Sequential Polypeptides. Part VIII.[1] Synthesis of Sequential Polypeptides containing Arginine †

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The suitability of the *p*-tolylsulphonyl group for protecting the guanidino-system in the synthesis of arginine-containing sequential polypeptides by the 2-hydroxyphenyl ester and pentachlorophenyl ester methods has been demonstrated by syntheses of poly-(L-arginyl-L-alanyl-L-alanine) and poly-(L-arginylglycyl-L-proline).

HOMOPOLYARGININE [2-5] and some simple oligoarginines [6] have been prepared for use in experiments [7] connected with studies of histone–nucleic acid interactions, but no general method for the synthesis of sequential polypeptides containing arginine, which would also be valuable models for work in this area, has been developed. At the time the work described here was done the only preparation [8] of an arginine-containing sequential polypeptide known to us involved guanidination of the side-chain amino-groups of poly(leucylornithyl-leucine). Since, however, only partial conversion of ornithine residues into arginine could be achieved (the degree of conversion being especially poor with high molecular weight samples) and extensive racemisation occurred, this approach is of restricted value. We have therefore investigated synthetic routes in which arginine residues are incorporated as arginine from the outset. Since this work was completed, Russian workers [9] have reported the preparation of some polydi- and polytripeptides containing arginine with the use of the nitro-group for protecting the side-chain, but only a brief account of the procedures and characterisation of the products was given.

We first studied the synthesis of poly(arginylalanylalanine) (1). The close relationship between this and some optically pure polypeptides we had prepared earlier [10,11] *via* benzyloxycarbonyltripeptide 2-benzyloxyphenyl esters suggested that this approach might be appropriate. This strategy involves conversion of a benzyloxycarbonylpeptide 2-benzyloxyphenyl ester, suitably protected in the side-chain, into a peptide 2-hydroxyphenyl ester by acidolysis or hydrogenolysis:

H-(-Arg-Ala-Ala-)$_n$-OH (1)

Z-Arg(R^1R^2)-OR^3

(4) R^1 = Tos, R^2 = R^3 = H
(6) R^1 = R^2 = Tos, R^3 = Na
(7) R^2 = Tos, R^2 = H, R^3 = Pcp

Z-Arg(R^1R^2)-Ala-Ala-O·C_6H_4(2-OBzl)

(2) R^1 = NO_2, R^2 = H
(3) R^1 = R^2 = Adoc
(8) R^1 = Tos, R^2 = H

† All amino-acid residues mentioned are L and abbreviated nomenclature follows the recommendations of the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature (Chem. Soc. Specialist Periodical Reports on Amino-acids, Peptides and Proteins, 1970, vol. 2, ch. 5); in addition, Adoc = adamantyloxycarbonyl; Pcp = pentachlorophenyl.

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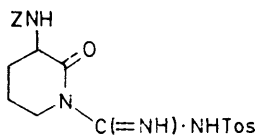
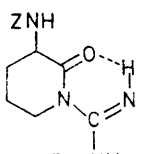
base-induced polymerisation of this activated intermediate followed by removal of the side-chain protecting group gives the required polymer. Extensive preliminary experiments on the possibility of using nitro [12] or adamantyloxycarbonyl [13] groups for protecting the guanidino-system in such an approach were uniformly unsuccessful. Neither of the critical intermediates (2) or (3) could be obtained pure, and appropriate deprotection–activation conditions [hydrogenolysis for (2) and acidolysis for (3)] followed by attempted polymerisation in the normal way gave, after treatment to remove side-chain protection, entirely dialysable products. We therefore turned our attention to the use of the tosyl group for protection,[14-16] the scope of which has recently been extended by the discovery [17] that it can be removed by treatment with liquid hydrogen fluoride.

We were dissatisfied with the convenience and yield of the procedure of Ramachandran and Li [14] for the preparation and purification of benzyloxycarbonyl-ω-tosylarginine (4) but were able to effect only a small improvement in the purification step. The crude acid contained several contaminants including the corresponding lactam (5) [15] and the ditosyl derivative which was characterised as its sodium salt (6). Of several methods of coupling the acid (4) to alanylalanine 2-benzyloxyphenyl ester which were examined, that *via* the pentachlorophenyl ester (7) was the most satisfactory. This active ester was never obtained quite pure, always being contaminated by the lactam (into which it was converted by treatment with base), but it coupled smoothly and the fully protected tripeptide (8) was obtained from it in pure condition without great difficulty.

Although Schabel and Li [15] reported that they never obtained any lactam when coupling the acid (4) by means of dicyclohexylcarbodi-imide, we find that this side-reaction invariably occurs to a significant extent with this and other common methods of activation. N.m.r. spectroscopy proved a convenient means of monitoring the presence of the lactam (5), since this



(5)

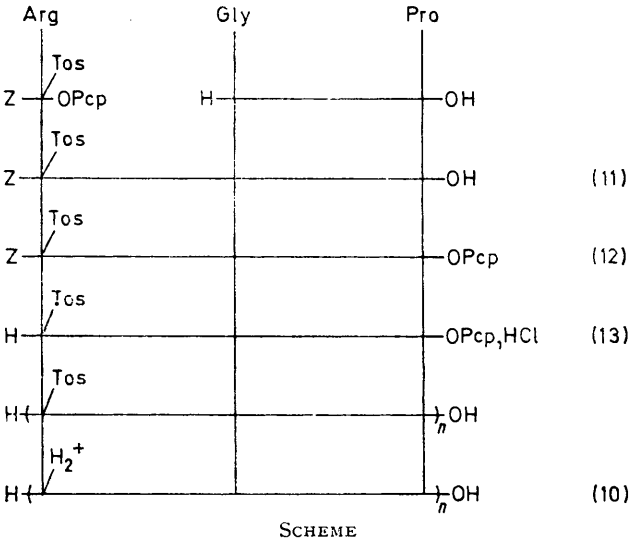


(9)

compound in deuteriochloroform gives a characteristic signal at τ 0·5 [probably due to the side chain NH deshielded by intramolecular hydrogen bonding as in (9)] which is not found in the spectra of any other ω-tosylarginine derivatives which we have examined. Also the sharp tosyl methyl group singlet at τ 7·61 is sufficiently downfield from the corresponding lines in the spectra of the acyclic compounds to be a useful indicator.

Simultaneous removal of the benzyloxycarbonyl and benzyl groups from (8) by means of hydrogen bromide in acetic acid or with hydrogen over palladium in acetic acid followed by polymerisation in dimethyl sulphoxide (concentrated solution) containing triethylamine gave the protected polymer: treatment with liquid hydrogen fluoride in the presence of anisole gave, after exhaustive dialysis and lyophilisation, the required polymer (1) as a white fluffy powder. Conventional analytical criteria gave satisfactory results although the weight average molecular weight was only *ca.* 3000—5000.

The second arginine-containing polymer investigated was poly(arginylglycylproline) (10). The presence of



SCHEME

proline here permitted a strategy involving formation of an active ester at the tripeptide level followed by $N(\alpha)$-deprotection and polymerisation. Since the pentachlorophenyl ester method had proved satisfactory for poly(alanylglycylproline),[18] this was the approach we chose: the tosyl group was again employed for protecting the guanidino-system (see Scheme). Although the active ester (7) gave only a mediocre yield, it provided a convenient means of preparing the tripeptide acid (11), which was converted into the corresponding pentachlorophenyl ester (12) without difficulty. In principle [16] hydrogen bromide in acetic acid should have been suitable for selective $N(\alpha)$-deprotection of (12) but in fact use of this reagent gave a two-component mixture which could not be resolved. We therefore turned to hydrogenolysis for this critical step. Johnson has described many examples [19] of hydrogenolytic removal of

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benzyloxycarbonyl substituents from pentachlorophenyl esters with t-butyl groups on the side-chains, using palladium–charcoal in methanol containg exactly 1 equiv. of hydrogen chloride. This procedure enabled essentially quantitative conversion of (12) into the partially protected active ester salt (13), which was obtained in pure condition. Polymerisation as in the first example, followed by detosylation, dialysis, and lyophilisation gave the polymer (10) as an analytically pure white fluffy powder with a weight average molecular weight in the region of 5000.

A tosyl substituent is therefore suitable for guanidino-group protection in conjunction with both 2-hydroxyphenyl esters and pentachlorophenyl esters, which are in our experience the two most satisfactory active esters for sequential polypeptide synthesis. Since the final step in the preparation of the activated tripeptide monomers can be hydrogenolysis in both of these approaches, the two examples described here provide in principle a general method for preparing sequential polypeptide histone models containing arginine together with any other amino-acid residues which occur frequently in histones.

EXPERIMENTAL

The general instructions given in Parts VII [1] and II [20] apply.

N(α)-*Benzyloxycarbonyl*-N(ω)-p-*tolylsulphonyl*-L-*arginine*.—This compound was prepared and purified at first *via* its cyclohexylammonium salt as described by Ramachandran and Li.[14] Yields of purified and chromatographically pure (TLC-3 R_F 0·70) product varied from 25 to 40%: it was obtained as a glass which could be crushed to a fine powder of indefinite m.p. (60—90°), which we were unable to crystallise (lit.,[14] m.p. 86—89°). In our hands the cyclohexylammonium salt was slow and difficult to crystallise and in later work it was found more convenient to purify the crude product by distribution between saturated sodium hydrogen carbonate solution and ethyl acetate. Variable amounts of white solid which separated at the interface were removed by filtration: washing with water and drying gave N(α)-*benzyloxycarbonyl*-N(ω)N(ω′)-*bis*-p-*tolylsulphonyl*-L-*arginine sodium salt*, m.p. 161—166°; τ [$(CD_3)_2SO$] 2·4—3·1 (16H, complex, all aromatic protons and NH groups), 4·97 (2H, s, PhCH_2), 5·9—6·3 (1H complex, NH·CH·CO), 6·7—7·2br (2H, NH·CH_2), 7·67 (3H, s, Me), and 7·9—8·5br (4H, CH_2·CH_2) (Found: C, 52·0; H, 5·0; N, 8·7; S, 9·75. $C_{28}H_{31}N_4NaO_8S_2$ requires C, 52·6, H, 4·9; N, 8·8; S, 10·0%). The aqueous layer was separated and acidified, and the precipitated oil was taken into ethyl acetate. Removal of solvent and trituration with light petroleum gave chromatographically pure non-crystalline acid in yields comparable to those obtained *via* the cyclohexylammonium salt. Attempts to obtain a crystalline dicyclohexylammonium salt failed.

N(α)*Benzyloxycarbonyl*-N(ω)-p-*tolylsulphonyl*-L-*arginine Lactam* (5).—Prepared by the method of Schnabel and Li,[15] this was obtained as a crystalline solid, m.p. 156—159° (lit.,[15] 156—157°); $\nu_{max.}$ ($CHCl_3$) 1720, 1700, and 1620 cm^{-1}; TLC-11 R_F 0·72; τ ($CDCl_3$) 0·55br (1H, s, C=NH), 1·97br (1H, s, TosNH), 2·1—3·0 (9H, complex, aromatic), 4·45 (1H, d, J 8 Hz, $PhCH_2$·O·CO·NH), 4·89 (2H, s, PhCH_2), 5·3—6·8 (3H, complex, NH·CH·CO and N·CH_2), 7·61 (3H, s, Me), and 7·8—8·7 (4H, complex, CH_2·CH_2·CH_2·N).

N(α)-*Benzyloxycarbonyl*-N(ω)-p-*tolylsulphonyl*-L-*arginine Pentachlorophenyl Ester*.—N(α)-Benzyloxycarbonyl-N(ω)-p-tolylsulphonyl-L-arginine (0·462 g, 1 mmol) and pentachlorophenol (0·266 g, 1 mmol) were dissolved in ethyl acetate (5 ml) and the solution was cooled to 0°. Dicyclohexylcarbodi-imide (0·206 g, 1 mmol) dissolved in ethyl acetate (5 ml) was added dropwise with stirring. The solution was stored at 0° overnight and then filtered. Removal of the ethyl acetate and trituration of the residue with petroleum gave crude active ester (0·67 g) contaminated with *ca.* 5—10% of the lactam (TLC-11 R_F 0·72; proportion estimated by n.m.r.) and traces of pentachlorophenol (TLC-11 R_F 0·35). This material, which was used in the coupling reactions described below, did not appear to undergo any chemical change at room temperature during a few weeks: t.l.c. investigations showed that the active ester was slowly converted into the lactam in solution. A small sample was reprecipitated several times by dripping a concentrated solution in ethyl acetate into a well-stirred large volume of petroleum to give the *active ester* as a white powder of indefinite m.p. (80—90°); $[\alpha]_D^{20}$ −2·1° (*c* 1 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1780, 1720, and 1620 cm^{-1}; TLC-11 R_F 0·56 (very slight traces of lactam and pentachlorophenol detectable); τ ($CDCl_3$) 2·2—3·0 (9H, complex, aromatic), 3·4—3·9br (3H, side-chain NH), 4·2 (1H, d, J 8 Hz, $PhCH_2$·O·CO·NH), 4·92 (2H, s, PhCH_2), 5·2—5·6 (1H, complex, NH·CH·CO), 6·4—7·1 (2H, complex, CH_2·NH), 7·61 (trace, s, lactam Me), 7·70 (3H, s, Me), and 7·8—8·6 (4H, complex, CH_2·CH_2·CH_2·NH) (Found: C, 46·0; H, 3·8; Cl, 24·7; N, 8·0; S, 4·8. $C_{27}H_{25}Cl_5N_4O_6S$ requires C, 45·6; H, 3·5; Cl, 25·0; N, 7·9; S, 4·5%).

Attempts were also made to prepare the corresponding *p*-nitrophenyl, trichlorophenyl, and succinimido esters by using dicyclohexylcarbodi-imide: all were obtained as grossly impure glasses which could not be purified.

N(α)-*Benzyloxycarbonyl*-N(ω)-p-*tolylsulphonyl*-L-*arginyl*-L-*alanyl*-L-*alanine* 2-*Benzyloxyphenyl Ester*.—t-Butoxycarbonyl-L-alanyl-L-alanine 2-benzyloxyphenyl ester [11] (0·44 g, 1 mmol) was treated with 90% trifluoroacetic acid as described elsewhere,[11] and the crude trifluoroacetate salt was dissolved with triethylamine (0·28 ml, 2 mmol) and the crude active ester described above (0·69 g, 1 mmol) in the minimum volume of dimethylformamide. After 36 h at room temperature, the mixture was diluted with ethyl acetate (50 ml), washed with N-hydrochloric acid, N-sodium hydrogen carbonate, and water, and dried. The solution was concentrated to a small volume and allowed to drip slowly into a large well-stirred volume of petroleum to give *protected tripeptide* as a white powder (0·63 g, 81%) of indefinite m.p. (100—120°); $[\alpha]_D^{20}$ −23·8° (*c* 1 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1770, 1720, and 1670 cm^{-1}; TLC-4 R_F 0·75; τ ($CDCl_3$) 2·0—3·3 (20H, complex, aromatic and peptide NH), 3·45—3·85br (3H, side-chain NH), 3·90 (1H, d, J 8 Hz, $PhCH_2O$·CO·NH), 4·96 and 5·02 (4H, s, s, both PhCH_2), 5·2—5·95br (3H, α-CH), 6·7—7·1br (2H, NH·CH_2), 7·69 (3H, s, CH_3·C_6H_4), and 7·9—8·9 (10H, complex, other protons) (Found: C, 60·8; H, 5·9; N, 10·4; S, 3·7. $C_{40}H_{46}N_6O_9S$ requires C, 61·1; H, 5·9; N, 10·7; S, 4·1%).

Poly-[N(ω)-p-*tolylsulphonyl*-L-*arginyl*-L-*alanyl*-L-*alanine*].—The fully protected tripeptide derivative described above (0·395 g, 0·5 mmol) was dissolved in acetic acid (0·2 ml), and hydrogen bromide in acetic acid (5·6N; 1 ml) was added.

[20] R. D. Cowell and J. H. Jones, *J.C.S. Perkin I*, 1972, 1809.

After 1 h at room temperature ether (50 ml) was added to precipitate the activated tripeptide, which was washed by trituration and decantation with ether (3 × 50 ml) and dried at 20° and 0·1 mmHg for 2 h. The residue was then dissolved in dimethyl sulphoxide (0·5 ml), triethylamine (0·14 ml, 1 mmol) was added, and the mixture was stirred. Within 24 h a viscous yellow mixture had formed and stirring was no longer possible. After 120 h the mixture was diluted with methanol (15 ml). The precipitate was collected by centrifugation and washed once with methanol and twice with ether to give *crude polymer* as a tan powder (0·103 g, 46%): $\eta_{sp.}/c$ 0·17 dl g^{-1} (*c* 0·50 in $CHCl_2{\cdot}CO_2H$), consistent [18,21] with a weight average molecular weight in the region 3000—5000; τ ($CF_3{\cdot}CO_2H$) 1·8—3·0 (10H, complex, aromatic, peptide NH, and side-chain NH), 4·95—5·55br (3H, all α-CH), 6·3—6·8br (2H, $CH_2{\cdot}NH$), 7·5 (3H, $CH_3{\cdot}C_6H_4$), and 7·6—8·8 (10H, complex, $CH{\cdot}CH_3$ and $CH_2{\cdot}CH_2{\cdot}CH$) [Found: C, 48·8; H, 6·4; N, 17·3. $(C_{19}H_{28}N_6O_5S)_n$ requires C, 50·4; H, 6·2; N, 18·6%].

Activation and N(α)-deprotection by hydrogenolysis as in previous examples,[11] followed by polymerisation as above, gave crude polymer with similar properties.

Poly-(L-*arginyl*-L-*alanyl*-L-*alanine*).— Poly-[*N*(ω)-*p*-tolylsulphonyl-L-arginyl-L-alanyl-L-alanine] (50 mg), anisole (0·07 ml), and anhydrous liquid hydrogen fluoride (5 ml) were kept for 1 h at 0°. The hydrogen fluoride was removed first with a water-pump for 1 h and then at 20° and 0·1 mmHg for 3 h. The residue was dissolved in acetic acid (1 ml) for removal from the reaction vessel and the solution was diluted with water (20 ml), filtered, and dialysed against water (5 l) for 30 h with changes of water every 10 h. Lyophilisation gave *polymer* as a fluffy white powder (27 mg, 82%); τ ($CF_3{\cdot}CO_2H$) 1·90—2·65br (3H, peptide NH), 3·2—4·25br (3H, side-chain NH), 5·05—5·65br (3H, α-CH), 6·4—6·85br (2H, $CH_2{\cdot}N$), 7·6—8·8 (13H, complex and s at 7·7, $CH_3{\cdot}CO_2H$, $CH_3{\cdot}CH$, and $CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}N$); amino-acid analysis: Arg 1·00, Ala 1·95. Gel chromatography estimates [22,23] of molecular weight gave $\bar{M}_n$ *ca.* 3000, $\bar{M}_w$ *ca.* 5000 [Found: C, 40·2; H, 7·4; N, 20·0. $(C_{12}H_{22}N_6O_3,CH_3CO_2H,3{\cdot}5H_2O)_n$ requires C, 40·0; H, 7·85; N, 20·0%].

N(α)-*Benzyloxycarbonyl*-N(ω)-p-*tolylsulphonyl*-L-*arginyl*-*glycyl*-L-*proline*.—Benzyloxycarbonylglycyl-L-proline (4·59 g, 15 mmol) was hydrogenated over palladium–charcoal (10%; 0·2 g) in acetic acid (90%; 150 ml) at atmospheric pressure for 4 h. The material obtained by filtration through Celite, evaporation, and addition and evaporation of benzene several times was dissolved in dimethylformamide (50 ml) together with triethylamine (4 g) and crude *N*(α)-benzyloxycarbonyl-*N*(ω)-*p*-tolylsulphonyl-L-arginine pentachlorophenyl ester (10·7 g). The mixture was stirred at room temperature for 24 h and then the dimethylformamide was removed. The residue was distributed between ethyl acetate and N-hydrochloric acid. The organic phase was washed with water and then extracted with N-sodium hydrogen carbonate. The sodium hydrogen carbonate extract was washed with ether and then acidified. The acidified aqueous solution was extracted with ethyl acetate. The ethyl acetate extract was washed with water and dried. Removal of the ethyl acetate and trituration with petroleum gave the required *protected tripeptide acid* (4·13 g, 50%) as a chromatographically pure glass which was crushed to a powder of indefinite m.p. (100—108°) from which the last traces of solvents could not be removed; $[\alpha]_D^{20}$ −33·6° (*c* 1 in $CHCl_3$); TLC-10 R_F 0·19; the n.m.r. spectrum was consistent with the required structure and purity apart from signals due to residual solvents (Found: C, 54·4; H, 6·0; N, 12·7; S, 5·2. $C_{28}H_{36}N_6O_8S$ requires C, 54·5; H, 5·9; N, 13·6; S, 5·0%).

N(α)-*Benzyloxycarbonyl*-N(ω)-p-*tolylsulphonyl*-L-*arginyl*-*glycyl*-L-*proline Pentachlorophenyl Ester*.—This was prepared in the usual manner from the above acid (3·5 g) and pentachlorophenol by using dicyclohexylcarbodi-imide with ethyl acetate–dioxan (3 : 1; 160 ml) as solvent. Reprecipitation from ethyl acetate by addition of petroleum gave *active ester* (4·1 g, 83%) as a powder of indefinite m.p. (90—110°); $[\alpha]_D^{20}$ −40·5° (*c* 1 in $CHCl_3$); TLC-11 R_F 0·50; $\nu_{max.}$ (Nujol) 1780, 1720, and 1650 cm^{-1}; the n.m.r. spectrum was consistent with the required structure apart from signals from residual solvent (Found: C, 47·7; H, 4·3; Cl, 19·3; N, 9·3; S, 3·3. $C_{34}H_{35}Cl_5N_6O_8S$ requires C, 47·2; H, 4·1; Cl, 20·5; N, 9·7; S, 3·7%).

N(ω)-p-*Tolylsulphonyl*-L-*arginylglycyl*-L-*proline Pentachlorophenyl Ester Hydrochloride*.—The above fully protected active ester (3·00 g, 3·46 mmol) was dissolved in pure dry methanol (200 ml) and a solution of hydrogen chloride in methanol (7·58 mg ml^{-1}; 17·0 ml, 3·50 mmol) was added. Hydrogenation over palladium–charcoal (10%; 830 mg) was continued at atmospheric pressure and room temperature until uptake ceased and t.l.c. indicated complete consumption of starting material (*ca.* 2 h). Filtration through Celite, removal of solvents, and reprecipitation from methanol by addition of ether gave the *active ester salt* (2·3 g, 87%) as a powder, m.p. 163—173°, $[\alpha]_D^{20}$ −37·2° (*c* 1 in $CHCl_3$) (Found: C, 40·8; H, 4·3; Cl, 27·3; N, 10·7; S, 4·3. $C_{26}H_{30}Cl_6N_6O_6S$ requires C, 40·7; H, 3·9; Cl, 27·7; N, 10·95; S, 4·2%).

Poly-(L-*arginylglycyl*-L-*proline*).—The active ester salt described above (760 mg, 1 mmol) was dissolved in dimethyl sulphoxide (0·75 ml), *N*-methylmorpholine (0·2 ml) was added, and the mixture was stirred at room temperature for 5 days. Ethanol (50 ml) was added and the crude polymer (389 mg, 84%) which separated was isolated by filtration and washing with more ethanol and finally with ether. Crude protected polymer (330 mg) was treated with liquid hydrogen fluoride (20 ml) in the presence of anisole (3 ml) for 1 h at 0°. The residue remaining after evaporation of the hydrogen fluoride was removed from the reaction vessel with 50% aqueous trifluoroacetic acid (50 ml). The solution was washed with ether (2 × 25 ml) and then evaporated. The residue was dissolved in water (10 ml) and dialysed against water (4·5 l) for 24 h with changes after 2, 4, and 8 h. Lyophilisation gave *polymer* (75 mg, *ca.* 25%) as a white fluffy powder: amino-acid analysis Arg 0·95, Gly 1·00, Pro 1·03; τ ($CF_3{\cdot}CO_2H$) 1·8—2·2br (2H, peptide NH), 3·0—4·5vbr (3H, side-chain NH), 4·9—6·8 (8H, complex, α-protons, $N{\cdot}CH_2$, and $NH{\cdot}CH_2$), and 7·3—8·3br (8H, other CH_2) (integration was satisfactory and no impurity or unexpected peaks were detected) [Found: C, 40·4; H, 6·1; N, 19·5. $(C_{13}H_{23}N_6O_3,CF_3{\cdot}CO_2H,H_2O)_n$ requires C, 40·6; H, 5·7; N, 19·0%]. Gel chromatography estimates [22,23] of molecular weight gave $\bar{M}_n$ *ca.* 3000, $\bar{M}_w$ *ca.* 5000.

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