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Novel Naphthalene Diimides as Activatable Precursors of Bisalkylating Agents, by Reduction and Base Catalysis

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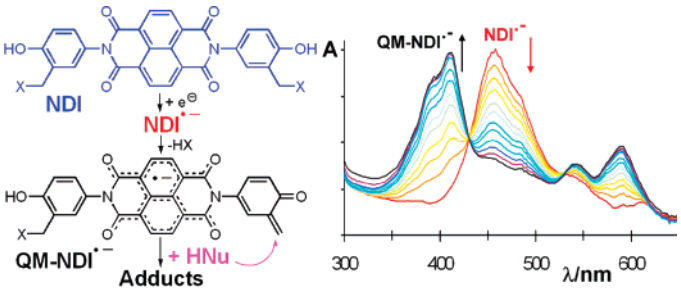

Mild activation of water-soluble naphthalene diimides (NDIs) as bisalkylating agents has been achieved by base catalysis and by chemical and electrochemical reductions. NDI activation by a single electron reduction represents a novelty in the field of activatable electrophiles. Under mild reduction, induced by $S_2O_4^{2-}$ in aqueous solution, the resulting NDI radical anion ($NDI^{\bullet-}$) undergoes a monomolecular fragmentation to yield a new transient species, where the NDI radical anion is tethered to a quinone methide moiety. The latter still retains electrophilic properties, reacting with amines, thiols, and ethyl vinyl ether. Owing to the NDI recognition properties, these results represent the first step toward selective and bioactivatable cross-linking agents.

Introduction

Derivatives of 1,8-naphthalimide, 1,4,5,8-naphthalene tetracarboxylic diimides (NDIs), and perylene analogues have been extensively studied in recent decades owing to their interesting photophysical properties[1] and their applications as molecular probes with recognition properties toward guanine-rich oligonucleotides by intercalation or end-stacking.[2] In many cases, such binding interactions function to stabilize duplex, triplex,[2c] or tetraplex structures, and it may contribute to stabilize complexes designed for gene-specific regulation of protein expression, as well as therapeutic agents.[3] Several naphthalimide-containing compounds have been described as photoinduced DNA cleaving agents,[4] and some of them entered into clinical trials owing to their strong anticancer activity.[5] Selective covalent modification of nucleotides has been achieved by tethering these naphthalimides with molecular moieties exhibiting alkylating reactivity.[6] On the other hand, several groups[7−10]

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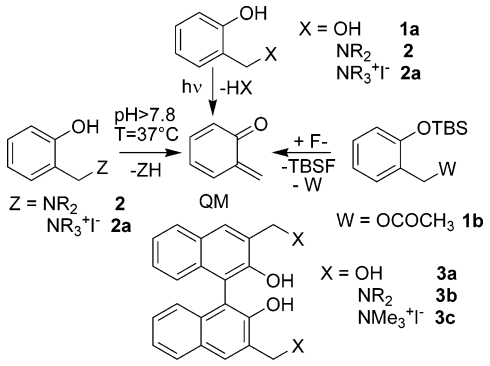
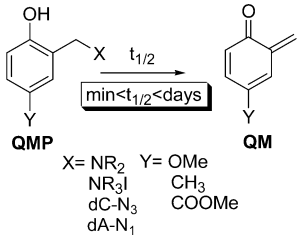
SCHEME 1. Generation of QMs from Phenol and Binol Derivatives



SCHEME 2. Substituent Effects on QM Precursor (QMP) Reactivity

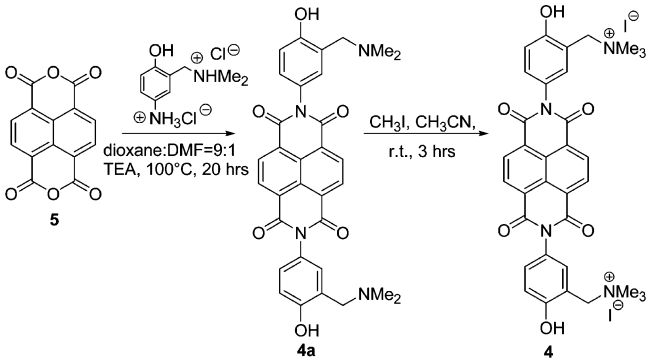


have recently shown that *o*-hydroxy benzylic alcohols (**1**), Mannich base derivatives of phenols (**2**), and binols (**3**) are capable of undergoing mono- and bisalkylation in water and DNA cross-linking by photoactivation,[8,10] base catalysis, and thermal activation under physiological conditions or in the presence of fluoride anion (Scheme 1).[9]

The key intermediates involved in these processes are transient quinone methides (QMs). The formation of a QM had previously demonstrated a dependence on the leaving group (Z or W) attached to the benzylic position of its precursor (QMP) when studying a model QM in the presence of biological nucleophiles. Furthermore, the generation of QMs has been shown to be highly responsive to the presence of electron-withdrawing and -donating groups (Y, in Scheme 2). Electron-donating groups greatly facilitate QM generation, while electron-withdrawing groups strongly suppress its formation.[11]

In the present work, we report the synthesis and the reactivity of bisalkylating QMPs directly tethered to the NDI (**4** and **4a**, Scheme 3). The latter moiety may act simultaneously as the molecular recognition structural element and substituent on a QMP, with electron donor/acceptor properties tunable by reduction (owing its redox properties) and upon base catalysis (due to its acidity). In fact, it is well-known that naphthalene diimide derivatives can be easily reduced to a radical anion by electrochemical means.[12]

SCHEME 3. Synthesis of 4



Results and Discussion

Synthesis. Attempts to elaborate the coupling procedure between the anhydride **5** and (5-amino-2-hydroxybenzyl)-trimethyl ammonium iodide, according to published procedure, were stymied by the thermal instability of the quaternary ammonium salts. In fact, it is known that quaternary ammonium salts of Mannich bases easily decompose into free QM and amine in the presence of electron-donating groups on the aromatic ring.[11] Consequently, at the beginning, the adduct **4** was synthesized by a three-step procedure starting from the coupling of the anhydride **5** to *p*-aminophenol, followed by Mannich reaction and CH_3I methylation. Due to solubility problems of the intermediate imide and also to poor yield in the Mannich reaction step (mainly due to the formation of both mono- and bis-CH_2NMe_2 adducts), the adduct **4** was synthesized in higher yield by a two-step procedure, starting from the coupling of the anhydride **5** to the preformed Mannich base 4-amino-2-dimethylaminomethylphenol generated in situ from its dichloride salt, followed by methylation (Scheme 3).

After the synthesis of **4** was achieved, we began to investigate the activation of the NDI **4** as bisalkylating agents under mild conditions. Two different activation protocols have been used: (i) base catalysis and (ii) a chemical reduction using $S_2O_4^{2-}$. In both cases, we performed the reaction of **4** at both 25 and 40 °C in the presence of several nucleophiles (thiols and amines) in aqueous solutions.

Base Catalytic Activation. Activation by base catalysis takes advantage of the low pK_a (8) of the quaternary ammonium salts of the Mannich bases, which allows the generation of a reactive zwitterionic form at pH ≥ 8, which generates alkylating QM (Scheme 4).

Basic amines such as piperidine, pyrrolidine, and diethylamine (pK_a > 10.7) gave almost quantitative conversion under mild conditions (25 °C, in 1:1 CH_3CN/H_2O; reaction condition A; see Experimental Section). Amines displaying lower basicity (pK_a ≤ 8), such as morpholine and L-proline methyl ester, require higher temperature (40 °C), carbonate-buffered conditions (pH ≥ 8.5, reaction condition B) and longer reaction times

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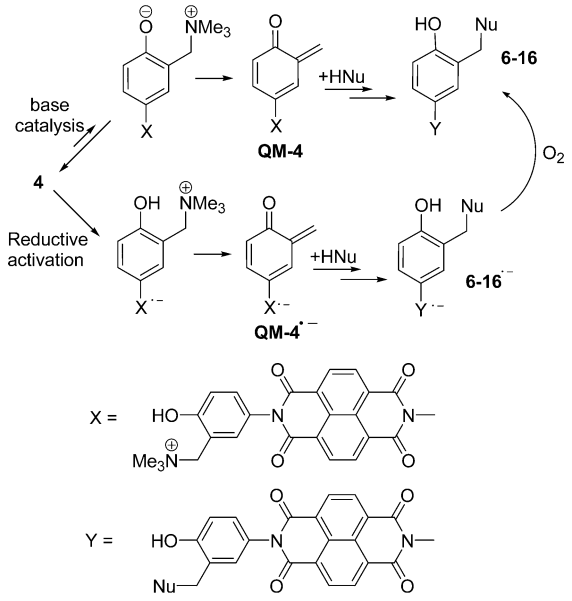
SCHEME 4. Base Catalytic and Reductive Activation of 4 as Bisalkylating Agent through the Generation of QM-4 and QM-4•−



(Table 1). **4** is much less reactive in the absence of basic nucleophiles, affording the diol **6** in low yield only in the presence of carbonate buffer at pH 8.5. In the presence of aniline (pK_a 4.6), adduct **16** formation was detected at 40 °C after 24 h, only under buffered conditions. In the absence of good nitrogen nucleophiles, the reaction was sluggish, and in addition to unreacted starting material, an uncharacterized oligomeric product was formed. Monoalkylated adducts were not detected by HPLC under the reaction condition described, and therefore, if they formed at all, their yields were very low (i.e., <5%).

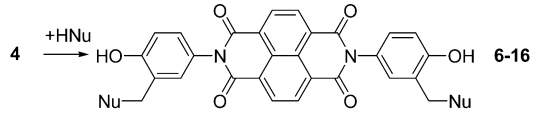
The results described above suggest that the activation of **4** as bisalkylating agent at rt is efficient when the phenol is mainly deprotonated. This suggests that, similar to the prototype quaternary ammonium salt **2a**, also for the QMP **4**, the zwitterionic form has to be populated to lower the barrier for the QM generation.

Reductive Activation. The second activation procedure should switch the electron properties of the NDI moiety from strongly withdrawing to donating by monoelectronic reduction, with formation of the NDI radical anion. Very interestingly, the activation of **4** as bisalkylating agent became even more facile under chemical reduction by dithionite anion ($S_2O_4^{2-}$), both in the absence and in the presence of nucleophiles, yielding the adducts **6**−**16**, after incubation at 25 °C, followed by workup with O_2. Oxygen oxidizes the adduct generated as radical anions (**6**$^{\bullet-}$−**16**$^{\bullet-}$) to the stable adducts (**6**−**16**), also quenching the excess of dithionite anion (Scheme 4).

The conversions into adducts **6**−**16** were obtained at lower temperature and shorter reaction time in comparison to the thermal incubations in the absence of dithionite. Data in Table 1 show that the reductive condition activates the bisalkylation process by diimide **4**. Such an activation is effective also with less basic nucleophiles such as L-proline methyl ester and aniline. Thus the diimide **4** does not react with aniline in aqueous acetonitrile at 40 °C for 1 day, but it undergoes bisalkylation under $S_2O_4^{2-}$ reducing conditions. **4a**, unlike **4**, is stable under both base catalysis and $S_2O_4^{2-}$ reduction.

The reactivity as bisalkylating agent of the NDI **4** is related to its favorable redox properties, which were characterized by

TABLE 1. Reactivity of 4 by Base Catalysis and $S_2O_4^{2-}$ Reduction



adduct	Nu	base catalyzed activation	reductive activation
		Conditions, (%, Yield)[a]	Conditions, (%, Yield)[b]
6	HO-	4; 40; (15)[c]	2; 25; (55)
		2; 25; (-)[c, d]	
7	Et_2N-	1; 40; (85)[e]	0.5; 25; (86)
		0.5; 25; (10)[e]	
8	Et, N, Bu	0.5; 40; (90)[e]	0.5; 25; (90)
		0.5; 25; (24)[e]	
9	iPr_2N-	0.5; 40; (90)[e]	0.5; 25; (93)
10	O, N	1; 40; (75)[c]	0.5; 25; (95)
		0.5; 25; (16)[c]	
11	*t*-BuNH-	4; 40; (65)[e]	0.5; 25; (70)
12	N	1; 40; (95)[e]	0.5; 25; (90)
13	N	1; 40; (85)[e]	0.5; 25; (90)
14	COOMe, N	4; 40; (65)[c]	l; 25; (75)
		1; 25; (10)[c]	
15	*t*-BuS-	0.5; 40; (85)[c]	0.5; 25; (80)
16	PhNH-	24; 40; (20)[c]	24; 40; (56)
		24; 40; (-)[e, d]	

[a] Reaction time/h, *T*/°C, CH_3CN/H_2O = 1:1, [**4**] = 5×10^{-3} M, [HNu] = 5×10^{-2} M. [b] Reaction time/h, *T*/°C, CH_3CN/H_2O = 1:1, in the presence of $Na_2S_2O_4$ 10^{-2} M, [**4**] = 5×10^{-4} M, [HNu] = 5×10^{-3} M. Reaction yields (%) are reported in parentheses. [c] Condition B, carbonate buffered (pH ≥ 8.5), as reported in the Experimental Section. [d] Undetected by HPLC. [e] Condition A, not buffered.

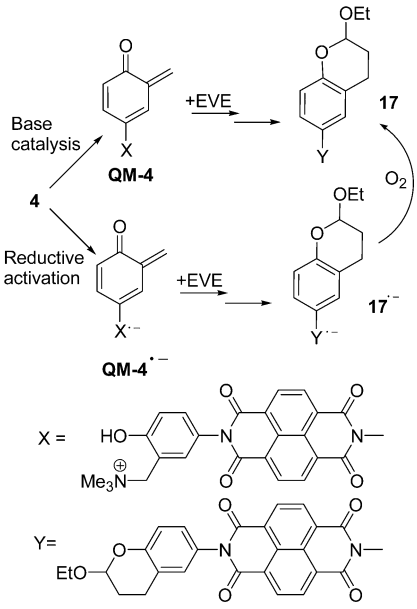
TABLE 2. Redox Properties of the NDI Derivatives 4 and 4a

	DMF Bu_4NBF_4 0.1 M		CH_3CN/H_2O = 1:1 Bu_4NBF_4 0.1 M	
NDI	$E°_{1/2}$[a]	$E°_{1/2}$[b]	$E°_{1/2}$[a]	$E°_{1/2}$[b]
4	−0.40	−0.85	−0.32	≤−0.65
4a	−0.46	−0.96	−0.44	≤−0.85

[a] First wave potential (NDI/NDI$^{\bullet-}$). [b] Second wave potential (NDI$^{\bullet-}$/NDI^{2-}). $E_{1/2}$ values in volts were taken as the center of the anodic and cathodic peak potentials vs Ag/AgCl/KCl (4 M KCl saturated with AgCl).

cyclic voltammetry (Table 2). Both the amine **4a** and its quaternary ammonium salt **4** exhibit two one-electron reduction peaks. In DMF, using Bu_4NBF_4, compound **4a**, used as dichloride salt, showed two redox couples at −0.46 and −0.96 V (vs Ag/AgCl/KCl). Although the first wave is perfectly reversible (in the sense that the peak separation for the reductions is exactly 60 mV), the second reduction is slightly less reversible (peak separation 120 mV). Apparent *E*° values in Table 2 were taken as the center of the anodic and cathodic peak potentials and are consistent with those reported for other aromatic

SCHEME 5. Reactivity of QM-4 and QM-4•− as Heterodiene in a [4 + 2] Cycloaddition



imides.[12b] Compounds **4**, with quaternary ammonium groups, had reduction potentials at slightly more positive potentials, −0.40 and −0.85 V, but both reduction processes were strongly irreversible, with dependence on the sweeping rate (peak separation >180 and 300 mV at 200 mV/s). Although in aqueous solution both substrates are more easily reduced (Table 2), the compounds **4a** and **4** behave similarly to that described for the DMF solution. The process is chemically reversible for the amine **4a** and chemically irreversible for the quaternary ammonium **4**. In fact, after a few redox cycles, the amine **4a** was recovered unreacted, unlike the quaternary ammonium **4** which was completely consumed, with diol **6** as the only detectable adduct (20%, yield). After a few redox cycles in a 1:1 acetonitrile/water solution containing morpholine (10^{-2} M), the quaternary ammonium **4** was converted into the adduct **10** (yield >80%).

Diels−Alder Reactivity of the Transient Electrophile. In order to clarify the nature of the electrophilic intermediate involved in the alkylation process, we ran two set of experiments in the presence of EVE (ethyl vinyl ether), under basic catalysis and reductive activation. **4** gave the adduct **17** as the main adducts under (i) basic conditions (pH 9, CH_3CN/H_2O) at 40 °C and (ii) in the presence of $S_2O_4^{2-}$ at rt, pH 7, followed by oxygen quenching (Scheme 5).

The formation of the adducts **6**−**17** by base catalysis is rationalized by the generation of a transient electrophilic QM (**QM-4**) from the reactive zwitterionic form, followed by nucleophile (Scheme 4) or EVE trapping in a hetero-Diels−Alder [4 + 2] cycloaddition (Scheme 5). The formation of the same adducts **6**−**17** by reduction, following oxygen quenching, reveals that it is possible to switch the electron properties of the NDI as a substituent to a QMP, from an electron-withdrawing to -donating group simply by monoelectronic reduction. In this way, it has been possible to generate a QM directly tethered to a NDI radical anion (**QM-4**•−), which retains electrophilicity at the exocyclic methylene group and heterodiene reactivity as the prototype *o*-QM.

Detection of the Transients by UV−Vis Spectroscopy. The QM generation by base catalysis had been previously rationalized,[10a]

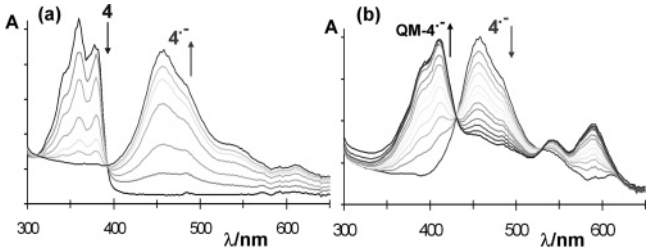


FIGURE 1. Generation from 0 to 20 min (a) and reactivity from the following 2 h (b) of **4**•− monitored by UV−visible spectroscopy.

TABLE 3. Activation Free Energy in the Gas Phase and in Aqueous Solution at the R(U)B3LYP/6-31+G(d,p) Level of Theory for the Generation of the QMs Starting from 18, Its Anion 18−, and Its Radical Anion 18•−

QMP	ΔE^a	$\Delta G_{gas}{}^b$	ΔG_{aq}
18	37.0	32.1	46.5[c]
			41.1[d]
			41.4[e]
18−	3.7	1.0	*f*
			8.5[d]
			8.5[e]
18•−	3.8	1.4	17.0[c]
			6.3[d]
			6.7[e]

[a] Activation electronic energies in kcal/mol. [b] Activation free energies in the gas phase. [c] Optimized in aqueous solution at R(U)B3LYP/6-31+G(d,p) using PCM (UA0 radii) solvation model. [d] Single-point calculation at B3LYP/6-31+G(d,p) on gas-phase geometries (UA0 radii). [e] Single-point calculation at B3LYP/6-31+G(d,p) on gas-phase geometries (UAHF radii). [f] We failed to locate the **TS-18**•− in aqueous solution.

thus we decided to corroborate the generation of a similar electrophilic intermediate by chemical reduction, producing further independent evidence. To this end, we managed to achieve the activation of **4** as bisalkylating agent by coulombometric reduction at $E = -0.5$V [vs Ag/AgCl/KCl (4 M KCl saturated with AgCl)]. In fact, under these conditions, the salt **4** was efficiently consumed, affording the diol **6** as the only detectable adduct in aqueous acetonitrile after O_2 workup. A similar experiment performed with morpholine (10^{-2} M) afforded only the adduct **10** in quantitative yield. The electrochemical reduction was also monitored as a function of time by UV−visible spectroscopy, which revealed two sequential transient species on the time scale of a few hours. The reduction of **4** in 1:1 CH_3CN/H_2O bleached the reactant absorbance (360 and 378 nm), generating a new species with maximum absorbance centered at 458 nm (Figure 1a). Such a transient was assigned to the radical anion of the diimide **4** (**4**•−) on the basis of (i) its rapid quenching in the presence of O_2, (ii) the spectroscopic similarity to NDI radical anions, bearing cationic substituents to the imide nitrogen atom [such as $-(CH_2)_nNMe_3^+$ (λ_{max} 449 nm)],[12b] generated by electrochemical reduction and by intramolecular photoinduced single electron transfer.[13] The radical anion **4**•− decays with a first-order kinetic ($k_1 = 8.1 \times 10^{-4}$ s^{-1}, $r^2 = 0.99$), in the absence of O_2, to generate another species with absorbance centered at 412, 542, and 590 nm (Figure 1b).

This UV−vis spectrum displays the features of both the radical anion NDI•− (542 and 590 nm) and the *ortho*-QM (410 nm).[10a] This species decays within a few hours under aqueous

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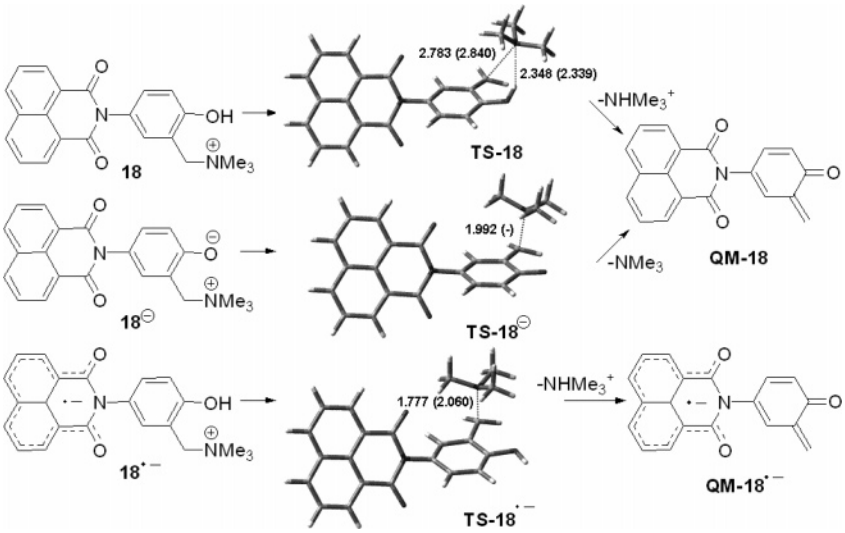
SCHEME 6. Generation of QM-18 and QM-18•− through the TSs TS-18, TS-18−, and TS-18•− (Bond Lengths are in Angstroms; Data in Parentheses are for Full R(U)B3LYP/6-31+G(d,p) Optimization in Aqueous Solution)



acetonitrile, in the absence of O_2, and it is rapidly quenched by O_2, morpholine, and EVE. The electrolyzed solution of **4** in the presence of morpholine (10^{-3} M) and EVE (10^{-2} M), after 30 min at rt and workup with O_2, gave the adducts **10** and **17**, respectively, in good yields (≥85%), with no hydration adduct **6** detected. Therefore, we assigned the absorbance of this species to a QM tethered to a NDI$^{\bullet-}$ (QM-**4**$^{\bullet-}$), displaying benzylating reactivity. Similarly, the NDI **4a** can be easily reduced to its radical anion (**4a**$^{\bullet-}$) with λ_{max} = 473 nm, which unlike **4**$^{\bullet-}$ is stable in the presence of nucleophiles in aqueous acetonitrile. Such an evidence is in accord with the lack of reactivity of **4a** in preparative experiments.

Computational Evaluation of the Basic and Reductive Activation Processes. The final proof that NDIs tethered to quaternary ammonium salts of a Mannich bases are activatable QMP by both base catalysis and monoelectronic reduction was given by a computational investigation at the R(U)B3LYP/6-31+G(d,p) level of theory both in the gas phase and in aqueous solution (by PCM solvation model) on the model imide **18** (Scheme 6).

The activation free energies computed both in the gas phase and in aqueous solution (Table 3) suggest that the generation of an alkylating QM (QM-18) becomes a much easier process, passing from the protonated quaternary ammonium salt **18** to its zwitterionic form **18**$^{-}$. The catalytic effect of the base is massive both in the gas phase and in solution (>30 kcal/mol). The reduction of the naphthalimide moiety to its radical anion **18**$^{\bullet-}$, which generates the QM tethered to the imide moiety **QM-18**$^{\bullet-}$, also induces a similar activation, lowering the barrier in the gas phase by 35 kcal/mol. Geometry optimization in the solvent bulk of the **TS-18** and **TS-18**$^{\bullet-}$ suggests a slightly lower catalytic effect since the activation free energy in aqueous solution is reduced by 29.5 kcal/mol. We were unable to optimize **TS-18**$^{-}$ in aqueous bulk; therefore, for this TS, we only compute the solvation by single-point calculation on B3LYP/6-31+G(d,p) gas-phase geometry.

Conclusion

In conclusion, we have described the activatable bisalkylating properties of the NDI moiety tethered to a quaternary ammonium salt of a Mannich base, exploring two protocols: (i) classic base catalysis and (ii) mild reduction. The involvement of QM as key electrophile of the bisalkylating process has been supported by product distribution analysis with nucleophiles and EVE and other methods, including UV−vis spectroscopy, electrochemical reduction, and computational modeling. The creation of a QMP requiring activation by single electron reduction represents a novelty in the field of the mild generation of QMs. In addition, owing to the NDI recognition properties, these results suggest bioapplications of **4** and its derivatives as triggerable and selective cross-linking agents toward guanine-rich oligonucleotides.[14]

Experimental Section

***N,N′*-Bis[3-(dimethylamino)methyl-4-hydroxyphenyl]-1,4,5,8-naphthalenetetracarboxylic diimide (4a):** 2.0 g of 4-amino-2-[(dimethylamino)methyl]phenol dihydrochloride, prepared according published procedure,[15] (8.4 mmol) and 1.07 g (4 mmol) of 1,4,5,8-tetracarboxylic dianhydride were suspended in 20 mL of a 9:1 mixture of dioxane/DMF. TEA (1 mL) was added to the suspension, and the mixture was allowed to reflux with vigorous stirring for 20 h under nitrogen. The reaction mixture was cooled and poured into 30 mL of water. The resulting suspension was filtered and washed first with water and then twice with anhydrous EtOH. The *N,N′*-bis[3-(dimethylamino)methyl-4-hydroxy]-1,4,5,8-naphthalenetetracarboxylic diimide was obtained as a pale green solid: mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 12H, Me_2), 3.60 (s, 4H, CH_2), 6.90 (d, 2H, J = 7.4 Hz), 7.20 (d, 2H, J = 7.4 Hz), 7.25 (s, 2H), 8.70 (s, 4H), 9.70 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 41.87, 54.43, 116.05, 116.68, 126.24, 126.64, 126.90, 130.55, 131.66, 132.93, 156.74, 163.03. Anal. Calcd for $C_{32}H_{28}N_4O_6$: C, 68.07; H, 5.00; N, 9.92; O, 17.00. Found: C, 68.14; H, 4.99; N, 9.90.

***N,N′*-Bis[3-(trimethylamino)methyl-4-hydroxyphenyl]-1,4,5,8-naphthalenetetracarboxylic diimide iodide (4):** 2.0 g (3.54 mmol) of diimide **4a** was suspended in CH_3CN (50 mL), and CH_3I (1.2 g, 8.5 mmol) was added. This suspension, refluxing under nitrogen, turned a dark red color in a few minutes. After 3 h, the reaction was chilled and Et_2O (50 mL) was added with formation of red crystals. The suspension was filtered and washed twice with CH_3-

(14) Reactivity of **4** and its duplex−quadruplex selectivity is under investigation.

(15) Werbel, M. *J. Med. Chem.* **1983**, *26*, 1258−1267.

CN to give **4** (2.5 g, 3 mmol, 84% yield) as a red-brownish solid: mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 3.1 (s, 18H), 4.5 (s, 4H), 7.10 (d, 2H, J = 8.6 Hz), 7.4 (d, 2H, J = 8.6 Hz), 7.50 (s, 2H), 8.80 (s, 4H), 10.9 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 52.04, 62.81, 114.84, 116.43, 126.34, 126.63, 126.97, 130.53, 132.60, 134.82, 157.35, 163.07. Anal. Calcd for $C_{34}H_{34}I_2N_4O_6$: C, 48.13; H, 4.04; I, 29.91; N, 6.60; O, 11.31. Found: C, 48.18; H, 4.02; I, 29.80; N, 6.63.

Activation by Base Catalysis. **General Procedure. Base Catalysis General Method:** The bisalkylating properties of the diimide **4** have been activated by base catalysis in the presence of several nucleophiles (amines and thiols), according the following procedures:

Procedure A (For nucleophile with pK_a > 8): To a solution of **4** (50 mL, CH_3CN/H_2O = 1:1, 5 × 10^{-3} M) oxygen-purged with a N_2 flux was added the nucleophile in order to reach a 5 × 10^{-2} M concentration. This solution was allowed to stand at 25 or 40 °C and after ∼0.5 h, a pale yellow solid begins to form. After a few hours, the solution was concentrated under reduced pressure and the resulting suspension was cooled at 4 °C for 12 h. The solid was filtered and washed with cold anhydrous EtOH to provide the bisalkylated adducts **6**−**9** and **11**−**13** in good yield (60−85%).

Procedure B (For nucleophile with pK_a < 8): To 50 mL of a solution of 5 × 10^{-3} M of the diimide **4** in CH_3CN/H_2O (1:1) buffered to pH 8.5 with a $Na_2CO_3/NaHCO_3$ buffer, outgassed with a N_2 flux, was added the nucleophile in order to reach a concentration of 5 × 10^{-2} M. This solution was heated at 40 °C for 12 h. After this time, the CH_3CN was evaporated under vacuum and the suspension was kept at 4 °C for 12 h. The solid was filtered and washed twice with cold water and anhydrous EtOH to provide the bisalkylated adducts **10** and **14**−**17** in good yield (55−70%).

Activation by $S_2O_4^{2-}$ Reduction. General Procedure. In order to trap the nucleophiles by reductive activation of **4**, a procedure similar to the base catalytic method was used. However, lower concentration of substrate is required in order to avoid π-stacking between **4** and its radical ion.

To 20 mL of an oxygen-purged solution of the diimide **4** (5 × 10^{-4} M) was added the nucleophile in order to reach a concentration of 5 × 10^{-3} M and 2 mL of a 0.1 M $Na_2S_2O_4$ solution. The resulting violet solution was allowed to stand at 40 °C for 4 h. After this time, the solution was chilled and treated with an air flux for 10 min in order to consume the unreacted $S_2O_4^{2-}$. The CH_3CN was evaporated under vacuum, and the product precipitated as a pale yellow solid.

Adduct 6: Pale yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 4.40 (s, 4H), 7.20 (d, 2H, J = 8.6 Hz), 7.4 (d, 2H, J = 8.6 Hz), 7.5 (s, 2H), 8.8 (s, 4H), 10.8 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 64.6, 115.9, 117.2, 126.2, 126.9, 127.7, 131.0, 131.4, 132.9, 156.7, 163.1. Anal. Calcd for $C_{28}H_{18}N_2O_8$: C, 65.88; H, 3.55; N, 5.49; O, 25.07. Found: C, 65.77; H, 3.58; N, 5.45.

Adduct 7: Yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (t, 12H), 2.75 (q, 8H), 3.70 (s, 4H), 6.90 (d, 2H, J = 9.2 Hz), 7.25 (d, 2H, J = 9.2 Hz), 7.25 (s, 2H), 8.6 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 11.2, 45.9, 54.8, 115.5, 123.2, 126.1, 126.6, 127.0, 128.4, 128.8, 130.39, 157.5, 163.1. Anal. Calcd for $C_{36}H_{36}N_4O_6$: C, 69.66; H, 5.85; N, 9.03; O, 15.47. Found: C, 69.69; H, 5.84; N, 9.10.

Adduct 8: Yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 12H), 1.10 (t, 12H), 1.30 (q, 8H), 1.5 (m, 8H), 2.75 (m, 8H), 3.75 (s, 4H), 6.75 (d, 2H, J = 8.8 Hz), 7.1 (d, 2H, J = 8.8 Hz), 7.15 (s, 2H), 8.7 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 10.9, 13.8, 20.0, 28.2, 46.4, 52.0, 55.4, 115.5, 123.2, 126.06, 126.5, 127.0, 128.4, 128.9, 130.4, 157.5, 163.1. Anal. Calcd for $C_{40}H_{44}N_4O_6$: C, 70.99; H, 6.55; N, 8.28; O, 14.18. Found: C, 71.02; H, 6.58; N, 8.25.

Adduct 9: Pale yellow needles; mp > 350 °C; ^{1}H NMR ($CDCl_3$) δ 2.1 (d, 12H), 2.5 (m, 2H), 3.75 (s, 4H), 6.9 (s, 2H), 7.0 (d, 2H, J = 8.6 Hz), 7.2 (d, 2H, J = 8.6 Hz), 8.8 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 30.8, 44.5, 62.5, 115.5, 117.0, 122.6, 125.1, 127.0, 127.9, 128.4, 131.3, 158.8, 163.2. Anal. Calcd for $C_{40}H_{44}N_4O_6$: C, 70.99; H, 6.55; N, 8.28; O, 14.18. Found: C, 71.09; H, 6.53; N, 8.21.

Adduct 10: Yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 20H), 6.90 (d, 2H, J = 8.6 Hz), 7.20 (d, 2H, J = 8.6 Hz), 7.25 (s, 2H), 8.75 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 52.8, 58.0, 66.1, 115.4, 122.8, 126.3, 126.6, 127.0, 128.5, 129.7, 130.4, 156.3, 163.1. Anal. Calcd for $C_{36}H_{32}N_4O_8$: C, 66.66; H, 4.97; N, 8.64; O, 19.73. Found: C, 66.73; H, 5.00; N, 8.59.

Adduct 11: Characterized as hydrochloride (**11**·2HCl); green needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 2.75 (s, 18H), 4.30 (s, 4H), 7.20 (d, 2H, J = 8.6 Hz), 7.4 (d, 2H, J = 8.6 Hz), 7.5 (s, 2H), 8.8 (s, 4H), 10.0 (s, 4H), 10.8 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 25.1, 41.9, 54.6, 115.8, 116.0, 126.3, 126.6, 126.9, 130.9, 131.7, 132.9, 156.7, 163.0. Anal. Calcd for $C_{36}H_{38}Cl_2N_4O_6$: C, 62.34; H, 5.52; Cl, 10.22; N, 8.08; O, 13.84. Found: C, 62.29; H, 5.61; Cl, 10.19; N, 8.06.

Adduct 12: Yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 12H), 3.5 (m, 8H), 3.85 (s, 4H), 6.80 (d, 2H, J = 8.8 Hz), 7.25 (d, 2H, J = 8.8 Hz), 7.25 (s, 2H), 8.8 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 25.5, 30.7, 53.3, 59.5, 115.5, 122.7, 126.3, 126.5, 126.90, 126.94, 127.0, 130.4, 156.3, 163.7. Anal. Calcd for $C_{38}H_{36}N_4O_6$: C, 70.79; H, 5.63; N, 8.69; O, 14.89. Found: C, 70.73; H, 5.65; N, 8.70.

Adduct 13: Yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 2.00 (m, 8H), 3.10 (m, 4H), 3.5 (m, 4H), 4.3 (s, 4H), 7.1 (d, 2H, J = 8.6 Hz), 7.35 (d, 2H, J = 8,6 Hz), 7.50 (s, 2H), 8.7 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 22.5, 51.5, 52.9, 116.0, 117.7, 126.3, 126.6, 126.9, 130.6, 131.4, 132.5, 156.4, 163.0. Anal. Calcd for $C_{36}H_{32}N_4O_6$: C, 70.12; H, 5.23; N, 9.09; O, 15.57. Found: C, 70.09; H, 5.25; N, 9.07.

Adduct 14: Yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 2.0 (m, 4H), 2.1 (m, 2H), 2.4 (m, 2H), 3.2 (m, 2H), 3.5 (m, 2H), 3.7 (m, 2H), 3.9 (s, 6H), 4.1 (s, 4H), 7.1 (d, 2H, J = 8.65 Hz), 7.35 (d, 2H, J = 8.65 Hz), 7.50 (s, 2H), 8.7 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 23.2, 29.4, 52.0, 52.6, 57.6, 65.0, 116.1, 117.2, 126.1, 126.4, 126.8, 130.7, 131.9, 132.6, 156.4, 163.0, 173.5. Anal. Calcd for $C_{40}H_{36}N_4O_{10}$: C, 65.57; H, 4.95; N, 7.65; O, 21.84. Found: C, 65.53; H, 4.99; N, 7.61.

Adduct 15: Yellow needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 18H), 4.30 (s, 4H), 7.20 (d, 2H, J = 8.8 Hz), 7.4 (d, 2H, J = 8.8 Hz), 7.5 (s, 2H), 8.8 (s, 4H), 10.8 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 30.2, 45.3, 60.3, 115.8, 116.0, 126.3, 126.6, 126.9, 130.9, 131.7, 132.9, 156.7, 163.0. Anal. Calcd for $C_{36}H_{34}N_2O_6S_2$: C, 66.03; H, 5.23; N, 4.28; O, 14.66; S, 9.79. Found: C, 66.09; H, 5.25; N, 4.23; S, 9.73.

Adduct 16: Characterized as hydrochloride (**16·2HCl**); pale green needles; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 18H), 4.30 (s, 4H), 6.8 (m, 6H), 7.10 (m, 4H), 7.20 (d, 2H, J = 8.7 Hz), 7.4 (d, 2H, J = 8.7 Hz), 7.5 (s, 2H), 8.8 (s, 4H), 10.0 (s, 2H), 10.8 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 55.3, 115.0, 115.8, 116.0, 118.4, 126.3, 126.6, 126.9, 129.3, 130.6, 131.0, 132.8, 147.0, 157.0, 163.1. Anal. Calcd for $C_{40}H_{30}Cl_2N_4O_6$: C, 65.49; H, 4.12; Cl, 9.67; N, 7.64; O, 13.09. Found: C, 65.43; H, 4.16; Cl, 9.69; N, 7.62.

Adduct 17: Yellow solid; mp > 350 °C; ^{1}H NMR (DMSO-d_6) δ 1.3 (t, 6H), 2.00 (m, 4H), 2.70 (m, 2H), 3.0 (m, 2H), 3.7 (m, 2H), 4.0 (m, 2H), 5.4 (dd, 2H, J = 2.7 Hz), 7.1 (d, 2H, J = 8.6 Hz), 7.35 (d, 2H, J = 8.6 Hz), 7.50 (s, 2H), 8.70 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 15.1, 22.5, 51.5, 52.9, 116.0, 117.7, 126.3, 126.6, 126.9, 130.6, 131.4, 132.5, 156.4, 163.0. Anal. Calcd for $C_{36}H_{30}N_2O_8$: C, 69.89; H, 4.89; N, 4.53; O, 20.69. Found: C, 69.94; H, 4.90; N, 4.49.

Electrochemical Measurements. Cyclic voltammetry (CV) was performed using an Amel 433/W polarographic analyzer equipped with a standard three-electrode cell with a platinum disk electrode as working electrode (2.0 mm diameter), a platinum wire as auxiliary electrode, and a Ag/AgCl/KCl (4 M KCl saturated with AgCl) reference electrode. All potentials in the text are reported versus Ag/AgCl/KCl (4 M KCl saturated with AgCl).

In degassed DMF using Bu_4NBF_4 (0.1 M), with a sweep rate = 100 mV s^{-1}, although the processes for both the compounds were not perfectly reversible, **4a** showed a more reversible behavior with a peak separation of 70 ± 10 and 120 ± 15 mV for the first and the second process, respectively (Supporting Information). A similar behavior has been found also in 0.1 M Bu_4NBF_4, CH_3CN/H_2O = 1:1, at 100 mV s^{-1}; in this case, ill-defined second reduction, especially for **4**, was obtained. As expected, less negative peak potentials have been found in CH_3CN:H_2O, with respect to DMF.

Coulometric reductions were performed on a BAS 100B/W Version 2.3 and a BAS C3 cell stand with a 200 mL glass cell, using a carbon sponge working electrode, a platinum wire as auxiliary electrode set in a separated quartz tube, and a Ag/AgCl/KCl (4 M KCl saturated with AgCl) reference electrode in degassed CH_3CN/H_2O = 1:1 solutions. Reduction was performed for the amine **4a** and its ammonium quaternary salt **4** at −600 and −500 mV, respectively. The formation of the anion radicals and their decay have been monitored as a function of time, recording the UV−vis spectra on a HP-8452A DAD spectrophotometer with a 661-500-QX Quarz Tauchsonde with 10 mm path length, HELLMA.

Methods and Computation Details. All calculations were carried out using revision D.02 of the Gaussian 03 program package.[16] The geometric structures of the reactants (**18**, **18**$^-$, and **18**$^{\bullet-}$) and the transition states (**TS-18**, **TS-18**$^-$, and **TS-18**$^{\bullet-}$) located were fully optimized in the gas phase using the hybrid density functional B3LYP[17] with the 6-31+G(d,p) basis set. It is known that diffuse functions are mandatory for a reliable evaluation of anion energies, and in our reactive system, a zwitterionic character is present in both the reactants **18**$^-$ and **18**$^{\bullet-}$ and related TSs (TS-18$^-$ and TS-18$^{\bullet-}$). Thermal contributions (δG) to activation free energy (ΔG; see Supporting Information) were computed from B3LYP/6-31G(d) structures and harmonic frequencies, by using the harmonic oscillator approximation and the standard expressions for an ideal gas in the canonical ensemble at 298.15 K and 1 atm. The optimization of the stationary points in the solvent bulk was calculated via the self-consistent reaction field (SCRF) method using PCM[18] as implemented in the D.02 version of Gaussian 03. The geometry optimizations in water solution for **TS-18** and **TS-18**$^{\bullet-}$ have been quite difficult; therefore, to solve the problem, the "loose" convergence criteria on geometry optimization were adopted. We were unable to optimize **TS-18**$^-$ in aqueous bulk; therefore, for this TS, we compute the solvation by single-point calculation on B3LYP/6-31+G(d,p) gas-phase geometry. The cavity is composed by interlocking spheres centered on non-hydrogen atoms with UA0 radii. Such a model includes the nonelectrostatic terms (cavitation, dispersion, and repulsion energy) in addition to the classical electrostatic contribution.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR for the NDIs **4**, **4a**, and the adducts **6**−**17**, electrochemical data (Figures S1 and S2), Cartesian coordinates of the reactants (**18**, **18**$^-$, and **18**$^{\bullet-}$), and TSs (**TS-18**$^-$ and **TS-18**$^{\bullet-}$) at the R(U)B3LYP/6-31+G(d,p) level (gas phase and aqueous solution). This material is available free of charge via Internet at http://pubs.acs.org.

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