Synthesis of A-Seco Derivatives of Betulinic Acid with Cytotoxic Activity

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In this study, the relationships between the chemical structure and cytotoxic activity of betulinic acid (**1**) derivatives were investigated. Eight lupane derivatives (**1**−**8**), one of them new (**6**), five diosphenols (**9**−**13**), four of them new (**10**−**13**), two new norderivatives (**14** and **15**), five seco derivatives (**16**−**20**), four of them new (**16**, **17**, **19**, and **20**), and three new seco-anhydrides (**21**−**23**) were synthesized from **1**, and their activities were compared with the activities of known compounds. The effects of substitution on the A-ring and esterification of the carboxyl group in position 28 on cytotoxicity were of special interest. Significant cytotoxic activity against the T-lymphoblastic leukemia cell line CEM was found in diosphenols **9** and **13** (TCS_{50} 4 and 5 μmol/L) and seco-anhydrides **22** and **23** (TCS_{50} 7 and 6 μmol/L). All compounds were also tested on cancer cell lines HT 29, K562, K562 Tax, and PC-3, and these confirmed activity of diosphenols **9**, **10**, and **11** and anhydride **22**. Diosphenols, as the most promising group of derivatives, were further tested on four more lines (A 549, DU 145, MCF 7, SK-Mel2).

Triterpenes are widespread in living organisms, and a number of biologically active derivatives were found in this group of compounds. This fact has created an interest in new derivatives with structures similar to known active natural compounds prepared either by organic synthesis[1] or by microbial transformations.[2,3] Betulinic acid (**1**) is a pentacyclic triterpene, which occurs in the bark of *Platanus acerifolius* and many other plant sources.[4] Betulinic acid (**1**) is a compound with anti-HIV[5,6] activity, cytotoxicity,[7] and antitumor[8] properties. The cytotoxic activity on human melanoma (MEL-2)[9] and lung carcinoma[10] (A549) cell lines was the first to be reported, and subsequent research showed that amides of **1** had even higher activity than the free acid, not only on human melanoma (MEL-2) and lung carcinoma (A549) but also on neuroblastoma,[11] medulloblastoma,[11] glioblastoma,[11] ovarian (OVCAR-3),[12] colon (HCT-15),[12] and central nervous system carcinoma (XF498)[12] cell lines. High in vivo activities on MEL-2,[13] the above-mentioned neuroectodermal tumors,[13,14] and Evings sarcoma[14] have also been observed. In more recent studies,[15−17] the cytotoxic activity of certain betulinic acid derivatives has been investigated. Their activity was usually associated with a free carboxylic or a carbonylic group in position C-28.[18] In contrast, all alkyl-esters were found to be inactive.[15,16] Moreover, hydrogenation of the 20(29) double bond caused significant increase in the cytotoxicity on HeLa and OVCAR-3 cell lines.[16] Analogous oxidation of the 3β-hydroxy group of **1** afforded derivatives with higher activity, and substitution with an amino group gave derivatives with properties similar to **1**. In contrast, acylation of the 3β-hydroxy group caused a decrease in cytotoxicity.

Despite the plenthora of recent research in this area, the influence of derivatization of the A-ring in betulinic acid (**1**) on cytotoxicity has not been studied in detail (with the exception of C-3 derivatives). In this paper, we describe both the synthesis and structure−activity relationships in a group of 14 new (**6**, **10**−**17**, **19**−**23**) and 9 known (**1**−**5**, **7**−**9**, **18**) compounds, derivatives of betulinic acid (**1**), which were modified in the A-ring.[18]

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Results and Discussion

3β-Hydroxylup-20(29)-en-28-oic acid (**1**) was extracted from the bark of plane trees, *Platanus acerifolius* (collected in the Czech Republic).

The seco derivatives **14**−**23** were prepared using three oxidative steps. Oxidation of acid **1** and ester **2** with CrO_3 in DMF[18] afforded ketones **7** and **8**. Further oxidation was performed by introducing air into the *tert*-butyl alcohol solution of ketones **7** and **8** in the presence of potassium 2-methyl-2-propoxide.[18] Two types of products resulted from the reaction; the major products were diosphenols **9** and **10**, and the minor ones were lactols **14** and **15**. Compounds **9** and **10** predominated by optimizing time for the oxidation (30−40 min). Prolonging the oxidation time to 48 h, under the same reaction conditions, gave lactols **14** and **15** as the major products. Using pure oxygen instead of air afforded lactols **14** and **15** in similar yields in 2 h. Subsequent treatment of diosphenol **9** with dimethyl sulfate in the presence of KOH afforded methyl ether **13**, and treatment of **9** with acetic anhydride in pyridine gave acetate **11** in high yields. Diosphenols **9** and **10** were then cleaved with a solution of hydrogen peroxide and KOH[18] in refluxing MeOH. The reactions afforded cleaved seco derivatives **16** and **17**. Reduction of triacid **16** with LAH in refluxing THF afforded triol **19**, and its treatment with acetic anhydride gave triacetate **20**. Treatment of A-seco derivatives **16** and **17** with acetic anhydride gave seven-membered cyclic anhydrides **21**−**23**. These anhydrides are not readily cleaved with water. Anhydride **23** alone lost an acetyl group from position 28. During the acetylation of acids with a free C-28-carbonyl group, **1** and **16**, formation of anhydrides **5** and **23** as byproducts were observed. Although these anhydrides were somewhat unstable, HPLC and crystallization afforded pure compounds **5** and **23**. A small amount of anhydride **6** was obtained after the acetylation of 150 g of betulinic acid (**1**), and this was stable owing to steric hindrance in the neighborhood of the anhydride functional group.Our cytotoxicity study was consistent with the literature.[15−17] Both acetylation of the 3β-OH and esterification of the carboxyl on C-17 caused a significant decrease in cytotoxicity (e.g., compounds **2**, **3**, **4**, and **8**). Betulinic acid (**1**) was active against CEM T-lymphoblastic leukemia and SK-Mel2 cell lines, but less

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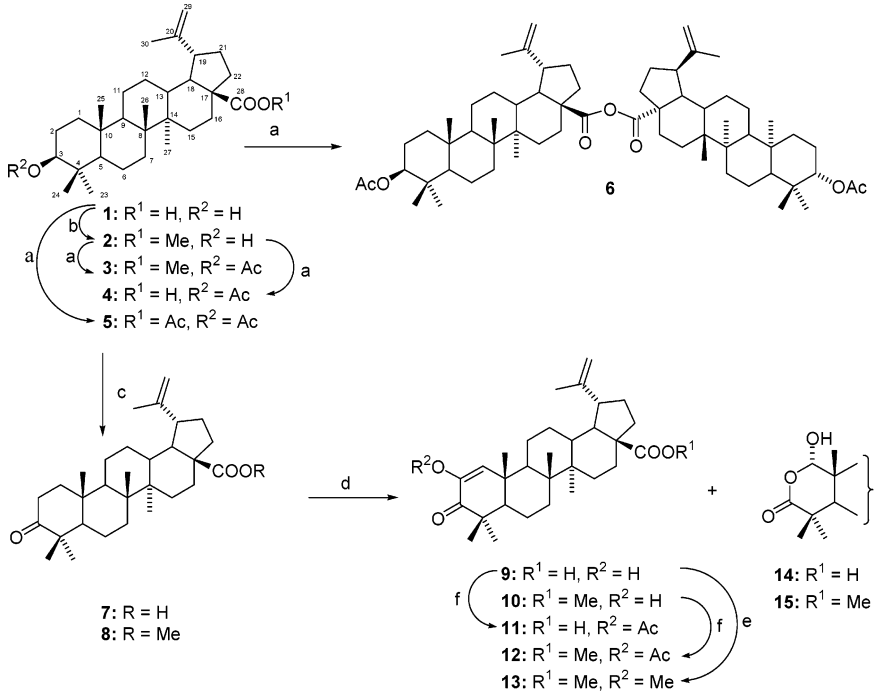
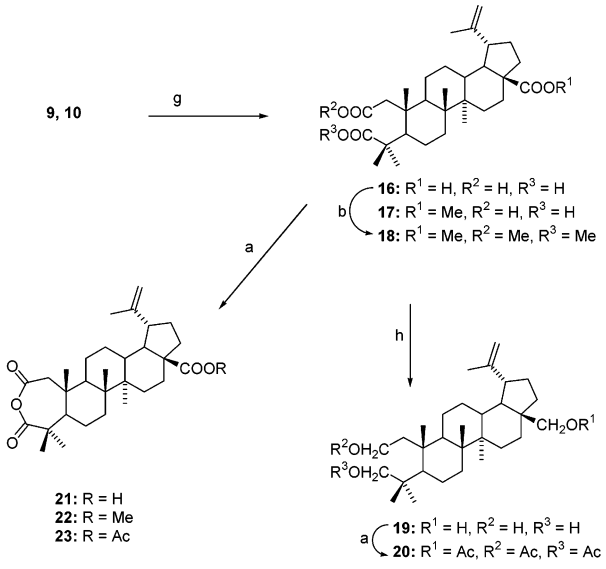
Scheme 1. Preparation of Diosphenois



(a) Ac_2O, pyridine, reflux; (b) Diazomethane, ether; (c) CrO_3, DMF, room temperature; (d) O_2, t-BuOK, t-BuOH, 40^0C; (e) Me_2SO_4, KOH, dioxan, H_2O, reflux, (f) Ac_2O, pyridine, DMAP, reflux.

Scheme 2. Oxidative Cleavage of Diosphenols and Further Derivatization



(a) Ac_2O, pyridine, reflux; (b) Diazomethane, ether; (g) H_2O_2, KOH, MeOH; (h) $LiAlH_4$, THF.

active in other lines we used. The α-diketones (diosphenols) **9**−**13** were more active than betulinic acid (**1**), even if they were transformed to enol ether **13**. The diosphenols showed activity in a number of cancer cell lines. In contrast, the activities of triacid **16** and triol **19** were surprisingly low. This may have been due to very low solubility of these compounds in water and organic solvents. Esterification and acetylation of these compounds did not lead to any active compounds either. In contrast, easily cleavable anhydrides **22** and **23** had the highest activities on CEM among all derivatives synthesized (TCS_{50} 7 μmol/L). Derivatization of **1** in the A-ring afforded derivatives with higher cytotoxicity on CEM cell lines than **1** and betulonic acid (**7**) used as standards. This led us to investigate all

Table 1. Cytotoxicity of A-Seco Derivatives of Betulinic Acid against HT 29, K562, K562 Tax, and PC-3 Cell Lines

	IC50 (μmol/L[a])				
compound	CEM	HT 29	K562	K562 Tax	PC-3
1	27	89	56	112	91
2	150	61	71	65	109
3	16	250	250	250	250
4	200	24	18	19	17
5	22	110	88	108	93
6	240	173	104	242	187
7	17	17	6	17	15
8	250	250	239	250	250
9	4	6	6	11	10
10	23	6	8	8	10
11	20	7	5	16	11
12	91	46	60	25	98
13	5	19	11	10	20
14	96	58	81	81	79
15	250	65	119	85	116
16	250	219	145	250	169
17	113	88	77	75	63
18	250	99	104	76	245
19	20	11	9	11	49
20	250				
21	101				
22	7	12	29	12	89
23	6				

[a] The lowest concentration that kills 50% of tumor cells.

compounds on four other cancer cell lines (Table 1) and the most promising on four more lines (Table 2). In conclusion, the cytotoxicity of new derivatives is not limited to the CEM line, since similar results were obtained on the following cell lines: HT 29, K562, K562 Tax, PC-3, A 549, DU 145, MCF 7, SK Mel2 (Tables 1, 2). Several compounds were not tested on the broader range of cell lines, mostly because of their poor availability in amounts large enough for these tests.

Experimental Section

General Experimental Procedures. Melting points were determined using a Kofler block and are uncorrected. Optical

Table 2. Cytotoxic Activity of Betulinic Acid (**1**), Betulonic Acid (**7**), and Selected Compounds (**9**, **10**, and **11**) on A 549, DU 145, MCF 7, and SK-Mel2 Cell Lines

	IC50 (μmol/L (n =4))			
compound	A 549	DU 145	MCF 7	SK-Mel2
betulinic acid (**1**)	146	196	143	21
betulonic acid (**7**)	15	36	29	26
9	10	23	14	23
10	11	9	6	11
11	11	18	17	24

rotations were measured using $CHCl_3$ solutions (unless otherwise stated) on an Autopol III (Rudolph Research, Flanders, NJ) polarimeter. NMR spectra were recorded on a Varian UNITY INOVA 400 instrument (^{1}H NMR spectra at 399.95 MHz) using $CDCl_3$ solutions (unless otherwise stated), with $SiMe_4$ as an internal standard. EIMS were recorded on an INCOS 50 (Finigan MAT) spectrometer at 70 eV and an ion source temperature of 150 °C. The samples were introduced from a direct exposure probe at a heating rate of 10 mA/s. Relative abundances stated are related to the most abundant ion in the region of m/z > 50. TLC was carried out using silica gel 60 F_{254}, and detection was by spraying with 10% aqueous H_2SO_4 and heating to 150−200 °C. Column chromatography was performed using silica gel 60 (Merck 7734). The HPLC system consisted of a Gilson high-pressure pump (model 361), a Rheodyne injection valve, preparative column (25 × 250 mm) with filling silica gel (Biospher 7 μm), differential-refractometrical detector (Laboratorni pristroje, Praha, CZ) connected with a PC (software Chromulan), and a Gilson automatic fraction collector (model 246). A mixture of ethyl acetate and hexane was used as the mobile phase, its composition specified in each experiment. TLC was carried out on Kieselgel 60 F_{254} plates (Merck) using toluene/diethyl ether, 5:1, unless otherwise stated.

Workup refers to pouring the reaction mixture into H_2O, extraction of the product with Et_2O, and washing the organic layer successively with H_2O, dilute aqueous HCl, H_2O, saturated aqueous $NaHCO_3$, and again H_2O, followed by drying over $MgSO_4$, filtration, and evaporation of the filtrate under reduced pressure. Analytical samples were dried over P_2O_5 under diminished pressure.

Potassium *tert*-butoxide, *tert*-butyl alcohol, dimethyl sulfate, dimethylformamide (DMF), tetrahydrofurane, lithium aluminum hydride, and chromium(VI) oxide were purchased from Sigma-Aldrich Company.

3β-Hydroxylup-20(29)-en-28-oic acid (1). The bark of plane trees *Platanus acerifolius* (5 kg) was extracted with MeOH (15 L, twice). Collected extracts were evaporated, and the residual crude betulinic acid (**1**) (150 g) was then purified by several crystallizations from MeOH (70 mL/g) to give white needles (50 g, 1% yield): R_f 0.16; mp 304−305 °C; $[\alpha]^{25}_D$ +8° (c 0.37); IR ($CHCl_3$) ν_{max} 1720vb, 1643 cm^{-1}.[19]

Methyl 3β-Hydroxylup-20(29)-en-28-oate (2). Diazomethane (50 mmol) in diethyl ether was added to a solution of **1** (5 g, 10.9 mmol) in $CHCl_3$ (400 mL). Organic solvents were evaporated under vacuum, and the crude product was chromatographed over silica gel (50 g) eluted with toluene and then crystallized from MeOH to afford **2** (4.5 g, 87% yield): R_f 0.25; mp 224−228 °C; $[\alpha]^{25}_D$ +3° (c 0.36); IR ($CHCl_3$) ν_{max} 1720vb, 1643 cm^{-1}.[19]

Methyl 3β-Acetoxylup-20(29)-en-28-oate (3). Acetic anhydride (6 mL, 60 mmol) was added to a suspension of methyl ester **2** (15 g, 31.9 mmol) in pyridine (3 mL). The mixture was worked up after 6 h. The crude product was chromatographed over silica gel (150 g) eluted with toluene and then crystallized from MeOH to afford **3** (14 g, 86% yield): R_f 0.65; mp 203−206 °C; $[\alpha]^{25}_D$ +17° (c 0.35); IR ($CHCl_3$) ν_{max} 1811, 1720vb, 1643 cm^{-1}.[20]

Acetylation of 1. Acetic anhydride (60 mL, 0.6 mol) was added to a suspension of 150 g of crude betulinic acid (**1**) in pyridine (30 mL). The mixture was vigorously stirred at 100 °C for 3 h. The solution was then cooled to 5 °C for 3 days. White crystals of **5** were removed from the solution by filtration under reduced pressure and washed with ethyl acetate and EtOH, and the filtrate was collected and yielded other compounds as described below. Crystallization from butanone afforded white crystals of the anhydride of 3β-acetoxylup-20-(29)-en-28-oic acid and acetic acid (**5**) (40 g, 23% yield): R_f 0.59; mp 177−178 °C; $[\alpha]^{25}_D$ +11° (c 0.42); IR ($CHCl_3$) ν_{max} 1811, 1720vb, 1643 cm^{-1}; ^{1}H NMR ($CHCl_3$) δ 0.83, 0.84, 0.85, 0.96, 0.97, 1.69 (18H, all s, 6 × CH_3), 1.90−2.02 (2H, m), 2.04 (3H, s, 3-OAc), 2.23 (3H, s, 28-COOAc), 2.19−2.31 (1H, m), 2.97 (1H, td, J(H-19β, H-18α) = 11.2 Hz, J(H-19β, H-21α) = 11.2 Hz, J(H-19β, H-21β) = 4.8 Hz, H-19 β), 4.47 (1H, m, $\sum J$ = 16.3 Hz, H-3α) 4.62 (1H, m, H-29 *pro*-E), 4.74 (1H, bd, J = 2.1 Hz, H-29 *pro*-Z); EIMS m/z 540 [M]$^+$ (0.2), 499 (1), 453 (15), 438 (5), 395 (5), 249 (4), 232 (5), 215 (5), 203 (10), 189 (38), 43 (100); *anal* C 75.73%, H 9.58%, calcd for $C_{34}H_{52}O_5$, C 75.51%, H 9.69%.

Decomposition of anhydride **5** using chromatography over silica gel eluted with toluene was observed, and this yielded acetate **4**.

The above-mentioned filtrate was worked up in the usual manner. The obtained 150 g of crude brown powder was then chromatographed over silica gel (1 kg); two compounds (**4** and **6**) were obtained: **3β-acetoxylup-20(29)-en-28-oic acid (4)** was the major chromatography product. Crystallization from MeOH afforded white crystals of **4** (50 g, 31% yield): R_f 0.51; mp 277−278 °C; $[\alpha]^{25}_D$ +22° (c 0.49).[20] The minor product was **3β-acetoxylup-20(29)-en-28-oic anhydride (6)** (3 g, 2%): R_f 0.59; mp 256−257 °C; $[\alpha]^{25}_D$ +6° (c 0.70); IR ($CHCl_3$) ν_{max} 1798, 1723, 1643 cm^{-1}; ^{1}H NMR ($CHCl_3$) δ 0.83, 0.84, 0.85, 0.97, (30H, all s, 10 × CH_3), 1.69 (6H, s, H-30), 1.93−2.03 (4H, m), 2.04 (6H, s, 2 × Ac), 2.15−2.30 (4H, m), 3.01 (2H, td, J(H-19β, H-18α) = 11.4 Hz, J(H-19β, H-21α) = 11.4 Hz, J(H-19β, H-21β) = 5.1 Hz, 2 × H-19β), 4.47 (2H, m, $\sum J$ = 16.2 Hz 2 × H-3α), 4.62 (2H, m, 2 × H-29 *pro*-E), 4.74 (2H, bd, J = 2.3 Hz, 2 × H-29 *pro*-Z); EIMS m/z 979 [M]$^+$ (not found), 498 (2), 483 (1), 453 (14), 438 (14), 423 (4), 393 (19), 377 (2), 351 (3), 249 (7), 233 (10), 203 (47), 189 (100); *anal* C 78.31%, H 10.13%, calcd for $C_{64}H_{98}O_7$, C 78.48%, H 10.08%.

3-Oxolup-20(29)-en-28-oic Acid (7). Chromium(VI) oxide (15.0 g, 150.1 mmol) and sulfuric acid (1 mL, 98%) were added to a solution of acid **1** (15.0 g, 32.8 mmol) in DMF (300 mL). The reaction mixture was stirred for 12 h at room temperature. The product was precipitated by pouring into vigorously stirred H_2O, filtered, and washed with H_2O. Column chromatography of crude material **7** (14.1 g) over silica gel (300 g) eluted with $CHCl_3$ afforded ketone **7** (10.7 g, 71% yield): R_f 0.39; mp 250−254 °C (MeOH), $[\alpha]^{25}_D$ +32° (c 0.37).[3]

Methyl 3-Oxolup-20(29)-en-28-oate (8). Sodium dichromate (37.0 g, 124.2 mmol) and sodium acetate (8.0 g) were added to a vigorously stirred solution of crude **2** (33.0 g, 70.2 mmol) in a mixture of dioxane (750 mL), glacial acetic acid (250 mL), and acetic anhydride (100 mL). The solution was then stirred for 28 h at room temperature and worked up in the usual manner. Column chromatography of the crude product (30.5 g) over silica gel (300 g) eluted with toluene afforded keto ester **8** (16.1 g, 49% yield): R_f 0.57; mp 161−165 °C (MeOH); α_D +28° (c 0.41).[21]

Diosphenols 9 and 10. Each ketone (**7** and **8**) (17.6 mmol) was dissolved in a mixture of potassium *tert*-butoxide (72 g) in *tert*-butyl alcohol (700 mL). Air was constantly introduced into the vigorously stirred solution at 40 °C for 40 min. The reaction mixture was then poured into dilute HCl and worked up in the usual manner. The crude product was chromatographed over silica gel (800 g) eluted with toluene/diethyl ether (15:1) and crystallized from diethyl ether to give compounds **9** and **10** in 75% and 85% yields.

2-Hydroxy-3-oxolupa-1,20(29)-dien-28-oic acid (9): R_f 0.45; mp 203−205 °C (diethyl ether); $[\alpha]^{25}_D$ +13° (c 0.75); IR ($CHCl_3$) ν_{max} 1730 sh, 1696, 1669, 1644 cm^{-1}; ^{1}H NMR δ 0.98, 1.01, 1.10, 1.13, 1.20 (15H, all s, 5 × CH_3), 1.70 m (3H, s, H-30), 1.74−1.82 (1H, dm, J = 13.0 Hz), 1.94−2.06 (2H, m), 2.24 (1H, ddd, J = 12.8, 11.6, 3.7 Hz), 2.30 (1H, dt, J = 12.5, 3.2, 3.2 Hz), 3.02 (1H, td, J(H-19β, H-18α) = 10.8 Hz, J(H-19β, H-21α)

= 10.8 Hz, J(H-19β, H-21β) = 4.8 Hz, H-19β), 4.64 (1H, m, H-29 *pro*-E), 4.76 (1H, bd, J = 2.3 Hz, (H-29 *pro*-Z), 5.91 (1H, bs, OH), 6.44 (1H, s, H-1); EIMS m/z 468 $[M]^+$ (43), 453 (2), 422 (22), 407 (5), 379 (3), 340 (23), 295 (5), 269 (12), 248 (7), 235 (15), 215 (100); *anal*. C 76.99%, H 9.22%, calcd for $C_{30}H_{44}O_4$, C 76.88%, H 9.46%.[5]

Methyl 2-hydroxy-3-oxolupa-1,20(29)-dien-28-oate (10): R_f 0.60; mp 122−124 °C (diethyl ether); α_D +3° (c 0.70); IR ($CHCl_3$) ν_{max} 1720 sh, 1643 cm^{-1}; ^1H NMR δ 0.96, 0.98, 1.10, 1.12, 1.20 (15H, all s, 5 × CH_3), 1.69 (3H, m, H-30), 1.74−1.82 (1H, dm, J = 13.3), 1.84−1.98 (2H, m), 2.20−2.30 (2H, m), 3.00 (1H, td, J(H-19β, H-18α) = 11.1 Hz, J(H-19β, H-21α) = 11.1 Hz, J(H-19β, H-21β) = 4.5 Hz, H-19β), 3.68 (1H, s, OCH_3), 4.62 (1H, m, H-29 *pro*-E), 4.75 (1H, bd, J = 2.3, H-29 *pro*-Z), 5.88 (1H, s, OH), 6.44 (1H, s, H-1); EIMS m/z 482 $[M]^+$ (91), 467 (4), 450 (6), 422 (50), 407 (9), 354 (18), 273 (19), 235 (12), 215 (100); *anal*. C 76.90%, H 9.88%, calcd for $C_{31}H_{46}O_4$, C 77.14%, H 9.61%.

2-Acetoxy-3-oxolupa-1,20(29)-dien-28-oic Acid (11). Acetic anhydride (3 mL, 30 mmol) and DMAP (0.3 g, 2.42 mmol) were added to a solution of **9** (1.0 g, 2.1 mmol) in pyridine (5 mL). The reaction mixture was worked up after 15 h. Chromatography on silica gel (150 g) eluted with toluene/diethyl ether (25:1) afforded acetate **11** (0.77 g, 71% yield): R_f 0.32; mp 149−151 °C (lyophyllizate from benzene); $[\alpha]^{25}_D$ +33° (c 1.30); IR ($CHCl_3$) ν_{max} 1758, 1686, 1644 cm^{-1}; ^1H NMR δ 1.00, 1.02, 1.11, 1.13, 1.19 (15H, all s, 5 × CH_3), 1.69 (3H, m, H-30), 1.79 (1H, m), 1.95−2.06 (2H, m), 2.19 (3H, s, Ac), 2.20−2.33 (2H, m), 3.02 (1H, td, J(H-19β, H-18α) = 10.7 Hz, J(H-19β, H-21α) = 10.7 Hz, J(H-19β, H-21β) = 4.9 Hz, H-19β), 4.63 (1H, m, H-29 *pro*-E), 4.75 (1H, bd, J = 2.3 Hz, H-29 *pro*-Z), 6.75 (1H, s, H-1); EIMS m/z 510 $[M]^+$ (3), 468 (30), 450 (8), 422 (10), 407 (2), 340 (2), 315 (3), 43 (100); *anal* C 75.42%, H 9.00%, calcd for $C_{32}H_{46}O_5$, C 75.26%, H 9.08%.

Methyl 2-Acetoxy-3-oxolupa-1,20(29)-dien-28-oate (12). Acetic anhydride (1 mL, 10 mmol) and DMAP (0.1 g, 0.81 mmol) were added to a solution of diosphenol **10** (300 mg, 0.6 mmol) in pyridine (2 mL). The reaction mixture was worked up after 15 h. HPLC in a 10% mixture of ethyl acetate in hexane afforded acetate **12** (150 mg, 45% yield): R_f 0.51; mp 95−97 °C (MeOH); $[\alpha]^{25}_D$ +43° (c 0.35); IR ($CHCl_3$) ν_{max} 1759, 1720, 1682, 1643 cm^{-1}; ^1H NMR δ 0.98, 0.99, 1.12, 1.13, 1.19 (15H, all s, 5 × CH_3), 1.69 (3H, s, H-30), 2.19 (3H, s, OAc), 2.23−2.40 (2H), 3.00 (1H, td, J(H-19β, H-18α) = 11.1 Hz, J(H-19β, H-21α) = 11.1 Hz, J(H-19β, H-21β) = 4.7 Hz (H-19β), 3.68 (3H, s, OCH_3), 4.61 (1H, m, H-29 *pro*-E), 4.74 (1H, bd, J = 2.3, H-29 *pro*-Z), 6.75 (1H, s, H-1); EIMS m/z 524 $[M]^+$ (29), 482 (40), 464 (39), 422 (31), 354 (6), 328 (11), 273 (100), 235 (14), 213 (64); *anal*. C 75.32%, H 9.46%, calcd for $C_{33}H_{48}O_5$, C 75.53%, H 9.22%.

Methyl 2-Methoxy-3-oxolupa-1,20(29)-dien-28-oate (13). Dimethyl sulfate (2 g, 16 mmol) was added to a vigorously stirred solution of diosphenol **9** (1 g, 2.1 mmol) in a solution of KOH (0.5 g, 8.8 mmol) in a mixture of dioxane (20 mL) and H_2O (10 mL). The mixture was then refluxed for 1 h and worked up in the usual manner. The crude product was crystallized from MeOH to give compound **13** (0.95 g, 87% yield): R_f 0.36; mp 105−107 °C (MeOH); $[\alpha]^{25}_D$ +46° (c 0.57); IR ($CHCl_3$) ν_{max} 1713, 1674, 1642, 1621 cm^{-1}; ^1H NMR δ 0.99, 0.99, 1.07, 1.19, 1.15, (15H, all s, 5 × CH_3), 1.71 (3H, m, H-30), 1.76−1.85 (1H), 1.85−1.98 (2H), 2.24−2.34 (2H), 3.02 (1H, td, J(H-19β, H-18α) = 11.0 Hz, J(H-19β, H-21α) = 11.0 Hz, J(H-19β, H-21β) = 4.7 Hz (H-19β), 3.55 (3H, s, 2-OCH_3), 3.68 (3H, s, 28-OCH_3), 4.63 (1H, m, H-29 pro-E), 4.76 (1H, bd, J = 2.3, H-29 pro-Z), 6.05 (1H, s, H-1); EIMS m/z 496 $[M]^+$ (97), 481 (4), 464 (2), 436 (24), 421 (5), 354 (17), 329 (3), 295 (6), 273 (47), 258 (6), 235 (5), 213 (62), 165 (100); *anal*. C 77.51%, H 9.62%, calcd for $C_{32}H_{48}O_4$, C 77.38%, H 9.74%.

2-Oxa-1α-hydroxy-3-oxolup-20(29)en-28-oic Acid (14). Diosphenol **9** (1.5 g, 3.3 mmol) was dissolved in a solution of potassium *tert*-butoxide (15 g) in *tert*-butyl alcohol (140 mL). Air was constantly introduced into the vigorously stirred solution at 40 °C for 48 h. The reaction mixture was then poured into dilute HCl and worked up in the usual manner. The crude product was chromatographed over silica gel (100 g) eluted with toluene/diethyl ether (3:1) to give acid **14** (0.8 g, 53% yield): R_f 0.08; mp 274−276 °C (MeOH); $[\alpha]^{25}_D$ −116° (c 0.02); IR ($CHCl_3$) ν_{max} 3280 vb, 1693, 1643 cm^{-1}; ^1H NMR δ 0.99, 1.03, 1.18, 1.25, 1.26 (15H, all bs, 5 × CH_3), 1.60 (1H, t, J = 11, 11 Hz, H-18α), 1.69 (3H, s, H-30), 3.00 (1H, td, J(H-19β, H-18α) = 10.8 Hz, J(H-19β, H-21α) = 10.8 Hz, J(H-19β, H-21β) = 4.2 Hz, H-19β), 4.59 (1H, m, H-29 *pro*-E), 4.72 (1H, bd, J = 2.1, H-29 *pro*-Z), 5.22 (1H, s, H-1β); EIMS m/z 472 $[M]^+$ (1), 454 (2), 444 (7), 426 (26), 411 (14), 397 (37), 385 (11), 367 (8), 357 (27), 339 (28), 327 (15), 316 (18), 301 (8), 259 (79), 248 (17), 213 (18), 201 (26), 189 (49), 55 (100); *anal*. C 73.81%, H 9.24%, calcd for $C_{29}H_{44}O_5$, C 73.69%, H 9.38%.

Methyl 2-Oxa-1α-hydroxy-3-oxolup-20(29)en-28-oate (15). Diosphenol **10** (1.5 g, 3.1 mmol) was oxidized analogously as described above. The crude product was chromatographed over silica gel (100 g) eluted with toluene/ether (10:1) to give lactol **15** (0.9 g, 60% yield): R_f 0.25; mp 143−146 °C (MeOH); $[\alpha]^{25}_D$ +13° (c 0.33); IR ($CHCl_3$) ν_{max} 3200−3550, 1720, 1642 cm^{-1}; ^1H NMR δ 0.96, 1.00, 1.01, 1.19, 1.26, (15H, all s, 5 × CH_3), 1.60 (1H, t, J(H-13β, H-18α) = 12.0 Hz, J(H-18α, H-19β) = 12.0 Hz, H-18α), 1.68 (3H, s, H-30), 2.98 (1H, td, J(H-19β, H-18α) = 11.2 Hz, J(H-19β, H-21α) = 11.2 Hz, J(H-19β, H-21β) = 4.4 Hz, H-19β), 3.67 (3H, s, OCH_3), 4.59 m, 1 H, (H-29 *pro*-E); 4.73 bd, 1H, J = 2.4 (H-29 *pro*-Z); 5.30 s, 1H, (H-1β). EIMS m/z 486 $[M]^+$ (5), 457 (14), 440 (8), 427 (11), 411 (6), 397 (24), 371 (18), 339 (47), 311 (9), 273 (86), 247 (18), 213 (22), 201 (37), 187 (78), 175 (94), 121 (100); *anal* C 74.15%, H 9.42%, calcd for $C_{30}H_{46}O_5$, C 74.04%, H 9.53%.

Using oxygen for the reaction instead of air allowed us to reduce the reaction time from 48 to 2 h without marked influence on yield.

Preparation of 2,3-Seco Derivatives 16−18. A solution of each diosphenol (**9** and **10**) (4.5 mmol) in a mixture of KOH (6.5 g) and MeOH (350 mL) was heated under reflux, and hydrogen peroxide (35 mL, 30%) was added during 100 min. The solution was then poured into cold H_2O, and the product was extracted with ethyl acetate (2 × 100 mL). The product was chromatographed over silica gel (200 g) eluted with $CHCl_3$/ethyl acetate/acetic acid (100:15:1) and crystallized to give seco-acids **16** and **17** in 35% and 80% yields, respectively.

2,3-Secolup-20(29)en-2,3,28-trioic acid (16): R_f 0.22 ($CHCl_3$/EtOAc/AcOH 10:2.5:0.15); mp 276−277 °C (MeOH/$CHCl_3$); $[\alpha]^{25}_D$ +10° (c 0.22); IR ($CHCl_3$) ν_{max} 2600−3400, 1725, 1643 cm^{-1}; ^1H NMR ($CDCl_3$/CD_3OD) δ 0.93, 0.95, 1.00, 1.23, 1.24 (15H, all s, 5 × CH_3), 1.67 (3H, m, H-30), 1.89−2.00 (2H, m), 2.20−2.31 (2H, m), 2.38−2.45 (2H, m), 2.46 (2H, s, H-1α, H-1β), 3.00 (1H, td, J(H-19β, H-18α) = 10.8 Hz, J(H-19β, H-21α) = 10.8 Hz, J(H-19β, H-21β) = 4.4 Hz, H-19β), 4.57 (1H, m, H-29 *pro*-E), 4.73 (1H, bd, J = 2.3 Hz, H-29 *pro*-Z); EIMS m/z 502 $[M]^+$ (1), 484 (3), 456 (17), 442 (12), 415 (13), 397 (24), 379 (7), 369 (100), 355 (32), 351 (16), 309 (8), 261 (20), 248 (17), 233 (16), 215 (35), 201 (34), 189 (46); *anal* C 71.92%, H 9.07%, calcd for $C_{30}H_{46}O_6$, C 71.68%, H 9.22%.

28-Methyl ester of 2,3-secolup-20(29)en-2,3,28-trioic acid (17): R_f 0.25 ($CHCl_3$/EtOAc/AcOH 10:2.5:0.15); mp 132−135 °C (hexane/diethyl ether); $[\alpha]^{25}_D$ +23° (c 0.31); IR ($CHCl_3$) ν_{max} 2400−3400, 1718, 1690, 1642 cm^{-1}; ^1H NMR ($CDCl_3$/CD_3-OD, 50 °C) δ 0.92, 0.93, 0.96, 1.17, 1.25 (15H, all s, 5 × CH_3), 1.68 (3H, s, H-30), 1.82−1.93 (2H), 2.14−2.26 (2H, m), 2.47 (1H, d, J(H-1α, H-1β) = 19.6 Hz, H-1α), 2.64 (1H, d, J(H-1β, H-1α) = 19.6 Hz, H-1β), 2.99 (1H, td, J(H-19β, H-18α) = 11.0 Hz, J(H-19β, H-21α) = 11.0 Hz, J(H-19β, H-21β) = 4.4 Hz, H-19β), 3.66 (3H, s, OCH_3), 4.60 (1H, m, H-29 *pro*-E), 4.73 (1H, bd, J = 2.3, H-29 *pro*-Z); EIMS m/z 516 $[M]^+$ (2), 498 (11), 470 (8), 456 (23), 439 (12), 429 (24), 411 (9), 397 (10), 384 (8), 369 (93), 351 (14), 275 (59), 262 (41), 249 (24), 215 (48), 201 (59), 189 (100); *anal* C 71.99%, H 9.42%, calcd for $C_{31}H_{48}O_6$, C 72.06%, H 9.36%.

Trimethyl 2,3-Secolup-20(29)en-2,3,28-trioate (18). An excess of diazomethane in diethyl ether was added to a solution of triacid **16** (200 mg, 0.4 mmol) in $CHCl_3$ (10 mL). The solvents were removed in vacuo, and the crude product was then chromatographed over silica gel (10 g) eluted with toluene to afford trimethyl ester **18** (150 mg, 69% yield): R_f 0.56; mp 186−188 °C (MeOH); $[\alpha]^{25}_D$ −8° (c 0.31); IR ($CHCl_3$) ν_{max}

1720vb, 1641 cm^{-1}; ^1H NMR δ 0.89, 0.92, 0.99, 1.22, 1.23 (15H, all s, 5 × CH_3), 1.69 (3H, m, H-30), 1.82−1.94 (2H, m), 2.18−2.27 (3H, m), 2.36−2.42 (2H, m), 3.00 (1H, td, J(H-19β, H-18α) = 11.0 Hz, J(H-19β, H-21α) = 11.0 Hz, J(H-19β, H-21β) = 4.7 Hz, H-19β), 3.60, 3.64, 3.66 (9H, all s, $COOCH_3$), 4.59 (1H, m, H-29 *pro*-E), 4.73 (1H, bd, J = 2.4, H-29 *pro*-Z); EIMS *m*/*z* 544 $[M]^+$ (1), 513 (1), 485 (5), 471 (16), 443 (9), 411 (7), 383 (33), 369 (64), 187 (24), 169 (100); *anal.* C 72.94%, H 9.46%, calcd for $C_{33}H_{52}O_6$, C 72.76%, H 9.62%.[22]

2,3-Secolup-20(29)-en-2,3,28-triol (19). LAH (1 g, 26.4 mmol) was added to a solution of triacid **16** (500 mg, 1 mmol) in tetrahydrofuran (30 mL), and the mixture was refluxed for 5 h. Ethyl acetate (50 mL) was slowly added after 5 h to decompose residual LAH, and the mixture was poured into cold H_2O and worked up as usual. The crude product was chromatographed over silica gel (50 g) eluted with diethyl ether and crystallized to afford triol **19** (350 mg, 76% yield): R_f 0.13 ($CHCl_3$/EtOAc/AcOH 10:2.5:0.15); mp 234−235 °C (hexane/diethyl ether); $[\alpha]^{25}_D$ +32° (*c* 0.25); IR ($CHCl_3$) ν_{max} 3626, 1641 cm^{-1}; ^1H NMR δ 0.93, 0.94, 0.97, 1.06, 1.08 (15H, all s, 5 × CH_3), 1.69 (3H, s, H-30), 2.40 (1H, td, J(H-19β, H-18α) = 10.9 Hz, J(H-19β, H-21α) = 10.9 Hz, J(H-19β, H-21β) = 5.9 Hz, H-19β), 3.30 (1H, bd, J = 10.8 Hz), 3.37 (1H, d, J = 11.1 Hz), 3.43 (1H, d, J = 11.1 Hz), 3.56 (1H, m), 3.74−3.82 (2H), 4.58 (1H, m, H-29 *pro*-E), 4.68 (1H, bd, J = 2.4, H-29 *pro*-Z); EIMS *m*/*z* 460 $[M]^+$ (1), 442 (2), 429 (4), 411 (4), 399 (1), 383 (2), 369 (5), 357 (3), 245 (12), 215 (13), 189 (19), 81 (100); *anal.* C 78.46, H 11.19%, calcd for $C_{30}H_{52}O_3$, C 78.21%, H 11.38%.

2,3-Secolup-20(29)-en-2,3,28-triyl Triacetate (20). Acetic anhydride (2 mL, 19.6 mmol) was added to a solution of triol **19** (80 mg, 0.17 mmol) in pyridine. The reaction mixture was worked up after 12 h, and the crude product was chromatographed using HPLC eluted with 15% ethyl acetate in hexane and lyophyllized to afford acetate **20** (50 mg, 43% yield): R_f 0.38; mp less than +50 °C (*tert*-butyl alcohol); $[\alpha]^{25}_D$ +17° (*c* 0.45); IR ($CHCl_3$) ν_{max} 1729, 1642 cm^{-1}; ^1H NMR δ 0.95, 0.97, 1.05, 1.13, 1.27 (15H, all s, 5 × CH_3), 1.68 (3H, s, H-30), 2.03, 2.07, 2.09 (9H, all s, 3 × Ac), 2.45 (1H, td, J(H-19β, H-18α) = 11.1 Hz, J(H-19β, H-21α) = 11.1 Hz, J(H-19β, H-21β) = 5.9 Hz, H-19β), 3.74 (1H, d, J = 10.8 Hz), 3.86 (1H, d, J = 11.1 Hz), 4.02 (1H, d, J = 10.8 Hz), 4.04−4.10 (1H), 4.20−4.28 (2H), 4.59 (1H, m, H-29 *pro*-E), 4.69 (1H, bd, J = 2.4 Hz, H-29 *pro*-Z); EIMS *m*/*z* 586 $[M]^+$ (1), 526 (3), 513 (1), 466 (5), 411 (5), 356 (8), 287 (7), 255 (5), 189 (14), 43 (100); *anal.* C 73.36%, H 10.14%, calcd for $C_{36}H_{58}O_6$, C 73.68%, H 9.96%.

Reaction of Seco Derivatives with Acetic Anhydride. Acetic anhydride (2 mL, 19.6 mmol) was added to a solution of each seco derivative **16** or **17** (250 mg, 0.5 mmol) in pyridine (3 mL). After 10 h, the mixture was poured into cold H_2O and worked up. HPLC in 15% ethyl acetate in hexane afforded two products (when **16** was the reactant). The first fraction was anhydride **23** (120 mg, 50% yield), and the second fraction was anhydride **21** (60 mg, 23% yield). Both were crystallized from hexane/diethyl ether. Reaction with **17** afforded only **22** (102 mg, 40% yield).

2,3-Anhydride of 2,3-secolup-20(29)-en-2,3,28-trioic acid (21): R_f 0.40; mp 242−244 °C (diethyl ether/hexane); $[\alpha]^{25}_D$ +42° (*c* 0.25); IR ($CHCl_3$) ν_{max} 3515, 1797, 1753, 1696, 1643 cm^{-1}; ^1H NMR δ 0.97, 0.99, 1.04, 1.26, 1.37 (15H, all s, 5 × CH_3), 1.70 (3H, m, H-30), 1.74−1.82 (2H, m), 1.95−2.03 (2H, m), 2.21 (1H, d, J = 13.7 Hz, H-1a), 2.86 (1H, d, J = 13.7 Hz, H-1b), 3.00 (1H, m, H-19β), 4.63 (1H, m, H-29 *pro*-E), 4.74 (1H, bd, J = 2.4 Hz, H-29 *pro*-Z); EIMS *m*/*z* 484 $[M]^+$ (3), 466 (2), 456 (12), 438 (15), 423 (6), 413 (3), 384 (8), 371 (6), 325 (13), 301 (8), 248 (42), 233 (15), 201 (37), 187 (42), 69 (100); *anal.* C 74.22%, H 9.27%, calcd for $C_{30}H_{44}O_5$, C 74.34%, H 9.15%.

2,3-Anhydride of 28-methyl ester of 2,3-secolup-20(29)-en-2,3,28-trioic acid (22): R_f 0.67; mp 206−208 °C (diethyl ether/hexane); $[\alpha]^{25}_D$ +43° (*c* 0.60); IR ($CHCl_3$) ν_{max} 1797, 1754, 1720, 1643 cm^{-1}; ^1H NMR δ 0.95, 0.98, 1.04, 1.26, 1.37 (15H, all s, 5 × CH_3), 1.69 (3H, m, H-30), 1.73−1.81 (2H, m), 1.84−1.93 (2H, m), 2.21 (1H, d, J = 13.7 Hz, H-1a), 2.20−2.28 (2H, m), 2.85 (1H, d, J = 13.7 Hz, H-1b), 2.99 (1H, m, H-19β), 3.67 (3H, s, OCH_3), 4.62 (1H, m, H-29 *pro*-E), 4.73 (1H, bd, J = 2.3 Hz, H-29 *pro*-Z); EIMS *m*/*z* 498 $[M]^+$ (35), 483 (3), 470 (11), 439 (22), 423 (10), 416 (18), 395 (11), 384 (25), 325 (21), 262 (41), 201 (32), 187 (60), 55 (100); *anal.* C 74.50%, H 9.47%, calcd for $C_{31}H_{46}O_5$, C 74.66%, H 9.30%.

Anhydride of 2,3-anhydride of 2,3-secolup-20(29)-en-2,3,28-trioic acid and acetic acid (23): R_f 0.63; mp 152−154 °C (diethyl ether/hexane); $[\alpha]^{25}_D$ +41° (*c* 0.50); IR ($CHCl_3$) ν_{max} 3526, 1809, 1796, 1753, 1702, 1643 cm^{-1}; ^1H NMR δ 0.99, 1.00, 1.05, 1.26, 1.37 (15H, all s, 5 × CH_3), 1.69 (3H, m, H-30), 1.74−1.82 (2H, m), 1.90−2.04 (m, 2H), 2.21 (1H, d, J = 13.7 Hz, H-1a), 2.23 (3H, s, Ac), 2.21−2.36 (2H, m), 2.86 (1H, d, J = 13.9 Hz, H-1b), 2.97 (1H, td, J(H-19β, H-18α) = 11.1 Hz, J(H-19β, H-21α) = 11.1 Hz, J(H-19β, H-21β) = 4.6 Hz, H-19β), 4.64 (1H, m, H-29 *pro*-E), 4.74 (1H, bd, J = 2.0 Hz, H-29 *pro*-Z); EIMS *m*/*z* 526 $[M]^+$ (1), 498 (2), 484 (6), 466 (3), 456 (6), 438 (29), 423 (5), 395 (7), 371 (5), 325 (8), 248 (15), 201 (17), 187 (24), 43 (100); *anal.* C 72.69%, H 9.08%, calcd for $C_{32}H_{46}O_6$, C 72.97%, H 8.80%.

Cell Lines. Cell lines A 549, DU 145, HT 29, K562, MCF 7, PC-3, and SK-Mel2 were all purchased from the American Tissue Culture Collection (ATTC). Paclitaxel-resistant subline of K562 cells was kindly provided by Dr. J. Dummont (University of Lyon, France). The human T-lymphoblastic leukemia cell line, CEM, was used for routine screening of compounds. To prove a common mechanism of action, selected compounds, which showed activity in the screening assay, were tested in a panel of cell lines. These lines were from different species and of various histogenetic origin, and they possess various alterations in their cell cycle-regulatory proteins and hormone receptor status (Tables 1 and 2). The cells were maintained in Nunc/Corning 80 cm^2 plastic tissue culture flasks and cultured in cell culture medium (DMEM/RPMI 1640 with 5 g/L glucose, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% fetal calf serum, and $NaHCO_3$).

Cytotoxicity Assay. Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2500−30 000 cells/well based on cell growth characteristics). Cells were added by pipet (80 μL) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37 °C and 5% CO_2 for stabilization. Four-fold dilutions, in 20 μL aliquots, of the intended test concentration were added at time zero to the microtiter plate wells. All tested compounds were dissolved in 10% DMSO, and concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for a further 1−4 h. After this incubation period formazan produced was dissolved by the addition of 100 μL/well of 10% aqueous SDS (pH = 5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Tumor cell survival (TCS) was calculated using the following equation: $TCS = (OD_{drug-exposed\ well}/\text{mean}\ OD_{control\ wells}) \times 100\%$. The TCS_{50} value, the drug concentration lethal to 50% of the tumor cells, was calculated from appropriate dose−response curves.

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Supporting Information Available: Tables of ^{13}C NMR data for compounds **1**−**23** and ^1H NMR data for compound **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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