Potential DNA Bis-Intercalating Agents. Bridged bis-(6-Chloropurines) and Related Compounds [1]

Cyril Párkányi*, Hui Liang Yuan and Meng-Kan Michael Tsai [2]

Department of Chemistry, Florida Atlantic University,
500 N. W. 20th Street, P. O. Box 3091,
Boca Raton, FL 33431-0991

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Two bis-(6-chloropurines) bridged by conformationally restricted tethers were synthesized as potential DNA bis-intercalating agents. Reduction of 4,6-dichloro-5-nitropyrimidine (**1**) afforded 5-amino-4,6-dichloropyrimidine (**2**) which was then used as the starting material. Reaction of **2** with 4,4'-diaminodiphenylmethane (**3**) and bis-(4-aminophenyl) ether (**4**) yielded bis-[4-(*N*-5-amino-4-chloro-6-pyrimidyl)aminophenyl]methane (**5**) and bis-[4-(*N*-5-amino-4-chloro-6-pyrimidyl)aminophenyl] ether (**6**), respectively. Acid-catalyzed condensation of the above pyrimidines, **5** and **6**, with triethyl orthoformate in *N,N*-dimethylacetamide gave bis-[4-(6-chloro-9-purinyl)phenyl]methane (**7**) and bis-[4-(6-chloro-9-purinyl)phenyl] ether (**8**). The spectral data on the new compounds will be discussed.

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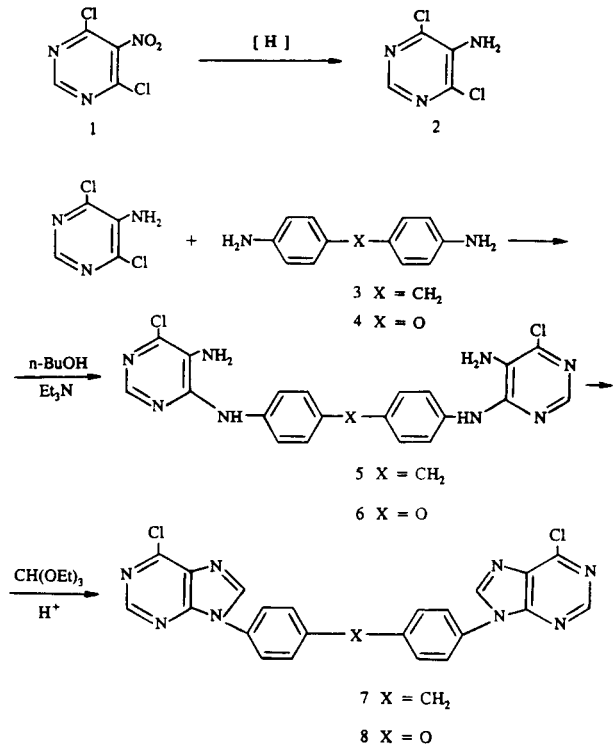
In connection with our systematic studies of novel heterocyclic compounds with potential biological activity [3-11], we have turned our attention to prospective bis-intercalating agents. The biological activity of compounds such as bis-(9-purinyl)ethane and the ethylenediamine derivatives [12-15] has been well established and in one of our recent contributions we have described the synthesis of dipurinyl derivatives and their 8-azapurine analogs bridged by polymethylene chains of different length [11]. In recent years, the inherent flexibility of the tethers has been addressed [16,17]. In general, bifunctional intercalators bridged by flexible chains exhibit a reduced affinity for the DNA. This can be explained as due to the fact that the self-stacking interactions effectively compete with the binding process [18,19]. Another possibility is that the flexible bifunctional intercalators can creep in a stepwise fashion along the DNA macromolecule, drastically lowering ligand residence lifetimes at any given site [20,21].

In the present contribution, we describe the synthesis of novel bis-(6-chloropurines) bridged by conformationally restricted diphenyl tethers (Scheme 1). The compounds were synthesized by a straightforward, two-step procedure described herein. 5-Amino-4,6-dichloropyrimidine (**2**) and 4,4'-diaminodiphenylmethane (**3**) refluxed in 1-butanol in the presence of triethylamine, using a previously described method [22-24], gave bis-[4-(*N*-5-amino-4-chloro-6-pyrimidyl)aminophenyl]methane (**5**). In an analogous reaction, **2** and bis-(4-aminophenyl) ether (**4**) yielded bis-[4-(*N*-5-amino-4-chloro-6-pyrimidyl)aminophenyl]ether (**6**). Subsequent acid-catalyzed condensation of **5** and **6** with triethyl orthoformate carried out in *N,N*-dimethylacetamide afforded bis-[4-(6-chloro-9-purinyl)phenyl]methane (**7**) and bis-[4-(6-chloro-9-purinyl)phenyl] ether (**8**), respectively, in good yield [22,25,26].

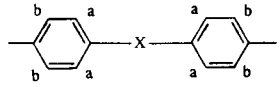
The structures of the new compounds were established on the basis of their elemental microanalyses and spectral data. For example, the ir spectra of the compounds **5** and **6** contain the characteristic NH stretching frequencies due to the presence of primary and secondary amino groups, 3350-3450 cm^{-1} and around 1650 cm^{-1}. The ir spectra of **7** and **8** are as expected, with the primary and secondary amino group frequencies in the 3350-3450 cm^{-1} range while there is no absorption at 1650 cm^{-1}.

Scheme 1



The ^1H nmr spectra of the new compounds measured in dimethylsulfoxide-d_6 (DMSO-d_6) show the presence of the expected protons, in agreement with the proposed structures. The primary and secondary amino group proton signals in **5** and **6**, appearing at 5.4 and 7.9 ppm, vanish in the spectra of the compounds **7** and **8** with a closed purine

ring system. Furthermore, a new signal at 9.1 ppm corresponding to -N=CH-N= is observed in the spectra of **7** and **8** in good agreement with the expected structures. A comparison of the positions of the signal of the H proton in the aromatic tethers linking the two heterocyclic units indicates a downfield shift in **6** and **8** with respect to **5** and



X= CH_2 , O

7, caused by higher electronegativity of the oxygen atom.

The uv spectrum of 5-amino-4,6-dichloropyrimidine (**2**) is that of a multiply substituted pyrimidine system [27]. The amino group, acting as an auxochrome, shifts the maxima of the unsubstituted pyrimidine at 210 and 240 nm towards longer wavelengths 260 and 320 nm, respectively. The uv spectra of the compounds **5-8** contain a maximum at 240 nm due to the presence of benzene rings. An additional maximum at 265 nm [28] can be found in the uv spectra of 6-chloropurines.

Detailed nmr, uv and ir spectra are presented in the Experimental.

EXPERIMENTAL

Melting points were determined in capillary tubes heated in a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H nmr spectra were obtained on Varian EM-360 (60 MHz) and IBM NR 200 AF (200 MHz) spectrometers using tetramethylsilane as the internal standard. The chemical shifts are expressed as δ values (ppm). The uv spectra were measured on a Perkin-Elmer Lambda 4C spectrophotometer, with a Perkin-Elmer 7700 professional computer. The ir spectra were recorded in pressed potassium bromide disks on a Perkin-Elmer 580B spectrophotometer with a Perkin-Elmer data station (intensity of absorption: s = strong, m = medium, w = weak). The purity of all the compounds was checked by thin-layer chromatography on silica gel 60 F-254 precoated plates and the spots were located in the uv light or by iodine vapor. Elemental microanalyses were performed by Desert Analytics, Tucson, AZ. All solvents used were reagent grade, except for the dimethyl sulfoxide used in spectroscopic measurements (spectrophotometric grade).

5-Amino-4,6-dichloropyrimidine (**2**) [29].

4,6-Dichloro-5-nitropyrimidine (5 g, 25.8 mmoles) was dissolved in 99% ethanol (500 ml) and Raney nickel (50% slurry in ethanol, 2 g) was added. The mixture was shaken for 12 hours at room temperature under hydrogen gas (30 psi). Then the ethanolic solution was filtered to remove the catalyst and the filtrate was evaporated under reduced pressure. The product, **2** (3.2 g, 76%) was obtained from the yellow residue by recrystallization from cyclohexane, mp 142-144° (lit mp 143-144° [29-31]).

Bis-[4-(*N*-5-amino-4-chloro-6-pyrimidyl)aminophenyl]methane (**5**).

A mixture of **2** (1.0 g, 6.1 mmoles), 4,4'-diaminodiphenylmethane (0.61 g, 3.1 mmoles), and triethylamine (0.73 g, 7.2 mmoles) in 1-butanol (30 ml) was heated to 85-90° for 60 hours under nitrogen. The reaction mixture was evaporated under reduced pressure and the oily residue was treated with cold water. The resulting dark brown solid was collected by filtration and recrystallized from dimethylformamide and water (1:3). The product was dried under reduced pressure to give **5** (0.3 g, 21%), mp 262-264° dec; ^{1}H nmr (DMSO-d_6): δ 3.90 (s, 2H, CH_2), 5.40 (s, 4H, Het-NH_2), 7.25 (d, 4H, J = 7.8 Hz, C_6H_4-H_a), 7.60 (d, 4H, J = 7.8 Hz, C_6H_4-H_b), 7.90 (s, 2H, -NH-), 8.55 ppm (s, 2H, Het-H); ir (potassium bromide): ν 3440 (s, -NH), 3350 (s, NH_2-), 1650 (s, NH_2-), 1600 (s), 1550 (s), 1500 (s), 1460 (s), 1395 (m), 1290 (s), 1160 (m), 990 cm^{-1} (m); uv (DMSO): λ max (log ε) 327 (3.55), 282 (3.35), 244 nm (3.24).

Anal. Calcd. for $C_{21}H_{18}Cl_2N_8$: C, 55.59; H, 3.97; N, 24.71. Found: C, 55.35; H, 3.94; N, 24.45.

Bis-[4-(*N*-5-amino-4-chloro-6-pyrimidyl)aminophenyl] Ether (**6**).

A mixture of 4,6-dichloro-5-aminopyrimidine (**2**) (1.0 g, 6.1 mmoles), bis-(4-aminophenyl) ether (0.62 g, 3.1 mmoles), and triethylamine (0.73 g, 7.2 mmoles) in 1-butanol (30 ml) was heated to 80-90° for 40 hours under nitrogen. The reaction mixture was evaporated under reduced pressure and the oily residue was triturated with cold water. The beige solid was collected, filtered off, and recrystallized from dimethylformamide and water (1:2.5). The product was dried under reduced pressure to give **6** (0.25 g, 18%), mp 230-231° dec; ^{1}H nmr (DMSO-d_6): δ 5.40 (s, 4H, -NH_2), 7.10 (d, 4H, J = 8.8 Hz, C_6H_4-H_a), 7.70 (d, 4H, J = 8.8 Hz, C_6H_4-H_b), 7.90 (s, 2H, -NH-), 8.60 ppm (s, 2H, Het-H); ir (potassium bromide): ν 3430 (m, -NH-), 3370 (s, NH_2-), 1650 (m, NH_2-), 1600 (s), 1560 (s), 1510 (s), 1460 (s), 1420 (m), 1230 (m), 1110 (w), 940 cm^{-1} (w); uv (DMSO): λ max (log ε) 327 (3.56), 281 (3.44), 244 nm (3.35).

Anal. Calcd. $C_{20}H_{16}Cl_2N_8O$: C, 52.75; H, 3.52; N, 24.62. Found: C, 52.45 (average from two determinations); H, 3.81; N, 24.62.

Bis-[4-(6-chloro-9-purinyl)phenyl]methane (**7**).

Freshly distilled triethyl orthoformate (1.34 g, 9.0 mmoles) was added to a solution of **5** (0.2 g, 0.44 mmole) in dimethylacetamide (1.5 ml). Concentrated hydrochloric acid (0.1 ml) was added to the solution and the resulting mixture was stirred at room temperature for 20 minutes. The beige solid was collected by filtration and recrystallized from dimethylformamide and water (1:3). The product was dried under reduced pressure to give **7** (0.18 g, 85%), white needles, mp 284-285° dec; ^{1}H nmr (DMSO-d_6): δ 4.15 (s, 2H, CH_2), 7.60 (d, 4H, J = 7.8 Hz, C_6H_4-H_a), 7.85 (d, 4H, J = 7.8 Hz, C_6H_4-H_b), 8.50 (s, 2H, Het-H), 9.10 ppm (s, 2H, -N=CH-N=); ir (potassium bromide): ν 3200-2900 (m, C-H), 1620 (s), 1610 (s), 1600 (s), 1560 (s), 1440 (m), 1420 (m), 1400 (m), 1290 (s), 1250 (s), 1100 (m), 990 (s), 820 cm^{-1} (s); uv (DMSO): λ max (log ε) 265 (3.36), 232 nm (sh) (3.16).

Anal. Calcd. for $C_{23}H_{14}Cl_2N_8$: C, 58.36; H, 2.98; N, 23.67; Cl, 14.98. Found: C, 58.46; H, 2.79; N, 23.79; Cl, 14.71.

Bis-[4-(6-chloro-9-purinyl)phenyl] Ether (**8**).

Freshly distilled triethyl orthoformate (1.34 g, 9.0 mmoles) was added to a solution of **6** (0.2 g, 0.44 mmole) in dimethylacetamide (1.5 ml). Concentrated hydrochloric acid (0.1 ml) was added to the solution and the resulting mixture was stirred at room temperature for 20 minutes. The beige solid was filtered off and recrystallized from dimethylformamide and water (1:4). The solid was dried under reduced pressure to give **8** (0.16 g, 77%), mp 300-301° dec; ^{1}H nmr (DMSO-d_6): δ 7.45 (d, 4H, J = 8.8 Hz, C_6H_4-H_a), 8.05 (d, 4H, J = 8.8 Hz, C_6H_4-H_b), 8.90 (s, 2H, Het-H), 9.15 ppm (s, 2H, -N=CH-N=); ir (potassium bromide): ν

3200-3150 (w, -CH), 1720 (w), 1630 (s), 1600 (s), 1550 (s), 1450 (m), 1380 (s), 1340 (m), 1300 (s), 1250 (s), 1070 (m), 980 (s), 880 cm^{-1} (m); uv (DMSO): λ max (log ε) 265 (3.49), 231 nm (sh) (3.25).

Anal. Calcd. for $C_{22}H_{12}Cl_2N_8O$: C, 55.58; H, 2.53; N, 23.58; Cl, 14.95. Found: C, 55.45; H, 2.45; N, 23.59; Cl, 14.30.

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[2] Present address: Department of Electrical Engineering, The University of Texas at El Paso, 500 W. University Avenue, El Paso, TX 79968.

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