Electronic Supplementary Information

Design and synthesis of gene-directed caged cyclic nucleotides exhibiting cell type selectivity

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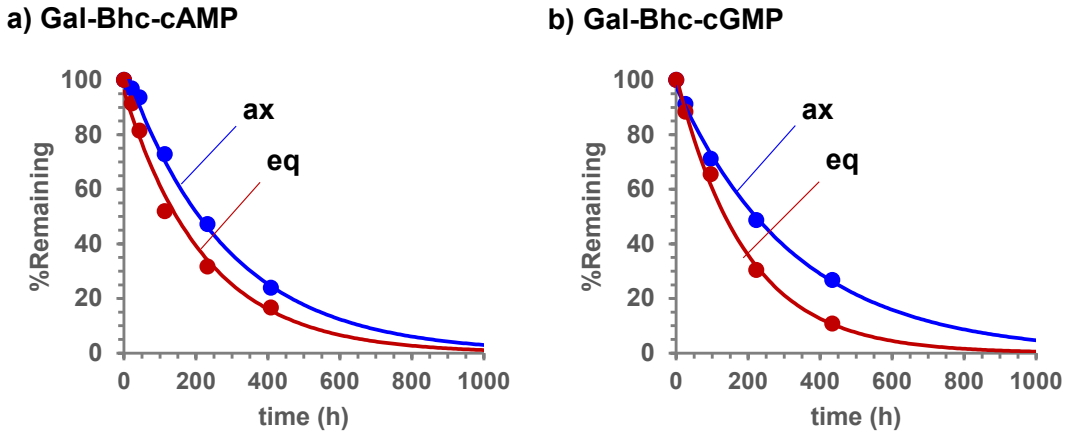


Fig. S1 Stability in the dark. Samples (10 μM in K-MOPS solution at pH 7.4) were kept in the dark place. The time course of the hydrolysis of the axial (blue) and equatorial (red) isomer of (a) Gal-Bhc-cAMP and (b) Gal-Bhc-cGMP. The solid lines are the best fit for single exponential decay.

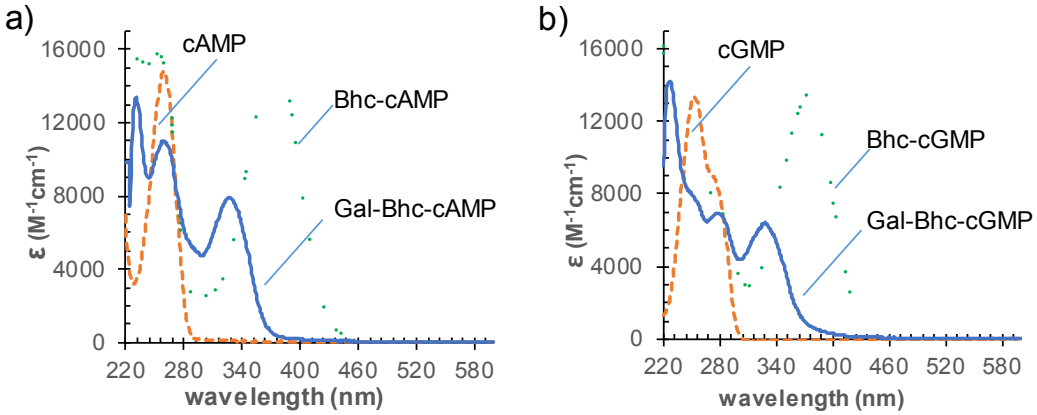


Fig. S2 Absorption spectra of caged cyclic nucleotides in 10 mM K-MOPS solutions (pH 7.4).

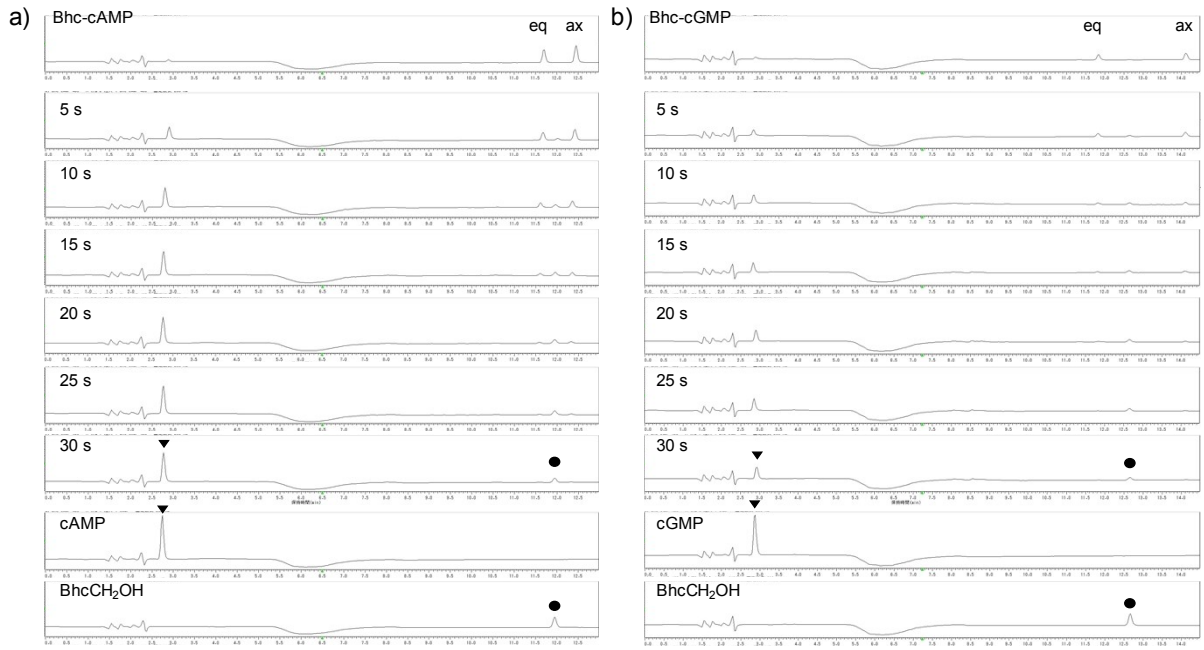


Fig. S3 HPLC traces for the photolysis of Bhc-cAMP (a) and Bhc-cGMP (b) at 405 nm. HPLC traces showing the photo-mediated production of cAMP from Bhc-cAMP (a) and cGMP from Bhc-cGMP (b) at 405 nm. Solid circles and triangles denote $BhcCH_2OH$ and cNMPs, respectively.

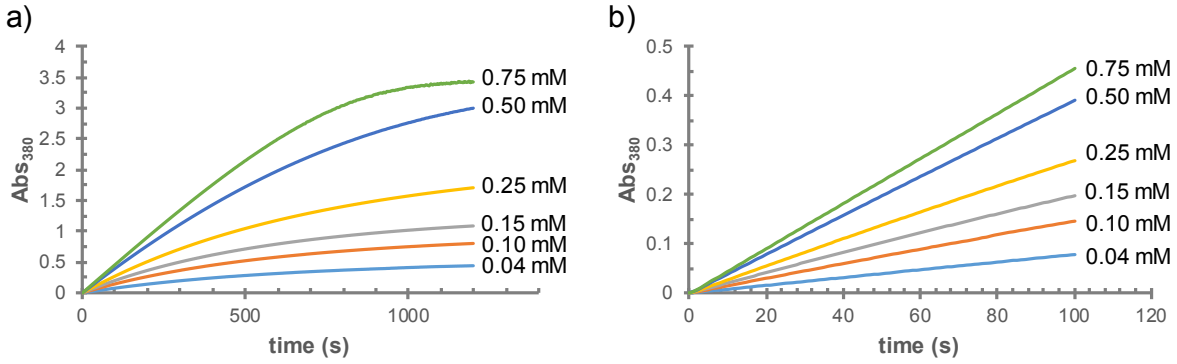


Fig. S4 Time course for the enzymatic deprotection of Gal-Bhc-cAMP. The indicated concentrations of Gal-Bhc-cAMP were incubated with 2.08 nM of *E. coli* β-Gal at 37 °C. Absorption changes of the reaction mixtures were measured at 380 nm. Data points for 1200 s (a) or 100 s (b) were indicated.

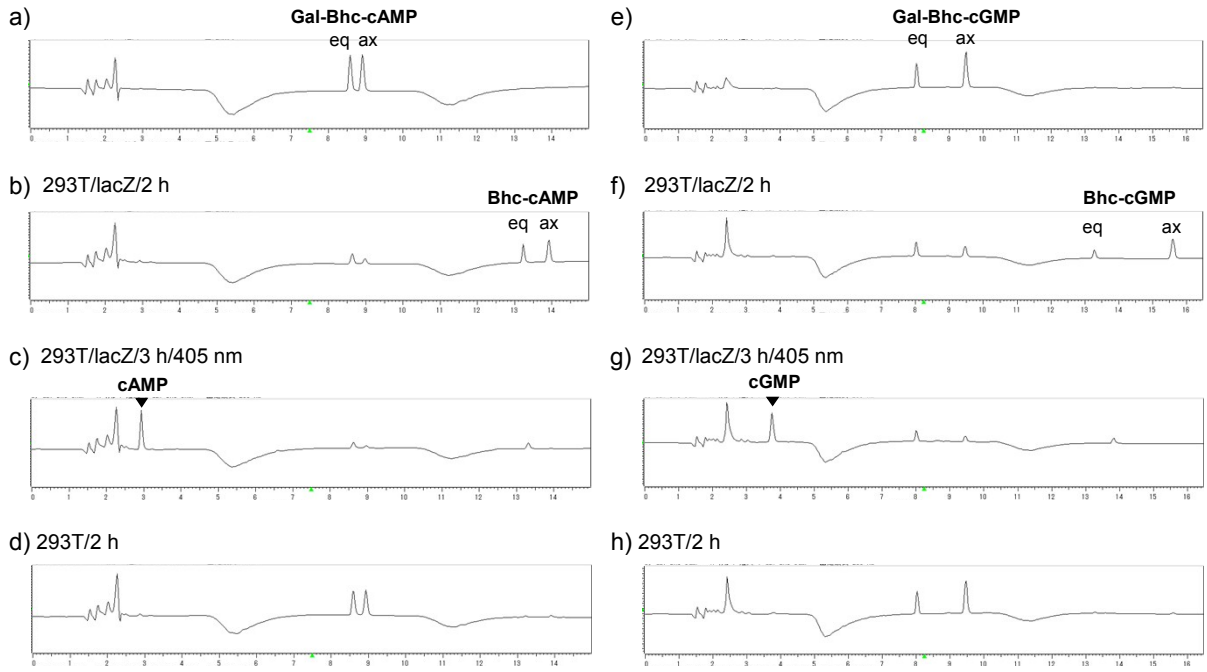


Fig. S5 Enzymatic reactions of Gal-Bhc-cNMPs in the presence of HEK293T cell lysates. Panels a to d are for caged cAMP and e to h are for cGMP. HPLC traces of Gal-Bhc-cNMP immediately after the treatment with HEK293T/lacZ cell lysates (a and e), after 2 hr of the treatment (b and f), with 405 nm irradiation (c and g), and 2 hr after the treatment with HEK293T cell lysates without lacZ expression (d and h).

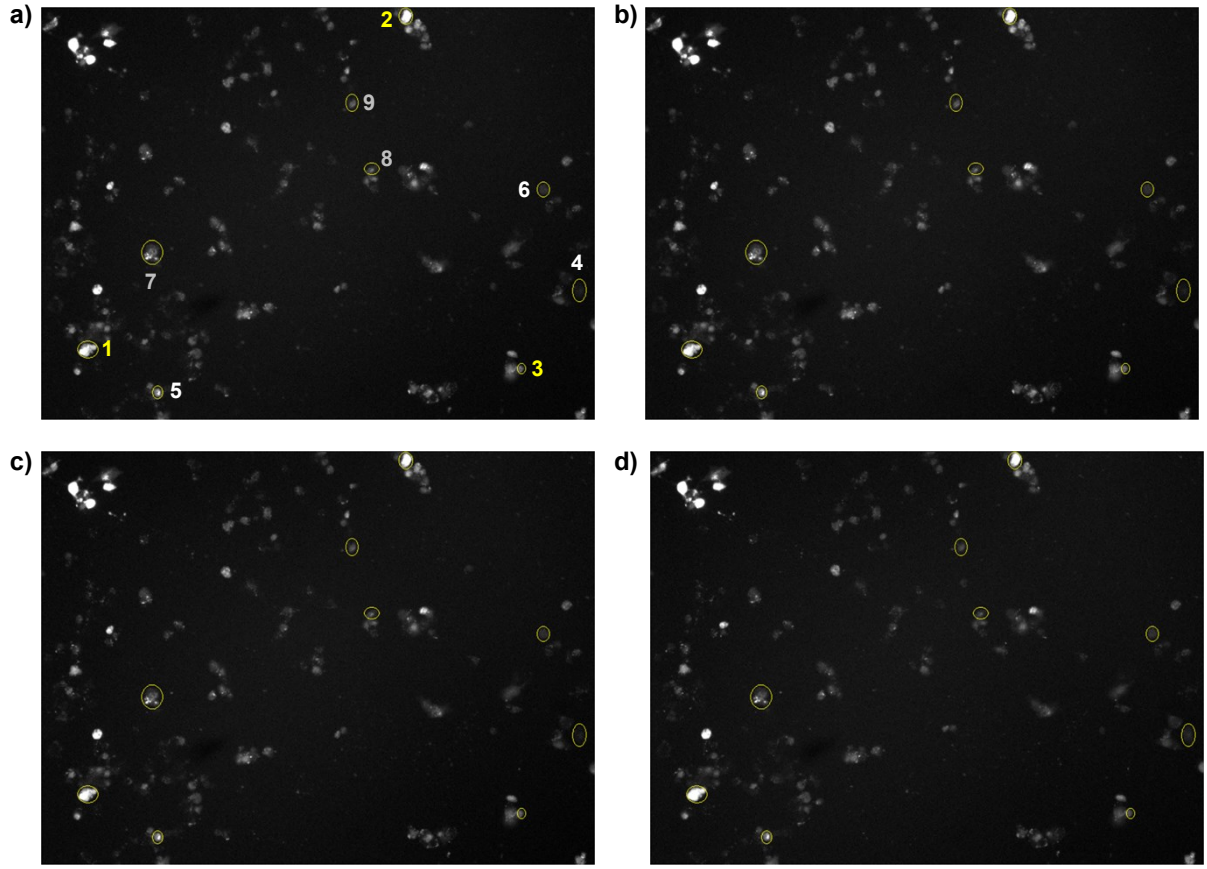


Fig. S6 Fluorescence images of HeLa cells co-transfected with R-FlincA and lacZ genes. R-FlincA fluorescence were recorded. ROIs 1-3 are examples of β-Gal(+) cells, ROIs 4-6 are β-Gal(−) cells and ROIs 7-9 are Forskolin(−) cells that express R-FlincA but not responded to forskolin (a). The change in fluorescence intensity of β-Gal(+) and β-Gal(−) cells was traced and quantified. (a) before 405 nm irradiation (30 s after the start point), (b) after photo-irradiation (125 s), (c) before forskolin addition (295 s), and (d) after forskolin addition (810 s).

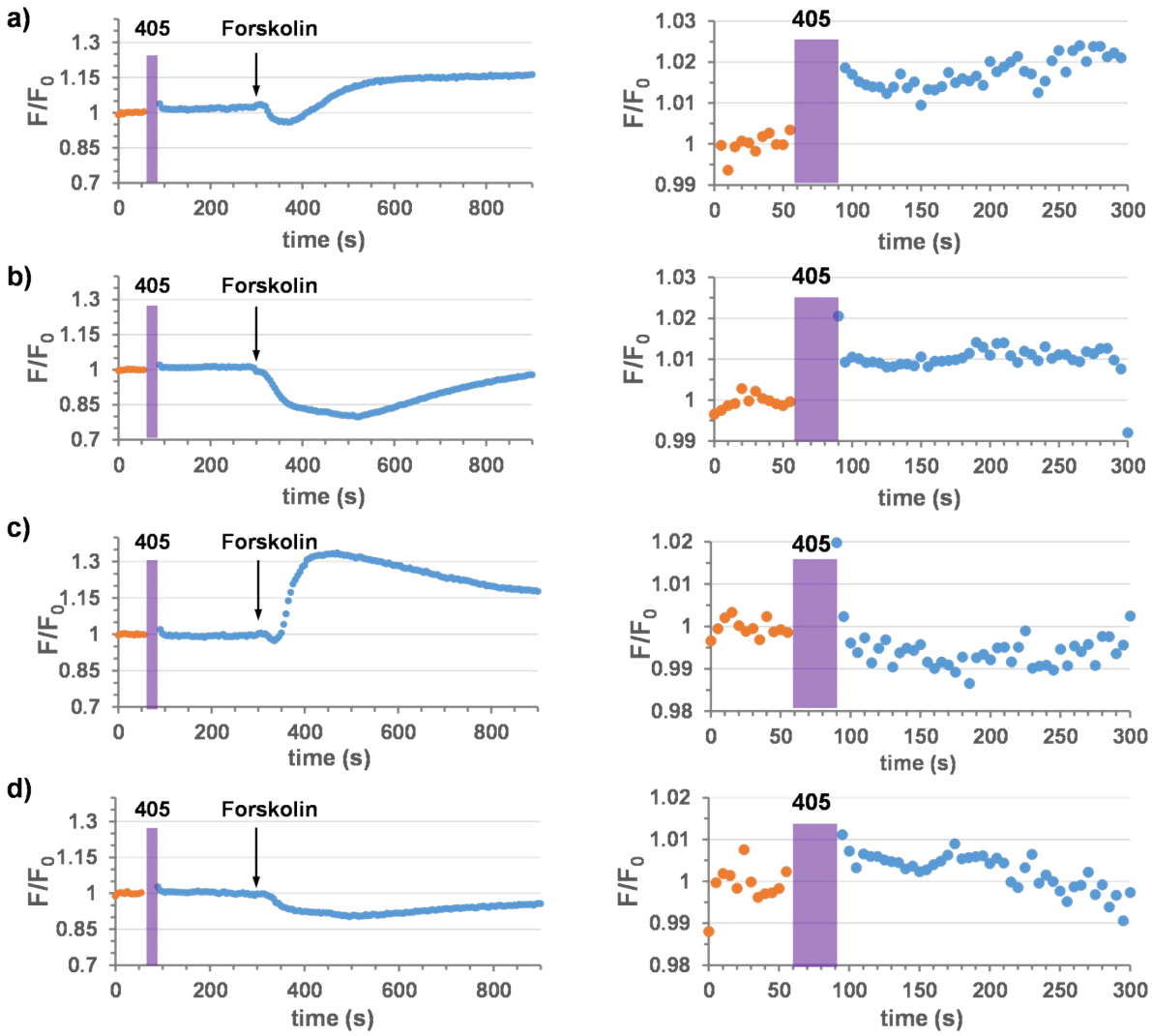


Fig. S7 Traces showing the fluorescence changes of R-FlincA in HeLa cells co-transfected with lacZ and R-FlincA. (a) and (b) β-Gal(+) cells. (c) and (d) β-Gal(−) cells. The traces on the right is a partially enlarged view of the left panel from 0 to 300 seconds. The traces in a and c are from the same cells in Fig. 5a and b. The cells were irradiated at 60 seconds after the start of observation, and forskolin was added at 300 seconds.

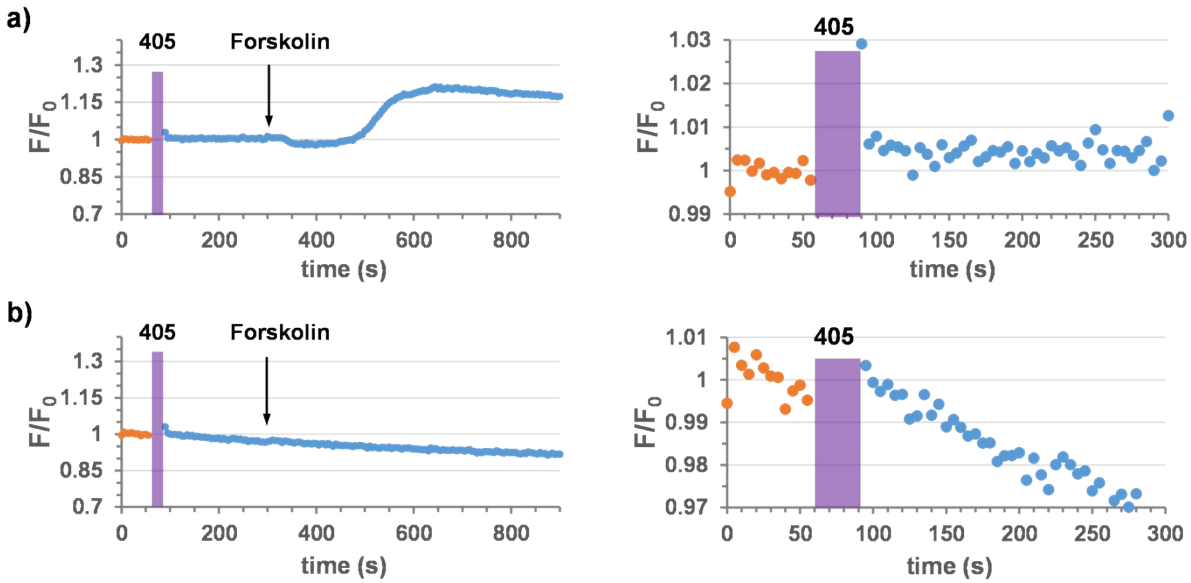


Fig. S8 Traces showing the fluorescence changes of R-FlincA in a control cell (a) and a Forskolin(−) cell (b).

Table S1 Photochemical and physical properties of Bhc-cNMPs and Gal-Bhc-cNMPs

Caged cNMPs	λ_{max}[a]	ε_{max}[b]	ε_{405}[c]	Φ_{dis}[d]	$\varepsilon\Phi$[e]	$t_{1/2}$[f]
Bhc-cAMP	374	15500	7100	0.40	2840	260/90[g]
Bhc-cGMP	374	13500	6000	0.43	2580	1240/420[g]
8	327	7900	100	nd[h]		209/146
9	328	6400	200	nd[h]		223/141

[a] absorption maximum (nm), [b] molar absorptivity at the absorption maximum (M^{-1} cm^{-1}), [c] molar absorptivity at 405 nm (M^{-1} cm^{-1}), [d] quantum yield of disappearance at 405 nm irradiation, [e] photolysis efficiency at 405 nm, [f] half-life (h) at 20 °C under dark conditions, axial/equatorial, [g] ref. 15, [h] photolysis was not detected.

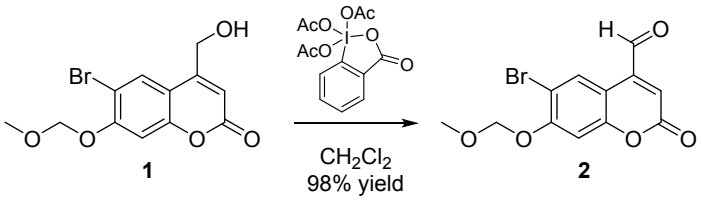
Synthetic Procedures

General Synthesis Methods

All reagents and solvents were purchased from commercial sources and used without further purification. Flash column chromatography was carried out on a YAMAZEN EPCLC-AI-580S system using universal column premium (30 μm silica gel). NMR spectra were recorded on a Brucker Biospin Avance 300M at 300 MHz for ^{1}H and 75 MHz for ^{13}C with a deuterated solvent as and TMS as an internal standard. IR spectra were recorded on a Thermo Nicolet Avatar 320 in ATR mode. Analytical HPLC was run on an Agilent HP 1100 system with DAD detection, on a Hitachi Chromaster system with DAD detection and semi-preparative HPLC on a Shimadzu LC system (pump: LC-6AD) with RI and DAD detection. ESI Mass spectra were recorded on a SHIMADZU LCMS-2010. HRMS spectra were recorded on a JEOL JMS-700 using *m*-nitrobenzyl alcohol as a matrix to facilitate sample ionization.

Synthesis of Gal-Bhc-diazo (7)

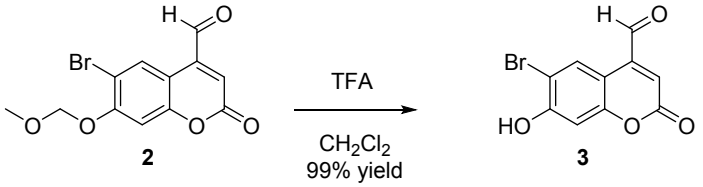
Synthesis of 6-bromo-7-methoxymethoxy-4-formylcoumarin (2):



To a stirred solution of **1**[1] (149.5 mg, 0.474 mmol) in anhydrous CH_2Cl_2 (25 mL) was added Dess-Martin periodinane (402.4 mg, 0.948 mmol). After 2 h of stirring at rt, the reaction mixture was evaporated and washed with saturated $NaHCO_3$ and 1.0 M $Na_2S_2O_3$ solutions. The mixture was then extracted with ethyl acetate, the organic layer was dried over $MgSO_4$, filtered, and evaporated under vacuum to yield **2** (145.0 mg, 0.463 mmol, 98% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ= 10.0 (1H, s; CHO), 8.83 (1H, s; Ar-H_5), 7.18 (1H, s, Ar-H_8), 6.78 (1H, s; C=C-H), 5.34 (2H, s; CH_2), 3.53 ppm (3H, s; OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ= 191.25 (formyl, C=O), 159.96 (alkene C_4), 156.95 (C=O), 155.10 (Ar-C_7), 142.60 (Ar-C_9), 130.32 (Ar-C_5), 123.85 (alkene C_3), 109.73 (Ar-C_{10}), 109.58 (Ar-C_8), 103.63 (Ar-C_6), 95.14 (O-CH_2-O), 56.77 ppm (CH_3); IR (neat) ν~ = 1725 (s) (formyl C=O, 1701 (s) (C=O) cm^{-1}; HRMS (ESI) calcd for $C_{12}H_{10}{}^{79}BrO_5$: 312.9706 $[M+H]^+$; found: 312.9711.

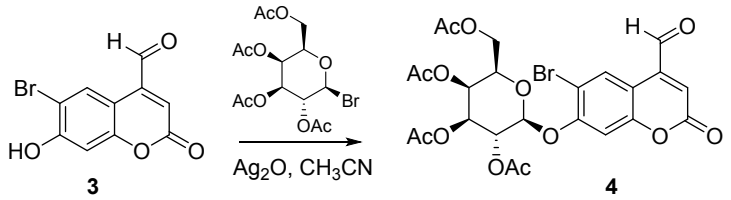
Synthesis of 6-bromo-7-hydroxy-4-formylcoumarin (3):



To a stirred solution **2** (545.9 mg, 1.74 mmol) in anhydrous CH_2CH_2 (2 mL) was added dropwise TFA (2 mL) at 0 °C. After 13 h of stirring at rt, the solvents were removed by a rotary evaporator. The trace mount of TFA was azeotropically removed by repeated treatment of toluene and evaporation under high vacuum to yield **3** (465.3 mg, 1.73 mmol, 99% yield).

^{1}H NMR (300 MHz, [D_6]DMSO) δ = 11.6 (1H, broad; Ar-OH), 10.1 (1H, s; CHO), 8.60 (1H, s, Ar-H_5), 6.99 (1H, s; Ar-H_8), 6.95 (1H, s; C=C-H); ^{13}C NMR (75 MHz, [D_6]DMSO) δ = 194.11 (formyl, C=O), 160.59 (alkene C_4), 158.13 (coumarin C=O), 155.22 (Ar-C_7), 143.06 (Ar-C_9), 129.94 (Ar-C_5), 121.96 (alkene C_3), 108.81 (Ar-C_{10}), 107.15 (Ar-C_8), 103.82 ppm (Ar-C_6); IR (neat) ν = 3500-3200 (OH), 1697 (s) (C=O) cm^{-1}; HRMS (ESI) calcd for $C_{10}H_4{}^{79}BrO_4$: 266.9298 [M-H]$^-$; found: 266.9309.

Synthesis of 6-bromo-7-(2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranosyl)-4-formylcoumarin (4):

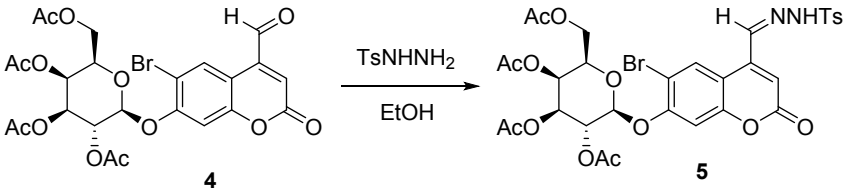


To a stirred mixture of **3** (503.4 mg, 1.871mmol) and acetobromo-α-D-galactose (1.154 g, 2.807 mmol) in acetonitrile (10 mL) was added Ag_2O (1.30 g, 5.61 mmol). After 20 min of stirring at 50 °C, the solvent was removed using vacuum evaporation. Then the residue was redissolved in ethyl acetate and filtered through silica gel. The filtrate was evaporated and purified by column chromatography (Yamazen Universal Column size L, $CHCl_3$/EtOAc 98:2 to 47:53) to give **4** (928.2 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.0 (1H, s; CHO), 8.85 (1H, s; Ar-H_5), 7.20 (1H, s; Ar-H_8), 6.82 (1H, s; C=C-H), 5.62-5.68 (dd, $^3J_{H\text{-}H}$ = 10.5 and 8.0 Hz, 1H), 5.50-5.51 (d, $^3J_{H\text{-}H}$ = 2.5 Hz, 1H), 5.12-5.17 (dd, $^3J_{H\text{-}H}$ = 10.5 and 2.5 Hz, 1H), 5.07-5.10 (d, $^3J_{H\text{-}H}$ = 8.0 Hz, 1H; $C_{1'}$-H_{anomer}), 4.22-4.24 (2H, m), 4.11-4.19 (1H, m), 2.20 (3H, s; O(C=O)CH_3), 2.17 (3H, s; O(C=O)CH_3), 2.11 (3H, s; O(C=O)CH_3), 2.03 (3H, s; O(C=O)CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 191.07(formyl C=O), 170.55 (acetyl C=O), 170.17 (acetyl C=O), 170.09 (acetyl C=O), 169.20 (acetyl C=O), 159.60 (alkene C_4), 156.42 (coumarin C=O), 154.79 (Ar-C_7), 142.40 (Ar-C_9), 130.59 (Ar-C_5), 124.68 (alkene C_3), 110.93 (Ar-C_{10}), 109.59 (Ar-C_8), 104.88 (Ar-C_6), 100.01 ($C_{1'anomer}$), 71.98 ($C_{5'}$), 70.48 ($C_{3'}$), 67.78 ($C_{2'}$), 66.83 ($C_{4'}$), 61.65 ($C_{6'}$), 20.85 (CH_3), 20.67

(CH_3), 20.62 (CH_3), 20.56 ppm (CH_3); IR (neat) ν = 1737 (s) (C=O) cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{27}{}^{79}BrN_1O_{13}$: 616.0660[M+$NH_4$]$^+$; found: 616.0659.

Synthesis of 6-bromo-7-(2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranosyl)-4-formylcoumarin p-tosylhydrazone (5):

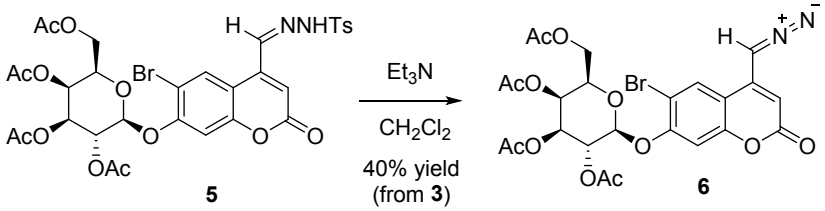


4 **5**

To a stirred solution of **4** (928.2 mg) in acetonitrile (2 mL) was added *p*-toluenesulfonyl hydrazide (112.5 mg, 0.604 mmol). After 3 h of stirring at 50 °C, *p*-toluenesulfonyl hydrazide (112.5 mg, 0.604 mmol) was added to the reaction mixture. After further stirring at 50 °C for 2 h and at rt for 40 h, the reaction mixture was evaporated to give the crude compound **5** (1.2956 g). The crude compound **5** was used directly for the next reaction without further purification.

^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.80 (s, 1H; C<u>H</u>=N-NHTs), 8.40 (1H, broad s; N-NH-Ts), 7.95 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H; Ts), 7.69 (1H, s; Ar-H_5), 7.38 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H; Ts), 7.14 (1H, s; Ar-H_8), 6.35 (1H, s; C=CH), 5.62-5.68 (dd, $^3J_{H\text{-}H}$ = 10.5 and 8.0 Hz, 1H), 5.50-5.51 (d, $^3J_{H\text{-}H}$ = 3.0 Hz, 1H), 5.13-5.18 (dd, $^3J_{H\text{-}H}$ = 8.5 and 3.4 Hz, 1H), , 5.07-5.10 (d, $^3J_{H\text{-}H}$ = 8.0 Hz, 1H; $C_{1'}$-H_{anomer}), 4.23-4.24 (2H, m), 4.15-4.21 (1H, m), 2.43 (3H, s; Ar-CH_3), 2.21 (3H, s; O(C=O)CH_3), 2.15 (3H, s; O(C=O)CH_3), 2.14 (3H, s; O(C=O)CH_3), 2.04 ppm (3H, s; O(C=O)CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 168.79 (acetyl C=O), 168.40 (acetyl C=O), 168.31 (acetyl C=O), 167.53 (acetyl C=O), 158.20 (coumarin C=O), 153.84 (CH=N), 152.36 (Ar-C7), 143.15 (alkene C4), 141.09 (Ar-C_9), 140.62(SO_2Ar-$C_{1'}$), 132.71(SO_2Ar-$C_{4'}$), 129.49 (Ar-C_5), 128.23 (SO_2Ar-$C_{3'}$), 126.33 (SO_2Ar-$C_{2'}$), 114.91 (Ar-C_{10}), 110.45 (alkene C_3), 106.63 (Ar-C_8), 102.83 (Ar-C_6), 97.91 ($C_{1'anomer}$), 69.91 ($C_{5'}$), 68.62 ($C_{3'}$), 66.03 ($C_{2'}$), 65.03 ($C_{4'}$), 59.70 ($C_{6'}$), 19.76 (CH_3), 19.05 (CH_3), 18.79 (CH_3), 18.75 (CH_3), 18.71 ppm (CH_3); IR (neat) ν = 1739 (broad) (C=O), 1600 (s) cm^{-1} (C=N); HRMS (ESI) calcd for $C_{31}H_{35}{}^{79}BrN_3O_{14}S$: 784.1018 [M+$NH_4$]$^+$; found: 784.1008.

Synthesis of 6-bromo-7-(2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranosyl)-4-diazomethylcoumarin (Ac$_4$Gal-Bhc-diazo, 6):

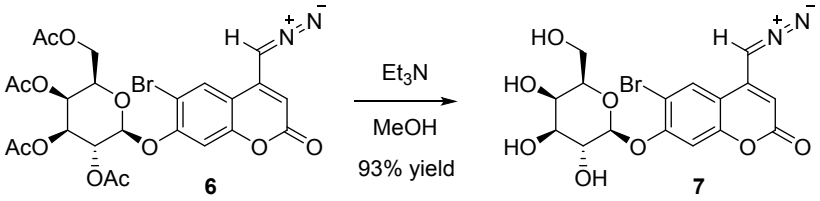


5 **6**

To a stirred solution of the crude compound **5** (1.2956 g) in CH_2Cl_2 (2 mL) was added Et_3N (139 µL, 1.00 mmol). After 2.5 h of stirring at rt, the reaction mixture was evaporated and suspended in MeOH. The precipitate was collected and dried under a vacuum to yield **6** (457.5 mg, 0.7483 mmol, 40% yield over 3 steps).

^{1}H NMR ($CDCl_3$) δ = 7.49 (1H, s; Ar-H_5), 7.15 (1H, s; Ar-H_8), 5.80 (1H, s; C=C-H), 5.60-5.66 (dd, $^3J_{H\text{-}H}$ = 10.5 and 8.0 Hz, 1H; $C_{1'}$-H_{anomer}), 5.49-5.50 (d, $^3J_{H\text{-}H}$ = 3.5 Hz, 1H), 5.25 (s, 1H, ; CH=N^+=N^-), 5.12-5.17 (dd, $^3J_{H\text{-}H}$ = 10.5 and 3.5 Hz, 1H), 5.06-5.09 (d, $^3J_{H\text{-}H}$ = 8.0 Hz, 1H), 4.21-4.24 (2H, m), 4.12-4.16 (1H, m), 2.20 (3H, s; O(C=O)CH_3), 2.16 (3H, s; O(C=O)CH_3), 2.11 (3H, s; O(C=O)CH_3), 2.03 ppm (3H, s; O(C=O)CH_3); ^{13}C NMR ($CDCl_3$) δ = 170.58 (acetyl C=O), 170.18 (acetyl C=O), 170.06 (acetyl C=O), 169.24 (acetyl C=O), 159.87 (coumarin C=O), 156.06 (Ar-C_7), 153.75 (Ar-C_4), 144.87 (Ar-C_9), 126.89 (Ar-C_5), 112.51 (Ar-C_{10}), 107.97 (alkene C3), 105.30 (Ar-C_8), 99.97 (Ar-C_6), 99.68 ($C^{1'}{}_{anomer}$), 71.93 ($C_{5'}$), 70.51 ($C_{3'}$), 67.86 ($C_{2'}$), 66.85 ($C_{4'}$), 61.56 ($C_{6'}$), 45.97 (CH=N^+=N^-), 20.87 (CH_3), 20.71 (CH_3), 20.64 (CH_3), 20.57 ppm (CH_3); IR (neat) ν = 2081 (C=N^+=N^-), 1747 (C=O), 1727 (vs) (C=O), 1715 (C=O), 1693 (C=O).1592 cm^{-1} (C=N)

Synthesis of 6-bromo-7-β-D-galactopyranosyl-4-diazomethylcoumarin (Gal-Bhc- diazo, 7):

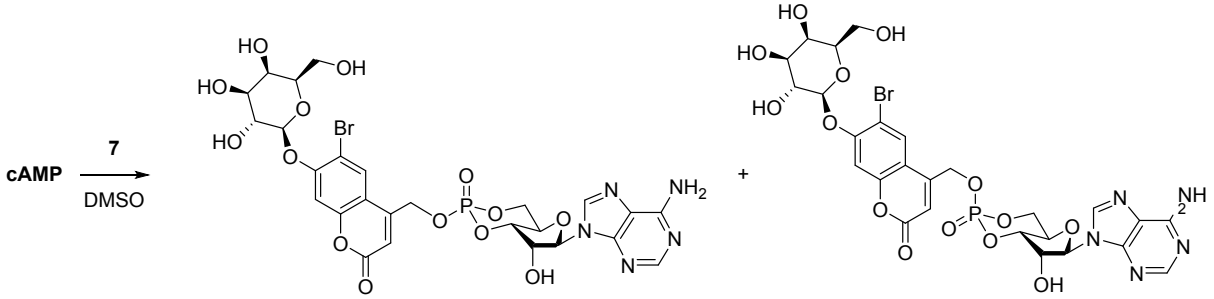


To a stirred suspension of **6** (49.2 mg, 80.5 µmol) in MeOH (1.0 mL) was added Et_3N (223 µL, 1.61 mmol). After 3.5 h and 7 h of stirring at 60 °C, 223 µL of Et_3N (1.61 mmol) was added to the reaction mixture one by one. After further stirring at 60 °C for 5 h, the reaction mixture was evaporated and suspended in CH_2Cl_2. The precipitate was collected by filtration, washed with CH_2Cl_2 and dried under vacuum to give **7** (33.3 mg, 75.1 µmol, 93%) as a solid

^{1}H NMR (300 MHz, [D_6]DMSO) δ = 8.06 (1H, s; Ar-H_5), 7.23 (1H, s; Ar-H_8), 6.66 (1H, s; C=C-H), 5.82 (1H, s; CH=N^+=N^-), 5.17-5.19 (d, $^3J_{H\text{-}H}$ = 5.5 Hz, 1H), 5.11-5.14 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1H; $C_{1'}$-H_{anomer}), 4.91-4.93 (d, $^3J_{H\text{-}H}$ = 5.9 Hz, 1H), 4.67-4.71 (dd, $^3J_{H\text{-}H}$ = 5.5 and 5.5 Hz, 1H), 4.61-4.62 (d, $^3J_{H\text{-}H}$ = 4.6 Hz, 1H), 3.41-3.72 ppm (6H, m); ^{13}C NMR (75 MHz, [D_6]DMSO) δ = 159.75 (coumarin C=O), 156.64 (Ar-C_7), 153.91 (alkene C_4), 146.78 (Ar-C_9), 128.04 (Ar-C_5), 111.60 (Ar-C_{10}), 107.29 (alkene C_3), 104.13 (Ar-C_8), 101.01 (Ar-C_5), 98.25 ($C^{1'}{}_{anomer}$), 76.25 ($C_{5'}$), 73.86 ($C_{3'}$), 70.39 ($C_{2'}$), 68.51 ($C_{4'}$), 60.78 ($C_{6'}$), 46.36 ppm (CH=N^+=N^-); IR (neat) ν = 3600-3100 (OH), 2101 (C=N^+=N^-), 1679 (C=O),1602 (C=N) cm^{-1}.

Synthesis of Gal-Bhc-cNMPs

Synthesis of 6-bromo-7-β-D-galactopyranosyl-4-ylmethyl adenosine cyclic 3',5'-monophosphate (Gal-Bhc-cAMP, 8)

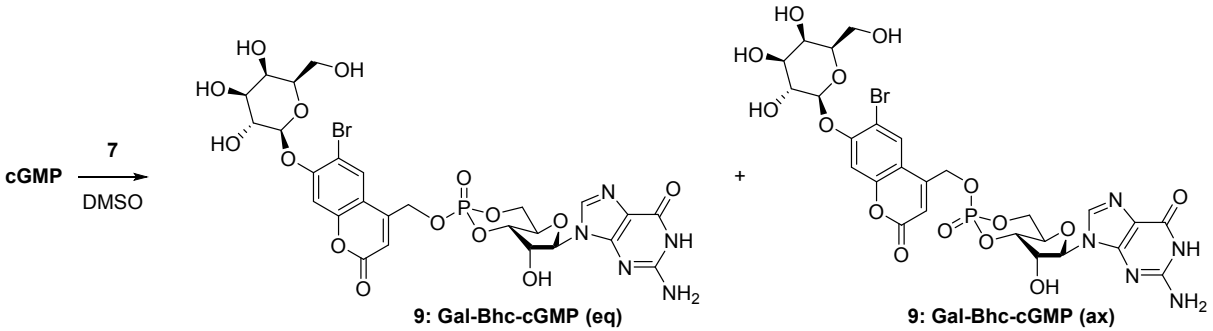


8: Gal-Bhc-cAMP (eq) **8: Gal-Bhc-cAMP (ax)**

To a stirred suspension of cAMP (142.2 mg, 0.4321 mmol) in DMSO (1 mL) was added **7** (191.5 mg, 0.4321 mmol). The reaction mixture was stirred at 50 °C for 4 h under an argon atmosphere. After cooling to ambient temperature, the mixture was centrifuged (4000 g for 5 min) to remove precipitated cAMP. Since substantial amount of **16** remained unreacted, the supernatant was reacted with another portion of cAMP (84.6 mg, 0.257 mmol) in DMSO (3 mL) and stirring was continued at 50 °C for 6.5 h. After the reaction mixture was centrifuged at 4000 g for 5 min, the supernatant was evaporated and the residue was purified by semi-preparative reversed-phase HPLC (column: COSMOSIL 5C18 AR-Ⅱ, 250×20, eluent: 20% ACN/H_2O) to yield **8** (15.7 mg, 21.1 μmol, 6.6% yield) as a stereo-isomeric mixture.

^{1}H NMR (300 MHz, [D_6]DMSO) (axial isomer) δ = 8.35 (1H, s), 8.13 (1H, s), 8.04 (1H, s), 7.38 (2H, broad), 7.33 (1H, s), 6.55 (1H, s), 6.36-6.42 (1H, m), 6.08-6.09 (1H, m), 5.34-5.53 (3H, m), 5.21-5.25 (1H, m), 5.15-5.18 (1H, m), 4.94-4.96 (1H, m), 4.45-4.80 (6H, m), 3.45-3.76 (6H, m); (equatorial isomer) δ = 8.40 (1H, s), 8.20 (1H, s), 8.00 (1H, s), 7.38 (2H, broad), 7.34 (1H, s), 6.44 (1H, s), 6.36-6.42 (1H, m), 6.08-6.09 (1H, m), 5.34-5.53 (3H, m), 5.21-5.25 (1H, m), 5.15-5.18 (1H, m), 4.94-4.96 (1H, m), 4.45-4.80 (6H, m), 3.45-3.76 (6H, m); ^{13}C NMR (75 MHz, [D_6]DMSO) (axial isomer) δ = 160.05, 156.63, 154.15, 153.36, 149.53, 149.16, 140.67, 128.93, 119.43, 112.20, 110.42, 107.75, 104.18, 101.06, 92.46, 79.29, 78.38, 76.30, 73.91, 71.73, 70.42, 70.05, 68.54, 65.62, 64.39, 60.80; (equatorial isomer) δ 159.96, 156.63, 154.21, 153.44, 150.08, 149.22, 140.47, 128.93, 119.64, 112.25, 111.13, 107.70, 104.18, 101.06, 92.19, 79.29, 78.38, 76.30, 73.91, 71.84, 70.42, 70.05, 68.54, 65.62, 64.39, 60.80; IR (neat) (mixture of two stereoisomers) ν = 3600-3100 (OH), 1729 (C=O), 1651, 1604, 1271 (P=O) cm^{-1}; UV/Vis (KMops, pH 7.4): λ_{max} (ε) 260 (11000), 327 (7900 mol^{-1}dm^{3}cm^{-1}) nm; HRMS (ESI) calcd for $C_{26}H_{28}{}^{79}BrN_5O_{14}P$: 744.0548 $[M+H]^+$; found: 744.0535.

Synthesis of 6-bromo-7-β-D-galactopyranosyl-4-ylmethyl guanosine cyclic 3',5'-monophosphate, (Gal-Bhc-cGMP, 9)



To a stirred suspension of cGMP (115.6 mg, 0.335 mmol) in DMSO (1 mL) was added **7** (148.4 mg, 0.335 mmol). The reaction mixture was stirred at 50 °C for 7 h under an argon atmosphere. After the reaction mixture was centrifuged at 4000 g for 5 min, the supernatant was evaporated and the residue was purified by semi-preparative reversed-phase HPLC (column: COSMOSIL 5C18 AR-Ⅱ, 250×20, eluent: 15% ACN/H_2O) to yield **9** (10.4 mg, 13.7 μmol, 4.1% yield) as a stereoisomeric mixture.

^{1}H NMR (300 MHz, [D_6]DMSO) (mixture of two stereoisomers) δ 10.7 (1H, broad), 7.95-8.04 (2H, m), 7.32 (1H, s), 6.39-6.59 (3H, m), 6.26-6.28 (1H, m), 5.86 (1H, s), 5.43-5.51 (2H, m), 5.15-5.23 (3H, m), 4.94-4.96 (1H, m), 4.50-4.72 (6H, m), 3.45-3.73 (6H, m); ^{13}C NMR (300 MHz, [D_6]DMSO) (axial isomer) δ 160.04, 157.15, 156.60, 154.20, 151.16, 149.64, 136.37, 128.85, 117.04, 112.27, 110.75, 107.71, 104.17, 101.06, 91.46, 79.42, 78.54, 76.30, 73.91, 71.77, 70.43, 69.81, 68.54, 65.38, 64.38, 60.80; (equatorial isomer) δ 159.98, 157.15, 156.60, 154.36, 151.09, 149.73, 136.69, 128.85, 117.17, 112.27, 111.11, 107.71, 104.17, 101.06, 91.87, 79.64, 78.54, 76.30, 73.91, 71.77, 70.43, 69.81, 68.54, 65.38, 64.38, 60.80; IR (neat) (mixture of two stereoisomers) ν = 3500-3100 (OH), 1729 (C=O), 1693, 1640, 1271 (P=O) cm^{-1}; λ_{max} (ε) 278 (6900), 328 (6400 mol^{-1}dm^{3}cm^{-1}) nm; HRMS (ESI) calcd for $C_{26}H_{28}{}^{79}BrN_5O_{15}P$: 760.0497 $[M+H]^+$; found: 760.0485.

Measurements of Photophysical and Chemical Parameters

All stock solutions of the samples were prepared in DMSO at concentration of 10 mM, then diluted with K-MOPS buffer (10 mM, pH 7.2). The final concentrations of samples were 10 μM. Absorption spectra shown in Fig. S1 were recorded in a quartz cuvette (10 × 10 mm) by using a UV–vis spectrophotometer (Shimadzu UV-2600). Photolysis time courses and stability in the dark were monitored with a HPLC (Chromaster 5110 Pump with 5430 Diode Array Detector, Hitachi, L-column 2 ODS, 3 μm (4.6 x 150 mm)). A 300 W xenon lamp (Asahi Spectra MAX-301) with a band pass filter (405 nm) was used for photolysis experiments. Aliquots of the photolysis mixtures were analyzed after the specified irradiation time by HPLC. For 405-nm photolysis of Bhc-cNMPs, the aliquots were analyzed with linear gradient of 2–15–27% (2–3.5–13 min) acetonitrile in water (0.1% TFA) and monitored at 260 nm. The light output for the quantum efficiencies measurement was performed using ferrioxalate actinometry.[2] Quantum yields of photolysis were calculated from the plot shown in Figure 3 following the method described in our previous report.[3]

Enzymatic reactions

Kinetic parameters measurements: Kinetic parameters of Gal-Bhc-cAMP toward β-Galactosidase were determined by monitoring the formation of Bhc-cAMP at 380 nm. The enzyme reactions were initiated by adding 7.3×10^{-4} U of β-galactosidase (Sigma G4155-1KU) to the 100 μL of reaction solution containing 110 mM 2-mercoptoethanol, 1 mM $MgCl_2$ and various concentrations of Gal-Bhc-cAMP (ranging from 0 to 750 μM) in 100 mM phosphate buffer (pH 7.4). The final concentration of the enzyme was determined to be 2.08 nM from the Bradford assay. The formation of Bhc-cAMP was continuously monitored for 1200 s at 37 °C by setting the cuvette (containing 100 μL of the reaction mixture) to a UV-vis spectrophotometer (Fig. S3a). The measurement was repeated for several times. Data points from 0 s to 100 s can be approximated by a linear equation. The initial velocity (V_0) of the reactions were calculated from the slopes of the plots shown in Fig S3b. Kinetic parameters K_m and V_{max} for the reaction of Gal-Bhc-cAMP and β-Gal were determined directly from the Michaelis-Menten plots following the equation: $V_0 = (V_{max}*[S])/(K_m+[S])$ and least squares method toward V_0 plots (Fig. 4a). The estimated kinetic parameters were K_m 361 μM, V_{max} 4.36×10^{-7} mol s^{-1}, k_{cat} 210 s^{-1} and k_{cat}/K_m 5.81×10^{-5} M^{-1} s^{-1}.

Cell lysates preparation: HEK293T cell was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, high glucose, Nacalai, Japan) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified 5% CO_2 incubator. To prepare cell lysates of β-Gal-expressing cells, HEK293T cells (passage number 12) in a 100-mm dish at 80% confluence were transfected with pSV-β-Galactosidase control vector (6 μg, Promega E1081). Transfections were performed with PEI (18 μg). After incubated at 37 °C for 19 h, cells were washed once with PBS (-), lysed with 1× lysis buffer (1 mL) by tapping the dish. Then the lysed cells were transferred into 1.5 mL microcentrifuge tubes, centrifuged at 13000 rpm at 4 °C for 10 min, the supernatants were used

as cell lysates. The cell lysates of β-Gal-expressing cells and control cells were stored at -80 °C, and thawed on ice before use.

Enzymatic reactions in the presence of β−Galactosidase expressing cell lysates: To the lysates (99 μL) was added a 10 mM stock solution of Gal-Bhc-cNMPs in DMSO (1 μL), then the solution was transferred into a micro cuvette, and incubated at 37 °C. Aliquots of the reaction mixture (10 μL) was transferred into an insert vial containing a 90 μL of K-MOPS buffer (10 mM, pH 7.2) once every hour. Enzymatic reactions were monitored by HPLC using the same conditions as described for the photolysis of caged cNMPs except for the following elution conditions: linear gradient of 1–13–20% (1.5–2.5, 7.5-8.5 min) for Gal-Bhc-cAMP and 2–13–22% (1.5–2.5, 7.5-8.5 min) acetonitrile in water (0.1% TFA) for Gal-Bhc-cGMP. Photolysis was done by using a 300 W xenon lamp (Asahi Spectra MAX-301) with a band pass filter (405 nm). Samples in the insert vials were directly irradiated and analyzed by HPLC.

Live cell experiments

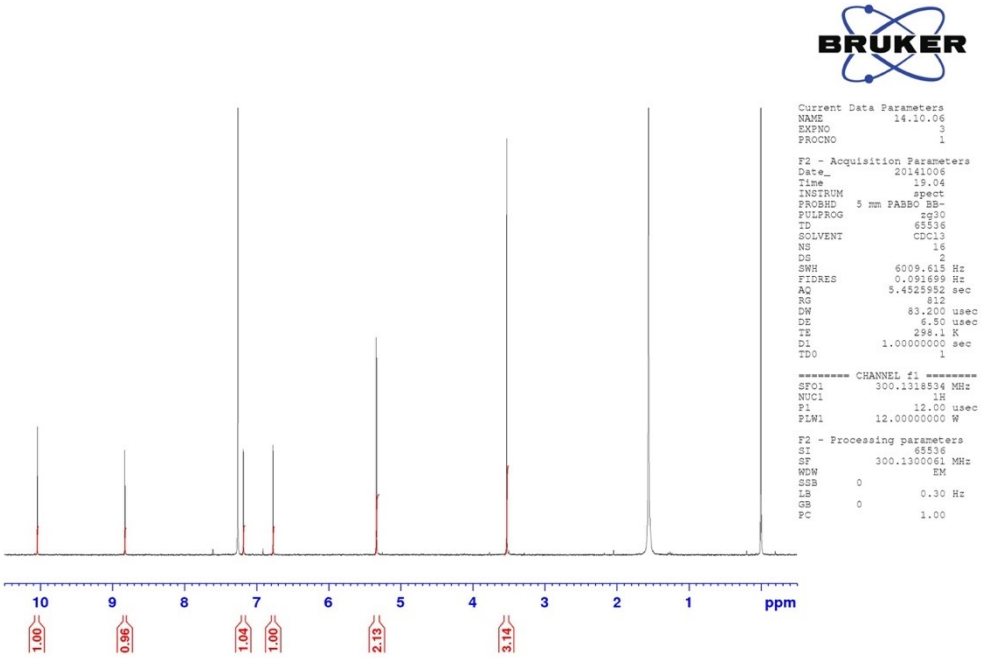
HeLa cell was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Cells were cultured at 37 °C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM, low glucose, Nacalai, Japan) supplemented with 10% fetal bovine serum (FBS). Cells were trypsinized, resuspended in DMEM and transferred into 35-mm glass-bottom dishes (3910-035, IWAKI, Japan) at an approximately 40% confluency in a volume of 2 mL. After 24 hr of growth, the media were changed to 2 mL/well of a reduced serum media Opti-MEM (GIBCO, USA). The cells were transfected with plasmid DNAs coding for a red fluorescent cAMP indicator (R-FlincA) (1.0 μg) and *E. coli* β-galactosidase (pSV-β-Galactosidase control vector) (3.6 μg) using Lipofectamine 2000 (Thermo Fisher Scientific, USA) as a lipofection reagent. The cells were cultured at 37 °C for an additional 24 hr, treated with 100 μM of Gal-Bhc-cAMP and incubated at 37 °C for 24 hr. The medium was aspirated and the cells were rinsed with 1 mL of a prewarmed Opti-MEM twice. After the addition of 1mL of Opti-MEM and IBMX (final 100 μM), the cells were placed at 25 °C for 15 min. Fluorescent images of the cells were taken every 5 seconds for 15 min using a laser scanning fluorescent confocal microscopy (FV 1200/IX 81, Olympus). Using the same 405 nm light source as above, the cells were exposed to 405 nm light for 30 s and treated with 25 μM forskolin 5 minutes after the irradiation. After measuring R-FlincA fluorescence, the cells were treated with a membrane-permeable fluorescent β-Gal indicator FDG-C12 (20 μM). The cells that responded to forskolin are marked as "Forskolin (+)". The cells that showed green fluorescence were marked as "FDG (+)".

Supplementary References

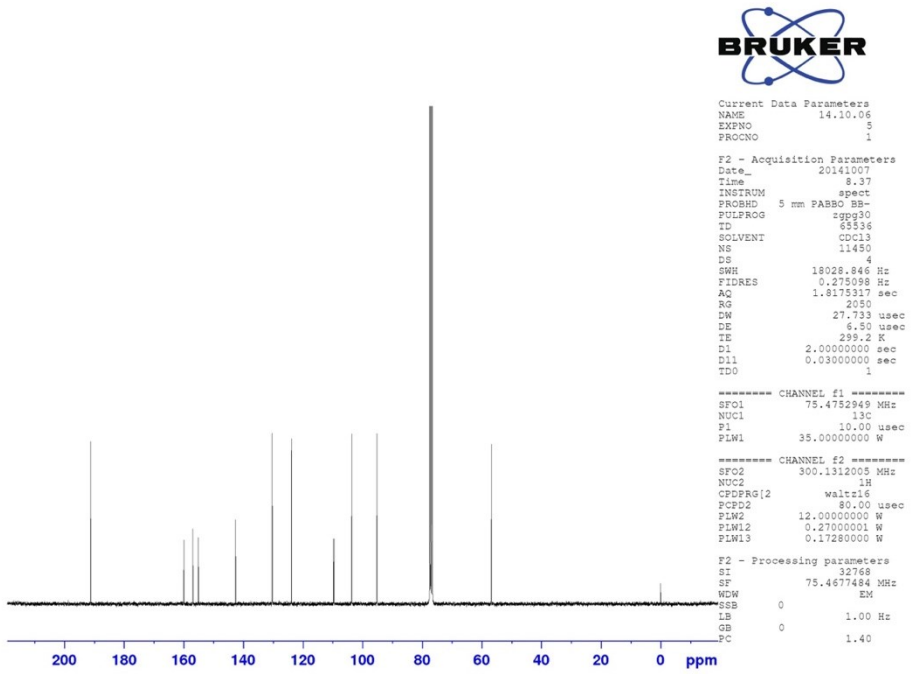
[1] A. Z. Suzuki, T. Watanabe, M. Kawamoto, K. Nishiyama, H. Yamashita, M. Ishii, M. Iwamura and T. Furuta, *Org. Lett.*, **2003**, *5*, 4867-4870.

[2] C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London Ser. A* **1956**, *235*, 518.

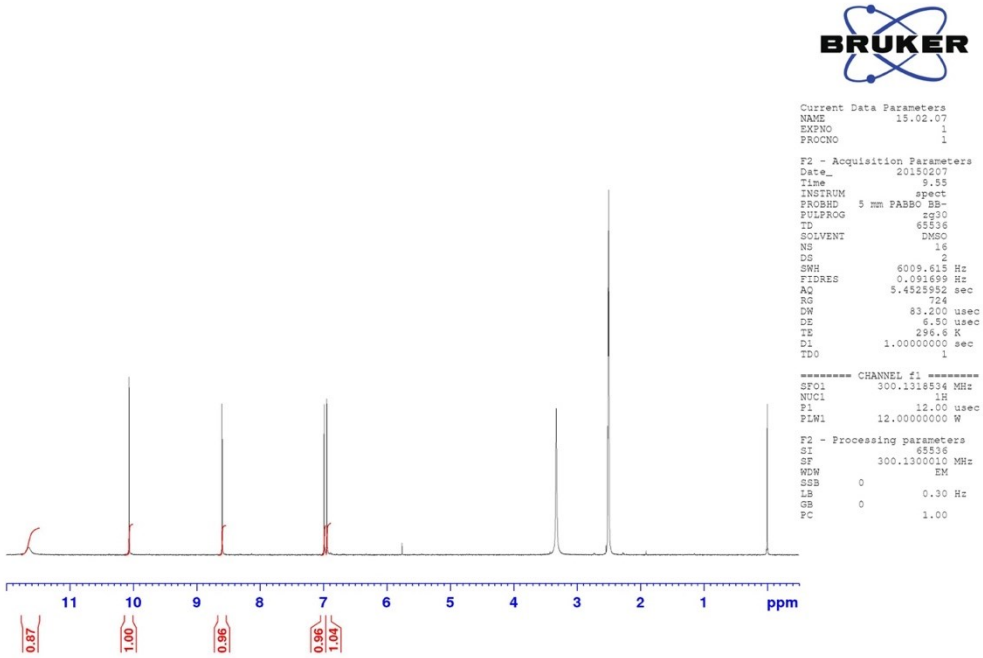
[3] T. Furuta, H. Takeuchi, M. Isozaki, Y. Takahashi, M. Kanehara, M. Sugimoto, T. Watanabe, K. Noguchi, T. M. Dore, T. Kurahashi, M. Iwamura and R. Y. Tsien, *ChemBioChem*, **2004**, *5*, 1119-1128.



^{1}H NMR spectrum of **2**



^{13}C NMR spectrum of **2**



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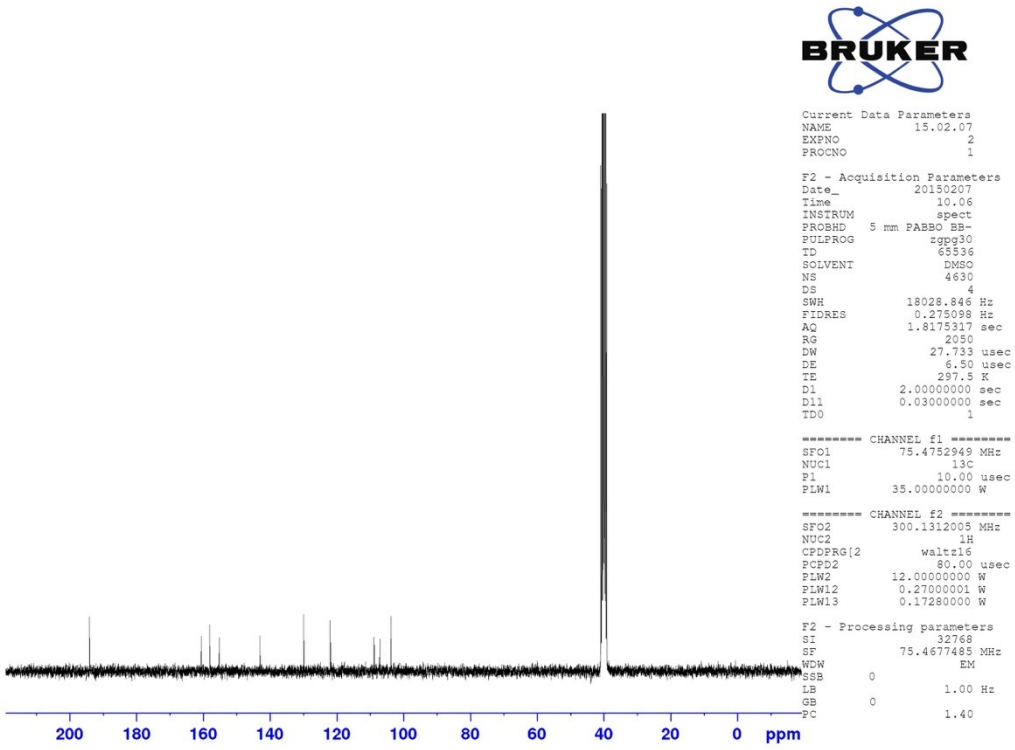

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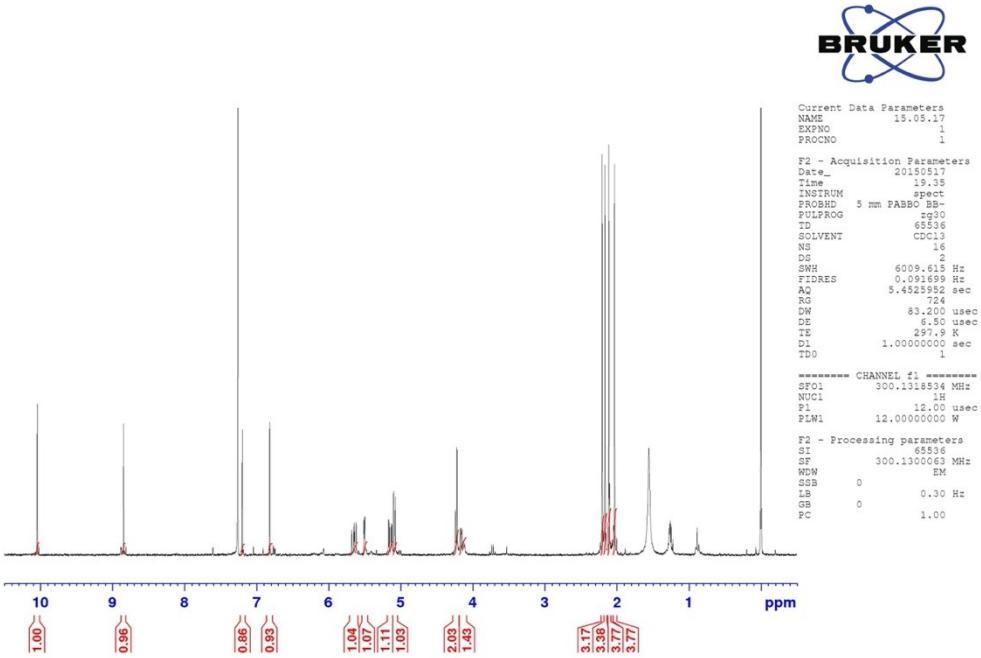

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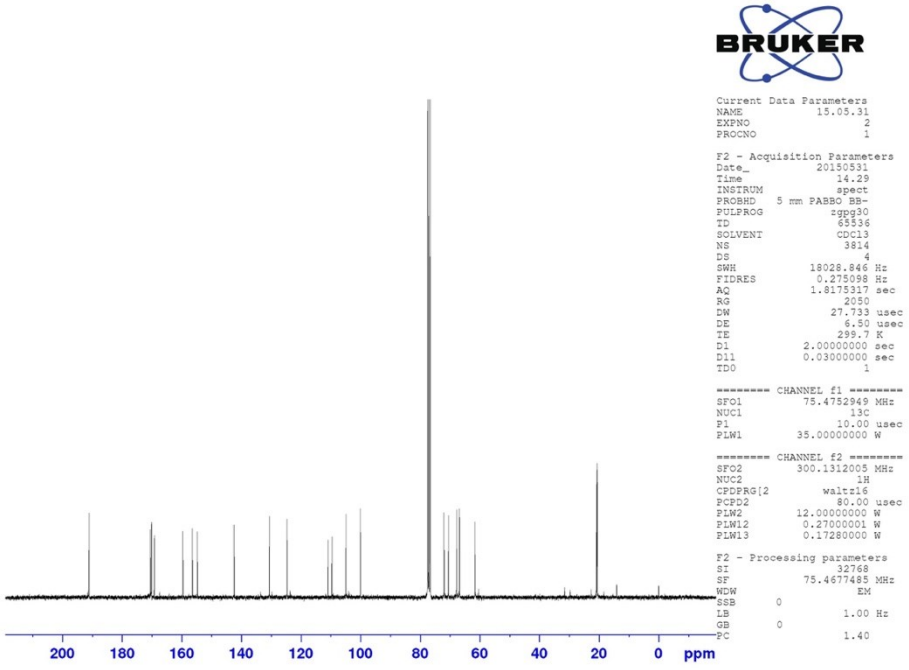

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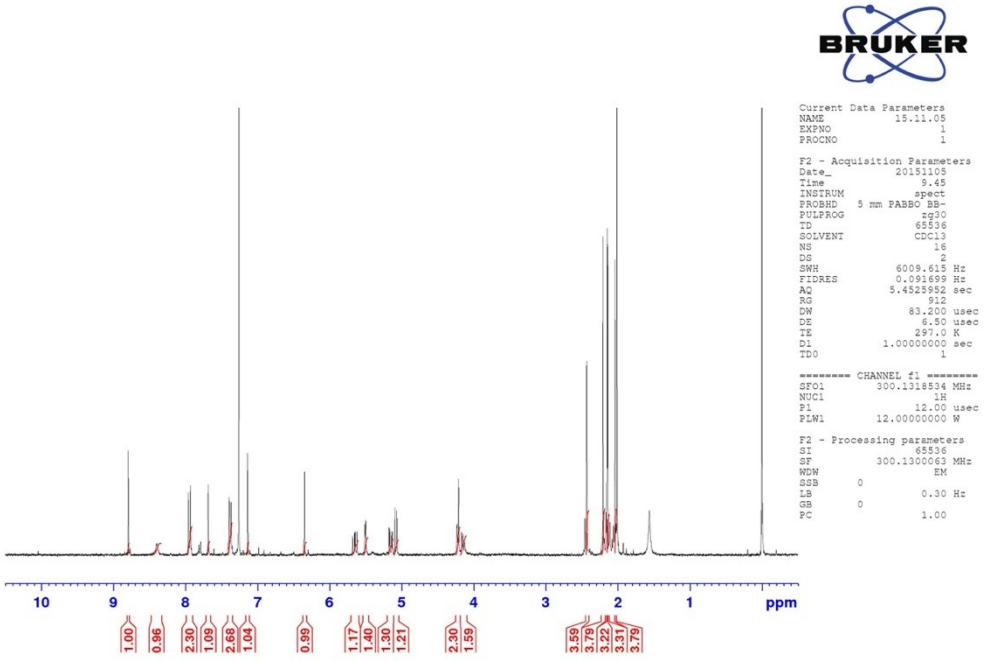

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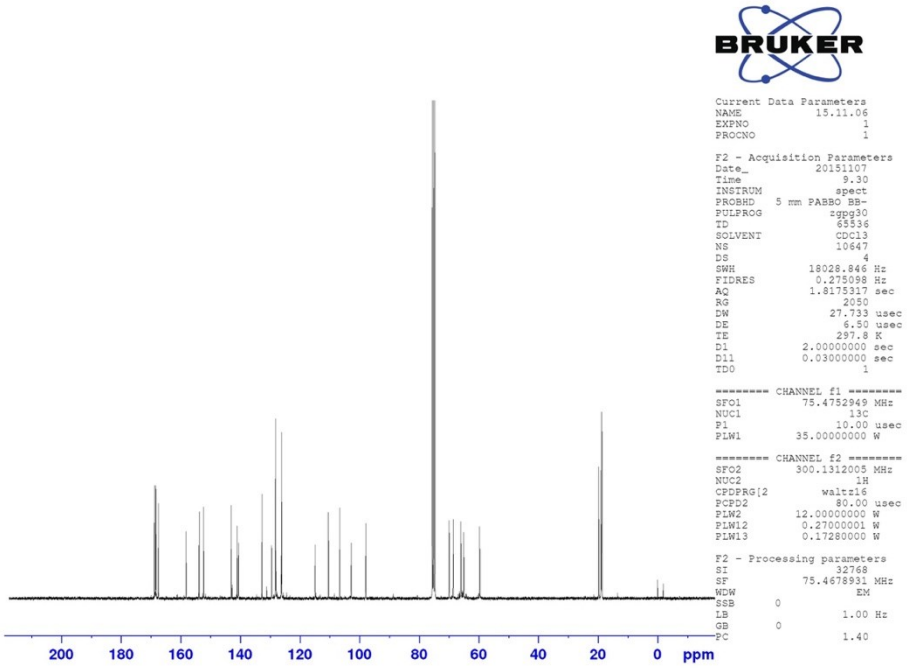
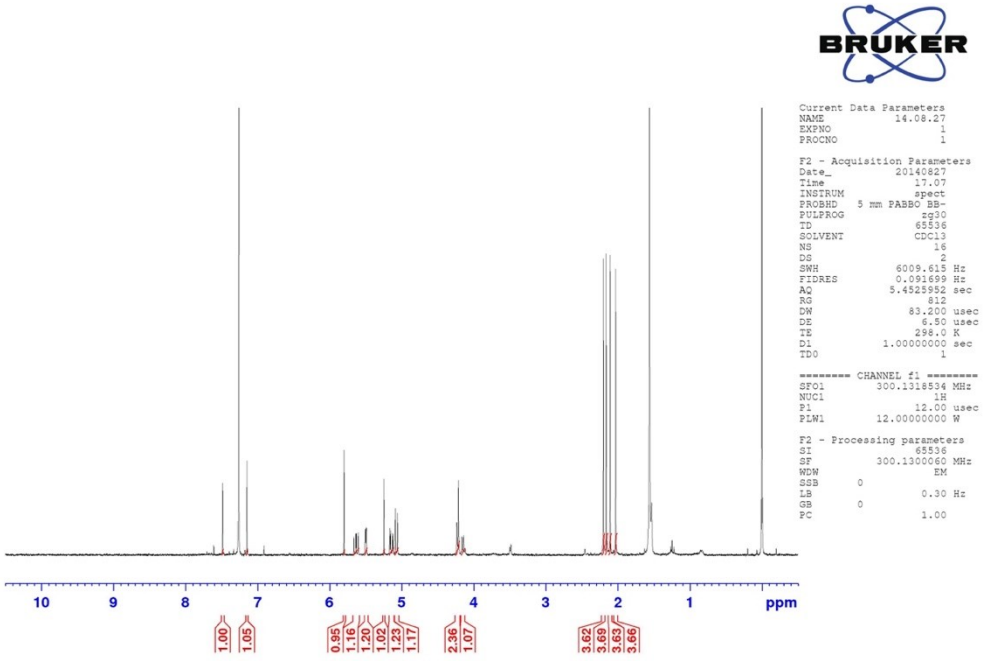


^{13}C NMR spectrum of **5**



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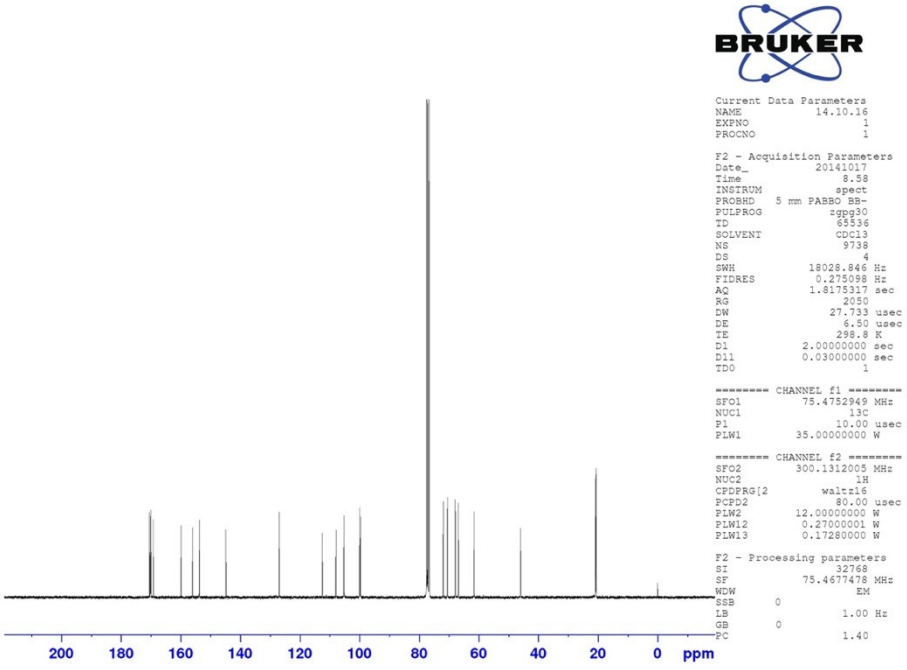
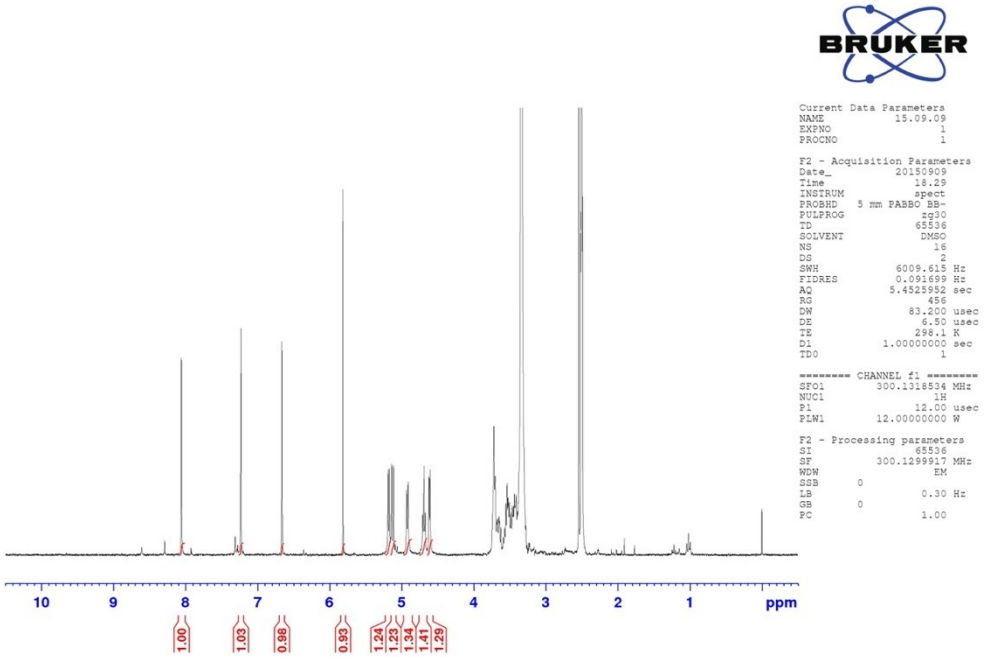


^{13}C NMR spectrum of **6**



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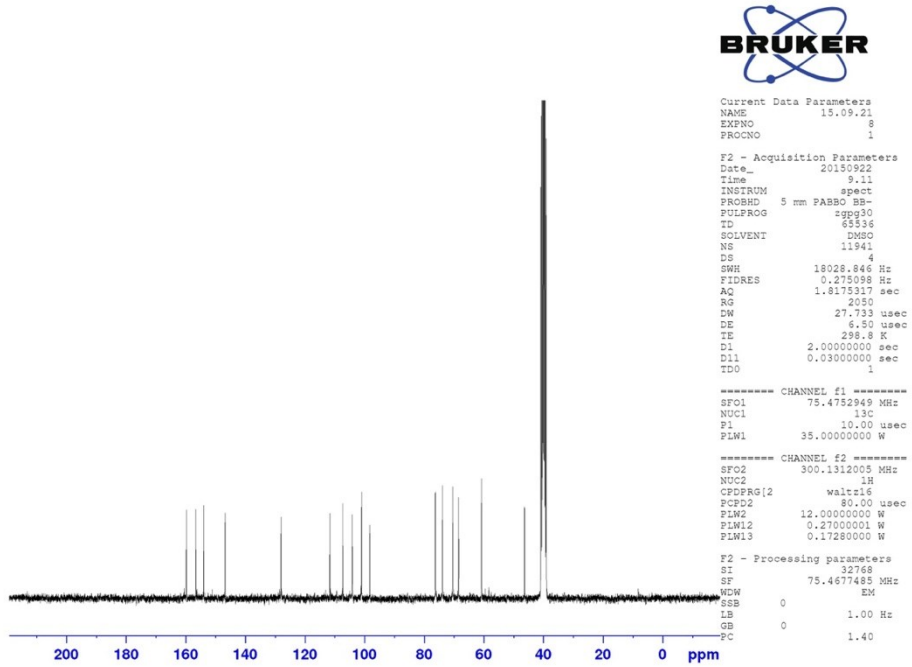

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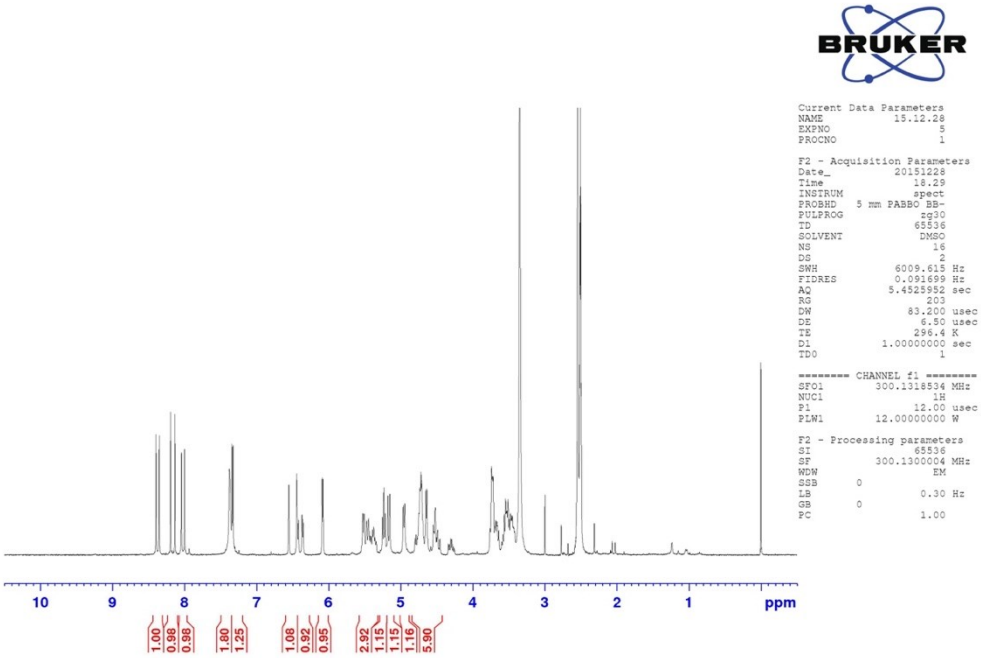

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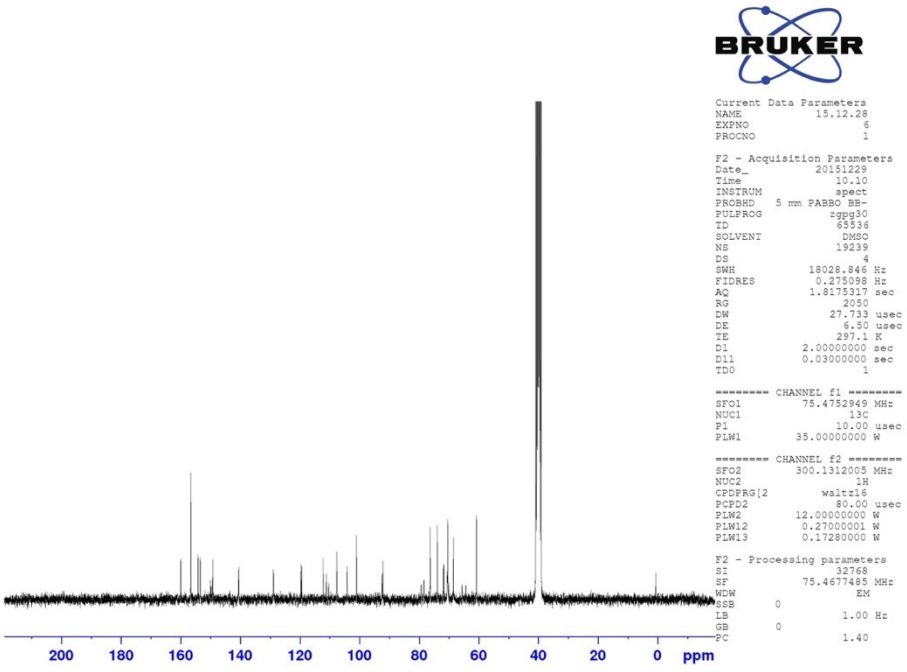

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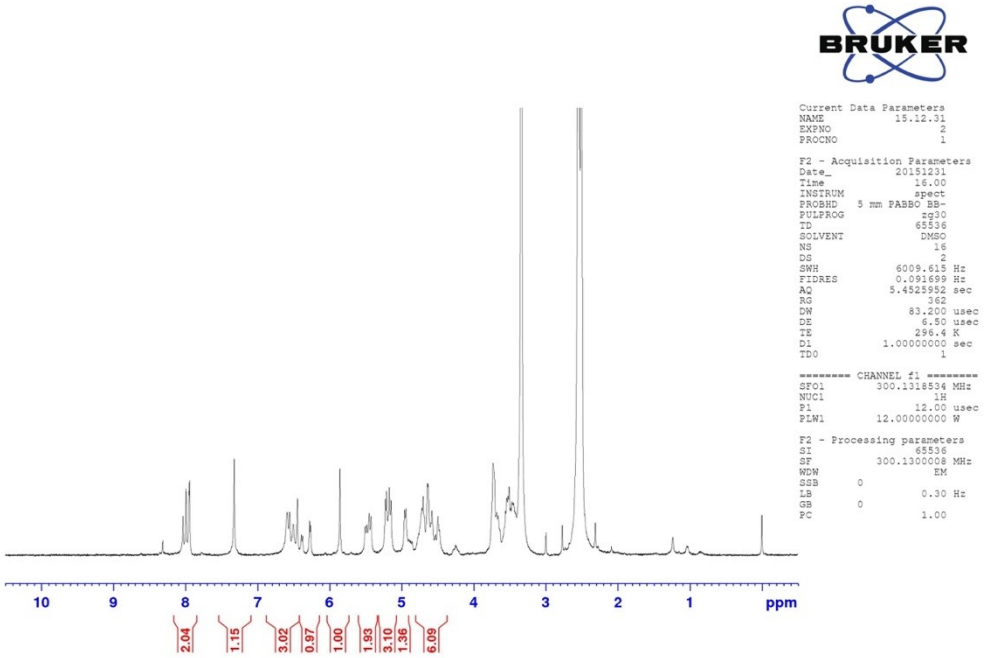

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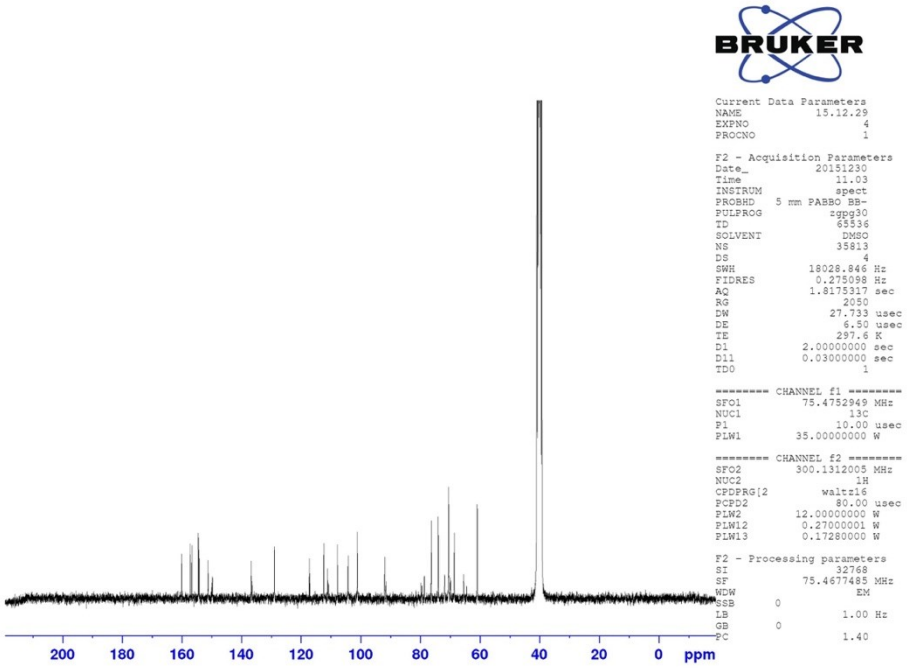

^{13}C NMR spectrum of **9**