Research Article

Journal of Physical Organic Chemistry

Received: 4 September 2007, Revised: 26 December 2007, Accepted: 27 December 2007, Published online in Wiley InterScience: 29 May 2008

(www.interscience.wiley.com) DOI 10.1002/poc.1334

Hydrolysis of aliphatic naphthalene diimides: effect of charge placement in the side chains

Michelle B. Kim[a] and Dabney W. Dixon[a]*

Water-soluble naphthalene diimides (NDIs) have found uses in a wide variety of applications including as electron acceptors in electron transfer reactions and as molecules that undergo spontaneous organization in aqueous solution. Many studies have looked at their interaction with nucleic acids including work with DNA duplexes, triplexes, quadruplexes, hairpins, and DNA–RNA heteroduplexes. In many of these interactions the NDIs serve as threading intercalators. Herein we describe the reversible hydroxide-catalyzed hydrolysis of NDIs bearing aliphatic side chains, with ring opening first to the monoimide and then to the diamide. Examples with *N*-methylpyrrolidinium groups placed two (1) and three (5) atoms from the central core were studied. The K_a values for the first and second hydrolyses for 1 were $2.5 \pm 0.2 \times 10^5$ and $2.0 \pm 0.1 \times 10^2\ M^{-1}$, respectively; for 5 they were $1.4 \pm 0.1 \times 10^5$ and $44 \pm 2\ M^{-1}$, respectively. NDI 1 hydrolyzed 6.8 times faster than 5. The rates for the first and second ring opening of 1 in 100 mM hydroxide measured by stopped-flow were 17.0 ± 0.2 and $0.53 \pm 0.01\ s^{-1}$, respectively. Capillary electrophoresis in borate buffer showed separation of the diimide and monoimide with the former eluting first. Nuclear magnetic resonance (NMR) showed both the *syn* and *anti* isomers of the diamide species. Overall, the rate of hydrolysis of the NDI is increased when the cationic charge is moved closer to the NDI core. Copyright © 2008 John Wiley & Sons, Ltd.

Supplementary electronic material for this paper is available in Wiley InterScience at http://www.mrw.interscience.wiley.com/suppmat/0894-3230/suppmat/

Keywords: hydrolysis; naphthalene diimide; diimide; kinetics; base catalyzed

INTRODUCTION

Water-soluble naphthalene diimides (NDIs) have found uses in a wide variety of applications. They are excellent electron acceptors in aqueous solutions;[1–3] the photophysics of these species has been well studied in water.[4–9] Photolysis of NDIs has been used to reduce heme proteins;[10] photoactivation of hydroperoxy derivatives has been used to oxidize both proteins[4,11,12] and DNA.[13–15] Selected NDI derivatives are examples of molecules that undergo spontaneous organization in aqueous solution.[16–18]

The majority of studies of NDI in aqueous solution have involved binding to nucleic acids. NDI derivatives have been known for many years to intercalate in duplex DNA.[19–21] Study of NDI binding to DNA is now extensive; leading references to studies in the last 10 years include those of binding to bulged duplexes,[22] triplexes,[23,24] quadruplexes,[25–27] hairpins,[23] and DNA–RNA heteroduplexes.[28] Molecules with the NDI moiety have been shown to thread into DNA, with the diimide groups intercalating and the side chains or conjugating moieties lying in the grooves.[29–33] The NDIs can carry reactive groups to the DNA including metal centers[34,35] and alkylating agents.[36] NDIs have been used to conjugate other nucleic acid binding species, thus increasing their binding,[37,38] and have been employed to stabilize DNA hairpins.[39] Diimides intercalated into or covalently bound to DNA have been used extensively in studies of photoinduced charge separation and charge recombination processes; leading references are given.[40–45] The facile reduction of NDI bound to DNA has allowed these species to be used in the electrochemical detection of DNA.[46–48]

In general, studies of NDIs with nucleic acids involve cationic substituents on the NDI side chains to favor electrostatic interactions between these side chains and the phosphate groups of the nucleic acid. Side chains with a methylene group adjacent to the imide nitrogen are necessary to prevent a steric clash between the side chains of the NDI and the DNA. A cationic center no farther than three atoms away from the central core prevents self-stacking in aqueous solution,[49] which can complicate DNA-binding measurements.

Recently, we have synthesized four new NDI derivatives (**1**–**4**) with the cationic center two atoms away from the central core (Fig. 1). Although such types of structures appear to be stable in neutral aqueous solution,[3,19,22,50,51] we find that they are quite sensitive to base. It has been known for some time that the diimide ring system can react with alkali.[52–55] Attack of hydroxide opens first one of the imide rings, and then the other, to give a diacid–diamide structure. Under forcing conditions, reaction of the diimide with base can lead to loss of CO_2 and contraction of one of the rings.[56,57]

Herein, we present a study of the base-catalyzed hydrolysis of NDI derivatives with the cationic center both two and three atoms away from the central core. The former is more labile to base both kinetically and thermodynamically. Stopped-flow and UV–visible absorbance studies as well as nuclear magnetic resonance (NMR) data show the two sequential hydrolyses.

* *Department of Chemistry, Box 4098, Georgia State University, Atlanta, GA 30302-4098, USA.*
E-mail: ddixon@gsu.edu

a *M. B. Kim, D. W. Dixon*
Department of Chemistry, Georgia State University, Atlanta, Georgia 30302-4098, USA

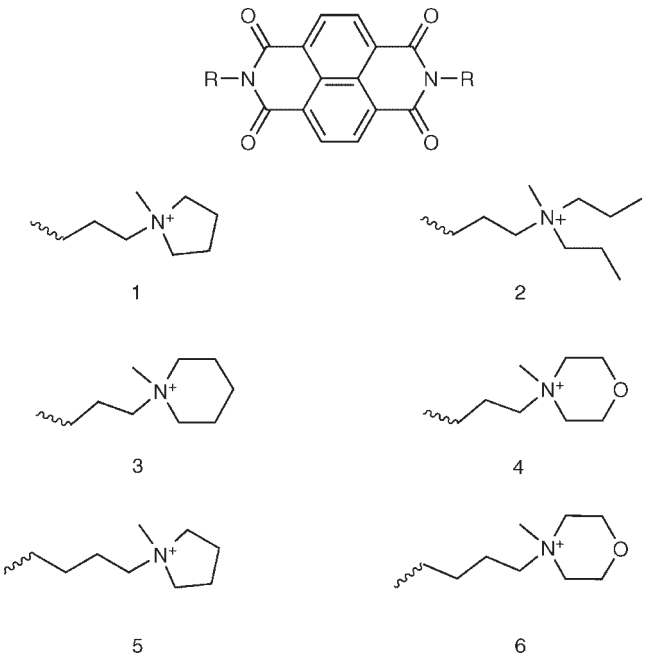

Figure 1. NDI derivatives in this study

RESULTS

Synthesis

The NDI derivatives (Fig. 1) were synthesized via condensation of one equivalent of naphthalene-1,4,5,8-tetracarboxylic dianhydride and two equivalents of the starting amine. The mixture was refluxed in toluene using a Dean Stark trap for 3 h.

The NDI derivatives were alkylated with iodomethane. Solvents including acetonitrile, chloroform, DMF, and ethanol were used. Generally, the best results were achieved with a large excess of iodomethane in chloroform with overnight stirring at room temperature. The morpholine derivatives, however, alkylated more slowly in chloroform. After stirring overnight at room temperature, the CH_2CH_2-morpholine derivative gave a mixture of the dialkylated (**4**, singlet at 8.96 ppm) and the monoalkylated (AB quartet at 8.83 ppm) products, as seen from appropriate peaks in the ^1H NMR spectrum in D_2O. Consistent with the NMR spectrum, capillary electrophoresis (CE) showed two peaks at 15.8 and 16.4 min with baseline separation. Complete alkylation for the CH_2CH_2-morpholine derivative was achieved in acetonitrile with a large excess of iodomethane at 60°C for 12 h.[34] For the $CH_2CH_2CH_2$-morpholine derivative, complete dialkylation did not occur after 2 days in a large excess of $CHCl_3$ and iodomethane at 60°C. Isolation of **4** was achieved by dissolving the mixture after alkylation in hot water and removing the residue by filtration.

Hydrolysis followed by UV–visible absorbance spectroscopy

The hydrolysis of the rings of **1** was followed by UV–visible absorbance spectroscopy. The starting diimide had two bands at 360 and 380 nm. In a borate buffer solution at pH 9.0, these bands decreased in intensity over time with three isosbestic points at 271, 306, and 352 nm (Fig. 2).

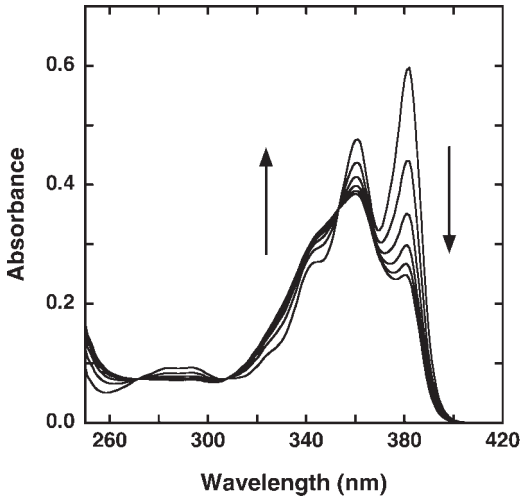

Figure 2. UV–visible absorbance spectra of **1** as a function of time (50 mM borate buffer at pH 9.0 with scans taken every 10 min for 50 min)

In a second experiment, increments of NaOH were added to a sample in 50 mM phosphate buffer to give pH values of 8.0–13.1. As the base was added, the spectra were first seen to change from the diimide to a species with a broad peak from 350 to 370 nm, assigned as the monoimide. Further addition of base gave conversion of the monoimide to the diamide, with a peak centered at 308 nm. An isosbestic point at 326 nm was observed for this second transition. The maximum concentration of the monoimide occurred at pH 10.2. Figure 3 shows the spectra of the diimide, monoimide, and diamide.

Solutions of **1** were prepared at pH values ranging from 7.5 to 12.3. In each case, the system was allowed to reach equilibrium. Figure 4 shows the fractions of the three species as a function of the log of the concentration of hydroxide in the solution. The K_a values for conversion of the diimide to the monoimide (K_{a1}) and monoimide to the diamide (K_{a2}) were $2.5 \pm 0.2 \times 10^5$ and $2.0 \pm 0.1 \times 10^2\ M^{-1}$, respectively. A similar experiment was performed for **5**, in which the cationic charge is separated by

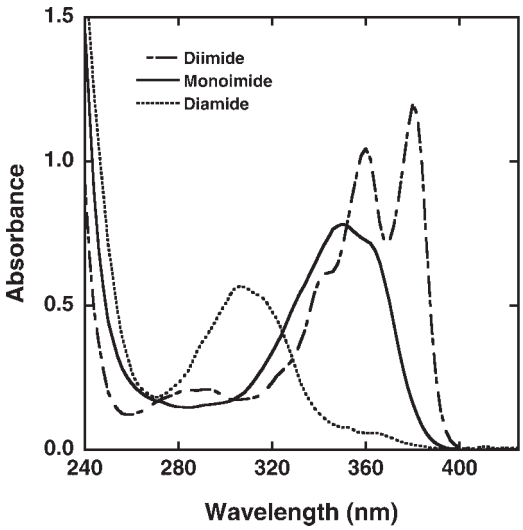

Figure 3. UV–visible absorbance spectra of the diimide, monoimide, and diamide in 50 mM phosphate buffer solution

Journal of Physical Organic Chemistry

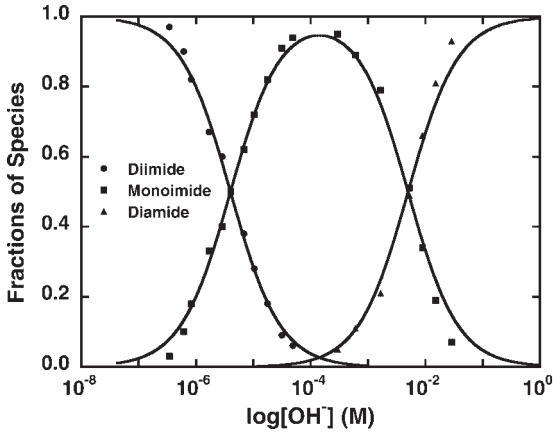

Figure 4. Fractions of the diimide, monoimide, and diamide as a function of pH. Samples of **1** were equilibrated in a buffer containing 100 mM sodium phosphate and 100 mM sodium tetraborate

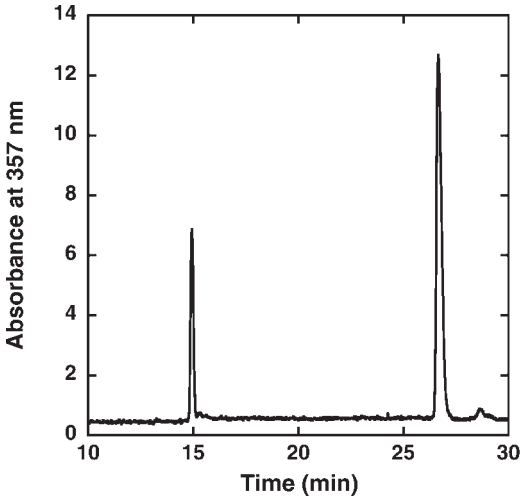

Figure 6. Electropherogram of NDI **1** (15.0 min) and its monoimide (28.6 min) using 25 mM sodium tetraborate buffer, pH 9.0, 11 kV

an additional methylene group from the imide center. The K_a values were $1.4 \pm 0.1 \times 10^5$ and $44 \pm 2\,M^{-1}$, respectively.

NMR and capillary electrophoresis studies

NMR spectra as a function of pH were consistent with the proposed structures of the three species. For example, the aromatic protons of the diimide **1** appeared as a singlet at 8.97 ppm in D_2O. At a pH of approximately 12, the monoimide was seen as two doublet of doublets at 7.62 and 8.28 ppm (Fig. 5). The diamide was seen as a series of six peaks at approximately 7.4 ppm. Two singlets at 7.39 and 7.43 ppm were ascribed to the *syn* isomer. An AB-quartet centered at 7.4 ppm with a coupling constant of 7.2 Hz was ascribed to the *anti* isomer. At 30°C the compound was a 44/56 mixture of the *syn* and *anti* isomers. In addition to the peaks above, small amounts of unidentified hydrolysis products were observed. When the NMR tube was allowed to stand at room temperature for 5 days, it was found that the pH had decreased and that the monoimide concentration had increased. This observation was consistent with previous studies that ring opening is reversible.[52,53,58]

A sample of **1** at pH 9.0 was also evaluated by CE with diode array detection. Two peaks were seen in the electropherogram (Fig. 6). The peak with the shorter migration time was the diimide and that with the longer migration time was the monoimide as determined from their absorbance spectra.

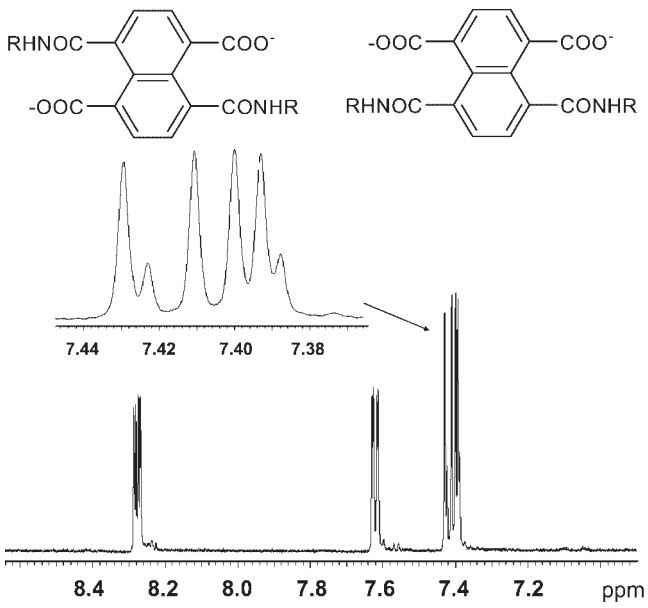

Figure 5. ^{1}H NMR at 600 MHz of compound **1** in D_2O at pH 12. The inset is the set of peaks at 7.4 ppm which is the diamide species (see text)

Kinetics of ring hydrolysis: diimide to monoimide

The hydrolysis of **1** from the diimide to the monoimide was followed by UV–visible absorbance spectroscopy as a function of pH in borate buffer. A high concentration of buffer was used so that the concentration of hydroxide would remain constant throughout the experiment, thus allowing treatment of the hydrolysis as a first-order reaction. At a pH of 9.6, the approximate half-life was 5 min at room temperature. The observed rate constant k_{obs}, calculated from the change in absorbance at 380 nm, increased linearly with the concentration of hydroxide (Fig. 7). Because the reaction is reversible, the kinetics are expressed in

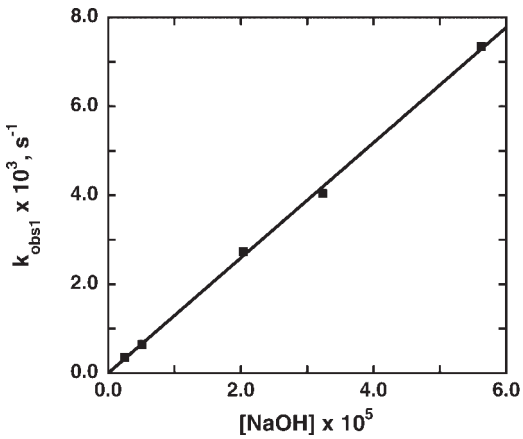

Figure 7. Rates as a function of the concentration of hydroxide for the first hydrolysis of NDI **1**. Reactions were run at 25°C in 100 mM sodium tetraborate buffer

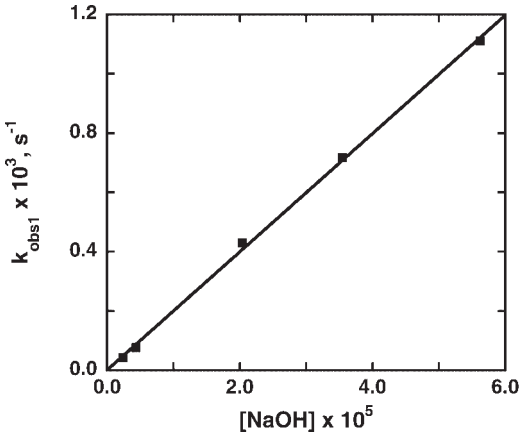

Figure 8. Rates as a function of the concentration of hydroxide for the first hydrolysis of NDI **5**. Reactions were run at 25°C in 100 mM borate buffer

terms of the sum of the forward (k_f) and reverse (k_r) rate constants

$$k_{obs} = k_f[OH^-] + k_r$$

A plot of k_{obs} *versus* $[OH^-]$ gave a second-order rate constant of $130 \pm 3\ M^{-1}\,s^{-1}$. The intercept, corresponding to k_r, was less than $10^{-4}\,s^{-1}$. The reverse rate constant was also calculated from the forward rate (k_f) and the fractions of diimide and monoimide from the equilibrium data shown in Fig. 4. The average k_r for the five points shown in Fig. 7 was $5.2 \pm 0.2 \times 10^{-4}\,s^{-1}$. The similarity of these two methods of calculating k_r indicates that the contribution of water itself to the hydrolysis of the NDI is small.

A similar measurement for homolog **5** (Fig. 8) gave a second-order rate constant of $20.0 \pm 0.4\ M^{-1}\,s^{-1}$. The intercept was less than $2 \times 10^{-5}\,s^{-1}$. The calculated reverse rate constant was $8.6 \pm 1.6 \times 10^{-5}\,s^{-1}$.

Kinetics of ring hydrolysis: monoimide to diamide

At the concentrations of hydroxide necessary to observe significant amounts of the diamide (>0.005 M), the hydrolysis reactions are too fast to measure by conventional UV–visible absorbance spectroscopy. Therefore, the reaction was studied using a stopped-flow spectrophotometer. Figure 9 shows a plot of the absorbance at 372 nm as a function of time at 100 mM NaOH in the absence of buffer. At this wavelength the absorbance changes for the diimide to monoimide and the monoimide to diamide are the same. Data are the average of nine individual kinetic runs, fit to two consecutive first-order reactions. The observed rates for the first (k_{obs1}) and second (k_{obs2}) hydrolysis processes were 17.0 ± 0.2 and $0.53 \pm 0.01\ s^{-1}$, respectively.

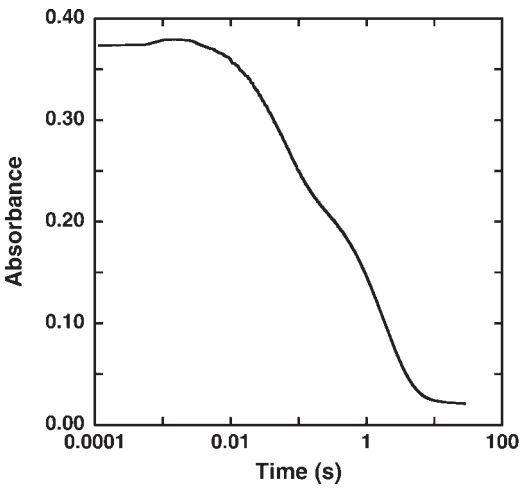

Figure 9. Stopped-flow trace of the absorbance of NDI **1** as a function of log (time) in 100 mM NaOH. Data were taken at 372 nm

DISCUSSION

The kinetics and equilibria of ring opening

In this work, we have compared the ring opening reactions of **1** and **5**. The conversion of the diimide to the monoimide is 6.8-fold faster for compound **1** with the charge closer to the NDI central core. The faster reaction for **1** is presumably due to electrostatic stabilization of the negatively charged transition state.

Equilibrium studies show that **1** converts from the diimide to the monoimide with a K_{a1} of $2.5 \pm 0.2 \times 10^5\ M^{-1}$ and from the monoimide to the diamide with a K_{a2} of $2.0 \pm 0.1 \times 10^2\ M^{-1}$. Expressed as apparent pK_a values, these are 8.6 and 11.8, respectively. The conversion from the diimide to the monoimide for NDI **5** had a K_{a1} of $1.4 \pm 0.1 \times 10^5\ M^{-1}$ and that from the monoimide to the diamide had a K_{a2} of $44 \pm 2\ M^{-1}$. Expressed as apparent pK_a values, these values are 8.8 and 12.4, respectively. Comparison of the equilibria for **1** and **5** shows that the ring-opened form of **1** is favored by a factor of 1.8 for the first hydrolysis and 4.5 for the second hydrolysis.

Our work may be compared with that of Kheifets and Martyushina.[52] They reported an apparent pK_a of 9.3 for the NDI with $CH_2CH_2CH_2CH_2CH_2CO_2^-$ side chains. They also reported that neither the NDI with positively charged ($CH_2CH_2CH_2N^+Me_3$) nor with negatively charged ($CH_2CH_2CH_2SO_3^-$) aliphatic side chains showed ring-opened forms at pH 7.5. In our study, **1** is about 3% in the ring-opened form at pH 7.5. Thus, although the extent of ring opening is small, the NDI is interconverting between the ring-closed and ring-opened forms at physiological pH.

Aliphatic NDI derivatives are significantly more stable toward base than their aromatic counterparts. For example, the dicationic NDI with *N*-ethylpiperdinylphenyl [—C_6H_4—N^+(Et)C_5H_{10}] side chains was about half converted to the monoimide at pH 6.7.[52] The monoimide in turn was about half converted to the diamide at about pH 9.9. The corresponding apparent pK_a values for the hydrolyses of the dianionic NDI (—C_6H_4—SO_3^- side chains) were 7.0 and 10.4. Thus, the presence of a cationic center in the side chain facilitates ring opening slightly in these systems; both are significantly more prone to ring opening in basic solution than are the aliphatic derivatives.

Our study is the first to measure the rate of ring opening of the monoimide to the diamide. For **1** at 100 mM hydroxide, the rates of the first and second hydrolysis were 17.0 ± 0.2 and $0.53 \pm 0.01\ s^{-1}$, respectively. Thus, the first reaction is about 30 times faster than the second. A recent study of the hydrolysis of naphthalene-1,4,5,8-tetracarboxylic dianhydride also showed that the first hydrolysis was faster than the second; in this case the difference was approximately a factor of 190.[59]

The calculated k_r from the forward rate (k_f) and fractions of each species from the equilibrium data were similar to the k_r derived from the intercepts of the plot of the rates as a function of

the concentration of hydroxide. This indicates that contribution of water itself to the hydrolysis of the NDI is small. A similar conclusion regarding the participation of water in the hydrolysis was reached earlier.[53] A minimal contribution of water has also been shown in the hydrolysis of the naphthalene-1,4,5,8-tetracarboxylic dianhydride.[59]

Borate can sometimes act as a catalyst in hydrolysis reactions.[60] The kinetics studies of **1** at 2.4×10^{-6}–5.6×10^{-5} M hydroxide were run with borate buffer, whereas the stopped-flow kinetics were run without buffer. Using the second-order rate constant of $130 \pm 3\ M^{-1}\ s^{-1}$ from the former data set, one can calculate an expected rate constant of $13\ s^{-1}$ at 0.10 M hydroxide. The measured value was $17\ s^{-1}$. The similarity of these two numbers argues that borate does not catalyze the ring opening in this diimide system.[53]

Syn and *anti* isomers of the diamide

The hydrolysis reaction was freely reversible as has been observed previously.[52,53,58] NMR spectroscopy allowed visualization of all three species in solution. In previous work, Kheifets and Martyushina recorded NMR spectra of an NDI with an aromatic side chain as a function of pH.[52] Data were difficult to interpret, however, due to the overlap of the naphthalene protons and the side chain protons at the 100 MHz field then available. In the current study, the NMR of **1** at 600 MHz allowed clear visualization of each of the aromatic peaks. The diimide and monoimide were the expected singlet and AB quartet, respectively. The diamide showed a series of six overlapping peaks, all of which were resolved at 600 MHz. The spectra were consistent with a mixture of the *syn* and *anti* isomers of this compound. To our knowledge, this is the first report of both isomers. The amounts of the *syn* and *anti* isomers were similar, indicating that there is very little energy difference between these two species.

Conclusions

The various uses of NDIs demand many different structures of the side chains. In some studies, for example, in some investigations of NDI binding to nucleic acids, it is desirable that the molecules should be freely water soluble. This in turn necessitates a charge no further than three atoms away from the central core; derivatives with charges further away tend to self-stack in solution. In this report, we have shown that moving the cationic charge closer to the NDI core by one methylene unit increases the rate of base-catalyzed hydrolysis. NDI **1** with an ethyl linker hydrolyses 6.8-fold faster than the derivative with a propyl linker **5**. The equilibrium constants for the reactions with hydroxide also show **1** to be less stable than **5**; at pH 7.5, **1** exists as 3% in the monoimide form. This study provides fundamental reactivity data which will aid the design of NDIs for a wide variety of applications.

EXPERIMENTAL

Materials

NMR spectra were recorded on Varian Unity+ 300 and 600 MHz spectrometers. NMR samples for the parent NDIs were prepared in 0.5 ml of $CDCl_3$ (Aldrich) in 5 mm NMR tubes. NMR samples for alkylated derivatives were prepared in 0.5 ml of D_2O (Aldrich) in 5 mm NMR tubes. CE was carried out on a Beckman PACE 5500 instrument on a fused-silica capillary (60 cm × 75 μm i.d.) with a P/ACE diode array detector. The voltage used for electrophoresis was 11 kV and the sample was injected into the capillary using high pressure injection for 6 s. Reverse polarity was used where the sample was injected at the cathode. A mixture of NDI **4** and its corresponding monoalkylated species was injected in deionized water; the running buffer was 50 mM sodium phosphate at pH 3.0. A mixture of NDI **1** and its monoimide was injected in 50 mM sodium tetraborate buffer at pH 9.0; the running buffer was 50 mM sodium phosphate at pH 3.0. UV–visible absorbance spectra were recorded on a Varian Cary 50 spectrophotometer. The high resolution mass spectra were taken on a Micromass Q-TOF spectrometer.

Determination of K_a

To determine the equilibrium constants, samples of **1** and **5** were equilibrated in buffer containing 100 mM sodium phosphate and 100 mM sodium tetraborate at approximately 10 pH values. Fractions of each of the diimide, monoimide, and diamide were determined by taking linear combinations of the spectra of these three species. The fraction of the monoimide as a function of the concentration of hydroxide was fit using a nonlinear least squares fitting algorithm (Kaleidagraph, version 4.01, Synergy Software, Reading, PA) to determine K_{a1} and K_{a2} using Eqn (1).

$$[\text{Monoimide}] = K_{a1}[OH^-] \times [\text{monoimide}]_0/(1 + K_{a1}[OH^-] + K_{a1}K_{a2}[OH^-]^2) \quad (1)$$

Stopped-flow kinetics

A HiTech Scientific SF-61 DX2 Double Mixing Stopped-Flow system equipped with temperature control was used to measure the absorption as a function of time after mixing and to fit the kinetic data. The temperature was equilibrated to 25°C. A xenon lamp was utilized to monitor absorbance at 372 nm. A solution of 0.2 M NaOH was filtered twice using a 0.45 μm Nalgene filter (Rochester, NY) prior to use. Compound **1** was dissolved in 18 MΩ water.

Synthesis of naphthalene diimide derivatives

The parent NDI derivatives were synthesized using naphthalene-1,4,5,8-tetracarboxylic dianhydride (Alfa Aesar, Ward Hill, MA) with the appropriate amine: *N,N*-di-*n*-propyl-ethylenediamine (Karl Industries, Aurora, OH), 2-(*N*-piperidino)ethylamine (Karl Industries), 4-(2-aminoethyl)morpholine (Alfa Aesar), *N*-(2-aminoethyl)pyrrolidine (Alfa Aesar), 1-(3-aminopropyl)pyrrolidine (Alfa Aesar), and *N*-(3-aminopropyl)morpholine (Fisher). Toluene (99.9%, Fischer Scientific, Fair Lawn, NJ) was used as the solvent. The parent NDIs were alkylated directly using iodomethane (Aldrich) in the indicated solvent. The purity of the derivatives was established using ^{1}H NMR (see Supplementary Material). Spectra in water were referenced to the HOD line at δ 4.70.

*N,N′-Bis[(2-N-pyrrolidinyl-N-methyl)ethyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide (**1**)*

To a mixture of *N,N′*-bis[2-(*N*-pyrrolidinyl)ethyl]-1,4,5,8-naphthalenetetracarboxylic-1,8:4,5-diimide[61] (0.10 g, 0.22 mmol) and acetonitrile (10 ml), iodomethane (4.6 g, 32 mmol) was added.

After a few minutes, the orange–yellow mixture turned into a red–orange solution which was allowed to stir overnight at room temperature. The precipitate was filtered under vacuum and washed with acetonitrile to give an orange solid.

^{1}H NMR (D_2O): δ 2.46 [br s, 8H, $N^+(CH_2CH_2CH_2CH_2)$], 3.47 [s, 6H, N^+CH_3], 3.86 [m, 12H, $N^+(CH_2CH_2CH_2CH_2) + NCH_2CH_2N^+$], 4.86 [t, 4H, $NCH_2CH_2N^+$), 8.97 (s, 4H, Ar). HRMS (ESI): m/2z calcd. for $C_{28}H_{34}N_4O_4$: 245.1290; found: 245.1298.

*N,N′-Bis[(2-N,N-dipropyl-N-methylamino)ethyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide (**2**)*

N,N′-Bis[(2-*N,N*-dipropylamino)ethyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide[61] (0.12 g, 0.23 mmol) was dissolved in chloroform (10 ml) and iodomethane (4.6 g, 32 mmol) was added. The dark brown solution was allowed to stir overnight at room temperature. The precipitate was filtered under vacuum and washed with chloroform; the resulting solid was dark red in color.

^{1}H NMR (D_2O): δ 1.21 [m, 12H, $N^+(CH_2CH_2CH_3)_2$], 2.06 [m, 8H, $N^+(CH_2CH_2CH_3)_2$], 3.39 [s, 6H, N^+CH_3], 3.59 [m, 8H, $N^+(CH_2CH_2CH_3)_2$], 3.79 [m, 4H, $NCH_2CH_2N^+$], 4.77 (m, 4H, $NCH_2CH_2N^+$), 8.94 (s, 4H, Ar). HRMS (ESI): m/2z calcd. for $C_{32}H_{46}N_4O_4$: 275.1760; found: 275.1768.

*N,N′-Bis[(2-N-piperidinyl-N-methyl)ethyl]-1,4,5,8-naphthalenetetracarboxylic-1,8:4,5-diimide (**3**)*

N,N′-Bis[(2-*N*-piperidinyl)ethyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide[61] (0.11 g, 0.23 mmol) was dissolved in chloroform (20 ml). Iodomethane (4.6 g, 32 mmol) was added to the light brown solution which was allowed to stir overnight at room temperature. The precipitate was filtered under vacuum and washed with chloroform to give a purple solid.

^{1}H NMR (D_2O): δ 1.90 [br s, 4H, $N^+(CH_2\ CH_2CH_2CH_2CH_2)$], 2.16 [br s, 8H, $N^+(CH_2CH_2CH_2CH_2CH_2)$], 3.47 [s, 6H, N^+CH_3], 3.66 [m, 8H, $N^+(CH_2CH_2CH_2CH_2CH_2)$], 3.89 [m, 4H, $NCH_2CH_2N^+$], presumably under D_2O peak (4H, $NCH_2CH_2N^+$), 8.99 (s, 4H, Ar). HRMS (ESI): m/2z calcd. for $C_{30}H_{38}N_4O_4$: 259.1447; found: 259.1441.

*N,N′-Bis[2-(4-morphyl-N-methyl)ethyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide (**4**)*

N,N′-Bis[2-(4-morpholinyl)ethyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide[62] (0.12 g, 0.24 mmol) was suspended in acetonitrile (10 ml). Iodomethane (2.3 g, 16 mmol) was added to the orange mixture and the mixture was allowed to stir overnight at 60°C. The mixture was filtered under vacuum and was washed with acetonitrile to give a red-orange solid.

^{1}H NMR (D_2O): δ 3.72 [s, 6H, N^+CH_3], 3.96 [m, 8H, $N^+(CH_2CH_2OCH_2CH_2)$], 4.25 [m, 4H, $NCH_2CH_2N^+$], δ 4.41 [br s, 8H, $N^+(CH_2CH_2OCH_2CH_2)$], 4.95 [t, 4H, $NCH_2CH_2N^+$), 9.09 (s, 4H, Ar). HRMS (ESI): m/2z calcd. for $C_{28}H_{34}N_4O_6$: 261.1239; found: 261.1233.

*N,N′-Bis[1-(3-pyrrolidinyl)propyl]-1,4,5,8-naphthalenetetracarboxylic-1,8:4,5-diimide (precursor to **5**)*

Naphthalene-1,4,5,8-tetracarboxcylic dianhydride (0.61 g, 2.3 mmol) and *N*-(3-aminopropyl)pyrrolidine (1.2 g, 9.4 mmol) were mixed in toluene (75 ml) and allowed to reflux with a Dean-Stark trap for 3 h. The orange–brown mixture was filtered and the filtrate was removed under vacuum. The orange solid was recrystallized from ethanol and dried.

^{1}H NMR ($CDCl_3$): δ 1.65 [br s, 8H, $N(CH_2CH_2CH_2CH_2)$], 1.98 [m, 4H, $NCH_2CH_2CH_2N$], 2.48 [br s, 8H, $N(CH_2CH_2CH_2CH_2)$], 2.61 [t, 4H, $NCH_2CH_2CH_2N(CH_2)_4$], 4.30 [t, 4H, $NCH_2CH_2CH_2N(CH_2)_4$), 8.75 (s, 4H, Ar). UV ($CHCl_3$): 360 nm (ε) $21.4 \pm 0.3 \times 10^3$ and 380 nm (ε) $26.3 \pm 0.3 \times 10^3\ M^{-1}\ cm^{-1}$. HRMS (ESI): m/z calcd. for $C_{28}H_{33}N_4O_4$: 489.2502; found: 489.2490.

*N,N′-Bis[1-(3-N-pyrrolidinyl-N-methyl)propyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide (**5**)*

N,N′-Bis[1-(3-pyrrolidinyl)propyl]-1,4,5,8-naphthalenetetracarboxylic-1,8:4,5-diimide[61] (66 mg, 0.14 mmol) was dissolved in chloroform (40 ml). Iodomethane (3.4 g, 24 mmol) was added to the dark brown solution which was allowed to stir overnight at room temperature. The precipitate was filtered under vacuum and washed with chloroform to give an orange-red solid.

^{1}H NMR (D_2O): δ 2.42 [br s, 8H, $N^+(CH_2CH_2CH_2CH_2)$], 2.51 [m, 4H, $NCH_2CH_2CH_2N^+$], 3.29 [s, 6H, N^+CH_3], 3.78 [m, 12H, $NCH_2CH_2CH_2N^+ + N^+(CH_2CH_2CH_2CH_2)$], 4.50 [t, 4H, $NCH_2CH_2CH_2N^+$], 8.89 [s, 4H, Ar]. HRMS (ESI): m/2z calcd. for $C_{30}H_{38}N_4O_4$: 259.1447; found: 259.1459.

*N,N′-Bis[(3-morpholinyl-N-methyl)propyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide (**6**)*

N,N′-Bis[*N*-(3-morpholinyl)propyl]-1,4,5,8-napthalenetetracarboxylic-1,8:4,5-diimide[61] (0.47 g, 0.90 mmol) was dissolved in chloroform (60 ml). Iodomethane (4.6 g, 32 mmol) was added to the red–orange mixture and was allowed to stir at 60°C for 2 days. The mixture was evaporated and washed with hot water (10 ml). An insoluble yellow solid was filtered and the filtrate was collected and allowed to crystallize. The crystals were filtered to give a yellow solid.

^{1}H NMR (D_2O): δ 2.52 [m, 4H, $NCH_2CH_2CH_2N^+$], 3.43 [s, 6H, N^+CH_3], 3.75 [m, 8H, $N^+(CH_2CH_2OCH_2CH_2)$], 3.90 [t, 4H, $NCH_2CH_2CH_2N^+$], 4.27 [br s, 8H, $N^+(CH_2CH_2OCH_2CH_2)$], 4.60 [t, 4H, $NCH_2CH_2CH_2N^+$), 8.97 (s, 4H, Ar). HRMS (ESI): m/2z calcd. for $C_{30}H_{38}N_4O_6$: 275.1396; found: 275.1402.

Acknowledgements

We thank Dr Giovanni Gadda for the stopped flow experiments, Christina Williams for help with the synthesis and Dr S. Chandrasekaran for NMR assistance. The NMR facilities were provided by the National Science Foundation and the Georgia Research Alliance.

REFERENCES

[1] L. L. Miller, K. R. Mann, *Acc. Chem. Res.* **1996**, *29*, 417–423.
[2] L. L. Miller, R. G. Duan, D. C. Tully, D. A. Tomalia, *J. Am. Chem. Soc.* **1997**, *119*, 1005–1010.
[3] X. Y. Zhao, M. R. Pinto, L. M. Hardison, J. Mwaura, J. Muller, H. Jiang, D. Witker, V. D. Kleiman, J. R. Reynolds, K. S. Schanze, *Macromolecules* **2006**, *39*, 6355–6366.
[4] B. M. Aveline, S. Matsugo, R. W. Redmond, *J. Am. Chem. Soc.* **1997**, *119*, 11785–11795.
[5] T. C. Barros, S. Brochsztain, V. G. Toscano, P. Berci, M. J. Politi, *J. Photochem. Photobiol. A* **1997**, *111*, 97–104.
[6] J. E. Rogers, L. A. Kelly, *J. Am. Chem. Soc.* **1999**, *121*, 3854–3861.
[7] M. A. Rodrigues, S. Brochsztain, T. C. Barros, M. S. Baptista, M. J. Politi, *Photochem. Photobiol.* **1999**, *70*, 35–39.

[8] J. E. Rogers, S. J. Weiss, L. A. Kelly, *J. Am. Chem. Soc.* **2000**, *122*, 427–436.
[9] B. Abraham, S. McMasters, M. A. Mullan, L. A. Kelly, *J. Am. Chem. Soc.* **2004**, *126*, 4293–4300.
[10] K. M. Figueiredo, R. O. Marcon, I. B. Campos, I. L. Nantes, S. Brochsztain, *J. Photochem. Photobiol. B, Biol.* **2005**, *79*, 1–9.
[11] S. Matsugo, L. J. Yan, D. Han, L. Packer, *Biochem. Biophys. Res. Commun.* **1995**, *206*, 138–145.
[12] I. B. Campos, I. L. Nantes, M. J. Politi, S. Brochsztain, *Photochem. Photobiol.* **2004**, *80*, 518–524.
[13] S. Matsugo, S. Kawanishi, K. Yamamoto, H. Sugiyama, T. Matsuura, I. Saito, *Angew. Chem. Int. Ed.* **1991**, *30*, 1351–1353.
[14] S. Matsugo, K. Kodaira, I. Saito, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1671–1674.
[15] M. Nakajima, T. Takeuchi, K. Ogino, K. Morimoto, *Jpn. J. Cancer Res.* **2002**, *93*, 247–252.
[16] M. Tomasulo, D. M. Naistat, A. J. P. White, D. J. Williams, F. M. Raymo, *Tetrahedron Lett.* **2005**, *46*, 5695–5698.
[17] A. J. Zych, B. L. Iverson, *Helv. Chim. Acta* **2002**, *85*, 3294–3300.
[18] G. J. Gabriel, S. Sorey, B. L. Iverson, *J. Am. Chem. Soc.* **2005**, *127*, 2637–2640.
[19] E. J. Gabbay, R. DeStefano, C. S. Baxter, *Biochem. Biophys. Res. Commun.* **1973**, *51*, 1083.
[20] S.-F. Yen, E. J. Gabbay, W. D. Wilson, *Biochemistry* **1982**, *21*, 2070–2076.
[21] F. A. Tanious, S.-F. Yen, W. D. Wilson, *Biochemistry* **1991**, *30*, 1813–1819.
[22] S. Takenaka, M. Yokoyama, H. Kondo, *Chem. Commun.* **1997**, 115–116.
[23] S. Bevers, S. Schutte, L. W. McLaughlin, *J. Am. Chem. Soc.* **2000**, *122*, 5905–5915.
[24] D. A. Gianolio, L. W. McLaughlin, *Bioorg. Med. Chem.* **2001**, *9*, 2329–2334.
[25] O. Y. Fedoroff, M. Salazar, H. Y. Han, V. V. Chemeris, S. M. Kerwin, L. H. Hurley, *Biochemistry* **1998**, *37*, 12367–12374.
[26] H. Y. Han, R. J. Bennett, L. H. Hurley, *Biochemistry* **2000**, *39*, 9311–9316.
[27] S. Sato, H. Kondo, T. Nojima, S. Takenaka, *Anal. Chem.* **2005**, *77*, 7304–7309.
[28] S. Sato, S. Fujii, K. Yamashita, M. Takagi, H. Kondo, S. Takenaka, *J. Organomet. Chem.* **2001**, *637*, 476–483.
[29] M. M. Murr, M. T. Harting, V. Guelev, J. S. Ren, J. B. Chaires, B. L. Iverson, *Bioorg. Med. Chem.* **2001**, *9*, 1141–1148.
[30] V. Guelev, J. Lee, J. Ward, S. Sorey, D. W. Hoffman, B. L. Iverson, *Chem. Biol.* **2001**, *8*, 415–425.
[31] V. Guelev, S. Sorey, D. W. Hoffman, B. L. Iverson, *J. Am. Chem. Soc.* **2002**, *124*, 2864–2865.
[32] J. Lee, V. Guelev, S. Sorey, D. W. Hoffman, B. L. Iverson, *J. Am. Chem. Soc.* **2004**, *126*, 14036–14042.
[33] Y. J. Chu, S. Sorey, D. W. Hoffman, B. L. Iverson, *J. Am. Chem. Soc.* **2007**, *129*, 1304–1311.
[34] V. Steullet, D. W. Dixon, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2935–2940.
[35] D. W. Dixon, N. B. Thornton, V. Steullet, T. L. Netzel, *Inorg. Chem.* **1999**, *38*, 5526–5534.
[36] A. Okamoto, T. Nakamura, K. Yoshida, K. Nakatani, I. Saito, *Org. Lett.* **2000**, *2*, 3249–3251.
[37] J. B. H. Tok, J. Fenker, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2987–2991.
[38] A. Mokhir, B. Zohm, A. Fuessl, R. Kraemer, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2489–2492.
[39] J. Michel, K. Bathany, J. M. Schmitter, J. P. Monti, S. Moreau, *Tetrahedron* **2002**, *58*, 7975–7982.
[40] D. A. Vicic, D. T. Odom, M. E. Nuñez, D. A. Gianolio, L. W. McLaughlin, J. K. Barton, *J. Am. Chem. Soc.* **2000**, *122*, 8603–8611.
[41] M. E. Nuñez, K. T. Noyes, D. A. Gianolio, L. W. McLaughlin, J. K. Barton, *Biochemistry* **2000**, *39*, 6190–6199.
[42] F. D. Lewis, R. L. Letsinger, M. R. Wasielewski, *Acc. Chem. Res.* **2001**, *34*, 159–170.
[43] T. Takada, K. Kawai, M. Fujitsuka, T. Majima, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 14002–14006.
[44] F. D. Lewis, M. R. Wasielewski, *Top. Curr. Chem.* **2004**, *236*, 45–65.
[45] S. Nakajima, K. Akiyama, K. Kawai, T. Takada, T. Ikoma, T. Majima, S. Tero-Kubota, *ChemPhysChem* **2007**, *8*, 507–509.
[46] S. Takenaka, Y. Uto, M. Takagi, H. Kondo, *Chem. Lett.* **1998**, *10*, 989–990.
[47] N. C. Tansil, H. Xie, F. Xie, Z. Q. Gao, *Anal. Chem.* **2005**, *77*, 126–134.
[48] M. Komatsu, T. Nojima, S. Takenaka, *Electrochemistry* **2006**, *74*, 65–67.
[49] V. Steullet, D. W. Dixon, *J. Chem. Soc. Perkin Trans.* **1999**, *2*, 1547–1558.
[50] C. J. Zhong, W. S. Kwan, L. L. Miller, *Chem. Mater.* **1992**, *4*, 1423–1428.
[51] C. Sissi, L. Lucatello, A. P. Krapcho, D. J. Maloney, M. B. Boxer, M. V. Camarasa, G. Pezzoni, E. Menta, M. Palumbo, *Bioorg. Med. Chem.* **2007**, *15*, 555–562.
[52] G. M. Kheifets, N. V. Martyushina, *Zh. Org. Khim.* **1982**, *18*, 1528–1535.
[53] G. M. Kheifets, N. V. Martyushina, *Zh. Org. Khim.* **1982**, *18*, 1537–1544.
[54] H. Langhals, H. Jaschke, H. Bastani-Oskoui, M. Speckbacher, *Eur. J. Org. Chem.* **2005**, *20*, 4313–4321.
[55] E. F. Bondarenko, V. A. Shigalevskii, *Zh. Org. Khim.* **1986**, *22*, 1282–1286.
[56] H. Langhals, P. von Unold, *Angew. Chem. Int. Ed.* **1995**, *34*, 2234–2236.
[57] P. Ponce, L. Fomina, F. Perez, S. Fomine, *J. Mol. Struct.—Theochem.* **2001**, *541*, 131–139.
[58] S. Brochsztain, M. J. Politi, *Langmuir* **1999**, *15*, 4486–4494.
[59] T. C. Barros, I. M. Cuccovia, J. P. S. Farah, J. C. Masini, H. Chaimovich, M. J. Politi, *Org. Biomol. Chem.* **2006**, *4*, 71–82.
[60] M. L. Ernst, G. L. Schmir, *J. Am. Chem. Soc.* **1966**, *88*, 5001–5009.
[61] S. Schuetz, J. Kurz, H. Pluempe, M. Bock, H. Otten, *Arzneim. Forsch.* **1971**, *21*, 739–763.
[62] S. Erten, Y. Posokhov, S. Alp, S. Icli, *Dyes Pigments* **2005**, *64*, 171–178.