Formaldehyde-Induced DNA Cross-Link of Indolizino[1,2-*b*]quinolines Derived from the A−D Rings of Camptothecin

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Camptothecin consists of a lactone E ring adjacent to tetracyclic A−D rings of a planar chromophore, which are essential for topoisomerase I inhibition and DNA interaction. The A−D rings can be exploited to develop DNA-sequence-reading molecules. Indolizino[1,2-*b*]-quinoline derivatives substituted with a piperidinoethyloxy side chain and an aminomethyl function on rings A and D, respectively, were synthesized, and their DNA binding and formaldehyde-mediated bonding properties were investigated.

Introduction

Camptothecin (CPT) is a monoterpene indole alkaloid that inhibits topoisomerase I, an essential enzyme for the regulation of DNA structures and functions. CPT binds strongly to the covalent topoisomerase I−DNA complex but shows little interaction with duplex DNA or topoisomerase I alone. However, CPT was initially reported to intercalate into poly(dGC),[1] and studies with the positively charged topotecan (TPT) and irinotecan (CPT-11) congeners, i.e., two clinically useful anticancer drugs of the CPT family, have established that DNA interactions play a role in their biological activity.[2,3] TPT displays a sequence preference of $(dG\text{-}dC)_{15}$ over $(dA\text{-}dT)_{15}$ and behaves as an intercalating agent in the absence of topoisomerase I.[2] Moreover, photoactivated CPT interacts specifically and intimately with guanine residues in double-stranded DNA.[4]

In the ternary topoisomerase I−CPT−DNA complex, the drug also engages direct contacts with the double helix at the cleavage site. Different models have been proposed for the configuration of the ternary complex,[5−7] and in all cases, the indolizino[1,2-*b*]quinoline moiety of CPT, represented by the A−D rings, provides the necessary framework for the DNA interaction whereas the lactone E ring interacts essentially, if not exclusively, with the enzyme through the Arg364 and Asp533 residues of human topoisomerase I.[5]

Thus far, all drug design approaches in the CPT series have been oriented toward the discovery of potent topoisomerase I poisons, generally with little or no consideration of the DNA binding aspect. Here, we report a different approach that consists of deleting the lactone E ring, therefore prohibiting the targeting of topoisomerase I but exploiting the indolizino[1,2-*b*]-quinoline structure to develop DNA-sequence-reading molecules (Chart 1).

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Results

The designed drugs lack the lactone E ring of CPT but preserve the A−D indolizino[1,2-*b*]quinoline rings, which can be considered as the DNA binding unit of CPT. Two types of substitutions were considered to potentially promote the drug−DNA interaction. On one hand, the A quinoline ring was substituted at position 2 or 3 (Scheme 1) with a positively charged piperidine side chain susceptible to interaction with the DNA phosphates. This side chain also confers hydrosolubility, and compounds **10a**,**b** and **12a**,**b** are fully water-soluble. An ether linkage was chosen to mimic the hydroxyl group found in TPT or SN38 (the active metabolite of CPT-11). On the other hand, the D ring was substituted with a methyl group at position 7 and either an aminomethyl or an amide group at position 8. The amide was selected for its hydrogen binding capacity, whereas the amine was incorporated to generate formaldehyde-mediated drug−DNA cross-links.

Chemistry. The 9,11-dihydroindolizino[1,2-*b*]quinolines **10** and **11** were synthesized on the basis of the Friedländer reaction, requiring the appropriate 2-aminobenzaldehydes **3a**,**b** (and the imine surrogate **4**[8,9]) and the enolizable indolizinones **8** and **9** (Scheme 1). The 2-aminobenzaldehydes **3a**,**b** were obtained in two steps by O-alkylation of phenols **1a**[10] and **1b** (K_2CO_3, DMF) followed by a Bechamp reduction of the nitro group of **2a**,**b**. The synthesis of indolizines **6** and **7** was found to be effective from indolizine **5**,[11,12] whose cyano group led to carboxamide **6** by hydrolysis in basic medium (without affecting the lactame moiety). The protected amine **7** resulted from the catalytic hydrogenation of **5** in acylating medium. These reaction conditions prevent overhydrolysis of the carboxamide function into carboxylic acid and nonregioselective reaction of the primary amine during the Friedländer cyclization. Moreover, the acetal group in **5** was found to be essential for protecting the ketone during the hydrogenation process. Deketalization of **6** and **7** enabled the Friedländer cyclization[13] of aldehydes **3a**,**b** (and **4)** with indolizines **8** and **9** to produce the tetracyclic compounds **10** and **11**; acetic acid acts as solvent and catalyst as well as a

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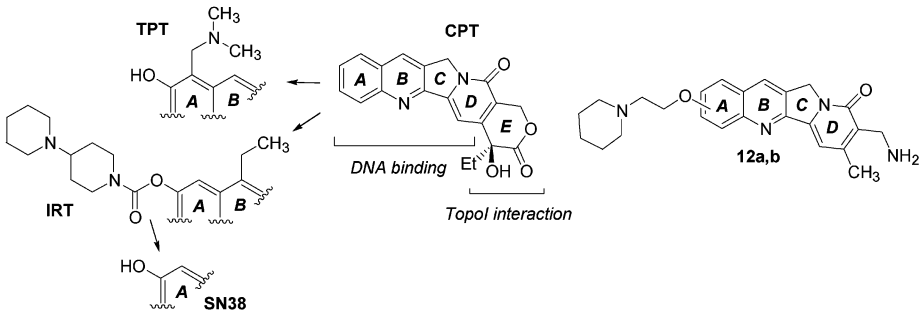
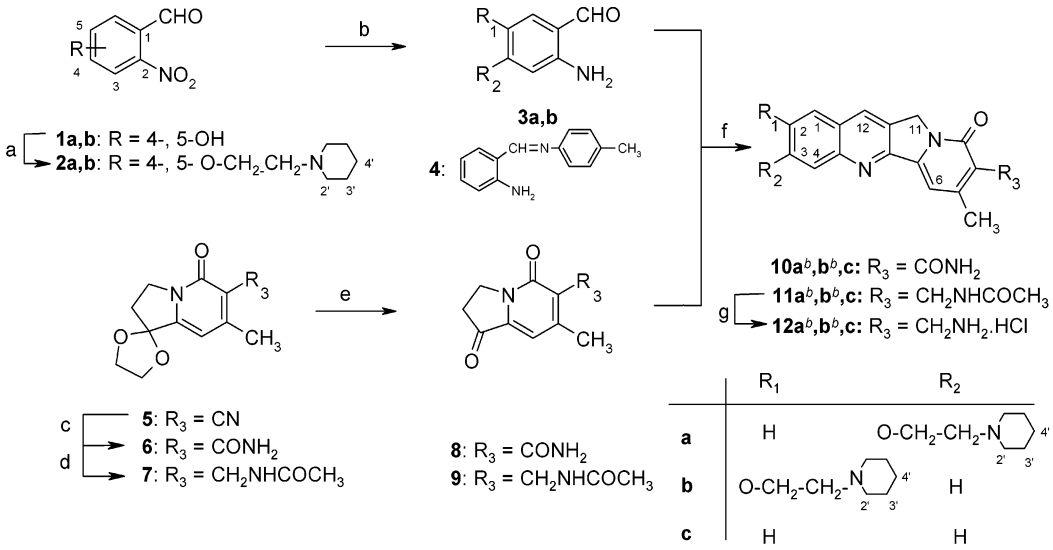
Chart 1



Scheme 1[a]



[a] Reagents and conditions: (a) *N*-2-chloroethylpiperidine, K_2CO_3, DMF, 80 °C; (b) Fe, HCl, AcOH, EtOH, H_2O, reflux; (c) NaOH, MeOH, H_2O, reflux; (d) Raney Ni, Ac_2O, AcOH, H_2 at 45 °C and 50 psi; (e) TFA 80%, room temp; (f) AcOH, reflux; (g) 6 N HCl, reflux. [b] Hydrochloride.

deprotective agent of the aldehydic function originating from **4**. Compounds **12a**−**c** were classically obtained from amides **11a**−**c** by acidic hydrolysis.

DNA Interaction. Addition of DNA induces marked changes of the absorption spectra of compounds **12a**−**c** (Figure S1 in Supporting Information). Good isosbestic behavior and large bathochromic and hypochromic shifts were observed with compounds **12a**−**c** (Table 1). With **12a**, the absorption maximum is shifted from 381 to 392 nm and the red shift amounts to 9 nm with **12b** (from 377 to 386 nm). The amide compounds **10a**−**c** showed weaker spectral changes compared to the aminomethyl analogues (Table 1). Thermal denaturation analyses, carried out with calf thymus DNA and the polynucleotide poly(dAT)$_2$, gave the ΔT_m values ($\Delta T_m = T_m^{complex} - T_m^{DNA}$) collated in Table 1. There are large differences between the compounds. In the aminomethyl series, **12a**,**b** stabilize duplex DNA against heat denaturation much more strongly than **12c**, indicating that the side chain plays a significant role in the drug−DNA interaction. The position of the side chain on the chromophore is also important because the T_m shifts are more pronounced with **12a** than with **12b**. The side chain introduced on the chromophore at position 3 is preferred over position 2. Binding affinities were determined by fluorescence using an ethidium bromide displacement assay. **12a** was found to bind strongly to calf thymus DNA with an apparent binding constant about 4 times higher than that determined with **12b** and 40 times superior to those calculated with the amides **10a**,**b** (Table 1). The mode of binding of **12a**−**c** to DNA was probed by electric linear dichroism (ELD) using calf thymus DNA and the polynucleotides poly(dAT)$_2$ and poly(dGC)$_2$ (Figure S2 in Supporting Information). In all cases, the reduced dichroism $\Delta A/A$ was negative in the drug absorption band that reflects the orientation

Table 1. DNA Binding Parameters

	ΔT_m [a] (°C)		Abs[b]		
	CT	dAT	$\Delta\lambda$ (nm)	H (%)	K_{app} [c] × 10^6 (M^{-1})
10a	4.6	12.8	8	35.8	0.20 ± 0.002
10b	1.2	5.8	9	33.0	0.29 ± 0.003
10c	0	0	0	2.7	*d*
12a	19.4	28.6	11	38.6	8.4 ± 0.12
12b	13.6	19.8	9	35.3	2.5 ± 0.08
12c	1.9	3	8	33.1	*d*

[a] Variation in melting temperature ($\Delta T_m = T_m^{complex} - T_m^{DNA}$). T_m measurements were performed in BPE buffer, pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA), using 10 μM drug and 20 μM calf thymus DNA (CT) or poly(dAdT)$_2$ (dAT) at 260 nm with a heating rate of 1 °C/min. [b] Absorption spectral changes recorded upon addition of 200 μM calf thymus DNA to a drug solution at 20 μM in 1 mM Na cacodylate buffer, pH 7.0. H and $\Delta\lambda$ refer to the hypochromic and bathochromic shifts, respectively. [c] Binding constants calculated from the concentration required to reduce by 50% the fluorescence of ethidium bromide bound to calf thymus DNA. [d] No displacement of DNA-bound ethidium bromide at 50 μM.

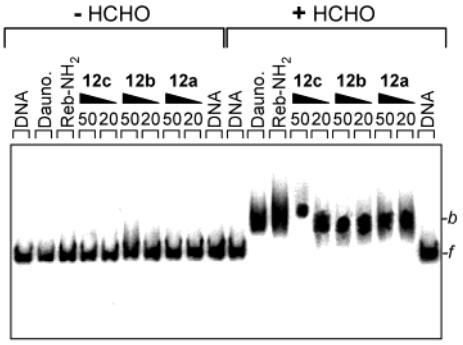


Figure 1. Mobility shifts of DNA cross-linked to **12a**−**c** in the presence of formaldehyde. The 117 base pair DNA fragment was reacted with the test drugs (20 or 50 μM) in the presence of 1% HCHO in 50 mM borate buffer at pH 8.2. After 1 h of incubation at 37 °C, the samples were precipitated with ethanol and the cross-linked DNA samples were subjected to electrophoresis on 6% polyacrylamide. The lane marked DNA contained no drug. Daunomycin (lane Dauno.) and the aminorebeccamycin derivative (lane Reb-NH_2) (20 μM each) cross-link the DNA in the presence of HCHO only, and 1% formaldehyde has no effect on the mobility of the DNA in the absence of the drug. The free (f) and bound (b) DNA forms are indicated.

of the chromophore perpendicular to the helix axis, as expected for an intercalative binding. Unwinding of supercoiled DNA was observed with **12a**, thus confirming that this compound is a DNA intercalator.

DNA Sequence Recognition. Footprinting experiments were performed with four restriction fragments (Figure S3 in Supporting Information). Clear modifications of the patterns of cleavage by DNase I were observed with **12a**, whereas **12b** and **12c** showed little, if any, effect on the enzyme activity. A few sites of protection from DNase I cutting (i.e., footprints) adjacent to regions of enhanced cleavage can be detected with **12a** as the ligand concentration is raised but not with **12b**,**c**. **12a** was found to bind selectively to many sequences with a high GC content, such as 5′-GGCCAGT, 5′-CGCC, and 5′-GCGTG. Binding to a few AT-GC mixed sequences, such as 5′-ACGT and 5′-AGTG, was also detected (Figure S4 in Supporting Information). In contrast, DNase I cutting at AT-rich sequences was found to be enhanced in the presence of **12a**, and this effect can be attributed to intercalation-induced perturbations of the double helical structure of DNA. It appears that **12a** is sensitive to GC-rich sequences and those containing GpT (ApC) and TpG (CpA) steps.

Formaldehyde-Mediated Cross-Linking. Formaldehyde can be used to cross-link drugs to DNA, providing that they contain a reactive amino function that is located adjacent to an amino group of the DNA bases. For example, the anticancer drugs daunomycin,[14] mitoxantrone,[15] and a rebeccamycin derivative[16] can be efficiently cross-linked to DNA in the presence of formaldehyde. This strategy was used here to probe the reactivity of the 8-aminomethyl function of compounds **12a**−**c** upon binding to DNA. The results of the gel mobility shift assay presented in Figure 1 indicate clearly that formaldehyde does promote the formation of covalent complexes that migrate more slowly into the gel. We can therefore conclude that in the drug−DNA complex, the amino group of compound **12** is located at a short distance from an amino group of the bases, most likely the 2-amino group of guanine exposed in the minor groove.

Conclusion

This study shows that the A−D rings of CPT is particularly well adapted for potent and selective recognition of GC-rich DNA sequences. The DNA binding data can be rationalized by a model in which the indolizino[1,2-*b*]quinoline chromophore stacks on G-C pairs with the 8-aminomethyl substituent located a short distance from an amino group on the DNA base pair so that a covalent drug−DNA methylene bridge can be created in the presence of formaldehyde. Incorporation of a cationic piperidinoethyloxy side chain reinforces significantly the DNA interaction as is the case with IRT. Beyond the camptothecins, the present data provide important information to better understand the mechanism of action of indolizino[1,2-*b*]quinolines such as batracyclins, which are highly potent antitumor agents.[17]

Experimental Section

General Procedure for the Synthesis of 7-Methyl-1,5-dioxo-1,2,3,5-tetrahydroindolizine-6-carboxamide (8) and *N*-(7-Methyl-1,5-dioxo-1,2,3,5-tetrahydroindolizin-6-ylmethyl)acetamide (9). A solution of **6** or **7** (4 mmol) in 80% aqueous TFA (10 mL) was stirred at room temperature for 3 h in a nitrogen atmosphere. Removal of the solvent gave an oil that crystallized from Et_2O. **8**: yellow solid (715 mg, 85% yield). R_f = 0.38 (CH_2Cl_2/MeOH 9:1). Mp: 185 °C. IR: 3301 (NH), 1735 (C═O ketone), 1647 (C═O amide, pyridone) cm^{-1}. EI MS *m*/*z* (relative intensity): 206 (M^+, 66%). Anal. ($C_{10}H_{10}N_2O_3$) C, H, N. **9**: pale solid (770 mg, 93% yield). R_f = 0.46 (CH_2Cl_2/MeOH 9:1). Mp: 161 °C. IR: 3328 (NH_2), 3130 (NH_2), 1745 (C═O ketone), 1672 (C═O amide, pyridone) cm^{-1}. EI MS *m*/*z* (relative intensity): 234 (M^+, 70%). Anal. ($C_{12}H_{14}N_2O_3$) C, H, N.

General Procedure for the Synthesis of 9,11-Dihydroindolizino[1,2-*b*]quinolines 10a−c and 11a−c. Tetrahydroindolizine **8** or **9** and 2-aminobenzaldehyde **3a**, **3b**, or **4**[8,9] were added to acetic acid (20 mL) and heated while stirring under reflux for 8 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. Methanol saturated HCl was added to the residual solid dissolved in MeOH. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 8:2 → 7:3).

7-Methyl-9-oxo-3-(2-piperidin-1-ylethoxy)-9,11-dihydroindolizino[1,2-*b*]quinoline-8-carboxamide Hydrochloride (10a). Pale solid (340 mg, 51% yield) from **8** (320 mg, 1.6 mmol) and **3a** (385 mg, 1.6 mmol). R_f = 0.31 (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3300 (NH), 1676 (C═O pyridone, amide), 1619 (C═N) cm^{-1}. Anal. ($C_{24}H_{26}N_4O_3 \cdot 1HCl \cdot 2.5H_2O$) C, H, N, Cl.

7-Methyl-9-oxo-2-(2-piperidin-1-ylethoxy)-9,11-dihydroindolizino[1,2-*b*]quinoline-8-carboxamide Hydrochloride (10b). Pale solid (330 mg, 49% yield) from **8** (320 mg, 1.6 mmol) and **3b** (385 mg, 1.6 mmol). R_f = 0.48 (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3360 (NH), 1658 (C═O pyridone, amide) cm^{-1}. Anal. ($C_{24}H_{26}N_4O_3 \cdot 1HCl \cdot 2.5H_2O$) C, H, N, Cl.

7-Methyl-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinoline-8-carboxamide (10c). Brown solid (290 mg, 42% yield) from **8** (500 mg, 2.4 mmol) and **4** (510 mg, 2.4 mmol). R_f = 0.49 (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3275 (NH), 1668 (C═O pyridone, amide) cm^{-1}. Anal. ($C_{17}H_{13}N_3O_2 \cdot 0.5HCl \cdot 0.5H_2O$) C, H, N.

***N*-[7-Methyl-9-oxo-3-(2-piperidin-1-ylethoxy)-9,11-dihydroindolizino[1,2-*b*]quinolin-8-ylmethyl]acetamide Hydrochloride (11a).** Orange solid (1.0 g, 45% yield) from **3a** (1.2 g, 5 mmol) and **9** (1.2 g, 5 mmol). R_f = 0.39 (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3300 (NH),

1664 (C═O pyridone, amide), 1618 (C═N) cm^{-1}. EI MS *m/z* (relative intensity): 446 (M^+, 14%), 403 ($M^+ - COCH_3$, 22%), 304 ($M^+ - COCH_3$ − piperidine, 17%), 275 ($M^+ - COCH_3$ − piperidine − CH_2NH, 14%).

***N*-[7-Methyl-9-oxo-2-(2-piperidin-1-ylethoxy)-9,11-dihydroindolizino[1,2-*b*]quinolin-8-ylmethyl]acetamide Hydrochloride (11b).** Brown solid (1.0 g, 44% yield) from **3b** (1.2 g, 5 mmol) and **9** (1.2 g, 5 mmol). $R_f = 0.22$ (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3418 (NH), 1655 (C═O pyridone, amide), 1621 (C═N) cm^{-1}. EI MS *m/z* (relative intensity): 446 (M^+, 15%), 403 ($M^+ - COCH_3$, 20%), 304 ($M^+ - COCH_3$ − piperidine, 8%), 275 ($M^+ - COCH_3$ − piperidine − CH_2NH, 6%).

***N*-[7-Methyl-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinolin-8-ylmethyl]acetamide (11c).** Brown solid (290 mg, 48% yield) from **4** (450 mg, 1.9 mmol) and **9** (400 mg, 1.9 mmol). $R_f = 0.63$ (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3329 (NH), 1666 (C═O pyridone, amide) cm^{-1}. EI MS *m/z* (relative intensity): 319 (M^+, 10%), 276 ($M^+ - COCH_3$, 100%).

General Procedure for the Synthesis of 9,11-Dihydroindolizino[1,2-*b*]quinolines 12a−c. Quinoline **11a**, **11b**, or **11c** was added to 6 N HCl, and the mixture was heated with stirring under reflux for 24 h. After the mixture was cooled to room temperature and after removal of the solvent under reduced pressure, the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 7:3).

8-Aminomethyl-7-methyl-9-oxo-3-(2-piperidin-1-ylethoxy)-9,11-dihydroindolizino[1,2-*b*]quinoline Dihydrochloride (12a). Yellow solid (130 mg, 76% yield) from **11a** (150 mg, 3.4 mmol) and HCl (15 mL). $R_f = 0.39$ (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3386 (NH_3^+), 1653 (C═O), 1617 (C═N) cm^{-1}. Anal. ($C_{24}H_{28}N_4O_2 \cdot 2HCl \cdot 3H_2O$) C, H, N, Cl.

8-Aminomethyl-7-methyl-9-oxo-2-(2-piperidin-1-ylethoxy)-9,11-dihydroindolizino[1,2-*b*]quinoline Dihydrochloride (12b). Yellow solid. (150 mg, 70% yield) from **11b** (200 mg, 4.4 mmol) and HCl (20 mL). $R_f = 0.36$ (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3417 (NH_3^+), 1655 (C═O), 1621 (C═N) cm^{-1}. Anal. ($C_{24}H_{28}N_4O_2 \cdot 2HCl \cdot 3.5H_2O$) C, H, N, Cl.

8-Aminomethyl-7-methyl-9-oxo-9,11-dihydroindolizino-[1,2-*b*]quinoline Hydrochloride (12c). Pale solid (850 mg, 58% yield) from **11c** (150 mg, 4.7 mmol) and HCl (15 mL). $R_f = 0.59$ (CH_2Cl_2/MeOH 9:1, ammonia 1%). Mp: >250 °C (EtOH/H_2O). IR: 3422 (NH_3^+), 1656 (C═O), 1609 (C═N) cm^{-1}. Anal. ($C_{17}H_{15}N_3O_1 \cdot 1.5HCl \cdot 2.25H_2O$) C, H, N, Cl.

Biochemical and Spectrophotometric Measurements. Absorption and melting temperature studies were performed as previously described.[18] Binding constants were determined using a competitive displacement fluorometric assay with DNA-bound ethidium.[19] The experimental procedures for the electric linear dichroism[20] and DNase I footprinting[21] and formaldehyde-mediated cross-linking[16] experiments have been previously reported.

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Supporting Information Available: Synthesis of **2a**,**b**, **3a**,**b**, **6**, and **7**; ^{1}H NMR data for **2a**,**b**, **3a**,**b**, **6-9**, **10a-c**, **12a−c**, and four figures showing spectrophotometric DNA titration, electric linear dichroism data, DNase I footprinting gels, and differential cleavage plots for **12a−c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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