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Unconventional Amphiphilic Polymers Based on Chiral Polyethylene Oxides**

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In recent years the coiled-coil superstructure of α-helical polymers has received enormous attention.[1] Not only naturally occurring proteins[2] but also synthetic, designed polypeptides[3] have been investigated in order to distinguish between the different factors that are involved in the formation of coiled coils. It is now generally accepted that the most important characteristic of polypeptides that form coiled coils is the regular repeat of hydrophobic amino acids in a hydrophilic sequence. This is expressed in a $[PAPPAPP]_n$ primary structure, in which P stands for a polar residue and A stands for an apolar residue (typically leucine). The repeat of polar and apolar residues gives rise to a hydrophobic ribbon on the α-helix of the polypeptide, which causes dimerization of two amphiphilic α-helices in water into a coiled coil with the leucine-zipper motif.[4] Despite numerous studies of synthetic amphiphilic polymers[5] and polymer complexes,[6] this special type of ribbon amphiphiles has never been addressed. Here we present the synthesis and properties of the first potential ribbon-type amphiphilic polymers based on chiral modified polyethylene oxides (PEOs). These polymers, as given in Figure 1, can provide the link between "complicated" biological systems and "simple" synthetic systems.[7]

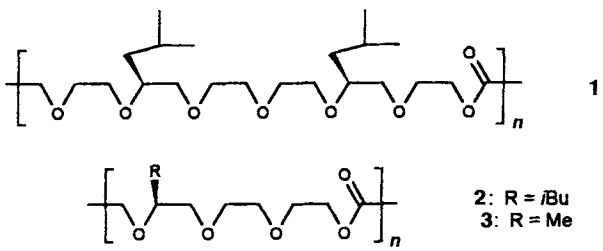

Figure 1. Designed synthetic coiled-coil analogues based on PEO. Isobutyl and methyl side groups are chosen in analogy to the leucine moiety in peptides that form coiled-coil structures and the methyl side group in PEO/PPO/PEO block-copolymers, respectively.

The choice of PEO-based target polymers **1**–**3** (for synthetic feasibility we introduced an ester functionality in the backbone) is inspired by the solubility of PEO in water and its 7_2-helical conformation in dilute aqueous solutions.[8] In contrast, polypropylene oxide (PPO) and polybutylene oxide (PBO) are insoluble in water, while both PEO/PPO/PEO and PEO/PBO/PEO block-copolymers show distinct amphiphilic behavior.[9] Therefore, the stereospecific placement of isobutyl side chains at every second and fifth unit of a seven-unit repeat of PEO, as shown in polymer **1**, results in a synthetic analogue for coiled-coil forming polypeptides, in the sense that the design of the primary structure is identical. Polymers **2** and **3** have

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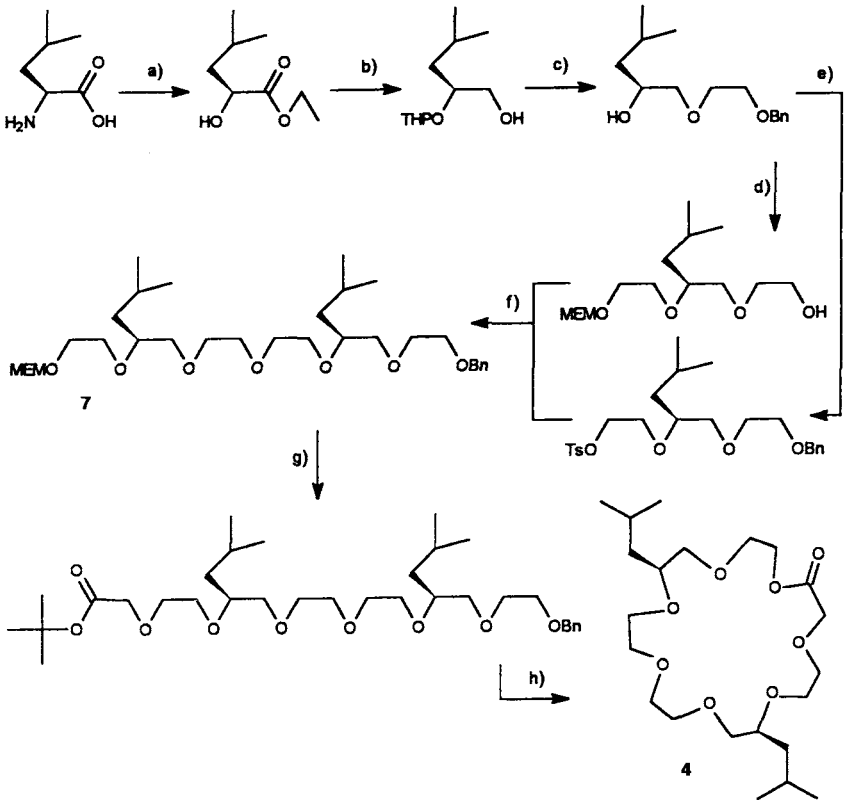

Scheme 1. Synthetic route to monomer **4**. a) 1. $NaNO_2$, H_2SO_4, H_2O (54%), 2. EtOH, toluene, HCl (75%); b) 1. dihydropyran, TsOH, ether (87%), 2. $LiAlH_4$, ether (94%) [19]; c) $TsOCH_2CH_2OBn$, KOH, THF; then TsOH, MeOH (77%); d) 1. $MEMOCH_2CH_2OTs$, KOH, THF (67%), 2. Pd/C, MeOH, H_2 (91%); e) 1. $THPOCH_2CH_2OTs$, KOH, THF; then TsOH, MeOH (40%), 2. TsCl, pyridine (90%); f) KOH, THF (68%); g) 1. $ZnBr_2$, CH_2Cl_2 (44%), 2. $BrCH_2COOtBu$, *t*BuOK, *t*BuOH (85%); h) 1. TFA (94%), 2 Pd/C, dioxane/water, H_2; then PPh_3, dithiodipyridine, xylene (50%). Ts = *p*-toluenesulfonyl, Bn = benzyl, MEM = methoxyethoxymethyl, THP = tetrahydropyran, TFA = trifluoroacetic acid.

also been synthesized, both of which have a [PPAP'] repeat in the primary structure. This repeat resembles the [PAPPAPP'] repeat in **1**.

Polymer **1** was obtained by the ring-opening polymerization of the 2-oxo[21]crown-7 **4**. The synthesis of optically pure **4** is outlined in Scheme 1.[10] (*S*)-Leucine, the starting compound, is the basis for the chiral, apolar residue in the final monomer. A short reaction sequence, including protection and deprotection steps and Williamson syntheses with glycol compounds, provided the ω-hydroxycarboxylic acid precursor of **4**. Subsequent cyclization using a modification of a procedure reported by Corey and Nicolaou gave **4** in 50% yield.[11]

Both 2-oxo[12]crown-4 monomers **5** and **6** (Figure 2) were synthesized in analogy to **4**.[10] Cyclization affording **5** and **6** was achieved in high yields by heating the α-hydroxycarboxylic acid precursors in a Kugelrohr apparatus in the presence of $CoCl_2$ at 250 °C and ≈ 5 mbar.[12] The distillate was purified by column chromatography with alumina giving **5** and **6** in yields of 72 and 56%, respectively. The optically pure, oxocrown ethers **4**–**6** were characterized by ^1H and ^{13}C NMR spectroscopy, mass spectrometry, and chromatography (HPLC with a chiral stationary phase); all data are in agreement with the proposed structures.

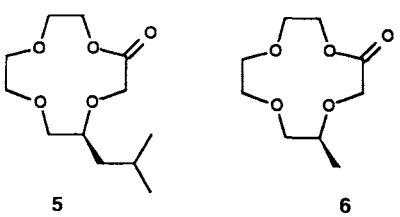

Figure 2. Substituted 2-oxocrown ethers **5** and **6**.

Stannous octanoate ($SnOct_2$) catalyzed polymerization[13] of the monomers **4**–**6** yielded polymers **1**–**3**. The reactions were carried out at 130 °C with monomer/$SnOct_2$ molar ratios of 20/1 and without any solvent. Polymers **1**–**3** were isolated as sticky, viscous oils by precipitation in hexane and/or ether and are soluble in a wide variety of solvents such as acetonitrile, ethyl acetate, THF, and methanol. All spectroscopic data of the polymers are in full agreement with the structures assigned. Selected physical and spectroscopic data for **1**–**3** are given in Table 1.

The behavior of the polymers was studied in water and in aqueous solvent mixtures. Solutions of **1**–**3** in water were transparent only at low concentrations (<0.0005, <0.1, and <1 mg mL^{-1} for **1**, **2**, and **3**, respectively). At higher concentrations of approximately 10 mg mL^{-1}, stable turbid solutions were obtained. A critical association concentration of approximately 3×10^{-6} M was measured for polymer **2** by application of pyrene-probe luminescence techniques.[14] This result implies that these newly designed polymers have a truly amphiphilic character. Transmission electron microscopy (TEM) studies of samples from both the dilute transparent and the concentrated turbid solutions did, however, not show any organized structures.

Table 1. Selected physical and spectroscopic data for **1**–**3**.

1: ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 4.3$ (m, 2H; CH_2CH_2CO), 4.2 (s, 2H; $COCH_2O$), 3.9–3.5 (m, 20H; further backbone protons), 1.75 (m, 2H; $CH_2CH(CH_3)_2$), 1.45 (m, 2H; $CH'H''CH(CH_3)_2$), 1.25 (m, 2H; $CH'H''CH(CH_3)_2$), 0.90 (m, 12H; $4 \times CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 170.5$ (CO), 77.6, 77.5, 74.5, 74.2, 71.3, 70.9, 70.8, 70.6, 69.4(2), 69.1, 68.5, 63.7 (backbone carbons), 41.2, 41.1, 24.5(2), 23.3(2), 22.4, 22.3 (isobutyl carbons); GPC [a]: $M_n = 5.2$ kg mol^{-1}, $M_w = 9.0$ kg mol^{-1}, $D = 1.7$.

2: ^1H NMR (400 MHz, CD_3CN, 25 °C): $\delta = 4.2$ (m, 4H; CH_2CH_2-CO and $COCH_2O$), 3.7–3.4 (m, 9H; further backbone protons), 1.75 (m, 1H; $CH_2CH(CH_3)_2$), 1.4 (ddd, $^2J(H,H) = 13.9$ Hz, $^3J(H,H) = 8.1$ Hz, $^3J(H,H) = 5.9$ Hz, 1H; $CH'H''CH(CH_3)_2$), 1.25 (ddd, $^2J(H,H) = 13.9$ Hz, $^3J(H,H) = 8.1$ Hz, $^3J(H,H) = 4.8$ Hz, 1H; $CH'H''CH(CH_3)_2$), 0.9 (d, $^3J(H,H) = 6.6$ Hz, 3H; CH_3'), 0.88 (d, $^3J(H,H) = 6.6$ Hz, 3H; CH_3''); ^{13}C NMR (100 MHz, CD_3CN, 25 °C): $\delta = 171.5$ (CO), 78.3, 74.9, 71.2, 71.0, 69.5, 68.0, 64.4 (backbone carbons), 41.6, 25.1, 23.5, 22.7 (isobutyl carbons); GPC [a]: $M_n = 7.3$ kg mol^{-1}, $M_w = 17.3$ kg mol^{-1}, $D = 2.4$.

3: ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 4.3$ (m, 2H; CH_2CH_2CO), 4.25 (s, 2H; $COCH_2O$), 3.8–3.45 (m, 9H; further backbone protons), 1.2 (d, $^3J(H,H) = 6.6$ Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 170.5$ (CO), 75.1 (2), 70.3, 70.1, 68.6, 66.6, 63.3 (backbone carbons), 16.6 (methyl carbon); GPC [a]: $M_n = 3.7$ kg mol^{-1}, $M_w = 7.4$ kg mol^{-1}, $D = 2.0$.

[a] The gel permeation chromatography measurements were performed in THF with polystyrene standards.

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We used optical rotatory dispersion (ORD) spectroscopy to investigate the possible conformational changes of the polymers upon the addition of water to THF solutions, prior to phase separation. All polymers showed the same trend: increasing the water content induced a less negative specific rotation. The effect was most striking for the methyl-substituted polymer **3**: an inversion of optical rotation was observed (Figure 3). The

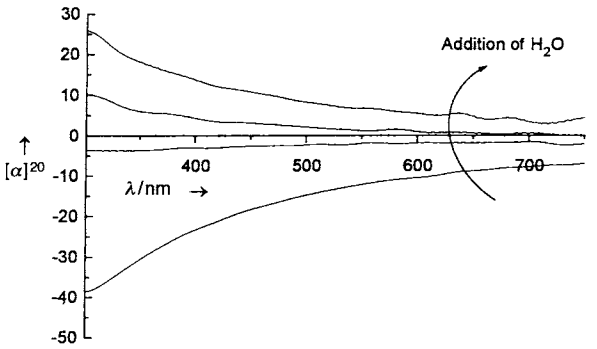

Figure 3. ORD spectra of polymer **3** in THF and THF/water mixtures. $[\alpha]^{20}$ in degrees vs wavelength in nm. Concentrations of **3**: 10–20 mg mL^{-1}; water content: 0, 18, 32, and 47%.

isobutyl-substituted polymers **1** and **2** show phase separation before inversion of optical rotation can occur. The observed inversion is seldomly seen for polymers and has always been related to a well-defined ordering process of the polymers.[15] Here, we propose that the inversion in optical rotation is caused by a distinct conformational transition. However, it is not possible to attribute the effects found in these experiments to the formation of a 7_2-helical conformation and subsequent association of the chiral substituted polyethylene oxides.

Indications for the nature of the conformational transition were found by studying the complexation of the polymers with cations in solution. Oligoethylene glycols form helical structures upon complexation with a variety of cations in the solid state.[16] The "monomeric" precursor of polymer **1**, compound **7** in Scheme 1, formed a 1:1 complex with KSCN in methanol.[17] A titration experiment monitored with ORD showed an inversion of optical rotation as a result of the complexation process (Figure 4). A similar result, inversion of optical rotation, was obtained when polymer **2** was titrated with KSCN. We interpret these inversions as the result of a conformation change from a random coil to a helical conformation, assuming that the behavior of the complexes in the solid state and in solution is similar.

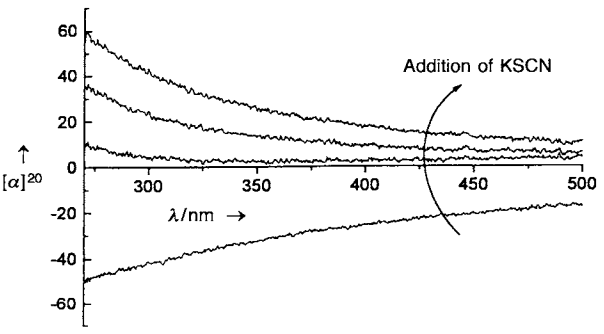

Figure 4. KSCN titration of a methanolic solution of **7** monitored by ORD spectroscopy. $[\alpha]^{20}$ in degrees vs wavelength in nm. Concentration of **7**: 8.3 mg mL^{-1}; equivalents of KSCN added: 0, 1.7, 4.0, and 10.9 equiv.

Consequently, we propose that the transitions in the ORD spectra of polymers **1**–**3** induced by increasing water content (see Figure 3) should also be interpreted as transitions from a random coil to a helical conformation.[18]

In summary, a new class of synthetic amphiphilic polymers has been synthesized and characterized. Further studies are in progress to determine how polymers **1**–**3** associate in aqueous solutions and whether the proposed random coil to helix transition is accompanied by the formation of coiled coils.

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[17] A K-value of approximately 35 M^{-1} was determined from the KSCN titration of **7** monitored by ^{13}C NMR spectroscopy (in CD_3OD).

[18] This hypothesis is in agreement with the fact that the balance between stereoelectronic *gauche* effects and electrostatic repulsion, which depends on the solvent applied, directs PEO structures into helical conformations in aqueous solutions: A. Abe, J. E. Mark, *J. Am. Chem. Soc.* **1976**, *98*, 6468; G. D. Smith, D. Y. Yoon, R. L Jaffe, *Macromolecules* **1993**, *26*, 5213, and references therein.

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Immunization of Mice with a Fully Synthetic Globo H Antigen Results in Antibodies against Human Cancer Cells: A Combined Chemical—Immunological Approach to the Fashioning of an Anticancer Vaccine

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Mobilization of the unsurpassed resourcefulness and ingenuity of the human immune system to do battle against cancer is an enduring hope.[1] Perhaps the ideal approach would be through a vaccine that would educate the immune apparatus to provide surveillance against a particular tumor-associated antigen or even a range of such signature antigens.[1, 2] Success along these lines would require proper identification of antigens and their presentation to patients in an effective immunostimulating context. Based on this principle, a number of completely synthetic vaccines have been prepared.[3] A construct containing a synthetic T-antigen disaccharide covalently attached to a carrier protein is of particular interest since a specific immune response in human cancer patients was observed.[4]

We started from the known tendency of transformed cells to express on their surfaces rather selective carbohydrate constellations in the form of glycoproteins or glycolipids. In principle, it should be possible to key the human immune system to identify the presence of such carbohydrate antigens. Toward this goal, we have merged the resources of chemistry and immunology. In describing our program we begin with the chemical synthesis of the carbohydrate sector. We made full use of the logic of glycal assembly[5] to obtain the complex carbohydrate moiety in homogeneous form. A spacer region was then inserted in a structurally defined way with a linkage arm for conjugation to carrier protein.[6] The extent of the conjugation is determined. The structurally defined protein–spacer-conjugated carbohydrate was administered to a mouse along with an appropriate immunoadjuvant (Figure 1). We demonstrate here that one such

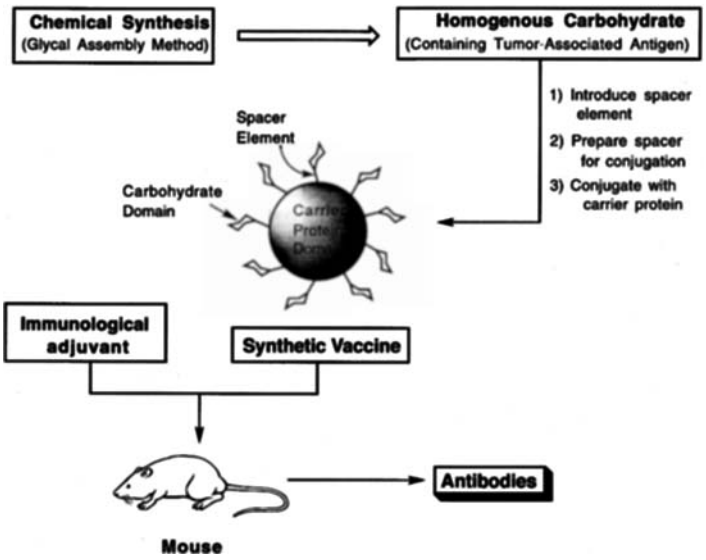

Figure 1. General strategy for the preparation of carbohydrate-based antitumor vaccines.

synthetic vaccine immunoconjugate combination activates the immune system of a mouse to produce antibodies that bind to human cancer cells expressing the epitope around which the carbohydrate region was fashioned.

The breast tumor glycolipid associated antigen **2** isolated by Hakomori and co-workers from MCF-7 cells, and termed globo H, constituted a promising possibility for exploration.[7] Early studies in this area were facilitated by the immunocharacterization of the antigen by the murine monoclonal antibody MBr1.[8] Previously, we had described the total synthesis of the hexacyclic globo H glycal **1** by the method of glycal assembly (Scheme 1).[9] This system was carried forward to reach antigen **2**, whose structure assignment was verified by unambiguous spectroscopic corroboration through this synthesis. Furthermore, the synthetic compound **2** did, indeed, bind to the murine monoclonal antibody (mAb) MBr1 and inhibited its binding to MCF-7 cells as measured by flow cytometry.[10]

We then built upon the logic of our synthesis to prepare congeners of the compound and to use them to map the structural requirements for binding to the mAb MBr1. Thus, allyl glycoside **3**, corresponding in its epitope region to **2**, was found to bind well (Figure 2).[10] These studies also revealed that for this monoclonal antibody both the fucose appendage[10, 11] (compound **4** is not bound) and the β-glycoside linkage between the C and D sectors are critical. Compound **5** containing an α linkage at this locus is only weakly bound. Both the A ring and the AB sector can be deleted (see compounds **6** and **7**, respectively). The β linkage of the glycosidic bond between the B and C rings is not of large effect (see compound **8**, which is well recognized).

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