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Original Article

Synthesis of 2,5- and 3,5-diphenylpyridine derivatives for DNA recognition and cytotoxicity

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Abstract

A series of 2,5- and 3,5-diphenylpyridine derivatives was synthetised in high yields. A versatile chemical strategy allows the design of diphenylpyridines differently substituted with cationic or neutral side chains. The interaction of the molecules with DNA was investigated by biophysical and biochemical methods and an AT-binder (**20**) was characterised. A few cytotoxic molecules were identified but their antiproliferative activity does not correlate with DNA binding. Two compounds **18** and **22** showed significant antiproliferative activity and provide a novel route to potential anticancer agents.

Keywords: Pyridine; Cytotoxicity; Cycle cell analysis; DNA; Binding

1. Introduction

The structure, dynamics and expression of gene can be controlled with small molecules designed to fit into the minor groove of the DNA double helix. Different series of drugs susceptible to regulate replication and transcription of DNA have been developed in recent years, including the hairpin polyamides [1], bis-benzimidazoles derived from the dye Hoechst 33258 [2–4] as well as diphenylfuran derivatives typified by the lead product furamidine [5]. This latter series shows great promises as antiparasitic agents with the amidoxime prodrug DB289 which is currently undergoing clinical trials for the treatment of African trypanosomiasis and *Pneumocystis carinii* pneumonia [6]. This is the case also with certain bisamidino-carbazole dications which form tight DNA minor groove complexes and exhibit a pronounced selectivity for AT-rich sequences [7,8].

Recently, we showed that the replacement of the terminal amidino groups with substituted phenyl groups provides new series of DNA targeted cytotoxic agents [9]. To extend this strategy, we now report our study aimed at synthesising similar compounds for which the central condensed tricyclic nucleus (carbazole) has been de-condensed in the form of a phenyl–pyridine–phenyl core.

A series of bis-phenylpyridine derivatives substituted with different side chains, neutral or cationic, has been elaborated. Both 2,5 and 3,5 di-substituted pyridine derivatives were prepared to explore the influence of linear or curved compounds on DNA recognition and cytotoxicity. These compounds were obtained from dibromopyridines as indicated in Scheme 1.

2. Chemistry

The first step of the synthesis of the diphenypyridine core was performed starting from the commercially available 3,5-dibromopyridine **1** and the 3- or 4-methoxy- phenylboronic acids **2** and **3** using Suzuki biphasic conditions (Scheme 2). Previously, the nude 3,5-diphenylpyridine skeleton was obtained from phenylacetaldehyde and ammonia [10].

The double coupling procedure was also optimised [11–15] to give **4** or **5** in the presence of toluene, ethanol and an aque-

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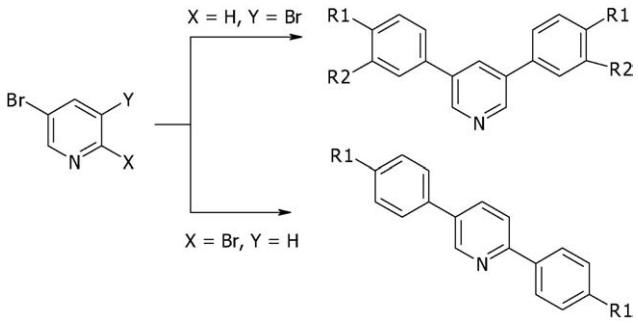
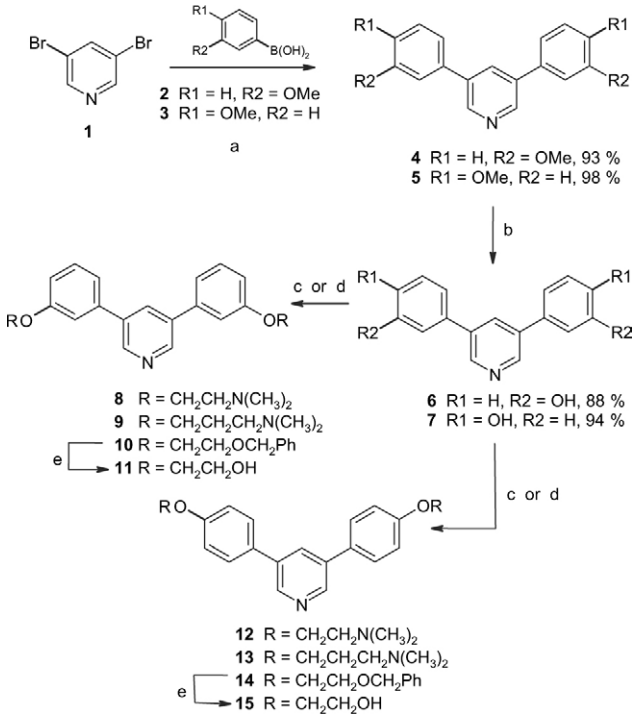


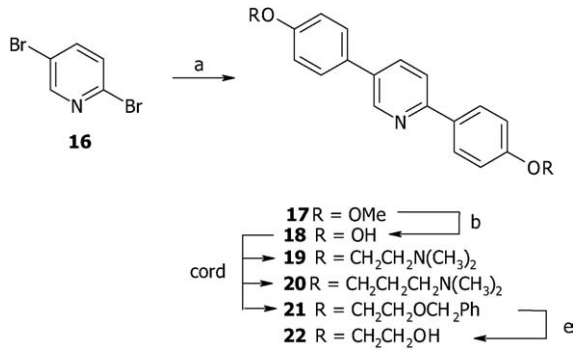
Scheme 1.



Scheme 2. a) Boronic acid 2 or 3 (2.6 eq.), toluene, EtOH, $NaHCO_3$ satd., 14 h.; b) BBr_3, CH_2Cl_2, 0 °C to r.t., 3 h.; c) 2-dimethyl aminoehtylchloride or 3-dimethylaminopropyl chloride hydrochloride salts (2.2 eq.), Cs_2CO_3 (4.4 eq.), DMF, r.t. to 100 °C, 2 h.; d) 2-bromoethoxymethylbenzene (2.2 eq.), Cs_2CO_3 (2.2 eq.), DMF, r.t. to 100 °C, 1 h.; e) BBr_3, CH_2Cl_2, 0 °C to r.t., 2 h.

ous saturated $NaHCO_3$ solution as solvents and base whereas 2.6 equivalents (eq.) of the desired boronic acid was added. After 14 h, the bis coupling reaction was completed affording compounds **4** and **5** in 93% and 98% yields from **1**, respectively. Deprotection of the methyl groups with BBr_3 led to compounds **6** and **7** in 88% and 94% yields, respectively.

The two products **6** and **7** were then *O*-alkylated with three different side chains using Williamson conditions. 2-Dimethyl aminoethylchloride or 3-dimethylaminopropyl chloride hydrochloride salts were treated with 2.2 eq. of Cs_2CO_3. This suspension was added to a solution of compounds **6** or **7** in dry DMF containing an additional amount of Cs_2CO_3 (2.2 eq.). After 2 h at 100 °C, the starting material fully disappeared and compounds **8**, **9**, **12** and **13** were produced in a range of 50–75%. Alkylations using 2-bromoethoxymethyl benzene were performed using only 2.2 eq. of base, starting from **6** or **7** for 1 h. In this case, the purification was easier



Scheme 3. For experimental conditions see Scheme 2.

and compounds **10** and **14** were obtained in 70% and 76%, respectively. Removing of the benzyl groups was first realised at room temperature under a slight pressure of hydrogen. After 12 h, the reaction was completed but several compounds were formed and the desired products **11** and **15** were obtained in low yields. Similar deprotection performed with BBr_3 proved to be faster (1 h) and more efficient. Compounds **11** and **15** were isolated in 88% and 94% yields, respectively.

The same reaction sequence was applied starting from 2,5-dibromopyridine **16** (Scheme 3). Suzuki coupling using boronic acid and further demethylation led to compound **18** in an 88% overall yield [15,16]. The three alkylation reactions using the hydrochloride salts of 2-dimethylamino ethylchloride or 3-dimethylamino propylchloride and 2-bromoethoxymethylbenzene afforded compounds **19**, **20** and **21** in 50%, 65%, 61% yields, respectively. Final debenzylation of **21** gave the alcohol **22** with an 87% yield.

3. Pharmacology

3.1. DNA interaction

The binding of the different compounds to double stranded DNA was first investigated by absorption spectroscopy but the spectral changes proved to be relatively modest. Small bathochromic and hypochromic effects were observed with all compounds upon interaction with calf thymus DNA.

Their affinity for DNA was quantified in two ways. Fluorescence quenching measurements of ethidium bromide-bound to DNA [17] were performed to as to produce displacement curves from which binding constants can be determined (K_{app} in Table 1). In parallel, we measured the variations of the helix-to-coil transition of the alternating polynucleotide poly(dAT)$_2$ in the presence of the drugs. The ΔT_m values ($T_m^{\text{drug–DNA complex}} - T_m^{\text{DNA alone}}$) increase with the drug/DNA ratio (Fig. 1) and by this method the relative affinity of the compounds for DNA can be compared (ΔT_m in Table 1). A satisfactory agreement was observed between the two techniques, despite the different experimental conditions and the different types of DNA used.

Unsurprisingly, relatively high ΔT_m values (> 10 °C) were measured with the compounds equipped with dimethy-

Table 1
DNA binding and cytotoxicity

Compounds	ΔT_m (°C)[a]	K_{app} (10^3 M^{-1})[b]	IC_{50} (μM)[c]
7	0	< 1	> 30
6	0	nd	13.9 ± 0.5
11	1	nd	> 40
9	13.2	17.9 ± 2.1	4.8 ± 0.8
15	nd	nd	> 70
13	17.8	24.8 ± 2.7	5.4 ± 0.5
8	12.3	60.4 ± 5.9	10.8 ± 1.5
12	1	< 1	16.3 ± 1.4
18	0	< 1	0.25 ± 0.2
19	11.3	1.1 ± 0.12	12.6 ± 3.1
20	15.4	12.2 ± 1.6	4.3 ± 0.3
22	0	< 1	0.4 ± 0.4

[a] T_m measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM $Na_2H_2PO_4$, 1 mM EDTA) using 20 μM drug and 20 μM calf thymus DNA (nucleotide concentration) with a heating rate of 1 °C min^{-1}.
[b] Apparent binding constant measured by fluorescence ($n = 3$).
[c] Drug concentration (μM) that inhibits CEM leukaemia cell growth by 50% after incubation in liquid medium for 72 h.

lamino side chains whereas the uncharged compounds showed little, if any, effect on T_m increase. There are clear differences between the linear and curved compounds. The *para*-3,5-bispyridine compound **13** provided higher ΔT_m than the related *meta*-3,5-bispyridine analogue **9** and the *para*-2,5-bispyridine linear molecule **20** showed an intermediate effect. However, in all cases the overall binding affinity measured by the conventional fluorescence quenching method remains very modest, in the 5×10^4 M^{-1} range (Table 1). The position of the dimethylaminoethyl side chains on the phenyl rings has a major impact on DNA interaction. The translation of the two alkyl chain from the meta position to the para position (e.g. **8** → **12**) practically abolishes the interaction with DNA. It is well known that the shape of such dicationic ligands plays a critical role for DNA recognition [18] but DNA can also accommodate molecules that are not optimally structured for DNA interaction [19].

The nature of the sequence preferentially recognised by these compounds was determined by DNase I footprinting using a 265-bp ^{32}P-labelled DNA restriction fragment. A typical footprinting gel is presented in Fig. 2 and differential cleavage plots obtained for compounds **19** and **20** are given in Fig. 3. The extent of DNase I protection varies considerably from one compound to another. The linear compounds such as **19** and **20** produced clearer footprints at AT-rich sequences than the curved molecules but here again, the low affinity of the compounds resulted in relatively discrete footprints, much less marked than those previously found with related diamidines for examples [18,19]. Nevertheless, compound **20** appeared as a AT-selective DNA ligand.

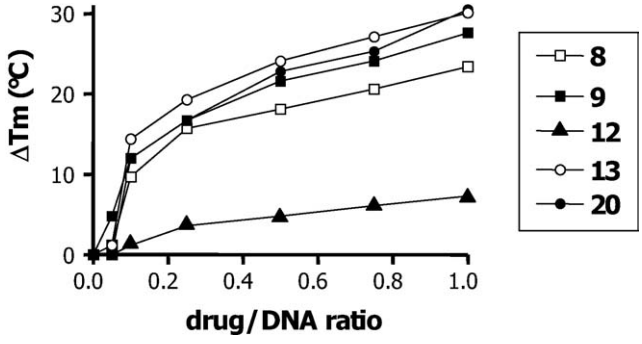


Fig. 1. Melting temperature variation ΔT_m ($T_m^{\text{drug–DNA complex}} - T_m^{\text{DNA alone}}$, in °C) of poly(dAT)$_2$ after incubation with the diphenylpyridines at increasing drug/DNA-phosphate. T_m measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA), in 1 cm quartz cuvettes at 260 nm with a heating rate of 1 °C min^{-1}. The T_m values were obtained from first-derivative plots.

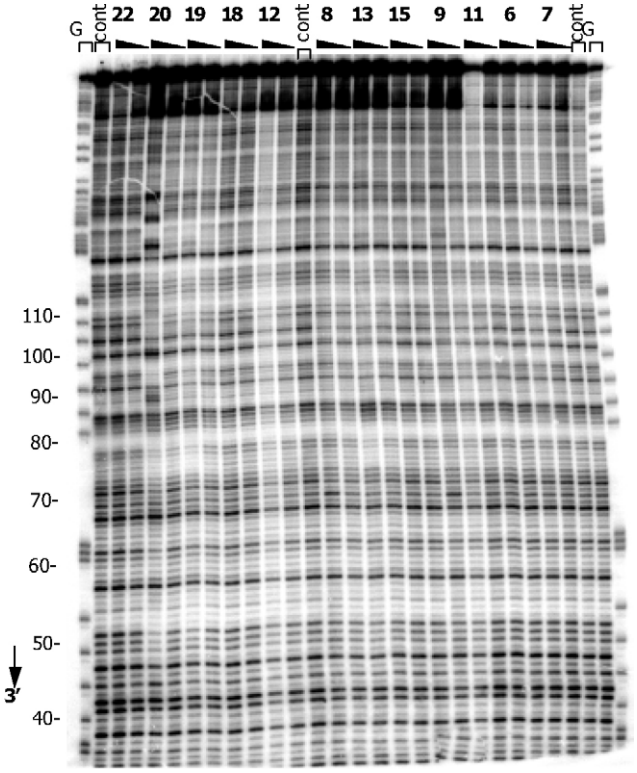


Fig. 2. DNase I footprinting of the diphenylpyridines on the 265-bp *Eco*RI-*Pvu*II DNA fragment from pBS. The DNA was 3′-end labelled with [α-^{32}P]dATP in the presence of AMV reverse transcriptase. The products of nuclease digestion were resolved on an 8% polyacrylamide gel containing 8 M urea. Each drug was tested at 5 and 20 μM. Control tracks (Cont) contained no drug. The tracks labelled "G" represent dimethylsulphate-piperidine markers specific for guanines.

3.2. Cytotoxicity

The antiproliferative activity of the molecules was investigated using human leukaemia CEM cells after treatment for 72 h with the test drugs. IC_{50} values are collated in Table 1.

There is clearly no direct correlation between DNA interaction and cytotoxicity in this series. Some of the DNA interacting ligands, such as **8** and **19** showed little effect on the growth of CEM cells whereas other molecules that do not bind well to DNA, such as **18** and **22** proved to be significantly cytotoxic. Sub-micro molar IC_{50} were found for these two latter compounds which will warrant further biological investigations to determine their mechanism of action and in vivo activity. The lack of cytotoxicity may in some cases be

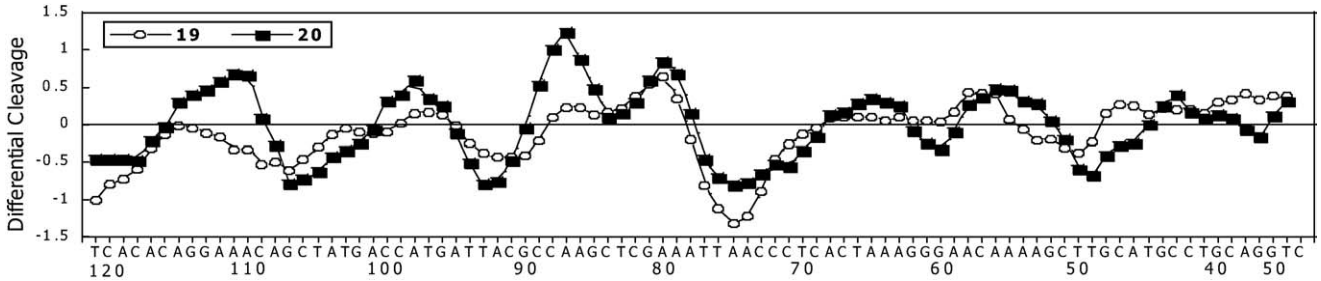


Fig. 3. Differential cleavage plots comparing the susceptibility of the 265-mer DNA fragment to DNase I cutting in the presence of 20 μM **19** or **20**. Negative values correspond to a ligand-protected site and positive values represent enhanced cleavage. Vertical scales are in units of ln(fa) – ln(fc), where fa is the fractional cleavage at any bond in the presence of the drug and fc is the fractional cleavage of the same bond in the control, given closely similar extents of overall digestion. Each line drawn represents a 3-bond running average of individual data points, calculated by averaging the value of ln(fa) – ln(fc) at any bond with those of its two nearest neighbours. Only the region of the restriction fragment analysed by densitometry is shown.

exploited for the search of antiparasitic compounds, as it is the case with certain diamidines. But our primary goal is to discover anticancer agents and in this respect, we will now focus our attention on compounds **18** and **22**, two uncharged bis-hydroxyl compounds with a common 2,5-bis-(4-oxyphenyl)-pyridine core. Interestingly, the linear extended tricyclic molecule **18** is considerably more cytotoxic than the V-shaped 2,4-bis-phenyl-pyridine analogues **6** and **7**.

In conclusion, we have successfully elaborated a series of extended diphenylpyridine derivatives. The architecture of the molecules can be manipulated so as to generate either DNA binding agents, which are generally non-cytotoxic, or non-DNA interacting compounds endowed with a potent cytotoxic potential. The archetype compound **18** represents a useful starting point to design potential anticancer agents.

4. Experimental section

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 250 instrument using $CDCl_3$ or DMSO-d_6. The chemical shifts are reported in ppm (δ scale) and all coupling constants (J) values are in hertz (Hz). The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet doublet). Melting points are uncorrected. IR absorption spectra were obtained on a Perkin Elmer PARAGON 1000 PC and values were reported in cm^{-1}. MS spectra (Ion Spray) were performed on a Perkin Elmer Sciex PI 300. Monitoring of the reactions was performed using silica gel TLC plates (silica Merck 60 F254). Spots were visualised by UV light at 254 and 356 nm. Chromatography columns were performed using silica gel 60 (0.063–0.200 mm, Merck).

4.1.1. General procedure A for Suzuki cross coupling reaction

A solution of dibromopyridine (1.0 mmol) and methoxyphenyl boronic acid derivatives (2.5 mmol) in a mixture of toluene (20.4 ml), ethanol (12.3 ml) and aqueous saturated $NaHCO_3$ solution (8 ml) was degassed under vigorous stirring by argon bubbling for 20 min. $Pd(PPh_3)_4$ (10 mol%) was then added and the mixture immediately transferred in a preheated oil bath and refluxed for 14 h. After cooling water was added (35 ml) and the aqueous layers were extracted with ethyl acetate (2 × 30 ml). The combined organic layers were successively washed with brine (35 ml), dried with $MgSO_4$ and filtered off. The solvents were removed under reduced pressure and the crude material was purified by flash chromatography.

4.1.2. General procedure B for demethylation

To a solution of methoxylated compound (1.0 mmol) in CH_2Cl_2 (7.2 ml) at 0 °C, BBr_3 (3.0 mmol) was added dropwise under stirring at room temperature, the reaction mixture was poured into ice, extracted with EtOAc then dried over $MgSO_4$, and filtered off. The solvents were removed under reduced pressure and the crude material was purified by flash chromatography.

4.1.3. General procedure C for the synthesis of aminoalkyl compounds

Two solutions were prepared separately under argon at room temperature. One contained the bis hydroxyphenyl pyridine compound (1.0 mmol) in dry DMF (10 ml) and Cs_2CO_3 (2.2 mmol), the other one contained the hydrochloride salt of chloroalkyldimethylamine (2.2 mmol) in dry DMF (10 ml) and another amount of Cs_2CO_3 (2.5 mmol). After 30 min, the second solution was added to the previous one solution. The mixture was warmed up to 100 °C for 2 h. After cooling, water was added and the aqueous layers were extracted successively with ethyl acetate (2 × 25 ml) and CH_2Cl_2 (2 × 25 ml). The combined organic layers were dried with $MgSO_4$, and filtered. The solvents were removed under reduced pressure and the crude material was purified by flash chromatography.

4.1.4. General procedure D for the synthesis of ethoxyalkyl compounds

A solution containing bis hydroxyphenyl pyridine (1.0 mmol) and Cs_2CO_3 (2.2 mmol) in DMF (10 ml) was

stirred under argon at room temperature for 30 min. 2-bromoethoxymethylbenzene (2.2 mmol) was then added in one portion and the mixture was warmed up to 100 °C for 1 h. After cooling, water was added and the aqueous layers were extracted successively with ethyl acetate (2 × 30 ml) and CH_2Cl_2 (2 × 30 ml). The combined organic layers were dried with $MgSO_4$, and filtered off. The solvents were removed under reduced pressure and the crude material was purified by flash chromatography.

*4.1.5. 3,5-Bis-(3-methoxyphenyl)-pyridine (**4**) [23]*

Following general **procedure A**. 3,5-Dibromopyridine **1** (4.22 mmol) and 3-methoxyphenyl boronic acid **2** (10.97 mmol), toluene (86 ml), ethanol (52 ml) and aqueous saturated $NaHCO_3$ solution (34 ml). Flash chromatography (dichloromethane/methanol 96:4); compound **4** was obtained as a white solid (1.14 g, 93%). Rf (dichloromethane/methanol 96:4): 0.58; m.p. 111 °C; IR (KBr, cm^{-1}) ν 3041, 1606, 1584, 1413, 1284, 1221, 1047, 1024, 867, 785; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.85 (s, 6H), 6.95 (dd, 2H, J = 2.2, 7.9 Hz), 7.15 (t, 2H, J = 2.2 Hz), 7.20 (d, 2H, J = 7.9 Hz), 7.39 (t, 2H, J = 7.8 Hz), 8.02 (t, 1H, J = 2.2 Hz), 8.81 (d, 2H, J = 2.2 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 55.5 (2 × CH_3), 113.2 (2 × CH), 113.7 (2 × CH), 119.8 (2 × CH), 130.3 (2 × CH), 133.1 (CH), 136.6 (2 × Cq), 139.3 (2 × Cq), 147.2 (2 × CH), 160.3 (2 × Cq); MS (IS): 292 $(M + 1)^+$; Anal. Calc. for $C_{19}H_{17}NO_2$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.70; H, 5.88; N, 4.94.

*4.1.6. 3,5-Bis-(4-methoxyphenyl)-pyridine (**5**) [11–15]*

Following general **procedure A**. 3,5-Dibromopyridine **1** (4.22 mmol) and 4-methoxyphenyl boronic acid **3** (10.97 mmol), toluene (86 ml), ethanol (52 ml) and aqueous saturated $NaHCO_3$ solution (34 ml). Flash chromatography (dichloromethane/methanol 96:4); compound **5** was obtained as a white solid (2.41 g, 98%). Rf (dichloromethane/methanol 96:4): 0.41; m.p. 198 °C; IR (KBr, cm^{-1}) ν 3029, 1695, 1576, 1453, 1374, 1070, 745; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.87 (s, 6H), 7.03 (d, 4H, J = 8.7 Hz), 7.58 (d, 4H, J = 8.7 Hz), 7.96 (t, 1H, J = 2.0 Hz), 8.73 (d, 2H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 55.6 (2 × CH_3), 114.7 (4 × CH), 128.5 (4 × CH), 130.4 (CH), 132.1 (2 × Cq), 146.2 (2 × CH), 160.0 (4 × Cq); MS (IS): 292 $(M + 1)^+$; Anal. Calc. for $C_{19}H_{17}NO_2$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.11; H, 6.03; N, 4.65.

*4.1.7. 3,5-Bis-(3-hydroxyphenyl)-pyridine (**6**) [11]*

Following general **procedure B**. Compound **4** (800 mg, 2.75 mmol), CH_2Cl_2 (20 ml), BBr_3 (8.5 ml, 1 M in CH_2Cl_2, 8.52 mmol), 3 h, flash chromatography (dichloromethane/methanol 96:4); compound **6** was obtained as a white solid (720 mg, 98%). Rf (dichloromethane/methanol 9:1): 0.43; m.p. > 250 °C; IR (KBr, cm^{-1}) ν 3202, 1613, 1586, 1491, 1325, 1288, 1198, 852; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 6.97 (dd, 2H, J = 3.6, 6.5 Hz), 7.32 (s, 2H), 7.38 (d, 4H, J = 6.5 Hz), 8.85 (s, 1H), 9.11 (s, 2H); ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 114.5 (2 × CH), 116.8 (2 × CH), 118.4 (2 × CH), 130.4 (2 × CH), 135.2 (2 × Cq), 139.0 (2 × Cq), 138.9 (2CH), 139.8 (CH), 158.2 (2 × Cq); MS (IS): 264 $(M + 1)^+$; Anal. Calc. for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.84; H, 5.17; N, 5.16.

*4.1.8. 3,5-Bis-(4-hydroxyphenyl)-pyridine (**7**) [11,15]*

Following general **procedure B**. Compound **5** (1 g, 3.73 mmol), CH_2Cl_2 (20 ml), BBr_3 (15.67 ml, 1 M in CH_2Cl_2, 15.67 mmol), 3 h, flash chromatography (petroleum ether/ethyl acetate 1:9); compound **7** was obtained as a white solid (900 mg, 80%). Rf (petroleum ether/ethyl acetate 1:9): 0.51; m.p. > 250 °C; IR (KBr, cm^{-1}) ν 3216, 1607, 1588, 1511, 1217, 1275, 1183, 829; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 6.95 (d, 4H, J = 8.3 Hz), 7.84 (d, 4H, J = 8.5 Hz), 8.82 (s, 1H), 9.00 (s, 2H); ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 116.2 (4 × CH), 124.4 (2 × Cq), 129.1 (4 × CH), 136.6 (2 × CH), 137.7 (CH), 138.9 (2 × Cq), 159.2 (2 × Cq); MS (IS): 264 $(M + 1)^+$; Anal. Calc. for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.79; H, 4.83; N, 5.44.

*4.1.9. 3,5-Bis-[3-(2-dimethylamino-ethoxy)-phenyl]-pyridine (**8**)*

Following general **procedure C**. Compound **6** (100 mg, 0.38 mmol), Cs_2CO_3 (273 mg, 0.84 mmol), 2-chloroethyldimethylamine hydrochloride (121 mg, 0.84 mmol), Cs_2CO_3 (310 mg, 0.95 mmol), flash chromatography (dichloromethane/methanol/triethylamine 1:1:0.01); compound **8** was obtained as a white solid (105 mg, 68%). Rf (dichloromethane/methanol/triethylamine 1:1:0.01): 0.24; m.p. 184 °C; IR (KBr, cm^{-1}) ν 2942, 2760, 1585, 1417, 1214, 1165, 1042, 790; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.37 (s, 12H), 2.79 (t, 4H, J = 5.6 Hz), 4.16 (t, 4H, J = 5.6 Hz), 6.98 (dd, 2H, J = 2.0, 7.6 Hz), 7.20–7.24 (m, 4H), 7.40 (t, 2H, J = 8.2 Hz), 8.03 (t, 1H, J = 2.2 Hz), 8.81 (d, 2H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 45.8 (4 × CH_3), 58.2 (2 × CH_2), 66.0 (2 × CH_2), 113.9 (2 × CH), 114.2 (2 × CH), 119.9 (2 × CH), 130.3 (2 × CH), 133.1 (CH), 136.5 (2 × Cq), 139.2 (2 × Cq), 147.1 (2 × CH), 159.5 (2 × Cq); MS (IS): 406 $(M + 1)^+$; Anal. Calc. for $C_{25}H_{31}N_3O_2$: C, 74.04; H, 7.70; N, 10.36. Found: C, 73.76; H, 7.88; N, 10.51.

*4.1.10. 3,5-[3-(3-Dimethylamino-propoxy)-phenyl]-pyridine (**9**)*

Following general procedure C. Compound **6** (100 mg, 0.38 mmol), Cs_2CO_3 (273 mg, 0.84 mmol), 3-chloropropyldimethylamine hydrochloride (133 mg, 0.84 mmol), Cs_2CO_3 (310 mg, 0.95 mmol), flash chromatography (dichloromethane/methanol/triethylamine 40:60:0.01); Compound **9** was obtained as a white solid (124 mg, 75%). Rf (dichloromethane/methanol/triethylamine 40:60:0.01): 0.16; m.p. 61 °C; IR (KBr, cm^{-1}) ν 2942, 2807, 2768, 1606, 1581, 1457, 1289, 1206, 1063, 783; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.99 (q, 4H, J = 6.8 Hz), 2.26 (s, 12H), 2.48 (t, 4H, J = 7.4 Hz), 4.09 (t, 4H, J = 6.4 Hz), 6.96 (dd, 2H, J = 1.8, 8.1 Hz), 7.17–7.22 (m, 4H), 7.39 (t, 2H, J = 7.9 Hz), 8.02 (t, 1H, J = 1.8 Hz),

8.80 (d, 2H, J = 1.8 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 27.6 (2 × CH_2), 45.6 (4 × CH_3), 56.4 (2 × CH_2), 66.4 (2 × CH_2), 113.7 (2 × CH), 114.1 (2 × CH), 119.6 (2 × CH), 130.2 (2 × CH), 132.9 (CH), 136.5 (2 × Cq), 139.1 (2 × Cq), 147.1 (2 × CH), 159.6 (2 × Cq); MS (IS): 434 $(M + 1)^+$; Anal. Calc. for $C_{27}H_{35}N_3O_2$: C, 74.79; H, 8.14; N, 9.69. Found: C, 75.13; H, 8.07; N, 9.54.

*4.1.11. 3,5-Bis-[3-(2-benzyloxy-ethoxy)-phenyl]-pyridine (**10**)*

Following general **procedure D**. Compound **6** (904 mg, 3.44 mmol), Cs_2CO_3 (2.5 g, 7.56 mmol), DMF (30 ml), 2-bromoethoxymethyl benzene (1.2 ml, 7.56 mmol), flash chromatography (ethyl acetate/ether petroleum 4:6 to 1:1); compound **10** was obtained as a white solid (1.38 g, 76%). Rf (ethyl acetate/ether petroleum 6:4): 0.52; m.p. 92 °C; IR (KBr, cm^{-1}) ν 2870, 1594, 1451, 1288, 1203, 1128, 802; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.86 (dd, 4H, J = 3.6, 5.0 Hz), 4.22 (t, 4H, J = 4.6 Hz), 4.65 (s, 4H), 6.98 (dd, 2H, J = 2.2, 7.9 Hz), 7.19–7.43 (m, 16H), 8.01 (t, 1H, J = 2.0 Hz), 8.80 (d, 2H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 67.7 (2 × CH_2), 68.6 (2 × CH_2), 73.5 (2 × CH_2), 113.9 (4 × CH), 114.3 (2 × CH), 120.0 (2 × CH), 127.8 (4 × CH), 128.5 (4 × CH), 130.2 (2 × CH), 133.0 (CH), 136.5 (2 × Cq), 138.1 (2 × Cq), 139.2 (2 × Cq), 147.2 (2 × CH), 159.5 (2 × Cq); MS (IS): 532 $(M + 1)^+$; Anal. Calc. for $C_{35}H_{33}NO_4$: C, 79.07; H, 6.26; N, 2.63. Found: C, 79.32; H, 6.15; N, 2.51.

*4.1.12. 3,5-Bis-[3-(2-hydroxy-ethoxy)-phenyl]-pyridine (**11**)*

Following general **procedure D**. Compound **10** (103 mg, 0.19 mmol), CH_2Cl_2 (15 ml), BBr_3 (0.97 ml, 1 M in CH_2Cl_2, 0.97 mmol), 2 h, flash chromatography (dichloromethane/methanol 90:10); compound **11** was obtained as a white solid (60 mg, 88%). Rf (dichloromethane/methanol 90:10): 0.52, m.p. 145 °C; IR (KBr, cm^{-1}) ν 3386, 2604, 1588, 1572, 1404, 1224, 1050, 782; ^{1}H NMR (DMSO-d_6, D_2O, 250 MHz): δ 3.76 (t, 4H, J = 5.2 Hz), 4.12 (t, 4H, J = 4.8 Hz), 7.06–7.11 (m, 2H), 7.47–7.50 (m, 6H), 8.76 (s, 1H), 9.08 (s, 2H); ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 59.5 (2 × CH_2), 69.8 (2 × CH_2), 113.6 (2 × CH), 115.8 (2 × CH), 119.8 (2 × CH), 130.4 (2 × CH), 135.8 (2 × Cq), 138.1 (2 × Cq), 138.9 (CH), 140.6 (2 × CH), 159.4 (2 × Cq); MS (IS): 352 $(M + 1)^+$; Anal. Calc. for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.42; H, 5.96; N, 4.14.

*4.1.13. 3,5-Bis-[4-(2-dimethylamino-ethoxy)-phenyl]-pyridine (**12**)*

Following general **procedure C**. Compound **7** (100 mg, 0.38 mmol), Cs_2CO_3 (273 mg, 0.84 mmol), 2-chloroethyldimethylamine hydrochloride (121 mg, 0.84 mmol), Cs_2CO_3 (310 mg, 0.95 mmol), flash chromatography (dichloromethane/methanol/triethylamine 4:6:0.01); compound **12** was obtained as a white solid (103 mg, 67%). Rf (dichloromethane/methanol/triethylamine 4:6:0.01): 0.28; m.p. 158 °C; IR (KBr, cm^{-1}) ν 2924, 2817, 2775, 2361, 1607, 1512, 1454, 1287, 1244, 1032, 830; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.37 (s, 12H), 2.78 (t, 4H, J = 5.6 Hz), 4.14 (t, 4H, J = 5.6 Hz), 7.04 (d, 4H, J = 8.8 Hz), 7.56 (d, 4H, J = 8.5 Hz), 7.95 (t, 1H, J = 2.2 Hz), 8.73 (d, 2H, J = 2.2 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 46.0 (4 × CH_3), 58.3 (2 × CH_2), 66.1 (2 × CH_2), 115.3 (4 × CH), 128.4 (4 × CH), 130.5 (2 × Cq), 132.0 (CH), 136.2 (2 × Cq), 146.2 (2 × CH), 159.1 (2 × Cq); MS (IS): 406 $(M + 1)^+$; Anal. Calc. for $C_{25}H_{31}N_3O_2$: C, 74.04; H, 7.70; N, 10.36. Found: C, 74.32; H, 7.86; N, 10.20.

*4.1.14. 3,5-Bis-[4-(3-dimethylamino-propoxy)-phenyl]-pyridine (**13**)*

Following general **procedure C**. Compound **7** (100 mg, 0.38 mmol), Cs_2CO_3 (273 mg, 0.84 mmol), 3-chloropropyldimethylamine hydrochloride (133 mg, 0.84 mmol), Cs_2CO_3 (310 mg, 0.95 mmol), flash chromatography (dichloromethane/methanol/triethylamine 1:1:0.01); compound **13** was obtained as a white solid (83 mg, 50%). Rf (dichloromethane/methanol/triethylamine 1:1:0.01): 0.22; m.p. 104 °C; IR (KBr, cm^{-1}) ν 2942, 2817, 2764, 1607, 1513, 1289, 1250, 1055, 821; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.87 (q, 4H, J = 6.4 Hz), 2.16 (s, 12H), 2.37 (t, 4H, J = 7.1 Hz), 4.06 (t, 4H, J = 6.5 Hz), 7.06 (d, 4H, J = 8.5 Hz), 7.77 (d, 4H, J = 8.7 Hz), 8.18 (s, 1H), 8.77 (d, 2H, J = 2.2 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 27.6 (2 × CH_2), 45.6 (4 × CH_3), 56.5 (2 × CH_2), 66.5 (2 × CH_2), 115.2 (4 × CH), 128.4 (4 × CH), 130.3 (2 × Cq), 132.0 (CH), 136.3 (2 × Cq), 146.2 (2 × CH), 159.4 (2 × Cq); MS (IS): 434 $(M + 1)^+$ Anal. Calc. for $C_{27}H_{35}N_3O_2$: C, 74.79; H, 8.14; N, 9.69. Found: C, 75.09; H, 8.03; N, 9.47.

*4.1.15. 3,5-Bis-[4-(2-benzyloxy-ethoxy)-phenyl]-pyridine (**14**)*

Following general **procedure D**. Compound **7** (100 mg, 0.38 mmol), Cs_2CO_3 (272 mg, 0.84 mmol), DMF (10 ml), 2-bromoethoxymethylbenzene (132 µl, 0.84 mmol), flash chromatography (ethyl acetate/ether petroleum 1:1); compound **14** was obtained as a white solid (141 mg, 70%). Rf (ethyl acetate/ether petroleum 4:6): 0.28; m.p. 151 °C; IR (KBr, cm^{-1}) ν 2878, 1608, 1513, 1453, 1287, 1244, 833, 747; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.86 (dd, 4H, J = 3.6, 5.0 Hz), 4.21 (dd, 4H, J = 4.8, 6.1 Hz), 4.65 (s, 4H), 7.05 (d, 4H, J = 8.7 Hz), 7.40–7.29 (m, 10H), 7.56 (d, 4H, J = 8.9 Hz), 7.96 (t, 1H, J = 2.0 Hz), 8.73 (s, 2H); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 67.7 (2 × CH_2), 68.6 (2 × CH_2), 73.6 (2 × CH_2), 115.4 (4 × CH), 127.9 (5 × CH), 128.4 (4 × CH), 128.6 (5 × CH), 130.5 (2 × Cq), 132.1 (CH), 136.3 (2 × Cq), 138.1 (2 × Cq), 146.1 (2 × CH), 159.2 (2 × Cq); MS (IS): 532 $(M + 1)^+$; Anal. Calc. for $C_{35}H_{33}NO_4$: C, 79.07; H, 6.26; N, 2.63. Found: C, 78.82; H, 6.40; N, 2.54.

*4.1.16. 3,5-Bis-[4-(2-hydroxy-ethoxy)-phenyl]-pyridine (**15**)*

Following general **procedure B**. Compound **14** (144 mg, 0.27 mmol), CH_2Cl_2 (15 ml), BBr_3 (1.35 ml, 1 M in CH_2Cl_2, 1.35 mmol), 2 h, flash chromatography (dichloromethane/

methanol 90:10) compound **15** was obtained as a white solid (90 mg, 94%). Rf (dichloromethane/methanol 90:10): 0.35; m.p. 198 °C; IR (KBr, cm^{-1}) *v* 3312, 1607, 1512, 1291, 1248, 1184, 1051, 828; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 3.74 (t, 4H, *J* = 5.2 Hz), 4.05 (t, 4H, *J* = 5.0 Hz), 7.08 (d, 4H, *J* = 8.7 Hz), 7.77 (d, 4H, *J* = 8.7 Hz), 8.19 (t, 1H, *J* = 2.2 Hz), 8.78 (d, 2H, *J* = 2.0 Hz); ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 59.5 (2 × CH_2), 69.8 (2 × CH_2), 115.2 (4 × CH), 127.1 (2 × Cq), 128.8 (4 × CH), 135.7 (CH), 137.3 (2 × Cq), 140.2 (2 × CH), 159.7 (2 × Cq); MS (IS): 352 $(M + 1)^+$; Anal. Calc. for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.97; H, 5.91; N, 4.14.

*4.1.17. 2,5-Bis-(4-methoxyphenyl)-pyridine (**17**) [11,16]*

Following general **procedure A**. 2,5-Dibromopyridine **16** (4.22 mmol) and 4-methoxyphenyl boronic acid **3** (10.97 mmol), toluene (86 ml), ethanol (52 ml) and aqueous saturated $NaHCO_3$ solution (34 ml). Flash chromatography (ether petroleum/ethyl acetate 7:3); compound **17** was obtained as a yellow solid (1.13 g, 92%). Rf (petroleum ether/ethyl acetate 7:3): 0.32; m.p. 210 °C; IR (KBr, cm^{-1}) *v* 3051, 1607, 1521, 1279, 1251, 1179, 1028, 819; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.87 (s, 3H), 3.88 (s, 3H), 7.02 (dd, 4H, *J* = 8.9, 2.4 Hz), 7.56 (d, 2H, *J* = 8.7 Hz), 7.71 (d, 1H, *J* = 8.2 Hz), 7.87 (dd, 1H, *J* = 8.4, 2.4 Hz), 7.99 (d, 2H, *J* = 8.9 Hz), 8.85 (d, 1H, *J* = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 55.5 (2 × CH_3), 114.3 (2 × CH), 114.7 (2 × CH), 119.7 (2 × CH), 128.1 (CH), 128.2 (CH), 130.3 (Cq), 131.9 (CH), 134.0 (CH), 134.7 (CH), 147.7 (2 × Cq), 155.4 (Cq), 159.8 (Cq), 160.6 (Cq); MS (IS): 292 $(M + 1)^+$; Anal. Calc. for $C_{19}H_{17}NO_2$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.66; H, 5.74; N, 4.69.

*4.1.18. 2,5-Bis-(4-hydroxyphenyl)-pyridine (**18**) [11,14]*

Following general **procedure B**. Compound **17** (950 mg, 3.26 mmol), CH_2Cl_2 (20 ml), BBr_3 (32.6 ml, 1 M in CH_2Cl_2, 32.6 mmol), 3 h, flash chromatography (dichloromethane/methanol 96:4); compound **18** was obtained as a yellow solid (817 mg, 95%). Rf (dichloromethane/methanol 96:4): 0.43; m.p. 155 °C; IR (KBr, cm^{-1}) *v* 3112, 1611, 1597, 1509, 1459, 1265, 1186, 823, 504; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 6.95 (d, 2H, *J* = 8.5 Hz), 7.02 (d, 2H, *J* = 8.5 Hz), 7.75 (d, 2H, *J* = 8.7 Hz), 7.96 (d, 2H, *J* = 8.5 Hz), 8.28 (d, 1H, *J* = 8.7 Hz), 8.68 (dd, 1H, *J* = 2.2, 8.7 Hz), 8.89 (d, 1H, *J* = 2.0 Hz); ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 116.2 (2 × CH), 116.3 (2 × CH), 122.1 (Cq), 23.8 (CH), 124.3 (Cq), 128.5 (2 × CH), 129.7 (2 × CH), 135.4 (Cq), 139.1 (CH), 141.2 (CH), 149.6 (Cq), 158.9 (Cq), 160.8 (Cq); MS (IS): 264 $(M + 1)^+$; Anal. Calc. for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.78; H, 4.83; N, 5.46.

*4.1.19. 2,5-Bis-[4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-3-yl}-phenoxy)-ethyl]-dimethyl amine (**19**)*

Following general **procedure C**. Compound **18** (100 mg, 0.38 mmol), Cs_2CO_3 (273 mg, 0.84 mmol), 2-chloroethyldimethylamine hydrochloride (121 mg, 0.84 mmol), Cs_2CO_3 (310 mg, 0.95 mmol), flash chromatography (dichloromethane/methanol/triethylamine 80:20:0.01); compound **19** was obtained as a white solid (77 mg, 50%). Rf (dichloromethane/methanol/triethylamine 80:20:0.01) 0.29; m.p. 184 °C; IR (KBr, cm^{-1}) *v* 2952, 2817, 2769, 1606, 1475, 1283, 1254, 1225, 1032, 819; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.36 (s, 12H), 2.76 (t, 4H, *J* = 5.7 Hz), 4.12 (t, 4H, *J* = 7.4 Hz), 7.03 (d, 4H, *J* = 7.6 Hz), 7.54 (d, 2H, *J* = 8.5 Hz), 7.70 (d, 1H, *J* = 8.2 Hz), 7.85 (dd, 1H, *J* = 2.2, 8.2 Hz), 7.97 (d, 2H, *J* = 8.8 Hz), 8.84 (s, 1H); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 46.0 (4 × CH_3), 58.4 (2 × CH_2), 66.2 (2 × CH_2), 114.9 (2 × CH), 115.3 (2 × CH), 119.6 (CH), 128.0 (2 × CH), 128.1 (2 × CH), 130.3 (Cq), 131.9 (Cq), 133.9 (Cq), 134.6 (CH), 147.6 (CH), 155.3 (Cq), 159.0 (Cq), 159.8 (Cq); MS (IS): 406 $(M + 1)^+$; Anal. Calc. for $C_{25}H_{31}N_3O_2$: C, 74.04; H, 7.70; N, 10.36. Found: C, 74.27; H, 7.60; N, 10.42.

*4.1.20. 2,5-Bis-[4-(3-dimethylamino-propoxy)-phenyl]-pyridine (**20**)*

Following general **procedure C**. Compound **18** (100 mg, 0.38 mmol), Cs_2CO_3 (273 mg, 0.84 mmol), 3-chloropropyldimethylamine hydrochloride (133 mg, 0.84 mmol), Cs_2CO_3 (310 mg, 0.95 mmol), flash chromatography (dichloromethane/methanol/triethylamine 80:20:0.01); compound **20** was obtained as a white solid (107 mg, 65%). Rf (dichloromethane/methanol/triethylamine 80:20:0.01): 0.26; m.p. 174 °C; IR (KBr, cm^{-1}) *v* 2946, 2764, 2362, 1607, 1468, 1251, 1222, 1057, 818; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.99 (q, 4H, *J* = 6.6 Hz), 2.27 (s, 12H), 2.48 (t, 4H, *J* = 7.2 Hz), 4.07 (td, 4H, *J* = 3.1, 6.3 Hz), 7.00 (d, 4H, *J* = 8.8 Hz), 7.53 (d, 2H, *J* = 8.5 Hz), 7.68 (d, 1H, *J* = 8.2 Hz), 7.84 (dd, 1H, *J* = 2.2, 8.2 Hz), 7.96 (d, 2H, *J* = 8.5 Hz), 8.84 (d, 1H, *J* = 2.2 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 27.6 (2 × CH_2), 45.6 (4 × CH_3), 56.4 (CH_2), 56.5 (CH_2), 66.4 (CH_2), 66.4 (CH_2), 114.8 (2 × CH), 115.2 (2 × CH), 119.6 (CH), 128.0 (2 × CH), 128.1 (2 × CH), 130.1 (Cq), 131.7 (Cq), 133.9 (Cq), 134.6 (CH), 147.6 (CH), 155.3 (Cq), 159.2 (Cq), 159.9 (Cq); MS (IS): 434 $(M + 1)^+$; Anal. Calc. for $C_{27}H_{35}N_3O_2$: C, 74.79; H, 8.14; N, 9.69. Found: C, 75.11; H, 8.24; N, 9.50.

*4.1.21. 2,5-Bis-[4-(2-benzyloxy-ethoxy)-phenyl]-pyridine (**21**)*

Following general **procedure D**. Compound **18** (523 mg, 1.99 mmol), Cs_2CO_3 (1.42 g, 4.37 mmol), 2-bromoethoxymethylbenzene (1.03 g, 4.77 mmol), flash chromatography (dichloromethane/ether petroleum 9:1); compound **21** was obtained as a brown solid (646 mg, 61%). Rf (dichloromethane/ether petroleum 8:2): 0.49; m.p. 147 °C; IR (KBr, cm^{-1}) *v* 2869, 1605, 1473, 1248, 1120, 1035, 823, 739; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 3.86 (t, 4H, *J* = 4.7 Hz), 4.22 (t, 4H, *J* = 5.4 Hz), 4.66 (s, 4H), 7.04 (dd, 4H, *J* = 2.3, *J* = 8.8 Hz), 7.29–7.38 (m, 10H), 7.55 (d, 2H, *J* = 8.8 Hz), 7.71 (d, 1H *J* = 8.3 Hz), 7.87 (dd, 1H, *J* = 2.3, *J* = 8.3 Hz), 7.98 (d, 2H, *J* = 8.6 Hz), 8.85 (d, 1H, *J* = 2.3 Hz); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 67.7 (2 × CH_2), 68.6 (2 × CH_2), 73.6 (2 × CH_2), 115.0 (2 × CH), 115.4 (2 × CH), 119.7 (CH), 127.9

(4 × CH), 128.1 (8 × CH), 128.6 (2 × CH), 130.5 (Cq), 132.0 (Cq), 134.0 (Cq), 134.6 (CH), 138.1 (Cq), 138.2 (Cq), 147.7 (CH), 155.4 (Cq), 159.0 (Cq), 159.8 (Cq); MS (IS): 532 $(M + 1)^+$; Anal. Calc. for $C_{35}H_{33}NO_4$: C, 79.07; H, 6.26; N, 2.63. Found: C, 79.41; H, 6.38; N, 2.54.

*4.1.22. 2,5-Bis-[4-(2-hydroxy-ethoxy)-phenyl]-pyridine (**22**)*

Following general **procedure B**. Compound **21** (546 mg, 1.03 mmol), CH_2Cl_2 (20 ml), BBr_3 (5.2 ml, 1 M in CH_2Cl_2, 5.14 mmol), 2 h, flash chromatography (dichloromethane/methanol 90:10); compound **22** was obtained as a yellow solid (320 mg, 87%). Rf (dichloromethane/methanol 90:10): 0.32; m.p. 191 °C; IR (KBr, cm^{-1}) ν 3422, 1610, 1509, 1263, 1191, 823; ^{1}H NMR (DMSO-d_6, D_2O, 250 MHz): δ 3.75 (t, 4H, J = 5.0 Hz), 4.10 (q, 4H, J = 5.2 Hz), 6.96 (d, 1H, J = 8.7 Hz), 7.13 (d, 1H, J = 8.7 Hz), 7.21 (d, 2H, J = 8.9 Hz), 7.77 (d, 1H, J = 8.7 Hz), 7.87 (d, 1H, J = 8.7 Hz), 8.04 (dd, 2H, J = 2.4, 8.9 Hz), 8.37 (d, 1H, J = 8.5 Hz), 8.74-8.80 (m, 1H), 9.01 (dd, 1H, J = 1.8 Hz); ^{13}C NMR (DMSO-d_6, 62.5 MHz): δ 59.4 (CH_2), 59.5 (CH_2), 69.8 (CH_2), 70.0 (CH_2), 115.4 (2 × CH), 116.3 (2 × CH), 123.1 (Cq), 123.4 (Cq), 124.5 (CH), 128.6 (CH), 128.6 (CH), 129.8 (2 × CH), 138.8 (CH), 141.8 (CH), 148.9 (Cq), 159.1 (Cq), 160.0 (Cq), 161.7 (Cq); MS (IS): 352 $(M + 1)^+$; Anal. Calc. for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 72.03; H, 5.88; N, 3.85.

4.2. Biological studies

4.2.1. Absorption spectroscopy and melting temperature studies

Absorption spectra and melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. The T_m measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette (10 mm pathlength) was increased over the range 20–100 °C with a heating rate of 1 °C min^{-1}. The "melting" temperature T_m was taken as the mid-point of the hyperchromic transition.

4.2.2. Fluorescence measurements

Binding studies were carried out through a competitive displacement fluorometric assay with DNA-bound ethidium [17,20]. Fluorescence data were recorded at room temperature with a SPEX Fluorolog fluorometer. Excitation was set at 515 nm and fluorescence emission was monitored over the range 550–700 nm. Experiments were performed with an [ethidium]:[DNA] molar ratio of 12.6:10 and a drug concentration range of 0.01–100 μM in a BPE buffer pH 7.1. C_{50} values for ethidium displacement were calculated using a fitting function incorporated into Prism 3.0 and the apparent equilibrium binding constants (K_{app}) were calculated as follows: $K_{app} = (1.26\ \mu M/C_{50}) \times K_{ethidium}$, with $K_{ethidium} = 10^7\ M^{-1}$.

4.2.3. DNase I footprinting

The experimental procedure has been previously described [21,22].

4.2.4. Cell cultures and survival assay

CEM human leukaemia cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium, supplemented with 10% foetal bovine serum, glutamine (2 mM), penicillin (100 UI ml^{-1}) and streptomycin (100 μg ml^{-1}). The cytotoxicity of the studied molecules was assessed using a cell proliferation assay developed by Promega (CellTiter 96® AQ_{ueous} one solution cell proliferation assay). Briefly, 2×10^4 exponentially growing cells were seeded in 96-well microculture plates with various drug concentrations in a volume of 100 μl. After 72 h incubation at 37 °C, 20 μl of the tetrazolium dye solution were added to each well and the samples were incubated for a further 2 h at 37 °C. Plates were analysed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

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