Ligands derived from C-aryl substituted derivatives of cyclen: formation of kinetically unstable complexes with lanthanide(III) ions

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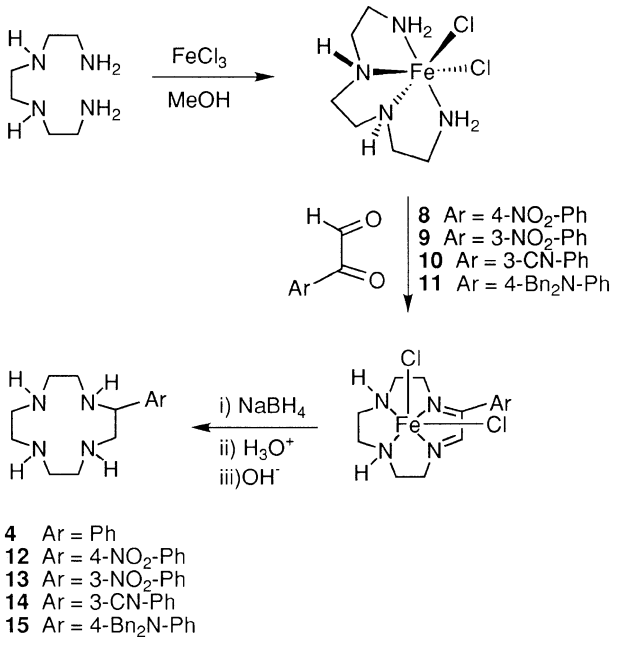
The synthesis of C-aryl monosubstituted derivatives of 1,4,7,10-tetraazacyclododecane (cyclen) by metal templated reductive amination of triethylenetetramine with aryl glyoxals has been developed and shown to be widely applicable. Octadentate ligands derived from these C-substituted macrocycles form relatively labile eight-coordinate complexes, which dissociate when challenged with EDTA in aqueous solution at pH 5.5, suggesting their use in metal-transport processes rather than for applications *in vivo*.

In recent years, lanthanide complexes of ligands derived from the aza-macrocycle tetraazacyclododecane (cyclen), **1**, have found a variety of diagnostic applications, particularly as luminescent probes[1,2] and as contrast agents for magnetic resonance imaging (MRI).[3,4] The use of lanthanide complexes *in vivo* imposes certain restrictions on the properties of the complexes. The complexes formed must be kinetically robust and physiologically well tolerated, owing to the inherent toxicity of free lanthanide ions, which tend to accrete in skeletal tissue and bone.[5] 1,4,7,10-Tetraazacyclododecane, or 'cyclen', provides a rigid skeleton to which pendent donor groups are readily attached. Examples of such ligands include the tetraacetate 'dota' (1,4,7,10-tetraazacyclododecane tetraacetate) **2**,[6] and the phosphinate analogue, **3**.[7] In both of these systems, the lanthanide is held in an eight-coordinate square antiprismatic environment and the ring adopts a regular square [3333] conformation. The lanthanide complexes of such octadentate ligands are kinetically as well as thermodynamically stable owing to the cooperative binding of the ring nitrogen and pendent arm oxygen donors.[8]

The published syntheses of substituted derivatives of cyclen are all rather lengthy and relatively inefficient,[9] rendering diagnostics based on such systems very expensive. We have recently published a single-step synthesis of C-aryl substituted azamacrocycles,[10] which relies on a metal-templated reaction between triethylenetetramine and aryl glyoxals (Scheme 1). This synthesis proceeds in good yield (40–70%) for a wide range of aryl glyoxals. We report herein details of the ring synthesis and the results of studies on the solution complexation behaviour of selected octadentate ligands.

Results and Discussion

2-Phenyl-1,4,7,10-tetraazacyclododecane, **4**, was prepared using the general procedure outlined in Scheme 1. Reaction of phenyl glyoxal with the iron(III) complex of triethylenetetraamine, **5**, prepared *in situ* by addition of triethylenetetraamine to a methanolic solution of iron(III) chloride, yielded the diimine, **6**. The cyclisation step is likely to proceed *via* the *cis*-dichloro complex, **7**.[11] In this configuration, the two terminal nitrogen atoms are situated close to one another, ideally placed to form the macrocyclic diimine. In early experiments, the diimine was isolated and its existence confirmed by IR ($\nu_{C=N}$ 1636 cm^{-1}), UV (λ_{max} = 254 nm) and electrospray mass spectrometry (*m*/*z* 245.24). However, the diimine complex tends to degrade on exposure to air and moisture, and it was found that the isolated yields of the macrocycles were significantly improved when the complex was not isolated, and reduction was carried out *in situ* using sodium borohydride. Reduction of the diimine complex also results in the reduction of the metal centre from Fe^{III} to Fe^{II}, associated with a change in colour from deep orange to pale green. After acidic work-up to remove the coordinated iron(III) ion, extraction of the free base into chloroform was effected at pH 14. Subsequent filtration through a short plug of activated alumina, allowed the 2-phenyl cyclen, **4**, to be isolated in good yield. The use of other transition-metal ions as templates, specifically nickel(II) and chromium(III), was also investigated. However, although the cyclisation processes proceeded efficiently in all cases, removal of the metal ion proved considerably more difficult with these ions and accordingly their use was not investigated further. In theory, cyclisation of an aryl glyoxal with triethylenetetraamine may yield four possible products, including the 12-ring cycle **4** (Fig. 1). Each of the other three possibilities is a primary amine. Reaction of **4** and **13** with acetyl chloride–Et_3N–CH_2Cl_2 yielded an amide which was purified by reverse phase high performance liquid chromatography (HPLC). Analysis by mass spectrometry (*e.g.*,



Scheme 1

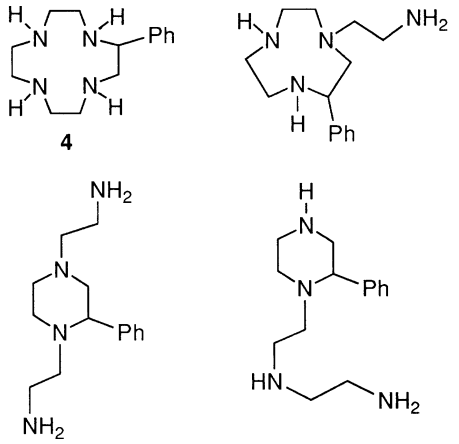
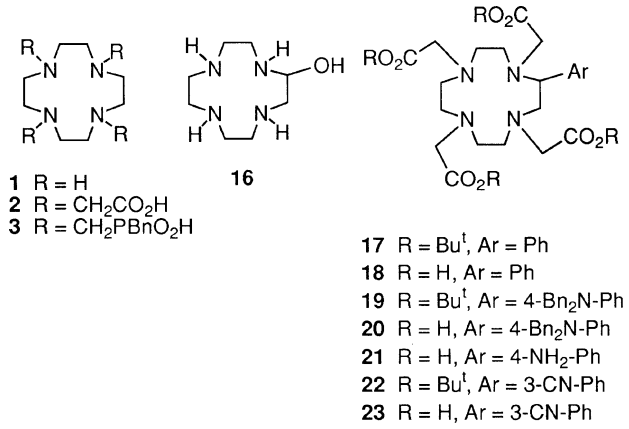


Fig. 1



Scheme 2

presence of molecular ion in ESMS at 417.15 for $[HAc_{44}]^+$) and IR (absence of amide NH band at 3250 cm^{-1}) confirmed that a tetraamide had been formed , consistent only with the formation of the 12-ring isomer .

A series of substituted C-aryl cyclens never prepared previously was also synthesised by this pathway with a view to simplifying conjugation of the ligand to various targeting vectors. Nitro-, cyano- and dibenzylamino-phenyl substituted cyclens were chosen for this purpose, as they present readily modified functionalities which can be used to attach the macrocycle to a suitable targeting vector. In these cases, the precursor substituted aryl glyoxals **8**–**11** were synthesised by oxidation of the corresponding acetophenones using selenium dioxide, according to literature procedures.[12] Owing to the equilibria that exist between the keto, enol and hydrate forms of these compounds, characterisation and purification of these compounds proved rather difficult. The desired glyoxals eventually crystallised in a partially hydrated form from aqueous solution. Templated cyclisation reactions of **8**–**11** with triethylene tetramine yielded the macrocycles **12** to **15** in good yields. Indeed, the cyclisation reaction would appear to be widely applicable to aryl glyoxal systems. However, the 4-substituted nitrophenyl cyclen **12** was found to be rather unstable in air and in aqueous solution. This instability was ascribed to the enhanced lability of the 2° benzylic hydrogen leading to ring opening of the macrocycle assisted by the electron accepting capacity of the nitro group. By contrast, each of the *meta*-substituted macrocycles (**13**, **14**), were stable in air and aqueous solution, consistent with this hypothesis.

Extension of this cyclisation procedure to the preparation of cyclen itself was then investigated. However, attempted cyclisations using either glyoxal itself, its hydrate or its sulfite addition compound did not yield cyclen in any significant quantity. No clear evidence for the formation of the diimine intermediate could be obtained by ^1H NMR or electrospray mass spectrometry. It is likely that the aryl group is necessary to stabilise the intermediate diimine: the increased conjugation resulting from formation of a planar array may play a part, as the glyoxals themselves exist mainly as their hydrates. This hypothesis is supported, in part, by the fact that after borohydride reduction, ESMS showed a small peak corresponding to the hemiaminal **16** (m/z = 189, $[M + H]^+$).

Synthesis of octadentate ligands

Formation of C-substituted analogues of dota was investigated using procedures similar to those employed with the parent compound. Treatment of the C-phenyl derivative **4**, with an aqueous solution containing 4 molar equiv. of sodium chloroacetate, using sodium hydroxide solution (1 M) to maintain the pH in the range 9–10, resulted in formation of the pentaalkylation product as the major product. This problem was overcome by slow addition of only 3 equiv. of *tert*-butyl bromoacetate to the macrocycle. Following ester cleavage with trifluoroacetic acid, the desired phenyl-dota analogue **18** was isolated in moderate yield.

The tetra *tert*-butyl esters, **17** and **19** were prepared similarly by alkylation with *tert*-butyl bromoacetate in acetonitrile solution. Once again, formation of the quaternary salt occurred readily and the addition of 3 equiv. of alkylating agent was found to be a reasonable compromise between a lower conversion and the avoidance of over alkylation. Purification of the tetra-esters was achieved by column chromatography on alumina, and subsequent hydrolysis using trifluoroacetic acid in dichloromethane yielded the desired octadentate ligands in an acceptable yield.†

The complexation properties of ligand **18** were then investigated. The europium and terbium complexes were prepared by treatment of an aqueous solution of the ligand with the lanthanide(III) nitrate salt while maintaining the pH around 5.5. Subsequent trituration with acetone yielded the complex, which was identified by electrospray mass spectrometry.

Luminescence measurements in H_2O and D_2O were used to probe the hydration state of the complexes. Quenching of the excited state of a bound lanthanide ion by proximate O—D oscillators is much less efficient than by O—H oscillators owing to the reduced Franck–Condon overlap of the higher vibrational harmonics of the O—D oscillator with the energy levels of the metal centre. Hence, assuming all other factors remain constant, the number of bound water molecules in solution may be related to the observed rate constants for the decay of the metal-based luminescence in H_2O and D_2O (k_{H_2O} and k_{D_2O} respectively), according to:

$$q = A(k_{H_2O} - k_{D_2O}) \quad (1)$$

where A is an empirically determined proportionality constant (A_{Eu} = 1.05 s, A_{Tb} = 4.2 s).[13]

The observed luminescent rate constants are shown in Table 1. They demonstrate that the solution state behaviour of $[Ln \cdot \mathbf{18}]^-$ is analogous to that of dota: each lanthanide ion is 9-coordinate, and possesses one bound water molecule. Deviations from this integral number can by explained by invoking contributions from outer-sphere water molecules, which also contribute to quenching.[14]

However, when the europium complexes were challenged with a tenfold excess of EDTA at pH 5.5, their behaviour diverged considerably from that which was expected. The observed lifetimes [τ_{H_2O}(Eu) < 0.1 ms and τ_{D_2O}(Eu) 0.5 ms]

† In the case of compound **20** a biphasic alkylation (CH_2Cl_2–NaOH) yielded **19** with a yield of 35%, after purification by silica chromatography. Subsequent hydrolysis using trifluoroacetic acid in CH_2Cl_2 gave **20** in quantitative yield.

Table 1 Radiative rate constants for decay of the metal-based emission in H_2O and D_2O for europium and terbium complexes (293 K, pH 5.5, $\lambda_{exe} = 250$ nm)[a]

Complex	k_{H_2O}/ms^{-1}	k_{D_2O}/ms^{-1}	$\Delta k/ms^{-1}$	q'[b]
$[Eu \cdot \mathbf{18}]^-$	1.75	0.63	1.12	1.09
$[Eu \cdot dota]^-$	1.56	0.41	1.15	1.12
$[Tb \cdot \mathbf{18}]^-$	1.89	1.61	0.28	1.15
$[Tb \cdot dota]^-$	0.71	0.49	0.22	0.92

[a] Errors on k values are $\pm 10\%$. [b] A subtraction of 0.25 ms^{-1} has been applied, in the case of the Eu complexes only, to account for the effect of outer-sphere (closely diffusing) OH oscillators, followed by multiplication by 1.25 (A′).

were consistent with dissociation of the macrocyclic complex, suggesting that the [Ln EDTA]$^-$ complex was being formed preferentially. This behaviour clearly indicates that these complexes do not have the kinetic stability associated with their dota analogues, and implies that their thermodynamic stability is considerably reduced ($\beta_{ML} < 10^{10}$, *cf.* 10^{25} for dota) since EDTA is able to extract the metal. These results were confirmed with $[Gd \cdot \mathbf{21}]^-$ following a challenge with a ten-fold excess of EDTA at pH 5.5 followed by ESMS mass spectrometric analysis. Only [Gd EDTA]$^-$ was observed in the negative-ion mass spectral analysis, after 30 min. In fact in the positive-ion mode the appearance of the free ligand $[\mathbf{21} \cdot H]^+$ was evident. This clearly precludes the use of such complexes for *in vivo* applications as diagnostic agents, but suggests that they may find application in the *transport* of lanthanide ions, given that complex lipophilicity may be readily tuned by C-aryl substitution.

By considering the crystal structures of the lanthanide complexes of dota,[14] it is possible to hypothesise that the lability of the complex may arise from unfavourable steric interactions between the aryl group attached to the macrocyclic ring and the adjacent methylene protons on the ring. These interactions may prevent the complex from taking up the favourable square antiprismatic geometry and will inhibit ring inversion. In an attempt to verify this hypothesis, complexation reactions were carried out under conditions of high temperature and pressure (up to 180 °C in a sealed tube for 72 h). However, no formation of the putative thermodynamically stable complex was observed, and it is possible that the activation barrier to formation of such a complex is too high to be readily overcome.

Conclusion

Lanthanide complexes formed by C-aryl functionalised cyclen derivatives are prevented from adopting a stable square [3333] ring conformation by the large steric bulk of the aryl group. Though the complexes are unsuitable for use as imaging and contrast agents, they may have applications in lanthanide extraction and transport, which could merit further investigation.

Experimental

Details of the experimental techniques used in this work, together with the instrumentation employed for product characterisation have been described previously.[15,16]

2-phenyl-1,4,7,10-tetraazacyclododecane, 4

A solution of triethylenetetraamine, **5** (400 mg, 2.73 mmol) in methanol (20 ml) was added dropwise over 10 min to a solution of iron(III) chloride (739 mg, 2.73 mmol) in methanol (40 ml). The mixture was stirred at room temperature for 2 h. A solution of phenyl glyoxal (366 mg, 2.73 mmol) in methanol (45 ml) was then added to the reaction and the mixture stirred for a further 4 h. Sodium borohydride (10 equiv. 1000 mg, 27.3 mmol) was added and the mixture heated to 50 °C under reflux with stirring (2 h). The solvent was then removed under reduced pressure and the resulting orange/brown foam was dissolved in water (50 ml) and the pH adjusted to 1 (conc. HCl). The aqueous layer was extracted with dichloromethane (3 × 50 ml) and the pH readjusted to 14 using solid NaOH. The basified aqueous layer was then exhaustively extracted with chloroform, the organic extracts dried (K_2CO_3) and filtered. Removal of the solvent under reduced pressure gave a yellow oil (612 mg, 90%). This was further purified by filtration through a plug of basic alumina ($MeOH–CH_2Cl_2$, 15 : 100). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.3 (2H, d, $^3J = 7.2$ Hz), 7.24 (2H, t, $^3J = 7.6$ Hz), 7.17 (1H, t, $^3J = 7.2$ Hz), 3.77 (1H, dd, $^3J = 10.4$ Hz, $^3J = 2.8$ Hz, H-2), 2.99 (1H, $J = 11.6$ Hz, $^3J = 2.8$ Hz), 2.94 (1H, dt, $J = 11.2$ Hz, $^3J = 2.80$ Hz), 2.80 (1H, br t, $^3J = 10.8$ Hz, H-3), 2.80 (1H, t, $J = 10.8$ Hz), 2.73 (2H, t, $^3J = 5.60$ Hz), 2.65 (2H, t, $^3J = 6.4$ Hz), 2.6 (2H, t, $^2J = 5.6$ Hz), 2.44 (2H, t, $^3J = 5.6$ Hz), 2.09 (1H, dt, $J = 11.2$ Hz, $^3J = 3.3$ Hz), 1.97 (1H, t, $^3J = 10.4$ Hz, H-3′), 1.9 (4H, s, NH). ^{13}C NMR ($CDCl_3$) : 142.32 (C-l′), 128.19 (C-3′), 127.28 (C-4′), 126.81 (C-2′), 61.37 (C-3), 60.15 (C-2), 57.99, 53.17, 52.30, 46.02, 45.99, 41.44. *m/z* (ESMS, +) 249.14 (100%, $[M + H]^+$).

4-Nitrophenyl glyoxal, 8

To a solution of 1,4-dioxane (18 ml) and water (2.4 ml) was added *para*-nitroacetophenone (5 g, 30.2 mmol) and selenium dioxide (3.35 g, 30.2 mmol). The reaction mixture was heated under reflux for 5 h. The mixture was allowed to cool and left at room temperature overnight. The resulting mixture was redissolved in dioxane (20 cm^3), filtered and the residue washed with hot dioxane. Solvent was then removed under reduced pressure to produce a viscous orange oil. The oil was dissolved in the minimum volume of boiling water and left to cool, yielding pale-yellow crystals. The supernatant was then concentrated and the process repeated. This procedure was repeated until no further crystals were obtained (3.63 g, 67%). NMR analysis revealed a mixture of the pure compound and its monohydrate. *m/z* 151.96 (31%, $MH^+ - CO_2$), 179.47 (2.5%, LH^+). ^1H NMR ($CDCl_3$) δ 9.67 (0.24H, s, CHO), 8.35 (5.24H, m, aromatic), 5.96 [1H, s, broad, $C(OH)_2H$], 4.0 [2H, s, broad, $C(OH)_2H$]. ^{13}C NMR (CD_3OD) : 195.53, 152.84, 150.09, 148.71, 140.72, 132.82, 131.42, 125.47, 124.50, 102.03, 93.53.

3-Nitrophenyl glyoxal, 9

Selenium(IV) oxide (3.35 g, 30.2 mmol) and 3-nitroacetophenone (3.71 g, 27.5 mmol) were dissolved in a 1,4-dioxane(18 ml)–water(2.4 ml) mixture. The solution was heated under reflux for 18 h and then allowed to cool to room temperature. The selenous salts were removed by filtration through celite and the solvents removed under vacuum. The brown residue was purified by column chromatography (20% tetrahydrofuran in dichloromethane). The solvents were removed *in vacuo* and the orange residue distilled twice (Kugelröhr) (120 °C, 0.5 mbar) to yield a bright yellow waxy

solid (2.1 g, 39%); *m/z* (EI^+) 179 (M^+, 6%), 150 ($[M - HCO]^+$, 100%), 133 ($[M - NO_2]^+$, 28%), 104 ($[M - NO_2 - HCO]^+$, 52%). $R_f = 0.3$ (5% tetrahydrofuran in dichloromethane, SiO_2). ^1H NMR ($CDCl_3$, 250 MHz) δ 7.75 (1H, t, $^3J = 8.0$ Hz, H-5), 8.52 (2H, m, H-4 and 6), 9.00 (1H, t, $^4J = 1.5$ Hz, H-2), 9.64 (1H, s, CHO). ^{13}C NMR ($CDCl_3$, 50 MHz) : 123.8 (d), 127.4 (d), 128.7 (s), 128.8 (d), 134.4 (d), 147.1 (s, C-N), 183.8 (s, Ar-C=O), 197.2 (d, CHO); ν_{max} (film)/cm^{-1} 3452 br (H_2O), 3089, 1705 (C=O), 1614, 1531 (NO_2), 1480, 1439, 1351 (NO_2), 1086, 1000, 910, 811, 728. Anal. calcd for $C_8H_5NO_4 \cdot 0.2H_2O$: C, 52.6; H, 3.0, N, 7.7. Found: C, 52.5; H, 3.4; N, 7.3%.

3-Cyanophenyl glyoxal, 10

Selenium(IV) oxide (3.35 g, 30.2 mmol) and 3-acetylbenzonitrile (3.98 g, 27.5 mmol) were dissolved in a 1,4-dioxane(18 ml)–water(2.4 ml) mixture. The solution was heated under reflux for 18 h and then allowed to cool to room temperature. The selenous salts were removed by filtration through celite and the solvents removed under vacuum. The brown residue was eluted over silica gel with 5% tetrahydrofuran in dichloromethane. The solvents were removed *in vacuo* and the orange residue distilled twice (Kugelröhr; 140 °C, 0.5 mbar) to yield a bright-yellow waxy solid (3.32 g, 76%). mp, 65–73 °C. $R_f = 0.4$ (SiO_2, 5% tetrahydrofuran in dichloromethane). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.66 (1H, t, $^3J = 7.8$ Hz, H-5), 7.92 (1H, dt, $^3J = 7.8$ Hz, $^4J = 1.5$ Hz, H-4), 8.43 (1H, dt, $^3J = 7.8$ Hz, $^4J = 1.5$ Hz, H-6), 8.47 (1H, t, $^4J = 1.5$ Hz, H-2), 9.60 (1H, s, aldehyde). ^{13}C NMR ($CDCl_3$, 75 MHz) : 113.8 (nitrile), 117.7 (*aromatic*-CN), 130.3 (C-5), 131.8 (C-1), 134.4 (C-4), 134.5 (C-6), 137.8 (C-2), 185.5 (CO), 188.8 (CHO). ν_{max} (thin film)/cm^{-1} 3078, 2979, 2936, 2234 (CN), 1700 (br, C=O), 1599, 1579, 1479, 1421, 1291, 1269, 1102, 1047, 984, 912, 786, 732, 678. *m/z* (CI^+) = 336 (100%, $2M + NH_4^+$). Anal. calcd for $C_9H_5NO_2 \cdot 0.2H_2O$: C, 66.7; H, 3.3; N, 8.6. Found: C, 66.7; H, 3.4; N, 8.6%.

N,*N*-Dibenzyl-*p*-aminophenyl glyoxal, 11

Selenium(IV) oxide (27 g, 0.063 mol) and *N*,*N*-dibenzyl-*p*-aminoacetophenone (20 g, 0.063 mol) were dissolved in a 1,4-dioxane(130 ml)–water(7 ml) mixture. The solution was heated under reflux for 18 h and then allowed to cool to room temperature. The selenous salts were removed by filtration through celite and the solvents removed under reduced pressure. The residue was purified by filtration through SiO_2 with CH_2Cl_2–heptane (70 : 30) and finally CH_2Cl_2, in order to obtain the unsolvated 1,2-dicarbonyl as a yellow-orange oil (12.5 g, 60%). ^1H NMR ($CDCl_3$, 200 MHz) δ 9.63 (1H, s, CHO), 6.77–7.98 (14H, m, ArH), 4.73 (4H, s, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz): 190.2 (CO), 190.0 (CHO), 153.7 (C—N); 136.7, 133.4, 131.6, 129.6, 129.0, 128.1, 120.8, 112.0, 53.9 (CH_2N).

2-(4'-Nitrophenyl)-1,4,7,10-tetraazacyclododecane, 12

To a stirring solution of iron(III) chloride (739 mg, 2.73 mmol) in methanol (40 ml) was added dropwise over 10 min a solution of triethylenetetramine (400 mg, 2.73 mmol) in methanol (20 ml) which was left to stir at room temperature for 2 h. To the reaction mixture was added a solution of *para*-nitrophenyl glyoxal (489 mg, 2.73 mmol) in methanol (45 ml) and the mixture was left for a further 4 h. Sodium borohydride (10 equiv. 1000 mg, 27.3 mmol) was added and the mixture heated under reflux (16 h). The methanol was removed under reduced pressure, the resulting brown foam was dissolved in water (50 ml) and the pH adjusted to 1 (conc. HCl). The aqueous layer was extracted with dichloromethane (3 × 50 ml), and the pH was readjusted to pH 14 with solid NaOH. The aqueous layer was extracted exhaustively with dichloromethane, the organic extracts were combined, dried (K_2CO_3) and the dichloromethane removed under reduced pressure to give a pale-brown oil (560 mg). This was further purified by reverse phase HPLC using Hypersil ODS semi-preparative column, λ = 258 nm, $t = 0$ min, A = 90% H_2O (0.1% trifluoroacetic acid), B = 10% MeCN (0.1% trifluoroacetic acid); $t = 20$ min, A = 100% MeCN (0.1% trifluoroacetic acid); $t = 25$ min end; $t_R = 5.81$ min. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.10 (2H, d, $^3J = 8.8$ Hz), 7.51 (2H, d, $^3J = 8.8$ Hz), 3.92 (1H, dd, $^3J = 10.4$ Hz, $^3J = 2.8$ Hz, H-2), 3.04 (1H, tt, $J = 8.8$ Hz, $^3J = 2.4$ Hz), 2.95 (1H, dt, $J = 11.2$ Hz, $^3J = 2.80$ Hz), 2.81 (1H, t, $J = 10.8$ Hz), 2.81 (1H, t, $J = 10.8$ Hz, H-3), 2.75 (2H, t, $^3J = 5.60$ Hz), 2.65 (2H, t, $^3J = 6.4$ Hz), 2.63 (2H, t, $^3J = 6.0$ Hz), 2.47 (2H, t, $^3J = 6.4$ Hz), 2.11 (1H, dt, $^2J = 14.4$ Hz, $^3J = 3.2$ Hz), 1.95 (1H, br t, $J = 10.4$ Hz, H-3'), 1.83 (4H, s, NH). ^{13}C NMR ($CDCl_3$, 75 MHz): 149.94, 147.24, 127.84, 123.61, 61.26, 59.71 (CHN), 58.07, 53.01, 52.49, 46.11, 45.87, 41.61. *m/z* (ESMS, +), 294.16 (100%, LH^+).

2-(3'-Nitrophenyl)-1,4,7,10-tetraazacyclododecane, 13

This was prepared using a procedure analogous to that reported for **12**, using iron(III) chloride (760 mg, 2.8 mmol.), triethylenetetraamine (400 mg, 2.73 mmol.), *m*-nitrophenylglyoxal (490 mg, 2.73 mmol) and sodium borohydride (800 mg, 21.8 mmol). The product was purified by chromatography on silica gel (83% CH_2Cl_2, 15% MeOH, 2% NH_4OH) to yield a pale-yellow oil (180 mg, 26%); *m/z* (ES+) 294.13 (MH^+, 100%), 251, 246. ^1H NMR ($CDCl_3$, 300 MHz) δ 8.28 (1H, s, H-2'), 8.10 (1H, d, $J = 7.5$ Hz, H-4'), 7.71 (1H, d, H-6'), 7.46 (1H, t, H-5'), 3.95 (1H, dd, $J = 11.5$, 2.6 Hz, CHN), 3.12–2.71 (14H, m, ring CH_2N), 1.90 (4H, br s, NH). ^{13}C NMR ($CDCl_3$, 75 MHz) 148.9, 145.4, 133.9, 129.9, 123.1, 122.7; 62.0 (CH_2N); 60.1 (CHN); 58.8, 53.8, 53.3, 46.7, 46.6, 42.4 (ring CH_2N).

2-(3'-cyanophenyl)-1,4,7,10-tetraazacyclododecane, 14

Iron(III) chloride hexahydrate (0.74 g, 2.7 mmol) was dissolved in methanol (20 ml). Under argon a solution of triethylenetetraamine (0.40 g, 2.7 mmol) in methanol (10 ml) was added dropwise over 30 min, resulting in an orange-brown precipitate. *meta*-Cyanophenyl glyoxal, **10**, (0.40 g, 2.7 mmol) was dissolved in methanol (20 ml) and added to the triethylenetetraamine iron(III) complex. The reaction was left to stir at room temperature for 4 h under argon before $NaBH_4$ (3 × 0.4 g pellets, 32 mmol) was added over 30 min. The reaction was stirred for a further 2 h after which 2M HCl (*ca.* 10 ml) was added until a clear-yellow solution was obtained. The volatiles were removed under vacuum and the remaining solution was washed with dichloromethane (50 ml). The pH was then raised to 14 by addition of NaOH pellets and extracted with $CHCl_3$ (3 × 200 ml). The organics were combined, dried over K_2CO_3 and the solvents removed. The residue was purified by column chromatography over silica gel (10% methanol, 10% ammonia, 80% tetrahydrofuran) to give a pale-brown oil, (0.4 g, 54%). ^1H NMR ($CDCl_3$, 250 MHz) δ 2.0–3.2 (14H, br m, CH_2N), 2.37 (4H, br s, NH), 3.90 (1H, dd, CHN), 7.45 (1H, t, $^3J = 7.8$ Hz, H-5'), 7.56 (1H, d, $^3J = 7.8$ Hz, H-6') 7.64 (1H, d, $^3J = 7.8$ Hz, H-4'), 7.73 (1H, s, H-2'). ^{13}C NMR ($CDCl_3$, 50 MHz) : 41.5 (CH_2N), 45.8 (CH_2N), 46.0 (CH_2N), 52.4 (CH_2N), 53.0 (CH_2N), 58.0 (CH_2N), 59.4 (CHN), 61.2 (CH_2N), 112.3 (CN), 118.7 (C-1'), 129.1 (C-6'), 130.7 (C-5'), 131.1 (C-2'), 131.6 (C-4'), 143.9 (C-3'). ν_{max} (film)/cm^{-1}; 3540 (NH), 3018, 2844 (CH), 2236 (CN), 1434, 1202, 1136, 904, 837, 806, 718, 694. *m/z* (ES+): 274 (100% , $M + H^+$).

2-(*N*,*N*-dibenzyl-*p*-aminophenyl)-1,4,7,10-tetraazacyclododecane, 15

Iron(III) chloride hexahydrate (1.35 g, 4.99 mmol) was dissolved in MeOH (80 ml). A solution of triethylenetetraamine

(0.73 g, 5 mmol) in MeOH (40 ml) was added dropwise. The mixture was stirred for 2 h and then a solution of *N*,*N*-dibenzyl-*p*-aminophenyl glyoxal (1.65 g, 3 mmol) in THF(35 ml)/MeOH(55 ml) was added dropwise. The reaction was left to stir at room temperature for 6 h under Ar before $NaBH_4$ (1.9 g, 50 mmol) was added. The mixture was heated under reflux for 16 h and then allowed to cool to room temperature. The salts were removed by filtration through charcoal. The volatiles were removed under reduced pressure and the residue was dissolved in water at pH 10. This solution was extracted with CH_2Cl_2 (3 × 100 ml), the organic extracts were combined, dried over Na_2SO_4 and the solvent removed. The residue was dissolved in water and acidified with HCl (6 M) and then washed with CH_2Cl_2 (3 × 50 ml). Water was removed under reduced pressure and the remaining solution was added dropwise to 300 ml of EtOH to yield pale-brown crystals of the tetrahydrochloride salt which were filtered off and dried *in vacuo* (0.9 g, 31%). *m*/*z* (ESMS, +) 444 ($[M + H^+]^+$, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.83 (14H, m), 4.61 (4H, s, CH_2Ph), 3.85 (1H, dd, H-2), 3.11 (14H, br m, CH_2N), 2.4 (4H, br s, NH). t_R [C_{18}-5μ-'Lichrosphere' (Merck); KH_2PO_4(10 mM)–(CH_3CN) 50 : 50; pH 2.7, 1 ml min^{-1}] 3.4 min.

N,*N*′,*N*″,*N*‴-Tetrakis(*tert*-butoxycarbonylmethyl)-2-phenyl-1,4,7,10-tetraazacyclododecane, 17

2-Phenyl cyclen, **4**, (0.37 g, 1.5 mmol) and caesium carbonate (1.74 g, 5 mmol) were dissolved in acetonitrile (5 ml) under argon. *tert*-Butyl bromoacetate (0.88 g, 4.5 mmol) was dissolved in acetonitrile (10 ml) under argon and added to the solution over 10 min by cannula transfer at room temperature. The reaction mixture was left stirred for 3 h and was monitored by ESMS analysis, showing the extent of tetraester formation and the onset of pentaalkylation. The solvents were then removed under reduced pressure, the residue taken up into dichloromethane (25 ml) and the inorganics removed by filtration. The solvents were removed under reduced pressure and the residue purified by column chromatography over silica gel. The column was washed with dichloromethane before the title compound was eluted with 20% tetrahydrofuran in dichloromethane. Removal of the solvents under reduced pressure yielded a colourless oil (0.26 g, 25%). R_f = 0.6 (SiO_2, 15% tetrahydrofuran in dichloromethane). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.44 (36H, s, Bu^t), 2.0–3.25 [16, m, CH_2N (ring) + CH_2N], 3.40 (2H, s, CH_2NCO), 3.53 (4H, s, CH_2NCO), 3.70 (1H, dd, ^3J = 7.5 Hz, ^3J = 2.5 Hz, CHN), 7.1–7.3 (5H, m, arom. CH). ^{13}C NMR ($CDCl_3$, 50 MHz) : 26.4 (CH_3), 50.4 (ring CH_2N), 50.8 (CH_2N), 51.7 (CH_2N), 51.9 (CH_2N), 54.6 (CH_2N), 55.3 (CH_2N), 55.6 (CH_2N), 60.3 (NCH_2CO + CHN), 63.5 (NCH_2CO), 79.0 (s, *C*—Me), 79.2 (s, *C*—Me), 79.4 (s, *C*—Me), 126.9 (C-2′), 127.3 (C-4′), 128.6 (C-3′), 142.3 (s, C-13′), 168.4 (CO), 169.6 (CO), 169.8 (CO); ν_{max} (thin film)/cm^{-1} 2970, 2814, 1733 (CO_2Bu^t), 1475, 1455, 1390, 1367, 1152, 910. *m*/*z* (ES+) 704 (100%, $M + H^+$), 727 (95%, $M + Na^+$). Anal. calcd for $C_{39}H_{63}N_5O_8 \cdot 2H_2O$: C, 63.2; H, 9.14, N, 7.76. Found: C, 62.8; H, 9.38; N, 7.51%.

N,*N*′,*N*″,*N*‴-Tetrakis(carboxymethyl)-2-phenyl-1,4,7,10, tetraazacyclododecane, 18

The tetra-*tert*-butyl ester **17** (141 mg, 0.2 mmol) was dissolved in 50% trifluoroacetic acid in dichloromethane (3 ml) and the solution was stirred for 24 h. The solvents were then removed under vacuum and dichloromethane (3 × 5 ml) added and then removed under vacuum. Methanol (10 ml) was added and removed under vacuum before the oily residue was triturated twice from diethyl ether. After decanting the solvents and drying under vacuum a colourless solid was obtained in quantitative yield, mp 133–135 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 2.45–3.33 (16H, m, ring NCH and CH_2CO), 3.43 (4H, s, CH_2CO), 3.65 (2H, s, CH_2CO), 3.83 (1H, dd overlapping, ^3J = 8.0 Hz, ^3J = 7.5 Hz, CHAr), 6.96 (1H, t, ^3J = 7.8 Hz, H-5′), 7.08 (1H, br d, ^3J = 7.8 Hz, H-6′), 7.22 (1H, d, ^3J = 7.8 Hz, H-4′), 7.31 (1H, s, H-2′); ν_{max} (Nujol mull)/cm^{-1} 2975, 2820, 1715 (CO_2H), 1646 (CO_2^-), 1304, 1202, 978, 966, 918. *m*/*z* (ESMS) 481 ($[M + H]^+$), 504 ($[M + Na]^+$).

N,*N*′,*N*″,*N*‴-Tetrakis(*tert*-butoxycarbonylmethyl)-2-(*N*,*N*-dibenzyl-*p*-aminophenyl)-1,4,7,10-tetraazacyclododecane, 19

To an emulsion of 20 g (34 mmol) 2-(*N*,*N*-dibenzyl-*p*-aminophenyl)-1,4,7,10-tetraazacyclododecane in water (110 ml) and dichloromethane (100 ml), at 10 °C, an aqueous solution of NaOH (90.5 ml, 3 mol dm^{-3}) was added slowly. The mixture was stirred at 15 °C for 15 min before a solution of *tert*-butyl bromoacetate (27.14 g, 0.139 mol) in 100 ml CH_2Cl_2 was added dropwise. The reaction was stirred for a further 4 h at the same temperature and then at room temperature for a further hour. After decanting, the organic layer was washed with water (2 × 90 ml) and dried over Na_2SO_4 and the solvent removed under reduced pressure. The residue was purified by column chromatography over silica gel (CH_2Cl_2–MeOH, 96 : 4). A second purification step was necessary involving column chromatography over silica gel (AcOEt–MeOH, 95 : 5) after which an orange oil was obtained (11 g, 36%). *m*/*z* (ES+) 450.5 ($[M + 2H]^{2+}$, 100%); 900 ($[M + H]^+$, 18%). Anal. calcd for $C_{52}H_{77}N_5O_8 \cdot H_2O$: C, 68.2; H, 8.6; N, 7.6. Found: C, 68.1; H, 8.6; N, 7.6%. t_R [5μ-diol (Merck), 98 : 2,heptane–ethanol; 1 ml min^{-1}] 7.3 min.

N,*N*′,*N*″,*N*‴-Tetrakis(carboxymethyl)-2-(*N*,*N*-dibenzyl-*p*-aminophenyl)-1,4,7,10-tetraazacyclododecane, 20

A solution of the tetra-*tert*-butyl ester, **19**, (11 g, 0.012 mol) in CF_3COOH (110 ml) was stirred overnight under Ar at room temperature. The solvent was then removed and the residue dissolved in water in order to be purified by filtration on reverse phase silica (R.P. 2, Merck) eluting with water(40%)–MeOH(60%). The solvents were removed under reduced pressure and acetone was added to the remaining oil. White crystals were obtained after filtration (6.5 g, 79.3%). *m*/*z* (ES+), 676 ($[M + H]^+$ 100%); t_R [C_{18}-5 μ, Lichrosphere (Merck); KH_2PO_4(10 mM)–CH_3CN, 60 : 40; pH 3.1; flow 1 ml min^{-1}] 5.1 min.

Tetrakis(carboxymethyl)-2-(*p*-aminophenyl)-1,4,7,10-tetraazacyclododecane, 21

To a solution of the tetraacid (2.5 g, 3.7 mmol) in MeOH (105 ml) and water (45 ml), 0.5 g of Pd/C (10%) was added and the mixture maintained at room temperature under a pressure of hydrogen of 22 psi,‡ with vigorous stirring for 12 h. The catalyst was removed by filtration through charcoal and the solvents were removed under reduced pressure. A white solid was obtained (1.8 g, 100%).

[Gd.21]$^-$

To a stirred solution of the aminoacid (1.7 g, 3.43 mmol) in water (30 ml) was added $GdCl_3 \cdot 6H_2O$ (0.8 g, 3.43 mmol). The pH was raised with NaOH (5 M) and adjusted to 6. The mixture was heated at 60 °C for 5 h and left at room temperature overnight. The pH was readjusted to 6 with NaOH (1 M). To this solution 9 ml of the ion exchange resin 'CHELEX' was added. One hour later, the pH was raised to 7. The mixture was concentrated under vacuum and the remaining solution added dropwise to MeOH (100 ml). The product was filtered and washed with MeOH (3 × 20 ml). This pro-

‡ 1 psi ≈ 6.89 × 10^3 Pa.

cedure was repeated twice. White crystals were obtained (1.3 g, 59%). *m/z* (ES−) 829 ([M − H]$^-$, 100%). t_R [C_{18}-Lichrosphere, 5μ (Merck); KH_2PO_4(10 mM)–CH_3CN, 70 : 30; pH 2.8; flow 1 ml min^{-1}] 27.8 min.

N,*N*′,*N*″,*N*‴-Tetrakis(*tert*-butoxycarbonylmethyl)-2-(3′-cyanophenyl)-1,4,7,10-tetraazacyclododecane, 22

meta-Cyanophenyl cyclen, **14**, (0.37 g, 1.3 mmol) and caesium carbonate (1.74 g, 5 mmol) were dissolved in acetonitrile (5 ml) under argon. *tert*-Butyl bromoacetate (0.78 g, 4 mmol) was dissolved in acetonitrile (10 ml) under argon and added to the solution over 10 min by cannula transfer at room temperature. The reaction was stirred for 4 h and followed by ESMS analysis. The solvents were then removed *in vacuo* and the residue taken up into dichloromethane (20 ml), and the inorganics were removed by filtration. The solvents were removed under vacuum and the residue purified by column chromatography over silica gel. The column was washed with dichloromethane before the title compound was eluted with 20% tetrahydrofuran in dichloromethane. Removal of the solvents under reduced pressure yielded a colourless oil (0.21, 22%). $R_f = 0.7$ (SiO_2, 20% tetrahydrofuran in dichloromethane); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.47 (36H, s, Bu^t), 2.0–3.2 [16, m, CH_2N (ring) + CH_2N], 3.42 (2H, s, CH_2NCO), 3.51 (4H, s, CH_2NCO), 3.74 (1H, dd, ^3J = 7.5 Hz, ^3J = 2.5 Hz, CHN), 7.48 (1H, t, ^3J = 8.0 Hz, H-5′), 7.63 (1H, d, ^3J = 8.0 Hz, H-6′), 7.70 (1H, d, ^3J = 8.0 Hz, H-2′), 7.77 (1H, s, H-2′). ^{13}C NMR ($CDCl_3$, 50 MHz) : 26.7 (CH_3), 50.1 (ring CH_2N), 50.7 (CH_2N), 51.7 (CH_2N), 51.9 (CH_2N), 54.8 (CH_2N), 55.1 (CH_2N), 55.3 (CH_2N), 60.5 (NCH_2CO + CHN), 63.1 (NCH_2CO), 79.3 (s, *C*—Me), 79.4 (s, *C*—Me), 79.5 (s, *C*—Me), 111.2 (CN), 117.3 (C-1′), 127.9 (C-6′), 129.4 (C-6′), 130.3 (C-5′), 131.2 (C-4′), 141.3 (s, C-3′), 168.1 (CO), 169.2 (CO), 169.4 (CO). ν_{max} (thin film)/cm^{-1} 2977, 2817, 2229 (CN), 1731 (CO_2Bu^t), 1479, 1455, 1392, 1367, 1156, 916, 851, 801, 735, 697. *m/z* (ES+) 730 (100%, M + H$^+$), 752 (95%, M + Na$^+$); Anal. calcd for $C_{39}H_{63}N_5O_8 \cdot 2H_2O$: C, 61.1; H, 8.7, N, 9.15. Found: C, 60.6; H, 8.30; N, 9.00%.

N,*N*′,*N*″,*N*‴-Tetrakis(carboxymethyl)-2-(3′-cyanophenyl)-1,4,7,10-tetraazacyclododecane, 23

The tetra-*tert*-butyl ester **22** (112 mg, 0.15 mmol) was dissolved in 50% trifluoroacetic acid in dichloromethane (3 ml) and the solution was stirred for 24 h. The solvents were then removed under vacuum and dichloromethane (2 × 5 ml) added and then removed under vacuum. Methanol (5 ml) was added and removed under vacuum before the oily residue was triturated twice from diethyl ether. After decanting the solvents and drying under vacuum a colourless solid was obtained in quantitative yield, mp 119–122 °C. ^{1}H NMR (CD_3OD, 300 MHz) 2.50–3.37 (16H, m, cyclen ring and 1 × acetate), 3.43 (4H, s, 2 × acetates), 3.65 (2H, s, 1 × acetate), 3.89 (1H, dd overlapping, ^3J = 8.2 Hz, ^3J = 7.9 Hz, CHAr), 6.96 (1H, t, ^3J = 7.8 Hz, H-5′), 7.08 (1H, br d, ^3J = 7.8 Hz, H-6′), 7.22 (1H, d, ^3J = 7.8 Hz, H-4′), 7.31 (1H, s, H-2′). ν_{max} (Nujol mull)/cm^{-1} 2234 (CN), 1718 (CO_2H), 1650 (CO_2^-), 1304, 1202, 978, 966, 918, 834, 800, 720; *m/z* (ESMS) 506 ([M + H]$^+$).

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