Synthesis of Bis-2,7-substituted 1,2,3,6,7,8-Hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraones

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A series of bis-2,7-substituted 1,2,3,6,7,8-hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraones (naphthalene diimides) has been synthesized. The key intermediate required for synthesis of the naphthalene diimides, 2,3,6,7-naphthalnetetracarboxylic dianhydride (**2**) was prepared starting from allene and maleic anhydride. The bis-anhydride **2** was converted to the naphthalene diimides using conventional methodology by the reaction with amines in organic solvents.

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Despite almost three decades of investigation [1-3] the relationship between structure of intercalators and their binding mode to nucleic acids continues to be intensely studied [4-10]. Structural features which control whether a molecule will be a groove binder or an intercalator are not yet fully understood [11,12]. Recently, it was demonstrated that small structural changes can result in binding mode changes from groove binder to classical intercalator to threading intercalator [13]. Factors which determine whether a planar aromatic molecule with two cationic side chains will bind by classical intercalation (both side chains extending into the same groove) or by a threading intercalation mode (one side chain extending into each groove) remain unclear [11] (see illustration in Figure 1). The threading interaction seems kinetically disfavored since one side chain must slide through the DNA-helix. Apparently the threading mode results when greater stacking interactions can occur between the intercalator and the base pairs than is achievable by other orientations. Threading intercalators have been shown to dissociate more slowly from a ligand-DNA complex than classical intercalators [7,13] and this slow off rate has been suggested to be important in determining the biological activity of such intercalators [14].

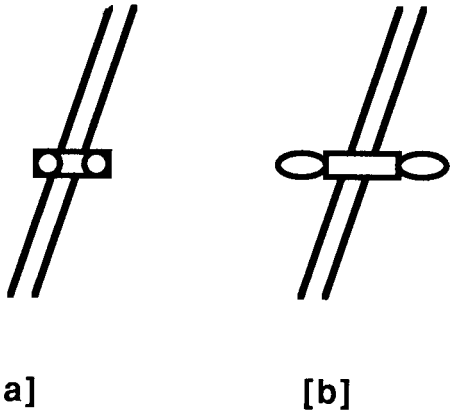
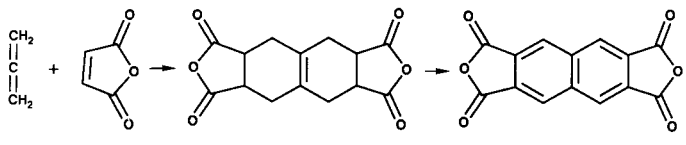


Figure 1. Depiction of [a] classical intercalation, both side chains (ovals) in major groove and [b] threading intercalation, one side chain (oval) in each groove; rectangle represents aromatic rings of an intercalator stacked between base pairs.

The first compounds reported to bind to DNA by threading were the *N,N'*-bis-substituted-1,4,5,8-naphthalenetetracarboxdiimides [15]. The intercalation of these molecules by threading demonstrated that the thermally induced breathing motions of DNA create large openings in the double helix even at relatively low temperatures. As part of an effort to further the understanding of factors controlling threading intercalation, we have prepared a series of 2,7-bis-substituted-1,2,3,6,7,8-hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraones (naphthalene diimides).

The synthetic approach employed to prepare these naphthalene diimides is outlined in Scheme 1. The initial step in this synthetic scheme involves the mechanistically involved, but synthetically straightforward (one reaction vessel) 2:2 dimerization of allene in the presence of maleic anhydride to form 1,2,3,4,5,6,7,8-octahydronaphthalene-2,3,6,7-tetracarboxylic dianhydride (**1**) [16]. The reaction of **1** with bromine in 1,2,4-trichlorobenzene readily results in aromatization to form the key bis-anhydride **2** [17]. Typically the *N,N'*-substituted diimides **3** were prepared by reaction of the appropriate diamine with the bis-anhydride **2** in refluxing ethanol [18]. In addition to compounds with simple *N,N*-dialkylamino cationic centers distal from the ring, we have also prepared an uncharged analog **3a** and analogs with both charged **3d** and uncharged **3e** bulky side chains. It will be interesting to determine

Scheme 1



1 2

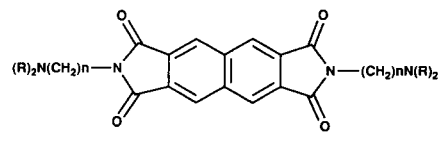


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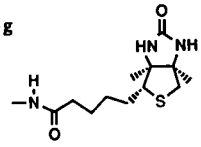
Table 1. Physical Data for Bis-2,7-substituted 1,2,3,6,7,8-hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraones

Compound No. [a]	R	Yield (%)	Analysis Mp (°C)	Molecular Formula	Calcd. (Found) C	H	N	^{1}H NMR Chemical Shifts (ppm)
3a	$-(CH_2)_2CH_3$	86	>320	$C_{20}H_{18}N_2O_4$	68.57 (68.46)	5.14 (5.21)	8.00 (8.06)	8.6 (4H, s), 3.9 (4H, t), 2.0 (4H, m), 1.05 (6H, t) [b]
3b	$-(CH_2)_2N(CH_3)_2$ [c]	52	323-325	$C_{22}H_{26}N_4O_4Cl_2 \cdot 0.5H_2O$	53.89 (53.81)	5.51 (5.45)	11.43 (11.31)	8.4 (4H, s), 4.15 (4H, t), 3.6 (4H, t), 3.05 (12H, s) [d]
3c	$-(CH_2)_2N(iPr)_2$ [c]	77	303-305	$C_{30}H_{42}N_4O_4Cl_2$	60.72 (60.44)	7.08 (7.10)	9.44 (9.34)	8.5 (4H, s), 4.75 (4H, t), 2.8 (6H, m), 0.9 (12H, d) [e]
3d	$-(CH_2)_2N^+(CH_3)_3$	73	>320	$C_{24}H_{30}N_4O_4I_2$	41.62 (41.47)	4.33 (4.37)	8.09 (8.04)	8.75 (4H, s), 4.1 (4H, t), 3.6 (4H, t), 3.1 (18H, s) [g]
3e	$-(CH_2)_3N(CH_3)_2$ [c]	28	310-312	$C_{24}H_{30}N_4O_4Cl_2$	56.59 (56.34)	5.89 (6.01)	11.00 (10.88)	8.1 (4H, s), 3.9 (4H, t), 3.4 (4H, t), 3.1 (12H, s), 2.3 (4H, m) [d]
3f	$-(CH_2)_3$NHAd [h]	22	290-295	$C_{40}H_{48}N_4O_4 \cdot 0.5H_2O$	73.01 (72.94)	7.46 (7.35)	8.52 (8.61)	8.8 (4H, s), 4.2 (4H, t), 3.1 (4H, t), 1.9-2.5 (36H, m) [b]
3g		49	>320	$C_{34}H_{36}N_8O_8S_2 \cdot 0.5H_2O$	53.89 (53.64)	4.89 (4.78)	14.79 (14.79)	10.5 (2, s), 8.9 (4H, s), 6.1 (4H, s), 4.3 (2H, m), 4.2 (2H, m), 3.15 (2H, m), 2.85 (4H, m), 2.4 (4H, t), 1.4-1.6 (12H, m) [g]

[a] Compound **3a** recrystallized from acetic acid; **3b**, **3e**, **3f** from ethanol; **3c** from methanol; **3d** from water; **3g** precipitated from the reaction solvent (N,N-dimethylformamide) and was washed with diethyl ether. [b] NMR solvent chloroform-d. [c] Dihydrochloride salt. [d] NMR solvent deuterium oxide. [e] NMR data obtained for free base from chloroform-d. [f] Diiodide. [g] NMR solvent dimethyl sulfoxide-d_6. [h] Ad=2-Adamantyl; MS, molecular ion=648.4.

if there is a significant difference in off-rate for these latter two compounds. Table 1 contains data for all the diimides, **3**, prepared for binding studies.

EXPERIMENTAL

Melting points were recorded on a Mel-Temp and/or a Thomas-Hoover Unimelt apparatus and all are uncorrected. Elemental analyses were performed by Atlantic Microlab, Atlanta, Georgia. The nmr spectra were recorded in deuteriochloroform or dimethyl sulfoxide-d_6 or deuterium oxide with a Varian VXR 400 or EM360L spectrophotometer.

2,3,6,7-Naphthalenetetracarboxylic Acid Dianhydride (**2**).

A mixture of allene (5 g, 0.125 mole), maleic anhydride (12.25 g, 0.125 mole) in 60 ml of benzene containing a trace amount of hydroquinone (0.02 g, 0.2 mmole) was heated in a Parr high pressure apparatus at ca. 175° for 40 hours essentially according to the published procedure [16]. After allowing the reaction mixture to cool to room temperature, the resulting solid, 1,2,3,4,5,6,7,8-octahydronaphthalene-2,3,6,7-tetracarboxylic acid anhydride (**1**), was collected by filtration [1.8 g, 6% yield, mp 239-241 (lit 240-242°) after recrystallization from ethyl acetate]. Compound **1** was aromatized as previously described [17] by first heating a solution of 0.55 g (0.002 mole) of **1** in 4 ml of 1,2,4-trichlorobenzene containing 0.02 ml of thionyl chloride at reflux for 0.5 hours, followed by dropwise addition of bromine (1.32 g, 0.008 mole), to the stirred suspension over a 7 hours period. During the addition of bromine the temperature of the mixture was held just below the boiling point of 1,2,4-dichlorobenzene (*ca.* 210°). The temperature of the mixture was maintained near 210° for an additional 3 hours after completion of bromine addition. After allowing the mixture to cool to room temperature the crude 2,3,6,7-naphthalenetetracarboxylic acid dianhydride **2** was collected (0.4 g, 75%). Purification of **2** was achieved as previously described [17] by conversion to tetramethyl 2,3,6,7-naphthalenetetracarboxylate, followed by hydrolysis to the corresponding tetracarboxylic acid and finally reconversion to the bis-anhydride **2** [mp $<350°$].

2,7-Dipropyl-1,2,3,6,7,8-hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraone (**3a**).

A solution of 0.27 g (0.001 mole) of the bisanhydride **2**, 1.5 ml of *n*-propyl amine and 3 ml of acetic acid was heated at reflux for 2 hours. The mixture was allowed to cool to room temperature, it was filtered and the resulting solid was recrystallized from acetic acid; yield 0.3 g (86%), mp $>320°$.

2,7-Bis[2-dimethylaminoethyl]-1,2,3,6,7,8-hexahydroisoindolo-[5,6-*f*]isoindole-1,3,6,8-tetraone Dichloride (**3b**).

A mixture of 1.1 g (0.004 mole) of bisanhydride **2**, 1.2 g (0.012 mole) of dimethylaminoethylamine and 15 ml of ethanol was allowed to reflux for 15 minutes. The reaction mixture was allowed to cool and the solid which formed was collected by filtration. The solid was treated with ethanolic hydrogen chloride and the salt was recrystallized from ethanol to yield 1.0 g (52%) mp $>350°$. Compound **3c** was prepared following essentially the same procedure.

2,7-Bis[2-trimethylammonium]ethyl-1,2,3,6,7,8-hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraone **3d**.

A solution of 0.2 g (0.5 mmole) of the free base of **3b**, 5 ml of methyl iodide and 15 ml of acetone was heated and refluxed for 15 hours. The mixture was allowed to cool and the solid which formed was filtered, washed with 2 x 5 ml of cold acetone and

recrystallized from water. The yield of **3d** was 0.25 g (73%), mp >320°.

2,7-Bis[3-(2-adamantylamino)propyl-1,2,3,6,7,8-hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraone (**3f**).

A mixture of 0.54 g (1.8 mmoles) of **2**, 0.95 g (4.8 mmoles) of *N*-(2-adamantyl)-1,3-diaminopropane (prepared by reductive amination [19] of 2-adamantanone with 1,3-diaminopropane) in 15ml of *N,N*-dimethylformamide was refluxed for 0.5 hour. After allowing the reaction mixture to cool to room temperature, the product was filtered and recrystallized from ethanol. The yield was 0.26 g (22%) of solid **3f**, mp 290-295°.

2,7-Bis(hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanamido)-1,2,3,6,7,8-hexahydroisoindolo[5,6-*f*]isoindole-1,3,6,8-tetraones (**3g**).

A mixture of 0.1 g (0.37 mmole) of **2**, 0.2 g (1.1 mmoles) of biotin hydrazide (Sigma) in 3 ml of anhydrous *N,N*-dimethylformamide was heated at reflux for 0.5 hour. The reaction mixture was allowed to cool to room temperature and the solid which formed was filtered, washed with diethyl ether and dried over phosphorus pentoxide *in vacuo*, yield 0.12 g (49%), mp >350°.

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