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Article

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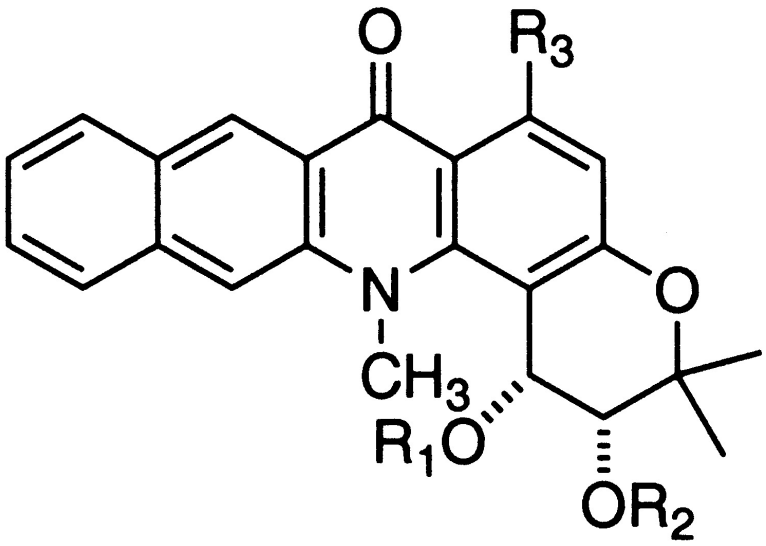

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Structure−Activity Relationships and Mechanism of Action of Antitumor Benzo[*b*]pyrano[3,2-*h*]acridin-7-one Acronycine Analogues

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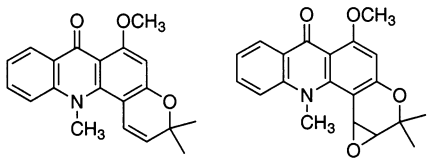
The cytotoxic and antitumor activities of *cis*-1,2-diacyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,-14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one derivatives **3**, **6**−**9** were strongly correlated with their ability to give covalent adducts with purified, as well as genomic, DNA. Such adducts involve reaction between the exocyclic N-2 amino group of guanines exposed in the minor groove of double helical DNA and the leaving ester group at the benzylic position 1 of the drug. A transesterification process of the ester group from position 2 to position 1 in aqueous medium accounted for the intense activity of the *cis*-1-hydroxy-2-acyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one derivatives **10**−**13**. Compounds without acyloxy or hydroxy group at position 1, such as **15**, **17**, **18**, and **22**, were inert with respect to DNA and almost devoid of significant cytotoxic activity. Condensation of 5-amino-2,2-dimethyl-2*H*-chromene (**26**) with 3-bromo-2-naphthoic acid (**27**), followed by cyclization, gave access to 6-demethoxy analogues. Diacetate **32** and cyclic carbonate **33**, both belonging to the latter series, were less reactive toward DNA and less cytotoxic than their 6-methoxy counterparts **3** and **34**. DNA alkylation appears thus to play an important role in the antitumor properties of benzo[*b*]pyrano[3,2-*h*]acridin-7-one derivatives.

Introduction

The acridone alkaloid acronycine (**1**) (Chart 1), first isolated from *Acronychia baueri* Schott (*Rutaceae*) in 1948,[1,2] was subsequently shown to exhibit a broad spectrum of activity against numerous experimental tumors models, including sarcoma, myeloma, carcinoma, and melanoma.[2,3] Nevertheless, clinical trials gave only poor results,[4] probably due to the moderate potency of this alkaloid. Moreover, the mechanism of action of acronycine at both the cellular and molecular levels has not yet been clearly established.[2]

The isolation of the unstable acronycine epoxide (**2**) from several New-Caledonian *Sarcomelicope* species led to the hypothesis of bioactivation of acronycine by transformation of the 1,2-double bond into the corresponding oxirane in vivo.[2,5] Consequently, there was interest in the search for new acronycine derivatives modified in the pyran ring and having a similar reactivity at the benzylic position 1 toward nucleophilic agents as acronycine epoxide, but having an improved stability.[6a] A series of 1,2-dihydroxy-1,2-dihydroacronycine diesters exhibited marked antitumor properties with a broadened spectrum and increased potency when compared with acronycine.[6] Further on, structural analogues with an additional aromatic ring linearly fused on the natural alkaloid skeleton were developed, and several *cis*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one diesters proved even more potent.[7] Among them, diacetate **3**, currently under preclinical development under the code S23906-1, displays a particularly impressive broad antitumor spectrum. Indeed, when evaluated against aggressive orthotopic models of human ovarian, lung, and colon cancers, compound **3** demonstrated comparable and/or better activity than paclitaxel, vinorelbine, and irinotecan, respectively.[8] Compound **3** was recently shown to alkylate purified DNA at the N-2 of guanine residue.[9] Covalent binding to genomic DNA of tumor cells in culture was also observed.[9] However, the precise molecular mechanism of DNA alkylation, as well as its involvement in the cytotoxic and antitumor properties, of **3** remained unresolved. The aim of the present work is to study the structure−activity relationships in the 3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-

Chart 1. Acronycine (**1**) and Acronycine Epoxide (**2**)



1 **2**

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Table 1. Cytotoxicity and DNA Alkylation

compound	cytotoxicity (L1210 cells, IC_{50}, μM)[a]	cytotoxicity (KB-3−1 cells, IC_{50}, μM)[a]	L1210 cell % of cells in S phase (μM)[b]	in vitro DNA alkylation[c,e]	cell DNA alkylation[d,e]
(±)-**3**	0.8	0.09	72 (5 μM)	++	++
4	14.9	3.4	n.t.[f]	0	0
(±)-**5**	41	6	28 (100 μM)	0	0
(±)-**6**	0.6	n.t.	69 (5 μM)	0	++
(±)-**7**	0.7	n.t.	65 (2.5 μM)	++	++
(±)-**8**	0.15	0.11	64 (2.5 μM)	+	+
(±)-**9**	1.3	0.14	73 (5 μM)	++	++
(±)-**10**	1.9	0.22	78 (10 μM)	++	++
(±)-**11**	0.6	0.32	78 (5 μM)	++	++
(±)-**12**	1.1	n.t.	75 (5 μM)	0	0
(±)-**13**	0.5	0.18	72 (2.5 μM)	++	++
(±)-**15**	17	n.t.	n.t.	0	0
(±)-**17**	22	n.t.	n.t.	0	0
(±)-**18**	8	n.t.	n.t.	0	0
(±)-**19**	9.7	10.8	n.t.	0	0
(±)-**22**	45	n.t.	n.t.	0	0
(±)-**24**	0.6	0.13	70 (5 μM)	++	++
(±)-**25**	0.7	0.13	65 (20 μM)	0	+
(±)-**32**	1.3	0.10	34 (10 μM)	0	+
(±)-**33**	1.7	0.27	58 (20 μM)	0	+
(±)-**34**	0.014	0.053	75 (0.2 μM)	+	+++

[a] Inhibition of cell proliferation measured by the MTT assay (mean of at least three values obtained in separate experiments). [b] Highest percentage of L1210 cells arrested in S phase after a 21 h exposure to the indicated concentration. Untreated control: 32% on average. [c] The capacity of the tested compounds to form complexes with purified DNA was investigated by a gel shift assay. [d] Alkylation of genomic DNA was measured by fluorescence. The experimental procedures are given in the Experimental Section. [e] Symbols ++++, ++, and + refer to very strong, strong, and weak alkylation, respectively, whereas 0 means no DNA alkylation at all. [f] n.t.: not tested.

Chart 2. Compounds **3**−**14** and **34**

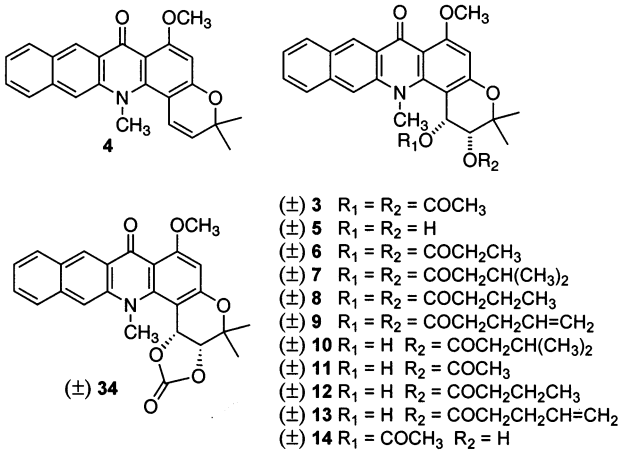


Table 2. Antitumor Activity against C-38 Colon Adenocarcinoma Implanted in Mice[a]

compound	optimal dose, mg/kg[b]	*T*/*C* tumor growth, %
(±)-**3**	6.25	0
(±)-**6**	6.25	0.8
(±)-**7**	12.5	120
(±)-**8**	12.5	0
(±)-**9**	25	12
(±)-**10**	6.25	2.6
(±)-**11**	1.56	5.3
(±)-**12**	1.56	9.4
(±)-**13**	6.25	0
(±)-**24**	6.25	20
(±)-**25**	25	59
(±)-**34**	3.12	12

[a] Tumor fragments were implanted sc on day 0, and the compounds administered iv on days 10 and 20. The tumor volume was measured twice a week [b] Dose (mg/kg) giving the optimal therapeutic effect (lowest *T*/*C* measured one week after the second administration) without major signs of toxicity.

acridin-7-one series in order to clarify the molecular mechanism of action of **3**.

Mechanism of Action of *cis*-1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one Diesters. These diesters were obtained by catalytic osmic oxidation of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one (**4**), followed by treatment of the resulting *cis*-diol **5** with an excess of acylating reagent.[7] In addition to the previously described compounds **3**, **6**, and **7**, the new diesters **8** and **9** were prepared (Chart 2). The compounds were tested in vitro against two tumor cell lines, a murine leukemia (L1210) and a human solid tumor cell line, KB-3-1. All the *cis*-diesters showed cytotoxic properties with IC_{50} values in the μM range (Table 1). Interestingly, these cytotoxic compounds were globally more potent on the solid tumor cell line than on the leukemia (10-fold in the case of **3**). According to our rationale,[5,6] the diesters **3** and **6**-**9** were markedly more potent than 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**4**).[7] The antitumor activity of the acronycine derivatives was first revealed on the murine C38 colon adenocarcinoma,[6a] and induction of tumor regression in this model was subsequently shown to be a good indicator of a marked antitumor activity of acronycine derivatives in more relevant orthotopic models of human solid tumor.[8a] Consequently, the derivatives were evaluated in vivo on established C38 colon adenocarcinoma (Table 2). The most potent compounds in vitro (**3**, **6**, and **8**) were highly active in vivo, inducing a significant rate of complete regressions. As previously reported, the diol **5** was about 100-fold less cytotoxic and inactive in vivo.[7] The cytotoxicity of **3** was previously shown to be linked to inhibition of DNA synthesis leading to an irreversible arrest in the S phase of the cell cycle, followed by apoptosis.[10] The effect of the compounds on the cell phase is shown in Table 1: all cytotoxic derivatives induce an accumulation in the S phase. DNA has been recently identified as a pharmacological target for the diacetate

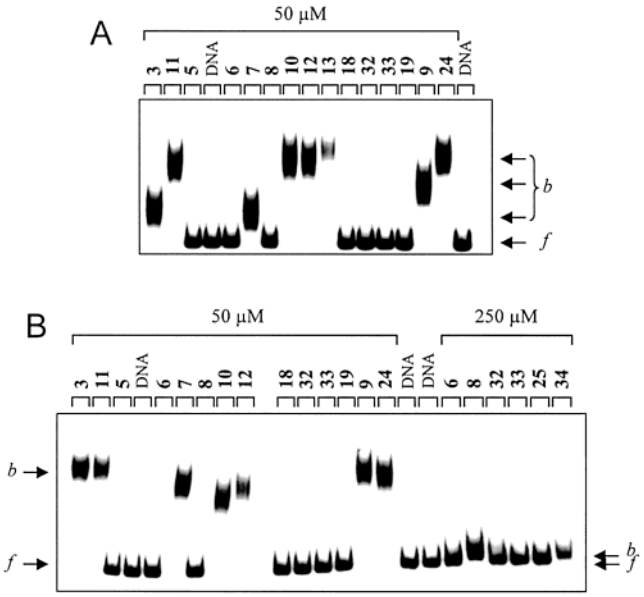


Figure 1. Covalent binding to DNA. The gel shift assay shows the alkylation of a 117 bp radiolabeled DNA fragment by the different compounds incubated (A) at 50 μM for 1.5 h or (B) 24 h at room temperature or (C) at 250 μM for 16 h at 37 °C to maximize the alkylation reaction. Experiments were performed in a 1 mM sodium cacodylate buffer at pH 7.0. Free (*f*) and bound (*b*) DNAs were separated by electrophoresis on denaturing 6% polyacrylamide gels. Control tracks labeled "DNA" contained no drug.

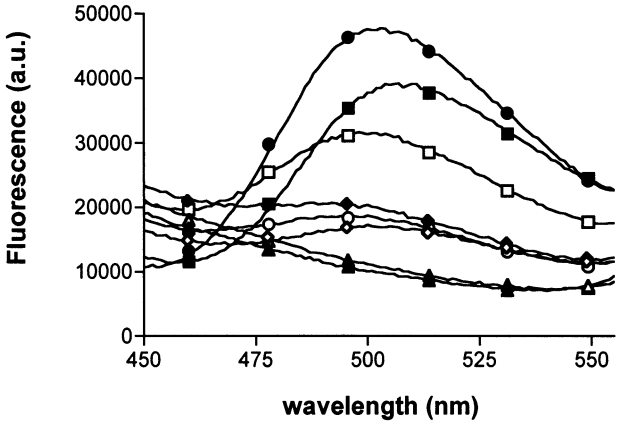


Figure 2. Formation of drug−DNA covalent complexes in KB-3-1 breast carcinoma cells. Fluorescence emission spectra of DNA extracted from KB-3-1 cells (10^6) treated with **5** (●), **8** (○), **6** (□), **32** (■), **19** (▲), **18** (△), **33** (XXXXX), or **25** (◆) for 24 h at 37 °C (10 μM each). The excitation wavelength was 300 nm.

lead compound **3**. Indeed, gel retardation experiments performed with DNA fragments and mass spectrometry of adducts obtained using short 7-base pairs hairpin oligonucleotides indicated that this compound links covalently to the exocyclic amino group of guanines exposed in the minor groove of double helical DNA.[9]

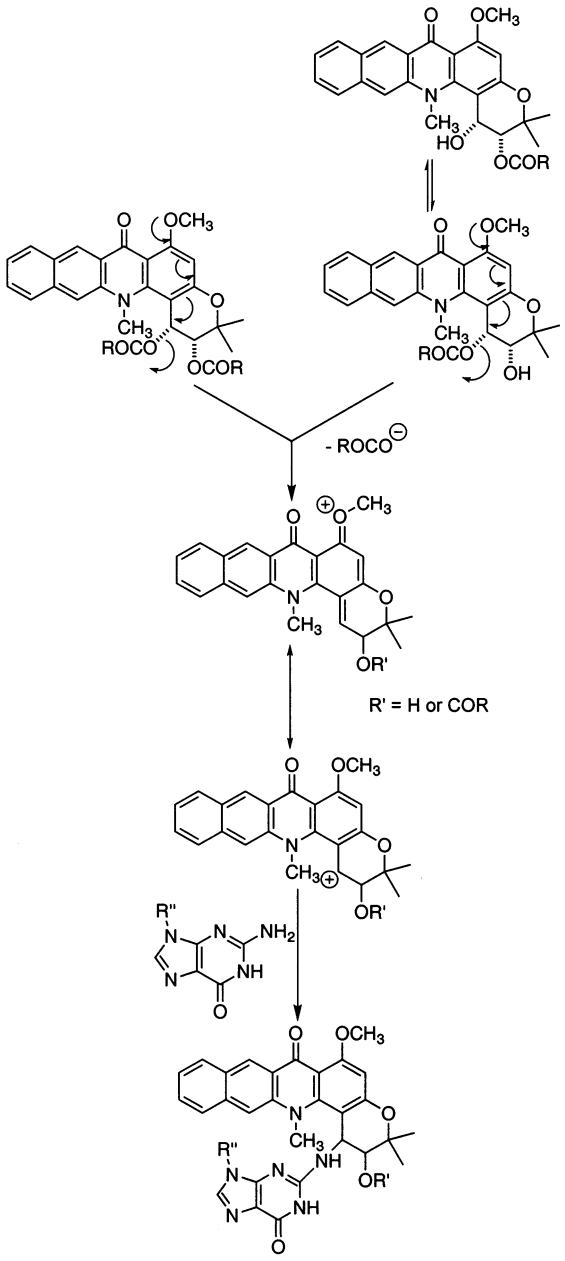
We compared the capacity of the newly synthesized derivatives to form covalent complexes with DNA, using the same gel shift assay as developed previously with **3**. The different compounds were incubated with a ^{32}P-radiolabeled DNA substrate, and the resulting products were resolved on polyacrylamide gels. A typical set of data is presented in Figure 1, and results are summarized in Table 1. Interestingly, not all of the diesters reduce the electrophoretic mobility of the DNA. For example, a prominent shift is seen with the dipentenyloxy derivative **9**, whereas, surprisingly, the dipropionate **6** and dibutanoate **8** analogues failed to shift the DNA band. However, a very weak effect was noticed with these two compounds when tested at a high concentration (250 μM, Figure 1B). Therefore, the capacity of the drugs to alkylate purified DNA depends on the nature of the ester groups. The alkylation of genomic DNA in KB-3-1 carcinoma cells was investigated by a fluorescence assay. Cells were treated with each compound at 10 μM for 24 h prior to extracting the DNA. The fluorescence of drug-bound molecules was then measured. Representative fluorescence spectra of different drug−DNA complexes are shown in Figure 2. A large fluorescence signal in the benzoacronycine band centered at 510 nm was detected with the diacetate **3**, whereas the fluorescence intensity remained very weak with the dibutanoate **8**, in agreement with the experiments performed with purified DNA (Figure 2). Conversely, the dipropionate **6**, which showed no reaction with purified DNA appears to form covalent complexes with DNA in cells. A summary of the data is given in Table 1, and, except in a few cases, a satisfactory correlation was found between the molecular and cellular data. The compounds that showed a high reactivity with purified DNA also bind strongly to genomic DNA. In addition, the good correlation between cytotoxicity and alkylation of DNA in cells should be emphasized.

Structure−Activity Relationships and Mechanism of Action in the 2-Acyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano-[3,2-*h*]acridin-7-one Series. *cis*-2-Acyloxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one derivatives, esterified only at the less hindered 2-position, were obtained almost quantitatively when diol **5** was treated with 1 equiv of acylating reagent. In addition to the known isovalerate **10**,[7] several new monoesters were prepared, exemplified by acetate **11**, butanoate **12**, and pentenoate **13**. Surprisingly, despite the lack of an ester leaving group at position 1, all these compounds exhibited cytotoxic activities within the same order of magnitude as the corresponding diesters, when tested against L1210 cells in vitro (Table 1). In vivo, antitumor activities similar to that of **3** were obtained with monoesters **11**−**13** (Table 2). The fact that compounds lacking a leaving group at position 1 possess marked cytotoxic and antitumor activities could lead to the conclusion that DNA alkylation is not involved in their mechanism of action. Nevertheless, a series of gel retardation experiments performed on monoesters **10**−**13** demonstrated that these compounds were also able to link covalently to DNA. Moreover, the gel shift is significantly more pronounced with the monoacetate **11** than with the diacetate **3**. As a general rule, the monoesters, such as isovalerate **10**, butanoate **12**, and pentenoate **13** react with DNA more efficiently than the corresponding diesters. This is clearly illustrated in the acetate pair of compounds (**3** less reactive than **11**), as well as with the isovalerate derivatives (compare the low reactivity of **7** versus **10**) (Figure 1). Mass spectral analysis revealed that the molecular weight of the adducts obtained with 7-base pairs hairpin oligonucleotides differed by one acyl group per alkylated guanine

residue from those of the heaviest adducts obtained with the corresponding diesters (not shown). These results led us to postulate that *cis*-monoesters at position 2 could spontaneously lead to the corresponding more reactive *cis*-monoesters at position 1, by a transesterification process. Indeed, this reaction could explain the similar reactivity observed in both series and the difference of molecular mass of the adducts obtained with DNA. Experimental evidence of spontaneous transesterification was obtained by careful NMR study of *cis*-monoacetate **11**. When kept at 20 °C in DMSO containing 15% D_2O, the ^1H NMR data of **11** evolved, revealing an equilibrium between the 2-monoacetate **11** and the 1-monoacetate **14** under those conditions. A stable 80:20 equilibrium between **11** and **14** was obtained within 48 h and did not evolve further when the duration of the experiment was increased ($t_{1/2} \approx 14$ h). Therefore, the mechanism of action of monoesters **10**−**13** can be rationalized according to Scheme 1. In this context, the fact that some diesters, exemplified by dipropionate **6**, exhibit a higher propensity to alkylate DNA in cells than with purified DNA may be attributed to specific metabolic processes, leading to the corresponding more reactive monoesters. In agreement with this statement, it was generally observed that the kinetics of the DNA bonding reactions of most of the diesters are faster in cells compared to reactions performed in test tubes using purified materials. Different esterases may contribute to the drug activation pathways.

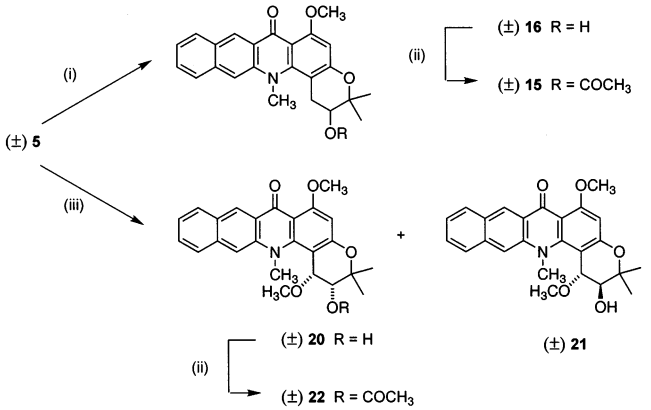
Structure−activity relationships in the 2-acyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one series are in full agreement with the above-described mechanism. Indeed, compounds bearing an ester at C-2, but lacking a free hydroxy group at C-1 exhibited cytotoxic activities dramatically decreased when compared with those of *cis*-2-acyloxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,-14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one. For instance, 2-acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,-14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**15**), obtained by reduction of diol **5** to the corresponding homobenzylic alcohol **16** (Scheme 2) by the use of sodium cyanoborohydride in the presence of zinc iodide followed by acetylation,[6c] was more than 10 fold less cytotoxic than both **3** and **11**. Weak activities were also observed with various 2-acyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-1,7-diones, exemplified by acetate **17** and propionate **18**, which were prepared by permanganate oxidation of **4** to the keto alcohol **19**, followed by esterification (Scheme 3). When treated with methanol in a strong acidic medium, the *cis*-diol **5** gave in almost quantitative yield a 1:1 mixture of *cis*- and *trans*-1-methoxy-2-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones, **20** and **21**, easily separated after column chromatography.[11] Acylation of **20** smoothly afforded 1-methoxy-2-acyloxy-6-methoxy-3,3,-14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano-[3,2-*h*]acridin-7-ones, such as acetate **22**, which were almost devoid of cytotoxic activity. Interestingly, derivatives **15**, **17**, **18**, and **22** were found to be inert with respect to DNA (Table 1). No reaction was observed with those compounds, even when tested at a concentration as high as 250 μM. This is a strong indication that a good leaving group at C-1 is actually required for covalent binding to DNA. In contrast, the ester group at C-2 is not implicated in the bonding to DNA. Compounds **15**, **17**, **18**, and **22** also failed to react with DNA in cells. Another important aspect is that these molecules are weakly cytotoxic compared to the DNA-reactive analogues. Overall, in the 6-methoxy-3,3,14-trimethylbenzo[*b*]pyrano[3,2-*h*]acridin-7-one series, the ability to reduce the electrophoretic mobility of DNA appears to be associated with cytotoxicity and antitumor activity, suggesting that DNA alkylation plays a major role in the antitumor properties of these derivatives.

Scheme 1. Mechanism of Action of 1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one Esters and Diesters



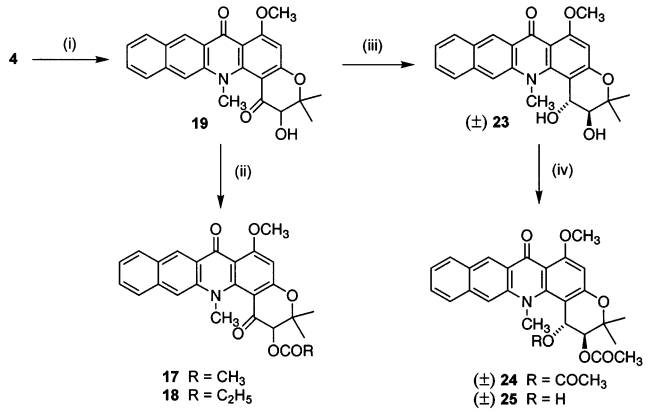
Activity of *trans*-1,2-Dihydroxy-6-methoxy-3,3,-14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one Esters and Diesters. The desired *trans*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-

Scheme 2. Synthesis of Compounds **15** and **22**[a]



[a] Reagents and conditions: (i) $NaBH_3CN/ZnI_2$, $ClCH_2CH_2Cl$, rt (31%); (ii) Ac_2O/C_5H_5N, rt (71−82%); (iii) MeOH/HCl, rt.

Scheme 3. Synthesis of Compounds **17**−**19** and **23**−**25**[a]



[a] Reagents and conditions: (i) $KMnO_4$, Me_2CO/H_2O, rt (76%); (ii) $(RCO)_2O/C_5H_5N$, rt (83%); (iii) $NaBH_4$, MeOH, 0 °C (49%); (iv) Ac_2O/C_5H_5N, rt.

1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**23**) was obtained, in 49% yield, by borohydride reduction of the keto alcohol **19**.[6b] As observed for the *cis*-diol **5**, treatment with excess acetic anhydride in pyridine afforded the corresponding diacetate **24**, whereas the monoacetate at the less hindered 2 position **25** was obtained following reaction of **23** with 1 equiv of acylating reagent under the same conditions. As expected, the *trans*-diacetate **24** exhibited cytotoxic activity of the same magnitude as that of the *cis*-diacetate **3**, although it was less active in vivo against C38 colon adenocarcinoma. The trans monoester **25**, in which intramolecular transesterification into the corresponding monoester at position 1 is not possible, was almost as cytotoxic as its cis counterpart **11**, but was markedly less potent and less active in vivo. Interestingly, although the two compounds were similarly cytotoxic toward L1210 cells, **25** is significantly less potent and less active to arrest cells in the S phase of the cell cycle. Cell cycle arrest in the S phase therefore appears to be better indicator of antitumor activity than the IC_{50} value. The *trans*-diacetate **24** easily forms covalent complexes with DNA, as illustrated in Figure 1. On the other hand, the *trans*-monoacetate **25** does not bind to purified DNA at all, as shown in the concentration-dependent gel shift in Figure 3B. The high reactivity of the *cis*-monoacetate **11** contrasts with the nonreactivity of the trans isomer **25**. This observation is consistent with the mechanism

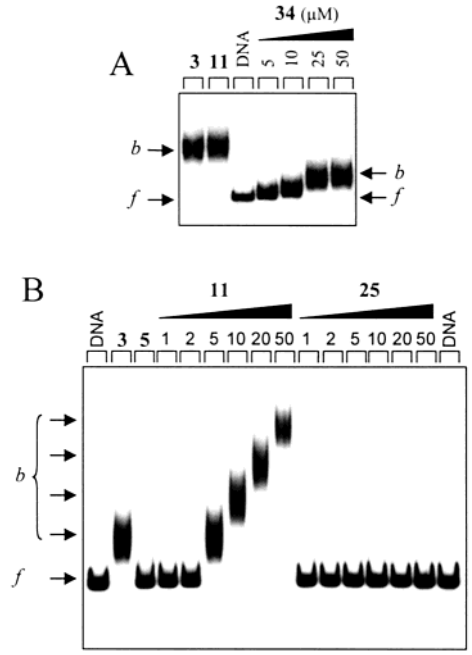
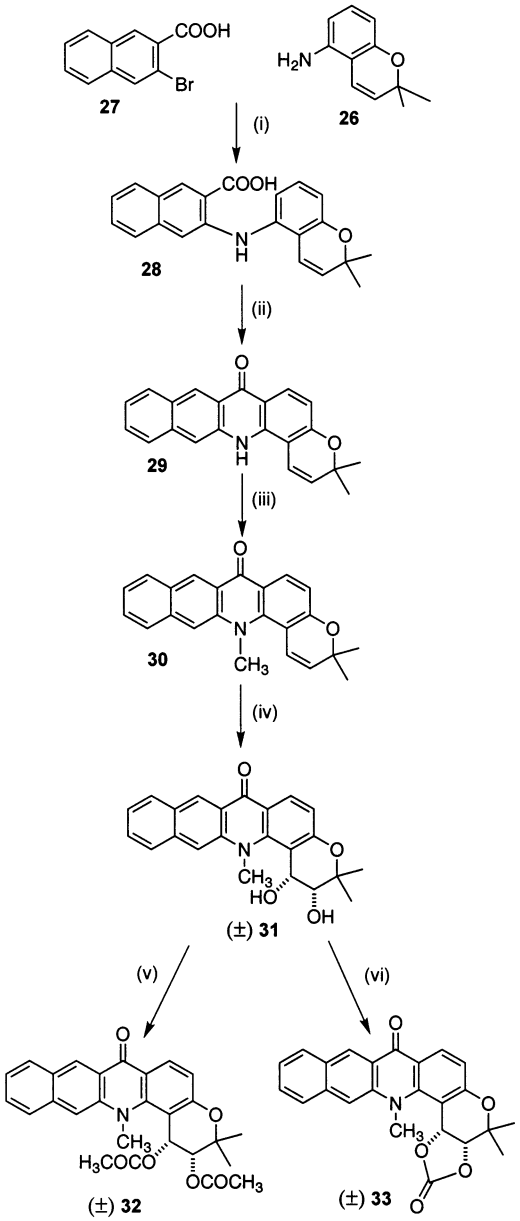


Figure 3. Concentration-dependence for the binding of (A) **34** or (B) **11** and **25** to DNA. Compound **5** was used as control. The compounds were incubated with the 117 bp radiolabeled DNA fragment for 15 h at room temperature, in a 1 mM sodium cacodylate. In gel (A), compounds **3** and **11** were used at 25 and 50 μM, respectively and in (B), both **3** and **5** were used at 50 μM. Other details as for Figure 1.

of DNA alkylation. Indeed, a transesterification reaction can occur with the cis compound **11** to bring the leaving acetate group to position 1, whereas it cannot occur in the case of the trans monoester **25**.

Influence of the Methoxy Group at C-6. To determine the influence of substitution at C-6 on the biological activity, compounds without a methoxy group at this position were prepared. Ullmann condensation of 5-amino-2,2-dimethyl-2*H*-chromene (**26**)[12] with 3-bromo-2-naphthoic acid (**27**)[13] afforded the corresponding carboxylic diarylamine **28** in 48% yield (Scheme 4). Cyclization to 3,3-dimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one (**29**) was achieved in 60% yield by the use of trifluoroacetic anhydride in dichloromethane, which had previously given good results in the course of syntheses in the acronycine series.[14] Methylation at N-14 was ensured by use of methyl iodide in the presence of sodium hydride in dimethylformamide to give the desired 3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**30**) in 86% yield. The (±)-*cis*-diol **31** was conveniently obtained in 69% yield by catalytic osmium tetroxide oxidation of **30**, using *N*-methylmorpholine *N*-oxide to regenerate the oxidative agent.[7,15] Treatment of **31** with excess acetic anhydride gave the corresponding diacetate **32** in 91% yield. In a similar way, acylation of diol **31** with *N*,*N*′-carbonyldiimidazole in 2-butanone under reflux afforded almost quantitatively the cyclic carbonate **33**, whose counterpart in the 6-methoxy series **34** had been previously prepared.[7] In the diacetate series, the 6-methoxy **3** and the 6-demethoxy **32** are similarly cytotoxic, as shown by their IC_{50} values on the two cell lines (Table 1). However, L1210 cells treated by **32** were not arrested in the S phase, but in the G2 + M phase of the cell cycle (not shown). In the carbonate series, the 6-methoxy **34**

Scheme 4. Synthesis of Compounds **32** and **33**[a]



[a] Reagents and conditions: (i) $Cu(OAc)_2 \cdot H_2O/K_2CO_3/Et_3N$, reflux (48%); (ii) $(CF_3CO)_2O$, CH_2Cl_2, rt (60%); (iii) NaH/MeI, DMF, rt (86%); (iv) OsO_4/NMO, BuOH/THF/H_2O, 40 °C (69%); (v) Ac_2O/C_5H_5N, rt (91%); (vi) *N*,*N*′-carbonyldiimidazole, MeCOEt, reflux (97%).

is about 10-fold more potent than the demethoxy **33** in terms of IC_{50}, and 100-fold more in terms of S phase accumulation. The cyclic carbonate **34** is at first sight an atypical case in the sense that it is poorly reactive toward purified DNA, giving only a slight gel shift (Figure 3A), but it exhibits an extremely high cytotoxic activity. Nevertheless, experiments at the cellular level indicated that this compound avidly alkylates genomic DNA. Indeed, a very intense fluorescence signal was measured with **34** after extraction of the DNA from the treated cells (Table 1). It could reflect the facile opening of the cyclic carbonate function into a highly reactive intermediate in whole cells. Interestingly, this opening process is dependent on the methoxy group at position 6, since the demethoxy analogue **33** shows almost no reactivity toward genomic DNA (Figure 2), despite the presence of the same carbonate group. The methoxy group at position 6 also reinforces the DNA alkylation potential of the compounds in the diacetate series, as indicated by the superior reactivity of **3** when compared with its demethoxy counterpart **32**.

Conclusion

In conclusion, the cytotoxic and antitumor activities of 1,2-dihydroxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one esters and diesters appear to be strongly correlated with their ability to give covalent DNA adducts. This correlation is stronger regarding DNA alkylation in whole cells. Adducts involve reaction between the exocyclic N-2 amino group of guanines exposed in the minor groove of double helical DNA and the leaving ester group at the benzylic position 1 of the drug. The intense activity observed with *cis*-1,2-diol monoesters at position 2 can be rationalized on the basis of a transesterification process of the ester group to position 1 in aqueous medium. The methoxy group at C-6, which facilitates the formation and stabilization of a carbocation at the benzylic position 1, has a marked influence on the activity. Therefore, the presence of an electron-donating group at position 6 appears as an important structural requirement in order to observe potent cytotoxic effects in the series.[16]

Experimental Section

Chemistry. Melting points were determined on a hot stage Reichert microscope and are uncorrected. Mass spectra were recorded with ZQ 2000 Waters and Q-Tof1 Micromass spectrometers using electrospray ionization (ESI-MS; Vc = 30 V), or with a Nermag R-10−10C spectrometer using desorption-chemical ionization (DCI-MS; reagent gas: NH_3). UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium bromide pellets or sodium chloride films on a Perkin-Elmer 257 instrument. ^1H NMR (δ-[ppm], *J*[Hz]) spectra were run at 400 MHz and ^{13}C NMR spectra at 75 MHz, using Bruker AVANCE-400 and AC-300 spectrometers, respectively. When necessary, the structures of the novel compounds were ensured and the signals unambiguously assigned by 2D NMR techniques: $^1H-^1H$ COSY, $^1H-^1H$ NOESY, $^{13}C-^1H$ HETCOR, and $^{13}C-^1H$ COLOC. These experiments were performed using standard Bruker microprograms. Of particular interset were (i) $^{13}C-^1H$ COLOC correlations observed between the ester carbonyl and H-2 in monoester derivatives **11**, **12**, and **13**, which unambiguously demonstrated the position of the acyl group (ii) in the presence of intense cross-peaks between the signals of H-1 and H-2 on the $^1H-^1H$ NOESY spectra of *cis*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one esters and diesters, which were not observed in the case of *trans*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one conterparts.[17] Column chromatography was carried out with silica gel 20−45 μm. Flash column chromatography was conducted using silica gel 60 Merck (35−70 μm) with an overpressure of 300 mbars. Microanalyses were in agreement with calculated values ± 0.4%.

Cell Culture and Cytotoxicity. L1210 and KB-3-1 cells were cultivated in RPMI 1640 or DMEM medium, respectively (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 (MTA) as described.[18] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for four doubling times (48 h for L1210 cells and 96 h for KB-3-1 cells). Results are expressed as IC_{50}, the concentration which reduced by 50%

the optical density of treated cells with respect to the optical density of 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 50 μg/mL propidium iodide for 30 min at 20°C. For each sample, 10 000 cells were analyzed on an XLMCL flow cytometer (Beckman Coulter, France). Results are given in the highest % of cells arrested in the S phase of the cell cycle.

Antitumor Activity. The antitumor activity of the compounds was evaluated on the murine colon 38 adenocarcinoma implanted in B6D2F1 (C57B1/6 x DBA2) mice. The colon adenocarcinoma C38 (NCI, Frederick) was introduced by sc implantation of a tumor fragment into the dorsal flank. The drugs were solubilized in 10% solutol HS15 and administered by iv injection on days 10 and 20. The tumor volume was measured twice a week, and the results are expressed as percent *T*/*C* (median tumor volume in treated animals divided by median tumor volume of controls) × 100. Results are expressed at the lowest *T*/*C* (highest antitumor activity) obtained at the highest non toxic dose. A dose is considered as toxic when the weight loss is higher than 20% or when it induces toxic deaths.

Binding to DNA. Gel Shift Studies. A typical cross-linking reaction consisted of incubating 8 μL of radiolabeled DNA, 2 μL of buffer (10 mM Na cacodylate, pH 7.0; Tris buffer must be avoided due to the presence of reactive amine functions), and 10 μL of the drug at the desired concentration in the dark at room temperature, during the period specified in the legend, prior to adding 5 μL of a 50% glycerol solution containing tracking dyes. DNA samples were resolved by electrophoresis under nondenaturing conditions in 6% acrylamide gels for about 5 h at 300 V at room temperature in TBE buffer (89 mM boric acid, 2.5 mM Na_2EDTA, pH 8.3). Gels were transferred to Whatman 3MM paper, dried under vacuum at 80 °C, and then analyzed on a phosphorimager (Molecular Dynamics 445SI).

Detection of DNA Adducts in KB-3-1 Cells. 10^6 KB-3-1 cells were grown for 24 h in 100 mm diameter dishes with 5 mL of culture medium, prior to the addition of 10 μM of the various compounds for 24 h. The genomic DNA was extracted from the cells as described previously.[9] Briefly, treated cells were collected by centrifugation (1000 rpm, 5 mn), washed twice with 10 mL PBS buffer, and resuspended in 2 mL of PBS containing 5 mM $MgCl_2$ prior to the addition of 200 μL of 10% SDS and mild agitation for 5 min. Proteinase K (80 μL at 10 mg/mL) was added for a further 5 mn of mild agitation, and finally 200 μL of 0.1 M EDTA pH 7.5 was added and the mixture was incubated 4 h at 37 °C. After addition of 80 μL 5M NaCl, the DNA was extracted using 3 mL of phenol/chloroform/isoamylic alcohol (25:24:1) and centrifugated at 4000 rpm for 10 min, followed by two extractions with 3 mL of chloroform/isoamylic alcohol (24:1). Finally, the DNA was precipitated with cold ethanol followed by centrifugation at 11 000 rpm for 30 min. The dry pellet was then dissolved in 200 μL of H_2O and treated for 2 h with 5 μM of RNase (10 mg/mL) to avoid RNA contamination. The absorption of the final solution was measured at 260 nm to estimate the quantity of collected DNA. The fluorescence of the various compounds covalently linked to DNA was measured using a SPEX Fluorolog spectrofluorimeter with an excitation wavelength at 300 nm and an emission range from 420 to 555 nm.

(±)-*cis*-1,2-Dibutanoyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (8) and (±)-*cis*-1-Hydroxy-2-butanoyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (12). To a solution of **5** (0.30 g, 0.74 mmol) in dry pyridine (7 mL) was added 4-butyryl chloride (0.55 mL, 5.2 mmol). The reaction mixture was stirred at room temperature for 6 days and then evaporated under reduced pressure (t < 40°C). Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 95:5), afforded successively **8** (0.11 g, 27%) and **12** (0.17 g, 48%), as yellow amorphous solids. Compound **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (m, 6H, 2 × $OCOCH_2CH_2CH_3$), 1.46 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.57 (m, 4H, 2 × $OCOCH_2CH_2CH_3$), 2.13 (t, J = 7,Hz, 2H, $OCOCH_2CH_2CH_3$), 2.26 (t, J = 7 Hz, 2H, $OCOCH_2CH_2CH_3$), 3.72 (s, 3H, NCH_3), 4.02 (s, 3H, OCH_3), 5.51 (d, J = 5 Hz, 1H, H-2), 6.30 (s, 1H, H-5), 6.61 (d, J = 5 Hz, 1H, H-1), 7.40 (td, J = 8, 1.5 Hz, 1H, H-10), 7.52 (s, 1H, H-13), 7.54 (td, J = 8, 1.5 Hz, 1H, H-11), 7.82 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.11 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.89 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5 ($OCOCH_2CH_2CH_3$), 13.7 ($OCOCH_2CH_2CH_3$), 18.1 ($OCOCH_2CH_2CH_3$), 18.2 ($OCOCH_2CH_2CH_3$), 23.7 (CH_3), 24.4 (CH_3), 35.8 ($OCOCH_2CH_2CH_3$), 36.1 ($OCOCH_2CH_2CH_3$), 43.2 (NCH_3), 56.3 (OCH_3), 65.5 (C1), 69.3 (C_2), 76.2 (C_3), 94.5 (C5), 98.3 (C_{14b}), 111.8 (C13), 112.2 (C6a), 124.5 (C10), 125.8 (C7a), 126.6 (C12), 127.9 (C8), 128.2 (C11), 128.7 (C8a), 129.6 (C9), 135.7 (C12a), 142.5 (C13a), 150.3 (C14a), 160.2 (C4a), 162.9 (C6), 172.9 ($OCOCH_2CH_2CH_3$), 173.5 ($OCOCH_2CH_2CH_3$), 177.6 (C7); DCI-MS m/z 546 $[MH]^+$; IR (KBr) ν cm^{-1} 3050, 2964, 2939, 1743, 1652, 1622, 1589, 1571, 1489, 1462, 1398, 1368, 1325, 1185, 1158, 1085, 1033, 862, 805, 747; UV λ nm (MeOH) (log ϵ) 236 (4.49), 287 (4.97), 338 (4.17), 433 (3.83). Anal. ($C_{32}H_{35}NO_7$) C, H, N. Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 7 Hz, 3H, $OCOCH_2CH_2CH_3$), 1.46 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.60 (sxt, J = 7 Hz, 2H, $OCOCH_2CH_2CH_3$), 2.27 (br. d, J = 9 Hz, 1H, OH-C1), 2.35 (t, J = 7 Hz, 2H, $OCOCH_2CH_2CH_3$), 3.88 (s, 3H, NCH_3), 3.93 (s, 3H, OCH_3), 5.36 (dd, J = 9, 5 Hz, 1H, H-1), 5.43 (d, J = 5 Hz, 1H, H-2), 6.10 (s, 1H, H-5), 7.41 (td, J = 8, 1.5 Hz, 1H, H-10), 7.53 (td, J = 8, 1.5 Hz, 1H, H-11), 7.65 (s, 1H, H-13), 7.84 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.02 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.84 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5 ($OCOCH_2CH_2CH_3$), 18.4 ($OCOCH_2CH_2CH_3$), 22.6 (CH_3), 25.3 (CH_3), 35.9 ($OCOCH_2CH_2CH_3$), 42.3 (NCH_3), 56.2 (OCH_3), 63.9 (C1), 71.7 (C_2), 76.7 (C_3), 93.9 (C5), 100.3 (C_{14b}), 110.2 (C6a), 111.9 (C13), 124.4 (C10), 125.8 (C7a), 126.8 (C12), 127.9 (C8), 128.2 (C11), 128.6 (C8a), 129.6 (C9), 135.8 (C12a), 143.1 (C13a), 148.0 (C14a), 159.3 (C4a), 162.5 (C6), 173.5 ($OCOCH_2CH_2CH_3$), 178.4 (C7); DCI-MS m/z 476 $[MH]^+$; IR (KBr) ν cm^{-1} 3420, 3052, 2959, 2924, 1730, 1641, 1614, 1589, 1493, 1390, 1204, 1149, 1094, 1032, 812, 733; UV λ nm (MeOH) (log ϵ) 236 (4.40), 287 (4.90), 342 (4.07), 440 (3.74). Anal. ($C_{28}H_{29}NO_6$) C, H, N.

(±)-*cis*-1,2-Bis(4-pentenoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (9) and (±)-*cis*-1-Hydroxy-2-(4-pentenoyloxy)-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (13). To a solution of **5** (0.60 g, 1.48 mmol) in dry pyridine (15 mL) was added 4-pentenoyl chloride (0.8 mL, 7.4 mmol). The reaction mixture was stirred at room temperature for 6 h and then evaporated under reduced pressure (t < 40 °C). Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 98:2) gave successively **9** (0.30 g, 36%) and **13** (0.12 g, 17%), as yellow crystals from cyclohexane/acetone 7:3. Compound **9**: mp 152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 2.27 (m, 4H, 2 × $OCOCH_2CH_2CHCH_2$), 2.39 (m, 4H, 2 × $OCOCH_2CH_2CHCH_2$), 3.71 (s, 3H, NCH_3), 4.02 (s, 3H, OCH_3), 4.98 (m, 4H, 2 × $OCOCH_2CH_2CHCH_2$), 5.51 (d, J = 5 Hz, 1H, H-2), 5.73 (m, 2H, 2 × $OCOCH_2CH_2CHCH_2$), 6.30 (s, 1H, H-5), 6.62 (d, J = 5 Hz, 1H, H-1), 7.42 (td, J = 8, 1.5 Hz, 1H, H-10), 7.53 (s, 1H, H-13), 7.55 (td, J = 8, 1.5 Hz, 1H, H-11), 7.83 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.02 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.88 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7 (CH_3), 24.4 (CH_3), 28.4 ($OCOCH_2CH_2CHCH_2$), 28.5 ($OCOCH_2CH_2CHCH_2$), 33.1 ($OCOCH_2CH_2CHCH_2$), 33.5 ($OCOCH_2CH_2CHCH_2$), 43.2 (NCH_3), 56.3 (OCH_3), 65.7 (C1), 69.5 (C_2), 76.4 (C_3), 94.5 (C5), 98.1 (C_{14b}), 111.2 (C6a), 111.9 (C13), 115.7 ($OCOCH_2CH_2CHCH_2$), 115.8 ($OCOCH_2CH_2CHCH_2$), 124.5 (C10), 125.8 (C7a), 126.6 (C12), 128.0 (C8), 128.2 (C11), 128.7 (C8a), 129.6 (C9), 135.7 ($OCOCH_2CH_2CHCH_2$), 136.1 ($OCOCH_2CH_2CHCH_2$), 136.4 (C12a), 142.3 (C13a), 150.3 (C14a), 160.3 (C4a), 162.9 (C6), 172.2 ($OCOCH_2CH_2CHCH_2$), 172.9 ($OCOCH_2CH_2CHCH_2$), 178.2 (C7); DCI-MS m/z 570 $[MH]^+$; IR (KBr) ν cm^{-1} 3050, 2971, 2930, 1743, 1617, 1589, 1493, 1460, 1400,

1204, 1150, 1087, 1030, 916, 875, 811; UV λ nm (MeOH) (log ϵ) 236 (4.43), 288 (4.89), 338 (4.09), 432 (3.74). Anal. ($C_{34}H_{35}NO_7$) C, H, N. Compound **13**: mp 194°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 1.65 (br. s, 1H, OH-C1), 2.31 (m, 2H, $OCOCH_2CH_2CHCH_2$), 2.49 (m, 2H, $OCOCH_2CH_2CHCH_2$), 3.90 (s, 3H, NCH_3), 3.93 (s, 3H, OCH_3), 4.82 (m, 2H, $OCOCH_2CH_2CHCH_2$), 5.33 (d, J = 5 Hz, 1H, H-1),, 5.43 (d, J = 5 Hz, 1H, H-2), 5.67 (ddt, J = 18, 10, 7 Hz, 1H, $OCOCH_2CH_2CHCH_2$), 6.12 (s, 1H, H-5), 7.41 (td, J = 8, 1.5 Hz, 1H, H-10), 7.53 (td, J = 8, 1.5 Hz, 1H, H-11), 7.65 (s, 1H, H-13), 7.84 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.02 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.84 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5 (CH_3), 25.3 (CH_3), 28.7 ($OCOCH_2CH_2CHCH_2$), 33.3 ($OCOCH_2CH_2CHCH_2$), 43.2 (NCH_3), 56.1 (OCH_3), 63.9 (C1), 72.0 (C_2), 77.0 (C_3), 93.7 (C5), 101.9 (C_{14b}), 110.9 (C6a), 111.8 (C13), 115.9 ($OCOCH_2CH_2CHCH_2$), 124.4 (C10), 125.6 (C7a), 126.8 (C12), 127.9 (C8), 128.2 (C11), 128.6 (C8a), 129.5 (C9), 135.8 ($OCOCH_2CH_2CHCH_2$), 136.6 (C12a), 142.0 (C13a), 149.6 (C14a), 159.3 (C4a), 162.5 (C6), 172.7 ($OCOCH_2CH_2CHCH_2$), 178.3 (C7); DCI-MS m/z 488 $[MH]^+$; IR (KBr) ν cm^{-1} 3430, 3049, 2974, 2926, 1734, 1639, 1610, 1588, 1496, 1458, 1436, 1398, 1204, 1150, 1090, 1036, 878, 811; UV λ nm (MeOH) (log ϵ) 236 (4.29), 287 (4.79), 341 (3.95), 440 (3.65). Anal. ($C_{29}H_{29}NO_6$) C, H, N.

(±)-*cis*-1-Hydroxy-2-acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (11). To an iced-cooled solution of **5** (0.81 g, 2 mmol) in dry pyridine (5 mL) was added acetic anhydride (0.21 mL, 2.2 mmol). After being stirred at room temperature for 3 h, the reaction mixture was evaporated under reduced pressure (t < 40 °C). Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) gave **11** (0.64 g, 71%) as a yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.75 (br. s, 1H, OH-C1), 2.19 (s, 3H, $OCOCH_3$), 3.70 (s, 3H, OCH_3), 3.92 (s, 3H, NCH_3), 5.37 (d, J = 5 Hz, 1H, H-1), 5.48 (d, J = 5 Hz, 1H, H-2), 5.90 (s, 1H, H-5), 7.40 (td, J = 8, 1.5 Hz, 1H, H-10), 7.52 (td, J = 8, 1.5 Hz, 1H, H-11), 7.61 (s, 1H, H-13), 7.80 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.03 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.81 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0 ($OCOCH_3$), 22.4 (CH_3), 25.4 (CH_3), 42.2 (NCH_3), 55.7 (OCH_3), 63.9 (C1), 72.0 (C_2), 77.2 (C_3), 93.5 (C5), 102.0 (C_{14b}), 111.2 (C6a), 111.6 (C13), 124.2 (C10), 124.6 (C7a), 126.7 (C12), 128.0 (C8), 128.2 (C11), 128.5 (C8a), 129.5 (C9), 135.7 (C12a), 144.8 (C13a), 149.7 (C14a), 159.3 (C4a), 162.3 (C6), 171.1 ($OCOCH_3$), 178.9 (C7); DCI-MS m/z 448 $[MH]^+$; IR (KBr) ν cm^{-1} 3370, 3052, 2978, 2928, 1743, 1646, 1615, 1588, 1495, 1394, 1231, 1207, 1153, 1087, 1029, 819, 745; UV λ nm (MeOH) (log ϵ) 236 (4.33), 287 (4.83), 342 (4.00), 440 (3.72). Anal. ($C_{26}H_{25}NO_6$) C, H, N.

(±)-2-Hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (16). To a solution of **5** (0.06 g, 0.15 mmol) in 1,2-dichloroethane (12 mL) were added solid zinc iodide (0.11 g, 0.33 mmol) and sodium cyanoborohydride (0.14 g, 2.22 mmol). The reaction mixture was stirred at room temperature for 48 h, filtered through Celite, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 95:5) gave **16** (0.018 g, 31%) as yellow needles: mp 260 °C (recrystallized from CH_2Cl_2/Me_2CO 7:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 2.96 (dd, J = 16, 6 Hz, 1H, H-1a), 3.17 (dd, J = 16, 6 Hz, 1H, H-1b), 3.81 (s, 3H, NCH_3), 3.88 (t, J = 6 Hz, 1H, H-2), 3.94 (s, 3H, OCH_3), 6.25 (s, 1H, H-5), 7.38 (ddd, J = 9, 8, 1 Hz, 1H, H-10), 7.46 (s, 1H, H-13), 7.48 (ddd, J = 9, 8, 1 Hz, 1H, H-11), 7.73 (dd, J = 8, 1 Hz, 1H, H-12), 7.99 (dd, J = 8, 1 Hz, 1H, H-9), 8.80 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.8 (CH_3), 25.1 (CH_3), 31.4 (C1), 44.7 (NCH_3), 55.9 (OCH_3), 69.3 (C_2), 76.2 (C3), 94.5 (C5), 98.9 (C14b), 110.0 (C6a), 112.0 (C13), 124.2 (C10), 125.6 (C7a), 126.7 (C12), 127.5 (C8), 128.0 (C11), 128.4 (C8a), 129.3 (C9), 135.5 (C12a), 143.0 (C13a), 151.1 (C14a), 159.0 (C4a), 162.9 (C6), 178.3 (C7); DCI-MS m/z 390 $[MH]^+$; IR (KBr) ν cm^{-1} 3429, 3050, 2967, 2919, 1656, 1639, 1608, 1587, 1497, 1456, 1435, 1394, 1201, 1139, 1084, 1025, 873, 804; UV λ nm (MeOH) (log ϵ) 235 (4.26), 280 (4.74), 344 (3.98), 433 (3.56). Anal. ($C_{24}H_{23}NO_4$) C, H, N.

(±)-2-Acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (15). Acetic anhydride (1 mL, 10.5 mmol) was added to an iced-cooled solution of **16** (0.018 g, 0.046 mmol) and 4-dimethylaminopyridine (0.005 g) in dry pyridine (1.5 mL). After being stirring at room temperature for 14 h, the reaction mixture was evaporated under reduced pressure (t < 40°C). Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) gave **15** (0.014 g, 71%) as yellow prisms: mp 228 °C (recrystallized from hexane/EtOAc 1:4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 2.04 (s, 3H, CH_3CO), 2.91 (dd, J = 16, 6 Hz, 1H, H-1a), 3.27 (dd, J = 16, 6 Hz, 1H, H-1b), 3.83 (s, 3H, NCH_3), 4.00 (s, 3H, OCH_3), 5.05 (t, J = 6 Hz, 1H, H-2), 6.30 (s, 1H, H-5), 7.40 (ddd, J = 9, 8, 1 Hz, 1H, H-10), 7.54 (ddd, J = 9, 8, 1 Hz, 1H, H-11), 7.64 (s, 1H, H-13), 7.85 (dd, J = 8, 1 Hz, 1H, H-12), 8.02 (dd, J = 8, 1 Hz, 1H, H-9), 8.85 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.8 (CH_3), 22.7 (CH_3CO), 25.0 (CH_3), 28.4 (C1), 44.9 (NCH_3), 56.2 (OCH_3), 70.6 (C_2), 75.6 (C3), 94.7 (C5), 98.4 (C14b), 110.5 (C6a), 112.3 (C13), 124.3 (C10), 125.1 (C7a), 126.6 (C12), 127.7 (C8), 128.1 (C11), 128.6 (C8a), 129.5 (C9), 135.6 (C12a), 143.3 (C13a), 150.9 (C14a), 158.6 (C4a), 161.2 (C6), 170.4 (CH3*C*O), 178.4 (C7); DCI-MS m/z 432 $[MH]^+$; IR (KBr) ν cm^{-1} 3054, 2953, 2914, 1735, 1646, 1620, 1588, 1495, 1459, 1395, 1230, 1198, 1079, 1026, 807, 739; UV λ nm (MeOH) (log ϵ) 234 (4.32), 280 (4.83), 341 (4.05), 429 (3.62). Anal. ($C_{26}H_{25}NO_5$) C, H, N.

(±)-2-Hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridine-1,7-dione (19). A suspension of powered $KMnO_4$ (1.28 g, 8.1 mmol) in H_2O (15 mL) was added dropwise within 30 min to a solution of **4** (0.5 g, 1.35 mmol) in Me_2CO (25 mL). The reaction mixture was stirred at room temperature for 8 h and extracted with 2-butanone (3 × 15 mL). The organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2/MeOH, 98/2) gave **19** (0.413 g, 76%) as yellow needles: mp 272 °C (recrystallized from Me_2CO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.39 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 3.62 (s, 3H, NCH_3), 3.94 (s, 3H, OCH_3), 4.14 (d, J = 5 Hz, 1H, H-2), 6.36 (s, 1H, H-5), 6.76 (d, J = 5 Hz, 1H, OH-C2), 7.47 (td, J = 8, 1.5 Hz, 1H, H-10), 7.62 (td, J = 8, 1.5 Hz, 1H, H-11), 8.05 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.12 (s, 1H, H-13), 8.14 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.75 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.9 (CH_3), 26.3 (CH_3), 46.2 (NCH_3), 57.7 (OCH_3), 75.6 (C3), 84.1 (C_2), 94.7 (C5), 104.0 (C14b), 109.3 (C6a), 115.0 (C13), 125.7 (C7a), 126.2 (C10), 127.8 (C12), 128.3 (C8), 129.4 (C8a + C11), 130.2 (C9), 136.4 (C12a), 141.1 (C13a), 150.0 (C14a), 166.7 (C4a), 167.7 (C6), 176.3 (C7), 190.1 (C1); DCI-MS m/z 404 $[MH]^+$; IR (KBr) ν cm^{-1} 3448, 2927, 1660, 1642, 1588, 1503, 1390, 1072, 808; UV λ nm (MeOH) (log ϵ) 240 (3.95), 266 (4.56), 299 (4.21), 328 (3.78), 359 (3.68), 417 (3.25), 429 (3.42). Anal. ($C_{24}H_{21}NO_5$) C, H, N.

(±)-2-Acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridine-1,7-dione (17). An ice-cooled mixture of acetic anhydride (0.4 mL, 4.2 mmol) and dry pyridine (0.4 mL) was added to **19** (50 mg, 0.12 mmol). After being stirred at room temperature for 24 h, the mixture was poured on cold water (5 mL). The precipitate was filtered, washed with water (2 × 5 mL), and dried in vacuo over P_2O_5 to afford **17** (44 mg, 83%) as yellow prisms: mp 259 °C (recrystallized from Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 2.28 (s, 3H, CH_3CO), 3.69 (s, 3H, NCH_3), 4.09 (s, 3H, OCH_3), 5.56 (s, 1H, H-2), 6.38 (s, 1H, H-5), 7.46 (td, J = 8, 1 Hz, 1H, H-10), 7.57 (td, J = 8, 1 Hz, 1H, H-11), 7.81 (s, 1H, H-13), 7.91 (dd, J = 8, 1 Hz, 1H, H-12), 8.04 (dd, J = 8, 1 Hz, 1H, H-9), 8.94 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.4 (CH_3), 20.7 (CH_3CO), 26.0 (CH_3), 45.4 (NCH_3), 56.8 (OCH_3), 77.0 (C3), 81.8 (C_2), 93.6 (C5), 103.3 (C14b), 109.5 (C6a), 113.7 (C13), 125.1 (C10), 125.2 (C7a), 127.1 (C12), 128.0 (C8), 128.4 (C11), 129.1 (C8a), 129.5 (C9), 135.7 (C12a), 140.5 (C13a), 149.1 (C14a), 165.8 (C4a), 167.8 (C6), 169.8 (CH3*C*O), 177.2 (C7), 183.3 (C1); DCI-MS m/z 446

[MH]$^+$; IR (KBr) ν cm^{-1} 2938, 1745, 1660, 1644, 1588, 1503, 1390, 1075, 913, 808; UV λ nm (MeOH) (log ϵ) 240 (4.11), 266 (4.62), 300 (4.32), 328 (3.69), 359 (3.74), 417 (3.35), 428 (3.50). Anal. ($C_{26}H_{23}NO_6$) C, H, N.

(±)-2-Propioxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridine-1,7-dione (18). An ice-cooled mixture of propionic anhydride (0.6 mL, 4.5 mmol) and dry pyridine (1 mL) was added to **19** (50 mg, 0.12 mmol). After being stirred at room temperature for 24 h, the mixture was poured onto cold water (5 mL). The precipitate was filtered, washed with water (2 × 5 mL), and dried in vacuo over P_2O_5 to afford **18** (46 mg, 84%) as yellow prisms: mp 218°C (recrystallized from Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J = 8 Hz, 3H, C*H*$_3$CH$_2$CO), 1.48 (s, 3H, CH$_3$), 1.63 (s, 3H, CH$_3$), 2.57 (q, J = 8 Hz, 2H, CH$_3$C*H*$_2$CO), 3.69 (s, 3H, NCH$_3$), 4.09 (s, 3H, OCH$_3$), 5.58 (s, 1H, H-2), 6.29 (s, 1H, H-5), 7.46 (td, J = 8, 1 Hz, 1H, H-10), 7.57 (td, J = 8, 1 Hz, 1H, H-11), 7.81 (s, 1H, H-13), 7.91 (dd, J = 8, 1 Hz, 1H, H-12), 8.04 (dd, J = 8, 1 Hz, 1H, H-9), 8.93 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.2 (*C*H$_3$CH$_2$CO), 20.4 (CH$_3$), 26.0 (CH$_3$), 27.3 (CH$_3$*C*H$_2$CO), 45.5 (NCH$_3$), 56.8 (OCH$_3$), 77.0 (C3), 82.0 (C$_2$), 93.6 (C5), 103.4 (C14b), 109.4 (C6a), 113.7 (C13), 125.1 (C10), 125.2 (C7a), 127.1 (C12), 128.0 (C8), 128.4 (C11), 129.1 (C8a), 129.5 (C9), 135.7 (C12a), 140.5 (C13a), 149.5 (C14a), 165.8 (C4a), 167.8 (C6), 173.3 (CH$_3$CH$_2$*C*O), 177.3 (C7), 183.5 (C1); DCI-MS m/z 460 [MH]$^+$; IR (KBr) ν cm^{-1} 2942, 1746, 1660, 1644, 1588, 1513, 1390, 1075, 917, 809; UV λ nm (MeOH) (log ϵ) 240 (4.29), 266 (4.75), 300 (4.56), 328 (3.62), 362 (3.61), 417 (3.30), 428 (3.46). Anal. ($C_{27}H_{25}NO_6$) C, H, N.

(±)-*cis*-1-Methoxy-2-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (20) and (±)-*trans*-1-Methoxy-2-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (21). To a solution of **5** (0.1 g, 0.24 mmol) in MeOH (2 mL) was added 1 N aqueous HCl (0.5 mL). The reaction mixture was stirred for 21 h at room temperature and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) successively afforded **21** (0.03 g, 29%) and **20** (0.028 g, 27%), as yellow amorphous solids. Compound **21**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (s, 3H, CH$_3$), 1.51 (s, 3H, CH$_3$), 2.70 (s, 3H, C$_1$-OCH$_3$), 3.10 (br. s, 1H, OH-C2), 3.73 (d, J = 8 Hz, 1H, H-2), 3.84 (s, 3H, C$_6$-OCH$_3$), 3.92 (s, 3H, NCH$_3$), 4.95 (d, J = 8 Hz, 1H, H-1), 6.19 (s, 1H, H-5), 7.36 (ddd, J = 9, 8, 1 Hz, 1H, H-10), 7.49 (ddd, J = 9, 8, 1 Hz, 1H, H-11), 7.66 (s, 1H, H-13), 7.83 (dd, J = 9, 1 Hz, 1H, H-12), 7.96 (dd J = 9, 1 Hz, IH, H-9), 8.10 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.3 (CH$_3$), 26.4 (CH$_3$), 42.1 (NCH$_3$), 50.1 (C$_1$-O*C*H$_3$), 55.8 (C$_6$-O*C*H$_3$), 69.8 (C$_1$), 76.1 (C$_2$), 78.3 (C$_3$), 93.8 (C$_5$), 99.4 (C$_{14b}$), 109.9 (C$_{6a}$), 111.6 (C$_{13}$), 124.3 (C$_{10}$), 125.2 (C$_{7a}$), 126.6 (C$_8$), 127.6 (C$_{12}$), 128.1 (C$_{11}$), 128.4 (C$_{8a}$), 129.3 (C$_9$), 135.8 (C$_{12a}$), 142.7 (C$_{13a}$), 149.0 (C$_{14a}$), 160.8 (C$_{4a}$), 162.6 (C$_6$), 178.7 (C$_7$); DCI-MS m/z 420 [MH]$^+$; IR (KBr) ν cm^{-1} 3438, 3055, 3048, 2969, 2925, 1637, 1610, 1580, 1559, 1491, 1393, 1369, 1206, 1118, 1084, 1029, 815, 737; UV λ nm (MeOH) (log ϵ) 236 (4.25), 288 (4.74), 342 (3.93), 438 (3.61). Anal. ($C_{25}H_{25}NO_5$) C, H, N. Compound **20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 3H, CH$_3$), 1.62 (s, 3H, CH$_3$), 2.53 (d, J = 2 Hz, 1H, OH-C2), 3.58 (s, 3H, C$_1$-OCH$_3$), 3.80 (s, 3H, NCH$_3$), 3.98 (s, 3H, C$_6$-OCH$_3$), 4.06 (dd, J = 5, 2 Hz, 1H, H-2), 4.85 (d, J = 5 Hz, 1H, H-1), 6.27 (s, 1H, H-5), 7.39 (ddd, J = 9, 8, 1 Hz, 1H, H-10), 7.53 (ddd, J = 9, 8, 1 Hz, 1H, H-11), 7.62 (s, 1H, H-13), 7.87 (dd, J = 9, 1 Hz, 1H, H-12), 8.01 (dd J = 9, 1 Hz, 1H, H-9), 8.87 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.9 (CH$_3$), 25.1 (CH$_3$), 41.8 (NCH$_3$), 55.4 (C$_1$-O*C*H$_3$), 56.2 (C$_6$-O*C*H$_3$), 66.8 (C$_2$), 74.4 (C$_1$), 76.2 (C$_3$), 93.8 (C$_5$), 98.5 (C$_{14b}$), 110.9 (C$_{6a}$), 111.3 (C$_{13}$), 124.3 (C$_{10}$), 125.7 (C$_{7a}$), 126.6 (C$_{12}$), 127.9 (C$_8$), 128.0 (C$_{11}$), 128.4 (C$_{8a}$), 129.6 (C$_9$), 135.7 (C$_{12a}$), 141.7 (C$_{13a}$), 150.4 (C$_{14a}$), 159.9 (C$_{4a}$), 162.7 (C$_6$), 178.1 (C$_7$); DCI-MS m/z 420 [MH]$^+$; IR (KBr) ν cm^{-1} 3427, 3052, 2924, 1637, 1613, 1589, 1492, 1458, 1269, 1201, 1137, 1080, 1032, 812, 745; UV λ nm (MeOH) (log ϵ) 236 (4.25), 288 (4.74), 343 (3.92), 440 (3.61). Anal. ($C_{25}H_{25}NO_5$) C, H, N.

(±)-*cis*-1-Methoxy-2-acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (22). Acetic anhydride (2 mL, 21 mmol) was added to a solution of **21** (0.022 g, 0.047 mmol) and 4-dimethylaminopyridine (0.005 g) in dry pyridine (2 mL). After being stirred at room temperature for 3 h, the reaction mixture was evaporated under reduced pressure (t < 40 °C). Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) gave **22** (0.018 g, 82%) as pale yellow prisms: mp 190 °C (recrystallized from CH_2Cl_2/cyclohexane 3:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 3H, CH$_3$), 1.54 (s, 3H, CH$_3$), 2.07 (s, 3H, CH$_3$CO), 3.46 (s, 3H, C$_1$-OCH$_3$), 3.81 (s, 3H, NCH$_3$), 4.00 (s, 3H, C$_6$-OCH$_3$), 4.94 (d, J = 5 Hz, 1H, H-1), 5.51 (d, J = 5 Hz, 1H, H-2), 6.26 (s, 1H, H-5), 7.39 (ddd, J = 9, 8, 1 Hz, 1H, H-10), 7.54 (ddd, J = 9, 8, 1 Hz, 1H, H-11), 7.62 (s, 1H, H-13), 7.86 (dd, J = 9, 1 Hz, 1H, H-12), 8.01 (dd J = 9, 1 Hz, IH, H-9), 8.89 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.9 (CH$_3$), 23.2 (*C*H$_3$CO), 25.2 (CH$_3$), 41.9 (NCH$_3$), 56.2 (C$_1$-O*C*H$_3$), 56.3 (C$_6$-O*C*H$_3$), 69.6 (C$_2$), 72.9 (C$_1$), 76.4 (C$_3$), 93.9 (C$_5$), 99.7 (C$_{14b}$), 110.9 (C$_{6a}$), 111.5 (C$_{13}$), 124.3 (C$_{10}$), 125.7 (C$_{7a}$), 126.6 (C$_{12}$), 127.9 (C$_8$), 128.0 (C$_{11}$), 128.5 (C$_{8a}$), 129.6 (C$_9$), 135.7 (C$_{12a}$), 141.8 (C$_{13a}$), 150.0 (C$_{14a}$), 159.7 (C$_{4a}$), 162.8 (C$_6$), 170.8 (CH$_3$*C*O), 178.4 (C$_7$); DCI-MS m/z 462 [MH]$^+$; IR (KBr) ν cm^{-1} 3055, 2979, 2925, 1730, 1645, 1584, 1490, 1460, 1392, 1365, 1226, 1206, 1152, 1081, 1027, 811, 747; UV λ nm (MeOH) (log ϵ) 236 (4.30), 288 (4.80), 340 (3.95), 440 (3.64). Anal. ($C_{27}H_{27}NO_6$) C, H, N.

(±)-*trans*-1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (23). Sodium borohydride (3.5 g, 92.6 mmol) was added at 0 °C to a suspension of **19** (2 g, 5.39 mmol) in MeOH (65 mL), and the reaction mixture was stirred at 0 °C for 24 h under argon. After addition of water (100 mL), the mixture was extracted with CH_2Cl_2 (100 mL) to eliminate the unreacted starting material. A precipitate obtained from the aqueous layer upon cooling was filtered, washed with water (2 × 5 mL), and dried in vacuo over P_2O_5 to afford **23** (1.06 g, 49%) as a yellow amorphous solid ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (s, 3H, CH$_3$), 1.39 (s, 3H, CH$_3$), 3.31 (m, 1H, H-2), 3.82 (s, 3H, OCH$_3$), 3.94 (s, 3H, NCH$_3$), 4.70 (t, J = 8 Hz, 1H, H-1), 4.96 (d, J = 8 Hz, 1H, OH-C1), 5.69 (d, J = 5.5 Hz, 1H, OH-C2), 6.21 (s, 1H, H-5), 7.42 (td, J = 8, 1.5 Hz, 1H, H-10), 7.57 (td, J = 8, 1.5 Hz, 1H, H-11), 7.88 (s, 1H, H-13), 8.01 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.08 (dd J = 8, 1.5 Hz, IH, H-9), 8.65 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.1 (CH$_3$), 27.3 (CH$_3$), 42.3 (NCH$_3$), 56.4 (OCH$_3$), 69.2 (C$_1$), 76.7 (C$_2$), 79.1 (C$_3$), 93.9 (C$_5$), 106.1 (C$_{14b}$), 110.2 (C$_{6a}$), 112.5 (C$_{13}$), 124.8 (C$_{10}$), 125.7 (C$_{7a}$), 126.8 (C$_8$), 127.4 (C$_{12}$), 128.2 (C$_{8a}$), 128.6 (C$_{11}$), 129.7 (C$_9$), 135.9 (C$_{12a}$), 142.1 (C$_{13a}$), 149.7 (C$_{14a}$), 159.3 (C$_{4a}$), 162.0 (C$_6$), 177.0 (C$_7$); ESI-MS m/z 428 [MNa]$^+$; IR (KBr) ν cm^{-1} 3479, 3398, 3056, 2970, 2932, 1638, 1611, 1580, 1553, 1495, 1390, 1095, 1025, 815. UV λ nm (MeOH) (log ϵ) 236 (4.35), 286 (4.85), 345 (4.02). Anal. ($C_{24}H_{23}NO_5$) C, H, N.

(±)-*trans*-1,2-Diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (24). Acetic anhydride (2.9 mL, 31 mmol) and 4-(dimethylamino)pyridine (1 mg) were added to an ice-cooled solution of **23** (500 mg, 1.23 mmol) in dry pyridine (17 mL). After being stirred at room temperature for 4 h, the mixture was poured onto cold water (20 mL). The precipitate was filtered, washed with CH_2Cl_2/Me_2CO (1/1, v/v, 2 × 10 mL), and dried in vacuo over P_2O_5 to afford **24** (388 mg, 64%) as an amorphous yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 3H, CH$_3$), 1.54 (s, 3H, CH$_3$), 1.87 (s, 3H, CH$_3$CO), 2.16 (s, 3H, CH$_3$CO), 3.80 (s, 3H, NCH$_3$), 4.01 (s, 3H, OCH$_3$), 5.26 (d, J = 6.5 Hz, 1H, H-2), 6.29 (s, 1H, H-5), 6.49 (d, J = 6.5 Hz, 1H, H-1), 7.43 (td, J = 8, 2 Hz, 1H, H-10), 7.55 (s, 1H, H-13), 7.55 (td, J = 8, 2 Hz, 1H, H-11), 7.86 (dd, J = 8, 2 Hz, 1H, H-12), 8.02 (dd, J = 8, 2 Hz, 1H, H-9), 8.86 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.5 (CH$_3$), 21.0 (*C*H$_3$CO), 21.3 (*C*H$_3$CO), 26.0 (CH$_3$), 44.1 (NCH$_3$), 56.5 (OCH$_3$), 70.0 (C1), 75.0 (C$_2$), 76.9 (C$_3$), 94.6 (C5), 100.2 (C$_{14b}$), 111.7 (C6a), 112.2 (C13), 124.8 (C10), 126.2 (C7a), 126.8 (C12), 128.1 (C8), 128.4 (C11), 128.9 (C8a), 129.8 (C9), 135.9 (C12a), 142.8 (C13a), 150.3 (C14a), 160.1 (C4a), 163.3 (C6), 170.2 (CH3*C*O), 171.2 (CH3*C*O), 178.5 (C7); ESI-MS m/z 528 [MK]$^+$, 512 [MNa]$^+$; IR (KBr) ν cm^{-1} 3009, 2978, 2955, 2935, 1746, 1648, 1617, 1588, 1495, 1462, 1386, 1372, 1241,

1219, 1198, 1137, 1127, 1084, 1056, 1032, 877, 810, 754; UV λ nm (MeOH) (log ϵ) 237 (4.57), 257 (sh.), 288 (4.97). Anal. ($C_{28}H_{27}NO_7$) C, H, N.

(±)-*trans*-1-Hydroxy-2-acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (25). To a solution of **23** (1.283 g, 3.17 mmol) in dry pyridine (68 mL) was added acetic anhydride (0.3 mL, 3.3 mmol). The reaction mixture was stirred at room temperature for 48 h and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2/MeOH, 99.5/0.5) gave **25** (0.921 g, 65%) as a yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 2.14 (s, 3H, CH_3CO), 3.83 (s, 3H, OCH_3), 3.86 (s, 3H, NCH_3), 4.86 (d, J = 8 Hz, 1H, H-2), 4.92 (t, J = 8 Hz, 1H, H-1), 5.31 (d, J = 8 Hz, 1H, OH-C1), 6.28 (s, 1H, H-5), 7.43 (td, J = 8, 2 Hz, 1H, H-10), 7.58 (td, J = 8, 2 Hz, 1H, H-11), 7.88 (s, 1H, H-13), 8.00 (dd, J = 8, 2 Hz, 1H, H-12), 8.09 (dd, J = 8, 2 Hz, 1H, H-9), 8.65 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.8 (CH_3), 21.9 (CH_3CO), 27.1 (CH_3), 43.0 (NCH_3), 57.0 (OCH_3), 67.1 (C1), 77.9 (C_2), 78.1 (C_3), 94.7 (C5), 105.6 (C_{14b}), 110.8 (C6a), 113.2 (C13), 125.5 (C10), 126.4 (C7a), 127.5 (C8), 128.1 (C12), 129.0 (C8a), 129.3 (C11), 130.3 (C9), 136.6 (C12a), 142.8 (C13a), 150.4 (C14a), 159.7 (C4a), 162.9 (C6), 171.1 (CH3*C*O), 178.0 (C7); ESI-MS m/z 486 $[MK]^+$, 470 $[MNa]^+$, 448 $[MH]^+$; IR (KBr) ν cm^{-1} 3332, 3052, 2978, 2943, 2846, 1743, 1650, 1619, 1588, 1495, 1394, 1359, 1223, 1196, 1141, 1087, 1048, 858, 807, 745; UV λ nm (MeOH) (log ϵ) 236 (4.33), 259 (4.26), 287 (4.83), 339 (4.00), 440 (3.70). Anal. ($C_{26}H_{25}NO_6$) C, H, N.

3-[(2,2-Dimethyl-2*H*-chromen-5-yl)amino]-2-naphthoic Acid (28). A mixture of **26** (1.6 g, 9.1 mmol), **27** (2.3 g, 9.1 mmol), potassium acetate (1.98 g), and cupric acetate monohydrate (0.06 g) in triethylamine (4 mL) was heated under reflux for 48 h. The reaction mixture was evaporated under reduced pressure, and the residue was partitionned between CH_2Cl_2 and 1 N aqueous HCl. The aqueous phase was extracted with CH_2Cl_2 (4 × 50 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 90:10) gave **28** (1.5 g, 48%) as yellow needles: mp 208 °C (recrystallized from CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 6H, $C(CH_3)_2$), 5.63 (d, J = 10 Hz, 1H, H-3′), 6.50 (d, J = 10 Hz, 1H, H-4′), 6.72 (dd, J = 8, 1.5 Hz, 1H, H-8′), 6.96 (dd, J = 8, 1.5 Hz, 1H, H-6′), 7.10 (s, 1H, H-4), 7.17 (t, J = 8 Hz, 1H, H-7′), 7.22 (td, J = 8, 1.5 Hz, 1H, H-6), 7.40 (td, J = 8, 1.5 Hz, 1H, H-7), 7.47 (dd, J = 8, 1.5 Hz, 1H, H-8), 7.77 (dd, J = 8, 1.5 Hz, 1H, H-5), 8.72 (s, 1H, H-1), 8.90 (br. s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.7 (2C, $C(CH_3)_2$), 75.6 ($C_{2'}$), 108.2 ($C_{4'a}$), 108.4 (C_4), 113.2 (C_2), 113.4 (C8′), 117.2 (C6′), 118.3 (C4′), 123.1 (C6), 125.7 (C4a), 125.9 (C8), 129.3 (C5 + C7 + C7′), 130.8 (C3′), 135.2 (C1), 136.7 (C5′), 137.8 (C8a), 144.9 (C3), 154.2 (C8′a), 173.3 (*C*OOH); DCI-MS m/z 346 $[MH]^+$; IR (KBr) ν cm^{-1} 3444, 3360, 3059, 2978, 2968, 2917, 1743, 1656, 1635, 1573, 1519, 1457, 1435, 1347, 1277, 1217, 1118, 1076, 761; UV λ nm (MeOH) (log ϵ) 227 (4.70), 268 (4.52), 320 (4.21), 337 (3.98), 410 (3.40). Anal. ($C_{22}H_{19}NO_3$) C, H, N.

3,3-Dimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one (29). Trifluoroacetic anhydride (2.4 mL) was added to a solution of **28** (1.2 g, 3.47 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred at room temperature for 23 h, evaporated under reduced pressure, and taken up by CH_2Cl_2 (75 mL) and saturated aqueous $NaHCO_3$ (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was shaken for 5 min with 1 N NaOH (mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 90:10) gave **29** (0.68 g, 60%) as an orange amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 6H, $C(CH_3)_2$), 5.78 (d, J = 10 Hz, 1H, H-2), 6.72 (d, J = 10 Hz, 1H, H-1), 6.74 (d, J = 9 Hz, 1H, H-5), 7.40 (td, J = 8, 1.5 Hz, 1H, H-10), 7.53 (td, J = 8, 1.5 Hz, 1H, H-11), 7.69 (s, 1H, H-13), 7.80 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.02 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.07 (br. s, 1H, NH), 8.32 (d, J = 9 Hz, 1H, H-6), 9.03 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.7 (2C, $C(CH_3)_2$), 76.8 (C_3), 105.7 (C_{14b}), 111.6 (C13), 111.8 (C5), 114.5 (C6a), 115.1 (C1), 121.3 (C7a), 124.1 (C2), 126.2 (C10), 127.9 (C12), 128.2 (C8), 128.5 (C8a), 128.9 (C11), 129.2 (C9), 129.6 (C6), 136.0 (C12a), 137.4 (C13a), 138.3 (C14a), 158.1 (C4a), 178.9 (C7); DCI-MS m/z 328 $[MH]^+$; IR (KBr) ν cm^{-1} 3316, 3049, 2968, 2916, 1629, 1587, 1555, 1502, 1458, 1436, 1293, 1280, 1237, 1199, 1046, 891, 757; UV λ nm (MeOH) (log ϵ) 232 (4.03), 274 (4.71), 300 (4.28), 356 (3.57), 443 (3.50). Anal. ($C_{22}H_{17}NO_2$) C, H, N.

3,3,14-Trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (30). Sodium hydride (0.3 g of 50% oil dispersion, 12 mmol) was added to an ice-cooled solution of **29** (0.65 g, 2 mmol) in dry *N*,*N*-dimethylformamide (25 mL). The mixture was stirred for 30 min at room temperature, and methyl iodide (0.75 mL, 12 mmol) was added. After 90 min, the reaction mixture was diluted with ice water and extracted with ethyl acetate. The organic layer was washed with 1 M NaOH aqueous solution, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2 then CH_2Cl_2/MeOH 99:1) afforded **30** (0.582 g, 86%) as an orange amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 6H, $C(CH_3)_2$), 3.92 (s, 3H, NCH_3), 5.61 (d, J = 10 Hz, 1H, H-2), 6.66 (d, J = 10 Hz, 1H, H-1), 6.75 (d, J = 9 Hz, 1H, H-5), 7.40 (td, J = 8, 1.5 Hz, 1H, H-10), 7.53 (td, J = 8, 1.5 Hz, 1H, H-11), 7.67 (s, 1H, H-13), 7.85 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.00 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.32 (d, J = 9 Hz, 1H, H-6), 8.97 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9 (2C, $C(CH_3)_2$), 43.7 (NCH_3), 76.1 (C_3), 99.9 (C_{14b}), 109.3 (C6a), 111.9 (C13), 112.4 (C5), 117.9 (C7a), 121.9 (C1), 123.4 (C8a), 123.6 (C2), 125.2 (C10), 127.9 (C12), 128.3 (C8), 128.4 (C11), 129.4 (C9), 129.6 (C6), 136.1 (C12a), 142.6 (C13a), 145.0 (C14a), 159.9 (C4a), 178.5 (C7); DCI-MS m/z 342 $[MH]^+$; IR (KBr) ν cm^{-1} 3056, 2972, 1637, 1626, 1610, 1579, 1488, 1438, 1417, 1299, 1245, 1218, 1199, 1114, 1087, 1016, 827, 787, 743; UV λ nm (MeOH) (log ϵ) 232 (4.35), 270 (4.79), 303 (4.73), 358 (3.91), 446 (3.79). Anal. ($C_{23}H_{19}NO_2$) C, H, N.

(±)-*cis*-1,2-Dihydroxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (31). Compound **30** (0.25 g, 0.73 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol) (0.7 mL) and *N*-methylmorpholine *N*-oxide dihydrate (0.163 g, 1.2 mmol) in *t*-BuOH/THF/H_2O (10/3/1, v/v/v, 50 mL). The reaction mixture was stirred at 40 °C for 4 days. After addition of saturated aqueous $NaHSO_3$, the mixture was stirred for 1 h and then extracted with CH_2Cl_2 (5 × 100 mL). The combined organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1 to 90:10) gave **31** (0.19 g, 69%) as yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.70 (t, 1H, J = 4.5 Hz, H-2), 4.01 (s, 3H, NCH_3), 4.78 (d, J = 9 Hz, 1H, OH-C1), 5.13 (d, J = 4.5 Hz, 1H, OH-C2), 5.23 (dd, J = 9, 4.5 Hz, 1H, H-1), 6.67 (d, J = 9 Hz, 1H, H-5), 7.44 (td, J = 8, 1.5 Hz, 1H, H-10), 7.60 (td, J = 8, 1.5 Hz, 1H, H-11), 8.09 (m, 4H, H-6, H-9, H-12, H-13), 8.83 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.4 (CH_3), 26.2 (CH_3), 42.4 (NCH_3), 65.4 (C_1), 70.9 (C_2), 78.6 (C_3), 111.5 (C_{14b}), 112.9 (C_{13}), 114.0 (C_5), 118.7 (C_{6a}), 123.9 (C_{7a}), 125.6 (C_{10}), 127.8 (C_{12}), 128.2 (C_8), 128.4 (C_{11}), 128.7 (C_{8a}), 129.5 (C_9), 130.3 (C_6), 136.9 (C_{12a}), 143.5 (C_{13a}), 148.9 (C_{14a}), 160.7 (C_{4a}), 178.4 (C_7); ESI-MS m/z 398 $[MNa]^+$; IR (KBr) ν cm^{-1} 3397, 3073, 3046, 2972, 2935, 1640, 1586, 1552, 1498, 1431, 1400, 1350, 1289, 1215, 1141, 1094, 1013, 864, 777, 743. UV λ nm (MeOH) (log ϵ) 232 (4.35), 258 (4.43), 293 (4.87), 348 (3.98), 454 (3.78). Anal. ($C_{23}H_{21}NO_4$) C, H, N.

(±)-*cis*-1,2-Diacetoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (32). Acetic anhydride (2 mL, 21 mmol) was added to an iced-cooled solution of **31** (0.086 g, 0.22 mmol) and 4-dimethylaminopyridine (0.005 g) in dry pyridine (2 mL). After being stirred at room temperature for 3 h, the reaction mixture was poured onto cold water (10 mL). The precipitate was filtered, washed with water (3 × 5 mL), and dried in vacuo over P_2O_5 to afford **32** (0.095 g, 91%) as an amorphous yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 2.02 (s,

3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 3.79 (s, 3H, NCH_3), 5.53 (d, J = 5 Hz, 1H, H-2), 6.68 (d, J = 5 Hz, 1H, H-1), 6.83 (d, J = 9 Hz, 1H, H-5), 7.46 (td, J = 8, 1 Hz, 1H, H-10), 7.60 (td, J = 8, 1 Hz, 1H, H-11), 7.67 (s, 1H, H-13), 7.89 (dd, J = 8, 1 Hz, 1H, H-12), 8.06 (dd, J = 8, 1 Hz, 1H, H-9), 8.45 (d, J = 9 Hz, 1H, H-6), 9.00 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7 (CH_3CO), 21.1 (CH_3CO), 23.5 (CH_3), 24.6 (CH_3), 42.4 (NCH_3), 66.0 (C1), 69.3 (C_2), 76.3 (C_3), 104.6 (C_{14b}), 112.4 (C13), 112.9 (C5), 119.3 (C6a), 123.7 (C7a), 124.9 (C10), 127.0 (C12), 128.3 (C8), 128.6 (C8a), 128.7 (C11), 129.7 (C9), 130.0 (C6), 136.3 (C12a), 143.3 (C13a), 147.8 (C14a), 160.3 (C4a), 170.6 (CH3*C*O), 171.0 (CH3*C*O), 178.7 (C7); DCI-MS m/z 460 $[MH]^+$; IR (KBr) ν cm^{-1} 3066, 2975, 2965, 2925, 1755, 1647, 1616, 1589, 1495, 1431, 1411, 1370, 1229, 1212, 1148, 1084, 1016, 912, 868, 736; UV λ nm (MeOH) (log ϵ) 232 (4.41), 258 (4.51), 291 (4.92), 343 (3.99), 436 (3.84). Anal. ($C_{27}H_{25}NO_6$) C, H, N.

(±)-*cis*-1,2-Di-*O*-carbonyl-1,2-dihydroxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (= (±)-*cis*-(3a*R,15c*R**)-4,4,15-trimethyl-15,15c-dihydro-4*H*-benzo[*b*][1,3]dioxolo[4,5]pyrano[3,2-*h*]acridine-2,8(3a*H*)-done) (33).** To a solution of **31** (0.065 g, 0.17 mmol) in 2-butanone (4 mL) was added *N*,*N*′-carbonyldiimidazole (0.14 g, 0.86 mmol). The reaction mixture was refluxed for 30 min under argon, and after cooling, 5% aqueous $NaHCO_3$ (10 mL) was added. The solution was extracted with EtOAc (4 × 40 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99:1) afforded **33** (0.067 g, 97%) as a yellow amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 4.06 (s, 3H, NCH_3), 4.86 (d, J = 8 Hz, 1H, H-2), 6.42 (d, J = 8 Hz, 1H, H-1), 6.83 (d, J = 9 Hz, 1H, H-5), 7.46 (td, J = 8, 1.5 Hz, 1H, H-10), 7.58 (td, J = 8, 1.5 Hz, 1H, H-11), 7.79 (s, 1H, H-13), 7.90 (dd, J = 8, 1.5 Hz, 1H, H-12), 8.04 (dd, J = 8, 1.5 Hz, 1H, H-9), 8.48 (d, J = 9 Hz, 1H, H-6), 8.95 (s, 1H, H-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.9 (CH_3), 24.2 (CH_3), 43.4 (NCH_3), 70.9 (C1), 74.2 (C_3), 79.0 (C_2), 104.5 (C_{14b}), 113.2 (C13), 113.5 (C5), 119.7 (C6a), 123.5 (C7a), 125.0 (C10), 126.9 (C12), 128.1 (C8), 128.7 (C8a), 128.8 (C11), 129.6 (C9), 131.5 (C6), 136.1 (C12a), 143.1 (C13a), 147.5 (C14a), 153.3 (O-*C*O-O), 159.5 (C4a), 178.8 (C7); ESI-MS m/z 424 $[MNa]^+$, 402 $[MH]^+$; IR (KBr) ν cm^{-1} 3052, 2969, 2930, 1798, 1647, 1624, 1586, 1499, 1441, 1412, 1371, 1294, 1210, 1181, 1104, 1021, 995, 873, 783, 745; UV λ nm (MeOH) (log ϵ) 231 (4.22), 258 (4.36), 288 (4.76), 340 (3.70), 434 (3.67). Anal. ($C_{24}H_{19}NO_5$) C, H, N.

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