Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 2387–2391
Tetrahedron

# Solid-phase synthesis of $^{15}$N-labeled acylpentamines as reference compounds for the MS/MS investigation of spider toxins

Nikolay Manov, Manuel Tzouros, Laurent Bigler and Stefan Bienz*

*Institute of Organic Chemistry, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland*

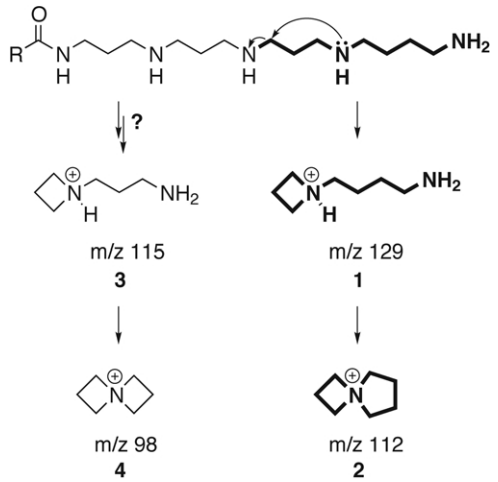
Received 16 June 2003; revised 19 November 2003; accepted 24 December 2003

**Abstract**—A solid-phase route for synthesis of $^{15}$N-labeled acylpolyamines is described. Utilizing alkylation at benzylic N-atom as a key step, $^{15}$N-atoms are incorporated by stepwise construction of the polyamine framework on the solid support. The derivatives were used as reference compounds for the investigation of the MS/MS behavior of spider toxins.


## 1. Introduction

The venoms of spiders are typically complex mixtures of free amino acids, heterocyclic bases, and large proteinaceous toxins, but also of relatively small polyamine toxins. The latter—usually consisting of a polyazaalkane backbone conjugated to an aromatic head moiety—arose particular interest over the last two or three decades, due to their interesting neurotoxic activities.[6,11] We also contributed to the research in the field of spider toxins by the isolation and structure elucidation of such compounds, as well as their synthesis. We were particularly interested in the venom of *Agelenopsis aperta*, wherein as many as 38 polyamine toxins have been identified so far by on-line coupled HPLC–MS/MS.[2,5] The characterization of these venom components was rather difficult (and is still not complete) because the several compounds differing by structural isomerisms within the polyamine backbones, their site of derivatization, or within the aromatic head portions are difficult to differentiate. The unequivocal characterization of some of the toxins with isomeric polyamine backbones was only possible through their comparison with reference samples that were specifically synthesized to this end.[5]

The synthetic acylpentamines investigated so far have not only allowed the direct comparison of structurally secured compounds with the components of the venom of *A. aperta* but have also permitted the study of the fragmentation behavior of isomeric acylpolyamines upon collision induced decomposition (CID) in MS/MS.[5] Whereas the fragmentation patterns of the differently constructed acylpentamines proved distinct enough for the unequivocal correlation of the natural products with the synthetic samples, the fragmentation mechanisms were not completely understood. It was originally assumed that the structures of the polyamine backbones could be deduced by the identification of diagnostic signals deriving from the polyamine termini. For IndAc3334, for instance, a fragment doublet for **1** and **2** at *m/z* 129/112 deriving from the terminal PA34 unit as indicated in Scheme 1 was expected, and the respective signals were in fact found. But in addition to these signals, a doublet at *m/z* 115/98 was also detected. This doublet, however, corresponds to fragments **3** and **4** that would be considered as diagnostic for a polyamine derivative possessing a PA33 rather than a PA34 terminus. Its occurrence in the spectrum of the natural toxin fraction was initially interpreted as deriving from an isomeric co-eluting minor venom component, and only with the




**Scheme 1.**

*Keywords*: $^{15}$N-labeled; Polyamine; Spider toxin; Solid-phase synthesis; MS/MS.
* Corresponding author. Tel.: +41-1-635-42-45; fax: +41-1-635-68-12; e-mail address: sbienz@oci.unizh.ch

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.12.066

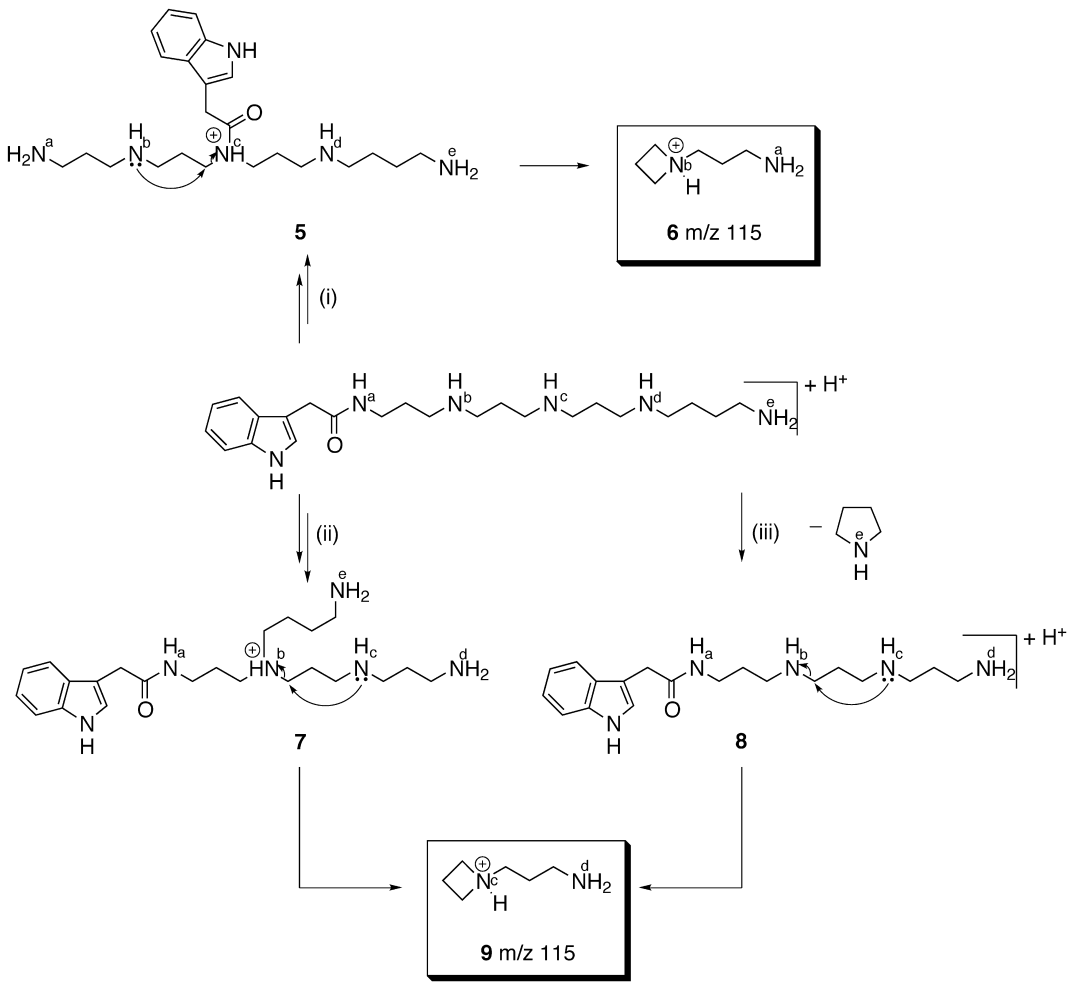
synthetic reference samples at hand it was recognized as a real fragment doublet deriving from IndAc3334. Since the detailed knowledge of the fragmentation behavior of polyamine derivatives might be helpful for the unequivocal structure elucidation of new polyamine compounds (particularly of such arising in very small amounts), we addressed a more detailed study to the deduction of the MS/MS behavior of polyamine compounds.

## 2. Results and discussion

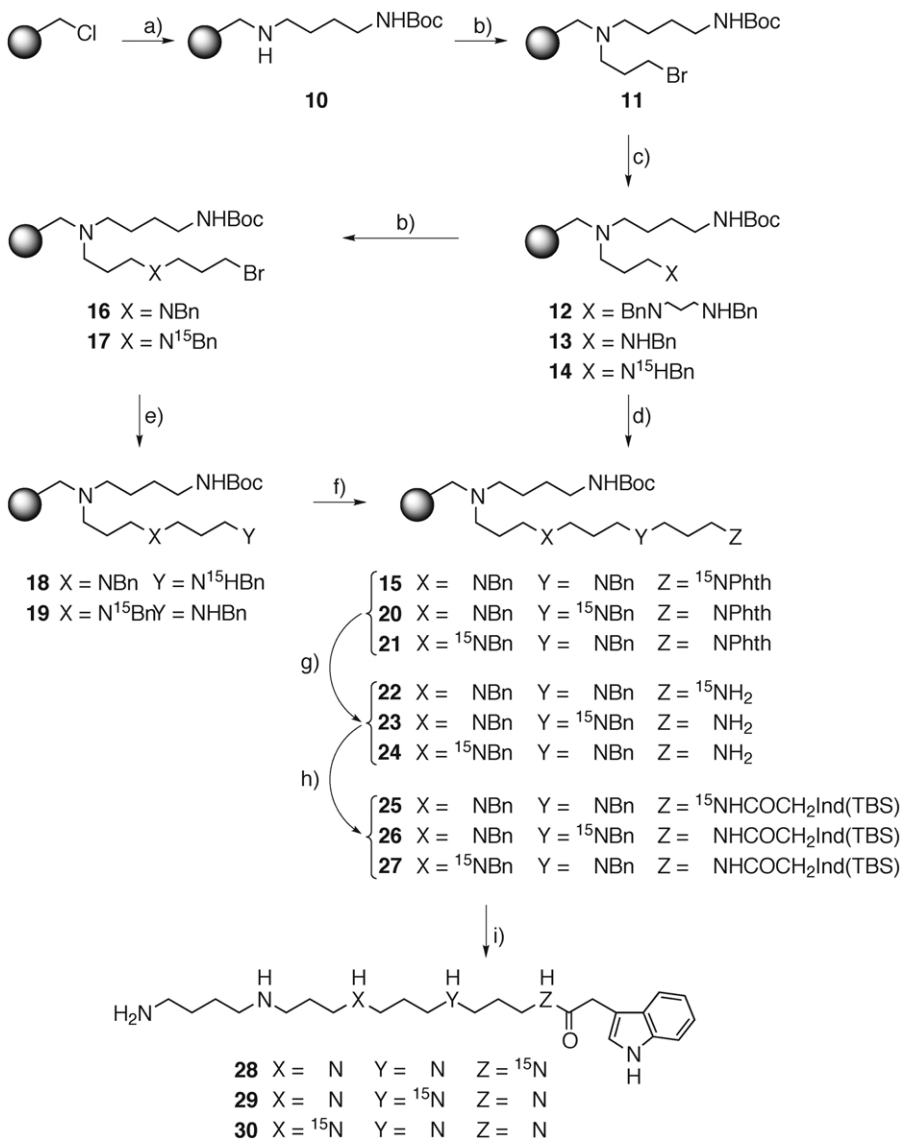
For the formation of the unexpected signals, two mechanisms were proposed (Scheme 2): for IndAc3334, for example, mechanisms involving (i) repetitive transamidations[1] or (ii) a cascade of transaminations[3] would lead to structures **5** and **7**, respectively, both containing a terminal PA33 unit prone to loose the observed fragments. The two ZIP-reactions have already been discussed previously for the explanation of the fragmentation behavior of polyamine derivatives.[5] An alternative to the ZIP-reaction/fragmentation mechanisms would be (iii) a stepwise fragmentation of the sample molecule, initiated by the loss of pyrrolidine to give **8**; a structure possessing a terminal PA33 moiety, too. To determine, which moiety of IndAc3334 is contained in the fragments **3** and **4**, and thus to distinguish between fragmentation mechanism (i) and mechanisms (ii) or (iii), we have synthesized labeled compounds **28**–**30** (IndAc$^{15}$N3334, IndAc3$^{15}$N334, and IndAc33$^{15}$N34, Scheme 3).

In analogy to our previous report, Merrifield resin (200–400 mesh, 1% divinylbenzene, 0.8 mmol g$^{-1}$ loading capacity) was reacted with mono-Boc-protected putrescine[7] to form intermediate **10**, which was then alkylated with 1,3-dibromopropane to obtain resin **11**.[5] Resin **11** was then derivatized by treatment with either *N*,*N*′-dibenzylpropane-1,3-diamine[8] or benzylamine to obtain resins **12** and **13**, respectively. A portion of resin **11** was used as the starting material for the introduction of the first $^{15}$N-label into the future pentamine backbone.

This was done by reacting the intermediate with commercially available $^{15}$*N*-benzylamine to yield resin **14**. Resin **13** and labeled resin **14** were further alkylated with 1,3-dibromopropane to prolongate the chain. The obtained intermediates **16** and **17** were reacted with $^{15}$*N*-benzylamine and benzylamine, respectively, to access compounds **18** and **19**. Alkylation of the terminal secondary amine with *N*-(3-bromopropyl)phthalimide yielded finally pentaminic resins **20** and **21**. The remaining intermediate **12** was treated with $^{15}$*N*-(3-bromopropyl)phthalimide[9] to yield resin **15** which bears the label at the terminal N-atom. As described earlier, the phthaloyl protective groups were removed from resins




**Scheme 2.**




**Scheme 3.** (a) *tert*-Butyl *N*-(4-aminobutyl)carbamate. (b) 1,3-dibromopropane, DIEA. (c) *N*,*N*′-dibenzylpropane-1,3-diamine, benzylamine, or $^{15}N$-benzylamine, DIEA. (d) $^{15}N$-(3-bromopropyl)phthalimide, DIEA. (e) $^{15}N$-benzylamine or benzylamine, DIEA. (f) *N*-(3-bromopropyl)phthalimide, DIEA. (g) $N_2H_4{\times}H_2O$. (h) (TBS)IndAcOH, DIC. (i) 1-chloroethyl chloroformate (ACE-Cl), then MeOH.

**15**, **20**, and **21** by treatment with $N_2H_4{\cdot}H_2O$ to yield compounds **22**, **23**, and **24**. Their acylation with TBS-protected 3-indoleacetic acid gave resins **25**, **26**, and **27**, and their treatment with ACE-Cl in $CH_2Cl_2$, followed by refluxing in MeOH, afforded finally the three labeled IndAc$^{15}$N3334, IndAc3$^{15}$N334 and IndAc33$^{15}$N34.

The MS/MS investigation of these materials revealed that the internal portion of the acylpolyamine, as indicated in Figure 1, is the moiety of the molecule giving rise to the fragments **3** and **4**.[12] Thus, mechanism (i) for their formation can be excluded, but the two mechanism (ii) and (iii) can still not be distinguished with the result.

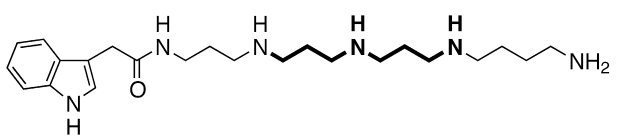


**Figure 1.**

## 3. Experimental

### 3.1. General

Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. As the solid support, Merrifield peptide resin 200–400 mesh, 1% divinylbenzene, loading 0.8 mmol $g^{-1}$ from Advanced ChemTech was used. Instrumentation for the solid phase reactions: PLS 1×6 Organic Synthesizer. IR spectra as KBr presslings; Perkin–Elmer 781; in $cm^{-1}$. $^1H$ NMR spectra in $D_2O$; Bruker AC-300 (300 MHz); $\delta$ in ppm rel. to TSP ($\delta$ 0.00), $J$ in Hz. $^{13}C$ NMR spectra in $D_2O$; Bruker ARX-300 (75.5 MHz); $\delta$ in ppm rel. to TSP ($\delta$ 1.7); multiplicities from DEPT-135 and DEPT-90 experiments. Preparative chromatographic conditions (HPLC): columns Kromasil KR100-10C18 (4.6×250 mm) and Kromasil KR100-10C18 (50.8×250 mm); the $H_2O$ was purified with a Milli-$Q_{RG}$ apparatus. Proof of structures and purities of the final polyamine derivatives is provided by their $^1H$ NMR

and $^{13}C$ NMR spectra and by their HPLC analysis. ESI-MS experiments were carried out on a Finnigan-TSQ-700 triple-stage quadrupole instrument equipped with an electrospray (ESI) ion source (Finnigan, San José, CA, USA). Elemental analyses and HRMS is not appropriate for polyamine derivatives since the compounds arise, as free bases, as waxy or glassy solids only, from which the last solvent molecules can hardly be removed. The hydrochloric salts are rather hygroscopic, and the uptake of water falsifies the elemental analyses. The compounds are not stable enough to survive distillation and show heavy fragmentation in EI-MS. HRMS on the molecular ions is thus not possible, and HRMS on fragment ions are not informative enough to prove the overall structures.

### 3.2. Construction of the polyamine backbones on the resin

**3.2.1. Derivatization of Merrifield resin with *tert*-butyl *N*-(4-aminobutyl)carbamate (resin 10).** Merrifield resin (4.00 g, 3.20 mmol) was swelled in 1-methyl-2-pyrrolidone (NMP) (40 ml). *tert*-Butyl-*N*-(4-aminobutyl)carbamate (3.61 g, 19.20 mmol,[7]) was added and the mixture was stirred for 21 h at 50 °C. The resin was filtered off, washed successively with NMP and $CH_2Cl_2$, and dried in vacuo at 50 °C. The loading, 0.65 mmol $g^{-1}$ (100%), was measured by Volhard titration.[4]

**3.2.2. Alkylation of resin 10 with 1,3-dibromopropane (resin 11).** Resin **10** (2.00 g, 1.30 mmol) was suspended in NMP (15 ml). 1,3-Dibromopropane (1.33 ml, 13.00 mmol) and DIEA (2.23 ml, 13.00 mmol) were added, and the mixture was agitated for 20 h at 50 °C. The resin was filtered off, washed with NMP and $CH_2Cl_2$, and dried in vacuo at 50 °C.

**3.2.3. Substitution of resin 11 with *N*,*N*′-dibenzylpropane-1,3-diamine (resin 12).** Resin **11** (1.30 mmol) was swelled in NMP (15 ml), and *N*,*N*′-dibenzylpropane-1,3-diamine[8] (1.98 g, 7.80 mmol) and DIEA (2.23 ml, 13.00 mmol) were added. After agitation for 24 h at 50 °C, the resin was filtered off, washed with NMP and $CH_2Cl_2$, and dried in vacuo at 50 °C.

**3.2.4. Substitution of resin 11 with benzylamine (resin 13).** Resin **11** (0.65 mmol) was substituted with benzylamine (0.71 ml, 6.50 mmol) analogously to Section 3.2.3.

**3.2.5. Substitution of resin 11 with $^{15}N$-benzylamine (resin 14).** Resin **11** (0.65 mmol) was substituted with $^{15}N$-benzylamine (0.42 g, 3.90 mmol) analogously to Section 3.2.3.

**3.2.6. Alkylation of resin 12 with $^{15}N$-(3-bromopropyl)phthalimide (resin 15).** Resin **12** (1.30 mmol) was suspended in NMP (15 ml). $^{15}N$-(3-bromopropyl)phthalimide[9] (1.20 g, 4.46 mmol) and DIEA (2.23 ml, 13.00 mmol) were added, and the mixture was agitated for 26 h at 50 °C. The resin was filtered off, washed with NMP and $CH_2Cl_2$, and dried in vacuo at 50 °C.

**3.2.7. Alkylation of resin 13 with 1,3-dibromopropane (resin 16).** Resin **13** (0.65 mmol) was alkylated with 1,3-dibromopropane (0.66 ml, 6.50 mmol) analogously to Section 3.2.2

**3.2.8. Alkylation of resin 14 with 1,3-dibromopropane (resin 17).** Resin **14** (0.65 mmol) was alkylated with 1,3-dibromopropane (0.66 ml, 6.50 mmol) analogously to Section 3.2.2.

**3.2.9. Substitution of resin 16 with $^{15}N$-benzylamine (resin 18).** Resin **16** (0.65 mmol) was substituted with $^{15}N$-benzylamine (0.42 g, 3.90 mmol) analogously to Section 3.2.3.

**3.2.10. Substitution of resin 17 with benzylamine (resin 19).** Resin **17** (0.65 mmol) was substituted with benzylamine analogously to Section 3.2.3.

**3.2.11. Alkylation of resin 18 with *N*-(3-bromopropyl)phthalimide (resin 20).** Resin **18** (0.65 mmol) was alkylated with *N*-(3-bromopropyl)phthalimide (0.87 g, 3.25 mmol) analogously to Section 3.2.6.

**3.2.12. Alkylation of resin 19 with *N*-(3-bromopropyl)phthalimide (resin 21).** Resin **19** (0.65 mmol) was alkylated with *N*-(3-bromopropyl)phthalimide (0.87 g, 3.25 mmol) analogously to Section 3.2.6.

### 3.3. Deprotection of the phthalimide group

**3.3.1. Deprotection of the phthalimide group from resin 15 (resin 22).** Resin **15** (1.30 mmol) was swelled in NMP (20 ml), and $N_2H_4{\cdot}H_2O$ (6.00 ml, 0.123 mol) was added. The mixture was agitated for 3 h at 80 °C, the resin was filtered off, washed with NMP, NMP/$H_2O$ (1:1), NMP, and $CH_2Cl_2$, and dried in vacuo at 50 °C.

**3.3.2. Deprotection of the phthalimide group from resin 20 (resin 23).** The phthalimide group from resin **20** (0.65 mmol) was removed according to Section 3.3.1.

**3.3.3. Deprotection of the phthalimide group from resin 21 (resin 24).** The phthalimide group from resin **21** (0.65 mmol) was removed according to Section 3.3.1.

### 3.4. Acylation of the terminal amino group with TBSIndAcOH

**3.4.1. Acylation of resin 22 (resin 25).** Resin **22** (1.30 mmol) was swelled in $CH_2Cl_2$ (20 ml). 1-[(*tert*-Butyl)dimethylsilyl]-1*H*-indole-3-acetic acid (TBSIndAcOH)[5] (3.76 g, 13.00 mmol) and *N*,*N*′-diisopropylcarbodiimide (1.01 ml, 6.50 mmol) were added and the mixture was agitated for 35 h at 23 °C. The product resin was filtered off, washed successively with $CH_2Cl_2$, NMP, NMP/DIEA (10:1), NMP, and $CH_2Cl_2$, and dried in vacuo at 50 °C. The Kaiser test[10] was performed to prove the absence of primary amino groups.

**3.4.2. Acylation of resin 23 (resin 26).** Resin **23** (0.65 mmol) was acylated with TBSIndAcOH (1.88 g, 6.50 mmol) according to Section 3.4.1.

**3.4.3. Acylation of resin 24 (resin 27).** Resin **24**

(0.65 mmol) was acylated with TBSIndAcOH (1.88 g, 6.50 mmol) according to Section 3.4.1.

### 3.5. Cleavage of the polyamine derivatives from the resins

**3.5.1. $^{15}N$-(16-Amino-4,8,12-triazahexadecyl)-1H-indole-3-acetamide; IndAc$^{15}$N3334 (28).** Resin **25** (1.30 mmol) was swelled in 1,2-dichloroethane (15 ml) and ACE-Cl (2.83 ml, 26.00 mmol) was added. After agitation for 3 h at 23 °C, the product resin was filtered off and washed with $CH_2Cl_2$. The organic solutions were combined and evaporated to dryness. The residues were dissolved in MeOH and the resulting solution was refluxed for 3 h. Finally the solvent was removed. This provided the respective polyamine IndAc$^{15}$N3334·4 HCl (95 mg, 0.17 mmol, 13% overall yield with respect to resin **10**) 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 21.0–30.0 min). IR (KBr): 3400s, 3330m, 2960s, 2760s, 2540m, 2420m, 1660s, 1535m, 1460m, 1260w, 1230w, 745m. $^1$H NMR ($D_2O$): 7.71–7.59 (m, 2 arom. H); 7.42–7.22 (m, 3 arom. H); 3.82 (s, $ArCH_2$); 3.33 (t, $J$=6.4 Hz, $CH_2$); 3.27–3.07 (m, 10 H); 3.02–2.88 (m, 4 H); 2.26–2.00 (m, 4 H); 1.95–1.76 (m, 6 H). $^{13}$C NMR ($D_2O$): 180.4 (s×d, $J_{C,^{15}N}$=16.1 Hz, CO); 140.8, 131.2 (2s, 2 arom. C); 129.7, 126.7, 124.1, 122.9, 116.6 (5d, 5 arom. C); 112.2 (s, 1 arom. C); 51.6, 49.6, 49.2, 49.1, 49.0, 48.9, 43.4 (7t); 40.5 (t×d, $J_{C,^{15}N}$=10.6 Hz, 1 C); 37.0 (t×d, $J_{C,^{15}N}$=7.0 Hz, 1 C); 30.1, 28.5, 27.3, 27.2, 27.1 (5t). ESI-MS: 418 (100, $[M+H]^+$).

**3.5.2. *N*-(16-Amino-4-$^{15}N$,8,12-triazahexadecyl)-1H-indole-3-acetamide; IndAc3$^{15}$N334 (29).** Treatment of resin **26** (0.64 mmol) according to Section 3.5.1 (in presence of DIEA; 0.55 ml, 3.20 mmol) afforded IndAc3$^{15}$N334·4HCl (51 mg, 0.091 mmol, overall 14% with respect to resin **10**) after purification by HPLC (solvent A: 0.05% HCl in MeOH; solvent B: 0.05% HCl in $H_2O$; 8% A; $\lambda$=220 nm; flow rate 20 ml min$^{-1}$; the product was collected at 32.0–51.0 min). Finally the residue was washed with MeOH (to remove DIEA·HCl, which was carried through chromatography within the sample). IR (KBr): 3390s, 3330m, 2950s, 2750s, 2530m, 2410m, 1655s, 1535m, 1460m, 1260w, 1225w, 740m. $^1$H NMR ($D_2O$): 7.73–7.61 (m, 2 arom. H); 7.44–7.24 (m, 3 arom. H); 3.85 (s, $ArCH_2$); 3.36 (t, $J$=6.6 Hz, $CH_2$); 3.29–3.08 (m, 10 H); 3.04–2.91 (m, 4 H); 2.27–2.01 (m, 4 H); 1.97–1.77 (m, 6 H). $^{13}$C NMR ($D_2O$): 180.5 (s, CO); 140.9, 131.1 (2s, 2 arom. C); 129.7, 126.7, 124.1, 122.9, 116.6 (5d, 5 arom. C); 112.2 (s, 1 arom. C); 51.7 (t); 49.7 (t×d, $J_{C,^{15}N}$=4.9 Hz, 1 C); 49.2 (t×d, $J_{C,^{15}N}$=4.4 Hz, 1 C); 49.0, 48.93, 48.86, 43.4, 40.5, 36.7, 30.1, 28.5, 27.3, 27.2, 27.1 (11t). ESI-MS: 418 (100, $[M+H]^+$).

**3.5.3. *N*-(16-Amino-4,8-$^{15}N$,12-triazahexadecyl)-1H-indole-3-acetamide; IndAc33$^{15}$N34 (30).** Treatment of resin **27** (0.64 mmol) according to Section 3.5.1 (in presence of DIEA; 0.55 ml, 3.20 mmol) afforded IndAc33$^{15}$N34·4HCl (47 mg, 0.083 mmol, overall 13% with respect to resin **10**) 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 24.0–34.0 min). Finally the residue was washed with MeOH (to remove DIEA·HCl, which was carried through chromatography within the sample). IR (KBr): 3400s, 3340m, 2960s, 2760s, 2530m, 2420m, 1655s, 1540m, 1460m, 1260w, 1230w, 745m. $^1$H NMR ($D_2O$): 7.73–7.61 (m, 2 arom. H); 7.45–7.24 (m, 3 arom. H); 3.86 (s, $ArCH_2$); 3.37 (t, $J$=6.5 Hz, $CH_2$); 3.29–3.09 (m, 10H); 3.04–2.92 (m, 4H); 2.27–2.01 (m, 4H); 1.97–1.77 (m, 6H). $^{13}$C NMR ($D_2O$): 180.6 (s, CO); 140.9, 131.1 (2s, 2 arom. C); 129.7, 126.7, 124.1, 122.8, 116.6 (5d, 5 arom. C); 112.2 (s, 1 arom. C); 51.6, 49.6, 49.1, 49.0 (4t); 48.9 (t×d, $J_{C,^{15}N}$=1.9 Hz, 1 C); 48.8 (t×d, $J_{C,^{15}N}$=2.1 Hz, 1 C); 43.4, 40.4, 37.0, 30.1, 28.5, 27.2, 27.1, 27.0 (8t). ESI-MS: 418 (100, $[M+H]^+$).


## Acknowledgements

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


## References and notes


1. Bigler, L.; Schnider, C. F.; Hu, W.; Hesse, M. *Helv. Chim. Acta* **1996**, *79*, 2152–2163.
2. Chesnov, S.; Bigler, L.; Hesse, M. *Helv. Chim. Acta* **2001**, *84*, 2178–2197.
3. De Maaijer-Gielbert, J.; Gu, C.; Somogyi, A.; Wysocki, V. H.; Kistemaker, P. G.; Weeding, T. L. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 414–422.
4. Lu, G.; Mojsov, S.; Tam, J.; Merrifield, R. *J. Org. Chem.* **1981**, *46*, 3433–3436.
5. Manov, N.; Tzouros, M.; Chesnov, S.; Bigler, L.; Bienz, S. *Helv. Chim. Acta* **2002**, *85*, 2827–2846.
6. Mueller, A. L.; Roeloffs, R.; Jackson, H. Pharmacology of polyamine toxins from spiders and wasps. *The alkaloids*; Cordell, G. A., Brossi, A., Eds.; Academic: San Diego, CA, 1995; Vol. 46, pp 63–94.
7. Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. *J. Org. Chem.* **1997**, *62*, 411–416.
8. Niitsu, M.; Samejima, K. *Chem. Pharm. Bull.* **1986**, *34*, 1032–1038.
9. Samejima, K.; Takeda, Y.; Kawase, M.; Okada, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1984**, *32*, 3428–3435.
10. Sarin, V. K.; Kent, S. B.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147–157.
11. Schäfer, A.; Benz, H.; Fiedler, W.; Guggisberg, A.; Bienz, S.; Hesse, M. Polyamine toxins from spiders and wasps. *The alkaloids*; Cordell, G. A., Brossi, A., Eds.; Academic: New York, 1994; Vol. 45, pp 1–125.
12. Tzouros, M.; Bienz, S.; Bigler, L. In preparation.