Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 664–666

Anticancer activity of novel 3-azaxanthenes

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Contents

The proposed mechanisms of the formation of 3-azaxanthenes.

The proposed mechanism of the formation of 3-azaxanthenes from pyridoxal-5'-phosphate **1a** involves the formation of benzylic alcohol **C** as the first step via the conventional electrophilic aromatic substitution. Further reaction of this compound with the second molecule of the phenol results in the intermediate **F**, which undergoes the dehydration to give the target compound **2**.

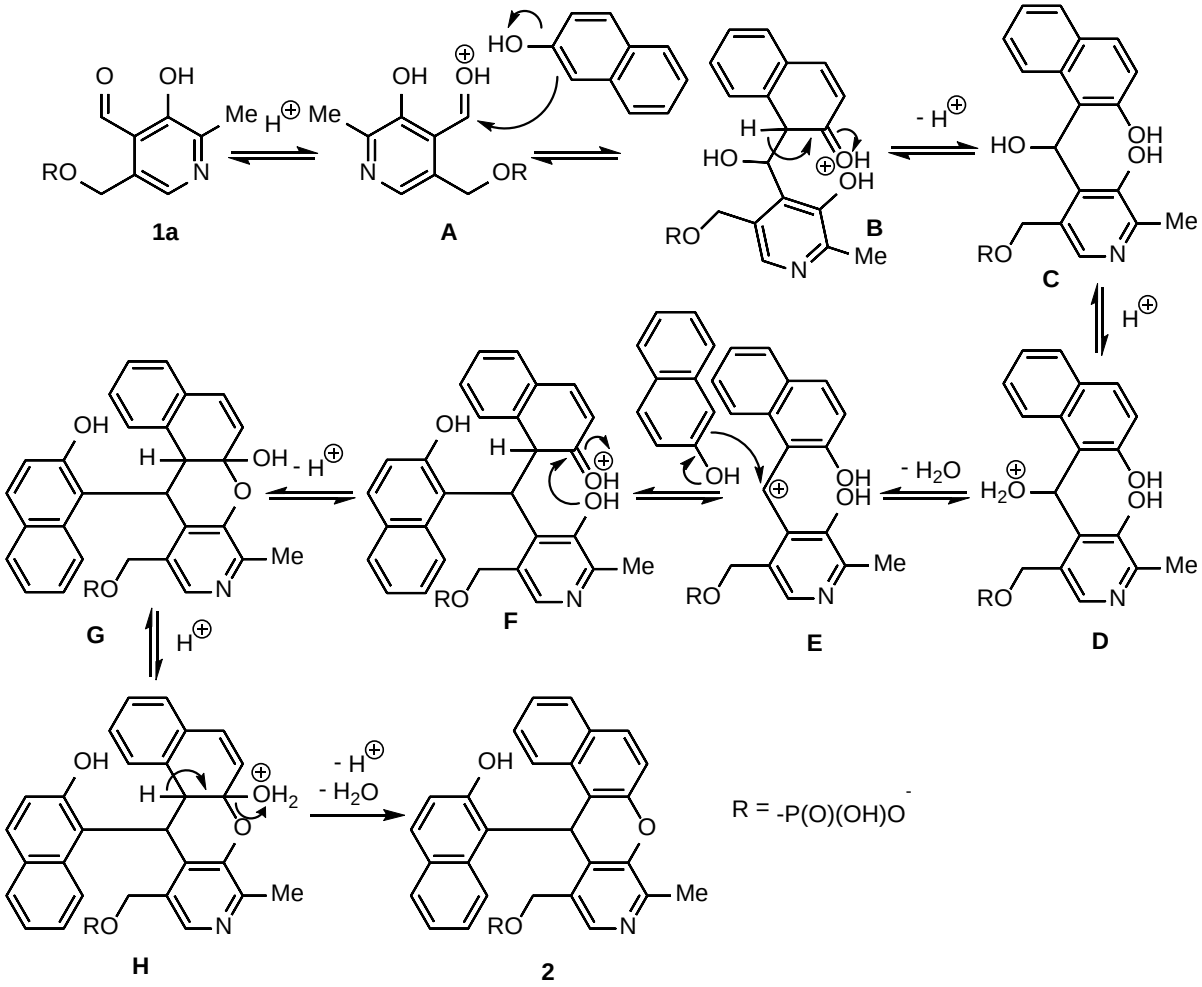


Figure S1 The proposed mechanism of 3-azaxanthenes formation from pyridoxal-5'-phosphate.

The formation of 3-azaxanthenes from pyridoxal **1b** presumably starts from the intramolecular cyclization of pyridoxal to give hemiacetal **C**. Further protonation of the hydroxy group and the elimination of water molecule furnishes cyclic oxonium ion **E**. Subsequent electrophilic aromatic substitution results in the furopyridine derivative **G**. The next stage is the protonation of the furan oxygen followed by the furan ring cleavage. The carbocation **J** thus generated undergoes the reaction with the second molecule of 2-naphthol. The subsequent transformations do not differ from the above scheme and result in the 3-azatxanthene **2** formation via the dehydration step.

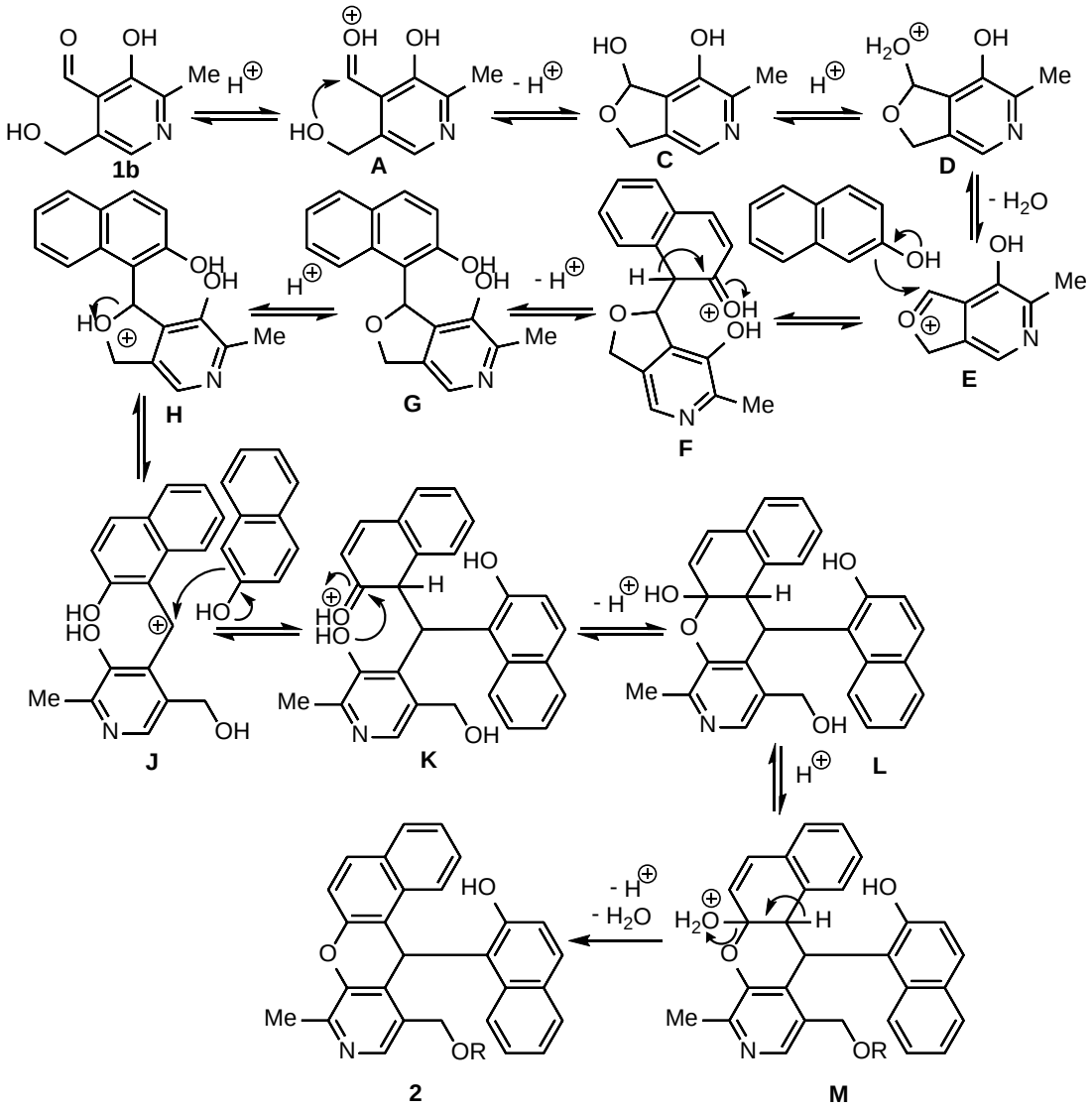


Figure S2 The proposed mechanism of 3-azaxanthenes formation from pyridoxal.

Experimental section

Spectroscopic measurements

NMR experiments were performed on a Bruker AVANCE-400 spectrometer at 303 K equipped with 5 mm broadband probehead working at 400.13 MHz in ^{1}H and 100.61 MHz in ^{13}C experiments. Chemical shifts in ^{1}H spectra were reported relative to the solvent as internal standard (DMSO-d_6). MALDI mass spectra were obtained on an ULTRAFLEX III mass spectrometer. Measurements were conducted with the use of plastic and metal plates. 2,5-Dihydroxybenzoic acid (2,5-DHB) was used as matrix. IR spectra were recorded on a Tensor-27 spectrometer of Bruker Company in the range of 400-3600 cm^{-1} in KBr pellets.

The X-ray diffraction data for compound 5.

The crystallographic data of compound **5** are as follows: ($C_{22}H_{21}NO_8P$ ×4(C_2H_6O), M = 642.64) monoclinic, at 100 K a = 12.448(2), b = 22.191(5), c = 12.727(3)Å, β = 107.399(10)°, V = 3354.8(12)$Å^3$, Z = 4, space group $P2_1/c$, d_{calc} = 1.272 $g \cdot cm^{-3}$, μ = 0.142 $мм^{-1}$, F(000) = 1372. The cell parameters and the experimental data were obtained on an automatic Bruker Smart APEX II CCD diffractometer [λ(MoK_α) = 0.71073 Å, ω-scanning], 2θ < 52°, R_{int} = 0.148. 27790 reflections were collected, 6202 of them were independent; the number of the observed reflections with $I > 2\sigma(I)$ was 2671. The absorption correction was applied using the SADABS program[S1]. The structure was solved by the direct method using the SIR program[S2], and it was refined by the full-matrix least-squares method using the SHELXL97 program package.[S3]. The hydrogen atoms of the hydroxyl groups were revealed by means of the difference electron density maps and refined in the isotropic approximation. The coordinates of the other hydrogen atoms were calculated geometrically and refined in a riding model. All the calculations were carried out using the WinGX[S4] and APEX2[S5] programs; the final values of the divergence factors were R 0.0808, $_wR_2$ 0.2359, GOF = 1.02; the number of parameters to be refined was 412. The crystallographic data of the structure of **5** have been deposited with the Cambridge Crystallographic Data Centre (http://www.ccdc.cam.ac.uk; the deposition code is CCDC 2084681)

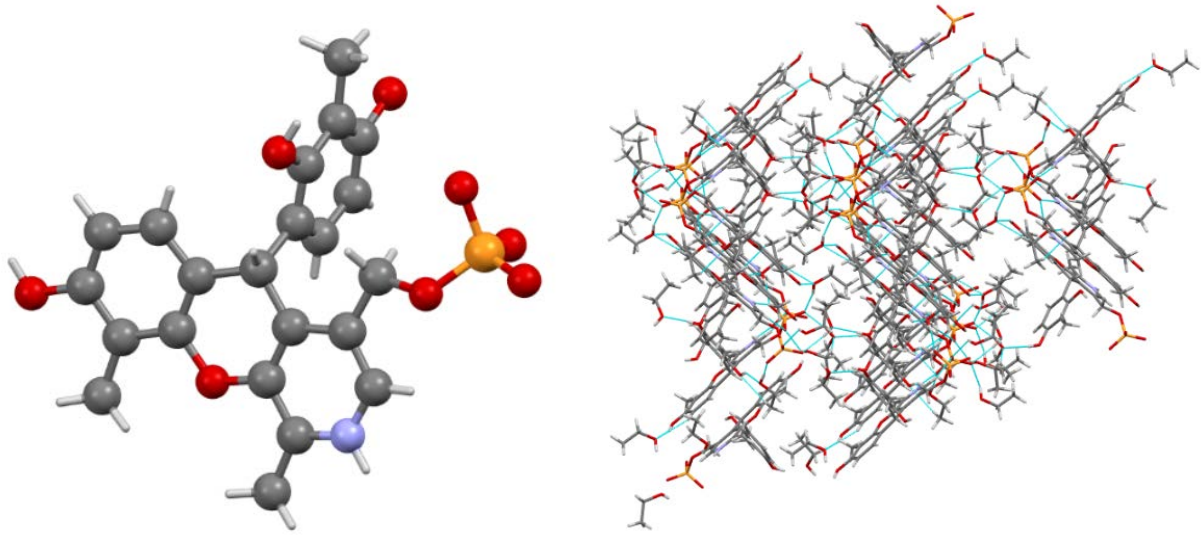


Figure S3 Molecular structure of compound **5** in crystals.

Table S1 Parameters of intra- and intermolecular contacts in crystals of compound **5**.

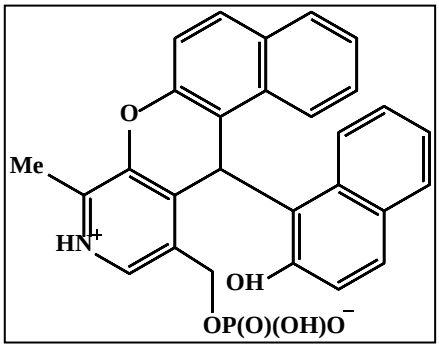
D—H···A	*D—H, Å*	*H…A, Å*	*D…A, Å*	*DHA, deg.*	*symmetry operations*
O30—H30…O50	*0.8200*	*1.9400*	*2.654(6)*	*146.00*	-
O32—H32…O40	*0.8200*	*1.9000*	*2.717(6)*	*175.00*	-
O60—H60…O15	*0.8200*	*2.0900*	*2.546(8)*	*115.00*	-
C6—H6…O11	*0.9300*	*2.3300*	*2.687(6)*	*102.00*	-
C9—H9…O30	*0.9800*	*2.2900*	*2.770(6)*	*109.00*	-
C23—H23C…O8	*0.9600*	*2.3500*	*2.772(7)*	*106.00*	-
C31—H31A…O32	*0.9600*	*2.3600*	*2.815(8)*	*109.00*	-
C52—H52C…O30	*0.9600*	*2.4700*	*3.210(14)*	*133.00*	-
N1—H1…O13	*0.8600*	*1.8400*	*2.695(5)*	*169.00*	*x,1/2-y,-1/2+z*
O22—H22…O70	*0.8200*	*1.8900*	*2.676(6)*	*159.00*	*1+x,y,z*
O40—H40…O14	*0.8200*	*1.9400*	*2.748(5)*	*170.00*	*1+x,1/2-y,1/2+z*
O50—H50…O13	*0.8200*	*1.9500*	*2.698(6)*	*151.00*	*x,1/2-y,1/2+z*
O70—H70…O14	*0.8200*	*1.9100*	*2.713(6)*	*166.00*	*-x,1/2+y,3/2-z*
C6—H6…O15	*0.9300*	*2.3900*	*3.114(6)*	*135.00*	*x,1/2-y,-1/2+z*

Cytotoxicity assay

Cytotoxic effects of the test compounds on human cancer and normal cells were estimated by means of the multifunctional Cytell Cell Imaging system (GE Health Care Life Science, Sweden) using the Cell Viability Bio App which precisely counts the number of cells and evaluates their viability from fluorescence intensity. Two fluorescent dyes that selectively penetrate the cell membranes and fluoresce at different wavelengths were used in the

experiments. DAPI is able to penetrate intact membranes of living cells and colors nuclei in blue and Propidium iodide dye penetrates only dead cells with damaged membranes, staining them in yellow. IC_{50} was calculated using an online tool: MLA—"Quest Graph™ IC50 Calculator." AAT Bioquest, Inc, 25 July, 2019, https://www.aatbio.com/tools/ic50-calculator. DAPI and propidium iodide were purchased from Sigma. The M-HeLa clone 11 human, epithelioid cervical carcinoma, strain of HeLa, clone of M-HeLa; human duodenal cancer cell line **(**HuTu 80) from the Type Culture Collection of the Institute of Cytology (Russian Academy of Sciences) and Chang liver cell line (Human liver cells) from N. F. Gamaleya Research Center of Epidemiology and Microbiology were used in the experiments. The cells were cultured in a standard Eagle's nutrient medium manufactured at the Chumakov Institute of Poliomyelitis and Virus Encephalitis (PanEco company) and supplemented with 10% fetal calf serum and 1% nonessential amino acids. The cells were plated into a 96-well plate (Nunc) at a concentration of 1×10^5 cells/ml, 150 μl of medium per well, and cultured in a CO_2 incubator at 37 °C. Twenty-four hours after seeding the cells into wells, the compound under study was added at a preset dilution, 150 μl to each well. The dilutions of the compounds were prepared immediately in nutrient media; 5% DMSO that does not induce inhibition of cells at this concentration was added for better solubility. The experiments were repeated three times. Intact cells cultured in parallel with experimental cells were used as a control.

Synthesis

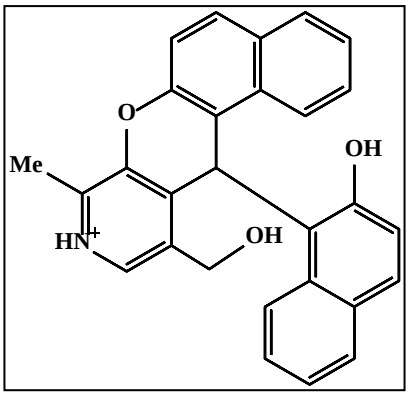


(12-(2-Hydroxynaphthalen-1-yl)-8-methyl-12*H*-benzo[5,6]chromeno[2,3-*c*]pyridin-9-ium-11-yl)methyl hydrogen phosphate (2a)

To a solution of pyridoxal-5-phosphate monohydrate (0.5 g, 1.89 mmol) in ethanol (5 ml) and concentrated hydrochloric acid (2 ml) was added 2-naphthol (0.54 g, 3.78 mmol) of. The mixture was refluxed for 4 h. After cooling, the solvent was removed, the solid residue was washed with ether. The product **2a** was obtained by recrystallization from ethanol. Yield 0.57 g (56%). M.p. > 300 ^oC. IR spectrum, ν, cm^{-1}: 754, 813, 929, 1078, 1232, 1286, 1367, 1405, 1437, 1517, 1626, 3056, 3413. ^1H NMR spectrum (400 MHz, DMSO–d_6), δ: 2.71 s (3H, CH_3), 4.55-4.70 m (1H, CH_2), 5.16-5.55 m (1H, CH_2), 6.88 s (1H, CH), 7.07-7.16 m (2H, $CH_{naph.}$), 7.26-7.51 m (4H, $CH_{naph.}$), 7.65-7.69 m (2H, $CH_{naph.}$), 7.76-7.86 m (3H, $CH_{naph.}$), 8.10 br.s (1H, $CH_{naph.}$), 8.20 s (1H, $CH_{arom.}$). ^{13}C NMR(DMSO-d_6), δ: 19.05, 30.01, 61.99, 115.88, 117.80, 118.46, 119.37, 121.98, 122.72, 123.09, 123.83, 124.95, 126.73, 127.15, 128.22, 128.99, 129.68, 129.98, 130.51, 131.02, 131.77, 131.99, 132.74, 140.54,

145.18, 145.80, 148.25, 151.89. ^{31}P NMR(DMSO-d_6), δ: -1.26. Found, %: C, 67.75; H, 4.75; N, 2.88; P, 6.24. $C_{28}H_{21}NO_6P$. Calculated, %: C, 67.47; H, 4.25; N, 2.81; P, 6.21. MS (MALDI-TOF), *m/z*: 499.9 $[M+H]^+$.

General procedure for the preparation of compounds 2b,c. A mixture of pyridoxal hydrochloride or 1-ethoxyfuropyridine and the corresponding naphthol conc. hydrochloric acid (1 ml) in anhydrous ethanol (10 ml) was refluxed for 2 hours. After cooling, the precipitate was separated, washed with ethyl alcohol, diethyl ether, and dried in a vacuum.



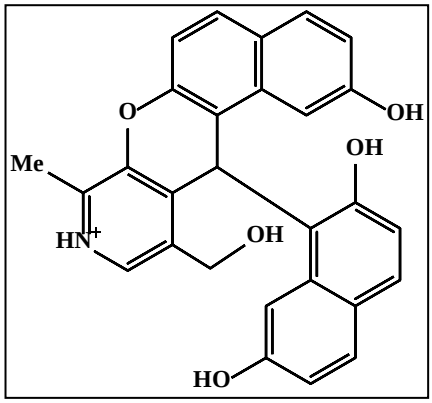
11-(Hydroxymethyl)-12-(2-hydroxynaphthalen-1-yl)-8-methyl-12*H*-benzo[5,6]chromeno[2,3-*c*]pyridin-9-ium chloride (2b)

a) from pyridoxal hydrochloride (0.73 g, 4.37 mmol) and 2-naphthol (1.26 g, 8.74 mmol). Yield 1.33 g (67%).

b) from 1-ethoxyfuropyridine hydrochloride (0.5 g, 2.16 mmol) and 2-naphthol (0.62 g, 4.32 mmol). Yield 0.63 g (65%).

c) from 1-diethylaminofuropyridine (0.50 g, 2.25 mmol) and 2-naphthol (0.65 g, 4.51 mmol). Yield 0.64 g (62%).

M.p. = 284°C. IR spectrum, ν, cm^{-1}: 745, 756, 818, 849, 931, 994, 1050, 1079, 1157, 1236, 1263, 1289, 1366, 1402, 1435, 1464, 1515, 1536, 1625, 1973, 2665, 3370. ^{1}H NMR spectrum (400 MHz, DMSO–d_6), δ: 2.87 s (3H, CH_3), 4.15 dd (1H, CH_2, J=73.3, 15.5 Hz), 5.05 d (1H, CH_2, J=15.5 Hz), 6.89–8.13 m (13H, CH $_{naph.}$ + CH), 8.22 s (1H, CH $_{arom.}$). ^{13}C NMR(DMSO-d_6), δ: 14.75, 19.04, 31.17, 56.54, 58.70, 113.57, 115.42, 117.50, 118.17, 119.55, 121.57, 122.95, 123.35, 123.83, 124.85, 125.51, 126.92, 127.38, 128.09, 128.21, 129.14, 129.70, 130.53, 131.00, 131.21, 131.67, 132.39, 132.69, 136.51, 136.97, 139.60, 140.10, 141.55, 147.37, 148.62, 152.37, 156.41. Found, %: C, 73.38; H, 5.12; Cl, 8.03; N, 2.91. $C_{28}H_{22}ClNO_3$. Calculated, %: C, 73.76; H, 4.86; Cl, 7.78; N, 3.07. MS (MALDI-TOF), *m/z*: 420 $[M-HCl]^+$.

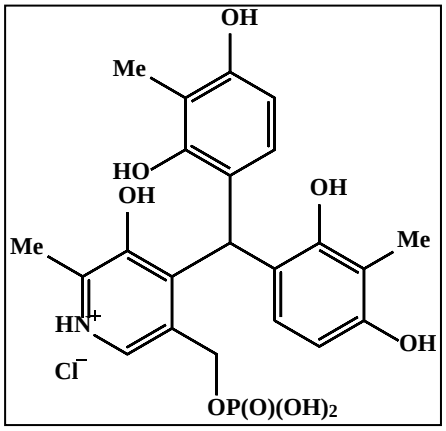


12-(2,7-Dihydroxynaphthalen-1-yl)-2-hydroxy-11-hydroxymethyl-8-methyl-12*H*-benzo[5,6]chromeno-[2,3-*c*]pyridin-9-ium chloride (2c)

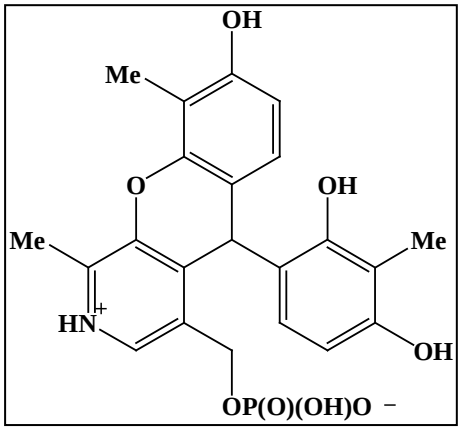
a) from pyridoxal hydrochloride (1.0 g, 4.90 mmol) and naphthalene-2,7-diol (1.57 g, 9.80 mmol). Yield 1.50 g (63%).

b) from 1-diethylaminofuropyridine (0.5 g, 2.16 mmol) and naphthalene-2,7-diol (0.69 g, 4.32 mmol). Yield 0.74 (70%).

M.p. = 270 °C (dec.). IR spectrum, ν, cm^{-1}: 1038, 1516, 1634, 3201. ^{1}H NMR spectrum (400 MHz, DMSO–*d*$_6$), δ: 1 isomer: 2.84 s (3H, CH_3), 4.15 d (1H, CH_2, *J*=15.3 Hz), 4.96 d (1H, CH_2, *J*=15.3 Hz), 6.70 s (1H, CH), 6.73 d (1H, CH_2, *J*=9.2 Hz), 6.88 s (1H, CH), 6.98 d (1H, CH, *J*=8.7 Hz), 7.17 d (1H, CH, *J*=8.8 Hz), 7.28 d (1H, CH, *J*=8.8 Hz), 7.51 tr (1H, CH, *J*=9.2 Hz), 7.59 d (1H, CH, *J*=8.8 Hz), 7.67 s (1H, CH), 8.20 s (1H, CH), 8.22 s (1H, CH), 8.33 s (1H, $CH_{arom.}$), 11.16 s (1H, OH). 2 isomer: 2.90 s (3H, CH_3), 4.23 d (1H, CH_2, *J*=16.3 Hz),5.05 d (1H, CH_2, *J*=16.3 Hz), 6.47 s (1H, CH), 6.66 d (1H, CH, *J*=8.7 Hz), 6.98 d(1H, CH, *J*=8.8 Hz), 7.04 d (1H, CH, *J*=8.7 Hz), 7.21 d (1H, CH, *J*=8.9 Hz), 7.44 s (1H, CH), 7.52 tr (1H, CH, *J*=9.2 Hz), 7.65 s (1H, CH), 7.67 s (1H, CH), 7.73 d (1H, CH, *J*=8.8 Hz), 7.76 d (1H, CH, *J*=8.8 Hz), 7.88 s (1H, $CH_{arom.}$), 9.82 s (1H, OH). ^{13}C NMR(DMSO-*d6*), δ: 1 isomer: 14.8, 30.9, 58.8, 104.6, 106.9, 113.4, 113.9, 115.2, 115.8, 116.3, 116.9, 124.4, 125.8, 130.0, 130.4, 130.6, 131.0, 131.2, 133.7, 137.0, 139.9, 140.2, 147.9, 148.7, 152.6, 156.2, 156.9. 2 isomer: 15.2, 31.4, 57.3, 105.9, 107.1, 112.2, 114.0, 115.8, 117.3, 117.6, 122.9, 125.6, 129.3, 130.6, 131.0, 131.9, 133.7, 134.4, 134.5, 137.4, 139.5, 141.2, 147.5, 148.9, 156.5, 156.6, 157.7. Found, %: C, 68.76; H, 4.40; Cl, 7.77; N, 2.83. $C_{28}H_{22}ClNO_5$. Calculated, %: C, 68.92; H, 4.54; Cl, 7.27; N, 2.87. MS (MALDI-TOF), *m/z*: 452 $[M - HCl]^+$.



4-[Bis(2,4-dihydroxy-3-methylphenyl)methyl]-3-hydroxy-2-methyl-5-(phosphonooxymethyl)pyridin-1-ium chloride (4) To a solution of pyridoxal-5-phosphate monohydrate (0.6 g, 2.26 mmol) in ethanol (10 ml) and concentrated hydrochloric acid (2 ml) was added 2-methylresorcinol (0.56 g, 4.52 mmol). The mixture was refluxed for 6 hours. Then the solvent was removed to leave a solid foam. Yield 1.0 g (86%). IR spectrum, ν, cm^{-1}: 796, 954, 1071, 1164, 1288, 1462, 1540, 1607, 3292. ^{1}H NMR spectrum (400 MHz, DMSO–*d*$_6$), δ: 1.94 s (3H, CH_3), 2.18 s (3H, CH_3), 2.83 s (3H, CH_3), 4.60 dd (1H, CH_2, *J*=14.0, 8.5 Hz), 5.11 dd (1H, CH_2, *J*=14.0, 8.5 Hz), 5.66 s (1H, CH), 6.32 d (1H, Ph, *J*=8.2 Hz), 6.60 д (1H, Ph, *J*=8.3 Hz), 6.68 d (1H, Ph, *J*=8.2 Hz), 6.90 d (1H, Ph, *J*=8.3 Hz), 8.24 s (1H, $CH_{arom.}$). ^{13}C NMR(DMSO-*d*$_6$), δ: 8.72, 9.73, 14.83, 35.89, 61.41, 107.68, 111.30, 112.10, 112.57, 113.75, 123.06, 126.33, 126.71, 131.85, 134.06, 139.29, 142.43, 147.71, 148.75, 153.11, 155.40, 155.98. ^{31}P NMR(DMSO-*d*$_6$), δ: -0.30. Found, %: C, 51.23; H, 5.14; Cl, 6.14; N, 2.80; P, 6.07. $C_{22}H_{25}ClNO_9P$. Calculated, %: C, 51.42; H, 4.90; Cl, 6.90; N, 2.73; P, 6.03.



[5-(2,4-Dihydroxy-3-methylphenyl)-8-hydroxy-1,9-dimethyl-5*H*-chromeno[2,3-*c*]pyridin-2-ium-4-yl]methyl hydrogen phosphate (5)

It was obtained upon recrystallization of compound **4** from absolute ethanol. Yield 0.79 g (89%) ^{1}H NMR spectrum (400 MHz, DMSO–d_6), δ: 1.97 s (3H, CH_3), 2.19 s (3H, CH_3), 2.61 s (3H, CH_3), 4.56 dd (1H, CH_2, J=12.9, 7.0 Hz), 4.90 dd (1H, CH_2, J=12.8, 8.5 Hz), 5.66 s (1H, CH), 6.19 d (1H, Ph, J=8.4 Hz), 6.38 d (1H, Ph, J=8.3 Hz), 6.52 d (1H, Ph, J=8.3 Hz), 6.98 d (1H, Ph, J=8.4 Hz), 8.13 s (1H, $CH_{arom.}$). Спектр ЯМР ^{13}C (ДМСО-d_6), δ_C, м.д.: 8.93, 9.81, 18.86, 34.19, 62.13, 107.60, 111.23, 112.32, 115.70, 124.49, 126.05, 126.22, 128.77, 129.85, 132.29, 141.26, 145.99, 147.25, 148.85, 152.66, 155.01, 155.23. ^{31}P NMR(DMSO-d_6), δ: -1.25. Found, %: C, 57.43; H, 4.71; N, 2.98; P, 6.59. $C_{22}H_{22}NO_8P$. Calculated, %: C, 57.52; H, 4.83; N, 3.05; P, 6.74. MS (MALDI-TOF), *m/z*: 497.9 $[M+K]^+$.

Acknowledgment

The work was supported by Russian Science Foundation (grant no 21-13-00022), https://rscf.ru/project/21-13-00022/.

The authors are grateful to the Collective Spectral-Analytical Center of Physicochemical studies of structure, properties, and composition of substances and materials of the Federal Research Center of the Kazan Scientific Center of RAS for technical support of the studies. X-ray studies were performed using the equipment of the Federal Research Center Kazan Scientific Center of RAS.

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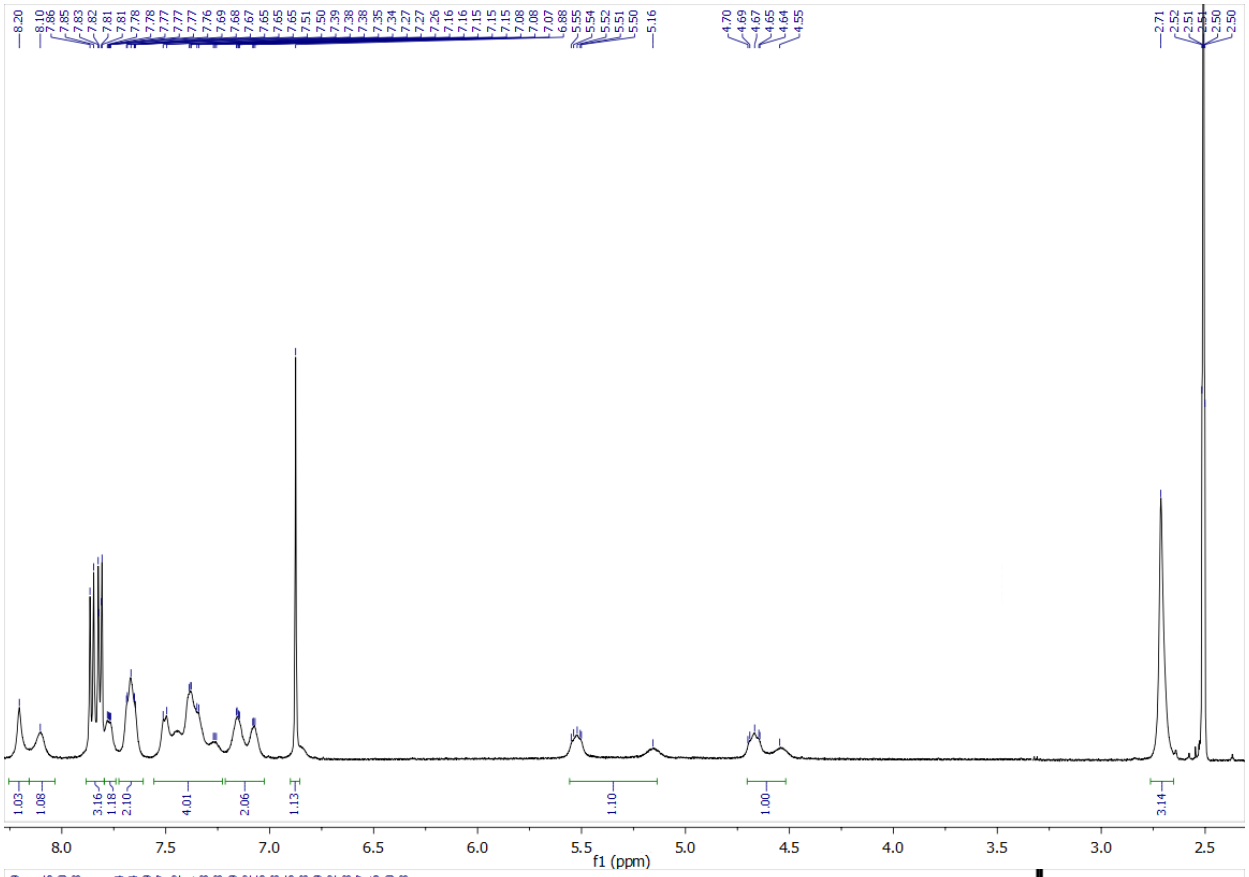
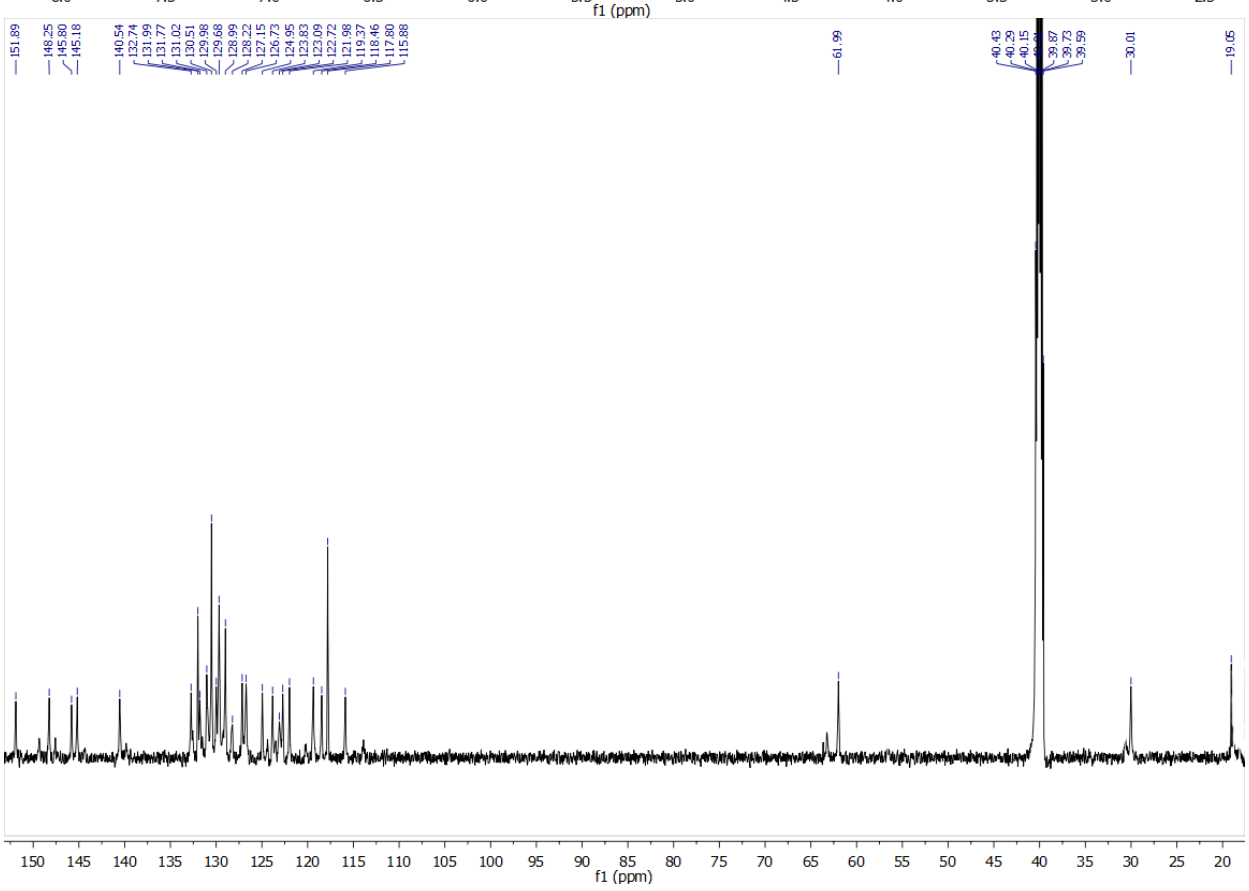
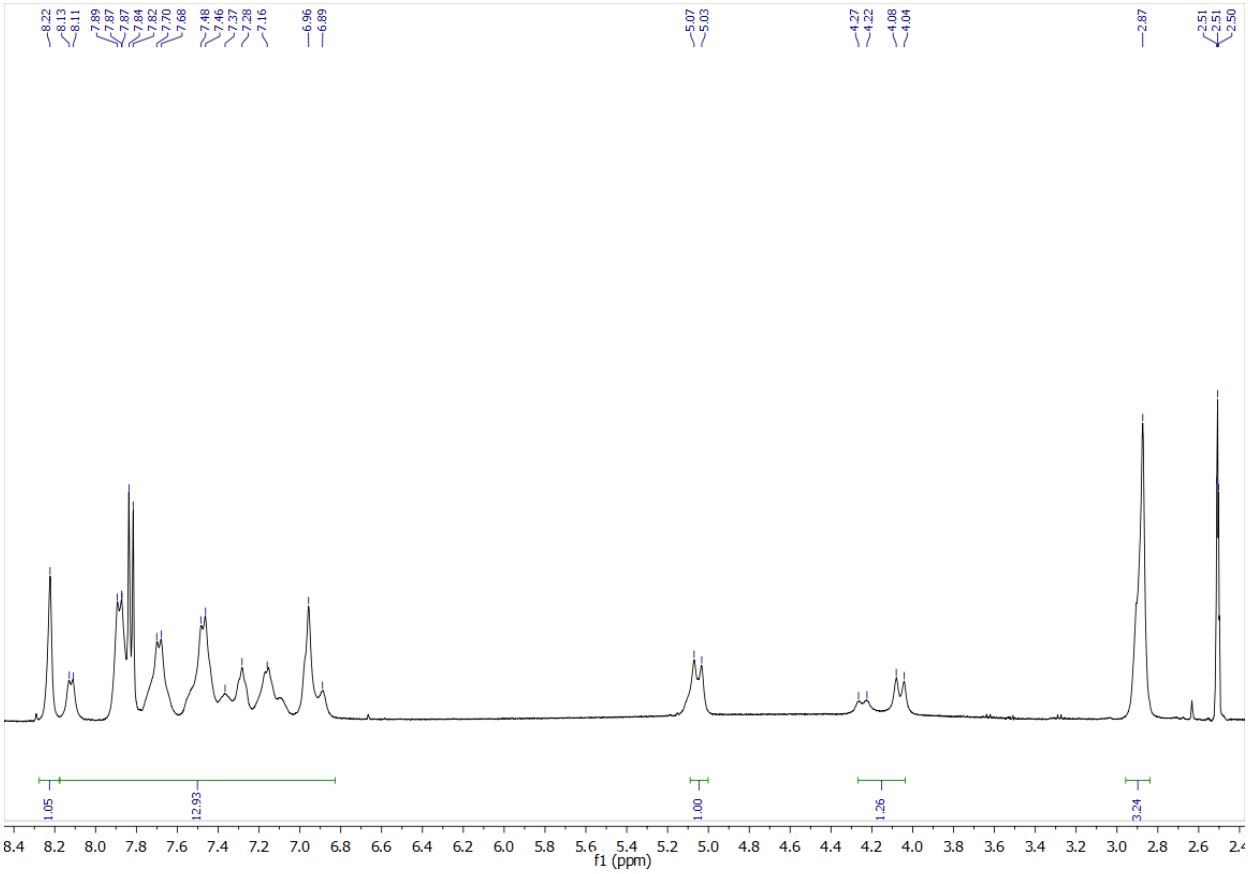
Copies of NMR spectra for the compounds

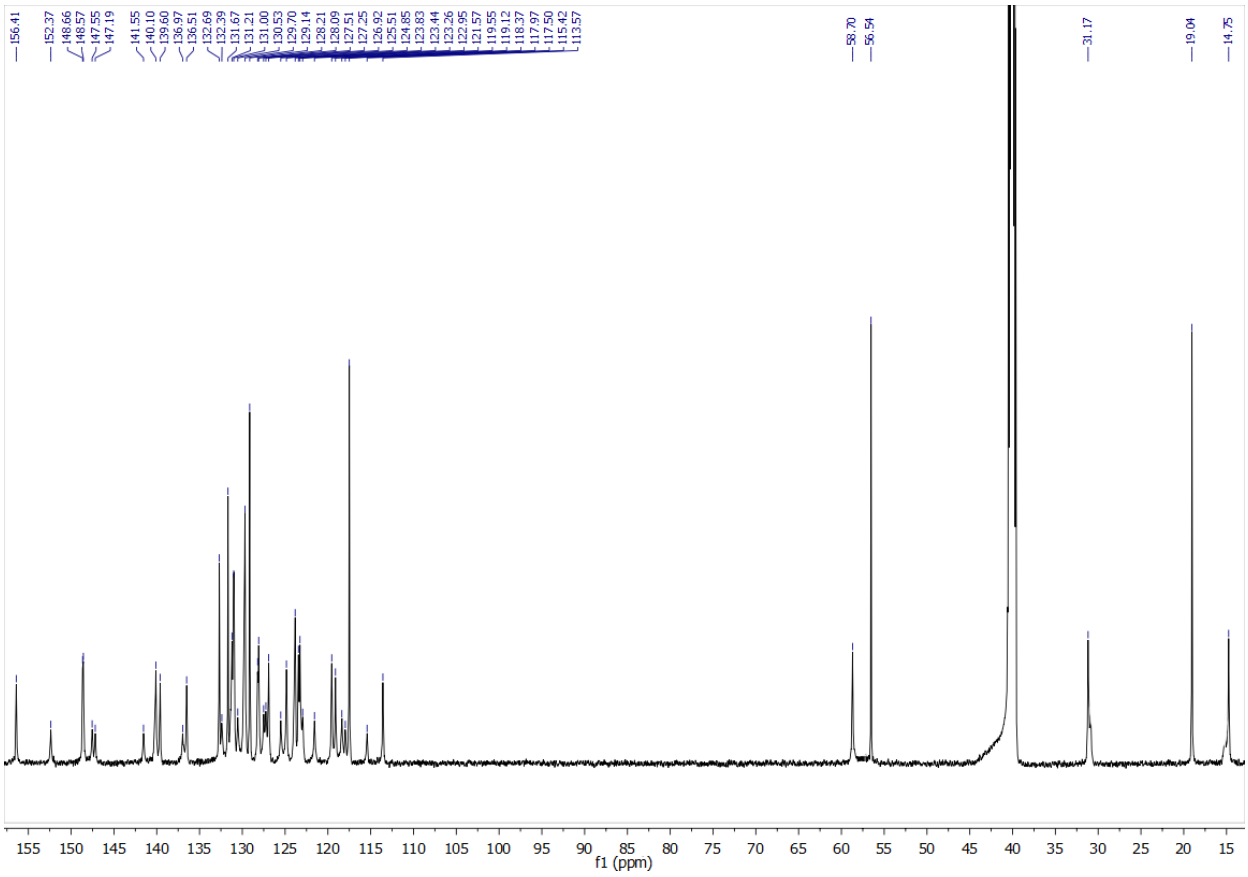
[12-(2-Hydroxynaphthalen-1-yl)-8-methyl-12*H*-benzo[5,6]chromeno[2,3-*c*]pyridin-9-ium-11-yl]methyl hydrogen phosphate (2a)



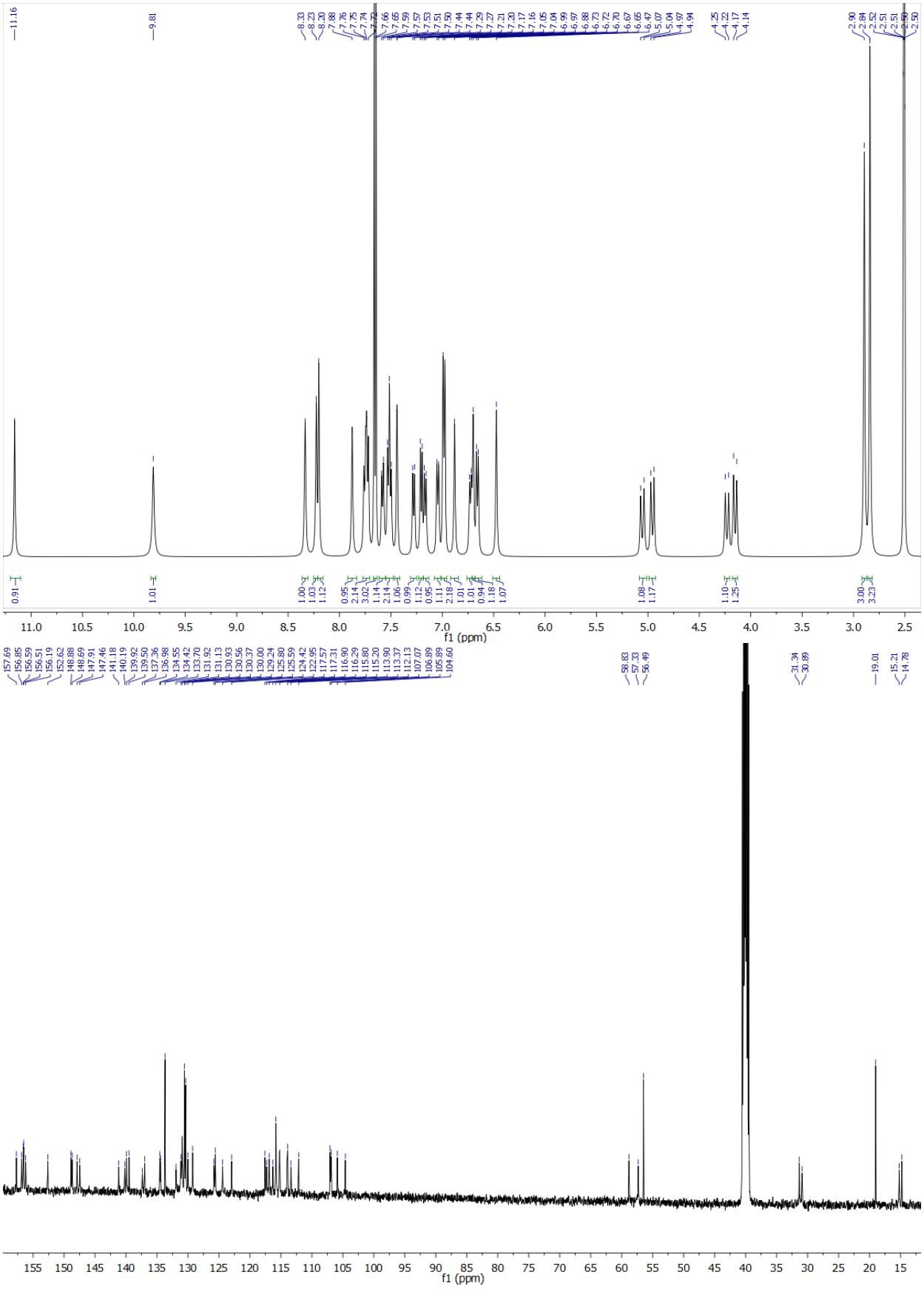


11-(Hydroxymethyl)-12-(2-hydroxynaphthalen-1-yl)-8-methyl-12*H*-benzo[5,6]chromeno[2,3-*c*]pyridin-9-ium chloride (2b)

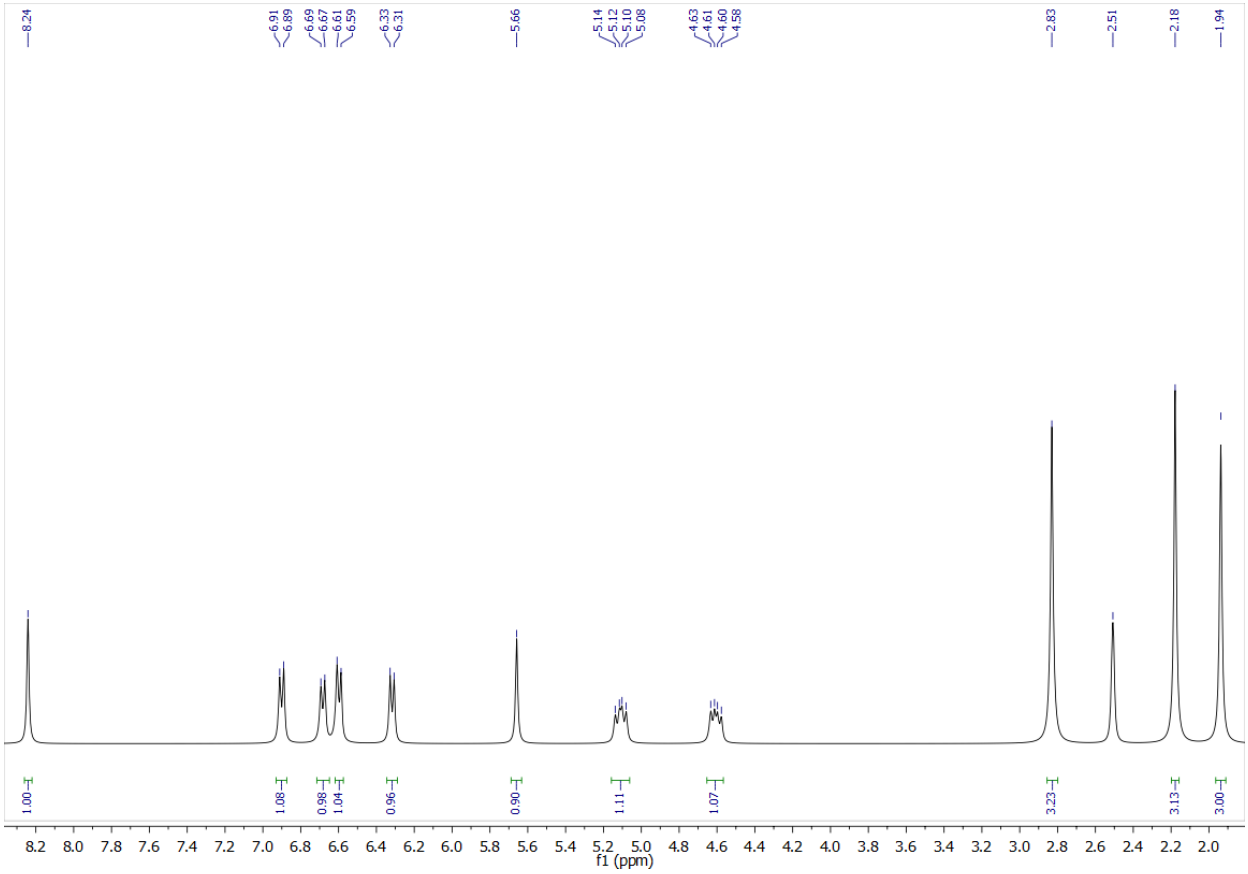


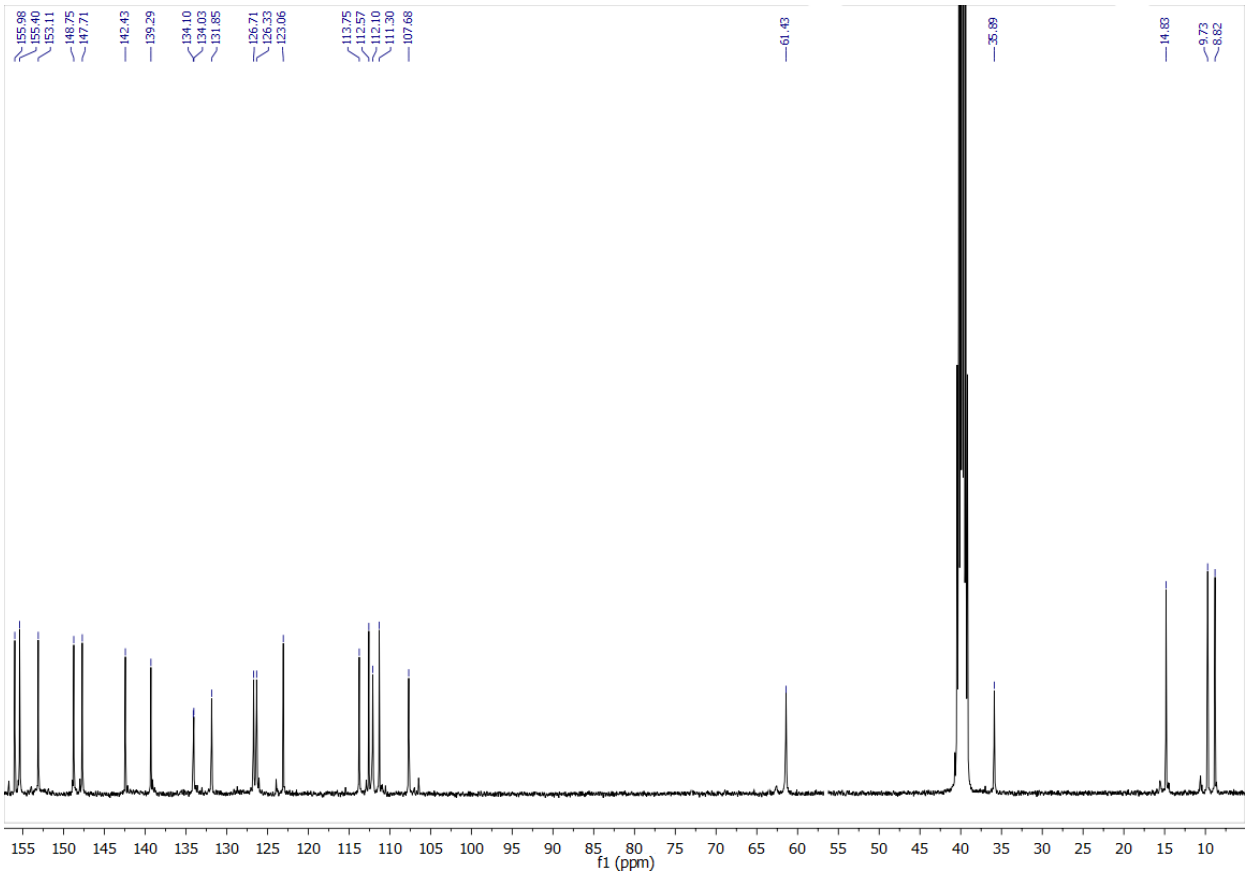


12-(2,7-Dihydroxynaphthalen-1-yl)-2-hydroxy-11-hydroxymethyl-8-methyl-12*H*-benzo[5,6]chromeno[2,3-*c*]pyridin-9-ium chloride (2c)



4-[Bis(2,4-dihydroxy-3-methylphenyl)methyl]-3-hydroxy-2-methyl-5-(phosphonooxymethyl)pyridin-1-ium chloride (4)





[5-(2,4-Dihydroxy-3-methylphenyl)-8-hydroxy-1,9-dimethyl-5*H*-chromeno[2,3-*c*]pyridin-2-ium-4-yl]methyl hydrogen phosphate (5)