Pergamon

Tetrahedron Letters 41 (2000) 1095–1098

TETRAHEDRON
LETTERS

Solid-phase synthesis of symmetrical and unsymmetrical polyamine analogues of philanthotoxins using a Dde-linker

Siri Ram Chhabra, Azra N. Khan and Barrie W. Bycroft *

School of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

Received 30 September 1999; accepted 29 November 1999

Abstract

The efficient construction of symmetrical & unsymmetrical polyamine analogues of philanthotoxins has been accomplished using the new Dde based linker. The linker allows the selective attachment of primary amines and the resulting linkage displays excellent stability towards the conditions required for the removal of Fmoc and Boc groups as well as the reductive alkylation conditions used for the elaboration of polyamines. The final cleavage of the polyamine or a derivative thereof from the solid support is readily achieved via transamination using a volatile primary amine with the regeneration of the linker.

Keywords: solid-phase synthesis; polyamines; philanthotoxins; Dde-linker.

L-Glutamate receptors regulate synaptic transmission in the mammalian CNS and are also linked to higher neural functions such as memory and learning. Overstimulation of these receptors can lead to neural cell death and has been implicated in a number of neurodegenerative diseases as well as epilepsy and stroke.[1,2] Various naturally occurring polyamines including the philanthotoxins isolated from the wasp *Philanthus triangulum*, and others isolated from spider venoms, are reported to act as antagonists at these receptors.[3–6] However, their detailed pharmacological evaluation and potential as neuroprotective agents have been hindered because of the limited quantities of the toxins available from natural sources.

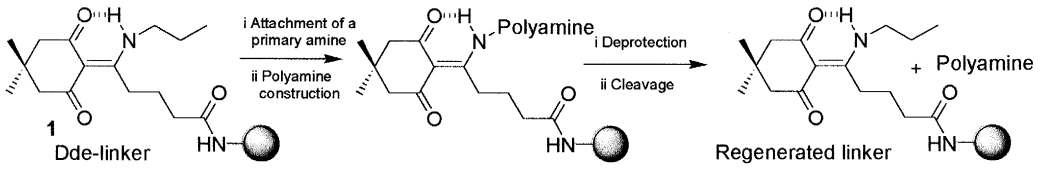
While solution[7–10] and solid phase[11–13] methods for the synthesis of polyamine toxins have been reported, we have focussed on developing a range of flexible solid phase approaches[14,15] which exploit the versatility of the Dde(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl) group and particularly its selectivity for primary amines.[16,17] Here we describe solid phase synthetic protocols for the preparation of symmetrical PhTX-3.4.3 and unsymmetrical PhTX-3.3.4 analogues employing Fmoc and Boc protection.† The key steps are the mono-attachment of a symmetrical diamine to our recently reported

* Corresponding author. Tel: +44 115 9515101; fax: +44 115 9515049; e-mail: barrie.bycroft@nottingham.ac.uk (B. W. Bycroft)

† **Abbreviations:** Amino acids and peptides follow the IUPAC-IUB nomenclature where applicable (*Eur. J. Biochem.* **1984**, 9–37); Boc, *t*-butoxycarbonyl; *t*Bu, *tert*-butyl; DCM, dichloromethane, DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; ES-MS, electrospray mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; RPHPLC, reverse phase high performance liquid chromatography; TBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; THF, tetrahydrofuran; TFA, trifluoroacetic acid; TIPS, triisopropylsilane.

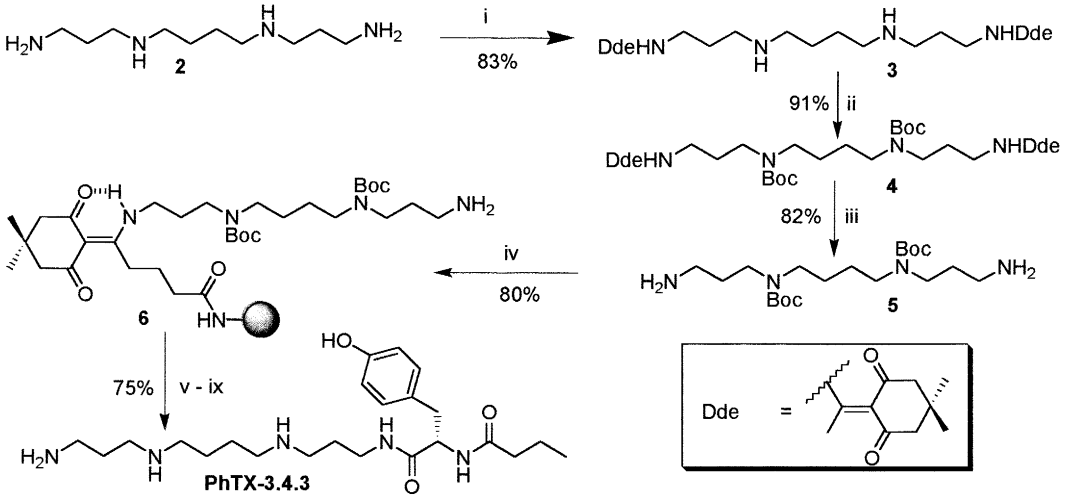
0040-4039/00/$ - see front matter
PII: S0040-4039(99)02238-8

Dde-linker **1**[18] followed by on resin reactions under acid, base and reductive alkylation conditions. Final cleavage via transamination with 10% *n*-propylamine not only releases the end product but also regenerates the linker (Scheme 1).



Scheme 1. General outline of construction and cleavage of a polyamine with simultaneous regeneration of Dde-linker

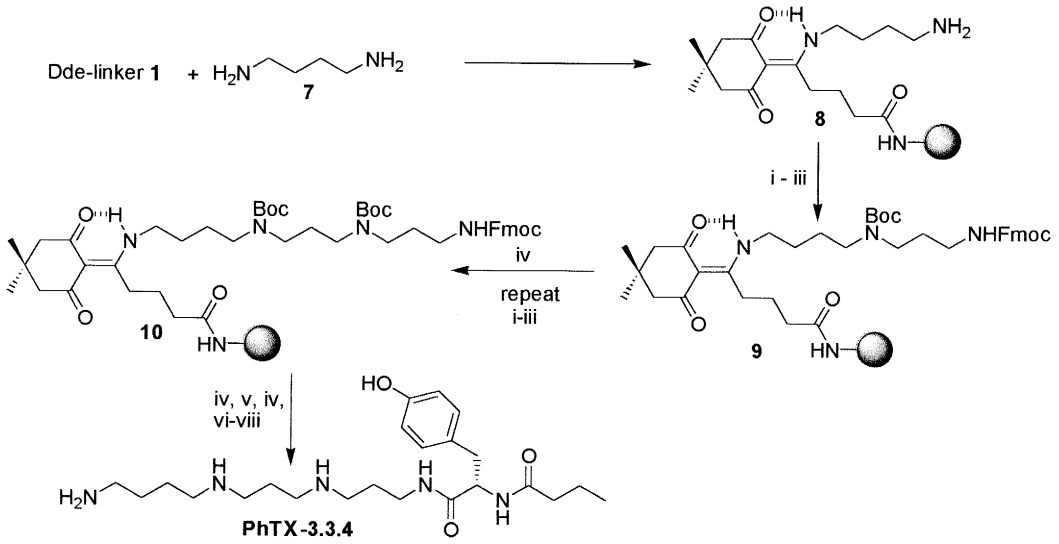
For the synthesis of the symmetrical philanthotoxin PhTX-3.4.3 (Scheme 2), spermine **2** was an ideal starting material, as the required polyamine backbone is already assembled. However, selective protection of the secondary amine functions at positions 4 and 9 was needed before the attachment of one of the equivalent primary amine functions to the solid support. The selectivity of Dde-OH was exploited to prepare N^1,N^{12}-bis-Dde spermine **3** which then allowed the protection of the secondary amines using Boc anhydride to give N^4,N^9-bis-Boc-N^1, N^{12}-bis-Dde spermine **4**.[19] Subsequent deprotection of the Dde groups with 5% v/v hydrazine afforded N^4,N^9-bis-Boc spermine **5** which was readily attached to the Dde-linker **1** in the presence of DIEA. This resin bound polyamine **6** was then acylated with Fmoc-Tyr(*t*-Bu)-OH using TBTU/HOBt/DIEA. At this stage a resin loading of 80% was determined spectrophotometrically from the Fmoc-derived chromophore liberated upon treatment with 20% piperidine in DMF. The on-resin synthesis was completed by removal of the Fmoc group and acylation of the resulting amine with *n*-butyric acid under the conditions indicated. After removing Boc and *t*-butyl groups with 50% v/v TFA in DCM containing TIPS and H_2O as scavengers, the toxin was cleaved from the solid support using 10% v/v propylamine in DMF.



Scheme 2. Synthesis of philanthotoxin-3.4.3. Reagents and conditions. (i) DdeOH, EtOH, reflux, 8 h; (ii) $(Boc)_2O$, DCM, 20 h; (iii) 5% $NH_2NH_2 \cdot H_2O$ in 50% THF/H_2O, 2 h; (iv) Dde-linker **1**, DIEA, DMF, 2 h; (v) Fmoc-Tyr(*t*Bu)-OH/TBTU/HOBt/DIEA, 4 h; (vi) 20% v/v piperidine in DMF, 10 min; (vii) *n*-butyric acid/TBTU/HOBt/DIEA, 4 h; (viii) 50% TFA in DCM/TIPS/H_2O, 3 h; (ix) 10% v/v *n*-propylamine in DMF, 6×20 min

Solvents were removed in vacuo to give an oil which was triturated with ether and then dissolved in water and lyophilised to afford philanthotoxin-3.4.3, ES-MS ($C_{23}H_{41}N_5O_3$) *m/z* 436 (M+H), as a white powder in 75% overall yield and with excellent purity as determined by a single peak eluted at 9.2 min in RPHPLC analysis.[20]

The unsymmetrical philanthotoxin analogues presented a greater synthetic challenge and we chose to construct the polyamine backbone appropriately protected using a stepwise approach on the solid support. This is illustrated for PhTX-3.3.4 in Scheme 3 but in principle the procedure can be adapted to combine a wide range of units. Reductive alkylation using $NaBH_3CN$[21] was the method selected to attach the individual units, since we anticipated that the mild acidic conditions required would be compatible with the stability of the linker and the Fmoc and Boc groups. Synthesis of PhTX-3.3.4 was initiated by anchoring 1,4-butanediamine (putrescine) **7** to the Dde-linker **1** to form the resin bound scaffold **8**. This was condensed with 3-Fmoc-aminopropanal[22] in 1% AcOH/DMF, the resin was washed to remove excess aldehyde, re-swollen in DMF and then reduced with $NaBH_3CN$ in the presence of acetic acid.



Scheme 3. Synthesis of philanthotoxin-3.3.4. Reagents and conditions. (i) Fmoc-$NHCH_2CH_2CHO$, 1% AcOH/DMF, 1.5 h; (ii) $NaBH_3CN$, 1% AcOH/DMF, 10 min; (iii) $(Boc)_2O$, DIEA, DMF, 20 h; (iv) 20% v/v piperidine in DMF, 10 min; (v) Fmoc-Tyr(*t*Bu)-OH/TBTU/HOBt/DIEA, 4 h; (vi) *n*-butyric acid/TBTU/HOBt/DIEA, 4 h; (vii) 50% TFA in DCM/TIPS/H_2O, 3 h; (viii)10% v/v *n*-propylamine in DMF, 6×30 min

The newly introduced secondary amine function was protected as a Boc-derivative to give **9**. Deprotection of the Fmoc group with 20% v/v piperidine was followed by a repeat cycle with 3-Fmoc-aminopropanal condensation, $NaBH_3CN$ reduction and Boc protection to afford the polyamine backbone **10** with the desired sequence (3.3.4). Protocols similar to those previously described for the successive acylations with Fmoc-Tyr(*t*-Bu)-OH and *n*-butyric acid were used to complete the synthesis. Resin loading assays were conducted after both the alkylation and the first acylation steps; these were 79%, 78% and 76%, respectively. Deprotection of the Boc and *t*-Bu groups followed by cleavage from the resin yielded the unsymmetrical philanthotoxin-3.3.4 in 65% yield and with 90% purity demonstrated by RPHPLC[19] by a major single peak eluted at 9.8 min that gave satisfactory ES-MS ($C_{23}H_{41}N_5O_3$) *m/z* 436 (M+H).

The solid phase methods developed above to prepare analogues of philanthotoxin are generic in nature and potentially can be applied to the synthesis of a variety of polyamines. The procedure is rapid and can be automated. Furthermore the high yields obtained clearly demonstrate the stability of our linker not only towards acid and base but also to the reducing and coupling conditions employed. While the overall process worked well, the introduction of C_4 and C_5 units by reductive alkylation presented unexpected problems. A method by which these particular difficulties can be circumvented is described in the following communication.[23]

Acknowledgements

We thank the Commonwealth Scholarship Commission, UK for a research studentship to A. N. Khan.

References

1. McCulloch, J.; Bullock, R.; Teesdale, G. M. In *Excitatory Amino Acid Antagonists*; Meldrum, B. S., Ed.; Blackwell Scientific Publications: Oxford, 1991; pp. 287–326.
2. Collingridge, G. L.; Lester, R. A. *Pharmacol. Rev*. **1989**, *40*, 143–210.
3. Goodnow, R.; Konno, K.; Niwa, M.; Kallimopoulos, T.; Buknownik, R.; Lenares, D.; Nakanishi, K. *Tetrahedron* **1990**, *46*, 3267–3286.
4. Piek, T. *Am. Chem. Soc., Symp. Series* **1993**, *524*, 233–238.
5. Blagbrough, I. S.; Usherwood, P. N. R. *Proc. Roy. Soc. Edin*. **1992**, *99B*, 67–81.
6. Eldefrawi, A. T.; Elderfrawi, E. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4910–4913.
7. Choi, S. K.; Nakanishi, K.; Usherwood, P. N. R. *Tetrahedron* **1993**, *49*, 5777–5790.
8. Choi, S. K.; Goodnow, R. A.; Kalivretenos, A.; Chiles, G. W.; Fushiya, K.; Nakanishi, K. *Tetrahedron* **1992**, *48*, 4793–4822.
9. Hidai, Y.; Kan, T.; Fukuyama, T. *Tetrahedron Lett.* **1999**, *40*, 4711–4714.
10. Blagbrough, I. S.; Moya, E. *Tetrahedron Lett*. **1994,** *35*, 2057–2060.
11. Marsh, I. R.; Bradley, M. *J. Chem. Soc., Chem. Commun*. **1996**, 941–942.
12. Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Tetrahedron* **1999**, *55*, 335–344.
13. Carrington, S.; Renault, J.; Tomasi, S.; Corbel, J.-C.; Uriac, P.; Blagbrough, I. S. *Chem. Commun*. **1999**, 1341–1342.
14. Bycroft, B. W.; Chan, W. C.; Hone, N. D.; Millington, S.; Nash, I. A. *J. Am. Chem. Soc.* **1994**, *116*, 7415–7416.
15. Nash, I. A.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett*. **1996**, *37*, 2625–2628.
16. Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Hone, N. D. *J. Chem. Soc., Chem. Commun*. **1993**, 778–779.
17. Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett*. **1998**, *39*, 1603–1606.
18. Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett*. **1998**, *39*, 3585–3588.
19. N^4,N^9-bis-Boc-N^1,N^{12}-bis-Dde spermine **4**: m.p. 94–96°C; R_f 0.5 (SiO_2, 1% MeOH/EtOAc). Found: C, 65.56; H, 9.23; N, 7.65. $C_{40}H_{66}N_4O_8$ requires: C, 65.72; H, 9.10; N, 7.67%; δ_H (250 MHz, $CDCl_3$) 1.03 (12H, s, ring 2×Me_2), 1.43 (18H, s, 2×Me_3), 1.55 (4H, s, 6, 7-$(CH_2)_2$), 1.84–199 (4H, m, 2, 11-$(CH_2)_2$), 2.36 (8H, s, ring 4×CH_2), 2.55 (6H, s, 2×=CMe), 3.18–3.23 (4H, m, 5, 8-$(CH_2)_2$), 3.25–3.34 (4H, m, 3,10-$(CH_2)_2$), 3.35–3.44 (4H, m, 1,12-$(CH_2)_2$), 13.49 (2H, s, 2×NH); δ_c (62.9 MHz, $CDCl_3$) 18.25 (2, 6, 7, 11-CH_2), 28.62 (ring Me_2), 28.78 (Me_3), 30.46 (ring *C*Me_2), 41.47 (=C*C*H_3), 44.68 (2×ring CH_2), 53.20 (1, 3, 5, 8, 10, 12-CH_2), 80.11 (Me_3*C*=O), 108.25 (alkene C), 155.82 (=C-NH), 173.86 (carbamate C=O), 198.26 (ring C=O). ES-MS *m/z* 731.6 (M+H, $C_{40}H_{66}N_4O_8$ requires: *m/z* 730.96).
20. RPHPLC was performed using a Hypersil Pep-100 C_{18} column (4.6×150 cm) at a constant flow rate of 1.2 ml min^{-1}. Mobile phase: (A) 0.06% $TFA_{(aq)}$ and (B) 0.06% TFA in 90% $MeCN_{(aq)}$; gradient: 20 to 100% B in 30 min linearly.
21. Brown, E. G.; Nuss, J. M. *Tetrahedron Lett*. **1997**, 38, 8457–8460 and the references cited therein.
22. 3-Fmoc-Aminopropanal was prepared from *N*-Fmoc-β-alanine by its conversion to *N*-methoxy-*N*-methyl-3-(Fmoc-amino)propionamide (80%) followed by reduction with $LiAlH_4$ in dry diethyl ether (76%). (Nahm, S.; Weinreb, S. M. *Tetrahedron Lett*. **1981**, *22*, 3815–3818).
23. Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett*. **2000**, *41*, 1099–1102.