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Regioselective solid-phase synthesis of *N*-mono-hydroxylated and *N*-mono-methylated acylpolyamine spider toxins using an 2-(*ortho*-nitrophenyl)ethanal-modified resin†

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A recently introduced new SPS resin, possessing a 2-(*ortho*-nitrophenyl)ethanal linker, was used for the regioselective on-resin synthesis of *N*-mono-hydroxylated and *N*-mono-methylated polyamine spider toxins of *Agelenopsis aperta* and *Larinioides folium*. The polyamine backbones of the target compounds were efficiently constructed from the center by reductive amination of the aldehyde linker, followed by stepwise alkylation and acylation on solid support. Depending on the cleavage conditions, employing either oxidation/Cope elimination or methylation/Hofmann elimination, regioselectively the respective *N*-hydroxyl or *N*-methyl products were obtained. Employing this methodology, a number of acylpolyamine spider toxins were synthesized and identified as venom components by UHPLC and ESI-MS/MS.

Introduction

Spider venoms are complex mixtures of diverse compounds such as proteins, peptides, nucleic acids, polyamines, and polyamine derivatives.[1] Since the early 1990s, particular attention has been given to the acylpolyamine derivatives, which exhibit interesting and diverse biological activities.[2–14] These compounds share, with a few exceptions, the same general structure (Fig. 1):[15] as a core, they all possess a linear α,ω-diamino polyazaalkane (polyamine) backbone, which is, in the simplest examples, modified at just one end with a lipophilic head unit, usually an aromatic acyl group. Some more complex representatives contain in addition one or more amino acid moieties as linker in-between the aromatic head group and the polyaza core, and the most complex members are furthermore modified at the tail with a guanidyl or an additional basic amino acid tail portion. While the polyamine backbones of the majority of the compounds are no further derivatized, some spider toxins are hydroxylated or methylated at one or more of their polyamine N-atoms.

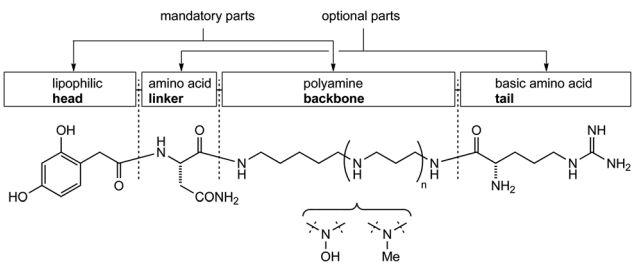

Fig. 1 General structure of polyamine spider toxins.

Due to the high interest into the polyamine spider toxins as biologically active compounds, not only access to larger amounts of such substrates is demanded but also synthetic flexibility to efficiently obtain structural variations. The initially used classical synthetic approaches to obtain spider toxins by in-solution chemistry proved feasible,[15] but they have been progressively replaced by methods of solid phase chemistry.[6–12,15–31] Such methods proved to be more flexible and more efficient. In particular, laborious work-up and purification procedures with the usually rather polar synthetic intermediates could be avoided.

With the resins and protocols that have been used so far, however, mostly polyamine toxins with non-modified internal amino groups have been prepared: *N*-hydroxylated and *N*-methylated derivatives have been accessed by solid phase synthesis (SPS) only recently,[14] by the application of an orthogonal protecting group strategy. The 2-(*ortho*-nitrophenyl) ethanal resin **1**, lately introduced by us,[32] is suitable for the assembly of acylpolyamine products of the type **A** (Scheme 1).

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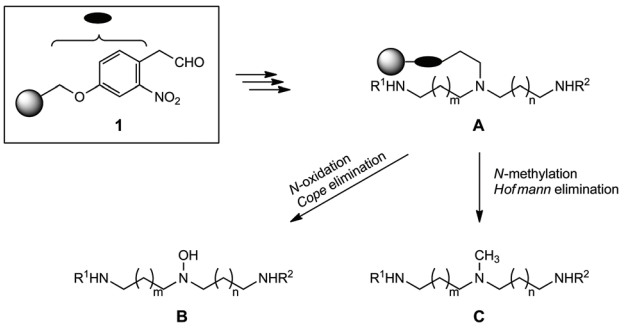
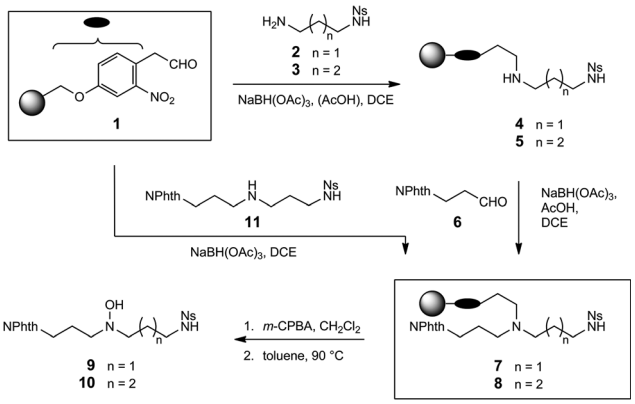
†Electronic supplementary information (ESI) available: Copies of the ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra of the final products **AG395a**, **AG432g**, 4-OH-Bz3(OH)433, **LF448A**, and **LF487A** and of the ^{1}H, ^{13}C, COSY, and HSQC spectra of the precursors **29**, **30**, **31**, **40**, and **41** as well as the comparison of the chromatographic and MS/MS behavior of the natural and synthetic toxins of *L. folium*. See DOI: 10.1039/c5ob00108k



Scheme 1 Use of resin **1** with a 2-(*ortho*-nitrophenyl)ethanal linker for the SPS preparation of *N*-hydroxylated and *N*-methylated secondary amines.



Scheme 2 Construction of the polyamine starter unit on the resin *versus* direct attachment of a secondary amine unit to the solid support.

By N-oxidation and subsequent Cope elimination or by N-methylation followed by Hofmann elimination, such precursor resins **A** can be cleaved to deliver *N*-hydroxylated products of the type **B** or *N*-methylated products of the type **C**. Resin **1** thus offers an alternative and efficient SPS tool for the divergent preparation of *N*-hydroxylated and *N*-methylated secondary amines, and herein, its application for the preparation of structurally related *N*-hydroxylated and *N*-methylated spider toxins is described.

Results and discussion

Synthesis of *N*-hydroxylated polyamine toxins

Compounds 4-OH-Bz3(OH)334 (**AG395a**), IndAc3(OH)334 (**AG432g**), and 4-OH-Bz3(OH)433, an isomer of **AG395a**, were chosen as the *N*-hydroxylated polyamine target structures (Fig. 2). The former two substances are proposed constituents of the venom of the spider *Agelenopsis aperta*,[33] for which we considered synthesis as appropriate to substantiate our structural assignments done by on-line coupled HPLC-UV(DAD)-MS and -MS/MS,[33] and the latter—due the underlying PA3433 polyamine framework, which is known for other compounds of the venom[34,35]—is suggested as a potential constituent of the same venom, which might have escaped detection and identification in our previous investigation.

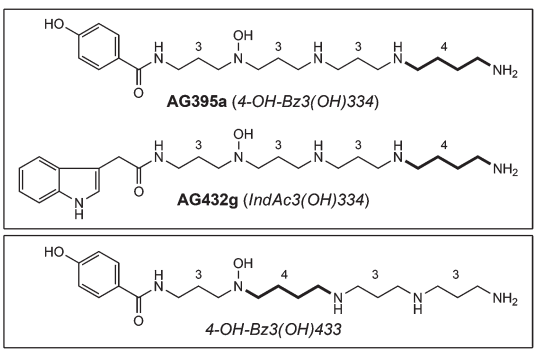


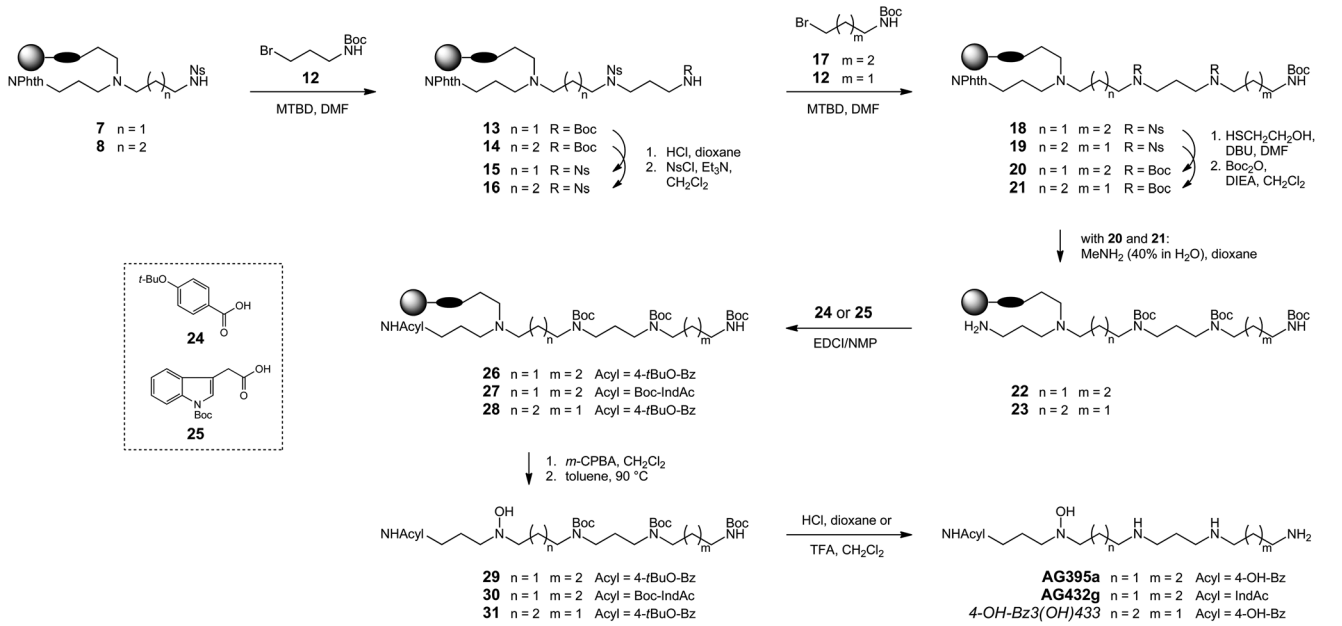
Fig. 2 Target structures of the *N*-hydroxylated polyamine toxin type. Toxins and analog of *Agelenopsis aperta*.

The three target structures are characterized by two different polyamine frameworks, PA3334 and PA3433 that had to be assembled separately. The respective starting resins **7** and **8** were prepared by reductive amination of aldehyde resin **1** with the two mono-nosyl-protected diamines **2** and **3**,[36] followed by reductive alkylation of the secondary amine resins **4** and **5** with phthalimido aldehyde **6** (Scheme 2). Liberation of the *N*-hydroxylated products **9** (32%) and **10** (71%) by N-oxidation and Cope elimination reveal synthetic success but also an inefficient formation of resin **7**. Competing reduction of the aldehyde function of resin **1** during the reductive aminations was considered to cause this problem, but the use of non-acidic conditions in the reductive amination of resin **1**[37] did not solve the problem. Thus, resin **7** was alternatively prepared by reductive amination of aldehyde resin **1** with bis-protected triamine derivative **11**.[38,39] Resin **7** obtained in this way delivered compound **9** in a respectable 88% yield upon oxidative cleavage.

The polyamine backbones PA3334 and PA3433 required for the target compounds were then assembled by N-alkylation of the nosyl amides in resins **7** and **8** with bromopropylamine derivative **12** (**7** → **13** and **8** → **14**),[20,24] exchange of the Boc by Ns protecting groups (**13** → **15** and **14** → **16**), and, finally, by alkylation of the terminal nosyl amides with the bromobutylamine and bromopropylamine derivatives **17** and **12**, respectively (**15** → **18** and **16** → **19**) (Scheme 3).

To avoid problems during the purification of the final products, the Ns groups of resins **18** and **19** were replaced at this stage with the tracelessly removable Boc groups. The phthal groups of the resulting resins **20** and **21** were then removed by transimidation, and the free amino groups of the resins **22** and **23** were acylated with the acid derivatives **24**[40] and **25**[41] to deliver the resins **26–28**, containing the complete frameworks of the target molecules.

N-oxidation of the resins **26–28**, followed by Cope elimination, liberated the ultimate toxin precursors **29–31** in 42%,



Scheme 3 Construction of the polyamine backbones of the target structures on the resin and completion of the syntheses of **AG395a**, **AG432g**, and analog 4-OH-Bz3(OH)433.

39%, and 21% yield, respectively, which correspond to average yields of 85–90% in the 8 or 9 steps performed on the solid supports. Removal of all protecting groups by acid treatment and purification of the already virtually clean products by preparative HPLC delivered **AG395a** and 4-OH-Bz3(OH)433 in 81% and 62%, and **AG432g** in 43% yield.

The lower yields of 4-OH-Bz3(OH)433 and **AG432g** are due to an undesired oxidation of higher extent that occurred during the preparative chromatography.[42] However, while it is known that indole derivatives are prone to get oxidized upon exposure to oxygen, it is not clear why 4-OH-Bz3(OH)433 decomposed to a greater extent than its isomeric counterpart **AG395a**.

Synthesis of *N*-methylated polyamine toxins

The two toxins **LF448A** and **LF487A** (Fig. 3) were found in the venom of *Larinioides folium*, and their structures were deduced on the basis of HPLC-UV(DAD)-MS/MS experiments combined with HR-MS, on column H/D exchange, and amino acid analysis.[43,44] They share the PA343 polyamine sub-unit with the toxin analog 4-OH-Bz3(OH)433 discussed above, and thus could be synthesized starting with the resin intermediate **14** already used above.

Exchange of the Ns group of resin **14** by the Boc group and subsequent removal of the phthal group of resin **32** afforded resin **33** with a free primary amine (Scheme 4). Condensation of this amine resin with *N*-protected asparagine derivative **34** (**33** → **35**), followed by selective removal of the Fmoc protecting group, provided resin **36**, which was acylated with acids **37** and **25**, respectively, to deliver resins **38** and **39** with the full toxin frameworks assembled.

The syntheses of the free toxins **LF448A** and **LF487A** were then completed in parallel. N-methylation and Hofmann elimination liberated the two fully protected tertiary amines **40** and **41** in 51% and 48% yields, which correspond to average yields of approximately 90% per step performed on the solid supports. Removal of the protecting groups[7,41,45] delivered finally the virtually pure compounds **LF448A** and **LF487A** in excellent 98% yields.

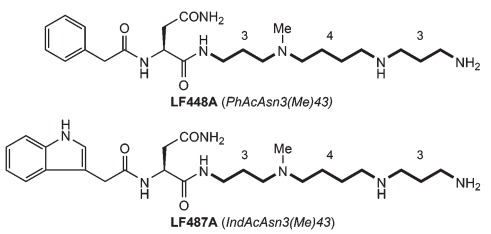
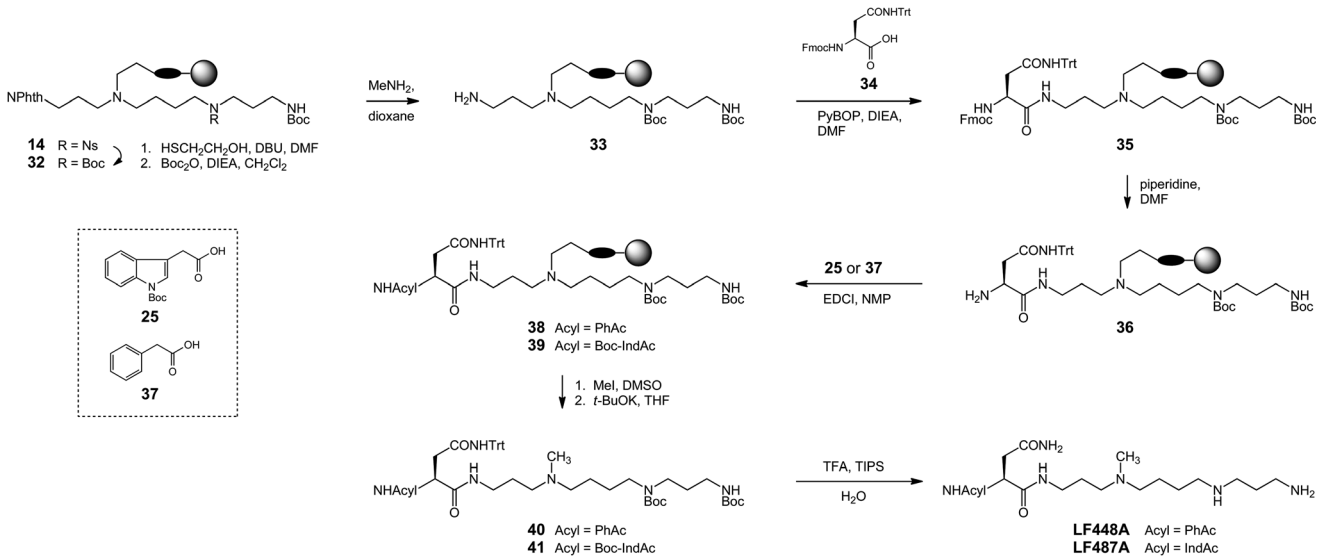


Fig. 3 Target structures of the *N*-methylated polyamine toxin type. Toxins of *Larinioides folium*.

Structural identity of the synthetic toxins and correlation with natural spider venoms

The synthetic *N*-hydroxylated polyamine toxins **AG395a**, **AG432g**, and analog 4-OH-Bz3(OH)433 as well as the *N*-methylated toxins **LF448A** and **LF487A** have been fully characterized by UV (on-line during UHPLC analysis), ^{1}H-NMR, ^{13}C-NMR (broadband decoupled and DEPT-90/DEPT-135), COSY, HSQC, HMBC, ESI-MS, and HRMS and HRMS/MS. In particular the data obtained from the 2D-NMR experiments allowed the com-



Scheme 4 Construction of the polyamine backbones of the target structures on the resin and completion of the syntheses of **LF448A** and **LF487A**.

plete assignment of all ^{1}H- and ^{13}C-signals and the verification of the given structures.

The synthetic samples were correlated with the venoms of *A. aperta* and *L. folium* by UHPLC-MS/MS. Fig. 4 shows the base peak chromatogram (BPC, chromatogram a) and the extracted ion chromatograms (EIC, chromatograms b) at *m/z* 396 (black) and at *m/z* 433 (red) of the native venom. The EIC of the synthetic compounds are shown in part c of Fig. 4: **AG395a** (black EIC at *m/z* 396), 4-OH-Bz3(OH)433 (green EIC at *m/z* 396), and **AG432g** (red EIC at *m/z* 433).

The comparison of the chromatograms of the native venom with those of the synthetic samples reveals that matching signals are found for **AG395a** (R_t = 4.18 min/4.23 min) and **AG432g** (R_t = 7.03 min/7.09 min), but not for 4-OH-Bz3(OH)433 (R_t = —/4.06 min). This already suggests that **AG395a** and **AG432g** might in fact be constituents of the venom of *A. aperta*, while compound 4-OH-Bz3(OH)433—isomeric to **AG395a**—is not.

This interpretation is supported by the MS/MS spectra acquired from the parent ions with *m/z* 396 and *m/z* 433, collected at the labelled peaks of the five EICs. The spectral comparison in Fig. 5 shows that the MS/MS of synthetic **AG395a** finds largely its match in the spectrum obtained from the natural sample. Not only all signals of spectrum b are found in spectrum a too, but also the complete signal pattern. Of particular relevance are the signals of the $\mathbf{t_1}$ and $\mathbf{a_3}$ ions at *m/z* 325 and *m/z* 308 that are diagnostic for a polyamine derivative with a terminal diaminobutane moiety (see fragmentation scheme

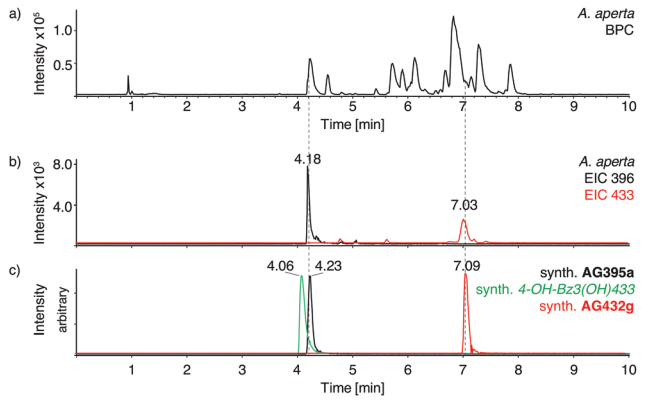


Fig. 4 Comparison of HPLC-MS: (a) base peak chromatogram (BPC) of native venom of *A. aperta*, (b) extracted ion chromatograms at *m/z* 396 and *m/z* 433 (EIC 396 and EIC 433) of native *A. aperta* venom, and (c) EIC 396 of synthetic **AG395a**, EIC 396 of synthetic 4-OH-Bz3(OH)433, and EIC 433 of synthetic **AG432g**.

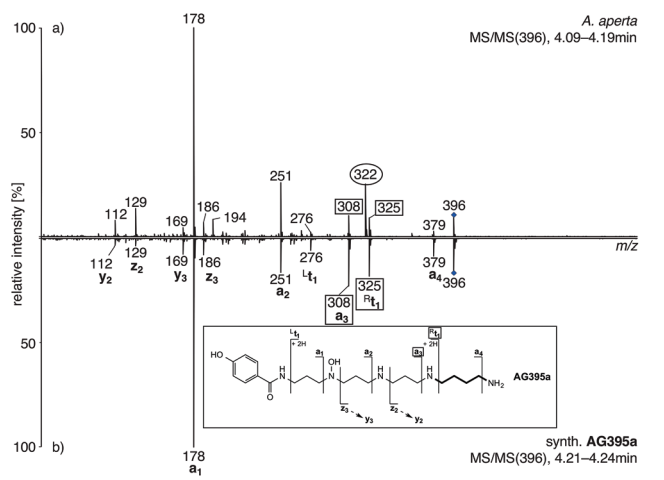


Fig. 5 Comparison of the MS/MS spectra (a) of the fraction of the native venom with precursor ions *m/z* 396 and (b) of synthetic **AG395a**.

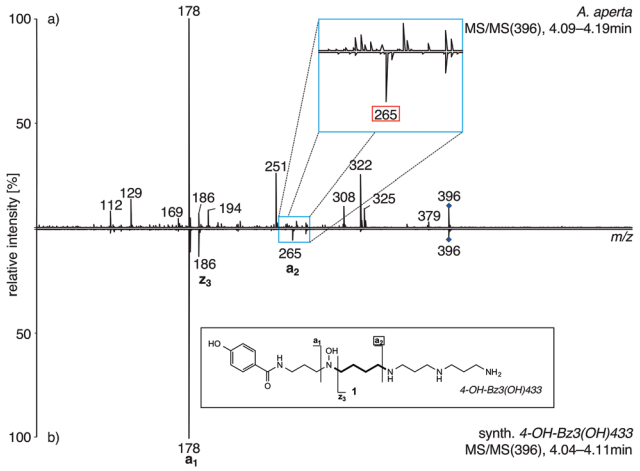


Fig. 6 Comparison of the MS/MS spectra (a) of the fraction of the native venom with precursor ions *m/z* 396 and (b) of synthetic analog 4-OH-Bz3(OH)433.

for **AG395a**; for the nomenclature of the fragment ions see ref. 46). The minor differences in the two spectra arise from co-eluting isomers of **AG395a** that were proposed already before.[33] The rather prominent signal at *m/z* 322, for instance, was assigned to the $\mathbf{a_3}$ ions deriving from **AG395a** (4-OH-Bz3-(OH)334).

The major signals of 4-OH-Bz3(OH)433 at *m/z* 178 and *m/z* 186 (spectrum b, Fig. 6) are not diagnostic because they are also expected for **AG395a** and other isomers. Thus, the rather weak signal at *m/z* 265 had to be considered to exclude the presence of 4-OH-Bz3(OH)433 in the natural venom. Despite its low intensity, it is indicative for 4-OH-Bz3(OH)433, and its absence in spectrum a, together with the different chromatographic behavior of the compound, implies the absence of 4-OH-Bz3(OH)433 in the venom of *A. aperta*.

Analogously to **AG395a**, the identities of **AG432g** as a component of *A. aperta* and of the two *N*-methylated compounds **LF448A** and **LF487A** as constituents of *L. folium* were ascertained. The respective spectral and chromatographic comparisons are found in the ESI.†

Conclusion

We have shown with the synthesis of the spider toxins **AG395a**, **AG432g**, **LF448A**, **LF487A**, and toxin analog 4-OH-Bz3(OH)433 that the new 2-(*ortho*-nitrophenyl)ethanal linker proposed recently can be used to efficiently synthesize rather complex *N*-hydroxylated and *N*-methylated polyamine derivatives in a divergent manner on the solid support. With the synthesized polyamine derivatives, the presence of **AG395a** and **AG432g** in the venom of *A. aperta* and of **LF448A** and **LF487A** in the venom of *L. folium*, as previously proposed, was confirmed. It was also shown that compound 4-OH-Bz3(OH)433, an isomer of **AG395a**, is not a constituent of the venom of *A. aperta* and was not just missed to be detected in previous investigations. This finding has its implication with regards to the biosynthesis of *N*-hydroxylated polyamine toxins: since polyamine toxins with the general structure Acyl3433 but none with the structure Acyl3(OH)433 are found in the venom of *A. aperta*, either the enzyme responsible for N-hydroxylation of acylpolyamines is selectively ignoring the Acyl34 partial structure or polyamines of the type PA3(OH)4... are not acylated at the PA3-(OH)4 end.

Experimental section

General

Unless otherwise stated, starting materials were purchased from commercial suppliers and used without further purification. Aldehyde resin **1** was synthesized according to ref. 32 from Merrifield Peptide Resin (Advanced ChemTech, 200–400 Mesh with 1% DVB, 0.8 mmol g^{-1} loading), and its loading was determined according to ref. 32. Lyophilized venom of the spiders *Agelenopsis aperta* and *Larinioides folium* was purchased from Spider Pharm. Inc. (Yarnell, AZ, USA). All reactions were carried out under an Ar atmosphere with dried apparati and in dry solvents (puriss. grade over molecular sieve sealed with a crown cap as purchased from Sigma-Aldrich). Solid-phase reactions: Advanced ChemTech PLS 6 synthesizer. Column chromatography: silica gel (pore size 60 Å, particle size 40–63 μm, 0.1% Ca) from Fluka with freshly distilled solvents of technical grade. The final products **AG395a**, **AG432g**, 4OH-Bz3(OH)433, **LF448A**, and **LF487A** were purified by preparative HPLC connected to a UV-Vis detector (detection at λ = 254 nm) and fraction collector on an Interchrom UP5HDO-250/212 column. For the solvent systems and gradients see the descriptions of the respective experiments. $[\alpha]_D$: Perkin-Elmer Polarimeter 241 MC; measured at 23°. UV-Vis (λ_{max} in nm): measured online during UHPLC-UV-(DAD)-MS (see below). IR spectra: Spectrum Two FT-IR Spectrometer (Perkin-Elmer) equipped with a Specac Golden Gate™ ATR (attenuated total reflection) accessory; applied as neat samples; $1/\lambda$ in cm^{-1} (for resins, only the diagnostic signals are reported). NMR spectra: in $CDCl_3$ on Bruker instruments at the given frequencies; chemical shifts δ in ppm relative to peaks of residual solvents ($CHCl_3$: ^1H: δ 7.26 ppm; ^{13}C: δ 77.16 ppm, HDO: ^1H: rel. to HDO δ 4.78 ppm; ^{13}C rel. to MeOH δ 49.5 ppm (which was added to the sample); coupling constants J in Hz; multiplicities of ^{13}C signals from DEPT-135 and DEPT-90 experiments; signal assignments based on COSY-, HSQC-, and HMBC-experiments. ESI-MS: Bruker ESQUIRE-LC quadrupole ion trap instrument (Bruker Daltonik GmbH) with a combined Hewlett-Packard Atmospheric Pressure Ion (API) source; continuous introduction of the sample solutions (0.1–1 μmol ml^{-1}) through the electrospray interface by using a syringe infusion pump at a flow rate of 5 μl min^{-1}; acquisitions in positive mode at normal resolution (0.6 u at half peak height) in the mass range from *m/z* 100–2000 with 8 scans averaged. HRMS and HRMS/MS: Bruker maXis quadrupole

time-of-flight instrument (Bruker Daltonik GmbH) with a combined Hewlett-Packard Atmospheric Pressure Ion (API) source; introduction of the sample solutions either continuously (10–100 nmol ml^{-1} at a flow rate of 3 μl min^{-1}) or online after separation by UHPLC (see below); acquisition in positive mode at 20 000 resolution (full width at half maximum) and 1.0 Hz spectra rate in the mass range from *m*/*z* 50 to 2000; calibration below 2 ppm accuracy between *m*/*z* 158 and 1450 with HCO_2NH_4; signals of intensities ≥5 rel% as well as molecular ions and characteristic fragments are reported with their *m*/*z* values (in mass units, u) and with their intensities in rel% in brackets. UHPLC-UV(DAD): Acquity BEH C18 HPLC column (1.7 μm, 2 × 100 mm); H_2O + 0.1% HCO_2H (A) and CH_3CN + 0.1% HCO_2H (B) solvent (0.3 ml min^{-1} flow, 1 min isocratic with 3% B, then linear gradient to 20% B within 10 min followed by flushing with 98% B for 3 min). UV spectra were recorded online between 190 and 500 nm at 1.2 nm resolution and 20 points s^{-1}.

Resin 4

Resin **1**[32] (500 mg, 142 μmol) was swelled in dry 1,2-dichloroethane (DCE, 5 ml). *N*-(3-Aminopropyl)-2-nitrobenzenesulfonamide[36] (**2**, 338 mg, 1.3 mmol) was added, and it was agitated at 23 °C for 1 h. Finely ground $NaBH(OAc)_3$ (321 mg, 1.51 mmol) was added, and it was agitated for 17 h before MeOH (5 ml) was added. After 5 min, the resin was filtered off, sequentially washed with MeOH, DMF–AcOH (100 : 1), DMF–Et_3N (10 : 1), CH_2Cl_2, and MeOH, and dried *in vacuo* to give resin **4** (batch A). Resin **4** (batch B) was also prepared by the reduction of the imine in the presence of AcOH (70 μl, 1.22 mmol) analogous to our previous report.[32] Chloranil-test[47] positive. IR: 1528, 1342, 1166.

Resin 5

Resin **1** (500 mg, 142 μmol) was swelled in dry DCE (5 ml). *N*-(4-Aminobutyl)-2-nitrobenzenesulfonamide[36] (**3**, 340 mg, 1.24 mmol) was added, and it was agitated at 23 °C for 1 h. Finely ground $NaBH(OAc)_3$ (300 mg, 1.42 mmol) was added, and it was shaken for 12 h before MeOH (5 ml) was added. After 5 min, the resin was filtered off, sequentially washed with MeOH, DMF–AcOH (100 : 1), DMF–Et_3N (10 : 1), CH_2Cl_2, CH_2Cl_2–MeOH (1 : 1), and MeOH, and dried *in vacuo* to deliver resin **5** (batch A). Resin **5** (batch B) was also prepared by the reduction of the imine in the presence of AcOH (70 μl, 1.22 mmol) analogous to our previous report.[32] Chloranil-test[47] positive. IR: 1528, 1345, 1166.

Resin 7[32]

A. By reductive alkylation of resins **4** *with aldehyde* **6**—Resin **4** (batch A or batch B, 142 μmol) was swelled in dry DCE (5 ml) and treated at 23 °C with 3-phthalimidopropanal[48] (**6**, 264 mg, 1.30 mmol). After 1 h, finely ground $NaBH(OAc)_3$ (317 mg, 1.50 mmol) was added, and it was agitated for 10 min. AcOH (70 μl, 1.22 mmol) was added, and it was shaken at 23 °C for 3 h. MeOH was added and, after 5 min, the resin was filtered off. It was washed sequentially with MeOH, DMF–AcOH (100 : 1), DMF–Et_3N (10 : 1), CH_2Cl_2, CH_2Cl_2–MeOH (1 : 1), and MeOH and dried *in vacuo* to deliver resin **7** (batch A or batch B, respectively).

B. By reductive amination of resin **1** *with secondary amine* **11** (for the preparation of **11**, see below)—Resin **1** (500 mg, 142 μmol) was swelled in dry DCE (5 ml) and treated at 23 °C with *N*-[7-(2-nitrobenzenesulfonylamido)-4-aminoheptyl]phthalimide (**11**, 570 mg, 1.28 mmol) for 1 h before finely ground $NaBH(OAc)_3$ (340 mg, 1.60 mmol) was added. It was agitated for 16 h. MeOH was added, and after 5 min, the resin was filtered off, sequentially washed with MeOH, DMF–AcOH (100 : 1), DMF–Et_3N (10 : 1), CH_2Cl_2, CH_2Cl_2–MeOH (1 : 1), and MeOH, and dried *in vacuo* to deliver resin **7** (batch C). Chloranil-test[47] negative. IR: 1770, 1712, 1528, 1343, 1167.

Resin 8[32]

Resin **5** (batch A or batch B, 142 μmol) was swelled in dry DCE (5 ml) and treated at 23 °C with 3-phthalimidopropanal[48] (**6**, 254 mg, 1.25 mmol). After 1 h, finely ground $NaBH(OAc)_3$ (310 mg, 1.46 mmol) was added, and it was agitated for 15 min. AcOH (70 μl, 1.22 mmol) was added, and it was shaken at 23 °C for 3 h. It was quenched with MeOH, and after 5 min, the resin was filtered off, sequentially washed with MeOH, DMF–AcOH (100 : 1), DMF–Et_3N (10 : 1), CH_2Cl_2, CH_2Cl_2–MeOH (1 : 1), and MeOH, and dried *in vacuo* to deliver resin **8** (batch A or batch B, respectively). Chloranil-test[47] negative. IR: 1771, 1712, 1528, 1347, 1167.

N-[4-Hydroxy-7-(2-nitrobenzenesulfonylamido)-4-azaheptyl]-phthalimide (9)

Resin **7** (142 μmol, batch C) was swelled in dry CH_2Cl_2 and cooled to 0 °C. *m*-CPBA (77%, 327 mg, *ca.* 1.45 mmol) was added, and it was agitated at 0 °C for 2.5 h. The resin was filtered off, sequentially washed with ice-cooled CH_2Cl_2, MeOH, CH_2Cl_2–MeOH (1 : 1), and CH_2Cl_2, and dried *in vacuo* for 30 min before it was again swelled in dry toluene (10 ml). It was heated to 90 °C for 2 h and then allowed to cool by interrupting the heating. When the temperature had fallen below 40 °C, the liquid was filtered off. The resin was washed additionally with toluene, CH_2Cl_2, and MeOH, and the filtrate and the rinsing solutions were combined. The volatiles were evaporated *in vacuo*, and column chromatography of the residue (CH_2Cl_2–MeOH 100 : 1) delivered **9** (58 mg, 125 μmol, 88%) as a colorless, amorphous solid. Batches A and B of resin **7** delivered product **9** in <45% (crude) and 32% (purified) yields, respectively. IR: 3464w (br.), 3320w (br.), 3096w, 2939w, 2873w, 1769w, 1705s, 1613w, 1593w, 1539m, 1439w, 1398m, 1363m, 1338m, 1269w, 1165m, 1125w, 1070w, 1032w, 892w, 853w, 784w, 721s, 655w, 588m, 530w. ^{1}H-NMR (400 MHz): 8.14–8.09 (m, 1 arom. H); 7.85–7.66 (m, 7 arom. H); 6.49 (br. s, 1 H); 3.79 (t, J = 6.9, PhthNCH_2); 3.25 (br. t, J = 5.7, NsNHCH_2); 2.71 (t, J = 5.9, NsNH(CH$_2$)$_2$CH_2); 2.67 (t, J = 6.7, PhthN(CH$_2$)$_2$CH_2); 1.96 (quint., J = 6.8, PhthNCH$_2$CH_2); 1.80 (br. s, NsNHCH$_2$CH_2). ^{13}C-NMR (100 MHz): 168.7 (s, 2 C=O); 148.1 (s); 134.08 (d, 2 C); 134.07 (s); 133.5 (d); 132.7 (d); 132.2 (s, 2 C); 131.1 (d); 125.2 (d); 123.4 (d, 2 C); 58.7 (t, NsHN(CH$_2$)$_2$–

CH_2); 58.0 (t, PhthN$(CH_2)_2CH_2$); 43.1 (t, NsHNCH_2); 35.8 (t, PhthNCH_2); 26.5 (t, NsHNCH_2CH_2); 26.3 (t, PhthNCH_2CH_2). ESI-MS: 485.1 (100, $[M + Na]^+$). HRMS (ESI-TOF): calcd for $C_{20}H_{22}N_4NaO_7S$ ($[M + Na]^+$): 485.11014; found 485.10996.

N-[4-Hydroxy-8-(2-nitrobenzenesulfonylamido)-4-azaoctyl]-phthalimide (10)

Analogous to the preparation of **9**, hydroxylamine **10** was cleaved from resin **8** (batch A, 142 μmol) by oxidation with *m*-CPBA (1.33 mmol) and heating to 90 °C in toluene. Column chromatography (CH_2Cl_2–MeOH 100 : 1.5) delivered **10** (50 mg, 105 μmol, 74%) as a colorless, amorphous solid. Resin **8** of batch B (142 μmol) gave the same product **10** (48 mg, 101 μmol, 71%). IR: 3465w (br.), 3315w (br.), 3095w, 2943w, 2870w, 1769w, 1706s, 1612w, 1593w, 1540m, 1440w, 1398m, 1363m, 1339m, 1166m, 1125w, 1076w, 1037w, 892w, 854w, 784w, 722m, 655w, 588m, 530w. ^{1}H-NMR (500 MHz): 8.15–8.11 (m, 1 arom. H); 7.84–7.80 (m, 3 arom. H); 7.75–7.68 (m, 4 arom. H); 6.00 (br. s, 1 H); 3.77 (t, J = 7.0, PhthNCH_2); 3.10 (br. t, J = 6.0, NsHNCH_2); 2.68 (t, J = 6.7, PhthN$(CH_2)_2CH_2$); 2.58 (br. t, J = 6.1, NsHN$(CH_2)_2CH_2$); 1.95 (quint., J = 6.8, PhthNCH_2CH_2); 1.58–1.55 (br. m, NsHNC$H_2CH_2CH_2$). ^{13}C-NMR (125 MHz): 168.7 (s, 2 C=O); 148.2 (s); 134.1 (d, 2 C); 134.0 (s); 133.5 (d); 132.8 (d); 132.2 (s, 2 C); 131.2 (d); 125.3 (d); 123.3 (d, 2 C); 60.1 (t, NsHN$(CH_2)_3CH_2$); 58.2 (t, PhthN$(CH_2)_2CH_2$); 43.8 (t, NsHNCH_2); 36.2 (t, PhthNCH_2); 27.7 (t); 26.2 (t, PhthNCH_2CH_2); 24.3 (t). ESI-MS: 477.1 (100, $[M + H]^+$); 499.1 (78, $[M + Na]^+$). HRMS (ESI-TOF): calcd for $C_{21}H_{24}N_4NaO_7S$ ($[M + Na]^+$): 499.12579; found 499.12615.

N-[7-(2-Nitrobenzenesulfonylamido)-4-aminoheptyl]-phthalimide (11)[38]

Norspermidine (80 ml, 0.57 mol) was dissolved in dry CH_2Cl_2 (250 ml) and the solution was cooled to 0 °C. A solution of NsCl (12.58 g, 56.7 mmol) in dry CH_2Cl_2 (200 ml) was added slowly over a period of 2 h. It was stirred for 10 min, the precipitate that was formed during the reaction was filtered off, and the filtrate was extracted with H_2O (1×). Aq. HCl (1 M) was added to the organic phase, and the formed precipitate was filtered off. The combined aqueous phases were alkalized by the addition of aq. NaOH (4 M) and subsequently extracted with CH_2Cl_2 (8×). The combined organic fractions were dried with $MgSO_4$, and the solvent was removed *in vacuo*, which delivered the terminally mono-Ns-protected triamine intermediate (12.09 g, 38.2 mmol, 67%) as an orange oil. A portion of this oil (9.40 g, 29.7 mmol) was dissolved in dry THF (40 ml), and a solution of *N*-carbethoxyphthalimide (6.65 g, 30.3 mmol) in dry THF (50 ml) was added over a period of 10 min at 23 °C. After it was stirred at 23 °C for 30 min, the solvent was evaporated, and the residue was purified twice by column chromatography (CH_2Cl_2–MeOH–NH_4OH (25% aq.), 100 : 10 : 1 and CH_2Cl_2–MeOH, 100 : 7) to deliver **11** (3.71 g, 8.3 mmol, 28%) as an orange oil. IR: 3317w (br.), 3066w (br.), 2947w, 2872w, 1770w, 1709s, 1617w, 1593w, 1541s, 1439m, 1397m, 1367m, 1339m, 1164m, 1126w, 1088w, 1038w, 853w, 780w, 724m, 654w, 586m. ^{1}H-NMR (300 MHz): 8.15–8.10 (m, 1 H); 7.87–7.66 (m, 7 arom. H); 3.77 (t, J = 6.8, 2 H); 3.23 (t, J = 6.1, 2 H); 2.71 (t, J = 5.8, 2 H); 2.61 (t, J = 6.8, 2 H); 1.90 (quint., J = 6.8, 2 H); 1.70 (quint., J = 6.0, 2 H). ^{13}C-NMR (75 MHz): 168.7 (s, 2 C=O); 148.2 (s); 134.1 (d, 2 C overlaying with s, 1 C); 133.3 (d); 132.6 (d); 132.2 (s, 2 C); 131.2 (d); 125.2 (d); 123.4 (d, 2 C); 48.5 (t); 46.6 (t); 43.8 (t); 35.7 (t); 28.7 (t); 28.4 (t). ESI-MS: 447.1 (100, $[M + H]^+$); 410.1 (19); 355 (31). HRMS (ESI-TOF): calcd for $C_{20}H_{23}N_4O_6S$ ($[M + H]^+$): 447.13328; found 4447.13365.

Resin 13

Resin **7** (batch C, 142 μmol) was swelled in dry DMF (5 ml), and the suspension was heated to 60 °C, MTBD (215 μl, 1.50 mmol) was added, followed by *tert*-butyl *N*-(3-bromopropyl)carbamate (**12**, 314 mg, 1.29 mmol). It was agitated for 22 h at 60 °C, and then allowed to cool to 23 °C. The resin was filtered off, washed sequentially with DMF, CH_2Cl_2, MeOH, and CH_2Cl_2, and dried *in vacuo* to give resin **13**. IR: 1770, 1712, 1527, 1364, 1246, 1163.

Resin 14

Analogous to the preparation of resin **13**, resin **8** (batch A, 142 μmol) was treated with MTBD (1.50 mmol) and *tert*-butyl *N*-(3-bromopropyl)carbamate (**12**, 1.29 mmol) to give resin **14**. IR: 1770, 1712, 1527, 1364, 1347, 1247, 1162.

Resin 15

Resin **13** (142 μmol) was treated with HCl in dioxane (4 M, 5 ml, 20 mmol) at 23 °C for 1.5 h. The resin was filtered off, washed sequentially with dioxane, $CHCl_3$, $CHCl_3$–Et_3N (10 : 1), $CHCl_3$, and CH_2Cl_2, and dried *in vacuo* before it was again swelled in dry CH_2Cl_2 (5 ml). Et_3N (0.25 ml, 1.80 mmol) was added and, after agitating for 10 min, NsCl (320 mg, 1.51 mmol). It was agitated for 2 h at 23 °C, the resin was filtered off, washed sequentially with MeOH, CH_2Cl_2–Et_3N (10 : 1), CH_2Cl_2, MeOH, MeOH–CH_2Cl_2 (1 : 1), and MeOH, and dried *in vacuo* to give resin **15**. Kaiser-test[49] negative. IR: 1769, 1712, 1540, 1533, 1347, 1164.

Resin 16

Analogous to the preparation of resin **15**, resin **14** (142 μmol) was treated with HCl in dioxane (4 M, 20 mmol), followed by the treatment of the resulting resin with Et_3N (5.0 mmol) and NsCl (2.6 mmol) to give resin **16**. Kaiser-test[49] negative. IR: 1770, 1712, 1540, 1347, 1164.

Resin 18

Analogous to the preparation of resin **13**, resin **15** (142 μmol) was treated with MTBD (1.50 mmol) and *tert*-butyl *N*-(4-bromobutyl)carbamate (**17**, 1.44 mmol) to give resin **18**. IR: 1770, 1711, 1544, 1527, 1344, 1248, 1160.

Resin 19

Analogous to the preparation of resin **13**, resin **16** (142 μmol) was treated with MTBD (1.50 mmol) and *tert*-butyl *N*-(3-bromopropyl)carbamate (**12**, 1.43 mmol) to give resin **19**. IR: 1771, 1712, 1542, 1527, 1363, 1348, 1247, 1160.

Resin 20

Resin **18** (142 μmol) was swelled in dry DMF (5 ml) and treated with DBU (1.5 ml, 10.0 mmol) and 2-thioethanol (0.35 ml, 5.0 mmol) at 23 °C. After it was agitated for 30 min, the resin was filtered off, sequentially washed with DMF, NMP (*N*-methyl-2-pyrrolidone), CH_2Cl_2, MeOH and CH_2Cl_2, and dried *in vacuo* for 10 min. This procedure was repeated until the filtrate of the reaction mixture was colorless, which is usually the case after two reaction cycles. Then, the resulting resin was swelled in dry CH_2Cl_2 (5 ml). Boc_2O (1.12 g, 5.14 mmol) and DIEA (200 μl, 1.11 mmol) were added, and it was agitated at 23 °C for 24 h. The resin was filtered off, washed sequentially with CH_2Cl_2, DMF, CH_2Cl_2, and MeOH, and dried *in vacuo* to give resin **20**. Chloranil-test[47] negative. IR: 1771, 1712, 1692, 1246, 1166.

Resin 21

Analogous to the preparation of resin **20**, resin **19** (142 μmol) was treated with DBU (10.0 mmol) and 2-thioethanol (5.0 mmol), followed by the treatment of the resulting resin with Boc_2O (4.72 mmol) and DIEA (1.15 mmol), to give resin **21**. Chloranil-test[47] negative. IR: 1772, 1712, 1692, 1254, 1154.

Resin 22

Resin **20** (142 μmol) was swelled in dioxane (5 ml), and the resulting suspension was heated to 60 °C. An aqueous solution of $MeNH_2$ (40% w/w, 1.7 ml) was added, and it was agitated at 60 °C for 40 h. It was allowed to cool to 23 °C, the resin was filtered off, sequentially washed with H_2O–dioxane (1 : 1), dioxane, DMF, CH_2Cl_2, MeOH, CH_2Cl_2–MeOH (1 : 1), and CH_2Cl_2, and dried *in vacuo* to give resin **22**. Kaiser-test[49] positive. IR: 1686, 1247, 1164.

Resin 23

Analogous to the preparation of resin **22**, resin **21** (142 μmol) was treated with an aqueous solution of $MeNH_2$ (40% w/w, 1 ml) to give resin **23**. Kaiser-test[49] positive. IR: 1689, 1247, 1156.

Resin 26

Resin **22** (142 μmol) was swelled in dry NMP (5 ml). 4-(*tert*-Butoxy)benzoic acid[40] (**24**, 489 mg, 2.52 mmol) and EDCI (587 mg, 3.06 mmol) were added, and it was agitated for 18 h at 23 °C. The resin was filtered off, sequentially washed with DMF, DMF–H_2O (1 : 1), DMF–Et_3N (10 : 1), CH_2Cl_2, and MeOH, and dried *in vacuo* to give resin **26**. Kaiser-test[49] negative. IR: 1691, 1245, 1160.

Resin 27

Analogous to the preparation of resin **26**, resin **22** (142 μmol) was treated with *N*-(*tert*-butoxycarbonyl)indolacetic acid[41] (**25**, 689 mg, 2.50 mmol) and EDCI (586 mg, 3.06 mmol) to give resin **27**. Kaiser-test[49] negative. FT-IR: 1728, 1686, 1527.

Resin 28

Analogous to the preparation of resin **26**, resin **23** (142 μmol) was treated with 4-(*tert*-butoxy)benzoic acid (**24**, 2.42 mmol) and EDCI (3.06 mmol) to give resin **28**. Kaiser-test[49] negative. IR: 1686, 1246, 1158.

***N*-[8,12-Bis(*tert*-butoxycarbonyl)-16-*tert*-butoxycarbonylamino-4-hydroxy-4,8,12-triazahexadecyl]-4-(*tert*-butoxy)benzamide (29)**

Analogous to the preparation of **9**, hydroxylamine **29** was cleaved from resin **26** (142 μmol) by oxidation with *m*-CPBA (1.49 mmol) and heating to 90 °C in toluene. The crude product was purified by chromatography (CH_2Cl_2–MeOH 100 : 6) to yield **29** (45 mg, 60 μmol, 42%) a slightly yellowish, amorphous solid. IR: 3444w (br.), 2975w, 2932w, 2873w, 1678s, 1606w, 1536w, 1499m, 1478m, 1418m, 1390w, 1365m, 1301m, 1248m, 1160s, 898w, 865w, 773w, 732m, 648w. ^{1}H-NMR (400 MHz): 7.71 (d-like m, J = *ca*. 8.6, 2 arom. H, *o* to CONH); 6.96 (d-like m, J = *ca*. 8.6, 2 arom. H, *m* to CONH); 4.72 (br. s, 1 H); 3.52 (q, J = 6.0, CONHCH_2); 3.28–3.22 (br. m, NOH$(CH_2)_2$CH_2NBoc); 3.17–3.06 (br. m, 4 CH_2NBoc); 2.81–2.76 (br. m, CONH$(CH_2)_2$CH_2NOH); 2.71–2.66 (br. m, NOHC$H_2(CH_2)_2$N-Boc); 1.92–1.89 (br. m, CONHCH$_2$CH_2); 1.86–1.82 (br. m, NOHCH$_2$CH_2CH$_2$NBoc); 1.73–1.69 (br. m, 2 H); 1.52–1.48 (br. m, 2 H); 1.44–1.38 (m, 3 $(H_3C)_3$C and CH_2); 1.34 (s, $(H_3C)_3$C). ^{13}C-NMR (100 MHz): 167.4 (s, CONH); 158.5 (br. s, arom. C, *p* to CONH); 156.2 (s, C=O of Boc); 155.8 (br. s, 2 C=O of 2 Boc); 129.3 (s, arom. C, i to CONH); 128.2 (d, 2 arom. C, *o* to CONH); 123.1 (d, 2 arom. C, *m* to CONH); 80.0–79.0 (4 br. overlaying s, 4 C, 4 $(H_3C)_3C$); 58.9 (t, CONH$(CH_2)_2$CH_2); 58.1 (br. t, NOHC$H_2(CH_2)_2$NBoc); 46.9 (br. t, CH_2NBoc); 45.7–44.8 (2 br. overlaying t, 3 CH_2NBoc); 40.3 (t, CH_2NBoc); 39.0 (br. t, ArCONHCH_2); 29.0 (q, $(H_3C)_3$COAr); 28.6, 28.5 (2 q, 9 C, Boc); 27.8 (br. t); 27.5 (t); 26.7–25.6 (br. t, 3 C). ESI-MS: 774.5 (100, $[M + Na]^+$). HRMS (ESI-TOF): calcd for $C_{39}H_{69}N_5NaO_9$ ($[M + Na]^+$): 774.49875; found 774.49871.

***N*-[8,12-Bis(*tert*-butoxycarbonyl)-16-*tert*-butoxycarbonylamino-4-hydroxy-4,8,12-triazahexadecyl](1-*tert*-butoxycarbonyl-1*H*-indol-3-yl)acetamide (30)**

Resin **27** (142 μmol) was swelled in dry CH_2Cl_2 (5 ml) and cooled to −20 °C. Then, *m*CPBA (77%, 203 mg, approx. 0.91 mmol) was added and it was agitated at −20 °C for 2 h. It was allowed to warm to 0 °C, the resin was filtered off, and washed at 0 °C with cooled solvents: CH_2Cl_2, MeOH, CH_2Cl_2–MeOH (1 : 1), and CH_2Cl_2. The resin was dried *in vacuo*. Cope elimination was performed analogous to the preparation of **9**, the crude product was purified by chromatography (CH_2Cl_2–MeOH, 100 : 5), and the fully protected *N*-OH acylpolyamine derivative **30** (46 mg, 55 μmol, 39%) was obtained as a slightly yellowish, amorphous solid. IR: 3337w (br.), 2975w, 2932w, 2873w, 1732m, 1686s, 1527w, 1476m, 1453m, 1419m, 1367s, 1305m, 1255m, 1160s, 1086w, 1016w, 861w, 766w, 749w. ^{1}H-NMR (400 MHz): 8.15 (d, J = 7.8, indole-C(4)H); 7.56 (s, indole-C(2)H); 7.53 (d, J = 7.6, indole-C(7)H); 7.34 (t, J = 7.8, indole-C(5)H); 7.25 (t, J = 7.4, indole-C(6)); 6.56, 6.30 (2 br. s,

1H); 4.71 (br. s, 1H); 3.65 (s, IndCH_2); 3.33–3.28 (br. m, CONHCH_2); 3.21–3.07 (br. s, BocNHCH_2(CH_2)$_2$CH_2NBocCH_2CH_2-CH_2NBocCH_2); 2.61, 2.55 (2 br. overlaying s, CH_2NOHCH_2); 1.72–1.67 (br. m overlaying with s, CONHCH_2CH_2CH$_2$NOHCH$_2$-CH_2CH$_2$NBocCH$_2$CH_2 and (H_3C)$_3$C); 1.53–1.43 (br. m overlaying with s, BocNHCH$_2$CH_2CH_2 and 3 (H_3C)$_3$C). ^{13}C-NMR (100 MHz): 170.3 (s, CH_2*C*O); 156.1, 155.7 (2s, 3 C=O of Boc); 149.6 (s, C=O of Boc); 135.7 (s, arom. C, *o* to CH_2CO); 130.0 (s, arom. C, i to NBoc); 125.0 (2 d, 2 arom. C, *o* and *p* to NBoc); 123.0 (d, arom. C, *m* to NBoc); 119.1 (d, arom. C, *o* to NBoc); 115.5 (d, arom. C, *m* to NBoc); 114.3 (s, arom. C, i to CH_2CO); 84.0, 79.9, 79.6, 79.2 (4s, 4× (H_3C)$_3$*C*); 58.5 (t, CONH(CH_2)$_2$*C*H$_2$); 57.9 (br. t, NOH*C*H$_2$(CH_2)$_2$NBoc); 46.9, 45.1, 40.3 (3 br. t, BocNH*C*H$_2$(CH_2)$_2$-*C*H$_2$NBoc*C*H$_2$CH$_2$*C*H$_2$NBoc*C*H$_2$); 38.5 (t, CONH*C*H$_2$); 33.3 (t, *C*H$_2$CONH); 28.6, 28.5, 28.3 (3 q, 4× (H_3*C*)$_3$C); 27.8 (br. t); 27.5 (t); 26.7 (br. t); 26.1 (br. t, 2C). ESI-MS: 1005.4 (9, [M + NaI + Na]$^+$); 855.5 (100, [M + Na]$^+$); 833.5 (10, [M + H]$^+$). HRMS (ESI-TOF): calcd for $C_{43}H_{72}N_6NaO_{10}$ ([M + Na]$^+$): 855.52021; found 855.52063.

N-[9,13-Bis(*tert*-butoxycarbonyl)-16-*tert*-butoxycarbonylamino-4-hydroxy-4,9,13-triazahexadecyl]-4-(*tert*-butoxy)benzamide (31)

Analogous to the preparation of **9**, hydroxylamine **31** was cleaved from resin **28** (142 μmol) by oxidation with *m*-CPBA (1.44 mmol) and heating to 90 °C in toluene. Chromatography (CH_2Cl_2–MeOH–NH_4OH (25% aq.) 100 : 6 : 0.6) delivered **31** (23 mg, 30 μmol, 21%) as a colorless oil. IR: 3342w (br.), 2975m, 2933w, 2873w, 1689s, 1606w, 1533w, 1500m, 1478m, 1419m, 1390w, 1366m, 1300m, 1250m, 1164s, 897w, 866w, 774w. ^{1}H-NMR (400 MHz): 7.73 (d-like m, *J* = *ca.* 8.6, 2 arom. H, *o* to CONH); 6.98 (d-like m, *J* = *ca.* 8.6, 2 arom. H, *m* to CONH); 5.28 (br. s, 0.5 H); 4.81 (br. s, 0.5 H); 3.55 (q, *J* = 6.0, CONHCH_2); 3.25–3.06 (3 br. overlaying m, 5 CH_2NBoc); 2.91–2.88 (br. m, CONH(CH_2)$_2$CH_2); 2.83–2.79 (br. m, NOHCH_2(CH_2)$_3$NBoc); 2.02–1.94 (br. m, CONHCH$_2$CH_2); 1.76–1.57 (m, 4 CH_2); 1.44, 1.424, 1.419 (3 overlaying s, 3 (H_3C)$_3$C); 1.36 (s, (H_3C)$_3$C). ^{13}C-NMR (100 MHz): 167.5 (s, CONH); 158.7 (br. s, arom. C, *p* to CONH); 156.2, 155.9, 155.6 (3 br. overlaying s, 3 (H_3C)$_3$*C*ON); 129.2 (s, arom. C, i to CONH); 128.2 (d, 2 arom. C, *o* to CONH); 123.3 (d, 2 arom. C, *m* to CONH); 80.0–79.0 (4 br. overlaying s, 4 (H_3C)$_3$*C*); 60.7 (br. t, NOH*C*H$_2$(CH_2)$_3$NBoc); 58.7 (br. t, CONH(CH_2)$_2$*C*H$_2$); 46.9 (br. t, *C*H$_2$NBoc); 45.4–43.7 (2 br. overlaying t, 3 *C*H$_2$NBoc); 38.7 (br. t, CONH*C*H$_2$); 37.8 (br. t, *C*H$_2$NBoc); 29.0 (q, (H_3*C*)$_3$COAr); 28.62, 28.59, 28.57 (3 overlaying q, 3 (H_3*C*)$_3$CON); 28.0–23.0 (4 br. overlaying t, 4 CH_2). ESI-MS: 924.4 (11, [M + NaI + Na]$^+$); 774.5 (100, [M + Na]$^+$); 752.5 (16, [M + H]$^+$); 617.4 (14, [M − $C_8H_{15}NO_2$ + Na]$^+$). HRMS (ESI-TOF): calcd for $C_{39}H_{69}N_5NaO_9$ ([M + Na]$^+$): 774.49875; found 774.49817.

N-(16-Amino-4-hydroxy-4,8,12-triazahexadecyl)-4-hydroxybenzamide (AG395a, 4-OH-Bz3(OH)334)

To **29** (10.5 mg, 14.0 μmol) under an Ar atmosphere, HCl (4 M in dioxane, 4 ml, 16 mmol) was added, and it was stirred for 15 min at 23 °C. The volatiles were removed *in vacuo* at 23 °C, the residue was dissolved in degassed H_2O (2 ml), and subsequently purified by prep. HPLC yielded *N*-OH acylpolyamine **AG395a**·2.6HCO_2H (5.8 mg, 11.3 μmol, 81%) as a colorless, highly hygroscopic solid. UHPLC: R_t = 4.23 min. UV(DAD) (H_2O): λ_{max} 199, 251. ^{1}H-NMR (D_2O + 0.5 μl MeOH, 400 MHz): 8.48 (s, 2.6 H, *H*CO_2H); 7.71 (d-like m, *J* = 8.7, 2 H, arom., *o* to CONH); 6.98 (d-like m, *J* = 8.7, 2 H, arom., *m* to CONH); 3.46 (t, *J* = 6.9, ArCONHCH_2); 3.18–3.10 (m, H_2N(CH_2)$_3$CH_2NHCH_2CH$_2$-CH_2NHCH_2); 3.05 (t, *J* = 7.1, H_2NCH_2); 2.85 (q-like m, *J* = *ca.* 7.2, CH_2NOHCH_2); 2.16–2.08 (m, NHCH$_2$CH_2CH$_2$NH); 2.00 (quint., *J* = 7.1, NOHCH$_2$CH_2CH$_2$NH); 1.91 (quint., *J* = 7.0, ArCONHCH$_2$CH_2); 1.81–1.73 (m, H_2NCH$_2$CH_2CH_2). ^{13}C-NMR (D_2O + 0.5 μl MeOH, 100 MHz): 171.7 (d, 2.6 C, HCO_2H); 171.0 (s, CONH); 159.9 (s, arom. C, *p* to CONH); 129.9 (d, 2 arom. C, *o* to CONH); 126.1 (s arom. C, i to CONH); 116.1 (d, 2 arom. C, *m* to CONH); 58.4 (t, ArCONH(CH)$_2$CH_2); 57.7 (t, ArCONH-(CH_2)$_3$NOH*C*H$_2$); 47.7 (t, NH*C*H$_2$(CH_2)$_3$NH$_2$); 47.0 (t, NOH-(CH_2)$_2$*C*H$_2$NH); 45.1, 45.0 (2 t, NH*C*H$_2$CH$_2$*C*H$_2$NH); 39.4 (t, *C*H$_2$NH$_2$); 38.3 (t, ArCONH*C*H$_2$); 26.7 (t, ArCONHCH$_2$*C*H$_2$); 24.5 (t, CH$_2$*C*H$_2$NH$_2$); 23.7 (t, NOHCH$_2$*C*H$_2$CH$_2$NH); 23.38 (t, *C*H$_2$CH$_2$NH$_2$); 23.35 (t, NHCH$_2$*C*H$_2$CH$_2$NH). ESI-MS: 418.3 (8, [M + Na]$^+$); 396.3 (100, [M + H]$^+$). HRMS (ESI-TOF): calcd for $C_{20}H_{38}N_5O_3$ ([M + H]$^+$): 396.29692; found 396.29707.

N-(16-Amino-4-hydroxyl-4,8,12-triazahexadecyl)-(1*H*-indol-3-yl)-acetamide (AG432g, IndAc3(OH)334)

A solution of **30** (6.1 mg, 7.3 μmol) in dry CH_2Cl_2 (2.5 ml) was added to TFA (6 ml, 78.4 mmol) under an Ar atmosphere over a period of 10 min at 23 °C. It was stirred for 1 h, and the volatiles were removed *in vacuo*. The residue was triturated with dry CH_2Cl_2, filtered, and washed with dry CH_2Cl_2. The solid was dissolved in degassed H_2O (2 ml), stirred for 4 h at 23 °C, and then purified by HPLC (grad. 3 to 15% B in 8 min, 15 to 100% B in 7 min, solvent A: H_2O + 0.1% HCOOH, solvent B: MeCN + 0.1% HCOOH, flow 20 ml min^{-1}), which delivered **AG432g**·1.3HCOOH (1.55 mg, 3.15 μmol, 43%) as a colorless solid. Purity: 96% (UHPLC, 220 nm). UV(DAD) (H_2O): λ_{max} 193, 216, 219, 279, 287. UHPLC: R_t = 5.71 min (2.1 × 100 mm BEHC18, linear 1% B for 3 min, grad. 3 to 100% B in 15 min, solvent A: H_2O + 0.1% HCOOH, solvent B: MeCN + 0.1% HCOOH, flow 0.3 ml min^{-1}, detection at λ = 220 nm). ^{1}H-NMR (D_2O, 600 MHz): 8.49 (s, 1.3H, *H*COOH); 7.66 (m, indole-C(4)H); 7.57 (m, indole-C(7)H); 7.37 (s, indole-C(2)H); 7.31 (m, indole-C(6)H); 7.22 (m, indole-C(5)H); 3.78 (s, IndCH_2); 3.27 (br. t, *J* = 6.2, CONHCH_2); 3.12–3.05 (m, H_2NCH_2(CH_2)$_2$CH_2NHCH_2-CH$_2$CH_2NH); 3.03 (t, *J* = 7.5, NOH(CH_2)$_2$CH_2NH); 2.65 (t, *J* = 6.7, NOHCH_2(CH_2)$_2$NH); 2.62 (t, CONH(CH_2)$_2$CH_2); 2.11–2.05 (m, NHCH$_2$CH_2CH$_2$NH); 1.89 (quint., *J* = 7.0, NOHCH$_2$CH_2-CH$_2$NH); 1.80–1.72 (m, H_2NCH$_2$(C*H*)$_2$CH$_2$NH and CONHCH$_2$-CH_2CH$_2$NOH). ^{13}C-NMR (D_2O, 150 MHz): 175.9 (s, CONH); 171.7 (d, HCOOH); 136.9 (s, indole-C(3)); 127.2 (s, indole-C(3a)); 125.7 (d, indole-C(2)); 122.7 (d, indole-C(6)); 120.2 (d, indole-C(5)); 119.0 (d, indole-C(4)); 112.6 (d, indole-C(7)); 108.5 (s, indole-C(7a)); 58.0 (t, CONH(CH_2)$_2$CH_2); 57.7 (t, NOHCH_2-(CH_2)$_2$NH); 47.7 (t); 47.1 (t, NOH(CH_2)$_2$*C*H$_2$NH); 45.1 (t); 39.4 (t); 37.7 (t, CONH*C*H$_2$); 33.2 (t, Ind*C*H$_2$); 26.4 (t, CONHCH$_2$*C*H$_2$); 24.6 (t); 23.6 (t, NOHCH$_2$*C*H$_2$CH$_2$NH); 23.5 (t, 2C). ESI-MS: 433.3

(9, $[M + H]^+$); 217.2 (100, $[M + 2H]^{2+}$); 208.7 (16, $[M - NH_3 + 2H]^{2+}$). HRMS (ESI-TOF): calcd for $C_{23}H_{41}N_6O_2$ ($[M + H]^+$): 433.32855; found 433.32857.

N-(16-Amino-4-hydroxy-4,9,13-triazahexadecyl)-4-hydroxybenzamide (4-OH-Bz3(OH)433)

Analogous to the preparation of **AG395a**, **31** (6.0 mg, 8.0 μmol) was treated with HCl (4 M in dioxane, 20 mmol). Purification of the crude mixture by preparative HPLC gave *N*-OH acylpolyamine 4-OH-Bz3(OH)433·2.6HCO_2H (2.55 mg, 5.0 μmol, 62%) as a colorless, highly hygroscopic solid. UHPLC: R_t = 4.06 min. UV(DAD) (H_2O): λ_{max} 199, 251. ^{1}H-NMR (D_2O + 0.5 μl MeOH, 400 MHz): 8.48 (s, 2.6 H, HCO_2H); 7.74–7.70 (d-like m, J = 8.7, 2 arom. H, *o* to CONH); 7.01–6.97 (d-like m, J = 8.7, 2 arom. H, *m* to CONH); 3.46 (t, J = 6.9, ArCONHCH_2); 3.17–3.08 (m, 4 NHCH_2 and CH_2NH_2); 2.86–2.80 (m, CH_2NOHCH_2); 2.14–2.05 (m, 2 NHCH_2CH_2CH_2NH); 1.96–1.89 (m, ArCONHCH_2CH_2); 1.79–1.67 (m, NOHCH_2CH_2CH_2CH_2NH). ^{13}C-NMR (D_2O + 0.5 μl MeOH, 100 MHz): 171.7 (d, 2.6 C, HCO_2H); 171.0 (s, CONH); 160.0 (s, arom. C, *p* to CONH); 129.9 (d, 2 arom. C, *o* to CONH); 126.1 (s, arom. C, i to COHN); 116.1 (d, 2 arom. C, *m* to CONH); 59.8 (t, NOH$CH_2(CH_2)_3$NH); 58.2 (t, ArCONH-$(CH_2)_2CH_2$); 48.1 (t, NOH$(CH_2)_3CH_2$NH); 45.4, 45.3, 45.0 (3 t, 3 NCH_2); 38.4 (t, ArCONHCH_2); 37.2 (t, NCH_2); 26.6 (t, ArCONHCH$_2CH_2$); 24.6 (t, NHCH$_2CH_2$CH$_2$NH); 24.1 (t, NOH-$(CH_2)_2CH_2$CH$_2$NH); 23.7 (t, NOHCH$_2CH_2(CH_2)_2$NH); 23.5 (t, NHCH$_2CH_2$CH$_2$NH). ESI-MS: 418.3 (15, $[M + Na]^+$); 396.3 (100, $[M + H]^+$); 276.3 (15); 178.1 (14); 145.1 (15); 128.1 (22); 121.0 (92). HRMS (ESI-TOF): calcd for $C_{20}H_{38}N_5O_3$ ($[M + H]^+$): 396.29692; found 396.29676.

Resin 32

Resin **14** (142 μmol) was swelled in dry DMF (5 ml). DBU (1 ml, 6.69 mmol) and 2-thioethanol (0.2 ml, 2.85 mmol) were subsequently added. It was agitated at 23 °C for 30 min. The resin was filtered off, sequentially washed with DMF, NMP, CH_2Cl_2, MeOH, and CH_2Cl_2, and dried *in vacuo* at 40 °C for 10 min. This procedure was repeated until the reaction filtrate was colorless, which is usually the case after two reaction cycles. The resin (Chloranil-test[47] positive; IR: 1771, 1712, 1528) was swelled in dry CH_2Cl_2 (5 ml), and then Boc_2O (746 mg, 3.42 mmol) and DIEA (0.2 ml, 1.15 mmol) were added, and it was agitated at 23 °C for 24 h. The resin was filtered off, sequentially washed with CH_2Cl_2, DMF, CH_2Cl_2, and MeOH (3×), and dried *in vacuo* to give resin **32**. Chloranil-test[47] negative. IR: 1769, 1712, 1528.

Resin 33

Resin **32** (142 μmol) was swelled in dioxane (5 ml). It was heated to 60 °C, an aqueous solution of $MeNH_2$ (40% w/w, 1.5 ml) was added, and it was agitated for 40 h at 60 °C. It was allowed to cool to 23 °C, and the resin was filtered off, sequentially washed with H_2O–dioxane (1 : 1), dioxane, DMF, CH_2Cl_2, MeOH, CH_2Cl_2–MeOH (1 : 1), and CH_2Cl_2, and dried *in vacuo* to give resin **33**. Kaiser-test[49] positive. IR: 1692, 1528.

Resin 35

Fmoc-Asn(Trt)-OH (**34**, 753 mg, 1.26 mmol) was dissolved in dry DMF (3 ml), and a solution of PyBOP (655 mg, 1.26 mmol) in dry DMF (2 ml) and DIEA (0.22 ml, 1.25 mmol) were added, and it was stirred for 5 min at 23 °C. This solution was added to resin **33** (142 μmol) that had previously been swelled in dry DMF (3 ml) for 20 min. After agitation for 2 h at 23 °C, the resin was filtered off, sequentially washed with DMF, MeOH, and CH_2Cl_2, and dried *in vacuo* to give resin **35**. Kaiser-test[49] negative. IR: 1683, 1528, 843.

Resin 36

Resin **35** (142 μmol) was washed with DMF (2×), a solution of piperidine in DMF (5 ml, 1 : 4) was added, and it was agitated for 5 min at 23 °C. The resin was filtered off, sequentially washed with DMF, and treated again with piperidine–DMF (5 ml, 1 : 4) for 10 min. The resin was filtered off, washed with DMF, MeOH, CH_2Cl_2, MeOH, and CH_2Cl_2, and dried *in vacuo* to give resin **36**. Kaiser-test[49] positive. IR: 1684, 1527.

Resin 38

Resin **36** (142 μmol) was swelled in dry NMP (5 ml). Phenylacetic acid (**37**, 353 mg 2.59 mmol) and EDCI (619 mg, 3.23 mmol) were added, and it was agitated at 23 °C for 19 h. The resin was filtered off, sequentially washed with DMF, DMF–NEt_3 (10 : 1), DMF, MeOH, CH_2Cl_2, and MeOH, and dried *in vacuo* to give resin **38**. Kaiser-test[49] negative. IR: 1683, 1527.

Resin 39

Analogous to the preparation of resin **38**, resin **36** (142 μmol) was treated with *N*-Boc-indolacetic acid[41] (**25**, 705 mg 2.56 mmol) and EDCI (596 mg, 3.11 mmol) to give resin **39**. Kaiser-test[49] negative. IR: 1684, 1527.

(*S*)-*N*-{12-*tert*-Butoxycarbonyl-15-*tert*-butoxycarbonylamino-7-methyl-1-[*N*-(triphenylmethylcarbamoyl)methyl]-3,7,12-triaza-2-oxopentadecyl}phenylacetamide (40)

Resin **38** (142 μmol) was swelled in dry DMSO (5 ml), MeI (0.5 ml, 8.00 mmol) was added, and it was agitated at 23 °C for 21 h. The resin was filtered off, sequentially washed with DMF, DMF–MeOH (1 : 1), MeOH, CH_2Cl_2, MeOH, CH_2Cl_2–MeOH (1 : 1), and CH_2Cl_2, and dried *in vacuo*. The resin was swelled in dry THF (5 ml), *t*-BuOK (1 M in *t*-BuOH, 0.5 ml, 0.50 mmol) was added, and it was agitated for 15 min. An aqueous solution of HCO_2NH_4 (10 M, 0.15 ml), THF (5 ml), and MeOH (5 ml) were added, and it was agitated for 5 min at 23 °C. The resin was filtered off and subsequently rinsed with THF, CH_2Cl_2, and MeOH. The filtrate and the rinsing solutions were combined, and the volatiles were removed *in vacuo*. Chromatography (CH_2Cl_2–MeOH–NH_4OH (25% aq.) 100 : 6 : 0.6) delivered **40** (65 mg, 73 μmol, 51%) as a colorless oil. $[\alpha]_D^{26}$ = +12.1 (c 0.56 in $CHCl_3$). IR: 3300w (br.), 3058w, 3030w, 2973w, 2935w, 2871w, 2795w, 1668s, 1520s, 1495s, 1449m, 1419m, 1389m, 1365m, 1250m, 1169s, 1073w, 1035w, 917w, 766m,

731m, 700s, 638m, 572w. ^{1}H-NMR (400 MHz, 315 K): 7.29–7.15 (m, 20 H, Trt and Ph); 7.03 (br. s, 1 H); 4.66 (*A* of *AMX*, *J* = 11.0, 6.4, PhCH$_2$CONHC*H*); 3.56 (s, PhCH_2); 3.28–3.03 (m, *M* of *AMX* and 3 × N(Boc)CH_2 and AsnCONHCH_2); 2.53 (*X* of *AMX*, *J* = 15.3, 5.8, TrtNHCOC*H*) partly overlaying with 2.38 (br. s, CH_2NMeCH_2); 2.21 (s, CH_3), 1.67–1.57 (m, 4 H); 1.45, 1.43 (2s, 2 × $(H_3C)_3$C overlaying with br. s, 4 H). ^{13}C-NMR (100 MHz, 315 K): 171.4 (s, PhCH$_2$*C*O); 170.8 (s, TrtNH*C*O); 170.3 (s, *C*ONHCH$_2$); 156.0 (s); 144.4 (s, 3 C); 134.9 (s); 129.2 (d, 2 C); 128.7 (d, 8 C); 127.9 (d, 6 C); 127.0 (d, 4 C); 79.6, 79.0 (2s, 2 × $(H_3C)_3C$); 70.8 (s, Ph$_3$*C*); 56.6, 55.4 (2 t, *C*H$_2$NMe*C*H$_2$); 50.1 (d, PhCH$_2$CONH*C*H); 46.7 (t); 44.1 (t); 43.6 (t, Ph*C*H$_2$); 41.0 (q, H_3C); 38.4 (t); 37.5 (t, 2 C); 28.45 (t), 28.43 (q, 2× $(H_3C)_3$C); 25.8 (t, 2 C); 23.0 (t). ESI-MS: 913.5 (47, [M + Na]$^+$); 891.5 (100, [M + H]$^+$); 357.2 (26); 535.4 (14); 468.3 (13); 401.2 (11). HRMS: calcd for $C_{52}H_{71}N_6O_7$ ([M + H]$^+$): 891.53788; found 891.53890.

(*S*)-*N*-{12-*tert*-Butoxycarbonyl-15-*tert*-butoxycarbonylamino-7-methyl-1-[*N*-(triphenylmethylcarbamoyl)methyl]-3,7,12-triaza-2-oxopentadecyl}-(1*H*-indol-3-yl)acetamide (41)

Analogous to the preparation of **40**, **41** was cleaved from resin **39** (142 μmol) by methylation with MeI (0.5 ml, 8.00 mmol) and treatment with *t*-BuOK (1 M in *t*-BuOH, 0.5 ml, 0.50 mmol). Chromatography (CH_2Cl_2–MeOH–NH_4OH (25% aq.) 100 : 8 : 0.8) delivered **41** (70 mg, 68 μmol, 48%) as a colorless oil. $[\alpha]_D^{26}$ = −5.3 (*c* 1.1 in $CHCl_3$). IR: 3289w (br.), 3057w, 2974w, 2934w, 2793w, 1729m, 1682s, 1646s, 1525m, 1451m, 1417m, 1367s, 1304m, 1254s, 1225m, 1158s, 1084m, 1019m, 860w, 766m, 745m, 700m, 630w, 569w. ^{1}H-NMR (500 MHz, 313 K): 8.14 (br. d, *J* = 7.7, 1 H, indole); 7.53 (s, 1 H, indole-C(2)H); 7.49 (d, *J* = 7.8, 1 H, indole); 7.37.33–7.16 (m, 17 arom. H); 7.01 (br. s, 1 H); 3.62 (s, IndCH_2); 3.22–3.00 (br. m, 9 H, CONHCH_2 and BocNHCH_2CH$_2$CH_2NBocCH_2 and TrtNH-COC*H*); 2.50–2.46 (br. m, TrtNHCOC*H*); 2.28 (br. s, CH_2N-MeCH_2); 2.12 (s, NMe); 1.66–1.63 (m, 4 H) overlaying with 1.65 (s, $(H_3C)_3$C); 1.46–1.40 (m, 4 H) overlaying with 1.45 (s, $(H_3C)_3$C and 1.43 (s, $(H_3C)_3$C). ^{13}C-NMR (125 MHz, 313 K): 170.7, 170.63, 170.55 (3s, 3 × CONH); 156.2 (br. s, 2 × C═O of Boc); 149.6 (s, C═O of Boc); 144.5 (s, 3 C); 135.8 (s); 130.0 (s); 128.8 (d, 6 C); 128.1 (d, 6 C); 127.2 (d, 3 C); 124.9 (d); 124.8 (d); 122.9 (d); 119.0 (d); 115.6 (d); 113.7 (s); 83.9 (s, $(H_3C)_3C$); 79.6, 79.1 (2 br. s, 2 × $(H_3C)_3C$); 71.0 (s, Ph$_3$*C*); 57.5, 55.3 (2 br. t, *C*H$_2$NMe*C*H$_2$); 50.2 (d, IndAcNH*C*H); 47.0 (br. t, BocN*C*H$_2$); 44.0 (br. t, BocN*C*H$_2$); 41.9 (br. q, MeN); 38.5 (t, 2 C, TrtHNCOCH_2 and BocN*C*H$_2$); 37.7 (br. t, BocN*C*H$_2$); 33.5 (t, Ind*C*H$_2$); 29.0 (br. t); 28.63, 28.61, 28.4 (3 q, 3× $(H_3C)_3$C); 26.7 (br. t, 2 C); 24.5 (br. t). ESI-MS: 1030.6 (100, [M + H]$^+$); 1052.6 (9, [M + Na]$^+$). HRMS: calcd for $C_{59}H_{80}N_7O_9$ ([M + H]$^+$): 1030.60120; found 1030.60050.

(*S*)-*N*-[15-Amino-1-(carbamoylmethyl)-7-methyl-3,7,12-triaza-2-oxopentadecyl]phenylacetamide (LF448A, PhAcAsn3(Me)43)

To fully protected acylpolyamine **40** (8.9 mg, 10.0 μmol) in dry CH_2Cl_2 (0.5 ml), a solution of TFA, TIPS and H_2O (10 ml, 95 : 2.5 : 2.5) was added. It was stirred at 23 °C for 1 h, the volatiles were evaporated, the residue was dissolved in H_2O (15 ml), and the mixture was lyophilized. Preparative HPLC (gradient from 3% to 10% B in 15 min, then to 100% B in 10 min, solvent A: H_2O + 0.1% HCOOH, solvent B: MeCN + 0.1% HCOOH, flow 20 ml min^{-1}) delivered **LF448A**·1.3HCOOH (4.9 mg, 9.7 μmol, 98%) as a colorless, highly hygroscopic solid. Purity: 97% (UHPLC, 220 nm). UHPLC: R_t = 5.27 min (2.1 × 100 mm BEHC18, linear 1% B for 3 min, then gradient from 35 to 100% B in 15 min, solvent A: H_2O + 0.1% HCO_2H, solvent B: MeCN + 0.1% HCOOH, flow 0.3 ml min^{-1}, detection at λ = 220 nm). $[\alpha]_D^{25}$ = −8.9 (*c* 0.14 in 0.1 M aq. HCOOH). UV (DAD) (H_2O): λ_{max} 197, 256. ^{1}H-NMR (D_2O + 1 μl MeOH, 500 MHz): 8.48 (s, 1.3 H, *H*COOH); 7.47–7.35 (m, 5 H, Ph); 4.61 (*X* of *ABX*, J_{AX} = 6.4, J_{BX} = 7.5, PhCH$_2$CONHC*H*, partly overlaying with HDO-peak); 3.69 (s, PhCH_2); 3.32 (t, *J* = 6.4, AsnNHCH_2); 3.16–3.05 (m, CH_2NMeCH_2(CH$_2$)$_2$–CH_2NHCH_2CH$_2$CH_2NH$_2$); 2.83, 2.76 (*AB* of *ABX*, J_{AB} = 15.5, J_{AX} = 6.4, J_{BX} = 7.5, CH_2CONH$_2$) overlaying with 2.79 (s, NMe); 2.13–2.06 (m, CH_2CH$_2$NH$_2$); 1.94–1.88 (m, CONHCH$_2$CH_2); 1.81–1.72 (m, NMeCH$_2$CH_2CH_2CH$_2$NH). ^{13}C-NMR (D_2O + 1 μl MeOH, 125 MHz): 175.3 (s, PhCH$_2$*C*O); 175.0 (s, H$_2$N*C*O); 173.7 (s, PhCH$_2$CH(R)*C*ONH); 171.6 (s, 1.3 C, HCOOH); 135.4 (s, arom., i to CH$_2$CONH); 129.8 (d, 2 arom. C, *o* to CH$_2$CONH); 129.6 (d, 2 arom. C, *m* to CH$_2$CONH); 128.0 (d, arom. C, *p* to CH$_2$CONH); 55.8 (t, NMe*C*H$_2$(CH$_2$)$_3$NH); 54.0 (t, NH(CH$_2$)$_2$*C*H$_2$NMe); 51.8 (d, PhCH$_2$CONH*C*H); 47.6 (t, NMe(CH$_2$)$_3$*C*H$_2$NH); 45.2 (t, NH*C*H$_2$(CH$_2$)$_2$NH$_2$); 42.6 (t, Ph*C*H$_2$); 40.2 (q, NMe); 37.19 (t, CH$_2$NH$_2$); 36.68 (t, CONH*C*H$_2$); 36.66 (t, *C*H$_2$CONH$_2$); 24.5 (t, NHCH$_2$*C*H$_2$CH$_2$NH$_2$); 24.3 (t, CONHCH$_2$*C*H$_2$); 23.4 (t, NMe(CH$_2$)$_2$*C*H$_2$CH$_2$NH); 21.4 (2 t, NMeCH$_2$*C*H$_2$(CH$_2$)$_2$NH). ESI-MS: 255.2 (100, [M + 2 H]$^{2+}$); 216.7 (9, [M + 2 H − NH$_3$]$^{2+}$); 449.3 (6, [M + H]$^+$). HRMS: calcd for $C_{23}H_{41}N_6O_3$ ([M + H]$^+$): 449.32347; found 449.32367.

(*S*)-*N*-[Amino-1-(carbamoylmethyl)-7-methyl-3,7,12-triaza-2-oxopentadecyl)-(1*H*-indol-3-yl)acetamide (LF487A, IndAcAsn3-(Me)43)

Compound **41** (8.3 mg, 8.1 μmol) was dissolved in dry CH_2Cl_2 (0.5 ml), and a solution of TFA, TIPS, and H_2O (10 ml, 95 : 2.5 : 2.5) was added. It was stirred at 23 °C for 1 h, and the volatiles were evaporated. CH_2Cl_2 (5 ml) was added to triturate the crude product. It was filtered and washed two additional times with CH_2Cl_2 (5 ml each time). The solvents were evaporated *in vacuo*, the residue was dissolved in H_2O (5 ml), and the mixture lyophilized. Preparative HPLC (gradient from 3% to 15% B in 8 min, then to 100% B in 15 min, solvent A: H_2O + 0.1% HCOOH, solvent B: MeCN + 0.1% HCOOH, flow 20 ml min^{-1}) delivered **LF487A**·1.1HCOOH (4.3 mg, 7.9 μmol, 98%) as a colorless, highly hygroscopic solid. Purity: 99% (UHPLC, 220 nm). UHPLC: R_t = 5.49 min (2.1 × 100 mm BEHC18, linear 1% B for 3 min, then gradient from 3% to 100% B in 15 min, solvent A: H_2O + 0.1% HCOOH, solvent B: MeCN + 0.1% HCOOH, flow 0.3 ml min^{-1}, detection at λ = 220 nm). $[\alpha]_D^{26}$ = −0.4 (*c* 0.21 in 0.1 M aq. HCOOH). UV(DAD) (H_2O): λ_{max} 193, 216, 219, 279, 287. ^{1}H-NMR (D_2O + 0.5 μl MeOH, 500 MHz):

8.48 (s, 1.1 H, *H*COOH); 7.66 (d, J = 7.9, indole-C(4)H); 7.57 (d, J = 8.1, indole-C(7)H); 7.38 (s, indole-C(2)H); 7.31 (dt, J = 8.1, 1.0, indole-C(6)H); 7.23 (dt, J = 7.9, 0.8, indole-C(5)H); 4.62 (*X* of *ABX*, J_{AX} = 7.9, J_{BX} = 6.0, 1H); 3.84 (s, IndCH_2); 3.26 (t, J = 6.0, CONHCH_2); 3.14–3.03 (m, H_2NCH_2CH_2CH_2NHCH_2); 2.96–2.91 (m, CH_2NMeCH_2); 2.80 (*A* of *ABX*, J_{AB} = 15.6, J_{AX} = 6.0, 1 H); 2.76 (*B* of *ABX*, J_{AB} = 15.6, J_{BX} = 7.9, 1 H); 2.67 (s, H_3C); 2.07 (quint., J = 7.9, H_2NCH$_2$CH_2); 1.81 (quint., J = 7.1, CONH-CH$_2$CH_2); 1.67 (br. s, NMeCH$_2$CH_2CH_2CH$_2$NH). ^{13}C-NMR (D_2O + 0.5 μl MeOH, 125 MHz): 175.8 (s, IndCH$_2$*C*O); 175.0 (s, CONH$_2$); 173.7 (s, *C*ONH(CH$_2$)$_3$N); 171.6 (d, 1.1 C, HCOOH); 136.9 (s, indole-C(7a)); 127.2 (s, indole-C(3a)); 125.6 (d, indole-C(2)); 122.8 (d, indole-C(6)); 120.2 (d, indole-C(5)); 119.0 (d, indole-C(4)); 112.7 (d, indole-C(7)); 108.3 (s, indole-C(3)); 55.7 (t, NMe*C*H$_2$(CH$_2$)$_3$NH); 53.8 (t, CONH(CH$_2$)$_2$*C*H$_2$NMe); 51.7 (d, IndCH$_2$CONH*C*H); 47.5 (t, NMe(CH$_2$)$_3$*C*H$_2$NH); 45.1 (t); 40.2 (q, NMe); 37.1 (t); 36.6 (t, CONH*C*H$_2$); 36.5 (t, *C*H$_2$CONH$_2$); 32.8 (t, Ind*C*H$_2$); 24.3 (t, *C*H$_2$CH$_2$NH$_2$); 24.2 (t, CONHCH$_2$*C*H$_2$); 23.3, 21.3 (2 t, NMeCH$_2$*C*H$_2$*C*H$_2$CH$_2$NH). ESI-MS: 244.7 (100, $[M + 2H]^{2+}$); (11, $[M + H]^+$). HRMS: calcd for $C_{25}H_{42}N_7O_3$ ($[M + H]^+$): 488.33436; found 488.33440.

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