New mustard-linked 2-aryl-bis-benzimidazoles with anti-proliferative activity

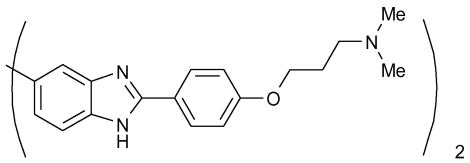
Christine Le Sann,[a] Anne Baron,[a] John Mann,*[a] Hendrik van den Berg,[b] Mekala Gunaratnam[c] and Stephen Neidle*[c]

Received 17th January 2006, Accepted 9th February 2006
First published as an Advance Article on the web 9th March 2006
DOI: 10.1039/b600567e

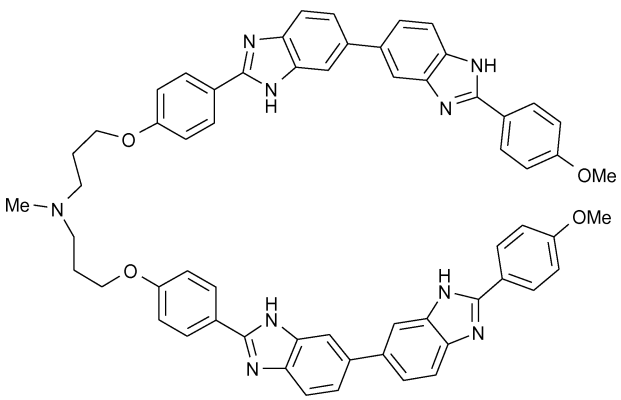
We describe new methodology for the synthesis of symmetric bis-benzimidazoles carrying 2-aryl moieties, including 2-[4-(3′-aminopropoxy)phenyl] and 2-[4-(3′-aminopropanamido)phenyl] substituents, together with the synthesis of novel hybrid molecules comprising bis-benzimidazoles in ester and amide combination with the *N*-mustard chlorambucil. The *in vitro* activities of these compounds against five cancer cell lines are also provided.

Introduction

We have recently described the synthesis and DNA-binding affinities of the symmetrical bis-benzimidazole **1**[1] and the dimeric bis-benzimidazole **2**.[2] The former exhibited high sequence-selectivity for four consecutive A/T base pairs in the dodecanucleotide sequence d[CGCGAATTCGCG], which is a model for longer oligonucleotide sequences in the A/T region of the minor groove. It also exhibited sub-micromolar activity against nine cancer cell lines *in vitro*, including IC_{50} values of 50 nM against MCF-7 (breast cancer) and 80 nM against H838 (lung cancer) cells.[3] In contrast, compound **2** exhibits levels of activity at least ten times less than exhibited by **1**, but has high affinity for ten base pairs[4] of the DNA sequence d[A.T]$_4$–[G.C]–[A.T]$_4$. This site-size selectivity compares well with the best selectivities reported by Dervan[5] and Bruice.[6] In this paper we report on bis-benzimidazole molecules with covalent-binding functionality and similar site-size requirements to compound **2**, that further define the structural requirements for potent anticancer activity in this series.



1



2

Results and discussion

We decided initially to investigate the coupling of the well-established alkylating agent chlorambucil to the bis-benzimidazole motif. Chlorambucil, in common with the majority of DNA alkylating compounds, has a preference for cross-linking *via* N7 of guanine in the DNA major groove,[7] but can also bind to phosphate groups and to N3 in the minor groove, as has been found with a chlorambucil–polyamide conjugate.[8] We have used computer modelling to provide a guide to the optimal length of linker groups between bis-benzimidazole moieties and chlorambucil, assuming alkylation at N3.

Molecular modelling has been used to develop a stereochemically-plausible structural model that would guide the synthesis of possible targets, and we have not at this stage employed extensive simulation methods to produce a set of detailed structures. The initial, energy-minimised structure of a DNA complex with compound **18** is shown in Fig. 1. The ligand binding site-size is 10 base pairs, with one arm of each alkylating group positioned to interact covalently with N3 sites on purines. Inter-strand cross-linking is also feasible. The ester–$(CH_2)_3$-linkers are in stereochemically acceptable, extended conformations, suggesting that this linker length is appropriate. We also find that the replacement of the ester group in the linkage by an amide group is similarly feasible, now with the potential for a hydrogen bond between the amide nitrogen atom and a thymine O2 atom (Fig. 2).

Our general synthetic strategy is shown in Scheme 1 and involved condensation of the commercially available 3,3′,4,4′-tetraaminobiphenyl **3** with 4-(3′-bromopropoxy)benzaldehyde **4** (prepared by a Mitsunobu reaction[9] between 3-bromopropanol and 4-hydroxybenzaldehyde) in the presence of the oxidising agent Oxone®.[10] This reagent proved to be more convenient than the nitrobenzene that we used in our earlier work.[1] The resultant bis-benzimidazole **5** was then reacted with the requisite amine

[a]*School of Chemistry, Queen's University Belfast, Stranmillis Road, Belfast, UK BT9 5AG. E-mail: j.mann@qub.ac.uk; Fax: +44 (0)28 9097 4890; Tel: +44 (0)28 9097 5525*
[b]*Department of Oncology, Queen's University Belfast, City Hospital, Belfast, UK BT9 7AR*
[c]*CRUK Biomolecular Structure Group, The School of Pharmacy, University of London, 29–39 Brunswick Square, London, UK WC1N 1AX. E-mail: stephen.neidle@pharmacy.ac.uk*

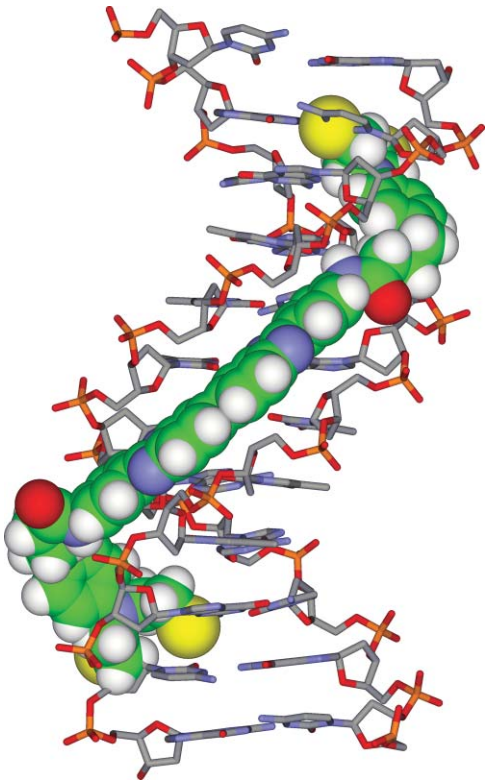


Fig. 1 Molecular model of compound **18** bound to a 12-mer DNA sequence. The ligand is shown in space-filling mode.

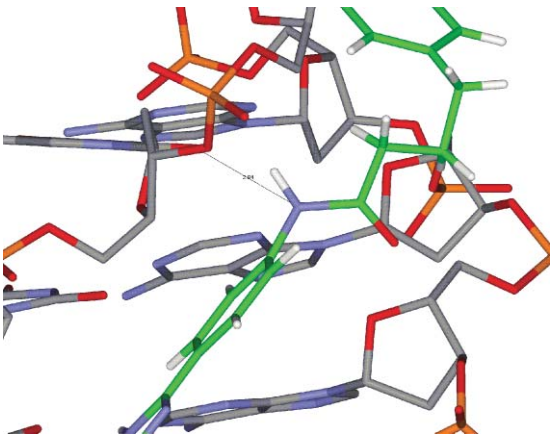


Fig. 2 View of the modelled structure of compound **19** bound to the 12-mer DNA sequence, showing a potential hydrogen bond between the linker amide nitrogen atom and a thymine O2 atom.

(piperidine or *N*-methylpiperazine) to provide the required bis-benzimidazoles **6a**,**b**. For pyrrolidine, a higher yield was obtained using a sequence involving Michael addition of the amine to methylacrylate, followed by reduction ($LiAlH_4$), chloride formation ($SOCl_2$), and reaction of the 3-(*N*-pyrrolidinyl)propyl chloride **7** with 4-hydroxybenzaldehyde (Cs_2CO_3, NaI)[11] to yield the 4-(*N*-pyrrolidinyl)propoxybenzaldehyde **8**, ready for condensation with 3,3′,4,4′-tetraaminobiphenyl in the presence of Oxone® to yield bis-benzimidazole **6c**. These compounds were tested *in vitro* against four cancer cell lines and the inhibition curves are shown in Fig. 3–5.

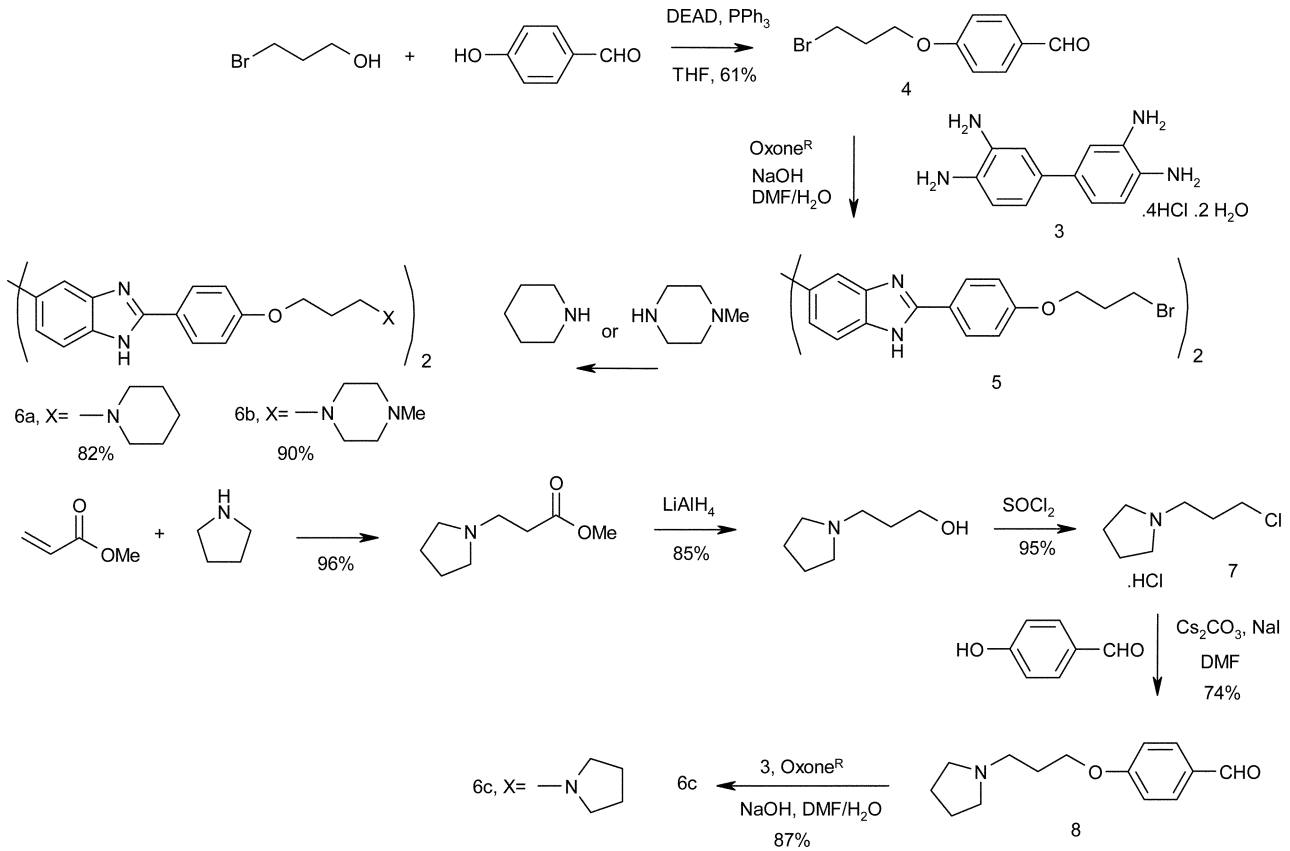
The relatively high activities of compounds **6a–c** prompted an examination of similar compounds, *e.g.* **9** wherein an aniline replaced the phenol, since such compounds would allow us to probe the effect of an O to N replacement in terms of efficacy of binding to the minor groove of DNA. These new compounds were prepared from the N–BOC derivative **10** of 4-aminobenzyl alcohol[12] which, after oxidation with MnO_2 was condensed with 3,3′,4,4′-tetraaminobiphenyl in the presence of Oxone® to produce the expected bis-benzimidazole **11**. Upon treatment with TFA this provided the key 2-(4′-aminophenyl)-bis-benzimidazole **12**, and this was reacted with chloropropionyl chloride and then dimethylamine[13] to yield the dimethylaminopropyl derivative **13**, and this chemistry is shown in Scheme 2. Unfortunately, we have not yet been able to synthesise the reduced compound **9**.

Alternative routes to the key 2-(4′-aminophenyl)-bis-benzimidazole **12** were explored as shown in Scheme 3. Thus condensation of the tetraaminobiphenyl **3** with 4′-nitrobenzaldehyde in the presence of Oxone® provided the 2-(4′-nitrophenyl)-bis-benzimidazole **14** (83% yield) and condensation of **3** with 4-fluorobenzaldehyde provided the 2-(4′-fluorophenyl-bis-benzimidazole) **15** (84%). Unfortunately, attempted reduction of **14** to produce **12** using a number of reducing agents (H_2/Pd, transfer hydrogenation with ammonium formate and Pd, Zn/HOAc, and Fe/HCl) all failed. In addition, attempted displacement of fluoride from compound **15** with 3-dimethylaminopropylamine was also non-productive. Despite these failures, this new methodology for producing 2-aryl-bis-benzimidazoles has proved to be both high-yielding and compatible with a variety of 4-substituted benzaldehydes.

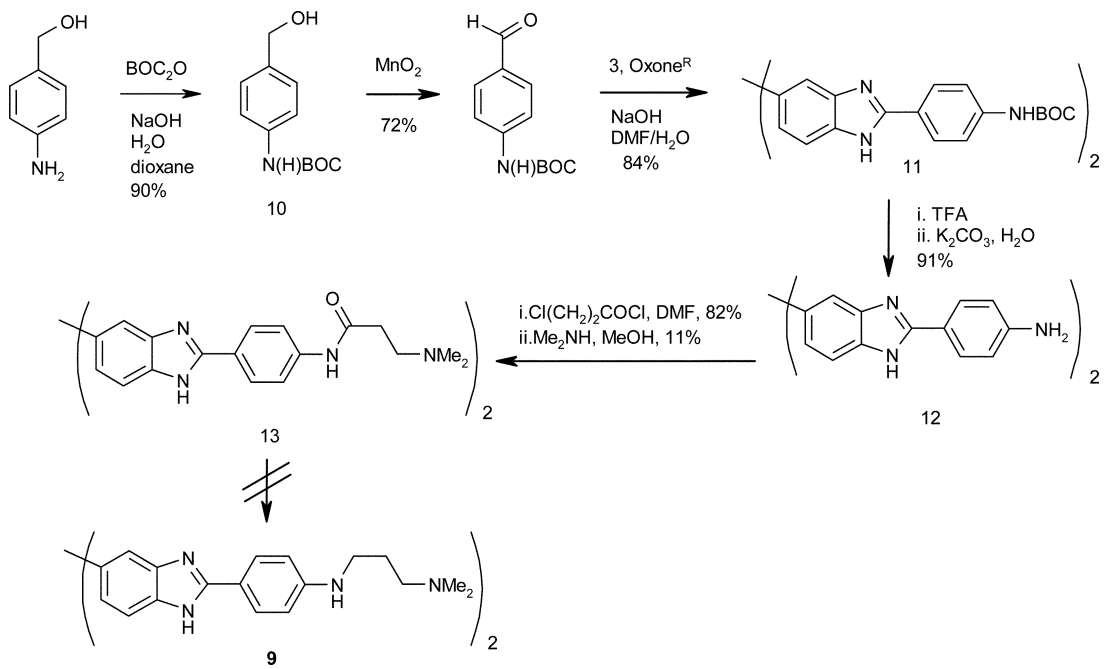
As a first foray into the production of hybrid molecules that combine a bis-benzimidazole (for binding to the DNA minor groove) and a known anticancer agent, we have prepared two novel hybrids that incorporate chlorambucil (Scheme 4). Thus reaction of 2-(4′-hydroxyphenyl)-bis-benzimidazole **16** (already prepared by us,[1] but now prepared more efficiently from **3** and 4-hydroxybenzaldehyde in conjunction with Oxone®) with the acid chloride of chlorambucil **17** (oxalyl chloride in DMF) provided the novel ester hybrid **18**. Similarly, reaction of 2-(4′-aminophenyl)-bis-benzimidazole **12** with the acid chloride of chlorambucil provided the novel amide hybrid **19**. Interestingly, this amide hybrid **19** was around 12-fold more cytotoxic (IC_{50} of 0.47 μM) than the ester hybrid **18** (IC_{50} 6.2 μM), possibly due to greater metabolic stability, though the possibility of more effective binding to the DNA minor groove by means of hydrogen bonding as shown in Fig. 2 cannot be excluded. A range of similar compounds with different side-chains will be prepared to explore further the SARs for these hybrids.

These IC_{50} values were obtained in MCF-7 breast carcinoma cell lines, in which the non-covalently-binding analogues **6a–c** have also been evaluated (Fig. 3–5). These also have an IC_{50} of *ca.* 1 μM, which suggests that the mustard group in compounds **18** and **19** is not contributing significantly to the cytotoxicity, probably on account of the known preference of this group for binding to N7 of guanine in the major groove of DNA, rather than the minor groove.

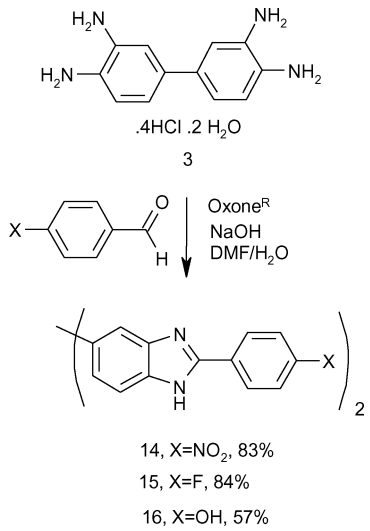
These results suggest that it could be profitable to attach alternative anticancer drugs with different modes of action (*e.g.* anthracyclinones) to the side-chains of bis-benzimidazoles in order to probe the efficacy of these molecules as drug delivery agents, and this work is in progress.



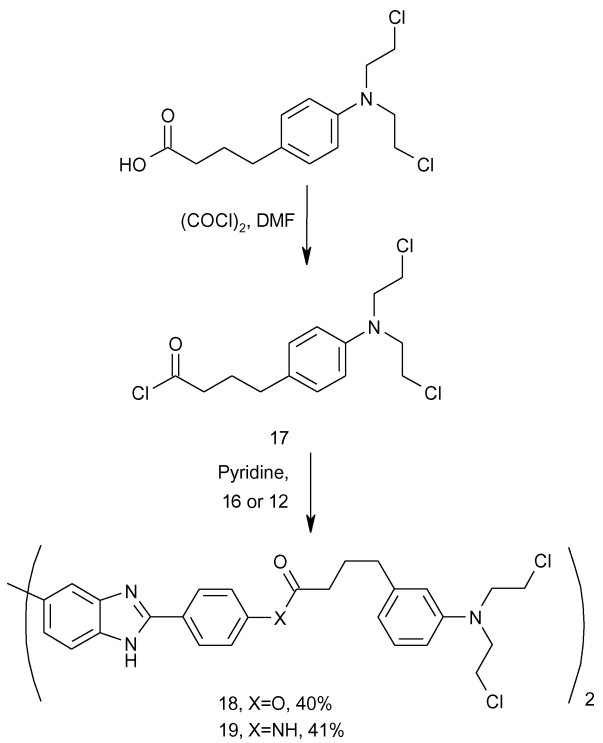
Scheme 1 Preparation of analogues of bis-benzimidazoles **1** using Oxone®.



Scheme 2 Preparation of a nitrogen isostere of **1**.



Scheme 3 An alternative method of preparing bis-benzimidazoles.



Scheme 4 Coupling of bis-benzimidazoles **12** and **16** with chlorambucil.

Experimental

General

^{1}H-NMR spectra were obtained using a Bruker 300 NMR Avance DPX spectrometer or a Bruker DRX Avance spectrometer at 300 MHz and 500 MHz respectively. ^{13}C-NMR spectra were recorded on a Bruker Avance DPX or Avance DRX spectrometer at a frequency of 75 MHz or 125 MHz respectively. ^{1}H–^{13}C-NMR correlation experiments were carried out on a Bruker Avance DRX spectrometer. Chemical shifts (δ) are given in parts per million (ppm). Mass spectra were recorded on a VG Autospec instrument

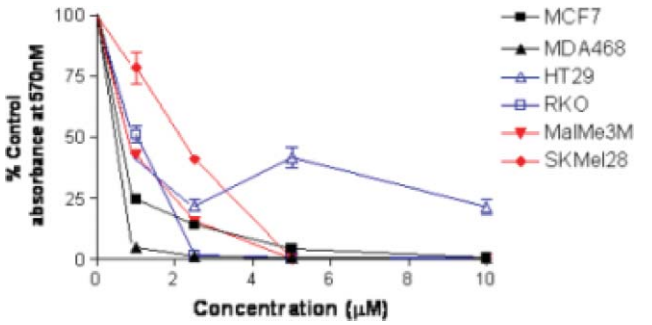


Fig. 3 The effect of piperidinyl analogue (compound **6a**) on the proliferation of human cancer cells *in vitro*.

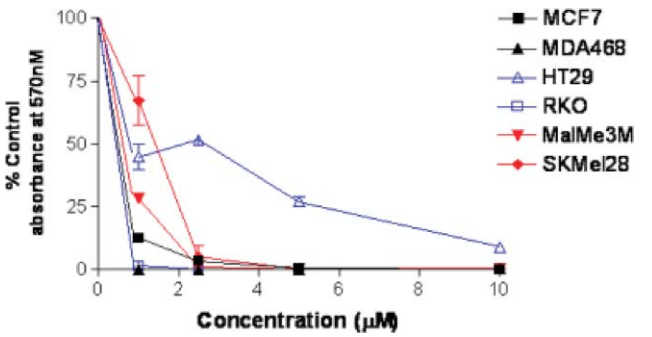


Fig. 4 The effect of *N*-methylpiperazinyl analogue (compound **6b**) on the proliferation of human cancer cells *in vitro*.

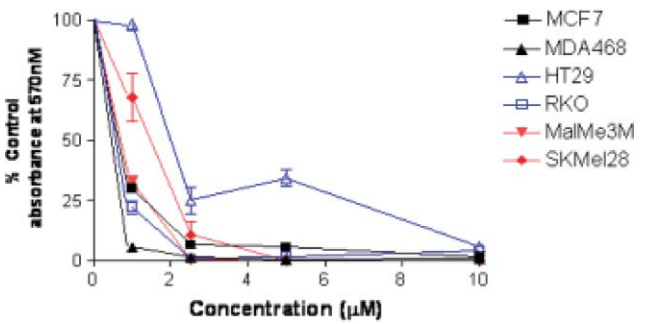


Fig. 5 The effect of pyrrolidyl analogue (compound **6c**) on the proliferation of human cancer cells *in vitro*. Human cells used were: MCF-7, MDA468-Breast carcinoma; HT29, RKO-Colon carcinoma; MalMe3M, SKMe128-Melanoma.

for electron impact (EI) and chemical ionisation (CI) techniques, or a VG Quattro spectrometer for the electrospray method. Infrared spectra were obtained on a Perkin Elmer Spectrum RX I FTIR System instrument. Melting points were taken on a Gallenkamp® apparatus and are uncorrected.

Methyl-3-pyrrolidin-1′-yl propanoate. A solution of methyl acrylate (1.21 g, 1.26 mL, 14.06 mmol) in dichloromethane (15 mL) at 0 °C was treated dropwise with pyrrolidine (1 g, 14.06 mmol). The mixture was left stirring and warming to room temperature for 4 h, and was then evaporated to yield the desired product as a pale yellow oil (2.12 g, 96%). ν_{max} (film)/cm^{-1} 2953, 2790, 1740, 1653, 1436, 1205; δ_H (300 MHz,

$CDCl_3$, Me_4Si) 1.80 (4H, m, $CH_2 \times 2$), 2.54 (4H, m, $CH_2N \times 2$), 2.54 (2H, t, *J* 7 Hz, CH_2CO), 2.76 (2 H, t, *J* 7 Hz, CH_2N), 3.68 (3H, s, CH_3); δ_C (125 MHz, $CDCl_3$, Me_4Si) 23.8 (CH_3), 34.3 ($CH_2 \times 2$), 51.8 (CH_2CO), 51.9 (CH_2N), 54.4 (CH_2N), 173.3 (C=O); *m*/*z* (CI) 158 (MH^+, 100%), 130 (47), 84 (30), 70 (15).

3-Pyrrolidin-1′-yl propan-1-ol. A suspension of lithium aluminium hydride (525 mg, 13.84 mmol) in dry diethyl ether (10 mL) at 0 °C was treated dropwise with a solution of methyl-3-pyrrolidin-1′-yl propanoate (2.12 g, 13.84 mmol) in diethyl ether (10 mL). The reaction mixture was left to stir for 1 hour. Water (525 μL) was then added slowly, followed by a solution of sodium hydroxide (15%, 525 μL), and finally water (1.58 mL). A white precipitate formed. Dichloromethane (15 mL) was added to solubilise the alcohol, the suspension was filtered and the filtrate was concentrated *in vacuo* to give the title product as a colourless oil (1.52 g, 85%). ν_{max} (film)/cm^{-1} 3367, 2944, 2877, 2803, 1457, 1134, 1065; δ_H (300 MHz, $CDCl_3$, Me_4Si) 1.56–1.78 (6H, m, 2-H_2 and $CH_2 \times 2$), 2.56 (4H, $CH_2N \times 2$), 2.73 (2 H, t, *J* 6 Hz, 3-H_2), 3.81 (2H, t, *J* 6 Hz, 1-H_2), 5.50 (1H, s, OH); δ_C (125 MHz, $CDCl_3$, Me_4Si) 23.7 ($CH_2 \times 2$), 29.5 (C-2), 54.6 ($CH_2N \times 2$), 56.8 (C-3), 65.1 (C-1); *m*/*z* (CI) 130 (MH^+, 100%), 84 (22), 58 (100).

4-(3′-Pyrrolidin-1″-ylpropoxy)benzaldehyde 8. A solution of 3-pyrrolidin-1′-yl propan-1-ol (250 mg, 1.93 mmol) in dichloromethane was cooled to 0 °C, then treated dropwise with thionyl chloride (3 mL, 41 mmol). The reaction mixture was stirred in the cold for 2.5 h. The solvent and excess reagent were then removed *in vacuo* and the solid residue was then dried under high vacuum to give the desired chloride **7** as a white solid that was used fresh and without further purification in the next step.

A solution of 4-hydroxybenzaldehyde (207 mg, 1.70 mmol) in dry DMF (15 mL) was treated with caesium carbonate (1.4 g, 4.3 mmol), followed after 5 minutes by sodium iodide (220 mg, 1.46 mmol) and the chloride **7** (270 mg, 1.46 mmol). The reaction mixture was stirred vigorously and heated to 100 °C, in the dark, for 10 hours. After cooling, the mixture was treated with a solution of sodium hydroxide (2 M, 50 mL). Sodium chloride was added to help the separation of the layers, and the aqueous mixture was extracted with ethyl acetate (4 × 50 mL). The combined organic layer was washed with brine (100 mL), and a solution of sodium hydroxide (2 M, 100 mL). It was then dried over magnesium sulfate, concentrated, and dried under high vacuum to give the desired product as an orange oil. (253 mg, 74%). ν_{max} (KBr disc)/cm^{-1} 2957, 2796, 1690, 1601, 1259 and 1159; δ_H (300 MHz, $CDCl_3$, Me_4Si) 1.82 (4H, m, $CH_2 \times 2$), 2.04 (2H, quintet, *J* 6.5 Hz, 2-H_2), 2.52 (4H, m, $CH_2N \times 2$), 2.63 (2H, t, *J* 7 Hz, 3-H_2), 4.12 (2H, t, *J* 6.5 Hz, 1-H_2), 7.04 (2H, d, *J* 7 Hz, 2′-H and 6′-H), 7.82 (2H, d, *J* 7 Hz, 3′-H and 5′-H), 9.87 (1H, s, CHO); δ_C (125 MHz, $CDCl_3$, Me_4Si) 23.8 (C-3″ and C-4″), 29.1 (C-2′), 53.3 (C-3′), 54.6 (C-2″ and C-5″), 67.2 (C-1′), 115.2 (C-3 and C-5), 130.2 (C-1), 132.36 (C-2 and C-6), 164.5 (C-4), 191.2 (C=O); *m*/*z* (CI^+) 234.1489 (MH^+, $C_{14}H_{20}NO_2$ requires 234.1494), 234 (100%), 112 (12), 84 (43).

4-(3′-Bromo-1″-propoxy)benzaldehyde 4. A solution of bromopropanol (500 mg, 3.6 mmol), triphenylphosphine (1.575 g, 5.4 mmol) and 4-hydroxybenzaldehyde (440 mg, 3.6 mmol) in dry tetrahydrofuran (15 mL) at 0 °C was treated with diethyl azodicarboxylate (DEAD) (0.62 mL, 5.4 mmol), dropwise. The reaction mixture was left to stir and warm to room temperature for 4 hours. The reaction mixture was then evaporated to dryness to give a thick yellow oil that was purified by column chromatography using 10% ethyl acetate in petroleum ether on neutralised silica gel. The title compound **4** was obtained as a pale yellow oil (536 mg, 61%). ν_{max} (film)/cm^{-1} 2940, 2880, 2828, 1688, 1600, 1468 and 1159; δ_H (300 MHz, $CDCl_3$, Me_4Si) 2.36 (2H, quintet, *J* 6 Hz, 2′-H_2), 3.62 (2H, t, *J* 6 Hz, 3′-H_2), 4.20 (2H, t, *J* 6 Hz, 1′-H_2), 7.02 (2H, d, *J* 8.5 Hz, 3-H and 5-H), 7.69 (2H, d, *J* 8.5 Hz, 2-H and 6-H), 9.89 (1H, s, CHO); δ_C (125 MHz, $CDCl_3$, Me_4Si) 25.7 (C-2′), 29.9 (C-3′), 66.0 (C-1′), 115.2 (C-3 and C-5), 130.6 (C-1), 132.4 (C-2 and C-6), 164.1 (C-1), 191.2 (CHO); *m*/*z* (EI) 241.9940 (M+, $C_{10}H_{11}O_2Br$ requires 241.9942), 244 (40%), 242 (41), 138 (40), 122 (47), 121 (100), 77 (23), 65 (33).

2,2-Bis[4′-(3″-pyrrolidinopropoxy)phenyl]-5,5-bi-1*H*-benzimidazole 6c. A solution of 3,3′-diaminobenzidine tetrahydrochloride dehydrate (200 mg, 541 mmol), in *N*,*N*-dimethylformamide (15 mL) was treated with sodium hydroxide (80 mg) in water (1 mL), followed by a solution of aldehyde **8** (252 mg, 1.082 mmol) in DMF (14 mL), and finally Oxone® (432 mg, 0.703 mmol). The reaction mixture was left stirring for 18 hours. A solution of potassium carbonate in water (1 M, 2 mL) was added, followed by water (30 mL). The solid that precipitated was then filtered under vacuum, and washed with water. It was then left to dry in the air. The title compound was obtained as a tan-coloured solid (301 mg, 87%). Mp 192–194 °C (decomp.); ν_{max} (KBr disc)/cm^{-1} 3400, 2959, 1612, 1254 and 1179; δ_H (500 MHz, DMSO-d_6) 1.66 (8H, m, 3‴-H_2 and 4‴-H_2), 1.89 (4H, quintet, *J* 7 Hz, 2″-H_2), 2.48 (8H, m, 2‴-H_2 and 5‴-H_2), 2.52 (4H, t, *J* 7 Hz, 3″-H_2), 4.08 (4H, t, *J* 6.5 Hz, 1″-H_2), 7.10 (4H, d, *J* 8.5 Hz, 3′-H_2 and 5′-H_2), 7.50 (2H, d, *J* 8.5 Hz, 7-H), 7.61 (2H, d, *J* 8.5 Hz, 6-H), 7.78 (2H, s, 4-H), 8.12 (4H, d, *J* 8.5 Hz, 2′-H and 6′-H); δ_C (125 MHz, DMSO-d_6) 24.0, 29.1, 53.1, 54.4, 67.0, 115.7, 122.4, 123.5, 128.9, 136.4, 152.9, 160.9; *m*/*z* (ES) 641.6 (M, 17), 321.6 (100).

General procedure for the preparation of 2,2-bis[4′-(3″-piperidinylpropoxy)phenyl]-5,5-bi-1*H*-benzimidazole 6a and 2,2-bis[4′-(3″-*N*-methylpiperazinylpropoxy)phenyl]-5,5-bi-1*H*-benzimidazole 6b

A solution of 3,3′-diaminobenzidine tetrahydrochloride dehydrate (1 equivalent), in *N*,*N*-dimethylformamide (29 mL) was treated with sodium hydroxide (3 equivalents) in water (1 mL), followed by a solution of 4-bromopropoxybenzaldehyde (2 equivalents) in DMF, and Oxone® (1.3 equivalents). The reaction mixture was left to stir for 18 hours, then excess methyl piperazine or piperidine was added. The mixture was left to stir for an additional hour. A solution of potassium carbonate in water (1 M, 1.5 mL/100 mg starting tetraamine) was added, followed by water (30 mL/100 mg starting tetraamine). The solid that precipitated was filtered under vacuum, and washed with water. It was then left to dry in air.

Bis-benzimidazole derivative 6a. The reaction was carried out following the procedure described above with tetraamine **3** (394 mg), aldehyde **4** (480 mg), Oxone® (782 mg), sodium hydroxide (163 mg), DMF (29 mL), water (1 mL), and piperidine (2.5 mL). The title compound was obtained as a tan-coloured solid (541 mg, 82%). Mp 164–166 °C (decomp.); ν_{max} (KBr disc)/cm^{-1} 3400, 3152, 2854, 2818, 1611, 1492, 1180, 1038 and 803; δ_H (500 MHz, DMSO-d_6) 1.37 (4H, m, 4″-H_2), 1.49 (8H, m, 3″-H_2

and 5″-H_2), 1.89 (4H, *J* 7 Hz, $CH_2CH_2CH_2$), 2.32 (8H, m, 2″-H_2 and 6″-H_2), 2.38 (4H, t, *J* 7 Hz, CH_2N), 4.07 (4H, t, *J* 6.5 Hz, CH_2O), 7.12 (4H, d, *J* 8.5 Hz, 3′-H and 5′-H), 7.53–7.91 (6H, m, 4′-H, 6′-H, 7′-H), 8.14 (4H, d, *J* 8.5 Hz, 2′-H and 6′-H); δ_C (75 MHz, DMSO-d_6) 24.5, 25.9, 26.6, 54.3, 55.4, 67.0, 115.1, 122.9, 128.4, 136.1, 145, 152, 160.4; *m/z* (ES) 669.5 (M^+, 100%).

Bis-benzimidazole derivative 6b. The reaction was carried out following the procedure described above with tetraamine **3** (448 mg), aldehyde **4** (540 mg), Oxone® (890 mg), sodium hydroxide (180 mg), DMF (29 mL), water (1 mL), and methylpiperazine (3 mL). The title compound was obtained as a tan-coloured solid (701 mg, 90%). Mp 158–160 °C (decomp.); ν_{max} (KBr disc)/cm^{-1} 3400, 2939, 2807, 1610, 1490, 1177, 1048, 837 and 808; δ_H (300 MHz, DMSO-d_6) 1.90 (4H, m, 2″-H_2), 2.19 (6H, s, CH_3N), 2.23–2.41 (20H, m, 3″-H_2 and CH_2N), 4.08 (4H, t, *J* 6 Hz, 1″-H_2), 7.11 (4H, d, *J* 8.5 Hz, 3′-H_2 and 5′-H_2), 7.52 (2H, d, *J* 8 Hz, 6-H), 7.64 (2H, d, *J* 8 Hz, 7-H), 7.80 (2H, s, 4-H), 8.13 (4H, d, *J* 8.5 Hz, 2′H and 6′-H); δ_C (125 MHz, DMSO-d_6) 26.5 (2C, 2″-C), 31.1 (10C, 3″-C and CH_2N), 54.8 (2C, 1″-C), 115.2 (4C, C-3′ and C-5′), 122.9 (2-C, C-6), 128.4 (2C, C-2′ and C-6′), 135.0 (4C, C-3a and C-7a), 152.3 (2C, C-2), 160.4 (2C, C-4′); *m/z* (ES) 699.4 (M^+, 28%), 680 (100), 617 (52).

General procedure for the preparation of bis-benzimidazoles 14, 15 and 16

A solution of 3,3′-diaminobenzidine tetrahydrochloride dehydrate (1 equivalent) in DMF (20 mL) was treated with sodium hydroxide (3–4 equivalents) in water (1 mL). Oxone® (1.3 equivalents) was then added to the resulting red solution, followed by 4-nitrobenzaldehyde, 4-fluorobenzaldehyde or 4-hydroxybenzaldehyde (2 equivalents) in solution in DMF (10 mL), using a syringe pump, over 3 hours. The reaction mixture was left to stir for 18 hours after the end of the addition. It was then treated with a solution of potassium carbonate in water (1 M, 1.5 mL/100 mg starting tetraamine), and poured in water (60 mL/200 mg starting tetraamine). The resulting suspension was filtered, washed with water and diethyl ether, and left to dry in air on the filter paper. It was subsequently collected and dried *in vacuo*.

2,2-Bis(4′-nitrophenyl)-5,5-bi-1*H*-benzimidazole 14. The reaction was carried out as described above using 3,3′-diaminobenzidine tetrahydrochloride dehydrate (200 mg), 4-nitrobenzaldehyde (152 mg), DMF (29 mL), water (1 mL), sodium hydroxide (80 mg) and Oxone® (400 mg). The product was precipitated in water after addition of a solution of potassium carbonate. The bis-benzimidazole **14** was obtained as a brown solid (200 mg, 83%). Mp >300 °C; ν_{max} (KBr disc)/cm^{-1} 3400, 1603, 1515, 1346 and 856; δ_H (500 MHz, DMSO-d_6) 7.69 (2H, d, *J* 8.5 Hz, 6-H), 7.81 (2H, d, *J* 8.5 Hz, 7-H), 7.98 (2H, s, 4-H), 8.49 (4H, d, *J* 8.5 Hz, 3′-H and 5′-H), 8.52 (4H, d, *J* 8.5 Hz, 2′-H and 6′-H); δ_C (125 MHz, DMSO-d_6) 121.7, 123.3, 126.3, 135.2, 135.3, 146.6, 148.9; *m/z* (ES) 477.0 (M^+, 25%), 223 (100).

2,2-Bis(4′-fluorophenyl)-5,5-bi-1*H*-benzimidazole 15. The reaction was carried out as described above using 3,3′-diaminobenzidine tetrahydrochloride dehydrate (200 mg), 4-fluorobenzaldehyde (125 mg), DMF (29 mL), water (1 mL), sodium hydroxide (80 mg) and Oxone® (400 mg). The product was precipitated in water after addition of a solution of potassium carbonate. The title compound was obtained as a brown solid (179 mg, 84%). Mp 158–160 °C (decomp.); ν_{max} (KBr disc)/cm^{-1} 3400, 3109, 1608, 1505, 1250 and 840; δ_H (300 MHz, DMSO-d_6) 7.45 (4H, m, 3′-H and 5′-H), 7.62 (2H, d, *J* 8 Hz, 6-H), 7.71 (2H, d, *J* 8 Hz, 7-H), 7.88 (2H, s, 4-H), 8.29 (4H, m, 2′-H and 6′-H); δ_C (125 MHz, DMSO-d_6) 115.8, 116.8, 117.2, 123.2, 126.92, 129.8, 129.9, 136.9, 137.7, 148.9, 151.6; 162; *m/z* (ES) 422.9 (M+, 90%), 318 (100), 172 (41).

4′,4′-(5,5-Bi-1*H*-benzimidazol)-2,2-diylbis(phenol) 16. The reaction was carried out as described above using 3,3′-diaminobenzidine tetrahydrochloride dehydrate (400 mg), 4-hydroxybenzaldehyde (246 mg), DMF (29 mL), water (1 mL), sodium hydroxide (160 mg) and Oxone® (800 mg). The product was precipitated in water without addition of the solution of potassium carbonate. The bis-benzimidazole **16** was obtained as a tan-coloured solid (238 mg, 57%). (Spectroscopic data identical to that previously described.[1])

4-[*N*-(*tert*-Butoxycarbonyl)amino]benzyl alcohol 10. (As synthesised in reference 12.)

4-[*N*-(*tert*-Butoxycarbonyl)amino]benzylaldehyde. A solution of alcohol **10** (200 mg, 0.95 mmol) in dry dichloromethane (5 mL) was treated with manganese dioxide (165 mg, 1.9 mmol). The reaction mixture was regularly monitored by TLC. Once no more progress of the reaction was observed, the mixture was filtered over Celite® to remove the residues of oxidising agent and the resulting filtrate was treated with an additional portion of manganese dioxide. After 2 h, TLC revealed that the reaction had gone to completion. The reaction mixture was filtered through Celite® and the filtrate was concentrated *in vacuo*. The title compound was obtained as a white crystalline solid (152 mg, 72%). Mp 130–132 °C (from ethyl acetate–hexane) (lit.[8] 138 °C); ν_{max} (KBr disc)/cm^{-1} 3253, 1734, 1674, 1150 and 836; δ_H (300 MHz, $CDCl_3$, Me_4Si) 1.57 (9H, s, $(CH_3)_3$), 7.56 (2H, d, *J* 8.5 Hz, 2-H and 6-H), 7.86 (2H, d, *J* 8.5 Hz, 3-H and 5-H), 9.92 (1H, s, CHO); *m/z* (CI) 222 (MH^+, 62%), 166 (100), 122 (9).

2,2-Bis(4′-[*N*-(*tert*-butoxycarbonyl)amino]phenyl)-5,5-bi-1*H*-benzimidazole 11. A solution of 3,3′diaminobenzidine tetrahydrochloride dehydrate (188 mg, 0.475 mmol) in DMF (15 mL) was treated with a solution of sodium hydroxide (75 mg) in water (1 mL), followed by a solution of BOC-protected aminobenzaldehyde (200 mg, 0.95 mmol) in DMF (14 mL), and Oxone® (376 mg, 0.61 mmol). A solution of potassium carbonate in water (1 M, 2 mL) was added, followed by water (30 mL). The solid that precipitated was filtered off under vacuum, and washed with water. It was then left to dry in air. The title compound was obtained as a yellowish solid (246 mg, 84%). Mp 252–254 °C (decomp.); ν_{max} (KBr disc)/cm^{-1} 3421, 2977, 1697, 1614 and 1158; δ_H (500 MHz, DMSO-d_6) 1.52 (18H, s, $(CH_3)_3$), 7.52–7.96 (6H, m, 4-H, 6-H, 7-H), 7.65 (4H, d, *J* 8.5 Hz, 3′-H and 5′-H), 8.10 (4H, d, *J* 8.5 Hz, 2′-H and 6′-H); δ_C (125 MHz, DMSO-d_6) 28.9, 80.3, 112.1, 118.9, 127.9, 142.0, 153.4, 257.4; *m/z* (ES) 617.5 (M^+, 100%), 309.4 (94).

4′,4′-(5,5-Bi-1*H*-benzimidazol)-2,2-diylbis(aniline) 12. The solid, **11**, was then treated with trifluoroacetic acid (5 mL) and left to stir for 20 minutes. The acid was evaporated at room temperature and the resulting solid was triturated in a solution of potassium carbonate (1 M, 10 mL). The resulting dark brown

solid was filtered off and dried under high vacuum (180 mg, 91%). Mp 242–244 °C (decomp.); ν_{max} (KBr disc)/cm^{-1} 3380, 1610, 1489 and 804; δ_H (500 MHz, DMSO-d_6) 5.62 (4H, s, NH_2), 6.67 (4H, d, J 8.5 Hz, 3′-H_2 and 5′-H_2), 7.44 (2H, dd, J 8, 1.5 Hz, 6-H), 7.55 (2H, d, J 8 Hz, 7-H), 7.87 (4H, d, J 8.5 Hz, 2′-H and 6′H); δ_C (125 MHz, DMSO-d_6) 114.5, 118.2, 121.9, 128.7, 136.1, 151.5, 154.1; m/z (ES) 417.04 (M^+, 100%).

Coupling of bis-benzimidazole 12 and bis-benzimidazole 16 with chlorambucil. Oxalyl chloride (50 μL, 0.58 mmol) was added dropwise to dry DMF (4 mL) at −20 °C under argon. A solution of chlorambucil (73 mg, 0.24 mmol) in dry DMF 90.5 mL) was added to the resulting slurry and the reaction mixture was left to warm to room temperature. After two hours, the solution was cooled to −20 °C and a solution of bis-benzimidazole **12** or **16** (50 mg, 0.12 mmol) in dry pyridine (0.5 mL) was added. The reaction mixture was left to warm to room temperature, then to stir for 48 h. The mixture was then poured into water (40 mL). After a few seconds a dark brown solid precipitated. It was filtered off and rinsed with water (3 × 10 mL). The dry solid was then triturated in acetone (1 mL). The resulting suspension was filtered, and the filtrate was concentrated under vacuum. Both adducts were prepared on the same scale.

2,2-Bis{4′-[4″-(*p*-*N*,*N*-(chloroethyl)aminophenyl)butanamido]-phenyl}-5,5-bi-1*H*-benzimidazole 19. The bis-benzimidazole **19** was obtained as a light brown solid (53 mg, 41%). Mp 108–112 °C; ν_{max} (KBr disc)/cm^{-1} 3400, 2957, 1666, 1613, 1518 and 804; δ_H (500 MHz, DMSO-d_6) 1.71 (4H, quintet, J 7 Hz, 3″-H_2), 2.17 (4H, t, J 7.5 Hz, 2″-H_2 or 4″-H_2), 2.45 (4H, J 7.5 Hz, 2″-H_2 or 4″-H_2), 3.66 (8H, m, NCH_2CH_2Cl), 5.58 (2H, s, NH), 6.64 (4H, d, J 8.5 Hz, Ar CHCN and CHCC), 6.66 (4H, d, J 8.5 Hz, Ar CHCC), 7.01 (4H, d, J 8.5 Hz, 3′-H and 5′-H), 7.43 (2H, dd, J 8.5, 1.5 Hz, 6-H), 7.53 (2H, d, J 8.5 Hz, 7-H), 7.70 (2H, s, 4-H), 7.85 (4H, d, J 8.5 Hz, 2′-H and 6′-H); δ_C (125 MHz, DMSO-d_6) 27.5, 33.9, 34.2, 42.1, 53.1, 112.8, 114.4, 118.2, 121.9, 128.7, 130.2, 130.6, 136.1, 145.4, 151.5, 153.9, 175.2; m/z (ES) 703.4 (M^+ − $C_{14}H_{16}NOCl_2$, 13%), 417 (100).

2,2-Bis{4′-[4″-(*p*-*N*,*N*-(chloroethyl)aminophenyl)butanoyl(oxy)]-phenyl}-5,5-bi-1*H*-benzimidazole 18. The bis-benzimidazole **18** was obtained as a golden solid (48 mg, 40%). Mp 111–114 °C; ν_{max} (KBr disc)/cm^{-1} 3401, 2929, 1612, 1179 and 803; δ_H (500 MHz, MeOD) 1.89 (4H, quintet, J 7 Hz, 3″-H_2), 2.27 (4H, t, J 7.5 Hz, 2″-H_2 or 4″-H_2), 2.55 (4H, J 7.5 Hz, 2″-H_2 or 4″-H_2), 3.71 (8H, m, NCH_2CH_2Cl), 6.69 (4H, d, J 8.5 Hz, Ar CHCN), 6.97 (4H, d, J 8.5 Hz, Ar CHCC), 7.06 (4H, d, J 8.5 Hz, 3′-H and 5′-H), 7.58 (2H, dd, J 8.5, 1.5 Hz, 6-H), 7.65 (2H, d, J 8.5 Hz, 7-H), 7.84 (2H, s, 4-H), 7.98 (4H, d, J 8.5 Hz, 2′-H and 6′-H); δ_C (75 MHz, DMSO-d_6) 26.8, 33.3, 33.6, 41.5, 52.6, 112.3, 121.9, 122.9, 128.0, 129.8, 130.1, 134.9, 135.9, 151.5, 152.0, 159.4, 171.9; m/z (ES) 998.4 (21%), 990.6 (M^+, 54).

2,2-Bis(4′-[3″-chloropropanamido]phenyl)-5,5-bi-1*H*-benzimidazole. A solution of chloropropionyl chloride (0.3 mL, 400 mg, 3.14 mmol) in dry DMF (3 mL) at −10 °C was treated dropwise with a solution of bis-benzimidazole **12** (118 mg, 0.28 mmol) in dry pyridine (2 mL). The reaction mixture was left stirring for 30 min. A solution of potassium carbonate (1 M, 5 mL) was then added. A precipitate formed and was filtered off under reduced pressure. The desired bis-benzimidazole was obtained as a light brown powder (138 mg, 82%). Mp > 250 °C ν_{max} (KBr disc)/cm^{-1} 3460, 1675, 1602, 841, 738; δ_H (300 MHz, DMSO-d_6) 2.90 (4H, t, J 7 Hz, 3″-H_2), 3.92 (4H, t, J 7 Hz, 2″-H_2), 7.54–7.86 (6H, m, 4-H, 6-H, 7-H), 7.81 (4H, d, J 8.5 Hz, 3′-H and 5′-H), 8.16 (4H, d, J 8.5 Hz, 2′-H and 6′-H); δ_C (75 MHz, DMSO-d_6) 57.2, 113.5, 119.5, 119.8, 124.3, 125.5, 127.5, 128.2, 132.1, 136.5, 145.7, 149.9, 163.8, 168.7; m/z (ES) 600.1/597.1 (M^+, 5/19%), 563.4/562.1 (25/100), 527 (48).

2,2-Bis(4′-[3″-*N*,*N*-dimethylaminopropanamido]phenyl)-5,5-bi-1*H*-benzimidazole 13. The reaction mixture containing bis-benzimidazole prepared above was treated with excess dimethylamine in solution in methanol (2 M, 2 mL) before work up. The reaction mixture was left stirring overnight at room temperature. Methanol was removed under vacuum, then a solution of potassium carbonate (1 M, 10 mL) was added, followed by water (60 mL). The precipitate that formed was filtered off and dried under reduced pressure. The desired bis-benzimidazole was obtained as a dark brown powder (18 mg, 11%). Mp > 250 °C, ν_{max} (KBr disc)/cm^{-1} 3423, 1664, 1602, 1536; δ_H (500 MHz, DMSO-d_6) 2.15 (12H, s, $N(CH_3)_2$), 2.48 (4H, m, 3″-H_2), 2.55 (4H, t, J 7 Hz, 2″-H_2), 7.51 (2H, d, J 8.5 Hz, 6-H), 7.62 (2H, d, J 8.5 Hz, 7-H), 7.75 (4H, d, J 8.5 Hz, 3′-H and 5′-H), 7.79 (2H, s, 4-H), 8.12 (4H, d, J 8.5 Hz, 2′-H and 6′-H); δ_C (75 MHz, DMSO-d_6) 35.7, 45.8, 55.9, 114.4, 119.9 (4 C), 122.5, 125.75, 127.9 (4 C), 136.5, 141.5, 152.8, 163.1, 171.4; m/z (ES) 615.3 (M^+, 12%), 308.2 (100), 279.6 (30).

Molecular modelling

The crystal structure[1] of the 12-mer duplex DNA, of sequence d(CGCGAATTCGCG), with the bis-benzimidazole compound **1** bound (PDB id number 1FTD), was used as a starting-point for modelling. The chlorambucil groups and linkers were added using the HYPERCHEM program,[14] and conformations manually adjusted in order to bring the mustard groups into close proximity to base edges. The conformation of the resulting ligands and DNA were minimised using the AMBER force-field as implemented in HYPERCHEM.

Sulforhodamine B (SRB) short term cytotoxicity assay

Short term growth inhibition was measured using the SRB assay as described previously.[1,3] Briefly, cells were seeded (4000 cells/wells) into the wells of a 96 well-plate in Dulbecco modified Eagle medium (DMEM) and incubated overnight as before to allow the cells to attach. Subsequently cells were exposed to freshly made compounds at increasing concentrations of 0, 0.1, 1, 5 and 25 μM in quadruplicate and incubated for a further 96 h. Following this the cells were fixed with ice-cold trichloroacetic acid (TCA) (10% w/v) for 30 min and stained with 0.4% SRB dissolved in 1% acetic acid for 15 min. All incubations were carried out at room temperature. The IC_{50} values, concentrations required to inhibit cell growth by 50%, were determined from the mean absorbances at 540 nm for each drug concentration expressed as a percentage of the control untreated well absorbance.

Acknowledgements

C. Le Sann acknowledges support from the EU Framework 6 Programme: Molecules and Cancer.

References

1 J. Mann, A. Baron, Y. Opoku-Boahen, E. Johansson, G. Parkinson, L. R. Kelland and S. Neidle, *J. Med. Chem.*, 2001, **44**, 138.
2 X.-W. Sun, S. Neidle and J. Mann, *Tetrahedron Lett.*, 2002, **43**, 7239.
3 A. Seaton, J. Mann, A. Baron, C. Bailly, S. Neidle and H. van den Berg, *Eur. J. Cancer*, 2003, **39**, 2548.
4 A. Joubert, X.-W. Sun, E. Johansson, C. Bailly, J. Mann and S. Neidle, *Biochemistry*, 2003, **42**, 5984.
5 P. B. Dervan, *Bioorg. Med. Chem.*, 2001, **9**, 2215.
6 A. L. Satz and T. C. Bruice, *Acc. Chem. Res.*, 2002, **35**, 86.
7 J. A. Hartley, in *Molecular Aspects of Anticancer Drug-DNA Interactions*, ed. S. Neidle and M. J. Waring, Macmillan Press, London, 1993, vol 1, p. 1.
8 Y.-D. Wang, J. Dziegielewski, N. R. Wurtz, B. Dziegielewska, P. B. Dervan and T. A. Beerman, *Nucleic Acids Res.*, 2003, **31**, 1208–1215.
9 Y. Ueda, J. M. Chuang, L. B. Crast and R. A. Partyka, *J. Antibiot.*, 1989, **42**, 1379.
10 P. L. Beaulieu, B. Haché and E. von Moos, *Synthesis*, 2003, 1683.
11 (*a*) B. F. Gisin, *Helv. Chim. Acta*, 1973, **56**, 1476; (*b*) C. Le Sann and A. D. Abell, *Aust. J. Chem.*, 2004, **57**, 355.
12 R. Rai and J. A. Katzenellenbogen, *J. Med. Chem.*, 1992, **35**, 4150.
13 P. J. Perry, S. M. Gowan, A. P. Reszka, P. Polucci, T. C. Jenkins, L. R. Kelland and S. Neidle, *J. Med. Chem.*, 1998, **41**, 3253.
14 HYPERCHEM, *release 7.1*, Hypercube Inc., Gainesville, Florida, 32601, USA, 2002.

