Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Systematic Synthetic and Biophysical Development of Mixed Sequence DNA Binding Agents

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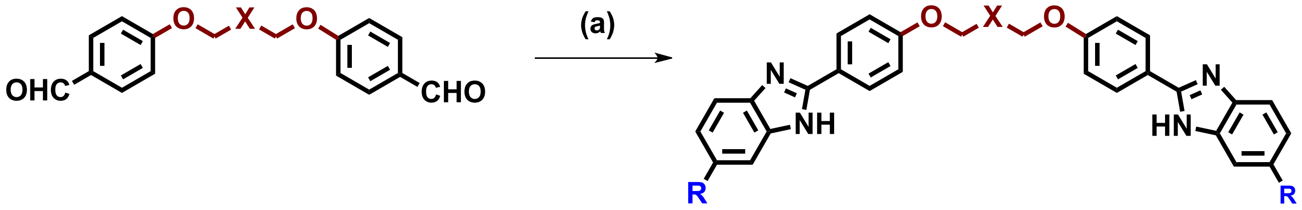
EXPERIMENTAL METHODS

Synthesis



Scheme 1: Reagents and conditions; (a) 4-Amidino-1,2-pheneylene diamine hydrochloride, 1,4-benzoquinone, ethanol.

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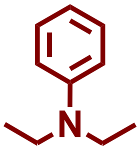
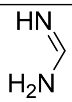
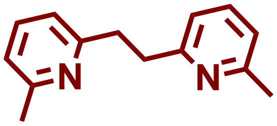
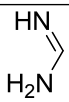
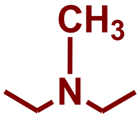
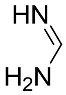
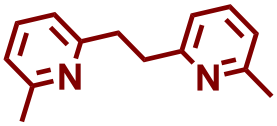
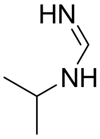
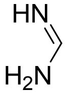
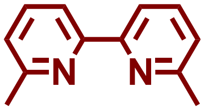
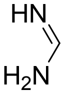
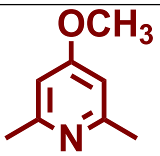
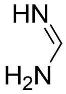
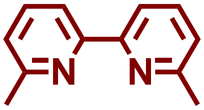
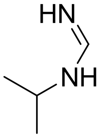
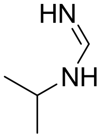
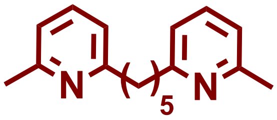
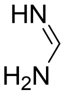
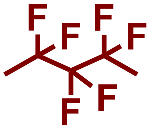
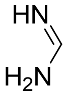
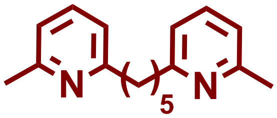
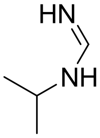
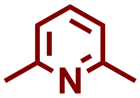
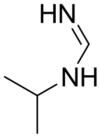
DB 2149			DB 2298		
DB 2150			DB 2299		
DB2146			DB 2300		
DB 2291			DB 2301		
DB 2295			DB 2314		
DB2355			DB 2315		
DB2302			---	---	---

General materials and methods

Melting points (uncorrected) were determined on a Mel-Temp 3.0 melting point apparatus. TLC analysis was performed on silica gel 60 F254 precoated aluminum sheets; UV light was used for detection. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer (^{1}H data was obtained at 400MHz and ^{13}C data obtained at 100.6 MHz) using the indicated solvents. Mass spectra were obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. Elemental analysis was performed by Atlantic Microlab Inc., Norcross, GA, and are within ±0.4 of the theoretical values. The compounds reported as salts frequently analyzed correctly for fractional moles of water and/or other solvents; in each case ^{1}H NMR spectra was consistent with the analysis. Reactants and solvents were purchase from Aldrich Chemical Co., VWR International, or Combi-Blocks, Inc.

N, ***N*****-Bis{4[4(5) amidinobenzimidazolyl] phenoxyethyl]} aniline dihydrochloride (DB 2149)**

N, *N*-Bis(chloroethyl)aniline (1) (1.12 g, 0.005 mol), 4-hydroxybenzaldehyde (1.02 g, 0.01 mol) and anhydrous. K_2CO_3 (2.07 g, 0.015 mol) in 5-6 ml DMF was heated at 80°C for 4 h (tlc Hexane:EtOAc 7:3) monitored], diluted with ice water 30 ml, the precipitated white solid filtered, washed with water, and dried. It was dissolved in DCM 75 ml), dried over anhydrous. $MgSO_4$, filtered, concentrated and triturated with cold hexane, filtered and dried in vac. at 60°C to yield *N*, *N*-bis (4-formylphenoxyethyl) aniline as an off-white solid 1.5 g (77%), mp 92-3°C.; ^{1}H NMR (DMSO-d_6): 9.86 (s, 2H), 7.83 (d, 4H, J = 8.5 Hz), 7.19 (t, 2H, J = 7.5 Hz), 7.09 (d, 4H, J = 8.5 Hz), 6.50 (d, 2H, J = 7.5 Hz), 6.51 (t, 1H, J = 7.5 Hz), 4.29 (t, 4H, J = 4.5 Hz), 3.86 (t, 4H, J = 4.5 Hz); ^{13}C NMR (DMSO-d_6): 191.7, 163.8, 147.7, 132.3, 130.2, 129.7, 116.6, 115.4, 112.3, 66.3, 50.2; MS: HR-ESI: calc'd for $C_{24}H_{24}NO_4$: 390.1705 (M^++1); found *m/z* 390.1700; Anal. calc'd. for $C_{24}H_{23}NO_4$: C, 74.01; H, 5.95; N, 3.59; Found: C, 74.21; H, 5.88; N, 3.47. *N, N*-bis (4-formylphenoxyethyl)aniline (0.194 g, 0.0005 mol) , 4-amidino-1,2-phenylenediamine hydrochloride (0.187 g, 0.001 mol), and 1,4-benzoquinone (0.108 g, 0.001 mol) in ethanol (30 ml), the mixture was heated at reflux under nitrogen for 24 h. The reaction mixture was concentrated to 5 ml and triturated with ether-acetone mixture, filtered, washed with acetone and dried. The solid was dissolved in 30 ml ethanol and treated with 2 ml ethanolic HCl (satd.), stirred and concentrated, triturated with dry ether and filtered, dried in vac. at 50 ^{0}C (12 h) to yield dihydrochloride salt as pinkish grey solid, 0.28 g (73%); mp >320°C dec.; ^{1}H NMR (DMSO-d$_6$): 9.31 (brs, 4H), 9.04 (brs, 4H), 8.21 (d, 4H, J = 8.5 Hz), 8.12 (brs, 2H), 7.73 (d, 2H, J = 8.5 Hz), 7.66 (d, 2H, J = 8.5 Hz), 7.21 (apparent triplet, 2H, J = 7.5 Hz), 7.14 (d, 4H, J = 8.5 Hz), 6.86 (d, 2H, J = 7.5 Hz), 6.58 (t, 1H, J = 7.5 Hz), 4.30 (t, 4H, J = 4.0 Hz), 3.90 (t, 4H, J = 4.0 Hz); MS: HR-ESI: calc'd. for $C_{38}H_{36}N_9O_2$: 650.2991 (M^++1), found *m/z* 650.3002; Anal. calc'd. for $C_{38}H_{35}N_9O_2$-2HCl-2.55H_20: C, 59.38; H, 5.52; N, 16.40; Found: C,59.52; H,5.29; N, 16.05.

***N, N*-Bis {4[4(5) amidino-benzimidazolyl] phenoxyethyl]} methylamine dihydrochloride (DB 2150)**

N, N-Bis (4-formylphenoxymethyl)methylamine (2) (0.163 g, 0.0005 mol) , 4-amidino-1,2-phenylenediamine hydrochloride (0.187 g, 0.001 mol), and 1,4-benzoquinone (0.108 g, 0.001 mol) in ethanol (30 ml) was heated at reflux for 8 h (under nitrogen) following standard workup yielded dihydrochloride salt as light grey solid, 0.26 g (74%); mp >320°C dec.; ^{1}H NMR (DMSO/D_2O): 8.13 (d, 4H, J = 8.5 Hz), 8.07 (brs, 2H), 7.72 (d, 2H, J = 8.0 Hz), 7.61 (d, 2H, J = 8.0 Hz), 7.13 (d, 4H, J = 8.5 Hz), 4.18 (t, 4H, J = 5.5 Hz), 2.89 (t, 4H, J = 5.5 Hz), 2.38 (s, 3H); MS: HR-ESI: calc'd. for $C_{33}H_{34}N_9O_2$: 588.2835 (M^++1), found *m/z* 588.2831; Anal. calc'd. for $C_{33}H_{33}N_9O_2$ -2.0HCl-3.5H_20: C, 56.00; H, 5.99; N, 17.81; Found: C,56.23; H,5.59; N, 17.55.

2,2'-(4,4'-(pyridine-2,6-diylbis(oxy))bis(4,1-phenylene))bis(1H-benzo[dimidazole-6-carboximidamide) tetrahydrochloride (DB2201).

The 2,6-difluoropyridine (0.57 gm, 5 mmol) was added to a solution of the p-hydroxybenzaldehyde (11 mmol), anhydrous K_2CO_3 (1.65 gm, 12 mmol) and anhydrous Cs_2CO_3 (0.65 gm, 2 mmol) in dry DMA (15 ml) under a nitrogen atmosphere. The reaction mixture was stirred at 100 ^{o}C for 24 h, cooled, then poured on ice-water (50 ml), and extracted with ethyl acetate, dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. Purification was by chromatography using ethyl acetate/

hexanes then recrystallization from ethanol to give a white solid of 4,4'-(pyridine-2,6-diylbis(oxy))dibenzaldehyde, 0.51 gm (32 %) mp 121-123 ^{o}C; ^{1}HNMR (DMSO-d_6): δ 9.95 (s, 2 H); 8.10 (d, *J* = 8.8 Hz, 1 H) 7.81 (m, 4 H), 7.30 (m, 4 H), 7.08 (br s, 2 H), , ^{13}CNMR (DMSO-d_6): 191.20; 159.8, 143.7, 133.4, 130.9, 130.7, 121.30, 117.9, ESI-HRMS: m/z calculated for $C_{19}H_{14}NO_4$: 320.0923, found: 320.0909 (M^+ + 1). The compound was used directly in the next step. To a stirred mixture of the above dialdehyde (0.127 gm, 0.4 mmol) and 4- amidino-1,2-phenylenediamine hydrochloride hydrate (0.148 gm, 0.8 mmol) in 50 ml anhydrous ethanol was added 1,4-benzoquinone (0.086 gm, 0.8 mmol) (under nitrogen). following standard workup yielded a brown solid, 0.18 gm (59 %) mp > 300 ^{o}C; ^{1}HNMR (DMSO-d_6): δ 9.65 (s, 4 H), 9.34 (s, 4 H), 8.52 (d, J = 8.0 Hz, 4 H), 8.24 (m, 2 H), 7.85 (m, 4 H), 7.46 (d, J = 8.0 Hz, 4 H), 7.20 (br s, 1 H), 6.94-6.95 (m, 2 H); ^{13}CNMR (DMSO-d_6): δ 166.0; 159.9, 156.3, 143.7, 137.6, 134.6, 130.6, 129.2, 128.3, 123.5, 120.6, 120.1, 119.5, 116.1, 114.8, ESI-HRMS: m/z calculated for $C_{33}H_{26}N_9O_2$: 580.2209, found: 580.2219 (amidine base M^+ + 1); Anal. calcd. for $C_{33}H_{25}N_9O_2$ -4 HCl-2.4 H_2O: C, 51.56; H, 4.43; N, 16.40. Found: C, 51.32; H, 4.51; N, 16.29.

2, 6-Bis {4[4(5) amidinobenzimidazolyl] phenoxymethyl]}-4-methoxypyridine pentahydrochloride (DB 2291)

A mixture of 2, 6-bis(bromomethyl)-4-methoxy pyridine (3) (0.59 g, 0.002 mol), 4-hydroxybenzaldehyde (0.488 g, 0.004 mol) and anhydrous K_2CO_3 (0.69 g, 0.005 mol) in 6-7 ml DMF was stirred at rt for 12 h (tlc Hexane:EtOAc 7:3), diluted with ice water, solid filtered, washed with water, dissolved in 70 ml DCM, dried over $MgSO_4$, filtered, concentrated and triturated with cold hexane, filtered and dried in vac., yielded 2, 6-bis (4-formylphenoxymethyl)pyridine as off white solid 0.54 g (71%), mp 151-2°C; ^{1}H NMR (DMSO-d_6): 9.88 (s, 2H), 7.88 (d, 4H, J = 8.8 Hz), 7.25 (d, 4H, J = 8.8 Hz), 7.10 (s, 2H), 5.27 (s, 4H), 3.86 (s, 3H); MS: HR-ESI: calc'd. for $C_{22}H_{20}NO_5$: 378.1341 (M^++1) found *m/z* 378.1344; Anal. calc'd. for $C_{22}H_{19}NO_{5.}$ 0.25H_2O: C, 69.17; H, 5.14; N, 3.66; Found: C,69.14; H,5.10; N, 3.76. A well stirred solution of 2, 6-bis(4-formylphenoxymethyl)pyridine (0.190 g, 0.0005 mol), 4- amidino-1, 2-phenylenediamine hydrochloride (0.187 g, 0.001 mol) and 1, 4-benzoquinone (0.108 g, 0.001 mol) in anhydrous ethanol (60 ml) (under nitrogen) was heated under reflux for 8-10 h. Following standard workup yielded hydrochloride salt as brown solid, 0.32 g (76%), mp >330°C dec.; ^{1}H NMR (DMSO-d$_6$/65^{0}C): 9.32 (brs, 4H), 9.07 (brs, 4H), 8.25 (d, 4H, J = 9.0 Hz), 8.14 (brs, 2H), 7.72 (d, 2H, J = 8.5 Hz), 7.61 (d, 2H, J = 8.5 Hz), 7.27 (d, 4H, J = 9.0 Hz), 7.10 (s, 2H), 5.26 (s, 4H), 3.87 (s, 3H); MS: HR-ESI: calc'd for $C_{36}H_{32}N_9O_3$: 638.2623 (M$^+$+1), found *m/z* 638.2601; Anal. calc'd for $C_{36}H_{31}N_9O_3$-5HCl-H_20: C, 51.72; H, 4.58; N,15.09; Found: C, 51.44; H, 4.85; N, 15.08.

1,7-Bis{4[4(5)-*N*-isopropylamidino-benzimidazolyl]pheny]}-1,4,7-trioxaheptane dihydrochloride (DB 2295)

1,7-Bis-(4-formylphenyl)-1,4,7-trioxaheptane (4) (0.162 g, 0.0005 mol) , 4-*N*-isopropylamidino-1,2-phenylenediamine hydrochloride (0.228 g, 0.001 mol), and 1,4-benzoquinone (0.108 g, 0.001 mol) in ethanol (30 ml) was heated at reflux for 8 h, (under nitrogen) following standard workup yielded dihydrochloride as violet grey solid**,** 0.30 g **(**74%); mp >320°C dec.; ^{1}H NMR (DMSO-d$_6$): 9.78 (brs, 1H), 9.76 (brs, 1H), 9.60 (brs, 2H), 9.21 (bs, 2H), 8.47 (d, 4H, J = 8.5 Hz), 8.10 (brs, 2H), 7.89 (d, 2H, J = 8.0 Hz), 7.72 (d, 2H, J = 8.0 Hz), 7.27 (d, 4H, J = 8.5 Hz), 4.31 (t, 4H, J = 4.5 Hz), 4.13 (septet, 2H, J = 6.5 Hz), 3.90 (t, 4H, J = 4.5 Hz), 1.32 (d, 12H, J = 6.5 Hz); MS: HRMS: calc'd. for $C_{38}H_{43}N_8O_3$: 659.3453 (M$^+$+1), found *m/z* 659.3431; Anal. calc'd. for $C_{38}H_{42}N_8O_3$-4.0HCl-1.0H_20: C, 55.48; H, 5.88; N, 13.62; Found: C,55.52; H,5.73; N, 13.86.

2,6-Bis{4[4(5)-N-isopropylamidinobenzimidazolyl]phenoxymethyl]}pyridinepenta hydrochloride (DB2302)

A well stirred solution of 2, 6-bis (4-formylphenoxymethyl)pyridine (0.173 g, 0.0005 mol), 4-N-isopropyl amidino-1, 2-phenylenediamine hydrochloride (0.228 g, 0.001 mol) and 1, 4-benzoquinone (0.108 g, 0.001 mol) in anhydrous ethanol (60 ml) (under nitrogen) was heated at reflux for 8-10 h. The reaction mixture following standard work-up and drying at 500C (12 h in vacuum) yielded greenish grey solid, 0.33 g (71%), mp >325°C dec.; 1H NMR (DMSO-d6/650C): 9.67 (s, 1H), 9.66 (s, 1H), 9.51 (br, 2H), 9.12 (brs, 2H), 8.46 (d, 4H, J = 8.5 Hz), 8.10 (s, 2H), 7.95 (t, 1H, J = 8.5 Hz), 7.87 (d, 2H, J = 8.5 Hz), 7.70

(d, 2H, J = 8.5 Hz), 7.57 (d, 2H, J = 8.5 Hz), 7.34 (d, 4H, J = 8.5 Hz), 5.38 (s, 4H), 4.15 (quint., 2H, J = 6.5Hz), 1.33 (d, 12H, J = 6.5 Hz); MS: HR-ESI: calc'd for C41H42N9O2; 692.3461 (M++1); found m/z 692.3466; Anal. calc'd. for C41H41N9O2-5HCl-3H2O: C, 53.17; H, 5.66; N,13.62; Found: C, 53.22; H, 5.49; N, 13.71.

1,2-bis-(6-({4[4(5)-amidinobenzimidazolyl]-phenoxymethyl)-pyridine-2-yl)ethane hexahydrochloride (DB 2298)

Reaction of 1, 2-bis-(6-(bromomethyl)-pyridine-2-yl) ethane (6) 0.734 (0.002 mol), 4-hydroxybenzaldehyde (0.488 g, 0.004 mole) and anhydrous K_2CO_3 (0.69 g, 0.005 mol) in 10 ml DMF at rt for 12 h (tlc Hexane:EtOAc 7:3), after standard work-up and drying 1,2-bis-(6-(4-formylphenoxymethyl)-pyridine-2-yl)ethane was obtained as a beige solid; mp 163-4°C; ^{1}H NMR(DMSO-d_6): 9.88 (s, 2H), 7.83 (d, 4H, J = 8.4 Hz), 7.60 (t, 2H, 8.0 Hz), 7.32 (d, 2H, J = 7.6 Hz), 7.09 (d, 4H, J = 8.4 Hz), 7.07 (d, 2H, J = 7.6 Hz), 5.3 (s, 4H), 3.72 (s, 4H); ^{13}C NMR(DMSO-d_6): 190.9, 163.6, 161.0, 155.8, 137.4, 132.2, 130.4, 122.4, 119.0, 115.4, 71.2, 38.0; MS: HR-ESI: calc'd for $C_{28}H_{25}N_2O_4$ *m/z*: 453.1809 (M^++1), found *m/z* 453.1788.

Reaction of 1,2-bis-(6-(4-formylphenoxymethyl)pyridine-2-yl)ethane (0.226 g, 0.0005 mol), 4-amidino-1, 2-phenylenediamine hydrochloride (0.187 g, 0.001 mol) and 1, 4-benzoquinone (0.108 g, 0.001 mol) in anhydrous ethanol (75 ml) (under nitrogen) was heated under reflux for 12-14h. The reaction mixture following standard work-up and drying at 50^{0}C (12 h in vac.) gave the hexahydrochloride salt as dark bluish grey solid; mp >330°C dec.; ^{1}H NMR(DMSO-d_6): 9.48 (brs, 4H), 9.20 (brs, 4H), 8.43 (d, 4H, J = 8.5 Hz), 8.16 (d, 2H, J = 1.2 Hz), 7.98 (apparent t, 2H, J = 8.0 Hz), 7.82 (d, 2H, J = 8.5 Hz), 7.74 (dd, 2H, J = 8.5 Hz, J =1.2Hz), 7.57 (d, 2H, J = 8.0 Hz), 7.47 (d, 2H, J = 8.0 Hz), 7.27 (d, 4H, J = 8.5 Hz), 5.35 (s, 4H), 3.38 (s, 4H); MS: HR-ESI: calc'd for $C_{42}H_{37}N_{10}O_2$ *m/z* (M^++1) 713.3100, found *m/z* 713.3119 Anal. calc'd for $C_{42}H_{36}N_{10}O_2$-6.0HCl-3.0H_2O:C, 51.18; H, 4.90; N, 14.21; Found: C,51.31; H,5.11; N, 14.09.

1,2-bis-(6-({4[4(5)-*N*-isopropylamidinobenzimidazolyl]phenoxymethyl)pyridine-2-yl) ethane hexahydrochloride (DB 2299)

Reaction of 1,2-bis-(6-(4-formylphenoxymethyl) pyridine-2-yl) ethane (0.226 g, 0.0005 mol), 4-*N*-isopropyl amidino-1, 2-phenylenediamine hydrochloride (0.228 g, 0.001 mole) and 1, 4-benzoquinone (0.108g, 0.001 mol) in anhydrous ethanol (75 ml) (under nitrogen) was heated under reflux for 12-14h. The reaction mixture following standard work-up and drying at 50^0C (12 h in vac.) gave hydrochloride salt as dark grey solid, mp >310°C dec.; ^{1}H NMR(DMSO-d_6): 9.67 (brs, 1H), 9.66 (brs, 1H), 9.51 (brs, 2H), 9.10. (brs, 2H), 8.44 (d, 4H, J = 9.0 Hz), 8.10 (s, 2H), 7.98 (apparent t, 2H, J = 7.5 Hz), 7.86 (d, 2H, J = 8.5 Hz), 7.69 (dd, 2H, J = 8.5, J = 1.2 Hz), 7.56 (d, 2H, J = 7.5 Hz), 7.46 (d, 2H, J = 7.5Hz), 7.31 (d, 4H, J = 9.0 Hz), 5.41 (s, 4H), 4.14 (septet, 2H, J = 6.5 Hz), 3.37 (s, 4H), 1.32 (d, 12H, J = 6.5 Hz); MS: HR-ESI: calcd. for $C_{48}H_{49}N_{10}O_2$: *m/z* 797.4034 (M^++1), found *m/z* 797.4011. Anal. calc'd. for $C_{48}H_{48}N_{10}O_2$-6HCl-3.75H_2O: C, 53.34; H, 5.74; N, 12.96; Found: C,53.55; H,6.01; N, 12.87.

6,6'-bis{4[4(5)-amidino-benzimidazolyl]phenoxymethyl]}-2,2'-bipyridine pentahydrochloride (DB 2300)

Stirring a mixture of 6,6'-bis-(chloromethyl)-2, 2'-bipyridine (5) (0.504 g, 0.002 mol), 4-hydroxybenzaldehyde (0.488 g, 0.004 mol) and anhydrous. K_2CO_3 (0.69 g, 0.005 mole) in 6-7 ml DMF at rt for 12 h (tlc Hexane:EtOAc 7:3). The reaction mixture was diluted with ice water, precipitated solid filtered, washed with water, and dried. The solid dissolved in hot methanol (85 ml), filtered, concentrated and triturated with cold hexane, filtered and dried in vac., afforded 6,6'-bis(4-formylphenoxymethyl)-2, 2'-bipyridine as light brown solid 0.63 g (74%); mp 247-8°C; ^{1}H NMR(DMSO-d_6): 9.89 (s, 2H), 8.32 (d, 2H, J = 7.5 Hz), 8.02 (t, 1H, J = 7.5 Hz), 7.90 (d, 4H, J = 8.5 Hz), 7.61 (t, 1H, J = 7.5 Hz), 7.29 (d, 4H, J = 8.5 Hz), 5.43 (s, 4H); MS: HR-ESI: Calc'd. for $C_{26}H_{21}N_2O_4$ *m/z*, 425.1496 (M^++1), found *m/z* 425.1480.

Reaction of 6,6'-bis(4-formylphenoxymethyl)-2, 2'-bipyridine (0.212 g, 0.0005 mol), 4-amidino-1, 2-phenylenediamine hydrochloride (0.187 g, 0.001 mol) and 1, 4-benzoquinone (0.108 g, 0.001 mol) in anhydrous ethanol (75 ml) (under nitrogen) by heating under reflux for 12-14 h. The reaction mixture following standard work-up and drying at 50^0C (12 h in vac.) gave penta hydrochloride salt as dark bluish green solid, 0.286 g (65%), mp >330°C dec.; ^{1}H NMR (DMSO-d_6/65^{0}C): 9.38 (brs, 4H), 9.06 (brs, 4H), 8.36 (d, 4H, J = 8.5 Hz), 8.34 (d, 2H, J = 8.0 Hz), 8.19 (s, 2H), 8.05 (t, 2H, J = 8.0 Hz), 7.84 (d, 2H, J = 8.0 Hz), 7.76 (d, 2H, J = 8.5 Hz), 7.65 (d, 2H, J = 8.0 Hz), 7.37 (d, 4H, J = 8.5 Hz), 5.45 (s, 4H); MS: HR-ESI: calc'd. for $C_{40}H_{33}N_{10}O_2$: *m/z* 685.2782 (M^++1), found *m/z* 685.2757. Anal. calc'd. for $C_{40}H_{32}N_{10}O_2$-5HCl-1.0H_2O: C, 54.28; H,4.44; N, 15.82; Found: C,54.52; H,4.73; N, 15.56.

6,6'-bis-{4[4(5)-*N*-isopropylamidino-benzimidazolyl] phenoxymethyl]}-2,2'-bipyridine penta hydrochloride (DB2301)

Reaction of 6,6'-bis(4-formylphenoxymethyl)-2, 2'-bipyridine (0.212 g, 0.0005 mol), 4-*N*-isopropylamidino-1, 2-phenylenediamine hydrochloride (0.228 g, 0.001 mole) and 1, 4-benzoquinone (0.108 g, 0.001 mol) in anhydrous ethanol (75ml) (under nitrogen) was heated at reflux for 12 h. The reaction mixture following standard work-up and drying at 50^{0}C (12 h in vac.) yielded dark bluish green hydrochloride salt, 0.336 g (69%); mp >320°C dec; ^{1}H NMR(DMSO-d_6): 9.64 (brs, 1H), 9.62 (brs, 1H), 9.48 (br, 2H), 9.07 (br, 2H), 8.43 (d, 4H, J = 8.5 Hz), 8.36 (d, 2H, J = 8.0 Hz), 8.09 (s, 2H), 8.05 (t, 2H, J = 8.0 Hz), 7.87 (d, 2H, J = 8.0 Hz), 7.69 (d, 2H, J = 8.0 Hz), 7.65 (d, 2H, J = 8.0 Hz), 7.37(d, 4H, J = 8.5 Hz), 5.46 (s, 4H), 4.12 (septet, 2H, J = 6.5 Hz), 1.32 (d, 12H, J = 6.5 Hz); MS: HR-ESI: calc'd. for $C_{46}H_{45}N_{10}O_2$: *m/z* 769.3721 (M^++1), found *m/z* 769.3694; Anal. calc'd. for $C_{46}H_{44}N_{10}O_2$-5HCl-1.5H_2O: C, 56.48; H,5.35; N, 14.32; Found: C,56.52; H,5.53; N, 14.56.

1,5-Bis (6-({4[4(5)-amidinobenzimidazolyl])pyridine-2-yl)-pentane hexahydrochloride (DB 2314)

LDA (12.8 g, 60 ml, 2M in THF) was added to a thoroughly degassed (nitrogen) solution of 2-bromo-6-methyl pyridine 17.2 g (0.1 mol) in 200 ml dry THF under nitrogen at -78^{0}C in 30 min the reaction mixture was stirred 2 h at this tempature followed by addition of 1, 3-dibromopropane 10.1 g (0.05 mol) in 50 ml THF at -78 ^{O}C in 20 min. The reaction mixture was slowly warmed to rt and stirred for 4 h, water 50 ml was added slowly with ice bath cooling, the solvent was removed under pressure and the residue was added to 200 ml DCM and 100 ml water. The organic layer was separated, washed with water, dried over anhydrous $MgSO_4$, concentrated and chromatographed over silica gel (tlc:Hexane:DCM 7:3) to yield 1, 5-bis (6-bromopyridine-2-yl)-pentane as a light brown solid 9.4 g (49%), mp 54-5°C; ^1H NMR(DMSO-d_6): 7.64 (apparent t, 2H, J = 7.5 Hz), 7.43 (d, 2H, J = 7.5 Hz),7.29 (d, 2H, J = 7.5 Hz), 2.66 (t, 4H, J = 7.6 Hz), 1.64 (quintet, 4H, J = 7.6 Hz), 1.29 (quintet, 2H, J = 7.6 Hz);^{13}C NMR(DMSO-d_6):163.9, 141.2, 140.1, 125.7, 122.5, 37.2, 29.1, 28.6; MS: HR-ESI: calc. for $C_{15}H_{17}Br_2N_2$ *m/z* 382.9758 (M^++1), found *m/z* 382.9736**.** In dry THF (300 ml) was dissolved 1, 5-bis (6-bromopyridine-2-yl) pentane 5.71 g (0.015 mol) and the mixture was degassed with nitrogen. To the solution was added *n*-BuLi (2.5 M in hexane, 12.8 ml, 0.032 mol) at -78^{0}C in 20 min. The solution was stirred at -78^{0}C for 1.5 h under N_2, followed by dropwise addition of DMF (4 ml, 0.05 mol) in 30 ml dry ether while keeping temperature at -78^{0}C. The mixture was warmed to rt and stirred for 6 h. To the mixture was added 40 ml 2M HCl, THF was removed under reduced pressure and diluted with 150 ml DCM and 100 ml H_2O, neutralized with Na_2CO_3, extracted with 2X 100 ml DCM, dried over anhydrous $MgSO_4$, concentrated and triturated with hexane to give pure 1, 5-bis (6-formyl)pyridine-2-yl)pentane as white solid 3.2 g (73%), mp 65-6°C; ^1H NMR(DMSO-d_6): 9.94 (s, 2H), 7.92 (apparent t, 2H, J = 7.6 Hz), 7.72 (d, 2H, J = 7.6 Hz),7.55 (d, 2H, J = 7.60 Hz), 2.83 (t, 4H, J = 7.6 Hz), 1.76 (quintet, 4H, J = 7.6 Hz), 1.39 (quintet, 2H, J = 7.60 Hz); ^{13}C NMR(DMSO-d_6): 194.3, 163.1, 152.3, 138.3, 127.9, 119.6, 37.5, 29.3, 28.7; MS: HR-ESI: calc. for $C_{17}H_{19}N_2O_2$ *m/z* 283.1447 (M^++1), found *m/z* 283.1433. To a cooled solution of the above dialdehyde 1.13 g (0.004 mol) in 65 ml anhydrous methanol while stirring was added $NaBH_4$ 0.30 g (0.008 mol) in 10 min, followed by stirring at rt for 12 h , tlc monitored. Acidified with 1N HCl to pH 6, solvent removed and the residue was extracted with 2X 40 ml DCM, dried over anhydrous $MgSO_4$. The solvent was removed and triturated with hexane to yield 1, 5-bis (6-hydroxymethyl)pyridine-2-yl) pentane, white solid 0.81 g (71%), mp 85-6°C; ^1H NMR(DMSO-d_6): 7.66 (apparent t, 2H, J = 7.6Hz), 7.26 (d, 2H, J = 7.60 Hz), 7.06 (d, 2H, J = 7.60 Hz), 5.34 (t, 2H, J = 5.6 Hz), 4.52 (t, 4H, J = 5.6 Hz), 2.67 (t, 4H, J = 7.6 Hz), 1.67 (quintet, 4H, J = 7.6 Hz), 1.34 (quintet, 2H, J = 7.6 Hz); ^{13}C NMR(DMSO-d_6): 161.7, 161.0, 137.2, 121.0, 117.8, 64.7,37.8, 29.6, 28.9; MS: HR-ESI: calc. for $C_{17}H_{23}N_2O_2$ *m/z* 287.1760 (M^++1), found *m/z* 287.1750. Thionyl chloride (0.59 g, 0.005 mole) was added to a solution of 1, 5-bis (6-hydroxymethyl)pyridine-2-yl)pentane 0.572 g (0.002 mol) in 25 ml dry DCM, followed by 2 drops of DMF, the reaction mixture was heated at reflux for 4 h, and solvent was removed in vac. followed by addition of ice water, neutralized with a saturated solution of $NaHCO_3$ to pH 8, extracted with 40 ml DCM, washed with water, dried over anhydrous $MgSO_4$, concentrated, and the residue was treated with hexane to yield 1, 5-bis (6-(chloromethyl)-pyridine-2-yl)-pentane as a beige solid 0.52 g (81%), mp 41-3°C; ^{1}H NMR(DMSO-d_6): 7.72 (apparent t, 2H, J = 7.60 Hz), 7.33(d, 2H, J= 7.60 Hz), 7.19 (d, 2H, J = 7.60 Hz), 4.73 (s, 4H), 2.71 (t, 4H, J = 7.60 Hz), 1.69 (quintet, 4H, J = 7.60 Hz), 1.34 (quint., 2H, J = 7.60 Hz); ^{13}C NMR(DMSO-d_6): 162.1, 156.1, 138.0, 122.6, 121.0, 47.5, 37.7, 29.5, 28.8; MS: HR-ESI: calc. for $C_{17}H_{21}N_2Cl_2$ *m/z* 323.1082 (M^++1), found *m/z* 323.1072. Reaction of 1, 5-bis (6-(chloromethyl)-pyridin-2-yl)-pentane 0.644 g, 0.002 mole), 4-hydroxybenzaldehyde (0.488 g, 0.004 mol) and anhydrous K_2CO_3 (0.69 g, 0.005 mol) in 6-7 ml DMF at rt for 12 h (tlc Hexane:EtOAc 7:3). The reaction mixture was diluted with ice water, precipitated solid filtered, washed with water, and dried. The solid dissolved in 85 ml DCM, dried over $MgSO_4$, filtered, conc. and triturated with cold hexane, filtered and dried in vac., afforded 1, 5-bis (6-(4-formyl-phenoxymethyl)-pyridin-2-yl)-pentane as white solid 0.63 g (64%); mp 100-1°C; ^{1}H NMR(DMSO-d_6): 9.86 (s, 2H), 7.87 (d, 4H, J = 8.8 Hz), 7.73 (apparent t, 2H, J = 7.6 Hz), 7.32 (d, 2H, J = 7.60 Hz), 7.22 (d, 4H, J = 8.8 Hz), 7.19 (d, 2H, J = 7.60 Hz), 5.25 (s, 4H), 2.73 (t, 4H, J = 7.60 Hz), 1.70 (quint., 4H, J = 7.60 Hz), 1.33 (quint., 2H, J = 7.6 Hz); ^{13}C NMR(DMSO-d_6): 191.8, 163.6, 161.9, 155.7, 137.8, 132.2, 130.4, 122.4, 119.5, 115.8, 71.2, 37.7, 29.5, 28.8; MS: HR-ESI: calc. for $C_{31}H_{31}N_2O_4$ *m/z* 495.2284 (M^++1), found *m/z* 495.2264. Anal. calc'd. for $C_{31}H_{30}N_2O_4$: C, 75.28; H, 6.11; N, 5.66; Found: C,75.04; H,6.08; N, 5.64. Reaction of 1, 5-bis (6-(4-formylphenoxymethyl) pyridine-2-yl)pentane (0.247 g, 0.0005 mol), 4- amidino-1, 2-phenylenediamine hydrochloride (0.187 g, 0.001 mol) and 1, 4-benzoquinone (0.108 g, 0.001 mol) in anhydrous ethanol (75 ml) (under nitrogen) was heated at reflux for 12-14h. The reaction mixture following standard work-up and drying at 50^0C (12 h in vac.) gave hydrochloride salt as bluish grey solid, 0.33 g (65%); mp >310°C dec.; ^{1}H NMR(DMSO-

d_6)**:** 9.57 (brs, 4H), 9.26 (brs, 4H), 8.47 (d, 4H, J= 8.8 Hz), 8.23 (brs, 2H), 8.21 (apparent t, 2H, J= 8.4 Hz), 7.91 (d, 2H, J= 8.4 Hz), 7.84 (d, 2H, J = 8.4 Hz), 7.76 (d, 2H, J = 8.0 Hz), 7.69 (d, 2H, J = 8.0 Hz), 7.38 (d, 4H, J = 8.8 Hz), 5.56 (s, 4H), 3.0 (t, 4H, J = 6.8 Hz), 1.80-1.77 (brm, 4H), 1.41-1.39 (m, 2H); MS: HR-ESI: calc. for $C_{45}H_{43}N_{10}O_2$ *m/z* 755.3565 (M^++1), found *m/z* 755.3547. Anal. calc'd. for $C_{45}H_{42}N_{10}O_2$-6HCl-2.5H_2O: C,53.05; H, 5.25; N, 13.74; Found: C, 52.91; H, 5.58; N, 13.85

1,5-bis(6-({4[4(5)N-isopropyl-amidino-benzimidazolyl])pyridin-2-yl)-pentane hexahydrochloride (DB 2315)

Reaction of 1, 5-bis(6-(4-formylphenoxymethyl)pyridin-2-yl)pentane (0.247 g, 0.0005 mol), 4-*N*-isopropyl amidino-1, 2-phenylenediamine hydrochloride (0.228 g, 0.001 mol) and 1, 4-benzoquinone (0.108 g, 0.001 mol) in anhydrous ethanol (75 ml) (under nitrogen) was heated at reflux for 12-14h. The reaction mixture following standard work-up and drying at 50^0C (12 h in vac.) gave hydrochloride salt as dark blackish grey solid, 0.35 g (62%); mp >325°C dec.; ^{1}H NMR(DMSO-d_6): 9.78 (brs, 1H), 9.77 (brs, 1H), 9.61 (br, 2H), 9.21 (br, 2H), 8.52 (d, 4H, J = 8.8Hz), 8.21 (apparent t, 2H, J = 8.0 Hz), 8.12 (brs, 2H), 7.91 (d, 2H, J = 8.0 Hz), 7.74 (brd, 4H, J = 8.0 Hz), 7.67 (d, 2H, J = 8.0 Hz), 7.38 (d, 4H, J = 8.8 Hz), 5.55 (s, 4H), 4.12 (sept., 2H, J = 6.5 Hz), 2.99 (t, 4H, J = 6.8 Hz), 1.81-1.77 (brm, 4H), 1.42-1.40 (brm, 2H,), 1.32 (d, 12H, J = 6.5 Hz); MS: HR-ESI: calc. for $C_{51}H_{54}N_{10}O_2$ *m/z* 839.4504 (M^++1), found *m/z* 839.4502. Anal.calc'd. for $C_{51}H_{54}N_{10}O_2$ -6HCl-4.0H_2O: C, 54.21; H, 6.06; N, 12.39; Found: C, 54.44; H, 6.04; N, 12.19.

2,2'-(((2,2,3,3,4,4-hexafluoropentane-1,5-diyl)bis(oxy))bis(4,1-phenylene))bis(1*H*-benzo[*d*]imidazole-6-carboximidamide) tetrahydrochloride (DB 2355).

p-Fluorobenzaldehyde (1.36 gm, 11 mmol) was added to a solution of the 2,2,3,3,4,4-hexafluoropentane-1,5-diol (1.06 gm, 5 mmol), anhydrous K_2CO_3 (1.65 gm, 12 mmol) and anhydrous cesium carbonate (0.65 gm, 2 mmol) in dry DMA (15 ml) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 24 h, cooled, poured on ice-water (50 ml), and extracted with ethyl acetate, dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. Purification by chromatography using ethyl acetate/ hexanes (product is the most polar fraction) gave a white solid of 4,4$^{'0}$-((2,2,3,3,4,4-hexafluoropentane-1,5-diyl)bis(oxy))dibenzaldehyde, yield 0.79 gm (38 %). mp 61-62 ^{o}C; ^{1}HNMR (DMSO-*d*$_6$) δ10.18 (s, 2H); 8.19 (d, *J* = 7.2 Hz, 4H), 7.54 (d, *J* = 7.2 Hz, 4 H), 5.21 (s, 4H). HRMS: m/z calculated for $C_{19}H_{15}F_6O_4$: 421.0875, found: 421.0871 (M^+ +1). The compound was used directly in the next step. To a stirred mixture of the above dialdehyde (0.168 gm, 0.4 mmol) and 4- amidino-1,2-phenylenediamine hydrochloride hydrate (0.148 gm, 0.8 mmol) in 50 ml anhydrous ethanol was added 1,4-benzoquinone (0.086 gm, 0.8 mmol) (under nitrogen). The mixture was heated at reflux for 24 h. The volume of the reaction mixture was reduced to approximately 10 ml under reduced pressure, cooled and 30 ml of ether was added. The resultant solid was collected by filtration and stirred with 40 ml dry acetone for 2-3 h, filtered, washed with ether and dried under reduced pressure. The solid was dissolved in 15 ml of ethanol and saturated with HCl gas, stirred for 2 h at 45-50^{o}C and stirred at room temp for overnight, filtered, washed with acetone and dried to give a brown solid, yield 161 mg (47 %); mp 300 dec. ^{o}C; ^{1}HNMR (DMSO-*d*$_6$) δ 9.56 (s, 4 H); 9.25 (s, 4 H), 8.48 (d, *J* = 7.6 Hz, 4 H), 8.24 (br s, 2H), 7.93 (d, *J* = 7.6 Hz, 2 H), 7.86 (br s, 2H), 7.41 (d, *J* = 7.6 Hz, 4 H), 4.99 (s, 4H). HRMS: m/z calculated for $C_{33}H_{27}F_6N_8O_2$: 681.2161., found: 681.2160 (M^+ +1); Anal. Calcd. For $C_{33}H_{26}F_6N_8O_2$-4 HCl-2 H_2O: C, 45.95; H, 3.97; N, 12.99; Found: C, 45.59; H, 4.21; N, 12.87.

Biophysical Experimental

Material

The syntheses of DB2114 and DB2119 are previously reported (10), and schemes for the new compounds are in Figure S1. DNA oligomers were purchased from Integrated DNA Technologies (Coralville, IA) with HPLC purification and mass spectrometry characterization. Stock solutions of the compounds and DNA were prepared at 0.5 - 1 mM concentrations in deionized H_2O and stored in -4 °C prior to use.

In the DNA thermal melting (T_m), circular dichroism (CD) and electrospray ionization mass spectrometry (ESI-MS) experiments, the following hairpin oligomer sequences were used with the hairpin loop underlined.

A4T4 [5'-CCAAAATTTTGCCTCTGCAAAATTTTGG-3'],

A4**G**T4 [5'-CCAAAA**G**TTTTGCTCTCAAAACTTTTGG-3'],

A4**GC**T4 [5'-CCAAAA**GC**TTTTGCTCTCAAAAGCTTTTGG-3'],

AATTAATT [5'-CCAATTAATTGCTCTCAATTAATTGG-3'],

AATT**G**AATT [5'-CCAATT**G**AATTGCTCTCAATTCAATTGG-3'],

AATT**GC**AATT [5'-CCAATT**GC**AATTGCTCTCAATTGCAATTGG-3'],

In SPR experiments, 5′-biotin labeled hairpin DNA oligomers were used including A4**I**T4 [5'-Biotin-CCAAAA**I**TTTTGCTCTCAAAACTTTTGG-3']. All DNA oligomers were obtained from Integrated DNA Technologies, Inc. (IDT, Coralville, IA) with reverse-phased HPLC purification and mass spectrometry characterization.

The buffer used in T_m, and CD spectroscopy experiments was 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4. The biosensor-surface plasmon resonance (SPR) experiments were performed in filtered, degassed 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4 with 0.05% (v/v) surfactant P20. 150 mM ammonium acetate buffer with 5% or 10% MeOH was used in ESI-MS experiments.

UV-vis Thermal Melting (T_m)

DNA thermal melting experiments were performed on a Cary 300 Bio UV-vis spectrophotometer (Varian). The concentration of each hairpin DNA sequence was 3 μM in Tris-HCl buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4) using 1 cm quartz cuvettes. The mixture solutions of DNA and ligands were tested with the ratio of

2:1 [ligand/DNA]. All samples were annealed prior to each experiment. The spectrophotometer was set at 260 nm with a 0.5 °C/min increase beginning at 25 °C, which is below the DNA melting temperature and ending above it at 95 °C. The absorbance of the buffer was subtracted, and a graph of normalized absorbance *vs.* temperature was created using KaleidaGraph 4.0 software. The ΔT_m values were calculated using a combination of the derivative function and estimation from the normalized graphs.

Biosensor-Surface Plasmon Resonance (SPR)

SPR measurements were performed with a four-channel Biacore T200 optical biosensor system (GE Healthcare, Inc., Piscataway, NJ). A streptavidin-derivatized (SA) CM5 sensor chip was prepared for use by conditioning with a series of 180 s injections of 1 M NaCl in 50 mM NaOH (activation buffer) followed by extensive washing with HBS buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, and 0.05% P20, pH 7.4). Biotinylated-DNA samples (A4T4, A4**G**T4, A4**GC**T4, A4**I**T4, AATT**G**AATT and AATT**GC**AATT hairpins) of 25-30 nM were prepared in HBS buffer and immobilized on the flow cell surface by noncovalent capture as previously described.(7, 8) Flow cell 1 was left blank as a reference, while flow cells 2−4 were immobilized separately by manual injection of biotinylated-DNA stock solutions (flow rate of 1 μL/min) until the desired amount of DNA response units (RU) were obtained (300−330 RU). Ligand solutions were prepared with degassed and filtered Tris-HCl buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4 with 0.05% (v/v) surfactant P20) by serial dilutions from a concentrated stock solution. Typically, a series of different ligand concentrations (2 nM to 500 nM) were injected over the DNA sensor chip at a flow rate of 100 μL/min until a constant steadystate response was obtained (180 s), followed by buffer flow for ligand dissociation (600−1800 s). After each cycle, the sensor chip surface was regenerated with a 10 mM glycine solution (pH 2.5) for 30 s followed by multiple buffer injections to yield a stable baseline for the following cycles. RU_{obs} was plotted as a function of free ligand concentration (C_{free}), and the equilibrium binding constants (K_A) were determined either with a one-site binding model ($K_2 = 0$) or with a two-site model, where $r = (RU_{obs}/RU_{max})$ represents the moles of bound compound/mol of DNA hairpin duplex and K_1 and K_2 are macroscopic binding constants.

$$r = (K_1 \cdot C_{free} + 2K_1 \cdot K_2 \cdot C_{free}^2)/(1 + K_1 \cdot C_{free} + K_1 \cdot K_2 \cdot C_{free}^2) \qquad (1)$$

RU_{max} can be used as a fitting parameter, and the obtained value compared to the predicted maximal response per bound ligand to independently evaluate the stoichiometry (9). Kinetic analyses were performed by globally fitting the binding results for the entire concentration series using a standard 1:1 kinetic model with integrated mass transport-limited binding parameters as described previously (10).

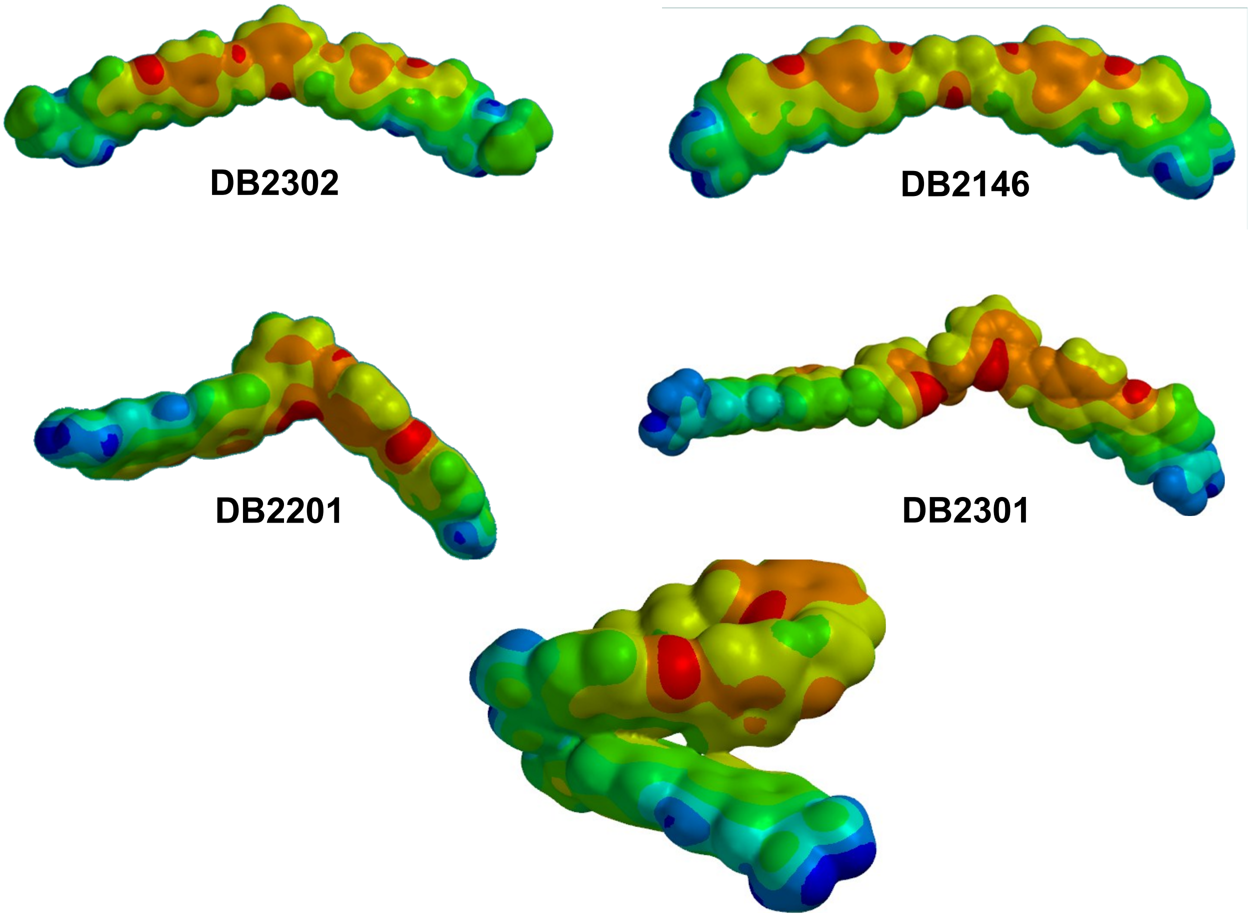
Circular Dichroism (CD)

Circular dichroism experiments were performed on a Jasco J-810 CD spectrometer in 1 cm quartz cuvette at 25 °C. A buffer scan as a baseline was collected first in the same cuvette and subtracted from the scan of following samples. The hairpin DNA sequence, A4**G**T4 and AATT**GC**AATT (5 μM) in Tris-HCl buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4) was added to the cuvette prior to the titration experiments and then the compound (DB2302, DB2146 or DB2295) was added to the DNA solution and incubated for 10 min to achieve equilibrium binding for the DNA-ligand complex formation. For each titration point, four spectra were averaged from 500 to 220 nm wavelength with scan speed 50 nm/min, with a response time of 1s. Baseline-subtracted graphs were created using the KaleidaGraph 4.0 software.

Electrospray Mass Spectrometry (ESI−MS)

ESI−MS experiments were performed on a Q-TOF micro (Waters Micromass, Manchester, U.K.) with its standard ESI source and generally published procedures for DNA (11). The electrospray source was operated in the negative ion mode with a needle voltage of −2.2 kV. The cone voltage was set to −30 V, and the RF lens1 voltage to −60 V. The hexapole collision voltage of 3 V and the ion energy of 1.5 V were used for full scan MS. Source block and desolvation temperatures were set to 70 and 100 °C, respectively. Spectra were acquired from 200 to 3200 m/z and only a portion of the mass range was shown for clarity. DNA oligomers were dialyzed in 150 mM ammonium acetate, pH 6.5, using Spectra/Por 7 dialysis membranes (molecular weight cut off: 1000 Da; Spectrum Laboratories, Inc., CA) to remove the nonvolatile ions. Experiments were generally conducted at a concentration of 5×10^{-6} M for hairpin DNA and the ratios of compound to DNA (1:1, 2:1, and 3:1) were obtained by adding the compound to DNA solution.

Figures and Tables



DB2302

DB2146

DB2201

DB2301

DB2314

Figure S1. Energy minimized structures of mentioned compounds at the 6-31G* (p,d) level of theory. In the electrostatic potential maps, red color indicates high electronegativity and blue indicates electron deficient/positively charged regions**.**

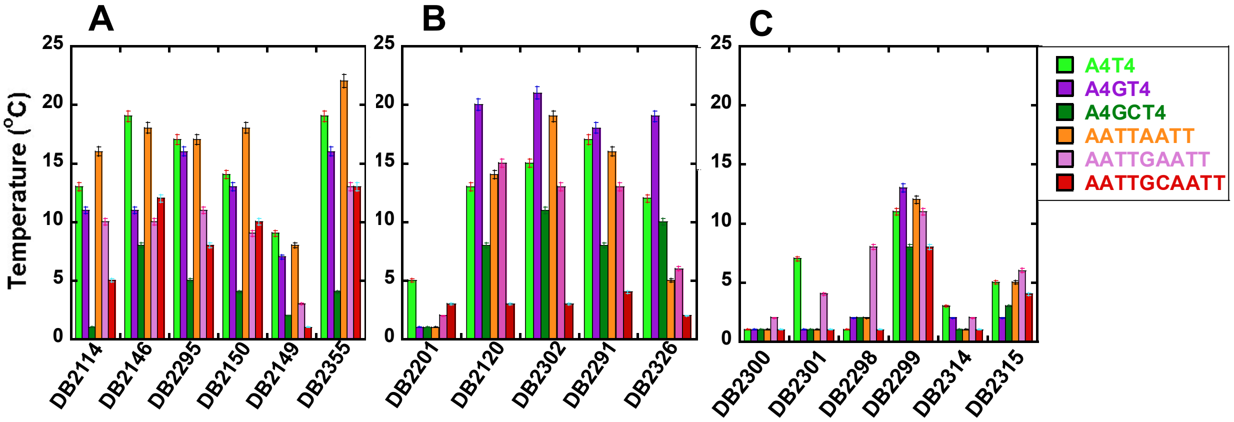


Figure S2. ΔT_m histogram of diamidine complexes with all AT and mixed sequence DNAs

Table S1: Thermal melting studies (ΔT_m; °C) of aliphatic linker linked bis-benzimidazole compounds with mixed DNA sequences.

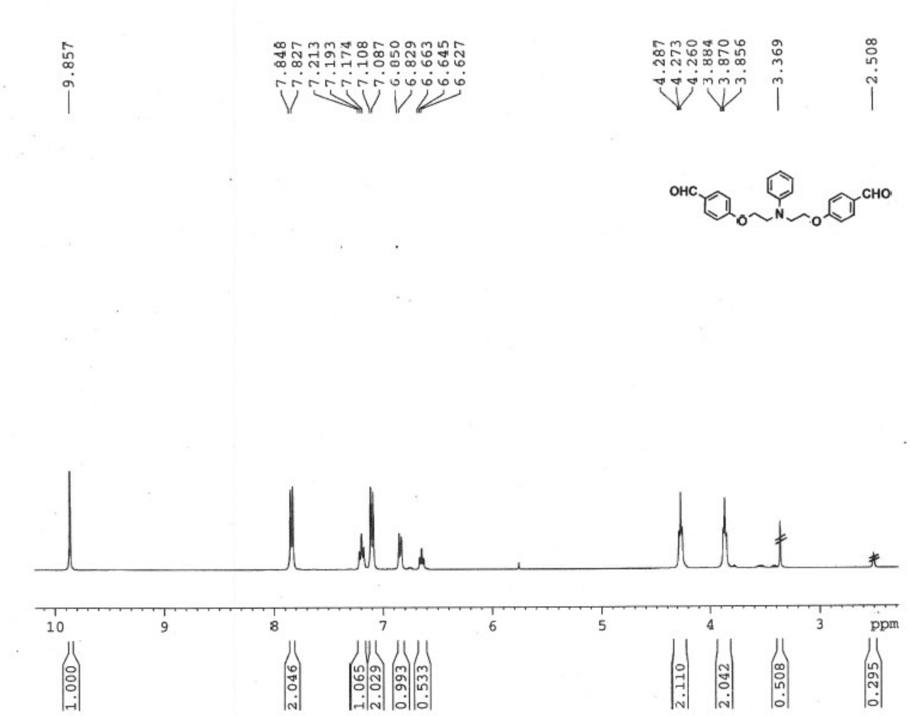
Compound	A4T4	A4 G T4	A4 GC T4	AATT AATT	AATT G AATT	AATT GC AATT
DB2114	13	11	1	16	10	5
DB2146	19	11	8	18	10	12
DB2295	17	16	5	17	11	8
DB2150	14	13	4	18	9	10
DB2149	9	7	2	8	3	1
DB2355	19	16	4	22	13	13

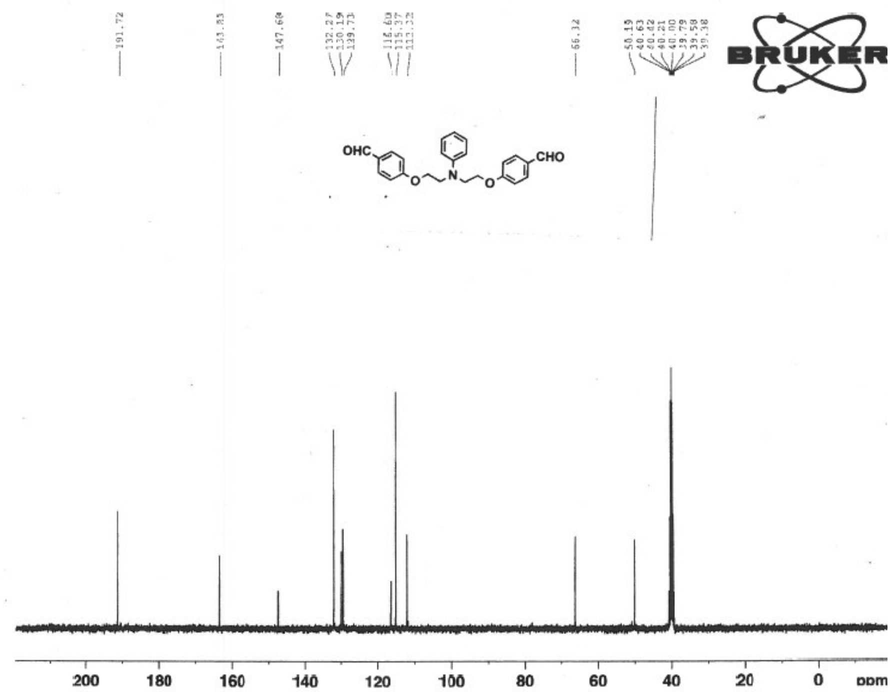
[a]ΔT_m = T_m (the complex) − T_m (the free DNA). The listed values are for 2:1 [ligand]/[DNA] ratio and an average of two independent experiments with a reproducibility of ±0.5 °C.

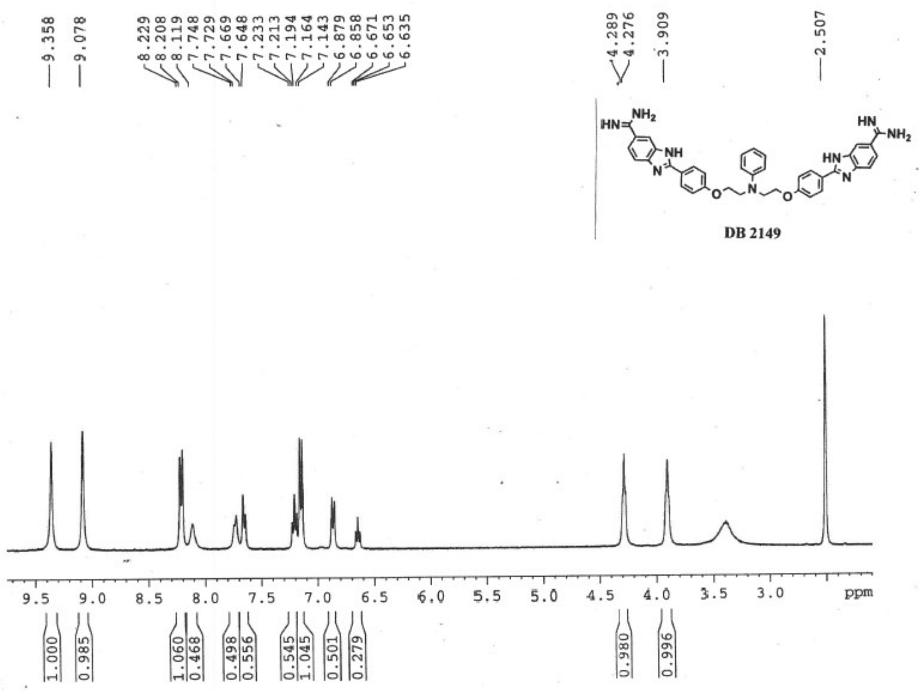
Table S2: Thermal melting studies (ΔT_m; °C) of *bi-pyridyl* linker linked bis-benzimidazole compounds with mixed DNA sequences.

Compound	A4T4	A4 G T4	A4 GC T4	AATT AATT	AATT G AATT	AATT GC AATT
DB2300	1	1	1	1	2	1
DB2301	7	1	1	1	4	1
DB2299*	11	13	8	12	11	8
DB2314	3	2	1	1	2	1
DB2315	5	2	3	5	6	4

${}^{a}\Delta T_m = T_m$ (the complex) − T_m (the free DNA). The listed values are for 2:1 [ligand]/[DNA] ratio and an average of two independent experiments with a reproducibility of ±0.5 °C. * DB2298 data has not shown due ation in solution.



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3.909
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O
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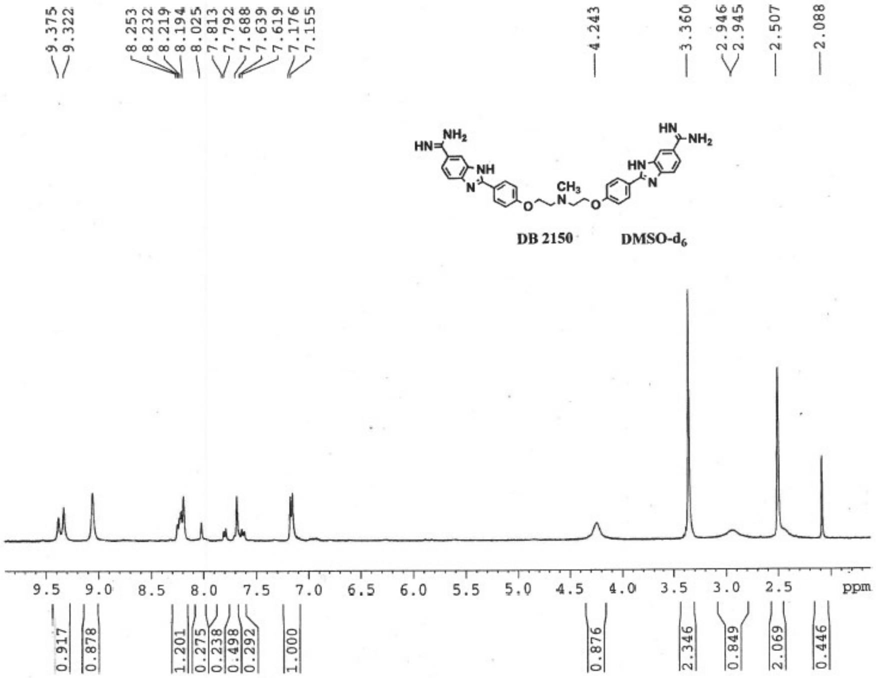
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2.946
2.945
2.507
2.088
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N
CH3
O
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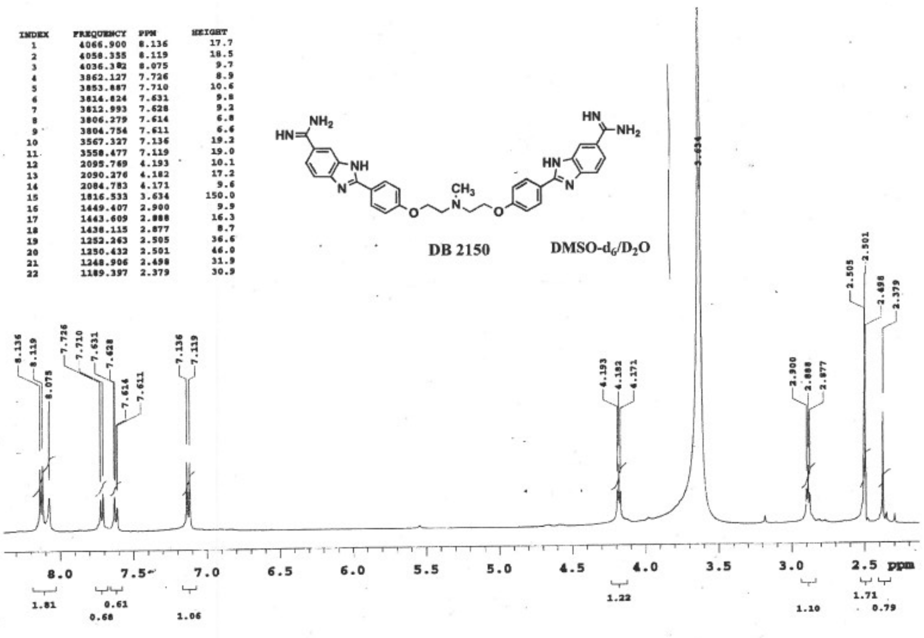

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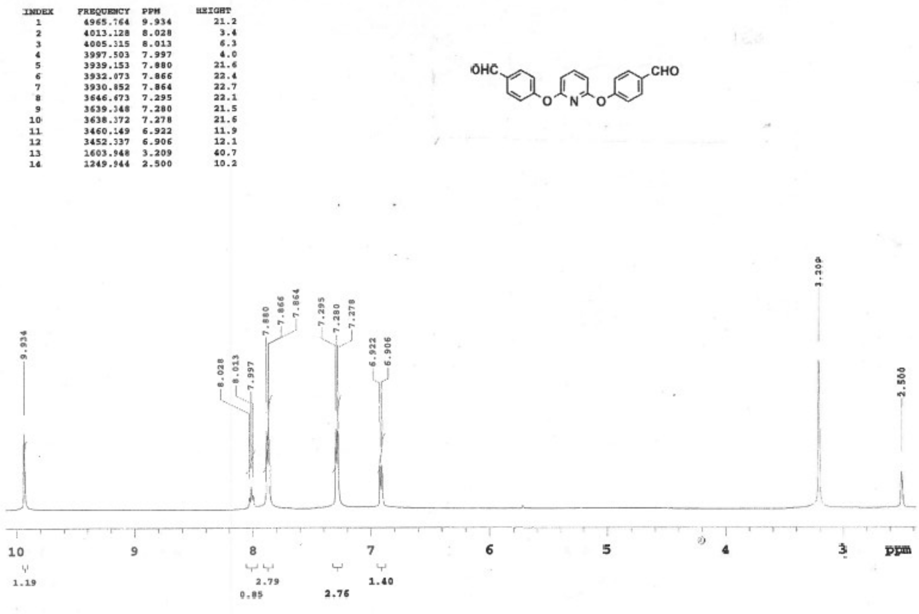
4	3862.127	7.726	8.9
5	3853.887	7.710	10.6
6	3814.824	7.631	9.8
7	3812.993	7.628	9.2
8	3806.279	7.614	6.8
9	3804.754	7.611	6.6
10	3567.327	7.136	19.2
11	3558.477	7.119	19.0
12	2095.769	4.193	10.1
13	2090.276	4.182	17.2
14	2084.783	4.171	9.6
15	1816.533	3.634	150.0
16	1449.407	2.900	9.9
17	1443.609	2.888	16.3
18	1438.115	2.877	8.7
19	1252.263	2.505	36.6
20	1250.432	2.501	46.0
21	1248.906	2.498	31.9
22	1189.397	2.379	30.9

DB 2150

DMSO-d_6/D_2O



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6	3932.073	7.866	22.4
7	3930.852	7.864	22.7
8	3646.673	7.295	22.1
9	3639.348	7.280	21.5
10	3638.372	7.278	21.6
11	3460.149	6.922	11.9
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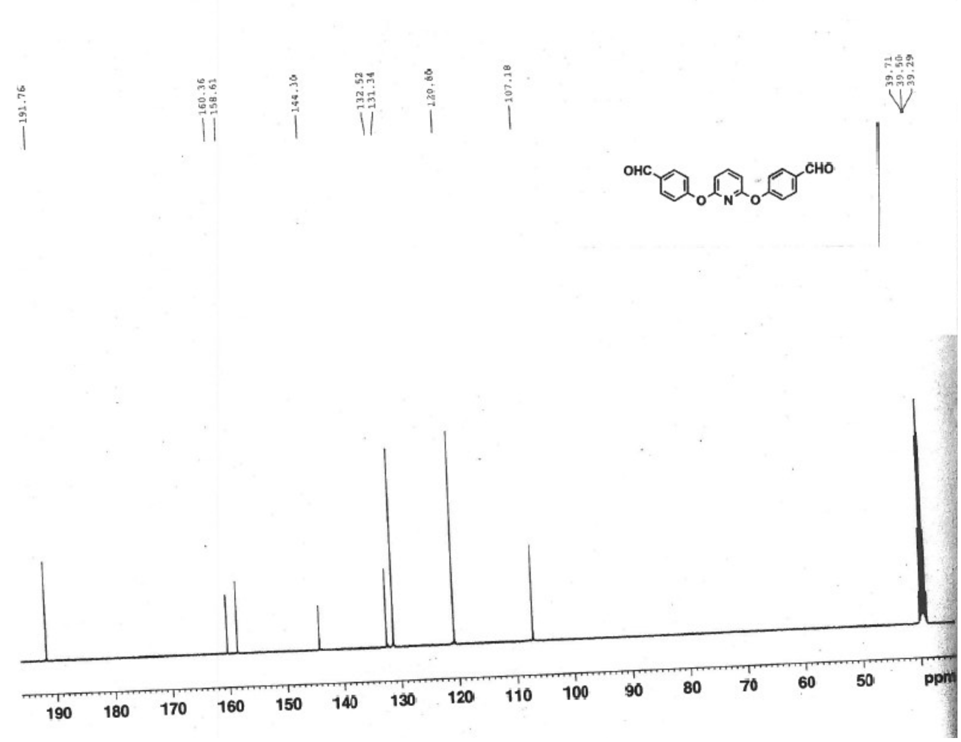
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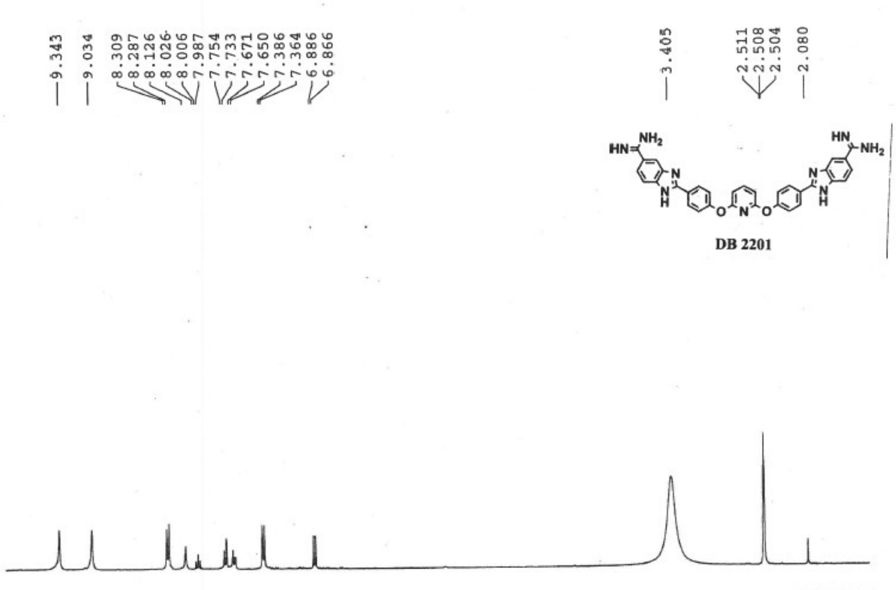
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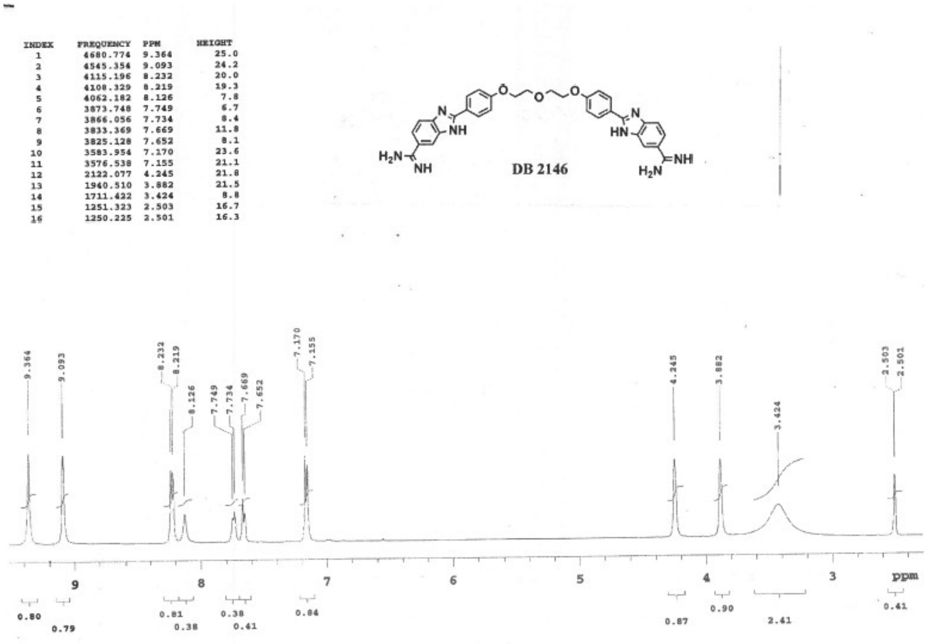

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  5      4062.182  8.126      7.8
  6      3873.748  7.749      6.7
  7      3866.056  7.734      8.4
  8      3833.369  7.669     11.8
  9      3825.128  7.652      8.1
 10      3583.954  7.170     23.6
 11      3576.538  7.155     21.1
 12      2122.077  4.245     21.8
 13      1940.510  3.882     21.5
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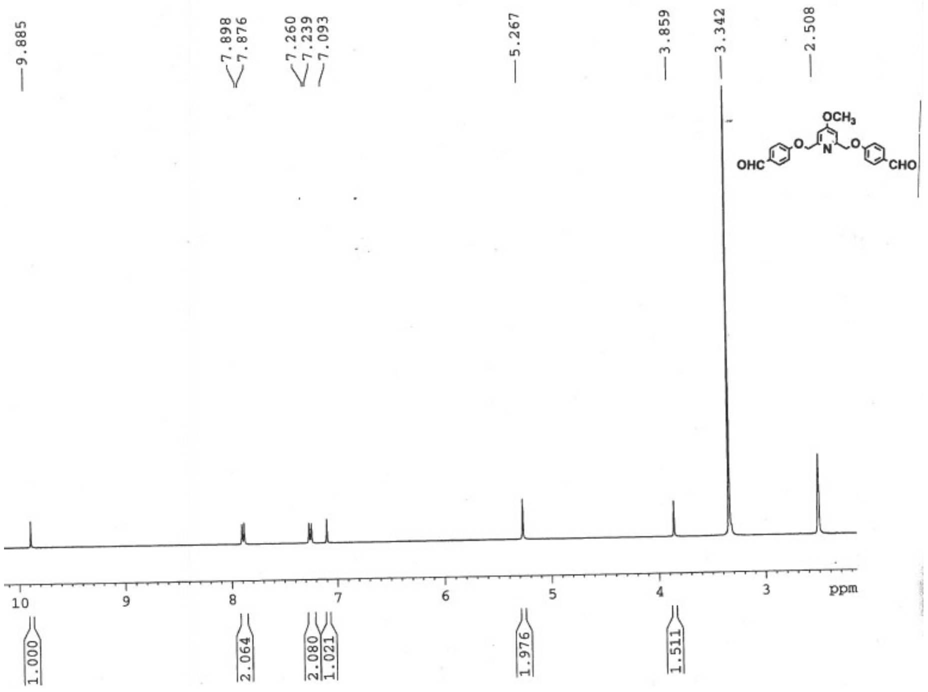

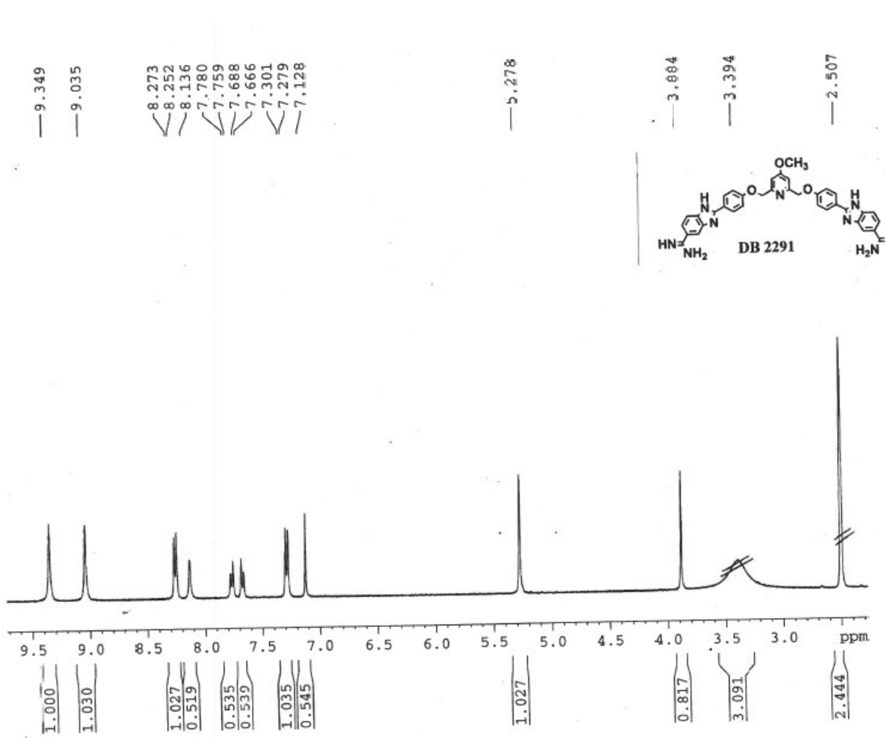
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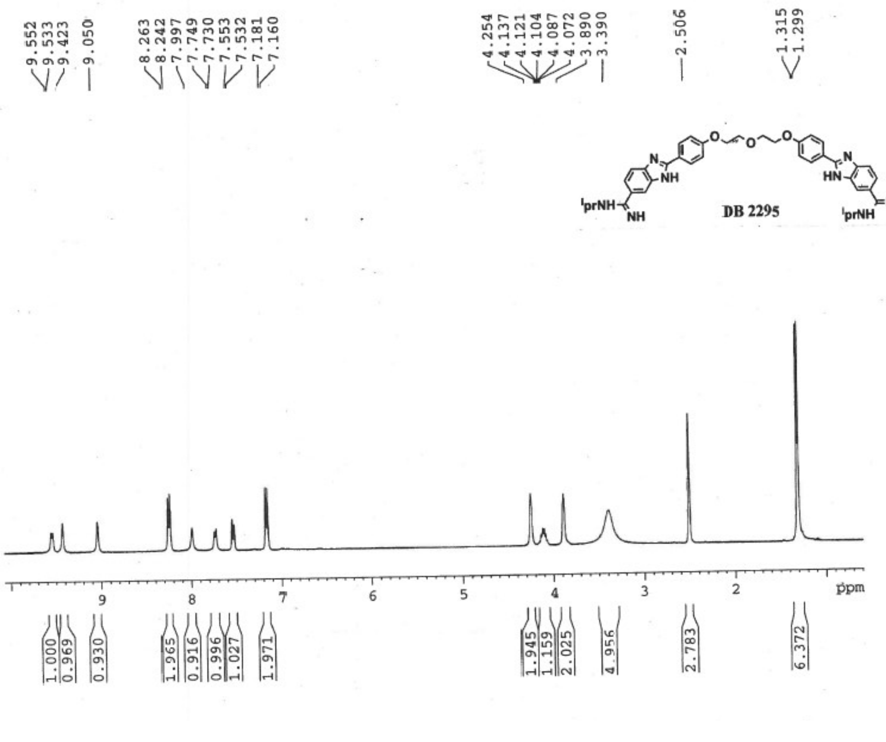
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DB 2295
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3
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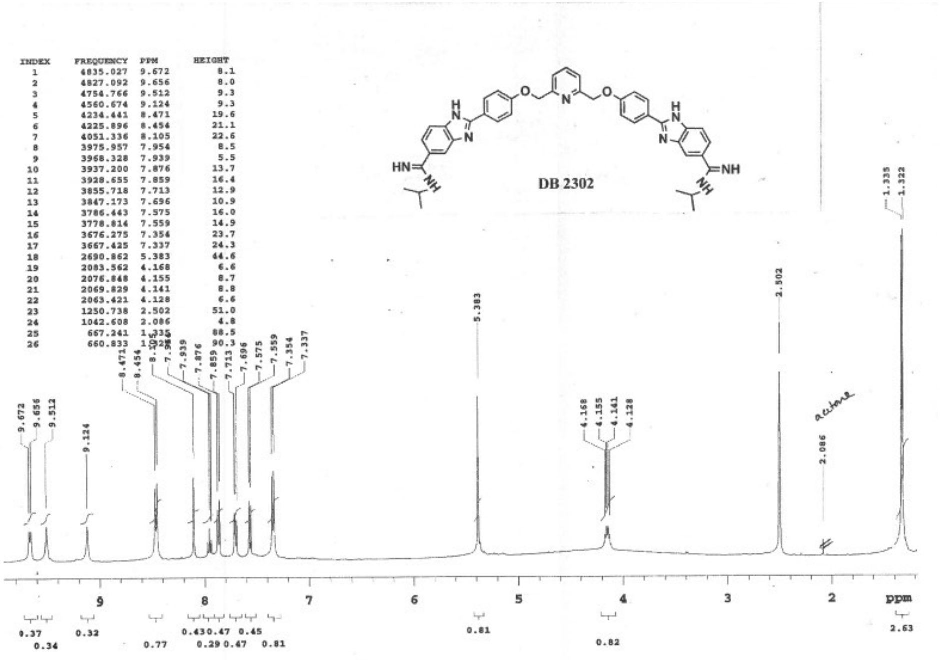

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6	4225.896	8.454	21.1
7	4051.336	8.105	22.6
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9	3968.328	7.939	5.5
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17	3667.425	7.337	24.3
18	2690.862	5.383	44.6
19	2083.562	4.168	6.6
20	2076.848	4.155	8.7
21	2069.829	4.141	8.8
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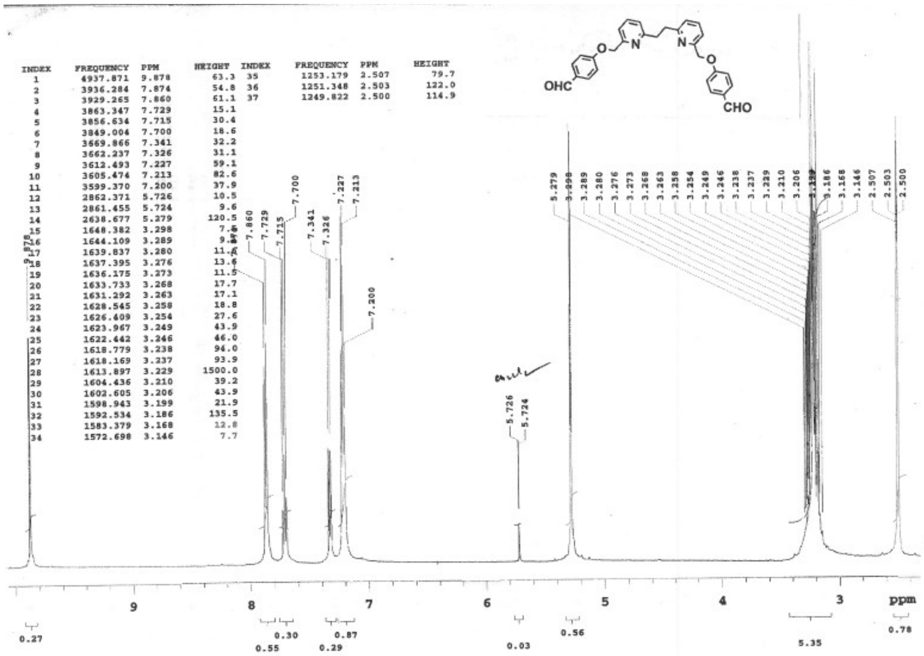

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14	2638.677	5.279	120.5
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16	1644.109	3.289	[illegible]
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19	1636.175	3.273	11.5
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26	1618.779	3.238	94.0
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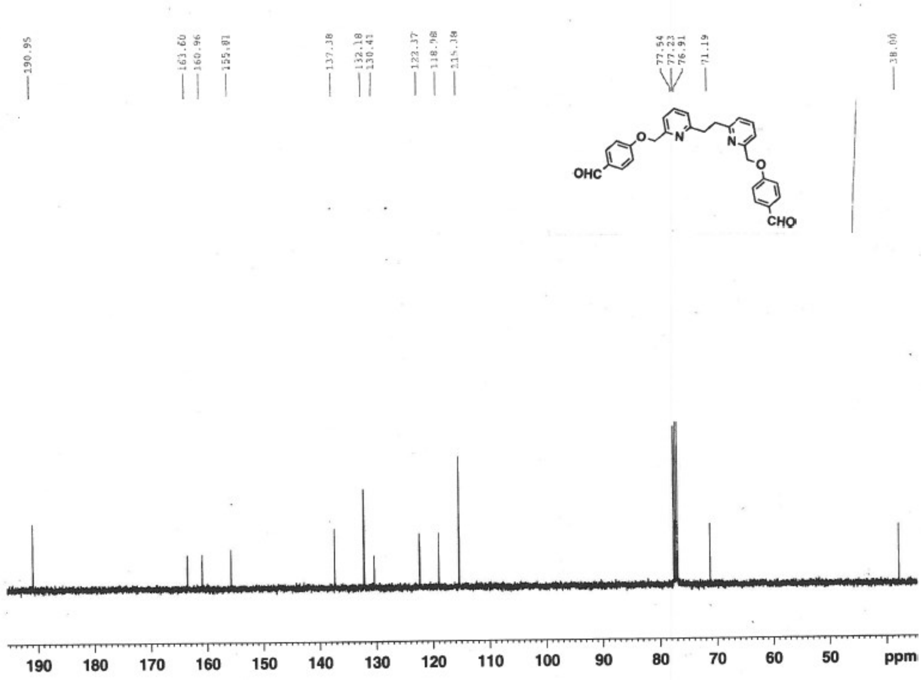

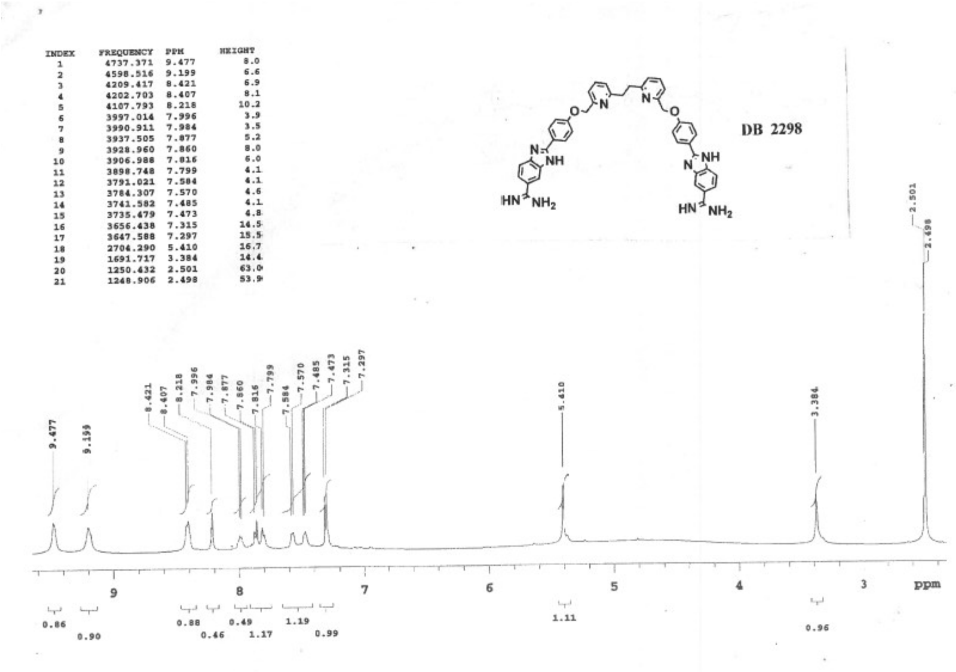

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6	3997.014	7.996	3.9
7	3990.911	7.984	3.5
8	3937.505	7.877	5.2
9	3928.960	7.860	8.0
10	3906.988	7.816	6.0
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12	3791.021	7.584	4.1
13	3784.307	7.570	4.6
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18	2704.290	5.410	16.7
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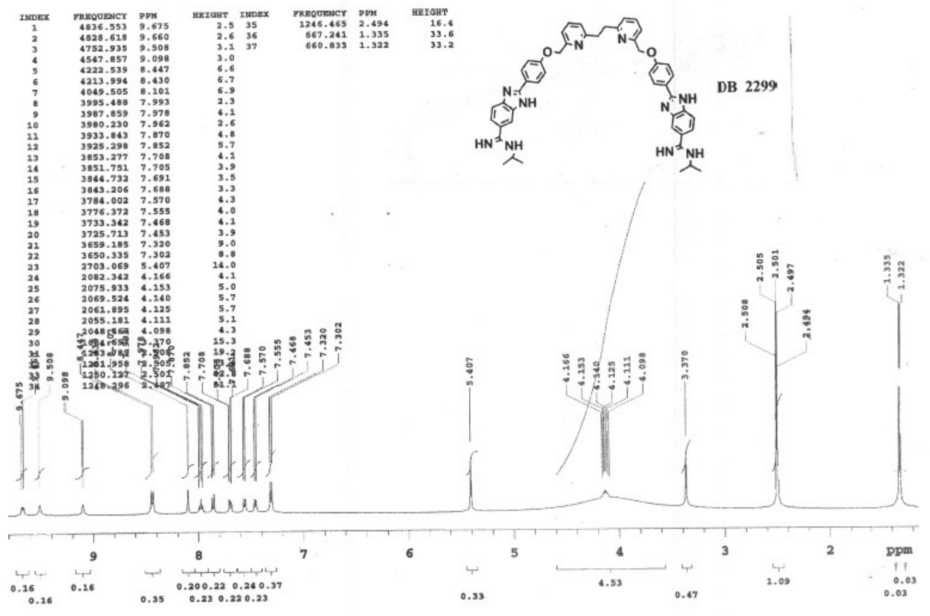
DB 2298



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11	3933.843	7.870	4.8
12	3925.298	7.852	5.7
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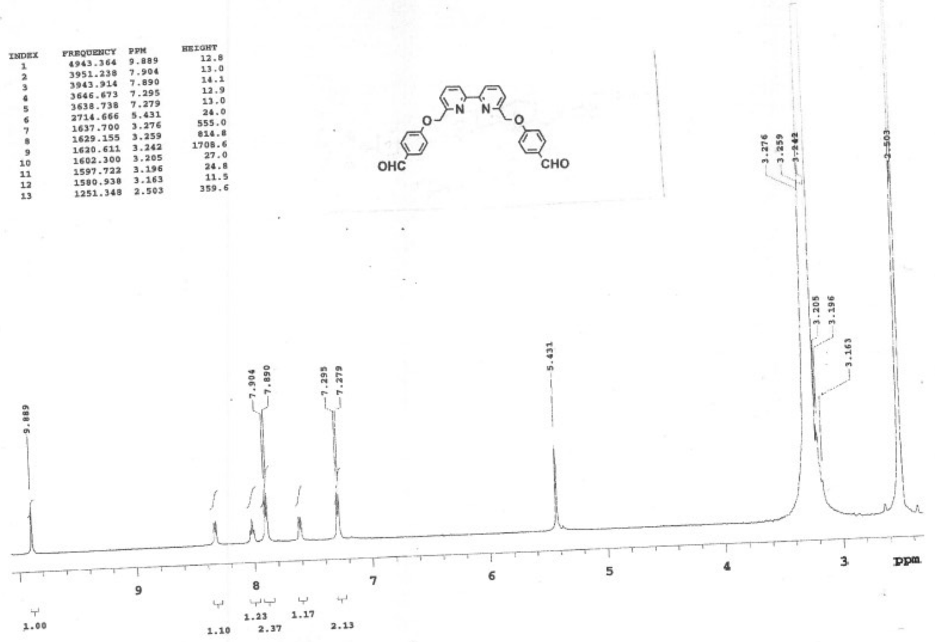
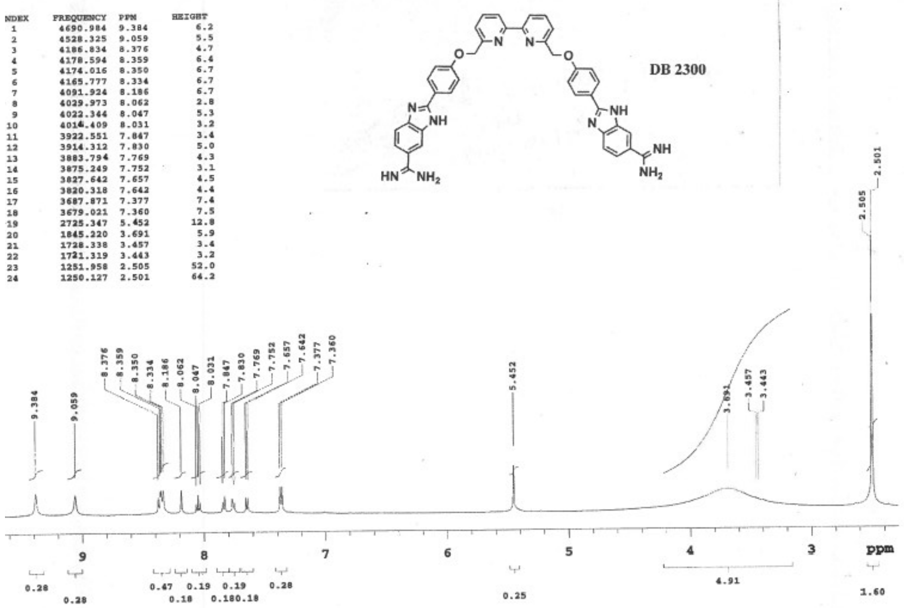
15	3844.732	7.691	3.5
16	3843.206	7.688	3.3
17	3784.002	7.570	4.3
18	3776.372	7.555	4.0
19	3733.342	7.468	4.1
20	3725.713	7.453	3.9
21	3659.185	7.320	9.0
22	3650.335	7.302	8.8
23	2703.069	5.407	14.0
24	2082.342	4.166	4.1
25	2075.933	4.153	5.0
26	2069.524	4.140	5.7
27	2061.895	4.125	5.7
28	2055.181	4.111	5.1
29	2048.468	4.098	4.3
30	[illegible]	3.370	15.3
31	[illegible]	2.508	19.2
32	1251.958	2.505	[illegible]
33	1250.137	2.501	[illegible]
34	1248.296	2.497	51.7

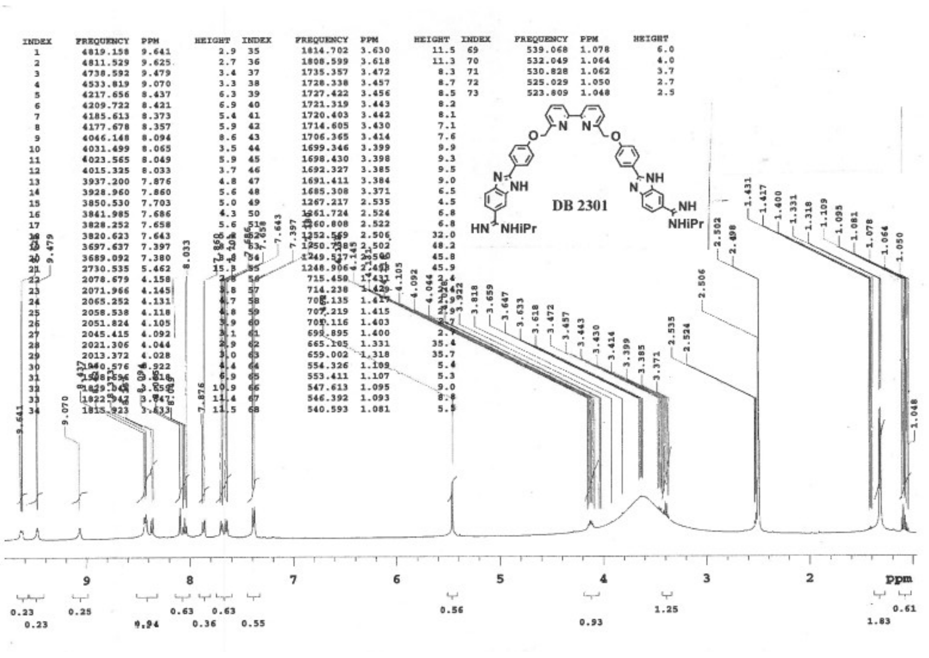
INDEX	FREQUENCY	PPM	HEIGHT
35	1246.465	2.494	16.4
36	667.241	1.335	33.6
37	660.833	1.322	33.2



INDEX	FREQUENCY	PPM	HEIGHT
1	4943.364	9.889	12.8
2	3951.238	7.904	13.0
3	3943.914	7.890	14.1
4	3646.673	7.295	12.9
5	3638.738	7.279	13.0
6	2714.666	5.431	24.0
7	1637.700	3.276	555.0
8	1629.155	3.259	814.8
9	1620.611	3.242	1708.6
10	1602.300	3.205	27.0
11	1597.722	3.196	24.8
12	1580.938	3.163	11.5
13	1251.348	2.503	359.6



NDEX	FREQUENCY	PPM	HEIGHT
1	4690.984	9.384	6.2
2	4528.325	9.059	5.5
3	4186.834	8.376	4.7
4	4178.594	8.359	6.4
5	4174.016	8.350	6.7
6	4165.777	8.334	6.7
7	4091.924	8.186	6.7
8	4029.973	8.062	2.8
9	4022.344	8.047	5.3
10	4014.409	8.031	3.2
11	3922.551	7.847	3.4
12	3914.312	7.830	5.0
13	3883.794	7.769	4.3
14	3875.249	7.752	3.1
15	3827.642	7.657	4.5
16	3820.318	7.642	4.4
17	3687.871	7.377	7.4
18	3679.021	7.360	7.5
19	2725.347	5.452	12.8
20	1845.220	3.691	5.9
21	1728.338	3.457	3.4
22	1721.319	3.443	3.2
23	1251.958	2.505	52.0
24	1250.127	2.501	64.2



INDEX	FREQUENCY	PPM	HEIGHT
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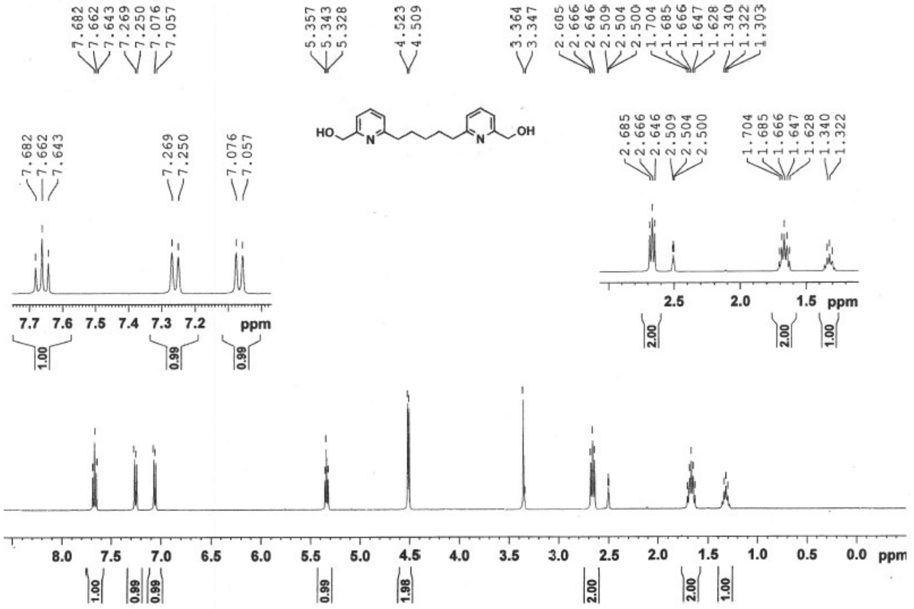
4	4533.819	9.070	3.3
5	4217.656	8.437	6.3
6	4209.722	8.421	6.9
7	4185.613	8.373	5.4
8	4177.678	8.357	5.9
9	4046.148	8.094	8.6
10	4031.499	8.065	3.5
11	4023.565	8.049	5.9
12	4015.325	8.033	3.7
13	3937.200	7.876	4.8
14	3928.960	7.860	5.6
15	3850.530	7.703	5.0
16	3841.985	7.686	4.3
17	3828.252	7.658	5.6
18	3820.623	7.643	[illegible]
19	3697.637	7.397	[illegible]
20	3689.092	7.380	[illegible]
21	2730.535	5.462	15.8
22	2078.679	4.158	2.8
23	2071.966	4.145	3.8
24	2065.252	4.131	4.7
25	2058.538	4.118	4.8
26	2051.824	4.105	3.9
27	2045.415	4.092	3.1
28	2021.306	4.044	2.9
29	2013.372	4.028	3.0
30	1960.576	3.922	4.4
31	1908.696	3.818	6.9
32	1829.04[illegible]	3.659	10.9
33	1822.942	3.647	11.4
34	1815.923	3.633	11.5
35	1814.702	3.630	11.5
36	1808.599	3.618	11.3
37	1735.357	3.472	8.3
38	1728.338	3.457	8.7
39	1727.422	3.456	8.5
40	1721.319	3.443	8.2
41	1720.403	3.442	8.1
42	1714.605	3.430	7.1
43	1706.365	3.414	7.6
44	1699.346	3.399	9.9
45	1698.430	3.398	9.3
46	1692.327	3.385	9.5
47	1691.411	3.384	9.0
48	1685.308	3.371	6.5
49	1267.217	2.535	4.5
50	1261.724	2.524	6.8
51	1260.808	2.522	6.8
52	1252.569	2.506	32.0
53	1250.738	2.502	48.2
54	1249.517	2.500	45.8
55	1248.906	2.498	45.9
56	715.450	1.431	2.4
57	714.238	1.429	2.4
58	708.135	1.417	2.9
59	707.219	1.415	2.9
60	701.116	1.403	2.7
61	699.895	1.400	2.7
62	665.105	1.331	35.4
63	659.002	1.318	35.7
64	554.326	1.109	5.4
65	553.411	1.107	5.3
66	547.613	1.095	9.0
67	546.392	1.093	8.8
68	540.593	1.081	5.5
69	539.068	1.078	6.0
70	532.049	1.064	4.0
71	530.828	1.062	3.7
72	525.029	1.050	2.7
73	523.809	1.048	2.5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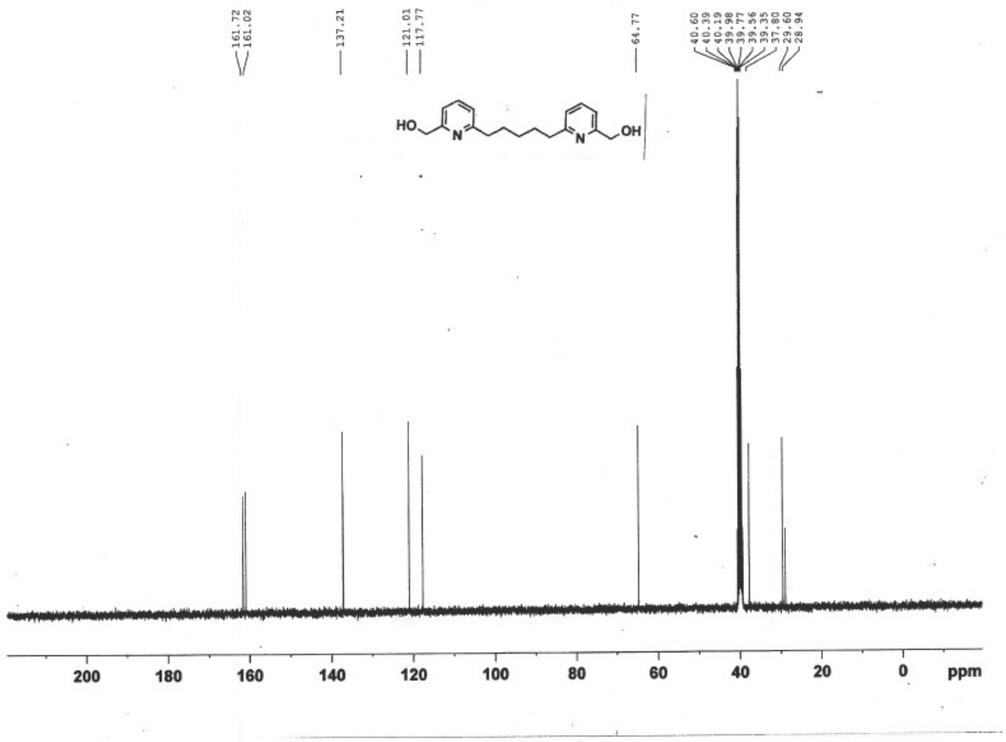
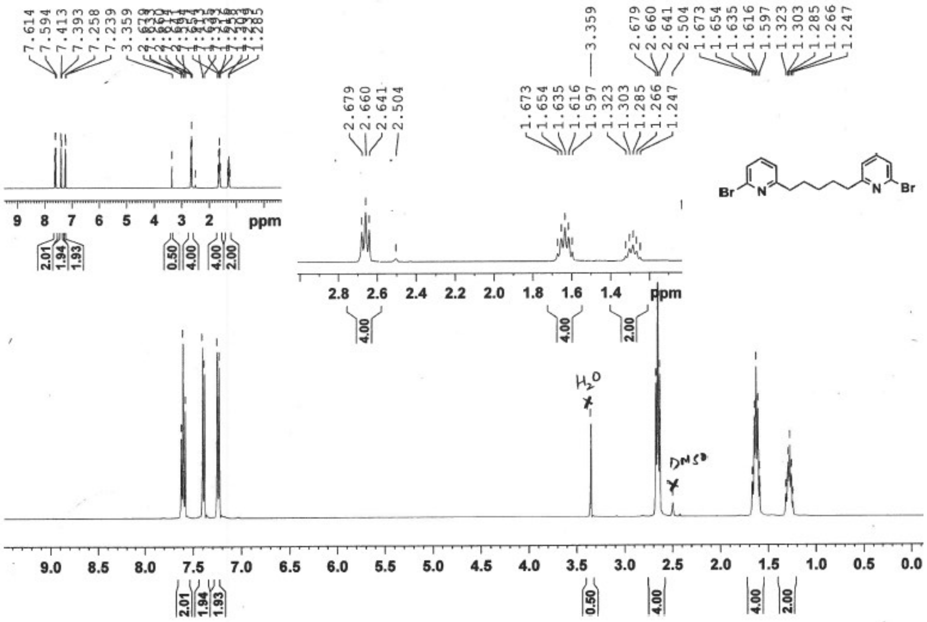
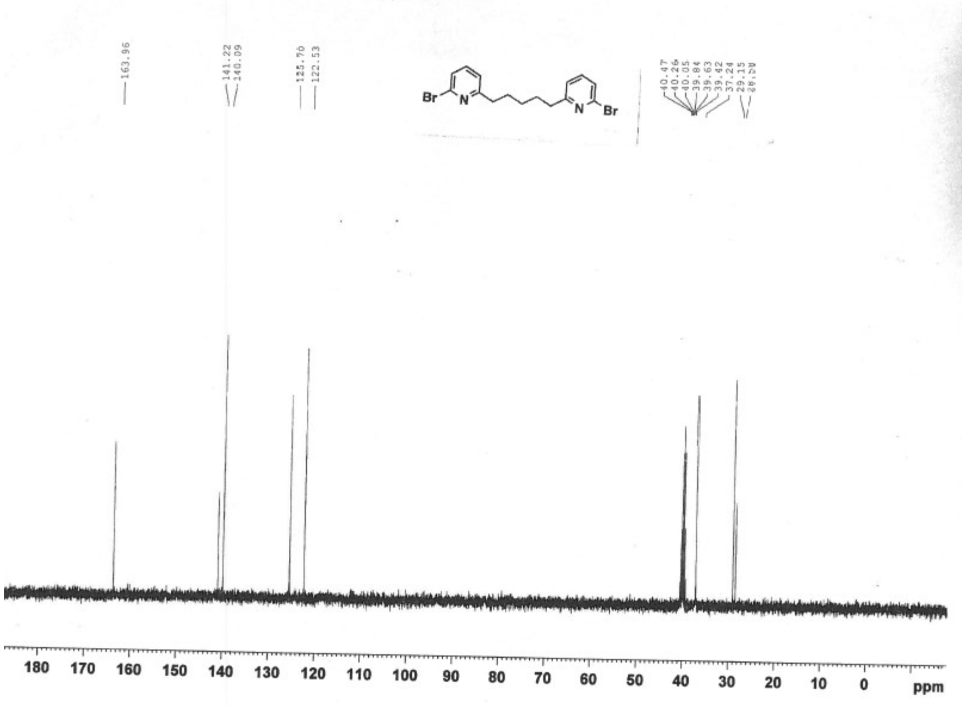
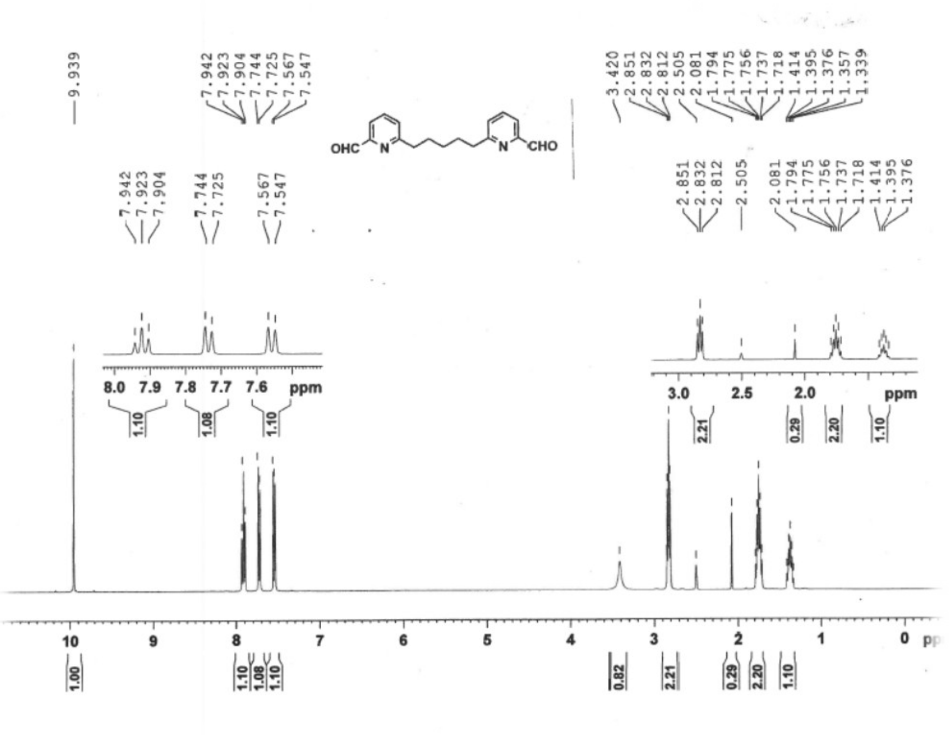
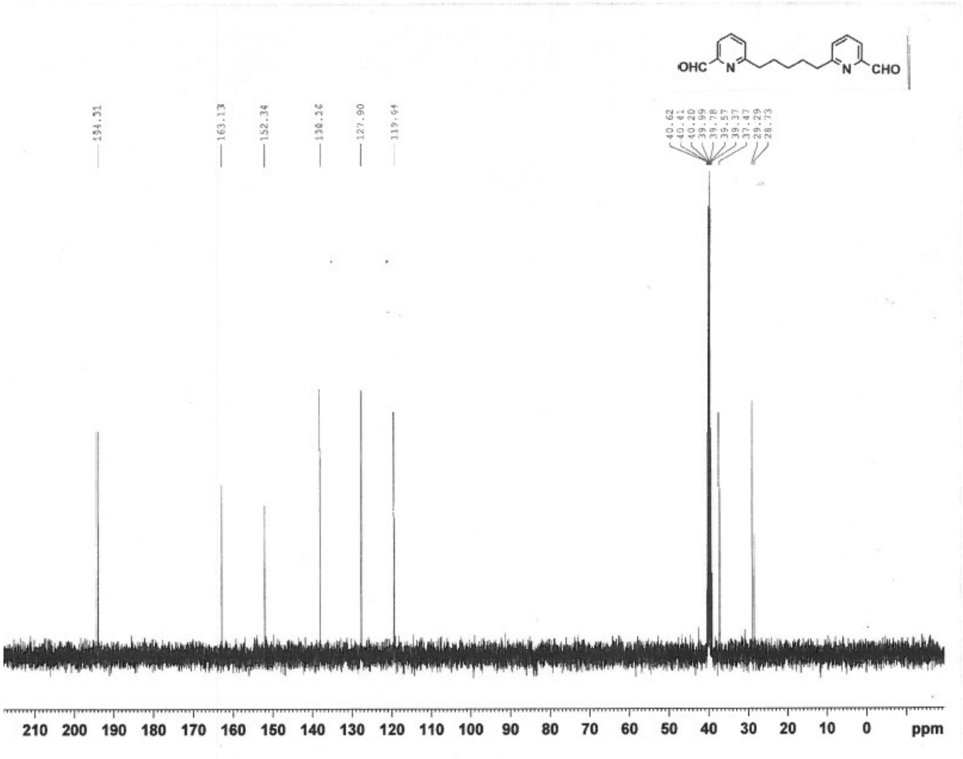
7.662
7.643
7.269
7.250
7.076
7.057
5.357
5.343
5.328
4.523
4.509
3.364
3.347
2.685
2.666
2.646
2.509
2.504
2.500
1.704
1.685
1.666
1.647
1.628
1.340
1.322
1.303
HO
N
N
OH
7.7 7.6 7.5 7.4 7.3 7.2 ppm
1.00
0.99
0.99
2.5 2.0 1.5 ppm
2.00
2.00
1.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
1.00
0.99
0.99
0.99
1.98
2.00
2.00
1.00

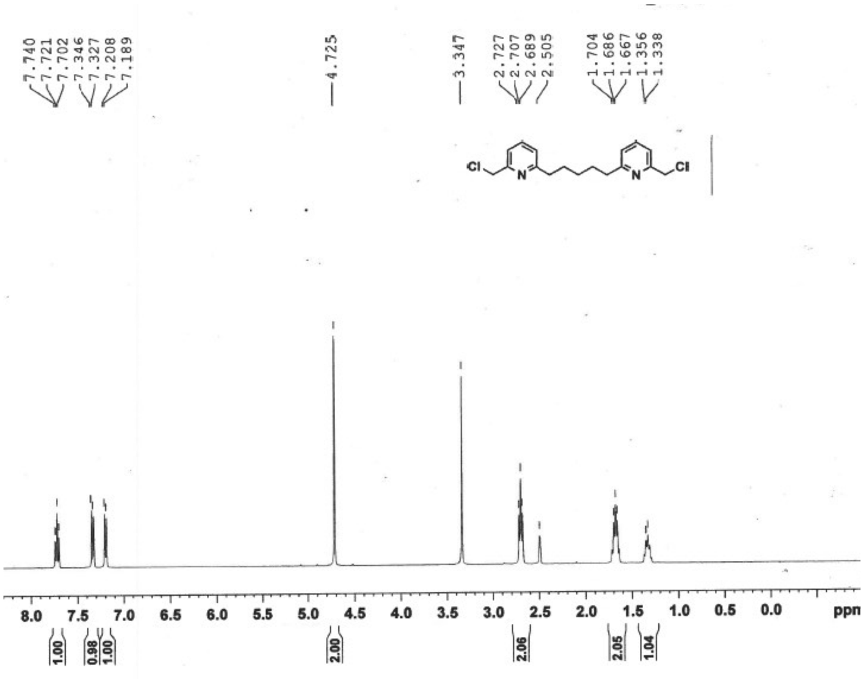

161.72
161.02
137.21
121.01
117.77
64.77
40.60
40.39
40.19
39.98
39.77
39.56
39.35
37.80
29.60
28.94
HO
N
N
OH
200 180 160 140 120 100 80 60 40 20 0 ppm

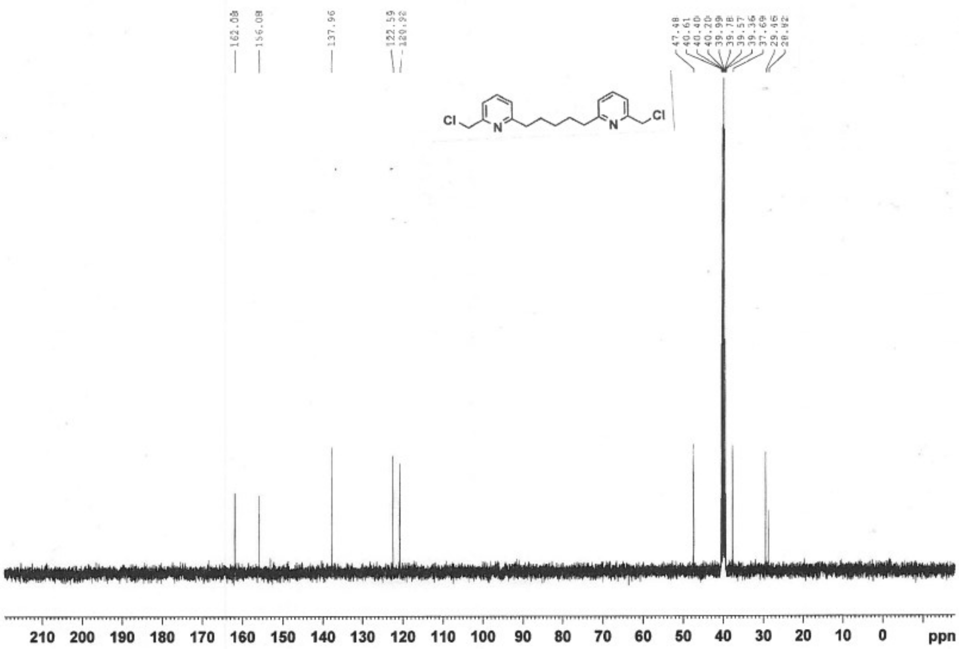

7.614
7.594
7.413
7.393
7.258
7.239
3.359
1.285
2.679
2.660
2.641
2.504
1.673
1.654
1.635
1.616
1.597
1.323
1.303
1.285
1.266
1.247
3.359
2.679
2.660
2.641
2.504
1.673
1.654
1.635
1.616
1.597
1.323
1.303
1.285
1.266
1.247
Br
N
N
Br
9
8
7
6
5
4
3
2
ppm
2.01
1.94
1.93
0.50
4.00
4.00
2.00
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
ppm
4.00
4.00
2.00
H₂O
DMSO
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2.01
1.94
1.93
0.50
4.00
4.00
2.00

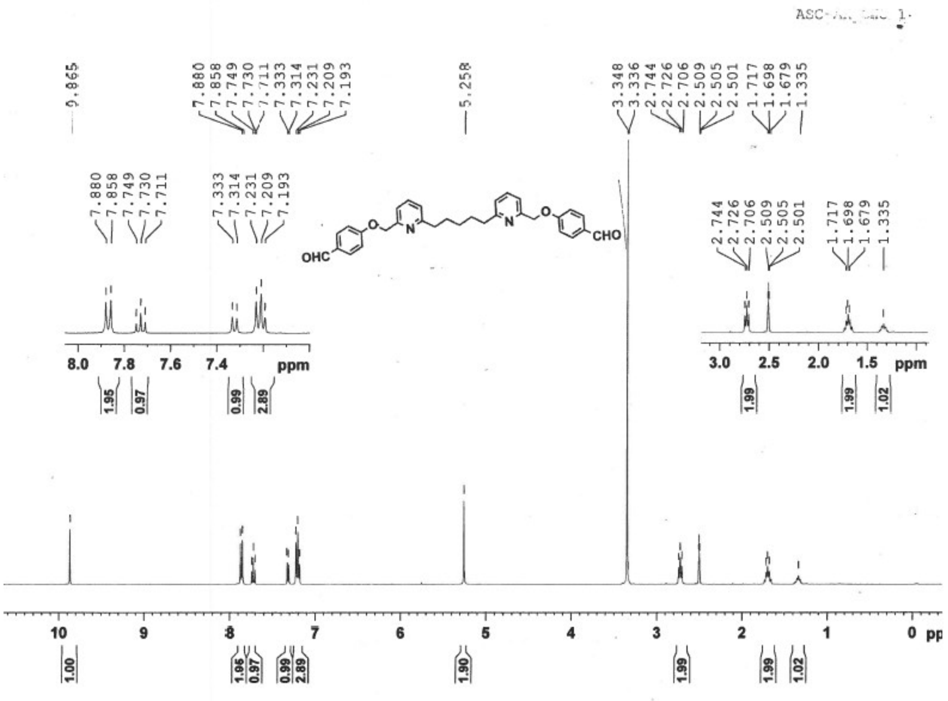

163.96
141.22
140.09
125.76
122.53
Br
N
N
Br
40.47
40.26
40.05
39.84
39.63
39.42
37.24
29.15
28.58
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

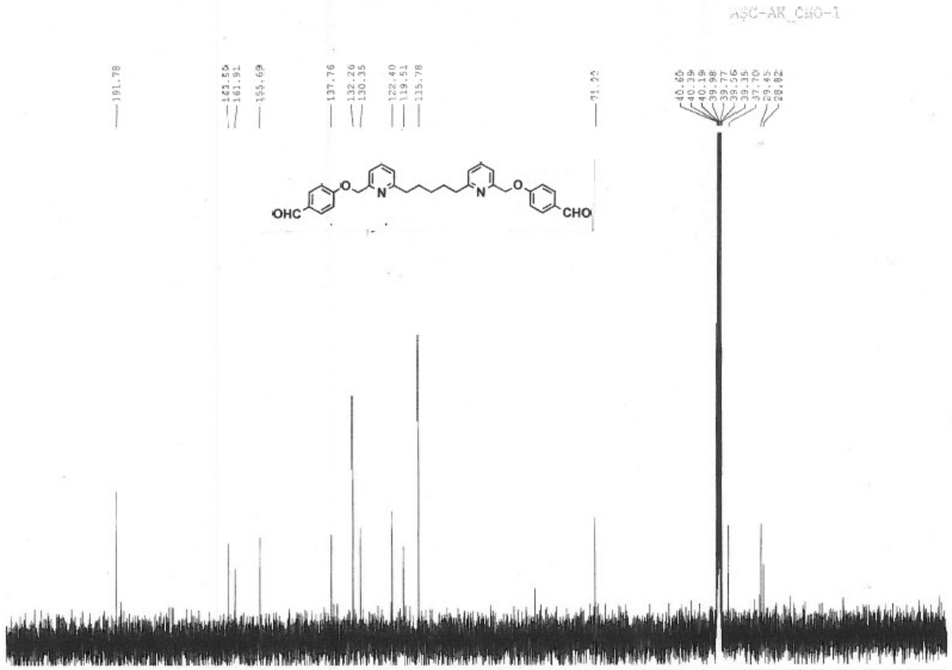

9.939
7.942
7.923
7.904
7.744
7.725
7.567
7.547
3.420
2.851
2.832
2.812
2.505
2.081
1.794
1.775
1.756
1.737
1.718
1.414
1.395
1.376
1.357
1.339
OHC
N
N
CHO
8.0 7.9 7.8 7.7 7.6 ppm
3.0 2.5 2.0 ppm
1.10
1.08
1.10
2.21
0.29
2.20
1.10
10 9 8 7 6 5 4 3 2 1 0 ppm
1.00
1.10
1.08
1.10
0.82
2.21
0.29
2.20
1.10

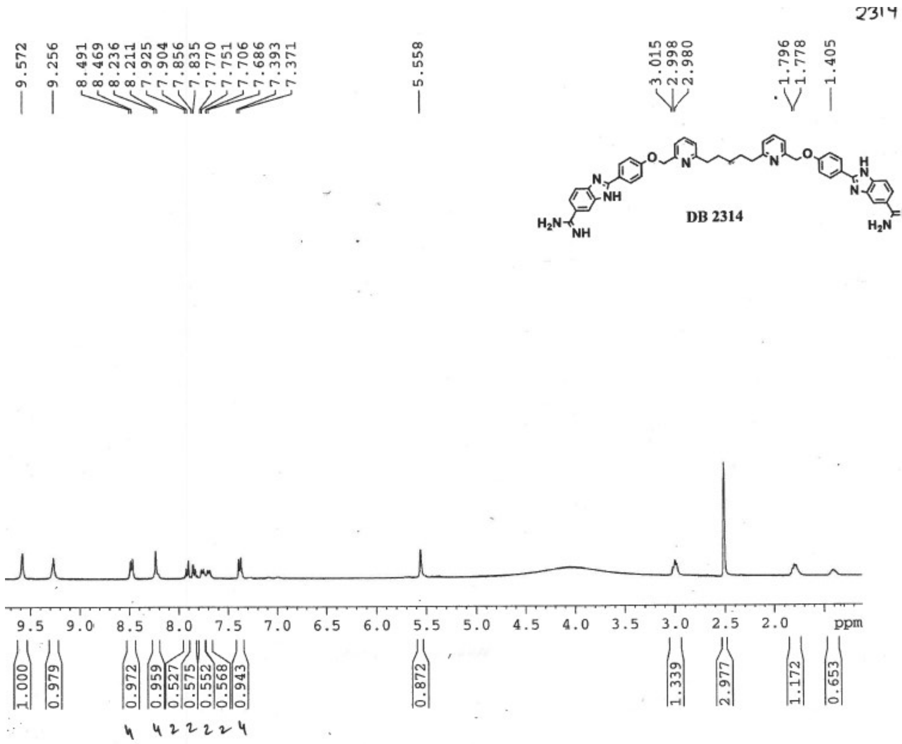

OHC
N
N
CHO
194.31
163.13
152.34
138.24
127.90
119.64
40.62
40.41
40.20
39.99
39.78
39.57
39.37
37.47
29.29
28.73
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

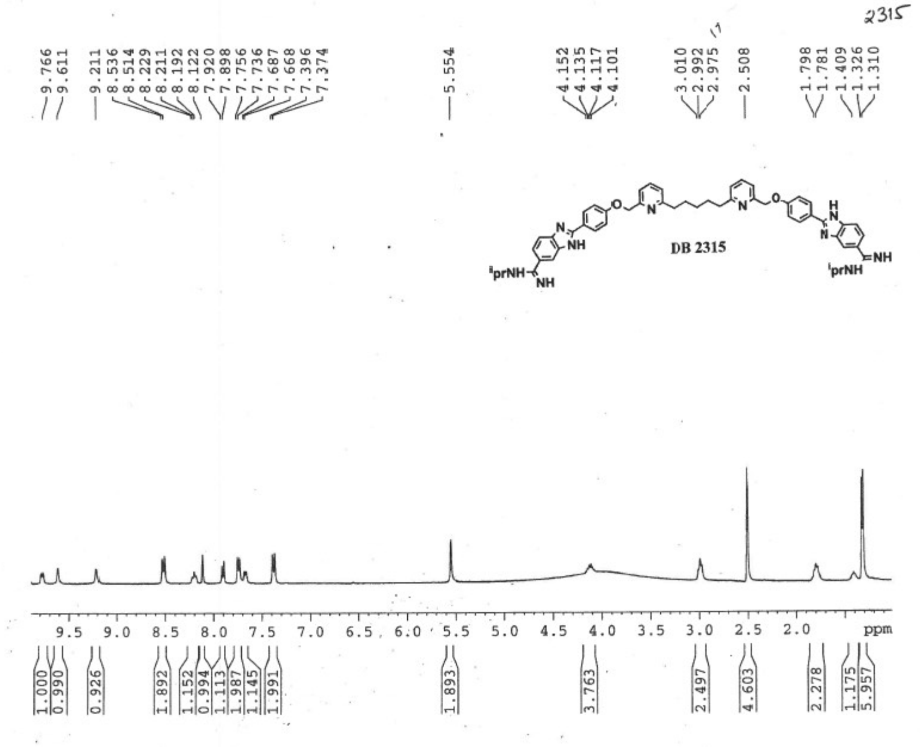

7.740
7.721
7.702
7.346
7.327
7.208
7.189
4.725
3.347
2.727
2.707
2.689
2.505
1.704
1.686
1.667
1.356
1.338
Cl
N
N
Cl
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1.00
0.98
1.00
2.00
2.06
2.05
1.04


162.08
156.08
137.96
122.59
120.92
47.48
40.61
40.40
40.20
39.99
39.78
39.57
39.36
37.69
29.46
28.92
Cl
N
N
Cl
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


ASC-AK_CHO 1
9.865
7.880
7.858
7.749
7.730
7.711
7.333
7.314
7.231
7.209
7.193
5.258
3.348
3.336
2.744
2.726
2.706
2.509
2.505
2.501
1.717
1.698
1.679
1.335
OHC
O
N
N
O
CHO
8.0 7.8 7.6 7.4 ppm
1.95 0.97 0.99 2.89
3.0 2.5 2.0 1.5 ppm
1.99 1.99 1.02
10 9 8 7 6 5 4 3 2 1 0 pp
1.00 1.96 0.97 0.99 2.89 1.90 1.99 1.99 1.02


ASC-AK_CHO-1
191.78
163.55
161.91
155.69
137.76
132.26
130.35
122.40
119.51
115.78
71.22
40.60
40.39
40.19
39.98
39.77
39.56
39.35
37.70
29.45
28.82
OHC
O
N
N
O
CHO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


2314
9.572
9.256
8.491
8.469
8.236
8.211
7.925
7.904
7.856
7.835
7.770
7.751
7.706
7.686
7.393
7.371
5.558
3.015
2.998
2.980
1.796
1.778
1.405
N
NH
O
N
N
O
H
N
N
H$_2$N
NH
DB 2314
=NH
H$_2$N
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.000
0.979
0.972
0.959
0.527
0.575
0.552
0.568
0.943
0.872
1.339
2.977
1.172
0.653
4 422224


2315
9.766
9.611
9.211
8.536
8.514
8.229
8.211
8.192
8.122
7.920
7.898
7.756
7.736
7.687
7.668
7.396
7.374
5.554
4.152
4.135
4.117
4.101
3.010
2.992
2.975
2.508
1.798
1.781
1.409
1.326
1.310
N
NH
O
N
N
O
H
N
N
^{i}prNH
NH
DB 2315
=NH
^{i}prNH
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.000
0.990
0.926
1.892
1.152
0.994
1.113
1.987
1.145
1.991
1.893
3.763
2.497
4.603
2.278
1.175
5.957

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