The synthesis of novel polyamine–nitroimidazole conjugates designed to probe the structural specificities of the polyamine uptake system in A549 lung carcinoma cells

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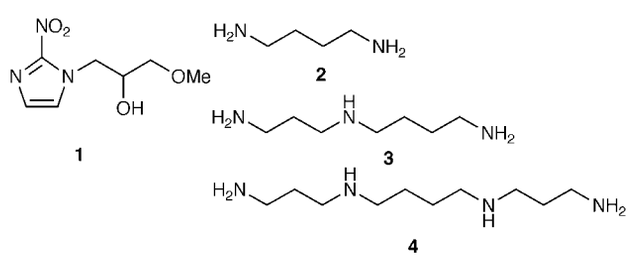
Synthetic routes were developed to synthesise an N^4-mono-derivatised spermidine–nitroimidazole conjugate and two novel structural isomers (N^1- and N^8-spermidine–nitroimidazole conjugates). A synthetic method was developed to synthesise an N^1,N^7-bis-derivatised norspermidine–nitroimidazole conjugate and further applied to the synthesis of an N^1,N^8-bis-derivatised spermidine–nitroimidazole conjugate. The compounds were examined for their ability to serve as substrates for the polyamine uptake system in A549 lung carcinoma cells, by measuring their inhibition of [^{14}C]spermidine uptake. Marked differences were observed between the nitroimidazole–polyamine conjugates. For maximum recognition as a substrate by the polyamine transport system, the aminobutyl unit of spermidine should remain underivatised. The preferred site(s) for spermidine amino derivatisation was in the order: $N^1 > N^8 \approx N^1$, $N^8 > N^4$.

Introduction

Hypoxic regions in certain tumour types often show problematic resistance to damage by ionising radiation due to the absence of the sensitising action of oxygen, known as the 'oxygen effect'.[1] Nitroimidazole-based drugs are able to sensitise hypoxic cells to the damaging effects of ionising radiation.[2] Misonidazole (**1**), for example, has been shown to



behave as an oxygen mimetic. Under the conditions of hypoxia, misonidazole may directly substitute for an oxygen molecule in the radical reaction pathway resulting from the oxidation of DNA, leading to DNA strand breakage.[3] Furthermore, nitroimidazoles can also exhibit a selective toxicity towards hypoxic cells, an effect possibly originating from their reduction by cellular nitroreductases.[4]

It has been proposed by ourselves and others that polyamines may be used as vectors for the cellular delivery of chemotherapeutic and/or DNA targeted drugs.[5,6] Found in all eukaryotic cells, polyamines are essential for the processes of cellular growth and differentiation, although their precise roles remain poorly defined. It is known that polyamines are required for the structural modification of DNA in the processes of transcription and recombination[7] and the reduction of repulsive forces between segments of DNA to facilitate DNA packaging, for example in phage heads.[8] The conjugation of nitroimidazoles to polyamines may target the drug to tumour cells by virtue of the high specific activity of the polyamine uptake system observed in a number of tumour cell lines.[9,10] The polyamine moiety would also be expected to increase the local concentration of the drug around DNA, the proposed site of action of nitroimidazoles, by virtue of the high affinity of the polyammonium cation for DNA (the binding constants of polyammonium cations to DNA are in the region of 10^3 M^{-1}).[6,7]

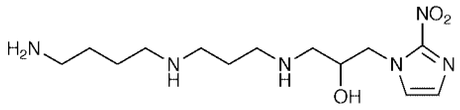
Polyamine derivatives and other structurally related compounds are known to be able to utilise cellular polyamine transport systems to cross the cell membrane.[5,11,12] The polyamine carrier protein has not yet been isolated in mammalian cells but its properties have been characterised for a variety of cell types including NB-15 mouse neuroblastomer cells, LoVo human colon adenocarcinoma cells and L1210 mouse leukemia cells.[9,13,14] Kinetic studies (reviewed by Seiler and Dezeure[15]) have shown the polyamine transport system to obey saturation kinetics and to be energy dependent. Furthermore, competition studies appear to suggest that a single transport mechanism is responsible for the uptake of putrescine, spermidine and spermine.[15] There have been a few previous studies that have made direct comparisons between the abilities of polyamines and structurally related compounds to be recognised as substrates.[5,12,16,17] Porter and co-workers using L1210 leukaemia cells were able to demonstrate a marked preference for certain polyamine inter-amino chain lengths by measuring the inhibition constants for a variety of di- and triamines.[16] Substrate recognition was found to be greatest for triamines containing a unit of two amino centres separated by four methylene groups.

In this study we report the synthesis of a range of spermidine–nitroimidazole derivatives and one norspermidine–nitroimidazole conjugate, *via* novel synthetic routes and, the common structural properties that optimise recognition by the polyamine uptake system in A549 lung carcinoma cells. In particular, we highlight the major differences that exist between regioisomeric spermidine derivatives, with a view to establishing the optimum site of spermidine derivatisation for these types of drug–polyamine conjugates.

Results

Synthesis of polyamine conjugates

To compare the effects of polyamine derivatisation at the three amino-centres of spermidine, we sought to synthesise the N^4-nitroimidazole–polyamine conjugate **7**[17] and two novel structural analogues, compounds **5** and **6**. To examine the



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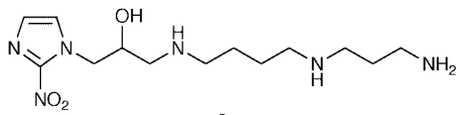

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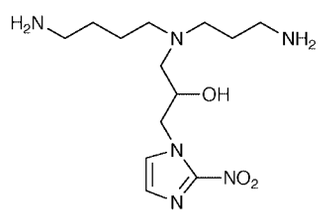

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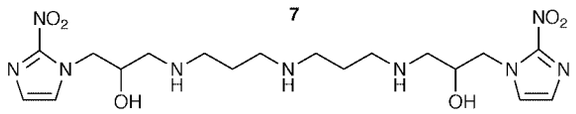

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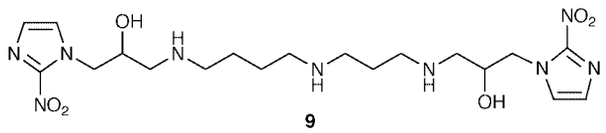

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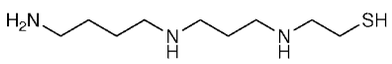

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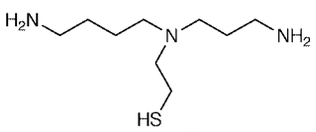

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importance of inter-polyamino chain-length a novel, efficient route was devised to synthesise compound **8**, an N^1,N^7-bis-derivatised norspermidine–nitroimidazole conjugate. This synthetic method was also applied to the synthesis of its longer chain homologue, the N^1,N^8-bis-derivatised spermidine–nitroimidazole conjugate **9**.[17] To provide evidence that the uptake inhibition studies on these compounds may provide a general guide for the optimal site of spermidine derivatisation, we also synthesised the known radioprotecting agents, N^1- and N^4-mercaptoethylspermidine, compounds **10** and **11** respectively, using similar synthetic procedures.

The synthesis of compounds **5** and **6** was accomplished *via* routes comprising of entirely novel synthetic intermediates. The starting point in both cases was the methylenespermidine derivative **12** synthesised previously by Ganem.[18] For compound **5** the introduction of the nitroimidazole moiety at the N^1 position was achieved *via* selective acylation of the primary N^8 nitrogen of **12** with *tert*-butoxycarbonyloxyimino-2-phenylacetonitrile (BOC-ON) at low temperature (Scheme 1). The resulting novel, orthogonally protected spermidine derivative **13** was alkylated using 1-(2′,3′-epoxypropyl)-2-nitroimidazole **14** (synthesised using the procedure described by Hoffmann[19]) at the N^1 position to give compound **15**. Deprotection of the compound was achieved in two steps and in high yield. Initially the N^1,N^8 methylene bridge was removed under Knoevenagel reaction conditions affording **16**. Subsequently, the Boc group was removed by acidolysis to give the desired compound as a hydrochloride salt **5**. For the synthesis of compound **6** both the N^1 and N^8 centres of **12** were phosphinylated using diphenylphosphinic chloride (DPPCl). This novel, orthogonally protected spermidine derivative was subsequently alkylated with epichlorohydrin using NaH as base. Addition of 2-nitroimidazole to the epoxide moiety of **18** under basic conditions afforded the correct regioisomeric adduct **19**. Deprotection was again accomplished in two steps, initially by removal of the methylene bridge under Knoevenagel conditions followed by acidolysis of the remaining DPP groups, to afford the desired product **6**. Synthesis of the N^4-nitroimidazole–spermidine conjugate was accomplished *via* alkylation of the N^1 N^8 Boc-protected spermidine derivative **21** with **14**, followed by deprotection under acidic conditions (Scheme 2). The N^1,N^7-norspermidine–nitroimidazole conjugate **8** was synthesised by alkylation of the triphosphinylated spermidine derivative **23** with two equivalents of epichlorohydrin (Scheme 3). One equivalent of 2-nitroimidazole was added to each epoxide moiety under basic conditions, followed by deprotection under acidic conditions to afford the desired compound **8**. The N^1,N^8-spermidine–bis-nitroimidazole conjugate **9** was synthesised by similar synthetic procedures. Both bis-epoxypropyl polyamine adducts **24** and **27** were formed as 1:1 diastereomeric mixtures resulting from chirality at the 2′-C and 2″-C centres of the hydroxypropyl linker. Evidence for this ratio derives from the ^{31}P NMR spectra of the subsequently formed bis-nitroimidazole adducts **25** and **28**. The diastereomers of **8** and **9** could not be separated by reverse-phase HPLC, consequently both **8** and **9** were evaluated as 1:1 diastereomeric mixtures.

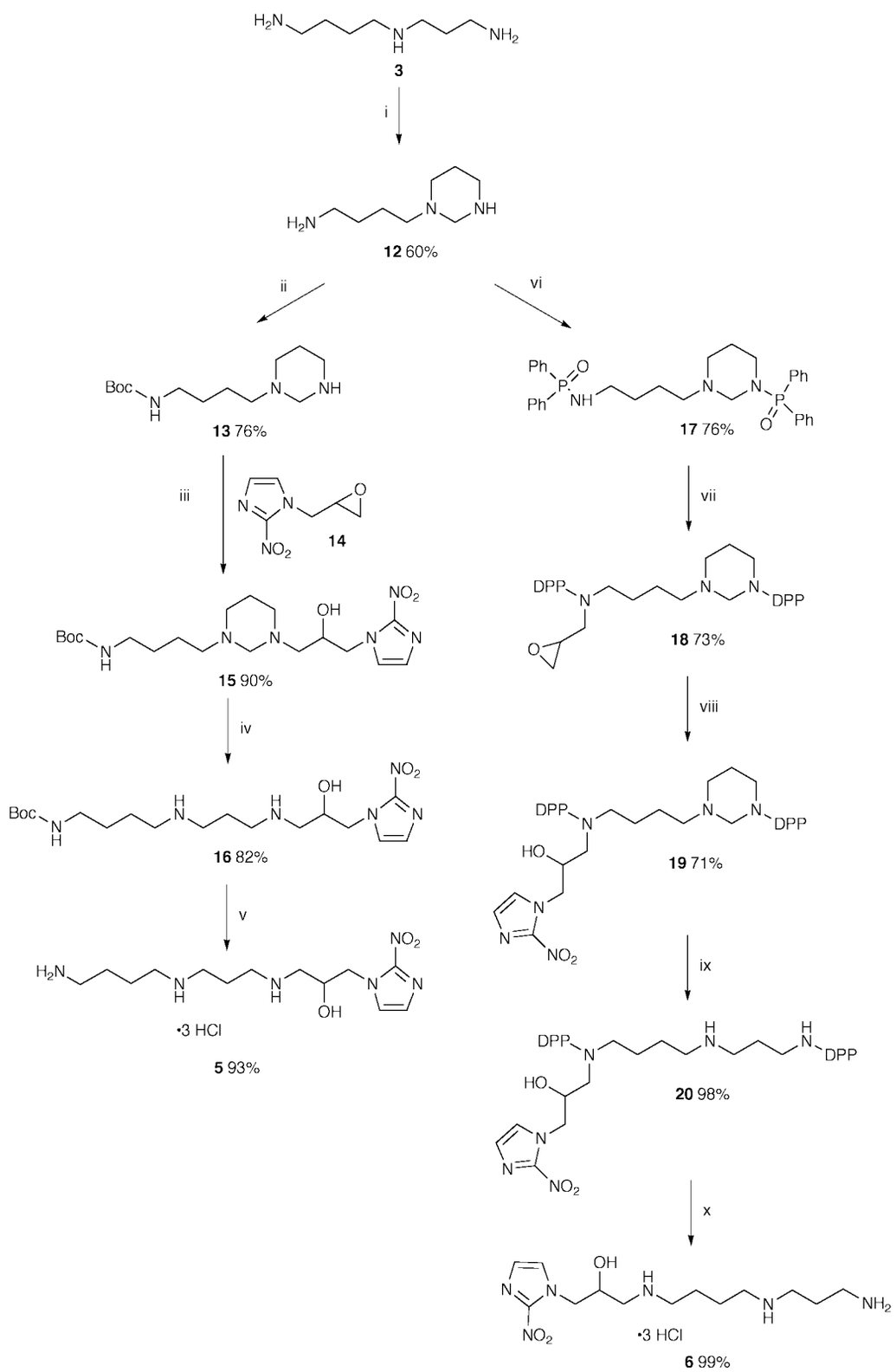
The synthesis of N^1-mercaptoethylspermidine **10** made use of the orthogonally protected spermidine derivative **13** (Scheme 4). Following alkylation of **13** with episulfide, the thiol functionality was protected by oxidation to the disulfide to prevent unwanted side-reactions during deprotection. Successive deprotection of the N^1,N^4-methylene bridge and N^8-Boc group was followed by reduction of the disulfide bond using dithiothreitol to afford the desired compound as a hydrochloride salt. The synthesis of N^4-mercaptoethylspermidine **11** was accomplished by a similar synthetic strategy, using the N^1,N^8-bis-Boc protected spermidine derivative **21** as a starting point (synthetic details given elsewhere[20]).

Inhibition of polyamine uptake

The inhibition of [^{14}C]spermidine uptake by the nitroimidazole–polyamine conjugates was determined as described in the Experimental section and the results are summarised in Table 1. As with simple enzyme inhibition, the lower the value of the inhibition constant (K_i) the more potent the conjugate was as an inhibitor of spermidine uptake. In all cases Lineweaver–Burk plots indicated simple competitive inhibition with respect to spermidine.

The comparison between the three structurally isomeric monoderivatised spermidine–nitroimidazole conjugates **5**, **6** and **7** was of particular interest. Both the N^8 and N^1 terminally-derivatised isomers **6** and **5** were substantially superior to the N^4 derivatised isomer **7** at inhibiting uptake of the normal substrate. Their K_i values (K_i **6**, 0.5 μM; K_i **5**, 0.09 μM) were 6 and 36 times lower respectively than the inhibition constant for the N^4 derivatised compound (K_i **7**, 3.1 μM). The adverse effects of derivatising at the N^4 position of spermidine could also be seen from comparison with the K_i value for the bis-derivatised nitroimidazole–spermidine conjugate **9** (K_i **9**, 0.6 μM), although in structure **9** *both* terminal amino centres were derivatised the inhibition constant was 5.3 times *lower* than that of the N^4 derivatised conjugate **7** (K_i **7**, 3.1 μM).

The difference between the K_i values of the two terminally mono-derivatised nitroimidazole–spermidine conjugates is also

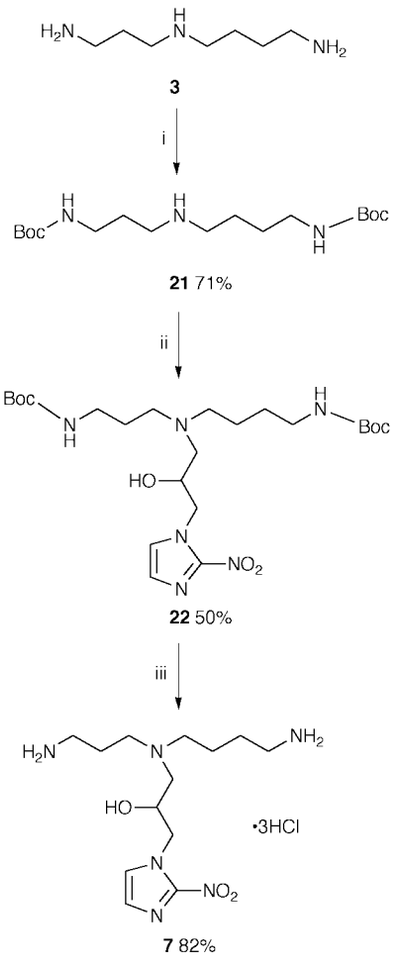


Scheme 1 *Reagents and conditions*: i, formaldehyde (37% aqueous soln.); ii, BOC-ON; iii, 1-(2′,3′-epoxypropyl)-2-nitroimidazole **14**, triethylamine; iv, malonic acid, pyridine; v, trifluoroacetic acid, triethylsilane; vi, DPPCl, triethylamine; vii, NaH, epichlorohydrin; viii, 2-nitroimidazole, triethylamine; ix, malonic acid, pyridine; x, HCl soln.–methanol.

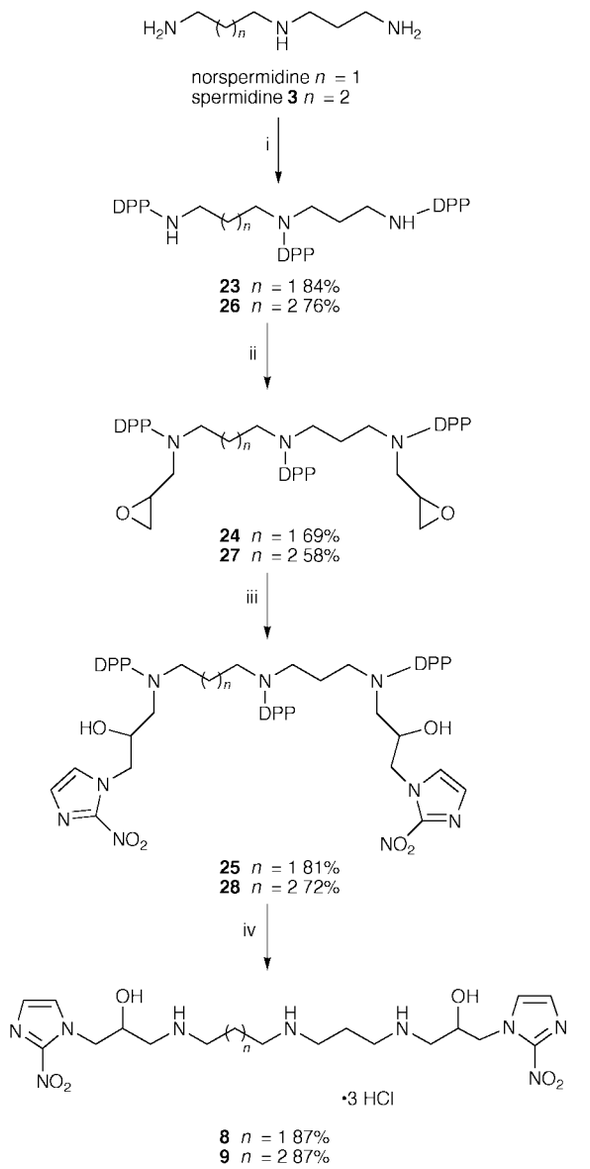
of note. The N^1-spermidine derivatised isomer **5** had a K_i value approximately 6 times lower than the K_i value for the N^8-spermidine derivatised isomer **6**. This suggests that to maximise substrate recognition, neither the N^4 nor N^8 position of the aminobutyl unit of spermidine should be derivatised.

The K_i values of the bis-derivatised N^1,N^8-spermidine–nitroimidazole conjugate **9** and the monoderivatised N^8-spermidine–nitroimidazole conjugate **6** were similar (K_i **9**, 0.6 μM; K_i **6**, 0.5 μM). This suggests, that in contrast to derivatisation at the N^1 position of spermidine, derivatising at the N^8 position has only a minimal effect on the derivatives ability to be recognised as a substrate.

A comparison between the inhibition constants for the two bis-derivatised polyamine–nitroimidazole conjugates **8** and **9** based on spermidine and norspermidine (K_i **9**, 0.6 μM; K_i **8**, 5.0 μM) show that spermidine-derived conjugates have a greater affinity for the polyamine receptor involved in uptake compared to those based on norspermidine. Unlike spermidine, norsperm-



Scheme 2 *Reagents and conditions*: i, BOC-ON; ii, 1-(2′,3′-epoxypropyl)-2-nitroimidazole, triethylamine; iii, trifluoroacetic acid, ion exchange resin, HCl aqueous soln.–methanol.



Scheme 3 *Reagents and conditions*: i, DPPCl, triethylamine; ii, NaH, epichlorohydrin; iii, 2-nitroimidazole, triethylamine; iv, HCl aqueous soln.

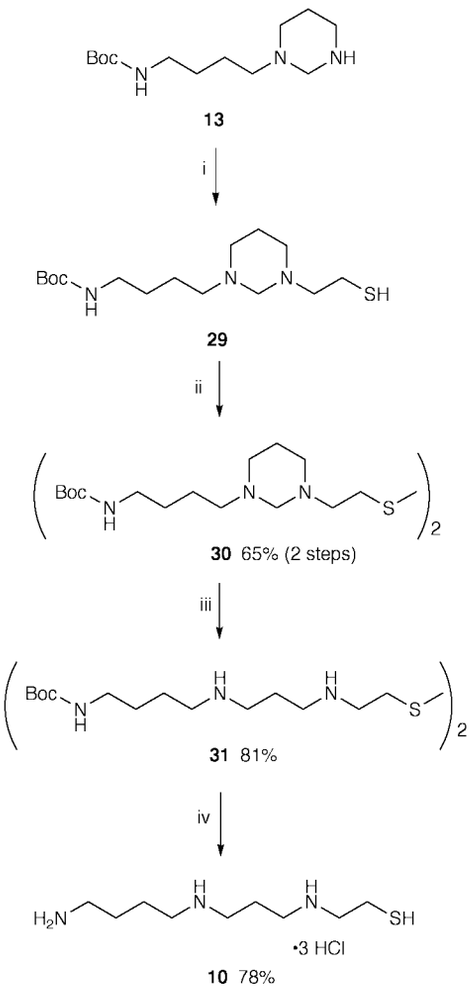
idine does not contain an aminobutyl unit, again emphasising the importance of this unit for substrate recognition.

Finally, a comparison between N^1-mercaptoethylspermidine **10** and N^4-mercaptoethylspermidine **11** further indicated that N^1-spermidine derivatives compete favourably with regioisomeric N^4-spermidine derivatives for recognition by the polyamine uptake system (K_i **10**, 0.6 μM; K_i **11**,1.1 μM). The difference between the inhibition constants of the N^1- and the N^4-mercaptoethylspermidine derivatives **10** and **11** was smaller than the difference observed between the N^1- and the N^4-nitroimidazole spermidine derivatives **5** and **7**. This may reflect differences in other properties involved in substrate binding, such as steric bulk, solvation/desolvation energies and possible differences in substrate–carrier intermolecular interactions.

Discussion

Porter and co-workers reported that the preferred site(s) of spermidine amino derivatisation for recognition by the polyamine uptake system in L1210 leukaemia cells was $N^4 > N^8 > N^1 > N^1$, N^8.[17] Their conclusions were based on comparisons made between derivatives having alkylated secondary amines and those where the terminal amines are acylated. However, the loss of basicity resulting from amine acylation would be expected to be of major significance in substrate recognition. Unpublished studies[20] have shown that successive methylation at the nitrogen centres of spermidine was tolerated by the polyamine transport system of *Ehrlich ascites* tumour cells up until but *not* including the quaternary ammonium derivative, which showed no inhibition of [^{3}H]-spermidine uptake. Our previous studies have suggested that the number of positive charges on the polyamine represents the single most important contribution to recognition by the polyamine receptor.[21] Hence, acylation at the terminal amine centres of spermidine would be expected to reduce affinity for the polyamine receptor.

Evidence that the inhibition constants reported in this study may reflect actual cellular uptake comes from several sources. Seppanen has reported the preference for N^1-spermidine derivatisation compared to N^8-spermidine derivatisation for actual cellular uptake in *Ehrlich ascites* tumour cells.[22] N^1-Acetylspermidine was shown to accumulate to levels approximately three times greater than those reached by N^8-acetylspermidine for both untreated and DFMO (α-difluoromethylornithine) treated cells. Similarly, direct evidence for the preferential uptake of N^1-mercaptoethylspermidine over N^4-mercaptoethylspermidine was provided in a study by Newton and co-workers.[23] The intracellular concentrations of various aminothiols were determined in DFMO pre-treated V79 cells. The intracellular concentrations of N^1-mercaptoethylspermidine and N^4-mercaptoethylspermidine after incubation with the pre-treated cells (50 μM drug, 30 minutes, 37 °C) were found to be 610 and 250 μM respectively.



Scheme 4 *Reagents and conditions*: i, ethylene sulfide; ii, O_2; iii, malonic acid, pyridine; iv, HCl aqueous soln. (5 M), dithiothreitol.

Table 1 Inhibition of spermidine uptake in A549 human lung carcinoma cells by polyamine conjugates[a]

Compound	K_i/μM	Structural characteristics
5	0.09 ± 0.09	Spermidine N^1 derivatisation
6	0.5 ± 0.3	Spermidine N^8 derivatisation
7	3.1 ± 0.4	Spermidine N^4 derivatisation
8	6.0 ± 1.6	Norspermidine N^1 and N^7 derivatisation
9	0.6 ± 0.2	Spermidine N^1 and N^8 derivatisation
10	0.6 ± 0.1	N^1-mercaptoethyl spermidine
11	1.1 ± 0.9	N^4-mercaptoethyl spermidine

[a] A549 cells were incubated at 37 °C for 30 min with [^{14}C]spermidine alone or in the presence of the conjugate. The accumulation of radiolabel into the cells was calculated and K_i values determined from Lineweaver–Burk plots. Values are the mean of at least three separate experiments and the errors are the standard deviations.

It is possible to conclude from the results presented here that, for maximum recognition of the substrate by the polyamine uptake system of A549 lung carcinoma cells, where charges are preserved, the preferred site(s) of conjugation to spermidine is in the order: $N^1 > N^8 \approx N^1$, $N^8 > N^4$. It would therefore appear to be preferable to derivatise spermidine at the N^1 position and not the N^4 position as has been suggested by others,[16] preserving the integrity of the aminobutyl unit, which appears to maximise recognition of the substrate. This study suggests that the conjugation of drugs to polyamines continues to have potential in the targeting of cells with active polyamine uptake systems, with conjugation at the N^1 position producing the most promising results.

Notwithstanding the above discussion, it is worth noting once more that despite the considerable structural variation in the conjugates described above, the variation in K_i values are comparatively modest. This again confirms the broad substrate tolerance of the mammalian polyamine transporter.[24]

Experimental

Chemicals

Radiolabelled spermidine (112 μCi mmol^{-1}) was purchased from Amersham International (Amersham, UK). All other chemicals unless otherwise stated were obtained from Sigma-Aldrich Company Ltd (Poole, UK). Compounds were analysed by HPLC using a reverse-phase column (C18, 5 μ, BDS column, Hypersil) connected to a Gilson 715 system. A Dynamax absorbance detector (model UV-1, Rainin) was used to detect the nitroimidazole–polyamine compounds. The solvent system used was eluent A; 0.1 M ammonium acetate pH 6, eluent B; acetonitrile (Far UV, HPLC grade, Fisher, Loughborough, UK), gradient 0 min (20% B); 20 min (60% B); 23 min (80% B); 26 min (80% B); 28 min (20% B); 30 min (20% B); flow rate was 1 cm^3 min^{-1} at room temperature. Fluorescence was detected at 326 nm. NMR spectra were recorded on a Bruker AM300 NMR spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz) and a Bruker 250 ARX NMR spectrometer (^{1}H at 250 MHz, ^{13}C at 62 MHz and ^{31}P at 101 MHz). ^{13}C NMR assignments (CH_3, CH_2, CH and C) have been made on the basis of interpretation of the DEPT spectra. *J* Values are given in Hz throughout. Mass spectra (low and high resolution) were recorded on a Kratos Concept 1H double focusing forward geometry mass spectrometer. IR spectra were recorded on a Perkin-Elmer 16 PC FT-IR spectrophotometer. UV spectra were recorded on a Beckman DU 7500 spectrophotometer.

Cell lines and bacterial strains

A549 human epithelial lung carcinoma cells were used throughout this study and were a gift from Dr C. Courage, CMHT, Leicester University. Cells were seeded at (5–10) × 10^4 cells cm^{-3} and maintained in Nut. Mix. F-12 (Hams) with Glutamax (Gibco BRL, cat 31765-027) supplemented with foetal calf serum, penicillin (100 iu cm^{-3}) and streptomycin (100 μg cm^{-3}).

Inhibition of polyamine uptake

To study the ability of a range of polyamines to inhibit the uptake of [^{14}C]spermidine *in vitro*, A549 cells were seeded into 24 well tissue culture plates (Nunc - 1 × 10^5 cells well^{-1}) and incubated for 16 h to form a monolayer. Cells were incubated for 30 min with ^{14}C radiolabelled spermidine (112 μCi mmol^{-1}, Amersham International) at various concentrations (0.125, 0.25, 0.5, 1 and 2 μM) alone or in the presence of a range polyamines/polyamine conjugates. After incubation, the plates were placed on ice and the cells were washed with cold 0.9% NaCl plus 1 mM spermidine, to displace any radiolabelled spermidine still attached to the cell surface. Cells were digested by the addition of 1 M NaOH (400 μl) and incubation at 60 °C for 30 min to 1 h. Samples were neutralised by the addition of an equal volume of 1 M HCl. Duplicate samples (400 μl) were added to 4 cm^3 Optiphase 'Safe' and radioactivity determined in a Wallac scintillation counter. The results were expressed as pmol spermidine uptake per min per 10^5 cells. The mechanism of inhibition of uptake was determined using Lineweaver–Burk plots.

N^1,N^4-Methylenespermidine 12[18]

Spermidine (4.00 g, 27 mmol) was dissolved in distilled water (10 cm^3). Aqueous formaldehyde solution (2.14 cm^3, 37%, 27 mmol) was added drop-wise to the rapidly stirring solution over

a period of 5 minutes. After stirring for an additional 20 minutes the product was removed from the reaction mixture by washing with chloroform (8 × 10 cm^3). The combined chloroform extracts were dried over Na_2SO_4 and the solvent removed *in vacuo* to leave an oily residue. This was further purified by vacuum distillation (150 °C, 2 mbar) to leave a clear, colourless oil (2.55 g, 60%); δ_H(250 MHz; $CDCl_3$) 4.80 (3 H, s, NH), 3.45 (2 H, s, 9-H), 2.92 (2 H, t, *J* 2, 1-H), 2.73 (4 H, m, 5-H, 7-H), 2.39 (2 H, t, *J* 3, 4-H), 1.66 (2 H, m, 6-H), 1.52 (4 H, m, 2-H, 3-H); δ_C(63 MHz; $CDCl_3$) 70.52 (9-C), 56.74, 54.52, 46.16, 43.00 (1-C, 3-C, 5-C, 8-C), 32.49 (2-C), 27.62, 25.61 (6-C, 7-C).

N^1,N^4-Methylene-N^8-(*tert*-butoxycarbonyl)spermidine 13

N^1,N^4-Methylenespermidine **12** (3.54 g, 22.5 mmol) was dissolved in dichloromethane (80 cm^3) and the solution suspended in an ice bath. After stirring for 15 minutes, BOC-ON (5.55 g, 22.5 mmol) dissolved in dichloromethane (40 cm^3) was added drop-wise to the solution over 1 h. After a further 1 h of stirring the solvent was removed *in vacuo* and the residue taken up in diethyl ether (80 cm^3). The solution was washed with saturated NaOH (4 × 10 cm^3) until removal of all yellow coloration. The solution was dried over Na_2SO_4, solvent evaporated *in vacuo* and the resulting residue was purified by flash chromatography [over silica eluting with 0.5% (concentrated NH_4OH solution)–MeOH]. The combined fractions had solvent removed *in vacuo* to leave a white crystalline solid (4.41 g, 76%); ν_{max}(neat)/cm^{-1} 3300m br (NH stretch), 2950s (C–H stretch), 1700s (C=O stretch), 1550m, 1450m, 1365m, 1300m, 1160m; δ_H(250 MHz; $CDCl_3$) 5.95 (1 H, br s, N^8-H), 3.22 (2 H, s, 9-H), 2.96 (2 H, br pseudo q, 8-H), 2.63 (2 H, t, *J* 7, 1-H), 2.40 (2 H, br t, 3-H), 2.09 (2 H, t, *J* 11, 5-H), 1.67 (1 H, br s, N^1-H), 1.47 (2 H, m, 2-H), 1.38 (4 H, m, 6-H, 7-H), 1.31 [9 H, s, $C(CH_3)_3$]; δ_C(63 MHz; $CDCl_3$) 156.4 (C=O), 79.0 [$C(CH_3)_3$], 70.1 (9-C), 55.6, 53.4, 45.5, 40.9 (1-C, 3-C, 5-C, 8-C), 28.8 [$C(CH_3)_3$], 28.5, 27.4, 24.8 (2-C, 6-C, 7-C); *m*/*z* (CI) 258 (MH^+, 100%), 200 (17, loss of *tert*-butyl + H^+), 99 (44), 85 (23), 70 (23), 57 (12, *tert*-butyl$^{+\bullet}$) (Found: MH^+, *m*/*z* 256.2024. $C_{13}H_{26}N_3O_2$ requires 256.2025).

N^1-[2′-Hydroxy-3′-(2″-nitroimidazol-1″-yl)propyl]-N^1,N^4-methylene-N^8-(*tert*-butoxycarbonyl)spermidine 15

Compound **13** (0.910 g, 3.54 mmol) and 1-(2′,3′-epoxypropyl)-2-nitroimidazole (595 mg, 3.54 mmol) were dissolved in methanol (5 cm^3) in a Young's tube. The reaction mixture was heated at 120 °C for 5 h. The volatiles were removed *in vacuo* and the residue was purified by flash chromatography (silica; 6% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a light yellow, viscous gum (1.359 g, 90%); ν_{max}($CHCl_3$)/cm^{-1} 3450m (O–H stretch), 3040m (aromatic C–H stretch) 2950s (aliphatic C–H stretch), 1700s (C=O stretch), 1480m, 1360m, 1365m, 1250m, 1160m, 900m, 7735m br; δ_H(250 MHz; $CDCl_3$) 7.24 (1 H, s, imidazole-ring CH), 7.06 (1 H, s, imidazole-ring CH), 4.91 (1 H, br s, N^8-H), 4.62 (1 H, dd, J^2 13.6 and J^3 1.6, 3′-H_aH_b), 4.56 (1 H, br s, D_2O exchange, OH), 4.08 (2 H, m, 3′-H_aH_b, 2′-H), 3.22 (2 H, pseudo q, 8-H), 3.04 (2 H, d, *J* 4.9, 9-H), 2.65 (4 H, m, 1-H, 1′-H), 1.59 (2 H, m, 2-H), 1.42 (4 H, m, 6-H, 7-H), 1.36 [9 H, s, $C(CH_3)_3$]; δ_C(63 MHz; $CDCl_3$) 156.0 (C=O), 128.4, 128.1 (2 × aromatic CH), 79.0 [$C(CH_3)_3$], 75.7 (9-C), 67.5 (2′-C), 59.1, 54.8, 53.9, 52.9, 40.7, 40.2 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 28.8 [$C(CH_3)_3$], 28.2, 24.3, 24.1 (2-C, 6-C, 7-C); *m*/*z* (EI) 425 (M − H^+, 13.9%), 409 (6.4), 369 (11.0, loss of *tert*-butyl), 254 (6.2), 149 (6.1), 114 (21), 70 (29), 57 (50.1), 51 (100) (Found: M − H^+, *m*/*z* 425.2511. $C_{19}H_{33}N_6O_5$ requires 425.2512).

N^1-[2′-Hydroxy-3′-(2″-nitroimidazol-1″-yl)propyl]-N^8-(*tert*-butoxycarbonyl)spermidine 16

Compound **15** (70 mg, 0.17 mmol), malonic acid (62.5 mg, 0.60 mmol) and pyridine (0.042 cm^3, 0.52 mmol) were dissolved in ethanol (10 cm^3). The reaction mixture was refluxed for 2 h at 100 °C. Following the removal of volatiles *in vacuo*, the residue was taken up in water (5 cm^3) and washed with chloroform (3 × 5 cm^3). The aqueous layer was adjusted to pH 11 by the addition of a few drops of 10% NaOH aqueous solution. The aqueous layer was then extracted with chloroform (5 × 5 cm^3) and the combined chloroform extracts were dried over Na_2SO_4. Removal of the solvent *in vacuo* isolated the product as a light yellow gum (56 mg, 82%); ν_{max}($CHCl_3$)/cm^{-1} 3050m (aromatic C–H stretch) 2990s (aliphatic C-H stretch), 1420s (C=O stretch), 1260s, 890m, 750s; δ_H(250 MHz; $CDCl_3$) 7.22 (1 H, s, imidazole-ring CH), 7.04 (1 H, s, imidazole-ring CH), 4.85 (1 H, br s, N^8-H), 4.59 (1 H, dd, J^2 13.6 and J^3 12.4, 3′-H_aH_b), 4.17 (1 H, dd, J^2 13.6 and J^3 8.0, 3′-H_aH_b), 3.92 (1 H, br m, OH), 3.02 (2 H, br m, 8-H), 2.58 (8 H, br m, 1-H, 3-H, 5-H, 1′-H), 1.58 (2 H, m, 2-H), 1.44 (4 H, m, 6-H, 7-H), 1.36 [9 H, s, $C(CH_3)_3$]; δ_C(63 MHz; $CDCl_3$) 156.5 (C=O), 128.3, 128.4 (2 × aromatic CH), 79.5 [$C(CH_3)_3$], 68.8 (2′-C), 54.1, 52.5, 49.5, 48.7, 48.5, 40.7, (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 28.9, 28.1, 26.8 (2-C, 6-C, 7-C), 28.8 [$C(CH_3)_3$]; *m*/*z* (FAB) 415 (MH^+, 59%), 368 (26, loss of HNO_2), 307 (10), 268 (7), 207 (10), 154 (100), 136 (90) (Found: MH^+, *m*/*z* 415.2669. $C_{18}H_{35}N_6O_5$ requires 415.2669).

N^1-[2′-Hydroxy-3′-(2″-nitroimidazol-1″-yl)propyl]spermidine hydrochloride 5

Compound **16** (0.761 g, 1.84 mmol) and triethylsilane (0.75 cm^3, 4.6 mmol) were dissolved in dichloromethane. Trifluoroacetic acid (15 cm^3) was added to the rapidly stirring solution and, after 3 h stirring, the volatiles were removed *in vacuo*. The product was then isolated by ion-exchange chromatography using Dowex 50W anionic exchange resin, eluting with a linear gradient of increasing concentration of aqueous HCl–MeOH (0.5–3.0 M, 100 cm^3 each). The product was isolated as a light yellow, brittle foam (0.725 g, 93%); δ_H(250 MHz; $CDCl_3$) 7.51 (1 H, s, imidazole-ring CH), 7.25 (1 H, s, imidazole-ring CH), 4.77 (1 H, m, 3′-H_aH_b), 4.42 (2 H, m, 3′-H_aH_b, 2′-H), 3.44 (1 H, pseudo d, 1′-H_aH_b), 3.15 (9 H, m, 1-H, 3-H, 5-H, 8-H, 1′-H_aH_b), 2.18 (2 H, m, 2-H), 1.78 (4 H, m, 6-H, 7-H); δ_C(63 MHz; $CDCl_3$) 129.2, 127.7 (2 × aromatic CH), 66.0 (2′-C), 53.1, 49.9, 47.4, 45.0, 44.7, 39.1 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 24.2, 23.0, 22.8 (2-C, 6-C, 7-C); *m*/*z* (FAB) 315 (MH^+, 100%), 268 (21), 205 (15), 176 (43), 146 (13) (Found: MH^+, *m*/*z* 315.2145. $C_{13}H_{27}N_6O_3$ requires 315.2146).

N^1,N^8-Bis(diphenylphosphinoyl)-N^1,N^4-methylenespermidine 17

N^1,N^4-Methylenespermidine **12** (0.946 g, 6.02 mmol) and triethylamine (2.1 cm^3, 15.1 mmol) were dissolved in dichloromethane (30 cm^3). The reaction mixture was suspended in an ice bath and DPPCl (2 equiv., 2.3 cm^3) was added by injection. After allowing the reaction mixture to warm to room temperature over 15 minutes, water (10 cm^3) was added to the reaction mixture and the organic solvent was removed *in vacuo*. The residue was partitioned between water and dichloromethane (40 cm^3 each) and the aqueous layer washed with dichloromethane (3 × 40 cm^3). The combined organic layers were dried over Na_2SO_4, after which the solvent was removed *in vacuo*. The residue was purified by flash chromatography (over silica eluting with 5% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a white, brittle foam (2.551 g, 76%); ν_{max}($CHCl_3$)/cm^{-1} 3370w, 2920m, 1440m, 1190s (P=O stretch), 1120s (P=O stretch), 1065m, 960w; δ_H(250 MHz; $CDCl_3$) 7.88 (8 H, m, *meta*-ring CH), 7.40 (12 H, m, *ortho*-ring CH, *para*-ring CH), 3.70 [2 H, d (s, ^{31}P decoupled), $J_{P\text{-}H}$ 8.18, 9-H], 3.64 [1 H, q (t, ^{31}P decoupled), *J* 6.73, NH], 3.08 [2 H, q (t, ^{31}P decoupled), *J* 6.87, 1-H], 2.89 [2 H, m (q, ^{31}P decoupled), 8-H], 2.65 (2 H, t, *J* 4.99, 3-H), 2.36 (2 H, t, *J* 6.93, 5-H), 1.55 (4 H, m, 7-H, 2-H), 1.26 (2 H, m, 6-H); δ_C(63 MHz; $CDCl_3$) 133.76, 130.372 (Ar-C), 132.43, 132.38, 132.24, 132.14, 132.00,

131.82, 131.70, 131.66, 128.62, 128.54, 128.42, 128.34 (Ar-CH), 66.32 (9-C), 52.73, 52.72, 44.82, 40.47 (1-C, 3-C, 5-C, 8-C), 29.91 (d, $J_{P\text{-}C}$ 6.9), 24.36, 23.20 (d, $J_{P\text{-}C}$ 4.6) (2-C, 6-C, 7-C); δ_P(101 MHz; $CDCl_3$) 27.91 (s, N^1-P), 23.52 (s, N^8-P); *m*/*z* (FAB) 558.22 (M^+, 35%), 356.17 (21; loss of $Ph_2P{=}O + H^+$), 270.09 (14), 201 (100, $Ph_2P{=}O^{+\bullet}$), 69.95 (11).

N^1,N^8-Bis(diphenylphosphinoyl)-N^1,N^4-methylene-N^8-(2′,3′-epoxypropyl)spermidine 18

Compound **17** (0.268 g, 0.48 mmol) and NaH (29 mg, 0.72 mmol) were added to THF (12 cm^3). After stirring the reaction mixture for 10 minutes, epichlorohydrin (0.2 cm^3, 2.4 mmol) was added and the mixture was heated at 90 °C for 3 h. Allowing the reaction mixture to cool to room temperature, further additions of NaH (0.72 mmol) and epichlorohydrin (5 equiv.) were made. The reaction mixture was then heated at 90 °C for a further 3 h. The mixture was allowed to cool to room temperature whereupon ethyl acetate and water (5 cm^3 each) were added. After 5 minutes stirring the solvent was removed *in vacuo*. The residue was partitioned between water and chloroform (30 cm^3 each) and the aqueous layer was washed with chloroform (3 × 20 cm^3). The combined organic layers were dried over Na_2SO_4, then solvent was removed *in vacuo.* The residue was purified by flash chromatography (over silica eluting with 5% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a colourless, viscous gum (215 mg, 73%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3375w, 2925m, 1190s (P=O stretch), 1120s (P=O stretch), 1035m, 960w; δ_H(250 MHz; $CDCl_3$) 7.80 (8 H, m, *meta*-ring CH), 7.31 (12 H, m, *ortho*-ring, *para*-ring CH), 3.55 [2 H, d (s, ^{31}P decoupled), $J_{P\text{-}H}$ 8.25, 9-H], 3.26 [1 H, m (dd, ^{31}P decoupled, J_{gem} 9.73 and J_{vic} 3.53), 3′-$H_a$$H_b$], 3.03 (4 H, m, 1-H, 7-H), 2.85 (2 H, m, 2′-H, 3′-$H_a$$H_b$), 2.59 (1 H, pseudo t, *J* 4.45, 1′-$H_a$$H_b$), 2.52 (2 H, br t, 5-H or 3-H), 2.23 (1 H, dd, J_{gem} 4.45 and J_{vic} 2.57, 1′-$H_a$$H_b$), 2.14 (2 H, br t, 3-H or 5-H), 1.49 (4 H, m, 6-H, 7-H), 0.97 (2 H, m, 2-H); δ_C(63 MHz; $CDCl_3$) 131.1, 131.0 (Ar-C), 136.3, 133.2, 133.0, 132.24, 132.8, 132.7, 132.6, 132.3, 132.2, 130.7, 129.1, 129.0 (Ar-CH), 67.1 (9-C), 51.8 (2′-C), 53.9, 53.3, 49.0, 47.4, 45.6, 45.2 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 24.7, 23.9, 21.6 (2-C, 6-C, 7-C); δ_P(101 MHz; $CDCl_3$) 31.31 (s, N^8-P), 28.03 (s, N^1-P); *m*/*z* (FAB) 614.25 (M^+, 63%), 412.20 (22, loss of $Ph_2P{=}O + H^+$), 201.05 (100, $Ph_2P{=}O^{+\bullet}$), 84.01 (17).

N^1,N^8-Bis(diphenylphosphinoyl)-N^1,N^4-methylene-N^8-[2′-hydroxy-3′-(2″-nitroimidazol-1″-yl)propyl]spermidine 19

Compound **18** (0.539 g, 0.88 mmol) and 2-nitroimidazole (45 mg, 1.23 mmol) were dissolved in methanol (10 cm^3) in a sealed tube. Following the addition of triethylamine (0.12 cm^3, 2.64 mmol) the reaction mixture was heated at 120 °C for 5 h. The volatiles were then removed *in vacuo* and the residue was purified by flash chromatography (over silica eluting with 5% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a light yellow, viscous gum (0.454 g, 71%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3050s, 2950m, 2300m, 1430m, 1370s, 1310s, 1200s (P=O stretch), 1125m (P=O stretch), 1070s, 903w, 804s, 670s br (out of plane aromatic CH stretch); δ_H(250 MHz; $CDCl_3$) 7.65 (8 H, m, *meta*-ring CH), 7.29 (12 H, m, *ortho*-ring CH, *para*-ring CH), 7.19 (1 H, d, *J* 0.7, imidazole ring C-H), 6.92 (1 H, d, *J* 0.7, imidazole ring C-H), 6.19 (1 H, br s, D_2O exchange, OH), 4.71 (1 H, dd, J_{gem} 13.6 and J_{vic} 1.95, 3′-$H_a$$H_b$), 4.27 (1 H, m, 2′-H), 3.98 (1 H, dd, J_{gem} 13.6 and J_{vic} 8.8, 3′-$H_a$$H_b$), 3.68 (1 H, pseudo t, *J* 10.1, 1′-$H_a$$H_b$), 3.52 (1 H, pseudo t, *J* 10.1, 1′-$H_a$$H_b$), 3.06 (6 H, m, 1-H, 8-H, 9-H), 2.55 (2 H, m, 3-H), 2.20 (2 H, m, 5-H), 1.55 (4 H, m, 6-H, 7-H), 1.07 (2 H, m, 2-H); δ_C(63 MHz; $CDCl_3$) 145.0, 130.7, 130.6 (Ar-C), 132.8, 132.7, 132.6, 132.4, 132.3, 132.1, 129.3, 129.2, 129.1, 129.0, 128.9, 128.6, 128.3 (Ar-CH), 67.7 (2′-C), 67.1 (9-C), 54.1, 53.3, 52.7, 50.3, 46.8, 45.7 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 25.5, 24.7, 23.8 (2-C, 6-C, 7-C); δ_P(101 MHz; $CDCl_3$) 34.59 (s, N^8-P), 28.47 (s, N^1-P); *m*/*z* (FAB) 727 (M^+, 41%), 646 (18), 258 (8), 201 (100, $Ph_2P{=}O^{+\bullet}$).

N^1,N^8-Bis(diphenylphosphinoyl)-N^8-[2′-hydroxy-3′-(2″-nitroimidazol-1″-yl)propyl]spermidine 20

Compound **19** (100 mg, 0.14 mmol), malonic acid (52.2 mg, 0.50 mmol) and pyridine (0.035 cm^3, 0.43 mmol) were dissolved in ethanol (10 cm^3). The reaction mixture was refluxed for 2 h at 100 °C. Following the removal of volatiles *in vacuo*, the residue was taken up in water (5 cm^3) which was adjusted to pH 11 by the addition of a few drops of aqueous NaOH (10% solution). The aqueous layer was then extracted with chloroform (5 × 5 cm^3) and the combined chloroform extracts were dried over Na_2SO_4. Removal of the solvent *in vacuo* isolated the product as a light yellow, viscous oil (97 mg, 98%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3220m br (N–H), 3050m, 2960m (aromatic or aliphatic C–H stretch), 2300w, 1530m, 1480s, 1425s, 1360s, 1210s (P=O stretch), 1125s (P=O stretch), 1120s, 950s, 710s br (out of plane aromatic C–H stretch); δ_H(250 MHz; $CDCl_3$) 7.74 (8 H, m, *meta*-ring CH), 7.37 (12 H, m, *ortho*-ring CH, *para*-ring CH), 7.19 (1 H, d, *J* 0.7, imidazole-ring C-H), 6.97 (1 H, d, *J* 0.7, imidazole-ring C-H), 6.18 (1 H, br s, D_2O exchange, OH), 4.59 (1 H, dd, J_{gem} 13.4 and J_{vic} 1.8, 3′-$H_a$$H_b$), 4.28 [1 H, pseudo q (t, ^{31}P decoupled), N^1-H], 4.08 (1 H, br m, 2′-H), 3.91 (1 H, dd, J_{gem} 13.4 and J_{vic} 8.6, 3′-$H_a$$H_b$), 2.95 (6 H, m, 1-H, 8-H, 1′-H), 2.58 (2 H, t, *J* 6.2, 3-H), 2.33 (2 H, t, *J* 6.7, 5-H), 1.62 (2 H, m, 2-H), 1.43 (2 H, m, 7-H), 1.12 (2 H, m, 6-H); δ_C(63 MHz; $CDCl_3$) 144.96, 132.1, 129.3 (Ar-C), 132.8, 132.7, 132.5, 132.4, 132.3, 132.1, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.5, 128.3 (Ar-CH), 68.03 (2′-C), 54.15, 50.67, 49.37, 48.72, 47.57, 40.50 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 31.42 (d, $J_{P\text{-}C}$ 7.0), 27.22, 26.53 (d, $J_{P\text{-}C}$ 4.2) (2-C, 6-C, 7-C); δ_P(101 MHz; $CDCl_3$) 35.36 (s, N^8-P), 23.84 (s, N^1-P); *m*/*z* (FAB) 715 (M^+, 64%), 634 (6), 307 (82), 201 (100, $Ph_2P{=}O^{+\bullet}$) (Found: M^+, *m*/*z* 715.2925. $C_{37}H_{45}O_5N_6P_2$ requires 715.2926).

N^8-[2′-Hydroxy-3′-(2″-nitroimidazol-1″-yl)propyl]spermidine hydrochloride 6

Compound **20** (89.1 mg, 0.125 mmol) was dissolved in 0.5 M HCl (16 cm^3; 50:50 MeOH–H_2O). The solution was subsequently stirred at 60 °C for 2 h. The product was then isolated by ion-exchange chromatography using Dowex 50W anionic exchange resin, eluting with a linear gradient of increasing concentration of aqueous HCl–MeOH (0.5–3.0 M, 100 cm^3 each). The product was isolated as a light yellow, brittle foam (39.0 mg, 99%); δ_H(250 MHz; $CDCl_3$) 7.56 (1 H, s, imidazole-ring CH), 7.30 (1 H, s, imidazole-ring CH), 4.82 (1 H, pseudo q, 3′-$H_a$$H_b$), 4.45 (2 H, m, 3′-$H_a$$H_b$, 2′-H), 3.46 (1 H, pseudo d, 1′-$H_a$$H_b$), 3.20 (9 H, m, 1-H, 3-H, 5-H, 8-H, 1′-$H_a$$H_b$), 2.15 (2 H, m, 2-H), 1.86 (4 H, m, 6-H, 7-H); δ_C(63 MHz; $CDCl_3$) 131.61, 130.29 (Ar-CH), 66.37 (2′-C), 55.43, 52.06, 49.61, 49.61, 47.15, 39.19 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 26.36, 25.37, 25.16 (2-C, 6-C, 7-C); *m*/*z* (FAB) 315 (MH^+, 100%), 299 (5), 268 (6), 234 (5), 158 (6) (Found: MH^+, *m*/*z* 315.2148. $C_{13}H_{27}N_6O_3$ requires 315.2146).

N^1,N^8-Bis(*tert*-butoxycarbonyl)spermidine 21

Spermidine (3.50 g, 24.13 mmol) was dissolved in THF (120 cm^3), to which was added BOC-ON (11.95 g, 48.5 mmol) at 0 °C. After 1 h stirring the THF was removed *in vacuo* and the residue taken up in diethyl ether (80 cm^3). The solution was washed with saturated NaOH (4 × 10 cm^3) until removal of yellow coloration. The solution was dried over Na_2SO_4, evaporated and the resulting white crystalline solid was recrystallised from diisopropyl ether (5.92 g, 71%) (Found: C, 59.0; H, 10.2; N, 12.2. $C_{17}H_{35}N_3O_4$ requires C, 59.1; H, 10.2; N, 12.2%); mp 82–84 °C; δ_H(300 MHz; $CDCl_3$) 5.39 (1 H, br t, NHCO), 5.37 (1 H, br t, NHCO), 3.19 (2 H, m, 1-H), 3.11 (2 H, m, 8-H), 2.65

(2 H, t, *J* 6.6, 3-H), 2.60 (2 H, t, *J* 6.5, 5-H), 1.65 (2 H, m, 2-H), 1.52 (4 H, m, 6-H, 7-H), 1.44 (18 H, br s, CMe_aMe_b, N^1-H, N^8-H); δ_C(75 MHz; $CDCl_3$) 156.10, 156.03 (C=O × 2), 78.78 [$C(CH_3)_3$ × 2], 49.42, 47.64, 40.40, 39.12 (1-C, 3-C, 5-C, 8-C), 29.87, 27.87, 27.35 (2-C, 6-C, 7-C), 28.43 [$C(CH_3)_3$ × 2]; *m/z* (CI) 346 (MH^+, 100%), 289 (31, loss of *tert*-butyl), 99 (28), 86 (21), 57 (27, *tert*-butyl$^{+\bullet}$).

N^1,N^8-Bis(*tert*-butoxycarbonyl)-N^4-[2″-hydroxy-3″-(2′-nitroimidazol-1′-yl)propyl]spermidine 22

Compound **21** (1.9 g, 5.5 mmol) and **14** (0.87 g, 5.2 mmol) were dissolved in methanol (40 cm^3). Triethylamine (0.73 cm^3, 5.5 mmol) was added and the mixture was subsequently refluxed at 100 °C for 1 hour. The solvent was removed *in vacuo* and the residue purified by flash chromatography (silica; 10% MeOH–CH_2Cl_2). The yellow–brown oil crystallised on standing to form a solid of the same colour (1.42 g, 50%); mp 58–61 °C; δ_H(300 MHz; $CDCl_3$) 7.31 (1 H, d, *J* 1.0, ring-CH), 7.10 (1 H, d, *J* 1.0, ring-CH), 4.98 (1 H, br s, N^1-H), 4.83 (1 H, br s, N^8-H), 4.75 (1 H, dd, *J* 2.0 and *J* 13.7, 3″-C$H_a$$H_b$), 4.16 (1 H, dd, *J* 8.3 and *J* 13.7, 3″-CH_aH_b), 3.13 (4 H, br m, 1-H, 8-H), 2.55 (4 H, m, 3-H, 5-H), 2.44 (2 H, m, 1″-CH_aH_b), 1.62 (2 H, m, 2-H), 1.46 (4 H, br s, 6-H, 7-H), 1.43 [9 H, s, $C(CH_3)_3$], 1.40 [9 H, s, $C(CH_3)_3$]; δ_C(75 MHz; $CDCl_3$) 156.1, 144.7 (C=O × 2), 127.8, 127.7 (2 × aromatic-CH), 79.2, 79.15 [$C(CH_3)_3$ × 2], 66.9 (2′-C), 57.7, 53.7, 53.6, 51.6, 40.2, 38.4 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 28.4, 28.35 [$C(CH_3)_3$ × 2], 27.7, 27.4, 23.9 (2-C, 6-C, 7-C); *m/z* (FAB) 515 (MH^+, 100%), 468 (23, loss of HNO_2), 458 (9, loss of *tert*-butyl), 70 (21), 57 (8, *tert*-butyl$^{+\bullet}$) (Found M^+ + 1, *m/z* (CI) 399.2971. $C_{20}H_{39}N_4O_4$ requires 399.2971).

N^4-[2″-Hydroxy-3″-(2′-nitroimidazol-1′-yl)propyl]spermidine hydrochloride 7

Compound **22** (0.13 g, 0.25 mmol) was dissolved in trifluoroacetic acid (2 cm^3) and the resulting solution stirred for 1 h. The acid was removed *in vacuo* and the residue dissolved in 0.5 M HCl (2 cm^3). Ion-exchange purification was accomplished using Dowex 50W anionic exchange resin (17 cm^3), eluting with a linear gradient of increasing concentration of aqueous HCl (0.5–3.0 M, 340 cm^3 each). Elution of the product was monitored by UV spectroscopy (315 nm). Combined fractions had solvent removed *in vacuo* to leave a light yellow foam (86 mg, 82%); δ_H(300 MHz; $CDCl_3$) 7.62 (1 H, br s, ring CH), 7.36 (1 H, br s, ring CH), 4.88 (1 H, dd, J^2 14.8 and J^3 2.4, 3′-$H_a$$H_b$), 4.61 (1 H, br m, 2′-H), 4.52 (1 H, dd, J^2 14.8 and J^3 8.7, 3′-H_aH_b), 3.65 (1 H, dd, J^2 13.0 and J^3 2.2, 1′-$H_a$$H_b$), 3.44 (5 H, br m, 1-H, 8-H, 1′-H_aH_b), 3.16 (4 H, m, 3-H, 5-H), 2.28 (2 H, m, 2-H), 1.91 (4 H, br m, 6-H, 7-H); δ_C(75 MHz; $CDCl_3$) 147.1 (Ar-C), 79.2, 131.5, 130.4 (Ar-CH), 66.8 (2′-C), 58.5, 56.6, 55.8, 54.0, 42.0, 41.0 (1-C, 3-C, 5-C, 8-C, 1′-C, 3′-C), 27.4, 25.7, 24.8 (2-C, 6-C, 7-C); *m/z* (FAB) 315 (MH^+, 100%), 307 (73), 289 (20), 259 (9), 234 (5), 158 (6) (Found: MH^+, *m/z* 315.21450. $C_{13}H_{27}N_6O_3$ requires 315.21446).

N^1,N^4,N^7-Tris(diphenylphosphinoyl)norspermidine 23

Norspermidine (0.923 g, 7.0 mmol) was dissolved in THF (50 cm^3). Triethylamine (3.4 cm^3, 21 mmol) was added to the solution which was subsequently stirred for 5 min. DPPCl (4.0 cm^3, 21 mmol) was added by injection to the rapidly stirring solution over a 5 minute period. After a further 1 h stir, water (10 cm^3) was added to the reaction mixture and the organic solvent was removed *in vacuo*. The residue was partitioned between water and dichloromethane (50 cm^3 each) and the aqueous layer washed with dichloromethane (3 × 50 cm^3). The combined organic layers were dried over Na_2SO_4, after which the solvent was removed *in vacuo.* The residue was purified by flash chromatography (silica; 5% MeOH–CH_2Cl_2). The combined fractions had their solvent removed *in vacuo* to leave a white, brittle foam (4.31 g, 84%); ν_{max}($CHCl_3$)/cm^{-1} 3400w (N–H stretch), 3200w, 2930w, 1730w, 1410m, 1190s (P=O stretch), 1125s (P=O stretch); δ_H(250 MHz; $CDCl_3$) 7.72 (12 H, m, *meta*-ring CH), 7.38 (18 H, m, *ortho*-ring, *para*-ring CH), 4.06 (2 H, q, $^3J_{H\text{-}H}$ 7.4 and $^2J_{H\text{-}P}$ 7.4, N^1-H, N^7-H), 3.07 (4 H, m, 1-H, 7-H), 2.89 (4 H, m, 3-H, 5-H), 1.74 (4 H, m, 2-H, 6-H); $\delta_{H\{31P\}}$(250 MHz; $CDCl_3$) 7.72 (12 H, m, *meta*-ring CH), 7.38 (18 H, m, *ortho*-ring CH, *para*-ring CH), 4.06 (2 H, t, $^3J_{H\text{-}H}$ 7.4, N^1-H, N^7-H), 3.07 (4 H, t, *J* 6.9, 1-H, 7-H), 2.89 (4 H, q, *J* 6.4, 3-H, 5-H), 1.74 (4 H, m, 2-H, 6-H); δ_C(63 MHz; $CDCl_3$) 133.74, 132.51, 130.46 (Ar-C), 132.28. 132.13, 132.09, 131.94, 131.86, 131.69, 131.65, 131.61, 128.68, 128.53, 128.50, 128.34 (Ar-CH) 42.91 (d, $^3J_{C\text{-}P}$ 3.4) (1-C, 7-C), 38.06 (3-C, 5-C), 30.28 (2-C, 6-C); δ_P(101 MHz; $CDCl_3$) 32.27 (s, N^4-P), 23.81 (s, N^1-P, N^7-P); *m/z* (FAB) 732 (MH^+, 56%), 475 (9), 201 (100, $Ph_2P{=}O^{+\bullet}$), 154 (55), 77 (25, $Ph^{+\bullet}$).

N^1,N^7-Bis(2′,3′-epoxypropyl)-N^1,N^4,N^7-tris(diphenylphosphinoyl)norspermidine 24

Compound **23** (1.573 g, 2.16 mmol) was dissolved in THF (40 cm^3). Additions of NaH (6 equiv., 12.96 mmol, 0.518 g) and epichlorohydrin (21.60 mmol, 1.68 cm^3) were made to the stirred solution and the resulting mixture was heated at 90 °C for 3 hours. The mixture was allowed to cool to room temperature and a further addition of NaH (12.96 mmol) was made. Following 15 minutes of stirring a further addition of epichlorohydrin (21.6 mmol) was made and the reaction mixture was re-heated to 90 °C where it was maintained for a further 3 h. The mixture was allowed to cool to room temperature whereupon ethyl acetate and water (5 cm^3 each) were added. After 5 minutes stirring the solvent was removed *in vacuo*. The residue was partitioned between water and chloroform (30 cm^3 each) and the aqueous layer was washed with chloroform (3 × 20 cm^3). The combined organic layers were dried over Na_2SO_4, then solvent was removed *in vacuo.* The residue was purified by flash chromatography (silica; 5% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a colourless, viscous gum (1.261 g, 69%); ν_{max}($CHCl_3$)/cm^{-1} 3400m, 2960m, 1440m, 1260m, 1190s (P=O stretch), 1120s (P=O stretch), 900m, 700m br (out of plane Ar–CH stretch); δ_H(250 MHz; $CDCl_3$) 7.79 (12 H, m, *meta*-ring CH), 7.42 (18 H, m, *ortho*-ring CH, *para*-ring CH), 3.27 (2 H, m, 2′-H, 2″-H), 3.01 (4 H, m, 1′-H, 1″-H), 2.79 (10 H, m, 1-H, 3-H, 5-H, 7-H, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 2.27 (2 H, m, 3′-$H_aH_b$, 3″-$H_aH_b$), 1.76 (4 H, m, 2-H, 6-H); δ_C(63 MHz; $CDCl_3$) 132.9, 132.6, 132.2, 131.9 (Ar-C), 132.5, 132.4, 132.3, 130.9, 130.6, 130.5, 128.7, 128.6, 128.5, 128.4 (Ar-CH), 53.5 (2′-C, 2″-C), 51.3, 48.6, 45.1, 44.8, 42.8 (1-C, 2-C, 3-C, 5-C, 6-C, 7-C, 1′-C, 1″-C, 3′-C, 3″-C); δ_P(101 MHz; $CDCl_3$) 31.32 (s, N^1-P, N^7-P), 30.45 (s, N^4-P); *m/z* (FAB) 844 (MH^+, 100%), 642 (8, loss of $Ph_2P{=}O$), 302 (6), 201 (84, $Ph_2P{=}O^{+\bullet}$), 77 (21, $Ph^{+\bullet}$).

N^1,N^7-Bis[2″-hydroxy-3″-(2′-nitroimidazol-1′-yl)propyl]-N^1,N^4,N^8-tris(diphenylphosphinoyl)norspermidine 25

Compound **24** (539 mg, 0.64 mmol) and 2-nitroimidazole (3 equiv., 217 mg) were dissolved in methanol (10 cm^3) in a sealed tube. Following the addition of triethylamine (0.54 cm^3, 3.84 mmol) the reaction mixture was heated at 120 °C for 5 h. The volatiles were then removed *in vacuo* and the residue was purified by flash chromatography (silica; 5% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a light yellow, viscous gum (556 mg, 81%); δ_H(250 MHz; $CDCl_3$) 7.69 (12 H, m, *meta*-ring CH), 7.43 (18 H, m, *ortho*-ring CH, *para*-ring CH), 7.23 (2 H, s, imidazole-ring CH), 7.02 (2 H, s, imidazole-ring CH), 5.94 (2 H, br s, OH), 4.66 (2 H, pseudo d, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 4.12 (2 H, br m, 2′-H, 2″-H), 3.95 (2 H, m, 3′-H_aH_b, 3″-H_aH_b), 3.09 (4 H, m, 1′-H, 1″-H), 2.88 (4 H, m, 1-H and 7-H or 3-H and 5-H), 2.70 (4 H, m, 3-H and 5-H or 1-H and 7-H), 1.75 (4 H, m, 2-H, 6-H); δ_C(63 MHz; $CDCl_3$) 144.9,

131.8, 131.5, 130.3, 129.7 (Ar-C), 132.7, 132.6, 132.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.5, 128.3 (Ar-CH), 68.4 (2′-C, 2″-C), 54.2 (3′-C, 3″-C), 50.5, 45.4, 43.6, 27.7 (1-C, 2-C, 3-C, 5-C, 6-C, 7-C, 1′-C, 1″-C); δ_P(101 MHz; $CDCl_3$) 34.96 (s, N^1-P), 34.82 (s, N^8-P), 31.64 (d, N^4-P); *m*/*z* (FAB) 1070 (MH^+, 29%), 915 (27), 834 (6), 289 (18), 201 (100, $Ph_2P{=}O^{+\bullet}$), 77 (92, $Ph^{+\bullet}$).

N^1,N^7-Bis[2″-hydroxy-3″-(2′-nitroimidazol-1′-yl)propyl]-norspermidine hydrochloride 8

Compound **25** (32 mg, 0.029 mmol) was dissolved in 0.5 M HCl–MeOH (50 : 50; 5 cm^3). The solution was subsequently stirred at 60 °C for 2 h. The product was then isolated by ion-exchange chromatography using Dowex 50W anionic exchange resin, eluting with a linear gradient of increasing concentration of aqueous HCl–MeOH (0.5–3.0 M, 100 cm^3 each). The product was isolated as a light yellow, brittle foam (15 mg, 87%); δ_H(250 MHz; $CDCl_3$) 7.43 (2 H, s, imidazole-ring CH), 7.15 (2 H, s, imidazole-ring CH), 4.78 (2 H, m, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 4.31 (4 H, m, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$, 2′-H, 2″-H), 3.35 (2 H, m, 1′-$H_a$$H_b$, 1″-$H_a$$H_b$), 3.16 (10 H, m, 1′-$H_a$$H_b$, 1″-$H_a$$H_b$, 1-H, 3-H, 5-H, 7-H), 2.09 (4 H, m, 2-H, 6-H); δ_C(63 MHz; $CDCl_3$) 144.50 (Ar-C), 129.10, 127.90 (Ar-CH), 66.00 (2′-CH, 2″-CH), 53.10 (3′-C, 3″-C), 49.90, 44.90, 44.89, 22.80 (1-C, 2-C, 3-C, 5-C, 6-C, 7-C, 1′-C, 1″-C); *m*/*z* (FAB) 470 (MH^+, 31%), 329 (60), 301 (14), 264 (14), 233 (16), 205 (12), 176 (100) (Found: MH^+, *m*/*z* 470.2475. $C_{18}H_{32}N_9O_6$ requires 470.2476).

N^1,N^4,N^8-Tris(diphenylphosphinoyl)spermidine 26

Spermidine (0.588 g, 4.0 mmol) was dissolved in THF (50 cm^3). Triethylamine (1.5 cm^3, 12 mmol) was added to the solution which was subsequently stirred for 5 minutes. DPPCl (2.4 cm^3, 9 mmol) was added by injection to the rapidly stirring solution over a 5 minute period. After a further 1 hour stir, water (10 cm^3) was added to the reaction mixture and the organic solvent was removed *in vacuo* . The residue was partitioned between water and dichloromethane (50 cm^3 each) and the aqueous layer washed with dichloromethane (3 × 50 cm^3). The combined organic layers were dried over Na_2SO_4, after which the solvent was removed *in vacuo.* The residue was purified by flash chromatography (silica; 5% MeOH–CH_2Cl_2). Combined fractions had their solvent removed *in vacuo* to leave a white, brittle foam (2.27 g, 76%); ν_{max}($CHCl_3$)/cm^{-1} 3405w (N–H stretch), 3205w, 2930w, 1730w, 1410m, 1190s (P=O stretch), 1125s (P=O stretch); δ_H(300 MHz; $CDCl_3$) 7.75 (12 H, m, *meta*-ring CH), 7.38 (18 H, m, *ortho*-ring CH, *para*-ring CH), 4.58 (1 H, br pseudo q, $^3J_{H\text{-}H}$ 7.4 and $^2J_{P\text{-}H}$ 7.4, N^1-H), 3.48 (1 H, br pseudo q, $^3J_{H\text{-}H}$ 6.9, $^2J_{P\text{-}H}$ 6.9, N^8-H), 3.12 (2 H, m, 1-H), 2.86 (6 H, m, 3-H, 7-H, 8-H), 1.74 (2 H, m, 2-H), 1.52 (2 H, m, 7-H), 1.34 (2 H, m, 6-H); δ_C(75 MHz; $CDCl_3$) 132.22, 132.07, 131.98, 131.48 (Ar-C), 131.92, 131.89, 131.80, 131.76, 131.68, 131.64, 128.59, 128.51, 128.44, 128.39, 128.31, 128.24 (Ar-CH), 45.61, 43.12, 40.31, 37.90 (1-C, 3-C, 5-C, 8-C), 30.30, 29.34, 25.92 (2-C, 6-C, 7-C); δ_P(101 MHz; $CDCl_3$) 31.80 (s, N^4-P), 23.72 (s, N^1-P), 23.61 (s, N^8-P); *m*/*z* (FAB) 744 (M − H^+, 100%), 684 (14.2), 544 (8.9, loss of Ph_2PO), 473 (17.9), 416 (2.9), 305 (16.8), 242 (14.9), 216 (74.0, $Ph_2PONH^{\cdot+}$).

N^1,N^8-Bis(2′,3′-epoxypropyl)-N^1,N^4,N^8-tris(diphenylphosphinoyl)spermidine 27

N^1,N^4,N^8-Tris(diphenylphosphinoyl)spermidine **26** (100 mg, 0.13 mmol) was dissolved in THF (10 cm^3). Additions of NaH (31 mg, 0.78 mmol) and epichlorohydrin (1.1 cm^3, 1.3 mmol) were made to the stirred solution and the resulting mixture was heated at 90 °C for 3 h. The mixture was allowed to cool to room temperature and a further addition of NaH (20 mg, 0.39 mmol) was made. Following 15 minutes of stirring a further addition of epichlorohydrin (10 equiv., 1.1 cm^3) was made and the reaction mixture re-heated to 90 °C where it was maintained for a further 3 h. The mixture was allowed to cool to room temperature, whereupon ethyl acetate and water (1 cm^3 each) were added. After 5 minutes stirring the solvent was removed *in vacuo*. The residue was partitioned between water and chloroform (5 cm^3 each) and the aqueous layer was washed with chloroform (3 × 5 cm^3). The combined organic layers were dried over Na_2SO_4, then solvent was removed *in vacuo.* The residue was purified by flash chromatography (silica; 5% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a colourless, viscous gum (65 mg, 58%); ν_{max}($CHCl_3$)/cm^{-1} 3400m, 2960m, 1440m, 1260m, 1190s (P=O stretch), 1120s (P=O stretch), 900m, 700m br (out of plane Ar-CH stretch); δ_H(250 MHz; $CDCl_3$) 7.73 (12 H, m, *meta*-ring CH), 7.42 (18 H, m, *ortho*-ring CH, *para*-ring CH), 3.29 (2 H, m, 2′-H, 2″-H), 3.04 (4 H, m, 1′-H, 1″-H), 2.76 (10 H, m, 1-H, 3-H, 5-H, 8-H, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 2.31 (2 H, m, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 1.72 (2 H, m, 2-H), 1.24 (4 H, m, 6-H, 7-H); δ_C(75 MHz; $CDCl_3$) 133.40, 133.07, 132.10, 131.40 (Ar-C), 133.00, 133.89, 132.80, 132.76, 132.68, 132.64, 132.59, 131.51, 129.44, 128.39, 128.31, 128.24 (Ar-CH), 51.8, 51.7 (2′-C, 2″-C), 48.94, 47.33, 45.52, 45.43, 45.24, 43.46 (1-C, 3-C, 5-C, 8-C, 1′-C, 1″-C, 3′-C, 3″-C), 27.61, 26.21, 26.23 (2-C, 6-C, 7-C); δ_P(101 MHz; $CDCl_3$) 31.58 (s, N^1-P), 31.46 (s, N^8-P), 30.78 (s, N^4-P); *m*/*z* (FAB) 858 (MH^+, 100%), 802 (5), 656 (10, loss of $Ph_2P{=}O$), 338 (30), 201 (96, $Ph_2P{=}O^{+\bullet}$), 77 (32, $Ph^{+\bullet}$) (Found: MH^+, *m*/*z* 858.3387. $C_{49}H_{55}N_3O_5P_3$ requires 858.3354).

N^1,N^8-Bis[2″-hydroxy-3″-(2′-nitroimidazol-1′-yl)propyl]-N^1,N^4,N^8-tris(diphenylphosphinoyl)spermidine 28

Compound **27** (40 mg, 0.047 mmol) and 2-nitroimidazole (21 mg, 0.188 mmol) were dissolved in methanol (1 cm^3) in a sealed tube. Following the addition of triethylamine (6 equiv., 0.39 cm^3) the reaction mixture was heated at 120 °C for 5 h. The volatiles were then removed *in vacuo* and the residue was purified by flash chromatography (silica; 5% MeOH–CH_2Cl_2). The combined fractions had solvent removed *in vacuo* to leave a light yellow, viscous gum (37 mg, 72%); δ_H(250 MHz; $CDCl_3$) 7.68 (12 H, m, *meta*-ring CH), 7.39 (18 H, m, *ortho*-ring CH, *para*-ring CH), 7.21 (2 H, s, imidazole-ring CH), 7.01 (2 H, s, imidazole-ring CH), 4.52 (2 H, pseudo d, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 4.05 (2 H, br m, 2′-H, 2″-H), 3.99 (2 H, m, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 3.02 (4 H, m, 1′-H, 1″-H), 2.69 (8 H, m, 1-H, 3-H, 5-H, 8-H), 1.68 (2 H, m, 2-H), 1.23 (4 H, m, 6-H, 7-H); δ_C(63 MHz; $CDCl_3$) 144.92, 133.47, 133.03, 132.24, 131.05 (Ar-CH), 133.00, 133.89, 132.70, 132.54, 132.06, 132.00, 131.39, 129.23, 128.37, 128.34, 128.23, 126.98 (Ar-CH), 68.3 (2′-C, 2″-C), 54.20 (3′-C, 3″-C), 50.65, 47.53, 45.61, 45.22, 43.44, 27.81, 25.91, 25.83 (1-C, 2-C, 3-C, 5-C, 6-C, 7-C, 1′-C, 1″-C); δ_P(101 MHz; $CDCl_3$) 35.75 (d, N^1-P), 35.47 (d, N^8-P), 31.14 (s, N^4-P); *m*/*z* (FAB) 1084 (MH^+, 22%), 1039 (2), 1003 (9), 915 (26), 834 (4), 289 (15), 201 (100, $Ph_2P{=}O^{+\bullet}$), 77 (92, $Ph^{+\bullet}$).

N^1,N^8-Bis[2″-hydroxy-3″-(2′-nitroimidazol-1′-yl)propyl]spermidine hydrochloride 9

Compound **28** (46 mg, 0.042 mmol) was dissolved in 0.5 M HCl–MeOH (50 : 50; 5 cm^3). The solution was subsequently stirred at 60 °C for 2 h. The product was then isolated by ion-exchange chromatography using Dowex 50W anionic exchange resin, eluting with a linear gradient of increasing concentration of aqueous HCl–MeOH (0.5–3.0 M, 100 cm^3 each). The product was isolated as a light yellow, brittle foam (22 mg, 87%); δ_H(300 MHz; $CDCl_3$) 7.46 (2 H, s, imidazole-ring CH), 7.22 (2 H, s, imidazole-ring CH), 4.75 (2 H, m, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$), 4.37 (4 H, m, 3′-$H_a$$H_b$, 3″-$H_a$$H_b$, 2′-H, 2″-H), 3.38 (2 H, m, 1′-$H_a$$H_b$, 1″-$H_a$$H_b$), 3.16 (10 H, m, 1′-$H_a$$H_b$, 1″-$H_a$$H_b$, 1-H, 3-H, 5-H, 8-H), 2.14 (2 H, m, 2-H), 1.79 (4 H, m, 6-H, 7-H); δ_C(63 MHz; $CDCl_3$) 131.4, 130.5 (Ar-CH), 68.4 (2′-C, 2″-C), 55.4 (3′-C, 3″-C), 52.2, 52.1 (1′-C, 1″-C), 49.7, 49.6, 47.3, 47.1, 25.4 (2-C), 25.2, 25.1 (6-C, 7-C); *m*/*z* (FAB) 484 (MH^+,

32%), 329 (60), 264 (23), 219 (26), 176 (100) (Found: MH^+, *m*/*z* 484.2632. $C_{19}H_{34}N_9O_6$ requires 484.2632).

N^1,$N^{1'}$-(Dithiodiethylene)bis[N^1,N^4-methylene-N^8-(*tert*-butoxycarbonyl)spermidine] 30

N^1,N^4-Methylene-N^8-(*tert*-butoxycarbonyl)spermidine **13** (205.4 mg, 0.8 mmol) was dissolved in acetonitrile (3 cm^3) and transferred to a sealed tube. Following the addition of ethylene sulfide (0.8 mmol, 48.5 cm^3), the tube was sealed and the reaction mixture heated at 80 °C for 3 days. After the removal of acetonitrile *in vacuo* the residue was purified by flash chromatography (silica; MeOH–1% conc. NH_4OH solution). The combined fractions had solvent removed *in vacuo* to leave the desired product, a colourless viscous oil (37%). A second product, the reduced form of the desired product, was collected in a similar fashion as a colourless oil. This was allowed to oxidise by stirring in dichoromethane (20 cm^3) for 120 h to yield the desired product which was subsequently combined with the amount already collected (329 mg, 65%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2940s (aliphatic C–H stretch), 1710s (C=O stretch), 1500m, 1335m, 1250s, 1165m, 700m; δ_H(250 MHz; $CDCl_3$) 4.86 (1 H, br s, NH), 3.12 (2 H, s, 9-H), 3.07 (2 H, br m, 8-H), 2.75 (2 H, m, 1′-H or 2′-H), 2.68 (2 H, m, 2′-H or 1′-H), 2.48 (4 H, m, 3-H, 1-H), 2.30 (2 H, m, 5-H), 2.62 (2 H, m, 2-H), 1.42 (4 H, m, 6-H, 7-H), 1.37 [9 H, m, $C(CH_3)_3$]; δ_C(63 MHz; $CDCl_3$) 156.43 (C=O), 79.32 [$C(CH_3)_3$], 76.15 (9-C, 9″-C), 54.90, 54.77, 52.68, 52.47, 40.74, 37.04, 28.84 [$C(CH_3)_3$], 28.26, 24.62, 23.44 (2-C, 6-C, 7-C); *m*/*z* (FAB) 633 (MH^+, 48%), 631 (57, $M - H^+$), 316 (100, monomer), 282 (14), 260 (44), 215 (57, loss of Boc), 159 (50) (Found: MH^+, *m*/*z* 633.4196. $C_{30}H_{61}N_6O_4S_2$ requires 633.4196).

N^1,$N^{1'}$-(Dithiodiethylene)bis[N^8-(*tert*-butoxycarbonyl)spermidine] 31

Compound **30** (45 mg, 0.07 mmol) was dissolved in ethanol (1 cm^3), followed by pyridine (0.22 mmol, 36 μl) and malonic acid (0.26 mmol, 54 mg). The reaction mixture was refluxed for two hours at 115 °C. Following the removal of volatiles *in vacuo*, the residue was taken up in water (5 cm^3) and washed with chloroform (3 × 5 cm^3). The aqueous layer was adjusted to pH 11 by the addition of a few drops of 10% aqueous NaOH. The aqueous layer was then extracted with chloroform (5 × 5 cm^3) and the combined chloroform extracts were dried over Na_2SO_4. Removal of the solvent *in vacuo* isolated the product as a faintly yellow, viscous oil (35 mg, 81%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2950s (aliphatic C–H stretch), 1710s (C=O stretch), 1450m, 1335m, 1250s, 710m; δ_H(250 MHz; $CDCl_3$) 4.90 (1 H, br s, OCNH), 3.07 (2 H, s, 8-H), 2.89 (2 H, m, 1′-H or 2′-H), 2.79 (2 H, m, 2′-H or 1′-H), 2.62 (6 H, m, 1-H, 3-H, 5-H), 1.88 (2 H, br s, D_2O exchange, NH), 1.62 (2 H, m, 2-H), 1.43 (4 H, m, 6-H, 7-H), 1.39 [9 H, m, $C(CH_3)_3$]; δ_C(63 MHz; $CDCl_3$), 156.4 (C=O), 79.3 [$C(CH_3)_3$], 50.0, 48.8, 48.4, 40.9, 40.3, 39.1 (1-C, 3-C, 5-C, 8-C, 1′-C, 2′-C, 1″-C, 2″-C), 30.5, 28.3, 27.7 (2-C, 6-C, 7-C), 28.8 [$C(CH_3)_3$]; *m*/*z* (FAB) 610 (MH_2^+, 100%), 304 (27, monomer), 272 (30, loss of $C_{14}H_{31}N_3O_2S_2$), 136 (74) (Found: MH_2^+, *m*/*z* 610.4274. $C_{28}H_{62}N_6O_4S_2$ requires 610.4274).

N^1-(2-Mercaptoethyl)spermidine hydrochloride 10

A solution of **31** (327 mg, 0.54 mmol) in 5 M HCl (5 cm^3) was stirred for 30 min. Following the removal of water *in vacuo* the residue was taken up in triethylammonium acetate buffer (5 cm^3, pH 7.4), to which was added dithiothreitol (0.32 mmol, 50 mg). The reaction mixture was then stirred for 30 minutes before removal of water *in vacuo*. The product was purified by ion-exchange chromatography using Dowex 50W anionic exchange resin, eluting with a linear gradient of increasing concentration of aqueous HCl–MeOH (0.5–3.0 M, 200 cm^3 each). The product was isolated as a white, hygroscopic solid (133 mg, 78%); δ_H(250 MHz; $CDCl_3$) 3.03 (2 H, t, *J* 7.5, 1′-H), 2.91 (8 H, m, 1-H, 3-H, 5-H, 8-H), 2.62 (2 H, t, *J* 7.5, 2′-H), 1.90 (2 H, m, 2-H), 1.56 (4 H, m, 6-H, 7-H); δ_C(63 MHz; $CDCl_3$) 50.20, 47.35, 44.73, 44.62, 39.10 (1-C, 3-C, 5-C, 8-C, 1′-C), 24.15 (2-C), 22.99, 22.84 (6-C, 7-C), 20.20 (2′-C); *m*/*z* (FAB) 206 (MH^+, 100%), 180 (15), 165 (26) (Found: MH^+, *m*/*z* 206.1691. $C_9H_{24}N_3S$ requires 206.1691).

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