[Table 1]

Table 1-1

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
1	S1C(disialo)-SRIF28	5427.7	$[M+3H]^{3+}$	1810.0	5427.0
			$[M+4H]^{4+}$	1357.5	
			$[M+5H]^{5+}$	1086.4	
2	N5C(disialo)-SRIF28	5400.6	$[M+3H]^{3+}$	1801.0	5400.1
			$[M+4H]^{4+}$	1350.8	
			$[M+5H]^{5+}$	1081.2	
3	A9C(disialo)-SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5443.1
			$[M+4H]^{4+}$	1361.8	
			$[M+5H]^{5+}$	1089.6	
4	E12C(disialo)-SRIF28	5385.6	$[M+3H]^{3+}$	1796.0	5384.0
			$[M+4H]^{4+}$	1347.0	
			$[M+5H]^{5+}$	1078.0	
5	R13C(disialo)-SRIF28	5358.6	$[M+3H]^{3+}$	1787.0	5358.0
			$[M+4H]^{4+}$	1340.5	
			$[M+5H]^{5+}$	1072.6	
6	K14C(disialo)-SRIF28	5386.6	$[M+3H]^{3+}$	1796.4	5386.0
			$[M+4H]^{4+}$	1347.5	
			$[M+5H]^{5+}$	1078.2	
7	A15C(disialo)-SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5443.1
			$[M+4H]^{4+}$	1361.8	
			$[M+5H]^{5+}$	1089.6	
8	G16C(disialo)-SRIF28	5457.7	$[M+3H]^{3+}$	1820.0	5457.1
			$[M+4H]^{4+}$	1365.3	
			$[M+5H]^{5+}$	1092.2	
9	K18C(disialo)-SRIF28	5386.6	$[M+3H]^{3+}$	1796.4	5386.1
			$[M+4H]^{4+}$	1347.5	
			$[M+5H]^{5+}$	1078.2	
			$[M+6H]^{6+}$	898.7	
			$[M+7H]^{7+}$	770.5	

[Table 2]

Table 1 — 2

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
10	N19C(disialo)-SRIF28	5400.6	$[M+3H]^{3+}$	1801.0	5400.1
			$[M+4H]^{4+}$	1351.0	
			$[M+5H]^{5+}$	1080.8	
11	F21C(disialo)-SRIF28	5367.6	$[M+3H]^{3+}$	1790.0	5366.9
			$[M+4H]^{4+}$	1342.7	
			$[M+5H]^{5+}$	1074.4	
12	T26C(disialo)-SRIF28	5413.6	$[M+3H]^{3+}$	1354.3	5412.1
			$[M+4H]^{4+}$	1083.4	
			$[M+5H]^{5+}$	903.0	
			$[M+6H]^{6+}$	774.3	
13	29C(disialo)-SRIF28	5514.7	$[M+3H]^{3+}$	1839.1	5514.1
			$[M+4H]^{4+}$	1379.5	
			$[M+5H]^{5+}$	1103.8	
14	30C(disialo)-SRIF28	5615.8	$[M+3H]^{3+}$	1872.7	5615.1
			$[M+4H]^{4+}$	1404.8	
			$[M+5H]^{5+}$	1124.0	
15	S1C(disialo)-D-Trp22 -SRIF28	5427.7	$[M+3H]^{3+}$	1810.1	5427.2
			$[M+4H]^{4+}$	1357.8	
			$[M+5H]^{5+}$	1086.4	
			$[M+6H]^{6+}$	905.5	
16	A9C(disialo)-D-Trp22 -SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5442.9
			$[M+4H]^{4+}$	1361.8	
			$[M+5H]^{5+}$	1089.6	
17	C(disialo)-SRIF14	4004.1	$[M+2H]^{2+}$	2002.9	4003.3
			$[M+3H]^{3+}$	1335.4	
			$[M+4H]^{4+}$	1001.8	
18	C(disialo)-R-K-SRIF14	4288.4	$[M+3H]^{3+}$	1430.2	4287.5
			$[M+4H]^{4+}$	1072.9	
19	C(disialo)-C12linker-SRIF14	4201.4	$[M+3H]^{3+}$	1401.2	4200.5
			$[M+4H]^{4+}$	1051.2	
			$[M+5H]^{5+}$	841.1	

[Table 3]

Table 1 — 3

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
20	S1-2C(disialo)-SRIF28	7793.8	$[M+4H]^{4+}$	1949.4	7793.4
			$[M+5H]^{5+}$	1559.7	
			$[M+6H]^{6+}$	1299.9	
21	S1C(disialo) ・ N5C(disialo)-SRIF28	7679.7	$[M+3H]^{3+}$	1920.4	7678.7
			$[M+4H]^{4+}$	1536.8	
			$[M+5H]^{5+}$	1280.9	
22	S1C(disialo) ・ R13C(disialo)-SRIF28	7637.7	$[M+3H]^{3+}$	1910.2	7636.6
			$[M+4H]^{4+}$	1528.3	
			$[M+5H]^{5+}$	1273.8	
23	N5C(disialo) ・ A9C(disialo)-SRIF28	7695.7	$[M+5H]^{5+}$	1540.1	7695.2
			$[M+6H]^{6+}$	1283.6	
			$[M+7H]^{7+}$	1100.3	
			$[M+8H]^{8+}$	962.9	
24	S1-3C(disialo)-SRIF28	10160.0	$[M+3H]^{3+}$	3387.6	10158.2
			$[M+4H]^{4+}$	2540.9	
			$[M+5H]^{5+}$	2032.7	
			$[M+6H]^{6+}$	1694.2	
			$[M+7H]^{7+}$	1452.3	
25	S1C(disialo) ・ N5C(disialo) ・ A9C(disialo)-SRIF28	9974.8	$[M+5H]^{5+}$	1995.9	9974.3
			$[M+6H]^{6+}$	1663.2	
26	S1C(monosialo)-SRIF28	5136.4	$[M+3H]^{3+}$	1713.0	5134.9
			$[M+4H]^{4+}$	1284.7	
			$[M+5H]^{5+}$	1028.2	
			$[M+6H]^{6+}$	856.8	

[Table 4]

Table 1 — 4

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
27	S1C(asialo)-SRIF28	4845.1	$[M+3H]^{3+}$	2615.7	4843.9
			$[M+4H]^{4+}$	1212.0	
			$[M+5H]^{5+}$	969.8	
28	S1-2C(asialo)-SRIF28	6628.8	$[M+4H]^{4+}$	1658.1	6627.2
			$[M+5H]^{5+}$	1326.7	
			$[M+6H]^{6+}$	1105.6	
			$[M+7H]^{7+}$	947.8	
			$[M+8H]^{8+}$	829.4	
29	S1-3C(asialo)-SRIF28	8412.5	$[M+4H]^{4+}$	2103.7	8411.7
			$[M+5H]^{5+}$	1683.3	
			$[M+6H]^{6+}$	1403.0	
30	N5N(disialo)-SRIF28	5354.5	$[M+3H]^{3+}$	1785.7	5354.0
			$[M+4H]^{4+}$	1339.5	
			$[M+5H]^{5+}$	1071.8	
31	S1N(disialo)-SRIF28	5381.6	$[M+3H]^{3+}$	1794.7	5380.9
			$[M+4H]^{4+}$	1346.2	
			$[M+5H]^{5+}$	1077.2	
32	S1C(disialo) + N19C(GlcNAc)-SRIF28	5676.9	$[M+3H]^{3+}$	1893.5	5676.4
			$[M+4H]^{4+}$	1420.1	
			$[M+5H]^{5+}$	1136.3	
33	S1C(disialo) + N19C(diMan)-SRIF28	6366.6	$[M+3H]^{3+}$	2122.9	6366.7
			$[M+4H]^{4+}$	1592.5	
			$[M+5H]^{5+}$	1274.4	
34	S1-5C(disialo)-SRIF28	14892.4	$[M+4H]^{4+}$	3723.7	14891.3
			$[M+5H]^{5+}$	2979.1	
			$[M+6H]^{6+}$	2482.9	
			$[M+7H]^{7+}$	2128.2	
			$[M+8H]^{8+}$	1862.4	
35	S1-10C(disialo)-SRIF28	26723.2	$[M+7H]^{7+}$	3818.3	26721.8
			$[M+8H]^{8+}$	3341.0	
			$[M+9H]^{9+}$	2970.1	
			$[M+10H]^{10+}$	2673.1	

[Table 5]

Table 1−5

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
36	C(disialo)-SRIF28	5514.7	$[M+4H]^{4+}$	1379.7	5514.6
			$[M+5H]^{5+}$	1103.9	
			$[M+6H]^{6+}$	920.1	
			$[M+7H]^{7+}$	788.8	
37	R11C(disialo)-SRIF28	5358.5	$[M+3H]^{3+}$	1787.4	5358.6
			$[M+4H]^{4+}$	1340.7	
			$[M+5H]^{5+}$	1072.7	
38	F20C(disialo)-SRIF28	5367.6	$[M+3H]^{3+}$	1790.1	5367.1
			$[M+4H]^{4+}$	1342.8	
			$[M+5H]^{5+}$	1074.4	
39	T24C(disialo)-SRIF28	5413.6	$[M+3H]^{3+}$	1805.7	5412.6
			$[M+4H]^{4+}$	1354.2	
			$[M+5H]^{5+}$	1083.7	
40	F25C(disialo)-SRIF28	5367.6	$[M+3H]^{3+}$	1790.1	5367.1
			$[M+4H]^{4+}$	1342.8	
			$[M+5H]^{5+}$	1074.4	
			$[M+6H]^{6+}$	895.4	
41	S27C(disialo)-SRIF28	5427.7	$[M+3H]^{3+}$	1810.1	5427.4
			$[M+4H]^{4+}$	1357.8	
42	C(disialo)-K-SRIF14	4131.2	$[M+2H]^{2+}$	2066.9	4131.7
			$[M+3H]^{3+}$	1378.2	
			$[M+4H]^{4+}$	1033.9	
43	S1C(disialo)-F25Y-SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5443.1
			$[M+4H]^{4+}$	1361.6	
			$[M+5H]^{5+}$	1089.6	
44	S1C(disialo)-SRIF28-amide	5426.7	$[M+3H]^{3+}$	1809.8	5426.2
			$[M+4H]^{4+}$	1357.6	
			$[M+5H]^{5+}$	1086.2	
			$[M+6H]^{6+}$	905.2	
45	C(disialo)-PEGlinker-SRIF14	4207.3	$[M+3H]^{3+}$	1403.2	4206.6
			$[M+4H]^{4+}$	1052.7	
			$[M+5H]^{5+}$	842.3	
46	Biotin-S1C(disialo)-SRIF28	5654.0	$[M+3H]^{3+}$	1885.4	5653.2
			$[M+4H]^{4+}$	1414.3	
			$[M+5H]^{5+}$	1131.7	
			$[M+6H]^{6+}$	943.2	
47	Biotin-PEGlinker -S1C(disialo)-SRIF28	5857.2	$[M+3H]^{3+}$	1953.2	5856.4
			$[M+4H]^{4+}$	1465.1	
			$[M+5H]^{5+}$	1172.1	
			$[M+6H]^{6+}$	977.1	
			$[M+7H]^{7+}$	837.6	

[Table 6]

Table 1—6

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
48	Azido-S1C(disialo)-SRIF28	5552.8	$[M+3H]^{3+}$	1851.8	5552.3
			$[M+4H]^{4+}$	1389.1	
			$[M+5H]^{5+}$	1111.7	
49	S1C(disialo)・E12C(disialo)-SRIF28	7664.7	$[M+4H]^{4+}$	1917.1	7664.1
			$[M+5H]^{5+}$	1533.8	
			$[M+6H]^{6+}$	1278.4	
50	2C(disialo)-R-K-SRIF14	6654.6	$[M+3H]^{3+}$	2219.2	6653.4
			$[M+4H]^{4+}$	1664.4	
			$[M+5H]^{5+}$	1331.9	
			$[M+6H]^{6+}$	1109.9	
51	3C(disialo)-R-K-SRIF14	9020.8	$[M+4H]^{4+}$	2256.1	9020.2
			$[M+5H]^{5+}$	1805.1	
			$[M+6H]^{6+}$	1504.4	
52	S1C(diGlcNAc)-SRIF28	4520.9	$[M+3H]^{3+}$	1507.7	4519.9
			$[M+4H]^{4+}$	1131.0	
			$[M+5H]^{5+}$	905.0	
53	S1C(diMan)-SRIF28	4114.5	$[M+3H]^{3+}$	1372.3	4113.8
			$[M+4H]^{4+}$	1029.5	
			$[M+5H]^{5+}$	823.8	
54	N19C(diMan)-SRIF28	4087.5	$[M+3H]^{3+}$	1363.3	4086.8
			$[M+4H]^{4+}$	1023.0	
55	S1C(GlcNAc)-SRIF28	3424.9	$[M+2H]^{2+}$	1713.3	3424.5
			$[M+3H]^{3+}$	1142.5	
			$[M+4H]^{4+}$	856.9	
			$[M+5H]^{5+}$	686.1	
56	N19C(GlcNAc)-SRIF28	3397.8	$[M+2H]^{2+}$	1699.8	3397.5
			$[M+3H]^{3+}$	1133.5	
			$[M+4H]^{4+}$	850.4	
57	S1C(trisialo)-SRIF28	6084.2	$[M+3H]^{3+}$	2028.8	6083.4
			$[M+4H]^{4+}$	1521.9	
			$[M+5H]^{5+}$	1217.7	
			$[M+6H]^{6+}$	1014.9	
58	S1C(tetrasialo)-SRIF28	6740.8	$[M+3H]^{3+}$	2247.7	6739.6
			$[M+4H]^{4+}$	1685.9	
			$[M+5H]^{5+}$	1349.1	
			$[M+6H]^{6+}$	1124.3	
			$[M+7H]^{7+}$	963.8	
59	S1C(disialo(aminoethylamide))-SRIF28	5511.8	$[M+3H]^{3+}$	1838.0	5511.1
			$[M+4H]^{4+}$	1378.5	
			$[M+5H]^{5+}$	1103.2	
			$[M+6H]^{6+}$	919.5	
			$[M+7H]^{7+}$	788.2	

[Table 7]

Table 1—7

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
60	S1C(disialo(amide))-SRIF28	5425.7	$[M+3H]^{3+}$	1809.3	5423.9
			$[M+4H]^{4+}$	1357.0	
			$[M+5H]^{5+}$	1085.8	
			$[M+6H]^{6+}$	905.2	
61	S1C(disialo(Bn))-SRIF28	5607.9	$[M+3H]^{3+}$	1870.0	5605.9
			$[M+4H]^{4+}$	1402.5	
			$[M+5H]^{5+}$	1122.2	
			$[M+6H]^{6+}$	935.5	
62	S1C(disialo(hexadecylamide))-SRIF28	5874.5	$[M+3H]^{3+}$	1958.9	5873.6
			$[M+4H]^{4+}$	1469.4	
			$[M+5H]^{5+}$	1175.7	
			$[M+6H]^{6+}$	979.9	
63	S1-2C(disialo(amide))-SRIF28	7789.9	$[M+4H]^{4+}$	1948.2	7789.9
			$[M+5H]^{5+}$	1559.0	
			$[M+6H]^{6+}$	1299.2	
64	S1-2C(disialo(Bn))-SRIF28	8154.3	$[M+4H]^{4+}$	2039.3	8153.0
			$[M+5H]^{5+}$	1631.6	
			$[M+6H]^{6+}$	1360.2	
65	S1C(Asn(disialo))-SRIF28	5542.7	$[M+3H]^{3+}$	1848.4	5542.2
			$[M+4H]^{4+}$	1386.6	
			$[M+5H]^{5+}$	1109.4	
			$[M+6H]^{6+}$	924.9	
66	S1N(disialo)·N19C(diMan)-SRIF28	6320.5	$[M+3H]^{3+}$	2107.9	6319.5
			$[M+4H]^{4+}$	1580.9	
			$[M+5H]^{5+}$	1265.1	
67	C(disialo(aminoethylamide))·S1C(disialo)-SRIF28	7878.0	$[M+4H]^{4+}$	1970.3	7878.1
			$[M+5H]^{5+}$	1576.4	
			$[M+6H]^{6+}$	1313.9	
			$[M+7H]^{7+}$	1126.4	
			$[M+8H]^{8+}$	985.5	
			$[M+9H]^{9+}$	876.1	
68	S1-4C(disialo)-SRIF28	12526.2	$[M+5H]^{5+}$	2506.1	12525.5
			$[M+6H]^{6+}$	2088.6	
			$[M+7H]^{7+}$	1790.4	

[0258]

Example 69-1 Calculation of receptor binding affinity

Competitive binding assay was performed with the method below to calculate receptor binding affinity.

Reagents employed in the competitive binding assay and their proper chemical names are as follows: HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and BSA (bovine serum albumin).

Competitive binding assay was consigned to Ricerca Biosciences, and experiment and data analysis was performed.

The receptor subtypes and receptor membrane samples employed are shown in Table 2. Common in each binding assay, [^{125}I] Tyr^{11}-Somatostatin 14 (Tyr11-SRIF14) was used as the labeled ligand, Somatostatin-14 (SRIF14) was used as the unlabeled ligand, and 25 mM HEPES containing 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.1% BSA, pH 7.4 was used as the buffer. The test substance was used at a concentration of 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM, or 1000 nM, and mixed with the membrane sample into a reaction solution. Moreover, the concentrations of the labeled and unlabeled ligands added to the reaction solution are shown in Table 2. The incubation condition of the reaction solution was at 25°C for 4 hours for SSTR2 and at 25°C for 2 hours for SSTR1, SSTR3, SSTR4, and SSTR5. For each experiment run, SRIF14 was used as the positive control. For data analysis, 50% inhibitory concentration (IC_{50} value) was determined using MathIQ™ (ID Business Solutions, UK) in a nonlinear least squares method based on the numerical data of the binding inhibition rate. The binding inhibition constant (Ki value) was calculated by the method of Cheng, Y et al. (Biochem Pharmacol, 22, 3099-3108, 1973).

[Table 8]

Table 2

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Labeled ligand	0.1nM	0.03nM	0.1nM	0.1nM	0.1nM
Unlabeled ligand	1μM	1μM	1μM	1μM	1μM
Source	Human recombinant CHO-K1 cells	Human recombinant CHO-K1 cells	Human recombinant CHO-K1 cells	Human recombinant Chem-1 cells	Human recombinant Chem-1 cells

[0259]

Compounds subjected to binding assay and the results of the binding experiment are shown in Table 3A. Moreover, octreotide, SRIF14 and SRIF28 were similarly evaluated as control compounds. Note that for octreotide, IC_{50} values could not be calculated for SSTR1 and SSTR4 since the maximum concentration was 100 nM, and is thus shown as >100 nM.

Figures 1A and 1B show examples of the structures of glycosylated peptides (glycosylated forms) corresponding to the compound names in Table 3A.

[0260]

[Table 9]

Table 3A

[0261]

The control octreotide bound to each of receptors SSTR2, SSTR3, and SSTR5, and SRIF14 and SRIF28 bound to all SSTRs. As shown in Table 3A, the compounds according to

Table 3B

Example		SSTR1		SSTR2		SSTR3		SSTR4		SSTR5	
		IC50 (nM)	Ki (nM)	IC50 (nM)	Ki (nM)	IC50 (nM)	Ki (nM)	IC50 (nM)	Ki (nM)	IC50 (nM)	Ki (nM)
1	S1C(disalic)—SRIF28	7.71	0.45	0.043	0.289	1.98	0.676	2.10	6.38	1.61	0.911
7	A1BC(disalic)-SRIF28	11.4	9.43	0.938	0.846	3.49	1.19	8.18	7.36	2.84	2.17
8	G16C(disalic)-SRIF28	17.4	14.4	1.22	0.846	3.12	1.07	12.2	11.0	5.67	3.21
9	K18C(disalic)-SRIF28	45.4	37.6	12.2	8.31	14.7	5.03	62.7	56.5	160	85.0
12	T26C(disalic)-SRIF28	21.5	17.9	3.10	1.50	21.7	7.44	25.8	24.1	400	230
26	S1C(amide)-SRIF28	5.38	4.46	0.213	0.112	1.18	0.405	2.35	2.12	0.816	0.466
27	S1C(acid)-SRIF28	3.97	3.29	0.200	0.105	1.02	0.349	2.98	2.68	1.04	0.768
32	S1C(disalic)-N19C(GlcNAc)-SRIF28	<10	<8.27	2.20	1.17	3.05	1.04	8.55	7.69	2.37	1.34
33	S1C(disalic)-N19C(dMan)-SRIF28	44.0	37.3	3.73	1.98	2.63	1.24	23.0	23.0	5.69	3.22
34	S1 10C(disalic)-SRIF28	110	91.0	12.7	6.38	21.3	7.29	62.2	58.4	7.23	4.05
35	S1 10C(disalic)-SRIF28	117	96.6	10.1	5.35	40.4	13.8	90	172	34.8	19.1
36	Cid salic)-SRIF28	5.04	4.51	0.428	0.225	2.56	0.870	6.65	7.79	2.62	1.48
37	R11C(disalic)-SRIF28	22.5	18.2	1.36	0.705	6.34	2.17	20.2	18.1	3.85	2.07
40	T25C(disalic)-SRIF28	>1000	>826	>1000	>531	>1000	>342	130	116	58.0	33.0
41	S27C(disalic)-SRIF28	28.4	23.5	5.26	2.70	6.77	2.31	33.4	33.1	4.94	2.80
42	Cid salic)-K SRIF-4	20.1	16.2	1.91	1.05	3.56	1.25	11.0	9.47	3.02	1.99
43	S1C(Acetic)-F27Y-SRIF28	1.77	1.48	0.310	0.180	0.491	0.190	1.55	1.38	1.09	0.620
44	S1C(Acetic)-SRIF28 amide	4.87	4.03	1.32	0.780	5.91	2.02	6.88	6.18	3.75	2.12
45	Cid salic)-PEGlinker SRIF14	36.0	29.9	4.67	2.48	8.71	2.98	21.0	19.0	>10	>5.65
46	Biotin-S1C(disalic)-SRIF28	11.0	9.05	1.66	0.890	2.92	1.00	9.28	8.35	2.42	1.37
47	Biotin-PEGlinker-S1C(disalic)-SRIF28	9.41	7.79	1.06	1.04	3.41	1.17	9.31	8.38	2.00	1.13
48	biotin-S1C(disalic)-SRIF28	8.82	7.30	1.79	0.690	3.17	1.09	5.34	4.81	0.840	0.470
49	S1C(disalic)-E12C(disalic)-SRIF28	22.0	18.2	2.70	1.44	4.62	1.58	20.0	18.0	2.11	1.19
50	2C(disalic)-RK-SRIF14	48.0	40.5	5.45	2.84	9.74	3.33	49.0	44.0	6.16	3.46
51	3C(disalic)-RK-SRIF14	270	230	44.0	17.0	40.0	14.0	>300	>270.0	41.0	23.0
52	S1C(AcGlcNAc)-SRIF28	5.04	4.17	0.730	0.100	2.02	0.690	3.91	3.52	1.45	0.820
53	S1C(dMan)-SRIF28	5.08	4.48	0.940	0.0700	1.32	0.450	3.01	2.70	0.990	0.560
54	N19C(dMan)-SRIF28	6.58	5.44	1.17	0.620	1.65	0.530	8.02	7.22	2.94	1.66
55	S1C(GlcNAc)-SRIF28	5.91	4.89	0.735	0.120	1.65	0.565	2.89	2.60	1.15	0.650
56	N19C(GlcNAc)-SRIF28	2.60	2.15	0.540	0.530	41	<0.342	2.38	2.15	1.89	1.07
57	S1C(hexose)-SRIF28	1.70	1.40	0.380	0.410	5.58	1.91	1.60	1.40	1.36	1.10
58	S1C(hexose)-SRIF28	1.73	1.42	1.12	0.307	5.69	1.93	1.43	1.26	2.12	1.20
59	S1C(Acetamidomethylamide)-SRIF28	2.35	1.94	0.101	0.0536	0.796	0.284	2.24	2.01	2.06	1.18
60	S1C(disalic)amide-SRIF28	5.00	4.21	0.297	0.110	1.53	0.522	6.12	5.30	2.60	1.47
61	S1C(disalic/Bn)-SRIF28	6.20	5.12	0.247	0.130	2.15	0.733	6.01	5.41	3.25	1.83
62	S1C(disalic/hexadecylamide)-SRIF28	5.63	4.67	1.04	0.350	11.0	3.75	26.0	24.0	37.0	21.0
63	S1 2C(disalic/amide)-SRIF28	8.92	5.73	0.830	0.440	2.06	0.710	4.76	4.28	2.67	1.51
64	S1 2C(disalic/Bn)-SRIF28	9.01	4.67	0.830	0.440	2.58	0.890	8.20	7.38	2.80	1.47
65	S1C(Acid/disalic)-SRIF28	15.8	13.0	0.610	0.330	5.57	1.91	15.0	14.0	1.61	0.910
66	S1Mdisalic-NH2GlcNAc-SRIF28	36.0	29.8	2.40	3.00	3.90	1.35	25.0	22.0	8.53	4.82
67	S1disalic-dimethylamide)-S1C(disalic)-SRIF28	6.20	5.13	0.690	0.610	1.89	0.650	6.12	5.51	1.81	1.02
68	S1 4C(disalic)-SRIF28	130	100	3.76	2.10	10.0	3.43	66.0	60.0	10.0	5.82
	SRIF28	0.633	1.83	0.0040	0.0365	0.230	0.116	0.776	1.23	0.189	0.099
	SRIF-14	1.25	2.69	0.0326	0.0093	0.0697	0.493	0.823	1.95	0.416	0.813

As shown in Table 3B, the controls SRIF14 and SRIF28 bound to all receptors SSTR1 - SSTR5. Since the number of

forskolin, and allowed to react at room temperature for 30 minutes. After the reaction, the cells were dissolved with 0.2 N HCl, and the amount of cAMP accumulated in the cell was measured with Cayman cyclic AMP EIA kit (Cayman, 582002).

Evaluation of SSTR1 was consigned to Cerep, and the experiment was performed. HBSS containing 20 mM HEPES (pH 7.4) and 500 μM IBMX was used as the buffer, and SSTR1 receptor expression cells were seeded at 10^4 cells/well. The test substance was treated by mixing at a concentration of 0.001 nM, 0.01 nM, 0.1 nM, 1 nM, 10 nM, or 100 nM with 1 μM NKH477, and allowed to react at 37°C for 20 minutes. After the reaction, the amount of cAMP accumulated in the cell was measured with Cisbio cAMP dynamic2 kit (Cisbio, 62AM4PE).

[Table 11]

Table 3C

	SSTR2	SSTR3	SSTR4	SSTR5
Distributer	Perkin Elmer			
Serial Number	ES-521-CF	ES-523-CF	ES-524-CF	ES-522-CF
Derived cell	CHO-K1 cells			

[0264]

The experimental results (IC_{50} (nM)) of the agonist activity evaluation test when employing the compounds of Examples 1, 18, 27, 28, 59, and 60 as the glycosylated form are shown in Table 3D and Figure 1E. IC_{50} of the control compounds SRIF14 and SRIF28 was 0.83 - 0.89 nM against SSTR1, and 0.0076 - 0.073 nM, 0.029 - 0.21 nM, 0.015 - 0.074 nM, and 0.066 - 0.12 nM against SSTR2, SSTR3, SSTR4, and SSTR5, respectively. IC_{50} of the agonist activity of the glycosylated compounds against SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 were 1.0 - 2.4 nM, 0.041 - 0.10 nM, 0.12 - 0.30 nM, 0.039 - 0.085 nM, and 0.043 - 0.081 nM, respectively, and potent agonist activity was shown against all receptors SSTR1 - SSTR5. From the results shown in Examples 69-1 and 69-2, it is apparent that these compounds have receptor binding affinity, and it became clear that these compounds exert proper agonistic action by binding to a receptor.

[Table 12]

Table 3D

Example		SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
-	SRIF14	0.83 ± 0.52	0.0076 ± 0.0012	0.029 ± 0.017	0.015 ± 0.006	0.12 ± 0.03
-	SRIF28	0.69 ± 0.41	0.073 ± 0.014	0.21 ± 0.08	0.074 ± 0.058	0.066 ± 0.033
1	S1C(disialo)-SRIF28	2.2 ± 1.1	0.075 ± 0.030	0.13 ± 0.06	0.065 ± 0.042	0.056 ± 0.028
18	Cys(disialo)-R-K-SRIF14	1.2 ± 0.6	0.055 ± 0.015	0.18 ± 0.10	0.050 ± 0.025	0.052 ± 0.032
27	S1C(asialo)-SRIF28	1.6 ± 0.6	0.041 ± 0.006	0.12 ± 0.05	0.039 ± 0.021	0.055 ± 0.031
28	S1-2C(asialo)-SRIF28	1.5 ± 0.6	0.079 ± 0.033	0.15 ± 0.08	0.051 ± 0.046	0.064 ± 0.029
59	S1C(disialo(aminoethylamide))-SRIF28	1.0 ± 0.9	0.10 ± 0.02	0.30 ± 0.15	0.085 ± 0.011	0.081 ± 0.019
60	S1C(disialo(amide))-SRIF28	2.4 ± 1.7	0.055 ± 0.013	0.14 ± 0.06	0.043 ± 0.023	0.043 ± 0.015

[0265]

Example 69-4 Agonist activity evaluation employing receptor expression cells - 2

Agonist activity evaluation test was carried out similarly to Example 69-3 employing each of the compounds described in Table 3E as the glycosylated form. The experimental results are shown in Table 3E. In Table 3E, fields with "- (hyphen)" shown indicates that the test was not performed.

[Table 13]

Table 3E

(-: Not performed)

Experiment		SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
–	SRIF14	0.83	0.0076	0.029	0.015	0.12
–	SRIF28	0.89	0.073	0.21	0.074	0.055
4	E12C(disialo)-SRIF28	2.3	–	–	–	–
10	N19C(disialo)-SRIF28	22	–	–	–	–
15	S1C(disialo)-D-Trp22-SRIF28	3.5	–	–	–	–
17	C(disialo)-SRIF14	–	0.029	0.18	0.10	0.23
19	C(disialo)-C12linker-SRIF14	–	–	–	0.11	–
20	S1-2C(disialo)-SRIF28	–	–	0.55	0.14	0.22
21	S1C(disialo)-N5C(disialo)-SRIF28	–	0.072	0.37	0.041	0.090
23	N5C(disialo)-A9C(disialo)-SRIF28	–	0.096	0.29	0.091	0.13
24	S1-3C(disialo)-SRIF28	7.5	0.19	0.79	0.36	0.97
25	S1C(disialo)-N5C(disialo)-A9C(disialo)-SRIF28	–	1.4	2.4	0.16	1.1
26	S1C(monosialo)-SRIF28	1.5	0.059	0.15	0.046	0.052
29	S1-3C(asialo)-SRIF28	–	0.19	–	0.10	–
31	S1N(disialo)-SRIF28	2.3	–	–	–	–
32	S1C(disialo)-N19C(GlcNAc)-SRIF28	–	0.080	0.039	0.033	0.088
34	S1-5C(disialo)-SRIF28	18	0.81	2.0	0.27	–
42	C(disialo)-K-SRIF14	–	0.019	0.10	0.050	–
44	S1C(disialo)-SRIF28-amide	1.7	–	–	–	–
47	C(disialo)-PEG2linker-SRIF14	–	0.034	0.15	0.056	0.18
50	2C(disialo)-R-K-SRIF14	–	0.15	0.55	1.1	–
51	3C(disialo)-R-K-SRIF14	–	1.4	3.8	1.6	–
52	S1C(diGlcNAc)-SRIF28	1.2	0.045	0.14	0.050	0.033
53	S1C(diMan)-SRIF28	1.2	0.035	0.11	0.043	0.037
54	N19C(diMan)-SRIF28	–	–	–	0.11	–
55	S1C(GlcNAc)-SRIF28	1.4	0.040	0.14	0.049	0.031
56	N19C(GlcNAc)-SRIF28	–	–	–	0.092	–
57	S1C(trisialo)-SRIF28	4.9	0.11	0.35	0.092	0.066
58	S1C(tetrasialo)-SRIF28	2.8	0.13	0.25	0.081	0.11
61	S1C(disialo(Bn))-SRIF28	1.4	–	–	–	–
62	S1C(disialo(hexadecylamide))-SRIF28	1.3	–	–	–	–
65	S1C(Asn(disialo))-SRIF28	2.6	–	–	0.13	–
67	C(disialo(aminoethylamide))-S1C(disialo)-SRIF28	–	0.039	0.17	0.047	0.034

[0266]

In Table 3E, IC_{50} of the agonist activity of the glycosylated compounds against SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 were 1.2 - 22 nM, 0.019 - 1.4 nM, 0.039 - 3.8 nM, 0.033 - 1.6 nM, and 0.031 - 1.1 nM, respectively, and agonist activity was shown against receptors SSTR1 - SSTR5. From the results shown in Examples 69-1 and 69-2, it became clear that these compounds have receptor binding affinity,

[Table 14]

Table 4

	Intravenous administration				Subcutaneous administration			
	$t_{1/2}$	AUC	MRT	C_0	$t_{1/2}$	AUC	MRT	C_{max}
S1C(disialo)-SRIF28	18.1	5086	20.4	780	26.0	1273	43.8	19.9
SRIF28	0.8	358	2.3	678	1.5	15	3.2	4.4

($t_{1/2}$: min, AUC : min·nM, MRT : min, C_0 : nM, C_{max} : nM)

As is clear from the results shown in Figure 2 and Table 4, S1C(disialo)-SRIF28 has significantly extended $t_{1/2}$ and MRT compared to the non-glycosylated SRIF28, and an increase in AUC and C_{max} was recognized. These are thought to be due to the increase in resistance to degradation activity in blood by glycosylation. It is clear that the glycosylated form has improved stability *in vivo* compared to the non-glycosylated form. Moreover, as factors for the increase in AUC and C_{max}, the improvement of bioavailability according to the present invention, as well as improvement of stability thereof *in vivo* are thought to be factors.

[0272]

Example 71 Pharmacokinetics test with rats 2

A pharmacokinetics test was carried out similarly to Example 70, except that S1C(disialo)-SRIF28, N5C(disialo)-SRIF28, and S1C(disialo)·N5C(disialo)-SRIF28 were employed as the glycosylated forms. As a control, non-glycosylated SRIF28 was employed instead of the glycosylated form. The compound plasma concentration transition obtained is shown in Figure 3.

[0273]

Example 72 Pharmacokinetics test with rats 3

A pharmacokinetics test was carried out similarly to Example 70, except that S1C(disialo)-SRIF28, S1C(disialo)·R13C(disialo)-SRIF28, and S1C(disialo)·N5C(disialo)·A9C(disialo)-SRIF28 were employed as the glycosylated forms. As a control, non-glycosylated SRIF28 was employed instead of the glycosylated form. The compound plasma concentration transition obtained is shown in Figure 4.

[0274]

Example 73 Pharmacokinetics test with rats 4

A pharmacokinetics test was carried out similarly to Example 70, except that S1C(disialo)-SRIF28, S1C(disialo)·N5C(disialo)·A9C(disialo)-SRIF28, S1-3C(disialo)-SRIF28, S1-5C(disialo)-SRIF28, and S1-10C(disialo)-SRIF28 were employed as the glycosylated forms.

Table 5A

	Compound	The number of sugar chains	value of n	Intravenous administration				Subcutaneous administration				BA
				$t_{1/2}$	AUC	MRT	C_0	$t_{1/2}$	AUC	MRT	C_{max}	
-	SRIF28	None	6	1.00 ±0.20	472 ±166	2.20 ±0.11	554 ±221	2.94 ±1.59	25.5 ±13.8	4.76 ±1.80	4.47 ±1.21	5
1	S1C(disialo)-SRIF28	1	15	16.9 ±1.02	4855 ±1030	18.6 ±1.72	686 ±363	29.8 ±2.68	1773 ±281	47.9 ±2.68	28.9 ±3.23	37
2	N5C(disialo)-SRIF28	1	6	20.1 ±1.49	4421 ±1366	22.9 ±2.16	496 ±312	28.1 ±3.72	2392 ±443	51.9 ±5.92	32.4 ±4.87	54
3	A9C(disialo)-SRIF28	1	3	19.9 ±0.33	4083 ±515	25.2 ±0.38	547 ±211	30.3 ±2.45	2462 ±64.0	55.6 ±5.81	30.2 ±5.05	60
	Average value			19.0	4453	22.2	576.3	29.4	2209	51.8	30.5	50
20	S1-2C(disialo)-SRIF28	2 (dense)	3	28.8 ±3.11	7306 ±625	23.3 ±2.63	1382 ±321	40.9 ±12.2	4046 ±248	67.9 ±15.0	46.3 ±9.75	55
21	S1C(disialo) - N5C(disialo)-SRIF28	2 (with intervals)	6	25.8 ±3.41	4099 ±631	24.6 ±5.97	558 ±248	38.4 ±4.93	3344 ±663	67.9 ±8.39	35.5 ±6.38	82
22	S1C(disialo) - R13C(disialo)-SRIF28	2 (with intervals)	3	23.4 ±0.66	4029 ±184	20.6 ±1.32	822 ±106	39.6 ±2.50	3407 ±436	67.7 ±5.39	38.7 ±8.73	85
23	N5C(disialo) - A9C(disialo)-SRIF28	2 (with intervals)	3	32.4 ±1.66	4465 ±833	26.6 ±0.27	664 ±104	42.7 ±9.76	3450 ±784	75.0 ±12.4	32.5 ±7.06	77
				27.2	4198	23.9	681.3	40.2	3400	70.2	35.6	81
24	S1-3C(disialo)-SRIF28	3 (dense)	6	38.5 ±2.83	5660 ±1354	24.0 ±3.92	1061 ±209	87.7 ±21.4	3884 ±543	126 ±31.7	27.5 ±10.8	69
25	S1C(disialo)- N5C(disialo) A9C(disialo)-SRIF28	3 (with intervals)	9	39.8 ±1.87	4402 ±999	25.8 ±2.87	938 ±145	87.4 ±17.1	4015 ±944	132 ±19.8	24.8 ±9.03	91
34	S1-5C(disialo)-SRIF28	5 (dense)	3	99.5 ±14.0	6016 ±873	49.0 ±6.06	752 ±81.4	271 ±38.7	5524 ±633	389 ±50.0	13.5 ±2.85	92
35	S1-10C(disialo)-SRIF28	10 (dense)	3	219 ±6.27	100259 ±10463	272 ±14.3	821 ±264	>480	-	-	45.9 ±2.32	-

($t_{1/2}$: min , AUC : min·nM , MRT : min , C_0 : nM , C_{max} : nM , BA : %)

[0278]

As is clear from the results shown in Table 5A, the bioavailability of the glycosylated form of the present

Table 5B

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRI	C_0	$T_{1/2}$	AUC	MRI	Cmax	
-	SRIF14	0.500	119	2.00	819	2.30	8	4.30	1.80	7
	SRIF28	1.32	467	2.46	518	2.38	22	4.18	4.65	5
1	S1C(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	30.1	38
4	E12C(disialo)-SRIF28	19.5	10521	27.5	1086	38.2	2891	57.5	34.7	27
5	R13C(disialo)-SRIF28	20.3	4926	22.8	481	35.4	5528	55.9	65.9	78
6	K14C(disialo)-SRIF28	20.4	6278	20.0	884	35.1	3945	55.1	57.5	62
10	N19C(disialo)-SRIF28	18.8	3854	21.5	407	37.9	2624	55.3	41.7	56
13	29C(disialo)-SRIF28	23.7	3736	25.3	359	39.3	2176	57.6	29.4	51
15	S1C(disialo)-D-Trp-SRIF28	18.3	4924	20.6	588	31.9	2220	51.9	34.0	45
18	C(disialo)-R-K-SRIF14	18.9	6571	22.5	429	28.4	1996	45.2	32.5	30
42	C(disialo)-K-SRIF14	18.8	5277	19.4	646	26.4	2446	43.7	40.5	46
17	C(disialo)-SRIF14	16.5	5614	18.3	725	27.2	3641	40.0	73.6	66
19	C(disialo)-C12linker-SRIF14	11.0	1686	7.60	500	14.4	345	22.0	13.4	21
45	C(disialo)-PEGlinker-SRIF14	16.4	3538	18.8	412	20.7	2725	39.2	47.8	77

Table 5C

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
–	SRIF28	1.32	467	2.46	518	2.38	22	4.18	1.65	5
55	SIC(GlcNAc)-SRIF28	1.80	690	3.00	666	5.90	63	7.90	7.10	9
53	SIC(diMan)-SRIF28	5.50	1445	5.20	423	9.10	124	13.9	7.30	9
52	SIC(diGlcNAc)-SRIF28	10.5	3588	12.3	495	16.5	445	25.7	13.2	12
27	SIC(asialo)-SRIF28	13.4	4602	12.5	749	19.7	710	29.0	17.2	16
26	SIC(monosialo)-SRIF28	15.9	6618	15.3	1061	24.5	995	37.8	19.4	17
1	SIC(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	30.1	38
57	SIC(trisialo)-SRIF28	21.1	4428	22.0	423	36.6	1979	56.9	24.9	45
58	SIC(tetrasialo)-SRIF28	20.9	4851	23.0	471	40.5	2624	59.4	32.2	54
65	SIC(Asn(disialo))-SRIF28	27.9	6488	35.9	365	34.0	2935	55.3	33.9	45
59	SIC(disialo(aminoethylamide))-SRIF28	18.6	3473	12.1	746	27.1	284	33.2	9.60	8
60	SIC(disialo(amide))-SRIF28	18.0	5814	12.6	1048	30.0	961	42.5	19.1	17
61	SIC(disialo(Bn))-SRIF28	14.4	3708	14.5	526	45.5	1047	52.6	18.7	28

[0293]

As is clear from the results shown in Table 5C, when the size of the modifying sugar chain is altered to GlcNAc (monosaccharide), diMan (5 sugars), diGlcNAc (7 sugars), asialo (9 sugars), monosialo (10 sugars), disialo (11 sugars), trisialo (14 sugars), and tetrasialo (17 sugars), the $t_{1/2}$, AUC, and bioavailability when administered subcutaneously increased to 2 - 17-folds, 3 - 120-folds, and 2 - 11-folds, respectively, according to the size of the modifying sugar chain. From this, it became clear that altering the size of the modifying sugar chain not only improved the stability in blood, but allowed change in its increase rate. Moreover, since it is known that dimannose or asialo sugar chains etc. among these sugar chains interact with particular proteins, they are thought to be utilizable for targeting a particular protein or organs and cells having the particular protein. Moreover, as modification of the sialic acid at the non-reducing terminal, S1C(disialo(aminoethylamide))-SRIF28, S1C(disialo(amide))-SRIF28, and S1C(disialo(Bn))-SRIF28 having added a sugar chain with altered charge by e.g. the introduction of an aminoethylamide, amide, and Bn to the carboxy group extended $t_{1/2}$ by 11 - 14-folds and increased AUC by 7 - 12-folds, and improved stability in blood compared to the non-glycosylated form when administered intravenously. This shows that the carboxy group of the sialic acid has a large influence on retentivity in blood, and shows the possibility for (usage in) blood clearance or body distribution control by abstraction of the negative charge of the carboxy group (disialo(Bn) and disialo(amide)) or conversion into a positive charge (disialo(aminoethylamide)).

[Table 18]

Table 5D

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
-	SRIF14	0.50C	119	2.00	819	2.30	6	4.30	1.80	7
-	SRIF28	1.32	467	2.46	518	2.36	22	4.18	4.65	5
18	C(disialo)-R-K-SRIF14	18.9	6571	22.5	429	28.4	1996	45.2	32.5	30
50	2C(disialo)R-K-SRIF14	31.7	5041	24.1	704	47.7	3310	73.4	36.7	66
51	3C(disialo)R-K-SRIF14	35.7	4208	22.2	1013	70.6	3515	112	23.5	82
1	S1C(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	39.1	38
20	S1-2C(disialo)-SRIF28	28.2	5804	23.0	950	40.8	3091	65.3	34.8	53
24	S1-3C(disialo)-SRIF28	39.5	5834	23.6	1083	79.3	4174	115	30.7	72
66	S1-4C(disialo)-SRIF28	63.5	7070	25.2	1007	119	4554	172	22.0	64

[0294]

As is clear from the results shown in Table 5D, C(disialo)-R-K-SRIF14 modified with one disialosugar chain, 2C(disialo)-R-K-SRIF14 modified with two disialosugar

chains, and 3C(disialo)-R-K-SRIF14 modified with three disialosugar chains on SRIF14 each extended $t_{1/2}$ by 38, 63, and 71-folds and increased AUC by 55, 42, and 36-folds compared to the non-glycosylated form when administered intravenously. Similarly, S1C(disialo)-SRIF28 modified with one disialosugar chain, S1-2C(disialo)-SRIF28 modified with two disialosugar chains, S1-3C(disialo)-SRIF28 modified with three disialosugar chains, and S1-4C(disialo)-SRIF28 modified with four disialosugar chains on SRIF28 each extended $t_{1/2}$ by 13, 21, 30, and 48-folds and increased AUC by 11, 12, 12, and 15-folds compared to the non-glycosylated form when administered intravenously. In either of SRIF14 and SRIF28, it became clear that stability in blood is improved according to the number of modifying disialosugar chain.

[Table 19]

Table 5E

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
-	SRIF28	1.32	467	2.46	518	2.36	22	4.18	4.65	5
27	S1C(asialo)-SRIF28	13.4	4602	19.5	749	19.7	710	29.0	17.2	16
28	S1-2C(asialo)-SRIF28	8.30	2604	9.60	741	19.2	1049	36.4	19.8	39
29	S1-3C(asialo)-SRIF28	6.60	2733	6.50	1147	26.4	885	42.1	15.0	32

[0295]

As is clear from the results shown in Table 5E, S1C(asialo)-SRIF28 modified with one asialosugar chain, S1-2C(asialo)-SRIF28 modified with two asialosugar chains, and S1-3C(asialo)-SRIF28 modified with three asialosugar chains on SRIF28 each extended $t_{1/2}$ by 10, 6, and 5-folds and increased AUC by 10, 6, and 6-folds compared to the non-glycosylated form when administered intravenously. It became clear that stability in blood is also improved when the modifying sugar chain is an asialosugar chain.

[Table 20]

Table 5F

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
-	SRIF28	1.32	467	2.46	518	2.38	22	4.18	4.65	5
1	S1C(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	30.1	38
20	S1-2C(disialo)-SRIF28	28.2	5804	23.0	950	40.8	3091	65.3	34.6	53
28	S1-2C(asialo)-SRIF28	8.30	2664	9.60	741	19.2	1049	36.4	19.8	39
63	S1-2C(disialo(amide))-SRIF28	23.8	5268	20.5	737	33.1	1297	55.8	16.3	25
64	S1-2C(disialo(Bn))-SRIF28	25.6	5525	20.7	807	33.9	1843	59.4	21.5	33
67	C(disialo(aminoethylamide))・S1C(disialo)-SRIF28	22.5	3742	20.5	518	42.3	1289	64.7	16.2	34

Table 6

Example		PS index
1	S1C(disialo)-SRIF28	4.5
2	N5C(disialo)-SRIF28	4.7
3	A9C(disialo)-SRIF28	6.7
4	E12C(disialo)-SRIF28	6.2
5	R13C(disialo)-SRIF28	15.9
6	K14C(disialo)-SRIF28	19.1
10	N19C(disialo)-SRIF28	>20
13	29C(disialo)-SRIF28	>20
14	30C(disialo)-SRIF28	16.8
15	S1C(disialo)-D-Trp-SRIF28	12.3
16	A9C(disialo)-D-Trp-SRIF28	17.2
21	S1C(disialo) · N5C(disialo)-SRIF28	17.8
22	S1C(disialo) · R13C(disialo)-SRIF28	>20
24	S1-3C(disialo)-SRIF28	>20
25	S1C(disialo)·N5C(disialo)·A9C(disialo)-SRIF28	>20
26	S1C(monosialo)-SRIF28	3.5
27	S1C(asialo)-SRIF28	2.6
-	Octreotide	>20
-	SRIF28	1

[0300]

As is clear from the results shown in Figure 20, the glycosylated polypeptide of the present invention was increased in plasma stability compared to SRIF28. In other words, this was 4.5 - 6.7-folds in those having one sugar chain added at positions 1, 5, 9, and 12 (the compounds of Examples 1, 2, 3, and 4), 15.9-folds for those having a sugar chain added at position 13 (the compound of Example 5), 19.1-folds for those having a sugar chain added at position 14 (the compound of Example 6), and 16.8-folds for those having a sugar chain added at position 30 (the compound of Example 14), compared to that of SRIF28. Moreover, this was 20-folds or more for those having one glycosylated amino acid further added at position 19 which is the C-terminal side (the compounds of Examples 10 and 13). It was shown that in regards to the position for glycosylation, plasma stability increases from position 1 towards the C-terminal.

Table 7

Example		Dose	GH (ng/mL)
1	S1C(disialo)-SRIF28	1nmol/kg	490
1	S1C(disialo)-SRIF28	10nmol/kg	17.1
10	N19C(disialo)-SRIF28	10nmol/kg	563
10	N19C(disialo)-SRIF28	100nmol/kg	1.5
13	29C(disialo)-SRIF28	10nmol/kg	497
13	29C(disialo)-SRIF28	100nmol/kg	88.5
21	S1C(disialo)-N5C(disialo)-SRIF28	1nmol/kg	776
21	S1C(disialo)-N5C(disialo)-SRIF28	10nmol/kg	181
25	S1C(disialo)-N5C(disialo)-A9C(disialo)-SRIF28	10nmol/kg	833
25	S1C(disialo)-N5C(disialo)-A9C(disialo)-SRIF28	100nmol/kg	151
-	vehicle	-	861

[0305]

As is clear from the results shown in Table 7, the glycosylated form of the present invention suppressed GH production in rats. The ratio between the Ki value of glycosylated forms of Examples 21 and 25 against each receptor and the Ki value of unglycosylated SRIF14 is shown to be in the range of 15.7:1 - 1.2:1 (the compound of Example 21) and 158:1 - 7.1:1 (the compound of Example 25), respectively, as measured in the method of Example 69-1. Moreover, the glycosylated forms of Examples 21 and 25 are shown to have 25-folds or more (the compound of Example 21) or 30-folds or more (the compound of Example 25) increase in half-life in blood, respectively, as measured in the method of Example 70. On the other hand, from the present Example having two sugar chains, it was shown that the glycosylated form effectively exerts pharmacological action even *in vivo*.

Moreover, the glycosylated form of the present invention had GH production suppressing action even when administered 1 hour before GRF administration.

It was shown that the effective administration dose for rat GH production ability between the glycosylated forms of Examples 1 and 21 and Example 25 was different by approximately 10-folds. This is similar to the receptor affinities thereof having a difference of approximately 10-folds in terms of Ki value, as measured in the method of Example 69-1. It was shown that they have pharmacological activity even when the affinity of the glycosylated form was somewhat attenuated.

[0306]

Table 8

Example		Dose	GH (ng/mL)	The number of runs
1	S1C(disialo)-SRIF28	1nmol/kg	787	4
		3nmol/kg	182	3
		10nmol/kg	15	5
		30nmol/kg	8	1
		100nmol/kg	7	2
2	N5C(disialo)-SRIF28	1nmol/kg	969	1
		10nmol/kg	12	1
3	A9C(disialo)-SRIF28	1nmol/kg	577	1
		10nmol/kg	20	1
4	E12C(disialo)-SRIF28	10nmol/kg	10	1
5	R13C(disialo)-SRIF28	10nmol/kg	136	1
6	K14C(disialo)-SRIF28	10nmol/kg	55	1
15	S1C(disialo)-D-Trp22-SRIF28	0.3nmol/kg	986	3
		1nmol/kg	335	3
		3nmol/kg	50	1
		10nmol/kg	6	2
		30nmol/kg	2	1
18	C(disialo)-R-K-SRIF14	1nmol/kg	912	4
		3nmol/kg	291	4
		10nmol/kg	30	4
		30nmol/kg	12	1
21	S1C(disialo)-N5C(disialo)-SRIF28	1nmol/kg	776	1
		10nmol/kg	181	1
25	S1C(disialo)-N5C(disialo)-A9C(disialo)-SRIF28	100nmol/kg	151	1
26	S1C(monosialo)-SRIF28	10nmol/kg	47	1
27	S1C(asialo)-SRIF28	1nmol/kg	879	4
		3nmol/kg	515	4
		10nmol/kg	146	4
28	S1-2C(asialo)-SRIF28	3nmol/kg	997	3
		10nmol/kg	175	3
34	S1-5C(disialo)-SRIF28	10nmol/kg	842	1
59	S1C(disialo(aminoethylamide))-SRIF28	10nmol/kg	314	1
60	S1C(disialo(amide))-SRIF28	10nmol/kg	14	1
61	S1C(disialo(Bn))-SRIF28	10nmol/kg	49	1
-	vehicle	-	1012	19

[0307]

As is clear from the results shown in Table 8, the glycosylated polypeptide of the present invention suppressed GH production in rats. In the present test line, S1C(disialo)-SRIF28 suppressed GH production from 1 nmol/kg, and showed 82 - 99% of the GH production suppression effect at 3 - 10 nmol/kg. This is thought to be due to the gain in affinity towards SSTR1 - SSTR5 and improvement in the

Employing S1C(disialo)-SRIF28, C(disialo)-R-K-SRIF14, and S1-2C(asialo)-SRIF28 as the glycosylated forms, these were dissolved in Japanese Pharmacopeia saline (from Otsuka Pharmaceutical factory, Inc.) to prepare a 40 μM solution as the administration solution. Eighteen hours after the gastrointestinal obstruction surgery, this was subcutaneously administered into the dorsal cervix at a dosage of 1 mL/kg with a glass syringe and a 25 G injection needle (all from Terumo Corporation). As the vehicle group, saline without glycosylated polypeptide was similarly administered. Moreover, octreotide was administered as the control group.

[0313]

Example 91-3 Measurement of intestinal fluid weight

One hour after compound administration, a median incision was made in the abdomen under inhalation anesthesia, and 1.5 mL of blood was collected from the abdominal vena cava. Then, the ligated jejunum on the musculus suspensorius duodeni side was resected. The fluid and blood on the jejunum surface were removed with a paper towel, nerves, blood vessels, and fat attached to the jejunum was removed, this was cut into 6 cm lengths, and wet weight was measured. This was then dried at 36 degrees for 24 hours, and dry weight was measured. The intestinal fluid weight (mg) was calculated by wet weight - dry weight. The intestinal fluid weight of the jejunum obtained is shown in Table 9.

[Table 24]

Table 9

	Intestinal fluid weight(mg)
Sham treatment	285 ± 27
vehicle	481 ± 31
octreotide	693 ± 48
S1C(disialo)-SRIF28	630 ± 83
C(disialo)-R-K-SRIF14	584 ± 95
S1-2C(asialo)-SRIF28	566 ± 34

[0314]

As apparent from Table 9, the vehicle had significantly increased intestinal fluid weight compared to

Table 10

	Amylase (IU/L)	LDH (IU/L)	CPK (IU/L)
Sham treatment	669 ± 164	168 ± 82	353 ± 68
vehicle	1672 ± 743	341 ± 68	377 ± 57
octreotide	1732 ± 774	269 ± 141	472 ± 215
S1C(disialo)-SRIF28	1164 ± 224	338 ± 97	455 ± 202
C(disialo)-R-K-SRIF14	985 ± 238	226 ± 155	415 ± 137
S1-2C(asialo)-SRIF28	1594 ± 573	318 ± 44	728 ± 665

[0316]

As apparent from Table 10, the vehicle had increased amylase activity and LDH activity compared to the sham treatment, and it was speculated that tissue disorder of the gastrointestinal system had developed accompanying gastrointestinal obstruction. In S1C(disialo)-SRIF28 and C(disialo)-R-K-SRIF14, the amylase activity had a low value compared to the vehicle. On the other hand, the amylase activity of octreotide was equivalent to the vehicle. As apparent from Examples 69-1 and 69-2, S1C(disialo)-SRIF28, C(disialo)-R-K-SRIF14, and S1-2C(asialo)-SRIF28 had binding affinity toward all the receptors from SSTR1 - SSTR5, whereas octreotide is a compound having specific affinity towards SSTR2, SSTR3, and SSTR5. In rat pancreas, since there is a report that all the receptors SSTR1 - SSTR5 are expressed (J. Histochem. Cytochem. 2004 Mar; 52 (3): 391-400), the possibility that the glycosylated form alleviated the tissue disorder by acting on a receptor different from that of octreotide was conceived. Moreover, in octreotide and C(disialo)-R-K-SRIF14, the LDH activity had a low value compared to the vehicle. There were no others that reduced each parameter by administration of the glycosylated form.

Sequence Listing
OCKP1103F sequence listing.txt

[Table 1]

Table 1-1

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
1	S1C(disialo)-SRIF28	5427.7	$[M+3H]^{3+}$	1810.0	5427.0
			$[M+4H]^{4+}$	1357.5	
			$[M+5H]^{5+}$	1086.4	
2	N5C(disialo)-SRIF28	5400.6	$[M+3H]^{3+}$	1801.0	5400.1
			$[M+4H]^{4+}$	1350.8	
			$[M+5H]^{5+}$	1081.2	
3	A9C(disialo)-SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5443.1
			$[M+4H]^{4+}$	1361.8	
			$[M+5H]^{5+}$	1089.6	
4	E12C(disialo)-SRIF28	5385.6	$[M+3H]^{3+}$	1796.0	5384.0
			$[M+4H]^{4+}$	1347.0	
			$[M+5H]^{5+}$	1078.0	
5	R13C(disialo)-SRIF28	5358.6	$[M+3H]^{3+}$	1787.0	5358.0
			$[M+4H]^{4+}$	1340.5	
			$[M+5H]^{5+}$	1072.6	
6	K14C(disialo)-SRIF28	5386.6	$[M+3H]^{3+}$	1796.4	5386.0
			$[M+4H]^{4+}$	1347.5	
			$[M+5H]^{5+}$	1078.2	
7	A15C(disialo)-SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5443.1
			$[M+4H]^{4+}$	1361.8	
			$[M+5H]^{5+}$	1089.6	
8	G16C(disialo)-SRIF28	5457.7	$[M+3H]^{3+}$	1820.0	5457.1
			$[M+4H]^{4+}$	1365.3	
			$[M+5H]^{5+}$	1092.2	
9	K18C(disialo)-SRIF28	5386.6	$[M+3H]^{3+}$	1796.4	5386.1
			$[M+4H]^{4+}$	1347.5	
			$[M+5H]^{5+}$	1078.2	
			$[M+6H]^{6+}$	898.7	
			$[M+7H]^{7+}$	770.5	

[Table 2]

Table 1 — 2

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
10	N19C(disialo)-SRIF28	5400.6	$[M+3H]^{3+}$	1801.0	5400.1
			$[M+4H]^{4+}$	1351.0	
			$[M+5H]^{5+}$	1080.8	
11	F21C(disialo)-SRIF28	5367.6	$[M+3H]^{3+}$	1790.0	5366.9
			$[M+4H]^{4+}$	1342.7	
			$[M+5H]^{5+}$	1074.4	
12	T26C(disialo)-SRIF28	5413.6	$[M+3H]^{3+}$	1354.3	5412.1
			$[M+4H]^{4+}$	1083.4	
			$[M+5H]^{5+}$	903.0	
			$[M+6H]^{6+}$	774.3	
13	29C(disialo)-SRIF28	5514.7	$[M+3H]^{3+}$	1839.1	5514.1
			$[M+4H]^{4+}$	1379.5	
			$[M+5H]^{5+}$	1103.8	
14	30C(disialo)-SRIF28	5615.8	$[M+3H]^{3+}$	1872.7	5615.1
			$[M+4H]^{4+}$	1404.8	
			$[M+5H]^{5+}$	1124.0	
15	S1C(disialo)-D-Trp22 -SRIF28	5427.7	$[M+3H]^{3+}$	1810.1	5427.2
			$[M+4H]^{4+}$	1357.8	
			$[M+5H]^{5+}$	1086.4	
			$[M+6H]^{6+}$	905.5	
16	A9C(disialo)-D-Trp22 -SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5442.9
			$[M+4H]^{4+}$	1361.8	
			$[M+5H]^{5+}$	1089.6	
17	C(disialo)-SRIF14	4004.1	$[M+2H]^{2+}$	2002.9	4003.3
			$[M+3H]^{3+}$	1335.4	
			$[M+4H]^{4+}$	1001.8	
18	C(disialo)-R-K-SRIF14	4288.4	$[M+3H]^{3+}$	1430.2	4287.5
			$[M+4H]^{4+}$	1072.9	
19	C(disialo)-C12linker-SRIF14	4201.4	$[M+3H]^{3+}$	1401.2	4200.5
			$[M+4H]^{4+}$	1051.2	
			$[M+5H]^{5+}$	841.1	

[Table 3]

Table 1 — 3

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
20	S1-2C(disialo)-SRIF28	7793.8	$[M+4H]^{4+}$	1949.4	7793.4
			$[M+5H]^{5+}$	1559.7	
			$[M+6H]^{6+}$	1299.9	
21	S1C(disialo) ・ N5C(disialo)-SRIF28	7679.7	$[M+3H]^{3+}$	1920.4	7678.7
			$[M+4H]^{4+}$	1536.8	
			$[M+5H]^{5+}$	1280.9	
22	S1C(disialo) ・ R13C(disialo)-SRIF28	7637.7	$[M+3H]^{3+}$	1910.2	7636.6
			$[M+4H]^{4+}$	1528.3	
			$[M+5H]^{5+}$	1273.8	
23	N5C(disialo) ・ A9C(disialo)-SRIF28	7695.7	$[M+5H]^{5+}$	1540.1	7695.2
			$[M+6H]^{6+}$	1283.6	
			$[M+7H]^{7+}$	1100.3	
			$[M+8H]^{8+}$	962.9	
24	S1-3C(disialo)-SRIF28	10160.0	$[M+3H]^{3+}$	3387.6	10158.2
			$[M+4H]^{4+}$	2540.9	
			$[M+5H]^{5+}$	2032.7	
			$[M+6H]^{6+}$	1694.2	
			$[M+7H]^{7+}$	1452.3	
25	S1C(disialo) ・ N5C(disialo) ・ A9C(disialo)-SRIF28	9974.8	$[M+5H]^{5+}$	1995.9	9974.3
			$[M+6H]^{6+}$	1663.2	
26	S1C(monosialo)-SRIF28	5136.4	$[M+3H]^{3+}$	1713.0	5134.9
			$[M+4H]^{4+}$	1284.7	
			$[M+5H]^{5+}$	1028.2	
			$[M+6H]^{6+}$	856.8	

[Table 4]

Table 1—4

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
27	S1C(asialo)-SRIF28	4845.1	$[M+3H]^{3+}$	2615.7	4843.9
			$[M+4H]^{4+}$	1212.0	
			$[M+5H]^{5+}$	969.8	
28	S1-2C(asialo)-SRIF28	6628.8	$[M+4H]^{4+}$	1658.1	6627.2
			$[M+5H]^{5+}$	1326.7	
			$[M+6H]^{6+}$	1105.6	
			$[M+7H]^{7+}$	947.8	
			$[M+8H]^{8+}$	829.4	
29	S1-3C(asialo)-SRIF28	8412.5	$[M+4H]^{4+}$	2103.7	8411.7
			$[M+5H]^{5+}$	1683.3	
			$[M+6H]^{6+}$	1403.0	
30	N5N(disialo)-SRIF28	5354.5	$[M+3H]^{3+}$	1785.7	5354.0
			$[M+4H]^{4+}$	1339.5	
			$[M+5H]^{5+}$	1071.8	
31	S1N(disialo)-SRIF28	5381.6	$[M+3H]^{3+}$	1794.7	5380.9
			$[M+4H]^{4+}$	1346.2	
			$[M+5H]^{5+}$	1077.2	
32	S1C(disialo)・N19C(GlcNAc)-SRIF28	5676.9	$[M+3H]^{3+}$	1893.5	5676.4
			$[M+4H]^{4+}$	1420.1	
			$[M+5H]^{5+}$	1136.3	
33	S1C(disialo)・N19C(diMan)-SRIF28	6366.6	$[M+3H]^{3+}$	2122.9	6366.7
			$[M+4H]^{4+}$	1592.5	
			$[M+5H]^{5+}$	1274.4	
34	S1-5C(disialo)-SRIF28	14892.4	$[M+4H]^{4+}$	3723.7	14891.3
			$[M+5H]^{5+}$	2979.1	
			$[M+6H]^{6+}$	2482.9	
			$[M+7H]^{7+}$	2128.2	
			$[M+8H]^{8+}$	1862.4	
35	S1-10C(disialo)-SRIF28	26723.2	$[M+7H]^{7+}$	3818.3	26721.8
			$[M+8H]^{8+}$	3341.0	
			$[M+9H]^{9+}$	2970.1	
			$[M+10H]^{10+}$	2673.1	

[Table 5]

Table 1—5

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
36	C(disialo)-SRIF28	5514.7	$[M+4H]^{4+}$	1379.7	5514.6
			$[M+5H]^{5+}$	1103.9	
			$[M+6H]^{6+}$	920.1	
			$[M+7H]^{7+}$	788.8	
37	R11C(disialo)-SRIF28	5358.5	$[M+3H]^{3+}$	1787.4	5358.6
			$[M+4H]^{4+}$	1340.7	
			$[M+5H]^{5+}$	1072.7	
38	F20C(disialo)-SRIF28	5367.6	$[M+3H]^{3+}$	1790.1	5367.1
			$[M+4H]^{4+}$	1342.8	
			$[M+5H]^{5+}$	1074.4	
39	T24C(disialo)-SRIF28	5413.6	$[M+3H]^{3+}$	1805.7	5412.6
			$[M+4H]^{4+}$	1354.2	
			$[M+5H]^{5+}$	1083.7	
40	F25C(disialo)-SRIF28	5367.6	$[M+3H]^{3+}$	1790.1	5367.1
			$[M+4H]^{4+}$	1342.8	
			$[M+5H]^{5+}$	1074.4	
			$[M+6H]^{6+}$	895.4	
41	S27C(disialo)-SRIF28	5427.7	$[M+3H]^{3+}$	1810.1	5427.4
			$[M+4H]^{4+}$	1357.8	
42	C(disialo)-K-SRIF14	4131.2	$[M+2H]^{2+}$	2066.9	4131.7
			$[M+3H]^{3+}$	1378.2	
			$[M+4H]^{4+}$	1033.9	
43	S1C(disialo)-F25Y-SRIF28	5443.7	$[M+3H]^{3+}$	1815.4	5443.1
			$[M+4H]^{4+}$	1361.6	
			$[M+5H]^{5+}$	1089.6	
44	S1C(disialo)-SRIF28-amide	5426.7	$[M+3H]^{3+}$	1809.8	5426.2
			$[M+4H]^{4+}$	1357.6	
			$[M+5H]^{5+}$	1086.2	
			$[M+6H]^{6+}$	905.2	
45	C(disialo)-PEGlinker-SRIF14	4207.3	$[M+3H]^{3+}$	1403.2	4206.6
			$[M+4H]^{4+}$	1052.7	
			$[M+5H]^{5+}$	842.3	
46	Biotin-S1C(disialo)-SRIF28	5654.0	$[M+3H]^{3+}$	1885.4	5653.2
			$[M+4H]^{4+}$	1414.3	
			$[M+5H]^{5+}$	1131.7	
			$[M+6H]^{6+}$	943.2	
47	Biotin-PEGlinker -S1C(disialo)-SRIF28	5857.2	$[M+3H]^{3+}$	1953.2	5856.4
			$[M+4H]^{4+}$	1465.1	
			$[M+5H]^{5+}$	1172.1	
			$[M+6H]^{6+}$	977.1	
			$[M+7H]^{7+}$	837.6	

[Table 6]

Table 1—6

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
48	Azido-S1C(disialo)-SRIF28	5552.8	$[M+3H]^{3+}$	1851.8	5552.3
			$[M+4H]^{4+}$	1389.1	
			$[M+5H]^{5+}$	1111.7	
49	S1C(disialo)・E12C(disialo)-SRIF28	7664.7	$[M+4H]^{4+}$	1917.1	7664.1
			$[M+5H]^{5+}$	1533.8	
			$[M+6H]^{6+}$	1278.4	
50	2C(disialo)-R-K-SRIF14	6654.6	$[M+3H]^{3+}$	2219.2	6653.4
			$[M+4H]^{4+}$	1664.4	
			$[M+5H]^{5+}$	1331.9	
			$[M+6H]^{6+}$	1109.9	
51	3C(disialo)-R-K-SRIF14	9020.8	$[M+4H]^{4+}$	2256.1	9020.2
			$[M+5H]^{5+}$	1805.1	
			$[M+6H]^{6+}$	1504.4	
52	S1C(diGlcNAc)-SRIF28	4520.9	$[M+3H]^{3+}$	1507.7	4519.9
			$[M+4H]^{4+}$	1131.0	
			$[M+5H]^{5+}$	905.0	
53	S1C(diMan)-SRIF28	4114.5	$[M+3H]^{3+}$	1372.3	4113.8
			$[M+4H]^{4+}$	1029.5	
			$[M+5H]^{5+}$	823.8	
54	N19C(diMan)-SRIF28	4087.5	$[M+3H]^{3+}$	1363.3	4086.8
			$[M+4H]^{4+}$	1023.0	
55	S1C(GlcNAc)-SRIF28	3424.9	$[M+2H]^{2+}$	1713.3	3424.5
			$[M+3H]^{3+}$	1142.5	
			$[M+4H]^{4+}$	856.9	
			$[M+5H]^{5+}$	686.1	
56	N19C(GlcNAc)-SRIF28	3397.8	$[M+2H]^{2+}$	1699.8	3397.5
			$[M+3H]^{3+}$	1133.5	
			$[M+4H]^{4+}$	850.4	
57	S1C(trisialo)-SRIF28	6084.2	$[M+3H]^{3+}$	2028.8	6083.4
			$[M+4H]^{4+}$	1521.9	
			$[M+5H]^{5+}$	1217.7	
			$[M+6H]^{6+}$	1014.9	
58	S1C(tetrasialo)-SRIF28	6740.8	$[M+3H]^{3+}$	2247.7	6739.6
			$[M+4H]^{4+}$	1685.9	
			$[M+5H]^{5+}$	1349.1	
			$[M+6H]^{6+}$	1124.3	
			$[M+7H]^{7+}$	963.8	
59	S1C(disialo(aminoethylamide))-SRIF28	5511.8	$[M+3H]^{3+}$	1838.0	5511.1
			$[M+4H]^{4+}$	1378.5	
			$[M+5H]^{5+}$	1103.2	
			$[M+6H]^{6+}$	919.5	
			$[M+7H]^{7+}$	788.2	

[Table 7]

Table 1—7

Example	Compound	Calculated molecular weight	Observed ions (m/z)		Observed molecular weight
60	S1C(disialo(amide))-SRIF28	5425.7	$[M+3H]^{3+}$	1809.3	5423.9
			$[M+4H]^{4+}$	1357.0	
			$[M+5H]^{5+}$	1085.8	
			$[M+6H]^{6+}$	905.2	
61	S1C(disialo(Bn))-SRIF28	5607.9	$[M+3H]^{3+}$	1870.0	5605.9
			$[M+4H]^{4+}$	1402.5	
			$[M+5H]^{5+}$	1122.2	
			$[M+6H]^{6+}$	935.5	
62	S1C(disialo(hexadecylamide))-SRIF28	5874.5	$[M+3H]^{3+}$	1958.9	5873.6
			$[M+4H]^{4+}$	1469.4	
			$[M+5H]^{5+}$	1175.7	
			$[M+6H]^{6+}$	979.9	
63	S1-2C(disialo(amide))-SRIF28	7789.9	$[M+4H]^{4+}$	1948.2	7789.9
			$[M+5H]^{5+}$	1559.0	
			$[M+6H]^{6+}$	1299.2	
64	S1-2C(disialo(Bn))-SRIF28	8154.3	$[M+4H]^{4+}$	2039.3	8153.0
			$[M+5H]^{5+}$	1631.6	
			$[M+6H]^{6+}$	1360.2	
65	S1C(Asn(disialo))-SRIF28	5542.7	$[M+3H]^{3+}$	1848.4	5542.2
			$[M+4H]^{4+}$	1386.6	
			$[M+5H]^{5+}$	1109.4	
			$[M+6H]^{6+}$	924.9	
66	S1N(disialo)·N19C(diMan)-SRIF28	6320.5	$[M+3H]^{3+}$	2107.9	6319.5
			$[M+4H]^{4+}$	1580.9	
			$[M+5H]^{5+}$	1265.1	
67	C(disialo(aminoethylamide))·S1C(disialo)-SRIF28	7878.0	$[M+4H]^{4+}$	1970.3	7878.1
			$[M+5H]^{5+}$	1576.4	
			$[M+6H]^{6+}$	1313.9	
			$[M+7H]^{7+}$	1126.4	
			$[M+8H]^{8+}$	985.5	
			$[M+9H]^{9+}$	876.1	
68	S1-4C(disialo)-SRIF28	12526.2	$[M+5H]^{5+}$	2506.1	12525.5
			$[M+6H]^{6+}$	2088.6	
			$[M+7H]^{7+}$	1790.4	

[0258]

Example 69-1 Calculation of receptor binding affinity

Competitive binding assay was performed with the method below to calculate receptor binding affinity.

Reagents employed in the competitive binding assay and their proper chemical names are as follows: HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and BSA (bovine serum albumin).

Competitive binding assay was consigned to Ricerca Biosciences, and experiment and data analysis was performed.

The receptor subtypes and receptor membrane samples employed are shown in Table 2. Common in each binding assay, [^{125}I] Tyr^{11}-Somatostatin 14 (Tyr11-SRIF14) was used as the labeled ligand, Somatostatin-14 (SRIF14) was used as the unlabeled ligand, and 25 mM HEPES containing 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.1% BSA, pH 7.4 was used as the buffer. The test substance was used at a concentration of 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM, or 1000 nM, and mixed with the membrane sample into a reaction solution. Moreover, the concentrations of the labeled and unlabeled ligands added to the reaction solution are shown in Table 2. The incubation condition of the reaction solution was at 25°C for 4 hours for SSTR2 and at 25°C for 2 hours for SSTR1, SSTR3, SSTR4, and SSTR5. For each experiment run, SRIF14 was used as the positive control. For data analysis, 50% inhibitory concentration (IC_{50} value) was determined using MathIQ™ (ID Business Solutions, UK) in a nonlinear least squares method based on the numerical data of the binding inhibition rate. The binding inhibition constant (Ki value) was calculated by the method of Cheng, Y et al. (Biochem Pharmacol, 22, 3099-3108, 1973).

[Table 8]

Table 2

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Labeled ligand	0.1nM	0.03nM	0.1nM	0.1nM	0.1nM
Unlabeled ligand	1μM	1μM	1μM	1μM	1μM
Source	Human recombinant CHO-K1 cells	Human recombinant CHO-K1 cells	Human recombinant CHO-K1 cells	Human recombinant Chem-1 cells	Human recombinant Chem-1 cells

[0259]

Compounds subjected to binding assay and the results of the binding experiment are shown in Table 3A. Moreover, octreotide, SRIF14 and SRIF28 were similarly evaluated as control compounds. Note that for octreotide, IC_{50} values could not be calculated for SSTR1 and SSTR4 since the maximum concentration was 100 nM, and is thus shown as >100 nM.

Figures 1A and 1B show examples of the structures of glycosylated peptides (glycosylated forms) corresponding to the compound names in Table 3A.

[0260]

[Table 9]

[0261] The control octreotide bound to each of receptors SSTR2, SSTR3, and SSTR5, and SRIF14 and SRIF28 bound to all SSTRs. As shown in Table 3A, the compounds according to

Table 3A

Example		SSTR1		SSTR2		SSTR3		SSTR4		SSTR5	
		IC_{50}(nM)	Ki(nM)	IC_{50}(nM)	Ki(nM)	IC_{50}(nM)	Ki(nM)	IC_{50}(nM)	Ki(nM)	IC_{50}(nM)	Ki(nM)
1	S1C(disialo)-SRIF28	2.19	1.81	0.109	0.0576	0.480	0.164	2.30	2.07	0.442	0.250
2	N5C(disialo)-SRIF28	1.10	0.912	0.0945	0.0502	0.278	0.0950	1.30	1.17	0.458	0.259
3	A9C(disialo)-SRIF28	2.08	1.72	0.0741	0.0394	0.593	0.203	1.72	1.54	0.471	0.266
4	E12C(disialo)-SRIF28	1.70	1.40	0.112	0.0596	0.362	0.124	2.04	1.84	0.376	0.212
5	R13C(disialo)-SRIF28	16.4	13.6	0.310	0.165	2.87	0.981	6.43	5.79	3.28	1.85
6	K14C(disialo)-SRIF28	13.8	11.4	0.481	0.256	2.59	0.887	8.02	7.22	4.01	2.27
10	N19C(disialo)-SRIF28	16.1	13.3	1.21	0.645	1.47	0.503	12.2	11.0	3.69	2.09
13	29C(disialo)-SRIF28	4.43	3.67	0.738	0.392	7.00	2.39	6.11	5.50	3.73	2.11
14	30C(disialo)-SRIF28	4.21	3.48	0.517	0.275	6.91	2.36	2.53	2.28	1.86	1.05
15	S1C(disialo)-D-Trp22-SRIF28	2.23	1.85	0.0324	0.0172	0.834	0.285	5.85	5.26	0.565	0.319
16	A9C(disialo)-D-Trp22-SRIF28	6.92	5.73	0.0396	0.0210	0.256	0.0876	4.87	4.38	0.194	0.110
17	C(disialo)-SRIF14	32.3	26.7	0.336	0.179	4.62	1.58	8.24	7.41	5.70	3.22
18	C(disialo)-R-K-SRIF14	3.09	2.56	0.104	0.0551	0.231	0.0791	1.06	0.951	0.187	0.106
19	C(disialo)-C12linker-SRIF14	15.5	12.8	0.502	0.267	3.12	1.07	2.92	2.62	2.29	1.29
20	S1-2C(disialo)-SRIF28	18.8	15.5	0.338	0.179	1.28	0.439	5.61	5.05	0.607	0.343
21	S1C(disialo) · N5C(disialo)-SRIF28	6.89	5.70	0.224	0.119	1.58	0.539	4.00	3.60	0.728	0.411
22	S1C(disialo) · R13C(disialo)-SRIF28	177	147	6.28	3.33	11.0	3.77	45.0	40.5	14.7	8.33
23	N5C(disialo) · A9C(disialo)-SRIF28	30.0	24.9	0.793	0.421	4.09	1.40	14.2	12.8	1.81	1.02
24	S1-3C(disialo)-SRIF28	20.4	16.9	0.421	0.224	3.30	1.13	7.69	6.92	0.927	0.524
25	S1C(disialo) · N5C(disialo) · A9C(disialo)-SRIF28	69.2	57.3	0.985	0.523	7.93	2.71	25.2	22.7	4.14	2.34
26	S1C(monosialo)-SRIF28	1.43	1.19	0.0465	0.0247	0.322	0.110	1.14	1.03	0.372	0.210
27	S1C(asialo)-SRIF28	0.851	0.704	0.0224	0.0119	0.155	0.0531	0.627	0.564	0.460	0.260
28	S1-2C(asialo)-SRIF28	0.860	0.712	0.0632	0.0336	0.177	0.0604	0.780	0.702	0.190	0.107
29	S1-3C(asialo)-SRIF28	4.21	3.49	0.0978	0.0519	0.581	0.199	1.44	1.29	0.369	0.209
30	N5N(disialo)-SRIF28	3.02	2.50	0.0685	0.0364	0.579	0.198	2.86	2.57	0.465	0.263
31	S1N(disialo)-SRIF28	2.68	2.21	0.0754	0.0401	0.314	0.107	1.07	0.960	0.272	0.154
-	SRIF14	0.438	0.362	0.0142	0.00755	0.131	0.0450	0.303	0.273	0.578	0.326
-	Octreotide	>100	-	0.0628	0.0334	6.41	2.19	>100	-	9.39	5.31
-	SRIF28	0.353	0.292	0.0271	0.0144	0.0868	0.0297	0.294	0.265	0.423	0.239

As shown in Table 3B, the controls SRIF14 and SRIF28 bound to all receptors SSTR1 - SSTR5. Since the number of

Table 3B

Example		SSTR1		SSTR2		SSTR3		SSTR4		SSTR5	
		IC_{50}(nM)	Ki (nM)	IC_{50}(nM)	Ki (nM)	IC_{50}(nM)	Ki (nM)	IC_{50}(nM)	Ki (nM)	IC_{50}(nM)	Ki (nM)
1	S1C(disialo)—SRIF28	7.71	6.45	0.543	0.289	1.98	0.676	7.10	6.39	1.61	0.911
7	A15C(disialo)-SRIF28	11.4	9.43	0.938	0.498	3.49	1.19	8.18	7.36	3.84	2.17
8	G16C(disialo)-SRIF28	17.4	14.4	1.22	0.646	3.12	1.07	12.2	11.0	5.67	3.21
9	K18C(disialo)-SRIF28	45.4	37.6	12.2	6.51	14.7	5.03	62.7	56.5	160	95.0
12	T26C(disialo)-SRIF28	21.6	17.9	310	160	21.7	7.44	26.8	24.1	400	230
26	S1C(monosialo)-SRIF28	5.38	4.46	0.213	0.112	1.18	0.405	2.35	2.12	0.816	0.460
27	S1C(asialo)-SRIF28	3.97	3.29	0.200	0.105	1.02	0.349	2.98	2.68	1.04	0.588
32	S1C(disialo)・N19C(GlcNAc)-SRIF28	<10	<8.27	2.20	1.17	3.05	1.04	8.55	7.69	2.37	1.34
33	S1C(disialo)・N19C(diMan)-SRIF28	44.0	37.0	3.73	1.98	3.63	1.24	25.0	23.0	5.69	3.22
34	S1-5C(disialo)-SRIF28	110	91.9	12.7	6.58	21.3	7.29	62.3	56.4	7.23	4.09
35	S1-10C(disialo)-SRIF28	117	96.5	10.1	5.35	40.4	13.8	191	172	34.8	19.7
36	C(disialo)-SRIF28	5.57	4.61	0.478	0.255	2.55	0.870	8.65	7.79	2.62	1.48
37	R11C(disialo)-SRIF28	22.0	18.2	1.38	0.735	6.34	2.17	20.2	18.1	3.65	2.07
40	F25C(disialo)-SRIF28	>1000	>828	>1000	>531	>1000	>342	130	110	58.0	33.0
41	S27C(disialo)-SRIF28	28.4	23.5	0.326	0.170	6.77	2.31	33.4	30.1	4.94	2.80
42	C(disialo)-K-SRIF14	20.7	17.2	1.97	1.05	3.55	1.21	11.0	9.77	3.52	1.99
43	S1C(disialo)-F25Y-SRIF28	1.77	1.46	0.310	0.160	0.480	0.160	1.55	1.39	1.09	0.620
44	S1C(disialo)-SRIF28-amide	4.87	4.03	1.32	0.700	5.91	2.02	6.88	6.19	3.75	2.12
45	C(disialo)-PEGlinker-SRIF14	36.0	30.0	4.67	2.48	8.71	2.98	21.0	19.0	>10	>5.65
46	Biotin-S1C(disialo)-SRIF28	11.0	9.05	1.68	0.890	2.92	1.00	9.28	8.35	2.42	1.37
47	Biotin-PEGlinker-S1C(disialo)-SRIF28	9.41	7.79	1.96	1.04	3.41	1.17	9.31	8.38	2.00	1.13
48	azido-S1C(disialo)-SRIF28	8.82	7.30	1.29	0.690	3.17	1.09	5.34	4.81	0.840	0.470
49	S1C(disialo)・E12C(disialo)-SRIF28	22.0	19.0	2.70	1.44	4.62	1.58	20.0	18.0	2.11	1.19
50	2C(disialo)-RK-SRIF14	48.0	40.0	5.35	2.84	9.74	3.33	49.0	44.0	6.16	3.48
51	3C(disialo)-RK-SRIF14	270	230	32.0	17.0	40.0	14.0	>100	>90.0	41.0	23.0
52	S1C(diGlcNAc)-SRIF28	5.04	4.17	0.190	0.100	2.02	0.690	3.91	3.52	1.45	0.820
53	S1C(diMan)-SRIF28	5.06	4.18	0.140	0.0750	1.32	0.450	3.01	2.70	0.550	0.310
54	N19C(diMan)-SRIF28	6.58	5.44	1.17	0.620	1.55	0.530	8.02	7.22	2.94	1.66
55	S1C(GlcNAc)-SRIF28	5.91	4.89	0.225	0.120	1.65	0.565	2.89	2.60	1.15	0.650
56	N19C(GlcNAc)-SRIF28	2.60	2.15	0.940	0.500	<1	<0.342	2.38	2.15	1.89	1.07
57	S1C(trisialo)-SRIF28	17.0	14.0	0.780	0.410	5.59	1.91	15.0	14.0	1.95	1.10
58	S1C(tetrasialo)-SRIF28	17.3	14.2	1.12	0.597	5.63	1.93	14.3	12.6	2.12	1.20
59	S1C(disialo(aminoethylamide))-SRIF28	2.35	1.94	0.101	0.0536	0.766	0.264	2.24	2.01	2.08	1.18
60	S1C(disialo(amide))-SRIF28	5.09	4.21	0.207	0.110	1.53	0.522	6.12	5.50	2.60	1.47
61	S1C(disialo(Bn))-SRIF28	6.20	5.12	0.247	0.130	2.15	0.733	6.01	5.41	3.25	1.83
62	S1C(disialo(hexadexylamide))-SRIF28	5.53	4.57	1.04	0.550	11.0	3.75	26.0	24.0	37.0	21.0
63	S1-2C(disialo(amide))-SRIF28	6.92	5.73	0.830	0.440	2.06	0.710	4.76	4.28	2.67	1.51
64	S1-2C(disialo(Bn))-SRIF28	6.01	4.97	0.830	0.440	2.58	0.880	8.20	7.38	2.60	1.47
65	S1C(Asn(disialo))-SRIF28	15.0	13.0	0.610	0.330	5.57	1.91	15.0	14.0	1.61	0.910
66	S1N(disialo)・N19C(diMan)-SRIF28	35.0	29.0	5.65	3.00	3.90	1.33	25.0	22.0	8.53	4.82
67	C(disialo(aminoethylamide))・S1C(disialo)-SRIF28	6.20	5.13	0.960	0.510	1.89	0.650	6.12	5.51	1.81	1.02
68	S1-4C(disialo)-SRIF28	130	100	3.76	2.00	10.0	3.43	66.0	59.0	10.0	5.82
-	SRIF28	0.533	1.80	0.0680	0.0565	0.206	0.116	0.776	1.23	0.189	0.666
-	SRIF14	1.25	2.69	0.0329	0.0993	0.0697	0.403	0.823	1.95	0.416	0.813

forskolin, and allowed to react at room temperature for 30 minutes. After the reaction, the cells were dissolved with 0.2 N HCl, and the amount of cAMP accumulated in the cell was measured with Cayman cyclic AMP EIA kit (Cayman, 582002).

Evaluation of SSTR1 was consigned to Cerep, and the experiment was performed. HBSS containing 20 mM HEPES (pH 7.4) and 500 µM IBMX was used as the buffer, and SSTR1 receptor expression cells were seeded at 10^4 cells/well. The test substance was treated by mixing at a concentration of 0.001 nM, 0.01 nM, 0.1 nM, 1 nM, 10 nM, or 100 nM with 1 µM NKH477, and allowed to react at 37°C for 20 minutes. After the reaction, the amount of cAMP accumulated in the cell was measured with Cisbio cAMP dynamic2 kit (Cisbio, 62AM4PE).

[Table 11]

Table 3C

	SSTR2	SSTR3	SSTR4	SSTR5
Distributer	Perkin Elmer			
Serial Number	ES-521-CF	ES-523-CF	ES-524-CF	ES-522-CF
Derived cell	CHO-K1 cells			

[0264]

The experimental results (IC_{50} (nM)) of the agonist activity evaluation test when employing the compounds of Examples 1, 18, 27, 28, 59, and 60 as the glycosylated form are shown in Table 3D and Figure 1E. IC_{50} of the control compounds SRIF14 and SRIF28 was 0.83 - 0.89 nM against SSTR1, and 0.0076 - 0.073 nM, 0.029 - 0.21 nM, 0.015 - 0.074 nM, and 0.066 - 0.12 nM against SSTR2, SSTR3, SSTR4, and SSTR5, respectively. IC_{50} of the agonist activity of the glycosylated compounds against SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 were 1.0 - 2.4 nM, 0.041 - 0.10 nM, 0.12 - 0.30 nM, 0.039 - 0.085 nM, and 0.043 - 0.081 nM, respectively, and potent agonist activity was shown against all receptors SSTR1 - SSTR5. From the results shown in Examples 69-1 and 69-2, it is apparent that these compounds have receptor binding affinity, and it became clear that these compounds exert proper agonistic action by binding to a receptor.

[Table 12]

Table 3D

Example		SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
-	SRIF14	0.83 ± 0.52	0.0076 ± 0.0012	0.029 ± 0.017	0.015 ± 0.006	0.12 ± 0.03
-	SRIF28	0.89 ± 0.41	0.073 ± 0.014	0.21 ± 0.08	0.074 ± 0.058	0.066 ± 0.033
1	S1C(disialo)-SRIF28	2.2 ± 1.1	0.075 ± 0.030	0.13 ± 0.06	0.065 ± 0.042	0.056 ± 0.028
18	Cys(disialo)-R-K-SRIF14	1.2 ± 0.6	0.055 ± 0.015	0.18 ± 0.10	0.050 ± 0.025	0.052 ± 0.032
27	S1C(asialo)-SRIF28	1.6 ± 0.6	0.041 ± 0.006	0.12 ± 0.05	0.039 ± 0.021	0.055 ± 0.031
28	S1-2C(asialo)-SRIF28	1.5 ± 0.6	0.079 ± 0.033	0.15 ± 0.08	0.051 ± 0.046	0.064 ± 0.029
59	S1C(disialo(aminoethylamide))-SRIF28	1.0 ± 0.9	0.10 ± 0.02	0.30 ± 0.15	0.085 ± 0.011	0.081 ± 0.019
60	S1C(disialo(amide))-SRIF28	2.4 ± 1.7	0.055 ± 0.013	0.14 ± 0.06	0.043 ± 0.023	0.043 ± 0.015

[0265]
Example 69-4 Agonist activity evaluation employing receptor expression cells - 2

Agonist activity evaluation test was carried out similarly to Example 69-3 employing each of the compounds described in Table 3E as the glycosylated form. The experimental results are shown in Table 3E. In Table 3E, fields with "- (hyphen)" shown indicates that the test was not performed.

[Table 13]

Table 3E

(-: Not performed)

Experiment		SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
–	SRIF14	0.83	0.0076	0.029	0.015	0.12
–	SRIF28	0.89	0.073	0.21	0.074	0.066
4	E12C(disialo)-SRIF28	2.3	–	–	–	–
10	N19C(disialo)-SRIF28	22	–	–	–	–
15	S1C(disialo)-D-Trp22-SRIF28	3.5	–	–	–	–
17	C(disialo)-SRIF14	–	0.029	0.18	0.10	0.23
19	C(disialo)-C12linker-SRIF14	–	–	–	0.11	–
20	S1-2C(disialo)-SRIF28	–	–	0.55	0.14	0.22
21	S1C(disialo)・N5C(disialo)-SRIF28	–	0.072	0.37	0.041	0.090
23	N5C(disialo)・A9C(disialo)-SRIF28	–	0.096	0.29	0.091	0.13
24	S1-3C(disialo)-SRIF28	7.5	0.19	0.79	0.36	0.97
25	S1C(disialo)・N5C(disialo)・A9C(disialo)-SRIF28	–	1.4	2.4	0.16	1.1
26	S1C(monosialo)-SRIF28	1.5	0.059	0.15	0.046	0.062
29	S1-3C(asialo)-SRIF28	–	0.19	–	0.10	–
31	S1N(disialo)-SRIF28	2.3	–	–	–	–
32	S1C(disialo)・N19C(GlcNAc)-SRIF28	–	0.080	0.039	0.033	0.068
34	S1-5C(disialo)-SRIF28	18	0.81	2.0	0.27	–
42	C(disialo)-K-SRIF14	–	0.019	0.10	0.059	–
44	S1C(disialo)-SRIF28-amide	1.7	–	–	–	–
47	C(disialo)-PEG2linker-SRIF14	–	0.034	0.15	0.066	0.18
50	2C(disialo)-R-K-SRIF14	–	0.15	0.55	1.1	–
51	3C(disialo)-R-K-SRIF14	–	1.4	3.8	1.6	–
52	S1C(diGlcNAc)-SRIF28	1.2	0.045	0.14	0.050	0.033
53	S1C(diMan)-SRIF28	1.2	0.035	0.11	0.043	0.037
54	N19C(diMan)-SRIF28	–	–	–	0.11	–
55	S1C(GlcNAc)-SRIF28	1.4	0.040	0.14	0.049	0.031
56	N19C(GlcNAc)-SRIF28	–	–	–	0.092	–
57	S1C(trisialo)-SRIF28	4.9	0.11	0.38	0.092	0.096
58	S1C(tetrasialo)-SRIF28	2.8	0.13	0.25	0.081	0.11
61	S1C(disialo(Bn))-SRIF28	1.4	–	–	–	–
62	S1C(disialo(hexadecylamide))-SRIF28	1.3	–	–	–	–
65	S1C(Asn(disialo))-SRIF28	2.6	–	–	0.13	–
67	C(disialo(aminoethylamide))・S1C(disialo)-SRIF28	–	0.039	0.17	0.047	0.034

[0266]
In Table 3E, IC_{50} of the agonist activity of the glycosylated compounds against SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 were 1.2 - 22 nM, 0.019 - 1.4 nM, 0.039 - 3.8 nM, 0.033 - 1.6 nM, and 0.031 - 1.1 nM, respectively, and agonist activity was shown against receptors SSTR1 - SSTR5. From the results shown in Examples 69-1 and 69-2, it became clear that these compounds have receptor binding affinity,

[Table 14]

Table 4

	Intravenous administration				Subcutaneous administration			
	$t_{1/2}$	AUC	MRT	C_0	$t_{1/2}$	AUC	MRT	C_{max}
S1C(disialo)-SRIF28	18.1	5086	20.4	780	26.0	1273	43.8	19.9
SRIF28	0.8	358	2.3	678	1.5	15	3.2	4.4

($t_{1/2}$: min, AUC : min · nM, MRT : min, C_0 : nM, C_{max} : nM)

As is clear from the results shown in Figure 2 and Table 4, S1C(disialo)-SRIF28 has significantly extended $t_{1/2}$ and MRT compared to the non-glycosylated SRIF28, and an increase in AUC and C_{max} was recognized. These are thought to be due to the increase in resistance to degradation activity in blood by glycosylation. It is clear that the glycosylated form has improved stability *in vivo* compared to the non-glycosylated form. Moreover, as factors for the increase in AUC and C_{max}, the improvement of bioavailability according to the present invention, as well as improvement of stability thereof *in vivo* are thought to be factors.

[0272]

Example 71 Pharmacokinetics test with rats 2

A pharmacokinetics test was carried out similarly to Example 70, except that S1C(disialo)-SRIF28, N5C(disialo)-SRIF28, and S1C(disialo)·N5C(disialo)-SRIF28 were employed as the glycosylated forms. As a control, non-glycosylated SRIF28 was employed instead of the glycosylated form. The compound plasma concentration transition obtained is shown in Figure 3.

[0273]

Example 72 Pharmacokinetics test with rats 3

A pharmacokinetics test was carried out similarly to Example 70, except that S1C(disialo)-SRIF28, S1C(disialo)·R13C(disialo)-SRIF28, and S1C(disialo)·N5C(disialo)·A9C(disialo)-SRIF28 were employed as the glycosylated forms. As a control, non-glycosylated SRIF28 was employed instead of the glycosylated form. The compound plasma concentration transition obtained is shown in Figure 4.

[0274]

Example 73 Pharmacokinetics test with rats 4

A pharmacokinetics test was carried out similarly to Example 70, except that S1C(disialo)-SRIF28, S1C(disialo)·N5C(disialo)·A9C(disialo)-SRIF28, S1-3C(disialo)-SRIF28, S1-5C(disialo)-SRIF28, and S1-10C(disialo)-SRIF28 were employed as the glycosylated forms.

[0278]

Table 5A

	Compound	The number of sugar chains	Value of n	Intravenous administration				Subcutaneous administration				BA
				$t_{1/2}$	AUC	MRT	C_0	$t_{1/2}$	AUC	MRT	C_{max}	
-	SRIF28	None	6	1.00 ±0.20	472 ±166	2.20 ±0.11	554 ±221	2.94 ±1.59	25.5 ±13.8	4.76 ±1.80	4.47 ±1.21	5
1	S1C(disialo)-SRIF28	1	15	16.9 ±1.02	4855 ±1030	18.6 ±1.72	686 ±363	29.8 ±2.68	1773 ±281	47.9 ±2.68	28.9 ±3.23	37
2	N5C(disialo)-SRIF28	1	6	20.1 ±1.49	4421 ±1366	22.9 ±2.16	496 ±312	28.1 ±3.72	2392 ±443	51.9 ±5.92	32.4 ±4.87	54
3	A9C(disialo)-SRIF28	1	3	19.9 ±0.33	4083 ±515	25.2 ±0.38	547 ±211	30.3 ±2.45	2462 ±64.0	55.6 ±5.81	30.2 ±5.05	60
		Average value		19.0	4453	22.2	576.3	29.4	2209	51.8	30.5	50
20	S1-2C(disialo)-SRIF28	2 (dense)	3	28.8 ±3.11	7306 ±625	23.3 ±2.63	1382 ±321	40.9 ±12.2	4046 ±248	67.9 ±15.0	46.3 ±9.75	55
21	S1C(disialo)・N5C(disialo)-SRIF28	2 (with intervals)	6	25.8 ±3.41	4099 ±634	24.6 ±5.97	558 ±248	38.4 ±4.93	3344 ±663	67.9 ±8.39	35.5 ±6.38	82
22	S1C(disialo)・R13C(disialo)-SRIF28	2 (with intervals)	3	23.4 ±0.66	4029 ±184	20.6 ±1.32	822 ±106	39.6 ±2.50	3407 ±436	67.7 ±5.39	38.7 ±8.73	85
23	N5C(disialo)・A9C(disialo)-SRIF28	2 (with intervals)	3	32.4 ±1.66	4465 ±833	26.6 ±0.27	664 ±104	42.7 ±9.76	3450 ±784	75.0 ±12.4	32.5 ±7.06	77
				27.2	4198	23.9	681.3	40.2	3400	70.2	35.6	81
24	S1-3C(disialo)-SRIF28	3 (dense)	6	38.5 ±2.83	5660 ±1354	24.0 ±3.92	1061 ±209	87.7 ±21.4	3884 ±543	126 ±31.7	27.5 ±10.8	69
25	S1C(disialo)・N5C(disialo)・A9C(disialo)-SRIF28	3 (with intervals)	9	39.8 ±1.87	4402 ±999	25.8 ±2.87	938 ±145	87.4 ±17.1	4015 ±944	132 ±19.8	24.8 ±9.03	91
34	S1-5C(disialo)-SRIF28	5 (dense)	3	99.5 ±14.0	6016 ±873	49.0 ±6.06	752 ±81.4	271 ±38.7	5524 ±633	389 ±50.0	13.5 ±2.85	92
35	S1-10C(disialo)-SRIF28	10 (dense)	3	219 ±6.27	100259 ±10463	272 ±14.3	821 ±264	>480	-	-	45.9 ±2.32	-

($t_{1/2}$: min , AUC : min・nM , MRT : min , C_0 : nM , C_{max} : nM , BA : %)

As is clear from the results shown in Table 5A, the bioavailability of the glycosylated form of the present

Table 5B

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
–	SRIF14	0.500	119	2.00	819	2.30	8	4.30	1.80	7
–	SRIF28	1.32	467	2.46	518	2.38	22	4.18	4.65	5
1	S1C(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	30.1	38
4	E12C(disialo)-SRIF28	19.5	10521	27.5	1086	38.2	2891	57.5	34.7	27
5	R13C(disialo)-SRIF28	20.3	4926	22.8	481	35.4	5528	55.9	65.9	78
6	K14C(disialo)-SRIF28	20.4	6278	20.0	884	35.1	3945	55.1	57.5	62
10	N19C(disialo)-SRIF28	18.8	3854	21.5	407	37.9	2624	55.3	41.7	56
13	29C(disialo)-SRIF28	23.7	3736	25.3	359	39.3	2176	57.6	29.4	51
15	S1C(disialo)-D-Trp-SRIF28	18.3	4924	20.6	588	31.0	2220	51.9	34.0	45
18	C(disialo)-R-K-SRIF14	18.9	6571	22.5	429	28.4	1996	45.2	32.5	30
42	C(disialo)-K-SRIF14	18.8	5277	19.4	646	26.4	2446	43.7	40.5	46
17	C(disialo)-SRIF14	16.5	5614	18.3	725	27.2	3641	40.0	73.6	66
19	C(disialo)-C12linker-SRIF14	11.0	1686	7.60	500	14.4	345	22.0	13.4	21
45	C(disialo)-PEGlinker-SRIF14	16.4	3538	18.8	412	20.7	2725	39.2	47.8	77

Table 5C

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
–	SRIF28	1.32	467	2.46	518	2.38	22	4.18	4.65	5
55	S1C(GlcNAc)-SRIF28	1.80	690	3.00	666	5.90	63	7.90	7.10	9
53	S1C(diMan)-SRIF28	5.50	1445	5.20	423	9.10	124	13.9	7.30	9
52	S1C(diGlcNAc)-SRIF28	10.5	3588	12.3	495	16.5	445	25.7	13.2	12
27	S1C(asialo)-SRIF28	13.4	4602	12.5	749	19.7	710	29.0	17.2	16
26	S1C(monosialo) -SRIF28	15.9	6618	15.3	1061	24.5	995	37.8	19.4	17
1	S1C(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	30.1	38
57	S1C(trisialo)-SRIF28	21.1	4428	22.0	423	36.6	1979	56.9	24.9	45
58	S1C(tetrasialo)-SRIF28	20.9	4851	23.0	471	40.5	2624	59.4	32.2	54
65	S1C(Asn(disialo))-SRIF28	27.9	6488	35.9	365	34.0	2935	55.3	33.9	45
59	S1C(disialo(aminoethylamide))-SRIF28	18.6	3473	12.1	746	27.1	284	33.2	9.60	8
60	S1C(disialo(amide))-SRIF28	18.0	5814	12.6	1048	30.0	961	42.5	19.1	17
61	S1C(disialo(Bn))-SRIF28	14.4	3708	14.5	526	45.5	1047	52.6	18.7	28

[0293]

As is clear from the results shown in Table 5C, when the size of the modifying sugar chain is altered to GlcNAc (monosaccharide), diMan (5 sugars), diGlcNAc (7 sugars), asialo (9 sugars), monosialo (10 sugars), disialo (11 sugars), trisialo (14 sugars), and tetrasialo (17 sugars), the $t_{1/2}$, AUC, and bioavailability when administered subcutaneously increased to 2 - 17-folds, 3 - 120-folds, and 2 - 11-folds, respectively, according to the size of the modifying sugar chain. From this, it became clear that altering the size of the modifying sugar chain not only improved the stability in blood, but allowed change in its increase rate. Moreover, since it is known that dimannose or asialo sugar chains etc. among these sugar chains interact with particular proteins, they are thought to be utilizable for targeting a particular protein or organs and cells having the particular protein. Moreover, as modification of the sialic acid at the non-reducing terminal, S1C(disialo(aminoethylamide))-SRIF28, S1C(disialo(amide))-SRIF28, and S1C(disialo(Bn))-SRIF28 having added a sugar chain with altered charge by e.g. the introduction of an aminoethylamide, amide, and Bn to the carboxy group extended $t_{1/2}$ by 11 - 14-folds and increased AUC by 7 - 12-folds, and improved stability in blood compared to the non-glycosylated form when administered intravenously. This shows that the carboxy group of the sialic acid has a large influence on retentivity in blood, and shows the possibility for (usage in) blood clearance or body distribution control by abstraction of the negative charge of the carboxy group (disialo(Bn) and disialo(amide)) or conversion into a positive charge (disialo(aminoethylamide)).

[Table 18]

Table 5D

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
–	SRIF14	0.500	119	2.00	819	2.30	8	4.30	1.80	7
–	SRIF28	1.32	467	2.46	518	2.38	22	4.18	4.65	5
18	C(disialo)-R-K-SRIF14	18.9	6571	22.5	429	28.4	1996	45.2	32.5	30
50	2C(disialo)R-K-SRIF14	31.7	5041	24.1	704	47.7	3310	73.4	36.7	66
51	3C(disialo)R-K-SRIF14	35.7	4288	22.2	1013	70.6	3515	112	23.5	82
1	S1C(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	30.1	38
20	S1-2C(disialo)-SRIF28	28.2	5804	23.0	950	40.8	3091	65.3	34.6	53
24	S1-3C(disialo)-SRIF28	39.5	5834	23.8	1083	79.3	4174	115	30.7	72
68	S1-4C(disialo)-SRIF28	63.5	7070	25.2	1007	119	4554	172	22.0	64

[0294]

As is clear from the results shown in Table 5D, C(disialo)-R-K-SRIF14 modified with one disialosugar chain, 2C(disialo)-R-K-SRIF14 modified with two disialosugar

chains, and 3C(disialo)-R-K-SRIF14 modified with three disialosugar chains on SRIF14 each extended $t_{1/2}$ by 38, 63, and 71-folds and increased AUC by 55, 42, and 36-folds compared to the non-glycosylated form when administered intravenously. Similarly, S1C(disialo)-SRIF28 modified with one disialosugar chain, S1-2C(disialo)-SRIF28 modified with two disialosugar chains, S1-3C(disialo)-SRIF28 modified with three disialosugar chains, and S1-4C(disialo)-SRIF28 modified with four disialosugar chains on SRIF28 each extended $t_{1/2}$ by 13, 21, 30, and 48-folds and increased AUC by 11, 12, 12, and 15-folds compared to the non-glycosylated form when administered intravenously. In either of SRIF14 and SRIF28, it became clear that stability in blood is improved according to the number of modifying disialosugar chain.

[Table 19]

Table 5E

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
–	SRIF28	1.32	467	2.46	518	2.38	22	4.18	4.65	5
27	S1C(asialo)-SRIF28	13.4	4602	12.5	749	19.7	710	29.0	17.2	16
28	S1-2C(asialo)-SRIF28	8.30	2664	9.60	741	19.2	1049	36.4	19.8	39
29	S1-3C(asialo)-SRIF28	6.60	2733	6.50	1147	26.4	885	42.1	15.0	32

[0295]

As is clear from the results shown in Table 5E, S1C(asialo)-SRIF28 modified with one asialosugar chain, S1-2C(asialo)-SRIF28 modified with two asialosugar chains, and S1-3C(asialo)-SRIF28 modified with three asialosugar chains on SRIF28 each extended $t_{1/2}$ by 10, 6, and 5-folds and increased AUC by 10, 6, and 6-folds compared to the non-glycosylated form when administered intravenously. It became clear that stability in blood is also improved when the modifying sugar chain is an asialosugar chain.

[Table 20]

Table 5F

Example		Intravenous administration				Subcutaneous administration				BA
		$T_{1/2}$	AUC	MRT	C_0	$T_{1/2}$	AUC	MRT	Cmax	
-	SRIF28	1.32	467	2.46	518	2.38	22	4.18	4.65	5
1	S1C(disialo)-SRIF28	17.7	5190	19.1	633	31.2	1946	49.2	30.1	38
20	S1-2C(disialo)-SRIF28	28.2	5804	23.0	950	40.8	3091	65.3	34.6	53
28	S1-2C(asialo)-SRIF28	8.30	2664	9.60	741	19.2	1049	36.4	19.8	39
63	S1-2C(disialo(amide))-SRIF28	23.8	5268	20.5	737	33.1	1297	55.8	16.3	25
64	S1-2C(disialo(Bn))-SRIF28	25.6	5525	20.7	807	33.9	1843	59.4	21.5	33
67	C(disialo(aminoethylamide))・S1C(disialo)-SRIF28	22.5	3742	20.5	518	42.3	1289	64.7	16.2	34

Table 6

Example		PS index
1	S1C(disialo)-SRIF28	4.5
2	N5C(disialo)-SRIF28	4.7
3	A9C(disialo)-SRIF28	6.7
4	E12C(disialo)-SRIF28	6.2
5	R13C(disialo)-SRIF28	15.9
6	K14C(disialo)-SRIF28	19.1
10	N19C(disialo)-SRIF28	>20
13	29C(disialo)-SRIF28	>20
14	30C(disialo)-SRIF28	16.8
15	S1C(disialo)-D-Trp-SRIF28	12.3
16	A9C(disialo)-D-Trp-SRIF28	17.2
21	S1C(disialo) · N5C(disialo)-SRIF28	17.8
22	S1C(disialo) · R13C(disialo)-SRIF28	>20
24	S1-3C(disialo)-SRIF28	>20
25	S1C(disialo)·N5C(disialo)·A9C(disialo)-SRIF28	>20
26	S1C(monosialo)-SRIF28	3.5
27	S1C(asialo)-SRIF28	2.6
-	Octreotide	>20
-	SRIF28	1

[0300]

As is clear from the results shown in Figure 20, the glycosylated polypeptide of the present invention was increased in plasma stability compared to SRIF28. In other words, this was 4.5 - 6.7-folds in those having one sugar chain added at positions 1, 5, 9, and 12 (the compounds of Examples 1, 2, 3, and 4), 15.9-folds for those having a sugar chain added at position 13 (the compound of Example 5), 19.1-folds for those having a sugar chain added at position 14 (the compound of Example 6), and 16.8-folds for those having a sugar chain added at position 30 (the compound of Example 14), compared to that of SRIF28. Moreover, this was 20-folds or more for those having one glycosylated amino acid further added at position 19 which is the C-terminal side (the compounds of Examples 10 and 13). It was shown that in regards to the position for glycosylation, plasma stability increases from position 1 towards the C-terminal.

Table 7

Example		Dose	GH (ng/mL)
1	S1C(disialo)-SRIF28	1nmol/kg	490
1	S1C(disialo)-SRIF28	10nmol/kg	17.1
10	N19C(disialo)-SRIF28	10nmol/kg	563
10	N19C(disialo)-SRIF28	100nmol/kg	1.5
13	29C(disialo)-SRIF28	10nmol/kg	497
13	29C(disialo)-SRIF28	100nmol/kg	88.5
21	S1C(disialo)-N5C(disialo)-SRIF28	1nmol/kg	776
21	S1C(disialo)-N5C(disialo)-SRIF28	10nmol/kg	181
25	S1C(disialo)-N5C(disialo)-A9C(disialo)-SRIF28	10nmol/kg	833
25	S1C(disialo)-N5C(disialo)-A9C(disialo)-SRIF28	100nmol/kg	151
-	vehicle	-	861

[0305]

As is clear from the results shown in Table 7, the glycosylated form of the present invention suppressed GH production in rats. The ratio between the Ki value of glycosylated forms of Examples 21 and 25 against each receptor and the Ki value of unglycosylated SRIF14 is shown to be in the range of 15.7:1 - 1.2:1 (the compound of Example 21) and 158:1 - 7.1:1 (the compound of Example 25), respectively, as measured in the method of Example 69-1. Moreover, the glycosylated forms of Examples 21 and 25 are shown to have 25-folds or more (the compound of Example 21) or 30-folds or more (the compound of Example 25) increase in half-life in blood, respectively, as measured in the method of Example 70. On the other hand, from the present Example having two sugar chains, it was shown that the glycosylated form effectively exerts pharmacological action even *in vivo*.

Moreover, the glycosylated form of the present invention had GH production suppressing action even when administered 1 hour before GRF administration.

It was shown that the effective administration dose for rat GH production ability between the glycosylated forms of Examples 1 and 21 and Example 25 was different by approximately 10-folds. This is similar to the receptor affinities thereof having a difference of approximately 10-folds in terms of Ki value, as measured in the method of Example 69-1. It was shown that they have pharmacological activity even when the affinity of the glycosylated form was somewhat attenuated.

[0306]

Table 8

Example		Dose	GH (ng/mL)	The number of runs
1	S1C(disialo)-SRIF28	1nmol/kg	787	4
		3nmol/kg	182	3
		10nmol/kg	15	5
		30nmol/kg	8	1
		100nmol/kg	7	2
2	N5C(disialo)-SRIF28	1nmol/kg	969	1
		10nmol/kg	12	1
3	A9C(disialo)-SRIF28	1nmol/kg	577	1
		10nmol/kg	20	1
4	E12C(disialo)-SRIF28	10nmol/kg	10	1
5	R13C(disialo)-SRIF28	10nmol/kg	136	1
6	K14C(disialo)-SRIF28	10nmol/kg	55	1
15	S1C(disialo)-D-Trp22-SRIF28	0.3nmol/kg	986	3
		1nmol/kg	335	3
		3nmol/kg	50	1
		10nmol/kg	6	2
		30nmol/kg	2	1
18	C(disialo)-R-K-SRIF14	1nmol/kg	912	4
		3nmol/kg	291	4
		10nmol/kg	30	4
		30nmol/kg	12	1
21	S1C(disialo)·N5C(disialo)-SRIF28	1nmol/kg	776	1
		10nmol/kg	181	1
25	S1C(disialo)·N5C(disialo)·A9C(disialo)-SRIF28	100nmol/kg	151	1
26	S1C(monosialo)-SRIF28	10nmol/kg	47	1
27	S1C(asialo)-SRIF28	1nmol/kg	879	4
		3nmol/kg	515	4
		10nmol/kg	146	4
28	S1-2C(asialo)-SRIF28	3nmol/kg	997	3
		10nmol/kg	175	3
34	S1-5C(disialo)-SRIF28	10nmol/kg	842	1
59	S1C(disialo(aminoethylamide))-SRIF28	10nmol/kg	314	1
60	S1C(disialo(amide))-SRIF28	10nmol/kg	14	1
61	S1C(disialo(Bn))-SRIF28	10nmol/kg	49	1
-	vehicle	-	1012	19

[0307]

As is clear from the results shown in Table 8, the glycosylated polypeptide of the present invention suppressed GH production in rats. In the present test line, S1C(disialo)-SRIF28 suppressed GH production from 1 nmol/kg, and showed 82 - 99% of the GH production suppression effect at 3 - 10 nmol/kg. This is thought to be due to the gain in affinity towards SSTR1 - SSTR5 and improvement in the

Employing S1C(disialo)-SRIF28, C(disialo)-R-K-SRIF14, and S1-2C(asialo)-SRIF28 as the glycosylated forms, these were dissolved in Japanese Pharmacopeia saline (from Otsuka Pharmaceutical factory, Inc.) to prepare a 40 μM solution as the administration solution. Eighteen hours after the gastrointestinal obstruction surgery, this was subcutaneously administered into the dorsal cervix at a dosage of 1 mL/kg with a glass syringe and a 25 G injection needle (all from Terumo Corporation). As the vehicle group, saline without glycosylated polypeptide was similarly administered. Moreover, octreotide was administered as the control group.

[0313]

Example 91-3 Measurement of intestinal fluid weight

One hour after compound administration, a median incision was made in the abdomen under inhalation anesthesia, and 1.5 mL of blood was collected from the abdominal vena cava. Then, the ligated jejunum on the musculus suspensorius duodeni side was resected. The fluid and blood on the jejunum surface were removed with a paper towel, nerves, blood vessels, and fat attached to the jejunum was removed, this was cut into 6 cm lengths, and wet weight was measured. This was then dried at 36 degrees for 24 hours, and dry weight was measured. The intestinal fluid weight (mg) was calculated by wet weight - dry weight. The intestinal fluid weight of the jejunum obtained is shown in Table 9.

[Table 24]

Table 9

	Intestinal fluid weight(mg)
Sham treatment	285 ± 27
vehicle	481 ± 31
octreotide	693 ± 48
S1C(disialo)-SRIF28	630 ± 83
C(disialo)-R-K-SRIF14	584 ± 95
S1-2C(asialo)-SRIF28	566 ± 34

[0314]

As apparent from Table 9, the vehicle had significantly increased intestinal fluid weight compared to

Table 10

	Amylase (IU/L)	LDH (IU/L)	CPK (IU/L)
Sham treatment	669 ± 164	168 ± 82	353 ± 68
vehicle	1672 ± 743	341 ± 68	377 ± 57
octreotide	1732 ± 774	269 ± 141	472 ± 215
S1C(disialo)-SRIF28	1164 ± 224	338 ± 97	455 ± 202
C(disialo)-R-K-SRIF14	985 ± 238	228 ± 155	415 ± 137
S1-2C(asialo)-SRIF28	1594 ± 573	318 ± 44	728 ± 665

[0316]

As apparent from Table 10, the vehicle had increased amylase activity and LDH activity compared to the sham treatment, and it was speculated that tissue disorder of the gastrointestinal system had developed accompanying gastrointestinal obstruction. In S1C(disialo)-SRIF28 and C(disialo)-R-K-SRIF14, the amylase activity had a low value compared to the vehicle. On the other hand, the amylase activity of octreotide was equivalent to the vehicle. As apparent from Examples 69-1 and 69-2, S1C(disialo)-SRIF28, C(disialo)-R-K-SRIF14, and S1-2C(asialo)-SRIF28 had binding affinity toward all the receptors from SSTR1 - SSTR5, whereas octreotide is a compound having specific affinity towards SSTR2, SSTR3, and SSTR5. In rat pancreas, since there is a report that all the receptors SSTR1 - SSTR5 are expressed (J. Histochem. Cytochem. 2004 Mar; 52 (3): 391-400), the possibility that the glycosylated form alleviated the tissue disorder by acting on a receptor different from that of octreotide was conceived. Moreover, in octreotide and C(disialo)-R-K-SRIF14, the LDH activity had a low value compared to the vehicle. There were no others that reduced each parameter by administration of the glycosylated form.

Sequence Listing
OCKP1103F sequence listing.txt