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Dde - A Selective Primary Amine Protecting Group: A Facile Solid Phase Synthetic Approach To Polyamine Conjugates

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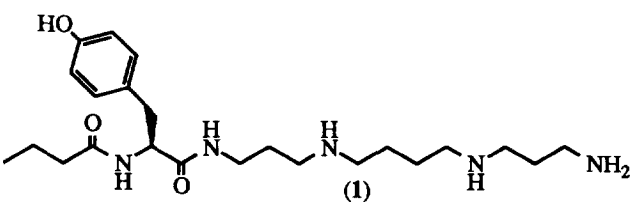
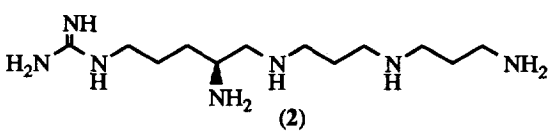
Abstract: The ability of the 2-chlorotrityl chloride resin and Dde to act as selective and orthogonal primary amine protecting groups has been utilised in a novel solid phase synthesis of protected symmetrical polyamines from which philanthotoxin-343 and sFTX-3.3 have been synthesised. Copyright © 1996 Published by Elsevier Science Ltd

Ionotropic glutamate receptors (i-GluR) mediate signal transmission in the central nervous system (CNS) of vertebrates[1] and at the neuromuscular junction of invertebrates.[2] The blockade of the latter by polyamine toxins from certain species of spiders and wasps is the means by which they paralyse their prey.[3] These same toxins also inhibit neural transmission *via* the mammalian CNS receptors,[4] overstimulation of which *e.g.* after cerebral ischaemia and trauma, can result in irreversible brain damage.[5] The neuroprotective potential of compounds of this type is of considerable interest in neuropharmacology. However, the isolation of these toxins from natural sources is difficult and low yielding.[6] Furthermore existing procedures for the general synthesis of polyamine conjugates are lengthy and not particularly efficient.[7]



(1)



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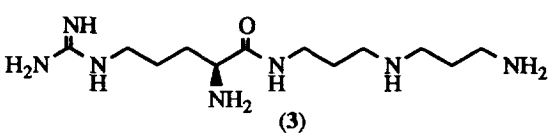
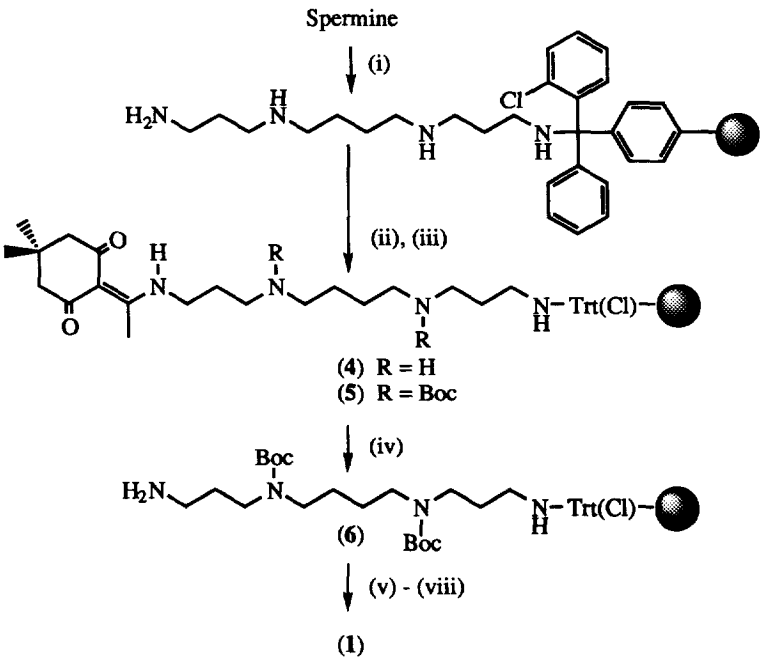


(3)

We recently described a novel solid phase approach to the synthesis of the polyamine peptides nephilatoxin-9 and -11[8] which exploits the orthogonal amine protecting capacities of *N*-Dde[9] and *N*-Fmoc. We now report a further facile and efficient procedure which allows the individual and multiple synthesis of more complex polyamine conjugates. This is illustrated with the specific synthesis of philanthotoxin-343 (PhTX-343)[6] (**1**), a potent analogue of the wasp toxin philanthotoxin-433 (PhTX-433) and the calcium channel blocker FTX-3.3 (**2**)[10].

The strategy employs 2-chlorotrityl chloride polystyrene resin[11] as both a primary amine protecting group and solid support, and exploits the ability of 2-acetyldimedone to react specifically with primary amines. We were already aware that even with a large excess of 2-acetyldimedone, polyamines such as spermine and *N*-(3-aminopropyl)-1,3-diaminopropane react in solution to give only the primary amine protected bis-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] (Dde) derivatives.[12] This selectivity is presumably a function of the stabilisation provided by a strong intramolecular hydrogen bond (N*H* δ = 12-15 ppm) which is not possible with secondary amines (see Scheme 1). These observations also served as a means of establishing that symmetrical polyamines can be coupled to a 2-chlorotrityl chloride resin exclusively through one or other of their two terminal amine groups.



Scheme 1

(i) 2-chlorotrityl chloride polystyrene resin, CH_2Cl_2; (ii) 2-acetyldimedone, DMF; (iii) Boc_2O, DIPEA; (iv) 2% hydrazine in DMF; (v) Activated Fmoc-L-Tyr(O^{t}Bu)-OH; (vi) 20% piperidine in DMF; (vii) Activated butyric acid; (viii) TFA: $^i Pr_3SiH$: H_2O.

Attachment of spermine was achieved by addition of a tenfold excess of spermine in a minimal amount of CH_2Cl_2, and followed after 30 min by quenching the unreacted 2-chlorotrityl groups with methanol. Any exposed primary amine groups of the attached spermine were then capped with Dde and the product released from the solid support with TFA. RP-HPLC analysis revealed the presence of essentially pure product (yield and

purity >99%) which was identified as the mono *N*-Dde derivative.[13] Having established that *N*-Dde spermine was attached in the manner shown (**4**, R=H), it was possible to exploit the stability and deprotection conditions of the Dde group to construct PhTX-343 (**1**) on the polymer support. The secondary amines were capped with *tert*-butyloxycarbonyl groups using an excess of Boc_2O (**5**, R=Boc) and the Dde protected primary amine selectively unmasked with 2% v/v hydrazine in DMF (**6**). The assembly of (**1**) was completed by sequential coupling with carboxyl activated Fmoc-L-Tyr(OtBu)-OH and butyric acid using standard procedures[14] (Scheme 1).

Deprotection and simultaneous cleavage from the resin yielded after trituration with Et_2O, a white solid (purity >90%) which was finally purified by RP-HPLC[15] (Figure 1). The LC-MS data, 250MHz (D_2O, Figure 2) and 500MHz NMR spectra[16] are fully consistent with the structure of PhTX-343. The whole sequence of operations can be fully automated and completed within 24 h.

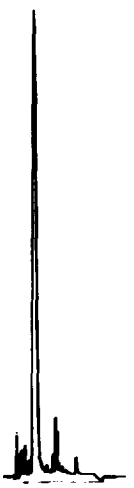


Figure 1

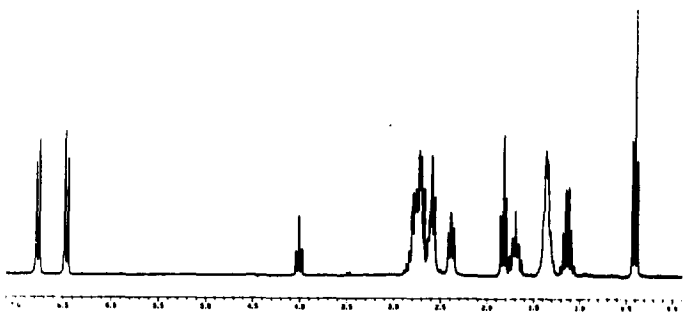


Figure 2

As further illustration of the efficacy of the procedures, the precursor sFTX-3.3 (**3**) of the calcium channel blocker (**2**)[10] has been constructed. The 2-chlorotrityl chloride resin was attached with *N*-(3-aminopropyl)-1,3-diaminopropane, the primary amine protected as described with Dde and the secondary amine capped with Boc_2O. Following removal of the *N*-Dde group, *N*-(3-aminopropyl)-*N*-Boc-*N'*-(2-chlorotrityl polystyrene)-1,3-diaminopropane was elaborated by coupling the free amine with carboxyl-activated Fmoc-L-Arg(Pmc)-OH. Cleavage from the resin gave (**3**) as a single compound[17] which can be reduced in solution to (**2**)[10]. We are currently investigating on resin reduction procedures which should allow the total synthesis of not only compounds of this type but also unsymmetrical polyamine derivatives. Nevertheless, these methodologies are now sufficiently robust and flexible for the synthesis of a wide range of polyamine peptide and polyamine conjugate libraries, as well as specific radiolabelled and photolabile toxins.

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References and Notes

Abbreviations: Boc, *tert*-butyloxycarbonyl; Dde, *N*-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl); DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; HBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; LC-MS, Liquid chromatography mass spectometry; NMR, nuclear magnetic resonance spectroscopy; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulphonyl; RP-HPLC, reverse-phase high performance liquid chromatography; TFA, trifluoroacetic acid.

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12 Bycroft, B. W.; Chan, W. C.; Nash, I. A. unpublished results.
13 The absence of the bis *N*-Dde derivative clearly established that none of the spermine had coupled to the resin via one of the secondary amines. This route now offers a very effective method of preparing mono *N*-Dde symmetrical polyamines which can in turn be elaborated into more complex building blocks for solid phase and other synthetic procedures.[8,12]
14. Free amines were acylated using fourfold excess of the appropriate acid activated by HBTU (1 equiv), HOBt (1 equiv) and DIPEA (2 equiv) in DMF (1 ml). The Fmoc group was removed by treatment with 20% piperidine in DMF for 7 min (flow rate 2.5 ml min^{-1}).
15. The compound was deprotected and cleaved from the resin by treatment with TFA, 95: H_2O, 2.5: $^{i}Pr_3SiH$, 2.5 %v/v for 2 h. PhTX-343 was purified on a semi-preparative column Kromasil KR100-5C8 (8 x 250 mm, flow rate 2.5 mL min^{-1}). The elution gradient was 10 to 50%B in 30 minutes. (*A* = 0.06% aqueous TFA, *B* = 0.06% TFA in 90% aqueous acetonitrile).
16. PhTX-343: Electrospray-MS, MH^+ calc. 436.6, found 435.6; ^1H NMR (500MHz, 90% H_2O:10%D_2O): Butyryl; 0.41 (3H, t, *J* = 7.5 Hz, CH_3), 1.14 (2H, m, *J* = 7.5 Hz, $CH_3CH_2CH_2$), 1.83 (2H, t, *J* = 7.5 Hz, $CH_3CH_2CH_2$). Tyrosyl; 2.58 (2H, m, βCH_2), 4.01 (1H, t, *J* = 8 Hz, αCH), 6.48 (2H, d, *J* = 8 Hz, 2-*H*, 6-*H*), 6.77 (2H, d, *J* = 8 Hz, 3-*H*, 5-*H*), 7.89 (1H, s, N*H*). Spermine; 7.74 (1H, d, *J* = 7 Hz, CHCON*H*), 2.82 (2H, m, $CONHCH_2$), 1.38 (2H, m, $CONHCH_2CH_2$), 2.40 (2H, t, *J* = 7.5 Hz, $CONHCH_2CH_2CH_2$), 1.38 (4H, m, $NHCH_2CH_2CH_2CH_2NH$), 2.72, 2.62 (4H, 2 x m, $NHCH_2CH_2CH_2CH_2NH$), 2.62, 2.76 (4H, 2 x m, $NHCH_2CH_2CH_2NH_2$), 1.70 (2H, m, $NHCH_2CH_2CH_2NH_2$).
17. sFTX-3.3: Electrospray-MS, MH^+ calc. 214.5, found 214.5.

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