Highly Versatile Synthesis of Polyamines by Ns-strategy on a Novel Trityl Chloride Resin

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Abstract: A solid-phase synthesis of polyamines on 4-alkoxytrityl chloride resin **5** is described. By alkylation of nitrobenzenesulfonamide **8** with alkyl halide **7** prepared from the resin **5**, spermine bound resin **10** was efficiently synthesized. Condensation of **10** and tyrosine derivative followed by deprotection and cleavage from the resin afforded highly pure philanthotoxin-343 (**4**).

Key words: polyamines, solid-phase synthesis, 2-nitrobenzenesulfonamide, 4-alkoxytrityl chloride resin, philanthotoxin-343

Polyamines, such as spermidine (**1**) and spermine (**2**) are natural products found in microorganism, plants, and animals, and are responsible for a variety of important biological activities.[1] They interact with nucleic acids and play an important role in DNA synthesis and cell proliferation. Furthermore, many of their conjugates and/or synthetic analogues are candidates for anticancer drugs such as **3**[2] and neuroactive agents such as **4**,[3] to name a few (Figure 1). Due to their biological significance, practical and versatile methods for their synthesis are sorely needed. However conventional synthesis of polyamines requires extremely tedious purification procedures owing to their high polarity. With an aim to solve the purification problems, solid-phase syntheses of the polyamines have been reported. However, the reported syntheses are not very efficient because they require multi-step processes and employ rather expensive resins.[4]

We have recently developed a novel 4-(chlorodiphenylmethyl)phenoxymethylated polystyrene **5**, which can be prepared readily from Merrifield resin.[5] The alkoxytrityl chloride resin **5** offers significant advantages over the commercially available 2-chlorotrityl chloride resin; it is more reactive because an alkoxy group is attached to the 4-position of the trityl chloride and because the reactive site is separated by a phenol unit away from the bulky polystyrene backbone. On the other hand, we have developed an efficient synthetic methodology for secondary amines by using nitrobenzenesulfonamide as protecting and activating group (Ns-strategy).[6] We envisioned that the combination of Ns-strategy with easy-to-use resin **5** (Figure 2) would enable us to develop a practical and versatile synthesis of polyamines. To test the feasibility of

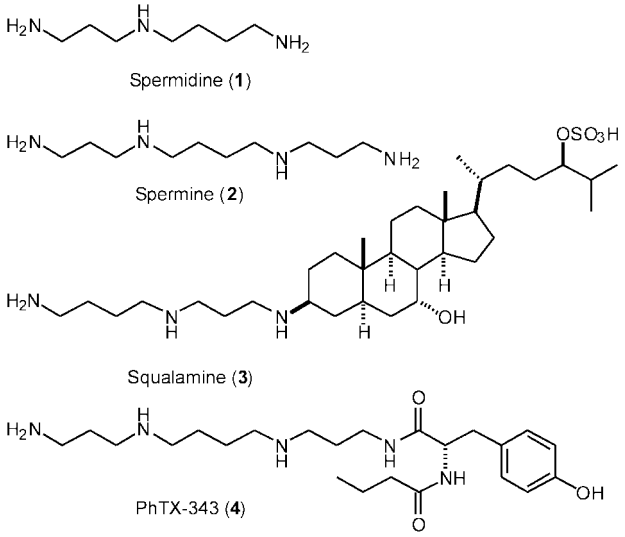

Figure 1 Polyamines and those conjugates

such a protocol, we decided to synthesize the spermine backbone **10**.

Firstly, 1,3-diaminopropane was attached to the resin **5** in the presence of *i*-Pr_2NEt and subsequent activation of the less-hindered amine with NsCl afforded **6** (Scheme 1). Elongation of the polyamine chain was performed by alkylation of Ns-amide with alkyl halide.[7] Thus, treatment of the resin-bound sulfonamide **6** with excess K_2CO_3 and dibromobutane provided **7**. The coupling between **7** with sulfonamide **8**[8] afforded the spermine backbone **9**, which, upon removal of the Alloc group gave the primary amine **10**. The polymer-bound **10** is a valuable intermediate for the synthesis of numerous spermine conjugates, since alkylation with R^1-X via the Ns-strategy or acylation with R^2-COX of primary amine **10** is expected to proceed smoothly. After deprotection of the Ns group, acidic cleavage from the resin would readily provide the sper-

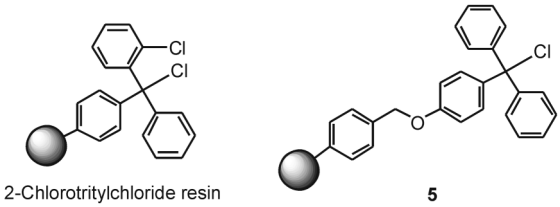

Figure 2 Structure of alkoxytritylchloride resin **5**

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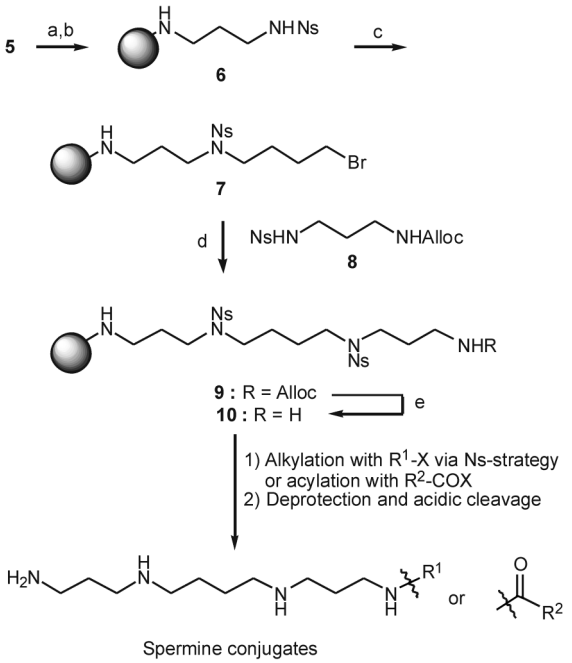
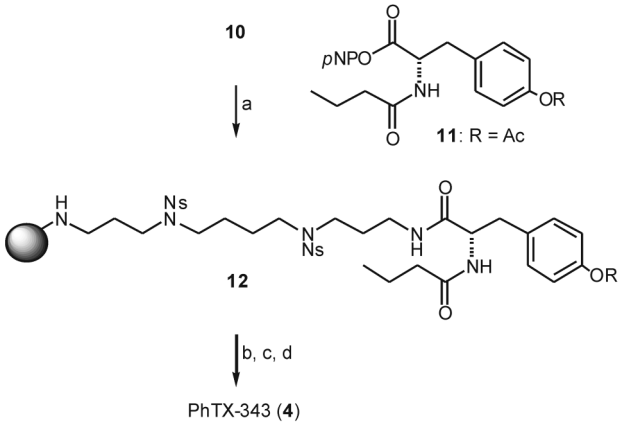


Scheme 1 a) 1,3-diaminopropane (5.5 equiv), *i*-Pr_2NEt (6.0 equiv), CH_2Cl_2, r.t.; b) NsCl (6.0 equiv), collidine (12 equiv), CH_2Cl_2, r.t.; c) 1,4-dibromobutane (20 equiv), K_2CO_3 (10 equiv), DMF, 60 °C; d) **8** (3 equiv), K_2CO_3 (10 equiv), DMF, 60 °C; e) $Pd(PPh_3)_4$ (0.4 equiv), pyrrolidine (2.5 equiv), DMF, r.t.



Scheme 2 a) **11** (2.0 equiv), CH_2Cl_2, r.t.; b) K_2CO_3 (20 equiv), MeOH; c) $HO(CH_2)_2SH$ (20 equiv), DBU (20 equiv), DMF, r.t.; d) TFA, CH_2Cl_2, r.t.

mine conjugate. To demonstrate the utility of this protocol, synthesis of PhTX-343 (**4**) was investigated.

PhTX-343 (**4**) is a synthetic analogue of natural polyamine toxin PhTX-433 isolated from digger wasp *Philanthus triangulum* which was discovered by Nakanishi[3] and Piek.[9] The spermine conjugate **4** has been known as a noncompetitive antagonist for nicotinic acetylcholine receptors (nAChRs) and ionotropic glutamate receptors (iGluRs). Due to their interesting biological activities, many synthetic studies on **4** and its analogues have been reported.[10] Our synthesis began with the condensation of **10**[16] with activated ester **11**,[11] which proceeded smoothly to give **12** (Scheme 2).[12] Following methanolysis of the acetate, the deprotection of the Ns group was accomplished by treatment with 2-mercaptoethanol and DBU.[13] The final cleavage from the resin was performed under acidic condition (1% TFA/CH_2Cl_2).[14] Upon removal of the solvent, **4** was obtained in high purity. The whole sequence of transformations from **5** to **4** was carried out without any purification procedures. All spectroscopic data (^1H and ^{13}C NMR spectra and MS) of synthetic **4** were identical with those reported in literature.[3] Tandem FAB MS-MS spectroscopic data also supported the results. Thus, highly efficient synthesis of PhTX-343 (**4**)[17] was accomplished in 9 steps and 75% overall yield, based on resin **5**.

In conclusion, an efficient solid-phase synthesis of a spermine conjugate was accomplished by utilizing the Ns-strategy in combination with the resin **5**. It should be noted that solid-phase alkylation of Ns-amide with alcohol under Mitsunobu conditions has also been reported.[15] Thus, combination of appropriate fragments (halides, alcohols and Ns-amides) would enable facile construction of the diverse polyamine libraries. Further investigations on the use of the newly developed resin **5** are currently underway in our laboratory.

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(12) While amide formation of *N*-acyl-amino acids is often accompanied in racemization, no appreciable racemization took place during the condensation of *p*-nitrophenyl ester **11** with primary amine as demonstrated in the following experiments. Condensation reaction with **11** and (*S*)-(–)-α-methylbenzylamine gave a single isomer. However, treatment of DCC with corresponding carboxylic acid of **11** and (*S*)-(–)-α-methylbenzylamine gave a 1:1 mixture of the diastereomers.
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(16) **Synthetic Procedure for the Polymer Bound Spermine (10)**. To a suspension of the freshly prepared resin **5** (1.40 g, 0.90 mmol/g) and 1,3-diaminopropane (0.60 mL, 7.20 mmol) in anhyd CH_2Cl_2 (12.5 mL) was added *i*-Pr_2NEt (1.20 mL, 6.87 mmol) at r.t. under an argon atmosphere. After shaking for 16 h, the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 8 h to give the resin (1.71 g). To a suspension of the above resin (1.71 g) and 2-nitrobenzenesulfonyl chloride (1.80 g, 8.12 mmol) in anhyd CH_2Cl_2 (12.5 mL) was added 2,4,6-collidine (2.00 mL, 15.2 mmol) at r.t. under an argon atmosphere. After shaking for 16 h, the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 7 h to give the resin **6** (1.86 g). To a suspension of the above resin **6** (1.38 g) and 1,4-dibromobutane (3.00 mL, 25.3 mmol) in DMF (10.0 mL) was added K_2CO_3 (1.80 g, 13.0 mmol) at 60 °C. After stirring for 16 h, the mixture was then cooled to r.t. Then the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 8 h to give the resin **7** (1.43 g). To a suspension of the above resin **7** (1.26 g) and **8** (1.30 g, 3.79 mmol) in DMF (10.0 mL) was added K_2CO_3 (1.75 g, 12.7 mmol) at 60 °C. After stirring for 16 h, the mixture was then cooled to r.t. Then the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 4 h to give the resin **9** (1.36 g). Confirmation of the structure of the spermine skeleton of **9** was performed by following acidic cleavage from the resin. To a mixture of the resulting resin **9** (60.0 mg) in CH_2Cl_2 (2.5 mL) was added TFA (10 μL, 0.130 mmol) at r.t. After shaking for 5 min, the resin was filtered and washed with MeOH:CH_2Cl_2 (1:2). The combined washings were evaporated with benzene and dried in vacuo to provide N^1-Alloc-N^4,N^8-di-Ns-spermine (23.5 mg, 91% from **5**) as the TFA salt. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.23 (2 H, m), 1.36 (2 H, m), 1.56 (2 H, m), 1.78 (2 H, m), 2.72 (2 H, m), 2.90 (2 H, m), 3.15–3.32 (8 H, m), 4.44 (2 H, d, *J* = 6.0 Hz), 5.19 (2 H, dd, *J* = 10.8 Hz, 16.8 Hz), 5.89 (1 H, m), 7.19 (1 H, m), 7.78–8.00 (10 H, m). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 24.8, 25.0, 28.4, 36.5, 37.7, 44.7, 45.2, 46.8, 47.1, 64.2, 116.9, 124.3, 124.4, 129.6, 129.6, 131.6, 131.7, 132.4, 132.5, 133.8, 134.5, 134.6, 147.5. MS: *m/z* = 657 (MH^+); HR MS: Found 657.2032 (MH^+). Calcd 657.2013 ($C_{26}H_{37}N_6O_{10}S_2$, MH^+). To a suspension of the resin **9** (220 mg) and $Pd(PPh_3)_4$ (100 mg, 0.087 mmol) in anhyd DMF or THF (2.5 mL) was added pyrrolidine (0.25 mL, 3.02 mmol) at r.t. under an argon atmosphere. After shaking for 16 h, the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 8 h to give the resin **10** (217 mg).
(17) **Synthetic Procedure for Philanthotoxin-343 (4)**. To a suspension of the resin **10** (217 mg) in CH_2Cl_2 (2.5 mL) was added **11** (168 mg, 0.405 mmol) at r.t. After shaking for 2 d, the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 4 h to give the resin **12** (316 mg). To a suspension of **12** (64 mg) in MeOH (1.0 mL) was added K_2CO_3 (60 mg, 0.434 mmol) at r.t. After shaking for 16 h, the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 4 h to afford the resin (60 mg). To a suspension of the above resin (50 mg) and 2-mercaptoethanol (30 μL, 0.426 mmol) in DMF (1.0 mL) was added DBU (60 μL, 0.402 mmol) at r.t. After shaking for 6 h, the resin was filtered, washed with 10% H_2O in THF, THF, and ether, and then dried in vacuo for 3 h to give the resin (41 mg). To a mixture of the resulting resin in CH_2Cl_2 (2.5 mL) was added TFA (30 μL, 0.389 mmol) at r.t. After shaking for 5 min, the resin was filtered and washed with MeOH:CH_2Cl_2 (1:2). The combined washings were evaporated with benzene and dried in vacuo to provide Philanthotoxin-343(**4**) (18.0 mg, 75% from **5**) as the TFA salt. $[\alpha]_D$ +5.1 (*c* 0.14, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ = 0.77 (3 H, t, *J* = 7.5 Hz), 1.46 (2 H, q, *J* = 7.5 Hz), 1.70 (6 H, m), 1.99 (2 H, t, *J* = 8.0 Hz), 2.09 (2 H, dt, *J* = 7.5 Hz, *J* = 2.0 Hz), 2.75–3.21 (14 H, m), 4.28 (1 H, t, *J* = 7.6 Hz), 6.61 (2 H, d, *J* = 8.5 Hz), 6.95 (2 H, d, *J* = 8.5 Hz). ^{13}C NMR (100 MHz, D_2O): δ = 13.4, 19.7, 23.6, 24.5, 26.1, 36.7, 36.9, 37.3, 38.0, 45.3, 45.7, 45.8, 47.8, 56.6, 116.3, 129.0, 131.3, 155.3, 174.7, 178.0. IR: 722, 799, 837, 1133, 1202, 1433, 1517, 1678, 2965 cm^{-1}. MS: *m/z* = 436 (MH^+); HR MS: Found 436.3264 (MH^+). Calcd 436.3288 ($C_{23}H_{42}O_3N_5$, MH^+).