyl-1-aza-2,5,-disilacyclopentane. After stirring at room temperature for 4 h, the solvents were evaporated and the residue was redissolved in THF. Then, the protecting group was removed by the addition of a 0.1 M HCl(aq) solution. The solution was stirred at room temperature for 2 h and then evaporated to dryness. The residue was dissolved in CH_2Cl_2 and repeatedly washed with a saturated $NaHCO_3$(aq) solution. The organic phase was separated, dried over $MgSO_4$, filtered, and evaporated to dryness. TLC analysis (SiO_2:CH_2Cl_2/MeOH 100/10 (v/v)) showed that, in addition to the desired compound, the crude product also contained some unfunctionalized oligo(styrene). These impurities were removed by means of flash chromatography (SiO_2). First the column was eluted with toluene to separate the unfunctionalized oligo(styrene). Then the eluent was changed to THF, and the desired primary amine end-functionalized oligo(styrene) was collected. Evaporation of the solvent and vacuum-drying at 50 °C finally afforded the primary amine end-functionalized oligo(styrene) as a white solid in 85% yield. R_f(SiO_2:CH_2Cl_2/MeOH 100/10): 0.67. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.0$ (b, 5H × m; Ph−H), 2.5 (b, 2H; −CH_2NH$_2$), 1.6 (b, 3H × m; −CH_2CH(Ph)−). Note: m = number-average degree of polymerization of the oligo(styrene). GPC analysis (toluene, PS standards) of a sample that was taken before the addition of the end-capper and quenched with MeOH revealed $M_n = 1230$ g/mol and $M_w/M_n = 1.04$. FD-MS analysis of the amine end-functionalized oligomer yielded $M_n = 1030$ g/mol and $M_w/M_n = 1.05$.

Scheme 1



Preparation of the (Styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ Oligomers. A Schlenk flask fitted with a stir bar and a drying tube was charged with the appropriate amount of Bn-Glu NCA in dry DMF (∼0.2 g/mL). Then, a DMF solution containing the calculated amount of (PS)$_{10}$−NH$_2$ was added, and the reaction mixture was stirred at room temperature for 5 days. Finally, the diblock oligomers were precipitated in acetone, filtered, and vacuum-dried at 50 °C. ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 8.3$ (b, 1H × n; NH), 7.0 (b, 5H × m + 5H × n; Ph−H + Bn−H), 5.0 (b, 2H × n; Bn−CH_2−), 4.0 (b, 1H × n; −(C═O)CHRNH−), 2.2 (b, 3H × m + 4H × n; −CH_2CH(Ph)− + −CH_2CH_2−). Note: m = number-average degree of polymerization of the oligo(styrene); n = number-average degree of polymerization of the oligo(peptide).

Preparation of the (γ-Benzyl-L-glutamate)$_n$ Oligomers. A Schlenk flask fitted with a stir bar and a drying tube was charged with the appropriate amount of Bn-Glu NCA in dry DMF (∼0.2 g/mL). Then, the required amount of *n*-hexylamine was added, and the reaction mixture was stirred at room temperature. After 5 days, the peptides were precipitated in Et_2O, filtered, and vacuum-dried at 50 °C. ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 8.3$ (b, 1H × n; NH), 7.25 (b, 5H × n; Bn−H), 5.0 (b, 2H × n; Bn-CH_2−), 4.0 (b, 1H × n; −(C═O)CHRNH−), 2.10 (b, 4H × n; −CH_2CH_2−), 0.80 (t, 3H; CH_3−). Note: n = number-average degree of polymerization of the oligo(peptide).

Results and Discussion

Synthesis. The synthesis of the rod−coil oligomers is outlined in Scheme 1 and starts with the preparation of a primary amine end-functionalized styrene oligomer with a number-average degree of polymerization of 10. The oligomerization of styrene is carried out in cyclohexane at room temperature using *sec*-butyllithium (*sec*-BuLi) as the initiator. Quenching the oligostyryllithium with 1-(3-chloropropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane followed by acidolysis and aqueous workup affords the crude primary amine end-functionalized oligo(styrene)$_{10}$ coil.[8] After flash chromatography to remove residual amounts of unsubstituted oligo(styrene), the primary amine-functionalized oligomer is

Table 1. Yields, Molecular Weights, and Rod Volume Fractions of the PS_{10}-*b*-$PBLG_n$ Diblock Oligomers and the Corresponding $PBLG_n$ Oligopeptides

	yield[a] (%)	M_w[b] (g/mol)	M_n[b] (g/mol)	M_w/M_n[b]	n_{GPC}[c]	n_{NMR}[d]	f_{rod}[e]
$PS_{10}-NH_2$	85	1280	1230	1.04	11		
$PBLG_{10}$	70	2990	2820	1.06	12	8	
$PBLG_{20}$	71	4800	3970	1.21	18	16	
PS_{10}-*b*-$PBLG_{10}$	60	3610	3500	1.03	10	11	0.75
PS_{10}-*b*-$PBLG_{20}$	70	6800	5100	1.33	18	24	0.86

[a] Isolated yield, after precipitation and drying. [b] From GPC. GPC experiments were performed in DMF. GPC data of the $(PS)_{10}-NH_2$ refer to a sample that was withdrawn from the oligo(styryl)lithium solution and quenched with MeOH, prior to the addition of the capping agent. This sample was analyzed by means of GPC using THF as the eluent. Mass spectroscopy analysis (FD) of the amine-terminated oligo(styrene) gave $M_n = 1030$ g/mol and $M_w/M_n = 1.05$, corresponding to a number-average degree of oligomerization of 9. [c] Number-average degree of polymerization of the peptide segment, determined from GPC. [d] Number-average degree of polymerization of the peptide segment, determined from ^{1}H NMR (DMSO-d_6). [e] $f_{rod} = R^2 \times L/(R^2 \times L + r^2 \times l)$, where R = the hard core radius of the rod, L = the extended length of the rod, r = the hard core radius of the oligo(styrene) segment, and l = the length of the oligo(styrene) segment. L was calculated according to $L = n \times 1.50$ Å, where n is the number-average degree of polymerization of the oligo(peptide) and 1.50 Å corresponds to the axial rise per α-amino acid residue of an α-helical poly(γ-benzyl-L-glutamate) chain.[5] R, r, and l were taken from computer simulation experiments (Cerius2, Macromodel) and estimated to 20, 10, and 20 Å, respectively.

obtained in 85% yield and used to initiate the oligomerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (Bn-Glu NCA), which is performed in DMF solution at room temperature under the exclusion of moisture over a period of 5 days. The length of the γ-benzyl-L-glutamate segment can be controlled by the molar ratio between the Bn-Glu NCA and the primary amine end-functionalized oligo(styrene)$_{10}$ initiator. In this way, two different (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ oligomers containing either 10 or 20 α-amino acid repeat units have been prepared. In addition to the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ oligomers, also the corresponding homooligopeptides have been synthesized using *n*-hexylamine as the initiator for the ring-opening polymerization of Bn-Glu NCA. These materials serve as reference compounds to investigate the effect of the attachment of the styrene segment on the supramolecular organization of the oligopeptides. The final products have been characterized by means of ^{1}H NMR and GPC, the results of which are summarized in Table 1.

Characterization. The supramolecular organization and properties of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ diblock oligomers were investigated by means of differential scanning calorimetry (DSC), optical microscopy, Fourier transform infrared spectroscopy (FTIR), and small-angle X-ray scattering (SAXS). DSC heating traces of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ and (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock molecules are presented in Figure 1. For comparison, also the heating traces of the primary amine end-functionalized oligo(styrene) coil and that of a (γ-benzyl-L-glutamate)$_{20}$ homooligopeptide are included in Figure 1. Optical microscopy experiments between crossed polarizers show that the first-order transitions at 61 and 98 °C for the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ and (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock molecules, respectively, are not the isotropization temperatures of these materials but represent a transition into a liquid-crystalline phase. The liquid-crystalline textures of these diblock oligomers, which are shown in Figure 2, start to develop at these first-order transitions and remain largely unchanged until about 200 °C. At this temperature, the materials start to degrade, and no isotropization is observed. The DSC traces shown in Figure 1 were recorded during the first heating cycle. After cooling, the observed first-order transitions do not reoccur during the second heating run. However, the liquid-crystalline textures (see Figure 2) that were observed for the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ and (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomers above 61 and

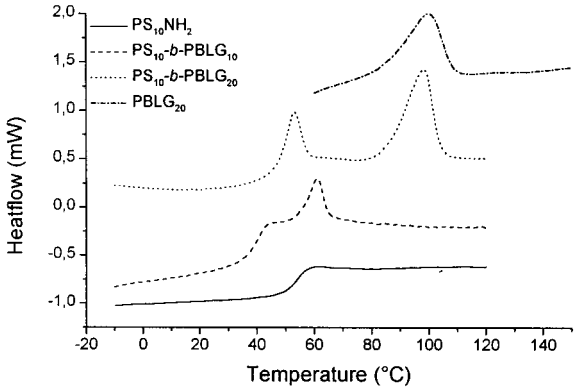


Figure 1. DSC-heating traces (first heating cycle, 10 °C/min) for the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ (PS_{10}-*b*-$PBLG_{10}$) and (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ (PS_{10}-*b*-$PBLG_{20}$) diblock oligomers, as well as for a (γ-benzyl-L-glutamate)$_{20}$ homooligopeptide ($PBLG_{20}$) and the primary amine end-functionalized oligo(styrene)$_{10}$ coil ($PS_{10}-NH_2$).

98 °C, respectively, remain present upon cooling to room temperature. These observations suggest that the liquid-crystalline order is frozen in upon cooling the samples from the liquid-crystalline state to room temperature. This is probably due to the relatively high glass-transition temperature of the oligo(styrene) segment (∼50 °C for $n = 10$, see Figure 1), which causes vitrification of the samples before any crystallization can occur.

The first-order transitions of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer and the (γ-benzyl-L-glutamate)$_{20}$ homooligopeptide around 100 °C are reminiscent of the first-order transition that is observed for high-molecular-weight poly(γ-benzyl-L-glutamate) at 84 °C and which has been attributed to an irreversible transition from a 7/2 to an 18/5 α-helical conformation.[9] In agreement with this supposition, FTIR experiments (vide infra) show that the (γ-benzyl-L-glutamate)$_{20}$ homooligopeptide and the peptide segment of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer predominantly adopt an α-helical conformation at 120 °C. The endotherm around 55 °C in the DSC trace of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer is an enthalpy relaxation peak that is accompanying the glass transition of the oligo(styrene)$_{10}$ segment.[10] For the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ diblock oligomer, the transition into the liquid-crystalline phase drops to 61 °C. Since at room temperature a considerable fraction of the peptide segments of this diblock oligomer possess a β-sheet conformation (vide infra), at least two interpre-

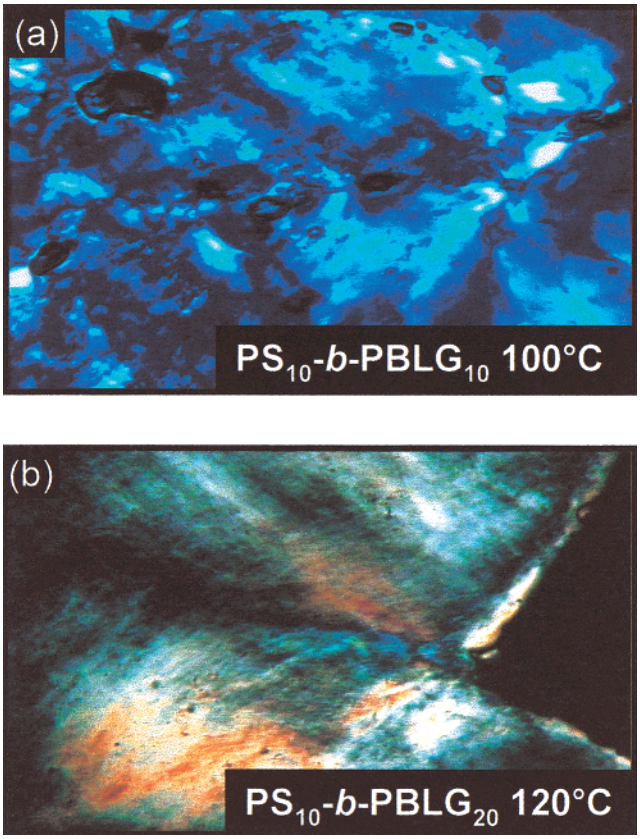


Figure 2. Polarized optical micrographs of the liquid-crystalline textures of (a) the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ diblock oligomer (PS$_{10}$-*b*-PBLG$_{10}$) at 100 °C and (b) the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer (PS$_{10}$-*b*-PBLG$_{20}$) at 120 °C.

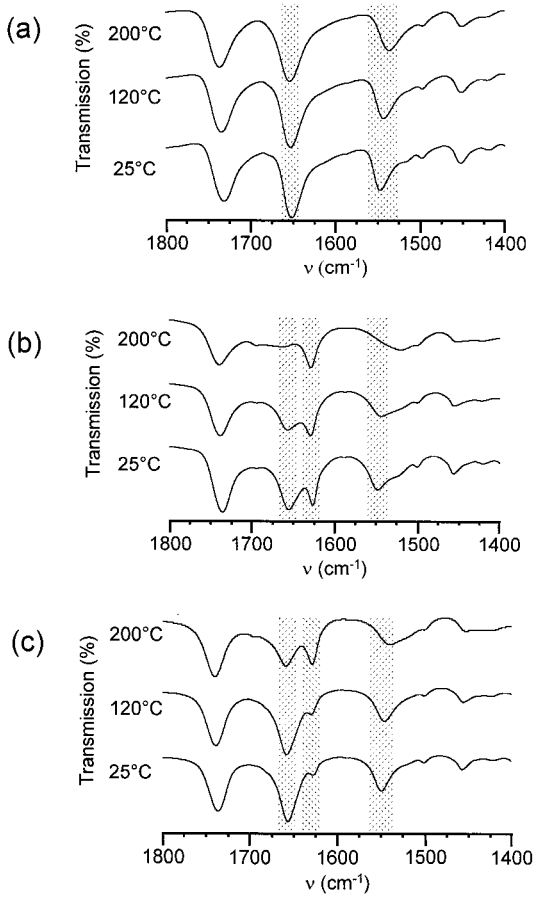


Figure 3. Temperature-dependent FTIR-spectra of (a) a commercial poly(γ-benzyl-L-glutamate) sample, (b) a (γ-benzyl-L-glutamate)$_{10}$ homooligopeptide, and (c) a (γ-benzyl-L-glutamate)$_{20}$ homooligopeptide. The amide bands indicative for the secondary structure of the peptide are marked with shaded bars.

tations can be proposed to explain this observation. A first possibility would be that the decrease in the first-order transition is due to the destabilization of the α-helix as a result of the limited degree of polymerization of the peptide segment. A second explanation could be that the observed first-order transition is not related to a conformational change of the α-helical peptide-segments but is due to changes in the β-sheet type peptide segments. Detailed experiments to answer this question are part of ongoing research.

The conformation of the (γ-benzyl-L-glutamate) oligopeptides and that of the peptide segment of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ diblock oligomers was investigated by means of variable-temperature FTIR spectroscopy. In Figure 3, the FTIR spectra of (γ-benzyl-L-glutamate)$_{10}$ and (γ-benzyl-L-glutamate)$_{20}$ at 25, 120, and 200 °C are compared with those of a commercial high-molecular-weight poly(γ-benzyl-L-glutamate) sample ($M_n \approx 70\,000$ g/mol). The amide I and amide II bands at 1655 and 1550 cm^{-1}, respectively, confirm the α-helical conformation of the commercial poly(γ-benzyl-L-glutamate) sample (Figure 3a).[11] Within the investigated temperature range, no change in the conformation of this polypeptide can be observed in the FTIR spectra. For the shorter (γ-benzyl-L-glutamate)$_{10}$ and (γ-benzyl-L-glutamate)$_{20}$ oligomers an additional band appears at ∼1630 cm^{-1}, indicating a β-sheet conformation of the polypeptide backbone (Figure 3b,c). The β-sheet amide I band at 1630 cm^{-1} is most pronounced for the (γ-benzyl-L-glutamate)$_{10}$ oligomer, reflecting the instability of the α-helical conformation for such short degrees of polymerization. At 200 °C, this peptide almost exclusively adopts a β-sheet conformation. In agreement with previous observations, the (γ-benzyl-L-glutamate)$_{20}$ oligomer almost exclusively adopts the α-helical conformation at 25 °C.[11] The fraction of this oligopeptide that is present in the β-sheet conformation increases significantly upon heating the sample to 200 °C.

The FTIR spectra of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ and (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomers are shown in Figure 4. Comparison of these spectra with those of the corresponding (γ-benzyl-L-glutamate) oligomers (see Figure 3) indicates a significant stabilization of the α-helical conformation upon attachment of a short oligo(styrene) segment. Whereas a large fraction of the polypeptide chains of (γ-benzyl-L-glutamate)$_{20}$ at 200 °C possesses a β-sheet conformation, the peptide segments in the corresponding diblock oligomer almost exclusively adopt an α-helical secondary structure at that temperature. This effect is even more pronounced for the (γ-benzyl-L-glutamate)$_{10}$ oligomer, which solely possesses a β-sheet conformation at 200 °C. Although the peptide segment contains on average only 10 α-amino acid repeat units, attachment of a styrene block of similar degree of polymerization significantly stabilizes the α-helical secondary structure, even up to 200 °C.

Kataoka et al. reported similar observations on poly(L-lysine)-*b*-poly(ethylene oxide) diblock copolymers, where the degree of polymerization of the peptide

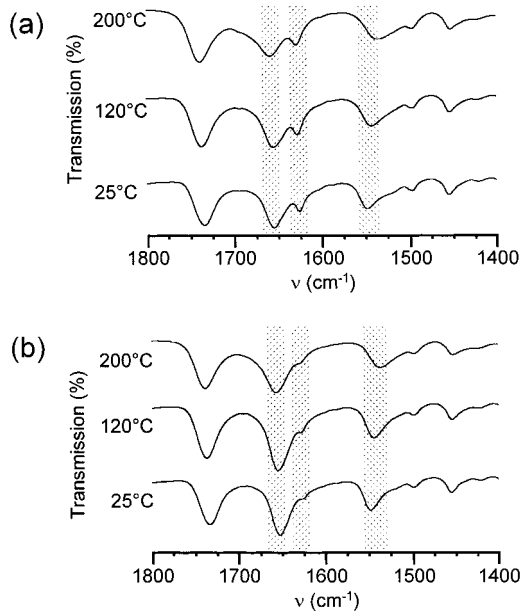


Figure 4. Temperature-dependent FTIR-spectra of (a) the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ diblock oligomer and (b) the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer. The amide bands indicative for the secondary structure of the peptide are marked with shaded bars.

segment alone was not sufficient to allow the formation of a stable α-helical secondary structure.[12] The stabilization of the α-helical secondary structure was explained by micellization of the diblock copolymers in aqueous solution. Similarly, the increased stability of the α-helical conformation of the peptide segments of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ molecules in comparison with the corresponding oligopeptides could also provide a first hint toward phase separation of the peptide-based diblock oligomers. Additional experiments to substantiate these suppositions are in progress.

SAXS experiments were performed to elucidate the supramolecular organization of the peptide-based diblock oligomers. The experiments were performed in the liquid-crystalline phase of the materials, i.e., at $T > 120$ °C. To facilitate the interpretation of the experimental data of the diblock oligomers and to investigate the effect of the oligo(styrene) coil, SAXS experiments were also performed on the corresponding (γ-benzyl-L-glutamate) homooligopeptides as well as on a high-molecular-weight commercial poly(γ-benzyl-L-glutamate) sample. The SAXS patterns of these samples, recorded at 120 and 200 °C, are shown in Figure 5.

The SAXS patterns of the commercial polypeptide shown in Figure 5a are in agreement with previously published data on the so-called C-form of poly(γ-benzyl-L-glutamate).[13] Above 120 °C, this solid-state modification is a nematic-like paracrystalline phase in which the 18/5 α-helical polypeptide chains are packed in a near-hexagonal lattice. The broad peak around $q = 1.20$ Å^{-1} can be assigned to the pitch (~5.4 Å) of the α-helical peptide-chain.[13] Increasing the temperature to 200 °C does not reveal any significant changes in the SAXS pattern. This is in agreement with the FTIR data (vide supra), which also indicate that the 18/5 α-helical conformation of the poly(γ-benzyl-L-glutamate) is stable up to these temperatures.

In addition to the Bragg peaks that are typical for a (near-)hexagonal packing of α-helical poly(γ-benzyl-L-glutamate) chains, the SAXS pattern of the (γ-benzyl-

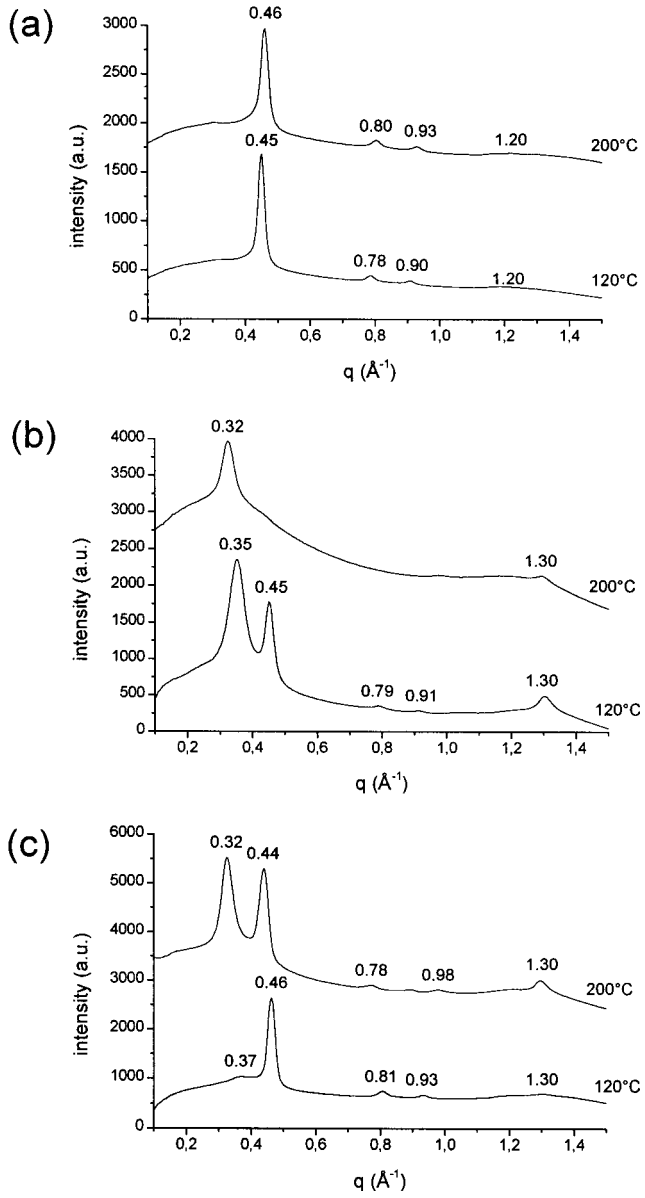


Figure 5. SAXS patterns recorded at 120 and 200 °C of (a) a commercial poly(γ-benzyl-L-glutamate) sample, (b) a (γ-benzyl-L-glutamate)$_{10}$ homooligopeptide, and (c) a (γ-benzyl-L-glutamate)$_{20}$ homooligopeptide. The scattering angles corresponding to the relevant Bragg peaks are indicated in the SAXS patterns.

L-glutamate)$_{10}$ oligomer at 120 °C (Figure 5b) also reveals a peak at $q = 0.35$ Å^{-1}. Upon increasing the temperature to 200 °C, the (near-)hexagonal order completely disappears, and only a diffraction-peak at $q = 0.32$ Å^{-1} is observed. According to the FTIR experiments discussed above, only a part of the peptide chains of this sample adopt an α-helical conformation at 120 °C. A large fraction of this material already possesses a β-sheet conformation at this temperature. Upon further increasing the temperature to 200 °C, virtually all of the peptide chains adopt a β-sheet conformation. Thus, the Bragg peak at $q = 0.32$ Å^{-1} that is observed for the (γ-benzyl-L-glutamate)$_{10}$ oligomer at 200 °C is most likely related to a regular packing of oligo(γ-benzyl-L-glutamate) chains in a β-sheet assembly. This diffraction maximum is in agreement with the observations reported by Komoto et al., who investigated the structure of crystals of low-molar-mass (γ-benzyl-L-glutamate) oligomers with the β-sheet conformation and

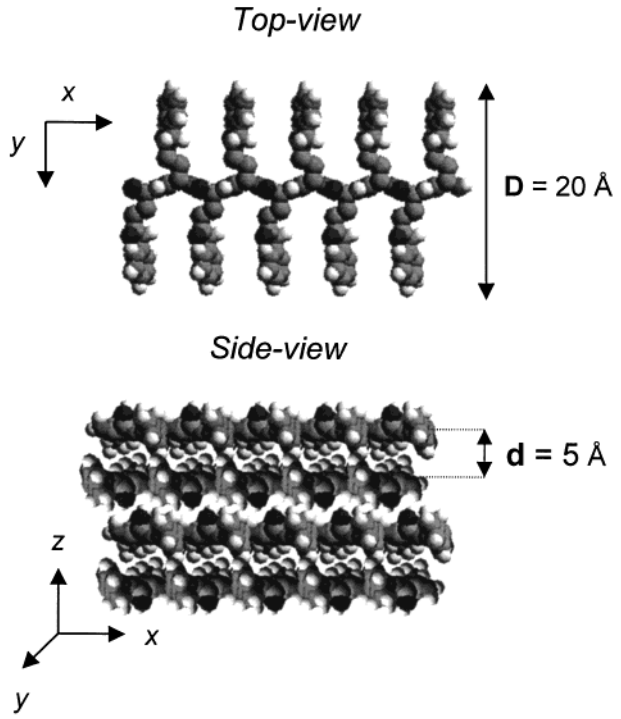


Figure 6. Computer-generated representation (Cerius2, Macromodel) of a proposed model for the β-sheet-type self-assembly of poly(γ-benzyl-L-glutamate)-chains.

assigned the reflection corresponding to a spacing of 16.7 Å to the (020) plane of the oligo(γ-benzyl-L-glutamate) unit cell.[14] A possible model for the packing of β-sheet (γ-benzyl-L-glutamate)$_{10}$ oligomers that is in agreement with these observations is proposed in Figure 6. In this model, the β-sheet (γ-benzyl-L-glutamate) oligomers are stacked on top of each other in the z direction, with the amide bonds lying within the $x-z$ plane. The stacking of the (γ-benzyl-L-glutamate) oligomers results in a lamellar type organization where the thickness of a lamellae is given by the distance (D) between the benzylester groups on both sides of the peptide chain within the $x-y$ plane. Using computer modeling, this distance can be estimated to 20 Å, which corresponds well with the spacing that can be calculated from the observed Bragg peak at $q = 0.32$ Å^{-1}. Comparison of the SAXS patterns of the commercial poly(γ-benzyl-L-glutamate) at 200 °C (Figure 5a) with that of the (γ-benzyl-L-glutamate)$_{10}$ oligomer also reveals that the conformational transition from purely α-helical to β-sheet (γ-benzyl-L-glutamate) chains causes a change in the Bragg peak at large angles from a broad signal centered around $q \sim 1.20$ Å^{-1} to a somewhat sharper peak at $q = 1.30$ Å^{-1}. The distance (d), which can be calculated from this peak (~4.8 Å), fits well with the intermolecular distance between neighboring (γ-benzyl-L-glutamate) chains within a lamellae, as is illustrated in Figure 6.

The SAXS patterns of the (γ-benzyl-L-glutamate)$_{10}$ at 120 °C (Figure 5b) and those of the (γ-benzyl-L-glutamate)$_{20}$ at 200 °C, and to a lesser extent at 120 °C (Figure 5c), display features of both a (near-)hexagonal packing of α-helical (γ-benzyl-L-glutamate) chains as well as of a β-sheet assembly of peptide chains. These observations are in agreement with the FTIR spectra (Figure 3), which show that at the temperatures mentioned above the peptides partly adopt an α-helical and a β-sheet conformation. Most likely, the materials are

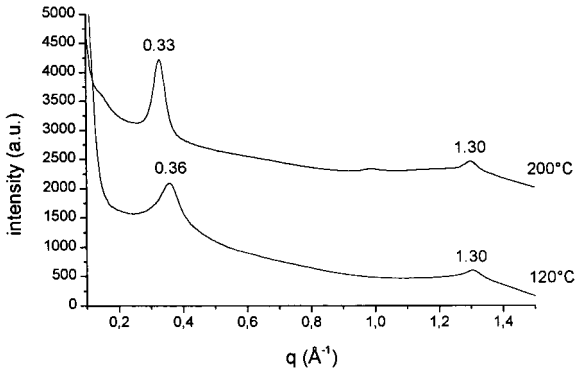


Figure 7. SAXS patterns of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ diblock oligomer recorded at 120 and 200 °C. The scattering angles corresponding to the relevant Bragg peaks are indicated in the SAXS patterns.

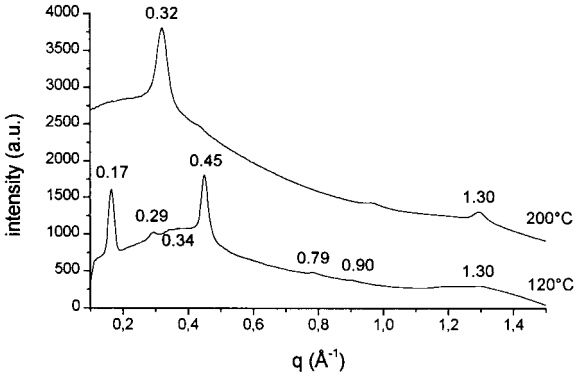


Figure 8. SAXS patterns of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer recorded at 120 and 200 °C. The scattering angles corresponding to the relevant Bragg peaks are indicated in the SAXS patterns.

composed of domains that consist of (near-)hexagonally packed α-helical peptides, next to domains in which the peptides are organized in a β-sheet fashion.

The SAXS patterns of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ diblock oligomer at 120 and 200 °C are presented in Figure 7. Whereas the SAXS patterns of the homooligopeptides that coexisted in the α-helical and the β-sheet conformation displayed features of both secondary structures (see Figure 5), the SAXS patterns shown in Figure 7 do not reveal any Bragg peaks that point toward a (near-)hexagonal packing of α-helical peptides, even though FTIR spectroscopy indicated that a considerable fraction of the peptide segments of this diblock oligomer possesses an α-helical conformation at these temperatures. This might be tentatively explained by the amorphous character of the oligo(styrene) coil, which apparently frustrate a regular packing of the α-helical fraction of the peptide segments of these diblock oligomers. The β-sheet type (γ-benzyl-L-glutamate)$_{10}$ segments most likely induce self-organization of the diblock oligomers in a way similar to the model proposed in Figure 6.

The SAXS pattern of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer recorded at 120 °C (Figure 8) is markedly different from that of the corresponding homooligopeptide as well as the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ diblock oligomer and reveals two sets of Bragg peaks with each of them in a ratio of 1:$\sqrt{3}$:2. The set of diffraction peaks at the largest angles ($d = 16$ Å) resembles the SAXS pattern of the commercial poly(γ-benzyl-L-glutamate) sample (Figure 5a) and indicates

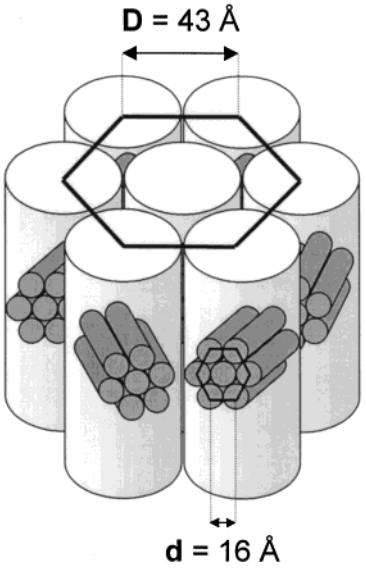


Figure 9. Proposed model for the self-assembly of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer at 120 °C. The small rods represent the helical oligo(γ-benzyl-L-glutamate)$_{20}$ segments. The oligo(styrene) coils have been omitted for reasons of clarity but are thought to protrude in a random fashion from both sides of the hexagonally packed oligo(peptide) clusters.

a (near-)hexagonal packing of the diblock oligomers with the peptide segments adopting an 18/5 α-helical conformation. The second set of diffraction maxima at lower angles points toward an additional hexagonal periodicity with $D \approx 43$ Å. A schematic illustration of a packing model of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ oligomers that is in agreement with these data is proposed in Figure 9. In this model, the diblock oligomers are assembled in a (near-)hexagonal fashion into long columns, with the peptide segments oriented perpendicularly to the director of the columns. The observed spacing of 16 Å corresponds well with the intermolecular distance in a (near-)hexagonal lattice of α-helical poly(γ-benzyl-L-glutamate chains).[5] The columns are packed in a superlattice with hexagonal periodicity parallel to the α-helical peptide-segments of the diblock oligomers. Assuming a random orientation of the oligo(γ-benzyl-L-glutamate) segments between the columns, full interdigitation of the oligo(styrene) coils, and a random placement of these coils on both sides of the hexagonally packed clusters, the center-to-center distance between two neighboring columns can be estimated to 50 Å,[15] which is close to the observed 43 Å. This estimate, however, assumes an extended chain conformation of the oligo(styrene) segments. Since the oligo(styrene) segment is not very likely to be present in the extended chain conformation, but will be "coiled" to a certain extent, 50 Å is probably an overestimate of the center-to-center distance between two neighboring columns. This agreement between the estimated and observed intercolumnar distance, as well as the absence of any additional signals in the SAXS pattern shown in Figure 8, supports the assumption of a random orientation of the diblock oligomers with respect to each other, both within and between the different columns.

Apparently, the amorphous oligo(styrene) coil plays an important role in the self-assembly of these (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomers and prevents a simple (near-)hexagonal packing, as was observed for the commercial poly(γ-benzyl-L-glutamate) and the (γ-benzyl-L-glutamate)$_{20}$ reference sample. Heating the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer to 200 °C disrupts the hexagonal order both within and between the columns. At this temperature, only a Bragg peak at $q = 0.32$ Å^{-1} is observed. As was already discussed above for the (γ-benzyl-L-glutamate)$_{10}$ and (γ-benzyl-L-glutamate)$_{20}$ reference compounds, as well as for the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ diblock oligomer, this Bragg peak indicates a regular packing of those diblock oligomers whose peptide segment has adopted a β-sheet conformation. Although the FTIR experiments at 200 °C (Figure 4) clearly indicate a considerable stabilization of the α-helical conformation of the peptide segment upon conjugation to an oligo(styrene) coil, comparison of the SAXS patterns at this temperature reveals a significantly higher degree of order in the (γ-benzyl-L-glutamate)$_{20}$ sample. Thus, despite stabilizing the α-helical conformation of the peptide segment, the oligo(styrene) coil disrupts long-range order at high temperatures.

Conclusions

In this contribution, we have described the synthesis and supramolecular organization of a novel set of diblock oligomers comprised of a conformationally dynamic oligo(γ-benzyl-L-glutamate) segment and an oligo(styrene)$_{10}$ coil. The diblock oligomers have been prepared via ring-opening polymerization of Bn-Glu NCA initiated by a primary amine-terminated oligo(styrene)$_{10}$. Variation of the molar ratio between Bn-Glu NCA and the initiator allows the preparation of diblock oligomers that contain (on average) either 10 or 20 repeat units of γ-benzyl-L-glutamate.

Both of the investigated diblock oligomers form thermotropic liquid-crystalline phases with morphologies that are different from the lamellar structures that are known for the high-molecular-weight homologues. Comparison of the FTIR spectra of the diblock oligomers with those of the corresponding homooligopeptides indicates a significant stabilization of the α-helical secondary structure upon conjugation of the oligopeptide to a short oligo(styrene) segment. At 120 °C the peptide segments of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{10}$ oligomers partly possess an α-helical conformation and partly adopt a β-sheet secondary structure. However, probably due to the amorphous nature of the oligo(styrene) coil, a regular organization of the α-helical oligo(γ-benzyl-L-glutamate) chain is prevented, and the SAXS patterns solely indicate a β-sheet type assembly of these diblock oligomers. Changing the length of the peptide segment to 20 α-amino acid repeat units results in a strong increase in the fraction of diblock oligomers possessing an α-helical peptide-segment. At 120 °C the interplay between the amorphous oligo(styrene)$_{10}$ coil and the tendency toward aggregation of the α-helical (γ-benzyl-L-glutamate)$_{20}$ segments results in an unconventional "double-hexagonal" organization. Upon increasing the temperature to 200 °C, the hexagonal order both within and between the columns is disrupted, and the SAXS patterns only indicate a β-sheet type assembly of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ oligomers.

Current research efforts focus on expanding the present set of diblock oligomers to other block length ratios, as well as to different α-amino acids. Furthermore, experiments are in progress to prepare films from these materials and to evaluate to which extent the permeability can be altered by manipulation of the

secondary structure of the peptide block and/or the supramolecular organization of the molecules.

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(15) Under the assumptions stated in the text, the intercolumnar distance (D) should be equal to the length of the (γ-benzyl-L-glutamate)$_{20}$ segment (R) plus the length of the oligo(styrene)$_{10}$-coil (C). The length of the peptide segment can be calculated according to $n \times 1.50$ Å, where n is the degree of polymerization and 1.50 Å the axial rise per α-amino acid residue of an helical poly(γ-benzyl-L-glutamate) chain.[5] Thus, for $n = 20$, R is 30 Å. The length of the oligo(styrene) coil (C) in the extended chain conformation was taken from computer-simulation experiments (Cerius2, Macromodel) and estimated to be 20 Å. Combination of these two lengths gives a estimation of the intercolumnar distance: $D = R + C = 30$ Å + 20 Å = 50 Å.

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