A new synthesis of alkene-containing minor-groove binders and essential hydrogen bonding in binding to DNA and in antibacterial activity†

Nahoum G. Anthony,[b] David Breen,[a] Gavin Donoghue,[a] Abedawn I. Khalaf,[a] Simon P. Mackay,[b] John A. Parkinson[a] and Colin J. Suckling*[a]

Received 29th January 2009, Accepted 16th February 2009
First published as an Advance Article on the web 12th March 2009
DOI: 10.1039/b901898k

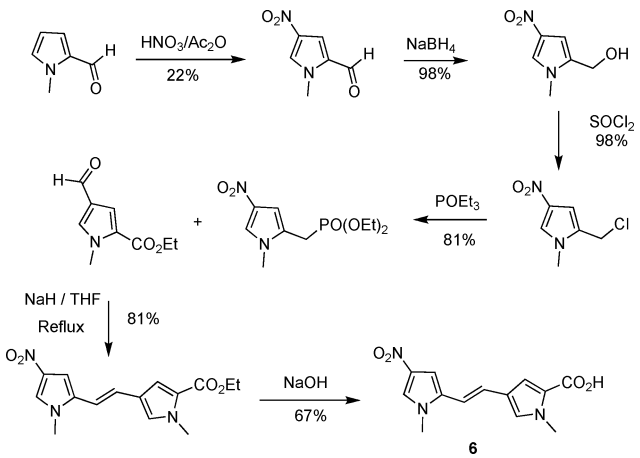
A practical synthesis of alkene-containing minor-groove binders for DNA, related to distamycin, with potential for wide structural diversity is described, based upon the Wittig chemistry of *N*-alkylpyrrole aldehydes. The compounds prepared have been evaluated for binding to DNA by physical methods (melting temperature and NMR) and for their antibacterial activity. Significantly, it was found that alkenes linking the aryl head group of the minor-groove binder promote strong binding to DNA and high antibacterial activity against Gram-positive bacteria. Conversely, a minor-groove binder containing an alkene located towards the alkylamino tail group has a low affinity for DNA and does not show antibacterial activity. These observations suggest an important role for specific hydrogen bonds in the binding of compounds of this type to DNA, and in their antibacterial activity.

Introduction

Minor-groove binders related to the natural product distamycin (**1**) have attracted much attention as antibacterial compounds.[1] Structural variation has typically involved increasing the hydrophobicity of the analogues by introducing larger *N*-alkyl groups than methyl, and larger and more hydrophobic head groups than formyl. The importance of a low pK_a basic tail group has also been emphasised.[2] Recently, we reported that the replacement of an amide by the isosteric, but non hydrogen bonding, alkene at the head-group link led to a series of highly active antibacterial minor-groove binders of which **2** and **3** are examples.[3] These compounds are approximately 100-fold more active than their corresponding amide isosteres, **4** and **5**. The question was therefore raised whether isosteric substitutions of other amide links in the distamycin template would be acceptable. To answer this, it was necessary to develop new synthetic methods for such compounds, which we refer to as 'internal alkenes' to distinguish them from the head-group alkenes of **2** and **3**.

Results and discussion

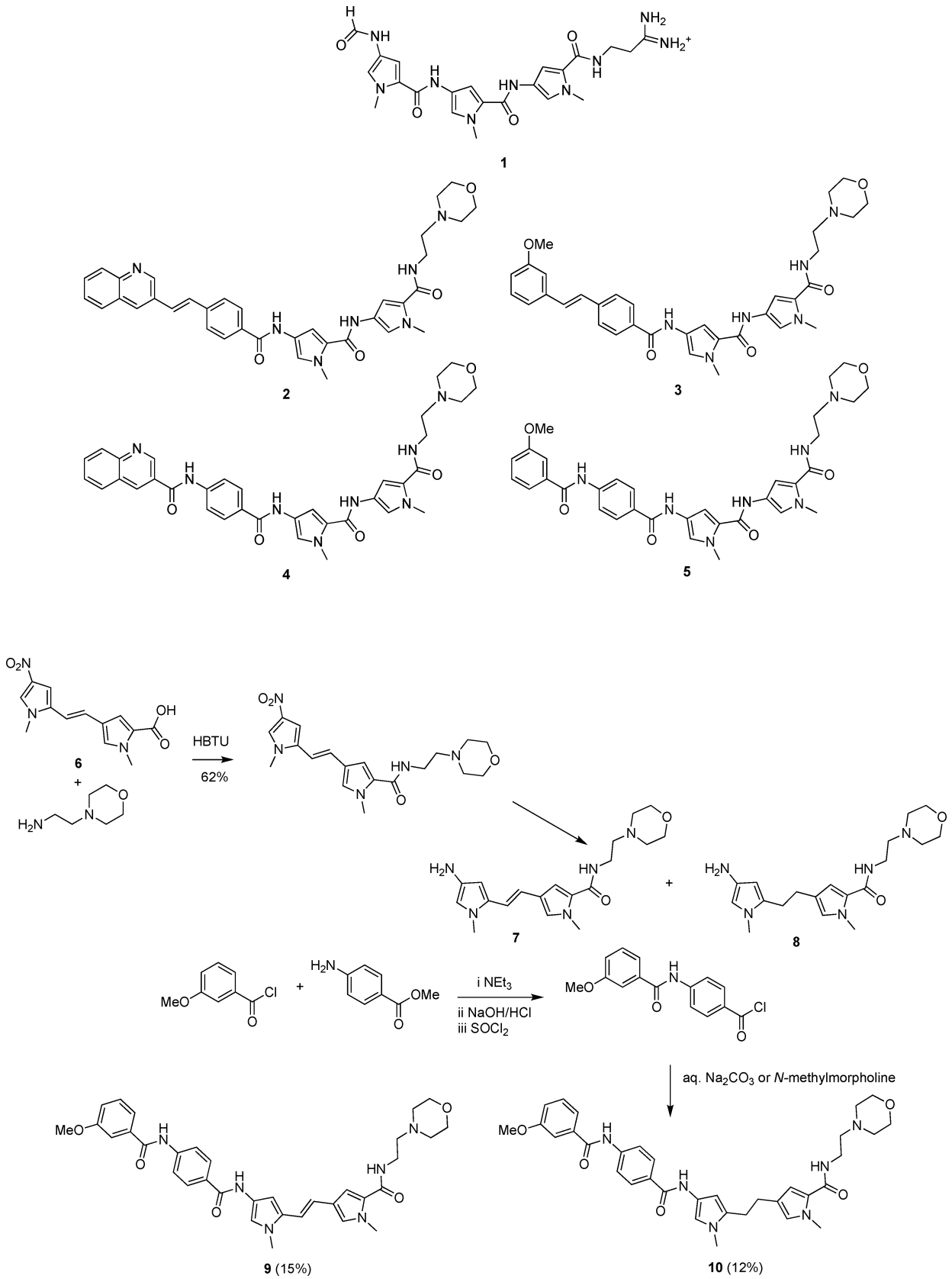
Although minor-groove binders with internal alkenes have been prepared before,[4] the method did not lend itself to the flexible synthesis of our target compounds in adequate yields. A practical route to the key intermediate, **6**, was developed from 2-formyl-*N*-methylpyrrole in six steps using standard functional group transformations (Scheme 1).[5] Also using established methods,[3] the morpholinoethyl side chain was added (Scheme 2).



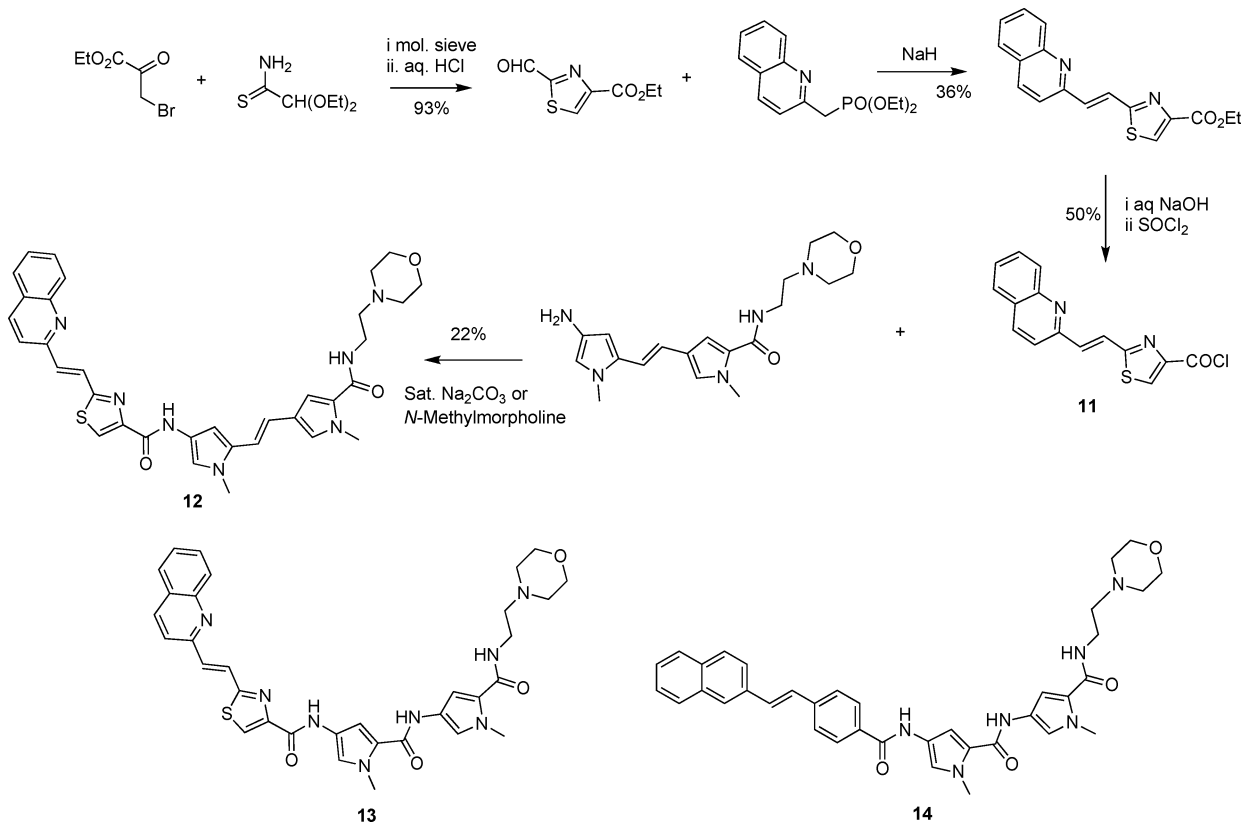
Scheme 1 Synthesis of a key intermediate for the preparation of internal alkene minor-groove binders.

The incorporation of **6** into the required minor-groove binders necessitated the development of reaction conditions for the selective reduction of the nitro group in the presence of the alkene. After much experimentation, it was found that reduction with sodium borohydride in the presence of palladium-on-charcoal[6] afforded clean reduction of the nitro group with no reduction of the alkene, provided the reaction time was controlled; extended reaction times led to reduction of the alkene also. The aminopyrrole alkene dimer **7** was then coupled with a range of head groups to afford the required internal alkene minor-groove binders which were purified by HPLC (Schemes 2 and 3: see also ESI†); in this way, the internal alkene analogue **9** of compounds **3** and **5** was prepared. The over-reduced dimer **8** was also coupled, affording compound **10** with a flexible link between two pyrrole rings; **10** provides an additional point of comparison for determining the structural features required for antibacterial activity. In addition to the amide

[a]*WestCHEM, Department of Pure & Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, Scotland. E-mail: c.j.suckling@strath.ac.uk; Fax: +44 141 548 5743; Tel: +44 141 548 2271*
[b]*Strathclyde Institute of Pharmacy and Biomedical Sciences, The John Arbuthnott Building, 27 Taylor Street, Glasgow, G4 0NR, Scotland. E-mail: simon.mackay@strath.ac.uk; Tel: +44 141 548 2866*
† Electronic supplementary information (ESI) available: Scanned ^{1}H NMR spectra of new compounds. See DOI: 10.1039/b901898k



Scheme 2 Elaboration of intermediate **6** into an internal alkene minor-groove binder, **9**, and its reduced alkyl analogue, **10**.



Scheme 3 Synthesis of the first bis-alkenyl minor-groove binder.

head group, that provides the direct analogue of **3**, an alkenyl head group containing a thiazole was also attached to **7** *via* the thiazole–quinoline dimer **11** (Scheme 3).[7] This provides, to our knowledge, the first bis-alkenyl minor-groove binder, **12**, based upon the distamycin template. The head-group alkenyl minor-groove binder, **13**, was also prepared.

The antibacterial activity of the internal alkene minor-groove binders **9** and **12** and the alkyl-linked compound **10** was compared with that of the corresponding head-group alkene minor-groove binders (MGBs) **3** and **13** against a group of Gram-positive bacteria, the type of bacterium that is most susceptible to this class of MGB. The lack of activity of the flexible alkyl-linked compound **10** was to be expected but emphasised the importance of an intrinsically and stably curved ligand for binding to DNA. Surprisingly, however, the internal alkene MGBs **9** and **12** were essentially inactive (Table 1). It is notable that in **12**, the beneficial presence of the head-group alkene does not compensate for the deleterious effect of the internal alkene, suggesting the importance of a specific interaction between DNA and the MGB at the position of the internal alkene.

If DNA is the crucial target for antibacterial activity of these compounds, it would be expected that the binding to DNA might be affected by such structural changes. Studies of the melting temperatures (T_m) suggest that this is a contributor to the lack of antibacterial activity of the internal alkenes.[8] Using a DNA target of GCGATATATGCG/CGCTATATACGC (target 1), the results shown in Table 2 were obtained. The target was chosen to have a T_m sufficiently above room temperature to give reliable data and to have a binding site appropriate for predominantly pyrrole-containing MGBs, which prefer AT-rich regions. These data show that the increase in T_m caused by binding the internal alkene MGB **9** is very much lower than that of the corresponding compound **3** with the internal amide. Such a result could be explained either by a molecular interaction such as a specific hydrogen bond or by a significant change of shape of the internal alkene MGB. With respect to the possibility of hydrogen bonding, a similar phenomenon was evident in the antibacterial activity of the quinolyl MGB **2** and its naphthyl analogue **14**;[9] in this case, the

Table 1 Antibacterial activity (MIC, μg mL^{-1}) of alkenyl MGBs against two strains of *Staphylococcus aureus* and one of *Streptococcus faecalis*

Compound	*S. aureus* 1	*S. aureus* 2	*S. faecalis*
9	>100	>100	100
10	>100	>100	>100
12	>100	>100	100
3	6.25	12.5	50
13	3.12	6.25	25

Table 2 T_m measurements to target 1

Compound	T_m Observed/°C	ΔT_m/°C
No ligand	47.0	—
5	59.0	10.0
9	47.0	0
2	67.5	20.5
14	47.0	0

non-hydrogen-bonding naphthyl compound **14** was also inactive. In parallel with the antibacterial activity, a significant stabilisation of target 1 oligomer was only found with **2**, which contains the hydrogen-bonding quinolyl group. A similar comparison of **12** and **13** by T_m measurement has not yet been possible because the DNA target of these MGBs is not known.

To evaluate the possibility that the shape of the internal alkene MGB might differ significantly from its amide prototype, molecular modeling was carried out. Structures for compounds **3** and **9** were calculated using Gaussian 03 using a b3lyp Hamiltonian and a 6-31G* basis set[10] It is known that the morpholinoethyl side-chain of these MGBs does not make ordered contacts with DNA when bound[3] and consequently, superimposition of the amide and alkene isosteres concentrated upon the regions containing the head group and the first two pyrroles (stopping at the alkene/amide isostere). Fig. 1 shows the result and indicates that the head group and first two pyrroles are essentially superimposable, with conformational change starting at the alkene/amide bond towards the morpholino tail. This confirms the expectation that there is no substantial change in conformation between internal alkene compounds compared with their isosteres and conformational change is therefore unlikely to account for the reduced DNA binding of internal alkene MGBs. Attention then focused on the possibility of specific hydrogen bonds being involved. This has been investigated by NMR.

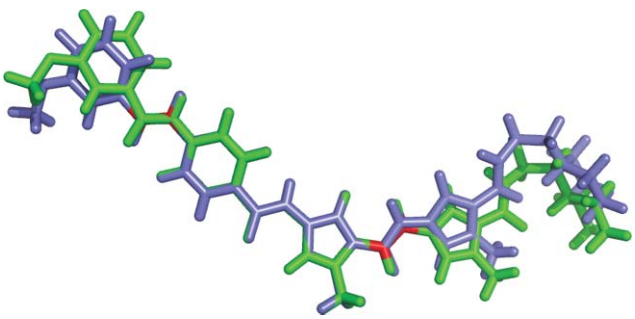


Fig. 1 Superimposition of **3** (green) on **9** (blue) maximising the overlap of the head and central part of the MGBs. The key alkene and amide bonds are coloured red. The major differences are to be seen at the tail (right-hand side) which is known to be disordered from NMR studies.[3]

The head-group alkene MGB **3** was studied bound 2 : 1 to a similar recognition sequence as target 1, but a shorter sequence more suited to NMR experiments (target 2, the self-complementary oligonucleotide, CGA^{3}TAT^{6}A^{7}T^{8}CG) showed significant NOEs between ligand N*H* of the inter-pyrrole peptide bond (replaced in **9** by an alkene) and DNA hydrogens T^{6}H1′, A^{7}H4′, A^{7}H1′, A^{7}H2 and T^{8}H1′ (Fig. 2). Importantly, these data establish the peptide bond as oriented with N*H* on the concave edge of the ligand, thus making it available to hydrogen-bond acceptors on the floor of the DNA minor groove. Whilst NOEs do not prove the presence of hydrogen bonds, our NMR-based solution structure calculated for the DNA complex of **3** implies that the most likely H-bond of this N*H* is to the H-bond acceptor T^{8}O2 (model distance = 2.16 Å). In addition, the proton chemical shift of 9.8 ppm is consistent with expectations for a hydrogen-bonded peptide N*H*, and is in agreement with chemical shift values for H-bonded N*H* in ligands of a similar nature bound to DNA.[9] Further, and consistent with

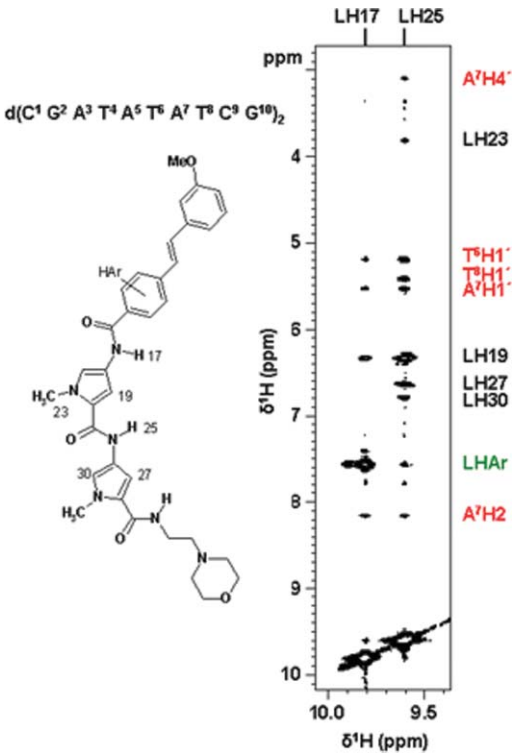


Fig. 2 NOEs to H25 and H17 recorded for the 2 : 1 complex formed between **3** and the decamer self-complementary oligonucleotide sequence shown. Assignments are colour-coded as follows: ligand-to-DNA contact (red); intra-ligand contact (black); inter-ligand contact (green). NMR data were acquired at 500 MHz using a mixing time of 50 ms and recorded at a sample temperature of 7 °C. Atom numbering is shown only for those atoms associated with NOEs in this figure.

this interpretation, as noted above in previous work[3,9] we found that a quinolyl head group (**1**) gave high antibacterial activity, but the corresponding compound with a naphthyl head group (**14**) was inactive. This again suggests the importance of a hydrogen-bond acceptor in this region of the molecule as we have argued before.[2] Moreover, there is evidence for such a hydrogen bond in the NMR study of **3**, which also shows a significant NOE between the aryl methoxy group and A^{3}NH.

Taken together, these results indicate that potent antibacterial activity of alkene-containing MGBs requires a contribution not only from hydrophobic interactions, as shown by the effect of head groups, but also specific hydrogen bonds, as shown by the amide link between the two pyrrolyl amino acids next to the alkylamino tail group.

Experimental

General

^{1}H-, ^{13}C-, and ^{31}P-NMR were carried out on a Bruker DPX-400 MHz spectrometer with chemical shifts given in ppm (δ values), relative to proton and carbon traces in solvent or, in the case of ^{31}P, phosphoric acid was used as an external standard ($\delta = 0$). Coupling constants are reported in Hz. IR spectra were recorded on a Perkin Elmer, 1 FT-IR spectrometer. Elemental analysis was carried out on a Perkin Elmer 2400, analyser series 2.

Table 3 Programme for HPLC purification of MGBs

Time/min	A	B	Flow rate/mL min^{-1}
0	90	10	4
28	30	70	4
33	10	90	4
38	90	10	4
40	90	10	0

Mobile phase, A = Water + 0.1% TFA, B = Acetonitrile + 0.1% TFA.

Mass spectra were obtained on a Jeol JMS AX505. Anhydrous solvents were obtained from a Puresolv purification system, from Innovative Technologies, or purchased as such from Aldrich. Melting points were recorded on a Reichert hot-stage microscope, and are uncorrected. Chromatography was carried out using 200–400 mesh silica gels, or using reverse-phase HPLC on a water system using a C18 Luna column with the gradient given in Table 3.

(1-Methyl-4-nitro-1*H*-pyrrol-2-yl)methanol[11]

1-Methyl-4-nitro-1*H*-pyrrole-2-carbaldehyde **6** (0.40 g, 2.08 mmol) was placed in anhydrous ethanol (50 mL) under nitrogen. Sodium borohydride (0.04 g, 1.04 mmol) was added in small portions over 5 min and the solution allowed to stir for 20 min. Water (10 mL) was then added slowly to quench the reaction and the solution was then extracted with ethyl acetate (2 × 20 mL). The organic layer was then dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to yield the required product as a light-brown solid (0.32 g, 98%), m.p. = 89–90 °C, (Lit. 90.5–91.5 °C).[11] ν_{max} (KBr): 3521, 3131, 2934, 2888, 1490, 1412, 1520, 1337 cm^{-1}. δ_H ($CDCl_3$): 3.67 (3H, s, N-Me), 4.40 (2H, d, CH_2, J = 5.4), 5.18 (1H, t, OH, J = 3.0), 6.57 (1H, d, Ar-H, J = 1.6), 7.92 (1H, d, Ar-H, J = 1.6).

Diethyl (1-methyl-4-nitro-1*H*-pyrrol-2-yl)methylphosphonate

(1-Methyl-4-nitro-1*H*-pyrrol-2-yl)methanol (0.10, 0.64 mmol) was dissolved in dichloromethane (5 mL), and thionyl chloride (5 mL) was added slowly. The solution was then heated under reflux for 15 min, and the excess thionyl chloride was removed under reduced pressure. The residue was then heated under reflux in triethylphosphite (3 mL) for 1 h at 160 °C, and the excess triethylphosphite was removed under vacuum (1.5 mmHg at 70 °C) to give the desired product initially as a brown oil, which solidified after 48 h at 0–4 °C to a brown semi-solid (0.17 g, 98%). ν_{max} (NaCl): 3137, 2985, 1556, 1438, 1519, 1346, 1308, 1163 cm^{-1}. δ_H ($CDCl_3$): 1.20 (6H, t, CH_3, J = 6.8), 3.08 (2H, d, (CH_2)P, J = 20.4), 3.65 (3H, s, N-Me), 4.01 (4H, q, $(CH_2)CH_3$, J = 6.8), 6.54 (1H, d, Ar-H, J = 1.6), 7.39 (1H, d, Ar-H, J = 1.6), δ_P ($CDCl_3$), 23.44. HRFABMS: Found 276.0875; $C_{10}H_{17}N_2O_5P$ requires 276.0873.

Ethyl 1-methyl-4-[(*E*)-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethenyl]-1*H*-pyrrole-2-carboxylate

(1-Methyl-4-nitro-1*H*-pyrrol-2-yl)methylphosphonate (0.50 g, 1.81 mmol) and 4-formyl-1-methyl-1H-pyrrole-2-carboxylate 8 (0.28 g, 1.84 mmol) were taken up in THF (4 mL) under nitrogen, sodium hydride (0.26 g, 10.83 mmol) was then added in small portions over 5 min and the solution heated under reflux for 1 h. Ice-water (5 mL) was then added, at which point the product precipitated and was collected by filtration, before being dried under reduced pressure (0.44 g, 81%) m.p. = 158–161 °C. ν_{max} (NaCl): 3129, 2971, 2620, 1680, 1554, 1532, 1488, 1512, 1318, 1258, 1209, 1441 cm^{-1}. δ_H ($CDCl_3$): 1.36 (3H, t, CH_2CH_3, J = 7.1), 3.69 (3H, s, N-Me), 3.93 (3H, s, N-Me), 4.31 (2H, q, CH_2CH_3, J = 7.1), 6.52 (1H, d, CH=CH, J = 16.0), 6.77 (1H, d, CH=CH, J = 16.0), 6.83 (1H, d, Ar-H, J = 1.6), 6.88 (1H, d, Ar-H, J = 1.6), 7.10 (1H, d, Ar-H, J = 1.6), 7.45 (1H, d, Ar-H, J = 1.6). HRFABMS: Found 303.1221; $C_{15}H_{17}N_3O_4$ requires 303.1219.

1-Methyl-4-[(*E*)-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethenyl]-1*H*-pyrrole-2-carboxylic acid 6

Ethyl 1-methyl-4-[(*E*)-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethenyl]-1*H*-pyrrole-2-carboxylate (0.200 g, 0.63 mmol) was dissolved in ethanol (3 mL). To this solution, sodium hydroxide (0.072 g, 1.81 mmol) in water (5 mL) was added and the solution was heated under reflux for 1 h. The solution was then cooled to 0 °C at which point a precipitate formed. This was collected by filtration and dissolved in water (10 mL) the solution was then cooled to 0 °C and dilute hydrochloric acid added until a pH of 2 was reached, at which point the product **6** precipitated as a yellow solid (0.110 g, 67%) m.p. = no distinct m.p., decomposes at >230 °C. ν_{max} (NaCl): 3200–2800, 3134, 2923, 2613, 1670, 1557, 1536, 1493, 1517, 1312, 1259 cm^{-1}. δ_H ($CDCl_3$): 3.71 (3H, s, N-Me), 3.83 (3H, s, N-Me), 6.74 (1H, d, C*H*=CH, J = 16.0), 6.89 (1H, d, Ar-H, J = 1.6), 6.90 (1H, d, CH=C*H*, J = 16.0), 7.13 (1H, d, Ar-H, J = 1.6), 7.23 (1H, d, Ar-H, J = 1.6), 7.92 (1H, d, Ar-H, J = 1.6), 12.30 (1H, s, COOH). HRFABMS: Found 275.0905; $C_{13}H_{13}N_3O_4$ requires 275.0906. Found: C, 56.28; H, 4.51.; N, 15.64; $C_{13}H_{13}N_3O_4$ requires C, 56.73; H, 4.76; N, 15.27%.

1-Methyl-4-[(*E*)-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethenyl]-*N*-[2-(4-morpholinyl)ethyl]-1*H*-pyrrole-2-carboxamide

1-Methyl-4-[(*E*)-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethenyl]-1*H*-pyrrole-2-carboxylic acid **6** (0.400 g, 1.51 mmol) and ethylaminomorpholine (196 µL, 1.52 mmol) were dissolved in DMF (2.0 mL), HBTU (1.137 g, 3.00 mmol) was added and the solution was allowed to stir overnight. Water (5.0 mL) was then added and the solution extracted with ethyl acetate (2 × 10 mL); the organic layer was dried over ($MgSO_4$), and the solvent removed under reduced pressure. The residue was dissolved in ethyl acetate, and hexane was added until the product precipitated as an orange solid (0.360 g, 62%) m.p. >230 °C. ν_{max} (KBr): 3429, 3143, 2925, 1630, 1518, 1300, 845 cm^{-1}. δ_H (DMSO): 3.15 (2H, d, CH_2, J = 11.5), 3.28 (2H, m, CH_2), 3.32 (4H, m, 2(CH_2)), 3.65 (2H, t, CH_2, J = 11.5), 3.68 (3H, s, N-Me), 3.84 (3H, s, N-Me), 4.02 (2H, d, CH_2, J = 11.5), 6.60 (1H, d, (C=CH), J = 16.2), 6.93 (3H, m, 2Ar-H and C=CH), 7.20 (1H, d, Ar-H, J = 1.8), 7.96 (1H, d, Ar-H, J = 1.8), 8.33 (1H, broad s, NH). HRFABMS: Found 388.1983; $C_{19}H_{26}N_5O_4^+$ requires 388.1985. Found: C, 58.19; H, 6.07; N, 18.42; $C_{19}H_{25}N_5O_4$ requires C, 58.90; H, 6.50; N, 18.08%.

Methyl 4-[(3-methoxybenzoyl)amino]benzoate

m-Anisoylchloride (1.038 g, 5.86 mmol) was dissolved in dichloromethane (25 mL), methyl 4-aminobenzoate (0.886 g,

5.86 mmol) and triethylamine (1.3 mL, 10.0 mmol). Dichloromethane (10 mL) was then added dropwise at 0 °C and the solution allowed to return to room temperature overnight, during this time the product precipitated as a white solid (1.236 g, 74%) m.p. = 79–81 °C. ν_{max} (KBr): 3467, 3356, 3070, 2846, 2831, 1709, 1500, 1450 cm^{-1}. δ_H (DMSO): 3.83 (6H, s, 2OCH$_3$), 7.17 (1H, d of d, Ar-H, J = 2.5 and 8.1), 7.49 (3H, m, Ar-H), 7.93 (4H, m, Ar-H), 10.52 (1H, s, NH). LRMS: Found 286.0; $C_{16}H_{15}NO_4^+$ requires 286.1.

4-[(3-Methoxybenzoyl)amino]benzoic acid[3]

Methyl 4-[(3-methoxybenzoyl)amino]benzoate (1.03 g, 3.80 mmol) was dissolved in ethanol (5 mL); a solution of sodium hydroxide (0.152 g, 3.80 mmol) in water (15 mL) was then added and the solution allowed to stir overnight. The solution was then cooled to 0 °C and acidified using dilute hydrochloric acid solution, until the product precipitated as a white solid (1.236 g, 74%) m.p. = 89–91 °C. ν_{max} (KBr): 3381, 2961, 1690, 1583, 1311 cm^{-1}. δ_H (DMSO): 3.85 (3H, s, OCH$_3$), 7.17 (1H, d of d, Ar-H, J = 2.5 and 8.1), 7.50 (3H, m, Ar-H), 7.93 (4H, m, Ar-H), 10.54 (1H, s, NH). LRMS: Found 271.9; $C_{15}H_{14}NO_4^+$ requires 272.1. Found: C, 65.98; H, 4.80; N, 5.16; $C_{15}H_{13}NO_4$ requires C, 66.41; H, 4.83; N, 5.16%.

4-[(3-Methoxybenzoyl)amino]benzoyl chloride

4-[(3-Methoxybenzoyl)amino]benzoic acid (0.410 g, 1.42 mmol) was dissolved in dichloromethane (3 mL).Thionyl chloride (5 mL) was then added, and the solution was heated under reflux for 1 h. The solvent and thionyl chloride were removed under reduced pressure to yield the product **20** as an off-white solid (1.236 g, 74%) m.p. = 65–67 °C ν_{max} (KBr): 3464, 3349, 3063, 2839, 2826, 1752, 1585, 1504, 1452 cm^{-1}. δ_H (DMSO): 3.84 (3H, s, OCH$_3$), 7.18 (1H, d of d, Ar-H, J = 2.5 and 8.1), 7.53 (3H, m, Ar-H), 7.92 (4H, m, Ar-H), 10.51 (1H, s, NH). LRMS: Found 291.0; $C_{15}H_{13}ClNO_3^+$ requires 291.1.

4-{(*E*)-2-[4-({4-[(3-Methoxybenzoyl)amino]benzoyl}amino)-1-methyl-1*H*-pyrrol-2-yl]ethenyl}-1-methyl-*N*-[2-(4-morpholinyl)ethyl]-1*H*-pyrrole-2-carboxamide trifluoroacetate 9 and 4-{2-[4-({4-[(3-methoxybenzoyl)amino]benzoyl}amino)-1-methyl-1*H*-pyrrol-2-yl]ethyl}-1-methyl-*N*-[2-(4-morpholinyl)ethyl]-1*H*-pyrrole-2-carboxamide trifluoroacetate 10

1-Methyl-4-[(*E*)-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethenyl]-*N*-[2-(4-morpholinyl)ethyl]-1*H*-pyrrole-2-carboxamide (0.111 g, 0.27 mmol) was dissolved in dioxane (5 mL). Pd/C (10%, 0.100 g) was then added, followed by a solution of sodium borohydride (0.030 g, 0.52 mmol) in water (2 mL). The solution was then allowed to stir for 20 min, before being filtered directly into a flask containing 4-[(3-methoxybenzoyl)amino]benzoyl chloride (0.078 g, 0.27 mmol). Saturated aqueous sodium carbonate (2 mL) was then added and the solution allowed to stir overnight. Water (5 mL) was added and the solution was extracted with ethyl acetate (2 × 20 mL). The organic layer was dried (MgSO$_4$) and the solvent removed under reduced pressure. The residue was purified by HPLC which allowed both products to be obtained as yellow/orange solids.

4-{(*E*)-2-[4-({4-[(3-Methoxybenzoyl)amino]benzoyl}amino)-1-methyl-1*H*-pyrrol-2-yl]ethenyl}-1-methyl-*N*-[2-(4-morpholinyl)-ethyl]-1*H*-pyrrole-2-carboxamide trifluoroacetate 9

Yield: 0.031 g, 15%, m.p.>230 °C, purity by HPLC = 97% ν_{max} (KBr): 3421, 2995, 1675, 1675, 1523 cm^{-1}. δ_H (DMSO): 3.20 (2H, m, CH$_2$), 3.34 (2H, m, CH$_2$), 3.58 (4H, m, 2(CH$_2$), 3.68 (3H, s, N-Me), 3.75 (2H, t, CH$_2$, J = 12.2), 3.90 (3H, s, N-Me), 3.92 (3H, s, N-Me), 4.02 (2H, d, CH$_2$, J = 12.9 Hz), 6.50 (1H, d, Ar-H, J = 1.7), 6.67 (1H, d, (C=CH), J = 16.1 Hz), 6.72 (1H, d, (C=CH), J = 16.1), 7.09 (1H, d, Ar-H, J = 1.7), 7.23 (1H, d, Ar-H, J = 1.7), 7.26 (1H, d, Ar-H, J = 1.7), 7.55 (2H, m, Ar-H), 7.63 (1H, m, Ar-H), 7.98 (4H, m, Ar-H), 8.33 (1H, t, NH, J = 5.5), 9.83 (1H, s, N$^+$H), 10.18 (1H, s, NH), 10.52 (1H, s, NH). HRFABMS: Found 611.3134; $C_{34}H_{39}N_5O_4^+$ requires 611.3133.

4-{2-[4-({4-[(3-Methoxybenzoyl)amino]benzoyl}amino)-1-methyl-1*H*-pyrrol-2-yl]ethyl}-1-methyl-*N*-[2-(4-morpholinyl)ethyl]-1*H*-pyrrole-2-carboxamide trifluoroacetate 10

Yield: 0.024 g, 12%, m.p. >230 °C, purity by HPLC = 98% ν_{max} (KBr): 3315, 3020, 1674, 1523 cm^{-1}. δ_H (DMSO): 2.73 (2H, m, CH$_2$), 2.77 (2H, m, CH$_2$), 3.20 (2H, m, CH$_2$), 3.31 (2H, m, CH$_2$), 3.54 (4H, m, 2(CH$_2$), 3.58 (3H, s, N-Me), 3.71 (2H, t, CH$_2$, J = 12.2), 3.86 (3H, s, N-Me), 3.91 (3H, s, N-Me), 4.08 (2H, d, CH$_2$, J = 12.9), 6.02 (1H, d, Ar-H, J = 1.7), 6.75 (1H, d, Ar-H, J = 1.7), 6.89 (1H, d, Ar-H, J = 1.7), 7.15 (1H, d, Ar-H, J = 1.7), 7.26 (2H, d of d, Ar-H, J = 2.5 and 9.1), 7.63 (1H, m, Ar-H), 7.98 (4H, m, Ar-H), 8.22 (1H, t, NH, J = 5.5), 9.62 (1H, s, N$^+$H), 10.04 (1H, s, NH), 10.49 (1H, s, NH). HRFABMS: Found 613.3156; $C_{34}H_{41}N_6O_5^+$ requires 613.3138.

Ethyl 2-(diethoxymethyl)-1,3-thiazole-4-carboxylate[12]

2,2-Diethoxyethanethioamide (0.654 g, 6.03 mmol) and ethyl bromopyruvate (1.28 g, 6.10 mmol) were dissolved in ethanol (10 mL) in the presence of a 4 Å mol. sieve (1.0 g). The solution was then heated under reflux for 45 min and the solvent removed under reduced pressure. The residue was then dissolved in ethyl acetate (20 mL) and extracted with sat. sodium bicarbonate solution (2 × 20 mL) and brine (2 × 20 mL). The organic fraction was dried (MgSO$_4$) and the solvent removed under reduced pressure to yield the required product as a white solid (1.452 g, 93%) m.p. = 70–72 °C. ν_{max} (KBr): 3108, 2981, 2885, 2856, 1735, 1505, 1042 cm^{-1}. δ_H (CDCl$_3$): 1.29 (9H, m, 2CH$_3$), 3.71 (4H, m, 2CH$_2$), 4.47 (2H, q, CH$_2$ J = 7.8), 5.70 (1H, s, CH), 8.19 (1H, s, Ar-H). HRFABMS: Found 260.0956 (M + H); $C_{11}H_{17}NO_4S$ requires 259.0878.

Ethyl 2-formyl-1,3-thiazole-4-carboxylate[12]

Ethyl 2-(diethoxymethyl)-1,3-thiazole-4-carboxylate (1.34 g, 5.17 mmol) was dissolved in acetone (100 mL). Hydrochloric acid solution (12.8 mL, 1 M) was then added and the solution heated under reflux for 45 min. The solvents were then removed under reduced pressure and the residue dissolved in ethyl acetate (40 mL) and extracted with sat. sodium bicarbonate solution (2 × 40 mL), and brine (2 × 40 mL). The organic fraction was then dried (MgSO$_4$) and the solvent removed under reduced pressure to yield ethyl 2-formyl-1,3-thiazole-4-carboxylate as a light-brown solid (0.937 g, 98%) m.p. = 65–67 °C (Lit. 67–68 °C).[12] ν_{max} (KBr):

3116, 2983, 2910, 2814, 1730, 1513, 1060 cm^{-1}. δ_H ($CDCl_3$): 1.47 (3H, t, CH_3 $J = 8.0$), 4.50 (2H, q, CH_2 $J = 8.0$), 8.52 (1H, d, Ar-H $J = 1.2$), 10.08 (1H, d, Ar–COH $J = 1.2$). HRFABMS: Found 186.0228 (M + H); $C_7H_7NO_3S$ requires 185.0147.

Ethyl 2-[(*E*)-2-(2-quinolinyl)ethenyl]-1,3-thiazole-4-carboxylate

Diethyl 2-quinolinylmethylphosphonate (0.580 g, 2.08 mmol) was dissolved in anhydrous THF (2 mL). Sodium hydride (0.273 g, 11 mmol) was then added in small portions and the resulting solution allowed to stir for 10 min. Ethyl 2-formyl-1,3-thiazole-4-carboxylate (0.387 g, 2.08 mmol) in anhydrous THF (3 mL) was added dropwise and the solution allowed to stir for 16 h. Water (5 mL) was then added (dropwise initially) during which time the required product precipitated as a light-brown/yellow solid (0.232 g, 36%) m.p. = 183–186 °C. ν_{max} (KBr): 3124, 3043, 2955, 2925, 2899, 2853, 1730, 1612, 1627, 1592, 1553, 1479 cm^{-1}. δ_H ($CDCl_3$): 1.34 (3H, t, CH_3 $J = 8.0$), 4.34 (2H, q, CH_2 $J = 8.0$), 7.62 (1H, t, Ar-H $J = 7.2$), 7.74 (1H, d, C=C alkene $J = 16.1$), 7.79 (1H, t, Ar-H $J = 6.8$), 7.98 (3H, m, Ar-H), 8.05 (1H, d, C=C alkene $J = 16.1$), 8.42 (1H, d, Ar-H $J = 8.5$), 8.56 (1H, s, Ar-H). HRFABMS: Found 310.0772; $C_{17}H_{14}N_2O_2S$ requires 310.0776.

2-[(*E*)-2-(2-Quinolinyl)ethenyl]-1,3-thiazole-4-carboxylic acid

2-[(*E*)-2-(2-Quinolinyl)ethenyl]-1,3-thiazole-4-carboxylate (0.137 g, 0.44 mmol) was suspended in ethanol (2 mL). Sodium hydroxide (0.052 g, 1.32 mmol) in water (5 mL) was added and the solution heated under reflux for 2 h. The reaction was then filtered and then cooled to 0 °C. Dilute hydrochloric acid was added dropwise until the required product precipitated as a yellow solid (0.103 g, 83%) m.p. = 218–220 °C. ν_{max} (KBr): 3469, 3105, 3068, 2923, 2853, 1715, 1640, 1632, 1599, 1541, 1493, 1320 cm^{-1}. δ_H ($CDCl_3$): 7.74 (1H, t, Ar-H $J = 7.2$), 7.76 (1H, d, C=C alkene $J = 16.1$), 7.93 (1H, t, Ar-H $J = 7.2$), 8.11 (1H, d, Ar-H $J = 8.5$), 8.05 (1H, d, Ar-H $J = 8.5$), 8.19 (2H, m, Ar-H), 8.24 (1H, d, C=C alkene $J = 16.1$), 8.58 (1H, s, Ar-H), 8.65 (1H, d, Ar-H $J = 8.5$). HRFABMS: Found 282.0465; $C_{15}H_{10}N_2O_2S$ requires 282.0463. Found: C, 63.49; H, 4.06; N, 9.49; S, 11.53; $C_{15}H_{10}N_2O_2S$ requires C, 63.81; H, 3.57; N, 9.92; S, 11.36%.

4-[2-({[1-Methyl-4-((*E*)-2-{1-methyl-4-[({2-[(*E*)-2-(2-quinolinyl)-ethenyl]-1,3-thiazol-4-yl}carbonyl)amino]-1*H*-pyrrol-2-yl}-ethenyl)-1*H*-pyrrol-2-yl]carbonyl}amino)ethyl]morpholin-4-ium trifluoroacetate 12

2-[(*E*)-2-(2-Quinolinyl)ethenyl]-1,3-thiazole-4-carboxylic acid **11** (0.078 g, 0.26 mmol) was dissolved in dichloromethane (2 mL), thionyl chloride (5 mL) added and the solution heated under reflux for 1 h, after which time the solvent was removed under reduced pressure to give 2-[(*E*)-2-(2-quinolinyl)ethenyl]-1,3-thiazole-4-carbonyl chloride as a dark-green/brown solid. 1-Methyl-4-[(*E*)-2-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethenyl]-*N*-[2-(4-morpholinyl)ethyl]-1*H*-pyrrole-2-carboxamide **6** (0.105 g, 0.26 mmol) was dissolved in dioxane (5 mL). Pd/C (10%, 0.100 g) was then added, followed by a solution of sodium borohydride (0.030 g, 0.52 mmol) in water (2 mL). The solution was then allowed to stir for 15 min, and filtered directly into the flask containing the previously prepared 2-[(*E*)-2-(2-quinolinyl)ethenyl]-1,3-thiazole-4-carbonyl chloride. Saturated sodium carbonate (2 mL) was then added and the solution allowed to stir overnight. Water (5 mL) was added and the solution extracted with ethyl acetate (2 × 20 mL), the organic layer was dried and the solvent removed under reduced pressure. The residue was purified by HPLC which allowed the product to be obtained as a dark-green solid (0.044 g, 22%) m.p. >230 °C, purity by HPLC = 98%. ν_{max} (KBr): 3347, 2925, 1685, 1535, 1204 cm^{-1}. δ_H (DMSO): 3.13 (2H, m, CH_2), 3.29 (2H, m, CH_2), 3.55 (4H, m, 2(CH_2), 3.57 (3H, s, N-Me), 3.67 (2H, t, CH_2, $J = 12.5$), 3.84 (3H, s, N-Me), 4.01 (2H, d, CH_2, $J = 11.16$), 6.60 (1H, d, Ar-H, $J = 1.7$), 6.63 (1H, d, (C=CH), $J = 16.1$), 6.70 (1H, d, (C=CH), $J = 16.1$), 7.01 (1H, d, Ar-H, $J = 1.8$), 7.18 (1H, d, Ar-H, $J = 1.7$), 7.25 (1H, d, Ar-H, $J = 1.7$), 7.64 (1H, t of d, Ar-H, $J = 1.2$ and 8.4), 7.82 (1H, t of d, Ar-H, $J = 1.2$ and 8.4), 7.92 (1H, d, (C=CH), $J = 16.0$), 7.98 (1H, d, Ar-H, $J = 8.6$), 8.01 (1H, d, Ar-H, $J = 8.4$), 8.04 (1H, d, Ar-H, $J = 8.5$), 8.10 (1H, d, (C=CH), $J = 16.0$), 8.34 (1H, t, NH, $J = 5.5$), 8.38 (1H, s, Ar-H), 8.46 (1H, d, Ar-H, $J = 8.6$), 9.55 (1H, s, N^+H), 10.26 (1H, s, NH). HRFABMS: Found 622.2598; $C_{34}H_{39}N_5O_4^+$ requires 622.2595.

N-[1-Methyl-5-({[1-methyl-5-({[2-(4-morpholinyl)ethyl]-amino}carbonyl)-1*H*-pyrrol-3-yl]amino}carbonyl)-1*H*-pyrrol-3-yl]-2-[(*E*)-2-(2-quinolinyl)ethenyl]-1,3-thiazole-4-carboxamide trifluoroacetate 13

N-[1-Methyl-5-({[1-methyl-5-({[2-(4-morpholinyl)ethyl]amino}-carbonyl)-1*H*-pyrrol-3-yl]amino}carbonyl)-1*H*-pyrrol-3-yl]-2-[(*E*)-2-(2-quinolinyl)ethenyl]-1,3-thiazole-4-carboxamide trifluoroacetate **13** was prepared using an analogous procedure to the coupling reaction for compound **12** above (0.020 g, 21%), no distinct m.p., purity by HPLC = 97%. ν_{max} (KBr): 3421, 3116, 2928, 1677, 1647, 1638, 1556, 1465, 1241, 1204, 1131, 722 cm^{-1}. δ_H ($CDCl_3$): 3.17 (2H, m, CH_2), 3.39 (2H, m, CH_2), 3.57 (4H, m, CH_2), 3.84 (3H, s, NMe), 3.88 (3H, s, NMe), 4.00 (2H, m, CH_2), 7.00 (1H, d, Ar-H $J = 1.6$), 7.22 (2H, m, Ar-H), 7.35 (1H, d, Ar-H $J = 1.6$), 7.63 (1H, t, Ar-H $J = 6.9$ Hz), 7.80 (1H, t, Ar-H $J = 6.9$), 7.90 (1H, d, HC=CH $J = 16.2$), 7.96 (1H, d, Ar-H $J = 8.6$), 8.01 (2H, m, Ar-H), 8.09 (1H, d, HC=CH $J = 16.2$), 8.24 (1H, t, NH $J = 5.6$), 8.40 (1H, s, Ar-H), 8.45 (1H, d, Ar-H $J = 8.6$), 9.66 (1H, s, NH^+), 9.98 (1H, s, NH), 10.39 (1H, s, NH). HRFABMS: Found 639.2505; $C_{33}H_{35}N_8O_4S$ requires 639.2502.

Melting temperature measurement

DNA oligomers and their complements were melted at a rate of 0.5 °C min^{-1} in 10 mM PBS buffer solution (pH 7.4) with 50 mM NaCl on a Cary 300 BIO UV–visible spectrophotometer. Each oligomer (made to a concentration of 6×10^{-6} M) was mixed with sufficient MGB to give the appropriate ratio. Samples were heated to 80 °C and cooled to 10 °C. The melting temperatures (T_m) of the hybrids were determined from the derivative maxima.

Antibacterial activity measurement

Carried out as described previously.[3,9]

References

1 R. W. Bürli, Y. Ge, S. White, S. M. Touami, M. Taylor, J. A. Kaizerman and H. E. Moser, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2591; R. W. Bürli, D. McMinn, J. A. Kaizerman, W. Hu, Y. Ge, Q. Pack, V. Jiang,

M. Gross, M. Garcia, R. Tanaka and H. E. Moser, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1253; R. W. Bürli, P. Jones, D. McMinn, Q. Le, J.-X. Duan, J. A. Kaizerman, S. Difuntorum and H. E. Moser, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1259; R. W. Bürli, J. A. Kaizerman, J.-X. Duan, P. Jones, K. W. Johnson, M. Iwamoto, K. Truong, W. Hu, T. Stanton, A. Chen, S. Touami, M. Gross, V. Jiang, Y. Ge and H. E. Moser, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2067.
2 C. J. Suckling, *J. Phys. Org. Chem.*, 2008, **21**, 575.
3 C. J. Suckling, D. Breen, A. I. Khalaf, E. Ellis, I. S. Hunter, G. Ford, C. G. Gemmell, N. Anthony, J.-J. Helsebeux, S. P. Mackay and R. D. Waigh, *J. Med. Chem.*, 2007, **50**, 6116.
4 Y. Yamamoto, T. Kimachi, Y. Kanaoka, S. Kato, T. Matsumoto, T. Kusakabe and Y. Sugiura, *Tetrahedron Lett.*, 1996, **37**, 7801.
5 P. Grammaticakis, *Bull. Soc. Chim. Fr.*, 1964, **5**, 924; C. J. Suckling, A. I. Khalaf, A. R. Pitt and M. Scobie, *Tetrahedron*, 2000, **56**, 5225.
6 T. Nielson, H. C. S. Wood and A. G. Wylie, *J. Chem. Soc.*, 1962, 371.
7 K. Inami and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 352.
8 I. Afonina, M. Zivarts, I. Kutyavin, E. Lukhtanov, H. Gamper and R. B. Meyer, *Nucleic Acids Res.*, 1997, **25**, 2657.
9 A. I. Khalaf, R. D. Waigh, A. J. Drummond, B. Pringle, I. McGroarty, G. G. Skellern and C. J. Suckling, *J. Med. Chem.*, 2004, **47**, 2133.
10 http://www.gaussian.com/citation_g03.htm.
11 C. Hotzel, A. Marotto and U. Pindur, *Eur. J. Med. Chem.*, 2003, **38**, 189.
12 K. Inami and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 352.

