Synthesis of *C*- and *N*-Functionalised Derivatives of 1,4,7-Triazacyclononane-1,4,7-triyltriacetic acid (NOTA), 1,4,7,10-Tetra-azacyclododecane-1,4,7,10-tetrayltetra-acetic Acid (DOTA), and Diethylenenetriaminepenta-acetic Acid (DTPA): Bifunctional Complexing Agents for the Derivatisation of Antibodies

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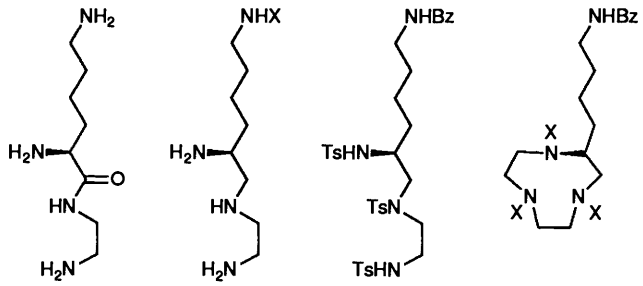
Using (2*S*)-lysine as a precursor, the syntheses of aminobutyl derivatives of 1,4,7-triazacyclononane, 1,4,7,10-tetra-azacyclododecane, and 3-azapentane-1,5-diamine are described. Transformation into their reactive maleimide derivatives is described and alternative strategies for synthesising the title complexing agents involving nitrogen functionalisation are defined.

An essential feature of tumour targeting with radiolabelled monoclonal antibodies is that the radioisotope should be irreversibly bound to the protein. If this condition is met then the tissue specificity and the biological half-life of the antibody *in vivo* and the physical half-life of the decaying radioisotope will determine the total radiation dose delivered to both cancerous and healthy cells.[1] If this condition is not met—most importantly with β^--emitting radioisotopes—then the premature release of the radionuclide invariably leads to localisation of the activity in sensitive organs, *e.g.* kidney, liver, and the bone-marrow. This latter process can result in lethal radiotoxic effects, such as myelosuppression. The most common approach used to radiolabel antibodies has involved the modification of the protein with bifunctional complexing agents.[2] Initial work concentrated on the attachment of acyclic chelating agents such as *C*-functionalised EDTA[3] or DTPA.[4] It has become increasingly apparent that the complexes of these ligands (*e.g.* with ^{111}In[γ; $t_{\frac{1}{2}}$ 2.83 d], ^{67}Ga[γ; $t_{\frac{1}{2}}$ 3.25 d], ^{67}Cu-[β^-; $t_{\frac{1}{2}}$ 61.5 h], and 90Y[β^-; $t_{\frac{1}{2}}$ 64 h]) are insufficiently stable *in vivo*. Acid catalysed dissociation and/or cation promoted release of the radioisotope occurs and is revealed by careful analysis of radiolabel biodistribution data.[5–7]

The introduction of macrocyclic complexing agents has led to much more promising results. With ^{67}Cu (and ^{64}Cu—a β^+ emitter of potential use in positron emission tomography), use of the ligand TETA (1,4,8,11-tetra-azacyclotetradecane 1,4,8,11-tetrayltetra-acetic acid) has led to some improvements in stability *in vivo* compared to EDTA and DTPA.[2,6] Much more robust are complexes of cyclam (1,4,8,11-tetra-azacyclotetradecane) and several *C*-functionalised cyclam derivatives have been prepared and their stability *in vivo* demonstrated unequivocally.[8,9] With indium and gallium, 1,4,7-triazacyclononane-1,4,7-triyltriacetic acid (NOTA) forms strong complexes, and the stability of these parent complexes with respect to acid-catalysed dissociation has been demonstrated both *in vitro*[10] and *in vivo*.[11] Similar studies have shown that ^{90}Y may be effectively bound by the macrocyclic complexing agent DOTA (1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetrayltetra-acetic acid).[12,13] Accordingly, we have synthesised a set of functionalised derivatives of these parent macrocyclic ligands bearing aminoalkyl sidechains to permit protein linkage. Both *C*- and *N*-substituted ligands have been prepared in expeditious syntheses that are amenable to scale-up to gram quantities. The synthesis of maleimide derivatives in particular is reported, although the thiol-specific vinylpyridine derivatives—among others—may also be prepared in an analogous manner. For purposes of comparison, a *C*-functionalised DTPA compound was also prepared. Parts of this work have been reported in a preliminary manner,[10,12] and fuller details of the kinetic stability of both the radiolabelled macrocyclic complexes and of the antibody–macrocycle conjugates *in vivo* will be discussed subsequently in separate publications.[11,13]

Synthesis of C-Functionalised NOTA, DOTA, and DTPA.—The amino acid (2*S*)-lysine was used as the starting material for the syntheses of both the 9- and the 12-membered ring ligands. An important feature of this approach was to protect selectively the ε-amino group, permitting cyclisation reactions with polyamine functionality introduced at the chiral centre. Reaction of the methyl ester of lysine (as the dihydrochloride) with warm neat ethylenediamine (in excess) afforded the monoamide (**1**). Under these conditions, the competitive formation of the 7-ring lactam by intramolecular cyclisation was suppressed, although it did occur with other solvents present, *e.g.* methanol. The



(1) (2) X = H (3) X = Bz (4) (5) X = Ts (6) X = H (7) X = CH_2CO_2H (8) X = CH_2CO_2Et

amide was reduced with borane–tetrahydrofuran to yield the tetra-amine (**2**) and reaction with copper(II) ions (as basic copper carbonate) in aqueous solution generated the blue 'diethylenetriamine'–copper(II) complex. The stability constant of the 1:1 complex is log K = 16.1 (298 K),[14] so the three

nitrogens are effectively protected from electrophilic acylation. The remote amino group may bind weakly to copper (although the UV–visible spectrum of $[(2)\cdot Cu]^{2+}$ was almost identical with that of [diethylenetriamine–$Cu]^{2+}$ recorded under the same conditions) but an 8-membered chelate ring is generated by such an interaction which is disfavoured both entropically and enthalpically. Reaction with benzoyl chloride in the presence of base permitted acylation of the remote amino group and treatment of the copper complex with H_2S permitted isolation of the free benzamide (**3**) (58%). Tosylation (TsCl, Et_3N, CH_2Cl_2) afforded the key intermediate (**4**).

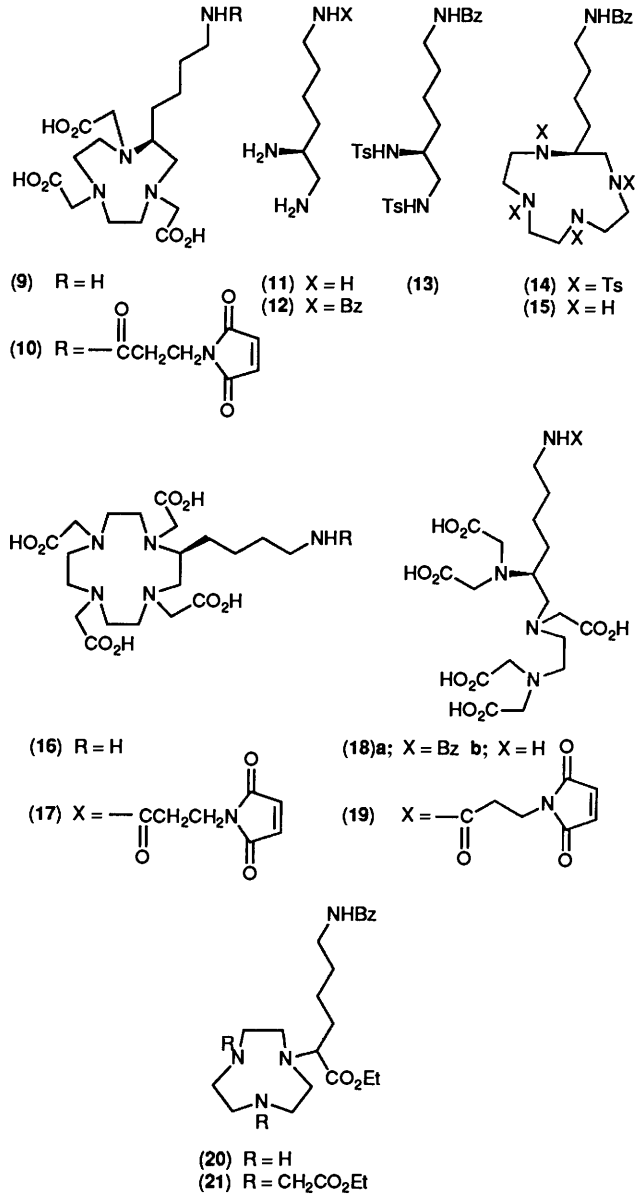
Co-condensation of this tritosylamide with ethylene glycol ditosylate under standard conditions (Cs_2CO_3, DMF, 65 °C, 18 h) afforded the 9-membered ring compound (**5**) in 71% yield. Detosylation may be achieved using 98% sulphuric acid to give the triamine (**6**). An alternative detosylation procedure under reductive conditions (Li, NH_3, MeOH–THF) also yielded the desired amine, but was accompanied by some amide reduction (*ca.* 12%) to give an *N*-benzyl tetra-amine. Some modified sulphonamide protecting groups were also examined; the tri(*p*-methoxybenzenesulphonyl) analogue of (**5**) was prepared and deprotection was possible with hydrogen bromide in acetic acid in the presence of phenol (110 °C, 48 h, 70%). No advantage in using this method was perceived although the enhanced sensitivity of the *p*-methoxybenzenesulphonamide to acid deprotection (HBr–AcOH in lieu of concentrated H_2SO_4) may prove useful in other situations, and has been reported previously.[15] The introduction of carboxymethyl groups was effected in two ways: reaction of (**6**) with ethyl bromoacetate (Cs_2CO_3, EtOH) afforded the triester (**8**) (77%) which was purified by alumina chromatography, while direct alkylation of (**5**) with bromoacetic acid (pH *ca.* 10) gave the triacid (**7**) which required ion-exchange (Sepharose DEAE) followed by reverse-phase chromatography before pure product could be obtained (79%). Acid hydrolysis (6M HCl, 48 h) of (**7**) or (**8**) gave the amino acid (**9**) quantitatively.

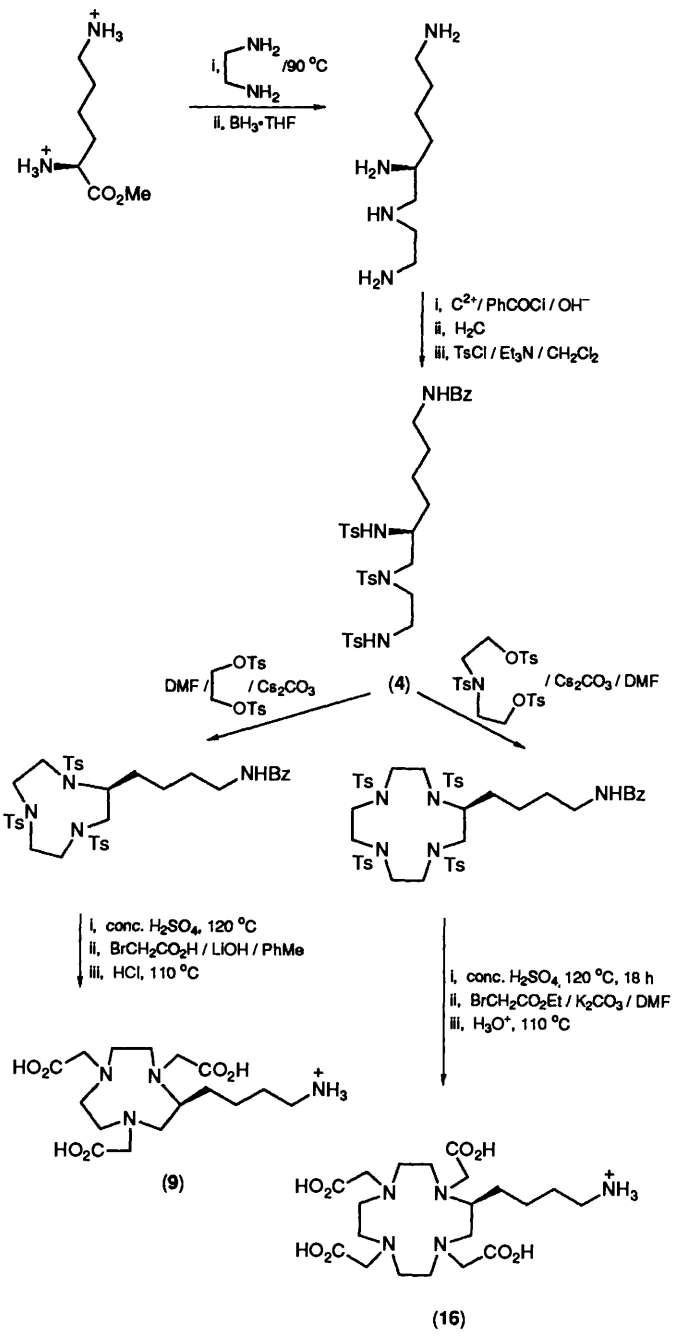
An alternative synthetic route to the cyclic tritosylamide (**5**) was also considered and evaluated. Reduction of lysine amide with borane–tetrahydrofuran gave the triamine (**11**), and selective protection of the ethylenediamine moiety using Cu^{2+} in aqueous solution permitted the benzoylation of the ε-amino group to yield (**12**) (49%). Tosylation of (**12**) afforded the ditosylamide and co-cyclisation with *N*-toluene-*p*-sulphonyl-1,5-bis(toluene-*p*-sulphonato)-3-azapentane (Cs_2CO_3, DMF) gave the cyclic tritosylamide (35%). The slowness of the amide reduction step (180 h) and the inferior cyclisation yield with this alternate route clearly indicated that the initially described method was preferred (Scheme 1).

The synthesis of *C*-functionalised EDTA and DPTA complexing agents is also permissible with this synthetic strategy. Reaction of (**3**), for example, with bromoacetic acid in a two-phase toluene–aqueous base system[4] afforded the penta-acid (**18**). This was purified by anion-exchange chromatography (52% recovered yield) and acidic hydrolysis (6M HCl, 110 °C, 48 h) yielded the amino acid (**18**) quantitatively. Other methods of introducing the desired carboxymethyl substituents of (**18**) were tested (*e.g.* the use of $BrCH_2CO_2H$ or $ClCH_2CO_2H$ with LiOH in H_2O, $BrCH_2CO_2Me$–K_2CO_3–DMF at 80 °C, or $BrCH_2CO_2Et$–Cs_2CO_3, or K_2CO_3–EtOH at 70 °C) but none gave satisfactory results and mixtures of unidentified (partially alkylated) products resulted.

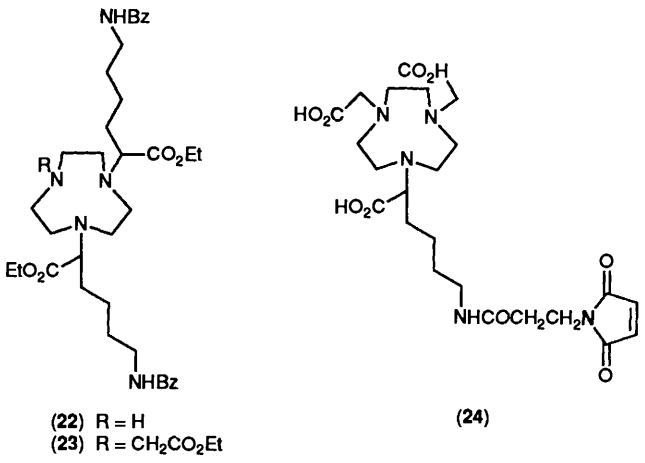
Finally in this series, co-condensation of (**4**) with *N*-toluene-*p*-sulphonyl-1,5-bis(toluene-*p*-sulphonato)-3-azapentane under standard conditions (Cs_2CO_3–DMF) yielded the tetratosylamide (**14**) in 72% yield. Detosylation using either 98% H_2SO_4 or Li–NH_3–THF (1% MeOH) at −78 °C gave the tetra-amine (**15**) (*ca.* 55%), with the reductive deprotection again accompanied by about 10% amide reduction [^{1}H NMR analysis of the benzylic singlet at $\delta_H(CDCl_3)$ 3.75 ($PhCH_2NH$) compared to the doublet of triplets observed for the $PhCONHCH_2$ at 3.46 ppm]. Alkylation of (**15**) may be effected either with $BrCH_2CO_2H$ in the aqueous–toluene two-phase reaction described for (**18**) or by reaction with ethyl bromoacetate in DMF in the presence of potassium carbonate. Acid hydrolysis of either product afforded the amino acid (**16**).

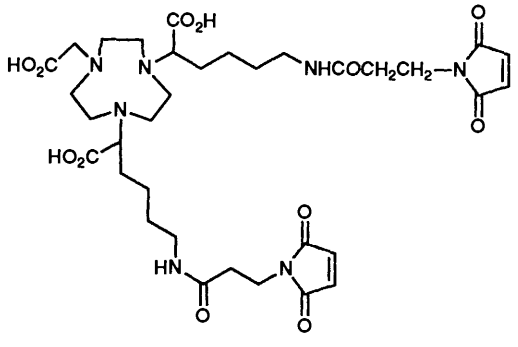
In order to link the aminoalkyl substituted complexing agents (**9**), (**16**), and (**18**) to a protein, a bifunctional linker molecule was sought which could permit formation of an amide bond to the exocyclic amine and leave a reactive group for antibody linkage, *e.g.* to the ε-amino groups of lysine residues. As our initial work was concerned with attachment to thiol residues on the protein (introduced either by reaction of the antibody with 2-iminothiolane or by using a recombinant antibody with a cysteine residue engineered close to the CH 1 domain) then bifunctional maleimides and vinylpyridine linking molecules were considered. Details of the thiol specificity of these vinylpyridine linkers have been reported elsewhere[8] and the preparation of vinylpyridine conjugates of (**9**) and (**16**) has been described.[16] The use of active esters (*e.g.*

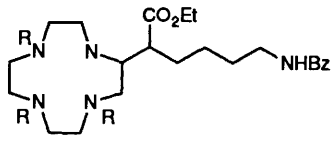


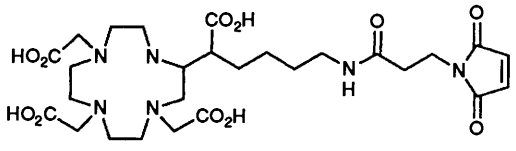


Scheme 1.



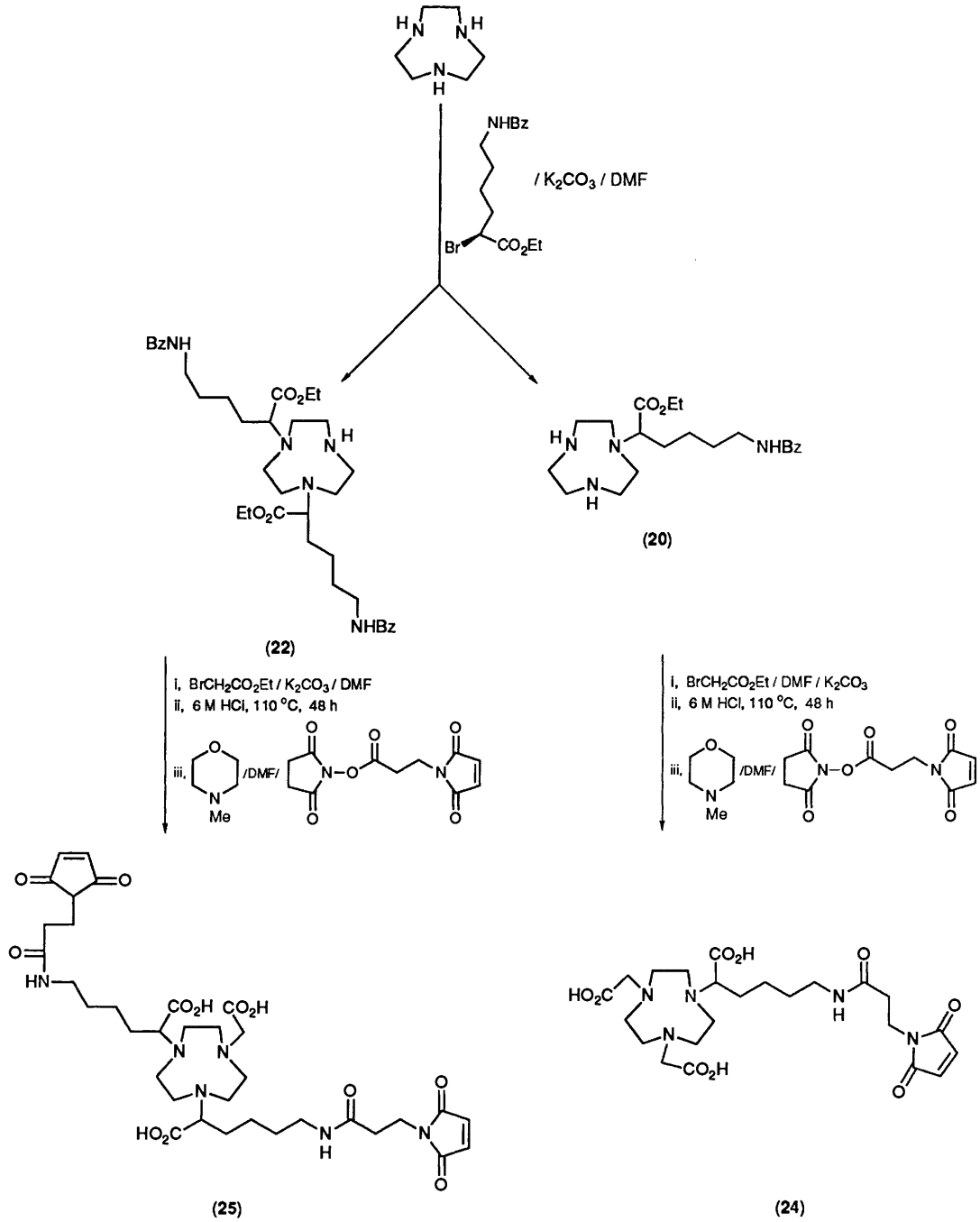






N-hydroxysuccinimide) of *N*-substituted maleimides as bifunctional linker molecules was first reported by Kitagawa.[17] A typical ester is 3-maleimidopropionic acid *N*-hydroxysuccinimide ester (MPHS) and this was allowed to react with (**9**), (**16**), and (**18**) under standard conditions (*N*-methylmorpholine, DMF or DMSO; 20 °C) to yield the desired maleimide-substituted complexing agents, (**10**), (**17**), and (**19**). Reaction was monitored by HPLC and the maleimides were purified by reverse-phase HPLC.

Synthesis of N-*Functionalised NOTA and DOTA*.—Shorter syntheses of functionalised macrocyclic ligands are possible if linkage through one of the ring nitrogens is used. Furthermore, disubstitution of two of the nitrogens in the 9-membered ring series permits the synthesis of a complexing agent with two linkage points, *i.e.* a cross linking agent. In the 12-membered ring series, such disubstituted compounds may also be prepared but two constitutionally isomeric compounds result which are difficult to separate, *i.e.* 1,4 and 1,7 disubsituted macrocycles. This reaction has not been attempted. Alkylation of 1,4,7-triazacyclononane with racemic 6-benzamido-2-bromohexanoate[18] in DMF in the presence of potassium carbonate afforded a 1:2 mixture (using a 1:1 reaction stoicheiometry) of the mono- and di-alkylated compounds (**20**) and (**22**). Using a larger excess of the free amine, increased amounts of the monoalkylated compound were readily obtained. The separation of (**20**) and (**22**) was achieved by cation exchange HPLC, although no separation of the diastereoisomers [*RR*, *SS*, and *RS*] was attempted. Reaction with ethyl bromoacetate (K_2CO_3–DMF) yielded the



Scheme 2.

triesters (**21**) and (**23**) which were separable by careful chromatography on alumina, obviating the need for the previous separation of (**20**) and (**21**) by HPLC. Acid hydrolysis (6M HCl, 48 h) followed by reaction with the maleimide active ester MPHS afforded the desired compounds (**24**) and (**25**) which were purified by reverse phase HPLC. Using controlled column conditions (see Experimental section) retention times of 10.5 min for (**25**) (2 closely spaced peaks due to the diastereoisomers *RR*, *SS*, and *RS*) and 8.7 min for (**24**) indicated that separation of mono- and di-alkylated material could realistically be withheld until this final step, providing a quicker route to the target compounds (Scheme 2).

In the 12-membered ring series, alkylation of 1,4,7,10-tetraazacyclododecane with ethyl (±)-6-benzamido-2-bromohexanoate afforded the monoalkylated compound, (**26**), in 40% yield (K_2CO_3–DMF for 64 h). Alkylation with ethyl bromoacetate gave the tetraester (**27**) (63%) and hydrolysis followed by reaction with the maleimide active ester MPHS (DMSO, *N*-methylmorpholine, 20 °C) yielded the maleimide functionalised macrocycle (**28**).

Conclusions

The syntheses of the *C*-substituted complexing agents are relatively short and amenable to structural variation. In principle, any chiral polyazamacrocycle (9- to 36-membered ring) bearing aminoalkyl functionality may be prepared using obvious variations of this strategy. The routes to the *N*-

substituted compounds are shorter but rely on using the parent polyazamacrocycle as a precursor: these are not always readily available. Although the compounds prepared in this work were attached to monoclonal antibodies for use in tumor targeting, they could equally easily be linked at specific sites[19,20] to oligonucleotides or DNA using phosphorothioate esters.

Experimental

M.p.s were determined on a Kofler block and are uncorrected. Column chromatography was carried out using either 'gravity' silica (Merck Art 7734), 'flash' silica (Merck Art 9385), or neutral alumina (Merck Art 1077) which had previously been treated with ethyl acetate. Analytical and semi-preparative HPLC was performed with a Varian Vista 5500/Polychrome 9060 instrument fitted with either cation exchange ('Synchropak' CM 300), anion exchange ('Synchropak' AX 100) or reverse phase columns ('Spherisorb' 5 ODS2). Flow rates of 1.4 ml min^{-1} and 4.0 ml min^{-1} were used for analytical and semi-preparative columns respectively. Column and gradient elution conditions were as follows: cation exchange, t = 0 min, 80% H_2O, 0% aqueous ammonium acetate (1.0M, pH 5.6), 20% MeCN; t = 5 min, 60% H_2O, 20% ammonium acetate, 20% MeCN; t = 10 min, 0% H_2O, 80% ammonium acetate, 20% MeCN. For anion exchange: t = 0 min, 70% H_2O, 10% ammonium acetate, 20% MeCN; t = 20 min, 0% H_2O, 80% ammonium acetate, 20% MeCN. For reverse phase: t = 0 min, 95% H_2O, 0% ammonium acetate, 5% MeCN, t = 20 min, 5% H_2O (0.1% trifluoroacetic acid) 0% ammonium acetate, 95% MeCN (0.1% trifluoroacetic acid). Solvents used were dried from an appropriate drying agent, and water was purified by the Milli Q system. IR spectra were recorded with a Perkin–Elmer 577 spectrometer, ^1H and ^{13}C NMR spectra were obtained with a Bruker AC 250 operating at 250.13 and 62.90 MHz, respectively. Mass spectra were recorded with a VG 7070E spectrometer operating in CI, DCI, or FAB modes with DCI samples presented as dilute CH_2Cl_2 or MeOH solutions and ammonia as the impingent gas. Optical rotations were recorded on Optical Activity AA-10 automatic polarimeter. All reactions were carried out under an atmosphere of dry nitrogen.

(2S)-N-(2-*Aminoethyl*)-2,6-*diaminohexanamide* (**1**).—(+)-(2*S*)-Lysine methyl ester hydrochloride (7.09 g, 30.4 mmol) was added in small batches over 2 h to ethylenediamine (100 ml) at 90 °C, with continuous stirring. The reaction mixture was heated to 120 °C (6 h) and the ethylenediamine removed by distillation under reduced pressure. The residue was taken into aqueous sodium hydroxide (4M; 25 ml), the solvent was evaporated, and the residue was dissolved in methanol (30 ml). The solution was filtered and the filtrate evaporated to leave a residue; this process was then repeated from the methanol stage. The subsequent residue was treated with dichloromethane (100 ml), filtered, and the filtrate evaporated to give a clear pale yellow oil (5.32 g, 93%) of the *title compound* (Found: M^+, 188.1640. $C_8H_{20}N_4O$ requires 188.1637); ν_{max}(film) 3 340br (NH), 3 280br s (NH), 1 650 (CO), and 1 560 cm^{-1} (NH); $\delta_H(CDCl_3)$ 1.37–1.60 (10 H, m, NH_2CH_2), 1.86 (2 H, m, CH_2), 2.71 (2 H, t, J 6.5 Hz, NCH_2), 2.83 (2 H, t, J 6.0 Hz, NCH_2), 3.33 (2 H, t, J 5.7, CH_2NHCO), 3.38 (1 H, t, J 3.8 Hz, CH), and 7.56 (1 H, br s, CONH); $\delta_C(D_2O)$ 22.2, 31.4, 34.0 (CH_2), 39.6, 40.2, 41.4, 42.7 (NCH_2), 54.6 (CH), and 178.0 (CO); m/z (DCI) 189 (M^+ + 1), 188 (M^+), 171 (M^+ − NH_3), 156, and 129.

(5S)-3-*Azanonane*-1,5,9-*triamine* (**2**).—The amide (**1**) (3.75 g, 20.0 mmol) was refluxed in borane–THF (130 ml, 130 mmol) for 20 h. Excess of borane was quenched with methanol (100 ml) and solvents were evaporated to leave a residue which was heated to reflux in hydrochloric acid (6M; 60 ml) for 3 h. Evaporation of solvent, entraining in methanol (2 × 20 ml), and finally slow evaporation of an ethanol solution (1% conc. HCl) gave the *title compound* as a hygroscopic white tetrahydrochloride salt (6.28 g, 98%) (Found: M^+, 174.1850. $C_8H_{22}N_4$ requires 174.1849); ν_{max}(film) 3 300br cm^{-1} (NH, NH_2); $\delta_H(D_2O)$ 1.46–1.58 (2 H, m, $CH_2CH_2CH_2$), 1.66–1.92 (4 H, m, $CH_2CH_2CH_2$), 3.01 (2 H, t, J 7.5 Hz, NCH_2), 2.70–2.79 (1 H, m, NCHH), 3.38–3.53 (5 H, m, NCHH, NCH_2), and 3.71 (1 H, quin, J 6.4 Hz, CH); $\delta_C(D_2O)$ 21.1, 26.1, 29.5 (CH_2), 35.2, 38.7, 44.8, 48.4, and 48.9 (CH, NCH_2); m/z (DCI) 175 (M^+ + 1), 174 (M^+), 157 (M^+ − NH_3), and 133.

(5S)-N-(5,9-*Diamino*-7-*azanonyl*)*benzamide* (**3**).—The tetra-amine tetrahydrochloride (**2**) (13.8 g, 43.0 mmol) was dissolved in water (100 ml) and converted into the free tetra-amine by addition of potassium hydroxide (9.65 g, 172 mmol). Addition of copper carbonate (5.71 g, 25.8 mmol) to the stirred solution at 50 °C gave an intense blue colour. After continued stirring (30 min, 50 °C), benzoyl chloride (6.5 ml, 55.9 mmol) was added over 1 h to the cooled solution (0 °C), the pH being maintained above 9 with periodic addition of potassium hydroxide pellets. The solution was allowed to warm to room temperature and stirring was continued for 1 h. The dark solution was filtered and the filtrate treated with hydrogen sulphide over 30 min. Filtration followed by partial evaporation and exhaustive extraction with dichloromethane, with subsequent drying and evaporation of the combined extracts, gave the *title compound* as a pale yellow oil (6.40 g, 54%) [Found: (M^+ + 1), 279.2020. $C_{15}H_{27}N_4O$ requires 279.2027]; ν_{max}(film) 3 300br (NH, NH_2, CONH), 3 060, 1 640 (CO), and 1 600 cm^{-1}; $\delta_H(CDCl_3)$ 1.15–1.45 (6 H, m, CH_2), 1.64 (5 H, br s, NH + NH_2), 2.29 (1 H, dd, J 12, 8.7 Hz, CH), 2.51–2.74 (6 H, m, NCH_2), 3.37 (2 H, dt, J 2 × 6.5 Hz, $CONHCH_2$), 6.38 (1 H, br t, CONH), 7.39–7.50 (3 H, m, ArH), and 7.76 (2 H, AB system, J_{AB} 6.6 Hz *ortho* ArH); $\delta_C(CDCl_3)$ 23.4, 29.5, 35.6 (CH_2); 39.7, 41.6, 50.8, 52.4, 56.4 (CH, NCH_2), 126.8, 128.3, 131.1; 134.7, and 167.5 (CO); m/z (DCI) 280 (M^+ + 2) and 279 (M^+ + 1).

(5S)-N-(5,9-*Bistoluene*-p-*sulphonamido*-7-*toluene*-p-*sulphonyl*-7-*azanonyl*)*benzamide* (**4**).—A solution of the benzamide (**3**) (1.82 g, 6.55 mmol) in dichloromethane (35 ml) was added dropwise to a solution of toluene-p-sulphonyl chloride (4.48 g, 23.6 mmol) and triethylamine (2.38 g, 23.6 mmol) in dichloromethane (50 ml) with stirring over a period of 1 h. The reaction mixture was stirred at room temperature for 12 h, after which it was washed with distilled water (25 ml), dried (K_2CO_3), filtered and evaporated under reduced pressure. The brown residue was redissolved in dichloromethane (12 ml) and after several minutes a white precipitate resulted. The solid was collected by filtration, washed with cold dichloromethane (2 × 5 ml), and dried *in vacuo* (10^{-2} mmHg) (3.3 g, 68%); R_F 0.4 [silica gel; CH_2Cl_2–MeOH (95:5)]; m.p. 149–151 °C (Found: C, 58.1; H, 5.98; N, 7.25. $C_{36}H_{44}N_4O_7S_3$ requires C, 58.4; H, 5.94; N, 7.56%); m/z (DCI): 741 (M^+ + 1), 740 (M^+), and 373; $\delta_H(CDCl_3)$ 7.83 (2 H, d, J 7.1 Hz, aromatic H), 7.72 (4 H, d, J 7.9 Hz, part of AA′XX′ system, aromatic H), 7.61 (2 H, d, J 8.1 Hz, part of AA′XX′ system, aromatic H), 7.44 (3 H, m, aromatic H), 7.31 (4 H, d, J 8.0 Hz, part of AA′XX′ system, aromatic H), 7.18 (2 H, d, J 8.1 Hz part of AA′XX′ system, aromatic H), 6.49 (1 H, br t, NHCO), 5.35 (1 H, br t, NHTS), 5.30 (1 H, d, J 6.3, NHTs), 3.40 (2 H, br m, CH_2NHCO), 3.30 (2 H, br m, CH_2N), 3.17 (5 H, m, CH_2N and CHN), 2.44 (6 H, s, CH_3), 2.32 (3 H, s, CH_3), 1.44 (2 H, m, CH_2C), 1.25 (2 H, m, CH_2C), and 1.06 (2 H, m, CH_2 − C); ν_{max}(KBr) 3 290br (NH), 3 060, 3 030 (CH), 1 642 (CO), 1 600 (aromatic ring), 1 580, 1 530 (NH), 1 320 (SO_2), and 1 160 cm^{-1} (SO_2).

2-(4-*Benzamidobutyl*)-1,4,7-*tris*(*toluene*-p-*sulphonyl*)-1,4,7-*triazacyclononane* (**5**).—Caesium carbonate (3.26 g, 10 mmol)

was added to a solution of the tritosylamide (**4**) (3.40 g, 5.0 mmol) in anhydrous DMF (200 ml) under dry nitrogen. A solution of ethylene glycol bis(toluene-*p*-sulphonate) (1.85 g, 5.0 mmol) in anhydrous DMF (50 ml) was added slowly over a period of 4 h, with efficient stirring. After the mixture had been stirred for 12 h (20 °C), its temperature was raised to 65 °C for 3 h. Solvent was removed under reduced pressure and the residue dissolved in chloroform (200 ml) and washed with water (3 × 50 ml). The organic layer was dried (K_2CO_3) filtered, and evaporated under reduced pressure. The pale brown residue was dissolved in the minimum volume of dichloromethane (15 ml) and ethanol added until a turbidity remained. The mixture was cooled to −20 °C for 12 h to give a colourless glassy solid (2.7 g, 71%), m.p. 106–108 °C (Found: C, 59.8; H, 5.88; N, 7.02. $C_{38}H_{46}N_4O_7S_3$ requires C, 59.5; H, 6.00; N, 7.31%); *m/z* (DCI) 768 ($M^+ + 2$), 767 ($M^+ + 1$), 611, 570, 457, 395, 373, and 301; $[\alpha]_D^{20}$ +9.2 (*c* 1.0 in CH_2Cl_2); $\delta_H(CDCl_3)$ 7.85–7.62 (8 H, m, ArH), 7.49–7.28 (9 H, m, aromatic), 6.46 (1 H, br s, NHCO), 3.77–2.98 (13 H, m, CH_2N + CHN), 2.46, 2.44, 2.42 (9 H, s + s + s, CH_3), and 1.64–1.20 (6 H, br m, CH_2C); $\delta_C(CDCl_3)$ 167.5 (CONH), 144.1, 143.8, 136.9, 134.5, 134.3, 134.2, 131.3, 129.9, 128.5, 127.6, 127.5, 127.3, 126.9 (arom.), 59.5 (CHN), 53.7, 52.6, 50.8, 46.0 (CH_2N), 39.0 (CH_2NHCO), 29.3, 28.9, 23.8 (CH_2C), and 21.5 (CH_3).

2-(4-*Benzamidobutyl*)-1,4,7-*triazacyclononane* (**6**).—The tritosylamide (**5**) (0.91 g, 1.19 mmol) was treated with concentrated sulphuric acid (10 ml) at 115 °C with stirring for 36 h. To the cooled solution (0 °C; ice bath) was added aqueous NaOH (30%; 5 ml). The sodium sulphate precipitate was filtered off and washed with distilled water (3 ml). The combined filtrate and washings were extracted with dichloromethane (5 × 10 ml) and the organic layer was dried (K_2CO_3), filtered, and evaporated under reduced pressure to give a very pale yellow oil (146 mg, 41%); HPLC t_R 13.7 min observed at λ = 254 nm (CM 300 'Synchropak') [Found: ($M^+ + 1$) 305.2351. $C_{17}H_{28}N_4O$ requires 305.2341]; *m/z* (DCI) 306 ($M^+ + 2$), 305 ($M^+ + 1$) 279, 262, 201, and 188; $\delta_H(CDCl_3)$ 7.82 (2 H, dd, *ortho*, aromatic H), 7.42 (3 H, m, aromatic H), 7.00 (1 H, br s, NHCO), 3.45 (2 H, m, CH_2NHCO), 3.07 (3 H, br s, NH), 2.76 (11 H, m, CH_2N, CHN), 1.63 (2 H, br m, CH_2 − C), and 1.40 (4 H, br m, CH_2 − C); $[\alpha]_D^{20}$ +4.1 (*c* 0.5 in CH_2Cl_2); $\delta_C(CDCl_3)$ 167.6 (CONH), 134.5 (ArCCO), 131.2, 128.4, 127.3 (ArCH), 58.8 (CHN), 56.0, 48.3, 45.2, 43.9, 39.5 (CH_2N), 32.0, 29.4, and 25.6 (CH_2C).

2-[4-*Benzamidobutyl*)-1,4,7-*triazacyclononane*-1,4,7-*triyltriacetic Acid* (**7**).—Bromoacetic acid (0.392 g, 2.82 mmol) was added to a solution of the triamine (**6**) (0.0855 g, 0.28 mmol) in water (5.0 ml) with stirring at 60 °C. Lithium hydroxide (0.118 g, 2.82 mmol) was added in portions to maintain a pH ⩾ 10. More bromoacetic acid (0.784 g, 5.64 mmol) was added (in two portions after 23 and 39 h) and again lithium hydroxide (0.236 g, 5.64 mmol) was added to the reaction mixture to maintain the pH ⩾ 10. After 46 h the reaction mixture was allowed to cool and then evaporated under reduced pressure to give a yellow residue which was taken up in Milli Q water (5 ml). The triacid product was separated by ion exchange chromatography (Sepharose DEAE) with gradient elution from aqueous NH_4OAc (0.25mM, pH 5.6)–MeCN (9:1) to aqueous NH_4OAc (0.25M, pH 5.6)–CH_3CN (9:1). De-salting was achieved by reverse phase chromatography eluting with water (0.1% trifluroacetic acid; 50 ml) followed by acetonitrile (0.1% trifluoroacetic acid; 50 ml). Solvent was removed from the eluates under reduced pressure to give a clear oil (0.106 g, 79%); HPLC R_t = 6.5 min, observed λ = 254 nm ('Synchropak' A101); elution, A = H_2O, B = 1.0M NH_4OAc, pH 5.6, C = MeCN, gradient from 70% A, 10% B, 20% C to 0% A, 80% B, 20% C over 20 min; t_R = 12.0 min, observed λ = 254 nm ('Spherisorb' S50DS); elution A = H_2O (0.1% TFA), B = MeCN (0.1% TFA), gradient from 95% A, 5% B to 5% A, 95% B over 20 min; *m/z* (FAB, *p*-NBA) 479 (M + 1); $\delta_H(D_2O)$ 7.81 (2 H, d, *J* 7.1 Hz, aromatic H), 7.62 (3 H, m, aromatic H), 4.05–3.60 (6 H, m, CH_2CO_2), 3.55–2.90 (13 H, m, CH_2N), 1.71 (3 H, m, CH_2 − C), and 1.52 (3 H, m, CH_2 − C); $[\alpha]_D^{20}$ +7.5 (*c* 1.0 in H_2O).

Triethyl 2-(4-*Benzamidobutyl*)-1,4,7-*triazacyclononanetriyltriacetate* (**8**).—To a solution of the triamine (**6**) (360 mg, 1.18 mmol) in dry ethanol (5 ml) was added ethyl bromoacetate (610 mg, 3.60 mmol) and caesium carbonate (1.2 g, 3.66 mmol) and the mixture was heated to 70 °C for 18 h. The mixture was cooled, filtered, and evaporated under reduced pressure and the residue was taken up in dichloromethane (50 ml). The solution was washed with water (3 × 15 ml), dried (K_2CO_3), and evaporated and the residue was chromatographed on neutral alumina [(2→5%) MeOH in CH_2Cl_2] to yield a colourless oil (405 mg, 61%); *m/z* (DCI) 563 ($M^+ + 1$), 477, 447, 316, and 119; $\delta_C(CDCl_3)$ 173.4, 172.1 (CO_2Et), 167.5 (CONH), 134.9 (s, Ar), 131.0, 128.3, 126.9 (CH), 61.5, 60.2, 58.8 (CH_2O), 58.7 (CHN), 55.9, 55.7, 55.3, 52.4 (CH_2N), 39.6 (CH_2NHCO), 30.4, 29.2, 24.1 (CH_2C), and 14.2 (CH_3); $\delta_H(CDCl_3)$ 7.81 (2 H, d, *J* 7.4), 7.40 (3 H, m, *meta* + *para* aromatic CH), 6.81 (1 H, br t, NHCO), 4.12, 4.08, 4.07 (6 H, q + q + q, CH_2O) 3.41 (8 H, m, CH_2NHCO + CH_2O), 3.09–2.68 (11 H, m, CH_2N + CHN), 1.56–1.34 (6 H, m, CH_2C), and 1.20 (9 H, t + t + t, CH_3); ν_{max}(film) 3 330br s (NHCO), 2 980, 2 930, 2 840 (CH), 1 742 (CO_2Et), 1 649 (NHCO), 1 538 (NH bend), 1 190vs, and 1 060 cm^{-1}.

2-(4-*Aminobutyl*)-1,4,7-*triazacyclononane*-1,4,7-*triyltriacetic Acid* (**9**).—Hydrolysis of either the *N*-benzoyl triacid (**7**) or the triester (**8**) was effected in the same manner. The benzamide (1.0 mmol) in hydrochloric acid (6M; 20 ml) was heated to reflux for 48 h. After cooling, the solution was washed with diethyl ether (3 × 10 ml) and evaporated to dryness (0.01 mmHg) to yield the hydrochloride salt as a colourless glass; $\delta_H(D_2O)$ 4.0–3.70 (6 H, br, CH_2NCO_2), 3.58–2.95 (13 H, br m, CH_2N + CHN), 1.85 (4 H, br m, CH_2C), and 1.60 (2 H, br m, CH_2C); *m/z* (FAB, *m*-nitrobenzyl alcohol) 377 ($M^+ + 2$), 376 ($M^+ + 1$), and 375 (M^+); $\delta_C(D_2O)$ 170.6 (CO_2H), 59.3, 59.1, 54.1, 51.9, 48.2, 39.2 (CH_2N + CH_2CO + CHN), 26.9, 26.7, and 22.8 (CH_2C).

2-[4-(3-*Maleimidopropionamido*)*butyl*]-1,4,7-*triazacyclononane*-1,4,7-*triyltriacetic Acid* (**10**).—To a solution of the amino acid (**9**) (70 mg, 0.118 mmol) in water (1 ml) was added a solution of aqueous potassium hydroxide (6M; *ca.* 50 µl) until the pH was 6, followed by a solution of piperazine-1,4-diylethanesulphonic acid (PIPES) buffer (0.5M, 1.0 ml) to maintain the pH at 6.7. A solution of the maleimide active ester MPHS (38 mg, 0.14 mmol) in dioxane (2 ml) was added and the mixture was stirred at room temperature for 16 h. After addition of hydrochloric acid (6M; 60 µl), the pH dropped to 2 and the mixture was centrifuged and the supernatant solution was washed with ether (5 × 2 ml), reduced to small volume, and purified by reverse-phase HPLC to yield the ditrifluoroacetate as a colourless solid (42 mg, 40%); t_R = 10.1 min (Dynamax (C18 60 Å); $\delta_H(D_2O)$ 6.77 (2 H, s, CH=CH), 4.0–3.6 (8 H, m, CH_2NHCO + CH_2CO_2H), 3.4–3.1 (11 H, br m, CH_2N + CHN ring), 3.02 (2 H, t, CH_2NCO), 2.42 (2 H, t, CH_2CONH), 1.40 (4 H, m, CH_2CH_2), and 1.25 (2 H, br, CH_2); *m/z* (FAB, *m*-nitrobenzyl alcohol) 526 (M^-).

Alternative Synthesis of 2-[4-*Benzamidobutyl*)-1,4,7-*tris*-(*toluene*-p-*sulphonyl*)-1,4,7-*triazacyclononane*.—(2S)-*Hexane*-1,2,6-*triamine* (**11**). To a suspension of lysineamide (5 g, 0.023 mol) in dry THF (250 ml) was added borane–dimethyl sulphide (10M; 35 ml) and the mixture was heated to reflux for 180 h. The reaction was quenched by adding methanol (50 ml) and solvent was removed under reduced pressure. The oily residue was

heated under reflux in hydrochloric acid (6M; 200 ml) for 4 h. The solvent was removed under reduced pressure, the residue taken up in methanol (2 × 75 ml), and the solvent was removed again to give a clear oil. The residue was taken up in aqueous KOH (100 ml; 6M) and extracted with dichloromethane (4 × 100 ml). The combined organic extracts were dried (K_2CO_3), filtered, and evaporated under reduced pressure to give a clear oil (3.0 g, 100%). The product was used without further purification [Found: (M^+ + 1) 132.1490. $C_6H_{18}N_3$ requires 132.1500]; ν_{max}(thin film) 3 400br s (NH), 1 570, and 1 470 cm^{-1}; *m/z* (DCI, NH_3) 132 (M^+ + 1); δ_H($CDCl_3$) 3.53 (1 H, m, CHN), 2.72–2.38 (4 H, m, CH_2N), and 1.72–1.21 (12 H, m, CH_2C, NHs); δ_C($CDCl_3$) 56.2, 48.6, 42.1 (CH_2N, CHN), 35.5, 33.8, and 23.5 (CH_2 – C).

N-(5,6-*Diaminohexyl*)*benzamide* (**12**). Copper(II) carbonate [$CuCO_3$·$Cu(OH)_2$, Aldrich] (2.95 g, 0.013 mol) was added to a solution of the triamine (**11**) (3.0 g, 0.023 mol) in distilled water and the solution stirred at room temperature for 30 min. The temperature was raised to 75 °C for 90 min to give a very deep blue solution. Benzoyl chloride (4.88 g, 0.035 mol) was added to the cooled solution (–1 °C, ice–salt–water bath) with vigorous stirring over a period of 1 h, with simultaneous addition of KOH (1.96 g, 0.035 mol) to maintain the pH ⩾9. After being stirred for a further 45 min, the solution was decanted from a viscous oily residue and treated with hydrogen sulphide gas (10 min) to give a brown precipitate which turned black after 20 min at room temperature. This was filtered off to leave a yellow–green solution. The volume of the aqueous layer was reduced (*ca.* 40 ml) under reduced pressure and the solution adjusted to pH 13 with KOH. After continuous extraction with dichloromethane (150 cm^3), the organic extracts were dried (K_2CO_3), filtered, and evaporated under reduced pressure to give a clear oil (2.98 g, 49%). This material was used without further purification [Found: (M^+ + 1) 236.1804. $C_{13}H_{22}N_3O$ requires 236.1763]; ν_{max}(thin film) 3 300br s (NH) 1 650 (amide I), and 1 540 cm^{-1} (amide II); δ_H($CDCl_3$) 7.70 (2 H, d, *J* 8.5 Hz, aromatic H), 7.33 (3 H, m, aromatic H), 7.13 (1 H, br s, NHCO), 3.32 (2 H, m, CH_2NHCO), 2.64–2.30 (3 H, m, CH_2N and CHN), 1.77 (4 H, br s, NH_2), and 1.60–1.10 (6 H, br m, C – CH_2).

N(5,6-*Bistoluene*-p-*sulphonamidohexyl*)*benzamide*. (**13**). A solution of *N*-(5,6-diaminohexyl)benzamide (2.3 g, 9.8 mmol) in dichloromethane (40 ml) was added dropwise over a period of 1 h to a stirred solution of toluene-*p*-sulphonyl chloride (4.48 g, 2.35 × 10^{-2} mol) and triethylamine (2.37 g, 23.5 mmol) in dichloromethane (75 ml). After being stirred at room temperature for 18 h the mixture was heated to 45 °C for 20 min. The volume of the solvent was reduced (40 ml) under reduced pressure and a white precipitate was filtered off. Solvent was removed under reduced pressure to give a brown oil from which the ditosylated product was separated by 'flash' silica gel chromatography; gradient elution dichloromethane–methanol (199:1 to 99:1) gave a 'glassy' solid (2.29 g, 43%); TLC R_F 0.2 [silica gel; CH_2Cl_2–MeOH (95:5)] (Found: C, 60.0; H, 6.45; N, 7.25. $C_{27}H_{33}N_3O_5S_2$ requires C, 59.7; H, 6.08; N, 7.73%); *m/z* (DCI, NH_3) 546 (M^+ + 2), 545 (M^+ + 1), 544 (M^+), 390, and 359; δ_H($CDCl_3$) 7.82 (2 H, d, *J* 6.9 Hz, aromatic H), 7.66 (4 H, m, aromatic H), 7.42 (3 H, m, aromatic H), 7.22 (4 H, m, aromatic H), 6.75 (1 H, t, *J* 5.8 Hz, NHCO), 5.87 (2 H, d + t, $NHSO_2$), 3.29 (3 H, m, CH_2N and CHN), 2.88 (2 H, t, CH_2NHCO), 2.40 (3 H, s, CH_3), 2.37 (3 H, s, CH_3), 1.42 (4 H, br m, CH_2 – C), and 1.10 (2 H, br m, CH_2 – C); δ_C($CDCl_3$) 167.9 (NHCO), 143.4, 137.2, 136.7, 131.5, 129.6, 128.5, 127.1, 127.0 (aromatic C), 53.2, 46.6, 38.7 (CH_2 – N), 31.3, 29.0, 21.8 (CH_2 – C), and 21.5 (CH_3).

2-[4-*Benzamidobutyl*)-1,4,7-*tris*(*toluene*-p-*sulphonyl*)-1,4,7-*triazacyclononane* (**5**). A solution of 1,5-bis(toluene-*p*-sulphonato)-3-(toluene-*p*-sulphonyl)-3-azapentane (1.67 g, 2.95 mmol) in dry DMF (40 ml) was added dropwise over a period of 4 h to a suspension of caesium carbonate (2.02 g, 6.20 mmol) in a solution of the ditosylamide (**13**) (1.60 g, 2.95 mmol) in dry DMF (200 ml) with vigorous stirring under an atmosphere of nitrogen. After being stirred at room temperature for 18 h, the mixture was heated to 60 °C for 3 h. Caesium carbonate (0.5 g, 1.5 mmol) was added to the reaction mixture which was stirred at 60 °C for a further 24 h. Solvent was removed under reduced pressure and the residue dried *in vacuo* (10^{-2} mmHg, 50 °C). The residue was taken up in dichloromethane (150 ml) and the solution washed with distilled water (3 × 150 ml), dried (K_2CO_3), filtered, and evaporated under reduced pressure to give a pale brown oil. The cyclised product was separated from the mixture by 'flash' silica gel chromatography with gradient elution, dichloromethane–methanol 399:1 to 199:1 to 99:1) (0.79 g, 35%); TLC R_F 0.7 [silica gel; CH_2Cl_2–MeOH (95:5)]; m.p. 106–108 °C (Found: C, 59.5; H, 6.25; N, 7.1. $C_{38}H_{46}N_4O_7S_3$ requires C, 59.5; H, 6.00; N, 7.31%); *m/z* (DCI, NH_3) 768 (M^+ + 2) and 767 (M^+ + 1). NMR data as reported above.

(+)-(2S)-2-(4-*Benzamidobutyl*)-1,4,7,10-*tetrakis*(*toluene*-p-*sulphonyl*)-1,4,7,10-*tetra-azacyclododecane* (**14**).—1,5-Bis(toluene-*p*-sulphonato)-3-(toluene-*p*-sulphonyl)-3-azapentane (2.79 g, 4.93 mmol) in DMF (50 ml) was added dropwise over 3 h to the tritosylamide compound (**4**) (3.65 g, 4.93 mmol) and caesium carbonate (4.01 g, 12.3 mmol) in DMF (200 ml) at room temperature. After being stirred at room temperature for 18 h, the reaction mixture was heated at 60 °C (72 h). A further batch (0.42 g, 7.39 mmol) of 1,5-bis(toluene-*p*-sulphonato)-3-(toluene-*p*-sulphonyl)-3-azapentane was added in one lot, and stirring continued (12 h, 60 °C). Solvent was removed under reduced pressure and the residue dissolved in dichloromethane (100 ml). The organic layer was washed with water (2 × 30 ml), dried and evaporated. Column chromatography (gradient eluant 0.5–1.5% methanol in dichloromethane) of the residue afforded the *title compound* as a glassy solid (3.41 g, 72%), m.p. 108–110 °C; $[\alpha]_D^{22}$ +14.0° (*c* 1.00 in CH_2Cl_2); t_R 2.9 min (Spherisorb 50DS2 reverse phase) (Found: C, 58.2; H, 5.6; N, 7.0. $C_{47}H_{57}N_5O_9S_4$ requires C, 58.6; H, 5.9; N, 7.3%); ν_{max}(film) 3 400 and 3 290 (NH), 3 040, 1 650 (CO), 1 600, 1 580, 1 530 (NH), 1 340, and 1 160 cm^{-1}; δ_H(CD_3CN; 333 K) 0.67 (2 H, br s, CH_2), 1.09–1.14 (2 H, m, CH_2), 1.27–1.45 (2 H, m, CH_2), 2.35, 2.41, 2.46, and 2.53 (12 H, 4 × s, $ArCH_3$), 2.57–4.22 (17 H, 7 × m, NCH_2, NCH, NHCH_2), 6.86 (1 H, br s, NH), 7.16–7.27 (4 H, AB system, ArH), 7.37–7.51 (9 H, AB system, ArH), and 7.63–7.83 (8 H, AB system, ArH); δ_C($CDCl_3$, 298 K) 21.4 (CH_3), 24.3, 28.7, 29.2 (CH_2C), 39.4, 44.5, 49.4, 50.5, 51.8, 51.9, 53.9, 55.4, 62.9 (br, NCH_2, NCH, CH_2), 126.8, 127.1, 128.2, 129.8 (CH), 131.0, 134.5, 135.6, 143.4, 143.9, 144.5 (quat.), and 167.3 (CO); *m/z* (CI) 965 (56, M^+ + 2), 965 (56, M^+ + 1), 964 (100, M^+ + 1), and 809 (21, M^+ – 154).

(+)-(2S)-2-(4-*Benzamidobutyl*)-1,4,7,10-*tetra-azacyclododecane* (**15**).—Ammonia (100 ml) was condensed into a mixture of the tetratosylated macrocycle (**14**), (0.50 g, 0.52 mmol) in THF (20 ml) and ethanol (2 ml) at –78 °C. Lithium (0.18 g, 25.9 mmol) was added in small portions to this mixture and an intense blue colour developed which was discharged over 1–10 min. The reaction mixture was allowed to warm to room temperature, water (20 ml) was added, and solvents were evaporated. The residue was dissolved in hydrochloric acid (6M; 20 ml), and the solution washed with diethyl ether (3 × 10 ml), and then evaporated. The residue was redissolved in aqueous potassium hydroxide (6M; 10 ml) and extracted with dichloromethane (4 × 5 ml). The combined extracts were evaporated to give a colourless oil. Preparative HPLC (cation exchange) afforded the *title compound* as a colourless oil (0.10 g, 57%); $[\alpha]_D^{25}$ +2.5° (*c* 0.80 in CH_2Cl_2); t_R (CM 300) 7.3 min (Found: M^+ + 1 348.2769. $C_{19}H_{34}N_5O$ requires 348.2763);

$\delta_H(CDCl_3)$ 1.41–1.48 (4 H, m, CH_2), 1.58–1.66 (2 H, m, CH_2), 2.38 (4 H, br s, NH), 2.46 (1 H, dd, *J* 12 and 7.7 Hz, NHCH*H*), 2.67 (14 H, s, NCH_2), 3.46 (2 H, dt, *J* 6.4 Hz, J_{NHCH} 6.2 Hz, CONHCH_2), 6.58 (1 H, br t, CONH), 7.39–7.49 (3 H, m, ArH), and 7.76–7.80 (2 H, AB system, J_{AB} 8.3 Hz, *ortho* CH); *m/z* (DCI) 348 ($M^+ + 1$). This compound may also be obtained—without the need for HPLC purification—by detosylation with conc. H_2SO_4 [as described for (**6**)] giving the amine in 60% yield.

(+)-(2S)-2-(4-*Aminobutyl*)-1,4,7,10-*tetra-azacyclododecane*-1,4,7,10-*tetrayltetra-acetic Acid Trihydrochloride* (**16**).—Ethyl bromoacetate (190 mg, 1.24 mmol) was added to the macrocyclic amine (**15**) (75 mg, 0.22 mmol) and potassium carbonate (176 mg, 1.28 mmol) in DMF (3 ml) and the mixture heated (90 °C, 18 h). Evaporation of solvent and preparative HPLC (cation exchange) gave the tetraester as a colourless oil, t_R 10.7 min. The tetraester was hydrolysed with 6M HCl according to the method for (**9**) to give the *title compound* as a white solid (97 mg, 77%); $[\alpha]_D^{22} + 2.7°$ (*c* 0.75 in H_2O (Found: C, 41.3; H, 5.9; N, 11.75. $C_{20}H_{37}N_5O_8 \cdot 3HCl$ requires C, 41.1; H, 6.3; N, 12.0%); $\delta_H(D_2O)$ 1.47 (2 H, br s, CH_2), 1.74 (2 H, br s, CH_2), 1.95 (2 H, br s, CH_2), 3.05, 3.47, and 4.00 (25 H, 3 × vbr s, CH, NCH_2, CH_2NH_2); *m/z* (FAB, glycerol) 476 ($M^+ + 1$).

(+)-(2S)-2-[4-(3-*Maleimidopropionamido)butyl*]-1,4,7,10-*tetra-azacyclododecane*-1,4,7,10-*tetrayltetra-acetic Acid* (**17**).—To the amino acid hydrochloride (**16**) (50 mg, 0.077 mmol) in water (25 ml) at pH 6.5 (KOH) was added PIPES buffer (0.5M, pH 6.8; 2.5 ml). A solution of the maleimide active ester MPHS (24.4 mg, 0.092 mmol) in dioxane (5 ml) was added and the mixture was maintained at 25 °C for 18 h. The mixture was acidified to pH 1.5 (conc. HCl), filtered, and extracted with ether (5 × 10 ml). The solution was concentrated under reduced pressure prior to purification using reverse phase HPLC (Spherisorb 50DS2) t_R 8.83 min; *m/z* (FAB, glycerol) 626 ($M^+ + 1$) and 625 (M^+); $\delta_H(D_2O)$ 6.83 (2 H, s, alkene CH), 4.0–3.0 (27 H, br m, CH_2N + CHN), 2.45 (2 H, t, *J* 6.3 Hz, NCH_2CH_2CO), and 1.5 (6 H, m, CH_2C).

N^9-*Benzoyl*-3-*azanonane*-1,5,9-*triamine*-N^1,N^1,N^3,N^5,N^5-*penta-acetic Acid* (**18a**).—*C-Functionalised* DTPA. A solution of potassium hydroxide (0.734 g, 13.1 mmol) in distilled water (3.0 ml) was added to a solution of bromoacetic acid (0.688 g, 4.81 mmol) in toluene (1.5 ml) at 0 °C (ice–salt bath). A solution of the triamine (**3**) (0.243 g, 0.874 mmol) in distilled water (0.5 ml) was added and the heterogeneous system stirred vigorously. More bromoacetic acid (0.688 g, 4.81 mmol) in toluene (0.75 ml), and potassium hydroxide (0.734 g, 13.1 mmol) in water (1.0 ml) were added after 24 h and again after 48 h. After 72 h the aqueous solution was adjusted to pH 1.5 with concentrated hydrobromic acid and washed with diethyl ether (3 × 10 ml). The aqueous layer was evaporated to dryness under reduced pressure to give a yellow solid residue. The product was separated from salts and hydrolysis products by ion exchange chromatography (Dowex-50W, H^+ form, dry mesh 100–200, cation exchange). The residue was loaded onto the column as a dry solid and eluted with distilled water (200 ml) followed by aqueous ammonia (0.2M; 250 ml). The latter fractions from the column were combined and solvent removed to give a colourless 'gummy' solid (0.26 g, 52%); HPLC, t_R 17.7 min observed at λ = 254 nm ('Synchropack' A100 anion exchange column) with gradient elution, where A = H_2O, B = 1.0M NH_4OAc, pH 5.6, C = MeCN, from 70% A, 10% B, 20% C to 0% A, 80% B, 20% C over 20 min [Found: ($M^+ + 1$) 639.3249. $C_{25}H_{37}N_4O_{11}$·pentamethyl ester requires 639.3241); *m/z* (DCI, NH_3, pentamethyl ester); 640 ($M^+ + 2$), 639 ($M^+ + 1$), 626, 625, and 611; $\delta_H(D_2O)$ 7.75 (2 H, dd, *J* 7.1 Hz, aromatic H), 7.56 (3 H, m, aromatic H), 3.90 (4 H, s, CH_2CO_2), 3.95–3.90 (6 H, m, CH_2CO_2), 3.50–2.84 (15 H, m, CH_2N), and 1.87–1.48 (6 H, m, CH_2–C); $\delta_C(D_2O)$ 177.3 (NHCO), 170.4, 170.0, 169.9, 169.8 (CO_2), 133.3, 131.6, 128.4, 126.6 (aromatic C), 61.1, 59.4, 57.1, 56.7, 54.9, 53.3, 53.0, 48.2 (C–O, C–N, C–CO), 27.8, 24.9, and 22.5 (CH_2–C).

N^9-(3-*Maleimidopropionyl*)-3-*azanonane*-1,5,9-*triamine*-N^1,N^1,N^3,N^5,N^5-*penta-acetic Acid* (**19**). Hydrolysis of (**18a**) with 6M HCl (48 h, 110 °C) was effected as described for (**9**), to yield (**18b**). To a solution of the amino acid (**18b**) (198 mg, 0.32 mmol) and *N*-methylmorpholine (210 μl, 2.91 mmol) in dry dimethyl sulphoxide (2 ml), was added a solution of the maleimide active ester MPHS (86 mg, 0.39 mmol) in DMSO (1 ml) and the mixture was warmed until homogeneous. After 0.5 h, solvent was removed under reduced pressure and the residue was purified by reverse phase HPLC (Dynamax) to yield the ditrifluoroacetate salt of the title compound as a colourless, glassy solid (80 mg, 53%); t_R (Dynamax C18 60 Å) 10.4 min; $\delta_H(D_2O)$ 6.38 (2 H, s, CH=CH), 4.05 + 3.93 (8 H, s + s, CH_2CO_2H), 3.75 (4 H, s + t, CH_2CO_2H + CH_2NCO), 3.6–3.0 (9 H, m, CHN + CH_2N), 2.45 (2 H, t, CH_2CH_2CONH), 1.43 (4 H, br, CH_2CH_2), and 1.26 (2 H, br, $CH_2CH_2CH_2$); *m/z* (FAB, *m*-nitrobenzyl alcohol), 615 (M^-).

1-(5-*Benzamido*-1-*ethoxycarbonylpentyl*)-1,4,7-*triazacyclononane* (**20**). Potassium carbonate (0.214 g, 1.54 mmol) was added to a solution of 1,4,7-triazacyclonane (0.380 g, 2.95 mmol) in anhydrous DMF (5 ml) under a nitrogen atmosphere and the mixture was heated to 60 °C. A solution of ethyl 6-benzamido-2-bromohexanoate (0.503 g, 1.48 mmol) in anhydrous DMF (5 ml) was added dropwise over a period of 5 h and the mixture stirred for a further 15 h at 60 °C. The cooled reaction mixture was filtered and solvent removed under reduced pressure to give a pale brown oil. A small amount of product (50 mg) was separated by preparative HPLC ('Synchropak' CM300 cation exchange), but the remaining product was used without further purification. Purified material: HPLC t_R 7.8 min observed at λ = 282 nm ('Synchropak' CM300 cation exchange) with gradient elution, 1.4 ml min^{-1}, A = H_2O, B = 1.0M NH_4OAc, C = MeCN; from *t* = 0 min, 80% A, 0% B, 20% C, to *t* = 5 min, 60% A, 20% B, 20% C, to *t* = 10 min, 0% A, 80% B, 20% C; ν_{max}(thin film) 3 500–3 300 (NH), 1 720 (C=O ester), and 1 635 cm^{-1} (NHCO); *m/z* (DCI, NH_3) 392 ($M^+ + 2$), 391 ($M^+ + 1$), 303, and 264; $\delta_H(CDCl_3)$ 8.63 (1 H, br t, NHCO), 8.01 (2 H, d, *J* 7.8 Hz), 7.41 (3 H, m, aromatic H), 4.14 (2 H, q, *J* 7.3 Hz, OCH_2), 3.63–3.29 (3 H, m, CH_2NCO and CHN), 2.85–2.67 (10 H, m, CH_2N), 2.45 (2 H, m, CH_2N), 1.76–1.45 (6 H, m, CH_2C), and 1.26 (3 H, t, *J* 7.1 Hz, CH_2CH_3); $\delta_C(CDCl_3)$ 173.4 (CO_2), 167.5 (NHCO), 134.7, 131.1, 128.2, 127.1 (aromatic), 64.2, 60.2, 57.8 (CH_2O, CH_2CO), 48.5, 46.9, 45.6, 45.3, 39.8 (CH_2N), 29.0, 23.5, 18.4 (CH_2–C), and 14.3 (CH_3).

1-(5-*Benzamido*-1-*ethoxycarbonylpentyl*)-4,7-*diethoxycarbonyl*-1,4,7-*triazacyclononane* (**21**). Caesium carbonate (1.20 g, 3.68 mmol) and ethyl bromoacetate (0.615 g, 3.68 mmol) were added to a solution of the crude monoalkylated triamine (**20**) [*ca.* 0.57 g, 1.5 mmol] in dry ethanol (6 ml) and the mixture stirred at 60 °C for 20 h. Solvent was removed under reduced pressure and the residue taken up in dichloromethane (6 ml) and a fine white precipitate removed by centrifuge. The organic layer was evaporated to dryness and the residue chromatographed on neutral alumina; gradient elution using dichloromethane–methanol from (100:0) to (199:1) to (99:1) (0.1 g, 12%); t_R 0.4 [alumina; CH_2Cl_2–MeOH (98:2)]; HPLC: t_R 5.3 min observed at λ = 254 nm ('Synchropak' CM300 cation exchange) gradient elution—as noted above [Found: ($M^+ + 1$) 563.3335. $C_{29}H_{47}N_4O_7$ requires 563.3227]; ν_{max}(thin film) 3 400, 3 240 br (NH), 1 725 (C=O ester), and 1 645 cm^{-1} (NHCO); $\delta_H(CDCl_3)$ 7.81 (2 H, d, *J* 6.8 Hz, aromatic H), 7.43 (3

H, m, aromatic H), 4.13 (6 H, q, *J* 6.9 Hz, OCH_2), 3.46 (7 H, m, CH_2CO_2, $CHCO_2$, and CH_2NCO), 3.02–2.76 (12 H, m, CH_2N), 1.50–1.28 (6 H, m, CH_2C), and 1.25 (9 H, t, *J* 7.1 Hz, CH_2CH_3); $\delta_C(CDCl_3)$ 173.5, 171.7 (CO_2), 167.4 (CONH), 134.4, 131.1, 128.3, 126.7 (aromatic) 66.3, 60.3, 60.2, 58.4, 55.4, 54.8, 52.9 (C–N, C–O, and C–CO), 39.7, 29.6, 29.0, 23.7, and 14.2 (C–CH_2, C–CH_3).

1,4-*Bis*(5-*benzamido*-1-*ethoxycarbonylpentyl*)-1,4,7-*triaza-cyclononane* (**22**). Potassium carbonate (0.117 g, 0.85 mmol) was added to a solution of 1,4,7-triazacyclononane (0.104 g, 0.81 mmol) in anhydrous DMF under a nitrogen atmosphere and the mixture heated to 55 °C. A solution of ethyl 6-benzamido-2-bromohexanoate (0.276 g, 8.1 mmol) in anhydrous DMF (4 ml) was added and the mixture stirred at 55 °C for 48 h. The cooled mixture was filtered and solvent removed under reduced pressure to give a pale yellow oil. The di-alkylated product was separated by preparative HPLC ('Synchropak' CM300 cation exchange 25 cm × 10 mm); gradient elution, 4.0 ml min^{-1}, A = H_2O, B = 1.0M NH_4OAc, pH 5.6, C = MeCN; *t* = 0 min, 80% A, 0% B, 20% C; *t* = 5 min, 60% A, 20% B, 20% C; *t* = 10 min, 0% A, 80% B, 20% C, held for a further 5 min. The collected fractions were combined and the solvent was removed under reduced pressure, followed by sublimation to remove the ammonium acetate, to give a pale yellow oil (0.11 g 21%); HPLC t_R = 5.7 min observed at λ = 254 nm—conditions as above; ν_{max}(thin film) 3 500–3 300 (NH), 1 720 (C=O ester), and 1 640 cm^{-1} (NHCO); *m/z* (DCI, NH_3) 653 (M^+ + 2) and 652 (M^+ + 1); $\delta_H(CDCl_3)$ 8.00–7.74 (4 H, m, aromatic H), 7.45–7.42 (6 H, m, aromatic H), 4.15 (4 H, m, OCH_2), 3.47–2.55 (18 H, m, CH_2N and CHN), 1.59 (12 H, m, CH_2C), and 1.24 (6 H, m, CH_2CH_3); $\delta_C(CDCl_3)$ 172.4 (C=O ester), 167.6 (CONH), 139.2, 131.1, 128.3, 127.3, 126.4 (aromatic), 65.3, 64.6, 60.6 (CH_2O, CH_2CO), 47.1, 46.6, 44.6, 43.2, 39.9 (CH_2N), 30.2, 28.9, 24.5 (CH_2–C), and 14.4 (CH_3).

1,4-*Bis*(5-*benzamido*-1-*ethoxycarbonylpentyl*)-7-*ethoxy-carbonyl*-1,4,7-*triazacyclononane* (**23**). Caesium carbonate (46.3 mg, 0.142 mmol) and ethyl bromoacetate (24.0 mg, 0.142 mmol) were added to a solution of the dialkylated triamine (**22**) (88.0 mg, 0.135 mmol) in anhydrous DMF. The mixture was stirred at 60 °C for 20 h, after which solvent was removed under reduced pressure. The residue was taken up in dichloromethane and a fine white precipitate removed by centrifuge. The solvent was removed under reduced pressure and the product purified by preparative HPLC ('Synchropak' CM300 cation exchange 25 cm × 10 mm)—conditions same as previous experiment. The collected fractions were combined, the solvent was removed under reduced pressure and the residue sublimed to remove ammonium acetate, leaving a pale brown 'gummy' solid (54 mg, 54%); t_R = 6 min observed at λ = 254 nm—conditions as above; ν_{min}(thin film) 3 500–3 300 (NH), 1 720 (C=O ester), and 1 640 (NHCO); *m/e* (DCI, NH_3) 738 (M^+ + 1), 621, and 420; $\delta_H(CDCl_3)$ 7.79 (4 H, d, *J* 7.0 Hz, aromatic H), 7.42 (6 H, m, aromatic H), 6.66 (2 H, br s, NHCO), 4.11 (6 H, m, OCH_2), 3.46–3.17 (8 H, m, CH_2CO, CHCO, and CH_2NCO), 3.09–2.63 (12 H, m, CH_2N), 1.68 (12 H, m, CH_2–C), and 1.24 (9 H, t, *J* 7.1 Hz, CH_2CH_3); $\delta_C(CDCl_3)$ 173.8, 172.4 (CO_2), 167.6 (NHCO), 134.8, 131.2, 128.4, 127.6, 127.0 (aromatic) 66.8, 60.2, 60.1, 56.0, 55.9, 53.4 (C–O, C–CO, C–N), 39.9, 30.1, 29.3, 23.9 (C–CH_2), and 14.7 (CH_3).

Maleimide Linkage.—7-(5-*Maleimido*-1-*ethoxycarbonyl-pentyl*)-1,4,7-*triazacyclononane*-1,4-*diylacetic acid* (**24**). The triester (**21**) (25 mg, 0.44 mmol) was dissolved in hydrochloric acid (6M; 2.0 ml) and heated under reflux for 16 h. Solvent was removed under reduced pressure and the residue dried *in vacuo* (40 °C, 10^{-2} mmHg). Hydrolysis of the ester and amide groups was seen to be complete by ^1H NMR (D_2O) and FAB (*p*-NBA matrix) mass spectrometry [*m/e* 375 (M^+ + 1)]. The residue was dissolved in anhydrous DMF (200 μl) and a solution of the maleimide active ester MPHS (14.1 mg, 0.053 mmol) in anhydrous DMF (60 μl) was added. *N*-Methylmorpholine (26.9 mg, 0.222 mmol) was added to the mixture, resulting in an immediate precipitation. Milli-Q purified water (25 μl) was added until the precipitate redissolved and the mixture allowed to stand at room temperature for 24 h. The product was separated by preparative HPLC ('Spherisorb' S50DS2 reverse phase); t_R = 8.5 min observed at λ = 282 nm gradient elution 1.4 ml min^{-1}, A = H_2O (0.1% TFA), B = MeCN (0.1% TFA), elution from 95% A, 5% B, to 5% A, 95% B in 20 min; *m/z* (FAB, *p*-NBA matrix) 526 (M^+ + 1), 453, 391, and 307; $\delta_H(D_2O)$ 6.85 (2 H, s, CH=CH), 3.95 (5 H, m, CH_2CO_2), 3.79 (4 H, m, CH_2NHCO and CH_2NCO), 3.00–3.50 (12 H, m, CH_2N), 2.49 (2 H, t, *J* 6.2 Hz, CH_2CONH_2), 1.69 (3 H, m, CH_2C), and 1.46 (3 H, m, CH_2C).

4,7-*Bis*[1-*carboxy*-5-(3-*maleimidopropionamido*)*pentyl*]-1,4,7-*triazacyclononan*-1-*ylacetic acid* (**25**). The triester (**23**) (38 mg, 0.051 mmol) was dissolved in hydrochloric acid (6M, 3.0 ml) and heated under reflux for 16 h. Solvent was removed under reduced pressure and the residue dried *in vacuo* (40 °C, 10^{-2} mmHg). Hydrolysis was seen to be complete by ^1H NMR (D_2O) and FAB mass spectrometry (*p*-NBA matrix) [*m/z* 444 (M^+ + 1)]. The procedure of the previous experiment was followed for the maleimide linkage step (2.5 equiv. of the maleimide active ester MPHS and 7 equiv. of *N*-methylmorpholine required in this case), yield (10 mg, 26%); t_R 10.4 min ('Spherisorb' S50DS2 reverse phase) conditions as for previous experiment; *m/z* (FAB, *p*-NBA matrix) 748 (M^+ + 1) and 596; $\delta_H(D_2O)$ 6.85 (4 H, s, CH=CH), 3.96 (4 H, m, CH_2CO_2), 3.79 (8 H, m, CH_2NCO and CH_2NHCO), 3.10–3.25 (12 H, m, CH_2N), 2.49 (4 H, t, *J* 6.3 Hz, CH_2CONH), 1.82 (3 H, m, CH_2C), and 1.46 (3 H, m, CH_2C).

1-(5-*Benzamido*-1-*ethoxycarbonylpentyl*)-1,4,7,10-*tetra-azacyclododecane* (**26**). To a stirred suspension of potassium carbonate (0.16 g, 1.16 mmol) and 1,4,7,10-tetra-azacyclododecane (0.20 g, 1.16 mmol) in dimethylformamide (40 ml) at 60 °C was added a solution of ethyl 6-benzamido-2-bromohexanoate (0.34 g, 1.0 mmol) in dimethylformamide (35 ml) over a period of 3 h. No significant product build-up was observed beyond 64 h (HPLC CM 300). Solvent was removed under reduced pressure and the crude residue was treated with dichloromethane (50 ml), filtered, and the solvent was removed under reduced pressure to yield a colourless oil which was purified by HPLC (CM 300) to yield a pale yellow oil (0.2 g, 40%), t_R = 7.5 min; *m/z* (CI) 434 (M^+ + 1); ν_{max}(thin film) 1 720 (ester CO) and 1 630 cm^{-1} (amide CO); $\delta_H(CDCl_3)$ 1.25 (3 H, t, *J* 7.6 Hz, CH_3), 1.30–1.74 (6 H, m, CH_2C), 2.55–2.92 (16 H, m, ring CH_2N), 3.34–3.49 (3 H, m, CH and $CONCH_2$), 4.14 (2 H, q, OCH_2), 7.41–7.47 (3 H, m, aromatic CH), 7.93 (2 H, dd, *ortho* CH), and 8.12 (1 H, br t, NHCO); $\delta_C(CDCl_3)$ 14.12 (CH_3), 24.43, 28.61, 28.95 (CH_2C), 39.66 ($CONHCH_2$), 44.30, 44.70, 46.31, and 48.11 (CH_2N), 60.42 (*N*CHCO), 63.25 (OCH_2), 127.11, 128.10 (CH arom), 130.67 ($COCC_5H_5$), 167.46 (CONH), and 176.57 (COO); [Found (CI): M^+, 433.3047. $C_{23}H_{39}N_5O_3$ requires 433.3053].

1-(5-*Benzamido*-1-*ethoxycarbonylpentyl*)-4,7,10-*tris*(*ethoxy-carbonylmethyl*)-1,4,7,10-*tetra-azacyclododecane* (**27**). To a stirred solution of the tetra-amine (**26**) (0.14 g, 0.33 mmol) in dry dimethylformamide (5 ml) was added potassium carbonate (0.15 g, 1.08 mmol) followed by a solution of ethyl bromoacetate (0.165 g, 0.99 mmol) in dry dimethylformamide (2 ml). The mixture was heated to 80 °C for 24 h, and solvent removed under reduced pressure to yield a residue which was treated with dichloromethane (20 ml), filtered, and solvent removed to give a pale yellow oil which was purified by chromatography on alumina (0→3% MeOH in CH_2Cl_2), followed by further purification by HPLC (CM 300) to yield a colourless oil (0.14 g,

63%); t_R 6.1 min; ν_{max}(thin film) 3 400, 3 240 (br, NH); 1 730 (ester 10), and 1 640 cm^{-1} (amide CO); $\delta_H(CDCl_3)$ 1.25 (12 H, t + t + t, CH_2CH_3), 1.30–1.90 (6 H, m, CH_2C), 2.13–3.20 (16 H, br m, ring CH_2N), 3.30–3.55 (9 H, m, CH_2CO + CHCO + CH_2NHCO), 4.08–4.18 (8 H, m, CH_2O), 7.40 (3 H, m, ArCH), and 7.94–8.07 (3 H, dd + br t, *ortho* CH + NHCO); *m/z* (DCI) 692 (M^+ + 1) (Found: M^+, 691.4163. $C_{35}H_{57}N_5O_9$ requires 691.4156).

10-[1-*Carboxy*-5-(3-*maleimidopropionamido*)*pentyl*]-1,4,7,10-*tetra-azacyclododecane*-1,4,7-*triyltriacetic acid* (**28**). The tetraester (30 mg, 4.34 × 10^{-5} mol) in hydrochloric acid (6M; 5 ml) was heated under reflux for 48 h. It was then cooled, washed with ether (3 × 5 ml), and evaporated under reduced pressure to yield the hydrochloride salt as a colourless glass (28 mg, 90%); $\delta_H(D_2O)$ 1.64 (6 H, br m, CH_2C) and 2.93–4.16 (25 H, m, CH_2N + CHN); *m/z* (FAB, *m*-nitrobenzyl alcohol) 476 (M^+). The residue was dissolved in dry DMSO (300 μl) and a solution of the maleimide active ester MPHS (14.0 mg, 0.05 mmol) in dry dimethylformamide (80 μl) was added, followed by *N*-methylmorpholine (40 mg, 0.32 mmol). After 3 h at 30 °C, solvent was removed under reduced pressure (0.01 mmHg) and the residue was purified by reverse-phase HPLC (Spherisorb 5-ODS-2) to yield a colourless glass (22 mg, 80%); t_R = 8.9 min; *m/z* (FAB, *m*-nitrobenzyl alcohol) 628 (M^+ + 1), 555, 493, and 409; $\delta_H(D_2O)$ 6.84 (2 H, s, CH alkene), 3.96–3.91 (7 H, m, CH_2N + CHN), 3.85–3.05 (20 H, br m, CH_2N ring + CH_2NHCO + CH_2NCO), 2.50 (2 H, t, *J* 6.4, NCH_2CH_2CONH), and 1.65 (6 H, m, CH_2C).

Acknowledgements

We thank SERC and MRC for support and the Royal Society of Chemistry for a Hickinbottom Fellowship (D. P.).

References

1 J. L. Humm, *J. Nucl. Med.*, 1986, **27**, 1490.
2 M. K. Moi, C. F. Meares, M. J. McCall, W. C. Cole, and S. J. DeNardo, *Anal. Biochem.*, 1985, **148**, 249.
3 C. F. Meares and T. G. Wensel, *Acc. Chem. Res.*, 1984, **17**, 202.
4 M. W. Brechbrel, O. A. Gansow, R. W. Archer, J. Schlom, J. Esteban, D. E. Simpson, and D. Colcher, *Inorg. Chem.*, 1986, **25**, 2772.
5 J. A. Carrasquillo, P. G. Abrams, R. W. Schroff, J. C. Reynolds, C. S. Woodhouse, A. C. Morgan, A. M. Keenan, K. A. Foon, P. Perentesis, S. Marshall, M. Horowitz, J. Englert, R. K. Oldham, and S. M. Larson, *J. Nucl. Med.*, 1988, **29**, 39.
6 S. V. Deshpande, S. J. De Nardo, C. F. Meares, M. J. McCall, G. P. Adams, M. K. Moi, and G. L. De Nardo, *J. Nucl. Chem.*, 1988, **29**, 217.
7 D. J. Hnatowich, M. Chinol, D. A. Siebecker, M. Gionet, T. Griffin, P. W. Doherty, R. Hunter, and K. R. Case, *J. Nucl. Med.*, 1988, **29**, 1428.
8 J. R. Morphy, D. Parker, R. Alexander, A. F. Carne, M. A. W. Eaton, A. Harrison, A. Millican, S. K. Rhind, R. Titmas, and D. Weatherby, *J. Chem. Soc., Chem. Commun.*, 1988, 156.
9 J. R. Morphy, D. Parker, R. Kataky, A. Harrison, M. A. W. Eaton, A. Millican, A. Phipps, and C. Walker, *J. Chem. Soc., Chem. Commun.*, 1989, 792.
10 A. S. Craig, I. M. Helps, K. J. Jankowski, D. Parker, N. R. A. Beeley, B. A. Boyce, M. A. W. Eaton, A. T. Millican, K. Millar, S. K. Rhind, A. Harrison, and C. Walker, *J. Chem. Soc., Chem. Commun.*, 1989, 794.
11 A. S. Craig, D. Parker, H. Adams, and N. R. Bailey, *J. Chem. Soc., Chem. Commun.*, 1989, 1792.
12 J. P. L. Cox, J. K. Jankowski, R. Kataky, D. Parker, N. R. A. Beeley, B. A. Boyce, M. A. W. Eaton, K. Miller, A. T. Millican, A. Harrison, and C. Walker, *J. Chem. Soc., Chem. Commun.*, 1989, 797.
13 J. P. L. Cox, K. J. Jankowski, D. Parker, A. Harrison, C. Walker, and J. Sansom, *Int. J. Nucl. Med. Biol.*, submitted, and unpublished results.
14 'Critical Stability Constants,' eds. A. E. Martel and R. M. Smith, Plenum (New York), 1974, vol. 2.
15 K. Kitagawa, K. Kitade, Y. Kizo, T. Akita, S. Funakoshi, N. Fujii, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1979, 955; T. Fuji and S. Sakakibara, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 3146.
16 D. Parker and A. T. Millican, Int. Pat. Appl. WO 8901, 476/1989.
17 T. Kitagawa and T. Aikawa, *J. Biochem.*, 1976, **79**, 233.
18 J. C. Eck and C. S. Marvel, *Org. Synth.*, Coll. Vol. 2, 1943, 74.
19 R. Hodges, N. E. Conway, and L. W. McLaughlin, *Biochemistry*, 1989, **28**, 261.
20 J. A. Fidanza and L. W. McLaughlin, *J. Am. Chem. Soc.*, 1989, **111**, 9117.

Paper 0/0117G
Received 13*th March* 1990
Accepted 12*th April* 1990