Anthraquinon-2-ylmethoxycarbonyl (Aqmoc): a new photochemically removable protecting group for alcohols

Toshiaki Furuta*,[Å]; Yuuki Hirayama[Å] and Michiko Iwamura[Å]

PRESTO, JST and Department of Biomolecular Science, Toho University, 2-2-1 Miyama, Funabashi, 274-8510 Japan

furuta@biomol.sci.toho-u.ac.jp

Synthesis

1,2,3,4-Di-O-isopropylidene-D-galactopyranosyl anthraquinon-2-ylmethoxycarbonate (1). (General Procedure) To a stirred solution of 236.3 mg (0.9919 mmol) of 2-hydroxymethyl anthraquinone in CH_2Cl_2 (6 mL) was added 132.8 mg (1.09 mmol) of 4-dimethylaminopyridine and 219.4 mg (1.09 mmol) of 4-nitrophenyl chloroformate. The reaction mixture was stirred for 12 hr at room temperature. Thin layer chromatography indicated that the formation of intermediate 4-nitrophenyl carbonate completed. Then, another 137.1 mg (1.12 mmol) of 4-dimethylaminopyridine and 1,2,3,4-di-O-isopropylidene-D-galactopyranose were added to the reaction mixture. After 10 hr, the mixture was diluted with $CHCl_3$, washed with sat. $NaHCO_3$, 1N HCl and sat. NaCl, and dried over $MgSO_4$. Purification by column chromatography (50 g of SiO_2, 25% ethyl acetate in n-hexane) gave 394.9 mg (0.7529 mmol. 76% yield) of **1**.

1. ^{1}H NMR ($CDCl_3$) δ 8.35-8.30 (4H, m), 8.30 (1H, s), 7.84-7.80 (3H, m), 5.54 (1H, d, J=5 Hz), 5.32 (2H, s), 4.63 (1H, dd, J=3 & 8 Hz), 4.36-4.32 (3H, m), 4.26 (1H, dd, J=2 & 8 Hz), 4.09 (1H, ddd, J=1.5, 5 & 8 Hz), 1.51 (3H, s), 1.46 (3H, s),1.35 (3H, s), 1.33 (3H, s): UV (50% THF-H2O) λ_{max}/nm (ε) 257 (37,400), 327 (4,100). ESI-MS m/z 547 ($M+Na^+$)

1,2,3,4-Di-O-isopropylidene-D-galactopyranosyl pyren-1-ylmethoxycarbonate (2). The same procedure as that for **1** was followed except for 219 mg (1.062 mmol) of 7-methoxycoumarin-4-ylmethanol was used. After work-up, purification by column chromatography (35 g of SiO_2, 50% ethyl acetate in n-hexane) gave 312.7 mg (0.6349 mmol. 60% yield) of **2**.

2. ^{1}H NMR ($CDCl_3$) δ 8.32 (1H, d, J=9 Hz), 8.25-8.02 (8H, m), 5.91 (2H, s), 5.52 (1H, d, J=5 Hz), 4.59 (1H, dd, J=2.5 & 8 Hz), 4.34-4.30 (3H, m), 4.24 (1H, dd, J=2 & 8 Hz), 4.06 (1H, ddd, J=1.5, 6 & 8 Hz), 1.45 (3H, s), 1.44 (3H, s),1.31 (3H, s), 1.29 (3H, s): UV (50% THF-H2O) λ_{max}/nm (ε) 276 (31,100), 343 (30,800).

1,2,3,4-Di-O-isopropylidene-D-galactopyranosyl 7-methoxycoumarin-4-ylmethoxycarbonate (3). The same procedure as that for **1** was followed except for 219 mg (1.062 mmol) of 7-methoxycoumarin-4-ylmethanol was used. After work-up, purification by column chromatography (35 g of SiO_2, 50% ethyl acetate in n-hexane) gave 312.7 mg (0.6349 mmol. 60% yield) of **3**.

3. ^{1}H NMR ($CDCl_3$) δ 7.40 (1H, d, J=9 Hz), 6.86 (1H, d, J=9Hz), 6.85 (1H, s), 6.39 (1H, s), 5.55 (1H, d, J=5 Hz), 5.33 (1H, d, J=14 Hz), 5.28 (1H, d, J=14 Hz), 4.64 (1H, dd, J=2 & 5.5 Hz), 4.40-4.33 (2H, m), 4.26 (1H, dd, J=0.5 & 9 Hz), 4.09 (1H, m), 1.52 (3H, s), 1.46 (3H, s),1.35 (3H, s), 1.34 (3H, s): UV (50% THF-H2O) λ_{max}/nm (ε) 218 (16,000), 323 (13,100).

1,2,3,4-Di-O-isopropylidene-D-galactopyranosyl phenanthrene-9-ylmethoxycarbonate (4). The same procedure as that for **1** was followed except for 426 mg (2.05 mmol) of phenanthrene-9-ylmethanol and 226.0 mg (0.8683 mmol) of 1,2,3,4-di-O-isopropylidene-D-galactopyranose were used. After work-up, purification by column chromatography (55 g of SiO_2, 1% methanol in CH_2Cl_2) gave 248.2 mg (0.5019 mmol. 58% yield) of **4**.

4. ^{1}H NMR ($CDCl_3$) δ 8.76-8.64 (2H, m), 8.08 (1H, m), 7.92-7.86 (2H, m), 7.72-7.58 (4H, m), 5.70 (1H, dd, J=0.5 & 13 Hz), 5.68 (1H, dd, J=0.5 & 13 Hz), 5.53 (1H, d, J=5 Hz), 5.33 (1H, d, J=14 Hz), 5.28 (1H, d, J=14 Hz), 4.60 (1H, dd, J=2.5 & 7.5 Hz), 4.36-4.30 (3H, m), 4.26 (1H, dd, J=2 & 7.5 Hz), 4.09 (1H, ddd, J=1.5, 6 & 6 Hz), 1.45 (3H, s), 1.44 (3H, s),1.32 (3H, s), 1.30 (3H, s): UV (50% THF-H_2O) λ_{max}/nm (ε) 297 (9,530).

5'-(Anthraquinon-2-ylmethoxycarbonyl)-2',3'-O-isopropylideneadenosine. To a stirred suspension of 240.1 mg (1.008 mmol) of 2-hydroxymethyl anthraquinone in CH_2Cl_2 (5 mL) were added 135.4 mg (1.108 mmol) of 4-dimethylaminopyridine and 224.9 mg (1.116 mmol) of 4-nitrophenyl chloroformate at room temperature. After 7.5 hr, another 137.7 mg (1.127 mmol) of 4-dimethylaminopyridine and 2',3'-isopropylideneadenosine were added. The reaction mixture was stirred at room temperature for 17 hr and at 45°C for 2 days. The mixture was cooled, diluted with $CHCl_3$, and washed with sat. $NaHCO_3$, 1N HCl and sat. NaCl. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotovap and high vacuum rotovap to yield 494.2 mg (0.8647 mmol, 86% yield) of 5'-(anthraquinon-2-ylmethoxycarbonyl)-2',3'-O-isopropylideneadenosine which can be used for the next reaction without further purification. The pure sample for the

spectroscopic measurements was obtained by column chromatography (75 g of SiO_2, 3.5% methanol in $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ 8.35-8.25 (5H, m), 7.90 (1H, s), 7.83-7.73 (3H, m), 6.15 (1H, d, J=1.5 Hz), 5.68 (2H, brs), 5.45 (1H, dd, J=2 &6 Hz), 5.26 (2H, s), 5.12 (1H, dd, J=3 & 6 Hz), 4.55-4.45 (2H, m), 4.36 (1H, dd, J=5 & 11 Hz), 1.62 (3H, s), 1.40 (3H, s).

5'-(Anthraquinon-2-ylmethoxycarbonyl)adenosine (5). To a stirred suspension of 99.5 mg (0.174 mmol) of 5'-(9,10-dioxo-anth-2-ylmethoxycarbonyl)-2',3'-O-isopropylideneadenosine in THF-H2O (1 mL-0.5 mL) was added 3 mL of trifluoroacetic acid at room temperature. After 13 hr, the solvents were evaporated by rotovap and high vacuum rotovap to yield 110.3 mg (0.171 mmol, 98% yield) of **5**.

5. ^{1}H NMR (CD_3OD) δ 8.38-8:20 (6H, m), 7.90-7.80 (3H, m), 6.10 (1H, d, J=5 Hz), 5.38 (1H, dÅ€13.5 Hz), 5.34 (1H, d, J=13.5 Hz), 4.61 (1H, m), 4.55-4.53 (2H, m), 4.37-4.32 (2H, m).

Photolysis.

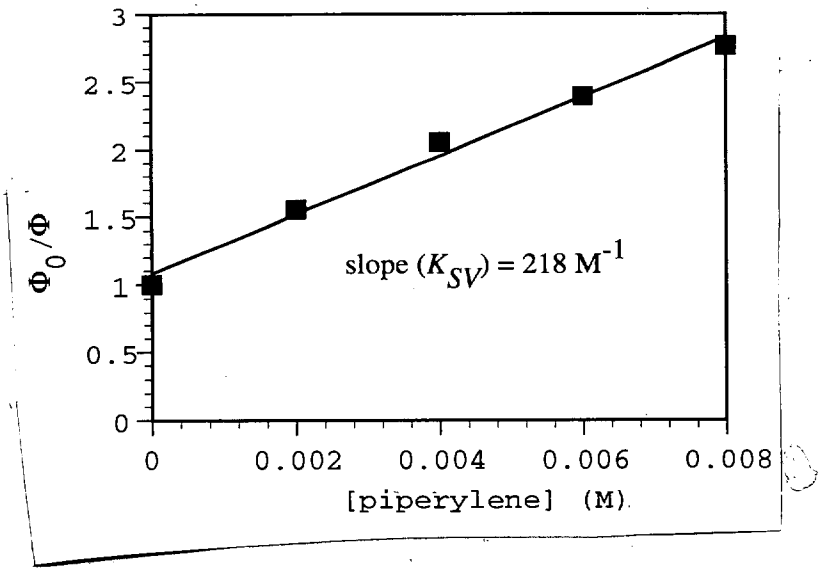
Preparative scale photolysis of 1. 100.1 mg (0.191 mmoml) of 1 was dissolved in 50 mL of THF (WAKO Pure Chemicals, no stabilizer included) and 50 mL of deionized water. Into each of 15 mL Pyrex test tubes (10Φ) were placed 14 mL of the resulting solution of 1 (2 X 10^{-3} M). The solution was irradiated at 350 nm using RPR 350 nm lamp X 8 for 150 min. The solvents were evaporated by rotovap and high vacuum rotovap to yield crude products. Purification by silica gel column chromatography gave 32.8mg (0.126 mmol, 66% yield) of 1,2,3,4-di-O-isopropylidene-D-galactopyranose and 8.3 mg (0.015 mmol, 16% yield) of Bis-(1,2,3,4-di-O-isopropylidene-D-galactopyranosyl) carbonate.

Quantum efficiency measurement. Into a pyrex test tube (12Φ) was placed 2 mL of 100 μM substrate solution in 50% THF-H_2O. The solution was irradiated at 350 nm using RPR 350 nm lamp X 16. Aliquots of 10μL were removed periodically and analyzed by HPLC. The light output for the quantum efficiencies measurement was performed using ferrioxalate actinometry.

Quenching studies of Aqmoc-Gal (1) in THF-H_2O with (E)-1,3-pentadiene (piperylene). Into each of 10 mL Pyrex tubes were placed 10 μL of 1 stock solution (10 mM in DMSO), 0 to 100 μL of piperylene stock solution (1M in DMSO) and 50% THF-H_2O to fill. Into each of six Pyrex test tubes was placed 2 mL of the above solutions. The solution was

irradiated at 350 nm using RPR 350 nm lamp X 4. Aliquots of 10μL were removed periodically and analyzed by HPLC.

Fig. Stern-Volmer plot for photolysis of 1 in 50% THF-H_2O at 350 nm.



Photolysis of 5. Into a 10 mL Pyrex tube was placed 10 μL of 5 stock solution (10 mM in DMSO) and 50% THF-KMOPS buffer (10 mM MOPS and 100 mM KCl, pH=7.2) to fill. The solution (10 μM 5 in 50% THF-KMOPS buffer) was irradiated at 350 nm using RPR 350 nm lamp X 4. Aliquots of 10μL were removed periodically and analyzed by reversed phase HPLC using Cosmosil 5C-18-AR-II (250 X 4, Nakalai) column and 60% CH_3CN-H_2O (containing 0.1% TFA) as an eluent. The HPLC traces were recorded by detecting UV absorption at 260 nm. The typical retention time of 5 is 7.5 min and that of adenosine is 3.0 min. After the photolysis was completed, the product yield was estimated by the following equation, (% yield) = 100 X [(the area of the produced adenosine peak)/(extinction coefficient of adenosine at 260 nm)]/[(the area of the starting 5 peak)/(extinction coefficient of 5 at 260 nm)]. At 260 nm, the extinction coefficients of adenosine and 5 are 1,410 $M^{-1}cm^{-1}$ and 4,190 M^{-1} cm^{-1}, respectively. The peak area s, taken from the HPLC traces, of the produced adenosine and the starting 5 were 49,654 and 162,407, respectively. Therefore, the estimated yield of the produced adenosine was 91%.