Synthesis of 9-*O*-Substituted Derivatives of 9-Hydroxy-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)ethyl)amide and Their 10- and 11-Methyl Analogues with Improved Antitumor Activity

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Division Chimie A and Division de Cancérologie Expérimentale, Institut de Recherches Servier, 11 rue des Moulineaux, 92150 Suresnes, France, and UMR176 CNRS−Institut Curie-Recherche, Bâtiment 110, 15 rue Georges Clémenceau, 91405 Orsay, France

Received October 11, 1998

Analogues of the antitumor drug S 16020-2 modified at the 9, 10, or 11 position were synthesized and evaluated in vitro and in vivo on the P388 leukemia and B16 melanoma models. Starting from 9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic acid ethyl ester, the 11-CH_3 analogue of 9-hydroxy-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic (2-(dimethylamino)ethyl)amide (**1**), compound **4**, was synthesized using a four-step sequence, whereas its 10-CH_3 analogue **5** was prepared using a two-step pathway, starting from compound **1**. Finally starting from the 9-OH compounds **1**, **4**, and **5**, a series of variously 9-*O*-substituted derivatives were synthesized. In these series, the most active compounds resulted from esterification of the 9-OH group with various aliphatic diacids, which led to 9-O-CO-( )-COOH derivatives of **1**, **4**, and **5**. For these compounds, the number of long-term surviving mice obtained at the optimal dose were 60−100% in the ip/iv P388 leukemia and 10−35% in the ip/ip B16 melanoma, corresponding to an improved therapeutic index with respect to **1** and **4**. This high antitumor activity, with curative examples in both models, was not due to a higher cytotoxicity since these compounds were equally or slightly less potent in vitro than **1** and **4.** The most active compounds were thus selected for further in vivo evaluation.

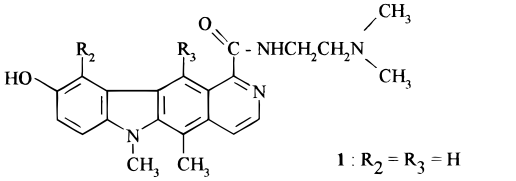
Introduction

In a preceding paper, we described a new series of cytotoxic olivacine derivatives, from which S 16020-2 (**1**) (Chart 1) was selected for further pharmacological and toxicological evaluation.[1] This new potent inhibitor of DNA topoisomerase II[2] was highly cytotoxic against a panel of 20 tumor cell lines, with a mean IC_{50} comparable to that of adriamycin and markedly lower than that of elliptinium acetate.[3] In vivo, S 16020-2 was very active against certain murine transplantable tumors and human xenografts in nude mice.[4,5] Due to its antitumor activity in experimental models, favorable pharmacokinetic characteristics, and acceptable toxicity,[6] S 16020-2 is currently studied in clinical trials.[7]

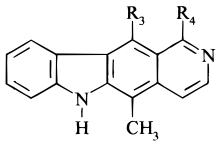
The aim of the present work was the synthesis of new analogues and derivatives possessing comparatively higher antitumor potency with an improved therapeutic index. We thus focused on three positions of the parent molecule that appear to be implicated in the biological activity and therefore worthy of modification: methylation at positions 10 and 11 and substitution of the free hydroxyl group at position 9.

Ellipticine (**2**), which bears a methyl group at position 11, was shown to be slightly more cytotoxic in vitro than olivacine (**3**) and also more toxic in mice as indicated by a lower LD_0.[8] Data available for a limited number of pairs of olivacine/ellipticine derivatives which differ only by the presence of the 11-CH_3 group suggest that these

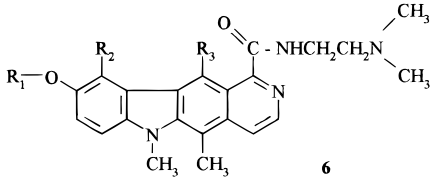
Chart 1



1 : $R_2 = R_3 = H$
4 : $R_2 = H$, $R_3 = CH_3$
5 : $R_2 = CH_3$, $R_3 = H$



2 : $R_3 = CH_3$, $R_4 = H$
3 : $R_3 = H$, $R_4 = CH_3$



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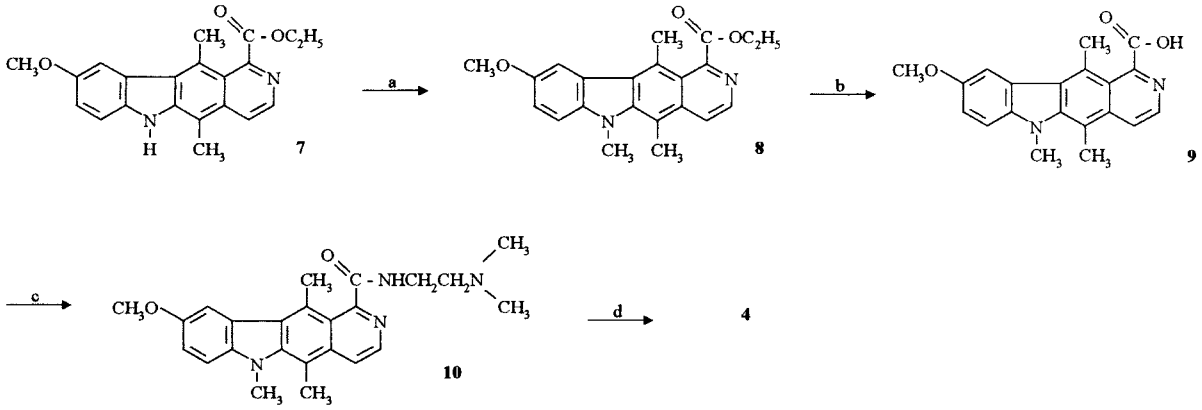
§ UMR176 CNRS−Institut Curie-Recherche.

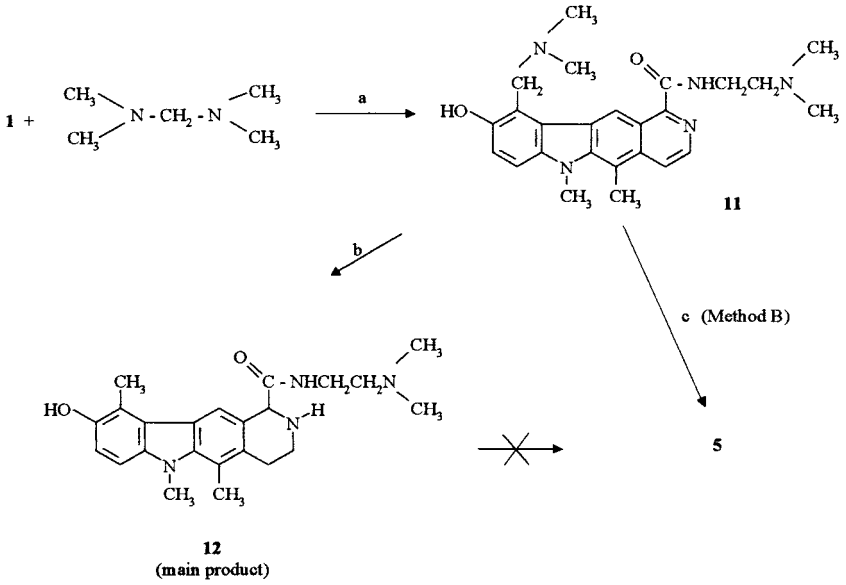
10.1021/jm981093m CCC: $18.00
Published on Web 06/02/1999

Scheme 1[a]



[a] Method A: (a) dimethyl carbonate, adogene 464, DMF, 89%; (b) NaOH, H_2O, ethanol, 52%; (c) $H_2N-CH_2CH_2N(CH_3)_2$, PyBOP, NMP, 86%; (d) BBr_3, CH_2Cl_2, 82%.

Scheme 2[a]



[a] (a) Acetic acid, dioxane, 86%; (b) 10% Pd/C, ethanol, H_2, 50%; (c) cyclohexene, 10% Pd/C, NMP, 80%.

properties might be general.[6] To have access to 11-CH_3 derivatives, we developed a synthetic route to compound **4**, herein described for the first time.

No data were available concerning derivatives bearing a methyl group at position 10 in these series of compounds. The possible involvement of a quinone imine, generating an electrophilic center at the 10 position, in either their antitumor activity or their toxicity[9] led us to synthesize the novel compound **5** and derivatives and to study their pharmacological properties.

Hydroxylation at the 9 position was previously shown to be a favorable modification in the ellipticine series: it increases the affinity for DNA,[10] stabilizes the DNA topoisomerase II cleavable complex,[11] and consequently increases the cytotoxicity and antitumor activity.[1,12] Moreover, it is interesting to note that many anticancer drugs interacting with topoisomerases possess a phenolic group. It can be hypothesized that this phenol plays an important role in the mechanism of antitumor action and also in the induction of some toxic side effects through the generation of the quinone imine in the case of ellipticines and olivacines.[13] Hence we decided to mask this function with hydrolyzable groups in order to decrease the general toxicity of the compounds and to allow gradual generation of the more active hydroxylated compounds. Compounds **1**, **4**, and **5** were therefore subjected to these modifications, to obtain various products corresponding to the general structure **6**.

Chemistry

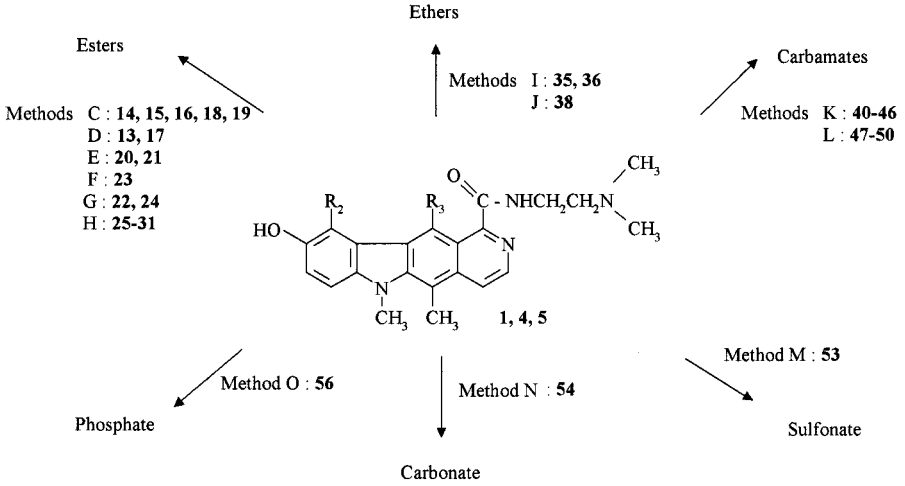
The general structure **6** is characterized by the presence of a fused tetracycle 6*H*-pyrido[4,3-*b*]carbazole substituted at position 1 by a 2-(dimethylamino)ethylcarbamoyl group and by methyl groups at positions 5 and 6. R_2 and R_3 substituents at positions 10 and 11 are either a proton or a methyl. In all cases, the variable group R_1 is bound to the 9 oxygen atom of the tetracycle and includes an ester, ether, carbamate, sulfonate, carbonate, or phosphate function.

The syntheses of original compounds **4** and **5** are summarized in Schemes 1 and 2 (methods A and B), whereas derivatives described in this publication were prepared from compounds **1**,[1] **4**, and **5** using various methods (Schemes 3−5, methods C−P).

The structures of all compounds corresponding to the general formula **6** are described in Table 1.

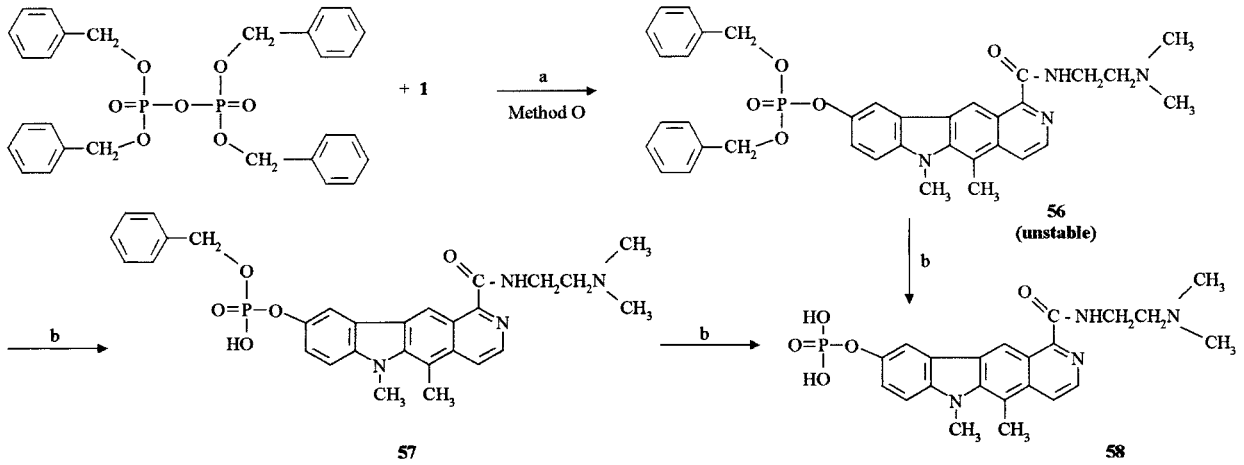
Methylation of the previously described ester **7**[1] using dimethyl carbonate as methylating agent gave com-

Scheme 3[a]



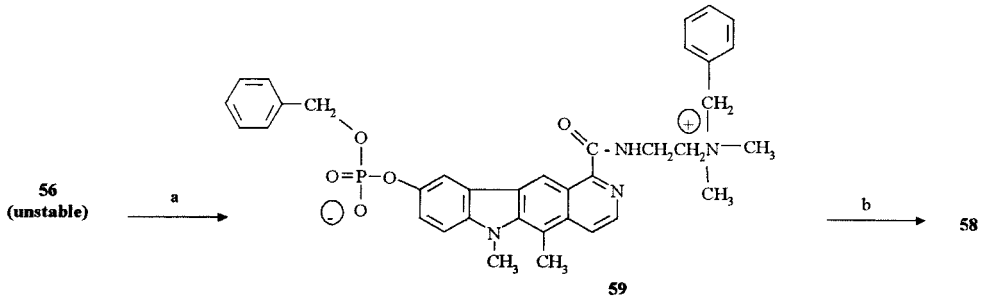
[a] Method C: acid chloride, $N(C_2H_5)_3$, THF, 25−65%. Method D: acid anhydride, pyridine, 28.5−75%. Method E: carboxylic acid, DCC, HOBT, DMF, 40−67.5%. Method F: carboxylic acid, DCC, DMAP, pyridine, 91%. Method G: carboxylic acid, PyBOP, NMP, 24−88%. Method H: cyclic anhydride, pyridine, 34−87%. Method I: alcohol, PPh_3, DEAD, THF, CH_2Cl_2, 32%. Method J: bromoalkyl ester, Cs_2CO_3, DMF, 69%. Method K: *N*-chloroformate, pyridine, 51−95%. Method L: isocyanate, DBU, pyridine, 42−92%. Method M: alkanesulfonyl chloride, $N(C_2H_5)_3$, THF, 63.5%. Method N: 3-(benzyloxycarbonyl)propyl chloroformate, pyridine, NMP, 39%. Method O: NaH, tetrabenzyl pyrophosphate, THF, 63%.

Scheme 4[a]



[a] (a) NaH, THF, 63%; (b) cyclohexene, Pd/C, NMP (method P); yields and reaction conditions indicated in Experimental Section.

Scheme 5[a]

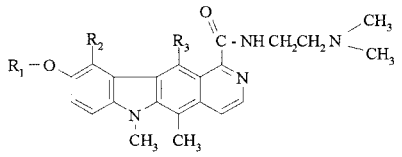


[a] (a) Room temperature (4 days); (b) cyclohexene, 10% Pd/C, NMP, 62.8%.

pound **8,** which on condensation with 2-(dimethylamino)-ethylamine failed to yield the amide **10** probably because of the steric hindrance due to the presence of the 11 methyl group. Indeed, at 120 °C no reaction occurred, but when the condensation was performed at a higher temperature, about 170 °C, compound **8** spontaneously lost its 1-$COOC_2H_5$ group and gave the 1-H analogue of **8**. Therefore, we tried to carry out the amidification from the acid **9**, obtained by saponification of **8**, using several classical coupling agents such as ethyl chloroformate, DCC−HOBT,[14] or DCC−DMAP[15] which totally failed. However, using PyBOP[16] in NMP, which generally works with hindered carboxylic acids, the amide **10** was obtained in good yield, and subsequent demethylation in the presence of BBr_3 gave the desired compound **4** (method A).

On the other hand, when performed with compound **1** using *N*,*N*,*N*′,*N*′-tetramethyldiaminomethane, a

Table 1. Biological Activity of Compounds



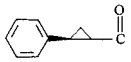
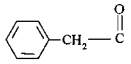
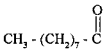
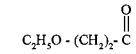
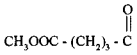
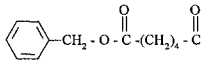
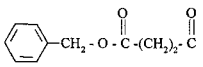
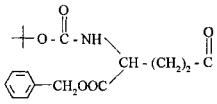
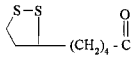
				Cytotoxicity IC 50 (nM)[a]		Antitumor activity against P388 leukemia[b]				Antitumor activity against B16 melanoma[f]		
Compounds	**R_1**	**R_2**	**R_3**	**L1210**	**B16**	**Schedule**	**Optimal dosage (mg/kg)[c]**	**T/C (%)[d]**	**LTS day 60[e]**	**Optimal dosage (mg/kg)[c]**	**T/C (%)[d]**	**LTS day 90[e]**
Adriamycin				34.3	7.6	D1 D1, 5, 9	20 10	258 332	2/10 4/10	2.5	456	3/7
Elliptinium				54.6	106.0	D1 D1, 5, 9	20 7.5	138 112	0/6 0/6	0.5	133	0/7
1	H	H	H	13.1	5.4	D1 D1, 5, 9	80 80	180-227 > 541	0/12 7/12	5	157-174	0/14
4	H	H	CH_3	13.4	3.4	D1 D1 , 5, 9	40 10	> 600 > 600	15/18 9/12	2.5	155-166	1/14
5	H	CH_3	H	3.5	0.2	D1 D1, 5, 9	20 10	180-211 220-223	0/12 0/12	0.3-0.6	138-144	0/13
13		H	H	5.1	6.1	D1 D1, 5, 9	60 40	214 275	1/6 0/6	10	182	0/6
14		H	H	30.9	14.1	D1 D1 , 5, 9	20 10	117-146 140-152	0/12 0/12	5	129	0/7
15		H	H	26.7	22.3	D1 D1, 5, 9	10 10	125 157	0/6 0/6	5	147	0/7
16		H	H	31.1	20.8	D1 D1, 5, 9	10 10	131 147	0/6 0/6	10	160	0/7
17		H	H	214.6	195.3	D1 D1, 5, 9	40 10	176 153	0/6 0/6	10	143-166	1/14
18		H	H	5.7 .	3.0	D1 D1, 5, 9	20 20	150 222	0/6 0/6	10	157	0/7
19		H	H	5.4	7.1	D1 D1, 5, 9	20 10	135-145 160-158	0/12 0/12	5	144	0/7
20		H	H	108.8	58.4	D1 D1, 5, 9	20 10	124 128	0/6 0/6	40	102	0/7
21		H	H	47.4	9.9	D1 D 1, 5, 9	10 5	111 96	0/6 0/6	10	169	1/6
22		H	H					Not tested				
23		H	H	74.5	2.3	D1 D 1, 5, 9	80 40	192-199 270-283	0/12 0/12	10	159-190	0/13
24		H	H	118.0	14.0	D1 D 1, 5, 9	10 10	118 129	0/6 0/6	5	140	0/7
25		H	H	19.7	14.8	D1 D 1, 5, 9	160-320 80	246->590 427-> 582	4/12 8/12	10	167-196	2/21

Table 1 (Continued)

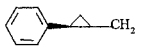
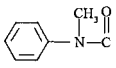
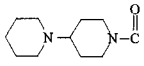
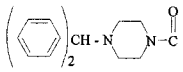
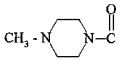
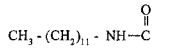
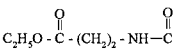
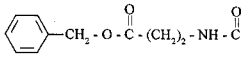
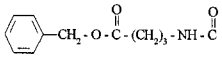
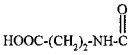
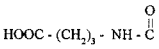
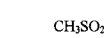
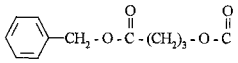
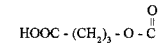
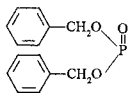
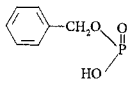
				Cytotoxicity IC 50 (nM)[a]		Antitumor activity against P388 leukemia[b]				Antitumor activity against B16 melanoma[f]		
Compounds	**R_1**	**R_2**	**R_3**	**L1210**	**B16**	**Schedule**	**Optimal dosage (mg/kg)[c]**	**T/C (%)[d]**	**LTS day 60[e]**	**Optimal dosage (mg/kg)[c]**	**T/C (%)[d]**	**LTS day 90[e]**
26	$HOOC-(CH_2)_4-C(=O)$	CH_3	H	24.7	6.4	D1 D 1, 5, 9	40 20	253-300 471-> 600	0/12 4/12	1.25	127	0/7
27	$HOOC-(CH_2)_4-C(=O)$	H	CH_3	45.9	6.3	D1 D 1, 5, 9	40 20	> 600 > 600	11/12 12/12	2.5-5	234-361	4/14
28	$HOOC-(CH_2)_3-C(=O)$	H	CH_3	34.0	1.4	D1 D 1, 5, 9	40 20	> 640 > 640	11/12 11/12	5	196-202	0/21
29	$HOOC-CH_2-C(CH_3)_2-CH_2-C(=O)$	H	H	2.7	3.7	D1 D 1, 5, 9	320 160	171-> 600 342-404	4/12 3/12	20-40	171->466	5/14
30	$HOOC-(CH_2)_5-C(=O)$	H	H	3.7	3.2	D1 D 1, 5, 9	320 80	270-> 600 370- > 600	5/12 5/12	10	175-246	1/14
31	$HOOC-(CH_2)_6-C(=O)$	H	H	53.2	3.1	D1 D 1, 5, 9	160 80	258-287 515-> 645	1/12 5/12	20	158	0/7
32	$HOOC-(CH_2)_4-C(=O)$	H	H	20.8	5.0	D1 D 1, 5, 9	240-320 80-160	> 607 > 607	16/19 15/19	10	155- > 352	4/12
33	$+O-C(=O)-NH-CH(COOH)-(CH_2)_2-C(=O)$	H	H	89.3	4.0	D1 D 1, 5, 9	320 80	>614 >614	3/6 3/6	20	188	0/7
34	CH_3	H	H	550.8	333.6	D1 D 1, 5, 9	40 20	94 96	0/6 0/6	10	126	0/7
35	$H_3C(CH_3)C(O)O-CH_2-CH-CH_2$	H	H					Not tested				
36		H	H	374.2	279.3	D1 D 1, 5, 9	20 20	102 99	0/6 0/6	5	94	0/ 7
37	$HO-CH_2-CHOH-CH_2$	H	H	500.0	954.5	D1 D 1, 5, 9	240 160	127 114	0/6 0/6	20	129	0/7
38	$C_2H_5OOC-(CH_2)_4$	H	H					Not tested				
39	$HOOC-(CH_2)_4$	H	H	4227.0	3466.0	D1 D 1, 5, 9	160 160	107 108	0/6 0/6	20	146	0/6
40	$(CH_3)_2N-C(=O)$	H	H	1850.0	2666.7	D1 D 1, 5, 9	80 40	124 119	0/6 0/6	20	161	0/7
41	$CH_3-(CH_2)_{11}-N(CH_3)-C(=O)$	H	H	2866.0	3256.0	D1 D 1, 5, 9	10 5	103 100	0/6 0/6	40	115	0/7
42		H	H	1168.0	1374.0	D1 D 1, 5, 9	10 5	100 106	0/6 0/6	20	101	0/7
43		H	H	696.5	2689.0	D1 D 1, 5, 9	40 40	133 169	0/6 0/6	10	161	0/7
44		H	H	163.9	589.9	D1 D 1, 5, 9	5 5	102 94	0/6 0/6	20	102	0/6
45		H	H	1296.5	2471.3	D1 D 1, 5, 9	160 80	146 134	0/6 0/6	20	159	0/7

Table 1 (Continued)

				Cytotoxicity IC 50 (nM)[a]		Antitumor activity against P388 leukemia[b]				Antitumor activity against B16 melanoma[f]		
Compounds	**R_1**	**R_2**	**R_3**	**L1210**	**B16**	**Schedule**	**Optimal dosage (mg/kg)[c]**	**T/C (%)[d]**	**LTS day 60[e]**	**Optimal dosage (mg/kg)[c]**	**T/C (%)[d]**	**LTS day 90[e]**
46		H	H	645.5	1766.5	D1 D 1, 5, 9	160 80	149 196	0/6 0/6	20	139	0/7
47		H	H	808.7	2299.5	D1 D 1, 5, 9	10 10	104 107	0/6 0/6	40	133	0/7
48		H	H	16.4	5.7	D1 D 1, 5, 9	40 20	97 95	0/6 0/6	10	115	0/7
49		H	H					Not tested				
50		H	H	304.5	29.4	D1 D 1, 5, 9	20 10	103 93	0/6 0/6	40	96	0/7
51		H	H	654.1	78.7	D1 D 1, 5, 9	240 160	109 109	0/6 0/6	80	138	0/7
52		H	H	522.1	45.7	D1 D 1, 5, 9	240 160	111 109	0/6 0/6	80	139	0/7
53		H	H	868.5	782.1	D1 D 1, 5, 9	160 40	96 106	0/6 0/6	20	142	0/6
54		H	H					Not tested				
55		H	H	64.0	4.8	D1 D 1, 5, 9	320 80	> 600 > 600	8/12 7/12	20	167	0/7
56		H	H					Not tested				
57		H	H	3999.0	511.0	D1 D 1, 5, 9	320 160	142 111	0/6 0/6	80	144	0/7
58		H	H	50.5	4.1	D1 D 1, 5, 9	160 40	239 405	1/6 1/6	10	171	0/7

[a] Inhibition of cell proliferation measured by the MTT assay (mean of ≥2 values obtained in independent experiments). [b] Mice were inoculated ip on day 0 with 10^6 P388 cells and treated iv by at least three doses of the compounds. [c] Dose (mg/kg) giving the best antitumor activity without major toxic effects (no lethality and body weight loss <20%). [d] Median survival time of treated/median survival time of control animals, obtained in one or two representative experiments. [e] Long-term survivors scored on day 60 (P388) or 90 (B16). [f] 0.5 mL of a tumor brei was injected ip on day 0, and compounds were administered ip on days 1−9.

Mannich reaction yielded compound **11** according to a previously described procedure.[17] Catalytic hydrogenation of derivative **11** gave the expected compound **5** (15% yield) besides the 1,2,3,4-tetrahydro-6*H*-pyrido[4,3-*b*]-carbazole derivative **12** (50% yield) as the major product. Aromatization of **12** over palladium on charcoal in xylene totally failed. Nevertheless, our study in order to find optimized conditions allowed us to obtain compound **5** in very good yield (80%) by replacing hydrogen gas by cyclohexene and palladium on charcoal in NMP.[18]

9-*O*-Substituted derivatives of compounds **1**, **4**, and **5**, described in Scheme 3, were readily prepared, using classical organic reactions from 9-OH compounds **1**, **4**, and **5**. Benzyl esters **20** and **21** were obtained from compound **1** and adipic and succinic monobenzyl ester,[19] respectively, using DCC−HOBT[14] as coupling agent (method E). Benzyl ester **22** was prepared from **1** and *N*-*t*BOC-L-glutamic acid α-benzyl ester using PyBOP[16] as coupling agent (method G). Debenzylation of **20** and **22** was achieved with cyclohexene and palladium on carbon as catalyst[18] in *N*-methylpyrrolidone (method P) to give monoesters **32** and **33,** respectively. It can be pointed out that debenzylation of benzyl ester **21**, using the same method P, failed to give the expected succinic acid monoester of compound **1**, this compound being probably susceptible to hydrolysis. Acidic hydrolysis of the protected glycerol ether **35** and saponification of ethyl ester **38** gave compounds **37** and **39**, respectively.

Condensation of compound **1** with (*R,S*)-*trans*-2-phenylcyclopropylmethanol[20] using the Mitsunobu procedure[21] gave compound **36**. Condensation of crude 3-(benzyloxycarbonyl)propyl chloroformate (prepared from 4-hydroxybutyric acid benzyl ester[22] and phosgene in toluene using dimethylaniline as base) with compound **1** in pyridine gave the carbonate **54** (method N). This compound was further debenzylated to give compound **55** (method P).

Reaction between tetrabenzyl pyrophosphate[23] with compound **1** gave unstable phosphate **56** (method O, Scheme 4) which was further monodebenzylated or didebenzylated, according to the reaction conditions, using method P[18] to give compounds **57** and **58**, respectively.

After 4 days at room temperature, the unstable compound **56** gave the zwitterion **59** resulting from the migration of one benzyl group from the 9-dibenzyl phosphate moiety to the dimethylamino part of the 1-substituent of compound **56**. Further debenzylation of **59**, using method P, gave compound **58**.

Pharmacology

In vivo evaluation was performed on two murine experimental models, the P388 leukemia and the B16 melanoma. The compounds were tested at three doses in the first experiment, and, if active, at five doses in a second experiment. To estimate the therapeutic index, the doses range includes a low, nontoxic, inactive dose and a high, toxic dose. Table 1 lists the antitumor activity obtained at the optimal dose, which is the dose giving the best T/C without major toxicity estimated by a body weight loss > 20% and/or toxic death in the experimental group. The optimal dose is generally immediately below the toxic dose.

Compound **1** was markedly active against the P388 leukemia following a day 1, 5, 9 schedule of administration, inducing on average 62% of long-term survivors (LTS) at 80 mg/kg (n = 40 experiments). It was less active on this model when administered on day 1 (4 % of LTS on average) and moderately active on the B16 melanoma, inducing a mean T/C of 157% and 3% of LTS (n = 60 experiments). Elliptinium acetate was only moderately active on the two models, without inducing LTS.

Hence, we selected the compounds giving a significant number of LTS on the P388 leukemia (day 1 schedule) and the B16 melanoma for further evaluation.They are **4**, **25**, **27**−**33**, and **55** for P388 and **4**, **21**, **25**, **27**, **29**, **30**, and **32** for B16. Some of these compounds have a broader therapeutic index than **1**, as illustrated on Figures 1 and 2. On P388 leukemia, the therapeutic index (TI) of **1** was 8 versus 16 for **32**, which induced 16 LTS over 19 mice at 240−320 mg/kg. On B16 melanoma, the TI of **1** was 8 versus 32 for **25** (Figure 2). All these compounds were either equally or slightly less cytotoxic than **1** and induced a massive accumulation of L1210 cells in the G2+M phase of the cell cycle at cytotoxic concentrations (not shown). This modification of the cell cycle reflects at the cellular level the inhibition of DNA topoisomerase II, as is the case for **1**.[2,3]

Structure−Activity Relationships

The present work was undertaken to determine the effect of methylation at positions 10 and 11 and substitution of the 9-hydroxy group of compound **1** and analogues **4** and **5** on cytotoxicity and antitumor activity.

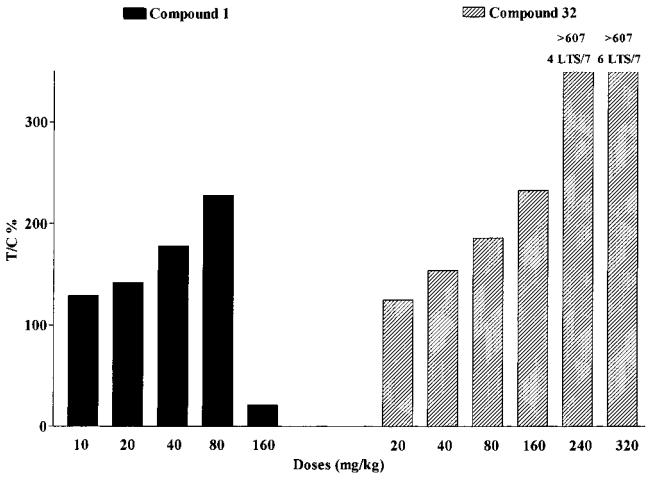


Figure 1. Antitumor activity against ip P388 leukemia. Compounds were administered by iv route on day 1 to P388 leukemia-bearing mice. Results are expressed as % T/C and long-term survivors (LTS) as indicated in the Experimental Section.

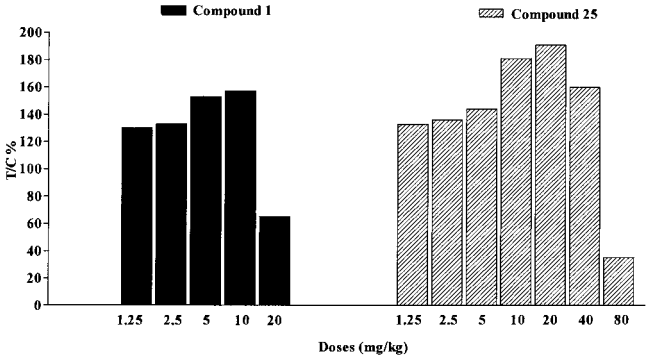


Figure 2. Antitumor activity against ip B16 melanoma. On day 0, 0.5 mL of B16 tumor brei at 1 g/10 mL was inoculated ip to B6D2F1 mice. Drugs were administered ip daily for a total of 9 injections (days 1−9). Results are expressed as % T/C.

Effect of Methylation at Position 10. Although **5** (10-CH_3) was more cytotoxic than **1** (10-H) on both cell lines, it was less active in vivo in the two models as no LTS were scored at the optimal doses. In addition, **5** was more toxic than **1** as shown by comparison of their iv optimal doses (day 1): 20 versus 80 mg/kg, respectively. The 10 methyl group, supposed to prevent the covalent binding of nucleophiles after formation of a quinone imine, thus led to a more cytotoxic and toxic compound. It is interesting to note that **1** and **5** showed a similar antitumor efficiency at low doses, which suggests that covalent binding to a cellular target like DNA is not involved in the mechanism of action of this family of compounds, at least through the 10 position of the 6*H*-pyrido[4,3-*b*]carbazole system.

A similar trend is observed for compound **26** (10-CH_3) which is less active in vivo and more toxic than compound **32** (10-H).

Effect of Methylation at Position 11. The cytotoxicity of compound **4** (11-CH_3) was similar to that of compound **1** (11-H) on both cell lines. The lack of effect of methylation at position 11 on cytotoxicity was also

observed with **27** versus **32** and with **28** versus **25** on L1210 cells only, **28** being surprisingly more cytotoxic than **25** on B16 cells.

In vivo, **4** was more potent and markedly more active than **1** following the day 1 schedule against the P388 leukemia (15 LTS/18 mice versus 0/12, respectively). Following the D1,5,9 schedule, both compounds were highly active, but **4** was 8-fold more potent than **1** (optimal doses 10 versus 80 mg/kg, respectively). Against the B16 melanoma, both compounds were equally active, but **4** was 2-fold more potent than **1**.

Similarly, a more pronounced antitumor activity and a higher potency of the 11-CH_3 derivatives were observed in the P388 model with the two pairs of derivatives: **28** versus **25** and **27** versus **32**. The two 11-CH_3 derivatives **28** and **27** were highly active following both schedules of administration, curing 90% of mice at doses 4−6-fold lower than those required for their 11-H derivatives **25** and **32**. Against the B16 melanoma, **28** and **27** were more potent than **25** and **32**.

Effect of Substitution of the 9-OH Group. Among the 9-OH-substituted derivatives, the most active compounds, in vitro and in vivo, corresponded to 9-O-CO-( )-COOH derivatives of the heterotetracyclic system. Derivatives of **1** (**25**, **29**, **32**) were generally more active than **1** in vivo and presented a better therapeutic index as shown by a higher optimal dose (Figure 2), although derivatives **27** and **28** (11-CH_3) were about as active as **4** at similar doses. It is thus clear that the substitution of the 9-OH group by an ester carrying a free COOH function is more favorable for 11-H than for 11-CH_3 derivatives. These 9-OH-substituted compounds can be cytotoxic by themselves or by generating the corresponding free hydroxyl group after cleavage of the ester bound by cellular esterases. Preliminary results show that **25** is less potent than **1** at inhibiting the catalytic activity of purified DNA topoisomerase II (not shown), which suggests that these esters are prodrugs of the 9-OH compounds.

The more lipophilic compounds containing one or two phenyl groups (**14**, **15**, **20**, **21**, **42**, **44**, **50**) or a linear polymethylene chain (**16**, **41**, **47**) were among the most toxic compounds studied as shown by their low optimal doses and were either slightly active or inactive.

Conclusion

Among the modifications of the olivacine/ellipticine derivatives studied in this work, methylation at the 11 position and aliphatic diacid monoesterification of the 9-OH position were shown to markedly improve the antitumor activity. The 11-methylated derivatives showed a more pronounced antitumor activity at lower doses than their corresponding 11-H derivatives. The most active compounds described in this study (**4**, **27**, **28**, **32**) are probably the most potently active ellipticine analogues described to date, dramatically more active than elliptinium acetate, in that they cured more than 80% of mice bearing the P388 leukemia after one iv injection. The monoesterification of the 9-OH of S16020-2 by an aliphatic diacid led to compounds with a very low toxicity, the optimal dose being in the range 160−320 mg/kg. Consequently, their therapeutic index was significantly improved. Highly active compounds (**4**, **25**−**32**) were selected for further evaluation in experimental models of solid tumors and for preliminary toxicological studies.

Experimental Section

Chemistry. Autonom 2 software was used to name compounds, used or prepared, in this publication.

Melting points (cap) were determined on a Mel-temp capillary apparatus and were uncorrected. Melting points (K) were determined on a Kofler apparatus. Fast atom bombardment mass spectra (FAB) and chemical ionization spectra (CI) were performed on a Nermag R10-10 C instrument. ^{1}H NMR spectra of compounds in solution in $CDCl_3$ or DMSO-d_6 were recorded using a Bruker AC 200 spectrometer. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet; AB, AB system), number of protons. Elemental analyses were performed on a Carlo Erba analyzer 1108. Column chromatography was performed using Amicon silica gel (0.035−0.07 mm) under a 1 bar nitrogen pressure (flash chromatography). All reactions were carried out under a nitrogen atmosphere. The methods of preparation, the yields, and the melting points for the final step products are reported. The formula indicates possible salification or solvation of products.

Dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBT), 4-(dimethylamino)pyridine (DMAP), *N*-methyl-2-pyrrolidone (NMP), triphenylphosphine (PPh_3), diethyl azodicarboxylate (DEAD), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyBOP) were obtained from Aldrich.

9-Hydroxy-5,6,11-trimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)ethyl)amide Dihydrochloride (4) (Method A, Scheme 1). A 1 M solution of boron tribromide in dichloromethane (230 mL, 230 mmol) was added to a stirred suspension of compound **10** (9.5 g, 23 mmol) and water (3 mL) in dichloromethane (380 mL) at −50 °C, and then the temperature was allowed to rise to 10 °C before pouring onto crushed ice and water. The mixture was made basic by addition of a 28% ammonia solution. The solid was collected by filtration, washed with water, and dried under vacuum. Column chromatography, eluting with a mixture of 1% ammonia solution (28%) and 10% methanol in dichloromethane, gave compound **4** as the free base (8.2 g). The product was suspended in ethanol (200 mL) and treated with 2 M solution of hydrogen chloride in ethanol. The solid was collected by filtration and washed with ethanol and ether before drying at 50 °C under vacuum to yield compound **4** (8.75 g, 82%): mp(cap) 288−290 °C; ^{1}H NMR (DMSO-d_6) δ 9.5−9.0 (m, 4H, exchangeable for D_2O), 8.5 (m, 2H), 7.8 (d, 1H), 7.6 (d, 1H), 7.2 (dd, 1H), 4.2 (s, 3H), 3.9 (m, 2H), 3.5 (m, 2H), 3.1 (2s, 6H), 2.95 (s, 6H). Anal. ($C_{23}H_{26}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$) C, H, N, Cl.

9-Hydroxy-5,6,10-trimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)ethyl)amide (5) (Method B, Scheme 2). Cyclohexene (20 mL) and 10% palladium on activated carbon (2 g) were added to a stirred solution of compound **11** (3.0 g, 6.9 mmol) in *N*-methylpyrrolidone (150 mL). The mixture was stirred for 10 h at 100 °C. A further three portions of cyclohexene (3 × 20 mL) and 10% palladium on activated carbon (3 × 2 g) were added during this period. After cooling, the mixture was filtered and the filtrate concentrated under vacuum. Column chromatography of the residue eluting with a mixture of 0.5% ammonia solution (28%) and 10% methanol in dichloromethane gave compound **5** (2.18 g, 80%): mp(K) 170 °C; ^{1}H NMR (DMSO-d_6) δ 10.0 (s, 1H), 9.1 (s, 1H), 8.85 (t, 1H), 8.5 (d, 1H), 8.2 (d, 1H), 7.35 (d, 1H), 7.2 (d, 1H), 4.15 (s, 3H), 3.55 (q, 2H), 3.15 (s, 3H), 2.75 (s, 3H), 2.55 (t, 2H), 2.3 (s, 6H). Anal. ($C_{23}H_{26}N_4O_2 \cdot 0.5H_2O$) C, H, N.

5,6,11-Trimethyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid Ethyl Ester (8) (Scheme 1). A mixture of compound **7** (5.0 g, 14.3 mmol), dimethyl carbonate (50 mL),

potassium carbonate (5.0 g), and adogen 464 (1.0 g) in dimethylformamide (25 mL) was stirred at reflux temperature for 3 h and then concentrated under vacuum. The residue was taken up with dichloromethane and the solution washed with water and a saturated aqueous solution of lithium chloride, dried (Na_2SO_4), and concentrated under vacuum. Chromatography of the residue, eluting with 3% ethanol in toluene, gave compound **8** (4.64 g, 89%): mp(cap) 130−133 °C; ^{1}H NMR ($CDCl_3$) δ 8.4 (d, 1H), 7.8 (d, 1H), 7.7 (s, 1H), 7.2−7.0 (m, 2H), 4.5 (q, 2H), 3.8 (2s, 6H), 3.0 (s, 3H), 2.9 (s, 3H), 1.5 (t, 3H). Anal. ($C_{21}H_{22}N_2O_3$) C, H, N.

9-Methoxy-5,6,11-trimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (9) (Scheme 1). A 2 N solution of sodium hydroxide (36 mL) was added to a solution of compound **8** (8.7 g, 24 mmol) in ethanol (900 mL). The mixture was stirred for 16 h at reflux temperature. After cooling, an aqueous solution of 1 N hydrochloric acid (72 mL) was added and the mixture concentrated under vacuum. The residue was taken up with water and the resulting solid collected by filtration and washed with water, methanol, and dichloromethane before drying under vacuum at 50 °C to give compound **9** (4.15 g, 52%): mp(K) 190 °C; ^{1}H NMR (DMSO-d_6) δ 8.25 (d, 1H), 8.0−7.1 (s, 1H, exchangeable for D_2O), 7.95 (d, 1H), 7.8 (d, 1H), 7.55 (d, 1H), 7.25 (dd, 1H), 4.1 (s, 3H), 3.9 (s, 3H), 3.1 (s, 3H), 3.0 (s, 3H). Anal. ($C_{20}H_{18}N_2O_3\cdot H_2O$) C, H, N.

9-Methoxy-5,6,11-trimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)ethyl)amide (10) (Scheme 1). PyBOP (25.8 g, 49.6 mmol) in *N*-methylpyrrolidone (50 mL) was added over a 1-h period to a solution of compound **9** (9.2 g, 27.5 mmol) and 2-(dimethylamino)ethylamine (4 mL) in *N*-methylpyrrolidone (75 mL) at 65 °C.[16] The mixture was stirred at 65 °C for 30 min and concentrated under vacuum. The residue was taken up with dichloromethane and the solution washed with a solution of sodium carbonate, dried (Na_2SO_4), and concentrated under vacuum. Column chromatography of the residue, eluting with a mixture of 1% ammonia solution (28%) and 10% methanol in dichloromethane, gave compound **10** (9.6 g, 86%): mp(cap) 132−135 °C; ^{1}H NMR (DMSO-d_6) δ 8.6 (t, 1H, exchangeable for D_2O), 8.35 (d, 1H), 8.05 (d, 1H), 7.8 (d, 1H), 7.55 (d, 1H), 7.25 (dd, 1H), 4.1 (s, 3H), 3.9 (s, 3H), 3.5 (q, 2H), 3.1 (s, 3H), 3.0 (s, 3H), 2.5 (t, 2H), 2.2 (s, 6H). Anal. ($C_{24}H_{28}N_4O_2\cdot 0.25H_2O$) C, H, N.

10-((Dimethylamino)methyl)-9-hydroxy-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)ethyl)amide (11) (Scheme 2). *N*,*N*,*N*′,*N*′-Tetramethyldiaminomethane[17] (3.25 g, 31.8 mmol) and acetic acid (0.3 mL) were added to a stirred solution of compound **1** (0.9 g, 2.39 mmol) in dioxane (72 mL). The mixture was then stirred at reflux for 30 min and concentrated under vacuum. The residue was taken up in water, the pH was made basic using ammonia solution, and the mixture was extracted with dichloromethane and ethanol. The organic layer was dried ($Mg SO_4$) and then concentrated under vacuum. The residue was stirred in ether and the solid collected by filtration, washed with ether, and dried at 30 °C under vacuum to give compound **11** (0.9 g, 86%): mp(K) 200 °C; ^{1}H NMR (DMSO-d_6) δ 9.8 (s, 1H), 8.7 (m, 1H exchangeable for D_2O), 8.4 (d, 1H), 8.15 (d, 1H), 7.4 (d, 1H), 7.1 (d, 1H), 4.1 (s, 5H), 3.6 (m, 2H), 3.1 (s, 3H), 2.6 (m, 2H), 2.35 (s, 6H), 2.2 (s, 6H). Anal. ($C_{25}H_{31}N_5O_2$) C, H, N.

9-Hydroxy-5,6,10-trimethyl-2,3,4,6-tetrahydro-1*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)ethyl)amide (12) (Scheme 2). To a solution of compound **11** (2.6 g, 6 mmol) in ethanol (1.2 L) was added 10% palladium on activated carbon (2.2 g). The mixture was stirred at 30 °C under hydrogen atmosphere at ambient pressure for 30 h. The mixture was filtered and the catalyst washed with a mixture of dichloromethane and ethanol. The combined filtrates were concentrated under vacuum. Column chromatography of the residue, eluting with a mixture of 1% triethylamine and 10% ethanol in dichloromethane, provided recovered starting material **11** (0.26 g, 10%), compound **5** (0.35 g, 15%), and compound **12** (1.15 g, 50%): ^{1}H NMR (DMSO-d_6) δ 8.7 (s, 1H), 8.0 (2s, 2H), 7.15 (d, 1H), 6.95 (d, 1H), 4.55 (s, 1H), 3.95 (s, 3H), 2.65 (s, 3H), 2.6 (s, 3H), 2.15 (s, 6H), 3.3−3.1 (m, 3H), 2.9 (m, 1H), 2.75 (m, 2H), 2.3 (m, 2H); MS (CI) *m*/*e* 394.

(*R*,*S*)-*trans*-2-Phenylcyclopropanecarboxylic Acid 1-[(2-(Dimethylamino)ethyl)carbamoyl]-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (14) (Method C). (*R*,*S*)-*trans*-2-Phenylcyclopropanecarbonyl chloride[24] (0.53 g, 2.9 mmol in tetrahydrofuran (50 mL) was added at 5 °C to a stirred solution of 9-hydroxy-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic acid (2-(dimethylamino)ethyl)amide (**1**) (1 g, 2.66 mmol) and triethylamine (0.29 g, 2.87 mmol) in tetrahydrofuran (50 mL). The mixture was stirred at 20 °C for 20 h and then concentrated under vacuum. The residue was dissolved in dichloromethane and the solution washed with water, dried (Na_2SO_4), and concentrated. Column chromatography of the residue, eluting with 10% methanol in dichloromethane, gave compound **14** (0.9 g, 65%): mp(cap) 176−178 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.75 (t, 1H), 8.4 (d, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.65 (d, 1H), 7.3 (m, 6H), 4.15 (s, 3H), 3.55 (q, 2H), 3.1 (s, 3H), 2.8 (m, 1H), 2.6 (m, 2H), 2.3 (s+m, 7H), 1.8−1.7 (m, 2H). Anal. ($C_{32}H_{32}N_4O_3\cdot 0.5C_2H_5OH$) C, H, N.

As described for **14**, using suitable starting materials, the following compounds were obtained. **15** (25%): mp(cap) 84−86 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.8 (t, 1H), 8.5 (d, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.65 (d, 1H), 7.5−7.3 (m, 6H), 4.2 (s, 3H), 4.1 (s, 2H), 3.5 (q, 2H), 3.1 (s, 3H), 2.5 (m, 2H), 2.3 (s, 6H); MS (FAB) *m*/*e* 494. Anal. ($C_{30}H_{30}N_4O_3\cdot H_2O$) C, H, N. **16** (58%): mp(cap) 122−125 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.9 (t, 1H), 8.5 (d, 1H), 8.25 (d, 1H), 8.1 (d, 1H), 7.7 (d, 1H), 7.4, (dd, 1H), 4.2 (s, 3H), 3.65 (q, 2H), 3.15 (s, 3H), 2.7 (m, 4H), 2.4 (s, 6H), 1.75 (m, 2H), 1.5−1.3 (m, 10H), 0.95 (t, 3H). Anal. ($C_{31}H_{40}N_4O_3\cdot 0.8H_2O$) C, H, N. **18** (64%): mp(cap) 116−118 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.8 (t, 1H), 8.45−8.2 (2d, 2H), 8.05 (d, 1H), 7.65 (d, 1H), 7.35 (dd, 1H), 4.15 (s, 3H), 3.8 (t, 2H), 3.55 (q+t, 4H), 3.1 (s, 3H), 2.9 (t, 2H), 2.6 (t, 2H), 2.25 (s, 6H), 1.2 (t, 3H). Anal. ($C_{27}H_{32}N_4O_4$) C, H, N. **19** (50%): mp(cap) 120−122 °C; ^{1}H NMR (DMSO-*d*6) δ 9.65 (s, 1H), 8.8 (t, 1H), 8.45 (d, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.6 (d, 1H), 7.35 (dd, 1H), 4.2 (s, 3H), 3.7 (s, 3H), 3.55 (q, 2H), 3.1 (s, 3H), 2.75 (t, 2H), 2.55 (m, 4H), 2.3 (s, 6H), 2.0 (q, 2H). Anal. ($C_{28}H_{32}N_4O_5$) C, H, N.

Tetradecanoic Acid 1-[(2-(Dimethylamino)ethyl)carbamoyl]-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (17) (Method D). Myristic anhydride (4.67 g, 10.64 mmol) was added at 5 °C to a stirred solution of compound **1** (2 g, 5.31 mmol) in pyridine (60 mL). The mixture was stirred at 20 °C for 16 h and concentrated under vacuum at a temperature below 40 °C. Column chromatography of the residue, eluting with a mixture of 10% methanol in dichloromethane, gave compound **17** (2.4 g, 28.5%): mp (K) 98 °C; ^{1}H NMR ($CDCl_3$) δ 10.1 (s, 1H), 8.4 (t+d, 2H), 7.9 (s+d, 2H), 7.2 (d, 2H), 4.0 (s, 3H), 3.7 (q, 2H), 3.0 (s, 3H), 2.7 (t+t, 4H), 2.4 (s, 6H), 1.9 (m, 2H), 1.4 (m, 20H), 0.9 (t, 3H). Anal. ($C_{36}H_{50}N_4O_3\cdot 0.7H_2O$) C, H, N.

Following a similar procedure as that described for **17**, using acetic anhydride and compound **1** as starting materials, compound **13** was obtained (75%): mp(K) 166 °C; ^{1}H NMR ($CDCl_3$) δ 10.1 (s, 1H), 8.4 (t, 1H), 8.3 (d, 1H), 7.9 (s, 1H), 7.8 (dd, 1H), 7.1 (m, 2H), 3.9 (s, 3H), 3.7 (q, 2H), 2.9 (s, 3H), 2.7 (t, 2H), 2.4 (s, 3H), 2.35 (s, 6H). Anal. ($C_{24}H_{26}N_4O_3$) C, H, N.

Hexanedioic Acid Benzyl Ester 1-[(2-(Dimethylamino)ethyl)carbamoyl]-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (20) (Method E). 1-Hydroxybenzotriazole (2.26 g, 16.8 mmol) and dicyclohexylcarbodiimide (4.0 g, 19.4 mmol) were added successively to a stirred solution of adipic acid monobenzyl ester (3.96 g, 16.8 mmol) in dimethylformamide (230 mL) at room temperature.[14] The mixture was stirred for 4 h before adding compound **1** (3.0 g, 7.97 mmol). The mixture was stirred for 48 h at room temperature and filtered. The filtrate was concentrated under vacuum, and the residue was taken up in dichloromethane, washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum. Column chromatography of the residue, eluting with a mixture of 0.5% triethylamine and 10% methanol in toluene, gave compound **20** (3.2 g, 67.5%): mp(cap) 88−

90 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.8 (t, 1H), 8.5 (d, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.7 (d, 1H), 7.3 (m, 6H), 5.15 (s, 2H), 4.2 (s, 3H), 3.55 (q, 2H), 3.1 (s, 3H), 2.7 (m, 2H), 2.5 (m, 4H), 2.3 (s, 6H), 1.75 (m, 4H). Anal. ($C_{35}H_{38}N_4O_5$) C, H, N.

Following a similar procedure as that described for **20**, using succinic acid monobenzyl ester and compound **1** as starting materials, compound **21** was obtained (40%): mp(cap) 174−176 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 1H), 8.70 (t, 1H), 8.50 (d, 1H), 8.20 (d, 1H), 8.0 (d, 1H), 7.70 (d, 1H), 7.40 (m, 5H), 7.30 (dd, 1H), 5.20 (AB, 2H), 4.20 (s, 3H), 3.50 (m, 2H), 3.20 (s, 3H), 2.95−2.80 (m, 4H), 2.50 (t, 2H), 2.20 (s, 6H). Anal. ($C_{33}H_{34}N_4O_5$) C, H, N.

Nicotinic Acid 1-[(2-(Dimethylamino)ethyl)carbamoyl]-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (23) (Method F). Dicyclohexylcarbodiimide (3.7 g, 18 mmol) and 4-(dimethylamino)pyridine (1.5 g, 12.3 mmol) were added to a stirred solution of compound **1** (3.0 g, 7.97 mmol) and nicotinic acid (1.5 g, 12.2 mmol) in pyridine[15] (100 mL). The mixture was stirred for 16 h at room temperature. Further amounts of dicyclohexylcarbodiimide (2.0 g, 9.7 mmol) and nicotinic acid (0.75 g, 6.1 mmol) were added; the mixture was stirred for a further 24 h and concentrated under vacuum. The residue was taken up in dichloromethane, washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum. Column chromatography of the residue, eluting with a mixture of 0.5% triethylamine and 10% ethanol in toluene, gave compound **23** (3.5 g, 91%): mp(cap) 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 9.35 (d, 1H), 9.0 (m, 1H), 8.8 (t, 1H), 8.6 (m, 1H), 8.5 (d, 1H), 8.35 (d, 1H), 8.25 (d, 1H), 7.75 (d, 1H), 7.7 (m, 1H); 7.6 (dd, 1H), 4.25 (s, 3H), 3.5 (q, 2H), 3.15 (s, 3H), 2.55 (t, 2H), 2.2 (s, 6H). Anal. ($C_{28}H_{27}N_5O_3$) C, H, N.

(*R,S*)-5-[1,2]Dithiolan-3-ylpentanoic Acid 1-[(2-(Dimethylamino)ethyl)carbamoyl]-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (24) (Method G). PyBOP (5.2 g, 10 mmol) was added, at room temperature, to a stirred solution of compound **1** (3.0 g, 8 mmol), (*R,S*)-thioctic acid (2.1 g, 10 mmol), and triethylamine (2.0 g, 19.8 mmol) in *N*-methylpyrrolidone (42 mL).[16] The mixture was stirred for 48 h, poured in water (400 mL), and extracted with dichloromethane. The organic phase was washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum. Column chromatography of the residue, eluting with 0.5% triethylamine and 5% methanol in dichloromethane, gave compound **24** (1.1 g, 24%): mp(cap) 140−142 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 1H), 8.95 (t, 1H), 8.45 (d, 1H), 8.20 (d, 1H), 8.10 (d, 1H), 7.70 (d, 1H), 7.45 (dd, 1H), 4.20 (s, 3H), 3.70 (m, 2H), 3.60 (m, 2H), 3.20 (m, 2H), 3.10 (s, 3H), 2.80 (m, 2H), 2.70 (t, 2H), 2.50 (m, 8H), 1.95 (m, 1H), 1.70 (m, 2H), 1.55 (m, 2H); MS (FAB) *m/e* 564. Anal. ($C_{30}H_{36}N_4O_3S_2$) C, H, N.

Following a similar procedure as that described for **24**, using *N*-*t*Boc-L-glutamic acid α-benzyl ester and compound **1** as starting materials, compound **22** was obtained (88%): mp(K) 120 °C; ^{1}H NMR (DMSO-d_6) δ 9.6 (s, 1H), 8.55−8.45 (2d, 2H), 8.25 (d, 1H), 7.75 (m, 1H), 7.2 (dd, 1H), 5.2 (AB, 2H), 4.35 (m, 4H), 3.85 (m, 2H), 3.45 (m, 2H), 3.2 (s, 3H), 2.9 (m, 6H), 2.8 (t, 2H), 2.2−2.0 (m, 2H), 1.4 (s, 9H).

Pentanedioic Acid Mono[1-((2-(dimethylamino)ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl] Ester Dihydrochloride (25) (Method H). Glutaric anhydride (4.56 g, 40 mmol) was added at room temperature to a stirred solution of compound **1** (7.52 g, 20 mmol) in pyridine (200 mL). The mixture was stirred at 55 °C for 16 h and concentrated under vacuum at a temperature below 40 °C. The residue was taken up in dichloromethane (150 mL) and stirred for 10 min. The solid was collected by filtration, washed with dichloromethane, dried at 50 °C under vacuum, and taken up in ethanol. A small excess of a 2 M solution of hydrogen chloride in ethanol was added and the suspension stirred for 2 min. The solid was collected by filtration, washed with ethanol, and dried at 50 °C under vacuum to give compound **25** (9.8 g, 87%): mp(cap) 189−195 °C; ^{1}H NMR (DMSO-d_6) δ 9.5 (s, 1H),, 8.55 (d, 1H), 8.45 (d, 1H), 8.2 (s, 1H), 7.8 (d, 1H), 7.45 (dd, 1H), 4.3 (s, 3H), 3.85 (m, 2H), 3.45 (m, 2H), 3.2 (s, 3H), 2.95 (d, 6H), 2.7 (t, 2H), 2.45 (t, 2H), 1.95 (m, 2H). Anal. ($C_{27}H_{30}N_4O_5\cdot 2HCl\cdot H_2O$) C, H, N, Cl.

As described for **25**, using suitable starting materials, the following compounds were obtained. **26** (62%): mp(cap) 215−225 °C; ^{1}H NMR (DMSO-d_6) δ 10.2 (s, 1H), 9.6 (s, 1H), 9.3 (t, 1H), 8.6 (d, 1H), 8.35 (d, 1H), 7.6 (d, 1H), 7.4 (d, 1H), 4.25 (s, 3H), 3.8 (q, 2H), 3.4 (q, 2H), 3.2 (s, 3H), 2.9 (2s, 6H), 2.8 (t+s, 5H), 2.35 (t, 2H), 1.8 (m, 4H). Anal. ($C_{29}H_{34}N_4O_5\cdot 2HCl\cdot 2H_2O$) C, H, N, Cl. **27** (36%): mp(cap) 185−195 °C; ^{1}H NMR (DMSO-d_6) δ 10.1−9.35 (2s, 2H), 8.5 (d, 1H), 8.35 (d, 1H), 8.1 (d, 1H), 7.8 (d, 1H), 7.45 (dd, 1H), 4.3 (s, 3H), 3.8 (q, 2H), 3.4 (m, 2H), 3.15 (2s, 6H), 2.9 (s, 6H), 2.7 (t, 2H), 2.35 (t, 2H), 1.75 (m, 4H). Anal. ($C_{29}H_{34}N_4O_5\cdot 2HCl\cdot H_2O$) C, H, N, Cl. **28** (46%): mp(K) 180 °C; ^{1}H NMR (DMSO-d_6) δ 10.3 (m, 1H exchangeable for D_2O), 9.3 (m, 1H exchangeable for D_2O), 8.5 (d, 1H), 8.3 (d, 1H), 8.1 (d, 1H), 7.75 (d, 1H), 7.45 (dd, 1H), 4.2 (s, 3H), 3.7 (m, 2H), 3.4 (m, 2H), 3.1 (2s, 6H), 2.7 (t, 2H), 2.6 (s, 6H), 2.4 (t, 2H), 1.9 (q, 2H). Anal. ($C_{28}H_{32}N_4O_5\cdot 2HCl\cdot 1.5H_2O$) C, H, N, Cl. **29** (34%): mp(cap) 190 °C; ^{1}H NMR (DMSO-d_6) δ 10.5 (s, 1H exchangeable for D_2O), 9.65 (t, 1H), 9.55 (s, 1H), 8.5 (2d, 2H), 8.2 (d, 1H), 7.7 (d, 1H), 7.4 (dd, 1H), 4.25 (s, 3H), 3.85 (q, 2H), 3.5 (q, 2H), 3.15 (s, 3H), 2.9 (2s, 6H), 2.8 (s, 2H), 2.45 (s, 2H), 1.3 (s, 6H). Anal. ($C_{29}H_{34}N_4O_5\cdot 2HCl\cdot 0.5H_2O$) C, H, N, Cl. **30** (42%): mp(cap) 190−196 °C; ^{1}H NMR (DMSO-d_6) δ 10.0 (s, 1H exchangeable for D_2O), 9.7 (s, 1H), 9.35 (t, 1H), 8.55 (d, 1H), 8.4 (d, 1H), 8.2 (d, 1H), 7.75 (d, 1H), 7.4 (dd, 1H), 4.5 (s, 1H exchangeable for D_2O), 4.25 (s, 3H), 3.85 (m, 2H), 3.45 (m, 2H), 3.15 (s, 3H), 2.9 (2s, 6H), 2.8 (t, 2H), 2.4 (t, 2H), 1.7−1.5 (m, 6H). Anal. ($C_{29}H_{34}N_4O_5\cdot 2HCl\cdot 0.5H_2O$) C, H, N, Cl. **31** (44%): mp(cap) 210−215 °C; ^{1}H NMR (DMSO-d_6) δ 10.3 (m, 1H exchangeable for D_2O), 9.5 (s, 1H), 8.5 (d, 1H), 8.4 (d, 1H), 8.2 (m, 1H), 7.7 (d, 1H), 7.35 (dd, 1H), 4.25 (s, 3H), 3.8 (q, 2H), 3.4 (m, 2H), 3.1 (s, 3H), 2.9 (m, 6H), 2.65 (t, 2H), 2.25 (t, 2H), 1.8−1.2 (m, 8H). Anal. ($C_{30}H_{36}N_4O_5\cdot 2HCl\cdot 0.6H_2O$) C, H, N, Cl.

Hexanedioic Acid Mono[1-((2-(dimethylamino)ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl] Ester (32) (Method P). 5% Palladium on activated carbon (3.0 g) was added to a solution of compound **20** (9.2 g, 15.47 mmol) and cyclohexene (20 mL) in *N*-methylpyrrolidone (200 mL) under nitrogen atmosphere.[18] The mixture was stirred at 80 °C for 30 min and filtered and the filtrate concentrated under vacuum. The residue was triturated in ethanol and the precipitate collected by filtration, washed with ethanol and ether, and dried at 50 °C under vacuum to give compound **32** (7.2 g, 92%): mp(cap) 200−202 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.8 (t, 1H, exchangeable for D_2O), 8.5 (d, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.7 (d, 1H), 7.3 (dd, 1H), 4.2 (s, 3H), 3.5 (q, 2H), 3.1 (s, 3H), 2.7 (t, 2H), 2.6 (t, 2H), 2.3 (t, 2H), 2.3 (s, 6H), 1.7 (m, 4H). Anal. ($C_{28}H_{32}N_4O_5\cdot 0.5H_2O$) C, H, N.

As described for **32**, using suitable starting materials, the following compounds were obtained. **33** (32%): mp(cap) 160−164 °C; ^{1}H NMR (DMSO-d_6) δ 10.6−9.7 (2s, 2H, exchangeable for D_2O), 9.5 (s, 1H), 8.55 (d, 1H), 8.48 (d, 1H), 8.25 (d, 1H), 7.75 (d, 1H), 7.4 (dd, 1H), 7.3 (d, 1H), 4.25 (s, 3H), 4.15 (m, 1H), 3.9 (q, 2H), 3.5 (m, 2H), 3.2 (s, 3H), 2.95 (2s, 6H), 2.75 (t, 2H), 2.2−2.0 (m, 2H), 1.45 (s, 9H). Anal. ($C_{32}H_{39}N_5O_7\cdot 2HCl\cdot H_2O$) C, H, N, Cl. **51** (55%): mp(cap) 195−198 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.8 (t, 1H), 8.5 (d, 1H), 8.25 (d, 1H), 8.05 (d, 1H), 7.85 (t, 1H), 7.7 (d, 1H), 7.35 (dd, 1H), 4.2 (s, 3H), 3.55 (q, 2H), 3.5 (q, 2H), 3.15 (s, 3H), 2.6 (2t, 4H), 2.3 (s, 6H). Anal. ($C_{26}H_{29}N_5O_5\cdot 2H_2O$) C, H, N. **52** (76%): mp(cap) 174−177 °C; ^{1}H NMR (DMSO-d_6) δ 9.60 (s, 1H), 8.75−7.85 (2s, 2H exchangeable for D_2O), 8.2 (d, 1H), 8.0 (d, 1H), 7.60 (dd, 1H), 7.20 (d, 1H), 4.20 (s, 3H), 3.50 (m, 2H), 3.20 (t, 2H), 3.10 (s, 3H), 2.60 (m, 2H), 2.25 (m, 2H), 2.20 (t, 2H), 1.75 (s, 6H). Anal. ($C_{27}H_{31}N_5O_5\cdot H_2O$) C, H, N. **55** (61%): mp(cap) 135−137 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.8 (t, 1H), 8.5 (d, 1H), 8.25 (2m, 2H), 7.7 (d, 1H), 7.5 (dd, 1H), 4.3 (t, 2H), 4.25 (s, 3H), 3.6 (q, 2H), 3.15 (s, 3H), 2.6 (t, 2H), 2.45 (t, 2H), 2.35 (s, 6H), 1.95 (q, 2H). Anal. ($C_{27}H_{30}N_4O_6\cdot H_2O$) C, H, N.

(*R,S*)-5,6-Dimethyl-9-(*trans*-2-phenylcyclopropylmethoxy)-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)ethyl)amide (36) (Method I). Diethyl azodicarboxylate (2.44 g, 14 mmol) was added over 20 min at

room temperature to a stirred suspension of compound **1** (5.3 g, 14 mmol), triphenylphosphine (3.67 g, 14 mmol), and (*R,S*)-*trans*-2-phenylcyclopropylmethanol[20] (2.1 g, 14 mmol) in tetrahydrofuran (330 mL). The mixture was stirred for 2 h at room temperature. Further aliquots of triphenylphosphine (3.67 g, 14 mmol) and diethyl azodicarboxylate (2.44 g, 14 mmol) were added (Mitsunobu reaction[21]); the mixture was stirred for 16 h and then concentrated under vacuum. Column chromatography of the residue, eluting with 10% methanol in dichloromethane, gave a crude product which was recrystallized from ethanol to give compound **36** (2.25 g, 32%): mp(cap) 120−123 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.8 (t, 1H, exchangeable for D_2O), 8.45 (d, 1H), 8.15 (d, 1H), 7.8 (d, 1H), 7.55 (d, 1H), 7.2 (m, 6H), 4.15 (m, 5H), 3.55 (m, 2H), 3.05 (s, 3H), 2.6 (t, 2H), 2.3 (s, 6H), 2.05 (m, 1H), 1.6 (m, 1H), 1.1 (m, 2H). Anal. ($C_{32}H_{34}N_4O_2$) C, H, N.

Following a similar procedure as that described for **36**, using (*R,S*)-2,2-dimethyl-1,3-dioxolane-4-methanol and compound **1** as starting materials, compound **35** was obtained (32%): mp(cap) 96−98 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 1H), 8.80 (t, 1H), 8.45 (d, 1H), 8.20 (d, 1H), 7.80 (d, 1H), 7.55 (d, 1H), 7.25 (dd, 1H), 4.50 (m, 1H), 4.20−3.85 (m, 2H), 4.20 (m, 5H), 3.50 (m, 2H), 3.15 (s, 3H), 2.50 (t, 2H), 2.20 (s, 6H), 1.45−1.30 (2s, 6H). Anal. ($C_{28}H_{34}N_4O_4\cdot0.5H_2O$) C, H, N.

(*R,S*)-9-(2,3-Dihydroxypropoxy)-5,6-dimethyl-6*H*-pyrido-[4,3-*b*]carbazole-1-carboxylic Acid (2-(Dimethylamino)-ethyl)amide (37) (Method Q). Compound **35** (2.4 g, 4.89 mmol) was dissolved in acetic acid (60 mL) and water (40 mL). The mixture was stirred for 8 h at reflux temperature and concentrated under vacuum. The residue was dissolved in dichloromethane; the solution was washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum. Column chromatography of the residue, eluting with a mixture of 0.75% triethylamine and 15% ethanol in toluene, gave compound **37** (0.75 g, 34%): mp(cap) 136−138 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.8 (t, 1H), 8.45 (d, 1H), 8.2 (d, 1H), 7.8 (d, 1H), 7.6 (d, 1H), 7.25 (dd, 1H), 5.0 (d, 1H), 4.7 (t, 1H), 4.2 (s, 3H), 4.2−4.1 (m, 2H), 3.9 (m, 1H), 3.55 (m, 4H), 3.15 (s, 3H), 2.6 (t, 2H), 2.3 (s, 6H); purity 98.3% (HPLC C18, 210 nm, $CH_3CN/H_2O/CH_3SO_3H$). Anal. ($C_{25}H_{30}N_4O_4$) C, H, N.

5-[1-((2-(Dimethylamino)ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yloxy]pentanoic Acid Ethyl Ester (38) (Method J). A suspension of compound **1** (3.0 g, 7.98 mmol), ethyl 5-bromopentanoate (3.75 g, 18.1 mmol), and cesium carbonate (5.85 g) in dimethylformamide (100 mL) was stirred at reflux temperature for 24 h at 50 °C and then concentrated under vacuum. The residue was taken up in dichloromethane, and the mixture was washed with an aqueous solution of sodium hydrogen carbonate. The organic layer was dried (Na_2SO_4) and concentrated under vacuum. Chromatography of the residue eluting with a mixture of 0.5% triethylamine and 10% ethanol in toluene gave compound **38** (2.8 g, 69%): mp(cap) 101−104 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.8 (t, 1H), 8.45 (d, 1H), 8.2 (d, 1H), 7.8 (d, 1H), 7.6 (d, 1H), 7.2 (dd, 1H), 4.2 (s, 3H), 4.2 (t, 2H), 4.1 (q, 2H), 3.55 (q, 2H), 3.15 (s, 3H), 2.55 (t, 2H), 2.4 (t, 2H), 2.3 (s, 6H), 1.8 (m, 4H), 1.2 (t, 3H). Anal. ($C_{29}H_{36}N_4O_4$) C, H, N.

5-[1-((2-(Dimethylamino)ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yloxy]pentanoic Acid (39) (Method R). A solution of **38** (2.8 g, 5.55 mmol) and an aqueous solution of 1 N sodium hydroxide (10 mL) in ethanol (50 mL) were stirred at reflux temperature for 24 h and then concentrated under vacuum. The residue was taken up in water, and an aqueous solution of 1 N hydrochloric acid (10 mL) was added. The solid was collected by filtration, washed with water and ethanol, and dried at 60 °C under vacuum to give compound **39** (2.1 g, 80%): mp(cap) 196−200 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.8 (t, 1H), 8.45 (d, 1H), 8.2 (d, 1H), 7.8 (d, 1H), 7.6 (d, 1H), 7.25 (dd, 1H), 4.2 (s+t, 5H), 3.6 (q, 2H), 3.15 (s, 3H), 2.6 (t, 3H), 2.4 (t, 2H), 2.3 (s, 6H), 1.8 (m, 4H). Anal. ($C_{27}H_{32}N_4O_4\cdot H_2O$) C, H, N.

[1,4′]Bipiperidinyl-1′-carboxylic Acid 1-((2-(Dimethylamino)ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]-carbazol-9-ylcarbamate (43) (Method K). [1,4′]Bipiperidinyl-1′-carbonyl chloride (1.5 g, 7.3 mmol) was added over 5 min at room temperature to a stirred solution of compound **1** (1.6 g, 4.25 mmol) in pyridine (80 mL). The mixture was stirred for 16 h and concentrated under vacuum. The residue was dissolved in dichloromethane, washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum. The residue was taken up with a mixture of ethyl acetate and ether. The solid was collected by filtration, washed with ether, and dried at 50 °C under vacuum to give compound **43** (2.3 g, 95%): mp(cap) 190−200 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.8 (t, 1H), 8.5 (d, 1H), 8.25 (d, 1H), 8.1 (s, 1H), 7.65 (d, 1H), 7.35 (dd, 1H), 4.2 (s, 3H), 4.3−4.15 (m, 2H), 3.6 (q, 2H), 3.35−3.0 (m, 3H), 3.15 (s, 3H), 2.6 (t+m, 5H), 2.3 (s, 6H), 1.9−1.5 (m, 10H). Anal. ($C_{33}H_{42}N_6O_3\cdot0.7H_2O$) C, H, N.

As described for **43**, using suitable carbamoyl chlorides prepared according to a previously described method,[25] the following compounds were prepared. **40** (75%): mp(cap) 188−190 °C; ^{1}H NMR ($CDCl_3$) δ 10.05 (s, 1H), 8.5 (t, 1H, exchangeable for D_2O), 8.3 (d, 1H), 8.0 (d, 1H), 7.9 (d, 1H), 7.3 (dd, 1H), 7.2 (d, 1H), 4.0 (s, 3H), 3.7 (q, 2H), 3.2 (s, 3H), 3.1 (s, 3H), 3.0 (s, 3H), 2.75 (t, 2H), 2.4 (s, 6H). Anal. ($C_{25}H_{29}N_5O_3\cdot0.5H_2O$) C, H, N. **41** (51%): mp(cap) 90−93 °C; ^{1}H NMR ($CDCl_3$) δ 10.1 (s, 1H), 8.5 (t, 1H, exchangeable for D_2O), 8.4 (d, 1H), 8.0 (m, 1H), 7.95 (d, 1H), 7.2 (m, 2H), 4.1 (s, 3H), 3.8 (q, 2H), 3.5 (m, 2H), 3.1 (2s, 3H), 3.0 (s, 3H), 2.65 (t, 2H), 2.4 (s, 6H), 1.8−1.2 (m, 20H), 0.9 (m, 3H). Anal. ($C_{36}H_{51}N_5O_3$) C, H, N. **42** (86%): mp(cap) 125−128 °C; ^{1}H NMR ($CDCl_3$) δ 10.1 (s, 1H), 8.5 (t, 1H, exchangeable for D_2O), 8.4 (d, 1H), 8.0 (m, 1H), 7.95 (d, 1H), 7.5−7.2 (m, 7H), 4.0 (s, 3H), 3.7 (q, 2H), 3.45 (s, 3H), 3.0 (s, 3H), 2.7 (t, 2H), 2.5 (s, 6H). Anal. ($C_{30}H_{31}N_5O_3$) C, H, N. **44** (78%): mp(cap) 135−138 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.8 (t, 1H), 8.45 (d, 1H), 8.2 (d, 1H), 8.05 (d, 1H), 7.6 (d, 1H), 7.55 (d, 6H), 7.4 (m, 4H), 7.3 (m, 2H), 4.45 (s, 1H), 4.15 (s, 3H), 3.8 (m, 2HJ), 3.6 (q, 2H), 2.6−2.4 (m, 8H), 3.1 (s, 3H), 2.3 (s, 6H). Anal. ($C_{40}H_{42}N_6O_3\cdot0.25H_2O$) C, H, N. **45** (87%): mp(cap) 134−137 °C; ^{1}H NMR ($CDCl_3$) δ 10.0 (s, 1H), 8.5 (t, 1H, exchangeable for D_2O), 8.3 (d, 1H), 8.0 (d, 1H), 7.8 (d, 1H), 7.3 (dd, 1H), 7.15 (d, 1H), 4.2−3.7 (m, 4H), 4.0 (s, 3H), 3.7 (q, 2H), 2.9 (s, 3H), 2.7 (t, 2H), 2.6 (m, 4H), 2.4 (s, 9H). Anal. ($C_{28}H_{34}N_6O_3\cdot0.5H_2O$) C, H, N. **46** (93%): mp(cap) 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.8 (t, 1H), 8.45 (d, 1H), 8.2 (d, 1H), 8.1 (d, 1H), 7.65 (d, 1H), 7.4 (dd, 1H), 4.2 (s, 3H), 3.6 (s, 8H), 3.5 (q, 2H), 3.1 (s, 3H), 2.5 (t, 2H), 2.25 (s, 6H). Anal. ($C_{27}H_{31}N_5O_4\cdot0.4H_2O$) C, H, N.

Dodecylcarbamic Acid 1-((2-(Dimethylamino)ethyl)-carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl-carbamate (47) (Method L). Dodecyl isocyanate (3.2 g, 15.2 mmol) in pyridine (10 mL) was added to a stirred solution of compound **1** (3.8 g, 10 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.05 g) in pyridine (40 mL). The mixture was stirred for 16 h at 50 °C and concentrated under vacuum. Column chromatography of the residue, eluting with a mixture of 1.5% ammonia solution (28%) and 5% methanol in dichloromethane, gave compound **47** (3.9 g, 66%): mp(cap) 162−164 °C; ^{1}H NMR (DMSO-d_6) δ 9.3 (s, 1H), 8.8−7.8 (2t, 2H), 8.45 (d, 1H), 8.2 (d, 1H), 8.0 (d, 1H), 7.6 (d, 1H), 7.3 (dd, 1H), 4.15 (s, 3H), 3.55 (q, 2H), 3.1 (m, 5H), 2.6 (t, 2H), 2.3 (s, 6H), 1.55 (m, 2H), 1.4−1.2 (m, 18H), 0.85 (t, 3H). Anal. ($C_{35}H_{49}N_5O_3$) C, H, N.

As described for **47**, using suitable isocyanates[26] and compound **1** as starting materials, the following compounds were obtained. **48** (92%): mp(cap) 120 °C; ^{1}H NMR (DMSO-d_6) δ 9.6 (s, 1H), 8.8 (t, 1H exchangeable for D_2O), 8.45 (d, 1H), 8.2 (d, 1H), 7.95 (d, 1H), 7.9 (t, 1H exchangeable for D_2O), 7.6 (d, 1H), 7.3 (dd, 1H), 4.2 (s, 3H), 4.15 (q, 2H), 3.6 (q, 2H), 3.4 (q, 2H), 3.1 (s, 3H), 2.6 (m, 4H), 2.25 (s, 6H), 1.2 (t, 3H). Anal. ($C_{28}H_{33}N_5O_5$), C, H, N. **49** (58%): mp(cap) 112−114 °C; ^{1}H NMR (DMSO-d_6) δ 9.63 (s, 1H), 8.75 (t, 1H exchangeable for D_2O), 8.45 (d, 1H), 8.18 (d, 1H), 7.97 (d, 1H), 7.90 (t, 1H exchangeable for D_2O), 7.60 (d, 1H), 7.40 (m, 5H), 7.3 (dd, 1H), 5.17 (s, 2H), 4.15 (s, 3H), 3.52 (q, 2H), 3.40 (q, 2H), 3.10 (s, 3H), 2.68 (t, 2H), 2.53 (t, 2H), 2.25 (s, 6H). Anal. ($C_{33}H_{35}N_5O_5$) C, H, N. **50** (42%): mp(cap) 125−128 °C; ^{1}H NMR (DMSO-d_6)

δ 9.6 (s, 1H), 8.8 (t, 1H exchangeable for D_2O), 8.4 (d, 1H), 8.2 (d, 1H), 8.0 (d, 1H), 7.7 (t, 1H, exchangeable for D_2O), 7.3 (m, 6H), 5.1 (s, 2H), 4.15 (s, 3H), 3.5 (q, 2H), 3.2 (q, 2H), 3.0 (s, 3H), 2.6 (m, 4H), 2.3 (s, 6H), 1.8 (m, 2H). Anal. ($C_{34}H_{37}N_5O_5$) C, H, N.

Methanesulfonic Acid 1-((2-(Dimethylamino)ethyl)-carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (53) (Method M). Methanesulfonyl chloride (1.2 g, 10.5 mmol) was added to a stirred solution of compound **1** (3 g, 7.97 mmol) and triethylamine (1 g) in tetrahydrofuran (200 mL) at 5 °C. The mixture was stirred at room temperature for 16 h and then concentrated under vacuum. The residue was dissolved in dichloromethane, and the mixture was washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum. Column chromatography of the residue, eluting with a mixture of 0.5% ammonia solution (28%) and 5% methanol in dichloromethane, gave compound **53** (2.3 g, 63.5%): mp(cap) 192−194 °C; ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 8.8 (t, 1H), 8.5 (d, 1H), 8.3 (d, 1H), 8.25 (d, 1H), 7.75 (d, 1H), 7.6 (dd, 1H), 4.2 (s, 3H), 3.55 (q, 2H), 3.5 (s, 3H), 3.15 (s, 3H), 2.6 (t, 2H), 2.3 (s, 6H). Anal. ($C_{23}H_{26}N_4O_4S$) C, H, N.

3-(Benzyloxycarbonyl)propyl Chloroformate. A mixture of 4-hydroxybutyric acid benzyl ester[22] (14.0 g, 72 mmol) and dimethylaniline (10.6 g) in toluene (47 mL) was added over 45 min at 0 °C to a 1.93 M solution of phosgene in toluene (41 mL). The mixture was stirred for 1 h at 5 °C, washed with iced water, 0.1 N hydrochloric acid, and water, dried (Na_2SO_4), and then concentrated to 37 mL under vacuum. This unstable compound (not isolated) was used immediately as a solution in toluene in the following procedure.

4-[[1-((2-(Dimethylamino)ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yloxy]carbonyloxy]-butyric Acid Benzyl Ester (54) (Method N). The above solution of 3-(benzyloxycarbonyl)propyl chloroformate in toluene (37 mL) was added over 30 min to a solution of compound **1** (2.6 g, 6.9 mmol) and pyridine (1.86 mL) in *N*-methylpyrrolidone (70 mL) at 5 °C. The mixture was stirred for 2 h at 5 °C followed by 16 h at room temperature and then concentrated under vacuum. The residue was dissolved in dichloromethane; the solution was washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum. Chromatography of the residue, eluting with a mixture of 0.5% ammonia solution (28%) and 5% methanol in dichloromethane, gave compound **54** as a gum (1.63 g, 39%): ^{1}H NMR (DMSO-d_6) δ 9.7 (s, 1H), 8.75 (t, 1H), 8.4 (d, 1H), 8.15 (m, 2H), 7.4 (m, 7H), 5.15 (s, 2H), 4.3 (t, 2H), 4.1 (s, 3H), 3.6 (m, 2H), 3.05 (s, 3H), 2.6 (m, 4H), 2.3 (s, 6H), 1.9 (m, 2H). Anal. ($C_{34}H_{36}N_4O_6$) C, H, N.

Phosphoric Acid Dibenzyl Ester 1-((2-(Dimethylamino)ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (56) (Method O, Scheme 4). Sodium hydride (60% dispersion in mineral oil, 0.6 g, 15 mmol) was added at room temperature to a stirred solution of compound **1** (3.76 g, 10 mmol) in tetrahydrofuran (200 mL). After 1 h, tetrabenzyl pyrophosphate[23] (6.5 g, 12 mmol) was added, and the stirring was continued for 6 h. The mixture was filtered, and the filtrate was concentrated under vacuum at a temperature below 35 °C. The residue was dissolved in dichloromethane, washed with an aqueous solution of sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under vacuum to give unstable compound **56** (4 g, 63%) which was used immediately in the following procedure: ^{1}H NMR ($CDCl_3$) δ 9.7 (s, 1H), 9.65 (t, 1H), 8.45 (d, 1H), 8.1 (d, 1H), 7.7−7.1 (m, 13H), 5.2 (m, 4H), 4.1 (s, 3H), 3.8 (m, 2H), 3.3 (s, 3H), 2.7 (m, 2H), 2.3 (s, 6H).

Phosphoric Acid Benzyl Ester 1-((2-(Dimethylamino)-ethyl)carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl Ester (57) (Method P, Scheme 4). 5% Palladium on carbon (1 g) was added to a solution of compound **56** (4 g, 6.28 mmol) and cyclohexene (10 mL) in *N*-methylpyrrolidone (100 mL).[18] The mixture was stirred for 35 min at 100 °C. After cooling, the solid was collected by filtration and extracted with water. Aqueous solution was lyophilized to give compound **57** (1.5 g, 43.5%): ^{1}H NMR (DMSO-d_6) δ 10.6 (s, 1H), exchangeable for D_2O), 9.6 (s, 1H), 9.20 (t, 1H), 8.25 (d, 1H), 8.0 (d, 2H), 7.5 (dd, 1H), 7.3−7.2 (m, 6H), 4.85 (s, 2H), 4.15 (s, 3H), 3.8 (m, 2H), 3.3 (m, 2H), 2.9 (s, 3H), 2.85 (s, 6H). Anal. ($C_{29}H_{31}N_4O_5P\cdot 2H_2O$) C, H, N.

Phosphoric Acid Mono[1-((2-(dimethylamino)ethyl)-carbamoyl)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-9-yl] Ester (58) (Method P, Scheme 4). 5% Palladium on activated carbon (2.5 g) was added to a stirred solution of compound **56** (4 g, 6.28 mmol) and cyclohexene (75 mL) in *N*-methylpyrrolidone (750 mL). The mixture was stirred for 1 h at 100 °C. After cooling, the solid was collected by filtration and extracted with water. The aqueous solution was concentrated under vacuum and the residue taken up with a mixture of 20% ethanol in ether. The solid was collected by filtration and dried at 50 °C under vacuum to give compound **58** (2.3 g, 80%): mp(cap) 220−224 °C; ^{1}H NMR (D_2O + NaOD) δ 8.8 (s, 1H), 8.1 (d, 1H), 8.0 (s, 1H), 7.7 (d, 1H), 7.5 (d, 1H), 7.15 (d, 1H), 3.9 (t, 2H), 3.6 (s, 3H), 2.9 (t, 2H), 2.6 (s, 3H), 2.5 (s, 6H). Anal. ($C_{22}H_{25}N_4O_5P\cdot 0.7H_2O$) C, H, N.

58 (Scheme 5). 10% Palladium on activated carbon (2 g) was added to a stirred solution of compound **59** (4.0 g, 6.28 mmol) and cyclohexene (50 mL) in *N*-methylpyrrolidone (400 mL). The mixture was stirred at reflux temperature for 1 h. A further 2-g portion of catalyst was added and the mixture stirred at reflux temperature for 4 h. After cooling, the solid was collected by filtration and extracted with water. The aqueous phase was concentrated under vacuum and the residue taken up with a mixture of 20% ethanol in ether. The solid was collected by filtration and dried at 60 °C under vacuum to give compound **58** (1.85 g, 64,5%), identified as the same product as that obtained in method P, Scheme 4.

Benzyl[2-(5,6-dimethyl-9-(benzyl phosphate)-6*H*-pyrido-[4,3-*b*]carbazol-1-yl)carbonylaminoethyl]methylammonium (59) (Scheme 5). Unstable compound **56** (6.37 g, 10 mmol) was left for 4 days at room temperature. Column chromatography, eluting with a mixture of 2% ammonia solution (28%) and 20% methanol in dichloromethane, gave compound **59** (4.0 g, 62.8%): ^{1}H NMR (DMSO-d_6) δ 9.6 (s, 1H), 9.4 (t, 1H, exchangeable for D_2O), 8.3 (d, 1H), 8.1 (m, 2H), 7.7−7.0 (m, 12H), 4.8 (d, 2H), 4.7 (s, 2H), 4.15 (s, 3H), 4.0 (m, 2H), 3.6 (m, 2H), 3.2 (s, 6H), 3.0 (s, 3H); MS (FAB) *m*/*e* 636.

Biology. 1. Cell Culture and Cytotoxicity. The murine L1210 leukemia and B16 melanoma cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[3,27] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for about four doubling times (48 h for L1210 and 96 h for B16). Results were expressed as IC_{50}, the concentration which reduced by 50% the optical density of treated cells with respect to untreated controls.

For the cell cycle analysis, L1210 cells (5×10^5 cells/mL) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg/mL RNase and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10 000 cells were analyzed on an ATC3000 flow cytometer (Brucker, Wissembourg, France).

2. Antitumor Activity. The antitumor activity of the compounds was evaluated on the P388 leukemia and the B16 melanoma, all provided by NCI, Frederick, MD. Groups of 6−7 mice were used. P388 cells were inoculated ip (10^6 cells/mouse) into B6D2F1 mice (Iffa credo, L'Arbresle, France) on day 0. The dihydrochloride salts of the compounds were dissolved in water and injected iv on day 1 or days 1, 5, and 9. The results are expressed in terms of % T/C survival (median survival time of treated animals/median survival time of control animals) $\times$ 100. For the ip B16 melanoma, 0.5 mL of a tumor brei (1 g of tumor in 10 mL of 0.9% NaCl) was injected ip on day 0, and compounds were administered ip on days 1−9. The optimal dose was the dose which gave the higher T/C without major

toxicity (no toxic death, weight loss < 20%). Results are expressed as % T/C survival. The therapeutic index TI was calculated by dividing the optimal dose by the dose giving a T/C of 125%.

Acknowledgment. We thank J. P. Volland and collaborators, especially C. Schaeffer (NMR), for analytical data, A. Nault for her excellent technical assistance in synthesis, the staff of the Division of Experimental Oncology for performing the biological assays, A. Morris and G. Poissonnet for critical reading of this manuscript, and F. Sainsaulieu and N. Brégou for preparing the manuscript.

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JM981093M