SYNTHESIS OF 2,4-BIS[4-(5-AMIDINO AND 5-SUBSTITUTEDAMIDINO-2-BENZIMIDAZOYL)PYRIMIDINES

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Abstract: The syntheses of 2,4-bis[4-(5-imidazolin-2yl-2-benzimidazolyl)phenyl]pyrimidine, 2,4-bis[4-(5-amidino-2-benzimidazolyl)phenyl]pyrimidine, and 2,4-bis[4-(5-N-isopropylamidino-2-benzimidazolyl)phenyl]pyrimidine from 2,4-bis-(4-cyanophenyl)pyrimidine are reported.

Introduction

We recently reported that dicationic 2,4-diarylpyrimidines bind strongly in the minor-groove of AT rich DNA (1). We demonstrated that by careful manulipation of structure highly DNA selective molecules could be developed. It has been further shown that dicationic 2,4-diarylpyrimidines have useful activity against the important AIDS associated pathogen *Pneumocystis carinii* pneumonia. Certain of the dicationic 2,4-diarylpyrimidines are quite active in the immunosuppressed rat model (2). Modeling studies suggest that the parent dicationic 2,4-diarylpyrimidine system occupies approximately 3 to 4 base pairs when binding in the DNA minor-groove. Potentially, base-pair selectivity can be increased by employing larger molecules which on binding to DNA would interact with more base pairs.

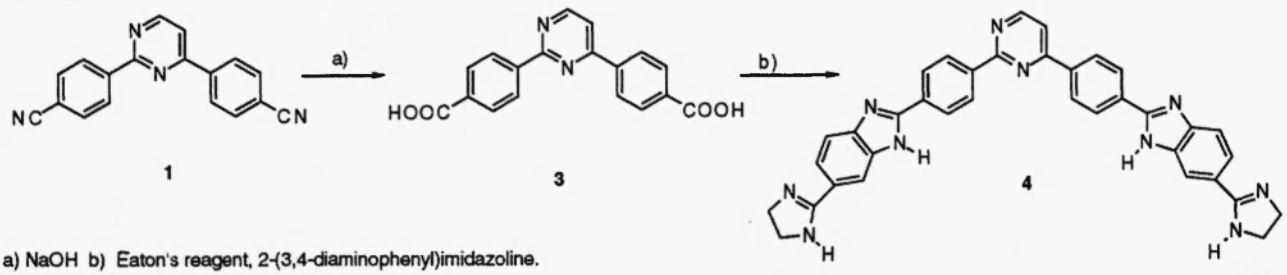
In a study of the influence of structural changes on the minor-groove binding affinity of the benzimidazole Hoechst 33258 and related compounds we found that DNA binding affinity was increased much more significantly by the addition of a second benzimidazole ring than by addition of a second cationic center (3). Based upon our work with the Hoechst analogs we expect that the binding affinity of the 2,4-diarylpyrimidine system should be enhanced by addition of benzimidazole rings to the parent structure. In order to make such compounds available for biophysical and antimicrobial evaluation, we undertook the synthesis of 2,4-bis[4-(5-substituted-2-benzimidazolyl)phenyl]pyrimidines, which are expected to show strong DNA binding affinity and occupy 6 or more base pairs on binding to DNA.

Results and Discussion

The synthesis of the extended 2,4-diarylpyrimidines is based upon our previously reported approach to 2,4-bis[diaryl]pyrimidines and uses 2,4-bis[4-cyanophenyl]pyrimidine(**1**) as the key starting molecule (1). We chose to incorporate benzimidazoles units in the 2,4-diarylpyrimidine parent system by using *ortho*-phenylenediamine methodology. These are two general approaches for formation of benzimidazole rings starting from *ortho*-phenylenediamines; one involves direct condensation with a carboxylic acid group (or other functional group of the same carbon oxidation level) (4) and the second employs condensation with an aldehyde and subsequent oxidation of the condensation product to form the benzimidazole ring (5). For the synthesis of dicationic aryl molecules it is typically desirable to make an appropriate bis-nitrile so that it can serve as precursor,

through the intermediacy of bis-imidate esters, for a number of different dicationic centers. Towards this end we synthesized 2,4-bis[4-(5-cyano-2-benzimidazolyl)phenyl]pyrimidine **2** by reacting 3,4-diaminobenzonitrile with the imidate ester prepared from 2,4-bis(4-cyanophenyl)pyrimidine. Unfortunately, in our hands, the bis-nitrile **2** was extremely insoluble and efforts to make and use the corresponding imidate ester lead to nonseparable mixtures.

SCHEME 1.

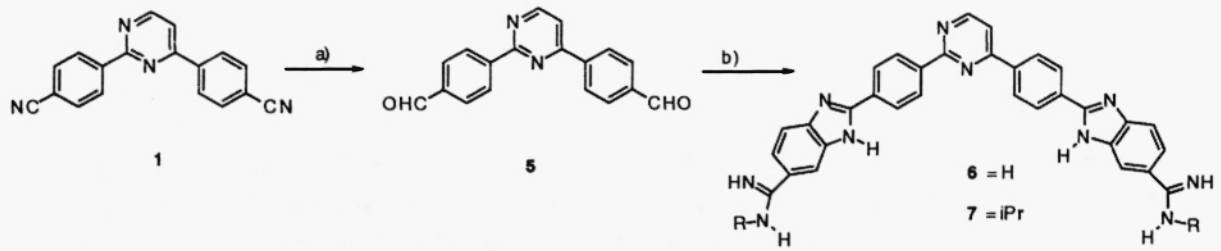


a) NaOH b) Eaton's reagent, 2-(3,4-diaminophenyl)imidazoline.

To circumvent this problem we obtained 2,4-bis(4-carboxyphenyl)pyrimidine **3** by base promoted hydrolysis of 2,4-bis(4-cyanophenyl)pyrimidine. Condensation of the diacid with 2-(3,4-diaminophenyl)imidazoline with aid of Eaton's reagent (6) formed the desired dicationic compound **4**, however only in approximately a 20% yield [Scheme 1].

In view of the modest yield obtained from the diacid route we decided to explore the aldehyde condensation approach in an effort to obtain enhanced yields. The bis-aldehyde, 2,4-bis(4-formylphenyl)pyrimidine **5**, was obtained by reduction of the bis-nitrile **1** with diisobutyl aluminum hydride (DIBALH) (7), however only in an approximately 35% yield. Efforts to improve the yield of the bis-aldehyde by employing shorter reaction times gave mixtures of unreacted bis-nitrile and the desired bis-aldehyde, longer reaction times resulted in formation of a product which was further reduced, presumably 2,4-bis(4-hydroxymethylphenyl)pyrimidine. Reaction of **5** with 3,4-diaminobenzamidine in the presence of 1,4-benzoquinone gave the desired diamidine, 2,4-bis[4-(5-amidino-2-benzimidazolyl)phenyl]pyrimidine hydrochloride **6**, in a 60% yield [Scheme 2]. The dicationic molecule, 2,4-bis[4-(5-N-isopropylamidino-2-benzimidazolyl)phenyl]pyrimidine **7** was obtained by reaction of 3,4-diamino-N-isopropylbenzamidine with **5**, although in a lower yield than obtained for **6.**

SCHEME 2.



a) DIBALH b) 3,4-diaminobenzamidine or 3,4-diamino-N-isopropylbenzamidine, 1,4-benzoquinone.

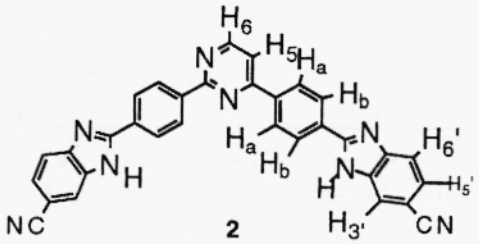
Experimental

Melting points were recorded using a Thomas Hoover(Uni-Melt) capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded employing a Varian GX400 spectrometer and chemica

shifts(δ) are in ppm relative to TMS. High resolution mass spectra were recorded with a VG Instruments 70-SE spectrometer (Georgia Institute of Technology, Atlanta, GA); others were recorded by a Shimadzu GC-MS 5000 instrument at 70ev chamber voltage on a direct inlet system. IR spectra were recorded using a Michelson 100 (Bomem, Inc.) instrument. Elemental analysis were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within ± 0.5 of the theoretical values. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific.

2,4-Bis[4-(5-cyano-2-benzimidazolyl)phenyl]pyrimidine 2

A suspension of 2,4-bis(4-cyanophenyl) pyrimidine (0.5 g, 1.77 mmol) in 50 mL absolute ethanol was cooled in ice-salt bath and was saturated with HCl (g). The flask was stoppered and the contents were stirred at room temperature until IR spectra indicated the disappearance of the nitrile peak. The imidate ester hydrochloride which formed was filtered, washed with anhydrous ether and dried in vacuum. The bis imidate ester hydrochloride and 3,4-diaminobenzonitrile (4,8) (0.52 g, 3.89 mmol) were suspended in glacial acetic acid (5 mL) and immediately heated to reflux. The solution becomes clear briefly, very rapidly a precipitate was formed. After 1 h the mixture was cooled to room temperture, suspended in water and filtered. The white solid was washed with water and with methanol. After drying the solid was suspended in absolute ethanol saturated with HCl and mixture was refluxed for 1 h. After cooling, the yellow crystals were filtered and recrystallized from dimethylformamide to yield 0.15 g (13%) of desired product, mp >300 °C. HRMS: calculated mass: 515.1733(M+H), observed mass: 515.1738.



2

^{1}H-NMR(DMSO-d$_6$, TMS) δ 9.08(d, 1H, H-6, J=5.2Hz), 8.76(d, 2H, H-a, J=8.8Hz), 8.62(d, 2H, H-a, J=8.8Hz), 8.45(d, 2H, H-b, J=8.4Hz), 8.43(d, 2H, H-b, J=8.4Hz), 8.21(d, 2H, H-3', J=3.6Hz), 8.19(d, 1H, H-5, J=5.2Hz), 7.82(dd, 2H, H-5', J=8.4Hz, J=2.6Hz), 7.66(td, 2H, H-6', J=8.4Hz, J=1.2Hz);[ethanol solvate 3.39(q, 1H, CH_2, J = 7.2 Hz),1.09(t, 1.5H, CH_3, J = 7.2 Hz)]. Anal. calcd. for: $C_{32}H_{18}N_8$•HCl•H_2O•O.5 C_2H_5OH, C,66.94; H,4.09; N, 18.93. Found: C,66.76; H,4.14; N,18.83.

2,4-Bis(4-carboxyphenyl)pyrimidine 3

A mixture of 2,4-bis(4-cyanophenyl)pyrimidine (1 g, 3.55 mmol), 20 mL of 20% aqueous sodium hydroxide solution and 25 mL of ethanol was refluxed for 4 h. After cooling the solid was filtered and the filtrate was acidified with acetic acid. After filtration the white powder was washed with water and methanol and dried in vacuum at 100 °C for 24 h. Yield: 1.02 g (90%), mp >300 °C. MS: m/z: 320 (M^+) ^{1}H-NMR(DMSO-d$_6$, TMS) δ 13.17(brs, 2H), 9.05(d, 1H, J=4.9Hz), 8.62(d, 2H, J=7.9Hz), 8.46(d, 2H, J=8.5Hz), 8.14(m, 5H). ^{13}C-NMR(DMSO-d$_6$) δ 167.0, 166.9, 162.6, 162.1, 159.0, 140.9, 139.8, 133.1, 132.8, 129.9, 129.7, 127.9, 127.3, 116.2. Anal. calcd. for: $C_{18}H_{12}N_2O_4$: C, 67.50; H, 3.78; N, 8.75; Found: C, 67.22, H, 3.88; N, 8.69.

2,4-Bis[4-(5-imidazolin-2yl-2-benzimidazolyl)phenyl]pyrimidine hydrochloride 4

To a suspension of 2,4-bis(4-carboxyphenyl)pyrimidine (0.5 g, 1.56 mmol) in Eaton's reagent (6) (50 mL), 2-(3,4-diaminophenyl)imidazoline (4,8) (0.56 g, 3.12 mmol) was added. The mixture was stirred under nitrogen at 170-180 °C for 4 h. The cooled mixture was poured into ice water (50 mL) and the resulting solution was neutralized to pH 8 with 25% ammonium hydroxide. The precipitate product was filtered and then dissolved in glacial acetic acid. After filtration conc. HCl was added to filtrate and formed precipitate was filtered off and was washed with acetone and methanol. The solid was dried in vacuum at 100 °C for 72 h. Yield 178 mg (21.8%) of yellow-green powder, mp >300 °C. HRMS: Calculated mass: 601.2576662 (M^++H), observed mass: 601.2573 (freebase). ^{1}H-NMR(DMSO-d_6, TMS) δ 10.44(d, 4H, N-H, J=3.9Hz), 9.07(d, 1H, H-6, J=5.3Hz), 8.76(d, 2H, H-a, J=8.5Hz), 8.59(d, 2H, H-a, J=8.5Hz), 8.50(d, 2H, H-b, J=8.5Hz), 8.47(d, 2H, H-b, J=8.4Hz), 8.37(d, 2H, H-3', J=4.0Hz), 8.15(d, 1H, H-5, J=5.3Hz), 7.85(s, 4H, H-5' + H-6'), 4.05(s, 8H, CH_2). Anal. calcd. for: $C_{36}H_{28}N_{10} \cdot 3HCl \cdot 6H_2O$: C, 52.85; H, 5.30; N, 17.12; Cl, 13.00. Found: C, 52.89; H, 5.29; N, 16.67; Cl, 13.26.

2,4-Bis(4-formylphenyl)pyrimidine 5

2,4-Bis(4-cyanophenyl)pyrimidine (2 g, 7.1 mmol) was stirred under nitrogen in 500 mL of dry benzene. To the suspension diisobutyl aluminum hydride(DIBALH) (2.22g, 15.62 mmol) was added slowly. The mixture warmed to 50 °C and the solid dissolved. After all DIBALH was added, the mixture was stirred for 3h at room temp. The complex was decomposed by careful addition of methanol. The mixture was then transferred to a 2L beaker and decomposed further with ice and 5% sulfuric acid. The layers were separated and the aqueous layer was extracted three times with ether. The organic layers were combined, dried over anhydrous magnesium sulfate, and solvent was evaporated. The crude product was recrystallized from ethanol to yield 0.71 g, (35%) of white crystals, mp >300°C. MS: m/z:288 (M^+) ^{1}H-NMR(DMSO-d_6, TMS) δ 10.15(s, 1H), 10.14(s, 1H), 9.07(d, 1H, J=5.2Hz), 8.71(d, 2H, J=8.0Hz), 8.54(d, 2H, J=8.4Hz), 8.14(d, 1H, J=5.2Hz), 8.11(d, 2H, J=8.4Hz), 8.09(d, 2H, J=8.4Hz). ^{13}C-NMR(DMSO-d_6): δ 192.3, 192.2, 162.3, 161.8, 158.8, 142.0, 140.9, 137.6, 137.5, 129.6, 129.3, 128.1, 127.5, 116.2. Anal. calcd. for: $C_{18}H_{12}N_2O_2$: C, 74.98; H, 4.20; N, 9.72. Found: C, 74.80; H, 4.16; N, 9.78.

The crude reaction mixture contained the desired aldehyde and unreacted nitrile. When reactions condition were changed: prolonged time of reaction, higher temperature and/or increased excess of DIBALH (25%) the corresponding bis-benzyl alcohol appeared to be formed as the main product. Although the compound was not isolated in pure form, the structure of 2,4-bis(4-hydroxymethylphenyl) pyrimidine was confirmed by ^{1}H NMR spectra and mass spectra. MS: m/z: 292(M^+) ^{1}H-NMR(DMSO-d_6, TMS) δ 8.82(d, 1H, J=6.1Hz), 8.57(d, 2H, J=8.6Hz), 8.22(d, 2H, J=8.5Hz), 7.58(d, 1H, J=4.9Hz), 7.52(m, 4H), 4.79(s, 4H), 1.73(s, 2H).

2,4-Bis[4-(5-amidino-2-benzimidazolyl)phenyl]pyrimidine hydrochloride 6

A solution of 2,4-bis(4-formylphenyl)pyrimidine (500 mg, 1.74 mmol), 3,4-diaminobenzamidine (4,8) (522 mg, 3.48 mmol) and 1,4-benzoquinone (365 mg, 3.48 mmol) in ethanol was refluxed under nitrogen with stirring for 4h. After cooling, the solid was filtered and was washed with ethanol, acetone and ether to yield 578

mg, (60.5%) dark purple powder, mp >300 °C. To a suspension of the free base (320 mg, 0.58 mmol) in absolute ethanol (25 mL), 50 mL of ethanol saturated with HCl (g) was added, and the mixture was refluxed for 2h. After cooling solid was filtered and was washed with methanol-ether (1:3) solution, and was dried in *vacuum* at 100 °C for 48 h. Yield 300 mg (70.5%) of dark blue powder, mp >300 °C. HRMS: Calculated mass: 549.226366 (M^++H); observed mass: 549.227005 (freebase). ^{1}H-NMR(DMSO-d_6, TMS) δ 9.50(s, 4H, N-H), 9.20(s, 4H, N-H), 9.08(d, 1H, H-6, J=5.2Hz), 8.75(d, 2H, H-a, J=8.4Hz), 8.63(d, 2H, H-a, J=8.8Hz), 8.56(d, 2H, H-b, J=8.4Hz), 8.53(d, 2H, H-b, J=8.4Hz), 8.26(s, 2H, H-3'), 8.21(d, 1H, H-5, J=5.2Hz), 7.89(d, 2H, H-5' or H-6', J=8.4Hz), 7.78(d, 2H, H-5' or H-6', J=8.4Hz). Anal. calcd for: $C_{32}H_{24}N_{10} \cdot 3HCl \cdot 4H_2O$: C,52.65; H,4.83; N, 19.19; Cl, 14.57. Found: C,52.64; H,4.95; N,18.66; Cl,14.03.

2,4-Bis[4-(5-N-isopropylamidino-2-benzimidazolyl)phenyl]pyrimidine hydrochloride 7

A solution of 2,4-bis(4-formylphenyl)pyrimidine (0.4 g, 1.39 mmol), 3,4-diamino-N-isopropylbenzamidine (4,8) (0.55 g, 2.78 mmol) and 1,4-benzoquinone (0.3 g, 2.78 mmol) in ethanol (100 mL) was refluxed under nitrogen for 4 h. After cooling, the solid was filtered, washed with methanol-ether (1:3) mixture, and was dried. To a suspension of free base in 300 mL absolute ethanol, 80 mL of absolute ethanol saturated with HCl (g) was added and mixture refluxed for 5 h. After cooling, the solid was filtered, washed with methanol-ether (1:3) mixture and was dried at 100 °C, in vacuum for 48 h. Yield 0.29 g, (25%) of bright yellow powder, mp
>300 °C. HRMS: Calculated mass: 633.3203 (M^++H), observed mass 633.3221 (freebase). ^{1}H-NMR(DMSO-d_6, TMS) δ 9.57(s, 1H, N-H), 9.55(s, 1H, N-H), 9.41(s, 2H, N-H), 9.10(d, 1H, H-6, J=5.3Hz), 8.98(s, 2H, N-H), 8.79(d, 2H, H-a, J=8.5Hz)' 8.65(d, 2H, H-a, J=8.5Hz), 8.56(d, 2H, H-b, J=8.6Hz), 8.50(d, 2H, H-b, J=8.6Hz), 8.21(d, 1H, H-5, J=5.4Hz), 8.09(brs, 2H, H-3')' 7.86(dd, 2H, H-5', J=8.4Hz, J=2.5Hz), 7.62(td, 2H, H-6', J=8.4Hz, J=1.6Hz), 4.10(m, 2H, CH), 1.33(d, 12H, CH_3, J=6.4Hz). Anal. calcd. for: $C_{38}H_{36}N_{10} \cdot 4HCl \cdot 2.5H_2O$: C,55.41; H, 5.51; N, 17.01; Cl, 17.22. Found: C, 55.53; H, 5.54; N, 16.93; CI, 17.12.

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