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Article

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A general approach for the liquid phase fragment synthesis of orthogonally protected naturally occurring polyamines and applications thereof

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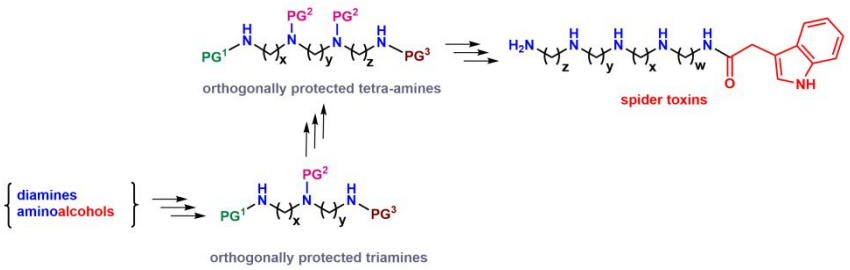


ABSTRACT

Orthogonally protected polyamines (PAs) have been synthesized using α,ω-diamines and ω-aminoalcohols as N-C_x-N and N-C_y synthons, respectively, and the Mitsunobou reaction as the key reaction for the assembly of the PA skeleta. The Trt, Dde and Phth groups have been employed for protecting the primary amino functions and the Ns group for activating the primary amino functions towards alkylation and

secondary amino function protection. The approach has been readily extended to accommodate the total synthesis of the spider toxins Agel 416 and HO-416b incorporating the 3-4-3-3 and the 3-3-3-4 PA skeleton, respectively.

Keywords: polyamines, orthogonal protection, fragment synthesis, conjugates, spider toxins

INTRODUCTION

Polymethylene polyamines (PAs), such as putrescine (Put), spermidine (Spd) and spermine (Spm), are naturally occurring small molecules found in all leaving organisms, either in their free polycationic form or conjugated to other biomolecules, and show interesting biological activities.[1-5] Less common PAs, such as the *nor* (Nsd and Nsm) and *homo* (Hsd and Hsm) analogs of Spd (a 3-4 PA) and Spm (a 3-4-3 PA) as well isomeric PAs, such as thermospermine (Tsm, a 3-3-4 PA) (Figure 1), are usually found in plant cells,[6-8] although the latter was first isolated from the extreme thermophile *Thermus thermophilus*.[9] Due to their biological significance and the fact that PAs could form the basis for the development of pharmaceuticals,[10,11] there is a constant interest in the synthesis of PA analogs and conjugates. Interestingly, a variety of conjugates of PAs with other biomolecules (herein abbreviated as PACs) with notable biological activities, e.g. PACs from spiders and wasps, bear the other biomolecule on one of the terminal amino functions of the PA skeleton.[2,3] This imposes interesting synthetic challenges in particular when the PA skeleton is not symmetrical, as it is the case with the PAs Spd and Tsm.

The synthesis of PA analogs and conjugates has followed two traditional pathways. The first one involves the selective direct or indirect functionalization of the desired amino function(s) of a PA skeleton and the second one the assembly of the PA skeleton from readily available fragments bearing suitable functionalized or protected amino functionalities.[2,3,12-19] We were recently interested in the selective mono-functionalization of the unsymmetrical Tsm and we therefore decided to attempt the synthesis of orthogonally protected Tsm using the fragment synthesis protocol. It should be noted that mono-functionalization of the symmetrical PA Spm

has been effected by using the N^1,N^4,N^8-Boc_3-Spm obtained via the low temperature selective mono-trifluoroacetylation of Spm.[20] Tsm has been first prepared by Ganem et al from Spd through a five-steps sequence.[21] Quite recently, the fragment synthesis of Tsm as well as Spm and Nsm was reported on a polymer support of the *o*-chlorotrityl-type and using the nosyl (Ns) protecting group for amino protection and activation and the base induced alkylation of the N-nosylated amines by suitable halides as the key-step for the assembly of the skeleton.[22] The Mitsunobu reaction has been also indicated by the same research group as an alternative for the alkylation of Ns-activated amines with alcohols[23,24] and was used on a solid support for the synthesis of the spider toxin Agel 416.[25]

Based on our long-standing experience in the use of the triphenylmethyl (trityl, Trt) group for the protection of amino functions of PAs, we reasoned that the Tsm skeleton incorporating orthogonally protected amino functionalities (**7**) could be assembled from readily available *N*-tritylated building blocks like the *N*-trityl-γ-aminobutyric acid (Trt-GABA) or *N*-tritylputrescine (Trt-Put, **9**) as N-C_4 and N-C_4-N synthons, respectively, and either 1,3-diaminopropane (Dap) or the corresponding Dde-protected 3-aminopropanol (**10a**)[25] as N-C_3-N or N-C_3 synthons, respectively. The Trt group, as an amino protecting group, offers a number of advantages due to its high lipophilicity, electron-releasing property, and steric bulk as well as its lability to mild acids. In particular, the lipophilicity of the Trt group facilitates aqueous work-up procedures for *N*-tritylated PAs and derivatives and their subsequent purification by routine flash column chromatography (FCC).

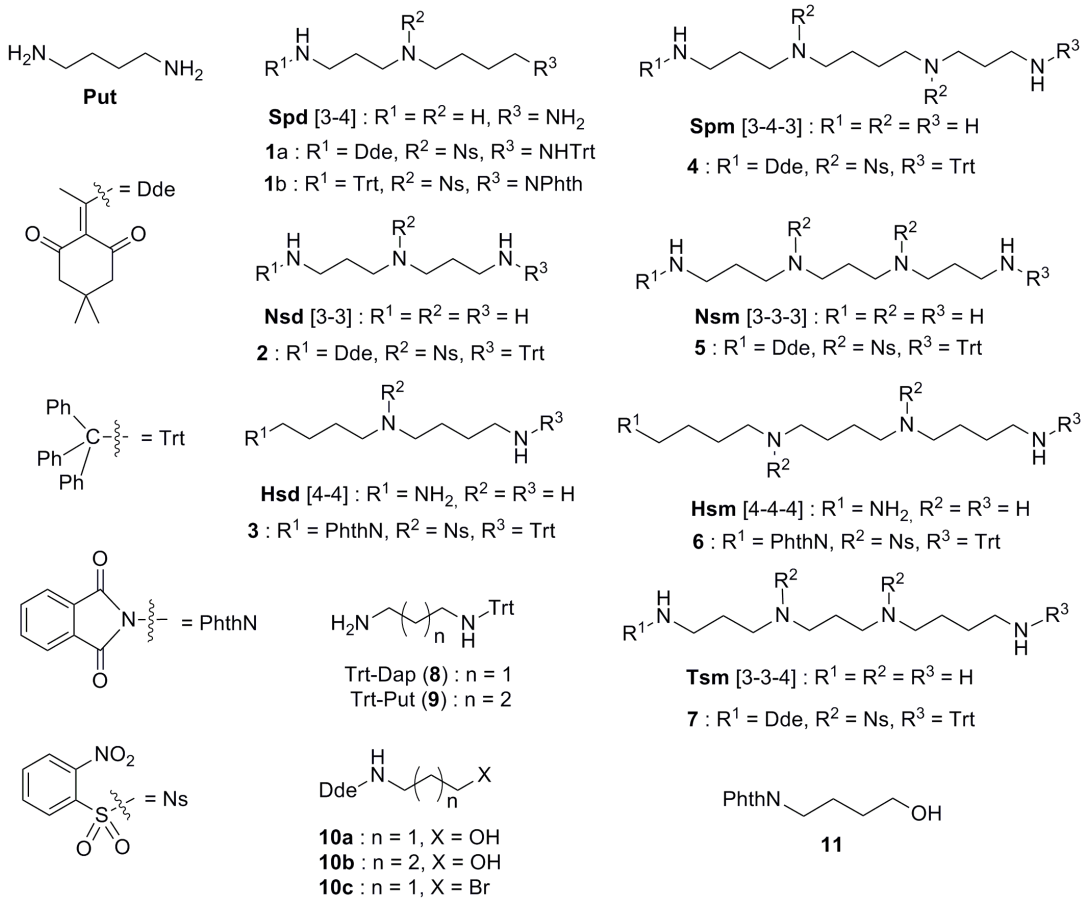


Figure 1. Structures of compounds and protecting groups encountered in this work.

On the other hand, the 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) group, used to protect selectively primary amino functions, is stable to acids and bases but is readily removed by a 2% solution of hydrazine in DMF at room temperature.[26] Finally, the Ns group was used for *N*-protection and *N*-activation and the Mitsunobu reaction for the assembly of the skeleton. The successful application of this methodology for the preparation of Tsm derivative **7** lead us to extent it, with some minor modifications, to the preparation of the orthogonally protected PA derivatives **1**-**6** depicted in Figure 1. Furthermore, the applicability of the tetra-amine derivatives **4**-**7** to the synthesis of PACs was exemplified with another syntheses of the spider toxins Agel 416[25] and HO-416b,[27] using the derivatives **4** of Spm and **5** of Nsm as key-intermediates, respectively.

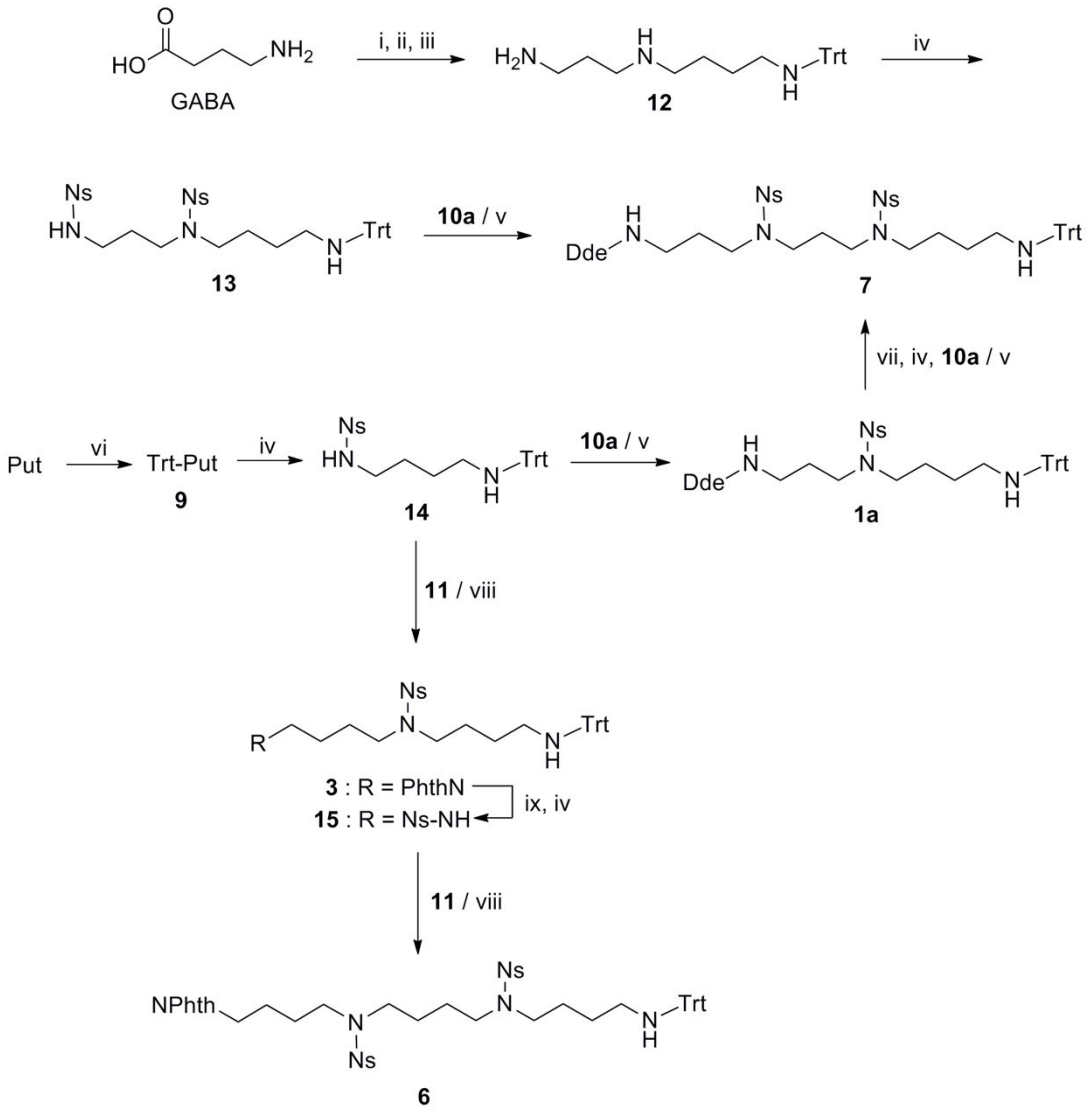
RESULTS AND DISCUSSION

We have reported in 2012 that *N*-tritylation of GABA, followed by coupling with Dap and finally $LiAlH_4$-mediated reduction, leads to N^8-tritylspermidine (**12**) in

52% overall yield.[28] Treatment of **12** with NsCl in the presence of diisopropyl ethylamine (DIPEA) produced the fully protected Spd derivative **13** in 55% yield which was then alkylated with the Dde-protected 3-aminopropanol (**10a**), in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (TPP), to give the orthogonally protected Tsm (3-3-4) derivative **7** in 84% yield (Scheme 1). Alternatively, compound **7** could be synthesized from Put as follows. Put was monotritylated according to a published procedure to provide N^1-tritylputrescine (Trt-Put, **9**) in 85% yield.[29] This compound was subsequently nosylated to give the fully protected Put derivative **14** in 88% yield. Mitsunobu reaction of compound **14** with alcohol **10a** produced the orthogonally protected Spd derivative **1a**. Because this compound was co-eluted with the by-product $^{i}PrO_2CNH\text{-}NHCO_2{}^{i}Pr$ ($DIADH_2$), it was used as such into the next step. Selective removal of the Dde group with 2% H_2NNH_2 in DMF, followed by nosylation and a second Mitsunobu reaction with the same alcohol finally provided the expected Tsm derivative **7** in 66% yield for the last three steps (Scheme 1). All intermediates and final products were purified by routine FCC.

We envisaged that intermediate **14** could be also useful for the preparation of the corresponding orthogonally protected Hsd (4-4) and Hsm (4-4-4) derivatives with a minor modification. Because the Dde protected 4-aminobutan-1-ol (**10b**) does not efficiently condense with *N*-nosylated functionalities under Mitsunobu reaction conditions (see below), we used instead the corresponding *N*-phthalyl protected 4-aminobutanol (**11**) as an N-C_4 synthon. That way, the expected orthogonally protected Hsd derivative **3** was obtained in 80% yield. From this intermediate, the Phth group was selectively removed with H_2NNH_2 in refluxing EtOH, the primary amino function thus exposed was nosylated and the resulting new intermediate **15** was finally condensed with a second molecule of compound **11** to give access to the orthogonally protected Hsm derivative **6** in 65% yield for the last three steps. Compound **6** contained $DIADH_2$ in the ratio 1:0.25 (see experimental).

Scheme 1. Synthesis of orthogonally protected Spd (1a), Tsm (7), Hsd (3) and Hsm (6)[α]

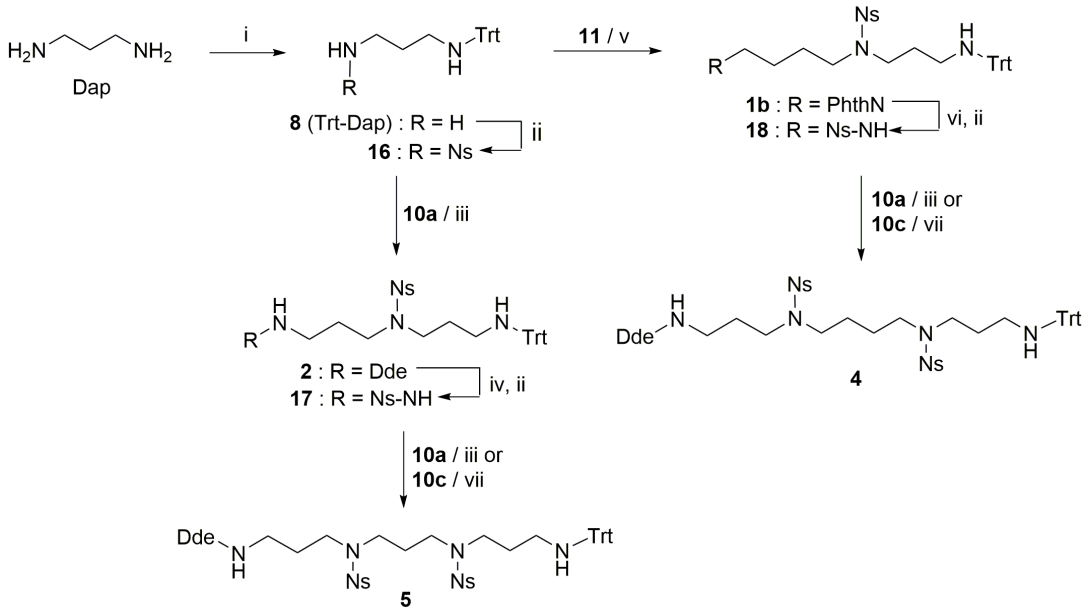


[a]Reagents and conditions: (i) (a) Me_3SiCl, (b) Et_3N, TrtCl, (c) MeOH, CH_2Cl_2/MeCN (5:1), 85%; (ii) Dap, PyBrOP, CH_2Cl_2, 0 ºC, 30 min, 81%; (iii) $LiAlH_4$, THF, 66 ºC, overnight, 75%; (iv) NsCl, DIPEA or Et_3N, CH_2Cl_2, 0 ºC for 30 min then 25 ºC for 2 h, 55%-90%; (v) TPP, DIAD, THF, 25 ºC, overnight, 77%-84%; (vi) TrtCl, CH_2Cl_2, 25 ºC, 2 h, 85%; (vii) 2% H_2NNH_2 in DMF, 25 ºC, 30 min, 87%; (viii) TPP, DIAD, THF, 25ºC-40ºC, overnight, 87%; (ix) H_2NNH_2, EtOH, 78 ºC, 2 h, 93%.

On the other hand, *N*-tritylation of 1,3-diaminopropane (Dap) in the way described for Trt-Put provided the N^1-tritylamino-3-aminopropane (Trt-Dap, **8**) in 85% yield. This compound was nosylated and the thus obtained fully protected Dap derivative **16** was condensed with alcohol **10a** under Mitsunobu reaction conditions to give the orthogonally protected Nsd derivative **2** in 74% overall yield (Scheme 2).

Dde removal and subsequent nosylation of the resulting primary amino function provided the dinosylated intermediate **17** in 60% yield. This was then reacted with a second molecule of alcohol **10a** in the presence of DIAD and TPP to provide the orthogonally protected Nsm derivative **5**. Due to the co-elution of derivative **5** with $DIADH_2$, alkylation of intermediate **17** with bromide **10c** was alternatively used. Nevertheless, derivative **5** containing $DIADH_2$ can be subjected to removal of the Dde group, as the thus obtained partially deprotected PA derivative can be obtained free of $DIADH_2$ by routine FCC (see below).

Scheme 2. Synthesis of orthogonally protected Spd (1b), Spm (4), Nsd (2) and Nsm (5) [α]



[α]Reagents and conditions: (i) TrtCl, CH_2Cl_2, 25 ºC, 2 h, 85%; (ii) NsCl, Et_3N, CH_2Cl_2, 0 ºC for 30 min then 25 ºC for 2 h, 66-84%; (iii) TPP, DIAD, THF, 25 ºC, overnight, 89%; (iv) 2% H_2NNH_2 in DMF, 25 ºC, 30 min, 72%; (v) TPP, DIAD, THF, 25 ºC-40 ºC, overnight, 82%; (vi) H_2NNH_2, EtOH, 78 ºC, 2 h, 94%; (vii) K_2CO_3, DMF, 60 ºC, 2.5 h, 76-88%.

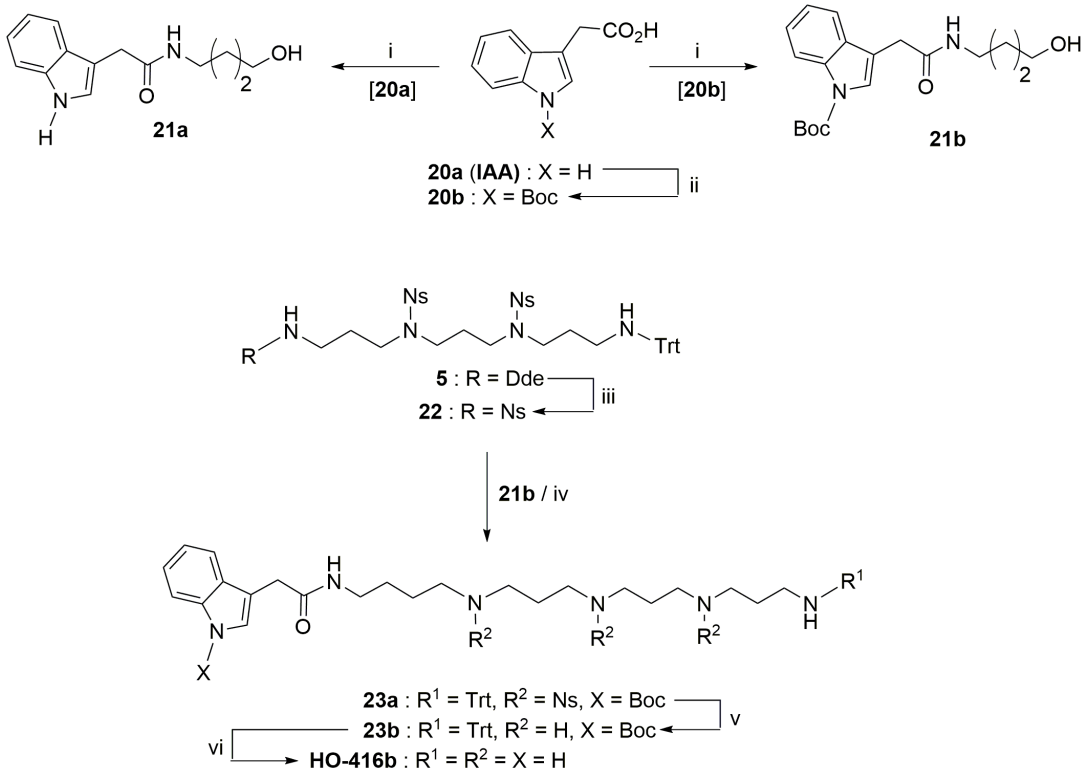
Compound **16** was also considered as a key-intermediate for the step-wise assembly of the Spm skeleton. However, attempted Mitsunobu reaction of compound **16** with alcohol **10b**[25] was sluggish and produced the expected Spd derivative in only 25% even after repeated additions of alcohol, DIAD and TPP. Therefore,we decided to use the corresponding 4-phthalimidobutan-1-ol (**11**), which cannot undergo

intramolecular alkylation, instead of alcohol **10b**. Indeed, using compound **11** as the alcohol component in the Mitsunobu reaction with intermediate **16**, we obtained the expected orthogonally protected Spd derivative **1b** in 82% yield (Scheme 2). Selective Phth-group removal and nosylation of the unmasked primary amino function produced the dinosylated intermediate **18** in 62% yield. Coupling compound of **18** with alcohol **10a** in the presence of DIAD and TPP produced the orthogonally protected Spm derivative **4**. This compound co-eluted with $DIADH_2$ and the side-product $^{i}PrO_2CNH\text{-}N(R)CO_2{}^{i}Pr$, where R is the alkyl group of alcohol **10a**. However, selective removal of the Dde group produces N^4,N^9-dinosyl-N^1-tritylspermine which can be readily purified and actually was used in the synthesis of toxin Agel-416 (see Scheme 4). Alternatively, the pure orthogonally protected Spm derivative **4** can be prepared in 88% yield by alkylation of intermediate **18** with bromide **10c**.

The applicability of the orthogonally protected PA derivatives **1**-**7** to the synthesis of PACs was exemplified with alternative syntheses of spider toxin HO-416b (a 4-3-3-3 PAC) using the Nsm derivative **5** (a 3-3-3 PA derivative) as key-intermediate and the spider toxin Agel 416 (a 3-3-4-3 PAC) using the Spm derivative **4** (a 3-4-3 PA derivative) as key-intermediate. These syntheses could be effected by either a convergent or a linear way. We used the former way in the synthesis of the HO-416b toxin and the latter in the synthesis of the Agel 416 toxin.

Accordingly, activation of (indol-3-yl)acetic acid (IAA, **20a**) with *N*-hydroxysuccinimide (HOSu) and *N*,*N*'-dicyclohexylcarbodiimide (DCC) and coupling with 4-aminobutanol provided the *N*-(4-hydroxybutyl)-2-(1*H*-indol-3-yl)acetamide (**21a**) in 65% yield. On the other hand Dde deprotection of intermediate **5**, followed by nosylation, gave the trinosylated Nsm derivative **22** in 76% yield (Scheme 3). Unfortunately, alcohol **21a** could not be coupled directly with compound **22** under Mitsunobu reaction conditions obviously due to the unprotected NH function of the indole nucleus. We therefore decided to protect the NH functionality of IAA (**20a**) with the acid-labile *tert*-butoxycarbonyl (Boc) group prior to coupling with 4-aminobutanol. Attempted introduction of the Boc group in IAA (**20a**) in a manner similar to the one-pot sequence described by our research group for the synthesis *N*-tritylamino acids,[30] involving silylation of the carboxyl function, *tert*-butoxycarbonylation in the presence of 4-dimethylaminopyridine (DMAP) and finally methanolysis of the resulting silyl ester, failed to produce the expected N^{ind}-Boc-IAA (**20b**).

Scheme 3. Synthesis of the spider toxin HO-416b. [a]



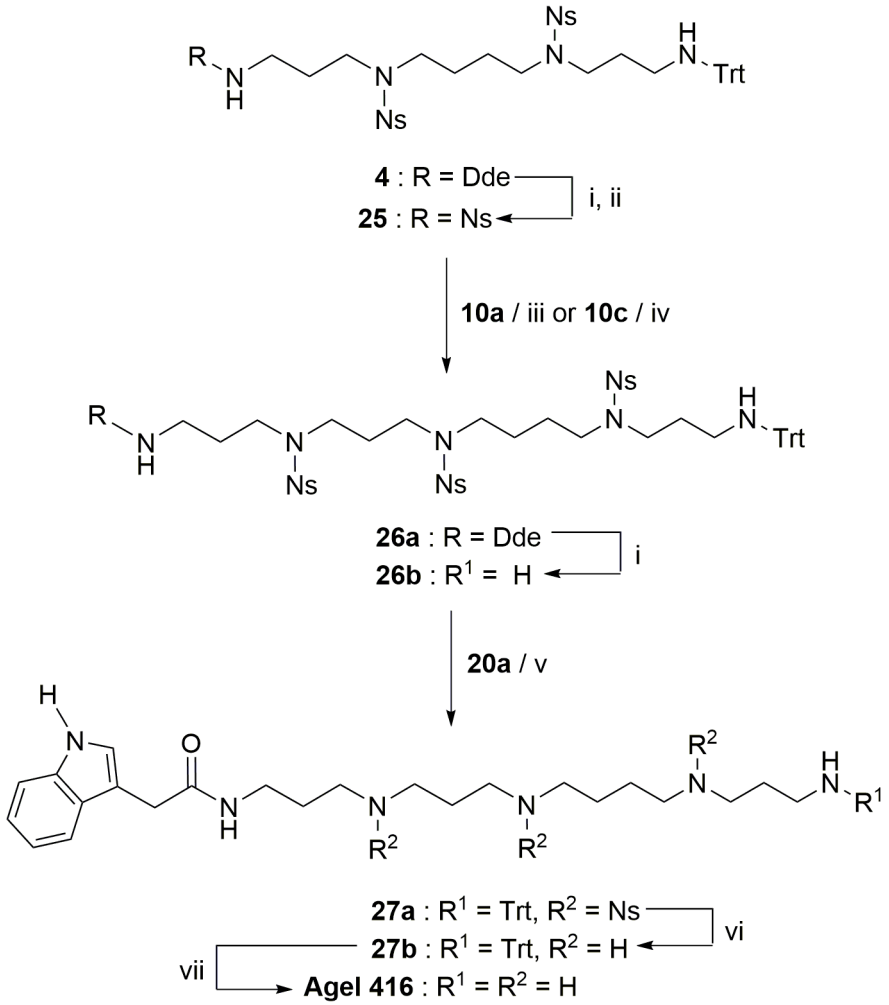
[a]*Reagents and conditions*: (i) (a) HOSu, DCC (b) 4-aminobutanol, DMF, 25 ºC, 2 h, 65-72%; (ii) (a) $PhCOCH_2Br$, Et_3N, DMF, 0 ºC for 30 min then 25 ºC for 2 h, 81%, (b) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 25 ºC, 3 h, 91%, (c) PhSH, Na_2CO_3, DMF, 60 ºC, 2 h, 84%; (iii) (a) 2% H_2NNH_2 in DMF, 25 ºC, 30 min, 94%, (b) NsCl, Et_3N, CH_2Cl_2, 0 ºC for 30 min then 25 ºC for 1.5 h, 81%; (iv) TPP, DIAD, THF, 40 ºC, overnight; (v) PhSH, Na_2CO_3, DMF, 25 ºC, overnight; (vi) 10% TFA in CH_2Cl_2 and PhSH, 25 ºC, 1 h, 70%.

Literature procedures for the preparation of N^{ind}-Boc-IAA (**20b**) use a three-steps sequence, involving protection of the carboxyl function as an isolable methyl[25] or allyl[31] ester, followed by reaction with Boc_2O in the presence of DMAP and finally carboxyl group deprotection. We used instead the phenacyl group for the protection of the carboxyl function of IAA (**20a**). Thus, reaction of IAA (**20a**) with phenacyl bromide in the presence of Et_3N produced the corresponding phenacyl ester in 81% yield. Reaction of this ester with Boc_2O in the presence of DMAP and Et_3N resulted

in *N*-protection and the thus obtained, in 91% yield, fully protected IAA was treated with thiophenol and Na_2CO_3 to produce N^{ind}-Boc-IAA (**20b**) in 84% yield. Activation of the thus obtained acid N^{ind}-Boc-IAA (**20b**) with HOSu and DCC and coupling with 4-aminobutanol produced the N^{ind}-Boc protected alcohol **21b** in 72% yield. This alcohol was then coupled directly with the trinosylated Nsm derivative **22** under Mitsunobu reaction conditions to give the expected fully protected crude HO-416b (**23a**) which contained triphenylphosphine oxide (TPPO), $DIADH_2$ and a side-product $^{i}PrO_2CNH\text{-}N(R')CO_2{}^{i}Pr$, where R' is the alkyl group of alcohol **21b**. Treatment of crude intermediate **23a** with PhSH and Na_2CO_3 in DMF resulted in the deprotection of the three secondary amino functions to give pure intermediate **23b** in 57% yield for the last two steps. From this compound, toxin HO-416b was readily obtained, as the corresponding penta-trifluoroacetate salt, in 70% yield and 97% purity according to HPLC analysis, upon deprotection with 10% trifluoroacetic acid (TFA) in CH_2Cl_2 in the presence of the cation scavenger PhSH.

For the synthesis of the Agel 416 toxin we decided to explore the viability of the linear way, which involves first the extension of the PA chain of Spm to the desired length. Thus, the Dde protecting group of Spm derivative **4** was selectively removed with 2% H_2NNH_2 in DMF and the resulting free primary amino function was nosylated to give the trinosylated Spm derivative **25** in 80%. This intermediate was then directly coupled with alcohol **10a** under Mitsunobu reaction conditions to produce the fully protected pentaamine **26a** which co-eluted with TPPO. The thus obtained crude **26a** was subjected to Dde group removal to give pure N^4,N^8,N^{13}-trinosyl-N^{16}-trityl-4,8,13-triazahexadeka-1,16-diamine following routine FCC. Alternatively, derivative **25** was alkylated with bromide **10c** to provide pure intermediate **26a** in 72% yield. From this intermediate, the Dde group was selectively removed and the resulting free primary amino function was acylated with IAA (**20a**), which had been preactivated with HOSu and *N*,*N*'-diisopropylcarbodiimide (DIC). That way, the partially protected Agel 416 (**27a**) was obtained, containing *N*,*N*'-diisopropylurea (DICU). Crude intermediate **27a** was then selectively deprotected at the secondary amino functions with PhSH and Na_2CO_3 in DMF to give pure intermediate **27b** in 49% yield for the two steps. Finally, trityl group removal with 10% TFA in CH_2Cl_2 in the presence PhSH, provided the expected toxin Agel 416, as the corresponding penta-trifluoroacetate salt, in 73% yield and 93% purity according to HPLC analysis.

Scheme 4. Synthesis of the spider toxin Agel 416. [α]



[α]*Reagents and conditions*: (i) 2% H_2NNH_2 in DMF, 25 ºC, 30 min, 83%-91%; (ii) NsCl, Et_3N, CH_2Cl_2, 0 ºC for 30 min then 25 ºC for 1.5 h, 87%; (iii) TPP, DIAD, THF, 25 ºC, overnight; (iv) K_2CO_3, DMF, 60 ºC, 2.5 h, 72%; (v) HOSu, DIC, DMF; (vi) PhSH, Na_2CO_3, DMF, 25 ºC, overnight; (vii) 10% TFA in CH_2Cl_2 and PhSH, 25 ºC, 1 h, 73%.

CONCLUSIONS

The mono-*N*-tritylated putrescine and propane-1,3-diamine were used as N-C_4-N and N-C_3-N synthons, respectively and the *N*-Dde or *N*-phthaloyl-protected 3-aminopropan-1-ol (or the corresponding bromide) and 4-aminobutan-1-ol were used as N-C_3 and N-C_4 synthons, respectively, in the liquid phase fragment synthesis of

the naturally occurring PAs spermidine, spermine and thermospermine and their lower and higher homologs nor- and homo-spermidine and -spermine. Key-figures in the assembly of PA chains in the required length were the use of the lipophilic trityl group, which facilitates aqueous work-up and purification by routine flash column chromatography, for *N*-protection and the nosyl group for the protection of secondary amino functions and activation of primary amino functions towards alkylation with alcohols in the presence of TPP and DIAD (Mitsunobu reaction) or the corresponding bromides in the presence of K_2CO_3.

The resulting orthogonally protected triamines and tetra-amines are well-suited, for example, for the preparation of unsymmetrically bisalkylated PA analogs and for the selective monoacylation of PAs, in particular asymmetric ones, leading to biologically interesting PACs. In addition, they can be extended to any direction by the selective removal of any of the protecting groups giving rise to higher order PAs. This extension may also provide access to a series of PA conjugates, such as the PA-type toxins from spiders and wasps. Indeed, this possibility was exemplified in the present work with alternative syntheses of the spider toxins Agel 416 and HO-416b, incorporating a pentaamine skeleton (3-4-3-3 and 3-3-3-4, respectively) and the lipophilic carboxylic acid IAA (**20a**). From the two alternative routes examined, the convergent one involving acylation of the ω-aminoalcohol with the *in situ* generated succinimidyl ester of *N*-Boc-protected IAA, followed by a Mitsunobu reaction gave purer final product than the linear one, involving first extension of the PA chain using the Mitsunobu reaction, followed by coupling with the succinimidyl ester of unprotected IAA. Both syntheses were finalized by routine PhSNa-mediated removal of the Ns groups, followed by TFA-mediated acidolysis of Boc and/or Trt groups. The present methodology can be also applied to the synthesis of a variety of PA analogs and PACs with changes in the aliphatic region of the PA skeleton by selecting the required α,ω-diaminoalkanes and ω-aminoalcohols, as N-C_n-N and N-C_n synthons, respectively. We are currently investigating the application of the present methodology to the synthesis of other PA analogs and conjugates and their biological evaluation as potential anticancer agents.

EXPERIMENTAL SECTION

General information. Melting points were determined with an Electrothermal apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained at 600

and 150 MHz, respectively, on Bruker AvanceIII HD spectrometer. Chemical shifts (δ) are reported for $CDCl_3$ solutions in parts per million (ppm) downfield from tetramethylsilane (TMS), used as internal standard, or for $(CD_3)_2SO$ solutions.

The HRMS-analysis for oily or foamy samples, using LC-MS grade MeOH as solvent, was performed using an ESI-LTQ-ORBITRAP XL unit (Thermo Scientific, Bremen, Germany). The Orbitrap Unit was operated in positive mode, with a spray voltage of 3.2 kV, while the sheath gas (N_2) flow rate and auxiliary gas (N_2) flow rate were adjusted to 12 and 2 arbitrary units, respectively. The capillary voltage and the tube lens voltage were set to 10 and 110 V, respectively. The scan ranged from m/z 150 up to 2000. Electron-spray ionization (ESI) mass spectra were recorded at 30 eV, on a Waters Micromass ZQ spectrometer using HPLC grade MeOH or MeCN as solvent, or MeCN/H_2O as solvent system.

Analytical reversed phase high-performance liquid chromatography was performed on Agilent Technologies, 1260 Affinity, Quaternary Pump VL system equipped with a photodiode array detector. The purity of the final conjugates was determined using UV detection (λ=254 nm). The chromatographic method employed the following: column, LeChrosorb RP18 (25 cm x 4.6 mm, 5 μm); mobile phase I, 0.08% TFA in water; mobile phase II, acetonitrile with 0.08% TFA; flow rate, 1.0 mL/min; elution profile, gradient elution from 5 to 50% II over 30 min. Elemental analyses for solid compounds were determined on a Carlo Erba EA 1108 CHNS elemental analyzer.

Flash column chromatography (FCC) was performed on Acros Organics silica gel 0.035-0.070 mm, 60 Å and TLC on Merck silica gel 60 F_{254} films (0.2 mm) precoated on aluminium foil. Spots were visualized with UV light at 254 nm and by spraying with a ninhydrine solution (0.3 g ninhydrin, 3 mL gl. acetic acid, 97 mL 1-butanol). The solvent systems used for the development of TLC or FCC are the following: (A) $CHCl_3$/MeOH (98:2), (B) $CHCl_3$/MeOH (95:5), (C) $CHCl_3$/MeOH (9:1), (D) $CHCl_3$/MeOH/NH_3 (99:1:0.1), (E) $CHCl_3$/MeOH/NH_3 (98:2:0.2), (F) $CHCl_3$/MeOH/NH_3 (97:3:0.3), (G) $CHCl_3$/MeOH/NH_3 (95:5:0.5), (H) $CHCl_3$/MeOH/NH_3 (9:1:0.1), (I) $CHCl_3$/MeOH/NH_3 (8.5:1.5:0.15), (J) $CHCl_3$/MeOH/NH_3 (8:2:0.2), (K) $CHCl_3$/MeOH/NH_3 (7:3:0.3), (L) $CHCl_3$/MeOH/NH_3 (6:4:0.4), (M) PhMe/EtOAc (93:7), (N) PhMe/EtOAc (9:1), (O) PhMe/EtOAc (8:2), (P) PhMe/EtOAc (7:3), (Q) PhMe/EtOAc (6:4), (R)

PhMe/EtOAc (1:1), (S) EtOAc/PhMe (7:3), (T) EtOAc/PhMe (8:2), (U) Hex/EtOAc (8:2), (V) Hex/EtOAc (7:3), (W) Hex/EtOAc (1:1).

All solvents were dried and/or purified according to standard procedures prior to use. Solvents were routinely removed at ca. 40 ºC under reduced pressure on Bücki Rotavapor RE 111 apparatus. Air-sensitive reagents were handled under inert atmosphere (Ar). All reagents employed in the present work were purchased from either Sigma-Aldrich or Alfa Aesar or Merck or Acros Organics or Fluorochem and were used without further purification. DIAD was used in the context of this work as a commercially available safer alternative to DEAD.

For the needs of the present work, the N^8-tritylspermidine (**12**) was prepared according to a published procedure.[28]

General procedure for the preparation of N^1-tritylated diamines

The N^1-tritylputrescine (Trt-Put, **9**) was prepared according to a published procedure.[29] Using identical procedure, the preparation of N^1-trityl-1,3-diaminopropane (Trt-Dap, **8**) was realized.

N^1-Trityl-1,3-diaminopropane (**8**). Colorless oil (1.29 g, 85%); R_f (G): 0.32; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, J = 7.2 Hz, 6H), 7.27 (t, J = 7.2 Hz, 6H), 7.18 (t, J = 7.2 Hz, 3H), 2.78 (t, J = 6.6 Hz, 2H), 2.20 (t, J = 6.6 Hz, 2H), 1.63 (quint., J = 6.6 Hz, 2H), 1.46 (br. s, 3H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 146.1 (3C), 128.6 (6C), 127.8 (6C), 126.2 (3C), 70.9, 41.4, 40.6, 34.7 ppm; MS (ESI, 30 eV): *m/z* 317.11 $[M+H]^+$, 243.17 $[Trt]^+$.

General procedure for the protection of ω-aminoalcohols with the Dde group

In general, the procedure described in the literature[25] was employed for the *N*-protection of 3-aminopropan-1-ol and 4-aminobutan-1-ol with the Dde group as follows:

To a stirred solution of Dde-OH (1.36 g, 7.46 mmol) in EtOH (3 mL), the ω-aminoalkanol (7.46 mmol) was added. The resulting mixture was heated under reflux for 30 min. Then it was left to attain room temperature and the solvent was evaporated

under reduced pressure to leave a crystalline residue. Recrystallisation from EtOAc gave the expected N-protected compound.

N-1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl-3-aminopropan-1-ol (**10a**).
White crystals (1.55 g, 87%); mp 106-108 °C; R_f (B): 0.21; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.45 (br. s, 1H), 3.78 (t, *J* = 6 Hz, 2H), 3.56 (q, *J* = 6 Hz, 2H), 2.57 (s, 3H), 2.35 (s, 4H), 2.00 (br. s, 1H), 1.92 (quint., *J* = 6 Hz, 2H), 1.02 (s, 6H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 197.8 (2C), 173.7, 107.9, 59.4, 52.9 (2C), 40.2 (2C), 31.6, 30.1 (2C), 28.3 (2C), 17.9 ppm; MS (ESI, 30 eV): *m/z* 517.44 $[2M+K]^+$, 262.48 $[M+Na]^+$, 240.52 $[M+H]^+$.

N-1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl-4-aminobutan-1-ol (**10b**).
Beige crystals (1.15 g, 91%); mp 105-107 °C; R_f (B): 0.35; ^{1}H NMR (600 MHz, $CHCl_3$) δ 13.45 (br. s, 1H), 3.70 (t, *J* = 6 Hz, 2H), 3.45 (q, *J* = 6 Hz, 2H), 2.56 (s, 3H), 2.35 (s, 4H), 1.79 (quint., *J* = 6 Hz, 2H), 1.74 (br. s, 1H), 1.68 (quint., *J* = 6 Hz, 2H) 1.02 (s, 6H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 197.7 (2C), 173.5, 107.9, 62.0, 52.9 (2C), 43.3, 30.1, 29.8, 28.3 (2C), 25.7, 17.9 ppm; MS (ESI, 30 eV): *m/z* 529.26 $[2M+Na]^+$, 276.20 $[M+Na]^+$, 254.26 $[M+H]^+$.

*Preparation of 1-bromo-3-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl-aminopropane (**10c**)*.
Triphenylphosphine (0.82 g, 3.12 mmol) was added portion-wise during a period of 2 h to a well-stirred solution of **10a** (0.66 g, 2.78 mmol) and carbon tetrabromide (1.38 g, 4.17 mmol) in dry CH_2Cl_2 (1.5 mL) at room temperature. After an additional 1 h of stirring the reaction mixture was applied on the top of a chromatography column and subjected to FCC, using EtOAc as eluant, to give bromide **10c**.

White solid (0.74 g, 88%); mp 63-66 °C; R_f (EtOAc): 0.41; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.56 (br. s, 1H), 3.60 (q, *J* = 6.6 Hz, 2H), 3.49 (t, *J* = 6.6 Hz, 2H), 2.59 (s, 3H), 2.37 (s, 4H), 2.21 (quint., *J* = 6.6 Hz, 2H), 1.03 (s, 6H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 198.3 (2C), 173.9, 108.1, 52.9 (2C), 41.4, 31.7, 30.1, 29.5, 28.3 (2C), 17.8 ppm; Anal. Calcd (%) for $C_{13}H_{20}BrNO_2$: C, 51.67; H, 6.67; N, 4.63. Found:

C, 51.87; H, 6.55; N, 4.48; MS (ESI, 30 eV): *m/z* 326.48 and 324.44 $[M+Na]^+$, 304.49 and 302.45 $[M+H]^+$.

*Preparation of 4-phthalimidobutan-1-ol (**11**)*.

A solution of 4-aminobutan-1-ol (0.5 mL, 5.5 mmol) in THF (2.5 mL) was added during a period of 20 min to a stirred slurry of *N*-(ethoxycarbonyl)phthalimide (1.1 g, 5 mmol) in THF (2.5 mL) at 0 °C. The mixture was further stirred for 2 h at room temperature. The completion of reaction was confirmed by TLC, using solvent system R. After removal of the solvent under reduced pressure, the residue diluted with CH_2Cl_2 and washed twice with H_2O and once with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness to leave a thick oil. Even after purification with FCC, using solvent system R for elution, the product **11** was found (by ^{1}NMR) to contain ethyl carbamate in the molar ratio **11**:ethyl carbamate = 1.4:1 and used as such into the following alkylation reactions.

Thick colorless oil (0.81 g, 67%); R_f (R): 0.30; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84-7.83 (m, 2H), 7.72-7.70 (m, 2H), 3.74 (t, *J* = 7.2 Hz, 2H), 3.69 (t, *J* = 7.2 Hz, 2H), 1.97 (br. s, 1H), 1.78 (quint., *J* = 7.2 Hz, 2H), 1.62 (quint., *J* = 7.2 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 168.5 (2C), 133.9 (2C), 132.2 (2C), 123.2 (2C), 62.2, 37.7, 29.8, 25.1 ppm.

General procedure for the nosylation of *N*-tritylated polyamines

A typical procedure for the mononosylation of *N*-tritylated PAs is the following: To an ice-cold solution of N^1-tritylputrescine (**9**) (1.18 g, 3.50 mmol) in dry CH_2Cl_2 (14 mL) and Et_3N (1.1 mL, 7.4 mmol), 2-nitrobenzenesulfonyl chloride (0.82 g, 3.70 mmol) was added portion-wise over 1.5 h under an inert atmosphere. The mixture was kept at 0 °C for further 30 min and then allowed to attain room temperature. The completion of the reaction was confirmed by TLC using the solvent system H. The reaction mixture was then diluted with CH_2Cl_2 and washed twice with 5% aqueous solution of Na_2CO_3, twice with H_2O and once with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The product **14** was obtained pure following FCC purification using solvent system N for elution.

In the case of the preparation of N^1,N^4-dinosyl-N^8-tritylspermidine (**13**) from N^8-tritylspermidine (**12**) were double nosylation was required, 4.4 equiv of DIPEA and 2.2 equiv of NsCl were employed.

N^1,N^4-Dinosyl-N^8-tritylspermidine (**13**). Reaction time: 2 h; slightly yellow oil (0.15 g, 55%); R_f (O): 0.28; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11-8.09 (m, 1H), 7.98 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.85-7.83 (m, 1H), 7.73-7.69 and 7.69-7.63 (two m, 4H), 7.60 (dd, *J* = 7.2 and 1.2 Hz, 1H), 7.44-7.41 (m, 6H), 7.29-7.23 (m, 6H), 7.20-7.15 (m, 3H), 5.59 (unresolv. t, 1H), 3.36 (t, *J* = 6.6 Hz, 2H), 3.24 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 6.6 Hz, 2H), 2.07 (unresolv. t, 1H), 1.82 (quint., *J* = 6.6 Hz, 2H), 1.53 (quint., *J* = 7.2 Hz, 4H), 1.40 (quint., *J* = 7.2 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.1, 148.0, 146.1 (3C), 133.7, 133.6, 133.5, 133.2, 132.8, 131.8, 131.0, 130.9, 128.6 (6C), 127.8 (6C), 126.3 (3C), 125.3, 124.2, 70.8, 47.9, 44.7, 43.1, 40.7, 28.9, 27.8, 26.2 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{38}H_{40}N_5O_8S_2$ $[M+H]^+$ 758.2318, found 758.2335.

N^1-Nosyl-N^4-tritylputrescine (**14**). Reaction time: 2 h; slightly yellow solid; (1.59 g, 88%); mp 149-152 °C; R_f (N): 0.36; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14-8.10 (m, 1H), 7.87-7.83 (m, 1H), 7.74-7.70 (m, 2H), 7.44-7.41 (m, 6H), 7.28-7.24 (m, 6H), 7.20-7.16 (m, 3H), 5.29 (t, *J* = 6.6 Hz, 1H), 3.08 (q, *J* = 6.6 Hz, 2H), 2.11 (t, *J* = 6.6 Hz, 2H) 1.61 (quint., *J* = 6.6 Hz, 2H), 1.55 (br. s, 1H), 1.48 (quint., *J* = 6.6 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.1, 146.0 (3C), 133.9, 133.5, 132.7, 131.1, 128.6 (6C), 127.8 (6C), 126.3 (3C), 125.4, 70.9, 43.9, 43.0, 27.8, 27.6 ppm; Anal. Calcd (%) for $C_{29}H_{29}N_3O_4S$: C, 67.55; H, 5.67; N, 8.15. Found: C, 67.84; H, 5.38; N, 8.09; MS (ESI, 30 eV): *m/z* 554.47 $[M+K]^+$, 538.60 $[M+Na]^+$, 516.62 $[M+H]^+$, 243.66 $[Trt]^+$.

N^1,N^5-Dinosyl-N^9-tritylhomospermidine (**15**). Reaction time: 2 h; yellow oil (0.3 g, 80%); R_f (O): 0.35; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12-8.10 (m, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.85-7.82 (m, 1H), 7.74-7.71 (m, 2H), 7.69-7.62 (m, 2H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.45-7.41 (m, 6H), 7.29-7.24 (m, 6H), 7.20-7.16 (m, 3H), 5.27 (t, *J* = 6 Hz, 1H), 3.28 (t, *J* = 7.2 Hz, 2H), 3.24 (t, *J* = 6 Hz, 2H), 3.10 (q, *J* = 6 Hz, 2H), 2.09 (t, *J* = 6.9 Hz, 2H), 1.61-1.51 (m, 7H), 1.42 (quint., *J* = 6.9 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.1, 148.0, 146.1 (3C), 133.63, 133.59, 133.57, 133.5, 132.9,

131.7, 131.1, 130.7, 128.6 (6C), 127.8 (6C), 126.3 (3C), 125.4, 124.2, 70.8, 47.3, 46.5, 43.12, 43.09, 27.8, 26.6, 26.0, 25.0 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{39}H_{42}N_5O_8S_2$ $[M+H]^+$ 772.2475, found 772.2439.

N[1]-Nosyl-*N*[3]-trityl-1,3-diaminopropane (**16**). Reaction time: 2 h; Slightly yellow solid (1.89 g, 83%); mp 185-189 °C; R_f (O): 0.29; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15-8.11 (m, 1H), 7.84-7.80 (m, 1H), 7.75-7.69 (m, 2H), 7.44-7.40 (m, 6H), 7.29-7.24 (m, 6H), 7.21-7.16 (m, 3H), 5.72 (br. s, 1H), 3.26 (t, *J* = 6.6 Hz, 2H), 2.19 (t, *J* = 6.6 Hz, 2H), 1.68 (quint., *J* = 6.6 Hz, 2H), 1.55 (br. s, 1H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.1, 145.7 (3C), 133.9, 133.4, 132.6, 131.1, 128.6 (6C), 127.9 (6C), 126.6 (3C), 125.3, 71.1, 42.6, 41.4, 30.6 ppm; Anal. Calcd (%) for $C_{28}H_{27}N_3O_4S$: C, 67.05; H, 5.43; N, 8.38. Found: C, 66.85; H, 5.58; N, 8.64; MS (ESI, 30 eV): *m/z* 540.57 $[M+K]^+$, 524.45 $[M+Na]^+$, 243.59 $[Trt]^+$.

N[1],*N*[4]-Dinosyl-*N*[7]-tritylnorspermidine (**17**). Reaction time: 2 h; Pale yellow solid (1.62 g, 84%); mp 166.0-168.4 °C; R_f (N): 0.25; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 7.2 and 1.7 Hz, 1H), 7.99 (dd, *J* = 7.8 and 0.9 Hz, 1H), 7.84 (dd, *J* = 7.2 and 1.7 Hz, 1H), 7.75-7.70 and 7.70-7.63 (two m, 4H), 7.59 (dd, *J* = 7.2 and 0.7 Hz, 1H), 7.43-7.39 (m, 6H), 7.28-7.24 (m, 6H), 7.20-7.16 (m, 3H), 5.60 (unresolv. t, 1H), 3.39-3.34 (m, 4H), 3.14 (q, *J* = 6.6 Hz, 2H), 2.08 (unresolv. t, 2H), 1.81 (quint., *J* = 6.6 Hz, 2H), 1.66 (unresolv. quint., 2H), 1.55 (br. s, 1H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.1, 148.0, 145.8 (3C), 133.7, 133.63, 133.60, 133.0, 132.8, 131.7, 130.90, 130.89, 128.6 (6C), 127.9 (6C), 126.4 (3C), 125.5, 124.3, 70.9, 46.2, 44.8, 40.9, 40.6, 29.6, 29.0 ppm; Anal. Calcd for $C_{37}H_{37}N_5O_8S_2$: C, 59.74; H, 5.01; N, 9.42. Found: C, 59.93; H, 4.89; N, 9.21; MS (ESI, 30 eV): *m/z* 782.42 $[M+K]^+$, 766.31 $[M+Na]^+$, 744.60 $[M+H]^+$, 243.54 $[Trt]^+$.

N[4],*N*[8]-Dinosyl-*N*[1]-tritylspermidine (**18**). Reaction time: 2 h; Pale yellow solid (0.86 g, 66%); mp 178-181 °C; R_f (O): 0.28; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 8.08 (t, *J* = 6.6 Hz, 1H), 7.99-7.96 (m, 1H), 7.96-7.92 (m, 3H), 7.88-7.85 and 7.85-7.82 (two m, 3H), 7.79 (td, *J* = 7.2 and 0.9 Hz, 1H), 7.36-7.33 (m, 6H), 7.29-7.24 (m, 6H), 7.18-7.14 (m, 3H), 3.25 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 6.6 Hz, 2H), 2.87 (q, *J* = 6.6 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 1H), 1.91 (q, *J* = 6.6 Hz, 2H), 1.61 (quint., *J* = 6.6 Hz, 2H), 1.44 (quint., *J* = 6.6 Hz, 2H), 1.37 (quint., *J* = 7.2 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (150

MHz, $(CD_3)_2SO$) δ 148.2, 148.0, 146.5 (3C), 134.8, 134.4, 133.2, 133.1, 132.8, 132.2, 130.1, 129.9, 128.8 (6C), 128.1 (6C), 126.5 (3C), 124.8, 124.7, 70.9, 47.2, 46.1, 42.7, 41.2, 29.2, 26.7, 25.5 ppm; Anal. Calcd for $C_{38}H_{39}N_5O_8S_2$: C, 60.22; H, 5.19; N, 9.24. Found: C, 60.35; H, 5.01; N, 9.1; MS (ESI, 30 eV): *m/z* 796.25 $[M+K]^+$, 780.32 $[M+Na]^+$, 243.52 $[Trt]^+$.

N^1,N^4,N^8-Trinosyl-N^{11}-tritylnorspermine (**22**). Reaction time: 1.5 h; Yellow foam (1.49 g, 81%); R_f (O): 0.17; ^1H NMR (600 MHz, $CDCl_3$) δ 8.11-8.07 (m, 1H), 8.01 (unresolv. dd, 1H), 7.97 (dd, *J* = 7.2 and 0.9 Hz, 1H), 7.86-7.82 (m, 1H), 7.74-7.70 and 7.70-7.64 (two m, 6H), 7.59 (dd, *J* = 7.8 and 1 Hz, 1H), 7.56 (dd, *J* = 7.2 and 0.8 Hz, 1H), 7.45-7.39 (m, 6H), 7.29-7.23 (m, 6H), 7.21-7.16 (m, 3H), 5.61 (t, *J* = 6.6 Hz, 1H), 3.40-3.35 (m, 4H), 3.31-3.24 (m, 4H), 3.14 (q, *J* = 6.6 Hz, 2H), 2.09 (t, *J* = 6.6 Hz, 2H), 1.83 (quint., *J* = 6.6 Hz, 4H), 1.66 (quint., *J* = 6.6 Hz, 2H), 1.60 (br. s, 1H) ppm; $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 148.1, 148.0 (2C), 145.9 (3C), 133.9, 133.7, 133.6, 133.5, 133.0, 132.9, 132.6, 132.1, 131.8, 130.94, 130.92, 130.7, 128.6 (6C), 127.8 (6C), 126.3 (3C), 125.4, 124.3, 124.2, 70.9, 46.0, 45.5, 45.2, 44.9, 40.8, 40.7, 29.3, 28.8, 27.6 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{46}H_{48}N_7O_{12}S_3$ $[M+H]^+$ 986.2523, found 986.2530.

N^1,N^4,N^8-Trinosyl-N^{12}-tritylspermine (**25**). Reaction time: 1.5 h; Yellow foam (0.93 g, 87%); R_f (O): 0.18; ^1H NMR (600 MHz, $CDCl_3$) δ 8.11-8.08 (m, 1H), 8.01-7.97 (m, 2H), 7.85-7.81 (m, 1H), 7.74-7.70, 7.70-7.67 and 7.67-7.62 (three m, 6H), 7.60-7.57 (m, 2H), 7.42-7.38 (m, 6H), 7.28-7.24 (m, 6H), 7.20-7.14 (m, 3H), 5.60 (t, *J* = 6 Hz, 1H), 3.37-3.31 (m, 4H), 3.30-3.26 (m, 4H), 3.13 (q, *J* = 6 Hz, 2H), 2.06 (t, *J* = 6 Hz, 2H), 1.83 (quint., *J* = 6 Hz, 2H), 1.64 (quint., *J* = 7.2 Hz, 2H), 1.54-1.50 (m, 5H) ppm; $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$), δ 148.1, 148.0, 147.9, 145.9 (3C), 133.7, 133.6, 133.55, 133.50, 133.3, 132.9, 132.8, 131.9, 131.7, 1310, 130.83, 130.80, 128.6 (6C), 127.8 (6C), 126.3 (3C), 125.4, 124.3, 124.2, 70.9, 47.3, 46.6, 46.5, 44.8, 40.9, 40.8, 29.3, 28.8, 25.04, 24.99 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{47}H_{50}N_7O_{12}S_3$ $[M+H]^+$ 1000.2680, found 1000.2568.

General procedure for the Mitsunobu reaction between nosylated PAs and *N*-protected ω-aminoalcohols

A typical procedure for the Mitsunobu reaction of *N*-nosylated PAs and *N*-protected ω-aminoalcohols is the following:

To an ice-cold solution of Spd derivative **13** (0.76 g, 1 mmol), alcohol **10a** (0.31 g, 1.30 mmol) and TPP (0.34 g, 1.30 mmol) in dry THF (2.9 mL), DIAD (0.26 mL, 1.30 mmol) was added under an inert atmosphere. The reaction mixture was allowed to attain room temperature where it was stirred for additional 2 h. The progress of the reaction was monitored by TLC using the solvent system P. Then, additional TPP (118 mg, 0.45 mmol) and DIAD (118 mg, 0.45 mmol) were added. The reaction mixture was further stirred for 2 h at room temperature and the completion of the reaction was confirmed by TLC. The solvent was evaporated and the product **7** was obtained pure following FCC purification using EtOAc as eluant.

The same procedure was used for the synthesis of all compounds tabulated below using the indicated for each case (a) different ratios of PA derivatives, alcohols, TPP and DIAD, (b) reaction time and (c) reaction temperature.

N^1-Dde-N^4-nosyl-N^8-tritylspermidine (**1a**). Quantities of reagents: **14** (1.03 g, 2.00 mmol), **10a** (0.62 g, 2.60 mmol), TPP (0.90 g, 3.33 mmol), DIAD (0.66 mL, 3.33 mmol); Reaction time: overnight; Reaction temperature: 25 °C; Yellow oil (1.13 g, 77%); R_f (Q): 0.18; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.48 (unresolv. t, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.62 (t, *J* =7.8 Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.45-7.41 (m, 6H), 7.29-7.23 (m, 6H), 7.20-7.14 (m, 3H), 3.43-3.38 (m, 4H), 3.28 (t, *J* = 7.5 Hz, 2H), 2.50 (s, 3H), 2.34 (s, 4H), 2.08 (t, *J* = 6.6 Hz, 2H), 1.92 (quint., *J* = 7.5 Hz, 2H), 1.65 (br. s, 1H), 1.57 (quint., *J* = 6.6 Hz, 2H), 1.43 (quint., *J* = 6.6 Hz, 2H), 1.01 (s, 6H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 173.7, 148.0, 146.1 (3C), 133.6, 133.2, 131.7, 131.0, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.2, 108.0, 70.8, 47.6, 44.6, 43.1, 40.6, 30.1, 28.3 (2C), 27.9, 27.8, 25.9, 17.9 ppm; MS (ESI, 30 eV): *m/z* 775.22 $[M+K]^+$, 759.47 $[M+Na]^+$, 737.55 $[M+H]^+$, 243.53 $[Trt]^+$.

N^4-Nosyl-N^8-phthaloyl-N^1-tritylspermidine (**1b**). Quantities of reagents: **16** (0.90 g, 1.80 mmol), **11** (0.51 g, 2.34 mmol), TPP (1.20 g, 4.50 mmol), DIAD (0.89 mL, 4.50 mmol); Reaction temperature and time: 25 °C, overnight then 40 °C, 2 h; Slightly yellow oil (1.04 g, 82%); R_f (N): 0.20; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00-7.97 (m, 1H), 7.83-7.79 (m, 2H), 7.73-7.69 (m, 2H), 7.65-7.60 (m, 2H), 7.56-7.54 (m, 1H), 7.46-7.40 (m, 6H), 7.30-7.25 (m, 6H), 7.21-7.16 (m, 3H), 3.68 (t, *J* = 6.6 Hz, 2H),

3.40 (t, *J* = 7.8 Hz, 2H), 3.33 (t, *J* = 7.8 Hz, 2H), 2.11 (t, *J* = 6.6 Hz, 2H), 1.74-1.65 (m, 4H), 1.60-1.55 (m, 3H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 168.3 (2C), 148.0, 145.9 (3C), 134.0 (2C), 133.6, 133.3, 132.1 (2C), 131.5, 130.8, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.1, 123.3 (2C), 70.9, 46.8, 45.8, 40.9, 37.2, 29.6, 25.7, 25.5 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{40}H_{39}N_4O_6S$ $[M+H]^+$ 703.2590, found 703.2612.

N^1-Dde-*N*^{4}-nosyl-*N*^{7}-tritylnorspermidine (**2**). Quantities of reagents: **16** (1.30 g, 2.60 mmol), **10a** (0.81 g, 3.38 mmol), TPP (1.13 g, 4.18 mmol), DIAD (0.82 mL, 4.18 mmol); Reaction time: overnight; Reaction temperature: 25 °C; Yellow oil (1.67 g, 89%); R_f (Q): 0.25; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.50 (unresolv. t, 1H), 8.01 (dd, *J* = 7.2 and 0.6 Hz, 1H), 7.67 (td, *J* = 7.2 and 0.6 Hz, 1H), 7.63 (td, *J* = 7.8 and 0.6 Hz, 1H), 7.59 (dd, *J* = 7.8 and 0.6 Hz, 1H), 7.46-7.37 (m, 6H), 7.29-7.23 (m, 6H), 7.20-7.14 (m, 3H), 3.44-3.37 (m, 6H), 3.52 (s, 3H), 3.31 (s, 4H), 2.11 (t, *J* = 6.6 Hz, 2H), 1.93 (quint., *J* = 7.2 Hz, 2H), 1.72 (quint., *J* = 6.6 Hz, 2H), 1.64 (br. s, 1H), 1.00 (s, 6H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 199.1 (2C), 173.8, 148.0, 145.9 (3C), 133.6, 133.0, 131.7, 131.0, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.2, 108.0, 70.9, 53.6, 52.2, 46.1, 44.8, 40.9, 40.6, 30.1, 29.4, 28.2 (2C), 28.0, 17.9 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{41}H_{47}N_4O_6S$ $[M+H]^+$ 723.3216, found 723.3201.

N^5-Nosyl-*N*^{1}-phthaloyl-*N*^{9}-tritylhomospermidine (**3**). Quantities of reagents: **14** (263 mg, 0.51 mmol), **11** (145 mg, 0.66 mmol), TPP (345 mg, 1.28 mmol), DIAD (0.25 mL, 1.28 mmol); Reaction temperature and time: 25 °C, overnight then 40 °C, 2 h; Slightly yellow oil (0.29 g, 80%); R_f (N): 0.20; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99-7.95 (m, 1H), 7.83-7.80 (m, 2H), 7.72-7.67 (m, 2H), 7.60-7.57 (m, 2H), 7.56-7.52 (m, 1H), 7.46-7.41 (m, 6H), 7.29-7.23 (m, 6H), 7.20-7.14 (m, 3H), 3.64 (t, *J* = 7.2 Hz, 2H), 3.31 (t, *J* = 7.2 Hz, 2H), 3.26 (t, *J* = 7.2 Hz, 2H), 2.71 (t, *J* = 6.6 Hz, 2H), 1.64 (quint., *J* = 7.2 Hz, 2H), 1.59-1.51 (m, 5H), 1.42 (quint., *J* = 6.6 Hz, 2H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 168.3 (2C), 148.0, 146.2 (3C), 134.0 (2C), 133.8, 133.3, 132.1 (2C), 131.5, 130.8, 128.6 (6C), 127.8 (6C), 126.2 (3C), 124.1, 123.3 (2C), 70.8, 42.5, 46.7, 43.1, 37.2, 27.9, 26.1, 25.7, 25.4 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{41}H_{41}N_4O_6S$ $[M+H]^+$ 717.2747, found 717.2722.

N^1-Dde-N^4,N^8-dinosyl-N^{12}-tritylspermine (**4**). Quantities of reagents: **18** (811 mg, 1.07 mmol), **10a** (333 mg, 1.39 mmol), TPP (468 mg, 1.74 mmol), DIAD (0.34 mL, 1.74 mmol); Reaction time: overnight; Reaction temperature: 25 °C; Yellow oil; R_f (R): 0.17; MS (ESI, 30 eV): *m/z* 1001.60 $[M+Na]^+$, 979.01 $[M+H]^+$, 243.57 $[Trt]^+$.
The NMR and HRMS spectra of compound **4** are provided below.

N^1-Dde- N^4,N^8-dinosyl-N^{11}-tritylnorspermine (**5**). Quantities of reagents: **17** (185 mg, 0.25 mmol), **10a** (77 mg, 0.32 mmol), TPP (172 mg, 0.64 mmol), DIAD (0.13 mL, 0.64 mmol); Reaction time: overnight; Reaction temperature: 25 °C; Yellow oil; R_f (Q): 0.15; MS (ESI, 30 eV): *m/z* 1003.51 $[M+K]^+$, 965.22 $[M+H]^+$.
The NMR and HRMS spectra of compound **5** are provided below.

N^5,N^{10}-Dinosyl-N^1-phthaloyl-N^{14}-tritylhomospermine (**6**). Quantities of reagents: **15** (295 mg, 0.38 mmol), **11** (107 mg, 0.49 mmol), TPP (226 mg, 0.84 mmol), DIAD (0.16 mL, 0.84 mmol). Compound **6** contained $DIADH_2$ in the ratio 1:0.25 (by ^{1}H-NMR); Reaction temperature and time: 25 °C, 2 h then 40 °C, 1.5 h and finally 25 °C, overnight; Yellow oil (0.32 g, 87%); R_f (O): 0.28; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99-7.94 (m, 2H), 7.85-7.81 (m, 2H), 7.72-7.69 (m, 2H), 7.68-7.58 (m, 5H), 7.54-7.51 (m, 1H), 7.46-7.41 (m, 6H), 7.29-7.23 (m, 6H), 7.20-7.14 (m, 3H), 3.64 (t, J = 6.6 Hz, 2H), 3.30-3.25 (m, 6H), 3.23 (t, J = 7.2 Hz, 2H), 2.08 (t, J = 6.6 Hz, 2H), 1.62 (quint., J = 7.2 Hz, 2H), 1.58-1.45 (m, 9H), 1.41 (quint., J = 6.6 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 168.3 (2C), 148.0 (2C), 146.1 (3C), 134.0 (2C), 133.7, 133.5, 133.4, 133.3, 132.1 (2C), 131.7, 131.6, 130.7, 130.6, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.14, 124.10, 123.2 (2C), 70.8, 47.2, 46.8, 46.7, 46.5, 43.2, 37.2, 27.9, 26.0, 25.7, 25.4, 25.1, 25.0 ppm; MS (ESI, 30 eV): *m/z* 995.68 $[M+Na]^+$, 973.80 $[M+H]^+$, 243.74 $[Trt]^+$.

N^1-Dde- N^4,N^8-dinosyl-N^{12}-tritylthermospermine (**7**). Quantities of reagents: **13** (0.76 g, 1 mmol), **10a** (0.31 mg, 1.30 mmol), TPP (0.47 mg, 1.75 mmol), DIAD (0.34 mL, 1.75 mmol); Reaction time: overnight; Reaction temperature: 25 °C; Yellow oil (0.82 g, 84%); R_f (EtOAc): 0.38; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.47 (unresolv. t, 1H), 8.05-8.01 (m, 1H), 7.96 (dd, J = 7.2 and 1.2 Hz, 1H), 7.69-7.61 (m, 4H), 7.59 (dd, J = 7.8 and 1.2 Hz, 1H), 7.58-7.55 (m, 1H), 7.45-7.41 (m, 6H), 7.29-7.24 (m, 6H), 7.20-

7.15 (m, 3H), 3.40 (t, J = 7.2 Hz, 2H), 3.37 (q, J = 7.2 Hz, 2H), 3.30 (t, J = 7.2 Hz, 2H), 3.27 and 3.24 (two t, J = 7.2 Hz, 4H), 2.48 (s, 3H), 2.35 (br. s, 4H), 2.06 (t, J = 7.2 Hz, 2H), 1.91 and 1.86 (two quint., J = 7.2 Hz, 4H), 1.61 (br. s, 1H), 1.53 (quint., J = 7.2 Hz, 1H), 1.40 (quint., J = 7.2 Hz, 2H), 1.02 (s, 6H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 173.8, 148.0, 147.9, 146.1 (3C), 133.8, 133.6, 133.2, 132.7, 132.0, 131.7, 131.2, 130.8, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.2, 124.1, 108.1, 70.8, 47.8, 45.2, 45.1, 44.9, 43.1, 40.4, 30.0, 28.3 (2C), 27.9, 27.8, 27.5, 26.0, 17.9 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{51}H_{59}N_6O_{10}S_2$ $[M+H]^+$ 979.3734, found 979.3718.

N^1-Dde-N^4,N^8,N^{13}-trinosyl-N^{16}-trityl-4,8,13-triazahexadecane-1,16-diamine (**26a**). Quantities of reagents: **23** (101 mg, 0.10 mmol), **10a** (31 mg, 0.13 mmol), TPP (105 mg, 0.40 mmol), DIAD (0.08 mL, 0.40 mmol); Reaction temperature and time: 25 °C, overnight then 40 °C, 2 h; Yellow oil; R_f (R): 0.18.

The NMR and HRMS spectra of compound **26a** are provided below.

Preparation of 2-(1-(*tert*-butoxycarbonyl)-1*H*-indol-3-yl)acetic acid (20b)

A. Protection of the carboxyl function of IAA with the phenacyl group

To an ice-cold solution of IAA (0.175 g, 1 mmol) and dry Et_3N (0.20 mL, 1.34 mmol) in dry DMF (3 mL), 2-bromoacetophenone (0.23 g, 1.16 mmol) was added under an inert atmosphere and the reaction was stirred at 0 °C for 30 min. The reaction mixture was then allowed to reach room temperature and further stirred for 2 h. The completion of the reaction was confirmed by TLC using the solvent system C. The reaction mixture was diluted with H_2O and extracted thrice with EtOAc. The organic layer washed twice with H_2O and once with brine and then was dried over Na_2SO_4 and evaporated to dryness. The desired product, that is phenacyl 2-(1*H*-indol-3-yl)acetate, was obtained pure following FCC and using solvent system V as eluant.

White solid (0.24 g, 81%); mp 87.8-90.0 °C; R_f (W): 0.44; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (br. s, 1H), 7.89 (dd, J = 8.1 and 1.3 Hz, 2H), 7.66 (d, J = 8.4 Hz, 1H), 7.59 (tt, J = 8.1 and 1.3 Hz, 1H), 7.46 (t, J = 8.1 Hz, 2H), 7.36 (d, J = 7.8 Hz, 1H), 7.26 (br. s, 1H), 7.22-7.19 (m, 1H), 7.16-7.14 (m, 1H), 5.36 (s, 2H), 3.98 (unresolv. d, 2H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 192.7, 171.9, 136.5, 134.7, 134.3,

129.2 (2C), 128.2 (2C), 127.7, 123.7, 122.6, 120.1, 119.3, 111.6, 108.4, 66.7, 31.3 ppm; MS (ESI, 30 eV): *m/z* 332.34 $[M+K]^+$, 316.40 $[M+Na]^+$.

*B. Protection of the amino function of phenacyl 2-(1*H*-indol-3-yl)acetate with the Boc-group*

To an ice-cold solution of phenacyl 2-(1*H*-indol-3-yl)acetate (0.24 g, 0.81 mmol), dry Et_3N (0.12 mL, 0.89 mmol) and DMAP (10 mg, 0.08 mmol) in dry CH_2Cl_2 (2 mL), Boc_2O (0.19 g, 0.89 mmol) was added under an inert atmosphere. The reaction was kept for 3 h at room temperature. The completion of the reaction was confirmed by TLC using the solvent system V. Then, the reaction mixture was diluted with CH_2Cl_2 and washed twice with H_2O and once with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The desired product, that is phenacyl 2-(1-(*tert*-butoxycarbonyl)-1*H*-indol-3-yl)acetate, was obtained pure following purification by FCC and using the solvent system U as eluant.

Yellow oil (0.29 g, 91%); R_f (U): 0.20; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 (br. s, 1H), 7.89 (dd, *J* = 8.4 and 1.2 Hz, 2H), 7.67 (br. s, 1H), 7.61-7.58 (m, 2H), 7.49-7.46 (m, 2H), 7.35-7.32 (m, 1H), 7.28-7.25 (m, 1H), 5.38 (s, 2H), 3.92 (unresolv. d, 2H), 1.67 (s, 9H) ppm; $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 192.4, 170.9, 150.0, 135.9, 134.6, 134.3, 130.5, 129.3 (2C), 128.2 (2C), 125.1, 125.0, 123.1, 119.5, 115.7, 113.1, 84.0, 66.9, 31.1, 28.6 (3C) ppm; MS (ESI, 30 eV): *m/z* 809.13 $[2M+Na]^+$, 416.15 $[M+Na]^+$.

*C. Deprotection of the carboxyl function of phenacyl 2-(1-(tert-butoxycarbonyl)-1*H*-indol-3-yl)acetate*

To a solution of phenacyl 2-(1-(*tert*-butoxycarbonyl)-1*H*-indol-3-yl)acetate (0.29 g, 0.74 mmol) in DMF (7 mL), Na_2CO_3 (0.41 g, 3.91 mmol) first and then PhSH (0.30 mL, 2.96 mmol) were added under an inert atmosphere. The reaction mixture was kept at 60 ºC for 2 h. The completion of the reaction was confirmed by TLC using the solvent system U. The reaction mixture was then diluted with 5% citric acid and extracted with EtOAc. The organic layer washed twice with H_2O and once with brine, dried over Na_2SO_4 and evaporated to dryness. The product N^{ind}-Boc-IAA **(20b)** was obtained pure following FCC purification and using solvent system R as eluant.

White solid (0.17 g, 84%); mp 117-120 ºC; R_f (R): 0.17; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (br. s, 1H), 7.58 (br. s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 3.75 (s, 2H), 1.66 (s, 9H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 176.9, 150.0, 135.8, 130.3, 129,4, 125.0, 123.1, 119.4, 115.7, 112.9, 84.2, 31.2, 28.6 (3C) ppm.

General procedure for coupling IAA (20a) or *N*-Boc-IAA (20b) with 4-aminobutan-1-ol

To an ice-cold solution of IAA or *N*-Boc-IAA (1 mmol) and HOSu (0.23 g, 2 mmol) in DMF (4 mL), DCC (0.23 g, 1.1 mmol) was added under an inert atmosphere. After 15 min at 0 ºC, the reaction mixture was further stirred at room temperature for 2 h. Completion of the activation reaction was confirmed by TLC using solvent system C. Then, 4-aminobutan-1-ol (0.3 mL, 3 mmol) was added and the reaction was completed within a few minutes. A few drops of H_2O and one drop of gl. acetic acid were added to the reaction mixture in order to destroy excess of DCC. The reaction mixture was then diluted with EtOAc and the precipitated urea was removed by filtration. The filtrate was diluted with EtOAc and washed twice with 5% $NaHCO_3$ and twice with H_2O, dried over Na_2SO_4 and evaporated to dryness. The product was obtained pure following FCC purification and using solvent system C as eluant.

*N-(4-Hydroxybutyl)-2-(1*H*-indol-3-yl)acetamide* (**21a**). Orange solid (0.16 g, 65%); mp 80.0-83.6 ºC; R_f (C): 0.23; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (br. s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.23 (td, *J* = 8.4 and 0.6 Hz, 1H), 7.15 (ddd, *J* = 8.4, 7.8 and 0.6 Hz, 1H), 7.12 (d, *J* = 2.4 Hz, 1H), 5.89 (unresolv. t, 1H), 3.73 (s, 2H), 3.52 (t, *J* = 6 Hz, 2H), 3.20 (q, *J* = 6.6 Hz, 2H), 1.48-1.40 (m, 5H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 171.8, 136.5, 127.1, 123.9, 122.6, 120.0, 118.7, 111.5, 109.0, 62.3, 39.3, 33.4, 29.6, 26.0 ppm; Anal. Calcd (%) for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.48; H, 7.52; N, 11.48.

tert-*Butyl 3-(2-((4-hydroxybutyl)amino)-2-oxoethyl)-1*H*-indole-1-carboxylate* (**21b**). Beige solid (0.13 g, 72%); mp 122-126 ºC; R_f (C): 0.43; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 (d, *J* = 6.6 Hz, 1H), 7.54 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.35 (td, *J* = 6.6 and 0.6 Hz, 1H), 7.26 (ddd, *J* = 7.8, 6.6 and 0.6 Hz, 1H), 5.78 (unresolv. t, 1H), 3.66 (s,

2H), 3.56 (t, *J* = 6 Hz, 2H), 3.23 (q, *J* = 6 Hz, 2H), 1.68 (s, 9H), 1.51-1.45 (m, 5H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 170.3 (2C), 149.5, 129.8, 125.0, 124.9, 122.9, 119.0, 115.4, 113.9, 84.1, 62.3, 39.4, 33.3, 29.6, 28.2 (3C), 26.1 ppm; Anal. Calcd (%) for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09. Found: C, 66.12; H, 7.38; N, 7.95.

Coupling of Nsm derivative 22 with alcohol 21b under Mitsunobu reaction conditions - Synthesis of the crude fully protected toxin HO-416b (23a). Quantities of reagents: **22** (197 mg, 0.20 mmol), **21b** (90 mg, 0.26 mmol), TPP (140 mg, 0.52 mg), DIAD (0.1 mL, 0.52 mmol), dry THF (0.6 mL); Reaction time: overnight; Reaction temperature: 40 °C; Slightly yellow oil; R_f (S): 0.29; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (unresolv. d, 1H), 8.00 (dd, *J* = 7.8 and 0.6 Hz, 1H), 7.97-7.93 (m, 2H), 7.72-7.63 (m, 4H), 7.60-7.54 (m, 5H), 7.53-7.48 (m, 2H), 7.44-7.40 (m, 6H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.30-7.23 (m, 7H), 7.21-7.15 (m, 3H), 5.80 (t, *J* = 6.6 Hz, 1H), 3.64 (s, 2H), 3.37 (t, *J* = 7.2 Hz, 2H), 3.39-3.24 and 3.24-3.20 (two m, 10H), 3.18 (q, *J* = 6.6 Hz, 2H), 2.09 (t, *J* = 6.6 Hz, 2H), 1.86-1.77 (m, 4H), 1.68 (s, 9H), 1.61 (br. s, 1H), 1.51-1.45 (m, 4H), 1.39 (quint., *J* = 7.2 Hz, 2H) ppm; MS (ESI, 30 eV): *m/z* 1314.32 $[M+H]^+$, 243.18 $[Trt]^+$.

General procedure for the alkylation of *N*-nosylated PAs with bromide 10c

A typical procedure for the alkylation of *N*-nosylated PAs with bromide **10c** is the following:

To a solution of dinosylated derivative **17** (1.31 g, 1.76 mmol) and bromide **10c** (0.59 g, 1.94 mmol) in dry DMF (3 mL), K_2CO_3 (0.53 g, 3.70 mmol) was added under an inert atmosphere. The reaction mixture was stirred at 60 °C for 2.5 h. The progress of the reaction was monitored by TLC using the solvent system R. The reaction mixture was then diluted with H_2O and extracted once with EtOAc. The organic layer was washed twice with H_2O and once with brine. It was then dried over Na_2SO_4 and it was evaporated to dryness. The product **5** was obtained pure following FCC purification using solvent system Q as eluant.

The same procedure was used for the synthesis of Spm derivative **4** and the penta-amine derivative **26a**.

N^1-Dde- N^4,N^8-dinosyl-N^{12}-tritylspermine (**4**). Yellow oil (0.43 g, 88%); R_f (R): 0.17; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 13.48 (unresolv. t, 1H), 8.04 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.99 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.71-7.63 (m, 4H), 7.62-7.58 (m, 2H), 7.45-7.39 (m, 6H), 7.31-7.25 (m, 6H), 7.22-7.16 (m, 3H), 3.44-3.38 (m, 4H), 3.37-3.33 (m, 4H), 3.31 (t, *J* = 6.6 Hz, 2H), 2.51 (s, 3H), 2.37 (s, 4H), 2.09 (t, *J* = 6.6 Hz, 2H), 1.94 (quint., *J* = 6.6 Hz, 2H), 1.67 (quint., *J* = 6.6 Hz, 2H), 1.62-1.51 (m, 5H), 1.04 (s, 6H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 173.7, 147.99, 147.96, 145.9 (3C), 133.7, 133.5, 133.4, 133.0, 131.9, 131.6, 131.0, 130.8, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.2 (2C), 108.0, 70.9, 53.5, 52.3, 47.0, 46.6, 45.6, 44.7, 40.9, 40.5, 30.1, 29.4, 28.3 (2C), 27.9, 25.0, 24.8, 17.8 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{51}H_{59}N_6O_{10}S_2$ $[M+H]^+$ 979.3734, found 979.3729.

N^1-Dde- N^4,N^8-dinosyl-N^{11}-tritylnorspermine (**5**). Yellow oil; (1.29 g, 76%); R_f (Q): 0.15; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.49 (unresolv. t, 1H), 8.07-8.03 (m, 1H), 7.98 (dd, *J* = 7.8 and 0.6 Hz, 1H), 7.70-7.63 (m, 4H), 7.59 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.58-7.55 (m, 1H), 7.43-7.38 (m, 6H), 7.29-7.23 (m, 6H), 7.20-7.14 (m, 3H), 3.44-3.39 (m, 4H), 3.39-3.35 (m, 2H), 3.31 (t, *J* = 7.2 Hz, 2H), 3.27 (t, *J* = 7.2 Hz, 2H), 2.50 (s, 3H), 2.36 (s, 4H), 2.08 (t, *J* = 6.6 Hz, 2H), 1.93 (quint., *J* = 7.2 Hz, 2H), 1.86 (quint., *J* = 7.2 Hz, 2H), 1.66 (quint., *J* = 6.6 Hz, 2H), 1.57 (br. s, 1H), 1.03 (s, 6H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 173.8, 148.0, 147.9, 145.7 (3C), 133.8, 133.6, 133.0, 132.7, 132.0, 131.7, 131.2, 130.8, 128.6 (6C), 127.9 (6C), 126.3 (3C), 124.22, 124.21, 108.1, 70.9, 46.0, 45.3, 45.1, 44.9, 40.9, 40.5, 30.1, 29.4, 28.3 (2C), 27.9, 27.5, 17.9 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{50}H_{57}N_6O_{10}S_2$ $[M+H]^+$ 965.3578, found 965.3541.

N^1-Dde-N^4,N^8,N^{13}-trinosyl-N^{16}-trityl-4,8,13-triazahexadecane-1,16-diamine (**26a**). Reaction time: 2.5 h; Reaction temperature: 60 °C; Yellow oil (0.85 g, 72%); R_f (R): 0.18; ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.45 (unresolv. t, 1H), 8.05-8.02 (m, 1H), 7.99-7.95 (m, 2H), 7.71-7.65 (m, 5H), 7.64 (td, *J* = 7.2 and 1.2 Hz, 1H), 7.61-7.56 (m, 3H), 7.42-7.38 (m, 6H), 7.28-7.23 (m, 6H), 7.20-7.14 (m, 3H), 3.41 (t, *J* = 7.2 Hz, 2H), 3.38 (q, *J* = 6.6 Hz, 2H), 3.33 (t, *J* = 7.8 Hz, 2H), 3.21-3.27 (m, 6H), 3.24 (t, *J* = 7.2 Hz, 2H), 2.48 (s, 3H), 2.36 (s, 4H), 2.06 (t, *J* = 6.6 Hz, 2H), 1.91 (quint., *J* = 7.8 Hz, 2H), 1.86 (quint., *J* = 6.6 Hz, 2H), 1.64 (quint., *J* = 7.2 Hz, 2H), 1.54-1.50 (m, 5H),

1.03 (s, 6H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 173.8, 148.0 (2C), 147.9, 145.9 (3C), 133.9, 133.7, 133.5, 133.3, 133.0, 132.7, 132.0, 131.9, 131.7, 131.1, 130.8, 130.7, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.24, 124.20 (2C), 108.0, 70.9, 47.2, 46.6, 45.6, 45.1, 45.0 (2C), 40.9, 40.5, 30.1, 29.4, 28.3 (2C), 27.8, 27.3, 25.0, 24.9, 17.9 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{60}H_{69}N_8O_{14}S_3$ $[M+H]^+$ 1221.4095, found 1221.4077.

General procedure for the selective removal of the Dde group

A typical procedure for the selective removal of the Dde group from fully protected PAs and conjugates is the following:

To a solution of N^1-Dde-N^4-nosyl-N^8-tritylspermidine (**1a**) (0.85 g, 1.16 mmol) in DMF (15 mL), $H_2NNH_2{\cdot}H_2O$ (0.30 mL, 6.15 mmol) was added. The reaction mixture was stirred at room temperature for 30 min. The progress of the reaction was monitored by TLC using EtOAc as eluant. The reaction mixture was then diluted with CH_2Cl_2 and washed twice with H_2O and once with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The product was obtained pure following FCC purification using initially solvent system E, to remove non-polar fractions, and then solvent system H as eluants.

The same procedure was used for the preparation of the partially protected PAs tabulated below. The solvent systems used for the purification of the following compounds are also provided.

N^4-Nosyl-N^8-tritylspermidine. Slightly yellow oil (0.58 g, 87%); R_f(E): 0.10; Solvent systems used for FCC purification: initially E, then H; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 7.8 and 1.8 Hz, 1H), 7.66-7.60 (m, 2H), 7.59 (dd, *J* = 7.8 and 1.8 Hz, 1H), 7.45-7.42 (m, 6H), 7.28-7.24 (m, 6H), 7.20-7.16 (m, 3H), 3.37 (t, *J* = 7.2 Hz, 2H), 3.27 (t, *J* = 7.2 Hz, 2H), 2.72 (t, *J* = 6.6 Hz, 2H), 2.08 (t, *J* = 6.6 Hz, 2H), 1.68 (quint., *J* = 6.6 Hz, 2H), 1.59-1.54 (m, 5H), 1.42 (quint., *J* = 7.2 Hz, 2H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 148.1, 146.1 (3C), 133.7, 133.3, 131.6, 130.7, 128.6 (6C), 127.8 (6C), 126.2 (3C), 124.1, 70.8, 47.5, 45.0, 43.1, 40.0, 31.6, 27.9, 26.0 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{32}H_{37}N_4O_4S$ $[M+H]^+$ 573.2536, found 573.2522.

N^4-Nosyl-N^1-tritylnorspermidine. Yellow oil (1.05 g, 72%); R_f (E): 0.15; Solvent systems used for FCC purification: initially E, then H; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (unresolv. dd, 1H), 7.67-7.60 (m, 2H), 7.58 (d, J = 7.8 Hz, 1H), 7.43-7.39 (m, 6H), 7.28-7.24 (m, 6H), 7.20-7.16 (m, 3H), 3.39 and 3.36 (two t, J = 7.2 Hz, 4H), 2.71 (t, J = 6.6 Hz, 2H), 2.09 (t, J = 6.6 Hz, 2H), 1.73-1.64 (m, 4H), 1.51 (br. s, 3H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.1, 145.9 (3C), 133.5, 133.3, 131.5, 130.8, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.2, 70.9, 45.7, 45.1, 40.9, 39.0, 31.7, 24.5 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{31}H_{35}N_4O_4S$ $[M+H]^+$ 559.2379, found 559.2402.

N^4,N^9-Dinosyl-N^1-tritylspermine. Yellow oil (0.79 g, 91%); R_f (G): 0.19; Solvent systems used for FCC purification: initially G, then H; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00-7.96 (m, 2H), 7.68-7.62 (m, 4H), 7.59-7.56 (m, 2H), 7.43-7.39 (m, 6H), 7.28-7.24 (m, 6H), 7.20-7.16 (m, 3H), 3.36-3.32 and 3.32-3.26 (two m, 8H), 2.69 (t, J = 6.6 Hz, 2H), 2.07 (t, J = 6.6 Hz, 2H), 1.70-1.63 (m, 4H), 1.53 (unresolv. m, 7H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.44, 148.39, 146.3 (3C), 133.9, 133.8, 132.1, 132.0, 131.12, 131.08, 129.0 (6C), 128.2 (6C), 126.7 (3C), 124.6, 124.5, 71.3, 47.3, 47.1, 46.0, 45.5, 41.3, 39.4, 32.1, 29.8, 25.5, 25.4 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{41}H_{47}N_6O_8S_2$ $[M+H]^+$ 815.2897, found 815.2868.

N^4,N^8-Dinosyl-N^1-tritylnorspermine. Yellow oil (1.51 g, 94%); R_f (G): 0.18; Solvent systems used for FCC purification: initially G then J; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02-7.97 (m, 2H), 7.69-7.63 (m, 4H), 7.60-7.57 and 7.57-7.54 (two m, 2H), 7.44-7.38 (m, 6H), 7.29-7.24 (m, 6H), 7.21-7.16 (m, 3H), 3.37 (t, J = 7.2 Hz, 4H), 3.29 and 3.27 (two t, J = 7.2 Hz, 4H), 2.70 (t, J = 6.6 Hz, 2H), 2.08 (t, J = 6.6 Hz, 2H), 1.86 (quint., J = 7.2 Hz, 2H), 1.70-1.63 (m, 4H), 1.44 (br. s, 3H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.44, 148.40, 146.3 (3C), 134.0, 133.9, 133.53, 133.47, 132.16, 132.09, 131.23, 131.22, 129.0 (6C), 128.2 (6C), 126.7 (3C), 124.6, 124.6, 71.3, 46.4, 45.9, 45.5, 45.4, 41.3, 39.3, 32.1, 29.8, 27.9 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{40}H_{45}N_6O_8S_2$ $[M+H]^+$ 801.2740, found 801.2752.

N^4,N^8,N^{13}-Trinosyl-N^{16}-trityl-4,8,13-triazahexadecane-1,16-diamine (**26b**). Orange oil; (0.85 g, 83%); R_f(G): 0.11; Solvent systems used for FCC purification: initially G, then H; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00-7.96 (m, 3H), 7.69-7.63 (m, 6H), 7.59-

7.56 (m, 3H), 7.42-7.38 (m, 6H), 7.27-7.23 (m, 6H), 7.19-7.15 (m, 3H), 3.37-3.32 (m, 4H), 3.28-3.27 (m, 6H), 3.23 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 6.6 Hz, 2H), 2.06 (t, J = 6.6 Hz, 2H), 1.83 (quint., J = 7.2 Hz, 2H), 1.65 (quint., J = 7.2 Hz, 4H), 1.54-1.45 (m, 7H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 148.03, 147.98, 147.96, 145.9 (3C), 133.63, 133.62, 133.5, 133.4, 133.04, 133.03, 131.9, 131.8, 131.7, 130.83, 130.75, 130.70, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.18 (2C), 124.17, 70.9, 47.1, 46.7, 45.6, 45.5, 44.96, 44.94, 40.9, 38.9, 31.7, 29.4, 27.4, 25.0, 24.9 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{50}H_{57}N_8O_{12}S_3$ $[M+H]^+$ 1057.3258, found 1057.3237.

General procedure for the selective removal of the Phth group

A typical procedure for the selective removal of the Phth group from fully protected PAs is the following:

To a solution of fully protected Spd **1b** (1.26 g, 1.80 mmol) in EtOH (12 mL) $H_2NNH_2{\cdot}H_2O$ (0.18 mL, 3.60 mmol) was added. The reaction mixture was then heated under reflux for 1 h. The progress of the reaction was monitored by TLC using the solvent system N. Then, additional $H_2NNH_2{\cdot}H_2O$ (0.18 mL, 3.6 mmol) was added and the reaction mixture was further refluxed for an additional 1 h. Following completion of the reaction, the resulting mixture was allowed to attain room temperature and the solvent was evaporated. The residue was diluted with 10% $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer washed once with brine and then was dried over Na_2SO_4. Filtration and evaporation of the filtrate to dryness provided a residue from which the desired product was obtained pure through FCC purification using the solvent systems indicated below.

The same procedure was used for the removal of the Phth group from the fully protected Hsp derivative **3**.

N^4-Nosyl-N^1-tritylspermidine. Slightly yellow oil (0.97 g, 94%); R_f(H): 0.34; Solvent system for FCC purification: initially G, then H; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (dd, J = 7.8 and 1.8 Hz, 1H), 7.67-7.60 (m, 2H), 7.58 (dd, J = 7.8 and 1.2 Hz, 1H), 7.44-7.39 (m, 6H), 7.30-7.24 (m, 6H), 7.20-7.16 (m, 3H), 3.38 (t, J = 7.2 Hz, 2H), 3.28 (t, J = 7.2 Hz, 2H), 2.68 (t, J = 7.2 Hz, 2H), 2.10 (t, J = 6.6 Hz, 2H), 1.69 (quint., J = 7.2 Hz, 2H), 1.57 (quint., J = 7.2 Hz, 2H), 1.48-1.41 (br. s, 3H), 1.41 (m, 2H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 148.0, 145.9 (3C), 133.7, 133.3, 131.5, 130.8, 128.6 (6C), 127.8 (6C), 126.3 (3C), 124.2, 70.9, 47.3, 45.5, 41.7, 40.9, 30.6,

29.5, 25.7 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{32}H_{37}N_4O_4S$ $[M+H]^+$ 573.2536, found 573.2561.

N^5-Nosyl-N^1-tritylhomospermidine. Yellow oil (0.28 g, 93%); R_f (H): 0.30; Solvent systems for FCC purification: initially G, then H; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.67-7.60 (m, 2H), 7.59 (dd, *J* = 7.8 and 0.6 Hz, 1H), 7.46-7.42 (m, 6H), 7.29-7.24 (m, 6H), 7.20-7.16 (m, 3H), 3.28 (two overlap. t, *J* = 7.8 Hz, 4H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.08 (t, *J* = 6.6 Hz, 2H), 1.57 (quint., *J* = 7.8 Hz, 4H), 1.46-1.34 (m, 7H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 148.4, 146.5 (3C), 134.2, 133.7, 131.9, 131.2, 129.0 (6C), 128.2 (6C), 126.6 (3C), 124.5, 71.2, 47.7, 47.6, 43.5, 42.1, 30.1, 28.3, 26.4, 26.1 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{33}H_{39}N_4O_4S$ $[M+H]^+$ 587.2692, found 587.2664.

Coupling of partially protected penta-amine 26b with IAA (20a) - Synthesis of toxin Agel 416 derivative 27a

To an ice-cold solution of IAA (**20a**) (0.15 mg, 0.87 mmol) and HOSu (0.20 g, 1.74 mmol) in dry DMF (3.5 mL), DIC (0.15 mL, 0.96 mmol) was added under an inert atmosphere. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for further 2 h. The completion of the activation reaction was confirmed by TLC using solvent system C. Then, the penta-amine derivative **26b** (0.84 g, 0.79 mmol) was added to the reaction mixture. The reaction was completed within 2 h. Completion of the reaction was confirmed by TLC using solvent system H. Then, the reaction mixture was diluted with EtOAc and washed twice with 5% $NaHCO_3$, twice with H_2O and once with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. FCC of the oily residue, using EtOAc as eluant, provided intermediate **27a**, co-eluted with DCIU, which was used as such into the next deprotection step

Reaction time: 4.5 h; Slightly yellow oil; R_f (EtOAc): 0.20; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (br. s, 1H), 7.95 (dd, *J* = 7.8 and 1.2 Hz, 1H), 7.93-7.91 (m, 1H), 7.70-7.60 (m, 8H), 7.59-7.54 (m, 4H), 7.41-7.37 (m, 6H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.28-7.22 (m, 6H), 7.21-7.15 (m, 4H), 7.11-7.07 (m, 1H), 6.11 (t, *J* = 6 Hz, 1H), 3.71 (s, 2H), 3.31 (t, *J* = 7.2 Hz, 2H), 3.24 and 3.22 (two t, *J* = 7.2 Hz, 4H), 3.18 (q, *J* = 6 Hz,

2H), 3.14 (t, *J* = 7.2 Hz, 2H), 3.09 and 3.09 (two t, *J* = 7.2 Hz, 4H), 2.07 (t, *J* = 6.6 Hz, 2H), 1.68-1.58 (m, 8H), 1.48-1.44 (m, 3H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 171.9, 147.97, 147.95, 147.93, 145.88 (3C), 136.4, 133.70, 133.66, 133.5, 133.3, 132.9, 132.6, 132.0, 131.9, 131.7, 130.68, 130.65, 130.4, 128.6 (6C), 127.8, (6C), 127.2, 126.3 (3C), 124.21, 124.16, 124.15, 122.3, 119.8, 118.6, 111.5, 109.0, 70.9, 47.1, 46.7, 45.6, 45.4, 44.9, 42.2, 40.9, 35.9, 33.5, 29.4, 28.1, 27.5, 25.0, 24.9 ppm; MS (ESI, 30 eV): *m/z* 1214.28 $[M+H]^+$.

General procedure for the removal of the Ns group – Preparation of the penta-amine derivatives 23b and 27b

A typical procedure for the selective removal of the Ns group from fully protected PAs is the following:

To a solution of crude **23a** (0.2 mmol) and PhSH (0.24 mL, 2.40 mmol) in DMF (2 mL) Na_2CO_3 (0.32 g, 3 mmol) was added, under an inert atmosphere, and the resulting suspension was stirred vigorously overnight. The completion of the reaction was confirmed by TLC using initially solvent system S and then solvent system H. The reaction mixture was diluted with H_2O and extracted twice with CH_2Cl_2. The organic layer was washed once with H_2O and once with brine, dried over Na_2SO_4 and evaporated to dryness. The product was obtained pure following FCC purification using solvent system I as eluant.

The same procedure was used for the removal of the Ns groups from the penta-amine derivative **27a**.

*Partially protected toxin HO-416b (**23b**)*. Slightly yellow oil; R_f (I): 0.15; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (unresolv. d, 1H), 7.55 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.49-7.45 (m, 6H), 7.38-7.33 (m, 1H), 7.30-7.24 (m, 7H), 7.20-7.16 (m, 3H), 6.02 (unresolv. t, 1H), 3.66 (s, 2H), 3.21 (q, *J* = 6.6 Hz, 2H), 2.70-2.60 (m, 8H), 2.58 (t, *J* = 6.6 Hz, 2H), 2.52 (t, *J* = 7.2 Hz, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 1.69 (s, 9H), 1.67 (quint., *J* = 7.2 Hz, 4H), 1.61 (quint., *J* = 6.6 Hz, 2H), 1.45 (quint., *J* = 6.6 Hz, 2H), 1.39 (quint., *J* = 7.2 Hz, 2H), 1.27 (br. s, 1H) ppm; $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$) δ 170.0 (2C), 149.5, 146.2 (3C), 129.9, 128.7 (6C), 127.8 (6C), 126.2 (3C), 124.9, 124.8, 122.9, 119.1, 115.4, 114.0, 84.0, 70.9, 49.4, 48.6, 48.5 (3C), 48.4, 42.1, 39.5, 33.4, 31.0, 30.4, 30.2, 28.2, 27.3 (3C), 27.3 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{47}H_{63}N_6O_3$ $[M+H]^+$ 759.4962, found 759.4985.

*Partially protected toxin Agel 416 (**27b**)*. Slightly yellow oil; R_f(L): 0.08; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.51 (br. s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.48-7.43 (m, 6H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.29-7.23 (m, 6H), 7.21-7.15 (m, 4H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.10 (br. s, 1H), 6.70 (unresolv. t, 1H), 3.72 (s, 2H), 3.28 (q, *J* = 6 Hz, 2H), 2.71 (t, *J* = 6.6 Hz, 2H), 2.65 (unresolv. t, 2H), 2.61 (unresolv. t, 2H), 2.50 (t, *J* = 6.6 Hz, 2H), 2.44 (t, *J* = 6 Hz, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 2.20 (t, *J* = 6 Hz, 2H), 1.68 (quint., *J* = 6 Hz, 2H), 1.59-1.53 (m, 4H), 1.48 (quint., *J* = 6 Hz, 2H), 1.39 (quint., *J* = 6.6 Hz, 2H), 1.26 (br. s, 1H) ppm; ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 171.6, 146.2 (3C), 136.6, 128.6 (6C), 127.8 (6C), 127.3, 126.2 (3C), 124.0, 122.3, 119.7, 118.8, 11.5, 109.1, 70.9, 49.8, 49.7, 48.5, 48.3, 48.10, 48.05, 42.1, 38.9, 33.5, 30.9, 29.7, 28.9, 28.0, 27.8 ppm; HRMS (Orbitrap-ESI) *m/z* calcd for $C_{42}H_{54}N_6O$ $[M+H]^+$ 659.4437, found 659.4421.

General procedure for the removal of the Trt and Boc *N*-protecting groups - Synthesis of toxins HO-416b and Agel 416

The penta-amine derivative **23b** (84 mg, 0.11 mmol) was dissolved in an ice-cold solution made up of TFA (0.1 ml) and PhSH (0.1 mL) in CH_2Cl_2 (0.9 mL) under an inert atmosphere. It was then allowed to attain room temperature where it was left to stand for 1 h. The completion of the reactions was confirmed by TLC using the solvent system L. Volatile components were then removed under vacuo and the residue was triturated with Et_2O and refrigerated overnight. The precipitated penta-trifluoroacetate salt of toxin HO-416b was obtained by filtration and drying under vacuo overnight.

Similarly, penta-amine derivative **27b** (0.2 g, 0.3 mmol) was dissolved in an ice-cold solution made up of TFA (0.27 ml) and PhSH (0.15 mL) in CH_2Cl_2 (2.55 mL) under an inert atmosphere. It was then allowed to attain room temperature where it was left to stand for 1 h. The completion of the reactions was confirmed by TLC also using the solvent system L. Then, evaporation of volatiles to dryness, trituration with Et_2O and cooling overnight provided the penta-trifluoroacetate salt of toxin Agel 416, following filtration and drying overnight under vacuo.

Toxin HO-416b·5CF$_3$CO$_2$H. White solid; (76 mg, 70%); mp 225-226 °C; RP-HPLC: t_R = 15.73 min; ^{1}H NMR (600 MHz, (CD$_3$)$_2$SO) δ 10.89 (br. s, 1H), 9.01 (br. s, 3H), 8.73 (br. s, 2H), 7.99 (br. s, 2H), 7.95 (t, *J* = 6.6 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.17 (unresolv. d, 1H), 7.06 (t, *J* = 7.7 Hz, 1H), 6.96 (t, *J* = 7.7 Hz, 1H), 3.49 (s, 2H), 3.06 (q, *J* = 6.6 Hz, 2H), 3.02-2.96 (m, 8H), 2.94 (t, *J* = 7.8 Hz, 2H), 2.91-2.86 (m, 4H), 2.00-1.87 (m, 6H), 1.56 (quint., *J* = 7.2 Hz, 2H), 1.44 (quint., *J* = 7.2 Hz, 2H) ppm; ^{13}C{^{1}H} NMR (150 MHz, (CD$_3$)$_2$SO) δ 171.2, 159.0 (q, *J* = 31.5 Hz, 5C), 136.6, 127.7, 124.2, 121.4, 119.1, 118.7, 117.6 (q, *J* = 297 Hz, 5C), 111.8, 109.3, 46.9, 45.4 (4C), 44.3, 38.4, 36.6, 33.2, 26.8, 24.3 23.4, 22.9, 22.8 ppm; Anal. Calcd (%) for C$_{33}$H$_{45}$F$_{15}$N$_6$O$_{11}$: C, 40.17; H, 4.60; N, 8.52. Found: C, 40.24; H, 4.75; N, 8.66; MS (ESI, 30 eV): *m/z* 417.33 [M+H]$^+$, 209.19 [M+2H]$^{2+}$.

Toxin Agel 416·5CF$_3$CO$_2$H. White solid (0.22 g, 73%); mp 199-201 °C; RP-HPLC: t_R = 14.68 min; ^{1}H NMR (600 MHz, (CD$_3$)$_2$SO) δ 10.90 (br. s, 1H), 8.89 and 8.73 (two br. s, 7H), 8.08 (t, *J* = 6.6 Hz, 1H), 7.98 (br. s, 2H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.18 (unresolv. d, 1H), 7.09-7.04 (m, 1H), 6.99-6.95 (m, 1H), 3.50 (s, 2H), 3.11 (q, *J* = 6.6 Hz, 2H), 3.01-2.95 (m, 4H), 2.93-2.87 (m, 8H), 2.83 (t, *J* = 6.6 Hz, 2H), 1.97-1.88 (m, 4H), 1.71 (quint., *J* = 7.2 Hz, 2H), 1.68-1.61 (m, 4H) ppm; ^{13}C{^{1}H} NMR (150 MHz, (CD$_3$)$_2$SO) δ 171.9, 159.1 (q, *J* = 31.5 Hz, 5C), 136.6, 127.6, 124.3, 121.4, 119.0, 118.8, 117.6 (q, *J* = 297 Hz, 5C), 111.9, 109.1, 46.57, 46.56, 45.2, 44.4, 44.34, 44.32, 36.7, 36.2, 33.2, 26.5, 24.2, 23.10, 23.08, 22.8 ppm; Anal Calcd. for C$_{33}$H$_{45}$F$_{15}$N$_6$O$_{11}$: C, 40.17; H, 4.60; N, 8.52. Found: C, 40.19; H, 5.06; N, 8.58; MS (ESI, 30 eV): *m/z* 417.34 [M+H]$^+$, 209.20 [M+2H]$^{2+}$.

ASSOCIATED CONTENT

Supporting Information ^{1}H- and ^{13}C-NMR figures for synthesized compounds, ^{1}H- and ^{13}C-NMR as well as RP-HPLC chromatograms for final products (spider toxins).

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Notes

The authors declare no competing financial interest.

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