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Phosphorylated flavonoids as selective carboxylesterase inhibitors

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Contents

1. Experimental section

1.1. Chemistry: General procedures

THF was dried according to standard procedures. Unless otherwise noted, all other reagents were obtained from commercial sources and used without further purification. Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. High-resolution mass spectra (HRMS) were recorded on a SolariX XR mass spectrometer. IR spectra were registered with a Shimadzu IRAffinity-1 instrument (KBr pellets). NMR spectra were obtained with a Bruker AVANCE III 400 spectrometer operating at 400 (^{1}H), 100.6 (^{13}C) or 162 (^{31}P) MHz. The reaction conditions were not optimized for reaction yields. All oxygen-sensitive or moisture-sensitive reactions were run under argon atmosphere. The reaction progress was monitored by thin layer chromatography (TLC) on EMD/Merck KGaA plates at 254 nm under a UV lamp using DCM/MeOH as eluent. Silica gel Merck 60 Å 70-230 mesh was used for column chromatography. 2-Chloro-4*H*-1,3,2-benzodioxaphosphinine 2-oxide, a starting material for the synthesis of **8**, was synthesized according to a known method.[1] The synthesis of compounds **1-3**,[2] **9**,[3] **10-11**[4] had been reported previously, and the characterization data we provided were in agreement with the published data. The synthesis of compounds **4**, **5**, **6** was described in our paper.[1] Compounds **7**, **8**, **12**, **13**, **14** are new.

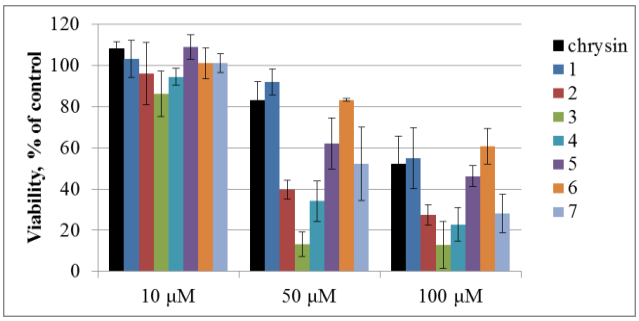
*1.2. General procedure for the synthesis of compounds **8-11**, **13***

A solution of $(RO)_2P(O)Cl$ (1.1 mmol) in THF (2 ml) was added dropwise to suspension of chrysin (7-hydroxyflavone) (1 mmol), DMAP (1 mmol) and triethylamine (1.5 mmol) in THF (5 ml) upon cooling and stirring. The reaction mixture was stirred at room temperature overnight, and then concentrated under reduced pressure using a high-vacuum pump. The residue was dissolved in ethyl acetate (25 ml), washed with 0.5 M HCl (20 ml), brine (20 ml) and dried over magnesium sulfate. After the solvent was removed, the residue was purified by column chromatography on silica gel (methylene chloride/methanol, 100:1 to 60:1) to give the corresponding product.

*1.3. General procedure for the synthesis of compounds **7**, **12**, **14***

A solution of $HP(O)(OR)_2$ (1.1 mmol) in carbon tetrachloride (35 mmol) was added to mixture of chrysin (7-hydroxyflavone) (1 mmol) and solution of triethylamine (35 mmol) in THF (20 ml) upon cooling and vigorous stirring. The reaction mixture was stirred at room temperature overnight, and then triethylammonium salt was filtered off. The filtrate was evaporated, and water (10 ml) was added to the residue. The product was extracted with ethyl acetate (20 ml), washed with 1 M HCl (20 ml), brine (20 ml) and dried over magnesium sulfate. After the solvent was removed, the residue was purified by column chromatography on silica gel (methylene chloride/methanol, 100:1 to 60:1) to give the corresponding product.

2. Anticancer activity of investigated compounds



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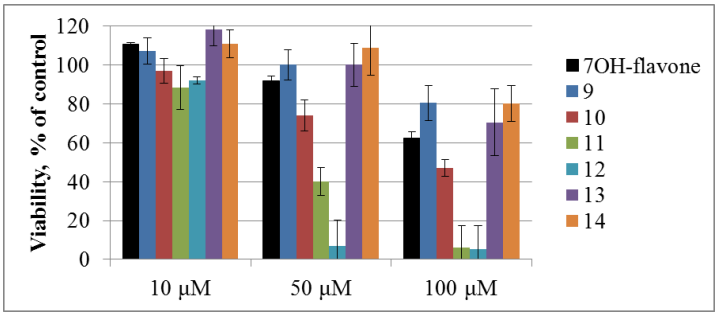

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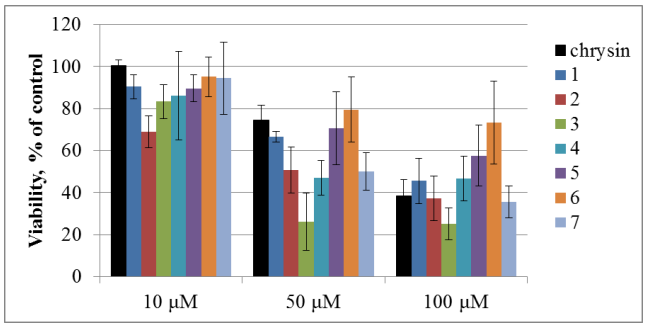

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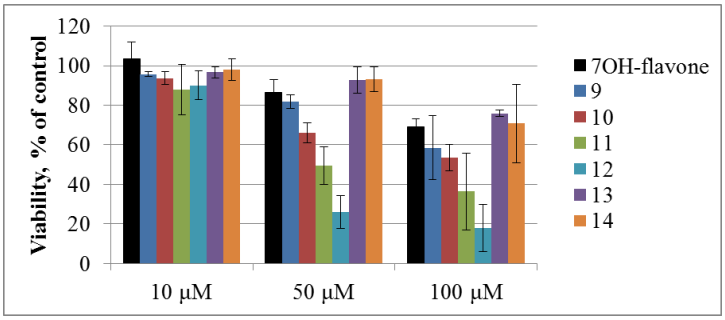

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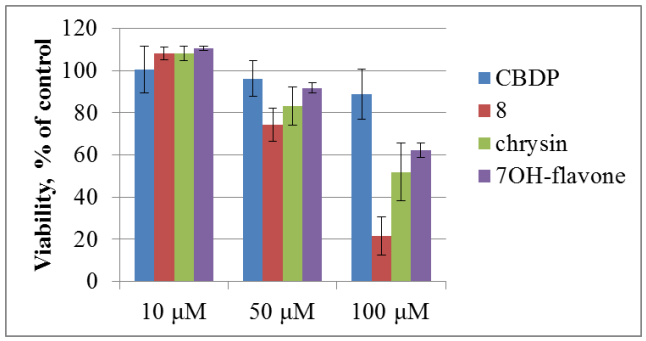

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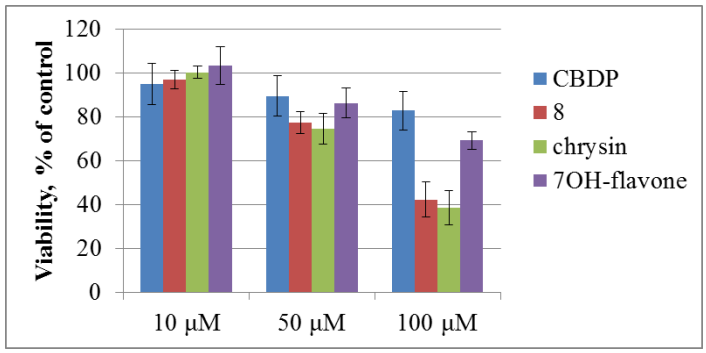

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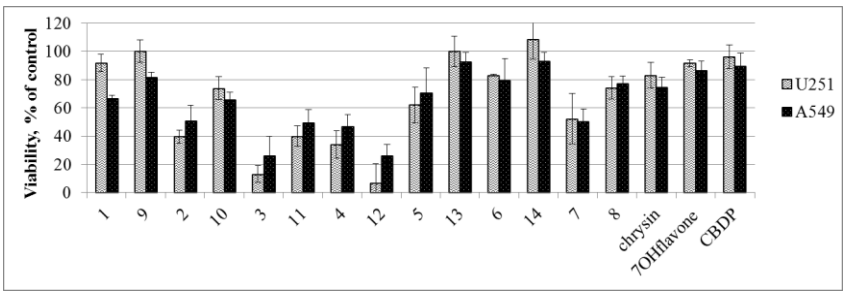

g

Figure S1 Anticancer activity of investigated compounds evaluated by SRB assay. Viability of: a) U251 cells after 48 h exposure to chrysin derivatives; b) U251 cells after 48 h exposure to 7-hydroxyflavone derivatives; c) A549 cells after 48 h exposure to chrysin derivatives; d) A549 cells after 48 h exposure to 7-hydroxyflavone derivatives; e) U251 cells after 48 h exposure to 7-hydroxyflavone, chrysin, CBDP and **8**; f) A549 cells after 48 h exposure to 7-hydroxyflavone, chrysin, CBDP and **8**; g) two cell lines after 48 h exposure to investigated compounds at 50 µM concentration. Results are reported relative to untreated cells and presented as the mean ± S.D.

3. *X-ray diffraction experiments*

For a single crystal X-ray diffraction experiment, a crystal of **7** was fixed on a micro mount, placed on Agilent Technologies SuperNova diffractometer and investigated at the temperature of 100 K using monochromated MoKα radiation. The unit cell parameters were refined by least square techniques using 4426 reflections in the 2θ range of 6.04–55.00. The structure has been solved by the direct methods and refined R_1 = 0.041 for 3816 unique reflections with $|F_o| \geq 4\sigma_F$ by means of the SHELXL–97 program[4] incorporated in the *OLEX2* program package.[5] The carbon-bound H atoms were placed in calculated positions and were included in the refinement in the 'riding' model approximation, with U_{iso}(H) set to 1.2U_{eq}(C) and C–H 0.97 Å for the CH_2 groups, U_{iso}(H) set to 1.2U_{eq}(N) and C–H 0.93 Å for the CH groups. Empirical absorption correction was applied by CrysAlisPro (Agilent Technologies, 2012) program complex using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. Supplementary crystallographic data for this paper have been deposited at Cambridge Crystallographic Data Centre (CCDC 1873194) and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

Table S1 Crystal data and structure refinement for 7.

Empirical formula	$C_{21}H_{19}O_7P$
Formula weight	414.33
Temperature/K	100(2)
Crystal system	triclinic
Space group	P-1
a/Å	8.2685(8)
b/Å	8.9080(6)
c/Å	13.9927(13)
α/°	78.860(8)
β/°	89.856(8)
γ/°	72.248(8)
Volume/Å^3	961.32(15)
Z	2
d_{calc}/g cm^{-3}	1.431
μ/mm^{-1}	0.185
F(000)	432.0
Crystal size/mm^3	0.21 × 0.18 × 0.15
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.818 to 54.986
Index ranges	-7 ≤ h ≤ 10, -11 ≤ k ≤ 11, -18 ≤ l ≤ 18
Reflections collected	8112
Independent reflections	4408 [R_{int} = 0.0284, R_{sigma} = 0.0433]
Data/restraints/parameters	4408/0/263
Goodness-of-fit on F^2	1.037
Final *R* indexes [*I*>=2σ (*I*)]	R_1 = 0.0406, wR_2 = 0.1077
Final *R* indexes [all data]	R_1 = 0.0482, wR_2 = 0.1131
Largest diff. peak/hole / e Å^{-3}	0.36/-0.41

Table S2 Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for 7. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
P1	284.9(5)	1894.5(5)	3789.5(3)	13.18(12)
O1	2958.7(14)	3816.2(13)	449.0(8)	14.6(2)
O2	211.4(15)	3720.7(13)	3444.4(8)	17.2(2)
O3	-1073.3(15)	1786.7(13)	3052.2(8)	16.7(2)
O4	1951.7(15)	699.6(14)	3856.2(9)	19.3(3)
O5	293.2(15)	8539.4(13)	-517.2(9)	19.0(3)
O6	-484.6(14)	1923.1(13)	4805.6(8)	15.7(2)
O7	-1293.2(15)	8486.7(13)	1093.1(9)	18.7(3)
C8	1787.7(19)	4596.1(18)	1028.8(11)	12.5(3)
C9	837.8(19)	6204.8(18)	721.3(11)	12.7(3)
C10	2335(2)	6183.3(19)	-781.2(11)	14.8(3)
C11	3201.7(19)	4625.4(18)	-441.6(11)	12.9(3)
C12	4495.7(19)	3607.0(19)	-967.6(11)	14.0(3)
C13	-346.7(19)	6940.0(18)	1353.1(11)	13.7(3)
C14	-544(2)	6065.8(19)	2249.1(12)	15.5(3)
C15	1623(2)	3691.5(19)	1926.8(11)	14.9(3)
C16	1094(2)	7093.4(18)	-219.2(11)	13.5(3)
C17	-2188(2)	2974(2)	4922.7(12)	18.0(3)
C18	444(2)	4473.0(19)	2514.8(11)	14.1(3)
C19	-1286(2)	219.2(19)	2997.1(12)	16.8(3)
C20	6931(2)	1695(2)	-1973.3(13)	23.5(4)
C21	6150(2)	3299(2)	-2383.3(13)	24.5(4)
C22	5299(2)	1993(2)	-560.6(12)	18.2(3)
C23	-2915(2)	130(2)	3429.8(13)	21.9(4)
C24	6504(2)	1047(2)	-1064.6(14)	22.8(4)
C25	4931(2)	4253(2)	-1884.2(12)	20.6(3)
C26	-2088(2)	4466(2)	5214.2(13)	20.6(3)
C2	-3060(3)	5918(2)	4818.5(15)	29.0(4)
C3	-4181(3)	27(3)	2920.2(17)	34.2(5)

Table S3 Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for 7. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
P1	16.5(2)	13.0(2)	10.9(2)	-1.88(15)	3.16(14)	-6.22(16)
O1	17.2(5)	11.4(5)	12.5(5)	-1.2(4)	3.7(4)	-1.2(4)
O2	26.9(6)	14.9(5)	12.3(6)	-3.2(4)	6.0(5)	-9.8(5)
O3	22.2(6)	13.0(5)	15.2(6)	-1.0(4)	-0.9(4)	-7.1(5)
O4	17.5(6)	19.0(6)	21.2(6)	-3.5(5)	4.6(5)	-5.7(5)
O5	24.9(6)	11.4(5)	17.2(6)	-0.4(4)	2.5(5)	-1.8(5)

O6	18.0(6)	15.5(5)	12.9(5)	-1.1(4)	3.4(4)	-5.1(5)
O7	23.9(6)	10.9(5)	18.1(6)	-2.1(4)	5.7(5)	-1.4(5)
C8	12.6(7)	13.2(7)	12.9(7)	-4.4(6)	1.8(5)	-4.3(6)
C9	13.4(7)	12.5(7)	13.3(7)	-2.6(6)	0.5(6)	-5.3(6)
C10	17.5(8)	15.7(7)	11.3(7)	-1.4(6)	2.8(6)	-6.2(6)
C11	13.6(7)	14.2(7)	11.8(7)	-2.7(6)	1.3(6)	-5.6(6)
C12	11.3(7)	17.3(8)	14.4(8)	-5.6(6)	0.9(6)	-4.6(6)
C13	14.8(7)	12.0(7)	15.9(8)	-4.6(6)	0.8(6)	-5.2(6)
C14	17.8(8)	16.1(8)	16.0(8)	-7.2(6)	4.0(6)	-8.0(6)
C15	18.3(8)	11.3(7)	14.6(8)	-1.1(6)	1.5(6)	-4.9(6)
C16	14.7(7)	12.8(7)	13.3(7)	-2.5(6)	-0.1(6)	-5.0(6)
C17	16.3(8)	19.3(8)	19.6(8)	-5.9(6)	7.5(6)	-6.1(6)
C18	18.4(8)	15.1(7)	11.4(7)	-2.1(6)	2.4(6)	-9.7(6)
C19	20.2(8)	14.2(7)	18.1(8)	-4.9(6)	1.5(6)	-7.3(6)
C20	14.6(8)	32.1(10)	27.7(9)	-17.6(8)	7.1(7)	-6.0(7)
C21	23.2(9)	33(1)	20.1(9)	-8.9(7)	8.2(7)	-10.6(8)
C22	16.7(8)	18.8(8)	17.8(8)	-3.9(6)	1.1(6)	-3.8(6)
C23	26.0(9)	19.2(8)	24.2(9)	-6.9(7)	8.1(7)	-10.6(7)
C24	17.4(8)	21.1(8)	28.1(10)	-9.7(7)	0.0(7)	-0.6(7)
C25	20.2(8)	22.9(8)	18.1(8)	-4.4(7)	4.9(6)	-5.6(7)
C26	24.2(9)	21.4(8)	17.8(8)	-5.4(7)	4.8(6)	-8.4(7)
C2	30.7(10)	21.1(9)	36.3(11)	-7.5(8)	3.1(8)	-8.7(8)
C3	24.4(10)	39.5(12)	42.0(12)	-11.6(10)	7.1(8)	-12.7(9)

Table S4 Bond Lengths for 7.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
P1	O2	1.5873(11)	C10	C16	1.436(2)
P1	O3	1.5635(11)	C11	C12	1.474(2)
P1	O4	1.4521(12)	C12	C22	1.391(2)
P1	O6	1.5579(12)	C12	C25	1.395(2)
O1	C8	1.3720(18)	C13	C14	1.380(2)
O1	C11	1.3612(19)	C14	C18	1.382(2)
O2	C18	1.3851(18)	C15	C18	1.384(2)
O3	C19	1.4760(18)	C17	C26	1.489(2)
O5	C16	1.2432(19)	C19	C23	1.493(2)
O6	C17	1.4633(19)	C20	C21	1.382(3)
O7	C13	1.3391(18)	C20	C24	1.383(3)
C8	C9	1.390(2)	C21	C25	1.389(2)
C8	C15	1.386(2)	C22	C24	1.384(2)
C9	C13	1.413(2)	C23	C3	1.304(3)
C9	C16	1.450(2)	C26	C2	1.310(3)
C10	C11	1.344(2)			

Table S5 Bond Angles for 7.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
O3	P1	O2	100.65(6)	C25	C12	C11	120.20(15)
O4	P1	O2	116.55(7)	O7	C13	C9	120.76(14)
O4	P1	O3	116.45(7)	O7	C13	C14	119.07(14)
O4	P1	O6	111.83(7)	C14	C13	C9	120.17(14)
O6	P1	O2	101.13(6)	C13	C14	C18	118.75(14)
O6	P1	O3	108.64(6)	C18	C15	C8	116.43(14)
C11	O1	C8	119.72(12)	O5	C16	C9	122.26(14)
C18	O2	P1	126.83(10)	O5	C16	C10	122.50(15)
C19	O3	P1	120.61(10)	C10	C16	C9	115.24(14)
C17	O6	P1	122.15(10)	O6	C17	C26	110.45(13)
O1	C8	C9	121.05(14)	C14	C18	O2	114.43(14)
O1	C8	C15	116.20(14)	C14	C18	C15	123.58(14)
C15	C8	C9	122.74(14)	C15	C18	O2	121.95(14)
C8	C9	C13	118.32(14)	O3	C19	C23	108.98(13)
C8	C9	C16	120.05(14)	C21	C20	C24	119.94(16)
C13	C9	C16	121.62(14)	C20	C21	C25	119.89(17)
C11	C10	C16	121.60(15)	C24	C22	C12	120.19(16)
O1	C11	C12	112.57(13)	C3	C23	C19	122.62(17)
C10	C11	O1	122.31(14)	C20	C24	C22	120.46(17)
C10	C11	C12	125.11(14)	C21	C25	C12	120.46(17)
C22	C12	C11	120.74(15)	C2	C26	C17	124.02(17)
C22	C12	C25	119.06(15)				

Table S6 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for 7.

Atom	*x*	*y*	*z*	U(eq)
H7	-992	8890	574	28
H10	2544	6685	-1395	18
H14	-1327	6540	2666	19
H15	2269	2617	2124	18
H17A	-2738	2409	5417	22
H17B	-2868	3255	4313	22
H19A	-340	-647	3352	20
H19B	-1308	105	2322	20
H20	7742	1053	-2308	28
H21	6441	3739	-2992	29
H22	5025	1549	52	22
H23	-3031	149	4089	26
H24	7032	-33	-790	27
H25	4402	5331	-2163	25
H26	-1285	4362	5707	25
H2A	-3875	6061	4324	35

H2B	-2936	6804	5032	35
H3A	-4090	6	2260	41
H3B	-5175	-26	3217	41

Experimental

the structure was solved with the ShelXS [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.
2. Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.
3. Sheldrick, G.M. (2015). Acta Cryst. C71, 3-8.

Refinement model description

Number of restraints - 0, number of constraints - unknown.
Details:
1. Fixed Uiso
At 1.2 times of:
All C(H) groups, All C(H,H) groups
At 1.5 times of:
All O(H) groups
2.a Secondary CH2 refined with riding coordinates:
C17(H17A,H17B), C19(H19A,H19B)
2.b Aromatic/amide H refined with riding coordinates:
C10(H10), C14(H14), C15(H15), C20(H20), C21(H21), C22(H22), C23(H23), C24(H24), C25(H25), C26(H26)
2.c X=CH2 refined with riding coordinates:
C2(H2A,H2B), C3(H3A,H3B)
2.d Idealised tetrahedral OH refined as rotating group:
O7(H7)

4. Biochemistry

4.1. Cell culture

The human lung adenocarcinoma A549 and human glioblastoma U251 cell lines were purchased from the Russian Academy of Sciences Cells Bank (Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russian Federation) and cultured at 37°C in a humidified atmosphere containing 5% CO_2 in DMEM complete medium supplemented with penicillin (100 U/ml), streptomycin (100 g/ml) and fetal calf serum (10%, HyClone). All experiments were performed on cells from 3 to 6 passages in the logarithmic phase of growth.

4.2. Preparation of stock solution

Each compound except CBDP and **8**, was initially solubilized in acetonitrile (ACN) at 10 mM concentration and stored in the fridge no more than one week. CBDP and **8** were solubilized in dimethyl sulfoxide (DMSO) at 10 mM concentration.

4.3. Cell treatment

To test the potential cytotoxicity of the synthesized compounds, A549 and U251 cells were seeded at a density of 7 500 cells/well and 15 000 cell/well accordingly into 96-well microplates and cultured for 24 h before the exposure. For each compound three dilutions were prepared in complete medium just before the experiment. The maximum final concentration of ACN or DMSO in wells was no more than 1%. It was found that this concentration didn't affect the cells. Solutions of the different concentrations (0.1 ml) were pipetted into separate wells of a microtiter plate containing cells with 0.1 ml complete medium, followed by incubation period of 48 hours at 37°C in an atmosphere of 5% CO_2. Non-exposed cells served as control in each experiment.

4.3.1. Estimation of cellular protein content by Sulforhodamine B (SRB) assay

Cells were fixed, washed, and stained for 30 minutes with 0.4% (w/v) SRB dissolved in 1% acetic acid according to the manufacture protocol (TOX6, Sigma). Excess unbound dye was removed by washing four times with 1% acetic acid and attached stain was recovered with 10 mM Tris buffer. Color intensity of wells was measured in an Epoch microplate spectrophotometer (BioTek Instruments, Inc., U. S.) at wavelength of 490 or 510 nm versus the blank sample, depending on the cell line. Percentage of viability was calculated from the experimental and control data.

4.3.2. Enzyme inhibition assay

To investigate the inhibitory activity of the compounds towards serine esterases the commercially available preparations of human AChE (C1682, Sigma), BChE (B4186, Sigma) and porcine liver CE (E3019, Sigma) were used. Enzymes were diluted in 10 mM phosphate buffered saline pH 7.4 containing 137 mM NaCl and 2,7 mM KCl (PBS).

Working solutions of investigated compounds were prepared from stock ACN solutions by dilution them firstly in ACN and then in PBS. Since compounds tested had significantly different range of activity and in view of their potentially different solubility in aqueous solutions we had to prepare some intermediate dilutions in ACN. Nevertheless, the final ACN concentration in working solutions never exceeded 1%. This concentration did not affect the activity of enzymes in selected experimental conditions.

In wells of a 96-well plate the solutions of enzymes with activity 25-30 U were poured, followed by addition of the solutions of investigated compounds of volume that was 1/9 of the volume of the enzyme solutions. Each inhibitor was assayed with six different concentrations around the IC50 values that were roughly estimated in the first round of experiments. In the control wells an equivalent volume of PBS was added instead of solutions of compounds. The

investigated compounds were incubated with the enzymes for 1 hour at 25 °C. Then plates were thermostated at 37 °C on a plate temperature-control shaker for 5 minutes and esterase activity assay was performed at the same temperature in the kinetic mode in the presence of excess substrate using conventional techniques with slight modifications.[6,7] When determining the BChE and AChE activity butyrylthiocholine and acethylthiocholine as substrates were used respectively. To evaluate the CE activity *p*-nitrophenylacetate (*p*-NPA) was used. All reactions were corrected for nonenzymatic hydrolysis of substrates.

4.3.3. Determination of inhibition rate constants

The measurements were performed with excess substrate and inhibitor concentrations. Only the initial time of the reaction was taken into account, therefore the concentration of substrate at the time of measurement could be considered constant. Enzymatic reaction was started by adding 50 µl of CE solution (85 U) to the wells containing 50 µl diluted **2, 3, 8, 9** and 100 µl *p*-NPA solution. The residual enzyme activity was then assayed in duplicate for each experiment. Substrate hydrolysis was monitored at 10 sec intervals for 5 min. The recorded curves were analyzed by two consecutive linear-regression analyses based on equation given in the Forsberg and Puu work.[8] Firstly the slopes (k') of each primary plot of ln (ΔV), change of velocity of substrate hydrolysis) versus time were calculated. Each value was obtained from a line through 8-9 points. These values of k' were then plotted against 1/[I] (I - inhibitor concentration) to obtain final simple equation from which k_2 and K_d could be calculated. K_d was determined as the reciprocal value of the intercept on the abscissa, and k_2 as the reciprocal value of the intercept on the ordinate. The bimolecular rate constant of inhibition (k_i) was calculated as ratio between these two constants, k_2/K_d.

K_m was calculated from Michaelis-Menten enzyme kinetics model equation using eight different concentrations of *p*-NPA (0.02-2.5 mM). Under the conditions employed K_m was 0.36 mM and α was 0.87. All reactions were corrected for nonenzymatic hydrolysis of substrates. Experiments were performed at 25 °C in PBS.

4.3.4. Statistical analysis

Three independent experiments were performed for each compound, cell line and enzyme. Measurements were performed in triplicate for each concentration and averaged before further calculations. Statistical calculations were performed by using GraphPad Prizm 5.0 software. To calculate IC50 (half-maximal effective concentration of test compounds in relation to the enzyme inhibition) we used nonlinear regression analysis. We used two-way ANOVA and Bonferroni post-test to reveal differences between data obtained for different compounds. The critical significance level (P) for the testing of statistical hypotheses was taken to be 0.05. Results were expressed as the mean ± S.D.

5. NMR-spectra

*Diallyl 5-hydroxy-4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**7**)*

Pale yellow solid. Yield: 40%. M.p.: 53°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.79 (s, 1H), 7.92 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.64 – 7.51 (m, 3H), 7.02 (d, *J* = 1.4 Hz, 1H), 6.75 (s, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 5.99 (ddd, *J* = 22.6, 10.9, 5.7 Hz, 2H), 5.44 (dd, *J* = 17.1, 1.3 Hz, 2H), 5.33 (dd, *J* = 10.4, 0.9 Hz, 2H), 4.78 – 4.64 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 182.74 (s), 164.74 (s), 162.25 (s), 157.08 (s), 155.84 (d, *J* = 5.7 Hz), 132.17 (s), 131.79 (d, *J* = 6.6 Hz), 130.97 (s), 129.18 (s), 126.43 (s), 119.17 (d, *J* = 2.4 Hz), 108.43 (s), 106.14 (s), 103.90 (d, *J* = 6.1 Hz), 99.11 (d, *J* = 4.4 Hz), 69.26 (d, *J* = 5.3 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ -7.09 (s). HRMS, m/z: calcd for $C_{21}H_{20}O_7P$ $(M+H)^+$, 415.0941; found, 415.0930. IR (KBr, cm^{-1}): 3076, 2855, 1659, 1614, 1491, 1454, 1348, 1285, 1196, 1152, 1030.

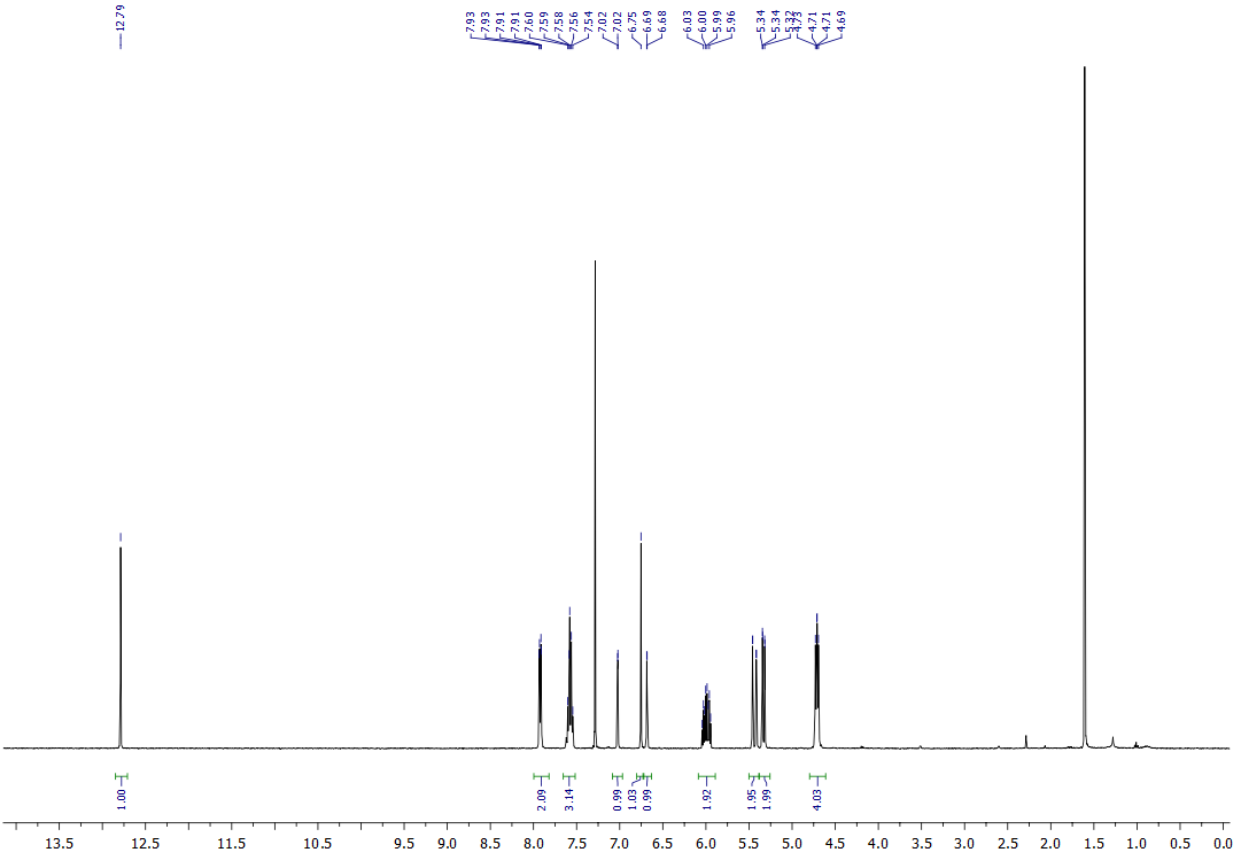

Figure S2. NMR ^{1}H-spectrum of *diallyl 5-hydroxy-4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**7**)*

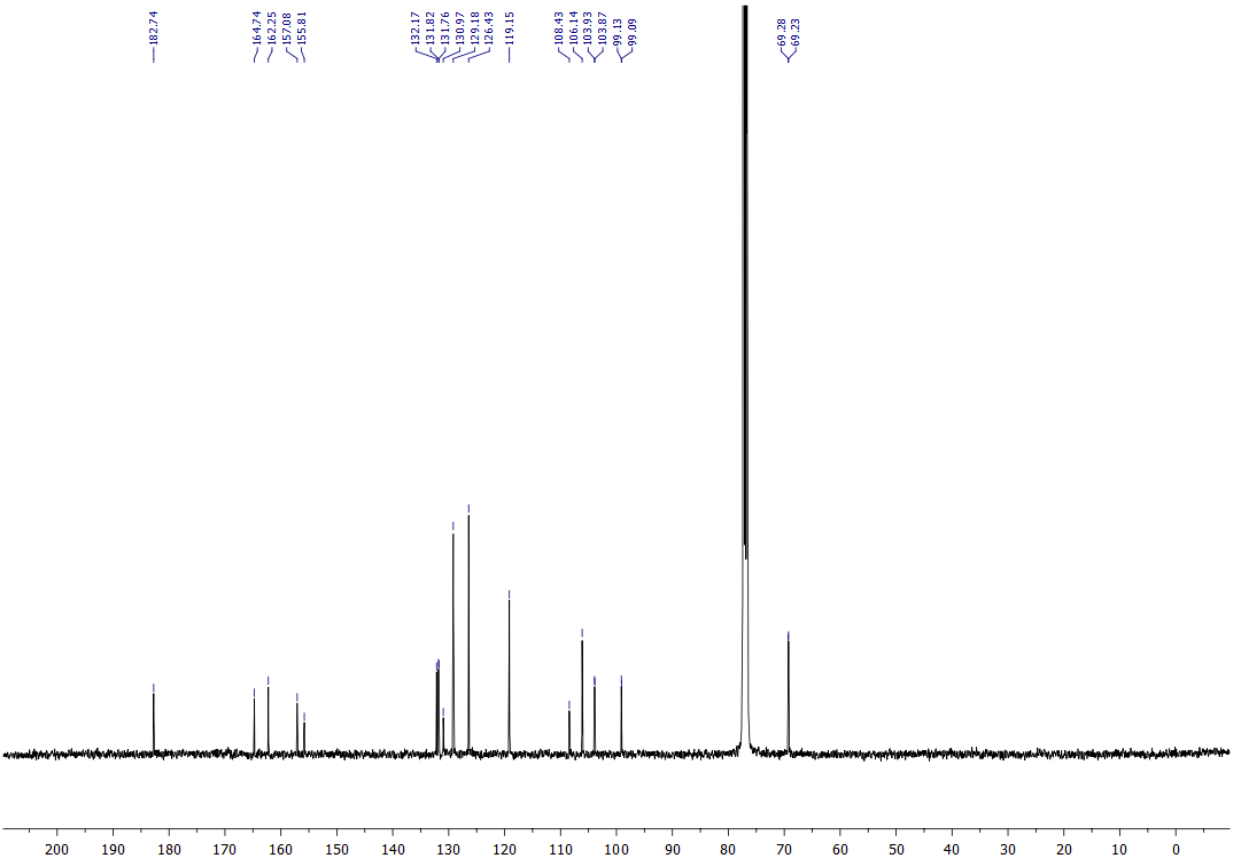

Figure S3. NMR ^{13}C-spectrum of *diallyl 5-hydroxy-4-oxo-2-phenyl-4H-chromen-7-yl phosphate (7)*

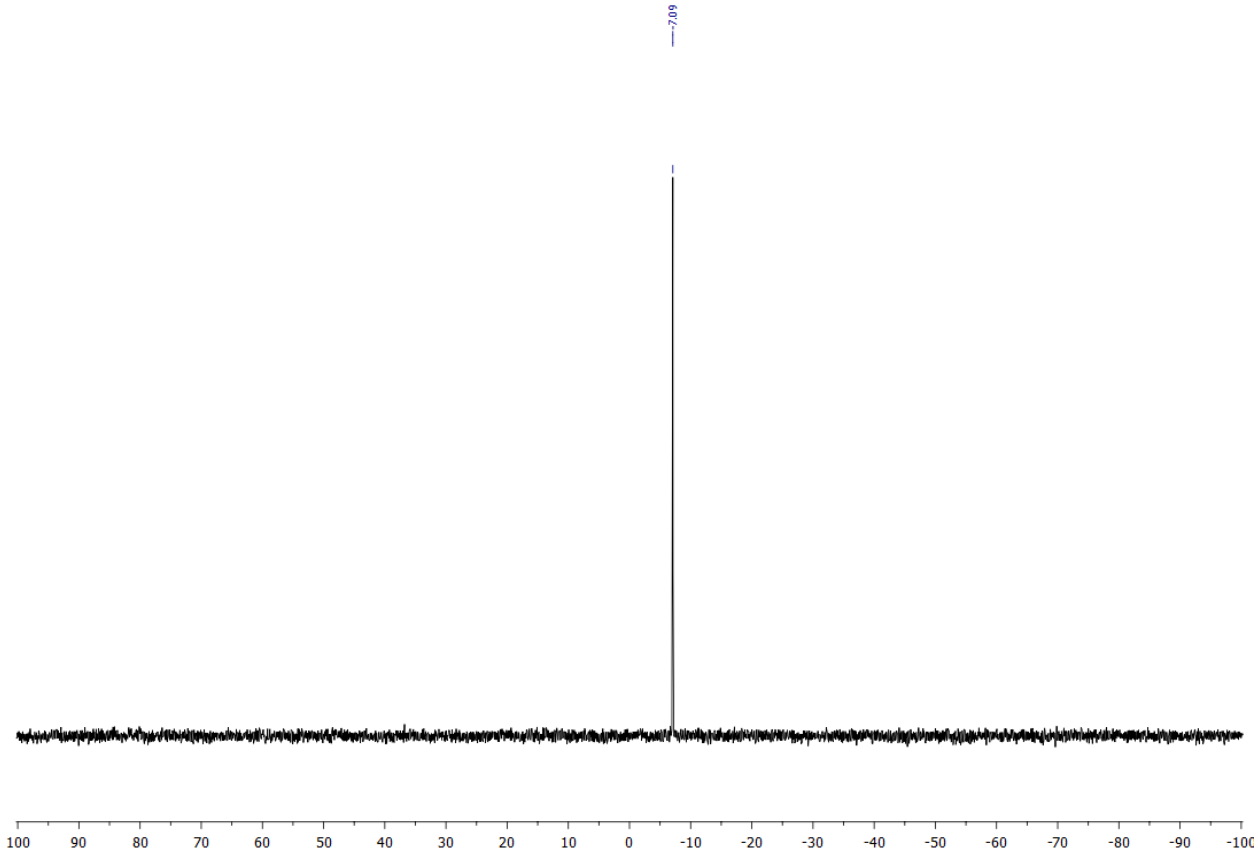

Figure S4. NMR ^{31}P-spectrum of *diallyl 5-hydroxy-4-oxo-2-phenyl-4H-chromen-7-yl phosphate (7)*

*5-Hydroxy-7-[(2-oxido-4H-1,3,2-benzodioxaphosphinin-2-yl)oxy]-2-phenyl-4H-chromen-4-one (**8**)*

Pale yellow solid. Yield: 45%. M.p.: 124°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.79 (s, 1H), 7.98 – 7.82 (m, 2H), 7.66 – 7.47 (m, 3H), 7.38 (t, *J* = 7.8 Hz, 1H), 7.26 – 7.19 (m, 1H), 7.15 (t, *J* = 7.6 Hz, 2H), 7.08 (dd, *J* = 2.2, 1.0 Hz, 1H), 6.74 (s, 1H), 6.61 (d, *J* = 1.6 Hz, 1H), 5.63 – 5.42 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 182.71 (s), 164.84 (s), 162.33 (s), 157.07 (s), 155.04 (d, *J* = 5.9 Hz), 149.72 (d, *J* = 7.3 Hz), 132.27 (s), 130.79 (s), 130.18 (s), 129.20 (s), 126.43 (s), 125.37 (s), 124.96 (s), 120.03 (d, *J* = 10.6 Hz), 118.95 (d, *J* = 9.1 Hz), 108.68 (s), 106.11 (s), 103.73 (d, *J* = 6.5 Hz), 99.15 (d, *J* = 4.1 Hz), 69.62 (d, *J* = 7.3 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ -16.90 (s). HRMS, m/z: calcd for $C_{22}H_{16}O_7P$ $(M+H)^+$, 423.0628; found, 423.0622. IR (KBr, cm^{-1}): 3574, 3073, 1651, 1624, 1489, 1450, 1314, 1190, 1144, 1107, 1028, 991, 961.

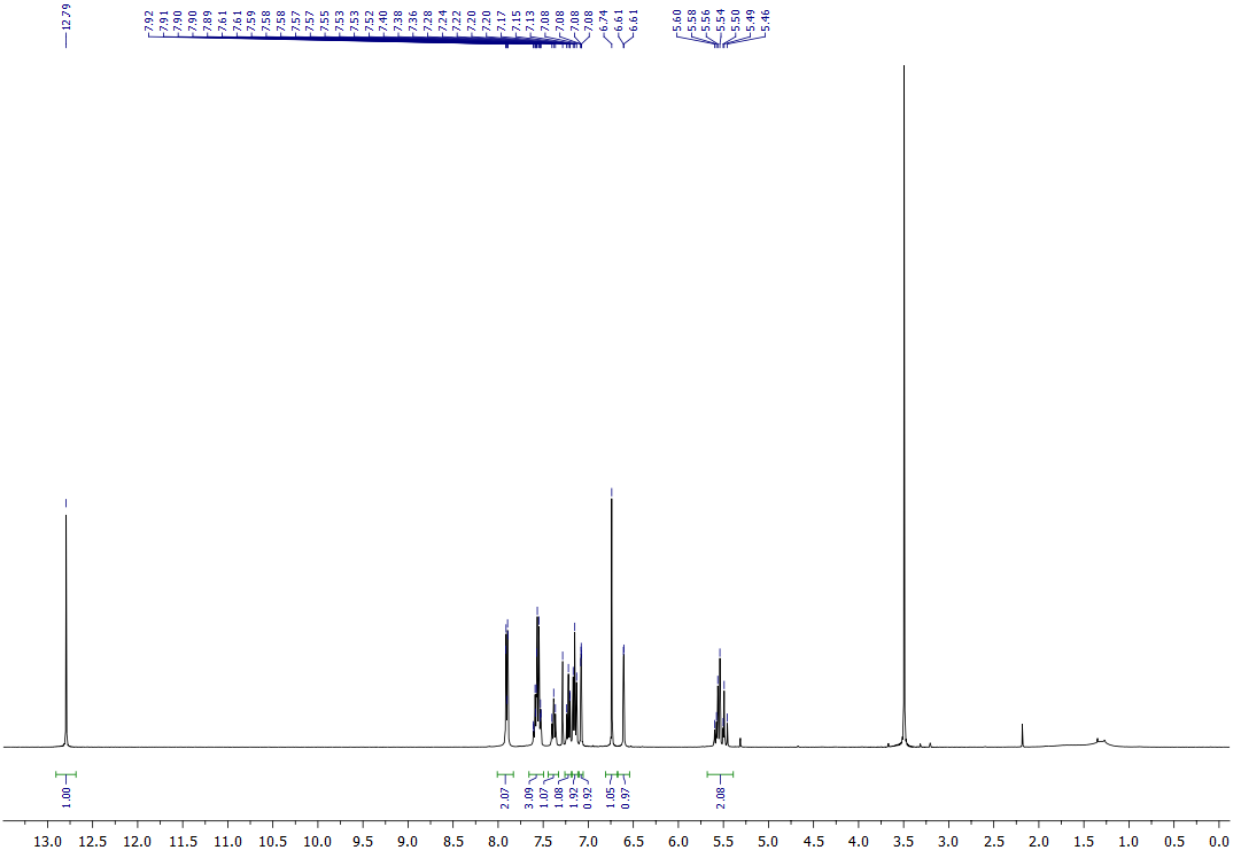

Figure S5. NMR ^{1}H-spectrum of *5-hydroxy-7-[(2-oxido-4H-1,3,2-benzodioxaphosphinin-2-yl)oxy]-2-phenyl-4H-chromen-4-one (**8**)*

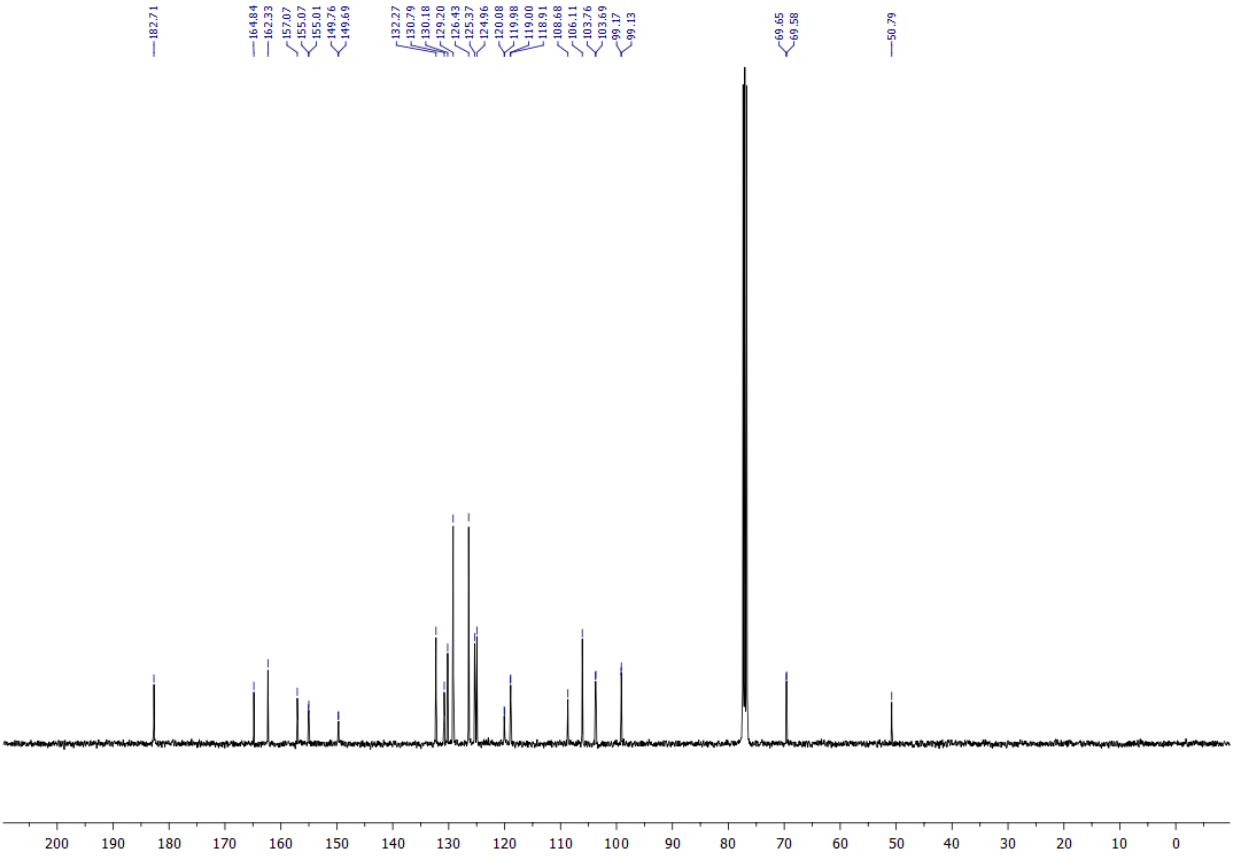

Figure S6. NMR ^{13}C-spectrum of *5-hydroxy-7-[(2-oxido-4H-1,3,2-benzodioxaphosphinin-2-yl)oxy]-2-phenyl-4H-chromen-4-one* (***8***)

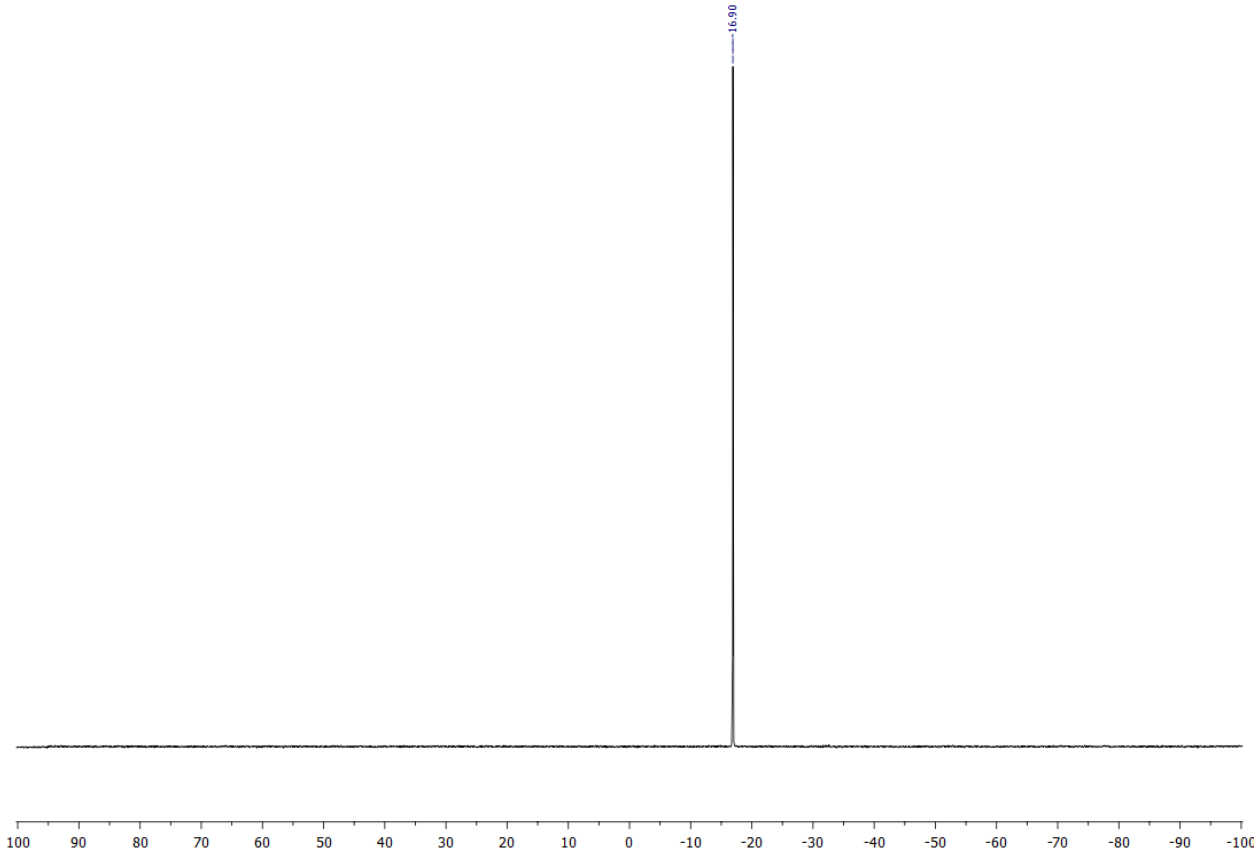

Figure S7. NMR ^{31}P-spectrum of *5-hydroxy-7-[(2-oxido-4H-1,3,2-benzodioxaphosphinin-2-yl)oxy]-2-phenyl-4H-chromen-4-one* (***8***)

*Dimethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**9**)*

Pale yellow solid. Yield: 80%. M.p.: 94°C (Lit.[9] mp 100–101°C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.7 Hz, 1H), 8.01 – 7.85 (m, 2H), 7.63 – 7.52 (m, 4H), 7.34 – 7.21 (m, 1H), 6.82 (s, 1H), 3.94 (d, *J* = 11.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.53 (s), 163.73 (s), 156.97 (s), 154.51 (d, *J* = 6.3 Hz), 131.76 (s), 131.46 (s), 129.10 (s), 127.69 (s), 126.29 (s), 121.26 (s), 117.87 (d, *J* = 5.7 Hz), 108.96 (d, *J* = 4.4 Hz), 107.65 (s), 55.27 (d, *J* = 6.2 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ -4.76 (s). HRMS, m/z: calcd for $C_{17}H_{16}O_6P$ $(M+H)^+$, 347.0679; found, 347.0675. IR (KBr, cm^{-1}): 3422, 3074, 2959, 2855, 1641, 1618, 1497, 1450, 1369, 1294, 1153, 1059.

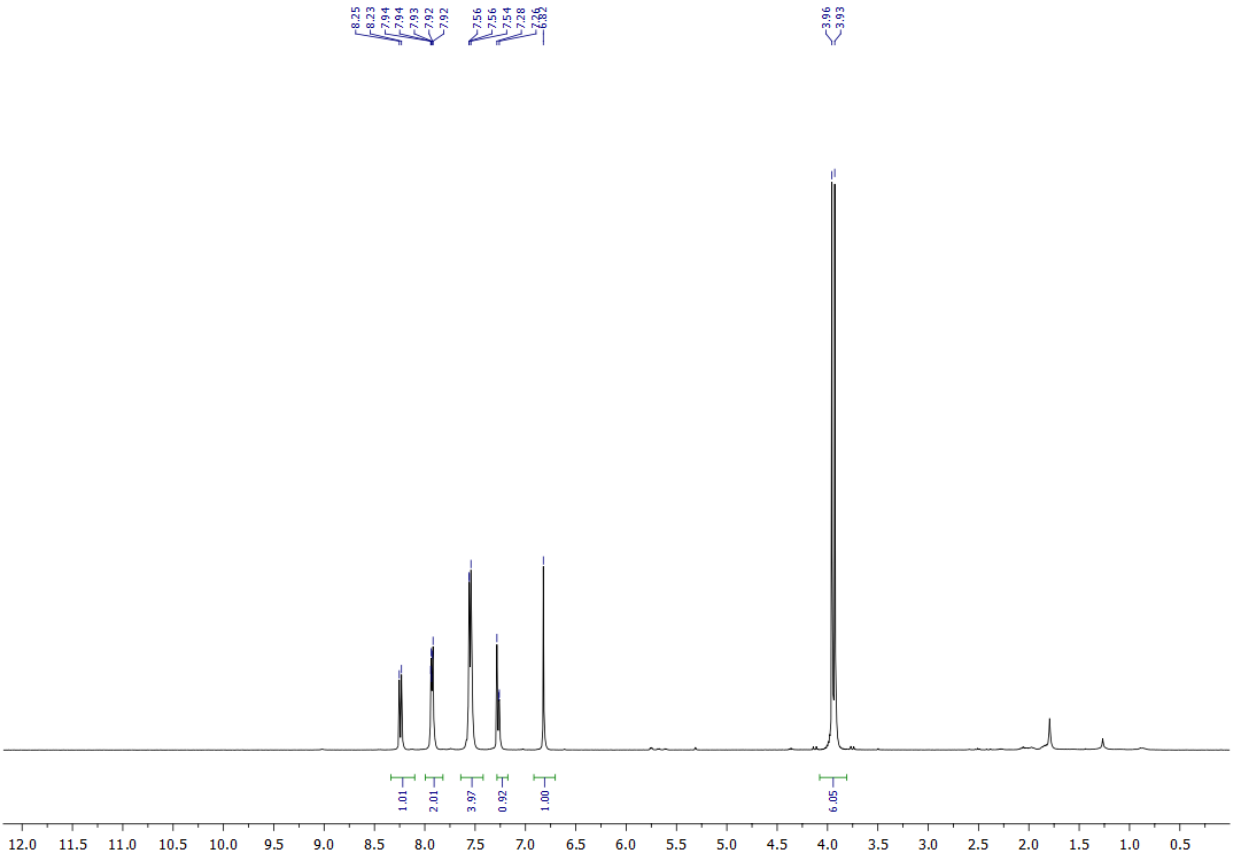

Figure S8. NMR ^{1}H-spectrum of *dimethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**9**)*

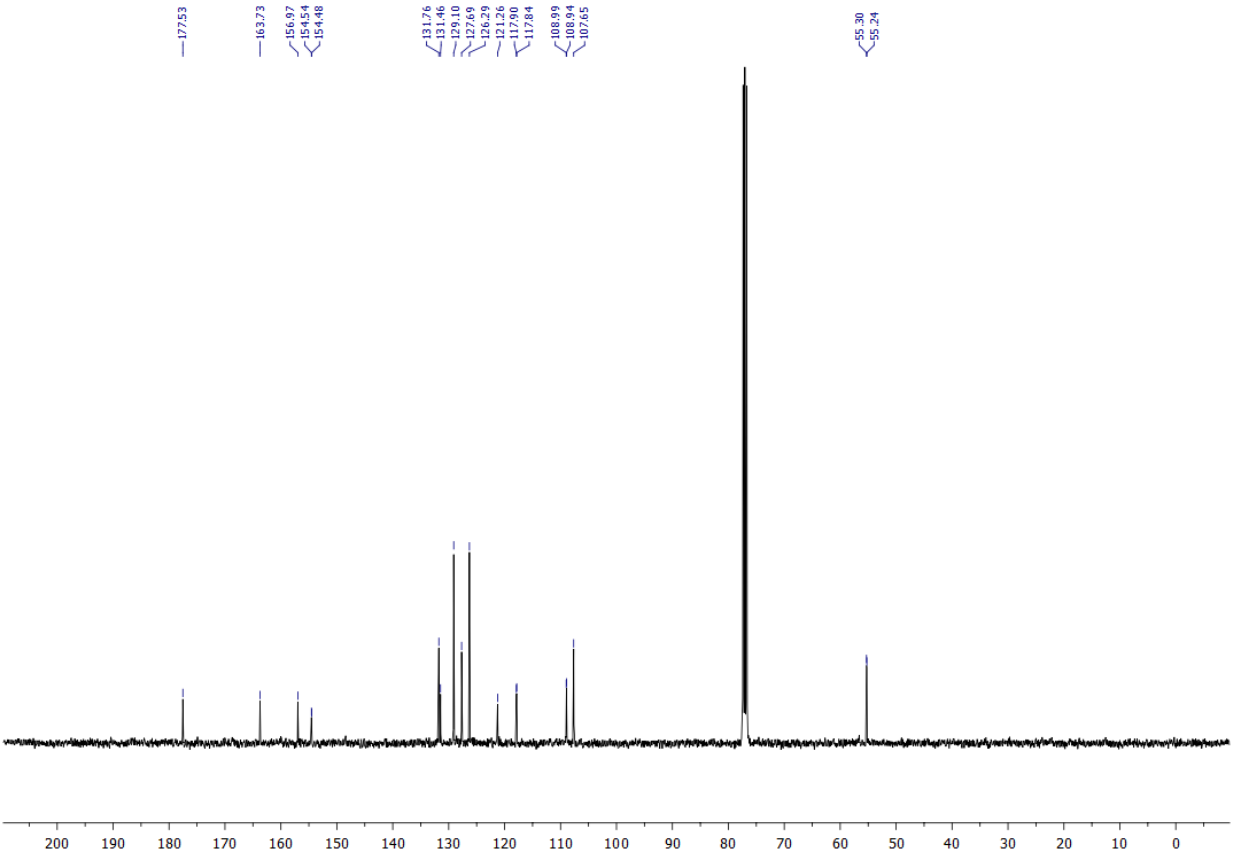

Figure S9. NMR ^{13}C-spectrum of *dimethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**9**)*

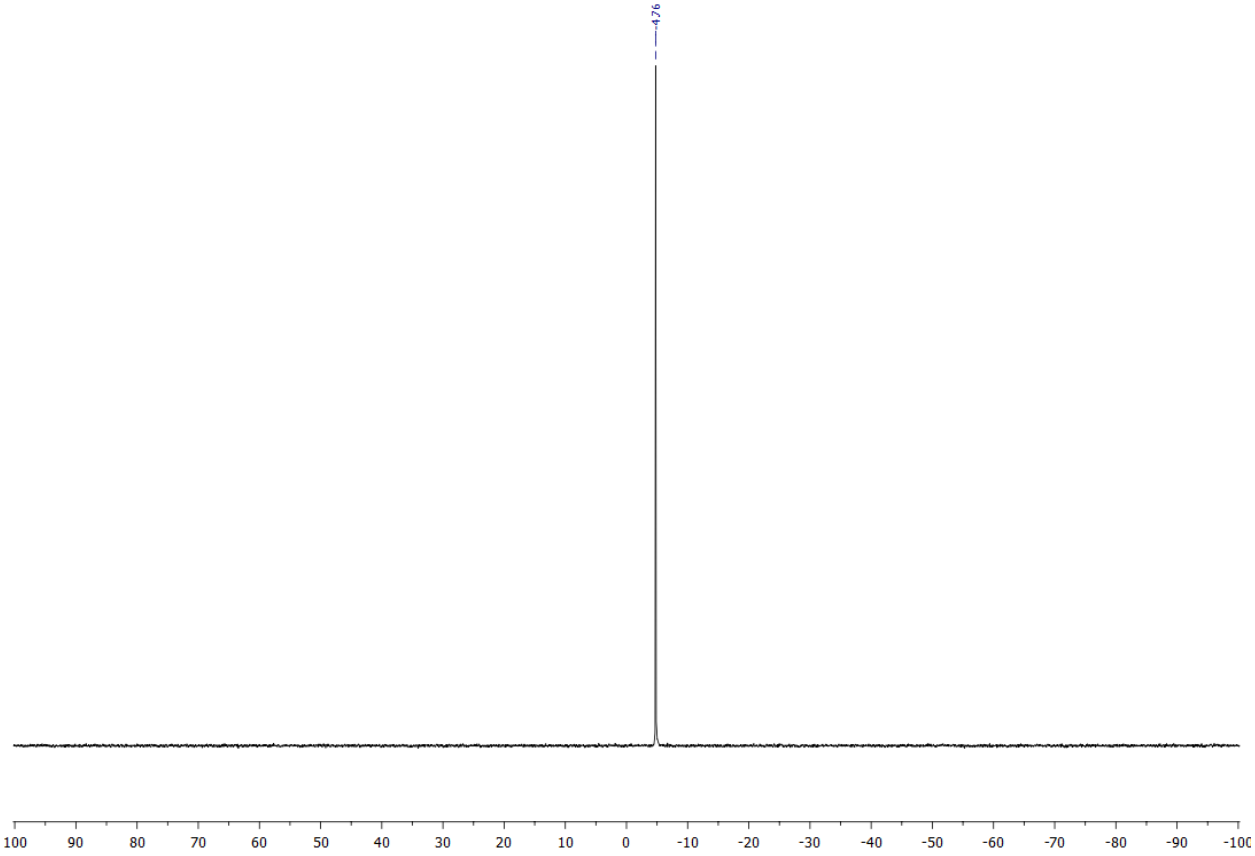

Figure S10. NMR ^{31}P-spectrum of *dimethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**9**)*

*Diethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**10**)*

Pale yellow solid. Yield: 61%. M.p.: 57-58°C (Lit.[10] mp 60–61°C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.8 Hz, 1H), 7.93 (dd, *J* = 7.6, 1.9 Hz, 2H), 7.58 – 7.51 (m, 4H), 7.28 – 7.26 (m, 1H), 6.82 (s, 1H), 4.39 – 4.20 (m, 4H), 1.41 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.57 (s), 163.70 (s), 156.99 (s), 154.75 (d, *J* = 6.3 Hz), 131.72 (s), 131.51 (s), 129.09 (s), 127.56 (s), 126.29 (s), 121.10 (s), 117.97 (d, *J* = 5.8 Hz), 108.96 (d, *J* = 4.6 Hz), 107.64 (s), 65.11 (d, *J* = 6.0 Hz), 16.10 (d, *J* = 6.4 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ -7.00 (s). HRMS, m/z: calcd for $C_{19}H_{20}O_6P$ $(M+H)^+$, 375.0992; found, 375.0996. IR (KBr, cm^{-1}): 3437, 3071, 2990, 1643, 1612, 1497, 1450, 1371, 1290, 1157, 1028.

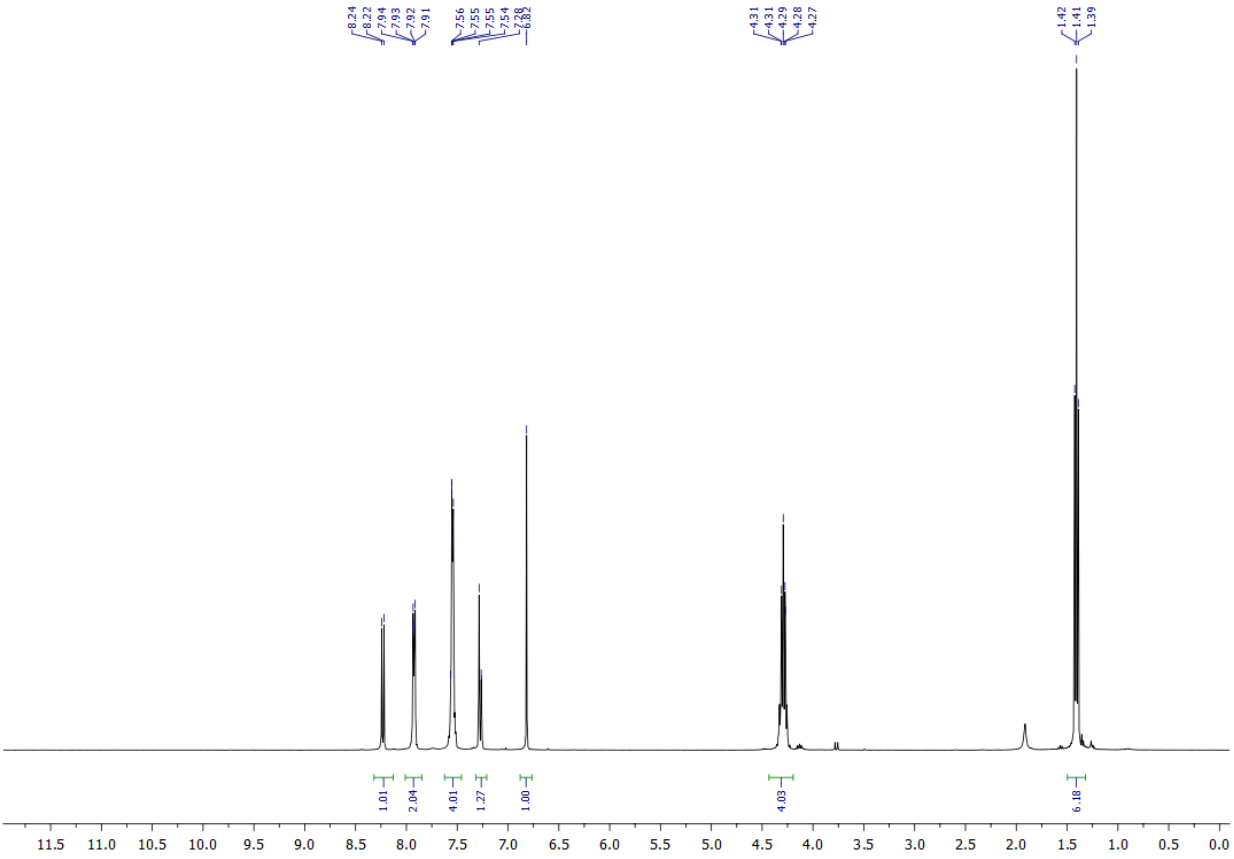

Figure S11. NMR ^{1}H-spectrum of *diethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**10**)*

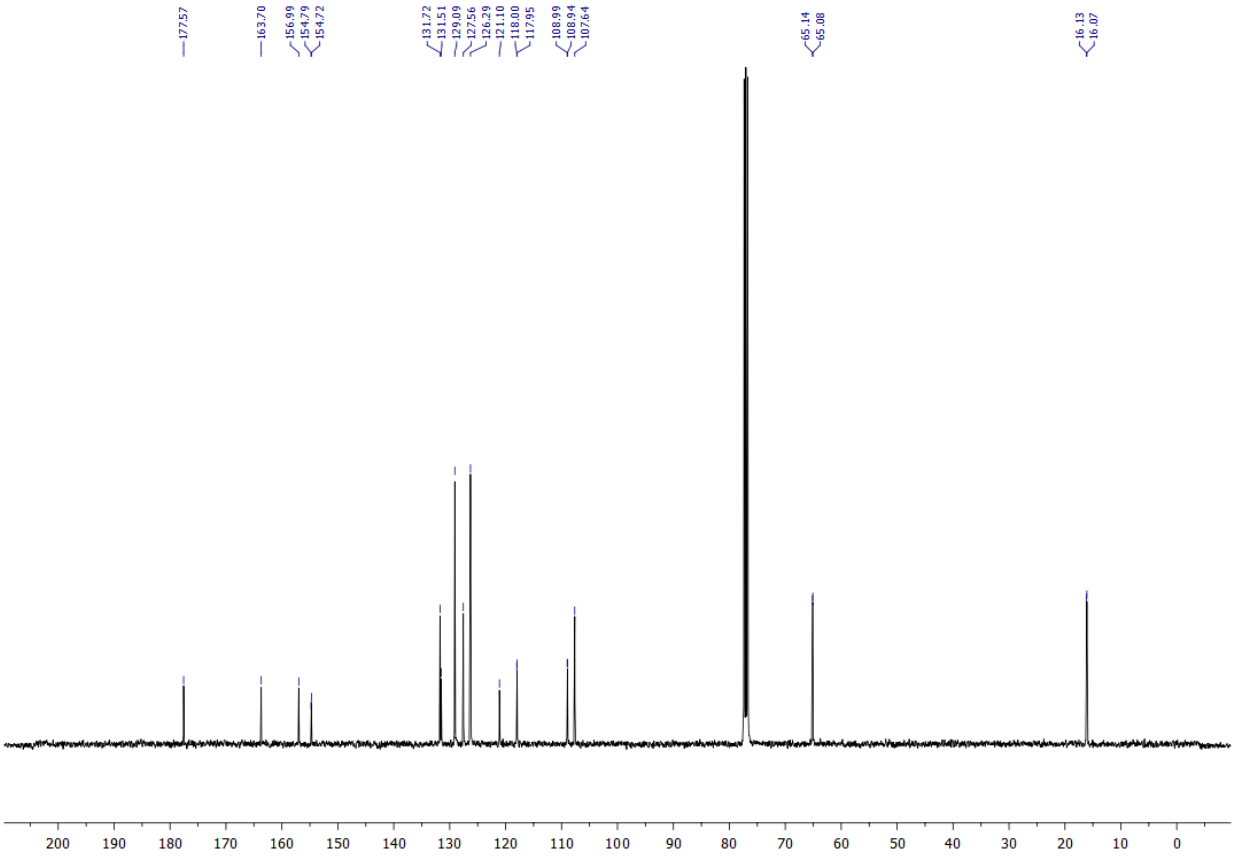

Figure S12. NMR ^{13}C-spectrum of *diethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**10**)*

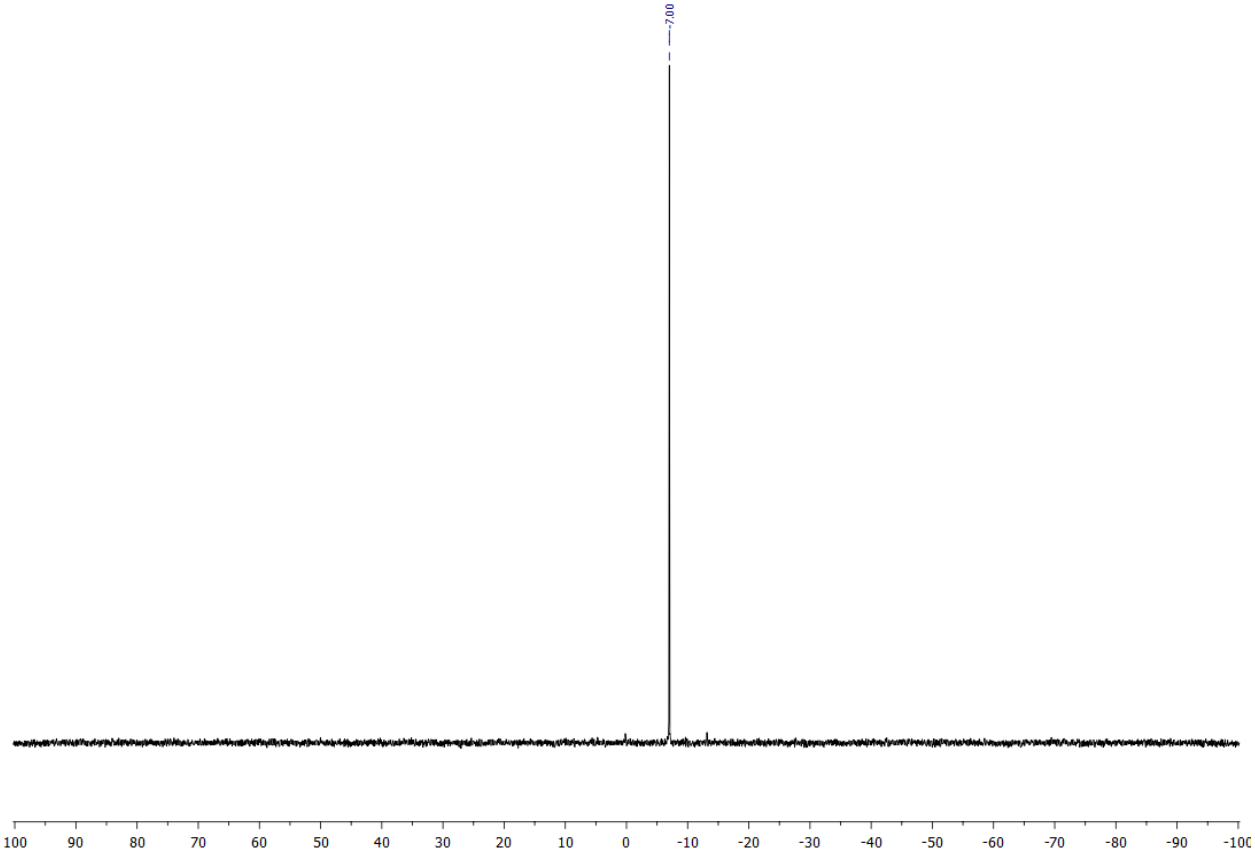

Figure S13. NMR ^{31}P-spectrum of *diethyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**10**)*

*Diisopropyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**11**)*

Pale yellow solid. Yield: 83%. M.p.: 77°C (Lit.[10] mp 58–59°C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, *J* = 8.8 Hz, 1H), 8.00 – 7.85 (m, 2H), 7.60 – 7.50 (m, 4H), 7.31 – 7.24 (m, 1H), 6.82 (s, 1H), 4.91 – 4.72 (m, 2H), 1.39 (dd, *J* = 16.8, 6.2 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.63 (s), 163.67 (s), 156.99 (s), 155.02 (d, *J* = 6.4 Hz), 131.62 (d, *J* = 14.9 Hz), 129.08 (s), 127.42 (s), 126.28 (s), 120.93 (s), 118.07 (d, *J* = 5.9 Hz), 108.95 (d, *J* = 4.8 Hz), 107.62 (s), 74.21 (d, *J* = 6.2 Hz), 23.60 (dd, *J* = 8.6, 5.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ -8.78 (s). HRMS, m/z: calcd for $C_{21}H_{24}O_6P$ $(M+H)^+$, 403.1305; found, 403.1296. IR (KBr, cm^{-1}): 3431, 3074, 2978, 1643, 1609, 1497, 1439, 1366, 1259, 1153, 1015.

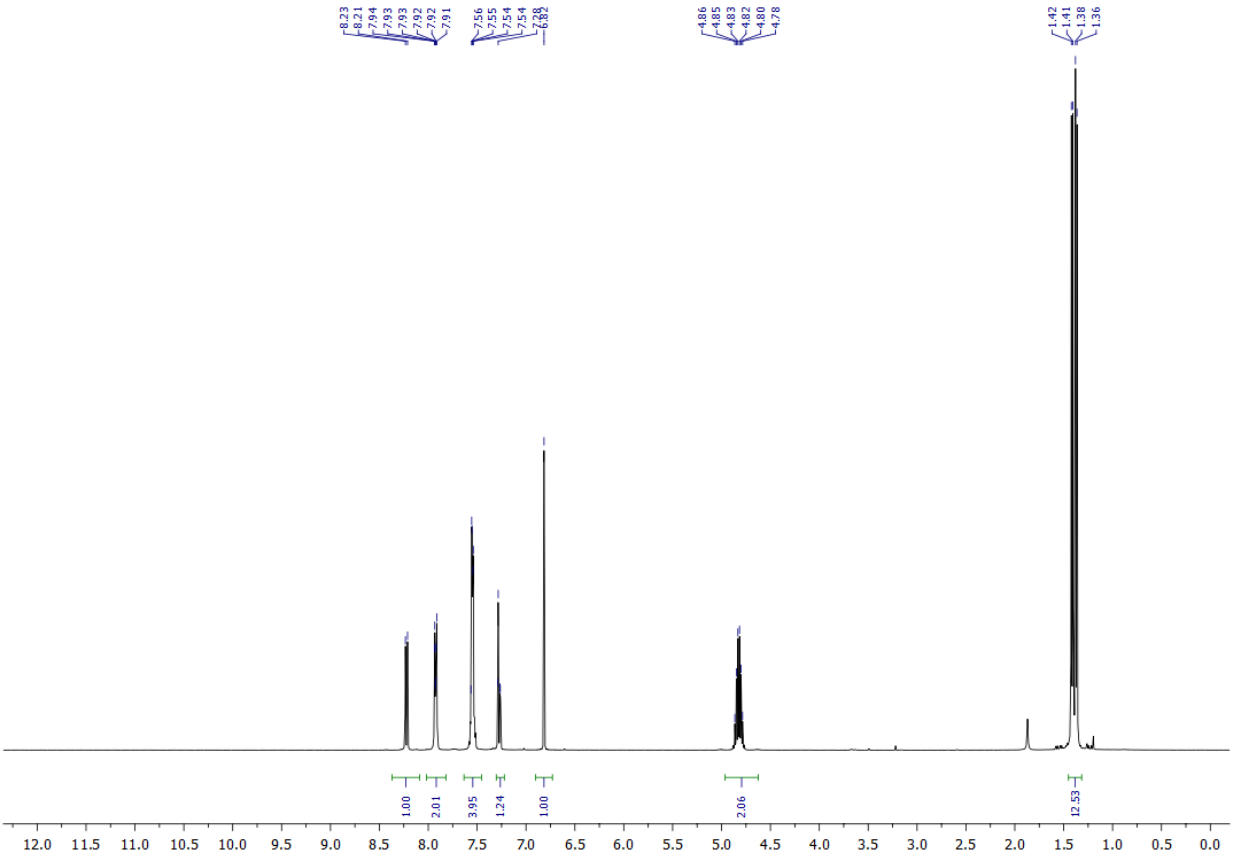

Figure S14. NMR ^{1}H-spectrum of *diisopropyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**11**)*

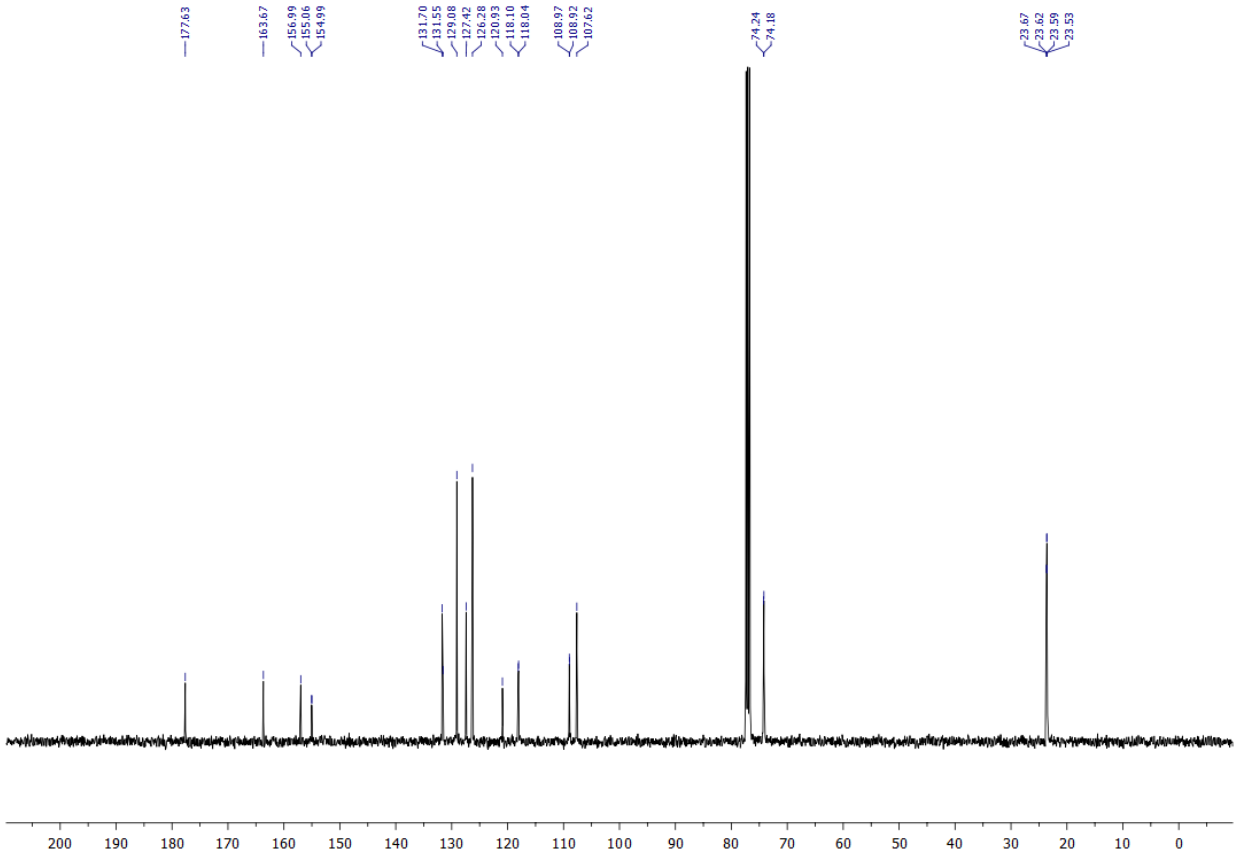

Figure S15. NMR ^{13}C-spectrum of *diisopropyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate **(11)***

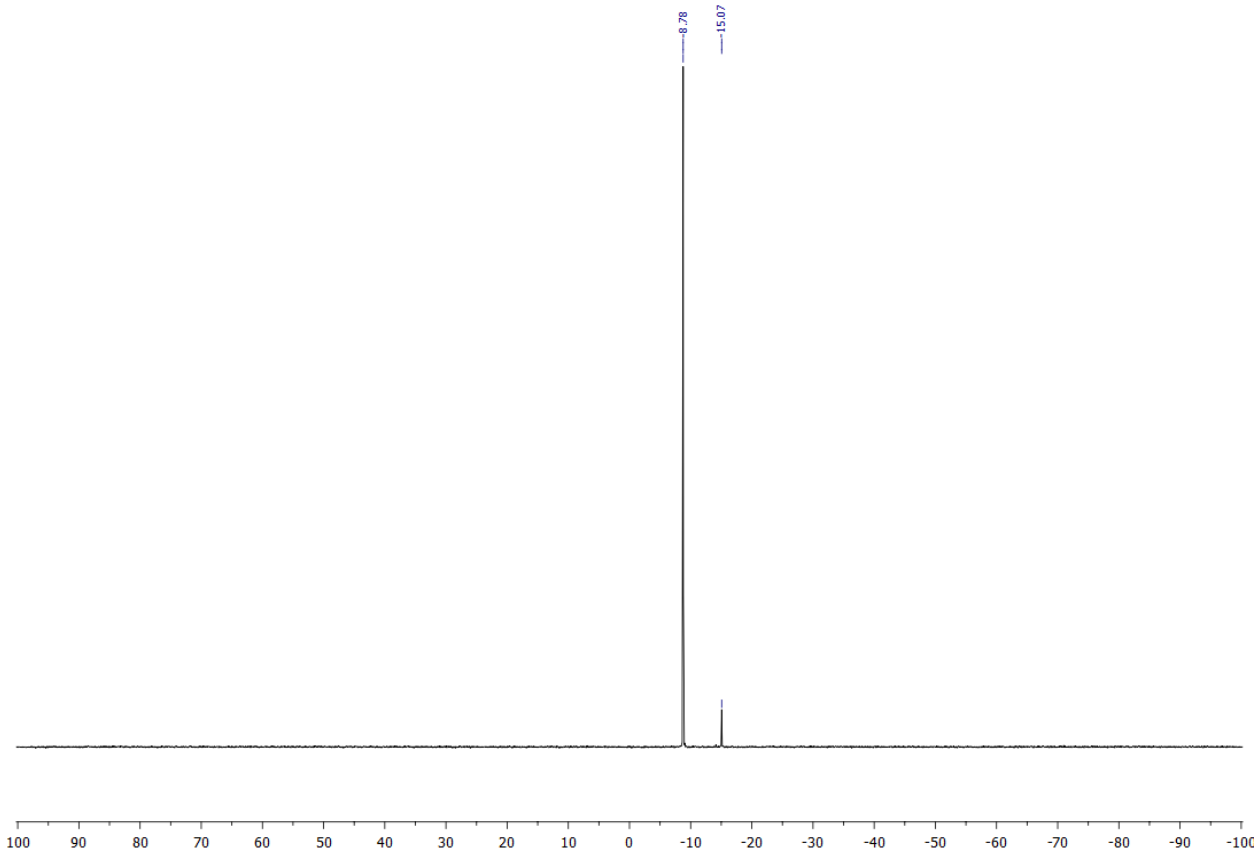

Figure S16. NMR ^{31}P-spectrum of *diisopropyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate **(11)***

*Dibutyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**12**)*

Yellow oil. Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.8 Hz, 1H), 7.98 – 7.89 (m, 2H), 7.62 – 7.51 (m, 4H), 7.29 – 7.26 (m, 1H), 6.83 (s, 3H), 4.29 – 4.15 (m, 4H), 1.78 – 1.68 (m, 4H), 1.51 – 1.38 (m, 4H), 0.96 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.59 (d, *J* = 4.3 Hz), 163.69 (s), 157.00 (s), 154.83 (d, *J* = 6.1 Hz), 131.63 (dd, *J* = 17.4, 2.0 Hz), 129.09 (s), 127.55 (s), 126.28 (s), 121.08 (s), 117.95 (d, *J* = 5.7 Hz), 108.95 (d, *J* = 4.5 Hz), 107.65 (s), 68.77 (d, *J* = 6.1 Hz), 32.18 (d, *J* = 6.6 Hz), 18.60 (s), 13.51 (s); ^{31}P NMR (162 MHz, $CDCl_3$): δ -6.77 (s). HRMS, m/z: calcd for $C_{23}H_{28}O_6P$ $(M+H)^+$, 431.1618; found, 431.1614. IR (KBr, cm^{-1}): 3480, 3067, 2961, 1651, 1611, 1450, 1368, 1288, 1157, 1026.

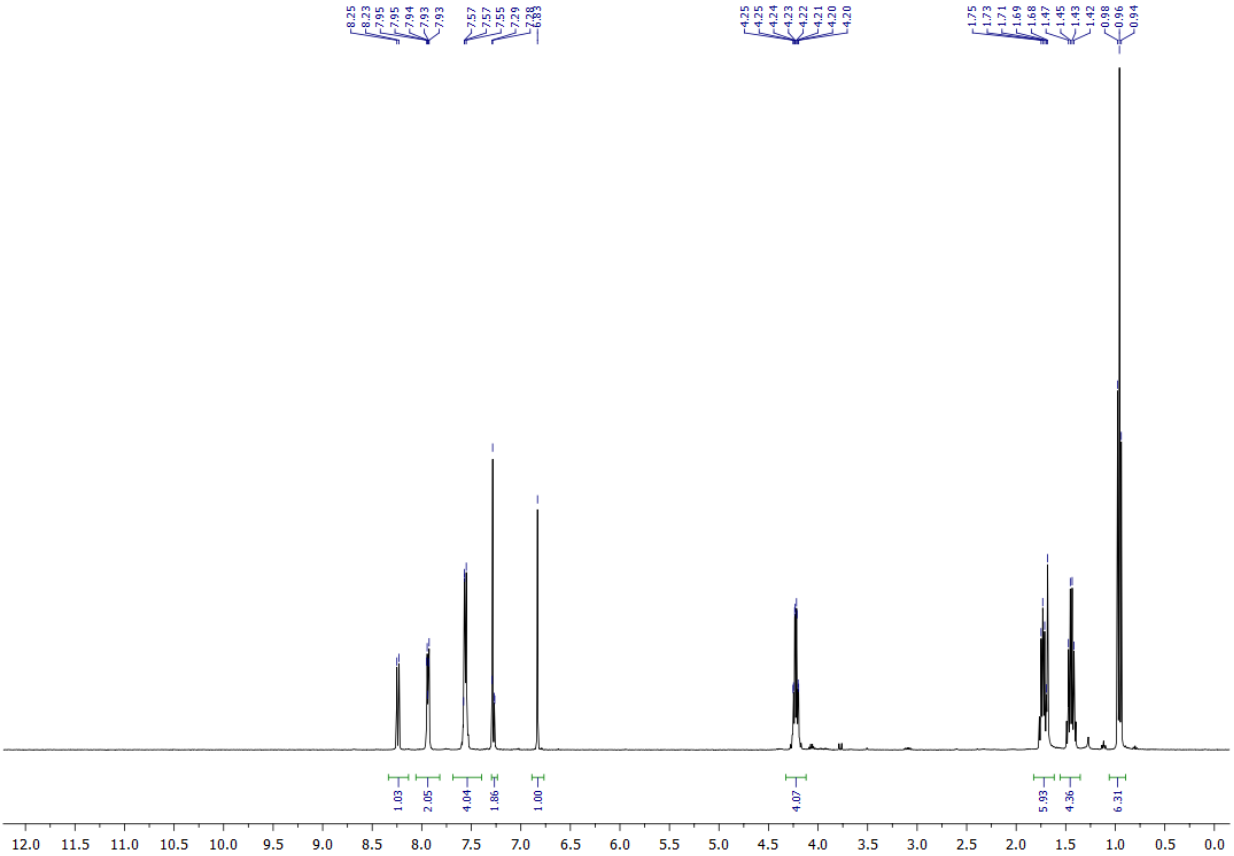

Figure S17. NMR ^{1}H-spectrum of *dibutyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate (**12**)*

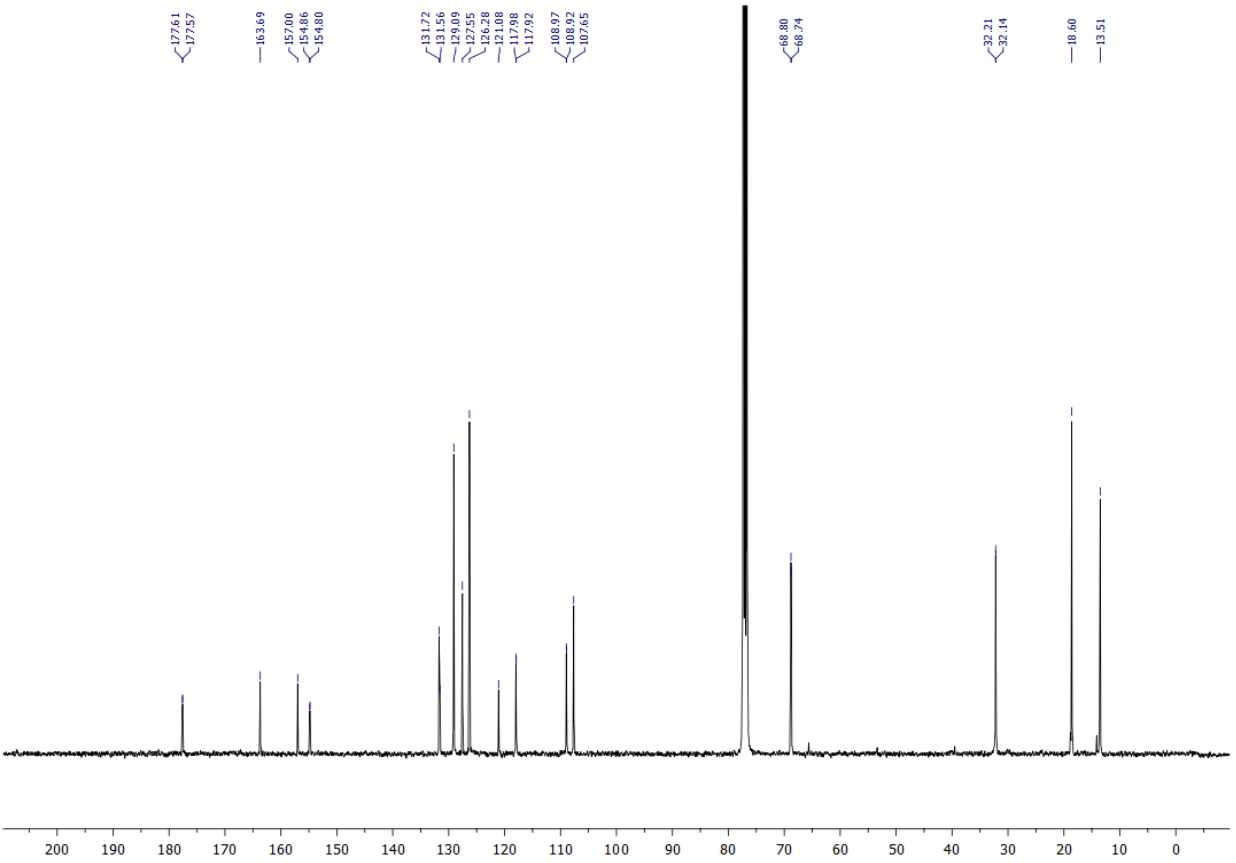

Figure S18. NMR ^{13}C-spectrum of *dibutyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate* ***(12)***

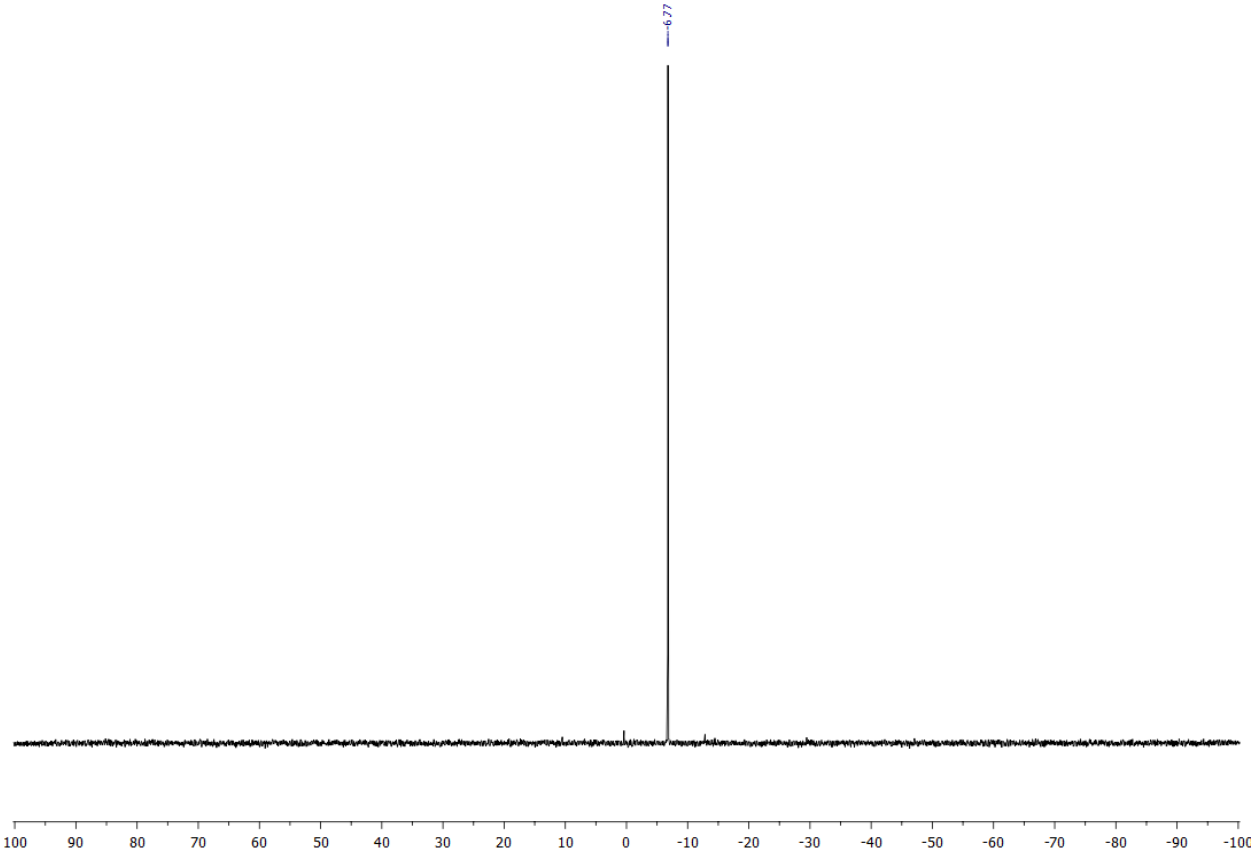

Figure S19. NMR ^{31}P-spectrum of *dibutyl 4-oxo-2-phenyl-4H-chromen-7-yl phosphate* ***(12)***

*4-Oxo-2-phenyl-4H-chromen-7-yl diphenyl phosphate (**13**)*

Pale yellow solid. Yield: 70%. M.p.: 61°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.8 Hz, 1H), 7.93 (dd, *J* = 7.7, 1.8 Hz, 2H), 7.66 – 7.51 (m, 4H), 7.47 – 7.35 (m, 4H), 7.30 – 7.25 (m, 7H), 6.84 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.46 (s), 163.82 (s), 156.91 (s), 154.17 (d, *J* = 6.4 Hz), 150.24 (d, *J* = 7.4 Hz), 131.81 (s), 131.41 (s), 130.04 (s), 129.11 (s), 127.82 (s), 126.31 (s), 125.98 (s), 121.62 (s), 120.07 (d, *J* = 4.7 Hz), 117.99 (d, *J* = 5.9 Hz), 109.41 (d, *J* = 4.6 Hz), 107.72 (s); ^{31}P NMR (162 MHz, $CDCl_3$): δ -18.38 (s). HRMS, m/z: calcd for $C_{27}H_{20}O_6P$ $(M+H)^+$, 471.0992; found, 471.0983. IR (KBr, cm^{-1}): 3443, 3055, 1655, 1609, 1489, 1450, 1369, 1286, 1188, 1148, 968.

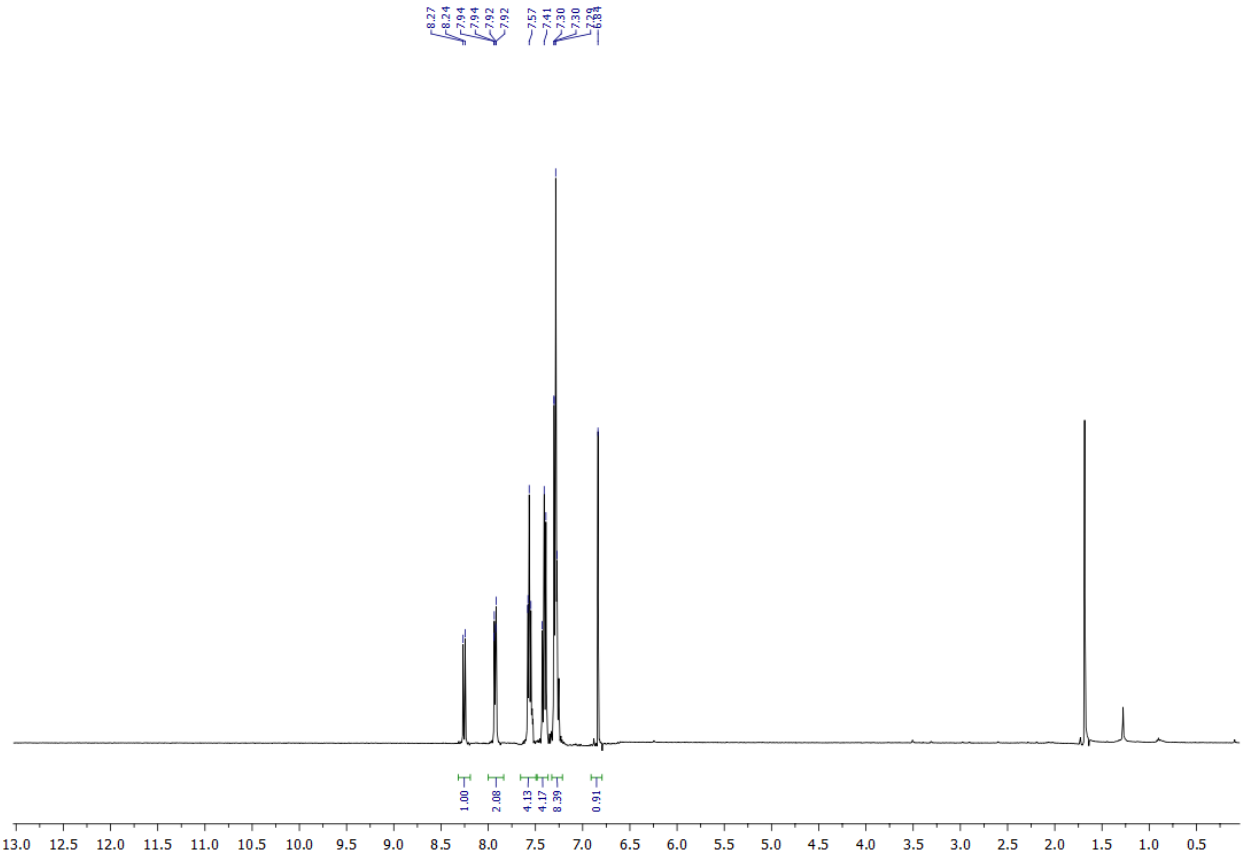

Figure S20. NMR ^{1}H-spectrum of *4-oxo-2-phenyl-4H-chromen-7-yl diphenyl phosphate (**13**)*

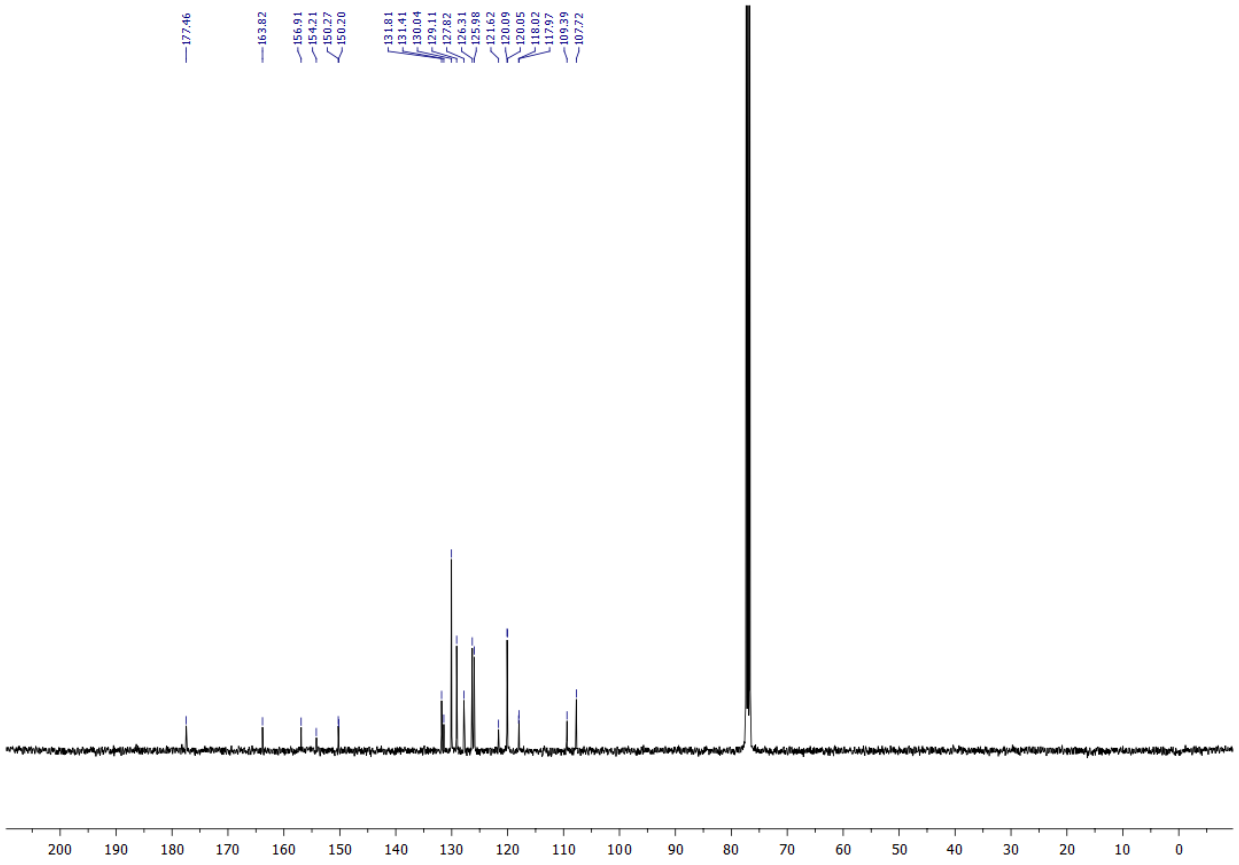

Figure S21. NMR ^{13}C-spectrum of *4-oxo-2-phenyl-4H-chromen-7-yl diphenyl phosphate (**13**)*

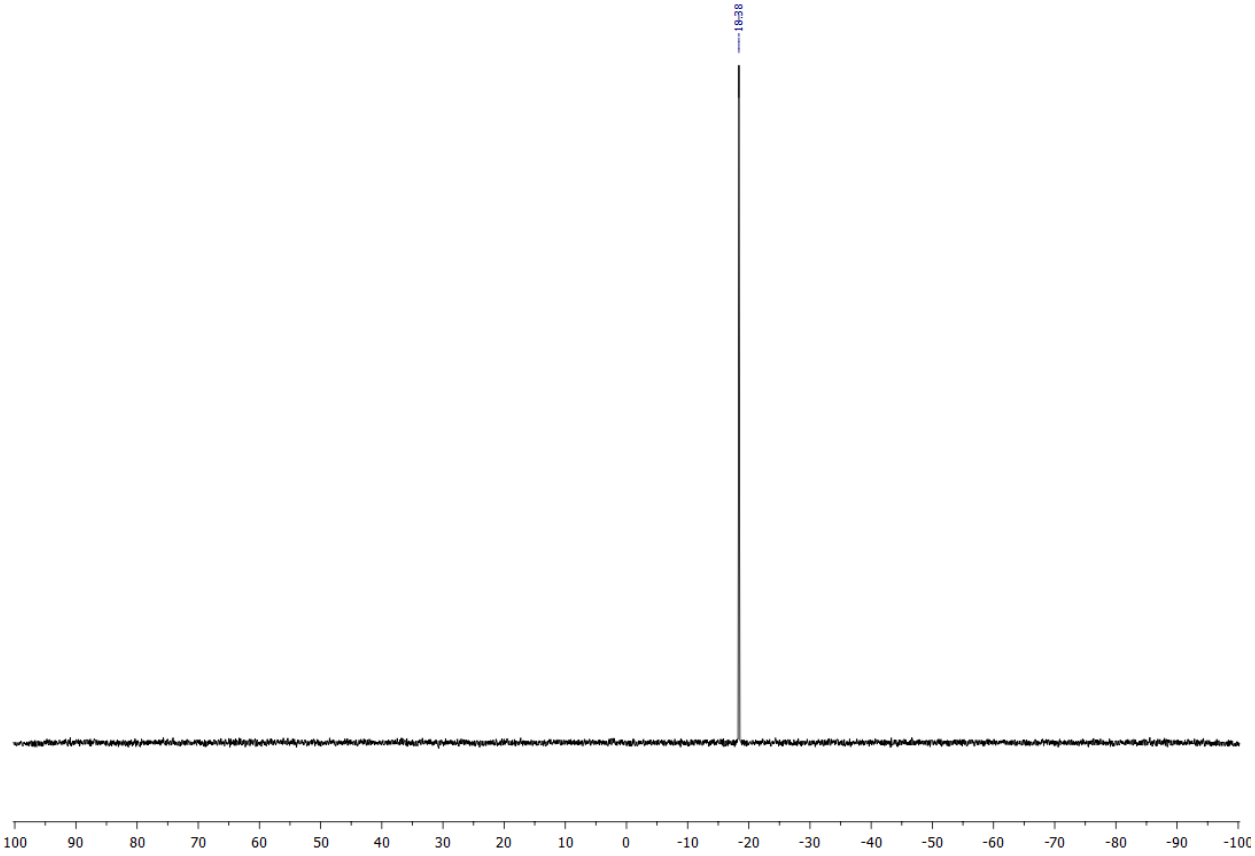

Figure S22. NMR ^{31}P-spectrum of *4-oxo-2-phenyl-4H-chromen-7-yl diphenyl phosphate (**13**)*

*4-Oxo-2-phenyl-4H-chromen-7-yl bis(2,2,2-trifluoroethyl) phosphate (**14**)*

Pale yellow solid. Yield: 78%. M.p.: 102°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (d, *J* = 8.8 Hz, 1H), 8.00 – 7.88 (m, 2H), 7.64 – 7.53 (m, 3H), 7.52 (dd, *J* = 2.1, 1.2 Hz, 1H), 7.34 – 7.25 (m, 2H), 6.85 (s, 1H), 4.61 – 4.48 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.29 (s), 163.93 (s), 156.85 (s), 153.16 (d, *J* = 6.3 Hz), 131.92 (s), 131.30 (s), 129.16 (s), 128.15 (s), 126.32 (s), 122.04 (s), 117.63 (d, *J* = 5.3 Hz), 109.35 (d, *J* = 4.7 Hz), 107.80 (s), 65.47 – 64.94 (m), 64.65 (dd, *J* = 38.7, 4.4 Hz); ^{31}P NMR (162 MHz, $CDCl_3$): δ -7.91 (s). HRMS, m/z: calcd for $C_{19}H_{14}F_6O_6P$ $(M+H)^+$, 483.0427; found, 483.0422. IR (KBr, cm^{-1}): 3431, 3022, 1647, 1622, 1441, 1373, 1283, 1190, 1169, 1152, 1080, 978.

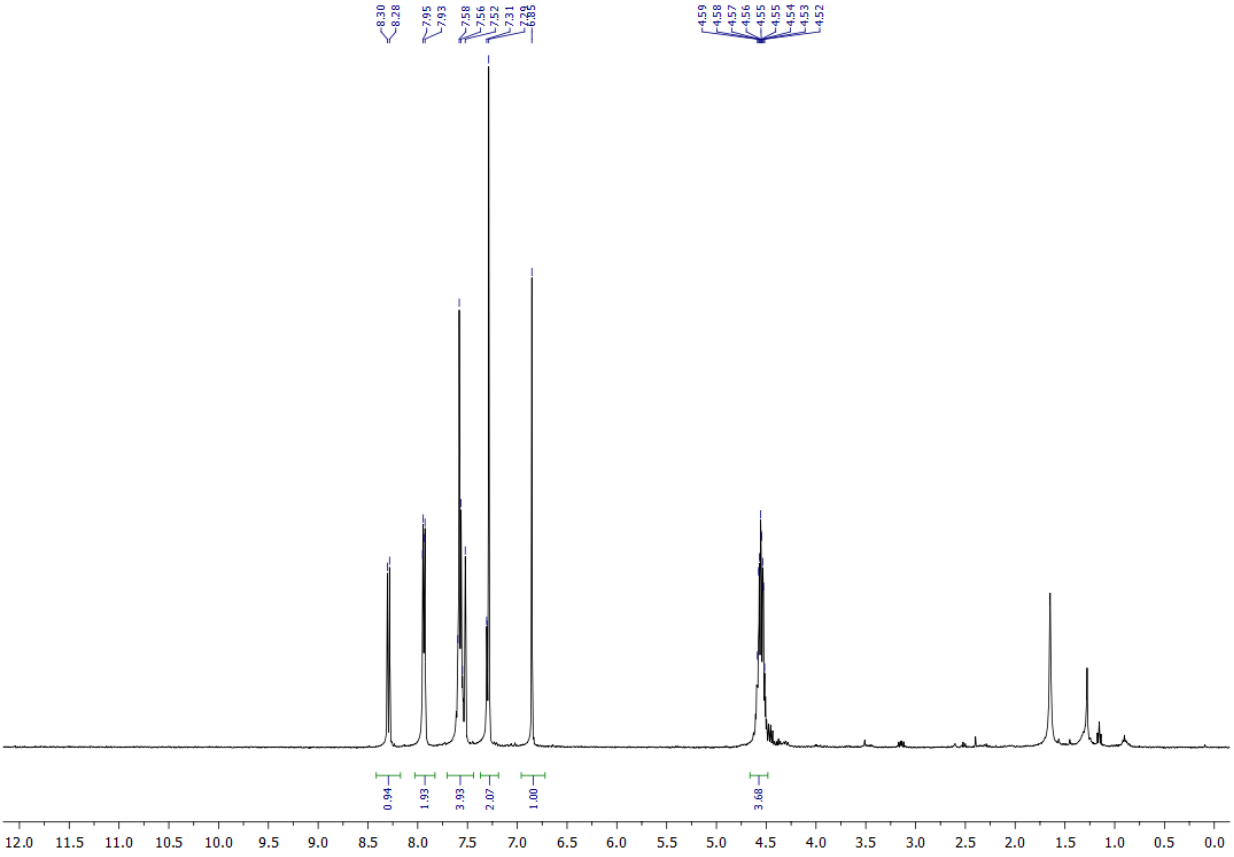

Figure S23. NMR ^{1}H-spectrum of *4-oxo-2-phenyl-4H-chromen-7-yl bis(2,2,2-trifluoroethyl) phosphate (**14**)*

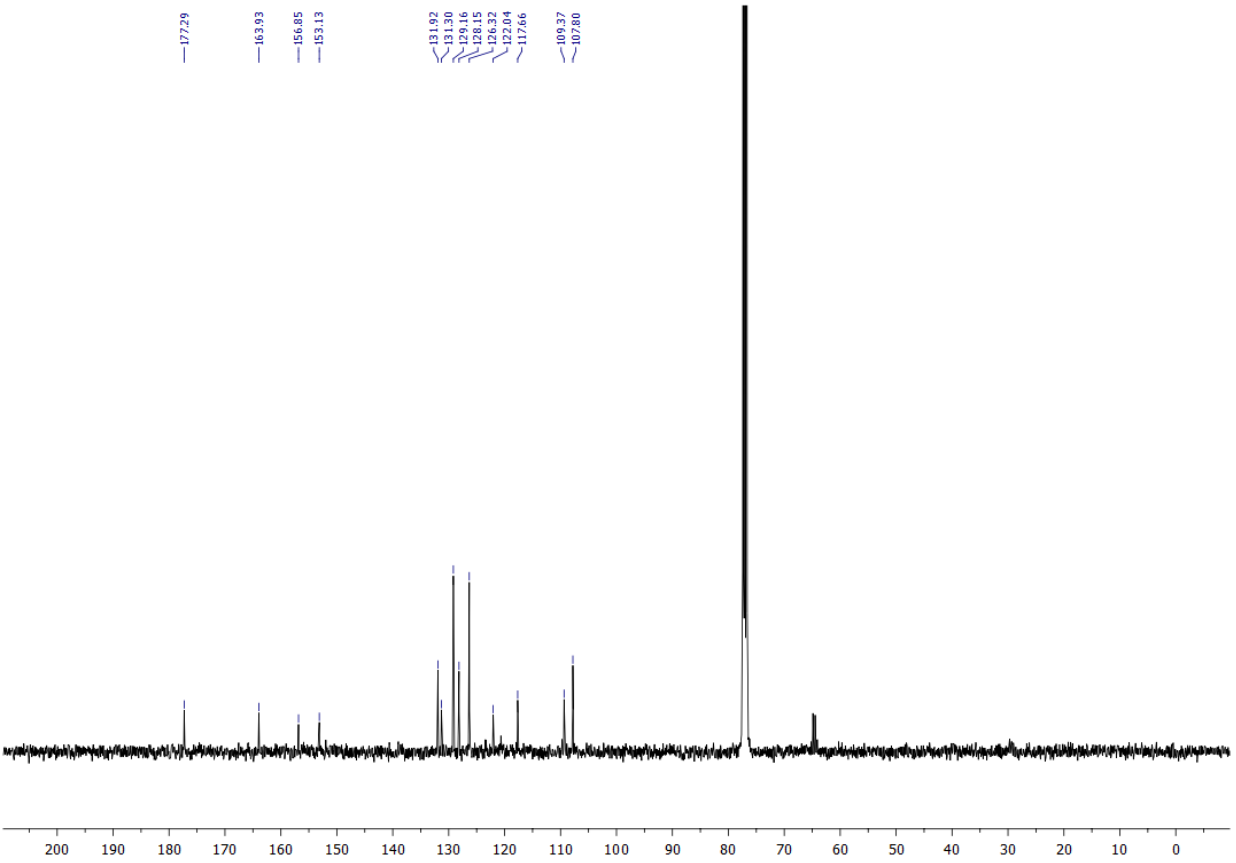

Figure S24. NMR ^{13}C-spectrum of *4-oxo-2-phenyl-4H-chromen-7-yl bis(2,2,2-trifluoroethyl) phosphate* ***(14)***

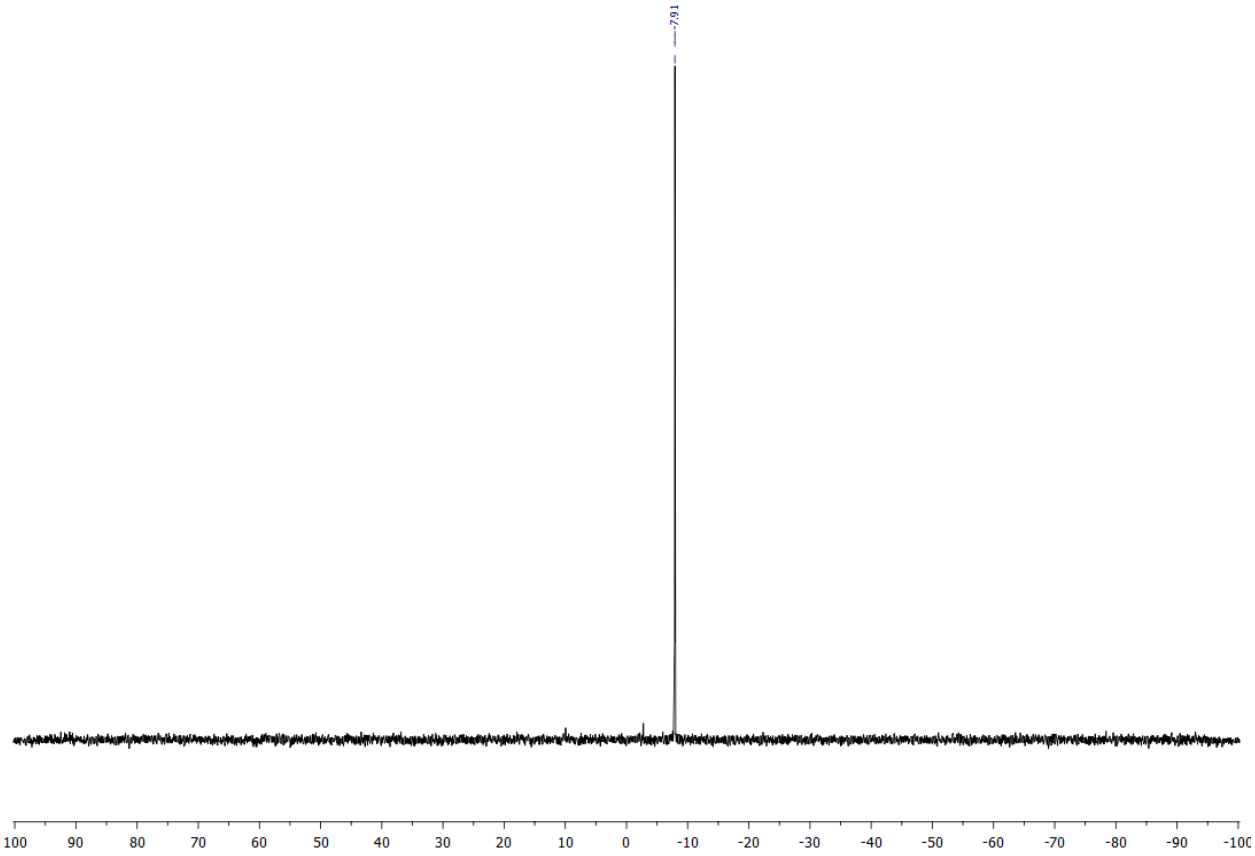

Figure S25. NMR ^{31}P-spectrum of *4-oxo-2-phenyl-4H-chromen-7-yl bis(2,2,2-trifluoroethyl) phosphate* ***(14)***

6. References

[1] E. H. Rios Morales, J. Balzarini, C. Meier, *J. Med. Chem.*, 2012, **55**, 7245-7252.

[2] V. V. Abzianidze, D. S. Prokofieva, A. S. Bogachenkov, Synthesis of phosphorylated chrysin derivatives and estimation of efficiency and selectivity of their inhibitory activity towards carboxylesterase, *Russ. J. Gen. Chem.*, 2016, **86**, 425-428. 10.1134/S1070363216020419.

[3] Y. Wei, A.-Y. Peng, B. Wang, L. Ma, G. Peng, Y. Du, J. Tang, Synthesis and biological evaluation of phosphorylated flavonoids as potent and selective inhibitors of cholesterol esterase, *Eur. J. Med. Chem.*, 2014, **74**, 751-758. https://doi.org/10.1016/j.ejmech.2013.03.025

[4] X. Chen, Y. Yu, L. Qu, X. Liao, Y. Zhao, Synthesis of Novel Phosphoric Esters of Flavone and Isoflavone by Atherton–Todd Reaction, *Synth. Commun.*, 2004, **34**, 493-499. 10.1081/SCC-120027289

[5] G. Sheldrick, A short history of SHELX, *Acta Crystallogr. A*, 2008, **64**, 112-122. doi:10.1107/S0108767307043930.

[6] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program, *J. Appl. Crystallogr.*, 2009, **42**, 339-341. doi:10.1107/S0021889808042726.

[7] (a) D. M. Maxwell, K. M. Brecht, B. L. O'Neill, The effect of carboxylesterase inhibition on interspecies differences in soman toxicity, *Toxicol. Lett.*, 1987, **39**, 35-42. https://doi.org/10.1016/0378-4274(87)90254-2; (b) F. Åke, P. U. U. Gertrud, Kinetics for the inhibition of acetylcholinesterase from the electric eel by some organophosphates and carbamates, *Eur. J. Biochem.*, 1984, 140, 153-156. 10.1111/j.1432-1033.1984.tb08079.x