General Methods. Melting points (uncorrected) were determined on a Stuart Scientific SMP3 apparatus. Infrared (IR) spectra were recorded with a Perkin-Elmer 1330 infrared spectrophotometer. ^{1}H and ^{13}C NMR: δ were recorded on a Bruker 300-AC instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (J) are in hertz. Mass spectra were run on a HP 5989A spectrometer. Elemental analyses (C, H, N) were performed on a Perkin Elmer 2400 CHN apparatus at the Microanalyses Service of the University Complutense of Madrid; unless otherwise stated all reported values are within ± 0.4% of the theoretical compositions. Thin-layer chromatography (TLC) was run on Merk silica gel 60 F-254 plates. Unless stated otherwise, starting materials used were high-grade commercial products.

***N,N'*-Bis[[(*tert*-butoxycarbonyl)amino]acetyl]-*N,N'*-dimethyl-1,3-propanediamine (16).** To a solution of *N*-(*tert*-butoxycarbonyl)glycine (4 g, 0.023 mol) in anhydrous CH_2Cl_2 (60 mL), was added freshly distilled 1,3-dicyclohexylcarbodiimide (4.7 g, 0.023 mol) and 1-hydroxybenzotriazole hydrate (0.62 g, 4.6 mmol) at 0 °C. After the mixture was stirred at this temperature for 1 hour, a solution of *N,N'*-dimethyl-1,3-propanediamine (1.46 g, 0.011 mol) in anhydrous CH_2Cl_2 (30 mL) was added dropwise for 1 hour at 0 °C. The mixture was stirred for 14 hours at room temperature and the resulting solid was filtered off, washed with CH_2Cl_2 and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography using AcOEt/Hexane (9:1) as eluent until elution of apolar impurities and then CH_2Cl_2/MeOH (8:2) to give **16** (3.9 g, 83%) as a colorless oil. IR (KBr): 3410, 3330, 1710, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.50 (br s, 2H, 2NH), 3.94 (m, 4H, $2COCH_2$), 3.35 and 3.24 (2m, 4H, $2NCH_2$), 2.98 and 2.95 (2s, 6H, $2NCH_3$), 1.80 (m, 2H, CH_2), 1.45 (s, 18H, 2^tBuO).

^{13}C-NMR ($CDCl_3$): δ 168.6, 168.3, 167.9, 155.7, 79.5, 46.2, 45.5, 45.4, 42.3, 41.9, 34.1, 33.9, 33.3, 28.3, 25.9, 24.8.

***N,N'*-Bis(aminoacetyl)-*N,N'*-dimethyl-1,3-propanediamine Dihydrochloride (17).** A solution of **16** (3.8 g, 9.2 mmol) in EtOH (dried by distillation from Mg/I_2, 100 mL) was saturated with HCl (g) for 1 hour at 0 °C. The mixture was stirred at room temperature overnight, and then the solvent was removed in vacuo. The residue was washed with Et_2O to give **17** (2.7 g, 98%) as a white solid, mp > 300 °C. IR (KBr): 2610, 1640 cm^{-1}. ^{1}H-NMR (D_2O): δ 3.80 and 3.77 (2s, 4H, $2COCH_2$), 3.20 and 3.09 (2m, 4H, $2NCH_2$), 2.79, 2.78, 2.76 and 2.74 (4s, 6H, $2NCH_3$), 1.66 (m, 2H, CH_2). ^{13}C-NMR (D_2O): δ 169.2, 169.1, 168.9, 49.1, 48.1, 48.0, 42.7, 42.6, 36.7, 36.6, 35.8, 27.1, 26.4

***N,N'*-Bis(2-aminoethyl)-*N,N'*-dimethyl-1,3-propanediamine (18).** To a suspension of **17** (2.7 g, 9.3 mmol) in anhydrous THF (77 mL) under argon, was added an 1 M Borane-THF complex (187 mL, 187 mmol). The mixture was heated at reflux for 30 minutes and then stirred overnight at room temperature. MeOH (43 mL) was added, and the solution was saturated with HCl (g) for 1 hour at 0 °C. The resulting mixture was stirred overnight at room temperature and the solvent was evaporated. The solid residue was washed with Et_2O under argon and the obtained hygroscopic white solid was added to a freshly prepared solution of sodium ethoxide in ethanol [sodium (1.0 g, 43.5 mmol) in 47 mL of EtOH]. After stirring mixture overnight at room temperature, the formed NaCl was removed by filtration and the solvent was evaporated, to give **18** (1.37 g, 78%) as a colorless oil which was further purified by Kugelrohr distillation, bp 120 °C/1.5 mmHg (lit.[1] bp 32/0.5 mmHg). IR (KBr): 3360, 3290 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.76 (t, 4H, J = 6.0 Hz, $2NCH_2$), 2.38 (m, 8H, $4NCH_2$), 2.21 (s, 6H, $2NCH_3$), 1.64 (m, 2H, CH_2), 1.52 (br s, 4H, $2NH_2$). ^{13}C-NMR ($CDCl_3$): δ 60.5, 55.9, 42.2, 39.5, 25.1.

3-Acetylamino-4-nitronaphthalene-1,8-dicarboxylic Anhydride (13). To a suspension of 3-acetylaminonaphthalene-1,8-dicarboxylic anhydride[2] (2 g, 7.8 mmol) in concentrated H_2SO_4 (9 mL) at −8 °C (ice/NaCl bath) was added slowly a mixture of fuming HNO_3 (7 mL) and concentrated H_2SO_4 (7 mL), keeping the temperature below 0 °C. The mixture was allowed to stand at room temperature for two hours, and the solution was poured into water and ice. The precipitate formed was filtered, washed with water and dried. Recrystallization from AcOEt gave **13** (1.46 g, 62%) as a yellow solid, mp 218-219 °C. IR (KBr): 3270, 1780, 1730, 1680 cm^{-1}. ^1H NMR (DMSO-d_6): δ 10.73 (s, 1H, NH), 8.66 (s, 1H, ArH), 8.57 (d, 1H, J = 7.3 Hz, ArH), 8.27 (d, 1H, J = 8.6 Hz, ArH), 8.04 (dd, 1H, J = 8.6 and 7.3 Hz, ArH), 2.16 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 169.4, 160.1, 159.4, 141.7, 131.9, 130.5, 129.6, 129.3, 128.2, 127.1, 123.5, 122.6, 120.0, 23.3. MS (EI): *m/z* (%) 300 (M^+, 1), 258 (62), 254 (100), 228 (27), 209 (95), 181 (40), 140 (36), 112 (32), 86 (24), 63 (15) 56 (11). Anal. ($C_{14}H_8N_2O_6$) C, H, N.

3-Acetylamino-4-aminonaphthalene-1,8-dicarboxylic Anhydride (14). A mixture of **13** (1.7 g, 5.7 mmol) and 10% Pd/C (121 mg) in DMF (120 mL) was shaken in a Parr hydrogenator under hydrogen at 50 PSI pressure for 24 hours. The catalyst was then filtered off and washed with DMF. The filtrate was concentrated, and water was added. The precipitate was then filtered and washed with water. Recrystallization from DMF gave **14** (1.15 g, 75%) as a red solid, mp >300 °C. IR (KBr): 3420, 3310, 3220, 1760, 1710, 1635 cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.37 (br s, 1H, NH), 8.76 (d, 1H, J = 8.5 Hz, ArH), 8.42 (d, 1H, J = 6.7 Hz, ArH), 8.23 (s, 1H, ArH), 7.72 (m, 1H, ArH), 7.42 (br s, 2H, NH_2), 2.13 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 169.4, 161.9, 160.3, 147.7, 133.5, 132.4, 130.8, 130.4, 124.8, 120.2, 118.4, 118.0, 102.2, 23.4. Anal. ($C_{14}H_{10}N_2O_4$) C, H, N.

2-Methyl-3*H*-naphtho[1,2-*d*]imidazole-5,6-dicarboxylic Anhydride (15). A suspension of **14** (1.20 g, 4.40 mmol) in AcOH (125 mL) was refluxed for 2 days. The hot suspension was filtered and the filtrate was left for crystallization. The precipitate was collected by filtration to give **15** (1.1 g, 98%) as a light brown solid, mp >300 °C (lit.[3] mp 340-342 °C). IR (KBr): 3180, 1760, 1710 cm^{-1}. ^1H NMR (CF_3CO_2D): δ 9.20 (s, 1H, ArH), 8.99 (m, 2H, ArH), 8.27 (dd, 1H, J = 8.0 and 7.4 Hz, ArH), 3.23 (s, 3H, CH_3). ^{13}C NMR (CF_3CO_2D): δ 164.59, 164.57, 156.0, 137.3, 134.0, 132.4, 132.0, 130.9, 130.4, 123.5, 121.7, 120.9, 119.5, 13.6. MS (EI): *m/z* (%) 253 (M^++1, 25), 252 (M^+, 100), 208 (57), 180 (36), 90 (17), 76 (5).

General Procedure for the Preparation of Mononaphthalimides 4-5 and their Salts. A suspension of anhydride **15** (1 equiv) in toluene was treated with the corresponding polyamine (1 equiv) in ethanol. The mixture was heated at reflux temperature until the reaction was completed (TLC). The precipitated solid was filtered and recrystallized from the appropriate solvent to provide the mononaphthalimide as a free base. This compound was suspended in ethanol and methanesulfonic acid (2.2 equiv) was added. The monoimide salt was isolated by filtration and washed with diethyl ether.

5-[2-(Dimethylamino)ethyl]-9-methyl-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (4). From **15** (200 mg, 0.79 mmol) in toluene (5 mL) and *N,N*-dimethylethylenediamine (69 mg, 0.79 mmol) in absolute EtOH (2 mL) yielded **4** (198 mg, 78%) as a yellow solid, mp 209-211 °C (AcOEt/hexane). The free base was converted into the corresponding dimethanesulfonate dihydrate (73%), mp 261-263 °C. IR (KBr): 3400, 2700, 1700, 1655 cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.17 (s, 1H, NH^+), 8.82 (d, 1H, J = 8.0 Hz, ArH), 8.74 (s, 1H, ArH), 8.56 (d, 1H, J = 7.3 Hz, ArH), 8.07 (dd, 1H, J = 8.0 and 7.3 Hz, ArH), 4.42 (t, 2H, J = 5.5 Hz, CH_2), 3.49 (m, 2H, CH_2), 2.94 (s, 3H, NCH_3), 2.92 (s, 3H, NCH_3), 2.89 (s, 3H, CH_3), 2.34 (s, 6H, $2CH_3SO_3^-$). ^{13}C

NMR (D_2O): δ 165.6, 165.1, 154.1, 132.9, 131.7, 129.4, 129.3, 128.9, 125.5, 122.2, 119.7, 119.4, 119.3, 56.2, 44.3, 39.4, 36.5, 13.1. Anal. ($C_{18}H_{18}N_4O_2 \cdot 2CH_3SO_3H \cdot 2H_2O$) C, H, N, S.

9-Methyl-5-[2-(pyrrolidin-1-yl)ethyl]-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (5). From **15** (113 mg, 0.45 mmol) in toluene (5 mL) and *N*-(2-aminoethyl)pyrrolidine (52 mg, 0.45 mmol) in absolute EtOH (2 mL) yielded **5** (153 mg, 97%) as a yellow solid, mp >300 °C (absolute EtOH). The free base was converted into the corresponding dihydromethanesulfonate monohydrate (62%), mp 216-220 °C. IR (KBr): 3380, 2700, 1690, 1650 cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.39 (s, 1H, NH^+), 8.84 (d, 1H, J = 8.0 Hz, ArH), 8.75 (s, 1H, ArH), 8.57 (d, 1H, J = 7.3 Hz, ArH), 8.07 (m, 1H, ArH), 4.42 (br s, 2H, NCH_2), 3.67 (m, 2H, NCH_2), 3.56 (m, 2H, NCH_2), 3.18 (m, 2H, NCH_2), 2.88 (s, 3H, CH_3), 2.35 (s, 6H, $2CH_3SO_3^-$), 2.03 (m, 2H, CH_2), 1.86 (m, 2H, CH_2). ^{13}C NMR (D_2O): δ 165.6, 165.0, 154.2, 133.0, 131.6, 129.5, 129.2, 128.9, 125.4, 122.2, 119.7, 119.4, 119.2, 58.4, 55.7, 53.3, 39.3, 37.6, 23.7, 17.7, 13.1. Anal. ($C_{20}H_{20}N_4O_2 \cdot 2CH_3SO_3H \cdot H_2O$) C, H, N, S.

General Procedure for the Preparation of Bisnaphthalimides 6-12 and their Salts. A suspension of anhydride **15** (2 equiv) in toluene was treated with the corresponding polyamine (1 equiv) in ethanol. The mixture was heated at reflux temperature until the reaction was completed (TLC). The precipitated solid was filtered and recrystallized from the appropriate solvent to provide the bisnaphthalimide as a free base, except for **12**, where column chromatography was used for purificaction. Bisimides were transformed into the corresponding methanesulphonate or hydrochloride salts

***N,N'*-Bis[2-(4,6-dioxo-9-methyl-4,5,6,8-tetrahydrobenz[*de*]imidazo[4,5-*g*]isoquinolin-5-yl)ethyl]-1,2-ethanediamine (6).** From **15** (200 mg, 0.79 mmol) in toluene (5 mL) and *N,N'*-bis(2-aminoethyl)-1,2-ethanediamine (58 mg, 0.40 mmol) in

absolute EtOH (2 mL) yielded **6** (196 mg, 80%) as a brown solid, mp >300 °C (EtOH/Et_2O). The free base was converted into the corresponding dihydromethanesulfonate monohydrate (72%), mp 287-290 °C. IR (KBr): 3400, 2650, 1700, 1655 cm^{-1}. ^1H NMR (D_2O): δ 7.83 (d, 2H, J = 6.6 Hz, ArH), 7.62 (br s, 2H, ArH), 7.26 (br s, 2H, ArH), 7.14 (br s, 2H, ArH), 4.11 (br s, 4H, $2CH_2$), 3.30 (br s, 4H, $2CH_2$), 3.38 (m, 4H, $2CH_2$), 2.52 (s, 12H, $4CH_3SO_3^-$), 2.39 (s, 6H, $2CH_3$). ^{13}C NMR (D_2O): δ 165.8, 165.4, 154.0, 133.7, 131.1, 129.7, 129.1, 128.6, 128.1, 124.7, 121.5, 119.3, 119.2, 47.9, 44.7, 39.4, 38.5, 13.26. Anal. ($C_{34}H_{30}N_8O_4 \cdot 4CH_3SO_3H \cdot H_2O$) C, H, N, S.

***N,N'*-Bis[2-(4,6-dioxo-9-methyl-4,5,6,8-tetrahydrobenz[*de*]imidazo[4,5-*g*]isoquinolin-5-yl)ethyl]-*N,N'*-dimethyl-1,2-ethanediamine (7).** From **15** (150 mg, 0.60 mmol) in toluene (5 mL) and *N,N'*-bis(2-aminoethyl)-*N,N'*-dimethylethanediamine (55 mg, 0.32 mmol) in absolute EtOH (2 mL) yielded **7** (173 mg, 90%) as a brown solid, mp 192-195 °C (EtOH/Et_2O). The free base was converted into the corresponding tetrahydromethanesulfonate sesquihydrate (69%), mp >300 °C. IR (KBr): 3400, 2600, 1700, 1655 cm^{-1}. ^1H NMR (D_2O): δ 7.87 (d, 2H, J = 7.1 Hz, ArH), 7.46 (d, 2H, J = 8.3 Hz, ArH), 7.38 (s, 2H, ArH), 7.25 (m, 2H, ArH), 4.15 (br s, 4H, $2CH_2$), 3.76 (br s, 4H, $2CH_2$), 3.49 (m, 4H, $2CH_2$), 2.99 (s, 6H, $2NCH_3$), 2.55 (s, 12H, $4CH_3SO_3^-$), 2.38 (s, 6H, $2CH_3$). ^{13}C NMR (D_2O): δ 165.6, 165.1, 154.5, 134.4, 131.4, 129.7, 128.6, 128.4, 124.5, 121.4, 119.5, 119.0, 117.3, 60.0, 51.3, 42.8, 39.4, 36.6, 13.4. Anal. ($C_{36}H_{34}N_8O_4 \cdot 4CH_3SO_3H \cdot 1.5H_2O$) C, H, N, S.

***N,N'*-Bis[2-(4,6-dioxo-9-methyl-4,5,6,8-tetrahydrobenz[*de*]imidazo[4,5-*g*]isoquinolin-5-yl)ethyl]-1,3-propanediamine (8).** From **15** (94 mg, 0.37 mmol) in toluene (2.5 mL) and *N,N'*-bis(2-aminoethyl)-1,3-propanediamine (30 mg, 0.19 mmol) in absolute EtOH (1 mL) yielded **8** (101 mg, 85%) as a brown solid, mp 157-159 °C (EtOH/Et_2O). The free base was converted into the corresponding

tetrahydromethanesulfonate trihydrate (94%), mp >300 °C. IR (KBr): 3400, 2740, 1700, 1660 cm^{-1}. ^1H NMR (D_2O): δ 7.95 (d, 2H, J = 7.1 Hz, ArH), 7.65 (m, 4H, ArH), 7.35 (dd, 2H, J = 7.7 and 7.1 Hz, ArH), 4.17 (br s, 4H, $2NCH_2$), 3.32 (m, 4H, $2NCH_2$), 3.23 (m, 4H, $2NCH_2$), 2.59 (s, 12H, $4CH_3SO_3^-$), 2.53 (s, 6H, $2CH_3$), 2.11 (m, 2H, CH_2). ^{13}C NMR (D_2O): δ 165.6, 165.4, 154.3, 133.4, 131.4, 129.7, 129.0, 128.6, 124.9, 121.7, 119.4, 119.2, 118.3, 46.8, 44.8, 39.4, 37.5, 22.5, 13.2. Anal. ($C_{35}H_{32}N_8O_4 \cdot 4CH_3SO_3H \cdot 3H_2O$) C, H, N, S.

***N,N'*-Bis[2-(4,6-dioxo-9-methyl-4,5,6,8-tetrahydrobenz[*de*]imidazo[4,5-*g*]isoquinolin-5-yl)ethyl]-*N,N'*-dimethyl-1,3-propanediamine (9).** From **15** (249 mg, 0.99 mmol) in toluene (5 mL) and *N,N'*-bis(2-aminoethyl)-*N,N'*-dimethyl-1,3-propnanediamine (93 mg, 0.49 mmol) in absolute EtOH (2 mL) yielded **9** (280 mg, 87%) as a yellow solid, mp 172-175 °C (absolute EtOH). The free base was converted into the corresponding tetrahydromethanesulfonate trihydrate (61%), mp 236 °C (dec). IR (KBr): 3400, 2650, 1700, 1660 cm^{-1}. ^1H NMR (D_2O): δ 8.07 (d, 2H, J = 7.7 Hz, ArH), 7.87 (s, 2H, ArH), 7.74 (d, 2H, J = 7.7 Hz, ArH), 7.45 (t, 2H, J = 7.7 Hz, ArH), 4.28 (br s, 4H, $2NCH_2$), 3.46 (br s, 8H, $4NCH_2$), 2.93 (s, 6H, $2NCH_3$), 2.62 (s, 6H, $2CH_3$), 2.60 (s, 12H, $4CH_3SO_3^-$), 2.28 (br s, 2H, CH_2). ^{13}C NMR (D_2O): δ 165.2, 164.9, 154.0, 132.0, 131.7, 129.3, 128.9, 128.5, 125.1, 121.9, 119.2, 119.1, 119.0, 53.1, 42.8, 41.0, 39.4, 35.9, 20.1, 13.0. Anal. ($C_{37}H_{36}N_8O_4 \cdot 4CH_3SO_3H \cdot 3H_2O$) C, H, N, S.

***N,N*-Bis[2-(4,6-dioxo-9-methyl-4,5,6,8-tetrahydrobenz[*de*]imidazo[4,5-*g*]isoquinolin-5-yl)ethyl]-*N*-methylamine (10).** From **15** (100 mg, 0.40 mmol) in toluene (2.5 mL) and N^2-methyldiethylenetriamine (23 mg, 0.20 mmol) in absolute EtOH (1 mL) yielded **10** (79 mg, 69%) as a yellow solid, mp >300 °C (DMF/AcOEt). The free base was converted into the corresponding trihydromethanesulfonate 2.75hydrate (74%), mp 284-285 °C. IR (KBr): 3400, 2580, 1700, 1660 cm^{-1}. ^1H NMR

(D_2O): δ 7.55 (m, 2H, ArH), 7.37 (br s, 2H, ArH), 7.28 (br s, 2H, ArH), 7.15 (m, 2H, ArH), 4.15 (m, 4H, $2CH_2$), 3.51 (m, 4H, $2CH_2$), 3.04 (s, 3H, NCH_3), 2.56 (s, 9H, $3CH_3SO_3^-$), 2.39 (s, 6H, $3CH_3$). ^{13}C NMR (D_2O): δ 165.2, 164.6, 154.4, 133.6, 130.8, 130.0, 128.7, 128.2, 124.3, 121.2, 119.5, 119.1, 117.4, 54.7, 42.4, 39.4, 36.0, 13.4. Anal. ($C_{33}H_{27}N_7O_4 \cdot 3CH_3SO_3H \cdot 2.75H_2O$) C, H, N, S.

***N,N*-Bis[3-(4,6-dioxo-9-methyl-4,5,6,8-tetrahydrobenz[*de*]imidazo[4,5-*g*]isoquinolin-5-yl)propyl]-*N*-methylamine (11).** From **15** (150 mg, 0.60 mmol) in toluene (3.7 mL) and 3,3'-diamino-*N*-methyldipropylamine(43 mg, 0.30 mmol) in absolute EtOH (1.5 mL) yielded **11** (61 mg, 33%) as a brown solid, mp 235-240 °C (DMF/AcOEt). The free base was converted into the corresponding trihydromethanesulfonate monohydrate (74%), mp 284-285 °C. IR (KBr): 3400, 2580, 1700, 1660 cm^{-1}. ^1H NMR (D_2O): δ 7.49 (br s, 2H, ArH), 6.95 (br s, 4H, ArH), 6.68 (br s, 2H, ArH), 3.64 (m, 4H, $2NCH_2$), 2.98 (s, 3H, NCH_3), 2.75 (m, 4H, $2NCH_2$), 2.59 (s, 9H, $3CH_3SO_3^-$), 2.23 (s, 6H, $2CH_3$), 1.82 (m, 4H, $2CH_2$). ^{13}C NMR (D_2O): δ 164.6, 163.8, 154.0, 133.6, 130.4, 129.2, 128.2, 127.4, 123.6, 121.2, 118.8, 117.5, 116.9, 54.4, 41.6, 39.5, 38.2, 23.9, 13.2. Anal. ($C_{35}H_{31}N_7O_4 \cdot 3CH_3SO_3H \cdot H_2O$) C, H, N, S.

***N,N'*-Bis[3-(4,6-dioxo-9-methyl-4,5,6,8-tetrahydrobenz[*de*]imidazo[4,5-*g*]isoquinolin-5-yl)propyl]-1,2-ethanediamine (12).** To a hot suspension of compound **15** (250 mg, 1.0 mmol) in toluene (2 mL), was added dropwise *N,N'*-bis(3-aminopropyl)ethylenediamine (86 mg, 0.5 mmol) in absolute EtOH (4 mL). After the mixture was refluxed for 6 hours, an excess of anhydride **15** (50 mg, 0.2 mmol) was added. The reaction mixture was refluxed for 2 additional hours, and then the solvent was evaporated. The residue was purified by flash chromatography using CH_2Cl_2/MeOH/NH_3 (5:5:0.1) as eluent to give **12** (248 mg, 77%) as an unstable orange solid. To a suspension of free base (202 mg, 0.31 mmol) in AcOEt (9 mL), was added a

saturated solution of HCl in AcOEt (8.3 mL). The obtained solid was filtered to give the corresponding tetrahydrochloride monohydrate (218 mg, 88%), mp 290-292 °C. IR (KBr): 3400, 2740 1700, 1660 cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.54 (br s, 4H, $2NH_2^+$), 8.81 (d, 2H, J = 7.9 Hz, ArH), 8.54 (s, 2H, ArH), 8.46 (d, 2H, J = 7.3 Hz, ArH), 7.95 (m, 2H, ArH), 4.15 (br s, 4H, $2NCH_2$), 3.41 (br s, 4H, $2NCH_2$), 3.18 (br s, 4H, $2NCH_2$), 2.96 (s, 6H, $2CH_3$), 2.14 (br s, 4H, $2CH_2$). ^{13}C NMR (D_2O): δ 163.9, 163.5, 153.4, 132.8, 130.1, 129.6, 128.4, 127.7, 124.4, 121.9, 119.2, 118.5, 118.3, 45.6, 43.0, 38.1, 25.0, 13.6. Anal. ($C_{36}H_{34}N_8O_4 \cdot 4HCl \cdot H_2O$) C, H, N.

Computational Methods

Model Building. Compounds **4**, **8**, **9** and **12** were model-built in Sybyl[4] using standard geometries, which were fully optimized by means of the ab initio quantum mechanical program Gaussian 94[5] and the 3-21G basis set. Atom-centered point charges for the optimized structures were derived[6] which best reproduced the electrostatic potential of the molecule calculated by means of a single point calculation using the larger 6-31G* basis set.[7]

The solution structure of the complex between $d(ATGCAT)_2$ and elinafide[8] was used as a template for modeling both a DNA dimer containing a d(TpG) step and the DNA bisintercalation site. Models were constructed for the four possible orientations of **4**, **8**, **9** and **12** relative to the DNA dimer and hexamer, respectively, as shown in Figures 1 and 2. The AMBER all-atom force field parameters[9] were used for the DNA dimer and hexamer, and covalent parameters for the intercalating chromophores and linking chains of the ligands were derived, by analogy or through interpolation,[10] from those already present in the AMBER database.

In order to achieve electrical neutrality in the complexes, an appropriate number of counterions (one for the dimers and six for the hexamers) resembling hexahydrated sodium

ions were placed in the bisector of the O-P-O groups located further from the positive charges of the ligand.[11]

Energy Minimization. The initial dimer and hexamer complexes were refined by progressively minimizing their potential energy. The optimizations were carried out in a continuum medium of relative permitivitty $e=4r_{ij}$ for simulating the solvent environment. For the first 3000 steps of the minimization all hydrogen bonds between the DNA base pairs, were reinforced with distance and angle restraining functions with force constants of 10 kcal mol^{-1} $Å^{-2}$ and 10 kcal mol^{-1} rad^{-2}, respectively.

Molecular Dynamics Simulations. In order to sample a larger extent of the conformational space, the four lowest energy complexes of **4**, **8**, **9** and **12** were subjected to molecular dynamics simulations at 300 K for 50 ps. In a 5 ps heating phase, the temperature was raised in steps of 10 K over 0.1 ps blocks, and the velocities were reassigned at each new temperature according to a Maxwell-Boltzmann distribution. This was followed by an equilibration phase of 15 ps at 300 K, in which the velocities were reassigned in the same way every 0.2 ps and by 30 ps sampling period during which system coordinates were saved every 0.2 ps. The time step used was 1fs during the heating period and 2fs for the rest of the simulations. All bonds involving hydrogens were constrained to their equilibrium values by means of the SHAKE algorithm,[12] and lists of nonbonded pairs were updated every 25 ps. For the first 20 ps of simulation the atoms of the phosphate-sugar backbone were restrained to their reference positions at 0 ps by means of a harmonic potential with a force constant of 10 Kcal mol^{-1} $Å^{-2}$. During the entire simulation time, the G:C and A:T hydrogen bonds were reinforced by means of an upper-bound harmonic restraining function with a force constant of 5 kcal mol^{-1} $Å^{-2}$ and 5 Kcal mol^{-1} rad^{-1} for distances and angles, respectively.

Analysis of the Dynamics Trajectories. Three dimensional structures were visually inspected using the computer graphics program Sybyl.[4] Trajectories were visualized by means of MDDISPLAY.[13] Root-mean-square (rms) deviations from the initial structures, interatomic distances, and distribution functions were monitored using the CARNAL module of Amber suite of programs.[14]

DNA Binding Experiments

DNA. Calf thymus DNA was purchased from Sigma Chemical Co. as the highly polymerized sodium salt. For viscometric experiments the DNA was sonicated to fragments of approx. 4.5 $\times 10^5$ D determined as described by Eigner and Doty.[15] The sonicated DNA sample displayed an A_{260}/A_{280} ratio of 1.92. This spectral data is consistent with published values.[16]

Viscometric Titrations. The viscometric measurements were performed in an Ubbelohde Microviscometer at 25 ± 0.05°C. Solutions of sonicated DNA and the selected compound were prepared in TRIS buffer (50 mM, pH = 6.9). These solutions had different molar ratio, r, of added compound to DNA nucleotides. Flow rates were measured with a Schott-Geräte Viscosystem AVS 350 to an accuracy of 0.01%. Time readings were recorded in triplicate to 0.01s.

UV-VIS. For spectrometric determinations was used an UV-1603 Schimadzu. The spectra of sonicated DNA, intercalator and DNA-intercalator complex were registered at different temperatures: 5, 10, 25 and 35 °C.

The UV-vis spectra of compound **4** were registered from pH 3 to 12 with ten different buffers, in order to find if the aliphatic nitrogen was protonated at physiological pH by recording the isosbestic point.

In Vitro Cytotoxicity Assays

The cell lines used were human colon carcinoma (HT-29) (ATCC, HTB 38), human cervical carcinoma (HeLa) (ATCC, CCL 2) and human prostate carcinoma (PC-3) (ECACC, 90112714). For each experiment, cultures were seeded from frozen stocks. Each cell line was maintained in its appropriated medium and was incubated at 37° C in a 5% CO_2 atmosphere.

All cell lines were in the logarithmic phase of growth when the assay of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was carried out. Cells were harvested and seeded into 96-well tissue culture plates at a density of 2.5×10^4 cells/well in 150 μL aliquots of medium. The concentrations tested were serial dilutions of a stock solution (1×10^{-5} M in DMSO) with phosphate-buffered saline (PBS) and were added 24 h later. The assay was ended after 72 h of drug exposure and PBS was used as a negative control and doxorubicine as a positive control.

After a 72 h exposure period, cells were washed twice with PBS, and then 50 μL/well of MTT reagent (1 mg/mL in PBS; Sigma) together with 150 μL/well of prewarmed medium were added. The plates were returned to the incubator for 4 h. Subsequently, DMSO was added as solvent. Absorbance was determined at 570 nm with a Microplate reader (Opsys MR).

All experiments were performed at least three times, and the average of the percentage absorbance was plotted against concentration. Then, the concentration of drug required to inhibit 50% of cell growth (IC_{50}) was calculated for each compound.

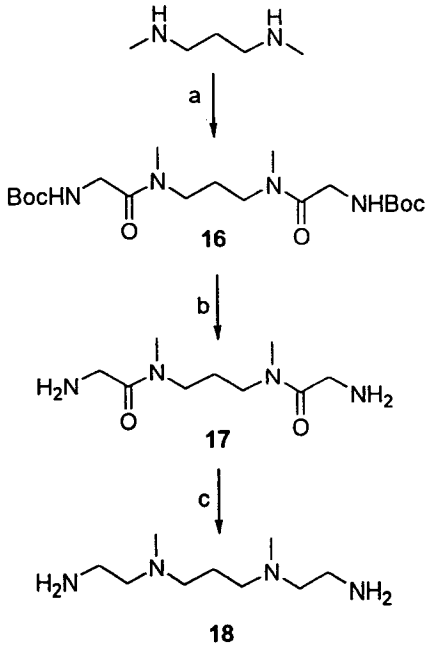
Alkaline Single-Cell Gel Electrophoresis Assay.

The alkaline single-cell gel electrophoresis assay (comet assay) detects DNA damage in individual cells embedded in agarose. The test was performed on HT-29 cells following the method described by Moinet-Hedin et al.[17] After 1h of treatment with the drug, cells

were centrifuged and resuspended in low-melting-point (LMP) agarose at 37 °C. The cell suspension was put on a slide precoated with normal agarose and a glass cover slip was added. After solidification at 0 °C the glass cover slip was gently removed and a third layer of 0.5% of LMP agarose in PBS was added and run for solidification.

The slides were put in a lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, pH 10, with freshly added 1% Triton X-100 and 10% DMSO) for 1h and were rinsed in the electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) for 40 min. Electrophoresis (300 mA, 0.7 V/cm) was then performed for 24 min in fresh buffer. The slides were washed twice in neutralization buffer (0.4 M Tris, pH 7.5) and stained with ethidium bromide (20 μg/mL). They were observed using a fluorescence microscope (Nikon) with an excitation filter of 515-560 nm and a barrier filter of 580 nm.

Scheme 2[a]



[a] Reagents: (a) *N*-Boc-glycine, DCC, HOBT, CH_2Cl_2; (b) HCl gas, MeOH; (c) BH_3-THF, THF

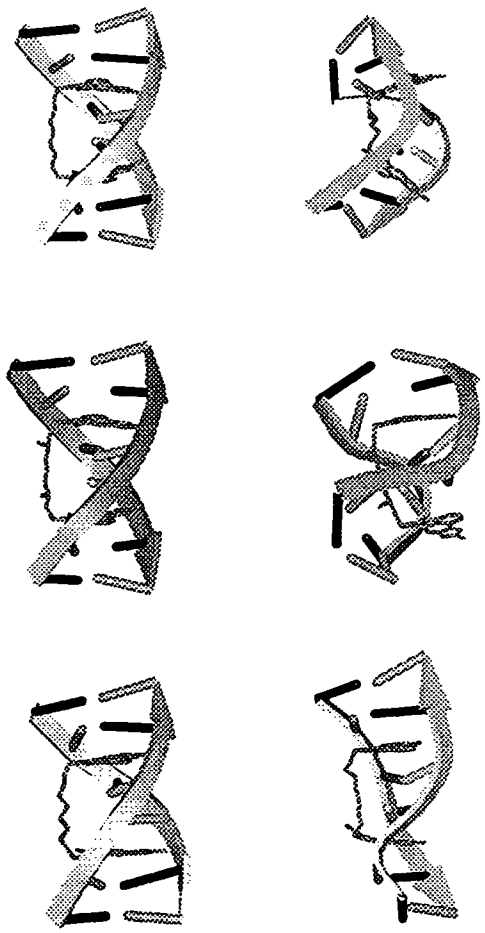


Figure 3. Side views of the energy-minimized initial (left) and average structure (right) of the last 10 ps of the molecular dynamics simulations for complexes **8b** (top), **9b** (center) and **12b** (bottom).

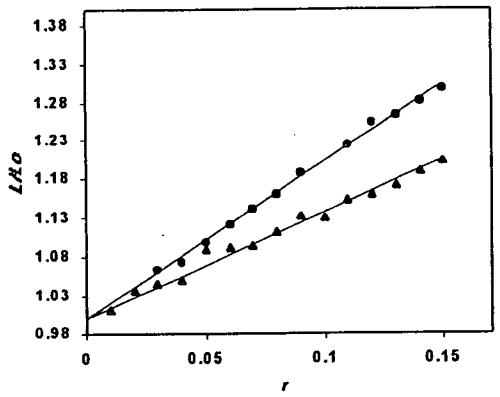


Figure 4. Relative length increase L/L_0 of **4** (▲) and **8** (●) DNA complexes as a function of the molar ratio of added compound to DNA nucleotides (r). The contour lengths in the presence (L) or absence (L_o) of the compounds were calculated from viscosity measurements on sonicated calf thymus DNA.

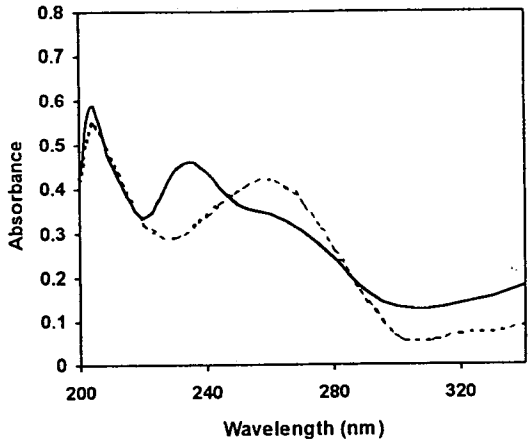


Figure 5. Effect of calf thymus on ultraviolet absorption spectrum of **4** in Tris·HCl (pH = 7.5). The solid line indicates the spectrum of **4** alone at a concentration of 5.25×10^{-6} M, and the dashed line represents the spectrum of **4** at the same concentration in the presence of 1.80×10^{-4} M DNA (nucleotide molarity).

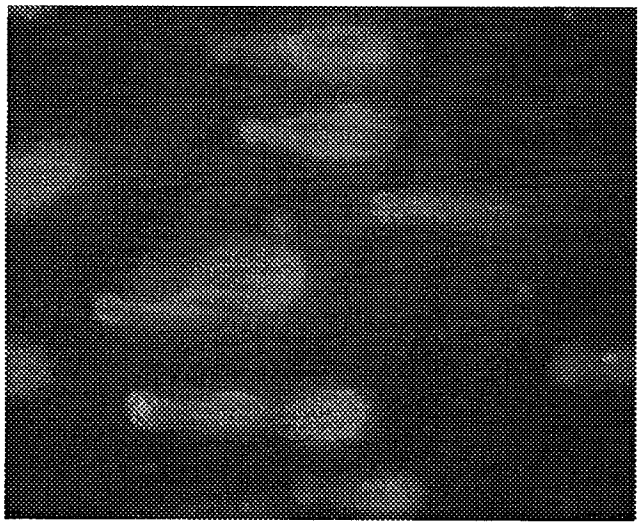
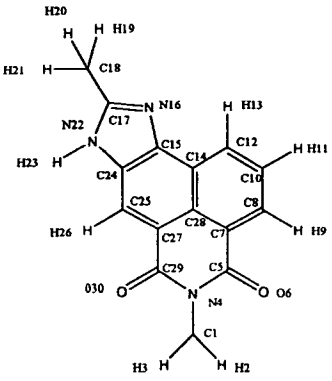


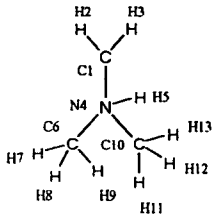
Figure 6. Single-cell gel electrophoresis assay for compound **5**

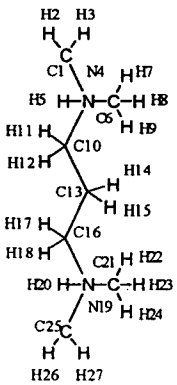
Table 2

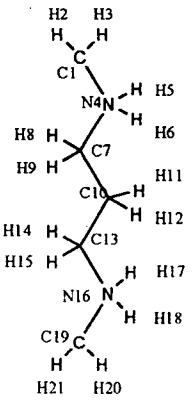
Ab Initio 6-31G*\\3-21G Electrostatic Potencial Atomic Charges for Chromophores and Linkers.

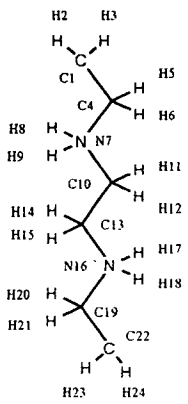
Chromophore compound 4, 8, 9 y 12			Linker compound 4			Linker compound 9			Linker compound 12		
Atm Nº	Atm type		Atm Nº	Atm type		Atm Nº	Atm type		Atm Nº	Atm type	
C1	CT	-0.19001	C1	CT	-0.36567	C1	CT	-0.04099	C1	CT	-0.26943
H2	HC	0.18156	H2	HC	0.28071	H2	HC	0.16774	H2	HC	0.15497
H3	HC	0.18156	H3	HC	0.28071	H3	HC	0.16774	H3	HC	0.15497
N4	N6	-0.51202	N4	N3	0.11807	N4	N3	-0.18194	C4	CT	0.09890
C5	CO	0.88780	H5	H	0.29478	H5	H	0.32021	H5	HC	0.09751
O6	O	-0.60347	C6	CT	-0.36568	C6	CT	0.32021	H6	HC	0.09815
C7	61	-0.53658	H7	HC	0.18340	H7	HC	-0.06653	N7	N3	-0.15329
C8	C6	0.13310	H8	HC	0.18340	H8	HC	0.14408	H8	H	0.34931
H9	HC	0.13548	H9	HC	0.19458	H9	HC	0.14408	H9	H	0.34944
C10	C6	-0.36914	C10	CT	-0.36568	C10	CT	-0.14409	C10	CT	0.05677
H11	HC	0.18990	H11	HC	0.18340	H11	HC	0.09745	H11	HC	0.06674
C12	C6	0.08230	H12	HC	0.19458	H12	HC	0.09745	H12	HC	0.07373
H13	HC	0.13683	H13	HC	0.18340	C13	CT	-0.06653	C13	CT	0.00292
C14	66	-0.32954	**Linker compound 8**			H14	HC	0.14408	H14	HC	0.08103
C15	CN	0.49315	C1	CT	-0.04099	H15	HC	0.14408	H15	HC	0.08573
N16	NB	-0.71679	H2	HC	0.16774	C16	CT	-0.18194	N16	N3	-0.13407
C17	CR	0.63227	H3	HC	0.16774	H17	HC	0.32021	H17	H	0.34931
C18	CN	-0.42887	N4	N3	-0.18194	H18	HC	0.32021	H18	H	0.34944
H19	HC	0.15964	H5	H	0.32021	N19	N3	-0.04099	C19	CT	0.07515
H20	HC	0.12474	H6	H	0.32021	H20	H	0.16774	H20	HC	0.09889
H21	HC	0.12491	C7	CT	-0.06653	C21	CT	0.16774	H21	HC	0.10136
N22	NA	-0.54326	H8	HC	0.14408	H22	HC	0.14016	C22	CT	-0.37113
H23	H	0.39044	H9	HC	0.14408	H23	HC	0.14016	H23	HC	0.14180
C24	CN	-0.03785	C10	CT	-0.14408	H24	HC	0.14016	H24	HC	0.14180
C25	C6	0.07904	H11	HC	0.09745	C25	CT	-0.08601			
H26	HC	0.18448	H12	HC	0.09745	H26	HC	0.16176			
C27	61	-0.79997	C13	CT	-0.06653	H27	HC	0.16176			
C28	66	0.52524	H14	HC	0.14408						
C29	CO	1.08212	H15	HC	0.14408						
O30	O	-0.65706	N16	N3	-0.18194						
			H17	H	0.32021						
			H18	H	0.32021						
			C19	CT	-0.04099						
			H20	HC	0.16774						
			H21	HC	0.16774						











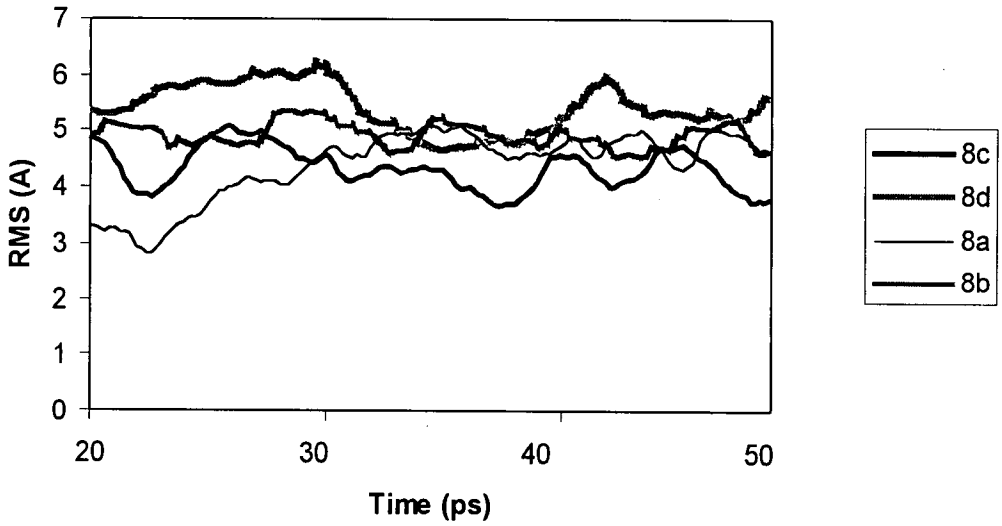


Figure 7. Time evolution of the root-mean-square deviations from the initial structures for **8**:$(ATGCAT)_2$ calculated for all non-hydrogen atoms after least-square fitting of the structures using the same atoms, from the dynamics simulations along the 50 ps sampling time.

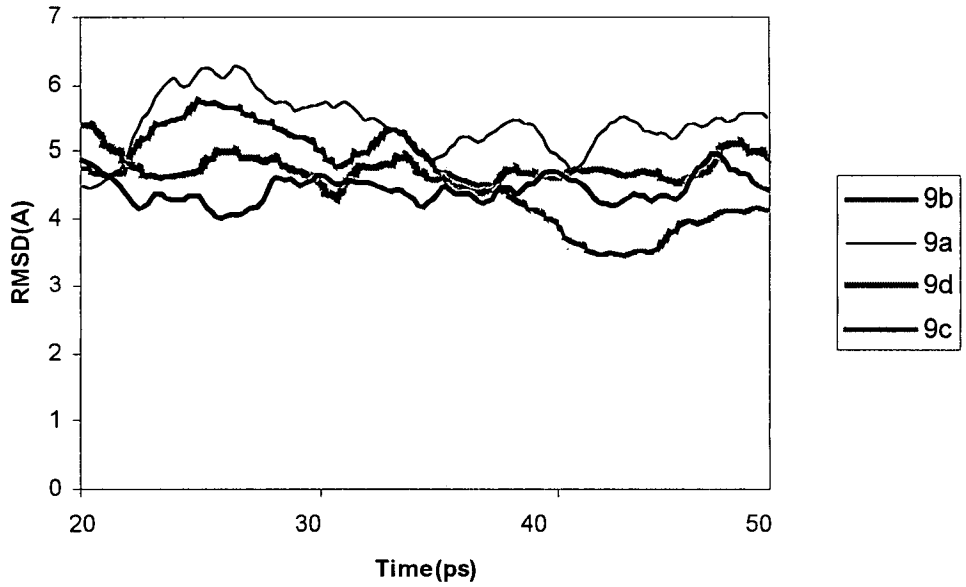


Figure 8. Time evolution of the root-mean-square deviations from the initial structures for **9**:$(ATGCAT)_2$ calculated for all non-hydrogen atoms after least-square fitting of the structures using the same atoms, from the dynamics simulations along the 50 ps sampling time.

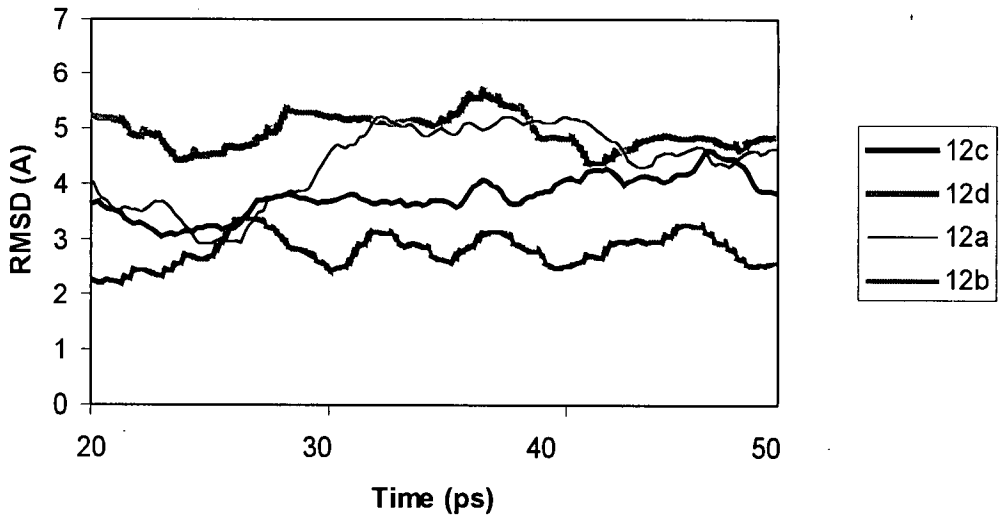
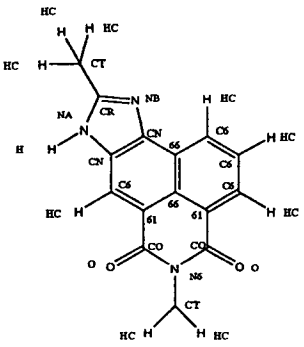


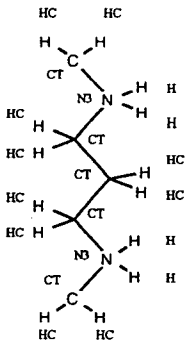
Figure 9. Time evolution of the root-mean-square deviations from the initial structures for **12**:$(ATGCAT)_2$ calculated for all non-hydrogen atoms after least-square fitting of the structures using the same atoms, from the dynamics simulations along the 50 ps sampling time.

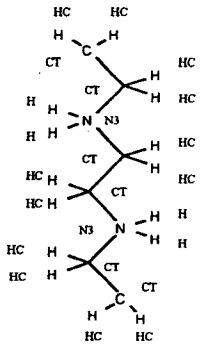
Table 3

Additional Force Field Parameters

Bond	Kr(kcal mol^{-1} A^{-2})	r_{eq} (A)
C6-C6	469.0	1.40
C6-HC	340.0	1.08
CO-N6	415.0	1.39
66-66	460.0	1.41
C6-66	446.0	1.42
C6-61	520.0	1.37
CO-61	342.0	1.49
61-66	460.0	1.41
CO-O	570.0	1.20
N6-CT	337.0	1.47
C6-CN	447.0	1.4
CR-CT	317.0	1.504
66-CN	447.0	1.419
H-N3	434.0	1.010







Angle	Kϕ(kcal mol^{-1} rad^{-2})	ϕ_{eq} (deg)
C6-C6-C6	85.	120.
C6-C6-66	85.	120.
C6-C6-61	85.	120.
C6-C6-HC	35.	120.
61-66-61	85.	120.3
N6-CO-O	80.	120.9
N6-CT-CT	50.	112.4
66-CN-NB	70.	132.8
C6-CN-NA	70.	132.8
CR-CT-HC	35.	109.5
NB-CR-CT	70.	120.
CN-CN-NA	70.	104.4
HC-C6-CN	35.	120.
CT-N3-H	50.	109.5

Angle	Kϕ(kcal mol^{-1} rad^{-2})	ϕ_{eq} (deg)
66-C6-HC	35.	120.
61-C6-HC	35.	120.
C6-66-C6	85.	123.
C6-66-66	85.	118.5
61-66-66	85.	119.85
61-CO-O	80.	121.8
N6-CT-HC	35.	109.5
66-CN-CN	85.	116.2
CN-C6-HC	35.	120.
CR-NA-CN	70.	120.
66-CN-NB	70.	132.8
CN-CN-C6	85.	117.3
CN-C6-61	85.	120.
H -N3-H	35.	109.5

Angle	Kϕ(kcal mol^{-1} rad^{-2})	ϕ_{eq} (deg)
C6-61-CO	85.	119.7
66-61-CO	85.	120.
C6-61-66	85.	120.3
CO-N6-CO	70.	125.
CO-N6-CT	70.	117.5
61-CO-N6	70.	117.3
C6-66-CN	85.	123.4
CN-66-66	85.	120.
CN-NB-CR	70.	120.
CT-CR-NA	70.	120.
CN-CN-NB	70.	110.4
C6-C6-CN	63.	120.
HC-CT-N3	50.	109.5

Dihedral Angle	idivf	$v_n/2$ (kcal mol^{-1})	γ (deg)	n
X-C6-C6-X	4	16.3	180.	2.
X-C6-66-X	4	4.4	180.	2.
X-C6-61-X	4	16.3	180.	2.
X-CO-61-X	4	0.9	180.	2.
X-66-66-X	4	4.9	180.	2.
X-66-61-X	4	4.9	180.	2.
X-CO-N6-X	2	5.2	180.	2.
X-66-CN-X	4	16.3	180.	2.

Dihedral Angle	idivf	$v_n/2$ (kcal mol^{-1})	γ (deg)	n
X-CR-CT-X	6	0.0	0.	2.
X-CN-NB-X	2	5.1	180.	2.
X-CN-CN-X	4	16.3	180.	2.
X-C6-CN-X	4	14.5	180.	2.
C6-N6-CT-HC	1	0.	0.	3.
CO-N6-CT-CT	1	0.	0.	2.
CO-N6-CT-HC	1	0.	0.	3.
C6-N6-CT-CT	1	0.5	0.	2.

Imp.Tor.Angles	$v_n/2$ (kcal mol^{-1})	γ (deg)	n
X-X-66-66	4.9	180.	2.
X-X-C6-C6	4.9	180.	2.
X-X-61-66	4.9	180.	2.
X-X-C6-61	4.9	180.	2.
X-X-CO-O	10.5	180.	2.
X-X-N6-CO	10.5	180.	2.
X-X-CN-CN	4.9	180.	2.
X-X-66-61	4.9	180.	2.

van der Waals	R* (A)	ε (kcal mol^{-1})
N6	1.75	0.16
C6	1.85	0.12
66	1.85	0.12
61	1.85	0.12
CO	1.85	0.12

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