Electronic supporting information for

Synthesis of nucleobase-caged peptide nucleic acids having improved photochemical properties

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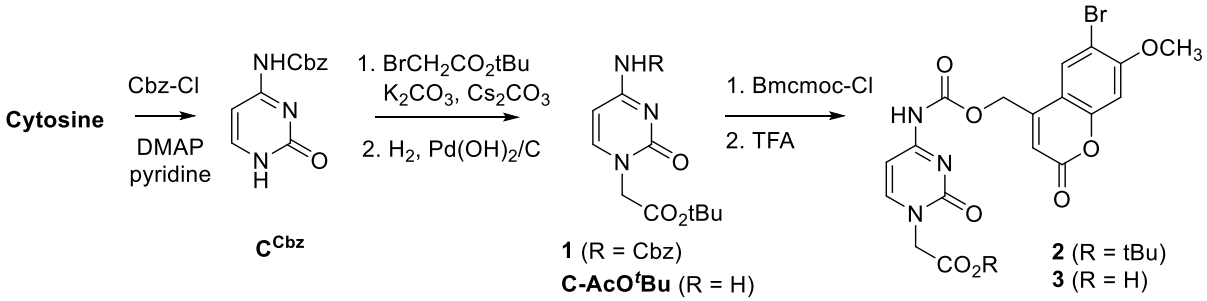
Synthesis

All reagents and solvents were purchased from commercial sources and were used without further purification. Flash column chromatography was conducted using 43–60 mesh silica gel. NMR spectra were recorded on GSX270 (JEOL) at 270 MHz for ^{1}H and at 67 MHz for ^{13}C and Avance 300M Biospin (Bruker Analytik GmbH) at 300 MHz for ^{1}H and 75 MHz for ^{13}C with a deuterated solvent and TMS as an internal standard. IR spectra were recorded Avatar 320 (Thermo Nicolet) in ATR mode. Analytical HPLC was conducted HP 1100 (Agilent) with DAD detection and preparative HPLC (PU 9800; Jasco Corp.) with UV detection. HRMS spectra were recorded on Exactive Plus (Thermo Fisher Scientific) or JMS-700 (JEOL).

Synthesis of C^{Bmcmoc}-AcOH (3)



4-N-(Benzyloxycarbonyl)cytosine (C^{Cbz})

The compound was synthesized using the reported procedure.[1)]

^{1}H NMR (DMSO-d$_6$) δ 11.20 (1H, brs, NH), 7.79 (1H, d, J=6.8 Hz, H6), 7.33–7.39 (5H, m), 6.92 (1H, d, J = 6.8 Hz, H5), 5.17 (2H, s)

^{13}C NMR (DMSO-d$_6$) δ 163.19, 155.76, 153.38, 146.71, 136.10, 128.46, 128.10, 127.89, 93.48, 66.37

FT-IR (neat) ν 2799, 1743, 1689, 1632, 1589, 1514, 1473, 1320, 1232, 1207, 1180, 1077, 1006, 808, 743, 696 cm^{-1}

***tert*-Butyl [4-N-(benzyloxycarbonyl)cytosine-1-yl]acetate (1)**

The compound was synthesized using the reported procedure.[2)]

To a stirred solution of the 4-N-(benzyloxycarbonyl)cytosine (1.276 g, 5.20 mmol) in dehydrated DMF (16 mL) was added K_2CO_3 (730.1 mg, 5.28 mmol) and Cs_2CO_3 (176.9 mg, 0.54 mmol). The mixture was stirred for 30 min at ambient temperature under Ar atmosphere. To this was added *tert*-butyl bromoacetate (764 μL, 5.20 mmol). The stirring was continued at an ambient temperature for 19 h. The reaction mixture was diluted with methanol (6 mL). Then the solvents were removed by evaporation. The residual material was dissolved in dichloromethane and washed with water. The organic layer was dried over Na_2SO_4 and concentrated to give crude **1**. To the crude product were added ethyl acetate and n-hexane. The resulted precipitates were collected using vacuum filtration to give **1** (1.259 g, 3.50 mmol, 67.4% yield).

^{1}H NMR ($CDCl_3$) δ 7.51 (1H, d, J=7.3 Hz, H6), 7.38 (5H, m), 7.24 (1H, d, J = 7.3 Hz, H5), 5.22 (2H, s), 4.51 (2H, s, -CH_2CO_2-), 1.48 (9H, s)

^{13}C NMR ($CDCl_3$) δ 166.43, 163.06, 155.45, 153.05, 148.42, 135.13, 128.61, 128.53, 128.29, 95.72, 83.26, 67.79, 51.14, 27.63

FT-IR (neat) ν 2995, 1745, 1665, 1614, 1556, 1504, 1379, 1369, 1352, 1228, 1150, 804, 786, 739 cm^{-1}

***tert*-Butyl (cytosin-1-yl)acetate (C-AcO^{t}Bu)**

In a 30-mL two-necked flask **1** (520.4 mg, 1.448 mmol) and $Pd(OH)_2$/C (105.0 mg, 20% w/w) were placed. The mixture was purged with hydrogen gas. To this was added ethanol (9 mL). Then the reaction mixture was stirred at an ambient temperature for 1 h. The catalyst was removed by filtration and the filtrate was concentrated under vacuum to give **C-AcO^{t}Bu** (305.7 mg, 1.448 mmol, 100% yield).

^{1}H NMR (CD_3OD) δ 7.50 (1H, d, J=7.5 Hz, H6), 5.85 (1H, d, J = 7.5 Hz, H5), 4.40 (2H, s, -CH_2CO_2-), 1.47 (9H, s)

^{13}C NMR ($CDCl_3$) δ 167.85, 166.33, 155.76, 146.39, 93.33, 81.15, 50.45, 27.69

FT-IR (neat) ν 3354, 3136, 1741, 1663, 1617, 1487, 1416, 1383, 1236, 1155, 790 cm^{-1}

***tert*-Butyl 4-*N*-[(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl]-cytosin-1-yl acetate (C^{Bmcmoc}-AcO^{t}Bu (2))**

In a 50-mL round-bottomed flask was placed **C-AcO^{t}Bu** (461.0 mg, 2.05 mmol), which was dried by azeotropic removal of water with toluene. To the flask were added Bmcmoc-Cl (639.7 mg, 1.86 mmol), N,N-dimethyl-4-aminopyridine (245.3 mg, 2.05 mmol) and CH_2Cl_2 (9 mL). The reaction mixture was stirred at an ambient temperature for 4 h under Ar atmosphere and was quenched by 0.5 M citric acid (9 mL). The precipitates were collected by centrifugation (4,000 rpm, 5 min), washed with methanol (3 mL), and then dried under vacuum to yield **C^{Bmcmoc}-AcO^{t}Bu** (**2**). The product was used for the next reaction without further purification.

^{1}H NMR (DMSO-d_6) δ 11.11 (1H, brs, NH), 8.07 (1H, d, J=6.8 Hz, H6), 7.98 (1H, s), 7.26 (1H, s, H8 Bmcmoc), 7.03 (1H, d, J=6.8 Hz, H5), 6.51 (1H, s, H3 Bmcmoc), 5.47 (2H, s), 4.52 (2H, s, -CH_2CO_2), 3.96 (3H, s), 1.42 (9H, s)

^{13}C NMR (DMSO-d_6) δ 166.93, 163.21, 159.60, 158.16, 154.87, 154.12, 152.35, 150.59, 149.64, 128.21, 111.09, 109.59, 106.73, 101.00, 93.89, 81.71, 62.06, 57.14, 51.14, 27.63

FT-IR (neat) 1752, 1740, 1723, 1657, 1604, 1488, 1360, 1348, 1274, 1240, 1194, 1153, 1095, 1053, 910, 898, 792 cm^{-1}

HRMS (ESI^+) Calcd for $C_{22}H_{23}N_3O_8Br^+$: 536.0663 Found: 536.0661

4-*N*-[(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl]-cytosin-1-yl acetic acid (C^{Bmcmoc}-AcOH (3))

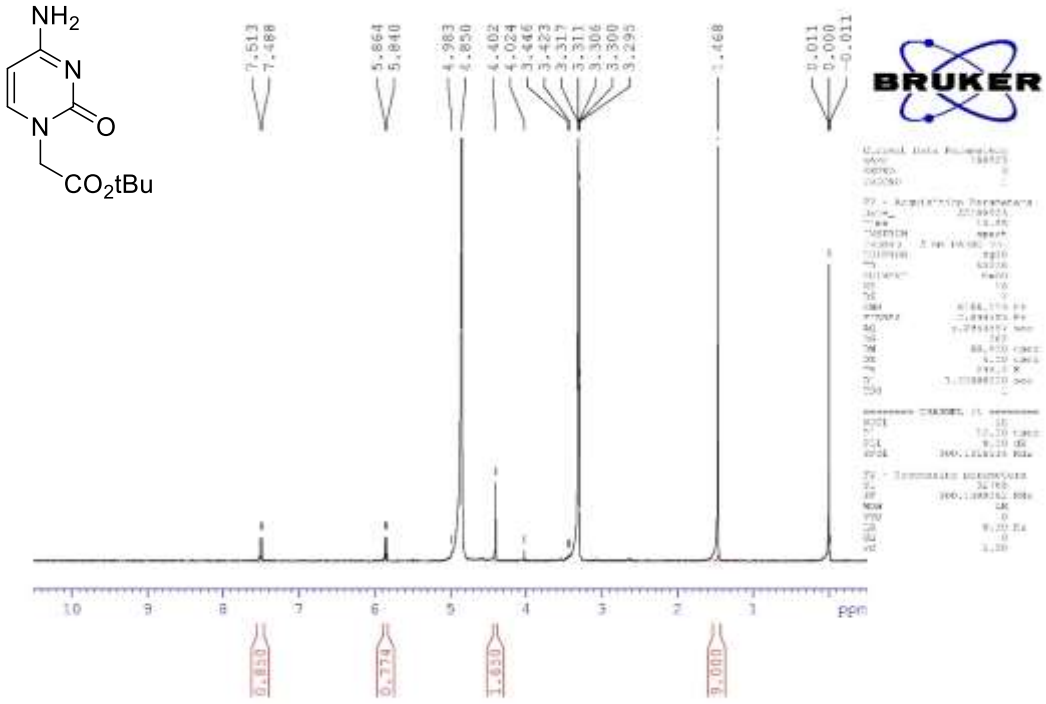
To the crude product **2** were added TFA (6 mL) and CH_2Cl_2 (6 mL). The solution was stirred at ambient temperature for 3 h. Solvents were removed using vacuum evaporation. Traces of solvents were removed azeotropically with toluene. The residue was resuspended in chloroform. The precipitate was collected using vacuum filtration to give **C^{Bmcmoc}-AcOH** (**3**) (410 mg, 1.19 mmol, 64% yield).

^1H NMR (DMSO-d_6) δ 8.06 (1H, d, J=7.3 Hz, H6), 7.98 (1H, s, H5 Bmcmoc), 7.25 (1H, s, H8 Bmcmoc), 7.01 (1H, d, J=7.3 Hz, H5), 6.50 (1H, s, H3 Bmcmoc), 5.47 (2H, s), 4.54 (2H, s, -CH_2CO_2), 3.96 (3H, s)

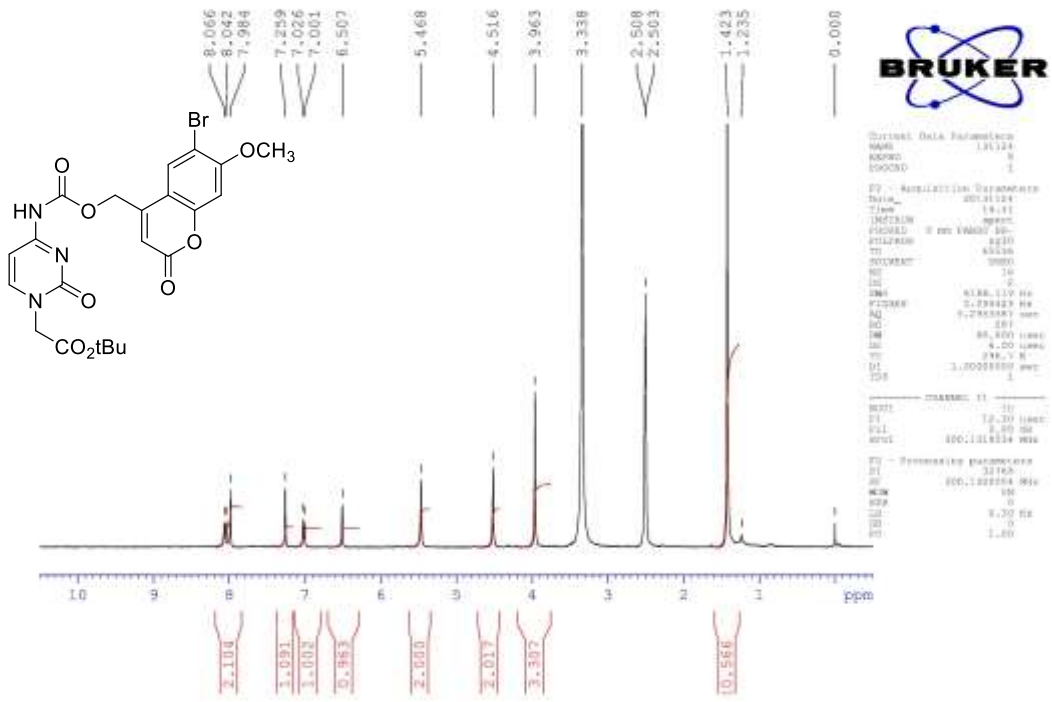
^{13}C NMR (DMSO-d_6) δ 169.32, 163.15, 159.62, 158.18, 154.94, 154.14, 152.40, 150.68, 149.62, 128.20, 111.09, 109.60, 106.78, 101.00, 93.94, 62.08, 57.16, 50.57

FT-IR (neat) 3081, 1715, 1658, 1605, 1498, 1409, 1387, 1366, 1276, 1209, 1194, 1159, 1096, 1043, 886, 789 cm^{-1}

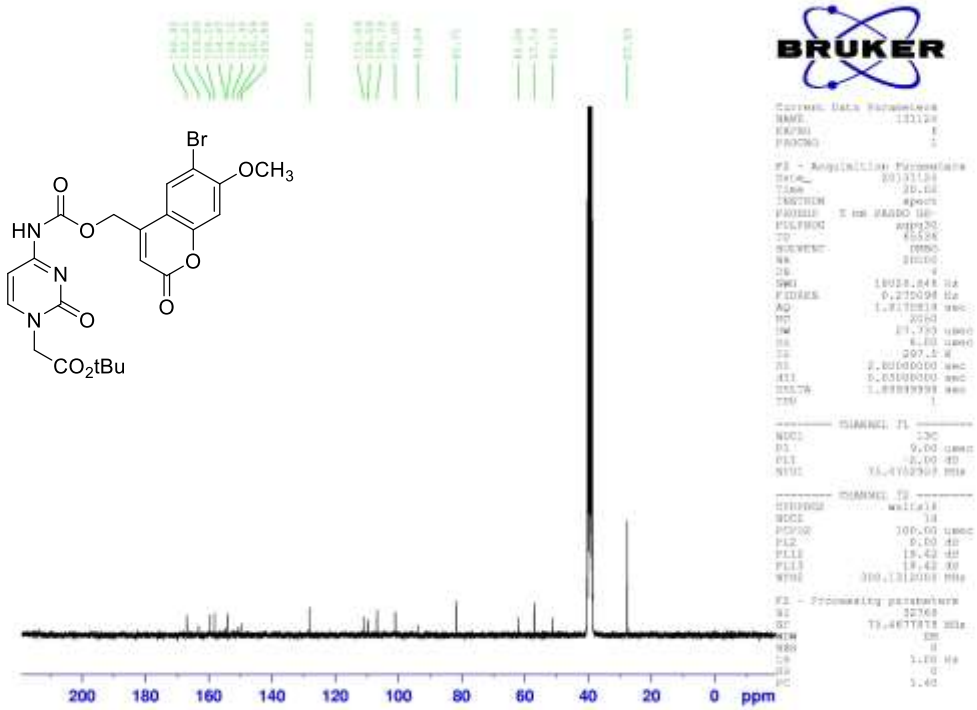
HRMS (ESI^+) Calcd for $C_{18}H_{15}N_3O_8Br^+$: 480.0037, Found: 480.0030



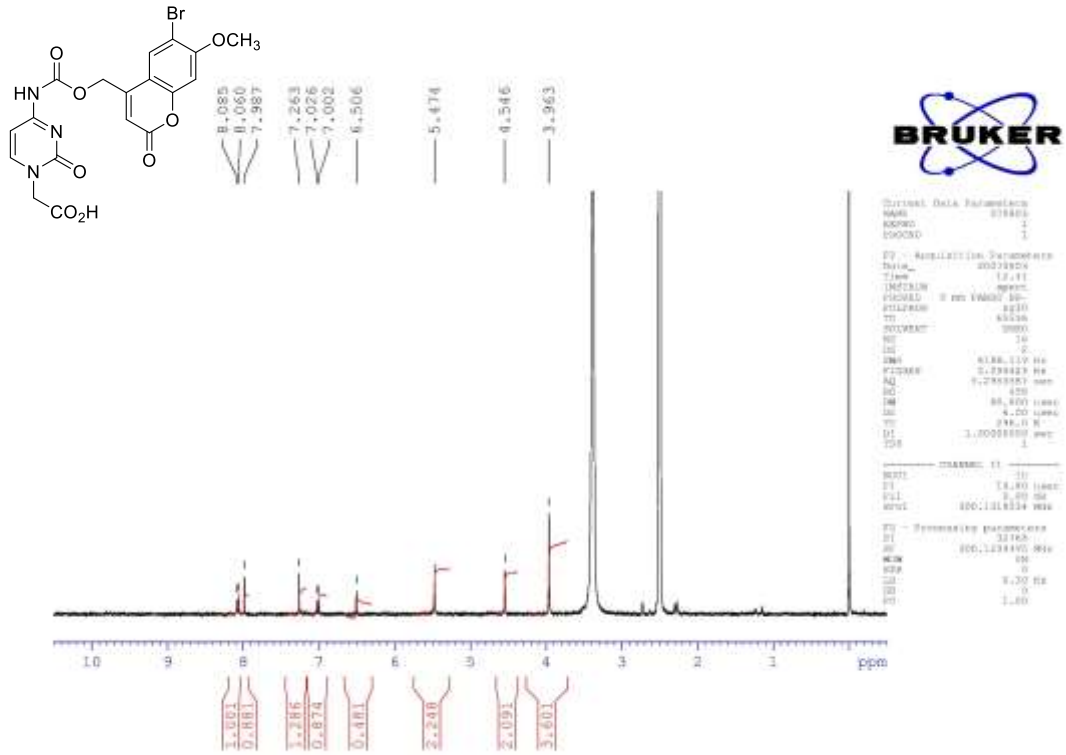
^1H NMR spectrum of **C-AcO^{t}Bu**



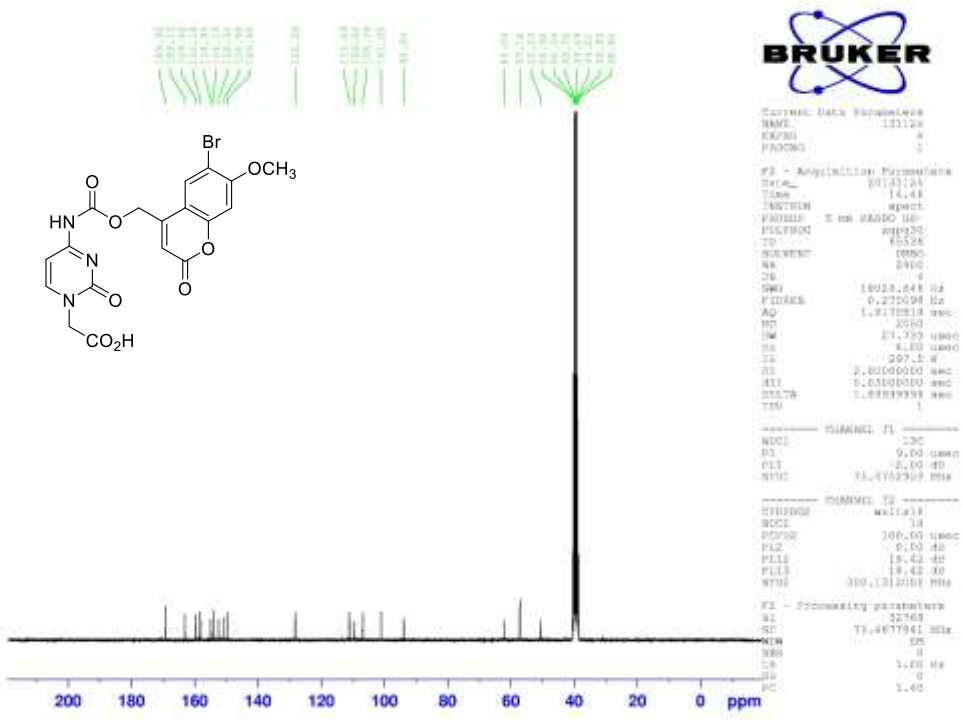
^{1}H NMR spectrum of **2**



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

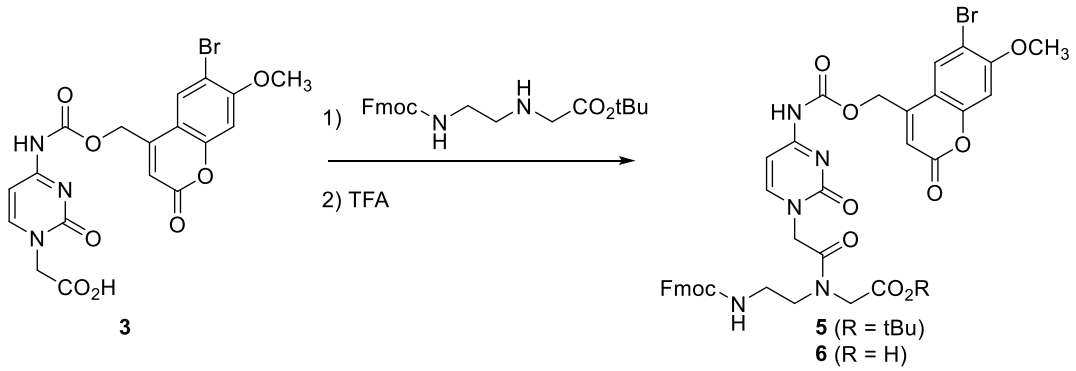


^{1}H NMR spectrum of **3**



^{13}C NMR spectrum of **3**

Synthesis of Fmoc-C^{Bmcmoc}-aeg-OH (6)



***tert*-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[{4-N-(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl-cytosin-1-yl}acetyl]glycinate (Fmoc-C^{Bmcmoc}-aeg-O^{t}Bu (5))**

To a stirred suspension of the **3** (210.4 mg, 0.438 mmol) and HATU (168.7 mg, 0.444 mmol) in DMF (7 mL) were added $^{i}Pr_2NEt$ (76.3 μL, 0.437 mmol) and 2,6-lutidine (76.5 mL, 0.657 mmol). Then stirring was continued at an ambient temperature for 5 min. The solution was transferred to a stirring solution of $FmocNH(CH_2)_2NHCH_2CO_2{}^{t}Bu$ (65.5 mg, 0.165 mmol) in DMF (3 mL) under Ar atmosphere. The reaction mixture was stirred at ambient temperature for 2 h. The solvent was removed using vacuum evaporation. Then the residue was redissolved in dichloromethane and washed with 0.5 M citric acid. The organic layer was dried over Na_2SO_4 and concentrated. Purification by column chromatography (57 g of CICA silica gel 60 (spherical), 40–50 μm, first 2.5% then 4% methanol in dichloromethane as eluents) gave **Fmoc-C^{Bmcmoc}-aeg-O^{t}Bu** (**5**) (103.5 mg, 0.121 mmol, 73.2% yield).

^{1}H NMR (DMSO-d_6) approximately 1 to 2 mixture of two rotational isomers around amide bond δ 11.10 (1H, brs), 7.97 (1H, s), 7.94-7.89 (3H, m), 7.69 (2H, m), 7.42-7.33 (5H, m), 7.24 (1H, s), 7.03 (1H, m), 6.51 (1H, s), 5.47 (2H, s), 4.84 (4.65)* (2H, s), 4.37-4.22 (3H, m), 3.96 (3H, s), 3.96 (1H, m), 3.49-3.42 (5H, m), 1.40 (1.47) (9H, s)

*chemical shifts in parenthesis are from the minor rotational isomer

^{13}C NMR (DMSO-d_6) δ (168.46)*, 168.03, (167.51), 167.11, 163.02, (162.94), 159.61, 158.16, 156.33 (156.12), 154.90, 154.12, 152.43, 151.12, 149.63, 143.86, 140.72, 128.18, 127.59, 127.04, 125.10, 120.10, 111.09, 109.57, 106.77, 100.98, 93.79, 81.94, 80.94, 65.48, (65.39), 62.04, 57.14, 54.89, (50.06), 49.54, 48.41, (47.05), 46.70, (27.66), 27.63

FT-IR (neat) 2954, 1733, 1706, 1669, 1629, 1606, 1506, 1450, 1414, 1371, 1346, 1276, 1210, 1153, 1094, 1051, 799, 758, 739 cm^{-1}

HRMS (ESI^{+}) Calcd for $C_{41}H_{41}N_5O_{11}Br^{+}$: 858.1980, Found: 858.1984

N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]-N-[{4-N-(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl-cytosin-1-yl}acetyl]glycinate (Fmoc-C^{Bmcmoc}-aeg-OH (6))

A solution of **5** (152.1 mg, 0.177 mmol) in TFA (10 mL) was stirred at rt for 2 h. The solvent was removed using vacuum evaporation and the trace of TFA was removed azeotropically with toluene. The residue was ground into small pieces, re-suspended in *n*-hexane and ethyl acetate, and then collected using vacuum

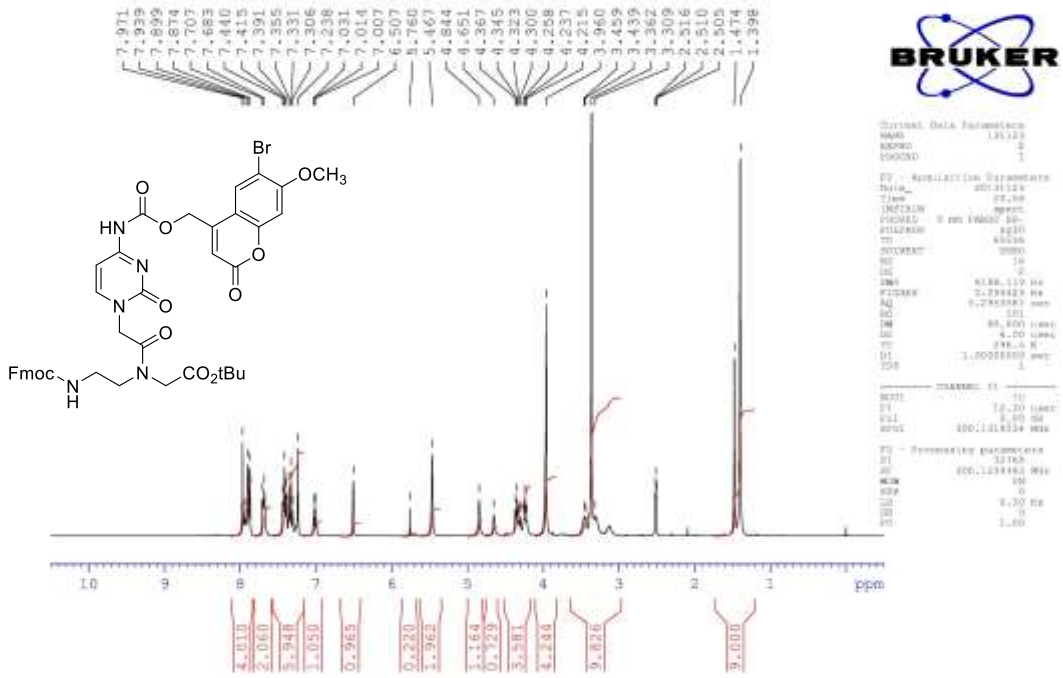
filtration to give **Fmoc-C^{Bmcmoc}-aeg-OH** (**6**) (128.3 mg, 0.160 mmol, 90.3% yield).

^{1}H NMR (DMSO-d_6) approximately 1 to 2 mixture of two rotational isomers around amide bond δ 11.08 (1H, brs), 7.99 (1H, s), 7.94 (1H, d, J=7 Hz, Cytosine), 7.91 (2H, d, J=7 Hz, minor), 7.89 (2H, d, J=7 Hz), 7.69 (2H, d, J=7 Hz), 7.41 (2H, dd, J=7 and 7 Hz), 7.33 (2H, dd, J=7 and 7 Hz), 7.27 (1H, s), 7.01 (1H, d, J=7 Hz, Cytosine, major), 6.98 (1H, J=7 Hz), 6.51 (1H, s), 5.47 (2H, s), 4.84 (2H, s, Cytosine-CH_2CON, major isomer), 4.65 (2H, s, Cytosine-CH_2CON, minor isomer), 4.37 (2H. d, J=7 Hz, FlCH_2OCO), 4.30 (1H, t, J=7 Hz, Fl), 4.21 (2H, m), 4.00 (2H, s), 3.96 (3H, s), 3.45 (2H, m), 3.10 (2H, m)

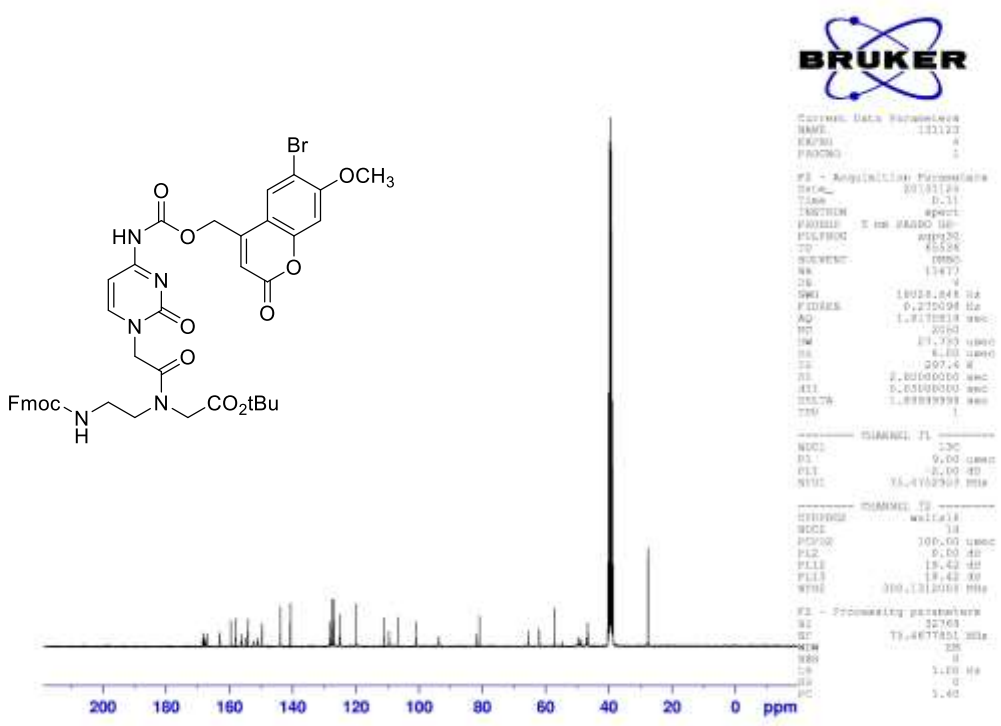
^{13}C NMR (DMSO-d_6) δ (170.81), 170.44, (167.51), 167.08, 163.04, (162.95), 159.65, 158.20, 156.37, 156.15, 154.94, 154.16, 152.47, 151.15, 149.70, 143.90, 140.75, 128.24, 127.62, 127.08, 125.15, 120.13, 111.14, 109.02, 106.80, 101.03, 93.80, 65.53, (65.40), 62.09, 57.19, 49.54, 47.77, 46.93, 46.72

FT-IR (neat) 3075, 1728, 1660, 1605, 1497, 1448, 1413, 1371, 1276, 1210, 1157, 1091, 1048, 796, 762, 740 cm^{-1}

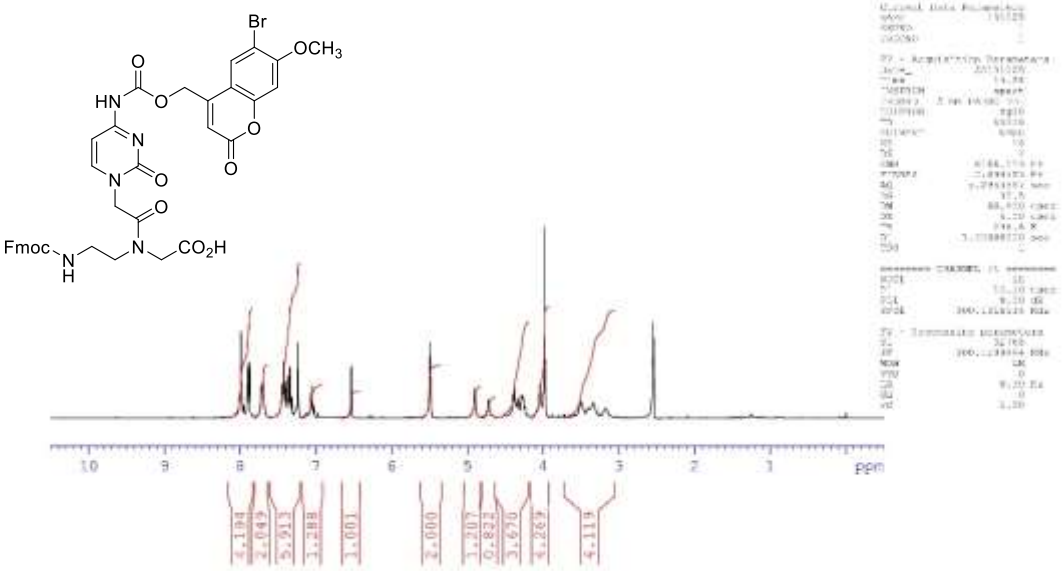
HRMS (ESI$^+$) Calcd for $C_{37}H_{33}N_5O_{11}Br^+$: 802.1354, Found: 802.1362



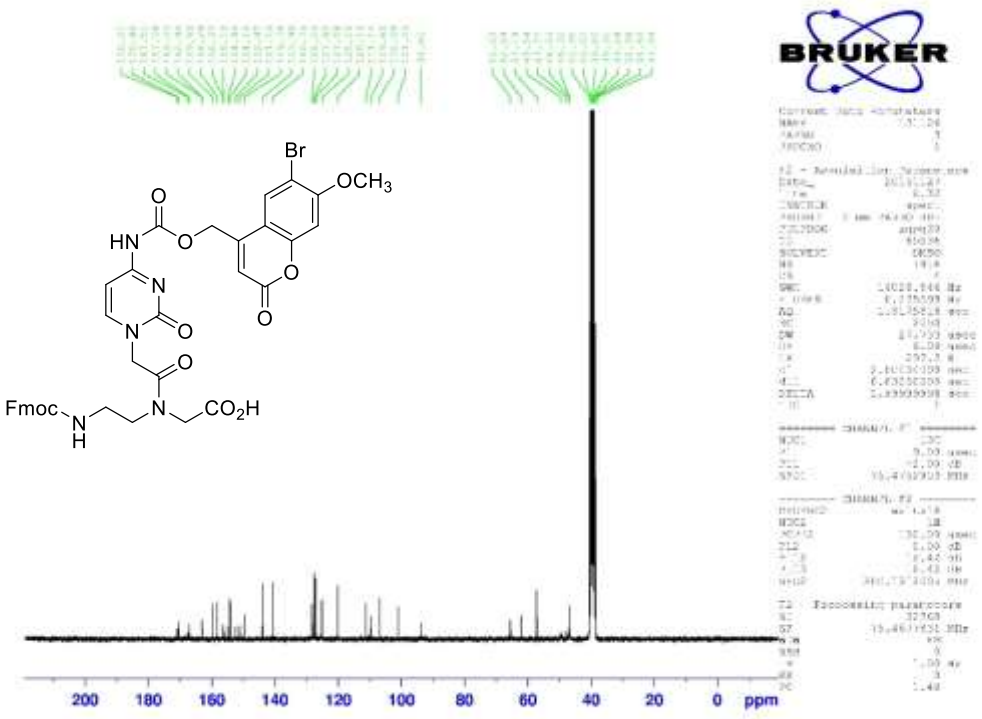
^{1}H NMR spectrum of **5**



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

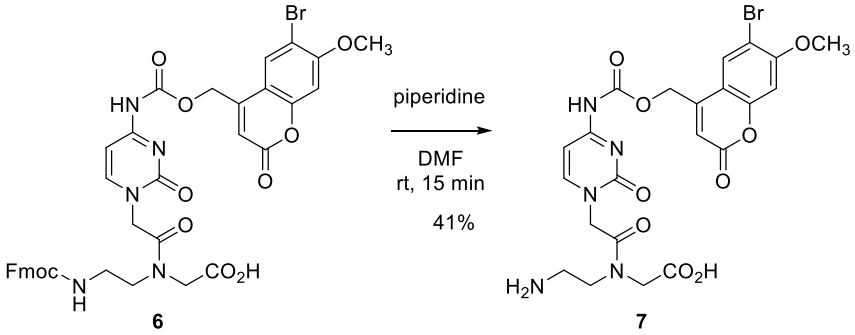


^{1}H NMR spectrum of **6**



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

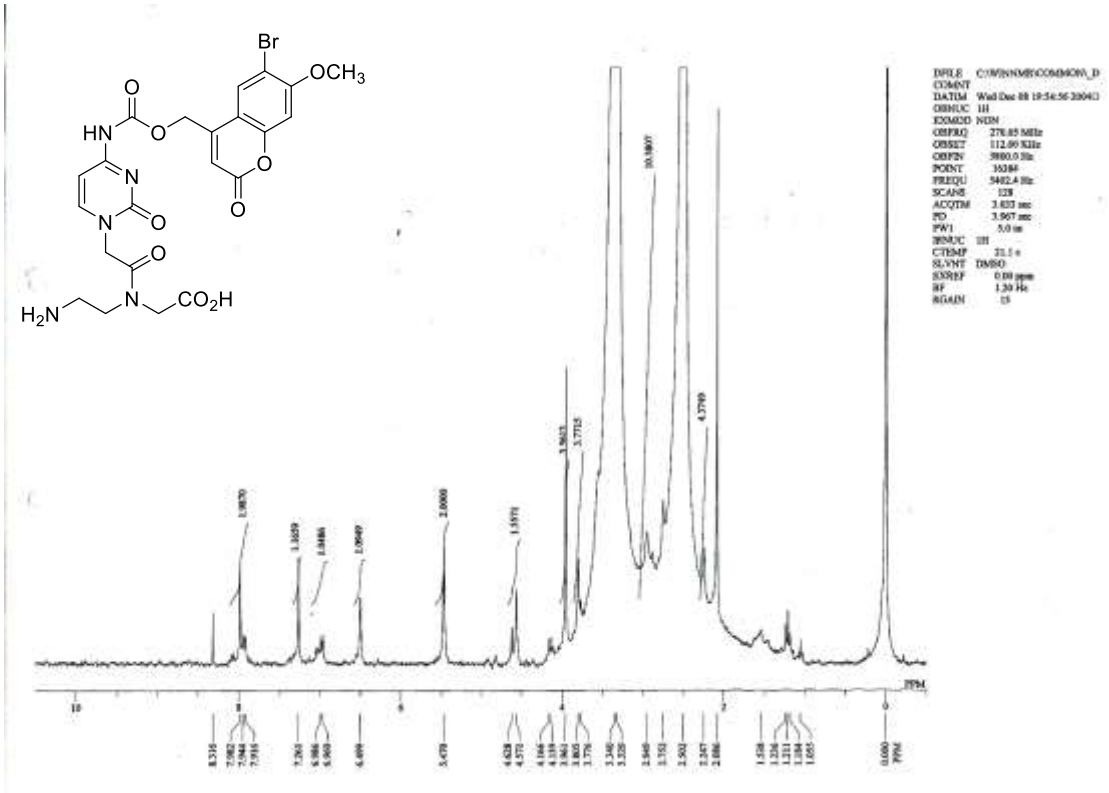
Synthesis of N-2-aminoethyl, N- [4-*N*-(6-Bromo-7-hydroxycoumarin-4-yl)methoxycarbonyl cytosin-1-yl]acetyl glycine (C^{Bmcmoc}-aeg-OH (7))



A solution of **6** (14.2 mg, 0.018 mmol) in 20% piperidine (1 mL) was stirred at rt for 15 min. The solvent was removed using vacuum evaporation. The residue was ground into small pieces, re-suspended in chloroform and methanol, and collected using vacuum filtration to give **C^{Bmcmoc}-aeg-OH** (**7**) (4.2 mg, 0. 0072 mmol, 41% yield).

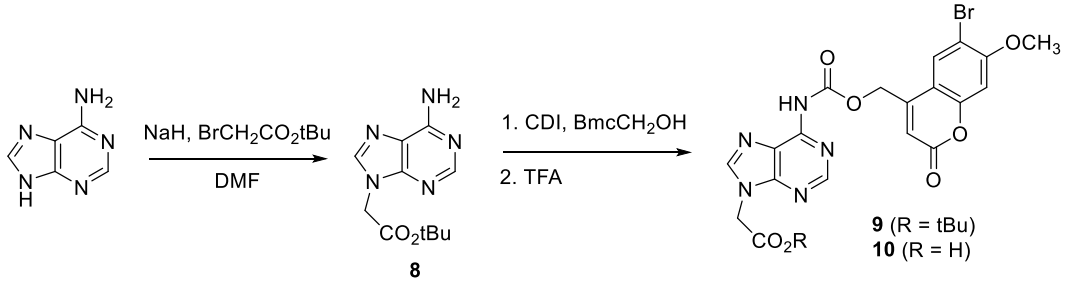
^{1}H NMR (DMSO-d_6) approximately 1 to 2 mixture of two rotational isomers around amide bond δ 7.98 (1H, s), 7.95 (1H, m), 7.26 (1H, s), 6.98 (1H, m), 6.50 (1H, s), 5.47 (2H, s), 4.84 (2H, s, major isomer), 4.65 (2H, s, minor isomer), 4.37-4.23 (5H, m), 3.96 (3H, s)

HRMS (FAB$^+$) Calcd for $C_{22}H_{23}N_5O_9Br$:580.0674, Found:580.0686



^{1}H NMR spectrum of **7**

Synthesis of A^{Bmcmoc}-AcOH (10)



***tert*-butyl 2-(6-amino-9H-purin-9-yl)acetate (8)**

The compound was synthesized using the reported procedure.[3)]

^{1}H NMR (DMSO-d_6) δ 8.13 (1H, s), 8.09 (1H, s), 8.10 (1H, s), 7.24 (2H, brs), 4.94 (2H, s), 1.42 (9H, s)

^{13}C NMR (DMSO-d_6) δ 167.44, 156.41, 153.07, 150.19, 141.78, 118.72, 82.50, 44.97, 28.12

FT-IR (neat) 3340, 3159, 1744, 1735, 1664, 1603, 1228, 1151 cm^{-1}

***tert*-Butyl 6-*N*-{[(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl]-adenin-9-yl}acetate (A^{Bmcmoc}-AcO^tBu (9))**

A solution of **8** (101.2 mg, 0.406 mmol) and *N,N'*-carbonyl diimidazole (96.6 mg, 0.596 mmol) in DMF (2 mL) was stirred at 105 °C for 2 h and then cooled to 95 °C. To the solution was added (6-bromo-7-methoxycoumarin-4-yl)methanol (171.7 mg, 0.602 mmol) and the reaction mixture was gradually cooled to an ambient temperature. The stirring was continued for 1.5 h. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4 and concentrated. Purification by column chromatography (20 g of silica gel 60, Merck, 43–60 μm, 3.2% methanol in dichloromethane as an eluent) gave **A^{Bmcmoc}-AcO^tBu (9)** (116.5 mg, 0.208 mmol, 51.2% yield).

^{1}H NMR ($CDCl_3$) δ 9.03 (1H, s), 8.80 (1H, s), 8.10 (1H, s), 7.76 (1H, s), 6.86 (1H, s), 6.52 (1H, s), 5.42 (2H, d, J = 1 Hz), 4.94 (2H, s), 3.97 (3H, s), 1.48 (9H, s)

^{13}C NMR ($CDCl_3$) δ 165.74, 160.10, 158.77, 154.67, 152.96, 151.84, 150.44, 148.85, 147.86, 143.58, 127.72, 121.58, 111.75, 111.55, 107.94, 100.55, 84.00, 62.61, 56.80, 45.00, 27.99

FT-IR (neat) 1738, 1726, 1610, 1296, 1163, 1099, 765 cm^{-1}

HRMS (ESI$^+$) Calcd for $C_{23}H_{23}N_5O_7Br^+$: 560.0775, Found: 560.0771

6-*N*-{[(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl]-adenin-9-yl}acetic acid (A^{Bmcmoc}-AcOH (10))

A solution of **9** (89.9 mg, 0.160 mmol) in TFA (3 mL) was stirred at an ambient temperature for 30 min. The reaction mixture was evaporated under vacuum to give 87.6 mg of **10**. The crude product **10** was used in the next reaction without further purification.

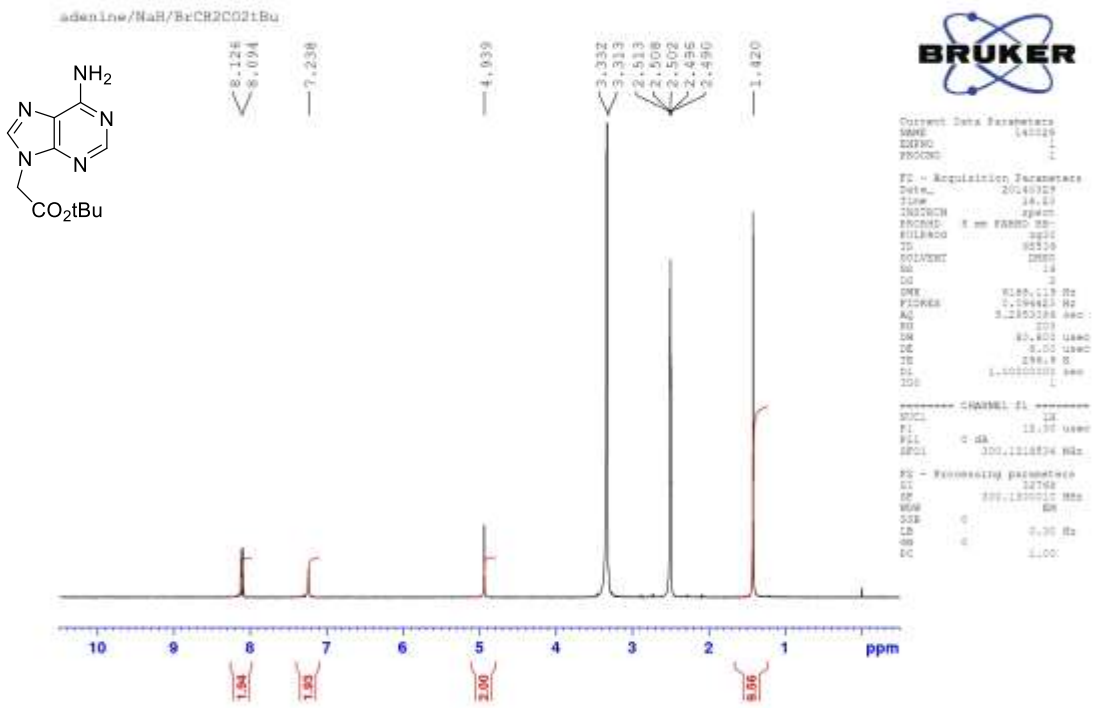
^{1}H NMR (DMSO-d_6) δ 11.05 (1H, brs), 8.65 (1H, s), 8.46 (1H, s), 8.01 (1H, s), 7.26 (1H, s), 6.70 (1H, s),

5.51 (2H, s), 5.10 (2H, s), 3.97 (3H, s)

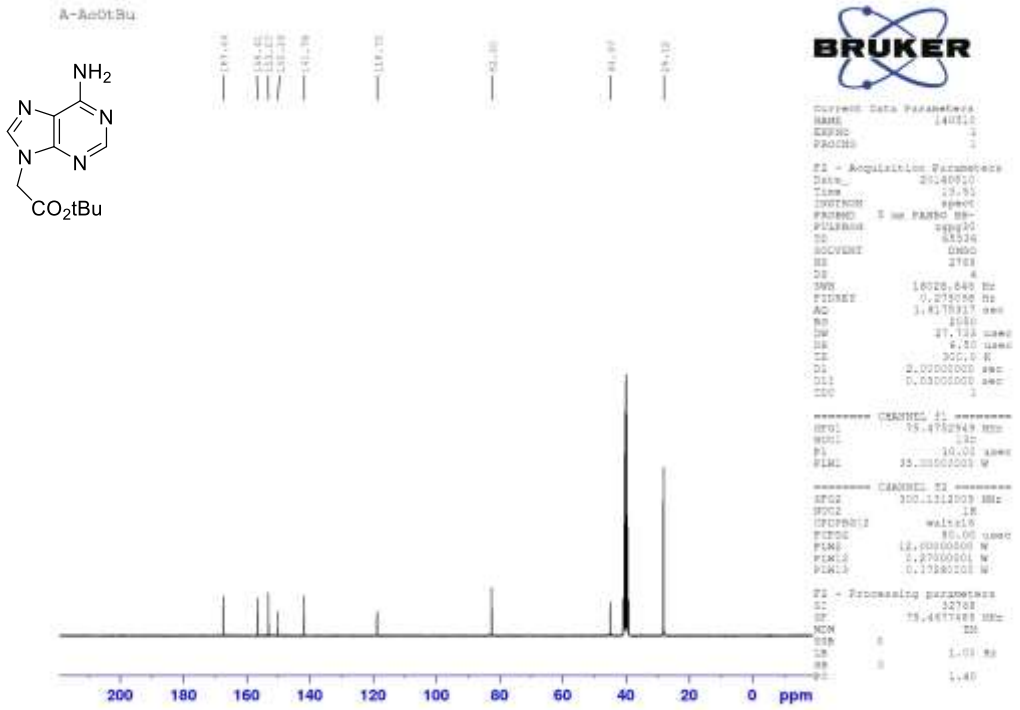
^{13}C NMR (DMSO-d_6) δ 168.96, 159.71, 158.12, 154.11, 152.06, 151.62, 151.19, 150.04, 149.08, 144.92, 128.18, 122.47, 111.15, 109.55, 106.72, 100.97, 61.87, 57.12, 44.25

FT-IR (neat) 1764, 1726, 1629, 1606, 1278, 1221, 1162, 1048 cm^{-1}

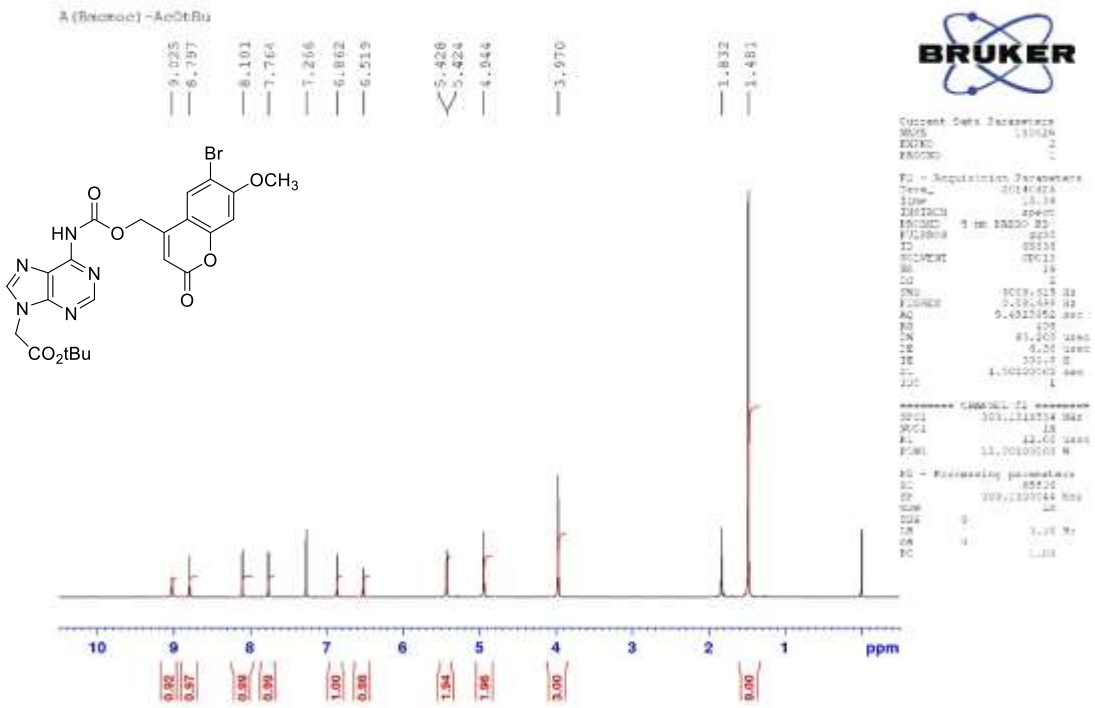
HRMS (ESI^+) Calcd for $C_{19}H_{15}N_5O_7Br^+$: 504.0149, Found: 504.0145



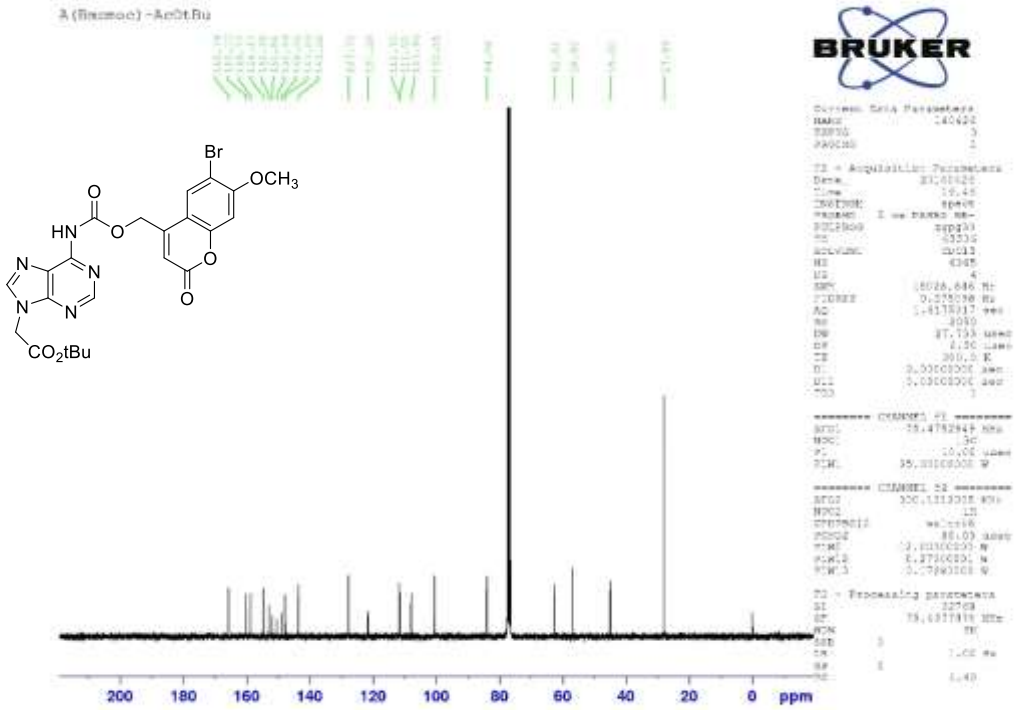
^{1}H NMR spectrum of **8**



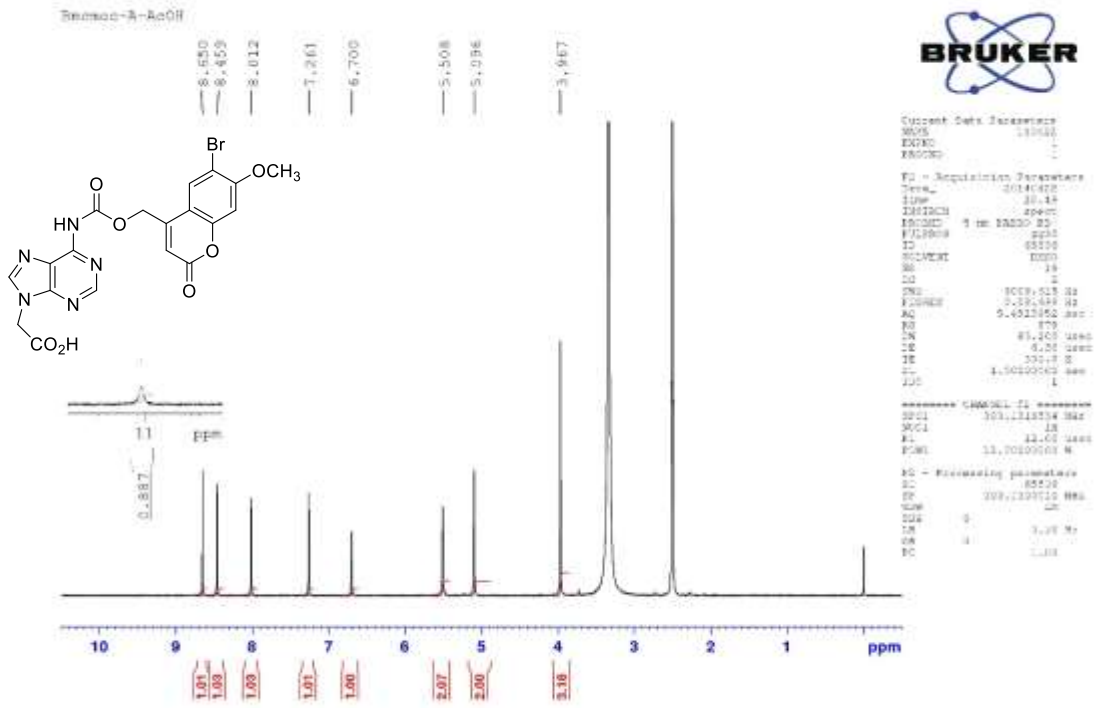
^{13}C NMR spectrum of **8**



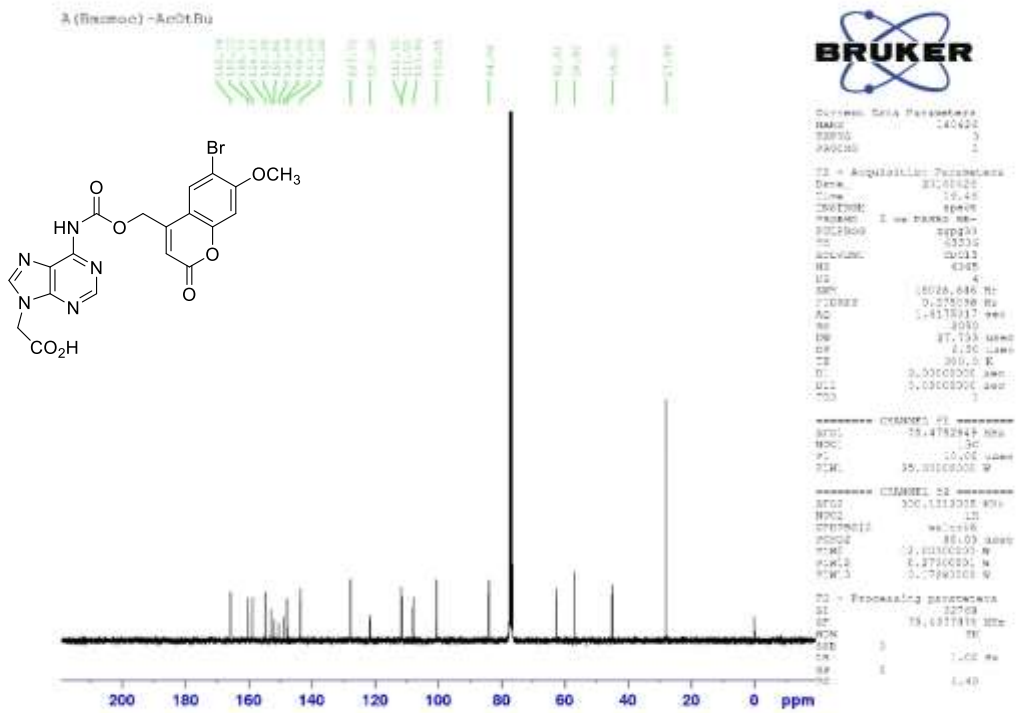
^{1}H NMR spectrum of **9**



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

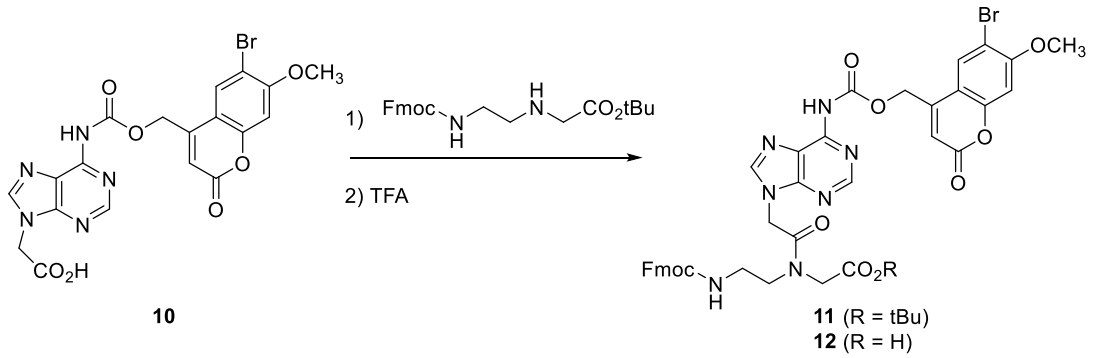


^{1}H NMR spectrum of **10**



^{13}C NMR spectrum of **10**

Synthesis of Fmoc-A^{Bmcmoc}-aeg-OH (12)



***tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[{6-*N*-(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl)adenin-9-yl}acetyl]glycinate (Fmoc-A^{Bmcmoc}-aeg-O^{t}Bu (11))**

To a stirred solution of FmocNH$(CH_2)_2$NH$CH_2CO_2{}^{t}$Bu (**4**) (81.5 mg, 0.205 mmol) in DMF (1 mL) was added **10** (111.2 mg, 0.220 mmol), HOBt H_2O (34.3 mg, 0.254 mmol), $^{i}Pr_2$NEt (84 μL, 0.48 mmol) and PyBOP (127.5 mg, 0.245 mmol). The reaction mixture was stirred at ambient temperature for 4 h, diluted with CH_2Cl_2 and washed with 1 M HCl and sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated. Purification by column chromatography (40 g of Merck silica gel 60, 43–60 μm, 3.2% methanol in dichloromethane as an eluent) gave **Fmoc-A^{Bmcmoc}-aeg-O^{t}Bu** (**11**) (127.1 mg, 0.144 mmol, 70.1% yield).

^{1}H NMR ($CDCl_3$) mixture of rotational isomers around amide bond δ 9.38 (9.45) (1H, s), 8.68 (8.66) (1H, s), 7.98 (8.16) (1H, s), 7.71 (1H, s), 7.71 (2H, m), 7.55 (2H, m), 7.36-7.23 (4H, m), 6.81 (1H, s), 6.45 (1H, s), 6.13 (5.44) (1H, m), 5.36 (2H, s), 5.05 (5.00) (2H, s), 4.48 (4.34) (2H, d, J = 6 Hz), 4.22-4.18 (2H, m), 3.95 (1H, m), 3.93 (3H, s), 3.66-3.39 (4H, m), 1.53 (1.44) (9H, s)

^{13}C NMR ($CDCl_3$) δ 168.62 (168.24), 166.27 (167.03), 160.17, 158.70, 156.74 (156.63), 154.58, 152.69, 151.75, 150.51, 148.84, 147.95, 144.38, 143.83 (143.68), 141.26, 127.76, 127.69, 127.07, 124.90 (125.03), 121.50 (121.39), 120.01 (119.97), 111.64, 111.51, 107.90, 100.49, 82.81 (83.97), 66.77, 62.49, 56.76, 49.99, 49.16 (48.96), 47.22, 43.69, 39.34 (38.93), 27.99 (28.05)

FT-IR (neat) 2926, 1737, 1726, 1712, 1606, 1276, 1154, 761, 742 cm^{-1}

HRMS (ESI$^+$) Calcd for $C_{42}H_{41}N_7O_{10}Br^+$: 882.2093, Found: 882.2087

***N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[{6-*N*-(6-Bromo-7-methoxycoumarin-4-yl)methoxycarbonyl)adenin-9-yl}acetyl]glycine (Fmoc-A^{Bmcmoc}-aeg-OH (12))**

A solution of **11** (127.1 mg, 0.144 mmol) in TFA (2 mL) was stirred at ambient temperature for 5 h. The solvent was removed using vacuum evaporation. The residue was re-dissolved in CH_2Cl_2 (ca 10 mL). *n*-Hexane was added dropwisely to the solution under sonication to get finely powdered precipitate, which was collected using vacuum filtration to give **Fmoc-A^{Bmcmoc}-aeg-OH** (**12**) (97.1 mg, 0.117 mmol, 81.6% yield).

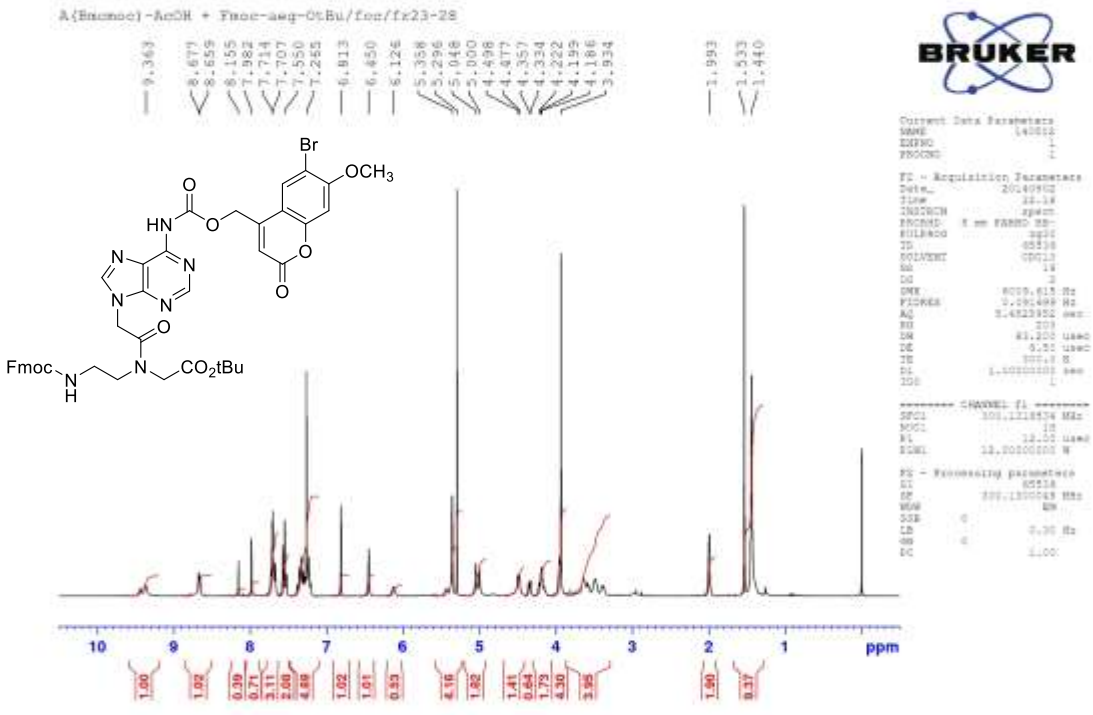
^{1}H NMR (DMSO-d_6) δ 11.01(1H, brs), 8.56 (8.60) (1H, s), 8.33 (1H, s), 8.01 (1H, s), 7.87 (2H, d, J = 7.5 Hz), 7.68 (7.66) (2H, d, J = 7.5 Hz), 7.50 (1H, m), 7.40 (2H, dd, J = 7.5 & 7.5 Hz), 7.30 (2H, dd, J = 7.5 &

7.5 Hz), 7.26 (1H, s), 6.70 (1H, s), 5.50 (2H, s), 5.35 (5.17) (2H, s), 4.38-4.22 (4H, m), 4.01 (2H, s), 3.97 (3H, s), 3.56-3.15 (4H, m)

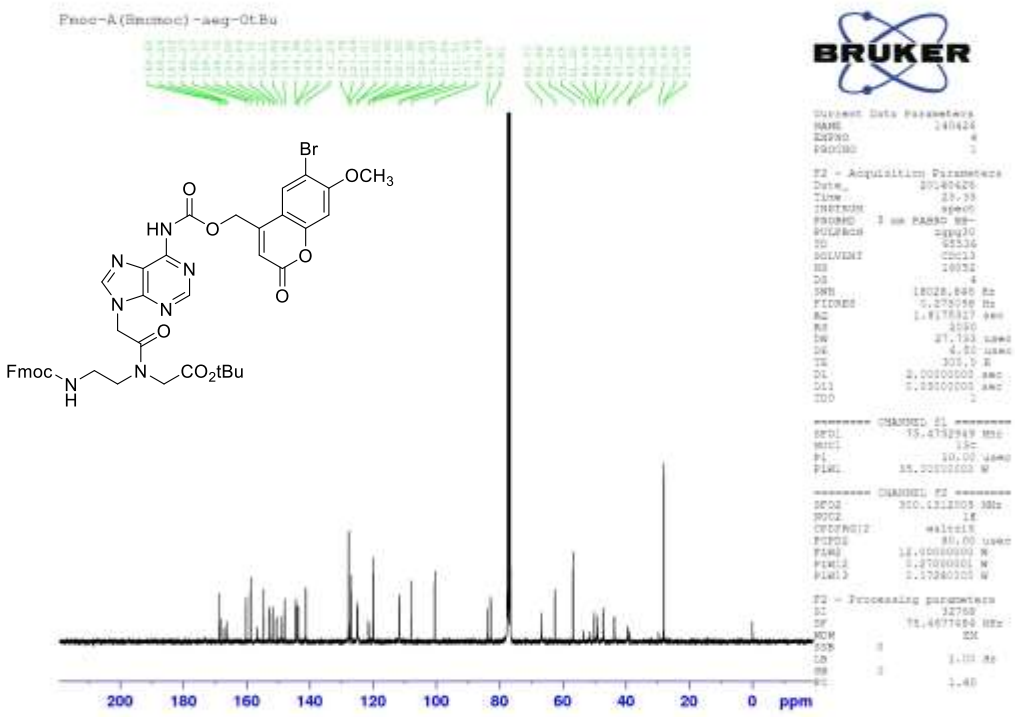
^{13}C NMR (DMSO-d_6) δ 170.77 (171.27), 166.98 (167.47), 160.23, 158.63, 156.91 (156.62), 154.61, 152.75, 151.97, 151.72, 150.53, 149.47, 145.81, 144.32, 141.18, 128.67, 128.07, 127.51, 125.55, 122.86 (122.78), 120.57, 111.66, 110.08, 107.24, 101.46, 66.00 (65.90), 62.38, 57.62, 49.65, 48.20, 47.46, 47.22, 44.62, (44.36)

FT-IR (neat) 3500- 3100 (br), 3057, 2950, 1726, 1711, 1665, 1653, 1619, 1605, 1209, 1156, 761, 743 cm^{-1}

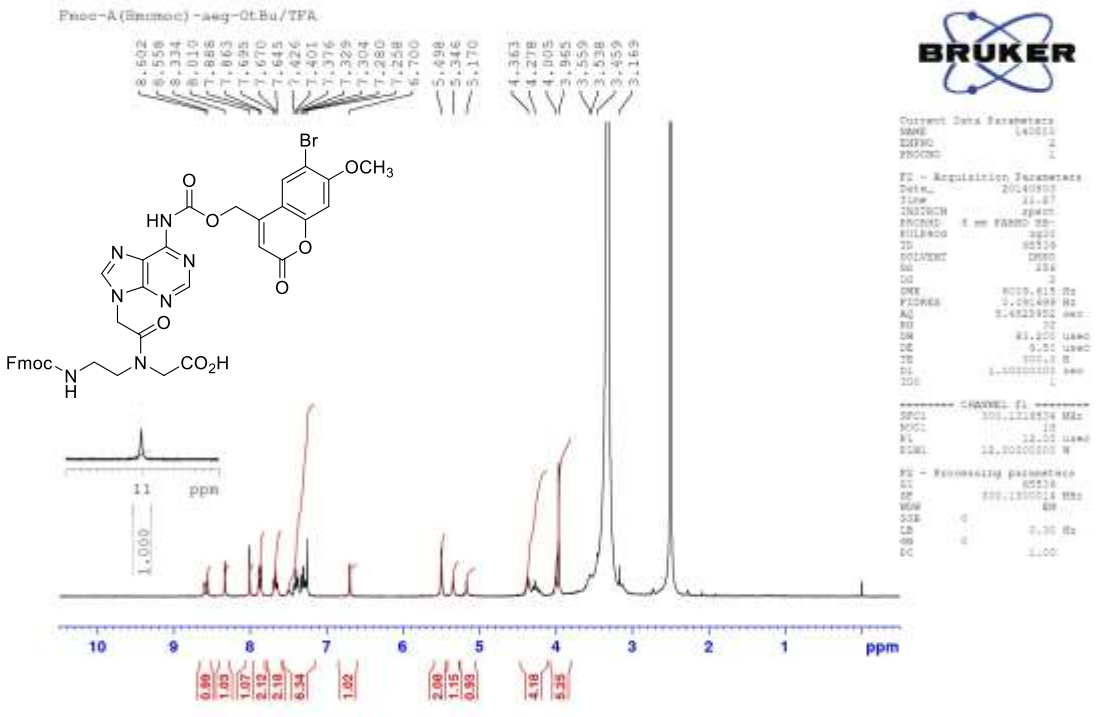
HRMS (ESI^+) Calcd for $C_{38}H_{33}N_7O_{10}Br^+$: 826.1467, Found: 826.1467



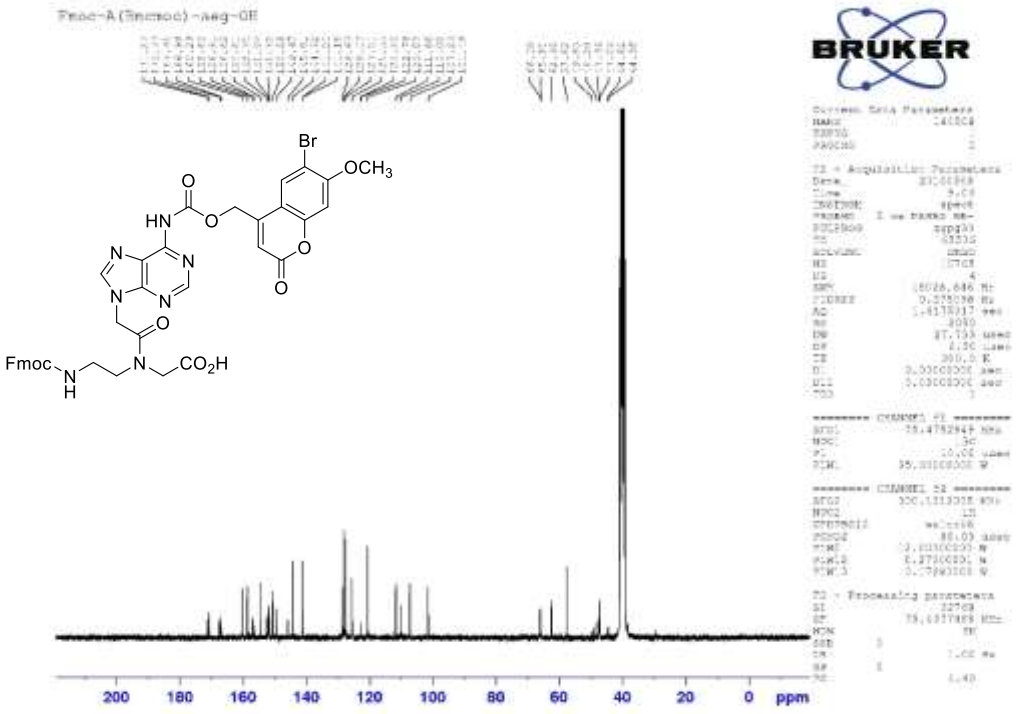
^{1}H NMR spectrum of **11**



^{13}C NMR spectrum of **11**

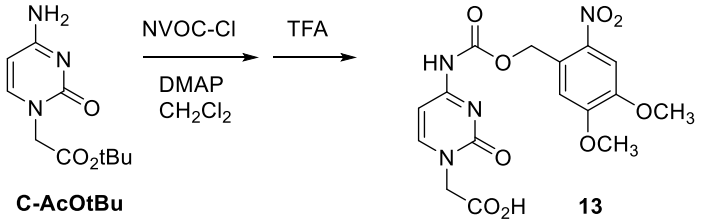


^{1}H NMR spectrum of **12**



^{13}C NMR spectrum of **12**

4-*N*-[(6-nitroveratryloxycarbonyl]-cytosin-1-yl acetic acid (C^{NVOC}-AcOH (13))



***tert*-butyl 4-*N*-[(6-nitroveratoryloxycarbonyl]-cytosin-1-yl acetate (C^{NVOC}-AcOtBu)**

In a 50-mL round-bottomed flask were placed **C-AcO^{t}Bu** (247.7 mg, 1.10 mmol), NVOC-Cl (275.4 mg, 1.00 mmol), and *N*,*N*-dimethyl-4-aminopyridine (134.3 mg, 1.10 mmol). After drying under vacuum, CH_2Cl_2 (19 mL) was added to the mixture. The reaction mixture was stirred at an ambient temperature for 5 h under Ar atmosphere and was quenched by 0.5 M citric acid. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using flash column chromatography (30 g of SiO_2, 2.7% MeOH in CH_2Cl_2) yielded **C^{NVOC}-AcO^{t}Bu** (417.3 mg, 0.90 mmol, 82% yield).

^{1}H NMR ($CDCl_3$) δ 7.75 (1H, s), 7.54 (1H, d, J=7.2 Hz), 7.25 (1H, d, J=7.2 Hz), 7.01 (1H, s), 5.65 (2H, s), 4.52 (2H, s), 4.00(3H, s), 3.97 (3H, s), 1.48 (9H, s)

^{13}C NMR (DMSO-d_6) δ 166.35, 162.84, 155.30, 153.84, 151.97, 149.22, 148.37, 139.66, 126.64, 109.92, 108.19, 95.05, 83.47, 64.63, 56.67, 56.44, 51.40, 27.98

HRMS (ESI^+) Calcd for $C_{20}H_{25}N_4O_9^+$: 465.1616, Found: 465.1611

4-*N*-[(6-nitroveratoryloxycarbonyl]-cytosin-1-yl acetic acid (C^{NVOC}-AcOH (13))

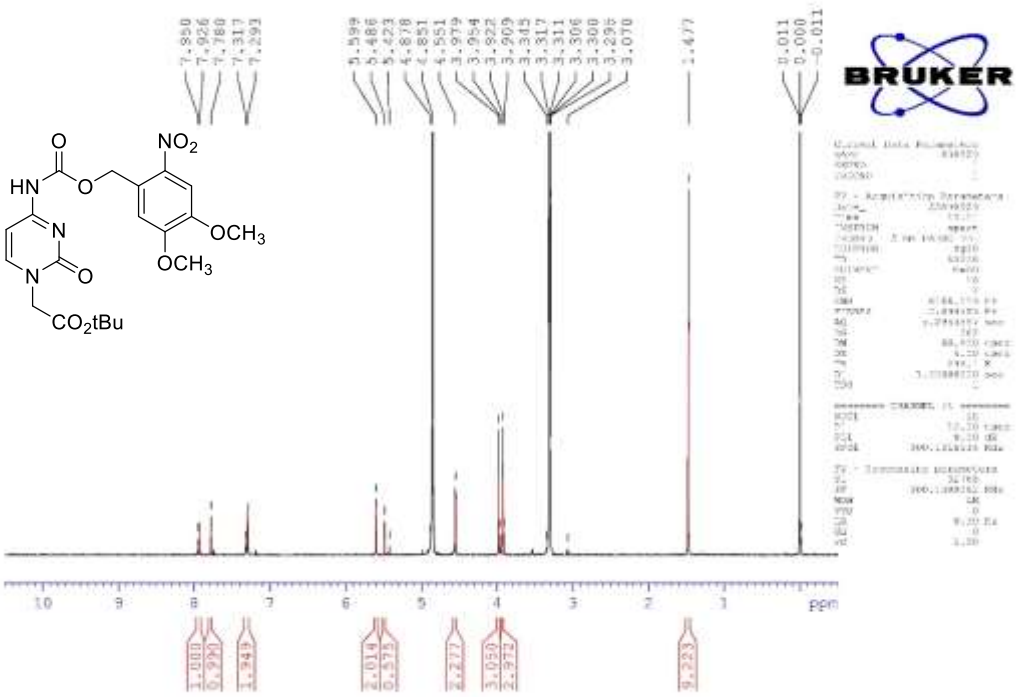
A solution of **C^{NVOC}-AcO^{t}Bu** (398.0 mg, 0.86 mmol) in trifluoroacetic acid (17 mL) was stirred at an ambient temperature for 75 min. The reaction mixture was evaporated under vacuum to give **C^{NVOC}-AcOH** (**13**) (391.3 mg, 0.75 mmol, 87% yield).

^{1}H NMR (DMSO-d_6) δ 8.07 (1H, d, *J*=7.2Hz), 7.45 (1H, s), 7.41 (1H, s), 7.04 (1H, d, *J*=7.2Hz), 5.52 (2H,s), 4.54 (2H, s), 3.96 (3H, s), 3.88 (3H, s)

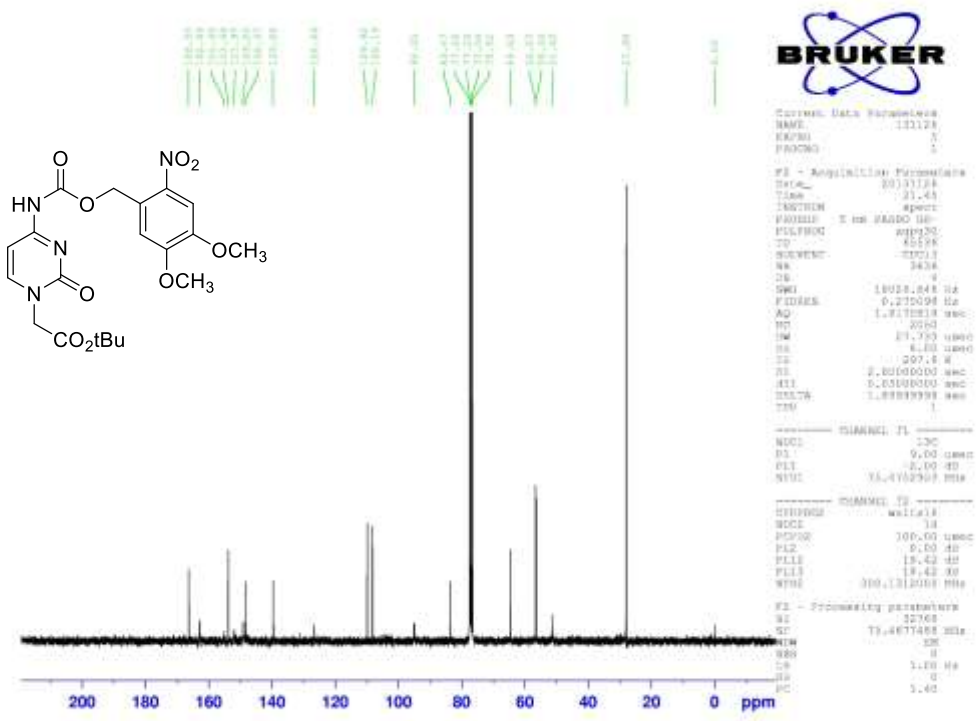
^{13}C NMR (DMSO-d_6) δ 169.37, 163.27, 155

.00, 153.65, 152.63, 150.68, 147.74, 138.86, 126.79, 110.34, 108.11, 93.90, 63.76, 56.52, 56.09, 50.56

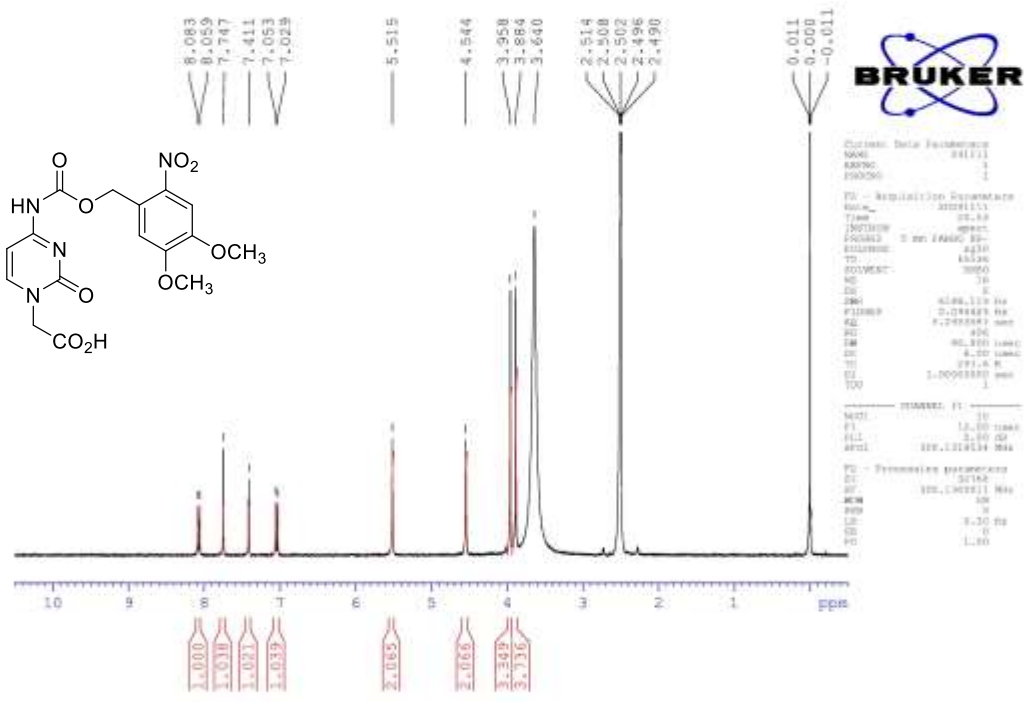
HRMS (ESI^+) Calcd for $C_{16}H_{17}N_4O_9^+$: 409.0990, Found: 409.0988



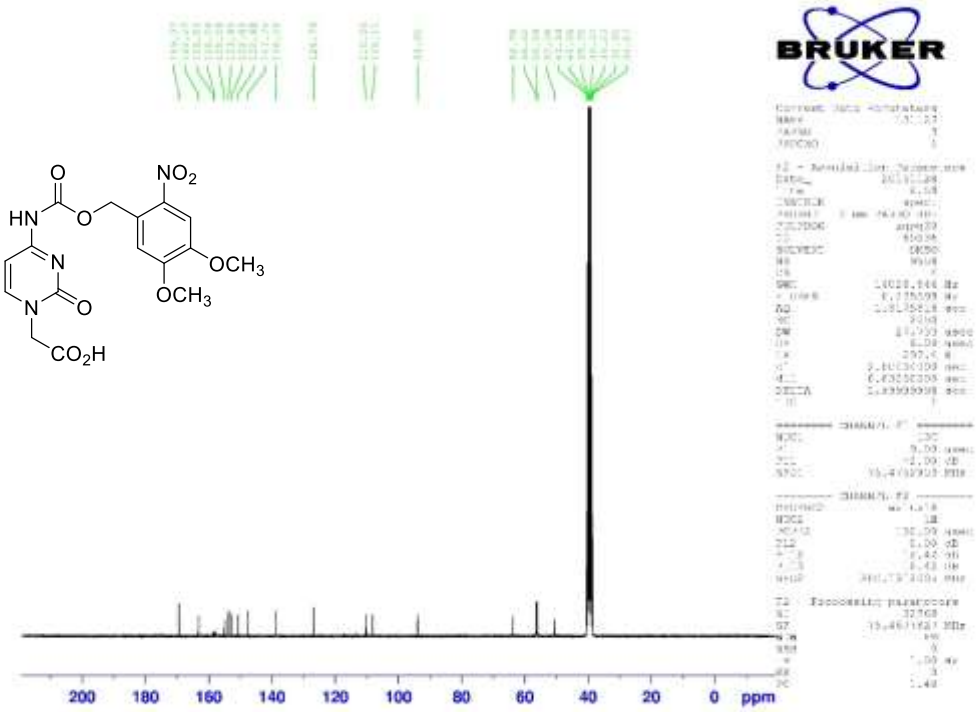
^{1}H NMR spectrum of **C^{NVOC}-AcOtBu**



^{13}C NMR spectrum of **C^{NVOC}-AcOtBu**



^{1}H NMR spectrum of **13**



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

Solid phase synthesis of caged PNAs

The caged PNAs having the C^{Bmcmoc}-aeg monomer were elongated manually on an Fmoc-Gly Wang-PEG-PS resin (0.28 mmol/g, Watanabe Chemical Industries Ltd., Japan) using a KMS-3 manual peptide synthesizer (Kokusan Corp., Tokyo, Japan). Nucleobase protected Fmoc monomers, Fmoc-C^{Bhoc}-aeg-OH, Fmoc-A^{Bhoc}-aeg-OH and Fmoc-G^{Bhoc}-aeg-OH were purchased from Applied Biosystems. Fmoc-T-aeg-OH was synthesized according to an explanation given by Thomson.[2)] The following is a general synthetic procedure. The resin (107.3 mg, 0.03 mmol) was swollen by washing three times with DMF (2 mL). Fmoc group was removed by application of 2 mL of 20% piperidine in DMF (2 × 3 min and 20 min). The resin was rinsed with DMF (2 mL, 4 × 1 min) and dichloromethane (2 mL, 1 min). Deprotection was checked by performing a positive Kaiser test. Before coupling pre-activation of Fmoc-PNA monomer was necessary. In a 10-mL round-bottomed flask an Fmoc-PNA monomer (0.09 mmol), DMF (3 mL) and HATU (0.084 mmol) were placed. The mixture was stirred under Ar atmosphere until a clear solution was obtained. To the solution were added $^{i}Pr_2NEt$ (0.09 mmol) and 2,6-lutidine (0.135 mmol). Then stirring was continued for 5 min at rt. The pre-activated Fmoc-PNA monomer was added to the resin. The coupling reaction proceeded for 20–25 min. The completion of the reaction was monitored using a negative Kaiser test. The resin was rinsed with DMF (2 mL, 3 × 1 min) and treated with a capping reagent, 5% acetic anhydride and 6% 2,6-lutidine in DMF (2 mL, 3 × 1 min). After washing with DMF (2 mL, 3 × 1 min), the resin was subjected to the next deprotection–coupling–capping cycle. After the last coupling, the Fmoc group was removed by application of 20% piperidine in DMF (2 × 3 min and 20 min). The resin was rinsed with DMF (2 mL, 4 × 1 min) and dichloromethane (2 mL, 3 × 1 min), dried in vacuum and then transferred to a 10-mL round-bottomed flask. Removal of the Bhoc group and cleavage of the PNA oligomers from the resin were performed by stirring with 1.5 mL of TFA/phenol (95/5) for 75 min at an ambient temperature. The resin was filtered off and washed with TFA. The combined filtrate and washings were concentrated to approximately 1 mL under vacuum and diluted with ice-cold ether. The obtained precipitates were collected by filtration, washed with ether and dried under vacuum. The crude PNA oligomer was purified using a semi-preparative RP-HPLC (Column: COSMOSIL 5C18-AR-II, 20 × 250 mm; Nacalai Tesque Inc.) and was analyzed using ESI-MS.

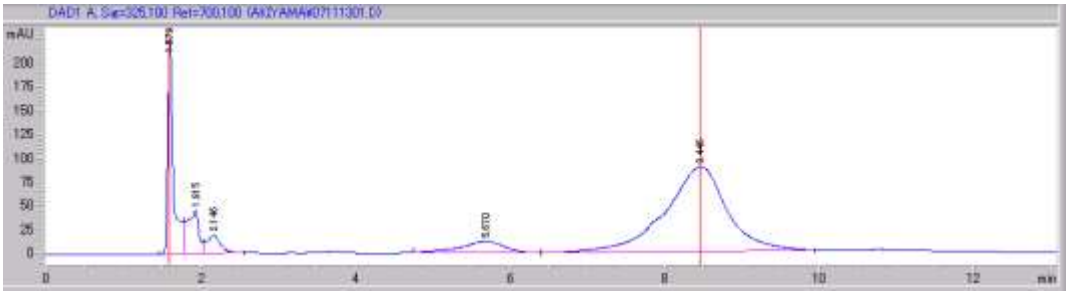
5-mer cPNA (14). RP-HPLC: Eluent: 25% acetonitrile in water (0.1% TFA), 7.5 mL/min, t_R = 15.4 min; ESI-MS (positive mode): Calcd for $C_{68}H_{81}{}^{79}BrN_{22}O_{26}$-$2H^+$ 851.2 $[M + 2H]^{2+}$, found 851.5.

10-mer cPNA (15). RP-HPLC: Eluent: 20% acetonitrile in water (0.1% TFA), 7.5 mL/min, t_R = 14.1 min; ESI-MS (positive mode): Calcd for $C_{122}H_{147}{}^{79}BrN_{52}O_{41}$-$3H^+$ 1026.91 $[M + 3H]^{3+}$, found 1027.55.

16-mer cPNA (16). RP-HPLC: Eluent: 20% acetonitrile in water (0.1% TFA), 7.5 mL/min, t_R = 17.7 min; ESI-MS (positive mode): Calcd for $C_{184}H_{230}{}^{79}BrN_{71}O_{65}$-$4H^+$ 1140.0 $[M + 4H]^{4+}$, found 1140.3.

All unmodified PNAs were synthesized by Fasmac Co. Ltd. (Japan).

(a)



(b)

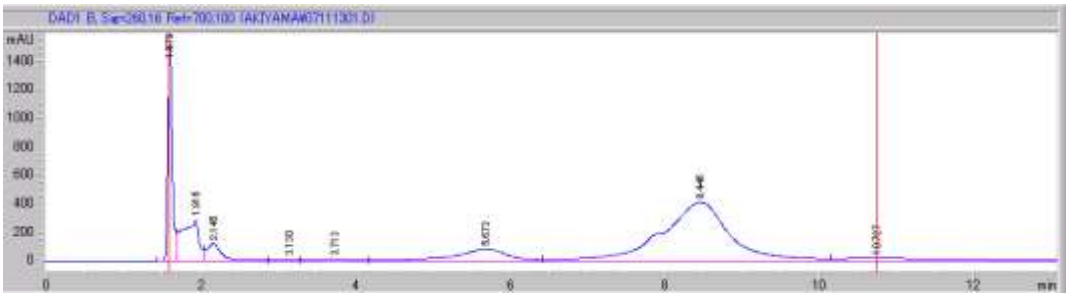


Figure S1. Analytical RP-HPLC traces for a crude mixture of 10-mer cPNA (**15**) synthesis (a) monitored at 325 nm, (b) monitored at 260 nm. The retention time of **15** was 8.4 min. Column: ZORBAX Eclipse XDB-C8 (4.6 × 150 mm), eluent: 18% acetonitrile (0.1% TFA), 1.0 mL/min.

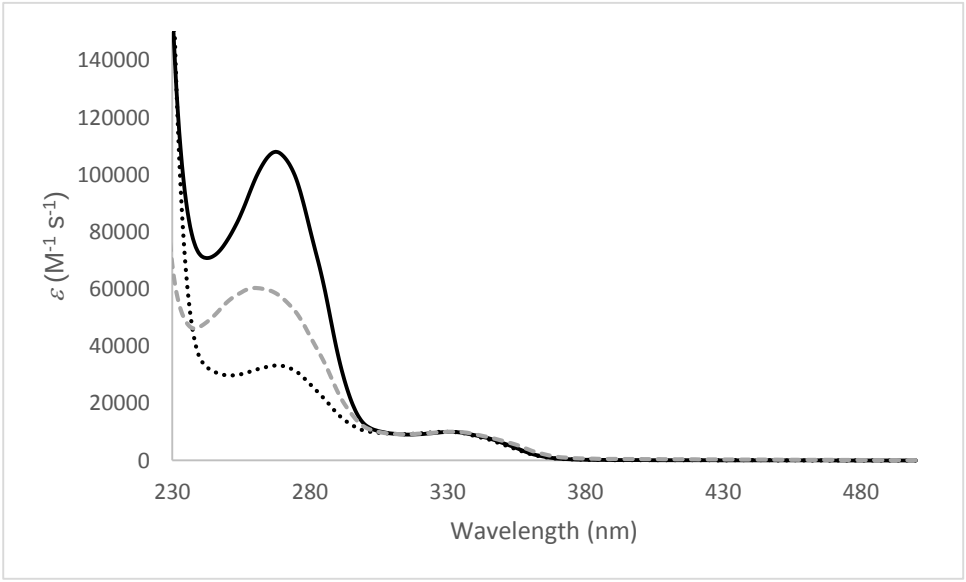


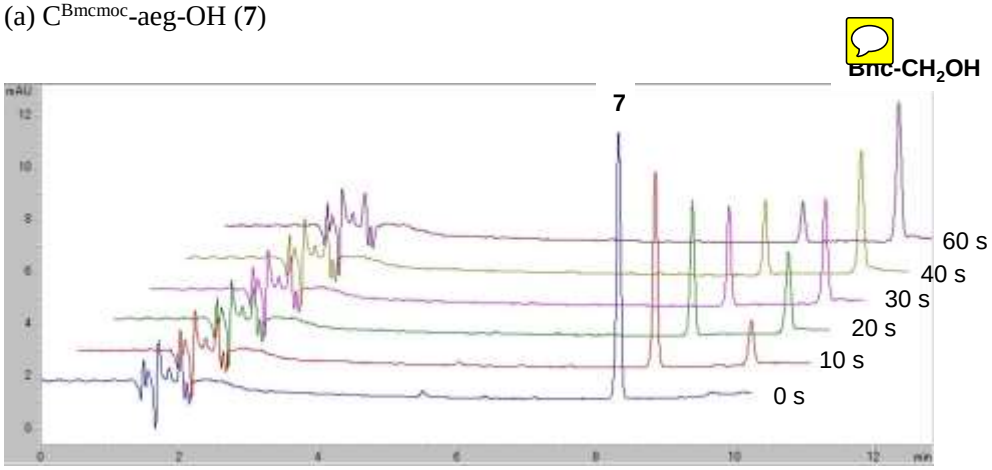
Figure S2. UV-vis spectra of the caged PNAs (KMOPS, pH 7.2). Dotted line: 5-mer cPNA (**14**). Broken line: 10-mer cPNA (**15**). Solid line: 16-mer cPNA (**16**).

Photolysis and quantum efficiency measurement

Into a 12-mm-diameter Pyrex test tube was placed 2 mL of 10 μM substrate solution in K-MOPS solution (pH 7.2) containing 0.1% DMSO. The solution was irradiated at 350 nm using four RPR 350 nm lamps (4 mJs^{-1}). Aliquots of 10 μL were removed periodically and analyzed using HPLC. The light output

for the quantum efficiencies measurement was performed using ferrioxalate actinometry. HPLC traces for the photolysis of caged PNAs are shown in **Fig. S3** and **S4**: ZORBAX Eclipse XDB-C8 (4.6 × 150 mm), 1.0 mL/min, linear gradient of acetonitrile in water (0.1% TFA), detection at 260 nm. From the HPLC traces, the consumption of the caged compounds and released 5-mer PNA from **14** and 16-mer PNA from **16** were quantified. They are shown against the irradiation time (**Fig. 2** in the main text and **Fig. S5**).

(a) C^{Bmcmoc}-aeg-OH (**7**)



(b) 5-mer cPNA (**14**)

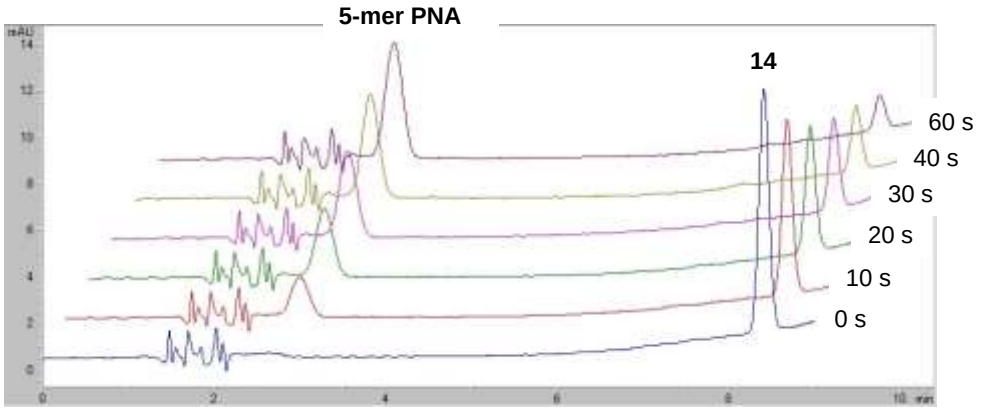


Figure S3. HPLC traces for the photolysis of **7** and **14**. (a) Photolysis of **7**. Aliquots of the photolysis mixtures were analyzed after the specified irradiation time by RP-HPLC with linear gradient of 12–50% (0–10 min) acetonitrile in water (0.1% TFA), detection at 325 nm. (b) Photolysis of **14**. Aliquots of the photolysis mixture were analyzed after the specified irradiation time by RP-HPLC with linear gradient of 15–40% (0-10 min) acetonitrile in water (0.1% TFA), detection at 260 nm.

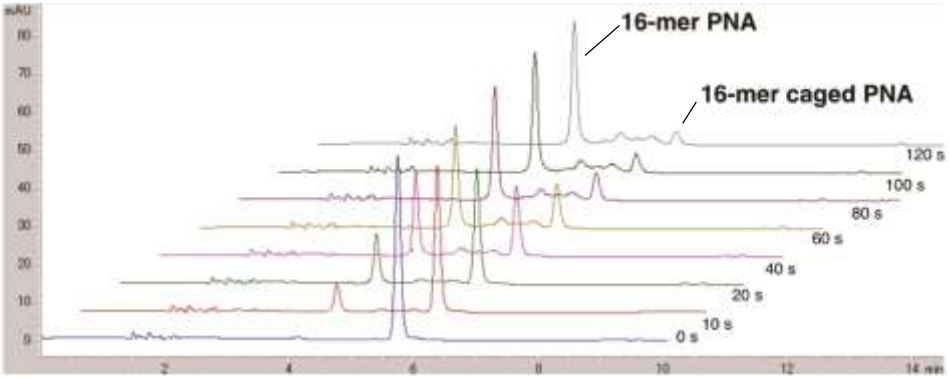


Figure S4. HPLC traces for the photolysis of **16**. Aliquots of the photolysis mixture were analyzed after the specified irradiation time by RP-HPLC with linear gradient of 10–55% (0–10 min) acetonitrile in water (0.1% TFA), detection at 260 nm.

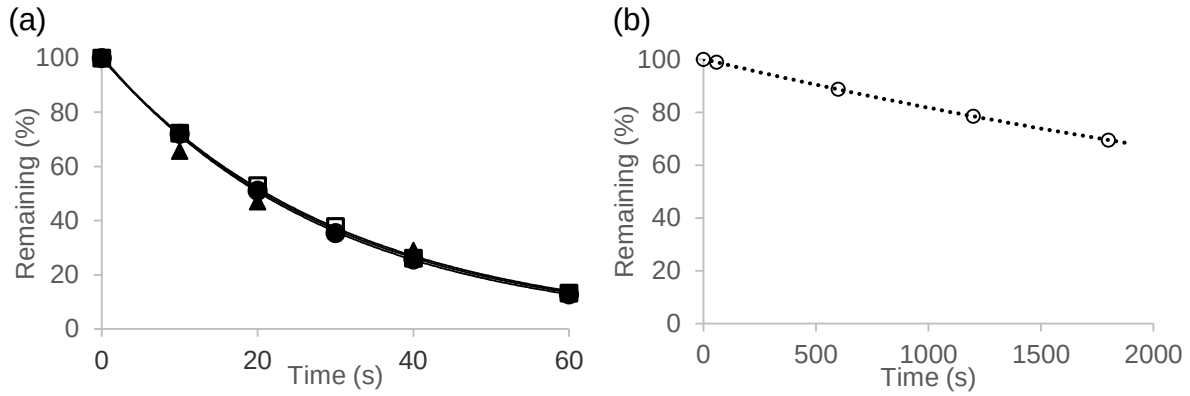


Figure S5. Time course for photolysis of the caged PNAs. Samples (10^{-5} M) were irradiated at 350 nm (4 mJ/s) under simulated physiological conditions (10 mM K-MOPS buffer at pH 7.2). (a) Open squares show consumption of **3**. Closed circles show consumption of **7**. Closed triangles show consumption of **15**. Solid lines show least-squares curve fit to a simple decaying exponential for **3**, **7** and **15**. (b) Open circles show consumption of **13**. Dotted line shows least-squares curve fit to a simple decaying exponential for **13**.

T_m measurement

The stability of PNA/DNA duplexes was determined spectrophotometrically. The temperature–absorbance profiles of the 10-mer PNA/DNA duplexes (1.0 μM each) in 5 mM NaH_2PO_4 were measured within temperatures of 8–95°C for the 10-mer PNA and 4–65°C for the 10-mer cPNA (**15**).

PCR clamping study

The following oligonucleotide primers were used in the PCR reactions. Primer forward (5'-TCATAGCTGTTTCCT-3'), primer reverse (5'-GCCAGCAACGCGGCCTTTTT-3'), primer 3 (5'-GAGCTAACTCACATT-3'). The primers were synthetic oligo DNAs purchased from FASMAC (Japan). Polymerase chain reactions (PCR) were conducted using Blend Taq (Toyobo Co. Ltd., Japan) including Blend Taq (2.5 U/μL), 10 × PCR Buffer for Blend Taq and 2 mM dNTPs. Each PCR reaction

mix (25 μL) contained 2.5 μL of the 10 × PCR buffer, 2.5 μL of 2 mM dNTPs, 0.75 μL of each primer (10 μM in TE buffer), 1 ng of the template pUC18 (10 ng/ μL), 0.5 U of the Blend Taq (2.5 U/μL), indicated concentration of the 10-mer PNA or cPNA (**15**) (100 μM in MilliQ water) and MilliQ water to fill. In photolysis experiments, the PCR reaction mixtures in 0.2 mL thin wall PCR tubes (Quality Scientific Plastics, USA) were exposed to 350-nm light (two RPR 350 nm lamps, 2 mJ/s) for 120 s prior to start PCR. The PCR conditions were 1 cycle of 2 min at 94°C followed by 20 cycles of denaturation (30 s at 94°C), annealing (30 s at 40°C), and extension (30 s at 72°C) using thermal cyclers (T100 Thermal Cycler and iCycler; Bio-Rad Laboratories, Inc., USA).

The amplified products were analyzed using agarose gel electrophoresis (2% agarose, TAE buffer, 100 V, 25 min) The dsDNAs were visualized using SYBR Gold (Molecular Probes Inc., USA) staining (Figs. 4(a) and 4(b)). The fluorescent band intensities were quantified using ImageJ software and a Molecular Imager (ChemiDoc XRS system, Bio-Rad Laboratories, Inc., USA).

Triplex formation

Formations of triplex invasion complexes were analyzed using a gel mobility shift assay. The 16-mer PNA was mixed with the annealed 50 bp dsDNA (0.2 μM) in TE buffer (10 mM Tris, 1 mM EDTA, pH 6.0). The solutions were incubated at 37°C for 24 h and analyzed using a 20% native acrylamide slab gel (TBE buffer, 10 mA, 80 min). The bands were detected by staining with SYBR Gold.

50 bp dsDNA (bold letters: 16-mer PNA binding sequence)

5'-AGCTAGTCATGCGATCTC**TTCTCTTCCTTCTCTT**CTAATGCACGTAACGG-3'
3'-TCGATCAGTACGCTAGAG**AAGAGAAGGAAGAGAA**GATTACGTGCATTGCC-5'

Reference

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