Design and Synthesis of Ellipticinium Salts and 1,2-Dihydroellipticines with High Selectivities against Human CNS Cancers *in Vitro*

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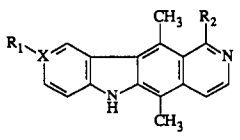
9-Methoxy-2-methylellipticinium acetate (**6**), along with the 9-methyl and 9-chloro derivatives (**7**, and **8**, respectively) have shown remarkable selectivities *in vitro* against the NCI human CNS cancer subpanel. In order to target these types of compounds to the CNS *in vivo*, a series of 1,2-dihydroellipticines was synthesized. 9-Methoxy-2-methyl-1,2-dihydroellipticine (**9**) retained the potency and selectivity of the parent compound **6** but was unstable toward oxidation to **6**. In order to improve the stability of **9**, it was converted to the vinylogous amide **33** by introduction of a formyl group in the 4-position. Compound **33** proved to be much more stable than **9**, but it was also less potent than **9** by about 1 order of magnitude, and it was less selective for the CNS subpanel than **9**. To overcome the limited water solubilities of the ellipticines and dihydroellipticines, several ellipticine analogues incorporating polar groups on the N-2 nitrogen were prepared. The 2-(methoxymethyl)ellipticinium salts **24** and **25**, as well as the (methylthio)methyl congener **26**, were relatively potent anticancer agents which displayed cytotoxicity selectivity profiles similar to compound **6**. The cytotoxic dihydroellipticines **9** and **10** exhibited potencies approaching that of ellipticine itself in facilitating the formation of a "cleavable complex", while the least cytotoxic ellipticine derivatives exhibited no cleavage above background.

Introduction

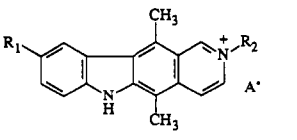
Following the initial reports on the synthesis and antitumor activity of ellipticine (**1**) and its analogues,[1] there has been a tremendous amount of effort directed toward the design and preparation of new analogues and the elucidation of structure–activity relationships.[2-4] The problems of aqueous insolubility and cardiovascular side effects that hindered the clinical application of ellipticine were offset by the introduction of highly potent analogues such as 9-hydroxy-2-methylellipticinium acetate (**2**, elliptinium),[5] 2-[2-(diethylamino)ethyl]-9-hydroxyellipticinium chloride (**3**, datelliptium),[6] 1-[[3-(diethylamino)propyl]amino]-9-methoxyellipticinium hydrochloride (**4**),[7] and pazellipticine **5**.[8] These compounds act by a combination of different mechanisms including DNA intercalation, inhibition of topoisomerase II, alkylation of macrobiomolecules, and redox generation of cytotoxic free radicals.[2-4]



1 R_1 = H X = C R_2 = H

4 R_1 = CH_3O X = C R_2 = $NH(CH_2)_3N(C_2H_5)_2$

5 R_1 = 2e⁻ X = N R_2 = $NH(CH_2)_3N(C_2H_5)_2$



2 R_1 = OH R_2 = CH_3 A = OAc

3 R_1 = OH R_2 = $CH_2CH_2N(C_2H_5)_2$ A = Cl

6 R_1 = OCH_3 R_2 = CH_3 A = OAc

7 R_1 = CH_3 R_2 = CH_3 A = OAc

8 R_1 = Cl R_2 = CH_3 A = OAc

One of the main goals of the *in vitro* screen recently devised at the National Cancer Institute (NCI) has been the identification of new antitumor agents that display selective cytotoxicities against particular types of human tumor cell lines.[9-11] Both elliptinium (**2**) and datelliptium (**3**) failed to show any selective cytotoxicity in the NCI screen, while 9-methoxy-2-methylellipticinium acetate (**6**) showed striking selectivity against the CNS subpanel.[12] Further studies showed that the 9-methyl and 9-chloro analogues (**7** and **8**, respectively) were also selective against the same subpanel.[13] The ionic nature of these compounds might be expected to limit their applicability *in vivo* due to the limited ability of ions in general to penetrate the blood–brain barrier (BBB) and the blood cerebrospinal fluid (CSF) barrier, although there is evidence that elliptinium is capable of crossing the BBB in rats.[14] Bodor has studied the distribution of berberine, a charged antineoplastic alkaloid, in rats and found minimal levels in the brain, but the levels rose dramatically when dihydroberberine chloride was used instead.[15] This indicates that dihydroberberine chloride, a neutral molecule at physiological pH, is able to cross the BBB and is then oxidized back *in vivo* to berberine. Oxidation to the ionic species is expected to trap it in the CNS due to the limited ability of the charged molecule to pass back out of the brain through the BBB. This suggests that a similar strategy employing 1,2-dihydroellipticines might target them to the CNS as well. In this paper we wish to report the preparation of neutral 2-alkyl-1,2-dihydroellipticines which retained the CNS selectivity of the parent ellipticinium salts and the efforts to address the problems of water insolubility and chemical instability that are associated with these compounds.

Several quaternary ellipticinium glycosides were previously reported to possess high antitumor activity.[16] These represent the only examples of ellipticinium salts in which a polar group (alkoxy) is separated from the N-2 by a

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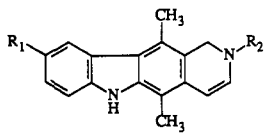
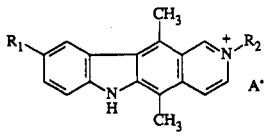
single methylene unit. In an attempt to increase the aqueous solubilities of the ellipticines in the present series, we have also prepared several such salts, and some of them have displayed good selectivities in the NCI screen.

Chemistry

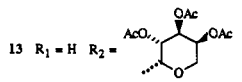
The ellipticinium salts **17–19**, **21**, and **24–26** were prepared by quaternizing ellipticine or 9-methoxyellipticine with an excess of the alkyl halide in DMF and were purified either by precipitation from methanol with ether or by chromatography on silica gel. Salt **20** was obtained by the acidic hydrolysis of **18**. Salt **22** was obtained from the reaction of ellipticine with 2,3,4-tri-*O*-acetyl-β-arabinopyranosyl bromide and cadmium carbonate in nitromethane and was converted to **23** by reaction with methanolic ammonia.[16] It is assumed that the product would have the α-configuration as previously observed for similar salts.[16] Salt **27** was isolated as a byproduct during the purification of **26**, obviously arising from air oxidation.



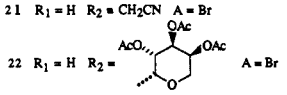
9 $R_1 = CH_3O$ $R_2 = CH_3$
10 $R_1 = CH_3$ $R_2 = CH_3$
11 $R_1 = H$ $R_2 = CH_2CO_2CH_2CH_2Si(CH_3)_3$
12 $R_1 = H$ $R_2 = CH_2CO_2^-$



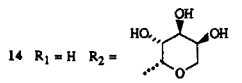
17 $R_1 = H$ $R_2 = CH_2CO_2CH_3$ A = Br
18 $R_1 = H$ $R_2 = CH_2CO_2$-*t*-C_4H_9 A = Br
19 $R_1 = H$ $R_2 = CH_2CO_2CH_2CH_2Si(CH_3)_3$ A = Br
20 $R_1 = H$ $R_2 = CH_2CO_2H$ A = Br
21 $R_1 = H$ $R_2 = CH_2CN$ A = Br



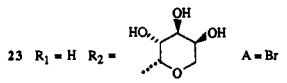
13 $R_1 = H$ $R_2 =$



22 $R_1 = H$ $R_2 =$ A = Br



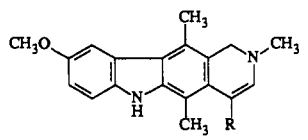
14 $R_1 = H$ $R_2 =$



23 $R_1 = H$ $R_2 =$ A = Br

15 $R_1 = H$ $R_2 = CH_2OCH_3$
16 $R_1 = H$ $R_2 = CH_3$

24 $R_1 = H$ $R_2 = CH_2OCH_3$ A = Cl
25 $R_1 = CH_3O$ $R_2 = CH_2OCH_3$ A = Cl
26 $R_1 = H$ $R_2 = CH_2SCH_3$ A = Cl
27 $R_1 = H$ $R_2 = CH_2S(O)CH_3$ A = Cl
28 $R_1 = H$ $R_2 = CH_2NO_2$ A = Br
29 $R_1 = H$ $R_2 = CH_2PO_3H_2$ A = Cl



32 $R = CO_2CH_3$
33 R = CHO

The dihydroellipticines **9** and **10** were prepared cleanly by the reduction of salts **6** and **7**, respectively, with excess lithium aluminum hydride (LAH) in ether.[17] A dilute solution of **9** in ethanol oxidized back to **6** with a $t_{1/2}$ of roughly 9 h at room temperature but could be stored as a solid under vacuum or argon atmosphere at −20 °C. Because of the limited stability of these compounds, they were submitted for screening without any further purification.

Dihydroellipticines **9** and **10** were not water soluble. However, the dihydroellipticines **12** and **14**, bearing hydrophilic substituents on the N-2 nitrogen, were expected to have higher water solubilities and were pursued as targets for synthesis. After extensive experimentation, the dihydroellipticine **11** was obtained by the reduction of salt **19** with sodium borohydride in anhydrous pyridine,[18] but the product was always contaminated with traces of pyridine or an impurity derived from pyridine. The use of polymer supported borohydride[19] gave the same results but the rate of reduction was slower. On the other hand, lithium tri-*tert*-butoxyaluminohydride in THF cleanly reduced **19** to **11**. This dihydroellipicine had superior stability toward oxidation when compared with **9** or **10**, as a solution of **11** in chloroform-*d* did not show any signs of decomposition after 5 days at −20 °C. The final conversion of **11** to **12** was not carried out, however, since at that time it was found that salt **20** (the oxidation product of **12**) was devoid of antitumor activity.

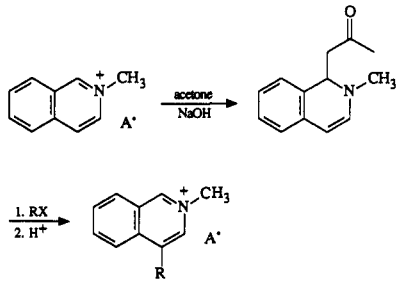
The reduction of **22** with LAH in ether or DME gave a complex mixture of products, and mass spectral evidence for the presence of **14** could not be obtained either in the chemical ionization (CI) or fast atom bombardment (FAB) modes. Salt **22** could be reduced cleanly to **13** with lithium tri-*tert*-butoxyaluminohydride in THF. Compound **13** in chloroform-*d* was stable for 5 days at −20 °C. Subsequently, **13** was briefly treated with amberlite IRA-400 (OH^-) ion-exchange resin to cleave the acetyl groups, but this only resulted in a complex mixture of products. Alternatively, **23** was reduced with polymer-supported borohydride in 2-propanol or pyridine, but in both cases complex product mixtures were also obtained. Apparently, while **13** yielded to synthesis and could be characterized, cleavage of the acetyl protecting groups gave a highly unstable product that underwent adverse side reactions.

The complexation of **9** with partially methylated β-cyclodextrin and γ-cyclodextrin[20] was tried as a noninvasive method of solubilization, but in neither case could significant complexation be demonstrated. Recently, it has been reported that ellipticine could be complexed to a nucleobase modified β-cyclodextrin, thus achieving good aqueous solubilities.[21]

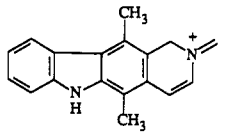
The stability of dihydroisoquinolines, and similarly dihydroellipticines, is affected by the basicity of the enamine function.[17] Thus the inductive electron withdrawal experienced by the nitrogen in **11** (from the ester group) and **13** (from the α-oxygen) causes a decrease in its basicity and an enhancement of stability for these dihydroellipticines. Also, N^2-acetyl-1,2-dihydroellipticine has been described as a stable isolable intermediate in a synthesis of ellipticine.[22] Considering that salt **24** was biologically active, it was postulated that the corresponding dihydroellipticine **15** might also be a biologically active compound. The reduction of **24** with LAH in ether did not lead to **15** but gave a mixture of products with **16** being the major constituent. A likely explanation for the conversion of **15** to **16** is that **15** can expel a methoxide ion to give the iminium salt **30**, which is further reduced by LAH to give **16**. The dihydroellipticine **15** could be obtained by the reduction of **24** with lithium tri-*tert*-butoxyaluminohydride in THF. The dihydroellipticine **15** was oxidized back to **24** on storage at room temperature for several days.

The stability of dihydroisoquinolines is also affected by the ease of protonation at C-4.[17] Consequently, an alkyl[17] or a conjugated electron-withdrawing group[23] (acyl, cyano, formyl) placed at C-4 increases stability. The alkylation of a dihydroisoquinoline has been reported to occur at C-4 but was not a very efficient process.[24] Better results were realized utilizing an adduct generated from an isoquinolinium salt and acetone under basic conditions (Scheme 1).[25] This approach was applied to the alkylation of the corresponding derivative of salt **6** with methyl iodide, but without success. Using NMR and UV analysis, it was

Scheme 1



found that the formation of the highly colored intermediate **31**[26] by abstraction of the indole proton from **6** under the basic reaction conditions occurred instead of addition of acetone enolate to the iminium ion.



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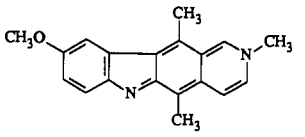

31

The reaction of freshly prepared **9** with methyl chloroformate did not lead to the desired product **32**.[27] However, the 4-formyl derivative **33** was obtained in moderate yield by formylation of freshly prepared **9** with $POCl_3$/DMF in chloroforom.[28] A solution of **33** in chloroform-*d* or acetone-d_6 showed only minor signs of decomposition when stored at room temperature for several days, thus indicating the high relative stability of this 1,2-dihydroellipticine derivative.

Biological Results and Discussion

The National Cancer Institutes antitumor drug discovery screen has been developed for the high flux *in vitro* cytotoxicity testing of new compounds and will ultimately lead to the identification of new antitumor agents.[9-11] The screen has been designed to distinguish between broad spectrum antitumor compounds and tumor- or subpanel-selective agents. The 2-methylellipticinium salts **6–8** offer remarkable examples of compounds that have shown selective cytotoxicity for the CNS cancer subpanel. A portion of the screening data available for compound **6** is shown in Table 1. In 17 assays, **6** showed selectivity for the CNS subpanel at every level of the dose–response curve down to 10^{-7} M. Both the 9-methyl and the 9-chloro analogues **7** and **8** also displayed good CNS selectivity at the levels for growth inhibition and cytostasis.

Some of the cytotoxicity screening results obtained for the present series of ellipticines are listed in Table 2. All of the compounds were examined in approximately 60 different cell lines, and the results in Table 2 were taken as being representative of those obtained in eight tumor subpanels. More specifically, the cell lines listed in Table 2 are CCRF-CEM leukemia cells, A549/ATCC non-small cell lung carcinoma cells, DMS small cell lung cancer cells, COLO 205 colon cancer cells, SF-268 CNS cancer cells, MALME-3M melanoma cells, IGROV1 ovarian cancer cells, and CAKI-1 renal cancer cells.

9-Methoxy-2-methyl-1,2-dihydroellipticine (**9**) retained the potency and selective cytotoxicity for the CNS subpanel found with the corresponding ellipticinium acetate **6** (Table 1). It has been observed that **9** readily oxidizes back to **6** in solution, and most likely, at least some of the activity observed for **9** may be due to the gradual generation of **6**. However, incorporation of a 4-formyl group into **9**, resulting in the stabilized vinylogous amide congener **33**, was associated with a decrease in general potency of approximately 1 order of magnitude (Table 2) and an attenuation of the selective cytotoxicity for CNS cancer cells seen with the parent compound. The activity of **33** is probably due to the compound itself since it is not readily oxidized to the corresponding ellipticinium species. Similarly, dihydroellipticine **10** retained the selectivity of its parent salt **7**, although the degree of selectivity for the CNS subpanel observed for **10** was slightly moderated relative to that of **9**. As suggested by the previous work on berberine,[15] these dihydroellipticines may prove useful in delivering the corresponding ellipticinium salts across the blood–brain barrier *in vivo*.

Our attempts to design and synthesize biologically active, water-soluble derivatives of the ellipticines have been met with limited success to date. The dihydroellipticine **12** was not expected to be interesting due to the lack of activity observed for its dehydrogenation product **20**, and the arabinopyranosyl derivative **14** was not obtained due to difficulties in the synthesis. On the other hand, the stable 2-(2,3,4-tri-*O*-acetyl-α-*L*-arabinopyranosyl)-1,2-dihydroellipticine (**13**) was not active. The related compound **15** was more cytotoxic than **13** but also did not exhibit any selectivity for CNS cancer. Both **13** and **15** were water insoluble.

In the ellipticinium series, the 2-(methoxycarbonyl)-methyl compound **17** and the 2-carboxymethyl compound **20** were essentially inactive. The apparent selective cytotoxicity observed with compound **17** for the SF-268 cell line was not general for the CNS subpanel, as compound **17** had GI_{50} values of >100 μM in the SF-295, SF-539, SNB-19, SNB-75, SNB-78, and XF 498 CNS cancer cell lines (data not shown). The cyanomethyl derivative **21** displayed higher general cytotoxicity than **17** or **20**, but was still only weakly active. However, the 2-methoxymethyl derivatives **24** and **25**, having analogy to the ellipticinium glycosides reported by Honda *et al.*,[9-11] and the methylthiomethyl congener **26**, were more potent and displayed cytotoxicity selectivity profiles similar to that of the 9-methoxy-2-methylellipticinium acetate **6**. Oxidation of the sulfide **26** to the sulfoxide **27** caused a general decrease in cytotoxicity, but the selectivity for the CNS subpanel was retained.

Twelve of these ellipticine derivatives were examined for their ability to facilitate the formation of "cleavable complex"[29-31] as an indication of the inhibition of mammalian topoisomerase II. The ellipticine family of compounds has been shown to exhibit a complex profile of inhibition, including intercalation, inhibition of topoisomerase II, and DNA binding.[32-35] The dihydroellipticines **9** and **10** exhibited potent cleavage complex formation (Figures 1 and 2), approaching that of ellipticine (Table 3). The least potent ellipticinium salts **17**, **20**, and **21** exhibited no cleavage above background, consistent with their limited cytotoxicities (Table 2). The other compounds had moderate potencies relative to ellipticine (Table 3). The ellipticine derivatives exhibiting cleavable complex formation demonstrated the hallmark biphasic response of the ellipticine family of compounds (Figures 1 and 2).[30,34]

The ellipticinium salts **17–21** and **24–27**, which contain polar side chains on the pyridine nitrogen, are a novel

Table I. Cytotoxicities of **6** and **9** in the NCI *In Vitro* Screen[a]

	6	9
Leukemia		
CCRF-CEM	> -4.0	-4.8
HL-60(TB)	> -4.0	-4.2
K-562	> -4.0	> -4.0
MOLT-4	> -4.0	> -4.0
RPMI-8226	> -4.0	> -4.0
SR	.	-4.2
Non-Small Cell Lung Cancer		
A549/ATCC	> -4.0	-4.3
EKVX	-4.6	.
HOP-18	> -4.0	-4.4
HOP-62	-5.2	-5.5
HOP-92	-4.1	-4.6
NCI-H226	.	-6.1
NCI-H23	-5.0	-5.4
NCI-H322M	.	-4.8
NCI-H460	-4.1	-5.0
NCI-H522	-5.1	-5.3
LXFL 529	-5.0	-5.3
Small Cell Lung Cancer		
DMS 114	-5.2	-5.6
DMS 273	-4.2	-4.9
Colon Cancer		
COLO 205	-4.4	-5.3
DLD-1	> -4.0	-4.7
HCC-2998	-4.2	-4.9
HCT-116	> -4.0	-4.8
HCT-15	> -4.0	> -4.0
HT29	> -4.0	-4.6
KM12	-4.6	-4.8
KM20L2	> -4.0	.
SW-620	> -4.0	-4.8
CNS Cancer		
SF-268	-5.3	-6.2
SF-295	-5.2	-5.1
SF-539	-5.2	-5.7
SNB-19	-5.0	.
SNB-75	-5.5	-6.1
SNB-78	-5.0	-5.3
U251	-5.3	-6.0
XF 498	-5.0	.
Melanoma		
LOX IMVI	> -4.0	-4.6
MALME-3M	-4.6	-5.3
M14	> -4.0	-4.6
M19-MEL	-4.6	-5.6
SK-MEL-2	-4.2	-5.0
SK-MEL-28	-4.3	-4.9
SK-MEL-5	-4.7	-5.0
UACC-257	> -4.0	-4.7
UACC-62	> -4.0	-4.7
Ovarian Cancer		
IGROV1	> -4.0	-4.4
OVCAR-3	-4.0	.
OVCAR-4	.	-4.6
OVCAR-5	> -4.0	-4.5
OVCAR-8	-4.5	-4.8
SK-OV-3	> -4.0	-4.2
Renal Cancer		
786-0	-5.3	-4.9
A498	> -4.0	-4.2
ACHN	> -4.0	-4.2
CAKI-1	> -4.0	-4.5
RXF-393	.	-4.5
RXF-631	> -4.0	-4.6
SN12C	.	-6.1
TK-10	> -4.0	-4.3
UO-31	> -4.0	> -4.0
MG_MID	> -4.42	> -4.89

[a] The numerical values listed are $\log_{10}$ TGI values, which are the logs of the molar concentrations required for total growth inhibition. Bars projecting to the right on the mean bar graph indicate greater sensitivity, while those projecting to the left indicate less sensitivity.

class of ellipticine congeners. As a generalization for compounds in this series, compounds **17**, **20**, and **21**, containing a carbon atom β to the nitrogen, were less potent and less selective than **24**, **25**, and **26**, having an oxygen or sulfur atom β to the nitrogen. These results suggest the salts **28** and **29** as possible candidates for future study.

Experimental Section

General. Melting points were determined under vacuum in sealed capillary tubes on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Varian XL-200A and Varian VXR-500 spectrometers. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer. Only

Table 2. Cytotoxicities of Ellipticines and 1,2-Dihydroellipticines (GI_{50} Values, μM)[a]

no.	CCRF-CEM	A549/ATCC	DMS 114	COLO 205	SF-268	MALME-3M	IGROV1	CAKI-1
1	0.415	0.410	0.552	0.588	0.816	2.52	0.862	1.01
6	4.22	1.52	0.811	2.40	0.409	5.27	3.62	48.2
9	2.64	0.993	0.525	1.32	0.186	1.44	1.68	20.1
10	2.34	0.380	0.438	0.306	0.395	1.72	0.774	1.21
13	29.0	64.0	15.6	36.7	31.0	30.2	24.1	>100
15	4.98	5.06	10.4	8.68	8.02	8.87	5.88	11.1
17	>100	>100	>100	>100	12.8	>100	>100	–
20	>100	77.3	76.8	>100	97.1	26.3	>100	–
21	100	97.5	44.7	34.6	13.0	27.2	35.6	>100
24	14.6	1.79	2.28	4.70	0.679	0.647	9.2	19.2
25	6.86	13.7	1.32	2.68	0.818	1.92	27.8	20.2
26	5.02	5.23	2.67	2.48	0.726	1.99	18.2	19.6
27	40.6	71.0	6.60	31.0	2.46	–	29.5	35.8
33	60.4	75.7	2.50	16.2	1.12	40.0	31.2	73.2

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition, and they are the averages of at least two determinations.

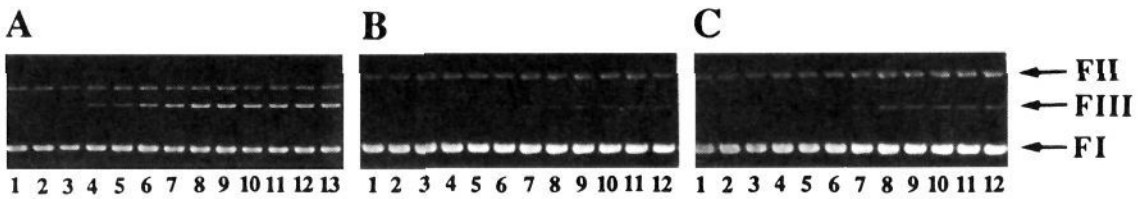

Figure 1. Facilitation of the cleavable complex of mammalian topoisomerase II by ellipticine (panel A), compound **9** (panel B), and compound **10** (panel C). A 1% agarose gel is shown. Panel A, line 1, no drug control; lanes 2–13, DNA cleavage carried out in the presence of 2-fold increasing concentrations of ellipticine from 0.015 to 32 μg/mL. Panel B, lanes 1–12, DNA cleavage carried out in the presence of 2-fold increasing concentrations of compound **9** from 0.015 to 32 μg/mL. Panel C, lanes 1–12, DNA cleavage carried out in the presence of 2-fold increasing concentrations of compound **10** from 0.015 to 32 μg/mL. The positions of the supercoiled DNA (F1), nicked circular DNA (FII), and linear DNA (FIII) are shown at right.

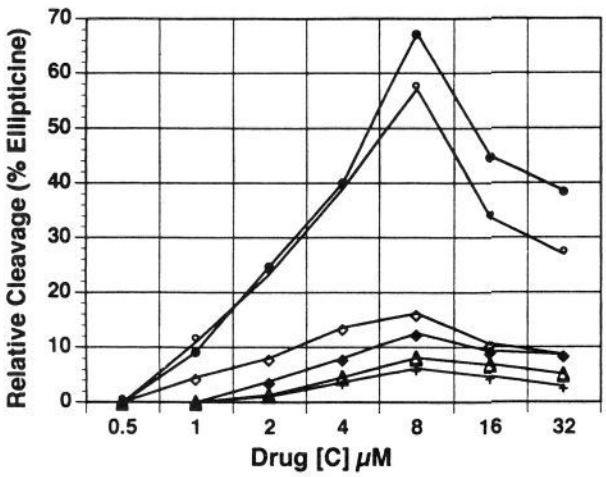

Figure 2. Concentration-dependent human topoisomerase II-mediated cleavage of analogs of ellipticine, relative to ellipticine. Shown are compounds **9** (●), **10** (○), **15** (◇), **24** (+), **25** (△), and **26** (◆), demonstrating the typical biphasic response of the ellipticine family of topoisomerase II inhibitors.

Table 3. Relative Cleavage of Ellipticine Analogs

compd	relative cleavage	compd	relative cleavage
9	0.571	24	0.062
10	0.673	25	0.082
13	0.014	26	0.126
15	0.162	27	0.016
17	NCD[a]	33	0.027
20	NCD	ellipticine	1.0
21	NCD		

[a] NCD: no cleavage detected above background to 32 μM.

functional group absorptions are reported. Low-resolution chemical ionization mass spectra (CIMS) were determined on a Finnigan 4000 spectrometer using 2-methylpropane as the reagent gas. High-resolution CIMS, low-resolution fast atom bombardment mass spectra (FABMS), and peak match FABMS were obtained on a Kratos MS50 spectrometer.

Analytical thin-layer chromatography was done on Whatman silica 60 K6F glass coated plates and Baker-flex aluminum oxide 1B-F sheets. Column chromatography was performed using Sigma 70–230 mesh silica gel and WN-6 neutral alumina.

Mallinckrodt anhydrous ether was used from freshly opened cans without further drying. *N,N*-Dimethylformamide and chloroform were distilled from calcium hydride. Pyridine was stored over potassium hydroxide pellets. Commercially available methyl bromoacetate, *tert*-butyl bromoacetate, chloromethyl methyl ether, and sulfide were used without further purification. β-Trimethylsilyl bromoacetate[36] and 2,3,4-tri-*O*-acetyl-β-L-arabinpyranosyl bromide[37] were prepared according to literature procedures.

9-Methoxy-2-methyl-1,2-dihydroellipticine (9). Lithium aluminum hydride powder (300 mg, 7.95 mmol) was added in one portion to an ice-cold suspension of 9-methoxy-2-methylellipticinium acetate (NSC 627505, 200 mg, 0.57 mmol) in anhydrous ether (50 mL). The ice bath was removed, and the mixture was stirred under an atmosphere of nitrogen for 18 h. The reaction mixture was cooled in an ice bath and quenched by the sequential addition of water (0.3 mL), 15% aqueous sodium hydroxide (0.3 mL), and water (0.9 mL). The granular aluminum salts thus formed were filtered off and washed with anhydrous ether (20 mL). The filtrate was concentrated on a rotary evaporator to give a greenish yellow solid (151 mg, 91%). This solid was stored either under vacuum or under an inert atmosphere: mp 205–207 °C (dec, determined under vacuum); UV (ethanol) 362 (ϵ = 36 040), 249 (ϵ = 55 000); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (br, 1 H), 7.65 (d, 1 H, J = 3 Hz), 7.31 (d, 1 H, J = 9 Hz), 6.98 (dd, 1 H, J = 9, 3 Hz), 6.20 (d, 1 H, J = 8 Hz), 5.54 (d, 1 H, J = 8 Hz), 4.38 (s, 2 H), 3.92 (s, 3 H), 2.84 (s, 3 H), 2.69 (s, 3 H), 2.37 (s, 3 H); low-resolution CIMS m/e (relative intensity) 293 (MH^+, 67), 292 (M^+, 100); high-resolution CIMS m/z calcd MH^+ 293.1654, found 293.1660.

2,9-Dimethyl-1,2-dihydroellipticine (10) was obtained from **7** in 83% yield as a yellow solid by the same procedure used for the preparation of **9**. This solid was stored either under vacuum or an inert atmosphere: mp 200–204 °C (dec, determined under vacuum); UV (ethanol) 356 (ϵ = 9039), 248 (ϵ = 17 492); ^{1}H NMR

(200 MHz, $CDCl_3$) δ 7.94–7.90 (m, 1 H), 7.74 (br, 1 H), 7.30 (d, 1 H, J = 9 Hz), 7.14 (ddd, 1 H, J = 8, 1.6, 0.6 Hz), 6.19 (d, 1 H, J = 8 Hz), 5.54 (d, 1 H, J = 8 Hz), 4.38 (s, 2 H), 2.84 (s, 3 H), 2.70 (s, 3 H), 2.52 (s, 3 H), 2.37 (s, 3 H); low-resolution CIMS m/e (relative intensity) 277 (MH^+, 48), 276 (M^+, 100); high-resolution CIMS m/z calcd MH^+ 277.1705, found 277.1699.

β-(Trimethylsilyl)ethyl 2-(Carboxymethyl)-1,2-dihydroellipticine (11). Under an atmosphere of argon, a 1 M solution of lithium tri-*tert*-butoxyaluminohydride (0.15 mL, 0.15 mmol) was added to a suspension of β-(trimethylsilyl)ethyl 2-(carboxymethyl)ellipticinium bromide (**19**, 5.6 mg, 0.011 mmol) in anhydrous THF (1 mL) that had been cooled to −30 °C in a dry ice–acetone bath. The mixture was allowed to warm slowly to −5 °C, at which time it became homogeneous. Solid sodium fluoride (80 mg) followed by water (20 μL) was added, and the mixture was stirred between −5 and 0 °C for 20 min. The mixture was diluted with benzene (3 mL) and filtered, the filtercake was washed with benzene (10 mL), and the filtrate was concentrated under vacuum at room temperature. The residual solid was triturated with benzene (2 × 5 mL) and filtered, and the filtrate was concentrated under vacuum at room temperature to give the product (3.1 mg, 69.4%) as a yellow solid: ^1H NMR (200 MHz, $CDCl_3$) δ 8.12 (d, 1 H, J = 8 Hz), 7.93 (br s, 1 H), 7.47–7.13 (m), 6.22 (d, 1 H, J = 8 Hz), 5.62 (d, 1 H, J = 8 Hz), 4.56 (s, 2 H), 4.33–4.24 (m, 2 H), 3.80 (s, 2 H), 2.66 (s, 3 H), 2.39 (s, 3 H), 1.10–1.01 (m, 2 H), 0.06 (s, 9 H); low-resolution CIMS m/z (relative intensity) 407 (MH^+, 57), 406 (M^+, 100), 247 (15); high-resolution CIMS m/e calcd M^+ 406.2077, found 406.2072.

2-(2,3,4-Tri-*O*-acetyl-α-L-arabinopyranosyl)-1,2-dihydroellipticine (13) was obtained from **22** as a yellow oil in 68.5% yield by exactly the same procedure used for **11**: ^1H NMR (200 MHz, $CDCl_3$) δ 8.16 (d, 1 H, J = 8 Hz), 7.98 (br s, 1 H), 7.47–7.13 (m, 1 H), 6.38 (d, 1 H, J = 8 Hz), 5.78 (d, 1 H, J = 8 Hz), 5.64 (apparent t, 1 H, J = 10 Hz), 5.37–5.32 (m, 1 H), 5.12 (dd, 1 H, J = 10, 3.5 Hz), 4.75 (d, 1 H, J = 13 Hz), 4.47 (d, 1 H, J = 13 Hz), 4.09 (dd, 1 H, J = 13, 2 Hz), 3.73 (dd, 1 H, J = 13, 1 Hz), 2.75 (s, 3 H), 2.40 (s, 3 H), 2.24 (s, 3 H), 2.03 (s, 3 H), 1.92 (s, 3 H); low-resolution CIMS m/e (relative intensity) 507 (MH^+, 100), 506 (M^+, 82), 259 (23), 247 (72); high-resolution CIMS m/z calcd M^+ 506.2053, found 506.2046.

2-(Methoxymethyl)-1,2-dihydroellipticine (15). Compound **15** was obtained from **24** as a yellow solid in 66.4% yield by exactly the same procedure used for **11**: ^1H NMR (200 MHz, $CDCl_3$) δ 8.15 (d, 1 H, J = 8 Hz), 7.88 (br s, 1 H), 7.44–7.14 (m, 3 H), 6.37 (d, 1 H, J = 8 Hz), 5.58 (d, 1 H, J = 8 Hz), 4.65 (s, 2 H), 4.50 (s, 2 H), 3.38 (s, 2 H), 2.71 (s, 3 H), 2.40 (s, 3 H); low-resolution CIMS m/e (relative intensity) 293 (MH^+, 74), 292 (M^+, 99), 261 (45), 247 (100); high-resolution CIMS m/z calcd M^+ 292.1576, found 292.1559.

4-Formyl-9-methoxy-2-methyl-1,2-dihydroellipticine (33). Freshly distilled $POCl_3$ (240 μL, 2.60 mmol) was added to dry DMF (810 μL), and the mixture was stirred at 0 °C for 30 min under nitrogen. A solution of freshly prepared **9** (167 mg, 0.572 mmol) in chloroform degassed and freshly distilled from CaH_2, 8 mL) was added by means of a double-tipped needle with stirring. After 5 min the ice bath was removed, and the mixture was heated under nitrogen at 68 °C for 17.5 h. The residue was cooled in an ice bath, and water (5 mL) was added cautiously followed by 15% NaOH (4 mL). The layers were separated, and the aqueous layer was extracted with chloroform (2 × 15 mL). The combined organic layers were washed with water (20 mL) and dried (Na_2SO_4). The drying agent was filtered off, and the filtrate was concentrated to give a dark green oil. The oil was chromatographed on neutral alumina (60 g, 1.5 × 7.5 cm) using a stepwise gradient starting with pure $CHCl_3$ up to 30% ethyl acetate–chloroform to give a brownish yellow solid (67.4 mg, 37%) along with a less pure fraction (13.6 mg): mp 205–210 °C (vacuum, rapid heating, dec, darkened at 150 °C); IR (KBr) 3259, 1738, 1599 cm^{-1}; UV (EtOH, ε) 240 (34 840), 256 (34 880), 323 (24 520), 343 (23 360), 364 (sh, 17 800); ^1H NMR (500 MHz, $CDCl_3$) δ 9.21 (s, 1 H), 7.96 (s, 1 H), 7.63 (d, 1 H, J = 2.5 Hz), 7.34 (dd, 1 H, J = 9, 0.5 Hz), 7.28 (s, 1 H), 7.02 (dd, 1 H, J = 9, 2.5 Hz), 4.54 (s, 2 H), 3.92 (s, 3 H), 3.19 (s, 3 H), 2.64 (s, 3 H), 2.47 (s, 3 H); low-resolution CIMS m/z (relative intensity) 321 (MH^+, 100), 320 (M^+, 40); high-resolution CIMS calcd MH^+ 321.1603, found 321.1585.

Methyl 2-(Carboxymethyl)ellipticinium Bromide (17). A mixture of ellipticine (20 mg, 0.08 mmol) and methyl bromoacetate (23 μL, 0.24 mmol) in DMF (0.4 mL) was heated at 70 °C for 1 h and allowed to stir at room temperature for 24 h. Methanol (0.6 mL) was added to solubilize the product, and the solution was added dropwise to anhydrous ether (30 mL) and left in the freezer for overnight. The yellow solid (31.4 mg, 97%) was filtered off, washed with ether (2 × 10 mL), and air-dried: ^1H NMR (200 MHz, DMSO-d_6) δ 12.28 (s, 1 H), 10.15 (s, 1 H), 8.55–8.30 (m, 3 H), 7.70–7.63 (m, 2 H), 7.41 (ddd, 1 H, J = 8, 5, 3 Hz), 5.75 (s, 2 H), 3.81 (s, 3 H), 3.29 (s, 3 H), 2.86 (s, 3 H); low-resolution FABMS m/z (relative intensity) 319 (M^+, 86), 289 (100); high-resolution FABMS calcd M^+ 319.1447, found 319.1440.

***tert*-Butyl 2-(Carboxymethyl)ellipticinium bromide (18)** was obtained in 86.5% yield by the procedure used for **17**: ^1H NMR (200 MHz, DMSO-d_6) δ 12.26 (s, 1 H), 10.14 (s, 1 H), 8.48 and 8.43 (s and d, 3 H, J_d = 8 Hz), 7.71–7.60 (m, 2 H), 7.40 (ddd, 1 H, J = 8, 5, 3 Hz), 5.64 (s, 2 H), 3.28 (s, 3 H), 2.85 (s, 3 H), 1.50 (s, 9 H); low-resolution FABMS m/z (relative intensity) 361 (M^+, 100), 305 (56), 289 (100).

β-(Trimethylsilyl)ethyl 2-(Carboxymethyl)ellipticinium bromide (19) was obtained in quantitative yield by the procedure used for **17**: ^1H NMR (200 MHz, DMSO-d_6) δ 12.31 (br s, 1 H), 10.17 (s, 1 H), 8.56–8.46 (m, 3 H), 7.73–7.64 (m, 2 H), 7.46–7.38 (m, 1 H), 5.72 (s, 2 H), 4.35–4.26 (m, 2 H), 3.32 (s, 3 H), 2.88 (s, 3 H), 1.09–1.00 (m, 2 H), 0.03 (s, 9 H); low-resolution FABMS m/z (relative intensity) 405 (M^+, 100), 377 (93), 260 (51), 245 (67); high-resolution FABMS calcd M^+ 405.1998, found 405.1975.

2-(Carboxymethyl)ellipticinium Bromide (20). A suspension of **18** (31 mg, 0.07 mmol) in 1 M hydrobromic acid (5 mL) was heated under reflux for 1 h and stirred at room temperature for 24 h. The reaction mixture was filtered, and the filtercake washed with water (5 mL) and acetone (5 mL) and air-dried to give a yellow solid (21.9 mg, 81%): ^1H NMR (200 MHz, DMSO-d_6) δ 12.30 (s, 1 H), 10.12 (s, 1 H), 8.46 and 8.42 (s and d, 3 H, J_d = 8 Hz), 7.72–7.60 (m, 2 H), 7.39 (ddd, 1 H, J = 8, 6, 3 Hz), 5.61 (s, 2 H), 3.26 (s, 3 H), 2.84 (s, 3 H); low resolution FABMS m/z (relative intensity) 305 (M^+, 100); high-resolution FABMS calcd M^+ 305.1290, found 305.1293.

2-(Cyanomethyl)ellipticinium bromide (21) was obtained in 81% yield by the procedure used for **17**: ^1H NMR (200 MHz, DMSO-d_6) δ 12.30 (s, 1 H), 10.24 (s, 1 H), 8.62–8.48 (m, 2 H), 8.41 (d, 1 H, J = 8 Hz), 7.65 (d, 2 H, J = 4 Hz), 7.46–7.34 (m, 1 H), 6.05 (s, 2 H), 3.28 (s, 3 H), 2.83 (s, 3 H); low-resolution FABMS m/z (relative intensity) 286 (M^+, 100); high-resolution FABMS calcd M^+ 286.1344, found 286.1340.

2-(2,3,4-Tri-*O*-acetyl-α-L-arabinopyranosyl)ellipticinium Bromide (22). Under an argon atmosphere a suspension of ellipticine (246 mg, 1.0 mmol), 2,3,4-tri-*O*-acetyl-β-L-arabinpyranosyl bromide (678 mg, 2.0 mmol), and cadmium carbonate (258 mg, 1.5 mmol) in dry nitromethane (30 mL) was heated under reflux for 15 min. The solids were filtered off and washed with nitromethane (5 mL), and the filtrate was concentrated to dryness. The residue was dissolved in a mixture of chloroform–methanol, adsorbed on sodium sulfate (1.7 g), and loaded on top of a silica gel column (60 g, 2 × 24.5 cm). The column was eluted with 10% methanol–methylene chloride to give the product (180 mg, 31%) as an orange-colored solid: ^1H NMR (500 MHz, DMSO-d_6) δ 12.47 (s, 1 H), 10.14 (s, 1 H), 8.60–8.52 (m, 3 H), 7.75–7.69 (m, 2 H), 7.47–7.44 (m, 1 H), 6.36–6.34 (m, 1 H), 5.52–5.51 (m, 2 H), 5.44–5.43 (m, 1 H), 4.33 (s, 2 H), 3.39 (s, 3 H), 2.89 (s, 3 H), 2.27 (s, 3 H), 2.00 (s, 3 H), 1.80 (s, 3 H); low-resolution FABMS m/z (relative intensity) 505 (M^+, 100), 247 (89), 233 (64); high-resolution FABMS calcd M^+ 505.1975, found 505.1973.

2-α-L-Arabinopyranosylellipticinium Bromide (23). A solution of **22** (50 mg, 0.085 mmol) in methanol (8 mL) was cooled to 0 °C, saturated with anhydrous ammonia, and stirred at 0 °C for 24 h. The mixture was filtered, and the filtrate was concentrated on a rotary evaporator. The residue was dissolved in methanol (10 mL), concentrated to dryness, and triturated with methylene chloride (20 mL). The red solid (27.2 mg, 70%) was filtered off, washed with methylene chloride, and dried under high vacuum: ^1H NMR (500 MHz, DMSO-d_6) δ 10.08 (s, 1 H), 8.55 (s, 2 H), 8.48 (d, 1 H, J = 8 Hz), 7.71–7.65 (m, 2 H), 7.42 (ddd, 1 H, J = 8, 6.5, 1.5 Hz), 5.78 (d, 1 H, J = 8.5 Hz), 5.63 (d, 1 H, J = 5.5 Hz), 5.27 (d, 1 H, J = 6 Hz), 5.15 (d, 1 H, J = 6 Hz), 4.10

(dd, 1 H, J = 12.5, 1.5 Hz), 3.96–3.86 and 3.91 (m overlapping d, 3 H, J_d = 12 Hz), 3.67 (ddd, 1 H, J = 8.0, 5.5, 3.0 Hz), 3.34 (s, 3 H), 2.87 (s, 3 H); low-resolution FABMS m/z (relative intensity) 379 (M^+, 68), 247 (29), 233 (100); high-resolution FABMS calcd M^+ 379.1658, found 379.1663.

2-(Methoxymethyl)ellipticinium chloride (24) was obtained in 98% yield by the procedure used for **17**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.50 (s, 1 H), 10.24 (s, 1 H), 8.60–8.43 (m, 3 H), 7.75–7.65 (m, 2 H), 7.42 (ddd, 1 H, J = 8, 6, 2 Hz), 6.03 (s, 2 H), 3.43 (s, 3 H), 3.36 (s, 3 H), 2.89 (s, 3 H); low-resolution FABMS m/z (relative intensity) 291 (M^+, 100); high-resolution FABMS calcd M^+ 291.1497, found 291.1491.

9-Methoxy-2-(methoxymethyl)ellipticinium chloride (25) was obtained in 77% yield by the procedure used for **17** and chromatography on silica gel (10% MeOH–CH_2Cl_2 then 15% MeOH–CH_2Cl_2): ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.35 (br, 1 H), 10.20 (s, 1 H), 8.50 (AB, 1 H, J = 8 Hz), 8.45 (AB, 1 H, J = 8 Hz), 7.86 (d, 1 H, J = 2 Hz), 7.61 (d, 1 H, J = 9 Hz), 7.29 (dd, 1 H, J = 9, 2 Hz), 6.01 (s, 2 H), 3.93 (s, 3 H), 3.42 (s, 3 H), 3.31 (s, 3 H), 2.82 (s, 3 H); low-resolution FABMS m/z (relative intensity) 321 (M^+, 100); high-resolution FABMS calcd M^+ 321.1603, found 321.1596.

2-[(Methylthio)methyl]ellipticinium Chloride (26) and 2-[(Methylsulfinyl)methyl]ellipticinium Chloride (27). The same procedure used for the preparation of **17** was followed. The crude product was chromatographed on silica gel (gradient elution from 5% MeOH–CH_2Cl_2 to 25% MeOH–CH_2Cl_2) to give **26** (37%) and **27** (10%) as yellow solids. For **26**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.43 (s, 1 H), 10.24 (s, 1 H), 8.63–8.44 (m, 3 H), 7.75–7.62 (m, 2 H), 7.41 (ddd, 1 H, J = 8, 6, 2 Hz), 5.94 (s, 2 H), 3.53 (s), 2.88 (s, 3 H), 2.19 (s, 3 H); low-resolution FABMS m/z (relative intensity) 307 (M^+, 100), 260 (42), 247 (67); high-resolution FABMS calcd M^+ 307.1269, found 307.1262. For **27**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.78 (s, 1 H), 10.15 (s, 1 H), 8.58–8.44 (m, 3 H), 7.78–7.63 (m, 2 H), 7.41 (td, 1 H, J = 8, 1 Hz), 6.26 (AB, 1 H, J = 12 Hz), 6.07 (AB, 1 H, J = 12 Hz), 3.32 (s, 3 H), 2.91 (s, 3 H), 2.71 (s, 3 H); low-resolution FABMS m/z (relative intensity) 323 (M^+, 100), 289 (96), 260 (75); high-resolution FABMS calcd M^+ 323.1218, found 323.1207.

Biological Screening Results. Screening was performed using the previously described protocols and reporting procedures.[9-11] DMSO solutions of the compounds were routinely diluted by a factor of 500 with aqueous media. Aliquots of the resulting aqueous solutions or suspensions were tested in the cell cultures for 48 h. Cell growth was monitored colorimetrically either by a metabolic assay in which a formazan dye is generated from a tetrazolium salt by reduction in the presence of viable cells or by a protein stain for total cellular protein.

Topoisomerase Inhibition Results. Topoisomerase inhibition activity by the ellipticinium compounds was measured by quantitating the formation of the "cleavable complex"[31] on pYRG plasmid DNA by mammalian topoisomerase II (purified from human placenta;[38] TopoGEN, Inc., Columbus, OH). The "cleavable complex" product, linear DNA, was quantitated [relative to ellipticine (Sigma Chemical Co., St. Louis, MO)] by densitometric analyses [area determination using Collage image analysis software (Fotodyne, Inc., New Berlin, WI)] of negatives from Polaroid from 665 photodocumentation of UV-fluorescence at 300 nm of EtBr-stained agarose gels.

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Supplementary Material Available: ^{1}H NMR spectra (32 pages). Ordering information is given on any current masthead page.

References

(1) Dalton, L. K.; Demerac, S.; Elmes, B. C.; Loder, J. W.; Swan, J. M.; Teitel, T. Synthesis of the Tumor-Inhibitory Alkaloids, Ellipticine, 9-Methoxyellipticine, and Related Pyrido[4,3-b]carbazoles. *Aust. J. Chem.* **1967**, *20*, 2715–2727.
(2) Gribble, G. W. Synthesis and Antitumor Activity of Ellipticine Alkaloids and Related Compounds. In *The Alkaloids*; Brossi, A., Ed.; Academic Press, Inc.: San Diego, 1990; Vol. 39; pp 239–352.
(3) Sainsbury, M. Ellipticines. In *Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed.; Chapman and Hall: New York, 1990; pp 410–435.
(4) Suffness, M.; Cordell, G. A. Antitumor Alkaloids. In *The Alkaloids*; Brossi, A., Ed.; Academic Press, Inc.: Orlando, 1985; Vol. 25; pp 89–142.
(5) Juret, P.; Tanguy, A.; Girard, A.; LeTalaer, J. Y.; Abbatucci, J. S. Preliminary Trial of 9-Hydroxy-2-methylellipticinium (NSC 264137) in Advanced Human Cancers. *Eur. J. Cancer* **1978**, *14*, 205–206.
(6) Auclair, C.; Pierre, A.; Voisin, E.; Pepin, O.; Cros, S.; Colas, C.; Saucier, J.-M.; Verschuere, B.; Gros, P.; Paoletti, C. Physicochemical and Pharmacological Properties of the Antitumor Ellipticine Derivative 2-(2-Diethylaminoethyl)-9-hydroxyellipticinium Chloride, HCl. *Cancer Res.* **1987**, *47*, 6254–6261.
(7) Atassi, G.; Dumont, P.; Pepin, O.; Gros, O.; Gros, P. SR95325B, A New Ellipticine Derivative Highly Active Against Established Murine Solid Tumors. *Proc. AACR* **1989**, *30*, A2458.
(8) Pierson, V.; Pierre, A.; Pommier, Y.; Gros, P. Production of Protein-associated DNA Breaks by 10-[diethylaminopropylamino]-6-methyl-5*H*-pyrido[3′,4′:4,5]pyrrolo[2,3-g]isoquinoline in Cultured L1210 Cells and in Isolated Nuclei: Comparison with Other Topoisomerase II Inhibitors. *Cancer Res.* **1988**, *48*, 1404–1409.
(9) Boyd, M. R. Status of the NCI Preclinical Antitumor Drug Discovery Screen. *Princ. Pract. Oncol.* **1989**, *3*, 1–12.
(10) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a High-Flux Anticancer Screen Using a Diverse Panel of Cultured Human Tumor Lines. *J. Natl. Cancer. Inst.* **1991**, *83*, 757–766.
(11) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Development of Mean Graph and COMPARE Algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088–1092.
(12) Acton, E. M.; Narayanan, V. L.; Cushman, M.; Haugwitz, R. D.; Risbood, P.; Monks, A.; Scudiero, D.; Shoemaker, R. H.; Boyd, M. R. Anticancer Specificity of some Ellipticinium Salts against Human Brain Tumors In Vitro. In *Proceedings of the Eighty-Third Annual Meeting of the American Association for Cancer Research*; San Diego, California, 1992; pp 488 Abstract Number 2917.
(13) Unpublished results.
(14) Ali-Osman, F.; Rosenblum, M. L.; Giannini, D. D.; Levin, V. A. Potential Antiglioma Activity of 9-Hydroxy-2-*N*-methylellipticine as Determined by Pharmacological and Human Tumor Clonogenic Cell Studies. *Cancer Res.* **1985**, *45*, 2988–2992.
(15) Bodor, N.; Brewster, M. E. Improved Delivery Through Biological Membranes XV-Sustained Brain Delivery of Berberine. *Eur. J. Med. Chem.* **1983**, *18*, 235–240.
(16) Honda, T.; Kato, M.; Inoue, M.; Shimamoto, T.; Shima, K.; Nakanishi, T.; Yoshida, T.; Hoguchi, T. Synthesis and Antitumor Activity of Quaternary Ellipticine Glycosides, a Series of Novel and Highly Active Antitumor Agents. *J. Med. Chem.* **1988**, *31*, 1295–1305.
(17) Dyke, S. F. 1,2-Dihydroisoquinolines. *Adv. Heterocycl. Chem.* **1972**, *14*, 279–330.
(18) Barton, D. H. R.; Hesse, R. H.; Kirby, G. W. Phenol Oxidation and Biosynthesis. Part VIII. Investigations on the Biosynthesis of Berberine and Protopine. *J. Chem. Soc.* **1965**, 6379–6389.
(19) Gibson, H. W.; Bailey, F. C. Chemical Modification of Polymers. Borohydride Reducing Agents Derived from Anion Exchange Resins. *J. Chem. Soc., Chem. Commun.* **1977**, 815.
(20) El Hage Chahine, J. M.; Bertigny, J.; Schwaller, M.-A. Kinetics and Thermodynamics of the Formation of Inclusion Complexes Between Cyclodextrins and DNA-intercalating Agents. Inclusion of Ellipticine in γ-Cyclodextrin. *J. Chem. Soc., Perkin Trans. 2* **1989**, 629–633.
(21) Djedaini-Pilard, F.; Perly, B.; Dupas, S.; Miocque, M.; Galons, H. Specific Interaction and Stabilization Between Host and Guest: Complexation of Ellipticine in a Nucleobase Functionalized Cyclodextrin. *Tetrahedron Lett.* **1993**, *34*, 1145–1148.
(22) Zee, S.-H.; Su, H.-P. A Convenient Method for the Preparation of Ellipticine. *J. Chin. Chem. Soc.* **1987**, *34*, 135–139.
(23) Brewster, M. E.; Kaminski, J. J.; Huang, M.; Bodor, N. Reactivity of Biologically Reduced Pyridines. 7. Energetics and Effect of Substitution on Hydride versus Electron Transfer in Dihydropyridines, Dihydroquinolines and Dihydroisoquinolines. *J. Org. Chem.* **1990**, *55*, 2361–2366.
(24) Sainsbury, M.; Brown, D. W.; Dyke, S. F.; Clipperton, R. D. J.; Tonkyn, W. R. 1,2-Dihydropyridines-XIV. Alkylation. *Tetrahedron* **1970**, *26*, 2239–2247.
(25) Chen, T.-K.; Bradsher, C. K. A New Method for Alkylating Isoquinolinium Salts at the 4-Position. *Tetrahedron* **1959**, *29*, 2951–2953.
(26) Goodwin, S.; Smith, A. F.; Horning, E. C. Alkaloids of *Ochrosia elliptica* Labill. *J. Am. Chem. Soc.* **1959**, *81*, 1903–1908.
(27) Dyke, S. F.; Sainsbury, M.; Brown, D. W.; Palfreyman, M. N. 1,2-Dihydroisoquinolines-IX. Acylation II. *Tetrahedron* **1968**, *24*, 6703–6717.

(28) Dyke, S. F.; Thorns, J. F.; Hedges, J. F.; Wiggins, D. W. 1,2-Dihydroisoquinolines-XXI. Vinylogous Meerwein Salts. *Tetrahedron* **1979**, *35*, 1861–1867.
(29) Monnot, M.; Mauffret, O.; Simon, V.; Lescot, E.; Psaume, B.; Saucier, J.-M.; Charra, M.; Belehradek, J.; Fermandjian, S. DNA-Drug Recognition and Effects of Topoisomerase II-mediated Cytotoxicity. *J. Biol. Chem.* **1991**, *266*, 1820–1829.
(30) Fosse, P.; Rene, B.; Charra, M.; Paoletti, C.; Saucier, J.-M. Stimulation of Topoisomerase II-mediated DNA Cleavage by Ellipticine Derivatives: Structure-activity Relationships. *Mol. Pharmacol.* **1992**, *42*, 590–595.
(31) Barrett, J. F.; Gootz, T. D.; McGuirk, P. R.; Farrell, C. A.; Sokolowski, S. A. Use of In Vitro Topoisomerase II Assays for Studying Quinolone Antibacterial Agents. *Antimicrob. Agents Chemother.* **1989**, *33*, 1697–1703.
(32) Robinson, M. J.; Osherhoff, N. Effects of Antineoplastic Drugs on the Post-strand-passage DNA Cleavage/religation Equilibrium of Topoisomerase II. *Biochemistry* **1991**, *30*, 1807–1813.
(33) Schwaller, M. A.; Aubard, J.; Dodin, G. Kinetic and Thermodynamic Studies on Drug-DNA Interactions in the Ellipticine Series. *Anti-Cancer Drug Design* **1990**, *5*, 77–87.
(34) Tewey, K. M.; Chen, G. L.; Nelson, E. M.; Liu, L. L. Intercalative Antitumor Drugs Interfere with the Breakage-reunion Reaction of Mammalian DNA Topoisomerase II. *J. Biol. Chem.* **1984**, *259*, 9182–9187.
(35) Nelson, E. M.; Tewey, K. M.; Liu, L. F. Mechanism of Antitumor Drug Design: Poisoning of Mammalian DNA Topoisomerase II on DNA by 4′-(9-acridinylamino)methanesulfon-*m*-anisidide. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1361–1365.
(36) Hungerbuhler, E.; Seebach, D.; Wasmuth, D. Enantiomerically Pure Building Blocks with Four C-Atoms and Two or Three Functional Groups from β-Hydroxy-butanoic, Malic, and Tartaric Acids. *Helv. Chem. Acta* **1981**, *64*, 1467–1287.
(37) Hudson, C. S.; Phelps, F. P. Relations Between Rotary Power and Structure in the Sugar Group. V. The Chloro- and Bromo-Acetyl Derivatives of Arabinose. The Nomenclature of Alpha and Beta Forms in the Sugar Group. Some Derivatives of 1,6-Bromo-Acetyl Glucose, Gentobiose, and Maltose. *J. Am. Chem. Soc.* **1924**, *46*, 2591–2604.
(38) Spitzner, J. R.; Muller, M. T. A. Consensus Sequence for Cleavage by Vertebrate DNA Topoisomerase II. *Nucleic Acids Res.* **1988**, *16*, 5533–5556.