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Synthesis, reactions and structure–activity relationships of 1-hydroxyspiro[2.5]cyclooct-4-en-3-ones: Illudin analogs with in vitro cytotoxic activity

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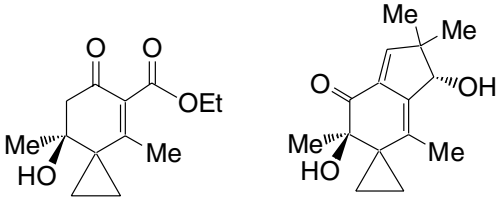
Abstract—1-Hydroxyspiro[2.5]cyclooct-4-en-3-ones—analogs of natural illudines—were prepared in good yields by cyclization of 1,3-dicarbonyl dianions or 1,3-bis-silyl enol ethers ('masked dianions') with 1,1-diacylcyclopropanes. Several spirocyclopropanes showed a significant antiproliferative activity against human leukemia HL60 cells in vitro. 1-Hydroxyspiro[2.5]cyclooct-4-en-3-ones represent highly reactive precursors of unstable spiro[5.2]cycloocta-4,7-dien-6-ones and reactions with a number of nucleophiles were studied.

1. Introduction

Cytotoxic compounds play an important role as therapeutic agents in the treatment of cancer. After the surgical removal of a solid tumor, the systemic chemotherapy is often used to assure a complete destruction of the remaining tumor cells in the patients.[1] Most of the chemotherapeutic agents used today belong to alkylating compounds (chlorambucil, melphalan, thiotepa, and busulfan), platinum derivatives (cisplatin, carboplatin), inhibitors of topoisomerases (camptothecin, etoposide, and doxorubicin), antimetabolic compounds (5-fluoruracil, methotrexate, and hydroxyurea) or inhibitors of mitosis (taxol, vinblastine). The latter are of special interest because of their selective damage of specific tumors in human. Like in bacterial infections tumors are able to aquire resistance to one or more chemotherapeutics.[2] Therefore, the search for new cytotoxic compounds is an important goal in medicinal chemistry. Cytotoxic natural products represent lead structures for the development of new anticancer agents.[3] Many natural products with cytotoxic properties were identified as the poisonous components in fungi. One example is the 'jack-o-latern' mushroom *Omphalotus illudens*.[4] The isolation of the illudins S and M (Chart 1) as cytotoxic constituents of *O. Illudens* was reported in 1950.[4] After the elucidation of their structures[5]—they possess a unique 1-hydroxyspiro[5.2]cyclooct-4-en-2-one skeleton—several related molecules were isolated from *O. illudens* and other mushrooms.[6] All compounds show cytotoxic effects in the range from very strong activity (for Illudins S and M) to weak activity (for dehydro and acylated derivatives).[7] Spiro[2.5]cycloocta-4,7-dien-



1-Hydroxyspiro[2.5]-cyclooct-4-en-3-one

Illudin M

Chart 1.

Keywords: Cyclopropanes; Cytotoxicity; Illudins; Spiro compounds; Structure–activity relationship.
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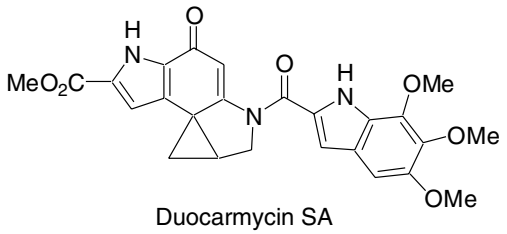


Duocarmycin SA

Chart 2.

6-ones containing aromatic rings fused to the double bonds are present, for example, in the cytotoxic natural products CC-1065 and duocarmycin SA (Chart 2).[8]

The synthesis of illudin analogs is of considerable pharmacological relevance, due to their potential cytotoxic and cancerostatic activity. The preparation of spiro[2.5]cycloocta-4,7-dien-6-ones and their reaction with nucleophiles, such as HBr, $LiAlH_4$ or NaOMe, has been previously studied.[9] Padwa et al. reported an interesting and efficient synthesis of illudins based on cyclization reactions of diazo compounds.[10] Recently, we have reported[11] the synthesis of 1-hydroxyspiro[5.2]cyclooct-4-en-3-ones—new illudin analogs—which represent highly reactive alkylating agents. Herein, we report full details of the synthesis of these compounds and their reaction with various nucleophiles. With regard to our preliminary communication, we optimized the synthesis of the spiro[5.2]cyclooct-4-en-3-ones (which are now available in up to 72% yield) and studied their antiproliferative activity against human leukemia HL60 cells.

2. Results and discussion

2.1. Synthesis of 1-hydroxyspiro[5.2]cyclooct-4-en-3-ones

The cyclization of dilithiated methyl acetoacetate (**1a**) with 1,1-diacetylcyclopropane (**3a**) afforded the 1-hydroxyspiro[2.5]cyclooct-4-en-3-one **4a** in up to 72% yield. During the optimization, the stoichiometry, reaction time and temperature proved to be important parameters. The reaction of **3a** with the dianions of ethyl and isopropyl acetoacetate gave the spirocyclopropanes **4b–c** in good yields. The cyclization of dilithiated acetylacetone with **3a** gave the 5-acetyl-1-hydroxyspiro[5.2]cyclooct-4-en-3-one **4f**. We have recently reported the synthesis of 4-(2-chloroethyl)salicylates by cyclization of 1,1-diacylcyclopropanes with 1,3-bis-silyl enol ethers—masked 1,3-dicarbonyl dianions—in the presence of $TiCl_4$ (2.0 equiv).[12] Based on these results, we developed an alternative, albeit less efficient, approach to 1-hydroxyspiro[2.5]cyclooct-4-en-3-ones. The reaction of 1,3-bis-silyl enol ether **2a**, prepared from methyl acetoacetate, afforded the 1-hydroxyspiro[5.2]cyclooct-4-en-3-one **4a** in 55% yield (Scheme 1, Table 1). During the optimization, the use of 0.3 equiv of $TiCl_4$ proved to be mandatory. In fact, the use of more than 0.5 equiv of $TiCl_4$ resulted in cleavage of the cyclopropane moiety and aromatisation. The employment of less than 0.3 equiv of $TiCl_4$ resulted in a significant decrease in yield. The cyclization of **3a** with 1,3-bis-silyl enol ethers **2b–d** afforded the alkoxycarbonyl-substituted 1-hydroxyspiro[5.2]cyclooct-4-en-3-ones **4b–d**. The reaction of 1,1-dipropionylcyclopropane (**3b**) with **2b** afforded **4e**. 5-Acetyl-1-hydroxyspiro[5.2]cyclooct-4-en-3-one **4f** was prepared, albeit in low yield, by cyclization of **3a** with 1,3-bis-silyl enol ether **2e** prepared from acetylacetone.

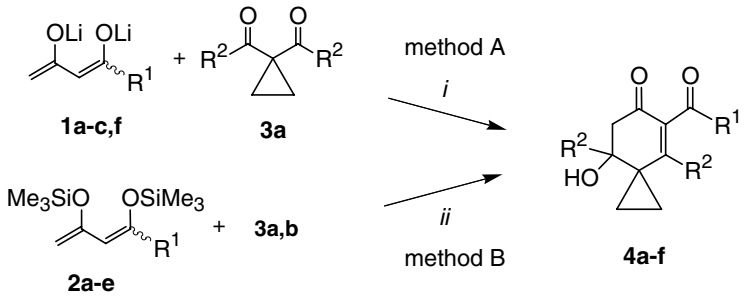
Table 1. Products and yields

1	2	3	4	R^1	R^2	% (**4**)[a] method A	% (**4**)[a] method B
a	**a**	**a**	**a**	OMe	Me	72	71
b	**b**	**a**	**b**	OEt	Me	70	48
c	**c**	**a**	**c**	O^{i}Pr	Me	68	48
	d	**a**	**d**	$O(CH_2)_2OMe$	Me	—[b]	40
	b	**b**	**e**	OEt	Et	—[b]	32
d	**e**	**a**	**f**	Me	Me	58	15

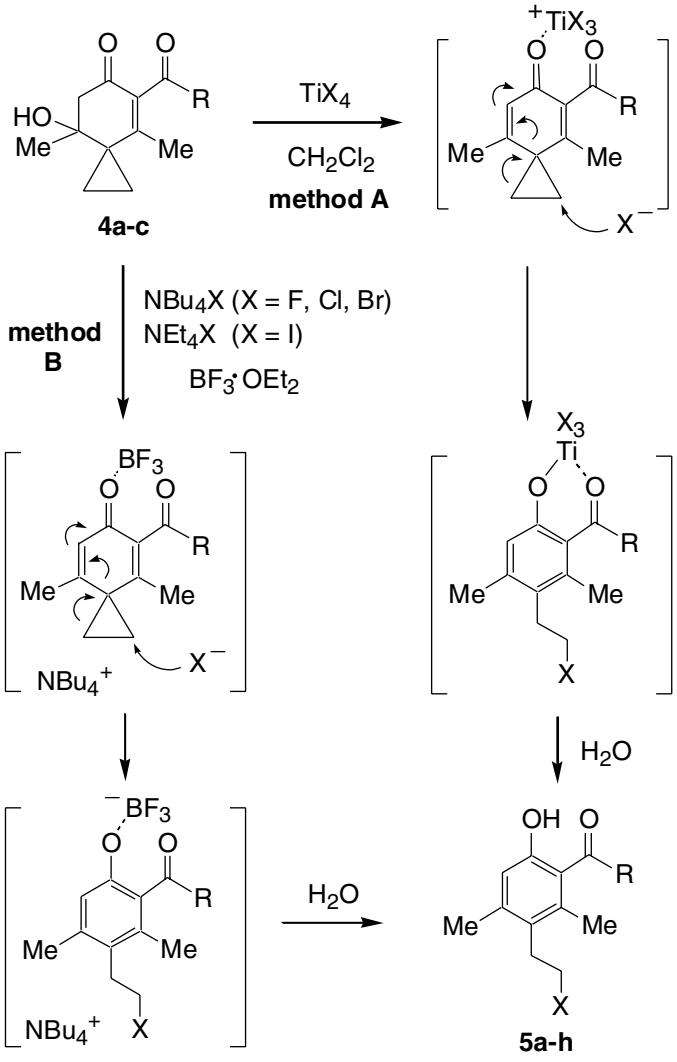
[a] Isolated yields.
[b] Experiment was not carried out.

2.2. Homo michael reactions of 1-hydroxyspiro[5.2]cyclooct-4-en-3-ones

Acceptor-substituted cyclopropanes represent important building blocks in homo Michael reactions with various nucleophiles.[13,14] 1-Hydroxyspiro[2.5]cyclooct-4-en-3-ones **4** represent direct precursors to spiro[2.5]cycloocta-4,7-dien-6-ones which are formed in situ by treatment with acid or Lewis acid and elimination of water. These intermediates are highly reactive since they represent vinylogous keto-substituted cyclopropanes which are transformed into stable aromatic phenols upon cleavage of the cyclopropane moiety. In fact, 1-hydroxyspi-



Scheme 1. Synthesis of 1-hydroxyspiro[2.5]cyclooct-4-en-3-ones; Reagents and conditions: (i) 1—LDA (2.3 equiv), **1a–c,f** (1.2 equiv), THF, 1 h, 0 °C, 2—**3a** (1.0 equiv), −78 °C, 1 h, −78 to 20 °C, 14 h; (ii), $TiCl_4$ (0.3 equiv), CH_2Cl_2, −78 to 20 °C, 12 h.



Scheme 2. Reaction of **4a–c** with TiX_4 (method A) and NBu_4X (method B).

ro[5.2]cyclooct-4-en-3-ones **4** represent highly reactive electrophiles and strong alkylating agents.

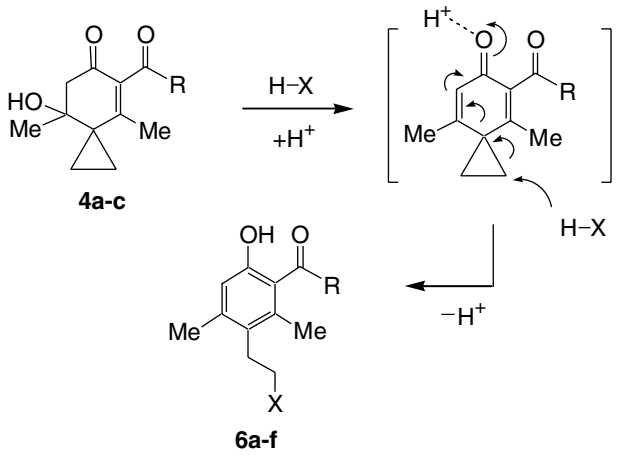
Treatment of 1-hydroxyspiro[5.2]cyclooct-4-en-3-ones **4** with titanium tetrahalides (method A) or with tetraalkylammonium halides in the presence of boron trifluoride (method B) resulted in the formation of 4-(2-haloethyl)salicylates (Scheme 2, Table 2). The reaction of **4c** with TiF_4 afforded the fluoride **5b**, albeit in low yield; the reaction of **4b** with Bu_4NF in the presence of BF_3OEt_2 failed. Treatment of **4c** with $TiCl_4$ afforded the chloride **5d** in moderate yield. The chlorination of **4b** with Bu_4NCl in the presence of a catalytic amount of $BF_3{\cdot}OEt_2$ afforded **5e** in very good yield. The reaction of **4a** and **4c** with $TiBr_4$ and TiI_4 afforded the halides **5f** and **5h**, respectively. Likewise, the reaction of **4b** and **4a** with Bu_4NBr and Bu_4NI afforded **5f** and **5h** in high yields, respectively.

Table 2. Products and yields

5	R	X	% (5)[a]	Method
a	O^{i}Pr	F	26	A
b	OEt	F	0	B
c	O^{i}Pr	Cl	53	A
d	OEt	Cl	84	B
e	OMe	Br	94	A
f	OEt	Br	96	B
g	O^{i}Pr	I	47	A
h	OMe	I	96	B

[a] Isolated yields.

The reaction of 1-hydroxyspiro[5.2]cyclooct-4-en-3-ones with acids was next studied (Scheme 3, Table 3). The reaction of **4c** with trifluoroacetic acid (TFA) afforded the trifluoroacetate **6a**. The latter was formed by acid-mediated elimination of water to give a spiro[2.5]cycloocta-4,7-dien-6-one and subsequent acid-mediated attack of TFA onto the spirocyclopropane. Treatment of a CH_2Cl_2 solution of **4b** with *p*-toluenesulfonic acid (TosOH) afforded the tosylate **6b**. This reaction is a rare example of a direct alkylation of *p*-toluenesulfonic acid which represents a very weak nucleophile. The reaction of **4b** with glacial acetic acid (AcOH) was unsuccessful, due to the low acidity of AcOH. However, the acetate **8c** was formed when the reaction was carried out in the presence of $BF_3{\cdot}OEt_2$. The reaction of a methanol solu-



Scheme 3. Reaction of **4a**–**c** with acids.

Table 3. Products and yields

6	R	X	% (6)[a]
a	O^{i}Pr	$O_2C(CF_3)$	95
b	OEt	OAc	79[b]
c	OEt	OTos	61
d	OEt	OH	61[c]
e	OMe	OPh	57[d]
f	O^{i}Pr	SPh	70[d]

[a] Isolated yields.
[b] Carried out in the presence of $BF_3{\cdot}OEt_2$.
[c] H_2SO_4, H_2O, MeOH.
[d] Carried out in the presence of TFA.

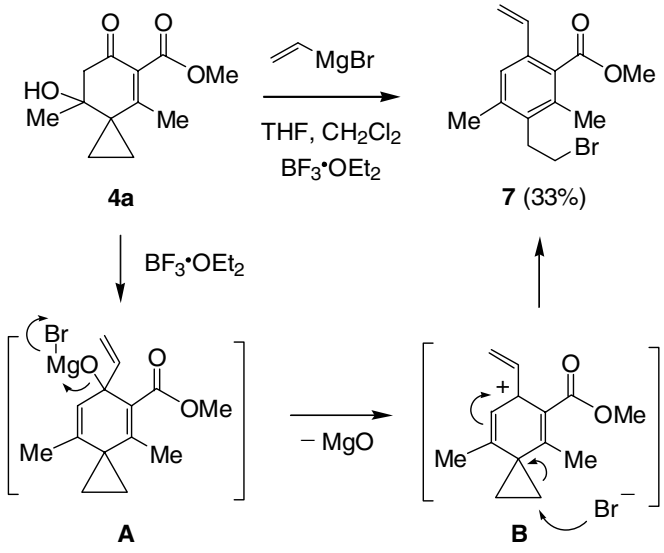
tion of **4b** with sulfuric acid afforded the alcohol **6d**. The replacement of $BF_3{\cdot}OEt_2$ by TFA also gave **6d**, however, in low yield. The reaction of **4a** with phenol, in the presence of TFA, afforded **6e**. Likewise, the TFA-mediated reaction of **4c** with thiophenol afforded the thioether **6f**.

The $BF_3{\cdot}OEt_2$-mediated reaction of **4a** with vinyl-magnesium bromide afforded the styrene **7** (Scheme 4). The formation of **7** can be explained by $BF_3{\cdot}OEt_2$-mediated elimination of water, attack of the Grignard reagent onto the carbonyl group, extrusion of magnesium oxide and of a bromide ion, attack of the latter onto the cyclopropane moiety and, finally, aromatization.

2.3. Biological evaluation of 1-hydroxyspiro[5.2]cyclooct-4-en-3-ones

The products prepared were tested for in vitro cytotoxic activity against the HL60 human acute promyeloid leukemia cell line.[15,16] Cells in the rapid phase of growth were exposed to test compounds for 48 h. The IC_{50} values were determined from data of the MTT assay, which measures cell viability.[17] The results are summarized in Table 4. For illustration, the corresponding values of established antitumor agents as well as the IC_{50} values for illudins S and M against human HL60 cells are shown.[7,18] For reasons of comparison, the cytotoxic activity of spiro[5.4]decenones **8a**–**c**[19] (containing a spirocyclopentane rather than a spirocyclopropane system) and of cyclopropane **8d** (containing a monocyclic rather than a spiro-anullated cyclopropane) was studied (Chart 3).

1-Hydroxyspiro[2.5]cyclooct-4-enes **4a**, **4b**, **4d**, and **4e** show a considerable antiproliferative activity comparable in potency to carboplatin and 6-fluoruracil, and stronger than busulfan. The activity of **4c** lies in the same range as busulfan. In contrast, spiro[5.4]decenones **8a**–**c** and cyclopropane **8d** exhibit only a weak activity. These results show that the presence of the 1-hydroxyspiro[2.5]cyclooct-4-ene moiety is mandatory for cytotoxic activity. The presence of an ester group appears to be important because a rather low cytotoxicity was observed for **4f** containing an acetyl rather than an ester group. The methyl, ethyl, and methoxyethyl ester groups of **4a**, **4b**, and **4d** are more prone toward hydrolysis than the isopropyl ester present in **4c** which may account for the low cell growth inhibitory activity of the latter. A



Scheme 4. Reaction of **4a** with vinylmagnesium bromide.

Table 4. Results of the in vitro cytotoxic testing against human HL60 cells

Compound	IC_{50}[a] (μM)
4a	4.17 ± 0.62
4b	3.31 ± 1.35
4c	122 ± 30
4d	9.90 ± 0.46
4e	19.5 ± 6.6
8a	270[b]
8b	119.3 ± 17.3
8c	140 ± 95
8d	437.16[b]
Illudin S	0.003 ± 0.001[c]
Illudin M	0.003 ± 0.001[c]
Cisplatin	0.41
Carboplatin	6.80
Busulfan	61.90
Doxorubicin	0.05
5-Fluoruracil	16.97

[a] Averages and standard deviations of four independent determinations.
[b] ($n = 1$).
[c] Ref. 7.

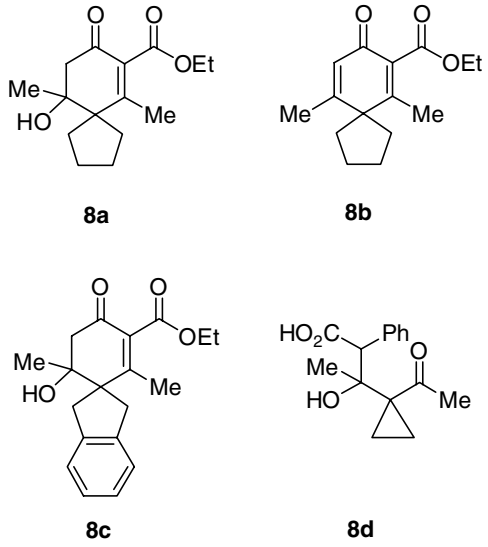


Chart 3.

very strong antiproliferative activity has been reported for illudins S and M.[7] It has been established that the compounds react as alkylating agents, following the reaction of a nucleophile (such as glutathione) with the unsaturated ketone moiety. The resultant cyclohexadiene intermediate rapidly aromatizes, with concurrent ring opening of the cyclopropane ring, which can trap DNA. The generation of such a cyclohexadiene intermediate is more difficult for **4a**–**e**, which is presumably the reason why these compounds are less active than the illudins. In addition, an important difference between **4a**–**e** and the illudins lies in the fact that the latter are double alkylation agents.[20] In conclusion, the cytotoxicities of readily accessible spirocyclopropanes **4a**, **b**, **d**, **e** are—albeit lower than those of the rather complex natural products—very promising.

3. Experimental

3.1. Typical procedure for the cyclization of 1,3-dicarbonyl dianions with 1,1-diacetylcyclopropane (method A)

To a THF solution (20 mL) of LDA, prepared by addition of *n*-BuLi (7.2 mL, 18.0 mmol, 2.5 M solution in hexane) to a THF solution of diisopropylamine (2.530 g, 18.0 mmol) at 0 °C. After stirring for 1 h, methyl acetoacetate (1.105 g, 9.52 mmol) was added at −78 °C and the solution was stirred for 1 h. To the solution was added 1,1-diacetylcyclopropane (**3a**) (1.000 g, 7.9 mmol) at −78 °C and the solution was allowed to warm to 20 °C during 14 h. To the reaction mixture was added an aqueous solution of HCl (1 M) and the organic and the aqueous layers were separated. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo. The residue was purified by chromatography (silica gel, hexane/EtOAc) to give **4a** as a colorless solid (1.275 g, 71%).

3.1.1. Methyl 8-hydroxy-4,8-dimethyl-6-oxospiro[5.2]oct-4-ene-5-carboxylate (4a). Mp = 108–109 °C; $R_f = 0.13$ (hexane/EtOAc = 4:1); IR (KBr): $\tilde{\nu} = 2950$ (w), 1715 (s), 1669 (s), 1606 (w), 1439 (m), 1383 (m), 1248 (m), 1160 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 3.81$ (s, 3H, OCH_3), 2.72 (d, 1H, $J = 15.6$ Hz, CH_2), 2.62 (d, 1H, $J = 15.6$ Hz, CH_2), 2.24 (s, 1H, OH), 1.69 (s, 3H, CH_3), 1.50–1.43 (m, 1H, CH_2), 1.26 (s, 3H, CH_3), 1.17–1.06 (m, 2H, CH_2), 0.89–0.85 (m, 1H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 194.1$, 167.6, 161.8, 132.6, 70.4 (C), 52.2 (CH_3), 51.3 (CH_2), 32.2 (C), 25.3, 17.0 (CH_3), 11.0, 9.5 (CH_2); MS (EI, 70 eV): *m*/*z* (%) = 224 (M^+, 40), 209 (30), 177 (48), 164 (69), 148 (48), 79 (30), 43 (100), 28 (37); Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 63.74; H, 7.71.

3.1.2. 5-Acetyl-8-hydroxy-4,8-dimethyl-6-oxospiro[5.2]oct-4-ene (4f). Starting with *n*-BuLi (2.74 mL, 4.3 mmol, 15% solution in hexane), diisopropylamine (0.62 g, 4.3 mmol), 1,1-diacetylcyclopropane (**3a**) (252 mg, 2.0 mmol), and acetylacetone (230 mg, 2.2 mmol) in THF (11 mL), **4f** was isolated as a yellow oil (0.242 g, 58%). IR (neat): $\tilde{\nu} = 3440$ (br), 2976 (m), 1700 (s), 1658 (s), 1597 (s), 1382 (s), 1354 (s), 1280 (m), 1160 (m), 1106 (m), 1067 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 3.10$ (br, 1H, OH), 2.73 (d, 1H, $J = 15.6$ Hz, CH_2), 2.59 (d, 1H, $J = 15.6$ Hz, CH_2), 2.32 (s, 3H, $COCH_3$), 1.63 (s, 3H, CH_3), 1.50–1.40 (m, 1H, CH_2), 1.25 (s, 3H, CH_3), 1.15–1.06 (m, 2H, CH_2), 0.82–0.88 (m, 1H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 205.0$ ($COCH_3$), 196.1 (CO), 161.4, 139.0 (C), 70.1 (*C*OH), 51.6 (CH_2), 32.4 (C, spiro), 31.6, 24.9, 16.1 (CH_3), 11.0, 9.5 (CH_2); MS (EI, 70 eV): *m*/*z* (%) = 208 (M^+, 10), 193 (24), 175 (8), 165 (21).

3.2. Typical procedure for the cyclization of 1,3-bis-silyl enol ethers with 1,1-diacylcyclopropanes (method B)

To a CH_2Cl_2 solution (100 mL) of 1,1-diacetylcyclopropane (**3a**) (0.190 g, 1.5 mmol) and of 1-methoxy-1,3-bis(trimethylsilyloxy)buta-1,3-diene (**2a**) (0.580 g,

2.2 mmol) in the presence of molecular sieves (4 Å, 1.0 g) was dropwise added a CH_2Cl_2 solution (1 mL) of $TiCl_4$ (0.05 mL 0.45 mmol) at −78 °C under argon atmosphere. The reaction mixture was allowed to warm to 20 °C over 6 h and was stirred for additional 6 h at 20 °C. The solution was filtered and the filtrate was poured into an aqueous solution of HCl (1.0 M, 100 mL). The organic and the aqueous layer were separated and the latter was extracted with CH_2Cl_2 (3× 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 4:1 → 1:1) to give **4a** (0.244 g, 72%) as a colorless solid (for spectroscopic data, see above).

3.2.1. Ethyl 8-hydroxy-4,8-dimethyl-6-oxospiro[5.2]oct-4-ene-5-carboxylate (4b). The reaction was carried out according to the procedure as given for the synthesis of **4a**. Starting with 1,1-diacetylcyclopropane (**3a**) (0.200 g, 1.6 mmol), 1-ethoxy-1,3-bis(trimethylsilanyloxy)buta-1,3-diene (**2b**) (0.650 g, 2.4 mmol), and $TiCl_4$ (0.05 mL in 1 mL CH_2Cl_2, 0.5 mmol), **4b** was isolated as a colorless solid (0.180 g, 48%), mp = 56–57 °C; R_f = 0.15 (hexane/ethyl acetate = 4:1); IR (KBr): $\tilde{\nu}$ = 3483 (m), 2980 (m), 1730 (s), 1666 (s), 1606 (m), 1439 (m), 1383 (m), 1248 (m), 1170 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.29 (d, 2H, J = 7.2 Hz, OCH_2), 2.72 (d, 1H, J = 15.6 Hz, CH_2), 2.62 (d, 1H, J = 15.6 Hz, CH_2), 2.06 (s, 1H, OH), 1.69 (s, 3H, CH_3), 1.49–1.42 (m, 1H, CH_2), 1.32 (t, 3H, J = 7.2 Hz, CH_3), 1.27 (s, 3H, CH_3), 1.16–1.06 (m, 2H, CH_2), 0.89–0.82 (m, 1H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 194.2, 167.2, 161.4, 132.7, 70.3 (C), 61.3, 51.3 (CH_2), 32.1 (C), 25.2, 16.8, 14.8 (CH_3), 10.9, 9.4 (CH_2); MS (EI, 70 eV): m/z (%) = 238 (M^+, 28), 223 (33), 193 (22), 177 (34), 164 (44), 149 (69), 43 (100); HRMS (EI, 70 eV): calcd for $C_{13}H_{18}O_4$: m/z = 238.1205; found: m/z = 238.1205 ± 2 mD (M^+); Anal. Calcd for $C_{13}H_{18}O_4$: C, 65.52; H, 7.61. Found: C, 65.98; H, 7.52.

3.2.2. Isopropyl 8-hydroxy-4,8-dimethyl-6-oxospiro[5.2]oct-4-ene-5-carboxylate (4c). The reaction was carried out according to the procedure as given for the synthesis of **4a**. Starting with 1,1-diacetylcyclopropane (**3a**) (0.131 g, 1.0 mmol), 1-isopropyloxy-1,3-bis(trimethylsilyloxy)buta-1,3-diene (**2c**) (0.450 g, 1.6 mmol) and $TiCl_4$ (0.03 mL in 1 mL CH_2Cl_2, 0.3 mmol), **4c** was isolated as a colorless oil (0.125 g, 48%); R_f = 0.18 (hexane/ethyl acetate = 1:1); IR (neat): $\tilde{\nu}$ = 3399 (br), 2983 (w), 1729 (s), 1659 (s), 1617 (m), 1380 (m), 1243 (s), 1024 (m), 745 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 5.17 (sept, 1H, J = 6.3 Hz, OCH), 2.71 (d, 1H, J = 15.6 Hz, CH_2), 2.59 (d, 1H, J = 15.6 Hz, CH_2), 2.40 (br, 1H, OH), 1.68 (s, 3H, CH_3), 1.49–1.37 (m, 1H, CH_2), 1.30 (d, 6H, J = 6.3 Hz, CH_3), 1.26 (s, 3H, CH_3), 1.15–1.04 (m, 2H, CH_2), 0.88–0.81 (m, 1H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 194.0, 166.8, 160.5, 133.2, 70.5 (C), 69.0 (CH), 51.5 (CH_2), 32.1 (C), 25.4, 21.7 (2C), 16.7 (CH_3), 10.8, 9.5 (CH_2); MS (EI, 70 eV): m/z (%) = 252 (M^+, 40), 237 (13), 193 (65), 177 (41), 164 (47), 148 (100), 91 (17), 43 (78); HRMS (EI, 70 eV): calcd for $C_{14}H_{20}O_4$: m/z = 252.1362; found: m/z = 252.1362 ± 2 mD.

3.2.3. Ethyl 8-hydroxy-4,8-diethyl-6-oxospiro[5.2]oct-4-ene-5-carboxylate (4e). The reaction was carried out according to the procedure as given for the synthesis of **4a**. Starting with 1,1-dipropionylcyclopropane (**3b**) (0.154 g, 1.0 mmol), 1-ethoxy-1,3-bis(trimethylsilyloxy)buta-1,3-diene (**2b**) (0.410 g, 1.5 mmol) and $TiCl_4$ (0.035 mL in 1 mL CH_2Cl_2, 0.3 mmol), **4e** was isolated as a yellow oil (0.085 g, 32%); R_f = 0.18 (hexane/ethyl acetate = 1:1); ^1H NMR (300 MHz, $CDCl_3$): δ = 4.15–4.40 (m, 2H, OCH_2), 2.72 (d, 1H, J = 15.6 Hz, CH_2), 2.65 (d, 1H, J = 15.6 Hz, CH_2), 2.10–2.25 (m, 1H, CH_2), 1.70–1.85 (m, 1H, CH_2), 1.50–1.65 (m, 2H, CH_2), 1.42–1.50 (m, 1H, CH_2), 1.32 (t, 3H, J = 7.0 Hz, CH_3), 1.15–1.25 (m, 1H, CH_2), 1.05–1.12 (m, 1H, CH_2), 1.10 (t, 3H, J = 7.0 Hz, CH_3), 0.92 (t, 3H, J = 7.0 Hz, CH_3), 0.60–0.70 (m, 1H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 194.2, 167.1, 166.3, 132.5, 72.1 (C), 61.3, 48.8 (CH_2), 30.3 (C), 29.6, 23.9 (CH_2), 14.2, 13.2 (CH_3), 10.5, 8.9 (CH_2), 7.4 (CH_3); MS (EI, 70 eV): m/z (%) = 266 (M^+, 12), 237 (25), 221 (20), 181 (85).

3.3. Typical procedure for the reaction of 1-hydroxyspiro[2.5]cyclooct-4-en-3-ones with titanium tetrahalides (method A)

To a CH_2Cl_2 solution (0.5 mL) of isopropyl-8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4c**) (0.035 g, 0.1 mmol) was dropwise added TiF_4 (0.017 g, 0.14 mmol) at −78 °C under argon atmosphere. The reaction mixture was allowed to warm to 20 °C over 6 h and was stirred for additional 6 h at 20 °C. The solution was filtered and the filtrate was poured into an aqueous solution of HCl (1.0 M, 100 mL). The organic and the aqueous layer were separated and the latter was extracted with CH_2Cl_2 (3× 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 4:1 → 1:1) to give **5a** (0.009 g, 26%) as a colorless oil (for spectroscopic data, see below).

3.3.1. Isopropyl 3-(2-fluoroethyl)-6-hydroxy-2,4-dimethylbenzoate (5a). Starting with isopropyl-8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4c**) (0.035 g, 0.14 mmol), TiF_4 (0.017 g, 0.14 mmol), and CH_2Cl_2 (0.5 mL), **5a** (0.009 g, 26%) was isolated as a colorless oil; R_f = 0.67 (hexane/EtOAc = 9:1); IR (neat): $\tilde{\nu}$ = 2979 (m), 1720 (w), 1655 (s), 1603 (m), 1574 (m), 1467 (s), 1370 (s), 1244 (s), 1105 (s), 804 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.68 (s, 1H, OH), 6.68 (s, 1H, ArH), 5.31 (sep, 1H, J = 6.2 Hz, OCH), 3.45 (t, 2H, J= 7.5 Hz, CH_2Cl), 2.92 (t, 2H, J = 7.5 Hz, CH_2), 2.49 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 1.39 (d, 6H, J = 6.3 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 159.7, 144.2, 138.9, 127.2 (C), 116.9 (CH), 112.3 (C), 69.8 (CH_2), 69.6 (CH), 29.8 (CH_2), 21.9 (C), 21.1, 18.7 (CH_3); elemental analysis: calcd (%) for $C_{14}H_{19}O_3F$: C, 66.12; H, 7.53; found: C, 66.51; H, 7.26.

3.3.2. Isopropyl 3-(2-chloroethyl)-6-hydroxy-2,4-dimethylbenzoate (5c). Starting with isopropyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4c**) (0.050 g, 0.20 mmol), $TiCl_4$ (0.014 mL, 0.14 mmol; dissolved in

1 mL of CH_2Cl_2), CH_2Cl_2 (3 mL), and molecular sieves (4 Å), **5c** was isolated (0.029 g, 53%) as a colorless solid, mp = 51–52 °C; R_f = 0.68 (hexane/EtOAc = 4:1); IR (KBr): $\tilde{\nu}$ = 2982 (m), 1731 (w), 1656 (s), 1601 (w), 1574 (m), 1467 (m), 1372 (s), 1238 (s), 1105 (m), 804 (w), 703 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.77 (s, 1H, OH), 6.70 (s, 1H, ArH), 5.32 (sep, 1H, J = 6.2 Hz, OCH), 3.53–3.46 (m, 2H, CH_2), 3.12–3.06 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 1.46 (d, 6H, J = 6.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8, 160.2, 143.9, 138.9, 127.0 (C), 117.2 (CH), 112.3 (C), 69.7 (OCH), 42.2, 33.0 (CH_2), 21.9 (C), 21.0, 18.6 (CH_3); MS (EI, 70 eV): m/z (%) = 272 (M^++2, 5), 270 (M^+, 15), 212 (26), 210 (74), 161 (100), 91 (8), 77 (7), 28 (35); elemental analysis: calcd (%) for $C_{14}H_{19}O_3Cl$: C, 62.10; H, 7.07; found: C, 62.07; H, 7.49.

3.3.3. Methyl 3-(2-bromoethyl)-6-hydroxy-2,4-dimethylbenzoate (5e). Starting with methyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4a**) (0.029 g, 0.20 mmol), $TiBr_4$ (0.048 mL, 0.13 mmol), and CH_2Cl_2 (1 mL), **5e** was isolated (0.035 g, 94%) as a colorless solid, mp = 73–74 °C; R_f = 0.63 (hexane/EtOAc = 4:1); IR (KBr): $\tilde{\nu}$ = 2950 (m), 1721 (w), 1656 (s), 1599 (m), 1574 (m), 1436 (s), 1355 (s), 1237 (s), 1071 (m), 805 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.69 (s, 1H, OH), 6.69 (s, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.35–3.29 (m, 2H, CH_2), 3.19–3.15 (m, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.8, 160.3, 144.0, 138.8, 128.2 (C), 117.3 (CH), 111.8 (C), 52.2 (OCH_3), 33.3, 29.7 (CH_2), 20.9, 18.5 (CH_3); MS (EI, 70 eV): m/z (%) = 288 (M^++2, 24), 286 (M^+, 26), 256 (63), 254 (62), 207 (62), 193 (23), 175 (31), 161 (100), 77 (12); elemental analysis: calcd (%) for $C_{12}H_{15}O_3Br$: C, 50.13; H, 5.26; found: C, 50.29; H, 5.43.

3.3.4. Isopropyl 6-hydroxy-3-(2-iodoethyl)-2,4-dimethylbenzoate (5g). Starting with isopropyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4c**) (0.025 g, 0.10 mmol), TiI_4 (0.054 mL, 0.10 mmol), and CH_2Cl_2 (1 mL), **5g** was isolated (0.018 g, 53%) as a colorless oil; R_f = 0.77 (hexane/EtOAc = 4:1); IR (neat): $\tilde{\nu}$ = 2980 (w), 1657 (s), 1603 (w), 1574 (w), 1464 (m), 1369 (s), 1236 (s), 1103 (m), 803 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.79 (s, 1H, OH), 6.69 (s, 1H, ArH), 5.32 (sep, 1H, J = 6.2 Hz, OCH), 3.19–3.15 (m, 2H, CH_2I), 3.12–3.06 (m, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 1.40 (d, 6H, J = 6.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8, 160.2, 143.4, 138.5, 130.6 (C), 117.3 (CH), 112.4 (C), 69.8 (CH_2), 34.8 (CH), 21.9 (2C), 20.9, 18.6 (CH_3), 1.8 (CH_2); MS (EI, 70 eV): m/z (%) = 362 (M^+, 11), 303 (14), 235 (61), 175 (100), 161 (14), 91 (10), 28 (10); the exact molecular mass for $C_{14}H_{19}O_3I$ m/z = 362.0379 ± 2 mD (M^+) was confirmed by HRMS (EI, 70 eV).

3.4. Typical procedure for the reaction of 1-hydroxyspiro[2.5]cyclooct-4-en-3-ones with tetraalkylammonium halides (method B)

To a CH_2Cl_2 solution (1 mL) of ethyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4b**) (0.031 g, 0.13 mmol) and of n-Bu_4NCl (0.037 g, 0.13 mmol) was dropwise added $BF_3{\cdot}OEt_2$ (0.01 mL, 0.07 mmol; dissolved in 0.2 mL of CH_2Cl_2) at −78 °C under argon atmosphere. The reaction mixture was allowed to warm to 20 °C over 6 h and was stirred for additional 6 h at 20 °C. The solution was filtered and the filtrate was poured into an aqueous solution of HCl (1.0 M, 100 mL). The organic and the aqueous layers were separated and the latter was extracted with CH_2Cl_2 (3× 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 4:1 → 1:1) to give **5d** (0.028 g, 84%) as a colorless solid (for spectroscopic data, see below).

3.4.1. Ethyl 3-(2-chloroethyl)-6-hydroxy-2,4-dimethylbenzoate (5d). Starting with ethyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4b**) (0.031 g, 0.13 mmol), n-Bu_4NCl (0.037 g, 0.13 mmol), CH_2Cl_2 (1 mL), and $BF_3{\cdot}OEt_2$ (0.01 mL, 0.07 mmol; dissolved in 0.2 mL of CH_2Cl_2), **5d** was isolated (0.028 g, 84%) as a colorless solid, mp = 53–54 °C; R_f = 0.64 (hexane/EtOAc = 4:1); IR (KBr): $\tilde{\nu}$ = 2978 (m), 1666 (s), 1606 (w), 1561 (m), 1467 (m), 1311 (s), 1072 (m), 699 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.74 (s, 1H, OH), 6.70 (s, 1H, ArH), 4.43 (q, 2H, J = 7.2 Hz, OCH_2), 3.52–3.47 (m, 2H, CH_2), 3.13–3.07 (m, 2H, CH_2), 2.51 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 1.42 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.3, 160.3, 144.0, 139.0, 127.0 (C), 117.2 (CH), 112.0 (C), 61.6, 42.2, 32.9 (CH_2), 21.0, 18.6, 14.1 (CH_3); MS (EI, 70 eV): m/z (%) = 258.4 (M^++2, 12), 256 (M^+, 38), 212 (34), 210 (88), 161 (100), 91 (5), 77 (4); elemental analysis: calcd (%) for $C_{13}H_{17}O_3Cl$: C, 60.82; H, 6.67; found: C, 60.81; H, 7.09.

3.4.2. Ethyl 3-(2-bromoethyl)-6-hydroxy-2,4-dimethylbenzoate (5f). Starting with ethyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4b**) (0.030 g, 0.13 mmol) and n-Bu_4NBr (0.048 g, 0.15 mmol), CH_2Cl_2 (1 mL), and $BF_3{\cdot}OEt_2$ (0.01 mL, 0.07 mmol; dissolved in 0.2 mL of CH_2Cl_2), **5f** was isolated (0.037 g, 95%) as a colorless solid, mp = 49–50 °C; R_f = 0.61 (hexane/EtOAc = 4:1); IR (KBr): $\tilde{\nu}$ = 2978 (m), 1666 (s), 1606 (w), 1561 (m), 1467 (m), 1311 (s), 1072 (m), 699 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.76 (s, 1 H, OH), 6.69 (s, 1H, ArH), 4.43 (q, 2H, J = 7.2 Hz, OCH_2), 3.36–3.30 (m, 2H, CH_2), 3.19–3.13 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 1.42 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.3, 160.3, 143.9, 138.9, 128.2 (C), 117.3 (CH), 112.0 (C), 61.6, 33.4, 29.7 (CH_2), 20.9, 18.5, 14.1 (CH_3); MS (EI, 70 eV): m/z (%) = 303 (M^++2, 11), 300 (M^+, 11), 256 (32), 254 (32), 221 (20), 175 (29), 161 (100), 91 (23), 77 (18), 29 (31); elemental analysis: calcd (%) for $C_{13}H_{17}O_3Br$: C, 60.82; H, 6.67; found: C, 60.81; H 7.09.

3.4.3. Methyl 6-hydroxy-3-(2-iodoethyl)-2,4-dimethylbenzoate (5h). Starting with methyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4a**) (0.032 g, 0.14 mmol), Et_4NI (0.036 g, 0.14 mmol), CH_2Cl_2 (1 mL), and $BF_3{\cdot}OEt_2$ (0.01 mL, 0.07 mmol; dissolved in 0.2 mL of CH_2Cl_2), **5h** was isolated (0.046 g, 96%) as a colorless solid, mp = 94–95 °C; R_f = 0.62 (hexane/EtOAc = 4:1); IR (neat): $\tilde{\nu}$ = 2980 (w), 1657 (s), 1603 (w), 1574 (w), 1464

(m), 1369 (s), 1236 (s), 1103 (m), 803 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.73 (s, 1H, OH), 6.70 (s, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.22–3.15 (m, 2H, CH_2I), 3.12–3.05 (m, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 1.40 (d, 6H, J = 6.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.8, 160.2, 143.7, 138.5, 130.7 (C), 117.3 (CH), 111.9 (C), 52.2 (CH_3), 34.7 (CH_2), 20.9, 18.5 (CH_3), 1.7 (CH_2); MS (EI, 70 eV): m/z (%) = 334 (M^+, 9), 303 (4), 207 (79), 175 (100), 161 (24), 147 (17), 119 (18), 91 (25), 77 (17), 28 (24); elemental analysis calcd (%) for $C_{12}H_{15}O_3I$: C, 43.13; H, 4.52; found: C, 42.81; H, 4.37.

3.4.4. Isopropyl 6-hydroxy-2,4-dimethyl-3-[2-(2,2,2-trifluoroacetoxy)ethyl]benzoate (6a). TFA (0.04 mL, 0.5 mmol) was added dropwise at 20 °C to a CH_2Cl_2 solution (1.0 mL) of isopropyl 8-hydroxy-4,8-dimethyl-6-oxospiro[5.2]oct-4-ene-5-carboxylate (**4c**) (0.064 g, 0.3 mmol) and the reaction mixture was stirred for 4 h (monitored by TLC). The solvent and TFA were removed in vacuo and the residue was purified by column chromatography (silica gel, hexane/EtOAc = 19:1) to give **6a** (0.084 g, 95%) as a colorless solid; mp = 32–33 °C; R_f = 0.58 (hexane/EtOAc = 19:1); IR (KBr): $\tilde{\nu}$ = 2959 (s), 1786 (m), 1657 (s), 1453 (s), 1375 (s), 1166 (s), 1105 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.77 (s, 1H, OH), 6.71 (s, 1H, ArH), 5.32 (sep, 1H, J = 6.3 Hz, OCH), 4.37 (t, 2H, J = 8.1 Hz, CH_2F), 3.08 (t, 2H, J = 8.1 Hz, CH_2), 2.53 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 1.40 (d, 3H, J = 6.3 Hz, CH_3), 1.39 (d, 3H, J = 6.3 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8 (2C), 160.3, 157.5 (q, J = 42.1 Hz, CF_3), 144.1, 139.3, 124.5 (C), 117.3 (CH), 112.5 (C), 69.8 (CH), 66.3, 27.9 (CH_2), 21.9 (C), 20.9, 18.6 (CH_3); MS (EI, 70 eV): m/z (%) = 348 (M^+, 68), 306 (41), 288 (100), 161 (93), 91 (31), 43 (34); the exact molecular mass for $C_{16}H_{19}O_5F_3$ m/z = 348.1185 ± 2 mD (M^+) was confirmed by HRMS (EI, 70 eV).

3.4.5. Ethyl 6-hydroxy-2,4-dimethyl-3-[2-(toluene-4-sulfonyloxy)ethyl]benzoate (6b). To a CH_2Cl_2 solution (1 mL) of ethyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4b**) (0.045 g, 0.19 mmol) was added a CH_2Cl_2 solution of PTSA (0.036 g, 0.19 mmol) at −78 °C under argon atmosphere. The reaction mixture was allowed to warm to 20 °C over 6 h and was stirred for additional 6 h at 20 °C. The solution was filtered and the filtrate was poured into an aqueous solution of HCl (1.0 M, 100 mL). The organic and the aqueous layer were separated and the latter was extracted with CH_2Cl_2 (3× 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, hexane/EtOAc = 4:1 → 1:1) to give **6b** (0.045 g, 53%) as a colorless oil which solidified upon standing; mp = 73–74 °C; R_f = 0.35 (hexane/EtOAc = 4:1); IR (neat): $\tilde{\nu}$ = 3432 (br), 1717 (w), 1654 (s), 1602 (m), 1574 (w), 1468 (m), 1351 (s), 1240 (s), 1179 (s), 965 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.71 (s, 1 H, OH), 7.67 (dd, 2H, J= 2.1 Hz, 8.1 Hz, ArH), 7.27 (dd, 2H, J = 2.1 Hz, 8.1 Hz, ArH), 6.62 (s, 1H, ArH), 4.41 (q, 2H, J = 7.2 Hz, OCH_2), 4.04 (t, 2H, J = 7.8 Hz, OCH_2), 2.99 (t, 2H, J = 7.8 Hz, CH_2), 2.42 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 1.41 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.2, 160.3, 144.7, 144.2, 139.1, 132.9 (C), 129.7 (C), 127.6 (CH), 124.7 (C), 117.2 (CH), 112.0 (C), 68.5, 61.6, 28.8 (CH_2), 20.9, 20.5, 18.4, 14.1 (CH_3); MS (EI, 70 eV): m/z (%) = 392 (M^+, 34), 346 (82), 207 (25), 174 (82), 161 (100), 91 (35), 28 (57).

3.4.6. Ethyl 3-(2-acetoxyethyl)-6-hydroxy-2,4-dimethylbenzoate (6c). The reaction was carried out following the procedure as given for the synthesis of **6b**. The $BF_3{\cdot}OEt_2$ was added last to the reaction mixture. Starting with ethyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4b**) (0.040 g, 0.17 mmol), glacial acetic acid (0.02 mL, 0.34 mmol), CH_2Cl_2 (1 mL), and $BF_3{\cdot}OEt_2$ (0.01 mL, 0.07 mmol), **6c** was isolated (0.038 g, 79%) as a colorless oil; R_f = 0.53 (hexane/EtOAc = 4:1); IR (neat): $\tilde{\nu}$ = 3420 (w), 2980 (m), 1739 (s), 1658 (s), 1605 (m), 1574 (w), 1467 (m), 1372 (m), 1244 (s), 1041 (m), 804 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.70 (s, 1H, OH), 6.70 (s, 1H, ArH), 4.42 (q, 2H, J = 7.2 Hz, OCH_2), 4.09 (t, 2H, J = 7.8 Hz, OCH_2), 2.96 (t, 2H, J = 7.8 Hz, CH_2), 2.53 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.42 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.4, 171.0, 160.6, 144.4, 139.2, 126.2 (C), 117.1 (CH), 112.0 (C), 62.9, 61.5, 28.5 (CH_2), 21.0, 21.0, 18.6, 14.2 (CH_3); MS (EI, 70 eV): m/z (%) = 288 (M^+, 19), 235 (11), 220 (31), 174 (100), 161 (83), 41 (17); elemental analysis: calcd (%) for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; found: C, 64.25; H, 7.59.

3.4.7. Ethyl 6-hydroxy-3-(2-hydroxyethyl)-2,4-dimethylbenzoate (6d). The reaction was carried out following the procedure as given for the synthesis of **6b**. Acetone rather than CH_2Cl_2 was used for the reaction. Starting with ethyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4b**) (0.045 g, 0.19 mmol), sulfuric acid (10%, 0.5 mL), and acetone (0.5 mL), **6d** was isolated (0.017 g, 39%) as a colorless oil; R_f = 0.13 (hexane/EtOAc = 4:1); IR (KBr): $\tilde{\nu}$ = 3339 (br), 2958 (m), 1722 (s), 1660 (s), 1603 (m), 1575 (w), 1442 (s), 1350 (m), 1244 (s), 1040 (s), 856 (w), 805 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.66 (s, 1H, OH), 6.70 (s, 1H, ArH), 4.42 (q, 2H, J = 7.2 Hz, OCH_2), 3.71 (t, 2H, J = 7.5 Hz, OCH_2), 2.93 (t, 2H, J = 7.5 Hz, CH_2), 2.51 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 1.44 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.5, 159.8, 144.4, 139.0, 127.0 (C), 117.0 (CH), 112.0 (C), 61.8, 61.5, 32.4 (CH_2), 21.2, 18.7, 14.2 (CH_3); MS (EI, 70 eV): m/z (%) = 238 (M^+, 66), 207 (69), 192 (71), 161 (100), 104 (25), 79 (19); the exact molecular mass for $C_{13}H_{18}O_4$: m/z = 238.1164 ± 2 mD was confirmed by HRMS (EI, 70 eV).

3.4.8. Methyl 6-hydroxy-2,4-dimethyl-3-(2-phenoxyethyl)benzoate (6e). The reaction was carried out following the procedure as given for the synthesis of **6a**. Starting with methyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4a**) (0.045 g, 0.20 mmol), phenol (0.025 g, 0.27 mmol), CH_2Cl_2 (1 mL), and TFA (two drops), **6e** was isolated (0.038 g, 79%) as a colorless solid, mp = 78–79 °C; R_f = 0.53 (hexane/EtOAc = 4:1); IR (neat): $\tilde{\nu}$ = 3398 (w), 2980 (m), 1738 (s), 1658 (s), 1605 (m), 1574 (w), 1465 (s), 1375 (m), 1244 (s), 1035 (s), 863 (w), 804 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.64 (s, 1H, OH), 7.29–7.24 (m, 2H, ArH), 6.96–

6.86 (m, 3H, ArH), 6.72 (s, 1 H, ArH), 3.97 (t, 2H, J = 7.2 Hz, OCH_2), 3.95 (s, 3H, OCH_3), 3.14 (t, 2H, J = 7.5 Hz, CH_2), 2.53 (s, 3H, CH_3), 2.37 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1, 160.2, 159.0, 144.8, 139.4 (C), 129.7 (CH), 127.1 (C), 121.0, 17.4, 114.7 (CH), 112.2 (C), 66.8 (CH_2), 52.3 (CH_3), 29.6 (CH_2), 21.4, 18.9 (CH_3); MS (EI, 70 eV): m/z (%) = 300 (M^+, 11), 269 (3), 207 (3), 193 (49), 175 (4), 161 (100), 133 (5), 105 (8), 77 (9), 28 (28); the exact molecular mass for $C_{18}H_{20}O_4$: m/z = 300.1362 ± 2 mD was confirmed by HRMS (EI, 70 eV); elemental analysis: calcd (%) for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71; found: C, 71.67; H, 7.09.

3.4.9. Isopropyl 6-hydroxy-2,4-dimethyl-3-(2-(thiophenoxy)ethyl)benzoate (6f). The reaction was carried out following the procedure as given for the synthesis of **6a**. Starting with isopropyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4c**) (0.035 g, 0.14 mmol), thiophenol (0.020 g, 0.18 mmol), CH_2Cl_2 (1 mL), and TFA (two drops), **6f** was isolated (0.033 g, 70%) as a colorless oil; R_f = 0.64 (hexane/EtOAc = 4:1); IR (neat): $\tilde{\nu}$ = 2980 (w). 1725 (w), 1654 (s), 1602 (w), 1576 (w), 1467 (m), 1370 (m), 1240 (m), 1100 (m), 740 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.72 (s, 1H, OH), 7.37–7.17 (m, 5 H, ArH), 6.66 (s, 1H, ArH), 5.30 (sep, 1H, J= 6.2 Hz, OCH), 2.96–2.83 (m, 4H, CH_2), 2.42 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 1.39 (d, 6H, J= 6.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 159.8, 143.6, 138.4, 136.0 (C), 130.1 (CH), 129.3 (C), 129.0, 128.9, 127.4, 126.4, 117.0 (CH), 112.3 (C), 69.6 (CH), 33.1, 29.6 (CH_2), 21.9 (2C), 20.8, 18.5 (CH_3); MS (EI; 70 eV) m/z (%) = 344 (M^+, 27), 285 (7), 235 (9), 221 (57), 179 (47), 161 (100), 123 (11), 77 (7), 28 (10); elemental analysis: calcd (%) for $C_{20}H_{24}O_3S$: C, 69.70; H, 7.02; found: C, 69.74; H, 7.20.

3.4.10. Methyl 3-(2-bromoethyl)-2,4-dimethyl-6-vinylbenzoate (9). The reaction was carried out following the procedure as given for the synthesis of **6b**. The $BF_3{\cdot}OEt_2$ was added last to the reaction mixture. Starting with methyl 8-hydroxy-4,8-dimethyl-6-oxospiro[2.5]oct-4-ene-5-carboxylate (**4a**) (0.030 g, 0.13 mmol), vinylmagnesium bromide (0.2 mL, 0.20 mmol; 1.0 M solution in THF), THF (1 mL), and $BF_3{\cdot}OEt_2$ (0.01 mL, 0.07 mmol; dissolved in 0.2 mL of THF), **7** was isolated (0.013 g, 33%) as a colorless oil which solidifies upon standing, mp = 62–63 °C; IR (neat): $\tilde{\nu}$ = 3435 (br), 2974 (w), 1728 (s), 1557 (s), 1442 (s), 1364 (m), 1149 (m), 1121 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.24 (s, 1H, ArH), 6.61 (dd, 1H, J = 11.1 Hz, 17.4 Hz, =CH), 5.68 (dd, 1H, J = 0.9 Hz, 17.4 Hz, $=CH_2$), 5.28 (dd, 1H, J = 0.9 Hz, 11.1 Hz, $=CH_2$), 3.91 (s, 3H, OCH_3), 3.38–3.12 (m, 2H, CH_2), 3.24–3.18 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 2.26 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 138.2, 136.0 (C), 133.9 (CH), 133.7, 133.2, 132.4 (C), 125.5 (CH), 116.7 (CH_2), 52.4 (CH_3), 33.9, 29.3 (CH_2), 20.4, 16.8 (CH_3); MS (EI, 70 eV): m/z (%) = 298 (M^++2, 79), 296 (M^+, 80), 267 (33), 265 (30), 217 (78), 203 (100), 171 (41), 128 (37), 114 (24), 28 (46); the exact molecular mass for $C_{14}H_{17}O_2Br$ m/z = 296.0412 ± 2 mD was confirmed by HRMS (EI, 70 eV); elemental analysis: calcd (%) for $C_{14}H_{17}O_2Br$: C, 56.58; H, 5.78; found: C, 56.31; H, 6.49.

3.5. Cytotoxicity assay

All chemicals were purchased from SIGMA and used without further purification. A human leukemia cell line HL60 (acute promyeloid leukemia, FAB-subtype M2) was used for the antitumor assay investigations (source: DSMZ, Braunschweig, Germany, No. ACC3). The growth medium consists of RPMI1640 medium including 10% FCS, 15 mg of penicillin and 27 mg of gentamicin-sulfate per 500 mL. The cells were incubated for 24 h at 37 °C, 5% CO_2, 95% humidity as a discrete cell suspension.[15,16] The final concentration was 100,000 cells/mL. The test compounds were dissolved in DMSO and serially diluted to five concentrations for testing (end concentration 1:1000 in medium). Negative controls were performed with just DMSO. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazoliumbromide) assay was performed according to a known procedure[17] with 2.5 mg of MTT per 1 mL of PBS (including 0.15 M NaCl and 0.02 M sodium phosphate); 20 μL was added per well. After an incubation time of 48 h at 37 °C, 5% CO_2, the optical densities (OD) were measured at λ = 570 nm with a microtiter plate reader (ANTHOS 2010). The OD values were used to calculate the T/C_{corr} (%) values based on the following equation T/C_{corr} (%) = $(OD_{test,48\ h} - OD_{blank,t0})/(OD_{blank,\ 48\ h} - OD_{test,\ t0}) \times 100$. T/C values between 90% and 10% were used to construct dose–response curves and the IC_{50} values were estimated by linear regression analysis of T/C versus the log concentration as previously described.[21]

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