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Halogenation and DNA cleavage via thermally stable arenediazonium camphorsulfonate salts

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ABSTRACT

A series of stable arenediazonium camphorsulfonate salts (**2a**–**2j**) were synthesized by simple diazotization of several aromatic amines in the presence of sodium nitrite and camphorsulfonic acid. All the new arenediazonium camphorsulfonates, which were characterized by multinuclear (^{1}H and ^{13}C) NMR, IR, DSC, and X-ray diffraction analysis (**2e** and **2f**) provide unambiguous proof for the molecular structures of **2e** and **2f**. The efficient application of these salts in halogenation reactions was studied in solvent and solvent-free conditions and the DNA cleavage activity was also assessed. These arenediazonium camphorsulfonate salts are noticed as efficient DNA cleaving agents.

1. Introduction

Arenediazonium salt reagents are required for a wide variety of classical organic reactions[1–7] and for many synthetic applications including the preparation of organic nano-compounds, the surface modification of carbon, and metal semiconductors.[8–14] Matsuda–Heck,[15] Suzuki–Miyaura,[16,17] Stille,[18] and Mizoroki–Heck[1,19] cross-coupling and carbonylation reactions are powerful synthetic applications associated with diazonium salts due to their mildness, simplicity, and high reactivity. They also provide economical alternatives to expensive aryl iodides and bromides. Moreover, diazonium salts have found applications in Gomberg–Bachmann–Hey[20] and Pschorr reactions and are also known to couple with fluorine-containing dicarbonyl compounds yielding corresponding hydrazones that can be used to fluorinate heterocyclic compounds with antifungal and anti-inflammatory activity.[21] The dediazotization of salt typically generates aryl cations, which are used in fluorination, photolithography, and DNA cleavage.[22–24]

Although extremely efficient, the main constraint for the use of diazonium salts in synthetic applications is their instability and high sensitivity toward heat, light, and shock that can lead to uncontrollable decomposition and even explosion. Thus, previous reports describe the synthesis of stable, explosion-proof diazonium salts with high applicability in organic reactions. The most well-recognized and stable diazonium salts are the arene tetrafluoroborates,[25] hexafluorophosphates,[26] arenediazonium *o*-benzenedisulfonimides,[27] and arenediazonium trifluoroacetates.[28]

Recently, we reported the synthesis of an exceptionally stable arenediazonium tosylate[29] with versatile synthetic applications.[30,31] In this study, we extended the research on diazonium salt synthesis and application by attempting to synthesize new arenediazonium camphorsulfonate salts and found that the resulting salts could be applied in halogenation reactions. The new diazonium salts were also studied for their biological applications in DNA cleavage reactions.

2. Results and discussion

Herein, we introduce a new series of novel arenediazonium camphorsulfonate salts (Scheme 1) of high purity and stability that were synthesized by a simple and appropriate method. Several aromatic amines were diazotized with camphorsulfonic acid and sodium nitrite in glacial acetic acid to produce arenediazonium camphorsulfonate salts **2a**–**2j**. Upon the addition of diethyl ether to

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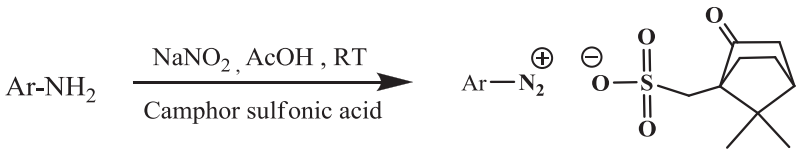


1 2

Here ; **1, 2**: **a**, Ar = 4-$NO_2C_6H_4$; **b**, Ar = 3-$NO_2C_6H_4$; **c**, Ar = 2-NO_2-C_6H_4; **d**, Ar = 4-CNC_6H_4; **e**, Ar = 2-CNC_6H_4; **f**, Ar = 4-BrC_6H_4; **g**, Ar = 2-BrC_6H_4; **h**, Ar =4-ClC_6H_4; **i**, Ar = 4-IC_6H_4; **j**, Ar = 4-$CH_3OC_6H_4$

Scheme 1. Synthesis of arenediazonium camphorsulfonate salts **2**.

the reaction mixture, pure diazonium salt precipitated immediately at an average 94% yield (Table 1). The optimal molar ratio of amine, camphorsulfonic acid, and sodium nitrite was 1:1.1:1.1.

Table 1
Arenediazonium camphorsulfonate salts **2**

Entry	Substrate	Yield of **2** (%)	Mp (°C)
1	4-$NO_2C_6H_4NH_2$ (**1a**)	97	118–122
2	3-$NO_2C_6H_4NH_2$ (**1b**)	95	118–126
3	2-$NO_2C_6H_4NH_2$ (**1c**)	92	138–140
4	4-$CNC_6H_4NH_2$ (**1d**)	95	106–110
5	2-$CNC_6H_4NH_2$ (**1e**)	92	148–150
6	4-$BrC_6H_4NH_2$ (**1f**)	94	140–146
7	2-Br C_6H_4 NH_2 (**1g**)	91	94–104
8	4-$ClC_6H_4NH_2$ (**1h**)	92	118–128
9	4-$IC_6H_4NH_2$ (**1i**)	91	136–138
10	4-$CH_3OC_6H_4NH_2$ (**1j**)	87	166–168

Salt formation was first observed by an azo coupling reaction with β-naphthol to yield (2-hydroxy-1-naphthyl)aryldiazene. IR, ^1H and ^{13}C NMR spectral results (Supplementary data) confirmed the structures of the newly synthesized products, and the structures of **2e** and **2f** were also established by single crystal X-ray analysis.

Thermal stability of these salts was tested by DSC between 0 and 600 °C under a N_2 atmosphere and no explosive activity was observed. The high stability and solubility of the new synthesized arenediazonium camphorsulfonate salts in protic as well as aprotic solvents identify them as very interesting chemical probes for various organic transformations.

To investigate stability of arenediazonium camphorsulfonate, **2e** and **2f** crystallized with suitable quality for X-ray diffraction were characterized by X-ray crystallography using synchrotron radiation. A close inspection of crystal structures reveals that one independent diazonium unit of arenediazonium is surrounded by sulfonate anions of two independent camphorsulfonates (Fig. 1). The close contact distances of **2e** and **2f** are 2.80 Å (O1/O3···N2)/ 2.55 Å (O1/O3···N1) and 2.77 Å (O1/O3···N2)/2.87 Å (O1/O3···N1) on average, respectively, which are shorter than the sum of van der Waals radii (2.90 Å) (Figs. 1 and 2). Therefore, we assume that exceptionally high thermal stability of arenediazonium camphorsulfonate is attributed to charge–charge interactions.

The reactivity of arenediazonium camphorsulfonate salts against bromination was tested by the reaction of **2d** with KBr in water by stirring the reaction mixture for 60 min at room temperature. The desired product was isolated in 71% yield attest the reactivity of arenediazonium camphorsulfonate salts for bromination reaction.

In order to avoid the isolation of pure diazonium salts we have attempted for halogenation of amine by utilizing in situ synthesis of diazonium salts. A variety of aromatic amines were efficiently transformed into their halogenated products in high yield via in situ formation of camphorsulfonate diazonium salts in both solvent and solvent-free grinding processes. These salts effectively reacted at room temperature with KI, TBAB, and BTAC, respectively, in solvent (Scheme 2, Table 2) or solvent-free conditions (Scheme 2, Table 3) to yield the corresponding halogenated products in high yields.

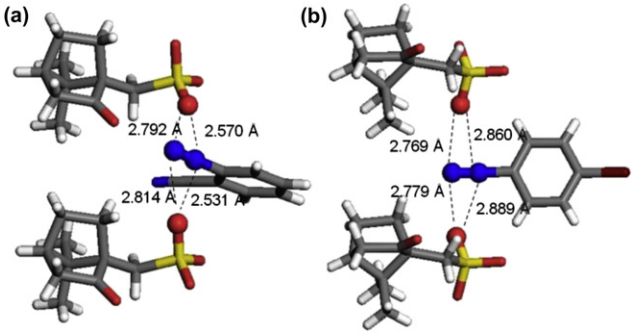


Fig. 1. X-ray crystal structures of **2e** (a) and **2f** (b). The close contact distances are marked with a ball model of atoms; (a) O1···N2: 2.792 Å, O3···N2: 2.814 Å, O1···N1: 2.570 Å, O3···N1: 2.531 Å; (b) O3···N2: 2.769 Å, O3···N2: 2.779 Å, O3···N1: 2.860 Å, O1···N1; 2.889 Å.

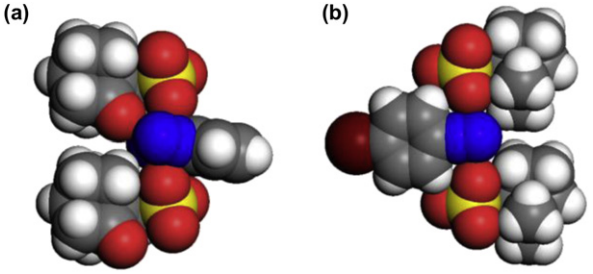
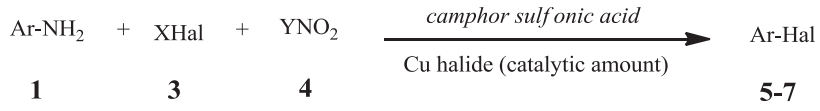


Fig. 2. X-ray crystal structures of **2e** (a) and **2f** (b) with a space filling model.

2.1. DNA cleavage activity

A survey of the literature revealed that diazonium salts are known to generate carbon-centered radicals that may directly modify DNA bases and/or cleave DNA strands and can potentially be used as anticancer drugs. To investigate the biological application, we assessed the DNA cleavage activity of these salts with exciting results. To confirm whether arenediazonium camphorsulfonate salts could convert supercoiled DNA (SC, form I) to the nicked circular relaxed (NC, form II) or linear (form III) DNA forms in vitro, 0.1,



Here ; **1, 5-7**: **a**, Ar = 4-$NO_2C_6H_4$; **b**, Ar = 3-$NO_2C_6H_4$; **c**, Ar = 2-NO_2-C_6H_4; **d**, Ar = 4-CNC_6H_4;
e, Ar = 2-CNC_6H_4; **f**, Ar = 4-BrC_6H_4; **g**, Ar = 2-BrC_6H_4;
h, Ar = 4-ClC_6H_4; **i**, Ar = 4-IC_6H_4; **j**, Ar = 4-$CH_3OC_6H_4$; **k**, Ar = 2-$CH_3OC_6H_4$;
l, Ar = 4-NO_2-2-$CH_3OC_6H_3$; **m**, Ar = 2-NO_2-4-$CH_3OC_6H_3$
3 : KI, tertabutylammonium bromide(TBAB), benzyltriethylammonium chloride(BTAC)
4 : *t*-butyl nitrite, sodium nitrite **5** : Hal = I **6** : Hal = Br **7**: Hal = Cl

Scheme 2. Halogenation of aryl amines in solvent and solvent-free conditions.

Table 2
Halogenation of aryl amines via arenediazonium salts as intermediates using camphorsulfonic acid

Entry	Substrate	Yield of **5**[a,d] (%)	Yield of **6**[a] (%)	Yield of **7**[a,g] (%)
1	4-$NO_2C_6H_4NH_2$ (**1a**)	100[b]	91[e]	74
		92[c]	82[f]	
2	3-$NO_2C_6H_4NH_2$ (**1b**)	58[b]	77[e]	65
			79[f]	
3	2-$NO_2C_6H_4NH_2$ (**1c**)	73[b]	83[e]	57
		83[c]	83[f]	
4	4-$NCC_6H_4NH_2$ (**1d**)	81[b]	78[e]	67
		81[c]	75[f]	
5	2-$NCC_6H_4NH_2$ (**1e**)	53[b]	64[e]	63[b]
		53[c]	72[f]	
6	4-$BrC_6H_4NH_2$ (**1f**)	69[b]	71[e]	43
		83[c]	71[f]	
7	4-$ClC_6H_4NH_2$ (**1h**)	68[b]	61[e]	4
			61[f]	
8	4-$IC_6H_4NH_2$ (**1i**)	71[b]	90[e]	33
		85[c]	64[f]	
9	4-$CH_3OC_6H_4NH_2$ (**1j**)	61[b]	76[e]	0
			9[f]	
10	2-$CH_3OC_6H_4NH_2$ (**1k**)	58[b]	98[e]	33
			0[f]	
11	4-NO_2–2-CH_3O–$C_6H_3NH_2$ (**1l**)	99[b]	94[e]	67
			74[f]	
12	2-NO_2–4-CH_3O–$C_6H_3NH_2$ (**1m**)	85[b]	83[e]	21
			40[f]	

[a] Isolated yield.
[b] $NaNO_2$, 24 h at rt, CH_3COOH.
[c] $NaNO_2$, 12 h at rt, CH_3COOH.
[d] *tert*-Butyl nitrite without Cu catalyst.
[e] *tert*-Butyl nitrite, CH_3CN, 24 h at rt in the presence of $CuBr_2$.
[f] CH_3CN, 24 h at rt without $CuBr_2$.
[g] *tert*-Butyl nitrite, CH_3CN, 24 h at rt in the presence of $CuCl_2$.

Table 3
Solvent-free bromination and chlorination of aryl amines via arenediazonium salts as the intermediate using camphorsulfonic acid

Entry	Substrate	Yield of **6**[a] (%)	Yield of **7**[a] (%)
1	4-$NO_2C_6H_4NH_2$ (**1a**)	79[b]	62[d]
		71[c]	
2	3-$NO_2C_6H_4NH_2$ (**1b**)	81[b]	65[d]
		59[c]	
3	2-$NO_2C_6H_4NH_2$ (**1c**)	73[b]	59[d]
		56[c]	
4	4-$NCC_6H_4NH_2$ (**1d**)	74[b]	37[d]
		70[c]	67[b]
5	2-$NCC_6H_4NH_2$ (**1e**)	58[b]	13[d]
		46[c]	
6	4-$BrC_6H_4NH_2$ (**1f**)	61[b]	24[d]
		21[c]	
7	4-$ClC_6H_4NH_2$ (**1h**)	48[b]	10[d]
		23[c]	
8	4-$IC_6H_4NH_2$ (**1i**)	88[b]	61[d]
		9[c]	
9	4-$CH_3OC_6H_4NH_2$ (**1j**)	73[b]	16[d]
		0[c]	
10	4-NO_2–2-CH_3O–$C_6H_3NH_2$ (**1l**)	84[b]	70[d]
		87[c]	
11	2-NO_2–4-CH_3O–$C_6H_3NH_2$ (**1m**)	71[b]	50[d]
		46[c]	

[a] Isolated yield, *tert*-butyl nitrite, reaction time within 1 h.
[b] In the presence of $CuBr_2$.
[c] Without $CuBr_2$.
[d] In the presence of $CuCl_2$.

0.2, 0.3, 0.5, and 1.0 mM arenediazonium camphorsulfonate salts were added to SC pUC19 DNA (Fig. 3). The first lane in Fig. 3a contained pUC19 DNA to demonstrate the electrophoresis pattern of unaltered DNA. Upon addition of 0.1 mM complex **2a**, no significant DNA cleavage was observed (lane 1); however, significant DNA cleavage activity was observed at 0.2, 0.3, and 0.5 mM complex **2a** as to the bands corresponding to nicked circular (NC) DNA (form II) were more intense as the complex concentration increased. At 1.0 mM, 100% DNA cleavage activity occurred as evidenced by a total disappearance of DNA bands (data not shown). To further confirm the behavior of arenediazonium camphorsulfonate with supercoiled DNA, a time dependent study was conducted. In the presence of 0.3 mM complex **2a**, the linear DNA (form III) was produced within 30 min (Fig. 3b). Interestingly, within 5 min, 0.3 mM complex **2a** converted SC DNA (form I) to NC DNA (II) by 80%. These results suggest that the newly synthesized arenediazonium camphorsulfonate initiated SC DNA cleavage very quickly. Similarly, DNA cleavage activities were assessed for the other complexes **2b**–**2h** at a fixed 0.5 mM concentration. The expected DNA cleavage activity was observed in all complexes (Fig. 3c).

The results suggested that the newly synthesized arenediazonium salts were able to cleave DNA. We used radiolabeled single-strand oliognucleotide (49 nt), linear duplex (19 bp), and forked duplex (34 bp and 15 nt) to detect the cleavage activity of complex **2f**. As shown in Fig. 4, any cleavage was not detected in the three substrates. In earlier study[22] of the reaction of arenediazonium salts with DNA resulted in DNA cleavage upon activation with cuprous salts or day light. On the basis of the results obtained in this study, the plausible mechanism could be suggested that the DNA cleavage mechanism is photochemical and probably hydrolytic cleavage of an intermediate phosphate trimester.[32] We carried out a cleavage reaction with pUC19 supercoiled plasmid and complex **2f**. The reactions were analyzed on an alkaline agarose gel to distinguish nicked circular plasmid from relaxed circular (open circular) plasmid. As shown in Fig. 5, supercoiled form (denatured duplex circle, sc) was decreased with the increasing concentration of complex, whereas cleaved DNAs with fast migration appeared. The cleaved DNAs are due to multiple nicks on both DNA strands of supercoiled Plasmid. No additional reagents, metals or electrochemistry are required for the reaction. Arenediazonium salts in aqueous solutions are known to be converted on illumination into

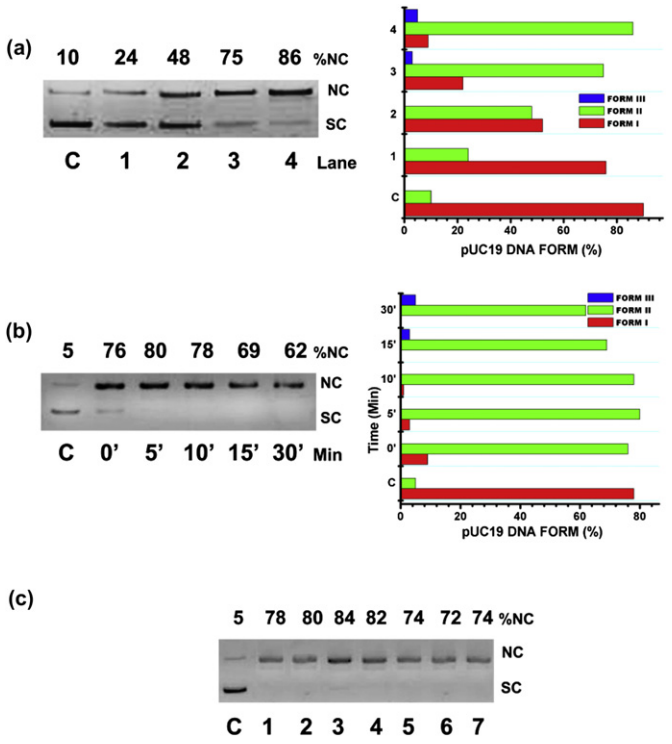


Fig. 3. (a) Gel electrophoresis diagrams showing cleavage of SC pUC19 DNA (5.0 μg) by diazonium salts in 0.1 M phosphate buffer. Lane C: control DNA, lanes 1–4: DNA+0.1, 0.2, 0.3, and 0.5 mM complex **2a**, respectively. (b) SC pUC19 DNA with 0.3 mM complex **2a** at 0, 5, 10, 15 and 30 min incubation showing DNA cleavage. (c) Lane C, control DNA; lanes 1 through 7 contain DNA+0.4 mM complex **2b** through **2h**, respectively.

aryl cations,[33] which are capable of behaving as alkylating agents. The alkylation of purine bases on DNA is used to trigger DNA cleavage or hydrolyze DNA both strands simultaneously, a camphorsulfonate may serve as very efficient vector to bring the molecular groups close to DNA.

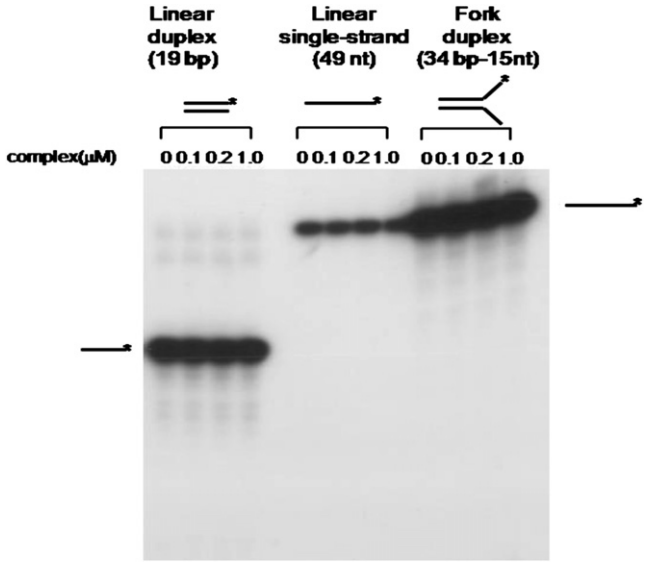


Fig. 4. For each substrates, a single oligonucleotide was labeled at the 5′-phosphate end with [γ-^{32}P]ATP. Radiolabeled DNAs (5 nM) were incubated with complex **2f** (0.1, 0.2, or 1 μM) in 10 mM phosphate buffer (pH 7.5) at room temperature for 15 min and then terminated with formamide stop dye. Samples were analyzed on a urea-denaturing polyacrylamide gel (16%). Autoradiography was obtained using Amersham Hyperfilm.

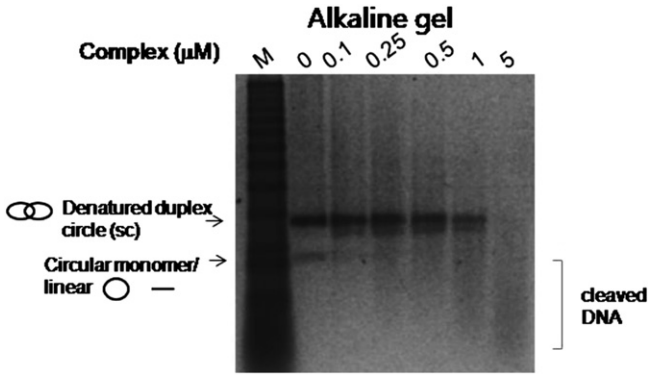


Fig. 5. pUC19 supercoiled circular DNA (140 ng) was treated with indicated amounts of complex **2f** in 10 μl of 10 mM phosphate buffer (pH 7.5) for 15 min at room temperature. Reactions were terminated by the addition of 6× alkaline electrophoresis loading buffer (180 mM NaOH, 6 mM EDTA, 18% Ficoll 400, 0.05% bromocresol green). Samples were heated at 70 °C for 5 min, then chilled on ice for 3 min and loaded on an agarose gel. Electrophoresis was run at 1 V/cm in alkaline electrophoresis buffer (30 mM NaOH, 2 mM EDTA) overnight. After electrophoresis, the gel was immersed in 100 ml of 0.5 M Tris–HCl buffer (pH 7.5) for 30 min and then was stained in a GelRed solution for 20 min. M; size marker-1 kb ladder.

The potential utility of these compounds is enormous and ranges from the generation of synthetic restriction enzymes for use by molecular biologists to the development of chemotherapeutic agents that might be effective against a variety of diseases including cancer.[34] Since DNA cleavage is one of the most important mechanisms to arrest the growth of bacteria and viruses, and is important in the control of cancer, there is considerable current interest in the development of reagents suitable for cleaving DNA.[35–37] Furthermore, natural products are most often shown to be toxic to cells; as such, great efforts have been directed toward the design of synthetic analogs capable of cleaving DNA in a similar manner without exhibiting the associated toxicity.[38]

3. Experimental

3.1. Materials and methods

The melting points were uncorrected. The IR spectra were recorded on Mattson (UNICAM). The ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer. IR spectra were recorded on Varian 2000 FT-IR spectrometer. Details for the reactions and yields of the pure isolated products are listed in Tables 1–3.

3.2. Determination of DNA cleavage activity

Plasmid pUC19 was extracted and the purity of pUC19 was confirmed via both agarose gel electrophoresis and UV spectroscopy by determining the ratio of absorbance at 260 nm to the absorbance at 280 nm. The concentration of DNA was determined from the absorbance at 260 nm (A_{260}=1.0 OD for 50 μg/ml). DNA cleavage activity of the diazonium complexes **2a**–**2h** was monitored using supercoiled pUC19 DNA as substrate and agarose gel electrophoresis for detection and quantification. The assay was performed in 20 μl of 0.1 M NaH_2PO_4/Na_2HPO_4 buffer pH 7.0 containing 5 μg DNA was treated with different concentrations of diazonium complexes **2a** (0 mM–0.5 mM). After incubation at room temperature for 10 min, the mixture was added to loading buffer (25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol) and loaded onto a 1.0% agarose gel containing 0.4 μg/ml ethidium bromide. Electrophoresis was conducted at 40 V for 30 min in TAE buffer. After electrophoresis, DNA cleavage activity was observed under UV light (254 nm) and photographed. The DNA in each

sample was quantified from band intensities using Bio-Rad Quantity One, version 2000 software. Similarly, supercoiled pUC19 DNA (5 μg) with 0.3 mM of **2a** with different time intervals was allowed to incubate and followed by agarose gel electrophoresis as described above.

3.3. Synthesis of arenediazonium camphorsulfonates 2

Sodium nitrite (1.1 mmol) was added at room temperature to a solution of camphorsulfonic acid (1.1 mmol) in AcOH (8 ml) and stirred for 5 min. The corresponding aniline (1.0 mmol) was added and the mixture was stirred for 5–20 min until TLC indicated the complete consumption of the aniline. The ether was poured into reaction solution to yield pure product. The resulting solid was filtered, washed by ether (20–40 ml), and dried under vacuum.

Caution! In our two laboratories there was no case of sudden decomposition during the preparation, purification, and handling of salts **2a**–**2j**. Nevertheless it must be kept in mind that in general diazonium salts in the dry state are potentially explosive. Therefore they must be carefully stored and handled.

*3.3.1. 4-Nitrobenzenediazonium camphorsulfonate (**2a**).* Cream yellow solid. Anal. Calcd for $C_{16}H_{19}N_3O_6S$: C, 50.39; H, 5.02; N, 11.02. Found: C, 50.16; H, 5.25; N, 11.31. ^{1}H NMR (300 MHz, CD_3OD) δ 8.95 (d, J=9.6 Hz, 2H), 8.74 (d, J=9.0 Hz, 2H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 218.4, 156.2, 135.9, 127.5, 122.4, 59.7, 48.6, 48.3, 44.2, 43.8, 27.9, 25.8, 20.6, 20.3; IR (cm^{-1}) 3471, 3270, 3106, 2959, 1741, 1599, 1521, 1347, 1240, 1172, 1040, 855, 749, 691, 518.

*3.3.2. 3-Nitrobenzenediazonium camphorsulfonate (**2b**).* Pale yellow solid. Anal. Calcd for $C_{16}H_{19}N_3O_6S$: C, 50.39; H, 5.02; N, 11.02. Found: C, 50.04; H, 5.19; N, 10.94. ^{1}H NMR (300 MHz, CD_3OD) δ 9.6 (s, 1H), 9.06 (d, J=8.4 Hz, 2H), 8.26 (t, J=8.4 Hz, 1H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 218.4, 148.5, 138.9, 136.5, 134.4, 129.5, 118.7, 59.7, 48.6, 48.3, 44.1, 43.8, 27.9, 25.9, 20.5, 20.2; IR (cm^{-1}) 3448, 3094, 2962, 1740, 1537, 1355, 1177, 1049, 670, 535.

*3.3.3. 2-Nitrobenzenediazonium camphorsulfonate (**2c**).* Cream yellow solid, Anal. Calcd for $C_{16}H_{19}N_3O_6S$: C, 50.39; H, 5.02; N, 11.02. Found: C, 50.45; H, 4.87; N, 11.27. ^{1}H NMR (300 MHz, CD_3OD) δ 9.13 (d, J=8.1 Hz, 1H), 8.87 (d, J=8.4 Hz, 1H), 8.59 (t, J=7.5 Hz, 1H), 8.42 (t, J=7.8 Hz, 1H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 218.4, 146.4, 143.3, 138.1, 137.8, 129.5, 113.8, 59.7, 48.6, 48.3, 44.1, 43.8, 27.9, 25.9, 20.5, 20.2; IR (cm^{-1}) 3456, 3087, 2959, 1738, 1550, 1455, 1191, 1042, 791, 601, 524.

*3.3.4. 4-Benzonitrilediazonium camphorsulfonate (**2d**).* Yellow solid. Anal. Calcd for $C_{17}H_{19}N_3O_4S$: C, 56.50; H, 5.30; N, 11.63. Found: C, 56.74; H, 5.08; N, 11.45. ^{1}H NMR (300 MHz, CD_3OD) δ 8.82 (d, J=8.7 Hz, 2H), 8.37 (d, J=8.7 Hz, 2H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 216.8, 134.8, 133.9, 123.8, 117.3, 112.0 58.2, 47.1, 46.8, 42.6, 42.3, 26.4, 24.4, 18.9, 18.7; IR (cm^{-1}) 3431, 3090, 2964, 2604, 2340, 1725, 1411, 1172, 1035, 855, 532.

*3.3.5. 2-Benzonitrilediazonium camphorsulfonate (**2e**).* Ivory brown solid. Anal. Calcd for $C_{17}H_{19}N_3O_4S$: C, 56.50; H, 5.30; N, 11.63. Found: C, 56.27; H, 5.02; N, 11.39. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.12 (s, 1H), 7.69 (d, J=7.8 Hz, 1H), 7.51 (d, J=7.8 Hz, 1H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 218.3, 142.9, 137.8, 136.7, 136.3, 134.5, 134.3, 130.6, 59.7, 48.6, 48.3, 44.2, 43.8, 27.9, 25.9, 20.6, 20.2; IR (cm^{-1}) 3702, 2925, 1730, 1574, 1436, 1206, 950, 760.

*3.3.6. 4-Bromobenzenediazonium camphorsulfonate (**2f**).* Yellow solid. Anal. Calcd for $C_{16}H_{19}BrN_2O_4S$: C, 46.27; H, 4.61; N, 6.75. Found: C, 45.97; H, 4.29; N, 6.47. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.55 (d, J=9.0 Hz, 2H), 8.23 (d, J=9.0 Hz, 2H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 216.9, 137.7, 134.9, 133.7, 114.4, 58.2, 47.1, 46.8, 42.6, 42.3, 26.4, 24.3, 19.0, 18.8; IR (cm^{-1}) 3473, 3120, 2958, 2620, 1744, 1555, 1529, 1156, 1044, 816, 616, 522.

*3.3.7. 2-Bromobenzenediazonium camphorsulfonate (**2g**).* Yellow solid. Anal. Calcd for $C_{16}H_{19}BrN_2O_4S$: C, 46.27; H, 4.61; N, 6.75. Found: C, 45.99; H, 4.30; N, 6.39. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.78 (d, J=8.4 Hz, 1H), 8.28 (d, J=8.1 Hz, 1H), 8.20 (t, J=8.0 Hz, 1H), 7.99 (t, J=8.0 Hz, 1H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 218.3, 143.6, 137.1, 136.6, 131.9, 126.2, 120.4, 59.6, 49.6, 48.3, 44.2, 43.7, 27.9, 25.9, 20.5, 20.2; IR (cm^{-1}) 3809, 3699, 2962, 1741, 1462, 1207, 1039, 766, 607.

*3.3.8. 4-Chlorobenzenediazonium camphorsulfonate (**2h**).* Brownish yellow solid. Anal. Calcd for $C_{16}H_{19}ClN_2O_4S$: C, 51.82; H, 5.16; N, 7.55. Found: C, 51.64; H, 5.37; N, 7.33. ^{1}H NMR (300 MHz, CD_3OD) δ 7.56 (m, 2H), 7.42 (m, 2H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 216.9, 138.1, 135.4, 132.9, 114.7, 59.6, 49.5, 48.2, 44.1, 43.7, 27.9, 25.9, 20.5, 20.2; IR (cm^{-1}) 3460, 2954, 2605, 2359, 1741, 1626, 1480, 1168, 1041, 809, 610.

*3.3.9. 4-Iodobenzenediazonium camphorsulfonate (**2i**).* Dark blue solid. Anal. Calcd for $C_{16}H_{19}IN_2O_4S$: C, 41.57; H, 4.14; N, 6.06. Found: C, 41.71; H, 4.32; N, 5.87. ^{1}H NMR (300 MHz, CD_3OD) δ 7.92 (d, J=7.8 Hz, 2H), 7.20 (d, J=8.4 Hz, 2H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 218.4, 140.7, 132.1, 126.4, 95.1, 59.7, 47.1, 46.8, 44.2, 43.7, 27.9, 25.9, 20.6, 20.3; IR (cm^{-1}) 3460, 2954, 2605, 2360, 1740, 1626, 1450, 1168, 1041, 808, 610.

*3.3.10. 4-Methoxybenzenediazonium camphorsulfonate (**2j**).* White solid. Anal. Calcd for $C_{17}H_{22}N_2O_5S$: C, 55.72; H, 6.05; N, 7.64. Found: C, 55.51; H, 6.23; N, 7.44. ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (d, J=6.9 Hz, 2H), 7.07 (d, J=6.6 Hz, 2H), 3.85 (s, 3H), 3.29 (d, J=14.7 Hz, 1H), 2.75 (d, J=14.7 Hz, 1H), 2.65–2.30 (m, 2H), 2.05 (m, 2H), 1.89 (d, J=18.3 Hz, 1H), 1.65–1.36 (m, 2H), 1.12 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 218.4, 161.6, 125.4, 124.4, 116.4, 59.7, 56.3, 49.6, 48.3, 44.2, 43.8, 27.9, 25.8, 20.6, 20.3; IR (cm^{-1}) 2957, 2636, 2064, 1741, 1624, 1514, 1185, 1043, 825, 606, 513.

3.4. Typical procedure for the subsequent bromination of arenediazonium camphorsulphonate salt 2d

3.4.1. 4-Bromobenzonitrile. Potassium bromide (0.52 g, 2.5 mmol) was added at room temperature to a solution of **2d** (0.36 g, 1.0 mmol) in water (10 ml), and the mixture was stirred for 60 min until a negative diazonium test with 2-naphthol. Pure 4-

bromobenzonitrile was precipitated and the precipitate was filtered and washed with water and the desired product was collected in 71% yield.

*3.4.2. Diazotization–halogenation to aryl iodide **5** via arenediazonium camphorsulfonate.* Potassium iodide (5.0 mmol) and sodium nitrite (3.0 mmol) was added at room temperature to a solution of aniline (2.5 mmol) and camphorsulfonic acid (3.0 mmol) in acetic acid (30 ml), and the mixture was stirred for 24 h. The evolution of N_2 was immediately observed. The solvent was removed by a rotary evaporator after completion of the reaction (confirmed by β-naphthol test and TLC). The solid was washed with water and extracted with CH_2Cl_2. The resulting solution was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The pure product was then collected by column chromatography using hexane/dichloromethane as eluting solvents. Physical and ^{1}H NMR data were identical to those of a commercially available sample of analytical purity.

*3.4.3. Diazotization–halogenation to aryl bromides **6** arenediazonium camphorsulfonate.* Catalytic amounts of copper bromide (1 mol %) were added to a solution of aniline (2.5 mmol) in acetonitrile (30 ml), camphorsulfonic acid (3.0 mmol), sodium nitrite or *tert*-butyl nitrite (3.0 mmol), and tetrabutylammonium bromide (5.0 mmol). The reaction mixture was stirred at 60 °C for 24 h (Tables 1 and 2). The evolution of N_2 was immediately observed. The solvent was removed by a rotary evaporator after completion of the reaction (confirmed by β-naphthol test and TLC). The solid was washed with water and extracted with CH_2Cl_2. The resulting solution was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The pure product was then collected by column chromatography using hexane/dichloromethane as eluting solvents. Physical and ^{1}H NMR data were identical to those of a commercially available sample of analytical purity.

*3.4.4. Diazotization–halogenation to aryl chlorides **7** arenediazonium camphorsulfonate.* Camphorsulfonic acid (3.0 mmol), *tert*-butyl nitrite (3.0 mmol), benzyltriethylammonium chloride (5.0 mmol), and catalytic amounts of copper chloride (1 mol %) were added to an acetonitrile solution (30 ml) of aniline (2.5 mmol). The reaction mixture was stirred at 60 °C for 24 h (Tables 1 and 2). The evolution of N_2 was immediately observed. The solvent was removed by rotary evaporator after completion of the reaction (confirmed by β-naphthol test and TLC). The crude residue was purified via column chromatography by using hexane/dichloromethane as the eluting solvent. Physical and ^{1}H NMR data were identical to those of a commercially available sample of analytical purity.

*3.4.5. Grinding method for diazotization–halogenation to aryl bromides **6** arenediazonium camphorsulfonate.* Into an agate mortar, aniline (1.25 mmol), camphorsulfonic acid (1.5 mmol), *tert*-butyl nitrite (3.0 mmol), TBAB (2.5 mmol), and a catalytic amount of copper(II) bromide (1 mol %) were added, and the mixture was ground vigorously. An immediate evolution of N_2 was observed. After completion of the reaction (confirmed by TLC), the solid was washed with water and extracted with CH_2Cl_2. The resulting solution was dried with anhydrous $MgSO_4$, and the solvent was removed in a rotary evaporator under reduced pressure. Further purification of the product was performed by column chromatography using hexane/dichloromethane as eluting solvents. All physical and ^{1}H NMR data of the products were identical to those from commercially available samples of analytical purity.

*3.4.6. Grinding method for diazotization–halogenation to aryl chlorides **7** arenediazonium camphorsulfonate.* Camphorsulfonic acid (1.5 mmol), *tert*-butyl nitrite (3.0 mmol), benzyltriethylammonium chloride (2.5 mmol), and a catalytic amount of copper (II) chloride (1 mol %) were combined in an agate mortar-containing aniline (1.25 mmol) and the mixture was vigorously ground for 15–20 min, until the evolution of N_2 completely stopped. After completion of the reaction (confirmed by TLC), the crude residue was worked up, as described for the aryl bromide reaction, and was purified by column chromatography using hexane/dichloromethane as eluting solvents. All physical and ^{1}H NMR data of the products were identical to those from commercially available samples of analytical purity.

3.5. X-ray diffraction studies

The diffraction quality crystals were obtained by vapor diffusion of diethyl ether in acetic acid solution of diazonium salts. The diffraction data for **2e** and **2f** were thus collected with synchrotron radiation (λ=0.70000 Å) at the Wiggler Beamline 4A, Pohang Accelerator Laboratory. Data reduction and adsorption correction were performed with HKL2000 package. The structures were solved by direct methods and refined by full-matrix least squares method with SHELXTL package. All the non-hydrogen atoms were refined anisotropically, and hydrogen atoms were added to their geometrically ideal positions.

*3.5.1. X-ray data for **2e**.* $C_{17}H_{19}N_3O_4S$, M=361.41, orthorhombic, $P2_12_12_1$ (no. 19), a=7.169(1) Å, b=8.185(2) Å, c=29.084(6) Å, V=1706.6(6) Å^3, Z=4, T=100 K, $\mu(\lambda$=0.70000 Å)=0.206 mm^{-1}, d_{calcd}=1.407 g/cm^3, 9559 reflections measured, 5046 unique (R_{int}=0.0735), R_1=0.0467, wR_2=0.1260 ($I>2\sigma(I)$), R_1=0.0470, wR_2=0.1262 (all data), GOF=1.094.

*3.5.2. X-ray data for **2f**.* $C_{16}H_{19}BrN_2O_4S$, M=415.30, Monoclinic, $P2_1$ (no. 4), a=10.569(2) Å, b=7.862(2) angst;, c=11.470(2) Å, V=903.0(3) Å^3, Z=2, T=100 K, $\mu(\lambda$=0.70000 Å)=2.403 mm^{-1}, d_{calcd}=1.527 g/cm^3, 4678 reflections measured, 2899 unique (R_{int}=0.0513), R_1=0.0723, wR_2=0.2149 ($I>2\sigma(I)$), R_1=0.0739, wR_2=0.2161 (all data), GOF=1.107.

CCDC 838649 (**2f**) and 838650 (**2e**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336 033; or deposit@ccdc.cam.ac.uk).

In summary, we report a series of newly synthesized stable arenediazonium camphorsulfonate salts that were characterized by NMR, IR, DSC, and X-ray analyses. We found that these salts are potential reagents for organic transformations in halogenation reactions under solvent or solvent-free conditions as well as having strong DNA cleavage activity under the physiological conditions studied. These newly synthesized arenediazonium camphorsulfonate salts may be capable of cleaving DNA specifically for biotechnological and medical applications.

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Supplementary data

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