(1H, t, J=5.5 Hz), 7.80 (1H, d, J=11.0 Hz), 8.03 (1H, t, J=5.8 Hz), 8.08 (1H, t, J=5.5 Hz), 8.14 (1H, d, J=7.9 Hz), 8.25 (1H, t, J=6.1 Hz), 8.46 (1H, d, J=8.5 Hz).

MS (APCI) m/z: 1032 $(M+H)^+$

[0232]

Process 4: Antibody-drug conjugate (1)

Reduction of the antibody: The M30-H1-L4P antibody produced in Reference Example 2 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure C-1 and Common procedure B (as absorption coefficient at 280 nm, 1.61 $mLmg^{-1}cm^{-1}$ was used) described in Production method 1. The solution (1.25 mL) was placed in a 1.5 mL polypropylene tube and charged with an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.025 mL; 3.0 equivalents per antibody molecule) and an aqueous solution of 1 M dipotassium hydrogen phosphate (Nacalai Tesque, Inc.; 0.0625 mL). After confirming that the solution had pH of 7.4 ± 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37°C for 1 hour.

Conjugation between antibody and drug linker: After adding dimethyl sulfoxide (Sigma-Aldrich Co. LLC; 0.109 mL) and a dimethyl sulfoxide solution containing 10 mM of the compound obtained in Process 3 above (0.039 mL; 4.6 equivalents per antibody molecule) to the above solution at room temperature, it was stirred by using a tube rotator (MTR-103, manufactured by AS ONE

Process 2: Antibody-drug conjugate (28)

Reduction of the antibody: The M30-H1-L4P antibody produced in Reference Example 2 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure C-1 and Common procedure B (as absorption coefficient at 280 nm, 1.61 $mLmg^{-1}cm^{-1}$ was used) described in Production method 1. The solution (1.25 mL) was placed in a 1.5 mL polypropylene tube and charged with an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.025 mL; 3.0 equivalents per antibody molecule) and an aqueous solution of 1 M dipotassium hydrogen phosphate (Nacalai Tesque, Inc.; 0.0625 mL). After confirming that the solution had pH of 7.4 ± 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37°C for 1 hour.

Conjugation between antibody and drug linker: After adding dimethyl sulfoxide (Sigma-Aldrich Co. LLC; 0.102 mL) and a dimethyl sulfoxide solution containing 10 mM of the compound obtained in Process 1 above (0.047 mL; 5.5 equivalents per antibody molecule) to the above solution at room temperature, it was stirred by using a tube rotator (MTR-103, manufactured by AS ONE Corporation) for conjugating the drug linker to the antibody at room temperature for 40 minutes. Next, an aqueous solution (0.009 mL) of 100 mM NAC (Sigma-Aldrich Co. LLC) was added thereto and stirred to

hexanoate to yield the titled compound as a pale yellow solid (77.1 mg, 71%).

^{1}H-NMR (DMSO-D_6) δ: 0.87 (3H, t, J=7.2 Hz), 1.21-1.38 (4H, m), 1.43-1.50 (2H, m), 1.55-1.63 (2H, m), 1.68-1.76 (2H, m), 1.80-1.91 (2H, m), 2.07-2.22 (6H, m), 2.40 (3H, s), 2.60-2.67 (2H, m), 2.76-2.84 (5H, m), 2.97-3.22 (5H, m), 3.56-3.76 (6H, m), 4.40-4.50 (1H, m), 5.20 (2H, q, J=18.8 Hz), 5.37-5.48 (2H, m), 5.53-5.62 (1H, m), 6.54 (1H, s), 7.15-7.28 (5H, m), 7.31 (1H, s), 7.71 (1H, t, J=5.5 Hz), 7.80 (1H, d, J=10.9 Hz), 8.04 (1H, t, J=5.9 Hz), 8.09 (1H, t, J=5.9 Hz), 8.14 (1H, d, J=7.8 Hz), 8.26 (1H, t, J=5.9 Hz), 8.47 (1H, d, J=8.6 Hz).

MS (ESI) m/z: 1092 $(M+H)^+$

[0354]

Process 2: Antibody-drug conjugate (36)

Conjugation between antibody and drug linker: The M30-H1-L4P antibody produced in Reference Example 2 was prepared to have antibody concentration of 20 mg/mL by replacing the medium with PBS6.5/EDTA by using the Common procedure C-2 and Common procedure B (as absorption coefficient at 280 nm, 1.61 $mLmg^{-1}cm^{-1}$ was used). The solution (0.25 mL) was placed in a 1.5 mL tube, charged with a DMSO solution containing 10 mM of the compound obtained in Process 1 above (0.025 mL; which corresponds to about 3.7 equivalents per antibody molecule) at room temperature, and stirred by using a tube rotator (MTR-103, manufactured by AS ONE

dd, J=8.9, 6.0 Hz), 5.10 (1H, d, J=18.9 Hz), 5.25 (1H, d, J=18.9 Hz), 5.35 (1H, d, J=16.6 Hz), 5.56 (1H, d, J=16.0 Hz), 5.60-5.64 (1H, m), 6.76 (2H, s), 7.12-7.24 (6H, m), 7.58 (1H, s), 7.60 (1H, d, J=10.9 Hz), 7.68 (1H, t, J=5.7 Hz).

MS (ESI) m/z: 1060 $(M+H)^+$

[0421]

Process 6: Antibody-drug conjugate (51)

Reduction of the antibody: The M30-H1-L4P antibody produced in Reference Example 2 was prepared to have antibody concentration of 10 mg/mL with PBS6.0/EDTA by using the Common procedure B (as absorption coefficient at 280 nm, 1.61 was used) and Common procedure C-1 described in Production method 1. The solution (1.0 mL) was collected into a 1.5 mL tube and charged with an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.0147 mL; 2.3 equivalents per antibody molecule) and an aqueous solution of 1 M dipotassium hydrogen phosphate (Nacalai Tesque, Inc.; 0. 050 mL). After confirming that the solution had pH of 7.4 ± 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37°C for 1 hour.

Conjugation between antibody and drug linker: After incubating the solution at 22°C for 10 minutes, a dimethyl sulfoxide solution containing 10 mM of the compound obtained in Process 5 above (0.0295 mL; 4.6 equivalents per antibody molecule) was added thereto and incubated for conjugating the drug linker to the

Reduction of the antibody: The M30-H1-L4P antibody produced in Reference Example 2 was prepared to have antibody concentration of 10 mg/mL by replacing the medium with PBS6.0/EDTA by using the Common procedure C-1 and Common procedure B (as absorption coefficient at 280 nm, 1.61 $mLmg^{-1}cm^{-1}$ was used) described in Production method 1. The solution (1.25 mL) was placed in a 1.5 mL polypropylene tube and charged with an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.025 mL; 3.0 equivalents per antibody molecule) and an aqueous solution of 1 M dipotassium hydrogen phosphate (Nacalai Tesque, Inc.; 0.0625 mL). After confirming that the solution had pH of 7.4 ± 0.1, the disulfide bond at hinge part in the antibody was reduced by incubating at 37°C for 1 hour.

Conjugation between antibody and drug linker: After adding dimethyl sulfoxide (Sigma-Aldrich Co. LLC; 0.109 mL) and a dimethyl sulfoxide solution containing 10 mM of the compound obtained in Process 8 above (0.039 mL; 4.6 equivalents per antibody molecule) to the above solution at room temperature, it was stirred by using a tube rotator (MTR-103, manufactured by AS ONE Corporation) for conjugating the drug linker to the antibody at room temperature for 40 minutes. Next, an aqueous solution (0.008 mL) of 100 mM NAC (Sigma-Aldrich Co. LLC) was added thereto and stirred to terminate the reaction of drug linker at room temperature for another 20 minutes.