Synthesis of 4,8-Substitiutednaphtho[2,1-*b*]thiophene Threading Intercalators

Sahar Badr[a,b], Mohamed M. El-Kerdawy[b], Farial A.Tanious[a], W. David Wilson[a] and David W. Boykin[a]*

[a]Department of Chemistry, Georgia State University, Atlanta, GA 30302, USA, [b]Department of Medicinal Chemistry, University of Mansoura, Mansoura, Egypt

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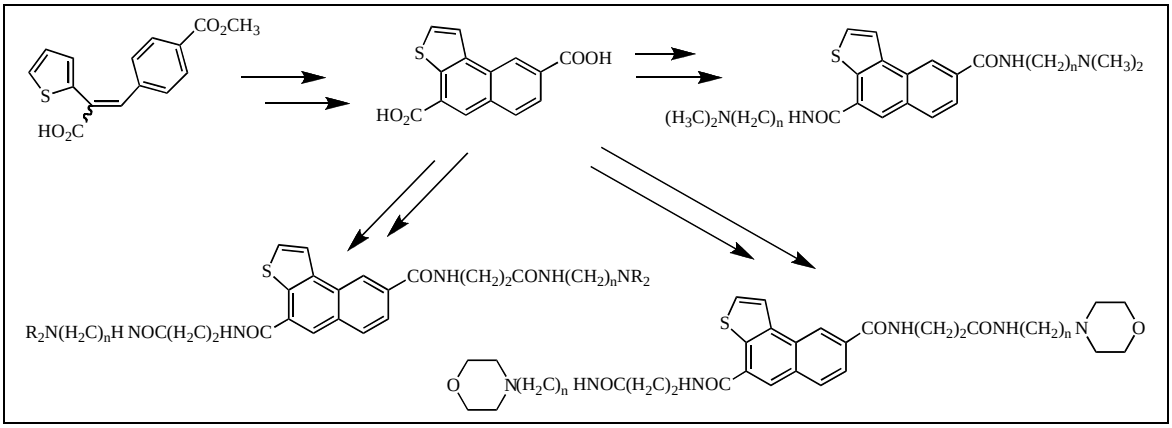

Eight 4,8-substitiutednaphtho[2,1-*b*]thiophenes with cationic containing side chains were synthesized as potential threading intercalators and their DNA binding affinity was evaluated. The key step in the synthesis of these systems was oxidative–photocyclization of α-(2-thienyl)-β-(4-methoxycarbonylphenyl) acrylic acid. Various *N*,*N*-dimethylaminoalkylamines were coupled with naphtho[2,1-*b*]thiophene 4,8-dicarboxylic acid to yield the title compounds.

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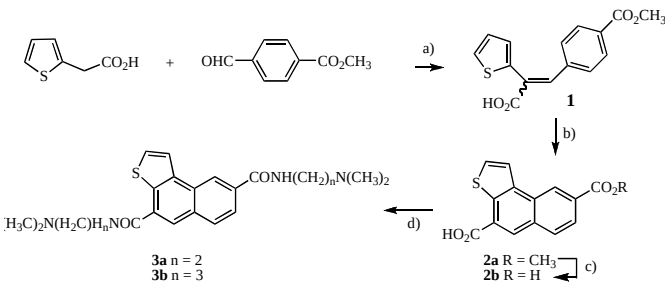
INTRODUCTION

Planer aromatic molecules which intercalate with DNA continue to be of great interest due to their therapeutic potential [1]. Many such molecules simply insert the planer aromatic ring between the DNA base pairs and often have a single cationic side chain which remains in one of the grooves frequently contributing to the binding affinity. A special class of intercalators which have two side chains on opposite sides of the planer aromatic ring requires that one of the side chains slide through the stacked base pairs in order to achieve maximum stacking interactions between the base pairs and the interacting aromatic ring. This class of molecules is referred to as threading intercalators [2]. These molecules are of interest for their ability to target both DNA [3,4] and RNA [5,6]. Some years ago we reported that naphthothiophenes with one cationic side chain were effective DNA intercalators [7,8]. In this report, we describe the synthesis of dicationic 4,8-substitiutednaphtho[2,1-*b*]thiophenes and the results of preliminary DNA binding interactions of these potential threading intercalators.

RESULTS AND DISCUSSION

The synthetic approach we employed to prepare the dicationic 4,8-Substitiutednaphtho[2,1-*b*]thiophenes is outlined in the following Schemes. The synthesis of the key intermediate 4,8-naphtho[2,1-*b*]thiophenedicarboxylic acid is shown in Scheme 1. This approach is patterned after ones we have used previously to prepare naphtha[2,1-*b*]thiophenes [7,8] and begins with a modified Perkin condensation [9] between 4-carbomethoxybenzaldehyde and 2-thiopheneacetic acid which forms α-(2-thienyl)-β-(4-methoxycarbonylphenyl)acrylic acid (**1**) in 70% yield. Since we have previously shown [10] that either geometric isomer of these type of diaryl acrylic acids readily undergo oxidative–photocyclization we did not determine which isomer (or mixture of isomers) were formed here. Photocyclization lead to **2a** in reasonable yield (56%). Base catalyzed hydrolysis of **2a** gave the dicarboxylic acid **2b** in a satisfactory yield

Scheme 1

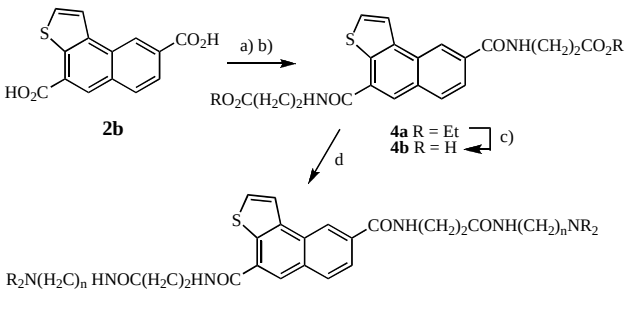


Reagents and Conditions: a) Ac_2O, Et_3N, reflux b) EtOH,I_2, air, hv c) NaOH, MeOH, H_2O, reflux d) CDI, amine, DMF, 20°C

(74%). Carbonyldiimidazole mediated coupling between **2b** and *N*,*N*-dimethylaminoalkylamines gave **3a** and **3b** in approximately 60% yields.

The synthesis of 4,8-Substitiutednaphtho[2,1-*b*]thiophenes with cationic centers that are more distant from the aromatic ring is outline in Scheme 2, starting from **2b**. The diacid **2b** was converted to its diacid chloride which was allowed to react directly with β-alanine ethyl ester to yield **4a** in an overall yield of 48%. The diester **4a** was hydrolyzed at 65°C by the action of methanolic sodium hydroxide to give the diacid **4b** in a 78% yield. Carbonyldiimidazole mediated coupling between **4b** and *N*,*N*-dimethylaminoalkylamines gave **5a-5d** in yields ranging from 60 to 75%.

Scheme 2

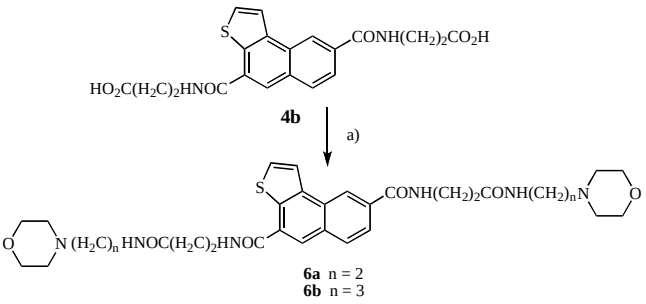


5a n = 2; R = CH_3
5b n = 3; R = CH_3
5c n = 2; R = Et
5d n = 3; R = Et

Reagents and Conditions: a) $SOCl_2$, reflux b) $NH_2(CH_2)_2CO_2Et$, Et_3N, CH_3CN, reflux c) NaOH,H_2O, 65°C d) CDI, amine, DMF, 20°C

The morpholino dicationic napthothiophenes **6a** and **6b** were prepared using carbonyldiimidazole mediated coupling between **4b** and the 4-(aminoalkyl)moropholino compounds in approximately 80% yields (Scheme 3).

Scheme 3



6a n = 2
6b n = 3

Reagents and Conditions: a) CDI, amine, DMF, 20°C

Table 1 contains the results from preliminary binding studies of the dicationic naphthothiophenes with calf thymus DNA. The change in melting temperature of DNA after complex formation with the dicationic naphthothiophenes (ΔTm) is a reliable reflection of the binding affinity [11]. The ΔTm values for **3a** and **3b** are 18.2 and 16.3, which represent moderately strong DNA affinities in comparison to the that of the symmetrical threading intercalator *N*,*N*'-bis[2-(dimethylamino)ethyl]-1,4,5,8-naphthaleneteracarboxylic 1,8,4,5-dimide which gave a ΔTm value of 24 under these conditions [3]. The ΔTm values for **5a-5d** range from 9.5 to 11.4 and represent a significant drop in binding affinity. This reduction in affinity may reflect greater difficulty in threading the side chain between the base pairs and/or weaker electrostatic interactions between the more distal cationic centers and the DNA backbone. Another possibility for the reduction is the loss of additional configurations for the longer side chains in **5a-5d** on moving from the free to the DNA bound state. The constricted helical space of the DNA grooves could result in an entropic penalty and lower the binding equilibrium constant and ΔTm. It is noteworthy that the somewhat more bulky *N*,*N*-diethylamino compounds **5c** and **5d** both exhibit lower ΔTm values than their *N*,*N*-methyl homologs **5a** and **5b**. The two molecules **6a** and **6b** essentially fail to bind to DNA as reflected by ΔTm values of approximately 2. This interesting result suggests that the rigid morpholino groups are more difficult to thread between the base pairs. The lower pKa of the morpholino unit, the pK of morpholine is more than two pK units lower than that of simple dialkyl amines [12], may also play a role in the binding affinity reduction. Further biophysical studies are required to elucidate the full details of the binding interactions of these dicationic naphthothiophenes with DNA.

Table 1

DNA Binding Affinity Results for Dicationic Naphtho[2,1-*b*]thiophenes[a]

Compound No.	ΔTm(°C)
3a	18.2
3b	16.3
5a	11.4
5b	10.9
5c	9.5
5d	9.5
6a	1.8
6b	2.0

[a] See experimental for details of these measurements.

EXPERIMENTAL

Tm Measurements. Thermal melting experiments were conducted with a Cary 300 spectrophotometer. For these measurements cuvettes are mounted in a thermal block and the solution temperatures are monitored by a thermistor in a reference cuvette. Temperatures are maintained under computer control and are increased at 0.5 °C/min. The thermal melting

studies with calf thymus DNA (Worthington Bichemicals) were performed in PIPES00 buffer (PIPES 10mM, EDTA 1mM) are conducted in 1 cm path length quartz cuvettes. The concentrations of DNA were determined by measuring the absorbance at 260 nm. A ratio of 0.1 molar equivalence of compound per that of base was used for the complex and DNA without compound was used as a control.

Chemistry. Melting points were determined in open capillary tubes with a Mel-Temp 3.0 melting point apparatus and are uncorrected. ^{1}H- and ^{13}C -NMR spectra were recorded on Varian Unity 300 and Varian VRX 400 instruments and chemical shifts are reported in ppm relative to TMS. Mass spectra (MS) were performed by the Georgia Tech Mass Spectra Laboratory at Georgia Institute of Technology in Atlanta, GA. Elemental analyses were performed by Atlantic Microlab in Norcross, GA. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific.

Synthesis of α-(2-thienyl)-β-(*p*-methoxycarbonylphenyl)-acrylic acid (1). A mixture of methyl-4-formyl benzoate 4.92 g (0.03 mole), 2-thienyl acetic acid 4.26 g (0.03 mole), 8 ml of distilled triethylamine and 16 ml of acetic anhydride was heated at reflux for 8 h. The mixture was poured into 1 L of water and made alkaline with sodium hydrogen carbonate with occasional external cooling. The alkaline solution was filtered and acidified with 18% hydrochloric acid with external cooling; the resulting yellow solid was collected and dried. It was purified by dissolving in warm chloroform, filtered and the solution was added to petroleum ether which gave 6 g (70% yield) of a pale yellow crystalline compound; mp 153-7°C. ^{1}H NMR ($CDCl_3$):δ 3.90(s, 3H); 6.96(d, J = 9Hz, 2H); 7.01(m, 1H); 7.22(d, J = 9Hz, 2H); 7.38(d, J = 6Hz, 1H); 7.87(d, J = 6Hz, 1H); 8.01(s, 1H,), 9.27(bs, 1H). ^{13}C NMR ($CDCl_3$): δ 52.2, 126.2, 126.9, 127.9, 128.3, 129.5, 129.8, 130.3, 139.6, 166.8, 171.7. Calcd mass: 288.32, observed mass: 288.0. Anal. Calcd. for: $C_{15}H_{12}O_4S$: C,62.48; H,4.19; Found:C,62.21;H,4.21.

Synthesis of 8-methoxycarbonylnaphtho[2,1-*b*]thiophene-4-carboxylic acid (2a). A solution of 4.32 g (0.015 mole) of α-(2-thienyl)-β-(p-methoxycarbonyl-phenyl) acrylic acid and 0.2 g of iodine in 600 ml of ethanol was irradiated for about 100 hours in a Rayonett reactor fitted with lamps that provided 3500 Å light. Air was bubbled through the solution during the irradiation period. The ethanol was concentrated to approximately 50 ml under reduced pressure; the precipitated compound was collected and washed with ether (2x100 ml) and hexane (2x100 ml). The yellow compound was purified by dissolving in boiling $CHCl_3$ and concentrated under reduced pressure to give 2.4 g (56% yield) of a pale yellow crystalline compound, mp 267-9°C. ^{1}H NMR (DMSO-d_6) δ 3.97 (s, 3H), 8.08 (d, J = 7Hz, 1H); 8.11 (d, J = 4Hz, 1H); 8.33(d, J = 7Hz, 1H,); 8.38(d, J = 4Hz, 1H); 8.67 (s, 1H); 9.11 (s, 1H); 13.8 (bs, 1H). ^{13}C NMR (DMSO-d_6): δ 52.4, 121.6, 124.8, 125.1, 125.5, 127.8, 129.2, 129.6, 130.4, 131.0, 132.3, 135.4, 137.5, 166.1, 166.8. Calculated mass: 286.3, observed mass: 286.0. Anal. Calcd. for: $C_{15}H_{10}O_4S$: C, 62.92; H, 3.52; Found: C, 62.82; H, 3.60.

Synthesis of Naphtho[2,1-*b*]thiophene-4,8-dicarboxylic acid (2b). A solution of 8-methoxycarbonylnaphtho[2,1-*b*]-thiophene-4-carboxylic acid (0.143 g; 0.0005 mole) in methanol (15 ml) and 10% aqueous sodium hydroxide (8 ml) was heated at reflux, with stirring. The progress of the reaction was monitored by TLC which indicated the reaction was complete after 5 h. The reaction mixture was cooled, filtered and acidified to pH 2-3 with 18% hydrochloric acid. The precipitated yellow compound was collected and washed with water, hot EtOH, ether and then dried. The obtained compound was crystallized from dimethylformamide to give a yellow crystalline compound, mp >300°C. Yield 0.1 g (74%). ^{1}H NMR (DMSO-d_6): δ 8.04 (d, J = 3.5Hz 1H,); 8.12 (d, J = 3.5Hz, 1H); 8.29 (d, J = 6Hz, 1H); 8.33 (d, J = 6Hz 1H,); 8.65 (s, 1H); 9.09 (s, 1H); 13.3 (bs, 2H). ^{13}C NMR (DMSO-d_6): δ 121.7, 124.9, 125.4, 125.7, 128.0, 129.8, 130.3, 130.7, 131.0, 132.3, 135.3, 137.6, 166.9, 167.2. Calculated mass: 272.268, observed mass: 272.1. Anal. Calcd. for: $C_{14}H_8O_4S$: C, 61.75; H, 2.96; Found: C, 61.59; H, 3.01.

General procedure for Synthesis of Naphtho[2,1-*b*]thiophene-4,8-bis(dimethylaminoalkyl carboxamides). A mixture of naphtho[2,1-*b*]thiophene-4,8-dicarboxylic acid 0.272 g (0.001mole), 1,1-carbonyldiimidazole 0.486 g (0.003 mole) in 3 ml of anhydrous *N*,*N*-dimethylformamide was stirred at 20°C, with exclusion of moisture, for 2 h. The mixture was then cooled to 5°C, the *N*,*N*-dialkylamino compound (0.05 mole) was added, the mixture was stirred for another 2 h at 20°C and all volatiles were removed under vacuum. The residue was partitioned between chloroform and 0.2 *M* sodium carbonate; the organic layer was washed with water, brine and the solvent removed under reduced pressure. The residue was crystallized twice from ethyl acetate to give the pure free base. This was dissolved in CH_2Cl_2 and dry HCl gas was bubbled until saturated. The contents were allowed to reflux for 3 hours, then the flask was stoppered, and the contents were stirred at 20°C overnight. The solid was collected by filtration, washed with anhydrous ether and dried under vacuum to give the dihydrochloride salt as white pure compound.

Naphtho[2,1-b]thiophene-4,8-bis(dimethylaminoethyl carboxamide) (3a). Yield 65 %; mp 207-9 °C.^{1}H NMR (DMSO-d_6): δ 2.22 (s, 6H); 2.23 (s, 6H); 2.52 (t, J = 4Hz, 4H); 3.48 (m, 4H); 8.03 (d, J = 7Hz, 1H); 8.07 (d, J = 4Hz, 1H); 8.16 (d, J = 7Hz, 1H); 8.26 (d, J = 4Hz, 1H); 8.45 (s, 1H); 8.61(bs, 1H); 8.75 (bs, 1H); 9.01 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 37.5, 37.6, 45.1, 45.2, 58.0, 58.2, 121.2, 122.7, 123.2, 124.4, 127.7, 128.9, 129.2, 130.9, 131.2, 133.5, 134.9, 137.4, 165.6, 165.8. Calculated mass: 413.542 (M^+ + H) ; observed mass; 413.10 Anal. Calcd. for: $C_{22}H_{28}N_4O_2S$: C, 64.04; H, 6.84; N, 13.58; Found: C, 64.00; H, 6.80; N, 13.56...

Dihydrochloride of 3a. Yield 62 %; mp 270-3°C; ^{1}H NMR (DMSO-d_6): δ 2.90 (s, 12H); 3.4 (t, J = 4Hz, 4H); 3.80 (m, 4H); 8.07 (d, J = 6Hz, 1H); 8.16 (bdd, 2H,); 8.54 (d, J = 6Hz, 1H); 8.86 (s, 1H); 9.43 (s, 1H,); 9.52 (bs, 2H). ^{13}C NMR (DMSO-d_6): δ 34.5, 42.2, 55.8, 56.1, 121.7, 123.3, 124.0, 124.4, 127.0, 129.0, 130.6, 131.3, 132.8, 134.7, 137.5, 166.1, 166.2. Anal. Calcd. for: $C_{22}H_{28}N_4O_2S$•2HCl•1H_2O: C, 52.47; H, 6.4; N, 11.12; Cl, 14.08; Found: C, 52.04; H, 6.44; N, 10.99; Cl, 14.04.

Naphtho[2,1-b]thiophene-4,8-bis(dimethylaminopropyl-carboxamide) (3b). Yield 70 %; mp 205-7°C; ^{1}H NMR (DMSO-d_6): δ 1.77 (m, 4H), 2.16 (s, 6H); 2.17 (s, 6H); 2.32 (t, J = 4Hz, 4H); 3.41 (m, 4H); 8.04 (d, J = 7 Hz, 1H); 8.07 (d, J = 4Hz, 1H); 8.14 (d, J = 7 Hz, 1H); 8.27 (d, J = 4Hz, 1H); 8.44 (s, 1H); 8.76 (bs, 1H); 8.93 (bs, 1H); 9.01 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.1, 27.2, 38.1, 38.2, 45.3, 57.1, 57.2, 121.5, 122.9, 123.4, 124.6, 128.1, 129.2, 129.7, 131.3, 131.6, 133.8, 135.2, 137.7, 166.1, 166.3. Anal. Calcd. for: $C_{24}H_{32}N_4O_2S$: C, 65.42; H, 7.32; N, 12.71; Found: C, 65.40; H, 7.31; N, 12.69. Calculated mass: 441.508 (M^+ +H), observed mass: 441.0

Dihydrochloride of 3b. Yield 65 %; mp 260-3°C; ^{1}H NMR (DMSO-d_6): δ 2.09 (m, 4H); 2.79 (s, 6H); 2.8 (s, 6H); 3.21 (t, J = 6Hz, 4H); 3.50 (m, 4H); 8.03 (d, J = 5Hz, 1H); 8.15 (d, J =

3Hz, 1H); 8.46 (d, J = 5Hz, 1H); 8.49 (d, J = 3Hz, 1H); 8.72 (s, 1H); 9.25 (bs, 2H); 9.29 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 23.9, 24.0, 36.4, 36.5, 41.8, 54.4, 121.6, 123.0, 123.6, 124.3, 127.3, 128.9, 129.0, 130.6, 131.3, 133.1, 134.7, 137.5, 165.8, 165.9. *Anal*. Calcd. for: $C_{24}H_{32}N_4O_2S$•2HCl•0.5H_2O: C, 55.16; H, 6.75; N, 10.72; Cl, 13.57; Found: C, 55.11; H, 6.72; N, 10.71; Cl, 13.60.

***N,N'*-Bis[2-(ethoxycarbonyl)ethyl]naphtho[2,1-*b*]thiophene-4,8-dicarboxamide (4a).** A suspension of the naphtho[2,1-*b*]-thiophene-4,8-dicarboxylic acid (1.36 g; 0.005 mole) and distilled thionyl chloride (130 ml) was allowed to reflux overnight. The excess thionyl chloride was distilled and the last traces of it were removed by codistillation with dry benzene. The solid when dried *in vaccuo* at room temperature gave a yellow crystalline diacid chloride 1.4 g (91 % yield) mp 175-7°C. The compound was used directly in the next step without further characterization. Naphtho[2,1-*b*]thiophene-4,8-diacid chloride 3.7 g (0.012 mole) was dissolved in 100 ml of anhydrous acetonitrile and cooled with vigorous stirring -10°C. The requisite β-alanine ethyl ester hydrochloride (3.68 g; 0.024 mole) and distilled triethylamine (9.05 ml; 0.065 mole) were dissolved in 50 ml of anhydrous acetonitrile and added dropwise. When the addition was completed, the reaction mixture was allowed to reflux with stirring until TLC indicated the reaction was completed. The reaction mixture was filtered; the filtrate was evaporated under vacuum. The residue was treated with water and extracted with chloroform. The chloroform extract was washed with cold 2% hydrochloric acid, water and brine. The chloroform solution was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to yield a solid which was crystallized from acetonitrile and recrystallized from ethanol/water to yield 3 g (53 % yield) mp 155-7°C. ^{1}H NMR (DMSO-d_6): δ 1.20 (t, J = 6Hz, 6H); 2.70 (t, J = 6Hz, 4H); 3.65 (m, 4H); 4.13 (q, J = 6Hz, 4H); 8.06 (d, J = 6Hz, 1H); 8.14 (d, J = 5Hz, 1H); 8.27 (d, J = 6Hz, 1H); 8.29 (d, J = 5Hz, 1H); 8.46 (s, 1H); 8.86 (bs, 1H); 8.99 (bs, 1H); 9.03 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.1, 33.7, 33.8, 35.8, 60.0, 121.3, 123.0, 123.5, 124.6, 127.6, 129.1, 129.4, 131.3, 131.4, 133.4, 135.0, 137.6, 166.0, 166.1, 171.3, 171.4. Calculated mass: 470.53, observed mass: 470.1. Anal. Calcd. for $C_{24}H_{26}N_2O_6S$: C, 61.25; H, 5.57; N, 5.95. Found: C, 61.07, H, 5.51; N, 5.91.

Synthesis of *N,N'*-Bis[2-carboxyethyl] naphtho[2,1-*b*]thiophene-4,8-dicarboxamide (4b). A solution of *N,N'*-bis[2-(ethoxycarbonyl)ethyl]naphtho[2,1-*b*]thiophene-4,8-dicarboxamide (2.63 g; 0.0056 mole) in methanol (25 ml) and sodium hydroxide (1.8 g in 18 ml of methanol/water, 20/2) was stirred at 65°C. The progress of the reaction was monitored by TLC and was thereby judged to be complete after 3 hours. The solvent was evaporated under reduced pressure and the residue was taken up in water. Impurities were extracted with ethyl acetate. The aqueous solution was cautiously acidified to pH 3 - 4 with cold 1 *N* hydrochloric acid and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated to give compound which was purified by trituration with small amount of ethylacetate, then warm $CHCl_3$ to yield 1.8 g (78 % yield) of creamy white compound, mp 230-2°C. ^{1}H NMR (DMSO-d_6): δ 2.66 (t, J = 5Hz, 4H); 3.64 (m, 4H,): 8.05 (d, J = 7Hz, 1H); 8.09 (d, J = 4Hz, 1H); 8.14 (d, J = 7Hz, 1H); 8.27 (d, J = 4Hz, 1H); 8.48 (s, 1H); 8.80 (bs, 1H); 8.93 (bs, 1H); 9.04 (s, 1H); 12.24 (bs, 2H). ^{13}C NMR (DMSO-d_6): δ 33.7, 33.9, 35.8, 121.3, 122.9. 123.5, 124.5, 127.6, 129.0, 129.3, 131.0, 131.4, 133.5, 134.9, 137.5, 165.9, 166.1, 172.7, 172.8. Calculated mass: 414.43, observed mass: 415.0. *Anal*. Calcd. for: $C_{20}H_{18}N_2O_6S$: C,57.95; H,4.37. Found: C, 57.75; H,4.35.

General procedure for Synthesis of *N,N'*-Bis[2-[[*n*-(dialkyl-amino)alkyl]carbamoyl]ethyl]naphtho[2,1-*b*]thiophene-4,8-dicarboxamides. A mixture of *N,N'*-bis[2-carboxyethyl]-naphtho[2,1-*b*]thiophene-4,8-dicarboxamide 0.414 g (0.001 mole), 1,1'-carbonyldiimidazole 0.486 g (0.003 mole) in 4 ml of anhydrous *N,N*-dimethylformamide was stirred at 20°C, with exclusion of moisture, for 1 hour. The mixture was then cooled to 0°C, the *N,N*-dialkylaminoalkylamine (0.05 mole) was added, the mixture was stirred for another 2 hours at 20°C, and all volatiles were removed under reduced pressure. The residue was triturated with 0.1 *M* sodium carbonate, collected, washed with water, chloroform, ether and dried to give a solid which was purified by trituration with warm ethylacetate to give the pure free base. The solid was dissolved in absolute ethanol by heating, then the solution was cooled in an ice bath and concentrated HCl was added dropwise to pH 2. The contents were stirred at room temperature for 2 h. In the case of **5a**; the solid was collected by filtration, washed with absolute ethanol and anhydrous ether and dried under vacuum to give the dihydrochloride salt as a white crystalline pure compound. For **5b-5d** slow dilution of the solution with anhydrous ether gave the dihydrochloride as white hygroscopic crystals, which were triturated with anhydrous ether and dried under vacuum to give pure compound.

***N,N'*–Bis[2-[[2-(dimethylamino)ethyl]carbamoyl]ethyl]-naphtho[2,1-*b*]thiophene-4,8-dicarboxamide(5a).** Yield 70 %, mp 246-8,°C (dec). ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 6H); 2.11 (s, 6H); 2.29 (t, J = 5Hz, 4H); 2.50 (t, J = 5Hz, 4H); 3.19 (m, 4H); 3.62 (m, 4H); 7.76 (bs, 2H); 8.04 (d, J = 7 Hz, 1H); 8.07 (d, J = 5Hz, 1H); 8.14 (d, J = 7Hz, 1H); 8.25 (d, J = 5Hz, 1H); 8.45 (s, 1H); 8.74 (bs, 1H); 8.88 (bs, 1H); 9.02 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 35.2, 35.3, 36.2, 36.7, 44.9, 58.1, 121.0, 122.7, 123.2, 124.3, 127.6, 128.9, 129.0, 130.8, 131.2, 133.5, 134.8, 137.3, 165.7, 165.9, 170.0, 170.1. Calculated mass; 555.702 (M^+ +H), observed mass; 555.3. *Anal*. Calcd. for: $C_{28}H_{38}N_6O_4S$: C,60.62; H,6.90; N,15.15; Found: C, 60.52; H,6.87; N,15.10.

Dihydrochloride of 5a. Yield 68%, mp 260-2°C .^{1}H NMR (DMSO-d_6): δ 2.58 (t, J = 7Hz, 4H); 2.79 (S, 12H); 3.19 (t, J = 5Hz, 4H); 3.48 (m, 4H); 3.65 (m, 4H); 8.06 (d, J = 7Hz, 1H); 8.14 (dd, 2H); 8.42 (bs, 2H); 8.48 (d, J = 5Hz, 1H); 8.69 (s, 1H); 9.21 (bs, 2H); 9.25 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 33.9, 35.3, 35.4, 36.2, 42.2, 55.8, 122.7, 123.2, 123.8, 124.7, 127.5, 129.1, 129.4, 131.1, 131.5, 133.3, 134.9, 137.7, 165.9, 166.0, 171.2, 171.3. *Anal*. Calcd. for: $C_{28}H_{38}N_6O_4S$•2HCl•1H_2O: C,52.08; H,6.55; N,13.01. Found: C, 52.34; H, 6.44; N, 12.96.

***N,N'*–Bis[2-[[3-(dimethylamino)propyl]carbamoyl]ethyl]-naphtho[2,1-*b*]thiophene-4,8-dicarboxamide(5b).** Yield 74%, mp 243-5 °C (dec) ^{1}H NMR (DMSO-d_6): δ 1.53 (m, 4H); 2.05 (s, 6H); 2.06 (s, 6H); 2.17 (t, J = 6Hz, 4H); 2.48 (t, J = 5Hz, 4H); 3.09 (m, 4H); 3.61 (m, 4H); 7.84 (bs, 2H); 8.03 (d, J = 7Hz, 1H); 8.07 (d, J = 4Hz, 1H); 8.11(d, J = 7Hz, 1H); 8.25 (d, J = 4Hz, 1H); 8.46 (s, 1H); 8.75 (bs 1H); 8.89 (bs, 1H); 9.02 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.0, 35.3, 35.4, 36.3, 36.8, 44.9, 56.6, 121.0, 122.7, 123.2, 124.2, 127.6, 128.9, 129.1, 130.8, 131.2, 133.5, 134.8, 137.3, 165.7, 165.9, 169.9, 170.0. Calculated mass; 583.762 (M^+ + H) , observed mass; 583.6. *Anal*. Calcd. for: $C_{30}H_{42}N_6O_4S$: C,61.82; H,7.26; N,14.42. Found: C, 61.80; H,7.20; N,14.32.

Dihydrochloride of 5b. Yield 70%; no mp obtained due to extreme hygroscopic properties. ^{1}H NMR(DMSO-d_6): δ 2.48

(m, 4H); 2.52(t, J = 6Hz, 4H); 2.69(s, 12H); 3.04(t, J = 6Hz, 4H); 3.62(m, 4H); 3.94(m, 4H); 8.06(d, J = 8Hz, 1H); 8.14(dd, 2H); 8.33(bs, 2H); 8.42(d, J = 5Hz, 1H); 8.65(s, 1H); 9.18(bs, 2H); 9.23(s, 1H). ^{13}C NMR(DMSO-d_6): δ 24.3, 35.4, 35.5, 35.7, 36.4, 41.9, 54.5, 121.8, 123.1, 123.7, 124.7, 127.6, 129.1, 129.4, 131.2, 131.5, 133.4, 134.9, 137.7, 165.9, 166.0, 170.8, 170.9. *Anal*. Calcd. for: $C_{30}H_{42}N_6O_4S$•2HCl•3H_2O: C, 50.76; H, 7.10; N, 11.84. Found: C, 50.55, H, 7.30; N,11.46.

***N,N'*–Bis[2-[[2-(diethylamino)ethyl]carbamoyl]ethyl]naphtho[2,1-*b*]thiophene-4,8-dicarboxamide(5c).** Yield 75%, mp 233-5 °C (dec), ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 4Hz, 6H); 0.91 (t, J = 4Hz, 6H); 2.41 (q, J = 4Hz, 4H); 2.43 (q, J = 4Hz, 4H); 2.46 (t, J = 4Hz, 4H); 3.13 (t, J = 4Hz, 4H); 3.40 (m, 4H); 3.61 (m, 4H); 7.87 (bs, 2H); 8.08 (d, J = 7Hz, 1H); 8.13 (d, J = 5Hz, 1H); 8.18 (d, J = 7Hz, 1H); 8.30 (d, J = 5Hz, 1H); 8.49 (s, 1H,); 8.88 (bs, 1H); 9.02 (bs, 1H); 9.06 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 11.7, 35.3, 35.5, 36.4, 37.0, 46.6, 51.6, 121.3, 122.9, 123.5, 124.5, 127.8, 129.1, 129.3, 131.2, 131.4, 133.5, 134.9, 137.5, 165.8, 166.0, 170.1, 170.3. Calculated mass: 611.814 (M^+ + H), observed mass; 611.4. *Anal.* Calcd. For: C_{32} H_{46} N_6 O_4S: C, 62.92; H, 7.59; N, 13.76 Found: C, 62.80; H, 7.50; N, 13.60.

Dihydrochloride of 5c. Yield 60%, no mp obtained due to extreme hygroscopic properties. ^{1}H NMR (DMSO-d_6): δ 1.21 (t, J = 5Hz, 12H); 2.61 (t, J = 5Hz, 4H); 3.15 (q, J = 4Hz, 8H); 3.51 (t, J = 5Hz, 4H); 3.70 (m, 4H); 3.85 (m, 4H); 8.09 (d, J = 8Hz, 1H); 8.12 (dd, 2H); 8.38 (d, J = 5Hz, 1H); 8.46 (bs, 2H); 8.62 (s, 1H); 9.12 (bs, 2H); 9.20 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 8.3, 33.5, 35.3, 35.4, 36.2, 46.5, 49.7, 121.7, 123.1, 123.8, 124.6, 127.5, 129.1, 129.3, 131.0, 131.5, 133.3, 134.9, 137.7, 165.9, 171.2, 171.3. *Anal*. Calcd. For: $C_{32}H_{46}N_6O_4S$•2 HCl•1H_2O: C, 54.76; H, 7.18; N, 11.97. Found: C, 54.57; H, 7.19; N, 11.77.

***N,N'*–Bis[2-[[3-(diethylamino)propyl]carbamoyl]ethyl]naphtho[2,1-*b*]thiophene-4,8-dicarboxamide(5d).** Yield 72%, mp 235-7 °C (dec), ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 4Hz, 6H); 0.90 (t, J = 4Hz, 6H); 1.52 (m, 4H); 2.35 (q, J = 3Hz, 4H); 2.38 (q, J = 3Hz, 4H); 2.40 (t, J = 4Hz, 4H); 2.50 (t, J = 4Hz, 4H); 3.11 (m, 4H); 3.61 (m, 4H); 7.84 (bs, 2H); 8.03 (d, J = 7.5Hz, 1H); 8.07 (d, J = 4.5Hz, 1H); 8.11 (d, J = 7.5Hz 1H); 8.25 (d, J = 4.5Hz, 1H); 8.46 (s, 1H); 8.75 (bs, 1H); 8.89 (bs, 1H); 9.02 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 11.5, 27.0, 35.2, 35.4, 36.2, 37.0, 46.1, 50.2, 120.9, 122.6, 123.1, 124.2, 127.6, 128.9, 129.0, 130.7, 131.2, 133.4, 134.8, 137.3, 165.7, 165.8, 169.9, 170.0. Calculated mass: 639.866 (M^+ + H), observed mass; 639.2 *Anal*. Calcd. for: $C_{34}H_{50}N_6O_4S$: C, 63.91, H, 7.88; N, 13.15. Found: C, 63.80; H, 7.80 N, 13.11.

Dihydrochloride of 5d: yield 65 %, no mp obtained due to extreme hygroscopic properties. ^{1}H NMR (DMSO-d_6): δ 1.22 (t, J = 4Hz, 6H); 1.23 (t, J = 4Hz, 6H); 1.81 (m, 4H); 2.51 (t, J = 4Hz, 4H); 3.00 (q, J = 4Hz, 8H); 3.51 (t, J = 4Hz, 4H); 3.81 (m, 4H,); 8.08 (d, J = 8 Hz, 1H); 8.12 (dd, 2H,); 8.32 (bs, 2H); 8.42 (d, J = 5Hz, 1H); 8.64 (s, 1H); 9.12 (bs, 1H); 9.18 (bs, 1H); 9.22 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 8.3, 23.4, 35.3, 35.4, 35.9, 36.4, 46.0, 48.4, 121.7, 123.1, 123.7, 124.7, 127.5, 129.1, 129.4, 131.2, 131.5, 133.4, 134.9, 137.7, 165.8, 165.9, 170.7, 170.8. *Anal*. Calcd. for: $C_{34}H_{50}N_6O_4S$•2HCl•3H_2O: C,53.31, H,7.63; N,10.97. Found: C, 53.78; H, 7.61; N, 10.96.

General procedure for synthesis of *N,N'*-Bis[2-(ω-4-morpholinylalkyl]carbamoyl]ethyl]naphtho[2,1-*b*]thiophene-4,8-dicarboxamides. A mixture of *N,N'*bis[2-carboxyethyl]-naphtho[2,1-*b*]thiophene-4,8-dicarboxamide 0.414 g (0.001 mole), 1,1'-carbonyldiimidazole 0.486 g (0.003 mole) in 4 ml of anhydrous *N,N*-dimethylformamide was stirred at 20°C, with exclusion of moisture, for 1 h. The mixture was then cooled to -5°C, the 4-(aminoalkyl) morpholino compound (0.05 mole) was added, the mixture was stirred overnight at 20°C under nitrogen, the precipitated compound was collected by filtration and was triturated with 0.1 *M* sodium hydrogen carbonate, collected, washed with water and dried to give a solid which was purified by trituration with warm ethanol to give a pure free base.

***N,N'*-Bis[2-(ω-4-morpholinylethyl]carbamoyl]ethyl]naphtho[2,1-*b*]thiophene-4,8- dicarboxamide (6a).** Yield 80 %, mp 252 - 4°C (dec) ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 16H); 2.51 (t, J = 4Hz, 4H); 3.26 (t, J = 5Hz, 4H); 3.50 (m, 4H); 3.64 (m, 4H); 7.76 (bs, 2H); 8.04 (d, J = 7Hz, 1H); 8.07 (d, J = 5Hz, 1H); 8.14 (d, J = 7Hz, 1H); 8.25 (d, J = 5Hz, 1H); 8.45 (s, 1H); 8.68 (bs, 1H); 8.88 (bs 1H); 9.02 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 35.1, 35.3, 35.7, 36.2, 53.0, 57.2, 65.8, 121.1, 122.6, 123.1, 124.2, 127.6, 128.8, 128.9, 130.7, 131.2, 133.5, 134.8, 137.3, 165.6, 165.8, 170.0, 170.1. Calculated mass: 639.78 (M^++H), observed mass: 639.3. *Anal*. Calcd. for: $C_{32}H_{42}N_6O_6S$: C, 60.16; H, 6.62; N, 13.15. Found: C, 60.20; H, 6.61; N, 13.08.

***N,N'*-Bis[2-(ω-4-morpholinylpropyl]carbamoyl]ethyl]-naphtho[2,1-*b*]thiophene-4,8-dicarboxamide (6b).** Yield 83 %, mp 227-30°C (dec) ^{1}H NMR (DMSO-d_6): δ 1.54 (m, 4H); 2.23 (s, 16H); 2.48 (t, J = 5Hz, 4H); 3.12 (t, J = 5Hz , 4H); 3.48 (m, 4H); 3.60 (m, 4H); 7.76 (bs, 2H); 8.02 (d, J = 7Hz, 1H); 8.06 (d, J = 5 Hz, 1H); 8.10 (d, J = 7 Hz, 1H); 8.24 (d, J = 5Hz, 1H); 8.45 (s, 1H); 8.72 (bs, 1H); 8.87 (bs, 1H); 9.02 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 25.9, 35.2, 35.4, 36.3, 36.8, 53.1, 55.7, 66.0, 121.1, 122.6, 123.2, 124.2, 127.6, 128.9, 129.0, 130.8, 131.2, 133.4, 134.8, 137.3, 165.7, 165.8, 169.9, 170.0. Calculated mass: 667.83 (M^++H), observed mass: 667.4. *Anal*. Calcd. for: $C_{34}H_{46}N_6O_6S$: C, 61.23; H, 6.95; N, 12.60. Found: C, 61.17; H, 6.90; N, 12.53.

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