Synthesis, characterisation and anti-protozoal activity of carbamate-derived polyazamacrocycles†

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Received 10th July 2007, Accepted 17th September 2007
First published as an Advance Article on the web 26th September 2007
DOI: 10.1039/b710487a

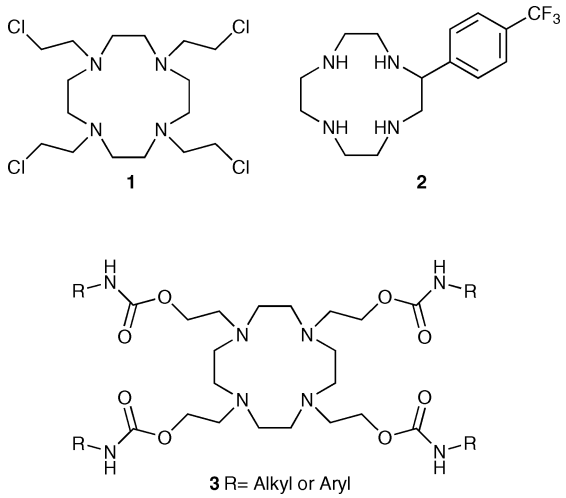
A short, highly efficient approach for the synthesis of a novel class of polyazamacrocycles containing *N*-functionalised carbamate side-chains has been developed. The key steps involved a phase-transfer mediated macrocyclisation to form the ring system as well as a tin-catalysed reaction with isocyanates to introduce the carbamate side-chains. X-Ray crystallography confirmed successful formation of the 1,4,7,10-tetraazacyclododecane ring and *N*-functionalisation of all the amine centres. Preliminary testing of the biological activity of the compounds revealed significant anti-parasitic activity against bloodstream form African trypanosomes.

Introduction

Macrocyclic polyamines have received much attention due to their ability to complex both organic and inorganic substrates.[1] Their structures are easily manipulated allowing the preparation of a variety of ring sizes as well as the addition of different side-chain arms through *N*-functionalisation.[2,3] Tuning the nature of these arms allows the creation of smart molecules with specific physical and chemical properties, and thus they have found many useful applications in fields such as medicinal[4] and analytical chemistry[5] and NMR imaging.[6]

Our interest in these compounds arose from the development of alkylating agents which could effectively cross-link DNA and thus act as anti-tumour agents. Accordingly, we synthesised a series of polyazamacrocycles with chloroethyl arms (**1**) which were as cytotoxic as the well-known anti-cancer agents, chlorambucil and melphalan.[4c,7] Complexation of these compounds with copper(II) allowed the generation of a series of bioreductive prodrugs which were selectively cytotoxic to hypoxic rather than oxic cells.[4b] More recently, we have synthesised a series of cyclen derived compounds with various alkyl and aryl groups attached to the carbon backbone of the macrocycle (*e.g.* **2**) and have shown that these compounds are toxic to both trypanosomes and to malarial parasites.[8] Having prepared biologically active polyazamacrocycles with either reactive side-chains or with pharmacokinetic tunable substituents, we were interested in designing a new class of tetraazamacrocycle which combined both of these concepts.

We now report the highly efficient synthesis of a small library of carbamate-derived polyazamacrocycles **3**, compounds which still contain cytotoxic side chains but whose pharmacokinetics can be easily manipulated by judicious choice of carbamate substituent.



Preliminary biological testing of these compounds as anti-parasitic agents is also described.

Results and discussion

Our first goal in this project was to develop an efficient synthesis of 1,4,7,10-tetraazacyclododecane which would provide the core for the new compounds. Although polyazamacrocycles are especially difficult to prepare, one method which has been commonly used for their synthesis is the Richman–Atkins cyclisation.[9] This approach generally involves the coupling of a dianion generated from a sulfonamide-protected linear polyamine with a ditosylate of a diol. Sulfonamides are used to protect the polyamine as they allow easy formation and stabilisation of the dianion as well as providing a Thorpe–Ingold-type effect on the transition state, promoting intramolecular cyclisation rather than intermolecular oligomerisation.[10] Using such an approach, 1,4,7,10-tetraazacyclododecane **9** was synthesised in three linear steps as shown in Scheme 1. Diethylene triamine **4** was tritosylated in 99% yield using *p*-toluenesulfonyl chloride in the presence of sodium hydroxide. The other coupling partner,

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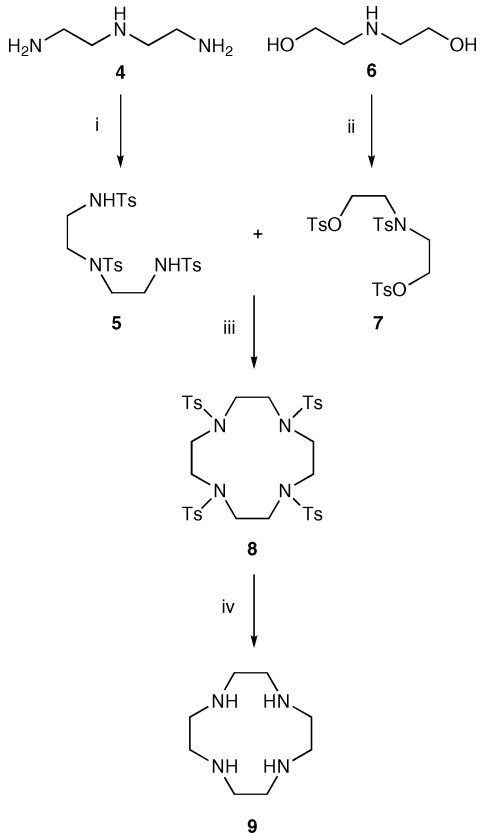
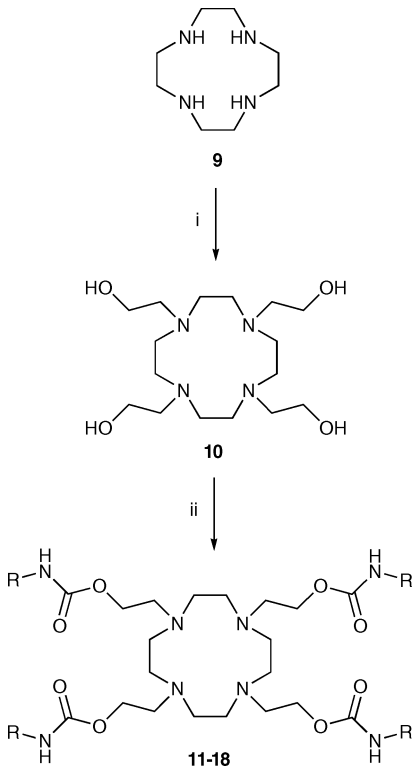
† Crystallographic data available for compound **11**. CCDC reference numbers 653591. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b710487a



Scheme 1 *Reagents and conditions*: i. TsCl, NaOH, H_2O, Et_2O, 99%; ii. TsCl, NaOH, TEBA, CH_2Cl_2, 91%; iii. method 1: Cs_2CO_3, DMF, 14 d, 79%, method 2: LiOH, Bu_4NBr, toluene, 14 h, 71%; iv. method 1: PhOH, HBr–AcOH, 46%, method 2: H_2SO_4, 110 °C, 13%, method 3: H_2SO_4, HBr then NaOH, toluene, Δ, 88%.

N,*O*,*O*′-tri(toluene-4-sulfonyl)diethanolamine **7** was prepared in a similar fashion from diethanolamine **6** in 91% yield. The two coupling partners were subjected to a Richman–Atkins cyclisation using caesium carbonate as the base. While this did generate the polyazamacrocycle **8** in 79% yield, reaction times were typically around 14 days. To overcome this, an alternative procedure involving a phase transfer catalyst was also investigated and this gave **8** in a similar yield after only 14 hours.[11] The next stage was deprotection of the polyazamacrocycle and, in an effort to optimise the yield of this transformation which normally requires very harsh conditions, a number of procedures were investigated. Direct deprotection using phenol and a hydrobromic acid–acetic acid mixture or hot sulfuric acid gave 1,4,7,10-tetraazacyclododecane **9** in modest or low yields.[12] However, a two-step procedure involving formation of the hydrobromide salt followed by treatment with sodium hydroxide under Dean–Stark conditions gave **9** in an excellent 88% yield.

Functionalisation of polyazamacrocycle **9** was easily achieved by reaction with gaseous ethylene oxide which gave the tetraol **10** in quantitative yield (Scheme 2).[4b] Initial synthesis of the phenyl carbamate **11** was accomplished in a quantitative yield using phenyl isocyanate and pyridine.[13] However, attempted synthesis of other carbamate analogues using this procedure led to decomposition of both the isocyanate and the tetraol **10**. An alternative procedure involving the reaction of **10** with the appropriate isocyanate in the presence of catalytic dibutyltin diacetate was then investigated.[14] This led to the formation of a range of alkyl and aryl derived polyazamacrocyclic derivatives in modest to excellent yield.

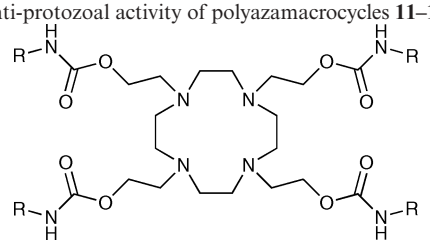


Scheme 2 *Reagents and conditions*: i. ethylene oxide, H_2O, 100%; ii. RNCO, $Bu_2Sn(OAc)_2$, CH_2Cl_2, Δ, R = Ph (**11**), 100%; iPr (**12**), 60%; *n*Pr (**13**), 41%; 4-MeOPh (**14**), 56%; 4-BrPh (**15**), 64%; 2-NO_2Ph (**16**), 79%; 3-NO_2Ph (**17**), 46%; 4-NO_2Ph (**18**), 60%.

All of the carbamate-derived polyazamacrocycles were purified by recrystallisation and showed spectroscopic data consistent with their structures. However, to confirm successful ring synthesis and *N*-functionalisation of all the amine centres, an X-ray crystal structure determination of the phenyl analogue **11** was undertaken. Compound **11** crystallises in the monoclinic space group $P21/a$ (Fig. 1, see also supporting information†) and the structure clearly shows formation of the tetraazacyclododecane ring and the four carbamate side-chains.

On successful preparation of this new class of polyazamacrocycles, we were interested in probing their anti-protozoal activity against bloodstream form African trypanosomes from *Trypanosoma brucei* (Table 1). The development of useful anti-parasitic agents requires the compounds to have low toxicity to human cells. Thus, the carbamates were also tested against human embryonic kidney (HEK) cells. As shown in Table 1, a number of these compounds do have significant trypanocidal activity in *in vitro* assays. Interestingly, the most potent anti-trypanosomal compounds (**16**, **17** and **18**) all possess a nitro-group and trypanosomes have previously been shown to be highly vulnerable to nitrocyclic compounds.[15] Moreover, carbamates **13** and **17** in particular are significantly less active against mammalian HEK cells and therefore these compounds provide some scope for the future development of more potent and selective analogues. Previous studies on the anti-protozoal activity of cyclic polyamines

Table 1 Anti-protozoal activity of polyazamacrocycles **11–18**



R	Estimated log P	*T. brucei* EC_{50}/μM	HEK EC_{50}/μM
iPr (**12**)	1.1	15.7	77.1
*n*Pr (**13**)	1.4	18.1	>200
Ph (**11**)	4.7	10.9	53.0
4-MeOPh (**14**)	5.0	4.2	18.1
4-BrPh (**15**)	8.2	6.4	24.9
2-NO_2Ph (**16**)	6.2	0.9	18.8
3-NO_2Ph (**17**)	6.2	2.4	87.0
4-NO_2Ph (**18**)	6.2	0.5	1.3[a]

[a] Compound **18** shows a biphasic curve: the EC_{50} value reported in the table refers to the first sigmoidal shift. The EC_{50} value of the second shift is 74 μM.

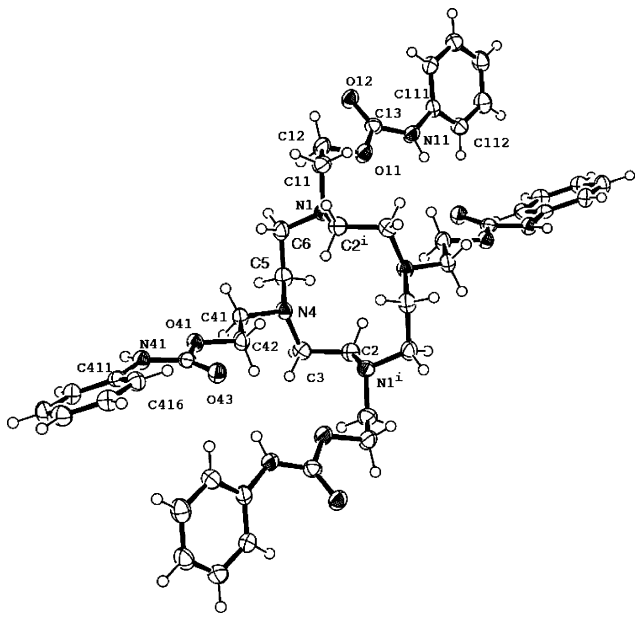


Fig. 1 Crystal structure of **11**.

have shown a dependence on the lipophilicity of the compound suggesting uptake might be limiting.[8] Surprisingly, the carbamate-derived polyazamacrocycles show no such dependence. At this stage it is not known by what mode of action these compounds exert their anti-protozoal activity, and thus future work will involve the preparation of structural analogues to probe both the transport of these compounds through the parasite's plasma membrane and the subsequent cytotoxic mechanism.

Conclusions

In summary, we have developed a short, highly efficient synthesis of a novel class of polyazamacrocycles using a phase-transfer mediated macrocyclisation and a tin-catalysed isocyanation of a tetraol to effect the key steps. The aim of this work was to produce macrocyclic structures which have both cytotoxic side-arms and groups which could be manipulated to tune the pharmacokinetic properties of these potential drugs. We have already shown these compounds to have significant cytotoxic activity against trypanosomes from *T. brucei* and future work will now investigate the biological mode of action of these compounds as well as the development of more potent and selective analogues.

Experimental

All reactions were carried out under an inert atmosphere unless otherwise stated, using oven-dried or flame-dried glassware. Solutions were added *via* syringe unless otherwise stated. Tetrahydrofuran and diethyl ether were freshly distilled from Na-benzophenone; dichloromethane, toluene, *N*,*N*-dimethylformamide and pyridine were distilled from CaH_2 prior to use. Petroleum ether refers to the fraction boiling at 40–60 °C. Reagents were obtained from Aldrich Chemical Company (Gillingham, Dorset, UK), Alfa Aesar Lancaster (Morecambe, Lancs., UK), or Alfa Aesar Avocado (Heysham, Lancs., UK) and used without further purification unless otherwise stated. Purification by column chromatography was carried out using Fisher Silica 60A silica gel (mesh size 35–70 μm) as the stationary phase. Melting points were measured using Gallenkamp apparatus and are uncorrected. IR spectra were recorded using Golden Gate, nujol or KBr on a JASCO FT/IR 410 spectrometer. NMR spectra were recorded using a Bruker AV400 or DPX/400 spectrometer. Chemical shifts are given in ppm relative to $SiMe_4$ where δ $SiMe_4$ = 0.00 ppm. Chemical shifts in ^{13}C NMR spectra are given in ppm relative to $CDCl_3$ as internal standard (77.00 ppm). All NMR *J* values are given in Hz. Mass spectra were recorded on a JEOL JMS700 spectrometer.

N,*N*′,*N*″-Tri(toluene-4-sulfonyl)diethylene triamine (5)[16]

Diethylene triamine **4** (20.65 g, 0.20 mol) was dissolved in distilled water (125 cm^3). Sodium hydroxide pellets (24.0 g, 0.60 mol) were added and the temperature was kept below 40 °C. Diethyl ether (125 cm^3) was added and the reaction mixture was stirred vigorously. Toluene-4-sulfonyl chloride (114.5 g, 0.6 mol) was added, the temperature was kept below 20 °C during addition. The reaction mixture was cooled to 0 °C and stirred for 1 h. The white precipitate was filtered and washed with diethyl ether (250 cm^3). Recrystallisation from chloroform gave the desired product **5** (112.60 g, 99%). Mp 176–178 °C (from chloroform), (lit.[16] 177–179 °C); δ_H (400 MHz, $CDCl_3$) 2.50 (9H, s, CH_3), 2.63 (2H, t, *J* 6.0, 2 × NH), 3.14–3.22 (8H, m, CH_2), 7.32–7.38 (6H, m, ArH), 7.64 (2H, d, *J* 8.4, ArH), 7.76–7.88 (4H, m, ArH); *m*/*z* (FAB) 566 (MH^+, 99%), 412 (71), 227 (53), 154 (50), 136 (35), 92 (31).

N,*O*,*O*′-Tri(toluene-4-sulfonyl)diethanolamine (7)[17]

A stirred solution of toluene-4-sulfonyl chloride (114.5 g, 0.6 mol) and dichloromethane (140 cm^3) was prepared and cooled to 0 °C. Diethanolamine **6** (21.03 g, 0.2 mol), benzyltriethylammonium chloride (18.22 g, 80.0 mmol) and 30% NaOH solution (24.0 g in 150 cm^3 of water) were added whilst stirring vigorously. The reaction mixture was allowed to return to room temperature and was stirred for 1 h. The reaction mixture was poured onto

water (300 cm^3). The organic phase was separated and washed with water (3 × 150 cm^3). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product **7** crystallised over a period of 2 weeks (102.85 g, 91%). Mp 97–99 °C (from methanol), (lit.[17] 101–103 °C); δ_H (400 MHz, $CDCl_3$) 2.45 (3H, s, CH_3), 2.49 (6H, s, CH_3), 3.40 (4H, t, *J* 6.0, CH_2), 4.14 (4H, t, *J* 6.0, CH_2), 7.31–7.34 (2H, m, ArH), 7.38 (4H, d, *J* 8.0, ArH), 7.63 (2H, d, *J* 8.4, ArH), 7.78 (4H, d, *J* 8.4, ArH); *m*/*z* (CI+) 568 (MH^+, 13%), 432 (12), 396 (12), 242 (100), 157 (35).

1,4,7,10-Tetrakis(toluene-4-sulfonyl)-1,4,7, 10-tetraazacyclododecane (8)[9]

Method 1. A stirred solution of *N*,*N*′,*N*″-tri(toluene-4-sulfonyl)diethylene triamine **5** (8.0 g, 11 mmol), caesium carbonate (13.68 g, 42 mmol) and *N*,*N*-dimethylformamide (300 cm^3) was prepared. A solution of *N*,*O*,*O*′-tri(toluene-4-sulfonyl)diethanolamine **7** (5.68 g, 11 mmol) in *N*,*N*-dimethylformamide (125 cm^3) was added dropwise over a period of 3 h. The reaction mixture was stirred for 5 d. The reaction was concentrated using a pump-assisted rotary evaporator. The residue was taken up in dichloromethane (150 cm^3) and distilled water (150 cm^3). The aqueous layer was extracted with dichloromethane (100 cm^3). The combined organic phases were washed with a saturated solution of sodium chloride (150 cm^3), dried ($MgSO_4$) and concentrated *in vacuo*. The light brown residue was recrystallised from methanol to give a colourless solid **8** (8.74 g, 79%). Mp 276–279 °C (from methanol), (lit.[9] 278–280 °C); δ_H (400 MHz, $CDCl_3$) 2.47 (12H, s, CH_3), 3.45 (16H, br s, CH_2), 7.36 (8H, d, *J* 7.0. ArH), 7.71 (8H, d, *J* 7.0, ArH); *m*/*z* (FAB) 789 (MH^+, 99%), 633 (40), 477 (16), 323 (20), 253 (37), 154 (38), 92 (54).

Method 2. A mixture of toluene (200 cm^3), tetrabutylammonium bromide (0.81 g, 2.5 mmol) and 2.5% lithium hydroxide solution (2.56 g in 100 cm^3 of water) was heated under reflux. *N*,*N*′,*N*″-Tri(toluene-4-sulfonyl)diethylene triamine **5** (5.65 g, 10 mmol), *N*,*O*,*O*′-tri(toluene-4-sulfonyl)diethanolamine **7** (5.68 g, 10 mmol) and toluene (400 cm^3) were added in small portions. The reaction mixture was heated under reflux overnight. The reaction mixture was cooled and the colourless precipitate was filtered and washed with methanol (100 cm^3) giving the desired product **8** (5.58 g, 71%). Spectroscopic data as described above.

1,4,7,10-Tetraazacyclododecane (9)[18]

Method 1. A stirred solution of 1,4,7,10-tetrakis(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane **8** (5.42 g, 6.87 mmol), phenol (12.80 g, 0.136 mol) and hydrobromic acid, 45% w/v solution in acetic acid (270 cm^3) was prepared. The round bottomed flask was fitted with a water-filled condenser and an air condenser. This allows the evolving HBr gas to escape to the top of the fumehood. The reaction mixture was heated under reflux for 36 h. The reaction mixture was cooled and concentrated *in vacuo*. The residue was redissolved in toluene (4 × 30 cm^3) and concentrated under vacuum. The dark purple residue was dissolved in water (150 cm^3), and dichloromethane (75 cm^3) was added. The layers were separated and the aqueous layer was washed with dichloromethane (4 × 75 cm^3). The aqueous layer was concentrated yielding a brown residue which was purified by an Amberlite IRA-400 resin anion exchange column. The product was then recrystallised from hot toluene yielding a colourless powder **9** (0.54 g, 46%). Mp 98–100 (from toluene), (lit.[18] 103–107 °C); δ_H (400 MHz, D_2O) 2.48 (16H, br s, CH_2); *m*/*z* (CI+) 173 (MH^+, 61%), 113 (8), 97 (9), 79 (100).

Method 2. A stirred solution of 1,4,7,10-tetrakis(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane **8** (9.66 g, 12.66 mmol) and concentrated sulfuric acid (25 cm^3) was prepared and stirred at 110 °C for 40 h. The brown–black solution was poured into a conical flask and cooled in an ice bath. Water (20 cm^3) was added slowly. Potassium hydroxide pellets (45 g) were added until the pH was 13. Ethanol (150 cm^3) was added and the mixture was filtered. The solid residue was washed with ethanol (5 × 20 cm^3) and the filtrate was concentrated. The residue was taken up in the minimum volume of 1 M hydrochloric acid (40 cm^3), and dichloromethane (30 cm^3) was added. The layers were separated and the aqueous layer was washed with dichloromethane (4 × 30 cm^3). The pH was raised to 13 by adding potassium hydroxide pellets. This was extracted with chloroform (4 × 25 cm^3). The organic layers were combined, dried (K_2CO_3) and concentrated yielding a yellow solid **9** (0.283 g, 13%). Spectroscopic data as described above.

Method 3. Concentrated sulfuric acid (11 cm^3) was heated to 165 °C. 1,4,7,10-Tetrakis(toluene-4-sulfonyl)-1,4,7,10-tetraazacyclododecane **8** (1.20 g, 2.42 mmol) was added in a single portion and the solution was stirred until the reaction mixture had turned black. The reaction mixture was cooled by transferring the mixture into a Buchner flask and submersing this flask in cold water. This mixture was added dropwise to a stirring solution of ethanol (36 cm^3). Diethyl ether (27 cm^3) was added and the solution was cooled to 0 °C in an ice–water bath. The solid was filtered and dissolved in a minimum volume of hot water (7 cm^3), and an equivalent volume of hydrobromic acid (48% aq., 7 cm^3) was added. Overnight the tetrahydrobromide salt crystallised. This was filtered and washed with hydrobromic acid (5 cm^3) and ethanol (5 cm^3). The white crystals were dried under high vacuum. The crystals were then added to a round bottomed flask charged with toluene (20 cm^3), water (5 cm^3) and sodium hydroxide pellets (0.4 g, 10 mmol). Dean–Stark apparatus was fitted and the reaction mixture was heated under reflux for 24 h. The toluene solution was then filtered and concentrated yielding colourless crystals of **9** (0.97 g, 88%). Spectroscopic data as decribed above.

1,4,7,10-Tetra(phenylaminocarbonyloxyethyl)-1,4,7, 10-tetraazacyclododecane (11)

General procedure. 1,4,7,10-Tetra(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane **10** (0.2 g, 0.29 mmol), phenyl isocyanate (0.50 cm^3, 4.64 mmol), dibutyltin diacetate (3 drops) and dichloromethane (5 cm^3) were stirred and heated under reflux for 24 h. The solvent was concentrated and the residue was filtered and washed with diethyl ether. Recrystallisation from hot methanol gave colourless crystals (0.69 g, 100%). Mp 150–152 °C (from methanol); ν_{max}(KBr)/cm^{-1} 3296 (NH), 2944 (CH), 1696 (CO), 1228, 797; δ_H (400 MHz, D_6-DMSO) 2.62 (24H, br s, CH_2N), 4.11 (8H, t, *J* 6.0, CH_2O), 6.96 (4H, t, *J* 8.0, ArH), 7.24 (8H, t, *J* 8.0, ArH), 7.45 (8H, d, *J* 8.0, ArH), 9.56 (4H, br s, NH); δ_C (100 MHz, D_6-DMSO) 52.4 (CH_2), 53.8 (CH_2), 62.1 (CH_2), 122.3 (CH), 128.7 (CH), 128.7 (CH), 128.9 (CH), 129.0 (CH),

134.8 (C), 153.5 (C); *m/z* (FAB) 825.4296 (MH^+. $C_{44}H_{57}N_8O_8$ requires 825.4299), 511(4), 358 (95), 307 (11), 155 (100), 109 (29).

1,4,7,10-Tetra(isopropylaminocarbonyloxyethyl)-1,4,7, 10-tetraazacyclododecane (12)

Using the general procedure above on a 0.58 mmol scale with isopropyl isocyanate gave colourless crystals (0.24 g, 60%). Found: C, 55.8; H, 9.5; N, 16.1. $C_{36}H_{64}N_8O_8$ requires C, 55.8; H, 9.4; N, 16.3%; mp 141–143 °C (from EtOAc–hexane); ν_{max}(KBr)/cm^{-1} 3279 (NH), 2969 (CH), 1684 (CO), 1539; δ_H (400 MHz, D_6-acetone) 1.00 (24H, d, *J* 6.8, CH_3), 2.44–2.49 (24H, m, CH_2N), 3.59–3.64 (4H, m, CH), 3.94 (8H, t, *J* 6.0, CH_2O); δ_C (100 MHz, D_6-DMSO) 22.5 (CH_3), 42.2 (CH), 52.7 (CH_2), 54.0 (CH_2), 61.6 (CH_2), 155.3 (C); *m/z* (FAB) 689 (MH^+, 100%), 586 (10), 503 (18), 331 (85), 174 (54), 131 (100), 90 (79), 72 (37).

1,4,7,10-Tetra(*n*-propylaminocarbonyloxyethyl)-1,4,7, 10-tetraazacyclododecane (13)

Using the general procedure above on a 0.58 mmol scale with propyl isocyanate gave colourless crystals (0.16 g, 41%). Mp 142–144 °C (from EtOAc–hexane); ν_{max}(KBr)/cm^{-1} 3312 (NH), 2960 (CH), 1686 (CO), 1549, 1273, 1008, 668; δ_H (400 MHz, D_6-acetone) 0.91 (12H, t, *J* 7.4, CH_3), 1.48–1.57 (8H, m, CH_2CH_3), 2.49–2.65 (24H, m, CH_2N), 3.09 (8H, t, *J* 7.0, CH_2NCO), 4.10 (8H, t, *J* 6.0, CH_2O); δ_C (100 MHz, D_6-DMSO) 11.2 (CH_3), 22.7 (CH_2), 42.0 (CH_2), 52.6 (CH_2), 54.0 (CH_2), 61.8 (CH_2) 156.2 (C); *m/z* (FAB) 689.4922 (MH^+. $C_{32}H_{65}N_8O_8$ requires 689.4925), 433 (4), 331 (2), 289 (20), 146 (100).

1,4,7,10-Tetra(4-methoxyphenyl)aminocarbonyloxyethyl-1,4,7, 10-tetraazacyclododecane (14)

Using the general procedure above on a 0.58 mmol scale with 4-methoxyphenyl isocycanate (23.2 mmol) gave a colourless precipitate that was filtered off and washed with methanol to give a colourless solid (0.55 g, 56%). Mp 166–168 °C (from methanol); ν_{max}(KBr)/cm^{-1} 3295 (NH), 2957 (CH), 1698 (CO), 1510, 1240, 827; δ_H (400 MHz, D_6-DMSO) 2.06 (24H, br s, CH_2N), 3.24 (12H, br s, OCH_3), 3.65 (8H, br s, CH_2O), 6.38 (8H, d, *J* 9.0, ArH), 6.90 (8H, d, *J* 9.0, ArH), 8.89 (4H, br s, NH); δ_C (400 MHz, D_6-DMSO) 52.5 (CH_2), 53.9 (CH_2), 55.1 (CH_3), 62.0 (CH_2), 113.8 (CH), 119.9 (CH), 132.2 (C), 153.7 (C), 154.7 (C); *m/z* (FAB) 945.4724 (MH^+. $C_{48}H_{65}N_8O_{12}$ requires 945.4722), 779 (4), 695 (3), 459 (4), 338 (5), 238 (22), 170 (51), 87 (100).

1,4,7,10-Tetra(4-bromophenyl)aminocarbonyloxyethyl-1,4,7, 10-tetraazacyclododecane (15)

Using the general procedure above on a 0.29 mmol scale with 4-bromophenyl isocycanate (11.6 mmol) gave a colourless precipitate that was filtered off and washed with methanol to give a colourless solid (0.21 g, 64%). Mp 212–214 °C (from methanol); ν_{max}(KBr)/cm^{-1} 3303 (NH), 2833 (CH), 1710 (CO), 1393, 1227, 820; δ_H (400 MHz, D_6-DMSO) 2.50 (24H, br s, CH_2N), 4.11 (8H, t, *J* 6.0, CH_2O), 7.41–7.46 (16H, m, ArH), 8.86 (4H, br s, NH); δ_C (100 MHz, D_6-DMSO) 52.5 (CH_2), 53.8 (CH_2), 62.3 (CH_2) 113.4 (C), 120.2 (CH), 131.5 (CH), 139.0 (C), 152.3 (C); *m/z* (FAB) 1143 [MH^+ (^{81}Br), 4%], 1141 [MH^+ (^{79}Br), 5%], 371 (5), 253 (9), 170 (100), 87 (95).

1,4,7,10-Tetra(2-nitrophenyl)aminocarbonyloxyethyl-1,4,7, 10-tetraazacyclododecane (16)

Using the general procedure above on a 0.29 mmol scale with 2-nitrophenyl isocycanate (11.6 mmol) gave a yellow precipitate that was filtered off and washed with methanol to give a pale yellow solid (0.29 g, 79%); mp 82–84 °C (from methanol); ν_{max}(KBr)/cm^{-1} 3350 (NH), 2359 (CH), 1718 (CO), 1428, 1237, 743; δ_H (400 MHz, D_6-DMSO) 2.59 (24H, bs, 12 × CH_2N), 4.11 (8H, bs, 4 × CH_2O), 7.28 (4H, t, *J* 8.0 Hz, 4 × ArH), 7.66 (4H, t, *J* 8.0 Hz, 4 × ArH), 7.73 (4H, d, *J* 8.0 Hz, 4 × ArH), 7.96 (4H, d, *J* 8.0 Hz, 4 × ArH), 9.77 (4H, bs, 4 × NH); δ_C (100 MHz, D_6-DMSO) 52.6 (CH_2), 53.6 (CH_2), 63.2 (CH_2), 123.8 (CH), 124.2 (CH), 125.7 (CH), 132.5 (C), 134.4 (CH), 140.7 (C), 153.4 (C); *m/z* (FAB) 1006 (MH^+, 97%), 842 (100), 826 (13), 678 (43), 660 (16), 490 (18), 326 (12), 232 (32), 158 (48), 81 (100).

1,4,7,10-Tetra(3-nitrophenyl)aminocarbonyloxyethyl-1,4,7, 10-tetraazacyclododecane (17)

Using the general procedure above on a 0.29 mmol scale with 3-nitrophenyl isocycanate (2.90 mmol) gave a yellow precipitate that was filtered off and washed with methanol to give a pale yellow solid (0.14 g, 46%). Mp 151–153 °C (from methanol); ν_{max}(KBr)/cm^{-1} 3389 (NH), 2797, 1720 (CO), 1526, 1081, 735; δ_H (400 MHz, D_6-DMSO) 2.61 (24H, br s, CH_2N), 4.14 (8H, t, *J* 6.0, CH_2O), 7.47–7.56 (4H, m, ArH), 7.80–7.96 (12H, m, ArH), 10.09 (4H, br s, NH); δ_C (100 MHz, D_6-DMSO) 52.6 (CH_2), 53.7 (CH_2), 62.6 (CH_2), 121.9 (CH), 122.3 (CH), 128.9 (CH), 133.7 (CH), 146.4 (C), 146.7 (C), 151.4 (C); *m/z* (FAB) 1005.3709 (MH^+. $C_{44}H_{53}N_{12}O_{16}$ requires 1005.3702), 391 (3), 322 (3), 238 (30), 170 (64), 87 (100).

1,4,7,10-Tetra(4-nitrophenyl)aminocarbonyloxyethyl-1,4,7, 10-tetraazacyclododecane (18)

Using the general procedure above on a 0.29 mmol scale with 4-methoxyphenyl isocycanate (2.90 mmol) gave a yellow precipitate that was filtered off and washed with methanol to give a yellow solid (0.18 g, 60%). Mp 179–181 °C (from methanol); ν_{max}(KBr)/cm^{-1} 3323 (NH), 2831 (CH), 1728 (CO), 1329, 855; δ_H (400 MHz, D_6-DMSO) 2.60 (24H, br s, CH_2N), 4.14 (8H, br s, CH_2O), 7.64 (8H, d, *J* 8.8, ArH), 8.12 (8H, d, *J* 8.8, ArH), 10.30 (4H, br s, NH); δ_C (100 MHz, D_6-DMSO) 52.6 (CH_2), 53.7 (CH_2), 62.7 (CH_2), 117.5 (CH), 124.9 (CH), 141.5 (C), 145.7 (C), 153.1 (C); *m/z* (FAB) 1005.3697 (MH^+. $C_{44}H_{53}N_{12}O_{16}$ requires 1005.3702), 238 (17), 170 (100), 87 (100).

Assays against *Trypanosoma brucei*

Bloodstream form *T. brucei* (strain 427) was cultivated in HMI-9 medium (Biosera) containing 10% foetal calf serum at 37 °C in a humidified CO_2 environment.[19] A derivative of the Alamar blue assay[20] was used to determine EC_{50} values against trypanosomes. Cells were seeded at 2 × 10^5 cells per cm^3 in 0.1 cm^3 volume to which was added 0.1 cm^3 of medium containing each compound in doubling dilution. Cells were incubated for 48 h at 37 °C

and then Alamar blue reagent (resazurin, Sigma; 20 μl of a 0.49 mM solution) was added and cells were incubated for a further 24 h before the reduction of Alamar blue was measured using a fluorimeter at 530 nm excitation and 590 nm emission wavelengths. Output was plotted using the EC_{50} determination algorithm of the Prism 3.0 software (GraphPad).[21]

Assays against HEK cells

The human embryonic kidney cell line (HEK 293T) was cultured in Dulbecco's Modified Eagle's Medium (Sigma) with penicillin (100 U cm^{-3}) and streptomycin (0.1 mg cm^{-3}), L-glutamine (2 mM), and 10% newborn calf serum in vented culture flasks at 37 °C in 5% CO_2 atmosphere, passaging when cells on the monolayer were 80–85% confluent. Developments of cultures were monitored by microscopy and cell numbers were determined using an improved Neubauer haemocytometer (counting chamber; Weber Scientific). The Alamar blue assay protocol was modified from the one used for live trypanosomes. Briefly, 100 μl of a 3×10^5 cells cm^{-3} suspension were added to each well of a 96-well plate and incubated at 37 °C for 3 h to allow cells to adhere to the bottom of the wells. Preparation of drug stocks (100 μl) in doubling dilution was added after the incubation period, incubated for a further 16 h before the addition of 10% resazurin (0.49 mM stock solution). After 24 h the plates were read fluorometrically (λ_{ex} = 530 nm and λ_{em} = 590 nm) and the data were analysed by Prism 3.0 software.

Acknowledgements

Financial support from the University of Glasgow (studentship for J.M.W.) and the Sir Halley Stewart Trust (studentship to F.G.) is gratefully acknowledged.

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