# Solid-Phase Synthesis of Polyamine Spider Toxins and Correlation with the Natural Products by HPLC-MS/MS

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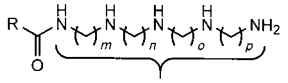
Dedicated to Prof. Dr. *Manfred Hesse* on the occasion of his retirement

A recently developed new and divergent approach for the solid-phase synthesis of polyamines and polyamine derivatives was extended to the preparation of linear pentamines, and it was applied to the synthesis of three quartets of isomeric polyamine spider toxins. The twelve synthetic acylpolyamines were investigated by HPLC-UV(DAD)-MS and HPLC-UV(DAD)-MS/MS and compared with the natural products in the complex mixture of the venom of *Agelenopsis aperta*. The comparative investigation supported the structures and assignments of seven previously found toxins and allowed the identification of an additional five polyamine derivatives in the natural sample. The MS/MS study of the isomerically pure polyamine derivatives revealed furthermore a characteristic pattern for the fragmentation of these compounds, which can possibly be used as evidence in the trace analysis of other polyamine derivatives.

**Introduction.** – Linear polyamines like putrescine, spermidine, and spermine, but also less common representatives, are widely distributed throughout the animal and plant kingdoms. They occur either free in protonated form or as conjugates connected to other biomolecules. Since they exhibit in either form a variety of biological activities [1–4], it is not surprising that polyamines as well as their analogs and derivatives are considered as therapeutic leads for the treatment of a variety of diseases. Unfortunately, natural polyamine derivatives arise often in small amounts and frequently within complex mixtures only. This makes their use in systematic biological investigations unviable. To study the detailed function and action of polyamine compounds, it is, thus, of particular interest not only to have access to a broad spectrum of different polyamine derivatives, preferably also to new substrates from natural sources, but also to sufficient amounts of pure samples. Highly sophisticated analytical methods to find new lead structures in nature combined with synthetic protocols to provide the corresponding compounds in ample amounts are, therefore, the optimal team players to support biological investigations related to the polyamines.

Recently, we have been increasingly engaged in the analysis and characterization of polyamine toxins from spiders, in particular from *Agelenopsis aperta* [5] and from *Paracoelotes birulai* [6], by on-line coupled high-performance liquid chromatography and atmospheric-pressure chemical-ionization mass spectrometry (HPLC-UV(DAD)-APCI-MS and MS/MS). In the venom of *A. aperta, e.g.*, as many as 33 acylpolyamines with 11 different molecular masses were detected by this method. Most of the structures were securely elucidated by MS/MS, but some evaded explicit determination. It was found that the MS/MS analysis of several venom 'components' (selected by their retention times in HPLC and their ion masses) do not conform completely to the

fragmentation patterns that would be expected for 'pure sample ions'. Such irregularities have been interpreted as the manifestation of overlapping spectra deriving from co-eluting compounds contained in the natural toxin mixture. For *IndAc* pentamines, it was, *e.g.*, cautiously concluded that *IndAc3334* (**AG 416a**, compound of type **A**) and *IndAc3343* (**AG 416**, compound of type **B**) are such co-eluting components of the venom (*Fig. 1*). The isomeric derivative *IndAc4333* (**AG 416b**, compound of type **D**) with a different chromatographic behavior, was also detected in the venom, while the remaining isomer *IndAc3433* (compound of type **C**), was not identified.



| | | trivial name | name of nat. product |
|---|---|---|---|
| Type **A** | *PA3334* | *IndAc3334* | (**AG 416a**) |
| | | *4-OH-Bz3334* | (**AG 379**) |
| | | *2,5-(OH)$_2$-Bz3334* | (**AG 395b**) |
| Type **B** | *PA3343* | *IndAc3343* | (**AG 416**) |
| | | *4-OH-Bz3343* | (—) |
| | | *2,5-(OH)$_2$-Bz3343* | (—) |
| Type **C** | *PA3433* | *IndAc3433* | (—) |
| | | *4-OH-Bz3433* | (—) |
| | | *2,5-(OH)$_2$-Bz3433* | (—) |
| Type **D** | *PA4333* | *IndAc4333* | (**AG 416b**) |
| | | *4-OH-Bz4333* | (**AG 379a**) |
| | | *2,5-(OH)$_2$-Bz4333* | (**AG 395c**) |

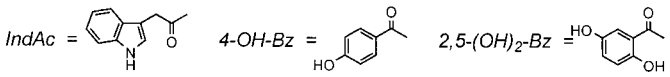


Fig. 1. *Typification, structures, and names of the acylpentamines found partially in the venom of the spider* A. aperta

While the structure elucidations for *IndAc3334* and *IndAc4333* appear quite conclusive, the evidence for the constitution of *IndAc3343* (**AG 416**) is rather weak. The assignment is based more on literature precedence – the compound was considered one of the major constituents of the venom of *A. aperta* [7] and of *H. curta* [8] – than on spectroscopic evidence. As a matter of fact, the MS/MS response at *m/z* 343, taken as the diagnostic signal for a fragment derived from *IndAc3343*, could likewise be attributed to a fragment of equal mass derived from *IndAc3433* (*Fig. 2*). Additionally, the signal at *m/z* 115, which was regarded as characteristic for the *PA33* terminus of *IndAc4333* (**AG 416b**), would indicate the presence of a structure also possessing a terminal *PA33* unit. Such a compound would be *IndAc3433* rather than *IndAc3343*. Since we have only marginal knowledge of the fragmentation behavior of the acylpolyamines, it cannot be excluded that the 'unusual' signals in the MS/MS spectra are not due to co-eluting isomeric compounds. They could have also been generated by unexpected fragmentation reactions occurring with the structurally secured *IndAc3334*.

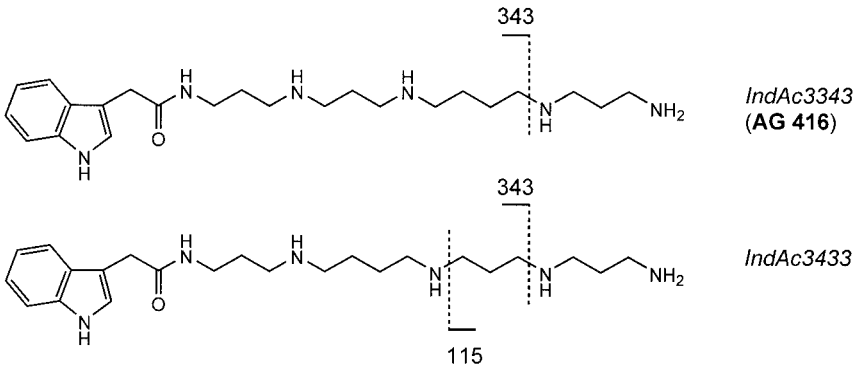


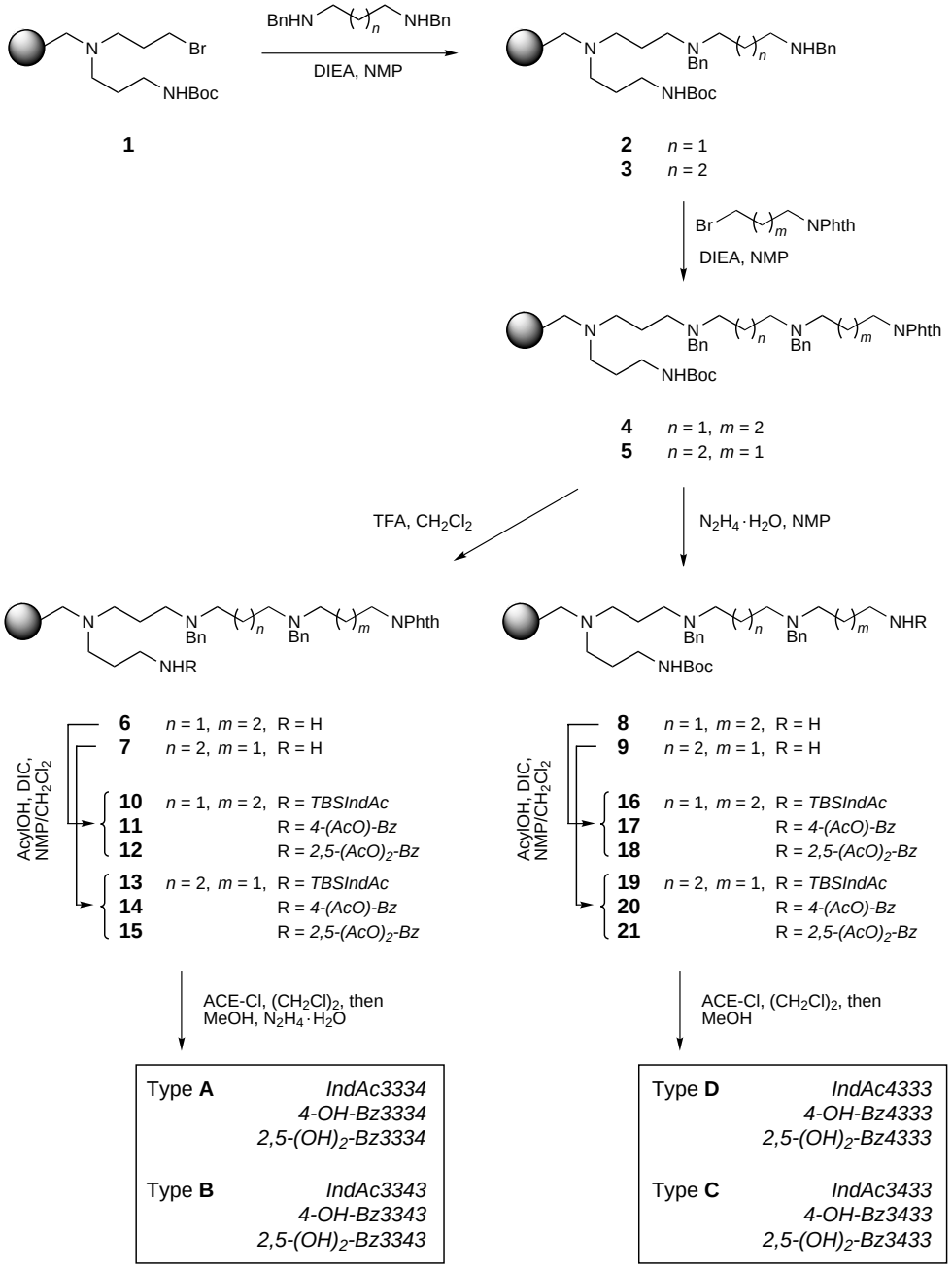

Fig. 2. *Proposed indicative fragment of* IndAc3343 (**AG 416**) *and fragments potentially derived from* IndAc3433

To unambiguously establish (or exclude) the presence and structures of the proposed indole-acetamides in the natural venom – eventually to find the remaining isomer *IndAc3433*, too – the respective compounds were synthesized, and they were analyzed separately and in comparison with the natural compounds by HPLC-UV(DAD)-MS and -MS/MS. Additionally, the analogous substrates with the 4-hydroxybenzoic (4-OH-Bz) and the 2,5-dihydroxybenzoic (2,5-$(OH)_2$-Bz) acid head portions were prepared as well. The corresponding isomers of type **A** and **D** were found also in the venom 'cocktail' of *A. aperta*, but the isomeric structures of type **B** and **C** have not been detected so far. For the synthesis of the twelve target structures, we had a powerful and efficient tool at hand, namely our recently developed new solid-phase methodology [9]. This methodology allows the construction of polyazaalkanes starting from their centers and the specific and separate modification of the linear polyamines at either end.

**Results.** – *Synthesis of the Polyamine Toxins.* As already described, *Merrifield* resin (200–400 mesh, 1% divinylbenzene, 0.9 mmol/g loading capacity) was converted to resin **1** by coupling with mono-Boc-protected 1,3-diamine followed by alkylation of the benzylic amine with 1,3-dibromopropane [9]. Elongation of the polyamine backbone was subsequently effected by treatment of resin **1** with *N,N′*-dibenzylpropane-1,3-diamine or *N,N′*-dibenzylbutane-1,4-diamine in 1-methylpyrrolidin-2-one (NMP) in presence of $EtN(i\text{-}Pr)_2$ (DIEA; *Scheme 1*). The resulting tetraminic resins **2** and **3** were further extended by alkylation of the terminal secondary amines with *N*-(4-bromobutyl)phthalimide or with *N*-(3-bromopropyl)phthalimide, respectively. This afforded the orthogonally protected resin-bound pentamine core structures **4** and **5**, respectively. The yields achieved so far amounted to *ca.* 30%, which was tested by removal of the polyamines from the resins and quantification of the liberated products.

To access the small library of selected target molecules, the protecting groups of the terminal amino functions were cleaved off specifically and replaced with the appropriate acid moieties. Thus, resins **4** and **5** were treated either with TFA or with $N_2H_4 \cdot H_2O$ to selectively remove the Boc or phthaloyl groups, respectively. Each of the product resins **6**–**9** was subsequently acylated with each of the three protected acids –

*Scheme 1*


BnHN
NHBn
DIEA, NMP
Br
NHBoc
1
2 n = 1
3 n = 2
Br
NPhth
DIEA, NMP
4 n = 1, m = 2
5 n = 2, m = 1
TFA, CH2Cl2
N2H4·H2O, NMP
NPhth
NHR
6 n = 1, m = 2, R = H
7 n = 2, m = 1, R = H
10 n = 1, m = 2, R = TBSIndAc
11 R = 4-(AcO)-Bz
12 R = 2,5-(AcO)2-Bz
13 n = 2, m = 1, R = TBSIndAc
14 R = 4-(AcO)-Bz
15 R = 2,5-(AcO)2-Bz
AcylOH, DIC, NMP/CH2Cl2
8 n = 1, m = 2, R = H
9 n = 2, m = 1, R = H
16 n = 1, m = 2, R = TBSIndAc
17 R = 4-(AcO)-Bz
18 R = 2,5-(AcO)2-Bz
19 n = 2, m = 1, R = TBSIndAc
20 R = 4-(AcO)-Bz
21 R = 2,5-(AcO)2-Bz
ACE-Cl, (CH2Cl)2, then MeOH, N2H4·H2O
ACE-Cl, (CH2Cl)2, then MeOH
Type A IndAc3334 4-OH-Bz3334 2,5-(OH)2-Bz3334
Type B IndAc3343 4-OH-Bz3343 2,5-(OH)2-Bz3343
Type D IndAc4333 4-OH-Bz4333 2,5-(OH)2-Bz4333
Type C IndAc3433 4-OH-Bz3433 2,5-(OH)2-Bz3433

*N*-[(*tert*-butyl)dimethylsilyl]-1*H*-indole-3-acetic acid (TBSIndAcOH), 4-acetoxybenzoic acid (4-(AcO)-Bz), and 2,5-diacetoxybenzoic acid (2,5-$(AcO)_2$-Bz) – by their mutual treatment with diisopropyl carbodiimide (DIC). Reaction of the resins **10**–**21** with 1-chloroethyl chloroformate (ACE-Cl), followed by MeOH [10] or MeOH/$N_2H_4 \cdot H_2O$ (for the *N*-phthaloyl derivatives **10**–**15**), provided the desired, properly derivatized twelve pentamine derivatives devoid of all protecting groups. The products were either purified by preparative HPLC or simply by rinsing with MeOH and were finally collected as their HCl or TFA salts in 10–30% overall yields. Alternatively to the procedure described above, the phthaloyl group of the resins **10**–**15** can be cleaved off the polyamine prior to its release from the polymer support. Without adding $N_2H_4 \cdot H_2O$ to the methanolic solution during the cleavage procedure, the phthaloyl group can also be retained as a protecting group in the final product.

During the preparation of the indole-acetamides, we encountered one noteworthy problem. It turned out that the choice of the acid derivative to be coupled with the polyamine backbone is not trivial. Indole-3-acetic acid itself as well as its *N*-Boc-protected derivative proved not to be the appropriate reagents. Upon cleavage of the corresponding final acylpolyamines from the resin, complete decomposition of the products was observed, possibly due to oxidation. Less decomposition was noticed when more stable protecting groups than Boc were used at the indole N-atom. At least, satisfactory yields of cleavage products were obtained with *N*-Bn-, *N*-Ts-, and *N*-TBDMS-protected compounds (17, 13, and 11%, respectively, for products derived from resin **8**). Since the hydrogenolytic removal of the Bn group was accompanied by partial reduction of the indole moiety, and, also, the reductive cleavage of the Ts group by the action of MeOH/Mg was not quantitative in yield (80%), the use of the TBDMS-protected indole-acetic acid in the acylation step was finally found to be appropriate.

*Correlation of the Synthetic Samples with the Natural Toxins within the Venom. Chromatographic Behavior.* Several synthetic polyamine derivatives were analyzed individually or as defined mixtures with each other and with the natural venom by HPLC-UV(DAD)-MS and HPLC-UV(DAD)-MS/MS under the standard conditions used previously [5]. *Fig. 3* shows representatively the extracted-ion chromatograms (EIC) of the quasi-molecular ion at *m/z* 417 for the natural venom and for mixtures of the natural venom with the four synthetic *IndAc*-polyamines. It is easily recognized from these chromatograms that the three isomeric compounds *IndAc3334*, *IndAc3343*, and *IndAc3433* all co-elute with the first fraction ($t_R$ 26.0 min) of the natural venom. The remaining isomer, *IndAc4333*, co-elutes with the second fraction of the native toxin mixture ($t_R$ 27.5 min). Similar pictures as with the *IndAc*-polyamines emerged with the other two groups of acylpolyamines (EICs of ions *m/z* 396 and 380 for the 4-OH-Bz and the 2,5-$(OH)_2$-Bz derivatives, resp.). Also for these compounds, the three isomers of types **A**–**C** (*Acyl3334*, *Acyl3343*, and *Acyl3433*) all co-elute with the first fractions of the natural venom components, whereas the remaining isomers of type **D** (*Acyl4333*) co-elute with the respective second fraction.

*MS/MS Fragmentation.* Due to the co-elution of several compounds in the HPLC, the single-ion detected chromatographic results do not allow a definitive structure correlation for all the toxins contained in the natural venom. Additional information, however, can be gained from the HPLC-MS/MS experiments. The respective spectra of the four isomeric *IndAc*-pentamines (*IndAc3334*, *IndAc3343*, *IndAc3433*, and

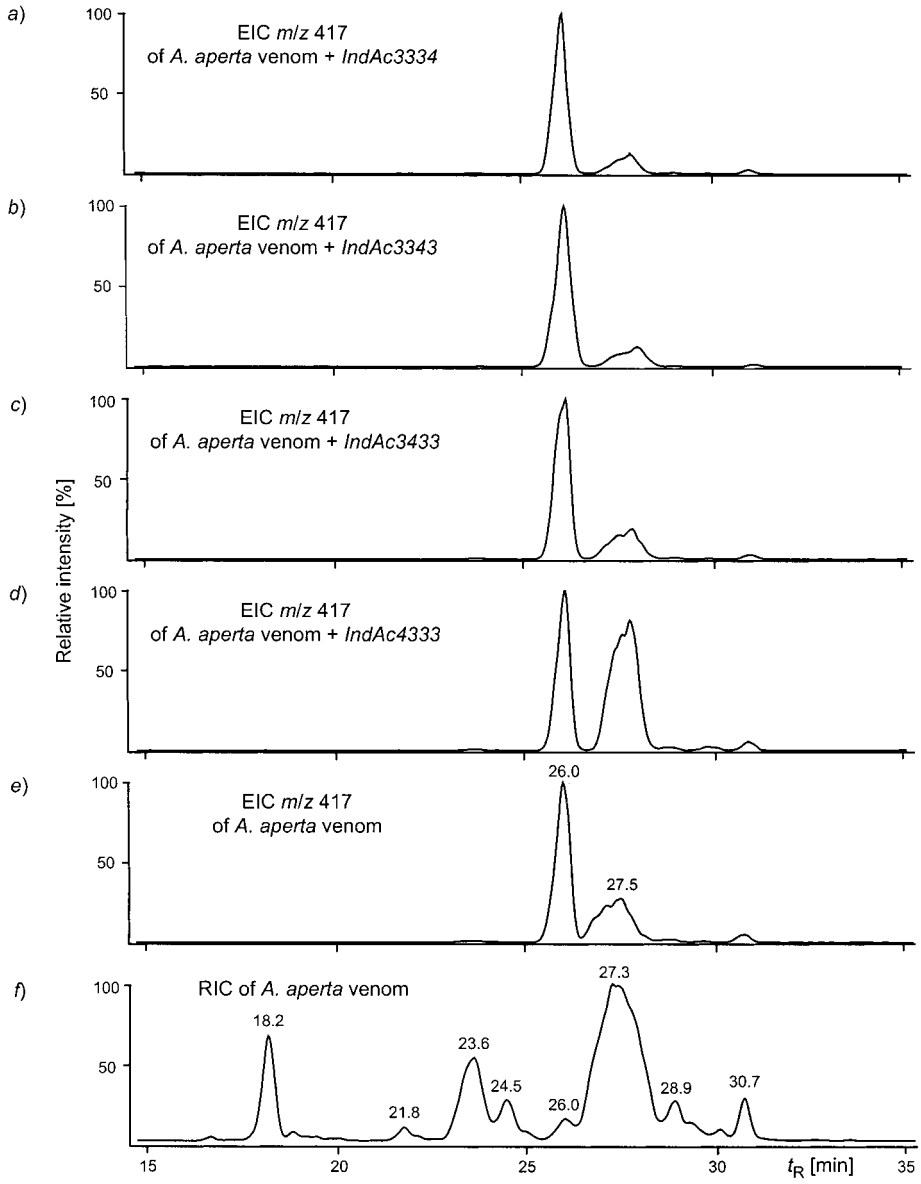



Fig. 3. *Extracted ion chromatograms* (EIC) *detected at* m/z *417 of the venom of* A. aperta *with admixed synthetic* IndAc3334 (*a*), IndAc3343 (*b*), IndAc3433 (*c*), *and* IndAc4333 (*d*) *as well as the EIC detected at* m/z *417* (*e*) *and the reconstructed ion chromatogram* (RIC) *of the natural venom* (*f*).

*IndAc4333*) and of two chromatographic fractions of the natural venom are shown in *Fig. 4*. The results obtained from the investigation of all synthetic polyamine derivatives and the corresponding natural-toxin fractions are given in the *Exper. Part*.

It is readily realized from the illustrations in *Fig. 4* that the collision-induced decompositions (CID) of the quasi-molecular ions of the several isomerically pure sample compounds lead to distinctively different peak patterns in the spectra. However, most of the signals (with respect to the *m/z* values) are shared by at least two of the four compounds. Nevertheless, characterization of the components of the natural venom by correlation with the synthetic compounds should be viable because of the distinctively different relative intensities of the signals manifested in the separate spectra. In fact, spectrum *f* of the second HPLC fraction of the biological sample matches well – except for the two signals at *m/z* 112 and 215 – with spectrum *e* of *IndAc4333*. This corroborates the earlier structure assignment for *IndAc4333*, which was recognized as the compound eluting at this position. Spectrum *d* of the first HPLC fraction, on the other hand, corresponds only largely to one of the remaining spectra, namely to spectrum *a* of *IndAc3334*. The differences in the relative peak intensities and the additional ion responses found in the spectrum of the natural probe, however, are distinctive enough to deduce that *IndAc3334* cannot be the sole component detected in the venom fraction. Analysis of the corresponding spectra of the 4-OH-Bz and 2,5-$(OH)_2$-Bz-polyamines revealed similar insights: the MS/MS of the second fraction of the natural toxin mixture correspond nicely to the *Acyl4333* derivatives and the spectra of the first fraction correlates largely, but not completely, to the *Acyl3334* compounds. The question arose, thus, whether it is possible to characterize one or the other of the remaining acylpolyamines as the additional constituents of the natural probe.

Scrutiny of the spectral data of the whole collection of synthetic compounds showed that only a few MS/MS signals are unique and thus characteristic for the individual isomeric acylpolyamines. Only these signals can be regarded as diagnostic for the different polyamine substructures and their attachments to the acyl groups. Of particular interest are the characteristic signals deriving from the compounds of type **A** – **C** within the three classes of isomers, because these substances co-elute in the HPLC. *Table 1* compiles these diagnostic signals obtained from the corresponding quasi-molecular ions at *m/z* 417, 380, and 396. Some other potentially characteristic peaks and the CID patterns of the respective first-eluting fractions of the natural venom are also included in *Table 1*.

The data in *Table 1* shows clearly that the spectra of the first-eluting venom fractions of all three classes of acylpolyamines are superimposable on the spectra of all three respective acylpentamines of type **A** – **C**. The major components of the natural toxin mixtures are, in all cases, recognized as the *Acyl3334* derivatives, as already realized above for *IndAc3334*. All four characteristic peaks for the fragments of type *a*, *b*, *c*, and $c - H_2O$ of these isomers are found in high abundance in the three spectra of the natural compound fractions. The presence of the *Acyl3343* derivatives in the natural mixture is manifested by the two diagnostic fragments of type *c* and $c - H_2O$, which were recorded with substantial intensities in all three spectra of the natural venom fractions. For the *Acyl3433* derivatives, only a single characteristic signal, corresponding to a fragment of type *e*, can be extracted from the data of the isomerically pure samples, and the corresponding responses for fragment *e* are detected also in the spectra of the natural-venom fractions. While the abundance of fragment *e* for *Acyl3433* is substantial in the spectrum of the venom (21 rel.%), the intensities for the related fragments of 4-OH-Bz3433 and 2,5-$(OH)_2$-Bz3433 are rather low (3 rel.%

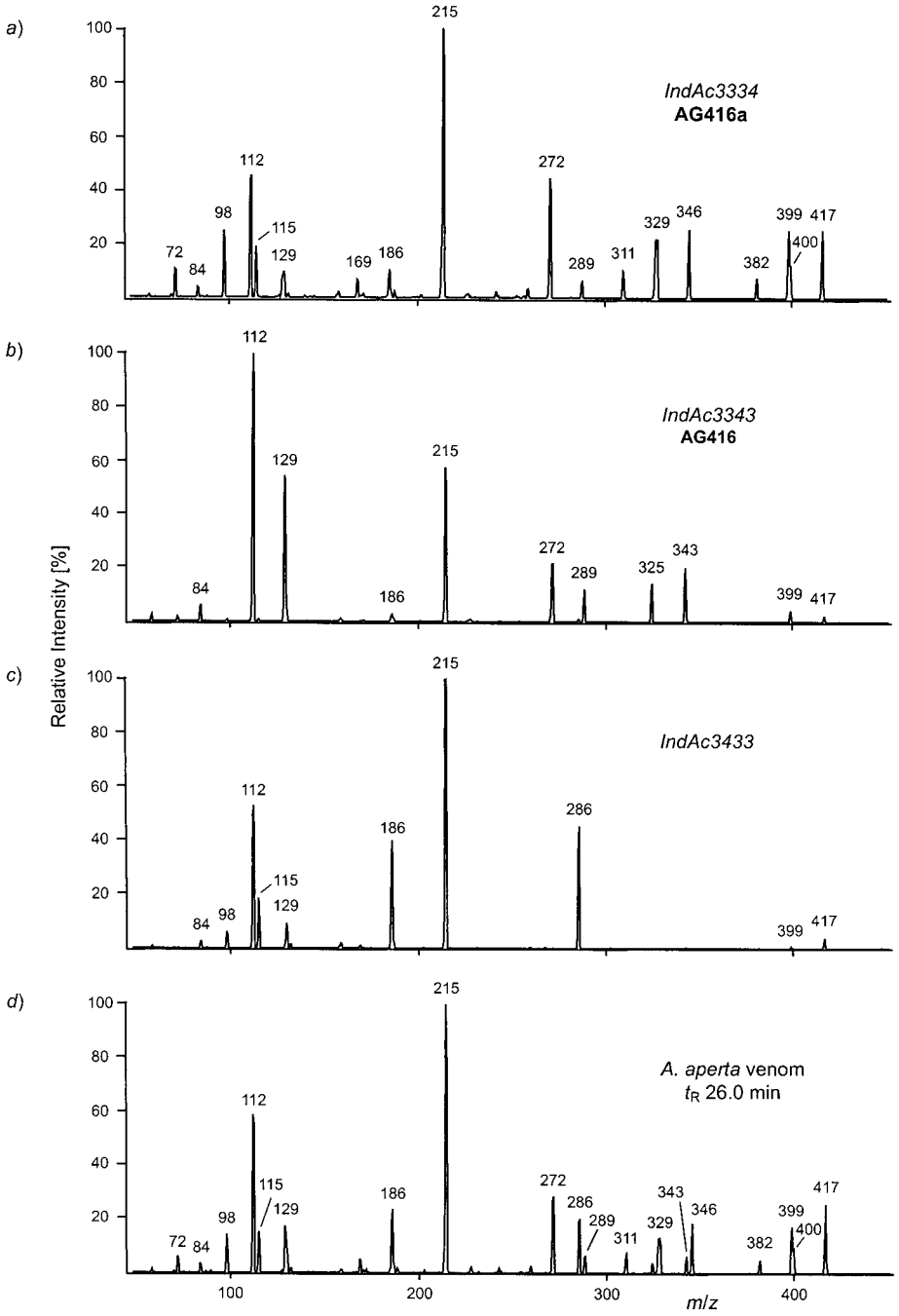



Fig. 4. *MS/MS Patterns of the quasi-molecular ions at* m/z *417 of the co-eluting synthetic samples of* IndAc3334 (*a*), IndAc3343 (*b*), IndAc3433 (*c*), *and of the fraction with* $t_R$ *26.0 min of the* A. aperta *venom* (*d*) *and the respective MS/MS of the synthetic sample of* IndAc4333 (*e*) *and of the fraction with* $t_R$ *27.2 min of the* A. aperta *venom* (*f*)

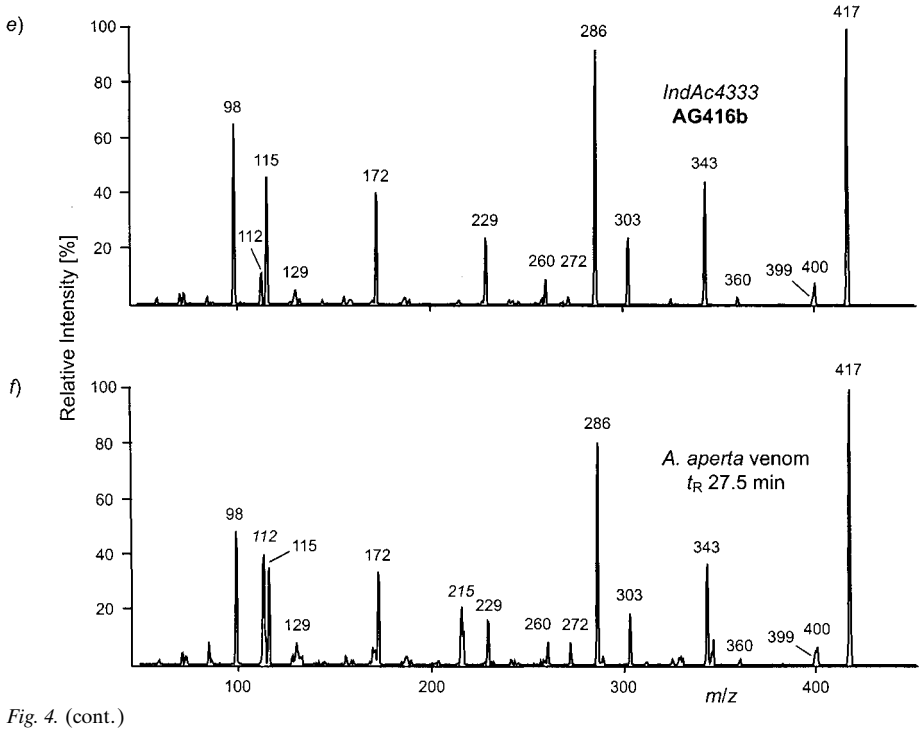


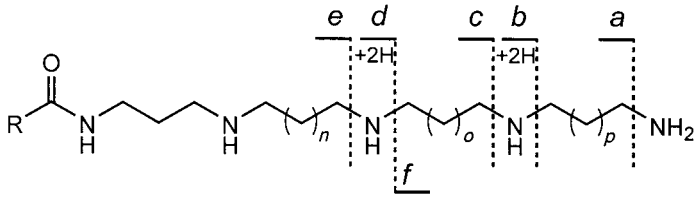

*Fig. 4.* (cont.)

each). Nevertheless, these signals are regarded as significant enough to conclude that all three *Acyl3433* derivatives are constituents of the natural spider venom.

**Discussion.** – Our comparative HPLC-MS/MS investigation of synthetic polyamine derivatives and of natural polyamine fractions of *A. aperta* venom largely supports the results and deductions published earlier [5]. It unambiguously confirms all the previously proposed compounds to be contained in the natural venom. It furthermore establishes the occurrence of five additional polyamine toxins that have not been identified so far. We have shown that the venom contains, for each of the three aromatic head groups, all four possible isomeric acylpolyamines related to the parent pentamines *PA3334* and *PA3343*, *i.e.*, all compounds of type **A**–**D**. The presence of all these isomeric acylpentamines is strongly indicative for the biosynthetic pathway of the compounds in the spiders. Even though the free polyamines themselves were not found in the venom of *A. aperta*, we assume that they represent the biosynthetic precursors of the several toxins. We propose that the final toxin cocktail of the spiders is derived from 'statistical' acylation of the two parent pentamines at either end of the molecules.

Besides the identification of the twelve synthetic acylpentamines as constituents of the venom of *A. aperta*, our MS/MS study exposed also some interesting spectrometric peculiarities. First, the spectra of the synthetic *IndAc3343* and *IndAc3433* revealed that the signal at *m/z* 343 for fragment *c* is, in fact, indicative for the former compound. As outlined earlier, the formation of a fragment with *m/z* 343 could principally also be due

Table 1. *Types and Relative Intensities of Relevant MS/MS Signals of the Quasi-Molecular Ions at* m/z *417, 380, and 396 of Synthetic and Natural Acylpolyamine Samples*[a])

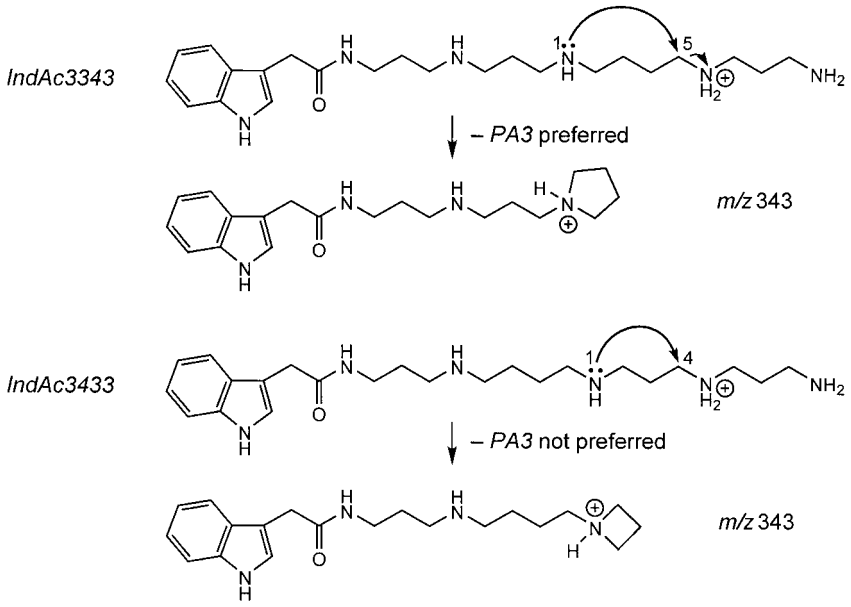



| Sample | | Fragments/*m/z* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *a* | *b* | | *c* | | *c* – $H_2O$ | | *d* | | *e* | | *f* | |
| | | *400* | *360* | *346* | *343* | *329* | *325* | *311* | *303* | *289* | *286* | *272* | *129* | *115* |
| *IndAc3334* | | 13 | – | 26 | – | 22 | – | 10 | – | 7 | – | 45 | 8 | – (19) |
| *IndAc3343* | (*m/z* 417) | 0 | 0 | – | 20 | – | 14 | – | – | 12 | – (1) | 22 | 55 | – |
| *IndAc3433* | | 0 | 0 | – | 0 | – | 0 | – | 0 | – | 46 | – | 3 | 19 |
| Venom ($t_R$ 26.0) | | 10 | 0 | 19 | 7 | 11 | 4 | 8 | 0 | 7 | 21 | 29 | 18 | 16 |
| | *m/z* | *363* | *323* | *309* | *306* | *292* | *288* | *274* | *266* | *252* | *249* | *235* | *129* | *115* |
| 4-OH-Bz3334 | | 9 | – | 11 | – | 19 | – | 7 | – | 11 | – | 34 | 12 | – (17) |
| 4-OH-Bz3343 | (*m/z* 380) | 0 | 0 | – | 11 | – | 4 | – | – | 5 | – | 18 | 46 | – |
| 4-OH-Bz3433 | | 0 | 0 | – | 0 | – | 0 | – | 0 | – | 25 | – | 1 | 18 |
| Venom ($t_R$ 16.7) | | 5 | 0 | 23 | 5 | 24 | 4 | 7 | 0 | 10 | 3 | 49 | 25 | 22 |
| | *m/z* | *379* | *339* | *325* | *322* | *308* | *304* | *290* | *282* | *268* | *265* | *251* | *129* | *115* |
| 2,5-$(OH)_2$-Bz3334 | | 10 | – | 29 | – | 28 | – | 10 | – | 5 | – | 49 | 15 | – (33) |
| 2,5-$(OH)_2$-Bz3343 | (*m/z* 396) | 0 | 0 | – | 17 | – | 6 | – | – | 4 | – | 19 | 50 | – (3) |
| 2,5-$(OH)_2$-Bz3433 | | 0 | 0 | – | 0 | – | 0 | – | 0 | – | 38 | – | 10 | 22 |
| Venom ($t_R$ 19.5) | | 21 | 0 | 27 | 8 | 46 | 3 | 7 | 0 | 11 | 3 | 82 | 36 | 28 |

[a]) – Denotes entry positions where no fragment intensities were expected based on the structure of the sample compound, in parentheses: effectively found intensities >1 rel.%; entries of 0 rel.% corresponds to the observation of no signal intensities (< 1 rel.%), even though fragments would principally be expected based to the structure of the sample compound.

to the decomposition of *IndAc3433*. However, the corresponding fragmentation of the latter is much less pronounced. This is possible because the reaction, in contrast to the reaction of *IndAc3343*, cannot proceed *via* a five-membered cyclic intermediate. The respective proposed fragmentation reactions are outlined in *Scheme 2*. The same effect as with *IndAc3343* and *IndAc3433* was observed for the two other pairs of acylpolyamines with the 4-OH-Bz and the 2,5-$(OH)_2$-Bz groups (fragments *c* at *m/z* 306 and 325, resp.). It is evidently also not restricted to the formation of fragments of type *c:* all fragmentations proceeding analogously *via* five-membered cyclic transition states are clearly favored reactions. This is demonstrated, for instance, with the abundant signals for fragments *a* observed for the *Acyl3334* derivatives. They are due to the loss of $NH_3$ from the quasi-molecular ions by intramolecular nucleophilic substitutions *via* five-membered transition states. The analogous reaction of the isomeric acylpolyamines would all have to proceed *via* four-membered transition structures and are, thus, less-pronounced; in fact, the respective signals are not observed at all.

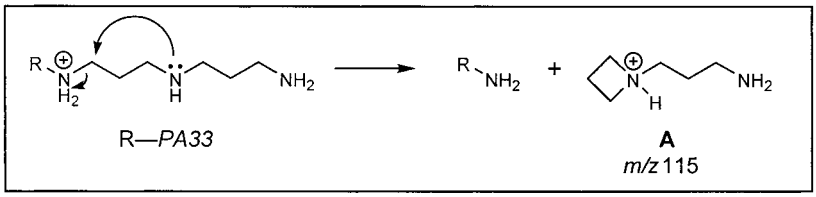
*Scheme 2*



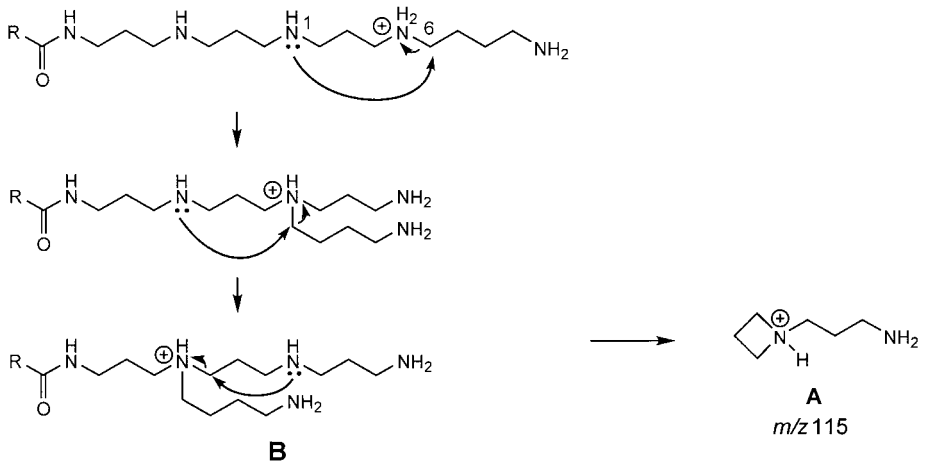

Another peculiarity in the fragmentation behavior of the acylpolyamines became evident. We would have expected, as mentioned earlier, to find fragment *f* at *m/z* 115 to be decisive for the *Acyl3433* derivatives (*Scheme 3*). The fragment, which was attributed to structure **A**, would be released from a polyamine with a terminal *PA33* unit. Fragment *f* at *m/z* 115, however, was also observed with high abundance in the MS/MS of the *Acyl3334* derivatives lacking the *PA33* termini. It was not found, or was found only with negligible intensity, however, in the *Acyl3343* derivatives possessing an inverted *PA34* unit at the end. The formation of the fragment with *m/z* 115 from *Acyl3334* derivatives can be explained by two processes, which involve a ZIP reaction (*Scheme 3*) [11]. The quasi-molecular ions could lead either by a cascade of intramolecular $S_N2$ reactions (transaminations) or transamidation processes – all proceeding *via* six-membered cyclic transition states – to intermediary structures **B** or **C**. These intermediates are both prone to lose fragment **A** corresponding to the observed signal. The analogous ZIP reactions with the *Acyl3343* derivatives are less advantageous, since they would involve at one stage a group transfer *via* an unfavorable seven-membered cyclic transition state [12–14]. Acid-catalyzed transamidation during MS analysis was already observed [15]; for the transamination reaction, no literature precedence is available. The latter reaction was nevertheless considered because relevant and expected fragmentation of the transamidation products was not observed. For instance, no loss of *PA33* from ion **C** was detected (*m/z* 286, 249, and 265, resp., for the three *Acyl3334* derivatives).

**Conclusions.** – Our study has shown that the conclusive and final analysis of trace components within complex mixtures of polyamine spider toxins needs synthetic

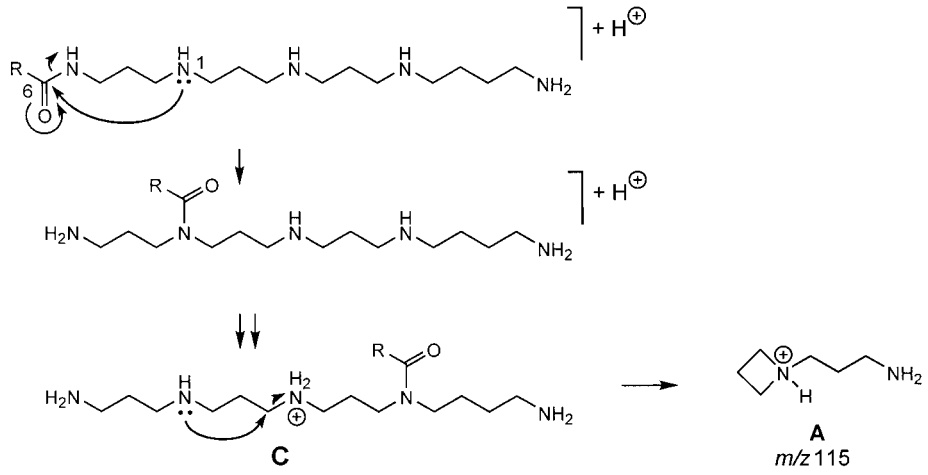
*Scheme 3*




*ZIP Reaction by transamination*




*ZIP Reaction by transamidation*



reference samples of known structures. With such compounds at hand, we were not only able to confirm seven proposed structures found previously in the venom of *A. aperta* but also to detect five additional acylpolyamines, completing the set of isomeric constituents of the natural sample. The solid-phase synthesis of the polyamine derivatives proved to be a viable way to rapidly prepare the small library of spider toxins, and we are confident that this strategy can be extended to the preparation of more complex molecules as well.

We would like to thank the *Swiss National Science Foundation* for their generous financial support.

## Experimental Part

1. *General*. Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. Lyophilized *Agelenopsis aperta* venom was purchased from *Spider Pharm. Inc.*, Yarnell, AZ, USA and was stored at $-80°$. As the solid support, *Merrifield* peptide resin 200–400 mesh, 1% divinylbenzene, loading 0.9 mmol/g from *Advanced ChemTech* was used. Instrumentation for the solid-phase reactions: *PLS 4 × 6* and *PLS 1 × 6 Organic Synthesizers*. IR Spectra: as KBr presslings; *Perkin-Elmer IR 'Spectrum One'* and *Perkin-Elmer 781*; in $cm^{-1}$. $^1$H-NMR Spectra: $D_2O$ or $CDCl_3$; *Bruker AC-300* (300 MHz); $\delta$ in ppm rel. to TSP ($\delta$ 0.00) or $CHCl_3$ ($\delta$ 7.26), *J* in Hz. $^{13}$C-NMR Spectra in: $D_2O$ or $CDCl_3$; *Bruker ARX-300* (75.5 MHz); $\delta$ in ppm rel. to TSP ($\delta$ 1.7) or $CDCl_3$ ($\delta$ 77.0); multiplicities from DEPT-135 and DEPT-90 experiments. HPLC-UV(DAD)-MS and HPLC-UV(DAD)-MS/MS: see detailed description in *7*. Prep. chromatographic conditions (HPLC): columns *Kromasil KR100-10C18* (4.6 × 250 mm) and *Kromasil KR100-10C18* (50.8 × 250 mm); $H_2O$ was purified with a *Milli-$Q_{RG}$* apparatus. Confirmation of structures and purities of the final polyamine derivatives are provided by their $^1$H-NMR spectra and by their HPLC/MS analysis. Elemental analyses and HR-MS are not appropriate methodologies for polyamine derivatives, since the compounds arise, as free bases only, as waxy or glassy solids, from which the last solvent molecules can hardly be removed. The HCl salts are rather hygroscopic, and the uptake of $H_2O$ falsifies the elemental analyses. The compounds are not stable enough to survive distillation and show heavy fragmentation in EI-MS. HR-MS on the molecular ions is thus not possible, and HR-MS on fragment ions are not informative enough to establish the overall structures.

2. *Construction of the Polyamine Backbones on the Resin.* 2.1. *Elongation of Resin* **1** *with* N,N′-*Dibenzylpropane-1,3-diamine* (→ *Resin* **2**). Resin **1** (4.1 mmol [9]) was swelled in 1-methylpyrrolidin-2-one (NMP; 30 ml). *N,N′*-Dibenzylpropane-1,3-diamine (6.25 g, 24.6 mmol [16]) and $EtN(i\text{-}Pr)_2$ (DIEA; 7.02 ml, 41 mmol) were added. After agitation for 24 h at 50°, resin **2** was filtered off, washed with NMP and $CH_2Cl_2$, and dried *in vacuo*.

2.2. *Elongation of Resin* **1** *with* N,N′-*Dibenzylbutane-1,4-diamine* (→ *Resin* **3**). Resin **1** (4.1 mmol [9]) was swelled in NMP (30 ml), and *N,N′*-dibenzylbutane-1,4-diamine (6.59 g, 24.6 mmol) and DIEA (7.02 ml, 41 mmol) were added. After agitation for 24 h at 50°, resin **3** was filtered off, washed with NMP and $CH_2Cl_2$, and dried *in vacuo*.

2.3. *Alkylation of Resin* **2** *with* N-*(4-Bromobutyl)phthalimide* (→ *Resin* **4**). Resin **2** (4.1 mmol) was suspended in NMP (30 ml). *N*-(4-Bromobutyl)phthalimide (5.784 g, 20.5 mmol) and DIEA (7.02 ml, 41 mmol) were added, and the mixture was agitated for 26 h at 50°. Resin **4** was filtered off, washed with NMP, $CH_2Cl_2$, and MeOH, and dried *in vacuo*. IR: 3426*w*, 1772*w*, 1714*s*.

2.4. *Alkylation of Resin* **3** *with* N-*(3-Bromopropyl)phthalimide* (→ *Resin* **5**). Resin **3** (4.1 mmol) was suspended in NMP (30 ml). *N*-(3-Bromopropyl)phthalimide (5.494 g, 20.5 mmol) and DIEA (7.02 ml, 41 mmol) were added, and the mixture was agitated for 26 h at 50°. Resin **5** was filtered off, washed with NMP, $CH_2Cl_2$, and MeOH, and dried *in vacuo*. IR: 3427*w*, 1772*m*, 1714*s*.

3. *Deprotection of the Terminal Amino Groups of the Resins.* 3.1. *Removal of the Boc Group from Resin* **4** (→ *Resin* **6**). To resin **4** (1.64 mmol), swelled in $CH_2Cl_2$ (16 ml), was added $CF_3COOH$ (TFA; 4.0 ml, 52 mmol). After agitation for 15 h at 23°, resin **6** was filtered off, washed with $CH_2Cl_2$, $CH_2Cl_2$/DIEA 3:1, NMP, and $CH_2Cl_2$, and dried *in vacuo* at 50°. IR: 3384*w*, 1771*w*, 1714*s*.

3.2. *Removal of the Boc Group from Resin* **5** (→ *Resin* **7**). To resin **5** (1.64 mmol), swelled in $CH_2Cl_2$ (16 ml) was added TFA (4.0 ml, 52 mmol). After agitation for 15 h at 23°, resin **7** was filtered off, washed with $CH_2Cl_2$, $CH_2Cl_2$/DIEA 3:1, NMP, and $CH_2Cl_2$, and dried *in vacuo* at 50°. IR: 3420*w*, 1771*w*, 1714*s*.

3.3. *Removal of the Phthaloyl Group from* **4** (→ *Resin* **8**). Resin **4** (1.64 mmol) was swelled in NMP (15 ml), and the mixture, after addition of $N_2H_4 \cdot H_2O$ (6.0 ml, 0.123 mol), was agitated for 3 h at 80°. Resin **8** was filtered off, washed with NMP, dioxane, dioxane/$H_2O$ 1:1, dioxane, and $CH_2Cl_2$, and dried *in vacuo* at 50°. IR: 3426*w*, 1714*s*.

3.4. *Removal of the Phthaloyl Group from* **5** (→ *Resin* **9**). Resin **5** (1.64 mmol) was swelled in NMP (15 ml), and the mixture, after addition of $N_2H_4 \cdot H_2O$ (6.0 ml, 0.123 mol), was agitated for 3 h at 80°. Resin **9** was filtered off, washed with NMP, dioxane, dioxane/$H_2O$ 1:1, dioxane, and $CH_2Cl_2$, and dried *in vacuo* at 50°. IR: 3427*w*, 1714*s*.

4. *Acylation of the Resins.* 4.1. *General Procedure* (*GP 4.1*). The resin (1.64 mmol) was swelled in NMP/$CH_2Cl_2$ 1:2 (15 ml). The appropriate carboxylic acid (16.4 mmol) and *N*,*N*′-diisopropylcarbodiimide (1.27 ml, 8.2 mmol) were added, and the mixture was agitated for 30 h at 23°. The product resin was filtered off, washed successively with $CH_2Cl_2$, NMP, NMP/DIEA 10:1, NMP, and $CH_2Cl_2$, and was dried *in vacuo* at 50°. The *Kaiser* test [17] was performed to confirm the absence of primary amino groups.

4.2. *Acylation of* **6** *with TBSIndAcOH* (→ *Resin* **10**). Resin **6** was acylated with 2-{1-[(*tert*-butyl)dimethylsilyl]-1*H*-indole-3-acetic acid according to *GP 4.1* to give resin **10**. IR: 3420*m*, 1770*w*, 1710*s*, 1665*s*.

4.3. *Acylation of* **6** *with 4-(AcO)-Bz* (→ *Resin* **11**). Resin **6** was acylated with 4-acetoxybenzoic acid according to *GP 4.1* to give resin **11**. IR: 3420*m*, 1760*m*, 1715*s*, 1660*m*.

4.4. *Acylation of Resin* **6** *with 2,5-(AcO)*$_2$*-Bz* (→ *Resin* **12**). Resin **6** was acylated with 2,5-diacetoxybenzoic acid according to *GP 4.1* to give resin **12**. IR: 3420*w*, 1765*s*, 1710*m*, 1665*m*.

4.5. *Acylation of* **7** *with TBSIndAcOH* (→ *Resin* **13**). Resin **7** was acylated with 2-{1-[(*tert*-butyl)dimethylsilyl]-1*H*-indole-3-acetic acid according to *GP 4.1* to give resin **13**. IR: 3420*w*, 1775*w*, 1715*s*, 1680*s*.

4.6. *Acylation of* **7** *with 4-(AcO)-Bz* (→ *Resin* **14**). Resin **7** was acylated with 4-acetoxybenzoic acid according to *GP 4.1* to give resin **14**. IR: 3420*m*, 1760*m*, 1715*s*, 1660*m*.

4.7. *Acylation of* **7** *with 2,5-(AcO)*$_2$*-Bz* (→ *Resin* **15**). Resin **7** was acylated with 2,5-diacetoxybenzoic acid according to *GP 4.1* to give resin **15**. IR: 3420*w*, 1765*s*, 1710*m*, 1665*m*.

4.8. *Acylation of* **8** *with TBSIndAcOH* (→ *Resin* **16**). Resin **8** was acylated with 2-{1-[(*tert*-butyl)dimethylsilyl]-1*H*-indole-3-acetic acid according to *GP 4.1* to give resin **16**. IR: 3420*w*, 1710*s*, 1690*s*.

4.9. *Acylation of* **8** *with 4-(AcO)-Bz* (→ *Resin* **17**). Resin **8** was acylated with 4-acetoxybenzoic acid according to *GP 4.1* to give resin **17**. IR: 3425*w*, 1760*m*, 1714*s*, 1663*m*.

4.10. *Acylation of* **8** *with 2,5-(AcO)*$_2$*-Bz* (→ *Resin* **18**). Resin **8** was acylated with 2,5-diacetoxybenzoic acid according to *GP 4.1* to give resin **18**. IR: 3420*w*, 1765*m*, 1715*s*, 1650*m*.

4.11. *Acylation of* **9** *with TBSIndAcOH* (→ *Resin* **19**). Resin **9** was acylated with 2-{1-[(*tert*-butyl)dimethylsilyl]-1*H*-indole-3-acetic acid according to *GP 4.1* to give resin **13**. IR: 3420*w*, 1710*s*, 1690*s*.

4.12. *Acylation of* **9** *with 4-(AcO)-Bz* (→ *Resin* **20**). Resin **9** was acylated with 4-acetoxybenzoic acid according to *GP 4.1* to give resin **20**. IR: 3425*m*, 1760*m*, 1715*s*, 1640*m*.

4.13. *Acylation of* **9** *with 2,5-(AcO)*$_2$*-Bz* (→ *Resin* **21**). Resin **9** was acylated with 2,5-diacetoxybenzoic acid according to *GP 4.1* to give resin **21**. IR: 3420*w*, 1765*m*, 1715*s*, 1650*m*.

5. *Cleavage of the Polyamine Derivatives from the Resins.* 5.1. *General Procedure* (*GP 5.1*). The resin (1.64 mmol) was swelled in 1,2-dichloroethane (15 ml), and 1-chloroethyl chloroformate (ACE-Cl; 3.57 ml, 32.8 mmol) was added. After agitation for 3 h at 23°, the product resin was filtered off and washed with $CH_2Cl_2$. The org. solns. were combined and evaporated to dryness. The residues were dissolved in MeOH, and the resulting soln. was refluxed for 3 h. For resins **10**–**15** (Phth-protected compounds), $N_2H_4 \cdot H_2O$ (2.0 ml, 41 mmol) was added, and refluxing was continued for an additional 2 h. The solvent was removed, and the solid residue, after washing of the residue with MeOH, was collected. This provided the corresponding polyamine derivative as the 4 HCl salt. Where appropriate, purification was followed by HPLC.

5.2. N-*(16-Amino-4,8,12-triazahexadecyl)-1*H-*indole-3-acetamide* (*IndAc3334*, **AG 416a**). Treatment of **10** (1.55 mmol) according to *GP 5.1* afforded *IndAc3334* · 4 HCl (115 mg, 0.20 mmol; overall yield 13% with respect to **1**) after purification by HPLC (solvent *A:* 0.05% HCl in MeOH; solvent *B:* 0.05% HCl in $H_2O$; 25% *A*; $\lambda =$ 254 nm; flow rate 20 ml min$^{-1}$; the product was collected at 18.2–20.8 min). IR: 3380*s*, 3320*m*, 2950*s*, 2750*s*, 2520*m*, 2405*m*, 1650*s*, 1610*w*, 1535*m*, 1455*m*, 1050*w*, 780*w*, 740*m*. $^1$H-NMR ($D_2O$): 7.68–7.53 (*m*, 2 arom. H); 7.38–7.15 (*m*, 3 arom. H); 3.77 (*s*, ArC*H*$_2$); 3.35–3.00 (*m*, 12 H); 2.98–2.82 (*m*, 4 H); 2.22–1.94 (*m*, 4 H); 1.91–1.70 (*m*, 6 H). $^{13}$C-NMR ($D_2O$): 180.5 (*s*, CO); 140.8, 131.2 (2*s*, 2 arom. C); 129.7, 126.7, 124.1, 122.8, 116.6 (5*d*, 5 arom. C); 112.2 (*s*, 1 arom. C); 51.6, 49.6, 49.17, 49.16, 49.1, 48.9, 43.4, 40.5, 37.0, 30.1, 28.4, 27.3, 27.2, 27.1 (14*t*). MS: see *7*.

5.3. N-*(16-Amino-4,8,12-triazahexadecyl)-4-hydroxybenzamide* (4-OH-Bz3334, **AG 379**). Treatment of **11** (1.62 mmol) according to *GP 5.1* afforded *4-OH-Bz3334* · 4 HCl (250 mg, 0.48 mmol; overall yield 30% with

respect to **1**) after purification by HPLC (solvent 0.05% HCl in $H_2O$; $\lambda =$ 254 nm; flow rate 25 ml $min^{-1}$; the product was collected at 31.5–41.0 min). IR: 3400*s*, 2955*s*, 2770*s*, 2550*s*, 2348*w*, 1635*s*, 1610*s*, 1505*s*, 1255*m*, 1088*s*, 1058*s*, 922*m*. $^1$H-NMR ($D_2O$): 7.69 (*d*, $J = 8.9$, 2 arom. H); 6.97 (*d*, $J = 8.9$, 2 arom. H); 3.48 (*t*, $J = 6.7$, 2 H); 3.27–3.02 (*m*, 14 H); 2.24–1.95 (*m*, 6 H); 1.84–1.72 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 174.9 (*s*, CO); 163.5 (*s*, 1 arom. C); 133.7 (*d*, 2 arom. C); 129.2 (*s*, 1 arom. C); 119.8 (*d*, 2 arom. C); 51.4 (*t*, 2 C); 49.9 (*t*); 49.0 (*t*, 2 C); 48.8, 42.9, 40.9, 30.0, 28.4, 27.1 (6*t*); 27.0 (*t*, 2 C). MS: see *7*.

5.4. N-*(16-Amino-4,8,12-triazahexadecyl)-2,5-dihydroxybenzamide* (*2,5-(OH)$_2$-Bz3334,* **AG 395b**). Treatment of **12** (1.62 mmol) according to *GP 5.1* afforded *2,5-(OH)$_2$-Bz3334* · 4 TFA (281 mg, 0.33 mmol; overall yield 20% with respect to **1**) after purification by HPLC (solvent *A:* 0.05% TFA in MeOH; solvent *B:* 0.05% TFA in $H_2O$; 11% *A*; $\lambda = 240$ nm; flow rate 25 ml $min^{-1}$; the product was collected at 41.0–62.0 min). IR: 3360*m*, 3095*m*, 2880*m*, 1670*s*, 1605*m*, 1490*m*, 1425*m*, 1200*s*, 1165*s*, 1130*s*, 835*m*, 800*m*, 720*s*. $^1$H-NMR ($D_2O$): 7.10 (*d*, $J = 3.1$, 1 arom. H); 6.92 (*dd*, $J = 3.1$, 8.8, 1 arom. H); 6.80 (*d*, $J = 8.8$, 1 arom. H); 3.43 (*t*, $J = 6.6$, 2 H); 3.19–2.93 (*m*, 14 H); 2.15–1.88 (*m*, 6 H); 1.80–1.61 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 174.1 (*s*, CONH); 167.1 (*q*, $J = 36$, 4 CO(TFA)); 154.9, 152.9 (2*s*, 2 arom. C); 125.9, 122.7 (2*d*, 2 arom. C); 121.3 (*s*, 1 arom. C); 120.7 (*q*, $J = 291$, 4 $CF_3$); 118.6 (*d*, 1 arom. C); 51.5, 49.7 (2*t*); 49.0, 48.8 (2*t*, 2 × 2 C); 43.2, 40.6, 30.1, 28.3, 27.2 (5*t*); 27.0 (*t*, 2 C). MS: see *7*.

5.5. N-*(16-Amino-4,8,13-triazahexadecyl)-1*H-*indole-3-acetamide* (*IndAc3343,* **AG 416**). Treatment of **13** (0.80 mmol) according to *GP 5.1* afforded *IndAc3343* · 4 HCl (45 mg, 0.08 mmol; overall yield 10% with respect to **1**) after purification by HPLC (solvent *A:* 0.05% HCl in MeOH; solvent *B:* 0.05% HCl in $H_2O$; 25% *A*; $\lambda =$ 254 nm; flow rate 20 ml $min^{-1}$; the product was collected at 17.9–19.5 min). IR: 3380*s*, 3325*m*, 2950*s*, 2750*s*, 2520*m*, 2405*w*, 1650*s*, 1535*m*, 1455*m*, 1060*w*, 780*w*, 740*m*. $^1$H-NMR ($D_2O$): 7.67–7.49 (*m*, 2 arom. H); 7.38–7.15 (*m*, 3 arom. H); 3.76 (*s*, Ar$CH_2$); 3.28 (*t*, $J = 6.5$, $NCH_2$); 3.23–3.01 (*m*, 10 H); 2.97–2.82 (*m*, 4 H); 2.19–1.92 (*m*, 4 H); 1.89–1.73 (*m*, 6 H). $^{13}$C-NMR ($D_2O$): 180.5 (*s*, CO); 140.8, 131.1 (2*s*, 2 arom. C); 129.7, 126.7, 124.1, 122.8, 116.6 (5*d*, 5 arom. C); 112.2 (*s*, 1arom. C); 51.5 (*t*, 2 C); 49.6, 49.1 (2*t*); 48.9 (*t*, 2 C); 41.1, 40.4, 37.0, 30.1, 28.3 (5*t*); 27.3 (*t*, 2 C); 27.1 (*t*). MS: see *7*.

5.6. N-*(16-Amino-4,8,13-triazahexadecyl)-4-hydroxybenzamide* (*4-OH-Bz3343*). Treatment of **14** (1.62 mmol) according to *GP 5.1* afforded *4-OH-Bz3343* · 4 HCl (100 mg, 0.19 mmol; overall yield 12% with respect to **1**) after purification by HPLC (solvent 0.06% HCl in $H_2O$; $\lambda = 254$ nm; flow rate 20 ml $min^{-1}$; the product was collected at 42.5–49.0 min). IR: 3320*m*, 2950*s*, 2780*s*, 1640*m*, 1610*m*, 1510*m*, 1460*m*, 845*m*. $^1$H-NMR ($D_2O$): 7.70 (*d*, $J = 8.8$, 2 arom. H); 6.96 (*d*, $J = 8.8$, 2 arom. H); 3.48 (*t*, $J = 6.6$, 2 H); 3.22–3.06 (*m*, 14 H); 2.22–1.94 (*m*, 6 H); 1.86–1.70 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 175.2 (*s*, CO); 163.8 (*s*, 1 arom. C); 133.9 (*d*, 2 arom. C); 129.6 (*s*, 1 arom. C); 120.0 (*d*, 2 arom. C); 51.5 (*t*, 2 C); 49.8, 49.1 (2*t*); 49.0 (*t*, 2 C); 41.1, 41.0, 30.3, 28.3 (4*t*); 27.3 (*t*, 2 C); 27.2 (*t*). MS: see *7*.

5.7. N-*(16-Amino-4,8,13-triazahexadecyl)-2,5-dihydroxybenzamide* (*2,5-(OH)$_2$-Bz3343*). Treatment of **15** (1.62 mmol) according to *GP 5.1* afforded *2,5-(OH)$_2$-Bz3343* · 4 HCl (110 mg, 0.20 mmol; overall yield 12% with respect to **1**) after purification by HPLC (solvent *A:* 0.06% HCl in MeOH; solvent *B:* 0.06% HCl in $H_2O$; 11% *A*; $\lambda = 254$ nm; flow rate 20 ml $min^{-1}$; the product was collected at 17.0–21.0 min). IR: 3425*m*, 3360*m*, 2950*s*, 2750*s*, 1645*w*, 1600*m*, 1490*s*, 1230*m*, 820*m*, 790*w*. $^1$H-NMR ($D_2O$): 7.09 (*d*, $J = 3.0$, 1 arom. H); 6.93 (*dd*, $J = 3.0$, 8.9, 1 arom. H); 6.82 (*d*, $J = 8.9$, 1 arom. H); 3.46 (*t*, $J = 6.7$, 2 H); 3.23–3.04 (*m*, 14 H); 2.23–1.95 (*m*, 6 H); 1.84–1.74 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 174.0 (*s*, CO); 155.2, 152.8 (2*s*, 2 arom. C); 126.0, 122.7 (2*d*, 2 arom. C); 121.4 (*s*, 1 arom. C); 118.5 (*d*, 1 arom. C); 51.5 (*t*, 2 C); 49.9, 49.10, 49.06, 49.0, 41.2, 40.8, 30.2, 29.3 (8*t*); 27.3 (*t*, 2 C); 27.2 (*t*). MS: see *7*.

5.8. N-*(16-Amino-4,9,13-triazahexadecyl)-1*H-*indole-3-acetamide* (*IndAc3433*). Treatment of **19** (1.55 mmol) according to *GP 5.1* afforded *IndAc3433* · 4 HCl (105 mg, 0.19 mmol; overall yield 12% with respect to **1**) after purification by HPLC (solvent *A:* 0.05% HCl in MeOH; solvent *B:* 0.05% HCl in $H_2O$; 12.5% *A*; $\lambda = 220$ nm; flow rate 20 ml $min^{-1}$; the product was collected at 25.5–36.5 min). IR: 3400*s*, 3300*m*, 2950*s*, 2750*s*, 2490*m*, 1650*m*, 1610*w*, 1455*m*, 740*m*. $^1$H-NMR ($D_2O$): 7.67–7.47 (*m*, 2 arom. H); 7.38–7.16 (*m*, 3 arom. H); 3.77 (*s*, Ar$CH_2$); 3.34–2.65 (*m*, 16 H); 2.22–2.07 (*m*, 4 H); 1.89–1.54 (*m*, 6 H). $^{13}$C-NMR ($D_2O$): 180.5 (*s*, CO); 140.9, 131.1 (2*s*, 2 arom. C); 129.7, 126.7, 124.1, 122.9, 116.6 (5*d*, 5 arom. C); 112.3 (*s*, 1 arom. C); 51.5, 51.3, 49.4, 49.3, 49.2, 49.0, 41.1, 40.5, 37.0, 30.1, 28.3 (11*t*); 27.2 (*t*, 2 C); 27.1 (*t*). MS: see *7*.

5.9. N-*(16-Amino-4,9,13-triazahexadecyl)-4-hydroxybenzamide* (*4-OH-Bz3433*). Treatment of **20** (1.62 mmol) according to *GP 5.1* afforded *4-OH-Bz3433* · 4 TFA (159 mg, 0.2 mmol; overall yield 12% with respect to **1**) after purification by HPLC (solvent *A:* 0.05% TFA in MeOH; solvent *B:* 0.05% TFA in $H_2O$; 0–65 min 5% *A*; 65–100 min 20% *A*; $\lambda = 254$ nm; flow rate 20 ml $min^{-1}$; the product was collected at 83.5–89.1 min). IR: 3400*m*, 3100*m*, 2880*m*, 1670*s*, 1605*m*, 1510*m*, 1475*m*, 1425*m*, 1200*s*, 1160*s*, 1130*s*, 835*m*, 800*m*, 720*s*. $^1$H-NMR ($D_2O$): 7.61 (*d*, $J = 8.8$, 2 arom. H); 6.88 (*d*, $J = 8.8$, 2 arom. H); 3.40 (*t*, $J = 6.6$, 2 H); 3.16–2.97

(*m*, 14 H); 2.18–1.86 (*m*, 6 H); 1.78–1.66 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 175.0 (*s*, CONH); 167.1 (*q*, $J = 36$, 4 CO(TFA)); 163.7 (*s*, 1 arom. C); 133.8 (*d*, 2 arom. C); 129.5 (*s*, 1 arom. C); 120.7 (*q*, $J = 291$, 4 $CF_3$); 119.9 (*d*, 2 arom. C); 51.4, 51.3, 49.6, 49.1, 49.0, 48.8, 41.0, 40.9, 30.2, 28.2 (10*t*); 27.2 (*t*, 2 C); 27.1 (*t*). MS: see *7*.

5.10. N-*(16-Amino-4,9,13-triazahexadecyl)-2,5-dihydroxybenzamide* (*2,5-(OH)$_2$-Bz3433*). Treatment of **21** (1.64 mmol) according to *GP 5.1* afforded *2,5-(OH)$_2$-Bz3433* · 4 TFA (182 mg, 0.21 mmol; overall yield 13% with respect to **1**) after purification by HPLC (solvent *A:* 0.05% TFA in MeOH; solvent *B:* 0.05% TFA in $H_2O$; 0–75 min 9% A; 75–100 min 20% *A*; $\lambda = 240$ nm; flow rate 25 ml min$^{-1}$; the product was collected at 86.5–90.0 min). IR: 3380*m*, 3095*m*, 2890*m*, 1670*s*, 1600*m*, 1480*w*, 1425*w*, 1200*s*, 1165*s*, 1135*s*, 835*w*, 800*m*, 720*m*. $^1$H-NMR ($D_2O$): 7.12 (*d*, $J = 3.1$, 1 arom. H); 6.96 (*dd*, $J = 3.1$, 9.0, 1 arom. H); 6.85 (*d*, $J = 9.0$, 1 arom. H); 3.47 (*t*, $J = 6.7$, 2 H); 3.23–3.04 (*m*, 14 H); 2.25–1.93 (*m*, 6 H); 1.85–1.71 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 174.0 (*s*, CONH); 167.0 (*q*, $J = 36$, 4 CO(TFA)); 155.1, 152.8 (2*s*, 2 arom. C); 126.4, 122.6 (2*d*, 2 arom. C); 121.4 (*s*, 1 arom. C); 120.6 (*q*, $J = 291$, 4 $CF_3$); 118.5 (*d*, 1 arom. C); 51.2, 49.5, 49.1 (3*t*); 49.0 (*t*, 2 C); 48.8, 40.9, 40.6, 30.1, 28.1 (5*t*, 5 C); 27.1 (*t*, 2 C); 27.0 (*t*). MS: see *7*.

5.11. N-*(16-Amino-5,9,13-triazahexadecyl)-1*H*-indole-3-acetamide* (*IndAc4333*, **AG 416b**). Treatment of **16** (1.55 mmol) according to *GP 5.1* afforded *IndAc4333* · 4 HCl (97 mg, 0.173 mmol; overall yield 11% with respect to **1**) after purification by HPLC (solvent *A:* 0.05% HCl in MeOH; solvent *B:* 0.05% HCl in $H_2O$; 12.5% *A*; $\lambda = 220$ nm; flow rate 20 ml min$^{-1}$; the product was collected at 31.5–42.0 min). IR: 3380*s*, 3320*m*, 2950*s*, 2750*s*, 2490*m*, 2410*m*, 1655*m*, 1610*w*, 1525*m*, 1455*m*, 1050*w*, 770*w*, 740*m*. $^1$H-NMR ($D_2O$): 7.55–7.40 (*m*, 2 arom. H); 7.25–7.03 (*m*, 3 arom. H); 3.63 (*s*, Ar$CH_2$); 3.14–2.95 (*m*, 12 H); 2.91–2.80 (*m*, 4 H); 2.10–1.87 (*m*, 6 H); 1.53–1.33 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 179.9 (*s*, CO); 140.9, 131.2 (2*s*, 2 arom. C); 129.6, 126.6, 124.0, 122.9, 116.6 (5*d*, 5 arom. C); 112.3 (*s*, 1 arom. C); 51.7, 49.3 (2*t*); 49.1 (*t*, 3 C); 48.6, 43.1, 41.1, 37.1, 30.1, 28.3, 27.3, 27.2, 27.1 (9*t*). MS: see *7*.

5.12. N-*(16-Amino-5,9,13-triazahexadecyl)-4-hydroxybenzamide* (*4-OH-Bz4333*, **AG 379a**). Treatment of **17** (1.64 mmol) according to *GP 5.1* afforded *4-OH-Bz4333* · 4 HCl (250 mg, 0.48 mmol; 30% with respect to **1**) without further purification. IR: 3320*s*, 2955*s*, 2755*s*, 2480*s*, 2415*s*, 2020*w*, 1860*w*, 1635*s*, 1610*s*, 1508*s*, 1450*s*, 1270*m*, 1165*m*, 1050*s*, 850*m*, 770*s*. $^1$H-NMR ($D_2O$): 7.66 (*d*, $J = 8.8$, 2 arom. H); 6.93 (*d*, $J = 8.8$, 2 arom. H); 3.37 (*t*, $J = 6.7$, 2 H); 3.26–3.05 (*m*, 14 H); 2.23–2.04 (*m*, 6 H); 1.82–1.59 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 174.7 (*s*, CO); 163.6 (*s*, 1 arom. C); 133.8 (*d*, 2 arom. C); 129.9 (*s*, 1 arom. C); 119.9 (*d*, 2 arom. C); 52.0, 49.2, 49.13 (3*t*); 49.09 (*t*, 2 C); 48.8, 43.4, 41.0, 30.3, 28.2, 27.5 (6*t*); 27.1 (*t*, 2 C). MS: see *7*.

5.13. N-*(16-Amino-5,9,13-triazahexadecyl)-2,5-dihydroxybenzamide* (*2,5-(OH)$_2$-Bz4333,* **AG 395c**). Treatment of **18** (1.62 mmol) according to *GP 5.1* afforded *4-OH-Bz3334* · 4 HCl (200 mg, 0.37 mmol; overall yield 23% with respect to **1**) without further purification. IR: 3423*s*, 2956*s*, 2756*s*, 2496*s*, 2415*s*, 1690*w*, 1650*m*, 1603*s*, 1590*s*, 1460*s*, 1265*m*, 1050*s*. $^1$H-NMR ($D_2O$): 7.13 (*d*, $J = 3.1$, 1 arom. H); 6.97 (*dd*, $J = 3.1$, 8.8, 1 arom. H); 6.87 (*d*, $J = 8.8$, 1 arom. H); 3.41 (*t*, $J = 6.5$, 2 H); 3.26–3.08 (*m*, 14 H); 2.22–2.05 (*m*, 6 H); 1.84–1.63 (*m*, 4 H). $^{13}$C-NMR ($D_2O$): 173.7 (*s*, CO); 155.0, 152.9 (2*s*, 2 arom. C); 125.9, 122.8 (2*d*, 2 arom. C); 122.0 (*s*, 1 arom. C); 118.6 (*d*, 1 arom. C); 51.9, 49.3 (2*t*); 49.1 (*t*, 3 C); 48.8, 43.3, 41.1, 30.1, 28.3, 27.5 (6*t*); 27.2 (*t*, 2 C). MS: see *7*.

6. *2-{1-[(*tert*-Butyl)dimethylsilyl]-1*H*-indole-3-acetic acid*. To 1*H*-Indole-3-acetic acid (1.3 g, 7.4 mmol) in DMF (15 ml) at 0° was added NaH (0.445 g, 18.6 mmol). After 30 min at 0°, (*t*-Bu)$Me_2$SiCl (1.23 g, 8.1 mmol) was added. The soln. was stirred for 1 h at 0°, poured into $H_2O$ (200 ml), and acidified with 10% aq. HCl soln. Crystallization of the acid formed was completed by cooling the soln. to 4° for 30 min. The colorless precipitate was filtered off, washed with $H_2O$, and dried *in vacuo* for 15 h to give *TBSIndAcOH* (2.00 g, 6.9 mmol; 93%). M.p. ($H_2O$): 144–145°. IR: 3400*s*, 2929*s*, 1709*s*, 1452*s*, 1316*s*, 1258*m*, 1216*m*, 1139*s*, 970*s*, 739*s*. $^1$H-NMR ($CDCl_3$): 10.08 (br *s*, COOH); 7.65–7.69 (*m*, 2 arom. H); 7.22–7.11 (*m*, 3 arom. H); 3.80 (*s*, $CH_2$); 0.94 (*s*, *t*-Bu); 0.60 (*s*, 2 Me). $^{13}$C-NMR ($CDCl_3$): 177.9 (*s*, CO); 141.3, 130.5 (2*s*, 2 arom. C); 130.0, 121.7 (2*d*, 2 arom. C); 119.8 (*s*, 1 arom. C); 118.8, 114.0 (*d*, 2 arom. C); 109.7 (*s*, $CH_2C$); 31.2 (*t*, $CH_2$); 26.3 (*q*, $Me_3C$); 19.4 (*s*, $Me_3C$); −4.0 (*q*, $Me_2Si$). CI-MS: 290 (100, $[M+H]^+$).

7. *HPLC-UV(DAD)-APCI-MS and -MS/MS Investigation.* 7.1. *Equipment and Conditions.* Solvents and reagents: MeCN (HPLC grade, *Scharlau*, E-Barcelona); TFA, *purum* (*Fluka*, CH-Buchs). $H_2O$ was purified with an *Milli-Q$_{RG}$* apparatus (*Millipore*, Milford, MA, USA). Venom preparation: lyophilized venom (5.0 mg) was dissolved at 23° in TFA soln. (1% TFA in $H_2O$/MeCN 3:2, 60 µl). The hazy soln. was filtered through a 0.45 µm filter (*Eppendorf*, D-Hamburg), and the filter was rinsed with $H_2O$ (20 µl). The combined filtrates provided the clear stock soln. that was stored at −20° and was used for the following HPLC/MS investigations. Samples of 1–5 µl of the venom stock soln., of 1–5 µl of a toxin stock soln. (1 mM synthetic polyamine derivatives in 0.75% TFA in $H_2O$/MeCN 7:3), or of a 1:1 mixture of venom/toxin stock soln. were injected for the individual runs and were analyzed by HPLC-UV(DAD), HPLC-UV(DAD)-APCI-MS, and HPLC-UV(DAD)-APCI-MS/MS.

Table 2. *MS/MS Signals of Natural and Synthetic Compounds with Quasi-Molecular Ions at* m/z *417* (IndAc-Pentamines)

| *m/z* | Signals and retention times $t_R$ | | | | | |
|---|---|---|---|---|---|---|
| | Natural venom | | Synthetic samples with the IndAc group attached to the polyamines | | | |
| | $t_R$ 26.0 | $t_R$ 27.5 | $t_R$ 26.0 *PA3334* (**AG 416a**) | $t_R$ 26.0 *PA3343* (**AG 416**) | $t_R$ 26.0 *PA3433* (–) | $t_R$ 27.5 *PA4333* (**AG 416b**) |
| 58 | 2 | 2 | 1 | 2 | – | 2 |
| 70 | – | 5 | – | – | – | 4 |
| 72 | 6 | 4 | 11 | 2 | – | 4 |
| 84 | 4 | 9 | 4 | 6 | 3 | 3 |
| 98 | 15 | 49 | 25 | – | 6 | 65 |
| 112 | 59 | 41 | 46 | 100 | 53 | 11 |
| 115 | 16 | 36 | 19 | – | 19 | 46 |
| 127 | – | 3 | – | – | – | – |
| 129 | 18 | 9 | 8 | 55 | 3 | 3 |
| 130 | 10 | 5 | 10 | 4 | 9 | 5 |
| 132 | 2 | 4 | 1 | – | 1 | 2 |
| 144 | – | 2 | – | – | – | 1 |
| 155 | – | 4 | – | – | – | 3 |
| 158 | 1 | 2 | 2 | 1 | – | 2 |
| 159 | 1 | 1 | 2 | 1 | 2 | 2 |
| 169 | 5 | 7 | 7 | – | 1 | - |
| 170 | – | 6 | – | – | – | 1 |
| 172 | 1 | 34 | 1 | – | - | 41 |
| 186 | 24 | 4 | 11 | 3 | 40 | 2 |
| 187 | 4 | 2 | – | 1 | 2 | 3 |
| 189 | 2 | 2 | 2 | – | – | 2 |
| 203 | 1 | 2 | 1 | – | – | – |
| 215 | 100 | 22 | 100 | 58 | 100 | 1 |
| 228 | 2 | 4 | 1 | – | - | 5 |
| 229 | – | 16 | – | – | – | 24 |
| 241 | – | 2 | – | – | – | 1 |
| 243 | 2 | 2 | 3 | – | – | 1 |
| 258 | – | 3 | – | – | – | 2 |
| 260 | 3 | 9 | 3 | – | – | 9 |
| 268 | – | – | – | – | – | 1 |
| 272 | 29 | 9 | 45 | 22 | – | 3 |
| 286 | 21 | 81 | – | 1 | 46 | 92 |
| 289 | 7 | 4 | 7 | 12 | – | – |
| 303 | – | 19 | – | – | – | 25 |
| 311 | 8 | 2 | 10 | – | – | – |
| 325 | 4 | 3 | – | 14 | – | 2 |
| 328 | 13 | 3 | 22 | – | – | – |
| 329 | 11 | 4 | 22 | – | – | – |
| 343 | 7 | 37 | – | 20 | – | 45 |
| 346 | 19 | 10 | 26 | – | – | – |
| 360 | – | 3 | – | – | – | 3 |
| 382 | 5 | 1 | 8 | – | – | – |
| 399 | 18 | 6 | 25 | 4 | – | 3 |
| 400 | 10 | 7 | 13 | – | – | 8 |
| 417 | 26 | 100 | 25 | 2 | 4 | 100 |

Table 3. *MS/MS Signals of Natural and Synthetic Compounds with Quasi-Molecular Ions at* m/z *380* (4-OH-Bz-Pentamines)

| *m/z* | Signals and retention times $t_R$ | | | | | |
|---|---|---|---|---|---|---|
| | Natural venom | | Synthetic samples with the 4-OH-Bz group attached to the polyamines | | | |
| | $t_R$ 16.7 | $t_R$ 17.8 | $t_R$ 16.7 *PA3334* (–) | $t_R$ 16.7 *PA3343* (**AG 379**) | $t_R$ 16.7 *PA3433* (–) | $t_R$ 17.8 *PA4333* (**AG 379a**) |
| 44 | 1 | 2 | – | – | – | 2 |
| 58 | 2 | 5 | 1 | 2 | – | 3 |
| 70 | 3 | 3 | – | – | – | 2 |
| 72 | 18 | 3 | 15 | 3 | – | 2 |
| 84 | 9 | 3 | 5 | 9 | 3 | 3 |
| 98 | 24 | 82 | 29 | – | 8 | 100 |
| 112 | 81 | 33 | 48 | 100 | 51 | 16 |
| 115 | 22 | 54 | 17 | – | 18 | 49 |
| 121 | 14 | 18 | 11 | 5 | 12 | 15 |
| 129 | 25 | 7 | 12 | 46 | 1 | 5 |
| 132 | 3 | 4 | 1 | – | 2 | 4 |
| 138 | – | 4 | – | – | – | – |
| 148 | – | – | 1 | – | – | – |
| 150 | 2 | 3 | 1 | – | – | – |
| 155 | – | 4 | – | – | – | 2 |
| 169 | 8 | 3 | 7 | – | – | – |
| 172 | 3 | 34 | 3 | – | – | 39 |
| 176 | – | 2 | – | – | – | – |
| 178 | 100 | 10 | 100 | 48 | 100 | 1 |
| 186 | 9 | 4 | 8 | 1 | 18 | 2 |
| 189 | 1 | 2 | 4 | – | – | |
| 191 | 5 | 3 | 2 | 2 | – | |
| 192 | – | 42 | – | – | – | 34 |
| 203 | 2 | – | – | – | – | – |
| 205 | – | 2 | – | – | – | – |
| 206 | – | – | 1 | – | – | – |
| 207 | 2 | – | 2 | – | – | – |
| 220 | – | – | 2 | – | – | – |
| 221 | 2 | 2 | – | – | – | – |
| 235 | 49 | 10 | 34 | 18 | – | 2 |
| 243 | 3 | 5 | 9 | – | – | 2 |
| 249 | 3 | 100 | – | – | 25 | 85 |
| 252 | 10 | 2 | 11 | 5 | – | – |
| 260 | 5 | 31 | 9 | – | – | 22 |
| 266 | – | 12 | – | – | – | 16 |
| 274 | 7 | – | 7 | – | – | – |
| 288 | 4 | 2 | – | 4 | – | 2 |
| 292 | 24 | 3 | 19 | – | – | – |
| 306 | 5 | 43 | – | 11 | – | 38 |
| 309 | 23 | 3 | 11 | – | – | – |
| 322 | – | – | – | – | – | 3 |
| 323 | – | 3 | – | – | – | – |
| 345 | 2 | – | 4 | – | – | – |
| 362 | 11 | 5 | 10 | 1 | – | 3 |
| 363 | 5 | 5 | 9 | – | – | 5 |
| 380 | 11 | 42 | 10 | – | – | 39 |

Table 4. *MS/MS Signals of Natural and Synthetic Compounds with Quasi-Molecular Ions at* m/z *396* (2,5-$(OH)_2$-Bz-Pentamines)

| *m/z* | Signals and retention times $t_R$ | | | | | |
|---|---|---|---|---|---|---|
| | Natural venom | | Synthetic samples with the 2,5-$(OH)_2$-Bz group attached to the polyamines | | | |
| | $t_R$ 19.5 | $t_R$ 21.8 | $t_R$ 19.5 *PA3334* (**AG 395b**) | $t_R$ 19.5 *PA3343* (–) | $t_R$ 19.5 *PA3433* (–) | $t_R$ 21.8 *PA4333* (**AG 395c**) |
| 44 | 3 | 8 | – | – | – | |
| 58 | 2 | 6 | 2 | 3 | 1 | 3 |
| 70 | 6 | 6 | 2 | – | – | 4 |
| 72 | 25 | – | 27 | 2 | – | 3 |
| 84 | 5 | 7 | 6 | 7 | 3 | 3 |
| 98 | 39 | 91 | 44 | – | 9 | 100 |
| 112 | 100 | 29 | 69 | 100 | 55 | 15 |
| 115 | 28 | 47 | 33 | 3 | 22 | 65 |
| 129 | 36 | 43 | 15 | 50 | 10 | 21 |
| 132 | 5 | – | 3 | – | 2 | 5 |
| 137 | 9 | 11 | 13 | 5 | 9 | 5 |
| 141 | – | – | 1 | – | – | – |
| 146 | – | – | – | – | – | 2 |
| 155 | – | 11 | – | – | – | 3 |
| 166 | 4 | – | 2 | 1 | 4 | – |
| 169 | 11 | – | 8 | – | – | 2 |
| 172 | 15 | 58 | 9 | – | – | 37 |
| 186 | 16 | 29 | 10 | 10 | 22 | 12 |
| 189 | 12 | 7 | 15 | – | – | 5 |
| 194 | 91 | 15 | 100 | 23 | 100 | – |
| 203 | 2 | – | 2 | – | – | – |
| 207 | 3 | 8 | 2 | – | – | 4 |
| 208 | – | 24 | – | – | – | 14 |
| 223 | 3 | – | 1 | – | – | – |
| 237 | – | 12 | – | – | – | 2 |
| 243 | 13 | 16 | 15 | – | – | 4 |
| 251 | 82 | 10 | 49 | 19 | – | 2 |
| 260 | 10 | 70 | 18 | – | 1 | 42 |
| 265 | 3 | 66 | – | – | 38 | 70 |
| 268 | 11 | – | 5 | 4 | – | – |
| 282 | – | 22 | – | – | – | 12 |
| 290 | 7 | – | 10 | – | – | – |
| 304 | 3 | 7 | – | 6 | – | – |
| 308 | 46 | 10 | 28 | – | – | – |
| 322 | 8 | 100 | – | 17 | – | 55 |
| 325 | 27 | 14 | 29 | – | – | – |
| 338 | – | – | – | – | – | – |
| 339 | – | 9 | – | – | – | 3 |
| 361 | 6 | – | 4 | – | – | – |
| 378 | 21 | – | 11 | – | – | 1 |
| 379 | 21 | 12 | 10 | – | – | 5 |
| 396 | 30 | 32 | 18 | – | 2 | 61 |

*HPLC-UV(DAD):* Instrumentation: *Waters 626-LC* system, fitted to a *996* photodiode-array detector, a *600S* controller, a *Millennium Chromatography Manager 2010* v. 2.15 (*Waters Corp.,* Milford, MA, USA.), and a *Rheodyne-Rotary-7725i* rotary valve with a 5-µl loop (*Rheodyne*, Cotati, CA, USA). Column and chromatographic conditions: *Macherey-Nagel $C_{18}$ HD* column (3 µm, 4.6 × 250 mm; *Macherey-Nagel*, F-Hoerdt); flow rate 0.5 ml $min^{-1}$. Mobile phase: step gradient over 5 min from 0 to 10% of solvent *B*, then over 75 min from 10 to 45% of *B*, and finally over 20 min from 45 to 100% of *B* (solvent *A:* 0.1% soln. of TFA in $H_2O$, solvent *B:* 0.1% soln. of TFA in MeCN).

*MS:* APCI-MS and APCI-MS/MS experiments were performed on a *Finnigan-TSQ-700* triple-stage quadrupole instrument equipped with an atmospheric-pressure chemical-ionization (APCI) ion source (*Finnigan,* San José, CA, USA). The APCI operating conditions in positive mode were: vaporizer temp. 450°; corona voltage 5 kV; heated capillary temp. 250°; sheath gas $N_2$ with an inlet pressure of 40 PSI; conversion dynode: −15 kV. For MS/MS experiments: collision gas Ar with a relative pressure of 2.5–3.3 mTorr; collision-induced dissociation offset (Coff): −27 eV.

7.2. *Results of the ESI-MS/MS Investigation. See Tables 2–4.*

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