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Synthesis of polyamines and polyamine toxins. An improved alkylation procedure

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Abstract—Polyamines and polyamine toxins are biologically important molecules, having modulatory effects on nucleotides and proteins. Here we present an improved alkylation procedure, which allows sequential synthesis of polyamines and polyamine toxins on solid phase using *N*-protected aminoalkyl halides and 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD) as base. The feasibility of the procedure is demonstrated with the synthesis of the native polyamine toxin, PhTX-433, as well as an analogue, PhTX-56, which is a very potent and subtype selective glutamate receptor antagonist.

Polyamines are present in the vast majority of cells. They play important roles in the synthesis of proteins, cell division and differentiation, and bind to nucleic acids resulting in their condensation, thereby affecting gene expression. These effects might have implications in cancer treatment.[1] More recently the binding of endogenous polyamines to several ion channel proteins has been discovered.[2] One such member of this group is the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor, which is a subtype of the ionotropic glutamate receptors (iGluRs).[3]

Polyamine-based ligands, particularly the naturally occurring polyamine-containing toxins and their derivatives, interact with ion channel proteins. An example is Philanthotoxin-433 (PhTX-433, **1**) isolated from the Egyptian digger wasp *Philanthus triangulum*[4] PhTX-433 (**1**) and its synthetic analogue PhTX-343 (**2**) (Fig. 1) which are antagonists of a broad range of ionotropic receptors such as iGluRs, but shows little selectivity between different subtypes of these receptors.[3] However, modification of the polyamine moiety of philanthotoxins has led to selective compounds with increased potency, exemplified by PhTX-56 (**3**), which is a very potent and highly selective antagonist of AMPA receptors.[5] This effect has therapeutic potential for a range of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.[6]

The biological interest in polyamines and polyamine toxins, has nourished an interest in the synthesis of these compounds, both native polyamines and polyamine toxins, but also derivatives of these for investigations of structure–activity relationship (SAR) studies.[13,7] The use of solid phase synthesis has greatly facilitated the synthesis of polyamines and polyamine derivatives, the major advantage being that purification of highly polar intermediates is avoided and use of protecting groups is reduced.[13a,7] Generally, two approaches have been used for the synthesis of polyamines on solid phase, either by reductive amination, or alkylation. The latter strategy has extensively used the Fukuyama amination,[8] where an amine is protected and activated as a nitrobenzene sulfonamide, which is subsequently alkylated either by conventional alkylation or by a Mitsunobu reaction.

We have previously shown how a small library of polyamine toxins could be synthesized by a sequential strategy using Fukuyama amination under Mitsunobu conditions.[9] This methodology has since been used for the synthesis of several philanthotoxin analogues.[5] Unfortunately, in order for the reaction to go to completion it has to be repeated three times, each time for 3 h. Since protected amino alcohols are used in five-fold excess each time, a total of 15 equiv are used, hence atom economy is very low. Moreover, the use of tributylphosphine (Bu_3P) was required and problems with oxidation

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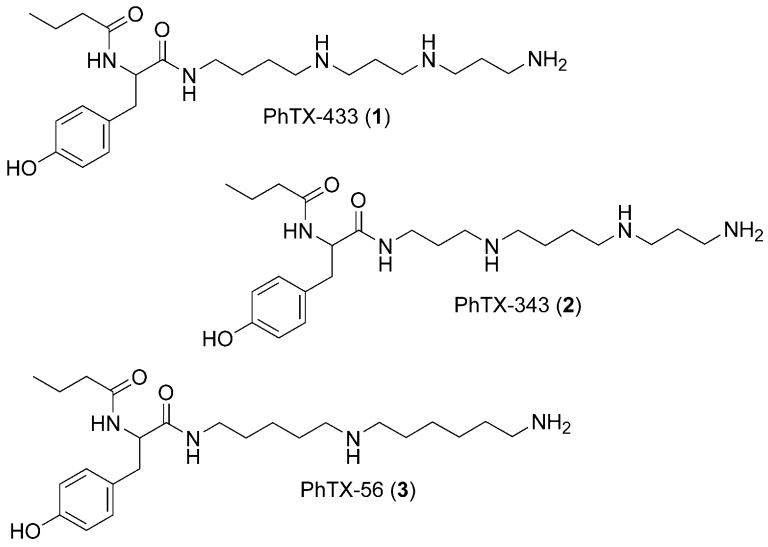


Figure 1. Structures of polyamine toxins. PhTX-433 (**1**) is the native philanthotoxin isolated from the wasp *Philanthus triangulum*, while PhTX-343 (**2**) and PhTX-56 (**3**) are derivatives, the latter being a very potent and highly selective AMPA receptor antagonist.

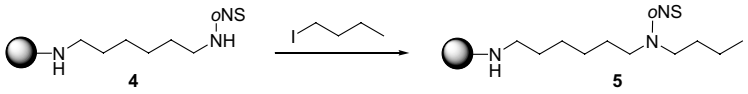
of Bu_3P are often encountered. Finally, the Mitsunobu reaction is generally very sensitive to moisture and has to be carried out under nitrogen.

Therefore, we decided to investigate alternative methods for *N*-alkylation of sulfonamides on solid phase.[10] Fukuyama and co-workers have used standard alkylation conditions (K_2CO_3 in DMF at 60 °C) for the alkylation of nitrobenzene sulfonamides on solid phase, either with the alkyl halide as the reagent in solution or bound to the solid phase.[8] However, when the same conditions were applied in the conversion of resin-bound nitrobenzene sulfonamide **4** to the corresponding alkylated product **5** using butyl iodide (Table 1) only a low conversion (36%) was observed. When carrying out the same reaction with *N*,*N*-diisopropylethylamine (DIPEA) as base (at room temperature) no detectable conversion was observed even after 16 h.

Instead, we became aware of an example of *N*-methylation of peptides, where the choice of base was crucial and the use of 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD) was required for satisfactory *N*-methylation.[11] The uncharged, sterically hindered guanidine base MTBD is among the strongest non-ionic bases known ($pK_{BH+} = 25$ in acetonitrile), comparable to the corresponding demethylated analogue 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TDB) ($pK_{BH+} = 26$), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) ($pK_{BH+} = 24$) (Fig. 2). These bases have proven to have pronounced effect on the outcome of various reactions such as Wittig and nitroaldol (Henry) reactions.[12]

Therefore, the use of MTBD for the alkylation of resin-bound *o*-nitrobenzene sulfonamide **4** was attempted using butyl iodide as alkylating agent. The reaction was carried out in DMF at room temperature for 16 h and gratifyingly the reaction provided the alkylated product in high yield. Based on this result, a more systematic investigation of this reaction was carried out. Initially the alkylation of resin-bound *o*-nitrobenzene sulfonamide **4** with butyl iodide to give **5** was investigated as a model system (Table 1). In contrast to the Mitsunobu alkylation,[9] the concentration of reagents was not important, as there was no difference using 100 or 200 mM concentration of reagents when MTDB

Table 1. Initial investigations of the alkylation of sulfonamides on solid phase[a]



4 5

Entry	Solvent	Concentration (mM)	Conversion (%)[b]	
			MTBD	DBU
1	DMF	100	>98	91
2	DMF	200	93	95
3	DMF–DCM (1:1)	100	94	65
4	DMF–DCM (1:1)	200	94	80

[a] Reactions were carried out using 4 equiv of butyl iodide and 6 equiv of base at room temperature reacting for 16 h.
[b] Ratios of starting material and product after cleavage from the resin as determined by HPLC–MS.

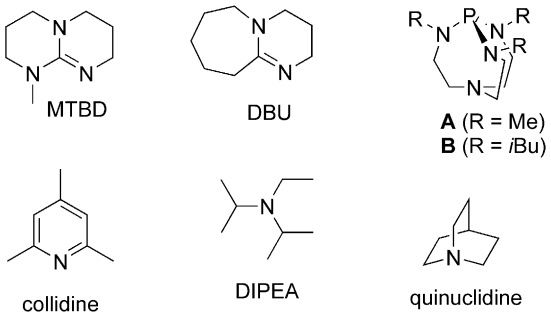


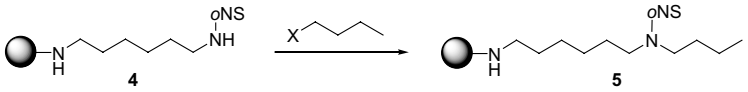
Figure 2. Bases employed in this study.

was used and only a small effect was noted for DBU (Table 1). Moreover, it was found that DMF as solvent was superior to a 1:1 mixture of DMF/DCM. The latter was employed due to the better swelling properties of the resin in DCM, which in this case is of minor importance (Table 1).

Next the effect of the leaving group, as well as reaction time was studied. Five different butyl derivatives with either Cl, Br, I, OMs or OTs as the leaving group were reacted with **4** for 1, 2, 3 or 16 h, respectively (Table 2), and the products were cleaved from the resin and analyzed by HPLC–MS. Reacting with butyl chloride did not lead to any detectable formation of the alkylated product, while complete conversion was observed with butyl bromide, but only after 16 h. Butyl iodide on the other hand, resulted in complete conversion after 3 h, whereas the mesylate and tosylate gave only 36% and 40% conversion, respectively, after 16 h. Thus, butyl iodide was clearly the alkylating agent required for complete conversion to the alkylated product in 3 h.

In light of the success of MTBD the use of other bases was considered, in particular it was envisaged that other bases may allow for the use of alkyl bromides or chlorides. Therefore, a range of different bases with a large span in their pK_{BH+} values were selected (Fig. 2): collidine (pK_{BH+} = 15), quinuclidine (pK_{BH+} = 19) and two proazaphosphatrane bases 2,8,9-trimethyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane (A, pK_{BH+} = 33) and 2,8,9-triisobutyl-2,5,8,9-tetraaza-1-phosphabicyclo-[3.3.3]undecane (B, pK_{BH+} = 33.5).[13] The alkylation reaction was carried out as described in Table 3, and for each of the four bases alkylation was carried out using either butyl chloride, bromide or iodide, respectively, for 3 and 16 h (Table 3). The use of collidine and quinuclidine did not lead to any detectable conversion in any of the reactions, which might be attributed to the relatively low pK_{BH+} values. Interestingly, neither of the proazaphosphatrane bases were competitive to

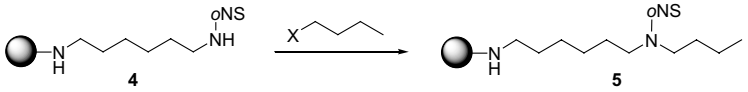
Table 2. Investigation of the leaving group and time of the reaction[a]



Entry	X	Conversion (%)[b]			
		1 h	2 h	3 h	16 h
1	Cl	<5	<5	<5	<5
2	Br	22	27	37	>98
3	I	78	93	>98	~98
4	OMs	19	18	32	36
5	OTs	6	9	14	40

[a] Reactions were carried out at room temperature using 4 equiv of alkylating agent at 100 mM concentration using 6 equiv of MTBD in DMF.
[b] Ratios of starting material and product after cleavage from the resin as determined by HPLC–MS.

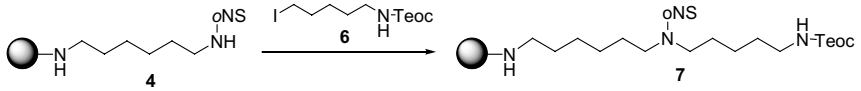
Table 3. Investigation of bases[a]



Entry	X	Time (h)	Conversion (%)[b]				
			Collidine	Quinuclidine	MTBD	**A**	**B**
1	Cl	3	<5	<5	<5	<5	<5
2	Cl	16	<5	<5	<5	<5	16
3	Br	3	<5	<5	37	52	65
4	Br	16	<5	<5	>98	44	72
5	I	3	<5	<5	>98	65	69
6	I	16	<5	<5	~98	60	76

[a] Reactions were carried out at room temperature using 4 equiv of alkylating agent at 100 mM concentration using 6 equiv of base in DMF.
[b] Ratios of starting material and product after cleavage from the resin as determined by HPLC–MS.

Table 4. Optimization of the alkylation reaction for the synthesis of polyamines[a]



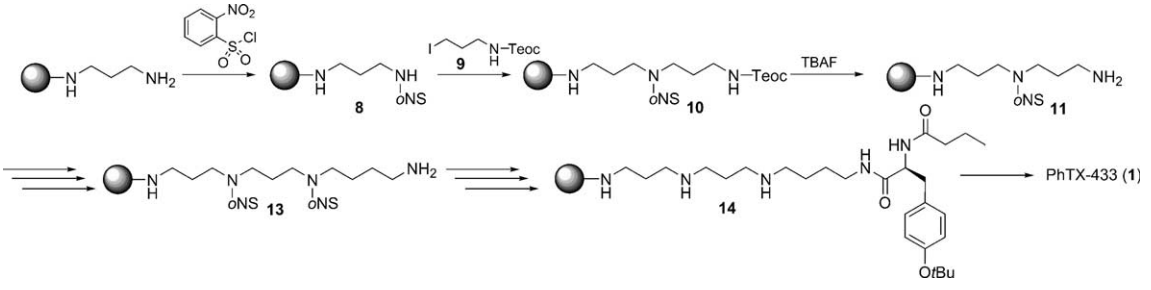
Entry	Temp	Time (h)	Conversion (%)[b]	
			MTBD	DBU
1	rt	3	>98	76
2	rt	2 × 3	>98	84
3	50 °C	3	>98	75
4	50 °C	2 × 3	>98	90

[a] Reactions were carried out using 4 equiv of alkylating agent at 100 mM concentration using 6 equiv of MTBD.
[b] Ratios of starting material and product after cleavage from the resin as determined by HPLC–MS.

MTBD; both gave 52–72% and 60–76% conversion (independent of reaction time) with butyl bromide and iodide, respectively (Table 3), thus significantly less conversion than MTBD. Obviously, base strength is not the only parameter determining the outcome of these reactions. One could speculate that the bulk of proazaphosphatrane bases are a limiting factor in this reaction, or that these bases are too nucleophilic leading to alkylation of the bases. Increased basicity could also lead to elimination (E2 reaction), but the increased reactivity for alkyl iodides indicated that this is not the case.

After optimization of the reaction conditions in the model system, the alkylation procedure was investigated in a system for the synthesis of the polyamine moiety in PhTX-56 (**3**). Thus resin **4** was reacted with *N*-Teoc aminopentyl iodide (**6**)[14] (Table 4), the latter being synthesized from the corresponding alcohol by treatment with iodine, triphenylphosphine and imidazole giving **6** in 76% yield.[15] The *N*-alkylation was carried out in DMF, using 4 equiv of the alkylating agent and 6 equiv of the base, while the temperature and reaction time were varied. Independent of temperature (rt or 50 °C) or reaction time (3 h or 2 × 3 h) using MTBD as base resulted in complete conversion, while DBU gave conversion in the range of 76–90%. Thus, the use of MTBD as a base in these alkylation reactions is superior to DBU, and reaction for 3 h at room temperature provided complete conversion. An obvious concern is that using a strong base such as MTBD might lead to alkylation of the nitrogen attached to the solid support by the trityl linker, as *N*-acylation has been observed when the resin is treated with reactive species such as acid chlorides. However, HPLC–MS analysis of the cleaved product of resin **7** showed no sign of the double alkylated product (M_w = 227.4 g/mol).

To demonstrate the versatility of this alkylation procedure, two target compounds were selected for synthesis, the native philanthotoxin PhTX-433 (**1**) and the derivative PhTX-56 (**3**). PhTX-433 (**1**) was synthesized on solid phase, as outlined in Scheme 1. Resin bound 1,3-diaminopropane was derivatized with *o*-nitrobenzenesulfonyl chloride to provide the corresponding sulfonamide **8**, which was subsequently alkylated with *N*-Teoc aminopropyl iodide (**9**)[14] using the established conditions, leading to resin **10**. The Teoc protecting group was selectively removed by treatment with tetrabutylammonium fluoride (TBAF) to give **11**. This three step procedure was repeated reacting with *N*-Teoc aminobutyl iodide (**12**, structure not shown),[14] thus leading to resin-bound, *o*NS-protected tetramine **13**, followed by standard peptide coupling procedures, providing **14**. Similarly, PhTX-56 (**3**) was synthesized from resin-bound, protected triamine **7** (Table 4). The final products were obtained by cleavage from the resins with concomitant deprotection of the phenol group, giving **1** and **3** as crude products. The compounds were purified by automated, preparative HPLC–MS and analyzed by HPLC–MS using simultaneous UV and evaporative light scattering detection (ELSD), the latter being capable of detecting the diamine impurities not observed by UV detection. Thus compounds **1** and **3** were obtained in 29% and 35% overall yield (11 and 8 steps), respectively, based on the starting resin and were >98% pure according to HPLC–MS.



Scheme 1.

In conclusion, an improved method for alkylation of nitrobenzene sulfonamides has been developed, using *N*-protected alkyl iodides and MTBD as base. The method is evidently superior to the corresponding Mitsunobu alkylation: the reaction time is reduced (from 3×3 h to 3 h), the number of equivalents is reduced (from 15 to 4 equiv), inert conditions are not required and the reaction is generally more robust. The versatility of the method was demonstrated by the synthesis of polyamine toxins, PhTX-433 (**1**) and its derivative, PhTX-56 (**3**), which were obtained in a higher overall yield compared to the Mitsunobu alkylation. The method is generally applicable to synthesis of polyamines and their derivatives.

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References and notes

1. (a) Thomas, T.; Thomas, T. J. *J. Cell. Mol. Med.* **2003**, *7*, 113–126; (b) Casero, R. A.; Woster, P. M. *J. Med. Chem.* **2001**, *44*, 1–26.
2. Williams, K. *Cell Signal* **1997**, *9*, 1–13.
3. (a) Strømgaard, K.; Mellor, I. R. *Med. Res. Rev.* **2004**, *24*, 589–620; (b) Mellor, I. R.; Usherwood, P. N. R. *Toxicon* **2004**, *43*, 493–508.
4. Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4910–4913.
5. Kromann, H.; Krikstolaityte, S.; Andersen, A. J.; Andersen, K.; Krogsgaard-Larsen, P.; Jaroszewski, J. W.; Egebjerg, K.; Strømgaard, K. *J. Med. Chem.* **2002**, *45*, 5745–5754.
6. (a) Weiss, J. H.; Sensi, S. L. *Trends Neurosci.* **2000**, *23*, 365–371; (b) Pellegrini-Giampietro, D. E.; Gorter, J. A.; Bennet, M. V. L.; Zukin, R. Z. *Trends Neurosci.* **1997**, *20*, 464–470.
7. Strømgaard, K.; Andersen, K.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Mini-Rev. Med. Chem.* **2001**, *1*, 317–338.
8. (a) Kan, T.; Kobayashi, H.; Fukuyama, T. *Synlett* **2002**, 1338–1340; (b) Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, 353–359.
9. Strømgaard, K.; Andersen, K.; Ruhland, T.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Synthesis* **2001**, 877–884.
10. All reactions on solid phase were carried out using MiniBlock™.
11. Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1997**, *119*, 2301–2302.
12. (a) Simoni, D.; Rondanin, R.; Morini, M.; Baruchello, R.; Invidiata, F. P. *Tetrahedron Lett.* **2000**, *41*, 1607–1610; (b) Simoni, D.; Rossi, M.; Rondanin, R.; Mazzali, A.; Baruchello, R.; Malagutti, C.; Roberti, M.; Invidiata, F. P. *Org. Lett.* **2000**, *2*, 3765–3768; (c) Reid, M.; Rowe, D. J.; Taylor, R. J. K. *Chem. Commun.* **2003**, 2284–2285.
13. (a) Verkade, J. G.; Kisanga, P. B. *Tetrahedron* **2003**, *59*, 7819–7858; (b) Kisanga, P. B.; Verkade, J. G.; Schwesinger, R. *J. Org. Chem.* **2000**, *65*, 5431–5432.
14. 5-{*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino}pentane iodide (**6**). Yield: 76%. ^{1}H NMR ($CDCl_3$): δ 0.03 (s, 9H), 0.96 (t, J = 8.4 Hz, 2H), 1.36–1.56 (m, 4H), 1.76–1.87 (m, 2H), 3.09–3.19 (m, 4H), 4.12 (t, J = 8.4 Hz, 2H); ^{13}C NMR: δ 1.4, 6.8, 17.8, 27.6, 29.1, 33.0, 40.6, 62.9, 156.6. 3-{*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino}propane iodide (**9**). Yield: 58%. ^{1}H NMR ($CDCl_3$): δ 0.01 (s, 9H), 0.92 (t, J = 8.2 Hz, 2H), 1.97 (m, 2H), 3.10–3.26 (m, 4H), 4.09 (t, J = 8.0 Hz, 2H), 5.00 (br s, NH); ^{13}C NMR: δ 1.4, 3.2, 17.8, 33.3, 41.3, 63.1, 156.6. 4-{*N*-[2-(Trimethylsilyl)ethoxycarbonyl]amino}butane iodide (**12**). Yield: 59%. ^{1}H NMR ($CDCl_3$): δ 0.05 (s, 9H), 0.98 (t, J = 8.2 Hz, 2H), 1.63 (m, 2H), 1.87 (m, 2H), 3.16–3.23 (m, 4H), 4.15 (t, J = 8.1 Hz, 2H); ^{13}C NMR: (δ −1.4, 6.2, 17.8, 30.5, 31.0, 39.7, 62.9, 156.6.
15. Mukai, C.; Nomura, I.; Kitagaki, S. *J. Org. Chem.* **2003**, *68*, 1376–1385.