Synthesis and Cytotoxic and Antitumor Activity of Benzo[*b*]pyrano[3,2-*h*]acridin-7-one Analogues of Acronycine

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Benzo[*b*]acronycine (6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one, **4**), an acronycine analogue with an additional aromatic ring linearly fused on the natural alkaloid basic skeleton, was synthesized in three steps, starting from 3-amino-2-naphthalenecarboxylic acid (**5**). Eight 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine esters and diesters (**17**−**24**) were obtained by catalytic osmic oxidation, followed by acylation. All these compounds were significantly more cytotoxic than acronycine, when tested against L1210 leukemia cells in vitro. The potency of the cyclic carbonate **24** was in the range of the most active drugs currently used in cancer chemotherapy. Two selected diesters (**17** and **24**) were evaluated in vivo against P388 leukemia and colon 38 adenocarcinoma implanted in mice. Both compounds were markedly active at doses 16-fold lower than the dose of acronycine itself. Against colon 38 adenocarcinoma, compounds **17** and **24** were highly efficient, inhibiting tumor growth by more than 80%. Diacetate **17** was the most active, inhibiting tumor growth by 96% at 6.25 mg/kg, with two of seven mice being tumor-free on day 43.

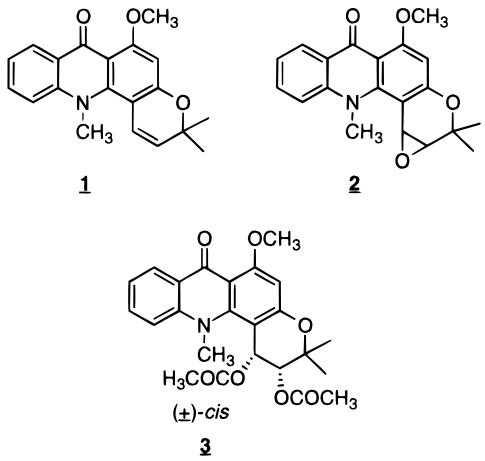
Introduction

The acridone alkaloid acronycine (**1**) (Chart 1), first isolated from *Acronychia baueri* Schott (*Rutaceae*) in 1948,[1,2] exhibits a broad spectrum of activity against numerous solid tumors including sarcoma, myeloma, carcinoma, and melanoma.[2,3] Nevertheless, clinical trials only gave poor results,[4] probably due to the moderate potency of this alkaloid. 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 improved stability but a similar reactivity toward nucleophilic agents as acronycine epoxide.[6a] Accordingly, we recently synthesized a series of *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroacronycine diesters which exhibited interesting antitumor properties with a broadened spectrum of activity and increased potency when compared with acronycine.[6] The cis isomers were the most promising, and (±)-*cis*-1,2-diacetoxy-1,2-dihydroacronycine (**3**) was of particular interest, because of its marked activity in vivo against P388 leukemia and against the resistant solid tumor C38 colon carcinoma.[6a]

Despite the broad antitumor spectrum of acronycine and its derivatives, the mechanism of their action at both cellular and molecular level has not yet been clearly established.[2] Early experiments suggested that this alkaloid acted primarily by alteration of membranous organelles and that its delayed effects were due at least in part to interference with the structure and function of cell-surface components.[7] Nevertheless, a more recent investigation of the DNA binding property of acronycine by Dorr and Liddil demonstrated that this alkaloid should interact with DNA, either by intercalation or by some other noncovalent process able to stabilize the double helix against thermal denaturation.[8]

Interaction with DNA is known to occur mainly for compounds with sufficiently large coplanar aromatic chromophores in the related acridine, ellipticine, and

Chart 1. Acronycine (**1**), Acronycine Epoxide (**2**), and (±)-*cis*-1,2-Diacetoxy-1,2-dihydroacronycine (**3**)



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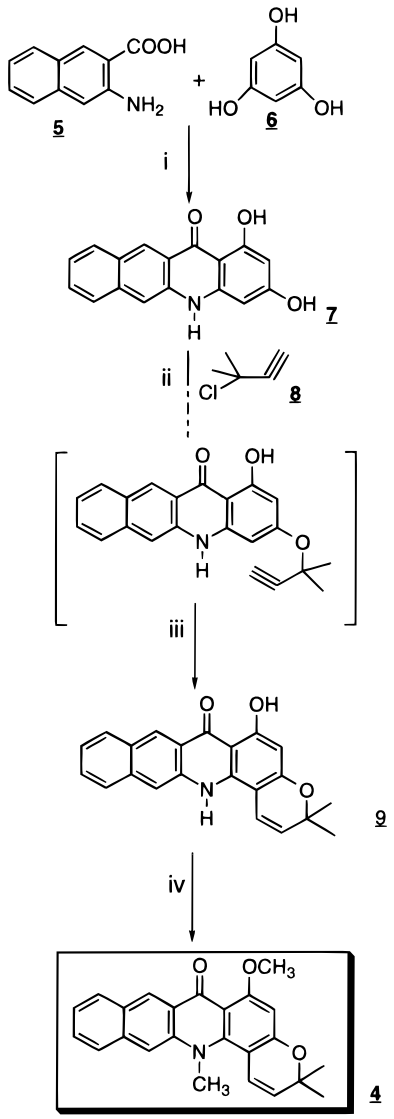
Scheme 1. Synthesis of 6-Methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one[a]



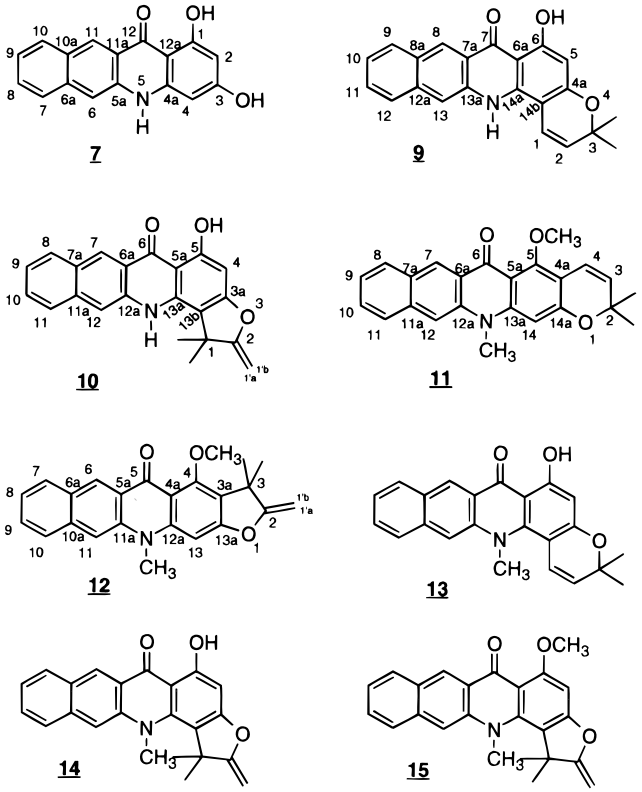
[a] Reagents and conditions: (i) refluxing heptan-1-ol, *p*-toluenesulfonic acid, 48 h; (ii) anhyd K_2CO_3 and KI in dry DMF under argon, 24 h at 65 °C; (iii) heating 3 h at 130 °C; (iv) NaH (2.5 equiv) and $(CH_3)_2SO_4$ (6 equiv) in dry DMF.

anthracene-dione series.[9] Therefore, the assumption of a step involving DNA intercalation in the mode of action of acronycine[8] prompted us to develop structural analogues with an additional aromatic ring linearly fused on the natural alkaloid basic skeleton. We describe here the synthesis and the biological properties of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one (**4**) and of related *cis*-1,2-dihydro-1,2-diol diesters.

Chemistry

The strategy used to build up the pentacyclic basic core was similar with that previously developed by Hlubucek et al. for the synthesis of acronycine.[10] Condensation of 3-amino-2-naphthalenecarboxylic acid (**5**) with phloroglucinol (**6**) carried out in 1-heptanol in the presence of 4-toluenesulfonic acid[11] afforded 1,3-dihydroxybenz[*b*]acridin-12(5*H*)-one (**7**) in 88% yield (Scheme 1). Construction of the dimethylpyran ring onto the

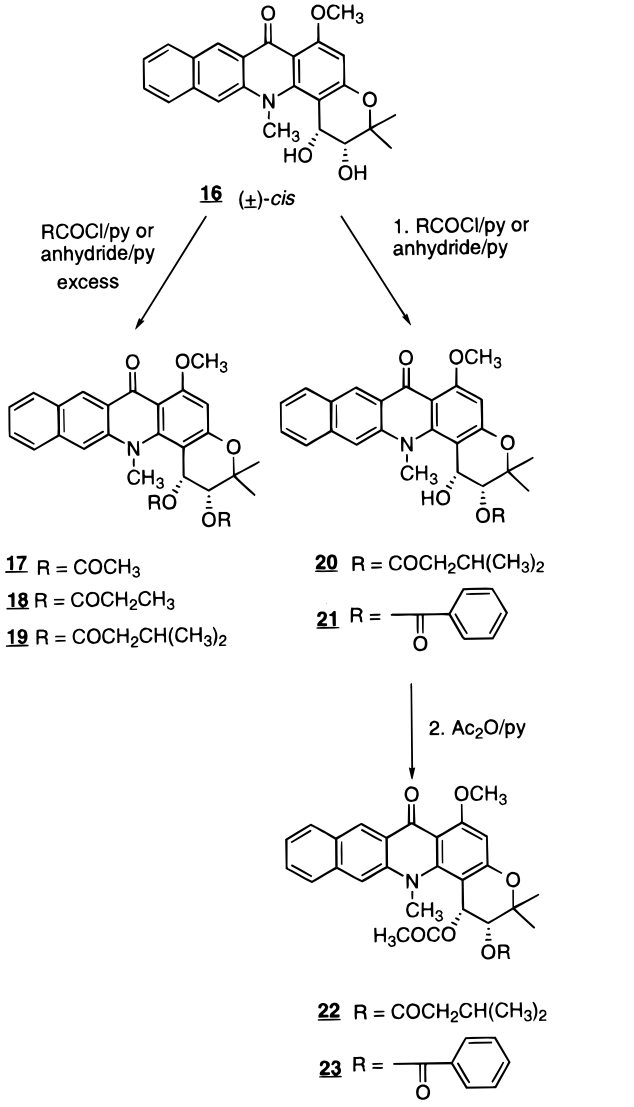
Chart 2. Compounds **7**, **8**, and **10**−**15**

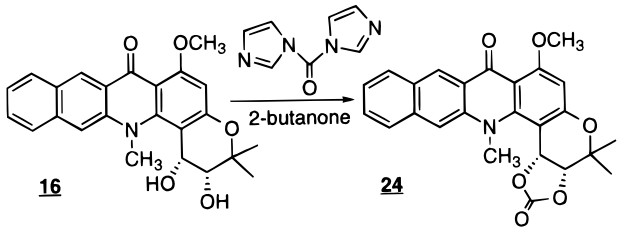


phenol at 3-position of **7** was performed by a Claisen rearrangement of the corresponding dimethylpropargyl ether. Thus, treatment of **7** with 3-chloro-3-methylbut-1-yne (**8**)[12] at 65 °C in dimethylformamide in the presence of potassium carbonate and potassium iodide, followed by heating at 130 °C, afforded the required 6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano-[3,2-*h*]acridin-7-one (**9**), isolated in 44% yield after purification by column chromatography. This compound was accompanied by 14% of 5-hydroxy-1,1-dimethyl-2-methylene-1,2-dihydrobenzo[*b*]furo[3,2-*h*]acridin-6(13*H*)-one (**10**), arising from cyclization in alkaline medium of a product of the C-alkylation of **7** by 3-chloro-3-methylbut-1-yne.[13] In addition, the corresponding pyrano and furano linear isomers were obtained in 2% overall yield as a mixture and could only be separated as their *O*,*N*-dimethyl derivatives **11** and **12**. Methylation of **9** with dimethyl sulfate afforded the *N*-methyl compound **13** when the reaction was carried out in the presence of 1 equiv of sodium hydride and afforded the desired 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**4**) when 2.5 equiv of sodium hydride was used. In a similar way, **10** was converted into the corresponding *N*-methyl derivative **14** and *O*,*N*-dimethyl derivative **15**. Phase-sensitive NOESY experiments performed on *O*,*N*-dimethyl derivatives permitted to ascribe unambiguously angular structures to compounds **4** and **15**, obtained from the major cyclization product **9** and **10**, and linear structures to minor derivatives **11** and **12**.[14]

The (±)-*cis*-diol **16** (Scheme 2) was conveniently obtained by catalytic osmium tetroxide oxidation of **4** using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent.[15] Treatment of diol **16** with excess acetic

Scheme 2. Synthesis 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





anhydride, propionic anhydride, or isovaleryl chloride afforded the corresponding diesters **17**, **18**, and **19**, respectively. Under controlled conditions, monoesters at the less hindered 2-position, exemplified by valerate **20** and benzoate **21**, were obtained. Treatment of monovalerate **20** and monobenzoate **21** with excess acetic anhydride led to the mixed esters **22** and **23**, respectively. Finally, treatment of diol **16** with *N*,*N*′-carbonyldiimidazole in 2-butanone under reflux afforded the cyclic carbonate **24**.

Table 1. Cytotoxicity and Antitumor Activity of the Compounds

compd	cytotoxicity IC_{50} L1210 (μM)[a]	P388 leukemia[b] optimal dose,[d] T/C (survival)	C38 adenocarcinoma[c] optimal dose,[d] T/C (tumor vol)
1 (acronycine)	19.9	200 mg/kg, ip, 125%	
3	3.4	12.5 mg/kg, ip, 224% 12.5 mg/kg, iv, 202%	
4	1.9		
7	6.0		
9	10.2		
10	3.2		
11	4.9		
12	8.5		
13	17.9		
14	9.0		
15	14.1		
16	40.8		
17	0.6	12.5 mg/kg, ip, 213% 12.5 mg/kg, iv, 178%	6.25 mg/kg, iv, 4%
18	0.9		
19	0.5		
20	1.9		
21	1.7		
22	0.5		
23	0.2		
24	0.02	12.5 mg/kg, ip, 327% 12.5 mg/kg, iv, 157%	3.12 mg/kg, iv, 18%

[a] Inhibition of L1210 cell proliferation measured by the MTA assay (mean of at least 2 values obtained in separate experiments). [b] Mice were inoculated ip on day 0 with 10^6 P388 cells, and the compounds were administered ip or iv on day 1. [c] Tumor fragments were implanted sc on day 0, and the compounds were administered iv on days 10 and 19. The tumor volume was measured on day 31. [d] Dose (mg/kg) giving the optimal therapeutic effect without toxicity.

Results and Discussion

These novel derivatives of acronycine were studied in vitro on L1210 leukemic cells. The results, expressed as IC_{50} values, are reported in Table 1. Compared to acronycine, compounds **17**−**19** and **22**−**24** were markedly more potent, the most cytotoxic derivative, compound **24**, being 1000-fold more potent than acronycine in inhibiting L1210 cell proliferation. All these cytotoxic compounds bear, in addition to the aromatic ring fused to the acronycine skeleton, a methoxy group at position 6 and two esters at positions 1 and 2. The importance of the esterification of positions 1 and 2 is illustrated by the lack of significant cytotoxicity of the diol derivative (compound **16**). The monoesterified compounds **20** and **21** show an intermediate cytotoxicity. The potency of compound **24** is noteworthy, being in the range of the most active cytotoxic drugs used in cancer chemotherapy.

The perturbation of the cell cycle induced by these compounds was studied on the same cell line. Acronycine induced a partial accumulation of cells in the G2+M phase of the cell cycle at a high concentration, as previously described[6] (Figure 1B). In contrast, the cytotoxic derivatives differently modified the DNA distribution, in that they induced a marked accumulation of cells in the S phase. Figure 1C shows the effect of 100 nM compound **24** which induced the accumulation of 74% of L1210 cells in the S phase (versus 36% for untreated cells). Moreover, a good relation was found in the series between cytotoxicity and potency in accumulating cells in the S phase of the cell cycle, which suggests that the cell death is the consequence of an irreversible arrest in S phase. Interestingly, the fact that these compounds induced a perturbation of the cell cycle different from that of acronycine suggests that they

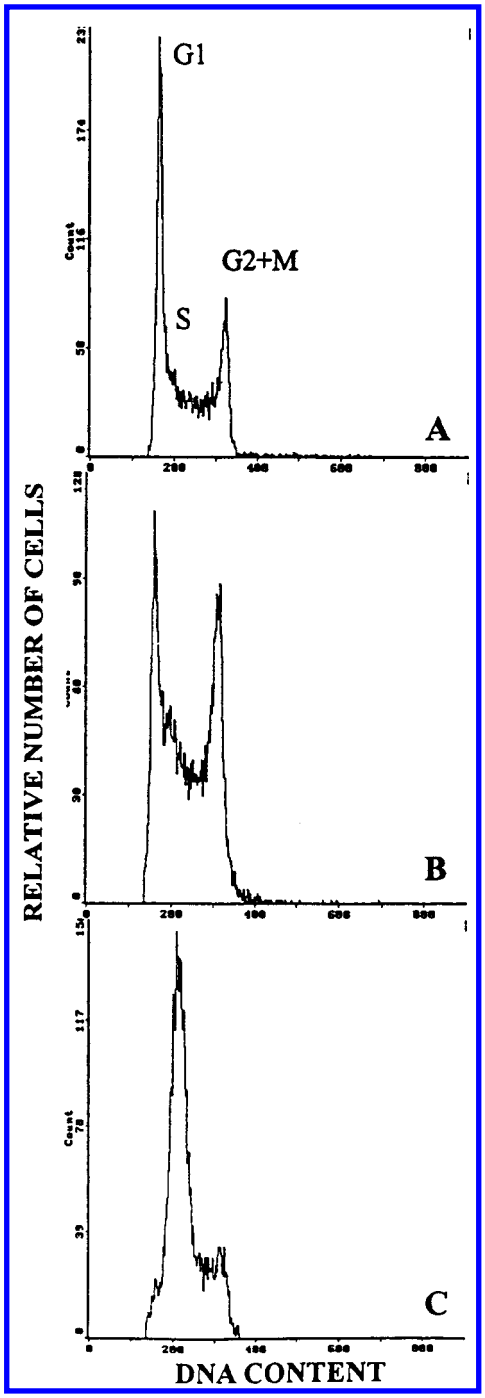


Figure 1. Typical DNA histogram and distribution into the different phases of the cell cycle of untreated L1210 cells (A) or L1210 cells treated for 21 h with 50 μM compound **1** (B) or 0.1 μM compound **24** (C).

act, at the molecular level, through a different mechanism of action.

In vivo, two standard experimental models were used, the sensitive ip P388 leukemia and the more resistant sc colon 38 adenocarcinoma. To make this latter model more resistant to chemotherapy, the compounds were administered when the tumor volume reached a weight of 60−100 mg, i.e., 10 days after tumor implant. Table 1 shows the results, in terms of percent T/C, obtained at the dose giving the best therapeutic effect without toxicity. Againt P388 leukemia, acronycine was only marginally active, while compounds **3**, **17**, and **24** were significantly active at doses 16-fold lower but not curative in that they did not induce long-term survivors. Interestingly, a significant antitumor activity was maintained following administration by the iv route, indicating a favorable distribution of these compounds. Against the colon 38 adenocarcinoma, compounds **17** and **24** were highly efficient, inhibiting tumor growth by more than 80%. The derivative **17** was the most active, since tumor growth was inhibited by 96% at 6.25 mg/kg and two of seven mice were tumor-free on day 43.

In conclusion, some of the derivatives described in this work are markedly more potent in vitro and in vivo and considerably more active in vivo than acronycine. The most potent derivative, compound **17**, is moderately active on P388 leukemia and induces tumor regression of the resistant C38 adenocarcinoma. The very favorable profile of this series, in terms of solid tumor selectivity, is currently under investigation in experimental models of solid tumors, including resistant human tumors, for the most active derivatives.

Experimental Section

Chemistry. Melting points were determined on a hot stage Reichert microscope and are uncorrected. Mass spectra were recorded with a Nermag R-10-10C spectrometer using electronic impact (EIMS) and/or chemical ionization (CIMS; reagent gas: NH_3) techniques. 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]) and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, using a Bruker AC-300 spectrometer. When necessary, the signals were unambiguously assigned by 2D NMR techniques: $^1H-^1H$ COSY, $^1H-^1H$ NOESY, $^{13}C-^1H$ HETCOR, and $^{13}C-^1H$ COLOC. These experiments performed using standard Bruker microprograms. Column chromatographies were carried out with silica gel 20−45 μm. Flash column chromatographies were conducted using silica gel 60 Merck (35−70 μm) with an overpressure of 300 mbars.[16] Microanalyses were in agreement with calculated values ±0.4%.

Biological Materials. Cell Culture and Cytotoxicity. L1210 cells were cultivated 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 (MTA) as described.[17] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for 48 h. 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 a XLMCL flow cytometer (Beckman Coulter, France).

Antitumor Activity. The antitumor activity of the compounds was evaluated on two experimental murine models: P388 leukemia and colon 38 adenocarcinoma. P388 cells (NCI, Frederick) were inoculated ip (10^6 cells/mouse) into B6D2F1 mice (Iffa credo) on day 0. The drugs were dissolved in DMSO, diluted in 5% Tween 80 in water, and injected ip or iv on day 1. The results are expressed in terms of percent T/C (median survival time of treated animals divided by median survival time of controls × 100). Colon adenocarcinoma 38 (NCI, Frederick, MD) was introduced by sc implantation of a tumor fragment into the dorsal flank. The drugs were administered by iv injection on days 10 and 19. The tumor volume was measured on day 31 and the results are expressed as percent

T/C (median tumor volume in treated animals divided by median tumor volume of controls × 100).

1,3-Dihydroxybenz[*b*]acridin-12(5*H*)-one (7). A solution containing 3-amino-2-naphthalenecarboxylic acid (**5**) (5 g, 26.7 mmol), dried phloroglucinol (**6**) (3.5 g, 27.7 mmol), and *p*-toluenesulfonic acid (63.5 g, 32.8 mmol) in heptanol (50 mL) was heated for 48 h, under reflux, using a Dean−Stark trap to remove water. The mixture was evaporated under reduced pressure and the dark brown residue purified by flash chromatography (solvent: cyclohexane then cyclohexane/acetone, 9:1 to 7:3) to give **7** (6.3 g, 88%) as orange needles: mp > 350 °C (cyclohexane/acetone, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) 5.97 (d, J = 2 Hz, 1H, H_2), 6.28 (d, J = 2 Hz, 1H, H_4), 7.41 (td, J = 8, 1 Hz, 1H, H_9), 7.56 (td, J = 8, 1 Hz, 1H, H_8), 7.82 (s, 1H, H_6), 7.96 (dd, J = 8, 1 Hz, 1H, H_7), 8.13 (dd, J = 8, 1 Hz, 1H, H_{10}), 8.85 (s, 1H, H_{11}), 10.68 (s, 1H, NH), 11.65 (s, 1H, OH_3), 14.08 (s, 1H, OH_1); ^{13}C NMR (75 MHz, DMSO-d_6) 92.4 (C_4), 96.4 (C_2), 103.4 (C_{12a}), 112.9 (C_6), 121.2 (C_{11a}), 125.8 (C_9), 127.9 (C_7 + C_{11}), 129.2 (C_{4a}), 130.2 (C_8), 131.0 (C_{10}), 137.3 (C_{10a}), 138.8 (C_{6a}), 145.9 (C_{5a}), 165.6 (C_3), 166.6 (C_1), 182.4 (C_{12}); CIMS m/z 278 $[MH]^+$; IR (KBr) 3350, 3080, 2980, 1647, 1591, 1546, 1510, 1474, 1428, 1346, 1272, 1176, 1101, 811; UV λ nm (MeOH) (log ϵ) 224 (4.17), 242 (sh), 279 (4.79), 351 (3.91).

Reaction of 7 with 3-Chloro-1-methylbut-1-yne (8). A solution of **7** (2 g, 7.22 mmol) in dry *N*,*N*-dimethylformamide (100 mL) was stirred and heated at 65 °C for 15 min, under nitrogen, in the presence of anhydrous potassium carbonate (2 g, 14.4 mmol). Then, dry potassium iodide (2.4 g, 14.4 mmol) and excess 3-chloro-3-methylbut-1-yne (5.9 g, 57 mmol) were added and the mixture was stirred for 24 h. Rearrangement of the propargylic ether occurred by heating the mixture at 130 °C for 1.5 h. The cooled reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with water and evaporated under reduced pressure. Purification by flash chromatography (solvent: cyclohexane then cyclohexane/acetone, 98:2 to 90:10) afforded **9** as an amorphous orange solid (1.1 g, 44%), **10** as a yellow solid (0.34 g, 14%), and an inseparable mixture of the corresponding linear pyrano and furano isomers (0.05 g, 2%).

6-Hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (9): ^{1}H NMR (300 MHz, DMSO-d_6) 1.45 (s, 6H), 5.74 (d, J = 10 Hz, 1H, H_2), 6.01 (s, 1H, H_5), 7.12 (d, J = 10 Hz, 1H, H_1), 7.43 (td, J = 9, 1.5 Hz, 1H, H_{10}), 7.60 (td, J = 9, 1.5 Hz, 1H, H_{11}), 7.96 (dd, J = 9, 1.5 Hz, 1H, H_{12}), 8.13 (dd, J = 9, 1.5 Hz, 1H, H_9), 8.16 (s, 1H, H_{13}), 8.86 (s, 1H, H_8), 11.05 (s, 1H, N−H), 14.51 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) 28.7 (2 × CH_3), 78.4 (C_3), 96.7 (C_5), 99.0 (C_{14b}), 103.8 (C_{6a}), 113.8 (C_{13}), 117.2 (C_1), 120.8 (C_{7a}), 125.7 (C_{10}), 126.9 (C_2), 127.5 (C_8), 127.9 (C_{12}), 129.2 (C_{14a}), 129.9 (C_{11}), 130.7 (C_9), 137.1 (C_{8a}), 138.5 (C_{12a}), 139.9 (C_{13a}), 161.2 (C_{4a}), 165.5 (C_6), 182.8 (C_7); EIMS m/z 343 $[M]^+$, 328; IR (NaCl) 3350, 3200−2500, 1636, 1582, 1552, 1474, 1345, 1168, 1136, 871, 822; UV λ nm (MeOH) (log ϵ) 250 (sh), 275 (4.92), 291 (4.81), 311 (sh), 367 (4.06).

5-Hydroxy-1,1-dimethyl-2-methylene-1,2-dihydrobenzo[*b*]furo[3,2-*h*]acridin-6(13*H*)-one (10): ^{1}H NMR (300 MHz, DMSO-d_6) 1.72 (s, 6H), 4.55 (d, J = 3 Hz, 1H, $H_{1'a}$), 4.70 (d, J = 3 Hz, 1H, $H_{1'b}$), 6.25 (s, 1H, H_4), 7.42 (td, J = 8, 1 Hz, 1H, H_9), 7.59 (td, J = 8, 1 Hz, 1H, H_{10}), 7.92 (dd, J = 8, 1 Hz, 1H, H_{11}), 8.11 (dd, J = 8, 1 Hz, 1H, H_8), 8.46 (s, 1H, H_{12}), 8.82 (s, 1H, H_7), 10.05 (s, 1H, NH), 14.82 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) 28.4 (2 × CH_3), 44.1 (C_1), 84.9 ($C_{1'}$), 91.2 (C_4), 104.7 (C_{5a}), 108.0 (C_{13b}), 114.3 (C_{12}), 120.7 (C_{6a}), 125.8 (C_9), 127.5 (C_7), 127.9 (C_{11}), 129.2 (C_{13a}), 130.0 (C_{10}), 130.7 (C_8), 137.0 (C_{7a}), 138.3 (C_{11a}), 139.6 (C_{12a}), 163.1 (C_{3a}), 166.4 (C_5), 173.3 (C_2), 183.1 (C_6); EIMS m/z 343 $[M]^+$; IR (KBr) 3054, 2966, 1646, 1595, 1509, 1162, 1074, 862; UV λ nm (MeOH) (log ϵ) 231 (4.22), 284 (4.68), 316 (sh), 354 (3.91).

6-Hydroxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (13). Sodium hydride (0.5 g of 50% oil dispersion, 1 mmol) was added to an ice-cooled solution of **9** (0.35 g, 1 mmol) in dry *N*,*N*-dimethylformamide (20 mL). The mixture was stirred under nitrogen for 15 min at 0 °C and dimethyl sulfate (0.28 mL, 3 mmol) was added. After 30 min, the reaction mixture was diluted with ice water and extracted with ethyl acetate. The organic layer, washed with 1 M NaOH solution and water was evaporated under reduced pressure. Purification by flash chromatography (solvent: cyclohexane then cyclohexane/acetone, 96:4 to 90:10) gave **13** as an orange amorphous solid (0.325 g, 89%): ^{1}H NMR (300 MHz, $CDCl_3$) 1.54 (s, 6H), 3.95 (s, 3H, NCH_3), 5.54 (d, J = 9 Hz, 1H, H_2), 6.22 (s, 1H, H_5), 6.59 (d, J = 9 Hz, 1H, H_1), 7.42 (td, J = 8, 1.5 Hz, 1H, H_{10}), 7.56 (td, J = 8, 1.5 Hz, 1H, H_{11}), 7.68 (s, 1H, H_{13}), 7.87 (dd, J = 8, 1.5 Hz, 1H, H_{12}), 7.98 (dd, J = 8, 1.5 Hz, 1H, H_9), 8.90 (s, 1H, H_8), 14.30 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) 26.9 (2 × CH_3), 44.0 (NCH_3), 76.6 (C_3), 97.3 (C_5), 101.0 (C_{14b}), 106.0 (C_{6a}), 112.2 (C_{13}), 121.6 (C_1), 121.7 (C_{7a}), 123.0 (C_2), 124.9 (C_{10}), 126.9 (C_{12}), 127.4 (C_8), 128.3 (C_{8a}), 128.8 (C_{11}), 129.4 (C_9), 136.3 (C_{12a}), 141.6 (C_{13a}), 145.1 (C_{14a}), 162.2 (C_{4a}), 165.6 (C_6), 182.0 (C_7); CIMS m/z 358 $[MH]^+$.

5-Hydroxy-1,1,13-trimethyl-2-methylene-1,2-dihydrobenzo[*b*]furo[3,2-*h*]acridin-6(13*H*)-one (14). Compound **14** was prepared from **10** under conditions similar with those described for the preparation of **13** from **10** (0.175 g, 0.5 mmol) using dry *N*,*N*-dimethylformamide (10 mL), sodium hydride (0.025 g of 50% oil dispersion, 0.5 mmol) and dimethyl sulfate (0.15 mL, 1.6 mmol. Purification by flash chromatography (solvent: cyclohexane then cyclohexane/acetone, 96:4 to 90:10) gave **14** as a solid product (0.150 g, 82%): ^{1}H NMR (300 MHz, $CDCl_3$) 1.75 (s, 6H), 4.01 (s, 1H, NCH_3), 4.40 (d, J = 3 Hz, 1H, $H_{1'a}$), 4.80 (d, J = 3 Hz, 1H, $H_{1'b}$), 6.32 (s, 1H, H_4), 7.40 (td, J = 8, 1 Hz, 1H, H_9), 7.56 (td, J = 8, 1 Hz, 1H, H_{10}), 7.57 (s, 1H, H_{12}), 7.80 (dd, J = 8, 1 Hz, 1H, H_{11}), 8.01 (dd, J = 8, 1 Hz, 1H, H_8), 8.89 (s, 1H, H_7), 15.42 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) 28.8 (2 × CH_3), 43.4 (NCH_3) 56.4 (C_1), 83.9 ($C_{1'}$), 97.5 (C_4), 107.6 (C_{5a}), 110.3 (C_{12} + C_{13b}), 124.3 (C_9), 124.4 (C_{6a}), 125.9 (C_7), 128.4 (C_{11}), 128.8 (C_{10}), 130.0 (C_8), 130.9 (C_{7a}), 135.8 (C_{11a}), 136.2 (C_{12a}), 140.6 (C_{13a}), 160.9 (C_{3a}), 164.1 (C_5), 173.7 (C_2), 179.5 (C_6); CIMS m/z 358 $[MH]^+$.

6-Methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (4). Compound **4** was obtained from **9** (0.445 g, 1.3 mmol) according to the procedure described for the preparation of **13**, using *N*,*N*-dimethylformamide (20 mL), sodium hydride (0.155 g of 50% oil dispersion, 3.2 mmol) and dimethyl sulfate (0.65 mL, 6.8 mmol). Silica gel column chromatography (solvent: cyclohexane then cyclohexane/acetone, 98:2 to 96:4) gave **4** (0.420 g, 94%) as small yellow needles: mp 267−268 °C ($CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 1.56 (s, 6H, 2 × CH_3), 3.90 (s, 3H, NCH_3), 4.00 (s, 3H, OCH_3), 5.54 (d, J = 9 Hz, 1H, H_2), 6.31 (s, 1H, H_5), 6.58 (d, J = 9 Hz, 1H, H_1), 7.40 (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}), 7.86 (dd, J = 8, 1.5 Hz, 1H, H_{12}), 8.10 (dd, J = 8, 1.5 Hz, 1H, H_9), 8.91 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 26.8 (2 × CH_3), 44.6 (NCH_3), 56.2 (OCH_3), 76.3 (C_3), 93.7 (C_5), 102.9 (C_{14b}), 109.4 (C_{6a}), 113.7 (C_{13}), 121.9 (C_1), 122.9 (C_2), 124.3 (C_{10}), 125.3 (C_{7a}), 126.6 (C_{12}), 127.9 (C_8), 128.0 (C_{11}), 128.4 (C_{8a}), 129.4 (C_9), 135.6 (C_{12a}), 141.7 (C_{13a}), 147.3 (C_{14a}), 159.7 (C_{4a}), 163.1 (C_6), 177.9 (C_7); CIMS m/z 372 $[MH]^+$; IR (NaCl) 3390, 2947, 1637, 1615, 1588, 1498, 1392, 1150, 1090, 808; UV λ nm (MeOH) (log ϵ) 240 (3.95), 275 (4.23), 296 (sh), 307 (4.26), 362 (3.50).

5-Methoxy-1,1,13-trimethyl-2-methylene-1,2-dihydrobenzo[*b*]furo[3,2-*h*]acridin-6(13*H*)-one (15). Compound **15** was obtained from **10** (0.100 g, 0.29 mmol) according to the procedure described for the preparation of **13**, using *N*,*N*-dimethylformamide (15 mL) sodium hydride (0.035 g of 50% oil dispersion, 0.73 mmol) and dimethyl sulfate (0.16 mL, 1.75 mmol). Silica gel column chromatography (solvent: cyclohexane then cyclohexane/acetone, 98:2 to 94:6) gave **15** (0.90 g, 83%) as a yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) 1.77 (s, 6H, 2 × CH_3), 3.97 (s, 3H, NCH_3), 4.00 (s, 3H, OCH_3), 4.33 (d, J = 3 Hz, 1H, $H_{1'a}$), 4.70 (d, J = 3 Hz, 1H, $H_{1'b}$), 6.43 (s, 1H, H_4), 7.41 (td, J = 8, 1 Hz, 1H, H_9), 7.55 (td, J = 8, 1 Hz, 1H, H_{10}), 7.65 (s, 1H, H_{12}), 7.85 (dd, J = 8, 1 Hz, 1H, H_{11}), 8.00 (dd, J = 8, 1 Hz, 1H, H_8), 8.82 (s, 1H, H_7); ^{13}C NMR (75 MHz, $CDCl_3$) 30.8 (2 × CH_3), 45.9 (NCH_3 + C_1), 56.4 (OCH_3), 83.0 ($C_{1'}$), 89.3 (C_4), 112.0 (C_{5a}), 112.3 (C_{12}), 113.1 (C_{13b}), 124.5 (C_9), 126.2 (C_{6a}), 126.6 (C_7), 127.6 (C_{11}), 128.2 (C_{7a} + C_{10}), 128.7

(C_{11a}), 129.5 (C_8), 135.6 (C_{12a}), 142.6 (C_{13a}), 162.7 (C_5 + C_{3a}), 173.4 (C_2), 179.5 (C_6); CIMS *m*/*z* 372 $[MH]^+$; UV λ nm (MeOH) (log ϵ) 238 (4.09), 252 (sh), 282 (4.38), 344 (3.77).

5-Methoxy-2,2,13-trimethyl-2,13-dihydro-6*H*-benzo-[*b*]pyrano[2,3-*i*]acridin-6-one (11) and 4-Methoxy-3,3,-12-trimethyl-2-methylene-2,3-dihydrobenzo[*b*]furo-[2,3-*i*]acridin-5(12*H*)-one (12). Compounds **11** and **12** were synthesized according to the procedure described for the preparation of **4**, from the mixture (0.65 g, 0.19 mmol) of the two nonisolated linear products obtained in the course of the synthesis of **9** using *N*,*N*-dimethylformamide (10 mL), sodium hydride (0.023 g of 50% oil dispersion, 0.48 mmol) and dimethyl sulfate (0.1 mL, 1.14 mmol). The crude product was purified by silica gel column chromatography (solvent: cyclohexane then cyclohexane/acetone, 99:1 to 98:2) to give **11** (0.039 g, 55%) and **12** (0.015 g, 22%) as solid products.

11: ^{1}H NMR (300 MHz, $CDCl_3$) 1.42 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 3.86 (s, 3H, NCH_3), 4.00 (s, 3H, OCH_3), 5.68 (d, J = 10 Hz, 1H, H_3), 6.65 (s, 1H, H_{14}), 6.81 (d, J = 10 Hz, 1H, H_4), 7.41 (td, J = 8, 1.5 Hz, 1H, H_9), 7.55 (td, J = 8, 1.5 Hz, 1H, H_{10}), 7.71 (s, 1H, H_{12}), 7.88 (dd, J = 8, 1.5 Hz, 1H, H_{11}), 8.02 (dd, J = 8, 1.5 Hz, 1H, H_8), 9.03 (s, 1H, H_7); ^{13}C NMR (75 MHz, $CDCl_3$) 28.3 (2 × CH_3), 34.5 (NCH_3), 62.3 (OCH_3), 77.5 (C_2), 97.1 (C_{14}), 109.1 (C_{5a}), 109.8 (C_{12}), 110.0 (C_{4a}), 116.2 (C_4), 123.7 (C_{6a}), 124.1 (C_9), 126.7 (C_{11}), 127.8 (C_{7a}), 128.0 (C_3), 128.2 (C_7), 128.7 (C_{10}), 129.1 (C_8), 135.5 (C_{11a}), 138.8 (C_{12a}), 146.4 (C_{13a}), 157.7 (C_{14a}), 160.6 (C_5), 177.0 (C_6); CIMS *m*/*z* 372 $[MH]^+$; IR (KBr) 3010, 2930, 1643, 1607, 1551, 1485, 1213, 1126, 1089, 776; UV λ nm (MeOH) (log ϵ) 245 (4.33), 303 (4.90).

12: ^{1}H NMR (300 MHz, $CDCl_3$) 1.63 (s, 6H, 2 × CH_3), 3.81 (s, 3H, NCH_3), 4.03 (s, 3H, OCH_3), 4.33 (d, J = 3 Hz, 1H, $H_{1'a}$), 4.73 (d, J = 3 Hz, 1H, $H_{1'b}$), 6.69 (s, 1H, H_{13}), 7.39 (t, J = 8 Hz, 1H, H_8), 7.52 (t, J = 8 Hz,1H, H_9), 7.65 (s, 1H, H_{11}), 7.82 (d, J = 8 Hz, 1H, H_{10}), 7.99 (d, J = 8 Hz, 1H, H_7), 9.01 (s, 1H, H_6); ^{13}C NMR (75 MHz, $CDCl_3$) 29.2 (2 × CH_3), 35.3 (NCH_3), 43.9 (C_3), 62.8 (OCH_3), 83.4 ($C_{1'}$), 91.0 (C_{13}), 110.0 (C_{11}), 111.1 (C_{4a}), 120.6 (C_{3a}), 124.0 (C_{5a}), 124.4 (C_8), 126.8 (C_6), 128.1 (C_{6a}), 128.3 (C_{10}), 128.5 (C_9) 129.3 (C_7), 135.8 (C_{10a}), 139.1 (C_{11a}), 147.8 (C_{12a}), 159.2 (C_{13a}), 161.7 (C_4), 172.1 (C_2), 177.3 (C_5); CIMS *m*/*z* 372 $[MH]^+$.

(+)-*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 (16). Compound **4** (2 g, 5.39 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol) (3.8 mL) and *N*-methylmorpholine *N*-oxide dihydrate (0.735 g, 5.4 mmol) in *t*-BuOH/THF/H_2O (10/3/1, v/v/v, 40 mL). The reaction mixture was stirred at room temperature for 2 days. After addition of saturated aqueous $NaHSO_3$, the mixture was stirred for 1 h and then extracted with CH_2Cl_2 (6 × 60 mL). The organic layers were evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane then cyclohexane/acetone, 95:5 to 85:15) afforded **16** (1.55 g, 71%) as yellow needles: mp 295 °C (recrystallized in CH_2Cl_2/acetone, 1:1); ^{1}H NMR (300 MHz, DMSO-d_6) 1.40 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.68 (t, J = 4.5 Hz, 1H, H-2), 3.81 (s, 3H, OCH_3), 3.90 (s, 3H, NCH_3), 4.62 (d, J = 9 Hz, 1H, OH−C_1), 5.07 (d, J = 4.5 Hz, 1H, OH−C_2), 5.09 (dd, J = 9, 4.5 Hz, 1H, H_1), 6.18 (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}), 7.90 (s, 1H, H_{13}), 8.02 (dd, J = 8, 1.5 Hz, 1H, H_{12}), 8.09 (dd J = 8, 1.5 Hz, IH, H_9) 8.65 (s, 1H, H_8); ^{13}C NMR (75 MHz, DMSO-d_6) 23.4 (CH_3), 26.2 (CH_3), 49.2 (NCH_3), 56.9 (OCH_3), 65.1 (C_1), 71.2 (C_2), 78.7 (C_3), 94.5 (C_5), 104.5 (C_{14b}), 110.8 (C_{6a}), 113.0 (C_{13}), 125.3 (C_{10}), 126.2 (C_{7a}), 127.3 (C_{12}), 128.0 (C_8), 128.7 (C_{8a}), 129.0 (C_{11}), 130.2 (C_9), 136.5 (C_{12a}), 142.7 (C_{13a}), 150.9 (C_{14a}), 160.8 (C_{4a}), 162.3 (C_6), 177.4 (C_7); CIMS *m*/*z* 406 $[MH]^+$; IR (NaCl) ν max cm^{-1} 3385, 2920, 1635, 1600, 1585, 1500, 1390, 1090, 810;UV λ nm (MeOH) (log ϵ) 236 (4.35), 286 (4.85), 345 (4.02).

(+)-*cis*-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 (17). An ice-cooled mixture of acetic anhydride (4 mL, 42 mmol) and dry pyridine (4 mL) was added to **16** (700 mg, 1.73 mmol). After stirring at room temperature for 1 week, the mixture was poured on cold water (20 mL). The precipitate was filtered, washed with water (2 × 10 mL), and dried in vacuo over P_2O_5 to afford **17** (816 mg, 96.5%) as an amorphous yellow-green solid: ^{1}H NMR (300 MHz, $CDCl_3$) 1.48 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.97 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 3.72 (s, 3H, NCH_3), 4.03 (s, 3H, OCH_3), 5.49 (d, J = 5 Hz, 1H, H_2), 6.30 (s, 1H, H_5), 6.58 (d, J = 5 Hz, 1H, H_1), 7.42 (td, J = 8, 2 Hz, 1H, H_{10}), 7.53 (s, 1H, H_{13}), 7.54 (td, J = 8, 2 Hz, 1H, H_{11}), 7.85 (dd, J = 8, 2 Hz, 1H, H_{12}), 8.02 (dd, J = 8, 2 Hz, 1H, H_9), 8.88 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 20.7 (CH_3CO), 21.1-(CH_3CO), 23.4 (CH_3), 24.5 (CH_3), 43.0 (NCH_3), 56.3 (OCH_3), 65.7 (C_1), 69.3 (C2), 76.6 (C3), 94.4 (C_5), 97.8 (C14b), 111.6 (C_{6a} + C_{13}), 124.5 (C_{10}), 125.8 (C_{7a}), 126.7 (C_{12}), 128.2 (C_8), 128.3 (C_{11}), 128.6 (C_{8a}), 129.6 (C_9), 135.8 (C_{12a}), 142.3 (C_{13a}), 150.3 (C_{14a}), 160.2 (C_{4a}), 162.9 (C_6), 170.5 (CH_3CO), 171.0 (CH_3CO), 178.0 (C_7); CIMS *m*/*z* 490 $[MH]^+$; IR (KBr) 3448, 2924, 2851, 1751, 1651, 1621, 1588, 1492, 1238, 1198, 1086, 1029, 913, 813, 742; UV λ nm (MeOH) (log ϵ) 237 (4.57), 257 (sh), 288 (4.97).

(+)-*cis*-6-Methoxy-3,3,14-trimethyl-1,2-dipropioxy-1,2,3,-14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (18). To a cooled solution (0 °C) of **16** (0.25 g, 0.62 mmol) in dry pyridine (5 mL) was added propionic anhydride (1.6 mL, 12.3 mmol). The mixture was stirred at room temperature for 2 days and evaporated in vacuo. Column chromatography on silica gel (solvent: cyclohexane then cyclohexane/acetone, 99:1 to 84:16) gave **18** (0.26 g, 81%) as bright yellow sheets: mp 202 °C (cyclohexane/acetone, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) 1.06 (m, 6H, 2 × CH_3), 1.47 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 2.18 (q, J = 8 Hz, 2H, CH_2), 2.30 (m, 2H, CH_2), 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), 6.60 (d, J = 5 Hz, 1H, H_1), 7.42 (td, J = 8, 1.5 Hz, 1H, H_{10}), 7.52 (s, 1H, H_{13}), 7.55 (td, J = 8, 1.5 Hz, 1H, H_{11}), 7.84 (dd, J = 8, 1.5 Hz, 1H, H_{12}), 8.02 (dd, J = 8, 1.5 Hz, 1H, H_9), 8.80 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 9.0 (2 × CH_3 CH_2), 23.5 (CH_3), 24.5 (CH_3), 27.3 (CH_2), 27.8 (CH_2), 44.1 (NCH_3), 56.3 (OCH_3), 65.6 (C_1), 69.2 (C2), 76.6 (C3), 94.4 (C_5), 98.0 (C14b), 111.2 (C_{6a}), 111.7 (C_{13}), 124.5 (C_{10}), 125.8 (C_{7a}), 126.7 (C_{12}), 128.0 (C_8), 128.2 (C_{11}), 128.6 (C_{8a}), 129.6 (C_9), 135.7 (C_{12a}), 142.3 (C_{13a}), 150.3 (C_{14a}), 160.3 (C_{4a}), 162.8 (C_6), 173.8 ($COCH_2$), 174.3 ($COCH_2$), 178.3 (C_7); CIMS *m*/*z* 518 $[MH]^+$; IR (NaCl) 3450, 3000, 2980, 2940, 1745, 1650, 1620, 1590, 1490, 1460, 1400, 1200, 1155, 1085; UV λ nm (MeOH) (log ϵ) 236 (4.42), 260 (sh), 288 (4.88), 335 (4.08).

(+)-*cis*-1,2-Diisovaleryloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (19). To an ice-cooled solution of **16** (0.175 g, 0.43 mmol) in dry pyridine (5 mL) was added isovaleryl chloride (0.5 mL, 4.16 mmol). The mixture was stirred for 15 min and evaporated in vacuo. Column chromatography on silica gel (solvent: cyclohexane then cyclohexane/acetone, 94:6 to 90:10) gave **19** (0.23 g, 93%) as a yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) 0.84−0.92 (m, 12H, 2 × $(CH_3)_2$), 1.44 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.94−2.09 (m, 4H, 2 × CH, CH_2), 2.15 (d, J = 7 Hz, 2H, CH_2), 3.70 (s, 3H, NCH_3), 3.98 (s, 3H, OCH_3), 5.49 (d, J = 5 Hz, 1H, H_2), 6.27 (d, J = 5 Hz, 1H, H_1), 6.61 (s, 1H, H_5), 7.37 (td, J = 8, 1.5 Hz, 1H, H_{10}), 7.51 (td, J = 8, 1.5 Hz, 1H, H_{11}), 7.52 (s, 1H, H_{13}), 7.79 (dd, J = 8, 1.5 Hz, 1H, H_{12}), 7.98 (dd, J = 8, 1.5 Hz, 1H, H_9), 8.85 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 22.2 (2 × $(CH_3)_2$), 23.8 (CH_3), 24.2 (CH_3), 24.9 (CH), 25.2 (CH), 42.8 (OCH_2), 43.0 (OCH_2), 43.2 (NCH_3), 56.2 (OCH_3), 65.3 (C_1), 69.3 (C_2), 76.4 (C_3), 94.4 (C_5), 98.2 (C_{14b}), 110.0 (C_{6a}), 111.8 (C_{13}), 124.4 (C_{10}), 125.6 (C_{7a}), 126.5 (C_{12}), 127.8 (C_8), 128.1 (C_{11}), 128.5 (C_{8a}), 129.4 (C_9), 135.6 (C_{12a}), 142.2 (C_{13a}), 150.2 (C_{14a}), 160.3 (C_{4a}), 162.7 (C_6), 172.2 (C_1OCO), 172.8 (C_2OCO), 178.8 (C_7); CIMS *m*/*z* 574 $[MH]^+$; IR (KBr) 3390, 3010, 2965, 1740, 1645, 1618, 1589, 1498, 1463, 1400, 1200, 1149, 1086; UV λ nm (MeOH) (log ϵ) 237 (4.47), 260 (sh), 288 (4.91), 338 (4.11).

(+)-*cis*-1-Hydroxy-2-isovaleryloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one (20). To an iced-cooled solution of **16** (0.120 g, 0.30 mmol) in dry pyridine (4 mL) was added isovaleryl chloride (0.16 mL, 1.33 mmol). The reaction mixture was stirred at 0 °C for 90 min and then evaporated under reduced pressure (T < 40 °C). Flash chromatography (solvent: cyclo-

hexane then cyclohexane/acetone, 94:6 to 90:10) gave **20** (0.126 g, 87%) as a yellow amorphous solid and a small amount of the disubstituted derivative **19** (0.020 g, 12%). **20:** ^{1}H NMR (300 MHz, $CDCl_3$) 0.86−0.92 (m, 6H, $(CH_3)_2$), 1.49 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 2.02−2.17 (m, 2H, CH + OH), 2.32 (d, J = 7 Hz, 2H, CH_2), 3.68 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 5.37 (m, 1H, H_1), 5.48 (d, J = 5 Hz, 1H, H_2), 5.86 (s, 1H, H_5), 7.39 (td, J = 8, 1.5 Hz, 1H, H_{10}), 7.50 (td, J = 8, 1.5 Hz, 1H, H_{11}), 7.59 (s, 1H, H_{13}), 7.79 (dd, J = 8, 1.5 Hz, 1H, H_{12}), 8.01 (dd, J = 8, 1.5 Hz, 1H, H_9), 8.76 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 22.2 (CH_3), 22.4 (2 × CH_3), 25.4 (CH_3+CH), 42.2 (OCH_2), 43.0 (NCH_3), 55.7 (OCH_3), 63.8 (C_1), 71.5 (C2), 76.6 (C3), 93.5 (C_5), 101.9 (C14b), 110.4 (C_{6a}), 111.6 (C_{13}), 124.2 (C_{10}), 125.0 (C_{7a}), 126.7 (C_{12}), 127.8 (C_8), 128.1 (C_{11}), 128.3 (C_{8a}), 129.4 (C_9), 135.6 (C_{12a}), 141.7 (C_{13a}), 149.5 (C_{14a}), 159.3 (C_{4a}), 162.2 (C_6), 173.1 (C_2OCO), 177.9 (C_7); CIMS m/z 490 $[MH]^+$; IR (KBr) 3450, 3274, 2930, 1734, 1640, 1613, 1590, 1498, 1395, 1152, 1090, 812, 734, 668; UV λ nm (MeOH) (log ϵ) 236 (4.39), 259 (sh), 287 (4.85), 343 (4.00).

(+)-*cis*-2-Benzoyloxy-1-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 **16** (0.200 g, 0.49 mmol) in dry pyridine (5 mL) was added benzoic anhydride (1.380 g, 5.72 mmol). The reaction mixture was stirred at room temperature for 5 days. After evaporation of the reaction mixture under reduced pressure, the residue was chromatographed on a silica gel column (solvent: cyclohexane then cyclohexane/acetone, 98:2 to 88:12) to give **21** (0.152 g, 61%) as a yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) 1.48 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 3.00 (br. s, 1H, C_1−OH), 3.88 (s, 3H, NCH_3), 3.98 (s, 3H, OCH_3), 5.46 (d, J = 5 Hz, 1H, H_1), 5.65 (d, J = 5 Hz, 1H, H_2), 6.26 (s, 1H, H_5), 7.30 (m, 3H, H_{10}, $H_{3'}$, $H_{5'}$), 7.43 (m, 2H, H_{11}, $H_{4'}$), 7.48 (s, 1H, H_{13}), 7.65 (d, J = 8 Hz, 1H, H_{12}), 7.90 (m, 3H, H_9, $H_{2'}$, $H_{6'}$), 8.72 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 22.6 (CH_3), 25.3 (CH_3), 41.1 (NCH_3), 56.0 (OCH_3), 64.1 (C_1), 72.6 (C2), 76.6 (C3), 93.5 (C_5), 101.7 (C14b), 110.7 (C_{6a}), 111.7 (C_{13}), 124.2 (C_{10}), 126.6 (C_{7a}), 127.6 (C_{12}), 127.9 (C_8), 128.3 ($C_{3'}$, $C_{5'}$, C_{11}), 129.0 ($C_{1'}$), 129.3 (C_9), 129.8 ($C_{2'}$, $C_{6'}$), 129.9 (C_{8a}), 133.3 ($C_{4'}$), 135.5 (C_{12a}), 141.7 (C_{13a}), 149.6 (C_{14a}), 159.4 (C_{4a}), 162.4 (C_6), 166.3 (C_2OCO), 178.1 (C_7); CIMS m/z 510 $[MH]^+$; IR (KBr) 3400−3100, 2926, 1720, 1643, 1590, 1493, 1397, 1276, 1118, 707, 668; UV λ nm (MeOH) (log ϵ) 232 (4.56), 258 (sh), 287 (4.87), 345 (4.07).

(+)-*cis*-1-Acetoxy-2-isovaleryloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (22). To an iced-cooled solution (0 °C) of **20** (0.086 g, 0.18 mmol) in dry pyridine (3 mL) was added acetic anhydride (3 mL, 31 mmol). The reaction mixture was stirred at room temperature during 3 days and evaporated under reduced pressure (T < 40 °C). Flash chromatography (solvent: cyclohexane/acetone, 94:6) gave **22** (0.070 g, 75%) as a yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) 0.86 (m, 6H, $(CH_3)_2$), 1.47 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.95 (s, 3H, CH_3CO), 2.01 (m, 1H, CH), 2.17 (d, J = 7 Hz, 2H, CH_2), 3.70 (s, 3H, NCH_3), 4.02 (s, 3H, OCH_3), 5.50 (d, J = 5 Hz, 1H, H_2), 6.30 (s, 1H, H_5), 6.56 (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}), 7.85 (dd, J = 8, 1.5 Hz, 1H, H_{12}), 8.02 (dd, J = 8, 1.5 Hz, 1H, H_9), 8.89 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 21.2 (CH_3), 22.2 $(CH_3)_2$, 23.4 (CH_3), 24.6 (CH_3), 25.3 (CH), 43.0 (NCH_3 + CH_2), 56.3 (OCH_3), 65.9 (C_1), 69.0 (C2), 76.4 (C3), 94.3 (C_5), 97.8 (C14b), 110.2 (C_{6a}), 111.6 (C_{13}), 124.5 (C_{10}), 125.8 (C_{7a}), 126.7 (C_{12}), 128.0 (C_8), 128.3 (C_{11}), 128.6 (C_{8a}), 129.6 (C_9), 135.8 (C_{12a}), 142.3 (C_{13a}), 150.3 (C_{14a}), 160.3 (C_{4a}), 162.9 (C_6), 171.0 (C_1OCO), 172.5 (C_2OCO), 178.3 (C_7); CIMS m/z 532 $[MH]^+$; IR (KBr) 3459, 3010, 2960, 1746, 1648, 1621, 1588, 1571, 1494, 1396, 1203, 1160, 1084, 912, 812; UV λ nm (MeOH) (log ϵ) 237 (4.46), 260 (sh), 288 (4.95), 338 (4.13).

(+)-*cis*-1-Acetoxy-2-benzoyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (23). To a solution of **21** (0.056 g, 1.10 mmol) in dry pyridine (2 mL) was added acetic anhydride (2 mL, 21 mmol). The reaction mixture was stirred at room temperature for 3 days and evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane then cyclohexane/acetone, 98:2 to 85:15) gave **23** (0.058 g, 96%) as an amorphous orange solid: ^{1}H NMR (300 MHz, $CDCl_3$) 1.52 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.87 (s, 3H, C_1−$OCOCH_3$), 3.72 (s, 3H, NCH_3), 4.06 (s, 3H, OCH_3), 5.76 (d, J = 5 Hz, 1H, H_2), 6.38 (s, 1H, H_5), 6.66 (d, J = 5 Hz, 1H, H_1), 7.37 (m, 3H, H_{10}, $H_{3'}$, $H_{5'}$), 7.51 (m, 3H, H_{11}, $H_{4'}$, H_{13}), 7.82 (d, J = 8 Hz, 1H, H_{12}), 7.85 (m, 2H, $H_{2'}$, $H_{6'}$), 7.99 (d, J = 8 Hz, 1H, H_9), 8.87 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 21.0 (CH_3CO), 23.0 (CH_3), 24.8 (CH_3), 42.8 (NCH_3), 56.3 (OCH_3), 66.0 (C_1), 69.6 (C_2), 76.5 (C_3), 94.1 (C_5), 97.6 (C_{14b}), 111.1 (C_{6a}), 111.4 (C_{13}), 124.4 (C_{10}), 125.6 (C_{7a}), 126.6 (C_{12}), 128.0 (C_8), 128.2 (C_{11}), 128.4 ($C_{3'}$, $C_{5'}$), 128.7 ($C_{1'}$), 129.5 (C_9), 129.6 ($C_{2'}$, $C_{6'}$), 130.8 (C_{8a}), 133.4 ($C_{4'}$), 135.7 (C_{12a}), 142.1 (C_{13a}), 150.2 (C_{14a}), 160.3 (C_{4a}), 162.9 (C_6), 165.8 (C_2OCO), 171.0 (C_1OCO), 178.3 (C_7); CIMS m/z 552 $[MH]^+$; IR (KBr) 3390, 2930, 1751, 1717, 1646, 1619, 1588, 1498, 1461, 1397, 1281, 1196, 1086, 770, 720; UV λ nm (MeOH) (log ϵ) 233 (4.39), 258 (sh), 288 (4.67), 340 (3.87).

(+)-*cis*-1,2-Di-*O*-carbonyl-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 (24). To a solution of **16** (1.05 g, 2.6 mmol) in 2-butanone (50 mL) was added *N*,*N*′-carbonyldiimidazole (2.10 g, 12.3 mmol). The reaction mixture was refluxed for 2 h under argon and after cooling, 5% aqueous $NaHCO_3$ (60 mL) was added. The solution was extracted with EtOAc (3 × 50 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. Flash chromatography (solvent: cyclohexane then cyclohexane/acetone, 98:2 to 96:4) afforded **24** (0.610 g, 55%) as a yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) 1.47 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 3.98 (s, 3H, NCH_3), 4.00 (s, 3H, OCH_3), 4.84 (d, J = 8 Hz, 1H, H_2), 6.31 (s, 1H, H_5), 6.33 (d, J = 8 Hz, 1H, H_2), 7.43 (td, J = 8, 2 Hz, 1H, H_{10}), 7.56 (td, J = 8, 2 Hz, 1H, H_{11}), 7.68 (s, 1H, H_{13}), 7.87 (dd, J = 8, 2 Hz, 1H, H_{12}), 8.01 (dd, J = 8, 2 Hz, 1H, H_9), 8.82 (s, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$) 21.9 (CH_3), 24.3 (CH_3), 44.3 (NCH_3), 56.4 (OCH_3), 71.0 (C_1), 74.1 (C3), 78.8 (C2), 95.3 (C_5), 97.5 (C14b), 111.6 (C_{6a}), 112.6 (C_{13}), 124.8 (C_{10}), 126.0 (C_{7a}), 126.8 (C_{12}), 127.6 (C_8), 128.4 (C_{11}), 128.9 (C_{8a}), 129.5 (C_9), 135.6 (C_{12a}), 142.3 (C_{13a}), 150.2 (C_{14a}), 153.4 (CO), 159.7 (C_{4a}), 163.7 (C_6), 178.6 (C_7); CIMS m/z 432 $[MH]^+$; IR (KBr) 3450, 2940, 1808, 1641, 1615, 1585, 1492, 1452, 1400, 1312, 1200, 1169, 1087, 980, 748; UV λ nm (MeOH) (log ϵ) 236 (4.57), 257 (sh), 288 (4.80), 335 (3.93).

References

(1) (a) Hughes, G. K.; Lahey, F. N.; Price J. R. Alkaloids of the Australian Rutaceae. *Nature* **1948**, *162*, 223−224. (b) Macdonald, P. L.; Robertson, A. V. The structure of acronycine. *Aust. J. Chem*. **1966**, *19*, 275−281. (c) Govindachari, T. R.; Pai, B. R.; Subramaniam, P. S. Alkaloids of *Glycosmis pentaphylla* (Retz.) Correa. *Tetrahedron* **1966**, *22*, 3245−3252. (d) Gougoutas, J. Z.; Kaski, B. A. The crystal and molecular structure of bromodihydroacronycine. *Acta Crystallogr*. **1970**, *B26*, 853−859.

(2) Tillequin, F.; Michel, S.; Skaltsounis A.-L. Acronycine-type alkaloids: Chemistry and biology. In *Alkaloids*: *Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Elsevier: Amsterdam, 1998; Vol. 12, pp 1−102.

(3) (a) Svoboda, G. H. Alkaloids of *Acronychia baueri*. Extraction of the alkaloids and studies of structure−activity relationships. *Lloydia* **1966**, *29*, 206−224. (b) Svoboda, G. H.; Poore, G. A.; Simpson, P. J.; Boder, G. B. Alkaloids of *Acronychia baueri*. Isolation of the alkaloids and study of the antitumor and other biological properties of acronycine. *J. Pharm. Sci*. **1966**, *55*, 758−768. (c) Dorr, R. T.; Liddil, J. D.; Von Hoff, D. D.; Soble, M.; Osborne, C. K. Antitumor activity and murine pharmacokinetics of parenteral acronycine. *Cancer Res*. **1989**, *49*, 340−344.

(4) Scarffe, J. H.; Beaumont, A. R.; Crowther, D. Phase I−II evaluation of acronycine in patients with multiple myeloma. *Cancer Treat. Rep*. **1983**, *67*, 93−94.

(5) Brum-Bousquet, M.; Mitaku, S.; Skaltsounis, A.-L.; Tillequin, F.; Koch, M. Acronycine epoxide: a new acridone alkaloid from several *Sarcomelicope* species. *Planta Med*. **1988**, *54*, 470−471.

(6) (a) Elomri, A.; Mitaku, S.; Michel, S.; Skaltsounis, A.-L.; Tillequin, F.; Koch, M.; Pierré, A.; Guilbaud, N.; Léonce, S.; Kraus-Berthier, L.; Rolland, Y.; Atassi, Gh. Synthesis and cytotoxic and antitumor activity of esters in the 1,2-dihydroxy-1,2-dihydroacronycine series. *J. Med. Chem*. **1996**, *39*, 4762−4766. (b) Magiatis, P.; Mitaku, S.; Skaltsounis, A.-L.; Tillequin, F.; Koch, M.; Pierré, A.; Atassi, Gh. Synthesis and biological activity of esters in the *trans*-1,2-dihydroxy-1,2-dihydroacronycine series.

J. Nat. Prod. **1998**, *61*, 198−201. (c) Costes, N.; Michel, S.; Tillequin, F.; Koch, M.; Pierré, A.; Atassi, Gh. Chiral dihydroxylation of acronycine: absolute configuration of natural *cis*-1,2-dihydroxy-1,2-dihydroacronycine and cytotoxicity of (1*R*,2*R*) and (1*S*,2*S*)-1,2-diacetoxy-1,2-dihydroacronycine. *J. Nat. Prod.* **1999**, *62*, 490−492.

(7) (a) Tan, P.; Auersperg, N. Effects of the antineoplastic alkaloid acronycine on the ultrastructure and growth pattern of cultured cells. *Cancer Res.* **1973**, *33*, 2320−2329. (b) Kessel, D. Effects of acronycine on cell-surface properties of murine leukemia cells. *Biochem. Pharmacol.* **1977**, *26*, 1077−1081. (c) Low, R. S.; Auersperg, N. Effects of acronycine and of cytochalasin B on the division of Rat leukemia cells. *Exp. Cell. Res.* **1981**, *131*, 15−24.

(8) Dorr, R. T.; Liddil, J. D. Development of a parenteral formulation of the antitumour agent acronycine. *J. Drug Dev.* **1988**, *1*, 31−39.

(9) (a) Cheng, C. C. Structural aspects of antineoplastic agents − A new approach. In *Progress in medicinal chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1988; Vol. 25, pp 35−83. (b) Gimenez-Arnau, E.; Missailidis, S.; Stevens, M. F. G. Antitumour polycyclic acridines. Part 4. Physicochemical studies on the interactions between DNA and novel tetracyclic acridine derivatives. *Anti-cancer Drug Des.* **1988**, *13*, 431−451. (c) Neidle, S.; Pearl, L. H.; Skelly, J. V. DNA structure and perturbation by drug binding. *Biochem. J.* **1987**, *243*, 1−13. (d) Gasiewicz, T. A.; Kende, A. S.; Rucci, G.; Whitney, B.; Willey, J. J. Analysis of structural requirements for Ah receptor antagonist activity: ellipticines, flavones, and related compounds. *Biochem. Pharmacol.* **1996**, *52*, 1787−1803. (e) Pullman, B. Molecular mechanisms of specificity in DNA-antitumour drug interactions. In *Advances in Drug Research*; Testa, B., Ed.; Academic Press: London, 1989; Vol. 18, pp 1−113.

(10) Hlubucek, J.; Ritchie, E.; Taylor, W. C. A synthesis of acronycine. *Aust. J. Chem.* **1970**, *23*, 1881−1889.

(11) Smolders, R. R.; Hanuise, J.; Voglet, N. Synthesis of some hydroxylated 9-acridanones. *Bull. Soc. Chim. Belg.* **1984**, *93*, 239−240.

(12) Hennion, G. F.; Boisselle, A. P. Preparation of *t*-acetylenic chlorides. *J. Org. Chem.* **1961**, *26*, 725−727.

(13) (a) Kirtany, J. K. Revised structure of a side-product obtained during synthesis of 11-hydroxynoracronycine. *Ind. J. Chem.* **1981**, *20B*, 614. (b) Evans, C. M.; Kirby, A. J. Intramolecular nucleophilic addition of phenolate to unactivated double and triple bonds. Relative reactivity, regiospecificity, stereochemistry and mechanism. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1269−1275.

(14) Mikros, E.; Mitaku, S.; Skaltsounis, A.-L.; Libot, F.; Tillequin, F.; Koch, M. Stereochemical effects in some acronycine derivatives. *Magn. Reson. Chem.* **1999**, *37*, 498−506.

(15) Rheenen, V. V.; Kelly, R. C.; Cha, D. Y. An improved catalytic OsO_4 oxidation of olefins to *cis*-1,2-glycols using tertiary amine oxides as the oxidant. *Tetrahedron Lett.* **1976**, *23*, 1973−1976.

(16) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.

(17) (a) Leonce, S.; Pierré, A.; Anstett, M.; Perez, V.; Genton, A.; Bizzari, J. P.; Atassi, Gh. Effects of a new triazinoaminopiperidine derivative on adriamycin accumulation and retention in cells displaying P-glycoprotein-mediated multidrug resistance. *Bio-Chem. Pharmacol.* **1992**, *44*, 1707. (b) Pierré, A.; Dunn, T. A.; Kraus-Berthier, L.; Léonce, S.; Saint-Dizier, D.; Regnier, G.; Dhainaut, A.; Berlion, M.; Bizarri, J. P.; Atassi, Gh. In vitro and in vivo circumvention of multidrug resistance by Servier 9788, a novel triazinoaminopiperidine derivative. *Invest. New Drugs* **1992**, *10*, 137.

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