Supplementary Material (ESI) for Chemical Communications

SUPPLEMENTARY INFORMATION

Target-selective photo-degradation of HIV-1 protease by a fullerene-sugar hybrid

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General Methods for Chemical Synthesis. Fullerene derivative **1** was purchased from Science Laboratories Inc. and used without further purification. ^{1}H-NMR spectra were recorded on a Varian MVX-300 (300 MHz) spectrometer using trimethylsilane as the internal standard, unless otherwise noted. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was conducted using a Bruker Ultraflex mass spectrometer with detection in the reflector mode. 2,5-Dihydroxybenzoic acid was used as the matrix, with positive ionization mode. UV/VIS spectrum was recorded on a JASCO V-650DS spectrophotometer. Silica gel TLC and column chromatography were performed using Merck TLC 60F-254 (0.25 mm) and Silica Gel 60 N (spherical, neutral) (Kanto Chemical Co., Inc.), respectively. Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere using

oven-dried glassware. In general, organic solvents were purified and dried using appropriate procedures, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

Synthesis of the protected fullerene glycoside 6. To a solution of **4**[1] (27.4 mg, 0.0346 mmol) dissolved in dry PhMe (11 mL), $CaCO_3$ (35.5 mg) and $CaSO_4$ (14.2 mg) were added at 0 °C. After addition of a solution of **5** (142 mg, 0.346 mmol) in dry PhMe (1.2 mL), $AgClO_4$ (70.9 mg, 0.346 mmol) was added to the solution at 0 °C, then stirred for 4 h at 25 °C.[2] The reaction mixture was filtered using Cerite, and the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography (PhMe:EtOAc = 8:1) to give **6** (17.3 mg) as a dark brown solid in 44% yield: R_f 0.30 (8:1 PhMe-EtOAc); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.02 (3/2H, s), 2.05 (3/2H, s), 2.06 (3H, s), 2.07 (3/2H, s), 2.12 (3/2H, s), 2.15 (3/2H, s), 2.20 (3/2H, s), 2.65-2.78 (1/2H, m), 2.88-3.00 (1/2H, m), 3.16-3.40 (2H, m), 3.50 (1/2H, dd, *J* = 13.0 and 5.6 Hz), 3.62-3.92 (7/2H, m), 4.19 (1/2H, dd, *J* = 12.1 and 2.0 Hz), 4.24-4.34 (1H, m), 4.39 (1/2H, dd, *J* = 12.1 and 4.6 Hz), 4.75 (1/2H, d, *J* = 8.0 Hz), 4.76 (1/2H, d, *J* = 8.0 Hz), 5.08-5.38 (4H, m); Anal. Calcd for $C_{78}H_{26}O_{10}$: C, 83.42; H, 2.33. Found: C, 83.29; H, 2.38. MALDI-TOF MS *m/z* 1145.16 (1145.14 calcd for $C_{78}H_{26}O_{10}$, M+Na^+).

Synthesis of the fullerene-sugar hybrid 2. To a solution of **6** (21.0 mg, 0.0187 mmol) dissolved in a mixture of dry PhMe (2.1 mL) and dry MeOH (1.1 mL), NaOMe/MeOH (14.4 μL, 0.0748 mmol) was added at 0 °C. After stirring for 30 min at 25 °C, the

reaction mixture was neutralized by addition of AmberLite CG-50. The resultant mixture was filtered and the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to give **2** (13.0 mg) as a dark brown solid in 73% yield: R_f 0.27 (10:1 $CHCl_3$-MeOH); ^{1}H-NMR (300 MHz, DMF-d_7) δ 3.24-3.98 (12H, m), 4.55 (1/2H, dd, J = 6.0 and 6.0 Hz), 4.65 (1/2H, dd, J = 6.0 and 6.0 Hz), 4.80 (1/2H, d, J = 8.0 Hz), 4.84 (1/2H, d, J = 8.0 Hz), 5.06-5.20 (5/2H, m), 5.20-5.36 (3/2H, m); Anal. Calcd for $C_{70}H_{18}O_6$•$3H_2O$: C, 83.33; H, 2.40. Found: C, 83.07; H, 2.44. MALDI-TOF MS *m/z* 997.10 (977.10 calcd for $C_{78}H_{26}O_{10}$, M+Na^+).

Photo-degradation of proteins. HIV-1 protease was purchased from Immuno Diagnostics, Inc. Bovine serum albumin (BSA) and hen egg lysozyme (Lyso) were purchased from Sigma-Aldrich Co. B-100A (UVP Inc., 365 nm, 100 W) and I-Sunsun (Wacom Sunray Lamp, 75-W xenon lamp) were used for photo-irradiation in the UV and visible regions, respectively. Protein cleavage experiments were performed with each protein (1.5 μM) in a volume of 10 μL containing 10% dimethylformamide in 50 mM PBS buffer (pH 7.0) or 50 mM PBS buffer (pH 7.0) at 25 °C for 2 h under irradiation by a lamp placed 10 cm from the sample. Protein concentrations in the samples were varied as indicated in the figure captions.

Protocol for electrophoresis. SDS/polyacrylamide gel electrophoresis (SDS-PAGE) experiments were performed as reported previously.[3] Electrophoresis buffer consisted of SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue

(0.007%, wt/vol); 4.8 μL of buffer was added to the photo-irradiated samples. Gels (8% for BSA and 12% for HIV-1 protease and Lyso) were run by applying 110 V for 1.5 h for BSA, or 2.5 h for HIV-1 and Lyso. The gels were stained with SYPRO Ruby Protein Gel Stain (Bio-Rad Lab. Inc.) for 3 h, destained in acetic acid (7%, vol/vol) and methanol (10%, vol/vol) for 0.5 h, and then washed with water. The gels were scanned with a Molecular Imager FX (Bio-Rad Lab. Inc.) and the images were processed using Adobe Photoshop software. Molecular weight markers were used in each gel for calibration purposes.

References

1 Y.-Z. An, C.-H. B. Chen, J. L. Anderson, D. S. Sigman, C. S. Foote and Y. Rubin, *Tetrahedron* **1996**, *52*, 5179.

2 Toshima, K. *O-Glycosidation methods*, In *Comprehensive Glycoscience from Chemistry to System Biology*, (Eds.; J. P. Kamerling, G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi and A. G. J. Voragen), Elsevier, Oxford, **2007**, p. 261.

3 H. Schägger and G. von Jagow, *Anal. Biochem.* **1987**, *166*, 368.