Solid-Phase Supported Polymer Synthesis of Sequence-Defined, Multifunctional Poly(amidoamines)

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Received November 21, 2005; Revised Manuscript Received December 21, 2005

A novel synthesis route toward multifunctional, sequence-defined polyamides is described. A fully automated, solid-phase supported polymer synthesis was developed and utilized to obtain linear poly(amidoamine) segments (PAAs) that exhibit the absence of molecular weight and chemical distribution. This was achieved by an alternating assembly of diacids and diamines, using a forced step-growth mechanism, and driving each coupling step to completion. Within the monodisperse PAA segment, functionalities can be precisely positioned along the polymer chain allowing local control of the chain properties. The versatility of the approach was demonstrated by the conjugation of the monodisperse PAA segment toward an oligopeptide, leading to a single component block copolymer as verified by mass spectrometry. Moreover, two different poly(ethylene oxide)−PAA conjugates were synthesized utilizing the direct, solid-phase supported route. By varying the PAA repeat unit, the cationic nature of the PAA segment was adjusted, demonstrating the potential of the approach. The products were characterized by means of ^{1}H NMR and matrix-assisted laser desorption mass spectrometry (MALDI-TOF-MS) methods, which confirmed the chemical structures conclusively.

Introduction

Linear poly(amidoamines) (PAA) combining amide connectivities with backbone amino groups can be classified as peptidomimetic polymers (Scheme 1).[1,2] Recently, PAAs have received attention due to their ease of synthesis, allowing the access of multifunctional polymers,[1,3] as well as due to their excellent biocompatibility.[4,5] In contrast to polypeptides, PAAs often exhibit the absence of an inherent immunogenicity.[6] Furthermore, if compared to established cationic polymers such as poly(L-lysine) or poly(ethylene imine), PAAs generally show a reduced cyto- and hemotoxicity.[7,8] Due to their versatile chemical structure and functionality, as well as to their variable aggregation behavior in solution, such as the formation of micelles or vesicles, PAAs are applicable as nanocarriers. Particularly, the broad diversity in functionalities of PAAs[1] allows the modulation of interactions with bioorganic macromolecules including DNA, RNA, proteins, and to some extent also functional polysaccharides. The potential to tailor-make the interactions makes this class of PAAs highly suitable for the reversible complexation and transport of biomacromolecules. For instance, such properties are demanded for protein or gene delivery applications,[1,9,10] especially because they are combined with a soft cationic character of the PAAs, allowing membrane interaction and barrier translocation in biological systems.[11]

However, the full potentials of this class of macromolecules as biocompatible, peptidomimetic polymers cannot be fully exploited, since linear PAAs are usually synthesized via polyaddition reactions.[12] Due to the statistical step-growth mechanism, ill-defined products with an $M_w/M_n \geq 2$ are obtained, thus limiting the introduction of additional functionalities either to a strict alternation or a statistical distribution, depending on the approach utilized.[13] On one hand this makes the rational design of multifunctional polymers difficult, since the position of functionalities within the polymer chain cannot be controlled. On the other hand, legislation is claiming more and more defined products for registration so that synthetic concepts resulting in well-defined structures are mandatory.

Furthermore, free polycondensation makes the access to more complex macromolecular architectures difficult, since additional reaction steps are required. For instance, linear AB-block copolymers would be of interest for diverse applications, and the combination of PAAs with poly(ethylene oxide) (PEO) could possibly increase the bioavailability of the system, as known for congener systems.[14,15] Alternatively, the conjugation of PAAs with oligopeptides would potentially result in polymers that exhibit advanced biofunctional properties, with the PAA segment extending the natural toolbox of segments.[16]

Here we present a straightforward strategy for the direct synthesis of well-defined PAA−polypeptide and PAA−PEO conjugates. The approach utilizes fully automated, solid-phase supported synthesis techniques, as known from peptide synthesis, and allows the access of monodisperse, sequence-defined PAA segments. Comparable synthetic methodologies have been already applied for diverse biopolymers such as peptides, DNA, and oligosaccharides as well as for the synthesis of low molecular weight biomolecules.[17−19] However, to the best of our knowledge solid-phase supported synthesis techniques have not been used to assemble PAA segments as peptidomimetic polymers and to conjugate these to oligopeptides or PEO.

Experimental Section

Materials. Succinic acid anhydride (Suc, 99%, Aldrich), 3,3′-diamino-*N*-methyl-dipropylamine (Damp, 96%, Aldrich), spermine (Spe, 99%, Aldrich), N^2,N^3-bis(*tert*-butoxycarbonyl-anhydride (tBoc$_2$O, IRIS Biotech GmbH), diisopropylethylamine (DIPEA, Acros, peptide

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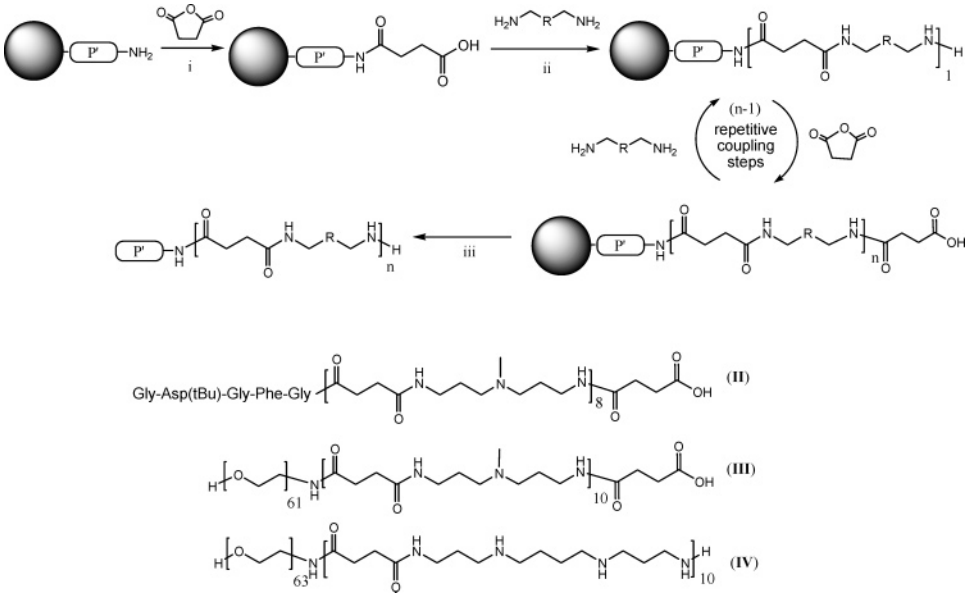
10.1021/bm050884k CCC: $33.50
Published on Web 03/08/2006

Scheme 1. Synthesis of the Peptide-*block*-PAA and the PEO-*block*-PAA Conjugates (**II** (Gly-Asp(*t*Bu)-Gly-Phe-Gly-*block*-(Suc-Damp)$_8$-Suc), **III** (PEO-*block*-(Suc-Damp)$_{10}$-Suc), and **IV** (PEO-*block*-(Suc-Spe)$_{10}$) with Suc = Succinic Acid Anhydride, Damp = 3,3′-Diamino-*N*-methyl-dipropylamine, and Spe = Spermine)[a]



[a] Reagents and conditions: (i) DIPEA, NMP/DMF, room temperature, 25 min; (ii) PyBOP/HOBt/DIPEA, DMF, room temperature, 30 min; (iii) TFA/DCM, room temperature, 30 min; P′ = peptide or PEO, R = $CH_2(NCH_3)CH_2$ or $(CH_2CH_2NtBocCH_2CH_2)_2$).

grade), trifluoroacetic acid (TFA, Acros, peptide grade), 1-benzotriazoyloxy-tris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP, NovaBiochem), and 1-hydroxybenztriazol (HOBt, IRIS Biotech GmbH) were used as received. All other reagents were used as received from Aldrich.

Fmoc-amino acid derivatives (Fmoc-Phe OH, Fmoc-Gly OH, Fmoc-Asp(*t*Bu) OH, Fmoc-Lys(Boc) OH), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-methyl-2-pyrrolidone (NMP, 99.9+%, peptide synthesis grade) were used as received from IRIS Biotech GmbH, Germany. Dichloromethane (DCM, IRIS Biotech GmbH, peptide grade) was distilled from CaH_2, and *N*,*N*-dimethylformamide (DMF, Aldrich, 99+%) was distilled prior to use. Hydroxymethylphenoxy (Wang) PEG-attached peptide resin (Wang PAP) (loading, 0.27 mmol/g; M_n = 2700, M_w/M_n =1.04 (GPC (THF, calibrated against linear PEO standards, PSS, Germany))) was synthesized by anionic polymerization of ethylene oxide initiated from a standard Wang resin, followed by chain end functionalization with an amine group. The synthesis was performed in collaboration with Rapp, Polymere GmbH, Tuebingen, Germany as described previously.[20]

Instrumentation. The synthesis of the poly(amidoamines) was performed on a fully automated ABI 433a peptide synthesizer by Applied Biosystems. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) measurements were performed on a Voyager-DE STR BioSpectrometry workstation MALDI-TOF mass spectrometer (Perseptive Biosystems, Inc., Framingham, MA). The samples were dissolved in concentrations of 0.1 mg/mL in methanol. A volume of 1 μL of the analyte solution was mixed with 1 μL of α-cyano-4-hydroxycinnamic acid matrix solution consisting of 10 mg of matrix dissolved in 1 mL of 0.1% trifluoroacetic acid in acetonitrile/water (1:1, v/v). From the resulting mixture 1 μL was applied to the sample plate. Samples were air-dried at ambient temperature. Measurements were performed at an acceleration voltage of 20 kV. Each spectrum obtained was the mean of 250 laser shots. Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker DPX-400 spectrometer at 400.1 MHz. Sedimentation coefficient measurements were performed on an Optima XLI ultracentrifuge (AUC) (Beckman Coulter, Palo Alto, CA) at 60.000 rpm. The distribution was calculated and diffusion-corrected with Sedfit by Peter Schuck. Small-angle X-ray scattering (SAXS) was measured in aqueous solution (4 wt %) with a NanoStar by Bruker AXS in a geometry-fixated capillary ø = 1 mm by Anton-Paar with λ = 0.154 nm (Cu Kα). Dynamic light scattering was measured in a commercial goniometer with temperature control, photomultiplier and Multiple-Tau digital correlator (ALV 5000) by ALV, Langen. A helium−neon laser (λ = 633 nm) by Polytec (model PL 3000) was used. The electrospray-MS (ESI) measurements were performed with nitrogen (4.5 L/min) in the positive modus with a detector voltage of 1.6 kV, the injector temperature at 150 °C, and a voltage of 4.5 kV.

***N*^{2},*N*^{3}-Bis(*tert*-butoxycarbonyl)spermine ((*t*Boc)Spe) (I).** The synthesis procedure was adapted from that reported previously.[21] Benzaldehyde (5.25 g, 0.05 mol) was added under stirring to a solution of spermine (5.00 g, 0.025 mol) dissolved in chloroform (100 mL). The mixture was refluxed for 5 h, and then cooled to 0 °C, followed by the addition of small portions of *t*Boc-anhydride (11.00 g, 0.05 mol). After the reaction mixture was stirred overnight at room temperature, the solvent was evaporated in vacuum, and the residue was stirred vigorously overnight with 150 mL of aq $KHSO_4$ (1 M). The mixture was extracted three times with ether (150 mL). After adjustment of the pH of the aqueous layer to strongly basic with NaOH and saturation with NaCl, the product was extracted with chloroform. The combined organic layers were dried over $MgSO_4$, filtered, and the solvent evaporated in vacuum yielding 9.1 g (91%).

Analysis. ^{1}H NMR ($CDCl_3$ (7.26 ppm); RT) δ = 1.28 (m, 4 H, β-CH_2−N*t*Boc), 1.39 (s, 18 H, CH_3-*t*Boc), 1.65 (m, 4 H, β-CH_2-amin), 2.64 (m, 6 H, α-CH_2-amin, α-CH_2−N*t*Boc), 3.16 (m, 6 H, α-CH_2−N*t*Boc) ppm. ^{13}C NMR ($CDCl_3$ (77.0 ppm); RT) δ = 26.1 (β-CH_2−N*t*Boc), 28.1 (*C*H_3-*t*Boc), 31.8 (β-*C*H_2-amin), 39.4 (α-*C*H_2-amin), 43.7 (α-*C*H_2−N*t*Boc), 46.5 (α-*C*H_2−N*t*Boc), 79.2 (*C*CH_3-*t*Boc), 155.5 (*C*=O) ppm.

Synthesis of Poly(amidoamine) (PAA) Conjugates. *General Synthesis Procedure.* The synthesis of the poly(amidoamines) was performed in a completely automated way using an ABI 433a peptide synthesizer by Applied Biosystems, Germany. The synthesizer was equipped with the necessary chemicals (cf., the Supporting Information). The standard peptide synthesis protocols (Fastmoc ABI) were modified to establish the following process.

The resin (833 mg, 0.3 mmol/g, 0.25 mmol) was placed in a 41 mL reaction vessel and washed three times with NMP. The succinic acid anhydride (250 mg, 2.5 mmol) was dissolved in 2.5 g of NMP and DIPEA (1 mL, 5.7 mmol). The mixture was transferred to the reaction vessel and agitated for 25 min. After washing with NMP and subsequently with DIPEA in NMP to hydrolyze eventually formed

linear anhydrides, the coupling procedure was repeated until a negative Kaiser test proved the quantitative capping of the amine with succinic acid anhydride.[22] A malachite-green color test was used to prove the completeness of the reaction.[23] After washing with DMF, a solution of PyBOP (1.0 g, 1.9 mmol) and HOBt (180 mg, 1.2 mmol) in 8 mL of DMF was transferred to the reaction vessel to activate the terminal carboxyl functions. After dissolving 2.5 mmol of the diamine in DMF and transferring it to the reaction vessel, the mixture was shaken for 30 min. After washing with DMF, the coupling procedure was repeated until a negative malachite-green test and a positive Kaiser test proved quantitative reaction of the carboxyl function with the diamine. All the subsequent couplings were performed following similar protocols, but NMP was substituted by DMF. To liberate the product from the resin, it was washed with DCM and then transferred into a flask. A solution of 5% TFA in DCM was prepared, and 100 mL was added to the resin and shaken for 30 min. In case of the protected spermine 30% TFA is necessary to cleave the tBoc group. The resin was collected in a filter funnel and transferred to the flask again to perform the cleavage another two times. The solutions were combined, and the solvent was evaporated in vacuum. The crude product was dried, dissolved in 10−15 mL of water, and dialyzed (MWCO, 3500 g/mol) against water, followed by freeze-drying to give the polymer as a colorless powder.

Peptide−PAA Conjugates (Peptide-*block*-(Suc-Damp)$_8$-Suc) (II). The amino acid sequence was synthesized via fully automated SPPS following standard Fmoc protocols on an ethylendiamine-2-chloro-trityl-resin as described previously.[24] Afterward, the PAA segment was assembled by following the general synthetic procedure, described above. **II** was obtained in 75% as crude product and could be isolated in up to 28% yield. A significant loss of material was observed during the purification procedure, probably caused by the inherent tendency of polycationic structures to adsorb on polar surfaces such as regenerated cellulose membranes and glassware.

Analysis. ESI-MS: m/z = 606 ($[M]^{4+}$ + 4H), 615 ($[M + 2Na + H]^{4+}$ + H), 629 ($[M]^{4+}$ + Na + 3H).

PEO−PAA Conjugates. For the synthesis of PEO-*block*-(Suc-Damp)$_{10}$-Suc (**III**) and PEO-*block*-(Suc-Spe)$_{10}$ (**IV**) the Wang PAP resin was used as the solid support. The synthesis followed the general procedure described above using Damp in the case of **III**, and **I** in the case of **IV** as the diamine component. **III** was obtained after dialysis in 32% (81% crude yield after liberation from the support and precipitation in diethyl ether) and **IV** in 27% (70% crude yield), as isolated yield.

*Analysis of **III.*** ^{1}H NMR (DMSO (2.49 ppm); 100 °C) δ = 1.74−1.98 (m, 10 H, β-C*H*-amin), 2.21−2.41 (m, 10 H, β-C*H*-amin), 2.67−3.30 (m, 70 H, α-C*H*$_3$-amin, α-C*H*$_2$-amin), 3.36−3.84 (m, 344 H, O−C*H*$_2$−C*H*$_2$, O═C−C*H*$_2$−C*H*$_2$, α-C*H*$_2$-amid, β-C*H*$_2$-amid), 7.42−7.80 (amid) ppm. The amide protons are visible in the ^{1}H NMR, but due to a proton exchange that occurs, integral intensities are not evaluated. ^{13}C NMR (DMSO (39.5 ppm); 100 °C) δ = 24.3 (β-*C*H$_2$-amin), 31.0 (O═C−*C*H$_2$−*C*H$_2$, α-*C*H$_3$-amin), 36.2 (α-*C*H$_2$-amid), 53.3 (α-*C*H$_3$-amin, α-*C*H$_2$-amin), 60.1 (HO−*C*H$_2$), 69.9 (O−*C*H$_2$−*C*H$_2$), 171.7 (*C*═O) ppm. FTIR (ν in cm^{-1}): 1643 (amid), 1465 (amin),1101 (ether). MALDI-TOF-MS: m/z = 5104 ($[M + H]^+$ + 3K). TGA: $T_{2(deg)}$ = 224 °C, $T_{1(max\ degredation)}$ = 409 °C, $T_{5\%}$ = 180 °C. DSC: T_m = 49 °C.

*Analysis of **IV.*** ^{1}H NMR (DMSO (2.49 ppm); 100 °C): δ = 1.36−1.98 (m, 20 H, β-C*H*$_2$-amin), 2.16−2.42 (m, 20 H, β-C*H*$_2$-amin), 2.73−3.33 (m, 80 H, α-C*H*$_2$-amin), 3.35−3.81 (m, 372 H, O−C*H*$_2$−C*H*$_2$, O═C−C*H*$_2$−C*H*$_2$, α-C*H*$_2$-amid, β-C*H*$_2$-amid), 6.95−8.30 (amid) ppm. The amide protons are visible in the ^{1}H NMR, but due to a proton exchange that occurs, integral intensities are not evaluated. ^{13}C NMR (DMSO (39.5 ppm); 100 °C) δ = 22.8 (CH2), 26.2 (β-*C*H$_2$-amin), 27.7 (β-*C*H$_2$-amin), 30.7 (O═C−*C*H$_2$−*C*H$_2$), 35.6 (α-*C*H$_2$-amid), 44.6 (α-*C*H$_2$-amin), 46.2 (α-*C*H$_2$-amin), 60.3 (HO−*C*H$_2$), 72.4 (O−*C*H$_2$−*C*H$_2$), 171.6 (*C*═O) ppm. FTIR (ν in cm^{-1}): 1645 (amid), 1465 (amin),-1101 (ether). MALDI-TOF-MS: m/z = 5512 ($[M + Na]^+$ + 2K).

Results and Discussion

To establish a synthesis route toward sequence-defined, monodisperse PAA segments a solid-phase supported synthesis strategy, modified from the classical Merrifield solid-phase supported peptide synthesis (SPPS) was applied.[25] However, in contrast to SPPS, where N-protected α-amino acids were repetitively coupled to a functionalized resin, the PAA segment is constituted by alternating condensation of dicarboxylates and diamines, respectively (Scheme 1).

In the ideal case each coupling step was forced to quantitative conversion, employing high excess of the particular monomer and the appropriate activating reagent. To prove that side reactions can be suppressed and coupling steps reach sufficient high conversion (≫98%), a model reaction was performed using an oligopeptide as a well-defined precursor to assemble the PAA segment. The oligopeptide segment simplifies the precise analysis of the products via electrospray ionization mass spectrometry (ESI-MS). The amino acid sequence Gly-Phe-Gly-Asp(*t*Bu)-Gly was synthesized via fully automated SPPS following Fmoc protocols as described previously.[24]

The dicarboxylate activated as succinic acid anhydride was coupled to the terminal amino group of the resin-bound oligopeptide (Scheme 1). High conversion was confirmed by colorimetric Kaiser and malachite-green tests: negative Kaiser tests proved the absence of amino groups and a positive malachite-green test proved the presence of free carboxylates.[22,23] By mass spectrometry neither the oligopeptide precursor nor any side products, e.g., formation of a terminal succinimide structure, were observed (data not shown), confirming that the reaction proceeds in a clean manner. The subsequent coupling of the diamine to the terminal carboxyl group was facilitated by PyBOP/HOBt/DIPEA, as is frequently used for carboxylate activation in SPPS.

In contrast to standard SPPS procedures, where the amino acid activation occurs in solution, the resin-bound carboxylates have to be activated (Scheme 1). This was achieved by modifying protocols of supported organic synthesis.[26] The quantitative conversion at this step was confirmed by a positive Kaiser test accompanied by a negative malachite-green test. ESI-MS results confirmed the clean monomer addition and the absence of any side product within the synthesis of the first repeating unit (data not shown). Via the subsequent repetition of the coupling of dicarboxylates and diamines, the PAA segment was stepwise assembled. After the completion of 8 coupling cycles and a final capping with succinic acid anhydride, the oligopeptide−PAA conjugate peptide-*block*-(Suc-Damp)$_8$-Suc (**II**) was liberated from the support under acidic conditions (Scheme 1). The chemical integrity of the product was confirmed by mass spectrometry, showing the characteristic signals that can be assigned to **II** (cf., the Supporting Information). The absence of detectable amounts of side products, e.g., deletion sequences, imidic structures, or dimerized sequences, indicates a controlled assembly process.

The stepwise PAA synthesis that was established to access the PAA−peptide conjugate **II** is fully compatible with standard peptide synthesis techniques. Therefore, PAA−PEO conjugates can be obtained by adapting routes to PEO−peptide conjugates. An elegant and straightforward approach toward such conjugates utilizes the PEO-attached polystyrene resin (PAP resin).[20,27] This consists of an α-hydroxy-ω-amino-functionalized PEO that is tethered via a benzyl-linker to the PS support (PS−[benzyl]−O−PEO−NH$_2$). Since the linker connects the PEO and the resin, a cleavage results in the direct liberation of the PEO conjugate. Usually strong acidic conditions (99% TFA, up to 1% bromo-

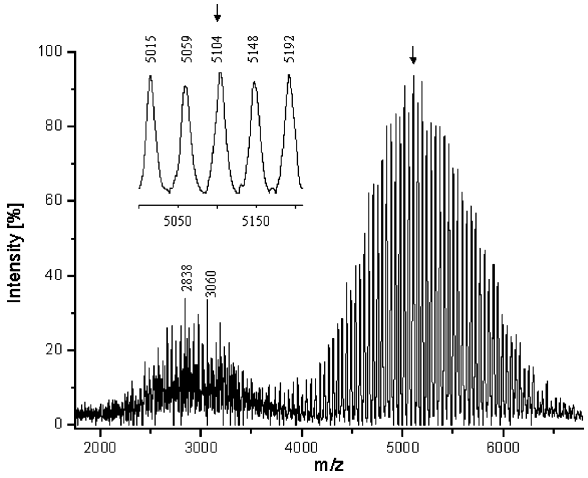


Figure 1. MALDI-TOF mass spectrum of **III**.

trimethylsilane, 2−6 h) are required for complete cleavage. These could be avoided by the substitution of the benzyl-linker with a hydroxymethylphenol-linker (Wang linker).[25] As a result an acid labile Wang PAP resin is obtained allowing the liberation of the conjugate with 2−5 vol % of TFA in DCM within 10−45 min (Scheme 1).

This novel acid labile Wang PAP resin was synthesized by grafting ethylene oxide from a Wang resin using anionic polymerization techniques, subsequently followed by the introduction of an amino chain end functionality.[20] The obtained HO−PEO−NH_2 shows an M_w/M_n of 1.04 and an $M_n = 2700$, as determined by size exclusion chromatography (SEC). Afterward, the resin was utilized to access two PEO-*block*-PAA conjugates, **III** and **IV**, differing in the type and number of main chain amine groups in the PAA repeating units. PEO-*block*-(Suc-Damp)$_{10}$-Suc (Scheme 1, **III**) exhibits a single tertiary amine group in each repeat unit and was accessed via 10-times repetitive coupling of succinic acid anhydride and the commercially available DAMP. PEO-*block*-(Suc-Spe)$_{10}$ (Scheme 1, **IV**) which comprises two secondary backbone amines per repeat unit, was synthesized by 10 times repetition of the coupling of succinic acid anhydride and selectively protected spermine (**I**). The latter is a derivative of a native polyamine, usually utilized to compress DNA, e.g., in spermatozoa.[28] To avoid branching, the secondary amine groups of spermine were temporary protected, using the *t*Boc protecting group. After liberation of **III** and **IV**, the PEO−PAA conjugates were isolated, purified via dialysis against Millipore water (3500 MWCO membrane), and analyzed by means of ^{1}H NMR spectroscopy as well as MALDI-TOF-MS measurements. The proton spectrum of **III** in DMSO at 100 °C shows the resonance of the PEO segment at $\delta = 3.60$ ppm (O−CH_2−CH_2−O) overlaid with signals from the PAA segment and the characteristic signal of the CH_x−N_{amine} at $\delta = 2.99$ ppm, representative of the PAA segment. The comparison of the integral intensities of these signals revealed a ratio of the number-average degree of polymerization of six PEO units to one PAA unit including one terminal succinic acid unit, therefore confirming the structural identity of the PEO$_{61}$-*b*-(Suc-Damp)$_{10}$-Suc conjugate. Similar results were observed in the ^{1}H NMR spectrum of **IV** ran in DMSO at 100 °C, verifying the structural identity of the PEO$_{63}$-*b*-(Suc-Spe)$_{10}$ by revealing a ratio of PEO/PAA of ca. 6:1.

The MALDI-TOF mass spectrometry of **III** confirms the chemical structure of the product by the presence of a dominant distribution, centered at 5059 *m*/*z* (Figure 1).

This high molecular weight fraction exhibits a characteristic signal spacing of 44 *m*/*z* assignable to the mass of a PEO repeat unit. Additionally, each peak can be correlated to 10 repeating units of the PAA segment (227.16 Da), one terminal succinic acid anhydride unit (101.02 Da), the α-ω-functionalities of the PEO block (16.02 Da), and *n* times EO repeat units, e.g., $n = 58$ for the signal marked by an arrow in Figure 1. Considering that singly charged peptide ions can form adduct cluster with sodium and/or potassium cations during the MALDI process,[29] the mass signals can be clearly assigned to **III** within ±1 *m*/*z* accuracy allowing for multiple potassium ion cluster peaks (three potassium ions and one proton).

Even though MALDI mass spectrometry can be considered as a rather soft desorption and ionization method, well-defined fragmentation pathways can be found if, e.g., PAA dendrimers are analyzed.[30] This is consistent with the observation that the second distribution, centered around 2700 *m*/*z*, could not be assigned to the PEO precursor, dihydroxy PEO, or to reasonable side products, e.g., deletion sequences or imide terminated structures. Furthermore, depending on the sample preparation for mass spectrometry a variation of the intensity of the low molecular weight distribution was found, frequently showing a conclusive fragmentation pattern (cf., the Supporting Information). It should be noted that the MALDI behavior of block copolymers has not been intensely investigated. This is due to the molecular polydispersity of both blocks that usually leads to complex mass spectra. Furthermore, a variety of synthetic polymers, e.g., aliphatic polyamides, show fragmentation during MALDI-TOF analysis.[30−32] Therefore, the low molecular weight distributions occurring in the mass spectra of **III** are considered to be products of fragmentation, caused during the ionization step in the MALDI process. This assumption is supported by ^{1}H NMR, showing consistent PEO/PAA ratios and by the fact that even intense dialysis using a membrane with an MWCO of 3.5 kDa (in water as well as in methanol) could not remove or reduce the low molecular fraction.

Analogous results could be obtained from the mass spectrum of **IV** (cf., the Supporting Information). Separated low as well as a high molecular weight distributions were observed centered around 2960 *m*/*z* and 5292 *m*/*z*, respectively. Both distributions were well-resolved, showing the characteristic 44 *m*/*z* peak spacing that corresponds to the PEO repeat unit. Conclusively, the signals of the high molecular weight distribution could be assigned to 10 repeat units of the PAA segment (284.22 Da), α-ω-functionalities of the PEO segment (16.02 Da), and *n* times EO repeat units, e.g., $n = 58$ for the signal marked with an arrow (cf., the Supporting Information) confirming the molecular structure of **IV**. Again, the low molecular weight distribution could not be assigned to any reasonable side product, making a fragmentation process very likely. Matching the end group mass with the chemical structure shows that the fragmentation occurs within the first spermine repeat unit, connecting the PEO with the PAA segment. This is supported by the fragmentation pattern of PAA dendrimers[30] and by the observation of only one homologue series which makes a statistical fragmentation process at different positions in the PAA segment implausible.

The lack of low molecular weight impurities in the products was verified by hydrodynamic techniques. SEC in NMP with LiBr additive as eluent could not be applied, due to strong interactions of **III** and **IV** with the stationary phase, as is typical for such cationic polymers. Therefore, a sedimentation velocity measurement was carried out with an analytical ultracentrifuge (AUC) to determine the sedimentation coefficients in water. Figure 2 shows the S-distributions of **III**, **IV**, and that of free PEO. The S-distribution function not only shows the presence of a single, homomolecular species but also proves that the

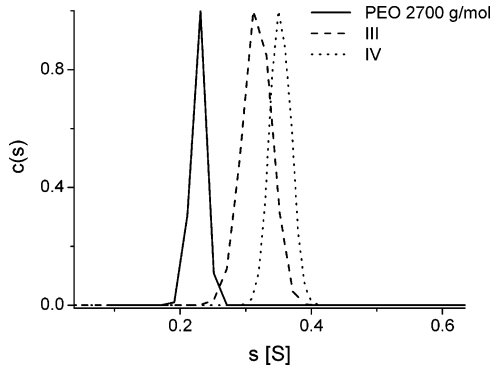


Figure 2. Sedimentation coefficient distribution of **III**, **IV**, and the PEO precursor.

PEO−PAA conjugates and the primary PEO segment are sufficiently different and could, in principle, be separated. For cross-examination, 5, 10, and 15 wt % PEO with respect to the concentration of **III** or **IV** were added to solutions of the PEO−PAA conjugate in order to estimate the detection limit of the method. Addition of already 5 wt % free PEO, results in an obvious broadening of the detected S-distributions if compared to that of the pure PEO−PAA samples (data not shown). Thus, the present sample is regarded to be free of lower molecular weight impurities, i.e., the amount of free PEO precursor can be estimated to be at least below 5 wt %.

The analysis of the PAA conjugates by HPLC and GPC measurements was not possible. Due to strong interactions of the PAA segment with the stationary phase incomplete elution of the samples and irreproducible measurements were observed.

With ^{1}H NMR analysis a solvent and temperature dependence of the calculated relative peak intensities of PEO/PAA was observed. Such behavior is frequently caused by a restriction of the mobility of the PAA segment compared to the well-solvated PEO block and indicates the existence of aggregates. To clarify this, aqueous solutions of **III** and **IV** were investigated by means of small-angle X-ray scattering (SAXS) and dynamic light scattering (DLS) methods. In contrast to **IV**, where no intermolecular aggregation could be found, sample **III** shows the formation of aggregates as could be conclusively observed by both DLS and SAXS methods (see the Supporting Information). The SAXS scattering curve of a 4 wt % solution of **III** shows the characteristic q^{-1} decay of the scattering intensity at higher scattering vectors, indicating the formation of one-dimensional structures with a high aspect ratio such as wormlike micelles.[33] It is noteworthy that the dominant driving force for the aggregation of **III** most likely does not result from preferential solvation (i.e., hydrophobic interactions), as the PEO−PAA conjugate can be classified as a double hydrophilic block copolymer. In fact, aggregation is probably induced by the tendency of the PAA segment to form stable hydrogen-bonding patterns. Such behavior is known for biopolymers, for instance proteins or polysaccharides, leading usually to a soft fixation of the polymer secondary and tertiary structures. Moreover, the capability to form aggregates, stabilized via hydrogen bonding, indicates the potential of the PAA segments for soft interactions with bioorganic macromolecules such as DNA or proteins. Such soft interactions are essential for the reversible complexation or incorporation of biomacromolecules, making the PEO−PAA systems useful as carriers for the delivery of drugs.

Conclusion

In this work an automated synthesis route toward monodisperse, sequence-defined poly(amidoamine) segments (PAA) was presented. The solid-phase supported synthesis approach includes the alternating addition of succinic acid and diamines, such as a spermine derivative or 3,3′-diamino-*N*-methyldipropylamine. Control of the monomer sequence and the molecular weight could be achieved by forcing each coupling step to completion. This novel route was applied for the synthesis of oligopeptide-*block*-PAA and poly(ethylene oxide)-*block*-PAA conjugates. The complete synthesis was performed on a standard peptide synthesizer allowing the fully automated synthesis of the different block copolymer systems potentially in gram or even multigram scales. Similar to solid-phase supported synthesis of peptides the attainable degree of repeat units of the PAA segment might be limited. This is mainly due to imperfections in the coupling step and to difficulties that arise from solid-phase support synthesis, such as inter- or intramolecular aggregation of PAAs or adsorption of PAA onto the support. However, up to now 10 repeat units can be easily realized without detectable amounts of side products.

The sequence-defined synthesis of PAA segments can be readily expanded toward the integration of diacids and diamines possessing diverse secondary functionalities that have to be orthogonally protected. The possibility to precisely position such monomers within a polymer chain allows the rational design of multifunctional polymers exhibiting highly purpose-defined properties. Furthermore, the sequence-defined integration of chiral compounds might lead to advanced structural control such as stereoselective folding or aggregation. These tailor-made polymers might be useful for applications in diverse fields such as drug and gene delivery, polymer therapeutics, as well as tissue engineering.

Acknowledgment. Jessica Brandt, Katharina Otte, Antje Völkel, Marlies Gräwert, Birgit Schonert, Helmut Cölfen, Reinhard Siegel, Bernd Smarsly, Erich C, and Wolfgang Rapp are thanked for the contributions to this project. Financial support was received from the German Research Foundation through the Emmy Noether Program (BO 1762/2-1) and the Max Planck Society.

Supporting Information Available. Set up of the ABI peptide synthesizer, detailed method description, ^{1}H NMR spectra of the PEO−PAA conjugates **III** and **IV**, MALDI-TOF mass spectra of the PEO−PAA conjugates **III** and **IV**, as well as SAXS data of an aqueous solution of **III**. This material is available free of charge via the Internet at http://pubs.acs.org.

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BM050884K