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Solid-Phase Synthesis of Rigid Acylpolyamines Using Temporary *N*-4,4′-Dimethoxytrityl Protection in the Presence of Trityl Linkers

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Abstract: An *N*-protection protocol employing the 4,4′-dimethoxytrityl (Dmt) group in combination with borane reduction of resin-bound polyamides was shown to be an efficient methodology that enables synthesis of novel analogues of natural acylpolyamine toxins. Thus, three philanthotoxins containing polyamine chains with piperidyl and cyclohexyl structural elements, which introduce conformational rigidity, increased lipophilicity, and altered proteolytic properties, were obtained in 39−44% overall yield.

Simple natural polyamines, such as spermidine and spermine, are ubiquitous in eukaryotic cells, and they play important roles in cellular physiology.[1] Polyamine derivatives have been the subject of extensive investigations due to their ability to interact with ion channels in the central and in the peripheral nervous system.[2−4] Among the naturally occurring polyamine derivatives, acylpolyamine toxins comprise a class of compounds that antagonize various types of ionotropic receptors, e.g., nicotinic acetylcholine receptors (nAChR) and ionotropic glutamate receptors (iGluR).[5−7] An example of a natural neuroactive acylpolyamine is philanthotoxin-433 (PhTX-433, **1**, Figure 1) from the venom of the female Egyptian digger wasp *Philanthus triangulum*.[8,9] Due to the therapeutic potential of such polyamine-containing compounds,[10−12] solution-phase synthesis[13,14] as well as solid-phase synthesis[6,7] (SPS) of acylpolyamine toxins has attracted considerable interest, and several structure−activity relationship (SAR) studies have been performed with philanthotoxins on various receptor types.[6,7]

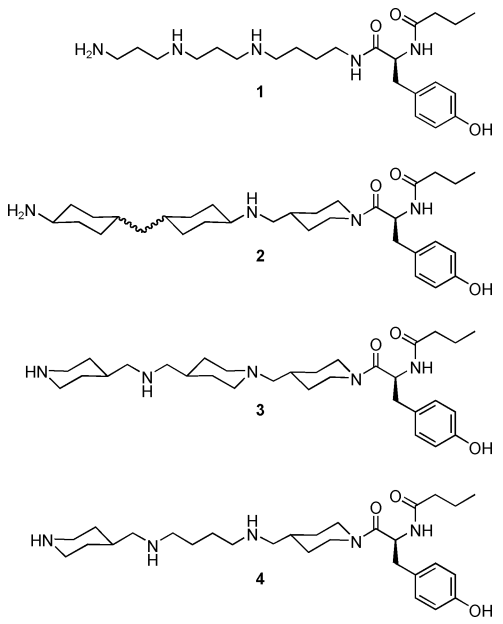

FIGURE 1. Structures of philanthotoxins**1**−**4**.

In particular, the development of sequential SPS methodologies has facilitated the synthesis of polyamine moieties, whereas the attachment of amino acid or acyl residues is readily achieved by conventional solid-phase peptide synthesis (SPPS) procedures. The synthetic strategies previously used for SPS of polyamines may be classified into three major groups: (i) *N*-alkylation reactions,[15−18] (ii) Mitsunobu reactions,[19−21] and (iii) reductive methods based on intermediary imines[22,23] or amides.[24]

In the present paper, we report on the development of a novel *N*-protection strategy, which in combination with

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borane reduction of polyamides supported on trityl resins enabled efficient SPS of three novel philanthotoxins **2**−**4** (Figure 1). This method allows SPS of compounds with a high degree of structural diversity of the polyamine chain, as the incorporation of piperidyl and cyclohexyl building blocks as well as acyclic aliphatic fragments was feasible.

Recently, two reports on solution-phase synthesis of monoacylated polyamines containing ring structures in the polyamine chain have been published, and incorporation of piperidyl and cyclohexyl moieties resulted indeed in interesting biological activities.[25,26] Another SPS protocol has been applied for the synthesis of philanthotoxins with similar structures; however, this approach is restricted to the synthesis of compounds with tertiary inner amino functionalities.[27]

A novel protecting group strategy was necessary in order to transform a resin-bound polyamide into the corresponding resin-bound polyamine having only the terminal secondary amine unprotected. Wang et al.[24] have employed a post-reductive bis-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] (Dde)[28] protection of the terminal primary amino group, followed by Boc protection of the inner secondary amino groups, and final selective liberation of the terminal primary amino group.[24] For the present purpose of SPS of polyamine derivatives having secondary internal and/or terminal amino groups in the resin-bound polyamine intermediate, this strategy is not applicable.

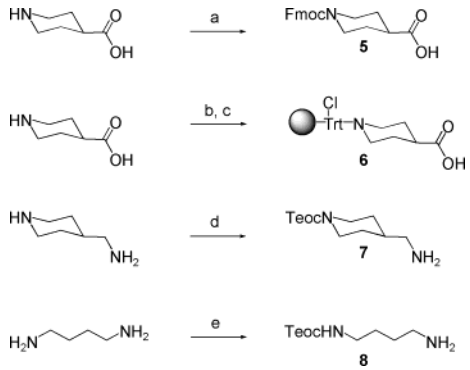
A previous analytical study has indicated that the 4-monomethoxytrityl (Mmt) and Trt groups might be useful as a protecting scheme during SPPS using acetic acid/trifluoroethanol/dichloromethane 1:2:7 for selective deprotection.[29] Considering this observation, it was envisioned that introduction of a Dmt group prior to borane reduction, followed by post-reductive Boc protection of the resulting secondary amines, and a final selective Dmt removal, would constitute a simple solution to the synthetic aim addressed. To implement this strategy, a mild and selective method for deprotection of the *N*-Dmt group on a polyamine attached to a solid support via trityl or 2-chlorotrityl linkers was developed. Qualitative TLC investigations in solution indicated that the desired selectivity could be obtained by using 0.1 M chloroacetic acid in dichloromethane (Table 1). Further studies showed that Boc groups were stable under these conditions. To address the possibility of premature cleavage from the resin during the chloroacetic acid treatment, a resin-bound diamine was acylated and treated with 0.1 M chloroacetic acid in dichloromethane. NMR analysis of the drained solvent pooled with MeOH washings showed no trace of amine.[30] These findings were utilized in the synthetic sequences leading to compounds **2**−**4**.

TABLE 1. Stability of Trt and Dmt Groups toward Selected Dilute Acids in Dichloromethane[a]

	Trt			Dmt		
acid (0.1 M)	10 min	30 min	20 h	10 min	30 min	20 h
TFA	−	−	−	−	−	−
DFA	±	−	−	−	−	−
ClAcOH	+	+	±	±	−	−
AcOH	+	+	+	+	±	±
PPTS	+	+	+	+	+	±

[a] Conditions: N^1-trityl-1,4-butanediamine and N^1-(4,4′-dimethoxytrityl)-N^4-trityl-1,4-butanediamine were each agitated with 0.1 M acid in CH_2Cl_2, and TLC was performed after 10 min, 30 min, and 20 h. DFA = difluoroacetic acid, ClAcOH = chloroacetic acid, AcOH = acetic acid, and PPTS = pyridinium *p*-toluenesulfonate; + denotes full stability (no cleavage), ± denotes partial stability, and − denotes full cleavage.

SCHEME 1. Building Block Synthesis[a]



[a] Reagents and conditions: (a) Fmoc-Cl, dioxane, aqueous Na_2CO_3, 16 h (91%); (b) TMS-Cl, DMF, N_2, 60 °C, 1 h; (c) Et_3N, 2-chlorotrityl chloride resin, N_2, 16 h; (d) ethyl trifluoroacetate, −60 → 0 °C, MeOH, N_2, then 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate, CH_2Cl_2, then 2 M aqueous NaOH (48% overall); (e) 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate, MeOH/CH_2Cl_2 (1:1), 16 h (93%).

The intermediate resin-bound polyamides for synthesis of structures **2**−**4** were obtained from building blocks and resins **5**−**8** (Scheme 1), along with a commercially available trityl resin preloaded with dicyclohexylmethane-4,4′-diamine (**9**). *N*-(9-Fluorenylmethoxycarbonyl) (Fmoc) protected isonipecotic acid (**5**) was obtained as previously described.[31] A 2-chlorotrityl chloride resin was loaded with *O*-trimethylsilyl (TMS)-protected isonipecotic acid to give resin **6** upon desilylation, as described by Barlos.[32] For the mono-*N*-protection of the two diamines, the 2-(trimethylsilyl)ethoxycarbonyl (Teoc)[33] group was chosen due to its stability under acylation conditions, and its conveniently mild removal with tetrabutylammonium fluoride (TBAF).[34] Preparation of compound **7** required a selective, temporary protection of the primary amine, for which trifluoroacetylation was employed.[27] Subsequent treatment with 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate and removal of the trifluoroacetyl group with 3 M aqueous NaOH afforded building block **7** (48%,

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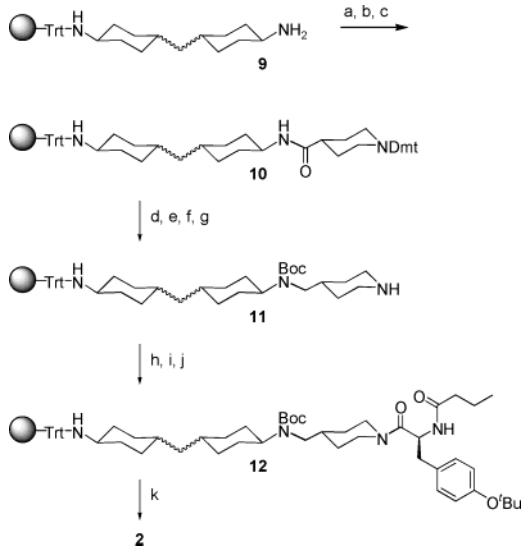
SCHEME 2. SPS of Philanthotoxin 2[a]

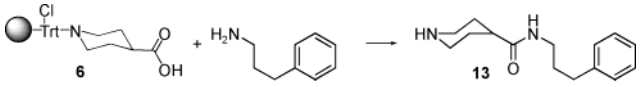


[a] Reagents and conditions: (a) **5**, DIC, HOBt, DMF, N_2, 3 h; (b) 20% piperidine in DMF, 2 × 10 min; (c) Dmt-Cl, DIPEA, CH_2Cl_2, N_2, 1.5 h; (d) BH_3·THF, 60 °C, 16 h; (e) piperidine, 60 °C, 3 × 2 h; (f) Boc_2O, DIPEA, CH_2Cl_2, N_2, 16 h; (g) chloroacetic acid (0.1 M in CH_2Cl_2), 3 × 30 min; (h) (*S*)-Fmoc-Tyr(ᵗBu)-OPfp, HODhbt, DIPEA, DMF, N_2, 5 h; (i) conditions as in (b); (j) $C_3H_7COOPfp$, HODhbt, DIPEA, DMF, N_2, 16 h; (k) TFA/CH_2Cl_2 (1:1), 1 h (39% overall).

three steps) in a one-pot procedure. 1,4-Butanediamine was treated with 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate in MeOH/CH_2Cl_2 (1:1) to give building block **8** in 93% yield.

Compound **2** was prepared by performing the sequence shown in Scheme 2 starting from a commercially available trityl resin preloaded with dicyclohexylmethane-4,4′-diamine (**9**). Initially, the resin was elongated with amino acid building block **5** using diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt). The Fmoc group was removed with piperidine, and introduction of a terminal Dmt group was achieved with Dmt-Cl to give resin **10**. Borane reduction was performed at 60 °C as previously described,[35−37] and subsequent dissociation of the borane−polyamine complex was accomplished by treatment with piperidine at 60 °C, a somewhat milder and faster treatment as compared to the conditions applied by Houghten et al. (piperidine, 65 °C, overnight).[36] The inner secondary amine thus formed was Boc protected, and then the terminal Dmt group was selectively removed with 0.1 M chloroacetic acid in dichloromethane to give resin **11**. Subsequent attachment of the amino acid (Tyr) and butanoyl residues was readily performed by pentafluorophenyl (Pfp) ester couplings[27] to give resin **12**. Finally, simultaneous deprotection and cleavage from the resin were achieved with trifluoroacetic acid (TFA/CH_2Cl_2 1:1), and philanthotoxin **2** was isolated by reversed-phase vacuum liquid chromatography (VLC) as its bis(TFA) salt in 39% overall yield.

TABLE 2. Test of Amide Coupling on Resin 6 Employing Selected Reagents



entry	reagents[a] (equiv)	solvent	time (h)	yield[b] (%)
1	DIC/HOBt (5:5)	DMF	18	90
2	HATU/DIPEA (5:10)	DMF	18	32
3	TBTU/DIPEA (5:10)	DMF	18	66
4	PyBOP/DIPEA (5:10)	DMF	18	91

[a] The crude material was cleaved from the resin using TFA/$CDCl_3$ (1:1) for 1 h. [b] Filtrates and washings with $CDCl_3$ were combined for each entry and analyzed quantitatively by ^1H NMR using toluene as internal standard. Yields were estimated by comparison with a sample of resin **6**, cleaved under identical conditions.

Various coupling reagents were examined in order to identify the optimal conditions for chain elongation by amide bond formation on resin **6** using primary amines (Table 2). The yield of **13** using the DIC/HOBt reagent pair was compared to the yields obtained when employing *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU),[38] *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU),[39] or benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)[40] in combination with diisopropylethylamine (DIPEA). Surprisingly, HATU/DIPEA afforded the lowest yield, and TBTU/DIPEA did not give an acceptable yield of **13** either. These results are contradictory to previous findings, where uronium salts such as HATU were reported to give better results than phosphonium salts and carbodiimide activation.[38,39] As DIC/HOBt and PyBOP/DIPEA appeared to perform equally well in this particular coupling, these reagents were employed in the total syntheses of **3** and **4** (Scheme 3), respectively, to compare the overall yields.

Starting from resin **6**, the amide bond formation (DIC and HOBt) using diamine building block **7** afforded resin **14** (sequence A). Removal of the Teoc group with TBAF, followed by acylation with **5**, and subsequent Fmoc group removal were performed as in the synthesis of **2**. Introduction of the terminal Dmt group was achieved with Dmt-Cl to give resin **15**. Then borane reduction was performed at 60 °C.

After dissociation of the borane−polyamine complex, the thus-formed inner secondary amine was Boc protected, and then the terminal Dmt group was selectively removed with 0.1 M chloroacetic acid in dichloromethane to give resin **16**. Attachment of the amino acid (Tyr) residue and the butanoyl group by Pfp ester couplings afforded resin **17**. Finally, simultaneous deprotection and cleavage from the linker with TFA/CH_2Cl_2 (1:1) afforded crude **3**, which was purified by reversed-phase VLC to give its tris(TFA) salt in 43% overall yield.

In sequence B (Scheme 3), the PyBOP/DIPEA reagent pair was applied in the first step of the total synthesis of

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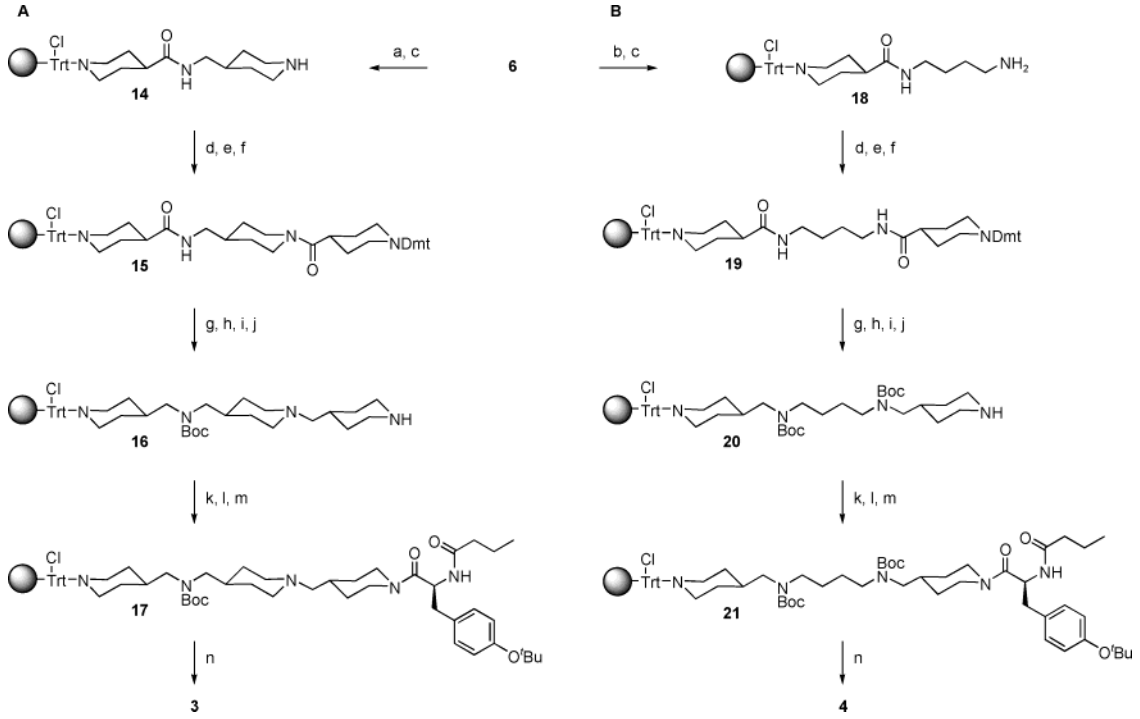
SCHEME 3. SPS of Philanthotoxin 3 and 4[a]



[a] Reagents and conditions: (sequence A) (a) **7**, DIC, HOBt, DMF, N_2, 16 h; (c) TBAF, DMF, 50 °C, 30 min; (d) **5**, DIC, HOBt, DMF, N_2, 3 h; (e) 20% piperidine in DMF, 2 × 10 min; (f) Dmt-Cl, DIPEA, CH_2Cl_2, N_2, 1.5 h; (g) BH_3·THF, 60 °C, 16 h; (h) piperidine, 60 °C, 3 × 2 h; (i) Boc_2O, DIPEA, CH_2Cl_2, N_2, 16 h; (j) chloroacetic acid (0.1 M in CH_2Cl_2), 3 × 30 min; (k) (*S*)-Fmoc-Tyr(^{t}Bu)-OPfp, HODhbt, DIPEA, DMF, N_2, 5 h; (l) conditions as in (e); (m) $C_3H_7COOPfp$, HODhbt, DIPEA, DMF, N_2, 16 h; (n) TFA/CH_2Cl_2 (1:1), 1 h (43% overall); (sequence B): (b) **8**, PyBOP, DIPEA, DMF, N_2, 16 h; (c) to (n) as above (44% overall).

4. The remaining steps from resin **18** to compound **4** were performed as described above, and the purified philanthotoxin **4** was obtained in 44% overall yield. As expected, the yields of philanthotoxins **3** and **4** were in the same range, in accordance with the coupling reagent test results (Table 2). Also, the overall yields were in the same range as those reported by Wang et al. using the Dde protection strategy.[24]

As described previously for example by Jacobson et al.,[41] tertiary amides such as **2**−**4** give rise to quite complex NMR spectra due to rotational isomerism with a relatively high energy barrier. Hence, NMR spectroscopic characterization of the products required a careful analysis of the 2D homo- and heteronuclear correlation spectra (COSY, HSQC, and HMBC). Pronounced splitting was observed for the signals of ^{1}H and ^{13}C nuclei in close proximity of the tertiary amide bond, as well as for the butanoyl resonances. The assignments of ^{1}H NMR and ^{13}C NMR resonances are given in detail in the Supporting Information.

In summary, the use of Dmt as a temporary *N*-protecting group in preparative SPS was demonstrated on polystyrene trityl and 2-chlorotrityl resins for the first time. Chloroacetic acid in dichloromethane (0.1 M) allowed a selective liberation of Dmt-protected amino groups of polyamines anchored via an acid-labile trityl or 2-chlorotrityl linker. Furthermore, Boc groups were shown to be compatible with these deprotection conditions. Accordingly, this methodology in combination with borane reduction of resin-bound polyamides constitutes a general SPS approach to acylpolyamine toxins. Moreover, the presented synthetic methodology described in this work extends the structural diversity of philanthotoxins with unique types of polyamines that enable further exploration of structure−activity relationships on ionotropic receptors.

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Supporting Information Available: General procedures, detailed experimental procedures (**5**−**8**), and full characterization of compounds **2**−**5**, **7**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO049278Q

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