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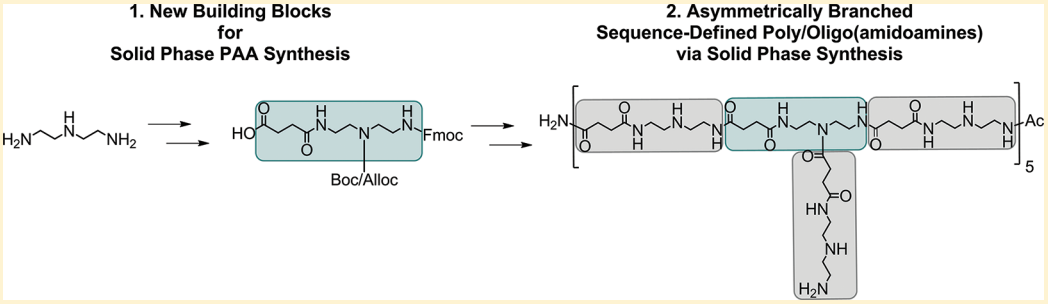
Solid-Phase Synthesis of Asymmetrically Branched Sequence-Defined Poly/Oligo(amidoamines)

Felix Wojcik, Simone Mosca, and Laura Hartmann*

Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany

Supporting Information



ABSTRACT: We present for the first time the synthesis of asymmetrically branched sequence-defined poly/oligo(amidoamines) (PAAs) using solid-phase synthesis with the capability of introducing diversity at the side chains. We introduce two new versatile (diethylenetriamine) building blocks for solid-phase synthesis bearing Fmoc/Boc and Fmoc/Alloc protecting groups expanding recently used Fmoc/Boc protecting group strategy for linear PAAs to an Fmoc/Alloc/Boc strategy. This allows for orthogonal on-resin cleavage of Fmoc and Alloc protecting groups during solid-phase synthesis of PAAs with backbones differing in chain length and sequence. With these structures we then demonstrate the potential for generating asymmetrical branching by automated multiple on-resin cleavage of Alloc protecting groups as well as the introduction of side chains varying in length and number. Such systems have high potential as nonviral vectors for gene delivery and will allow for more detailed studies on the correlation between the degree of branching of PAAs and their resulting biological properties.

INTRODUCTION

Recently, a novel class of peptidomimetic oligocations based on poly(amidoamines) have been recognized for their significant potential as nonviral vectors in gene delivery.[1−4] In contrast to classic polycationic carrier systems,[5−8] these are highly defined synthetic systems that allow for a step-by-step correlation between their chemical structure and the resulting biological properties of the carrier.[9,10] We previously presented a novel approach for the synthesis of sequence-defined linear poly/oligo(amidoamines) (PAAs) with high efficiency using solid-phase synthesis and showed their use as nonviral vectors in gene delivery.[11−13] In order to overcome side reactions observed during solid support synthesis, Schaffert et al.[14] improved on our approach[15,16] by exploiting Fmoc as a temporary protective group for Boc-protected triethylenetetramine acids and tetraethylenepentamine acids. They also used this process to illustrate the synthesis of symmetrical branched structures by using Fmoc-Lys(Fmoc)-OH. Furthermore, they were able to show that the introduction of branching points leads to improved carrier properties, resulting in more efficient transfection reagents as is also known for other branched polycationic systems[17] like poly(ethyleneimine)[18] and polydisperse PAAs[19] or dendritic PAAs.[20] This effect is often explained by cationic functionalities participating in the so-called proton sponge effect enabling an effective release of the cargo.[21,22] Since at the same time these cationic functionalities could also increase cytotoxicity, the degree of branching, as well as the number of cationic functionalities along the branches, seems to be of great importance for the design of new and improved PAA carrier systems.[18]

So far, only symmetrically branched PAAs have been obtained by applying Fmoc-Lys(Fmoc)-OH as a building block.[1,14] After Fmoc cleavage, the main chain can now grow in two directions leading to a symmetrical branching point. Here we now present a new set of building blocks for PAA synthesis allowing for the first time separate building of the main and side chains on the solid phase. We refer to these systems as asymmetrically branched sequence-defined PAAs. Suitable building blocks need to introduce a protective group that can be cleaved during solid-phase synthesis and which is orthogonal to Fmoc.[23] This protecting group strategy[24] will give access to a controlled number and length of side chains as well as main and side chains of different chemical compositions.

We chose to expand on the already known Fmoc/Boc protecting group strategy[14] to an Fmoc/Alloc/Boc strategy for the straightforward synthesis of asymmetric PAAs. The Fmoc protecting group is used as a temporary protecting group during synthesis of the main chain, while the Boc protecting group is only cleaved after complete synthesis of the desired

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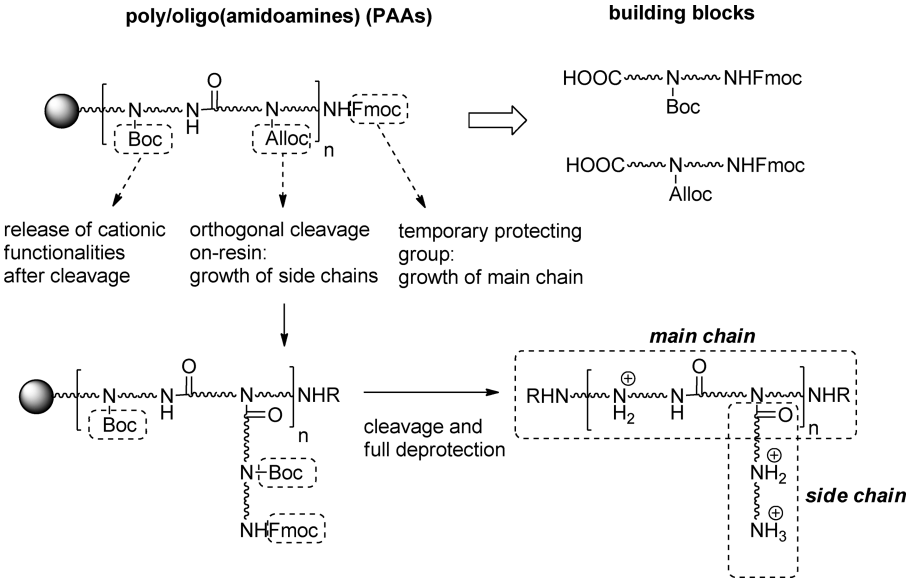
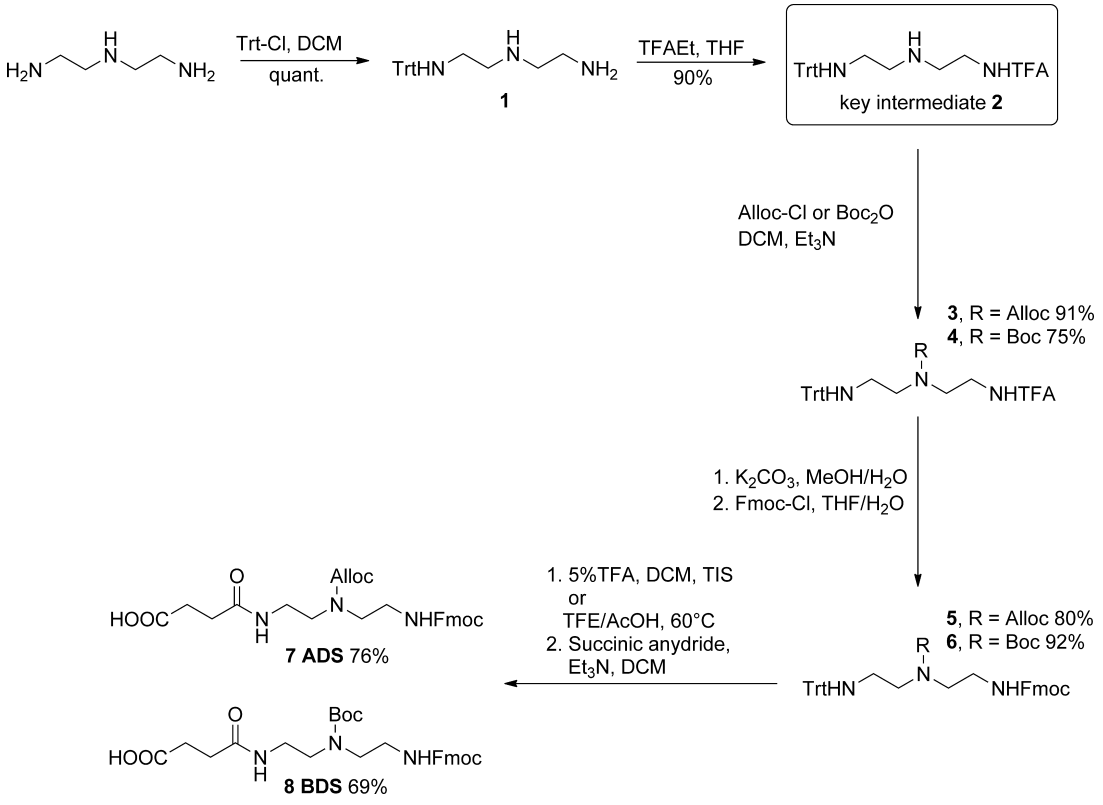


Figure 1. Novel approach for the synthesis of asymmetrically branched PAAs referring to a Fmoc/Alloc/Boc strategy.

Scheme 1. Building Block Synthesis Starting from Diethylenetriamine Leading to ADS 7 and BDS 8



PAA molecule. The Alloc protecting group[23,25] now allows for selective cleavage on resin and the introduction of side chains or branching points leading to asymmetrically branched sequence-defined PAAs (Figure 1).

RESULTS AND DISCUSSION

Building blocks suitable for PAA solid-phase synthesis have to fulfill certain requirements: they have to be accessible in large quantities and high purity and have to be compatible with standard solid-phase peptide synthesis (SPPS) coupling protocols; e.g., they need to be soluble in DMF or NMP. In general, building blocks should also consist of a backbone unit containing a protected amine and free carboxylate functionality with the capability of introducing additional functionalities into the backbone. This work specifically focuses on introducing a secondary amine carrying either a Boc or Alloc protecting group.

In general, we are interested in a synthetic approach for building blocks not only suitable for the Fmoc/Boc/Alloc approach but also for straightforward synthesis of various building blocks. Therefore, we chose an approach starting from a simple triamine, diethylenetriamine, and differentiating the

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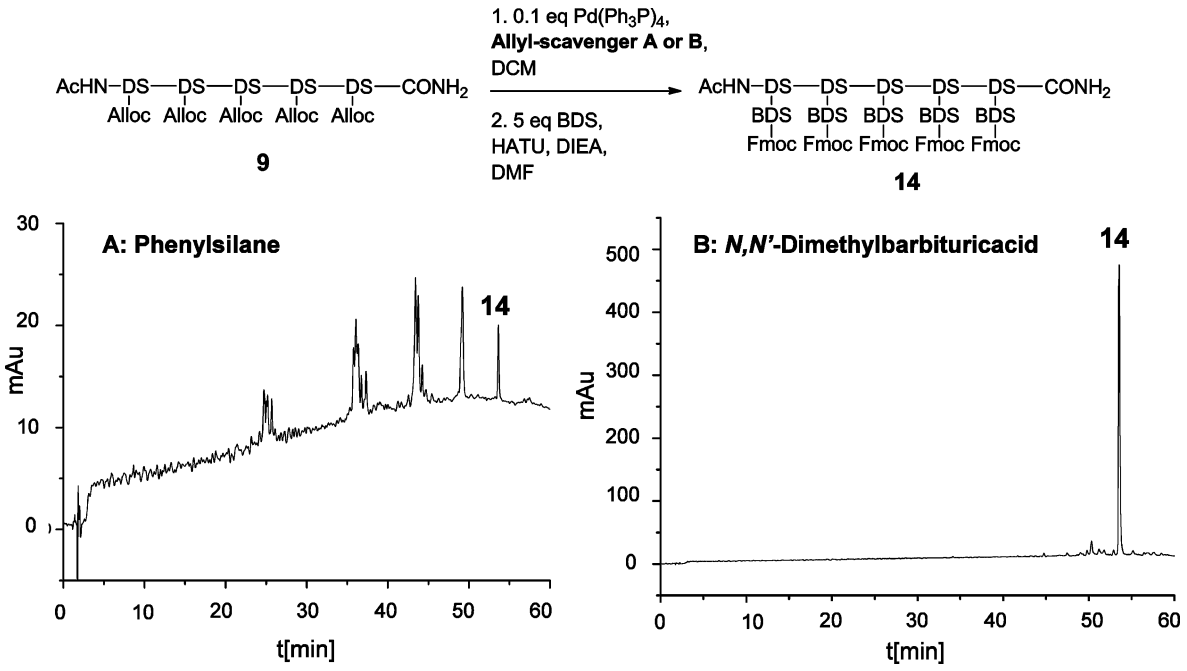

Figure 2. RP-HPLC analysis (5−50% MeCN in 60 min) of compound **14** referring to different allyl scavengers during Alloc deprotection. Sequential Alloc deprotection and BDS coupling using phenylsilane (A) and *N,N′*-dimethlybarbituric acid (B) as allyl scavenger.

three amine groups by a multistep protecting protocol, which can then be coupled to a diacid and produce the corresponding dimer building block referring to our original diacid and diamine building block approach.[11] During this synthesis, different protecting groups as well as functional side groups can be introduced at the secondary amine to give straightforward access to a library of building blocks from a key intermediate presented here for the first time.

For this key intermediate, the two primary amine groups of diethylenetriamine were differentiated by quantitative protection of just one amine group exploiting the bulky triphenylmethyl group in the presence of an excess of diethylenetriamine[26] to give the corresponding monotrityl-protected triamine **1**. Then ethyl trifluoroacetate was used to protect the remaining primary amine[27] of compound **1** to give the asymmetrical protected precursor **2** on a scale of up to 120 g (Scheme 1).

Compound **2** as a novel key intermediate can now be functionalized at the secondary amine with various protecting groups as well as through coupling of functional side chains for conjugaton chemistry[28] or the attachment of biological ligands or fluorescent labels. Here, for the synthesis of the Fmoc/Boc and Fmoc/Alloc building blocks, it was selectively functionalized with either a Boc or an Alloc group by reaction with Alloc-Cl or Boc anhydride providing compound **3** and **4**. The Boc- and Alloc-protected triamines were then converted into the final building blocks: First, the TFA group was converted to an Fmoc moiety using a one-pot protocol giving compound **5** and **6**. Then the trityl group was selectively cleaved, and the corresponding primary amine group was coupled to succinic anhydride affording the final building blocks for the Fmoc solid-phase synthesis, ADS **7** and BDS **8** with an overall yield of 50% and 43%, respectively. The complete synthesis can be carried out in large scale resulting in up to 41 g of final building block in one synthesis. Furthermore, no chromatographic purification is needed. At this point, it is important to describe our nomenclature for these groups. Our nomenclature for novel PAA building blocks indicates the side group, diamine, and diacid components: The two building blocks are functionalized with **A**lloc or **B**oc on the secondary amine and contain **D**iethylentriamine as the diamine unit and **S**uccinic acid as the diacid unit.

During trityl cleavage, we observed a shielding of the trityl protecting group, probably by the other protecting groups in the molecule. This effect is also known for *N*-trityl α-amino acid esters and requires a higher concentration of TFA for quantitative trityl cleavage.[29] Here, higher concentrations of TFA (>3%) in DCM had little impact on the stability of the Alloc-containing compound **5**, but already 4% TFA in DCM showed about 15% cleavage of the Boc protecting group of compound **6** after only 30 min. Therefore, a new protocol for the cleavage of shielded trityl protecting groups in the presence of Boc was necessary. We found that a mixture of acetic acid and trifluoroethanol 1:1 at 60 °C for 1 h resulted in exclusive deprotection of the trityl group without formation of the Boc-deprotected byproduct (see the Supporting Information). To our knowledge, this is a new method for the deprotection of such shielded *N*-trityl groups in the presence of Boc protecting groups.

After the complete building block synthesis, we proved by NMR and HPLC that ADS and BDS were obtained in very high purity (>99%) without referring to any chromatographic purification method. The building blocks were then directly applied for fully automated solid-phase PAA synthesis using an automated standard peptide synthesizer. In a first set of experiments, five backbones of different length and varying sequences containing the Boc as well as Alloc building block were synthesized, showing the suitability of the new building blocks for PAA solid-phase synthesis. All backbones were synthesized following Fmoc amino acid coupling protocols using double coupling with 5 equiv of building blocks **7** or **8** and coupling times of 1 h with HATU as an activation agent[30] (for the backbone coupling pattern, see Figure 4, Supporting Information). Although ADS and BDS are significantly longer

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Table 1. Overview of PAA Backbones and Asymmetrically Branched PAAs Including MALDI TOF MS Analysis and Purity by RP-HPLC Analysis (for HPLC and MS Spectra See the Supporting Information)

No	Sequence	m/z calcd.	MALDI m/z found*[1]	Purity by HPLC
	Backbones			
9	AcHN–ADS–ADS–ADS–ADS–ADS–$CONH_2$	$[M+Na]^+$ 1427.72	1427.70	>95%
10	AcHN–[ADS–BDS]$_4$–$CONH_2$	$[M+Na]^+$ 1899.04	1899.06	95%
11	AcHN–[BDS–ADS–BDS]$_3$–$CONH_2$	$[M+Na]^+$ 2000.14	2000.12	94%
12	AcHN–[BDS–ADS–BDS]$_4$–$CONH_2$	$[M+H]^+$ 2617.53	2617.53	85%
13	AcHN–[BDS–ADS–BDS]$_5$–$CONH_2$	$[M+Na]^+$ 3278.88	3278.84	83%
	1x asymmetrical side chain elongation			
14	AcHN–DS(BDS-Fmoc)–DS(BDS-Fmoc)–DS(BDS-Fmoc)–DS(BDS-Fmoc)–DS(BDS-Fmoc)–$CONH_2$	$[M+Na]^+$ 3043.53	3043.51	95%
15	AcHN–DS(BDS-NH_2)–DS(BDS-NH_2)–DS(BDS-NH_2)–DS(BDS-NH_2)–DS(BDS-NH_2)–$CONH_2$	$[M+H]^+$ 1911.21	1911.15	95%
16	AcHN–[DS(BDS-Fmoc)–BDS]$_4$–$CONH_2$	$[M+Na]^4$ 3191.70	3191.69	92%
17	AcHN–[DS(BDS-NH_2)–BDS]$_4$–$CONH_2$	$[M+Na]^+$ 2303.42	2303.43	92%*[2]
18	AcHN–[BDS–DS(BDS-Fmoc)–BDS]$_3$–$CONH_2$	$[M+Na]^+$ 2969.63	2969.70	91%
19	AcHN–[BDS–DS(BDS-NH_2)–BDS]$_3$–$CONH_2$	$[M+Na]^+$ 2303.42	2303.49	91%*[2]
20	AcHN–[BDS–DS(BDS-Fmoc)–BDS]$_4$–$CONH_2$	$[M+Na]^+$ 3932.16	3932.14	84%
21	AcHN–[BDS–DS(BDS-NH_2)–BDS]$_4$–$CONH_2$	$[M+Na]^+$ 3043.89	3043.81	84%*[2]
22	AcHN–[BDS–DS(BDS-Fmoc)–BDS]$_5$–$CONH_2$	$[M+Na]^+$ 4894.70	4894.28	83%
23	AcHN–[BDS–DS(BDS-NH_2)–BDS]$_5$–$CONH_2$	$[M+Na]^+$ 3784.36	3784.32	83%*[2]
	2x asymmetrical side chain elongation			
24	AcHN–[DS(BDS-BDS-Fmoc)–BDS]$_4$–$CONH_2$	$[M+Na]^+$ 3932.16	3932.07	82%
25	AcHN–[DS(BDS-BDS-NH_2)–BDS]$_4$–$CONH_2$	$[M+Na]^+$ 3043.89	3043.86	82%*[2]

*[1]The most abundant ion adduct is shown here; a detailed MALDI analysis and spectra can be found in the Experimental Section and in the Supporting Information. *[2]According to previously determined purity of the Fmoc-containing compound, followed by complete Fmoc-deprotection shown with ^{1}H NMR.

than α- or β-amino acids or even pseudoprolines[31] used in SPPS, solid-phase coupling gave PAA backbones in very high purity of up to 15 dimer building blocks (Figure 2a, Table 1). The backbones **9**–**13** were synthesized on solid support in purities of >95% for the homo oligomer consisting of 5 ADS building blocks (PAA **9**) and purities of 83% for a 15mer bearing 10 BDS building blocks and 5 ADS building blocks (PAA **13**), respectively. The decrease in purity for PAA chains bearing more then 10 repeating units can be attributed to a decrease in coupling efficiency for longer PAA chains; similar trends are known from standard SPPS. Boc as well as Alloc protecting groups are stable during backbone synthesis; thus, no undesired branching during backbone synthesis was observed, and different chain lengths as well as different sequences of Alloc and Boc building blocks within the chain were obtained (Table 1).

After synthesis of the desired PAA backbone, capping of the *N*-terminal side for the following side-chain elongation proved to be essential in order to avoid further backbone growth. For this, we exploited acetic anhydride. Notably, different acid bearing compounds can also be applied for the capping, e.g., allowing for the introduction of lipids, different temporary protecting groups, hydrophobic dyes, or the attachment of polymer chains.

This first set of backbones was then applied to test for the efficiency of Alloc cleavage and the elongation of side chains at the introduced branching points. The Alloc protecting groups introduced into the main chain were cleaved orthogonally by

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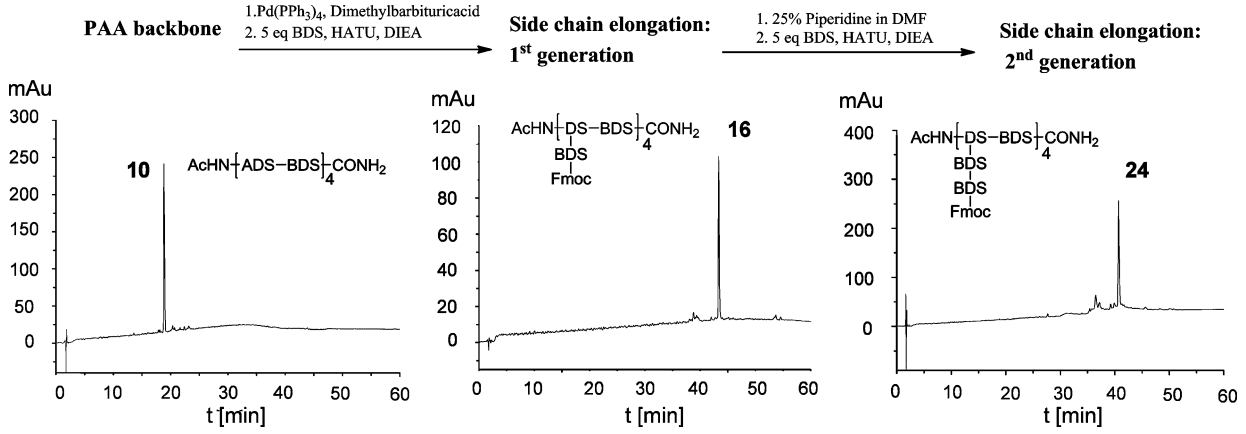

Figure 3. RP-HPLC analysis (5% to 50% MeCN in 60 min) of PAA backbone **10**, asymmetrically branched PAA **16** bearing 12 bearing block and asymmetrically branched PAA **24** bearing 16 building blocks with 2× side-chain elongation.

the use of palladium(0) and an appropriate scavenger for the generated allyl species (Figure 2), while the PAA chain was still attached to the solid support. In order to achieve a fully automated synthesis, this reaction was performed on a peptide synthesizer.[32] In a first attempt to cleave Alloc groups on solid support, we observed incomplete deprotection and the formation of undesired side products. We also showed that for the PAA systems, the secondary amines generated after Alloc cleavage could also serve as a nucleophile during Alloc cleavage resulting in undesired byproduct. This was especially critical for PAA backbones containing more than 2 Alloc building blocks (see PAA backbone **9**, Figure 2) when used in the presence of phenylsilane, one of the most common allyl-scavenger in SPPS (see Figure 2a). In addition, tandem deprotection/coupling[33] using DABCO, EDC, HOBT, and BDS in DCM proved to be inefficient and resulted in allylated byproduct. In order to avoid the formation of allylated byproduct, we used *N,N′*-dimethylbarbituric acid as the scavenger[34] (see Figure 2b). With these conditions, full deprotection of all Alloc groups was achieved releasing free secondary amines within the backbone while retaining backbone integrity.

Side-chain elongation was performed by applying the same fully automated coupling conditions used for the backbone synthesis. All five PAA systems were used for the introduction of side chains (Table 1) at the secondary amine groups released after Alloc cleavage. Therefore, BDS **8** was coupled to the PAA backbones resulting in branched PAAs with different numbers of side chains depending on the number of Alloc building blocks introduced during main-chain synthesis. Control over the chemical structure was obtained by ensuring close to complete conversion in every reaction step as was proven by NMR, HPLC, and MS (table 1, Figure 3b, Supporting Information).

The purity of all asymmetrically branched PAAs was determined by integration of the HPLC UV spectra at 214 nm. After introducing the asymmetrical branches, we proved that all peaks in the HPLC UV spectra contain Fmoc moieties by connecting a fluorescence detector (Ex 259, Em 311) to the HPLC system. Furthermore, we used LC/MS analysis of the Fmoc-protected stages, which show exclusively the MS of the desired asymmetrically branched PAAs within the main peak (for LC/MS spectra, see the Supporting Information). Finally, we were able to compare these purities obtained by RP-HPLC with those obtained by strong cation exchange HPLC of PAA **15** (**14** after Fmoc deprotection, for HPLC spectra see the Supporting Information) showing similar purity as PAA **14**.

At this point, we were able to install three (PAA **18** and **19**), four (PAA **17**, **18**, **20**, **21**), and five (PAA **14**, **15**, **22**, **23**) side chains simultaneously with different spacing along the PAA backbone: no spacing (PAA **14** and **15**), one BDS building block (PAA **16** and **17**), and two BDS building blocks (PAA **18**-**23**). All of these systems present a first generation branched PAA structure bearing one 1,2-diaminoethane motif[35] in the side chain.

In order to now allow for a further growth of side chains or attachment of additional moieties, Fmoc end groups in the side chain have to be cleaved. Again, this step was performed fully automated on the peptide synthesizer. Analysis by NMR and MS proved successful cleavage of all Fmoc protecting groups, now allowing for further coupling reactions to the primary amine groups in the side chains (Table 1).

In order to show that further elongation was feasible, we chose the PAA backbone **10** containing 4 ADS building blocks and 4 BDS building blocks (see Figure 3). After the successful synthesis of the first-generation branched PAA **16** (see Figure 3) and Fmoc deprotection of the side chains (PAA **17**), a second Boc building block BDS was coupled to the PAA side chains (see Figure 3, PAA **24**) followed by Fmoc deprotection to give the unprotected PAA **25**. Analysis via RP-HPLC, NMR, and MS proved the successful synthesis of a second generation branched PAA system now presenting two repeating units of the BDS motif in the side chains. As was observed for longer linear PAA chains, we also see a decrease in product purity to about 80% directly after resin cleavage, indicating a loss of coupling efficiency for larger PAA structures. During LC/MS analysis, a deletion sequence of one Fmoc-BDS-moiety after the last coupling step could be identified as the major impurity (about 9%) in PAA **24**. Here, this could be attributed to the polarity of the side chains rather than the chain length as was indicated by high purities of PAAs bearing all α-amino acid side chains of 90% (unpublished data).

All PAAs shown here are finally purified after cleavage from the resin and precipitation from diethylether. This is one prerequisite of our synthetic strategy as we want to have access to highly pure samples by an optimized synthetic procedure rather then by elaborate purification of a mixture of products, e.g., by preparative HPLC. Nevertheless, if highly pure samples (purity >95%) are required for special future biophysical and in vitro characterization, further purification of all presented

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asymmetrically branched PAA systems is possible via preparative RP-HPLC of the Fmoc protected structures.

In general, we showed in this work that solid-phase synthesis provides an unique tool for the synthesis of highly defined asymmetrically branched poly/oligo(amidoamines) within the molecular weight range of small polymers (20 repeating units).[36,37] Further optimization of this method could lead to larger structures, but due to the solid-phase approach there is an upper limit as already known, e.g., from peptide chemistry.[38] This could be overcome for structures >10 kDa by employing chemoselective ligation[28] or conjugation chemistry resulting in monodisperse systems. Furthermore, the poly/oligo(amidoamines) can be transformed into macromonomers,[39] thus providing access to larger polymers with highly controlled repeating patterns.

CONCLUSION

We introduced a new strategy toward the synthesis of asymmetrically branched PAAs for solid support synthesis. Therefore, a new versatile approach for the synthesis of dimer building blocks applicable for fully automated PAA synthesis was developed. Following this procedure, we synthesized a set of two novel building blocks, ADS and BDS, introducing either Boc or for the first time Alloc-protected amine groups within the PAA main chain. The Alloc protecting group now allows for multiple on-resin cleavage and release of secondary amines within the PAA backbone. The resulting secondary amine groups were then used for introduction and elongation of side chains by the addition of Boc building blocks (BDS). Here, also diversification of the side chains is possible by coupling to the secondary amines, e.g., with other building blocks such as amino acids. All systems are sequence-defined thus allowing for the precise control of the number and positioning of side chains along the PAA backbone. Furthermore, the complete synthesis including growth of the main chain, cleavage of the Alloc protecting groups, as well as elongation of the side chains was performed continuously and fully automated on a standard peptide synthesizer.

This synthetic approach now allows the synthesis of a library of differently multiple branched PAAs varying in the number and distance of branching points within the PAA backbone as well as chain length or chemical composition of main and side chain. In the future, these systems will be systematically analyzed for their potential as nonviral vectors as well as a novel platform for polymer bioconjugates for drug-targeting applications.

EXPERIMENTAL SECTION

General Methods. Unless otherwise noted, all solvents were HPLC grade. Regarding DCM, amylene-stabilized HPLC grade was chosen. The solid-support resin was purchased from Rapp Polymers. Reversed-phase HPLC (RP-HPLC) was performed with 214 nm UV detection on a C18 (Agilent Eclipse, 4.6 × 100 mm) analytical column at 60 °C and a flow rate of 1 mL/min. Eluent: (A) water + 0.1% TFA; (B) acetonitrile + 0.1% TFA. Strong cation-exchange HPLC (SCX-HPLC) was performed with a 214 nm UV detection on a PL-SCX (Agilent, 1000 Å 8 μm 150 × 4.6 mm) analytical column at 60 °C and a flow rate of 1 mL/min. Eluent: (A) 20 mmol NaCl in H_2O + 10 mmol HCl; (B) 1.5 mol of NaCl in H_2O + 10 mmol of HCl were used. The purity was determined by integration of the UV-signal with the software ChemStation for LC from Agilent Technologies.

Building Block Synthesis. *Synthesis of N^1-(2-Aminoethyl)-N^2-tritylethane-1,2-diamine (**1**).* Diethylenetriamine (135 mL, 1.25 mol) was dissolved in 3.75 L of DCM and cooled to 0 °C. Trityl chloride (69.7 g, 250 mmol) was dissolved in 250 mL of DCM and added dropwise to diethylenetriamine solution at 0 °C. After 2 h, the mixture was slowly warmed to room temperature and stirred for an additional 3 h. After 5 h reaction time, the mixture was concentrated under reduced pressure to 1 L and washed with 3 × 500 mL aqueous satd $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give **1** as a white, hygroscopic solid (86 g, 250 mmol) in quantitative yield: mp = 105−110 °C; IR (film) ν 3282, 1723, 1678, 1147 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (dd, *J*1=J2= 7.7 Hz, 6H), 7.27 (t, *J* = 7.7 Hz, 6H), 7.17 (t, *J* = 7.2 Hz, 3H), 2.75−2.72 (m, 4H), 2.60−2.57 (m, 2H), 2.29−2.26 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.1, 128.6, 127.7, 126.2, 70.7, 52.1, 49.9, 43.2, 41.6; ESI-HRMS calcd for $C_{23}H_{28}N_3$ $[M + H]^+$ 346.2283, found 346.2272.

*Synthesis of 2,2,2-Trifluoro-N-(2-((2-(tritylamino)ethyl)amino)-ethyl)acetamide (**2**).* Compound **1** (86 g, 250 mmol) was dissolved in 500 mL of THF and cooled to 0 °C. Under inert atmosphere, ethyl trifluoroaceate (31.3 mL, 263 mmol) was added slowly to the reaction mixture, and then the solution was stirred overnight. The solvent was evaporated under reduced pressure, and the resulting crude product was recrystallized from toluene. Compound **2** (99 g, 224 mmol) was isolated as colorless crystals with a yield of 90%: mp = 118−120 °C; IR (film) ν 3349, 2849, 1706, 1147 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.49−7.47 (m, 6H), 7.31−7.24 (m, 6H), 7.21−7.16 (m, 3H), 3.40−3.34 (m, 2H), 2.75 (t, *J* = 5.7 Hz, 2H), 2.70 (t, *J* = 6 Hz, 2H), 2.29 (t, *J* = 6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 157.3, 156.9, 156.6, 145.9, 128.5, 127.8, 126.3, 120.2, 117.3, 114.5, 111.6, 70.7, 49.4, 47.1, 43.2, 38.9; ESI-HRMS calcd for $C_{25}H_{27}F_3N_3O$ $[M + H]^+$ 442.2106, found 442.2111.

*Synthesis of Allyl (2-(2,2,2-Trifluoroacetamido)ethyl)(2-(tritylamino)ethyl)carbamate (**3**).* Compound **2** (44 g, 100 mmol) and triethylamine (27.9 mL, 200 mmol) were dissolved in 500 mL of DCM under inert atmosphere. The solution was cooled to 0 °C, and allyl chloroformate (11.2 mL, 105 mmol) was added dropwise. Then the reaction mixture was slowly warmed to room temperature and stirred for an additional 4 h. Afterward, the mixture was evaporated under reduced pressure, and the crude product was redissolved in 500 mL of diethyl ether. The organic layer was washed twice with 400 mL satd sodium bicarbonate solution, dried over $NaSO_4$, and evaporated under reduced pressure. Compound **3** (47.8 g, 91 mmol) was isolated as a hygroscopic white solid with a yield of 91%. The crude product was used for the next reaction without further purification: mp = 98−100 °C; IR (film) ν 3286, 1722, 1678, 1146 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.80−7.72 (bs, 1H), 7.37−7.32 (m, 6H), 7.22−7.15 (m, 6H), 7.13−7.07 (m, 3H), 5.80−5.70 (m, 1H), 5.20−5.08 (m, 2H), 4.48 (d, *J* = 5.1 Hz, 2H), 3.43−3.33 (m, 4H), 3.27 (t, *J* = 6.2 Hz, 2H), 2.30−2.23 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1, 157.7, 157.4, 157.3, 145.7, 132.2, 128.4, 127.9, 126.4, 125.3, 118.2, 117.2, 114.3, 70.8, 66.7, 48.4, 46.4, 42.3, 40.3; ESI-HRMS calcd for $C_{29}H_{31}F_3N_3O_3$ $[M + H]^+$ 526.2318, found. 526.2333.

*Synthesis of tert-Butyl (2-(2,2,2-Trifluoroacetamido)ethyl)(2-(tritylamino)ethyl)carbamate (**4**).* Compound **2** (88 g, 200 mmol) and triethylamine (55.8 mL, 400 mmol) were dissolved in 1 L of DCM under inert atmosphere. The solution was cooled to 0 °C, and di-*tert*-butyl dicarbonate (45.8 g, 210 mmol) was added in small portions. The reaction mixture was slowly warmed to room temperature and stirred for an additional 18 h. Afterward, the mixture was evaporated under reduced pressure, and the residue was redissolved in 800 mL of diethyl ether. The organic layer was washed twice with 500 mL of satd sodium bicarbonate solution, dried over $NaSO_4$, and evaporated under reduced pressure. The crude product was recrystallized from hexane. Compound **4** (81 g, 150 mmol) was isolated in form of a white crystalline solid with a yield of 75%: mp = 95−98 °C; IR (film) ν 3306, 1709, 1174, 1145 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (bs, 1H), 7.39−7.33 (m, 6H), 7.23−7.16 (m, 6H), 7.13−7.08 (m, 3H), 3.38−3.29 (m, 4H), 3.21 (t, *J* = 5.8 Hz, 2H), 2.24 (t, *J* = 5.7 Hz, 2H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2, 157.9, 157.7, 157.4, 145.7, 128.4, 127.9, 126.4, 120.1, 117.2, 114.4, 111.5, 81.0, 70.8,

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48.5, 45.6, 42.3, 40.8, 28.2; ESI-HRMS calcd for $C_{30}H_{35}F_3N_3O_3$ $[M + H]^+$ 542.2631, found 542.2606; calcd for $C_{30}H_{34}F_3N_3NaO_3$ $[M + Na]^+$ 564.2450, found 564.2424.

*Synthesis of Allyl (2-((((9H-Fluoren-9-yl)methoxy)carbonyl)-amino)ethyl)(2(tritylamino)ethyl)carbamate (**5**).* Compound **3** (45.6 g, 87 mmol) was dissolved in 72 mL of water and 720 mL of methanol. Then K_2CO_3 (60 g, 5 equiv) was added. The suspension was stirred for 18 h until the methanol was evaporated, and an additional 400 mL of water, 470 mL of THF, and 9-fluorenylmethyl chloroformate (Fmoc-Cl) (22.5 g, 27 mmol) were added. The mixture was stirred for an additional 18 h, and then THF was evaporated and the product extracted with diethyl ether. The organic layer was washed twice with satd sodium bicarbonate solution, dried over Na_2SO_4, and evaporated under reduced pressure. The carbamate **5** was recrystallized from toluene as a white solid (45 g, 69 mmol) with a yield of 80%: mp = 118−121 °C; IR (film) ν 3347, 1686 1239, 1146 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (bs, 1H), 7.75 (bs, 1H), 7.52−7.51 (m, 2H), 7.47−7.44 (m, 6H), 7.39 (t, J = 7.4 Hz, 2 H), 7.32−7.24 (m, 8H), 7.22−7.16 (m, 3H), 5.95−5.82 (m, 1H), 5.36−5.16 (m, 3H), 4.61−4.54 (m, 2H), 4.45−4.33 (m, 2H), 4.21 (t, J = 6.7 Hz, 1 H), 3.46−3.55 (m, 6H), 2.38−2.30 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 156.5 145.8, 143.9, 141.3, 132.7, 128.5, 127.8, 127.6, 127.0, 126.3, 119.9, 117.7, 70.7, 66.6, 66.2, 48.2, 47.4, 47,2, 42.4, 40.0; ESI-HRMS calcd for $C_{42}H_{42}N_3O_4$ $[M + H]^+$ 652.3175, found 652.3196.

*Synthesis of tert-Butyl (2-((((9H-Fluoren-9-yl)methoxy)carbonyl)-amino)ethyl) (2-(Tritylamino)ethyl)carbamate (**6**).* Compound **4** (80 g, 148 mmol) was dissolved in 57 mL of water and 680 mL of methanol. Then K_2CO_3 (102 g, 5 equiv) was added. The suspension was stirred for 18 h until methanol was evaporated, and an additional 310 mL of water and 370 mL of THF and Fmoc-Cl (40 g, 155 mmol) were added. The mixture was stirred for an additional 18 h, and then THF was evaporated and the product was extracted with 800 mL of diethyl ether. The organic layer was washed twice with 500 mL of satd $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was recrystallized from hexane. Compound **6** (91 g, 136 mmol) was obtained as a white solid with a yield of 92%: mp = 154−156 °C; IR (film) ν 3351, 1678 1238, 1143 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.3 Hz, 2H), 7.46 (d, J = 7.6 Hz, 6H), 7.38 (t, J = 7.4 Hz, 2 H), 7.31−7.16 (m, 8H), 7.21−7.15 (t, J = 7.2 Hz), 4.50−4.34 (m, 2H), 4.20 (t, J = 6.9 Hz, 1 H), 3.40−3.12 (m, 6H), 2.36−2.28 (m, 2H), 1.50−1.35 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 145.9, 143.9, 141.3, 128.5, 127.8, 127.6, 127.0, 126.3, 119.9, 80.1, 70.7, 66.7, 48.3, 47.3, 46,8, 42.4, 40.4, 28.4; ESI-HRMS calcd for $C_{43}H_{46}N_3O_4$ $[M + H]^+$ 668.3488, found 668.3462.

*Synthesis of ADS: 7-((Allyloxy)carbonyl)-1-(9H-fluoren-9-yl)-3,11-dioxo-2-oxa-4,7,10-triazatetradecan-14-oic Acid (**7**).* Compound **5** (43 g, 66 mmol) was dissolved in 627 mL of DCM and triethylsilane (31.6 mL, 198 mmol). After addition of 33 mL of TFA (5 vol% regarding the reaction mixture) at 0 °C, the mixture was allowed to reach rt. The reaction was complete after 30 min according to TLC monitoring (2:1 Hex/EtoAc). Then the reaction mixture was coevaporated with toluene. The resulting solid was dissolved in 30 mL of toluene and precipitated with 200 mL of diethyl ether. The white solid was dissolved with succinic anhydride (6.9 g, 69.3 mmol) in 660 mL of DCM, and triethylamine (27.6 mL, 198 mmol) was added carefully to the mixture. After 30 min, complete conversion was monitored on TLC (DCM/MeOH/AcOH 90:10:1). The reaction mixture was acidified with aqueous citric acid to pH 3 and washed with 500 mL of 5% aqueous citric acid. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was recrystallized from a mixture of toluene/ethanol 20:1. Compound **7** (25.6 g, 50 mmol) was obtained as a white crystalline solid with a yield of 76%: mp = 81−84 °C; IR (film) ν 3320, 1716, 1678, 1645, 1237, 1142 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.4 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2 H), 7.29 (t, J = 7.4 Hz, 2 H), 5.91−5.81 (m, 1H), 5.28−5.15 (m, 2H), 4.56−4.45 (m, 2H), 4.41−4.31 (m, 2H), 4.18 (t, J = 6.8 Hz, 1 H), 3.45−3.15 (m, 8H), 2.65 (t, J = 6.2 Hz, 2H), 2.49−2.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 172.9, 157.0, 156.7, 143.8, 141.2, 132.4, 127.8, 127.0, 125.0, 119.9, 117.9, 66.7, 66.5, 47.8, 47.1, 40.1, 39.9, 39.0, 30.6, 29.5; ESI-HRMS calcd for $C_{27}H_{31}N_3NaO_7$ $[M + Na]^+$ 532.2060, found 532.2067; RP-HPLC analysis, 5% to 95% MeCN in 10 min, t_R = 7.8 min

*Synthesis of BDS: 7-(tert-Butoxycarbonyl)-1-(9H-fluoren-9-yl)-3,11-dioxo-2-oxa-4,7,10-triazatetradecan-14-oic Acid (**8**).* Compound **6** (76.7 g, 115 mmol) was added to a mixture of 145 mL of acetic acid and 145 mL of trifluoroethanol at 60 °C. The reaction mixture was stirred for 60 min. Then the solution was coevaporated with toluene. The resulting solid was dissolved in 30 mL of toluene and precipitated with 250 mL of ice-cold diethyl ether. The white solid was taken without any purification and dissolved in 575 mL of DCM, followed by addition of succinic anhydride (12 g, 120 mmol). During stirring, triethylamine (48.1 mL, 345 mmol) was added slowly to the mixture. After 30 min, TLC monitoring (DCM/MeOH/AcOH 90:10:1) showed complete conversion. The reaction mixture was acidified with aqueous citric acid to pH 3 and then washed with 500 mL of 5% aqueous citric acid. The crude product was dissolved in acetone and precipitated with aqueous 1% citric acid. The highly viscous white oil was washed twice with 1% citric acid in water. Finally, the solid was redissolved in EtOAc and the organic layer was washed with water once, dried over Na_2SO_4, and evaporated under reduced pressure. Compound **8** (41.4 g, 79 mmol) was obtained as hygroscopic amorphous white solid with a yield of 69%: IR (film) ν 3285, 1743, 1695, 1170, 1145 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.4 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.29 (t, J = 7.3 Hz, 2H), 4.48−4.28 (m, 2H), 4.18 (t, J = 6.6 Hz, 1 H), 3.44−3.05 (m, 8H), 2.70−2.60 (m, 2H), 2.54−2.42 (m, 2H), 1.42 (bs, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 175.5, 156.6, 143.8, 141.2, 127.7, 127.0, 125.0, 119.9, 80.7, 66.6, 48.0, 47.1, 40.5, 40.1, 39.3, 30.7, 29.8, 28,3; ESI-HRMS calcd for $C_{28}H_{34}N_3O_7$ $[M - H]^-$ 524.2397, found 524.2399; RP-HPLC analysis, 5% to 95% MeCN in 10 min, t_R = 8.4 min.

Solid-Phase Synthesis. All solid-phase reactions were performed on an automated standard peptide synthesizer in 0.05 mmol scale referring to the following general solid-phase protocols. Tentagel S RAM resin (loading 0.23 mmol/g) was used as solid support, which was swollen twice for 15 min in DCM before starting the initial Fmoc-deprotection.

General Procedure. *Coupling/Fmoc-Deprotection Protocol.* ADS (5 equiv, 127 mg) or BDS (5 equiv, 131 mg) and *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) (93 mg, 4.9 equiv) were placed as powder in the amino acid vial and placed in the peptide synthesizer. The solids were dissolved in 2 mL of DMF with a gentle nitrogen stream. Then 10 equiv of 1 M DIEA solution in DMF was added. Preactivation was carried out for 3 min in the amino acid vial before the solution was transferred to the resin. Afterward the resin with the coupling solution was shaken carefully for 1 h, the reaction vessel was emptied and washed with DMF. Then the whole procedure was repeated once.

Fmoc deprotection was performed using 25% piperidine in DMF for 5 min and checked by UV monitoring for the fluorenyl piperidine adduct at 301 nm. This step was repeated until the deprotection was complete.

Acetylation of the N-Terminal Side. For acetylation of the *N*-terminal side 3 mL of Ac_2O was placed in the amino acid vial and transferred to the reaction vessel, which was shaken for 5 min. Afterward the resin was washed with DMF.

Automated Alloc Cleavage. Tetrakis(triphenylphosphine)-palladium(0) (0.1 equiv per Alloc moiety) and *N,N*-dimethylbarbituric acid (5 equiv per Alloc moiety) were placed in the amino acid vial flushed under argon. Then 4.5 mL of DCM was added to the amino acid vial, and the solids were dissolved by a gentle nitrogen stream for 4 min. The solution was transferred to the reaction vessel, which was shaken for 2 h. The whole procedure was repeated once before the resin was washed 3× with DCM, 3 × 0.2 M DIEA in DMF, and 6 × DMF.

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Side-Chain Elongation. The side-chain elongation was performed with the previously described protocol for coupling and deprotection, but 5 equiv of BDS, 4.9 equiv of HATU, and 10 equiv of 1 M DIEA per side chain were used. The solids were dissolved in 4 mL of DMF.

Final Cleavage from Solid Support. The final cleavage was performed by adding the cleavage cocktail (95% TFA, 2.5% triisopropylsilane, and 2.5% water 1 mL/50 mg resin) to the resin and allowing it to react for 70 min. The cleavage solution was filtered and purged into ice-cold diethyl ether. The precipitate was isolated and washed twice with diethyl ether. The residue was dissolved in water and lyophilized overnight, giving the final product and the corresponding yield.

AcHN-ADS-ADS-ADS-ADS-ADS-CONH$_2$ ***(9)***. Compound **9** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{62}H_{101}N_{16}O_{21}$ $[M + H]^+$ 1405.733 (monoisotopic), found 1405.711; calcd for $C_{62}H_{100}N_{16}NaO_{21}$ $[M + Na]^+$ 1427.72 (monoisotopic), found 1427.70; calcd for $C_{62}H_{100}KN_{16}O_{21}$ $[M + K]^+$ 1443.69 (monoisotopic), found 1443.68; RP-HPLC analysis 5% to 50% MeCN in 60 min, t_R = 29.2 min.

AcNH-(ADS-BDS)$_4$-CONH$_2$ ***(10)***. Compound **10** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{82}H_{142}N_{25}O_{25}$ $[M + H]^+$ 1877.06 (monoisotopic), found 1877.08; calcd for $C_{82}H_{141}N_{25}NaO_{25}$ $[M + Na]^+$ 1899.04 (monoisotopic), found 1899.06; calcd for $C_{82}H_{141}KN_{25}O_{25}$ $[M + K]^+$ 1915.02 (monoisotopic), found 1915.03; RP-HPLC analysis 5% to 50% MeCN in 60 min, t_R = 18.8 min.

AcNH-(BDS-ADS-BDS)$_3$-CONH$_2$ ***(11)***. Compound **11** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{86}H_{153}N_{28}O_{25}$ $[M + H]^+$ 1978.156 (monoisotopic), found 1978.131; calcd for $C_{86}H_{152}N_{28}NaO_{25}$ $[M + Na]^+$ 2000.14 (monoisotopic), found 2000.12; calcd for $C_{86}H_{152}KN_{28}O_{25}$ $[M + K]^+$ 2016.11 (monoisotopic), found 2016.09; RP-HPLC analysis 5% to 50% MeCN in 60 min, t_R = 12.9 min.

AcNH-(BDS-ADS-BDS)$_4$-CONH$_2$ ***(12)***. Compound **12** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions. MALDI-TOF calcd for $C_{114}H_{202}N_{37}O_{33}$ $[M + H]^+$ 2617.53 (monoisotopic), found 2617.53; calcd for $C_{114}H_{201}N_{37}NaO_{33}$ $[M + Na]^+$ 2639.51 (monoisotopic), found 2639.51; calcd for $C_{114}H_{201}KN_{37}O_{33}$ $[M + K]^+$ 2655.48 (monoisotopic), found 2655.49; RP-HPLC analysis 5% to 30% MeCN in 60 min, t_R = 22.9 min.

AcNH-(BDS-ADS-BDS)$_5$-CONH$_2$ ***(13)***. Compound **13** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{142}H_{251}N_{46}O_{41}$ $[M + H]^+$ 3256.90 (monoisotopic), found 3256.92; calcd for $C_{142}H_{250}N_{46}NaO_{41}$ $[M + Na]^+$ 3278.88 (monoisotopic), found 3278.84; calcd for $C_{142}H_{250}KN_{46}O_{41}$ $[M + K]^+$ 3294.85 (monoisotopic), found 3294.82; RP-HPLC analysis 5% to 30% MeCN in 60 min, t_R = 24.6 min.

AcNH-[DS(BDS-Fmoc)]$_5$-CONH$_2$ ***(14)***. Compound **14** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{157}H_{205}N_{31}NaO_{31}$ $[M + Na]^+$ 3043.53 (monoisotopic), found 3043.51; calcd for $C_{157}H_{205}KN_{31}O_{31}$ $[M + K]^+$ 3059.51 (monoisotopic), 3059.50; RP-LC/MS analysis 5% to 50% MeCN in 60 min, t_R = 53.5 min, calcd for $C_{157}H_{207}N_{31}O_{31}$ $[M + 2H]^{2+}$ 1512.3 (mean), found 1512.7; calcd for $C_{157}H_{208}N_{31}O_{31}$ $[M + 3H]^{3+}$ 1008.5 (mean), found 1008.7; calcd for $C_{157}H_{209}N_{31}O_{31}$ $[M + 4H]^{4+}$ 756.6 (mean), found 756.6; calcd for $C_{157}H_{210}N_{31}O_{31}$ $[M + 5H]^{5+}$ 605.5 (mean), found 605.7.

AcNH-[DS(BDS-NH$_2$)]$_5$-CONH$_2$ ***(15)***. Compound **15** (71 mg, 0.037 mmol) was obtained with a yield of 74%: ^{1}H NMR (400 MHz, D_2O) δ 3.71−3.29 (m, diamine unit, 80H), 2.75−2.49 (m, diacid unit, 40H), 1.98 (d, J = 13.7 Hz, acetyl, 3H); MALDI-TOF calcd for $C_{82}H_{156}N_{31}O_{21}$ $[M + H]^+$ 1911.21 (monoisotopic), found 1911.15; calcd for $C_{82}H_{155}N_{31}NaO_{21}$ $[M + Na]^+$ 1933.19 (monoisotopic), found 1933.09; SCX-HPLC analysis 20 mmol to 1.5 mol NaCl in 30 min, t_R = 20.2 min.

AcNH-[DS(BDS-Fmoc)-BDS]$_4$-CONH$_2$ ***(16)***. Compound **16** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{158}H_{226}N_{37}O_{33}$ $[M + H]^+$ 3169.71 (monoisotopic), found 3169.71; calcd for $C_{158}H_{225}N_{37}NaO_{33}$ $[M + Na]^+$ 3191.70 (monoisotopic), found 3196.69; calcd for $C_{158}H_{225}KN_{37}O_{33}$ $[M + K]^+$ 3207.67 (monoisotopic), found 3207.66; RP-LC/MS analysis 5% to 50% MeCN in 60 min, t_R = 43.4 min; calcd for $C_{158}H_{227}N_{37}O_{33}$ $[M + 2H]^{2+}$ 1586.4 (mean), found 1586.3; calcd for $C_{158}H_{228}N_{37}O_{33}$ $[M + 3H]^{3+}$ 1057.9 (mean), found 1058.1; calcd for $C_{158}H_{229}N_{37}O_{33}$ $[M + 4H]^{4+}$ 793.7 (mean); found 794.0; calcd for $C_{158}H_{230}N_{37}O_{33}$ $[M + 5H]^{5+}$ 635.1 (mean); found 635.5.

AcNH-[DS(BDS-NH$_2$)-BDS]$_4$-CONH$_2$ ***(17)***. Compound **17** (77 mg, 0.034 mmol) was obtained with a yield of 68%. ^{1}H NMR (400 MHz, D_2O) δ 3.70−3.20 (m, diamine unit, 96H), 2.74−2.50 (m, diacid unit, 48H), 2.02 (s, acetyl, 3H); MALDI-TOF calcd for $C_{98}H_{186}N_{37}O_{25}$ $[M + H]^+$ 2281.44 (monoisotopic); found 2281.47; calcd for $C_{98}H_{185}N_{37}NaO_{25}$ $[M + Na]^+$, 2303.42 (monoisotopic); found 2303.43; calcd for $C_{98}H_{185}KN_{37}O_{25}$ $[M + K]^+$, 2319.40 (monoisotopic), found 2319.41.

AcNH-[BDS-DS(BDS-Fmoc)-BDS]$_3$-CONH$_2$ ***(18)***. Compound **18** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{143}H_{216}N_{37}O_{31}$ $[M + H]^+$ 2947.65 (monoisotopic), found 2947.73; calcd for $C_{143}H_{215}N_{37}NaO_{31}$ $[M + Na]^+$ 2969.63 (monoisotopic), found 2281.70; calcd for $C_{143}H_{215}KN_{37}O_{31}$ $[M + K]^+$ 2985.60 (monoisotopic), found 2985.68; RP-LC/MS analysis 5% to 50% MeCN in 60 min, t_R = 36.5 min; calcd for $C_{143}H_{218}N_{37}O_{31}$ $[M + 3H]^{3+}$ 983.8 (mean), found 984.0; calcd for $C_{143}H_{219}N_{37}O_{31}$ $[M + 4H]^{4+}$ 738.1 (mean), found 738.4.

AcNH-[BDS-DS(BDS-NH$_2$)-BDS]$_3$-CONH$_2$ ***(19)***. Compound **19** (90 mg, 0.040 mmol) was obtained with a yield of 79%: ^{1}H NMR (400 MHz, D_2O) δ 3.70−3.20 (m, diamine unit, 96H), 2.74−2.51 (m, diacid unit, 48H), 2.01 (s, acetyl, 3H); MALDI-TOF calcd for $C_{98}H_{186}N_{37}O_{25}$ $[M + H]^+$ 2281.44 (monoisotopic), found 2281.52; calcd for $C_{98}H_{185}N_{37}NaO_{25}$ $[M + Na]^+$ 2303.42 (monoisotopic), found 2303.49.

AcNH-[BDS-DS(BDS-Fmoc)-BDS]$_4$-CONH$_2$ ***(20)***. Compound **20** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{190}H_{285}N_{49}NaO_{41}$ $[M + Na]^+$ 3932.16 (monoisotopic), found 3932.14; RP-LC/MS analysis, 5% to 50% MeCN in 60 min, t_R = 39.3 min; calcd for $C_{190}H_{288}N_{49}O_{41}$ $[M + 3H]^{3+}$ 1304.9 (mean), found 1305.1; calcd for $C_{190}H_{289}N_{49}O_{41}$ $[M + 4H]^{4+}$ 978.9 (mean), found 978.3; calcd for $C_{190}H_{290}N_{49}O_{41}$ $[M + 5H]^{5+}$ 783.3 (mean), found 783.7; calcd for $C_{190}H_{291}N_{49}O_{41}$ $[M + 6H]^{6+}$ 652.9 (mean), found 653.2.

AcNH-[BDS-DS(BDS-NH$_2$)-BDS]$_4$-CONH$_2$ ***(21)***. Compound **21** (107 mg, 0.036 mmol) was obtained with a yield of 71%: ^{1}H NMR (400 MHz, D_2O) δ 3.71−3.22 (m, diamine unit, 128H), 2.75−2.52 (m, diacid unit, 64H), 2.03 (s, acetyl, 3H); MALDI-TOF calcd for $C_{130}H_{246}N_{49}O_{33}$ $[M + H]^+$, 3021.91 (monoisotopic), found 3021.87; calcd for $C_{130}H_{245}N_{49}NaO_{33}$ $[M + Na]^+$ 3043.89 (monoisotopic), found 3043.81.

AcNH-[BDS-DS(BDS-Fmoc)-BDS]$_5$-CONH$_2$ ***(22)***. Compound **22** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{237}H_{356}N_{61}O_{51}$ $[M + H]^+$, 4872.51 (monoisotopic), found 4872.28; calcd for $C_{237}H_{355}N_{61}NaO_{51}$ $[M + Na]^+$ 4894.70 (monoisotopic), found 4894.28; RP-LC/MS analysis 5% to 50% MeCN in 60 min, t_R = 42.3 min; calcd for $C_{237}H_{359}N_{61}O_{51}$ $[M + 4H]^{4+}$ 1219.7 (mean), found 1219.6; calcd for $C_{237}H_{360}N_{61}O_{51}$ $[M + 5H]^{5+}$ 975.9 (mean), found 975.8; calcd for $C_{237}H_{361}N_{61}O_{51}$ $[M + 6H]^{6+}$ 813.5 (mean), found 813.4; calcd for $C_{237}H_{362}N_{61}O_{51}$ $[M + 7H]^{7+}$ 697.4 (mean), found 697.4; calcd for $C_{237}H_{363}N_{61}O_{51}$ $[M + 8H]^{8+}$ 610.3 (mean), found 610.3.

AcNH-[BDS-DS(BDS-NH$_2$)-BDS]$_5$-CONH$_2$ ***(23)***. Compound **23** (124 mg, 0.033 mmol) was obtained with a yield of 66%: ^{1}H NMR (400 MHz, D_2O) δ 3.71−3.21 (m, diamine unit, 160H), 2.75−2.53 (m, diacid unit, 80H), 2.02 (s, acetyl, 3H); MALDI-TOF calcd for

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$C_{162}H_{305}N_{61}NaO_{41}$ $[M + Na]^+$ 3784.36 (monoisotopic), found 3784.32.

*AcNH-[DS(BDS-BDS-Fmoc)-BDS]$_4$-CONH$_2$ (**24**).* Compound **24** was cleaved from the resin on an analytical scale. The resin-bound product was used for further reactions: MALDI-TOF calcd for $C_{190}H_{285}N_{49}NaO_{41}$ $[M + Na]^+$ 3932.16 (monoisotopic), found 3932.07; RP-LC/MS analysis 5% to 50% MeCN in 60 min, t_R = 40.7 min; calcd for $C_{190}H_{288}N_{49}O_{41}$ $[M + 3H]^{3+}$ 1304.9 (mean), found 1305.0; calcd for $C_{190}H_{289}N_{49}O_{41}$ $[M + 4H]^{4+}$ 978.9 (mean), found 978.3; calcd for $C_{190}H_{290}N_{49}O_{41}$ $[M + 5H]^{5+}$ 783.3 (mean), found 783.8; calcd for $C_{190}H_{291}N_{49}O_{41}$ $[M + 6H]^{6+}$ 652.9 (mean), found 653.3.

*AcNH-[DS(BDS-BDS-NH$_2$)-BDS]$_4$-CONH$_2$ (**25**).* Compound **25** (110 mg, 0.037 mmol) was obtained with a yield of 73%: ^{1}H NMR (400 MHz, D_2O) δ 3.71−3.24 (m, diamine unit, 128H), 2.75−2.50 (m, diacid unit, 64H), 2.02 (s, acetyl, 3H); MALDI-TOF calcd for $C_{130}H_{246}N_{49}O_{33}$ $[M + H]^+$ 3021.91 (monoisotopic), found 3021.91; calcd for $C_{130}H_{245}N_{49}NaO_{33}$ $[M + Na]^+$ 3043.89 (monoisotopic), found 3043.86.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

More experimental details, copies of the ^{1}H and ^{13}C NMR spectra, RP-HPLC analysis, SCX-HPLC analysis, LC/MS analysis, and mass spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: laura.hartmann@mpikg.mpg.de.

Notes

The authors declare no competing financial interest.

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