Novel Synthesis, Cytotoxic Evaluation, and Structure−Activity Relationship Studies of a Series of α-Alkylidene-γ-lactones and Lactams

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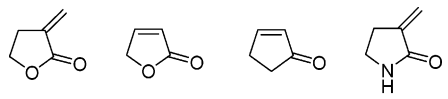
5-Alkyl- and 5-arylalkyl-3-methylenedihydrofuran-2-ones **13a**−**e**, 3-alkylidenedihydrofuran-2-ones **18a**−**c**, and 3-methylenepyrrolidin-2-ones **16a**−**e** were synthesized utilizing ethyl 2-diethoxyphosphoryl-4-nitroalkanoates **9a**−**e** as common intermediates. All obtained compounds were tested against L-1210, HL-60, and NALM-6 leukemia cells. The highest cytotoxic activity was observed for 3-methylenefuranones **13d**,**e** bearing benzyl or 3,4-dimethoxyphenylmethyl substituents at position 5, with IC_{50} values of 5.4 and 6.0 μM, respectively. Contrary to the literature reports, no enhancement in activity due to the presence of a hydroxy group was found when the cytotoxicity of furanones **13a**,**b**,**d** and 5-(1′-hydroxyalkyl)-3-methylenedihydrofuran-2-ones **6a**,**b**,**d** was compared. The anticancer activity of pyrrolidinones **16a**−**e** and 3-alkylidenefuranones **18a**−**c** was much weaker than that of furanones **13a**−**e**.

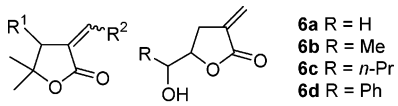
Introduction

Sesquiterpene lactones, found almost exclusively in the species of the Compositae (Asteraceae) family, are known to possess significant biological activity.[1] Cytotoxic, allergenic, anti-inflammatory, phytotoxic, and antimicrobial properties make them a desired target for many synthetic organic chemists.[1−3] Characteristic structural features of this class of compounds are α-methylene-γ-lactone **1**, butenolide **2**, and/or cyclopentenone **3** moieties. From the recent comparative studies it appears that the most important structural requirement for the activity of this class of compounds is an α-methylene-γ-lactone moiety.[4−6] It was also shown that compounds containing this moiety can act as Michael acceptors in the reaction with thiol groups of bionucleophiles.[7] Furthermore, an α-alkylidene-γ-lactone moiety readily forms a 2 + 2 cycloadduct with the DNA base thymine.[5] A number of possible drug candidates bearing this lactone motif have been synthesized and tested.[7−11] Much less common in nature are products with the α-methylene-γ-lactam framework **4**. Examples of a few naturally occurring compounds of this structure are pukeleimid E, isolated from cyanobacteria *Lyngbya majuscula*,[12] and two imidazole alkaloids anantin and isoanantin, found in the leaf tissue of *Cynometra*.[13] However, the biological activity of this class of compounds is scarcely recognized.[14]



1 **2** **3** **4**

The crucial role of the α-methylene-γ-lactone moiety in biological and especially cytotoxic activity of the sesquiterpene natural products prompted us to develop several new synthetic methods leading to this structural unit. Successful application of the Horner−Wadsworth−Emmons olefination reaction for the construction of alkylidene bond gave us access to a series of 3-alkylidene-5,5-dimethyldihydrofuran-2-ones **5**[15] and 3-methylidene-5-(1′-hydroxyalkyl)dihydrofuran-2-ones **6**.[16] The latter compounds were tested for their cytotoxic activity, and several of them proved to be very potent.[17]



5 **6a-d**

Here, we present the full account[18] of a novel synthesis of 3-alkylidenedihydrofuran-2-ones **13a**−**e** and **18a**−**c** as well as 3-methylenepyrrolidin-2-ones **16a**−**e** bearing an alkyl or arylmethyl substituent at position 5. Cytotoxic evaluation of the target compounds was performed on the mouse leukemia cell line L-1210 and two human leukemia cell lines NALM-6 and HL-60. Structure−activity relationships of the newly obtained compounds are discussed. Also, cytotoxicities of furanones **13** and furanones **6** containing a hydroxy group are compared.

Chemistry

3-Alkylidenedihydrofuran-2-ones **13a**−**e** and **18a**−**c** and 3-methylenepyrrolidin-2-ones **16a**−**e** were all synthesized starting from ethyl 2-diethoxyphosphoryl-4-nitroalkanoates **9a**−**e**, which are the key intermediates in our method (Scheme 1). Nitroalkanoates **9a**−**e** were conveniently prepared by addition of nitroalkanes **8a**−**e** to ethyl 2-diethoxyphosphorylacrylate (**7**) in the presence of NaH, using a 2-fold excess of nitroalkane (**7**/**8**/NaH = 1:2:1.1). Column chromatography afforded **9a**−**e** as mixtures of diastereoisomers with close to a 3:2 ratio.

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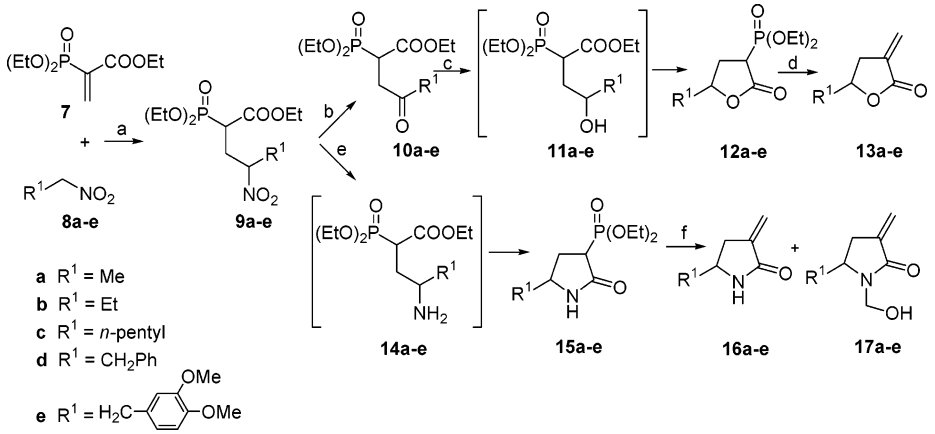
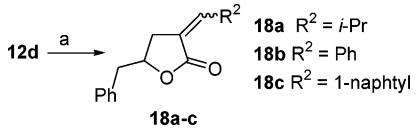
Scheme 1[a]



[a] Reagents and conditions: (a) NaH, THF, room temp, 24 h; (b) (1) MeONa/MeOH, room temp, 0.5 h, (2) conc H_2SO_4, MeOH, −60 °C, 2 h; (c) (1) $NaBH_4$, MeOH, NaOH, H_2O, room temp, 20 h, (2) 1 N HCl_{aq}; (d) K_2CO_3, 36% formalin, 0−5 °C, 15 min; (e) NH_4HCO_2, 10% Pd/C, MeOH/THF, 0 °C to room temp, 24 h; (f) (1) NaH, THF, (2) $(CH_2O)_n$, reflux, 1 h.

Scheme 2[a]



[a] Reagents and conditions: (a) (1) NaH, benzene, room temp, 0.5 h, (2) R^2CHO, benzene, reflux, 4 h.

Conversion of the nitro group in **9a**−**e** into a carbonyl group (Nef reaction) followed by reduction of the 4-oxoalkanoates **10a**−**e** gave 4-hydroxyalkanoates **11a**−**e**, which lactonized spontaneously to 3-(diethoxyphosphoryl)dihydrofuran-2-ones **12a**−**e**. These compounds were obtained as mixtures of diastereoisomers with close to a 1:1 ratio. Horner−Wadsworth−Emmons olefination of formaldehyde using **12a**−**e** and applying the Villieras procedure[19] (36% formaline, K_2CO_3, 0−5 °C, 15 min) gave the expected 3-methylenedihydrofuran-2-ones **13a**−**e**. Furanones **13a**,[20] **13b**,**c**,[21] and **13d**[2] have previously been prepared using other synthetic methods. Olefination of isobutyraldehyde, benzaldehyde, and 1-naphthylaldehyde was accomplished using the sodium derivative of furanone **12d** in boiling benzene (Scheme 2). 3-Alkylidenedihydrofuran-2-ones **18a**,**b** were obtained as mixtures of *E* and *Z* isomers in a 30:70 and 85:15 ratio, respectively, while furanone **18c** was formed as a single *E* isomer. Configurational assignments were made using the diagnostic deshielding effect of the carbonyl group exerted on the cis-oriented vinyl proton.[22]

Reduction of the nitro group in **9a**−**e** gave, after spontaneous lactamization, 3-(diethoxyphosphoryl)pyrrolidin-2-ones **15a**−**e** as mixtures of diastereoisomers with close to a 3:2 ratio (Scheme 1). Olefination of formaldehyde using **15a**−**e** proceeded smoothly when sodium hydride with paraformaldehyde in boiling THF was used. Under these conditions the expected 3-methylenepyrrolidin-2-ones **16a**−**e** were formed along with various amounts (6−26%) of 1-hydroxymethyl-3-methylenepyrrolidin-2-ones **17a**−**e**. Purification and separation of these mixtures by column chromatography afforded pure pyrrolidinones **16a**−**e**. Pyrrolidinones **16a**[23] and **16d**[24] have previously been prepared using other synthetic methods.

Table 1. Cytotoxic Activity and Lipophilicity of Compounds **13a**−**e**, **16a**−**e**, **18a**−**c**, and **6a**−**d**

compd	lipophilicity log(*P*)	cytotoxicity IC_{50} (μM)[a] L-1210	HL-60	NALM-6
13a	0.82	32.5 ± 4,8	77.4 ± 6.5	41.0 ± 1.8
13b	1.29	6.0 ± 1.6	39.5 ± 17.3	51.6 ± 24.0
13c	2.48	20.0 ± 3.2	99.4 ± 31.5	23.6 ± 12.9
13d	2.51	15.5 ± 2.9	42.7 ± 11.1	5.4 ± 0.3
13e	2.00	4.3 ± 0.8	46.3 ± 1.8	6.0 ± 1.4
16a	0.17	20.0 ± 5.7	640 ± 84	658 ± 47
16b	0.64	>100	894 ± 83	387 ± 71
16c	1.83	59.0 ± 6.9	397 ± 87	82.7 ± 8.4
16d	1.86	93.0 ± 12.4	490 ± 62	420 ± 48
16e	1.35	79.0 ± 7.5	402 ± 31	507 ± 26
18a	3.59	90.0 ± 6.9	168 ± 57	380 ± 24
18b	4.08	63.3 ± 10.0	60.6 ± 2.7	48.7 ± 2.8
18c	5.08	46.1 ± 9.2	49.8 ± 3.1	71.0 ± 4.4
6a	0.04	27.2 ± 6.1[b]	72.4 ± 16.9[b]	
6b	0.45	19.3 ± 3.7[b]	47.3 ± 3.7[b]	
6c	1.31	16.9 ± 3.9[b]	52.6 ± 4.9[b]	
6d	1.88	8.0 ± 2.1[b]	40.2 ± 2.4[b]	
carboplatin		9.7 ± 1.2	2.9 ± 0.1	0.7 ± 0.3

[a] IC_{50}, 50% inhibitory concentration represents the mean from dose response curves of at least three experiments. [b] From ref 17.

Results and Discussion

The cytotoxicity of all obtained compounds was assessed in vitro against three leukemia cell lines (mouse L-1210 and human HL-60 and NALM-6) and expressed as IC_{50} values. IC_{50} is the concentration (μM) required to inhibit tumor cell proliferation by 50% after 72 h of exposure of the cells to a tested compound. The measured IC_{50} values for 5-alkyl-3-methylenedihydrofuran-2-ones **13a**−**e**, 5-alkyl-3-methylenepyrrolidin-2-ones **16a**−**e**, and 5-alkyl-3-alkylidenedihydrofuran-2-ones **18a**−**c** are summarized in Table 1. Carboplatin was used as a reference compound.[25] Cytotoxicities of 5-(1′-hydroxyalkyl)-3-methylenedihydrofuran-2-ones **6a**−**d**, which are structurally related to furanones **13a**−**e**, are also given in Table 1.

As can be seen from the presented data, cytotoxicity of pyrrolidin-2-ones **16a**−**e** is generally low. IC_{50} values for these compounds against HL-60 and NALM-6 cell lines are greater than 300 (with one exception only), and they are 10−100 times less active than the corresponding furan-2-ones **13a**−**e**. Differences in activity between these two series of compounds against the L-1210 cell line are smaller but also apparent. These findings are

in agreement with the only other comparative study describing the differences in activity between α-methylene-γ-lactones and α-methylene-γ-lactams.[14] Replacement of the oxygen by a nitrogen atom in the furanone ring most likely reduces the efficacy of 3-methylenepyrrolidin-2-ones as Michael acceptors because of better conjugation between the carbonyl group and the unshared electrons on the nitrogen atom compared to unshared electrons on an oxygen atom. Comparison of the cytotoxicities of **13d** and **18a**−**c** shows clearly that substitution of the methylene group with the isopropyl or aryl substituent decreases the activity. Steric hindrance introduced by the substituent, which makes the double bond less vulnerable to the nucleophilic attack, seems the most obvious explanation for this observation. Furthermore, an isopropyl substituent is more deactivating than aryl substituents. Once again, the steric effect should be taken into account. Aryl substituents are conjugated with an α,β-unsaturated system and thus are coplanar with it, contrary to an isopropyl substituent, which can rotate freely around the C1′−C2′ bond. As a consequence, the double bond is less hindered by an aryl than by an isopropyl substituent. There is no clear relationship between the cytotoxicity of furanones **13a**−**e** against L-1210 or HL-60 cell lines and the nature of the R^1 substituents. However, cytotoxicities against the L-1210 cell line are generally higher than against the HL-60 cell line. On the other hand, cytotoxicities of furanones **13a**−**e** against NALM-6 cells are clearly higher when R^1 has an aromatic character. Furanones **13d**,**e** with benzyl and 3,4-dimethoxyphenylmethyl substituents have remarkably high cytotoxities against this cell line.

Recently we have synthesized and evaluated the cytotoxic activity of a series of 5-(1′-hydroxyalkyl)-3-methylenedihydrofuran-2-ones **6a**−**d** against L-1210 and HL-60 cell lines.[17] These investigations were encouraged by the reports[26] that the presence of a stereochemically defined carbinol unit may enhance the biological properties of α-methylene-γ-lactones. The present results gave the opportunity to verify this hypothesis because the only structural difference between the furanones **13a**,**b**,**d** described in this study and the corresponding furanones **6a**,**b**,**d** is the lack of the hydroxy group in the former ones. Disappointingly, a comparison of these two series of furanones shows no significant differences in cytotoxicities. Evidently, the presence of a hydroxy group in **6a**,**b**,**d** does not enhance their cytotoxic properties.

Structure−cytotoxicity relationship studies of sesquiterpene lactones revealed also that increased lipophilicity is often accompanied by increased cytotoxicity.[27] We calculated the lipophilicity of the newly obtained compounds,[28] and the results, expressed as log *P* where *P* is a partition coefficient in the 1-octanol/water system, are given in Table 1. In the series of furanones **6a**−**d** we have found a straightforward correlation between lipophilicity and cytotoxicity with the more lipophilic compounds being more cytotoxic. However, the cytotoxicity of compounds **13a**−**e** and **18a**−**c** does not show a clear correlation with their lipophilicity. Nevertheless, high cytotoxicities of **13d** and **13e** against the NALM-6 cell line can be, at least partially, attributed to the strongly lipophilic character of these compounds. Likewise, higher cytotoxicities of **18b**,**c** in comparison with **18a** can be rationalized not only in terms of the steric effect but also in terms of their more lipophilic character.

In conclusion, we have developed a novel, general, and straightforward route to 3-alkylidenefuran-2-ones **13a**−**e** and **18a**−**c** and 3-methylenepyrrolidin-2-ones **16a**−**e** starting from easily available common intermediates, ethyl 2-diethoxyphosphoryl-4-nitroalkanoates **9a**−**e**. For all obtained compounds, cytotoxic activity against the L-1210, HL-60, and NALM-6 cell lines was determined and lipophilicity was calculated. Two of the prepared furanones **13d** and **13e** exhibited remarkable cytotoxicity toward the NALM-6 cell line. Pyrrolidinones **16** proved to be generally much less active than furanones **13** and **18**. Also, 3-alkylidenefuranones **18** had weaker activity than 3-methylenefuranones **13**. No clear correlation between cytotoxicity and lipophilicity in these series of compounds was found, and it seems that observed differences in activity can be better rationalized in terms of steric and electronic effects. A comparison of the cytotoxicities of furanones **13a**,**b**,**d** and **6a**,**b**,**d** does not support the literature reports that the presence of a hydroxy group may enhance the activity of α-methylene-γ-lactones.

Experimental Section

Organic solvents and reagents were purified by the appropriate standard procedures. IR spectra were recorded on a Specord M80 spectrometer. ^{1}H NMR (250 MHz), ^{13}C NMR (62.9 MHz), and ^{31}P NMR (101 MHz) spectra were recorded on a Bruker DPX-250 spectrometer with TMS as an internal standard and 85% H_3PO_4 as an external standard. ^{31}P NMR spectra were recorded using broad-band proton decoupling. Column chromatography was performed on Fluka silica gel 60 (230−400 mesh).

Nitroethane, 1-nitropropane, and 1-nitrohexane were purchased from Fluka. 2-Phenylnitroethane,[29] 2-(3,4-dimethoxyphenyl)nitroethane,[29] and ethyl 2-diethoxyphosphorylacrylate (**7**)[18] were prepared according to the literature procedures.

General Procedure for the Preparation of Ethyl 2-diethoxyphosphoryl-4-nitroalkanoates 9. A solution of nitroalkane **8** (17.0 mmol) in THF (10 mL) was added to a stirred suspension of NaH (0.213 g, 8.9 mmol) in THF (40 mL) under argon atmosphere at 0−4 °C. The reaction mixture was stirred for 40 min at room temperature and cooled to 0−4 °C, and a solution of ethyl 2-diethoxyphosphorylacrylate (**7**) (2.0 g, 8.5 mmol) in THF (10 mL) was added. The mixture was then stirred for 24 h at room temperauture, THF was evaporated at room temperauture, and the residue was quenched with water (15 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The organic extracts were dried ($MgSO_4$) and evaporated at room temperauture to give a crude product that was purified by column chromatography (eluent, $CHCl_3$/acetone = 90:10 for **9a**−**c** and $CHCl_3$/acetone = 95:5 for **9d**,**e**).

Ethyl 2-diethoxyphosphoryl-5-(3,4-dimethoxyphenyl)-4-nitropentanoate (9e): ratio of diastereoisomers = 65:35; oil, 85% yield; IR (film) 1732, 1552, 1260 cm^{-1}; ^{1}H NMR[30] ($CDCl_3$) δ 1.28 (t, $^3J_{HH}$ = 7.0 Hz, 3H, major + minor), 1.31 (t, $^3J_{HH}$ = 7.0 Hz, 3H, major), 1.32 (td, $^3J_{HH}$ = 7.2 Hz, $^4J_{PH}$ = 0.5 Hz, 3H, minor), 1.33 (td, $^3J_{HH}$ = 7.5 Hz, $^4J_{PH}$ = 0.5 Hz, 3H, major), 1.34 (td, $^3J_{HH}$ = 7.0 Hz, $^4J_{PH}$ = 0.5 Hz, 3H, minor), 2.28−2.75 (m, 2H + 2H, major + minor), 2.84−3.04 (m, 1H + 1H, major + minor), 3.02 (dd, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 7.2 Hz, 1H, minor), 3.03 (dd, $^2J_{HH}$ = 14.5, $^3J_{HH}$ = 5.5 Hz, 1H, major), 3.22 (dd, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 8.8 Hz, 1H, major), 3.23 (dd, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 7.5 Hz, 1H, minor), 3.85 (s, 3H + 3H, major + minor), 3.86 (s, 3H + 3H, major + minor), 4.02−4.28 (m, 6H + 6H, major + minor), 4.67−4.81 (m, 1H, major), 4.90−5.03 (m, 1H, minor), 6.62−6.82 (m, 3H + 3H, major + minor);

^{13}C NMR[30] ($CDCl_3$) δ 13.82 (s), 16.07 (d, $^3J_{PC}$ = 6.0 Hz), 30.04 (d, $^2J_{PC}$ = 4.5 Hz), 30.26 (d, $^2J_{PC}$ = 3.5 Hz), 39.46 (s), 39.60 (s), 41.77 (d, $^1J_{PC}$ = 130.2 Hz), 42.06 (d, $^1J_{PC}$ = 130.6 Hz), 55.67 (s), 55.70 (s), 61.76 (s), 61.82 (s), 62.99 (d, $^2J_{PC}$ = 6.5 Hz), 87.39 (d, $^3J_{PC}$ = 8.4 Hz), 87.67 (d, $^3J_{PC}$ = 15.0 Hz), 111.26 (s), 111.73 (s), 111.81 (s), 120.88 (s), 121.02 (s), 127.10 (s), 148.28 (s), 148.93 (s), 167.58 (d, $^2J_{PC}$ = 5.7 Hz), 167.73 (d, $^2J_{PC}$ = 6.3 Hz); ^{31}P NMR[30] ($CDCl_3$) δ 20.46 (major), 21.12 (minor). Anal. ($C_{19}H_{30}NO_9P$): C, H, N, P.

General Procedure for the Preparation of Ethyl 2-diethoxyphosphoryl-4-oxoalkanoates 10. A solution of 2-diethoxyphosphoryl-4-nitroalkanoate **9** (4.0 mmol) in MeOH (4 mL) was added to a solution of sodium methoxide prepared from sodium (100 mg, 4.4 mmol) and MeOH (8 mL), and the reaction mixture was stirred under argon atmosphere at room temperauture for 0.5 h. Then it was cooled to −60 °C and a cold (0 °C) solution of H_2SO_4 (2.4 mL) in MeOH (12 mL) was added. Stirring was continued for 2 h at −60 °C, and water (30 mL) was added at such a rate that the temperature did not exceed 4 °C. The solvent was evaporated at room temperauture under reduced pressure, and the residue was extracted with CH_2Cl_2 (4 × 20 mL). Combined extracts were dried ($MgSO_4$) and evaporated, and the crude products **10** were purified by column chromatography (eluent, $CHCl_3$/acetone = 95:5).

Ethyl 2-diethoxyphosphoryl-5-(3,4-dimethoxyphenyl)-4-oxopentanoate (10e): oil, 62% yield; IR (film) 1732, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, $^3J_{HH}$ = 7.0 Hz; 3H), 1.30 (td, $^3J_{HH}$ = 7.0 Hz, $^4J_{PH}$ = 0.5 Hz, 3H), 1.33 (td, $^3J_{HH}$ = 7.0 Hz, $^4J_{PH}$ = 0.5 Hz, 3H), 2.91 (ddd, $^2J_{HH}$ = 17.8 Hz, $^3J_{PH}$ = 8.8 Hz, $^3J_{HH}$ = 2.5 Hz, 1H), 3.28 (ddd, $^2J_{HH}$ = 17.8 Hz, $^3J_{HH}$ = 11.0 Hz, $^3J_{PH}$ = 6.0 Hz, 1H), 3.47 (ddd, $^2J_{PH}$ = 23.8 Hz, $^3J_{HH}$ = 11.0 Hz, $^3J_{HH}$ = 2.5 Hz, 1H), 3.68 (s, 2H), 3.86 (s, 6H), 4.03−4.24 (m, 6H), 6.67−6.85 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 13.00 (s), 15.25 (d, $^3J_{PC}$ = 6.0 Hz), 15.30 (d, $^3J_{PC}$ = 5.7 Hz), 37.72 (d, $^2J_{PC}$ = 2.0 Hz), 39.07 (d, $^1J_{PC}$ = 131.8 Hz), 48.08 (s), 54.85 (s), 54.89 (s), 60.63 (s), 61.88 (d, $^2J_{PC}$ = 6.2 Hz), 61.91 (d, $^2J_{PC}$ = 6.8 Hz), 110.49 (s), 111.52 (s), 120.68 (s), 125.09 (s) 147.24 (s), 148.11 (s), 167.24 (d, $^2J_{PC}$ = 5.7 Hz), 204.14 (d, $^3J_{PC}$ = 15.1 Hz); ^{31}P NMR ($CDCl_3$) δ 22.69. Anal. ($C_{19}H_{29}O_8P$) C, H, P.

General Procedure for the Preparation of 3-Diethoxyphosphoryl-3,4-dihydro-2(5*H*)-furanones 12. A solution of $NaBH_4$ (38 mg, 1.0 mmol) and NaOH (4 mg, 0.1 mmol) in H_2O (0.5 mL) was added to a solution of 2-diethoxyphosphoryl 4-oxoalkanoate **10** (2.0 mmol) in MeOH (2.5 mL), and the reaction mixture was stirred for 20 h at room temperauture. The mixture was acidified to pH ∼ 1 using 1 M HCl, and MeOH was evaporated under reduced pressure. The residue was extracted with $CHCl_3$ (3 × 15 mL), combined extracts were washed with H_2O (10 mL), dried ($MgSO_4$), and evaporated. Crude products were purified by column chromatography (eluent, $CHCl_3$/acetone = 95:5) to give **12** as mixtures of diastereoisomers.

3-Diethoxyphosphoryl-5-(3,4-dimethoxyphenylmethyl)-3,4-dihydro-2(5*H*)-furanone (12e): ratio of diastereoisomers = 60:40; oil, 70% yield; IR (film) 1772, 1260 cm^{-1}, ^{1}H NMR[30] ($CDCl_3$) δ 1.32 (t, $^3J_{HH}$ = 7.0 Hz, 6H + 6H, major + minor), 2.12−2.37 (m, 1H + 1H, major + minor), 2.42−2.69 (m, 1H + 1H, major + minor), 2.81−3.24 (m, 3H + 3H, major + minor), 3.87 (s, 6H + 6H, major + minor), 4.09−4.30 (m, 4H + 4H, major + minor), 4.64 (dq, $^3J_{HH}$ = 8.8 Hz, $^3J_{HH}$ = 6.5 Hz, 1H, minor), 4.91 (dq, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 6.9 Hz, 1H, major), 6.72−6.85 (m, 3H + 3H, major + minor); ^{13}C NMR[30] ($CDCl_3$) δ 16.35 (d, $^3J_{PC}$ = 5.6 Hz), 29.45 (d, $^2J_{PC}$ = 3.4 Hz), 29.64 (d, $^2J_{PC}$ = 3.1 Hz), 39.71 (d, $^1J_{PC}$ = 150.8 Hz), 39.89 (d, $^1J_{PC}$ = 139.6 Hz), 40.53 (s), 40.61 (s), 55.9 (s), 62.73 (d, $^2J_{PC}$ = 6.7 Hz), 62.92 (d, $^2J_{PC}$ = 6.7 Hz), 63.51 (d, $^2J_{PC}$ = 6.7 Hz), 63.56 (d, $^2J_{PC}$ = 6.5 Hz), 79.84 (d, $^3J_{PC}$ = 3.2 Hz), 79.95 (d, $^3J_{PC}$ = 10.5 Hz), 111.45 (s), 112.67 (s), 112.75 (s), 121.52 (s), 121.67 (s), 127.84 (s), 128.31 (s), 148.09 (s), 148.16 (s), 149.02 (s), 171.28 (s), 171.48 (d, $^2J_{PC}$ = 4.4 Hz); ^{31}P NMR[30] ($CDCl_3$) δ 20.82 (minor), 21.03 (major). Anal. ($C_{17}H_{25}O_7P$) C, H, P.

General Procedure for the Preparation of 3-Methylenedihydro-2-furanones 13. A mixture of 3-diethoxyphosphoryltetrahydro-2-furanone **12** (1.0 mmol), K_2CO_3 (0.415 g, 3 mmol), and aqueous 36% formaldehyde solution (0.54 mL, 7.0 mmol) was stirred at 0−4 °C for 15 min. The mixture was next extracted with Et_2O (4 × 15 mL), dried ($MgSO_4$), and evaporated. Residue was purified by column chromatography (eluent, $CHCl_3$) to give pure **13**.

5-(3,4-Dimethoxyphenylmethyl)-3-methylene-4,5-dihydrofuran-2-one (13e): oil, 48% yield; IR (film) 1772, 1664 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.59 (ddt, $^2J_{HH}$ = 17.0 Hz, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 2.8 Hz, 1H), 2.81 (dd, $^2J_{HH}$ = 14.3 Hz, $^3J_{HH}$ = 6.0 Hz, 1H), 2.89 (ddt, $^2J_{HH}$ = 17.0 Hz, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 2.8 Hz, 1H), 2.95 (dd, $^2J_{HH}$ = 14.3 Hz, $^3J_{HH}$ = 6.0 Hz, 1H), 3.79 (s, 3H), 3.80 (s, 3H), 4.69 (dq, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 6.0 Hz, 1H), 5.49 (t, $^4J_{HH}$ = 2.8 Hz, 1H), 6.10 (t, $^4J_{HH}$ = 2.8 Hz, 1H), 6,65−6,78 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 32.50 (s), 41.27 (s), 55.90 (s), 55.92 (s), 77.21 (s), 111.32 (s), 112.74 (s), 121.68 (s), 127.89 (s), 148.14 (s), 149.02 (s), 121.98 (s), 134.37 (s), 170.13 (s). Anal. ($C_{14}H_{16}O_4$) C, H.

General Procedure for the Preparation of 3-Diethoxyphosphorylpyrrolidyn-2-ones 15. A mixture of 2-diethoxyphosphoryl 4-nitroalkanoate **9** (2.5 mmol), 10% Pd/C (0.118 g), and $HCOONH_4$ (0.843 g; 13.4 mmol) in MeOH (15 mL) and THF (15 mL) was stirred at 0−4 °C for 2 h, warmed to room temperature and stirred for an additional 22 h. The reaction mixture was filtered through a Celite bed, filtrate was evaporated, $CHCl_3$ (40 mL) was added to the residue, and chloroform solution was filtered through a Celite bed. Evaporation of the chloroform gave crude product that was purified by column chromatography (eluent, $CHCl_3$/MeOH = 97:3).

3-Diethoxyphosphoryl-5-(3,4-dimethoxyphenylmethyl)pyrrolidyn-2-one (15e): ratio of diastereoisomers 60:40; oil, 70% yield; IR (film) 3120, 1712, 1232 cm^{-1}; ^{1}H NMR[30] ($CDCl_3$) δ 1.29 (t, $^3J_{HH}$ = 7.0 Hz, 6H, major), 1.30 (t, $^3J_{HH}$ = 7.0 Hz, 3H, minor), 1.32 (t, $^3J_{HH}$ = 7.0 Hz, 3H, minor), 1.84−2.92 (m, 4H + 4H, major + minor), 3.01 (dd, $^2J_{HH}$ = 13.5 Hz, $^3J_{HH}$ = 3.5 Hz, 1H, major), 3.28 (dd, $^2J_{HH}$ = 13.5 Hz, $^3J_{HH}$ = 3.5 Hz, 1H, minor), 3.75−3.88 (m, 6H + 6H, major + minor), 3.95−4.23 (m, 5H + 5H, major + minor), 6.70−6.82 (m, 3H + 3H, major + minor), 9.50 (bs, 1H + 1H, major + minor); ^{13}C NMR[30] ($CDCl_3$) δ 15.50−16.20 (m), 22.30 (d, $^2J_{PC}$ = 3.6 Hz), 22.85 (d, $^2J_{PC}$ = 3.8 Hz), 36.36 (s), 38.0 (s), 36.79 (d, $^1J_{PC}$ = 139.8 Hz), 36.18 (d, $^1J_{PC}$ = 149.5 Hz), 55.54 (s), 55.71 (s), 58.31 (d, $^3J_{PC}$ = 1.2 Hz), 59.31 (d, $^3J_{PC}$ = 7.3 Hz), 62.12 (d, $^2J_{PC}$ = 6.5 Hz), 62.21 (d, $^2J_{PC}$ = 6.7 Hz), 62.95 (d, $^2J_{PC}$ = 6.5 Hz), 63.14 (d, $^2J_{PC}$ = 6.5 Hz), 111.06 (s), 112.45 (s), 112.57 (s), 121.26 (s), 121.45 (s), 127.94 (s), 128.78 (s), 147.51, 147.64 (s), 148.68 (s), 163.63 (d, $^2J_{PC}$ = 3.8 Hz), 165.53 (d, $^2J_{PC}$ = 3.8 Hz); ^{31}P NMR[30] ($CDCl_3$) δ 23.59 (minor) 24.45 (major). Anal. ($C_{17}H_{26}NO_6P$) C, H, N.

General Procedure for the Preparation of 3-Methylenepyrrolidyn-2-ones 16. A solution of 3-diethoxyphosphorylpyrrolidyn-2-one (1.0 mmol) in THF (7 mL) was added to a suspension of NaH (0.025 g, 1.05 mmol) in THF (3 mL), and the reaction mixture was stirred at room temperature for 0.5 h. Next, paraformaldehyde (0.033 g, 1.1 mmol) was added in one portion, and the mixture was refluxed for 1 h and cooled to 0−4 °C. Then H_2O (3 mL) was added, THF was evaporated under reduced pressure, and the residue was extracted with CH_2Cl_2 (3 × 15 mL). Combined organic extracts were washed with H_2O (5 mL), dried ($MgSO_4$), and evaporated to give crude **15** that were purified by column chromatography (eluent, $CHCl_3$).

5-(3,4-Dimethoxyphenylmethyl)-3-methylenepyrrolidyn-2-one (16e): oil, 40% yield; IR (film) 3100, 1684, 1662 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (ddt, $^2J_{HH}$ = 17.0 Hz, $^3J_{HH}$ = 4.0 Hz, $^4J_{HH}$ = 2.2 Hz, 1H), 2.73 (ddt, $^2J_{HH}$ = 17.0 Hz, $^3J_{HH}$ = 7.5 Hz, $^4J_{HH}$ = 2.2 Hz, 1H), 2.82 (dd, $^2J_{HH}$ = 13.8 Hz, $^3J_{HH}$ = 7.6 Hz, 1H), 3.15 (dd, $^2J_{HH}$ = 13.8 Hz, $^3J_{HH}$ = 3.4 Hz, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 4.02−4.20 (m, 1H), 5.18 (t, $^4J_{HH}$ = 2.2 Hz, 1H), 5.83 (t, $^4J_{HH}$ = 2.2 Hz, 1H), 6.53−6.57 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 28.13 (s), 37.41 (s), 55.81 (s), 58.20 (s), 115.92 (s), 111.14 (s), 112.69 (s), 121.72 (s), 128.17 (s), 147.89 (s), 148.93 (s), 135.58 (s), 163.89 (s). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

General Procedure for the Preparation of 3-Alkylidene-5-benzyldihydrofuran-2-ones 18. A solution of **12d**

(0.310 g, 1.0 mmol) in benzene (5 mL) was added to a suspension of NaH (0.033 g, 1.1 mmol) in benzene (3 mL), and the reaction mixture was stirred at room temperature for 0.5 h. Next, appropriate aldehyde (1.1 mmol) was added and the mixture was refluxed for 4 h. After cooling to room temperature, water (5 mL) was added, layers were separated, and water layer was washed with benzene (2 × 10 mL). Combined organic extracts were dried ($MgSO_4$) and evaporated to give crude **18** that was purified by column chromatography (eluent, $CHCl_3$/acetone = 95:5).

5-Benzyl-3-(2-methylpropylidene)-4,5-dihydrofuran-2-one (18a): ratio of diastereoisomers *E*/*Z* = 30:70, oil, 78% yield; IR (film) 1752, 1672 cm^{-1}; ^1H NMR[30] ($CDCl_3$) δ 0.96 (d, $^3J_{HH}$ = 6.8 Hz, 3H, major), 0,97 (d, $^3J_{HH}$ = 6.8 Hz, 3H, major), 1.01 (d, $^3J_{HH}$ = 6.8 Hz, 3H, minor), 1.02 (d, $^3J_{HH}$ = 6.8 Hz, 3H, minor), 2.34 (d sept, $^3J_{HH}$ = 9.8 Hz, $^3J_{HH}$ = 6.8 Hz, 1H, minor), 2.55 (ddd, $^2J_{HH}$ = 16.5 Hz, $^3J_{HH}$ = 6.5 Hz, $^4J_{HH}$ = 3.0 Hz, 1H, minor), 2.60 (ddd, $^2J_{HH}$ = 17.8 Hz, $^3J_{HH}$ = 6.5 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, major), 2.76−2.94 (m, 2H + 2H, major + minor), 3.08 (dd, $^2J_{HH}$ = 14.0 Hz, $^3J_{HH}$ = 6.5 Hz, 1H, major), 3.12 (dd, $^2J_{HH}$ = 13.8 Hz, $^3J_{HH}$ = 5.5 Hz, 1H, minor), 3.70 (d sept, $^3J_{HH}$ = 10.0 Hz, $^3J_{HH}$ = 6.8 Hz, 1H, major), 4.69 (dq, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 6.5 Hz, 1H, major), 4.67−4.83 (m, 1H, minor), 5.90 (dt, $^3J_{HH}$ = 10.0 Hz, $^4J_{HH}$ = 2.5 Hz, 1H, major), 6.51 (dt, $^3J_{HH}$ = 9.8 Hz, $^4J_{HH}$ = 3.0 Hz, 1H, minor), 7.20−7.37 (m, 5H + 5H, major + minor); ^{13}C NMR[30] ($CDCl_3$) δ 21.34 (s), 21.37 (s), 22.24 (s) 22.29 (s), 26.14 (s), 29.63 (s), 30.00 (s), 34.18 (s), 41.64 (s), 41.93(s), 77.10 (s), 77.37 (s), 122.03 (s), 123.71 (s), 126.75 (s), 126.81 (s), 128.45 (s), 128.50 (s), 129.40 (s), 135.57 (s), 135.72 (s), 146.18 (s), 150.45 (s), 169.33 (s), 171.01 (s). Anal. ($C_{15}H_{18}O_2$) C, H.

Cells and Cytotoxicity Assays. Mouse leukemia L-1210 cells were cultured in RPMI 1640 medium (Sigma) supplemented with heat-inactivated 10% fetal bovine serum (Gibco) in a 5% CO_2/95% air atmosphere. Cytotoxic effects were assayed by measuring the inhibitory effects on L-1210 cell proliferation. In this assay, cells were seeded in 2 mL aliquots in 6 mL tissue culture tubes (Corning) at a concentration of 5 × 10^3 cells/mL and exposed to drugs for 72 h at 37 °C. The cell number relative to control was then determined by the colorimetric tetrazolium dye method.[31]

Human promyelocytic leukemia HL-60 cells and lymphoblastic NALM-6 cells were grown in RPMI 1640 (Cambrex, Belgium) supplemented with heat-inactivated 10% fetal bovine serum (Cytogen, Germany), penicillin (100 U/mL), and streptomycin (100 μg/mL) under a 5% CO_2/95% air atmosphere. Exponentially growing HL-60 and NALM-6 cells were seeded at 0.2 × 10^6 cells per each well onto a 24-well plate (Nunc, Denmark), and cells were then exposed to the tested compounds or carboplatin for 72 h. The number of viable cells was counted in Bürker hemocytometer using the trypan-blue exclusion assay. Concentration−response curves were determined. IC_{50} values (the concentration of the tested compounds required to reduce a fraction of surviving cells to 50% of that observed in the control cells) were calculated from dose−response curves and used as an index of cellular sensitivity to a given treatment. All the data were expressed as the mean ± SD.

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Supporting Information Available: Elemental analysis results, IR, and full ^1H, ^{13}C, and ^{31}P NMR spectra for compounds **9a**−**d**, **10a**−**d**, **12a**−**d**, and **15a**−**d** and IR and full ^1H and ^{13}C NMR spectra for compounds **13a**−**d**, **16a**−**d**, and **18b**,**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hoffmann, H. M. R.; Rabe, J. Synthesis and Biological Activity of α-Methylene-γ-butyrolactones. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 94−110.

(2) Ballini, R.; Bosica, G.; Livi, D. A New Synthesis of *exo*-Methylene Butyrolactones from Nitroalkanes. *Synthesis* **2001**, 1519−1522 and references therein.

(3) GowriSankar, S.; Lee, Ch. G.; Kim, J. N. Facile Synthesis of Lactones and Dihydronaphthalenes from Methyl 2-Isobutenyl (or 2-Isopentenyl) Cinnamates as the Common Intermediates. *Tetrahedron Lett.* **2004**, *45*, 6949−6953 and references therein.

(4) Dirsch, V. M.; Stuppner, H.; Ellmerer-Mûller, E. P.; Vollmar, A. M. Structural Requirementes of Sesquiterpene Lactones To Inhibit LPS-Induced Nitric Oxide Synthesis in RAW 264. 7 Macrophages. *Bioorg. Med. Chem.* **2000**, *8*, 2747−2753.

(5) Heilmann, J.; Wasescha, M. R.; Schmidt, T. J. The influence of Glutathione and Cysteine Levels on the Cytotoxicity of Helenanolide Type Sesquiterpene Lactones against KB Cells. *Bioorg. Med. Chem.* **2001**, *9*, 2189−2194.

(6) Patel, B. P.; Waddel, T. G.; Pagni, R. M. Explaining Photodermatosis: Cyclopentenone vs α-Methylene-γ-lactone Natural Products. *Fitoterapia* **2001**, *72*, 511−515.

(7) González, A. G.; Silva, M. H.; Padrón, J. I.; León, F.; Reyes, E.; Álvarez-Mon, M.; Pivel, J. P.; Quintana, J.; Estévez, F.; Bermejo, J. Synthesis and Antiproliferative Activity of a New Compound Containing an α-Methylene-γ-Lactone Group. *J. Med. Chem.* **2002**, *45*, 2358−2361 and references therein.

(8) Wang, T.-Ch.; Zhao, Y.-L.; Kuo, D.-H. Synthesis and Vasorelaxing Evaluation of α-Methylidene-γ-butyrolactone Bearing Quinolin-2(1*H*)-one and 3,4-Dihydroquinolin-2(1*H*)-one Derivatives. *Eur. J. Med. Chem.* **2001**, *36*, 909−914.

(9) Lee, K.-H.; Huang, B.-R. Synthesis and Cytotoxic Evaluation of α-Methylene-γ-butyrolactone Bearing Naphthalene and Naphthol-[2,1-*b*]furan Derivatives. *Eur. J. Med. Chem.* **2002**, *37*, 333−338.

(10) Paintner, F. F.; Allmendinger, L.; Bauschke, G.; Berns, C.; Heisig, P. Synthesis and Antimicrobial Activity of Tetrodecamycin Partial Structures. *Bioorg. Med. Chem.* **2003**, *11*, 2823−2833.

(11) Baraldi, P. G.; Núnez, M. C.; Tabrizi, M. A.; Clerq, E.; Balzarini, J.; Bermejo, J.; Estévez, F.; Romagnoli, R. Design, Synthesis and Biological Evaluation of Hybrid Molecules Containing α-Methylene-γ-butyrolactanes and Polypyrrole Minor Groove Binders. *J. Med. Chem.* **2004**, *47*, 2877−2886.

(12) Cardellina, J. H.; Moore, R. E. The Structures of Pukeleimides A, B, D, E, F, and G. *Tetrahedron Lett.* **1979**, *22*, 2007−2010.

(13) Natio, T.; Honda, Y.; Miyata, O.; Ninomiya. T. Total Synthesis of (±) Anatine and (±) Isoanatine via Thiyl Radical Addition−Cyclization Reaction. *Chem. Pharm. Bull.* **1993**, *41*, 217−219.

(14) Belaud, Ch.; Roussakis, Ch.; Letourneux, Y.; El Alami, N.; Villieras, J. Synthesis of Potential Cytotoxic α-Methylene-γ-Lactams. *Synth. Commun.* **1985**, *15*, 1233−1243.

(15) Janecki, T.; Błaszczyk, E. A Convenient Synthesis of 3-Alkylidenetetrahydro-2-furanones from 3-Diethoxyphosphoryl-2,5-dihydro-2-furanones. *Synthesis* **2001**, 403−408.

(16) Janecki, T.; Błaszczyk, E. Stereocontrolled Synthesis of 5-(1′-Hydroxyalkyl)-3-methylidenetetrahydro-2-furanones. *Tetrahedron Lett.* **2001**, *42*, 2919−2922.

(17) Janecki, T.; Błaszczyk, E.; Studzian, K.; Różalski, M.; Krajewska, U.; Janecka, A. New Stereocontrolled Synthesis and Biological Evaluation of 5-(1-Hydroxyalkyl)-3-methylidenetetrahydro-2-furanones as Potential Cytotoxic Agents. *J. Med. Chem.* **2002**, *45*, 1142−1145.

(18) For a preliminary communication, see the following: Janecki, T.; Krawczyk, H.; Błaszczyk, E. 2-Diethoxyphosphoryl-4-nitroalkanoates—A Versatile Intermediates in the Synthesis of α-Alkylidene-γ-lactones and Lactams. *Synlett* **2004**, 2685−2688.

(19) Villieras, J.; Rambaud, M. Wittig−Horner Reactions in Heterogeneous Media; V. An Efficient Synthesis of α-Methylenecarboxylic Esters and α-Methyleneketones under Mild Conditions. *Synthesis* **1984**, 406−408.

(20) Consorti, C. S.; Ebeling, G.; Dupont, J. Carbonylation of Alkynals Catalyzed by Pd(II)/2-$PyPPh_2$ Dissolved in Organic Solvents and in Ionic Liquids: A Facile Entry to α-Methylene-γ- and δ-Lactones. *Tetrahedron Lett.* **2002**, *43*, 753−755.

(21) Talaga, P.; Schaeffer, M.; Benezra, C.; Stampf, J.-L. A New Synthesis of γ-Substituted α-Methylene-γ-butyrolactones (2-Methylene-4-alkanolides) Using Catalysis by $SnCl_2$/Amberlist 15. *Synthesis* **1990**, 530−530.

(22) Macomber, R. S. A. *Complete Introduction to Modern NMR Spectrascopy*; John Wiley & Sons: New York, 1998; p 416.

(23) Van Beylen, M.; Samyn, C. Synthesis, Polimerization and Copolimerization of 5-Methyl-3-methylene-2-pyrrolidone. *Makromol. Chem.* **1990**, *191*, 2485−2489.

(24) Horiike, M.; Kim, S.; Yamaguchi, I.; Sakata, K. Agrochemical Insecticides Containing γ-Butyrolactone Derivatives. *Jpn. Kokai Tokkyo Koho* JP 11 199,410[99 199,410] (*Chem. Abstr.* **1999**, *131*, 112724u).

(25) Canetta, R.; Goodlow, J.; Sinaldone, L. Pharamcologic Characteristic of Carboplatin: Clinical Experience, Current Perspectives and Future Directions. *Saunders* **1990**, 19−38.
(26) Nair, V.; Sinhabadu, A. K. Carbohydrate Models of α-Methylene-γ-butyrolactones. *J. Org. Chem.* **1980**, *45*, 1893−1897 and references therein.
(27) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. Tumor Inhibitors 69. Structure−Cytotoxicity Relationship among the Sesquiterpene Lactones. *J. Med. Chem.* **1971**, *14*, 1147−1152.
(28) Lipophilicities were calculated in the standard way using the following: *HyperChem Release 7.5 for Windows Molecular Modeling System*; Hypercube Inc.: Gainesville, FL, 2003.
(29) Bhattachrajya, A.; Mukhopadhyay, R.; Pakrashi, S. C. Sodium Borohydride Reduction of Nitrostyrenes by Reverse Addition: A Simple and Efficient Method for the Large-Scale Preparation of Phenylnitroethanes. *Synthesis* **1985**, 886−887.
(30) Values for specific diastereoisomer were taken from the spectrum of a mixture of diastereoisomers.
(31) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of Tetrazolium-Based Semiautomatic Colorimetric Assay: Assessment of Chemosensitivity Testing. *Cancer Res*. **1987**, *47*, 936−942.

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