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4-Methylideneisoxazolidin-5-ones—A new class of α-methylidene-γ-lactones with high cytostatic activity

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Abstract—A novel, general method of synthesis of 4-methylideneisoxazolidin-5-ones **10** is described. The target compounds were synthesized starting from ethyl 2-diethoxyphosphoryl-2-alkenoates **6** or dicyclohexylammonium 4-diethoxyphosphoryl-2-alkenoates **7**. Addition of *N*-methylhydroxylamine hydrochloride to these Michael acceptors, lactonization to 4-diethoxyphosphorylisoxazolidin-5-ones **9**, and Horner–Wadsworth–Emmons olefination of formaldehyde using **9** gave the title isoxazolidinones **10**. All obtained compounds were tested against L-1210, HL-60, and NALM-6 leukemia cell lines. Several isoxazolidinones **10** were found to be very potent with $IC_{50} < 1$ μM. The highest cytostatic activity against HL-60 was observed for **10a** and against NALM-6 for **10b** with IC_{50} values of 0.74 and 0.34 μM, respectively.

α-Methylidene-γ-lactones **1** are a well-known group of natural and synthetic compounds which possess a wide spectrum of biological activities such as cytotoxic, antimicrobial or antifungal.[1] This activity is mainly associated with the α,β-unsaturated ester moiety which can act as a Michael acceptor in the reactions with bionucleophiles, especially with sulfhydryl-containing enzymes and other functional proteins.[2] Also light-activated 2 + 2 additions of α-methylidene-γ-lactones to the DNA base, thymine, have been recently described.[3] On the other hand, lactams **2**, which are the nitrogen analogs of α-methylidene-γ-lactones, are much less common in nature[4] and according to a few reports available, also much less active against the cancer cells.[5]

In our search for highly cytotoxic, yet structurally simple, α-methylidene-γ-lactones as possible drug candidates,[5b,6] we envisioned the synthesis of 4-methylideneisoxazolidin-5-ones **3**, where one of the carbon atoms in the lactone ring is replaced by a nitrogen atom. To the best of our knowledge compounds of this structure are essentially unknown. The only compounds of the related structure mentioned in the literature were 4-arylaminomethylideneisoxazolidin-5-ones **4**[7] and isoxazolidinone of structure **5**.[8] Therefore, the development of a general synthetic route to **3** appeared to us as an important and challenging endeavor. Here, we report on our initial investigations in this area.

Synthesis of the target isoxazolidinones **10** was executed in a two-step reaction sequence shown in Scheme 1.

Starting ethyl 2-diethoxyphosphoryl-2-alkenoates **6**[9] or dicyclohexylammonium 4-diethoxyphosphoryl-2-alkenoates **7**[10] were prepared according to the methods described in the literature. These two Michael acceptors were next tested in the reaction with *N*-methylhydroxylamine hydrochloride. Adducts **8a**–**i** formed in these additions were not isolated and lactonized spontaneously to 4-diethoxyphosphorylisoxazolidin-5-ones **9a**–**i**. Crude products were purified by column chromatography. Yields of this step were only moderate or poor and are given in Table 1.[11] Esters **6a**, **b**, and **i** gave expected isoxazolidinones **9a**, **b**, and **i** in poor yields,

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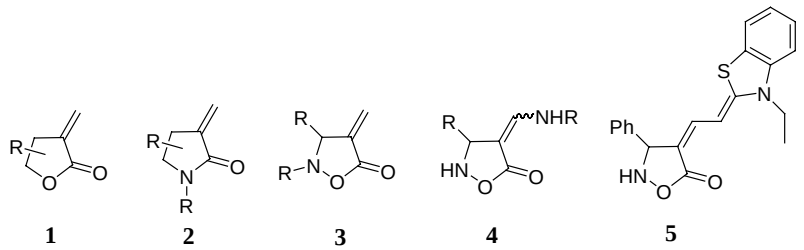
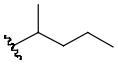
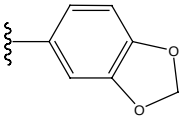


1 **2** **3** **4** **5**

whereas ammonium salts **7a**, **b**, and **i** in these reactions were even less effective (10–18% yield). Also ester **6c** gave **9c** with very low 10% yield. On the other hand, ammonium salts **7c**–**h** provided isoxazolidinones **9c**–**h** in moderate yields. Variations in reaction time and/or temperature did not improve the yields of **9**. Apparently, the conditions of this step still need to be optimized or other Michael acceptors should be tested. Compounds **9a**–**i** were obtained as single diastereoisomers. Only **9b** was formed as a mixture of diastereoisomers in close to 1:1 ratio, due to the additional stereogenic center in the R^2 substituent. Because in this type of Michael additions thermodynamic control is usually observed, we anticipated that *trans* isomers should be formed.[12] Pleasingly, structures and *trans* configurations of all obtained isoxazolidinones **9** were confirmed by ^1H, ^{13}C, and ^{31}P NMR data. In particular, $^3J_{H3\text{-}H4}$ and $^3J_{P\text{-}C}$ coupling constants were diagnostic, for example, for **9c** these coupling constants were 12.0 and 0 Hz, respectively, indicating the *trans*-relationship between protons H-3 and H-4.[13] Isoxazolidin-5-ones **9** when used in the Horner–Wadsworth–Emmons olefination of formaldehyde, in the presence of K_2CO_3 as a base, gave expected 4-methylideneisoxazolidin-5-ones **10** in good to excellent yields (Table 1).[14] All target compounds were purified by silica gel column chromatography and characterized by IR, ^1H, and ^{13}C NMR spectroscopy.

Table 1. Synthesis of 4-diethoxyphosphorylisoxazolidin-5-ones **9a**–**i** and 4-metylideneisoxazolidin-5-ones **10a**–**i**

Compound	Michael acceptor	R^2	**9** yield (%)[a]	**10** yield (%)[a]
a	**6**	*i*-Pr	24	46
b	**6**		25	80
c	**7**	Ph	36	57
d	**7**	*p*-MePh	45	53
e	**7**	*p*-MeOPh	34	83
f	**7**	*p*-BrPh	32	89
g	**7**	*p*-NO_2Ph	41	70
h	**7**		32	64
i	**6**	1-Naphthyl	25	55

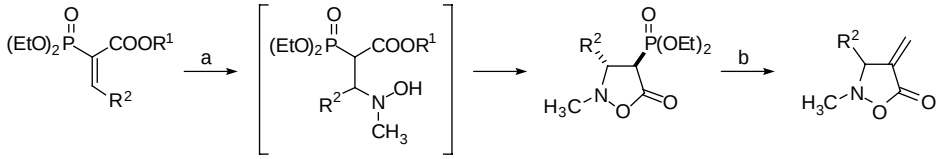
[a] Yields of pure, isolated products based on **6**, **7** or **9**, respectively.

Table 2. Cytostatic activity of 4-methylideneisoxazalidin-5-ones **10a**–**i**

Compound	Cytostatic activity IC_{50} (μM)[a]		
	L-1210	HL-60	NALM-6
10a	2.63 ± 0.31	0.74 ± 0.13	4.16 ± 0.24
10b	1.90 ± 0.15	4.70 ± 0.8	0.34 ± 0.04
10c	5.5 ± 0.4	34.8 ± 3.6	4.96 ± 0.31
10d	0.8 ± 0.01	5.4 ± 1.1	5.8 ± 0.5
10e	3.5 ± 0.21	5.4 ± 0.3	5.5 ± 0.4
10f	0.7 ± 0.02	5.1 ± 0.5	4.6 ± 0.5
10g	0.8 ± 0.02	7.4 ± 0.3	5.2 ± 0.4
10h	7.0 ± 0.3	6.6 ± 1.4	4.2 ± 1.2
10i	3.2 ± 0.4	5.6 ± 0.8	4.2 ± 1.0
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.

The final products **10a**–**i** were evaluated for in vitro cytostatic activity against L-1210 mouse leukemia[15] as well as HL-60 and NALM-6 human leukemia[16] cell lines. The activities, expressed as IC_{50} values (the concentration in μM required to inhibit tumor cell proliferation by 50% after 72 h of exposure of the cells to a tested compound), are given in Table 2. Carboplatin[17] was used as a reference compound. The results of the biological evaluation turned out to be extremely gratifying. Except for the derivative **10c**, all new compounds showed IC_{50} values against all three tested cell lines lower than 7.4 μM and can be considered highly potent according to *Kupchan's* classification ($IC_{50} \leqslant 15$ μM).[18] Activities of all tested compounds against the L-1210 cell line were considerably higher compared to that of the standard drug carboplatin. For the HL-60 cell line activities were usually comparable to, and for the



6, R^1 = Et
7, $R^1 = NH_2(C_6H_{11})_2$

8a–**i** **9a**–**i** **10a**–**i**

Scheme 1. Reagents and conditions: (a) $CH_3NHOH \times HCl$, CH_2Cl_2, rt 12 h; (b) K_2CO_3, 36% formalin, THF, 0 °C to rt 45 min.

NALM-6 cell line lower than that of, the activity of carboplatin. Furthermore, two of the obtained compounds, **10a** and **10b**, showed significantly improved activities against HL-60 and NALM-6 cell lines, respectively, in comparison with those of the other compounds in the series. This high biological activity might be due to the non-aromatic character of the substituents R^2 in **10a**, **b**. Determined IC_{50} values of 0.74 μM for **10a** and 0.34 μM for **10b** make these compounds the target for further biological evaluations as well as make them very interesting leads in the search for even more potent anticancer agents.

In conclusion, a simple and general method for the synthesis of, so far essentially unknown, 4-methylideneisoxazolidin-5-ones **10** has been developed. These compounds turned out to be very potent against mouse L-1210 as well as human HL-60 and NALM-6 leukemia cell lines. Currently, more specific biological evaluations of the most potent isoxazolidinones **10** are in progress. Also further synthetic efforts have been undertaken to improve the yields of Michael addition/lactonization step and to broaden the scope of the method.

Acknowledgment

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11. General procedure for the preparation of 4-diethoxyphosphorylisoxazolidin-5-ones **9a**, **b**, **i** from ethyl 2-diethoxyphosphoryl-2-alkenoates **6a**, **b**, **i**: To a suspension of MeNHOH × HCl (0.27 g, 3.23 mmol) in anhydrous CH_2Cl_2 (10 mL), ethyl 2-(diethoxyphosphoryl)acrylate **6** (2.16 mmol) and anhydrous triethylamine (0.45 mL, 3.23 mmol) were added. The mixture was stirred at room temperature for 12 h, followed by the addition of anhydrous $ZnCl_2$ (0.44 g, 3.23 mmol). Stirring was continued for another 8 h and the reaction was quenched with water (40 mL). The mixture was extracted with CH_2Cl_2 (3× 15 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give pure **9**. Sample data: 4-diethoxyphosphoryl-2-methyl-3-isopropylisoxazolidin-5-one **9a**: eluent EtOAc/hexane 7/3, oil; IR (film, cm^{-1}): 1772, 1256, 1024; ^1H NMR (250 MHz, $CDCl_3$): $\delta = 0.97$ (d, 3 H, $^3J_{HH} = 6.7$ Hz, CH_3CH), 0.98 (d, 3H, $^3J_{HH} = 6.7$ Hz, CH_3CH), 1.38 (td, 3H, $^3J_{HH} = 7.0$ Hz, $^4J_{PH} = 0.5$ Hz, CH_3CH$_2$OP), 1.39 (td, 3H, $^3J_{HH} = 7.0$ Hz, $^4J_{PH} = 0.5$ Hz, CH_3CH$_2$OP), 1.78–1.97 (m, 1H, (CH$_3$)$_2$CH), 3.03 (s, 3H, CH_3N), 3.13 (dd, 1H, $^2J_{PH} = 25.75$ Hz, $^3J_{HH} = 5.0$ Hz, PCHCH), 3.39 (ddd, 1H, $^3J_{PH} = 19.2$ Hz, $^3J_{HH} = 5.0$ Hz, $^3J_{HH} = 5.0$ Hz, PCHCH), 4.15–4.32 (m, 4H, (CH$_3$CH_2O)$_2$); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 16.06$ (d, $^3J_{CP} = 6.3$ Hz, CH$_3$CH$_2$O), 16.12 (d, $^3J_{CP} = 6.1$ Hz, CH$_3$CH$_2$O), 17.03 (s, CH$_3$CH), 17.93 (s, CH$_3$CH), 32.75 (d, $^3J_{CP} = 8.9$ Hz, (CH$_3$)$_2C$H), 43.80 (d, $^1J_{CP} = 142.2$ Hz, PCHCH), 48.43 (s, CH$_3$N), 62.76 (d,$^2J_{CP} = 6.9$ Hz, CH$_3C$H$_2$O), 63.99 (d, $^2J_{CP} = 6.7$ Hz, CH$_3C$H$_2$O), 72.17 (d, $^2J_{CP} = 2.1$ Hz, PCHCH), 171.22 (d$^2J_{CP} = 3.1$ Hz, C=O); ^{31}P NMR (101 MHz, $CDCl_3$): $\delta = 19.05$; Anal. Calcd for $C_{11}H_{22}NO_5P$: C, 47.31; H, 7.94; N, 5.02; P, 11.09. Found: C, 47.54; H, 7.82; N, 5.28; P, 10.79. General procedure for the preparation of 4-diethoxyphosphorylisoxazolidin-5-ones **9c**–**h** from dicyclohexylammonium 4-diethoxyphosphoryl-2-alkenoates **7c**–**h**: to a suspension of MeNHOH × HCl (0.261 g, 3.13 mmol) in anhydrous CH_2Cl_2 (10 mL), dicyclohexylammonium (*E*)-2-(diethoxyphosphoryl)acrylate **7** (2.08 mmol) was added. The mixture was stirred at room temperature for 12 h. After that time, the mixture was filtered and quenched with saturated $NaHCO_3$ (20 mL). The mixture was extracted with CH_2Cl_2 (3× 15 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give pure **9**. Sample data: 4-diethoxyphosphoryl-2-methyl-3-(4-methylphenyl)isoxazolidin-5-one **9d**: eluent EtOAc/hexane 1/1, white prisms (from Et_2O) mp 79–81 °C; IR (KBr, cm^{-1}): 1780, 1260, 1060; ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.09$ (t, 3H, $^3J_{HH} = 7.0$ Hz, CH_3CH$_2$O), 1.24 (t, 3H, $^3J_{HH} = 7.0$ Hz, CH_3CH$_2$O), 2.36 (s, 3H, CH_3C$_6$H$_4$), 2.78 (s, 3H, CH_3N), 3.56 (dd, 1H, $^2J_{PH} = 21.5$ Hz, $^3J_{HH} = 12.0$ Hz, PCHCH), 3.86–4.02 (m, 2H, CH$_3$CH_2O), 4.08–4.25 (m, 2H, CH$_3$CH_2O), 4.32 (dd, 1H, $^3J_{PH} = 12.0$ Hz, $^3J_{HH} = 12.0$ Hz, PCHCH), 7.17–7.34 (m, 4H, C$_6H_4$); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 15.89$ (d, $^3J_{CP} = 6.3$ Hz, CH$_3$CH$_2$O), 16.16 (d, $^3J_{CP} = 6.2$ Hz, CH$_3$CH$_2$O), 21.11 (s,CH$_3$C$_6$H$_4$), 43.87 (s, CH$_3$N), 50.11 (d, $^1J_{CP} = 152.3$ Hz, PCHCH), 62.60 (d, $^2J_{CP} = 6.8$ Hz, CH$_3C$H$_2$O), 63.80 (d, $^2J_{CP} = 6.3$ Hz, CH$_3C$H$_2$O), 74.58 (s, PCHCH), 128.10 (s, 2C, C_6H_4), 129.40 (s, 2C, C_6H_4), 131.78 (s, C_6H_4), 139.20 (s, C_6H_4), 168.11 (s, C=O); ^{31}P NMR (101 MHz, $CDCl_3$): $\delta = 16.79$; Anal. Calcd for $C_{15}H_{22}NO_5P$: C, 55.04; H, 6.77; N, 4.28; P, 9.46. Found: C, 55.18; H, 6.61; N, 4.45; P,9.60.
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14. General procedure for the preparation of 2-methyl-4-methylideneisoxazolidin-5-ones **10a–i** from 4-diethoxyphosphorylisoxazolidin-5-ones **9a–i**: to a solution of 2-methyl-4-diethoxyphosphorylisoxazolidin-5-one **9** (0.61 mmol) in THF (5 mL) an aqueous 36% solution of formaldehyde (0.33 ml, 4.3 mmol) was added. The mixture was cooled to 0–5 °C and the solution of potassium carbonate (0.253 g, 1.83 mmol) in H_2O (2 mL) was added. The solution was stirred for 45 min at room temperature (monitored by TLC) and then was extracted with Et_2O (3× 15 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The remaining oil was purified by column chromatography on silica gel to give pure **10**. Sample data: 2-methyl-4-methylene-3-(4-methylphenyl)isoxazolidin-5-one **10d**, eluent $CHCl_3$, white prisms (from EtOAc/hexane) mp 43–45 °C; IR (KBr, cm^{-1}): 1768, 1672; ^1H NMR (250 MHz, $CDCl_3$): $\delta = 2.38$ (s, 3H, CH_3C$_6$H$_4$), 2.89 (s, 3H, CH_3N), 4.50 (br s, 1H, H$_2$C=CCH), 5.28 (d, 1H, $^2J_{HH} = 3.0$ Hz, CHHC = CH), 6.27 (d, 1H, $^2J_{HH} = 3.0$ Hz, CHHC=CH), 7.19–7.27 (m, 4H, C$_6H_4$); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 21.17$ (s, CH$_3$C$_6$H$_4$), 45.24 (s, CH$_3$N), 76.17 (s, CH$_2$CCH), 122.60 (s, CH$_2$CCH), 128.56 (s, 2C, C_6H$_4$), 129.68 (s, 2C, C_6H$_4$), 132.76 (s, C_6H$_4$), 139.10 (s, C_6H$_4$), 140.15 (s, CH$_2C$CH), 167.31 (s, C=O); Anal. Calcd for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.73; H, 6.48; N, 6.62.
15. (a) Mouse leukemia L-1210 cells were cultured in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum (Gibco, Berlin, Germany), gentamycin (50 μg/mL), and 0.02 M HEPES buffer (Gibco). Cytostatic effects were assayed by measuring the inhibitory effects on L-1210 cell proliferation. In this assay, cells were seeded in 2 mL aliquots onto a 24-well plate (NUNC, Denmark) at a concentration of 1.5×10^3 cells/mL. After 24 h, drug solution was added and incubation was carried out for an additional 72 h. The cell number relative to control was determined by a tetrazolium dye method[15b]; (b) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
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