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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 5457–5479
Bioorganic & Medicinal Chemistry

Synthesis and pharmacological testing of polyaminoquinolines as blockers of the apamin-sensitive Ca^{2+}-activated K^+ channel (SK_{Ca})

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Received 2 August 2006; revised 19 May 2007; accepted 23 May 2007
Available online 26 May 2007

Abstract—The synthesis and pharmacological testing of a series of non-peptidic blockers of the SK_{Ca} (SK-3) channel is described. Target compounds were designed to mimic the spatial relationships of selected key residues in the energy-minimised structure of the octadecapeptide apamin, which are a highly potent blocker of this channel. Structures consist of a central unit, either a fumaric acid or an aromatic ring, to which are attached two alkylguanidine or two to four alkylaminoquinoline substituents. Potency was tested by the ability to inhibit the SK_{Ca} channel-mediated after-hyperpolarization (AHP) in cultured rat sympathetic neurones. It was found that bis-aminoquinoline derivatives are significantly more potent as channel blockers than are the corresponding guanidines. This adds to the earlier evidence that delocalisation of positive charge through the more extensive aminoquinolinium ring system is important for effective channel binding. It was also found that an increase in activity can be gained by the addition of a third aminoquinoline residue to give non-quaternized amines which have submicromolar potencies (IC_{50} = 0.13–0.36 μM). Extension to four aminoquinoline residues increased the potency to IC_{50} = 93 nM.

1. Introduction

Small conductance Ca^{2+} activated K^+ (SK_{Ca}) channels are found in a wide variety of cells, both electrically excitable and inexcitable. These include, for example, many neurones, intestinal myocytes, endothelial cells and hepatocytes[1–4]. Accordingly, there is much current interest in the synthesis of blockers of these channels for use as pharmacological tools[5] and in possible therapeutic applications.[6] The latter include effects on peripheral tissues (e.g., the insulin releasing cells of the pancreas[7]) as well as on the CNS where there is evidence for a role of SK_{Ca} channels in memory processes, both general[8] and specifically hippocampal.[9]

The work described herein extends an ongoing study of the pharmacology and medicinal chemistry of SK_{Ca} blockers which was begun before the composition and sequence of these channels had been determined. The principal measure of blocking potency utilised throughout has been the ability of the test agents to inhibit the SK_{Ca}-mediated after-hyperpolarization (AHP) that follows the action potential in rat sympathetic ganglion cells in short term tissue culture. Subsequent to the cloning and sequencing of SK_{Ca} channels,[10] of which three variants (SK1-3) were identified, it was established that this AHP is mediated by the SK3 subtype.[11] This occurs in the nerve endings[12] and cell bodies of many CNS neurones as well as in peripheral tissues.[4]

The first naturally occurring, highly potent, selective blocker of the SK_{Ca} channel discovered was apamin[13,14] (Fig. 1). Other peptidic toxins such as scyllatoxin

Keywords: Quinoline; Aminoquinoline; Guanidine; Apamin; Potassium ion channel; Calcium activated; SK_{Ca}; SK3; AHP; After-hyperpolarization.
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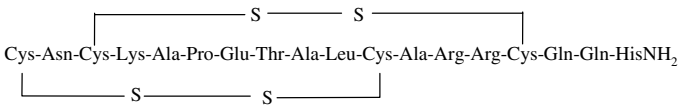
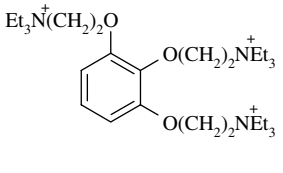
doi:10.1016/j.bmc.2007.05.054



apamin



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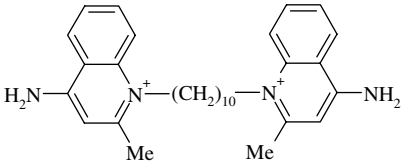
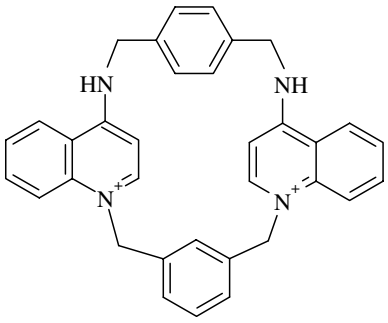
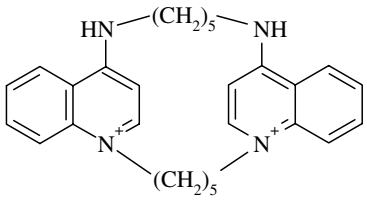


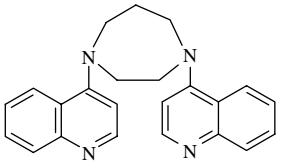
dequalinium



UCL 1684



UCL 1848



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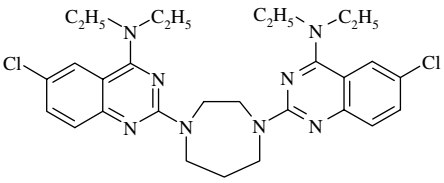

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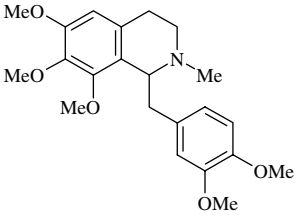

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Figure 1. Structures of some blockers (apamin, gallamine, dequalinium, UCL 1684, UCL 1848 and **1**–**3**) of SK_{Ca} channels (SK3).

(leiurotoxin 1)[15] and PO_5[16] also have similar affinity. Several non-peptidic compounds have been shown to be blockers of SK_{Ca} channels, such as tubocurarine,[17,18] pancuronium, gallamine (Fig. 1)[19] and dequalinium[20] (Fig. 1), albeit with affinity several orders of magnitude lower than the natural toxins. The latter provided a lead for development of several series of novel selective SK_{Ca} blockers.[21–24] The most active of these has been the bis-quinolinium cyclophanes, some of which are equipotent with apamin,[23,24], for example, UCL 1684 and UCL 1848 (Fig. 1).

Bridged bis-aminoquinolines and bridged bis-diamino-quinazolines (e.g., **1** and **2**, Fig. 1) have also been described[25a,b] in a series of patents from scientists at the Bayer company. Compounds **1** and **2** are tertiary diamines where the basic centres are the heterocyclic nitrogen atoms. These compounds and their analogues have been claimed to be useful for the treatment of degenerative diseases of the central nervous system. Another recent approach to the development of SK_{Ca} blockers takes as a starting point methylated derivatives of the monotertiary alkaloids bicuculline and laudanosine.[26] In this paper, the authors note that one of the compounds (**3**, Fig. 1) synthesized is a tertiary amine which may therefore be useful as a lead for the development of compounds able to cross the blood–brain barrier. The potency is, however, rather low, having a K_i of 5.8 μM.

We have also previously described[21,22] bis non-quaternized amines of similar potencies. These are based on 1,10-diaminodecane with bis substitution by nitrogen heterocycles such as pyridine, quinoline and acridine (IC_{50} values = 2.5–6.7 μM). The present paper identifies tris non-quaternized amines (**12**–**14**) which are more potent by at least an order of magnitude (IC_{50} values = 0.13–0.36 μM) and where the basic centres are the quinoline nitrogen atoms.

Structure–activity studies carried out on apamin,[28,29] leiurotoxin[30,31] and PO_5[16,32] analogues have indicated the importance for pharmacological activity of two

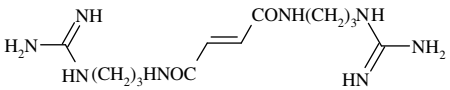
arginines (Arg-13 and Arg-14 in apamin), located on the same side of the α-helix section of the molecule, whose cationic guanidine groups are separated by approximately 11 Å.[19] However, the high affinity of these toxins for the binding sites does not appear to be explained exclusively by the presence of the two arginine groups.[29,33] Closely located Gln and His residues may also contribute. For example, the binding affinity is reduced to 1.3% of apamin on removal of His-18, and reduced to 0.01% without both His-18 and Gln-17. Removal of Gln-16, in addition, resulted in no further change in affinity.[29]

The evidence to date indicates that the nature of the binding is electrostatic, with a region of negative charge in the channel mouth.[2,31,32] Arg-13, Arg-14 and His-18 are all protonated at physiological pH. The Gln-17 residue is located on the helix one turn below Arg-14, and one may envisage that it could participate in binding through hydrogen bonding with the electronegative area. A common feature of the majority of the non-peptidic compounds prepared to date as apamin analogues is the possession of only two basic groups. In the selection of the present series of compounds, the nature of this interaction was explored by incorporating further positively charged residues to mimic the interaction of Gln-17 and His-18.

The spatial conformation of apamin has been well studied, and the structure optimised by computer molecular modelling.[34] One of our research group had built a CPK model according to this structure and target molecules were selected by overlaying on this and also by molecular modelling studies using ChemX.[45] We began with elaborations of a bis-guanidiniumalkyl diamide of fumaric acid **4** (Fig. 2), which had been originally synthesized to mimic the Arg-13-Arg-14 groups of apamin and had been found to exhibit only weak activity (30% inhibition at 300 M).[33] By inspection of the CPK model of apamin it could be seen that an appropriate spatial relationship of Gln-17 NH_2 to the two arginine residues could be achieved by incorporation of an octylamino group (assuming it to be in a fully extended *trans* conformation) on one of the amide nitrogens of **4** by rotation of the alkyl chain, leading to the selection of **5**. The *cis* isomer, based upon the diamide of maleic acid, was also prepared.

It should be noted that the exact position of the basic substituents may vary widely in solution due to *E*/*Z* conformational isomerisation about the amide bond and flexibility of the alkyl linkages. In order to explore this further a third target compound, **7**, was prepared having the *n*-octyl chain of **5** replaced by a biphenyl group, this unit being much more rigid but of similar length (15 Å) to the alkyl analogue in its fully extended conformation.

Much of our prior structure–activity studies have involved the synthesis and testing of dequalinium analogues. Dequalinium which contains two quinolinium rings is approximately 500 times more potent as an SK_{Ca} channel blocker than is decamethonium in which the positive charges are carried by two trimethylammonium groups. This observation, together with our early



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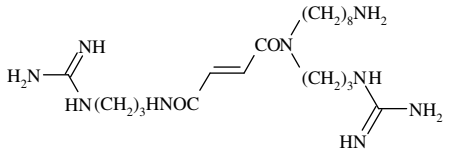

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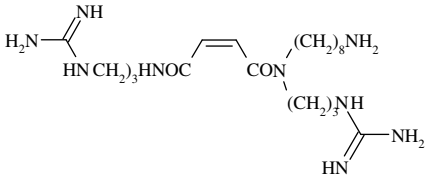

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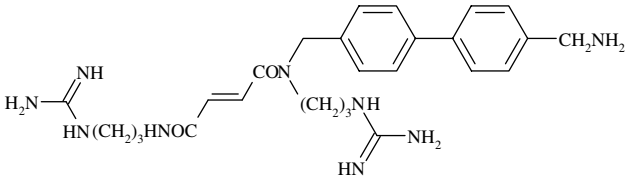

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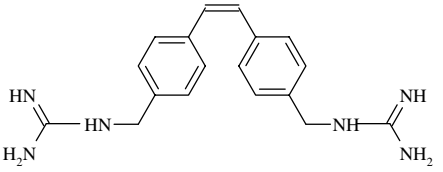

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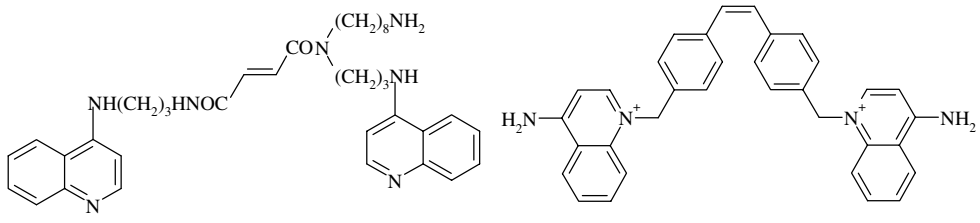
Figure 2. Structures of bisguanidines **4**–**8**.

structure–activity studies, suggested[21] that there is a need for a ring-shaped positive electrostatic field around the charged part of the molecule for blockade of the SK_{Ca} channel. It was of interest, therefore, to compare compounds with guanidine moieties with the corresponding aminoquinolines. Two examples were explored. In the aminooctyl bis-guanidiniumalkyl fumaric acid diamide **5** the two guanidine groups were replaced by aminoquinoline residues to give **9** (Fig. 3) as a synthetic accessible target; in the bis-aminoquinolino-*cis*-stilbene **10** (Fig. 3), that we had previously reported on,[35] the aminoquinoline groups were replaced by guanidines to give **8** (Fig. 2).

In the model compound **10**, the aminoquinoline groups are positively charged through being quaternized whereas in **9**, they are not quaternized. Although 4-aminoquinoline is less basic than guanidine, and non-quaternized, quaternization has been shown not to be an essential feature for channel blockade, as the heterocycle is sufficiently basic so that it is likely to be protonated at physiological pH.[21]



9 10

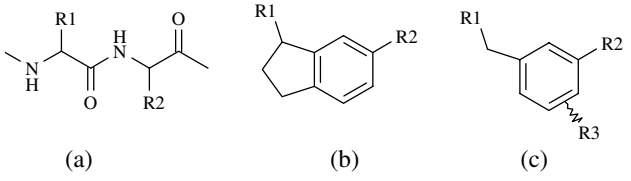
Figure 3. Structures of the bis-aminoquinoline derivatives **9** and **10** corresponding to the bisguanidines **5** and **8**.

As discussed in Section 4, inclusion of the 4-aminoquinoline group was demonstrated to provide a major improvement in blocking potency of compounds relative to those containing guanidine groups; it was of interest therefore to extend the bis-quinolinium structures by incorporating a third (or even a fourth) 4-aminoquinoline group. Since the positive charge in the ring structure is much more diffusely spread over a larger area than in guanidine, the apamin molecule as modelled with its two guanidine groups is no longer a reliable guide for showing the precise location to be taken by the aminoquinoline groups; the structural requirements were therefore probed more empirically.

A further series of target compounds was prepared in which an aromatic central unit was used as a framework for assembly of three or four aminoquinolinyl groups. Molecular modelling studies have indicated that a 1,6-disubstituted indane (Fig. 4b) is a good mimic for two adjacent residues of an α-helix (Fig. 4a).[36] A synthetically more accessible target is a *m*-substituted aromatic (Fig. 4c). The ring is able to act as a spacer approximately equivalent to one turn on the helix, with the result that it can provide a suitable template for the three key residues of interest in binding to the SK_{Ca} channel. The adjacent Arg-13 and Arg-14 residues in apamin are represented by *m*-substituents (R_1 and R_2), and Gln-17 by an appropriate substituent in the 4- or 5-position (R_3).

While the separation between the aminoquinolinyl substituents representing the two arginines was maintained at approximately 11 Å, the position of the third substituent was varied. The following test compounds (**11**–**13**, Fig. 5) were prepared from amongst the various potential alternatives by examining not only fulfilment of the appropriate structural requirements, but also in terms of accessibility of starting materials and ease of synthesis.

Compound **11**, as a benzylamine, accords with the structure depicted in Figure 4c with R_3 being in the 5-position relative to R_1; each R group has a five-atom chain to a quinoline ring. Compound **12** is the lower homologue where the amino group is present as an aniline instead of a benzylamine, thereby reducing considerably the basicity of this nitrogen link. Attempts to synthesize the lower homologues of **11** and **12**, with each R group having a four-atom chain, were thwarted by the ready elimination of the leaving group X from the reagent $X(CH_2)_2NH{\cdot}Boc$. Synthesis of an isomer of **12**, in which the oxypropylaminoquinoline chains were in the 2,4-positions, was investigated but was unsuccessful.



(a) (b) (c)

Figure 4. 1,6-Disubstituted indane (b) is a good mimic for two adjacent residues of an α-helix (a). This can be represented by a *m*-substituted benzene ring (c).

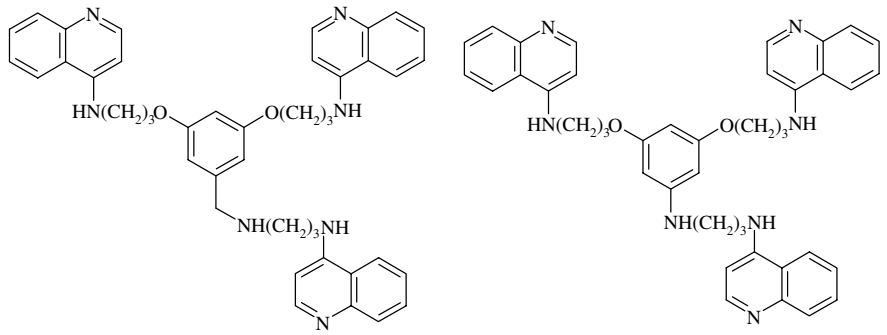
In compound **13**, a shorter chain to the quinoline group for R_1 was achieved by using dopamine as the starting material. In this case R_3 is in the 4-position. Here again, R_2 and R_3 have five-atom chains to the quinoline rings. Several attempts to synthesize lower homologues using a CH_2CH_2 link were unsuccessful.

A homologous analogue (**14**) of gallamine, having the quaternary ammonium groups replaced by aminoquinolinyl, was prepared from pyrogallol. Since gallamine is a tris-triethylammonium quaternary, the analogue (**14**) was also quaternized with ethyl iodide, giving **15** to provide further data for investigating the need for quaternization (Fig. 6). Isomers of **14**, having a 1,2,4 substitution pattern or the fully symmetrical 1,3,5 pattern, were planned but the syntheses posed problems that could not be overcome.

As a further measure of a potential statistical contribution to the binding, two target compounds **16** and **17** having four aminoquinolinyl substituents (Fig. 7) were prepared, by elaboration of the alkylamino linkage. The length of the additional linkage chain was selected to mimic His-18 in apamin based on the modelling results.

2. Biological testing

The potencies of compounds as blockers of the SK3 variant of the SK_{Ca} channel were determined from their ability to inhibit the after-hyperpolarization which follows the action potential in rat sympathetic neurones, as previously described.[23,38,43] The amplitude of the AHP was measured before, during and after the presence of the compound. Dequalinium was also applied, serving as a reference agent in each assay. Because the effectiveness of dequalinium varied a little between experiments, equieffective molar concentration ratios (EMR with respect to dequalinium) were determined.



11 **12**

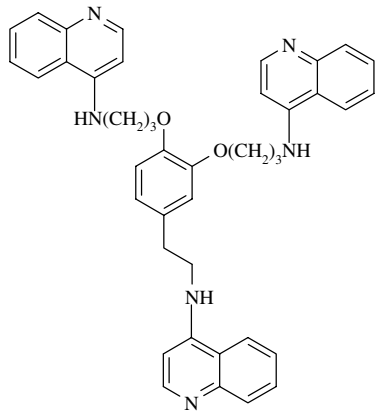


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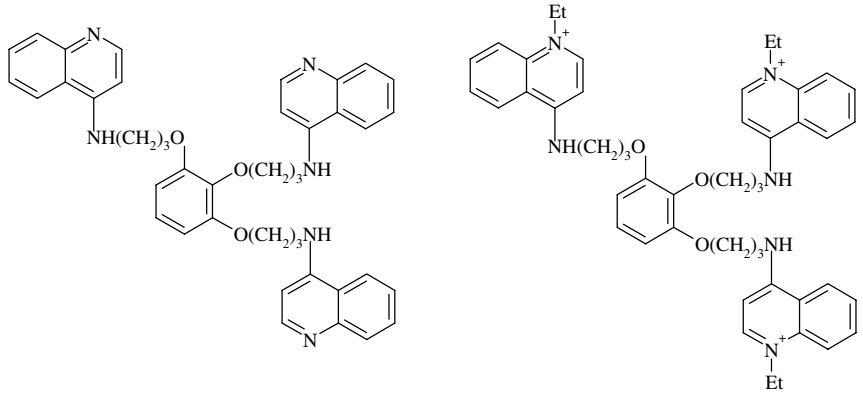
Figure 5. Trisubstituted benzene derivatives (**11**–**13**) having three aminoquinolinyl groups.



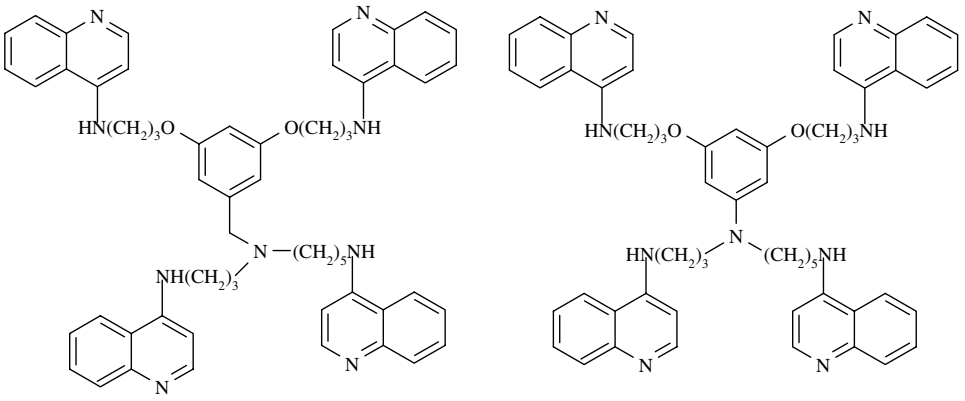
14 **15**

Figure 6. Gallamine analogues (**14**–**15**) in which the quaternary ammonium groups of gallamine are replaced by aminoquinolinyl or amino-*N*-ethylquinolinium.

The EMR values have been used for the comparisons between compounds. IC_{50} values, the concentration required to achieve 50% inhibition of the AHP, were also calculated.

3. Chemistry

Compound **5** was prepared via reaction between two key N-protected components, **5g**, derived from 1,8-diaminooctane, and aminopropyl fumaramide **5c** (Scheme 1). Appropriate choice of protecting groups allowed the two *t*-Boc groups to be removed under acidic conditions for subsequent guanidination (using 3,5-dimethylpyrazole-1-carboxamidine nitrate) before removing the trifluoroacetyl protecting group under basic conditions in the final step to leave the primary amine substituent. A similar synthetic strategy was adopted for **7** using the bis-protected biphenyl analogue **7e** (Scheme 3). An approach to **6** via attempted reaction of **5g** with protected aminopropyl maleamide resulted in complete isomerisation to the *trans* isomer, and so this target



16 **17**

Figure 7. Compounds (**16**–**17**) having four aminoquinolinyl groups.

compound was prepared by an alternative route via reaction of **5g** with maleic anhydride (Scheme 2). Compound **8** was synthesized via 4,4′-bis(aminomethyl)-*cis*-stilbene which had been prepared from the known[39] dibromide through a Gabriel synthesis, by treatment with potassium phthalimide followed by hydrazinolysis, and then by bis-guanidination. In all syntheses, guanidination of the deprotected triamines required careful pH control in order to achieve a balance between removal of the trifluoroacetyl group at high pH and protonation at low pH, which would inhibit reaction.

The general strategy for preparation of the aminoquinolinyl target compounds involved heating the amine precursor with 4-chloroquinoline and diisopropyl ethylamine in *n*-pentanol. In the synthesis of **9** this was carried out at early stages of the synthesis, since in subsequent transformations the aminoquinolinyl moiety is unreactive. After linking of the two key intermediates **9d** and **9e**, the trifluoroacetyl group was removed to give **9** (Scheme 4).

Target compounds (**11**–**14**) in which the alkylaminoquino linyl groups are linked to an aromatic ring as an ether were prepared by Williamson synthesis in which the phenol was treated with potassium *tert*-butoxide and a *t*-Boc-protected bromopropylamine. Subsequent removal of the protecting groups liberated the primary amines for N-alkylation as the final step (Schemes 5–8). Removal of the *t*-Boc protecting group was usually carried out with trifluoroacetic acid since isolation or further processing of the product was straightforward, following evaporation of the solvent under mild conditions, whereas using water-based acids, the high aqueous solubility of many of the primary amine compounds made them difficult to extract without decomposition.

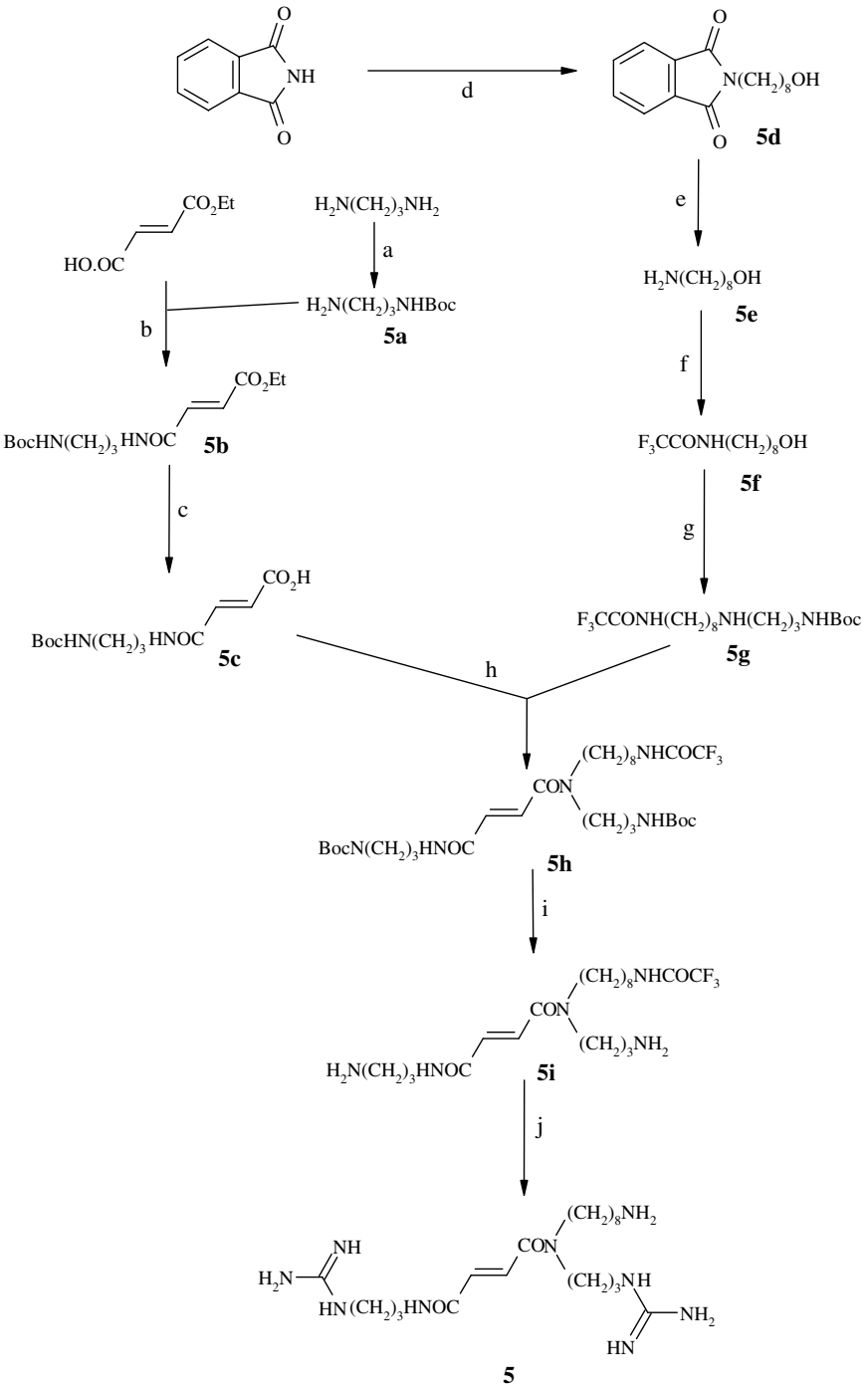
For **12** the amine linkage to the aromatic ring was formed by reaction of **5a** with phloroglucinol, achievable since the phenolic OH has sufficient ketonic character to be susceptible to nucleophilic attack. This produced a mixture of anilines, which was separated, and the mono-aniline was converted into **12** using previous methodology. Attempted conversion of the di-aniline **12b** was unsuccessful due to rapid aerial oxidation. Compound **15** was prepared by alkylation of **14** in ethyl iodide (Scheme 8), adding the minimum quantity of DMF throughout the reaction to ensure dissolution of the intermediate mono- and di-quaternary salts.

Preparation of **16** and **17** (Schemes 9, 10, respectively) followed strategies similar to those for **11** and **12**. Reaction of the protected secondary amine with phloroglucinol gave exclusively the monosubstituted product **17a**. For **16b** an effective procedure was developed for separating amines by selective extraction, utilising their different base strengths, in which the more basic component is extracted into the aqueous layer of a two-phase aqueous–organic mixture, by addition of a stoichiometric amount of acid.

Table 1. Results for inhibition of the after-hyperpolarization of cultured rat sympathetic neurones

Compound	IC_{50} (μM) ± SD	EMR[a] ± SD
Apamin	0.0023 ± 0.0007[12]	
Gallamine	68 ± 83[43]	
Dequalinium	0.6 ± 0.05[43]	1
4	>100[b]	
5	27[c]	
6	> > 30	
7	17	33 ± 6
8	25	59 ± 26
9	0.49 ± 0.02	0.59 ± 0.03
10	0.19 ± 0.03[35]	0.24 ± 0.08
11	0.36 ± 0.06	0.48 ± 0.14
12	0.22 ± 0.03	0.26 ± 0.07
13	0.27 ± 0.03	0.31 ± 0.07
14	0.13 ± 0.03	0.23 ± 0.05
15	0.086 ± 0.009	0.16 ± 0.02
16	0.13 ± 0.03	0.23 ± 0.05
17	0.093 ± 0.020	0.17 ± 0.04
18[d]	0.084 ± 0.020[37]	0.10 ± 0.005
UCL 1684	0.003 ± 0.001[44]	0.01 ± 0.001
UCL 1848	0.002 ± 0.0005[24]	0.003 ± 0.001

[a] Equieffective molar ratio: the ratio of the concentrations of the test compound and dequalinium to cause 50% inhibition of the AHP.
[b] 18 ± 3% inhibition at 100 μM.[33]
[c] Compound may have had additional actions.
[d] Ref. 37 wherein dequalinium had EMR 0.78 ± 0.05.

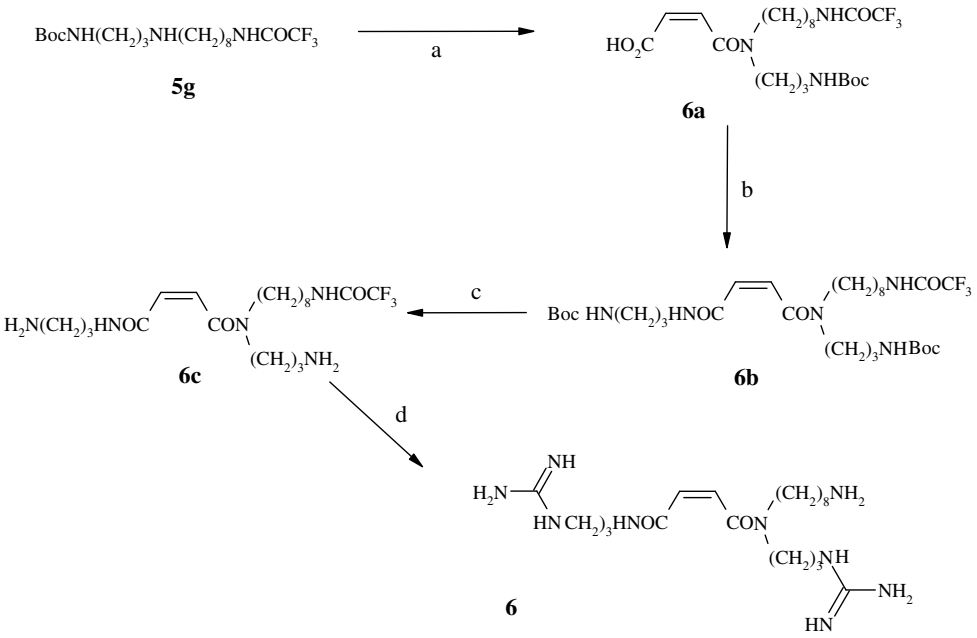


Scheme 1. Reagents: (a) $(t\text{-Boc})_2O$, MeOH; (b) DCC, HOBT, THF; (c) KOH, H_2O/EtOH; HCl; (d) $Br(CH_2)_8OH$, K_2CO_3, DMF; (e) NH_2NH_2, EtOH; (f) F_3CCO_2Me, MeOH; (g) TsCl, NEt_3 $CHCl_3$; **5a**, DMF; (h) DCC, HOBT, THF; (i) HCl, MeOH; (j) DMPCN, NaOH, H_2O (pH 7.0–8.5); NaOH; HNO_3.

4. Results and discussion

The results of the biological testing of the target compounds are summarised in Table 1. Although the bisguanidines with an additional basic group **5** and **7** were more potent than the simple bisguanidine **4**, used as the starting point, they were much less active than dequalinium. Direct replacement of the guanidine groups of **5** with 4-aminoquinolinyl, giving **9**, resulted in a substantial (approximately 50-fold) increase in potency to submicromolar activity. This is in accord with the even larger potency difference found between the guanidine **8** and the aminoquinoline **10** and supports the assertion[21] that delocalisation of positive charge is an important factor in determining potency, by improving the interaction with the negatively charged region in the channel.

Compound **9** was only some twofold more potent than dequalinium (based on comparison of equieffective



Scheme 2. Reagents: (a) Maleic anhydride, MeOH; (b) NHS, DCC, CH_2Cl_2; **5a**; (c) HCl, MeOH; (d) DMPCN, NaOH, H_2O (pH 7.0–8.5); NaOH, HNO_3.

molar ratios, EMRs) and equipotent with most other compounds prepared previously[35] which contain two aminoquinolinyl groups which are not rigidly held. Evidently, the aminooctyl group in **9** has not located a suitable additional binding site. It is noteworthy, however, that the conformation about the linking *trans* double bond in **5** and **7** is important since the alternative *cis* form **6** is much less active. This may be due to hydrogen bonding between the two *cis*-amide groups in the unbound molecule, which would be lost during channel binding. There is evidence in the ^{1}H NMR spectra of compounds **5**, **6** and **7** for the existence of rotamers about the amide bonds, which may impact on the energetic favourability of binding.

Though compound **11**, having three aminoquinoline groups, was only a little more potent than **9**, all the other target compounds (**12**–**15**) with three aminoquinolinyl groups were 2–3 times more active than **9**. This may be because of the statistical effect of having three rather than two potential binding groups present in the molecule, as previously suggested.[37] In keeping with this, the addition of yet another aminoquinoline group to give compounds having four quinoline rings provided a further twofold increase in potency. This is demonstrated with compounds **11** and **16**, or **13** and **17** where, in each case, the additional aminoquinoline group is linked to the rest of the molecule by a penta-methylene chain.

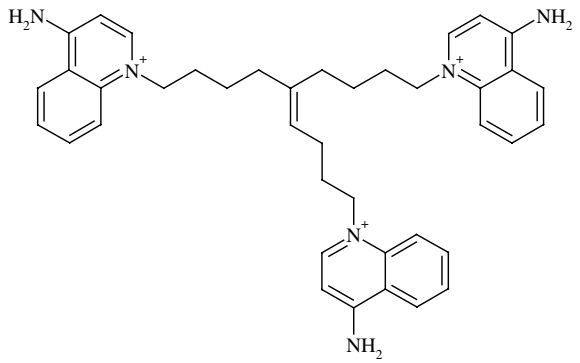
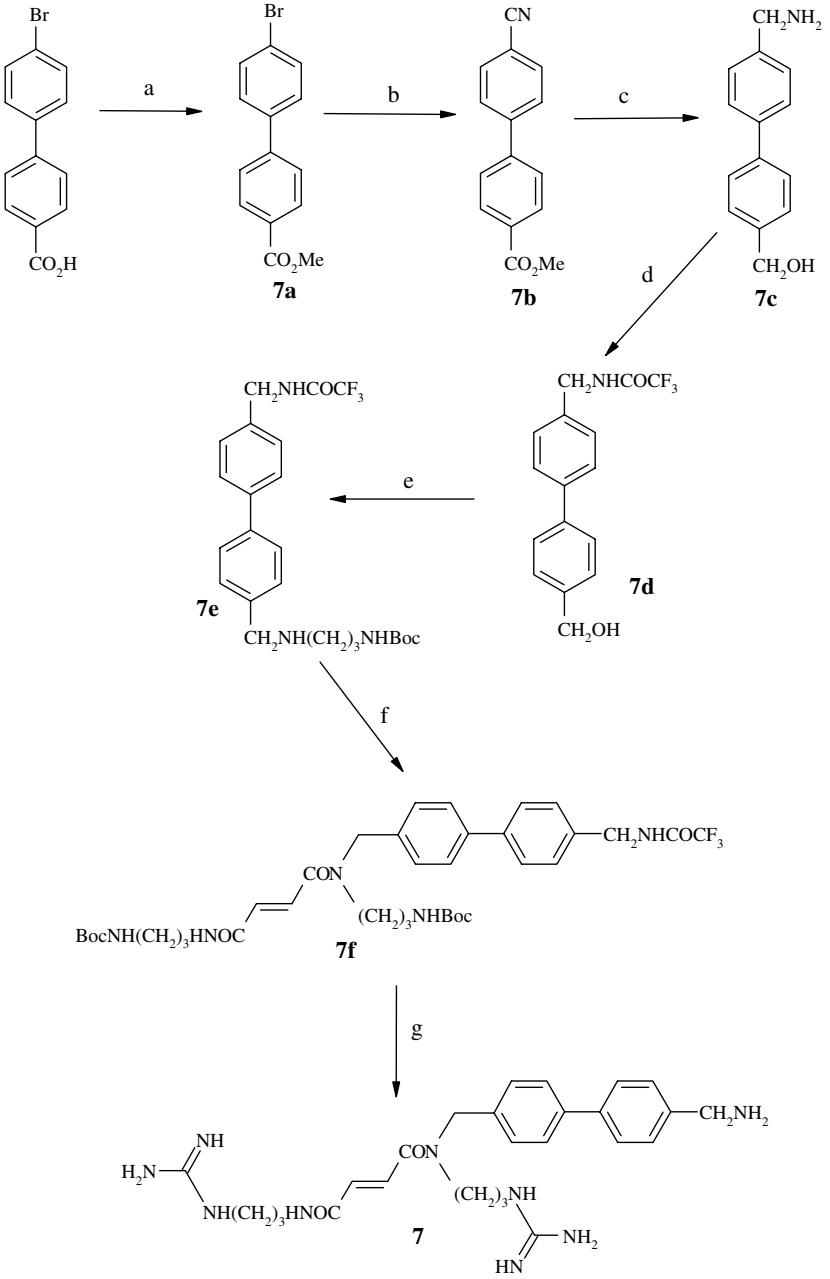


Figure 8. The tris-(4-aminoquinolinium) compound **18** described in Ref. 37.

The similarity in activity between **14** and **15** confirms the previous conclusion[21] that quaternization is not a prerequisite for activity. It is also of interest to compare **15** with gallamine where we again see a demonstration of the importance of the aminoquinoline group. In this case, 4-amino-1-ethyl-quinolinium moieties replace each of the three triethylammonium groups in gallamine to provide **15** which shows an approximately 800-fold increase in potency. This is consistent with our previously reported[21] observations on decamethonium.

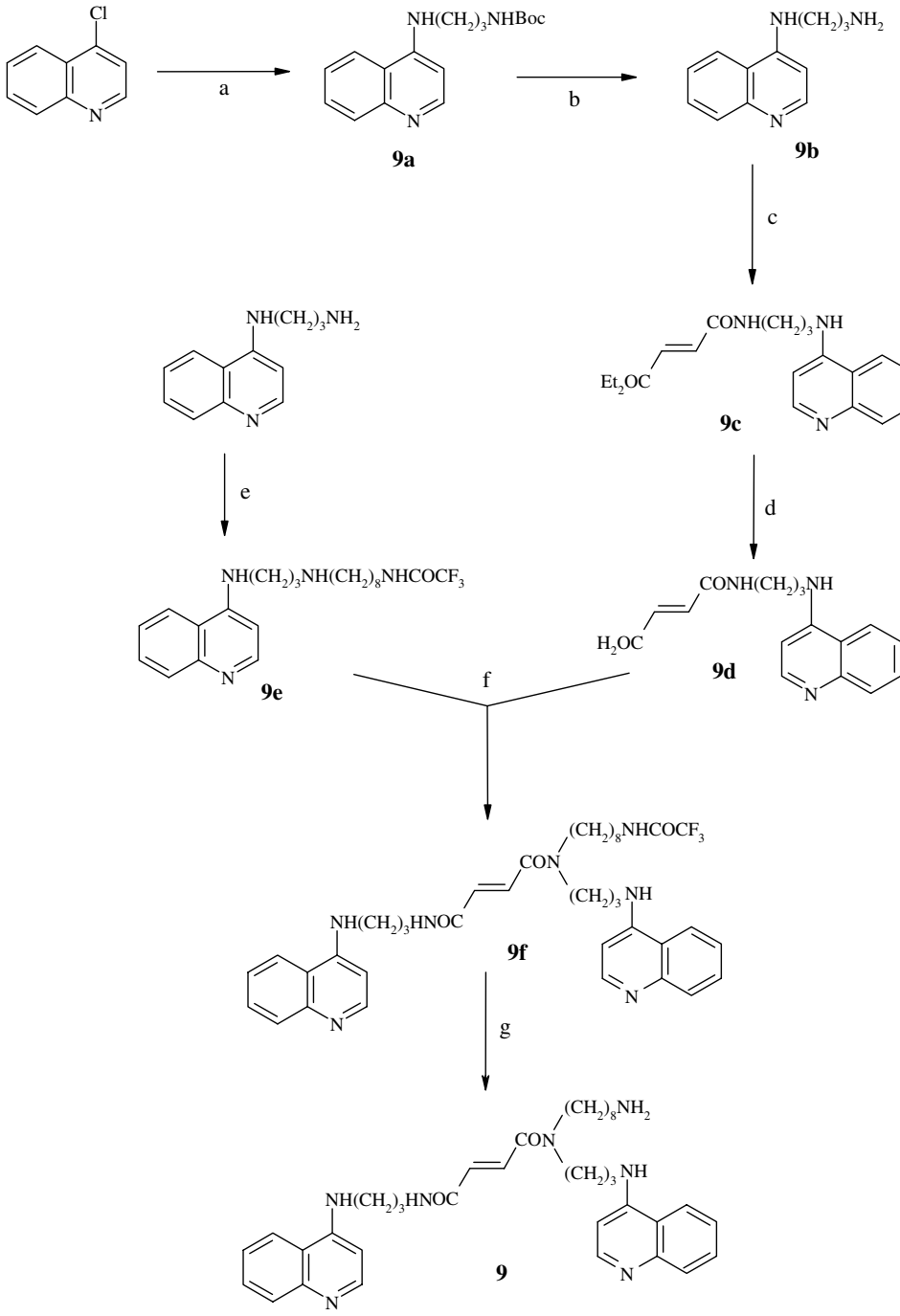
None of these polyquinolinic compounds (**11**–**17**) appears to be as potent as the tris-(4-aminoquinolinium) **18** (Fig. 8) which had[37] approximately 8 times the potency of dequalinium. There are two main changes to the compound structure which might account for the differences in potency. First, in **18**, each quinoline ring is on a four-carbon atom chain from the same central carbon atom (thus there are nine-carbon atoms bridging the quinoline rings), whereas for **11**–**17**, the quinoline rings are on five or six-atom chains (which include heteroatoms) that lead from a central benzene ring and therefore the molecules are less compact (**11**, for example, is bridged by thirteen atom chains). If we consider all the combinations for the bridging chains between all the quinoline rings then, for compounds **11**–**17**, they fall between 11- and 16-atom chains. The chain length does not seem to be a critical difference however since, in a series of dequalinium homologues, there was little influence of bridging chain length $(CH_2)_n$ for $n = 5$–12



Scheme 3. Reagents: (a) $SOCl_2$; MeOH; (b) CuCN, DMF; (c) $LiBH_4$, THF; (d) F_3CCO_2Me, MeOH; (e) TsCl, NEt_3, $CHCl_3$; **5a**, DMF, (f) **5c**, DCC, HOBT, DMF; (g) HCl, MeOH; DMPCN, NaOH, H_2O (pH 7.0–8.5); NaOH; HNO_3.

between the quinoline rings, although potency was reduced when the chain was shorter than five carbon atoms.[40] Second, in **18**, each quinoline ring is attached to the linking chain by the ring-nitrogen atom at position-1, whereas for compounds **11**–**17** the chain is attached at the 4-amino groups. It has been demonstrated that attaching the chain at the 4-amino group instead of at the 1-position to give a non-quaternized dequalinium isomer does indeed lower the potency by a few fold.[22]

It is of interest in this connection to observe that the most active non-peptidic K_{Ca} blockers so far described are cyclophanes which have two quinolinium groups joined by two bridges and are attached at both the 4-amino group and the nitrogen 1-position. The bridges can be aromatic groups, alkane chains or a combination of the two.[23,41,44] Counting the least number of carbon atoms between the points of attachment for the aromatic groups and including heteroatoms then: when the number of bridging atoms varies between 8 and 13, the EMR activities range over one order of magnitude, between 0.11 and 1.1. For shorter chains, however, potency rapidly and strikingly increases as the distances between the quinolinium rings become optimised so that UCL 1684 (Fig. 1) has two bridges of 8 and 5 atoms,[44] respectively, and UCL 1848 (Fig. 1) has correspondingly 7 and 5 atoms.[24] Molecular modelling studies on these compounds have been reported[23,27] but the compounds of the present study are too large and flexible to warrant such modelling.

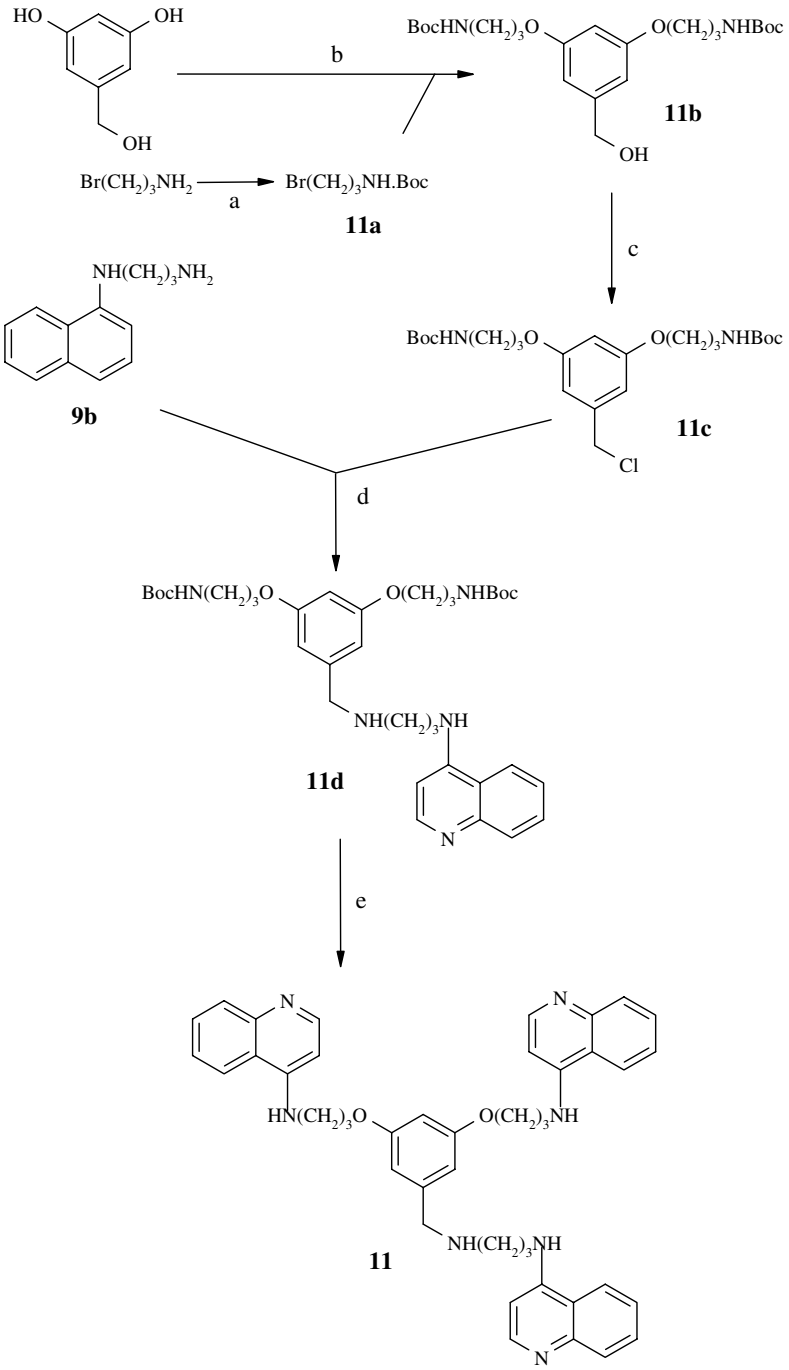


Scheme 4. Reagents: (a) **5a**, N^iPrEt_2, *n*-pentanol; (b) TFA; NaOH, H_2O; (c) monoethyl fumarate, DCC, THF; (d) NaOH, MeOH/H_2O; (e) $F_3CCO{\cdot}NH(CH_2)_8OTs$, NEt_3, DMF; (f) DCC, N^iPrEt_2, DMF; (g) NaOH, MeOH/H_2O.

5. Conclusion

The synthesis and pharmacological testing of novel blocking agents of the SK_{Ca} channel has been described. The results add to the structure–activity knowledge and are consistent with previous reported studies, in that delocalisation of positive charge is an important feature for potent binding, and the presence of more than two positively charged substituents causes a small increase in activity.

The variety in central linking units across the series indicates that this unit, whether it is an aromatic ring or a rigid aliphatic structure, provides an appropriate framework for the active aminoquinolinium substituents. The degree of similarity within this series and with compounds in the cyclophane series linked by 8–10 atoms indicates a relatively long range interaction, in which the compounds are not able to form a tight fit with the channel. This contrasts with the substantial increase in activity that was seen in the cyclophane[23,24,27,44] series



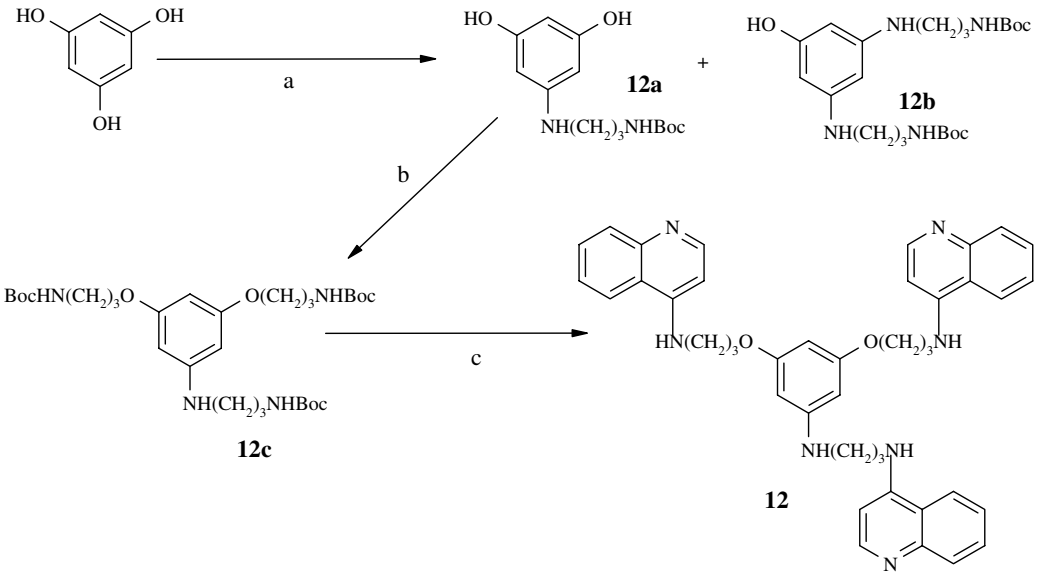
Scheme 5. Reagents: (a) $(t\text{-Boc})_2O$, NaOMe, MeOH; (b) KOBu, **11a**, DMF; (c) TsCl, NEt_3, $CHCl_3$; (d) DMF; (e) TFA; NaOH, MeOH/H_2O; 4ClQ, N^iPrEt_2, *n*-pentanol.

on reducing the number of linking atoms to give much more rigid structures.

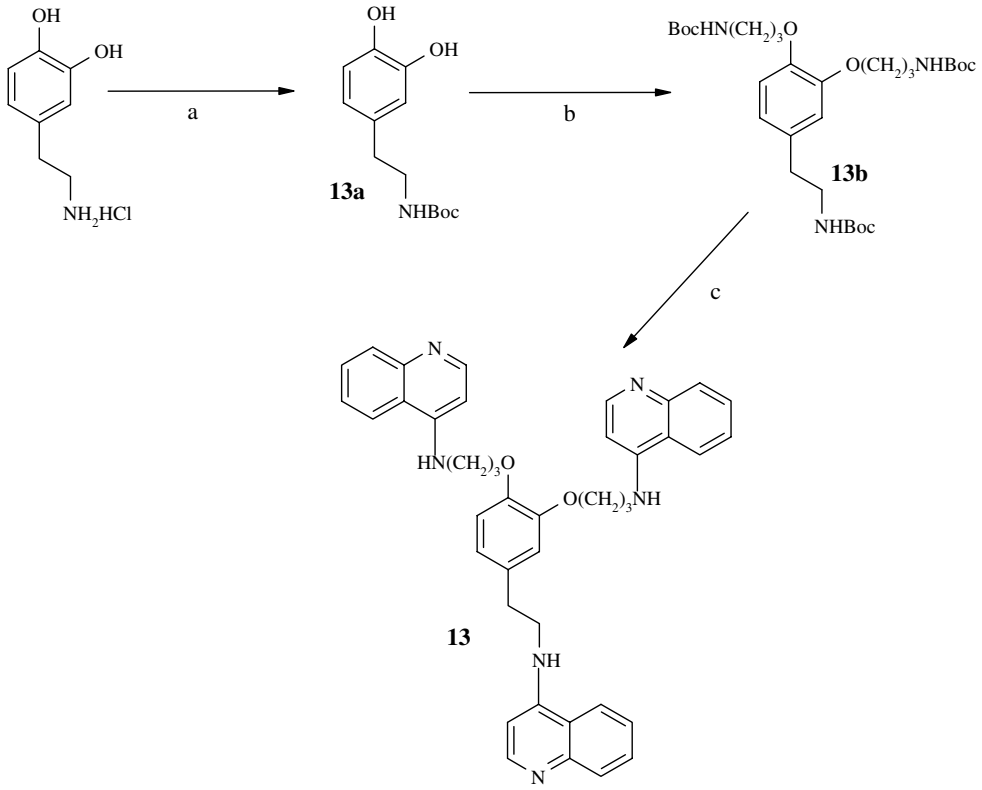
6. Experimental

6.1. General

Commercially available compounds were obtained from either Aldrich Chemical Company or Lancaster Synthesis. Nuclear magnetic resonance (NMR) spectra were recorded on either a Bruker AC200 (200 MHz) or AC400 (400 MHz) spectrometer, and chemical shifts (ppm) are reported relative to TMS. Signals are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; qu, quintet; m, multiplet. Infrared (IR) spectra were run on a Perkin-Elmer 16PC Fourier Transform spectrophotometer. Mass spectra (MS) were run on a V.G. Quattro spectrometer. Analytical high performance liquid chromatography (HPLC) was carried out on a Varian Star Work Station, using Varian 9065 Polychrome DAD detector,



Scheme 6. Reagents: (a) $(t\text{-Boc})_2O$, NaOMe, MeOH; (b) KOBu, **11a**, DMF; (c) TFA; NaOH, $MeOH/H_2O$; 4ClQ, N^iPrEt_2, *n*-pentanol.

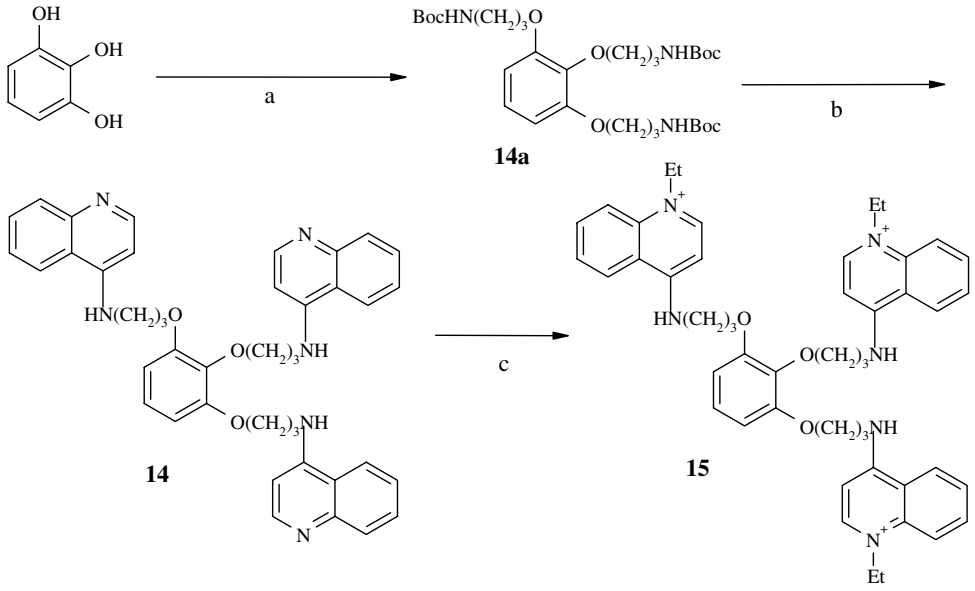


Scheme 7. Reagents: (a) **5a**, THF; (b) KOBu, **11a**, DMF; (c) TFA; NaOH, $MeOH/H_2O$; 4ClQ, N^iPrEt_2, *n*-pentanol.

9010 Pump and Rheodyne 7125 Injector valve with 20 μL loop. Melting points (mp) were obtained on an Electrothermal melting point apparatus and are uncorrected. All final products had satisfactory (within ±0.4%) C, H and N analyses unless otherwise indicated (Supplementary Table 2). Elemental analyses were performed by Wyeth Research (UK) analytical laboratories, Taplow, Berkshire, UK. Molecular modelling was carried out using ChemX.[45]

6.2. Abbreviations

DMF, dimethylformamide; MeOH, methanol; EtOH, ethanol; EtOAc, ethyl acetate; *t*-Boc, *tert*-butyloxycarbonyl; NEt_3, triethylamine; NaOMe, sodium methoxide; CMA, chloroform/methanol/0.91 w/w aq ammonia (100:20:5); Et_2O, diethyl ether; KOBu, potassium *tert*-butoxide; HOBT, 1-hydroxybenzotriazole; DCC, 1,3-dicyclohexylcarbodiimide; TsCl, *p*-toluenesulfonyl chloride; 4-ClQ,



Scheme 8. Reagents: (a) KOBu, **11a**, DMF; (b) TFA; NaOH, MeOH/H_2O; 4ClQ, N^{i}PrEt_2, *n*-pentanol; (c) EtI, DMF.

4-chloroquinoline; N^iPr_2Et, di-isopropylethylamine; TMS, trimethylsilane; tlc, thin layer chromatography.

6.3. *N*-(3-Guanidinopropyl)-*N*-(8-aminooctyl)-3-[(*N*′-(*N*″-3-guanidinopropyl)-carbamoyl)]-*trans*-propenamide trinitrate trihydrate (5)

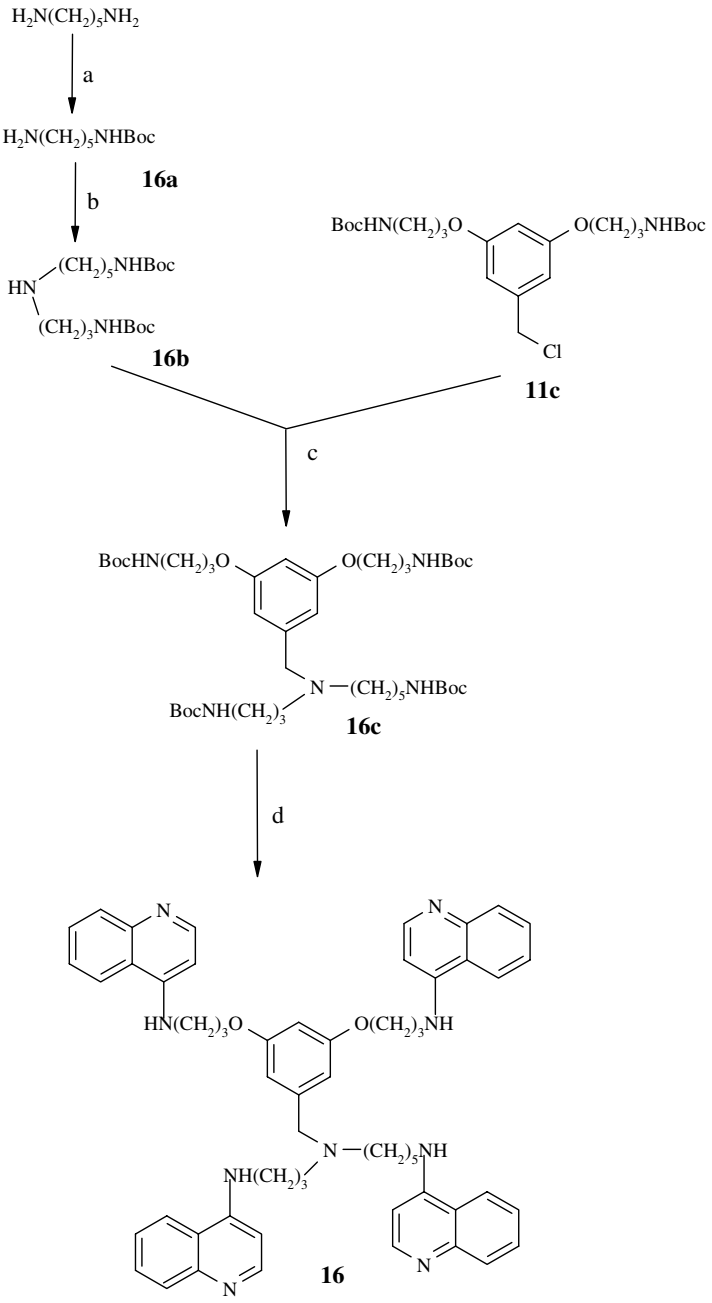
6.3.1. 3-(*tert*-Butoxycarbonylamino)-propylamine (5a). A solution of di *tert*-butyl dicarbonate (327 g, 1.5 mol) in MeOH (1.0 L) was added to 1,3-diaminopropane (450 mL, 7.0 mol) in MeOH (1.5 L) at 10 °C. The resulting suspension was stirred for 18 h and filtered. The filtrate was concentrated under vacuum to low volume, then diluted with water (2 L), filtered to remove di-(*t*-Boc)-1,3-diaminopropane and extracted with Et_2O (5× 500 mL). The extracts were combined and concentrated to give the product as a yellow oil (118 g, 55% yield). ^{1}H NMR ($CDCl_3$) δ 1.2 (s, 2H, NH_2), 1.3 (s, 9H, C(CH_3)$_3$), 1.6 (m, 2H, CH_2), 2.7 (t, 2H, CH_2NH_2), 3.15 (q, 2H, CH_2NH), 6.0 (br t, 1H, N*H*·CO).

6.3.2. Ethyl 3-[*N*-(3-(*tert*-butoxycarbonylamino)-propyl)]-carbamoyl-*trans*-propenoate (5b). A mixture of monoethyl fumarate (43.2 g, 0.3 mol), HOBT (40.5 g, 0.3 mol) and DCC (62.0 g, 0.3 mol) in THF (500 mL) was stirred at 20 °C for 18 h. A solution of **5a** (52.2 g, 0.3 mol) in THF (100 mL) was added and after stirring for 2 h the reaction mixture was concentrated to low volume, diluted with $CHCl_3$ (500 mL), acidified to pH 2 with 2 M citric acid and then filtered. The organic phase was washed with aqueous $NaHCO_3$ (300 mL), then the solvent removed under vacuum to give the product as an off-white powder (62 g, 70% yield): mp 95–97 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 12H, C(CH_3)$_3$ + CH_3), 1.65 (m, 2H, CH_2), 3.0 (q, 2H, CH_2N), 3.3 (q, 2H, CH_2N), 4.15 (q, 2H, CH_2O), 6.05 (br t, 1H, NH·CO), 6.5 (d, J = 15Hz, 1H, *H*C═CH), 6.9 (d, J = 15 Hz, 1H, HC═C*H*), 8.1 (br t, 1H, N*H*·CO); IR (Nujol) υ_{max} 3230, 1720, 1660 cm^{-1}.

6.3.3. 3-[*N*-(3-(*tert*-Butoxycarbonylamino)-propyl)]-carbamoyl-*trans*-propenoic acid (5c). A solution of **5b** (50 g, 0.17 mol) and KOH (11 g, 0.20 mol) in EtOH (300 mL) and water (25 mL) was stirred for 18 h at 20 °C, then acidified to pH 2 with concd HCl (25 mL), diluted with water (500 mL) and extracted with $CHCl_3$(4× 250 mL). The extracts were combined and concentrated to low volume to give an oil, which was diluted with EtOAc (500 mL) to precipitate the product as a white powder (32 g, 70% yield): mp 143–145 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (s, 9H, C(CH_3)$_3$), 1.55 (m, 2H, CH_2), 2.95 (q, 2H, CH_2N), 3.15 (q, 2H, CH_2N), 6.5 (d, J = 15 Hz, 1H, *H*C═CH), 6.9 (d, J = 15 Hz, 1H, HC═C*H*), 8.4 (t, 1H, N*H*·CO), 12.8 (br s, 1H, CO_2H); MS (FB$^+$) 273 (M$^+$ + 1).

6.3.4. 8-(*N*-Phthalimido)-octan-1-ol (5d). A mixture of phthalimide (42.8 g, 0.29 mol), 8-bromooctan-1-ol (60.5 g, 0.29 mol) and K_2CO_3 (140 g, 1.0 mol) was heated in DMF at 60 °C for 18 h, then cooled to 20 °C, filtered and concentrated under vacuum. The resulting oil was diluted with brine (300 mL) and extracted with CH_2Cl_2 (3× 250 mL). The extracts were combined and concentrated to low volume to give an oil, which was diluted with Et_2O, filtered, and the filtrate concentrated to give the product as a cream powder (33 g, 42% yield): mp 60–62 °C; ^{1}H NMR ($CDCl_3$) δ 1.3–1.7 (m, 13H, 6CH_2 +O*H*), 3.6 (m, 4H, 2CH_2), 7.8 (br s, 4H, 4Ar–*H*).

6.3.5. 8-Amino-octan-1-ol (5e). A solution of **5d** (33 g, 0.12 mol) and hydrazine monohydrate (23.5 g, 0.47 mol) in EtOH (200 mL) was stirred for 18 h at 20 °C. The reaction mixture was diluted with 4 M HCl (400 mL), filtered and the filtrate washed with CH_2Cl_2 (200 mL), before being basified to pH 14 with aq NaOH and extracted with

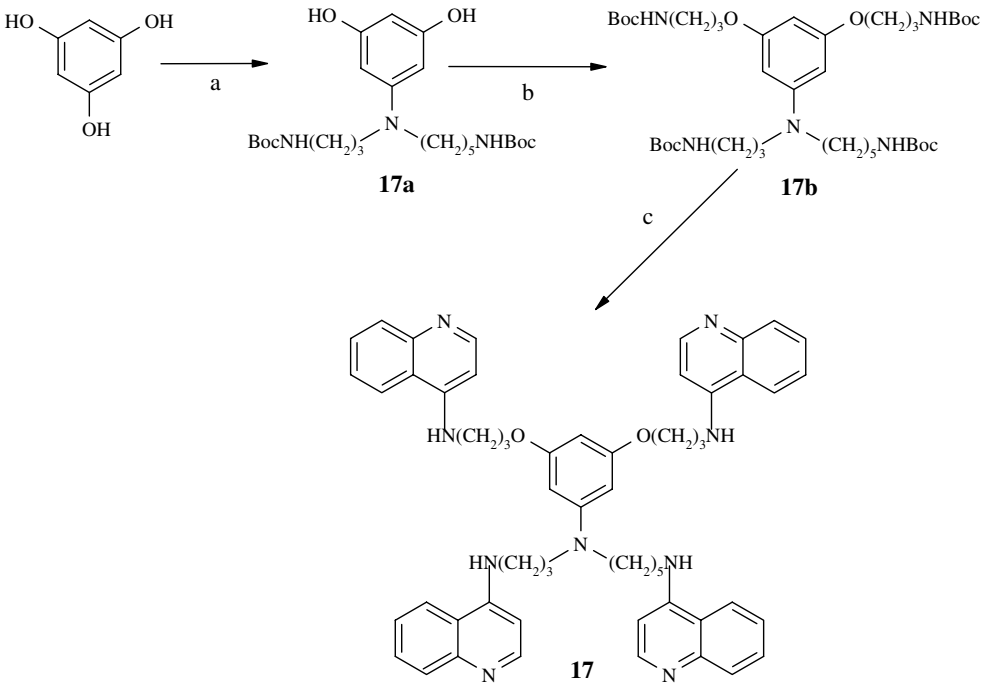


Scheme 9. Reagents: (a) $(t\text{-Boc})_2O$, MeOH; (b) **11a**, K_2CO_3, DMF; (c) K_2CO_3, DMF; (d) TFA; 4ClQ, NaOMe, N^iPrEt_2, *n*-pentanol.

CH_2Cl_2 (5× 150 mL). The extracts were combined and concentrated to give an oil, which was diluted with diisopropyl ether to precipitate the product as a white powder (15 g, 82% yield): mp 59–61 °C; ^{1}H NMR ($CDCl_3$) δ 1.3–1.7 (m, 12H, 6CH_2), 1.8 (br s, 3H, NH_2 and O*H*), 2.7 (t, 2H, CH_2N), 3.6 (t, 2H, CH_2O).

6.3.6. 8-(*N*-Trifluoroacetamido)-octan-1-ol (5f). A solution of methyl trifluoroacetate (7.1 g, 50 mmol) and **5e** (7.3 g, 50 mmol) in MeOH (25 mL) was stirred for 1 h at 15 °C, before being concentrated to give an oil, which was diluted with cyclohexane (100 mL) to precipitate the product as a cream powder (11.2 g, 93% yield): mp 48–50 °C; ^{1}H NMR ($CDCl_3$) δ 1.3–1.7 (br s, 12H, 6CH_2), 2.9 (s, 1H, O*H*), 3.2 (q, 2H, CH_2N), 3.5 (t, 2H, CH_2O), 7.5 (br s, 1H, N*H*·CO).

6.3.7. *N*-(Trifluoroacetyl)-*N*′-[3-(*tert*-butoxycarbonylamino)-propyl]-1,8-diaminooctane (5g). A solution of **5f** (10.6 g, 44 mmol) and TsCl (13.8 g, 73 mmol) in $CHCl_3$ (75 mL) was treated with NEt_3 (8.6 g, 85 mmol) at 20 °C. After 2 h the solvent was removed by distillation and the residue diluted with EtOAc (100 mL). The precipitated NEt_3·HCl was filtered off and the filtrate concentrated to give the crude tosylate as an oil, which was stirred in DMF (50 mL) with **5a** (33 g, 170 mmol) at 20 °C for 18 h. The reaction mixture was then diluted with 2 M HCl (300 mL) and extracted with CH_2Cl_2 (2× 100 mL). The extracts were combined, treated with excess NEt_3 (15 g) and washed with water (2× 100 mL) before concentrating under vacuum. The resulting oil was absorbed onto silica gel and chromatographed with MeOH/0.91 aq NH_3 (100:1) to give



Scheme 10. Reagents: (a) **16b**, THF/toluene; (b) KOBu, **11a**, DMF; (c) TFA; 4ClQ, NaOMe, N^{i}PrEt$_2$, *n*-pentanol.

the product as a cream powder, precipitated from diisopropyl ether (12 g, 68% yield): mp 55–57 °C; IR (Nujol) υ_{max} 3260, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.3 (br s, 8H, 4CH_2), 1.45 (s, 9H, C(CH_3)$_3$), 1.65 (m, 6H, 3CH_2), 2.55 (t, 2H, CH_2N), 2.75 (t, 2H, CH_2N), 3.2 (q, 2H, CH_2N), 3.4 (q, 2H, CH_2N), 5.3 (br s, 1H, NH·CO), 7.25 (br s, 1H, NH·CO); MS (EI$^+$) 397 (M$^+$), fragment at 324.

6.3.8. *N*-[3-(*tert*-Butoxycarbonylamino)-propyl]-*N*-(8-trifluoroacetamidooctyl)-3-[*N*′-3-(*tert*-butoxycarbonylamino)-propyl]-carbamoyl-*trans*-propenamide (5h). A mixture of **5c** (8.6 g, 32 mmol), HOBT (4.7 g, 35 mmol) and DCC (7.2 g, 35 mmol) was stirred in THF (150 mL) for 18 h at 20 °C to give a thick suspension, which was treated with **5g** (11.0 g, 28 mmol) in THF (50 mL). After 6 h the dicyclohexylurea was filtered off and the filtrate concentrated by distillation. The residue was diluted with CH_2Cl_2 (100 mL) and 1 M aq citric acid (75 mL). The organic phase was washed sequentially with aq K_2CO_3 (100 mL) and water (100 mL), before removing the solvent by distillation under vacuum to give an oily solid. This was absorbed onto silica gel and chromatographed with EtOAc/MeOH (20:1) to give **5h**, crystallised as white needles from Et_2O (17.4 g, 95% yield): mp 100–102 °C; ^{1}H NMR ($CDCl_3$) δ 1.3 (br s, 8H, 4CH_2), 1.45 (s, 18H, 2C(CH_3)$_3$), 1.55–1.75 (m, 8H, 4CH_2), 3.05–3.25 (m, 4H, 2CH_2N), 3.3–3.55 (m, 8H, 4CH_2N), 4.85 (br t, 1H, NH·CO), 5.4 (br t, 1H, NH·CO), 6.9 (d, J = 15 Hz, 1H, HC=CH), 7.1 (br s, 2H, 2NH·CO), 7.3 (d, J = 15 Hz, 1H, HC=CH).

6.3.9. *N*-(3-Guanidinopropyl)-*N*-(8-aminooctyl)-3-[(*N*′-(*N*″-3-guanidinopropyl)-carbamoyl)]-*trans*-propenamide trinitrate trihydrate (5). A solution of **5h** (2.0 g, 3 mmol) in 15% w/w HCl in MeOH (25 mL) was stirred for 2 h at 20 °C, then diluted with water, cooled to −5 °C, basified to pH 9 with 40% w/w aq NaOH and extracted with $CHCl_3$ (6× 50 mL). The extracts were combined and concentrated under vacuum to give **5i** (1.6 g) as a yellow oil. A solution of the oil and 3,5-dimethylpyrazole-1-carboxamidine nitrate (2.6 g, 13 mmol) in water (10 mL) was heated at 60 °C for 24 h, maintaining the pH at 7.0–8.5 by periodic addition of 1 M NaOH solution. The reaction mixture was then cooled, basified to pH 14 with 40% w/w NaOH solution (1 mL) and stirred for 2 h at 20 °C before being washed with $CHCl_3$ (2× 20 mL). 50% w/w aq nitric acid was then added to the aqueous phase to pH 1 and the solution concentrated under vacuum to dryness. The residue was stirred in EtOH, filtered at 70 °C, then cooled to produce an orange oil. Multiple precipitations from EtOH gave the product as a yellow glass (300 mg, 23% yield); ^{1}H NMR (DMSO-d_6) δ 1.35 (br s, 8H, 4CH_2), 1.65 (m, 4H, 2CH_2), 1.8 (m, 4H, 2CH_2), 2.75 (m, 2H, CH_2N), 3.2 (m, 6H, 3CH_2N), 3.45 (m, 4H, 2CH_2N), 6.85 (d, J = 15 Hz, 1H, HC=CH), 7.1 (br s, 8H, 4NH_2^+), 7.2 (d, J = 15 Hz, 1H, HC=CH), 7.5 (m, 2H, NH), 7.7 (br s, 3H, NH_3^+), 8.55 (m, 1H, NH·CO)—2 rotamers 2:1; IR (film) υ_{max} 3350, 3200, 1665, 1635 cm^{-1}; HPLC Lichrosorb *RP* Select B (MeOH/0.05% aq TFA, 80:20, 1.0 mL/min), 224 nm, product at 3.5 min; MS (LC/MS, TS$^+$) 440 (M$^+$+1), fragments at 398, 324, 244. Anal. ($C_{20}H_{41}N_9O_2$, $3HNO_3$, $3H_2O$) C, H, N. Anal. Calcd for $C_{20}H_{41}N_9O_2{\cdot}3HNO_3{\cdot}3H_2O$: C, 35.19; H, 7.38; N, 24.62. Found: C, 35.37; H, 7.31; N, 24.48.

6.4. *N*-(3-Guanidinopropyl)-*N*-(8-aminooctyl)-3-[*N*′-(*N*″-3-guanidino propyl)-carbamoyl]-*cis*-propenamide trinitrate trihydrate (6)

6.4.1. *N*-[3-(*tert*-Butoxycarbonylamino)-propyl]-*N*-(8-trifluoroacetamidooctyl)maleamide (6a). A solution of

maleic anhydride (1.4 g, 1.43 mmol) and **5g** (5.3 g, 1.34 mmol) in MeOH (30 mL) was stirred at 20 °C for 2 h, before concentrating then diluting with CH_2Cl_2 (100 mL) and 1 M HCl (50 mL). The organic phase was concentrated to low volume, and the product precipitated as a white powder from diisopropylether (6.5 g, 98% yield): mp 92–94 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (m, 8H, 4CH_2), 1.45 (s, 9H, C(CH_3)$_3$), 1.5 (m, 4H, 2CH_2), 1.75 (qu, 2H, CH_2), 3.1 (m, 2H, CH_2N), 3.25 (q, 4H, 2CH_2N), 3.45 (m, 2H, CH_2N), 5.0 (br t, 1H, N*H*·CO), 7.30 (d, J = 15 Hz, 1H, *H*C=CH), 7.45 (d, J = 15 Hz, 1H, HC=C*H*), 8.55 (bt t, 1H, N*H*·CO); IR (Nujol) υ_{max} 3380, 3250, 1680, 1650 cm^{-1}.

6.4.2. *N*-(3-(*tert*-Butoxycarbonylamino)-propyl)-*N*-(8-trifluoroacetamidooctyl)-3-[*N*″-3-(*tert*-butoxycarbonylamino)-propyl]-carbamoyl-*cis*-propenamide (6b). A solution of **6a** (6.5 g, 13.2 mmol), *N*-hydroxysuccinimide (1.8 g, 16 mmol) and DCC (3.0 g, 14.5 mmol) in CH_2Cl_2 (60 mL) was stirred for 16 h at 20 °C before adding **5a** (2.4 g, 14.5 mmol). After 3 h the reaction mixture was filtered and the filtrate was washed with water (30 mL), then concentrated under vacuum to give an oil. This was absorbed onto silica gel and chromatographed with EtOAc/MeOH 20:1 to give the product as a yellow gum (6.9 g, 80% yield); ^{1}H NMR ($CDCl_3$) δ 1.3 (m, 8H, 4CH_2), 1.45 (s, 18H, 2C(CH_3)$_3$), 1.5 (m, 4H, 2CH_2), 1.6 (m, 2H, CH_2), 1.7 (m, 2H, CH_2), 3.1 (m, 4H, 2CH_2N), 3.3 (m, 6H, 3CH_2N), 3.45 (m, 2H, CH_2N), 5.1 (br t, 1H, N*H*·CO), 5.4 (br t, 1H, N*H*·CO), 6.1 (d, J = 12.7 Hz, 1H, *H*C=CH), 6.4 (d, J = 12.7 Hz, 1H, HC=C*H*), 7.1 (br t, 1H, N*H*·CO), 8.0 (br t, 1H, N*H*·CO); IR (film) υ_{max} 3350, 1705, 1700 cm^{-1}; MS (TS$^+$) 652 (M$^+$).

6.4.3. *N*-(3-Guanidinopropyl)-*N*-(8-aminooctyl)-3-[*N*′-(*N*″-3-guanidinopropyl)-carbamoyl]-*cis*-propenamide trinitrate trihydrate (6). Compound **6b** (3.2 g, 5 mmol) was stirred in a solution of HCl in MeOH (22% w/w, 30 mL) at 20 °C for 2 h, before adjusting to pH 6 with 25% w/w NaOMe in MeOH and filtering. The MeOH was removed from the filtrate by distillation under vacuum and the resulting oil **6c** diluted with water (20 mL). 3,5-Dimethylpyrazole-1-carboxamidine nitrate (3.7 g, 18 mmol) was added and the pH adjusted to 8 with 1 M aq NaOH. The reaction mixture was heated at 60 °C for 26 h, maintaining the pH between 7.0 and 8.5 by periodic addition of 1 M aq NaOH. After cooling to −5 °C, 40% w/w aq NaOH (1 mL) was added (to pH 14), and the solution stirred for 1 h before washing with $CHCl_3$ (2× 30 mL). The aqueous phase was acidified to pH 1 with 50% w/w nitric acid and concentrated to dryness under vacuum. The resulting oil was stirred in MeOH (30 mL), filtered and reconcentrated. The residue was precipitated twice from EtOH (2× 30 mL) to give the product **6** as a yellow glass (100 mg, 5%); ^{1}H NMR (DMSO-d_6) δ 1.2 (br m, 8H, 4CH_2), 1.4 (m, 4H, 2CH_2), 1.8 (m, 4H, 2CH_2), 2.8 (m, 4H, 2CH_2N), 3.3 (m, 6H, 3CH_2N), 3.55 (m, 2H, CH_2N), 6.0 (m, J = 12.7 Hz, 1H, *H*C=CH), 6.5 (m, J = 12.7 Hz, 1H, HC=C*H*), 7.1 (br s, 8H, 4NH_2^+), 7.5 (m, 2H, 2N*H*), 7.7 (br s, 3H, NH_3^+), 8.40 (br s, 1H, N*H*·CO)—2 rotamers 1:1; HPLC Lichrosorb PR Select B (MeOH/0.5% aq TFA; 80:20, 1.0 mL/min), 224 nm, product at 2.9 min; MS (TS$^+$) 440 (M$^+$+1), fragment at 244. Anal. Calcd for $C_{20}H_{41}N_9O_2{\cdot}3HNO_3{\cdot}3.5H_2O$: C, 34.73; H, 7.43; N, 24.30. Found: C, 34.46; H, 7.13; N, 24.12.

6.5. *N*-(3-Guanidinopropyl)-*N*-[4-(4′-aminomethylphenyl)]-benzyl-3-*N*″-(3-guanidinopropyl)-carbamoyl-*trans*-propenamide trinitrate, (7)

6.5.1. Methyl 4-(4′-bromophenyl)-benzoate (7a). 4-(4′-Bromophenyl)benzoic acid[42] (257 g) was heated in $SOCl_2$ (2 L) under reflux for 1 h. The solvent was then replaced by distillation with toluene, before concentrating the solution under vacuum. The resulting oil was diluted with MeOH (1.5 L) to give the product as red–brown crystals (244 g, 90% yield): mp 138–142 °C; ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H, OCH_3), 7.50 (d, 2H, 2Ar–*H*), 7.55 (d, 2H, 2Ar–*H*), 7.65 (d, 2H, 2Ar–*H*), 8.15 (d, 2H, 2Ar–*H*); IR (Nujol) υ_{max} 1715 cm^{-1}.

6.5.2. Methyl 4-(4′-cyanophenyl)-benzoate (7b). Compound **7a** (240 g, 0.8 mol) was heated in DMF (1.5 L) with CuCN (170 g, 1.9 mol) at 130 °C for 16 h. The resulting suspension was cooled to 20 °C then added to water (3 L) and CH_2Cl_2 (2 L), and filtered. The organic phase was washed with water (2 L) then concentrated to give an oil, which was diluted with diisopropylether (1 L) to precipitate the product as a yellow powder (79 g, 40% yield): mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H, OCH_3), 7.65 (d, 2H, 2Ar–*H*), 7.75 (s, 4H, 4Ar–*H*), 8.15 (d, 2H, 2Ar–*H*); IR (Nujol) υ_{max} 2230, 1710 cm^{-1}.

6.5.3. 4-(4′-Aminomethylphenyl)-benzyl alcohol (7c). Compound **7b** (78 g) was treated in THF (800 mL) with 2 M $LiBH_4$ in THF (200 mL). The resulting solution was heated under reflux for 4 h to give a thick suspension, which was then cooled and added to water (1.5 L) and $CHCl_3$ (1 L). The mixture was filtered and the layers separated. The aqueous phase was extracted with $CHCl_3$/MeOH 5:1 (2× 1 L). The organic layers were combined and solvent removed by distillation to give an oily solid. This was absorbed onto silica gel, chromatographed with $CHCl_3$/MeOH 5:1 and the product precipitated as a yellow powder from diisopropylether (10 g, 14% yield): mp 164–168 °C; ^{1}H NMR ($CDCl_3$) δ 3.2 (br s, 2H, NH_2), 3.8 (s, 2H, CH_2N), 4.55 (s, 2H, CH_2O), 7.40 (d, 2H, 2Ar–*H*), 7.45 (d, 2H, 2Ar–*H*), 7.60 (d, 2H, 2Ar–*H*), 7.65 (d, 2H, 2Ar–*H*).

6.5.4. 4-[4′-(Trifluoroacetylamidomethyl)-phenyl]-benzyl alcohol (7d). A suspension of **7c** (7.8 g, 37 mmol) in MeOH (100 mL) was treated with methyl trifluoroacetate (7.0 g, 55 mmol) until a solution was obtained. The solvent was then removed by distillation under vacuum. The residue was absorbed onto silica gel and chromatographed with CH_2Cl_2/MeOH 5:1 to give the product as a white powder, precipitated from diisopropylether (5.9 g, 53% yield): mp 179–181 °C; ^{1}H NMR ($CDCl_3$) δ 4.45 (d, 2H, CH_2N), 4.55 (d, 2H, CH_2O), 5.25 (t, 1H, O*H*), 7.40 (d, 2H, 2Ar–*H*), 7.45 (d, 2H, 2Ar–*H*), 7.60 (d, 2H, 2Ar–*H*), 7.65 (d, 2H, 2Ar–*H*), 10.05 (br t, 1H, N*H*·CO); IR (Nujol) υ_{max} 3290, 1700, 1560 cm^{-1}.

6.5.5. 4-[4′-(Trifluoroacetamidomethyl)-phenyl]-*N*-[3-(*tert*-butoxycarbonyl)-aminopropyl]benzylamine (7e). DMF (8 mL) was added to a mixture of **7d** (3.7 g, 12 mmol),

TsCl (5.1 g, 27 mmol) and NEt_3 (1.8 g, 18 mmol) in $CHCl_3$ (30 mL) until a solution was obtained. This was heated to reflux and more NEt_3 (0.8 g, 0.7 mol/mol) added dropwise until the pH increased to 7. The solution was cooled, washed with 1 M HCl (50 mL), water (50 mL) and concentrated under vacuum to give an oil. This was dissolved in DMF and stirred with the amine **5a** (3.4 g, 20 mmol) for 2 h at 20 °C, then diluted with water and extracted with CH_2Cl_2 (2× 30 mL). The extracts were combined, washed with water (15 mL) and concentrated under vacuum to give an oil, which was absorbed onto silica gel and chromatographed with CH_2Cl_2/MeOH 3:1 to give the product as a white powder, precipitated from diisopropylether (1.6 g, 69% yield): mp 195–197 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H, C(CH_3)$_3$), 1.7 (m, 2H, CH_2), 2.75 (t, 2H, CH_2N), 3.35 (t, 2H, CH_2N), 3.85 (s, 2H, CH_2N), 4.45 (d, 2H, CH_2N), 5.35 (br t, 1H, N*H*·CO), 6.90 (br t, 1H, N*H*·CO), 7.35 (d, 2H, 2Ar–*H*), 7.40 (d, 2H, 2Ar–*H*), 7.50 (d, 2H, 2Ar–*H*), 7.60 (d, 2H, 2Ar–*H*); MS (FB^+) 466 (M^++1), fragments at 410, 292.

6.5.6. *N*-[3-(*tert*-Butoxycarbonylamino)-propyl]-*N*′-[4-(4′-trifluoroacetamidomethyl)-phenyl-benzyl]-3-*N*′-[3-(*tert*-butoxycarbonylamino)-propyl]-carbamoyl-*trans*-propenamide (7f). A solution of **5c** (0.7 g, 2.57 mmol), HOBT (0.35 g, 2.60 mmol) and DCC (0.55 g, 2.70 mmol) in DMF (7 mL) was stirred at 20 °C for 2 h. The resulting suspension was treated with **7e** (1.1 g, 2.35 mmol) before being stirred for a further 2 h. The reaction mixture was filtered, diluted with water (30 mL), extracted with CH_2Cl_2 (60 mL) and the extract was concentrated to low volume. The resulting oil was absorbed onto silica gel and chromatographed with EtOAc to give the product as a white powder, precipitated from diisopropyl ether (1.3 g, 77% yield): mp 134–136 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 18H, 2C(CH_3)$_3$)-satellite signals due to rotamers, 1.55 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 2.95 (m, 4H, 2CH_2N), 3.15 (m, 2H, CH_2N), 3.35 (m, 2H, CH_2N), 4.45 (d, 2H, CH_2N), 4.7 (s, 2H, CH_2N), 6.7 (d, J = 15 Hz, 1H, *H*C=CH), 7.15 (d, J = 15 Hz, 1H, HC=C*H*), 7.50 (d, 4H, 4Ar–*H*), 7.60 (d, 4H, 4Ar–*H*), 10.05 (t, 1H, N*H*·CO); IR (Nujol) υ_{max} 3320, 1705, 1680 cm^{-1}; MS (TS^+) 720 (M^++1), fragments at 292, 427.

6.5.7. *N*-(3-Guanidinopropyl)-*N*-[4-(4′-aminomethylphenyl)]-benzyl-3-*N*′-(3-guanidino propyl)-carbamoyl-*trans*-propenamide trinitrate (7). A solution of **7f** (1.2 g, 1.7 mmol) was stirred in HCl in MeOH (17% w/w, 10 mL) at 20 °C for 2 h, before adjusting the pH to 6 with 25% w/w NaOMe in MeOH and filtering. The MeOH was removed by distillation under vacuum, and the residue diluted with water (20 mL). 3,5-Dimethylpyrazole-1-carboxamidine nitrate (1.6 g, 8.0 mmol) was then added and the pH adjusted to 8 with 1 M aq NaOH before heating at 60 °C for 18 h, maintaining the pH between 7.5 and 8.5 by periodic addition of 1 M aq NaOH. After cooling to 20 °C, 10 M aq NaOH (0.5 mL) was added to pH 14, and the solution stirred for 1 h before washing with $CHCl_3$ (2× 25 mL). The aqueous phase was acidified to pH 1 with 50% w/w aq HNO_3 and concentrated under vacuum to give an oil, which was stirred in MeOH (30 mL), filtered and reconcentrated. The resulting gum was precipitated twice from EtOH (2× 40 mL) to give the product as a yellow glass (120 mg, 14% yield); ^{1}H NMR (DMSO-d_6) δ 1.90 (m, 4H, 2CH_2)-satellite signals due to rotamers, 3.00 (m, 2H, CH_2N), 3.20 (m, 2H, CH_2N), 3.35 (m, 2H, CH_2N), 3.55 (m, 2H, CH_2N), 4.10 (m, 2H, CH_2N), 4.70 (m, 2H, CH_2N), 7.10 (d, J = 15 Hz, 1H, *H*C=CH), 7.35 (d, J = 15 Hz, 1H, HC=C*H*), 7.40 (m, 2H, 2Ar–*H*), 7.70–7.55 (m, 8H, 4Ar–*H*), 7.85 (br s, 8H, 4NH_2), 8.35 (br s, 3H, NH_3^+), 8.70 (m, 1H, N*H*·CO)—2 rotamers 1:1; HPLC Lichrosorb RP Select B (MeOH/0.05M aq NH_4OAc, 95:5, 1.0 mL/min), 210 nm, product at 4.3 min; MS (FAB^+) 509 (M^++1), fragments at 467, 425. HPLC Lichrosorb RP Select B (MeOH/0.05 M aq NH_4OAc, 95/5, 1.0 mL/min), 210 nm, product at 4.3 min. Anal. Calcd for $C_{26}H_{37}N_9O_2$·3HNO_3·0.3EtOH·13% w/w inorganic material: C, 39.20; H, 5.12; N, 20.50. Found: C, 39.20; H, 5.10; N, 20.30.

6.6. 4,4′Bis(guanidinomethyl)-*cis*-stilbene trifluoroacetate (8)

4,4′-Bis (bromomethyl)-*cis*-stilbene[38] (2.65 g, 7.2 mmol) and potassium phthalimide (3.5 g, 19 mmol) in 10 mL of anhydrous DMF were heated with stirring for 12 h at 90 °C, then cooled, treated with 15 mL of water, filtered and washed with water. Crystallisation from MeOH afforded 3.2 g (89% yield) of the diphthalimide derivative **8a**, mp 180–181 °C.

The latter (**8a**), 3.2 g in ethanol (130 mL), was heated under reflux with 1 mL of hydrazine hydrate for 5 h. The mixture was cooled, water (15 mL) was added and the ethanol was removed by distillation. The residual solid was washed with water and then chromatographed on silica gel using a mixture of $CHCl_3$/EtOAc/MeOH to give the bis-amine product **8b** (1.21 g, 79% yield), mp 123–125 °C decomp.

The bis-amine **8b** (0.5 g) was stirred with 3,5-dimethylpyrazole-1-carboxamidine nitrate (1.2 g) in water (25 mL) at 80 °C. The mixture was cooled to 4 °C and the product was isolated by preparative chromatography using 30% aqueous methanol rising to 50% methanol over 20 min. and then taken into isopropanol, filtered cold and concentrated in vacuo to give **8** as an oil (0.05 g, 36% yield).^{1}H NMR (DMSO-d_6)δ 4.33 (br s, 4H, CH_2N), 6.62 (s, 2H, C*H*=C*H*), 7.17 (d, 4H, C_6H_4), 7.20 (br s, 8H, NH^+), 7.23 (d, 4H, C_6H_4), 7.99 (br s, 2H, N*H*). MS(FAB^+) 323 (M^++1), fragments at 306, 263, 149, 102. Anal. Calcd for $C_{18}H_{22}N_6$·2.3CF_3·CO_2H·CH_3OH: C, 45.95; H, 4.63; N, 13.63. Found: C, 46.14; H, 4.65; N, 13.52.

6.7. *N*-[3-(4-Quinolinyl)-aminopropyl]-*N*-(8-aminooctyl)-3-*N*′-[3-(4-quinolinyl)-aminopropyl]-carbamoyl-*trans* propenamide trihydrate (9)

6.7.1. *N*-(*tert*-Butoxycarbonyl)-*N*′-(4-quinolinyl)-1,3-diaminopropane (9a). A solution of **5a** (32.6 g, 0.187 mmol), N^iPr_2Et (25.2 g, 195 mmol) and 4-chloroquinoline (24.5 g, 150 mmol) in *n*-pentanol (200 mL) was heated un-

der reflux for 6 h, before concentrated to low volume. The resulting oil was diluted with CH_2Cl_2 (250 mL) and washed with 2 M NaOH (120 mL). After the second wash a precipitate was produced, which was filtered off and washed with toluene (50 mL). The organic phase from the filtrate was concentrated and the residue diluted with toluene (150 mL) to give a further precipitate, which was also filtered off and washed with toluene. The two crops were combined and recrystallised from toluene (300 mL) to provide the product as a cream powder (38 g, 84% yield): mp 165–167 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H, $C(CH_3)_3$), 1.85 (qu, 2H, CH_2), 3.30 (q, 2H, CH_2N), 3.45 (q, 2H, CH_2N), 4.85 (br s, 1H, N*H*), 6.0 (br s, 1H, N*H*·CO), 6.45 (d, 1H, Ar–*H*), 7.45 (m, 1H, Ar–*H*), 7.60 (m, 1H, Ar–*H*), 7.95 (d, 1H, Ar–*H*), 8.0 (d, 1H, Ar–*H*), 8.50 (d, 1H, Ar–*H*).

6.7.2. *N*-(4-Quinolinyl)-1,3-diaminopropane (9b). A solution of **9a** (30.1 g, 0.10 mol) in trifluoroacetic acid (200 mL) was stirred at 20 °C for 1 h, diluted with toluene (200 mL) and concentrated to remove the bulk of the solvent. The resulting oil was diluted with water (200 mL), basified to pH 14 with 40% w/w aq NaOH (50 mL) and extracted with $CHCl_3$ (2× 150 mL). The combined extracts were concentrated under vacuum to give the product as a white powder (19 g, 95% yield): mp 75–77 °C; ^{1}H NMR ($CDCl_3$) δ 1.90 (qu, 2H, CH_2), 3.05 (t, 2H, CH_2N), 3.45 (t, 2H, CH_2N), 6.35 (d, 1H, Ar–*H*), 7.25 (br t, 1H, N*H*), 7.40 (m, 1H, Ar–*H*), 7.65 (m, 1H, Ar–*H*), 7.80 (d, 1H, Ar–*H*), 7.95 (d, 1H, Ar–*H*), 8.55 (d, 1H, Ar–*H*).

6.7.3. Ethyl 3-[*N*-(4-quinolinylamino)-propyl]-carbamoyl-*trans*-propenoate (9c). Monoethylfumarate (8.6 g, 60 mmol) and DCC (13.6 g, 70 mmol) were stirred in THF (120 mL) for 2 h at 20 °C to give a thick suspension, to which a solution of **9b** (12.0 g, 60 mmol) in THF (50 mL) was added. After a further 2 h, the mixture was filtered and the filtrate concentrated under vacuum to give the product as a cream powder, precipitated from diisopropyl ether (11.7 g, 60% yield): mp 194–196 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, CH_3), 1.90 (m, 2H, CH_2), 3.40 (m, 2H, CH_2N), 3.55 (m, 2H, CH_2N), 4.30 (q, 2H, CH_2O), 6.30 (d, 1H, Ar–H), 6.50 (t, 1H, NH·CO), 6.90 (d, J = 15 Hz, 1H, *H*C═CH), 7.00 (d, J = 15 Hz, 1H, HC=C*H*), 7.50 (m, 1H, Ar–H), 7.65 (m, 1H, Ar–H), 7.80 (d, 1H, Ar–H), 8.00 (m, 2H, Ar–H), 8.50 (d, 1H, Ar–H).

6.7.4. 3-[*N*-(4-Quinolinylamino)-propyl]-carbamoyl-*trans*-propenoic acid hydrochloride (9d). A suspension of **9c** (9.85 g, 30 mmol) in MeOH (30 mL) and water (120 mL) was stirred at 20 °C while adding 40% w/w aq NaOH (30 mL, 30 mmol). The mixture was heated to 50 °C to give a solution, which was cooled to 20 °C, and concd HCl (60 g, 70 mmol) added to precipitate the product as white needles (3.0 g, 30% yield): mp 179–181 °C; ^{1}H NMR (DMSO-d_6) δ 1.90 (qu, 2H, CH_2), 2.70 (m, 2H, CH_2N), 2.95 (m, 2H, CH_2N), 6.50 (d, J = 15 Hz, 1H, *H*C CH), 6.80 (d, 1H, Ar–*H*), 6.95 (d, J = 15 Hz, 1H, HC═C*H*), 7.70 (m, 1H, Ar–*H*), 7.95 (m, 2H, 2Ar–*H*), 8.50 (d, 1H, Ar–*H*), 8.60 (d, 1H, Ar–*H*), 8.85 (t, 1H, N*H*·CO), 9.50 (t, 1H, CO_2H).

6.7.5. *N*-(Trifluoroacetamido)-*N*′-[3-(4-quinolinylamino)-propyl]-1,8-diaminooctane (9e). A solution of **9b** (5.0 g, 50 mmol), 8-trifluoroacetamido-1-octyl tosylate (10.0 g, 26 mmol) and N^{i}Pr$_2$Et (3.5 g, 27.5 mmol) in DMF was stirred for 16 h at 20 °C, before diluting with water (150 mL) and extracting with CH_2Cl_2 (2× 100 mL). The combined extracts were washed with water (100 mL) and concentrated to give an orange oil, which was absorbed onto silica gel and chromatographed with CMA to give the product as a pale yellow gum (3.5 g, 34% yield); ^{1}H NMR ($CDCl_3$) δ 1.25 (br s, 8H, $4CH_2$), 1.50 (m, 4H, $2CH_2$), 1.95 (qu, 2H, CH_2), 2.70 (m, 2H, CH_2N), 2.95 (m, 2H, CH_2N), 3.35 (q, 2H, CH_2N), 3.45 (m, 2H, CH_2N), 6.35 (d, 1H, Ar–*H*), 6.70 (br s, 1H, N*H*), 7.40 (m, 1H, Ar–*H*), 7.70 (m, 1H, Ar–*H*), 7.75 (br s, 1H, N*H*·CO), 7.85 (m, 1H, Ar–*H*), 7.95 (m, 1H, Ar–*H*), 8.50 (d, 1H, Ar–*H*).

6.7.6. *N*-[3-(4-Quinolinylamino)-propyl]-*N*-(8-trifluoroacetamidooctyl)-*N*′-[3-(4-quinolinylamino)-propyl]-carbamoyl-*trans* propenamide (9f). Compound **9d** (3.4 g, 10 mmol) and DCC (2.5 g, 12 mmol) were stirred in DMF (20 mL) for 3 h to give a thick suspension, which was treated with **9e** (4.3 g, 10 mmol) and N^{i}Pr$_2$Et (1.6 g, 12.5 mmol). After 2 h, the mixture was filtered to remove dicyclohexylurea, diluted with water (100 mL) and extracted with CH_2Cl_2 (2× 100 mL). The combined extracts were washed with water (50 mL) and concentrated to give a yellow oil, which was absorbed onto silica gel and chromatographed with toluene/EtOH/0.91 w/w aq NH_3 (80:20:1) to give the product as off-white needles, crystallised from Et_2O (1.5 g, 22% yield): mp 127–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (br s, 8H, $4CH_2$), 1.95 (m, 4H, $2CH_2$), 1.55 (m, 4H, $2CH_2$), 3.35 (m, 8H, $4CH_2N$), 3.55 (m, 4H, $2CH_2N$), 6.10 (t, 1H, N*H*·CO), 6.35 (d, 2H, 2Ar–*H*), 6.55 (t, 2H, 2N*H*), 7.05 (d, J = 15 Hz, 1H, *H*C═CH), 7.35 (m, 2H, 2Ar–*H*), 7.40 (d, J = 15 Hz, 1H, HC═C*H*), 7.40 (m, 2H, 2Ar–*H*), 7.60 (m, 2H, 2Ar–*H*), 7.85 (m, 1H, N*H*·CO), 7.95 (m, 4H, 2Ar–*H*), 8.50 (m, 2H, 2Ar–*H*); IR (Nujol) υ_{max} 3400, 1710, 1630 cm^{-1}.

6.7.7. *N*-[3-(4-Quinolinylamino)-propyl]-*N*-(8-aminooctyl)-3-*N*′-[3-(4-quinolinylamino)-propyl]-carbamoyl-*trans* propenamide trihydrate (9). 40% w/w aq NaOH (0.5 mL) was added to **9f** (0.4 g) in a mixture of MeOH (5 mL) and water (3 mL). After 1 h at 20 °C, the solution was extracted with $CHCl_3$ (2× 20 mL). The extracts were combined and concentrated to give a pale yellow gum, which was redissolved in $CHCl_3$/MeOH (20:1, 10 mL), washed with 0.5 M aq NaOH (2× 5 mL) and reconcentrated. The residue was dissolved in EtOH and diluted with Et_2O to precipitate the product as a clear glass (30 mg, 8% yield); ^{1}H NMR ($CDCl_3$) δ 1.30 (br s, 8H, $4CH_2$), 1.45 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.90 (m, 4H, $2CH_2$), 2.65 (m, 2H, CH_2N), 3.30 (m, 2H, CH_2N), 3.40 (m, 4H, $2CH_2N$), 3.55 (m, 4H, $2CH_2N$), 6.15 (t, 2H, NH_2), 6.40 (m, 2H, 2Ar–*H*), 6.55 (t, 1H, N*H*), 6.90 (t, 1H, N*H*),

7.05 (d, J = 15 Hz, 1H, *H*C═CH), 7.40 (m, 2H, 2Ar–*H*), 7.45 (d, J = 15 Hz, 1H, HC═C*H*), 7.60 (m, 2H, 2Ar–*H*), 7.95 (m, 4H, 4Ar–*H*), 8.55 (m, 2H, 2Ar–*H*); IR (Nujol) υ_{max} 3250, 1665 cm^{-1}; HPLC (Phenyl BDS, MeOH/0.05 M aq NH_4OAc 90:10, 215 nm, 1.0 mL/min), product at 3.5 min; MS (TS^+) 610 (M^++1). Anal. Calcd for $C_{26}H_{47}N_7O_2{\cdot}3.5H_2O{\cdot}0.3$EtOH: C, 63.20; H, 8.00; N, 14.01. Found: C, 63.24; H, 7.42; N, 13.86.

6.8. *N*-[3-(4-Quinolinylamino)-propyl]-3,5-di-[3-(4-quinolinylamino)-propoxy]-benzylamine, (11)

6.8.1. 3-Bromo-*N*-(*tert*-butoxycarbonyl)-propylamine (11a). A solution of 3-bromopropylamine·HBr (217 g, 1.0 mol) and di-*t*-butyl dicarbonate (229 g, 1.05 mol) in MeOH (400 mL) was treated with 25% w/w NaOMe in MeOH (450 mL, 2.1 mol) over 1 h at 25 °C. After stirring for 0.5 h the reaction mixture was diluted with water (3 L) and extracted with CH_2Cl_2 (3 L). The extract was washed with 1 M HCl (1.5 L) then with saturated $NaHCO_3$ (1.5 L), before being concentrated under vacuum to give the product as white crystals, precipitated from diisopropylether (204 g, 86% yield); ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H, C(C*H*$_3$)$_3$), 1.95 (qu, 2H, C*H*$_2$), 3.15 (q, 2H, C*H*$_2$N), 3.30 (t, 2H, C*H*$_2$Br), 5.00 (t, 1H, N*H*·CO); IR (Nujol) υ_{max} 3350, 1680 cm^{-1}.

6.8.2. 3,5-Di-[3-(*tert*-butoxycarbonylamino)-propoxy]-benzyl alcohol (11b). A solution of KOBu (25 g, 0.22 mol) in DMF (100 mL) was added to 3,5-dihydroxybenzyl alcohol (14 g, 0.1 mol) in DMF (50 mL) at 20–45 °C. The resulting suspension was treated with **11a** (50 g, 0.21 mol) to give a solution, which was diluted with water (300 mL) and extracted with EtOAc (3× 150 mL). The combined extracts were washed with 1 M aq NaOH (100 mL) then water (100 mL) and concentrated under vacuum to give the product as a cream powder, precipitated from diisopropylether (30.2 g, 71% yield); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 18H, 2C(C*H*$_3$)$_3$), 1.95 (qu, 4H, 2C*H*$_2$), 3.30 (q, 4H, 2C*H*$_2$N), 4.00 (t, 4H, 2C*H*$_2$O), 4.60 (s, 2H, C*H*$_2$O), 4.75 (br s, 2H, 2N*H*·CO), 6.35 (s, 1H, Ar–*H*), 6.50 (s, 2H, 2Ar–*H*); IR (Nujol) υ_{max} 3380, 1690 cm^{-1}.

6.8.3. 3,5-Di-[3-(*tert*-butoxycarbonylamino)-propoxy]-benzyl chloride (11c). A mixture of **11b** (11.3 g, 25 mmol), NEt_3 (3.3 g, 32 mmol) and TsCl (6.0 g, 31 mmol) in $CHCl_3$ (100 mL) was stirred at 20 °C for 16 h. The solvent was then replaced with EtOAc by distillation to precipitate NEt_3·HCl, which was filtered off and the filtrate concentrated. The residue was absorbed onto silica gel and chromatographed with EtOAc to give the product as a yellow oil, which was crystallised from diisopropylether (5.0 g, 42% yield): mp 129–131 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 18H, 2C(C*H*$_3$)$_3$), 1.95 (qu, 4H, 2C*H*$_2$), 3.30 (q, 4H, 2C*H*$_2$N), 4.00 (t, 4H, 2C*H*$_2$O), 4.50 (s, 2H, C*H*$_2$Cl), 4.75 (br s, 2H, N*H*·CO), 6.40 (s, 1H, Ar–*H*), 6.50 (s, 2H, Ar–*H*); IR (Nujol) υ_{max} 3380, 1690 cm^{-1}.

6.8.4. *N*-[3-(4-Quinolinylamino)-propyl]-3,5-di-[3-(*tert*-butoxycarbonylamino)-propoxy]-benzylamine (11d). A solution of **9b** (4.0 g, 20 mmol) and **11c** (2.35 g , 5 mmol) in DMF (20 mL) was stirred at 20 °C for 16 h, before diluting with water (80 mL) and extracting with EtOAc (160 mL). The extracts were combined, washed with water and concentrated under vacuum. The resulting residue was absorbed onto silica gel and chromatographed with CMA to give the product as a yellow oil (2.6 g, 83% yield); ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 18H, 2C(C*H*$_3$)$_3$), 1.80 (qu, 2H, C*H*$_2$), 1.85 (qu, 2H, C*H*$_2$), 2.80 (t, 2H, C*H*$_2$), 3.20 (q, 4H, 2C*H*$_2$N), 3.30 (t, 4H, 2C*H*$_2$N), 3.70 (s, 2H, C*H*$_2$N), 3.80 (t, 4H, 2C*H*$_2$O), 4.80 (br s, 2H, N*H*·CO), 6.25 (d, 1H, Ar–*H*), 6.30 (s, 1H, Ar–*H*), 6.40 (s, 2H, Ar–*H*), 7.25 (m, 2H, Ar–*H*), 7.60 (m, 1H, Ar–*H*), 7.90 (d, 1H, Ar–*H*), 8.45 (d, 1H, Ar–*H*); IR (film) υ_{max} 3280, 1700 cm^{-1}; MS (ES^+) 638 (M^++1), fragment at 356.

6.8.5. *N*-[3-(4-Quinolinylamino)-propyl]-3,5-di-[3-(4-quinolinylamino)-propoxy]-benzylamine dihydrate (11). A solution of **11d** (1.9 g, 3.0 mmol) in trifluoroacetic acid (20 mL) was stirred at 20 °C for 1 h. The excess acid was then removed by distillation and the resulting oil dissolved in MeOH. 25% w/w NaOMe in MeOH was added to pH 10 and the solution concentrated to low volume before diluting with *n*-pentanol (20 mL). N^{i}Pr$_2$Et (2.0 g, 15 mmol) and 4-chloroquinoline (2.0 g, 12 mmol) were added and the solution heated under reflux for 4 h, before concentrating, diluting with 1 M aq NaOH (50 mL) and extracting with CH_2Cl_2/MeOH (10:1, 2× 50 mL). The combined extracts were concentrated and the residue absorbed onto silica gel and chromatographed with CMA. The resulting oil was dissolved in CH_2Cl_2, filtered to clarify, then the solvent removed by distillation and the residue triturated with EtOAc to give the product as a pale yellow foam (0.2 g, 10% yield); ^{1}H NMR ($CDCl_3$) δ 1.90 (m, 2H, C*H*$_2$), 2.05 (m, 2H, C*H*$_2$), 2.85 (m, 2H, C*H*$_2$), 3.35 (m, 4H, 2C*H*$_2$N), 3.45 (m, 4H, 2C*H*$_2$N), 3.80 (s, 2H, ArC*H*$_2$N), 4.90 (t, 4H, 2C*H*$_2$O), 6.40 (m, 3H, 3Ar–*H*), 6.50 (s, 2H, 2Ar–*H*), 7.30 (m, 3H, 3Ar–*H*), 7.50 (m, 3H, 3Ar–*H*), 7.75 (m, 3H, 3Ar–*H*), 7.90 (d, 1H, Ar–*H*) 7.95 (m, 3H, 3Ar–*H*), 8.55 (m, 3H, 3Ar–*H*); IR (Nujol) υ_{max} 3250, 1710 cm^{-1}; MS (ES^+) 692 (M^++1). Anal. Calcd for $C_{43}H_{45}N_7O_2{\cdot}2.5H_2O$: C, 70.00; H, 6.78; N, 13.29. Found: C, 70.49; H, 6.77; N, 12.88.

6.9. *N*-[3-(4-Quinolinylamino)-propyl]-3,5-di-[3-(4-quinolinylamino)-propoxy]-aniline dihydrate (12)

6.9.1. *N*-(3,5-Dihydroxyphenyl)-*N*′-(*t*-butoxycarbonyl)-1,3-diaminopropane (12a) and 3,5-di-[3′-(*tert*-butoxycarbonylamino)-propyl]-aminophenol (12b). A solution of phloroglucinol (63 g, 0.50 mol) and **5a** (100 g, 0.58 mol) in THF was heated under reflux in (500 mL) for 0.5 h. Toluene (800 mL) was then added and the solvent removed under vacuum. The residue was absorbed onto silica gel and chromatographed with EtOAc/CH_2Cl_2 (1:1) to give an oil, which was stirred in EtOAc (500 mL) to precipitate **12b** as a dark green powder (28 g, 13% yield). The mother liquors were concentrated under vacuum then absorbed onto silica gel and chromatographed with Et_2O to give **12a** as a brown gum (80 g, 56% yield); ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 9H, C(C*H*$_3$)$_3$), 1.80 (m, 2H, C*H*$_2$), 3.00 (m, 2H, C*H*$_2$N), 3.10 (m, 2H, C*H*$_2$N), 5.30 (t, 1H, N*H*), 5.50 (m, 3H, 3Ar–*H*), 6.80

(t, 1H, N*H*·CO), 8.70 (br s, 2H, 2OH); IR (film) υ_{max} 3400, 1690, 1630 cm^{-1}.

6.9.2. *N*-[(*tert*-Butoxycarbonylamino)-propyl]-3,5-di-[(*tert*-butoxycarbonylamino)-propoxy]-aniline (12c). A solution of **12a** (5.7 g, 20 mmol) in DMF (50 mL) was treated sequentially with KOBu (5.3 g, 46 mmol) and **11a** (9.5 g, 40 mmol). After 1 h the solution was diluted with water (200 mL) and extracted with CH_2Cl_2 (2× 150 mL). The combined extracts were washed with 1 M aq NaOH and concentrated to give a brown oil. This was absorbed onto silica gel and chromatographed with Et_2O to give the product as cream crystals (2.3 g, 20% yield): mp 125–127 °C; ^1H NMR ($CDCl_3$) δ 1.40 (s, 27H, 3C(CH_3)$_3$), 1.80 (m, 2H, CH_2), 1.95 (m, 4H, 2CH_2), 3.10 (m, 2H, CH_2N), 3.20 (m, 2H, CH_2N), 3.30 (q, 4H, 2CH_2N), 3.95 (m, 4H, 2CH_2O), 4.80 (t, 1H, N*H*·CO), 4.85 (t, 2H, 2N*H*·CO), 5.80 (m, 2H, 2Ar–*H*), 5.85 (m, 1H, Ar–*H*); IR (Nujol) υ_{max} 3380, 1700, 1620 cm^{-1}; MS (ES^+) 597 (M^++1).

6.9.3. *N*-[3-(4-Quinolinylamino)-propyl]-3,5-di-[3-(4-quinolinylamino)-propoxy]-aniline dihydrate (12). A solution of **12c** (2.0 g, 3.3 mmol) in trifluoroacetic acid (30 mL) was stirred at ambient temperature for 1 h, then concentrated under vacuum, diluted with MeOH, and 25% w/w NaOMe in MeOH added until pH 12. This solution was concentrated to give a green oil, which was dissolved in *n*-pentanol (30 mL) and the solution heated with 4-chloroquinoline (2.2 g, 13 mmol) plus NPr_2Et (2.2 g, 16.5 mmol) for 4 h. The reaction mixture was then concentrated, diluted with 1 M aq NaOH (100 mL) and extracted with CH_2Cl_2/MeOH (10:1, 2× 100 mL). The combined extracts were washed with 1 M aq NaOH (50 mL) and concentrated. The residue was absorbed onto silica gel and chromatographed with CMA to give the product as a clear glass, precipitated from Et_2O (0.5 g, 23% yield): mp 102–107 °C; ^1H NMR (DMSO-d_6) δ 1.90 (qu, 2H, CH_2), 2.10 (qu, 4H, 2CH_2), 3.20 (t, 2H, CH_2N), 3.45 (t, 2H, CH_2N), 3.50 (t, 4H, 2CH_2N), 3.95 (t, 4H, 2CH_2O), 5.70 (t, 1H, N*H*), 5.80 (m, 2H, Ar–*H*), 5.85 (m, 1H, Ar–*H*), 6.45 (m, 3H, 3Ar–*H*), 7.20 (m, 3H, 3N*H*), 7.40 (m, 3H, 3Ar–*H*), 7.60 (m, 3H, 3Ar–*H*), 7.75 (m, 3H, 3Ar–*H*), 8.25 (d, 3H, 3Ar–*H*), 8.40 (m, 3H, 3Ar–*H*); HPLC (IB-SIL Phenyl-BDS, MeOH/0.05M aq NH_4OAc, 90:10, 215 nm, 1.0 mL/min), product at 2.6 min, impurity at 1.8 min; MS (EI^+) 678 (M^++1). Anal. Calcd for $C_{42}H_{43}N_7O_2{\cdot}2H_2O$: C, 70.58; H, 6.58; N, 13.72. Found: C, 70.30; H, 6.50; N, 13.38.

6.10. *N*-(4-Quinolinyl)-3,4-di-[3-(4-quinolinylamino)-propoxy]-phenethylamine dihydrate (13)

6.10.1. *N*-(*tert*-Butoxycarbonyl)-dopamine (13a). A solution of dopamine·HCl (38.0 g, 0.20 mol) and di-*tert*-butyl dicarbonate (48.0 g, 0.22 mol) in MeOH (200 mL) was treated with 25% w/w NaOMe in MeOH (50 mL, 0.23 mol) at 25 °C. After 0.5 h, the reaction mixture was concentrated to low volume, before diluting with EtOAc (800 mL). The precipitated inorganic salts were filtered off and the filtrate concentrated to give an oil, which was stirred in diisopropylether to precipitate the product as a grey powder (32.9 g, 65% yield): mp 132–134 °C; ^1H NMR (DMSO-d_6) δ 1.35 (s, 9H, C(CH_3)$_3$), 2.45 (t, 2H, ArCH_2) 3.10 (q, 2H, CH_2N), 6.35 (m, 1H, Ar–H), 6.60 (m, 1H, Ar–H), 6.65 (d, 1H, Ar–H), 6.80 (t, 1H, NH·CO), 8.80 (br s, 2H, 2OH). MS (EI^+) 253 (M^++1); IR (Nujol) υ_{max} 3500, 3380, 1680 cm^{-1}.

6.10.2. *N*-(*tert*-Butoxycarbonyl)-3,4-di-[3-(*tert*-butoxycarbonylamino)-propoxy]-phenethyl-amine (13b). A solution of **13a** (12.6 g, 50 mmol) in DMF (200 mL) was treated with KOBu (13.0 g, 115 mmol) and **11a** (25.0 g, 105 mmol) at 20 °C. After 1 h, the reaction mixture was diluted with water (800 mL) and extracted with CH_2Cl_2 (600 mL). The extract was washed with 1 M aq NaOH, then concentrated. The residue was absorbed onto silica gel and chromatographed with Et_2O to give the product as an off-white powder, precipitated from diisopropylether (8.5 g, 33% yield): mp 91–93 °C; ^1H NMR ($CDCl_3$) δ 1.40 (s, 18H, 2C(CH_3)$_3$), 2.00 (m, 4H, 2CH_2), 2.70 (t, 2H, CH_2Ar), 3.35 (m, 6H, 3CH_2N), 4.00 (m, 4H, 2CH_2O), 4.60 (br s, 1H, N*H*·CO), 5.30 (br s, 2H, N*H*·CO), 6.70 (m, 2H, 2Ar–*H*), 6.80 (d, 1H, Ar–*H*); IR (Nujol) υ_{max} 3350, 1680 cm^{-1}; MS (TS^+) 568 (M^++1).

6.10.3. *N*-(4-Quinolinylamino)-3,4-di-[3-(4-quinolinylamino)-propoxy]-phenethylamine dihydrate (13). A solution of **13b** (5.7 g, 10 mmol) in trifluoroacetic acid (30 mL) was stirred at 20 °C for 1 h, then concentrated under vacuum to give a yellow oil. This was dissolved in MeOH (50 mL) and 25% w/w NaOMe in MeOH added until pH 10. The MeOH was then removed by distillation and the residue diluted with *n*-pentanol (50 mL). 4-Chloroquinoline (3.3 g, 40 mmol) and N^iPr_2Et (3.0g, 40 mmol) were added and the reaction mixture heated under reflux for 7 h, before concentrating under vacuum. The residue was absorbed onto silica gel and chromatographed with CMA to give a gum, which was triturated with Et_2O to precipitate the product as a clear glass (30 mg, 5% yield); ^1H NMR (CD_3OD) δ 2.05 (m, 2H, CH_2), 2.15 (m, 2H, CH_2), 2.90 (t, 2H, CH_2Ar), 3.45 (m, 2H, CH_2N), 3.55 (m, 4H, 2CH_2N), 4.00 (t, 2H, CH_2O), 4.20 (t, 2H, CH_2O), 6.45 (m, 3H, 3Ar–*H*), 6.85 (m, 2H, 2Ar–*H*), 6.95 (d, 1H, Ar–*H*), 7.35 (m, 3H, 3Ar–*H*), 7.55 (m, 3H, 3Ar–*H*), 7.75 (m, 3H, 3Ar–*H*), 8.00 (m, 3H, 3Ar–*H*), 8.20 (d, 2H, 2Ar–*H*), 8.30 (d, 1H, Ar–*H*); IR (Nujol) υ_{max} 3250, 1700 cm^{-1}; HPLC (Partisil ODS3, MeOH/0.05 M aq NH_4OAc (pH 4) 90:10, 1.0 mL/min, 215 nm), product at 5.6 min, impurity at 4.1 min: MS (ES^+) 649 (M^++1). Anal. Calcd for $C_{41}H_{40}N_6O_2{\cdot}2H_2O$: C, 71.82; H, 6.42; N, 12.26. Found: C, 71.82; H, 6.17; N, 11.88.

6.11. 1,2,3-Tri-[3-(4-Quinolinylamino)-propoxy]-benzene hydrate (14)

6.11.1. 1,2,3-Tri-[3-(*tert*-butoxycarbonylamino)-propoxy]-benzene (14a). A solution of pyrogallol (3.2 g, 25 mmol) in DMF (20 mL) was treated sequentially at 40 °C with KOBu (9.3 g, 82 mmol) in DMF (50 mL) and **11a** (18.0 g, 75 mmol). The reaction mixture was then cooled to 20 °C and diluted with water (200 mL) and extracted with EtOAc (200 mL). The combined extracts were washed with 1 M aq NaOH (100 mL) then concentrated under vacuum to give an oil, which was absorbed onto

silica gel and chromatographed with diisopropyl ether/Et_2O, 1:1 to give the product, crystallised from Et_2O (3.0 g, 20% yield); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 27H, 3C(CH_3)$_3$), 1.90 (qu, 2H, CH_2), 2.00 (qu, 4H, 2CH_2), 3.30 (m, 4H, 2CH_2N), 3.40 (m, 2H, CH_2N), 4.05 (q, 6H, 3CH_2O), 5.15 (br s, 2H, N*H*·CO), 5.45 (br s, 1H, N*H*·CO), 6.55 (d, 2H, 2Ar–*H*), 6.95 (t, 1H, Ar–*H*); IR (Nujol) υ_{max} 3280, 1700 cm^{-1}; MS (ES$^+$) 598 (M$^+$+H).

6.11.2. 1,2,3-Tri-[3-(4-quinolinylamino)-propoxy]-benzene hydrate (14). A solution of **14a** (2.4 g, 4 mmol) in trifluoroacetic acid (20 mL) was stirred at 20 °C for 1 h, before concentrating under vacuum. The resulting oil was diluted with MeOH (50 mL) before basifying to pH 10 with 25% w/w NaOMe in MeOH. The solvent was then replaced with *n*-pentanol (80 mL) before adding 4-chloroquinoline (2.6 g, 16 mmol) and N^{i}Pr$_2$Et (2.6 g, 20 mmol). The solution was then heated under reflux for 6 h, concentrated, diluted with 1 M aq NaOH (100 mL) and extracted with CH_2Cl_2/MeOH (20:1; 2× 100 mL). The extracts were combined, concentrated under vacuum, and the residue absorbed onto silica gel and chromatographed with CMA to give an oil, which was recrystallised from EtOAc to give the product as a white powder (200 mg, 8% yield); ^{1}H NMR ($CDCl_3$) δ 2.15 (m, 6H, 3CH_2), 3.45 (m, 6H, 3CH_2N), 4.15 (t, 4H, 2CH_2O), 4.25 (t, 2H, CH_2O), 5.25 (t, 2H, 2NH), 6.20 (t, 1H, NH), 6.30 (m, 3H, 3Ar–*H*), 6.65 (d, 2H, 2Ar–*H*), 7.05 (t, 1H, Ar–*H*), 7.35 (m, 3H, 3Ar–*H*), 7.55 (m, 3H, 3Ar–*H*), 7.70 (m, 3H, 3Ar–*H*), 8.00 (m, 3H, 3Ar–*H*), 8.50 (m, 3H, 3Ar–*H*); MS (ES$^+$) 679 (M$^+$+H). Anal. Calcd for $C_{42}H_{42}N_6O_3$·1.25H_2O·0.2EtOAc: C, 71.50; H, 6.46; N, 11.69. Found: C, 71.49; H, 6.24; N, 11.68.

6.12. 1,2,3-Tri-[3-(4-(*N*-ethyl)-quinoliniumylamino)-propoxy]-benzene triiodide (15)

A solution of **14a** (10 g, 16.7 mmol) in trifluoroacetic acid (40 mL) was stirred at 20 °C for 1 h, before concentrating under vacuum to low volume, diluting with MeOH (50 mL) and basifying to pH 10 with 25% w/w NaOMe in MeOH. The solvent was then replaced with *n*-pentanol by distillation before adding 4-chloroquinoline (11.0 g, 67.5 mmol) and N^{i}Pr$_2$Et (10.8 g, 83.5 mmol). The solution was heated under reflux for 6 h, before concentrating under vacuum, diluting with 1 M aq NaOH (150 mL) and extracting with CH_2Cl_2/MeOH (10:1, 2× 100 mL). The combined extracts were concentrated and the residue absorbed onto silica gel and chromatographed with CMA. The crude product **14** was diluted with EtI (25 mL) and the suspension heated to reflux. DMF (2 mL) was added to the resulting oily suspension to give a clear solution. Reflux was then maintained for a further 10 h, adding more DMF, in 1 mL portions (total 10 mL), to redissolve the liberated oil, throughout the reflux period. The solution was then cooled before diluting with water (100 mL) to precipitate a gum. The supernatant liquor was removed, after which more water (100 mL) was added and the supernatant removed. The remaining residue was stirred in MeOH (80 mL) at 60 °C, filtered to remove insoluble material and the filtrate cooled to precipitate a red gum, which was further purified by reprecipitation from EtOH to give the product as a yellow powder (80 mg, 6% yield); ^{1}H NMR (DMSO-d_6) δ 1.40 (t, 9H, 3CH_3), 2.10 (qu, 6H, 3CH_2), 3.75 (q, 6H, 3CH_2N), 4.15 (t, 6H, 3CH_2O), 4.60 (q, 6H, 3CH_2N$^+$) 6.70 (d, 2H, 2Ar–*H*), 6.90 (m, 3H, 3Ar–*H*), 7.05 (t, 1H, Ar–H), 7.70 (m, 3H, 3Ar–*H*), 8.10 (m, 3H, 3Ar–*H*), 8.50 (m, 3H, 3Ar–*H*), 8.65 (m, 3H, 3r-*H*), 9.15 (m, 3H, 3Ar–*H*); MS (ES$^+$) 763 (M$^+$−2). Anal. Calcd for $[C_{48}H_{57}N_6O_3]^{3+}$·3I$^-$. 4$H_2O$: C, 47.29; H, 5.37; N, 6.89. Found: C, 47.12; H, 5.02; N, 6.86.

6.13. *N*-[3-(4-Quinolinylamino)-propyl]-*N*-[5-(4-quinolinylamino)-pentyl]-3,5-di- [3-(4-quinolinylamino)-propoxy]-benzylamine hydrate (16)

6.13.1. 5-(*tert*-Butoxycarbonylamino)-pentylamine (16a). A solution of di *tert*-butyl dicarbonate (60 g, 275 mmol) in MeOH (200 mL) was added to 1,5-diaminopentane (100 g, 1.0 mol) in MeOH (100 mL) at 20 °C. The resulting suspension was concentrated to 50% volume by distillation, then diluted with 1 M aq NaOH (1200 mL), filtered to remove di-(*t*-Boc)-1,5-diaminopentane and extracted with *t*-butyl methyl ether (5× 500 mL). The extracts were combined and concentrated under vacuum to give the product as a red oil (35 g, 64% yield); ^{1}H NMR ($CDCl_3$) δ 1.2 (s, 2H, NH_2), 1.30 (s, 9H, C(CH_3)$_3$), 1.60 (qu, 2H, CH_2), 1.95 (qu, 4H, 2CH_2), 2.40 (m, 4H, 2CH_2N), 6.0 (br t, 1H, N*H*·CO).

6.13.2. *N*-[(*tert*-Butoxycarbonylamino)-propyl]-*N*′-5-(*tert*-butoxycarbonylamino)-1,5-diaminopentane (16b). A solution of **11a** (35.6 g, 150 mmol) and **16a** (30.3 g, 150 mmol) in DMF (200 mL) was stirred with K_2CO_3 (27 g, 450 mmol) at 20 °C for 16 h, then diluted with water (600 mL) and extracted with *tert*-butyl methyl ether (2× 1 L). The extracts were combined and stirred with water (600 mL), adding in portions approximately 80% of the theoretical amount of concd HCl needed to neutralise the amine function in **16b** until tlc monitoring showed that the product had been extracted into the aqueous phase. This was separated off, basified to pH 14 with 40% w/w aq NaOH and extracted with *tert*-butyl methyl ether (2× 600 mL). The extracts were combined and concentrated under vacuum to give the product as an orange powder (20 g, 39% yield); ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 18H, 2C(CH_3)$_3$), 1.60 (qu, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.95 (qu, 4H, 2CH_2), 2.7 (m, 2H, CH_2N), 3.15 (m, 2H, CH_2N), 3.35 (q, 4H, 2CH_2N), 5.00 (br s, 1H, N*H*·CO), 5.15 (br s, 1H, N*H*·CO).

6.13.3. *N*-[(*tert*-Butoxycarbonylamino)-propyl]-*N*-[5-(*tert*-butoxycarbonylamino)-pentyl]-3,5,-di-[(*tert*-butoxycarbonylamino)-propoxy]-benzylamine (16c). A solution of **11c** (3.3 g, 7.0 mmol) and **16b** (2.7 g, 7.7 mmol) in DMF (30 mL) was heated with K_2CO_3 (1.3 g, 9.5 mmol) at 80 °C for 3 h. The mixture was then cooled, diluted with water (100 mL) and extracted with EtOAc (2× 100 mL). The extracts were combined, washed with water (100 mL) and concentrated under vacuum to give an oil, which was absorbed onto silica gel and chromatographed with EtOAc to give the product as a pale yellow oil (5.6 g, 100% yield); ^{1}H NMR ($CDCl_3$) δ 1.4 (s,

36H, 4C(CH_3)$_3$), 1.45 (m, 2H, CH_2), 1.60 (qu, 2H, CH_2), 1.75 (m, 4H, 2CH_2), 1.95 (qu, 4H, 2CH_2), 2.40 (m, 4H, 2CH_2N), 3.15 (m, 2H, CH_2N), 3.35 (q, 4H, 2CH_2N), 3.45 (s, 4H, 2CH_2N), 4.00 (t, 4H, 2CH_2O), 4.75 (t, 4H, 2N*H*·CO), 5.00 (br s, 1H, N*H*·CO), 5.15 (br s, 1H, N*H*·CO), 6.30 (s, 1H, Ar–*H*), 6.50 (s, 2H, 2Ar–*H*); MS (ES^+) 796 (M^++1).

6.13.4. *N*-[3-(4-Quinolinylamino)-propyl]-*N*-[5-(4-quinolinylamino)-pentyl]-3,5-di-[3-(4-quinolinylamino)-propoxy]-benzylamine hydrate (16). Compound **16c** (4.8 g, 6.0 mmol) was stirred in trifluoroacetic acid (40 mL) at 20 °C for 1 h, then concentrated under vacuum to low volume. The resulting oil was dissolved in MeOH (50 mL) and 25% w/w NaOMe in MeOH added until pH 10, before replacing the solvent with *n*-pentanol by distillation. 4-Chloroquinoline (4.9 g, 30 mmol) and N^{i}Pr$_2$Et (4.5 g, 35 mmol) were added and the solution heated under reflux for 8 h, before concentrating under vacuum to low volume, diluting with 1M aq NaOH (100 mL) and extracting with CH_2Cl_2(2× 100 mL). The extracts were combined and concentrated to give an oil, which was absorbed onto silica gel and chromatographed with CMA. The resulting crude product was reprecipitated twice from EtOAc, filtering the hot supernatant each time, to give a cream foam (100 mg, 2% yield); ^{1}H NMR ($CDCl_3$) δ 1.45 (m, 2H, CH_2), 1.65 (m, 4H, 2CH_2), 1.85 (m, 2H, CH_2), 2.00 (m, 4H, 2CH_2), 2.60 (m, 4H, 2CH_2N), 3.25 (m, 2H, CH_2N), 3.35 (m, 4H, 2CH_2N), 3.55 (m, 4H, 2CH_2N), 3.80 (m, 4H, 2CH_2O), 5.10 (t, 1H, N*H*), 5.65 (t, 2H, N*H*), 6.25 (t, 1H, Ar–*H*), 6.35 (m, 4H, 4Ar–*H*), 6.45 (s, 2H, 2Ar–*H*), 6.85 (t, 1H, N*H*), 7.35 (m, 4H, 4Ar–*H*), 7.55 (m, 4H, 4Ar–*H*), 7.75 (m, 4H, 4Ar–*H*), 7.95 (m, 4H, 4Ar–*H*), 8.50 (m, 4H, 4Ar–*H*); TLC CMA/silica gel, r.f 0.3; MS (ES^+) 905 (M^++1). Anal. Calcd for $C_{57}H_{61}N_9O_2$·1.75H_2O·0.66EtOAc: C, 72.08; H, 7.08; N, 12.68. Found: C, 71.98; H, 6.91; N, 12.69.

6.14. *N*-[3-(4-Quinolinylamino)-propyl]-*N*-[5-(4-quinolinylamino)-pentyl]-3,5-di-[3-(4-quinolinylamino)-propoxy]-aniline trihydrate (17)

6.14.1. *N*-[3-(*tert*-Butoxycarbonylamino)-propyl]-*N*′-[5-(*tert*-butoxycarbonylamino)-pentyl]-3,5-dihydroxyaniline (17a). Phloroglucinol (4.4 g, 35 mmol) and **16b** (12.0 g, 35 mmol) were heated in a mixture of THF (30 mL) and toluene (50 mL) under reflux for 2 h. The solution was then concentrated under vacuum to give an oil, which was absorbed onto silica gel and chromatographed with EtOAc, to give the product as an orange gum (10 g, 61% yield); ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 18H, 2C(CH_3)$_3$), 1.45 (qu, 2H, CH_2), 1.65 (qu, 4H, 2CH_2), 1.75 (m, 2H, CH_2), 2.90 (m, 4H, 2CH_2N), 3.15 (m, 4H, 2CH_2N), 5.55 (s, 1H, Ar–*H*), 5.65 (s, 2H, Ar–*H*), 6.68 (t, 1H, N*H*·CO), 6.85 (t, 1H, N*H*·CO), 8.80 (s, 2H, 2O*H*).

6.14.2. *N*-[(*tert*-Butoxycarbonylamino)-propyl]-*N*-[5-(*tert*-butoxycarbonylamino)-pentyl]-3,5-[3-(*tert*-butoxycarbonylamino)-propoxy]-aniline (17b). Compound **17a** (9.4 g, 20 mmol) was sequentially treated in DMF (100 mL) with KOBu (4.9 g, 44 mmol) at 30 °C and 11a (10.0 g, 42 mmol) at 60 °C. The solution was then cooled to 20 °C, diluted with water (400 mL) and extracted with EtOAc (3× 300 mL). The combined extracts were washed with 1 M aq NaOH (200 mL) and concentrated under vacuum. The residue was absorbed onto silica gel and chromatographed with Et_2O to give the product as a brown oil (7.0 g, 45%); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 38H, 4C(CH_3)$_3$ + CH_2), 1.65 (m, 4H, 2CH_2), 1.75 (m, 2H, CH_2), 1.95 (m, 4H, 2CH_2), 3.15 (m, 4H, 2CH_2N), 3.35 (m, 8H, 4CH_2N), 4.00 (m, 4H, 2CH_2O), 4.75 (br s, 1H, N*H*·CO), 4.85 (br s, 2H, N*H*·CO), 4.95 (br s, 1H, N*H*·CO), 5.85 (s, 2H, 2Ar–*H*), 6.05 (s, 1H, Ar–*H*); IR (film) ν_{max} 3380, 1700, 1620 cm^{-1}.

6.14.3. *N*-[3-(4-Quinolinylamino)-propyl]-*N*-[5-(4-quinolinylamino)-pentyl]-3,5-di-[3-(4-quinolinylamino)-propoxy]-aniline trihydrate (17). Compound **17b** (5.9 g, 7.5 mmol) was stirred in trifluoroacetic acid (30 mL) at 20 °C for 1 h, then concentrated under vacuum. The resulting oil was dissolved in MeOH (100 mL), 25% w/w NaOMe in MeOH added until pH 10, before replacing the solvent with *n*-pentanol by distillation. 4-Chloroquinoline (7.3 g, 45 mmol) and N^{i}Pr$_2$Et (6.8 g, 53 mmol) were added and the solution heated under reflux for 6 h, before concentrating to low volume, diluting with water (150 mL) and extracting with CH_2Cl_2/MeOH (20:1, 2× 100 mL). The extracts were combined, concentrated, and the residue absorbed onto silica gel and chromatographed with CMA. The resulting crude product was purified by precipitation twice from EtOAc, filtering the hot supernatant each time, to give a fawn foam (200 mg, 3%); ^{1}H NMR ($CDCl_3$) δ 1.35 (m, 2H, CH_2), 1.65 (m, 4H, 2CH_2), 2.10 (m, 6H, CH_2), 3.3–3.5 (m, 12H, 6CH_2N), 3.95 (t, 4H, 2CH_2O), 5.90 (s, 3H, 3Ar–*H*), 6.40 (m, 4H, 4Ar–*H*), 7.35 (m, 4H, 4Ar–*H*), 7.60 (m, 4H, 4Ar–*H*), 7.75 (m, 4H, 4Ar–*H*), 7.95 (m, 4H, 4Ar–*H*), 8.55 (m, 4H, 4Ar–*H*); MS (EI^+) 890 (M^++1). Anal. Calcd for $C_{56}H_{59}N_9O_2$·3.5H_2O·0.5EtOAc: C, 68.98; H, 7.07; N, 12.63. Found: C, 68.99; H, 6.77; N, 12.50.

6.15. Biological assay

Intracellular recordings were made, using microelectrodes filled with 1 M KCl, from rat sympathetic neurones incubated in tissue culture for 6–10 days. Action potentials were invoked by injection of depolarizing current pulses, and test compounds were applied in the external solution at 2–4 concentrations, on at least three neurones.

Equieffective molar concentration ratios (EMR with respect to dequalinium) were determined by simultaneous non-linear weighted least squares fitting (using Origin (versions 4–7), OriginLab, Northampton, Massachusetts, USA) of the data obtained with each compound, taken together with the values observed with dequalinium in that set of assays. The Hill equation was employed to fit the data: a common Hill coefficient and maximum inhibition were assumed for each assay; IC_{50} values and the concentration required to achieve 50% inhibition of the AHP were calculated.

Acknowledgments

This work was partially supported by the Wellcome Trust which provided a fellowship for P.M.D. D.F. is grateful to Wyeth Research, UK, for supporting his research and providing facilities for chemical synthesis, spectra, and chemical analysis. Thanks are also due to Dr. K. Heatherington for help with obtaining and interpreting the analytical and spectroscopic data. We also thank Vicky Taylor (née Richardson) for building the CPK model of apamin and Dr. J. G. Vinter for help with molecular modelling.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.05.054.

References and notes

1. Haylett, D. G.; Jenkinson, D. H. Calcium-activated potassium channels. In *Potassium Channels*; Cook, N. S., Ed.; Ellis Horwood: Chichester, 1990; pp 70–95.
2. Vergara, C.; Latorre, R.; Marrion, N. V.; Adelman, J. P. *Curr. Opin. Neurobiol.* **1998**, *8*, 321.
3. Castle, N. A. *Perspect. Drug Discovery Des.* **1999**, *16*, 131.
4. Stocker, M. *Nature Rev. Neurosci.* **2004**, *5*, 758.
5. Liégeois, J.-F.; Mercier, F.; Graulich, A.; Graulich-Lorge, F.; Scuvée-Moreau, J.; Seutin, V. *Curr. Med. Chem.* **2003**, *10*, 625.
6. Lawson, K.; McKay, N. G. *Curr. Pharm. Des.* **2006**, *12*, 459.
7. Tamarina, N. A.; Wang, Y.; Mariotto, L.; Kuznetsov, A.; Bond, C.; Adelman, J.; Philipson, L. H. *Diabetes* **2003**, *52*, 2000.
8. Deschaux, O.; Bizot, J. C. *Neurosci. Lett.* **2005**, *386*, 5.
9. Hammond, R. S.; Bond, C. T.; Strassmaier, T.; Ngo-Ahn, T. J.; Adelman, J. P.; Maylie, J.; Stackman, R. W. *J. Neurosci.* **2006**, *26*, 1844.
10. Kohler, M.; Hirschberg, B.; Bond, C. T.; Kinzie, J. M.; Marrion, N. V.; Maylie, J.; Adelman, J. P. *Science* **1996**, *273*, 1709.
11. Hosseini, R.; Benton, D. C. H.; Dunn, P. M.; Jenkinson, D. H.; Moss, G. W. J. *J. Physiol.* **2001**, *535*, 323.
12. Obermair, G. J.; Kaufmann, W. A.; Knaus, H. G.; Flucher, B. E. *Eur. J. Neurosci.* **2003**, *17*, 721.
13. Banks, B. E. C.; Brown, C.; Burgess, G. M.; Burnstock, G.; Claret, M.; Cocks, T. M.; Jenkinson, D. H. *Nature* **1979**, *282*, 415.
14. Blatz, A. L.; Magleby, K. L. *Nature* **1986**, *323*, 718.
15. Auguste, P.; Hugues, M.; Grave, B.; Gesquiere, J. C.; Maes, P.; Tartar, A.; Romey, G.; Schweitz, H.; Lazdunski, M. *J. Biol. Chem.* **1990**, *265*, 4753.
16. Zerrouk, H.; Mansuelle, P.; Benslimane, A.; Rochat, H.; Martin-Eauclaire, M. F. *FEBS Lett.* **1993**, *320*, 189.
17. Jenkinson, D. H.; Haylett, D. G.; Cook, N. S. *Cell Calcium* **1983**, *4*, 429.
18. Nohmi, M.; Kuba, K. *Brain Res.* **1984**, *301*, 146.
19. Cook, N. S.; Haylett, D. G. *J. Physiol.* **1985**, *358*, 373.
20. Castle, N. A.; Haylett, D. G.; Morgan, J. M.; Jenkinson, D. H. *Eur. J. Pharmacol.* **1993**, *236*, 201.
21. Galanakis, D.; Davis, C. A.; Del Rey Herrero, B.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **1995**, *38*, 595.
22. Galanakis, D.; Davis, C. A.; Ganellin, C. R.; Dunn, P. M. *J. Med. Chem.* **1996**, *39*, 359.
23. Campos, Rosa J.; Galanakis, D.; Piergentili, A.; Bhandari, K.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **2000**, *43*, 420.
24. Chen, J.Q. Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **2000**, *43*, 3478.
25. (a) U.S. Patent 5,866,562, 1999; (b) U.S. Patent 5,874,438, 1999.
26. Graulich, A.; Scuvée-Moreau, J.; Alleva, L.; Lamy, C.; Waroux, O.; Seutin, V.; Liégeois, J.-F. *J. Med. Chem.* **2006**, *49*, 7208.
27. Galanakis, D.; Ganellin, C. R.; Chen, J. Q.; Gunasekera, D.; Dunn, P. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4231.
28. Vincent, J. P.; Schweitz, H.; Lazdunski, M. *Biochemistry* **1975**, *14*, 2521.
29. Labbe-Jullie, C.; Granier, C.; Albericio, F.; Defendini, M.-L.; Ceard, B.; Rochat, H.; Van Rietschoten, J. *Eur. J. Biochem.* **1991**, *196*, 639.
30. Sabatier, J. M.; Fremont, V.; Mabrouk, K.; Crest, M.; Darbon, H.; Rochat, H.; Vanrietschoten, J.; Martin-Eauclaire, M. F. *Int. J. Pept. Protein Res.* **1994**, *43*, 486.
31. Inisan, A. G.; Meunier, S.; Fedelli, O.; Altbach, M.; Fremont, V.; Sabatier, J. M.; Thevan, A.; Bernassau, J. M.; Cambillau, C.; Darbon, H. *Int. J. Pept. Protein Res.* **1995**, *45*, 441.
32. Sabatier, J. M.; Zerrouk, H.; Darbon, H.; Mabrouk, K.; Benslimane, A.; Rochat, H.; Martin-Eauclaire, M. F.; Van Rietschoten, J. *Biochemistry* **1993**, *32*, 2763.
33. Demonchaux, P.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *Eur. J. Med. Chem.* **1991**, *26*, 915.
34. Freeman, C. M.; Catlow, C. R. A.; Hemmings, A. M.; Hider, R. C. *FEBS Lett.* **1986**, *197*, 289.
35. Campos Rosa, J.; Galanakis, D.; Ganellin, C. R.; Dunn, P. M. *J. Med. Chem.* **1996**, *39*, 4247.
36. Nolan, W. P.; Ratcliffe, G. S.; Rees, D. C. *Tetrahedron Lett.* **1992**, *33*, 6879.
37. Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *Arch. Pharm. Pharm. Med. Chem.* **1996**, *329*, 524.
38. Dunn, P. M. *Eur. J. Pharmacol.* **1994**, *252*, 189.
39. Otsubo, T.; Gray, R.; Boekelheide, V. *J. Am. Chem. Soc.* **1978**, *100*, 2449.
40. Galanakis, D.; Ganellin, C. R.; Malik, S.; Dunn, P. M. *J. Med. Chem.* **1996**, *39*, 3592.
41. Campos Rosa, J.; Beckwith-Hall, B. M.; Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 7.
42. Byron, D. J.; Gray, G. W.; Wilson, R. C. *J. Chem. Soc. (C)* **1966**, 840.
43. Dunn, P. M.; Benton, D. C. H.; Campos Rosa, J.; Ganellin, C. R.; Jenkinson, D. H. *Br. J. Pharmacol.* **1996**, *117*, 35.
44. Campos Rosa, J.; Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **1998**, *41*, 2.
45. Davies, K. ChemX Molecular Graphics Programme, developed and distributed by Chemical Design Ltd, Oxford, UK, **1986**.