Supporting Information

Asymmetrical Bi-antennary Glycans Prepared by a Stop-and-Go Strategy Reveal Receptor Binding Evolution of Human Influenza A Viruses

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Table of Contents Page

1. Preparation of compound 9

1.1 Materials and methods

Chemicals and solvents were purchased from Sigma-Aldrich unless noted otherwise. Pronase from *Streptomyces griseus* (#P5147) was also purchased from Sigma-Aldrich. Egg yolk powder was purchased from Natural Foods Inc, Toledo, OH (#40504) and stored at 4 ℃ before used. Active carbon, NORIT™ SA 2, decolorizing grade, catalog 40403 and Celite® 545 was purchased from Acros. *Clostridium perfringens* Neuraminidase (#P0720), *Flavobacterium meningosepticum* PNGase F (#P0704) and *Streptococcus pneumoniae* β-N-Acetylglucosaminidase S (#P0744S) were obtained from New England Biolabs. *Aspergillus niger* Galactosidase (#E-BGLAN) was purchased from Megazyme.

1.2 Sialyl glycopeptide (SGP) preparation

SGP was extracted, isolated and purified according to our previously reported method.[1,2] Briefly, commercialized egg yolk powder (2.27 Kg) was suspended twice in 95% ethanol (4 L) and mechanically stirred for 2 h at room temperature to remove lipids and other organic soluble components. The filtrate was discarded and the insoluble powder was suspended twice in aqueous ethanol (40% *v/v* ethanol, 3 L). The insoluble material was discarded and the filtrate was concentrated under reduced pressure at 40 °C. The collected liquid was concentrated *in vacuo* and purified using an active carbon/celite column (200 g of active carbon and 600 g celite). Impurities were removed by flushing the column with 3 L of water (trifluoroacetic acid (TFA) 0.1% *v/v*), 3 L of 5% acetonitrile in water (TFA, 0.1% *v/v*), and 3 L 10% acetonitrile in water (TFA, 0.1% *v/v*). The desired SGP was released from the column using a solution of 25% acetonitrile in water (TFA, 0.1% *v/v*), and fractions containing the product were pooled and concentraded *in vacuo*. The resulting crude SGP was subjected to size-exclusion chromatography (Bio-Rad® P-2, fine particle size 45 – 90 μm, column dimensions 5.0 cm x 80 cm, 250 mL fractions) eluting with 0.1 M ammonium bicarbonate to yield pure SGP (**5**) as a fluffy, white powder after be evaporated and lyophilized (1.6 g, or 0.7 mg SGP/g egg yolk powder). Additional P-2 bio-gel column purification was applied when necessary to obtain highly pure SGP.

1.3 Trimming and modification of SGP to prepare compound 9

SGP (500 mg) was dissolved in 50 mM sodium acetate buffer (5 mL, pH 5.5). 5 mM $CaCl_2$ and 40 μL *Clostridium perfringens* neuraminidase were added to reaction mixture solution and then the reaction was incubated overnight at 37 °C with shaking. When ESI-MS indicated all the sialic acid residues had been removed, the pH of the reaction mixture was adjusted to 4.5 with acetic acid. After that, 5 mg BSA and 200 μL β-galactosidase from *Aspergillus niger* were successively added. The resulting reaction mixture was incubated overnight with shaking at 37 °C. Additional 100 μL β-galactosidase will be needed if a spot of substrates were observed via ESI-MS. There are no any substrates in reaction solution and full galactose removal was monitored by ESI-MS, after which an equal volume of cooled alcohol was added to precipitate protein residuals. The result solution was concentrated *in vacuo* and loaded into P-2 Bio-gel size-exclusion chromatography eluting with 0.1 M ammonium bicarbonate buffer. The

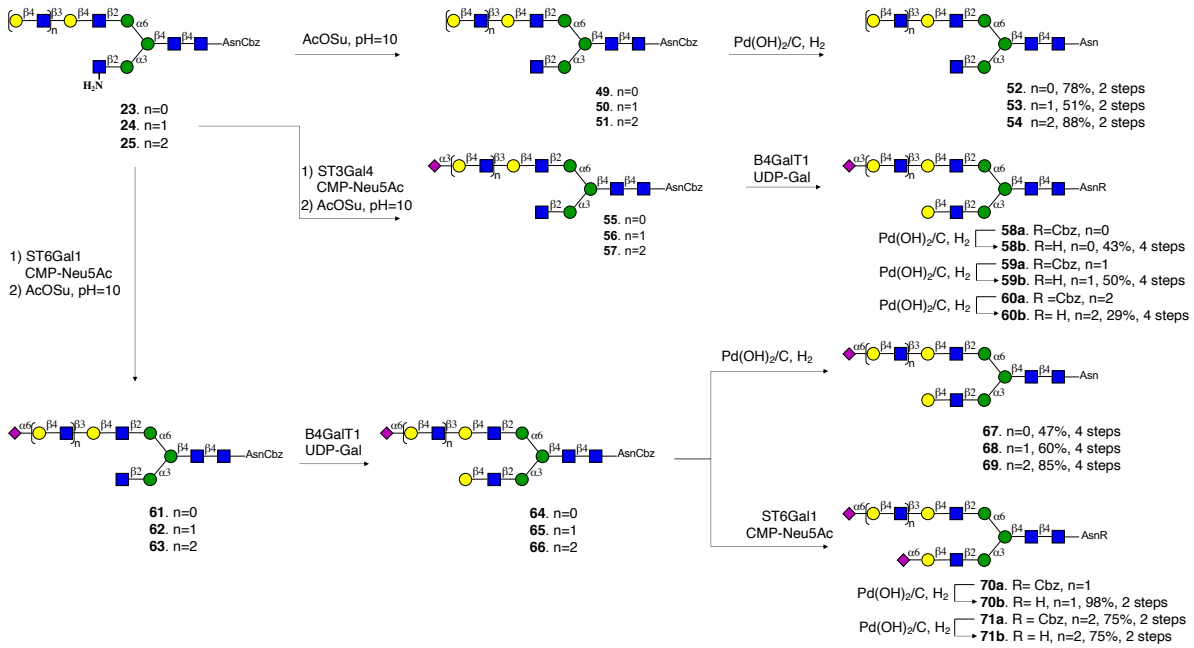
fractions containing product were collected and lyophilized. Finally, compound **17** was obtained as white amorphous powder (265 mg, 77% over 2 steps).

Compound **17** (265 mg, 0.14 mmol) was dissolved in 100 mM tris buffer with 5 mM $CaCl_2$. Pronase (130 mg) was added keeping a 1 mg/mL final concentration. The reaction was incubated overnight at 37 °C with shaking. The reaction was monitored by ESI-MS and an additional 50 mg of pronase was added an incubation continued for 8 h if partial product formation was observed. Finally, an equal volume of cooled (0 ºC) alcohol was added to precipitate residual protein. The resulting solution was concentrated *in vacuo* and loaded into P-2 Bio-gel size-exclusion chromatography which was eluting with 0.1 M ammonium bicarbonate buffer. The fractions containing product were collected, lyophilized and dissolved in water (5 mL). Next, K_2CO_3 (1.5 eq), and Cbz-Cl (1.5 eq) were added successively. Subsequently, the mixed solution was incubated at 37 °C with shaking until ESI-MS indicated complete installation of the Cbz group. The reaction mixture was diluted using water (20 mL) and extracted twice with ethyl acetate (30 mL). The aqueous phase was concentrated *in vacuo* and loaded into P-2 Bio-gel size-exclusion chromatography, which was eluted with 0.1 M ammonium bicarbonate buffer. The fractions containing product were collected and lyophilized giving 140 mg of **19** as a white powder (66% over 2 steps).

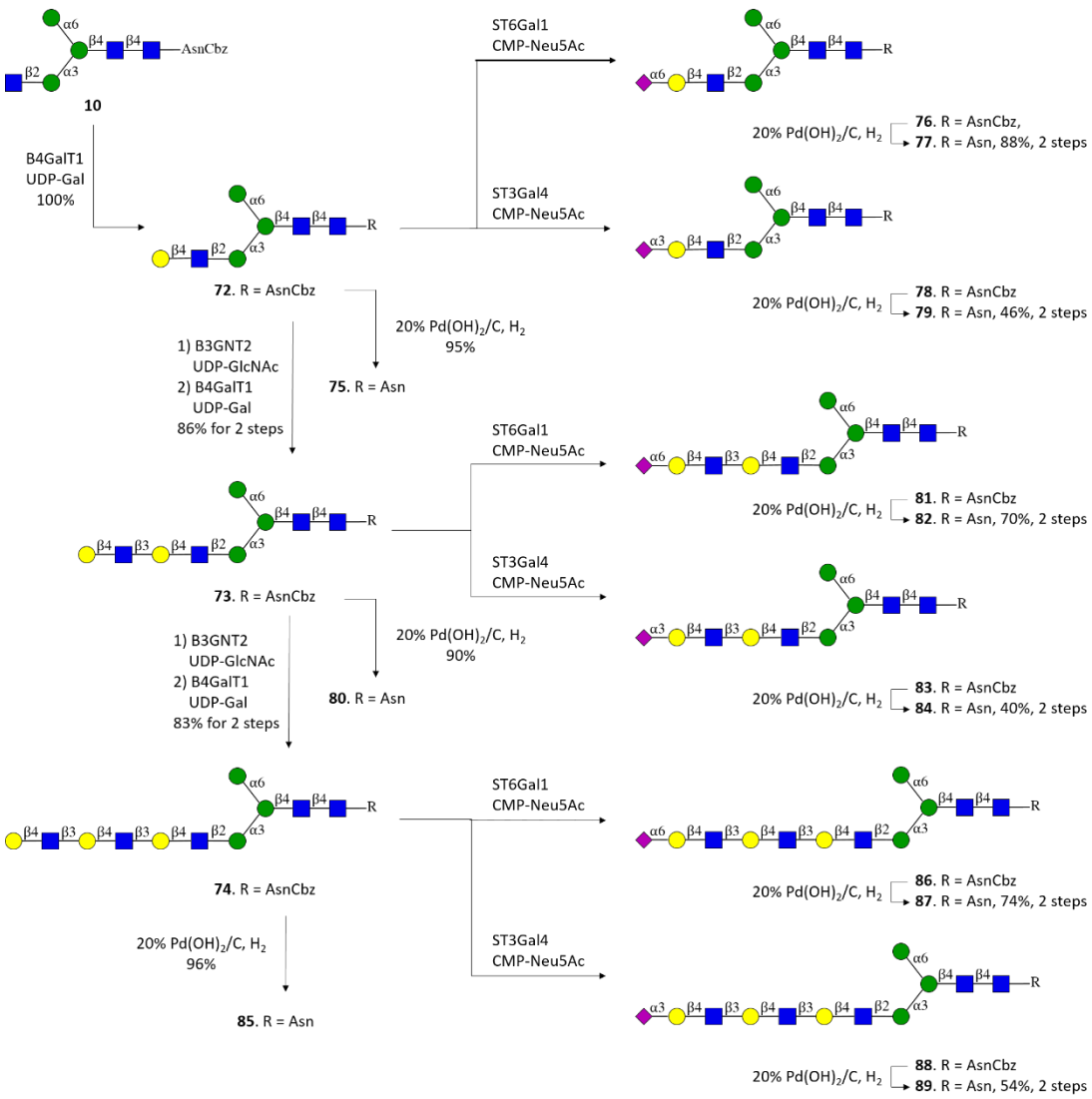
Compound **19** (140 mg, 0.09 mmol) was dissolved in 5 mL of 100 mM Tris-HCl buffer (pH = 8.0). 5 mM $CaCl_2$ and 50 U β-Acetylglucosaminidase S were added to reaction solution. The resulting reaction mixture was incubated overnight with shaking at 37 °C. The progress of the reaction was monitored by ESI-MS until full conversion. As a result, an equal volume of cooled alcohol was added to precipitate protein residuals. The result solution was concentrated *in vacuo* and loaded into P-2 Bio-gel size-exclusion chromatography eluting with 0.1 M ammonium bicarbonate buffer. The fractions containing product were collected and lyophilized. Compound **9** was obtained as white powder (86 mg, 83%).

2. Enzymatic synthesis

2.1 Schemes S1 and S2

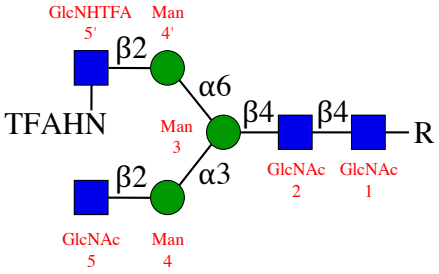


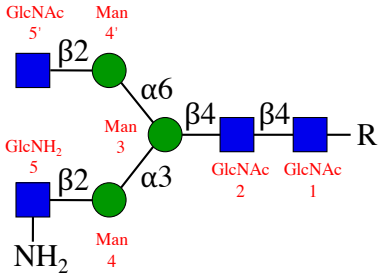
Scheme S1. Selective modification of termini at the α(1,3)- and α(1,6)-antennae starting for compounds **23**-**25**.

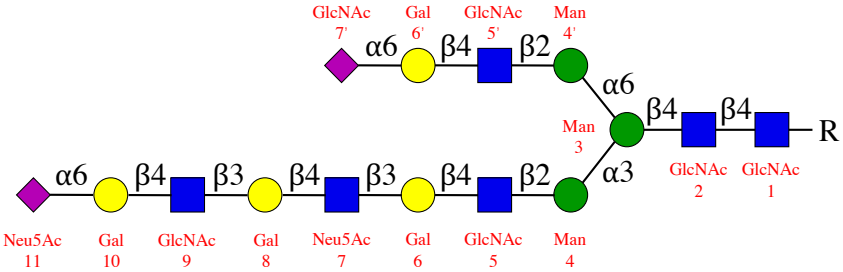


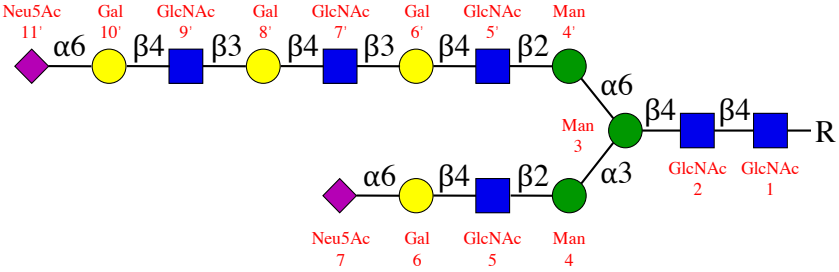
Scheme S2. Selective modification of termini of the α(1,3)-arm starting from **72**, **73** and **74**.

2.2 NMR nomenclature





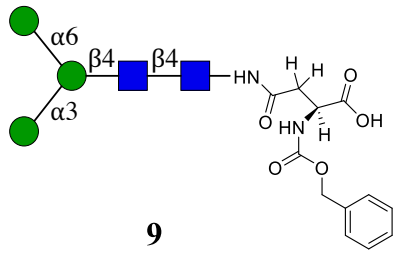




R = Asn or AsnCbz

2.3 Enzymatic reactions

Compound 9[1,2]



9

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.7 Hz, 1H)	3.82	3.73	3.64	3.54	3.80, 3.62
GlcNAc2	4.60 (d, *J* = 8.0 Hz, 1H)	3.79	3.79	3.72	3.60	3.87, 3.74
Man3	4.78 (s, 1H)	4.26 (d, *J* = 2.4 Hz, 1H)	3.76	3.76	3.65	3.92, 3.80
Man4	5.10	4.07 (dd, *J* = 3.4, 1.7 Hz, 1H)	3.88	3.63	3.80	3.91, 3.73
Man4'	4.91 (d, *J* = 1.7 Hz, 1H)	3.97 (dd, *J* = 3.5, 1.7 Hz, 1H)	3.88	3.64	3.64	3.88, 3.76

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.03	53.63	72.66	78.49	76.10	59.75
GlcNAc2	101.14	54.78	65.72	79.56	74.29	59.90
Man3	100.28	70.06	80.41	71.87	74.07	65.72
Man4	102.44	69.91	70.23	66.77	73.35	61.04
Man4'	99.51	69.78	70.31	66.69	72.58	60.86

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-[a]	174.68, 174.60
NHC(O)<u>CH_3</u>	2.07 (s, 3H), 1.91 (s, 3H)	22.09, 21.89
Aromatic	7.51 – 7.34 (m, 5H)	136.21, 128.67, 128.28, 127.73, 127.56
<u>CH_2</u>-Ph	5.19 – 5.08	66.93
NH-<u>C</u>OO-	-	157.70
NH-<u>CH</u>-COOH	4.38 (dd, *J* = 9.0, 4.3 Hz, 1H)	52.38
NH-CH-<u>C</u>OOH	-	176.86
C(O)-<u>CH_2</u>-CH	2.82 (dd, *J* = 15.6, 4.3 Hz, 1H), 2.63 (dd, *J* = 15.6, 9.1 Hz, 1H)	38.17

C(O)-CH$_2$-CH	-	173.36

[a] Not applicable

ESI TOF-MS *m/z* calculated for $C_{46}H_{69}N_4O_{30}$, $[M-H]^-$: 1157.4002, found 1157.3935.

Characterization of compound 9

Initial examination of the ^{13}C-^{1}H HSQC (Fig. S1) illustrated the proton signals of the anomeric region, the H_2 of Man 3, 4 & 4', the Asn and the Cbz group. Especially, five unique signals in the anomeric region could clearly be assigned, which corresponded to five monosaccharides (Fig. S2). After the H_1 of Man 3, 4 and 4' were confirmed, the COSY data led us to distinguish each H_2 of three mannosides. As shown in Fig. S3, the H_1 of Man 3 have a neighboring correlation with the H_2 from the Man 3 spin system. The cross peak at 4.36 ppm correspond to the H_2 of Man 3. Similarly, the H_2 of Man 4 and 4' are observed at 4.07ppm and 3.97 ppm, respectively.

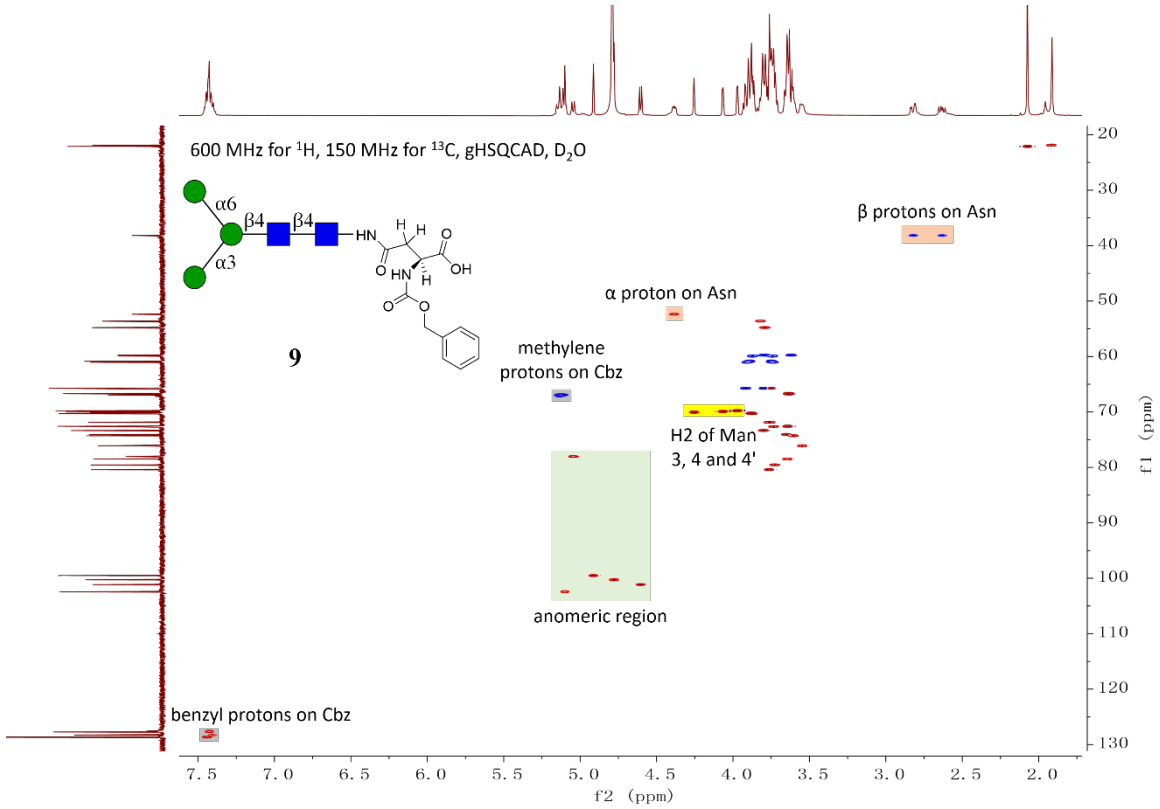


Figure S1. HSQC for compound **9**.

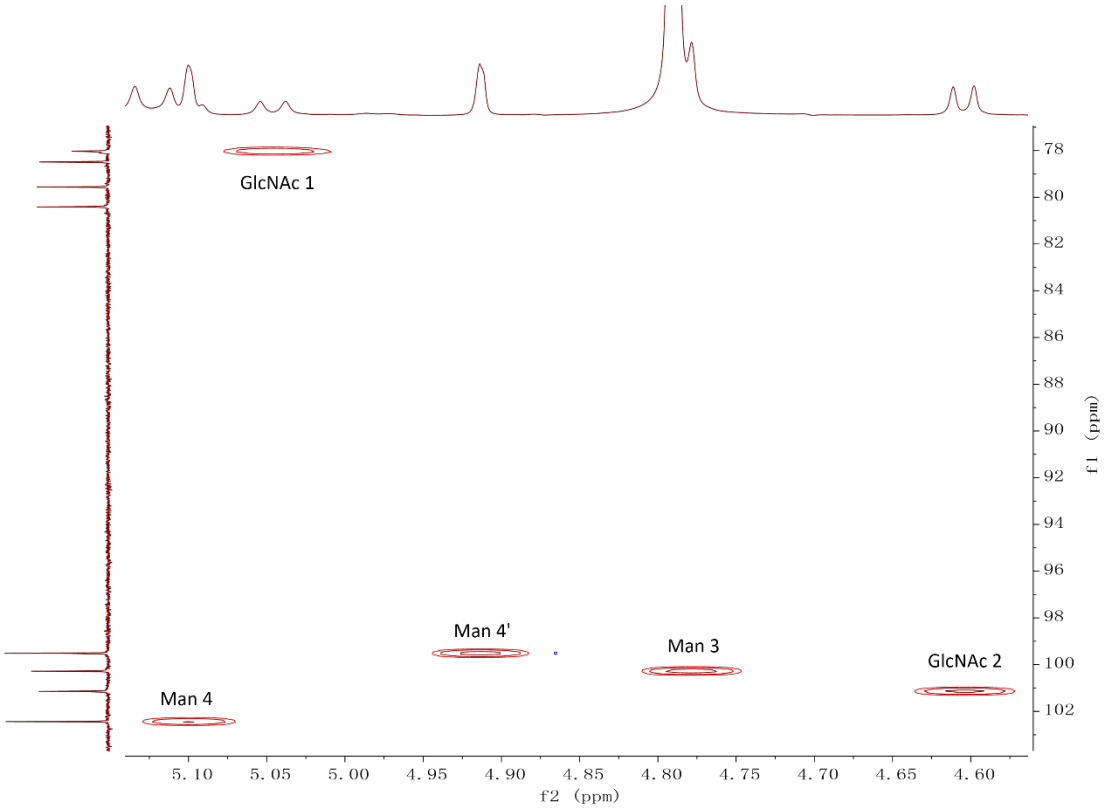


Figure S2. Expanded HSQC for anomeric region of compound **9**.

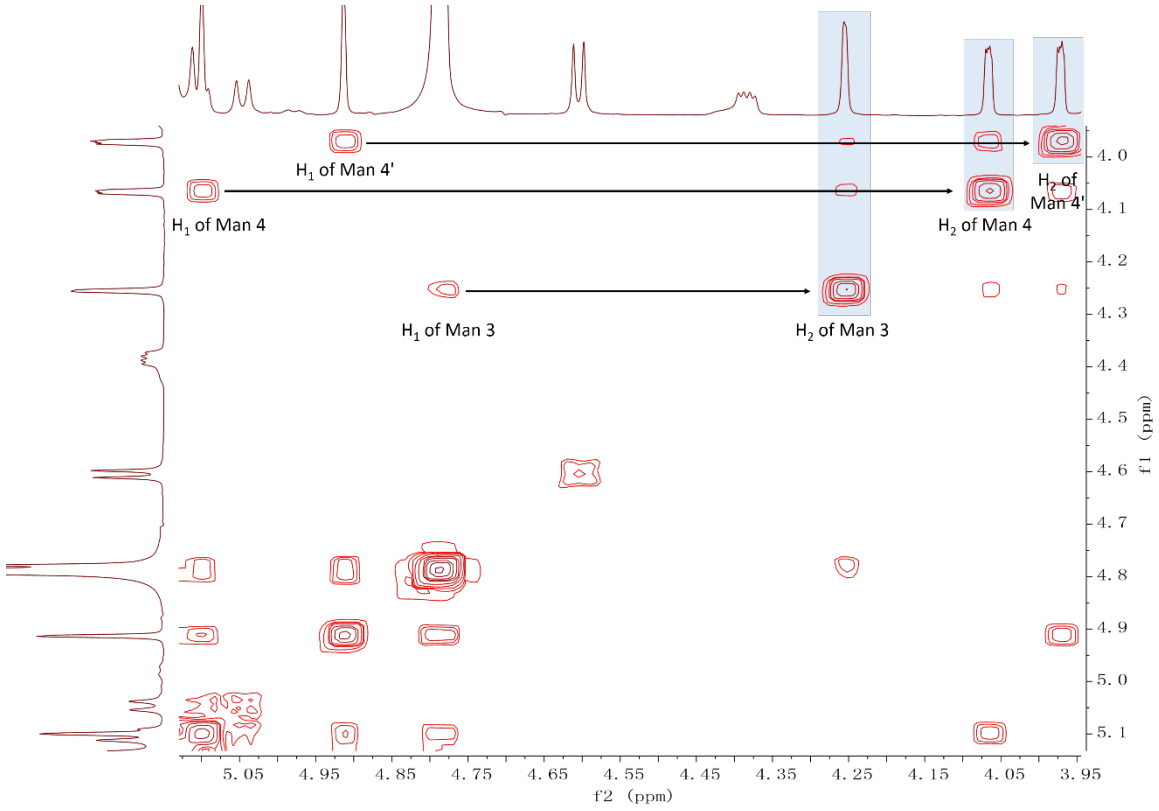


Figure S3. Expanded COSY for Mannose $H_{1\&2}$ region of compound **9**.

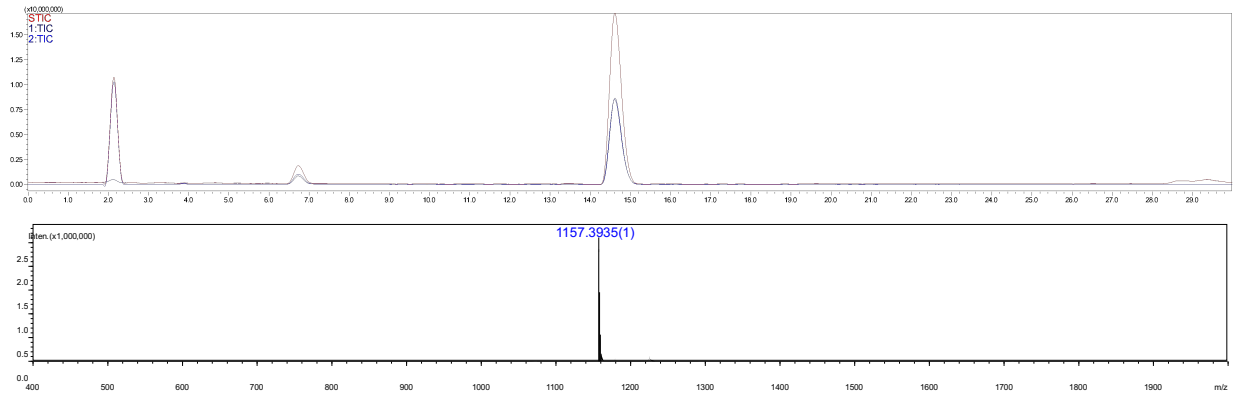
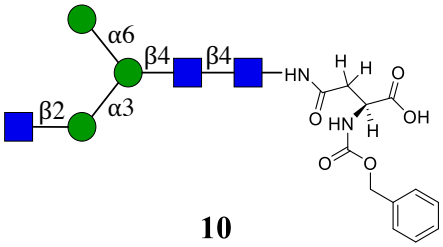


Figure S4. Analytical HPLC-MS chromatogram of compound **9**. The retention time = 14.6 min.

Compound 10

10 was synthesized from starting material **9** (10 mg) according to the general procedure **2.2 a** for the installation of GlcNAc moiety with UDP-GlcNAc and recombinant MGAT 1. The product was purified using a combined purification system **(2.2 j)** providing **10** as a white fluffy solid (10.7 mg, 91%).



10

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.7 Hz, 1H)	3.82	3.74	3.65	3.55	3.80, 3.62
GlcNAc2	4.61 (d, *J* = 8.0 Hz, 1H)	3.79	3.75	3.74	3.60	3.88, 3.75
Man3	4.78 (s, 1H)	4.25 (d, *J* = 2.5 Hz, 1H)	3.77	3.76	3.66	3.92, 3.80
Man4	5.17 – 5.09	4.19 (dd, *J* = 3.3, 1.6 Hz, 1H)	3.90	3.50	3.74	3.93, 3.62
Man4'	4.92 (d, *J* = 1.8 Hz, 1H)	3.97 (dd, *J* = 3.4, 1.7 Hz, 1H)	3.88	3.64	3.64	3.89, 3.75
GlcNAc5	4.55 (d, *J* = 8.5 Hz, 1H)	3.70	3.56	3.45	3.44	3.91, 3.76

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.05	53.65	72.63	78.48	76.11	59.75
GlcNAc2	101.15	54.77	65.80	79.58	74.26	59.86
Man3	100.31	70.10	80.29	71.87	74.06	65.75
Man4	99.53	76.32	69.30	67.19	73.46	61.61
Man4'	99.52	69.78	70.30	66.67	72.59	60.86
GlcNAc5	99.50	55.22	73.17	69.81	75.72	60.53

Signal	Proton	Carbon
NHC(O)CH_3	-[a]	174.67, 174.61
NHC(O)CH_3	2.08 (s, 3H), 2.05 (s, 3H), 1.92 (s, 3H)	22.23, 22.09, 21.88
Aromatic	7.57 – 7.29 (m, 5H)	136.18, 128.67, 128.29, 127.69
CH_2-Ph	5.17 – 5.09 (m, 2H)	67.00
NH-COO-	-	157.72
NH-CH-COOH	4.45 (dd, J = 8.6, 4.6 Hz, 1H)	51.79
NH-CH-COOH	-	176.07
C(O)-CH_2-CH	2.84 (dd, J = 15.7, 4.5 Hz, 1H), 2.69 (dd, J = 15.7, 8.6 Hz, 1H)	37.84
C(O)-CH_2-CH	-	173.11

[a] Not applicable

ESI TOF-MS *m/z* calculated for $C_{54}H_{82}N_5O_{35}$ $[M-H]^-$: 1360.4796, found 1360.4693.

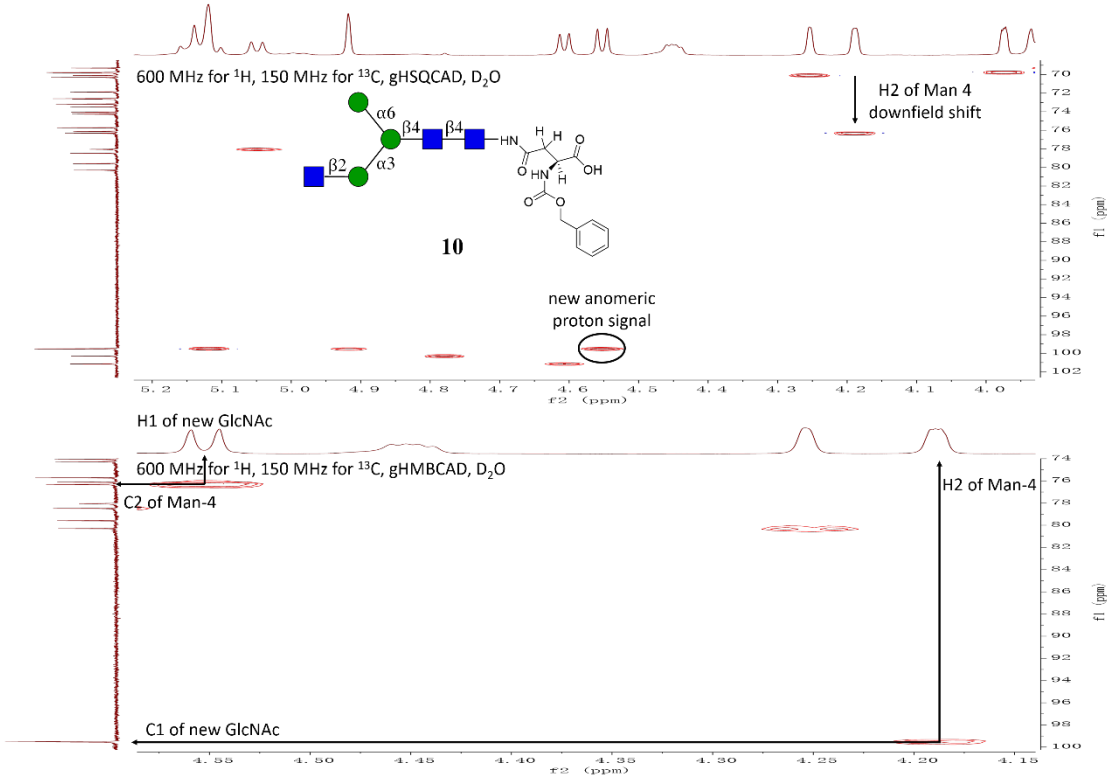
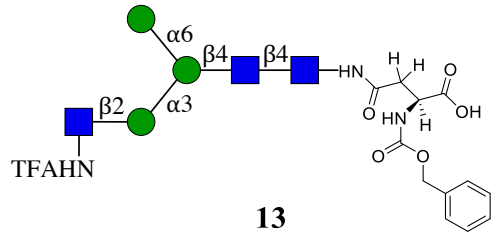


Figure S5. Expanded HSQC and HMBC of compound **10**.

Compound 13

13 was synthesized from starting material **9** (10 mg, 1.0 eq) according to the general procedure **2.2 a** for the installation of GlcNTFA moiety with UDP-GlcNTFA and MGAT 1. The product was purified using the combined purification system **(2.2 j)** providing **13** as a white fluffy solid (9.8 mg, 80%).



13

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 9.7 Hz, 1H)	3.81	3.72	3.64	3.54	3.79, 3.61
GlcNAc2	4.60 (d, *J* = 8.0 Hz, 1H)	3.78	3.74	3.73	3.59	3.87, 3.74
Man3	4.77 (s, 1H)	4.24 (s, 1H)	3.75	3.76	3.65	3.91, 3.79
Man4	5.07 (s, 1H)	4.21 (m, 1H)	3.90	3.45	3.72	3.89, 3.56
Man4'	4.77 (s, 1H)	3.96 (m, 1H)	3.87	3.63	3.64	3.88, 3.74
GlcTFA5	4.67 (d, *J* = 8.3 Hz, 1H)	3.80	3.67	3.47	3.47	3.92, 3.76

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.01	54.77	72.67	78.47	76.09	59.75
GlcNAc2	101.15	54.77	65.78	79.59	74.25	59.81
Man3	100.33	70.05	80.27	71.87	74.03	65.73
Man4	99.36	76.27	69.16	67.31	73.51	61.52
Man4'	99.53	69.77	70.30	66.66	72.59	60.85
GlcTFA5	98.71	55.80	72.51	69.77	75.75	60.47

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-[a]	174.68, 174.60
NHC(O)<u>CH_3</u>	2.07 (s, 3H), 1.90 (s, 3H)	22.08, 21.89
NH<u>C</u>(O)CF_3	-	159.63, 159.38 (d)
NHC(O)<u>CF_3</u>	-	116.69-111.38 (m)
Aromatic	7.48 – 7.36 (m, 5H)	136.23, 128.66, 128.27, 127.73

CH_2-Ph	5.16 – 5.09 (m, 2H)	66.60
NH-COO-	-	157.69
NH-CH-COOH	4.35 (dd, *J* = 8.1, 4.5 Hz, 1H)	52.66
NH-CH-COOH	-	177.23
C(O)-CH_2-CH	2.81 (dd, *J* = 15.3, 3.8 Hz, 1H), 2.60 (dd, *J* = 15.4, 9.4 Hz, 1H)	38.32
C(O)-CH_2-CH	-	173.49

[a] Not applicable

ESI TOF-MS *m/z* calculated for $C_{54}H_{79}F_3N_5O_{35}$, $[M-H]^-$: 1414.4513, found 1414.4509.

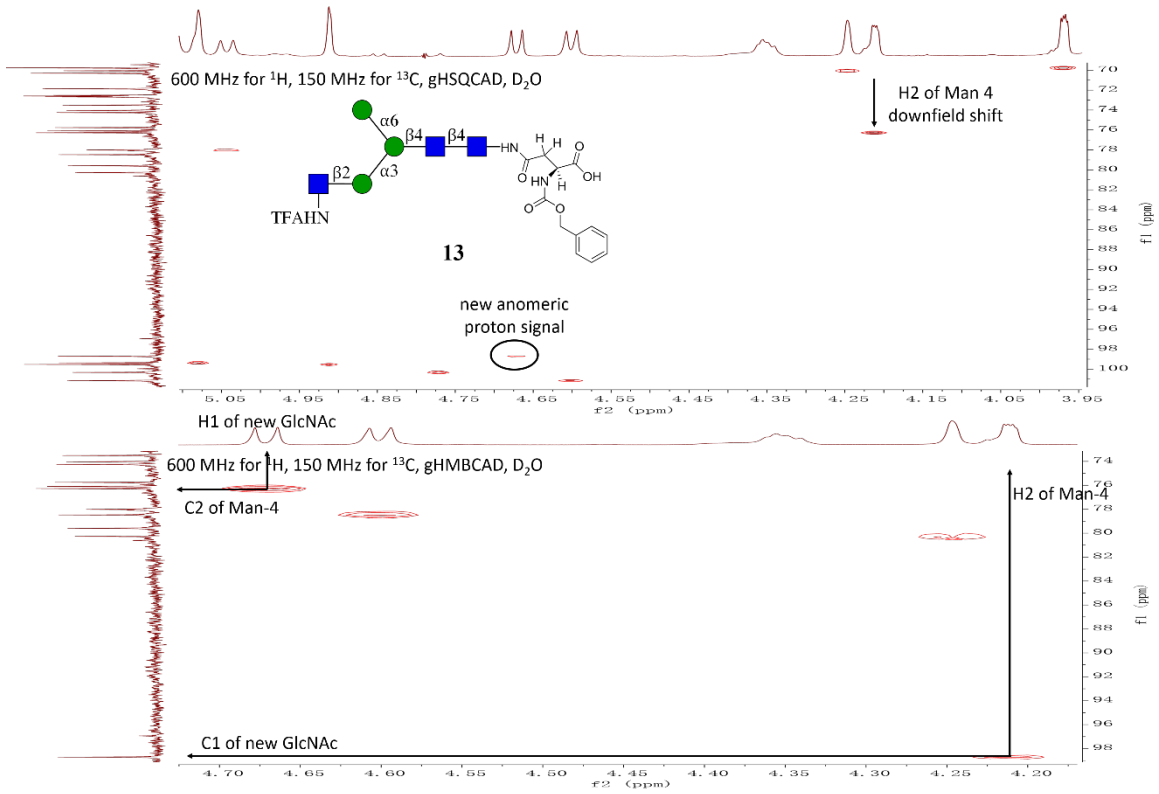
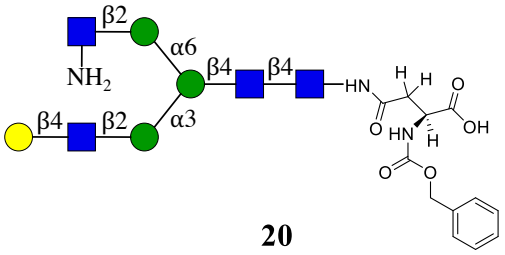


Figure S6. Expanded HSQC and HMBC of compound **13**.

Compound 20

Compound **10** (10.7 mg) was subjected procedures **2.2 b** to install GlcNTFA at the MGAT2 arm to provide intermediate **11** which was subjected to the purification protocol **2.2 j**. Compound **11** was subjected to the general procedures **2.2 g** and **c** for removal of the TFA moiety to give $GlcNH_2$ and galactosylation by B4GalT1. The product was purified using the described purification system **(2.2 j)** providing **20** as a white fluffy solid (11.0 mg, 83% yield over three steps).



20

NMR and MS analysis of compound **11**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 9.7 Hz, 1H)	3.81	3.73	3.64	3.55	3.79, 3.62
GlcNAc2	4.60 (d, *J* = 7.9 Hz, 1H)	3.78	3.77	3.72	3.60	3.86, 3.73
Man3	4.76 (s, 1H)	4.24 (d, *J* = 2.5 Hz, 1H)	3.76	3.75	3.73	3.94, 3.77
Man4	5.11 (s, 1H)	4.20 – 4.16 (m, 1H)	3.89	3.49	3.73	3.91, 3.60
Man4'	4.88 (s, 1H)	4.15 – 4.11 (m, 1H)	3.89	3.43	3.59	3.87, 3.56
GlcNAc5	4.55 (d, *J* = 8.4 Hz, 1H)	3.69	3.55	3.45	3.44	3.90, 3.75
GlcTFA5'	4.67 (d, *J* = 8.4 Hz, 1H)	3.80	3.65	3.49	3.44	3.92, 3.77

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.03	53.64	72.64	78.46	76.10	59.75
GlcNAc2	101.16	54.82	65.59	79.47	74.29	59.82
Man3	100.35	70.09	80.35	71.89	74.15	65.80
Man4	99.51	76.32	69.30	67.20	73.46	61.60
Man4'	96.81	76.24	69.24	67.39	72.80	61.48
GlcNAc5	99.51	55.23	73.17	69.81	75.72	60.53
GlcTFA5'	98.74	55.83	72.64	69.73	75.75	60.45

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-[a]	174.67, 174.55
NHC(O)<u>CH_3</u>	2.24 – 1.82 (m, 9H)	22.23, 22.09, 21.89
NH<u>C</u>(O)CF_3	-	159.62, 159.37
NHC(O)<u>CF_3</u>	-	116.69, 114.80
Aromatic	7.51 – 7.32 (m, 5H)	128.67, 128.28, 127.71
<u>CH_2</u>-Ph	5.16 – 5.07 (m, 2H)	66.95

NH-C̲OO-	-	157.70
NH-C̲H-COOH	4.42 – 4.38 (m, 1H)	52.20
NH-CH-C̲OOH	-	176.56
C(O)-C̲H$_2$-CH	2.82 (dd, *J* = 15.6, 4.4 Hz, 1H), 2.65 (dd, *J* = 15.5, 9.1 Hz, 1H)	38.06
C̲(O)-CH$_2$-CH	-	173.29

[a] Not applicable

ESI TOF-MS *m/z* calculated for $C_{62}H_{91}F_3N_6O_{40}$, $[M-2H]^{2-}$: 808.2617, found 808.2627.

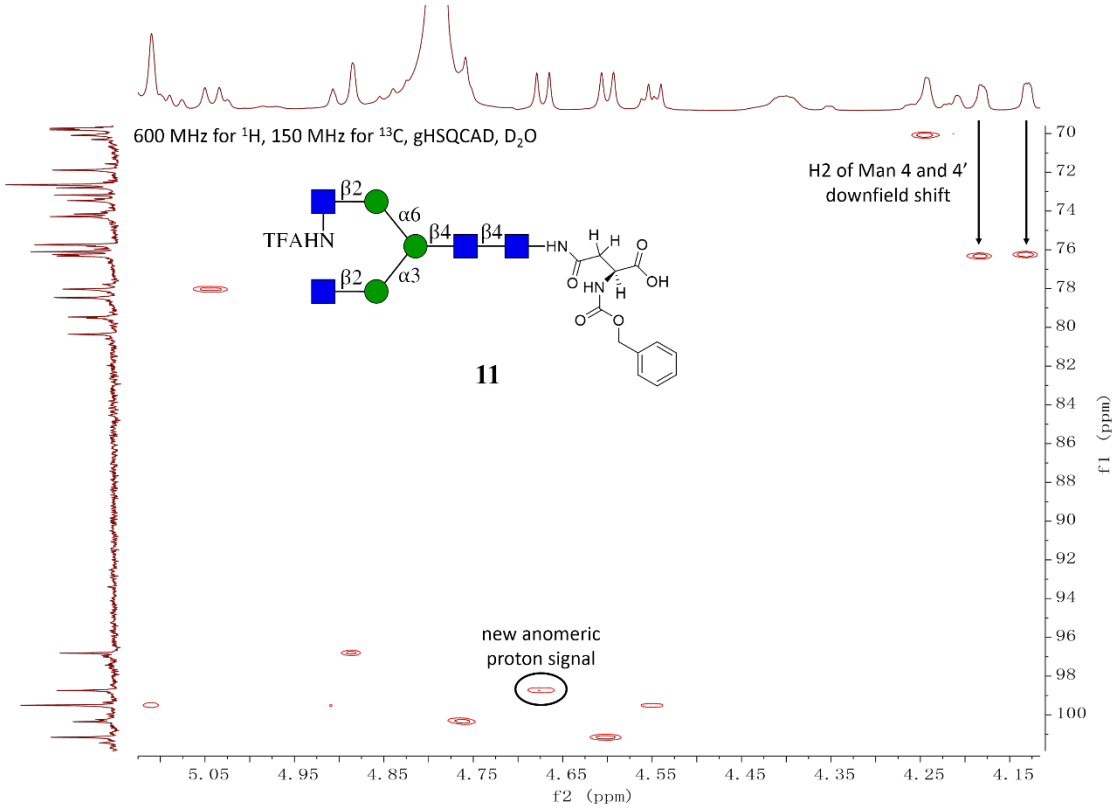


Figure S7. Expanded HSQC and HMBC of compound **11**.

NMR and MS analysis of compound **20**:

^{1}H (600 MHz, D$_2$O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04	3.82	3.73	3.64	3.55	3.79, 3.62
GlcNAc2	4.60	3.79	3.77	3.74	3.60	3.88, 3.75
Man3	4.77	4.26	3.77	3.77	3.64	3.96, 3.80

Man4	5.11	4.19 (d, *J* = 3.3 Hz, 1H)	3.90	3.50	3.75	3.93, 3.61
Man4'	5.03	4.26	3.96	3.70	3.66	3.87, 3.83
GlcNAc5	4.58	3.73	3.73	3.73	3.57	3.98, 3.85
GlcNH$_2$5'	4.79	3.09 (t, *J* = 9.5 Hz, 1H)	3.62	3.49	3.48	3.92, 3.77
Gal6	4.47 (d, *J* = 7.8 Hz, 1H)	3.54	3.66	3.92	3.73	3.77, 3.75

^{13}C (150 MHz, D$_2$O): δ (ppm)

	C1	**C2**	**C3**	**C4**	**C5**	**C6**
GlcNAc1	78.00	53.63	72.70	78.47	76.10	59.77
GlcNAc2	101.15	54.81	65.62	79.39	74.27	59.81
Man3	100.29	70.08	80.36	71.88	74.07	65.92
Man4	99.45	76.24	69.30	67.18	73.47	61.60
Man4'	97.44	76.07	69.26	66.48	72.44	60.08
GlcNAc5	99.32	54.79	71.85	78.40	74.65	59.90
GlcNH$_2$5'	97.58	55.23	72.29	69.45	76.24	60.26
Gal6	102.83	70.85	72.41	68.44	75.26	60.93

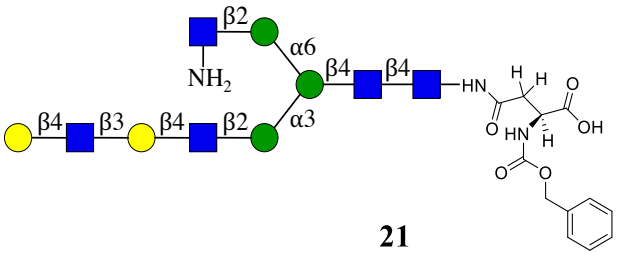
Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH$_3$	-[a]	174.61, 174.56
NHC(O)<u>CH$_3$</u>	2.08 (s, 3H), 2.05 (s, 3H), 1.91 (s, 3H)	22.25, 22.12, 21. 91
Aromatic	7.53 – 7.33 (m, 5H)	136.25, 128.67, 128.27, 127.75
<u>CH$_2$</u>-Ph	5.17 – 5.07 (m, 2H)	66.88
NH-<u>C</u>OO-	-	157.68
NH-<u>CH</u>-COOH	4.33 (dd, *J* = 9.5, 4.1 Hz, 1H)	52.94
NH-CH-<u>C</u>OOH	-	177.57
C(O)-<u>CH$_2$</u>-CH	2.81 (dd, *J* = 15.5, 4.2 Hz, 1H) 2.59 (dd, *J* = 15.4, 9.4 Hz, 1H)	38.47
<u>C</u>(O)-CH$_2$-CH	-	173.62

[a] Not applicable

ESI TOF-MS *m/z* calculated for $C_{66}H_{102}N_6O_{44}$, $[M-2H]^{2-}$: 841.2970, found 841.3034.

Compound 21

21 was prepared from **20** (11.0 mg, 1.0 eq) using the general procedures **2.2 d** and **c** for the installation of GlcNAc and Gal moieties. The product was purified using the described purification system **(2.2 j)** providing **21** as a white fluffy solid (11.6 mg, 87% yield over two steps).



21

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04	3.82	3.73	3.64	3.54	2.79, 3.62
GlcNAc2	4.60 (d, *J* = 7.8 Hz, 1H)	3.79	3.77	3.74	3.60	N/R[b]
Man3	4.77	4.25	3.77	3.77	3.64	3.96, 3.80
Man4	5.11	4.19 (d, *J* = 3.4 Hz, 1H)	3.90	3.50	3.74	3.92, 3.61
Man4'	5.04	4.24	3.96	3.69	N/R	N/R
GlcNAc5	4.58 (d, *J* = 7.0 Hz, 1H)	3.73	3.73	3.72	3.57	N/R
GlcNH$_2$5'	4.73 (d, *J* = 8.1 Hz, 1H)	3.02 (t, *J* = 9.4 Hz, 1H)	3.58	3.48	3.48	3.92, 3.77
Gal6	4.45	3.58	3.72	4.16 (d, *J* = 3.1 Hz, 1H)	3.71	N/R
GlcNAc7	4.70 (d, *J* = 8.4 Hz, 1H)	3.81	N/R	3.74	N/R	N/R
Gal8	4.48 (d, *J* = 7.8 Hz, 1H)	3.54	3.67	3.92	3.73	3.78, 3.74

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.00	53.63	72.69	78.47	76.09	59.76
GlcNAc2	101.15	54.82	65.64	79.42	74.27	59.81
Man3	100.29	70.08	80.33	71.87	74.08	65.91
Man4	99.45	76.27	69.29	67.18	73.46	61.60
Man4'	97.44	76.19	69.29	66.57	N/R	N/R
GlcNAc5	99.34	54.73	71.84	78.47	74.63	59.89
GlcNH$_2$5'	98.37	55.36	72.84	69.46	76.19	60.32
Gal6	102.86	69.85	82.01	68.19	74.78	N/R
GlcNAc7	102.68	55.09	N/R	78.04	N/R	N/R
Gal8	102.76	70.87	72.41	68.45	75.26	60.94

Signal	Proton	Carbon
NHC(O)CH_3	-[a]	174.81, 174.60, 174.55
NHC(O)CH_3	2.08 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.91 (s, 3H)	22.24, 22.11, 22.09, 21.90
Aromatic	7.49 – 7.36 (m, 5H)	136.25, 128.66, 128.26, 127.75
CH_2-Ph	5.17 – 5.07 (m, 2H)	66.87
NH-COO-	-	157.68
NH-CH-COOH	4.35 – 4.30 (m, 3H)	52.95
NH-CH-COOH	-	N/R
C(O)-CH_2-CH	2.83 – 2.78 (m, 1H) 2.58 (dd, *J* = 15.4, 9.3 Hz, 1H)	38.47
C(O)-CH_2-CH	-	173.62

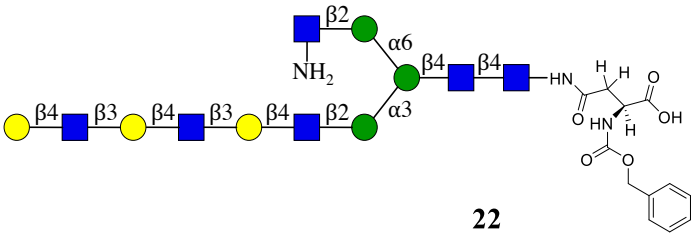
[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{80}H_{125}N_7O_{54}$, $[M-2H]^{2-}$: 1023.8631, found 1023.8725.

Compound 22

22 was synthesized from **21** (11.6 mg) using the general procedures **2.2 d** and **c** for the installation of GlcNAc and Gal moieties. The product was purified using the described purification system **(2.2 j)** providing **22** as a white fluffy solid (11.5 mg, 77% yield over two steps).



22

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04	3.81	3.73	3.64	3.54	N/R[b]
GlcNAc2	4.60 (d, *J* = 8.2 Hz, 1H)	3.80	3.77	3.74	N/R	N/R
Man3	4.77	4.25	3.77	3.77	N/R	3.96, 3.79
Man4	5.11	4.19 (d, *J* = 3.4 Hz, 1H)	3.89	3.50	3.74	3.92, 3.61
Man4'	5.03	4.25	3.96	3.69	N/R	N/R
GlcNAc5	4.58 (d, *J* = 7.1 Hz, 1H)	3.73	3.72	3.71	N/R	N/R
GlcNH$_2$5'		3.08 (t, *J* = 9.5 Hz, 1H)	N/R	3.49	N/R	N/R
Gal6	4.45	3.58	N/R	4.15	N/R	N/R
GlcNAc7	4.70	3.80	N/R	N/R	N/R	N/R

Gal8	4.47	3.58	N/R	4.15	N/R	N/R
GlcNAc9	4.70	3.80	N/R	N/R	N/R	N/R
Gal10	4.48	3.54	3.67	3.92	3.72	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	**C2**	**C3**	**C4**	**C5**	**C6**
GlcNAc1	78.00	53.62	72.69	78.45	76.09	N/R
GlcNAc2	101.14	54.81	65.63	79.39	N/R	N/R
Man3	100.28	70.07	80.33	71.88	N/R	65.92
Man4	99.45	76.22	69.29	67.17	73.46	61.59
Man4'	97.43	76.09	69.26	66.48	N/R	N/R
GlcNAc5	99.33	54.73	71.84	78.45	N/R	N/R
GlcNH$_2$5'	N/R	55.25	N/R	69.45	N/R	N/R
Gal6	102.87	69.85	N/R	68.21	N/R	N/R
GlcNAc7	102.67	55.04	N/R	N/R	N/R	N/R
Gal8	102.76	69.85	N/R	68.21	N/R	N/R
GlcNAc9	102.67	55.09	N/R	N/R	N/R	N/R
Gal10	102.76	70.87	72.40	68.45	75.26	60.94

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH$_3$	-[a]	174.80, 174.69, 174.60, 174.55, 174.50
NHC(O)<u>CH$_3$</u>	2.07 (s, 2H), 2.06 – 2.02 (m, 8H), 1.91 (s, 2H)	22.23, 22.08, 21.89
Aromatic	7.52 – 7.31 (m, 5H)	136.25, 128.66, 128.26, 127.75
<u>CH$_2$</u>-Ph	5.17 – 5.07 (m, 2H)	66.87
NH-<u>C</u>OO-	-	157.67
NH-<u>CH</u>-COOH	4.35 – 4.29 (m, 1H)	52.93
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.81 (dd, *J* = 15.7, 4.1 Hz, 1H), 2.58 (dd, *J* = 15.3, 9.4 Hz, 1H)	38.46
<u>C</u>(O)-CH$_2$-CH	-	173.61

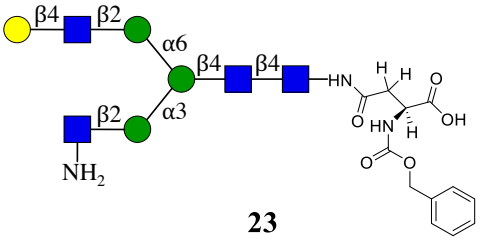
[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{94}H_{148}N_8O_{64}$, $[M-2H]^{2-}$: 1206.4292, found 1206.4386.

Compound 23

Compound **13** (9.8 mg) was subjected to MGAT2 and UDP-GlcNAc according to general procedure **2.2 b** to give **14**, which was purified according to **2.2 j**. Compound **14** was subjected to general procedures **2.2 g** and **c** to remove the TFA moiety and introduce Gal by B4GalT1 to provide after purification using general protocal **2.2 j** compound **23** as a white fluffy solid (10.3 mg, 88% yield for three steps).



23

NMR and MS analysis of compound **14**:

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.7 Hz, 1H)	3.83	3.74	3.65	3.55	3.81, 3.63
GlcNAc2	4.61 (d, *J* = 7.9 Hz, 1H)	3.80	3.81	3.74	3.62	3.88, 3.74
Man3	4.76 (s, 1H)	4.25 (d, *J* = 2.9 Hz, 1H)	3.76	3.75	3.62	3.97, 3.78
Man4	5.08 (s, 2H)	4.22 (dd, *J* = 3.3, 1.6 Hz, 1H)	3.91	3.51 or 3.47	3.74	3.93, 3.62
Man4'	4.92 (s, 1H)	4.11 (dd, *J* = 3.4, 1.7 Hz, 1H)	3.91	3.51 or 3.47	3.62	3.90, 3.58
GlcTFA5	4.68 (d, *J* = 8.4 Hz, 1H)	3.81	3.69	3.49	3.48	3.94, 3.79
GlcNAc5'	4.55 (d, *J* = 8.4 Hz, 1H)	3.71	3.55	3.45	3.44	3.91, 3.75

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.03	53.64	72.65	78.47	76.11	59.75
GlcNAc2	101.17	54.83	65.56	79.44	74.29	59.79
Man3	100.36	70.06	80.34	71.89	74.19	65.72
Man4	99.36	76.30	69.17	67.32	73.51	61.53
Man4'	96.92	76.23	69.37	67.24	72.75	61.53
GlcTFA5	98.72	55.81	72.52	69.76	75.77	60.48
GlcNAc5'	99.51	55.23	73.29	69.79	75.70	60.51

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.69, 174.54
NHC(O)<u>CH$_3$</u>	2.08 (s, 3H), 2.05 (s, 3H), 1.92 (s, 3H)	22.23, 22.11, 21.89
NH<u>C</u>(O)CF$_3$	-	159.63, 159.38
NHC(O)<u>CF$_3$</u>	-	116.70, 114.80
Aromatic	7.55 – 7.31 (m, 5H)	136.20, 128.67, 128.28, 127.71
<u>CH$_2$</u>-Ph	5.17 – 5.09 (m, 2H)	66.96
NH-<u>C</u>OO-	-	157.71
NH-<u>CH</u>-COOH	4.41 (dd, *J* = 9.0, 4.5 Hz, 1H)	52.16
NH-CH-<u>C</u>OOH	-	176.66
C(O)-<u>CH$_2$</u>-CH	2.83 (dd, *J* = 15.7, 4.3 Hz, 1H), 2.66 (dd, *J* = 15.3, 9.1 Hz, 3H)	38.05
<u>C</u>(O)-CH$_2$-CH	-	173.28

[a] Not applicable

ESI TOF-MS *m/z* calculated for $C_{62}H_{91}F_3N_6O_{40}$, $[M-2H]^{2-}$: 808.2617, found 808.2579.

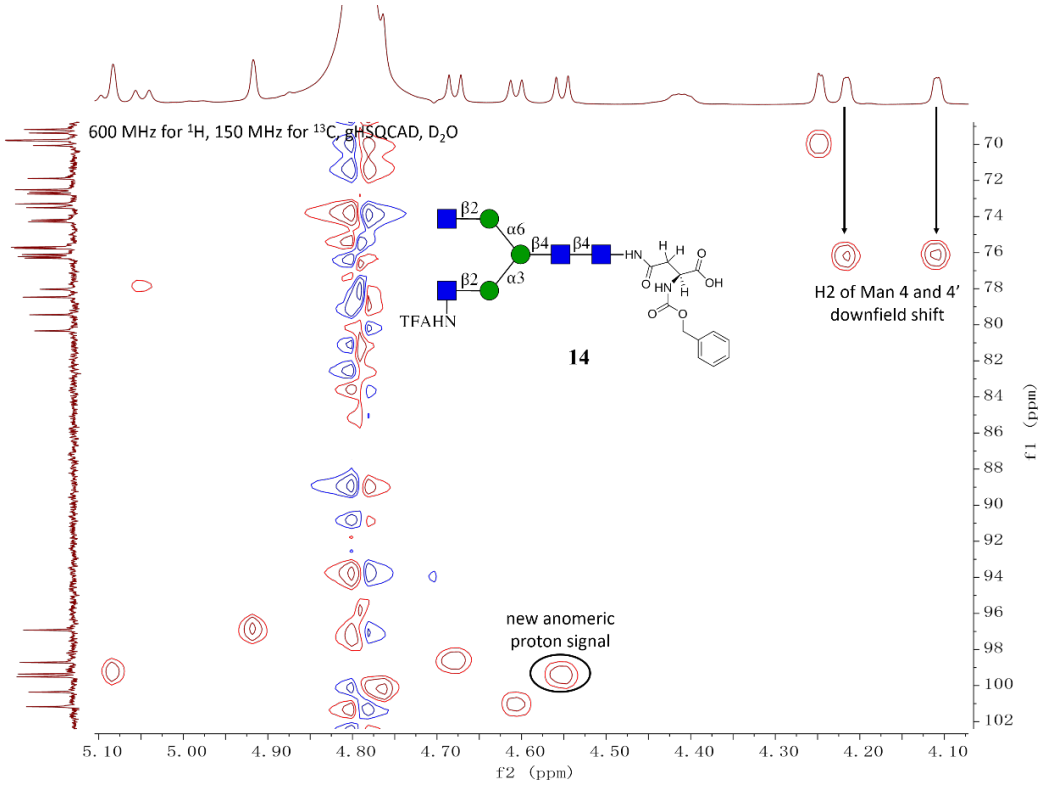


Figure S8. Expanded HSQC and HMBC of compound **14**.

NMR and MS analysis of compound **23**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 9.6 Hz, 1H)	3.82	3.73	3.65	3.54	N/R[b]
GlcNAc2	4.60 (d, *J* = 8.0 Hz, 1H)	3.79	3.81	N/R	N/R	N/R
Man3	4.76	4.23 (d, *J* = 2.7 Hz, 1H)	3.80	N/R	N/R	N/R
Man4	5.22 (s, 1H)	4.33 (d, *J* = 3.5 Hz, 1H)	3.97	N/R	N/R	N/R
Man4'	4.92 (s, 1H)	4.11 (d, *J* = 3.7 Hz, 1H)	3.88	N/R	N/R	N/R
$GlcNH_2$5	4.76	3.04 (t, *J* = 9.4 Hz, 1H)	3.59	N/R	3.48	N/R
GlcNAc5'	4.58 (d, *J* = 8.1 Hz, 1H)	3.74	N/R	3.73	N/R	N/R
Gal6'	4.47 (d, *J* = 7.8 Hz, 1H)	3.54	3.67	3.92	3.73	3.78, 3.74

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	77.98	53.62	72.69	78.41	76.08	N/R
GlcNAc2	101.13	54.88	65.40	N/R	N/R	N/R
Man3	100.26	70.08	80.70	N/R	N/R	65.55
Man4	100.00	76.13	69.18	N/R	N/R	N/R
Man4'	96.92	76.20	69.36	N/R	N/R	N/R
$GlcNH_2$5	98.04	55.26	72.74	N/R	76.20	N/R
GlcNAc5'	99.33	54.75	N/R	78.41	N/R	N/R
Gal6'	102.85	70.87	72.40	68.43	75.25	60.92

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.61, 174.53
NHC(O)<u>CH$_3$</u>	2.07 (s, 1H), 2.04 (s, 1H), 1.91 (s, 1H)	22.24, 22.12, 21.90
Aromatic	7.51 – 7.34 (m, 5H)	136.24, 128.66, 128.26, 127.74
<u>CH$_2$</u>-Ph	5.12 (q, *J* = 12.7, 12.3 Hz, 2H)	66.87
NH-<u>C</u>OO-	-	157.68
NH-<u>CH</u>-COOH	4.33	52.89
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.81 (d, *J* = 15.2 Hz, 1H) 2.58 (dd, *J* = 15.4, 9.1 Hz, 1H)	38.47
<u>C</u>(O)-CH$_2$-CH	-	173.61

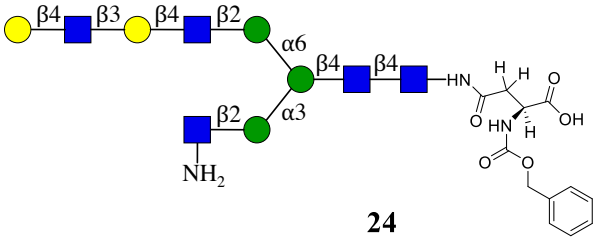
[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{66}H_{102}N_6O_{44}$, $[M-2H]^{2-}$: 841.2970, found 841.3032.

Compound 24

24 was prepared from **23** (10.5 mg) using the general procedures **2.2 d** and **c** for the installation of GlcNAc and Gal moieties. The product was purified using the described two-stage purification system **(2.2 j)** providing **24** as a white fluffy solid (9.1 mg, 71% yield for two steps).



24

^{1}H (600 MHz, D$_2$O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.7 Hz, 1H)	3.83	3.74	3.66	3.56	N/R[b]
GlcNAc2	4.62 (d, *J* = 8.0 Hz, 1H)	3.81	3.82	3.75	N/R	N/R
Man3	4.78	4.24 (s, 1H)	3.82	N/R	N/R	3.97 3.79
Man4	5.24 (d, *J* = 1.8 Hz, 1H)	4.38 – 4.35 (m, 1H)	3.99	N/R	N/R	N/R
Man4'	4.94 (d, *J* = 1.7 Hz, 1H)	4.12 (d, *J* = 3.0 Hz, 1H)	3.89	N/R	N/R	N/R
GlcNH$_2$5	4.85 (d, *J* = 8.5 Hz, 1H)	3.14	3.62	N/R	3.51	N/R
GlcNAc5'	4.59 (d, *J* = 8.2 Hz, 1H)	3.76	N/R	3.72	N/R	N/R
Gal6'	4.51 – 4.44 (m, 2H)	3.60	3.74	N/R	N/R	N/R
GlcNAc7'	4.71 (d, *J* = 8.4 Hz, 1H)	3.82	N/R	N/R	N/R	N/R

Gal8'	4.51 – 4.44 (m, 2H)	3.55	3.68	3.94	3.74	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.01	53.64	72.70	78.43	76.10	N/R
GlcNAc2	101.14	54.89	65.45	79.32	N/R	N/R
Man3	100.28	70.09	80.71	N/R	N/R	65.57
Man4	99.98	76.01	69.16	N/R	N/R	N/R
Man4'	96.94	76.23	69.38	N/R	N/R	N/R
GlcNH$_2$5	97.19	55.12	72.76	N/R	76.27	N/R
GlcNAc5'	99.36	54.73	N/R	78.49	N/R	N/R
Gal6'	102.90	69.87	82.02	N/R	N/R	N/R
GlcNAc7'	102.68	55.12	N/R	N/R	N/R	N/R
Gal8'	102.78	70.89	72.43	68.47	75.25	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.81, 174.70, 174.62, 174.54
NHC(O)<u>CH$_3$</u>	2.20 – 1.84 (m, 12H)	22.26, 22.15, 22.12, 21.92
Aromatic	7.52 – 7.35 (m, 5H)	136.26, 128.67, 128.27, 127.76
<u>CH$_2$</u>-Ph	5.18 – 5.08 (m, 2H)	66.89
NH-<u>C</u>OO-	-	157.68
NH-<u>CH</u>-COOH	4.34 (dd, *J* = 9.1, 3.8 Hz, 1H)	52.94
NH-CH-<u>C</u>OOH	-	177.54
C(O)-<u>CH$_2$</u>-CH	2.82 (dd, *J* = 15.4, 4.2 Hz, 1H) 2.60 (dd, *J* = 15.4, 9.4 Hz, 1H)	38.48
<u>C</u>(O)-CH$_2$-CH	-	173.61

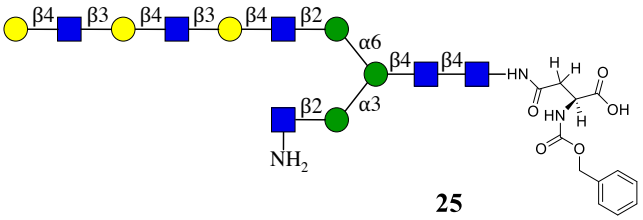
[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{80}H_{125}N_7O_{54}$, $[M-2H]^{2-}$: 1023.8631, found 1023.8719.

Compound 25

25 was prepared from **24** (9.1 mg) using the general procedures **2.2 d** and **c** for the installation of GlcNAc and Gal moieties. The product was purified using the described two-stage purification system **(2.2 j)** providing **25** as a white fluffy solid (9.4 mg, 88% yield for two steps).



25

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.08 (d, *J* = 9.3 Hz, 1H)	3.86	3.77	3.68	3.58	N/R[b]
GlcNAc2	4.65 (d, *J* = 7.8 Hz, 1H)	3.81	3.84	3.77	N/R	N/R
Man3	4.80	4.27	3.83	N/R	N/R	4.01 3.82
Man4	5.27 (s, 1H)	4.37	4.00	N/R	N/R	N/R
Man4'	4.96 (s, 1H)	4.14	3.92	N/R	N/R	N/R
GlcNH$_2$5	4.76	3.09 – 3.00 (m, 1H)	3.61	N/R	3.51	N/R
GlcNAc5'	4.61 (d, *J* = 8.0 Hz, 1H)	3.78	N/R	3.75	N/R	N/R
Gal6'	4.49	3.62	3.76	N/R	N/R	N/R
GlcNAc7'	4.75 – 4.72 (m, 1H)	3.84	N/R	N/R	N/R	N/R
Gal8'	4.50	3.62	3.76	N/R	N/R	N/R
GlcNAc9'	4.75 – 4.72 (m, 1H)	3.78	N/R	N/R	N/R	N/R
Gal10'	4.52	3.58	3.70	3.96	3.76	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.06	53.68	72.73	78.50	76.13	N/R
GlcNAc2	101.17	54.93	65.49	79.36	N/R	N/R
Man3	100.31	70.12	80.74	N/R	N/R	66.91
Man4	100.03	76.24	69.26	N/R	N/R	N/R
Man4'	96.99	76.28	69.42	N/R	N/R	N/R
GlcNH$_2$5	98.40	55.39	73.03	N/R	76.23	N/R
GlcNAc5'	99.41	54.76	N/R	78.54	N/R	N/R
Gal6'	102.94	69.91	82.03	N/R	N/R	N/R
GlcNAc7'	102.68	55.15	N/R	N/R	N/R	N/R
Gal8'	102.82	69.91	82.01	N/R	N/R	N/R
GlcNAc9'	102.68	55.11	N/R	N/R	N/R	N/R
Gal10'	102.84	70.92	72.47	68.50	75.30	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.84, 174.73, 174.65, 174.56
NHC(O)<u>CH$_3$</u>	2.33 – 1.79 (m, 15H)	22.29, 22.18, 22.14, 21.95
Aromatic	7.64 – 7.30 (m, 5H)	136.30, 128.71, 128.30, 127.79
<u>CH$_2$</u>-Ph	7.64 – 7.30 (m, 2H)	66.91
NH-<u>C</u>OO-	-	157.70
NH-<u>CH</u>-COOH	4.36 (s, 2H)	53.00
NH-CH-<u>C</u>OOH	-	177.57
C(O)-<u>CH$_2$</u>-CH	2.85 (d, *J* = 14.6 Hz, 1H), 2.62 (dd, *J* = 15.4, 9.5 Hz, 1H)	38.53
<u>C</u>(O)-CH$_2$-CH	-	173.65

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{94}H_{148}N_8O_{64}$, $[M-2H]^{2-}$: 1206.4292, found 1206.4188.

Table S1. The anomeric proton and carbon chemical shift of key intermediate compounds.

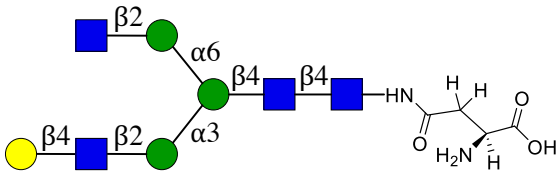
	Compound 20		**Compound21**		**Compound 22**	
	H1	**C1**	**H1**	**C1**	**H1**	**C1**
GlcNAc1	5.04	78.00	5.04	78.00	5.04	78.00
GlcNAc2	4.60	101.15	4.60	101.15	4.60	101.14
Man3	4.77	100.29	4.77	100.29	4.77	100.28
Man4	5.11	99.45	5.11	99.45	5.11	99.45
Man4'	5.03	97.44	5.04	97.44	5.03	97.43
GlcNAc5	4.58	99.32	4.58	99.34	4.58	99.33
GlcNH$_2$5'	4.79	97.58	4.73	98.37	N/R[b]	N/R
Gal6	4.47	102.83	4.45	102.86	4.45	102.87
GlcNAc7	--[a]	--	4.70	102.68	4.70	102.67
Gal8	--	--	4.48	102.76	4.47	102.76
GlcNAc9	--	--	--	--	4.70	102.67
Gal10	--	--	--	--	4.48	102.76
	Compound 23		**Compound24**		**Compound 25**	
	H1	**C1**	**H1**	**C1**	**H1**	**C1**
GlcNAc1	5.04	77.98	5.06	78.01	5.08	78.06
GlcNAc2	4.60	101.13	4.62	101.14	4.65	101.17
Man3	4.76	100.26	4.78	100.28	4.80	100.31
Man4	5.22	100.00	5.24	99.98	5.27	100.03
Man4'	4.92	96.92	4.94	96.94	4.96	96.99
GlcNH$_2$5	4.76	98.04	4.85	97.19	4.76	98.40
GlcNAc5'	4.58	99.33	4.59	99.36	4.61	99.41
Gal6'	4.47	102.85	4.51 – 4.44	102.90	4.49	102.94
GlcNAc7'	--	--	4.71	102.68	4.75 – 4.72	102.68
Gal8'	--	--	4.51 – 4.44	102.78	4.50	102.82
GlcNAc9'	--	--	--	--	4.75 – 4.72	102.68
Gal10'	--	--	--	--	4.52	102.84

[a] Not applicable

[b] Not reported

Compound 29

29 was prepared by acetylation of **20** (1.0 mg) to give intermediate **26** using general procedure **2.2 h** that was purified using protocal **2.2 j**. The CBz protecting group was removed by general procedure **2.2 i** to give the final product **29** as a white fluffy solid (0.9 mg, 92% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{60}H_{98}N_6O_{43}$, $[M-2H]^{2-}$: 795.2839, found 795.2831.



29

NMR and MS analysis of compound **26**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 9.8 Hz, 1H)	3.81	3.60	3.63	N/R[b]	N/R
GlcNAc2	4.59 (d, *J* = 7.8 Hz, 1H)	3.77	3.77	3.72	3.59	N/R
Man3	4.76	4.24 (d, *J* = 2.7 Hz, 1H)	3.76	3.76	3.61	3.95, 3.76
Man4	5.10	4.20 – 4.16 (m, 1H)	3.88	3.48	3.73	N/R
Man4'	4.90 (s, 1H)	4.11 – 4.08 (m, 1H)	3.88	3.48	N/R	N/R
GlcNAc5	4.57 (d, *J* = 7.2 Hz, 1H)	3.72	3.72	3.71	3.56	N/R
GlcNAc5'	4.54 (d, *J* = 8.5 Hz, 1H)	3.69	N/R	N/R	N/R	N/R
Gal6	4.45 (d, *J* = 7.8 Hz, 1H)	3.52	3.65	3.91	3.72	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.06	53.65	72.73	78.45	N/R	N/R
GlcNAc2	101.16	54.81	65.58	79.43	74.27	N/R
Man3	100.33	70.08	80.23	71.86	74.20	65.75
Man4	99.44	76.28	69.35	67.23	73.44	N/R
Man4'	96.91	76.19	69.27	67.17	N/R	N/R
GlcNAc5	99.34	54.76	71.84	78.35	74.62	N/R
GlcNAc5'	99.48	55.22	N/R	N/R	N/R	N/R
Gal6	102.81	70.85	72.38	68.43	75.24	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.67, 174.60
NHC(O)<u>CH$_3$</u>	2.09 – 1.85 (m, 12H)	22.23, 22.10, 21.86
Aromatic	7.50 – 7.32 (m, 5H)	136.12, 128.66, 128.30, 127.64
<u>CH$_2$</u>-Ph	5.16 – 5.08 (m, 2H)	67.07
NH-<u>C</u>OO-	-	157.75

NH-CH-COOH	4.52 (m, 1H)	50.99
NH-CH-COOH	-	177.09
C(O)-CH$_2$-CH	2.84 (dd, *J* = 14.7, 3.0 Hz, 1H), 2.74 (dd, *J* = 15.9, 7.8 Hz, 1H)	37.40
C(O)-CH$_2$-CH	-	172.78

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{68}H_{104}N_6O_{45}$, $[M-2H]^{2-}$: 862.3023, found 862.3026.

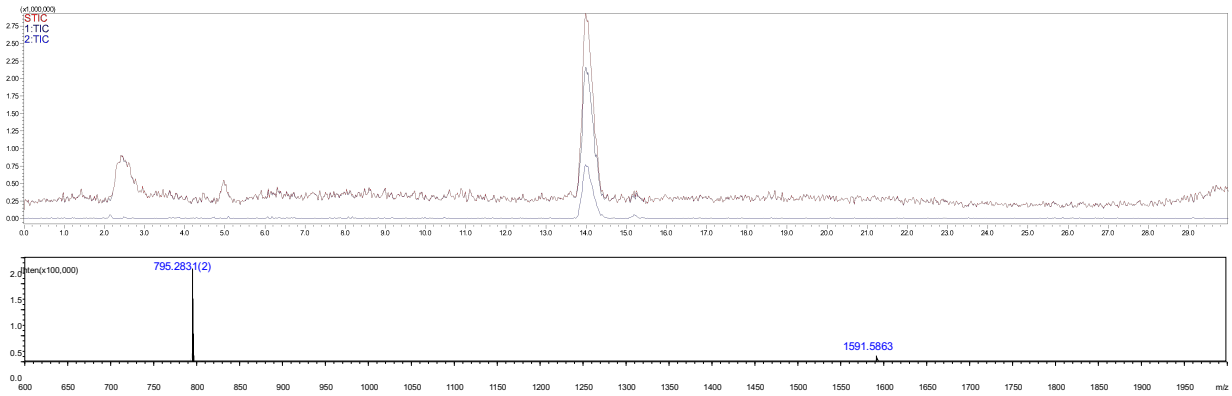
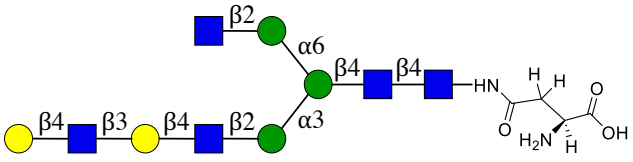


Figure S9. Analytical HPLC-MS chromatogram of compound **29**. The retention time = 14.0 min.

Compound 30

30 was prepared by acetylation of **21** (1.0 mg) by general procedures **2.2 h** to give intermediate **27**, which was purified using the combined purification approach **(2.2 j)**. The Cbz protecting group of **27** was removed by hydrogenaton according to **2.2 i** to give final product **30** as a white fluffy solid (0.8 mg, 86% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{74}H_{121}N_7O_{53}$, $[M-2H]^{2-}$: 977.8500, found 977.8472.



30

NMR and MS analysis of compound **27**

^{1}H (600 MHz, D$_2$O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 9.7 Hz, 1H)	3.81	3.61	3.64	N/R[b]	N/R
GlcNAc2	4.60 (d, *J* = 7.9 Hz, 1H)	3.77	3.78	3.73	3.61	N/R
Man3	4.76 (s, 1H)	4.25 (s, 1H)	3.76	3.77	3.62	3.96, 3.77

Man4	5.11	4.20 – 4.17 (m, 1H)	3.88	3.49	3.74	N/R
Man4'	4.91 (s, 1H)	4.10 (d, J = 3.0 Hz, 1H)	3.89	3.49	N/R	N/R
GlcNAc5	4.57 (d, J = 7.5 Hz, 1H)	3.73	3.72	3.71	3.57	N/R
GlcNAc5'	4.55 (d, J = 8.4 Hz, 1H)	3.70	N/R	N/R	N/R	N/R
Gal6	4.45 (d, J = 7.8 Hz, 1H)	3.57	3.72	4.15 (d, J = 2.6 Hz, 1H)	N/R	N/R
GlcNAc7	4.69 (d, J = 8.3 Hz, 1H)	3.80	N/R	N/R	N/R	N/R
Gal8	4.47 (d, J = 7.8 Hz, 1H)	3.53	3.66	3.92	3.72	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	**C2**	**C3**	**C4**	**C5**	**C6**
GlcNAc1	78.03	51.78	72.74	78.45	N/R	N/R
GlcNAc2	101.16	54.82	65.58	79.42	74.28	N/R
Man3	100.33	70.10	80.34	71.88	74.20	65.73
Man4	99.45	76.28	69.36	67.24	73.45	N/R
Man4'	96.92	76.20	69.28	67.18	N/R	N/R
GlcNAc5	99.35	54.72	71.83	78.39	74.62	N/R
GlcNAc5'	99.49	55.23	N/R	N/R	N/R	N/R
Gal6	102.85	69.85	81.97	68.21	N/R	N/R
GlcNAc7	102.67	55.08	N/R	N/R	N/R	N/R
Gal8	102.76	70.86	72.40	68.45	75.25	N/R

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH$_3$	-[a]	174.80, 174.68, 174.60, 174.54
NHC(O)<u>CH$_3$</u>	2.10 – 1.88 (m, 15H)	22.23, 22.10, 22.08, 21.87
Aromatic	7.52 – 7.31 (m, 5H)	136.17, 128.66, 128.29, 127.69
<u>CH$_2$</u>-Ph	5.18 – 5.08 (m, 2H)	67.00
NH-<u>C</u>OO-	-	157.72
NH-<u>CH</u>-COOH	4.44	51.78
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.87 – 2.80 (m, 1H), 2.68 (dd, J = 15.4, 8.9 Hz, 1H)	37.83
<u>C</u>(O)-CH$_2$-CH	-	173.12

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{82}H_{127}N_7O_{55}$, $[M-2H]^{2-}$: 1044.8684, found 1044.8703.

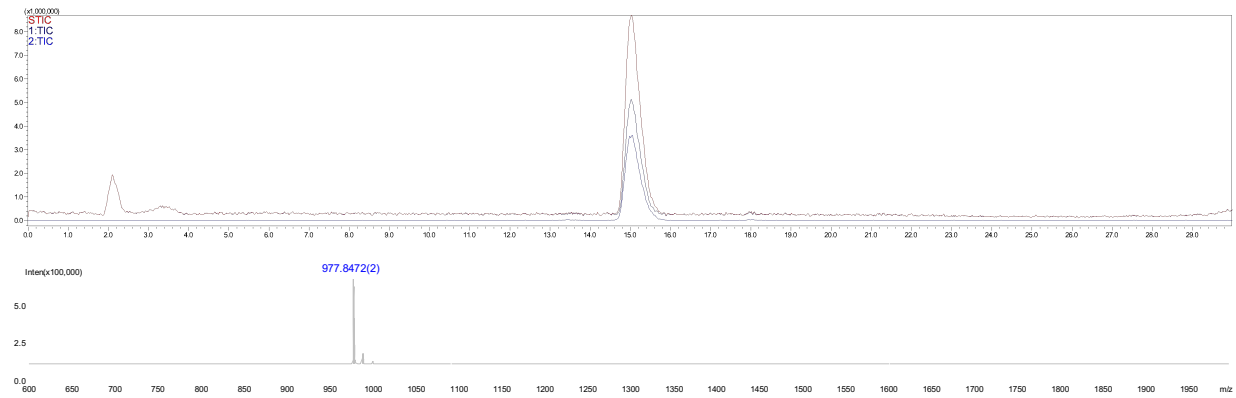
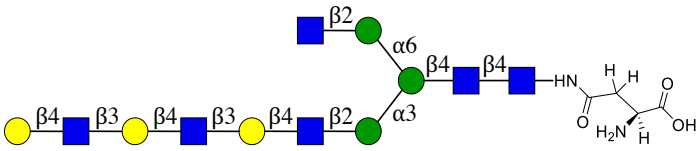


Figure S10. Analytical HPLC-MS chromatogram of compound **30**. The retention time = 15.0 min.

Compound 31

31 was prepared by acetylation of **22** (1.0 mg) according to the general procedure **2.2 h** to give, after purified using the described two-stage purification protocal **(2.2 j)**, intermediate **28**. The Cbz protecting group of **28** was removed by hydrogenation (**2.2 i**) to give the final product **31** as a white fluffy solid (1.0 mg, 99% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{88}H_{144}N_8O_{63}$, $[M-2H]^{2-}$: 1160.4161, found 1160.4127.



31

NMR and MS analysis of compound **28**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 10.0 Hz, 1H)	3.81	3.61	3.64	N/R[b]	N/R
GlcNAc2	4.60 (d, *J* = 7.8 Hz, 1H)	3.78	3.78	3.72	3.61	N/R
Man3	4.76	4.24 (s, 1H)	3.77	3.77	3.61	3.95, 3.77
Man4	5.18 – 5.08 (m, 3H)	4.18 (s, 1H)	3.88	3.49	3.73	N/R
Man4'	4.91 (s, 1H)	4.10 (s, 1H)	3.88	3.49	N/R	N/R
GlcNAc5	4.57 (d, *J* = 6.6 Hz, 1H)	3.72	3.72	3.71	3.57	N/R
GlcNAc5'	4.54 (d, *J* = 8.4 Hz, 1H)	3.69	N/R	N/R	N/R	N/R
Gal6	4.51 – 4.41 (m, 4H)	3.57	3.71	4.15 (s, 2H)	N/R	N/R
GlcNAc7	4.69 (d, *J* = 8.4 Hz, 2H)	3.73	N/R	N/R	N/R	N/R
Gal8	4.51 – 4.41 (m, 4H)	3.57	3.71	4.15 (s, 2H)	N/R	N/R
GlcNAc9	4.69 (d, *J* = 8.4 Hz, 2H)	3.79	N/R	N/R	N/R	N/R

Gal10	4.51 – 4.41 (m, 4H)	3.53	3.66	3.92	3.72	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.03	53.65	72.74	78.45	N/R	N/R
GlcNAc2	101.16	54.81	65.58	79.43	74.28	N/R
Man3	100.33	70.09	80.34	71.87	74.20	65.74
Man4	99.45	76.28	69.36	67.23	73.44	N/R
Man4'	96.91	76.20	69.27	67.18	N/R	N/R
GlcNAc5	99.35	54.72	71.83	78.37	74.61	N/R
GlcNAc5'	99.49	55.22	N/R	N/R	N/R	N/R
Gal6	102.76	69.85	81.96	68.21	N/R	N/R
GlcNAc7	102.66	55.09	N/R	N/R	N/R	N/R
Gal8	102.84	69.85	81.96	68.21	N/R	N/R
GlcNAc9	102.66	55.04	N/R	N/R	N/R	N/R
Gal10	102.76	70.86	72.40	68.44	75.25	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-[a]	174.80, 174.68, 174,60, 174.54
NHC(O)<u>CH_3</u>	2.10 – 1.87 (m, 18H)	22.23, 22.08, 21.87
Aromatic	7.52 – 7.32 (m, 5H)	136.15, 128.66, 128.29, 127.66
<u>CH_2</u>-Ph	5.18 – 5.08 (m, 3H)	67.03
NH-<u>C</u>OO-	-	157.73
NH-<u>CH</u>-COOH	4.51 – 4.41 (m, 4H)	51.44
NH-CH-<u>C</u>OOH	-	177.29
C(O)-<u>CH_2</u>-CH	2.84 (dd, *J* = 15.4, 3.6 Hz, 1H), 2.71 (dd, *J* = 16.1, 8.5 Hz, 1H)	37.65
<u>C</u>(O)-CH_2-CH	-	172.96

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{96}H_{150}N_8O_{65}$, $[M-2H]^{2-}$: 1227.4344, found 1227.4352.

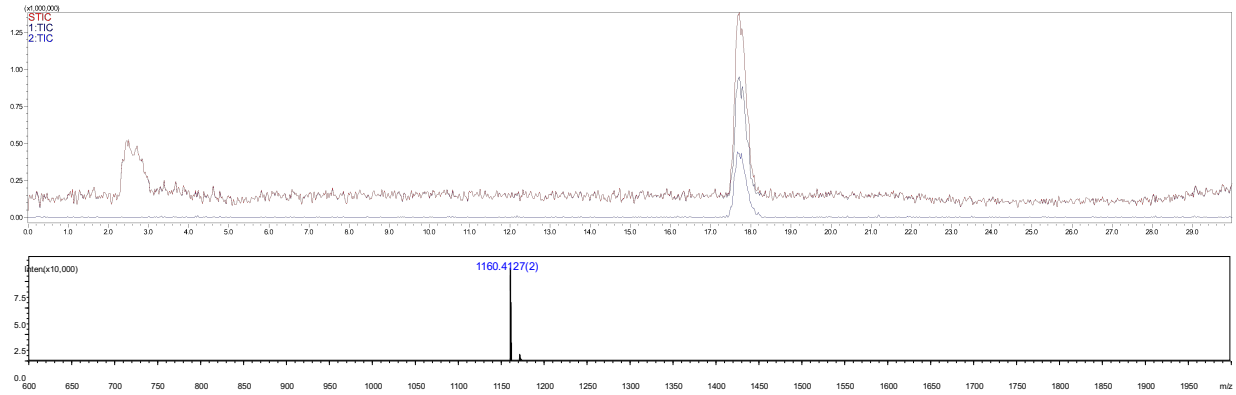
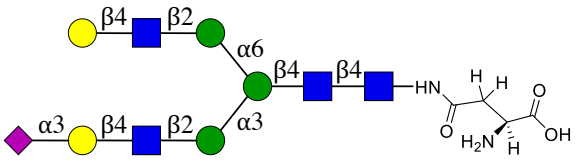


Figure S11. Analytical HPLC-MS chromatogram of compound **31**. The retention time = 17.8 min.

Compound 35

35 was synthesized from **20** (3 mg, 1.0 eq) using the general procedures **2.2 f**, **h**, **c** and **i** for the installation of α2,3-sialic acid with ST3Gal4, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give **32**, which was was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **32** was removed using method **2.2 i** to give final product **35** as a white fluffy solid (1.1 mg, 30% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{77}H_{125}N_7O_{56}$, $[M-2H]^{2-}$: 1021.8580, found 1021.8613.



35

NMR and MS analysis of compound **32**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.8 Hz, 1H)	3.82	3.74	3.65	3.56	N/R[b]
GlcNAc2	4.61 (d, *J* = 8.0 Hz, 1H)	3.80	3.79	N/R	N/R	N/R
Man3	4.77	4.25 (d, *J* = 2.7 Hz, 1H)	3.77	N/R	N/R	3.97, 3.80
Man4	5.13	4.20 (d, *J* = 3.5 Hz, 1H)	3.90	N/R	N/R	N/R
Man4'	4.93 (d, *J* = 7.4 Hz, 1H)	4.15 – 4.08 (m, 2H)	3.90	N/R	N/R	N/R
GlcNAc5	4.60 – 4.57 (m, 2H)	3.75	N/R	N/R	N/R	N/R
GlcNAc5'	4.60 – 4.57 (m, 2H)	3.75	N/R	N/R	N/R	N/R
Gal6	4.48 (d, *J* = 7.8 Hz, 1H)	3.55	N/R	4.15 – 4.08 (m, 2H)	N/R	N/R
Gal6'	4.55 (d, *J* = 7.8 Hz, 1H)	3.57	N/R	N/R	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac7	-[a]	-	2.76 (dd, *J* = 12.5, 4.6 Hz, 1H), 1.81 (t, *J* = 12.1 Hz, 1H)	3.70	3.85	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.07
GlcNAc2	101.18
Man3	100.34
Man4	99.47
Man4'	96.94
GlcNAc5	99.33
GlcNAc5'	99.41
Gal6	102.86
Gal6'	102.50

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac7	N/R	N/R	39.54	68.26	51.59	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-	174.92, 174.63, 174.55, 173.76
NHC(O)<u>CH_3</u>	2.19 – 1.87 (m, 15H)	22.25, 22.14, 21.95, 21.89
Aromatic	7.52 – 7.34 (m, 5H)	136.20, 128.68, 128.29, 127.71
<u>CH_2</u>-Ph	5.14 (q, *J* = 11.9 Hz, 2H)	66.99
NH-<u>C</u>OO-	-	157.73
NH-<u>CH</u>-COOH	4.44 (dd, *J* = 8.7, 4.6 Hz, 1H)	51.95
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH_2</u>-CH	2.84 (dd, *J* = 15.6, 4.4 Hz, 1H), 2.68 (dd, *J* = 15.7, 8.8 Hz, 1H)	37.93
<u>C</u>(O)-CH_2-CH	-	173.20

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{85}H_{131}N_7O_{58}$, $[M-2H]^{2-}$: 1088.8764, found 1088.8828.

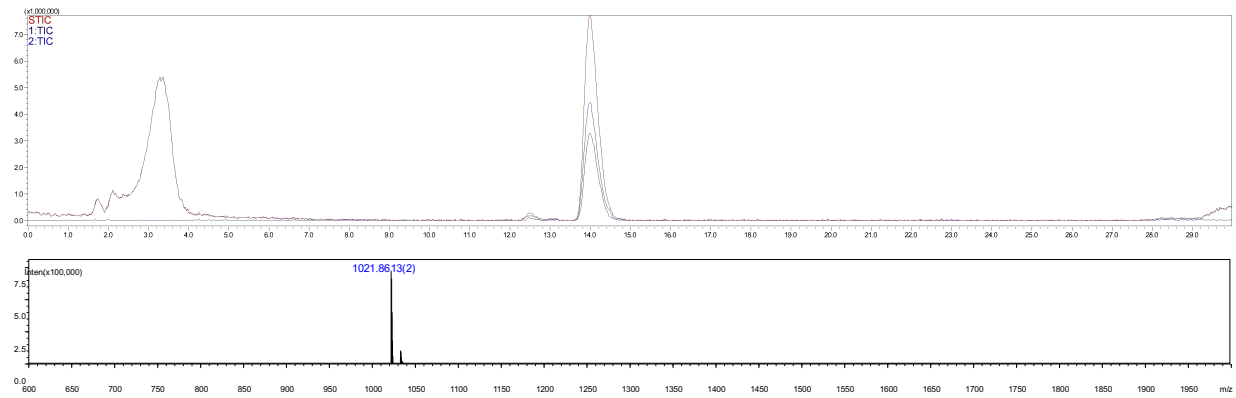
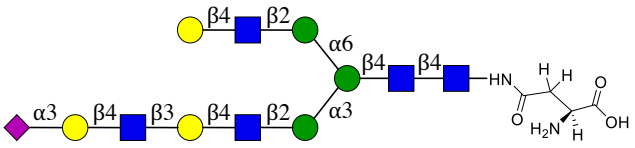


Figure S12. Analytical HPLC-MS chromatogram of compound **35**. The retention time = 14.0 min.

Compound 36

36 was synthesized from **21** (3.0 mg) using the general procedures **2.2 f**, **h**, **c** for the installation of α2,3-sialic acid with ST3Gal4, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give product **33**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **33** was removed by hydrogenation (**2.2 i**) to give final product **36** as a white fluffy solid (1.4 mg, 41% yield for four steps). ESI TOF-MS *m/z* calculated for $C_{91}H_{148}N_8O_{66}$, $[M-2H]^{2-}$: 1204.4241, found 1204.4245.



36

NMR and MS analysis of compound **33**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	4.99 (d, *J* = 9.7 Hz, 1H)	3.76	N/R[b]	N/R	N/R	N/R
GlcNAc2	4.54 (d, *J* = 8.0 Hz, 1H)	3.73	3.72	N/R	N/R	N/R
Man3	4.71 (d, *J* = 6.4 Hz, 1H)	4.19 (d, *J* = 2.6 Hz, 1H)	3.71	N/R	N/R	3.90, 3.73
Man4	5.05	4.13 (d, *J* = 3.5 Hz, 1H)	3.83	N/R	N/R	N/R
Man4'	4.87 (s, 1H)	4.05	3.89	N/R	N/R	N/R
GlcNAc5	4.53 – 4.47 (m, 3H)	3.68	N/R	N/R	N/R	N/R
GlcNAc5'	4.53 – 4.47 (m, 3H)	3.68	N/R	N/R	N/R	N/R
Gal6	4.44 – 4.36 (m, 3H)	3.47	3.66	4.10	N/R	N/R
Gal6'	4.53 – 4.47 (m, 3H)	3.51	N/R	N/R	N/R	N/R
GlcNAc7	4.62 (d, *J* = 8.3 Hz, 1H)	4.13 (d, *J* = 3.5 Hz, 1H)	N/R	N/R	N/R	N/R
Gal8	4.44 – 4.36 (m, 3H)	3.51	N/R	4.06	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac9	-[a]	-	2.69 (dd, *J* = 12.5, 4.6 Hz, 1H), 1.74 (t, *J* = 12.2 Hz, 1H)	3.62	3.79	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.01
GlcNAc2	101.09
Man3	100.28
Man4	99.40
Man4'	96.84
GlcNAc5	99.26
GlcNAc5'	99.26
Gal6	102.75
Gal6'	102.33
GlcNAc7	102.71
Gal8	102.75

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac9	N/R	N/R	39.44	68.21	51.48	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-	174.81, 174.73, 174.57, 174.48
NHC(O)<u>CH$_3$</u>	2.12 – 1.79 (m, 18H)	22.16, 22.04, 21.99, 21.86, 21.79
Aromatic	7.49 – 7.25 (m, 5H)	136.08, 128.59, 128.22, 127.60
<u>CH$_2$</u>-Ph	5.11 – 5.02 (m, 2H)	66.94
NH-<u>C</u>OO-	-	157.69
NH-<u>CH</u>-COOH	4.44 – 4.36 (m, 3H)	51.54
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.81 – 2.75 (m, 1H), 2.65 – 2.60 (m, 1H)	37.66
<u>C</u>(O)-CH$_2$-CH	-	173.69

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{99}H_{154}N_8O_{68}$, $[M-2H]^{2-}$: 1271.4425, found 1271.4403.

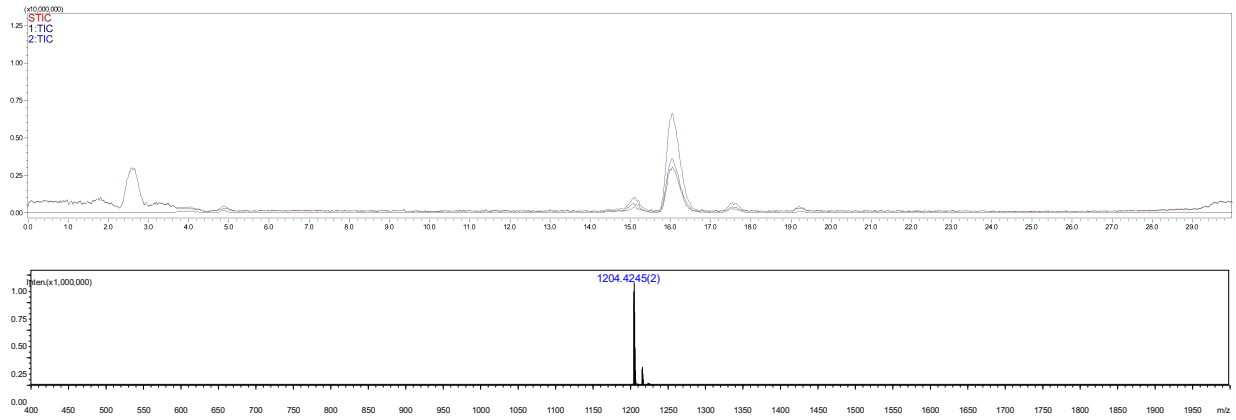
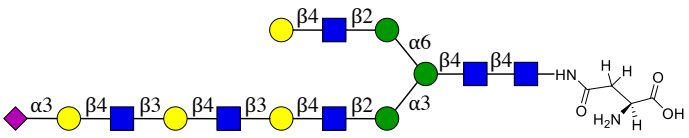


Figure S13. Analytical HPLC-MS chromatogram of compound **36**. The retention time = 16.0 min.

Compound 37

37 was synthesized from **22** (3 mg, 1.0 eq) using the general procedures **2.2 f**, **h**, **c** for the installation of α2,3-sialic acid with ST3Gal4, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give intermediate product **34**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz group protecting group of **34** was removed by hydrogenation to give final product **37** as a white fluffy solid (2.3 mg, 66% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{105}H_{171}N_9O_{76}$, $[M-2H]^{2-}$: 1386.9902, found 1386.9860.



37

NMR and MS analysis of compound **34**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.7 Hz, 1H)	3.82	3.75	3.65	3.56	N/R[b]
GlcNAc2	4.61 (d, *J* = 7.9 Hz, 1H)	3.79	3.80	N/R	N/R	N/R
Man3	4.47	4.25 (d, *J* = 2.8 Hz, 1H)	3.77	N/R	N/R	3.97, 3.80
Man4	5.12	4.19 (d, *J* = 3.5 Hz, 1H)	3.91	N/R	N/R	N/R
Man4'	4.93 (s, 1H)	4.14 – 4.08 (m, 2H)	3.89	N/R	N/R	N/R
GlcNAc5	4.60 – 4.53 (m, 3H)	3.75	N/R	N/R	N/R	N/R
GlcNAc5'	4.60 – 4.53 (m, 3H)	3.75	N/R	N/R	N/R	N/R
Gal6	4.51 – 4.41 (m, 4H)	3.58	3.73	4.16 (s, 2H)	N/R	N/R
Gal6'	4.60 – 4.53 (m, 3H)	3.75	N/R	N/R	N/R	N/R
GlcNAc7	4.70 (d, *J* = 8.4 Hz, 1H)	3.81	N/R	N/R	N/R	N/R

Gal8	4.51 – 4.41 (m, 4H)	3.59	3.73	4.16 (s, 2H)	N/R	N/R
GlcNAc9	4.70 (d, *J* = 8.4 Hz, 1H)	3.81	N/R	N/R	N/R	N/R
Gal11	4.51 – 4.41 (m, 4H)	3.55	N/R	4.14 – 4.08 (m, 2H)	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac9	-[a]	-	2.76 (dd, *J* = 12.5, 4.6 Hz, 1H), 1.80 (t, *J* = 12.1 Hz, 1H)	3.70	3.86	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.05
GlcNAc2	101.18
Man3	100.33
Man4	99.47
Man4'	96.95
GlcNAc5	99.35
GlcNAc5'	99.35
Gal6	102.86
Gal6'	102.46
GlcNAc7	102.68
Gal8	102.86
GlcNAc9	102.71
Gal10	102.79

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac11	N/R	N/R	39.55	68.25	51.60	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH$_3$	-	174.92, 174.81, 174.72, 174.62, 174.54, 173.78
NHC(O)<u>CH$_3$</u>	2.25 – 1.86 (m, 21H)	22.26, 22.14, 22.10, 21.96, 21.89
Aromatic	7.52 – 7.32 (m, 5H)	136.20, 128.68, 128.29, 127.71
<u>CH$_2$</u>-Ph	5.18 – 5.09 (m, 2H)	66.99
NH-<u>C</u>OO-	-	157.73
NH-<u>CH</u>-COOH	4.51 – 4.41 (m, 4H)	51.95
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.84 (dd, *J* = 15.6, 4.3 Hz, 1H), 2.68 (dd, *J* = 15.3, 8.6 Hz, 1H)	37.94
<u>C</u>(O)-CH$_2$-CH	-	173.19

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{113}H_{177}N_9O_{78}$, $[M-2H]^{2-}$: 1454.0086, found 1453.9947.

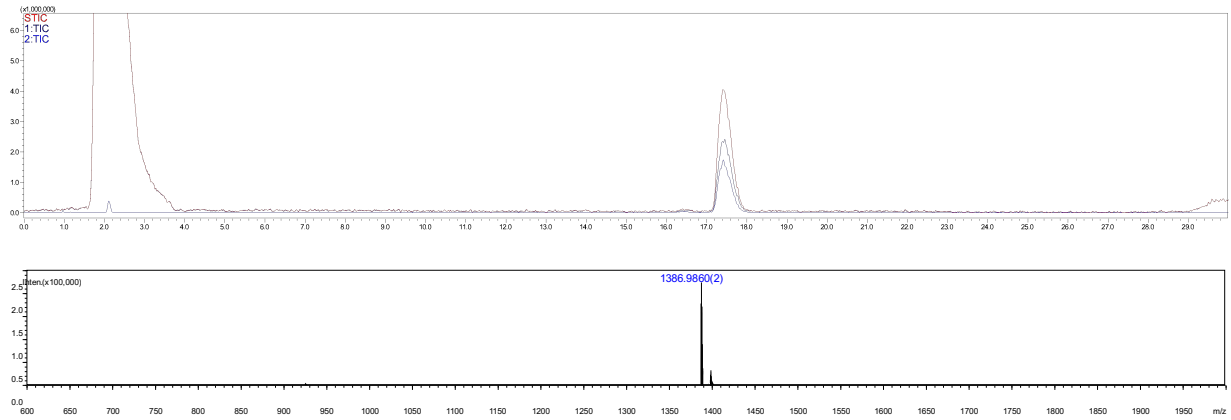
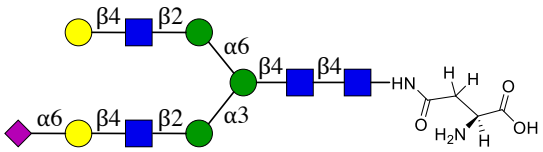


Figure S14. Analytical HPLC-MS chromatogram of compound **37**. The retention time = 17.5 min.

Compound 44

44 was synthesized from **20** (3.0 mg, 1.0 eq) using the general procedures **2.2 e**, **h**, **c** for the installation of α2,6-sialic acid with ST6Gal1, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give intermediate **41** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **41** was removed by hydrogentation (**2.2 i**) to give final product **44** as a white fluffy solid (1.9 mg, 52% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{77}H_{125}N_7O_{56}$, $[M-2H]^{2-}$: 1021.8580, found 1021.8526.



44

NMR and MS analysis of compound **41**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.02 (d, *J* = 9.9 Hz, 1H)	3.79	3.70	3.61	3.52	N/R[b]
GlcNAc2	4.61 – 4.52 (m, 3H)	3.74	3.76	N/R	N/R	N/R
Man3	4.74	4.23 (s, 1H)	3.75	N/R	N/R	3.93, 3.77
Man4	5.11	4.17 (s, 1H)	3.87	N/R	N/R	N/R
Man4'	4.90 (s, 1H)	4.11 – 4.06 (m, 1H)	3.87	N/R	N/R	N/R
GlcNAc5	4.61 – 4.52 (m, 3H)	3.74	N/R	N/R	N/R	N/R
GlcNAc5'	4.61 – 4.52 (m, 3H)	3.74	N/R	N/R	N/R	N/R
Gal6	4.42 (dd, *J* = 7.8, 1.7 Hz, 1H)	3.51	N/R	N/R	N/R	N/R
Gal6'	4.44 (d, *J* = 7.8 Hz, 1H)	3.51	N/R	N/R	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac7	-[a]	-	2.64 (dd, *J* = 12.4, 4.6 Hz, 1H), 1.69 (t, *J* = 12.2 Hz, 1H)	3.62	3.78	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.03
GlcNAc2	101.18
Man3	100.32
Man4	99.46
Man4'	96.94
GlcNAc5	99.23
GlcNAc5'	99.34
Gal6	103.45
Gal6'	102.85

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac7	N/R	100.03	39.96	68.13	51.79	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-	174.81, 174.69, 174.63, 174.53
NHC(O)<u>CH_3</u>	2.26 – 1.77 (m, 15H)	22.34, 22.25, 22.13, 21.95, 21.89
Aromatic	7.52 – 7.27 (m, 5H)	136.22, 128.66, 128.27, 127.73
<u>CH_2</u>-Ph	5.10 (d, *J* = 13.8 Hz, 2H)	66.92
NH-<u>C</u>OO-	-	157.70
NH-<u>CH</u>-COOH	4.34 (dd, *J* = 9.1, 4.2 Hz, 1H)	52.50
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH_2</u>-CH	2.82 – 2.76 (m, 1H), 2.61 – 2.56 (m, 1H)	38.23
<u>C</u>(O)-CH_2-CH	-	173.43

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{85}H_{131}N_7O_{58}$, $[M-2H]^{2-}$: 1088.8764, found 1088.8744.

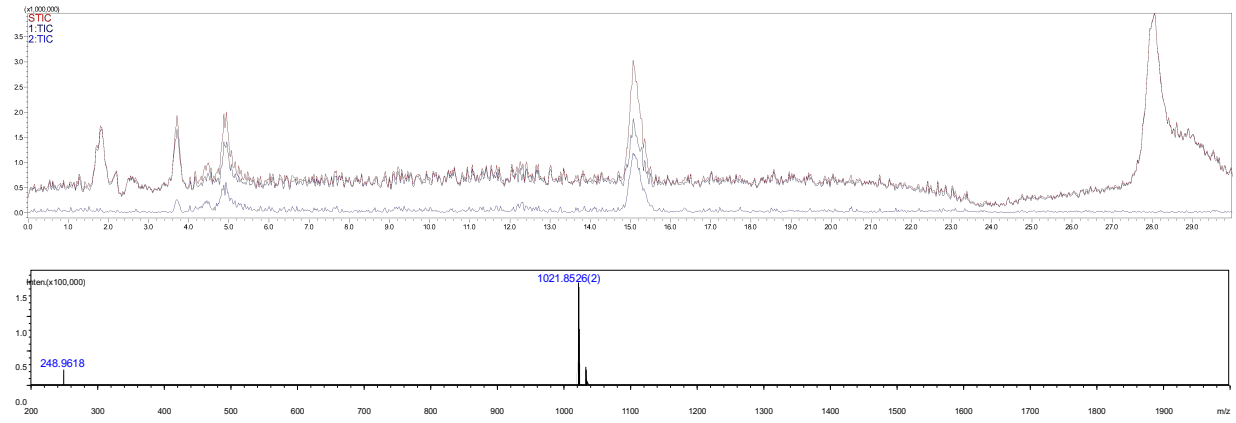
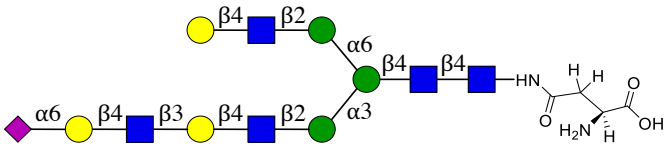


Figure S15. Analytical HPLC-MS chromatogram of compound **44**. The retention time = 15.2 min.

Compound 45

45 was synthesized from **21** (3 mg) using the general procedures **2.2 e**, **h**, **c** and **i** for the installation of α2,6-sialic acid with ST6Gal1, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give compound **42** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **42** was removed by hydrogentation (**2.2 i**) to give final product **45** as a white fluffy solid (2.2 mg, 61% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{91}H_{148}N_8O_{66}$, $[M-2H]^{2-}$: 1204.4241, found 1204.4341.



45

NMR and MS analysis of compound **42**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.9 Hz, 1H)	3.82	3.73	3.65	3.55	N/R[b]
GlcNAc2	4.64 – 4.53 (m, 3H)	3.79	3.79	N/R	N/R	N/R
Man3	4.76	4.25 (d, *J* = 2.7 Hz, 1H)	3.78	N/R	N/R	3.96, 3.79
Man4	5.12	4.19 (d, *J* = 3.3 Hz, 1H)	3.91	N/R	N/R	N/R
Man4'	4.93 (s, 1H)	4.13 – 4.09 (m, 1H)	3.90	N/R	N/R	N/R
GlcNAc5	4.64 – 4.53 (m, 3H)	3.74	N/R	N/R	N/R	N/R
GlcNAc5'	4.64 – 4.53 (m, 3H)	3.74	N/R	N/R	N/R	N/R

Gal6	4.51 – 4.42 (m, 3H)	3.56	3.73	4.16 (d, *J* = 3.1 Hz, 1H)	N/R	N/R
Gal6'	4.51 – 4.42 (m, 3H)	3.56	N/R	N/R	N/R	N/R
GlcNAc7	4.73 (d, *J* = 7.7 Hz, 1H)	3.80	N/R	N/R	N/R	N/R
Gal8	4.51 – 4.42 (m, 3H)	3.56	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac9	-[a]	-	2.67 (dd, *J* = 12.5, 4.6 Hz, 1H), 1.72 (t, *J* = 12.1 Hz, 1H)	3.65	3.81	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.01
GlcNAc2	101.17
Man3	100.34
Man4	99.47
Man4'	96.94
GlcNAc5	99.38
GlcNAc5'	99.38
Gal6	102.86
Gal6'	102.86
GlcNAc7	102.51
Gal8	103.38

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac9	N/R	N/R	40.00	68.14	51.79	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH$_3$	-	174.82, 174.65, 174.54
NHC(O)<u>CH$_3$</u>	2.36 – 1.79 (m, 18H)	22.25, 22.20, 22.14, 21.94, 21.90
Aromatic	7.55 – 7.31 (m, 5H)	136.24, 128.67, 128.27, 127.75
<u>CH$_2$</u>-Ph	5.18 – 5.07 (m, 2H)	66.87
NH-<u>C</u>OO-	-	N/R
NH-<u>CH</u>-COOH	4.34 (dd, *J* = 9.5, 3.9 Hz, 1H)	52.76
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.84 – 2.78 (m, 1H), 2.60 (dd, *J* = 15.4, 9.3 Hz, 1H)	38.40
<u>C</u>(O)-CH$_2$-CH	-	N/R

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{99}H_{154}N_8O_{68}$, $[M-2H]^{2-}$: 1271.4425, found 1271.4336.

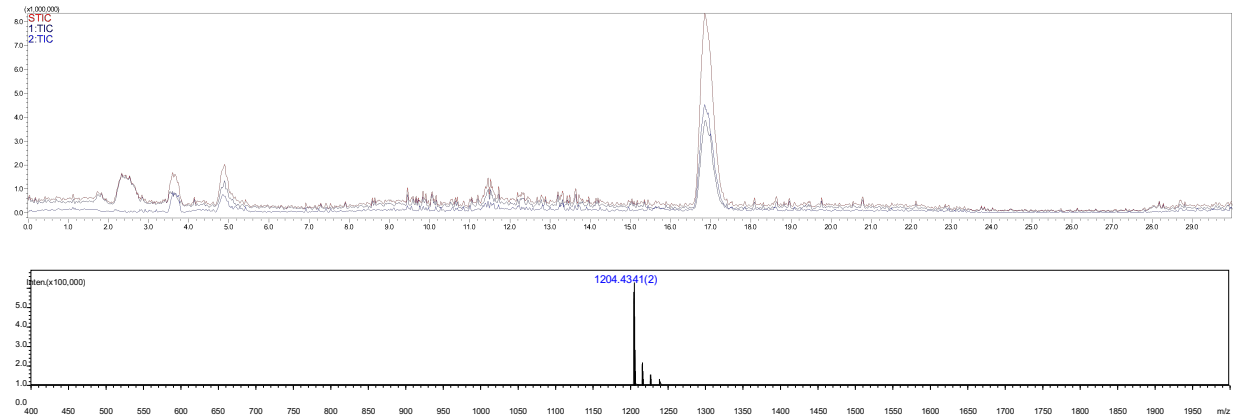
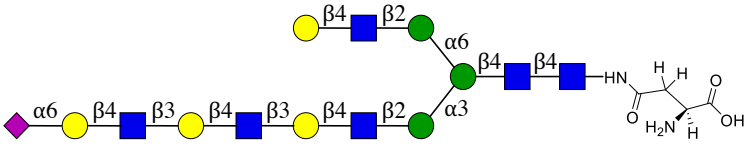


Figure S16. Analytical HPLC-MS chromatogram of compound **45**. The retention time = 17.0 min.

Compound 46

46 was synthesized from **22** (3 mg, 1.0 eq) using the general procedures **2.2 e**, **h**, **c** for the installation of α2,6-sialic acid with ST6Gal1, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give intermediate product **43** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **43** was removed by hydrogentation (**2.2 i**) to give final product **46** as a white fluffy solid (1.7 mg, 50% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{125}H_{171}N_9O_{76}$, $[M-2H]^{2-}$: 1386.9902, found 1386.9802.



46

NMR and MS analysis of compound **43**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 9.6 Hz, 1H)	3.81	3.72	3.64	3.54	N/R[b]
GlcNAc2	4.64 – 4.53 (m, 3H)	3.78	3.79	N/R	N/R	N/R
Man3	4.76	4.24 (d, *J* = 2.8 Hz, 1H)	3.76	N/R	N/R	3.96, 3.78
Man4	5.11	4.18 (d, *J* = 3.4 Hz, 1H)	3.89	N/R	N/R	N/R
Man4'	4.92 (s, 1H)	4.12 – 4.09 (m, 1H)	3.88	N/R	N/R	N/R
GlcNAc5	4.64 – 4.53 (m, 3H)	3.74	N/R	N/R	N/R	N/R
GlcNAc5'	4.64 – 4.53 (m, 3H)	3.74	N/R	N/R	N/R	N/R
Gal6	4.51 – 4.40 (m, 4H)	3.54	3.72	4.15 (d, *J* = 3.0 Hz,	N/R	N/R

				2H)		
Gal6'	4.51 – 4.40 (m, 4H)	3.54	N/R	N/R	N/R	N/R
GlcNAc7	4.72 (d, *J* = 7.6 Hz, 1H)	3.79	N/R	N/R	N/R	N/R
Gal8	4.51 – 4.40 (m, 4H)	3.53	3.72	4.15 (d, *J* = 3.0 Hz, 2H)	N/R	N/R
GlcNAc9	4.69 (d, *J* = 8.3 Hz, 1H)	3.79	N/R	N/R	N/R	N/R
Gal10	4.51 – 4.40 (m, 4H)	3.58	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac11	-[a]	-	2.66 (dd, *J* = 12.5, 4.5 Hz, 1H), 1.71 (t, *J* = 12.1 Hz, 1H)	3.64	3.80	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.03
GlcNAc2	101.21
Man3	100.31
Man4	99.46
Man4'	96.91
GlcNAc5	99.40
GlcNAc5'	99.40
Gal6	102.83
Gal6'	102.83
GlcNAc7	102.56
Gal8	99.40
GlcNAc9	102.71
Gal10	103.36

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac10	N/R	N/R	39.99	68.16	51.77	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH$_3$	-	174.81, 174.62, 174.53
NHC(O)<u>CH$_3$</u>	2.42 – 1.77 (m, 21H)	22.24, 22.19, 22.13, 22.08, 21.94, 21.90
Aromatic	7.51 – 7.33 (m, 5H)	136.23, 128.66, 128.26, 127.74
<u>CH$_2$</u>-Ph	5.16 – 5.06 (m, 2H)	66.89
NH-<u>C</u>OO-	-	N/R
NH-<u>CH</u>-COOH	4.32 (dd, *J* = 9.6, 4.2 Hz, 1H)	52.91
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.80 (dd, *J* = 15.5, 4.2 Hz, 1H), 2.58 (dd, *J* = 15.4, 9.5 Hz, 1H)	38.47

C(O)-CH$_2$-CH	-	173.45

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{133}H_{177}N_9O_{78}$, $[M-2H]^{2-}$: 1454.0086, found 1454.0188.

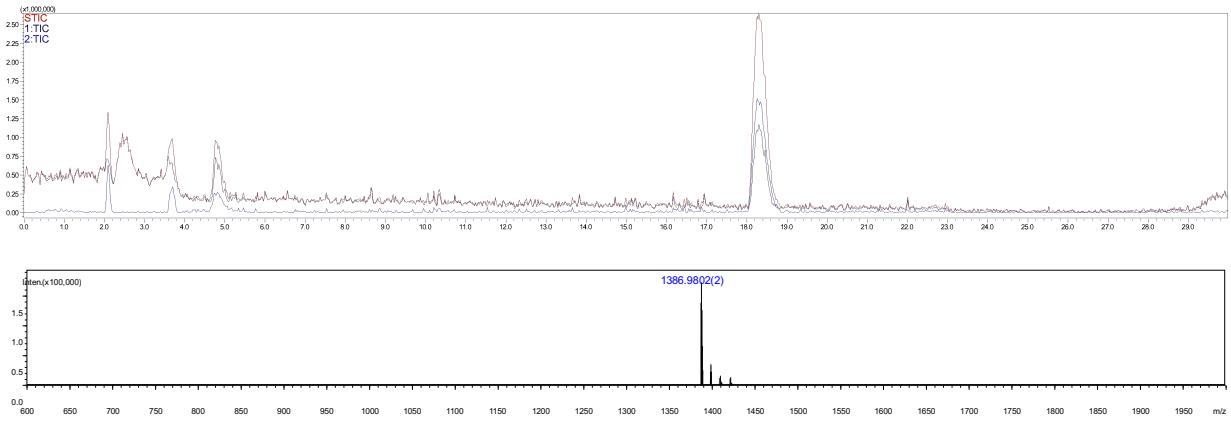
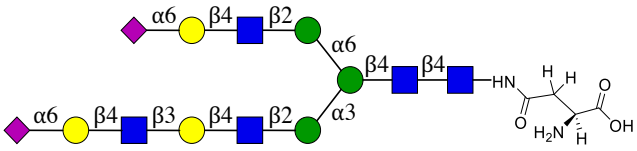


Figure S17. Analytical HPLC-MS chromatogram of compound **46**. The retention time = 18.3 min.

Compound 47b

47b was prepared from **42** (1.0 mg) using the general procedures **2.2 e** for the installation of α2,6-sialic acid with ST6Gal1 to give intermediate products **47a** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **47a** was removed by hydrogentation (**2.2 i**) to give final product **47b** as a white fluffy solid (1 mg, 90% yield for two steps). ESI TOF-MS *m/z* calculated for $C_{102}H_{165}N_9O_{74}$, $[M-2H]^{2-}$: 1349.9718, found 1349.9654.



47

NMR and MS analysis of compound **47a**

^{1}H (600 MHz, D$_2$O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.09 (d, *J* = 9.8 Hz, 1H)	3.86	3.77	3.68	3.59	N/R[b]
GlcNAc2	4.65	3.77	3.82	N/R	N/R	N/R
Man3	4.81	4.29 (s, 1H)	3.80	N/R	N/R	4.0, 3.83
Man4	5.15	4.23 (d, *J* = 3.5 Hz,	3.94	N/R	N/R	N/R

		1H)				
Man4'	4.98 (s, 1H)	4.17 – 4.13 (m, 1H)	3.92	N/R	N/R	N/R
GlcNAc5	4.64	3.77	N/R	N/R	N/R	N/R
GlcNAc5'	4.62	3.77	N/R	N/R	N/R	N/R
Gal6	4.49	3.63	3.77	4.19 (d, *J* = 3.2 Hz, 1H)	N/R	N/R
Gal6'	4.49	3.57	N/R	N/R	N/R	N/R
GlcNAc7	4.77	3.84	N/R	N/R	N/R	N/R
Gal8	4.49	3.57	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac7'	-[a]	-	2.71 (dd, *J* = 12.5, 4.7 Hz, 2H), 1.76 (t, *J* = 12.2 Hz, 2H)	3.70	51.84	N/R	N/R	N/R	N/R
Neu5Ac9	-	-	2.71 (dd, *J* = 12.5, 4.7 Hz, 2H), 1.76 (t, *J* = 12.2 Hz, 2H)	3.70	51.84	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.06
GlcNAc2	101.18
Man3	100.37
Man4	99.54
Man4'	96.88
GlcNAc5	99.21
GlcNAc5'	99.39
Gal6	102.87
Gal6'	103.43
GlcNAc7	102.50
Gal8	103.43

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac7'	N/R	N/R	40.00	68.16	51.82	N/R	N/R	N/R	N/R
Neu5Ac9	N/R	N/R	40.00	68.16	51.82	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH_3	-	174.87, 174.64
NHC(O)<u>CH_3</u>	2.15 – 1.93 (m, 21H)	22.25, 22.20
Aromatic	7.57 – 7.35 (m, 5H)	128.71, 128.33, 127.72
<u>CH_2</u>-Ph	5.21 – 5.12 (m, 2H)	67.04
NH-<u>C</u>OO-	-	N/R
NH-<u>CH</u>-COOH	4.52	51.48
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH_2</u>-CH	2.88 (d, *J* = 16.3 Hz, 1H),	37.75

	2.78 – 2.73 (m, 1H)	
C(O)-CH$_2$-CH	-	N/R

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{110}H_{171}N_9O_{76}$, $[M-2H]^{2-}$: 1416.9902, found 1416.9864.

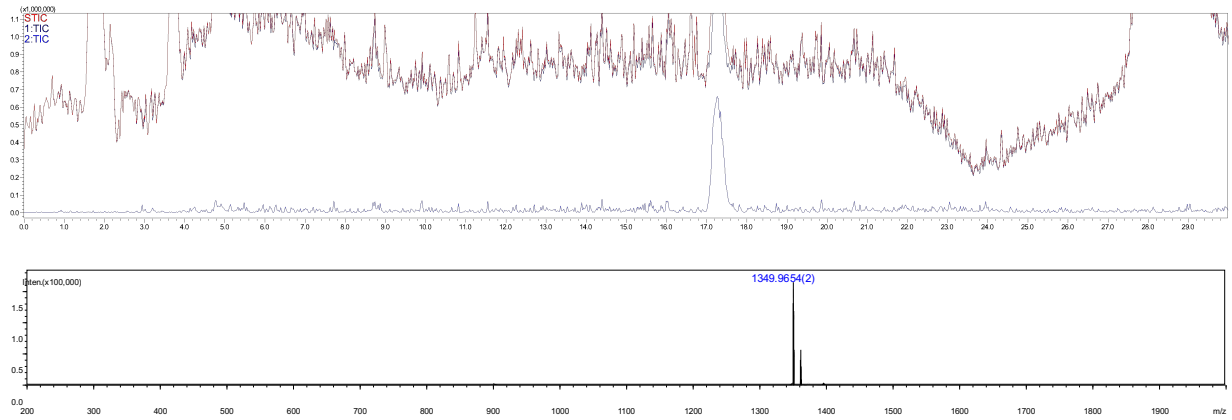
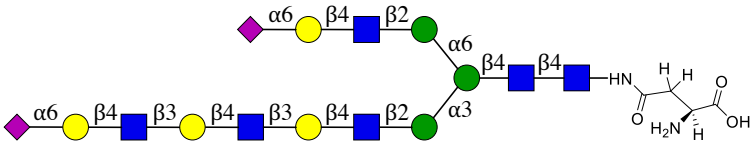


Figure S18. Analytical HPLC-MS chromatogram of compound **47b**. The retention time = 17.3 min.

Compound 48b

48b was prepared from **43** (1.0 mg) using the general procedures **2.2 e** for the installation of α2,6-sialic acid with ST6Gal1 to give intermediate product **48a**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **48a** was removed by hydrogentation (**2.2 i**) to give final product **48b** as a white fluffy solid (1.0 mg, 93% yield for two steps). ESI TOF-MS *m/z* calculated for $C_{116}H_{191}N_{10}O_{84}$, $[M-2H]^{2-}$: 1532.5379, found 1532.5301.



48

NMR and MS analysis of compound **48a**

^{1}H (600 MHz, D$_2$O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.08 (d, *J* = 9.7 Hz, 1H)	3.85	3.77	3.68	3.59	N/R[b]
GlcNAc2	4.64	3.78	3.82	N/R	N/R	N/R
Man3	4.80	4.28 (d, *J* = 2.6 Hz,	N/R	N/R	N/R	4.00,

		1H)				3.82
Man4	5.51	4.22 (d, *J* = 3.5 Hz, 1H)	N/R	N/R	N/R	N/R
Man4'	4.98 (s, 1H)	4.17 – 4.13 (m, 1H)	N/R	N/R	N/R	N/R
GlcNAc5	4.64	3.78	N/R	N/R	N/R	N/R
GlcNAc5'	4.61	3.78	N/R	N/R	N/R	N/R
Gal6	4.50	3.62	3.76	4.19 (d, *J* = 2.9 Hz, 3H)	N/R	N/R
Gal6'	4.48	3.57	N/R	N/R	N/R	N/R
GlcNAc7	4.76	3.83	N/R	N/R	N/R	N/R
Gal8	4.50	3.62	3.76	4.19 (d, *J* = 2.9 Hz, 3H)	N/R	N/R
GlcNAc9	4.73	3.83	N/R	N/R	N/R	N/R
Gal10	4.48	3.57	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac7'	-[a]	-	2.75 – 2.66 (m, 3H), 1.75 (t, *J* = 12.2 Hz, 2H)	3.70	3.84	N/R	N/R	N/R	N/R
Neu5Ac11	-	-	2.75 – 2.66 (m, 3H), 1.75 (t, *J* = 12.2 Hz, 2H)	3.70	3.84	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.12
GlcNAc2	101.20
Man3	100.43
Man4	99.51
Man4'	96.89
GlcNAc5	99.23
GlcNAc5'	99.41
Gal6	102.88
Gal6'	103.46
GlcNAc7	102.54
Gal8	102.88
GlcNAc9	102.65
Gal10	103.46

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac7'	N/R	N/R	40.02	68.15	51.84	N/R	N/R	N/R	N/R
Neu5Ac11	N/R	N/R	40.02	68.15	51.84	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NHC(O)CH_3	-	174.86, 174.78, 174.64

NHC(O)$\underline{CH_3}$	2.15 – 1.91 (m, 24H)	22.37, 22.29, 22.24, 22.20, 22.14, 22.00, 21.93
Aromatic	7.55 – 7.36 (m, 5H)	136.24, 128.71, 128.32, 127.75
$\underline{CH_2}$-Ph	5.21 – 5.11 (m, 2H)	67.01
NH-$\underline{C}$OO-	-	157.74
NH-$\underline{CH}$-COOH	4.46	52.09
NH-CH-$\underline{C}$OOH	-	N/R
C(O)-$\underline{CH_2}$-CH	2.90 – 2.84 (m, 1H), 2.75 – 2.66 (m, 3H)	38.04
$\underline{C}$(O)-CH_2-CH	-	N/R

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{124}H_{197}N_{10}O_{86}$, $[M-2H]^{2-}$: 1599.5563, found 1599.5423.

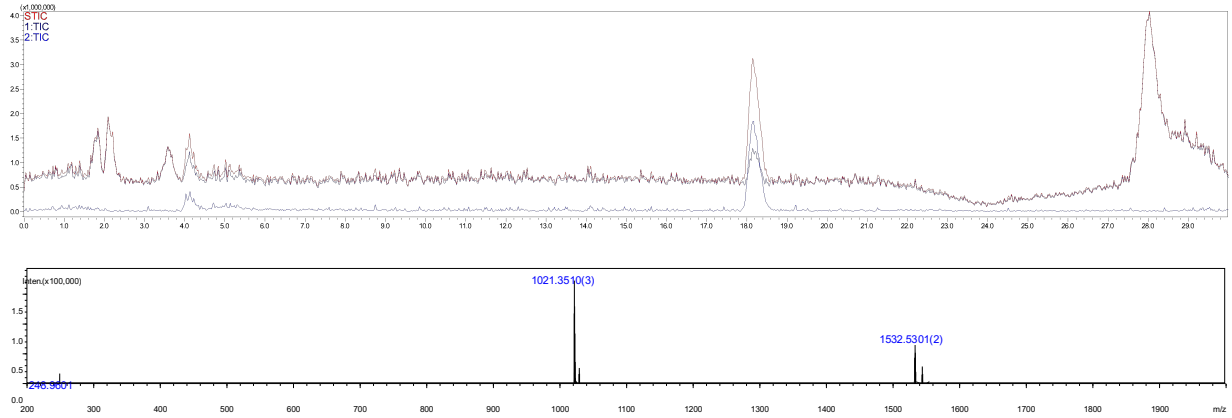
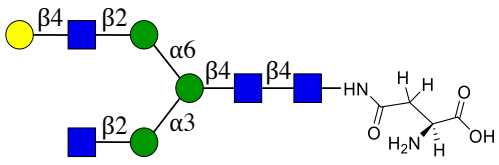


Figure S19. Analytical HPLC-MS chromatogram of compound **48b**. The retention time = 18.2 min.

Compound 52

52 was prepared by acetylation of **23** (1 mg, 1.0 eq) according to the general procedures **2.2 h** to give intermediate product **49**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **49** was removed by hydrogentation (**2.2 i**) to give final product **52** as a white fluffy solid (0.7 mg, 78% yield for two steps). ESI TOF-MS *m/z* calculated for $C_{60}H_{98}N_6O_{43}$, $[M-2H]^{2-}$: 795.2839, found 795.2879.



52

NMR and MS analysis of compound **49**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.7 Hz, 1H)	3.83	3.74	3.65	3.56	3.81 3.63
GlcNAc2	4.61 (d, *J* = 8.0 Hz, 1H)	3.79	3.80	3.74	3.61	N/R[b]
Man3	4.77	4.25 (s, 1H)	3.78	N/R	3.62	3.97 3.79
Man4	5.12	4.19 (s, 1H)	3.90	3.51	3.75	3.92 3.63
Man4'	4.93 (s, 1H)	4.12 (s, 1H)	3.89	3.50	3.62	N/R
GlcNAc5	4.56 (d, *J* = 8.4 Hz, 1H)	3.70	N/R	N/R	N/R	N/R
GlcNAc5'	4.58 (d, *J* = 8.1 Hz, 1H)	3.75	N/R	3.73	N/R	N/R
Gal6'	4.48 (d, *J* = 7.7 Hz, 2H)	3.54	3.67	3.93	3.74	3.77

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.06	53.66	72.74	78.48	76.12	59.76
GlcNAc2	101.17	54.87	65.55	79.38	74.31	N/R
Man3	100.35	70.10	80.35	N/R	74.28	65.55
Man4	99.52	76.34	69.31	67.21	73.47	61.55
Man4'	96.95	76.22	69.36	67.25	72.76	N/R
GlcNAc5	99.52	55.24	N/R	N/R	N/R	N/R
GlcNAc5'	99.35	54.77	N/R	78.42	N/R	N/R
Gal6'	102.86	70.87	72.42	68.44	75.26	60.92

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-[a]	174.67, 174.63, 174.54
NHC(O)<u>CH_3</u>	2.17 – 1.88 (m, 12H)	22.25, 22.24, 22.14, 21.89
Aromatic	7.53 – 7.32 (m, 5H)	136.18, 128.68, 128.30, 127.69
<u>CH_2</u>-Ph	5.18 – 5.10 (m, 2H)	67.02
NH-<u>C</u>OO-	-	157.73
NH-<u>CH</u>-COOH	4.48 (d, *J* = 7.7 Hz, 2H)	51.62
NH-CH-<u>C</u>OOH	-	175.88
C(O)-<u>CH_2</u>-CH	2.87 – 2.81 (m, 1H), 2.71 (dd, *J* = 15.7, 8.4 Hz, 1H)	37.76
<u>C</u>(O)-CH_2-CH	-	173.06

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{68}H_{104}N_6O_{45}$, $[M-2H]^{2-}$: 862.3023, found 862.2961.

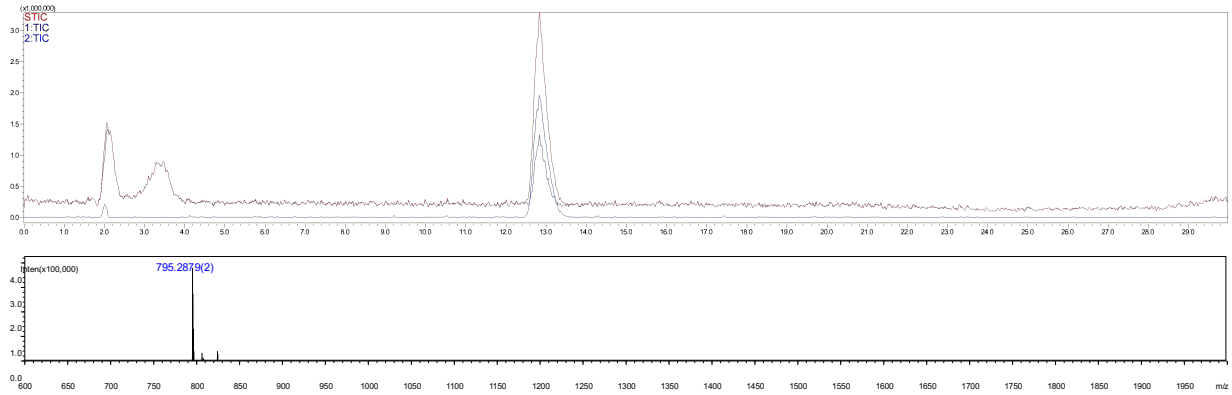
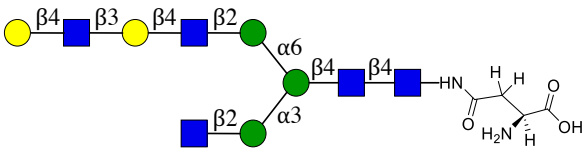


Figure S20. Analytical HPLC-MS chromatogram of compound **52**. The retention time = 12.8 min.

Compound 53

53 was prepared by acetylation of **24** (1 mg, 1.0 eq) by the general procedures **2.2 h** to give intermediate product **50**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **50** was removed by hydrogentation (**2.2 i**) to give final product **53** as a white fluffy solid (0.5 mg, 51% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{74}H_{121}N_7O_{53}$, $[M\text{-}2H]^{2-}$: 977.8500, found 977.8415.



53

NMR and MS analysis of compound **50**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.7 Hz, 1H)	3.83	3.74	3.66	3.55	N/R[b]
GlcNAc2	4.62 (d, *J* = 8.1 Hz, 1H)	3.80	3.80	3.75	N/R	N/R
Man3	4.77	4.26 (d, *J* = 2.7 Hz, 1H)	3.78	N/R	N/R	3.97, 3.80
Man4	5.12	4.20 (d, *J* = 3.6 Hz, 1H)	3.90	3.51	N/R	N/R
Man4'	4.93 (s, 1H)	4.11 (d, *J* = 3.6 Hz, 1H)	3.90	3.50	N/R	N/R
GlcNAc5	4.56 (d, *J* = 8.4 Hz, 1H)	3.70	N/R	N/R	N/R	N/R
GlcNAc5'	4.59 (d, *J* = 8.2 Hz, 1H)	3.75	N/R	3.72	N/R	N/R
Gal6'	4.47 (d, *J* = 8.0 Hz, 1H)	3.59	3.73	N/R	N/R	N/R
GlcNAc7'	4.71 (d, *J* = 8.4 Hz, 1H)	3.81	N/R	N/R	N/R	N/R
Gal8'	4.49 (d, *J* = 7.9 Hz, 1H)	3.55	3.67	3.93	3.74	3.76

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.06	53.65	72.66	78.46	76.11	N/R
GlcNAc2	101.16	54.86	65.56	79.38	N/R	N/R
Man3	100.35	70.10	80.38	N/R	N/R	65.66
Man4	99.51	76.33	69.30	67.21	N/R	N/R
Man4'	96.95	76.22	69.36	67.25	N/R	N/R
GlcNH$_2$5	99.51	55.24	N/R	N/R	N/R	N/R
GlcNAc5'	99.36	54.72	N/R	78.46	N/R	N/R
Gal6'	102.89	69.87	81.98	N/R	N/R	N/R
GlcNAc7'	102.67	55.10	N/R	N/R	N/R	N/R
Gal8'	102.77	70.88	72.42	68.46	75.26	60.94

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.81, 174.67, 174.62, 174.54
NHC(O)<u>CH$_3$</u>	2.23 – 1.83 (m, 15H)	22.25, 22.14, 22.10, 21.89
Aromatic	7.54 – 7.32 (m, 5H)	136.21, 128.67, 128.28, 127.72
<u>CH$_2$</u>-Ph	5.19 – 5.08 (m, 2H)	66.95
NH-<u>C</u>OO-	-	157.71
NH-<u>CH</u>-COOH	4.41 (dd, *J* = 8.9, 4.4 Hz, 1H)	52.26
NH-CH-<u>C</u>OOH	-	176.58
C(O)-<u>CH$_2$</u>-CH	2.84 (dd, *J* = 15.7, 4.4 Hz, 1H) 2.66 (dd, *J* = 12.3, 6.6 Hz, 1H)	38.10
<u>C</u>(O)-CH$_2$-CH	-	173.31

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{82}H_{127}N_7O_{55}$, $[M-2H]^{2-}$: 1044.8684, found 1044.8757.

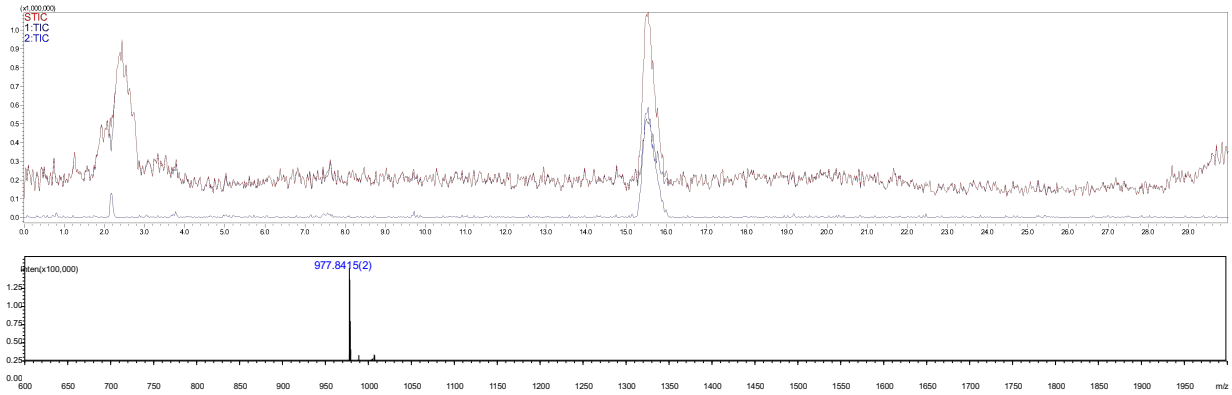
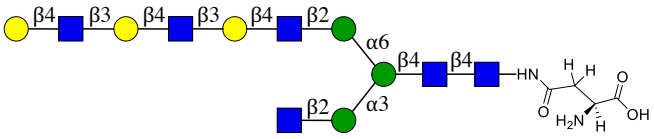


Figure S21. Analytical HPLC-MS chromatogram of compound **53**. The retention time = 15.6 min.

Compound 54

54 was prepared by acetylation of **25** (1.0 mg) according to the general procedures **2.2 h** to give intermediate product **51** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **51** was removed by hydrogentation (**2.2 i**) to give final product **54** as a white fluffy solid (0.8 mg, 88% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{88}H_{144}N_8O_{63}$, $[M\text{-}2H]^{2-}$: 1160.4161, found 1160.4119.



54

NMR and MS analysis of compound **51**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.7 Hz, 1H)	3.83	3.74	3.65	3.55	N/R[b]
GlcNAc2	4.61 (d, *J* = 8.2 Hz, 1H)	3.81	3.80	3.74	N/R	N/R
Man3	4.77	4.25 (d, *J* = 2.7 Hz, 1H)	3.78	N/R	N/R	3.97, 3.80
Man4	5.12	4.21 – 4.17 (m, 1H)	3.90	3.50	N/R	N/R
Man4'	4.93 (s, 1H)	4.11 (d, *J* = 3.6 Hz, 1H)	3.90	3.50	N/R	N/R
GlcNAc5	4.56 (d, *J* = 8.3 Hz, 1H)	3.70	N/R	N/R	N/R	N/R
GlcNAc5'	4.58 (d, *J* = 8.3 Hz, 1H)	3.75	N/R	3.72	N/R	N/R
Gal6'	4.52 – 4.42 (m, 3H)	N/R	3.73	N/R	N/R	N/R
GlcNAc7'	4.71 (d, *J* = 8.1 Hz, 2H)	3.80	N/R	N/R	N/R	N/R
Gal8'	4.52 – 4.42 (m, 3H)	N/R	3.73	N/R	N/R	N/R
GlcNAc9'	4.71 (d, *J* = 8.1 Hz, 2H)	3.80	N/R	N/R	N/R	N/R
Gal10'	4.52 – 4.42 (m, 3H)	3.55	3.67	3.93	3.73	3.75

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.06	53.66	72.64	78.47	76.12	N/R
GlcNAc2	101.17	54.87	65.56	79.38	N/R	N/R
Man3	100.35	70.10	80.37	N/R	N/R	65.65
Man4	99.51	76.33	69.31	67.21	N/R	N/R
Man4'	96.95	76.22	69.36	67.25	N/R	N/R
GlcNH$_2$5	99.51	55.24	N/R	N/R	N/R	N/R
GlcNAc5'	99.35	54.72	N/R	78.45	N/R	N/R
Gal6'	102.89	N/R	81.99	N/R	N/R	N/R
GlcNAc7'	102.67	55.10	N/R	N/R	N/R	N/R
Gal8'	102.80	N/R	81.99	N/R	N/R	N/R
GlcNAc9'	102.67	55.06	N/R	N/R	N/R	N/R
Gal10'	102.77	70.88	72.42	68.46	75.26	60.94

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.81, 174.67, 174.62, 174.53
NHC(O)<u>CH$_3$</u>	2.11 – 1.87 (m, 18H)	22.25, 22.14, 22.10, 21.89
Aromatic	7.54 – 7.34 (m, 5H)	136.19, 128.68, 128.30, 127.70
<u>CH$_2$</u>-Ph	5.18 – 5.09 (m, 2H)	67.01
NH-<u>C</u>OO-	-	157.72
NH-<u>CH</u>-COOH	4.46	51.78
NH-CH-<u>C</u>OOH	-	176.07
C(O)-<u>CH$_2$</u>-CH	2.84 (dd, *J* = 15.7, 4.5 Hz, 1H), 2.69 (dd, *J* = 15.9, 8.7 Hz, 1H)	37.85
<u>C</u>(O)-CH$_2$-CH	-	173.11

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{96}H_{150}N_8O_{65}$, $[M-2H]^{2-}$: 1227.4344, found 1227.4397.

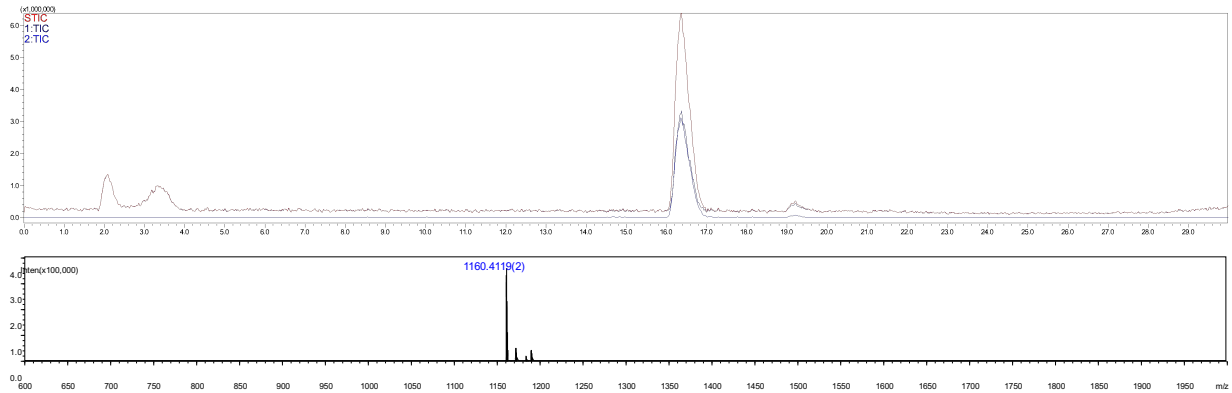
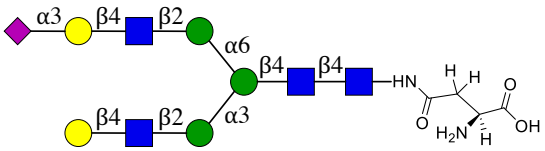


Figure S22. Analytical HPLC-MS chromatogram of compound **54**. The retention time = 16.4 min.

Compound 58b

58b was synthesized from **23** (3 mg, 1.0 eq) using the general procedures **2.2 f**, **h**, **c** for the installation of α2,3-sialic acid with ST3Gal4, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give the intermediate product **58a**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **58a** was removed by hydrogentation (**2.2 i**) to give final product **58b** as a white fluffy solid (1.6 mg, 43% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{77}H_{125}N_7O_{56}$, $[M-2H]^{2-}$: 1021.8580, found 1021.8615.



58

NMR and MS analysis of compound **58a**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.8 Hz, 1H)	3.82	3.74	3.65	3.57	N/R[b]
GlcNAc2	4.62 (d, *J* = 8.1 Hz, 1H)	3.80	3.80	N/R	N/R	N/R
Man3	4.77	4.26 (d, *J* = 2.9 Hz, 1H)	3.77	N/R	N/R	3.98, 3.79
Man4	5.13	4.20 (dd, *J* = 3.3, 1.6 Hz, 1H)	3.91	N/R	N/R	N/R
Man4'	4.93 (d, *J* = 1.7 Hz, 1H)	4.16 – 4.09 (m, 2H)	3.97	N/R	N/R	N/R
GlcNAc5	4.58 (dd, *J* = 7.8, 3.1 Hz, 2H)	3.76	N/R	N/R	N/R	N/R
GlcNAc5'	4.58 (dd, *J* = 7.8, 3.1 Hz, 2H)	3.76	N/R	N/R	N/R	N/R
Gal6	4.56 (d, *J* = 7.9 Hz, 1H)	3.55	N/R	N/R	N/R	N/R
Gal6'	4.47 (d, *J* = 7.7 Hz, 1H)	3.58	3.68	4.16 – 4.09 (m, 2H)	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac7'	-[a]	-	2.77 (dd, *J* = 12.5, 4.6 Hz, 1H), 1.81 (t, *J* = 12.1 Hz, 1H)	3.70	3,86	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.04
GlcNAc2	101.17
Man3	100.35
Man4	99.46

Man4'	97.02
GlcNAc5	99.36
GlcNAc5'	99.36
Gal6	102.53
Gal6'	102.83

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac7'	N/R	99.71	39.54	68.26	51.60	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NHC(O)CH_3	-	174.92, 174.67, 174.62, 174.55, 173.75
NHC(O)CH_3	2.21 – 1.88 (m, 15H)	22.25, 22.15, 21.95, 21.89
Aromatic	7.55 – 7.33 (m, 5H)	136.19, 128.68, 128.30, 127.70
CH_2-Ph	5.19 – 5.09 (m, 2H)	67.01
NH-COO-	-	157.74
NH-CH-COOH	4.45	51.76
NH-CH-COOH	-	N/R
C(O)-CH_2-CH	2.85 (dd, *J* = 15.6, 4.5 Hz, 1H), 2.70 (dd, *J* = 15.6, 8.6 Hz, 1H)	47.84
C(O)-CH_2-CH	-	173.11

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{85}H_{131}N_7O_{58}$, $[M-2H]^{2-}$: 1088.8764, found 1088.8769.

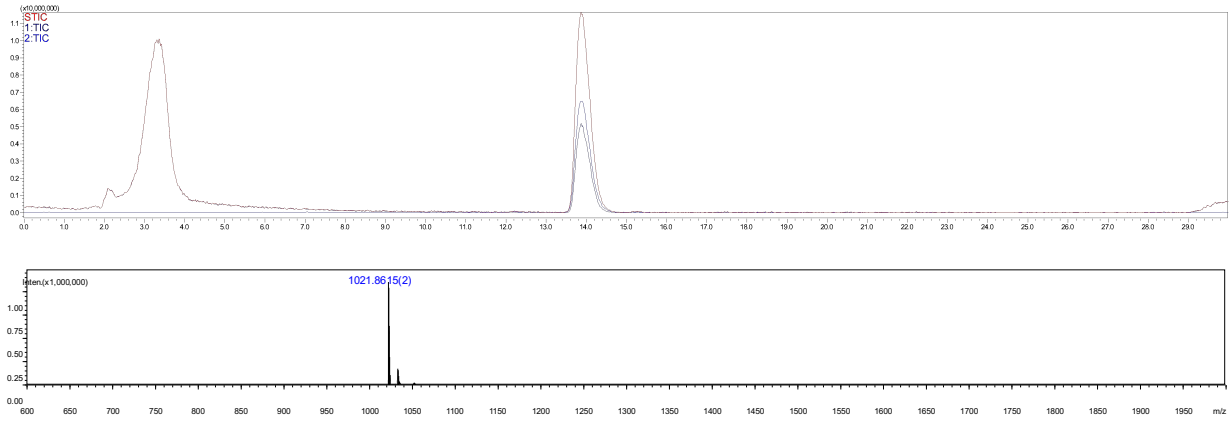
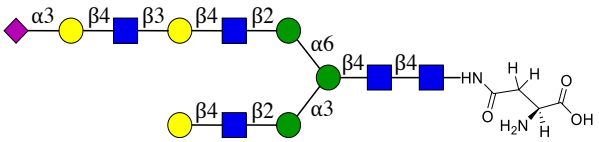


Figure S23. Analytical HPLC-MS chromatogram of compound **58b**. The retention time = 14.0 min.

Compound 59b

59b was synthesized from **24** (3 mg, 1.0 eq) using the general procedures **2.2 f**, **h**, **c** and **i** for the installation of α-2,3-sialic acid with ST3Gal4, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give **59a** that was purified using the described two-stage purification system **(2.2 j)**, The Cbz protecrting group of **59a** was removed by hydrogentation (**2.2 i**) to give final product the final product **59b** as a white fluffy solid (1.8 mg, 50% over four steps). ESI TOF-MS *m/z* calculated for $C_{91}H_{148}N_8O_{66}$, $[M-2H]^{2-}$: 1204.4241, found 1204.4299.



59

NMR and MS analysis of compound **59a** attaching Cbz group

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.03 (d, *J* = 9.7 Hz, 1H)	3.79	3.72	3.62	3.53	N/R[b]
GlcNAc2	4.58 (d, *J* = 8.1 Hz, 1H)	3.77	3.77	N/R	N/R	N/R
Man3	4.74	4.22 (d, *J* = 2.8 Hz, 1H)	3.75	N/R	N/R	3.94, 3.76
Man4	5.09	4.19 – 4.15 (m, 1H)	3.87	N/R	N/R	N/R
Man4'	4.90 (s, 1H)	4.12 – 4.05 (m, 2H)	3.86	N/R	N/R	N/R
GlcNAc5	4.57 – 4.52 (m, 3H)	3.72	N/R	N/R	N/R	N/R
GlcNAc5'	4.57 – 4.52 (m, 3H)	3.72	N/R	N/R	N/R	N/R
Gal6	4.57 – 4.52 (m, 3H)	3.55	N/R	N/R	N/R	N/R
Gal6'	4.47 – 4.40 (m, 2H)	3.56	3.70	4.14 (d, *J* = 3.2 Hz, 1H)	N/R	N/R
GlcNAc7'	4.67 (d, *J* = 8.4 Hz, 1H)	3.78	N/R	N/R	N/R	N/R
Gal8'	4.47 – 4.40 (m, 2H)	3.51	3.93	4.12 – 4.05 (m, 2H)	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac9'	-[a]	-	2.77 – 2.68 (m, 2H), 1.78 (t, *J* = 12.1 Hz, 1H)	3.67	3.83	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.06
GlcNAc2	101.17

Man3	100.36
Man4	99.46
Man4'	96.96
GlcNAc5	99.36
GlcNAc5'	99.36
Gal6	102.45
Gal6'	102.83
GlcNAc7'	102.72
Gal8'	102.88

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac9'	N/R	99.66	39.52	68.21	51.59	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NHC(O)CH_3	-	175.16, 174.92, 174.79, 174.65, 174.61, 174.54
NHC(O)CH_3	2.19 – 1.85 (m, 18H)	22.25, 22.14, 22.11, 21.95, 21.88
Aromatic	7.54 – 7.27 (m, 5H)	136.14, 128.68, 128.32, 127.67
CH_2-Ph	5.16 – 5.06 (m, 2H)	67.07
NH-COO-	-	157.76
NH-CH-COOH	4.50 (dd, *J* = 7.9, 4.8 Hz, 1H)	51.05
NH-CH-COOH	-	N/R
C(O)-CH_2-CH	2.83 (dd, *J* = 15.9, 4.7 Hz, 1H), 2.77 – 2.68 (m, 2H)	37.45
C(O)-CH_2-CH	-	172.81

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{99}H_{154}N_8O_{68}$, $[M-2H]^{2-}$: 1271.4425, found 1271.4428.

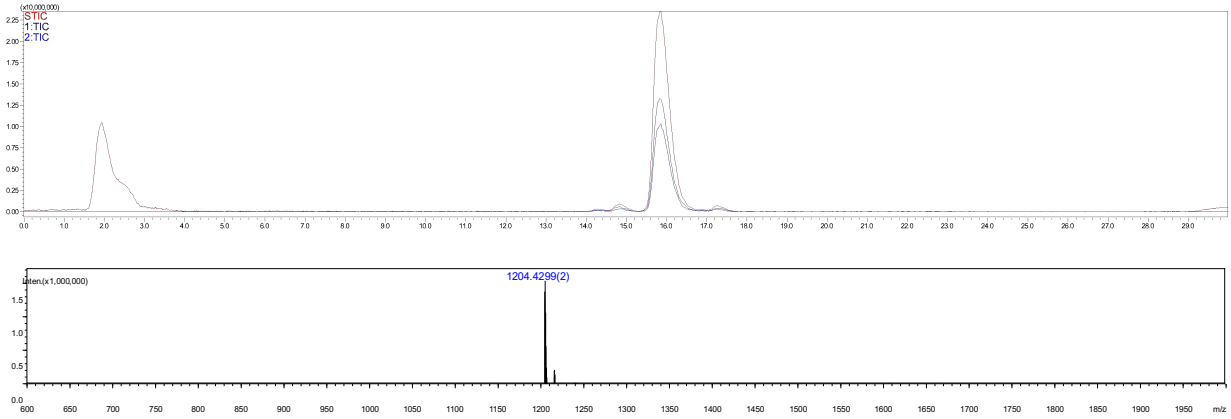
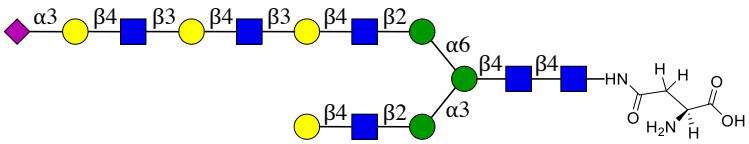


Figure S24. Analytical HPLC-MS chromatogram of compound **59b**. The retention time = 15.8 min.

Compound 60b

60a was synthesized from **25** (3 mg, 1.0 eq) using the general procedures **2.2 f**, **h**, **c** for the installation of α2,3-sialic acid with ST3Gal4, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give product **60a** which purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **60a** was removed by hydrogentation (**2.2 i**) to give final product the final product **60b** as a white fluffy solid (1 mg, 29% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{105}H_{171}N_9O_{76}$, $[M-2H]^{2-}$: 1386.9902, found 1386.9792.



60

NMR and MS analysis of compound **60a**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.7 Hz, 1H)	3.84	3.75	3.66	3.57	N/R[b]
GlcNAc2	4.62 (d, *J* = 8.0 Hz, 1H)	3.76	3.80	N/R	N/R	N/R
Man3	4.77	4.26 (d, *J* = 2.7 Hz, 1H)	3.78	N/R	N/R	3.97, 3.80
Man4	5.13	4.20 (d, *J* = 3.6 Hz, 1H)	3.91	N/R	N/R	N/R
Man4'	4.93 (s, 1H)	4.15 – 4.09 (m, 2H)	3.90	N/R	N/R	N/R
GlcNAc5	4.60 – 4.52 (m, 4H)	3.74	N/R	N/R	N/R	N/R
GlcNAc5'	4.60 – 4.52 (m, 4H)	3.74	N/R	N/R	N/R	N/R
Gal6	4.51 – 4.43 (m, 3H)	3.59	N/R	N/R	N/R	N/R
Gal6'	4.51 – 4.43 (m, 3H)	3.55	3.73	4.17 (d, *J* = 3.1 Hz, 2H)	N/R	N/R
GlcNAc7'	4.70 (d, *J* = 8.3 Hz, 2H)	3.76	N/R	N/R	N/R	N/R
Gal8'	4.51 – 4.43 (m, 3H)	3.55	3.73	4.17 (d, *J* = 3.1 Hz, 2H)	N/R	N/R
GlcNAc9'	4.70 (d, *J* = 8.3 Hz, 2H)	3.76	N/R	N/R	N/R	N/R
Gal10'	4.60 – 4.52 (m, 4H)	3.58	3.97	4.15 – 4.09 (m, 2H)	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac11'	-[a]	-	2.81 – 2.73 (m, 2H), 1.81 (t, *J* = 12.1 Hz, 2H)	3.70	3.86	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.07
GlcNAc2	101.17
Man3	100.35
Man4	99.45
Man4'	96.96
GlcNAc5	99.35
GlcNAc5'	99.35
Gal6	102.83
Gal6'	102.83
GlcNAc7'	102.72
Gal8'	102.83
GlcNAc9'	102.72
Gal10'	102.45

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac11'	N/R	99.63	39.51	68.21	51.60	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-	175.00, 174.92, 174.80, 174.62, 174.54
NHC(O)<u>CH_3</u>	2.30 – 1.90 (m, 21H)	22.25, 22.14, 22.10, 21.96, 21.88
Aromatic	7.53 – 7.34 (m, 5H)	136.14, 128.69, 128.32, 127.67
<u>CH_2</u>-Ph	5.19 – 5.10 (m, 2H)	67.10
NH-<u>C</u>OO-	-	157.77
NH-<u>CH</u>-COOH	4.60 – 4.52 (m, 4H)	50.95
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH_2</u>-CH	2.87 (dd, *J* = 15.9, 4.8 Hz, 1H), 2.81 – 2.73 (m, 2H)	3739
<u>C</u>(O)-CH_2-CH	-	172.76

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{113}H_{177}N_9O_{78}$, $[M-2H]^{2-}$: 1454.0086, found 1453.9978.

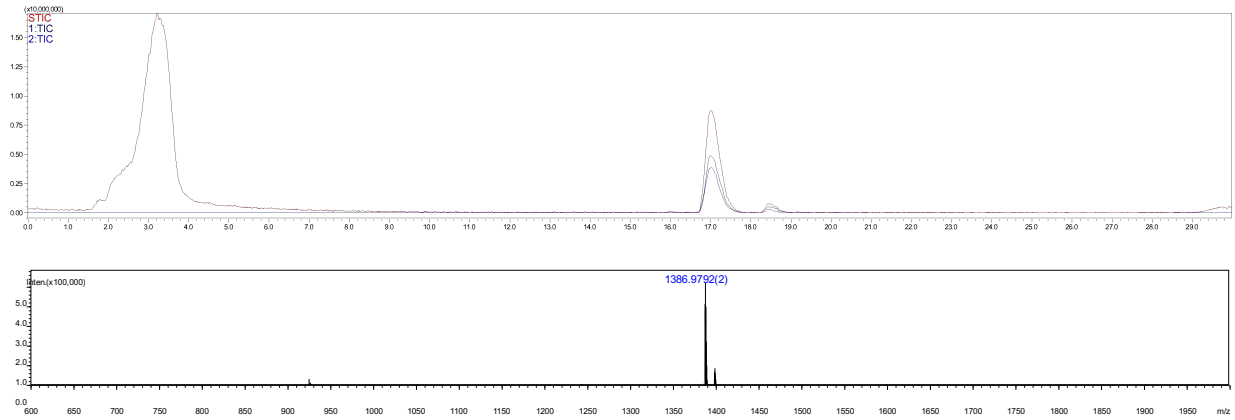
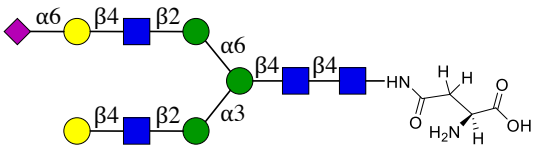


Figure S25. Analytical HPLC-MS chromatogram of compound **60b**. The retention time = 17.0 min.

Compound 67

67 was synthesized from **23** (3 mg, 1.0 eq) using the general procedures **2.2 e**, **h**, **c** for the installation of α2,6-sialic acid with ST6Gal1, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give the product **64** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **64** was removed by hydrogentation (**2.2 i**) to give final product **67** as a white fluffy solid (0.6 mg, 47% yield for four steps). ESI TOF-MS *m/z* calculated for $C_{77}H_{125}N_7O_{56}$, $[M\text{-}2H]^{2-}$: 1021.8580, found 1021.8581.



67

NMR and MS analysis of compound **64**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.8 Hz, 1H)	3.83	3.74	3.65	3.56	N/R[b]
GlcNAc2	4.62	3.78	3.79	N/R	N/R	N/R
Man3	4.78	4.26 (s, 1H)	3.79	N/R	N/R	3.97, 3.80
Man4	5.13	4.20 (d, *J* = 3.4 Hz, 1H)	3.92	N/R	N/R	N/R
Man4'	4.95 (s, 1H)	4.12 (d, *J* = 3.4 Hz, 1H)	3.91	N/R	N/R	N/R
GlcNAc5	4.59	3.78	N/R	N/R	N/R	N/R
GlcNAc5'	4.61	3.78	N/R	N/R	N/R	N/R
Gal6	4.47	3.55	N/R	N/R	N/R	N/R
Gal6'	4.46	3.55	N/R	N/R	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac7'	-[a]	-	2.72 – 2.60 (m, 2H), 1.73 (t, *J* = 12.1 Hz, 1H)	3.67	3.82	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.05
GlcNAc2	101.17
Man3	100.42
Man4	99.46
Man4'	99.37
GlcNAc5	99.20
GlcNAc5'	102.83
Gal6	103.48
Gal6'	100.07

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac7'	N/R	100.07	39.99	68.12	51.80	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-	174.83, 174.62, 174.52, 173.40
NHC(O)<u>CH$_3$</u>	2.28 – 1.83 (m, 15H)	22.34, 22.26, 22.16, 21.97, 21.90
Aromatic	7.59 – 7.29 (m, 5H)	136.21, 128.68, 128.29, 127.73
<u>CH$_2$</u>-Ph	5.19 – 5.09 (m, 2H)	66.95
NH-<u>C</u>OO-	-	157.72
NH-<u>CH</u>-COOH	4.41 (d, *J* = 8.1 Hz, 1H)	52.29
NH-CH-<u>C</u>OOH	-	176.36
C(O)-<u>CH$_2$</u>-CH	2.87 – 2.80 (m, 1H), 2.72 – 2.60 (m, 2H)	38.10
<u>C</u>(O)-CH$_2$-CH	-	173.30

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{85}H_{131}N_7O_{58}$, $[M-2H]^{2-}$: 1088.8764, found 1088.8733.

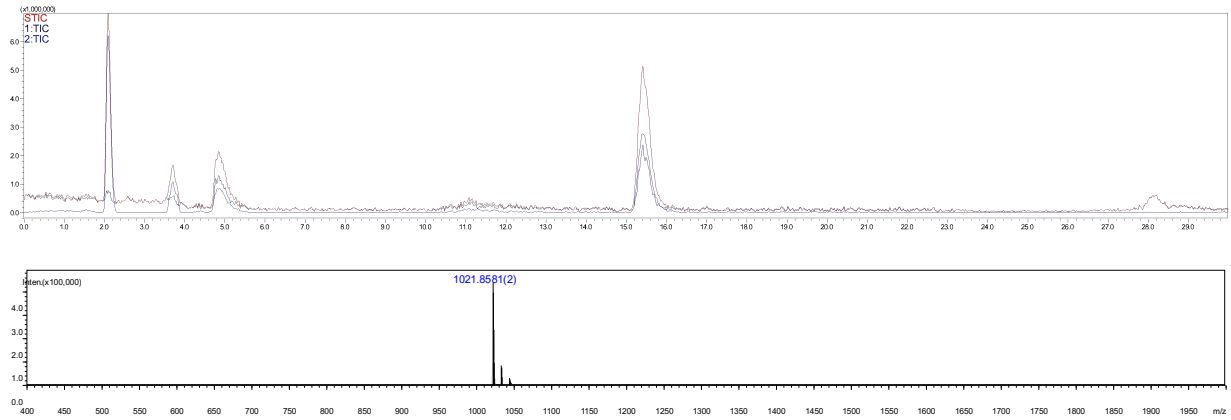
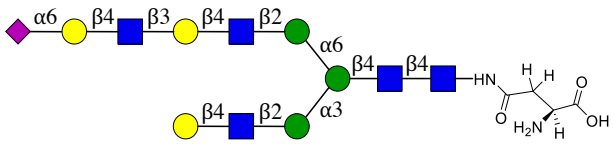


Figure S26. Analytical HPLC-MS chromatogram of compound **67**. The retention time = 15.4 min.

Compound 68

68 was synthesized from **24** (3.0 mg) using the general procedures **2.2 e**, **h**, **c** for the installation of α2,6-sialic acid with ST6Gal1, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give **65** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **65** was removed by hydrogentation (**2.2 i**) to give final product **68** as a white fluffy solid (0.7 mg, 60% yield for four steps). ESI TOF-MS *m/z* calculated for $C_{91}H_{148}N_8O_{66}$, $[M-2H]^{2-}$: 1204.4241, found 1204.4337.



68

NMR and MS analysis of compound **65**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.09 (d, *J* = 9.8 Hz, 1H)	3.86	3.77	3.69	3.59	N/R[b]
GlcNAc2	4.65 (d, *J* = 8.1 Hz, 1H)	3.78	3.83	N/R	N/R	N/R
Man3	4.80	4.29 (d, *J* = 2.7 Hz, 1H)	3.81	N/R	N/R	4.00, 3.83
Man4	5.16	4.25 – 4.22 (m, 1H)	3.94	N/R	N/R	N/R
Man4'	4.97 (s, 1H)	4.17 – 4.13 (m, 1H)	3.92	N/R	N/R	N/R
GlcNAc5	4.62 (d, *J* = 7.7 Hz, 2H)	3.78	N/R	N/R	N/R	N/R
GlcNAc5'	4.62 (d, *J* = 7.7 Hz, 2H)	3.78	N/R	N/R	N/R	N/R

Gal6	4.53 – 4.45 (m, 3H)	3.58	N/R	N/R	N/R	N/R
Gal6'	4.53 – 4.45 (m, 3H)	3.58	3.77	4.20 (d, *J* = 3.2 Hz, 1H)		
GlcNAc7'	4.77 (s, 1H)	3.84	N/R	N/R	N/R	N/R
Gal8'	4.53 – 4.45 (m, 3H)	3.64	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac9'	-[a]	-	2.77 – 2.67 (m, 2H), 1.76 (t, *J* = 12.2 Hz, 1H)	3.70	3.84	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.10
GlcNAc2	101.20
Man3	100.39
Man4	99.49
Man4'	97.02
GlcNAc5	99.40
GlcNAc5'	99.40
Gal6	102.92
Gal6'	102.87
GlcNAc7'	102.52
Gal8'	103.40

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac9'	N/R	100.07	40.02	68.15	51.85	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NHC(O)CH_3	-	174.86, 174.70, 174.64, 174.57
NHC(O)CH_3	2.32 – 1.89 (m, 18H)	22.29, 22.26, 22.18, 21.99, 21.93
Aromatic	7.63 – 7.34 (m, 5H)	136.23, 128.71, 128.33, 127.73
CH_2-Ph	5.21 – 5.12 (m, 2H)	67.04
NH-COO-	-	157.74
NH-CH-COOH	4.48	51.85
NH-CH-COOH	-	N/R
C(O)-CH_2-CH	2.88 (dd, *J* = 15.8, 4.4 Hz, 1H), 2.77 – 2.67 (m, 2H)	37.92
C(O)-CH_2-CH	-	173.42

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{99}H_{154}N_8O_{68}$, $[M-2H]^{2-}$: 1271.4425, found 1271.439.

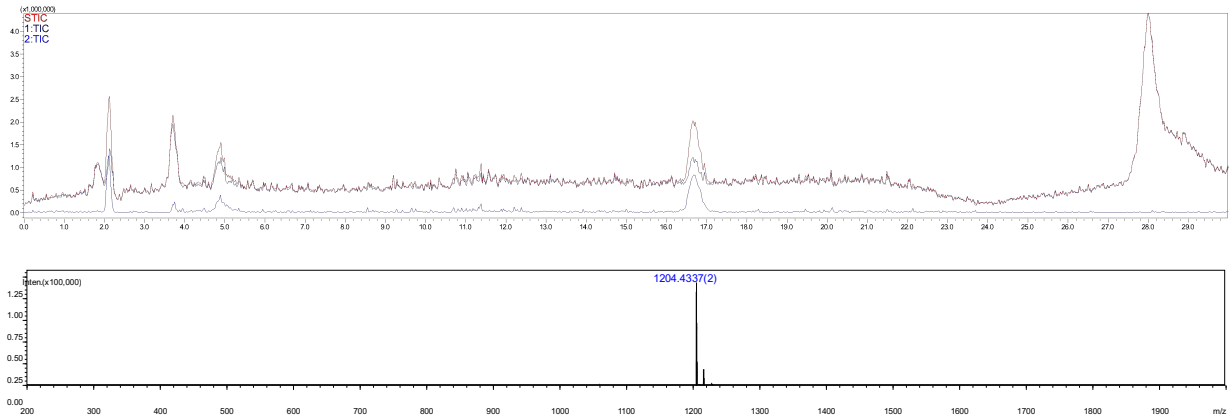
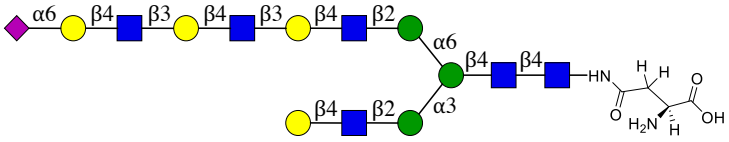


Figure S27. Analytical HPLC-MS chromatogram of compound **68**. The retention time = 16.7 min.

Compound 69

69 was synthesized from **25** (3 mg, 1.0 eq) using the general procedures **2.2 e**, **h**, **c** for the installation of α2,6-sialic acid with ST6Gal1, converting $GlcNH_2$ to GlcNAc with AcOSu, installation of β1,4-Gal with B4GalT1 to give **66**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **66** was removed by hydrogentation (**2.2 i**) to give final product **69** as a white fluffy solid (1.0 mg, 85% yield over four steps). ESI TOF-MS *m/z* calculated for $C_{105}H_{171}N_9O_{76}$, $[M-2H]^{2-}$: 1386.9902, found 1386.9943.



69

NMR and MS analysis of compound **66**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.01 (d, *J* = 9.8 Hz, 1H)	3.78	3.70	3.61	3.52	N/R[b]
GlcNAc2	4.57 (d, *J* = 8.2 Hz, 1H)	3.76	3.77	N/R	N/R	N/R
Man3	4.73 (s, 2H)	4.22 (d, *J* = 2.7 Hz, 1H)	3.74	N/R	N/R	3.93, 3.75
Man4	5.08	4.18 – 4.14 (m, 1H)	3.87	N/R	N/R	N/R
Man4'	4.89 (s, 1H)	4.07	3.85	N/R	N/R	N/R
GlcNAc5	4.55 (d, *J* = 7.8 Hz, 2H)	3.71	N/R	N/R	N/R	N/R
GlcNAc5'	4.55 (d, *J* = 7.8 Hz,	3.71	N/R	N/R	N/R	N/R

	2H)					
Gal6	4.48 – 4.37 (m, 4H)	3.56	N/R	N/R	N/R	N/R
Gal6'	4.48 – 4.37 (m, 4H)	3.50	3.70	4.13 (d, *J* = 3.1 Hz, 2H)		N/R
GlcNAc7'	4.70 (d, *J* = 7.6 Hz, 1H)	3.77	N/R	N/R	N/R	N/R
Gal8'	4.48 – 4.37 (m, 4H)	3.50	3.70	4.13 (d, *J* = 3.1 Hz, 2H)		N/R
GlcNAc9'	4.66 (d, *J* = 8.3 Hz, 1H)	3.77	N/R	N/R	N/R	N/R
Gal10'	4.48 – 4.37 (m, 4H)	3.56	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac9'	-[a]	-	2.64 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.69 (t, *J* = 12.2 Hz, 1H)	3.62	3.77	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.02
GlcNAc2	101.15
Man3	100.34
Man4	99.45
Man4'	96.95
GlcNAc5	99.35
GlcNAc5'	96.35
Gal6	102.81
Gal6'	102.81
GlcNAc7'	102.50
Gal8'	102.81
GlcNAc9'	102.69
Gal10'	103.38

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac9'	N/R	N/R	40.00	68.13	51.79	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NHC(O)CH_3	-	174.81, 174.62, 174.54
NHC(O)CH_3	2.17 – 1.81 (m, 33H)	22.24, 22.19, 22.13, 22.10, 21.94, 21.90
Aromatic	7.48 – 7.31 (m, 5H)	136.24, 128.66, 128.26, 127.75
CH_2-Ph	5.14 – 5.04 (m, 2H)	66.86
NH-COO-	-	N/R
NH-CH-COOH	4.29 (dd, *J* = 9.2, 4.1 Hz, 1H)	52.92
NH-CH-COOH	-	177.55

C(O)-$\underline{CH_2}$-CH	2.78 (dd, *J* = 15.7, 4.2 Hz, 1H), 2.55 (dd, *J* = 15.4, 9.5 Hz, 1H)	38.46
$\underline{C}$(O)-CH_2-CH	-	173.46

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{113}H_{177}N_9O_{78}$, $[M-2H]^{2-}$: 1454.0086, found 1453.9998.

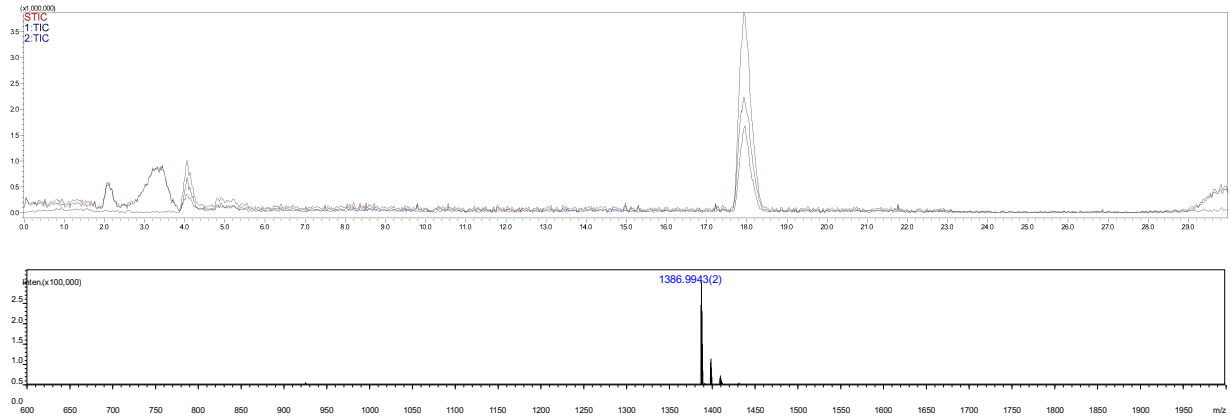
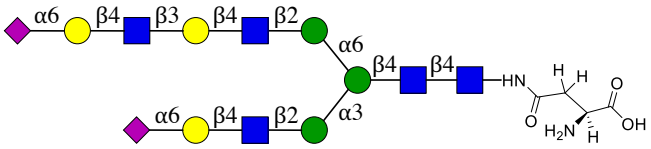


Figure S28. Analytical HPLC-MS chromatogram of compound **69**. The retention time = 18.0 min.

Compound 70b

70b was prepared from **65** (1.0 mg, 1.0 eq) using the general procedures **2.2 e** for the installation of α2, 6-sialic acid with ST6Gal1 to give the intermediate product **70a**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **70a** was removed by hydrogentation (**2.2 i**) to give final product **70b** as a white fluffy solid (1.0 mg, 98% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{102}H_{165}N_9O_{74}$, $[M-2H]^{2-}$: 1349.9718, found 1349.9790.



70

NMR and MS analysis of compound **70a**:

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.1 Hz, 1H)	3.82	3.74	3.65	3.56	N/R[b]
GlcNAc2	4.63 – 4.55 (m, 3H)	3.77	3.79	N/R	N/R	N/R

Man3	4.77	4.25 (s, 1H)	3.78	N/R	N/R	3.96, 3.78
Man4	5.13	4.20 (s, 1H)	3.90	N/R	N/R	N/R
Man4'	4.92 (s, 1H)	4.11 (s, 1H)	3.89	N/R	N/R	N/R
GlcNAc5	4.63 – 4.55 (m, 3H)	3.75	N/R	N/R	N/R	N/R
GlcNAc5'	4.63 – 4.55 (m, 3H)	3.75	N/R	N/R	N/R	N/R
Gal6	4.50 – 4.38 (m, 3H)	3.54	N/R	N/R	N/R	N/R
Gal6'	4.50 – 4.38 (m, 3H)	3.59	3.73	4.16 (s, 1H)	N/R	N/R
GlcNAc7'	4.73	3.80	N/R	N/R	N/R	N/R
Gal8'	4.50 – 4.38 (m, 3H)	3.54	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac7	-[a]	-	2.67 (d, *J* = 12.4 Hz, 3H), 1.72 (t, *J* = 12.0 Hz, 2H)	3.65	3.81	N/R	N/R	N/R	N/R
Neu5Ac9'	-	-	2.67 (d, *J* = 12.4 Hz, 3H), 1.72 (t, *J* = 12.0 Hz, 2H)	3.65	3.81	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.10
GlcNAc2	101.22
Man3	100.35
Man4	99.48
Man4'	97.03
GlcNAc5	99.27
GlcNAc5'	99.44
Gal6	103.47
Gal6'	102.94
GlcNAc7'	102.52
Gal8'	103.40

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac7	N/R	N/R	40.01	68.17	51.84	N/R	N/R	N/R	N/R
Neu5Ac9'	N/R	N/R	40.01	68.17	51.84	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH_3	-	174.81, 174.65, 174.62
NHC(O)<u>CH_3</u>	2.25 – 1.86 (m, 21H)	22.38, 22.29, 22.25, 22.17, 21.99, 21.93
Aromatic	7.56 – 7.30 (m, 5H)	128.70, 128.32, 127.74
<u>CH_2</u>-Ph	5.20 – 5.08 (m, 2H)	67.01
NH-<u>C</u>OO-	-	N/R
NH-<u>CH</u>-COOH	N/R	N/R
NH-CH-<u>C</u>OOH	-	N/R

C(O)-$\underline{CH_2}$-CH	2.83 (s, 1H), 2.67 (d, *J* = 12.4 Hz, 3H)	67.07
$\underline{C}$(O)-CH_2-CH	-	N/R

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{110}H_{171}N_9O_{76}$, $[M-2H]^{2-}$: 1416.9902, found 1416.9896.

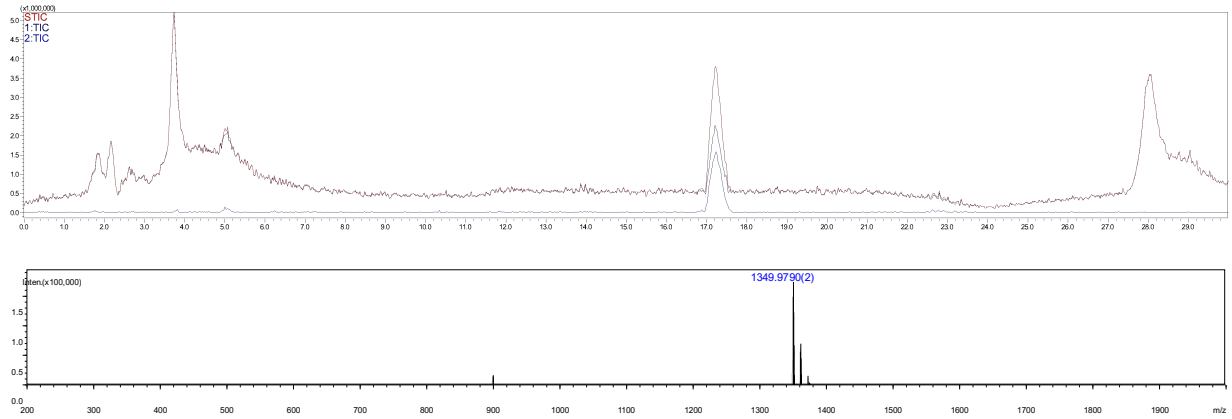
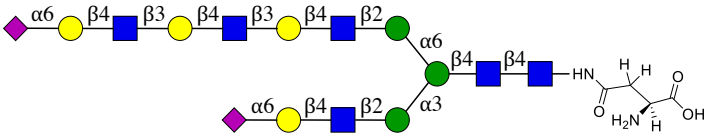


Figure S29. Analytical HPLC-MS chromatogram of compound **70b**. The retention time = 17.2 min.

Compound 71b

71b was prepared from **66** (1 mg, 1.0 eq) using the general procedures **2.2 e** for the installation of α2, 6-sialic acid with ST6Gal1 to give intermediate product **71a** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **71a** was removed by hydrogentation (**2.2 i**) to give final product **71b** as a white fluffy solid (0.8 mg, 75% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{116}H_{188}N_{10}O_{84}$, $[M-2H]^{2-}$: 1532.5379, found 1532.5241.



71

NMR and MS analysis of compound **71a**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.08 (d, *J* = 9.7 Hz, 1H)	3.86	3.77	3.68	3.59	N/R[b]
GlcNAc2	4.67 – 4.58 (m, 3H)	3.78	3.82	N/R	N/R	N/R

Man3	4.80	4.29 (d, *J* = 2.5 Hz, 1H)	3.82	N/R	N/R	3.99, 3.83
Man4	5.17	4.23 (d, *J* = 3.4 Hz, 1H)	3.93	N/R	N/R	N/R
Man4'	4.96 (s, 1H)	4.14 (d, *J* = 3.6 Hz, 1H)	3.92	N/R	N/R	N/R
GlcNAc5	4.67 – 4.58 (m, 3H)	3.78	N/R	N/R	N/R	N/R
GlcNAc5'	4.67 – 4.58 (m, 3H)	3.78	N/R	N/R	N/R	N/R
Gal6	4.57 – 4.44 (m, 5H)	3.62	N/R	N/R	N/R	N/R
Gal6'	4.57 – 4.44 (m, 5H)	3.57	3.77	4.19 (d, *J* = 3.1 Hz, 2H)	N/R	N/R
GlcNAc7'	4.75 – 4.71 (m, 2H)	3.84	N/R	N/R	N/R	N/R
Gal8'	4.57 – 4.44 (m, 5H)	3.57	3.77	4.19 (d, *J* = 3.1 Hz, 2H)	N/R	N/R
GlcNAc9'	4.75 – 4.71 (m, 2H)	3.84	N/R	N/R	N/R	N/R
Gal10'	4.57 – 4.44 (m, 5H)	3.62	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac7	-[a]	-	2.70 (dd, *J* = 13.3, 4.0 Hz, 2H), 1.76 (t, *J* = 12.1 Hz, 2H)	3.70	3.84	N/R	N/R	N/R	N/R
Neu5Ac11'	-	-	2.70 (dd, *J* = 13.3, 4.0 Hz, 2H), 1.76 (t, *J* = 12.1 Hz, 2H)	3.70	3.84	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1
GlcNAc1	78.12
GlcNAc2	101.22
Man3	100.34
Man4	99.52
Man4'	97.03
GlcNAc5	99.31
GlcNAc5'	99.43
Gal6	103.48
Gal6'	102.91
GlcNAc7'	102.54
Gal8'	102.91
GlcNAc9'	102.70
Gal10'	103.48

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac7	N/R	N/R	39.97	68.14	51.83	N/R	N/R	N/R	N/R
Neu5Ac11'	N/R	N/R	39.97	68.14	51.83	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NHC(O)CH$_3$	-	174.89, 174.70, 174.61, 174.54
NHC(O)CH$_3$	2.33 – 1.88 (m, 24H)	22.39, 22.29, 22.24, 22.18, 22.15, 21.99, 21.92
Aromatic	7.56 – 7.37 (m, 5H)	128.71, 128.34, 127.72
CH$_2$-Ph	5.21 – 5.13 (m, 2H)	67.13
NH-COO-	-	N/R
NH-CH-COOH	4.57 – 4.44 (m, 5H)	N/R
NH-CH-COOH	-	N/R
C(O)-CH$_2$-CH	2.88 (d, *J* = 16.4 Hz, 1H), 2.76 (dd, *J* = 15.5, 8.0 Hz, 1H)	37.70
C(O)-CH$_2$-CH	-	N/R

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{124}H_{194}N_{10}O_{86}$, $[M-2H]^{2-}$: 1599.5563, found 1599.5561.

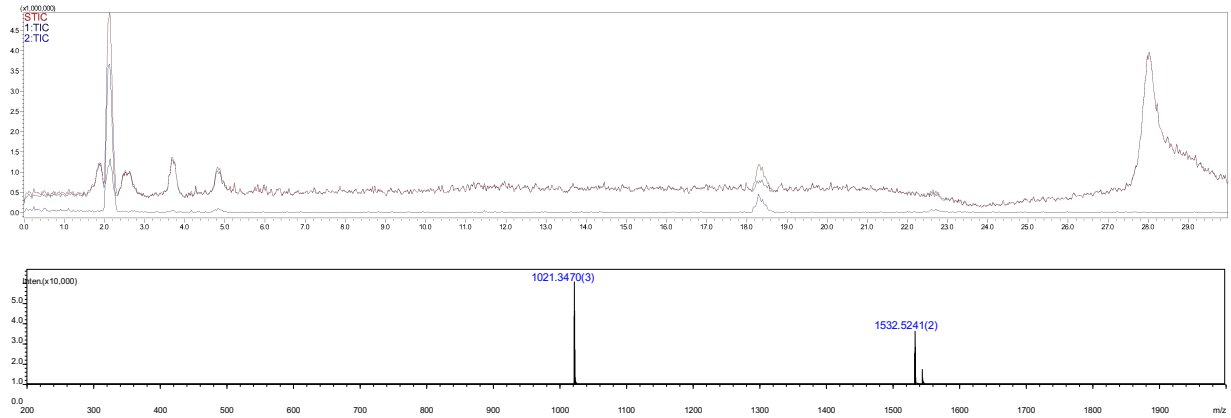
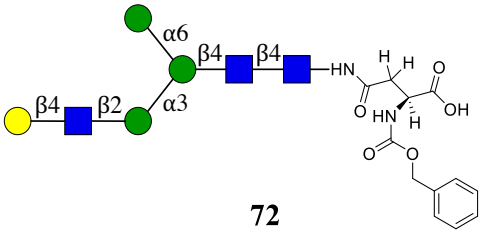


Figure S30. Analytical HPLC-MS chromatogram of compound **71b**. The retention time = 18.5 min.

Compound 72

72 was prepared by install a β1, 4-Gal in the extended arm of **10** (10 mg, 1.0 eq) according to the general procedure **2.2 c** with B4GalT1. The product was was purified using the described two-stage purification system **(2.2 j)** providing **72** as a white fluffy solid (12 mg, 100%).



72

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.5 Hz, 1H)	3.83	3.74	3.66	3.56	3.81, 3.63
GlcNAc2	4.62 (d, *J* = 7.9 Hz, 1H)	3.79	3.75	3.75	3.62	3.89, 3.76
Man3	4.79	4.27 (s, 1H)	3.78	3.78	3.67	3.93, 3.81
Man4	5.13	4.20 (s, 1H)	3.91	3.51	3.75	3.94, 3.63
Man4'	4.93 (s, 1H)	3.98	3.88	3.66	3.66	3.90, 3.77
GlcNAc5	4.59 (d, *J* = 7.3 Hz, 1H)	3.75	3.74	3.74	3.59	3.98, 3.85
Gal6	4.48 (d, *J* = 7.8 Hz, 1H)	3.55	3.68	3.94	3.74	3.79, 3.75

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.04	53.64	72.68	78.50	76.11	59.77
GlcNAc2	101.16	54.78	65.82	79.59	74.28	59.89
Man3	100.32	70.11	80.30	71.87	74.07	65.76
Man4	99.46	76.29	69.30	67.20	73.47	61.62
Man4'	99.55	69.79	70.32	66.69	72.61	60.88
GlcNAc5	99.36	54.78	71.87	78.39	74.65	59.89
Gal6	102.83	70.88	72.42	68.45	75.26	60.93

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-[a]	174.62
NHC(O)<u>CH_3</u>	2.09 (s, 3H), 2.06 (s, 3H), 1.93 (s, 3H)	22.26, 22.10, 21.90
Aromatic	7.59 – 7.26 (m, 5H)	136.23, 128.68, 128.28, 127.74
<u>CH_2</u>-Ph	5.19 – 5.09 (m, 2H)	66.94
NH-<u>C</u>OO-	-	157.71
NH-<u>CH</u>-COOH	4.43 – 4.37 (m, 1H)	52.42
NH-CH-<u>C</u>OOH	-	N/R[b]
C(O)-<u>CH_2</u>-CH	2.84 (d, *J* = 14.4 Hz, 2H) 2.64 (dd, *J* = 15.0, 9.5 Hz, 2H)	38.21
<u>C</u>(O)-CH_2-CH	-	173.39

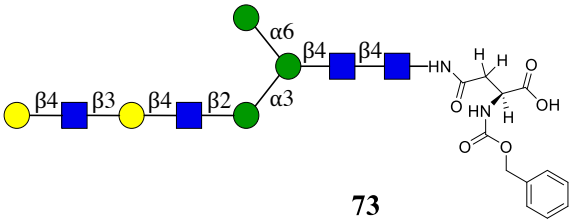
[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{60}H_{91}N_5O_{40}$, $[M-2H]^{2-}$: 760.7626, found 760.7662.

Compound 73

73 was prepared from **72** (8 mg, 1.0 eq) using the general procedures **2.2 d** and **c** for the installation of GlcNAc and Gal moieties. The product was purified using the described two-stage purification system **(2.2 j)** providing **73** as a white fluffy solid (8.5 mg, 86% yield over two steps).



73

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.8 Hz, 1H)	3.83	3.74	3.66	3.56	N/R[b]
GlcNAc2	4.62 (d, *J* = 8.0 Hz, 1H)	3.80	3.77	3.75	3.62	N/R
Man3	4.79	4.26 (d, *J* = 2.4 Hz, 1H)	3.78	3.78	3.67	3.93, 3.81
Man4	5.13	4.20 (d, *J* = 3.4 Hz, 1H)	3.89	3.51	3.75	3.93, 3.63
Man4'	4.93 (s, 1H)	3.98	3.89	3.65	3.65	N/R
GlcNAc5	4.58 (d, *J* = 7.4 Hz, 1H)	3.74	3.74	3.73	3.59	N/R
Gal6	4.46 (d, *J* = 7.9 Hz, 1H)	3.59	3.73	4.17 (d, *J* = 3.2 Hz, 1H)	N/R	N/R
GlcNAc7	4.71 (d, *J* = 8.3 Hz, 1H)	3.82	N/R	3.75	N/R	N/R
Gal8	4.49 (d, *J* = 7.9 Hz, 1H)	3.55	3.68	3.94	3.74	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.03	53.64	72.69	78.50	76.11	N/R
GlcNAc2	101.16	54.79	65.83	79.59	74.28	N/R
Man3	100.32	70.11	80.30	71.86	74.07	65.76
Man4	99.46	76.29	69.30	67.20	73.47	61.62
Man4'	99.55	69.79	70.32	66.69	72.61	N/R
GlcNAc5	99.38	54.74	71.89	78.42	74.64	N/R
Gal6	102.87	66.87	81.97	68.23	N/R	N/R
GlcNAc7	1.2.67	55.11	N/R	78.08	N/R	N/R
Gal8	102.78	70.89	72.43	68.47	75.27	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-[a]	174.83, 174.62
NHC(O)<u>CH$_3$</u>	2.22 – 1.83 (m, 12H)	22.25, 22.10, 21.91

Aromatic	7.53 – 7.35 (m, 5H)	136.24, 128.68, 128.28, 127.75
$\underline{CH_2}$-Ph	5.18 – 5.09 (m, 2H)	66.92
NH-$\underline{C}$OO-	-	157.72
NH-$\underline{CH}$-COOH	4.39 (s, 1H)	N/R
NH-CH-$\underline{C}$OOH	-	N/R
C(O)-$\underline{CH_2}$-CH	2.83 (d, *J* = 15.2 Hz, 1H) 2.63 (d, *J* = 6.8 Hz, 1H)	38.31
$\underline{C}$(O)-CH_2-CH	-	N/R

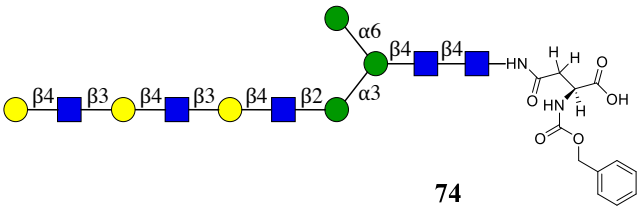
[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{74}H_{114}N_6O_{50}$, $[M-2H]^{2-}$: 943.3287, found 943.3307.

Compound 74

74 was prepared from **73** (4 mg) using the general procedures **2.2 d** and **c** for the installation of GlcNAc and Gal moieties. The product was purified using the described two-stage purification system **(2.2 j)** providing **74** as a white fluffy solid (4 mg, 83% yield for two steps).



74

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.04 (d, *J* = 9.8 Hz, 1H)	3.82	3.73	3.65	3.55	N/R[b]
GlcNAc2	4.60 (d, *J* = 8.1 Hz, 1H)	3.80	3.76	3.73	3.61	N/R
Man3	4.78	4.25 (s, 1H)	3.76	3.77	3.66	3.92, 3.80
Man4	5.12	4.19 (d, *J* = 3.3 Hz, 1H)	3.90	3.50	3.74	3.92, 3.61
Man4'	4.92 (s, 1H)	3.97	3.88	3.64	3.64	N/R
GlcNAc5	4.57 (d, *J* = 7.3 Hz, 1H)	3.73	3.73	3.72	3.57	N/R
Gal6	4.50 – 4.43 (m, 3H)	3.58	3.72	4.17 – 4.13 (m, 2H)	N/R	N/R
GlcNAc7	4.72 – 4.67 (m, 2H)	3.80		3.74	N/R	N/R
Gal8	4.50 – 4.43 (m, 3H)	3.58	3.72	4.17 – 4.13 (m, 2H)	N/R	N/R
GlcNAc9	4.72 – 4.67 (m, 2H)	3.80		3.74	N/R	N/R
Gal10	4.50 – 4.43 (m, 3H)	3.54	3.67	3.92	3.73	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.04	53.62	72.68	78.47	76.09	N/R
GlcNAc2	101.14	54.76	65.81	79.57	74.26	N/R
Man3	100.31	70.09	80.28	71.84	74.05	65.74
Man4	99.45	76.27	69.28	67.18	73.45	61.60
Man4'	99.53	69.77	70.30	66.67	72.59	N/R
GlcNAc5	99.34	54.72	71.87	78.37	74.62	N/R
Gal6	102.92 – 102.59 (m)	69.86	81.96	68.22	N/R	N/R
GlcNAc7	102.67	55.05	N/R	78.04	N/R	N/R
Gal8	102.92 – 102.59 (m)	69.86	81.96	68.22	N/R	N/R
GlcNAc9	102.67	55.09	N/R	78.04	N/R	N/R
Gal10	102.92 – 102.59 (m)	70.87	72.40	68.45	75.26	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH_3	-[a]	174.81, 174.69, 174.61
NHC(O)<u>CH_3</u>	2.24 – 1.77 (m, 15H)	22.24, 22.08, 21.89
Aromatic	2.24 – 1.77 (m, 5H)	136.24, 128.66, 128,26, 127.75
<u>CH_2</u>-Ph	5.18 – 5.07 (m, 2H)	66.87
NH-<u>C</u>OO-	-	157.68
NH-<u>CH</u>-COOH	4.32 (dd, *J* = 9.5, 4.1 Hz, 1H)	52.92
NH-CH-<u>C</u>OOH	-	177.57
C(O)-<u>CH_2</u>-CH	2.81 (dd, *J* = 15.8, 4.1 Hz, 1H), 2.58 (dd, *J* = 15.4, 9.5 Hz, 1H)	38.47
<u>C</u>(O)-CH_2-CH	-	173.60

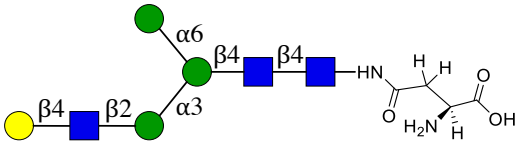
[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{88}H_{137}N_7O_{60}$, $[M-2H]^{2-}$: 1125.8948, found 1125.8906.

Compound 75

75 was prepared from **72** (1 mg) using the general procedure **2.2 i** for the removing of Cbz group with 20% $Pd(OH)_2/C$ and H_2. The final product **75** was obtained as a white fluffy solid (0.9 mg, 95%). ESI TOF-MS *m/z* calculated for $C_{52}H_{86}N_5O_{38}$, $[M-H]^-$: 1388.4956, found 1388.4899.



75

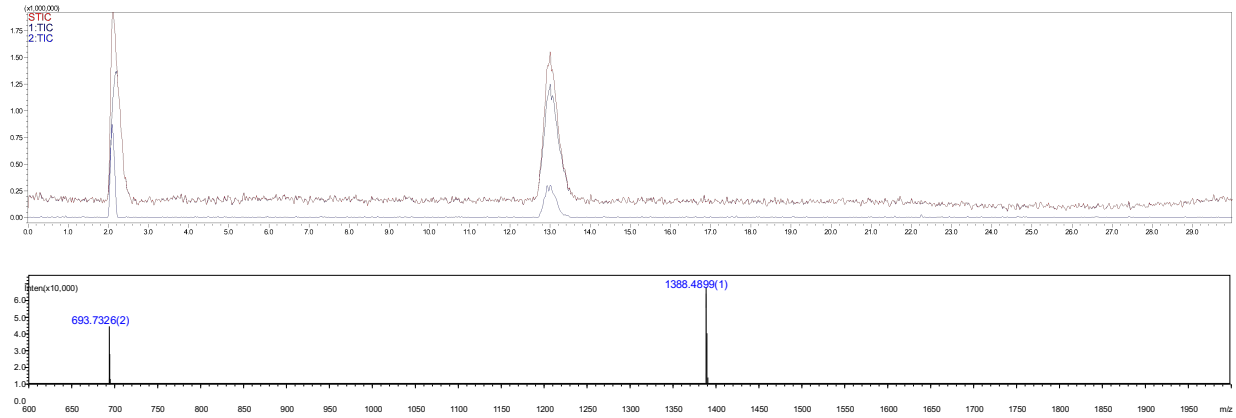
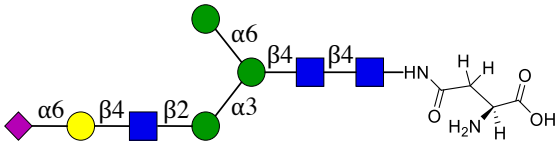


Figure S31. Analytical HPLC-MS chromatogram of compound **75**. The retention time = 13.0 min.

Compound 77

77 was synthesized from **72** (1 mg, 1.0 eq) through the installation of α2, 6-Neu5Ac using the general procedure **2.2 e** to give intermediate product **76** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **76** was removed by hydrogentation (**2.2 i**) to give final product **77** as a white fluffy solid (1 mg, 88% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{63}H_{102}N_6O_{46}$, $[M-2H]^{2-}$: 839.2919, found 839.2950.



77

NMR and MS analysis of compound **76**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.08 (d, *J* = 9.7 Hz, 1H)	3.86	3.77	3.68	3.59	N/R[b]
GlcNAc2	4.64 (dd, *J* = 7.9, 2.6 Hz, 4H)	3.82	3.79	3.77	N/R	N/R
Man3	4.82 (s, 1H)	4.29 (d, *J* = 2.8 Hz, 1H)	3.81	N/R	N/R	3.95, 3.84
Man4	5.17	4.23 (dd, *J* = 3.3, 1.6 Hz, 1H)	3.93	3.55	N/R	N/R
Man4'	4.95 (d, *J* = 1.7 Hz, 1H)	4.01	3.91	3.68	N/R	N/R
GlcNAc5	4.64 (dd, *J* = 7.9, 2.6 Hz, 4H)	3.79		3.69	N/R	N/R
Gal6	4.48 (d, *J* = 7.9 Hz, 1H)	3.57	3.69	3.96	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac7	-[a]	-	2.70 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.76 (t, *J* = 12.1 Hz, 1H)	3.70	3.84	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.11	53.69	72.67	78.58	76.16	N/R
GlcNAc2	101.21	54.57	65.86	79.60	N/R	N/R
Man3	100.31	70.14	80.35	N/R	N/R	65.82
Man4	99.50	76.36	69.38	67.23	N/R	N/R
Man4'	99.59	69.83	70.35	66.72	N/R	N/R
GlcNAc5	99.29	54.84	N/R	80.66	N/R	N/R
Gal6	103.47	70.69	N/R	68.31	N/R	N/R

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac7	N/R	100.08	39.99	68.13	51.83	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NHC(O)CH_3	-	174.86, 174.71, 174.63
NHC(O)CH_3	2.22 – 1.86 (m, 12H)	22.38, 22.14, 22.00, 21.92
Aromatic	7.54 – 7.37 (m, 5H)	136.21, 128.71, 128.34, 127.72
CH_2-Ph	5.22 – 5.13 (m, 2H)	67.06
NH-COO-	-	157.76
NH-CH-COOH	4.51	51.71
NH-CH-COOH	-	N/R
C(O)-CH_2-CH	2.88 (dd, *J* = 15.7, 4.4 Hz, 1H), 2.77 – 2.73 (m, 1H)	37.82
C(O)-CH_2-CH	-	173.08

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{71}H_{108}N_6O_{48}$, $[M-2H]^{2-}$: 906.3103, found 906.3120.

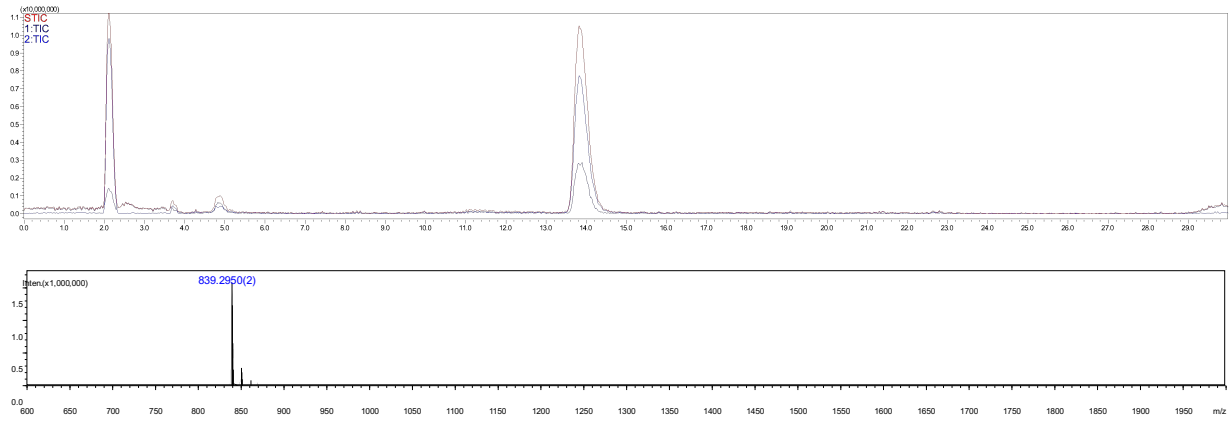
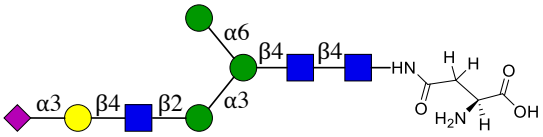


Figure S32. Analytical HPLC-MS chromatogram of compound **77**. The retention time = 13.8 min.

Compound 79

79 was synthesized from **72** (2 mg) through the installation of α2,3-Neu5Ac using the general procedure **2.2 f** to give intermediate product **78** after purification using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **78** was removed by hydrogentation (**2.2 i**) to give final product **79** as a white fluffy solid (0.5 mg, 46% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{63}H_{102}N_6O_{46}$, $[M-2H]^{2-}$: 839.2919, found 839.2884.



79

NMR and MS analysis of compound **78**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.05 (d, *J* = 9.8 Hz, 1H)	3.83	N/R[b]	3.65	3.56	N/R
GlcNAc2	4.61 (d, *J* = 8.0 Hz, 1H)	3.80	3.77	3.75	N/R	N/R
Man3	4.78	4.26 (d, *J* = 2.4 Hz, 1H)	3.77	N/R	N/R	3.92, 3.81
Man4	5.13	4.20 (d, *J* = 3.5 Hz, 1H)	3.90	3.51	N/R	N/R
Man4'	4.92 (s, 1H)	3.98	3.88	3.65	N/R	N/R
GlcNAc5	4.58 (d, *J* = 7.6 Hz, 1H)	3.75	N/R	3.72	N/R	N/R
Gal6	4.55 (d, *J* = 7.8 Hz, 1H)	3.57	3.96	4.12	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac7	-[a]	-	2.76 (dd, *J* = 12.5, 4.5 Hz, 1H), 1.81 (t, *J* = 12.1 Hz, 1H)	3.70	3.85	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.05	53.64	N/R	78.51	76.12	N/R
GlcNAc2	101.17	54.78	65.82	79.58	N/R	N/R
Man3	100.30	70.10	80.26	N/R	N/R	65.77
Man4	99.46	76.30	69.30	67.19	N/R	N/R
Man4'	99.55	69.78	70.31	66.68	N/R	N/R
GlcNAc5	99.42	54.78	N/R	75.08	N/R	N/R
Gal6	102.50	69.30	67.38	75.39	N/R	N/R

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac7	N/R	99.74	39.54	68.26	51.59	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NHC(O)CH$_3$	-	174.88, 174.78, 174.60
NHC(O)CH$_3$	2.10 – 1.89 (m, 12H)	22.25, 22.10, 21.95, 21.89
Aromatic	7.49 – 7.37 (m, 5H)	136.20, 128.67, 128.29, 127.71
CH$_2$-Ph	5.17 – 5.10 (m, 2H)	66.98
NH-COO-	-	157.73
NH-CH-COOH	4.44	52.08
NH-CH-COOH	-	N/R
C(O)-CH$_2$-CH	2.84 (d, *J* = 15.4 Hz, 1H), 2.67 (d, *J* = 14.3 Hz, 1H)	37.97
C(O)-CH$_2$-CH	-	N/R

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{71}H_{108}N_6O_{48}$, $[M-2H]^{2-}$: 906.3103, found 906.3124.

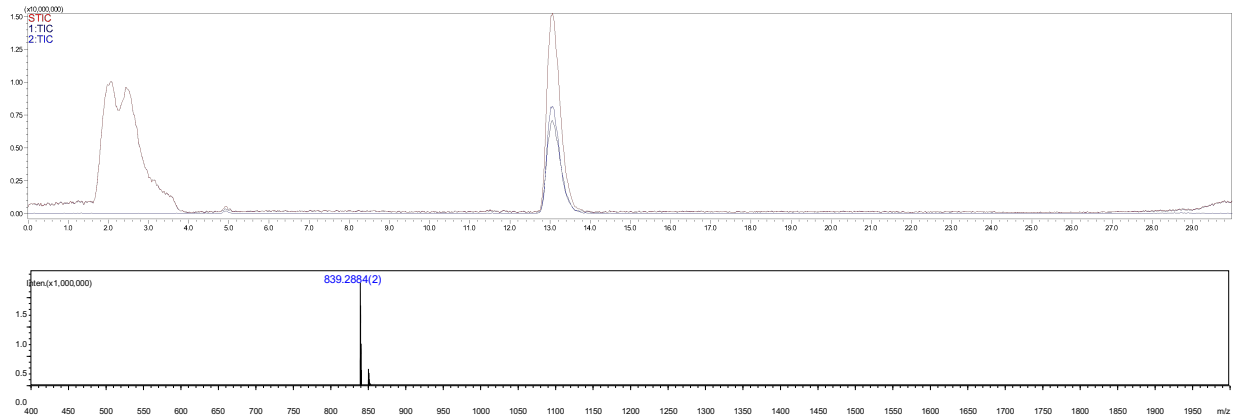
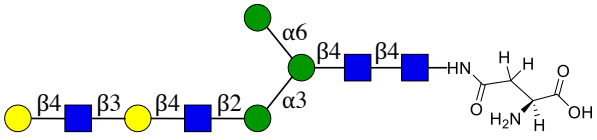


Figure S33. Analytical HPLC-MS chromatogram of compound **79**. The retention time = 13.0 min.

Compound 80

80 was prepared from **73** (1.0 mg) using the general procedure **2.2 i** for the removing of Cbz group with 20% Pd(OH)$_2$/C and H_2. The final product **80** was obtained as a white fluffy solid (0.8 mg, 90%). ESI TOF-MS *m/z* calculated for $C_{66}H_{108}N_6O_{48}$, $[M-2H]^{2-}$: 876.3103, found 876.3092.



80

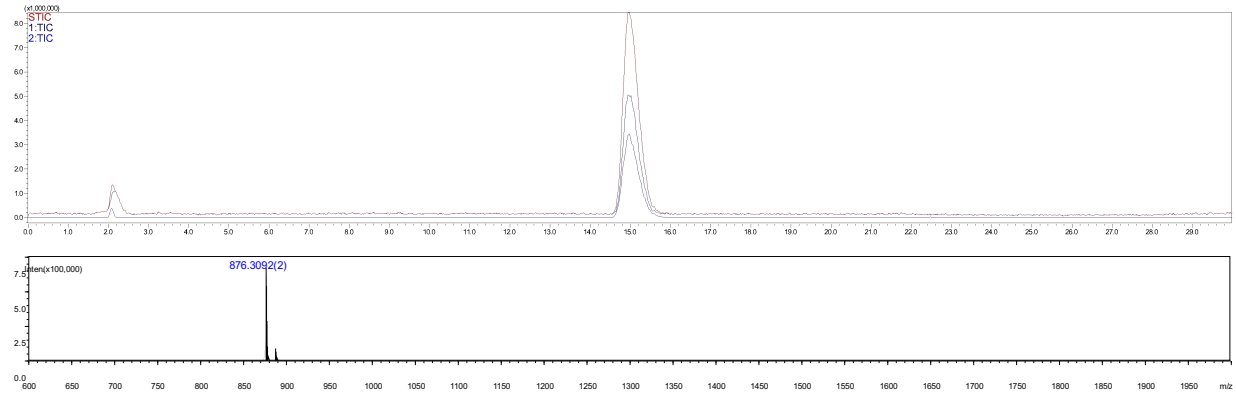
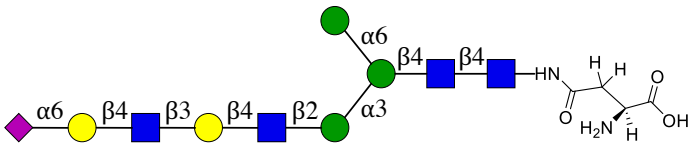


Figure S34. Analytical HPLC-MS chromatogram of compound **80**. The retention time = 15.0 min.

Compound 82

82 was synthesized from **73** (1.0 mg) through the installation of α2 6-Neu5Ac using the general procedure **2.2** to give the intermediate product **81** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **81** was removed by hydrogentation (**2.2 i**) to give final product **82** as a white fluffy solid (0.8 mg, 70% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{77}H_{125}N_7O_{56}$, $[M\text{-}2H]^{2-}$: 1021.8580, found 1021.8574.



82

NMR and MS analysis of compound **81**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.08 (d, *J* = 9.8 Hz, 1H)	3.86		3.68	3.58	N/R[b]
GlcNAc2	4.64	3.82	3.79	3.77	N/R	N/R
Man3	4.81 (s, 1H)	4.28 (d, *J* = 2.5 Hz, 1H)	3.80	N/R	N/R	3.95, 3.84
Man4	5.15	4.24 – 4.21 (m, 1H)		3.54	N/R	N/R
Man4'	4.95 (d, *J* = 1.8 Hz, 1H)	4.01	N/R	3.67	N/R	N/R
GlcNAc5	4.61 (d, *J* = 7.4 Hz, 1H)	3.77	N/R	3.75	N/R	N/R
Gal6	4.51 – 4.47 (m, 2H)	3.62	3.77	4.19 (d, *J* = 3.2 Hz, 1H)	N/R	N/R
GlcNAc7	4.76	3.82	N/R	N/R	N/R	N/R

Gal8	4.51 – 4.47 (m, 2H)	3.57	N/R	N/R	N/R	N/R

	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Neu5Ac9	-[a]	-	2.70 (dd, *J* = 12.3, 4.6 Hz, 2H), 1.75 (t, *J* = 12.2 Hz, 1H)	3.68	3.84	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	**C2**	**C3**	**C4**	**C5**	**C6**
GlcNAc1	78.09	53.68	N/R	78.56	76.15	N/R
GlcNAc2	101.19	54.83	65.86	79.61	N/R	N/R
Man3	100.33	70.14	80.31	N/R	N/R	65.80
Man4	99.50	76.36	N/R	67.23	N/R	N/R
Man4'	99.58	69.82	N/R	66.73	N/R	N/R
GlcNAc5	99.42	54.76	N/R	78.48	N/R	N/R
Gal6	103.40	69.92	81.95	68.24	N/R	N/R
GlcNAc7	102.52	54.90	N/R	N/R	N/R	N/R
Gal8	102.90	70.69	N/R	N/R	N/R	N/R

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac9	N/R	100.08	40.02	68.15	51.84	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NH<u>C</u>(O)CH$_3$	-	174.86, 174.64
NHC(O)<u>CH$_3$</u>	2.25 – 1.86 (m, 15H)	22.28, 22.24, 22.13, 21.98, 21.92
Aromatic	7.55 – 7.37 (m, 5H)	136.24, 128.71, 128.32, 127.74
<u>CH$_2$</u>-Ph	5.21 – 5.11 (m, 1H)	67.01
NH-<u>C</u>OO-	-	157.75
NH-<u>CH</u>-COOH	4.45 (dd, *J* = 8.9, 4.8 Hz, 1H)	52.11
NH-CH-<u>C</u>OOH	-	176.47
C(O)-<u>CH$_2$</u>-CH	2.87 (dd, *J* = 15.6, 4.3 Hz, 1H), 2.70 (dd, *J* = 12.3, 4.6 Hz, 2H)	38.05
<u>C</u>(O)-CH$_2$-CH	-	173.44

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{85}H_{131}N_7O_{58}$, $[M-2H]^{2-}$: 1088.8764, found 1088.8674.

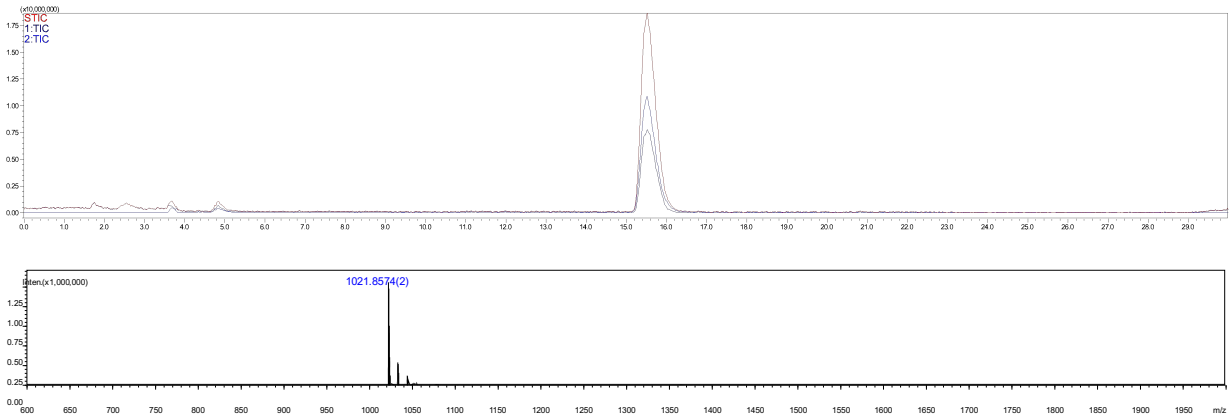
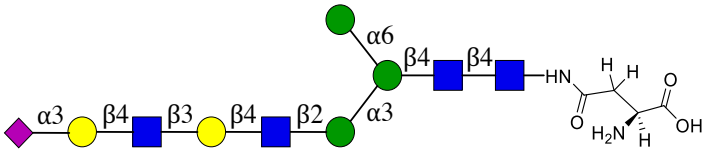


Figure S35. Analytical HPLC-MS chromatogram of compound **82**. The retention time = 15.6 min.

Compound 84

84 was synthesized from **73** (2 mg) through the installation of α2,3-Neu5Ac using the general procedure **2.2 f** to give the intermediate product **83** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **83** was removed by hydrogentation (**2.2 i**) to give final product product **84** as a white fluffy solid (0.4 mg, 44% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{77}H_{125}N_7O_{56}$, $[M\text{-}2H]^{2-}$: 1021.8580, found 1021.8552.



84

NMR and MS analysis of compound **83**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.8 Hz, 1H)	3.83	3.75	3.66	3.57	N/R[b]
GlcNAc2	4.61 (d, *J* = 8.0 Hz, 1H)	3.80	3.76	3.74	N/R	N/R
Man3	4.79	4.26 (d, *J* = 2.5 Hz, 1H)	3.77	N/R	N/R	3.92, 3.81
Man4	5.12	4.20 (d, *J* = 3.6 Hz, 1H)	3.90	3.51	N/R	N/R
Man4'	4.92 (d, *J* = 1.8 Hz, 1H)	3.98	3.88	3.65	N/R	N/R
GlcNAc5	4.60 – 4.55 (m, 2H)	3.75		3.72	N/R	N/R
Gal6	4.46 (d, *J* = 7.9 Hz, 1H)	3.59	3.73	4.17 (d, *J* = 3.2 Hz, 1H)	N/R	N/R
GlcNAc7	4.70 (d, *J* = 8.3 Hz, 1H)	3.81	N/R	N/R	N/R	N/R
Gal8	4.60 – 4.55 (m, 2H)	3.58	3.96	4.12	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac9	-[a]	-	2.79 – 2.71 (m, 2H), 1.81 (t, *J* = 12.2 Hz, 1H)	3.70	3.86	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.08	53.67	N/R	78.50	76.13	N/R
GlcNAc2	101.17	54.73	65.82	79.59	N/R	N/R
Man3	100.31	70.11	80.28	N/R	N/R	65.77
Man4	99.46	76.30	69.30	67.20	N/R	N/R
Man4'	99.55	69.78	70.31	66.69	N/R	N/R
GlcNAc5	99.38	54.79	N/R	75.09	N/R	N/R
Gal6	102.86	69.87	81.99	68.23	N/R	N/R
GlcNAc7	102.73	55.10	N/R	N/R	N/R	N/R
Gal8	102.45	69.30	67.39	75.40	N/R	N/R

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac9	N/R	99.67	39.53	68.23	51.59	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-	174.92, 174.80, 174.61
NHC(O)<u>CH$_3$</u>	2.24 – 1.88 (m, 15H)	22.25, 22.09, 21.95, 21.88
Aromatic	7.58 – 7.30 (m, 5H)	136.15, 128.68, 128.32, 127.68
<u>CH$_2$</u>-Ph	5.20 – 5.09 (m, 2H)	67.08
NH-<u>C</u>OO-	-	157.76
NH-<u>CH</u>-COOH	4.52 (dd, *J* = 7.9, 4.7 Hz, 1H)	51.19
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.86 (dd, *J* = 15.7, 4.6 Hz, 1H), 2.79 – 2.71 (m, 2H)	37.52
<u>C</u>(O)-CH$_2$-CH	-	N/R

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{85}H_{131}N_7O_{58}$, $[M-2H]^{2-}$: 1088.8764, found 1088.8734.

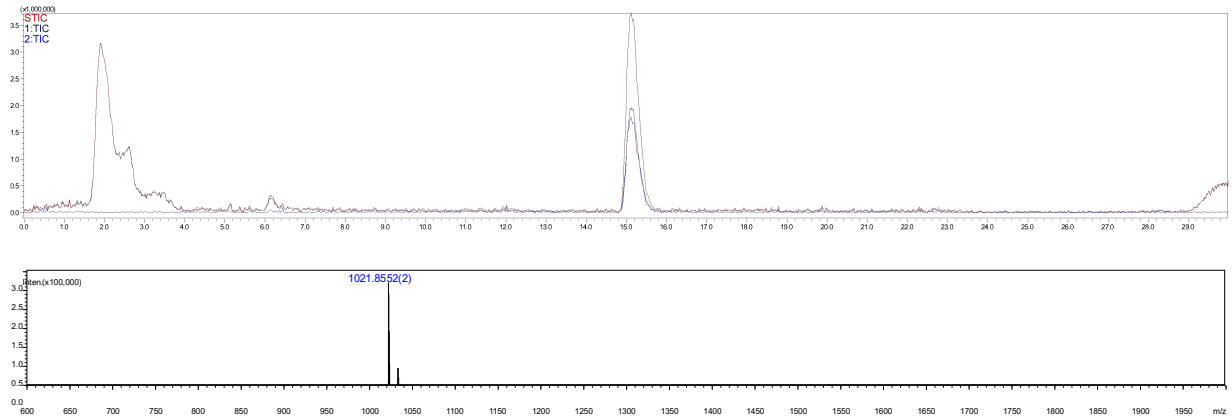
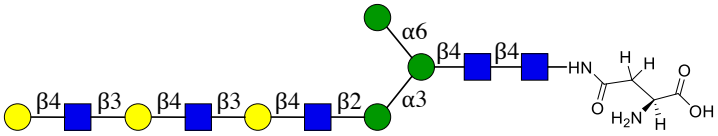


Figure S36. Analytical HPLC-MS chromatogram of compound **84**. The retention time = 15.2 min.

Compound 85

85 was prepared from **74** (1 mg) using the general procedure **2.2 i** for the removing of Cbz group with 20% $Pd(OH)_2/C$ and H_2. The final product **85** was obtained as a white fluffy solid (0.9 mg, 96%). ESI TOF-MS *m/z* calculated for $C_{80}H_{131}N_7O_{58}$, $[M-2H]^{2-}$: 1058.8764, found 1058.8682.



85

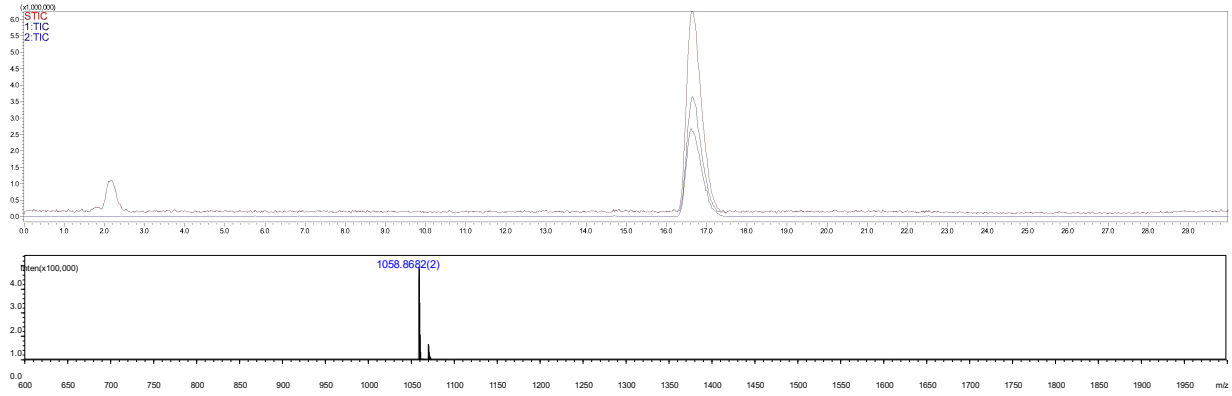
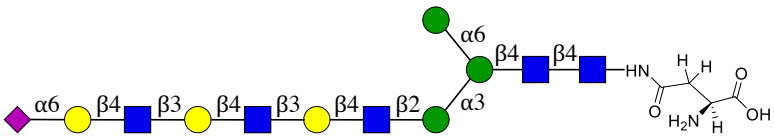


Figure S37. Analytical HPLC-MS chromatogram of compound **85**. The retention time = 14.0 min.

Compound 87

87 was synthesized from **74** (1 mg, 1.0 eq) through the installation of α2, 6-Neu5Ac using the general procedure **2.2 e** to give the intermediate product **86**, which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecting group of **86** was removed by hydrogentation (**2.2 i**) to give final product **87** as a white fluffy solid (0.8 mg, 74% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{91}H_{148}N_8O_{66}$, $[M-2H]^{2-}$: 1204.4241, found 1204.4175.



87

NMR and MS analysis of compound **86**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.08 (d, *J* = 9.8 Hz, 1H)	3.86	N/R[b]	3.68	3.59	N/R
GlcNAc2	4.64 (d, *J* = 7.9 Hz, 1H)	3.83	3.80	3.76	N/R	N/R
Man3	4.81	4.28 (d, *J* = 2.3 Hz, 1H)	3.80	N/R	N/R	3.95, 3.84
Man4	5.15	4.24 – 4.21 (m, 1H)	N/R	3.54	N/R	N/R
Man4'	4.95 (d, *J* = 1.7 Hz, 1H)	4.01	N/R	3.67	N/R	N/R
GlcNAc5	4.61 (d, *J* = 7.4 Hz, 1H)	3.77	N/R	3.75	N/R	N/R
Gal6	4.52 – 4.45 (m, 3H)	3.62	3.76	4.19 (s, 2H)	N/R	N/R
GlcNAc7	4.76 (s, 1H)	3.83	N/R	N/R	N/R	N/R
Gal8	4.52 – 4.45 (m, 3H)	N/R	3.76	4.19 (s, 2H)	N/R	N/R
GlcNAc9	4.73 (d, *J* = 8.4 Hz, 1H)	3.83	N/R	N/R	N/R	N/R
Gal10	4.52 – 4.45 (m, 3H)	N/R	N/R	N/R	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac9	-[a]	-	2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.76 (t, *J* = 12.2 Hz, 1H)	3.70	3.84	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.15	53.70	N/R	78.56	76.16	N/R
GlcNAc2	101.19	54.83	65.86	79.61	N/R	N/R
Man3	100.33	70.13	80.31	N/R	N/R	65.80
Man4	99.49	76.34	N/R	67.23	N/R	N/R
Man4'	99.58	69.82	N/R	66.72	N/R	N/R
GlcNAc5	99.40	54.77	N/R	78.46	N/R	N/R
Gal6	102.89	69.92	81.96	68.25	N/R	N/R

GlcNAc7	102.51	55.08	N/R	N/R	N/R	N/R
Gal8	102.83	N/R	82.00	68.25	N/R	N/R
GlcNAc9	102.69	54.90	N/R	N/R	N/R	N/R
Gal10	103.40	N/R	N/R	N/R	N/R	N/R

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**
Neu5Ac9	N/R	100.02	39.98	68.12	51.83	N/R	N/R	N/R	N/R

Signal	**Proton**	**Carbon**
NHC(O)CH_3	-	174.85, 174.69, 174.63
NHC(O)CH_3	2.34 – 1.86 (m, 18H)	22.28, 22.24, 22.13, 21.98, 21.91
Aromatic	7.56 – 7.36 (m, 5H)	128.71, 128.34, 127.71
CH_2-Ph	5.21 – 5.13 (m, 2H)	67.09
NH-COO-	-	157.75
NH-CH-COOH	4.54 (dd, *J* = 8.1, 4.8 Hz, 1H)	51.29
NH-CH-COOH	-	N/R
C(O)-CH_2-CH	2.89 (dd, *J* = 15.9, 4.7 Hz, 1H), 2.77 (dd, *J* = 15.8, 8.3 Hz, 1H)	37.60
C(O)-CH_2-CH	-	173.35

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{99}H_{154}N_8O_{68}$, $[M-2H]^{2-}$: 1271.4425, found 1271.4315.

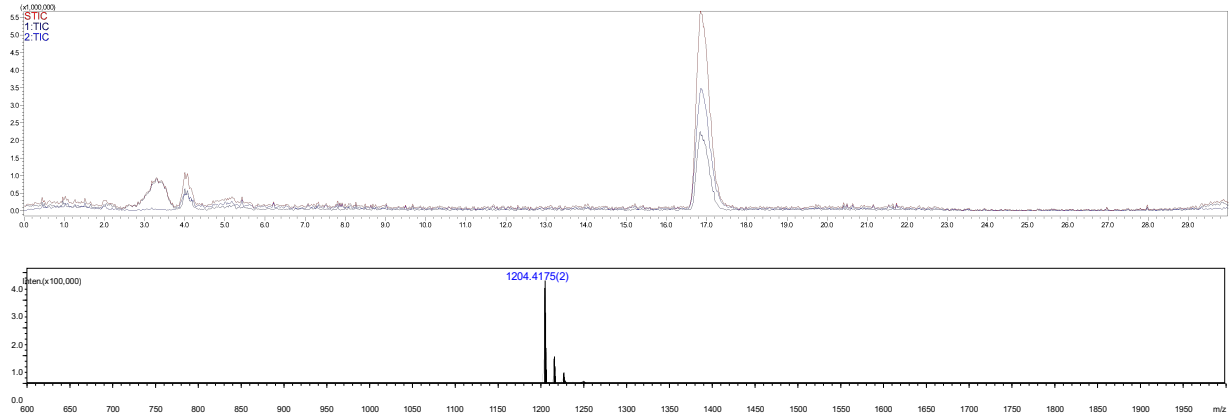
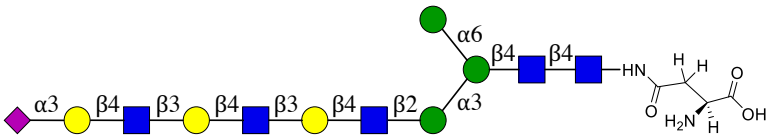


Figure S38. Analytical HPLC-MS chromatogram of compound **87**. The retention time = 17.0 min.

Compound 89

89 was synthesized from **74** (2 mg, 1.0 eq) through the installation of α2,3-Neu5Ac using the general procedure **2.2 f** to give intermediate product **88** which was purified using the described two-stage purification system **(2.2 j)**. The Cbz protecrting group of **88** was removed by hydrogentation (**2.2 i**) to give final product **89** as a white fluffy solid (0.6 mg, 54% yield over two steps). ESI TOF-MS *m/z* calculated for $C_{91}H_{148}N_8O_{66}$, $[M\text{-}2H]^{2-}$: 1204.4241, found 1204.4201.



89

NMR and MS analysis of compound **88**

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
GlcNAc1	5.06 (d, *J* = 9.8 Hz, 1H)	3.83	N/R[b]	3.66	N/R	N/R
GlcNAc2	4.61 (d, *J* = 8.0 Hz, 1H)	3.80	3.77	3.74	N/R	N/R
Man3	4.79	4.26 (d, *J* = 2.5 Hz, 1H)	3.77	N/R	N/R	3.92, 3.81
Man4	5.13	4.20 (d, *J* = 3.6 Hz, 1H)	3.91	3.51	N/R	N/R
Man4'	4.93 (d, *J* = 1.7 Hz, 0H)	3.98	3.89	3.65	N/R	N/R
GlcNAc5	4.60 – 4.53 (m, 1H)	3.75	N/R	3.72	N/R	N/R
Gal6	4.50 – 4.41 (m, 2H)	3.59	3.73	4.17 (d, *J* = 3.0 Hz, 1H)	N/R	N/R
GlcNAc7	4.70 (d, *J* = 8.3 Hz, 2H)	3.81	N/R	N/R	N/R	N/R
Gal8	4.50 – 4.41 (m, 2H)	3.59	3.73	4.17 (d, *J* = 3.0 Hz, 1H)	N/R	N/R
GlcNAc9	4.70 (d, *J* = 8.3 Hz, 2H)	3.81	N/R	N/R	N/R	N/R
Gal10	4.60 – 4.53 (m, 1H)	3.58	3.96	4.12 (dd, *J* = 9.9, 3.1 Hz, 1H)	N/R	N/R

	H1	H2	H3	H4	H5	H6	H7	H8	H9
Neu5Ac9	-[a]	-	2.77 (dd, *J* = 12.5, 4.6 Hz, 1H), 1.81 (t, *J* = 12.1 Hz, 1H)	3.70	3.85	N/R	N/R	N/R	N/R

^{13}C (150 MHz, D_2O): δ (ppm)

	C1	C2	C3	C4	C5	C6
GlcNAc1	78.07	53.65	N/R	78.49	76.12	N/R
GlcNAc2	101.16	54.79	65.82	79.59	N/R	N/R
Man3	100.31	70.11	80.28	N/R	N/R	65.76
Man4	99.47	76.29	69.30	67.19	N/R	N/R
Man4'	99.55	69.78	70.31	66.69	N/R	N/R
GlcNAc5	99.37	54.73	N/R	75.09	N/R	N/R
Gal6	102.86	69.88	81.98	68.25	N/R	N/R
GlcNAc7	102.72	55.10	N/R	N/R	N/R	N/R
Gal8	102.79	69.88	81.98	68.25	N/R	N/R
GlcNAc9	102.68	55.06	N/R	N/R	N/R	N/R
Gal10	102.45	69.30	67.39	75.41	N/R	N/R

	C1	C2	C3	C4	C5	C6	C7	C8	C9
Neu5Ac9	N/R	99.71	39.55	68.23	51.60	N/R	N/R	N/R	N/R

Signal	Proton	Carbon
NH<u>C</u>(O)CH$_3$	-	174.92, 174.81, 174.69, 174.61
NHC(O)<u>CH$_3$</u>	2.35 – 1.85 (m, 18H)	22.25, 22.09, 21.95, 21.89
Aromatic	7.55 – 7.33 (m, 5H)	136.19, 128.68, 128.30, 127.70
<u>CH$_2$</u>-Ph	5.19 – 5.09 (m, 2H)	65.76
NH-<u>C</u>OO-	-	157.73
NH-<u>CH</u>-COOH	4.45	51.94
NH-CH-<u>C</u>OOH	-	N/R
C(O)-<u>CH$_2$</u>-CH	2.85 (dd, *J* = 15.7, 4.5 Hz, 1H), 2.69 (dd, *J* = 15.6, 8.7 Hz, 1H)	37.89
<u>C</u>(O)-CH$_2$-CH	-	173.73

[a] Not applicable

[b] Not reported

ESI TOF-MS *m/z* calculated for $C_{99}H_{154}N_8O_{68}$, $[M-2H]^{2-}$: 1271.4425, found 1271.4318.

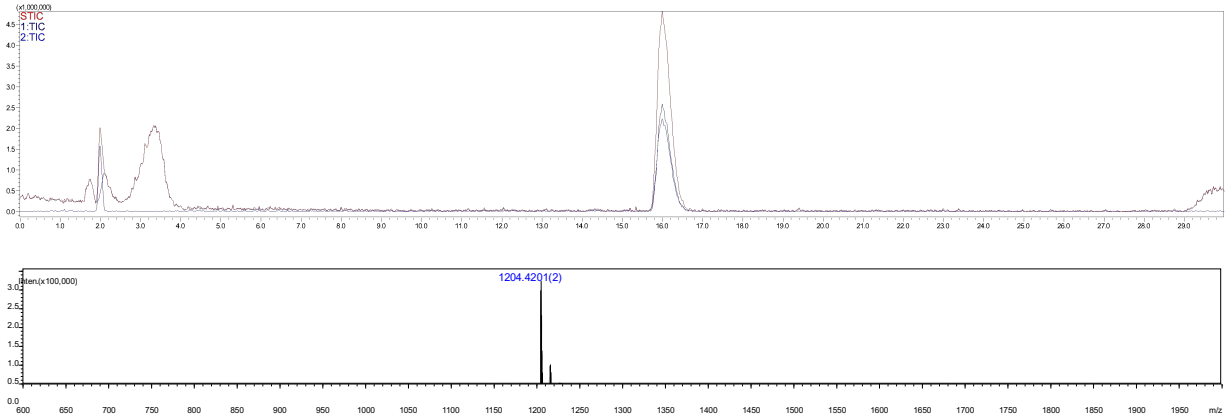


Figure S39. Analytical HPLC-MS chromatogram of compound **89**. The retention time = 1.0 min.

3. Microarray

3.1 Materials

Virus isolates were produced as described previously.[3] Oseltamivir was purchased from Sigma Aldrich [Cat# SML1606]. CR8020 group 2, and CR6261 group 1 stem antibodies provided by Dr. Dirk Eggink and expressed following previously published procedures.[4] Goat anti-human Alexa-647 [Cat# A21445] antibodies and streptavidin-AlexaFluor 635 [Cat# SA1011] were obtained from Thermo Fisher. Control lectins *Erythrina cristagalli* agglutinin (ECA) [Cat#B-1145], *Sambuca nigra* agglutinin (SNA) [Cat# B-1305], and *Maackia amurensis* lectin I (MAL-I) [Cat# B-1315] were purchased from Vector Labs.

3.2 Glycan printing surfaces

Compounds were printed on amine reactive, N-hydroxy succinimide (NHS)-activated glass slides (NEXTERION ® Slide H, Schott Inc) employing the free amine of the anomeric asparagine moiety of the *N*-glycans for immobilization. The printing was performed using a Scienion sciFLEXARRAYER S3 non-contact microarray printer equipped with a Scienion PDC80 nozzle (Scienion Inc). Glycans were dissolved in sodium phosphate buffer (250 mM, pH 8.5) at a concentration of 100 µM and printed in replicates of six (spot volume of ~400 pL, at 20 °C and 50% humidity). Slides were blocked with 5 mM ethanolamine in Tris buffer (pH 9, 50 mM) for 1 h at 50 °C and rinsed with DI water after printing.

3.3 Glycan microarray

Quality control was performed using the plant lectins SNA, ECA and MAL-I at 10 µg/mL precomplexed with 2 µg/mL Streptavidin-AlexaFluor 635.

Viral isolates (25 µL) diluted in PBS-T (PBS + 0.1% Tween, 25 µL) were applied to subarrays in the presence of oseltamivir (200 nM) in a humidified chamber for 1 h. Next, the microarray slide was rinsed with PBS-T (PBS + 0.1% Tween), PBS, and deionized water (2x) and dried by centrifugation. The slide was incubated for 1 h in the presence of the CR8020 A/H3N2 influenza hemaglutinin or CR6261 stem specific antibody (100 µL, 5 µg mL^{-1} in PBS-T) and washed as described above. A secondary goat anti-human AlexaFluor-647 antibody (100 µL, 2 µg mL^{-1} in PBS-T) was applied and the resulting slide was incubated for 1 h in a humidified chamber and then washed by the standard procedure. Slides were dried by centrifugation after the washing steps and scanned immediately using an Innopsys Innoscan 710 microarray scanner. Various gains and PMT values were used to ensure the signals were in the linear range and to avoid saturation of the signals. Images were analyzed with Mapix software (version 8.1.0 Innopsys) and processed with an Excel macro (https://github.com/enthalpyliu/carbohydrate-microarray-processing). The average fluorescence intensity and SD were determined for each compound after removal of the highest and lowest intensities from the spot replicates to give $n = 4$.

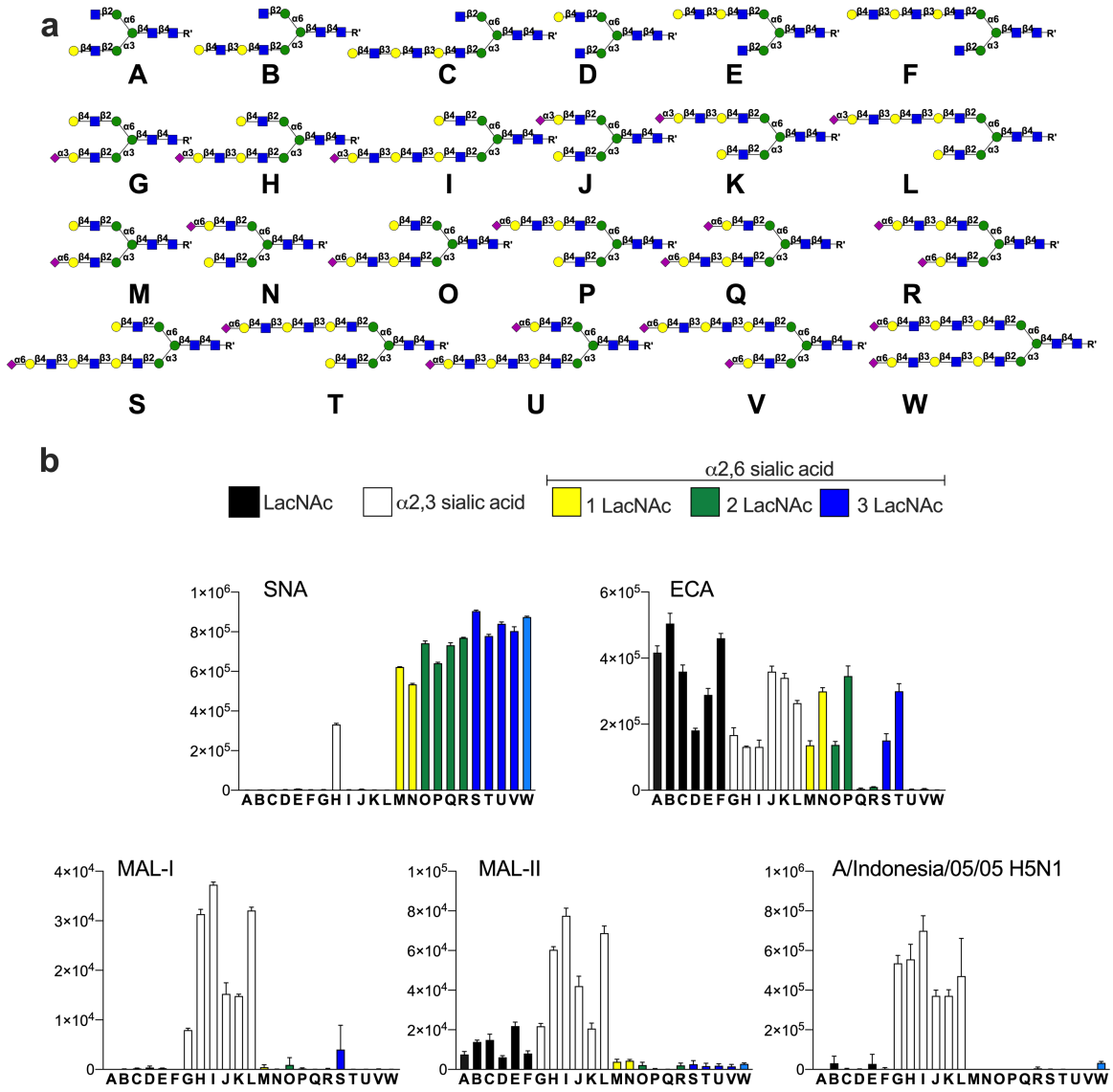


Figure S40. Validation of the microarray. **a**) Library of compounds. **b**) Microarray screening for lectins and and A/Indonesia/05/05 H5N1. Bars represent the background-subtracted average relative fluorescence units (RFU) of four replicates ±SD.

4. Hemagglutination and sequence alignment

4.1 Erythrocyte preparation

Fresh turkey blood was centrifuged (430 rcf, 10 min) followed by removal of the supernatant. Pellets were washed with PBS three times with intermittent centrifugation (430 rcf, 10 min). Erythrocyte suspensions were stored a 50% solution in PBS.

4.2 Enzymatic erythrocyte remodeling

Arthrobacter ureafaciens neuraminidase (12 U; New England Biolabs) was added to a suspension of turkey erythrocytes (250 µL, 50%) in PBS (900 µL) and incubated for 6 h at 37 °C with tilting. Next, recombinant B4GALT1 (37.5 µL, 1 mg/mL) and B3GnT2 (37.5 µL, 1 mg/mL), UDP-Gal (4.4 mM) and UDP-GlcNAc (4.4 mM), alkaline phosphatase (6 U), $MnCl_2$ (2 mM) and BSA (6 µL, 2 mg/mL) were added. This mixture was incubated for 18 h at 37 °C with gentle tilting. Next, the erythrocytes were washed with PBS (2x, 750 µL) and the pellet was reconstituted in PBS (900 µL). The erythrocytes were resialylated with ST6Gal1 (37 µL, 1 mg/mL) and CMP-Neu5Ac (4.5 mM) in the presence of alkaline phosphatase (6 U) and BSA (6 µL, 2 mg/mL) for 4 h at 37 °C with gentle tilting. The erythrocytes were washed with PBS (1x, 600 µL) and diluted to a 1% solution.

4.3 Hemagglutination assay

Hemagglutination assays were performed according to a standard procedure.[5.] Briefly, virus stocks were two-fold serial diluted in the presence of oseltamivir (20 nM) in PBS. Turkey erythrocytes (1%, 25 µL) were mixed with the serial diluted viruses and incubated for 1 h at 4 °C. Titers were expressed as the highest dilution of virus stock that completely agglutinated the turkey erythrocytes.

Table S2. Amino acid alignment of H3 proteins.

No.	Name	Clade	Year	98	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	155	156	157	158	159	160	161	162	163	164	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	219	220	221	222	223	224	225	226	227
1	A/Hong Kong/001/1968	Unknown	1968	Y	F	I	T	E	G	F	T	W	T	G	V	T	Q	N	G	G	S	N	A	C	K	R	G	P	G	S	G	T	K	S	G	S	T	Y	P	V	L	S	T	N	Q	E	Q	T	S	L	Y	V	Q	A	S	G	R	V	T	V	S	T	R	R	S	Q	Q	T	I	S	R	P	W	V	R	G	L	S
2	A/Bilthoven/16190/1968	Unknown	1968	Y	F	I	T	E	G	F	T	W	T	G	V	T	Q	N	G	G	S	N	A	C	K	R	G	P	G	S	G	T	K	S	G	S	T	Y	P	V	L	S	T	N	Q	E	Q	T	S	L	Y	V	Q	A	S	G	R	V	T	V	S	T	R	R	S	Q	Q	T	I	S	R	P	W	V	R	G	L	S
3	A/Beijing/353/1989	Unknown	1989	Y	F	I	N	E	D	F	N	W	T	G	V	A	Q	S	G	E	S	Y	A	C	K	R	G	S	V	K	S	H	E	S	E	Y	K	Y	P	A	L	S	T	D	R	E	Q	T	K	L	Y	V	R	A	S	G	R	V	T	V	S	T	K	R	S	Q	Q	T	V	S	R	P	W	V	R	G	L	S
4	A/Netherlands/816/1991	Unknown	1991	Y	F	I	N	E	D	F	N	W	T	G	V	A	Q	S	G	E	S	Y	A	C	K	R	G	S	V	K	S	H	E	S	E	Y	K	Y	P	A	L	S	T	D	R	E	Q	T	S	L	Y	V	R	A	S	G	R	V	T	V	S	T	K	R	S	Q	Q	T	V	S	R	P	W	V	R	G	L	S
5	A/Netherlands/109/2003	Unknown	2003	Y	F	N	N	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	A	C	K	R	R	S	N	K	S	T	H	L	K	Y	K	Y	P	A	L	G	T	D	S	D	Q	I	S	L	Y	A	Q	A	S	G	R	I	T	V	S	T	K	R	S	Q	Q	T	V	S	R	P	R	V	R	D	I	S
6	A/Wisconsin/67/2005	Unknown	2005	Y	F	N	D	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	S	C	K	R	R	S	N	N	S	T	H	L	K	F	K	Y	P	A	L	V	T	D	N	D	Q	I	F	L	Y	A	Q	A	S	G	R	I	T	V	S	T	K	R	S	Q	Q	T	V	S	R	P	R	I	R	N	I	P
7	A/Brisbane/10/2007	Unknown	2007	Y	F	N	N	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	A	C	I	R	R	S	N	N	S	T	H	L	K	F	K	Y	P	A	L	G	T	D	N	D	Q	I	F	P	Y	A	Q	A	S	G	R	I	T	V	S	T	K	R	S	Q	Q	T	V	S	R	P	R	V	R	N	I	P
8	A/Netherlands/761/2009	Unknown	2009	Y	F	N	N	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	A	C	I	R	R	S	N	N	S	T	H	L	R	F	K	Y	P	A	L	G	T	D	N	D	Q	I	F	L	Y	A	Q	A	S	G	R	I	T	V	S	T	K	R	S	Q	Q	T	V	S	R	P	R	V	R	N	I	P
9	A/Singapore/INFIMH-16-0019/2016	3c.2a1	2016	Y	F	K	N	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
10	A/Netherlands/2413/16	3C.2a1	2016	Y	F	K	N	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	A	C	M	R	R	S	S	S	S	T	H	L	H	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
11	A/Netherlands/1797/2017	3C.2a1	2017	Y	F	K	N	E	S	F	N	W	A	G	V	T	Q	N	G	K	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
12	A/Netherlands/371/2019	3C.2a1b	2019	Y	F	K	N	E	S	F	N	W	A	G	V	T	Q	N	G	K	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
13	A/Netherlands/10/2019	3C.2a1b.1	2019	Y	F	K	N	E	S	F	N	W	A	G	V	T	Q	N	G	K	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
14	A/Netherlands/314/2019	3C.2a1b	2019	Y	F	K	N	E	S	F	N	W	A	G	V	T	Q	N	G	K	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
15	A/Netherlands/751/2017	3C.2a1	2017	Y	F	K	N	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	A	C	M	R	R	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
16	A/Switzerland/8060/2017	3C.2a2	2017	Y	F	N	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	K	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
17	A/Netherlands/3466/2017	3C.2a	2017	Y	X	N	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	K	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
18	A/Netherlands/1802/2018	3C.2a2	2018	Y	F	N	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	K	S	R	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	F	R	P	R	I	R	D	I	P
19	A/Netherlands/295/2019	3C.2a1b	2019	Y	F	K	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	F	R	P	R	I	R	D	I	P
20	A/Netherlands/10616/2019	3C.2a2	2019	Y	F	N	N	E	N	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	K	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
21	A/Netherlands/10009/2019	3C.2a1b	2019	Y	F	K	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	R	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	F	R	P	R	I	R	D	I	P
22	A/Netherlands/110/2021	3C.2a1b.1a	2021	Y	F	K	N	E	S	F	N	W	A	G	V	T	Q	N	G	K	S	S	S	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	D	T	D	K	N	Q	I	S	L	Y	A	Q	P	S	G	R	I	T	V	F	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
23	A/Netherlands/7/2021	3C.2a1b.2a.2	2021	Y	F	K	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	G	S	S	S	S	T	S	L	N	N	I	Y	P	A	Q	D	T	D	K	N	Q	I	S	L	F	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
24	A/Netherlands/8/2021	3C.2a1b.2a.2	2021	Y	F	K	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	G	S	S	S	S	T	S	L	N	N	I	Y	P	A	Q	D	T	D	K	N	Q	F	S	L	F	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
25	A/Netherlands/92/2021	3C.2a1b.2a.2	2021	Y	F	K	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	G	S	S	S	S	T	S	L	N	N	I	Y	P	A	Q	D	T	D	K	N	Q	I	S	L	F	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
26	A/Netherlands/1/2022	3C.2a1b.2a.2	2022	Y	F	K	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	N	I	Y	P	A	Q	D	T	D	K	N	Q	I	S	L	F	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
27	A/Netherlands/832/2022	3C.2a1b.2a.2	2022	Y	F	K	N	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	K	R	G	S	S	S	S	T	H	L	N	N	I	Y	P	A	Q	D	T	D	K	N	Q	I	S	L	F	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	K	I	R	D	I	P
28	A/Netherlands/568/2023	3C.2a1b.2a.2	2023	Y	F	K	N	E	S	F	N	W	A	G	V	T	Q	N	G	K	S	S	A	C	I	R	G	S	S	S	S	T	H	L	N	Y	T	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
29	A/Netherlands/59/2023	3C.2a1b.2a.2	2023	Y	F	K	D	E	S	F	N	W	T	G	V	K	Q	N	G	T	S	S	A	C	K	R	G	S	S	S	S	T	S	L	N	N	I	Y	P	A	Q	D	T	D	K	N	Q	F	S	L	F	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	V	R	D	I	P
30	A/Texas/50/2012	3C.1	2012	Y	F	N	N	E	S	F	N	W	N	G	V	T	Q	N	G	T	S	S	A	C	I	R	R	S	N	N	S	T	H	L	N	F	K	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	P	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	N	I	P
31	A/Switzerland/9715293/2013	3c.3a	2013	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	S	C	I	R	G	S	N	S	S	T	H	L	N	S	K	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
32	A/Netherlands/622/2012	3C.3	2012	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	A	C	I	R	G	S	N	S	S	T	H	L	N	F	K	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	N	I	P
33	A/Netherlands/153/2016+B38	3C.3a	2016	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	S	C	I	R	G	S	N	S	S	T	H	L	N	S	K	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
34	A/Kansas/14/2017	3C.3a	2017	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	S	C	I	R	G	S	K	S	S	T	H	L	N	S	K	Y	P	A	L	G	T	D	K	D	Q	I	S	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
35	A/Netherlands/384/2019	3C.3a	2019	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	S	C	I	R	G	S	N	S	S	T	H	L	N	S	K	Y	P	A	L	G	T	D	K	D	Q	I	F	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
36	A/Netherlands/10002/2019	3C.3a	2019	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	S	C	I	R	G	S	K	S	S	T	H	L	N	S	K	Y	P	A	L	G	T	D	K	D	Q	T	S	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
37	A/Netherlands/10006/2019	3C.3a	2019	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	S	C	I	R	G	S	K	S	S	T	H	L	N	S	K	Y	P	A	L	G	T	D	K	D	Q	T	S	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P
38	A/Netherlands/322/2020	3C.3a1	2020	Y	F	N	N	E	S	F	N	W	A	G	V	T	Q	N	G	T	S	S	S	C	I	R	G	S	K	S	S	T	H	L	N	S	K	Y	P	A	L	G	T	D	K	D	Q	I	S	L	Y	A	Q	S	S	G	R	I	T	V	S	T	K	R	S	Q	Q	A	V	S	R	P	R	I	R	D	I	P

Table S3. Hemagglutination titers of additional A(H3N2) viruses isolated between 2021 and 2023 for wild type (WT) and glyco-remodeled (mod) turkey erythrocytes.

Name	GISAID	HA (WT) titer	HA (mod) titer
A/H3N2 viruses			
A/NL/7/21	EPI_ISL_3447260	48	64
A/NL/8/21	EPI_ISL_4551782	96	256
A/NL/11832/22	EPI_ISL_15895148	96	128
A/NL/12136/22	EPI_ISL_16494722	96	96
A/NL/1431/22	EPI_ISL_16147119	48	64
A/NL/1867/22	EPI_ISL_16334493	24	32
A/NL/10162/23	EPI_ISL_16812568	96	128
A/NL/496/23	EPI_ISL_16877488	48	32
A/NL/10142/23	EPI_ISL_16700790	48	64
A/NL/10290/23	EPI_ISL_17017548	96	128
A/NL/10277/23	EPI_ISL_17017534	192	256
A/NL/10132/23	EPI_ISL_16700787	96	256
A/NL/10226/23	EPI_ISL_16867389	192	128
A/NL/10228/23	EPI_ISL_16954700	192	192
A/NL/652/23	EPI_ISL_16955822	24	128
A/NL/442/23	EPI_ISL_16877479	24	192

5. References

(1) Liu, L.; Prudden, A. R.; Bosman, G. P.; Boons, G. J. Improved isolation and characterization procedure of sialylglycopeptide from egg yolk powder. *Carbohydr. Res.* **2017**, *452*, 122-128.

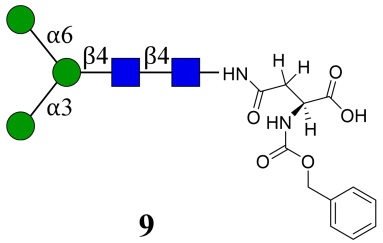
(2) Liu, L.; Prudden, A. R.; Capicciotti, C. J.; Bosman, G. P.; Yang, J. Y.; Chapla, D. G.; Moremen, K. W.; Boons, G. J. Streamlining the chemoenzymatic synthesis of complex *N*-glycans by a stop and go strategy. *Nat. Chem.* **2019**, *11*, 161-169.

(3) Broszeit, F.; van Beek, R. J.; Unione, L.; Bestebroer, T. M.; Chapla, D.; Yang, J. Y.; Moremen, K. W.; Herfst, S.; Fouchier, R. A. M.; de Vries, R. P.; Boons, G. J. Glycan remodeled erythrocytes facilitate antigenic characterization of recent A/H3N2 influenza viruses. *Nat. Commun.* **2021**, *12*, 5449.

(4) Ekiert, D. C.; Friesen, R. H.; Bhabha, G.; Kwaks, T.; Jongeneelen, M.; Yu, W.; Ophorst, C.; Cox, F.; Korse, H. J.; Brandenburg, B.; Vogels, R.; Brakenhoff, J. P.; Kompier, R.; Koldijk, M. H.; Cornelissen, L. A.; Poon, L. L.; Peiris, M.; Koudstaal, W.; Wilson, I. A.; Goudsmit, J. A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science* **2011**, *333*, 843-850.

(5) WHO Global Influenza Surveillance Network. *Manual for the laboratory diagnosis and virological surveillance of influenza*; World Health Organization, **2011**.

6. NMR spectra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9

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6.2
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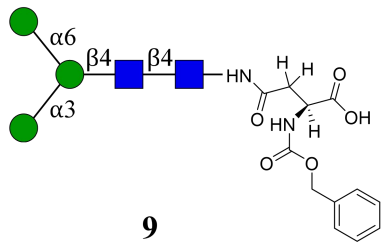
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9

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50
40
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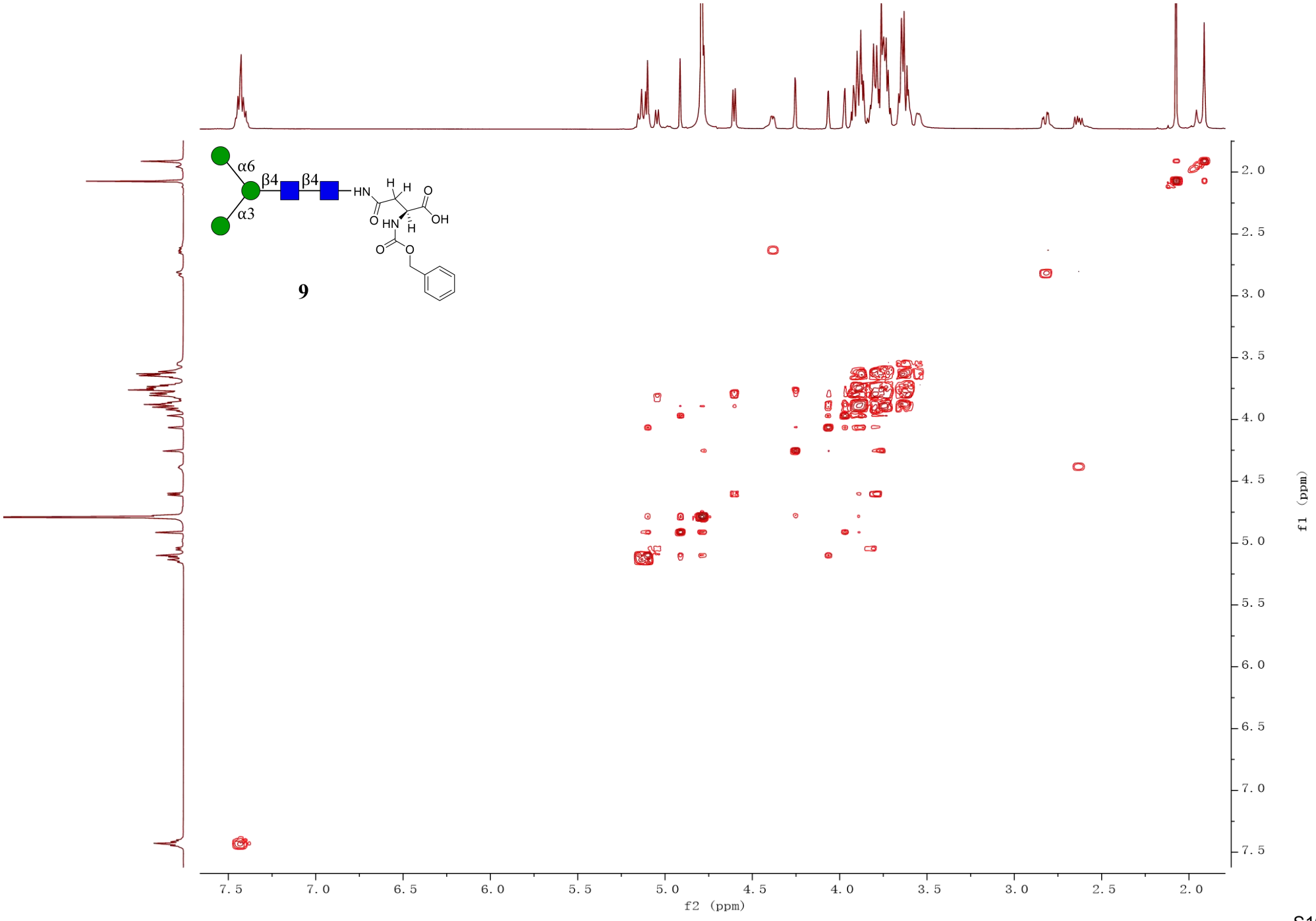
f1 (ppm)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4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
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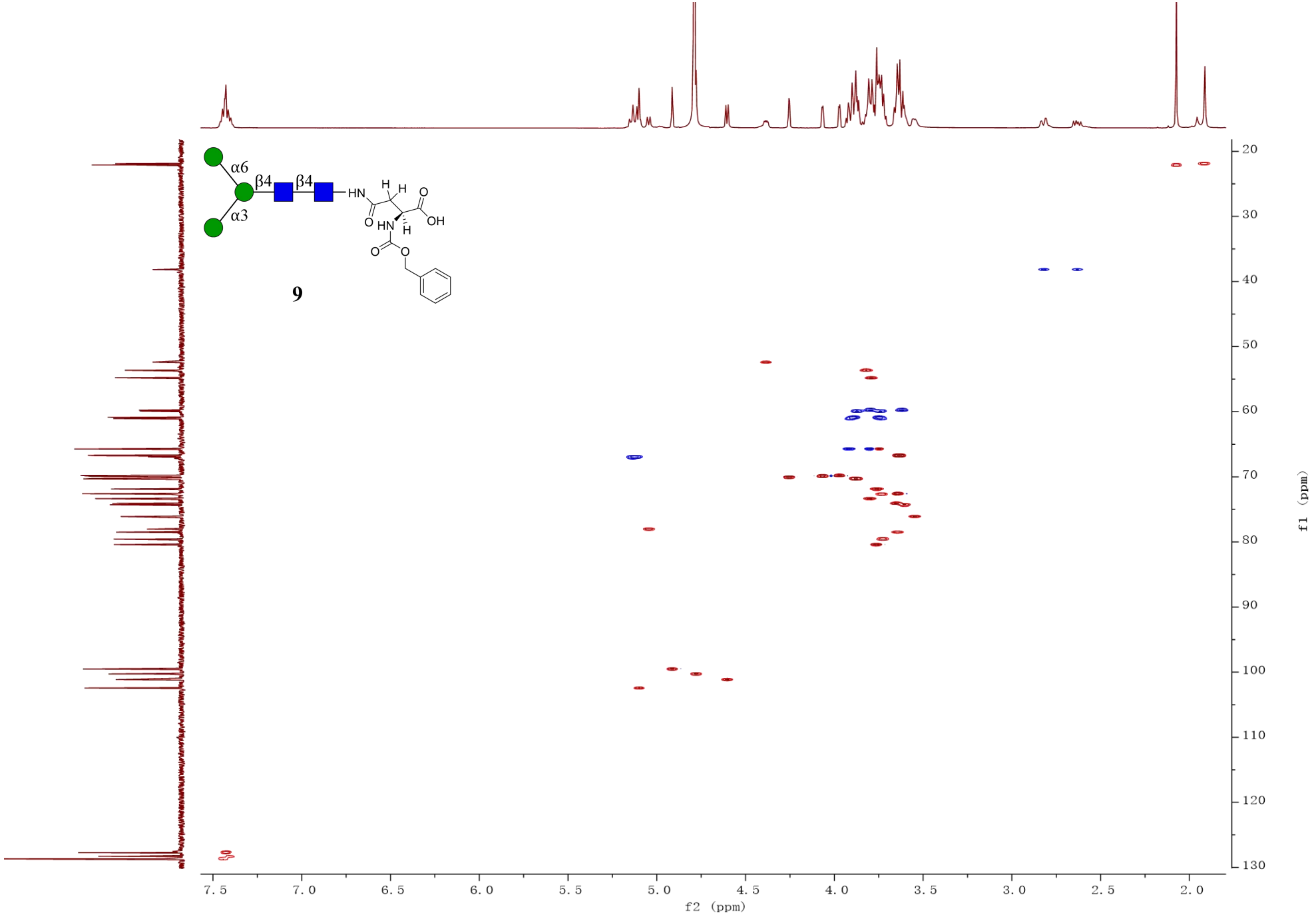
f1 (ppm)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5.0
4.5
4.0
3.5
3.0
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40
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80
90
100
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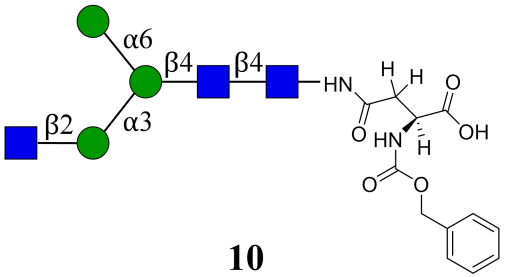
120
130
f1 (ppm)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O

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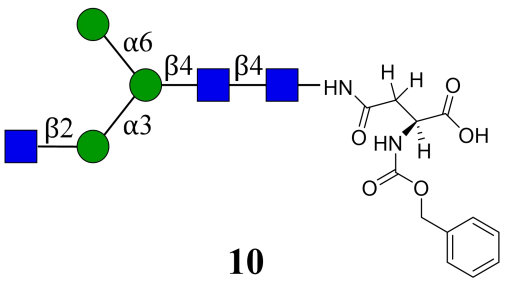
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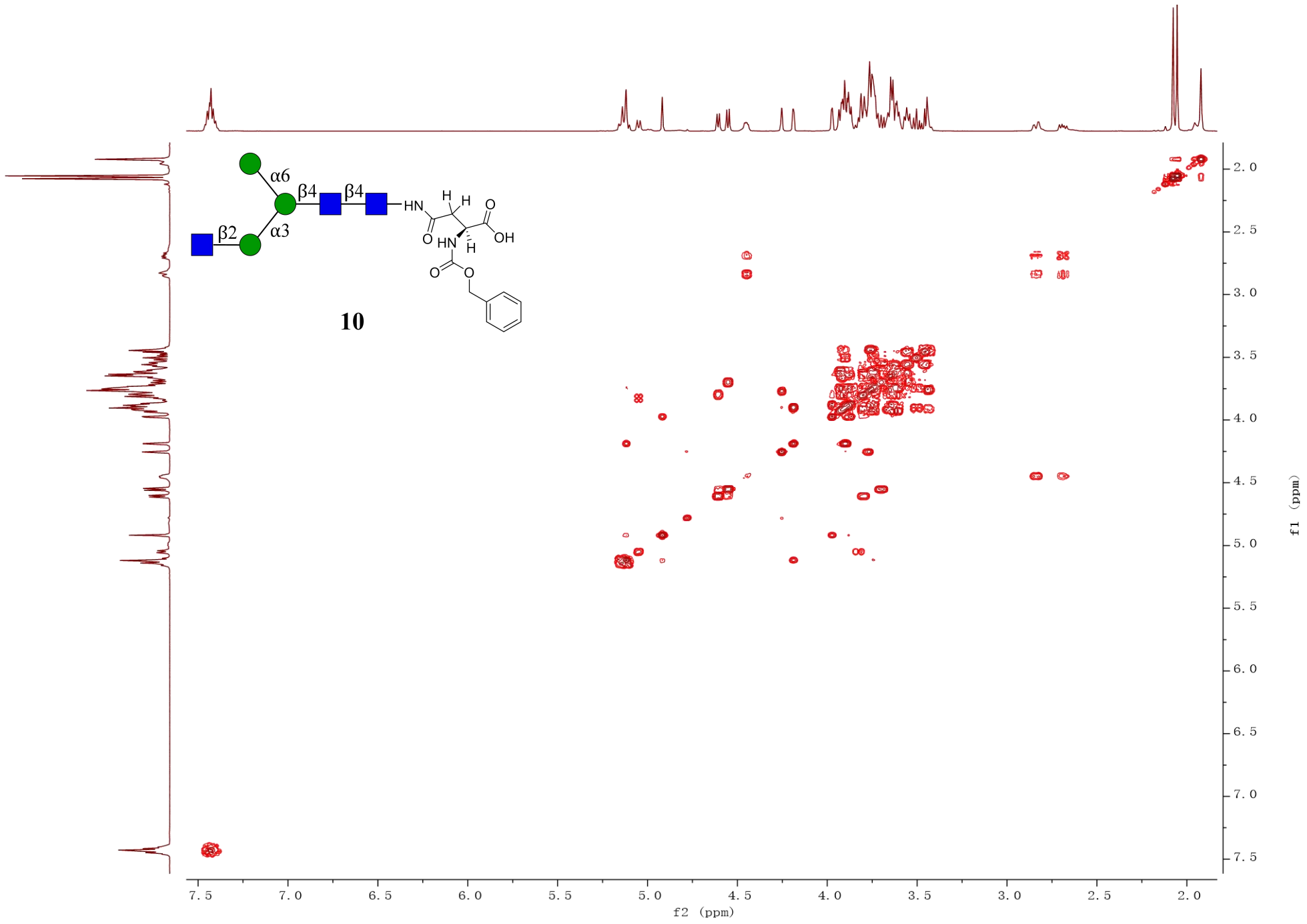
f1 (ppm)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7.5
f1 (ppm)
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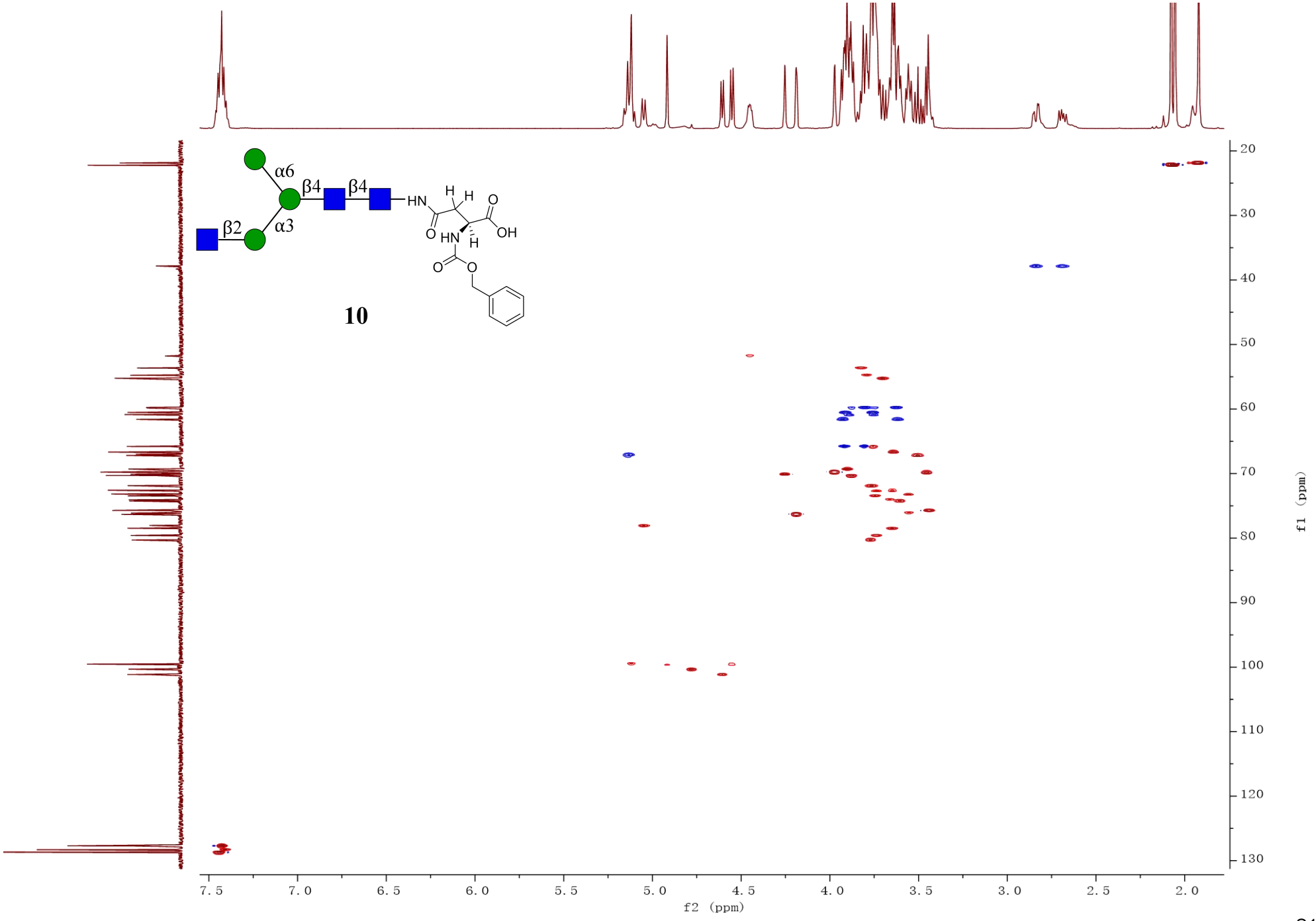
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O
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α3
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60
70
80
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100
110
120
130
f1 (ppm)
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5.0
4.5
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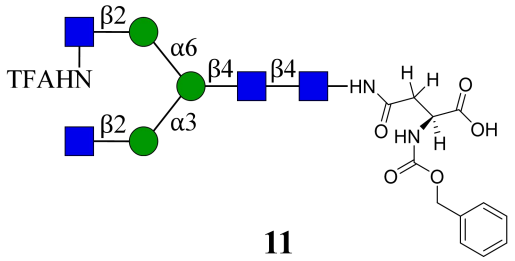
2.0
f2 (ppm)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β2
α3
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HN
H
OH
O
O

11

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7. 5
7. 0
6. 5
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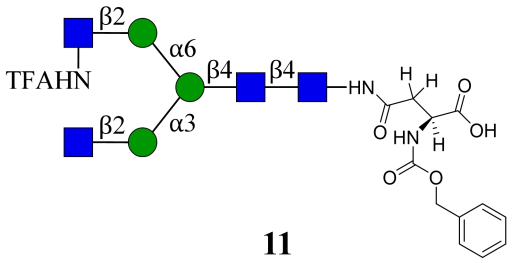
f1 (ppm)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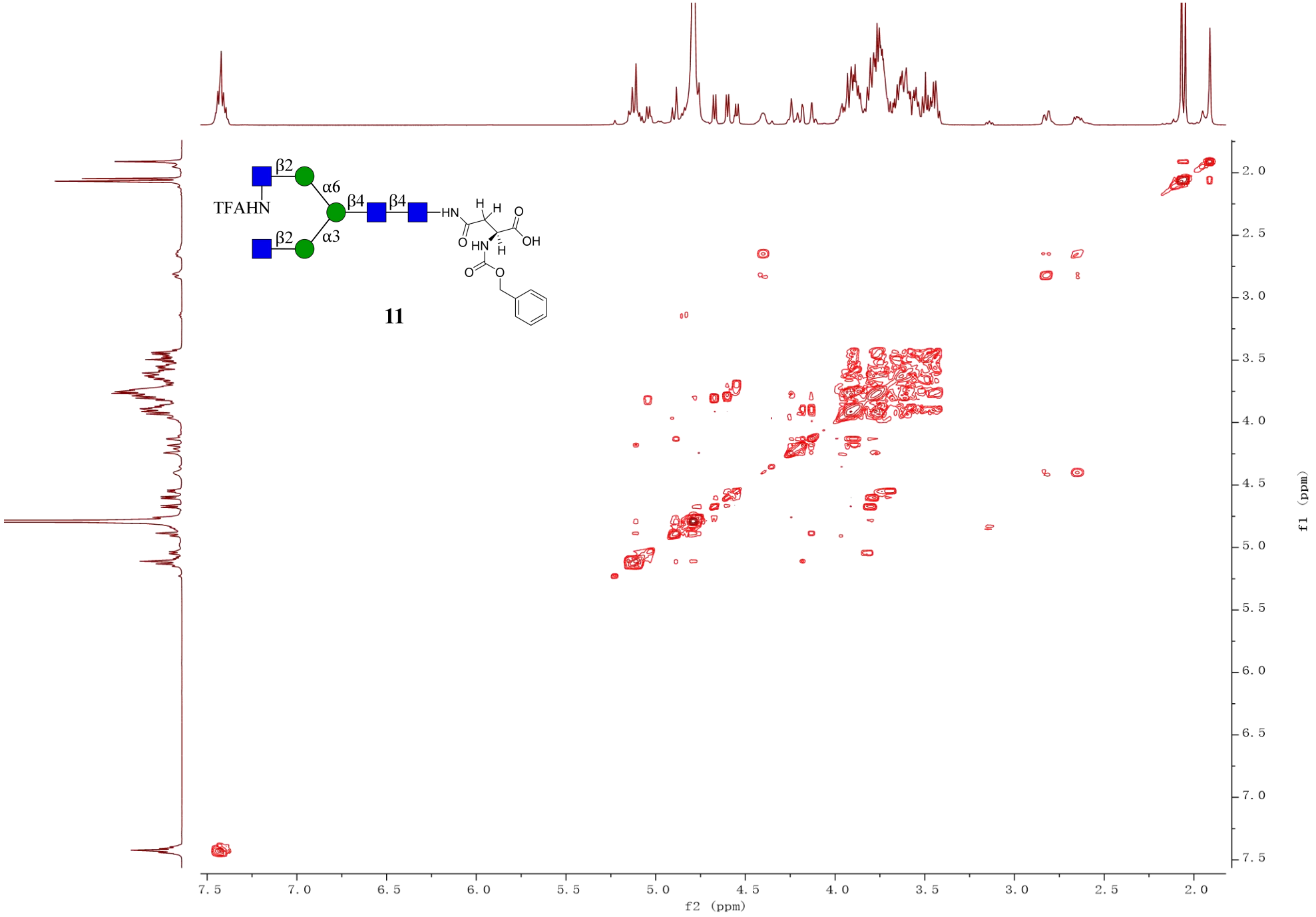
f1 (ppm)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4.5
5.0
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7.5
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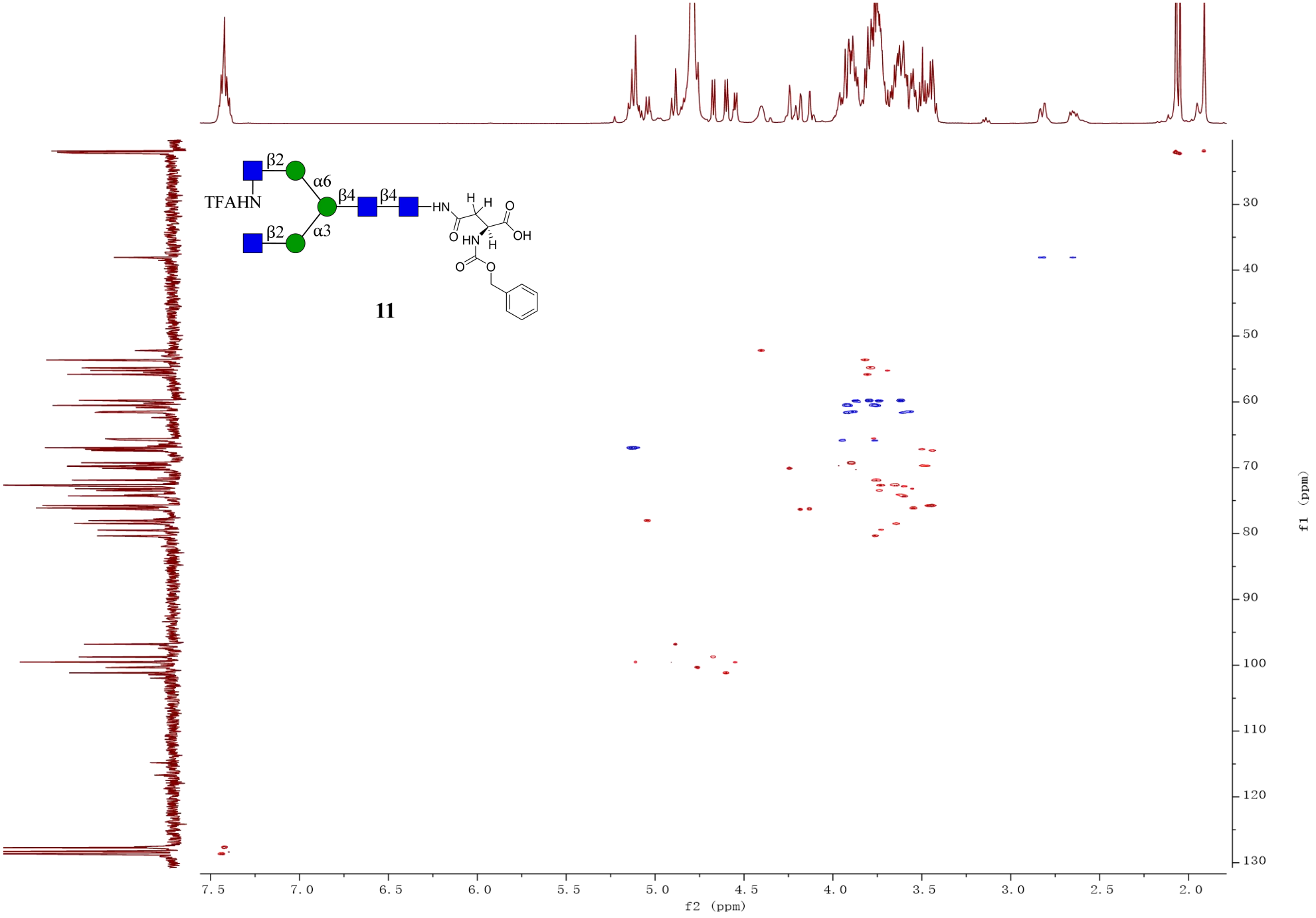
2.0
f2 (ppm)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5.0
4.5
4.0
3.5
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f2 (ppm)
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80
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100
110
120
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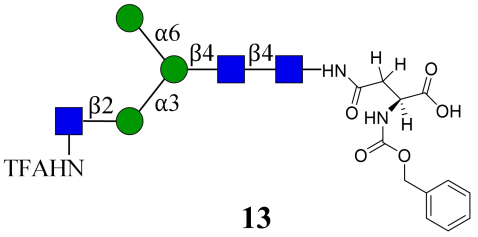
f1 (ppm)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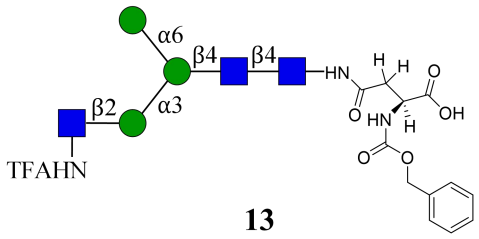
f1 (ppm)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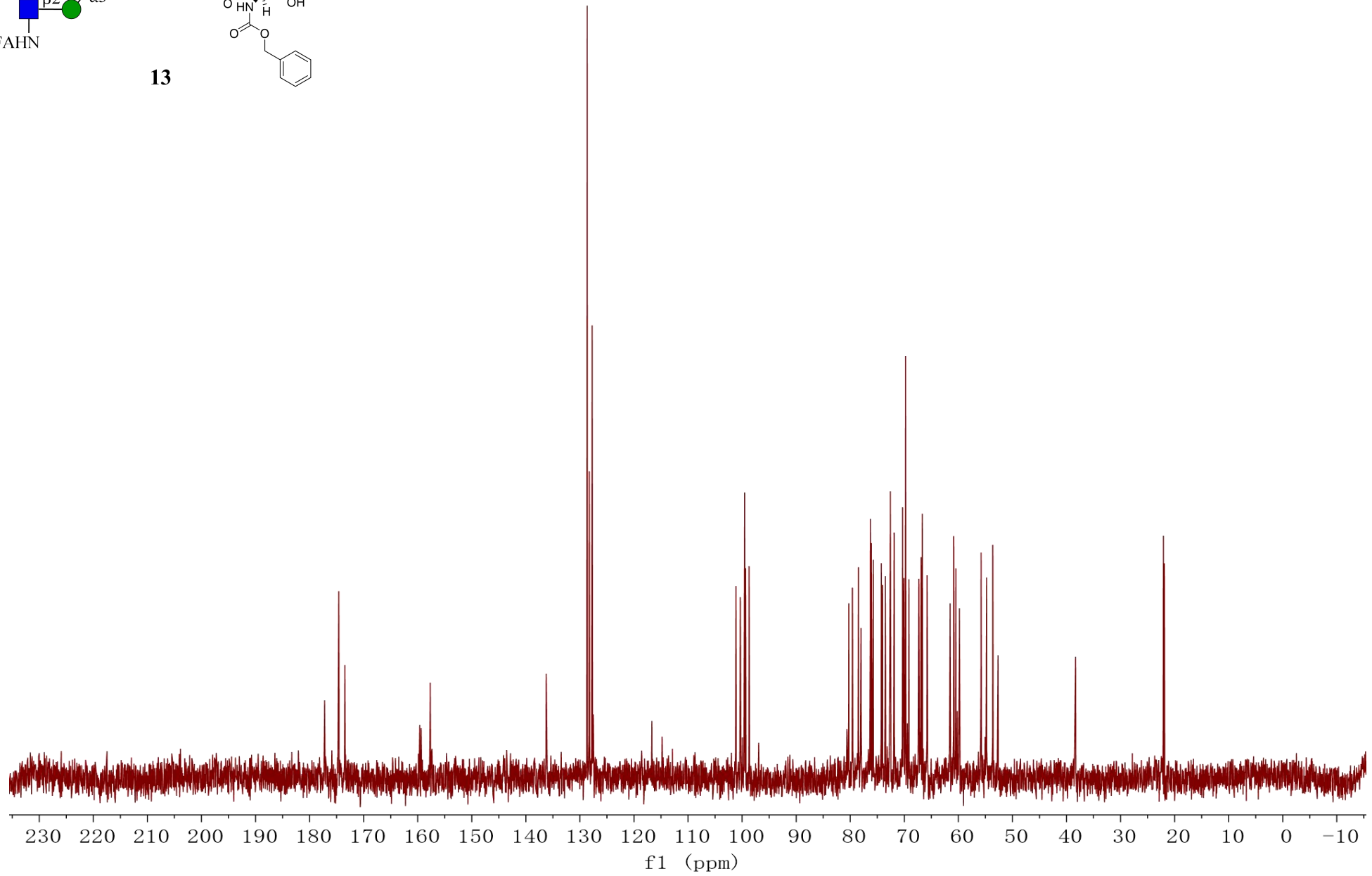
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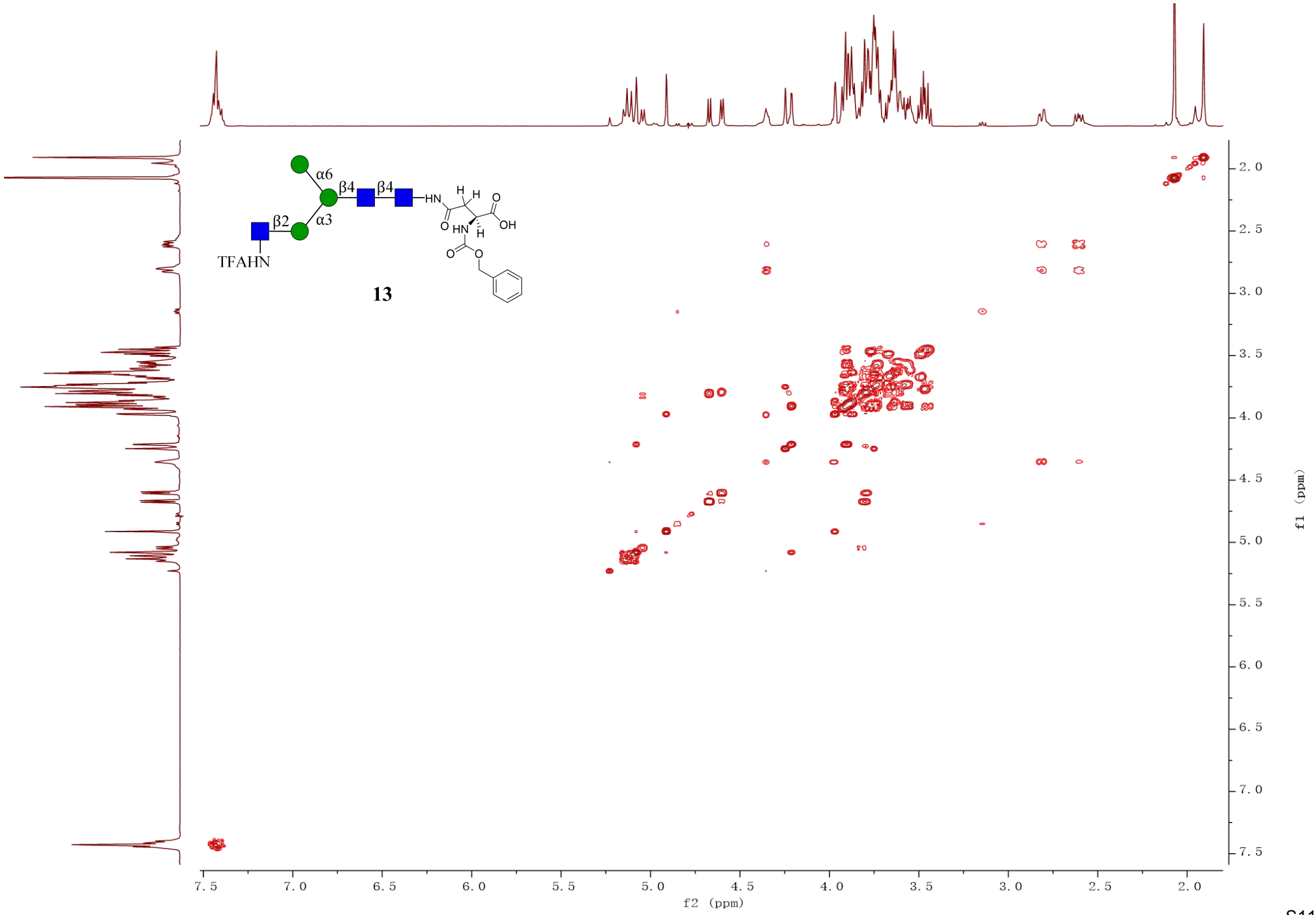
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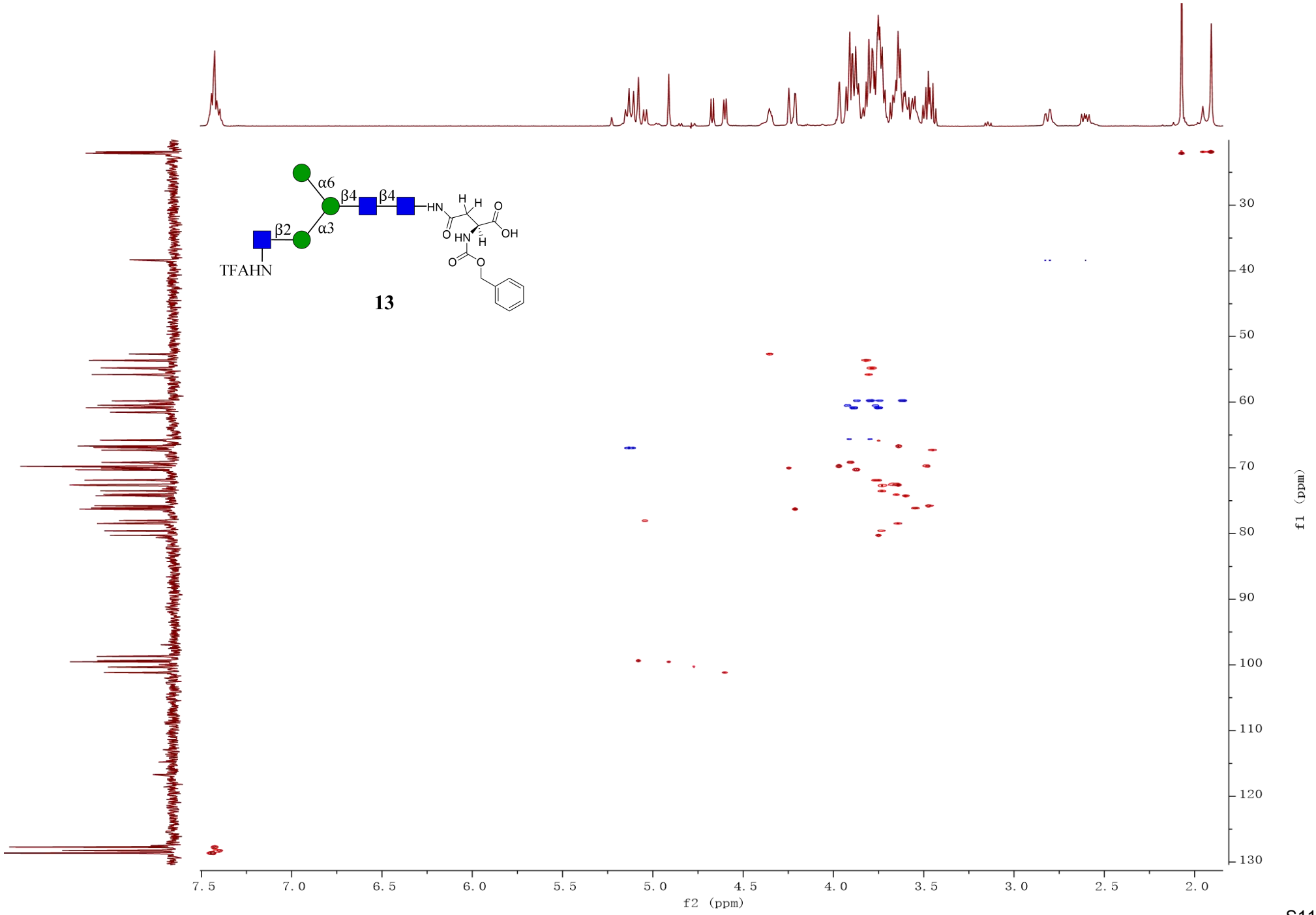
f1 (ppm)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60
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80
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100
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120
130
f1 (ppm)
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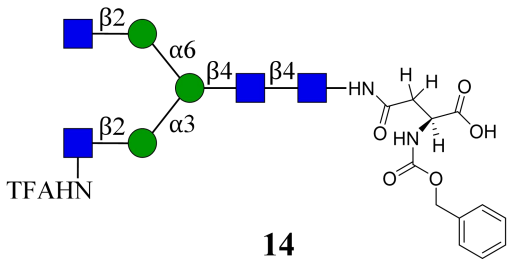
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14

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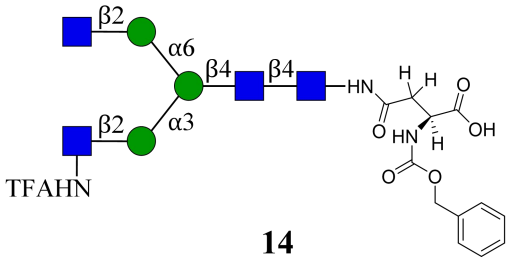
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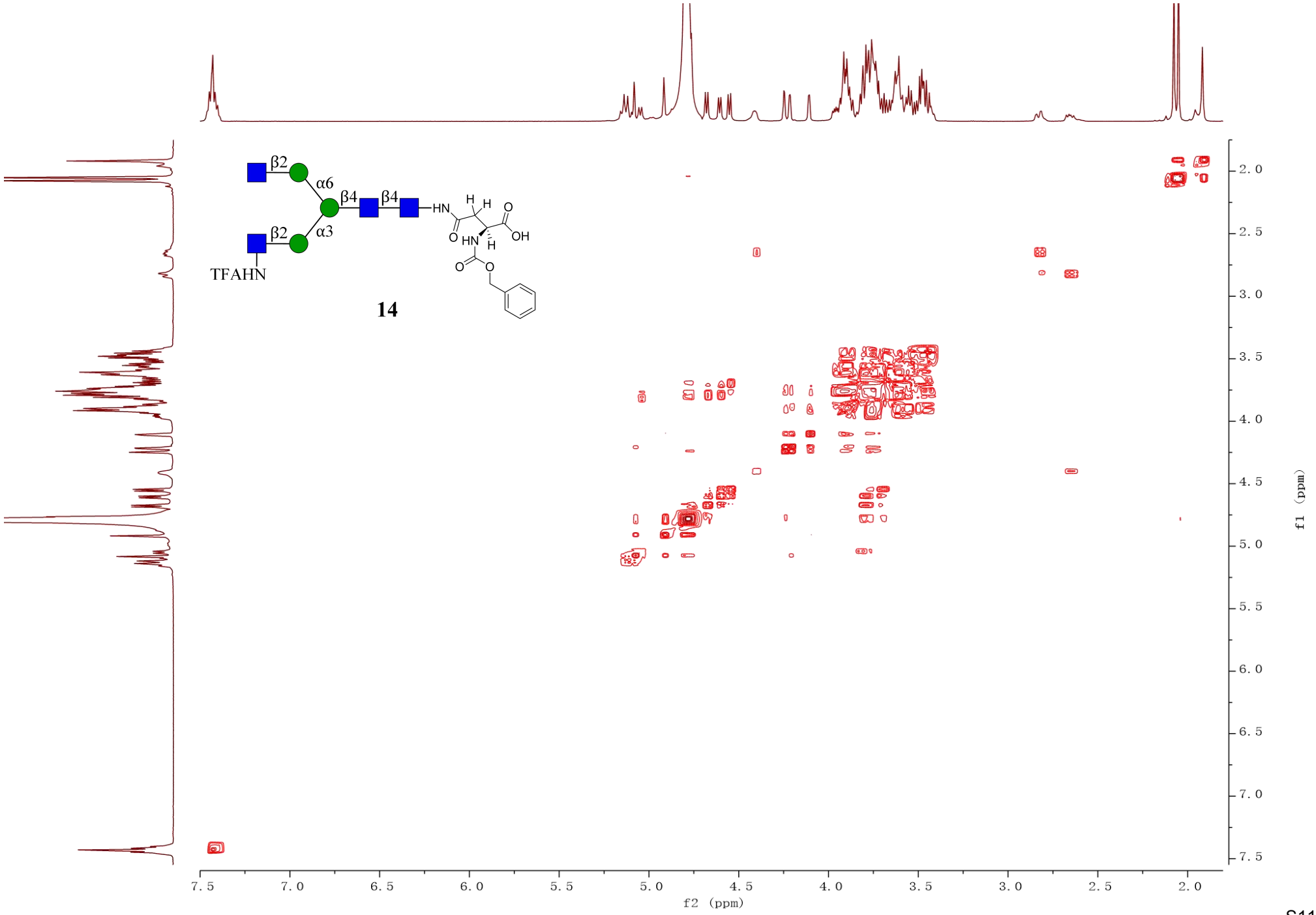
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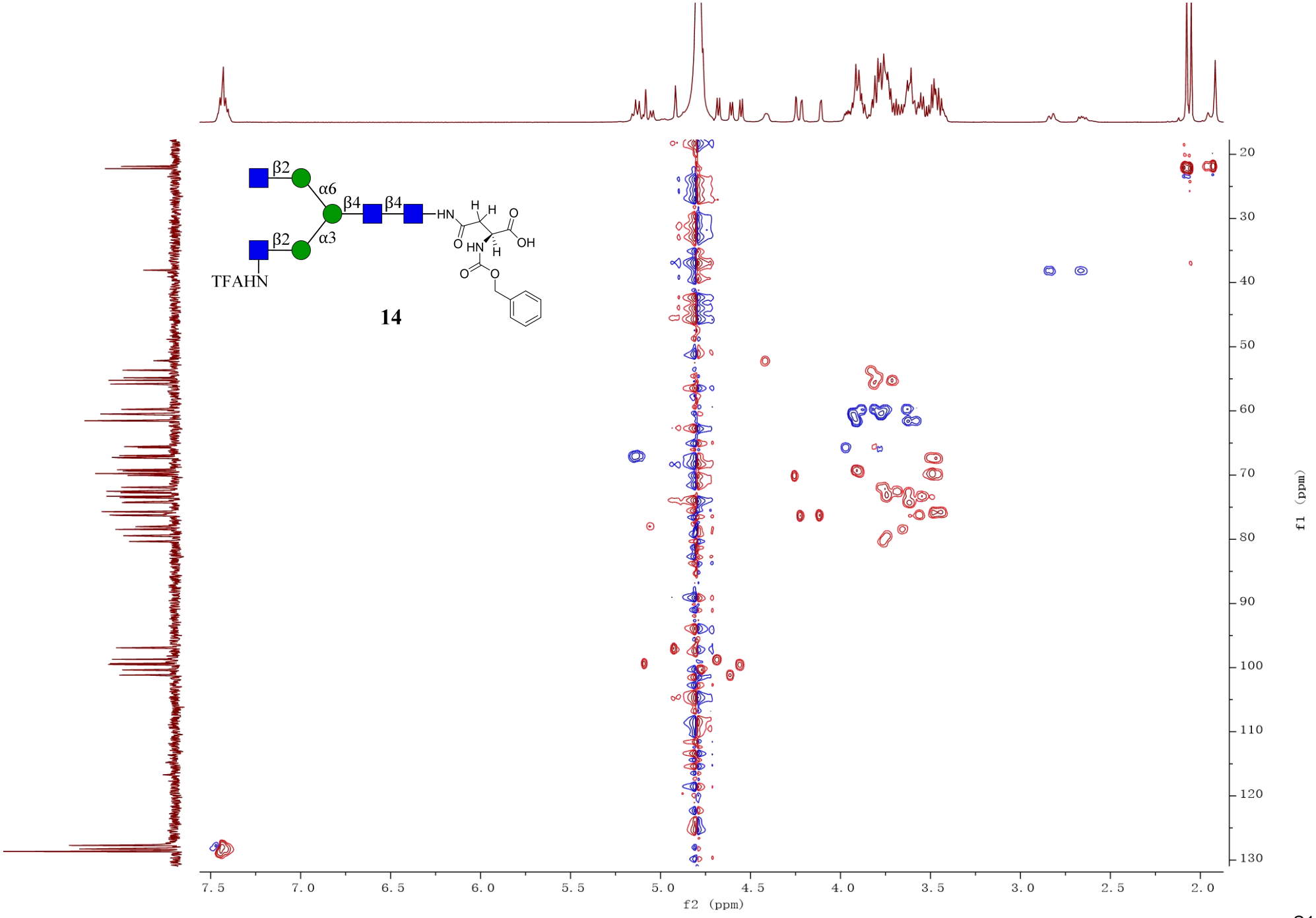
f2 (ppm)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6.5
6.0
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5.0
4.5
4.0
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3.0
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2.0
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40
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80
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120
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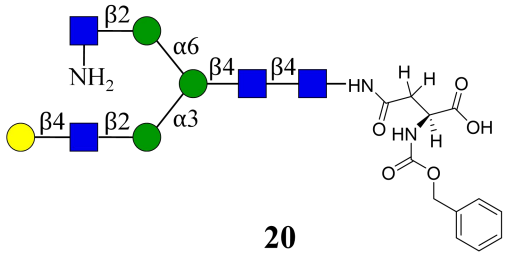
f1 (ppm)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O

20

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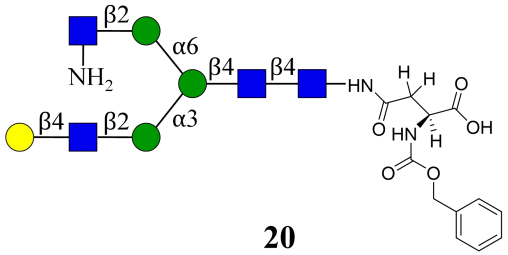
f1 (ppm)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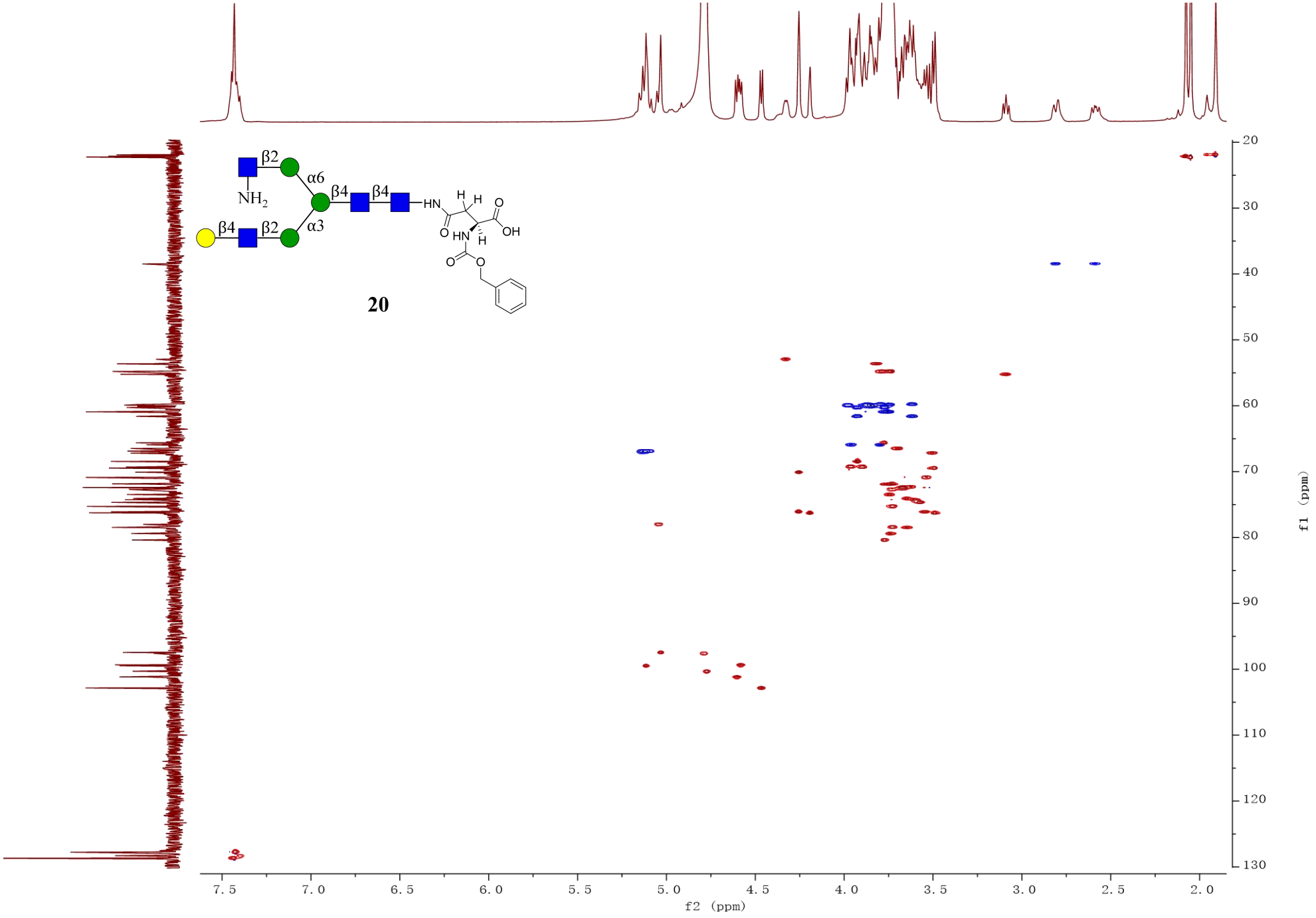
f1 (ppm)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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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2.0
f2 (ppm)
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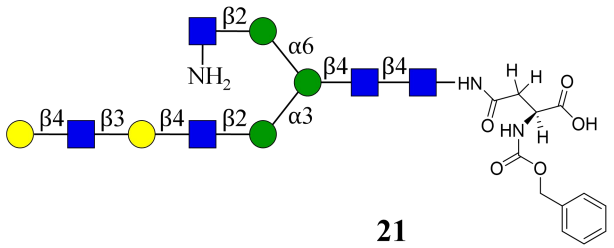
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)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β4
β2
α3
O
O

21

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6.5
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5.5
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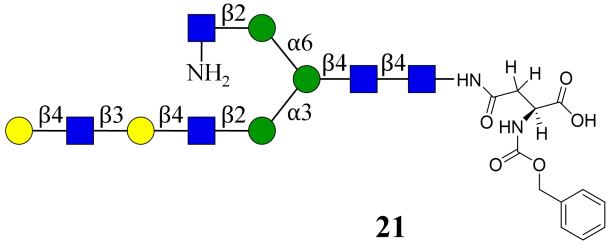
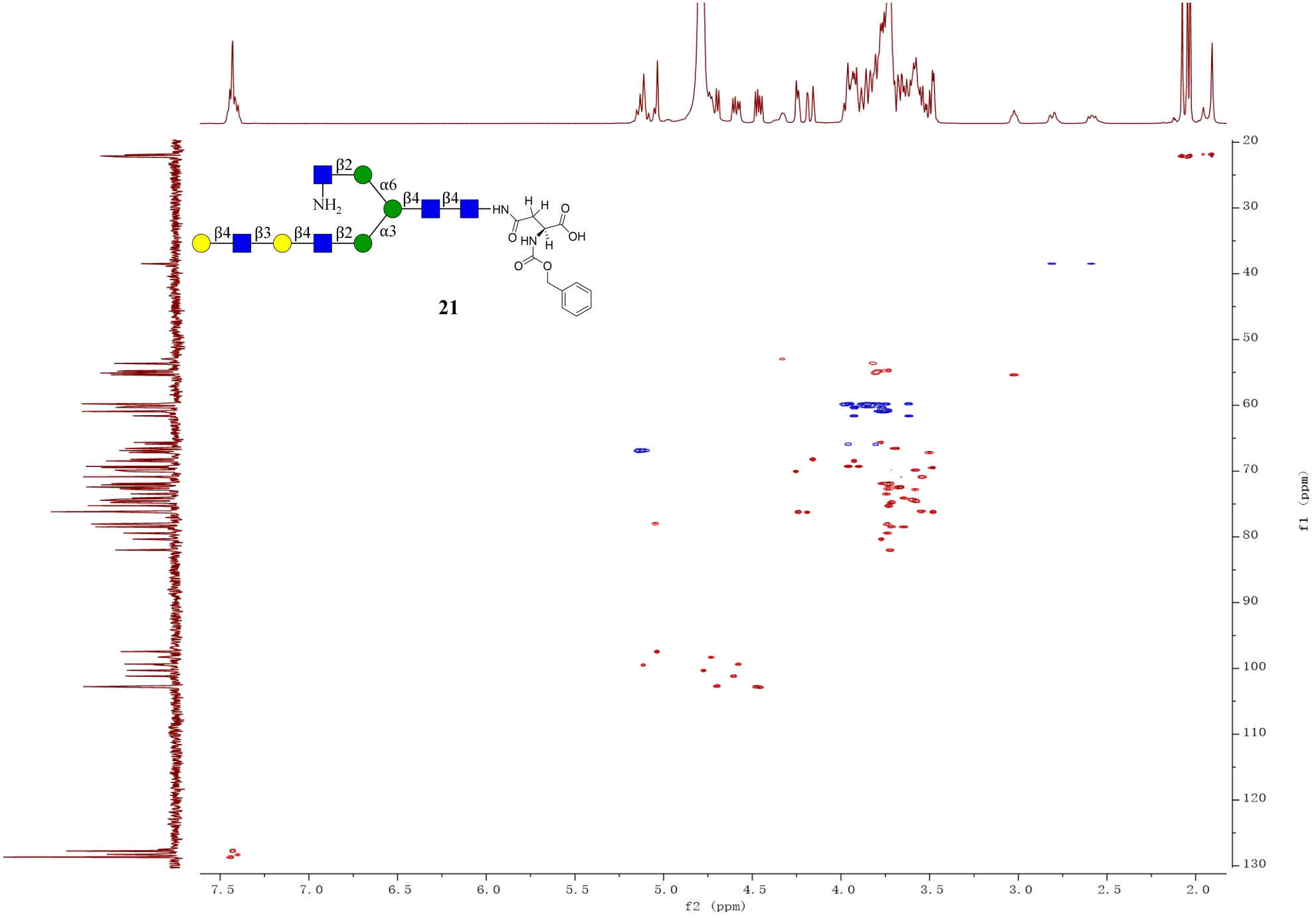
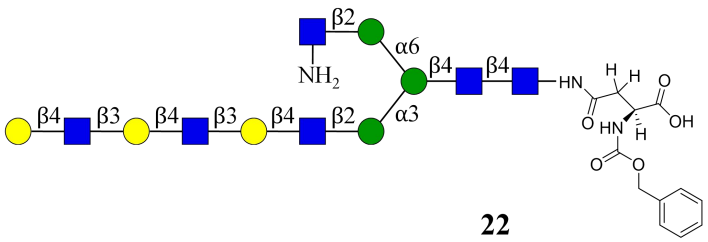
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
NH2
β4
β3
β4
β2
α3
O
HN
H
O
O

21

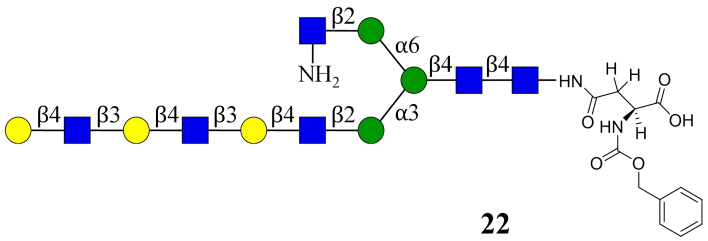
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
NH2
β4
β3
β4
β2
α3
O
HN
H
O
O
21
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

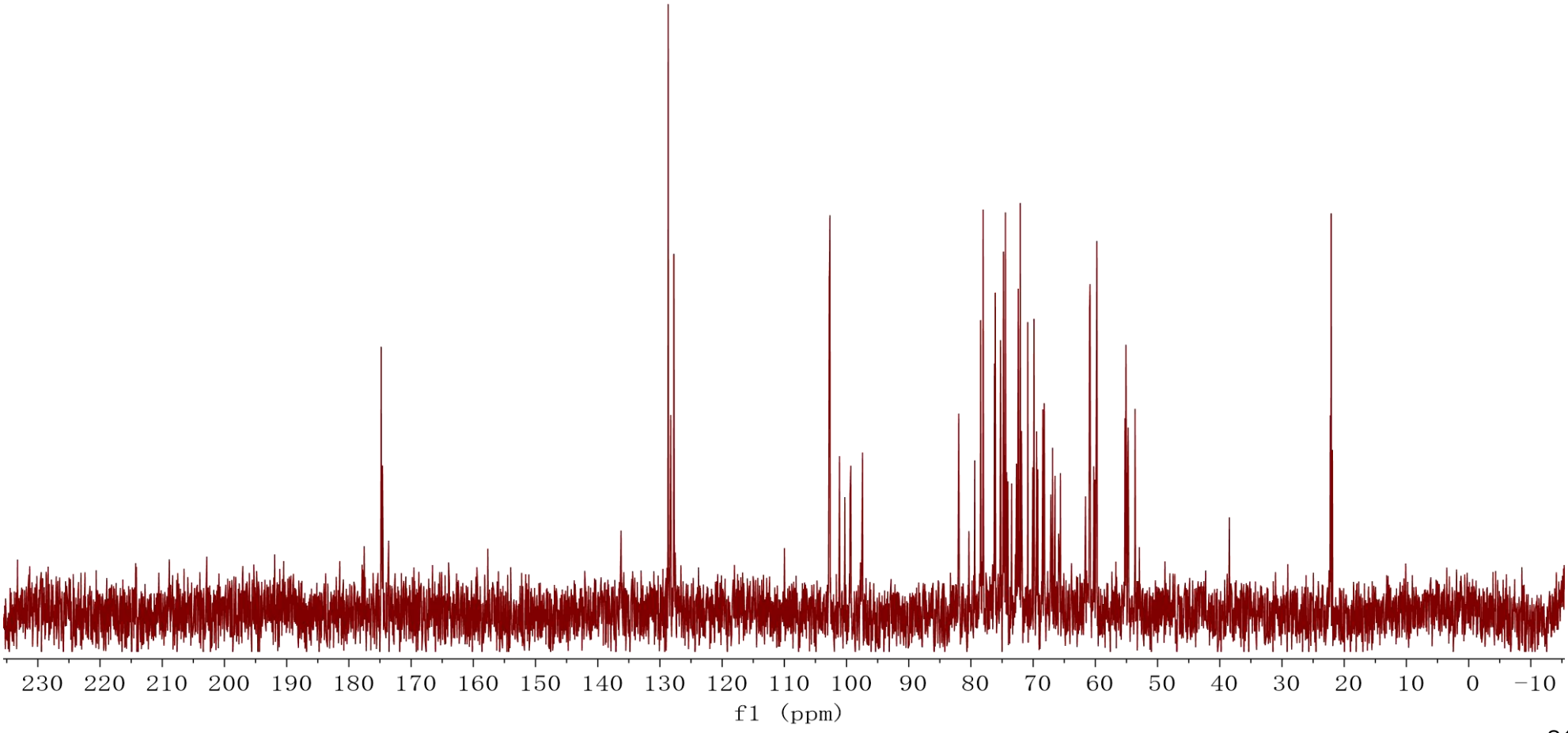

β2
α6
β4
β4
HN
H
H
O
OH
NH2
β4
β3
β4
β3
β4
β2
α3
O
HN
H
O
O

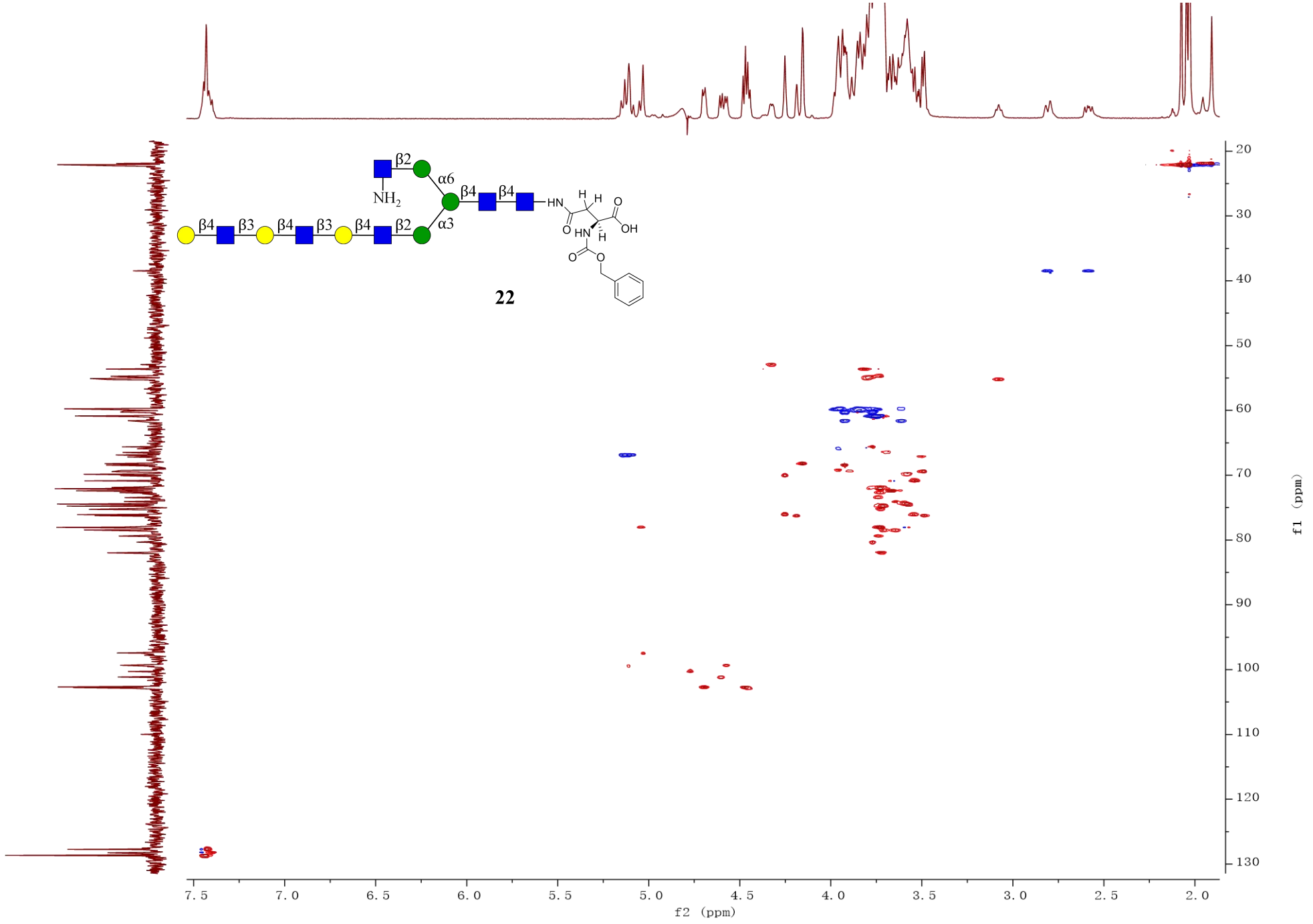
22

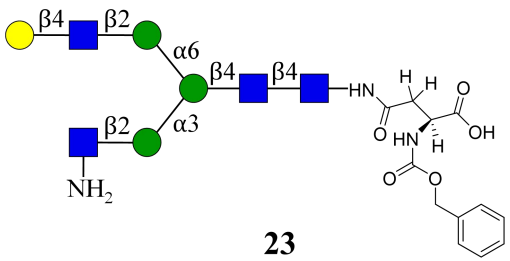
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
NH2
β4
β3
β4
β3
β4
β2
α3
HN
H
O
O
O

22

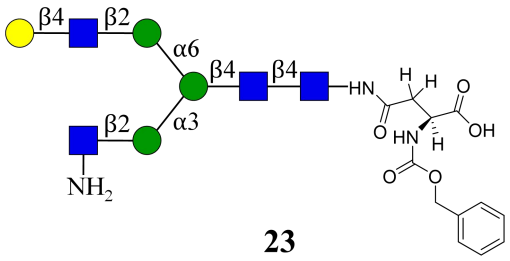

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β2
α6
β4
β4
HN
NH2
β4
β3
β4
β3
β4
β2
α3
O
OH
HN
O
O
22
f2 (ppm)
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
20
30
40
50
60
70
80
90
100
110
120
130


β4
β2
α6
β4
β4
HN
H
H
O
OH
β2
α3
HN
H
O
O
O
NH2

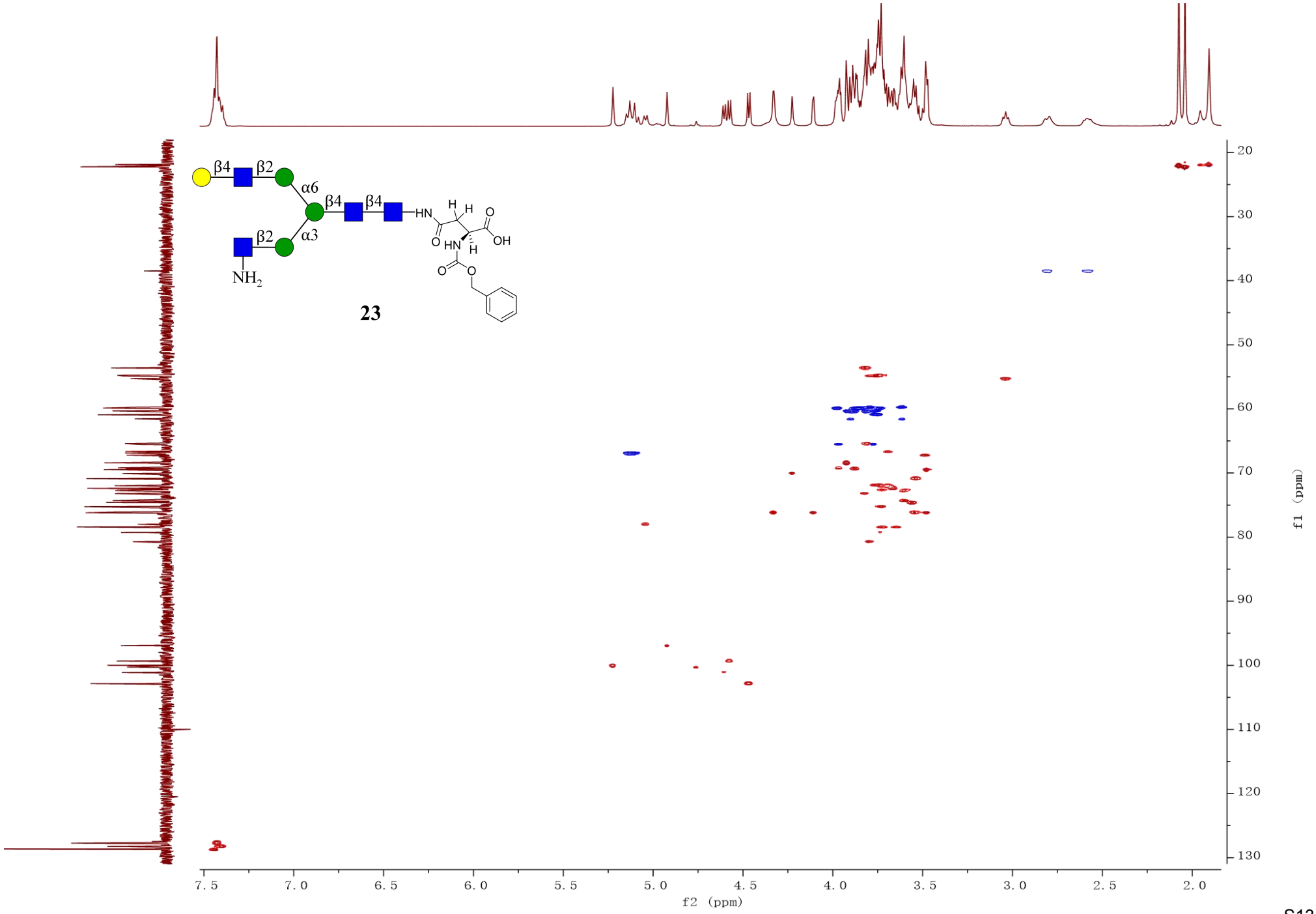
23

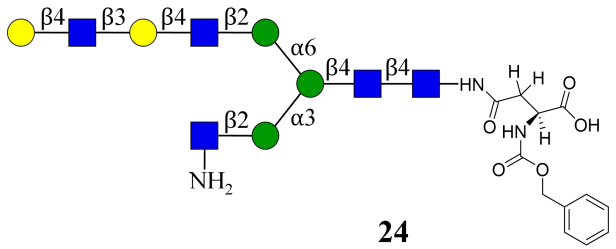
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3
NH2

23

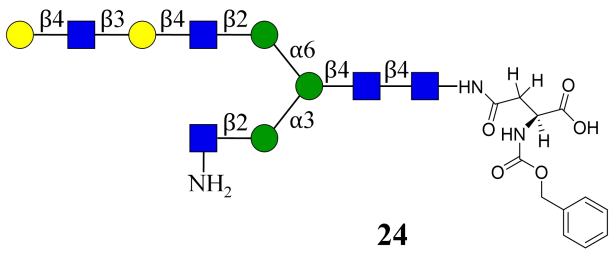
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3
NH2
23
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)


β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
β2
α3
NH2
O
O

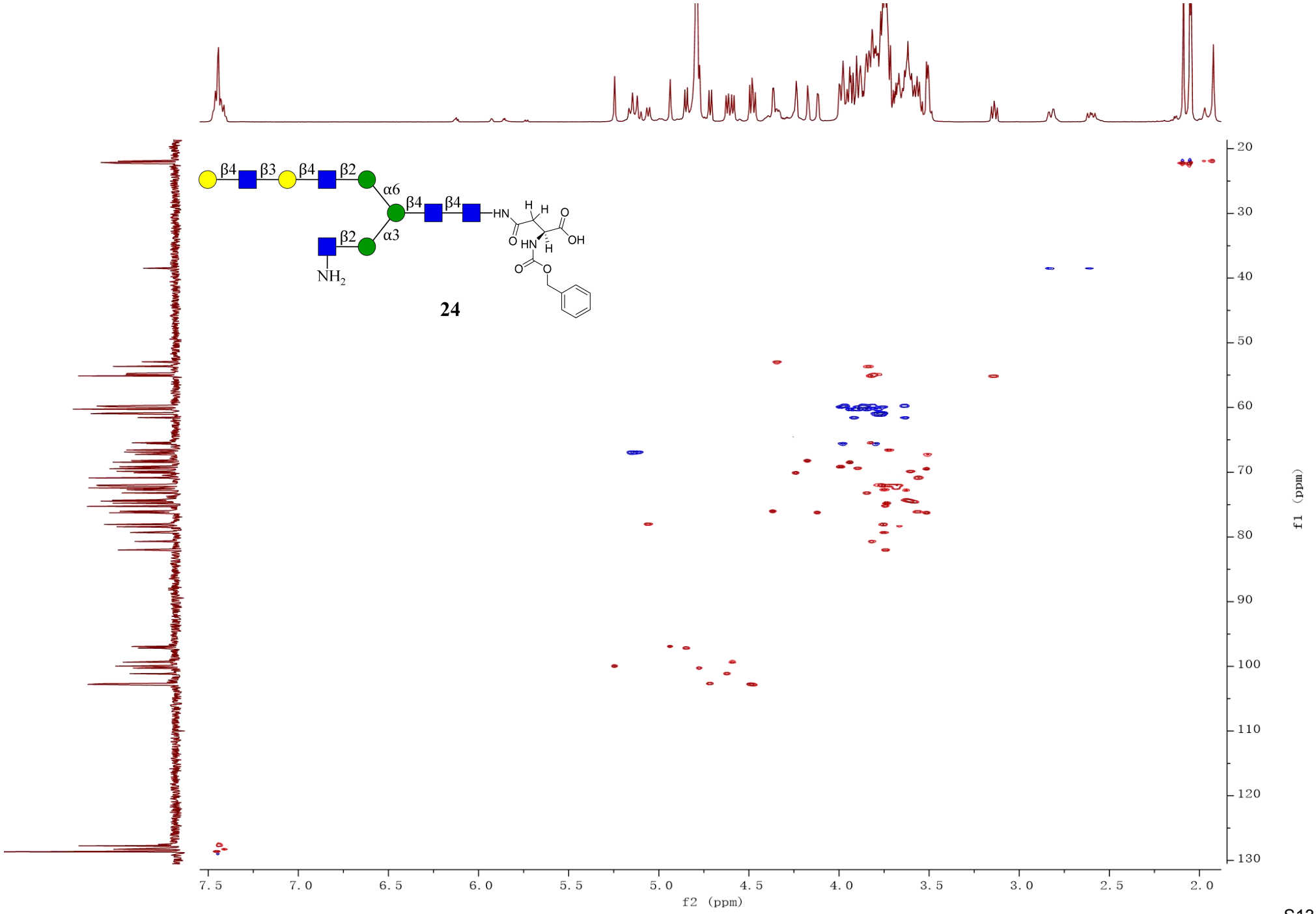
24

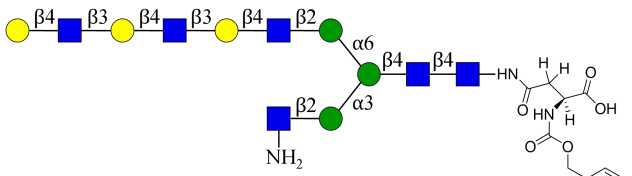
3.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3
NH2

24

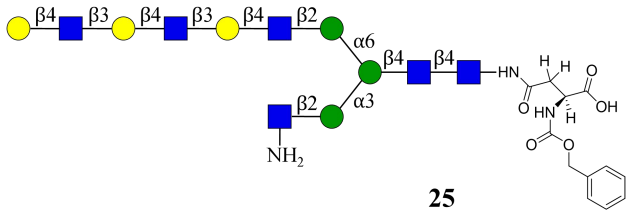
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3
NH2
24
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

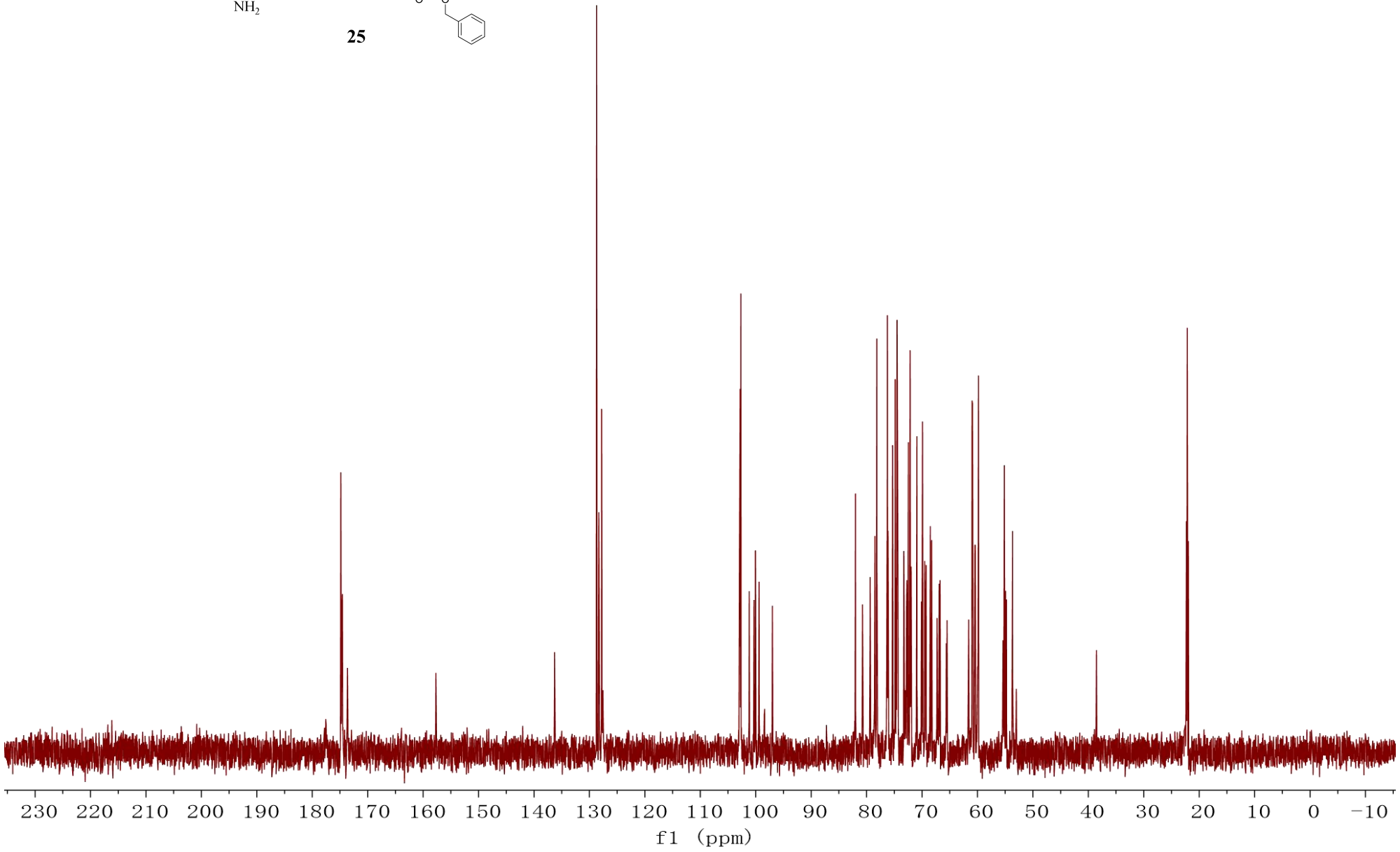

β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
O
OH
HN
H
O
O
β2
α3
NH2

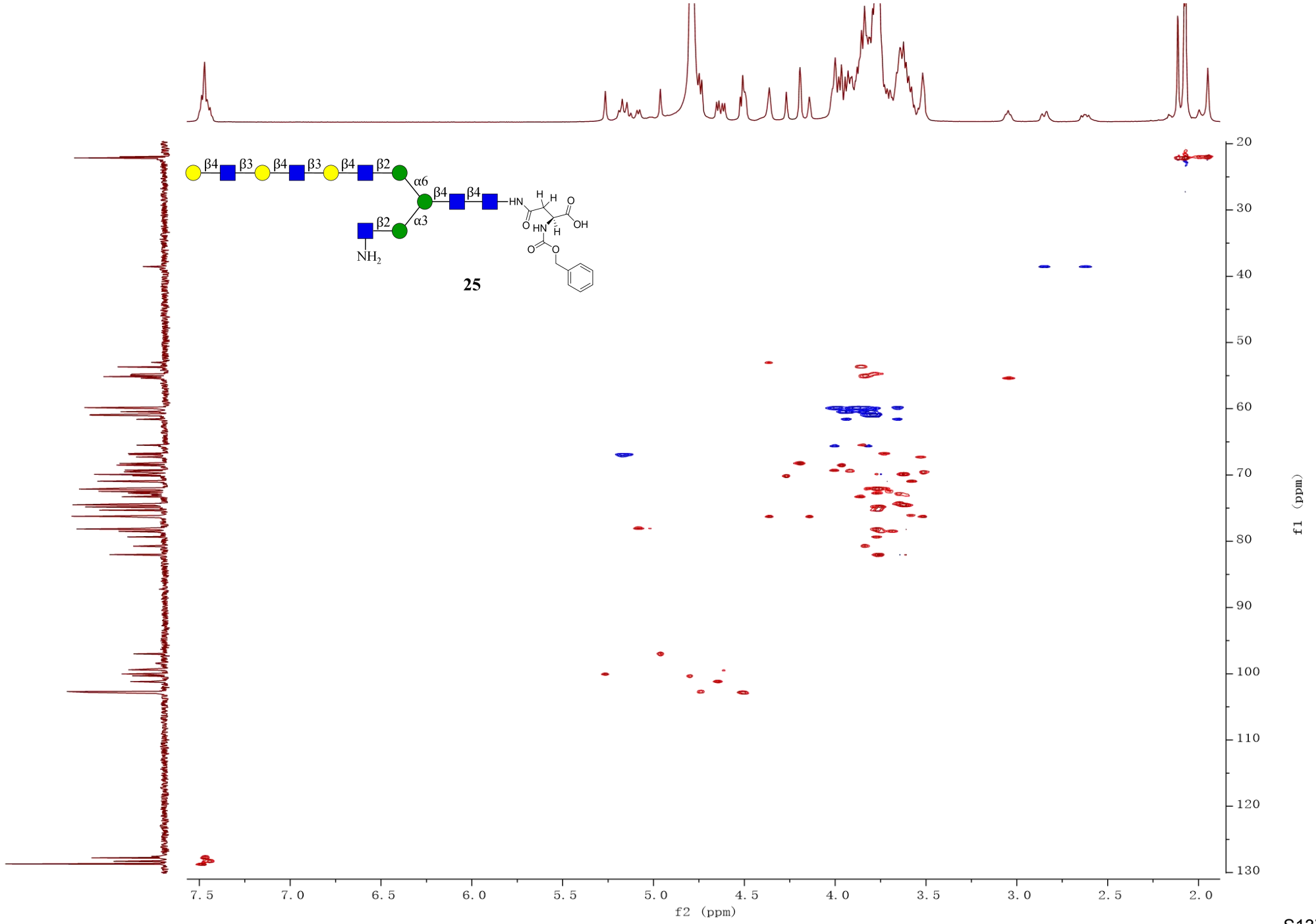
25

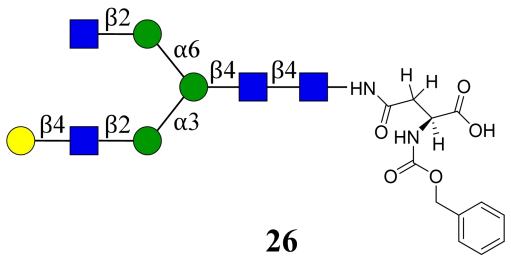
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3
NH2

25

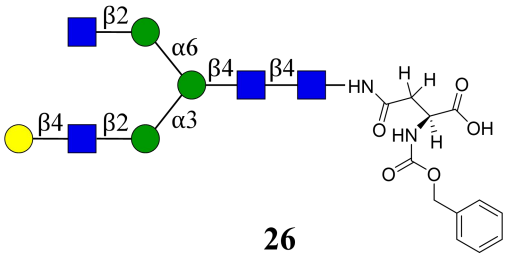

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3
NH2
25
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3

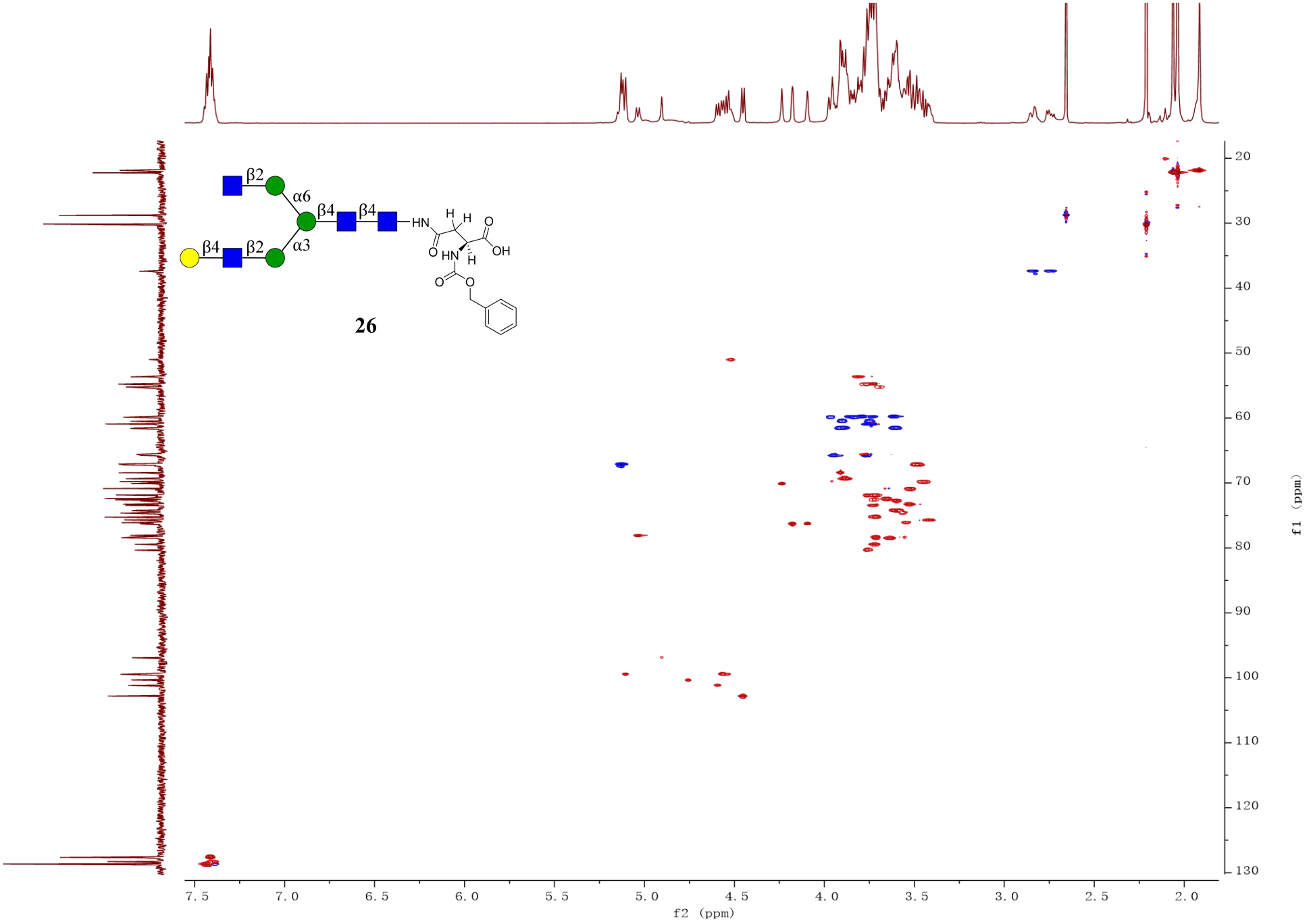
26

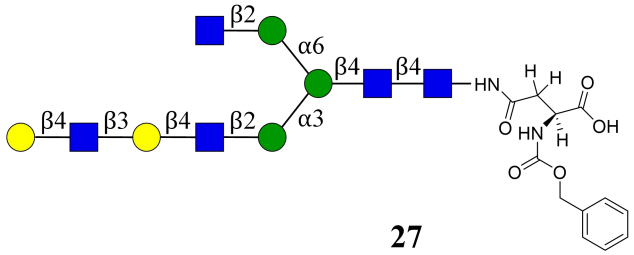
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
β4
β2
α3
O
HN
H
O
O

26

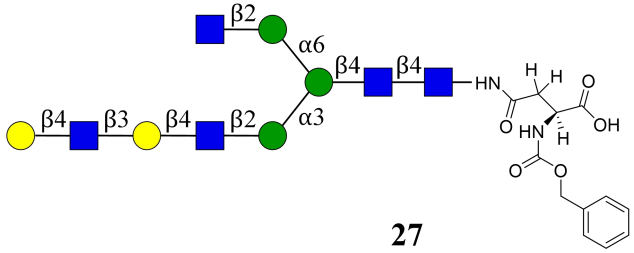
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
β4
β2
α3
O
HN
H
O
O
O
26
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)

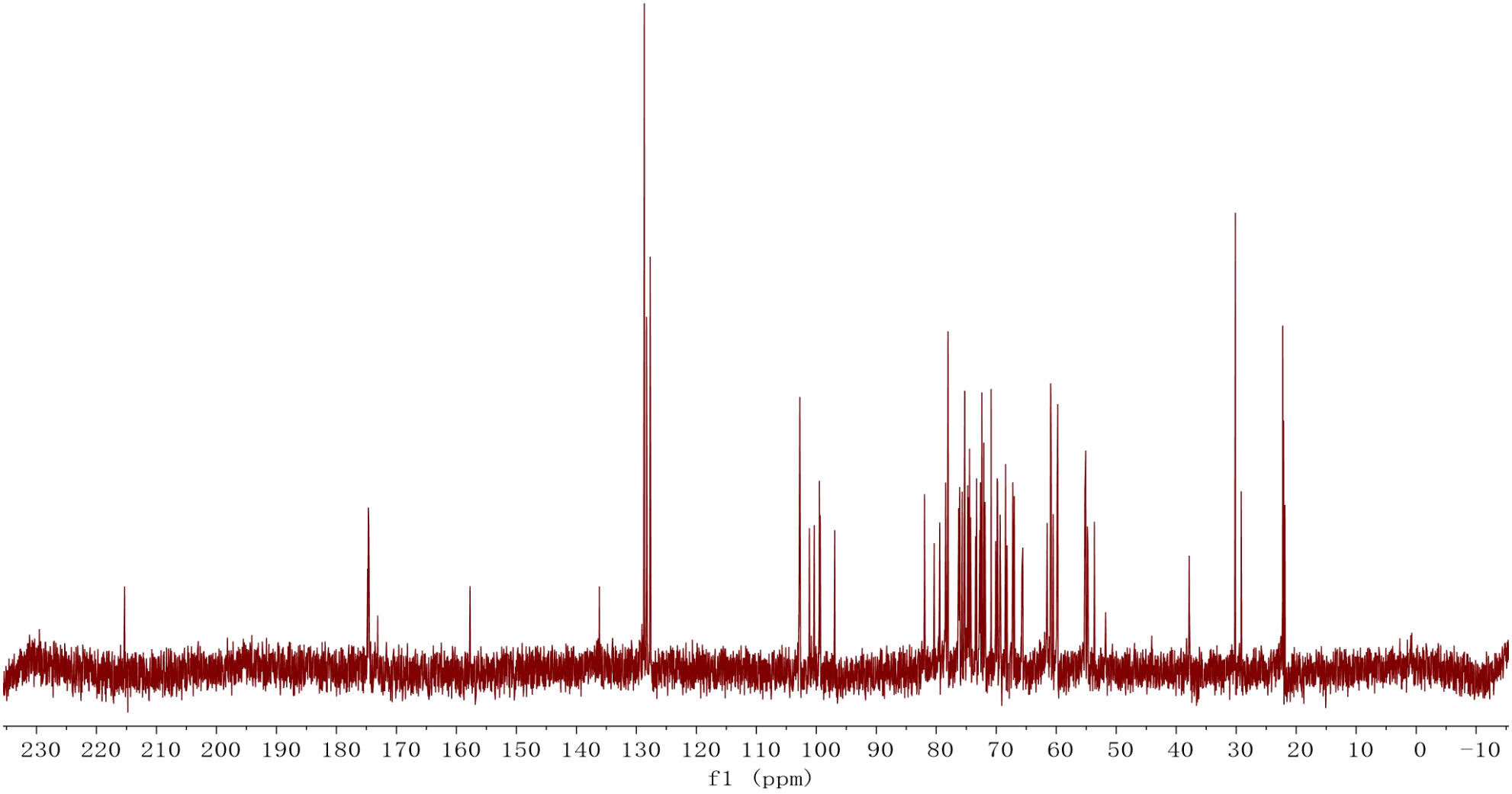

β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β2
α3

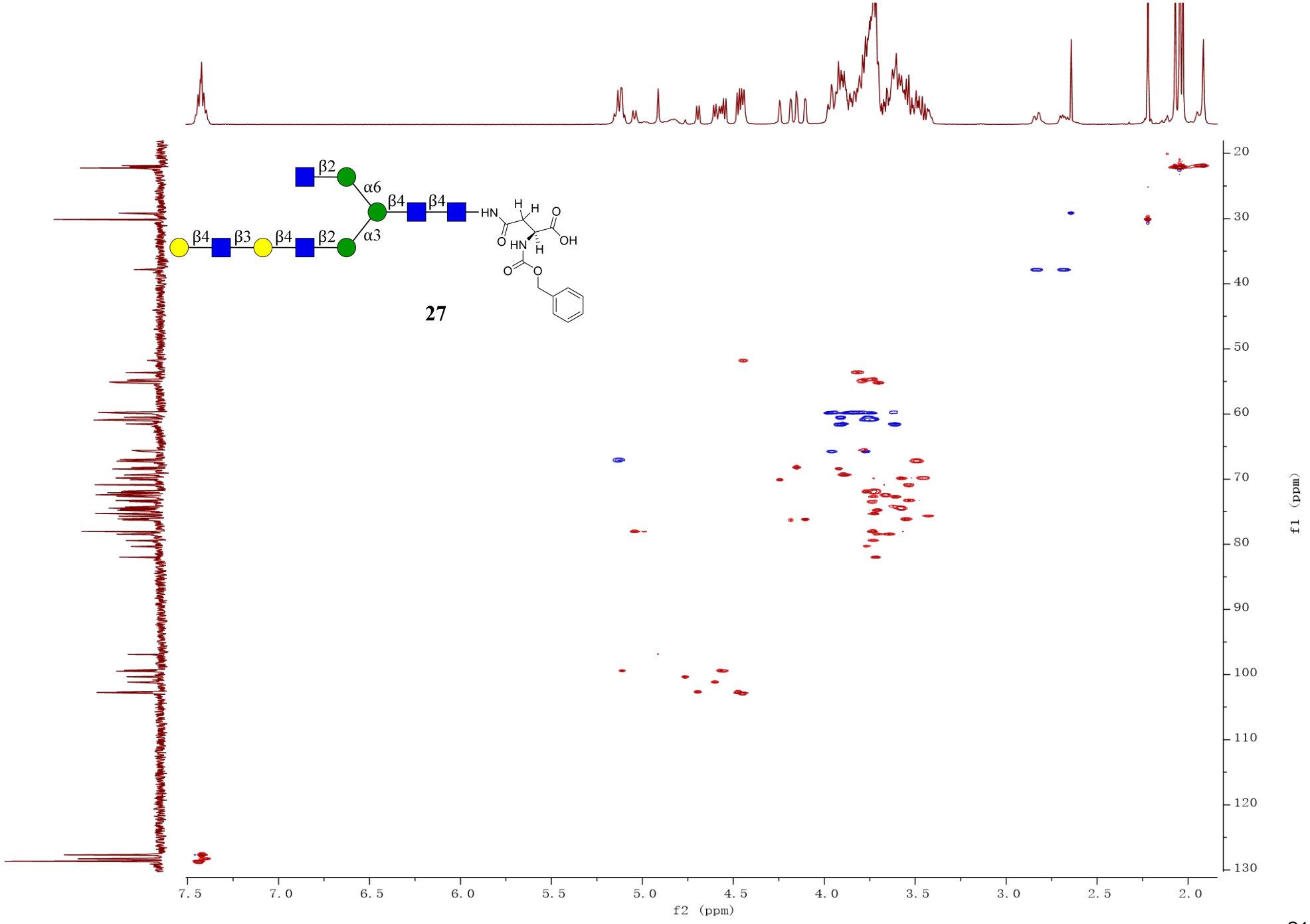
27

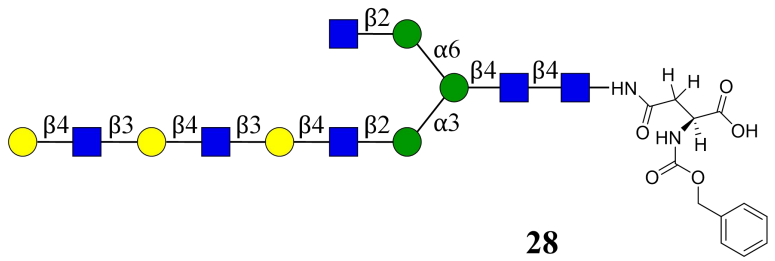
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β2
α3

27

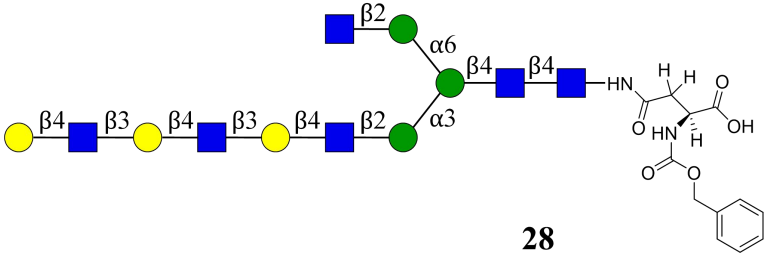

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β2
α3
27
f2 (ppm)
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
20
30
40
50
60
70
80
90
100
110
120
130

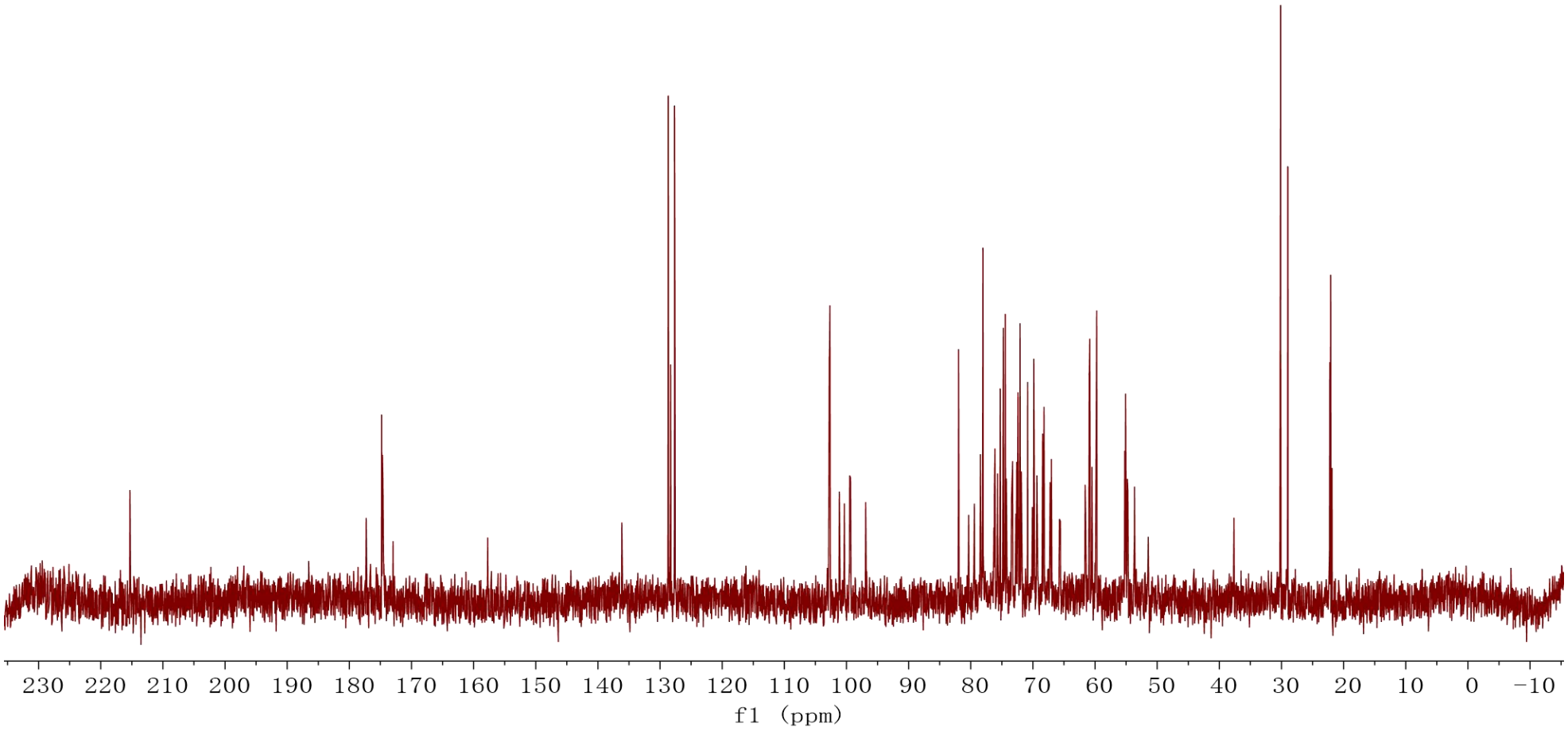

β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β3
β4
β2
α3

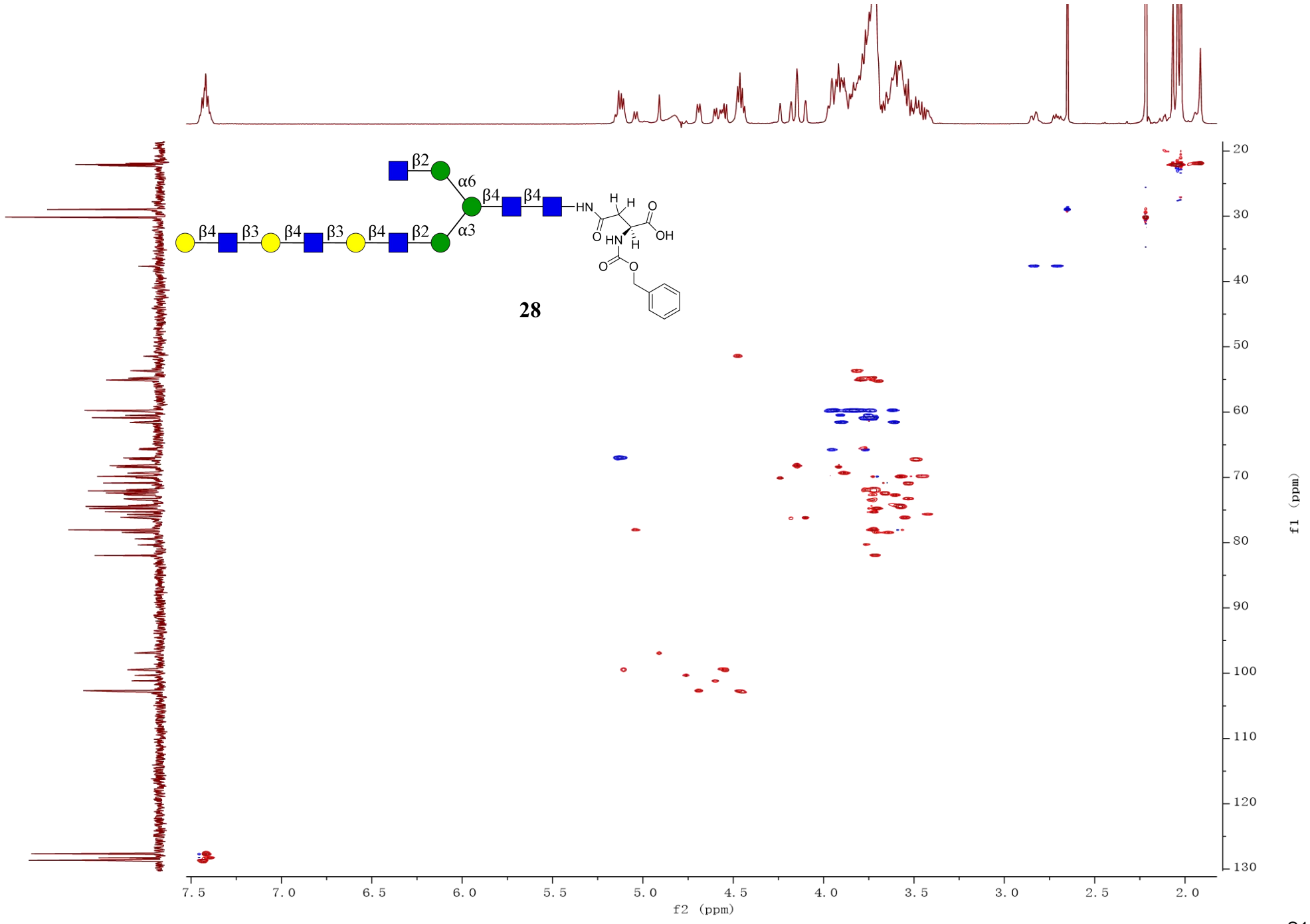
28

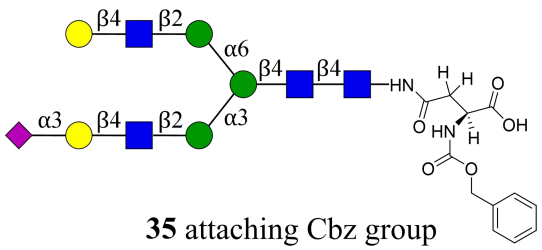
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β3
β4
β2
α3

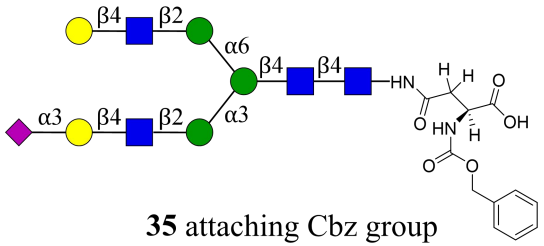
28


230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

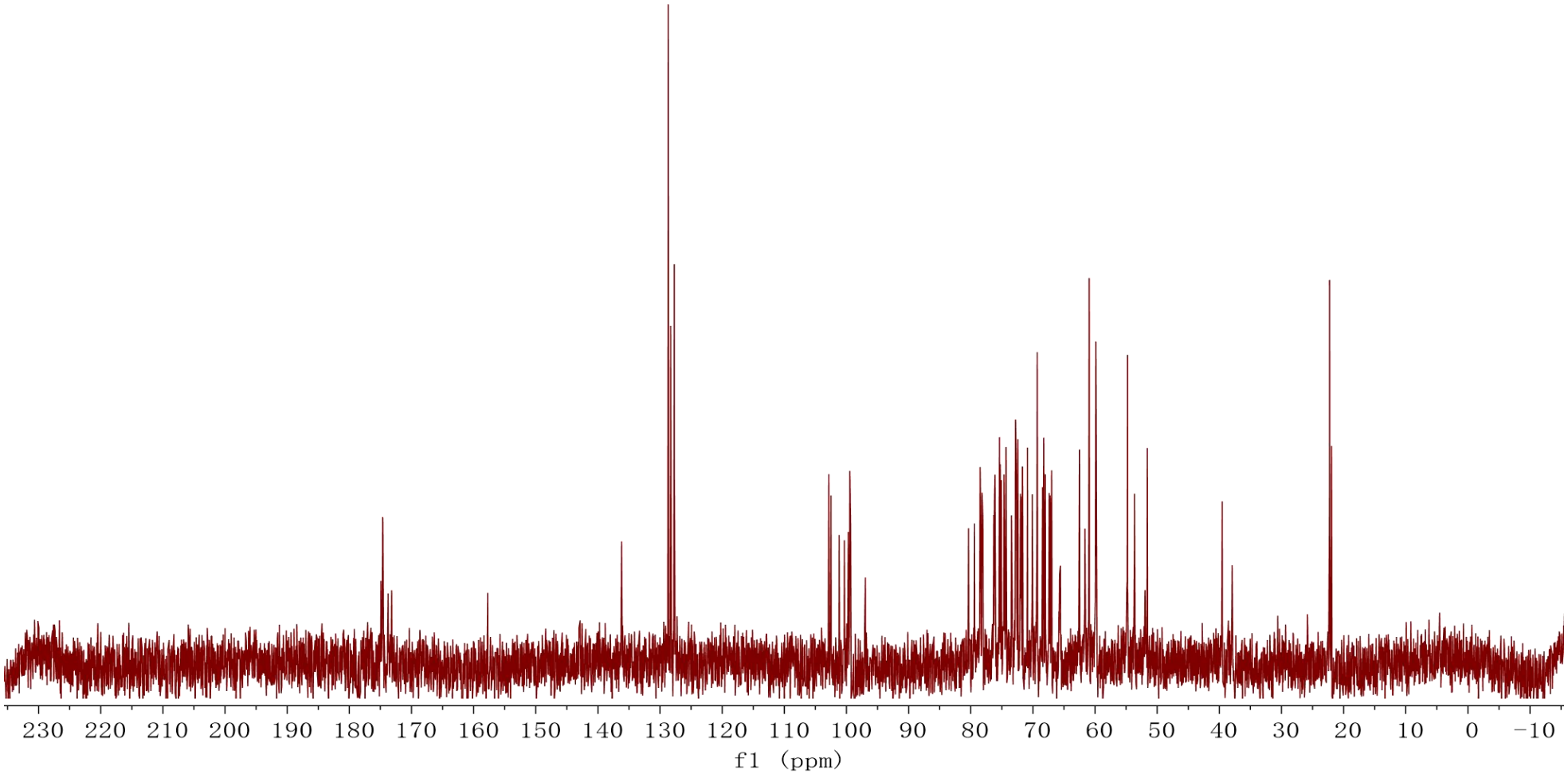

β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β3
β4
β2
α3
28
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

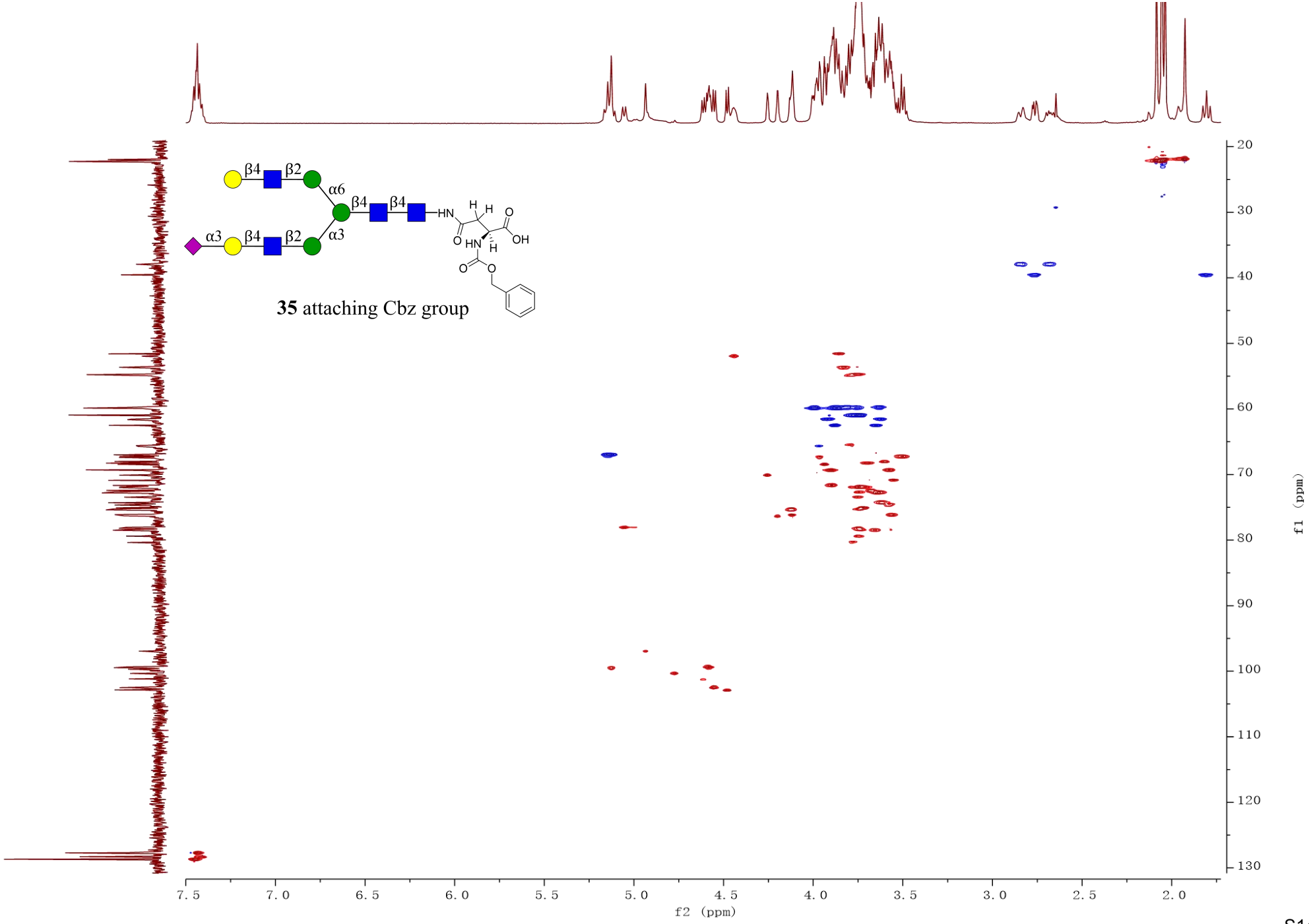


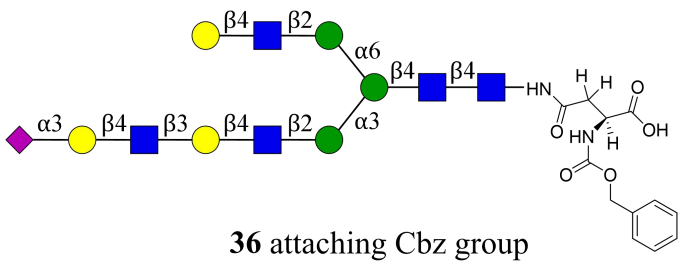
35 attaching Cbz group



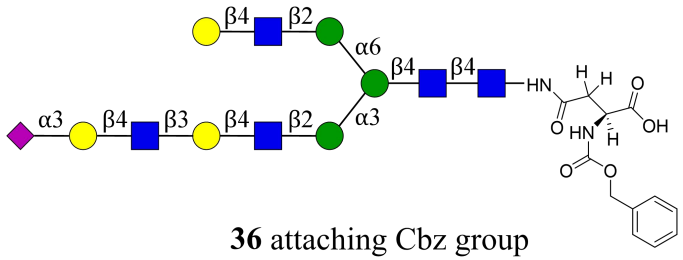
35 attaching Cbz group



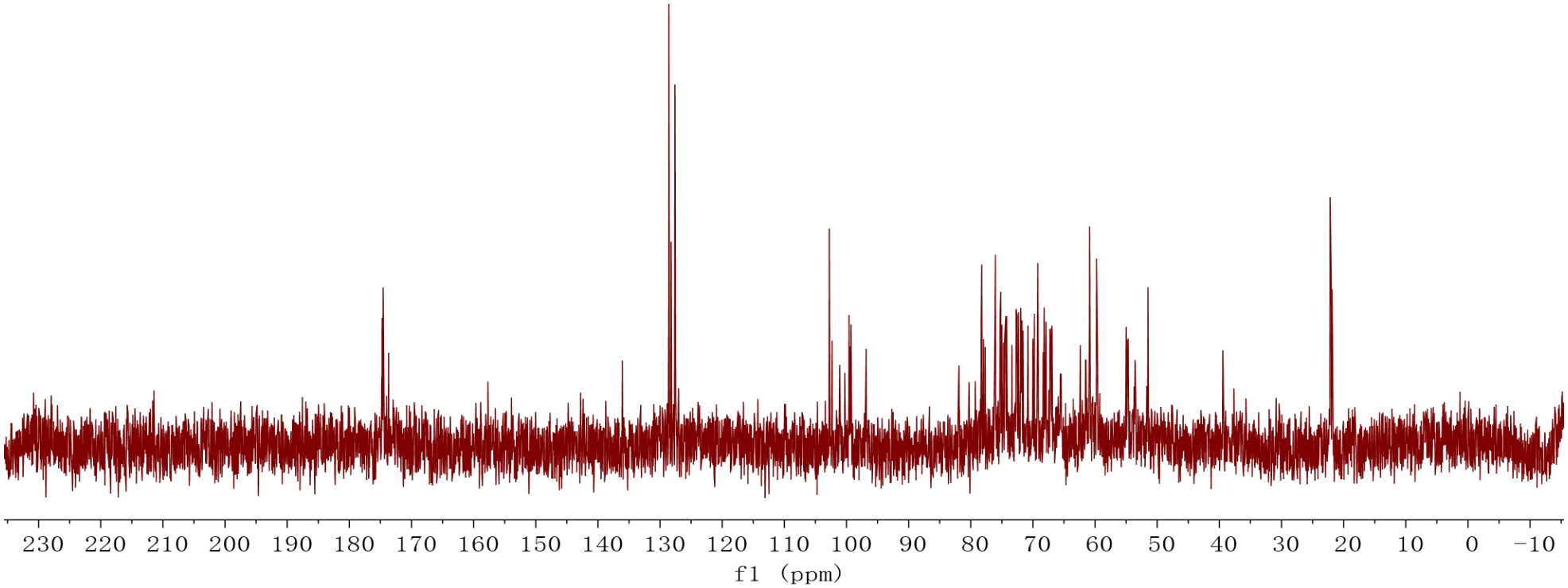

β4
β2
α6
β4
β4
HN
H
H
O
α3
β4
β2
α3
O
HN
H
OH
O
O
35 attaching Cbz group
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

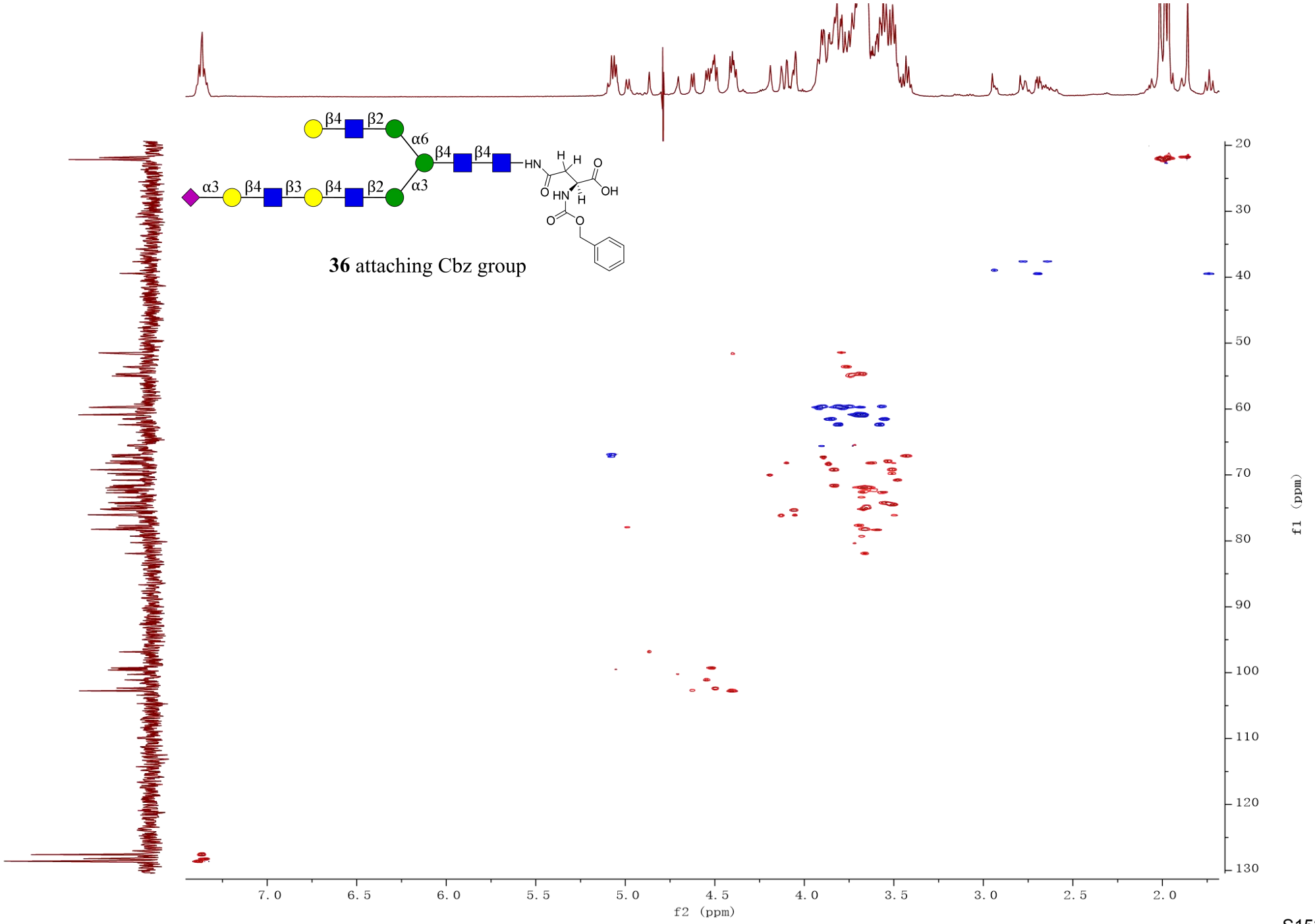


36 attaching Cbz group

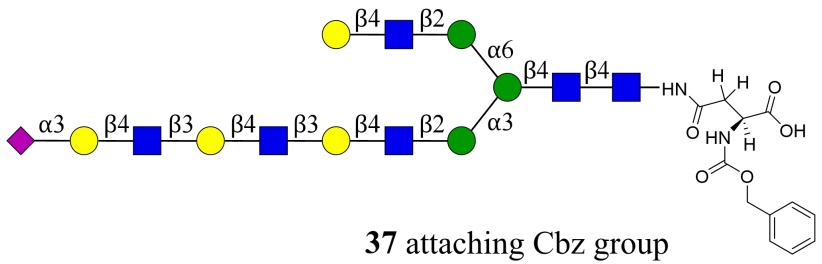


36 attaching Cbz group

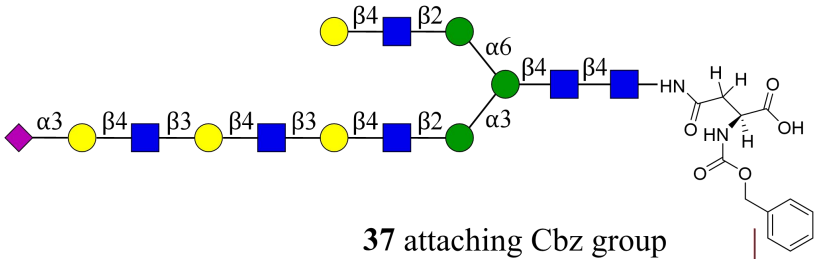




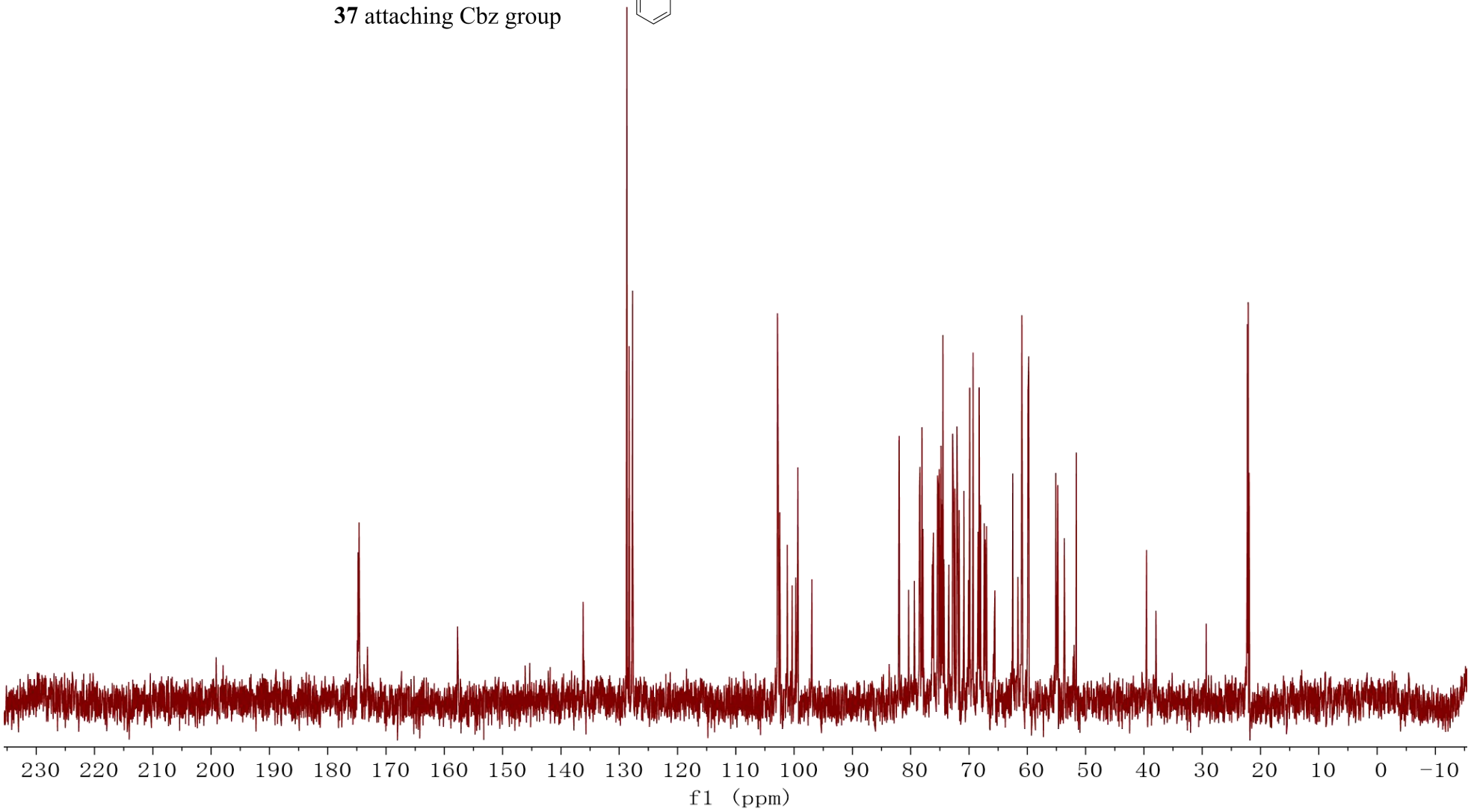
36 attaching Cbz group

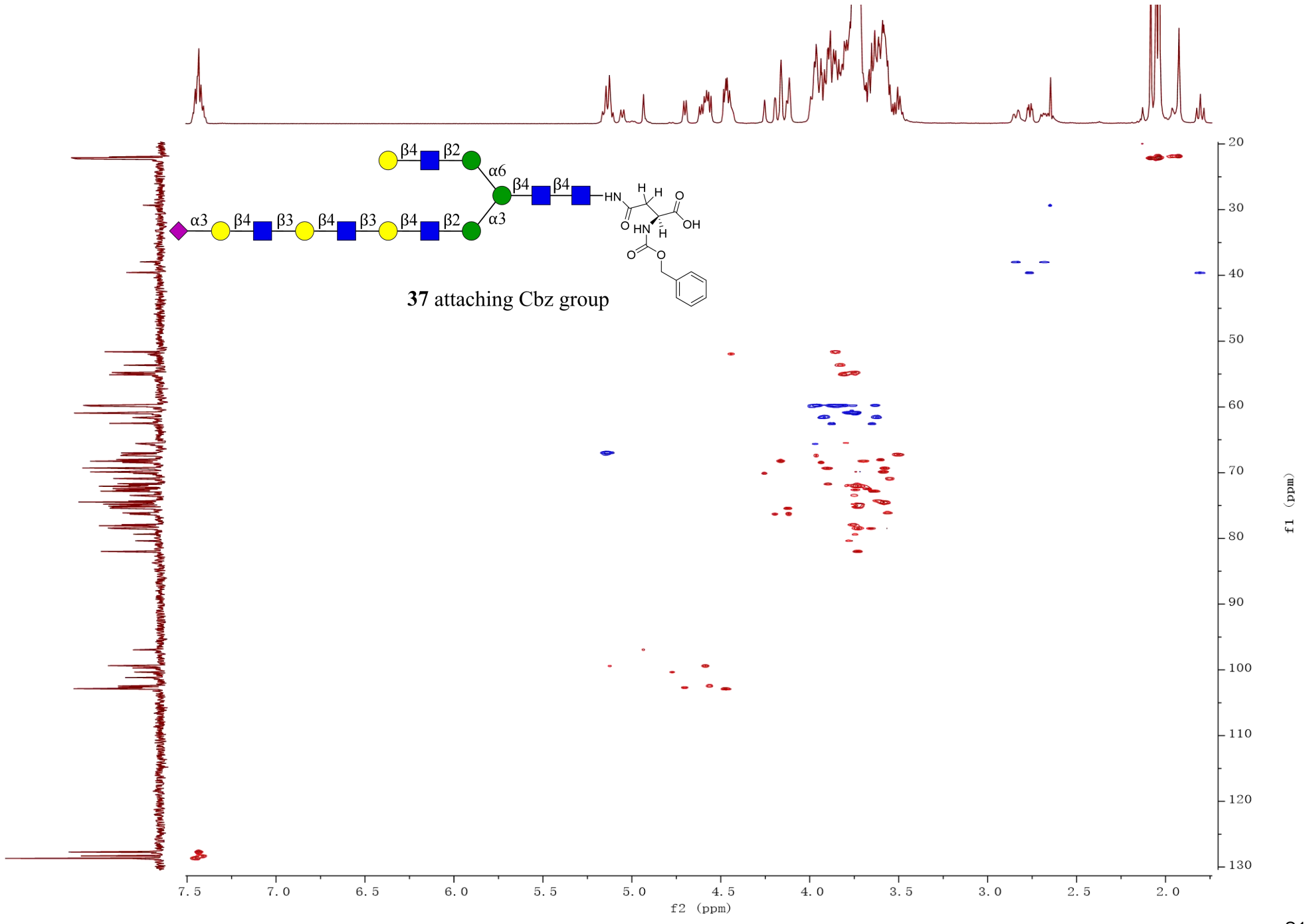


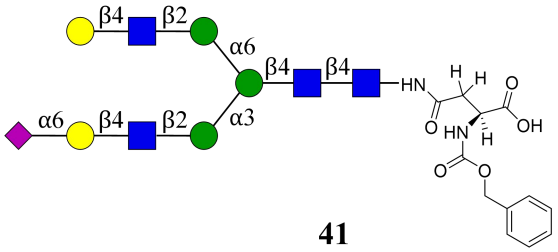
37 attaching Cbz group



37 attaching Cbz group

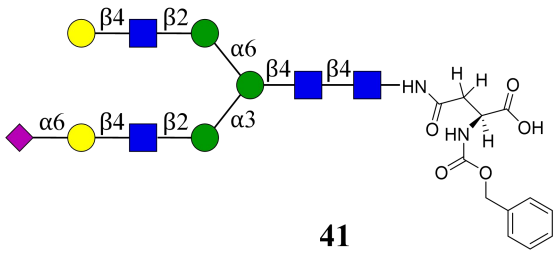



β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α3
β4
β3
β4
β3
β4
β2
α3
37 attaching Cbz group
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

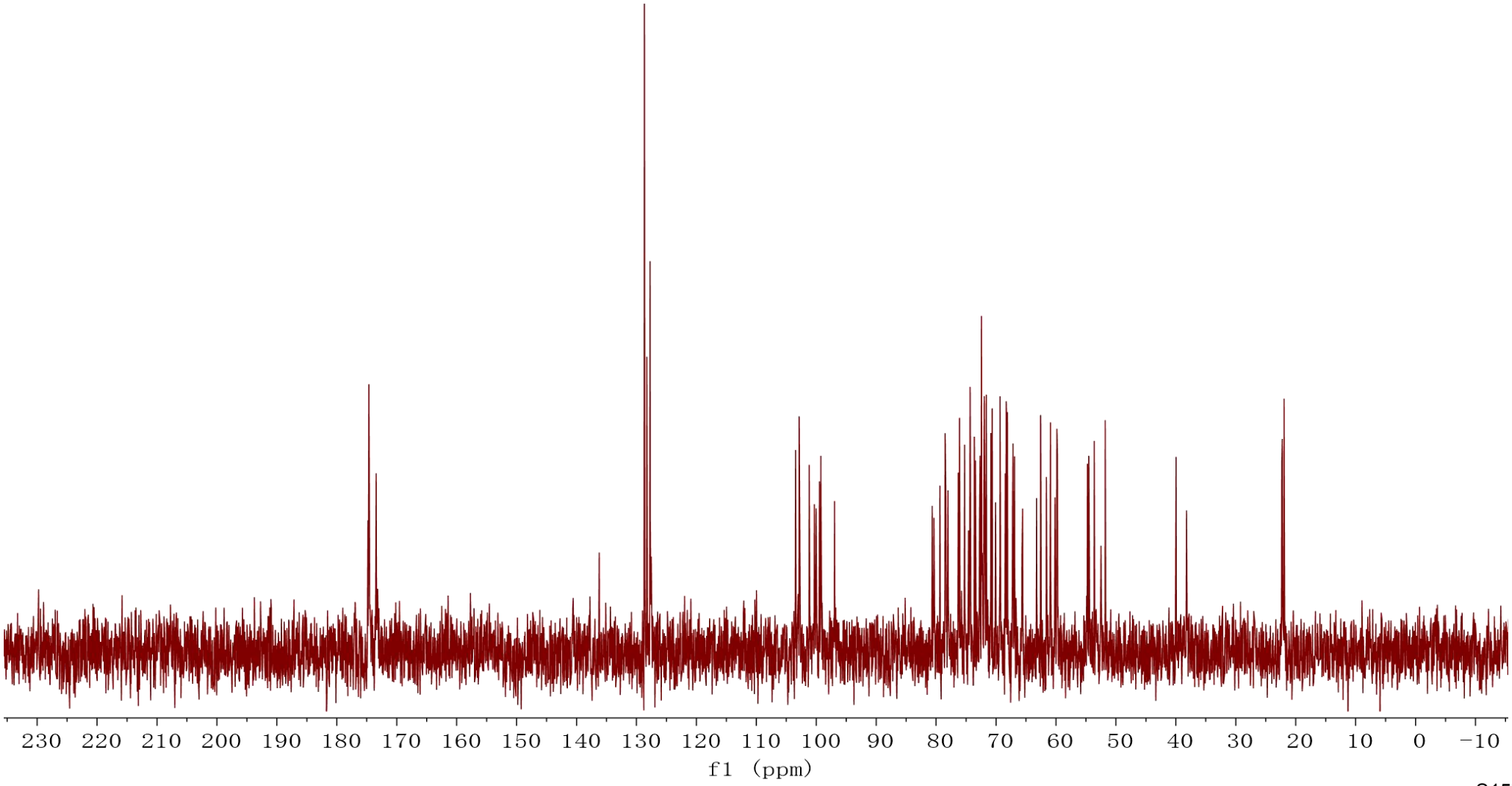

β4
β2
α6
β4
β4
HN
H
H
O
OH
α6
β4
β2
α3
O
HN
H
O
O

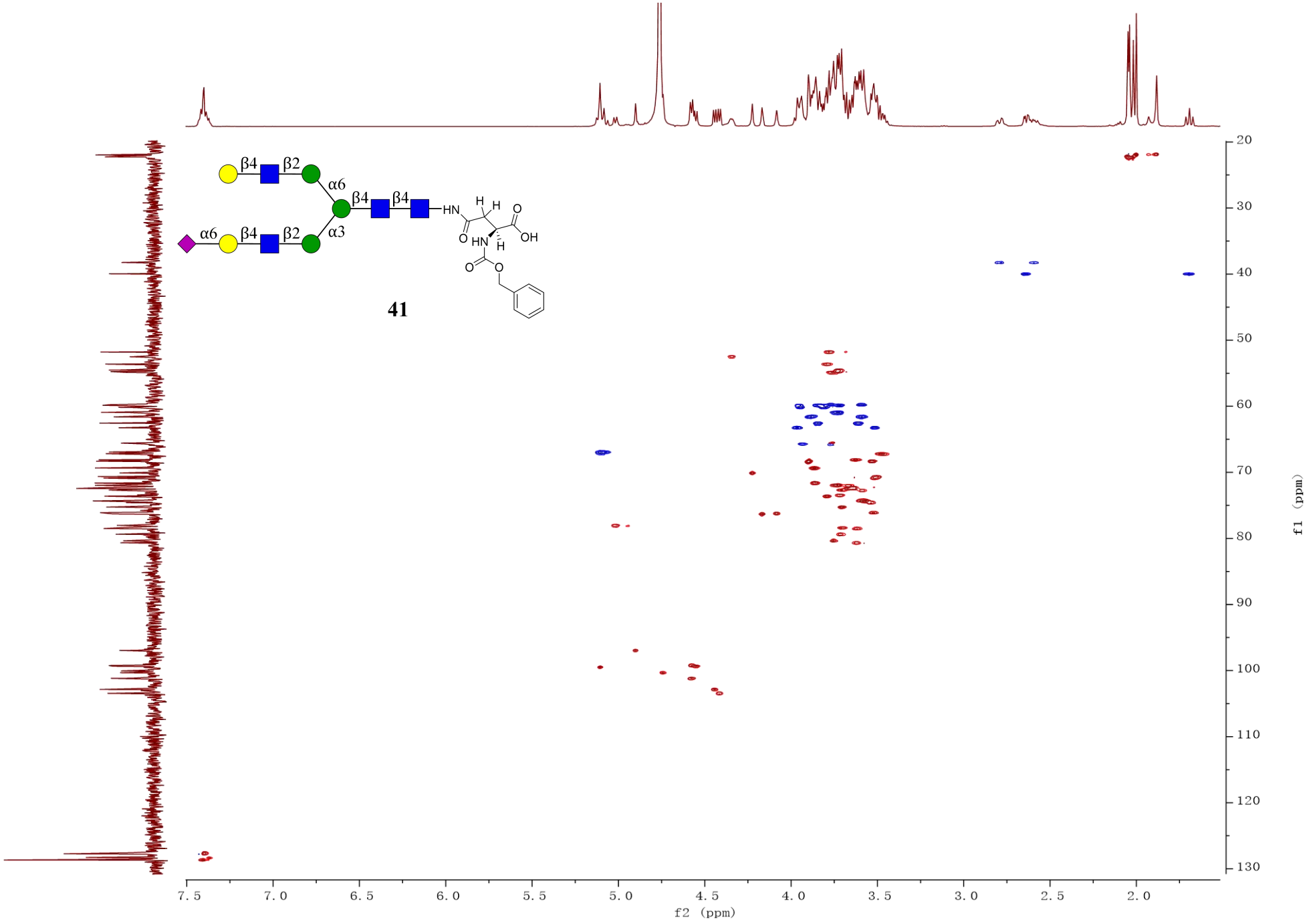
41

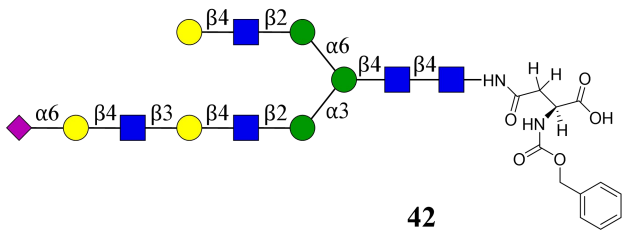
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
O
OH
HN
H
O
O
α6
β4
β2
α3

41

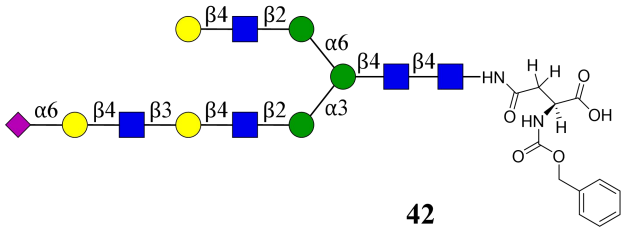

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
OH
α6
β4
β2
α3
O
HN
H
O
O
41
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

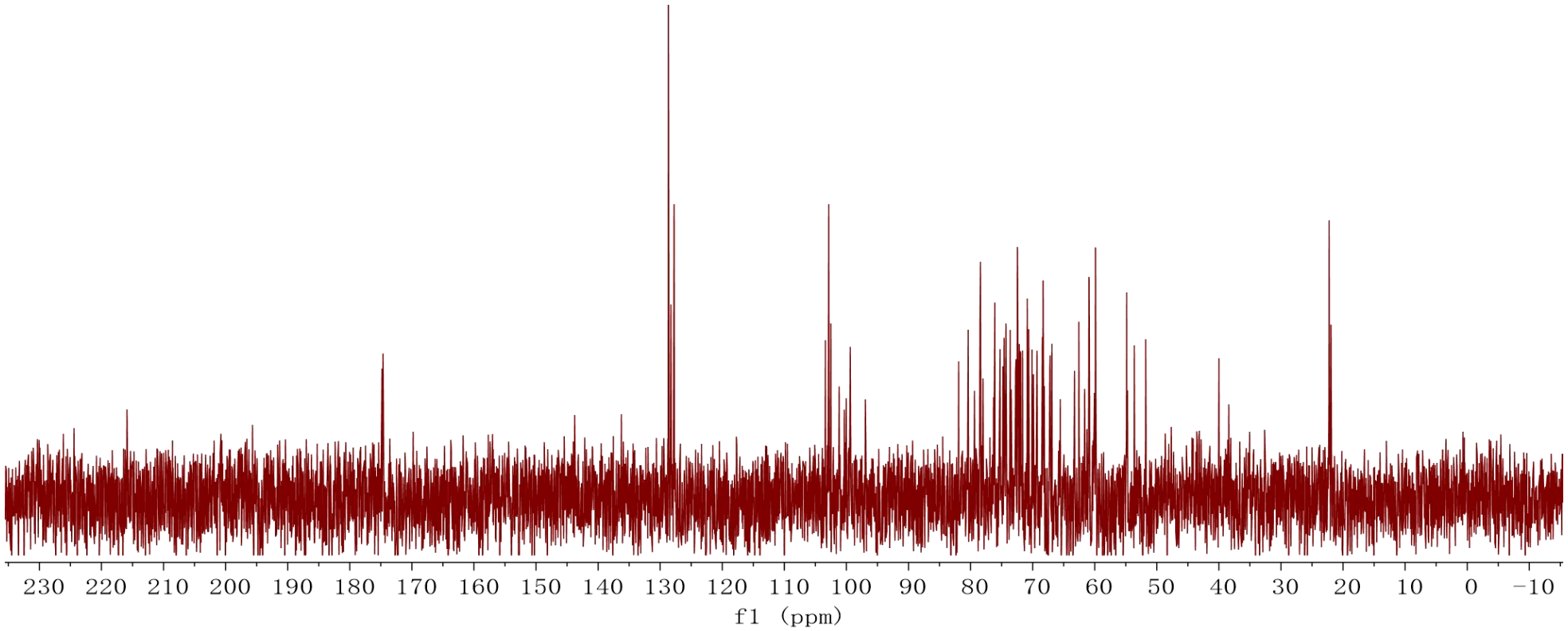

β4
β2
α6
β4
β4
HN
H
H
O
O
OH
HN
H
O
O
α6
β4
β3
β4
β2
α3

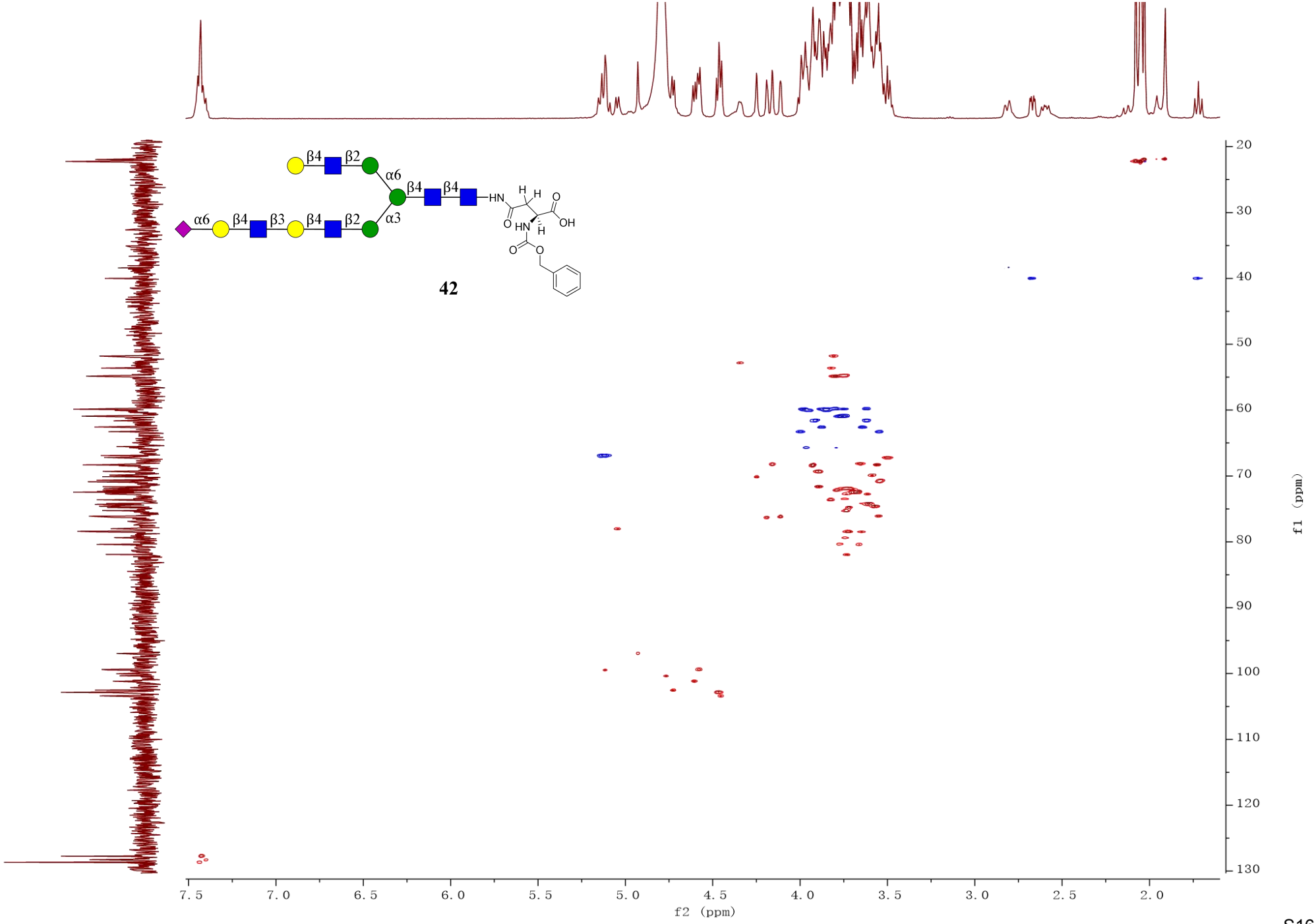
42

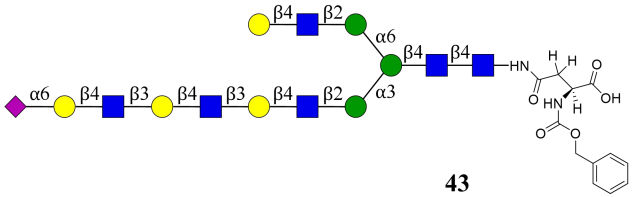
7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
1.
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β2
α3

42

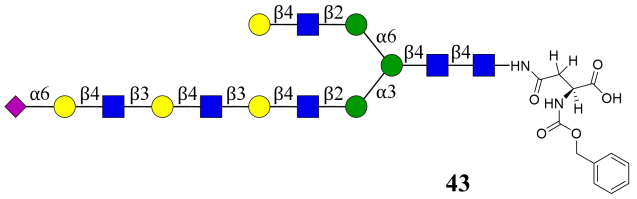

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β2
α3
42
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

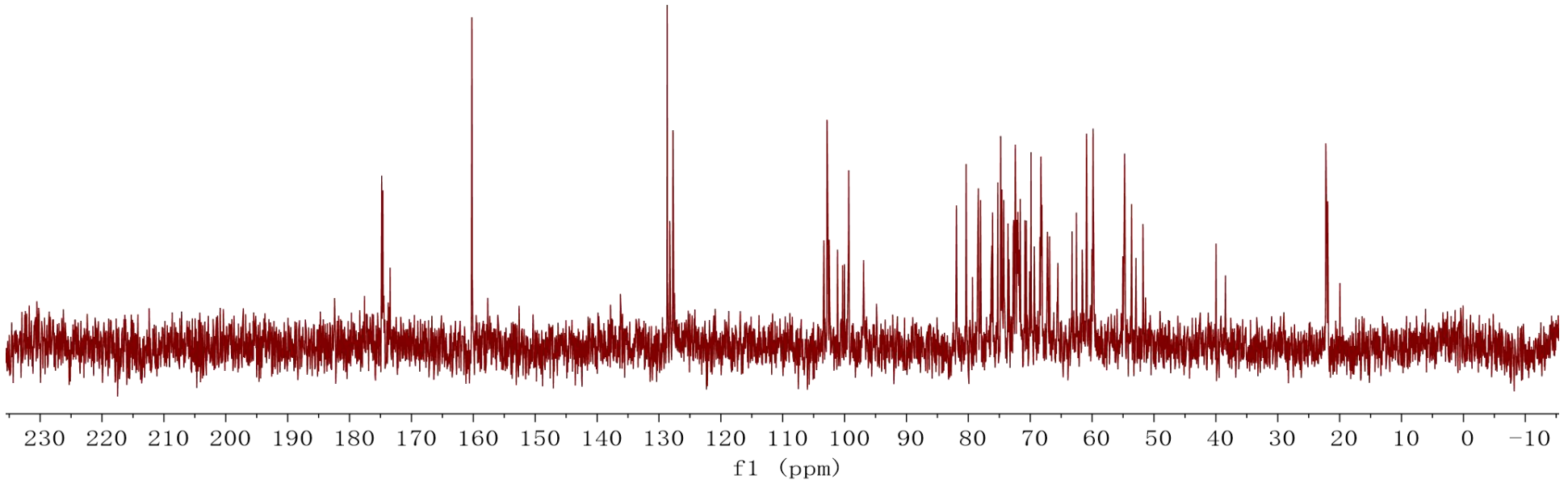

β4
β2
α6
β4
β4
HN
H
H
O
O
OH
α6
β4
β3
β4
β3
β4
β2
α3
HN
H
O
O
O

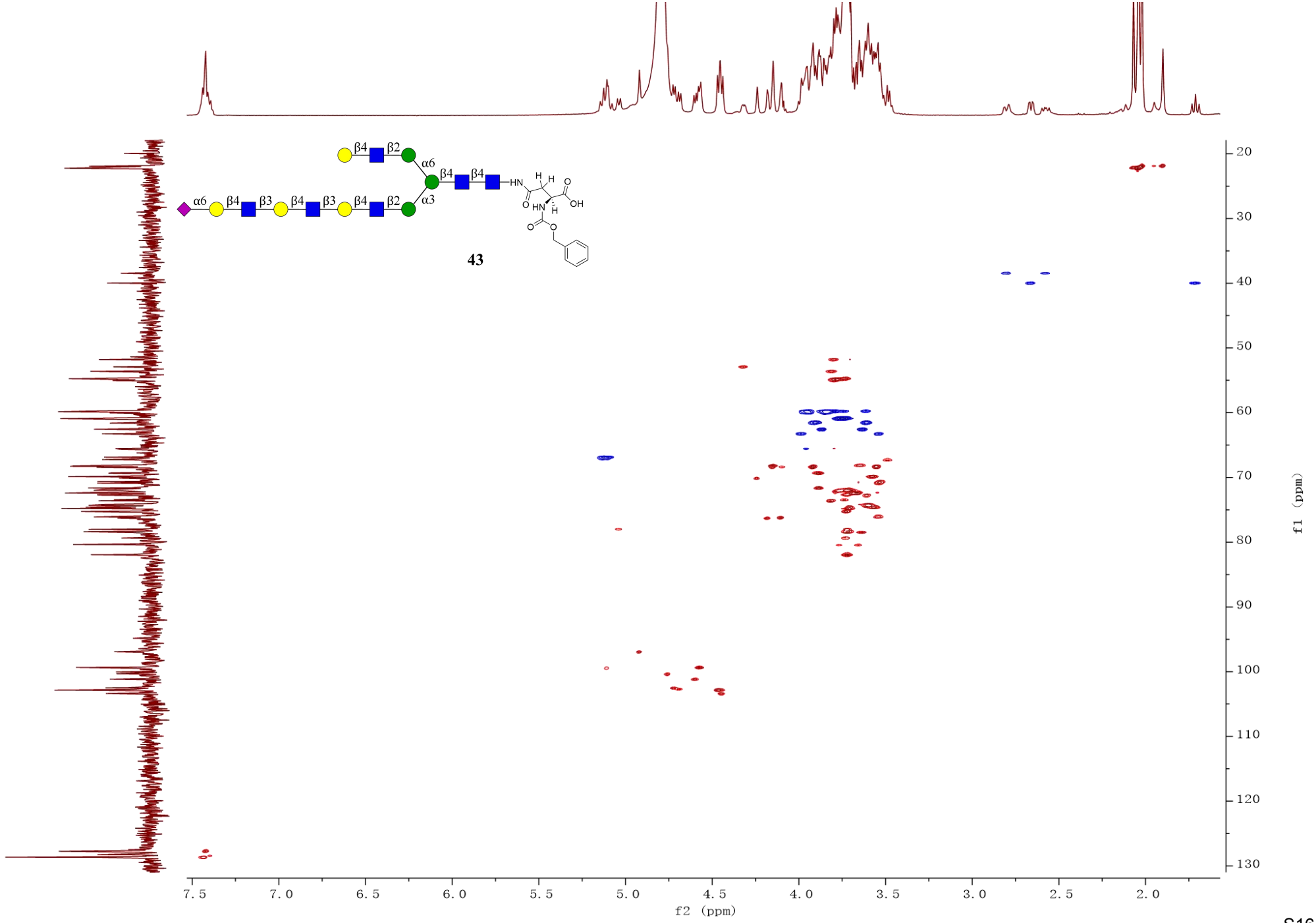
43

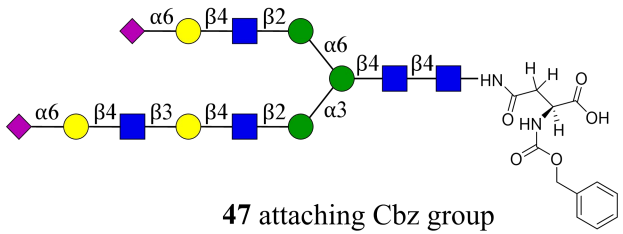
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β3
β4
β2
α3

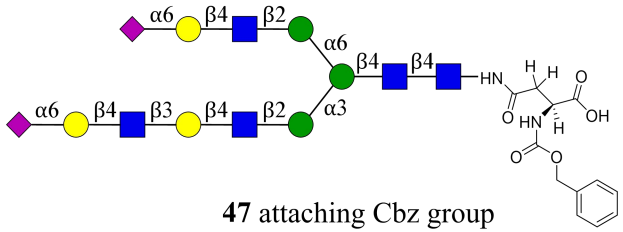
43


230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

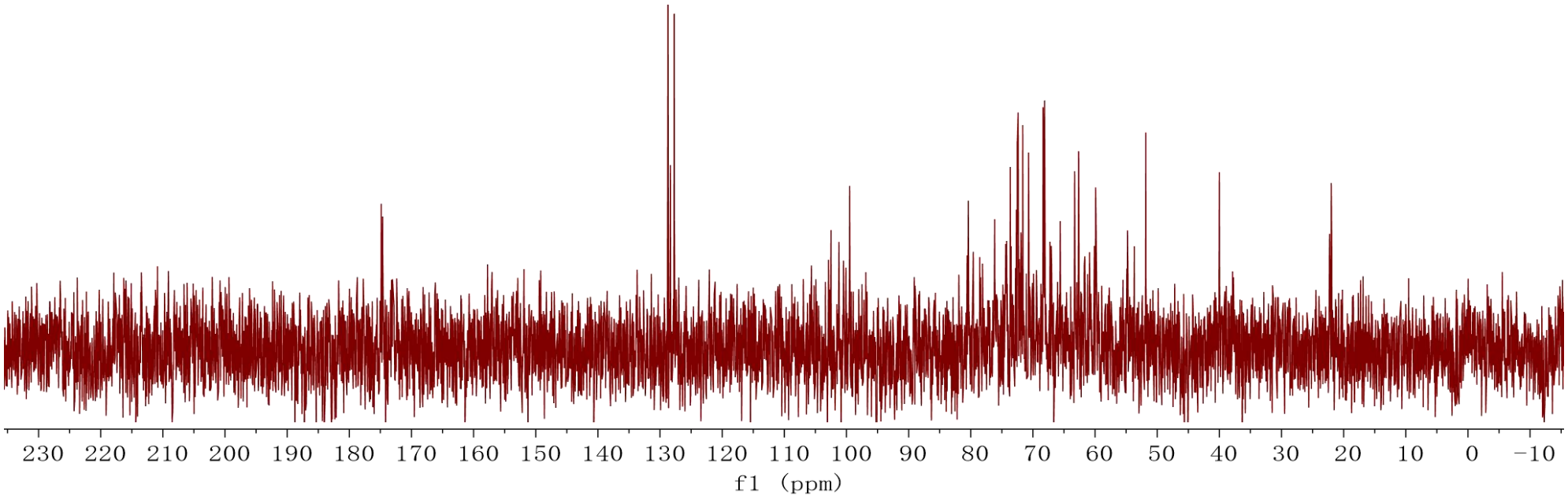

β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β3
β4
β2
α3
43
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

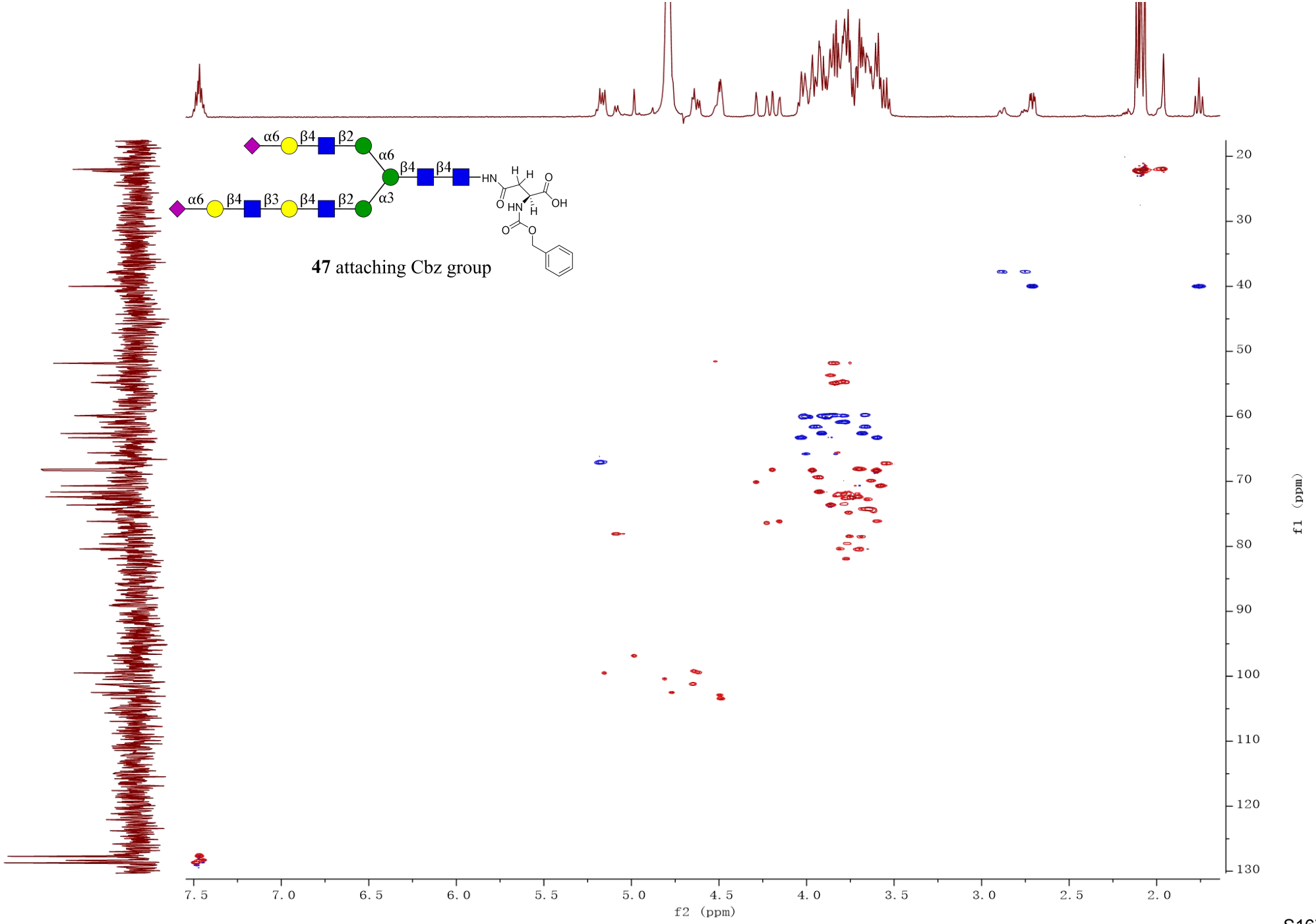


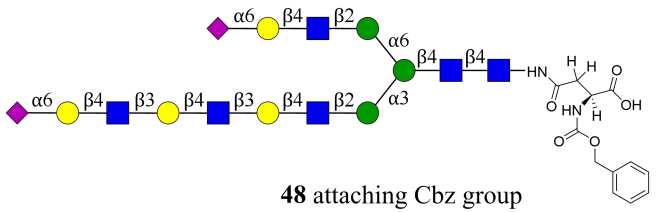
47 attaching Cbz group



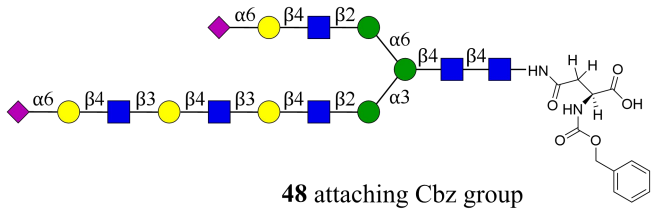
47 attaching Cbz group



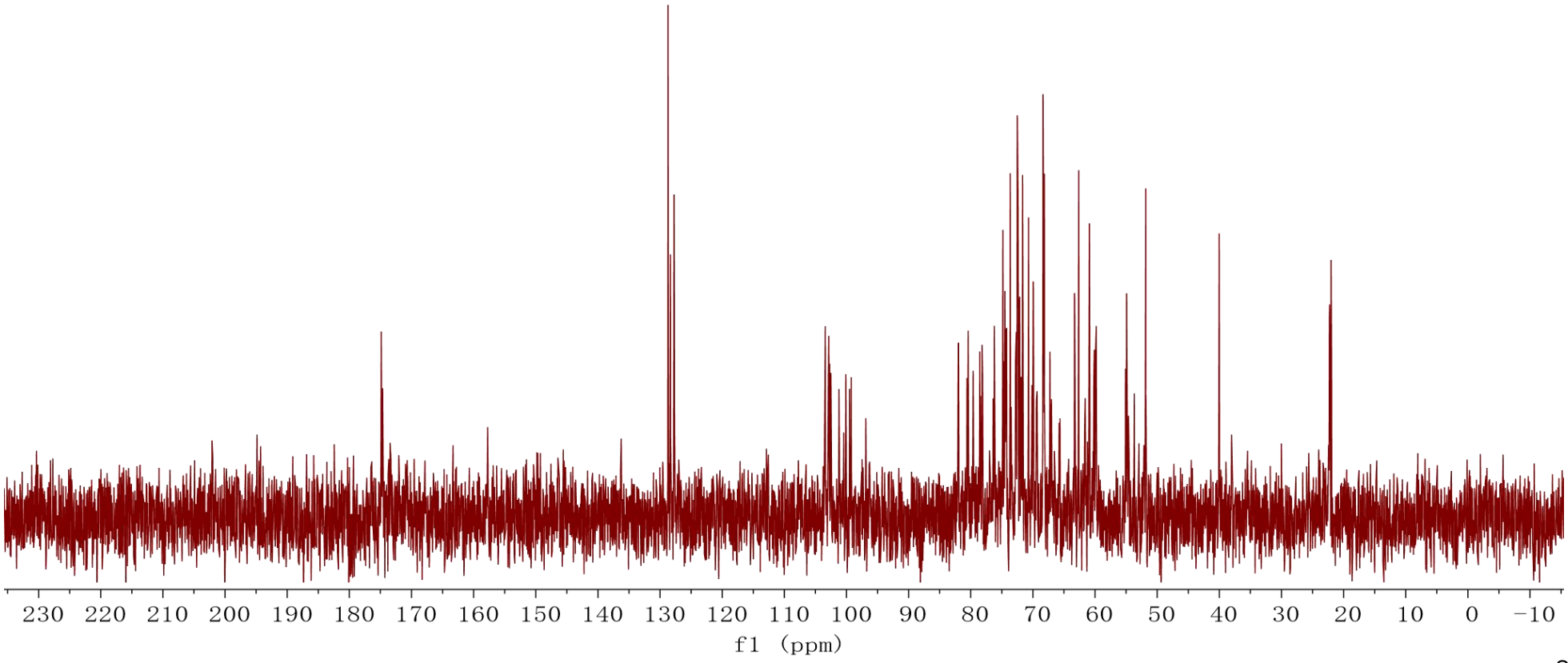

α6 β4 β2
α6
β4 β4
HN
α6 β4 β3 β4 β2
α3
OH
47 attaching Cbz group
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

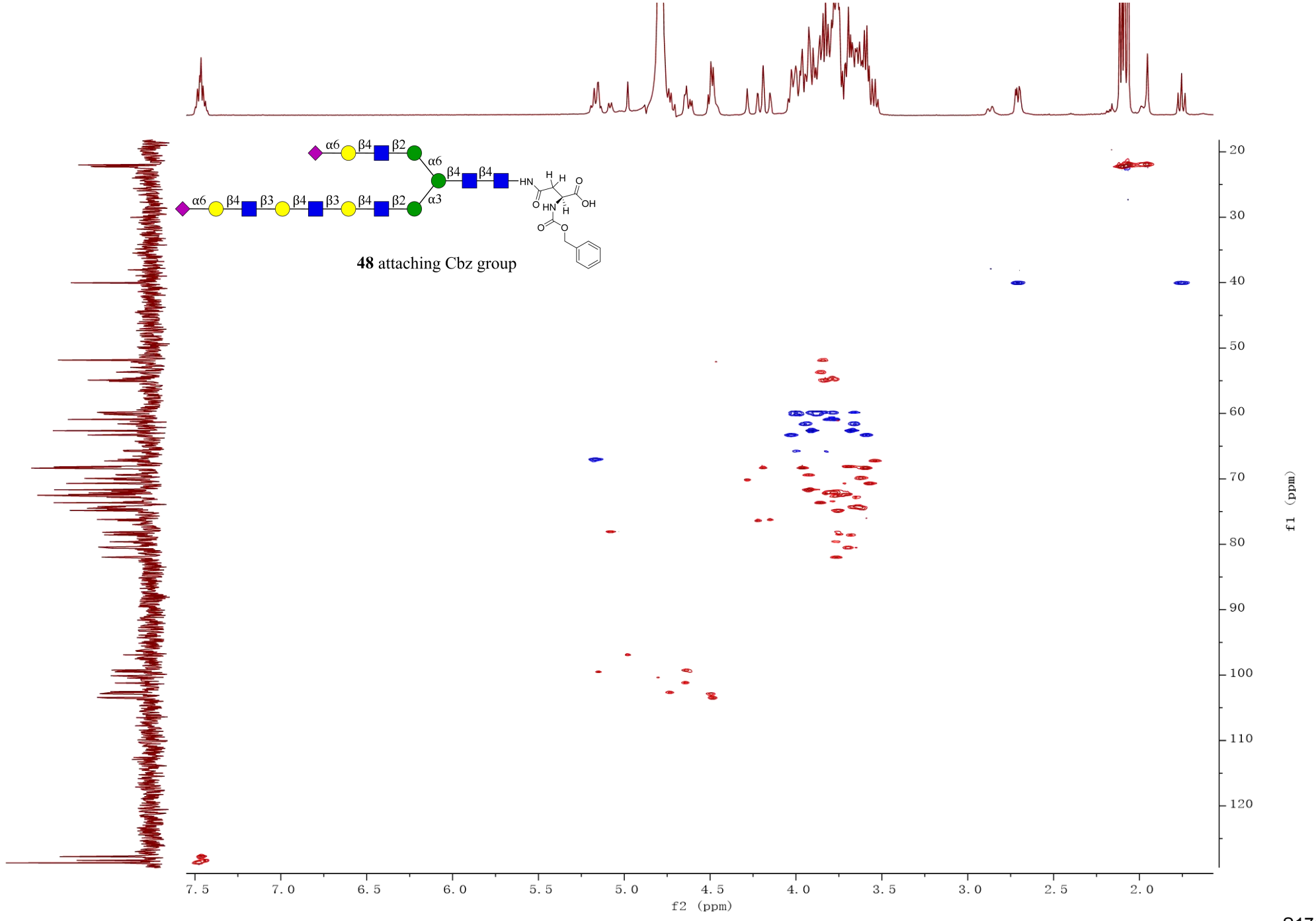


48 attaching Cbz group

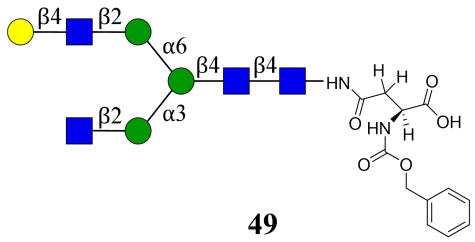


48 attaching Cbz group



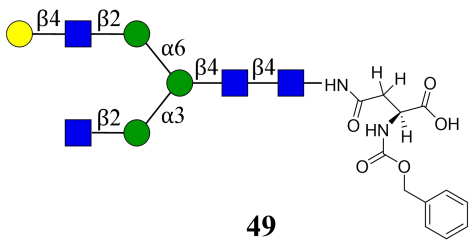


48 attaching Cbz group


β4
β2
α6
β4
β4
HN
H
H
O
OH
β2
α3
HN
O
O
O

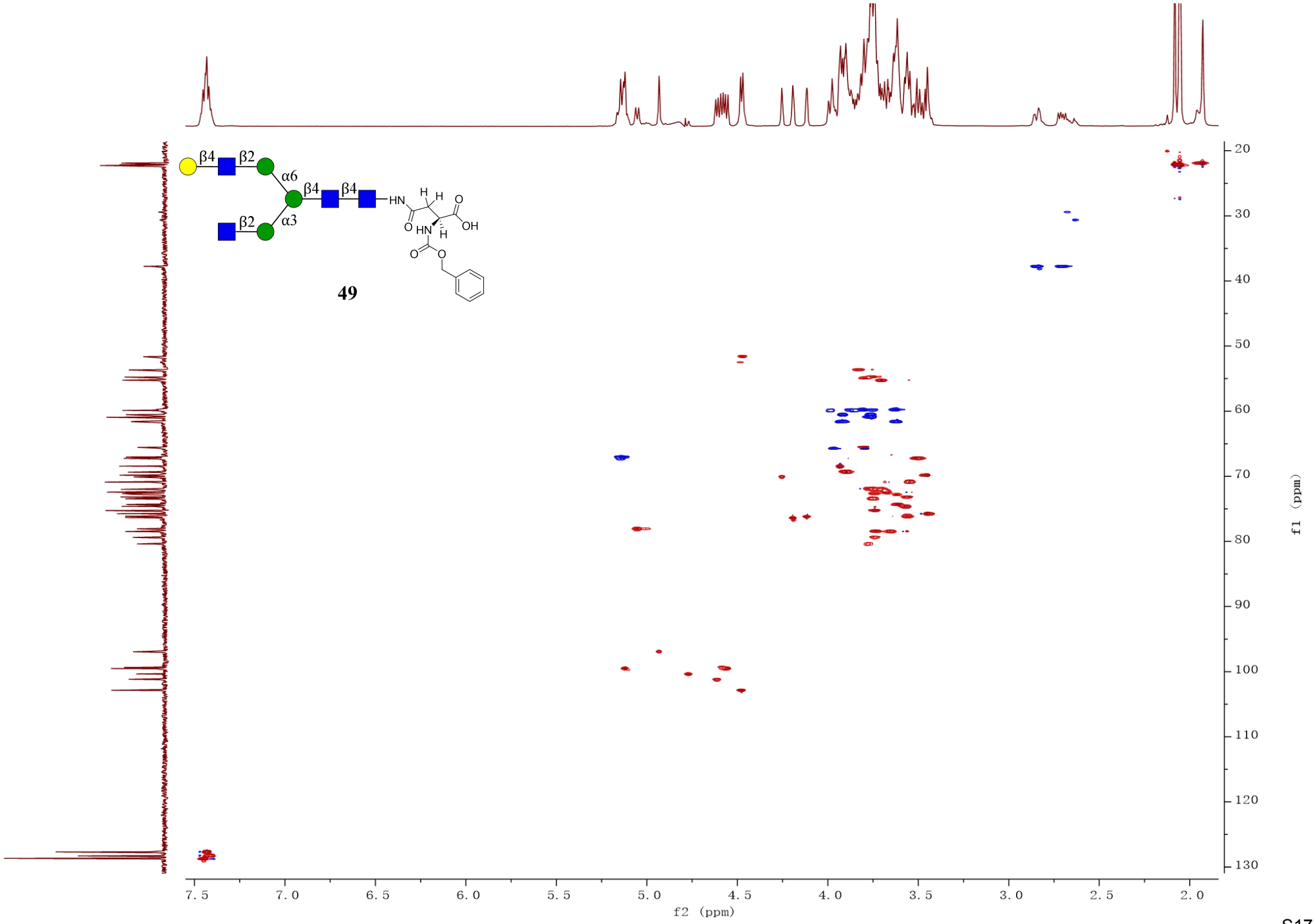
49

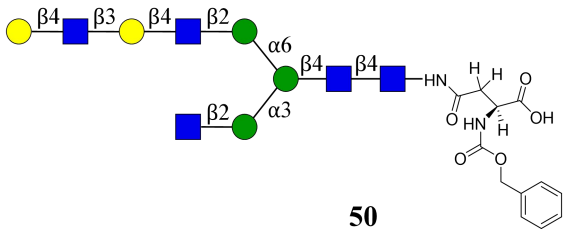
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
O
OH
β2
α3
O
HN
H
O
O

49

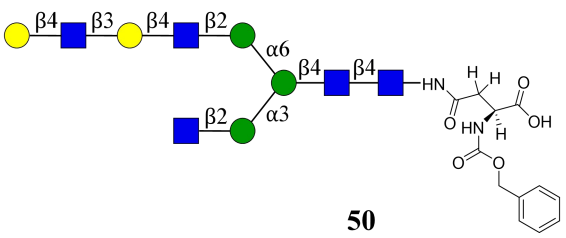
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β4
β2
α6
β4
β4
HN
H
H
O
OH
β2
α3
O
HN
H
O
O
49
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)


β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3

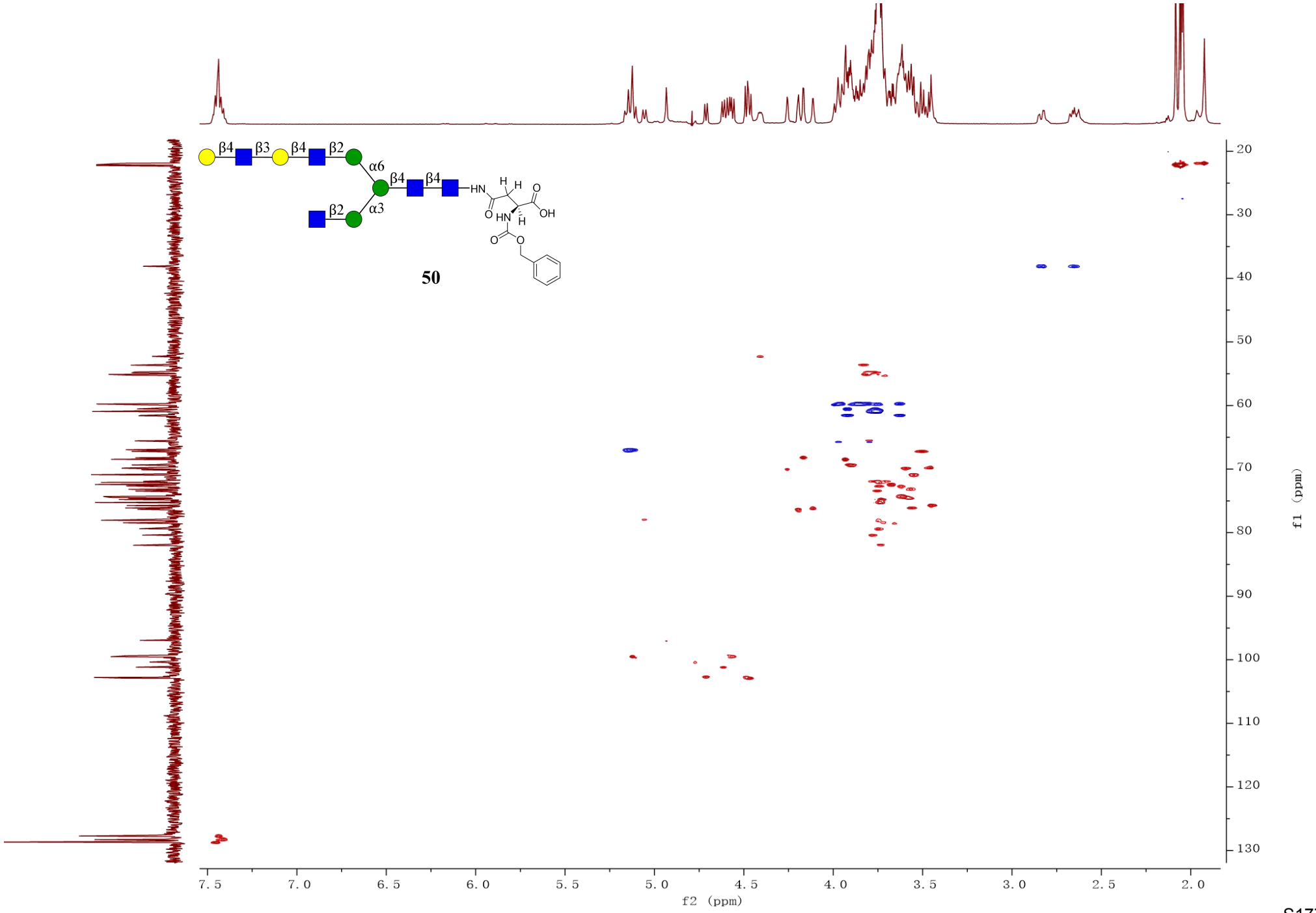
50

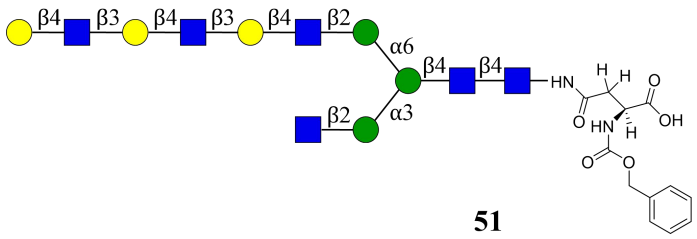
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3

50

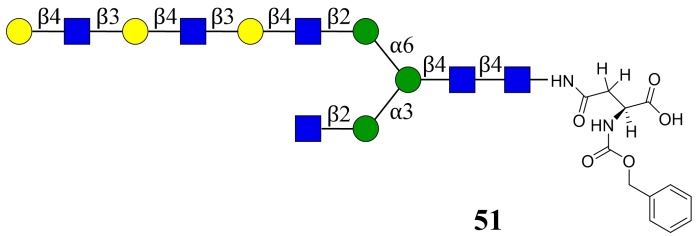
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
HN
H
O
O
O
β2
α3
50
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

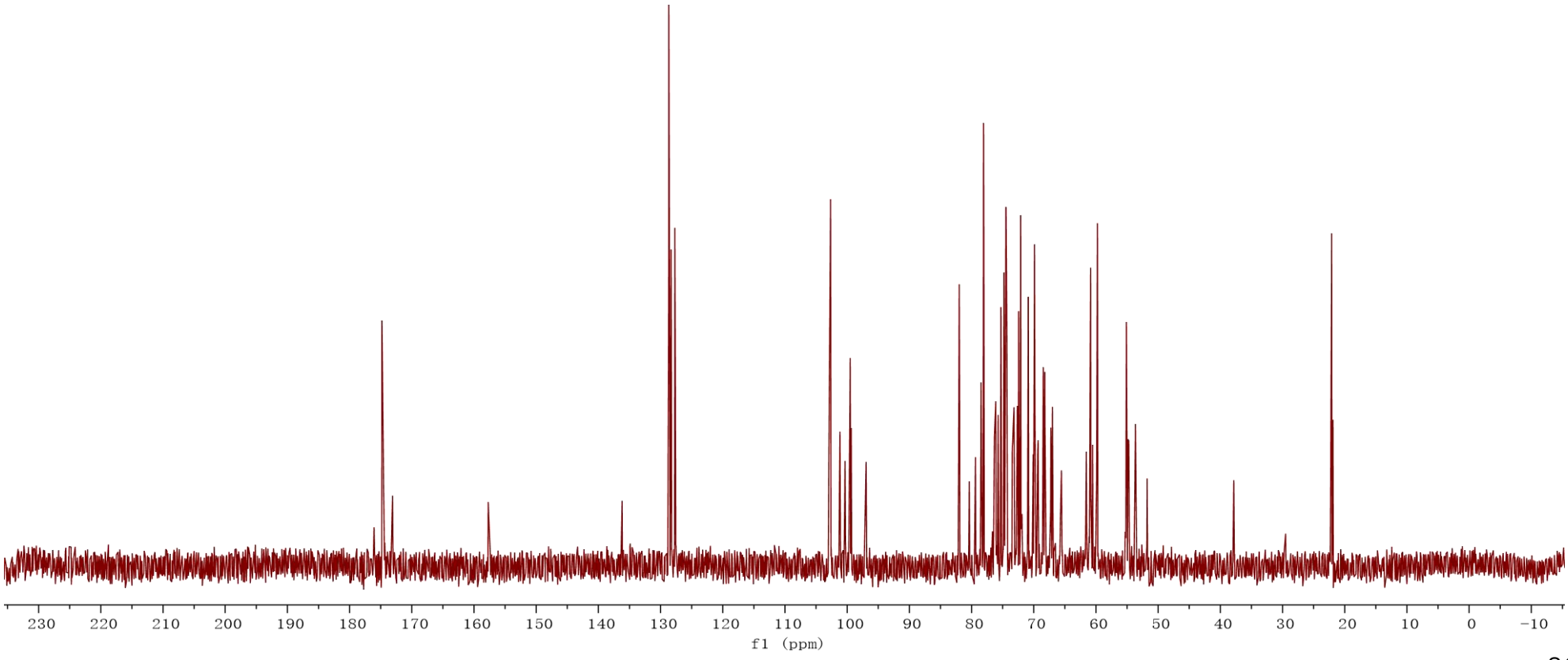

β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
O
β2
α3

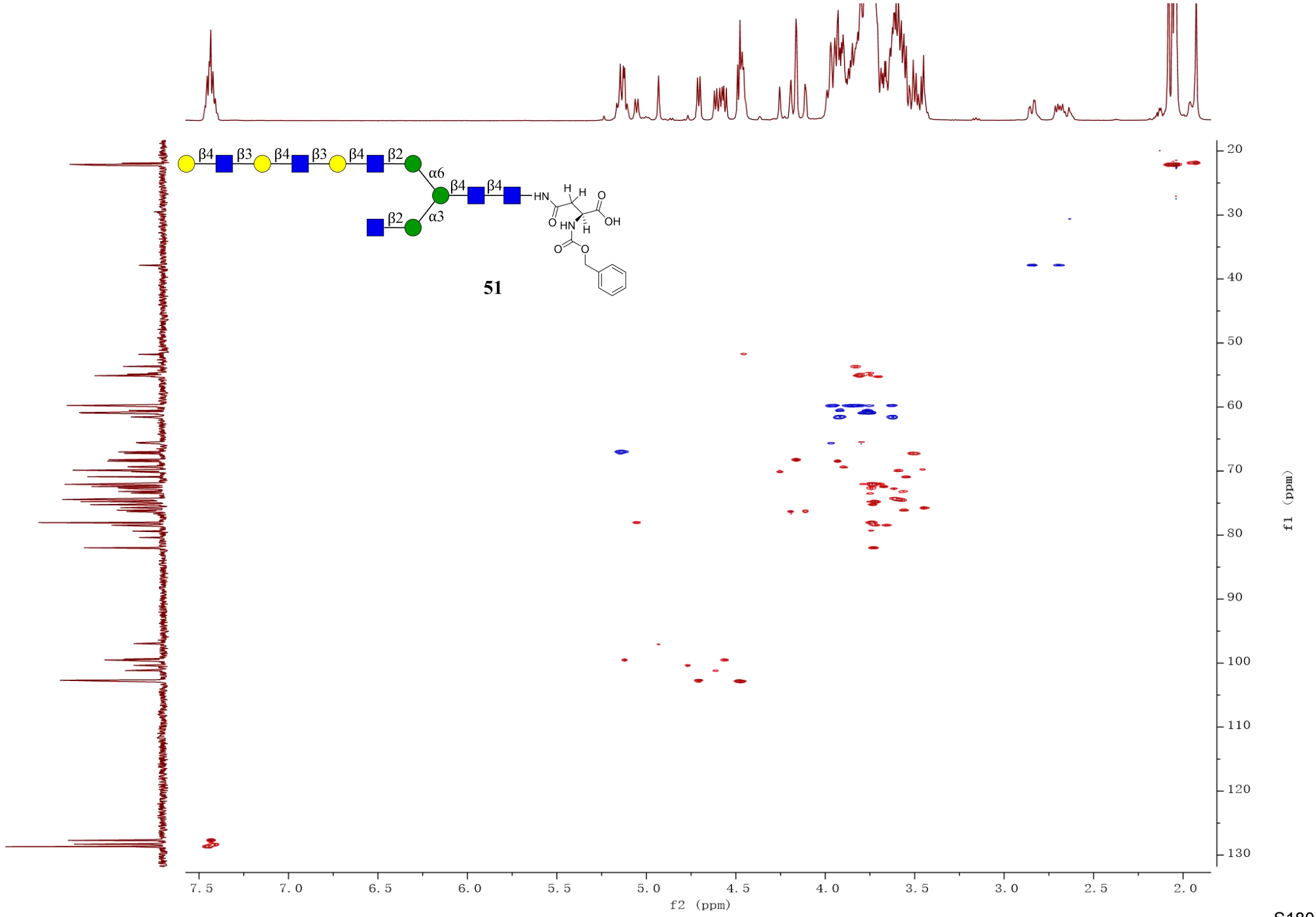
51

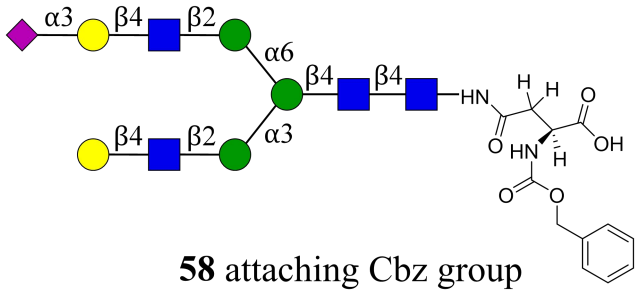
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
O
β2
α3

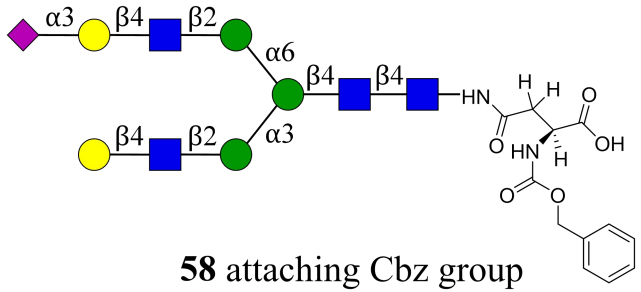
51


230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

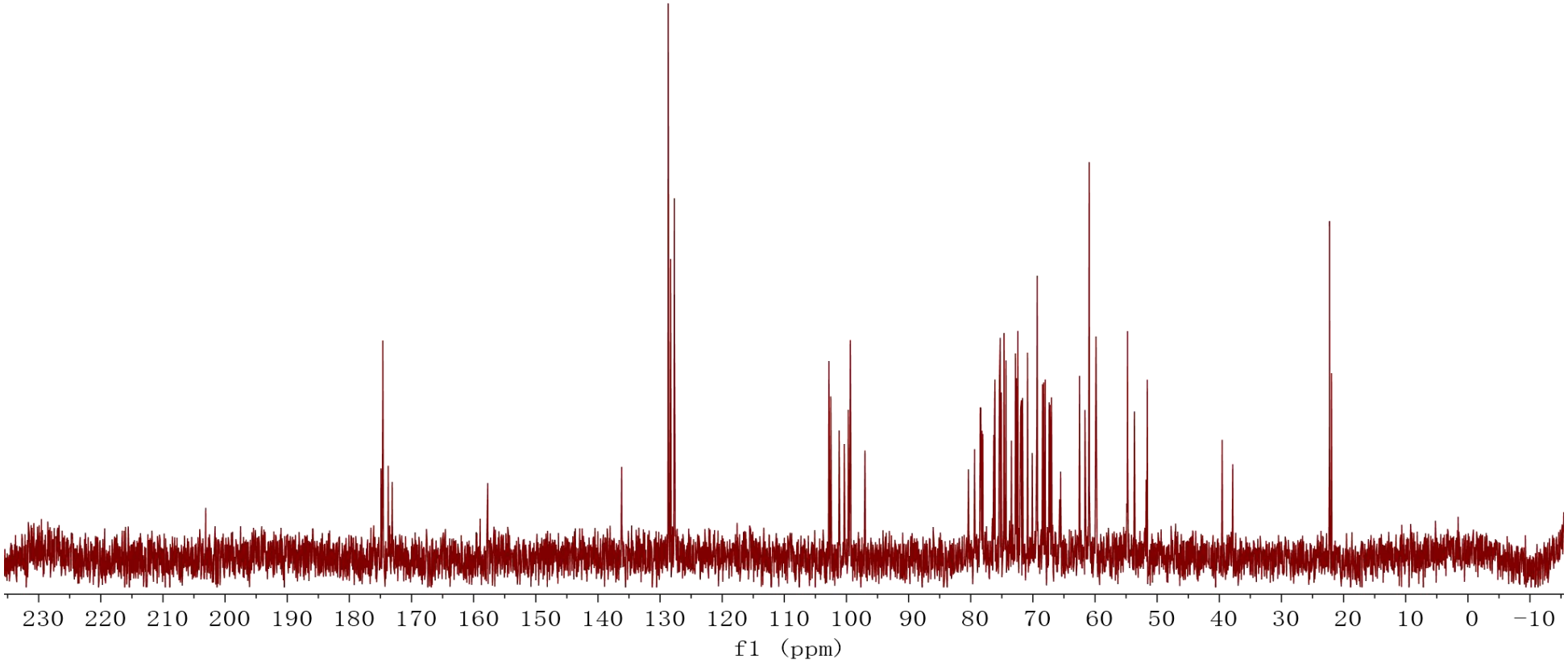

β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β2
α3
51
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

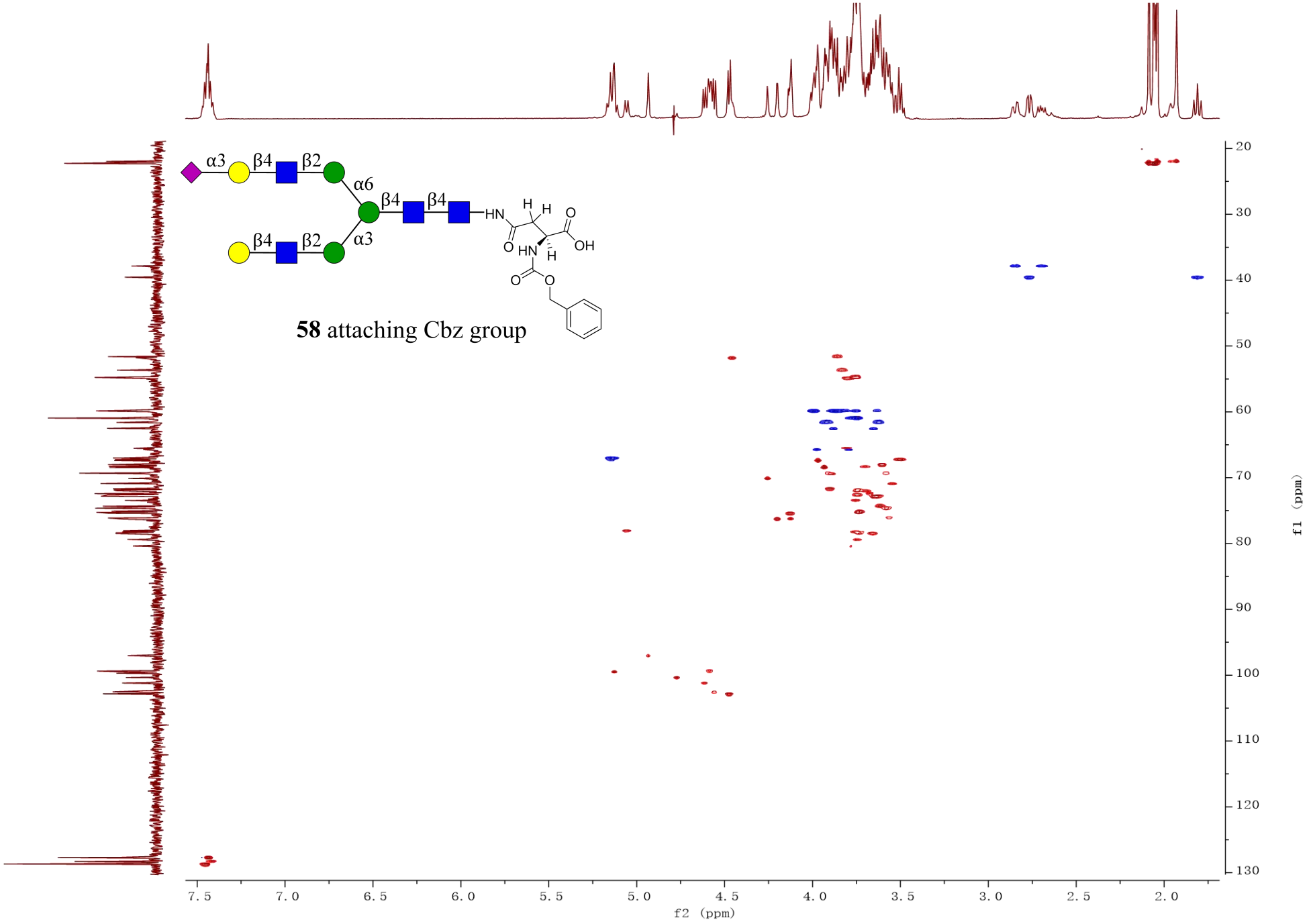


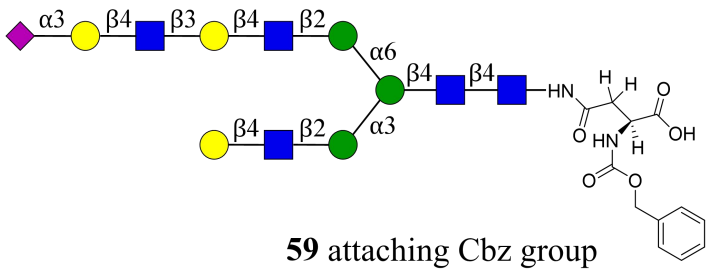
58 attaching Cbz group



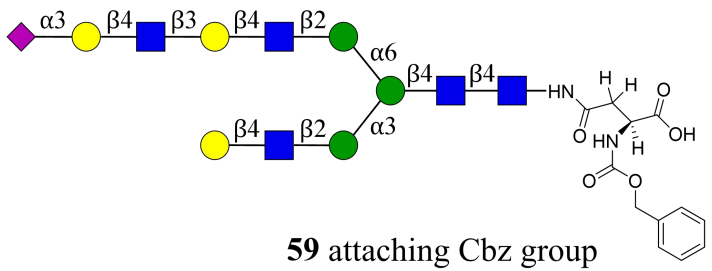
58 attaching Cbz group



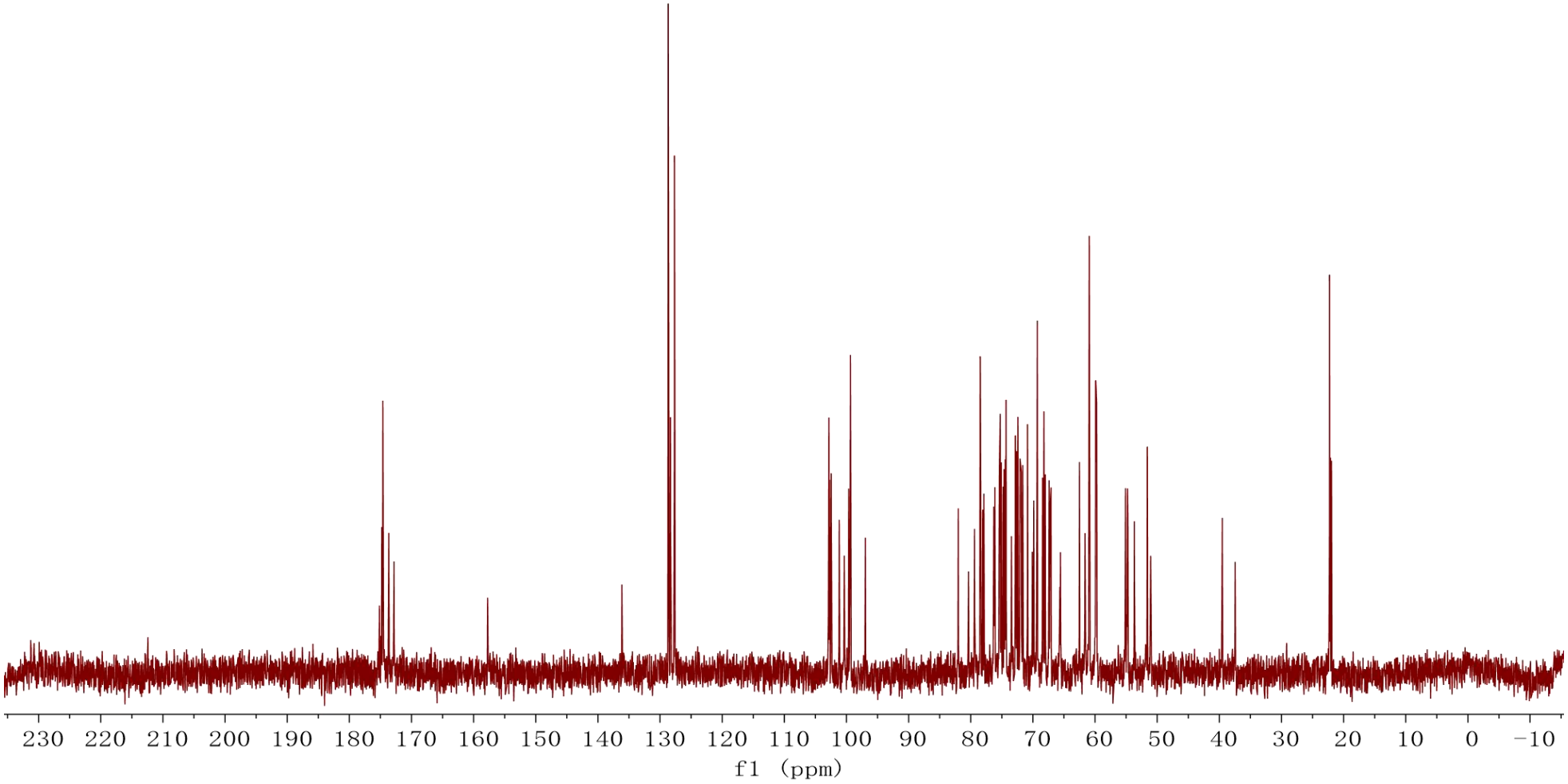

α3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3
58 attaching Cbz group
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)

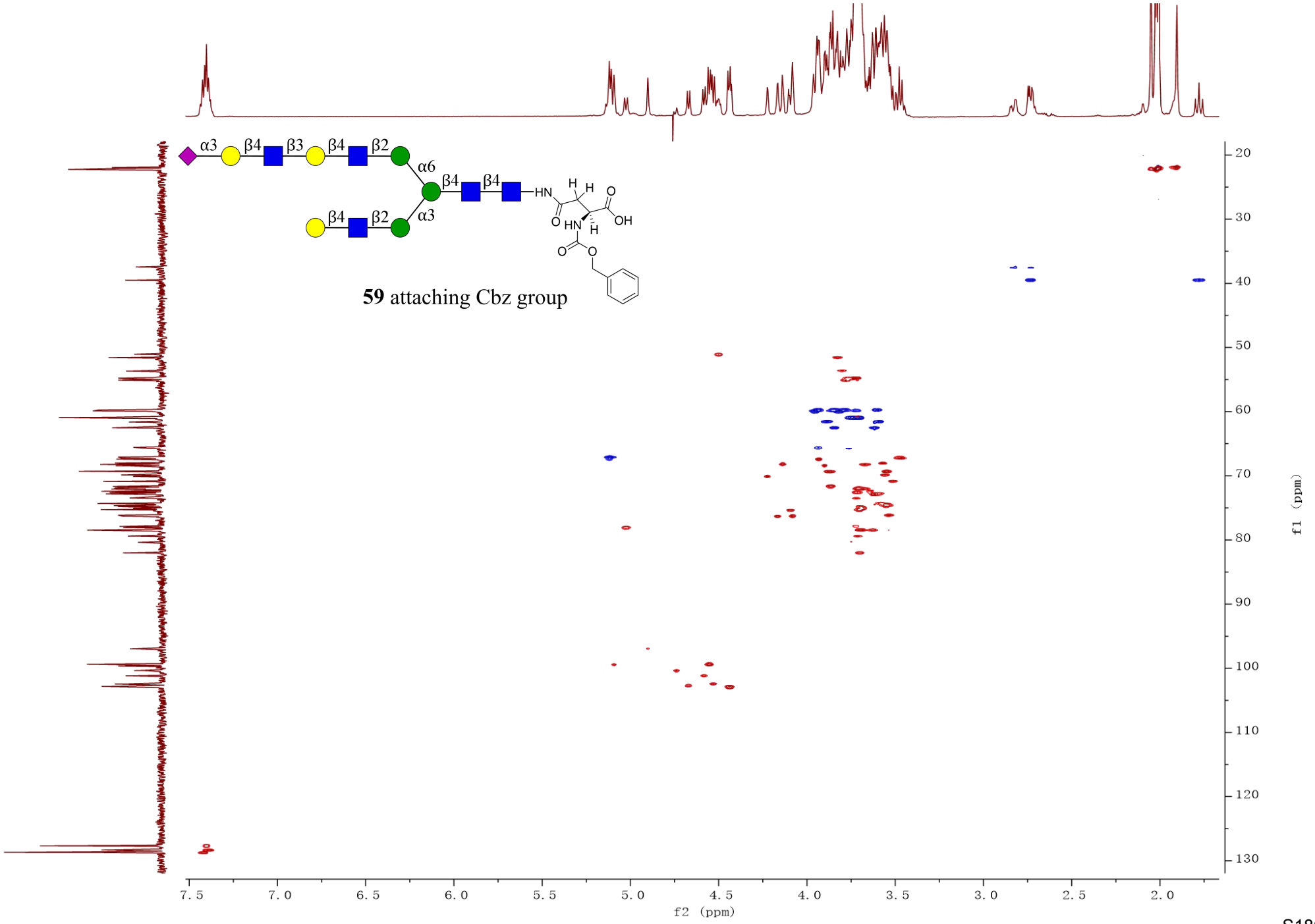


59 attaching Cbz group

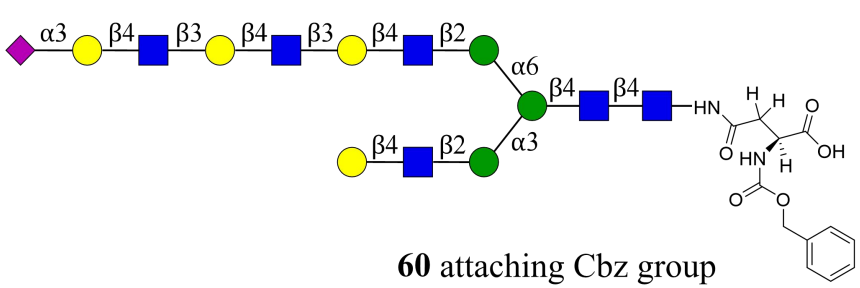


59 attaching Cbz group

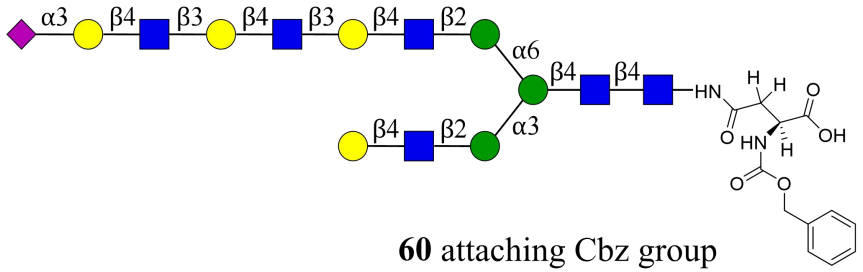




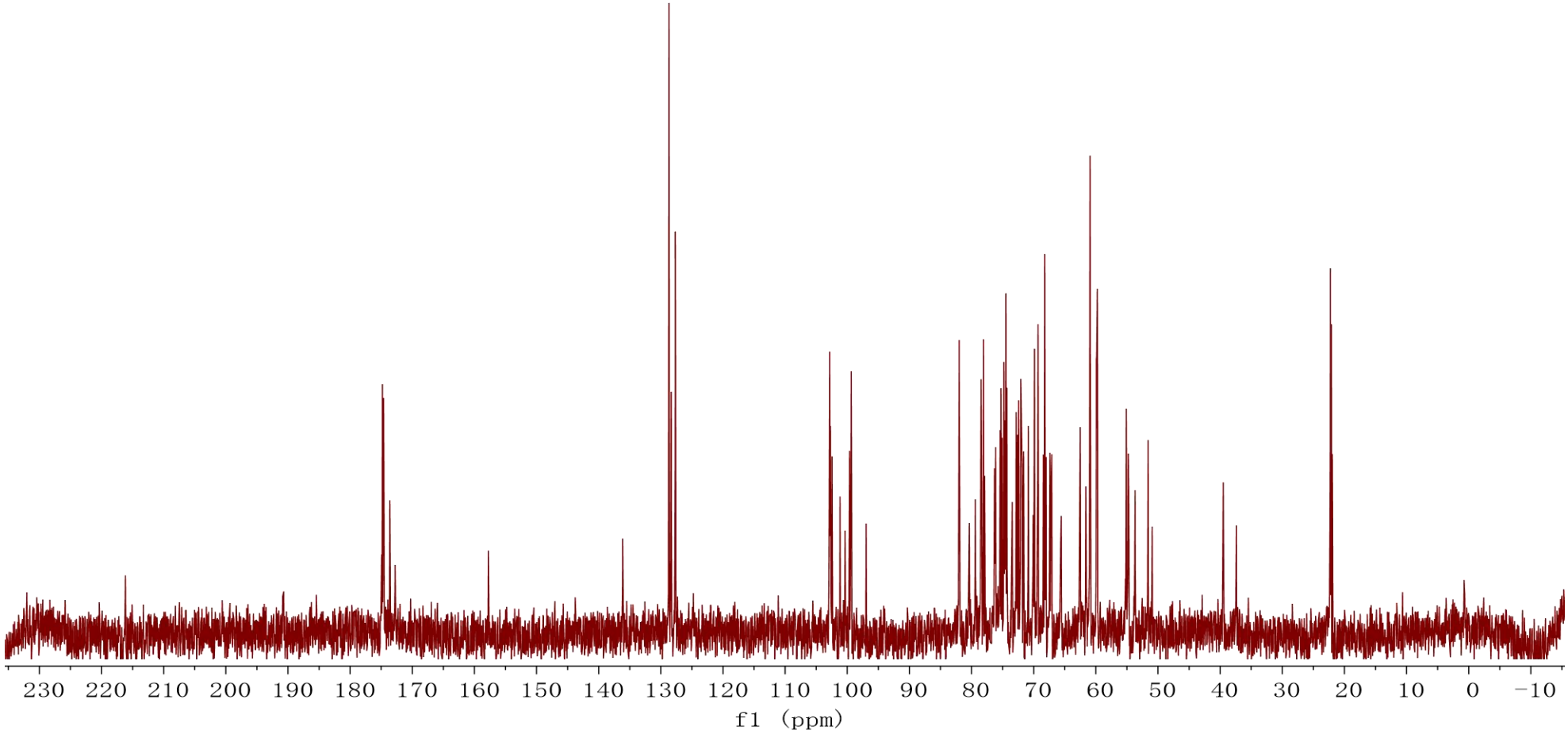
59 attaching Cbz group

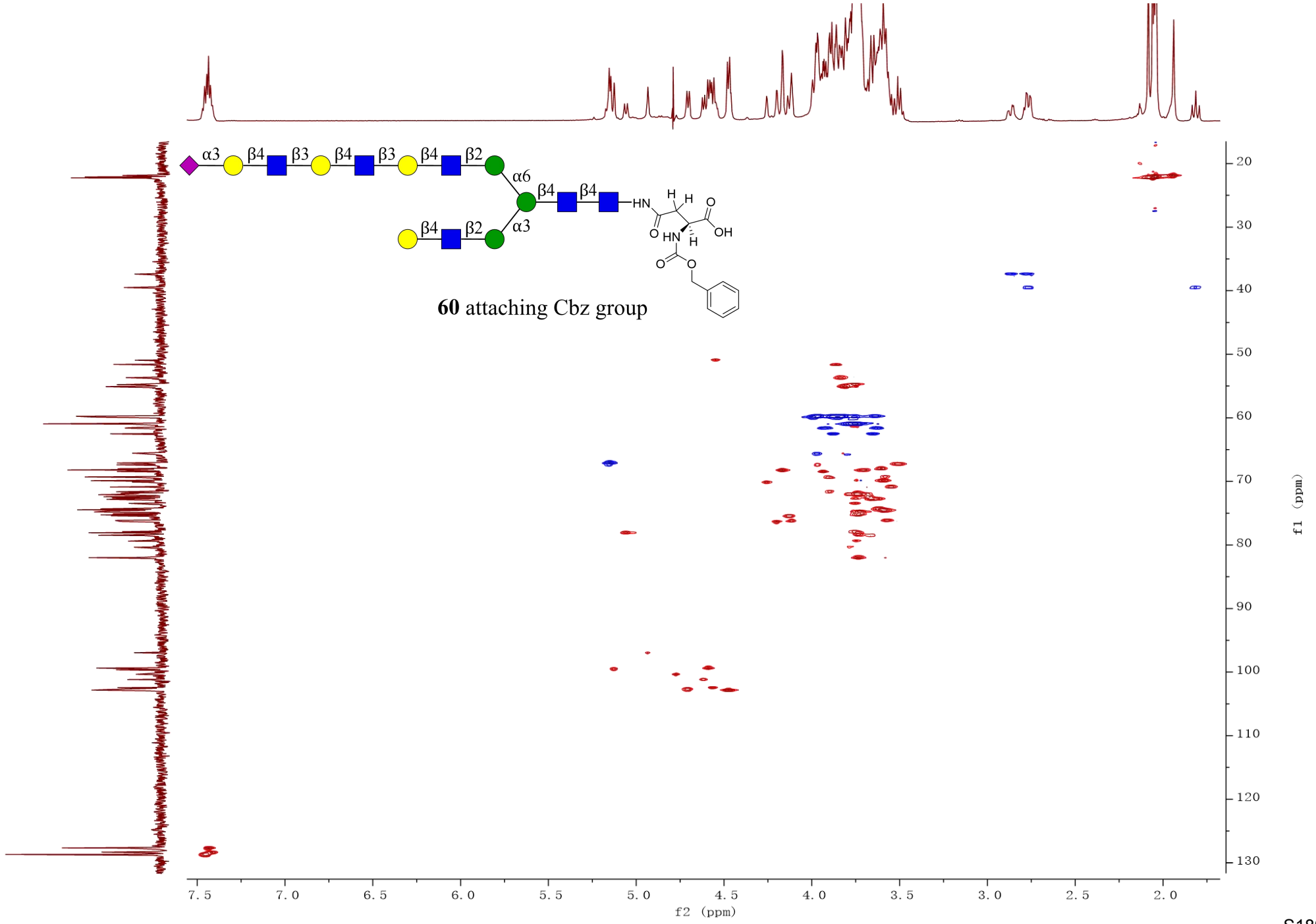


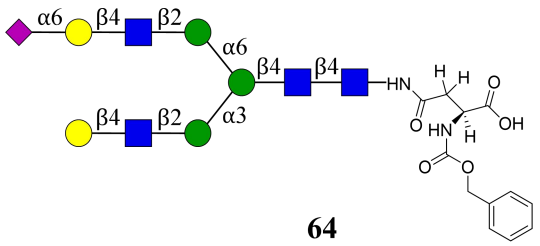
60 attaching Cbz group



60 attaching Cbz group

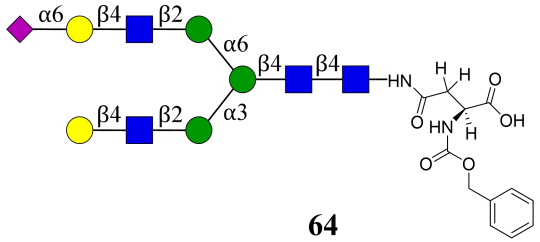



α3
β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3
60 attaching Cbz group
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

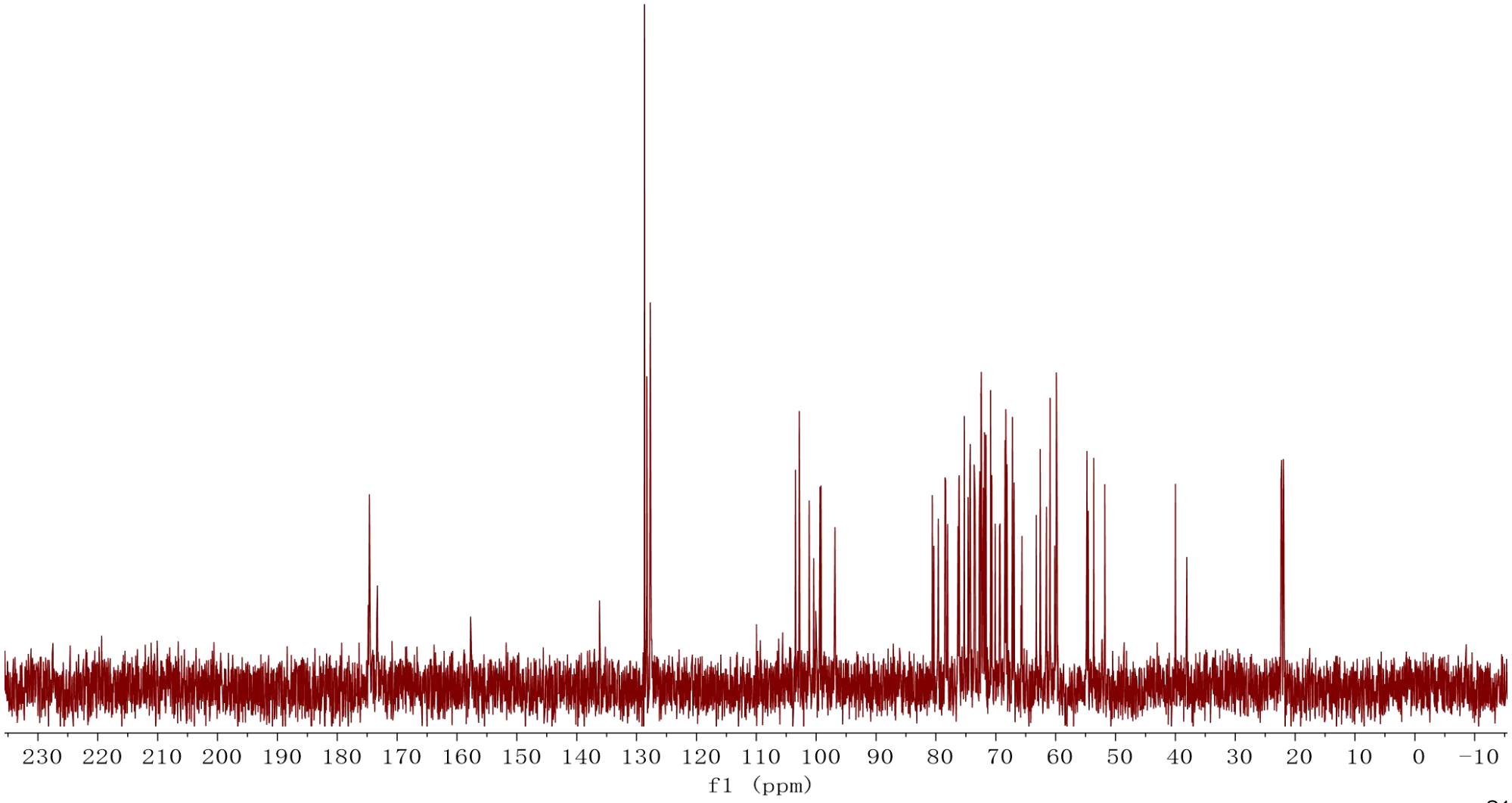

α6
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3

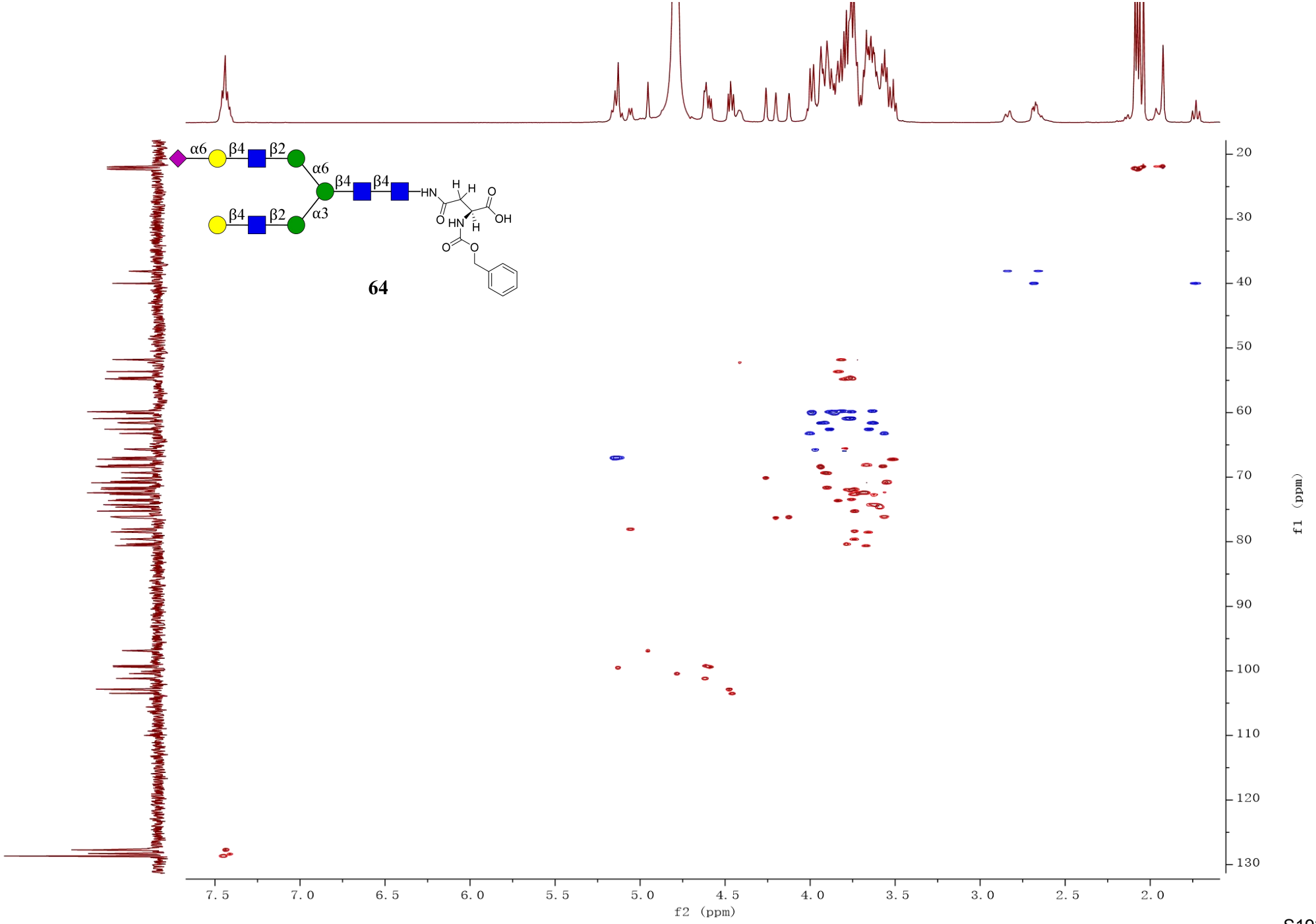
64

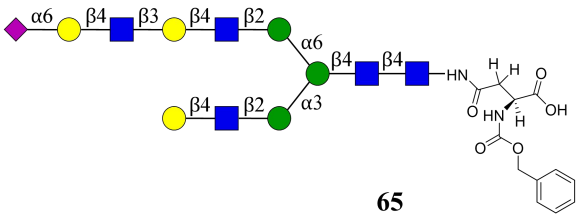
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


α6
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3

64

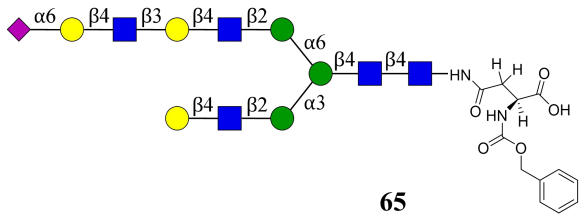

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


α6
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3
64
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

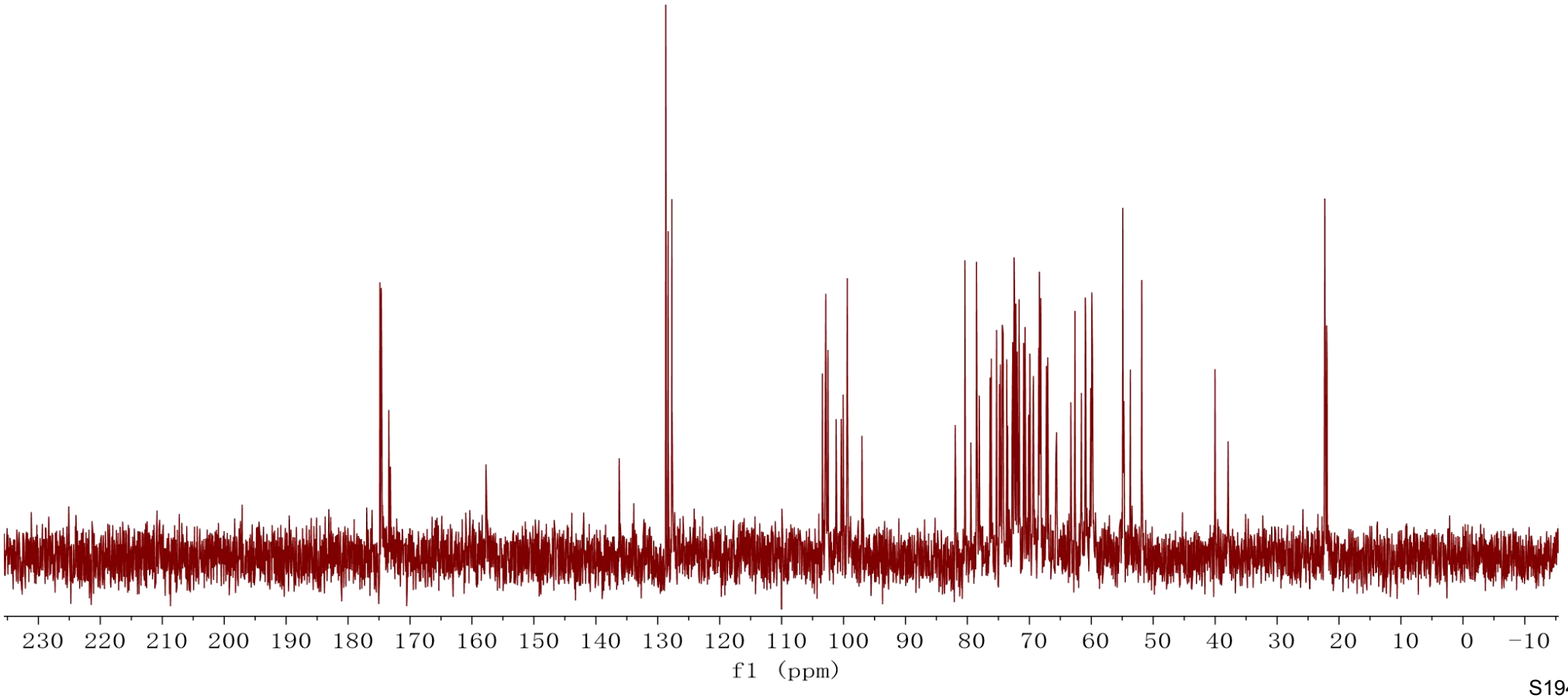

α6
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3

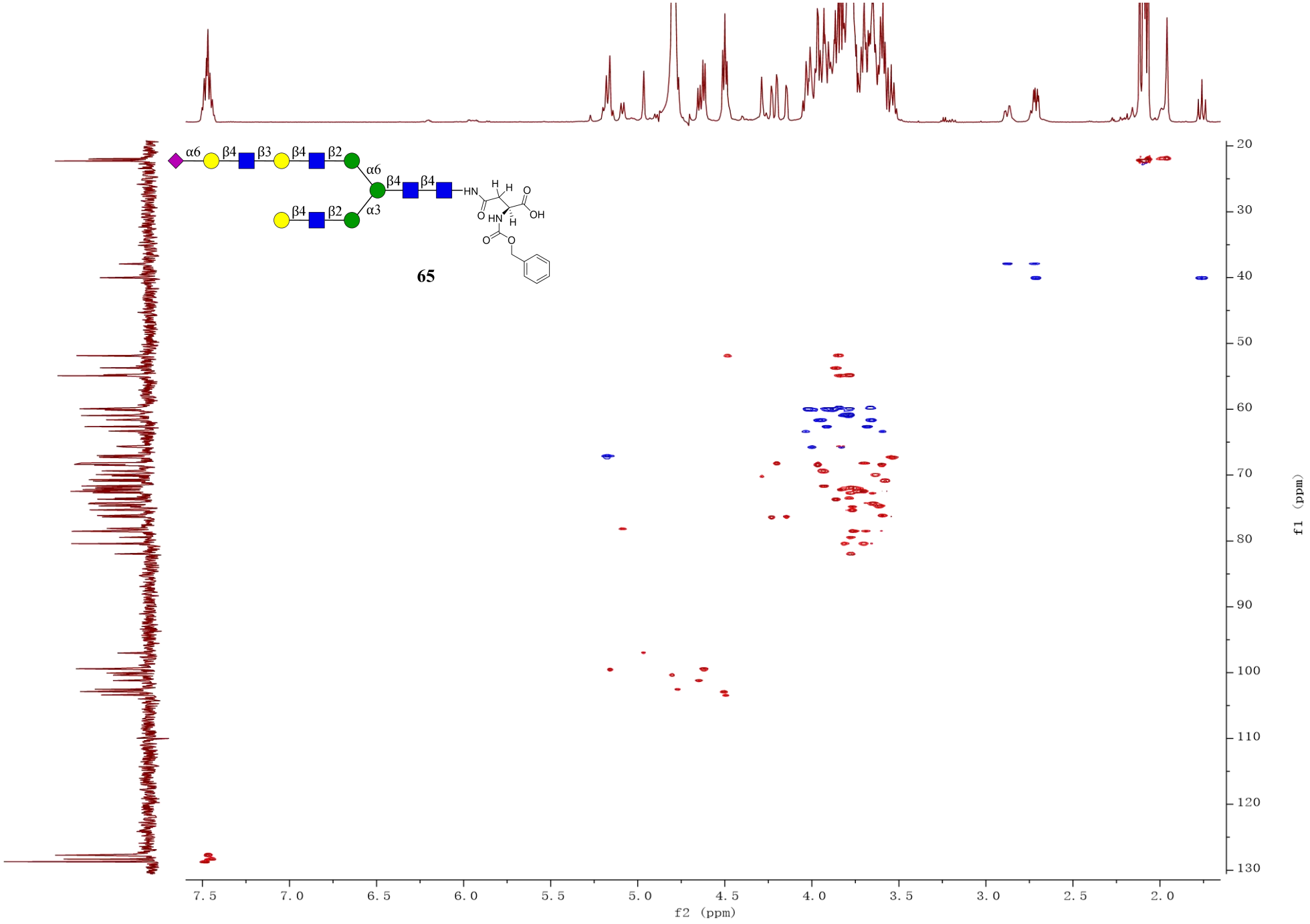
65

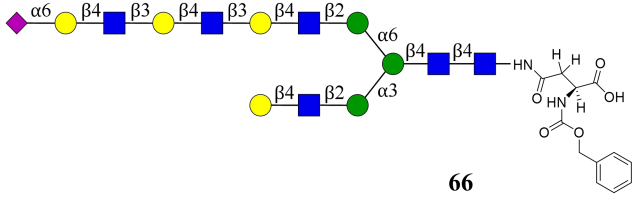
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


α6
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
O
HN
H
OH
β4
β2
α3
O
O

65

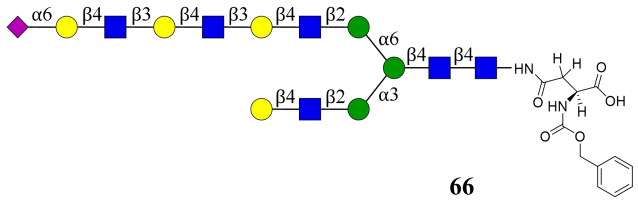

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


α6
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3
65
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

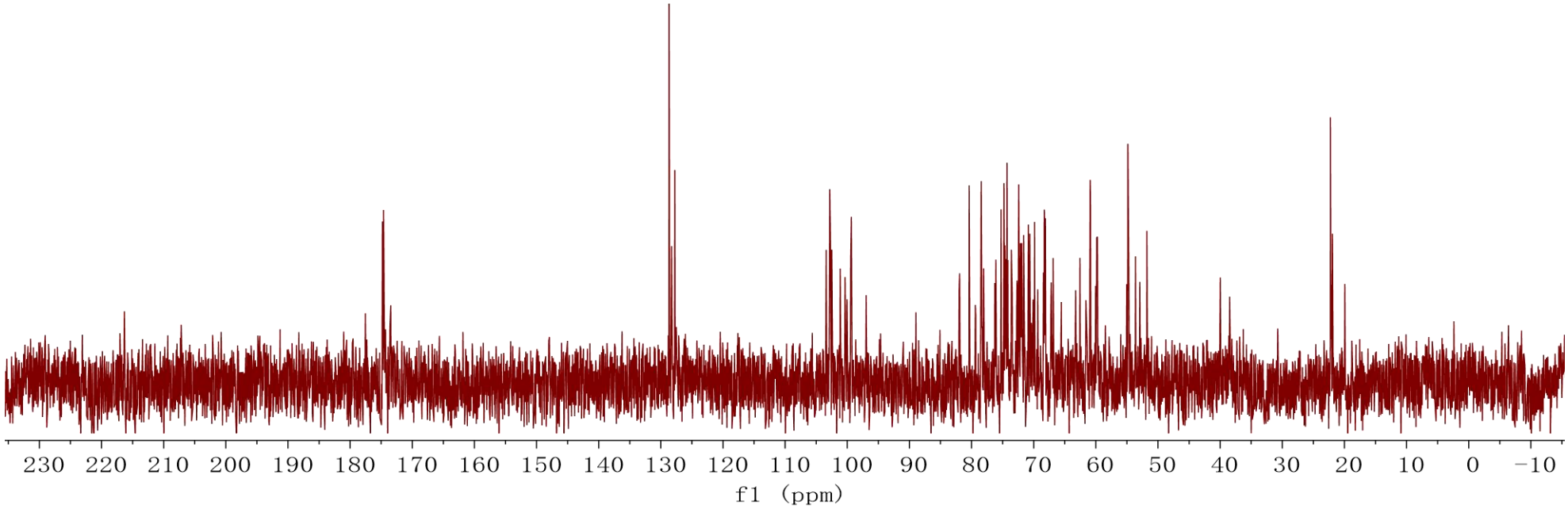

α6
β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
O
OH
HN
H
O
O
β4
β2
α3

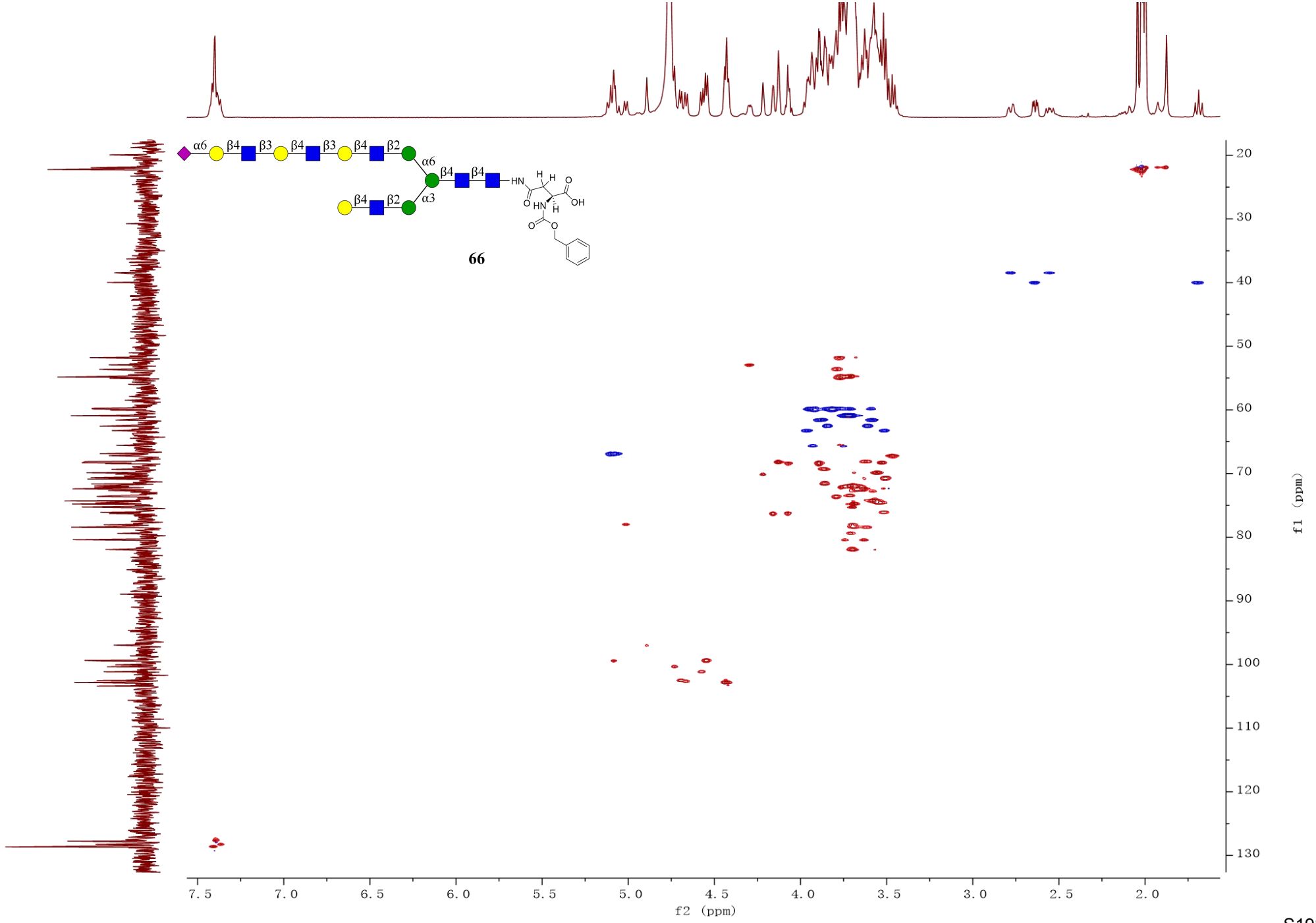
66

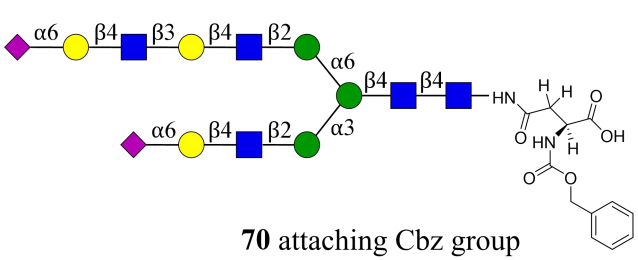
3.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


α6
β4
β3
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
O
OH
HN
H
O
O
β4
β2
α3

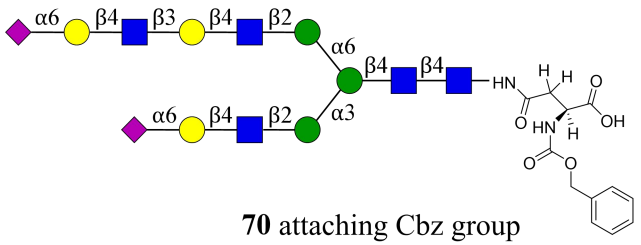
66


230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

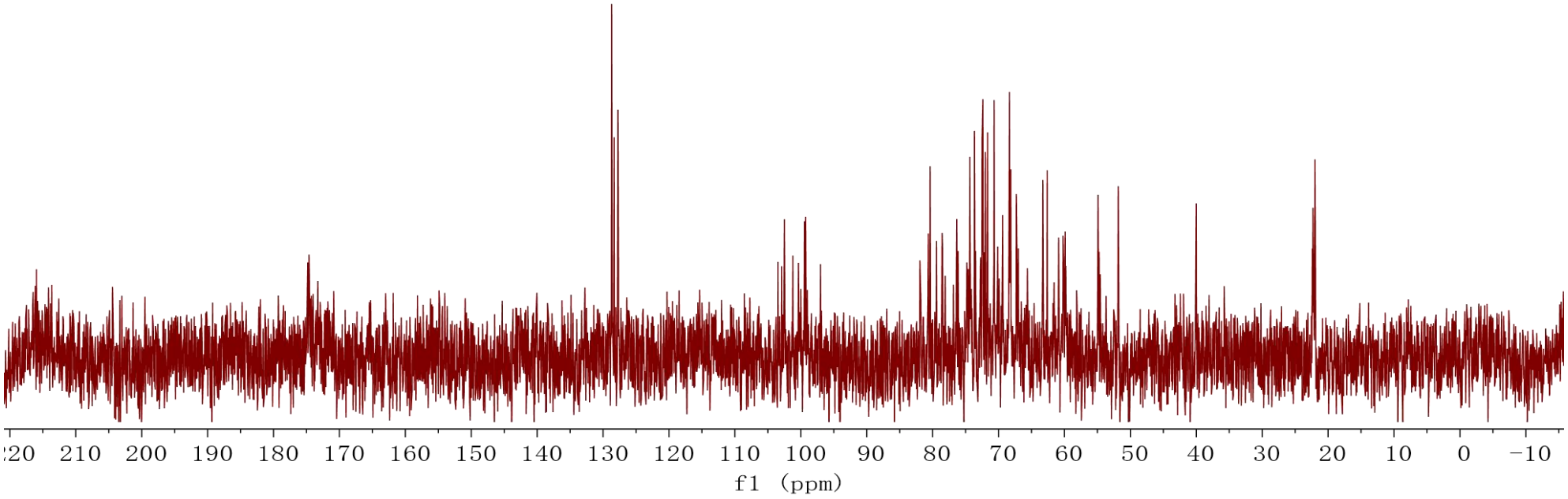

α6 β4 β3 β4 β3 β4 β2
α6 β4 β4 HN
β4 β2 α3
66
f2 (ppm)
f1 (ppm)

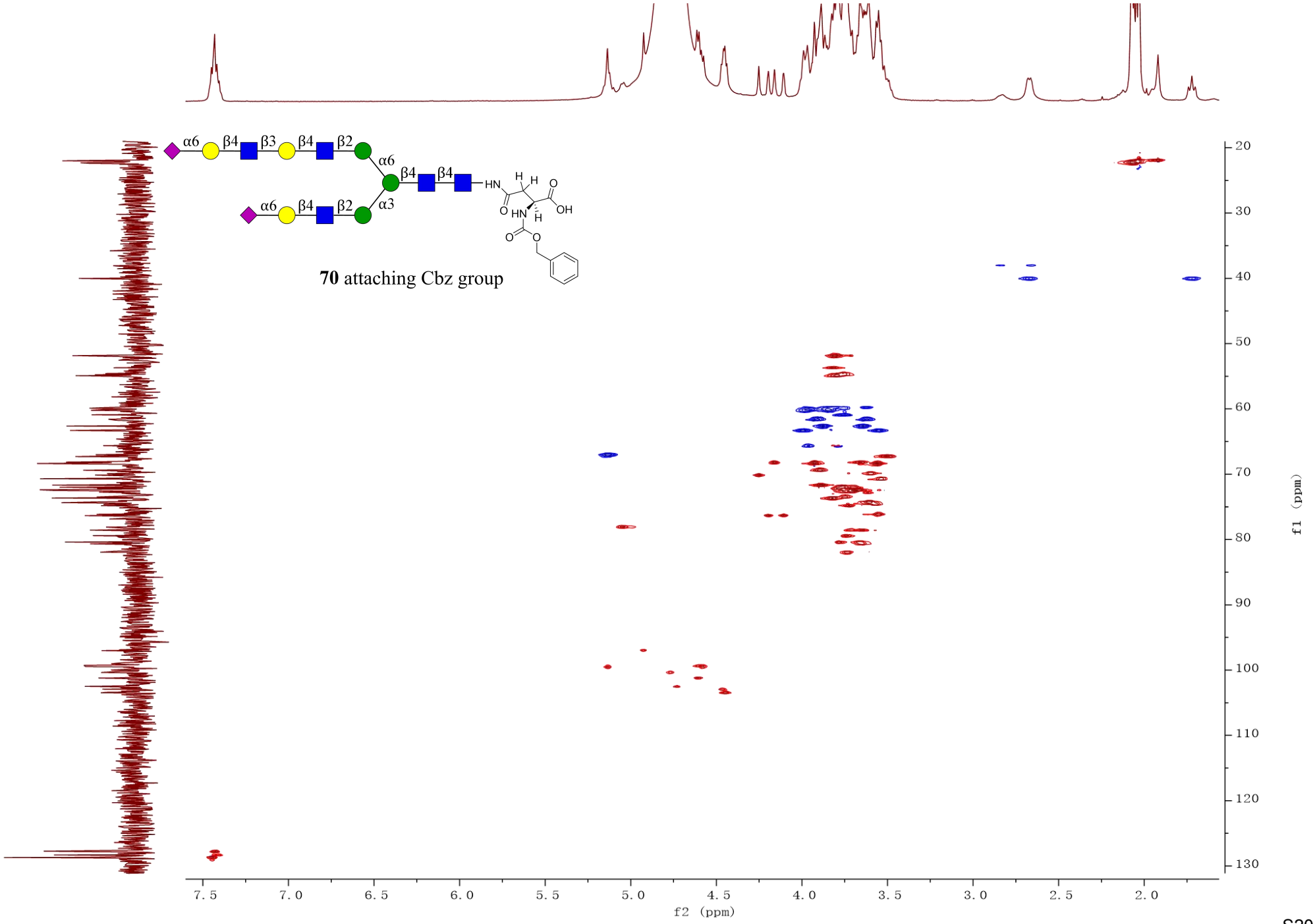


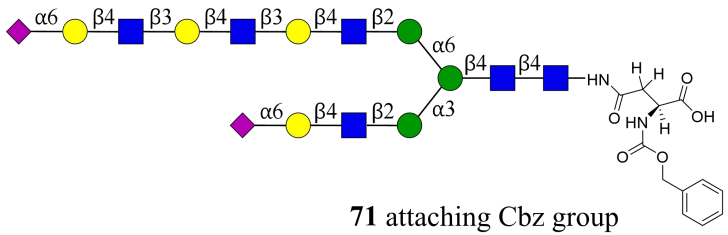
70 attaching Cbz group



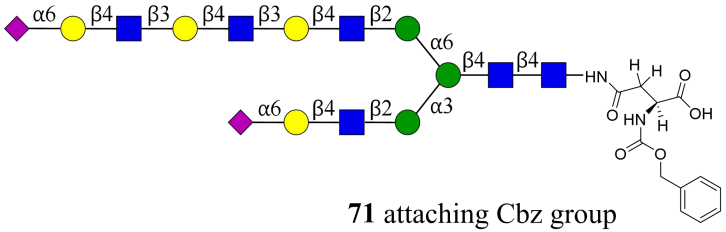
70 attaching Cbz group



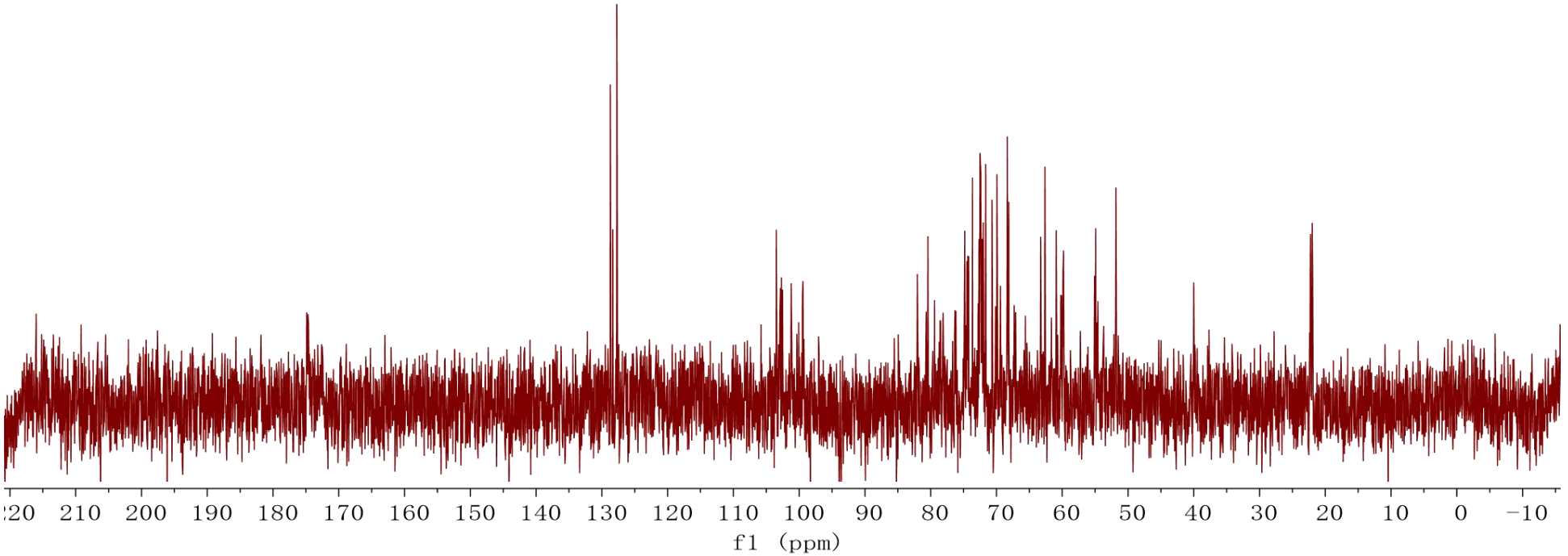

α6
β4
β3
β4
β2
α6
β4
β4
HN
H
H
O
OH
α6
β4
β2
α3
O
HN
H
O
O
70 attaching Cbz group
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

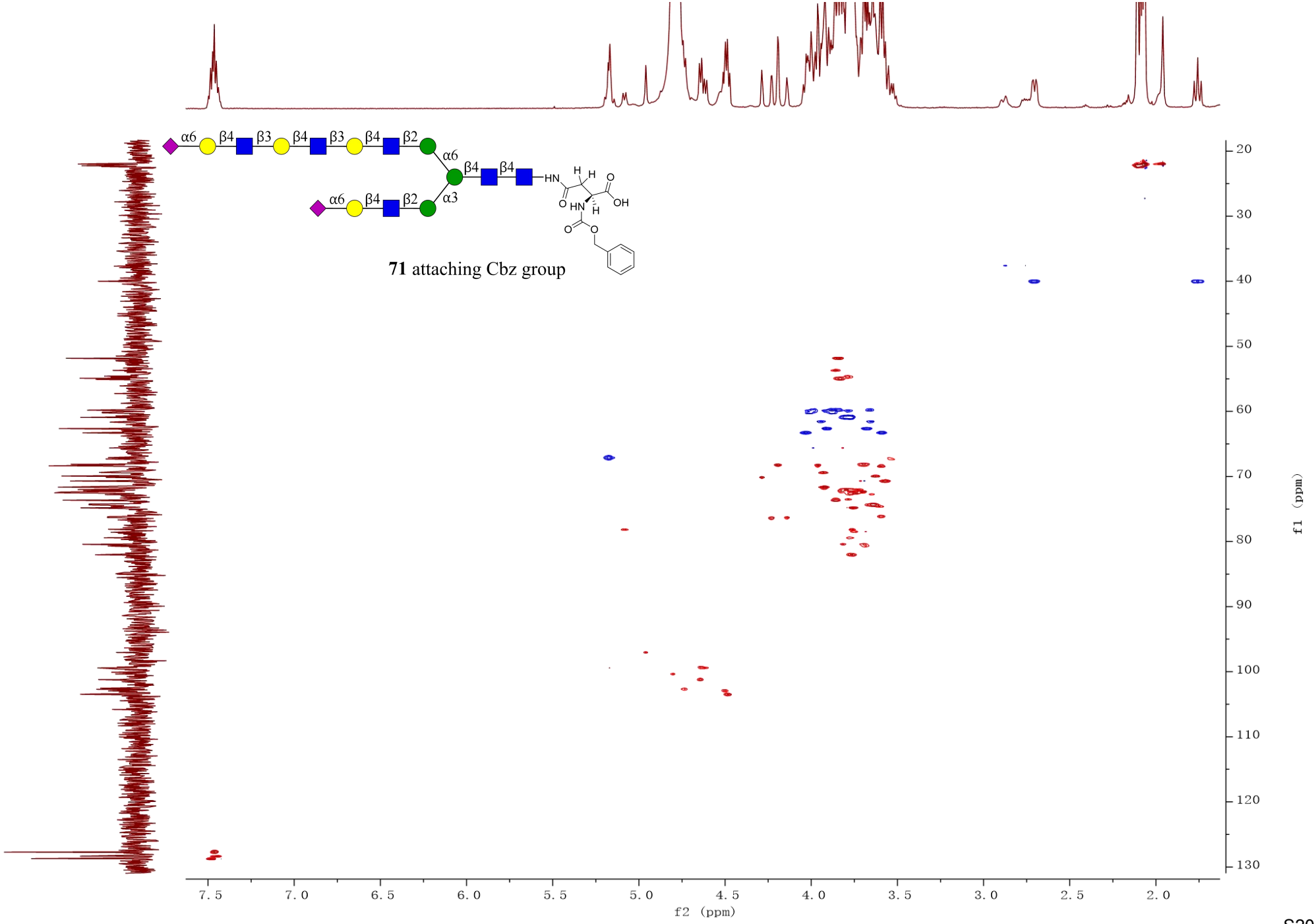


71 attaching Cbz group

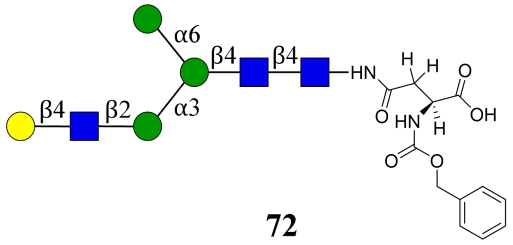


71 attaching Cbz group



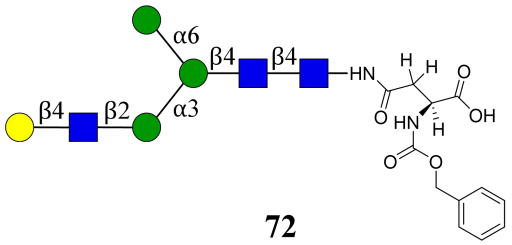


71 attaching Cbz group


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3

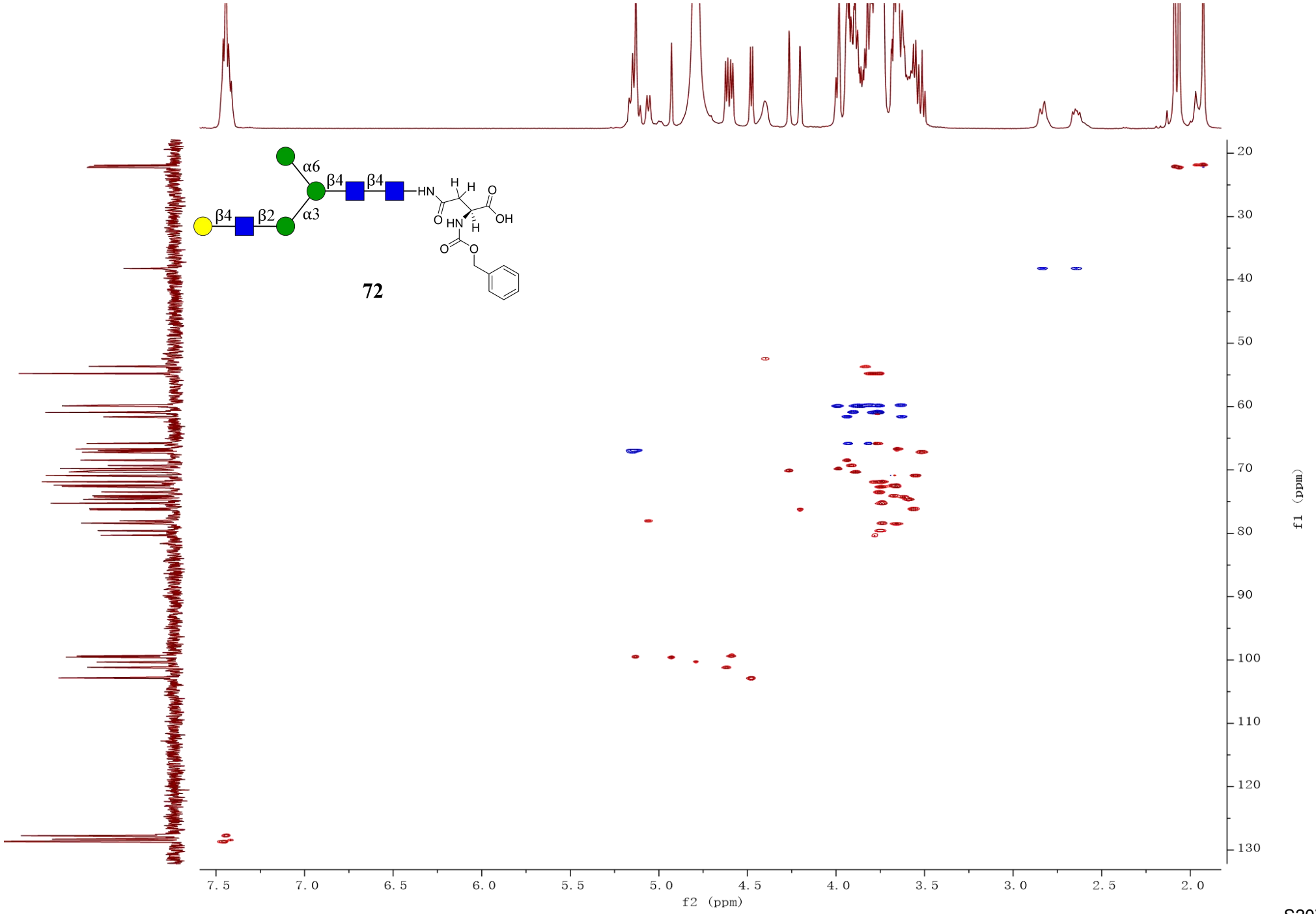
72

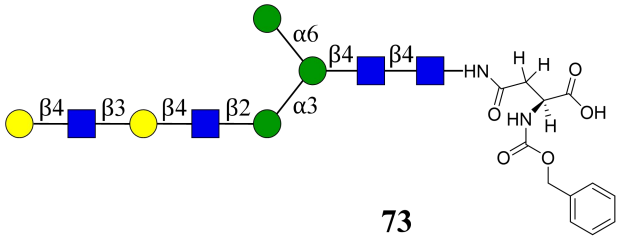
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
β4
β2
α3
O
HN
H
O
O

72

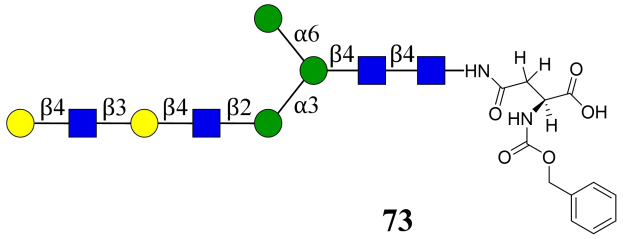
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β2
α3
72
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β2
α3

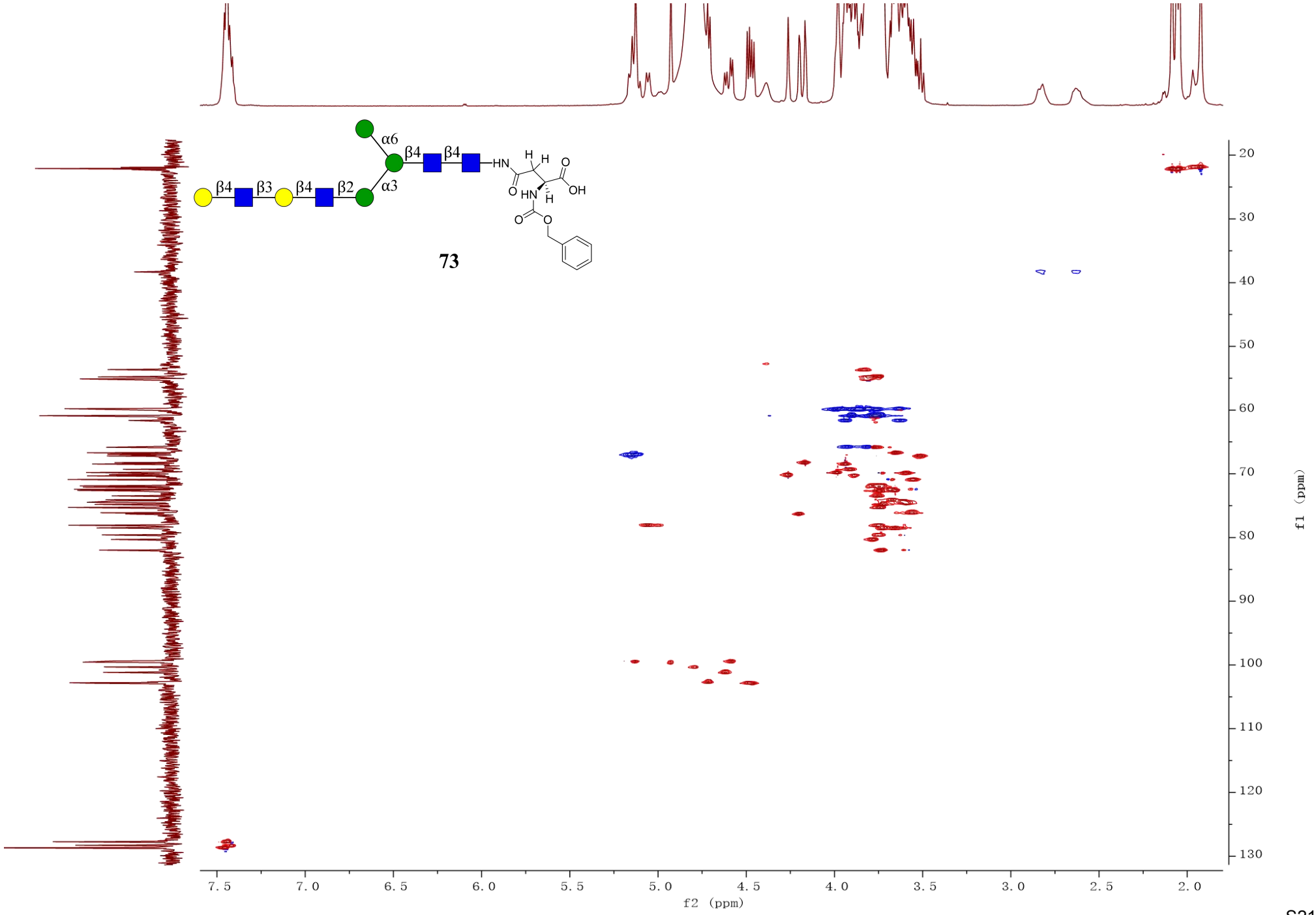
73

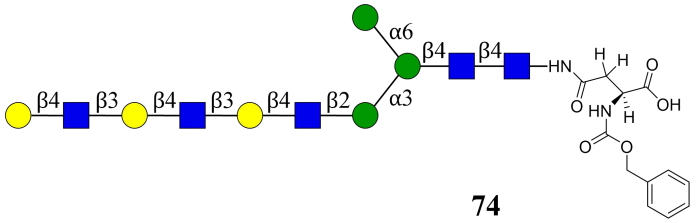
3.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
β4
β3
β4
β2
α3
O
HN
H
O
O

73

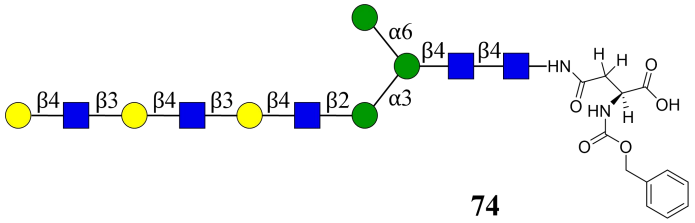
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β2
α3
73
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

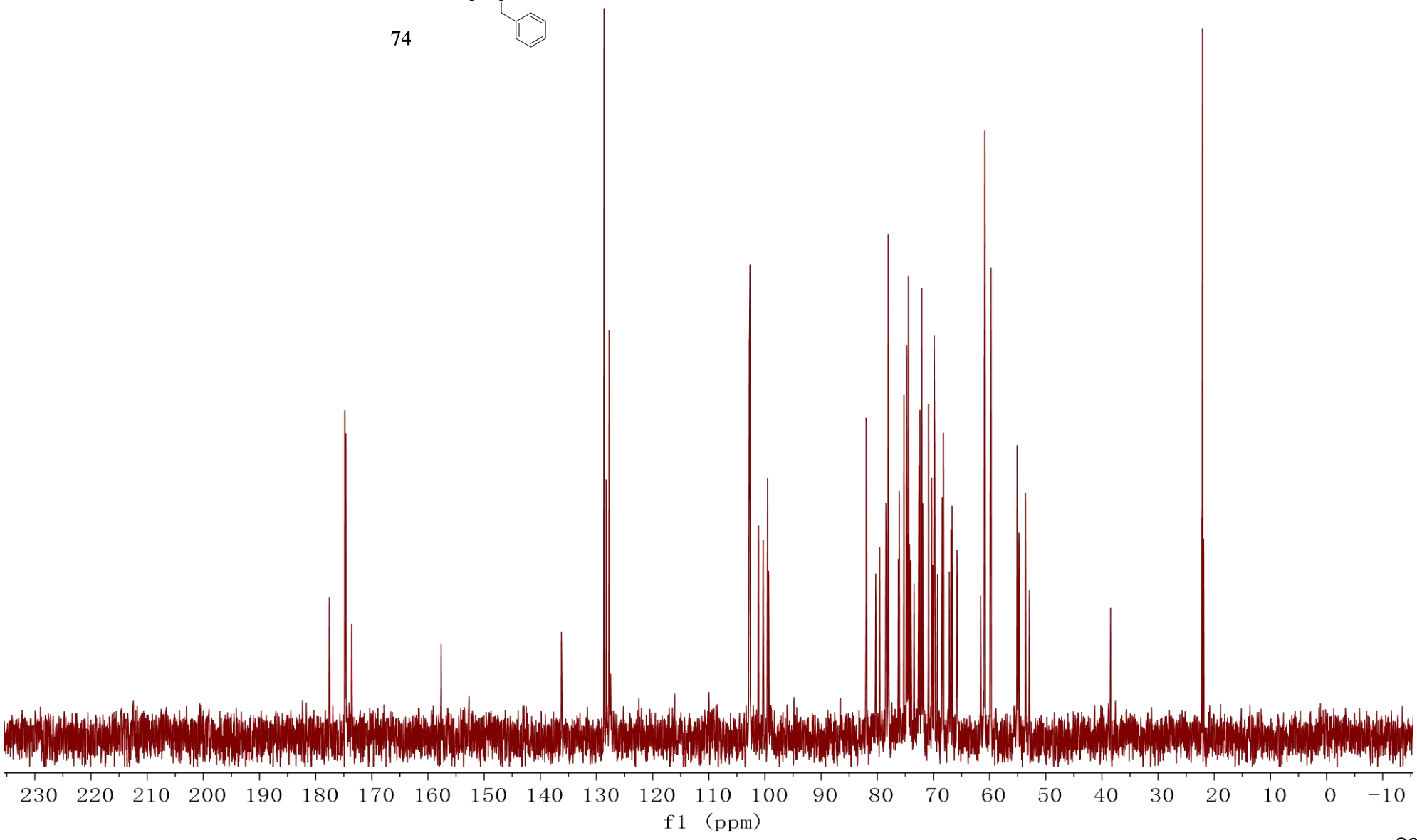

α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
β4
β3
β4
β3
β4
β2
α3

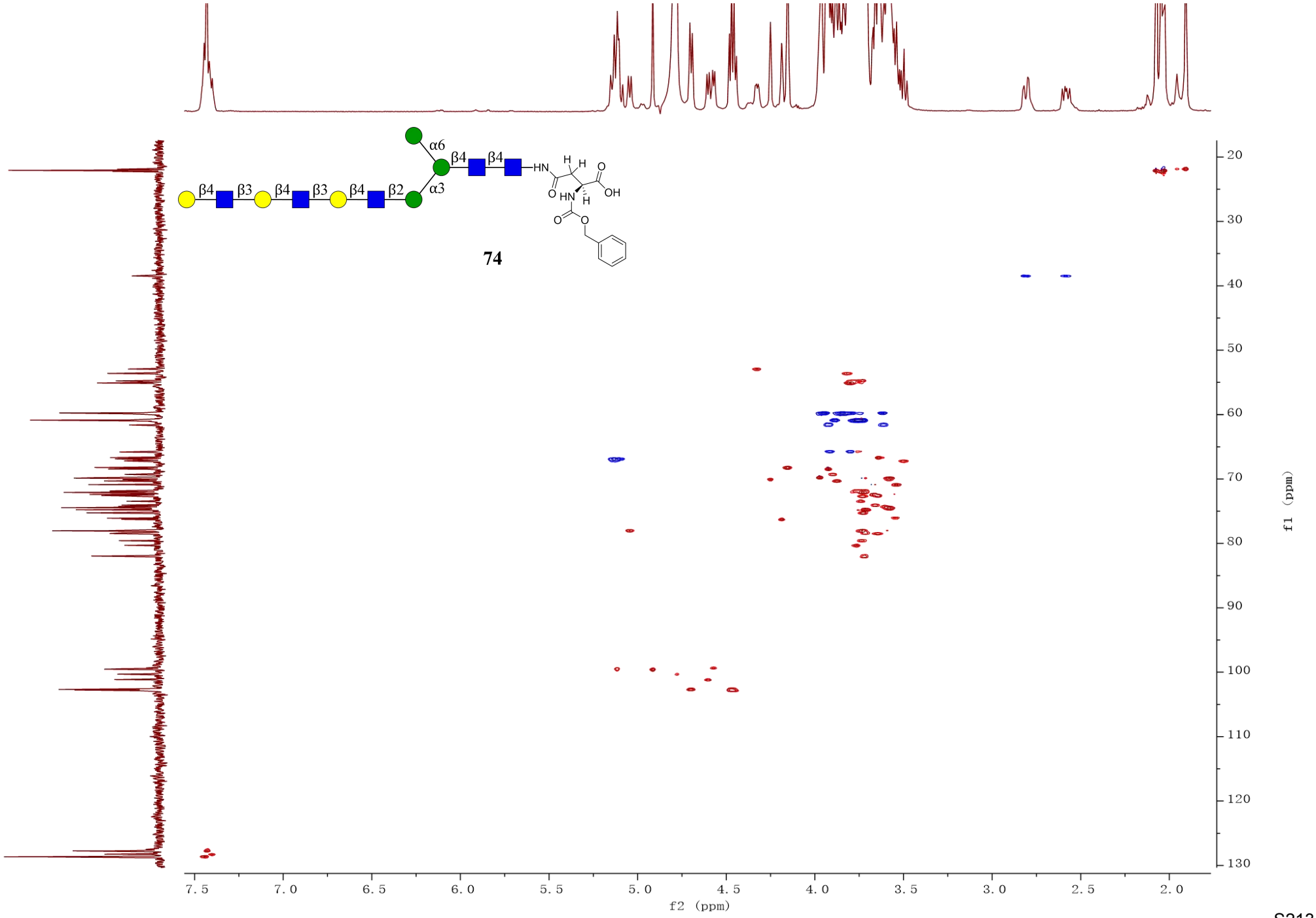
74

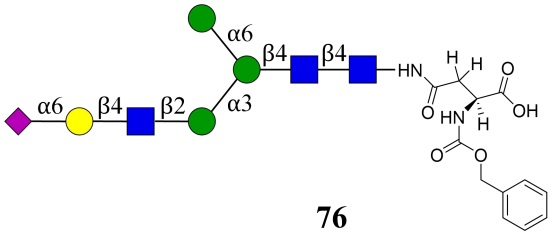
7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
1.
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
O
β4
β3
β4
β3
β4
β2
α3

74

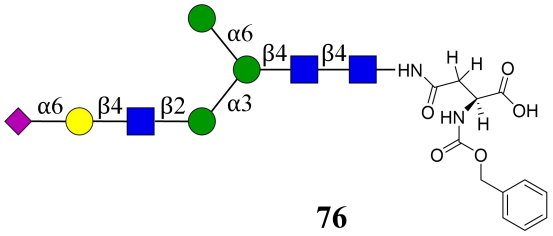

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


α6
β4
β4
HN
β4
β3
β4
β3
β4
β2
α3
74
f2 (ppm)
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β2
α3

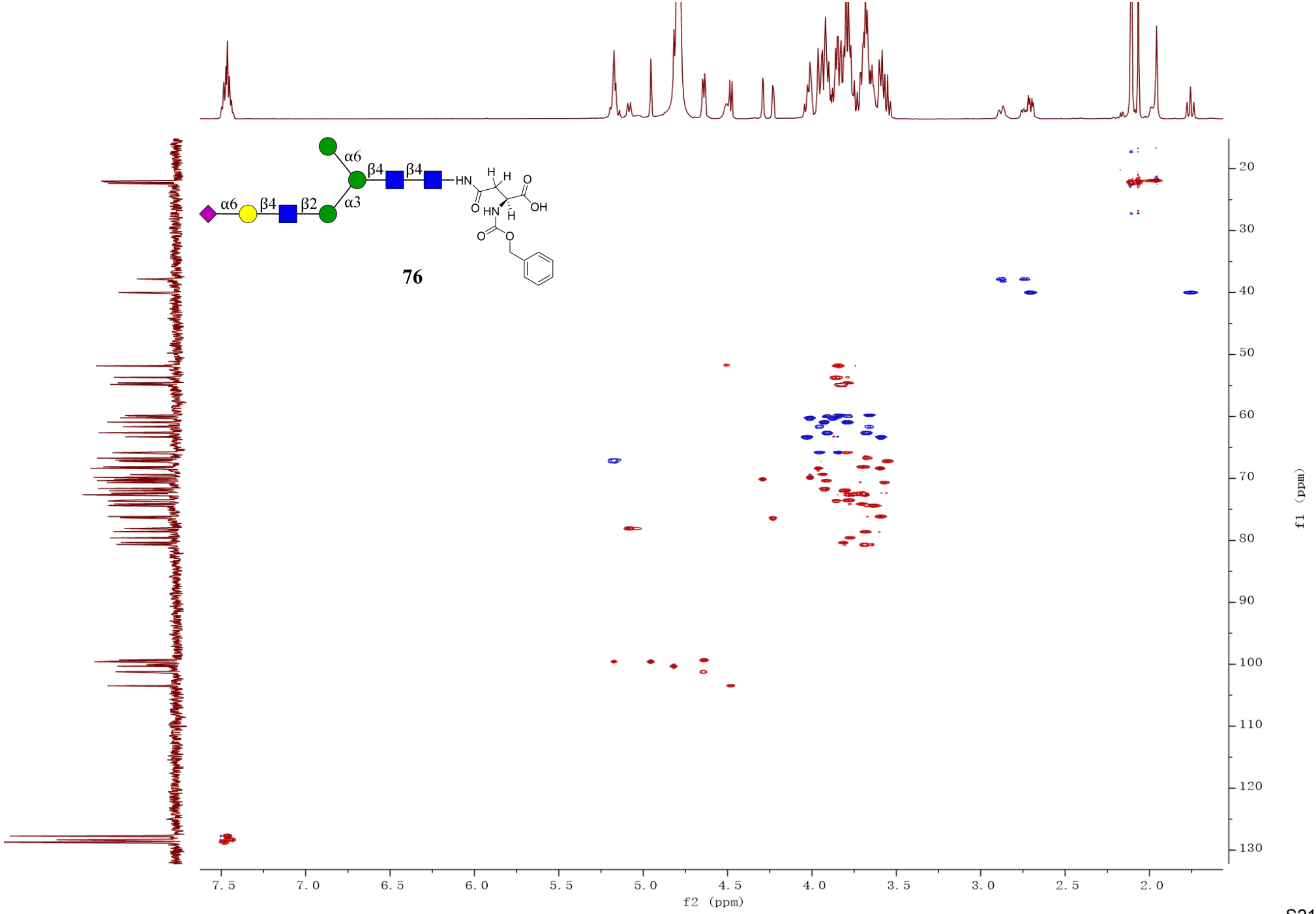
76

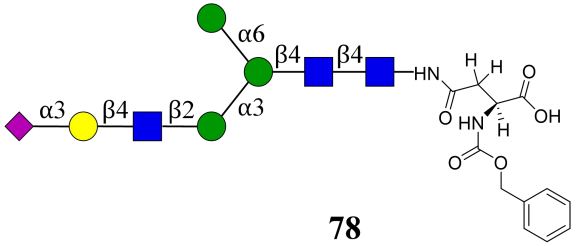
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β2
α3

76

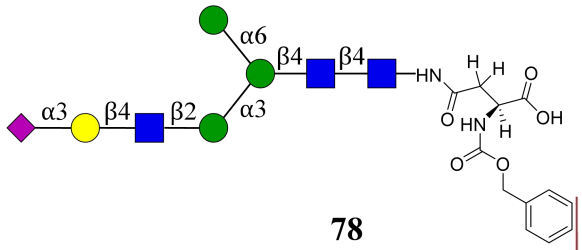
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β2
α3
76
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

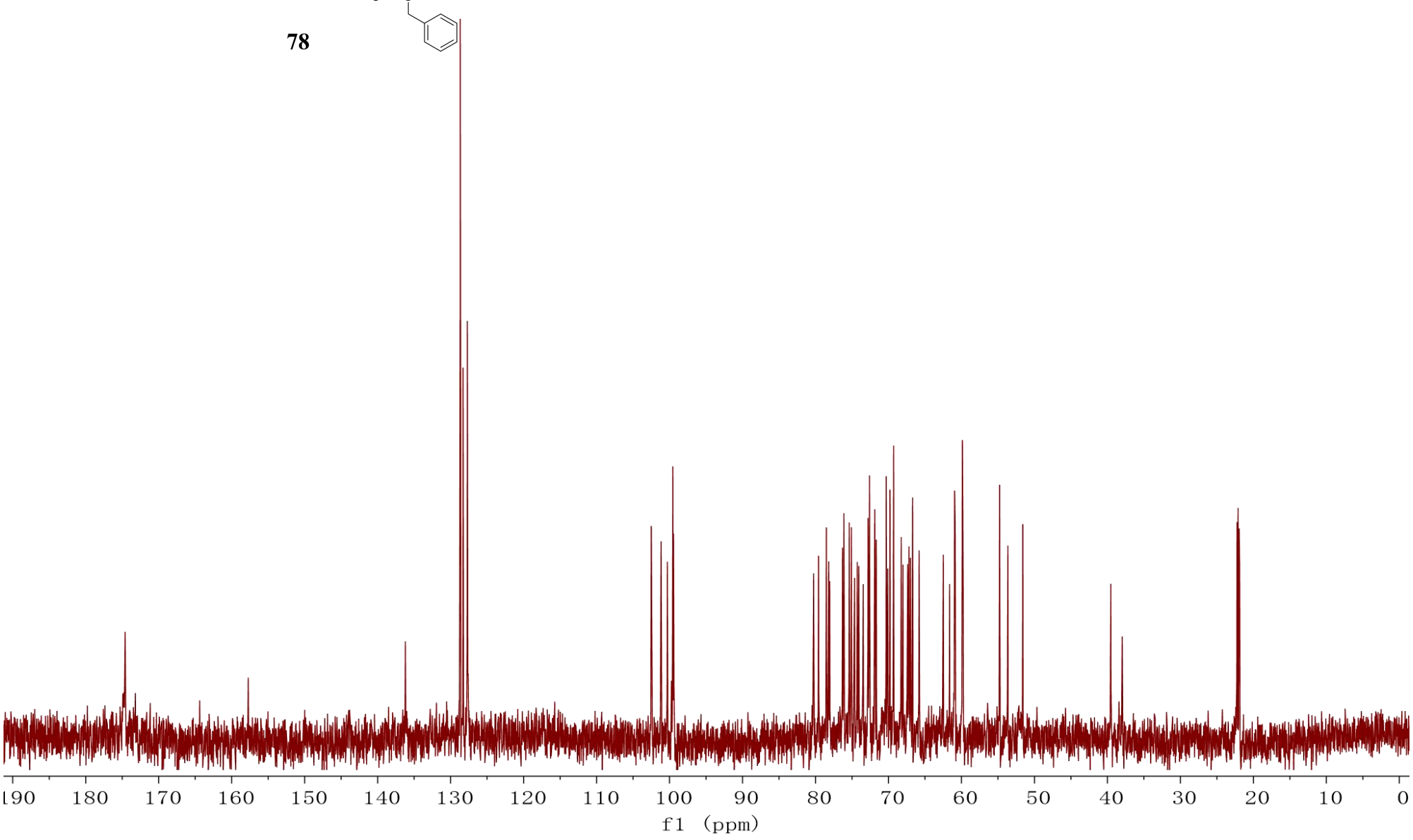

α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α3
β4
β2
α3

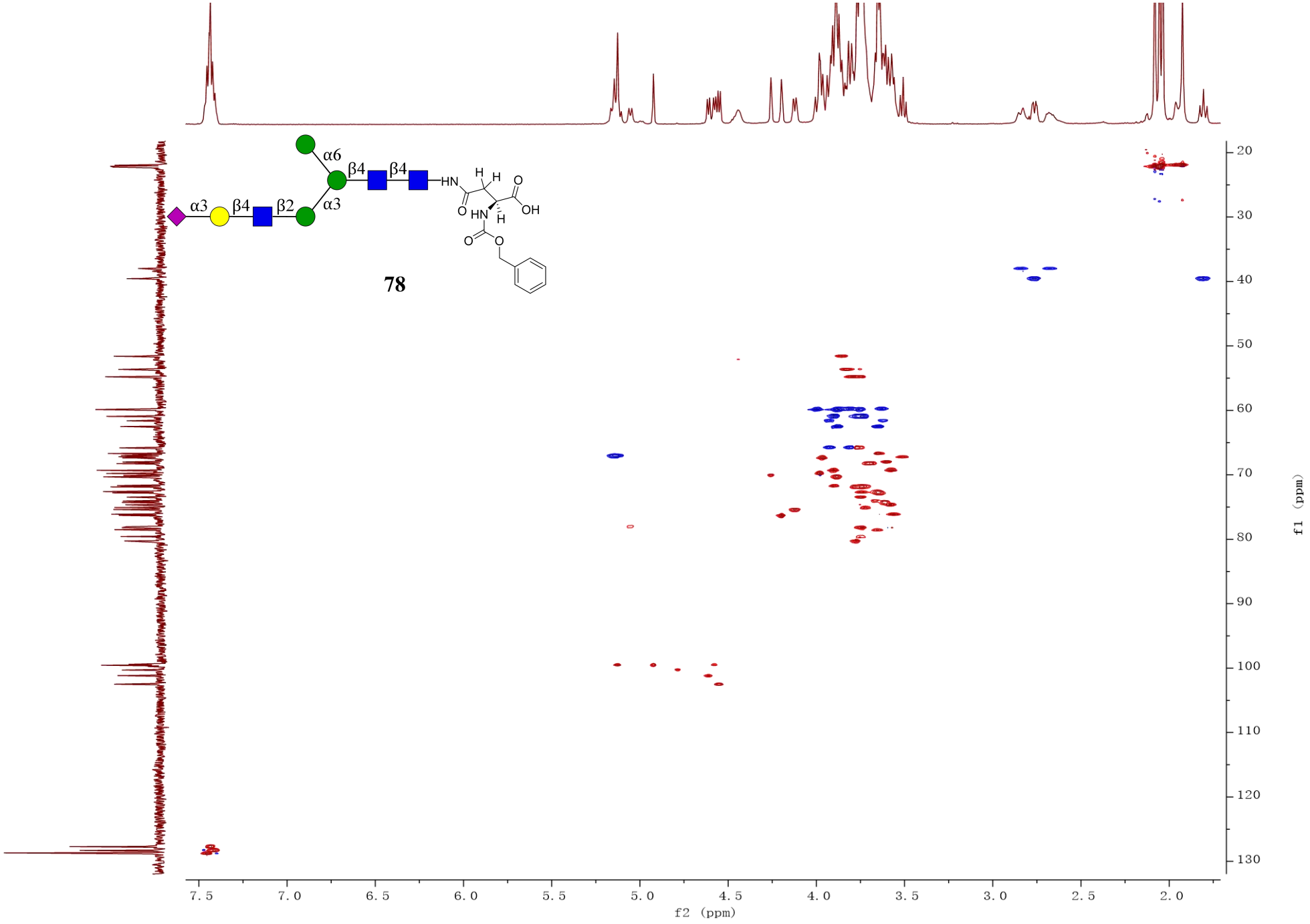
78

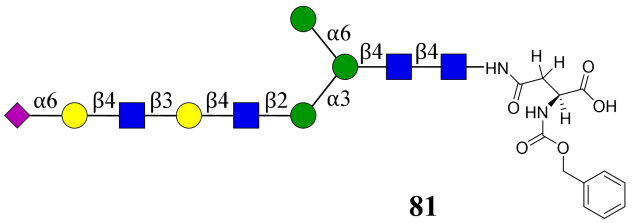
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α3
β4
β2
α3

78

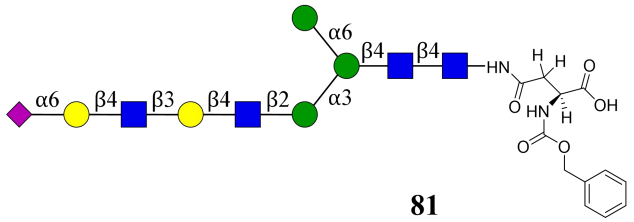

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


α6
β4
β4
HN
α3
β4
β2
α3
78
f2 (ppm)
f1 (ppm)

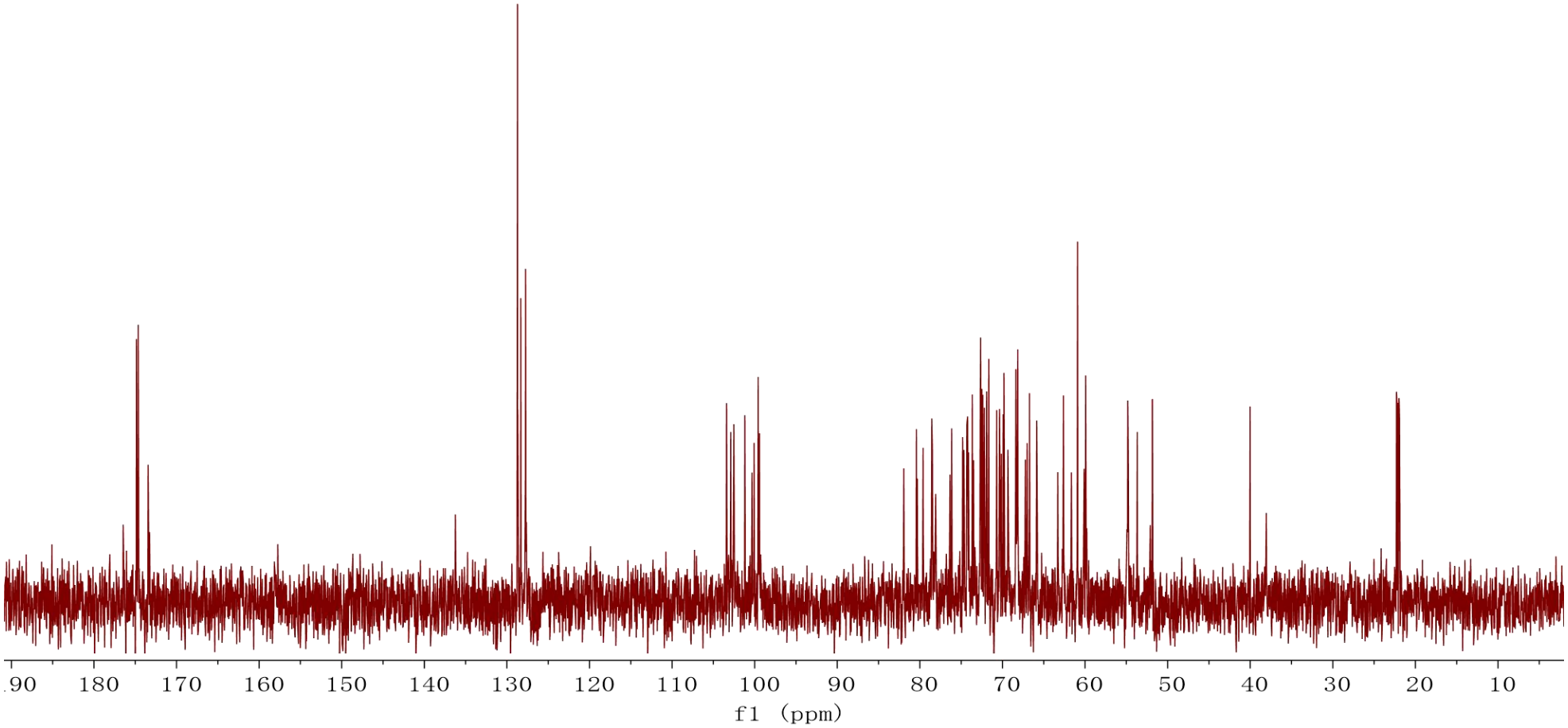

α6
β4
β4
HN
H
H
O
OH
α6
β4
β3
β4
β2
α3
O
HN
H
O
O

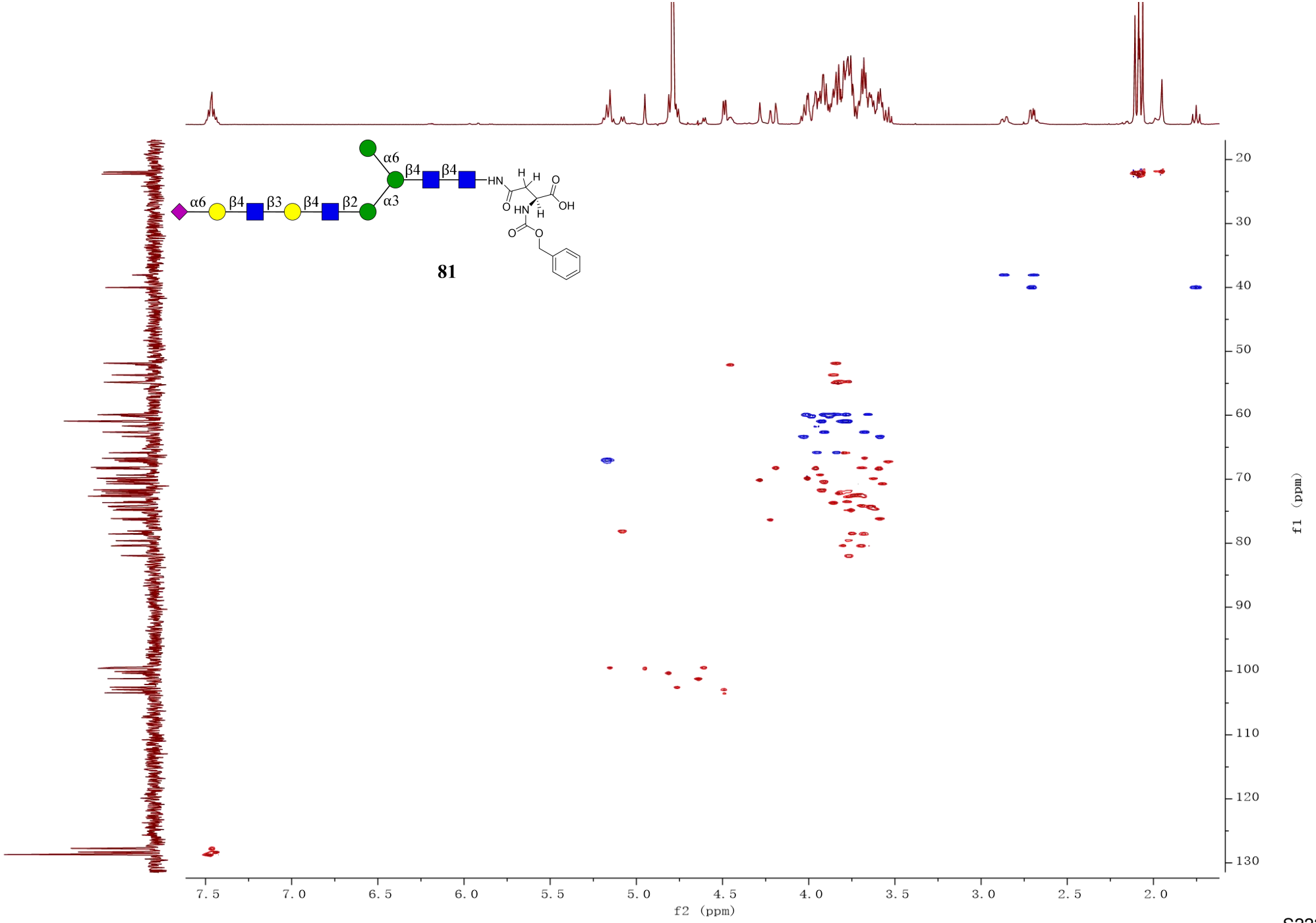
81

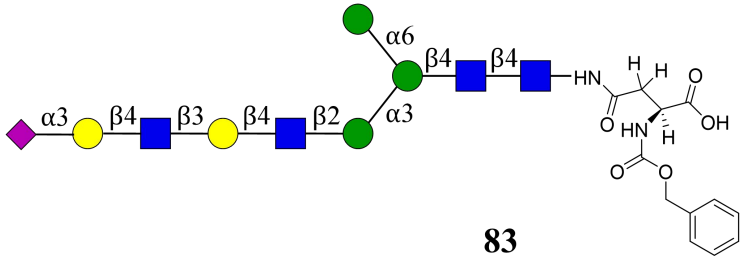
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
α6
β4
β3
β4
β2
α3
O
HN
H
O
O

81

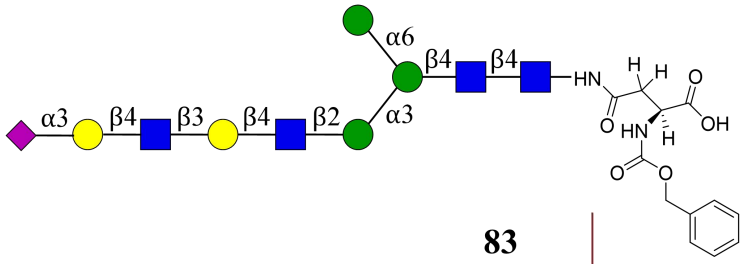

.90
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β2
α3
81
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

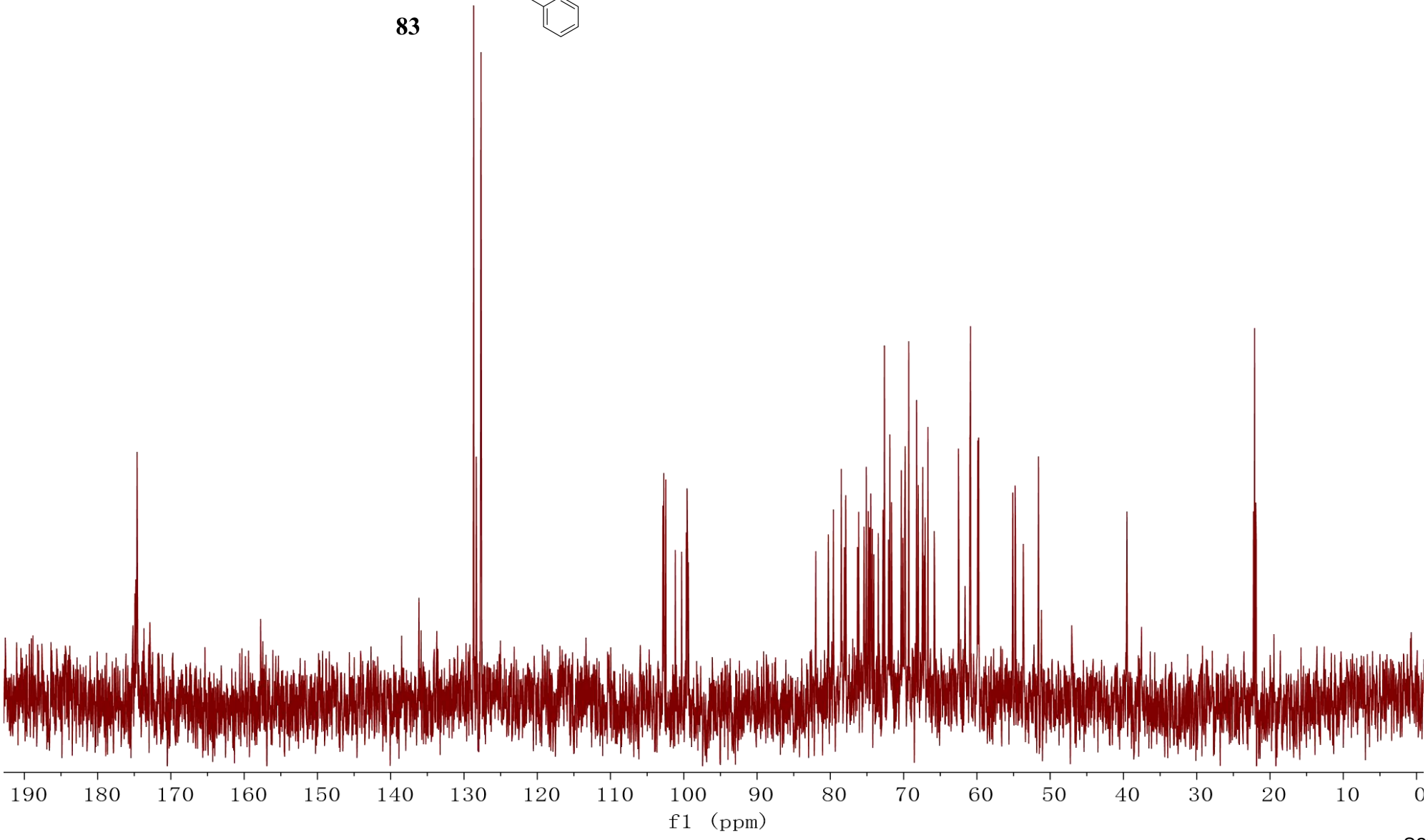

α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α3
β4
β3
β4
β2
α3

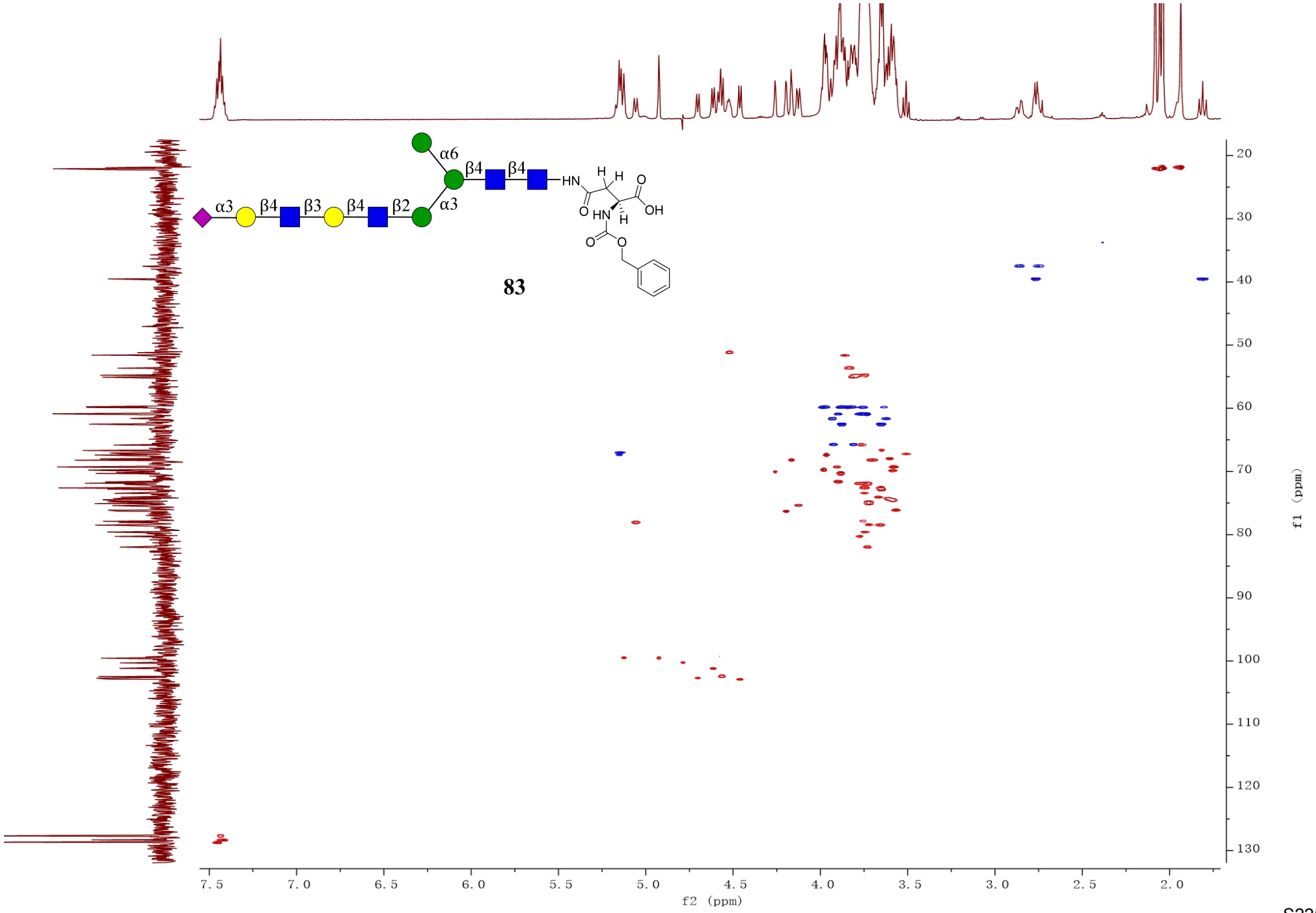
83

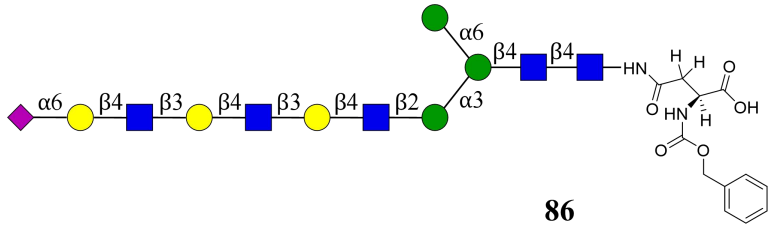
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α3
β4
β3
β4
β2
α3

83

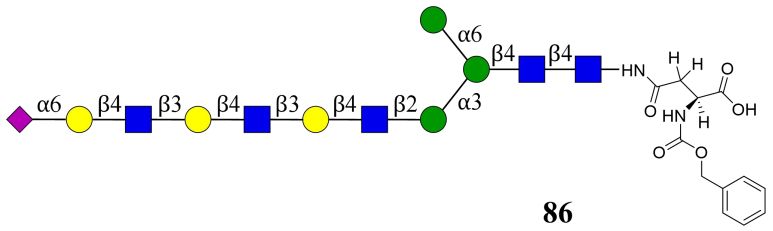

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


α6
β4
β4
HN
H
H
O
O
OH
O
HN
H
O
O
α3
β4
β3
β4
β2
α3
83
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

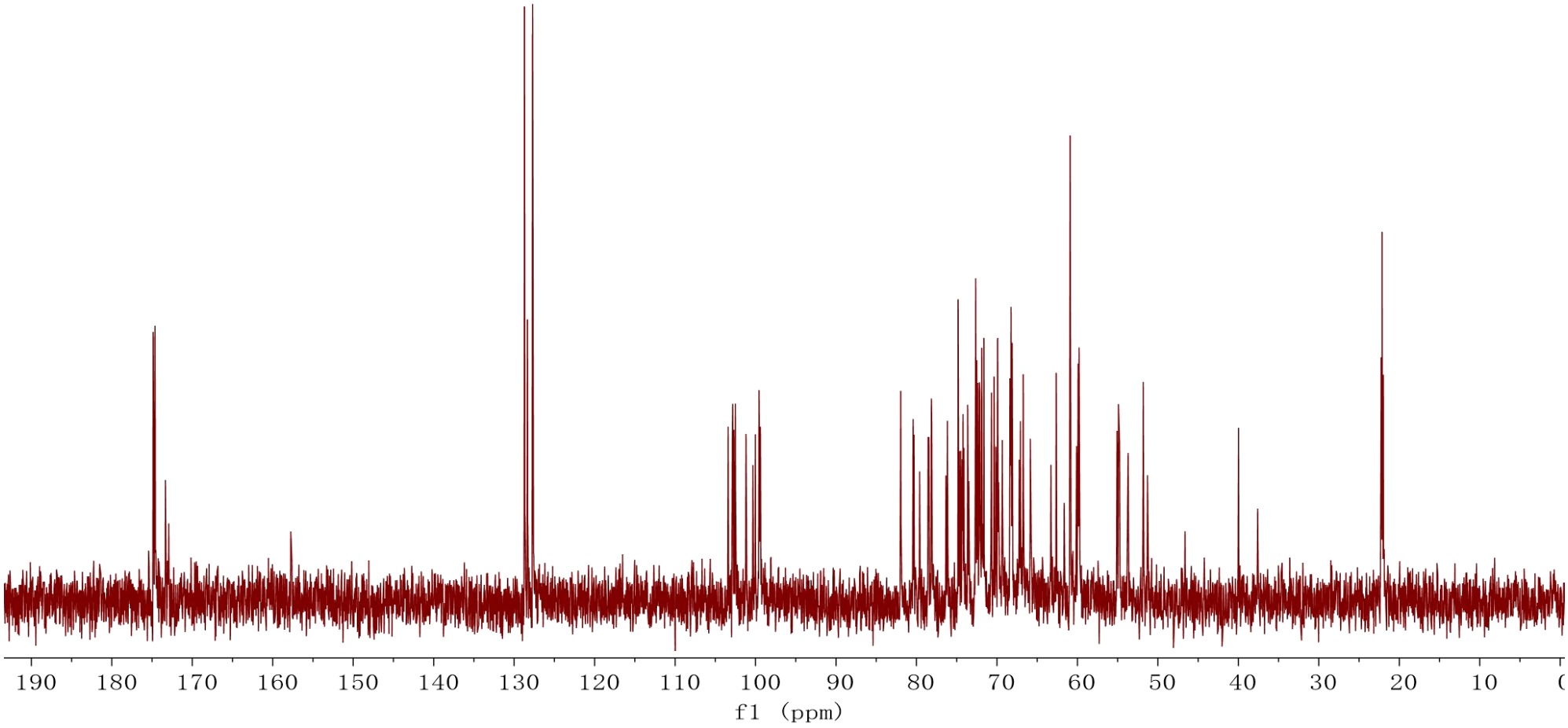

α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β3
β4
β2
α3

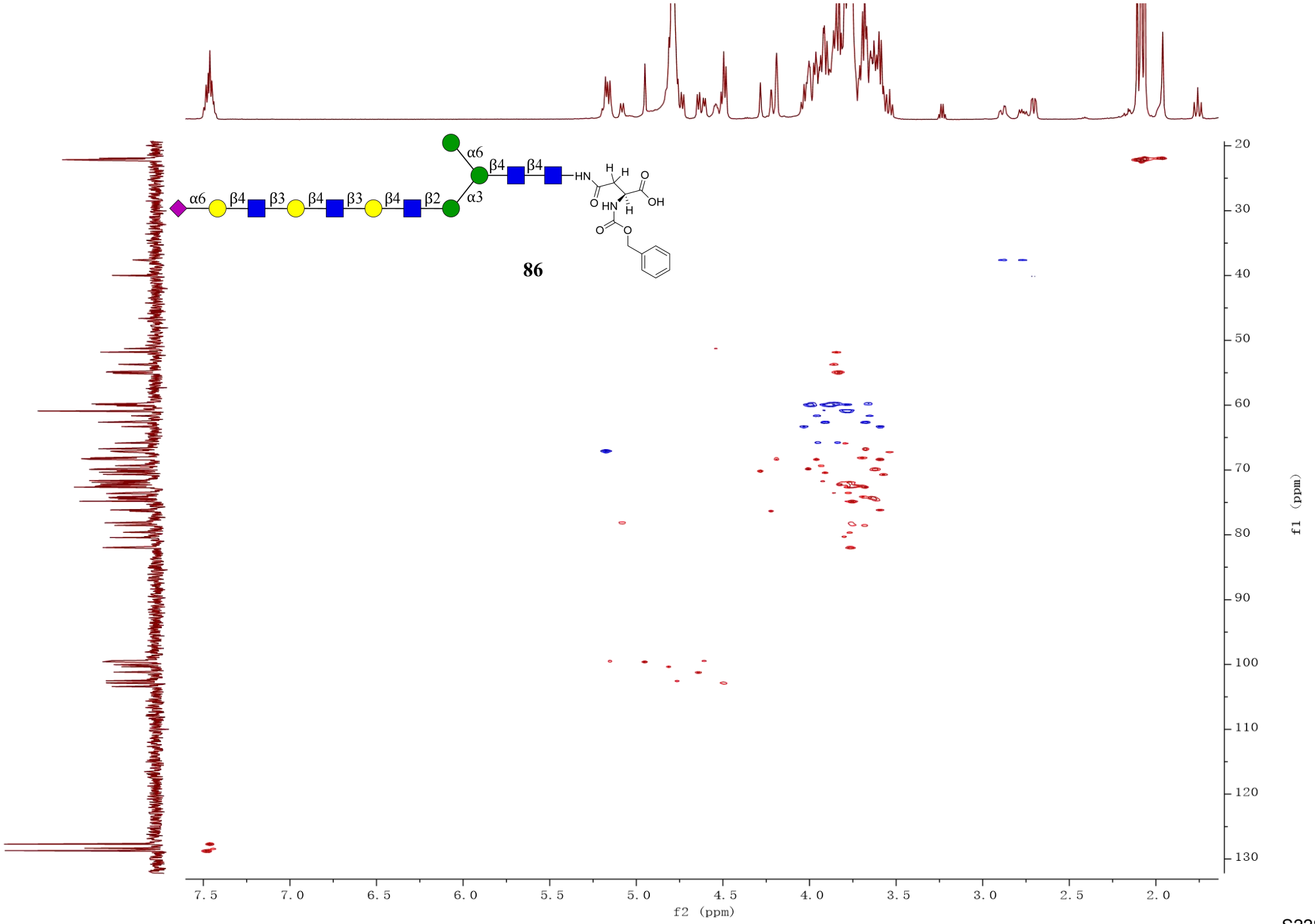
86

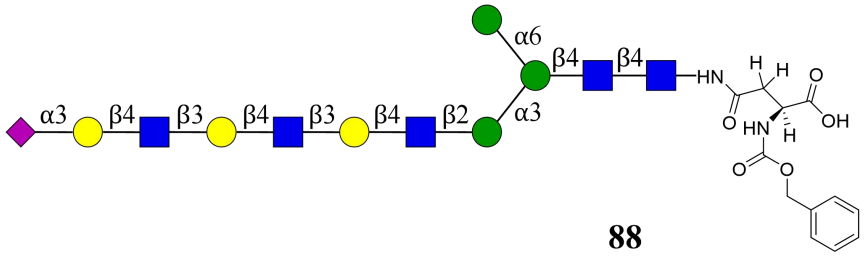
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β3
β4
β2
α3

86

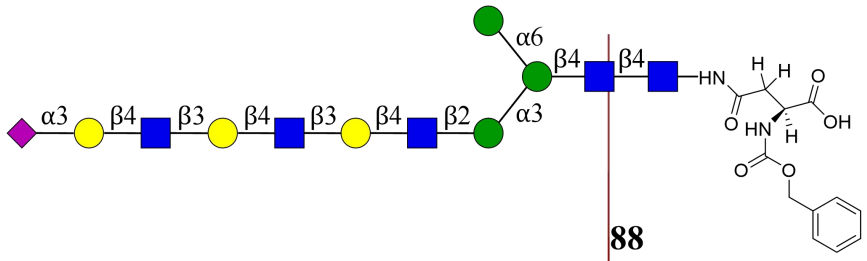

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
O
HN
H
O
O
α6
β4
β3
β4
β3
β4
β2
α3
86
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

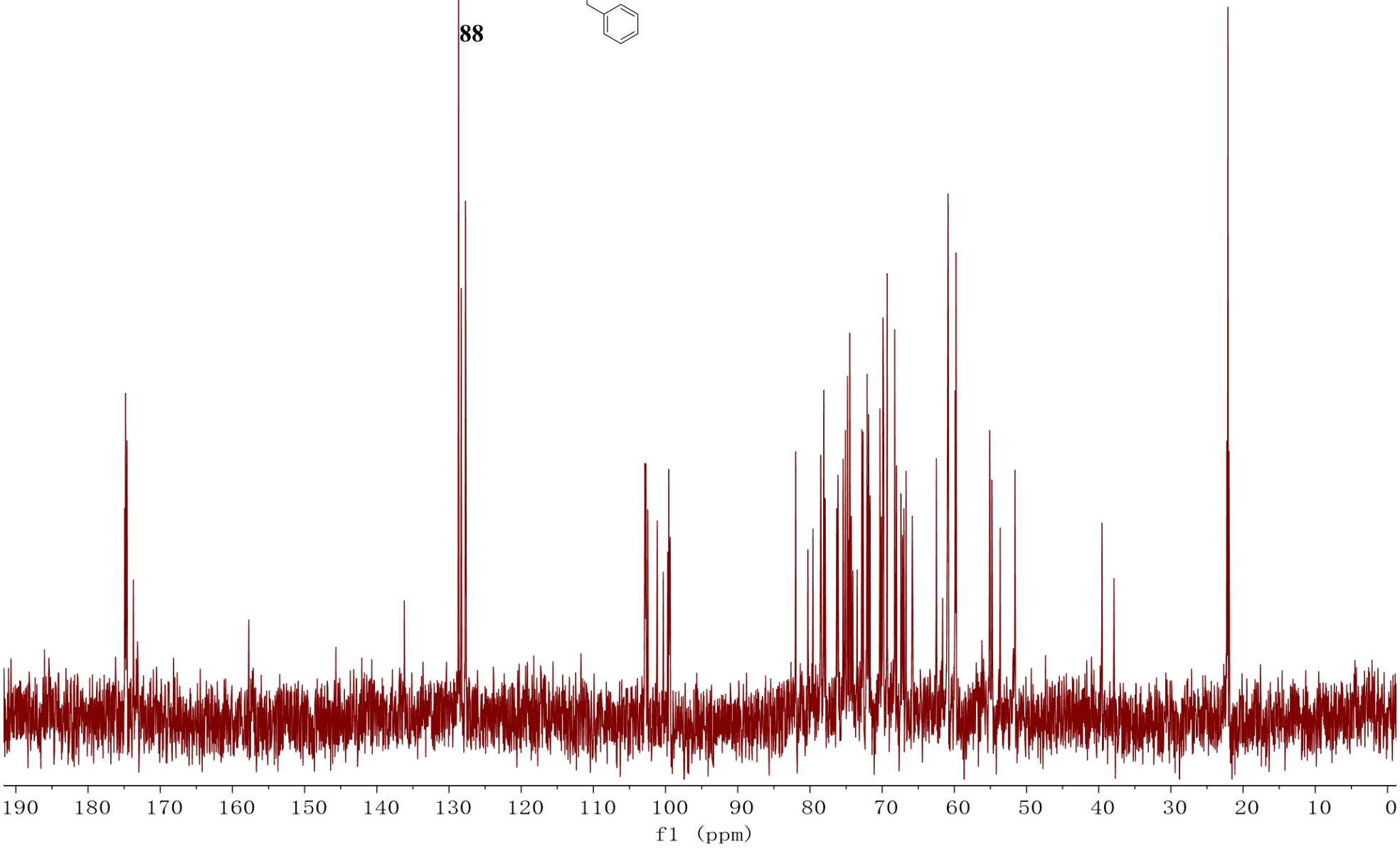

α6
β4
β4
HN
H
H
O
OH
α3
β4
β3
β4
β3
β4
β2
α3
HN
H
O
O

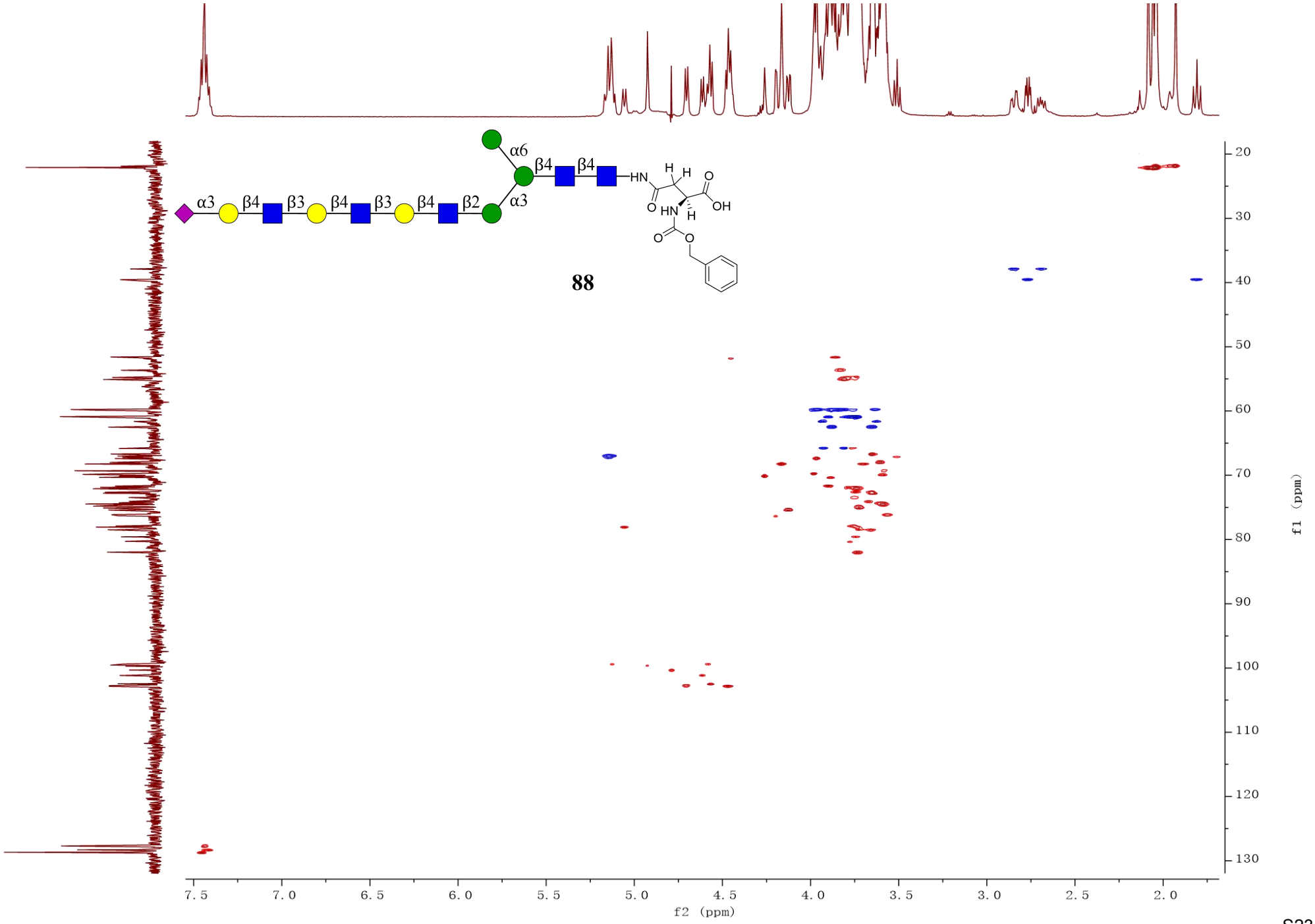
88

8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


α6
β4
β4
HN
H
H
O
OH
α3
β4
β3
β4
β3
β4
β2
α3
HN
H
O
O
O

88


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


α6
β4
β4
HN
α3
β4
β3
β4
β3
β4
β2
α3
O
OH
HN
O
O
88
20
30
40
50
60
70
80
90
100
110
120
130
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)