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Side-Chain-Anchored N^{α}-Fmoc-Tyr-OPfp for Bidirectional Solid-Phase Synthesis

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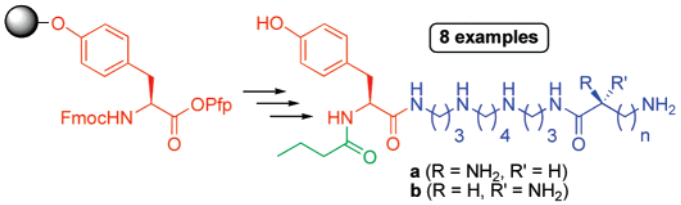
ABSTRACT



A mild resin-immobilization strategy employing a readily prepared trityl bromide resin for anchoring building blocks via a phenol group has been developed. With N^{α}-Fmoc-Tyr-OPfp as a starter building block, it was possible to prepare asymmetrically substituted hybrids of spider- and wasp-type polyamine toxins using solid-phase peptide synthesis conditions.

Since Merrifield's pioneering synthesis of a tetrapeptide in 1963,[1] the field of solid-phase synthesis (SPS) has undergone immense development, becoming a powerful tool for accessing large numbers of structures either in a parallel or in a combinatorial fashion.[2] Furthermore, the emergence of methods for high-throughput screening (HTS) in biological systems initiated the development of techniques for synthesis of libraries of drug-like small molecules,[3] often inspired by natural products[4] or designed by the diversity-oriented synthesis (DOS) approach.[5]

To plan a successful synthetic sequence on solid phase, the type of resin should be chosen carefully. Besides the stability toward reaction conditions, the physical and chemical properties of the polymer are important, as it may be regarded as a co-solvent.[6] Moreover, the linker used for anchoring the starting material to the polymer is especially important, since premature cleavage from the resin as well as undesirable sterical and chemical interference from the linker should be avoided.[7]

In the present work, a traditional Merrifield polymer (divinylbenzene cross-linked polystyrene) with a trityl linker[8] was employed in a novel approach to anchoring a phenolic amino acid to a solid support. The phenol functionality has

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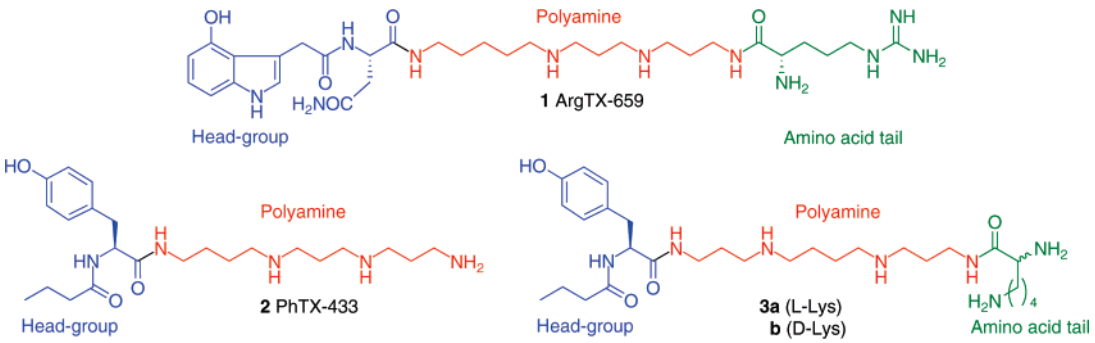

Figure 1. Structures of natural toxins and hybrid synthetic targets **3a**,**b**. The numbering in ArgTX-659 denotes the molecular mass. The digits in the name PhTX-433 denote the number of methylene groups that separate the amino groups, counting from the head-group.

previously been used as the point of attachment in SPS on Merrifield-OH[9] and Wang-OH[10] resins via the Mitsunobu reaction, as well as on Wang-Br,[11] dihydropyran,[12] and 2-chlorotrityl-Cl[13] resins.

The protocol described here employs a trityl bromide resin for mild immobilization of the active pentafluorophenyl (Pfp) ester, N^{α}-Fmoc-Tyr-OPfp (**4**), via its free phenolic functionality. Thus, concise bidirectional SPS, including synthesis of novel hybrid spider−wasp neurotoxin analogues (e.g., **3a**,**b**, Figure 1), is feasible from this internal residue, while the linker is compatible with TFA cleavage and concomitant Boc/Teoc deprotection.

Polyamine-containing toxins isolated from the venoms of spiders (e.g., argiotoxin-659, **1**)[14] and wasps (philanthotoxin-433, **2**)[15] are known to be noncompetitive inhibitors of various types of ionotropic receptors in the central nervous system (CNS).[16] Natural and synthetic polyamine derivatives have therefore proved to be valuable as probes in structural and functional studies of ligand-gated ion channels such as ionotropic glutamate receptors (iGluRs) and nicotinic acetylcholine receptors (nAChRs).[17] Recent developments in SPS methods have greatly facilitated the preparation of these types of compounds,[18] as they allow omission of several tedious purification steps inherent to solution-phase methods. As a result of recent synthetic efforts, structure−activity relationship (SAR) studies have been performed with compounds having altered position and number of NH groups in the polyamine chain,[19] branched sites in the polyamine chain,[20] bulky acyl[21] or amino acid[22] moieties in the headgroup, variation of the stereochemistry,[23] and rigidity of the headgroup.[22] The present method enables SPS of novel philanthotoxins elongated with amino acid tail moieties[24] through peptide SPS using Fmoc-[25] and 2-(trimethylsilyl)-ethoxycarbonyl (Teoc)-protected[26] building blocks. Since the tail group is introduced in the last synthetic step prior to

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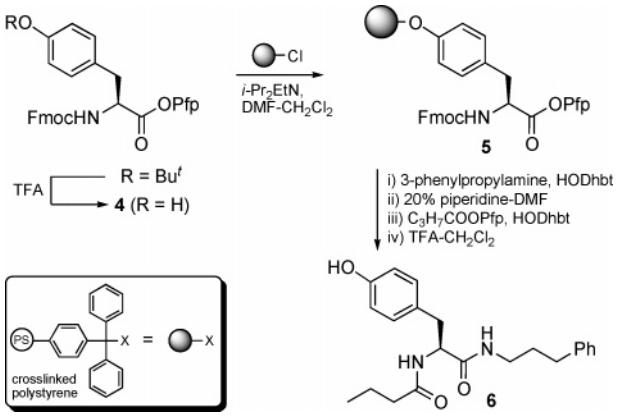
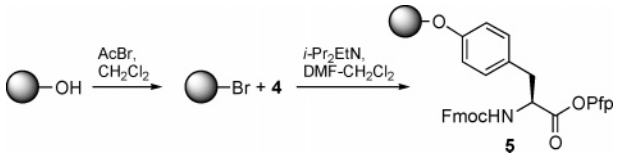
Scheme 1. SPS of Model Compound **6** on Trityl Chloride Resin



Scheme 2. Preparation of Resin **5** from Trityl Bromide Resin



deprotection and cleavage, diversification at the terminal amino position of PhTX-343 is possible.

Initially, model compound **6** was prepared starting from a conventional polystyrene trityl chloride resin. The appropriately protected starting material, N^{α}-Fmoc-Tyr-OPfp (**4**), was readily obtained from commercially available N^{α}-Fmoc-Tyr(*t*-Bu)-OPfp by treatment with TFA as previously described[27] and was attached to the resin under mild conditions in order to keep the activated ester group intact. The resulting resin **5** was treated with 3-phenylpropylamine, followed by Fmoc deprotection, acylation with Pfp butanoate, and finally cleavage with TFA-CH_2Cl_2 to afford **6** (Scheme 1). This model sequence was performed to ensure that both Pfp ester and NHFmoc functionalities were preserved during the loading procedure, and indeed compound **6** was obtained in high purity (>90%) but only in 12% overall yield.

The high purity showed that the functional groups were fully compatible with the reaction conditions, whereas the low yield could arise from premature cleavage from the linker or a low degree of loading. Since the resin linkage is acid-labile and all steps proceeded in basic or neutral (washings) media, it was assumed that more forcing conditions were required to achieve a higher degree of resin loading.

A polystyrene trityl bromide resin was chosen due to its higher reactivity as compared to the trityl chloride resin. Previously, a trityl alcohol has successfully been converted to a trityl bromide with acetyl bromide in solution,[28] and these conditions were adopted for functionalization of the trityl alcohol resin. Subsequent treatment with N^{α}-Fmoc-Tyr-OPfp (**4**) in DMF−CH_2Cl_2 afforded resin **5** (Scheme 2). When the previously mentioned sequence (Scheme 1) was performed with the thus obtained trityl bromide resin, the product (**6**) was again obtained not only in high purity (>90%) but now also in an overall yield of 66% (92% average per step).[29] This was considered satisfactory for the preparation of spider−wasp hybrid toxin analogues. For this purpose four portions of the resin **5** were prepared[30] and treated with building block **7**[31] in parallel to give resin **8** (Scheme 3). These four resin batches were Fmoc-deprotected and acylated with Pfp butanoate to give resin **9**. One batch was treated with dilute TFA to give PhTX-343 (**10**) in 43% yield, which is in the range of yields obtained in previous SPS methods.[23] The terminal amino groups of the three remaining resin batches were liberated with Bu_4NF. Subsequent acylation with N^{α}-Fmoc-L-Lys(Boc)-OH or N^{α}-Fmoc-D-Lys(Boc)-OH afforded resins **11a**,**b**, respectively. Deprotection and cleavage from the support furnished compounds **3a** (12%) and **3b** (21%) after reversed-phase HPLC purification.[32] Capillary electrophoresis experiments with **3a**,**b** and **10** showed that this tyrosine anchoring approach is suitable for preparation of polyamine amides with chiral tail moieties without racemization at the α-carbon of the amino acids.[31] The sequence in Scheme 3 also provided the guanidino analogue of PhTX-343 (**13**, 22% overall yield). *N*,*N*′-Bis-Boc-1*H*-pyrazole-1-carboxamidine[33] was chosen as the guanidinylating agent in favor of 1*H*-pyrazole-1-carboxamidine hydrochloride to avoid the potential risk of self-condensation in the presence of excess base.[34]

In summary, efficient protocols for preparation of trityl bromide resin and its subsequent loading with (*S*)-N^{α}-Fmoc-Tyr-OPfp under mild conditions were developed. This functionalized resin enabled a highly versatile new route to an array of spider−wasp toxin hybrids. The pharmacological evaluation of these compounds is in progress, the results of which will be published elsewhere. Furthermore, this synthetic approach may be used for bidirectional peptide synthesis from an internal tyrosine unit and for anchoring of other phenolic substrates for SPS of druglike small molecules and peptide analogues. Previously, backbone amide linker (BAL) resins[35] and other side-chain anchoring approaches have been utilized in bidirectional SPPS and

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(29) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24 (m, 3H), 7.07 (d, J = 7.1 Hz, 2H), 6.98 (d, J = 8.3 Hz, 2H), 6.70 (d, J = 8.3 Hz, 2H), 6.64 (br s, 1H), 6.19 (br s, 1H), 4.55 (m, 1H), 3.15 (m, 2H), 2.89 (m, 2H), 2.48 (t, J = 7.5 Hz, 2H), 2.15 (t, J = 7.4 Hz, 2H), 1.68 (p, J = 7.5 Hz, 2H), 1.56 (sextet, J = 7.4 Hz, 2H), (t, J = 7.4 Hz, 3H).
(30) Since the trityl bromide group is rather reactive, it was preferred to prepare small portions of the resin immediately prior to use.
(31) See Supporting Information for experimental details.
(32) Also, the hybrid toxins containing (*S*)-2,3-diaminopropanoic acid (**A**, 14%), (*S*)-2,4-diaminobutanoic acid (**B**, 10%), and (*S*)-ornithine (**C**, 17%) were prepared. See Supporting Information for characterization data.
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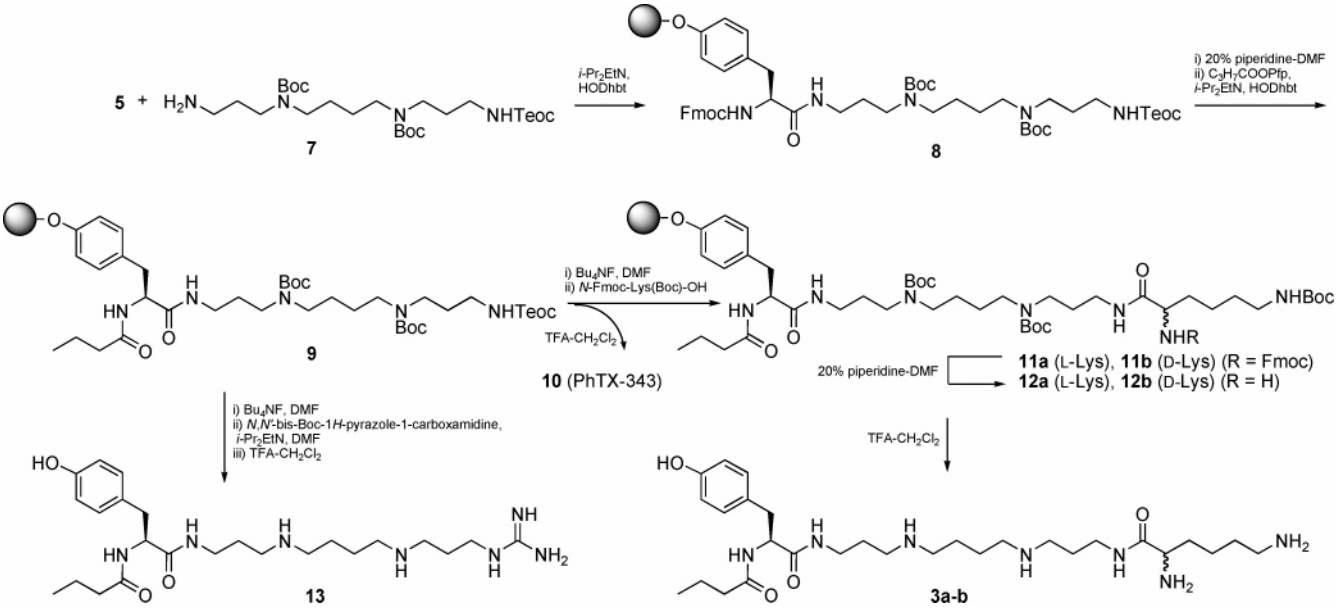
Scheme 3. Synthesis of Compounds **10** (PhTX-343), **3a**, **3b**, and **13**



in preparation of cyclic peptides,[10a,36] spider toxins,[37] and fluorogenic peptides.[38] Fluorogenic peptides are important substrates in the investigation of protease specificity,[39] and in addition to the work described herein, we envision that the present protocols may find use in the preparation of such substrates as well as homo- or heterodetic cyclic peptides.

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Supporting Information Available: Full experimental procedures, ^{1}H and ^{13}C NMR spectra of **3a**, **3b**, **13**, and **A**−**C**, as well as capillary electropherograms of **3a**, **3b**, **10**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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