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Practical Synthesis of Unsymmetrical Polyamine Amides

Ian S. Blagbrough* and Andrew J. Geall

**Department of Pharmacy and Pharmacology,
University of Bath, Bath BA2 7AY, U.K.**

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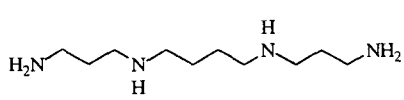
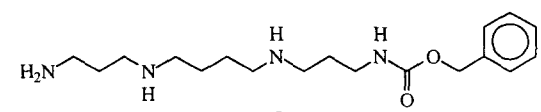
Abstract: Desymmetrisation of readily available symmetrical polyamines is an important first step in the synthesis of many polyamine containing natural products. Likewise in the synthesis of polyamine amides which are potentially useful for gene delivery and as neuroprotectants, based upon channel blocking toxins found in certain wasp and spider venoms. The application of trifluoroacetyl as a protecting group allows unsymmetrical polyamine amides to be easily prepared on a gram scale.

In our studies of polyamines and polyamine amides,[1-11] we are investigating spermine **1** which is a linear tetra-amine, naturally occurring in all cells and playing important roles *in vivo*. These roles include maintaining the 3D structure of DNA,[12-16] including condensing DNA,[11,17-18] modulating cell signalling via e.g. inward rectifying potassium channels,[19-20] and potentiating and channel-blocking subtypes of glutamate receptors.[1-3,21-23] Recently, we and others have shown that polyamines and polyamine amides can be prepared by reductive alkylation,[7-9,24,25] consecutive Michael additions to acrylonitrile,[25,26] or regioselective acylation of unsymmetrically protected polyamines.[1-4,24-27] Tetra-amine spermine **1** is readily available and is an ideal starting material to incorporate three (or four) positive charges in to a target molecule. However, the desymmetrisation protocol is by nature low yielding and involves laborious chromatographic purification. Such low yielding steps are not efficient on a gram scale. There are problems with efficient syntheses of N^1-mono-Z- **2** and N^1-mono-BOC-spermine **3**. Using either Z-Cl together with sensitive pH control, or $(BOC)_2O$ with the polyamine in large excess, was either not practical or required time-consuming chromatographic purification from the excess of unreacted polyamine.[28] In this *Letter*, we report the practical synthesis of unsymmetrical polyamine amides using trifluoroacetyl as a protecting group (see **4**) whose introduction and removal can be controlled under facile conditions.



spermine **1**



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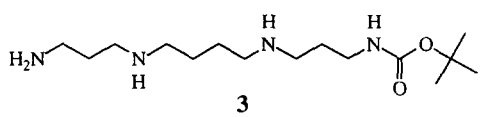

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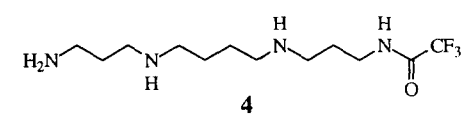

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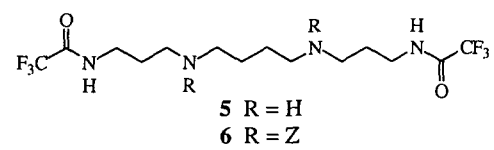
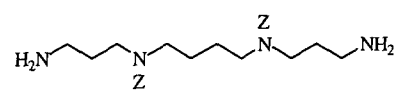
The ratio of primary amine to protecting group reagent is critical in order to avoid di-protection (of primary amines) and poly-protection (of secondary amines).[28] Presumably, the higher nucleophilicity of the secondary amines is masked by corresponding steric effects,[25] as there is always selectivity. The facile and specific (for primary over secondary amines) introduction of trifluoroacetyl using ethyl trifluoroacetate, as reported in recent *Letters*,[29] and its ready removal with aq. ammonia[30] (pH = 11) or with methanolic aq. K_2CO_3 solution[31] makes it a superior protecting group to carbobenzoxy (Z, CBZ) and to *tert*-butoxycarbonyl (BOC) for the purpose of gram scale protection of polyamines. Thus, trifluoroacetyl is the protecting group of choice, over Z and BOC, for practical routes to unsymmetrical polyamine amides and carbamates. Therefore, we have prepared N^1, N^2, N^3-tri-Z-spermine **9** and N^1, N^2, N^3-tri-BOC-spermine **15** by this strategy.

Spermine **1** was selectively protected on a primary amino functional group by reaction with ethyl trifluoroacetate (1.0 eq., MeOH, -78 °C for 1 h then to 0 °C over 1 h), to afford a mixture containing (by HPLC) predominantly mono-trifluoroacetamide **4**, but also di-trifluoroacetamide **5** (shown by Z protection to afford **6** and subsequent TFA selective deprotection to yield diamine **7** which was easily purified from monoamine **9**). Immediately, in this solution of **4**, the remaining amino functional groups were quantitatively protected with dibenzyl dicarbonate (4 eq., 0 °C to 25 °C over 1 h) to afford protected polyamine **8**, or with di-*tert*-butyl dicarbonate (4 eq., 0 °C to 25 °C over 1 h) to afford protected polyamine **14**. The TFA protecting group was then removed (*in situ*) by increasing the pH to 11, with conc. aq. ammonia,[30] stirring (25 °C, 15 h) to afford after one simple (the reaction mixtures do not contain any free spermine) chromatographic purification over silica gel (DCM-MeOH-conc. NH_4OH 70:10:1 to 50:10:1 v/v/v), N^1, N^2, N^3-tri-Z-spermine **9** (48 %) and N^1, N^2, N^3-tri-BOC-spermine **15** (50 %) respectively, from convenient, one-pot reactions. The utility of protected spermine **9** was demonstrated by a practical synthesis of the biologically important cation channel blocker philanthotoxin-3.4.3 (PhTX-3.4.3) **11** starting with *N*-acylation of L-Tyr methyl ester.HCl *O*-benzyl ether with n-butanoyl chloride (1.1 eq., 2.2 eq. TEA, DCM, 25 °C, 2 h, 83 %). Saponification of the methyl ester (3 eq. 1 M aq. NaOH, MeOH, 25 °C, 4 h, then 3 M aq. HCl) afforded the free carboxylic acid (96 %) which was coupled (1.5 eq. DCC, 0.2 eq. HOBt, DCM, 25 °C, 16 h) to tri-Z-spermine **9** to afford polyamine amide **10** (79 %). Deprotection of *O*-benzyl and three benzylcarbamate functional groups was achieved by hydrogenolysis (Pearlman's catalyst, H_2 50 psi, MeOH, 25 °C, 20 h) affording PhTX-3.4.3 **11** as its free base (85 %).[9,21]



5 R = H
6 R = Z



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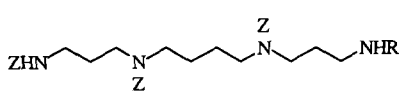
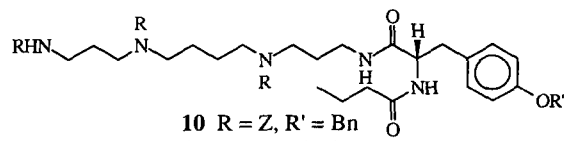
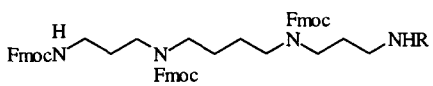


8 R = $COCF_3$
9 R = H

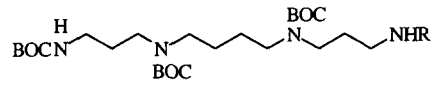


10 R = Z, R' = Bn
11 R = H, R' = H

The preparation of N^1, N^2, N^3-tri-fluorenylmethoxycarbonylspermine **13** was attempted following the above protocol by reaction with 9-fluorenylmethyl succinimidyl carbonate (4 eq., 0 °C to 25 °C, 15 h) to afford protected polyamine **12** (70 %). Selective deprotection was then investigated using potassium carbonate,[31] sodium borohydride, and hydrazine, but in all cases proved to be unsuccessful yielding mixtures of products.

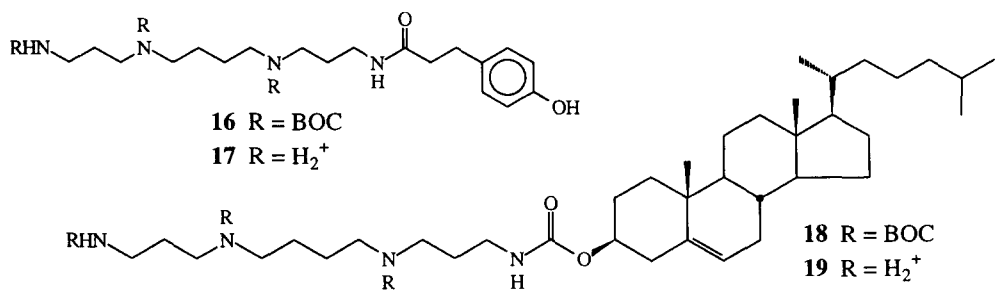


12 R = $COCF_3$
13 R = H



14 R = $COCF_3$
15 R = H

We have exemplified the gram scale use of N^1, N^2, N^3-tri-BOC-spermine **15** by preparing N^1-3-(4-hydroxyphenyl)-propanoyl-spermine **17**, acylating protected spermine **15** with 3-(4-hydroxyphenyl)-propanoic acid (1.5 eq. DCC, 0.2 eq. HOBt, DCM, 25 °C, 16 h) to afford protected polyamine amide **16** (79 %). Deprotection by treatment with TFA in DCM (DCM-TFA 90:10 v/v, 25 °C, 2 h) afforded the polytrifluoroacetate salt of channel blocking polyamine amide **17** (77 %).[2,23] This cation channel blocker is a potent analogue of PhTX-3.4.3 **11**.[2,23] 3β-(N^1-Spermine)-carbonylcholesterol **19** has recently been developed as a novel lead compound[11,32,33] for polyamine-mediated DNA condensation in gene delivery.[11] Therefore, we have prepared unsymmetrical polyamine carbamate **19** by reaction of protected spermine **15** with cholesteryl chloroformate (1.2 eq., 3.0 eq. TEA, DCM, 0 °C 10 min then 25 °C 12 h) to yield 3β-N^4-(N^1, N^2, N^3-tri-BOC-spermine)carbamoylcholesterol **18** (77 %). Deprotection (DCM-TFA 10:90 v/v, 0 °C, 2 h) and purification by RP-HPLC over Supelcosil ABZ+Plus (5 μm) (MeCN-0.1 % aq. TFA 50:50 v/v, λ = 220 nm) afforded the polytrifluoroacetate salt of polyamine carbamate **19** (50 %).[32] Polyamine-steroid (or more simply polyamine-lipid) conjugates such as polyamine carbamate **19** are biologically important as non-viral vectors for safe and efficient gene transfer with potential application in gene delivery.[11,32,33] The practical synthetic routes to unsymmetrical polyamine amides and carbamates, reported in this *Letter*, will find ready application.



16 R = BOC
17 R = H_2^+

18 R = BOC
19 R = H_2^+

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REFERENCES

1. Usherwood, P. N. R.; Blagbrough, I. S. *Pharmacol. Ther.* **1991**, *52*, 245-268.
2. Blagbrough, I. S.; Bruce, M.; Bycroft, B. W.; Mather, A. J.; Usherwood, P. N. R. *Pestic. Sci.* **1990**, *30*, 397-403; Blagbrough, I. S.; Brackley, P. T. H.; Bruce, M.; Bycroft, B. W.; Mather, A. J.; Millington, S.; Sudan, H. L.; Usherwood, P. N. R. *Toxicon* **1992**, *30*, 303-322.
3. Blagbrough, I. S.; Usherwood, P. N. R. *Proc. Roy. Soc. Edin.* **1992**, *99B*, 67-81.
4. Adlam, G.; Blagbrough, I. S.; Taylor, S.; Latham, H. C.; Haworth, I. S.; Rodger, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2435-2440.
5. Rodger, A.; Blagbrough, I. S.; Adlam, G.; Carpenter, M. L. *Biopolymers* **1994**, *34*, 1583-1593.
6. Rodger, A.; Taylor, S.; Adlam, G.; Blagbrough, I. S.; Haworth, I. S. *Bioorg. Med. Chem.* **1995**, *3*, 861-72.
7. Blagbrough, I. S.; Moya, E. *Tetrahedron Lett.* **1995**, *36*, 9393-9396.
8. Ashton, M. R.; Moya, E.; Blagbrough, I. S. *Tetrahedron Lett.* **1995**, *36*, 9397-9400.
9. Moya, E.; Blagbrough, I. S. *Tetrahedron Lett.* **1995**, *36*, 9401-9404.
10. Blagbrough, I. S.; Moya, E.; Walford, S. P. *Tetrahedron Lett.* **1996**, *37*, 551-554.
11. Blagbrough, I. S.; Carrington, S.; Geall, A. J. *Pharmaceutical Sci.* **1997**, *3*, 223-233.
12. Feuerstein, B. G.; Pattabiraman, N.; Marton, L. J. *Nucleic Acids Res.* **1990**, *18*, 1271-1282.
13. Behr, J.-P.; Demeneix, B.; Loeffler, J.-P.; Perez-Mutul, J. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6982-986.
14. Rowatt, E.; Williams, R. J. P. *J. Inorg. Biochem.* **1992**, *46*, 87-97.
15. Stewart, K. D.; Gray, T. A. *J. Phys. Org. Chem.* **1992**, *5*, 461-466.
16. Remy, J.-S.; Sirlin, C.; Vierling, P.; Behr, J.-P. *Bioconjugate Chem.* **1994**, *5*, 647-654.
17. Wilson, R. W.; Bloomfield, V. A. *Biochemistry* **1979**, *18*, 2192-2196.
18. Bloomfield, V. A. *Current Opinion in Structural Biology* **1996**, *6*, 334-341.
19. Ficker, E.; Taglialatela, M.; Wible, B. A.; Henly, C. M.; Brown, A. M. *Science* **1994**, *266*, 1068-1072.
20. Lopatin, A. N.; Makhina, E. N.; Nicholas, C. G. *Nature* **1994**, *372*, 366-369.
21. Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4910-4913; Nakanishi, K.; Goodnow, R.; Konno, K.; Niwa, M.; Bukownik, R.; Kallimopoulos, T. A.; Usherwood, P.; Eldefrawi, M. E. *Pure Applied Chem.* **1990**, *62*, 1223-1230; Choi, S.-K.; Nakanishi, K.; Usherwood, P. N. R. *Tetrahedron* **1993**, *49*, 5777-5790.
22. Carter, C. (ed.) *The Neuropharmacology of Polyamines*, Academic Press: London, 1994, pp. 1-318.
23. Bowie, D.; Mayer, M. L. *Neuron* **1995**, *15*, 453-462.
24. Huang, D.; Jiang, H.; Nakanishi, K.; Usherwood, P. N. R. *Tetrahedron* **1997**, *53*, 12391-12404.
25. Ganem, B. *Acc. Chem. Res.* **1982**, *15*, 290-298.
26. Bergeron, R. J. *Acc. Chem. Res.* **1986**, *19*, 105-113.
27. McCormick, K. D.; Meinwald, J. *J. Chem. Ecol.* **1993**, *19*, 2411-2451; Schäfer, A.; Benz, H.; Fiedler, W.; Guggisberg, A.; Bienz, S.; Hesse, M. *The Alkaloids* **1994**, *45*, 1-125.
28. Atwell, G. J.; Denny, W. A. *Synthesis* **1984**, 1032-1033; Saari, W. S.; Schwering, J. E.; Lyle, P. A.; Smith, S. J.; Engelhardt, E. L. *J. Med. Chem.* **1990**, *33*, 97-101; Krapcho, A. P.; Kuell, C. S. *Synth. Commun.* **1994**, *20*, 2559-2564.
29. O'Sullivan, M. C.; Dalrymple, D. M. *Tetrahedron Lett.* **1995**, *36*, 3451-3452; Xu, D.; Prasad, K.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **1995**, *36*, 7357-7360.
30. Imazawa, M.; Eckstein, F. *J. Org. Chem.* **1979**, *44*, 2039-2041.
31. Bergeron, R. J.; McManis, J. S. *J. Org. Chem.* **1988**, *53*, 3108-3111.
32. Guy-Caffey, J. K.; Bodepudi, V.; Bishop, J. S.; Jayaraman, K.; Chaudhary, N. *J. Biol. Chem.* **1995**, *270*, 31391-31396.
33. Geall, A. J.; Blagbrough, I. S. *Tetrahedron Lett.* **1998**, *39*, 443-446.