

**Fig. 7 RCA growth curve in HeLa cells.**

HeLa cells were infected with serial log dilution of RCA. Cells were harvested on day 1, 3, 6, and 9, and viral DNA were extracted by glass beads and then amount of RCA were determined by real-time quantitative PCR.

Data were the mean  $\pm$  S.D. (n=3).

**Table 3 Comparison of CPE assay and infectivity PCR in detecting RCA**

**A) CPE method**

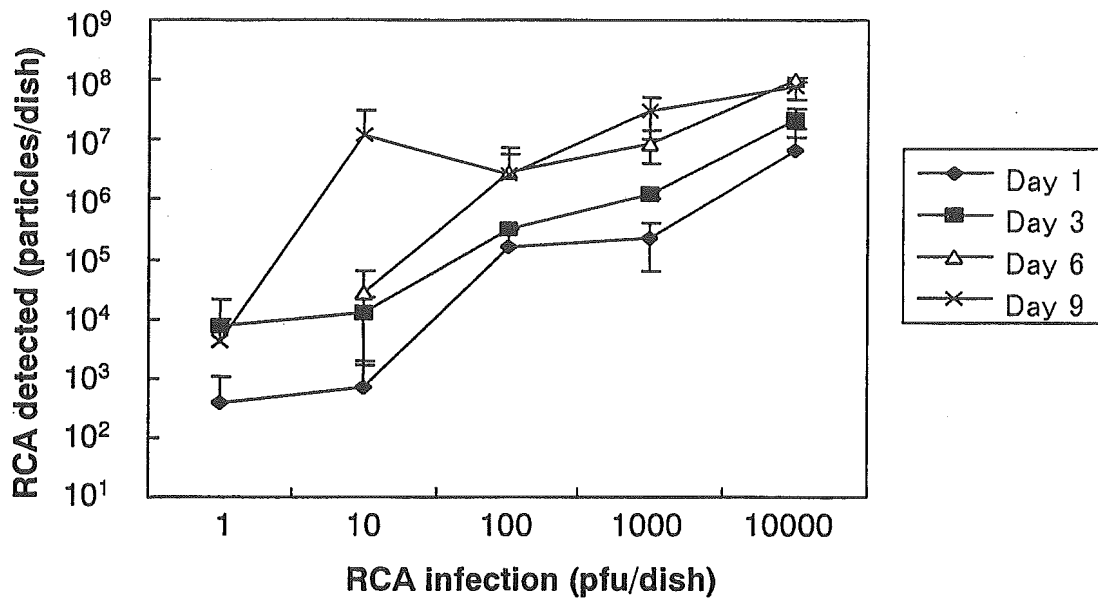
pfu/dish	Day1	Day3	Day6	Day9
10000	0/3	0/3	3/3	3/3
1000	0/3	0/3	3/3	3/3
100	0/3	0/3	0/3	3/3
10	0/3	0/3	0/3	0/3
1	0/3	0/3	0/3	0/3
0.1	0/3	0/3	0/3	0/3
0	0/3	0/3	0/3	0/3

**B) Infectivity PCR**

pfu/dish	Day1	Day3	Day6	Day9
10000	3/3	3/3	3/3	3/3
1000	3/3	3/3	3/3	3/3
100	3/3	3/3	3/3	3/3
10	0/3	2/3	3/3	3/3
1	0/3	1/3	2/3	2/3
0.1	0/3	0/3	0/3	0/3
0	0/3	0/3	0/3	0/3

Serial dilutions of RCA in medium were infected into HeLa cells. (A) CPE was observed under microscopy and (B) E1 DNA in DNA extracted with glass beads was detected by real-time quantitative PCR.

Number of CPE or E1 DNA positive samples were presented. (n=3)



**Fig. 8 Detection of RCA spiked in adenovirus vectors by infectivity PCR and glass beads extraction.**

HeLa cells were infected with serial log dilution of RCA spiked in AdHM10LacZ-3 adenovirus vectors. Cells were harvested on day 1, 3, 6, and 9, and then viral DNA were extracted with glass beads. Amount of RCA of each sample was determined by real-time quantitative PCR. Data were the mean  $\pm$  S.D. (n=3).

**Table 4 Comparison of CPE assay and infectivity PCR in detecting RCA spiked in adenovirus vectors**

**A) CPE method**

pfu/dish	Day1	Day3	Day6	Day9
10000	0/3	0/3	0/3	1/3
1000	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3
10	0/3	0/3	0/3	0/3
1	0/3	0/3	0/3	0/3
0.1	0/3	0/3	0/3	0/3
0	0/3	0/3	0/3	0/3

**B-1) Infectivity PCR (TaqMan PCR)**

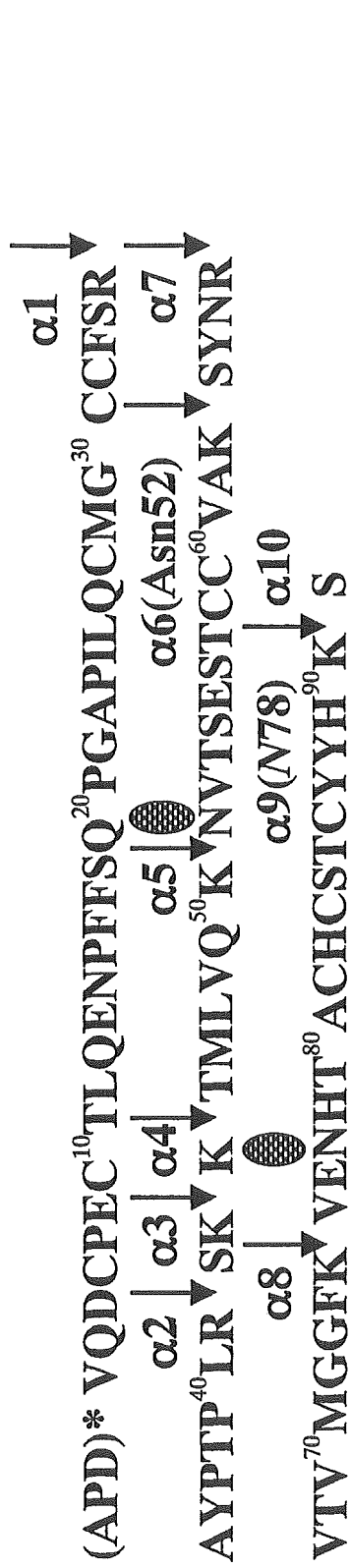
pfu/dish	Day1	Day3	Day6	Day9
10000	3/3	3/3	3/3	3/3
1000	3/3	3/3	3/3	3/3
100	3/3	3/3	3/3	3/3
10	1/3	2/3	3/3	3/3
1	1/3	1/3	0/3	2/3
0.1	0/3	0/3	0/3	0/3
0	0/3	0/3	0/3	0/3

**B-2) Infectivity PCR( Nested PCR)**

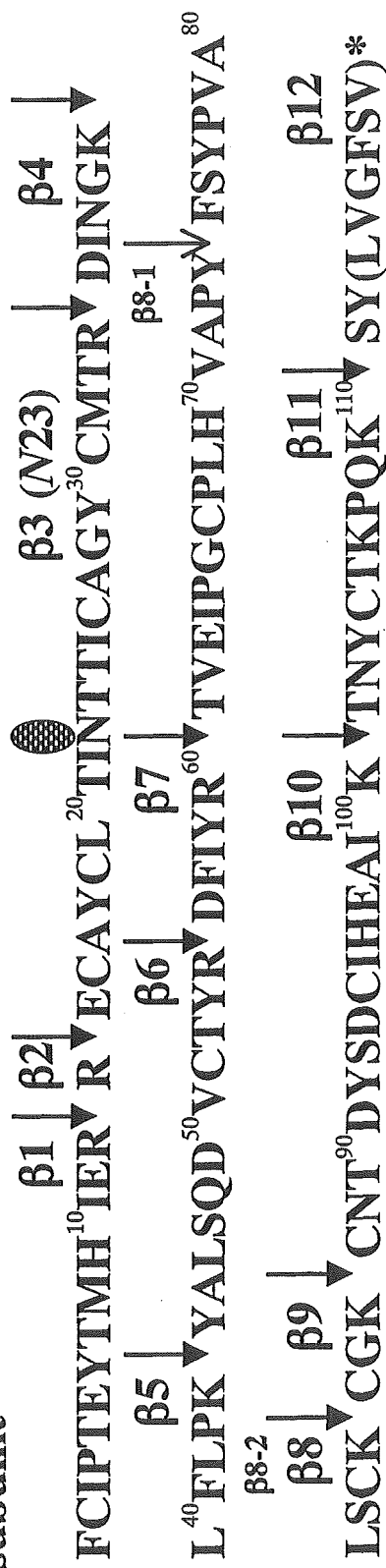
pfu/dish	Day1	Day3	Day6	Day9
1	2/3	2/3	1/3	2/3

Serial dilutions of RCA spiked in  $10^9$  particles of Adenovirus vectors were infected into HeLa cells. (A) CPE was observed under microscopy and (B-1) E1 DNA in DNA extracted with glass beads was detected by real-time quantitative PCR. (B-2) E1 DNA was detected by nested PCR using DNA extracted from HeLa cells infected with 1 pfu of RCA. Number of CPE or E1 DNA positive samples was presented (n=3).

**α subunit**

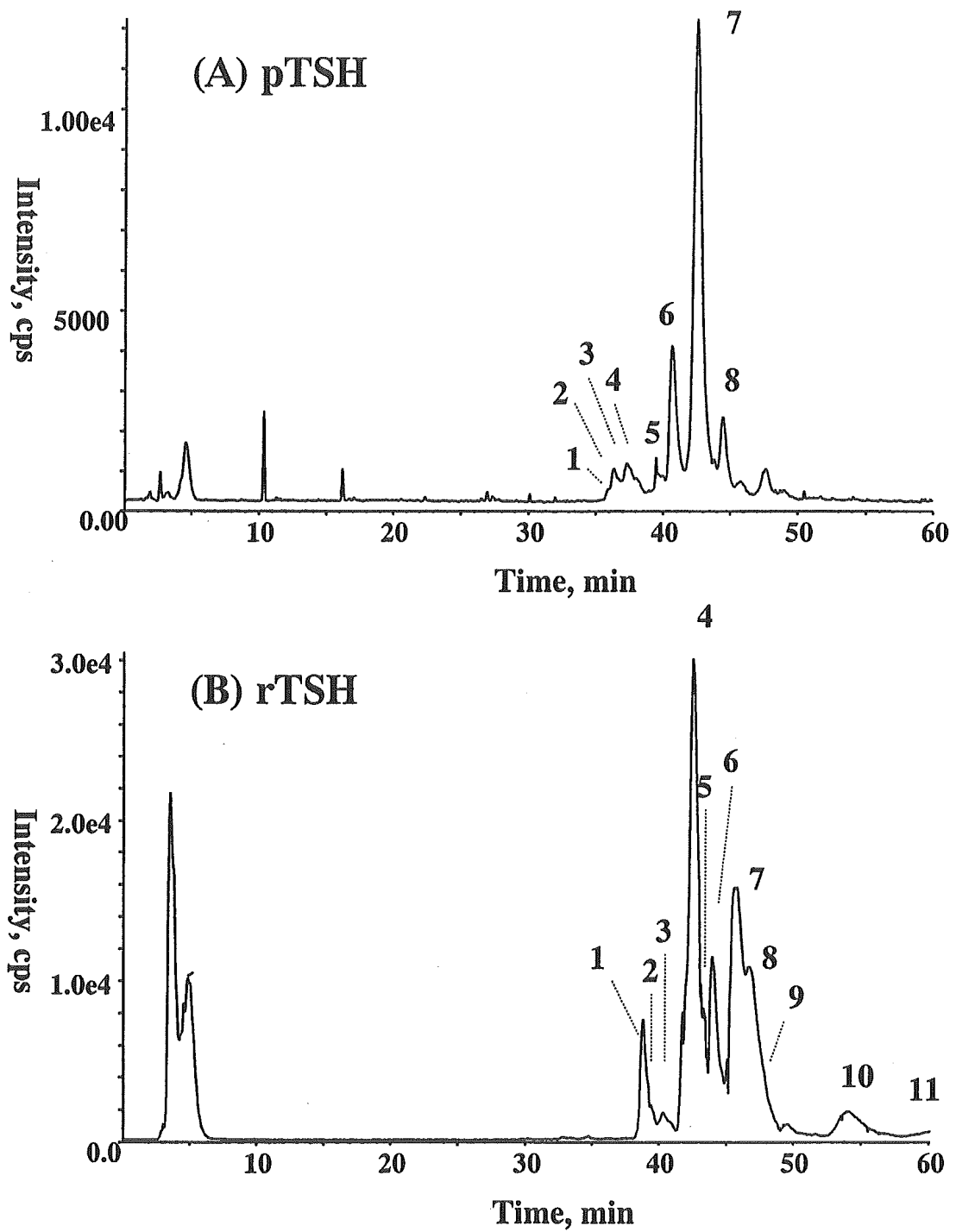


**β subunit**



● : Carbohydrate, (\*)\*: only in r-TSH.

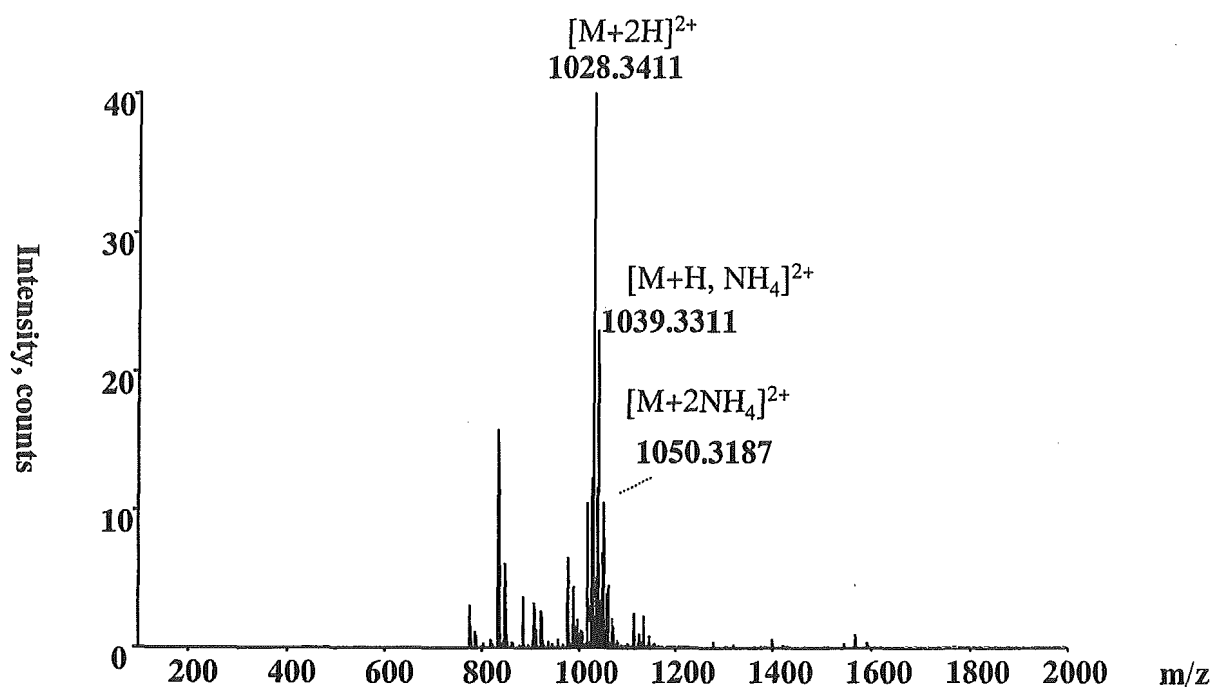
Fig. 9 Amino acid sequence and glycosylation sites of TSH



**Fig. 10** Oligosaccharide profiles of pTSH (A) and rTSH (B)

N-linked oligosaccharides from pTSH and rTSH were analyzed by CapLC-ESI-Q/TOFMS

(A) Mass spectrum of peak 7 in Fig. 10A



(B) Product ion spectrum of peak 7 in Fig. 10A

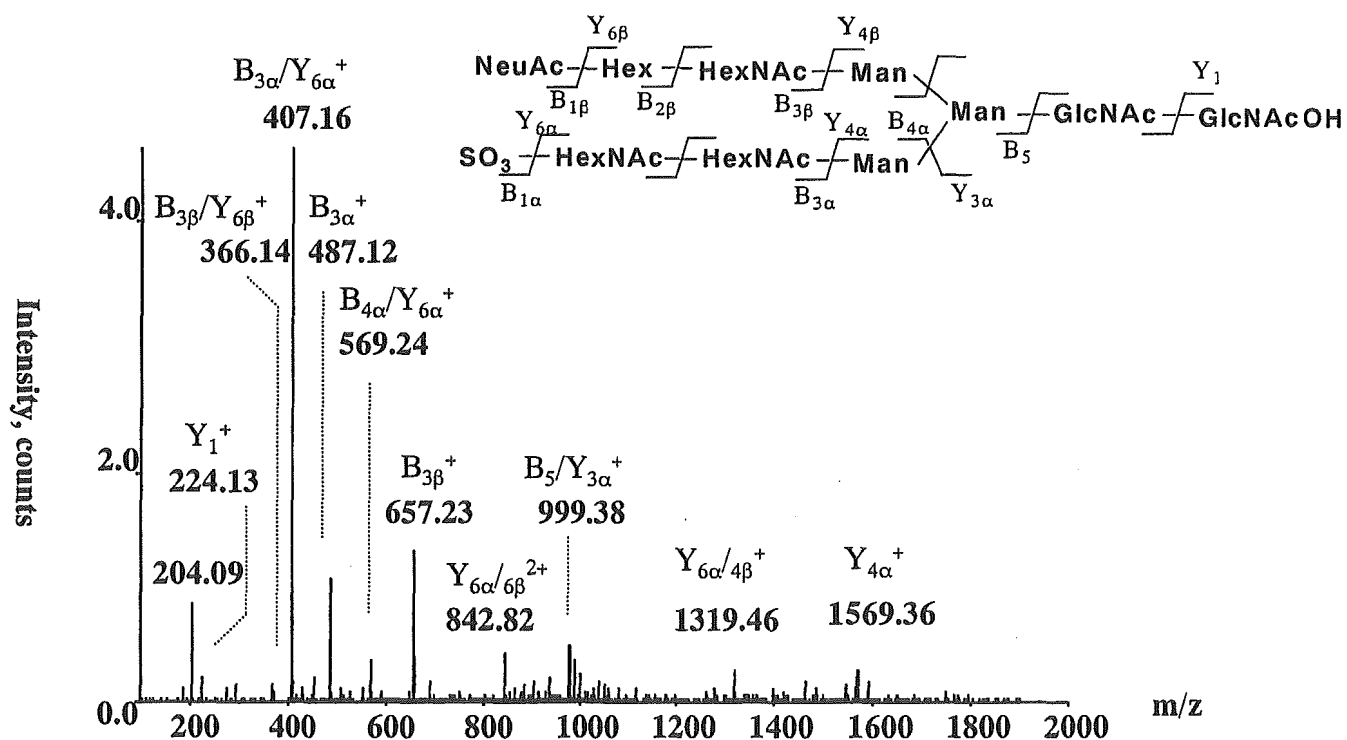


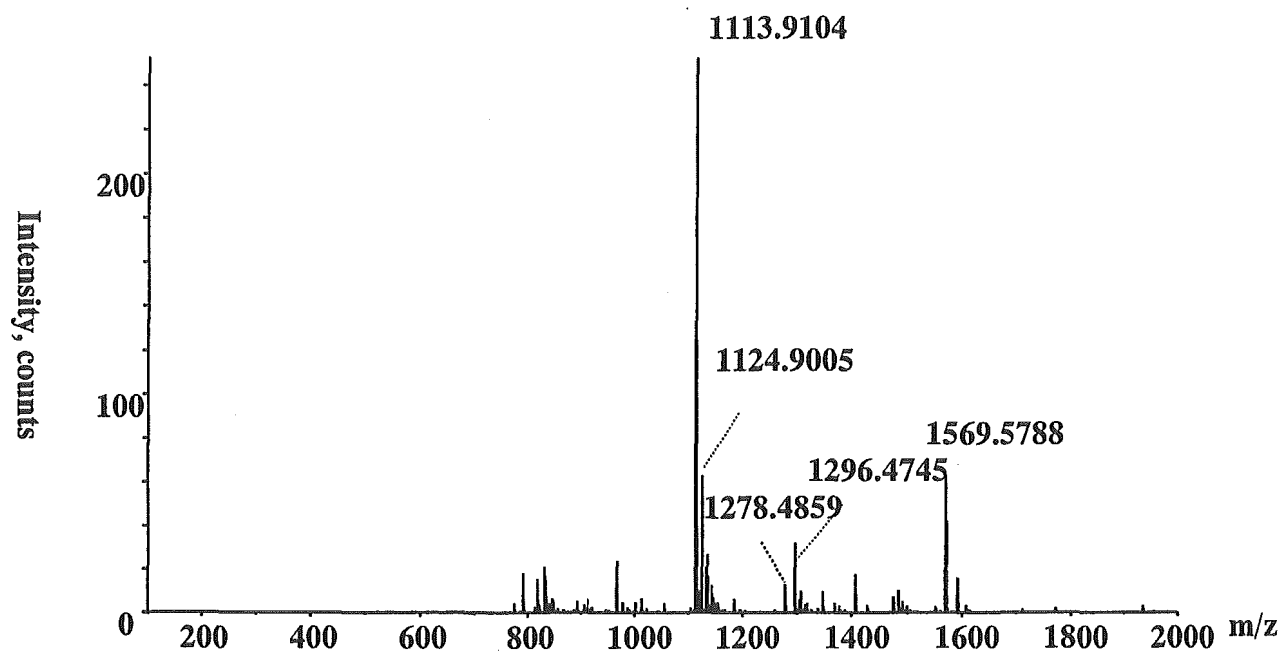
Fig. 11 Mass spectrum (A) and product ion spectrum (B) of peak 7 in Fig. 10A

**Table 5 Carbohydrate compositions, and theoretical and calculated masses of peaks in Fig. 10A**

Peak No.	Carbohydrate composition	Deduced carbohydrate structure <sup>a</sup>	Theoretical mass <sup>b</sup>	Calculated mass	Charge state	Observed <i>m/z</i>
1	[dHex] <sub>0</sub> [Hex] <sub>4</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>0</sub>	SO <sub>3</sub> -Hybrid(2)	1560.51	1560.53	1+	1561.53
2	[dHex] <sub>0</sub> [Hex] <sub>3</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>0</sub>	SO <sub>3</sub> -Bi(4)	1398.46	1398.47	1+	1399.48
3	[dHex] <sub>0</sub> [Hex] <sub>3</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>0</sub>	SO <sub>3</sub> -Bi(5)	1601.57	1601.57	1+	1602.58
	[dHex] <sub>0</sub> [Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>0</sub>	SO <sub>3</sub> -Hybrid(3)	1722.58	1722.58	2+	862.30
	[dHex] <sub>1</sub> [Hex] <sub>6</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>0</sub>	SO <sub>3</sub> -FBI(3)	1950.70	1950.70	2+	976.36
4	[dHex] <sub>0</sub> [Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>0</sub>	SO <sub>3</sub> -Bi(2)	1763.62	1763.62	2+	882.82
5	[dHex] <sub>1</sub> [Hex] <sub>3</sub> [HexNAc] <sub>2</sub> [NeuAc] <sub>0</sub>	FCore	1058.41	1058.41	1+	1059.42
	[dHex] <sub>0</sub> [Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>1</sub>	SO <sub>3</sub> -Bi(2)NA <sub>1</sub>	2054.69	2054.74	2+	1028.38
	[dHex] <sub>1</sub> [Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>0</sub>	SO <sub>3</sub> -FBI(2)	1909.69	1909.66	2+	955.84
	[dHex] <sub>1</sub> [Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>1</sub>	FBI(2)NA <sub>1</sub>	2120.79	2120.82	2+	1061.42
	[dHex] <sub>0</sub> [Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>0</sub>	(SO <sub>3</sub> ) <sub>2</sub> -Bi(2)	1843.55	1843.56	2+	922.79
6	[dHex] <sub>0</sub> [Hex] <sub>3</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>0</sub>	(SO <sub>3</sub> ) <sub>2</sub> -Bi(3)	1884.57	1884.60	2+	943.31
7	[dHex] <sub>0</sub> [Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>1</sub>	SO <sub>3</sub> -Bi(2)NA <sub>1</sub>	2054.69	2054.68	2+	1028.34
	[dHex] <sub>1</sub> [Hex] <sub>3</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>0</sub>	(SO <sub>3</sub> ) <sub>2</sub> -FBI(3)	2030.68	2030.66	2+	1016.34
8	[dHex] <sub>1</sub> [Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>1</sub>	SO <sub>3</sub> -FBI(2)NA <sub>1</sub>	2200.74	2200.78	2+	1101.40

<sup>a</sup>Deduced carbohydrate structures are presented in Table 9 ; <sup>b</sup> monoisotopic mass value.

(A) Mass spectrum of peak 4 in Fig. 10B  $[M+H]^{2+}$



(B) Product ion spectrum of peak 4 in Fig. 10B

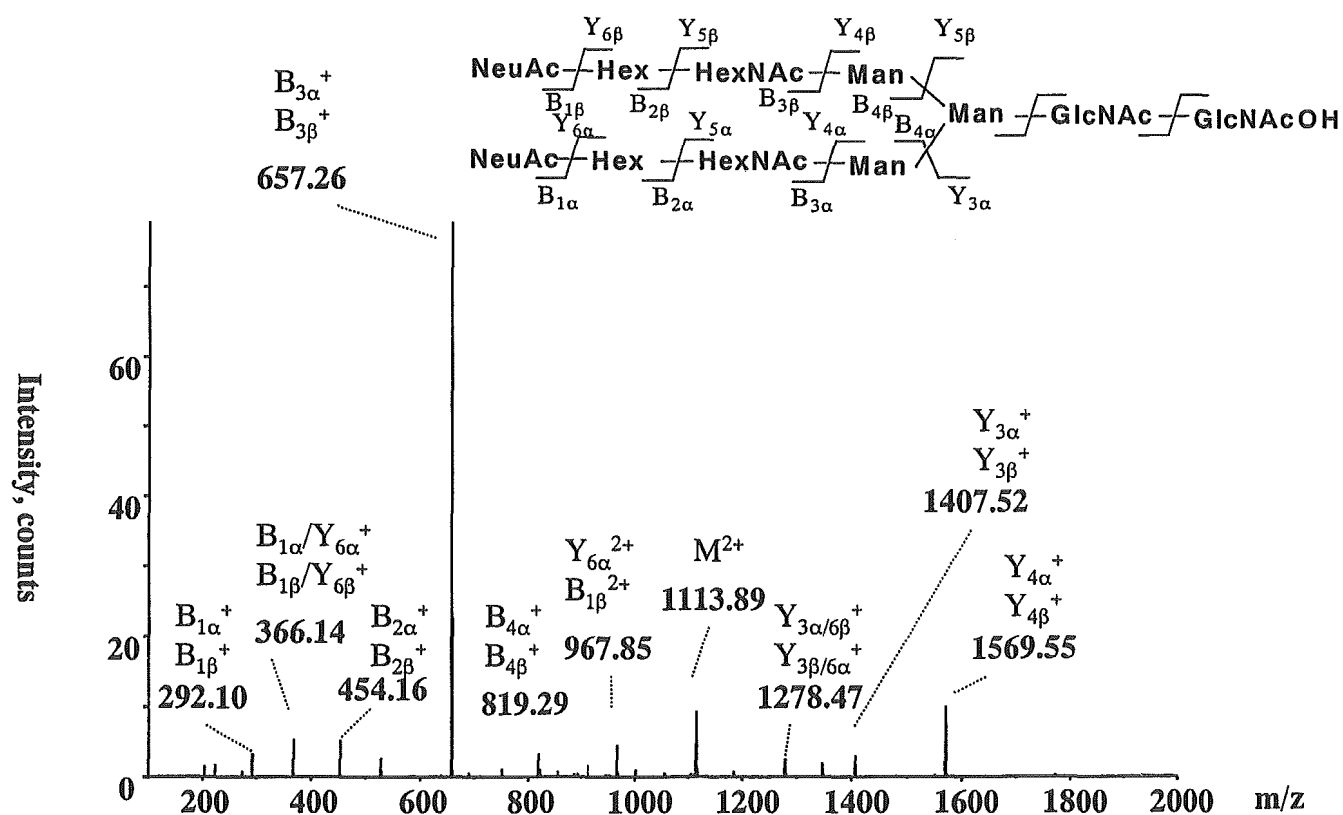


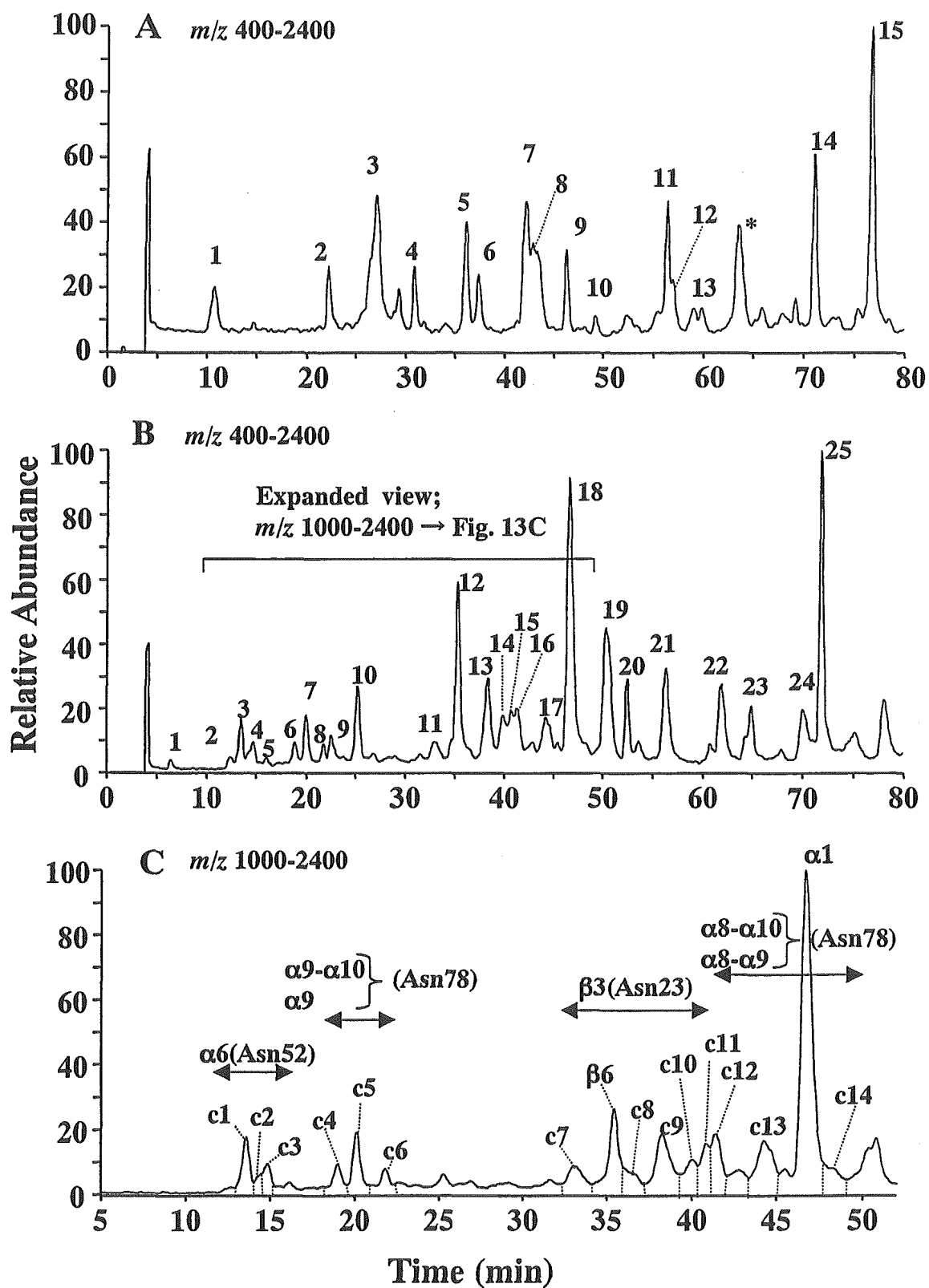
Fig. 12 Mass spectrum (A) and product ion spectrum (B) of peak 4 in Fig. 10B

**Table 6 Carbohydrate compositions, and theoretical and calculated masses of peaks in Fig. 10B**

Peak No.	Carbohydrate composition	Deduced carbohydrate structure <sup>a</sup>	Theoretical mass <sup>b</sup>	Calculated mass	Charge state	Observed $m/z$
1	[dHex] <sub>0</sub> [Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	1933.70	1933.68	1+	1934.69
2	[dHex] <sub>0</sub> [Hex] <sub>6</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	Hybrid(4)NA <sub>1</sub>	2095.76	2095.74	2+	1048.88
3	[dHex] <sub>1</sub> [Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	FBiNA <sub>1</sub>	2079.76	2079.78	2+	1040.90
4	[dHex] <sub>0</sub> [Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	2224.80	2224.80	2+	1113.41
5	[dHex] <sub>0</sub> [Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	FTriNA <sub>2</sub> , FBiLacNA <sub>2</sub>	2735.99	2735.98	2+	1369.00
6	[dHex] <sub>1</sub> [Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBiNA <sub>2</sub>	2370.86	2370.86	2+	1186.44
7	[dHex] <sub>0</sub> [Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	2881.03	2881.02	2+	1441.52
8	[dHex] <sub>1</sub> [Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	FTriNA <sub>3</sub>	3027.08	3027.08	2+	1514.55
9	[dHex] <sub>1</sub> [Hex] <sub>7</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>3</sub>	FTetraNA <sub>3</sub> , FTriLacNA <sub>3</sub>	3393.22	3393.26	2+	1697.64
10	[dHex] <sub>1</sub> [Hex] <sub>7</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>4</sub>	FTetraNA <sub>4</sub>	3683.31	3683.42	2+	1842.72
11	[dHex] <sub>1</sub> [Hex] <sub>8</sub> [HexNAc] <sub>7</sub> [NeuAc] <sub>4</sub>	FTetraLac <sub>1</sub> NA <sub>4</sub>	4048.44	4048.45	3+	1350.49

<sup>a</sup>Deduced carbohydrate structures are presented in Table 13 ; <sup>b</sup> monoisotopic mass value.





**Fig.13 (A)** Peptide/glycopeptide map of trypsin digested-rTSH obtained by elution with TFA  
**(B)** Peptide/glycopeptide map of trypsin digested-rTSH obtained by elution with ammonium acetate  
**(C)** Glycopeptide map of trypsin digested-rTSH obtained by elution with ammonium acetate

**Table 7 Structural assignments of peaks in Fig 13A**

Peak No.	Amino acid residues	Theoretical mass <sup>a</sup>	Observed m/z		
			M <sup>1+</sup>	M <sup>2+</sup>	M <sup>3+</sup>
1	$\alpha$ 7	538.6	539.3		
	$\beta$ 4	545.6	546.4		
2	$\beta$ 11	1140.2	1140.7	571.0	
3 <sup>b</sup>	$\alpha$ 6(Asn52)	–			
	$\alpha$ 9 (Asn78) and $\alpha$ 9- $\alpha$ 10 (Asn78)	–			
4	$\alpha$ 5	718.9	719.5		
5	$\alpha$ 2	817.0	817.6	409.3	
6	$\beta$ 7	712.8	713.4		
7	$\beta$ 6	1376.5	1377.4	689.0	
	$\beta$ 10	1727.8	1728.4	864.6	576.9
8 <sup>b</sup>	$\alpha$ 8	838.0	838.6	419.8	
	$\alpha$ 8- $\alpha$ 9 (Asn78) and $\alpha$ 8- $\alpha$ 10 (Asn78)	–			
9	$\beta$ 5	616.8	617.5		
10	$\beta$ 8-2 <sup>c</sup>	1172.4	1172.5	587.1	
11	$\beta$ 1	1697.9	1698.4	849.7	566.9
12 <sup>b</sup>	$\beta$ 3 (Asn23)	–			
	$\beta$ 8-1 <sup>c</sup>	1553.8	1554.3	777.6	
13	$\beta$ 12	871.0	871.6		
14	$\beta$ 8	2708.1		1354.8	903.5
15	$\alpha$ 1	4123.4		2062.8	1375.2

<sup>a</sup> average mass value, <sup>b</sup> glycopeptides,<sup>c</sup>  $\beta$ 8 was partly hydrolyzed to  $\beta$ 8-1 and  $\beta$ 8-2,  $\alpha$ 3,  $\alpha$ 4,  $\beta$ 2 and  $\beta$ 9 were not detected.

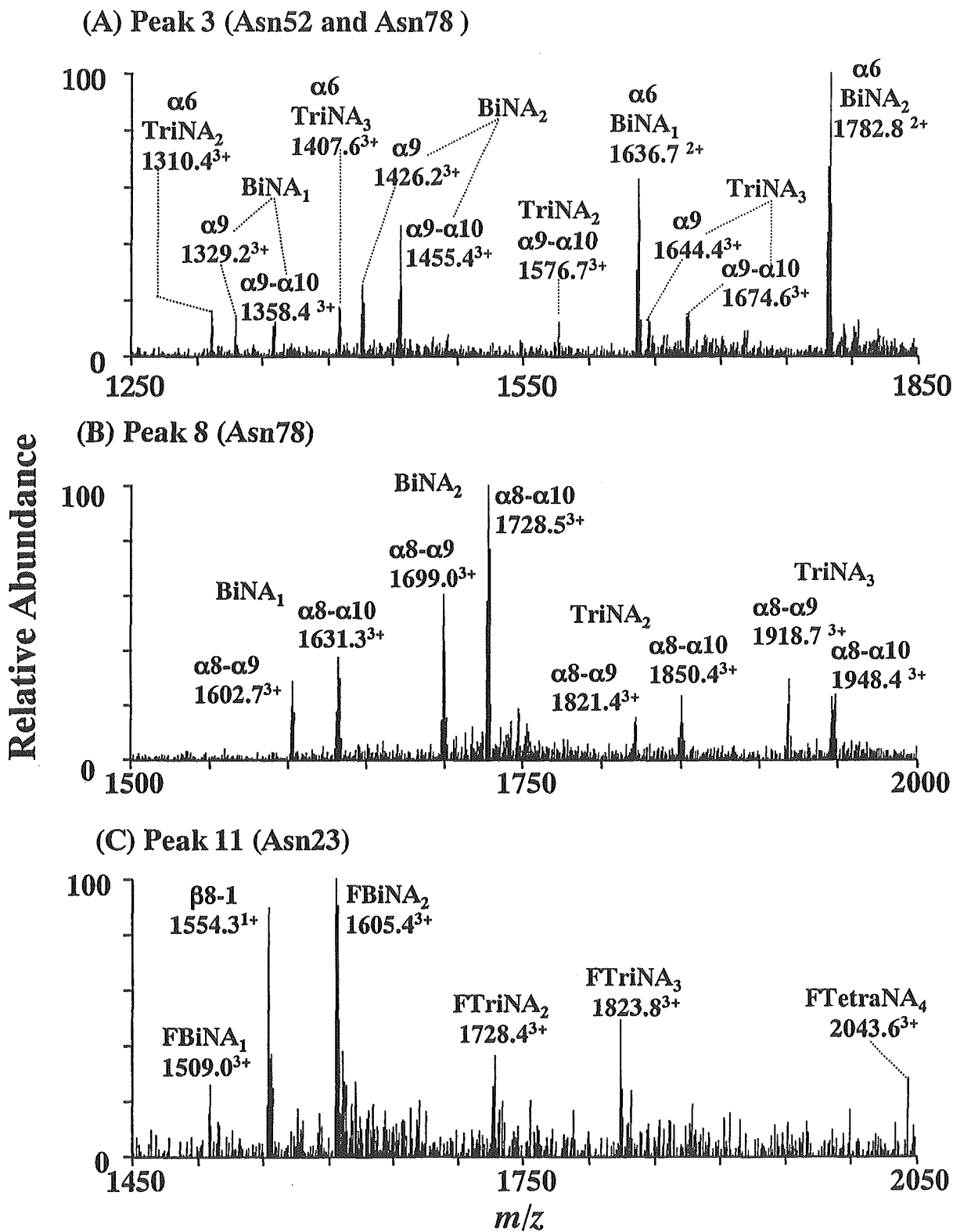


Fig.14 Mass spectra of peaks 3, 8 and 12 in Fig. 13A

**Table 8 Peptides and carbohydrate compositions of peaks 3, 8, and 12 in Fig. 13A**

Peak No. <sup>a</sup>	Glyco-peptides	Carbohydrate composition <sup>b</sup>	Deduced carbohydrate structure <sup>c</sup>	Observed <i>m/z</i>		
				Theoretical mass <sup>d</sup>	<i>M</i> <sup>2+</sup>	<i>M</i> <sup>4+</sup>
3	α6 (Asn52)	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	4,220.0	1,407.6	
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	3,563.4	1,189.1	1,782.8
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac,NA <sub>2</sub>	3,928.8	1,310.4	
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	3,272.2	1,091.5 <sup>e</sup>	
α9 (Asn78)		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	4,932.7	1,644.4	
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	4,276.1	1,426.2	1,070.0
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	3,984.9	1,329.2	
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	5,019.8	1,674.6	
α9-α10 (Asn78)		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	4,363.2	1,455.4	1,091.5 <sup>e</sup>
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac,NA <sub>2</sub>	4,728.5	1,576.7	
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	4,071.9	1,358.4	
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	5,752.7	1,918.7	1,439.4
8	α8-α9 (Asn78)	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	5,096.1	1,699.0	1,275.1
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac,NA <sub>2</sub>	5,461.5	1,821.4	1,366.7
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	4,804.9	1,602.7	
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	5,839.8	1,948.4	1,461.0
α8-α10 (Asn78)		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	5,183.2	1,728.5	1,296.6
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac,NA <sub>2</sub>	5,548.6	1,850.4	1,388.2
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	4,892.0	1,631.3	
		[Fuc][Hex] <sub>7</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>4</sub>	FTetra NA <sub>4</sub>	6,128.0	2,043.6	
12	β3 (Asn23)	[Fuc][Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	FTriNA <sub>3</sub>	5,471.5	1,823.8	
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBiNA <sub>2</sub>	4,814.9	1,605.4	
		[Fuc][Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	FTriNA <sub>2</sub> , FBiLac,NA <sub>2</sub>	5,180.2	1,728.4	
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	FBiNA <sub>1</sub>	4,523.6	1,509.0	2,262.2

<sup>a</sup> Mass spectra are shown in Fig. 14. <sup>b</sup> Hex, hexose; Fuc, fucose; HexNAc, *N*-acetylhexosamine; NeuAc, *N*-acetylneuramic acid. <sup>c</sup> See Table 13,

<sup>d</sup> average mass value.

**Table 9 Structural assignments of peaks in Fig. 13B**

Peak No.	Amino acid residues	Theoretical mass <sup>a</sup>	observed m/z		
			M <sup>1+</sup>	M <sup>2+</sup>	M <sup>3+</sup>
1	β4	545.6	546.2		
2	α7	538.6	539.2		
3 <sup>b</sup>	α6(Asn52)	–			
4 <sup>b</sup>	α6(Asn52)	–			
5 <sup>b</sup>	α6(Asn52)	–			
6 <sup>b</sup>	α9(Asn78) and α9-α10(Asn78)	–			
7 <sup>b</sup>	α9(Asn78) and α9-α10(Asn78)	–			
8 <sup>b</sup>	α9(Asn78) and α9-α10(Asn78)	–			
9	β11	1140.2	1140.6	570.9	
10	β10	1727.8	1728.0	864.5	
11 <sup>b</sup>	β3(Asn23)	–			
12 <sup>c</sup>	β6	1376.5	1377.2	689.0	
	β3(Asn23)	–			
13 <sup>c</sup>	β7	712.8	713.4		
	β3(Asn23)	–			
14 <sup>c</sup>	α5	718.9	719.4		
	β3(Asn23)	–			
15 <sup>b</sup>	β3(Asn23)	–			
16 <sup>b</sup>	α8-α9 (Asn78) and α8-α10 (Asn78)	–			
17 <sup>b</sup>	α8-α9 (Asn78) and α8-α10 (Asn78)	–			
18	α1	4123.4		2061.5	1375.6
19	α2	817.0	817.5	409.2	
20	β8-1 <sup>d</sup>	1553.8	1554.1	777.5	
21	β1	1697.9	1698.0	849.6	
	β8-2 <sup>d</sup>	1172.4	1172.5	586.9	
22	α8	838.0	838.5		
23	β12	871.0	871.5		
24	β5	616.8	617.4		
25	β8	2708.1		1355.4	903.5

<sup>a</sup> average mass value, <sup>b</sup> glycopeptide, <sup>c</sup> glycopeptide and peptide,

<sup>d</sup> β8 was partly hydrolyzed to β8-1 and β8-2, α3, α4,β2 and β9 were not detected.

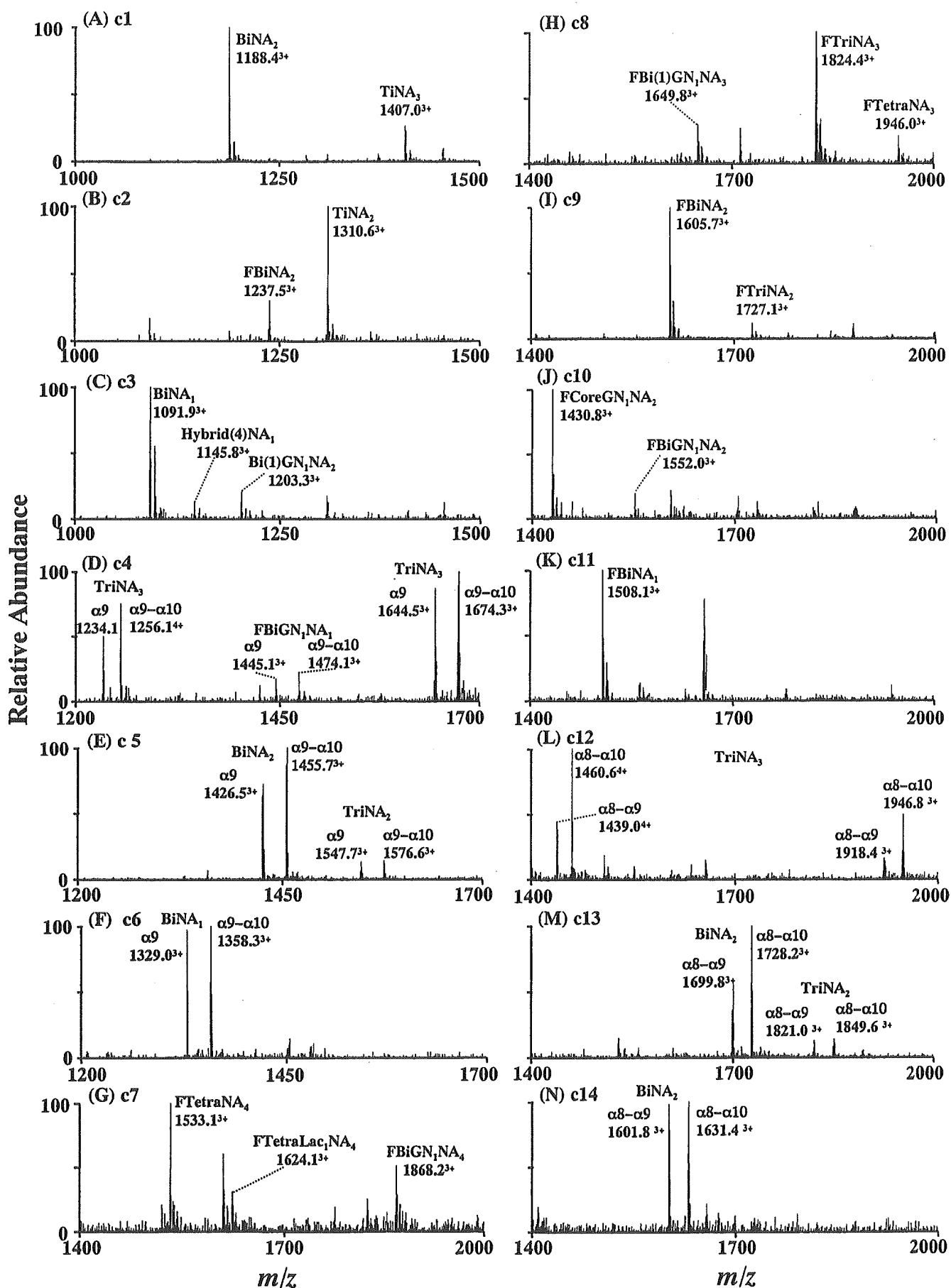


Fig. 15 Mass spectra of peaks c1-c14 in Fig. 13C

**Table 10 Peptides and carbohydrate compositions of peaks in Fig. 13C**

Peak No. <sup>a</sup>	Glyco-peptides	Carbohydrate composition <sup>b</sup>	Deduced carbohydrate structure <sup>c</sup>	Theoretical mass <sup>d</sup>		Observed <i>m/z</i>
				<i>M</i> <sup>2+</sup>	<i>M</i> <sup>3+</sup>	
c1	α6(Asn52)	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	4,220.0		1407.0
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	3,563.4	1781.7	1188.4
c2	α6(Asn52)	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac <sub>1</sub> NA <sub>2</sub>	3,928.8	1964.5	1310.6
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBiNA <sub>2</sub>	3,709.6	1854.8	1237.5
c3	α6(Asn52)	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	3,272.2	1637.3	1091.9
		[Hex] <sub>6</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	Hybrid (4)NA <sub>1</sub> , Hybrid (2)Lac <sub>1</sub> NA <sub>1</sub>	3,434.3	1718.6	1145.8
c4	α9(Asn78)	[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	Bi(1)GN <sub>2</sub> NA <sub>2</sub>	3,604.5	1203.3	1203.3
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	4,932.7	1644.5	1234.1
c5	α9-α10(Asn78)	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	5,019.8	1674.3	1256.1
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	FBiGN <sub>1</sub> NA <sub>1</sub>	4,334.2	1445.1	1105.9
c6	α9(Asn78)	[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>1</sub>	FBiGN <sub>1</sub> NA <sub>1</sub>	4,421.3	1474.1	1105.9
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	4,276.1	1426.5	1070.0
c7	β3(Asn23)	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	4,343.2	1455.7	1092.2
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac <sub>1</sub> NA <sub>2</sub>	4,641.5	1547.7	1183.3
c8	β3(Asn23)	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac <sub>1</sub> NA <sub>2</sub>	4,728.5	1576.6	1183.3
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	3,984.9	1329.0	1019.4
c9	β3(Asn23)	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	4,071.9	1358.3	1019.4
		[Fuc][Hex] <sub>7</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>4</sub>	FTetra NA <sub>4</sub>	6,128.0	2043.7	1533.1
c10	β3(Asn23)	[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>4</sub>	FBiGN <sub>1</sub> NA <sub>4</sub>	5,600.6	1868.2	1624.1
		[Fuc][Hex] <sub>8</sub> [HexNAc] <sub>7</sub> [NeuAc] <sub>4</sub>	FTetraLac <sub>1</sub> NA <sub>4</sub>	6,493.4	2165.5	1624.1
c11	β3(Asn23)	[Fuc][Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	FTriNA <sub>3</sub>	5,471.5	1824.4	1368.7
		[Fuc][Hex] <sub>7</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>3</sub>	FTetraNA <sub>3</sub> , FTriLac <sub>1</sub> NA <sub>3</sub>	5,836.8	1946.0	1649.8
c12	α8-α9(Asn78)	[Fuc][Hex] <sub>4</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>3</sub>	FBi(1)GN <sub>1</sub> NA <sub>3</sub>	4,944.0	1605.7	1605.7
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBiNA <sub>2</sub>	4,814.9	1605.7	1605.7
c13	β3(Asn23)	[Fuc][Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	FTriNA <sub>2</sub> , FBiLac <sub>1</sub> NA <sub>2</sub>	5,180.2	1727.1	1727.1
		[Fuc][Hex] <sub>3</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	FCore GN <sub>1</sub> NA <sub>2</sub>	4,287.4	1430.8	1430.8
c14	α8-α9(Asn78)	[Fuc][Hex] <sub>4</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBi(1) GN <sub>1</sub> NA <sub>2</sub>	4,652.7	1552.0	1552.0
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	FBiNA <sub>1</sub>	4,523.6	1508.1	1508.1
c15	α8-α9(Asn78)	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	5,752.7	1918.4	1439.0
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	5,839.8	1946.8	1460.6
c16	α8-α9(Asn78)	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	5,096.1	1699.8	1274.7
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	5,183.2	1728.2	1296.3
c17	α8-α9(Asn78)	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac <sub>1</sub> NA <sub>2</sub>	5,461.5	1821.0	1366.3
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac <sub>1</sub> NA <sub>2</sub>	5,548.6	1849.6	1388.1
c18	α8-α9(Asn78)	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	4,804.9	1601.8	1202.3
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	4,892.0	1631.4	1223.5

<sup>a</sup> Mass spectra are shown in Fig. 15. <sup>b</sup> See Table 8. <sup>c</sup> See Table 13. <sup>d</sup> average mass value.

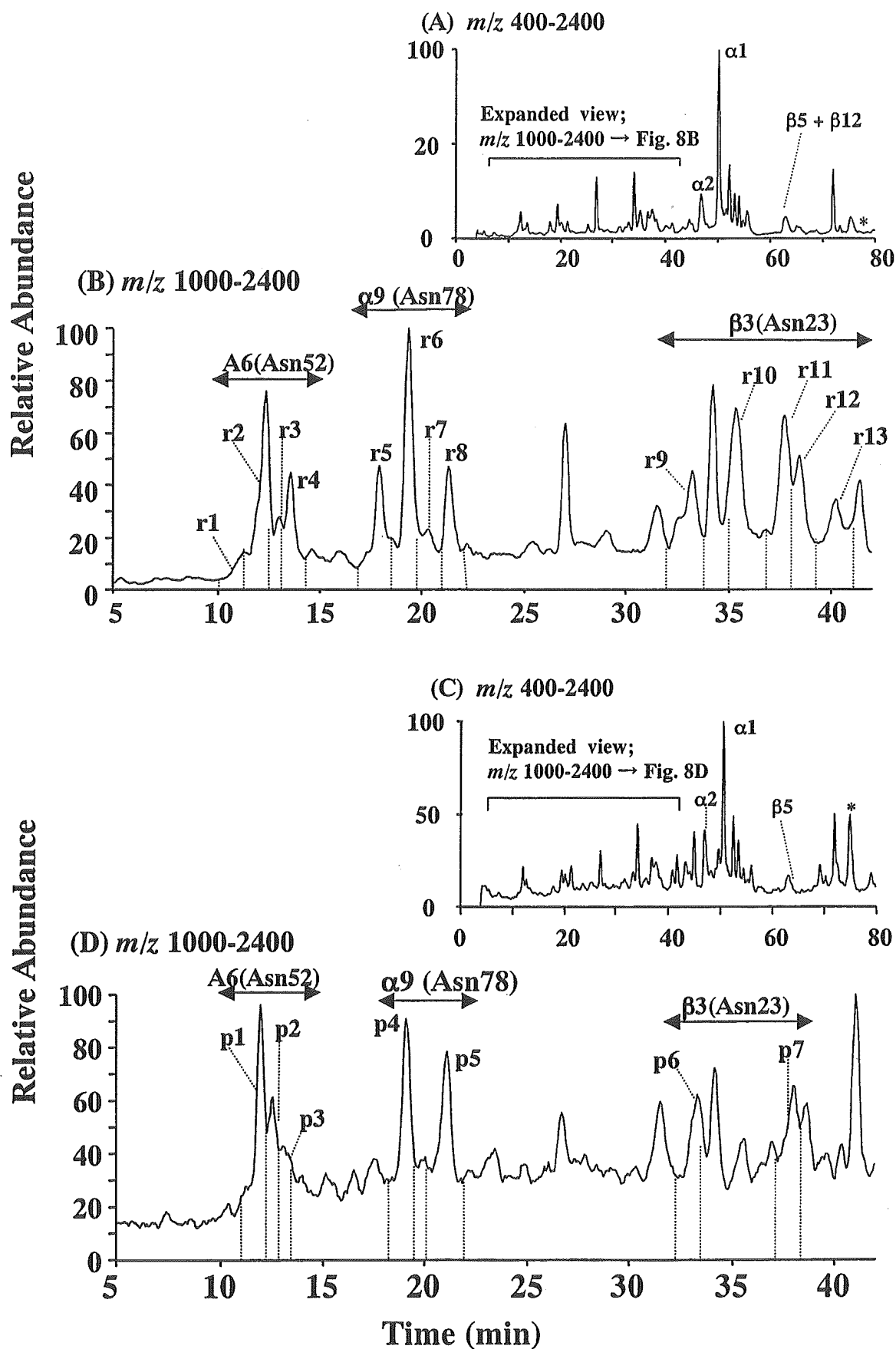


Fig. 16 Peptide/glycopeptide maps of rTSH (A) and pTSH (C), and Glycopeptide maps of rTSH (B) and pTSH (D)



Table 11 Peptides and carbohydrate compositions of peaks in Fig. 16B

Peak No.	Glyco-peptides	Carbohydrate composition <sup>a</sup>	Deduced carbohydrate structure <sup>b</sup>	Observed $m/z$			
				Theoretical mass <sup>c</sup>	$M^{2+}$	$M^{3+}$	$M^{4+}$
r1	$\alpha 6(\text{Asn}52)$	[Hex] <sub>9</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>3</sub>	Hybrid (7)NA <sub>3</sub> , Hybrid (1)Lac <sub>1</sub> NA <sub>3</sub>	4,503.2	1,925.1	1,501.6	
r2	$\alpha 6(\text{Asn}52)$	[Hex] <sub>8</sub> [HexNAc] <sub>3</sub> [NeuAc] <sub>2</sub>	Hybrid (1)NA <sub>2</sub>	3,846.6		1,283.4	
		[Hex] <sub>6</sub> [HexNAc] <sub>3</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	4,220.0		1,407.8	
r3	$\alpha 6(\text{Asn}52)$	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	3,563.4	1,782.0	1,188.6	
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac <sub>1</sub> NA <sub>2</sub>	3,928.8	1,965.2	1,310.9	
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBiNA <sub>2</sub>	3,709.6	1,855.2	1,237.5	
r4	$\alpha 6(\text{Asn}52)$	[Hex] <sub>7</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	Hybrid (8)NA <sub>2</sub> , Hybrid (4)Lac <sub>1</sub> NA <sub>2</sub> , Hybrid (2)Lac <sub>2</sub> NA <sub>2</sub>	4,090.9		1,365.1	
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	3,272.2	1,636.6	1,091.4	
r5	$\alpha 9(\text{Asn}78)$	[Hex] <sub>6</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	Hybrid (4)NA <sub>1</sub> , Hybrid (2)Lac <sub>1</sub> NA <sub>1</sub>	3,434.3	1,717.4	1,145.4	
		[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	Bi(1)GN <sub>2</sub> NA <sub>2</sub>	3,604.5	1,803.1	1,202.9	
r6	$\alpha 9(\text{Asn}78)$	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	TriNA <sub>3</sub>	4,932.7		1,644.7	1,233.9
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>1</sub>	FBiGN <sub>1</sub> NA <sub>1</sub>	4,334.2		1,445.1	
r7	$\alpha 9(\text{Asn}78)$	[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	BiNA <sub>2</sub>	4,276.1	2,139.3	1,426.0	1,069.8
		[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	TriNA <sub>2</sub> , BiLac <sub>1</sub> NA <sub>2</sub>	4,641.5		1,547.9	1,160.9
r8	$\alpha 9(\text{Asn}78)$	[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	Bi(1)GN <sub>2</sub> NA <sub>2</sub>	4,317.2		1,440.1	1,080.1
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	BiNA <sub>1</sub>	3,984.9	1,992.8	1,329.4	
r9	$\beta 3(\text{Asn}23)$	[Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>1</sub>	TriNA <sub>1</sub> , BiLac <sub>1</sub> NA <sub>1</sub>	4,350.2		1,450.6	
		[Fuc][Hex] <sub>7</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>4</sub>	FTetraNA <sub>4</sub>	6,128.0		2,043.1	1,532.7
r10	$\beta 3(\text{Asn}23)$	[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>4</sub>	FBiGN <sub>1</sub> NA <sub>4</sub>	5,600.6		1,868.0	
		[Fuc][Hex] <sub>8</sub> [HexNAc] <sub>7</sub> [NeuAc] <sub>4</sub>	FTetraLac <sub>1</sub> NA <sub>4</sub>	6,493.4		2,164.6	1,624.2
		[Fuc][Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	FTriNA <sub>3</sub>	5,471.5		1,824.8	1,368.5
		[Fuc][Hex] <sub>7</sub> [HexNAc] <sub>6</sub> [NeuAc] <sub>3</sub>	FTetraNA <sub>3</sub> , FTriLac <sub>1</sub> NA <sub>3</sub>	5,836.8		1,946.1	
r11	$\beta 3(\text{Asn}23)$	[Fuc][Hex] <sub>4</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>3</sub>	FBi(1)GN <sub>1</sub> NA <sub>3</sub>	4,944.0		1,649.1	
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>3</sub>	FBiGN <sub>1</sub> NA <sub>3</sub>	5,309.3		1,771.5	
r12	$\beta 3(\text{Asn}23)$	[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBiNA <sub>2</sub>	4,814.9		1,606.0	1,205.1
		[Fuc][Hex] <sub>6</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	FTriNA <sub>2</sub> , FBiLac <sub>1</sub> NA <sub>2</sub>	5,180.2		1,727.7	
r13	$\beta 3(\text{Asn}23)$	[Fuc][Hex] <sub>3</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	FCore GN <sub>1</sub> NA <sub>2</sub>	4,287.4	2,145.2	1,430.3	
		[Fuc][Hex] <sub>4</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBi(1)GN <sub>1</sub> NA <sub>2</sub>	4,652.7		1,552.7	
r13	$\beta 3(\text{Asn}23)$	[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>1</sub>	FBiNA <sub>1</sub>	4,523.6	2,263.5	1,508.7	
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBiNA <sub>2</sub>	4,814.9		1,606.0	

<sup>a</sup> See Table 8. <sup>b</sup> See Table 13. <sup>c</sup> average mass value.

**Table 12 Peptides and carbohydrate compositions of peaks in Fig. 16D**

Peak No.	Glyco-peptides	Deduced			Observed $m/z$	
		Carbohydrate composition <sup>a</sup>	carbohydrate composition <sup>b</sup>	Theoretical mass <sup>c</sup>	$M^{2+}$	$M^{3+}$
p1	$\alpha 6(\text{Asn}52)$	[Hex] <sub>3</sub> [HexNAc] <sub>6</sub> [SO <sub>3</sub> ] <sub>2</sub>	Bi(3)(SO <sub>3</sub> ) <sub>2</sub>	3,223.1	1,612.4	1,075.6
		[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>1</sub>	Bi(2)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub>	3,393.3	1,696.9	1,132.1
		[Hex] <sub>3</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>2</sub>	Bi(4)GN <sub>1</sub> (SO <sub>3</sub> ) <sub>2</sub>	3,019.9	1,510.8	
p2	$\alpha 6(\text{Asn}52)$	[Hex] <sub>4</sub> [HexNAc] <sub>4</sub> [SO <sub>3</sub> ] <sub>1</sub>	Hybrid(2)(SO <sub>3</sub> ) <sub>1</sub>	2,898.8	1,450.1	
		[Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [SO <sub>3</sub> ] <sub>1</sub>	Hybrid(3)(SO <sub>3</sub> ) <sub>1</sub>	3,061.0	1,531.1	
		[Hex] <sub>3</sub> [HexNAc] <sub>4</sub> [SO <sub>3</sub> ] <sub>1</sub>	Bi(4)(SO <sub>3</sub> ) <sub>1</sub>	2,736.7	1,368.7	
p3	$\alpha 6(\text{Asn}52)$	[Hex] <sub>3</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub>	Bi(4)GN <sub>1</sub> (SO <sub>3</sub> ) <sub>1</sub>	2,939.9	1,470.6	
		[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub>	Bi(2)(SO <sub>3</sub> ) <sub>1</sub> , Hybrid(2)GN <sub>1</sub> (SO <sub>3</sub> ) <sub>1</sub>	3,102.0	1,552.1	
		[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>1</sub>	Bi(2)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub>	4,106.0	2,053.8	1,369.7
p4	$\alpha 9(\text{Asn}78)$	[Hex] <sub>5</sub> [HexNAc] <sub>6</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>1</sub>	Tri(1)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub> , Bi(2)Lac(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub>	4,471.3	1,491.3	
		[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	Bi(2)NA <sub>2</sub>	4,317.2	1,440.6	
		[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>2</sub>	Bi(2)(SO <sub>3</sub> ) <sub>2</sub>	3,894.8	1,298.9	
p5	$\alpha 9(\text{Asn}78)$	[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub>	Bi(2)(SO <sub>3</sub> ) <sub>1</sub>	3,814.7	1,271.8	
		[Hex] <sub>3</sub> [HexNAc] <sub>6</sub> [SO <sub>3</sub> ] <sub>2</sub>	Bi(3)(SO <sub>3</sub> ) <sub>2</sub>	3,935.8	1,313.6	
		[Hex] <sub>6</sub> [HexNAc] <sub>7</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>1</sub>	Tri(1)Lac <sub>1</sub> (SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub> , Tetra(1)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub>	4,836.6	1,613.0	
p6	$\beta 3(\text{N}23)$	[Hex] <sub>3</sub> [HexNAc] <sub>4</sub> [SO <sub>3</sub> ] <sub>1</sub>	Bi(4)(SO <sub>3</sub> ) <sub>1</sub>	3,449.4	1,726.0	1,150.9
		[Hex] <sub>3</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub>	Bi(5)(SO <sub>3</sub> ) <sub>1</sub>	3,652.6	1,827.0	1,219.1
		[Fuc][Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub>	FBi(2)(SO <sub>3</sub> ) <sub>1</sub> , FHybrid(2)GN <sub>1</sub> (SO <sub>3</sub> ) <sub>1</sub>	3,960.9	1,321.4	
p7	$\beta 3(\text{N}23)$	[Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub>	Bi(2)(SO <sub>3</sub> ) <sub>1</sub> , Hybrid(2)GN <sub>1</sub> (SO <sub>3</sub> ) <sub>1</sub>	3,814.7	1,273.0	
		[Hex] <sub>4</sub> [HexNAc] <sub>6</sub> [SO <sub>3</sub> ] <sub>2</sub>	Hybrid(5)(SO <sub>3</sub> ) <sub>2</sub>	4,490.6	1,497.3	
		[Hex] <sub>5</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>1</sub>	Hybrid(6)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub>	4,660.7	1,554.2	
p8	$\beta 3(\text{N}23)$	[Fuc][Hex] <sub>3</sub> [HexNAc] <sub>6</sub> [SO <sub>3</sub> ] <sub>2</sub>	FBi(3)(SO <sub>3</sub> ) <sub>2</sub>	4,474.6	1,492.3	
		[Fuc][Hex] <sub>3</sub> [HexNAc] <sub>6</sub> [SO <sub>3</sub> ] <sub>1</sub>	FBi(3)(SO <sub>3</sub> ) <sub>1</sub>	4,394.5	1,465.6	
		[Fuc][Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>1</sub>	FBi(2)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub>	4,644.7	1,548.8	
p9	$\beta 3(\text{N}23)$	[Fuc][Hex] <sub>4</sub> [HexNAc] <sub>5</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>2</sub>	FBi(2)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>2</sub>	4,936.0	1,645.6	
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>4</sub> [NeuAc] <sub>2</sub>	FBINA <sub>2</sub>	4,814.9	1,606.5	
		[Fuc][Hex] <sub>5</sub> [HexNAc] <sub>6</sub> [SO <sub>3</sub> ] <sub>1</sub> [NeuAc] <sub>1</sub>	FTri(1)(SO <sub>3</sub> ) <sub>1</sub> NA <sub>1</sub>	5,010.1	1,671.4	
p10	$\beta 3(\text{N}23)$	[Fuc][Hex] <sub>3</sub> [HexNAc] <sub>4</sub> [SO <sub>3</sub> ] <sub>1</sub>	FBi(4)(SO <sub>3</sub> ) <sub>1</sub>	3,988.1	1,330.5	
		[Fuc][Hex] <sub>3</sub> [HexNAc] <sub>5</sub> [NeuAc] <sub>2</sub>	FCoreGN <sub>1</sub> NA <sub>2</sub>	4,287.4	1,430.1	

<sup>a</sup> See Table 8, <sup>b</sup> See Table 13, <sup>c</sup> average mass value.

**Table 13** Deduced carbohydrate structures presented in Table 5, 6, 8, and 10-12

Abbreviation <sup>a</sup>	Structure <sup>b</sup>
Hybrid(1)	
Hybrid(2) [FHybrid(2)]	
Hybrid(3)	
Hybrid(4)	
Hybrid(5)	
Hybrid(6)	
Hybrid(7)	
Hybrid(8)	
FCore	
Bi [FBi]	
Bi(1) [FBi(1)]	
Bi(2) [FBi(2)]	
Bi(3) [FBi(3)]	
Bi(4) [FBi(4)]	
Bi(5) [FBi(5)]	
Tri [FTri]	
Tri(1) [FTri(1)]	
FTetra	
Tetra (1)	

<sup>a</sup> F, fucose; Bi, biantennary; Tri, triantennary; Tetra, tetraantennary.

<sup>b</sup> Man, mannose; Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine.

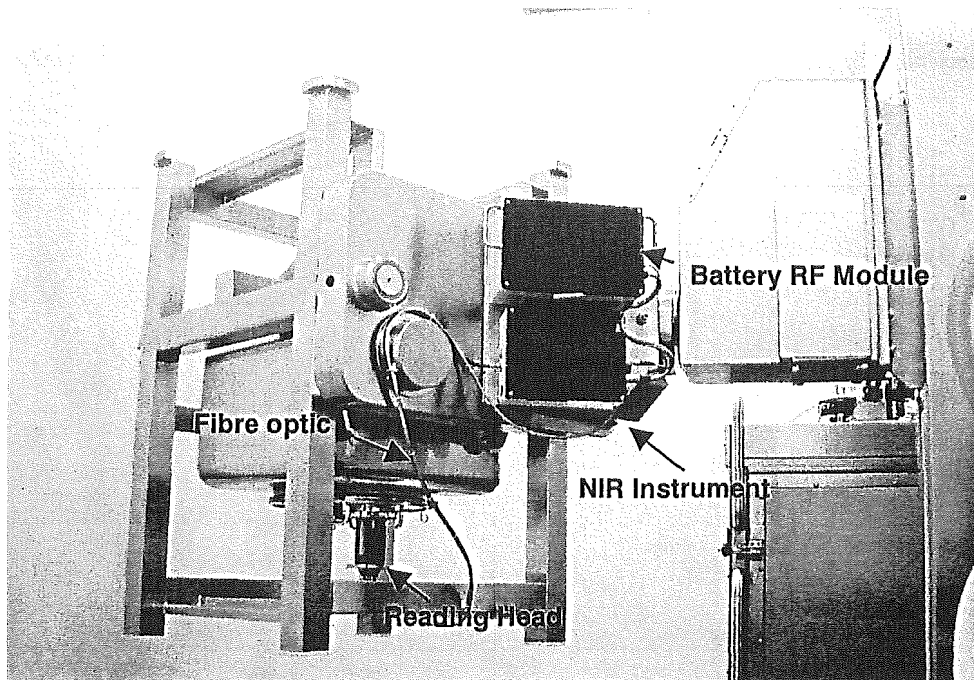


Fig. 17 On- line NIR bin blender

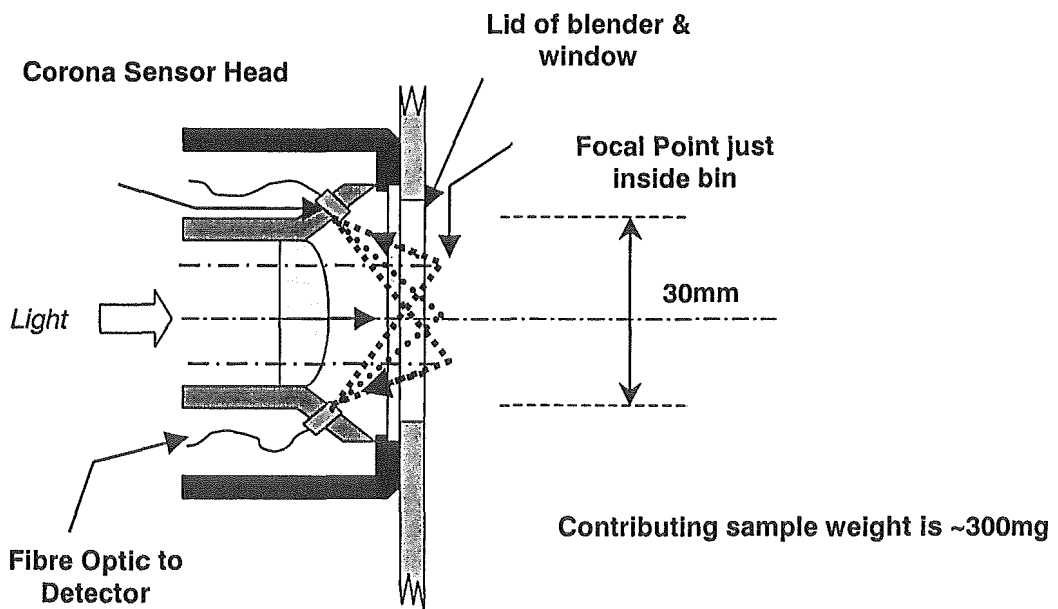


Fig. 18 Corona Sensor Head