Macrocyclic Polyamines [16]-N_3 and [21]-N_4: Synthesis and Study of their ATP Complexation by ^{31}P Nuclear Magnetic Resonance Spectroscopy

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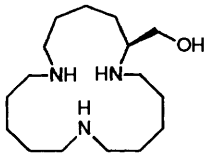
The macrocyclic polyamines [16]-N_3, compound **1**, and [21]-N_4, compound **2**, possessing a hydroxymethyl side-chain have been synthesized. The macrocyclisation was achieved by condensation of lysine ditosate **3b** with appropriate components **5** and **6** in the presence of Cs_2CO_3 to obtain **7** and **8** in 33 and 37% yield, respectively. The complexation of polyamines **1** and **2** with ATP was studied by ^{31}P NMR spectroscopy, which indicated a specific recognition of γ-phosphorus of ATP (NMe_4 salt) by the polyamines. The binding constant estimates indicated that ATP binds compound **2** *ca.* 35-times stronger than it does compound **1**. The binding curves indicated a definite 1:1 stoicheiometry for **2**:ATP complex, and not so well defined stoicheiometry for **1**:ATP binding. The mononucleotides, AMP and cAMP, and dinucleotide TpT did not show significant complexation with either compound **1** or **2**. The binding of ATP by macrocyclic polyamines **1** and **2** and the presence of a hydroxymethyl side-chain to link with a nucleophile may aid rational design of chemical nucleases.

Macrocyclic polyamines[1–7] have recently attracted attention as receptors for a variety of anions and, in particular, for halides,[1,8] carboxylates[9,10] and phosphates.[5–7] Compared with linear biogenic polyamines such as spermidine, spermine, *etc.*, macrocyclic polyamines offer several advantages for the complexation of anions. Macrocyclic polyamine systems have rigid conformations due to intramolecular N–H^+ ··· N hydrogen bonds. In contrast to the wide dispersion of cationic charge in linear polyamine systems, the geometric proximity of various nitrogens in macrocyclic polyammonium systems results in condensation of positive charge in a narrow space. Unlike other cationic systems such as metal ions, macrocyclic polyammonium ions can hydrogen bond with oxyanions *via* NH^+ protons, leading to stronger complexation through ion-pairs. Introduction of functionalised side-chains into the macrocyclic receptors in a defined way may provide means to impart additional features, such as catalysis, transport or other such processes to the complexones.



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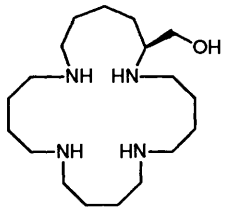

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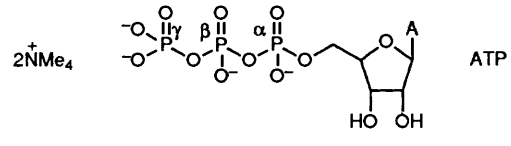


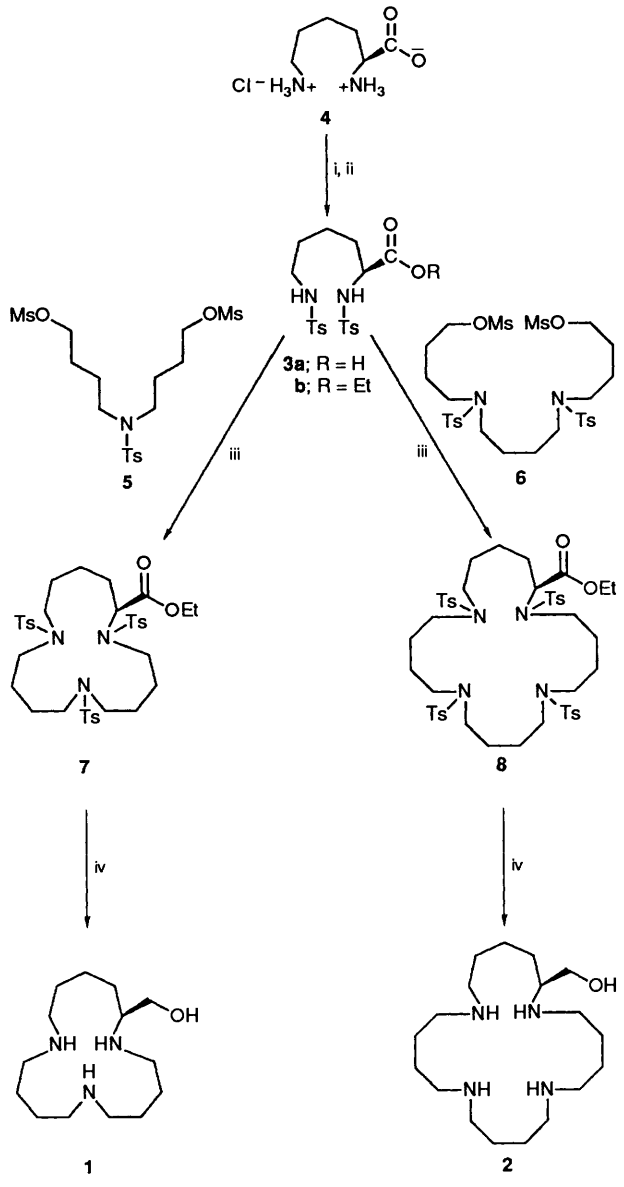
In order to understand and delineate the steric and electrostatic factors involved in molecular recognition of nucleotides, particularly ATP, by macrocyclic polyamines, we report here the synthesis of the hitherto unknown polyamine systems [16]-N_3 (**1**) and [21]-N_4 (**2**) which differ in ring size and the number of constituent nitrogens. Both contain a pendant hydroxymethyl side-chain which enables functionalisation of macrocycles to modulate the properties of these complexes. We also report here their differential binding characteristics towards ATP, as investigated by ^{31}P NMR spectroscopy. We notice that both complexes bind to ATP several orders of magnitude more strongly than that with mononucleotides such as AMP, 2′:3′-cAMP and ADP, and the dinucleotide TpT.

Results

Synthesis of Macrocyclic Polyamines **1** *and* **2**.—The macrocyclic polyamines were synthesized by identical routes but with appropriately different starting components, as shown in Scheme 1. The *N*,*N*′-ditosylamides **3**, common precursors for the syntheses, were prepared from lysine monohydrochloride **4**, in two steps. First, treatment with toluene-*p*-sulphonyl chloride in aq. solution (pH 12.0) and then esterification with ethanol–$SOCl_2$ gave ester **3b** in 84% yield. Macrocyclisation of compound **3b** in the presence of Cs_2CO_3 in dimethylformamide (DMF) with the alkylation partners **5** and **6** gave products **7** and **8**, respectively. Simultaneous detosylation and reduction of the side-chain carboxy group with sodium/liquid ammonia gave the macrocyclic polyamines **1** and **2**, respectively.

Compounds **5** and **6** were obtained by the route shown in Scheme 2. Alkylation of toluene-*p*-sulphonamide **10** and *N*,*N*′-ditosyltetramethylene-1,4-diamine **11** with 4-chloro-1-tetrahydropyran-2-yloxy butane **9** gave the bis-alkylated products **12a** and **13a**. These were transformed into the required compounds **5** and **6** by acid hydrolysis to remove tetrahydropyranyl groups, followed by mesylation to give products **5** and **6** in overall yields (starting from chloro compound **9**) of 22 and 25%, respectively.

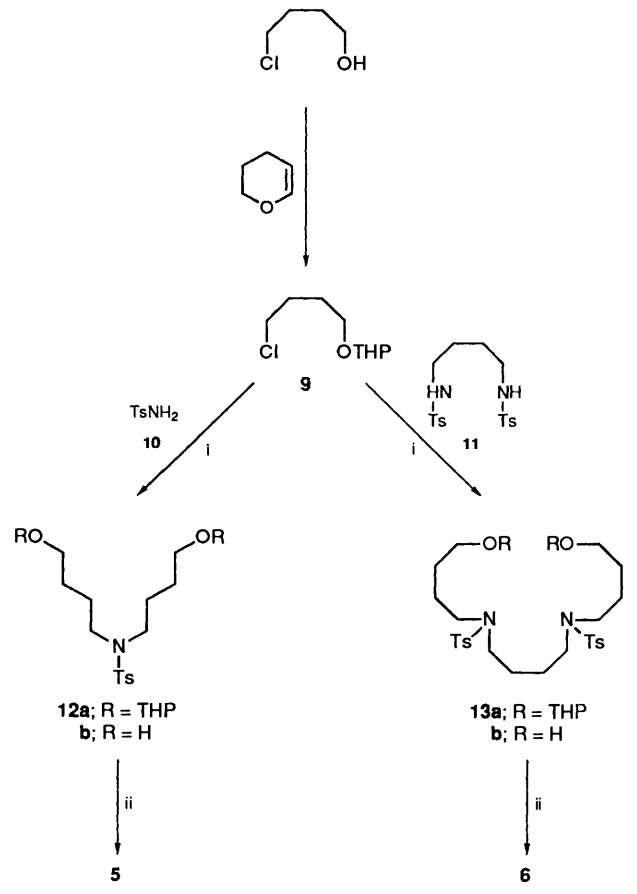
The macrocyclisation was achieved with Cs_2CO_3 by the procedure of Kellogg and co-workers,[11] which is a modification of the original Richman–Atkins[12] method of macrocyclic polyamine synthesis. The cyclisation was conducted by simultaneous dropwise addition of separate solutions of the required components **5** and **3**, or **6** and **3**, to a suspension of Cs_2CO_3 in DMF followed by heating overnight. The macrocycles **7** and **8** were produced in 33 and 37% yield,



Scheme 1 *Reagents and conditions:* i, aq. NaOH–Et_2O, *p*-TsCl, 14 h; ii, abs. EtOH, $SOCl_2$, 4 h; iii, Cs_2CO_3, DMF, 80 °C, 36 h; iv, Na–liq. NH_3, THF, −78 °C, 4 h

respectively, after column purification by silica gel chromatography. They were then detosylated with simultaneous reduction of side-arm carboxy groups to give hydroxymethyls by means of sodium/liquid ammonia to afford the target polyamines **1** and **2**, which were isolated and characterised as their corresponding tri- and tetra-hydrochloride salts.

^{31}P NMR Study of Polyamine–ATP Complexation.—^{31}P Chemical shifts are sensitive to changes in the electronic environment and the geometry of the phosphate ligand and hence the phosphate-binding ability of polyamines **1** and **2** was investigated by ^{31}P NMR spectroscopy. A solution of the tetramethylammonium salt of ATP, $(NMe_4)_2$·ATP·$3H_2O$, (20 mmol) in 20% D_2O–H_2O (pH 6.8) was titrated with stoicheiometric amounts of aq. solutions of cyclic polyamine hydrochlorides **1** and **2**. The observed changes in the ^{31}P NMR spectra of ATP at each successive addition of cyclic polyamine **2** are shown in Fig. 1. It was seen that upon addition of macrocyclic polyamine **2** only the γ-phosphorus of ATP exhibited significant upfield shifts, whose magnitude was a function of the added polyamine's concentration. The



Scheme 2 *Reagents and conditions:* i, NaH, DMF, 75–80 °C, 24 h; ii, 10% aq. HCl–MeOH; then MsCl–NEt_3, −5 °C

polyamine **1** also induced similar ^{31}P shift changes in ATP upon binding (not shown). The results of the binding studies of ATP with the polyamines **1** and **2** are represented in Fig. 2, with a plot of the ^{31}P chemical-shift differences as a function of polyamine concentration. It may be seen from these Figures that ATP binds strongly to polyamines **1** and **2**, with the stoicheiometry of the complexes being 1:1. A similar binding experiment with the disodium salt of ATP resulted in a 30% broadening of ^{31}P resonances of ATP without significant shifts for any of the phosphorus resonances (not shown). The complexation itself is a function of pH. Addition of a free base such as histamine to a polyamine–ATP complex in a stoicheiometric amount altered the pH to 9.0 and, at this point, the induced shifts were reversals of the initial complexation shifts but identical in magnitude.

The binding studies of the mononucleotides AMP and the 2′:3′-cyclic AMP and a dinucleotide, TpT, were similarly carried out in order to understand the specificity associated with binding. In all cases, broadening of the ^{31}P resonances of ATP from 9 Hz (linewidth at half-height) up to 30 Hz was observed without much change in chemical shift.

Discussion

Synthetic Strategy.—The required components **5** and **6** for the cyclisation reaction were synthesized by standard chain-extension alkylation (Scheme 2). Since these are symmetrical compounds they are easily obtained by bis-*N*-alkylation of appropriate precursor tosylamides. By a simple variation of the *N*-tosyl components **10** and **11**, different alkylating components for the macrocyclisation reaction are easily obtained. The bistosylamides **3** are common key intermediates for both syntheses. Compound **3a** was prepared easily from lysine

monohydrochloride and the α-carboxy group of compound **3a** is the progenitor of the side-arm in the final macrocycle. Its immediate lower homologue ornithine has been used previously[6,7] for synthesis of macrocycles [15]-N_3 and [18]-N_4. The extra methylene group of lysine affords an increase in the N • • • N distance in the 'northern' half of the macrocycle.

The synthesis of macrocyclic polyamines is easily effected through a cyclic bis-*N*-alkylation using metal ion-promoted templation. Among several methods known in the literature to effect macrocyclisation,[11–14] the Cs_2CO_3 method[11] seems to be a good choice for cyclisations involving 16–25-membered rings. The macrocyclisations proceeded smoothly with yields of ~33–37% and only small amounts of uncyclised derivatives

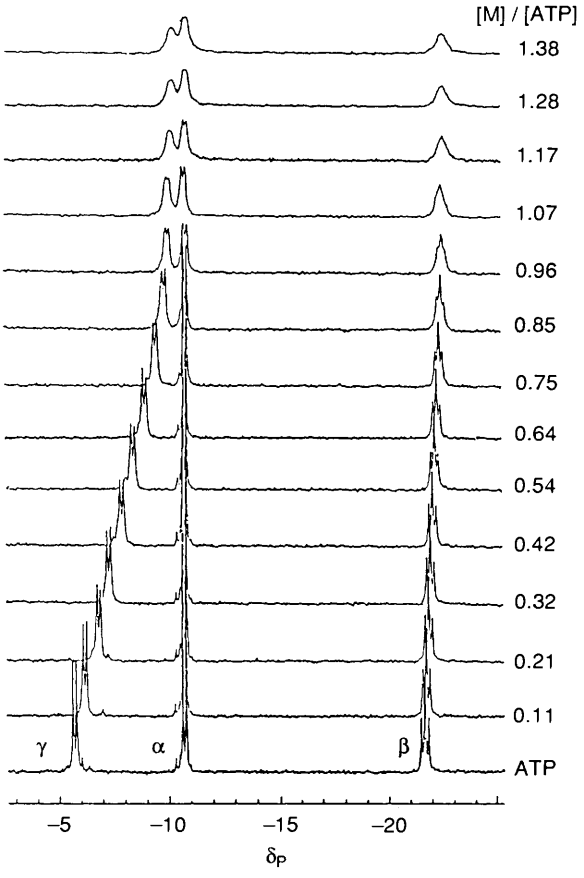


Fig. 1 ^{31}P NMR (121.5 MHz) spectra of (NMe_4)-ATP upon addition of polyamine [M], [21]-N_4, **2**. *Conditions:* 0.02 mol dm^{-3} ATP; 20% D_2O–H_2O; p*A* 6.8; 21 °C. The polyamine–ATP quotients ([M]/[ATP]) are indicated on the right side.

were isolable. The structural proof of cyclised products **7** and **8** was supported by ^1H NMR spectroscopy, which gave evidence for the presence of both components of the cyclisation: (i) the absence of methyl resonances due to the mesyl group at δ 3.0, (ii) the presence of protons due to the tosyl groups (ArMe) at δ 2.4, (iii) the resonance patterns and the increase in the integral values of various methylenes. Though we have not determined the enantiomeric purity, the stereogenic carbon is unlikely to epimerise under the cyclisation conditions.[11]

Among several reagents that were employed for detosylation of compounds **7** and **8**, the most effective was sodium in liquid ammonia. This reagent also caused the desired reduction of the pendant ethoxycarbonyl ester function into a hydroxymethyl group. The product macrocyclic polyamines **1** and **2** are extremely water soluble and care was taken to minimise isolation losses. Physical and chemical data are consistent with structures **1** and **2** (see Experimental section).

Complexation of Polyamines **1** *and* **2** *with Phosphates.*—In biological systems, phosphates are often found as complexed anions, particularly in transport systems.[1,10] To understand the molecular interactions involved in anion complexation, the polyammonium macrocyclic receptors **1** and **2** were used as model compounds. It was reported earlier[6,7] that [15]-N_3 and [18]-N_4 macrocyclic polyamines strongly associate with ATP. The association is attributed to charge–charge interactions of the polycation (polyamines) of high positive charge density with a polyanion (ATP^{4-}) of high negative charge. Since the polyamines are fully protonated at pH 7.0, a smaller ring size might be expected to be effective in binding as a consequence of its high charge density.

Figs. 1 and 2 depict the results of complexation studies as followed by ^{31}P NMR spectroscopy. The upfield shifts seen specifically for the γ-phosphorus of ATP indicate that this is sufficiently sensitive to polyamine binding and is the main target for macrocyclic polyamine–ATP complexation. The magnitude of the upfield shifts observed was a function of added polyamine concentration and, for γ-phosphorus, a limiting value of ~4.5 ppm was obtained. On further polyamine addition, no significant effect was observed. Compared with this, the limiting shifts for the α- and β-phosphorus atoms of ATP were ~1.0 and ~0.5 ppm, respectively.

The lower aza homologue [16]-N_3 gave, qualitatively, a similar upfield shift of the ^{31}P resonance from ATP, with the γ-phosphorus displaying shifts of a higher magnitude than the less significant shifts from the β- and α-phosphorus atoms. However, in contrast to [21]-N_4, no plateau was observed in the binding curve, even beyond the addition of molar equivalents of polyamines. The magnitude of the shift itself is about the same

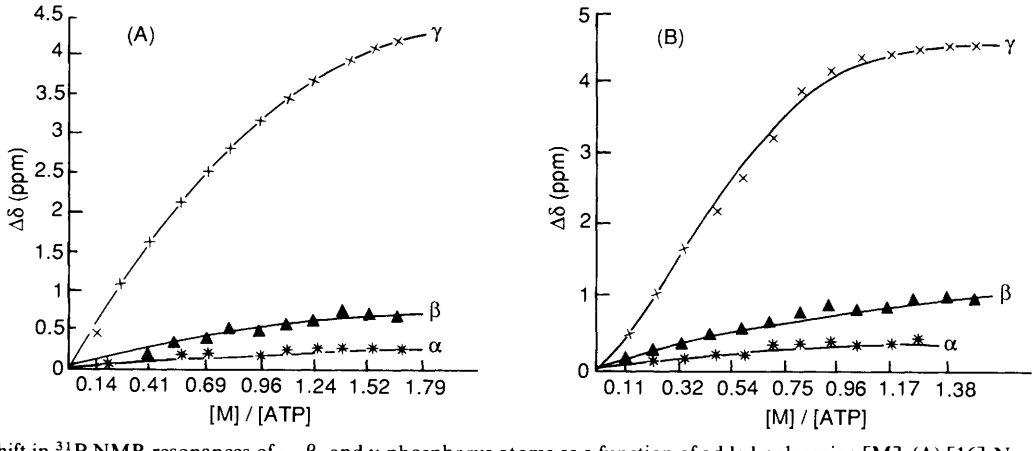


Fig. 2 Plot of shift in ^{31}P NMR resonances of α-, β- and γ-phosphorus atoms as a function of added polyamine [M]; (A) [16]-N_3 and (B) [21]-N_4. Other conditions as in Fig. 1.

order of magnitude (4.3 ppm) in both cases. The ^{31}P NMR data were analysed using HOSTEST-II software,* a program designed to assist investigations in molecular recognition and association constants. K_a-Values of 1.72×10^2 dm^3 mol^{-1} for **1** and 6.3×10^3 dm^3 mol^{-1} for **2** were obtained. These values interestingly indicate that the macrocycle [21]-N_4 **2** binds about 35-times more strongly to ATP than does [16]-N_3 **1**. Thus, although both polyamines act as receptors for the γ-P of ATP and induce upfield shifts of similar magnitude, the macrocycle [21]-N_4 **2** is a remarkably better receptor than is [16]-N_3 **1**. Further, it can be seen from the binding curves that compound **2** binds ATP at a definite stoicheiometry of 1:1, whereas that for compound **1** is not so well defined.

It has been reported[15] that the ^{31}P chemical shift of phosphorus is highly sensitive to the O–P–O bond angle, with a change of 1° inducing as much as 4 ppm shift difference. The complexation shifts in ATP may therefore be attributed to the conformational changes the triphosphate group undergoes upon association to the polyammonium cation of the macrocycle. The data in Fig. 2 indicate that though the overall shifts upon complexation to triamine **1** and tetramine **2** are similar in magnitude, the slopes of the binding curves are somewhat different. In the limit of infinitely strong binding, a straight line with a change to zero slope at 1:1 stoicheiometry would be expected. For the complexation of compound **2** with ATP, the binding curve is not only steeper but also resembles more closely the theoretically expected one for bimolecular association. In the case of macrocycle **1**, the binding curve has a slightly different profile, without showing a tendency towards a zero slope even beyond 1:1 stoicheiometry, indicating a possibility of higher order composition for the corresponding ATP complex. Because of the dependence of ^{31}P chemical shifts of phosphates on the O–P–O geometry in a major way compared with the electronic shielding parameters, the magnitude of the shift is not a true indication of strength of binding.

The weaker binding of [16]-N_3 **1** may be attributed to either or both of the following reasons: (i) the macrocycle is not in its fully protonated form at the reaction pH, (ii) the complexation is non-stoicheiometric, with multiple species contribution, apparently leading to an overall weak association. It has been reported previously that, compared to fully protonated species, non-fully protonated macrocyclic polyamines may show lower stability constants.[10] Our present results suggest that the sum of charge–charge interactions (electronic) may contribute to the association in a major way, causing the tetraprotonated† receptor **2** to interact strongly with the tetraanion ATP^{4-}. An assumption in suggesting such a possibility is that there are no inherent conformational differences in the polyamines **1** and **2**. Earlier work[6,7] has also indicated that the tetrammonium receptor [18]-N_4 with a larger ring size is a slightly better binding unit than its triammonium analogue [15]-N_3.

We have further noticed that the sodium salt of ATP does not bind strongly to the macrocycles **1** and **2**. In mixtures of the macrocycle and the salt, the phosphate resonances of ATP are only broadened, and are not shifted very much. However, the tetramethylammonium salt of ATP displayed significant binding to **1** and **2**. This counter-ion effect is perhaps caused by the fact that, while the bulky macrocycle cannot displace a 'hard' metal ion such as Na^+ from the anion shell, the spatially concentrated positive charge in macrocyclic polyamines can effectively compete with a 'soft' point-charged counter-ion such as N^+Me_4. We have also noticed similar counter-ion differences as well in the binding of the linear polyamines spermine and spermidine with ATP. Owing to the mechanistic nature of the interaction between ATP and a 'hard' metal ion,[16] the binding of polyammonium macrocycles to ATP may effect much larger conformational changes in O–P–O bond geometry than those seen due to binding of isocationic metal ions. Attempts to link the pendant hydroxymethyl group to side-chains containing nucleophilic groups such as imidazole, thiol, carboxylate or organometallics are in progress. Such compounds may effect catalytic hydrolysis of bound ATP, which is a topic of current interest due to its applications in the design of artificial chemical nucleases.[17]

Experimental

M.p.s were measured on a Yanaco micro melting point apparatus and are uncorrected and all compounds gave satisfactory C, H analysis. TLC analysis was carried out on precoated silica gel plates (E Merck, Art. No. 5554) and the solvent systems used were: A, $CHCl_3$–MeOH (10%), B, CH_2Cl_2. The spots were identified by iodine detection, ninhydrin spray or by acid spray (60% $HClO_4$–EtOH) depending on the nature of the compounds assayed. Lysine monohydrochloride, butane-1,4-diamine, 4-chlorobutan-1-ol, caesium carbonate (all from Aldrich) and 1,2-dihydropyran (Merck) were used without further purification. Tetrahydrofuran (THF) was distilled over sodium–benzophenone, and DMF was dried by storage overnight over KOH pellets, followed by distillation *in vacuo*. ^1H and ^{31}P NMR spectra were recorded on a Bruker MSL300 spectrometer; *J*-values are given in Hz. IR spectra were recorded with a Perkin-Elmer 599-B double-beam IR spectrometer. Mass spectra were run on a Finnigan MAT 1020 automated GC/MS instrument.

N,N-*Ditosylbutane*-1,4-*diamine* **11**.—To a cooled solution of butane-1,4-diamine (10 g, 113 mmol) in 1 mol dm^{-3} aq. NaOH (450 cm^3) was slowly added a solution of toluene-*p*-sulphonyl chloride (43.4 g, 230 mmol) in diethyl ether (150 cm^3). The reaction mixture was stirred for 4 h at room temperature. The separated ethereal layer was evaporated to yield crude *N,N'*-ditosylbutane-1,4-diamine. This was purified by column chromatography over silica gel (100–200 mesh) and eluted with EtOAc–hexane (1:1) to yield compound **11** (23.33 g, 85%), which was recrystallised from MeOH to a pale yellow solid, m.p. 139 °C; ν_{max}(Nujol)/cm^{-1} 3370 (N–H), 1600, 1480 (aromatic), and 1320 and 1160 (O=S=O); δ_H([$^2H_6$]acetone) 1.47 (4 H, m, 2 × CH_2), 2.38 (6 H, s, 2 × Ar*Me*), 2.78 (4 H, t, *J* 3, 2 × NCH_2), 7.31 (4 H, d, *J* 8, Ar*H*) and 7.67 (4 H, d, *J* 8, ArH).

1-*Chloro*-4-(*tetrahydropyran*-2-*yloxy*)*butane* **9**.—To an ice-cooled mixture of 4-chlorobutan-1-ol (10.85 g, 100 mmol) and dihydropyran (10.09 g, 120 mmol) was slowly added phosphorus trichloride oxide (0.5 cm^3). The reaction mixture was stirred at room temperature for 3 h, after which it was diluted with diethyl ether (50 cm^3) and washed with aq. KOH (5%; 2 × 50 cm^3). The ethereal layer was dried and evaporated to afford crude product **9** which was purified by distillation (b.p. 82–84 °C/2 mmHg) in 90% yield; δ_H(CCl_4) 4.53 (1 H, m, CH_2OCHO), 3.56 (6 H, overlapping, multiplets 2 × OCH_2, CH_2Cl) and 1.75 (10 H, m, 5 × CH_2).

N,N'-*Bis*(4-*hydroxybutyl*)*toluene*-p-*sulphonamide* **12b**.—To a solution of toluene-*p*-sulphonamide **10** (9.26 g, 54 mmol) in dry DMF (25 cm^3) was added NaH (1 g, 43 mmol) and the mixture

* We gratefully acknowledge Professor C. Wilcox, Chemistry Department, University of Pittsburgh for helpful discussions and for permission to use HOSTEST-II.

† Preliminary pH titration experiments have shown the first pK_a-value for macrocycle **2** to be 6.9, indicating that this compound remains in the tetraprotonated form under NMR experimental conditions. More accurate determinations of pK_a-values in this and compound **1** by potentiometric methods are in progress.

was stirred for 30 min. 1-Chloro-4-(tetrahydropyran-2-yloxy)-butane **9** (5 g, 26 mmol) was then added and the mixture was heated at 70–75 °C for 4 h. The reaction mixture was cooled, further batches of NaH (1 g, 43 mmol) and 1-chloro-4-(tetrahydropyran-2-yloxy)butane (5 g, 26 mmol) were added, and the mixture was heated overnight (70–75 °C). Reaction progress was monitored by the disappearance of compound **9** (TLC, solvent system A). The separated solid was filtered off and the filtrate, on concentration under reduced pressure, gave a brown oil. This was dissolved in 10% aq. HCl (150 cm^3; 36%) in MeOH and the mixture was stirred overnight to remove the tetrahydropyranyl group. Evaporation of the mixture under reduced pressure gave a residue, which was dissolved in CH_2Cl_2 (70 cm^3) and washed successively with aq. $NaHCO_3$ (2 × 75 cm^3) and water (2 × 75 cm^3). The organic phase was dried and concentrated to obtain a brown solid, which was purified by column chromatography and eluted with EtOAc–hexane to yield the pure product **12b** (5.1 g, 30%), m.p. 69–70 °C; ν_{max}(Nujol)/cm^{-1} 3300–3450 (O–H), 1150, 1330 (O=S=O), and 1040 (C–O); δ_H($CDCl_3$) 1.6 (8 H, m, 4 × CH_2), 2.4 (3 H, s, Ar*Me*), 3.18 (4 H, t, *J* 6, 2 × CH_2), 3.68 (4 H, t, *J* 6, 2 × OCH_2), 7.41 (2 H, d, *J* 8, ArH), and 7.77 (2 H, d, *J* 8, ArH); 314 [M − 1].

N,N′-*Bis*-(4-*hydroxybutyl*)-N,N′-*tetramethylenedi*(*toluene*-p-*sulphonamide*) **13b**.—*N,N′*-Ditosylbutane-1,4-diamine (7.6 g, 19 mmol) was dissolved in dry DMF (50 cm^3) and NaH (0.8 g) was added, and the mixture was stirred for 30 min. Compound **9** (3.64 g, 18.92 mmol) was added and the mixture was heated for 4 h at 75–80 °C. After the mixture had cooled, a second portion of NaH (0.8 g) was added followed by addition of more compound **9** (3.64 g, 18.92 mmol) and the mixture was heated at 75–80 °C for a further 40 h. Work-up was repeated as above to afford pure diamide **13b** (3.49 g, 34%) as a pale yellow gum; ν_{max}(neat)/cm^{-1} 3300–3500 (O–H), 1310 and 1140 (O=S=O), 1075 (C–N); δ_H($CDCl_3$) 1.57 (12 H, m, 6 × CH_2), 2.4 (6 H, s, 2 × Ar*Me*), 3.09 (8 H, t, *J* 5, 4 × NCH_2), 3.6 (4 H, t, *J* 5, 2 × OCH_2), 7.27 (4 H, d, *J* 8, ArH) and 7.64 (4 H, d, *J* 8, ArH).

2,6-*Bis*(*tosylamino*)*hexanoic Acid* **3a**.—To a stirred solution of lysine monohydrochloride (5 g, 82 mmol) in aq. NaOH (1 mol dm^{-3}; 390 cm^3) was gradually added a solution of toluene-*p*-sulphonyl chloride (39 g, 200 mmol) in diethyl ether (375 cm^3) and the reaction mixture was stirred for 14 h. The aq. layer was separated and acidified with aq. HCl (1 mol dm^{-3}). The precipitated oily material was extracted with CH_2Cl_2 (3 × 200 cm^3) and the residue obtained on evaporation was dissolved in NaOH (1 mol dm^{-3}; 100 cm^3) and precipitated with aq. HCl (1 mol dm^{-3}; 75 cm^3) to obtain compound **3a** as a solid (28.8 g, 75%), m.p. 152 °C; ν_{max}($CHCl_3$)/cm^{-1} 1730 (C=O) and 1320 and 1170 (O=S=O); δ_H($CDCl_3$) 1.31 (6 H, m, CH_2), 1.62 (2 H, m, CH_2), 2.4 (6 H, s, 2 × Ar*Me*), 2.75 (2 H, t, *J* 6, NCH_2), 3.82 (1 H, t, *J* 5.5, NCHCO), 7.44 (4 H, d, *J* 8, ArH) and 7.83 (4 H, d, *J* 8, ArH).

Ethyl 2,6-*Bis*(*tosylamino*)*hexanoate* **3b**.—Thionyl chloride (17.78 g, 150 mmol) was gradually added to a cooled suspension of 2,6-bis(ditosylamino)hexanoic acid **3a** (23.37 g, 50 mmol) in abs. EtOH (75 cm^3). The reaction mixture was refluxed for 4 h and the excess of EtOH was evaporated off. The residue was coevaporated three times with dry benzene to remove excess of thionyl chloride. The product was dissolved in $CHCl_3$ and washed successively with NH_4OH and water (2 × 100 cm^3). Removal of solvent from the organic layer gave crude product **3b**, which was purified by filtration through a column of silica gel (TLC grade) and eluted with ethyl acetate–MeOH (4:1) to give a solid (20.85 g, 84%), m.p. 124 °C; ν_{max}($CHCl_3$)/cm^{-1} 1740 (C=O), 1340 and 1170 (O=S=O) and 1230 (CO–O); δ_H($CDCl_3$) 1.1 (3 H, t, *J* 7, Me), 1.29–1.8 (6 H, m, 3 × CH_2), 2.4 (3 H, s, Ar*Me*), 2.42 (3 H, s, Ar*Me*), 2.9 (2 H, q, *J* 6, 3 × NCH_2), 3.9 (2 H, q, *J* 7, OCH_2), 3.76 (1 H, t, *J* 6, NCHCO), 7.25 (4 H, d, *J* 8, ArH) and 7.69 (4 H, d, *J* 8, ArH).

4,4′-*Toluene*-p-*sulphonyliminodibutyl Bismethanesulphonate* **5**.—*N,N*-Bis-(4-hydroxybutyl)toluene-*p*-sulphonamide **12b** (2.0 g, 6 mmol) and NEt_3 (2.3 g, 24 mmol) were dissolved in CH_2Cl_2 (25 cm^3) and the solution was chilled to −5 °C. Methanesulphonyl chloride was added dropwise while the temperature was kept below 0 °C. After 1 h the mixture was worked up by washing successively with ice–water (2 × 30 cm^3), 10% aq. HCl (2 × 30 cm^3), aq. $NaHCO_3$ (2 × 30 cm^3) and brine. On concentration of the CH_2Cl_2 layer a brown oil was produced, which was purified by column chromatography on silica gel (100–200 mesh). Elution with EtOAc–hexane (1:1) yielded diester **5** (2.2 g, 74%), m.p. 65–66 °C; ν_{max}($CHCl_3$)/cm^{-1} 1176 and 1351 (O=S=O); δ_H($CDCl_3$) 1.7 (8 H, m, 4 × CH_2), 2.4 (3 H, s, Ar*Me*), 3.0 (6 H, s, 2 × SO_2Me), 3.1 (4 H, t, *J* 6, 2 × CH_2), 4.2 (4 H, t, *J* 6, 2 × CH_2OSO_2), 7.4 (2 H, d, *J* 8, ArH) and 7.79 (2 H, d, *J* 8, ArH).

4,4′-[N,N′-*Tetramethylene*-N,N′-*bis*(*toluene*-p-*sulphonyl*)*diamino*]*dibutyl Bis*(*methanesulphonate*) **6**.—Compound **6** was prepared as above from compound **13b** (3.1 g, 6 mmol) and methanesulphonyl chloride (1.56 g, 14 mmol) in 73% yield, m.p. 118–120 °C; ν_{max}($CHCl_3$)/cm^{-1} 1176 and 1351 (O=S=O); δ_H($CDCl_3$) 1.42–2.0 (12 H, m, 6 × CH_2), 2.42 (6 H, s, 2 × Ar*Me*), 3.0 (6 H, s, SO_2Me), 3.1 (8 H, t, *J* 7, 4 × NCH_2), 4.24 (4 H, t, *J* 6, 2 × CH_2OSO_2), 7.31 (4 H, d, *J* 8, ArH) and 7.69 (4 H, d, *J* 8, ArH).

(S)-*Ethyl*-1,6,11-*Tritosyl*-1,6,11-*triazacyclohexadecane*-12-*carboxylate* **7**.—Ethyl *N,N′*-ditosyllysinate **3b** (2.85 g, 6 mmol) was dissolved in dry DMF (600 cm^3), Cs_2CO_3 (3.27 g, 17 mmol) was added, and the mixture was stirred for 30 min. To this was added a solution of compound **5** in dry DMF (240 cm^3), dropwise during 6–7 h. The mixture was then heated at 80 °C for 36 h. DMF was removed under reduced pressure, the residue was diluted with CH_2Cl_2 (40 cm^3), and the usual work-up gave a brown gum, which was purified by column chromatography with EtOAc–hexane (1:1) as eluent to obtain compound **7** (1.52 g, 33%), m.p. 97 °C; ν_{max}($CHCl_3$)/cm^{-1} 1740 (C=O) and 1140 and 1320 (O=S=O); δ_H($CDCl_3$) 1.25 (3 H, t, *J* 6, Me), 2.4 (9 H, s, Ar*Me*), 2.8–3.3 (10 H, m, NCH_2), 3.97 (3 H, overlapping ms, NCHCO, OCH_2), 7.25 (6 H, d, *J* 8, ArH) and 7.68 (6 H, d, *J* 8, ArH).

(S)-*Ethyl* 1,6,11,16-*Tetratosyl*-1,6,11,16-*tetraazacycloheneicosane*-17-*carboxylate* **8**.—Compound **8** was prepared according to the procedure described above, from ethyl *N,N′*-ditosyllysinate (2.61 g, 6 mmol) in dry DMF (570 cm^3), Cs_2CO_3 (5.03 g, 26 mmol), and compound **6** (4 g, 6 mmol) in dry DMF (228 cm^3) in 37% yield (1.82 g), m.p. 88 °C. $[\alpha]_{577}$ 5.41° (*c* 4.82, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 1740 (C=O), 1140 and 1320 (O=S=O) and 1080 (C–N); δ_H($CDCl_3$) 1.24 (3 H, t, *J* 7, Me), 1.6 (18 H, m, 9 × CH_2), 2.4 (12 H, s, 4 × ArMe), 3.09 (14 H, m, 7 × NCH_2), 3.9 (1 H, m, $NCHCO_2$), 4.13 (2 H, q, *J* 7, OCH_2), 7.28 (8 H, d, *J* 8, ArH) and 7.67 (8 H, d, *J* 8, ArH).

(S)-(1,6,11-*Triazacyclohexadecan*-12-*yl*)*methanol* **1**.—A solution of compound **7** (1 g, 1.3 mmol) in dry THF (30 cm^3) was slowly added to a solution of liquid NH_3 (260 cm^3) and sodium metal. When the blue colour had disappeared, more sodium was added until the blue colour persisted and the reaction mixture was stirred at −78 °C for 4 h. NH_4Cl (4 g) was added and NH_3 was slowly allowed to boil off. The solvent was evaporated off and the residue was dissolved in aq. AcOH (0.1 mol dm^{-3}; 30 cm^3); the solution was washed with diethyl ether (2 × 30 cm^3). The aq. layer was neutralised (pH 7.0) with

$NaHCO_3$ and the mixture was extracted with CH_2Cl_2 (1 × 25 cm^3) to compound **1** as a dark yellow gum (0.26 g, 77%). Dissolution of the gum in aq. HCl (1 mol dm^{-3}; 20 cm^3) followed by lyophilisation afforded the corresponding trihydrochloride a yellow solid, m.p. 266 °C (decomp.); ν_{max}(free amine, neat)/ cm^{-1} 3200–3440br (O–H, N–H) and 1050 (C–N); $\delta_H(D_2O)$ 1.6–1.9 (14 H, m), 3–3.3 (10 H, m, NCH_2) and 3.5–3.75 (3 H, m, NCH, CH_2OH); *m/z* 256 [M − 1].

(S)-(1,6,11,16-*Tetraazacycloheneicosan*-17-*yl*)*methanol* **2**.—Compound **2** was prepared by following the same procedure as above, from compound **8** (1.44 g, 2 mmol), dry THF (70 cm^3) and liquid NH_3 (300 cm^3) in 73% yield (0.46 g). The tetrahydrochloride had m.p. 280 °C (decomp.); $[\alpha]_D$ +6.98° (*c* 0.47, water); ν_{max} (free amine, neat)/cm^{-1}; 3300, 3190 (N–H, O–H), 1620 (N–H) and 1040 (C–N); $\delta_H(CDCl_3)$ 1.21–1.61 (18 H, m, 9 × CH_2), 2.88–3.15 (12 H, m, 6 × NCH_2) and 3.6 (2 H, t, CH_2OH).

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