Novel bifunctional probe for radioisotope-free photoaffinity labeling: compact structure comprised of photospecific ligand ligation and detectable tag anchoring units

Takamitsu Hosoya,[a,b] Toshiyuki Hiramatsu,[b] Takaaki Ikemoto,[c] Masayuki Nakanishi,[b] Hiroshi Aoyama,[b] Ayako Hosoya,[a] Tomoya Iwata,[a] Kei Maruyama,[c] Makoto Endo[c] and Masaaki Suzuki*[a,b]

[a] *Division of Regeneration and Advanced Medical Science, Gifu University Graduate School of Medicine, Yanagido 1-1, Gifu 501-1193, Japan. E-mail: suzukims@biomol.gifu-u.ac.jp*
[b] *Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*
[c] *Department of Pharmacology, Saitama Medical School, Moroyama-machi, Saitama 350-0495, Japan*

Supplementary Data

General remarks

THF was distilled over sodium benzophenone ketyl under Ar. Optically active statin side-chain unit **14** was prepared by Swern oxidation of the corresponding alcohol,[1] which was donated from Kaneka Co. All other chemical reagents used were commercial grade. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm) silica-gel plates (MERCK, Silica Gel 60 F_{254}, Cat. No. 1.05715 Kieselgel 60 F_{254} or MERCK, RP-18 F_{254S}, Cat. No. 1.15389). Preparative TLC was carried out using precoated (0.5 mm) silica-gel plates (MERCK, Silica Gel 60 F_{254}, Cat. No. 1.05744). Column chromatography was conducted using silica-gel (MERCK, Silica Gel 60 (40–63 μm), Cat. No. 109385), neutral silica-gel (Kanto, Silicagel 60N (spherical), Cat. No. 37563-79), reversed-phase silica-gel (Fuji Silysia, Chromatorex-ODS DM1020T, 100-200 mesh), or alumina (ICN, activity 1, Cat. No. 02084). ^{1}H, ^{13}C, and ^{19}F NMR spectra were obtained with a JEOL JNM AL-400 spectrometer. $CDCl_3$ (CIL), DMSO-d_6 (CIL), cyclohexane-d_{12} (CIL), or CD_3OD (ACROS) was used as a solvent for obtaining NMR spectra. Chemical shifts (δ) are given in parts per million (ppm) downfield from $(CH_3)_4Si$ (δ 0.00 for ^{1}H NMR in $CDCl_3$), CF_3COOH (δ 0.00 for ^{19}F NMR), or solvents (for ^{13}C NMR and ^{1}H NMR in DMSO-d_6, cyclohexane-d_{12}, and CD_3OD) as internal references with coupling constants (J) in Hz. The abbreviations s, d, t, q, m, and br signify singlet, doublet, triplet, quartet, multiplet, and broad respectively. IR spectra were recorded on a SHIMADZU FTIR-8100A spectrophotometer with the absorption band given in cm^{-1}. UV spectra were recorded on a JASCO Ubest-55 UV/VIS spectrophotometer. Fluorescence excitation and emission spectra were measured on a SHIMADZU RF-5300PC spectrofluorophotometer. High-resolution mass spectra (HRMS) were measured on a JEOL JMS-700 mass spectrometer at Nagoya University Chemical Instrument Center under electron impact ionization (EI) or positive fast atom bombardment (FAB^+) conditions.

CAUTION ! **Azido-containing compounds are presumed to be potentially explosive. Although we have never experienced such an explosion with azido-functionalized compounds used in this study, all manipulations should be carefully carried out behind a safety shield in a hood.**

Photo-decomposition study of phenyl azide in the presence of benzyl azide.

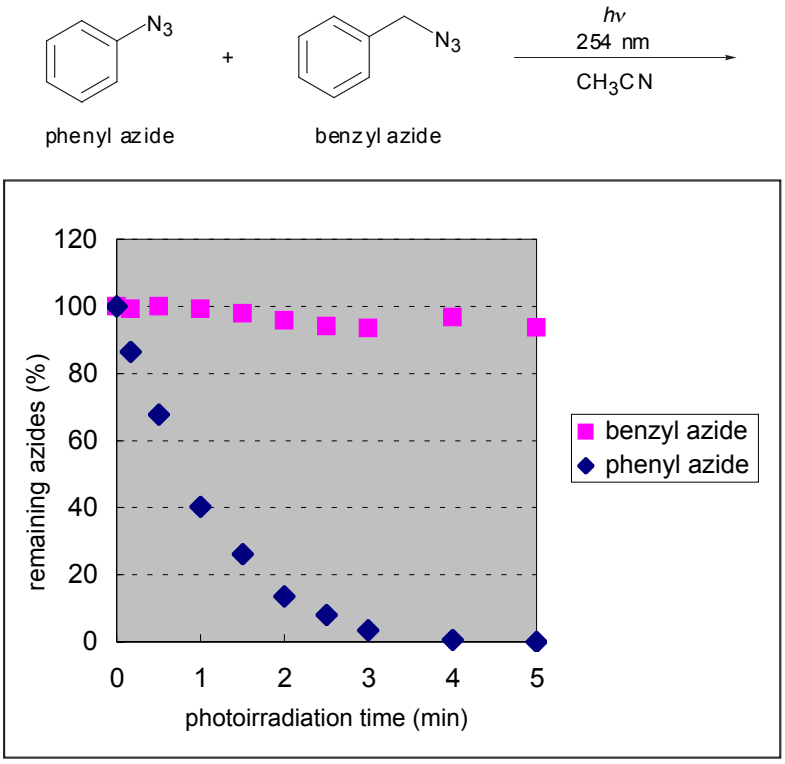
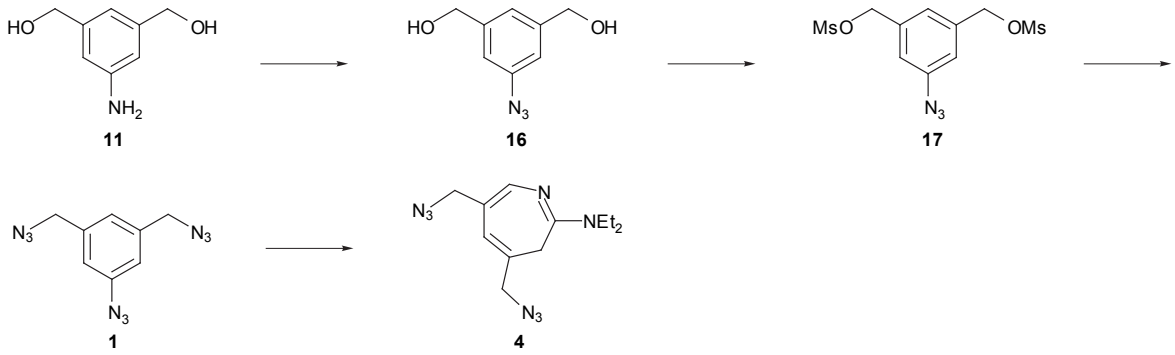


Figure S1. Time course of remaining amounts of phenyl and benzyl azides under photo-irradiation. A 1 mM solution of a 1:1 mixture of phenyl and benzyl azides in acetonitrile (40 μL) was exposed to UV with a wavelength of 254 nm (UVP, UVG-11, 4 W) at 22 °C from the distance of 1 cm. The amounts of remaining azide substrates were quantified by HPLC analysis by using anisole as an internal standard. The mean values for three independent experiments are shown. All of the SE mean values were within the range of the symbols thereby omitted. HPLC analysis was performed on JASCO GULLIVER instrument equipped with MD-910 multi-wavelength detector; Mightysil RP-18 GP column (Kanto), 150 × 4.6 mm i.d., eluted with CH_3CN/H_2O = 40/60, flow rate 1.0 mL/min, column oven temperature 40 °C; retention time: phenyl azide; 17.7 min, benzyl azide; 15.4 min (anisole, 9.8 min).

Synthesis and photoreaction of 1-azido-3,5-di(azidomethyl)benzene (1).



To a solution of 3,5-di(hydroxymethyl)aniline (**11**)[2] (306 mg, 2.00 mmol) in a 9:1 mixture of acetic acid and water (15 mL) was added $NaNO_2$ (207 mg, 3.00 mmol) at 0 °C and the mixture was stirred for 3 min. To this was added NaN_3 (195 mg, 3.00 mmol) at the same temperature and stirring was continued for 15 min. To this was added saturated aqueous $NaHCO_3$ solution followed by $NaHCO_3$ powder until the mixture reached ca. pH 7. The mixture was extracted with EtOAc (×3) and the combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was recrystallized from EtOAc to give 1-azido-3,5-di(hydroxymethyl)benzene (**16**) (314 mg, 87.6%); orange solid; TLC R_f = 0.45 (*n*-hexane/EtOAc = 1/6); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.48 (d, 4H, J = 5.8 Hz), 5.27 (t, 2H, J = 5.8 Hz), 6.91 (br s, 2H), 7.07 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 62.5 (2C), 114.9 (2C), 121.0, 138.9, 144.6 (2C); IR (KBr, cm^{-1}) 640, 706, 849, 882, 1005, 1014, 1069, 1308, 1458, 2114, 2859, 2903, 3225; HRMS (EI) *m/z* 179.0688, (M^+, $C_8H_9N_3O_2$ requires 179.0695).

To a solution of **16** (100 mg, 558 μmol) in DMF (1.5 mL) were successively added Et_3N (350 μL, 2.51 mmol) and CH_3SO_2Cl (175 μL, 2.23 mmol) at 0 ˚C. After stirring for 1 h at the same temperature, to this was added 2 M aqueous HCl solution and the mixture was extracted with EtOAc (×3). The combined organic extracts were successively washed with water (×3) and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 1/1) to give 1-azido-3,5-di(methanesulfonyloxymethyl)benzene (**17**) (132 mg, 70.6%); colorless solid; TLC R_f = 0.34 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.03 (s, 6H), 5.22 (s, 4H), 7.08 (br s, 2H), 7.22 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.3 (2C), 69.7 (2C), 119.4 (2C), 124.3, 136.2 (2C), 141.4; IR (KBr, cm^{-1}) 505, 529, 837, 932, 976, 1019, 1175, 1352, 2116; HRMS (EI) *m/z* 307.0177, ($(M - N_2)^+$, $C_{10}H_{13}NO_6S_2$ requires 307.0184).

To a solution of **17** (63.1 mg, 188 μmol) in DMF (2.0 mL) was added NaN_3 (30.6 mg, 470 μmol) at 0 ˚C and the mixture was stirred for 26 h at room temperature. To this was added water and the mixture was extracted with Et_2O (×3). The combined organic extracts were successively washed with water (×3) and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 9/1) to give 1-azido-3,5-di(azidomethyl)benzene (**1**) (37.7 mg, 87.2%); pale yellow oil; TLC R_f = 0.39 (*n*-hexane/EtOAc = 9/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.37 (s, 4H), 6.95 (br s, 2H), 7.04 (br s, 1H); ^{1}H NMR (400 MHz,

cyclohexane-d_{12}) δ 4.21 (s, 4H), 6.87 (br s, 2H), 6.95 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.9 (2C), 118.0 (2C), 123.6, 137.8 (2C), 141.0; IR (KBr, cm^{-1}) 847, 1233, 1248, 1297, 1308, 1348, 1456, 1597, 1609, 2104 cm^{-1}; UV (EtOH, nm) λ_{max} (log ε) 252 (4.11); HRMS (EI) *m/z* 229.0830, (M^+, $C_8H_7N_9$ requires 229.0824).

*Photoreaction of **1** in the presence of excess amounts of diethylamine.*

A 20 mM solution of triazido compound **1** in cyclohexane-d_{12} (700 μL, 14.0 μmol, in the presence of 10 mM phthalan as an internal standard) and Et_2NH (434 μL, 4.20 mmol) were put in a quartz NMR tube (Nihon Seimitsu Kagaku, N-5Q) under Ar atmosphere. The mixture was exposed to UV with a wavelength of 254 nm (Asahi Spectra, LAX-101, 100 W) side-by-side at 22 °C. The course of the reaction was monitored by ^{1}H NMR (Chart S1). The yields of the new product **4** and the remaining amount of **1** were quantified by comparing the values of integration of the peaks for these compounds (4H at δ 4.21 ppm for **1** and 1H at δ 6.14 ppm for **4**) with that of phthalan (4H at δ 4.97 ppm). The monitoring was continued for 12 h in total period of irradiation and the result is shown in Figure S2.

For the isolation purpose of **4**, a solution of **1** (24.4 mg, 106 μmol) and Et_2NH (3.30 mL, 32.0 mmol) in cyclohexane (10 mL) was distributed equally into two quartz test tubes, which were independently irradiated by UV with a wavelength of 254 nm (Asahi Spectra, LAX-101, 100 W) side-by-side at 22 °C for 5 h under continuous bubbling of Ar through the reaction mixture. Both of the reaction mixtures were collected and concentrated under reduced pressure to approximately one third of the total volume. To this was added *n*-hexane (ca. 10 mL) and the mixture was concentrated under reduced pressure to approximately one third of the total volume. This process was repeated five times and at the last time, the mixture was concentrated to approximately one fifth of the total volume. The crude product was purified by alumina column chromatography (*n*-hexane/EtOAc = 6/1), preparative TLC (*n*-hexane/EtOAc = 1/1 containing a small amount of Et_2NH), and silica-gel column chromatography (neutral, Et_2O) to give 4,6-di(azidomethyl)-2-diethylamino-3*H*-azepine (**4**) (5.9 mg, 20%). The starting material **1** (7.6 mg, 31%) was recovered; pale yellow oil; TLC R_f = 0.50 (*n*-hexane/EtOAc = 1/1); ^{1}H NMR (400 MHz, cyclohexane-d_{12}, observed at 50 °C because two methylene protons on the 3*H*-azepine ring were not observable clearly at room temperature owing to conformational exchange,[3] see Chart S2 for the temperature dependency) δ 1.12 (t, 6H, *J* = 6.7 Hz), 2.76 (br s, 2H), 3.41 (q, 4H, *J* = 6.7 Hz), 3.75 (s, 2H), 3.82 (s, 2H), 6.14 (s, 1H), 7.09 (s, 1H); ^{13}C NMR (100 MHz, cyclohexane-d_{12}) δ 27.0 (2C), 33.6 (2C), 43.9, 57.0, 57.5, 115.2, 119.8, 128.7, 143.1, 145.2; IR (KBr, cm^{-1}) 639, 851, 1022, 1080, 1113, 1144, 1177, 1198, 1260, 1316, 1347, 1360, 1379, 1433, 1524, 1566, 1626, 2102, 2207, 2872, 2930, 2977; HRMS (FAB$^+$) *m/z* 275.1741, ($(M + H)^+$, $C_{12}H_{19}N_8$ requires 275.1733).

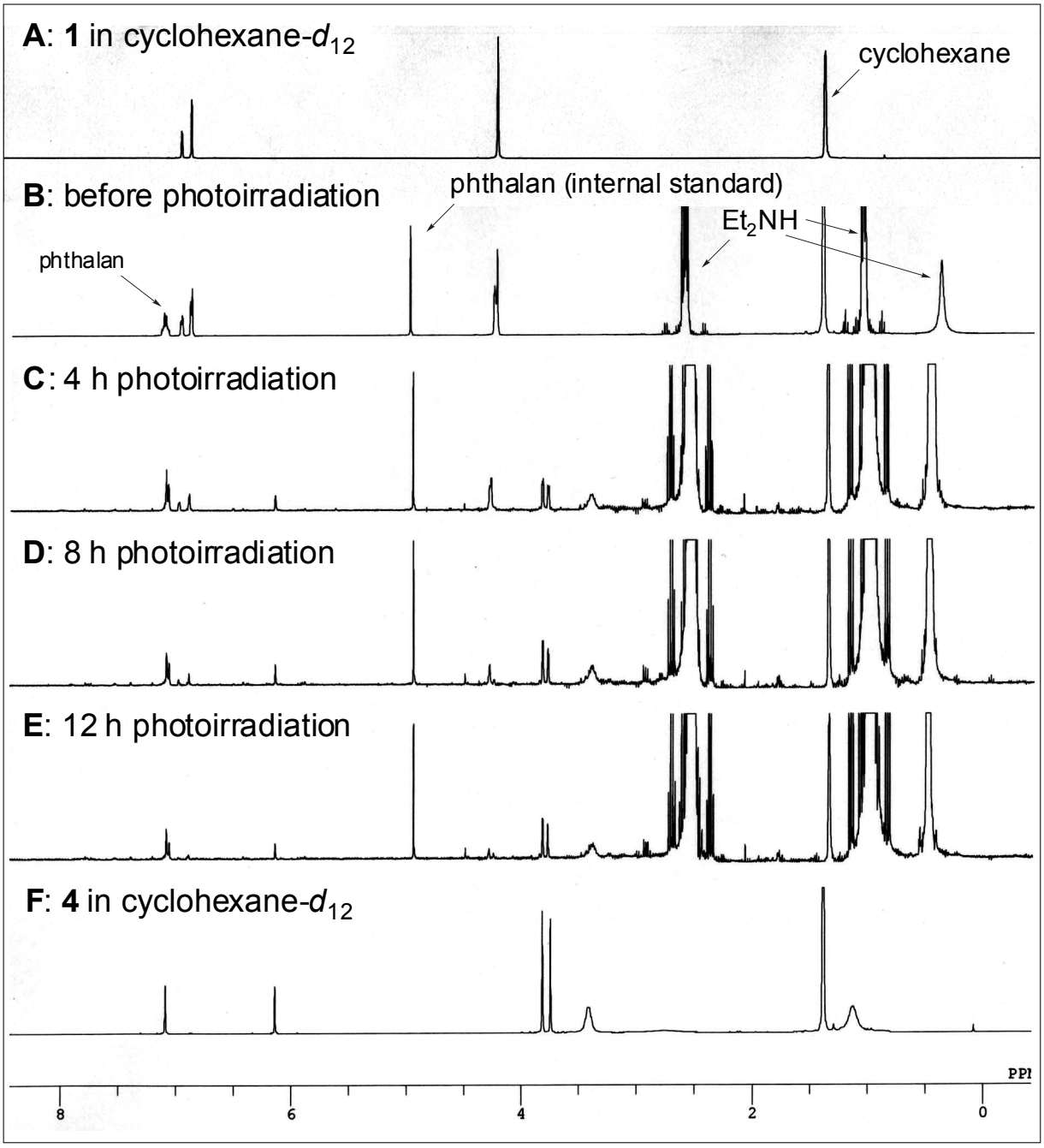


Chart S1. Photoreaction of **1** in cyclohexane-d_{12} monitored by ^{1}H NMR. The reaction was carried out at 22 °C in the presence of excess diethylamine using a quartz tube. Phthalan was used as an internal standard for quantification.

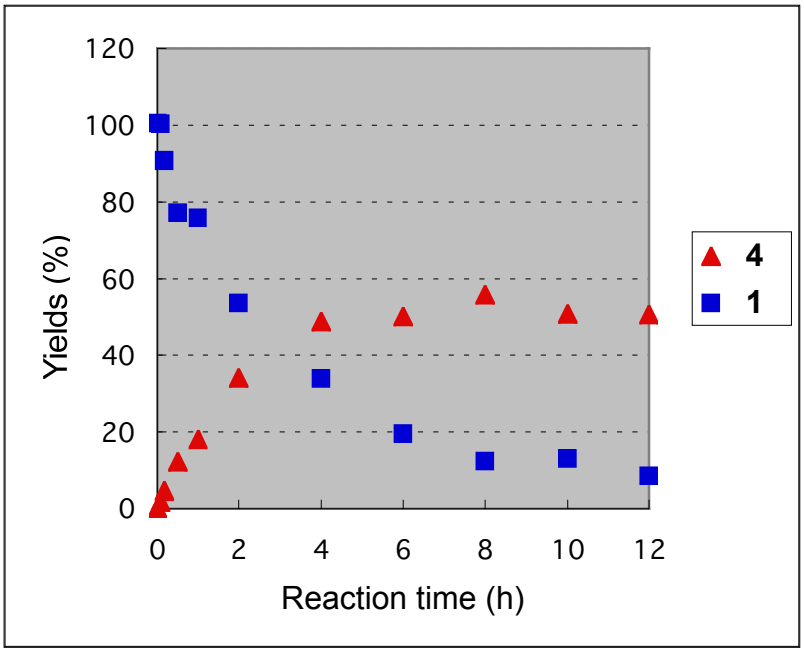


Figure S2. Time course of the photoreaction of **1** in cyclohexane-d_{12} at 22 °C in the presence of excess diethylamine. The reaction was monitored by ^{1}H NMR. The amounts of **1** and **4** were quantified using phthalan as an internal standard.

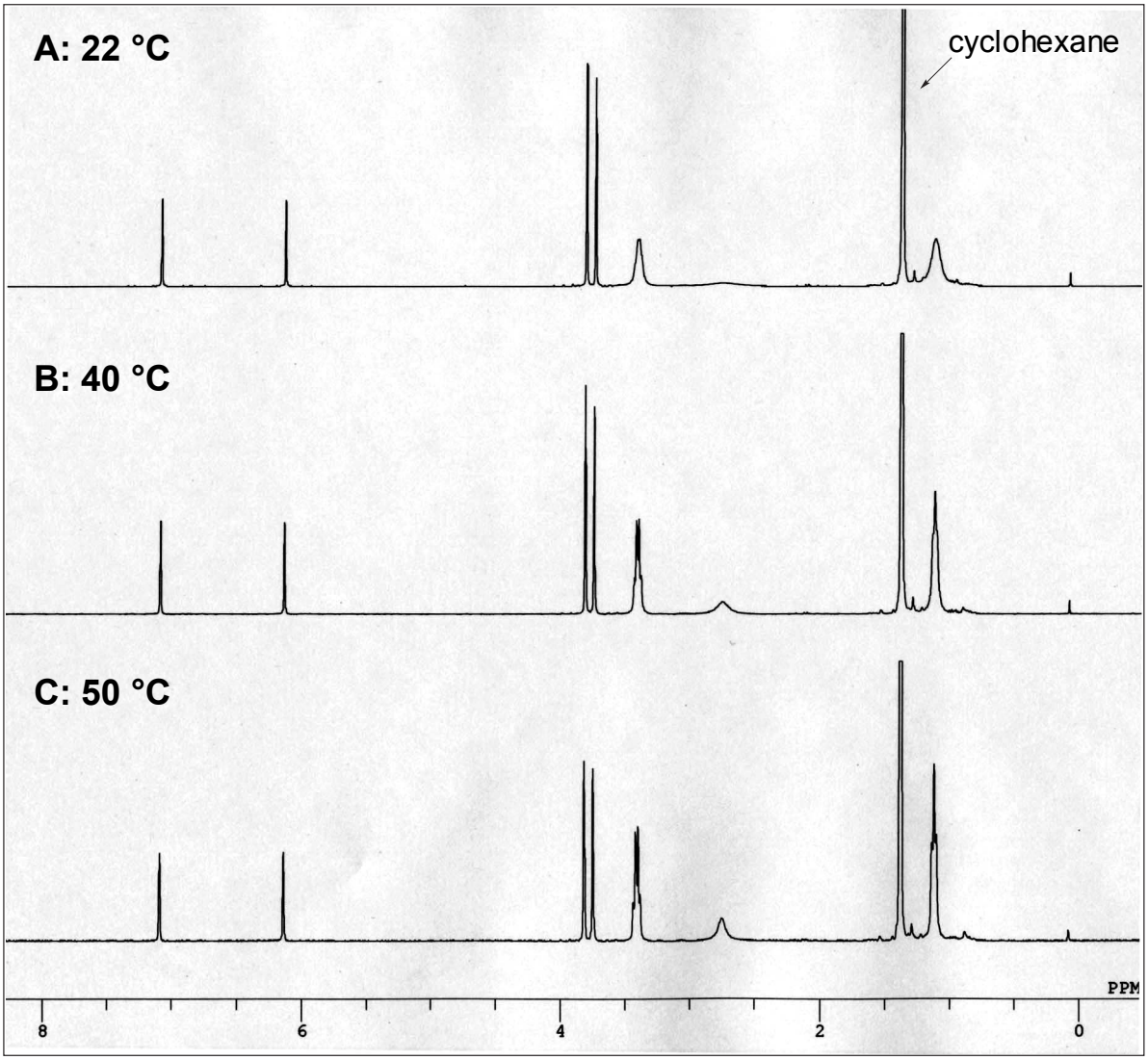
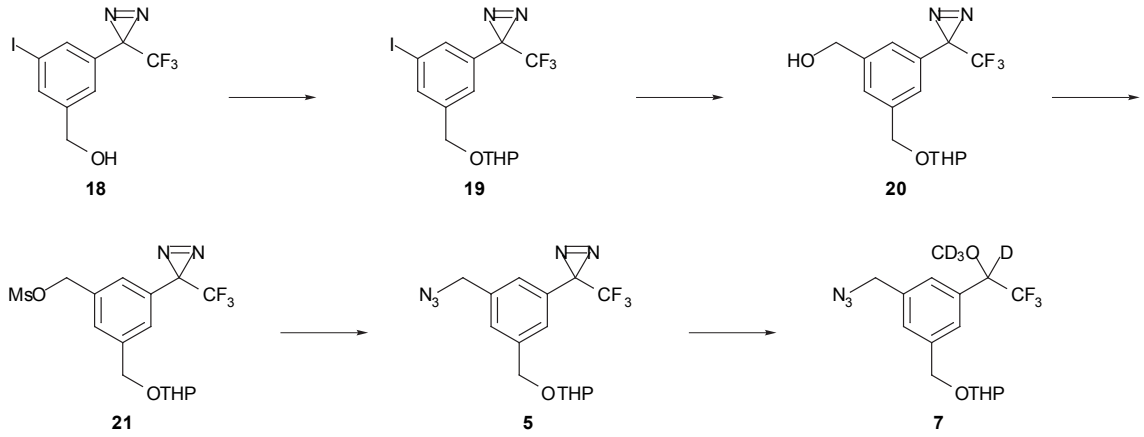


Chart S2. ^{1}H NMR spectra of **4** in cyclohexane-d_{12} measured at various temperatures.

Synthesis and photoreaction of 3-[3-azidomethyl-5-(tetrahydropyran-2-yloxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (5).



To a solution of 3-[3-iodo-5-(hydroxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (**18**)[4] (100 mg, 292 μmol) in CH_2Cl_2 (3.5 mL) were successively added 3,4-dihydro-2*H*-pyran (55.0 μL, 603 μmol) and pyridinium *p*-toluenesulfonate (7.3 mg, 29 μmol) and the mixture was stirred at 40 °C for 7 h. After cooling the reaction mixture to room temperature, the mixture was extracted with Et_2O (×3). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 20/1) to give 3-[3-iodo-5-(tetrahydropyran-2-yloxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (**19**) (124 mg, 99.7%); pale yellow oil; TLC R_f = 0.23 (*n*-hexane/EtOAc = 20/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54–1.90 (m, 6H), 3.52–3.58 (m, 1H), 3.86 (ddd, 1H, *J* = 3.1, 8.5, 11.4 Hz), 4.43 (d, 1H, *J* = 12.6 Hz), 4.67 (dd, 1H, *J* = 3.4, 3.4 Hz), 4.72 (d, 1H, *J* = 12.6 Hz), 7.16 (br s, 1H), 7.40 (br s, 1H), 7.76 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.4, 25.5, 27.9 (q, $^2J_{C–F}$ = 41.1 Hz), 30.5, 62.3, 67.3, 94.4, 98.1, 121.7 (q, $^1J_{C–F}$ = 274.7 Hz), 124.7, 131.0, 134.1, 137.6, 141.3; ^{19}F NMR (372 MHz, $CDCl_3$) δ 10.5 (s, 3F); IR (KBr, cm^{-1}) 1038, 1078, 1130, 1159, 1200, 1246, 1341, 2946; HRMS (FAB$^+$) *m/z* 448.9960, ((M + Na)$^+$, $C_{14}H_{14}F_3IN_2O_2Na$ requires 448.9950).

A solution of **19** (128 mg, 300 μmol), *n*Bu_3SnCH_2OH[5] (145 mg, 450 μmol), and $(Ph_3P)_4Pd$ (17.3 mg, 15.0 μmol) in 1,4-dioxane (3.0 mL) was stirred at 80 °C for 4 h under Ar atmosphere. After cooling the reaction mixture to room temperature, saturated aqueous KF solution was added and then the mixture was filtered through a pad of Celite using EtOAc. The filtrate was washed successively with a saturated aqueous KF solution (×2), saturated aqueous $NaHCO_3$ solution, water, and brine. The resulting solution was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (neutral, *n*-hexane/EtOAc = 4/1) to give 3-[3-hydroxymethyl-5-(tetrahydropyran-2-yloxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (**20**) (29.9 mg, 30.2%); brown oil; TLC R_f = 0.25 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55–1.90 (m, 7H), 3.52–3.58 (m, 1H), 3.89 (ddd, 1H, *J* = 3.4, 8.0, 11.4 Hz), 4.49 (d, 1H, *J* = 12.3 Hz), 4.69 (dd, 1H, *J* = 3.4, 3.4 Hz), 4.71 (d, 2H, *J* = 6.0 Hz), 4.78 (d, 1H, *J* = 12.3 Hz), 7.12 (br s, 2H), 7.41 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.4, 25.5, 28.5 (q, $^2J_{C–F}$ = 40.3 Hz), 30.6, 62.3, 64.6, 68.2, 98.0, 122.0 (q, $^1J_{C–F}$ = 273.8 Hz),

123.7, 124.6, 127.1, 129.4, 139.5, 141.8; ^{19}F NMR (372 MHz, $CDCl_3$) δ 10.5 (s, 3F); IR (KBr, cm^{-1}) 704, 814, 849, 872, 907, 949, 976, 1038, 1076, 1125, 1156, 1175, 1202, 1277, 1323, 1354, 1443, 1456, 1466, 1605, 2946, 3415; HRMS (FAB^+) *m/z* 353.1072, ($(M + Na)^+$, $C_{15}H_{17}F_3N_2O_3Na$ requires 353.1089).

To a solution of **20** (13.9 mg, 42.1 μmol) in CH_2Cl_2 (1.0 mL) were successively added Et_3N (17.5 μL, 126 μmol) and CH_3SO_2Cl (6.5 μL, 84 μmol) at 0 ˚C. After stirring for 2 h at the same temperature, to this was added water and the mixture was extracted with Et_2O (×3). The combined organic extracts were successively washed with water (×3) and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 9/1, 6/1, 3/1) to give 3-[3-methanesulfonyloxymethyl-5-(tetrahydropyran-2-yloxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (**21**) (15.2 mg, 88.4%); colorless solid; TLC R_f = 0.67 (*n*-hexane/EtOAc/CH_2Cl_2 = 1/1/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55–1.90 (m, 6H), 2.99 (s, 3H), 3.52–3.59 (m, 1H), 3.87 (ddd, 1H, *J* = 3.4, 8.0, 11.4 Hz), 4.51 (d, 1H, *J* = 12.6 Hz), 4.69 (dd, 1H, *J* = 3.4, 3.4 Hz), 4.80 (d, 1H, 12.6 Hz), 5.22 (s, 2H), 7.13 (br s, 1H), 7.22 (br s, 1H), 7.47 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.8, 25.8, 28.7 (q, $^2J_{C–F}$ = 40.3 Hz), 30.9, 38.7, 62.7, 68.1, 70.4, 98.5, 122.1 (q, $^1J_{C–F}$ = 273.9 Hz), 125.7, 126.4, 129.0, 130.3, 134.8, 140.7; ^{19}F NMR (372 MHz, $CDCl_3$) δ 10.5 (s, 3F); IR (KBr, cm^{-1}) 743, 816, 912, 939, 974, 1038, 1078, 1125, 1177, 1204, 1279, 1356, 2946; HRMS (FAB^+) *m/z* 431.0852, ($(M + Na)^+$, $C_{16}H_{19}F_3N_2O_5SNa$ requires 431.0864).

To a solution of **21** (21.3 mg, 52.2 μmol) in DMF (0.5 mL) was added NaN_3 (7.0 mg, 0.11 mmol) at 0 ˚C and the mixture was stirred for 4.5 h at room temperature. To this was added water and the mixture was extracted with Et_2O (×3). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 20/1) to give 3-[3-azidomethyl-5-(tetrahydropyran-2-yloxymethyl)phenyl]-3-trifluoromethyl-3*H*-diazirine (**5**) (11.9 mg, 64.2%); colorless oil; TLC R_f = 0.33 (*n*-hexane/EtOAc = 9/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54–1.93 (m, 6H), 3.52–3.59 (m, 1H), 3.88 (ddd, 1H, *J* = 3.4, 8.2, 11.4 Hz), 4.37 (s, 2H), 4.51 (d, 1H, *J* = 12.6 Hz), 4.69 (dd, 1H, *J* = 3.6, 3.6 Hz), 4.79 (d, 1H, *J* = 12.6 Hz), 7.04 (br s, 1H), 7.16 (br s, 1H), 7.37 (br s, 1H); ^{1}H NMR (400 MHz, CD_3OD) δ 1.40–1.82 (m, 6H), 3.41–3.47 (m, 1H), 3.78 (ddd, 1H, *J* = 3.1, 8.2, 11.4 Hz), 4.34 (s, 2H), 4.45 (d, 1H, *J* = 12.6 Hz), 4.61 (dd, 1H, *J* = 2.9, 4.1 Hz), 4.68 (d, 1H, *J* = 12.6 Hz), 7.04 (br s, 1H), 7.14 (br s, 1H), 7.38 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.4, 25.5, 28.5 (q, $^2J_{C–F}$ = 39.5 Hz), 30.6, 54.3, 62.3, 67.9, 98.1, 121.9 (q, $^1J_{C–F}$ = 274.7 Hz), 124.9, 125.2, 128.2, 129.8, 136.5, 140.1; ^{19}F NMR (372 MHz, $CDCl_3$) δ 10.6 (s, 3F); ^{19}F NMR (372 MHz, CD_3OD) δ 10.8 (s, 3F); IR (KBr, cm^{-1}) 872, 907, 972, 1038, 1078, 1125, 1157, 1177, 1202, 1275, 1323, 1385, 1456, 1607, 2103, 2872, 2946; UV (EtOH, nm) λ_{max} (log ε) 353 (2.55); HRMS (FAB^+) *m/z* 378.1148, ($(M + Na)^+$, $C_{15}H_{16}F_3N_5O_2Na$ requires 378.1154).

*Photoreaction of **5** in CD_3OD.*

A solution of **5** (3.0 mg, 8.4 μmol) in deaerated CD_3OD (0.75 mL) placed in a quartz NMR tube (Nihon Seimitsu Kagaku, N-5Q) under Ar atmosphere was continuously irradiated side-by-side by UV with a wavelength of 365 nm (UVP, UVL-56, 6 W) for 10 min and 302 nm (UVP, UVM-57, 6 W) for 8 min at 22 °C. The course of the reaction was monitored by ^{19}F and ^{1}H NMR (Charts S3 and S4). After concentration of the reaction mixture under reduced pressure, the crude product was purified by

preparative TLC (*n*-hexane/EtOAc = 6/1) to give 1-azidomethyl-3-(2,2,2-trifluoro-1-[$^2H_3$]methoxy[1-^{2}H]ethyl)-5-(tetrahydropyran-2-yloxymethyl)benzene (**7**) (2.5 mg, 82%); pale yellow oil; TLC R_f = 0.31 (*n*-hexane/EtOAc = 6/1); ^{1}H NMR (400 MHz, CD_3OD) δ 1.50–1.91 (m, 6H), 3.50–3.57 (m, 1H), 3.90 (ddd, 1H, *J* = 3.1, 8.5, 11.4 Hz), 4.42 (s, 2H), 4.56 (d, 1H, *J* = 12.3 Hz), 4.71 (dd, 1H, *J* = 3.1, 4.1 Hz), 4.79 (d, 1H, 12.3 Hz), 7.37 (br s, 1H), 7.42 (br s, 1H), 7.43 (br s, 1H); ^{19}F NMR (372 MHz, CD_3OD) δ –0.4 (s, 3F); IR (KBr, cm^{-1}) 737, 858, 870, 907, 976, 1038, 1069, 1136, 1169, 1184, 1202, 1264, 1288, 1320, 1343, 2101, 2855, 2872, 2944; HRMS (FAB$^+$) *m/z* 386.1617, ((M + Na)$^+$, $C_{16}H_{16}D_4F_3N_3O_3Na$ requires 386.1606).

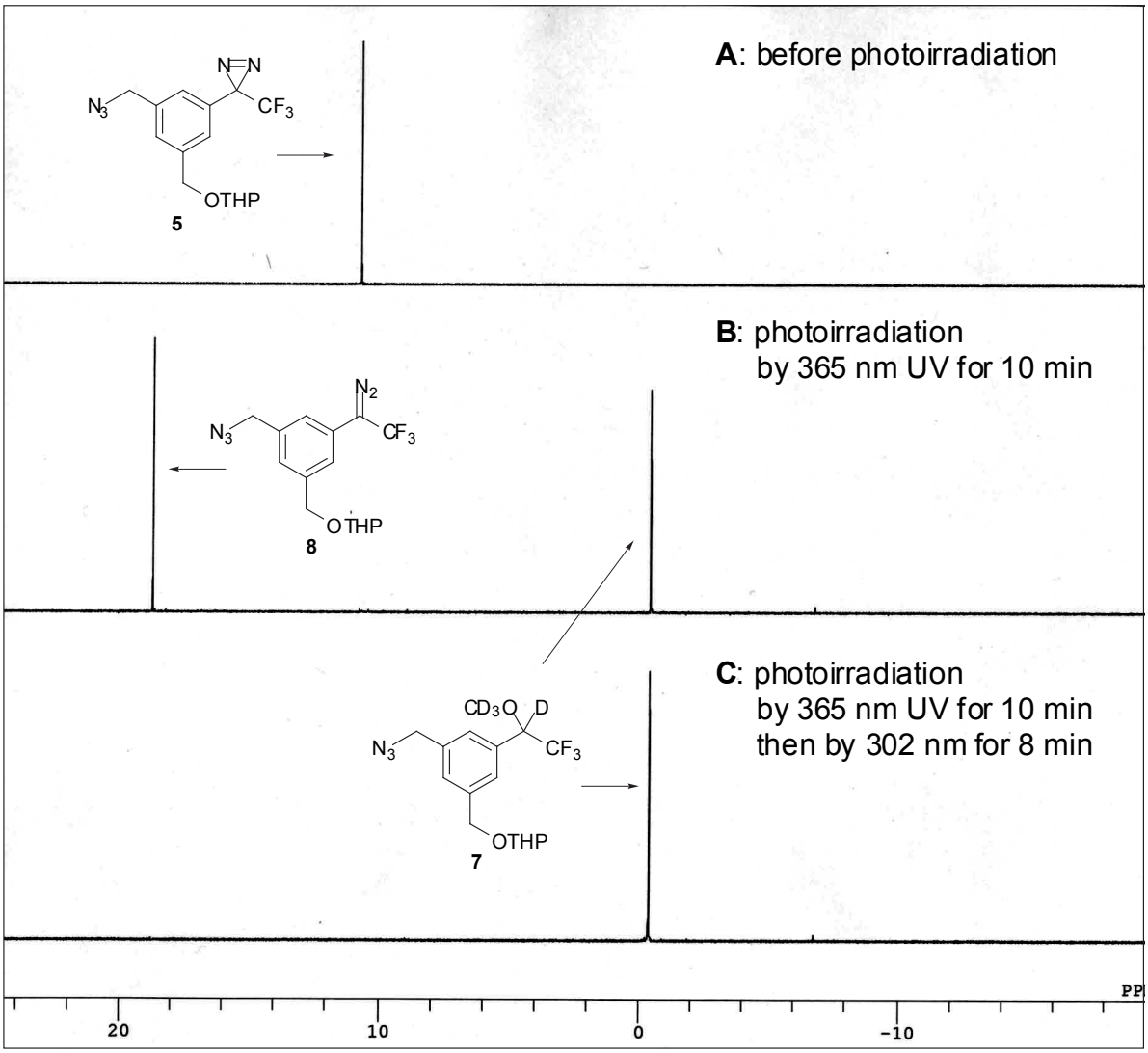


Chart S3. Photoreaction of **5** in CD_3OD monitored by ^{19}F NMR. The reaction was carried out at 22 °C using a quartz tube.

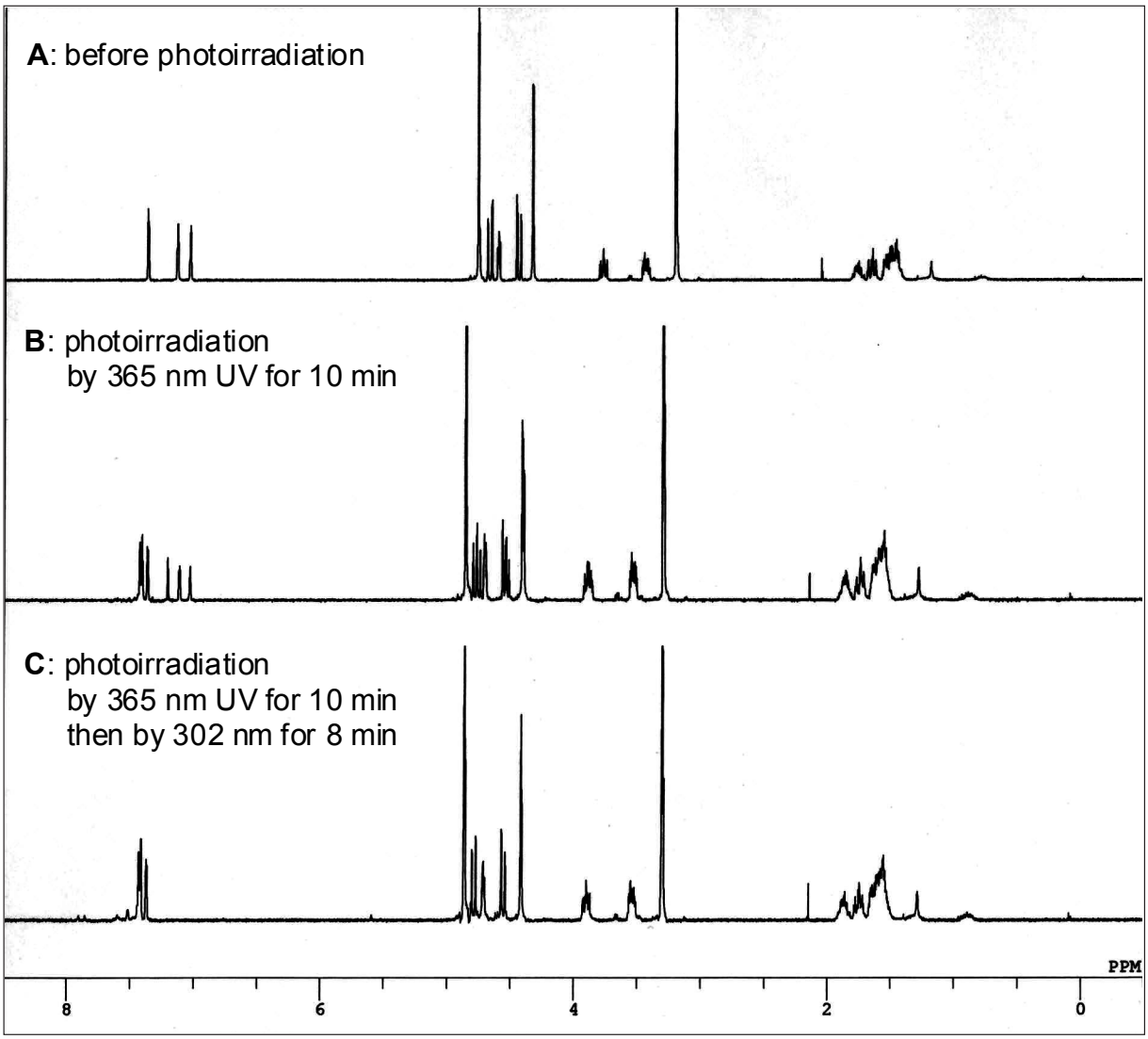
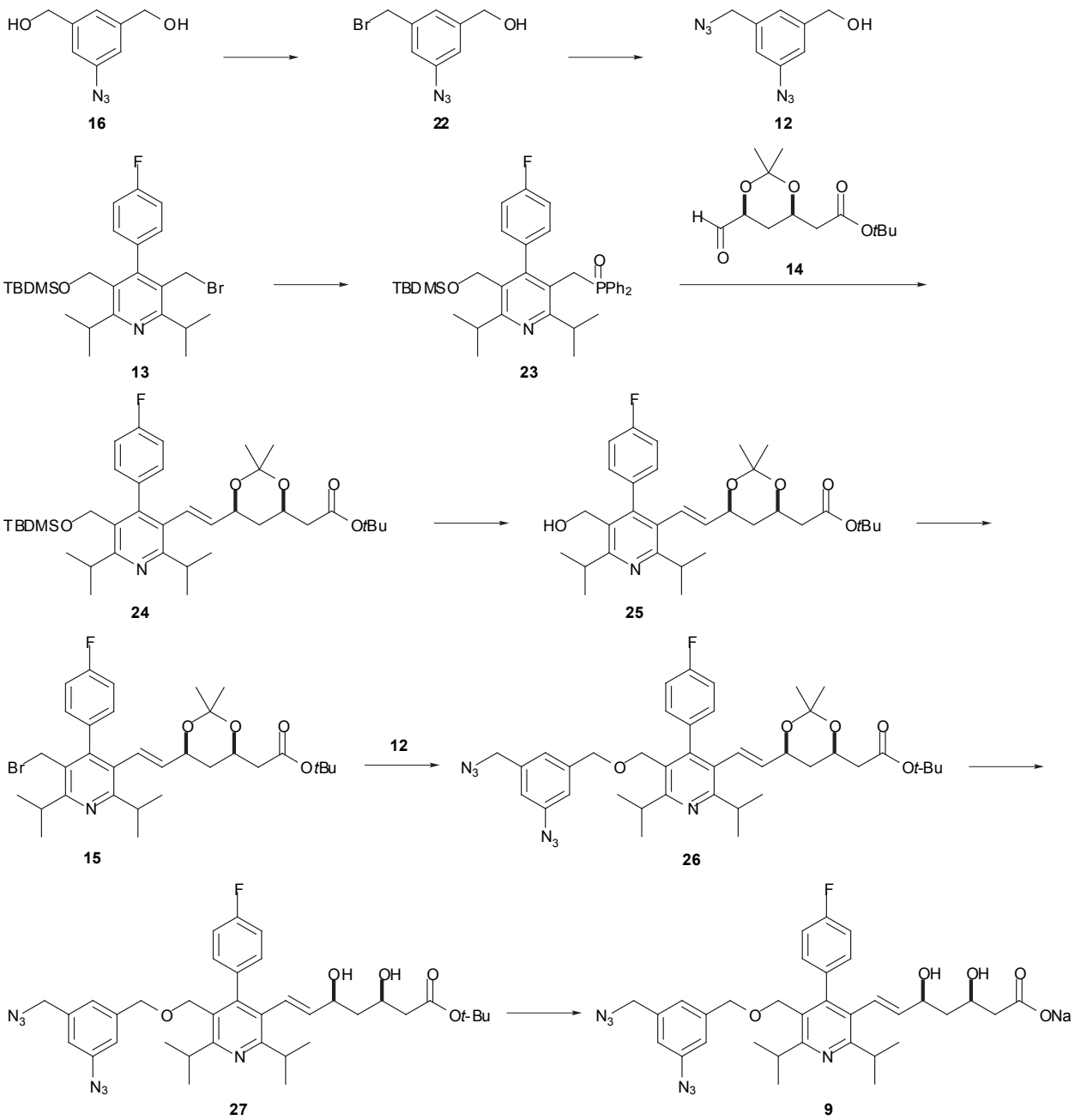


Chart S4. Photoreaction of **5** in CD_3OD monitored by ^1H NMR. The reaction was carried out at 22 °C using a quartz tube.

Synthesis of (3*R*,5*S*,6*E*)-7-({5-[3-azido-5-(azidomethyl)benzyloxymethyl]-4-(4-fluorophenyl)-2,6-diisopropyl}pyridin-3-yl)-3,5-dihydroxy-6-heptenoic acid sodium salt (9, Photovastatin CAA1).



To a solution of **16** (300 mg, 1.67 mmol) in DMF (4.0 mL) were successively added CBr_4 (832 mg, 2.51 mmol) and PPh_3 (483 mg, 1.84 mmol) at 0 °C. After stirring for 2 h at the same temperature, the product was purified directly from the reaction mixture by silica-gel column chromatography (*n*-hexane only, *n*-hexane/EtOAc = 4/1) to give 1-azido-3-bromomethyl-5-(hydroxymethyl)benzene (**22**) (215 mg, 53.2%). A dibrominated product (99.2 mg, 19.5%; colorless solid; TLC R_f = 0.71 (*n*-hexane/EtOAc = 4/1)) was also obtained; colorless solid; TLC R_f = 0.26 (*n*-hexane/EtOAc = 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75 (t, 1H, *J* = 6.0 Hz), 4.45 (s, 2H), 4.70 (d, 2H, *J* = 6.0 Hz), 6.97 (br s, 1H), 6.99 (br s, 1H), 7.16 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.5, 64.4, 117.1, 118.5, 123.5, 139.7, 140.7, 143.3; IR (KBr, cm^{-1}) 563, 696, 860, 1049, 1210, 1235, 1312, 1370, 1456, 1595, 1609, 2113, 3279; HRMS (EI) *m/z* 240.9862, (M^+, $C_8H_8BrN_3O$ requires 240.9851).

To a solution of **22** (213 mg, 880 μmol) in DMF (3.0 mL) was added NaN_3 (85.8 mg, 1.32 mmol) at 0 °C and the mixture was gradually warmed to room temperature and stirred for 6.5 h. To this was added water and the mixture was extracted with EtOAc (×3). The combined organic extracts were successively washed with water and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 2/1) to give 1-azido-3-azidomethyl-5-(hydroxymethyl)benzene (**12**) (173 mg, 96.3%); pale yellow oil; TLC R_f = 0.36 (*n*-hexane/EtOAc = 2/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82 (br s, 1H), 4.35 (s, 2H), 4.72 (br s, 2H), 6.89 (br s, 1H) , 7.03 (br s, 1H), 7.09 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.3, 64.4, 116.8, 117.5, 122.5, 137.5, 140.8, 143.4; IR (KBr, cm^{-1}) 849, 1028, 1057, 1233, 1308, 1343, 1456, 1597, 1609, 2109, 3332; HRMS (EI) *m/z* 204.0755, (M^+, $C_8H_8N_6O$ requires 204.0760).

A mixture of 3-bromomethyl-5-[(*tert*-butyldimethylsiloxy)methyl]-4-(4-fluorophenyl)-2,6-diisopropylpyridine (**13**)[6] (1.31 g, 2.65 mmol) and $Ph_2P(OEt)$ (1.44 mL, 6.63 mmol) was heated at 150 °C for 3 h under Ar atmosphere. After cooling to room temperature, the product was purified directly from the reaction mixture by silica-gel column chromatography (*n*-hexane/EtOAc = 4/1, 3/1, 2/1) to give 3-[(*tert*-butyldimethylsiloxy)methyl]-5-diphenylphosphinoylmethyl-4-(4-fluorophenyl)-2,6-diisopropylpyridine (**23**) (1.62 g, 98.5%); colorless solid; TLC R_f = 0.44 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ –0.15 (s, 6H), 0.80 (s, 9H), 1.20 (d, 6H, *J* = 6.5 Hz), 1.31 (d, 6H, *J* = 6.5 Hz), 3.33 (septet, 1H, *J* = 6.5 Hz), 3.36 (septet, 1H, *J* = 6.5 Hz), 3.63 (d, 2H, $^2J_{H–P}$ = 13.8 Hz), 4.12 (s, 2H), 6.68 (dd, 2H, *J* = 8.7, $^4J_{H–F}$ = 5.3 Hz), 6.84 (dd, 2H, *J* = 8.7, $^3J_{H–F}$ = 8.7 Hz), 7.26–7.37 (m, 8H), 7.44–7.50 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –5.6 (2C), 18.3, 22.4 (2C), 22.9 (2C), 25.9 (3C), 31.5, 32.6 (d, $^1J_{C–P}$ = 65.8 Hz), 32.9, 59.1, 114.6 (d, 2C, $^2J_{C–F}$ = 20.6 Hz), 119.4 (d, $^2J_{C–P}$ = 9.0 Hz), 127.2 (d, $^4J_{C–P}$ = 2.5 Hz), 128.2 (d, 4C, $^3J_{C–P}$ = 11.5 Hz), 130.9 (d, 4C, $^2J_{C–P}$ = 9.0 Hz), 131.1 (d, 2C, $^3J_{C–F}$ = 7.4 Hz), 131.4 (d, 2C, $^4J_{C–P}$ = 2.5 Hz), 132.8 (d, 2C, $^1J_{C–P}$ = 97.1 Hz), 133.9 (d, $^4J_{C–F}$ = 3.3 Hz), 149.4 (d, $^3J_{C–P}$ = 4.1 Hz), 161.7 (d, $^1J_{C–F}$ = 245.9 Hz), 164.0 (d, $^5J_{C–P}$ = 3.3 Hz), 164.4 (d, $^3J_{C–P}$ = 4.1 Hz); ^{19}F NMR (372 MHz, $CDCl_3$) δ –39.5 (tt, 1F, $^3J_{F–H}$ = 8.7, $^4J_{F–H}$ = 5.3 Hz); IR (KBr, cm^{-1}) 503, 527, 565, 696, 731, 776, 835, 857, 1071, 1092, 1117, 1159, 1194, 1221, 1252, 1379, 1437, 1471, 1509, 1151, 1603, 2859, 2928, 2959; HRMS (FAB^+) *m/z* 638.2976, ($(M + Na)^+$, $C_{37}H_{47}FNO_2PSiNa$ requires 638.2995).

Under Ar atmosphere, to a solution of **23** (300 mg, 487 μmol) in THF (6.0 mL) was added 1.58 M *n*-hexane solution of *n*BuLi (320 μL, 506 μmol) at 0 °C and the mixture was stirred for 20 min at the

same temperature. To this was added a solution of *tert*-butyl (3*R*,5*S*)-6-oxo-3,5-dihydroxy-3,5-*O*-isopropylidenehexanoate (**14**)[1] (131 mg, 506 μmol) in THF (2.0 mL) at 0 °C. After stirring the mixture for 4 h at the same temperature, to this was added saturated aqueous NH_4Cl solution and the mixture was extracted with Et_2O (×3). The combined organic extracts were washed successively with water and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 15/1, 9/1, 3/1) to give (3*R*,5*S*,6*E*)-7-({5-[(*tert*-butyldimethylsiloxy)methyl]-4-(4-fluorophenyl)-2,6-diisopropyl}pyridin-3-yl)-3,5-dihydroxy-3,5-*O*-isopropylidene-6-heptenoic acid *tert*-butyl ester (**24**) (210 mg, 65.7%). The starting material **23** (103 mg, 34.3%) was recovered; colorless solid; TLC R_f = 0.29 (*n*-hexane/EtOAc = 15/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ –0.10 (s, 6H), 0.82 (s, 9H), 0.88 (ddd, 1H, *J* = 11.6, 11.6, 11.6 Hz), 1.23 (d, 6H, *J* = 6.8 Hz), (1H included in 1.20–1.30), 1.29 (d, 6H, *J* = 6.8 Hz), 1.32 (s, 3H), 1.40 (s, 3H), 1.43 (s, 9H), 2.22 (dd, 1H, *J* = 6.0, 15.2 Hz), 2.37 (dd, 1H, *J* = 7.0, 15.2 Hz), 3.30 (septet, 1H, *J* = 6.8 Hz), 3.36 (septet, 1H, *J* = 6.8 Hz), 4.13–4.24 (m, 2H), 4.26 (d, 1H, *J* = 10.6 Hz), 4.30 (d, 1H, *J* = 10.6 Hz), 5.20 (dd, 1H, *J* = 6.0, 16.2 Hz), 6.16 (dd, 1H, *J* = 1.2, 16.2 Hz), 6.95–7.14 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –5.7 (2C), 18.2, 19.7, 22.29, 22.33, 22.7 (2C), 25.8 (3C), 28.0 (3C), 29.9, 31.3, 31.9, 36.2, 42.4, 58.9, 65.6, 69.6, 80.4, 98.4, 114.2 (d, 2C, $^2J_{C–F}$ = 20.6 Hz), 125.9, 126.4, 126.5, 131.1 (d, $^3J_{C–F}$ = 7.4 Hz), 131.5 (d, $^3J_{C–F}$ = 8.2 Hz), 134.5 (d, $^4J_{C–F}$ = 4.1 Hz), 136.2, 147.6, 161.5 (d, $^1J_{C–F}$ = 245.9 Hz), 162.0, 163.8, 169.8; ^{19}F NMR (372 MHz, $CDCl_3$) δ –40.0 (tt, 1F, $^3J_{F–H}$ = 8.7, $^4J_{F–H}$ = 5.3 Hz); IR (KBr, cm^{-1}) 776, 814, 837, 858, 951, 968, 1005, 1034, 1075, 1157, 1200, 1223, 1258, 1316, 1370, 1379, 1391, 1420, 1472, 1510, 1549, 1605, 1732, 2861, 2930, 2959; HRMS (FAB$^+$) *m*/*z* 656.4153, ((M + H)$^+$, $C_{38}H_{59}FNO_5Si$ requires 656.4147).

To a solution of **24** (100 mg, 152 μmol) in THF (1.5 mL) was added 1.0 M THF solution of nBu$_4$NF (760 μL, 760 μmol) at room temperature and the mixture was stirred for 5 h. To this was added water and the mixture was extracted with Et_2O (×3). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 6/1) to give (3*R*,5*S*,6*E*)-7-{[4-(4-fluorophenyl)-5-hydroxymethyl-2,6-diisopropyl]pyridin-3-yl}-3,5-dihydroxy-3,5-*O*-isopropylidene-6-heptenoic acid *tert*-butyl ester (**25**) (80.8 mg, 98.0%); colorless solid; TLC R_f = 0.44 (*n*-hexane/EtOAc = 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (ddd, 1H, *J* = 11.6, 11.6, 11.6 Hz), 1.25 (d, 6H, *J* = 6.5 Hz), (1H included in 1.20–1.30), 1.33 (d, 3H, *J* = 6.5 Hz), 1.34 (d, 3H, *J* = 6.5 Hz), 1.34 (s, 3H), 1.42 (s, 3H), 1.45 (s, 9H), 2.23 (dd, 1H, *J* = 6.0, 15.2 Hz), 2.39 (dd, 1H, *J* = 7.0, 15.2 Hz), 3.32 (septet, 1H, *J* = 6.5 Hz), 3.43 (qq, 1H, *J* = 6.5, 6.5 Hz), 4.14–4.26 (m, 2H), 4.38 (dd, 1H, *J* = 5.3, 10.6 Hz), 4.42 (dd, 1H, *J* = 5.3, 10.6 Hz), 5.23 (dd, 1H, *J* = 6.0, 16.2 Hz), 6.18 (dd, 1H, *J* = 1.2, 16.2 Hz), 7.01–7.15 (m, 4H), (hydroxy proton not identified); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.4, 22.5, 23.0 (2C), 28.2 (3C), 30.1, 31.7, 32.2, 36.4, 42.6, 58.8, 65.8, 69.7, 80.6, 98.6, 114.9 (d, 2C, $^2J_{C–F}$ = 21.4 Hz), 125.7, 126.5, 127.0, 130.9 (d, $^3J_{C–F}$ = 8.2 Hz), 131.2 (d, $^3J_{C–F}$ = 8.2 Hz), 134.5 (d, $^4J_{C–F}$ = 3.3 Hz), 136.6, 148.0, 161.8 (d, $^1J_{C–F}$ = 245.9 Hz), 162.8, 163.7, 169.9; ^{19}F NMR (372 MHz, $CDCl_3$) δ –38.9 (tt, 1F, $^3J_{F–H}$ = 8.7, $^4J_{F–H}$ = 5.3 Hz); IR (KBr, cm^{-1}) 523, 735, 843, 893, 951, 968, 1005, 1044, 1094, 1159, 1200, 1223, 1258, 1316, 1370, 1379, 1418, 1456, 1468, 1510, 1547, 1605, 1730, 2870, 2940, 2977, 3264; HRMS (FAB$^+$) *m*/*z* 564.3124, ((M + Na)$^+$, $C_{32}H_{44}FNO_5Na$ requires 564.3101).

To a solution of **25** (300 mg, 554 μmol) in CH_2Cl_2 (5.0 mL) were successively added CBr_4 (551 mg,

1.66 mmol) and PPh_3 (153 mg, 582 μmol) at room temperature. After stirring for 1 h, the product was purified directly from the reaction mixture by silica-gel column chromatography (*n*-hexane/EtOAc = 20/1, 9/1, 7/1) to give (3*R*,5*S*,6*E*)-7-{[5-bromomethyl-4-(4-fluorophenyl)-2,6-diisopropyl]pyridin-3-yl}-3,5-dihydroxy-3,5-*O*-isopropylidene-6-heptenoic acid *tert*-butyl ester (**15**) (239 mg, 71.3%). The starting material **25** (60.6 mg, 20.1%) was recovered; colorless solid; TLC R_f = 0.62 (*n*-hexane/EtOAc = 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (ddd, 1H, *J* = 11.8, 11.8, 11.8 Hz), 1.25 (d, 6H, *J* = 6.5 Hz), (1H included in 1.20–1.30), 1.33 (s, 3H), 1.340 (d, 3H, *J* = 6.5 Hz), 1.343 (d, 3H, *J* = 6.5 Hz), 1.42 (s, 3H), 1.45 (s, 9H), 2.23 (dd, 1H, *J* = 6.0, 15.2 Hz), 2.38 (dd, 1H, *J* = 7.0, 15.2 Hz), 3.31 (septet, 1H, *J* = 6.5 Hz), 3.38 (qq, 1H, *J* = 6.5, 6.5 Hz), 4.14–4.26 (m, 2H), 4.23 (d, 1H, *J* = 10.6 Hz), 4.27 (d, 1H, *J* = 10.6 Hz), 5.24 (dd, 1H, *J* = 6.0, 16.2 Hz), 6.15 (dd, 1H, *J* = 1.2, 16.2 Hz), 7.04–7.21 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5, 22.0, 22.1, 22.2 (2C), 27.9 (3C), 28.8, 29.8, 31.4, 32.0, 36.1, 42.3, 65.5, 69.3, 80.3, 98.3, 114.7 (d, 2C, $^2J_{C–F}$ = 21.4 Hz), 124.1, 125.0, 127.1, 130.3 (d, $^3J_{C–F}$ = 8.2 Hz), 130.6 (d, $^3J_{C–F}$ = 8.2 Hz), 133.4 (d, $^4J_{C–F}$ = 3.3 Hz), 136.6, 147.5, 161.6 (d, $^1J_{C–F}$ = 246.0 Hz), 163.16, 163.24, 169.6; ^{19}F NMR (372 MHz, $CDCl_3$) δ –38.9 (tt, 1F, $^3J_{F–H}$ = 8.4, $^4J_{F–H}$ = 5.3 Hz); IR (KBr, cm^{-1}) 845, 951, 968, 1040, 1057, 1094, 1132, 1159, 1202, 1223, 1256, 1316, 1339, 1370, 1379, 1393, 1420, 1456, 1470, 1509, 1545, 1603, 1732, 2870, 2940, 2977; HRMS (FAB$^+$) *m/z* 604.2417, ((M + H)$^+$, $C_{32}H_{44}BrFNO_4$ requires 604.2438).

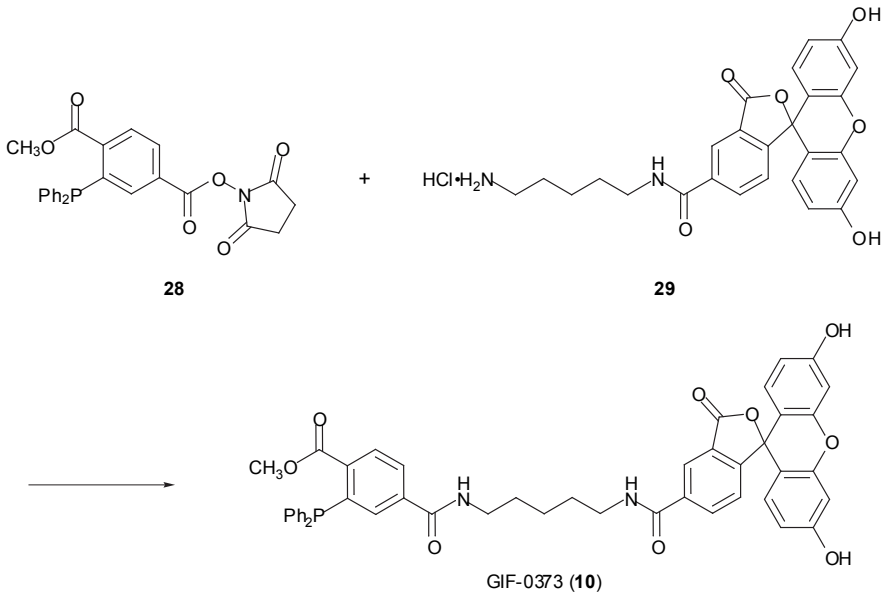
Under Ar atmosphere, to a solution of **12** (15.3 mg, 74.9 μmol) in DMF (1.0 mL) was added NaH (ca. 60% suspended in oil, 3.0 mg, 75 μmol) at 0 °C and the mixture was stirred for 15 min at the same temperature. To this was added a solution of **15** (45.3 mg, 74.9 μmol) in DMF (1.5 mL) at 0 °C and the mixture was stirred for 3 h at the same temperature. To this was added saturated aqueous NH_4Cl solution and the mixture was extracted with EtOAc (×3). The combined organic extracts were washed successively with water (×3) and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 15/1) to give (3*R*,5*S*,6*E*)-7-({5-[3-azido-5-(azidomethyl)benzyloxymethyl]-4-(4-fluorophenyl)-2,6-diisopropyl}pyridin-3-yl)-3,5-dihydroxy-3,5-*O*-isopropylidene-6-heptenoic acid *tert*-butyl ester (**26**) (31.9 mg, 58.5%); colorless oil; TLC R_f = 0.57 (*n*-hexane/EtOAc = 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (ddd, 1H, *J* = 11.8, 11.8, 11.8 Hz), 1.24 (d, 6H, *J* = 6.8 Hz), (1H included in 1.20–1.30), 1.319 (d, 3H, *J* = 6.8 Hz), 1.324 (d, 3H, *J* = 6.8 Hz), 1.34 (s, 3H), 1.42 (s, 3H), 1.45 (s, 9H), 2.23 (dd, 1H, *J* = 6.0, 15.2 Hz), 2.38 (dd, 1H, *J* = 7.0, 15.2 Hz), 3.32 (septet, 1H, *J* = 6.8 Hz), 3.34 (qq, 1H, *J* = 6.8, 6.8 Hz), 4.15–4.26 (m, 4H), 4.31 (s, 2H), 4.32 (s, 2H), 5.22 (dd, 1H, *J* = 6.0, 16.4 Hz), 6.18 (dd, 1H, *J* = 1.2, 16.4 Hz), 6.87 (br s, 1H), 6.89 (br s, 1H), 6.95 (br s, 1H), 6.98–7.12 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.8, 22.39, 22.44, 22.9 (2C), 28.2 (3C), 30.1, 31.7, 32.2, 36.4, 42.6, 54.2, 65.8, 66.6, 69.6, 72.1, 80.6, 98.6, 114.5, (d, 2C, $^2J_{C–F}$ = 20.6 Hz), 117.6, 117.8, 123.5, 123.9, 125.8, 126.9, 131.0 (d, $^3J_{C–F}$ = 7.4 Hz), 131.3 (d, $^3J_{C–F}$ = 8.2 Hz), 134.5 (d, $^4J_{C–F}$ = 3.3 Hz), 136.6, 137.3, 140.6 (2C), 148.5, 161.7 (d, $^1J_{C–F}$ = 246.0 Hz), 163.0, 164.0, 169.9; ^{19}F NMR (372 MHz, $CDCl_3$) δ –39.5 (tt, 1F, $^3J_{F–H}$ = 8.7, $^4J_{F–H}$ = 5.3 Hz); IR (KBr, cm^{-1}) 743, 845, 912, 1092, 1159, 1200, 1223, 1258, 1308, 1368, 1379, 1456, 1510, 1728, 2109, 2870; HRMS (FAB$^+$) *m/z* 728.3917, ((M + H)$^+$, $C_{40}H_{51}FN_7O_5$ requires 728.3936).

To a solution of **26** (106 mg, 146 μmol) in THF (8.0 mL) was added 10% aqueous HCl solution (2.0 mL) at room temperature. After stirring for 14 h, the mixture was extracted with EtOAc (×3). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated under

reduced pressure. The crude product was purified by silica-gel column chromatography (*n*-hexane/EtOAc = 7/3) to give (3*R*,5*S*,6*E*)-7-({5-[3-azido-5-(azidomethyl)benzyloxymethyl]-4-(4-fluorophenyl)-2,6-diisopropyl}pyridin-3-yl)-3,5-dihydroxy-6-heptenoic acid *tert*-butyl ester (**27**) (91.8 mg, 91.1%); colorless oil; TLC R_f = 0.18 (*n*-hexane/EtOAc = 3/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.235 (d, 3H, *J* = 6.8 Hz), (1H included in 1.15–1.30), 1.240 (d, 3H, *J* = 6.8 Hz), 1.32 (d, 6H, *J* = 6.8 Hz), (1H included in 1.30–1.45), 1.47 (s, 9H), 2.26–2.38 (m, 2H), 3.07 (d, 1H, *J* = 1.5 Hz), 3.30 (septet, 1H, *J* = 6.8 Hz), 3.34 (qq, 1H, *J* = 6.8, 6.8 Hz), 3.68 (d, 1H, *J* = 1.9 Hz), 3.99–4.07 (m, 1H), 4.18 (s, 2H), 4.24–4.30 (m, 1H), 4.31 (s, 2H), 4.32 (s, 2H), 5.24 (dd, 1H, *J* = 6.3, 16.2 Hz), 6.29 (dd, 1H, *J* = 1.2, 16.2 Hz), 6.87 (br s, 1H), 6.89 (br s, 1H), 6.95 (br s, 1H), 6.99–7.13 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.30, 22.33, 22.9 (2C), 28.2 (3C), 31.7, 32.4, 42.33, 42.35, 54.2, 66.6, 68.2, 72.1, 72.3, 81.6, 114.5 (d, 2C, $^2J_{C–F}$ = 21.4 Hz), 117.7, 117.9, 123.5, 124.0, 125.6, 127.1, 131.1 (d, $^3J_{C–F}$ = 8.2 Hz), 131.3 (d, $^3J_{C–F}$ = 7.4 Hz), 134.7 (d, $^4J_{C–F}$ = 3.3 Hz), 137.3, 138.4, 140.6 (2C), 148.4, 161.7 (d, $^1J_{C–F}$ = 246.0 Hz), 163.1, 164.1, 171.8; ^{19}F NMR (372 MHz, $CDCl_3$) δ –39.3 (tt, 1F, $^3J_{F–H}$ = 8.4, $^4J_{F–H}$ = 5.3 Hz); IR (KBr, cm^{-1}) 845, 1080, 1157, 1223, 1308, 1341, 1368, 1456, 1509, 1603, 2110, 2870, 2932; HRMS (FAB$^+$) *m/z* 688.3613, ((M + H)$^+$, $C_{37}H_{47}FN_7O_5$ requires 688.3623).

To a solution of **27** (67.0 mg, 97.4 μmol) in THF (4.0 mL) was added 0.10 M aqueous NaOH solution (0.98 mL, 98 μmol) at room temperature and the mixture was stirred for 11.5 h. After concentration of the reaction mixture under reduced pressure, the crude product was purified by reversed-phase silica-gel column chromatography (H_2O/CH_3OH = 1/4) to give (3*R*,5*S*,6*E*)-7-({5-[3-azido-5-(azidomethyl)benzyloxymethyl]-4-(4-fluorophenyl)-2,6-diisopropyl}pyridin-3-yl)-3,5-dihydroxy-6-heptenoic acid sodium salt (**9**, Photovastatin CAA1) (58.9 mg, 92.5%); colorless solid; TLC (reversed phase) R_f = 0.47 (H_2O/CH_3OH = 1/9); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95 (ddd, 1H, *J* = 5.1, 5.1, 13.5 Hz), 1.186 (d, 3H, *J* = 6.8 Hz), 1.191 (d, 3H, *J* = 6.8 Hz), 1.24 (d, 6H, *J* = 6.8 Hz), (1H included in 1.15–1.30), 1.67 (dd, 1H, *J* = 8.2, 14.7 Hz), 1.90 (dd, 1H, *J* = 3.9, 14.7 Hz), (2H included in 3.24–3.40), 3.42–3.50 (m, 1H), 3.95–4.03 (m, 1H), 4.12 (s, 2H), 4.32 (s, 2H), 4.45 (s, 2H), 4.88 (br, 1H), 5.25 (dd, 1H, *J* = 5.7, 16.2 Hz), 6.12 (dd, 1H, *J* = 1.2, 16.2 Hz), 6.93 (br s, 1H), 7.01 (br s, 1H), 7.04 (br s, 1H), 7.07–7.19 (m, 4H), 7.70 (br, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 22.8 (2C), 23.2 (2C), 32.7, 33.2, 44.8, 45.0, 54.9, 67.1, 68.0, 71.5, 72.9, 115.6 (d, $^2J_{C–F}$ = 21.4 Hz), 115.7 (d, $^2J_{C–F}$ = 22.3 Hz), 118.76, 118.84, 125.1, 125.6, 126.5, 128.7, 132.3 (d, $^3J_{C–F}$ = 8.2 Hz), 132.6 (d, $^3J_{C–F}$ = 7.4 Hz), 135.8 (d, $^4J_{C–F}$ = 3.3 Hz), 139.2, 140.3, 141.8, 141.9, 150.1, 163.1 (d, $^1J_{C–F}$ = 244.3 Hz), 164.2, 165.3, 179.7; ^{19}F NMR (372 MHz, CD_3OD) δ –46.7 (tt, 1F, $^3J_{F–H}$ = 8.4, $^4J_{F–H}$ = 6.1 Hz); IR (KBr, cm^{-1}) 843, 1080, 1159, 1223, 1308, 1341, 1358, 1399, 1418, 1456, 1509, 1559, 1595, 2109, 1869, 2963; UV (EtOH, nm) λ_{max} (log ε) 243 (4.35); HRMS (FAB$^+$) *m/z* 654.2830, ((M + H)$^+$, $C_{33}H_{38}FN_7NaO_5$ requires 654.2816).

Synthesis of *N*-({3',6'-dihydroxy-3-oxo-spiro[isobenzofuran-1(3*H*),9'-[9*H*]xanthen]-5-yl}carbonylaminopentyl)-2-(diphenylphosphino)terephthalamic acid methyl ester (10, GIF-0373)



Under Ar atmosphere, to a solution of *N*-(5-aminopentyl)fluorescein-5-carboxamide hydrochloride (**29**) (Molecular Probes) (10.8 mg, 21.7 μmol) in DMF (0.5 mL) were successively added 2-(diphenylphosphanyl)terephthalic acid 1-methyl ester 4-(*N*-succinimidyl) ester (**28**)[7] (15.0 mg, 32.6 μmol) and Et_3N (3.5 μL, 25 μmol) at room temperature and stirred for 20 h. To this was added pH 6.0 phosphate buffer and the mixture was extracted with a mixture of Et_2O and EtOAc (2:1) (×5). The combined organic extracts were successively washed with water and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography (CH_2Cl_2/CH_3OH = 15/1, 9/1) to give *N*-({3',6'-dihydroxy-3-oxo-spiro[isobenzofuran-1(3*H*),9'-[9*H*]xanthen]-5-yl}carbonylaminopentyl)-2-(diphenylphosphino)terephthalamic acid methyl ester (**10**, GIF-0373) (14.8 mg, 84.3%); yellow solid; TLC R_f = 0.44 (CH_2Cl_2/CH_3OH = 9/1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (tt, 2H, *J* = 7.3, 7.3 Hz), 1.49 (tt, 2H, *J* = 7.3, 7.3 Hz), 1.55 (tt, 2H, *J* = 7.3, 7.3 Hz), 3.16 (dt, 2H, *J* = 5.6, 7.3 Hz), 3.29 (dt, 2H, *J* = 5.6, 7.3 Hz), 3.63 (s, 3H), 6.52 (dd, 2H, *J* = 1.7, 8.7 Hz), 6.57 (d, 2H, *J* = 8.7 Hz), 6.66 (d, 2H, *J* = 1.7 Hz), 7.16–7.23 (m, 4H), 7.33 (d, 1H, *J* = 8.0 Hz), 7.34–7.41 (m, 7H), 7.88 (dd, 1H, *J* = 1.2, 8.0 Hz), 7.98 (dd, 1H, *J* = 3.6, 8.0 Hz), 8.20 (d, 1H, *J* = 8.0 Hz), 8.45 (s, 1H), 8.53 (t, 1H, *J* = 5.6 Hz), 8.79 (t, 1H, *J* = 5.6 Hz), 10.20 (br, 2H); IR (KBr, cm^{-1}) 473, 501, 531, 583, 606, 673, 698, 747, 791, 822, 851, 914, 995, 1057, 1092, 1115, 1156, 1183, 1250, 1279, 1364, 1436, 1496, 1509, 1541, 1588, 1637, 1647, 1717, 1744, 2861, 2932, 3304, 3071, 3298; UV (EtOH, nm) λ_{max} (log ε) 483 (4.24), 457 (4.23); Fluorescence (EtOH, nm) λ_{ex} 506, λ_{em} 525; HRMS (FAB$^+$) *m/z* 807.2462, ((M + H)$^+$, $C_{47}H_{40}N_2O_9P$ requires 807.2471).

Photoaffinity labeling of HMGR$^{426–888}$ with photovastatin CAA1 (9) and its fluorescence detection by GIF-0373 (10): Comparison study with or without UV irradiation.

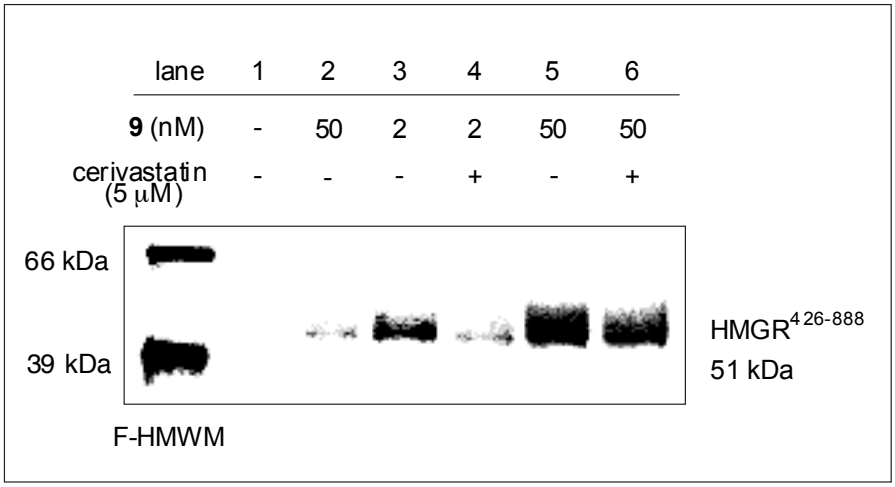


Figure S3. SDS-PAGE analysis of HMGR$^{426–888}$ (51 kDa) photolabeled with **9** in the absence (-) or presence (+) of cerivastatin (5 μM) followed by chemoselective ligation with **10** (100 μM). In lane 1 is shown the SDS buffer alone as a control. All other lanes shows experiments carried out under the same conditions described for Figure 2 in the text with the exception that the reaction mixture for lane 2 was not irradiated by UV. The fluorescent signal was visualized by laser-scanning of the electrophoresed gel with a fluorescent imaging analyzer (FluorImager SI, Molecular Dynamics, 488 nm excitation, 530±15 nm detection filter). For comparison, the fluorescent high molecular weight marker (F-HMWM, Sigma) is shown in the leftmost lane.

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