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[E0003]

Studies of Naphthalene Diimides as DNA-binding Agents

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Abstract

In this work we have focused on the synthesis of new derivatives of naphthalene diimides that bind to DNA by a threading intercalation mode. The advantage of having a threading intercalator as a "carrier" part of the drug is that it brings additional recognition pattern into the system by directing substituents into the opposite grooves of DNA. This allows the possibility of selectively varying substituents to develop medicinal agents as well as probes for large-amplitude DNA dynamics. A systematic set of naphthalene diimides, with side chains of increasing size, have been synthesized. Their physical properties as well as DNA interaction kinetics were studied to probe the factors which determine the rates of association and dissociation of DNA binding. We have used intercalators with large and rigid substituents to probe the opening of an intercalation site with disruption of an adjacent base pair.

Keywords: Intercalator, nucleic acid, binding agents.

Introduction

I. Chemistry of Naphthalene Diimides

1. Syntheses of NDI   2. Capillary Electrophoresis of NDI   3. NMR Studies of Self-stacking of NDI

II. Binding Studies of Naphthalene Diimides

1. Kinetic Measurements   2. Viscometric Titrations of CT DNA   3. Circular Dichroism Studies

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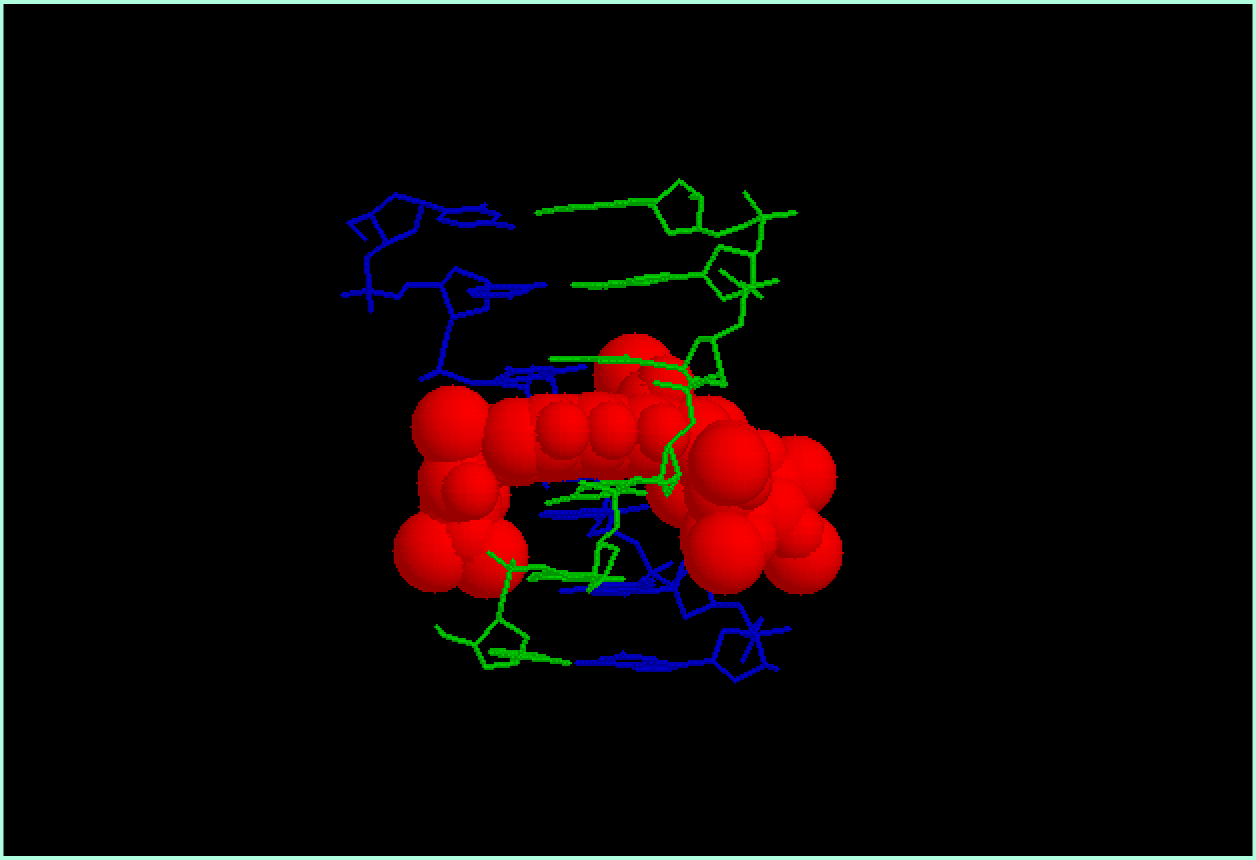
Introduction

The goal of our work is to probe how the size and shape of the side chain of a binding agent influences nucleic acid-drug interactions. We have focused on the synthesis of new derivatives of naphthalene diimides that bind to duplex DNA by a threading intercalation mode. In this mode, the drug is inserted into DNA such that the two side chains are forced to lie in the opposite grooves of DNA



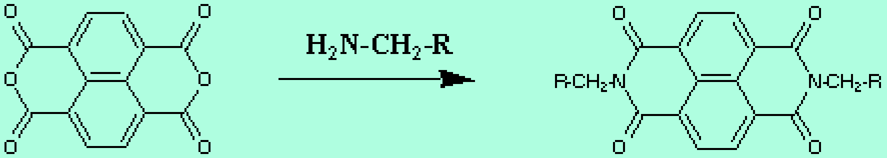
The advantage of having a threading intercalator as a 'carrier' in design of a drug is that it brings additional recognition patterns into the system by directing substituents into the opposite grooves of DNA. This allows the possibility of selectively varying substituents to develop medicinal agents as well as probes for large-amplitude DNA dynamics. We have used intercalators with large and rigid substituents to probe the opening of an intercalation site with disruption of an adjacent base pair. Our specific goals are:

- to determine whether large, rigid groups can thread through DNA
- to determine how the shape and net charge of the side chains affect the threading intercalation
- to develop analytical techniques which establish the purity of these large, cationic molecules

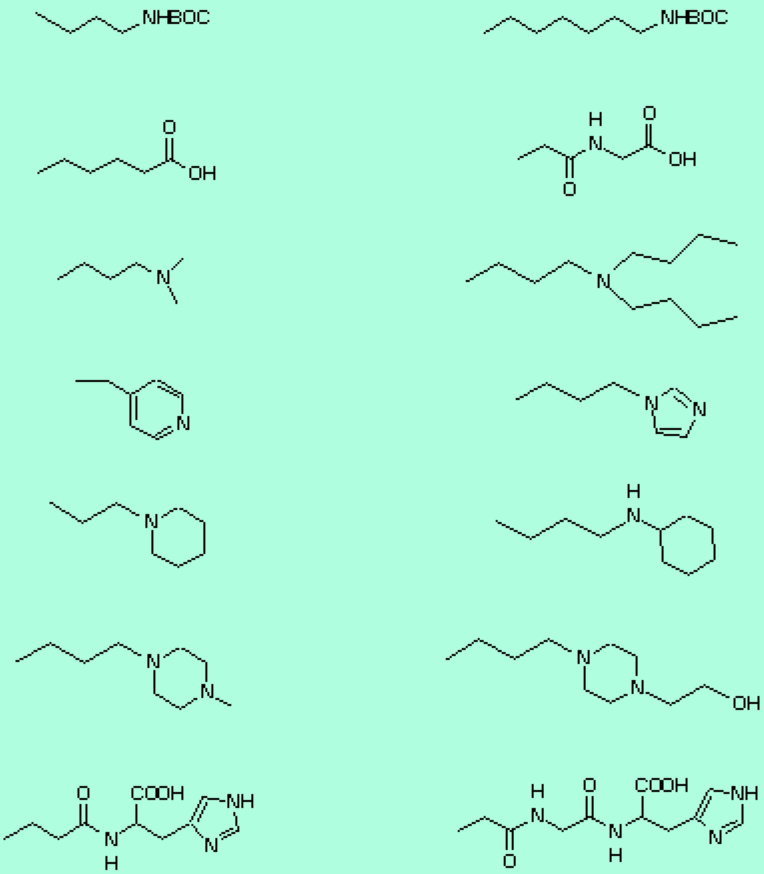
II. Chemistry of Naphthalene Diimides

1. Syntheses

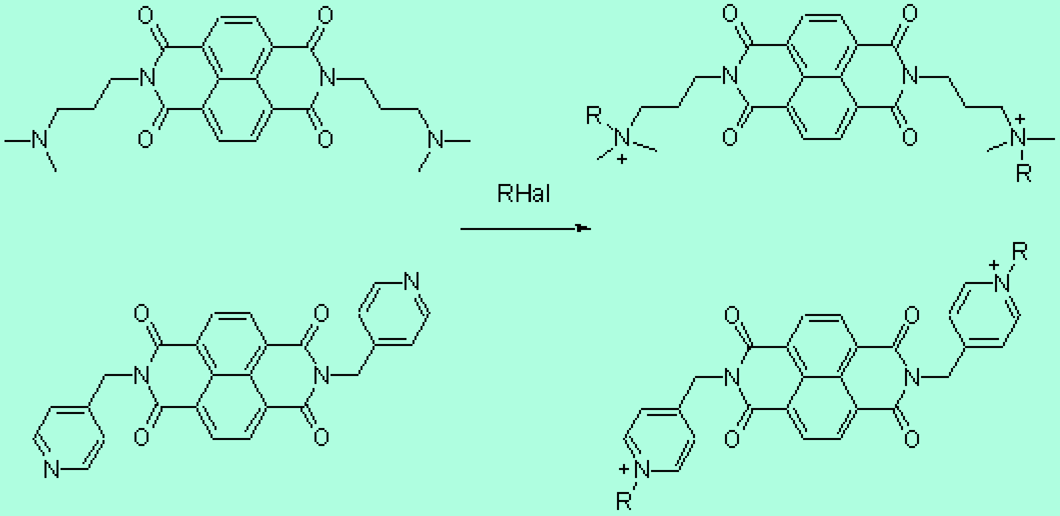
Naphthalene diimides are readily synthesized via condensation of the appropriate amine with the commercially available 1,4,5,8-naphthalenetetracarboxylic dianhydride using toluene or DMF as the solvent.



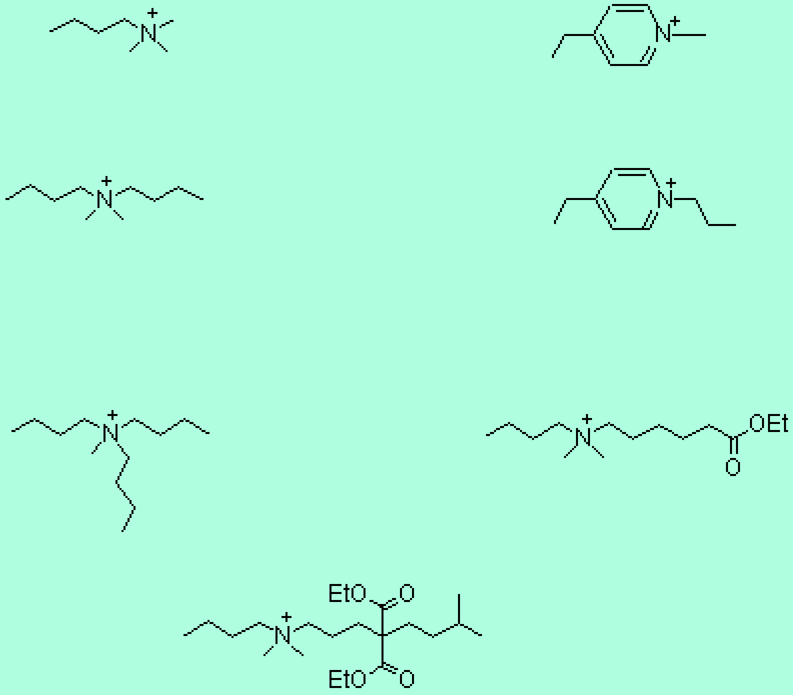
SIDE CHAIN



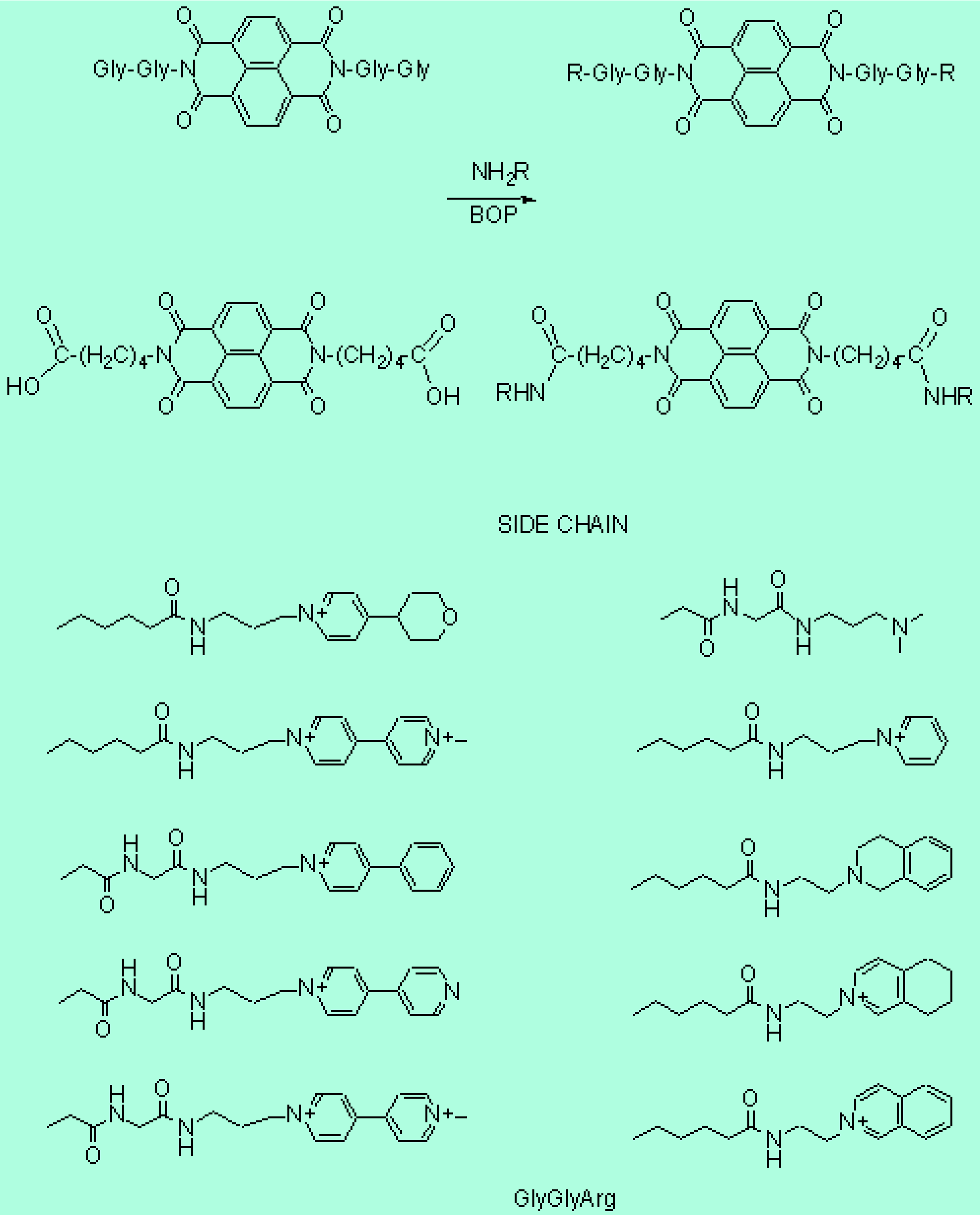
The quaternary amines were prepared by alkylation of the appropriate tertiary amine.



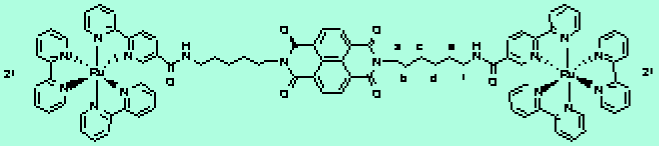
SIDE CHAIN



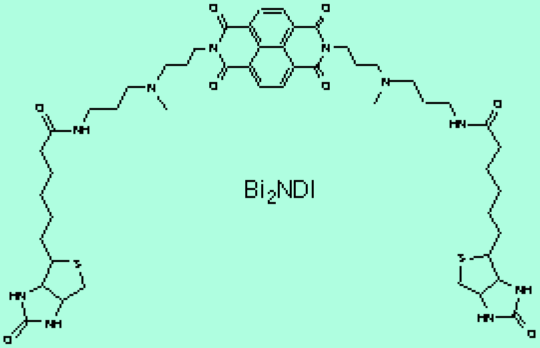
The resulting amine was deprotected if necessary and then condensed with the appropriate acid using benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as a reagent.



Condensation of the appropriate amine with biotin led to Bi$_2$NDI; condensation with 2,2'-bipyridine-5-carboxylic acid led to the bipyridyl derivative which was treated with Ru(bpy)$_2$ to give Ru$_2$NDI



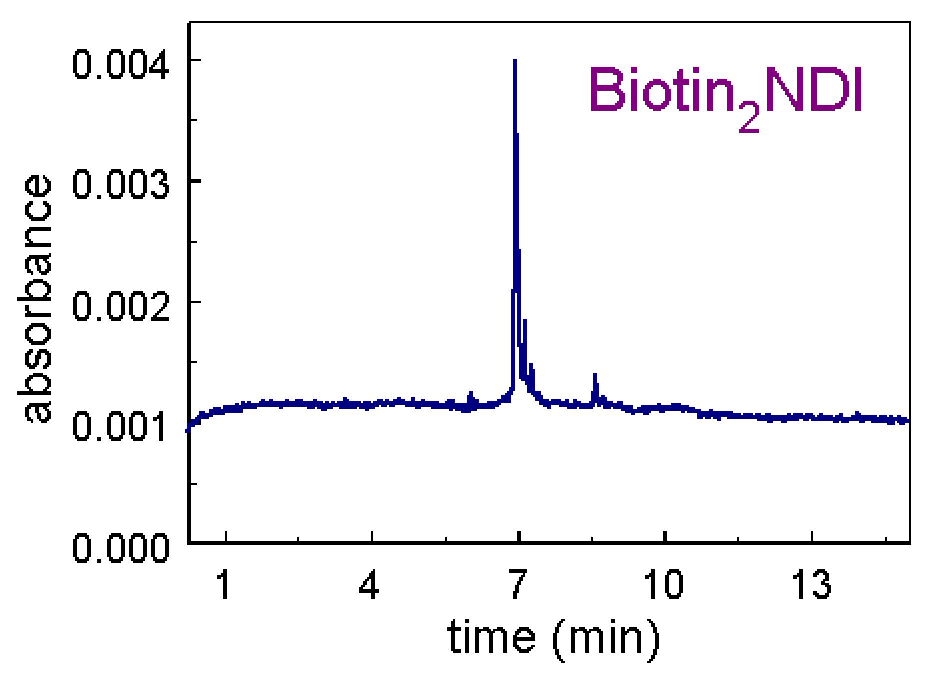
Ru$_2$NDI



Bi$_2$NDI

2. Capillary Electrophoresis

Capillary zone electrophoresis experiments were performed using a Beckman PACE 5510 instrument equipped with a fused-silica capillary (57 cm x 75 mm i.d.) and P/ACE diode array detector. Electrophoresis was performed at voltage of 13 kV. The sample was introduced into the capillary by high pressure injection for 5 s. A solution of 50 mM sodium phosphate, pH 2.06 was used as the buffer electrolyte.



3. NMR Studies of Self-stacking

a. Introduction

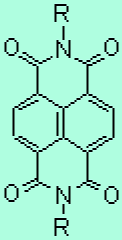
b. Temperature Dependence

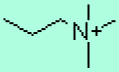
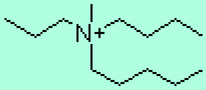
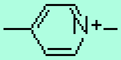
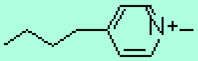
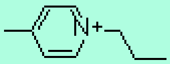
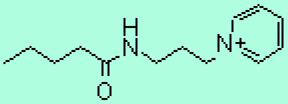
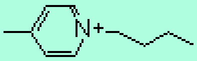
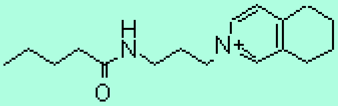
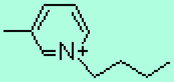
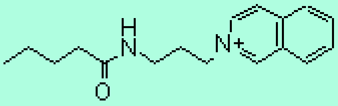
c. 2D-Data

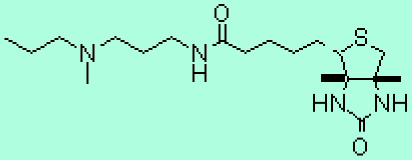
d. UV-VIS Studies

a. Introduction

The widespread use of the naphthalene diimide ring system in a variety of settings leads to an interest in the fundamental physical characteristics of this ring system such as self-stacking. Comparison of a large number of naphthalene diimides shows that the tendency to self-stack is influenced in large part by the placement of the positive charge on the side chain; derivatives with positive charges at greater distances from the NDI ring show a greater tendency to self-stack. This is presumably because electrostatic repulsion between the two cationic centers is reduced in the stacked geometry when the positively charged-groups are further from the NDI ring.

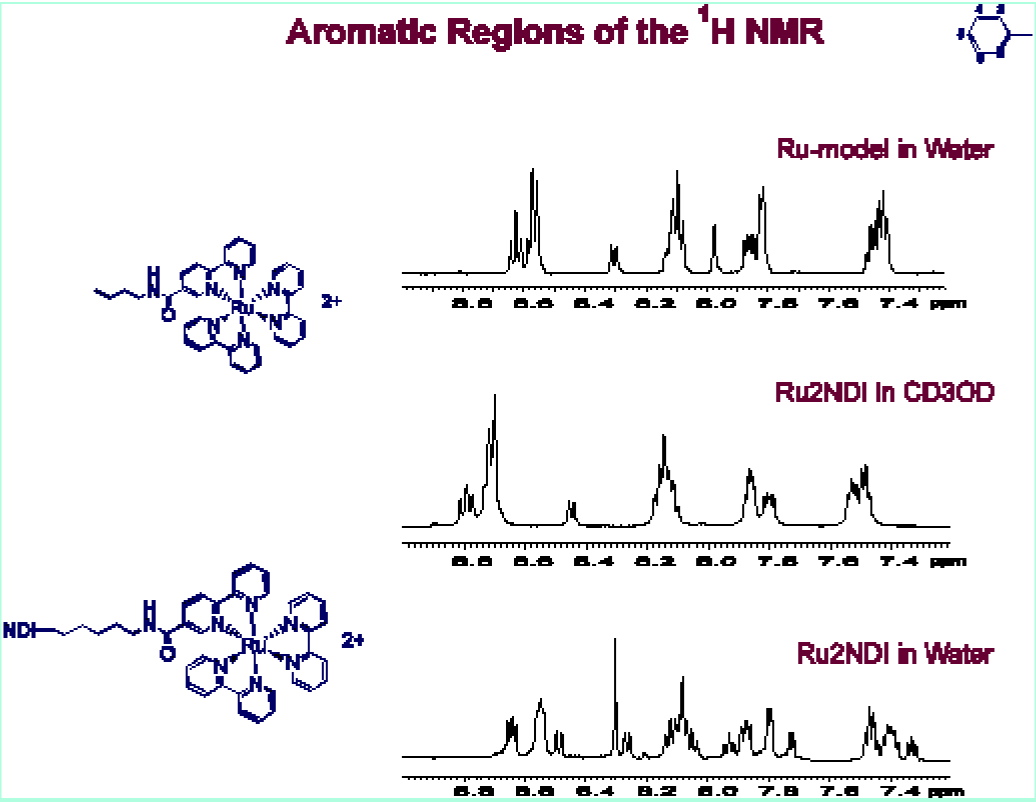


R	Chemical Shift	R	Chemical Shift
	8.70 (40 ^{o}C)		8.69 (50 ^{o}C)
	8.81 (30 ^{o}C)		8.26 (22 ^{o}C)
	8.76 (80 ^{o}C)		8.42 (25 ^{o}C)
	8.82 (60 ^{o}C)		8.20 (30 ^{o}C)
	8.80 (60 ^{o}C)		8.40 (30 ^{o}C)



8.75 (30 ^{o}C)

The ring current effect of the NDI can have a significant effect on the chemical shifts of specific side chains. This is seen most clearly in the derivative bearing side chains ending in Ru(bpy)$_3$ moieties.



Aromatic Regions of the ^{1}H NMR

Ru-model in Water

Ru2NDI in CD3OD

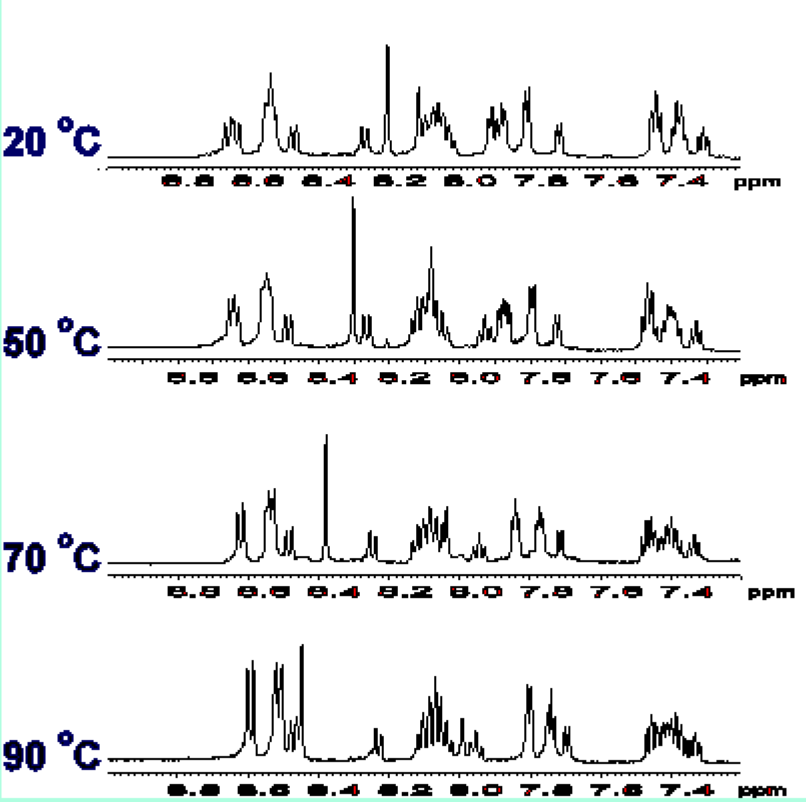
Ru2NDI in Water

In methanol, the shift patterns of the aromatic resonances of the bipyridine rings are very close to those of a model compound. However, a more complicated chemical shift pattern is seen in water. This indicates that the Ru(bpy)$_3$ moiety is constrained to lie, at least in part, in the shielding cone over the aromatic naphthalene diimide ring system. This results in each of the bipyridine protons experiencing a different magnetic environment, leading to a spread in the chemical shifts.

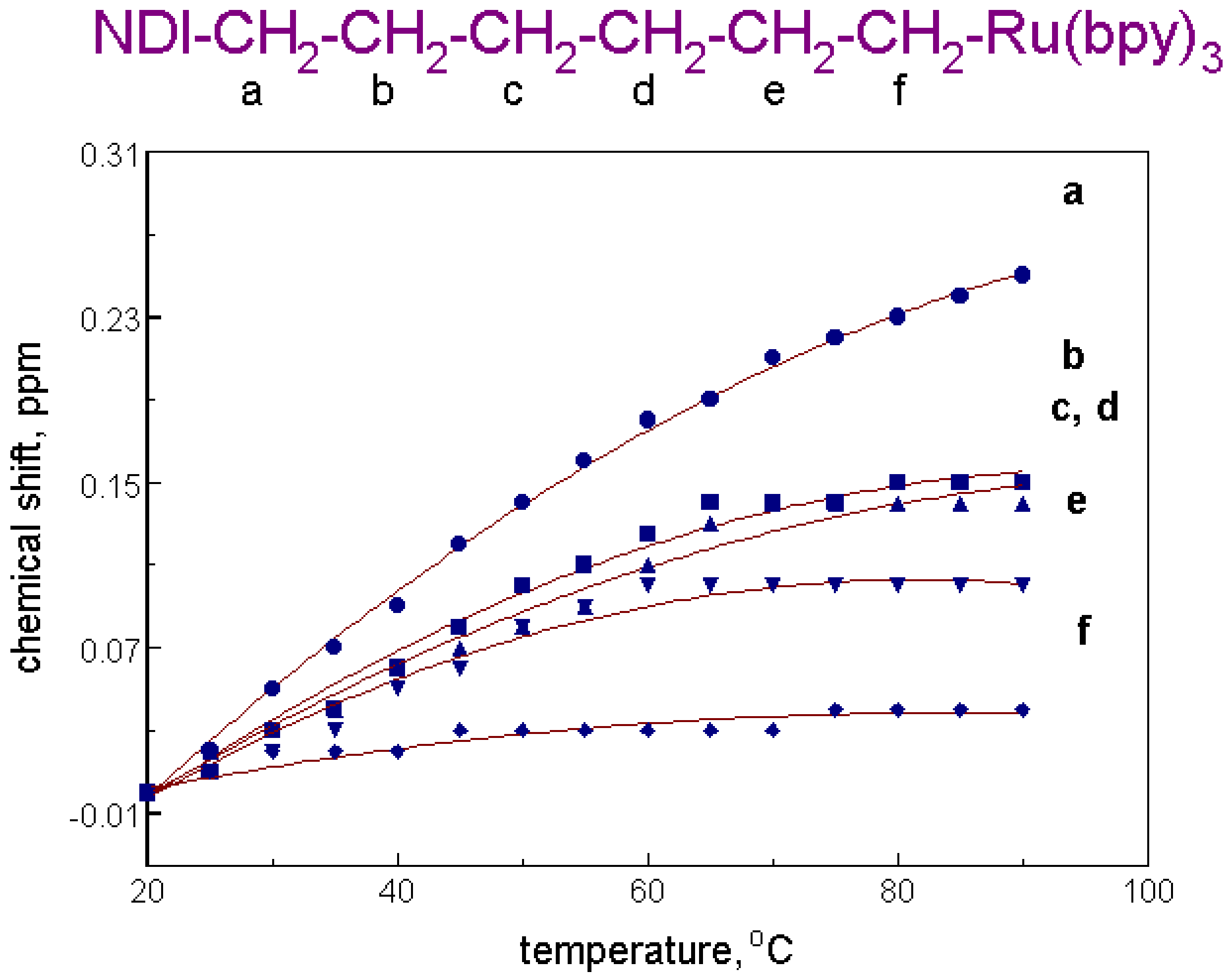
b. Temperature Dependence

The NDI resonance moves downfield as the temperature is increased (0.26 ppm from 20 to 90 ^{o}C) as would be expected for destacking of the aromatic rings with increasing temperature.

Aromatic Region of the ^1H NMR of Ru_2NDI as a Function of Temperature in D_2O



The chemical shifts of the aliphatic side chain resonances were also a function of temperature. The methylene group adjacent to the diimide ring is most sensitive to temperature; its chemical shift increases by 0.28 ppm as the temperature is raised from 20 to 90 ^{O}C. As one moves along the chain away from the diimide nucleus, the effect diminishes; the methylene group adjacent to the $Ru(bpy)_3$ group shows almost no change in chemical shift with increasing temperature.

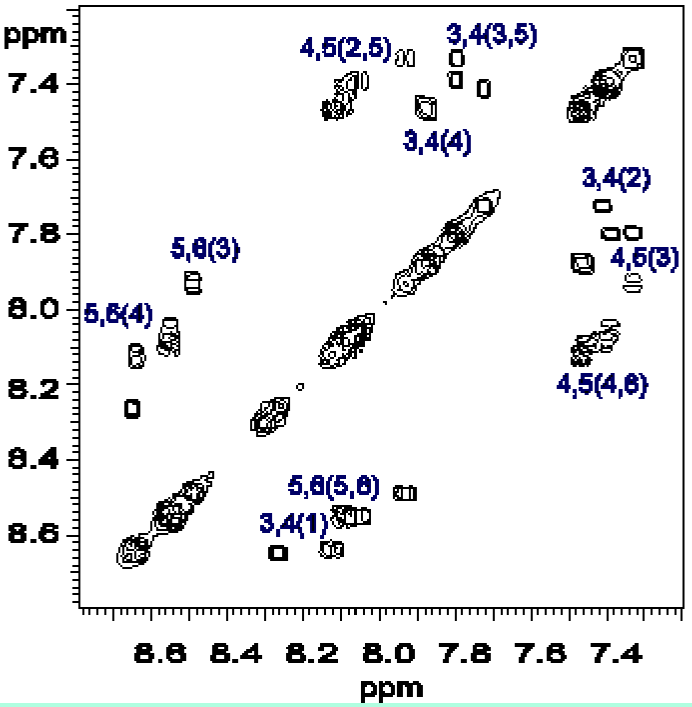


According to our studies, the extent of stacking does not correlate with the size of the side chain. Even the bisbiotin derivative showed no stacking in D_2O at NMR concentrations as indicated by the chemical shift of the NDI ring at 8.75 ppm (30 °C).

c. 2D-Data

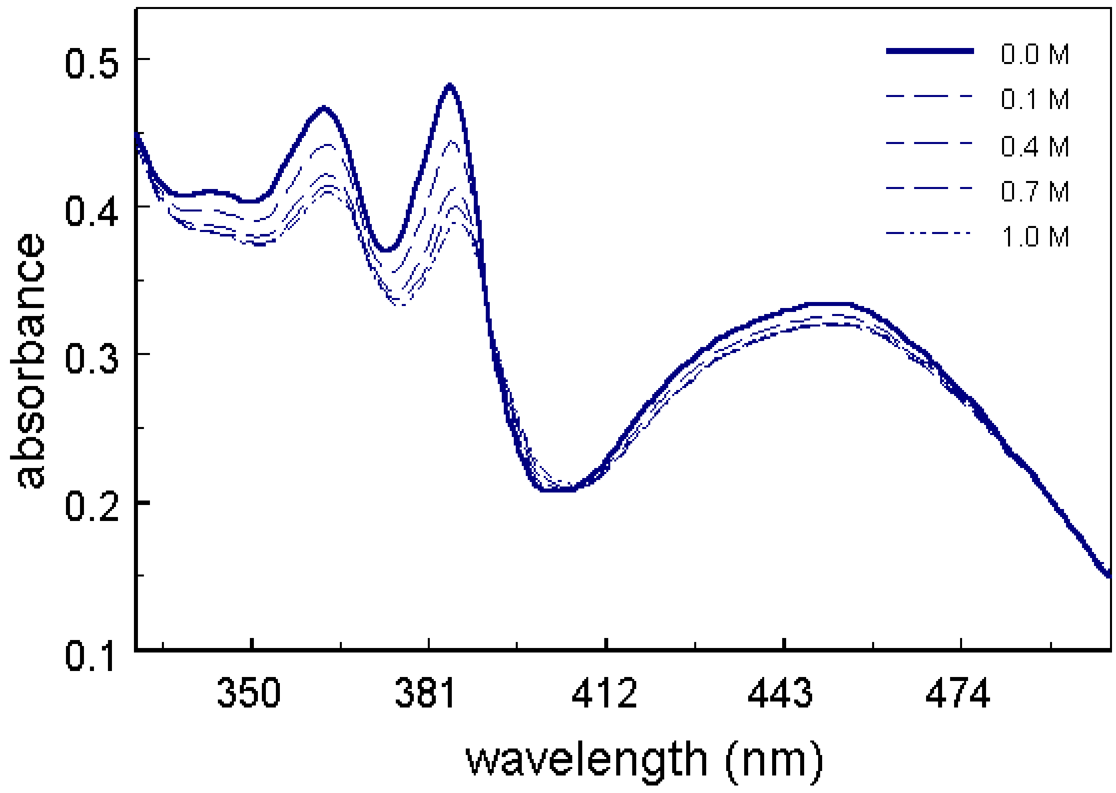
The assignments of the ring protons for Ru2NDI was made through the COSY data set.

Aromatic Region of the ^1H-COSY NMR of Ru_2NDI in D_2O at 50 °C



d. UV-VIS Studies

Increased ionic strength due to buffers and salts can induce certain aromatic molecules to stack. For Ru2NDI the intensities of the 383 (NDI) and 453 $[Ru(bpy)_3]$ nm bands decrease by 22 and 4 %, respectively, as the salt concentration is raised from 0 to 1 M NaCl. The decrease in the intensity of the NDI band as a function of [NaCl] indicates that the NDI participates in salt-induced stacking at optical concentrations.



II. Binding Studies of Naphthalene Diimides

1. Kinetic Measurements

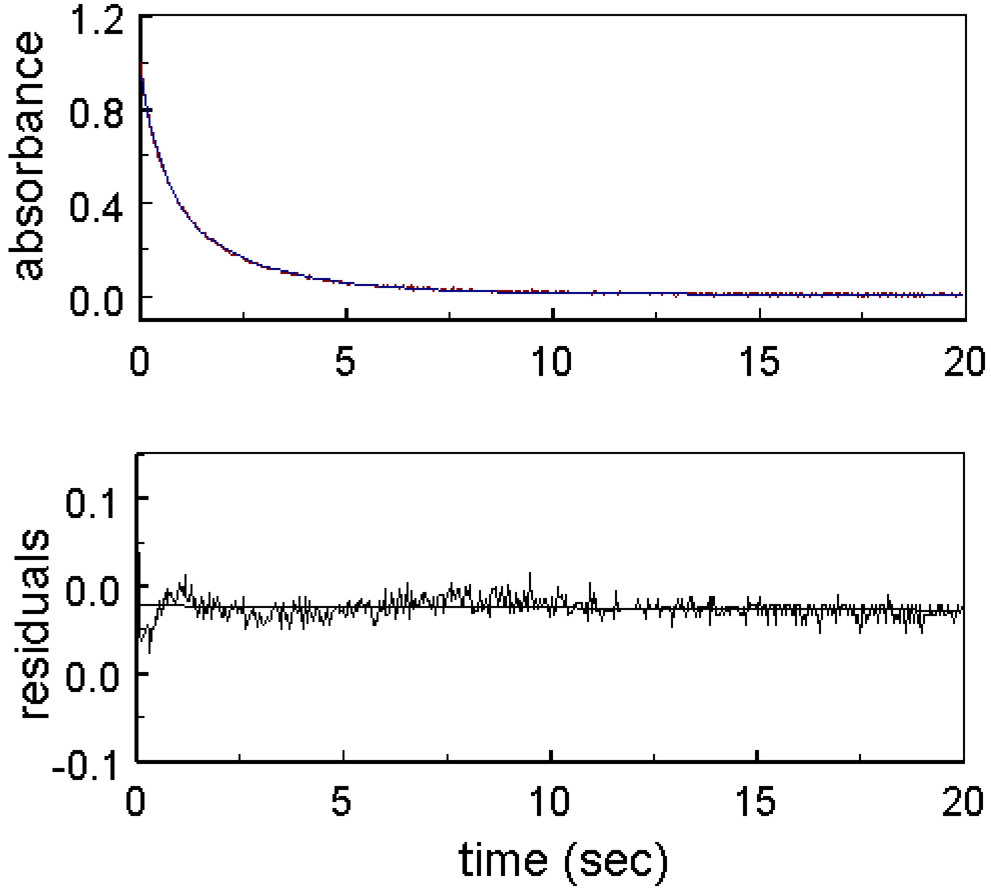
a. General

b. Dicationic NDI

c. Tetracationic

a. General

Absorbance of naphthalene diimides can be measured as a function of time after mixing with calf thymus DNA in a stopped flow instrument.



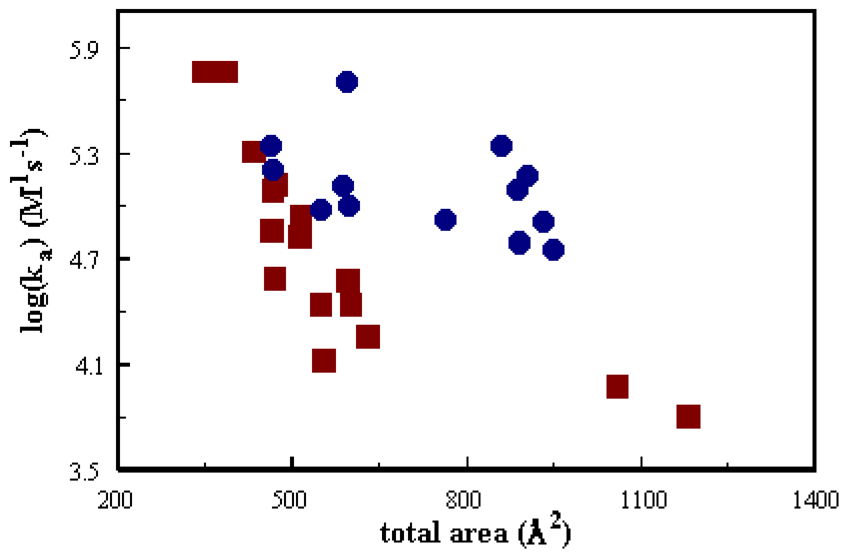
The smooth line in this plot is a fit of the data to two exponentials.

The association kinetics of NDIs with CT-DNA were measured as a function of DNA concentration with an approximately 10-fold molar exess of DNA base pairs over NDI. Dissociation kinetics of the NDIs from CT DNA were determined by rapid mixing of a NDI-DNA solution with a solution containing SDS (sodium dodecyl sulfate).

b. Dicationic NDI

Association and dissociation rate constants were plotted as a function of the surface area of the molecule (calculated using PC Model)

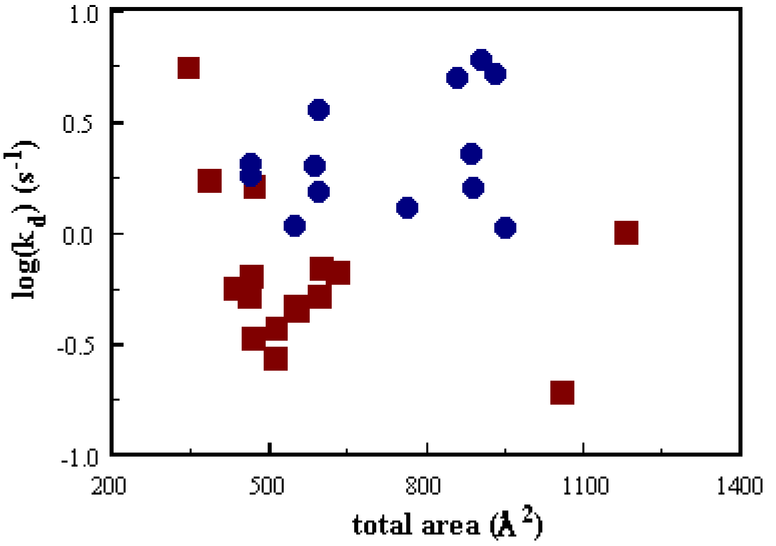
Association rates decrease as the size of the side chain increases for compounds with aliphatic side chains. Compounds with aromatic side chains show little dependence of association rates on the size of the side chain.



■ aliphatic side chain

● aromatic side chain

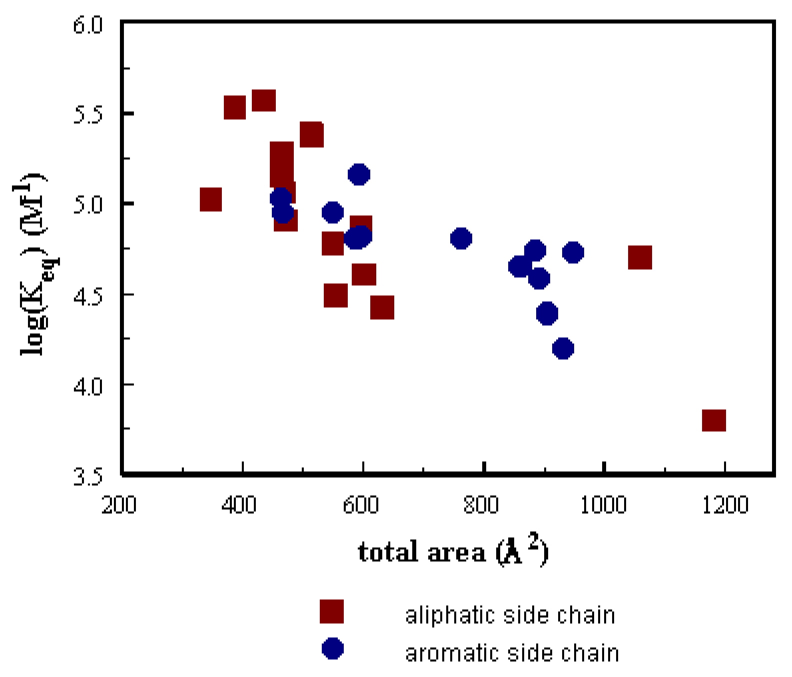
Dissociation rates show no clear relationship between the size of the side chain and the rate of dissociation.



■ aliphatic side chain

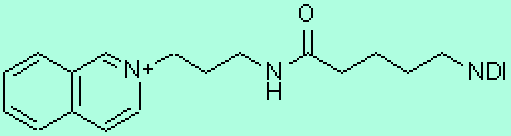
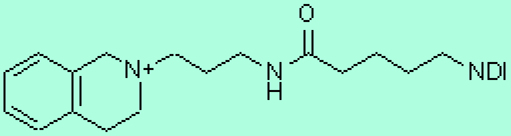
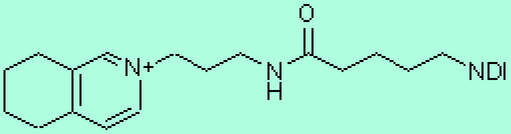
● aromatic side chain

Equilibrium constants for binding decrease as the size of the side chain increases.



For NDI with aromatic side chains, neither association nor dissociation rate constants are a function of the overall size of the molecule. This contrast with data on NDI with aliphatic side chains. Comparison of the relative rate constants of molecules in this data set led to the conclusion that aromatic rings at the termini of the side chains enhance the rate of association and dissociation.

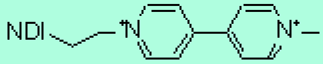
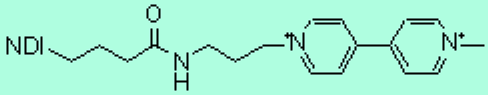
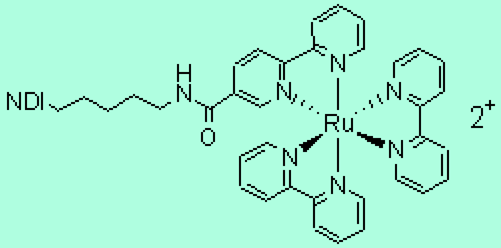
ASSOCIATION AND DISSOCIATION RATES CONSTANTS ARE SENSITIVE TO AROMATICITY AT THE "TIP" OF THE INTERCALATOR

	$k_a \times 10^{-5}$ $M^{-1}s^{-1}$	k_d s^{-1}	$K_{eq} \times 10^{-4}$ M^{-1}
 fastest	2.2	6.0	3.6
 intermediate	1.2	2.3	5.2
 slowest	0.61	1.6	3.8

- the association rate constant is higher for the compound with the fully aromatic system at the end of the side chain
- the fully aromatic isoquinoline derivative has the fastest dissociation rate
- overall, the derivative with fully aromatic systems at the ends of the side chains has faster on and off rates, but equilibrium constants for all three molecules are the same within a factor of two

c. Tetracationic NDI

Overall, the pattern of rate dependence on the size of the drug is the same for tetracationic as for the dicationic NDI: the bigger the molecule, the slower it intercalates into the DNA.

	$k_a \times 10^{-5}$ $M^{-1}s^{-1}$	k_d s^{-1}	$K_{eq} \times 10^{-4}$ M^{-1}
	4.1	0.58	7.0
	1.7	0.69	2.8
	0.32	3.2	0.10

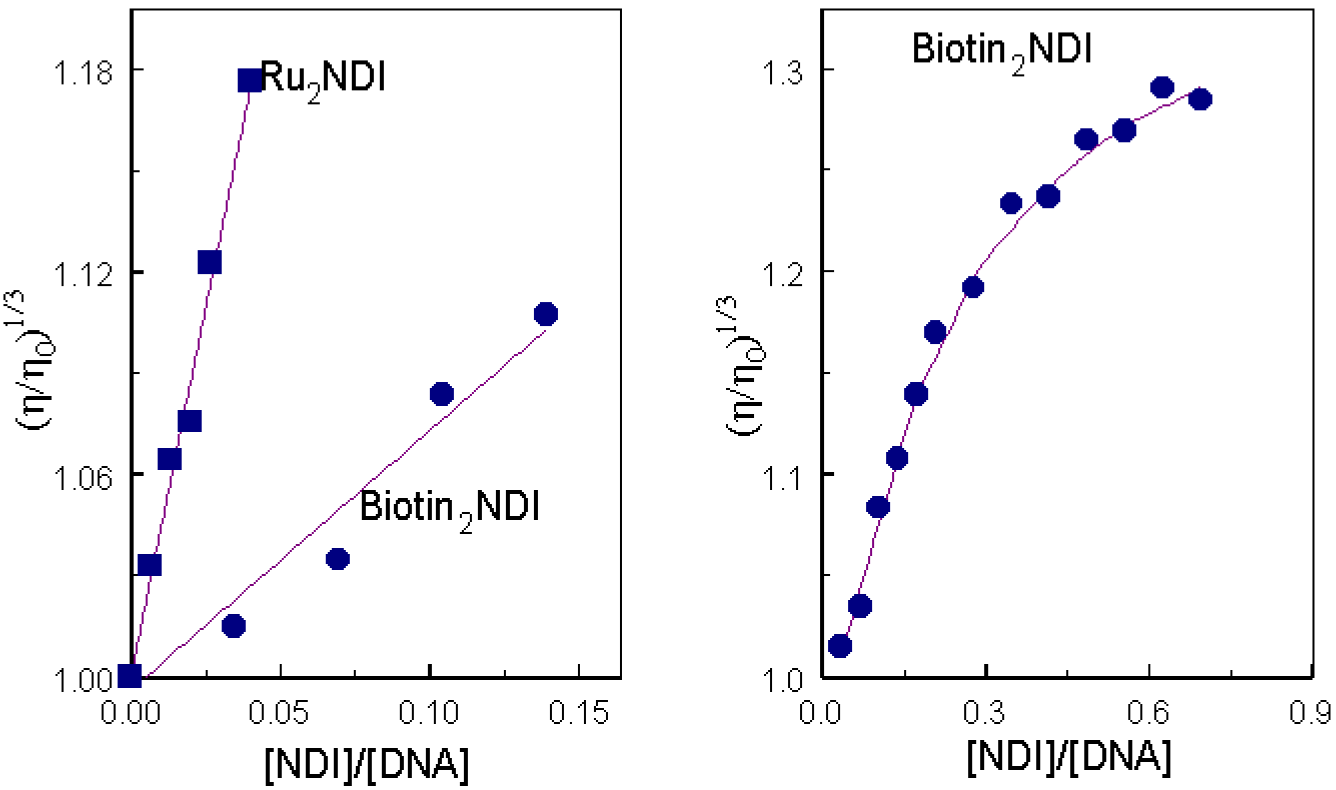
- association rates and the equilibrium constants decrease as the size of the side chain increases.
- dissociation rates show no clear relationship between the size of the compound and the rate of dissociation.

2. Viscometric Titrations of CT DNA

Intercalators are known to lengthen duplex DNA upon binding; groove-binders do not have any significant effect on the length of the helix. One of the "oldest" and still one of the most sensitive tests for determining the binding pattern of a drug is the increase in viscosity of the solution as the drug is added to the DNA.

Both Bi2NDI and Ru2NDI thread through DNA as seen in the increase of the solution viscosity as the concentration of drug increases.

Viscosity of CT DNA solution as a function of added NDI



· On the left data up to [NDI]/[DNA] ratios of 0.1 for both Bi2NDI (2.5 mM Na_2HPO_4/7.5 mM NaH_2PO_4, 0.2 M NaCl, pH 6.5, 25^oC) and Ru2NDI (same buffer with addition of 0.5 M NaCl). The viscosity of the Ru2NDI solution increases up to approximately [drug]/[DNA] = 0.1 and then begins to decrease. We attribute this to aggregation of the drug and DNA, possibly due to the 2+ positive charge on the Ru2NDI side chains. For this compound, aggregation is moved to higher [drug]/[DNA] ratios by increasing the salt concentration of the buffer.

· On the right Bi2NDI at higher [NDI]/[DNA] ratios. The viscosity of the biotin2-NDI solution increases monotonically up to [drug]/[DNA] = 1

· Ru2NDI at [NDI]/[DNA] ratios > 0.1 shows decreasing **h**/**h**$_o$ as a function of increasing [NDI]/[DNA] (data not shown).

3. Circular Dichroism Studies

Circular dichroism is widely used to indicate intercalation of molecules into duplex DNA. The induced CD spectra of Ru2NDI and Bi2NDI into duplex DNA were compared with that of the known intercalator NDI-$[(CH_2)_3\text{-}N(CH_3)_3]_2$ and appear to be almost identical (data are not shown). They all show a strong negative absorption band in the 330-440 nm region which indicates intercalation into duplex DNA.

For the tetracationic Ru2-NDI complex, as [NaCl] increases, the strength of its CD spectra also increases. We attribute this to reduced aggregation of the Ru2NDI-DNA under high salt conditions.

Conclusion

Threading intercalation is indicated by stop-flow kinetic measurements, circular dichroism and viscosity titrations.

Association rates decrease as the size of the side chain increases.

Dissociation rates show no clear relationship between the size of the side chain and the rate of dissociation.

Aromatic rings at the termini of the side chains of naphthalene diimides threading intercalators increase the rates of DNA association and dissociation.

Large, rigid groups (at least 11.5 Å in diameter) can thread through DNA as exemplified by side chains ending in biotin and Ru(bpy)$_3$.

Capillary electrophoresis is an excellent tool for establishing the purity of these large, cationic molecules.

Derivatives with side chains bearing positive charges close to the NDI ring do not stack at optical or even NMR concentrations. The extent of stacking does not correlate with the size of the side chain. Derivatives bearing side chains with the cationic centers at some distance to the NDI moiety show a greater tendency to self-stack. Self-stacking can also be induced by increasing the ionic strength of the solution.

References

1. Bourdouxhe-Housiaux, C., Colson, P., Houssier, C., and Bailly, C. Interaction of DNA-threading peptide-amsacrine conjugates with DNA and chromatin. *Anticancer Drug Des.* 11:509-525, **1996**.
2. Bourdouxhe-Housiaux, C., Colson, P., Houssier, C., Waring, M.J., and Bailly, C. Interaction of a DNA-threading netropsin-amsacrine combilexin with DNA and chromatin. *Biochemistry* 35:4251-4264, **1996**.
3. Searcey, M., Martin, R.B., Howarth, N.M., Madden, B., and Wakelin, L.P.G. DNA threading agents: Effect of sidechain bulk on DNA binding and cytotoxicity of 9-anilinoacridine-4-carboxamides. *Bioorg.Med.Chem.Lett.* 6(15):1831-1836, **1996**.
4. Hansen, M. and Hurley, L. Altromycin-b threads the DNA helix interacting with both the major and the minor grooves to position itself for site-directed alkylation of guanine-N7. *J.Am.Chem.Soc.* 117:2421-2429, **1995**.
5. Searle, M.S. and Bicknell, W. Interaction of the anthracycline antibiotic nogalamycin with the hexamer duplex d(5'- GACGTC)2. An NMR and molecular modelling study. *Eur.J.Biochem.* 205:45-58, **1992**.
6. Tanious, F.A., Yen, S.-F., and Wilson, W.D. Kinetic and equilibrium analysis of a threading intercalation mode: DNA sequence and ion effects. *Biochemistry* 30:1813-1819, **1991.**
7. Wakelin, L.P.G. and Denny, W.A. Kinetic and equilibrium binding studies of a series of intercalating agents that bind by threading a sidechain through the DNA helix. In: *Molecular Basis of Specificity in Nucleic Acid-Drug Interactions*, edited by Pullman, B. and Jortner, J.Dordrecht:Kluwer Academic Publishers, **1990,**p. 191-206.
8. Wakelin, L.P.G., Chetcuti, P., and Denny, W.A. Kinetic and equilibrium binding studies of amsacrine-4-carboxamides: a class of asymmetrical DNA-intercalating agents which bind by threading through the DNA helix. *J.Med.Chem.* 33:2039-2044, **1990.**
9. Denny, W.A. and Wakelin, L.P.G. Mode and kinetics of DNA binding of the anti-tumour agent bisantrene. *Anticancer Drug Des.* 2:71-77, **1987.**
10. Collier, D.A., Neidle, S., and Brown, J.R. Molecular models for the interactions of the anti-tumor drug nogalamycin with DNA. *Biochem.Pharmacol.* 33:2877-2880, **1984.**
11. Yen, S.-F., Gabbay, E.J., and Wilson, W.D. Interaction of aromatic imides with deoxyribonucleic acid.

Spectrophotometric and viscometric studies. *Biochemistry* 21:2070-2076, **1982.**

12. Gabbay, E.J., DeStefano, R., and Baxter, C.S. Topography of nucleic helices in solutions. XXVIII. Evidence for a dynamic structure of DNA in solution. *Biochem.Biophys.Res.Commun.* 51:1083, **1973.**

Comments

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