A Versatile Method for Solid-Phase Synthesis of Polyamines: Neuroactive Polyamine Toxins as Example

Kristian Strømggaard, Kim Andersen, Thomas Ruhland, Povl Krosgaard-Larsen, Jerzy W. Jaroszewski

Abstract: A general method for sequential synthesis of polyamines on solid-phase is described. Each polyamine chain elongation step is based on alkylation under Mitsunobu conditions of resin-bound amine, activated with a 2-nitrobenzenesulfonyl group. The Mitsunobu reaction was accomplished with 1,1’-(azadicarbonyl)dipiperidine and tributylphosphine, using 2-(trimethylsilyl)ethoxycarbonyl-protected amino alcohols as the chain extension elements. The yield of the Mitsunobu reaction was optimized with respect to reaction time, reagent ratio and concentration, order of addition of the reagents, and temperature; the optimized yield approached 100%. The method was used for synthesis of philanthotoxin-433, a natural polyamine wasp toxin, and of its seven analogs, with all possible combinations of trimethylene and tetramethylene units separating the nitrogen atoms of the polyamine chain. The yields of purified end products were 23–40%.

Key words: amines, philanthotoxins, parallel synthesis, solid-phase synthesis, Mitsunobu reaction

Polyamines play an important role in biology and have a growing practical interest.1 Polyamines such as spermine and spermidine are ubiquitous in eukariotic cells, playing important roles in DNA synthesis and cell proliferation, and their analogs are being explored as therapeutic leads.2–4 Polyamines are characteristic of thermophilic bacteria,5,6 and are found in a range of natural products, including antibiotics,7,8 plant polyamine alkaloids9,10 and animal toxins.11–13 Analogs of the latter are of considerable interest as receptor probes and potential neuroactive drugs.14–17 Other applications of synthetic polyamines include DNA vaccine delivery18,19 and use as supramolecular building blocks.20,21 All of these applications require efficient and versatile methods for the synthesis of polyamines.

The synthesis of polyamines in solution is a laborious task involving extensive use of protective groups22–25 as well as tedious purification procedures. It has already been demonstrated that the solid-phase approach greatly facilitates syntheses involving polyamines,16,17,26–34 including construction of combinatorial libraries of polyamine derivatives.17

A mild and selective strategy for the synthesis of secondary amines has recently been introduced by Fukuyama.35,36 In this procedure, primary amines are initially converted to the corresponding nitrobenzenesulfonamides, and the acidity of the latter is exploited in an alkylation reaction, either with alkyl halides or using the Mitsunobu procedure37,38 involving alcohols. The Fukuyamaamination has been successfully applied for the solid-phase synthesis of a variety of N-alkylated products.39–56 Here, we present a general procedure for Fukuyama amination-based synthesis of polyamines on solid support using 2-(trimethylsilyl)ethoxycarbonyl- (Teoc)-protected amino alcohols as the chain extension elements, and apply this procedure for the synthesis of a number of analogs of philanthotoxin-433 [PhTX-433 (1), Figure 1], a neuroactive constituent of the venom of Philanthus triangulum bigger wasp.57 The strategy presented herein allows a sequential construction of polyamines using building blocks of varying lengths, as well as further derivatization of the polyamine chain. Thus, eight philanthotoxins, 1 and 34–40, with all possible combinations of trimethylene or tetramethylene units in the polyamine moiety, were synthesized.

Figure 1

PhTX-433 (k = 2, l = 1, m = 1)
ary amino group remains protected as the 2-nitrobenzenesulfonamide (NS) group, and the primary amino group can be further derivatized with 2-nitrobenzenesulfonyl chloride (Scheme 2).

![Scheme 1](image)

Scheme 1 Reagents: (a) 2-(trimethylsilyl)ethyl 4-nitrophenyl carbonate

Commercially available trityl resins derivatized with 1,3-diaminopropane and 1,4-diaminobutane (6 and 7) were used as starting materials. Reaction with 2-nitrobenzenesulfonyl chloride in THF–CH₂Cl₂ (2:1) gave the resin-bound sulfonamides 8 and 9, respectively (Scheme 2).³³ The progress of the reaction was monitored either by the Kaiser test,⁶⁰ or by HPLC-MS analysis of cleaved products. The 2-nitrobenzenesulfonyl group was chosen in favor of 4-nitrobenzenesulfonyl group, since it has been reported that the former gives a cleaner deprotection step.⁶¹

The Mitsunobu reaction utilizing the Tsunoda protocol was applied for the resin-bound, NS-activated amines 8 and 9, using 1,1′-(azadicarbonyl)dipiperidine (ADDP), tributylphosphine (TBP)⁶² and the N-Teoc-protected 3-aminopropanol (4) or 4-aminobutanol (5). This gave the resin-bound intermediates 10–13. Use of unprotected amino alcohols 2 and 3 instead of the Teoc-protected substrates 4 and 5 was attempted, but the required products were not formed. Moreover, use of 9-fluorenylmethoxycarbonyl- (Fmoc-) protected amino alcohols was investigated using either, ADDP and TBP as redox reagents, or by traditional Mitsunobu conditions, utilizing diethylazodicarboxylate (DEAD) and triphenylphosphine. However, under neither of these conditions the formation of any detectable amounts of the required secondary amines was observed. Failure of this reaction could, at least in part, be due to the low solubility of the N-Fmoc-protected amino alcohol. Nevertheless, Bycroft and co-workers very recently presented a preliminary report on a solid-phase synthesis of PhTX-433 (1) involving Fukuyama amination in a single chain elongation step, using an Fmoc-protected amino alcohol and DEAD and triphenylphosphine as the redox reagents.³²

![Scheme 2](image)

Scheme 2 Reagents: (a) 2-nitrobenzenesulfonyl chloride; (b) 4 (m = 1) or 5 (m = 2), ADDP, TBP; (c) TBAF; (d) (S)-N-Fmoc-O-(tert-butyl)tyrosine, HATU, collidine; (e) piperidine in DMF; (f) butyric acid, HATU, collidine; (g) 2-mercaptoethanol, DBU; (h) CH₂Cl₂/TFA/triisopropylsilane/H₂O

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The conditions of the Mitsunobu reaction were optimized with respect to number of equivalents of the reagents (4, ADDP and TBP 1:1:1), as well as their absolute concentration, order of addition and the reaction time, using the conversion of 8 to 10 as an example. The results are shown in the Table. A mixture of CH₂Cl₂ and THF (1:1) was employed as solvent for the best compromise between swelling of the resin and progress of the Mitsunobu reaction. The reaction was performed at ambient temperature, since neither elevated temperature (reflux) nor cooling to 0°C prior to addition of the reagents improved the yield. As expected, higher concentrations of the reagents increased the yield of 10 (entries 1–4), as did an extension of the reaction time from 2 h to 16 h (entry 6). The order of addition of the reagents affected the outcome of the reaction only slightly, the yield being somewhat higher when 4 and TBP were added prior to ADDP (entries 6 and 7). However, the reaction appeared to be more reproducible in the latter case. The increase of the excess of the reagents from five-fold to ten-fold was not advantageous (entries 8–11).

Repetition of the reaction with a fresh portion of the reagents together with increased reaction time offered a major advantage in terms of yield. Thus, change from 2 × 1 h to 2 × 3 h gave a significantly improved conversion (entries 5 and 13). Although further increase of the reaction time to 2 × 16 h only gave a marginal improvement (entry 11), repetition of the reaction for 3 × 3 h gave a practically quantitative yield (entry 14). These conditions, i.e., five-fold excess of the reagents used at 200 mM concentration, an addition order of 4 followed by TBP and then by ADDP, and repetition of the reaction three times, each time for 3 h, were used in all coupling steps involving 4 as well as 5 (Scheme 2).

Use of 2,4-dinitrobenzenesulfonyl chloride for protection and activation of 6 was tested in order to determine whether an increased acidity of the sulfonamide was advantageous for the subsequent alkylation step. However, the 2,4-dinitrobenzenesulfonyl group was found to be incompatible with TBP, most likely because of the increased nucleophilicity of TBP as compared to triphenylphosphine, resulting in cleavage of the sulfonamide bond. In fact, the use of the 2,4-dinitrobenzenesul-

<table>
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<th>Entry</th>
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Table Optimization of the Conditions of the Mitsunobu Reaction of 8 with 4

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a Concentration of 4, ADDP and TBP (1:1:1).
b Number of equivalents of 4, ADDP and TBP (1:1:1) relative to 8.
c Order of addition A: ADDP, TBP, 4. Order of addition B: 4, TBP, ADDP.
d Yield of protected product cleaved from the resin 10 as determined by HPLC.
phosphate N-methylene]-S-philanthotoxin analogues thylpyridine (collidine). This coupling has previously been adopted for further work. HPLC-MS analysis of the reaction product cleaved from the resin was used to monitor the reaction. Thus, the deprotection was judged complete when the reaction mixture remained colorless upon repeated treatment with 2-mercaptoethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). This deprotection releases a yellow chromophore, which can be used to detect the formation of the polyamine chain. HPLC-MS spectra were recorded on a Bruker Avance DRX 500 or a Bruker AMX 400 instrument operating for $^1$H at 500.13 MHz and 400.13 MHz, respectively. Chemical shifts are reported in ppm, using tetramethylsilane (TMS) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as internal standard for organic solvents and D$_2$O solutions, respectively. Coupling constants ($^J$ values) are expressed as numerical values in Hz. $^1$H NMR spectra of polymer-bound compounds were recorded on the Advance DRX 500 spectrometer with a 4 mm $^1$H/$^1$C double resonance, pulsed field-gradient high-resolution MAS probe-head, with sample spinning at 4 kHz. The resin spectra were recorded in CD$_2$Cl$_2$, and chemical shifts are reported relative to the residual solvent signal.

Analytical and preparative high performance liquid chromatography-mass spectrometry (HPLC-MS) was performed on a Perkin Elmer API 150EX instrument equipped with Turbo Ionspray (electronspray ionization) source. The HPLC system consisted of two Shimadzu LC8A pumps. UV trace was obtained with a Shimadzu SPD10A detector operating at 274 nm. Evaporating light scattering (ELS) trace was obtained with Eurosep DDL 31 Light Scattering Detector, and was used for estimation of the purity of final products. Analytical HPLC-MS was performed on a 50 x 4.6 mm YMC RP18 column, using 2 mL/min of H$_2$O−CH$_3$CN−TFA (90:10:0.05 raising to 10:90:0.05 during 7 min), with 10 µL injections. Preparative HPLC-MS (split-flow MS detection) was run with 190 µL injections (50 mg samples in 1.0 mL MeOH) to a 50 x 20 mm YMC RP18 column eluted with the same solvent gradient at 22.7 mL/min. Accurate mass determinations (±2 ppm) were performed at the University of Southern Denmark, Odense University, Department of Chemistry, on an IonSpec Fourier Transform Mass Spectrometer, using Matrix Assisted Laser Desorption Ionization (MALDI) with 2,5-dihydroxybenzoic acid as matrix.

Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. Resin-bound diamines 6 and 7 (trityl chloride resin, 1% divinylbenzene, 200–400 mesh, loading 1.50 mmol/g and 1.20 mmol/g for 6 and 7, respectively) and (S)-N-Fmoc-O-((tert-butyl)tyrosine were obtained from Novabiochem (Läufelingen, Switzerland). Tetrahydrodronorufan (THF) was distilled under N$_2$ from sodium/benzophenone immediately before use.

N-(Trimethylsilyl)ethoxy carbonyl-Protected Amino Alcohols; Synthesis of 4 and 5 To a solution of the amino alcohol 2 (2.25 g, 30.0 mmol) in aq 2 M Na$_2$CO$_3$ (150 mL) was added a solution of 2-(trimethylsilyl)ethyl NMR spectra were recorded on a Bruker Avance DRX 500 or a Bruker AMX 400 instrument operating for $^1$H at 500.13 MHz and 400.13 MHz, respectively. Chemical shifts are reported in ppm, using tetramethylsilane (TMS) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as internal standard for organic solvents and D$_2$O solutions, respectively. Coupling constants ($^J$ values) are expressed as numerical values in Hz. $^1$H NMR spectra of polymer-bound compounds were recorded on the Advance DRX 500 spectrometer with a 4 mm $^1$H/$^1$C double resonance, pulsed field-gradient high-resolution MAS probe-head, with sample spinning at 4 kHz. The resin spectra were recorded in CD$_2$Cl$_2$, and chemical shifts are reported relative to the residual solvent signal.

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4-nitrophenyl carbonate (8.50 g, 30.0 mmol) in warm (50 °C) ethanol (30 mL). The mixture was boiled under reflux with vigorous stirring for 1 h, cooled to r.t., and evaporated. The resulting slurry was diluted with H2O (500 mL) and extracted with CH2Cl2 (3 × 100 mL). The combined organic phases were washed with 5%aq NaCl (3 × 200 mL), dried (MgSO4) and concentrated in vacuo. The resulting resin was filtered off and washed with DMF (3 × 5 mL) and TBAF (1 M in THF, 2.50 mL, 2.50 mmol) was added slowly, and the mixture was stirred at r.t. under nitrogen at r.t. for 3 h. The resin was drained, washed with MeOH (2 × 5 mL) and 2-mercaptoethanol (0.088 mL, 1.25 mmol) in DMF (1 mL). The resin was dried, and the colorless solvent confirmed that the deprotection was complete. The resin was washed with DMF (5 × 5 mL), and the procedure was repeated a second time for 5 min, using DBU (0.093 mL, 0.63 mmol) in DMF (1 mL) and 2-mercaptoethanol (0.088 mL, 1.25 mmol) in DMF (1 mL). The resin was dried, and the colorless solvent confirmed that the deprotection was complete. The resin was washed with DMF (3 × 5 mL), CH2Cl2 (3 × 5 mL), MeOH (3 × 5 mL) and CH2Cl2 (3 × 5 mL), and then treated with a solution of CH2Cl2-TFA-trisopropylsilane-H2O (47.5:47.5:2.5:2.5, v/v, 5 mL) for 2 h. The resin was drained and washed with MeOH (2 × 5 mL) and CH2Cl2 (2 × 5 mL). The solution of the cleaved product and the washings were combined and evaporated in vacuo to give a yellow sticky solid (0.060 g), which was triturated with EtO, and purified by flash chromatography (silica gel, CH2Cl2-MeOH-iPrNH2 (10:10:1 then 5:5:1), followed by automated preparative HPLC to give the final product 1 (0.037 g, 23%) as a clear gum.

Compounds 34–40 were prepared and purified according to the procedure described above.

Optimization of the Mitsunobu Reaction

Reactions of 8 with 4 were performed as described above according to protocols summarized in the Table. The product was cleaved from the resulting resins using CH2Cl2-TFA-trisopropylsilane-H2O (47.5:47.5:2.5:2.5, v/v), and its identity confirmed by HPLC-MS. Yield of the reaction was calculated by comparison of HPLC (UV trace at 274 nm) of the product cleaved from the resin 10 with that obtained with resin 8, which was not subjected to the Mitsunobu reaction.

(S)-N-[4-(4-[(3-Aminopropyl)amino]propyl)[amino]butyl]-4-hydroxy-α-(1-oxobutyl)amino]benzenepropanamide tris(trifluoroacetate) (PhTX-433, 1)

Yield: 23%.

1H NMR (CDOD): δ = 0.85 (t, 2H), 1.54 (s) and 2.16 (m) (respectively 4-CH2, 3-CH2, and 2-CH2 of the 1-oxobutyl moiety. J = 7.5 Hz), 2.80 and 2.96 (each dd, JAB = 13.7, JAX = 8.5, JAX = 6.6 Hz, CH2), 4.40 (dd, α-CH2), 6.70 and 7.05 (each, 2H, Ar), 1.50–1.60 (m, 4H, CH2), 2.07–2.17 (m, 4H, CH2), 2.99–3.16 (m, 12H, remaining CH2 of the polyamine).

13C NMR (H2DO/D2O, 9:1; pH 7.1): δ = 15.3, 21.5, 25.5 (2C), 26.6, 28.0, 39.0, 39.5, 39.9, 41.1, 47.2, 47.5 (2C), 50.1, 58.4, 118.1 (2C), 130.9, 133.1 (2C), 157.0, 175.9, 179.6. MS (ESI) m/z: 436.5 (M + 1).

HPLC-ELS: 98.5%.

HRMS (MALDI) m/z: [M + 1] calcld for C25H27N4O5, 436.3282; found, 436.3282.

(S)-N-[3-[(3-Aminopropyl)amino][propyl][amino]propyl]-4-hydroxy-α-(1-oxobutyl)amino]benzenepropanamide tris(trifluoroacetate) (PhTX-333, 34)

Yield: 26%.

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Yield: 29%.

**α**tris(trifluoroacetate) (PhTX-344, tris(trifluoroacetate) (PhTX-334, tris(trifluoroacetate) (PhTX-343, hydroxy-[\(\alpha\)]-[(1-oxobutyl)amino]benzenepropanamide tris(trifluoroacetate) (PhTX-343, 35)

Yield: 29%.

\(^1\)H and \(^{13}\)C NMR spectra as previously reported. 15,16,76

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-343, 36)

Yield: 27%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 37)

Yield: 32%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 38)

Yield: 30%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 39)

Yield: 32%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 40)

Yield: 40%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 41)

Yield: 40%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 42)

Yield: 40%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 43)

Yield: 30%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 44)

Yield: 30%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 45)

Yield: 30%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 46)

Yield: 30%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 47)

Yield: 30%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 48)

Yield: 30%.

**α**-[\(4-\text{oxo}\)-[\(1\)-oxobutyl]amino]benzenepropanamide tris(trifluoroacetate) (PhTX-344, 49)

Yield: 30%.
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References

(7) Savage, P. B.; Li, C. Expert Opin. Invest. Drugs 2000, 9, 263.


