Protecting-group strategies for the synthesis of N^4-substituted and N^1,N^8-disubstituted spermidines, exemplified by hirudonine

Bernard T. Golding,[a]* Andrew Mitchinson,[a] William Clegg,[b] Mark R. J. Elsegood[b] and Roger J. Griffin[a]

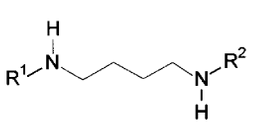
[a] *Department of Chemistry, Bedson Building, The University of Newcastle upon Tyne, Newcastle upon Tyne, UK, NE1 7RU*
[b] *Chemical Crystallography Unit, Department of Chemistry, Bedson Building, The University of Newcastle upon Tyne, Newcastle upon Tyne, UK, NE1 7RU*

Received (in Cambridge) 11th August 1998, Accepted 20th October 1998

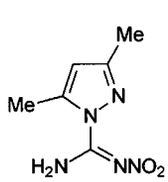
Methods are described for the preparation of derivatives of the polyamines 1,4-diaminobutane (putrescine) and *N*-(3-aminopropyl)-1,4-diaminobutane (spermidine) in which a particular amino group is modified with, *e.g.*, a guanidino function. Specific amino groups in these polyamines were protected as *N*-trifluoroacetyl and *N*-4-azidobenzyloxycarbonyl derivatives, which were unmasked chemoselectively using methanolic ammonia and dithiothreitol–triethylamine, respectively. Guanidino functions were introduced by an improved procedure in which an amino group was treated with 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide in methanol to give a nitroguanidine derivative, from which the nitro group was removed by catalytic transfer hydrogenation. The methodology is exemplified by the development of efficient preparative routes to agmatine and hirudonine. The integrity of the sequence of protection/deprotection leading to hirudonine was confirmed by a crystal-structure analysis of its sulfate. The effect of selected compounds on the uptake of putrescine into rat lung cells was determined and showed that N^4-(4-azidobenzyloxycarbonyl)spermidine was the best inhibitor (K_i = 3.4 μM).

Introduction

Polyamines are essential components of mammalian and plant cells that participate in a variety of biological functions, often in association with nucleic acids.[1] The common polyamines are putrescine (1,4-diaminobutane, **1**), spermidine [*N*-(3-aminopropyl)-1,4-diaminobutane, **2**] and spermine [*N*,*N*′-bis(3-aminopropyl)-1,4-diaminobutane, **3**] (for the numbering system for the nitrogen atoms of spermidine see structure **2** in Scheme 3). Cellular-uptake systems for polyamines have been characterised[2,3] and utilised for transporting toxic species into cells,[4–6] for example *via* their covalent attachment to N^4 of spermidine.[6] Numerous derivatives of polyamines have been described both as natural products[7] and as synthetic analogues.[8]



1 $R^1 = R^2 = H$
2 $R^1 = CH_2CH_2CH_2NH_2$, $R^2 = H$
3 $R^1 = R^2 = CH_2CH_2CH_2NH_2$



4

Primarily due to the therapeutic potential of polyamine derivatives, many studies have been concerned with their synthesis.[9] The strategy employed can be either the synthesis of the polyamine *de novo* or a partial synthesis starting from putrescine, spermidine or spermine. In the latter case, a major challenge is to discriminate efficiently between the nitrogen functions of the polyamine. We have previously reported a method for preparing spermidine derivatives by condensation of protected C_3 and C_4 fragments.[10,11] In this paper we describe a method which enables N^1,N^8-disubstituted derivatives **9** and **16** to be prepared reliably and efficiently from spermidine, either directly or *via* intermediate protection at N^4 with the 4-azidobenzyloxycarbonyl group[12] and/or at N^1 and N^8 with the trifluoroacetyl group (see compounds **13**–**15**). The natural products hirudonine **17** and agmatine **7** were used as test systems for exemplifying methodology, which included the use of the reagent 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide **4** for introducing *N*-nitroguanidino functions as precursors of guanidino functions. An improved crystal-structure determination for hydrated hirudonine sulfate is also presented. A preliminary communication on part of this work has appeared.[13]

We were also interested in the effect that the presence of guanidino functions in a polyamine structure would have on their cellular uptake. It has been surmised that the transporter system exhibits molecular recognition towards polyamines which have two positively charged nitrogen functions separated by ~7 Å.[3,14] This can account for the known selectivity of the transporter for diamines $H_2N(CH_2)_nNH_2$ where $n > 3$.[14] As guanidino functions bind strongly to carboxylates,[15] it was expected that polyamines bearing guanidino functions would also be efficiently transported. Guanylated polyamines may also be expected to compete with natural polyamines in other biologically important processes. Indeed, certain guanylated polyamines have been recently shown to inhibit uptake of spermidine by deoxyhypusine synthase, an enzyme implicated in cell proliferation.[16]

We describe preliminary results on the measurement of the uptake of hirudonine and agmatine into rat lung cells. We have also determined the uptake of N^4-(4-azidobenzyloxycarbonyl)spermidine **14** into cells, with a view to developing analogous spermidine derivatives as selective, intracellular enzyme inhibitors.

Results and discussion

Preparation of guanidine derivatives using 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide (DMNPC) 4

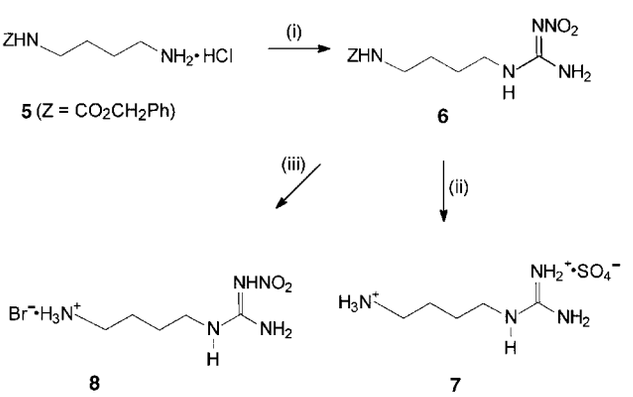
Amines can be converted directly into guanidines by treatment with *O*-methylisourea,[17] or *S*-methylisothiourea.[18] However, the basicity of guanidines may present difficulties in carrying a free guanidine group through several steps of a synthetic sequence. An alternative approach is to use a nitroguanidine as a masked guanidine. The nitroguanidino function is essentially non-basic and unaffected by most reaction conditions, which allows synthetic manipulations to be performed elsewhere in a molecule prior to reduction of the nitroguanidine to release the desired guanidine end-product. For the conversion of amines into nitroguanidines, we have used 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide (DMNPC) **4**.[10,19]

Treatment of nitroguanidine with aqueous hydrazine hydrate gave nitroaminoguanidine (1-amino-2-nitroguanidine),[20] which was condensed with pentane-2,4-dione in water, containing acetic acid as a catalyst, to yield DMNPC **4**.[19] Reactions of amines with DMNPC have been previously carried out in 1,4-dioxane,[19] which we sought to replace because of its toxicity and low polarity. As a model for nitroguanidinations, the reaction of benzylamine with DMNPC leading to *N*-benzyl-*N′*-nitroguanidine was studied under a variety of conditions of solvent, and in the presence or absence of a putative catalyst (metal ion, acid or base). The cleanest and fastest reactions were those in methanol (80% yield of *N*-benzyl-*N′*-nitroguanidine, which precipitated directly from the reaction mixture), for which no useful catalysts were found. Indeed, the use of either erbium(III) or holmium(III) trichloride as 'catalyst' diverted the reaction to afford *O*-methyl-*N*-nitroisourea.

Synthesis of agmatine 7

Agmatine is the mono-guanidinated derivative of putrescine, formed by arginine decarboxylation, and is distributed widely throughout Nature. It has been found in plants,[21] bacteria[22] and some higher life forms, including mammals.[23] Recently, this compound and analogues have assumed considerable importance because of the ability of agmatine to inhibit nitric oxide synthase,[24] its affinity for α-adrenoceptors, *e.g.* the I_1 receptor,[25] and its suppression of proliferation by reducing cellular levels of polyamines.[26]

To exemplify our methodology we have prepared agmatine as shown in Scheme 1. Nitroguanidination of *N*-benzyloxycarbonyl-1,4-diaminobutane **5** (obtained from the corresponding hydrochloride[27]) with DMNPC **4** in methanol gave the nitroguanidine **6** in 85% yield. The preparation of compound **6** in 47% yield has been reported in the patent literature, using similar reagents but under different reaction conditions.[28] Simultaneous removal of the benzyloxycarbonyl and nitro protecting groups from compound **6** was achieved using catalytic transfer hydrogenation,[29] with formic acid as the hydrogen donor. Addition of sulfuric acid to the reaction mixture precipitated agmatine sulfate **7** in 52% yield.



Scheme 1 Synthesis of agmatine sulfate **7** and intermediate **8**. *Reagents and conditions:* (i) Na_2CO_3 (10% w/v); 1.2 equiv. DMNPC **4**, MeOH, 25 °C, 3 days; (ii) HCO_2H–MeOH (5% v/v), 10% Pd/C (1 mass equiv.), 25 °C, 2 h; 1 equiv. H_2SO_4; (iii) HBr in AcOH (45% w/v), 25 °C, 1 h.

The benzyloxycarbonyl protecting group of compound **6** could be removed independently of reduction of the nitroguanidine group. This was originally attempted using trimethylsilyl iodide, either used directly,[30] or generated *in situ* from trimethylsilyl chloride and sodium iodide.[31] Neither of these methods was satisfactory, with the maximum yield attained being only 39%. The reaction was best performed using hydrobromic acid[28] (see Scheme 1), which generated 1-(4-aminobutyl)-2-nitroguanidine hydrobromide **8** in 88% yield.

Synthesis of N^4-substituted spermidines 10–12 and 14

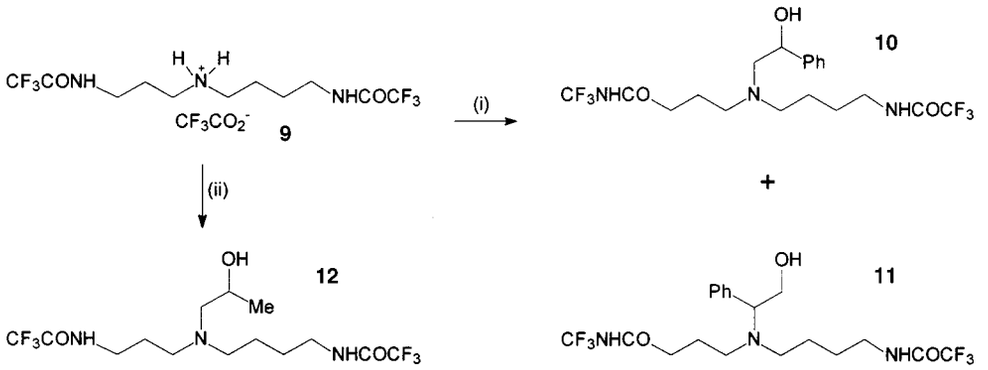
Reaction of spermidine with four equivalents of ethyl trifluoroacetate, in the presence of one equivalent of water in acetonitrile under reflux, afforded N^1,N^8-bis(trifluoroacetyl)-spermidine trifluoroacetate **9** as a crystalline solid in 89% yield.[13] Selective trifluoroacetylations of other amines and polyamines by this technique have been reported.[9,32–34] Although a number of methods have been reported[35–45] for the selective protection or reaction of primary amino groups in polyamines, our procedure offers technical simplicity and the use of readily available reagents. The reason for the selectivity observed in these reactions is not clear. Nagao and Fujita[35] observed selectivity for the primary amino groups of spermidine in reactions with 3-acyl-1,3-thiazolidine-2-thiones and explained this by postulating a conformation for spermidine that would reduce the nucleophilicity of N^4. However, the observed selectivities may be merely a reflection of the different basicities and hence nucleophilicities of the amino functions (with the first protonation occurring predominantly at the N^8 position, then at N^1 and finally at N^4).[46,47] The direct synthesis of N^1,N^8-disubstituted spermidines is further illustrated by the synthesis of hirudonine **17**, described below. However, the success of this method for preparing N^1,N^8-disubstituted spermidines depends on achieving direct crystallisation and/or easy separation of the desired product. Our method for preparing N^1,N^8-disubstituted spermidines, which necessarily has more steps, is completely reliable for securing the desired product.

Conversion of compound **9** into N^1,N^8-bis(trifluoroacetyl)-N^4-(4-azidobenzyloxycarbonyl) spermidine **13** was achieved in excellent yield by treatment of compound **9** with *N*-ethyldiisopropylamine (Hünig's base) and 4-azidobenzyl 4-nitrophenyl carbonate[12] in THF. Reaction of compound **13** with conc. ammonia in methanol gave N^4-(4-azidobenzyloxycarbonyl)spermidine **14** in 82% yield (see Scheme 3).

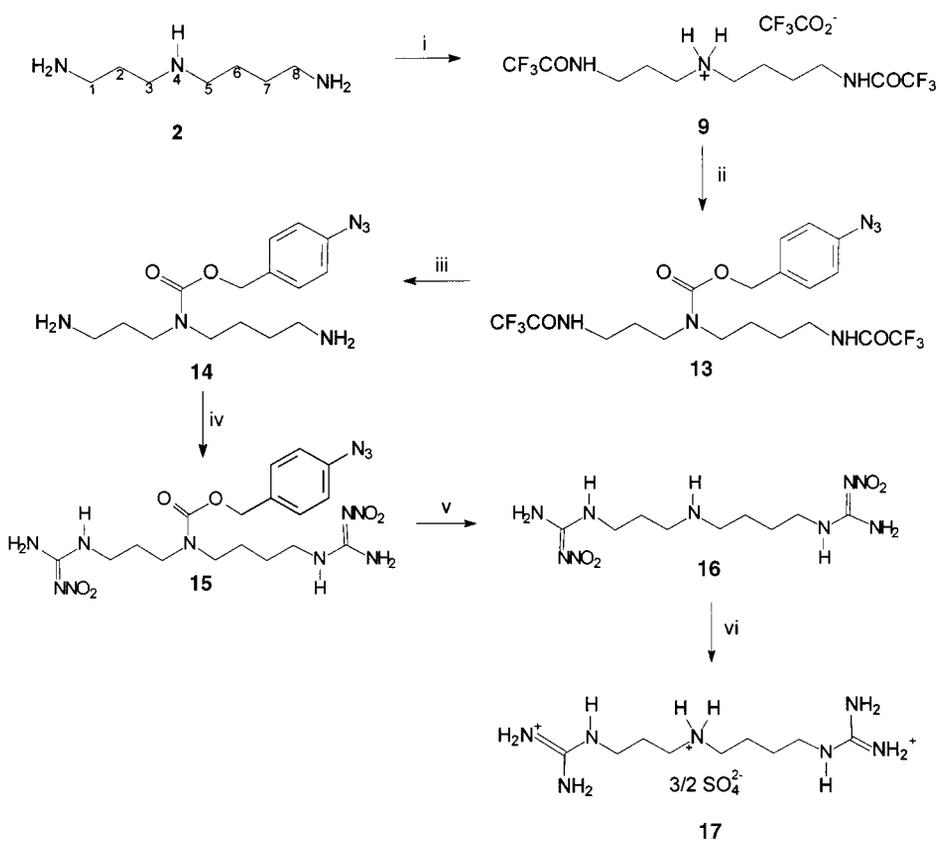
Compound **9** is an excellent starting material for the preparation of N^4-alkylated spermidines from epoxides. Although salt **9** reacts directly with epoxides in methanol, the yields are low, and catalysing the reaction using lithium perchlorate[48] in acetonitrile was found to give much better yields. Thus, alkylation of compound **9** with styrene oxide in the presence of catalytic lithium perchlorate and Hünig's base in acetonitrile gave a mixture of regioisomers **10** and **11** (ratio of 3 : 1, respectively) in 89% total yield (see Scheme 2). Similarly, reaction of salt **9** with propylene oxide gave compound **12** in 83% yield.

Synthesis of hirudonine 17

'Orthogonally' protected polyamines, in which each amino function is protected with a different group capable of being removed independently, are required for the synthesis of complex polyamine derivatives. Spermidine and related compounds are attractive templates for combinatorial synthesis,[49] but their exploitation, especially in parallel syntheses, requires efficient



Scheme 2 Alkylation of salt **9** with epoxides. *Reagents and conditions:* (i) EtN^iPr_2, styrene oxide, $LiClO_4$, CH_3CN, 25 °C, 24 h; (ii) EtN^iPr_2, propylene oxide, $LiClO_4$, CH_3CN, 25 °C, 24 h (*nb* all compounds in this scheme are racemic).



Scheme 3 Synthesis of hirudonine sulfate **17**. *Reagents and conditions:* (i) 4 equiv. CF_3CO_2Et, 1 equiv. water, CH_3CN, reflux, 7 h; (ii) 1.1 equiv. 4-azidobenzyl 4-nitrophenyl carbonate, 2.1 equiv. EtN^iPr_2, THF, 25 °C, dark, 24 h; (iii) conc. aq. NH_3, MeOH, 25 °C, dark, 6 days; (iv) 2 equiv. DMNPC **4**, MeOH, 25 °C, dark; (v) 4 equiv. DTT, 4 equiv. Et_3N, MeOH–water (9 : 1 v/v), 25 °C, dark, 4 h; (vi) HCO_2H–MeOH (5% w/v), 10% Pd/C (0.5 mass equiv.), 25 °C, 3 h; H_2SO_4.

methods for stepwise manipulation of the polyamine functionalities. Various syntheses of orthogonally protected polyamines have been reported, and are exemplified by the work of Bergeron and McManis.[50] Protecting groups with unusual conditions for removal are of great potential in polyamine synthesis. The use of the protecting group 4-azidobenzyloxycarbonyl for this purpose is illustrated (see Scheme 3) in the synthesis of the N^1,N^8-bis-guanidinated derivative of spermidine (hirudonine **17**), isolated as its sulfate. This substance has been found in the central nervous system of the leech *Hirudo medicinalis* L.[51,52]

Reaction of compound **14** (Scheme 3) with two equivalents of DMNPC in methanol, at saturating concentrations of the reactants, yielded N^1,N^8-bis(nitroguanidinyl)-N^4-(4-azidobenzyloxycarbonyl)spermidine **15** in 81% yield, and sufficiently pure for the next stage. Removal of the N^4-protecting group from carbamate **15** by reduction with dithiothreitol (DTT) in aq. methanol afforded N^1,N^8-bis(nitroguanidinyl)spermidine **16** in 50–60% yield. Compound **16** was reduced by catalytic transfer hydrogenation to give hirudonine **17**, isolated as its sulfate. This could be obtained by addition of sulfuric acid to the reaction mixture, once the Pd/C catalyst had been removed by filtration. In this way the desired product was isolated in 89% yield. The structure of this material was confirmed by an X-ray analysis (see below).

With an authentic specimen of hirudonine sulfate in hand, it was possible to explore two direct approaches to this compound. In one approach, spermidine was allowed to react with two equivalents of DMNPC in methanol. A solid precipitated from the reaction solution, which was subjected to catalytic transfer hydrogenation as before, to give hirudonine sulfate with an overall yield of 66%. The synthesis of hirudonine has been reported in a one-step procedure, by the reaction of spermidine directly with *O*-methylisothiourea sulfate.[53] We confirm that this reaction does indeed give hirudonine sulfate, which we obtained in 47% yield.

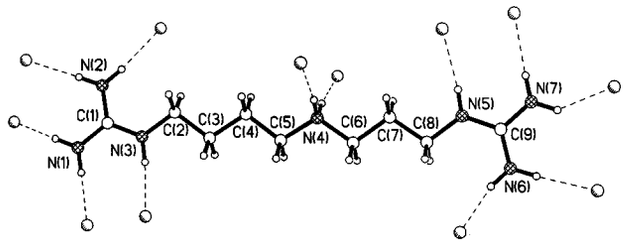


Fig. 1 Structure of the triprotonated hirudonine molecule with hydrogen bonds to twelve adjacent oxygen atoms of sulfate anions and water molecules, and crystallographic numbering scheme.

Crystal structure of hydrated hirudonine sulfate

It is conceivable that intramolecular group migration can occur between nitrogen functions in spermidine and it was therefore considered essential to confirm the regioselectivity of the sequences described. We also wished to determine the conformation of hirudonine **17**, which was of interest in the context of protein and DNA binding studies. The structure has been previously reported,[54] but the data were of poor quality and the structural results of low precision. We have obtained more satisfactory results from data collected at low temperature, facilitating a reasonable modelling of the disorder in the sulfate anions and water molecules.

The structure of the fully ordered cation is shown in Fig. 1, together with twelve neighbouring oxygen atoms to which it is hydrogen bonded. All the hydrogen atoms of the cation were clearly revealed in a difference synthesis and were refined subject to the constraints of a riding model with C–H and N–H distances appropriate to X-ray diffraction. The hirudonine molecule is unambiguously triprotonated in such a way that the central nitrogen is NH_2^+ and all four terminal nitrogen atoms are NH_2. The essentially planar guanidine groups have completely delocalised bonding with all their C–N bonds of similar length (Table 1). All twelve N–H bonds are involved in hydrogen bonding to oxygen atoms of sulfate anions and water molecules, with H···O distances in the range 1.81–2.10 Å, N···O 2.71–2.96 Å, and N–H···O angles of 162–174° except for one value of 139°.

The cation adopts a fully extended (all-*anti*) conformation in this crystal structure, with all torsion angles (excluding H-atoms) within 6.2° of 180° (or 0° in two N–C–N–C cases). A similar extended conformation has been found in several derivatives of spermine and spermidine,[55–58] while a single *gauche* segment occurs in others.[59–61]

The sulfate anions are each two-fold disordered. Disorder also affects most of the water molecules in the crystal structure. Our disorder model is consistent with a tetrahydrate formulation,[54] and with normal hydrogen-bonding geometries.

Inhibition of putrescine uptake into lung cells by spermidine derivatives

The inhibitory effect upon the uptake of ^{14}C-putrescine into rat lung slices was determined for agmatine **7**, hirudonine **17**, 1-(4-aminobutyl)-2-nitroguanidine **8** and N^4-(4-azidobenzyloxycarbonyl)spermidine **14** by using the method of Smith and Wyatt.[14] All of the compounds were inhibitors of putrescine uptake except for hirudonine **17**, which showed no significant effect (K_I-values/μM: 5.3 ± 0.2 for **7**, 65 ± 12 for **8** and 3.4 ± 1.3 for **14**). Identical V_{max}-values were obtained when calculated in the absence or presence of the compounds tested, indicating that competitive inhibition was occurring. Pre-incubation studies with compounds **7** and **14** confirmed the competitive nature of their inhibition of putrescine uptake.

Compound **8** was a much less effective inhibitor of putrescine uptake than was agmatine **7**, its close analogue. This was presumably because compound **8** contains a non-basic nitroguanidine group, which would not be protonated under physiological conditions, making it a poor substrate for the receptor. The best inhibitor was compound **14**, a spermidine derivative, in which the terminal amino groups had been left intact. This was accepted at the receptor despite the bulky group attached at the N^4-position.

The substrate-binding sites of polyamine transporters are thought to contain carboxylate groups,[13] which interact with protonated polyamines. In some lactate dehydrogenase enzyme–substrate complexes, replacement of an amino residue in the binding site with a guanidine residue results in stronger interactions to the carboxy group of the substrate.[62] The failure of hirudonine **17** to inhibit putrescine uptake was therefore surprising given the result for agmatine **7**, and that methylglyoxal bis(guanylhydrazone) (MGBG) and its analogues are well known guanidinated compounds taken up by these transporters.[14] It would be interesting to test hirudonine **17** and agmatine **7** in other models for polyamine uptake, such as the murine B16 melanoma cell line. Further studies upon compound **14** are also desirable, to test for its cytotoxic properties and for its behaviour upon irradiation with UV light.

Table 1 Geometry of the triprotonated hirudonine molecule, excluding H atoms

Bond length/Å			
N(1)–C(1)	1.323(7)	N(2)–C(1)	1.335(7)
C(1)–N(3)	1.323(7)	C(9)–N(6)	1.309(8)
N(5)–C(9)	1.322(8)		
C(9)–N(7)	1.343(7)		
Bond angle (°)			
N(1)–C(1)–N(3)	119.6(5)	N(1)–C(1)–N(2)	119.5(5)
N(3)–C(1)–N(2)	120.9(5)	N(6)–C(9)–N(5)	122.8(6)
N(6)–C(9)–N(7)	118.5(6)	N(5)–C(9)–N(7)	118.7(6)
Torsion angle (°)			
N(1)–C(1)–N(3)–C(2)	−174.0(5)	N(2)–C(1)–N(3)–C(2)	6.0(8)
C(1)–N(3)–C(2)–C(3)	−180.0(5)	N(3)–C(2)–C(3)–C(4)	−179.6(5)
C(2)–C(3)–C(4)–C(5)	−178.8(5)	C(3)–C(4)–C(5)–N(4)	179.9(5)
C(4)–C(5)–N(4)–C(6)	175.5(5)	C(5)–N(4)–C(6)–C(7)	−178.9(5)
N(4)–C(6)–C(7)–C(8)	179.6(5)	C(6)–C(7)–C(8)–N(5)	179.2(5)
C(7)–C(8)–N(5)–C(9)	−176.0(5)	C(8)–N(5)–C(9)–N(6)	3.4(8)
C(8)–N(5)–C(9)–N(7)	−178.0(5)		

Experimental

General procedures

All commercial chemical reagents used were of analytical grade or the highest available purity. When necessary, reagents and solvents were purified by standard procedures.[12,63] Light petroleum was the fraction boiling between 40 and 60 °C and ether refers to diethyl ether. Solvents were removed either on a rotary evaporator (ambient temperature; 10–15 mmHg) or by short-path distillation (ambient temperature; 0.001–0.01 mmHg). Water was removed with a freeze-dryer (LSL Secfroid, 0.05–0.2 mmHg).

TLC was performed on silica gel $60F_{254}$ (Merck Art. No. 1.05554) with visualisation of spots by UV light (254 nm), or by exposure to ninhydrin, potassium permanganate or molybdic acid solution, followed by heating to ~80 °C. For 'flash' chromatography, Fisons Matrix silica 60, mesh 0.035–0.070 mm was used. Mps were measured on a Kofler hot stage or Gallenkamp apparatus and are uncorrected. CHN analyses were performed with a Carlo-Erba Instrumentazione model 1106 analyser. IR spectra were recorded on a Nicolet 20PC Fourier transform spectrometer. NMR spectra were recorded on Bruker instruments, at 200, 300 or 500 MHz for ^{1}H, and with broad-band decoupling at 50, 75 or 125 MHz for ^{13}C. Chemical shifts and coupling constants are recorded in units of ppm and Hz, respectively. Chemical shifts labelled with an asterisk are exchangeable with D_2O. Electron impact (EI) and fast-atom

bombardment (FAB) mass spectra were recorded on a Kratos MS80RF spectrometer. FAB spectra were recorded using a matrix of 3-nitrobenzyl alcohol unless otherwise stated. The relative intensities of the peaks are given in parentheses.

Uptake of compounds into rat lung slices

Measurements of the uptake of compounds into rat lung slices were performed at the MRC Toxicology Unit, Leicester using the methods of Smith, Wyatt and co-workers.[3,14]

Nitroaminoguanidine (1-amino-2-nitroguanidine)[20]

To an agitated slurry of 2-nitroguanidine (2.83 g, 27 mmol) in water (40 ml) was added aq. hydrazine hydrate (1.32 ml, 1.36 g, 27.2 mmol) in water (20 ml) dropwise. The temperature in the flask was maintained at 60–65 °C until all the hydrazine had been added. The temperature was then held at 55–60 °C for a further 15 min with constant stirring. The slurry cleared to a solution, which turned from colourless to yellow, and finally to orange. The mixture was cooled rapidly to below 45 °C, neutralised with hydrochloric acid (10 M) to prevent further reaction, and cooled at 5 °C overnight. A precipitate was produced which was collected by filtration, washed with ice-cold water and air-dried. The crude solid was recrystallised twice from water to give nitroaminoguanidine as a solid (1.04 g, 32%) homogeneous by TLC (silica gel; 20% v/v methanol in dichloromethane; UV detection) R_f 0.34, mp 187–188 °C (decomp.) lit.,[20] 190 °C); δ_H(200 MHz; d_6-DMSO) 4.80 (2H, s, NH_2), 7.67 (1H, br s, N*H*NH_2), 8.39 (1H, br s, one of NH_2) and 9.45 (1H, br s, one of NH_2); δ_C(50 MHz; d_6-DMSO) 161.8 [$H_2NC(=NNO_2)NHNH_2$].

3,5-Dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide 4

To a solution of nitroaminoguanidine (0.70 g, 5.9 mmol) in boiling water (130 ml) were added glacial acetic acid (1 ml) and pentane-2,4-dione (0.77 ml, 0.75 g, 7.5 mmol) with vigorous stirring. The solution was allowed to cool to rt, and was stirred for 7 h. A precipitate was formed. This was filtered off, and recrystallised from ethanol to give 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide DMNPC **4** as crystals (0.86 g, 80%), homogeneous by TLC (silica gel; 20% v/v methanol in dichloromethane; UV detection) R_f 0.79, mp 127–128 °C (lit.[19] 125–126 °C); δ_H(200 MHz; $CDCl_3$) 2.18 (3H, s, CH_3), 2.55 (3H, s, CH_3), 5.98 (1H, s, CH), 8.14 (1H, br s, one of NH_2) and 9.04 (1H, br s, one of NH_2); δ_C(50 MHz; $CDCl_3$) 13.7 and 15.9 (2 × CH_3), 112.6, 144.8 and 153.8 (ring carbons) and 155.7 [$H_2NC(=NNO_2)NHNH_2$].

1-[4-(Benzyloxycarbonylamino)butyl]-2-nitroguanidine 6

N-Benzyloxycarbonyl-1,4-diaminobutane hydrochloride[27] (0.70 g, 2.7 mmol) was dissolved in aq. sodium carbonate (10% w/v; 10 ml) and the resultant solution was extracted with dichloromethane (3 × 5 ml). The organic layers were combined and concentrated to give the free amine. This was taken up in methanol (10 ml) and DMNPC **4** (0.57 g, 3.1 mmol) was added with stirring. The mixture was stirred at rt for 3 days. The solvent was removed and the crude product was purified by 'flash' chromatography (silica, elution with 5% v/v methanol in dichloromethane) to give 1-[4-(benzyloxycarbonylamino)butyl 2-nitroguanidine **6** as a solid (0.71 g, 85%) homogeneous by TLC (silica gel; 20% v/v methanol in dichloromethane; UV detection, R_f 0.72), mp 127–129 °C (lit.,[28] 123–124.5 °C); δ_H(200 MHz; d_6-acetone) 1.66 (4H, m, $CH_2CH_2CH_2CH_2$), 3.24 (2H, br s, NH_2), 3.41 [2H, m, $CH_2NHC(=NNO_2)NH_2$], 4.74 (2H, br s, $NHCO_2$), 6.52 [0.4H, br s, $CH_2NHC(NNO_2)NH_2$ tautomer A], 7.37 (5H, m, ArH) and 7.66 [0.6H, br s, $CH_2NC(NHNO_2)NH_2$, tautomer B]; *m*/*z* (FAB) 310 (M + 1, 100%) (Found: C, 50.9; H, 6.1; N, 22.6. $C_{13}H_{19}N_5O_4$ requires C, 50.5; H, 6.15; N, 22.65%).

Agmatine sulfate 7

To 1-[4-(benzyloxycarbonylamino)butyl]-2-nitroguanidine **6** (0.25 g, 0.81 mmol) in a formic acid–methanol mixture (5% w/v, 20 ml) was added 10% Pd/C (0.25 g). The mixture was stirred at room temperature under nitrogen for 2 h. The catalyst was filtered off and sulfuric acid (0.5 M; 1.61 ml, 0.81 mmol) was added to the solution. Upon cooling at 5 °C, crystals of agmatine sulfate **7** were produced (96 mg, 52%), homogeneous by TLC (silica gel; 4 : 1 : 1 : 2 v/v butan-1-ol–acetic acid–pyridine–water; ninhydrin detection, R_f 0.21), mp 227–228 °C (lit.,[64] 229 °C); ν_{max}/cm^{-1} (KBr disc) 3335 (NH_2^+ of guanidine), 3172 (NH of guanidine), 3023 (NH_3^+ of amine), 2965 and 2881 (CH_2) and 1636 (C=N); δ_H(200 MHz; D_2O) 1.69 (4H, m, $CH_2CH_2CH_2CH_2$), 3.01 (2H, br m, $CH_2NH_3^+$), 3.28 [2H, br m, $CH_2NHC(=NH_2)NH_2^+$], 7.73* (3H, br s, $CH_2NH_3^+$), 8.02* [2H, br s, $NHC(=NH_2)NH_2^+$], 8.67* [1H, br s, $NHC(=NH_2)NH_2^+$]; δ_C(50 MHz; D_2O) 25.5 and 26.5 ($CH_2CH_2CH_2CH_2$), 40.5 and 42.0 [$CH_2NH_3^+$ and $CH_2NHC(=NH_2)NH_2^+$] and 158.3 [$NHC(=NH_2)NH_2^+$]; *m*/*z* (FAB) 131 (M + 1, 100%).

1-(4-Aminobutyl)-2-nitroguanidine hydrobromide 8

1-[4-(Benzyloxycarbonylamino)butyl]-2-nitroguanidine **6** (0.50 g, 1.6 mmol) in hydrobromic acid–acetic acid (45% w/v, 4.4 ml) was stirred at rt for 1 h. Ether was added dropwise to the solution to give a precipitate, and the mixture was chilled overnight at 5 °C. The solid was filtered off, and recrystallised from methanol–ether, to give (4-aminobutyl)-nitroguanidine hydrobromide **8** as crystals (0.37 g, 88%), homogeneous by TLC (silica gel; 4 : 1 : 1 : 2 v/v butan-1-ol–acetic acid–pyridine–water, ninhydrin detection, R_f 0.40), mp 172–174 °C (lit.,[28] 173–175 °C); δ_H(200 MHz; d_6-DMSO) 1.63 (4H, m, $CH_2CH_2CH_2CH_2$), 2.91 (2H, m, $H_3N^+CH_2CH_2$), 3.28 [2H, m, $CH_2NHC(=NNO_2)NH_2$], 7.73* (2H, br s, $H_3N^+CH_2$), 8.02* [2H, br s, $NHC(=NNO_2)NH_2$] and 8.67* [1H, br s, $NHC(=NNO_2)NH_2$]; *m*/*z* (FAB) 176 (M + 1, 100%), 102 (M − H_2NNO_2, 18), 89 [M − $C(NNO_2)NH_2$, 25] (Found: C, 23.7; H, 5.4; N, 26.9. $C_5H_{13}N_5O_2$·HBr requires C, 23.4; H, 5.5; N, 27.3%).

N^1,N^8-Bis(trifluoroacetyl)spermidine trifluoroacetate 9

To a stirred solution of spermidine (1.01 g, 7.0 mmol) in acetonitrile (25 ml) were added water (0.126 ml, 7.0 mmol) and ethyl trifluoroacetate (3.4 ml, 4.1 g, 29 mmol). The mixture was heated at reflux for 7 h, and then cooled to 5 °C. The pure product which precipitated was collected by filtration under reduced pressure. The filtrate was concentrated to give a crude product, which was recrystallised from acetonitrile to give pure N^1,N^8-*bis*(*trifluoroacetyl*)*spermidine trifluoroacetate* **9** as crystals, which were combined with the initially precipitated material (combined yield 2.81 g, 89%). The substance was homogeneous by TLC (silica gel, 4 : 1 v/v dichloromethane–methanol; potassium permanganate detection, R_f 0.65), mp 145–146 °C: ν_{max}/cm^{-1} (KBr disc) 3319 (NH), 2960 and 2877 (CH_2), 1706 and 1671 (C=O), 1561 (CO_2^-) and 1193 (CF_3); δ_H(200 MHz; d_6-DMSO) 1.65 (4H, m, $CH_2CH_2CH_2CH_2$), 1.92 (2H, m, $NH_2^+CH_2CH_2CH_2NH$), 3.01 (4H, br t, $CH_2NH_2^+CH_2$), 3.32 (4H, m, 2 × $CF_3CONHCH_2$), 8.81 (2H, m, 2 × CF_3CONH) and 9.68 (2H, br s, $CH_2NH_2^+CH_2$); δ_C(50 MHz; d_6-DMSO) 26.9, 29.1 and 29.3 ($CH_2CH_2CH_2NH_2^+CH_2CH_2$), 40.6 and 42.2 (2 × $CF_3CONHCH_2$), 48.5 and 50.4 ($CH_2NH_2^+CH_2$), 120 (2 × overlapping q, J_{CF} 288, 2 × CF_3CONH), 120.7 (q, J_{CF} 296, CF_3COO^-), 160.4 (two overlapping q, J_{CF} 44, 2 × CF_3CONH) and 162.8 (q, J_{CF} 32, CF_3COO^-); *m*/*z* (EI) 338 (M^+, 100%), 197 (M − $CH_3CH_2NHCOCF_3$, 11) and 183 (M − $CF_3CONHCH_2CH_2CH_3$, 21) (Found: C, 34.7; H, 3.8; N, 9.1. $C_{13}H_{18}F_9N_3O_4$ requires C, 34.6; H, 4.0; N, 9.3%).

N^4-(4-Azidobenzyloxycarbonyl)-N^1,N^8-bis(trifluoroacetyl)-spermidine 13

To a mixture of N^1,N^8-bis(trifluoroacetyl)spermidine trifluoroacetate **9** (0.44 g, 0.97 mmol) in dry, stirred THF (6 ml) was added *N*-ethyldiisopropylamine (0.35 ml, 2.0 mmol), followed by a solution of 4-azidobenzyl 4-nitrophenyl carbonate (0.35 g, 1.11 mmol) in THF (3 ml) over a period of 5 min. The mixture was stirred at rt under nitrogen in the dark for 24 h. The solvent was removed to give a brown oil, which was taken up in dichloromethane (5 ml). The resulting solution was washed successively with aq. sodium hydroxide (0.1 M; 3 × 15 ml) followed by saturated sodium aq. chloride (3 × 30 ml). The organic layer was concentrated to give the crude product, which was purified by 'flash' chromatography (silica gel; 2% v/v methanol in dichloromethane; UV detection) to give N^1,N^8-*bis(trifluoroacetyl)*-N^4-*(4-azidobenzyloxycarbonyl)spermidine* **13** as a yellow oil (0.45 g, 92%), homogeneous by TLC (silica gel; 2% methanol in dichloromethane v/v; UV detection; R_f 0.31); ν_{max}/cm^{-1} (cap film) 3310 (NH), 3104 (ArH), 2946 and 2888 (CH_2), 2120 (N_3), 1705 (C=O) and 1169 (CF_3); δ_H(200 MHz; $CDCl_3$) 1.48 (4H, s, $CH_2CH_2CH_2CH_2$), 1.63 (2H, m, $NCH_2CH_2CH_2N$), 3.23 (8H, m, 2 × $CF_3CONHCH_2$, CH_2NCH_2), 5.03 (2H, s, OCH_2Ar), 6.36 [1H, br s, $CONH(CH_2)_4$], 6.95 (2H, d, *J* 8.5, 2 × ArH) and 7.27 [1H, d, *J* 8.5, 2 × ArH), 7.92 (1H, br s, $CONH(CH_2)_3$]; δ_C(50 MHz; $CDCl_3$) 25.4, 26.3 and 27.1 ($CH_2CH_2CH_2CH_2NCH_2CH_2$), 36.4 and 39.6 (2 × $CF_3CONHCH_2$), 44.2 and 46.5 (CH_2NCH_2), 67.3 (CO_2CH_2), 115.9 (two overlapping q, J_{CF} 289, 2 × CF_3CONH), 119.2 (ArC), 130.0 (ArC), 133.0 (ArC), 140.5 (ArC), 157.1 (CO_2CH_2), 157.5 (two overlapping q, J_{CF} 34, 2 × CF_3CONH); *m*/*z* (FAB) 513 (M + 1, 2%), 469 (M − N_3, 3), 364 (M − $OCH_2C_6H_4N_3$, 15) and 338 (M − $CO_2CH_2C_6H_4N_3$, 13) (Found: C, 45.6; H, 4.1; N, 16.5. $C_{19}H_{22}F_6N_6O_4$ requires C, 44.5; H, 4.3; N, 16.4%).

N^4-(4-Azidobenzyloxycarbonyl)spermidine 14

To a solution of N^4-(4-azidobenzyloxycarbonyl)-N^1,N^8-bis-(trifluoroacetyl)spermidine **13** (0.114 g, 0.22 mmol) in methanol (8 ml) was added conc. ammonia (2 ml). The mixture was stirred at rt in the dark for 24 h and more conc. ammonia was added (2 ml). The reaction mixture was stirred as before for 5 days. The methanol was removed *in vacuo*, and the resultant aqueous solution made up to 5 ml with distilled water, washed with dichloromethane (3 × 10 ml) and freeze-dried. The crude product was purified by 'flash' chromatography (silica gel; 10% v/v methanol in conc. ammonia; UV detection) to give N^4-(4-*azidobenzyloxycarbonyl*)*spermidine* **14** as an oil (58 mg, 82%) homogeneous by TLC (silica gel; 10% v/v methanol in conc. ammonia; UV detection; R_f 0.23): ν_{max}/cm^{-1} (cap film) 3395 (NH), 2942 and 2870 (CH_2), 2114 (N_3) and 1684 (C=O); δ_H(200 MHz; d_6-DMSO) 1.59 (4H, br s, $CH_2CH_2CH_2CH_2$), 1.86 (2H, m, $NCH_2CH_2CH_2N$), 2.86 (4H, m, 2 × CH_2NH_2), 3.32 (4H, m, CH_2NCH_2), 5.14 (2H, s, OCH_2), 7.22 (2H, d, *J* 8.4, Ar *meta* protons), 7.50 (2H, d, *J* 8.5, Ar *ortho* protons) and 7.96 (4H, br s, 2 × NH_2); δ_C(50 MHz; D_2O) 26.5, 28.5 and 30.0 ($CH_2CH_2CH_2CH_2NCH_2CH_2$), 40.1 and 40.9 (two CH_2NH_2), 45.9 and 47.9 (CH_2NCH_2), 68.4 (OCH_2), 120.5 (Ar *meta* carbons), 131.0 (Ar *ortho* carbons), 134.6 (Ar *ipso* carbon), 141.1 (Ar *para* carbon), 158.7 (CO); *m*/*z* (FAB) 321 (M + 1, 30%) (Found: C, 56.7; H, 7.5; N, 25.9. $C_{15}H_{24}N_6O_6$ requires C, 56.25; H, 7.5; N, 26.25%).

N^4-(4-Azidobenzyloxycarbonyl)-N^1,N^8-bis(nitroguanidino)-spermidine 15

To a stirred solution of N^4-(4-azidobenzyloxycarbonyl)-spermidine **14** (0.551 g, 1.72 mmol) in methanol (10 ml) was added DMNPC **4** (0.632 g, 3.45 mmol). The mixture was stirred in the dark at rt for 3 days. A precipitate of crude product was formed. This was filtered off, and purified by 'flash' chromatography (silica gel; 15% v/v methanol in dichloromethane; UV detection) to give N^4-(*4-azidobenzyloxycarbonyl*)-N^1,N^8-*bis(nitroguanidino)spermidine* **15** as a pale yellow solid (0.690 g, 81%) homogeneous by TLC (silica gel; 10% v/v methanol in dichloromethane; UV detection; R_f 0.26), mp 144–146 °C: ν_{max}/cm^{-1} (KBr disc) 3407 (NH_2), 2951 and 2881 (CH_2), 2119 (N_3), 1685 (CO), 1642 (C=N) and 1595 (NO_2); δ_H(200 MHz; d_6-DMSO) 1.55 (4H, br m, $CH_2CH_2CH_2CH_2$), 1.79 (2H, m, $NHCH_2CH_2CH_2N$), 3.25 (4H, m, CH_2NCH_2), 3.48 [4H, m, 2 × $CH_2NHC(NNO_2)NH_2$], 5.13 (2H, s, CH_2OCO), 7.21 (2H, d, *J* 8.3, 2 × ArH), 7.48 (2H, d, *J* 8.3, 2 × ArH), 8.03* (4H, br s, 2 × NH_2) and 8.67* (2H, br s, 2 × NH); δ_C(50 MHz; d_6-DMSO) 25.2, 25.9 and 28.2 ($CH_2CH_2CH_2CH_2NCH_2CH_2$), 44.1, 45.0, 46.5 and 47.1 [2 × $CH_2NHC(NNO_2)NH_2$ and CH_2NCH_2], 119.4 (ArC), 129.7 (ArC), 134.2 (ArC), 139.2 (ArC), 155.6 (CO) and 159.5 [2 × $NHC(NNO_2)NH_2$]; *m*/*z* (FAB) 495 (M + 1, 100%) and 320 (M − $CO_2CH_2C_6H_4N_3$, 17) (Found: C, 41.4; H, 5.2; N, 33.3. $C_{17}H_{26}N_{12}O_6$ requires C, 41.3; H, 5.3; N, 34.0%).

N^1,N^8-Bis(nitroguanidino)spermidine 16

To a suspension of N^4-(4-azidobenzyloxycarbonyl)-N^1,N^8-bis(nitroguanidino)spermidine **15** (0.318 g, 0.643 mmol) in a methanol–water mixture (9 : 1 v/v; 5 ml) were added DTT (0.398 g, 2.58 mmol) and triethylamine (0.260 g, 2.57 mmol). The mixture was stirred at rt in the dark for 4 h, to give a yellow solution. The solvent was removed and the residue was purified by 'flash' chromatography (silica gel; 10% v/v methanol in conc. ammonia; UV detection) to give an oil. This crystallised upon trituration with water, giving N^1,N^4-*bis*(*nitroguanidio*)-*spermidine* **16** as a solid (0.103 g, 50%) homogeneous by TLC (silica gel; 4 : 1 : 1 : 2 v/v butan-1-ol–acetic acid–pyridine–water; UV detection; R_f 0.44), mp 75–77 °C; ν_{max}/cm^{-1} (KBr disc) 3406 (NH_2), 2932 and 2860 (CH_2), 1636 (C=N) and 1598 (NO_2); δ_H(200 MHz; d_6-DMSO) 1.55 (4H, m, $CH_2CH_2CH_2CH_2$), 1.72 (2H, m, $NCH_2CH_2CH_2N$), 2.60 (4H, m, CH_2NHCH_2), 3.27 [4H, m, 2 × $CH_2NHC(NNO_2)NH_2$], 8.00* (4H, br s, 2 × NH_2), 8.63* [2H, br s, 2 × $NHC(NH_2)(NNO_2)$]; δ_C(50 MHz; d_6-DMSO) 26.4, 26.8 and 28.9 ($CH_2CH_2CH_2CH_2NHCH_2CH_2$), 39.4 ($NHCH_2CH_2CH_2NHCH_2$), 40.9 ($NHCH_2CH_2CH_2CH_2NHCH_2$), 46.3 and 49.0 [2 × $CH_2NHC(NNO_2)NH_2$] and 159.6 and 160.3 [2 × $NHC(NH_2)(NNO_2)$]; *m*/*z* (FAB) 320 (M + 1, 100%), 303 (M − NH_2, 16), 275 (M − NO_2, 27) and 258 (M − H_2NNO_2, 43) (Found: C, 34.2; H, 6.7; N, 39.9. $C_9H_{21}N_9O_4$ requires C, 33.9; H, 6.6; N, 39.5%).

Hirudonine sulfate 17 by catalytic transfer hydrogenation

To a slurry of N^1,N^8-bis(nitroguanidino)spermidine **16** (0.100 g, 0.314 mmol) in a 5% w/v formic acid–methanol mixture (7.5 ml) was added 10% Pd/C (50 mg). The mixture was stirred under nitrogen for 3 h. The catalyst was filtered off and the solvent was removed from the filtrate. The resultant residue was taken up in water (1.5 ml) and sulfuric acid (1 M) was added dropwise to the solution until the pH was ~2. The solution was cooled to 0 °C and crystals of hirudonine sulfate **17** were deposited. These were filtered off, and ethanol was added to the supernatent until the cloud point was reached. Upon cooling to 5 °C, a second batch of crystals was produced. These were filtered off, combined with the first batch, and the combined product was recrystallised from water, to yield hirudonine sulfate **17** as crystals (0.135 g, 89%) homogeneous by TLC (silica gel; 4 : 1 : 1 : 2 v/v butan-1-ol–acetic acid–pyridine–water; ninhydrin detection; R_f 0.20), mp 167–168 °C (lit.,[53] 169 °C): ν_{max}/cm^{-1} (KBr disc) 3405 (NH_3^+), 3173 (NH), 2958 and 2877 (CH_2) and 1640 (C=N); δ_H(200 MHz; D_2O) 1.72 (4H, m, $CH_2CH_2CH_2CH_2$), 2.00 (2H, m, $NCH_2CH_2CH_2N$), 3.10 (4H, m, $CH_2\overset{+}{N}H_2CH_2$) and 3.27 [4H, m, 2 × $CH_2NHC(NH)\overset{+}{N}H_3$]; δ_C(50 MHz; D_2O) 24.3 ($NCH_2CH_2CH_2N$), 26.5 ($CH_2CH_2CH_2CH_2CH_2$), 39.7 and 41.9 ($CH_2\overset{+}{N}H_2CH_2$), 46.3 and 48.7 [2 × $CH_2NHC(NH)\overset{+}{N}H_3$] and 158.1 [2 × $NHC(NH)\overset{+}{N}H_3$); *m*/*z*

(FAB) 230 (M + 1, 66%) and 100 [M − $NH(CH_2)_4NHC(NH)NH_2$, 42] (Found: C, 24.0; H, 7.6; N, 21.5. $C_9H_{26}N_7O_6S_{1.5}\cdot 4H_2O$ requires C, 24.1; H, 7.6; N, 21.9%).

Hirudonine sulfate 17 by direct guanidination of spermidine with *S*-methylisothiourea

To a solution of spermidine (0.315 g, 2.17 mmol) in aq. ammonia (20% w/v; 5 ml) was added *S*-methylisothiourea sulfate (0.946 g, 3.40 mmol) in portions at rt. The reaction solution was stirred at rt for 24 h and the resultant precipitate was filtered off, washed successively with ice-cold water and then ethanol, and air-dried. The solid was recrystallised from water–ethanol to give hirudonine sulfate **17** as crystals (0.488 g, 47%) homogeneous by TLC (silica gel; 4 : 1 : 1 : 2 v/v butan-1-ol–acetic acid–pyridine–water; ninhydrin detection; R_f 0.20), mp 169–170 °C (lit.,[53] 169 °C): ν_{max}/cm^{-1} (KBr disc) 3405 (NH_3^+), 3173 (NH), 2958 and 2877 (CH_2) and 1640 (C=N); δ_H(50 MHz; D_2O) 1.72 (4H, m, $CH_2CH_2CH_2CH_2$), 2.00 (2H, m, $NCH_2CH_2CH_2N$), 3.10 (4H, m, $CH_2\overset{+}{N}H_2CH_2$) and 3.27 [4H, m, 2 × $CH_2NHC(NH)\overset{+}{N}H_3$]; δ_C(50 MHz; D_2O) 24.3 ($NCH_2CH_2CH_2N$), 26.5 ($CH_2CH_2CH_2CH_2$), 39.7 and 41.9 ($CH_2\overset{+}{N}H_2CH_2$), 46.3 and 48.7 [2 × $CH_2NHC(NH)\overset{+}{N}H_3$] and 158.1 [2 × $NHC(NH)\overset{+}{N}H_3$]; *m/z* (FAB) 230 (M + 1, 70%) and 100 [M − $NH(CH_2)_4NHC(NH)NH_2$, 39] [Found (again for the tetrahydrate): C, 24.0; H, 7.45; N, 21.6%].

N^4-(2-Hydroxy-2-phenylethyl)-N^1,N^8-bis(trifluoroacetyl)spermidine 10

To a stirred mixture of lithium perchlorate (0.107 g, 1.01 mmol) in dry acetonitrile (0.75 ml) were added styrene oxide (0.114 ml, 0.120 g, 1.00 mmol), N^1,N^8-bis(trifluoroacetyl)spermidine trifluoroacetate **9** (0.452 g, 1.00 mmol), and *N*-ethyldiisopropylamine (0.175 ml, 0.130 g, 1.01 mmol) at rt. (**Warning**: lithium perchlorate is potentially explosive, and should be handled with extreme caution at all times. Safety screens and additional facial protection must be used.) The mixture was stirred under nitrogen at rt for 24 h. The solution was diluted with water (4 ml) and then extracted with ether (3 × 10 ml). The organic layers were combined, and the solvent was removed to give a crude oil. This was purified by 'flash' chromatography (silica gel; 5% v/v methanol in dichloromethane; molybdic acid detection. Note that a large excess of silica is required for good separation) to give N^4-(2-hydroxy-2-phenylethyl)-N^1,N^8-bis(trifluoroacetyl)spermidine **10** as an oil (0.309 g, 67%) homogeneous by TLC (silica gel; 10% v/v methanol in dichloromethane; molybdic acid detection) R_f 0.40. Isomeric N^4-(2-hydroxy-1-phenylethyl)-N^1,N^8-bis(trifluoroacetyl)spermidine **11** was also produced as an oil (0.103 g, 22%), homogeneous by TLC (silica gel; 10% v/v methanol in dichloromethane; molybdic acid detection) R_f 0.45. The overall yield of both isomers was 0.412 g, 89%.

N^4-(2-*Hydroxy*-2-*phenylethyl*)-N^1,N^8-bis(*trifluoroacetyl*)-*spermidine* **10**: ν_{max}/cm^{-1} (cap film) 3200–3400 (OH), 3100 and 3066 (Ar CH), 2949 and 2870 (sp^3 CH), 1708 (CO), 1162, 1185 and 1209 (CF); δ_H(200 MHz; $CDCl_3$) 1.41–1.60 (4H, m, $CH_2CH_2CH_2CH_2$), 1.61–1.74 (2H, m, $NCH_2CH_2CH_2N$), 2.32–2.69 [6H, m, $N(CH_2)_3$], 2.91 (1H, br s, OH), 3.24–3.45 (4H, m, 2 × CH_2NH), 2.84 [1H, dt, J_1 8.7, J_2 4.5, $CH_2CH(OH)Ph$], 6.90 (1H, br s, NH), 7.21–7.32 (5H, m, ArH) and 7.82 (1H, br s, NH); δ_C(50 MHz; $CDCl_3$) 24.0, 25.9 and 26.5 ($CH_2CH_2NCH_2CH_2CH_2CH_2$), 38.9 and 39.6 ($CH_2CH_2NCH_2CH_2$), 52.4 and 53.6 (2 × CH_2NH), 62.8 [$NCH_2CH(OH)Ph$], 70.8 [$CH_2CH(OH)Ph$], 115.9 (two overlapping q, J_{CF} 293, 2 × CF_3), 125.9 (Ar *ortho* carbons), 127.9 (Ar *para* carbon), 128.5 (Ar *meta* carbons), 142.2 (Ar *ipso* carbon) and 157.5 and 157.6 (two overlapping q, J_{CF} 38, 2 × CO); *m/z* (FAB) 458 (M + 1, 37%), 440 (M − OH, 25) and 350 (M − $PhCH_2OH$, 100) (Found: C, 48.1; H, 5.5; N, 8.7. $C_{19}H_{25}F_6N_3O_3\cdot H_2O$ requires C, 48.0; H, 5.7; N, 8.8%).

N^4-(2-*Hydroxy*-1-*phenylethyl*)-N^1,N^8-*bis*(*trifluoroacetyl*)-*spermidine* **11**: ν_{max}/cm^{-1} (cap film) 3150–3500 (OH), 3092 and 3033 (Ar CH), 2947 and 2871 (sp^3 CH), 1707 (CO), 1160, 1186 and 1210 (CF); δ_H(200 MHz; $CDCl_3$) 1.54–1.64 (4H, m, $CH_2CH_2CH_2CH_2$), 1.66–1.90 (2H, m, $NCH_2CH_2CH_2N$), 2.23–2.36 (2H, m, CH_2NCH_2), 2.36 (1H, br s, OH), 2.53–2.75 (2H, m, CH_2NCH_2), 3.35–3.52 (4H, m, 2 × CH_2NH), 3.85 -3.99 (2H, m, CH_2OH), 5.88 (1H, dd, J_1 9.28, J_2 3.9, $NCHPhCH_2OH$), 6.89 (1H, br s, NH), 7.12–7.17 (2H, m, Ar *ortho* protons), 7.28–7.38 (3H, Ar *meta* and *para* protons) and 7.82 (1H, br s, NH); δ_C(50 MHz; $CDCl_3$) 24.6, 26.2 and 26.5 ($CH_2CH_2NCH_2CH_2CH_2CH_2$), 38.7 and 39.7 ($CH_2NCH_2$), 47.4 and 49.1 (2 × CH_2NH), 61.2 (CH_2OH), 64.9 ($NCHPhCH_2OH$), 114.0 (two overlapping q, J_{CF} 290, 2 × CF_3), 128.1 (ArC), 128.5 (ArC), 128.8 (ArC), 136.0 (ArC) and 157.5 (two overlapping q, J_{CF} 37, 2 × CO); *m/z* (FAB) 458 (M + 1, 5%), 426 (M − CH_2OH, 100), 338 (M − $CHPhCH_2OH$, 5), 126 ($CF_3CONHCH_2^+$, 10) and 91 ($PhCH_2^+$, 10) (Found: C, 49.3; H, 5.1; N, 8.7. $C_{19}H_{25}F_6N_3O_3$ requires C, 49.9; H, 5.5; N, 9.2%).

N^4-(2-Hydroxypropyl)-N^1,N^8-bis(trifluoroacetyl)spermidine 12

To a stirred suspension of N^1,N^8-bis(trifluoroacetyl)spermidine trifluoroacetate **9** (0.802 g, 1.77 mmol) and lithium perchlorate (0.191 g, 1.77 mmol — *see warning above*) in dry acetonitrile (1.3 ml) was added *N*-ethyldiisopropylamine (0.310 ml, 0.232 g, 1.77 mmol) at rt. A solution formed immediately, to which was added propylene oxide (0.124 ml, 0.103 g, 1.77 mmol). The reaction mixture was stirred at rt for 24 h. Water (5 ml) was added and the solution was extracted with ether (3 × 10 ml). The organic layers were combined, and the solvent was removed *in vacuo*. The crude product was purified by 'flash' chromatography (silica gel; 10% v/v methanol in dichloromethane; molybdic acid detection) to give N^4-(2-*hydroxypropyl*)-N^1,N^8-*bis*(*trifluoroacetyl*)*spermidine* **12** as a pale yellow oil (0.582 g, 83%) homogeneous by TLC (silica gel; 10% v/v methanol in dichloromethane; molybdic acid detection; R_f 0.22); ν_{max}/cm^{-1} (cap film) 3302 and 3099 (NH), 3100–3500 (OH), 2946 and 2826 (sp^3 CH), 1708 (CO), 1210, 1187 and 1159 (CF); δ_H(200 MHz; $CDCl_3$) 1.10 (3H, d, *J* 6.1, CH_3), 1.42–1.60 (4H, m, $CH_2CH_2CH_2CH_2$), 1.64–1.77 (2H, m, $NCH_2CH_2CH_2N$), 2.27 [2H, d, *J* 6.1, $NCH_2CH(OH)CH_3$], 2.37–2.70 (4H, m, $CH_2CH_2NCH_2CH_2$), 3.02 (1H, br s, OH), 3.28–3.47 (4H, m, 2 × CH_2NHCO), 3.78 [1H, sextet, *J* 6.3, $CH_2CH(OH)CH_3$] and 7.27 and 8.01 (2H, 2 br s, 2 × $NHCOCF_3$); δ_C(50 MHz; $CDCl_3$) 20.5 (CH_3), 24.0, 25.9 and 26.5 ($CH_2CH_2CH_2CH_2NCH_2CH_2$), 38.8 and 39.6 (2 × CH_2NHCO), 52.4 and 53.7 ($CH_2CH_2CH_2CH_2NCH_2CH_2$), 62.3 [$NCH_2CH(OH)CH_3$], 64.2 [$CH_2CH(OH)CH_3$], 116.2 (two overlapping q, J_{CF} 287, 2 × CF_3) and 157.8 (two overlapping q, J_{CF} 60.4, 2 × CO); *m/z* (EI) 394 (M − 1, 2%), 364 (M − CH_3OH, 1), 350 (M − CH_3CH^+OH, 100), 211 [$CF_3CONH(CH_2)_4NHCH_2CH_2^+$, 18], 197 [$CF_3CONH(CH_2)_4NHCH_2^+$, 12], 154 [$CF_3CONH(CH_2)_2CH_2^+$, 35] and 126 ($CF_3COCH_2^+$, 26) (Found: C, 42.1; H, 5.7; N, 11.0. $C_{14}H_{23}F_6N_3O_3$ requires C, 42.5; H, 5.8; N, 10.6%).

Crystal-structure determination

Crystal data: $(C_9H_{26}N_7)^{3+}\cdot 3/2\ (SO_4)^{2-}\cdot 4H_2O$, M_r = 448.52, triclinic, $P\bar{1}$, a = 7.092(5), b = 11.736(9), c = 14.767(12) Å, α = 103.65(3), β = 103.42(3), γ = 106.15(6)°, V = 1087.3(14) Å^3, Z = 2, D_{calcd} = 1.370 g cm^{-3}, μ = 0.26 mm^{-1} (Mo-Kα, λ = 0.710 73Å), $F(000)$ = 482, T = 160 K. Crystal size 0.44 × 0.21 × 0.18 mm, Stoe-Siemens four-circle diffractometer, cell parameters from 2θ-values of 26 reflections measured at ±ω; 3045 intensities measured by ω/θ scans, θ_{max} = 22.5°, maximum indices 7, 12, 15; 2821 unique reflections, R_{int} = 0.159 (based on relatively weak high-angle data), corrections for ~1% intensity decay.

The structure was determined by direct methods and refined[65] on all F^2-values with weighting $w^{-1} = \sigma^2(F_o^2) + (0.1388P)^2 + 1.6381P$, where $P = (F_o^2 + 2F_c^2)/3$. Cation H

atoms were located in a difference synthesis and refined with a riding model; other atoms were assigned anisotropic displacement parameters. Both sulfate anions were found to be disordered, one as two orientations with a common S-atom [occupancies 61 : 39(2)%], the other over an inversion centre with two O-atoms in common. One ordered and seven disordered (variously 50% and 25%) positions were resolved and refined for water oxygen atoms, but H-atoms for these were not located; the total water content is $4H_2O$ per cation.

Final $R_w = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{\frac{1}{2}} = 0.239$ for all data, conventional $R = 0.074$ for F-values of 1830 reflections having $F_o^2 > 2\sigma(F_o^2)$, goodness of fit $S = 1.062$ for all F^2-values and 335 refined parameters. Final difference map extremes +0.93 and −0.37 e Å^{-3}. Full crystallographic details, excluding structure factor tables, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc*., *Perkin Trans. 1*, available *via* the RSC Web page (http://www.rsc.org/authors). Any request to the CCDC for this material should quote the full literature citation and the reference number 207/273.

Acknowledgements

We thank Lilly Pharmaceuticals and the MRC Toxicology Unit, Leicester for support of A. M., Dr R. D. Verschoyle (MRC Toxicology Unit, University of Leicester) for assistance with polyamine uptake assays, Professor L. L. Smith (MRC Toxicology Unit) for helpful discussions and the EPSRC for partial funding of the X-ray equipment.

References

1 A. E. Pegg, *Cancer Res.*, 1988, **48**, 759.
2 D. M. L. Morgan, *Biochem. Soc. Trans.*, 1990, **18**, 1080.
3 M. C. O'Sullivan, B. T. Golding, L. L. Smith and I. H. Wyatt, *Biochem. Pharmacol.*, 1991, **41**, 1839.
4 R. J. Bergeron, Y. Feng, W. R. Weimar, J. S. McManis, H. Dimova, C. Porter, B. Raisler and O. Phanstiel, *J. Med. Chem.*, 1997, **40**, 1475 and refs. cited therein.
5 W. D. W. Heston, K. A. Watanabe and K. W. Pankiewicz, *Biochem. Pharmacol.*, 1987, **36**, 1849.
6 R. D. Verschoyle, P. Carthew, J. L. Holley, P. Cullis and G. M. Cohen, *Cancer Lett.*, 1994, **85**, 217.
7 S. Sagner, Z. W. Shen and M. H. Zenk, *Tetrahedron Lett.*, 1997, **38**, 2443.
8 M. C. O'Sullivan, O. Zhou, Z. Li, T. B. Durham, D. Rattendi, S. Lane and C. J. Bacchi, *Bioorg. Med. Chem.*, 1997, **5**, 2145.
9 A. J. Geall, R. J. Taylor, M. E. Earll, M. A. W. Eaton and I. S. Blagbrough, *Chem. Commun.*, 1998, 1403.
10 J. Boukovalas, B. T. Golding, R. W. McCabe and P. K. Slaich, *Angew. Chem., Int. Ed. Engl. Suppl.*, 1983, **22**, 860.
11 B. T. Golding, M. C. O'Sullivan and L. L. Smith, *Tetrahedron Lett.*, 1988, **29**, 6651.
12 R. J. Griffin, E. Evers, R. Davison, D. Layton and W. J. Irwin, *J. Chem. Soc., Perkin Trans. 1*, 1996, 1205.
13 A. Mitchinson, B. T. Golding, R. J. Griffin and M. C. O'Sullivan, *J. Chem. Soc., Chem. Commun.*, 1994, 2613.
14 L. L. Smith and I. Wyatt, *Biochem. Pharmacol.*, 1981, **30**, 1053.
15 D. B. Wigley, A. Lyall, K. W. Hart and J. J. Holbrook, *Biochem. Biophys. Res. Commun.*, 1987, **149**, 927.
16 Y. B. Lee, M. H. Park and J. E. Folk, *J. Med. Chem.*, 1995, **38**, 3053.
17 W. L. Hughes, H. A. Saroff and A. L. Carney, *J. Am. Chem. Soc.*, 1949, **71**, 2476.
18 S. C. Yorke, J. W. Blunt, M. H. G. Munro, J. C. Cook and K. L. Rinehart, *Aust. J. Chem.*, 1986, **39**, 447.
19 A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1964, **93**, 533.
20 R. A. Henry, R. C. Makosky and G. B. L. Smith, *J. Am. Chem. Soc.*, 1951, **73**, 474.
21 S. Matsuzaki, K. Hamana and K. Isobe, *Phytochemistry*, 1990, **29**, 1313.
22 K. Hamana, T. Akiba, F. Uchino and S. Matsuzaki, *Can. J. Microbiol.*, 1989, **35**, 450.
23 W. Raasch, S. Regunathan, G. Li and D. J. Reis, *Life Sci.*, 1995, **56**, 2319.
24 E. Galea, S. Regunathan, V. Eliopoulos, D. L. Feinstein and D. J. Reis, *Biochem. J.*, 1996, **316**, 247.
25 R. R. Ruffolo, W. Bondinell and J. P. Hieble, *J. Med. Chem.*, 1995, **38**, 3681.
26 J. Satriano, S. Matsufuji, Y. Murakami, M. J. Lortie, D. Schwartz, C. J. Kelly, S. Hayashi and R. C. Blantz, *J. Biol. Chem.*, 1998, **273**, 15313.
27 W. B. Lawson, M. D. Leafer, A. Tewes and G. J. S. Rao, *Hoppe-Seyler's Z. Physiol. Chem.*, 1968, **349**, 251.
28 S. Yaginuma, A. Asahi, M. Takada, M. Hayashi and K. Fukukawa, *Eur. Pat.*, 196 189, 1986 (*Chem. Abstr.*, 1987, **106**, 67676a).
29 B. ElAmin, G. M. Anantharamaiah, G. P. Royer and G. E. Means, *J. Org. Chem.*, 1979, **44**, 3442.
30 R. S. Lott, V. S. Chauhan and C. H. Stammer, *J. Chem. Soc., Chem. Commun.*, 1979, 495.
31 T. Morita, Y. Okamoto and H. Sakurai, *J. Chem. Soc., Chem. Commun.*, 1978, 874.
32 M. C. O'Sullivan and D. M. Dalrymple, *Tetrahedron Lett.*, 1995, **36**, 3451.
33 D. Xu, K. Prasad, O. Repic and T. J. Blacklock, *Tetrahedron Lett.*, 1995, **36**, 7357.
34 I. S. Blagbrough and A. J. Geall, *Tetrahedron Lett.*, 1998, **39**, 439.
35 Y. Nagao and E. Fujita, *Heterocycles*, 1982, **17**, 537.
36 R. W. Doskotch, A. B. Ray, W. Kubelka, E. H. Fairchild, C. D. Hufford and J. L. Beal, *Tetrahedron*, 1974, **30**, 3229.
37 A. V. Joshua and J. R. Scott, *Tetrahedron Lett.*, 1984, **25**, 5725.
38 S.-I. Murahashi, T. Naota and N. Nakajima, *Chem. Lett.*, 1987, 879.
39 B. Garrigues and L. Vidaud, *Bull. Soc. Chim. Belg.*, 1988, **97**, 775.
40 Y. Kikugawa, K. Mitsui, T. Sakamoto, M. Kawase and H. Tamiya, *Tetrahedron Lett.*, 1990, **31**, 243.
41 G. M. Cohen, P. M. Cullis, J. A. Hartley, A. Mather, M. C. R. Symons and R. T. Wheelhouse, *J. Chem. Soc., Chem. Commun.*, 1992, 298.
42 C. Morin and M. Vidal, *Tetrahedron*, 1992, **48**, 9277.
43 V. Fauchet, L. Bourel, A. Tartar and C. Sergheraert, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2559.
44 S. Lemaireaudoire, M. Savignac and J. P. Genet, *Synlett*, 1996, 75.
45 I. Levchine, P. Rajan, M. Borloo, W. Bollaert and A. Haemers, *Synthesis*, 1994, 37.
46 Y. Takeda, K. Samejima, K. Nagano, M. Watanabe, H. Sugeta and Y. Kyogoku, *Eur. J. Biochem*, 1983, **130**, 383.
47 M. M. Kimberley and J. H. Goldstein, *Anal. Chem.*, 1981, **53**, 789.
48 M. Chini, P. Crotti and F. Macchia, *Tetrahedron Lett.*, 1990, **31**, 4661.
49 I. R. Marsh and M. Bradley, *Tetrahedron*, 1997, **53**, 17317.
50 R. J. Bergeron and J. S. McManis, *J. Org. Chem.*, 1988, **53**, 3108.
51 Y. Robin, N. van Thoai and L.-A. Pradel, *Biochim. Biophys. Acta*, 1957, **24**, 381.
52 A. Mori, M. Hiramatsu, A. Numoto and Y. Robin, *C. R. Seances Soc. Biol. Ses Fil.*, 1981, **175**, 755.
53 Y. Robin and N. van Thoai, *C. R. Hebd. Séances Acad. Sci.*, 1961, **252**, 1224.
54 M. Nethaji and V. Pattabhi, *Proc. Indian Acad. Sci., Chem. Sci.*, 1987, **99**, 403.
55 I. Labadi, R. Sillanpaa and H. Lonnberg, *J. Chem. Soc., Dalton Trans.*, 1992, 765.
56 Y. Huse and Y. Iitaka, *Acta Crystallogr., Sect. B*, 1969, **25**, 498.
57 E. Giglio, A. M. Liquori, R. Puliti and A. Ripamonti, *Acta Crystallogr.*, 1966, **20**, 683.
58 Y. Iitaka and Y. Huse, *Acta Crystallogr.*, 1965, **18**, 110.
59 M. Jaskolski, *Acta Crystallogr., Sect. C*, 1987, **43**, 1375.
60 A. Gharbi and A. Jouini, *Acta Crystallogr., Sect. C*, 1996, **52**, 1342.
61 E. Giglio, A. M. Liquori and A. Ripamonti, *Acta Crystallogr.*, 1966, **20**, 652.
62 K. W. Hart, A. R. Clarke, D. B. Wigley, A. D. B. Waldman, W. N. Chia, D. A. Barstow, T. Atkinson, J. B. Jones and J. J. Holbrook, *Biochim. Biophys. Acta*, 1987, **914**, 294.
63 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *The Purification of Laboratory Reagents*, Pergamon Press, Oxford, 2nd edn., 1980.
64 O. Keijiro, *J. Chem. Soc. Jpn.*, 1946, **67**, 132 (*Chem. Abstr*., 1952, **46**, 90e).
65 G. M. Sheldrick, SHELXTL Version 5, Bruker AXS, Madison, Wisconsin, USA, 1994.

Paper 8/06355I