Electronic Supplementary Information

for

Highly efficient biocatalytic cascade for the diversity-oriented synthesis of complex blood group Sd^a antigens

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Contents

1. Green chemistry metrics

In order to evaluate the "greenness" of the preparation processes, several metrics of green chemistry were calculated according to the equations below (table S1). The atom economy (AE) is a parameter which measures the amount of the reactants remain in the end products.[1] The Curzons reaction mass efficiency (RME) is the ratio of actual mass of final product to the mass of all reactants used.[2] It takes into consideration of both atom economy and chemical yield. However, the AE and Curzons RME only give information about the "greenness" of the reaction, but not of a process, therefore cannot reflect the waste and energy issues in the process. The Andraos RME accounts for all the materials involved in the chemical process, including the mass of catalysts, solvents, work-up and purification materials.[3] The environmental factor (*E*-factor) is the ratio of the mass of waste produced in the process to mass of final product, which incorporate yield, stoichiometry and solvent usage.[4] The effective mass yield is defined by the ratio of the mass of the desired product to the mass of all non-benign reactant, solvent, and catalyst, which have environmental risks.[5] The calculation of EcoScale takes into account the yield, cost, safety, technical set-up, energy and purification aspects, providing a general and simple parameter of the "greenness" of the whole preparation processes.[6]

Table S1 The green chemistry metrics and their calculations

Metrics	Expression	Aim	Ideal value
Atom economy (AE)[1]	$\frac{\text{molecular weight of the product}}{\text{sum of molecular weight of all the stoichiometric reagents}}$	Increase	1
Curzons reaction mass efficiency (RME)[2]	$\frac{\text{mass of the product}}{\text{total mass of all the reactants}}$	Increase	1
Andraos reaction mass efficiency (RME)[3]	$\frac{\text{mass of the product}}{\text{total mass of all input material relevant to the reaction}}$	Increase	1
Sheldon environmental factor (*E*-factor)[4]	$\frac{\text{mass of the waste}}{\text{mass of the product}}$	Decrease	0
Effective mass yield (EMY)[5,a]	$\frac{\text{mass of the product}}{\text{mass of non-benign reagents}}$	Increase	-
EcoScale[6]	100 minus penalty points the penalty points is calculated based on yield, cost, safety, technical set-up, energy and purification aspects.	Increase	100

[a] The EMY values were not provided in the below tables because there were no non-benign reagents in these enzymatic reaction system.

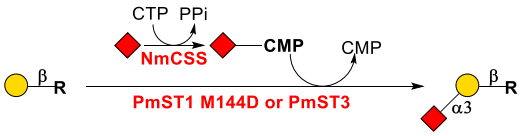


Table S2 The green chemistry metrics of two-enzyme cascade α2,3-sialylation system with PmST1 M144D or PmST3

Product	**28**	**29**	**34**	**35**	**31**	**32**	**36**	**37**	**38**	**39**	**41**	**42**
Substrate	**27**	**27**	**33**	**33**	**30**	**30**	**23**	**23**	**24**	**24**	**40**	**40**
Reaction conditions	**Two-enzyme cascade α2,3-sialylation system with PmST1 M144D:** Neu5Ac or Neu5Gc (1.2 equiv), CTP (1.2 equiv), Tris-HCl (100 mM), $MgCl_2$ (20 mM), **NmCSS**, and **PmST1** M144D, 37 ºC in one-pot.				**Two-enzyme cascade α2,3-sialylation system with PmST3:** Neu5Ac or Neu5Gc (1.2 equiv), CTP (1.2 equiv), Tris-HCl (100 mM), $MgCl_2$ (20 mM), **NmCSS**, and **PmST3**, 37 ºC in one-pot							
Atom economy	0.520	0.520	0.520	0.520	0.520	0.520	0.612	0.613	0.574	0.577	0.603	0.605
Curzons reaction mass efficiency (RME)	0.313	0.377	0.389	0.387	0.466	0.436	0.583	0.500	0.505	0.513	0.577	0.533
Andraos reaction mass efficiency (RME)	0.012	0.006	0.015	0.005	0.014	0.017	0.017	0.002	0.015	0.002	0.002	0.002
Sheldon *E*-factor	82.7	166	67.1	189	69.4	59.3	59.1	621	129	404	538	504
Ecoscale	59.5	64.5	66	65.5	73.5	70	76	72.5	71.5	74	73.5	74
Separation yield	0.630	0.730	0.760	0.750	0.910	0.840	0.960	0.890	0.870	0.920	0.910	0.920

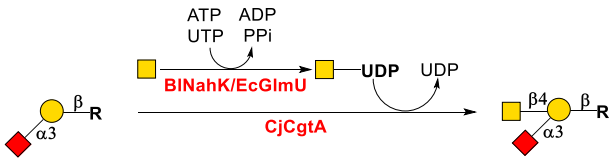


Table S3 The green chemistry metrics of three-enzyme cascade β1,4-*N*-acetylgalatosaminylation system

Product	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Substrate	**28**	**29**	**31**	**32**	**34**	**35**	**36**	**37**	**38**	**39**	**41**	**42**
Reaction conditions	**Three-enzyme cascade β1,4-*N*-acetylgalatosaminylation system:** GalNAc (1.2 equiv), ATP (1.2 equiv), UTP (1.2 equiv), Tris-HCl (100 mM), $MgCl_2$ (20 mM), **BlNahK/EcGlmU**, and **CjCgtA**, 37 ºC in one-pot.											
Atom economy	0.402	0.406	0.402	0.406	0.402	0.406	0.482	0.485	0.449	0.428	0.474	0.477
Curzons reaction mass efficiency (RME)	0.363	0.333	0.375	0.350	0.359	0.352	0.417	0.426	0.400	0.389	0.400	0.421
Andraos reaction mass efficiency (RME)	0.004	0.003	0.004	0.005	0.002	0.005	0.001	0.003	0.002	0.002	0.001	0.002
Sheldon *E*-factor	278	300	270	189	578	189	807	351	577	577	673	504
Ecoscale	73.5	72.5	75	71.5	74.5	71.5	74	72	74	74	73	73
Separation yield	0.910	0.890	0.940	0.870	0.930	0.870	0.920	0.880	0.920	0.920	0.900	0.900

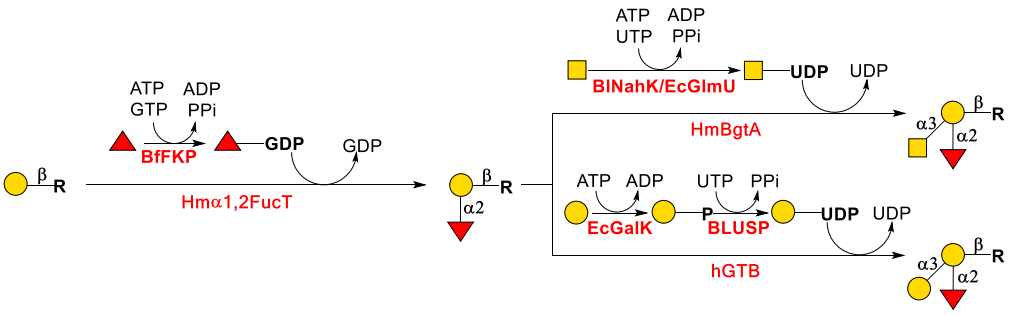
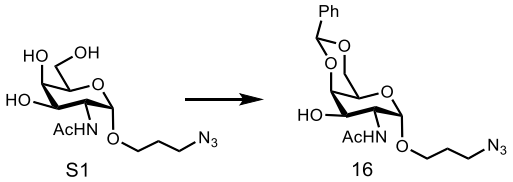


Table S4 The green chemistry metrics of two-enzyme cascade α1,2-fucosylation system, three-enzyme cascade α1,3-*N*-acetyl-galactosaminylation system, and Three-enzyme cascade α1,3-galactosylation system

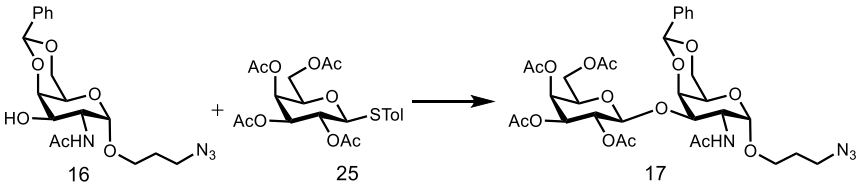
Product	**13**	**14**	**15**
Substrate	**7**	**13**	**13**
Reaction conditions	**Two-enzyme cascade α1,2-fucosylation system:** Fucose (1.2 equiv), ATP (1.5 equiv), GTP (1.5 equiv), Tris-HCl (100 mM), $MgCl_2$ (20 mM), 37 ºC, **BfFKP**, and **Hmα1,2FucT** in one-pot	**Three-enzyme cascade α1,3-*N*-acetyl-galactosaminylation system:** GalNAc (1.2 equiv), ATP (1.2 equiv), UTP (1.2 equiv), Tris-HCl (100 mM), $MgCl_2$ (20 mM), 37 ºC, **BlNahK/EcGlmU**, and **HmBgtA** in one-pot	**Three-enzyme cascade α1,3-galactosylation system:** Gal (1.2 equiv), ATP (1.2 equiv), UTP (1.2 equiv), Tris-HCl (100 mM), $MgCl_2$ (20 mM), **EcGalK**, **BLUSP**, and **hGTB** in one-pot
Atom economy	0.506	0.540	0.535
Curzons reaction mass efficiency (RME)	0.409	0.348	0.273
Andraos reaction mass efficiency (RME)	0.002	0.001	0.001
Sheldon *E*-factor	448.9	1008.8	896.7
Ecoscale	68.5	64	53
Separation yield	0.810	0.720	0.500

2. Experimental procedures of Chemical synthesis



3-azidopropyl 2-acetylamino-2-deoxy-4,6-*O*-phenylmethylene-α-D-galactopyranoside (16)

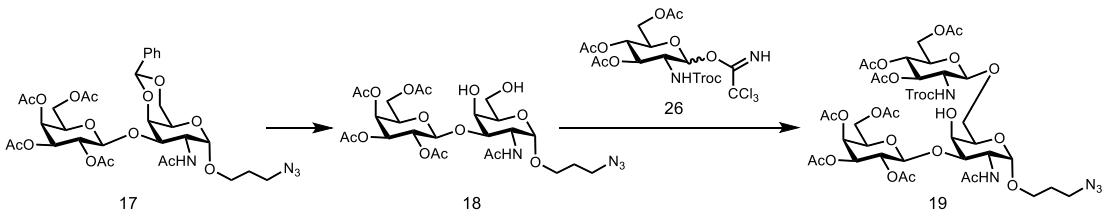
To a solution of 3-azidopropyl 2-acetylamino-α-D-galactopyranoside **S1**[7] (200 mg, 0.66 mmol) in CH_3CN, benzaldehyde dimethyl acetal (120 μL, 0.79 mmol) and CSA (46 mg, 0.20 mmol) were added and the resulting solution was stirred at room temperature overnight. The reaction was quenched with Et_3N, concentrated *in vacuo* and purified by silica gel chromatography (eluted with hexane:ethyl acetate, 1:6, *v*/*v*). Compound **16** was obtained as a white solid (81%, 210 mg); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (dd, J = 7.6, 2.1 Hz, 2H), 7.39 – 7.35 (m, 3H), 5.88 (d, J = 8.9 Hz, 1H), 5.57 (s, 1H), 4.96 (d, J = 3.5 Hz, 1H), 4.47 (ddd, J = 10.7, 8.9, 3.5 Hz, 1H), 4.28 (dd, J = 12.6, 1.5 Hz, 1H), 4.23 (d, J = 3.3 Hz, 1H), 4.08 (dd, J = 12.5, 1.7 Hz, 1H), 3.87 – 3.81 (m, 2H), 3.67 (d, J = 1.6 Hz, 1H), 3.54 (ddd, J = 10.0, 6.5, 5.4 Hz, 1H), 3.46 – 3.36 (m, 2H), 2.93 (d, J = 10.6 Hz, 1H), 2.03 (s, 3H), 1.94 – 1.86 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.29 , 129.18 , 128.23 , 126.32 , 101.27 , 98.37 , 75.44 , 69.30 , 69.01 , 65.23 , 63.10 , 50.33 , 48.73 , 28.52 , 23.37; HRMS (ESI) *m/z* calcd for $C_{18}H_{24}N_4O_6$ $[M+H]^+$ 393.1769, found 393.1791.



3-azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-acetylamino-α-D-galactopyranoside (17)

To a solution of 2-acetylamino-2-deoxy-4,6-*O*-phenylmethylene-α-D-galactopyranoside **16** (700 mg, 1.78 mmol) and *p*-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **25**[8] (968 mg, 2.14 mmol) in dry CH_2Cl_2 (15 mL), 4 Å molecular sieves (1.5 g) was added, and the reaction mixture was stirred under argon at room temperature for 30 min. The reaction mixture was cooled down to -25

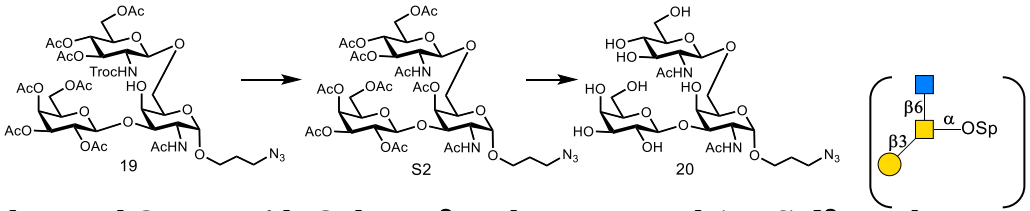
°C and *N*-iodosuccinimide (NIS, 803 mg, 3.57 mmol) was added. The reaction mixture was stirred at the same temperature for 10 min before TfOH (47 μL) was added. When thin-layer chromatography (TLC) showed complete consumption of the acceptor **16**, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and filtered through Celite®. The organic layer was washed with 5% aqueous $Na_2S_2O_3$, saturated $NaHCO_3$, and brine solution (10% aqueous NaCl). After dried with anhydrous Na_2SO_4, the organic layer was filtered and the solvent was removed by evaporation. The resulting residue was purified using silica gel chromatography (eluted with hexane:ethyl acetate, 1:3 *v*/*v*) to produce disaccharide **17** (850 mg, 66%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 – 7.53 (m, 2H), 7.38 – 7.33 (m, 3H), 5.70 (d, *J* = 8.7 Hz, 1H), 5.55 (s, 1H), 5.38 (td, *J* = 3.6, 1.1 Hz, 1H), 5.20 (dd, *J* = 10.3, 7.9 Hz, 1H), 5.03 (d, *J* = 3.6 Hz, 1H), 4.98 (dd, *J* = 10.3, 3.5 Hz, 1H), 4.76 (d, *J* = 7.9 Hz, 1H), 4.68 – 4.63 (m, 1H), 4.30 (d, *J* = 3.0 Hz, 1H), 4.26 (dd, *J* = 12.4, 1.6 Hz, 1H), 4.18 (dd, *J* = 11.3, 6.3 Hz, 1H), 4.13 (dd, *J* = 11.3, 6.7 Hz, 1H), 4.06 (dd, *J* = 12.5, 1.7 Hz, 1H), 3.96 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.90 (td, *J* = 6.6, 1.3 Hz, 1H), 3.86 – 3.81 (m, 1H), 3.65 (d, *J* = 1.5 Hz, 1H), 3.58 – 3.52 (m, 1H), 3.40 (qd, *J* = 6.1, 5.1, 2.1 Hz, 2H), 2.15 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.94 – 1.87 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.40, 170.27, 170.20, 169.64, 169.54, 137.55, 128.87, 128.15, 126.19, 126.03, 101.13, 100.74, 98.33, 75.52, 74.06, 70.95, 70.84, 69.24, 68.80, 66.92, 65.54, 63.20, 61.34, 48.89, 48.20, 28.39, 23.39, 20.70, 20.69, 20.68, 20.55; HRMS (ESI) *m/z* calcd for $C_{32}H_{42}N_4O_{15}$ $[M+H]^+$ 723.2719, found 723.2805.



3-azidopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino) -β-D-glucopyranoside-(1→6)-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)] -4,6-*O*-benzilidine-2-deoxy-2-acetylamino-α-D-galactopyranoside (19)

A solution of compound **17** (397 mg, 0.55 mmol) in 80% aqueous acetic acid (20 mL) was stirred at 70 ℃ for 3 h and the solvents were removed under reduced pressure. The crude compound was co-evaporated with toluene (3×10 mL) and purified using silica gel chromatography (eluted with hexane:acetone, 1:1, *v*/*v*) to produce

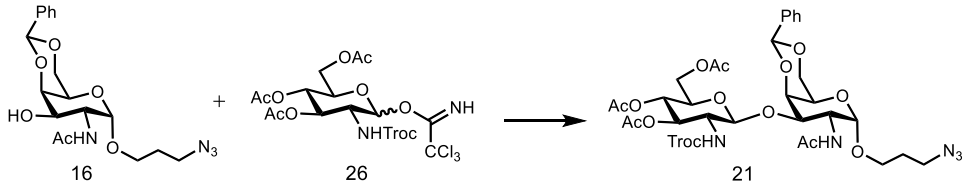
disaccharide diol **18** (257 mg) as a white solid. And then a solution of glycosyl donor **26**[9] (303 mg, 0.48 mmol), glycosyl acceptor **20** (257 mg, 0.40 mmol) and activated 4 Å molecular sieves (1.0 g) in anhydrous CH_2Cl_2 (10 mL) was stirred under argon at room temperature for 30 min. The reaction mixture was then cooled to -45 ^oC, followed by adding TfOH (11 μL, 0.12 mmol) and then warm to room temperature. The reaction mixture was stirred until complete consumption of the acceptor, as observed by TLC (dichloromethane:ethyl acetate, 1:6, *v*/*v*). The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite®. The organic layer was washed with saturated $NaHCO_3$ solution. After dried with anhydrous Na_2SO_4, the solution was filtered and the solvent was removed by evaporation. The resulting residue was purified using silica gel chromatography (eluted with dichloromethane:ethyl acetate, 1:3, *v*/*v*) to afford disaccharide **19** (355 mg, 58% for two steps); ^1H NMR (600 MHz, $CDCl_3$) δ 5.78 (d, *J* = 9.0 Hz, 1H), 5.65 (d, *J* = 9.5 Hz, 1H), 5.37 – 5.35 (m, 1H), 5.24 (t, *J* = 9.7 Hz, 1H), 5.18 (dd, *J* = 10.5, 7.9 Hz, 1H), 5.06 (t, *J* = 9.6 Hz, 1H), 4.97 (dd, *J* = 10.5, 3.5 Hz, 1H), 4.81 (d, *J* = 12.1 Hz, 1H), 4.75 (d, *J* = 3.7 Hz, 1H), 4.66 (d, *J* = 8.3 Hz, 1H), 4.58 (d, *J* = 7.9 Hz, 1H), 4.55 – 4.51 (m, 2H), 4.29 (dt, *J* = 13.1, 3.9 Hz, 1H), 4.17 (dd, *J* = 11.3, 6.7 Hz, 1H), 4.12 (dd, *J* = 12.6, 2.3 Hz, 1H), 4.05 (td, *J* = 10.9, 9.8, 5.0 Hz, 2H), 4.00 (s, 1H), 3.93 – 3.87 (m, 2H), 3.82 – 3.66 (m, 4H), 3.50 – 3.37 (m, 4H), 2.82 (s, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.90 (p, *J* = 6.3 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.70, 170.22, 170.14, 169.71, 169.48, 169.47, 154.21, 101.82, 101.16, 97.68, 95.52, 77.22, 77.01, 76.80, 74.35, 72.18, 71.75, 70.80, 70.62, 69.34, 68.55, 66.78, 65.36, 61.98, 61.18, 56.19, 49.03, 47.75, 29.67, 28.41, 23.37, 20.76, 20.72, 20.69, 20.65, 20.62, 20.55; HRMS (ESI) *m/z* calcd for $C_{40}H_{56}Cl_3N_5O_{24}$ $[M+H]^+$ 1096.2454, found 1096.2660.



3-azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-2-α-D-galactopyranoside (20)

Compound **19** (296 mg, 0.27 mmol) was dissolved in THF (15 mL), and then TBAF (283 mg, 1.08 mmol) was added. The mixture was stirred at room temperature

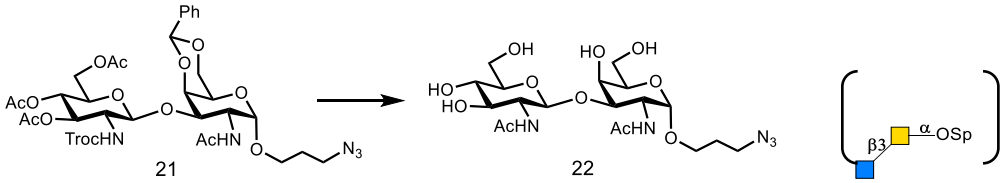
overnight. Without further purification, the solvent was removed under vacuum, and the residue was dissolved in pyridine (10 mL) and treated with acetic anhydride (76 μL, 1.2 equiv). The mixture was stirred at room temperature for 12 h and monitored by TLC (dichloromethane:methanol, 10:1, *v*/*v*). Then the mixture was washed with 1 M HCl and CH_2Cl_2 to remove pyridine. After dried and purified by silica gel chromatography, the trisaccharide **S2** was obtained in high yield. The product **S2** was dissolved in dry MeOH, and 30% NaOMe in MeOH solution was added to adjust the pH to 10. The resulting mixture was stirred at room temperature and monitored by TLC (EtOAc:MeOH:H_2O:HOAc, 8:3:1:0.2, *v*/*v*). After the completion of the reaction, the reaction mixture was neutralized with Dowex 50WX8 (H^+) resin. The mixture was concentrated and purified by Bio-Gel P2 gel filtration chromatography (eluted with H_2O) to afford the trisaccharide **20** (150 mg, 83% for 3 steps) as a white solid; ^{1}H NMR (600 MHz, D_2O) δ 4.83 (d, *J* = 3.8 Hz, 1H), 4.49 (d, *J* = 8.5 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.29 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.19 (d, *J* = 3.2 Hz, 1H), 4.03 (dd, *J* = 8.5, 3.1 Hz, 2H), 3.99 (dd, *J* = 11.1, 3.2 Hz, 1H), 3.90 (dd, *J* = 12.4, 2.0 Hz, 1H), 3.87 (dd, *J* = 3.5, 0.9 Hz, 1H), 3.75 – 3.65 (m, 6H), 3.61 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.59 (dd, *J* = 9.9, 3.4 Hz, 1H), 3.51 – 3.38 (m, 7H), 1.99 (s, 6H), 1.89 – 1.84 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.42, 174.13, 104.59, 101.41, 96.92, 76.83, 75.72, 74.86, 73.78, 72.34, 70.45, 69.84, 69.79, 69.24, 68.83, 68.44, 64.41, 60.85, 60.57, 55.35, 48.49, 48.08, 27.80, 22.07, 21.84; HRMS (ESI) *m/z* calcd for $C_{25}H_{43}N_5O_{16}$ $[M+H]^+$ 670.2778, found 670.2829.



3-azidopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-acetylamino-α-D-galactopyranoside (21)

A solution of glycosyl donor **26**[9] (1.08 g, 1.74 mmol), glycosyl acceptor **16** (455 mg, 1.16 mmol) and activated 4 Å molecular sieves (5.0 g) in anhydrous CH_2Cl_2 (50 mL) was stirred under argon at room temperature for 30 min. The reaction mixture was then cooled to 0 ^{o}C, followed by adding of TfOH (30 μL, 0.35 mmol). The reaction

mixture was stirred for 2 h until complete consumption of the acceptor, as observed by TLC (hexane:ethyl acetate, 1:3, *v*/*v*). The reaction mixture diluted with CH_2Cl_2 and filtered through Celite®. The organic layer was washed with saturated $NaHCO_3$. After dried with anhydrous Na_2SO_4, the solution was filtered and the solvent was removed by evaporation. The resulting residue was purified using silica gel chromatography (eluted with hexane:ethyl acetate, 1:1, *v*/*v*) to produce disaccharide **21** (805 mg, 81%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 – 7.51 (m, 2H), 7.40 – 7.33 (m, 3H), 5.84 (d, *J* = 8.9 Hz, 1H), 5.66 (d, *J* = 8.1 Hz, 1H), 5.56 (s, 1H), 5.28 (t, *J* = 9.8 Hz, 1H), 5.06 – 4.97 (m, 2H), 4.71-4.63 (m, 3H), 4.36 (d, *J* = 3.2 Hz, 1H), 4.30 (d, *J* = 11.9 Hz, 1H), 4.24 (dd, *J* = 12.4, 1.5 Hz, 1H), 4.09 (dd, *J* = 12.4, 4.0 Hz, 1H), 4.05 (dd, *J* = 12.6, 1.7 Hz, 1H), 3.99 – 3.93 (m, 2H), 3.81 (dt, *J* = 10.2, 6.1 Hz, 1H), 3.67 (d, *J* = 13.5 Hz, 1H), 3.61 (s, 1H), 3.52 (dq, *J* = 11.6, 8.4, 7.4 Hz, 2H), 3.44 – 3.34 (m, *J* = 6.2 Hz, 2H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.88 (p, *J* = 6.7, 6.2 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.57, 170.29, 169.43, 154.04, 137.47, 129.13, 128.31, 126.23, 100.98, 99.62, 98.37, 95.41, 77.21, 77.00, 76.79, 75.63, 74.34, 71.89, 71.55, 69.29, 68.40, 65.36, 63.06, 61.55, 56.42, 48.71, 28.48, 23.40, 20.77, 20.63; HRMS (ESI) *m/z* calcd for $C_{33}H_{42}Cl_3N_5O_{15}$ $[M+H]^+$ 854.1816, found 854.1887.

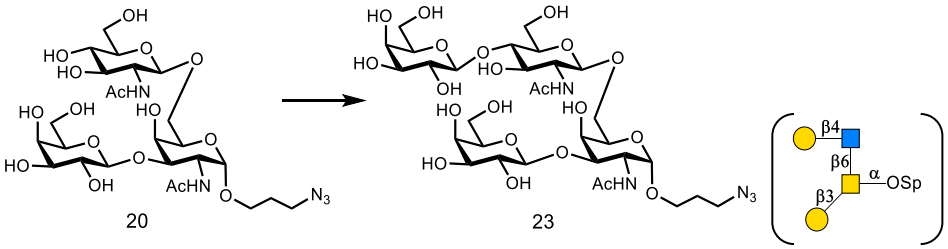


3-azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-2-α-D-galactopyranoside (22)

Compound **21** (41 mg, 0.05 mmol) was dissolved in THF (5 mL), and then TBAF (51 mg, 0.2 mmol) was added. The mixture was stirred at room temperature overnight. Without further purification, the solution was removed under vacuum, and the residue was dissolved in pyridine (5 mL) and treated with acetic anhydride (1.2 equiv). The mixture was string at room temperature for 12 h and monitored by TLC (dichloromethane:methanol, 10:1, *v*/*v*). Then the mixture was washed with 1 M HCl to remove pyridine. The crude disaccharide was then dissolved in 80% AcOH (5 mL) and string at 70 ºC for 2 h until the complete consumption of starting material. The solvent was removed under vacuum, followed by co-evaporation with toluene (5 mL × 4). And the resulting residue was directly used for the next reaction. Finally, the crude product was dissolved in dry MeOH, and 30% methanolic NaOMe was added to the

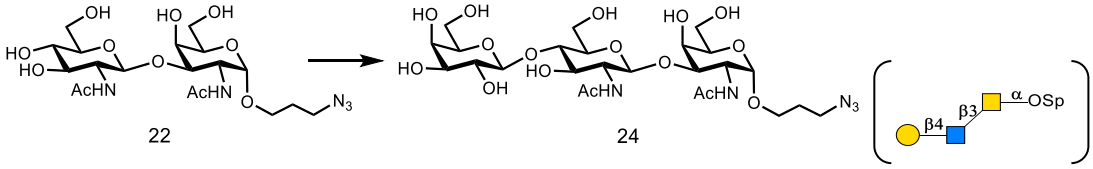
solution to adjust the pH to 10. The resulting mixture was stirred at room temperature and monitored by TLC (EtOAc:MeOH:H_2O:HOAc, 8:3:1:0.2, *v*/*v*) and neutralized with Dowex 50WX8 (H^+) resin. The mixture was concentrated and purified by Bio-Gel P2 gel filtration chromatography (eluted with H_2O) to afford the disaccharide **22** (19 mg, 78% for 4 steps) as a white solid. ^{1}H NMR (600 MHz, D_2O) δ 4.80 (d, J = 3.9 Hz, 1H), 4.53 (d, J = 8.4 Hz, 1H), 4.21 (dd, J = 11.1, 3.8 Hz, 1H), 4.19 – 4.17 (m, 1H), 3.96 – 3.91 (m, 2H), 3.86 (dd, J = 12.4, 2.2 Hz, 1H), 3.78 – 3.68 (m, 4H), 3.66 (dd, J = 10.4, 8.4 Hz, 1H), 3.53 – 3.47 (m, 2H), 3.46 – 3.36 (m, 4H), 2.01 (s, 3H), 1.98 (s, 3H), 1.89 – 1.84 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.30, 173.49, 102.33, 97.01, 76.33, 75.46, 73.26, 70.34, 69.55, 68.66, 64.77, 61.03, 60.25, 55.42, 48.36, 48.07, 27.82, 22.04, 21.89; HRMS (ESI) *m/z* calcd for $C_{19}H_{35}N_5O_{11}$ $[M+H]^+$ 508.2249, found 508.2289.

3. Experimental Procedures of Enzymatic synthesis



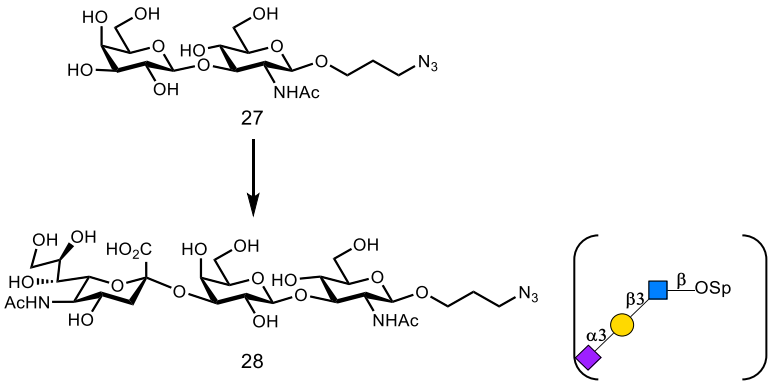
3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (23)

Tetrasaccharide **23** was prepared according to general procedure of multienzyme cascade β1,4-galactosylation system with GalK, BLUSP and NmLgtB. After lyophilization, **23** was obtained as white solid (79 mg, 78%); ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, *J* = 3.8 Hz, 1H), 4.53 (d, *J* = 8.3 Hz, 1H), 4.45 (d, *J* = 7,8Hz, 1H), 4.43 (d, *J* = 9 Hz, 1H), 4.30 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.20 (d, *J* = 3.1 Hz, 1H), 4.05 (dd, *J* = 8.8, 2.7 Hz, 2H), 3.99 (ddd, *J* = 15.4, 11.7, 2.7 Hz, 2H), 3.89 (dd, *J* = 10.4, 3.4 Hz, 2H), 3.81 (dd, *J* = 12.3, 5.2 Hz, 1H), 3.78 – 3.56 (m, 14H), 3.54 – 3.39 (m, 5H), 2.00 (d, *J* = 1.5 Hz, 6H), 1.91–1.85 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.45, 174.12, 104.61, 102.76, 101.34, 96.95, 78.34, 76.85, 75.24, 74.89, 74.64, 72.41, 72.37, 70.84, 70.49, 69.84, 69.26, 68.85, 68.47, 68.43, 64.47, 60.91, 60.89, 59.93, 54.90, 48.51, 48.12, 27.83, 22.12, 21.88; HRMS (ESI) *m/z* calcd for $C_{31}H_{53}N_5O_{21}$ $[M+H]^+$ 832.3306, found 832.3359.



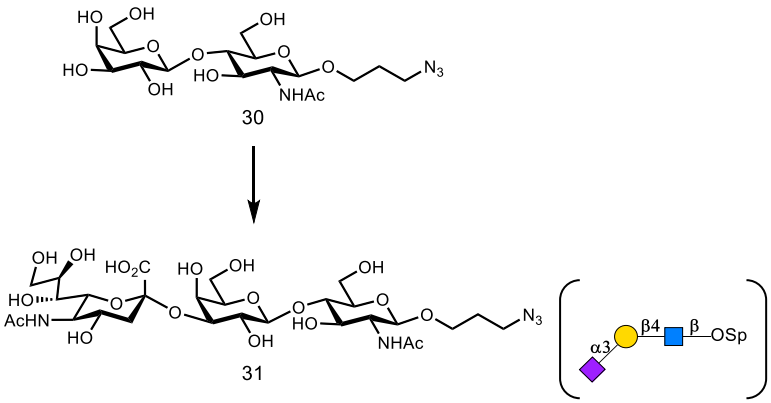
3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (24)

Trisaccharide **24** was prepared according to general procedure of multienzyme cascade β1,4-galactosylation system with GalK, BLUSP and NmLgtB. After lyophilization, **24** was obtained as white solid (120 mg, 91%); ^{1}H NMR (600 MHz, D_2O) δ 4.81 (d, J = 3.9 Hz, 1H), 4.55 (d, J = 7.9 Hz, 1H), 4.44 (d, J = 7.8 Hz, 1H), 4.22 (dd, J = 11.1, 3.8 Hz, 1H), 4.18 (d, J = 2.5 Hz, 1H), 3.96 – 3.87 (m, 4H), 3.83 – 3.61 (m, 12H), 3.54 – 3.47 (m, 2H), 3.47 – 3.38 (m, 2H), 2.01 (s, 3H), 1.98 (s, 3H), 1.86 (p, J = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.27, 173.48, 102.73, 102.21, 97.01, 78.10, 76.39, 75.22, 74.37, 72.35, 71.90, 70.82, 70.33, 68.64, 68.39, 64.79, 61.03, 60.88, 59.65, 54.96, 48.35, 48.08, 27.82, 22.06, 21.89; HRMS (ESI) *m/z* calcd for $C_{25}H_{43}N_5O_{16}$ $[M+H]^+$ 670.2778, found 670.2856.



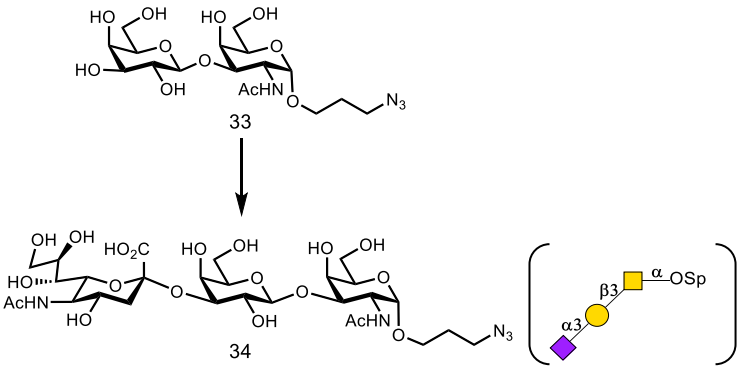
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (28)

Trisaccharide **28** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST1 M144D. After lyophilization, **28** was obtained as white solid (100 mg, yield 63%); ^{1}H NMR (600 MHz, D_2O) δ 4.56 (d, *J* = 8.3 Hz, 1H), 4.50 (d, *J* = 7.8 Hz, 1H), 4.09 (dd, *J* = 9.8, 3.2 Hz, 1H), 3.98 (dt, *J* = 10.9, 5.6 Hz, 1H), 3.95 – 3.91 (m, 2H), 3.89 – 3.81 (m, 4H), 3.80 – 3.71 (m, 4H), 3.70 – 3.59 (m, 6H), 3.54 (td, *J* = 9.0, 7.8, 2.7 Hz, 2H), 3.49 (ddd, *J* = 10.1, 5.7, 2.2 Hz, 1H), 3.38 (td, *J* = 6.6, 2.4 Hz, 2H), 2.76 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.03 (d, *J* = 6.2 Hz, 6H), 1.85 (p, *J* = 6.4 Hz, 2H), 1.78 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.93, 174.54, 173.86, 103.41, 100.87, 99.60, 82.48, 75.58, 75.34, 75.06, 72.76, 71.80, 69.04, 68.70, 68.35, 68.00, 67.20, 67.11, 62.41, 60.98, 60.70, 54.40, 51.61, 47.75, 39.73, 28.06, 22.27, 22.00; HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{19}$ $[M-H]^-$ 756.2792, found 756.2778.



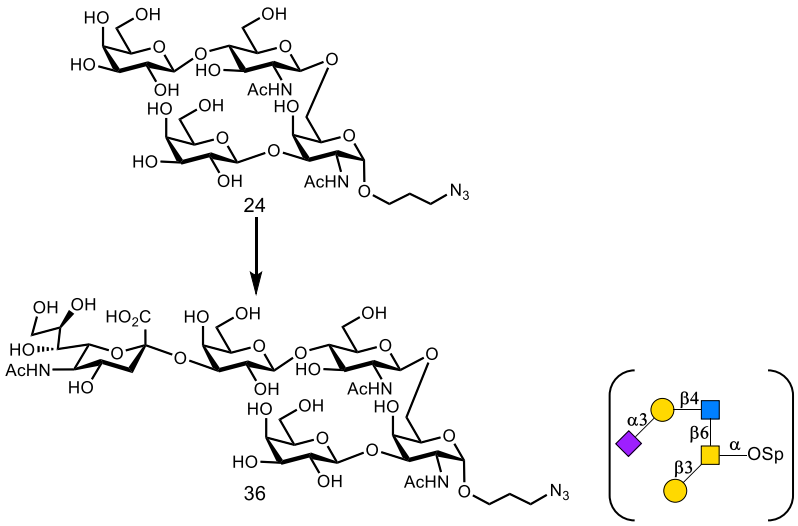
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (31)

Trisaccharide **31** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **31** was obtained as white solid (118 mg, yield 91%); ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, J = 7.9 Hz, 1H), 4.51 (d, J = 8.1 Hz, 1H), 4.10 (dd, J = 9.9, 3.1 Hz, 1H), 4.00 – 3.92 (m, 3H), 3.89 – 3.81 (m, 4H), 3.76 – 3.52 (m, 13H), 3.36 (td, J = 6.5, 3.8 Hz, 2H), 2.74 (dd, J = 12.5, 4.7 Hz, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 1.83 (q, J = 6.4 Hz, 2H), 1.78 (t, J = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.97, 174.45, 173.85, 102.54, 101.12, 99.78, 78.27, 75.44, 75.14, 74.72, 72.85, 72.31, 71.74, 69.35, 68.32, 68.06, 67.44, 67.10, 62.55, 61.00, 60.00, 55.05, 51.65, 47.75, 39.60, 28.08, 22.14, 22.02; HRMS (ESI) *m/z* calcd for $C_{28}H_{47}N_5O_{19}$ $[M-H]^-$ 756.2792, found 756.2771.



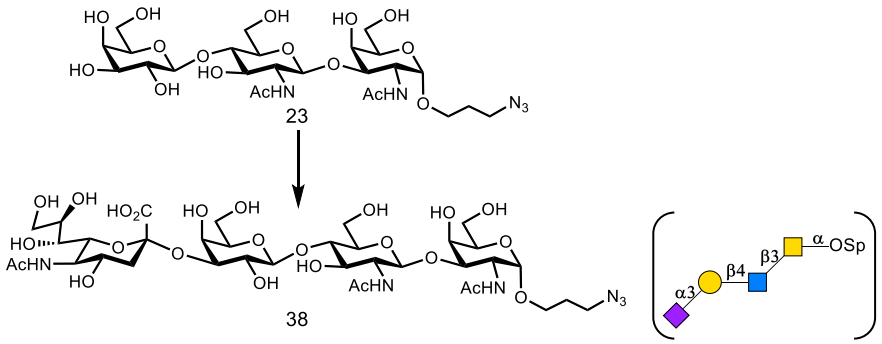
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galacopyranoside (34)

Trisaccharide **34** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST1 M144D. After lyophilization, **34** was obtained as white solid white solid after lyophilization (123 mg, 76%); ^{1}H NMR (600 MHz, D_2O) δ 4.91 (d, J = 3.7 Hz, 1H), 4.54 (d, J = 7.8 Hz, 1H), 4.32 (dd, J = 11.1, 3.7 Hz, 1H), 4.25 (d, J = 3 Hz, 1H), 4.08 (dd, J = 9.8, 3.2 Hz, 1H), 4.05 (dd, J = 11.1, 3.1 Hz, 1H), 3.99 (dd, J = 7.3, 5.9 Hz, 1H), 3.93 (d, J = 3.2 Hz, 1H), 3.90 – 3.58 (m, 13H), 3.55 (ddd, J = 9.8, 7.0, 2.9 Hz, 2H), 3.52 – 3.42 (m, 2H), 2.75 (dd, J = 12.4, 4.6 Hz, 1H), 2.03 (d, J = 1.4 Hz, 6H), 1.93 – 1.88 (m, 2H), 1.79 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.95, 174.54, 173.87, 104.42, 99.68, 97.15, 77.36, 75.62, 74.75, 72.77, 71.80, 70.60, 69.06, 68.54, 68.35, 68.02, 67.36, 64.89, 62.47, 61.19, 60.95, 51.62, 48.66, 48.15, 39.69, 27.94, 22.02, 22.01; HRMS (ESI) *m/z* calcd for $C_{28}H_{47}N_5O_{19}$ $[M-H]^-$ 756.2792, found 756.2777.



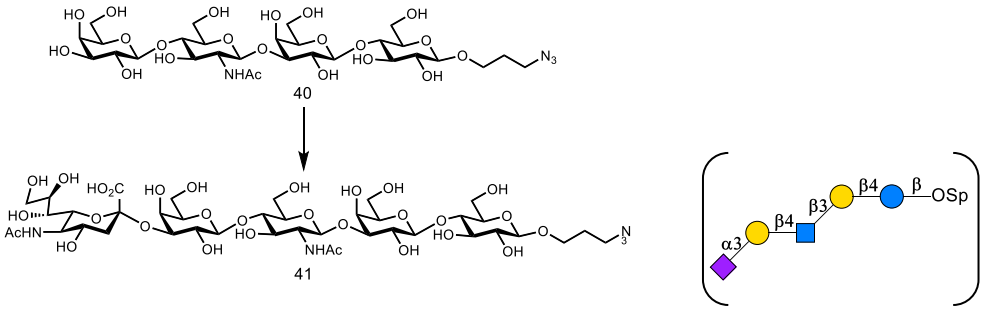
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (36)

Pentasaccharide **36** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **36** was obtained as white solid (137 mg, 96%); ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, J = 3.8 Hz, 1H), 4.53 (d, J = 7.2 Hz, 1H),4.53 (d, J = 9 Hz, 1H) 4.43 (d, J = 7.8 Hz, 1H), 4.30 (dd, J = 11.1, 3.7 Hz, 1H), 4.20 (d, J = 3.2 Hz, 1H), 4.09 (dd, J = 9.9, 3.1 Hz, 1H), 4.07 – 4.03 (m, 2H), 4.00 (ddd, J = 11.9, 8.3, 2.7 Hz, 2H), 3.93 (d, J = 3.2 Hz, 1H), 3.89 – 3.81 (m, 4H), 3.77 – 3.53 (m, 16H), 3.51 – 3.40 (m, 4H), 2.74 (dd, J = 12.4, 4.6 Hz, 1H), 2.01 (d, J = 0.8 Hz, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.91 – 1.85 (m, 2H), 1.78 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.88, 174.43, 174.11, 173.76, 104.60, 102.45, 101.39, 99.67, 96.95, 78.19, 76.86, 75.34, 75.06, 74.88, 74.64, 72.76, 72.37, 71.65, 70.48, 69.86, 69.28, 69.26, 68.83, 68.47, 68.25, 67.96, 67.34, 64.48, 62.45, 60.92, 60.87, 59.91, 54.90, 51.55, 48.51, 48.12, 39.51, 27.82, 22.11, 21.92, 21.87; HRMS (ESI) *m/z* calcd for $C_{42}H_{70}N_6O_{29}$ $[M-H]^-$ 1121.4114, found 1121.4071.



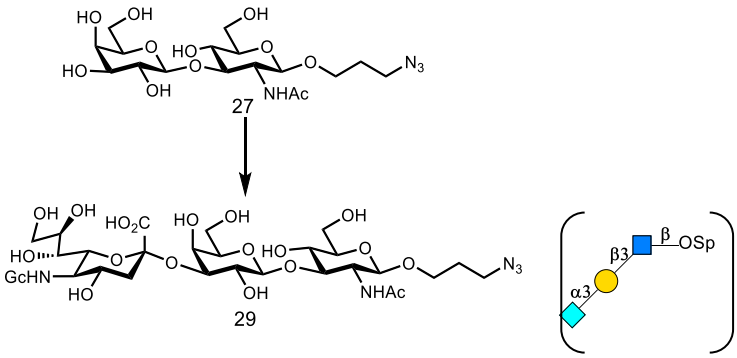
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (38)

Tetrasaccharide **38** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **38** was obtained as white solid (63 mg, 87%); ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, J = 7.9 Hz, 1H), 4.51 (d, J = 7.8 Hz, 1H), 4.20 (dd, J = 11.1, 3.8 Hz, 1H), 4.17 (d, J = 3 Hz, 1H) 4.07 (dd, J = 9.9, 3.1 Hz, 1H), 3.92 (ddd, J = 13.0, 7.4, 3.9 Hz, 4H), 3.86 – 3.37 (m, 23H), 2.71 (dd, J = 12.4, 4.6 Hz, 1H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.85 (p, J = 6.5 Hz, 2H), 1.75 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.63, 174.27, 173.75, 173.47, 102.36, 102.27, 99.66, 96.99, 77.86, 76.29, 75.31, 75.03, 74.36, 72.44, 71.85, 71.67, 70.34, 69.24, 68.65, 67.94, 67.82, 67.28, 64.74, 62.36, 61.04, 60.88, 60.80, 59.60, 54.93, 51.21, 48.36, 48.04, 39.53, 27.81, 22.03, 21.86; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{24}$ $[M-H]^-$ 959.3586, found 959.3548.



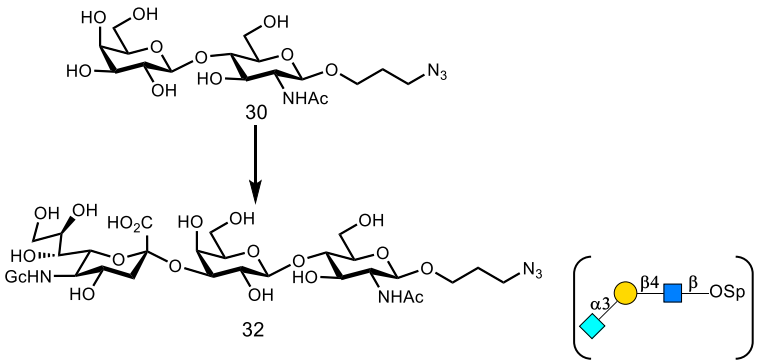
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (41)

Pentasaccharide **41** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **41** was obtained as white solid (15 mg, 91%); ^{1}H NMR (600 MHz, D_2O) δ 4.70 (d, J = 8.3 Hz, 1H), 4.56 (d, J = 7.9 Hz, 1H), 4.49 (d, J = 8.0 Hz, 1H), 4.44 (d, J = 7.9 Hz, 1H), 4.16 (d, J = 3.3 Hz, 1H), 4.12 (dd, J = 9.9, 3.1 Hz, 1H), 4.02 – 3.94 (m, 5H), 3.91 – 3.55 (m, 25H), 3.46 (t, J = 6.7 Hz, 2H), 3.33 – 3.29 (m, 1H), 2.76 (dd, J = 12.4, 4.6 Hz, 1H), 2.03 (s, 6H), 1.91 (p, J = 6.6 Hz, 2H), 1.80 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.98, 174.86, 173.83, 102.91, 102.77, 102.50, 102.07, 99.77, 82.01, 78.32, 77.94, 75.45, 75.14, 74.86, 74.74, 74.52, 74.32, 72.85, 72.75, 72.11, 71.73, 69.92, 69.35, 68.32, 68.28, 68.04, 67.43, 67.33, 62.54, 61.00, 60.93, 60.01, 59.80, 55.14, 51.64, 47.83, 39.60, 28.19, 22.13, 22.00; HRMS (ESI) *m/z* calcd for $C_{40}H_{67}N_5O_{29}$ $[M-H]^-$ 1080.3849, found 1080.3819.



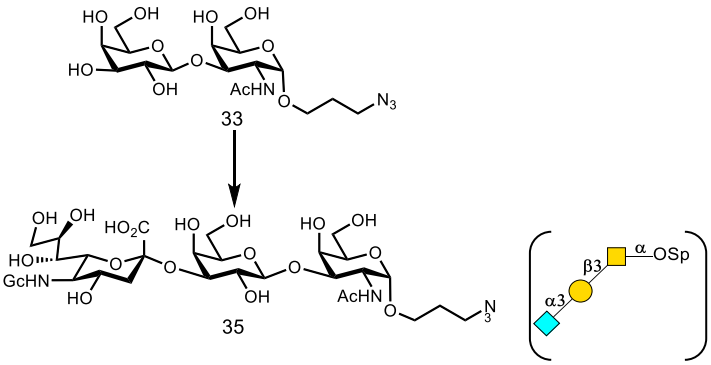
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (29)

Trisaccharide **29** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST1M144D. After lyophilization, **29** was obtained as white solid (49 mg, yield 73%); ^{1}H NMR (600 MHz, D_2O) δ 4.54 (d, J = 8.4 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.10 (s, 2H), 4.08 (dd, J = 9.9, 3.2 Hz, 1H), 3.96 (dt, J = 10.9, 5.6 Hz, 1H), 3.93 – 3.90 (m, 2H), 3.86 (ddd, J = 8.9, 6.1, 2.5 Hz, 1H), 3.84 – 3.61 (m, 12H), 3.58 (dd, J = 9.1, 2.0 Hz, 1H), 3.55 – 3.51 (m, 2H), 3.48 (dd, J = 5.6, 2.2 Hz, 1H), 3.37 (td, J = 6.6, 2.4 Hz, 2H), 2.76 (dd, J = 12.4, 4.7 Hz, 1H), 2.02 (s, 3H), 1.83 (p, J = 7.2, 6.8 Hz, 2H), 1.78 (t, J = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.63, 174.47, 173.83, 103.35, 100.81, 99.54, 82.40, 75.51, 75.28, 74.99, 72.41, 71.81, 68.98, 68.63, 68.02, 67.85, 67.13, 67.05, 62.31, 60.92, 60.87, 60.63, 54.35, 51.26, 47.69, 39.74, 28.00, 22.21; HRMS (ESI) *m/z* calcd for $C_{28}H_{47}N_5O_{20}$ $[M-H]^-$ 772.2742, found 772.2730.



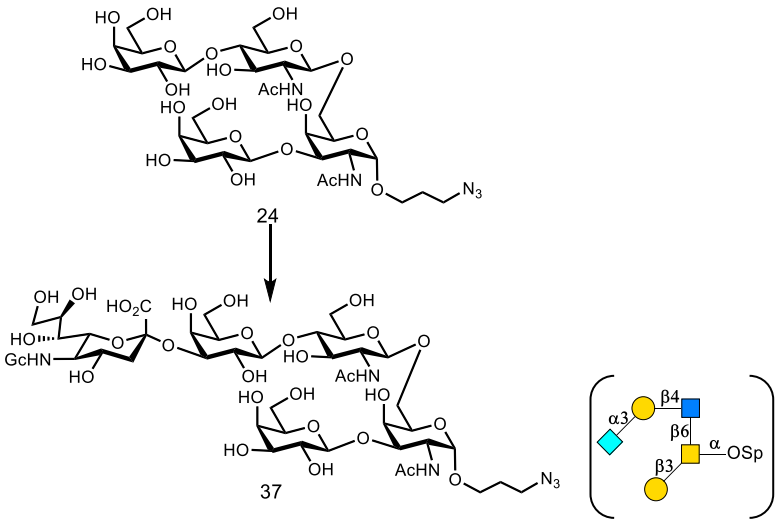
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (32)

Trisaccharide **32** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **32** was obtained as white solid (139 mg, yield 83%); ^{1}H NMR (600 MHz, D_2O) δ 4.54 (d, *J* = 7.9 Hz, 1H), 4.51 (d, *J* = 8.1 Hz, 1H), 4.13 – 4.09 (m, 3H), 4.01 – 3.61 (m, 17H), 3.59 – 3.54 (m, 3H), 3.36 (td, *J* = 6.5, 3.7 Hz, 2H), 2.76 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.03 (s, 3H), 1.85 – 1.77 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 175.67, 174.38, 173.82, 102.47, 101.05, 99.72, 78.19, 75.36, 75.07, 74.65, 72.50, 72.24, 71.72, 69.29, 68.00, 67.91, 67.36, 67.03, 62.43, 60.94, 60.87, 59.94, 54.98, 51.28, 47.67, 39.59, 28.01, 22.07; HRMS (ESI) *m/z* calcd for $C_{28}H_{47}N_5O_{20}$ $[M-H]^-$ 772.2742, found 772.2718.



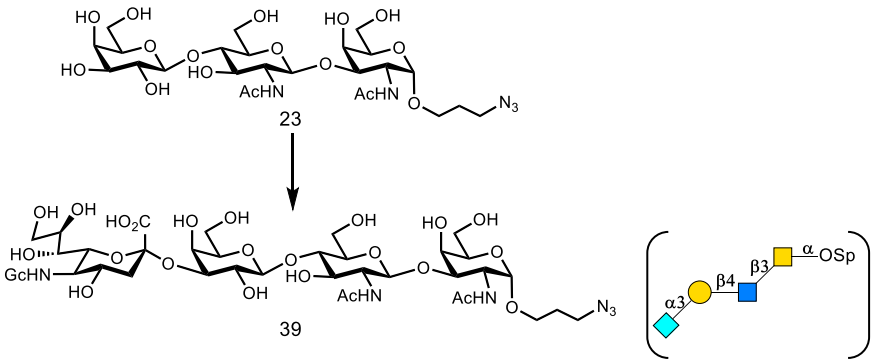
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galacopyranoside (35)

Trisaccharide **35** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST1M144D. After lyophilization, **35** was obtained as white solid (42.90 mg, 75%); ^{1}H NMR (600 MHz, D_2O) δ 4.90 (d, J = 3.7 Hz, 1H), 4.53 (d, J = 7.9 Hz, 1H), 4.31 (dd, J = 11.1, 3.7 Hz, 1H), 4.24 (d, J = 3.0 Hz, 1H), 4.12 – 4.01 (m, 4H), 4.00 – 3.90 (m, 3H), 3.90 – 3.69 (m, 9H), 3.66 – 3.40 (m, 7H), 2.76 (dd, J = 12.4, 4.7 Hz, 1H), 2.02 (s, 3H), 1.89 (p, J = 6.4 Hz, 2H), 1.79 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.65, 174.47, 173.84, 104.36, 99.62, 97.08, 77.28, 75.53, 74.68, 72.41, 71.80, 70.53, 68.98, 68.47, 68.02, 67.86, 67.27, 64.81, 62.35, 61.12, 60.88, 59.22, 51.25, 48.59, 48.07, 39.68, 27.87, 21.95; HRMS (ESI) *m/z* calcd for $C_{28}H_{47}N_5O_{20}$ $[M-H]^-$ 772.2742, found 772.2721.



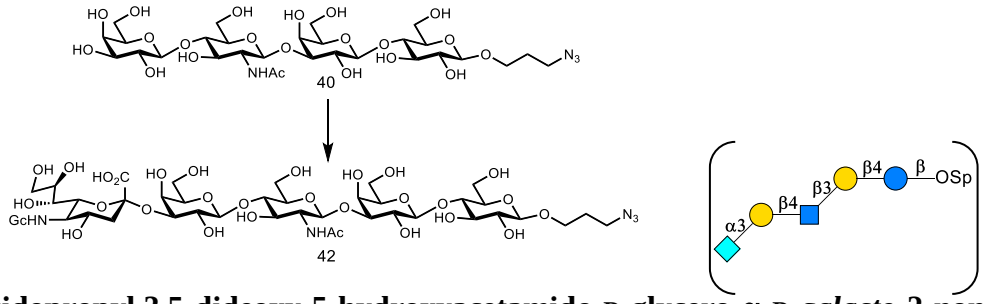
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (37)

Pentasaccharide **37** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **37** was obtained as white solid (13 mg, 89%); ^{1}H NMR (600 MHz, D_2O) δ 4.86 (d, J = 3.8 Hz, 1H), 4.55 (d, J = 7.8 Hz, 1H), 4.54 (d, J = 8.4Hz, 1H) 4.45 (d, J = 7.8 Hz, 1H), 4.31 (dd, J = 11.0, 3.7 Hz, 1H), 4.22 (d, J = 3.1 Hz, 1H), 4.13 – 4.10(m, 3H), 4.06 (dq, J = 7.9, 3.1 Hz, 2H), 4.01 (ddd, J = 11.0, 7.4, 2.6 Hz, 2H), 3.96 – 3.41 (m, 28H), 2.77 (dd, J = 12.5, 4.7 Hz, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 1.94 – 1.86 (m, 2H), 1.81 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.68, 174.46, 174.13, 173.81, 104.62, 102.49, 101.42, 99.72, 96.98, 78.26, 76.89, 75.37, 75.09, 74.91, 74.67, 72.51, 72.40, 71.73, 70.52, 69.87, 69.30, 69.29, 68.85, 68.50, 68.01, 67.93, 67.37, 64.53, 62.45, 60.94, 60.90, 59.96, 59.59, 54.93, 51.29, 48.54, 48.15, 39.61, 27.85, 22.15, 21.91; HRMS (ESI) *m/z* calcd for $C_{42}H_{70}N_6O_{30}$ $[M-H]^-$ 1137.4064, found 1137.4024.



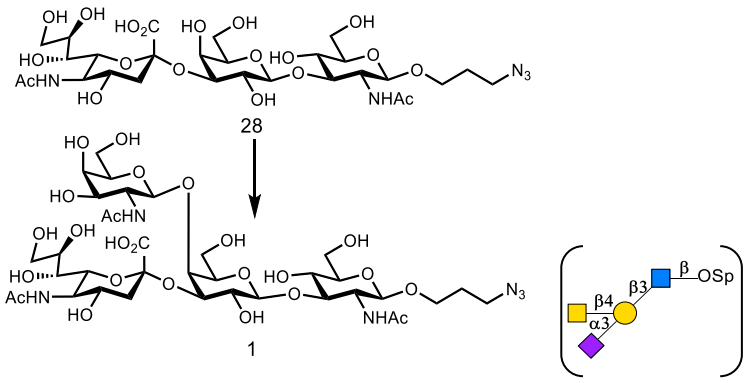
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (39)

Tetrasaccharide **39** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **39** was obtained as white solid (20 mg, 92%); ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, J = 8.0 Hz, 1H), 4.51 (d, J = 7.9 Hz, 1H), 4.20 (dd, J = 11.1, 3.8 Hz, 1H), 4.18 (d, J = 3 Hz, 1H), 4.09 – 4.06 (m, 3H), 3.95 – 3.80 (m, 9H), 3.77 – 3.64 (m, 11H), 3.60 (dd, J = 11.9, 6.3 Hz, 1H), 3.56 – 3.45 (m, 4H), 3.41 (tq, J = 12.6, 6.1 Hz, 2H), 2.73 (dd, J = 12.4, 4.7 Hz, 1H), 2.00 (s, 3H), 1.96 (s, 3H), 1.85 (p, J = 6.5 Hz, 2H), 1.77 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.85, 174.27, 173.72, 173.48, 102.36, 102.27, 99.65, 97.00, 77.87, 76.30, 75.33, 75.03, 74.37, 72.73, 71.85, 71.62, 70.35, 69.24, 68.66, 68.21, 67.91, 67.30, 64.75, 62.40, 61.05, 60.88, 59.60, 54.94, 51.51, 48.37, 48.05, 39.47, 27.82, 22.04, 21.87; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{25}$ $[M-H]^-$ 975.3535, found 975.3499.



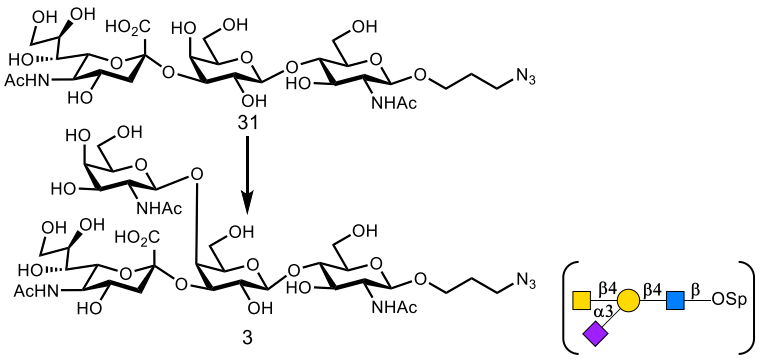
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (42)

Pentasaccharide **42** was prepared according to general procedure of multienzyme cascade α2,3-sialylation system with NmCSS and PmST3. After lyophilization, **42** was obtained as white solid (16 mg, 92%); ^{1}H NMR (600 MHz, D_2O) δ 4.68 (d, J = 8.4 Hz, 1H), 4.55 (d, J = 7.9 Hz, 1H), 4.47 (d, J = 8.0 Hz, 1H), 4.42 (d, J = 7.9 Hz, 1H), 4.14 (d, J = 3.3 Hz, 1H), 4.10 (m, 3H), 4.01 – 3.84 (m, 9H), 3.81 – 3.69 (m, 12H), 3.65 – 3.54 (m, 9H), 3.44 (t, J = 6.7 Hz, 2H), 3.31 – 3.28 (m, 1H), 2.76 (dd, J = 12.4, 4.6 Hz, 1H), 2.02 (s, 3H), 1.90 (p, J = 6.6 Hz, 2H), 1.80 (t, J = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.68, 174.80, 173.80, 102.84, 102.71, 102.44, 102.01, 99.72, 81.95, 78.25, 77.87, 75.38, 75.08, 74.80, 74.68, 74.46, 74.26, 72.68, 72.51, 72.05, 71.73, 69.86, 69.29, 68.23, 68.00, 67.91, 67.36, 67.27, 62.43, 60.94, 60.87, 59.95, 59.74, 55.08, 51.28, 47.77, 39.60, 28.13, 22.07; HRMS (ESI) *m/z* calcd for $C_{40}H_{67}N_5O_{30}$ $[M-H]^-$ 1096.3798, found 1096.3748.



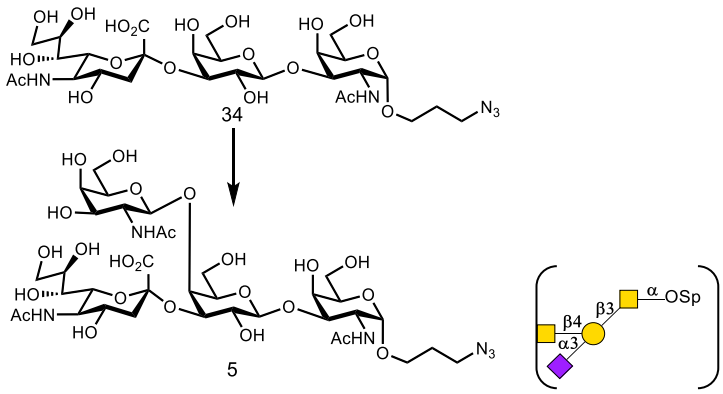
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

Tetrasaccharide **1** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **1** was obtained as white solid (29 mg, yield 91%); ^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.5 Hz, 1H), 4.55 (d, *J* = 8.4 Hz, 1H), 4.52 (d, *J* = 7.9 Hz, 1H), 4.13 (dd, *J* = 9.8, 3.1 Hz, 1H), 4.10 (d, *J* = 3.1 Hz, 1H), 3.98 (dt, *J* = 11.0, 5.6 Hz, 1H), 3.95 – 3.46 (m, 23H), 3.40 – 3.33 (m, 3H), 2.67 (dd, *J* = 12.6, 4.7 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.91 (t, *J* = 12.1 Hz, 1H), 1.85 (p, *J* = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.97, 174.82, 174.51, 174.03, 103.00, 102.68, 101.38, 100.91, 82.38, 76.77, 75.30, 74.65, 74.44, 73.99, 72.97, 72.23, 71.17, 69.59, 68.64, 68.62, 67.95, 67.73, 67.10, 62.72, 61.11, 60.72, 60.48, 54.38, 52.30, 51.53, 47.75, 37.16, 28.07, 22.55, 22.27, 22.01; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{24}$ $[M-H]^-$ 959.3586, found 959.3578.



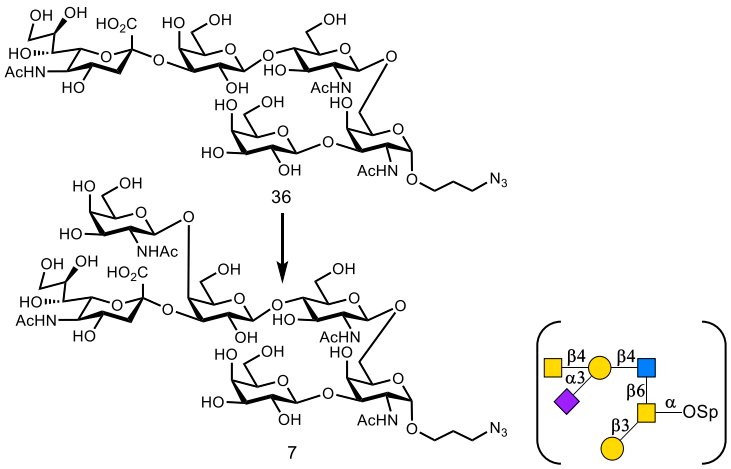
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (3)

Tetrasaccharide **3** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **3** was obtained as white solid (30 mg, 94%); ^{1}H NMR (600 MHz, D_2O) δ 4.73 (d, J = 8.5 Hz, 1H), 4.55 (d, J = 7.9 Hz, 1H), 4.52 (d, J = 7.9 Hz, 1H), 4.15 (dd, J = 9.8, 3.1 Hz, 1H), 4.12 (d, J = 3.1 Hz, 1H), 4.01 – 3.95 (m, 2H), 3.93 – 3.56 (m, 21H), 3.48 (dd, J = 10.1, 2.1 Hz, 1H), 3.39 – 3.34 (m, 3H), 2.66 (dd, J = 12.6, 4.6 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.93 (t, J = 12.1 Hz, 1H), 1.84 (p, J = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.96, 174.79, 174.45, 174.04, 102.72, 102.56, 101.60, 101.13, 78.84, 77.14, 74.66, 74.26, 73.96, 73.03, 72.34, 72.24, 71.22, 69.99, 68.67, 67.96, 67.72, 67.08, 62.79, 61.11, 60.48, 60.06, 54.92, 52.29, 51.55, 47.74, 36.88, 28.07, 22.56, 22.13, 22.01; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{24}$ $[M-H]^-$ 959.3586, found 959.3572.



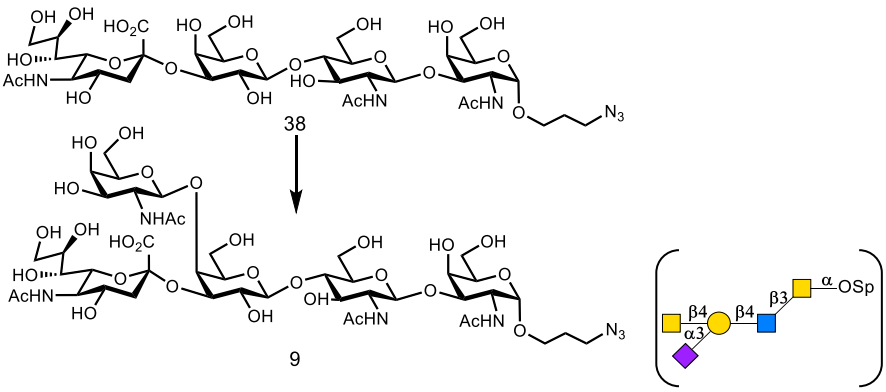
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galacopyranoside (5)

Tetrasaccharide **5** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **5** was obtained as white solid (14 mg, 93%); ^{1}H NMR (600 MHz, D_2O) δ 4.86 (d, *J* = 3.8 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.52 (d, *J* = 7.9 Hz, 1H), 4.30 (dd, *J* = 11.0, 3.7 Hz, 1H), 4.17 (d, *J* = 3.1 Hz, 1H), 4.10 – 4.06 (m, 2H), 3.99 (dd, *J* = 11.1, 3.1 Hz, 1H), 3.96 (dd, *J* = 7.0, 5.5 Hz, 1H), 3.91 – 3.86 (m, 2H), 3.84 (dd, *J* = 12.0, 2.3 Hz, 1H), 3.81 – 3.64 (m, 13H), 3.59 (dd, *J* = 12.0, 6.7 Hz, 1H), 3.56 (dd, *J* = 9.2, 2.2 Hz, 1H), 3.51 (dt, *J* = 10.2, 6.0 Hz, 1H), 3.48 – 3.40 (m, 3H), 3.31 (dd, *J* = 9.6, 7.9 Hz, 1H), 2.63 (dd, *J* = 12.7, 4.7 Hz, 1H), 2.00 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.91 – 1.85 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 174.93, 174.86, 174.50, 174.04, 104.30, 102.65, 101.43, 97.19, 77.29, 76.83, 74.62, 74.29, 73.56, 72.93, 72.22, 71.08, 70.53, 69.57, 68.62, 68.61, 67.93, 67.68, 64.82, 62.74, 61.15, 61.09, 60.33, 52.27, 51.49, 48.55, 48.10, 37.00, 27.92, 22.53, 21.98, 21.96; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{24}$ $[M-H]^-$ 959.3586, found 959.3544.



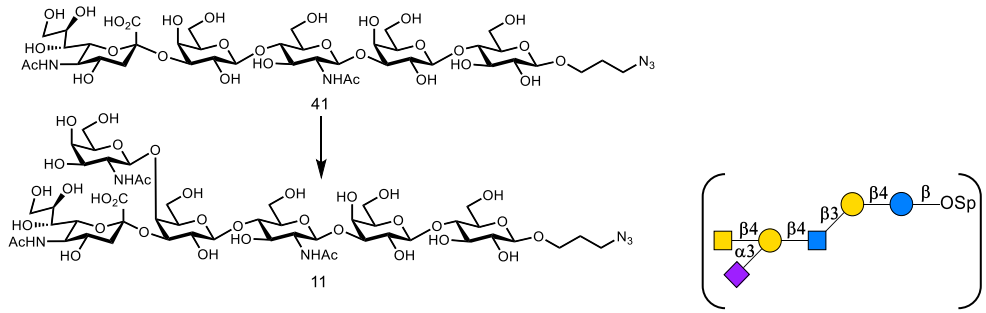
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (7)

Hexasaccharide **7** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **7** was obtained as white solid (10 mg, 92%); ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, *J* = 3.8 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.52 (d, *J* = 7.8 Hz, 1H), 4.52 (d, *J* = 9 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.30 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.20 (d, *J* = 3.2 Hz, 1H), 4.13 (dd, *J* = 9.8, 3.0 Hz, 1H), 4.09 (d, *J* = 3.1 Hz, 1H), 4.06 – 4.03 (m, 2H), 3.99 (td, *J* = 12.7, 12.0, 2.7 Hz, 2H), 3.90 – 3.55 (m, 26H), 3.50 – 3.40 (m, 6H), 3.33 (dd, *J* = 9.7, 7.9 Hz, 1H), 2.63 (dd, *J* = 12.6, 4.6 Hz, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.99 (s, 3H), 1.93 – 1.84 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 174.92, 174.76, 174.49, 174.17, 174.03, 104.66, 102.69, 102.53, 101.56, 101.44, 97.00, 78.79, 77.10, 76.91, 74.93, 74.64, 74.22, 73.93, 72.99, 72.42, 72.22, 71.17, 70.54, 69.95, 69.85, 69.32, 68.88, 68.64, 68.53, 67.92, 67.69, 64.52, 62.76, 61.09, 60.93, 60.45, 60.02, 54.84, 52.26, 51.51, 48.56, 48.17, 36.84, 27.88, 22.53, 22.17, 21.98, 21.93; HRMS (ESI) *m/z* calcd for $C_{50}H_{83}N_7O_{34}$ $[M-H]^-$ 1324.4908, found 1324.4894.



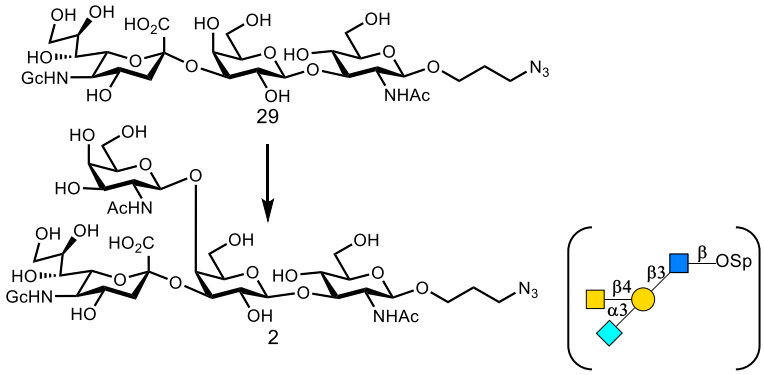
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (9)

Pentasaccharide **9** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **9** was obtained as white solid (14 mg, 92%); ^{1}H NMR (600 MHz, D_2O) δ 4.85 (d, J = 3.8 Hz, 1H), 4.73 (d, J = 8.5 Hz, 1H), 4.59 (d, J = 7.8 Hz, 1H), 4.56 (d, J = 7.9 Hz, 1H), 4.26 (dd, J = 11.1, 3.8 Hz, 1H), 4.22 (d, J = 3.1 Hz, 1H), 4.16 (dd, J = 9.8, 3.1 Hz, 1H), 4.12 (d, J = 3.1 Hz, 1H), 4.00 – 3.67 (m, 23H), 3.63 (dd, J = 12.1, 6.7 Hz, 1H), 3.60 (dd, J = 9.1, 2.1 Hz, 1H), 3.57 – 3.43 (m, 5H), 3.36 (dd, J = 9.8, 7.9 Hz, 1H), 2.67 (dd, J = 12.6, 4.6 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 1.94 – 1.88 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 174.97, 174.79, 174.39, 174.03, 173.60, 102.73, 102.55, 102.39, 101.58, 97.13, 78.65, 77.12, 76.45, 74.66, 74.45, 74.28, 73.99, 73.04, 72.24, 72.03, 71.20, 70.47, 70.00, 68.77, 68.66, 67.96, 67.72, 64.91, 62.79, 61.16, 61.12, 60.50, 59.83, 54.94, 52.30, 51.57, 48.49, 48.20, 36.91, 27.94, 22.58, 22.18, 22.02, 22.00; HRMS (ESI) *m/z* calcd for $C_{44}H_{73}N_7O_{29}$ $[M-H]^-$ 1162.4380, found 1162.4315.



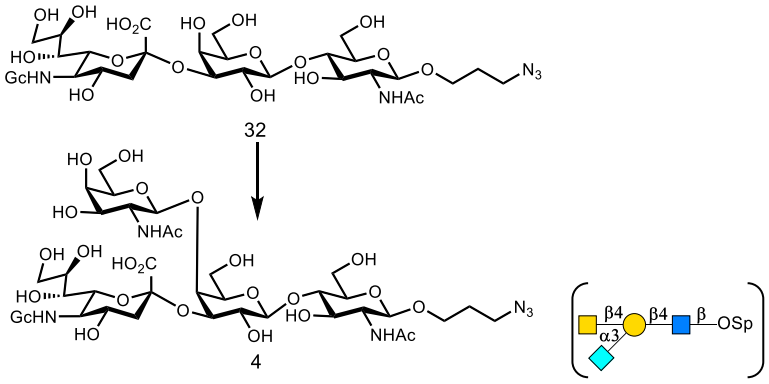
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (11)

Hexasaccharide **11** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **11** was obtained as white solid (12 mg, 90%); ^{1}H NMR (600 MHz, D_2O) δ 4.70 (d, J = 8.5 Hz, 1H), 4.67 (d, J = 8.3 Hz, 1H), 4.53 (d, J = 8.0 Hz, 1H), 4.46 (d, J = 8.0 Hz, 1H), 4.41 (d, J = 7.9 Hz, 1H), 4.13 (q, J = 4.6 Hz, 2H), 4.09 (d, J = 3.1 Hz, 1H), 4.00 – 3.53 (m, 32H), 3.47 – 3.45 (m, 1H), 3.43 (t, J = 6.8 Hz, 3H), 3.33 (dd, J = 9.8, 7.9 Hz, 1H), 3.30 – 3.26 (m, 1H), 2.63 (dd, J = 12.6, 4.6 Hz, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.93 – 1.86 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 174.92, 174.84, 174.76, 174.02, 102.88, 102.76, 102.69, 102.49, 102.04, 101.56, 81.97, 78.45, 78.26, 77.10, 74.83, 74.71, 74.64, 74.44, 74.29, 74.23, 73.93, 73.00, 72.71, 72.22, 72.11, 71.17, 69.96, 69.90, 68.65, 68.26, 67.92, 67.69, 67.30, 62.75, 61.09, 60.91, 60.46, 59.97, 59.81, 54.98, 52.26, 51.51, 47.79, 36.84, 28.17, 22.53, 22.10, 21.97; HRMS (ESI) *m/z* calcd for $C_{50}H_{83}N_7O_{34}$ $[M-H]^-$ 1283.4643, found 1283.4608.



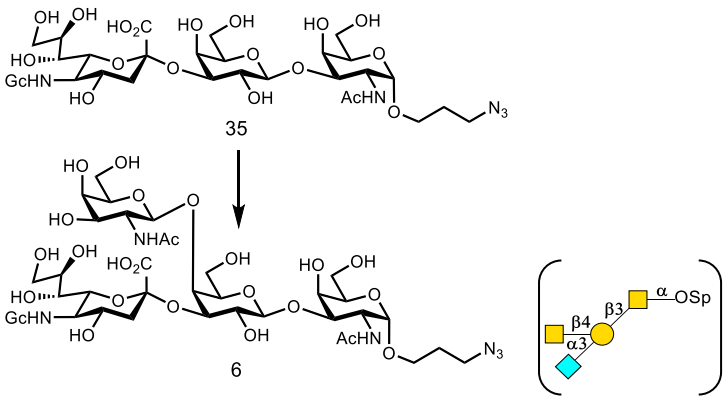
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (2)

Tetrasaccharide **2** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **2** was obtained as white solid (27 mg, 89%); ^{1}H NMR (600 MHz, D_2O) δ 4.70 (d, J = 8.5 Hz, 1H), 4.52 (d, J = 8.4 Hz, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.12 (dd, J = 9.7, 3.1 Hz, 1H), 4.09 (d, J = 8.1 Hz, 3H), 3.95 (dt, J = 10.9, 5.6 Hz, 1H), 3.92 – 3.56 (m, 21H), 3.52 (dd, J = 9.9, 8.4 Hz, 1H), 3.45 (ddd, J = 9.9, 5.7, 2.3 Hz, 1H), 3.37 – 3.31 (m, 3H), 2.66 (dd, J = 12.6, 4.7 Hz, 1H), 2.02 (s, 3H), 2.00 (s, 3H), 1.90 (t, J = 12.1 Hz, 1H), 1.82 (p, J = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 175.64, 174.76, 174.44, 173.99, 102.94, 102.62, 101.31, 100.85, 82.30, 76.67, 75.24, 74.59, 74.38, 73.93, 72.62, 72.24, 71.09, 69.53, 68.58, 68.30, 67.80, 67.67, 67.03, 62.61, 61.05, 60.88, 60.66, 60.42, 54.32, 52.24, 51.17, 47.68, 37.18, 28.01, 22.49, 22.21; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{25}$ $[M-H]^-$ 975.3535, found 975.3518.



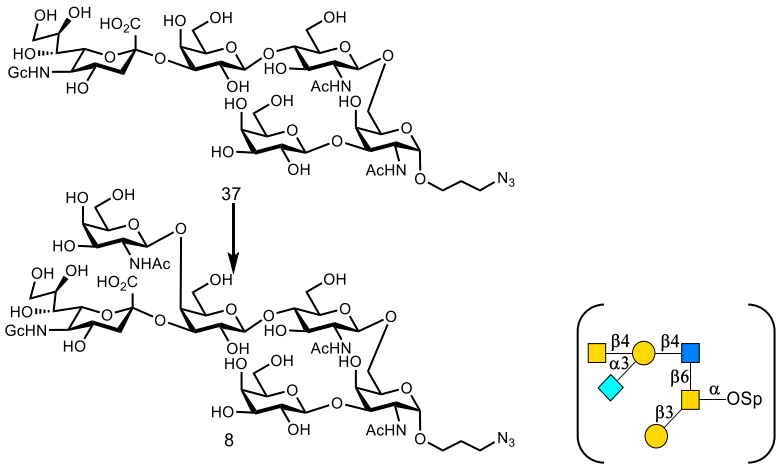
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (4)

Tetrasaccharide **4** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **4** was obtained as white solid (43 mg, 87%); ^{1}H NMR (500 MHz, D_2O) δ 4.74 (d, J = 8.5 Hz, 1H), 4.56 (d, J = 8.0 Hz, 1H), 4.53 (d, J = 8.1 Hz, 1H), 4.17 (dd, J = 9.8, 3.0 Hz, 1H), 4.12 (s, 3H), 4.03 – 3.57 (m, 24H), 3.41 – 3.35 (m, 3H), 2.68 (dd, J = 12.6, 4.4 Hz, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 1.95 (t, J = 11.8 Hz, 1H), 1.85 (p, J = 6.5 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 175.68, 174.76, 174.43, 174.06, 102.69, 102.53, 101.58, 101.10, 78.78, 77.09, 74.64, 74.23, 73.93, 72.71, 72.31, 72.28, 71.17, 69.97, 68.39, 67.84, 67.69, 67.05, 62.72, 61.09, 60.90, 60.45, 60.02, 54.90, 52.26, 51.22, 47.70, 36.91, 28.05, 22.54, 22.10; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{25}$ $[M-H]^-$ 975.3535, found 975.3509.



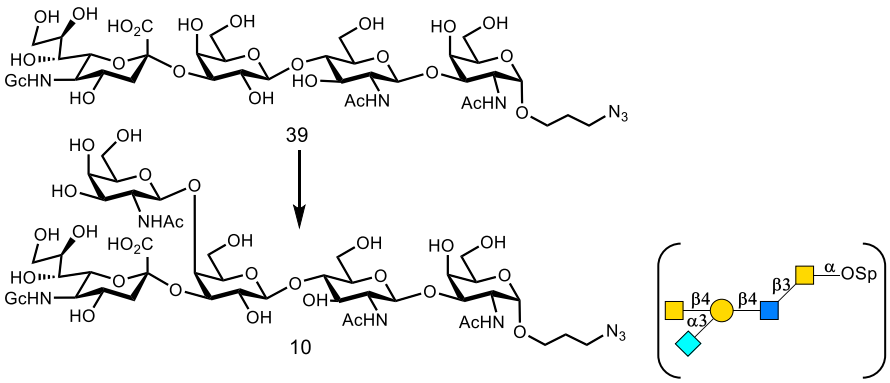
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galacopyranoside (6)

Tetrasaccharide **6** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **6** was obtained as white solid (43 mg, 87%); ^{1}H NMR (500 MHz, D_2O) δ 4.90 (d, *J* = 3.8 Hz, 1H), 4.74 (d, *J* = 8.5 Hz, 1H), 4.56 (d, *J* = 7.9 Hz, 1H), 4.33 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.20 (d, *J* = 3.0 Hz, 1H), 4.15 – 4.10 (m, 4H), 4.02 (dd, *J* = 11.1, 3.1 Hz, 1H), 3.99 (t, *J* = 6.3 Hz, 1H), 3.94 – 3.68 (m, 16H), 3.65 – 3.58 (m, 3H), 3.55 (dt, *J* = 10.3, 6.1 Hz, 1H), 3.52 – 3.42 (m, 2H), 3.35 (dd, *J* = 9.6, 7.9 Hz, 1H), 2.68 (dd, *J* = 12.6, 4.5 Hz, 1H), 2.03 (s, 3H), 2.02 (s, 3H), 1.96 – 1.88 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 175.68, 174.87, 174.50, 174.07, 104.31, 102.65, 101.43, 97.19, 77.29, 76.80, 74.63, 74.30, 73.57, 72.65, 72.30, 71.07, 70.53, 69.58, 68.63, 68.36, 67.85, 67.69, 64.83, 62.70, 61.15, 61.09, 60.91, 60.33, 52.27, 51.19, 48.55, 48.11, 37.09, 27.92, 22.54, 21.97; HRMS (ESI) *m/z* calcd for $C_{36}H_{60}N_6O_{25}$ $[M-H]^-$ 975.3535, found 975.3523.



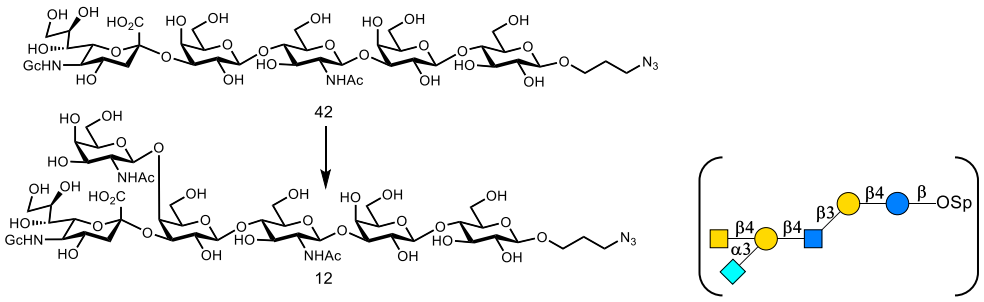
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (8)

Pentasaccharide **8** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **8** was obtained as white solid (23 mg, 88%); ^{1}H NMR (600 MHz, D_2O) δ 4.88 (d, J = 3.8 Hz, 1H), 4.75 (d, J = 8.5 Hz, 1H), 4.56 (d, J = 7.8 Hz, 1H), 4.55 (d, J = 8.4 Hz, 1H) 4.46 (d, J = 7.8 Hz, 1H), 4.33 (dd, J = 11.1, 3.7 Hz, 1H), 4.23 (d, J = 3.2 Hz, 1H), 4.17 (dd, J = 9.8, 3.1 Hz, 1H), 4.13 (s, 2H), 4.09 – 4.00 (m, 5H), 3.95 – 3.58 (m, 27H), 3.54 – 3.43 (m, 5H), 3.37 (dd, J = 9.6, 7.8 Hz, 1H), 2.69 (dd, J = 12.5, 4.5 Hz, 1H), 2.03 (d, J = 2.6 Hz, 9H), 1.95 (t, J = 12.0 Hz, 1H), 1.92 – 1.86 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 175.72, 174.80, 174.52, 174.20, 174.07, 104.68, 102.72, 102.58, 101.61, 101.47, 97.04, 78.87, 77.11, 76.95, 74.97, 74.68, 74.28, 73.98, 72.75, 72.47, 72.31, 71.23, 70.59, 69.99, 69.87, 69.36, 68.91, 68.57, 68.42, 67.89, 67.74, 64.60, 62.76, 61.12, 60.96, 60.49, 60.08, 54.88, 52.30, 51.27, 48.61, 48.22, 36.98, 27.91, 22.58, 22.21, 21.97; HRMS (ESI) *m/z* calcd for $C_{50}H_{83}N_7O_{35}$ $[M-H]^-$ 1340.4857, found 1340.4823.



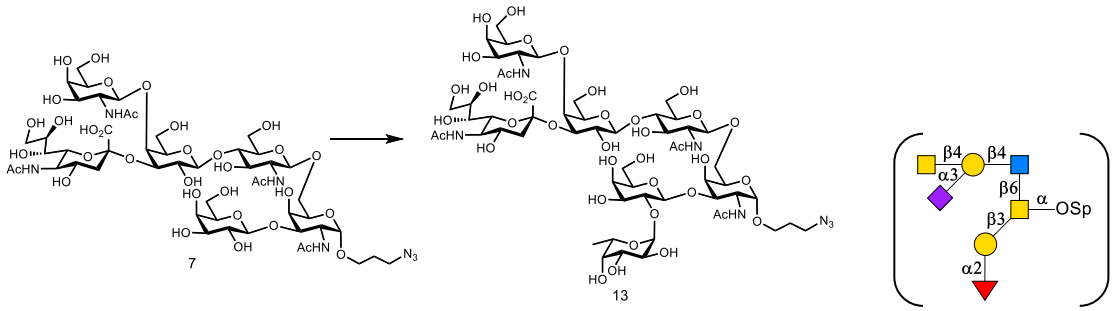
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (10)

Pentasaccharide **10** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **10** was obtained as white solid (14 mg, 92%); ^{1}H NMR (600 MHz, D_2O) δ 4.85 (d, J = 3.8 Hz, 1H), 4.74 (d, J = 8.6 Hz, 1H), 4.59 (d, J = 7.7 Hz, 1H), 4.57 (d, J = 7.9 Hz, 1H), 4.26 (dd, J = 11.1, 3.8 Hz, 1H), 4.22 (d, J = 3.1 Hz, 1H), 4.17 (dd, J = 9.8, 3.1 Hz, 1H), 4.13 (s, 3H), 4.00 – 3.67 (m, 23H), 3.65 – 3.60 (m, 3H), 3.58 – 3.51 (m, 2H), 3.50 – 3.43 (m, 2H), 3.37 (dd, J = 9.8, 7.9 Hz, 1H), 2.69 (dd, J = 12.6, 4.5 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.97 – 1.93 (m, 1H), 1.93 – 1.89 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 175.72, 174.80, 174.40, 174.06, 173.60, 102.73, 102.56, 102.40, 101.60, 97.13, 78.66, 77.10, 76.45, 74.67, 74.45, 74.29, 74.00, 72.76, 72.31, 72.04, 71.20, 70.47, 70.01, 68.78, 68.41, 67.88, 67.73, 64.91, 62.76, 61.17, 61.12, 60.96, 60.50, 59.84, 54.94, 52.30, 51.27, 48.49, 48.20, 36.98, 27.94, 22.59, 22.18, 22.01; HRMS (ESI) *m/z* calcd for $C_{44}H_{73}N_7O_{30}$ $[M-H]^-$ 1178.4329, found 1178.4283.



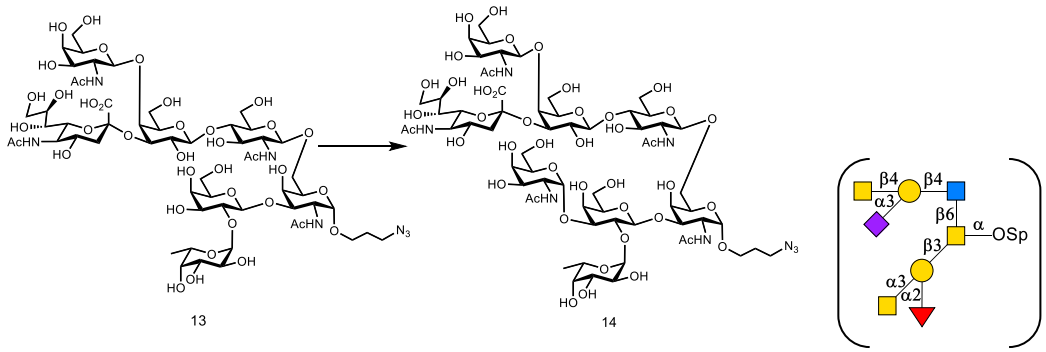
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (12)

Pentasaccharide **12** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with NahK/GlmU and CjCgtA. After lyophilization, **12** was obtained as white solid (16 mg, 90%); ^{1}H NMR (600 MHz, D_2O) δ 4.71 (d, J = 8.5 Hz, 1H), 4.67 (d, J = 8.3 Hz, 1H), 4.54 (d, J = 7.9 Hz, 1H), 4.46 (d, J = 8.0 Hz, 1H), 4.41 (d, J = 7.9 Hz, 1H), 4.16 – 4.12 (m, 3H), 4.10 (d, J = 5.4 Hz, 2H), 4.00 – 3.53 (m, 34H), 3.44 (t, J = 6.7 Hz, 2H), 3.34 (dd, J = 9.7, 7.9 Hz, 1H), 3.31 – 3.27 (m, 1H), 2.65 (dd, J = 12.6, 4.7 Hz, 1H), 2.01 (s, 3H), 1.99 (s, 3H), 1.94 – 1.86 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 175.64, 174.80, 174.72, 174.00, 102.84, 102.71, 102.65, 102.47, 102.00, 101.53, 81.94, 78.45, 78.26, 77.04, 74.79, 74.68, 74.60, 74.41, 74.26, 74.21, 73.91, 72.68, 72.24, 72.08, 71.14, 69.93, 69.87, 68.35, 68.22, 67.81, 67.66, 67.27, 62.68, 61.05, 60.87, 60.43, 59.95, 59.79, 54.96, 52.23, 51.19, 47.77, 36.90, 28.13, 22.51, 22.07; HRMS (ESI) *m/z* calcd for $C_{48}H_{80}N_6O_{35}$ $[M-H]^-$ 1299.4592, found 1299.4550.



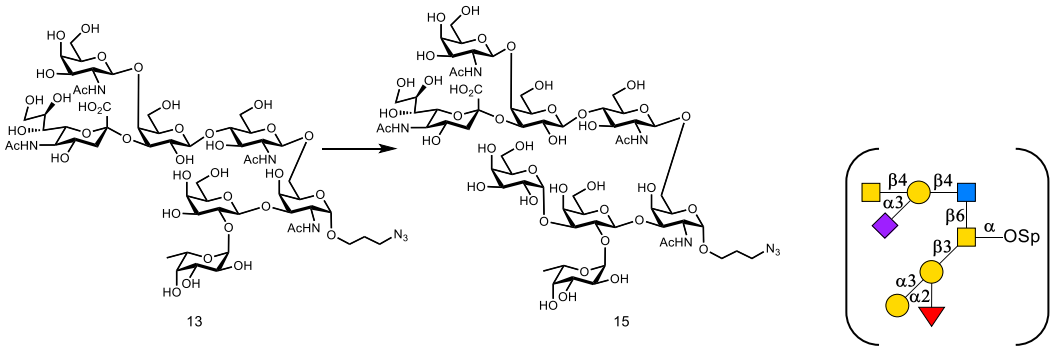
3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (13)

Heptasaccharide **13** was prepared according to general procedure of multienzyme cascade α1,2-fucosylation system with FKP and Hmα1,2FucT. After lyophilization, **13** was obtained as white solid (18 mg, 81%) as a white solid; ^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 4.1 Hz, 1H), 4.86 (d, *J* = 3.5 Hz, 1H), 4.74 (d, *J* = 8.5 Hz, 1H), 4.63 (d, *J* = 7.7 Hz, 1H), 4.56 (d, *J* = 7.8 Hz, 2H), 4.23 (q, *J* = 6.6 Hz, 1H), 4.19 – 4.07 (m, 7H), 4.01 (dd, *J* = 12.3, 2.2 Hz, 1H), 3.94 – 3.59 (m, 30H), 3.51 – 3.42 (m, 5H), 3.36 (dd, *J* = 9.8, 7.9 Hz, 1H), 2.67 (dd, *J* = 12.6, 4.6 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (d, *J* = 1.0 Hz, 6H), 1.96 – 1.92 (m, 1H), 1.89 (q, *J* = 6.3 Hz, 2H), 1.20 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, D_2O) δ 174.97, 174.79, 174.23, 174.04, 173.60, 102.72, 102.58, 101.99, 101.60, 101.53, 99.21, 96.57, 78.85, 77.14, 76.19, 75.00, 74.67, 74.27, 73.98, 73.84, 73.50, 73.04, 72.48, 72.25, 71.80, 71.23, 70.07, 69.99, 69.55, 69.35, 69.27, 69.07, 68.67, 68.04, 67.97, 67.73, 66.77, 64.41, 62.80, 61.12, 60.92, 60.49, 60.07, 59.42, 54.89, 52.30, 51.56, 49.42, 48.15, 36.91, 27.98, 22.57, 22.21, 22.01, 21.87, 15.35; HRMS (ESI) *m/z* calcd for $C_{56}H_{93}N_7O_{38}$ $[M-H]^-$ 1470.5487, found 1470.5464.



3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-{2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)}-2-acetamido-2-deoxy-α-D-galactopyranoside (14)

Octasaccharide **14** was prepared according to general procedure of multienzyme cascade β1,4-*N*-acetygalactosaminylation system with Nahk/GlmU and BgtA. After lyophilization, **14** was obtained as white solid (8 mg, yield 72%); ^{1}H NMR (600 MHz, D_2O) δ 5.25 (d, *J* = 4.2 Hz, 1H), 5.14 (d, *J* = 3.9 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.64 (d, *J* = 7.6 Hz, 1H), 4.43 (d, *J* = 7.2 Hz, 1H), 4.42 (d, *J* = 8.4 Hz, 1H), 4.26 (td, *J* = 8.3, 7.8, 5.5 Hz, 2H), 4.22 – 3.52 (m, 45H), 3.49 – 3.39 (m, 4H), 3.33 (dd, *J* = 9.7, 7.9 Hz, 1H), 2.63 (dd, *J* = 12.6, 4.6 Hz, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (d, *J* = 1.0 Hz, 6H), 1.93 – 1.83 (m, 3H), 1.17 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, D_2O) δ 174.92, 174.75, 174.20, 174.02, 173.56, 102.69, 102.52, 102.05, 101.56, 101.48, 98.68, 96.44, 91.15, 78.77, 77.10, 75.31, 74.77, 74.63, 74.21, 73.93, 72.99, 72.74, 72.43, 72.22, 71.73, 71.17, 70.88, 70.06, 69.95, 69.88, 69.51, 69.34, 69.27, 68.64, 68.46, 67.91, 67.68, 67.65, 67.57, 66.83, 64.27, 62.91, 62.75, 61.33, 61.08, 60.93, 60.45, 60.00, 54.84, 52.25, 51.51, 49.50, 49.30, 48.06, 36.83, 27.95, 22.53, 22.17, 21.97, 21.88, 15.27; HRMS (ESI) *m/z* calcd for $C_{62}H_{103}N_7O_{43}$ $[M-H]^-$ 1673.6281, found 1673.6248.

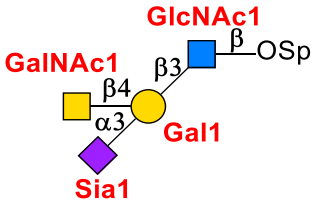


3-Azidopropyl 3,5-dideoxy-5-hydroxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-[2-acetamido-2-deoxy-β-D-galacopyranosyl]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-{α-D-galactopyranosyl-(1→3)-[L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)}-2-acetamido-2-deoxy-α-D-galactopyranoside (15)

Octasaccharide **15** was prepared according to general procedure of multienzyme cascade β1,4-galactosylation system with GalK, BLUSP and GTB. After lyophilization, **15** was obtained as white solid (9 mg, yield 50%); ^{1}H NMR (600 MHz, D_2O) δ 5.23 (d, *J* = 4.3 Hz, 1H), 5.21 (d, *J* = 3.7 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.66 (d, *J* = 7.6 Hz, 1H), 4.39 (d, *J* = 7.8 Hz, 1H), 4.38 (d, *J* = 9 Hz, 1H), 4.27 – 4.04 (m, 9H), 3.99 – 3.52 (m, 38H), 3.47 – 3.39 (m, 4H), 3.33 (dd, *J* = 9.7, 7.9 Hz, 1H), 2.63 (dd, *J* = 12.6, 4.6 Hz, 1H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 6H), 1.93 – 1.83 (m, 3H), 1.16 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, D_2O) δ 174.92, 174.75, 174.19, 174.02, 173.55, 102.69, 102.52, 102.14, 101.56, 101.49, 98.75, 96.42, 92.82, 78.76, 77.10, 75.84, 74.63, 74.57, 74.27, 74.21, 73.93, 72.99, 72.74, 72.43, 72.22, 71.73, 71.17, 70.96, 70.16, 69.95, 69.52, 69.39, 69.29, 69.22, 68.64, 68.03, 67.91, 67.69, 67.58, 66.80, 64.25, 63.33, 62.75, 61.29, 61.09, 60.90, 60.45, 60.00, 54.85, 52.25, 51.51, 49.29, 48.06, 36.83, 27.96, 22.53, 22.17, 21.98, 21.88, 15.27; HRMS (ESI) *m/z* calcd for $C_{62}H_{103}N_7O_{43}$ $[M-H]^-$ 1632.6015, found 1632.5996.

4. NMR assignment data for final products

NMR assignment of compound 1



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GlcNAc1	4.55	3.82	3.77	3.54	N/A[a]	N/A	2.02-2.04	100.92
Gal1	4.52	3.36	4.12	4.09	N/A	N/A	-	102.99
GalNAc1	4.72	3.90	3.67	3.89	N/A	N/A	2.02-2.04	102.68
Sia1[c]	- [b]	-	2.67, 1.91	3.70	3.81	3.49	2.02-2.04	101.38

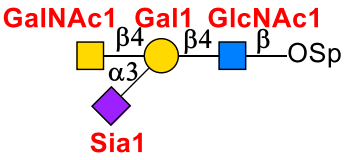
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.98, 3.68	1.85	3.38
C	67.06	28.07	47.71

NMR assignment of compound 3



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GlcNAc1	4.52	3.73	3.58	3.68	N/A[a]	N/A	2.01-2.05	101.13
Gal1	4.55	3.36	4.17	4.13	N/A	N/A	-	102.56
GalNAc1	4.73	3.93	3.69	3.83	N/A	N/A	2.01-2.05	102.72
Sia1[c]	- [b]	-	2.66, 1.93	3.78	3.82	3.49	2.01-2.05	101.60

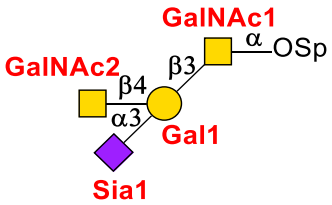
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.97, 3.67	1.84	3.38
C	67.08	28.07	47.74

NMR assignment of compound 5



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.86	4.30	4.00	4.17	N/A[a]	N/A	1.99 or 2.00	97.19
GalNAc2	4.70	3.90	3.67	3.89	N/A	N/A	1.99 or 2.00	102.65
Gal1	4.52	3.33	4.09	4.08	N/A	N/A	-	104.30
Sia1[c]	- [b]	-	2.63, 1.88	3.75	3.80	3.47	1.99 or 2.00	101.43

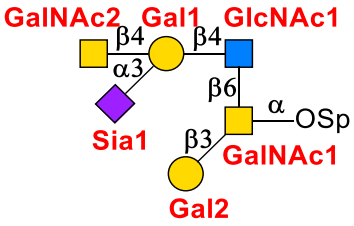
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.79, 3.52	1.89	3.45
C	64.82	27.92	48.10

NMR assignment of compound 7



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.84	4.29	4.01	4.20	N/A[a]	3.70	1.99-2.01	97.00
GalNAc2	4.70	3.89	3.66	3.90	N/A	N/A	1.99-2.01	102.69
GlcNAc1	4.52	3.74	3.57	3.66	N/A	N/A	1.99-2.01	101.44
Gal1	4.52	3.33	4.13	4.09	N/A	N/A	-	102.53
Gal2	4.43	3.48	3.60	3.88	N/A	N/A	-	104.66
Sia1[c]	- [b]	-	2.63, 1.91	3.74	3.80	3.46	1.99-2.01	101.56

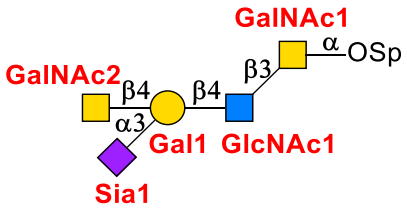
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.72, 3.48	1.88	3.42
C	64.52	27.88	48.17

NMR assignment of compound 9



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.76	4.26	3.97	4.22	N/A[a]	N/A	2.02-2.05	97.13
GalNAc2	4.64	3.90	3.70	3.92	N/A	N/A	2.02-2.05	102.73
GlcNAc1	4.50	3.75	3.56	3.71	N/A	N/A	2.02-2.05	102.39
Gal1	4.47	3.37	4.15	4.11	N/A	N/A	-	102.55
Sia1[c]	- [b]	-	2.67, 1.91	3.77	3.83	3.49	2.02-2.05	101.60

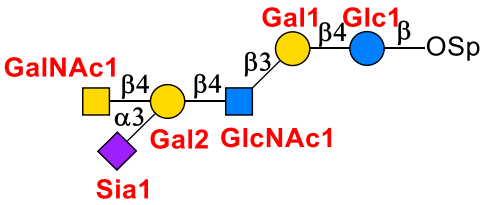
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.80, 3.63	1.91	3.47
C	64.91	27.94	48.20

NMR assignment of compound 11



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.46	3.28	3.62	3.62	N/A[a]	N/A	-	102.04
Gal1	4.41	3.57	3.70	4.14	N/A	N/A	-	102.88
Gal2	4.53	3.33	4.13	4.09	N/A	N/A	-	102.49
GlcNAc1	4.67	3.77	3.67	3.69	N/A	N/A	1.99 or 2.01	102.76
GalNAc1	4.70	3.89	3.66	3.86	N/A	N/A	1.99 or 2.01	102.69
Sia1[c]	- [b]	-	2.63, 1.90	3.75	3.81	3.46	1.99 or 2.01	101.56

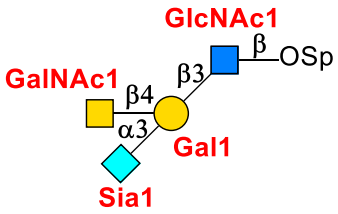
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.98, 3.75	1.90	3.45
C	67.30	28.17	47.79

NMR assignment of compound 2



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GlcNAc1	4.52	3.78	3.74	3.52	3.46	3.83, 3.60	2.00 or 2.02	100.92
Gal1	4.49	3.32	4.12	4.08	N/A[a]	N/A	-	102.99
GalNAc1	4.70	3.89	3.67	3.86	N/A	N/A	2.00 or 2.02	102.68
Sia1[c]	- [b]	-	2.66, 1.90	3.83	3.89	3.60	4.09 (NGc)	101.38

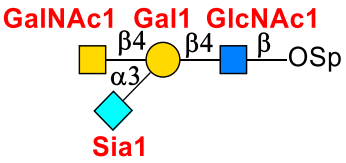
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.95, 3.65	1.82	3.36
C	67.03	28.01	47.68

NMR assignment of compound 4



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GlcNAc1	4.50	3.71	3.56	3.65	N/A[a]	N/A	2.00 or 2.02	101.10
Gal1	4.53	3.33	4.15	4.10	N/A	N/A	-	102.53
GalNAc1	4.71	3.89	3.65	N/A	N/A	N/A	2.00 or 2.02	102.70
Sia1[c]	- [b]	-	2.65, 1.92	3.85	3.89	3.60	4.10 (NGc)	101.58

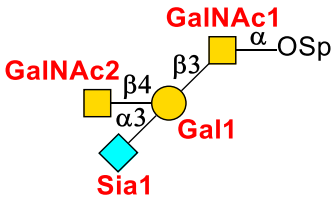
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.95, 3.65	1.82	3.35
C	67.05	28.05	47.70

NMR assignment of compound 6



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.87	4.31	4.00	4.18	N/A[a]	N/A	1.99 or 2.00	97.19
GalNAc2	4.71	3.89	3.67	3.89	N/A	N/A	1.99 or 2.00	102.65
Gal1	4.53	3.32	4.10	4.08	N/A	N/A	-	104.31
Sia1[c]	- [b]	-	2.65, 1.89	3.84	3.89	3.60	4.10 (NGc)	101.43

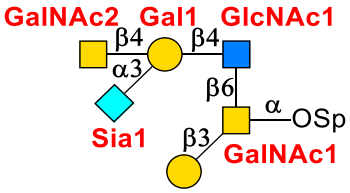
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.79, 3.53	1.89	3.46
C	64.82	27.92	48.11

NMR assignment of compound 8



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.88	4.34	4.03	4.24	N/A[a]	3.73	2.03	97.04
GalNAc2	4.75	3.92	3.70	3.91	N/A	N/A	2.03	102.72
GlcNAc1	4.56	3.77	3.61	3.69	N/A	N/A	2.03	101.47
Gal1	4.56	3.37	4.18	4.13	N/A	N/A	-	102.58
Gal2	4.46	3.53	3.63	3.93	N/A	N/A	-	104.68
Sia1[c]	- [b]	-	2.69, 1.92	3.88	3.92	3.63	4.13 (NGc)	101.61

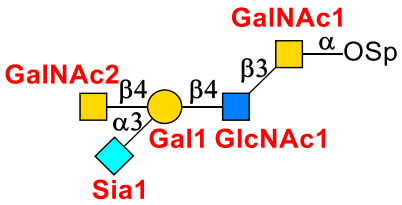
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.76, 3.52	1.92	3.50
C	64.59	27.91	48.22

NMR assignment of compound 10



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.85	4.27	3.99	4.23	N/A[a]	N/A	2.02-2.06	97.13
GalNAc2	4.74	3.92	3.69	3.93	N/A	N/A	2.02-2.06	102.73
GlcNAc1	4.59	3.74	3.56	3.71	N/A	N/A	2.02-2.06	102.40
Gal1	4.57	3.35	4.17	4.12	N/A	N/A	-	102.56
Sia1[c]	- [b]	-	2.69, 1.94	3.86	3.92	3.63	4.13 (NGc)	100.57

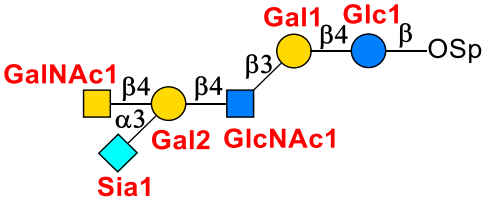
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.80, 3.54	1.91	3.47
C	64.91	27.94	48.20

NMR assignment of compound 12



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.46	3.29	3.62	3.62	N/A[a]	N/A	-	102.00
Gal1	4.41	3.57	3.70	4.14	N/A	N/A	-	102.84
Gal2	4.54	3.34	4.15	4.09	N/A	N/A	-	102.47
GlcNAc1	4.67	3.76	3.73	3.69	3.57	N/A	1.99 or 2.01	102.71
GalNAc1	4.71	3.89	3.66	3.90	N/A	N/A	1.99 or 2.01	102.65
Sia1[c]	- [b]	-	2.65, 1.90	3.87	3.88	3.60	4.09 (NGc)	101.53

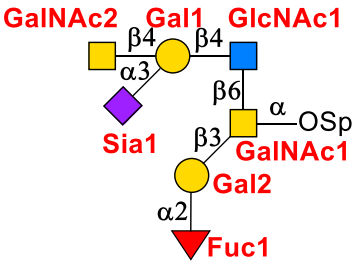
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.99, 3.74	1.91	3.44
C	67.27	28.13	47.77

NMR assignment of compound 13



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.86	4.17	4.12	4.14	N/A[a]	3.70	2.02-2.05	96.57
GalNAc2	4.74	3.95	3.67	3.93	N/A	N/A	2.02-2.05	102.72
GlcNAc1	4.55	3.76	3.57	3.70	N/A	N/A	2.02-2.05	101.53
Gal1	4.55	3.36	4.15	4.12	N/A	N/A	-	102.58
Gal2	4.63	3.66	3.83	3.90	N/A	N/A	-	101.99
Fuc1	5.24	3.78	3.76	3.67	4.23	1.20	-	99.21
Sia1[c]	- [b]	-	2.67, 1.94	3.78	3.83	3.48	2.02-2.05	101.60

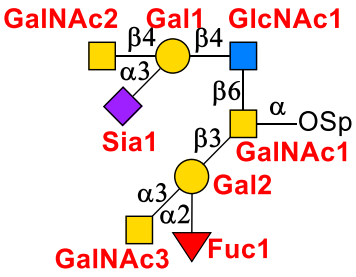
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.74, 3.46	1.90	3.47
C	64.41	27.98	48.15

NMR assignment of compound 14



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.85	4.10	4.11	4.17	N/A[a]	3.70	1.99-2.02	96.44
GalNAc2	4.70	3.88	3.64	3.86	N/A	N/A	1.99-2.02	102.69
GalNAc3	5.14	4.25	3.89	4.20	N/A	N/A	1.99-2.02	91.16
GlcNAc1	4.52	3.73	3.57	3.65	N/A	N/A	1.99-2.02	101.48
Gal1	4.52	3.33	4.12	4.08	N/A	N/A	-	102.52
Gal2	4.64	3.84	3.91	4.19	N/A	N/A	-	102.05
Fuc1	5.25	3.73	3.54	3.45	4.27	1.70	-	98.68
Sia1[c]	- [b]	-	2.63, 1.90	3.75	3.91	3.46	1.99-2.02	101.56

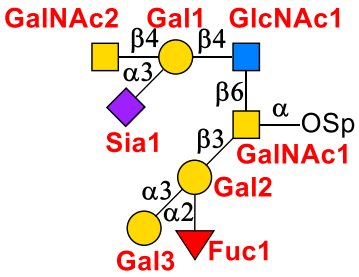
[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.7, 3.42	1.85	3.42
C	64.27	27.95	48.06

NMR assignment of compound 15



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
GalNAc1	4.85	4.10	4.10	4.17	N/A[a]	3.70	1.99-2.02	96.42
GalNAc2	4.70	3.88	3.65	3.92	N/A	N/A	1.99-2.02	102.68
GlcNAc1	4.52	3.73	3.55	3.65	N/A	N/A	1.99-2.02	101.49
Gal1	4.52	3.33	4.13	4.10	N/A	N/A	-	102.52
Gal2	4.66	3.88	3.92	4.25	N/A	N/A	-	102.14
Gal3	5.21	3.85	3.93	4.18	N/A	N/A	-	92.82
Fuc1	5.23	3.74	3.56	3.45	4.24	1.16	-	98.75
Sia1[c]	- [b]	-	2.63, 1.91	3.74	3.97	3.46	1.99-2.02	101.56

[a] Not applicable

[b] Not assigned

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH_2N_3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
H	3.69, 3.42	1.86	3.41
C	64.25	27.96	48.05

5. References

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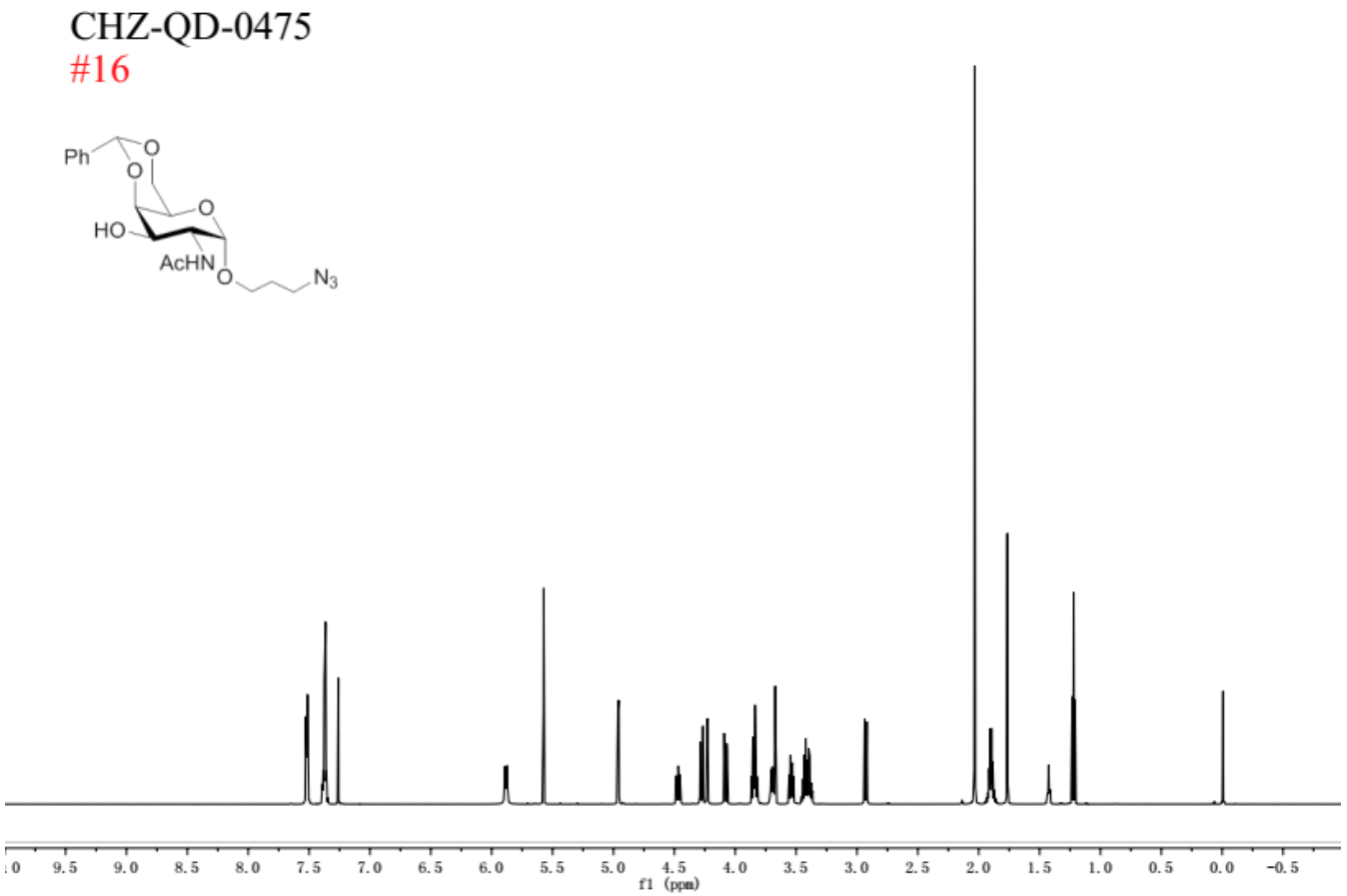
6. NMR Spectra



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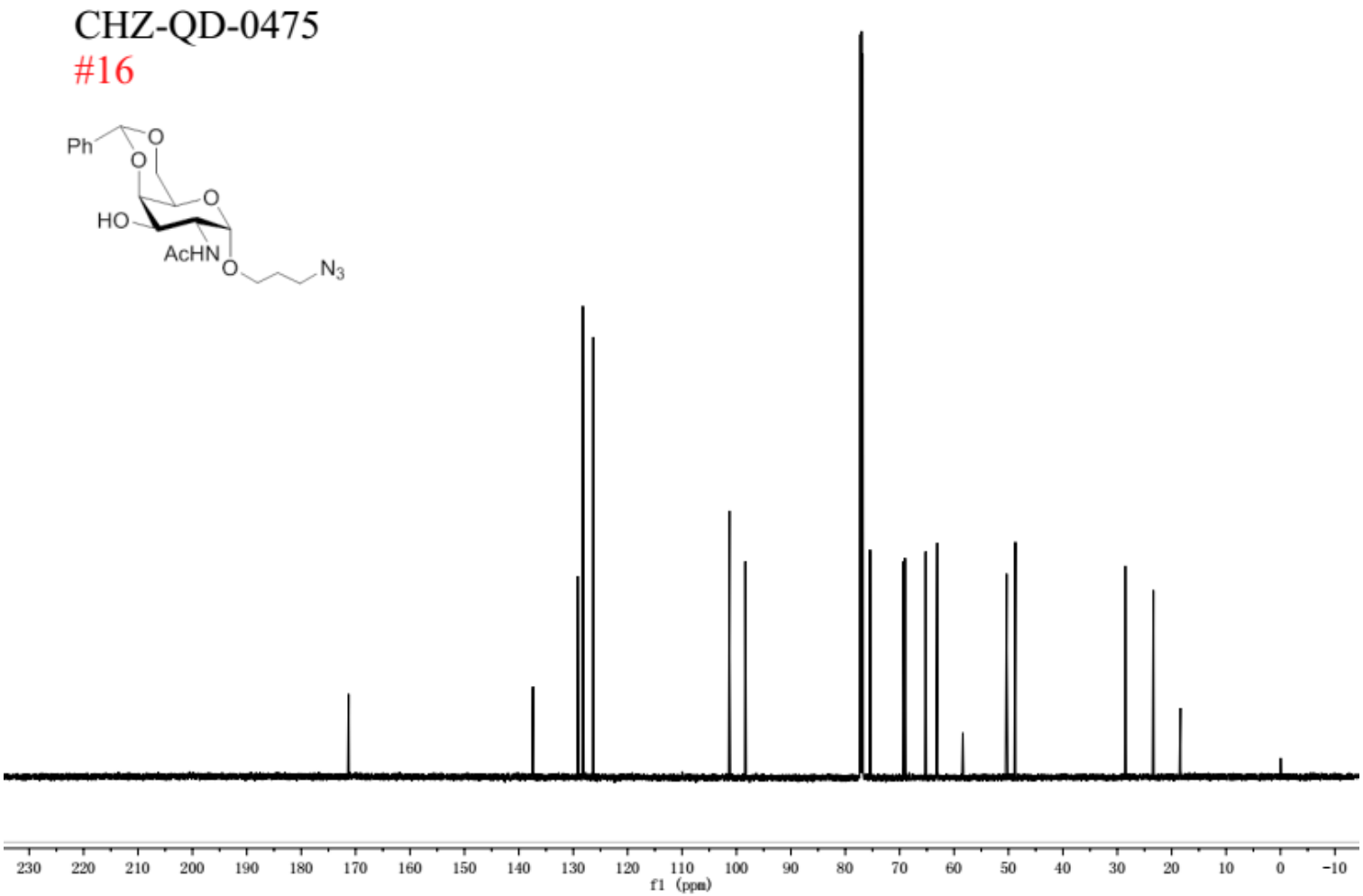
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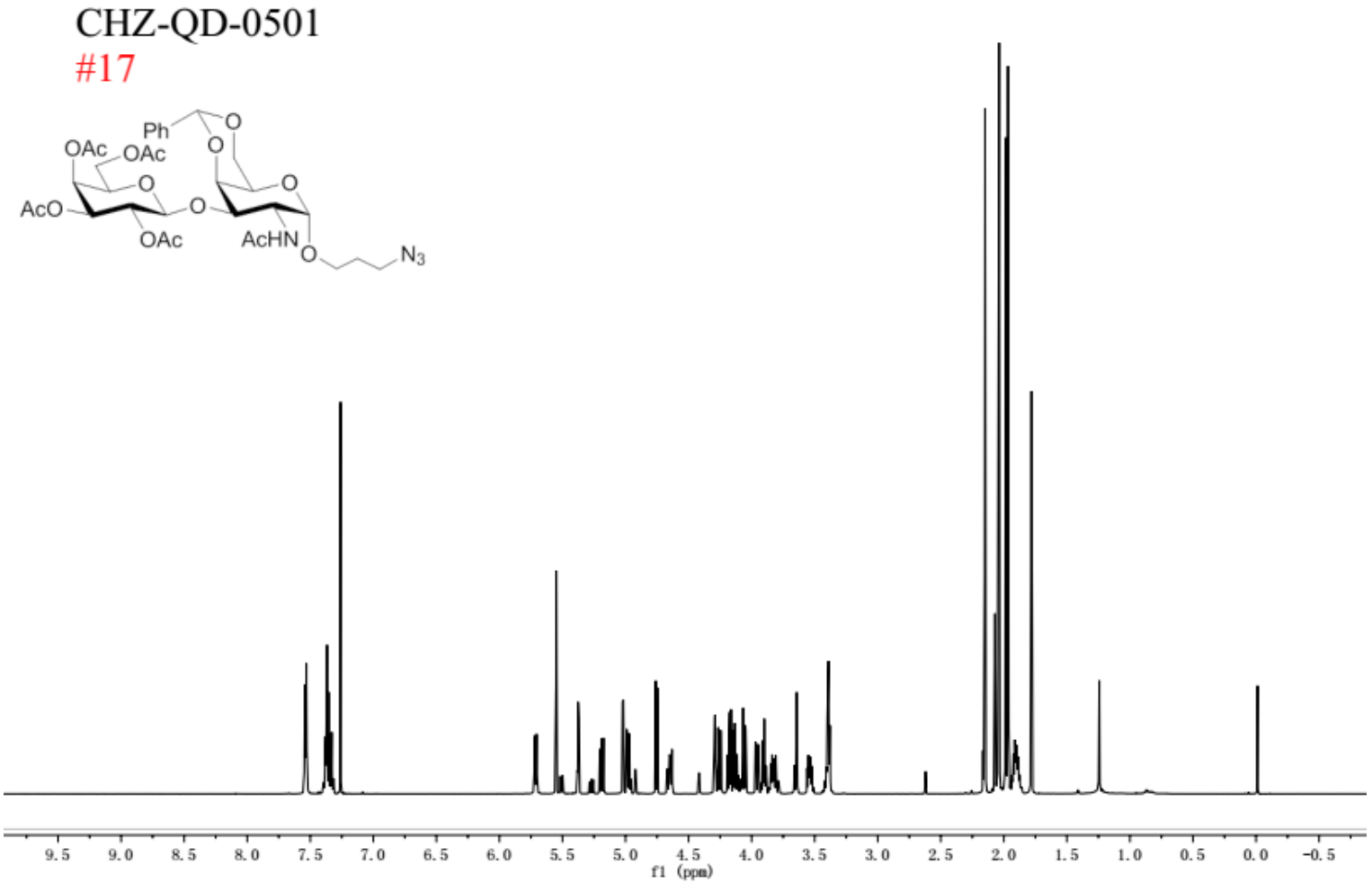
^{13}C NMR of 16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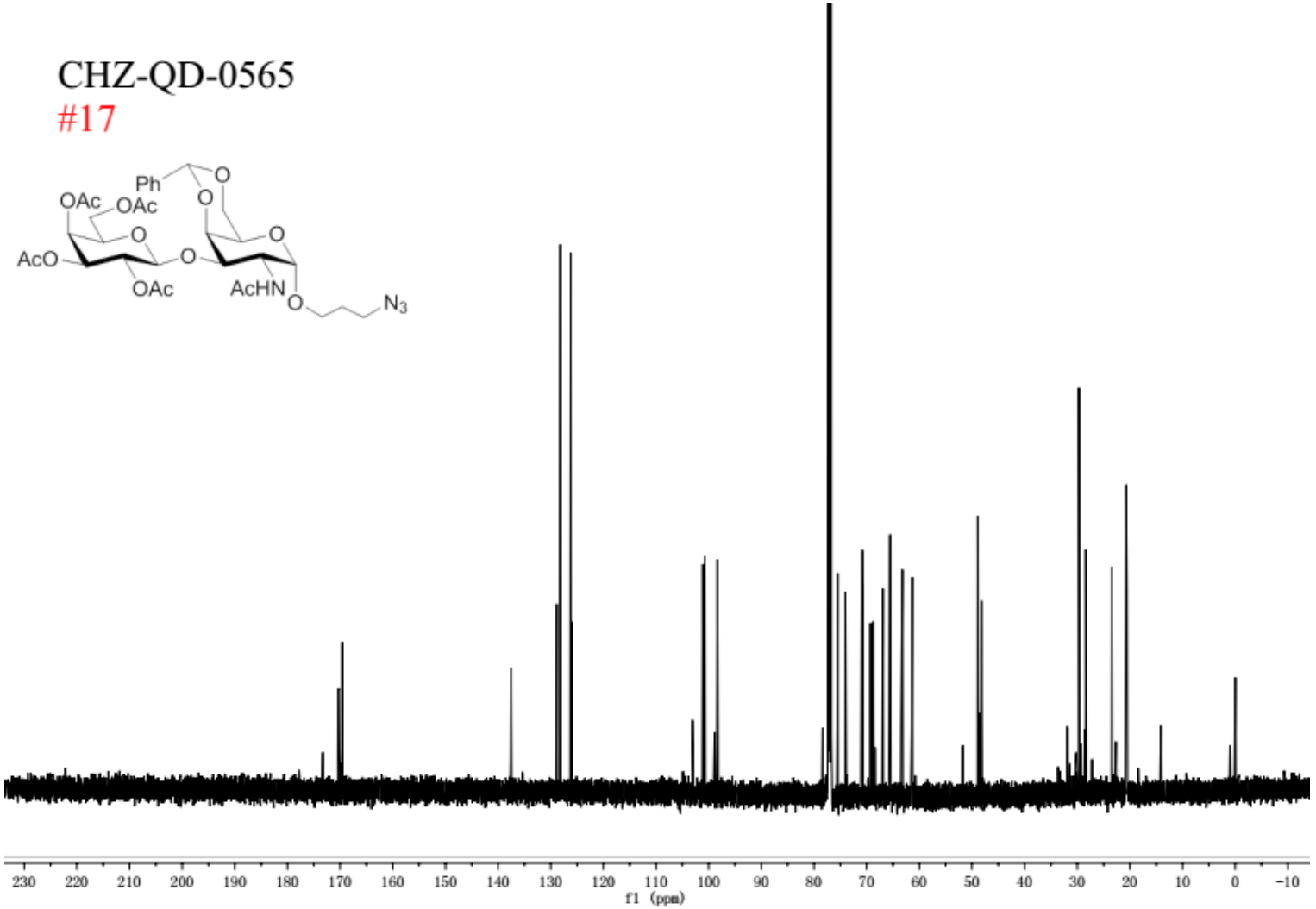
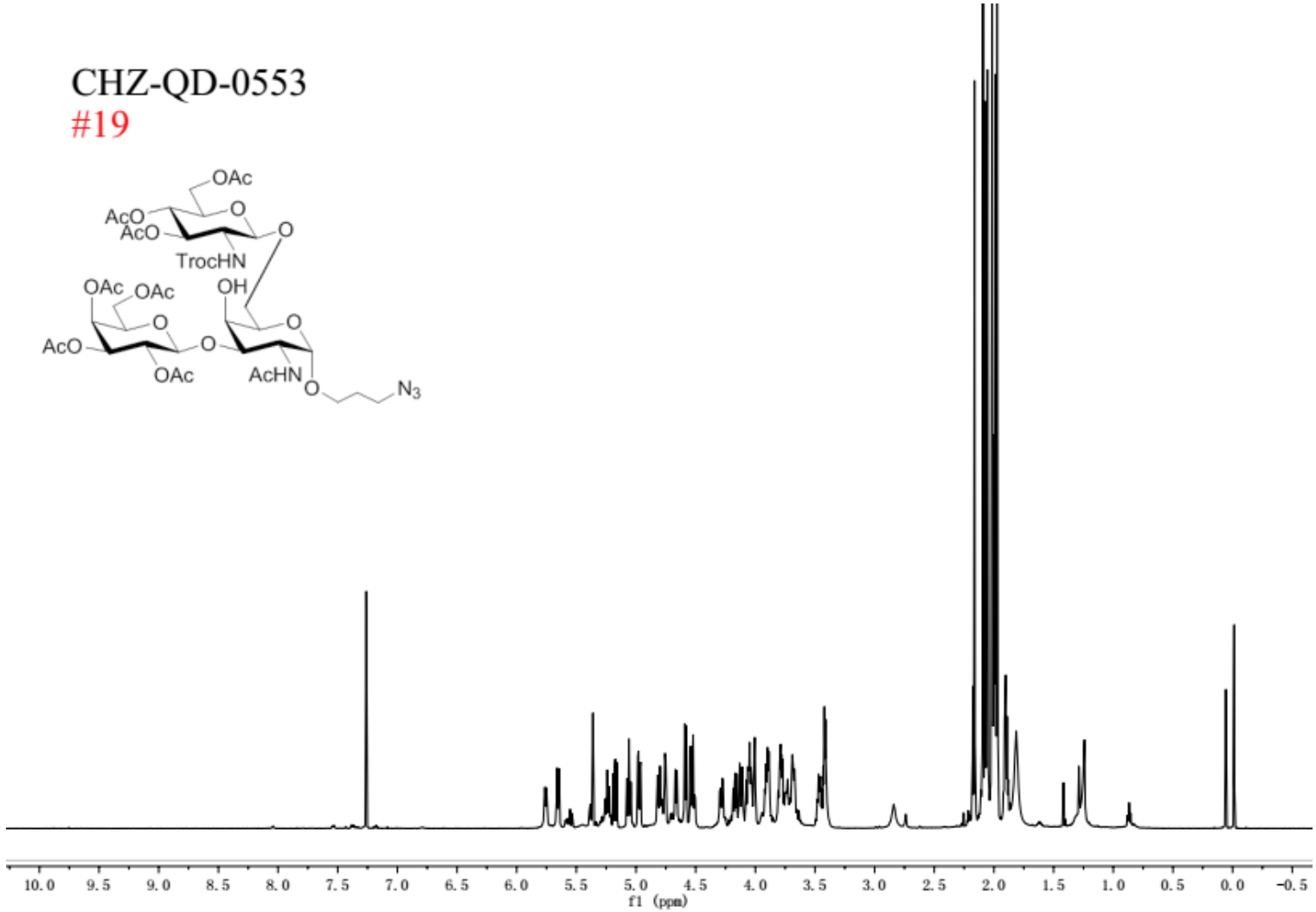
#17
Ph
OAc
OAc
AcO
OAc
AcHN
N3
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

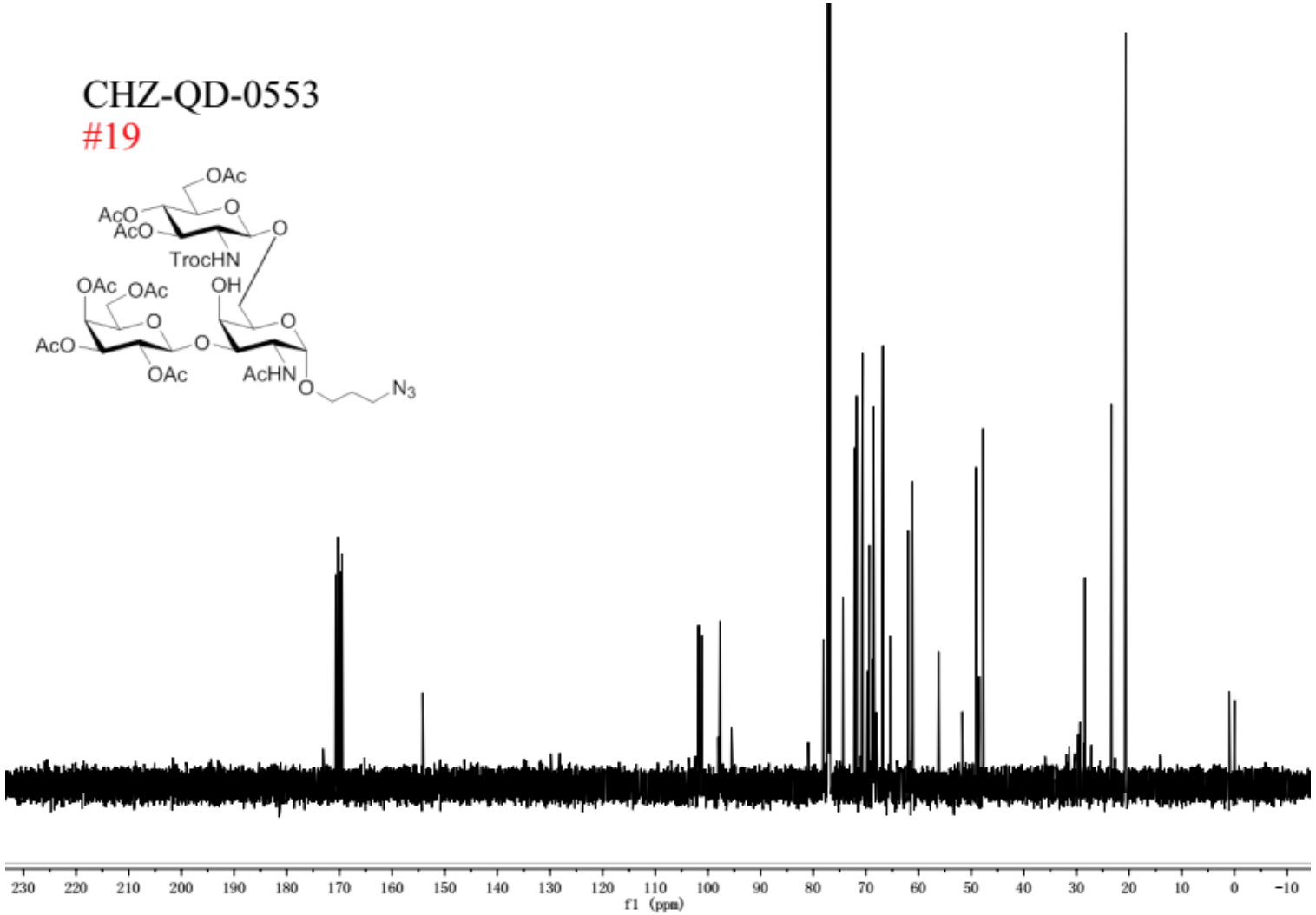
^{1}H NMR of 17


CHZ-QD-0565
#17
Ph
O
OAc
OAc
O
O
O
AcO
O
OAc
AcHN
O
N3
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)

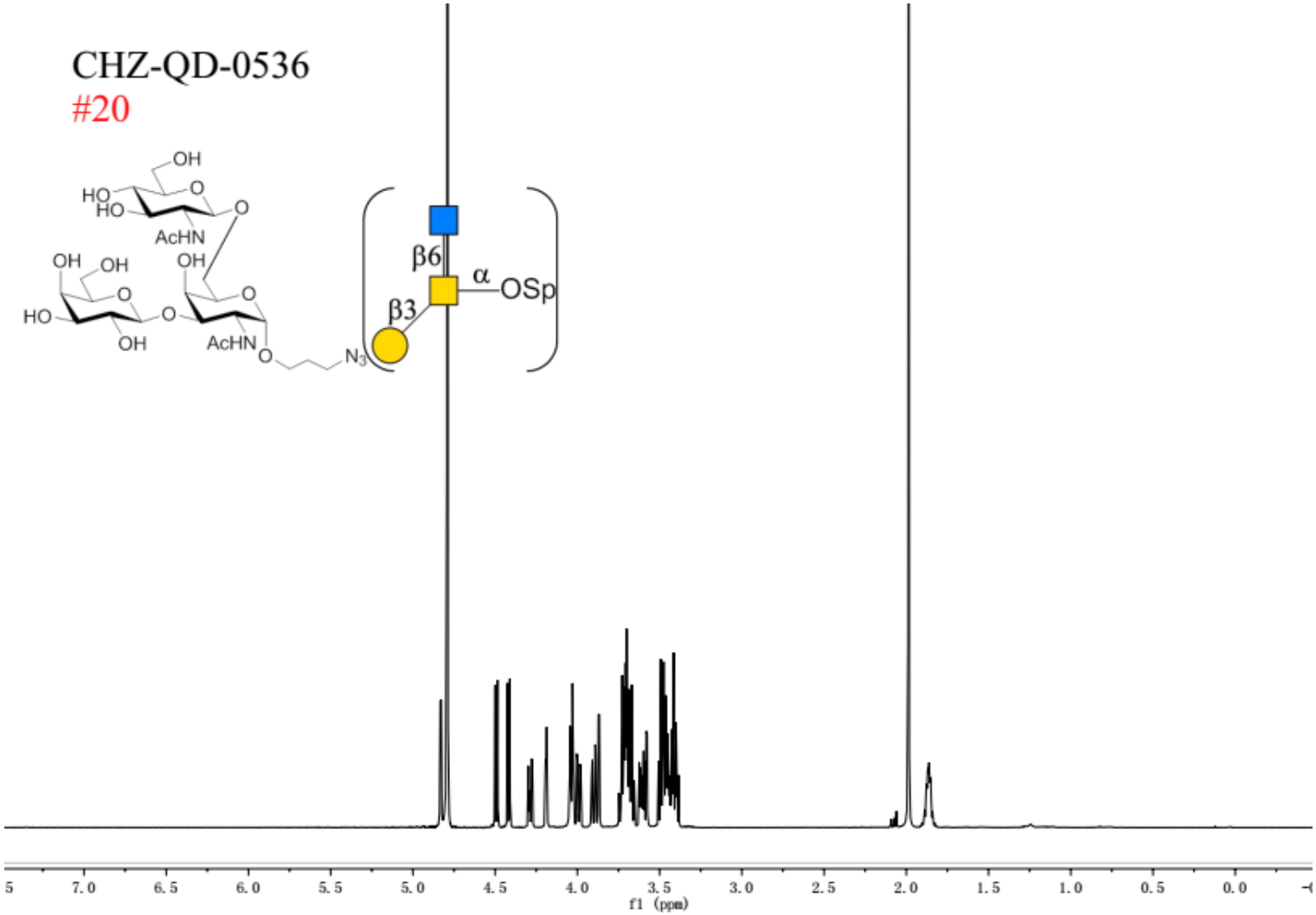
^{13}C NMR of 17


CHZ-QD-0553
#19
OAc
AcO
AcO
O
O
TrocHN
OAc
OAc
OH
AcO
O
O
O
OAc
AcHN
O
N3
10.0
9.5
9.0
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
0.0
-0.5
f1 (ppm)

^{1}H NMR of 19


CHZ-QD-0553
#19
OAc
AcO
AcO
O
O
TrocHN
OAc
OAc
OH
O
O
AcO
O
OAc
AcHN
O
N3
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)

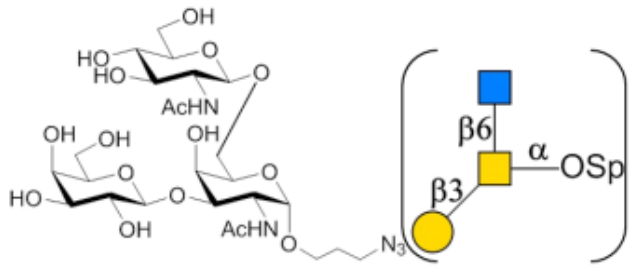
^{13}C NMR of 19

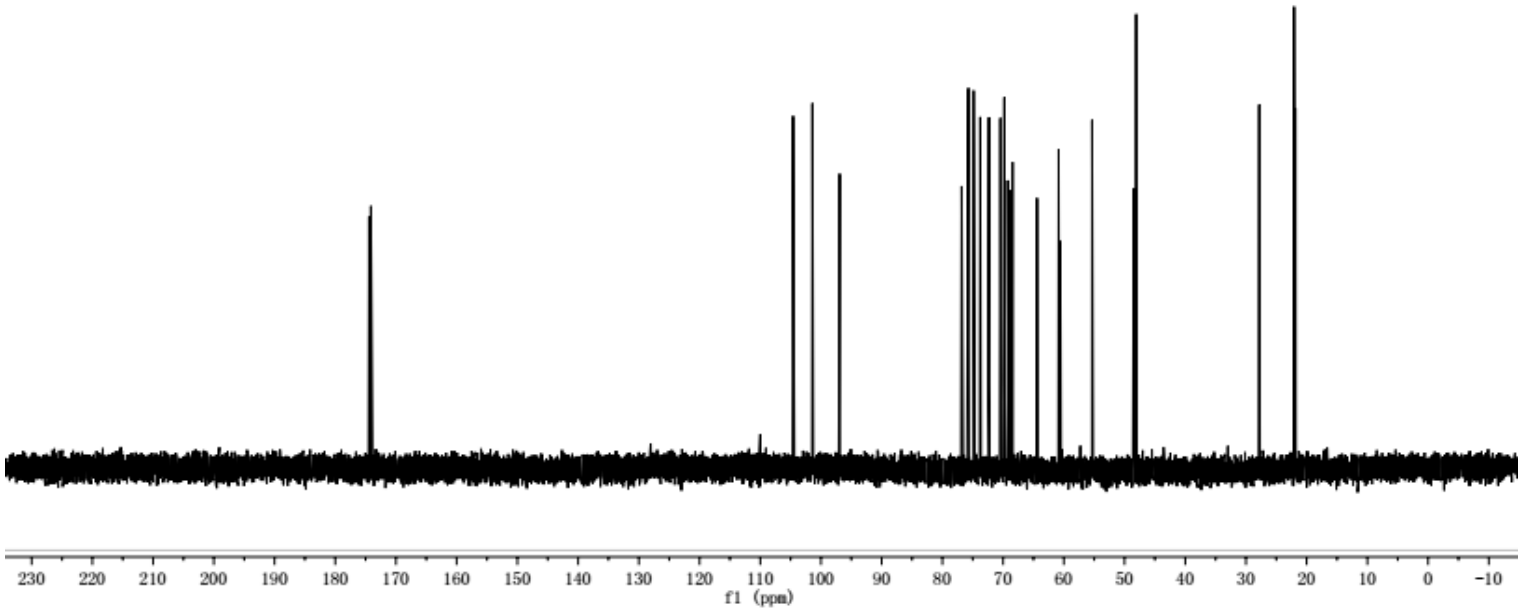

CHZ-QD-0536
#20
OH
HO
HO
O
AcHN
OH
OH
HO
O
OH
OH
AcHN
O
O
N3
β6
α
OSp
β3
5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{1}H NMR of 20

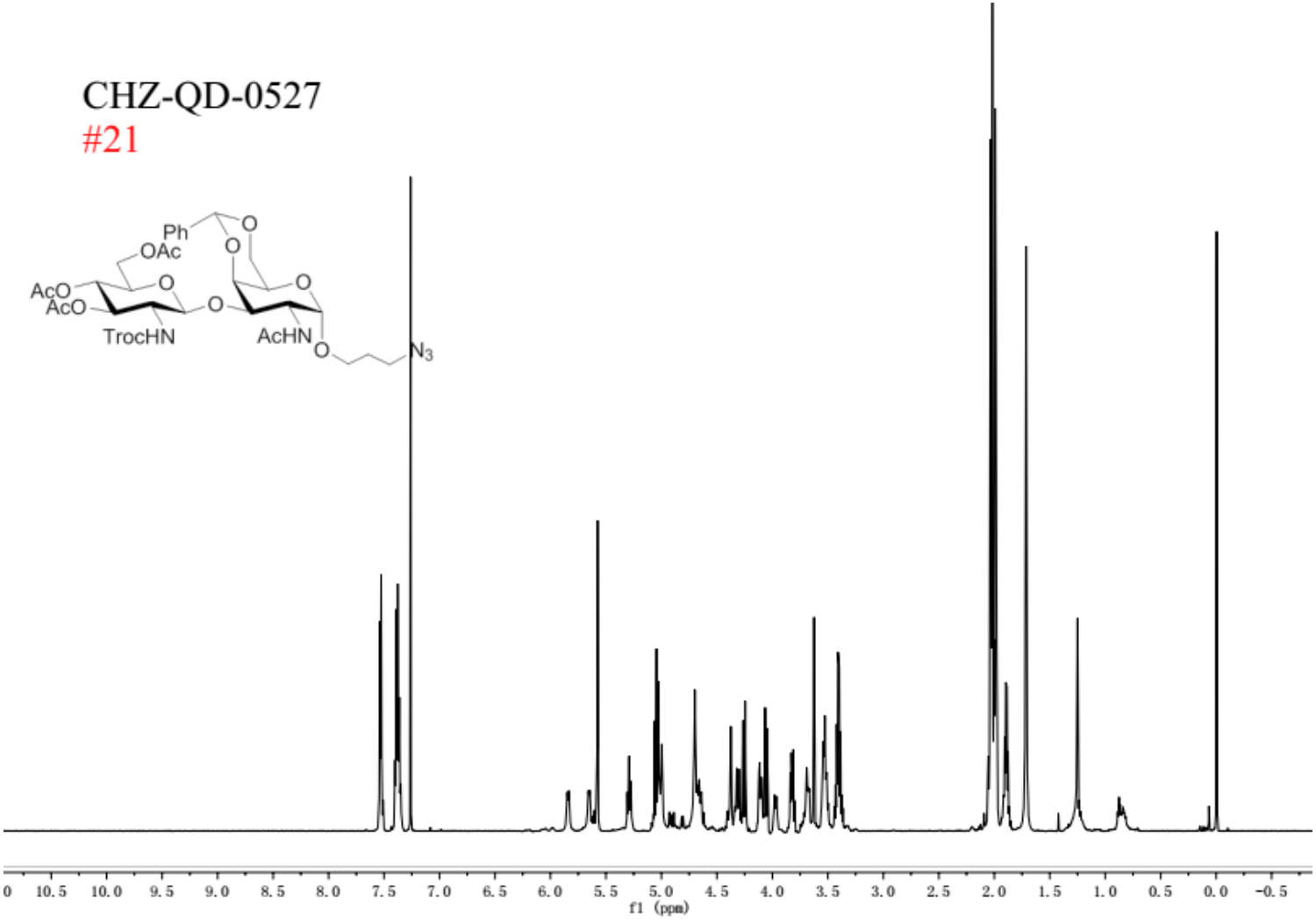
CHZ-QD-0536

#20

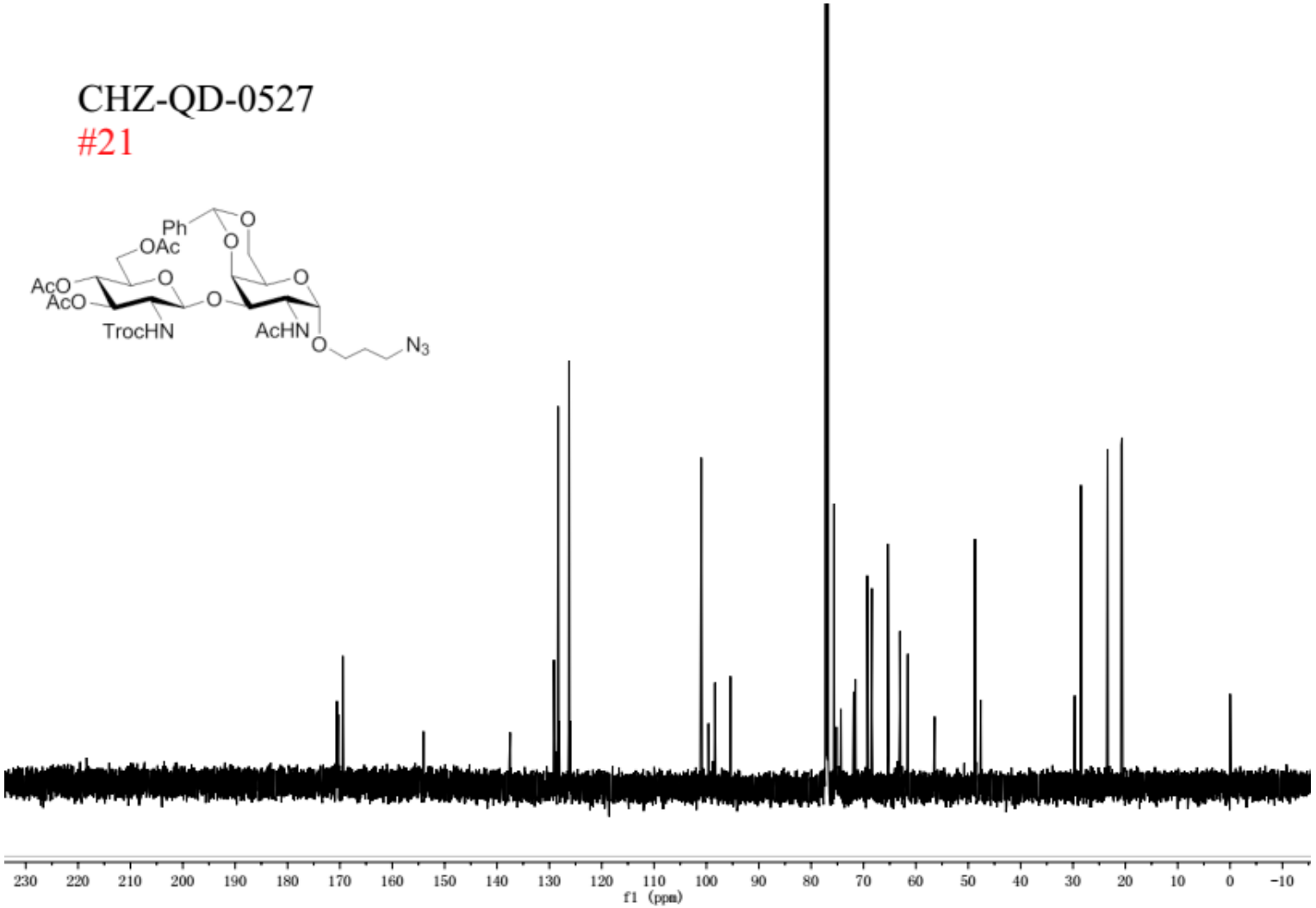




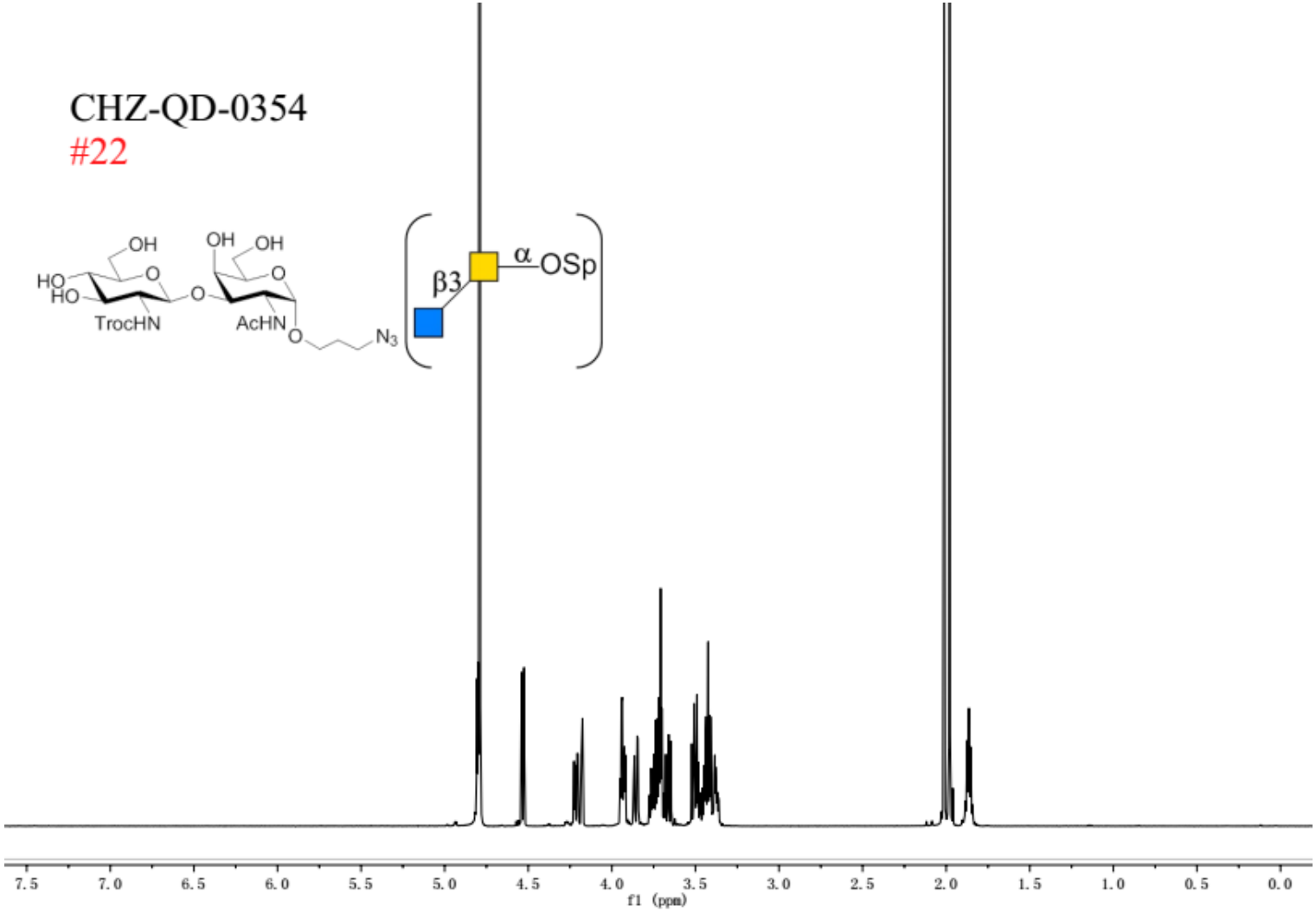
^{13}C NMR of 20



^{1}H NMR of 21

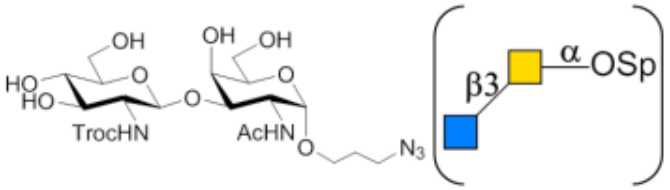

CHZ-QD-0527
#21
Ph
OAc
AcO
AcO
TrocHN
AcHN
N3
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

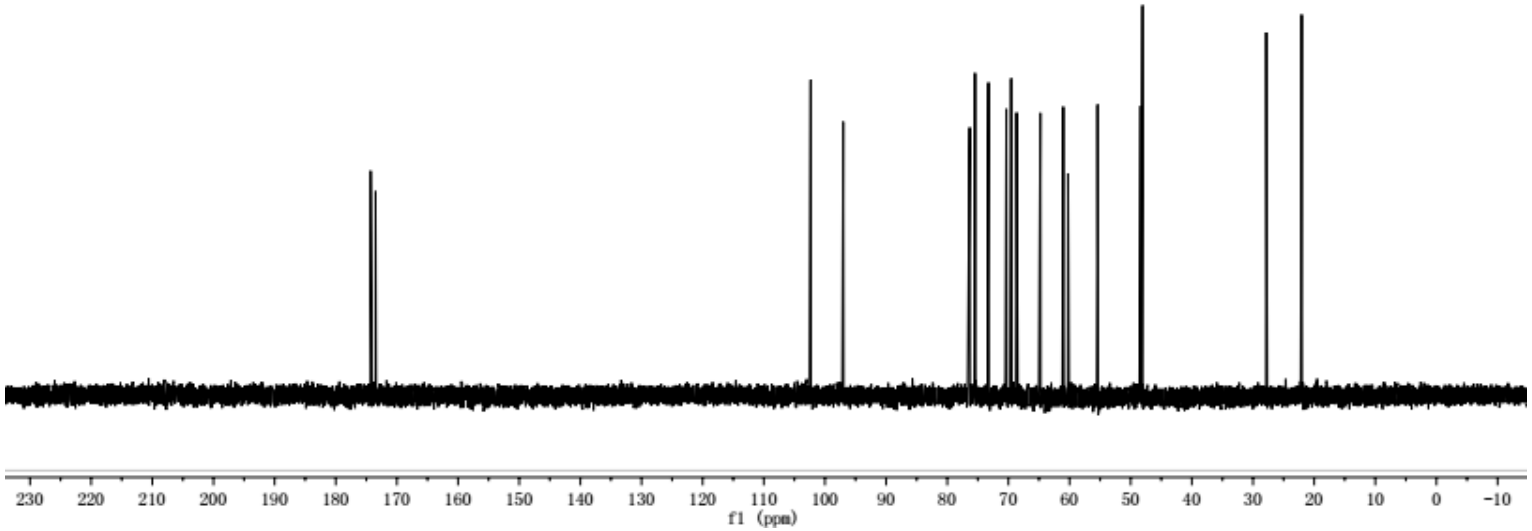
^{13}C NMR of 21


CHZ-QD-0354
#22
OH
OH
OH
HO
HO
O
O
O
TrocHN
AcHN
O
N3
β3
α
OSp
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
0.0
f1 (ppm)

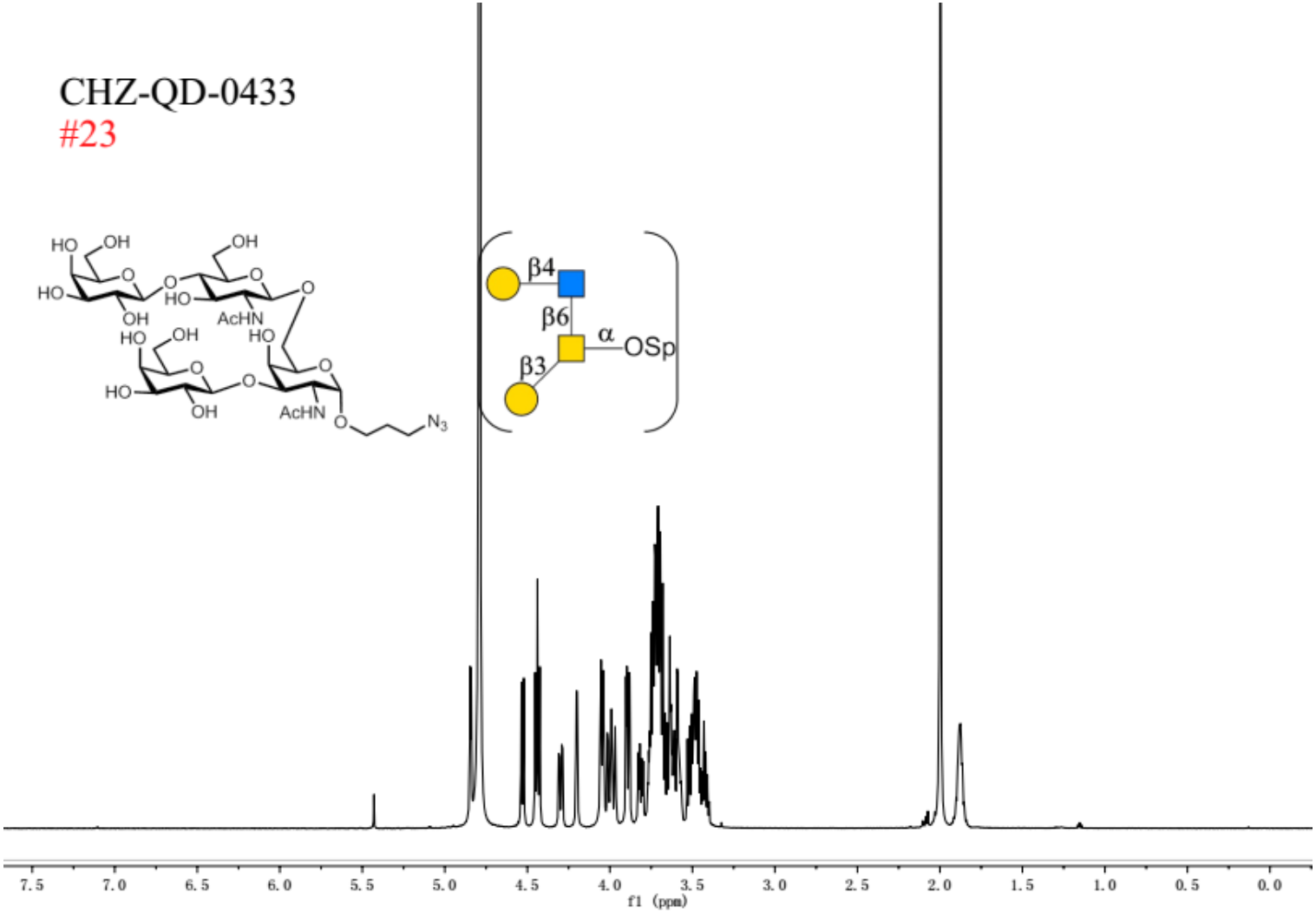
^{1}H NMR of 22

CHZ-QD-0354
#22

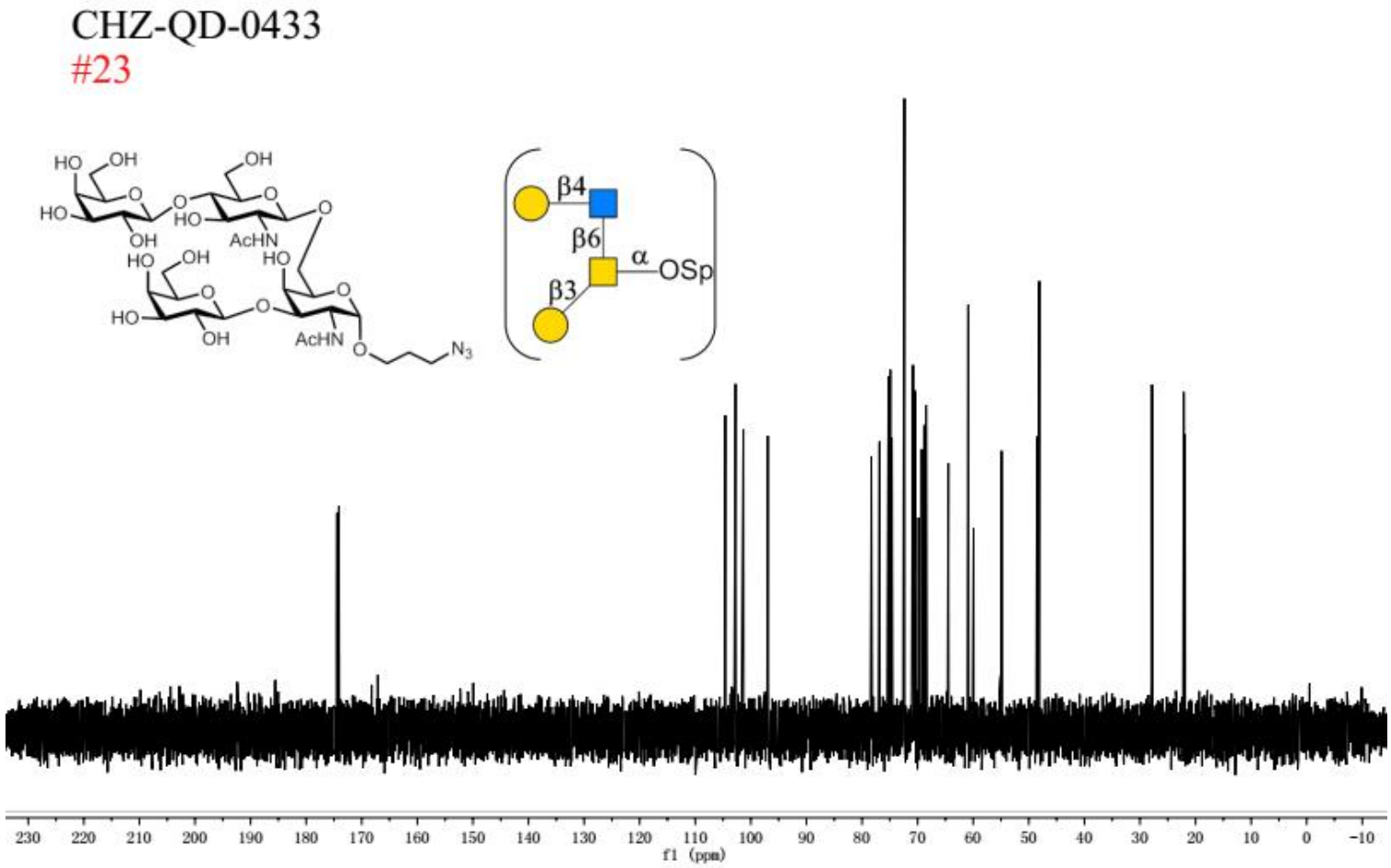




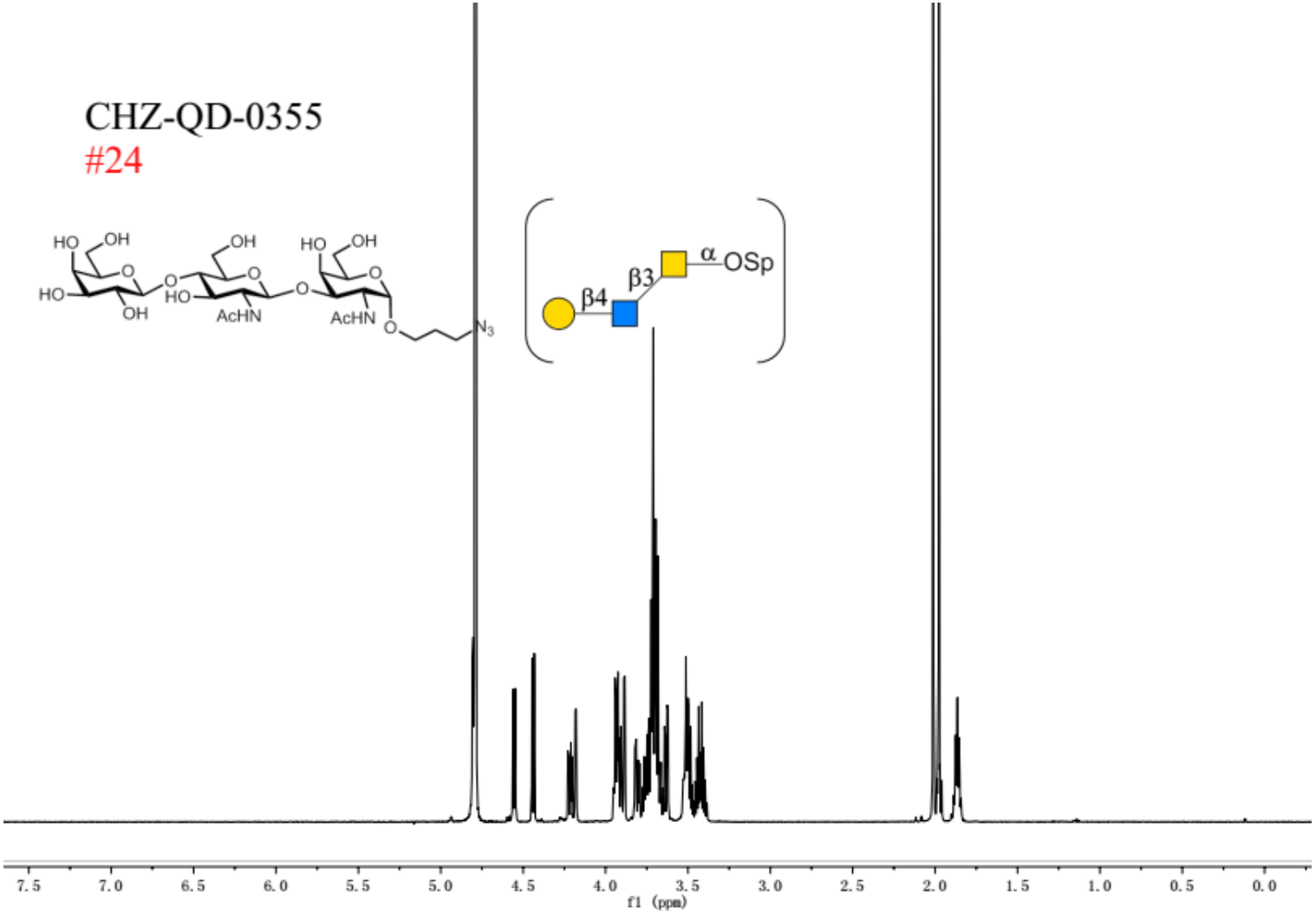
^{13}C NMR of 22


CHZ-QD-0433
#23
HO
OH
AcHN
N3
β4
β6
α
OSp
β3
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
0.0
f1 (ppm)

^{1}H NMR of 23

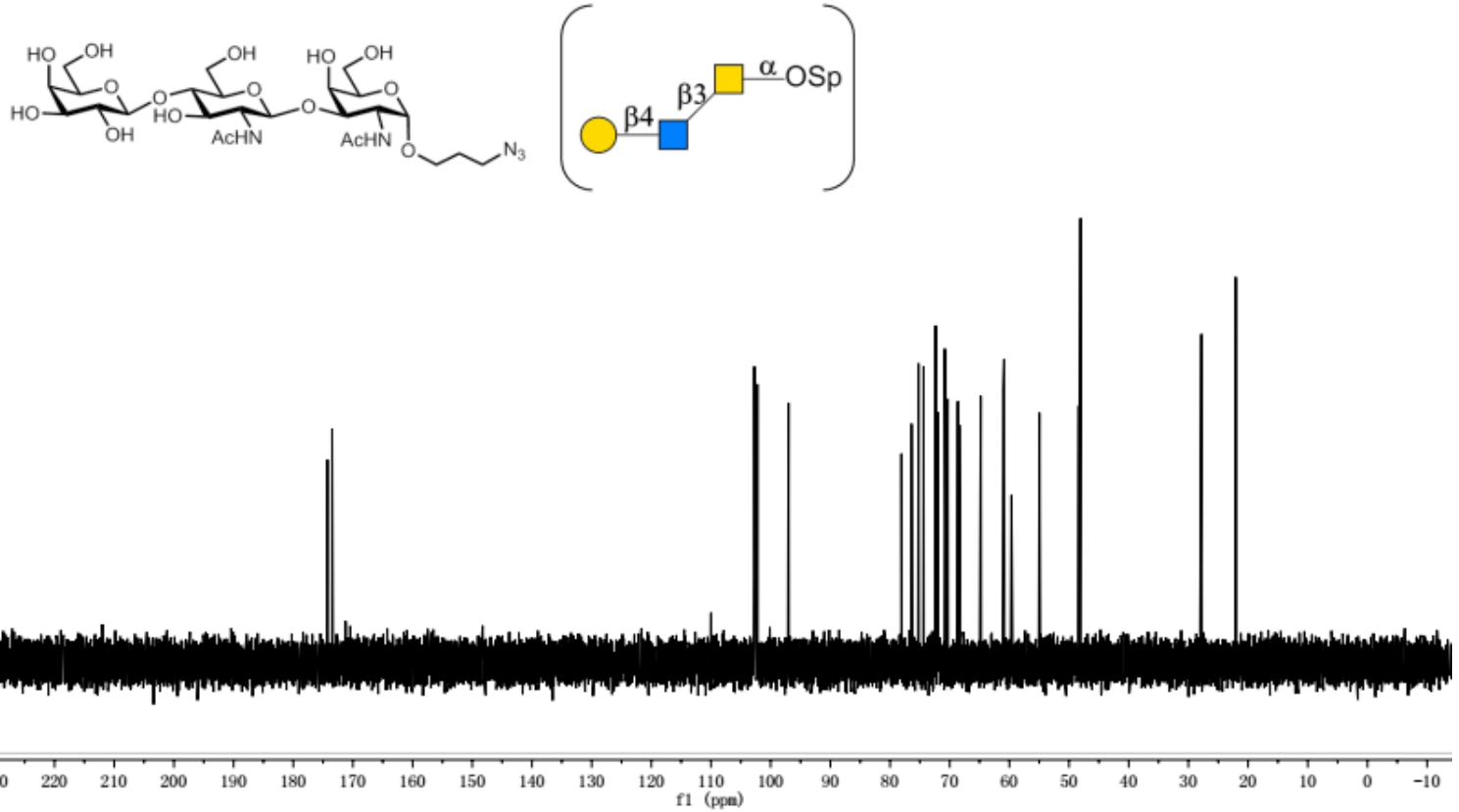

CHZ-QD-0433
#23
HO
OH
OH
HO
O
O
HO
AcHN
OH
HO
HO
OH
O
O
HO
OH
AcHN
O
N3
β4
β6
α
OSp
β3
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

^{13}C NMR of 23

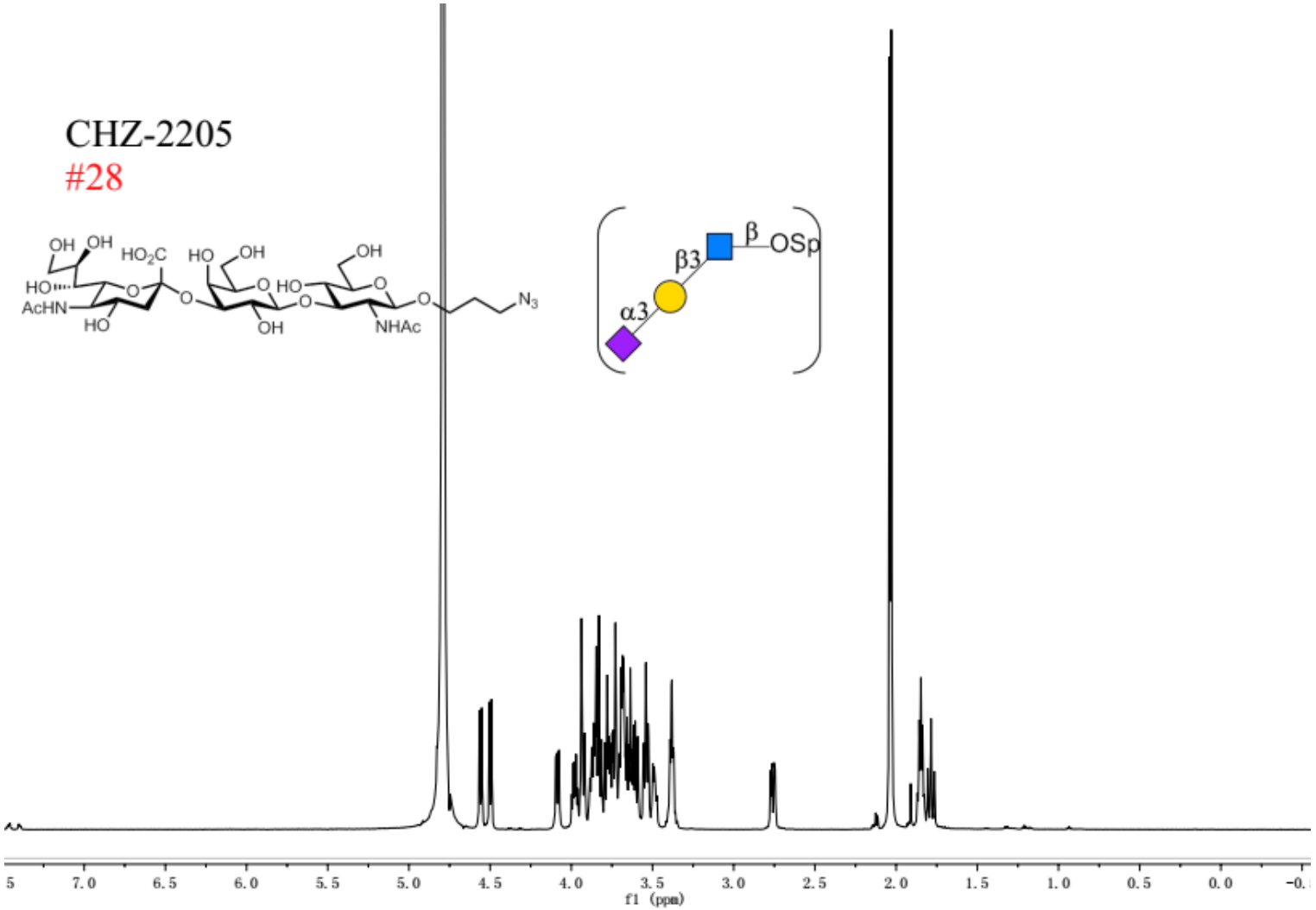

CHZ-QD-0355
#24
HO
OH
O
HO
OH
AcHN
HO
OH
O
HO
OH
AcHN
O
N3
β4
β3
α
OSp
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{1}H NMR of 24

CHZ-QD-0355
#24



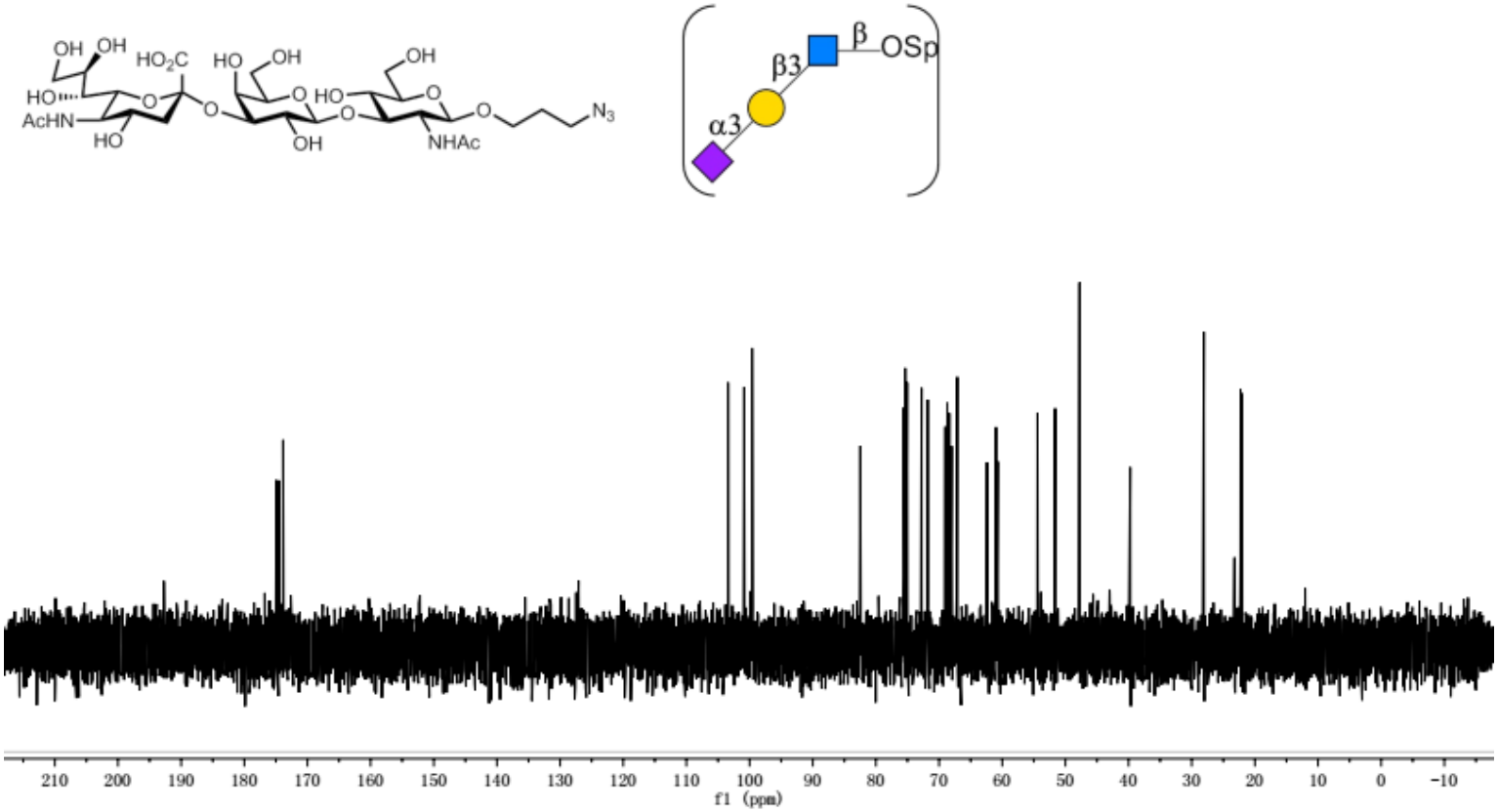
^{13}C NMR of 24



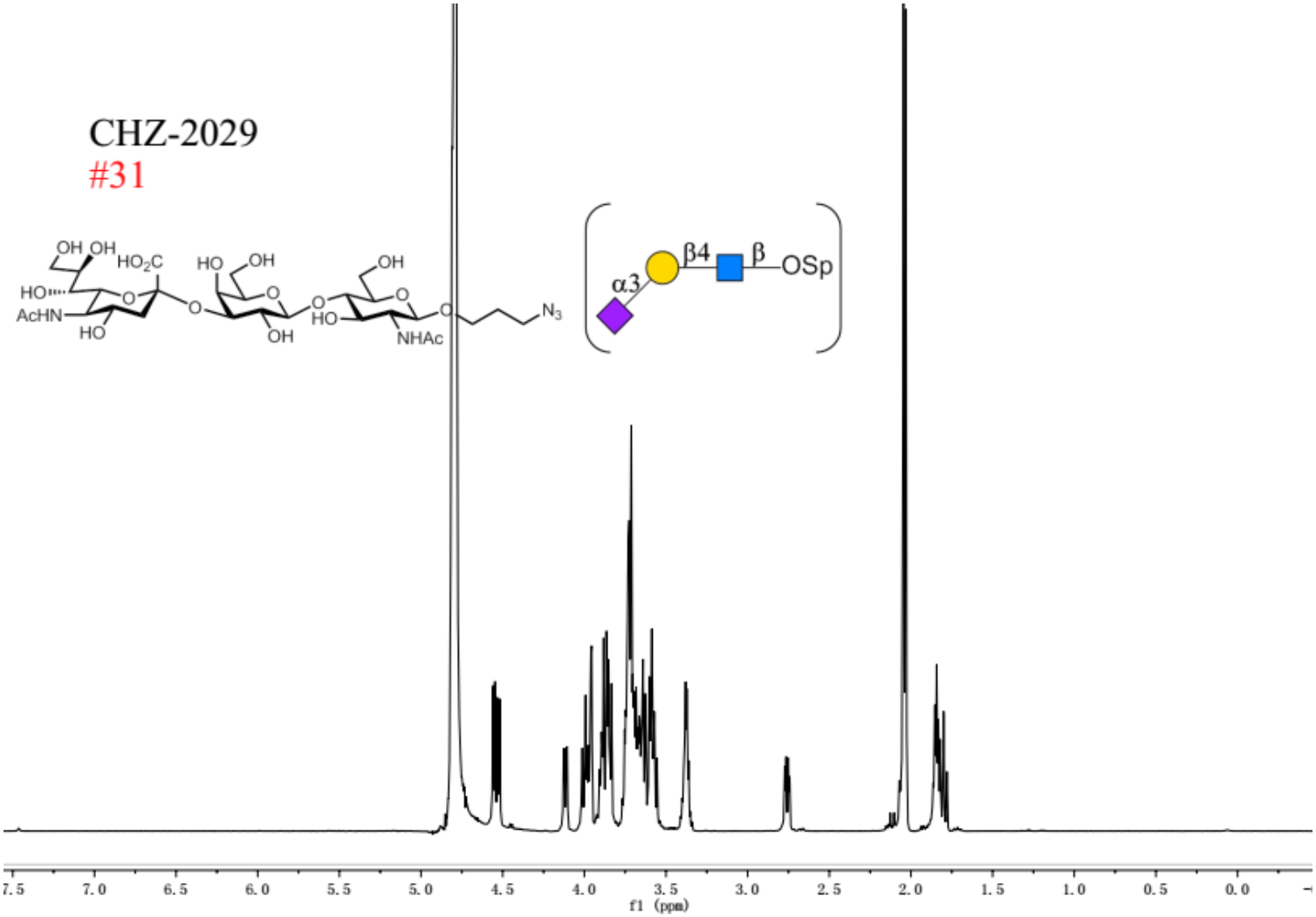
^{1}H NMR of 28

CHZ-2174

#28

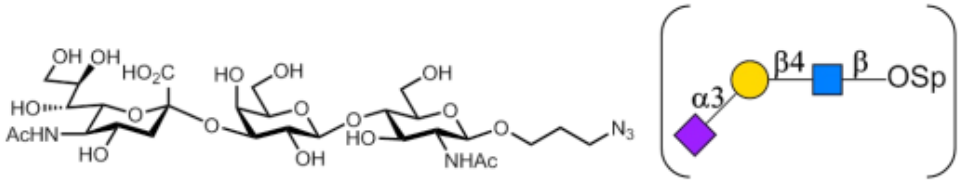


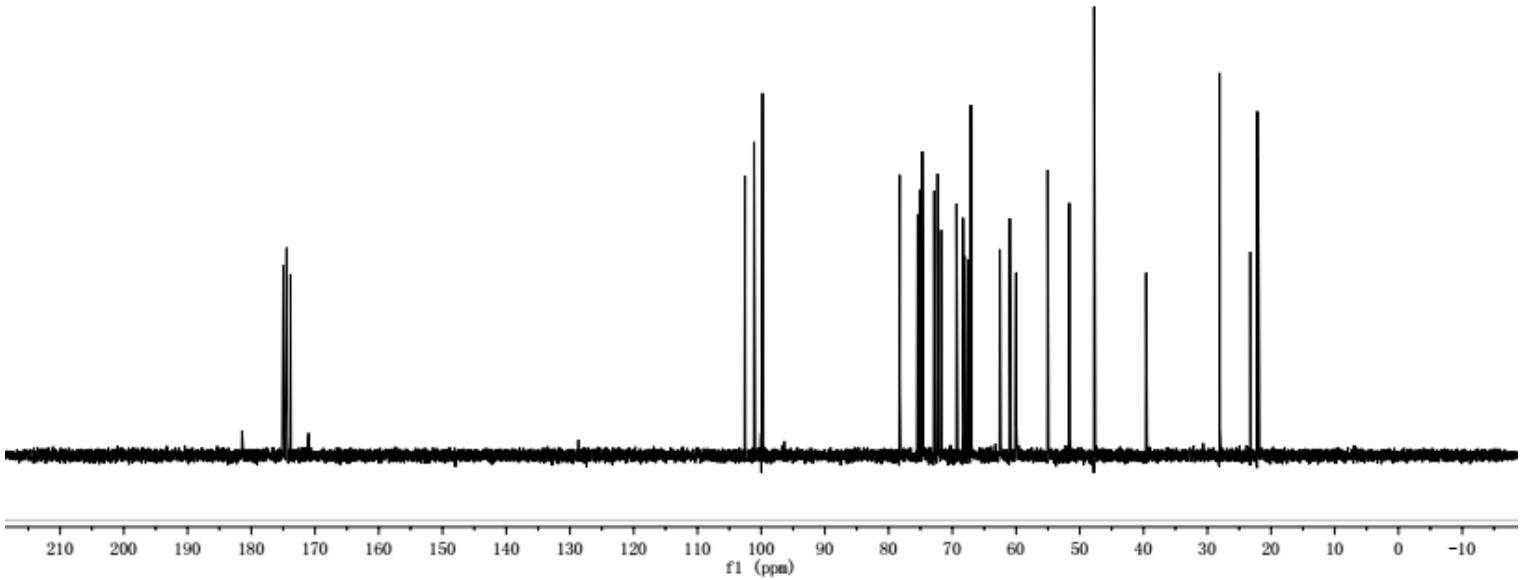
^{13}C NMR of 28


CHZ-2029
#31
OH OH
HO2C
HO
OH
HO
O
AcHN
HO
O
O
HO
OH
O
O
NHAc
O
N3
β4
β
OSp
α3
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
0.0
f1 (ppm)

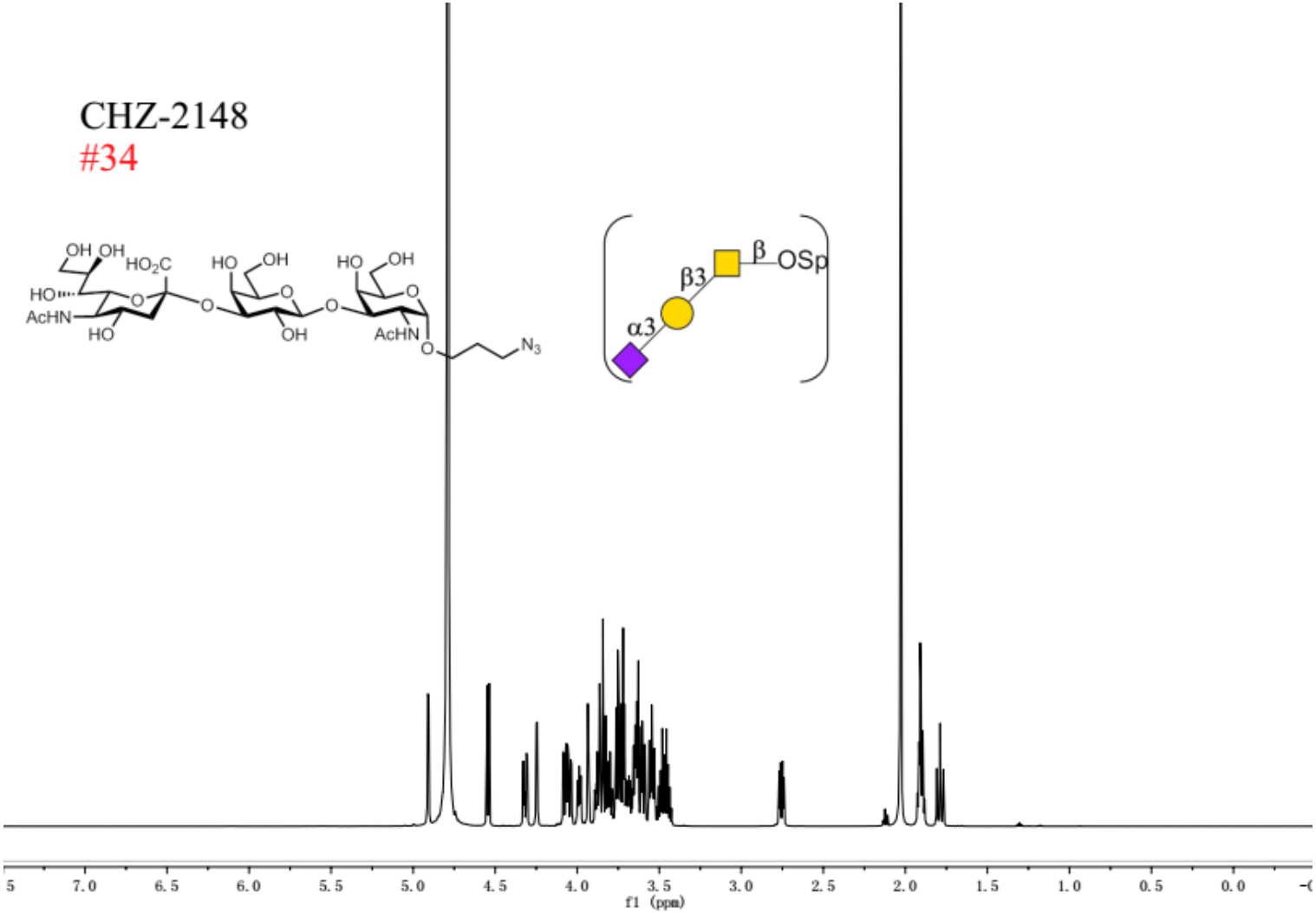
^{1}H NMR of 31

CHZ-2181
#31



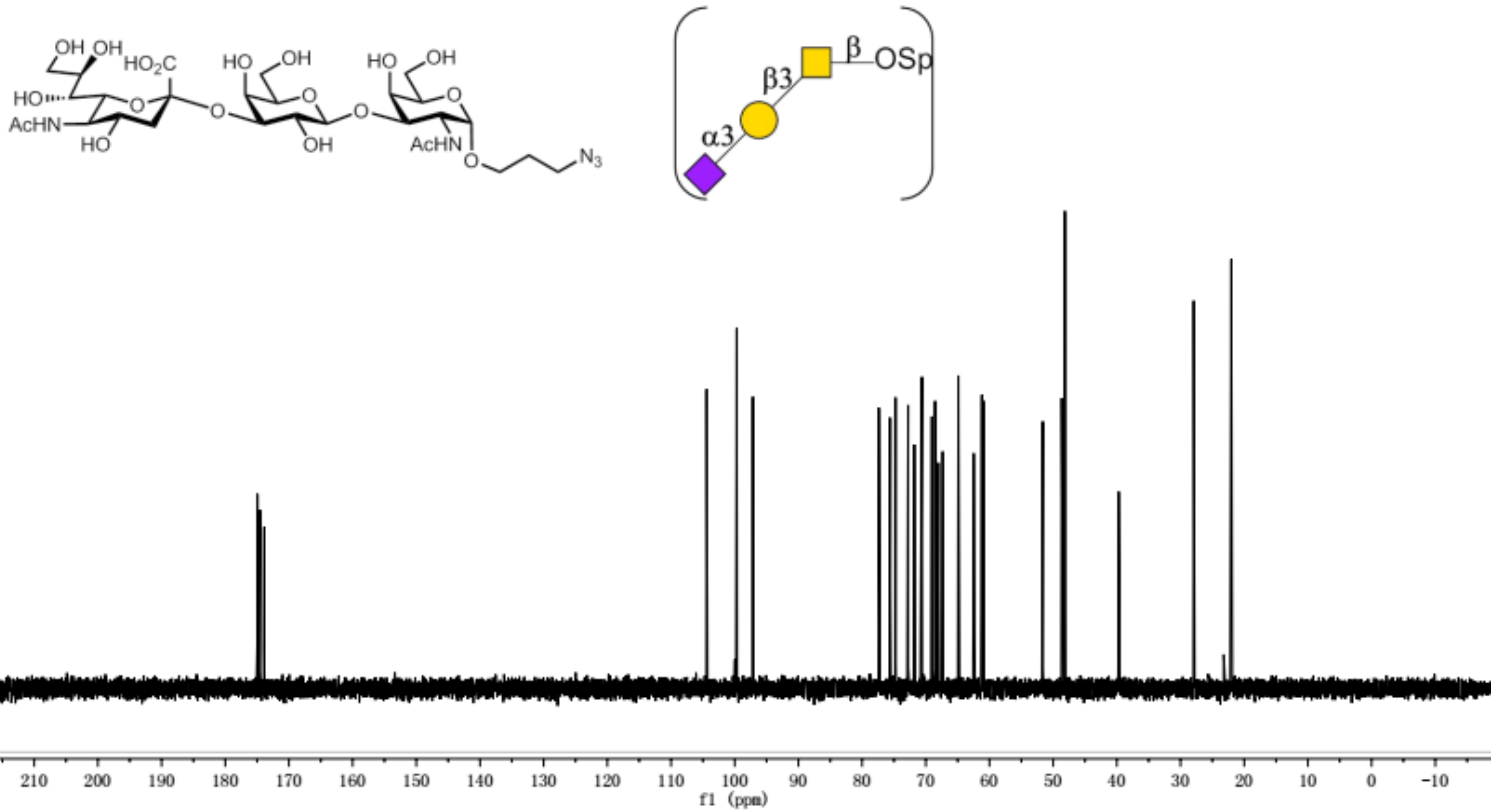


^{13}C NMR of 31

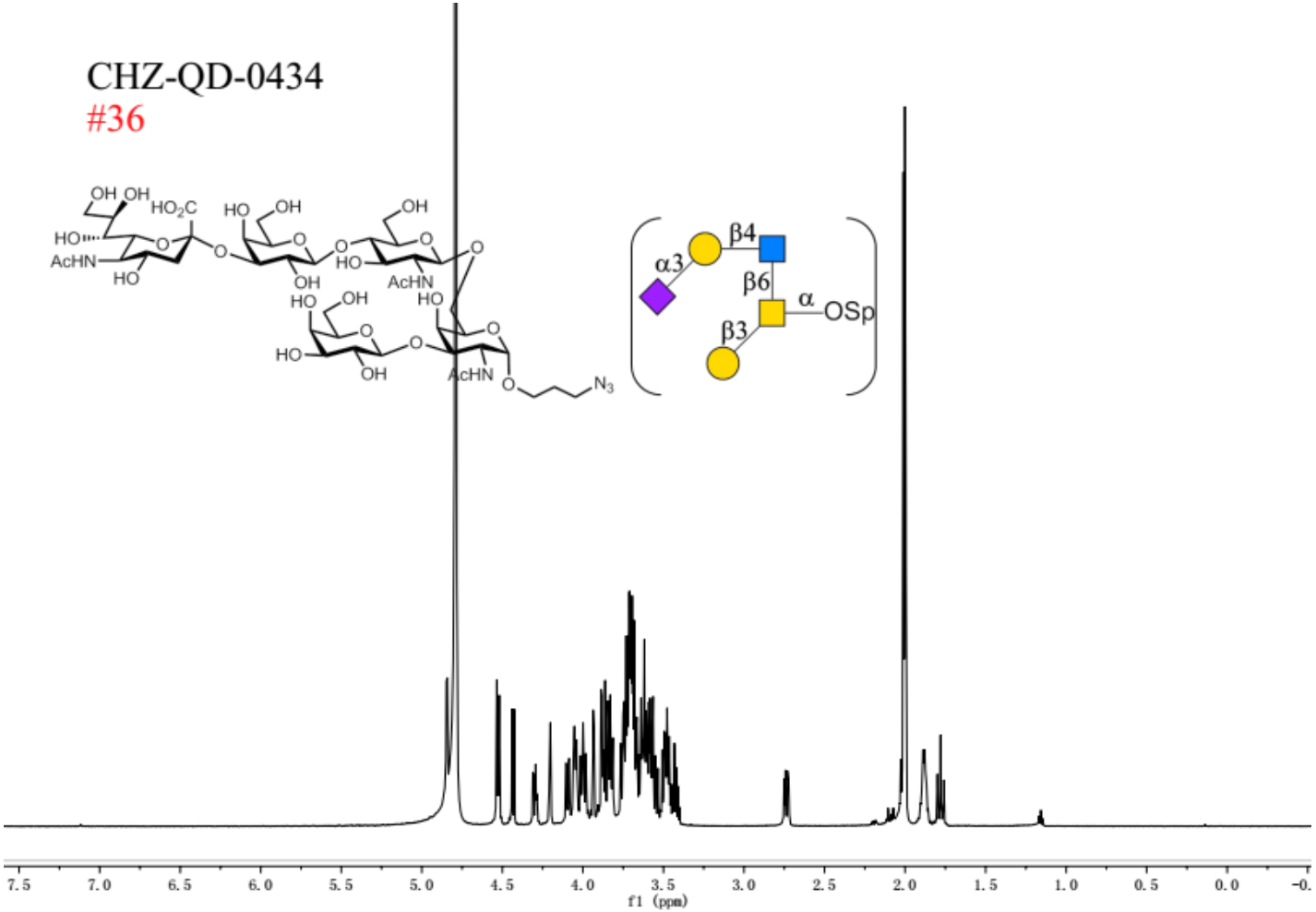

CHZ-2148
#34
OH OH
HO2C
HO
AcHN
HO
OH
HO
OH
HO
OH
AcHN
O
N3
β
OSp
β3
α3
5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{1}H NMR of 34

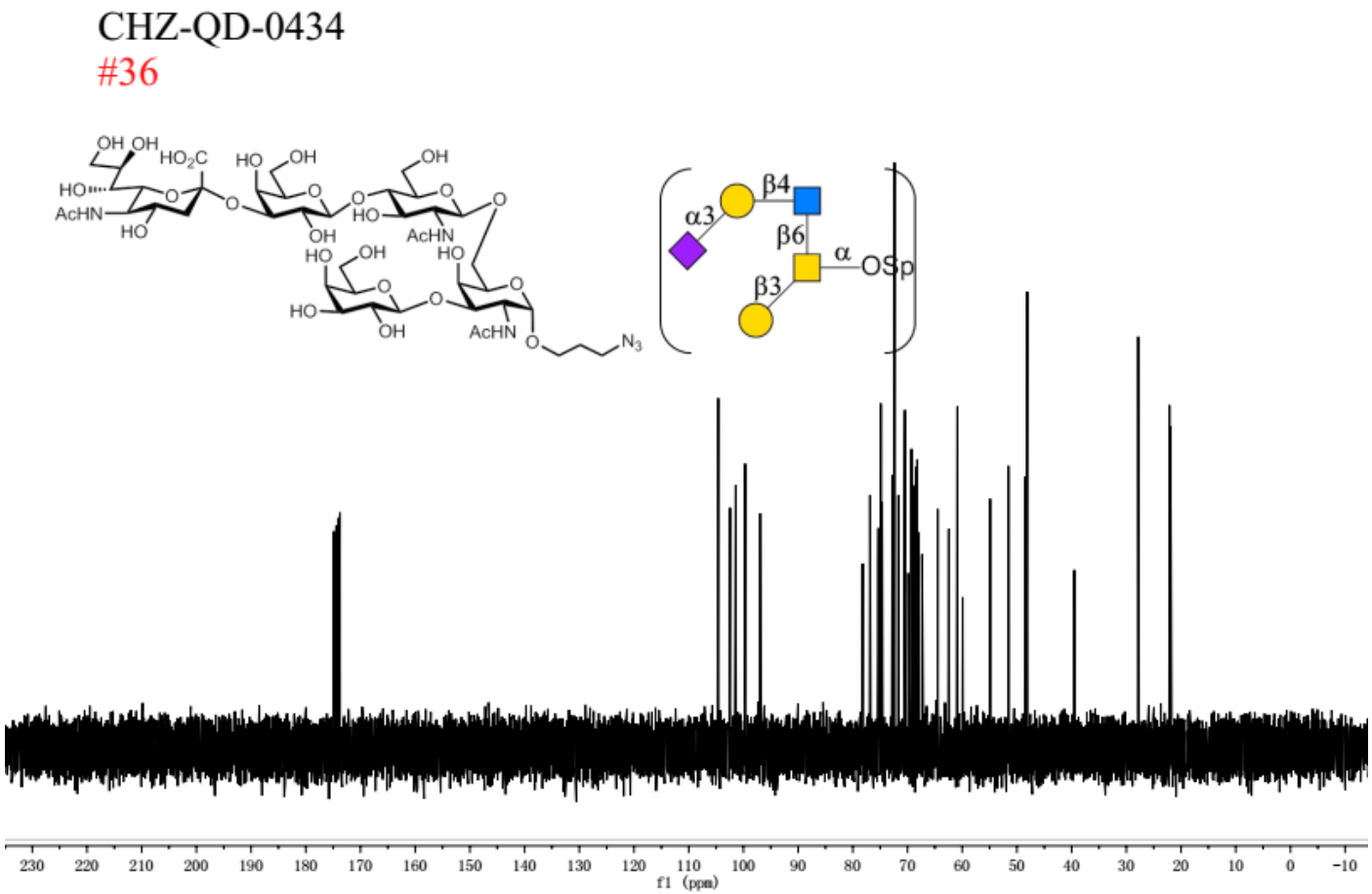
CHZ-2148
#34



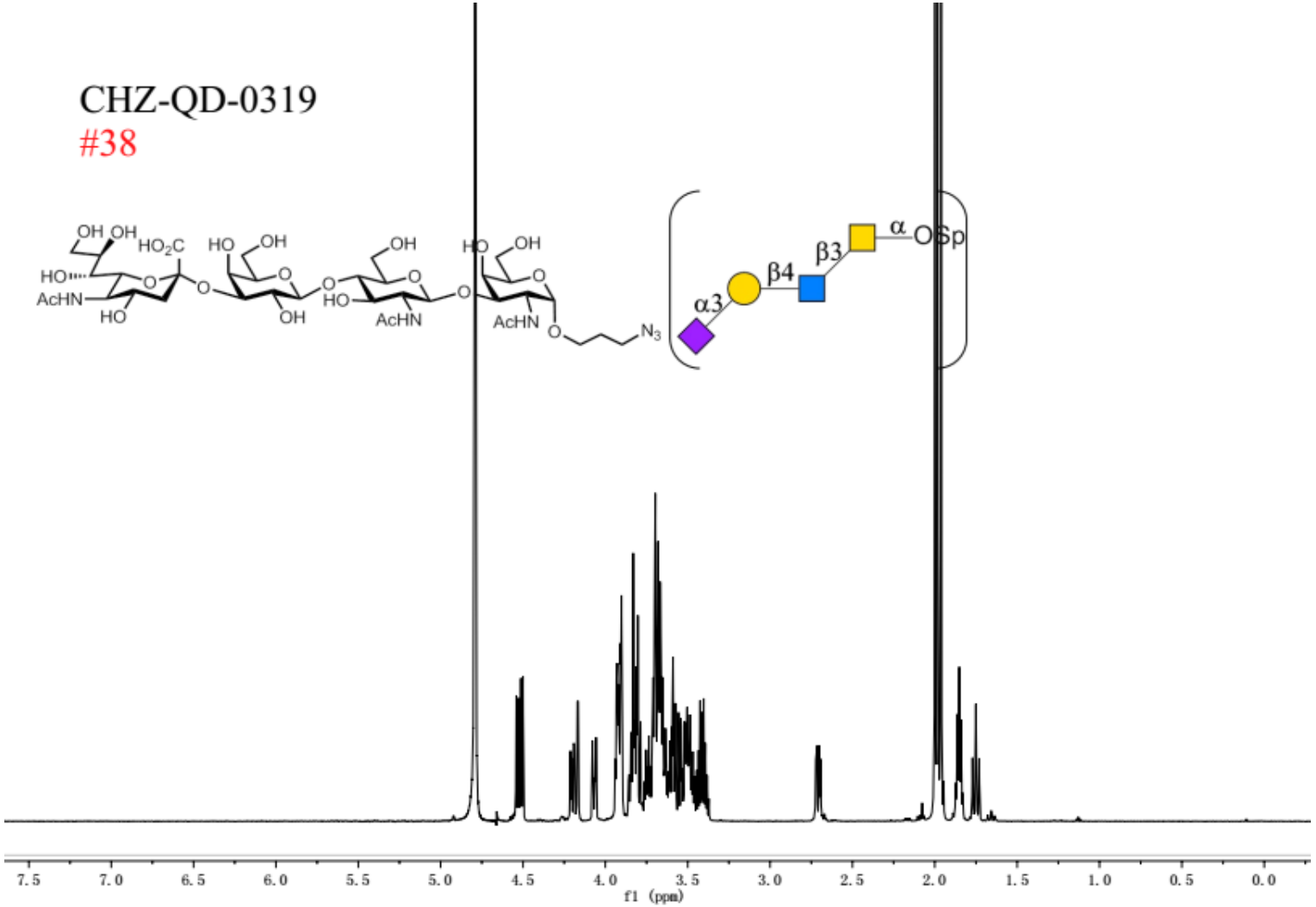
^{13}C NMR of 34


CHZ-QD-0434
#36
β4
α3
β6
α
OSp
β3
AcHN
HO
OH
HO2C
N3
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
0.0
f1 (ppm)

^{1}H NMR of 36

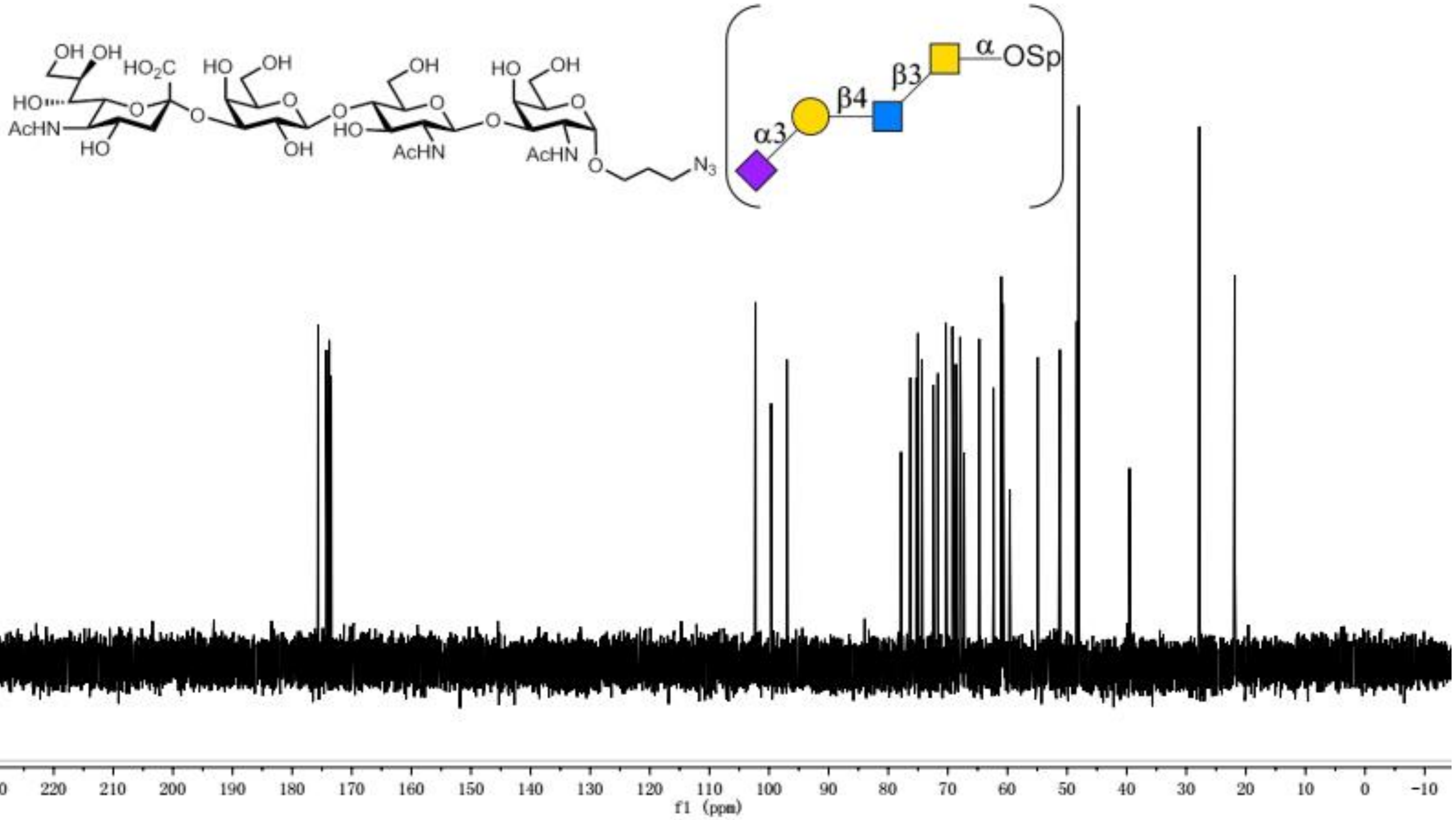

CHZ-QD-0434
#36
β4
α3
β6
α
OSp
β3
AcHN
HO
OH
HO2C
N3
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

^{13}C NMR of 36

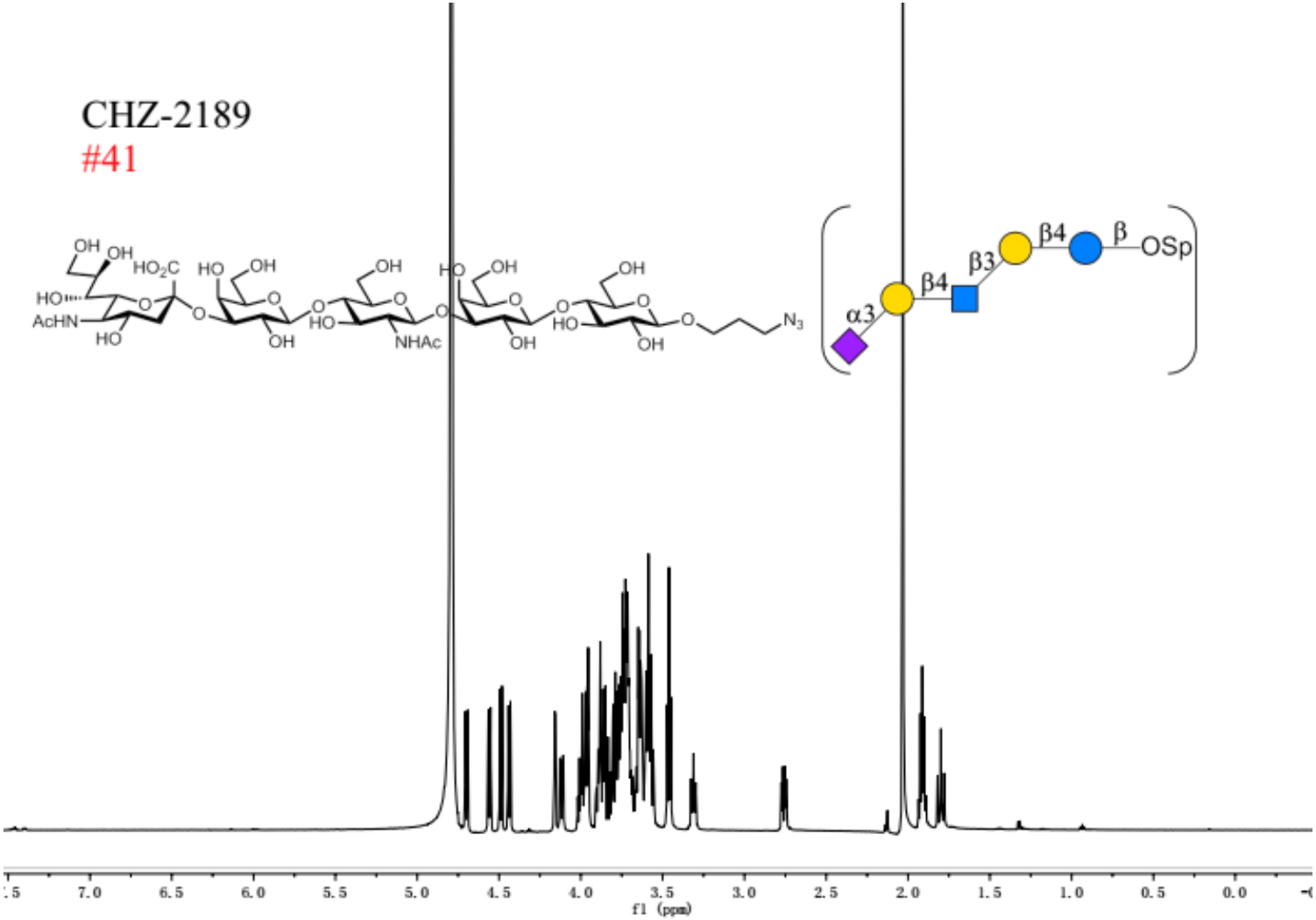

CHZ-QD-0319
#38
OH OH
HO2C
HO
OH
HO
AcHN
HO
O
OH
HO
AcHN
AcHN
N3
α3
β4
β3
α
OSp
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{1}H NMR of 38

CHZ-QD-0319
#38



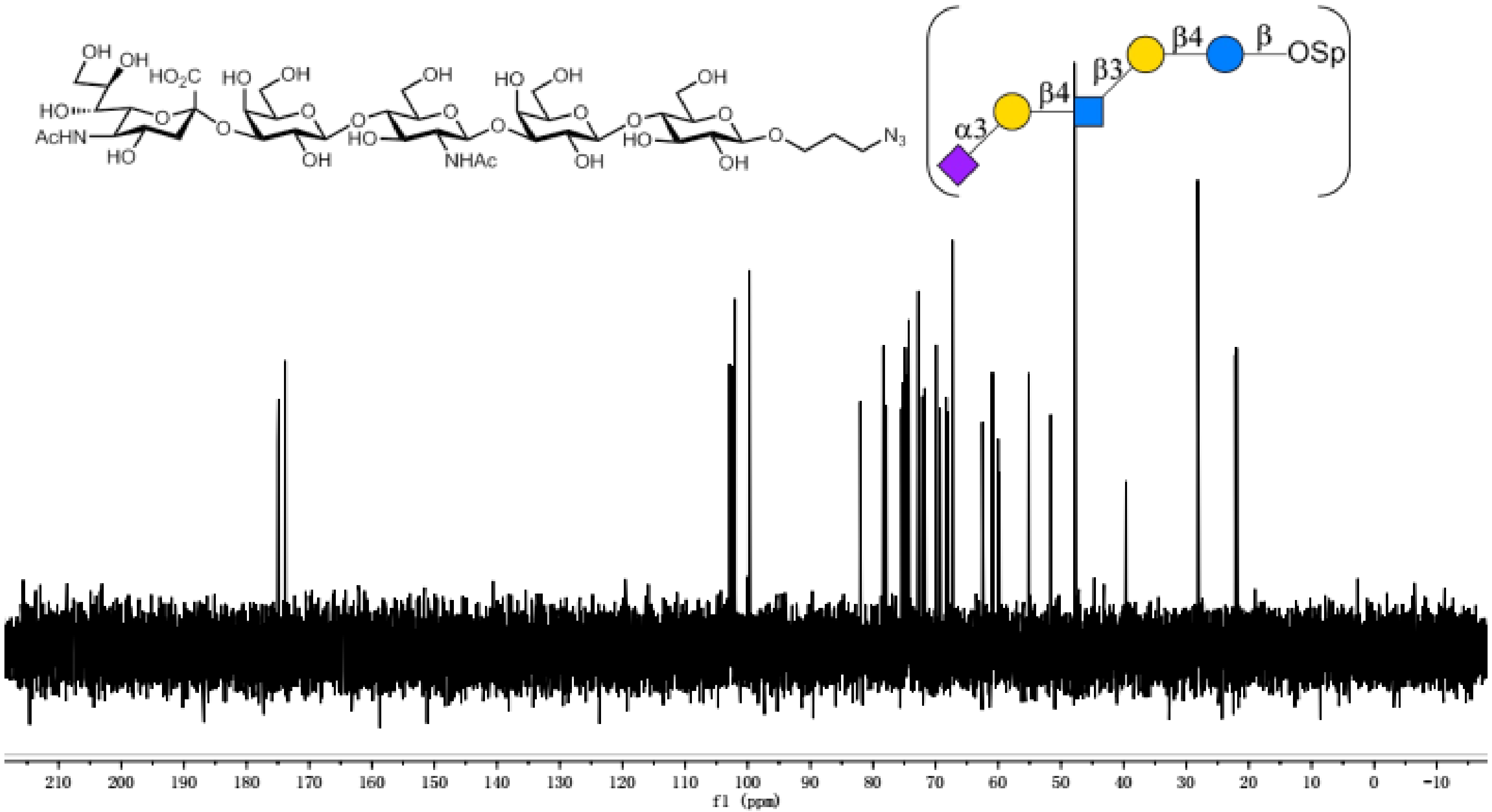
^{13}C NMR of 38



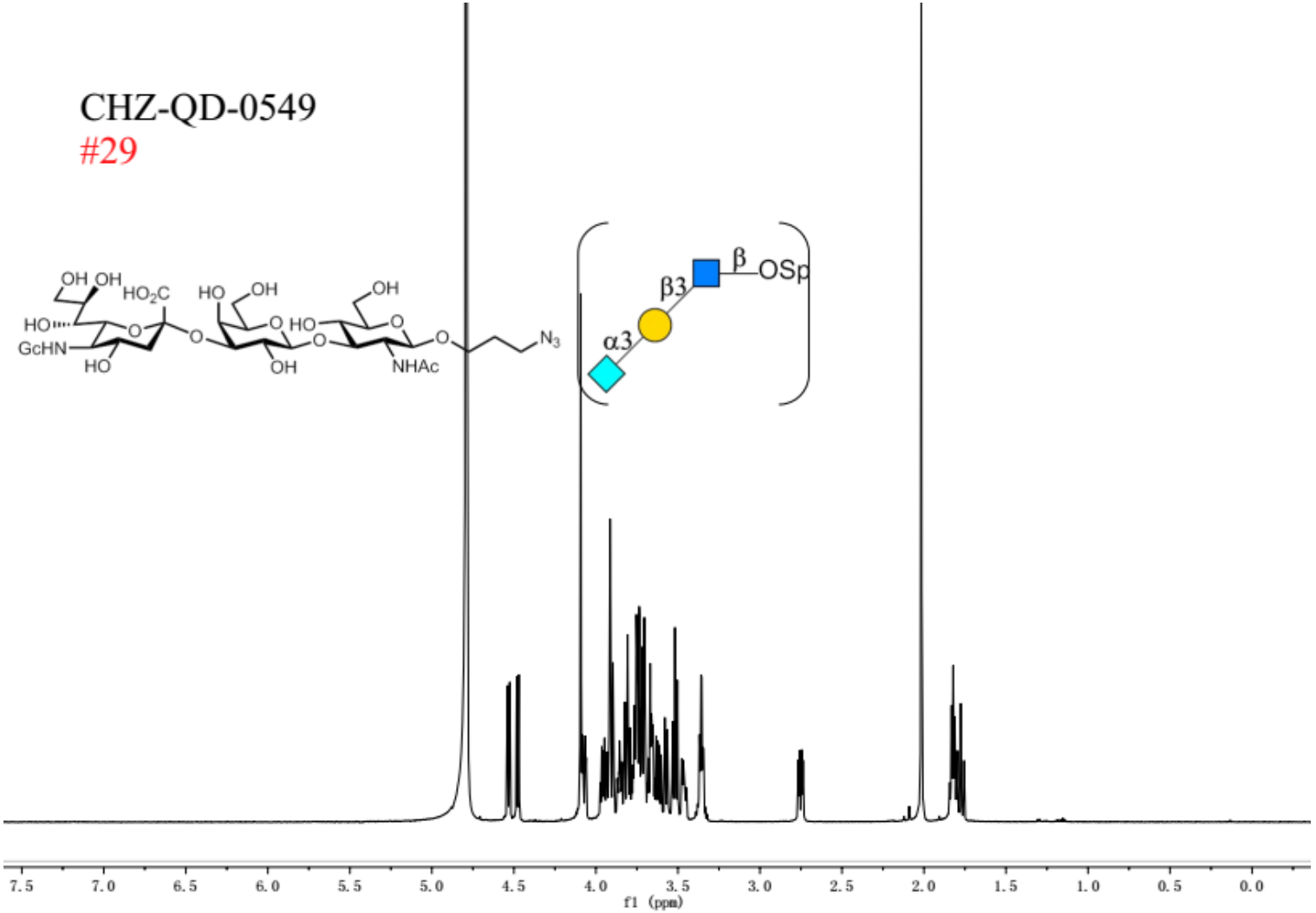
^{1}H NMR of 41

CHZ-2189

#41

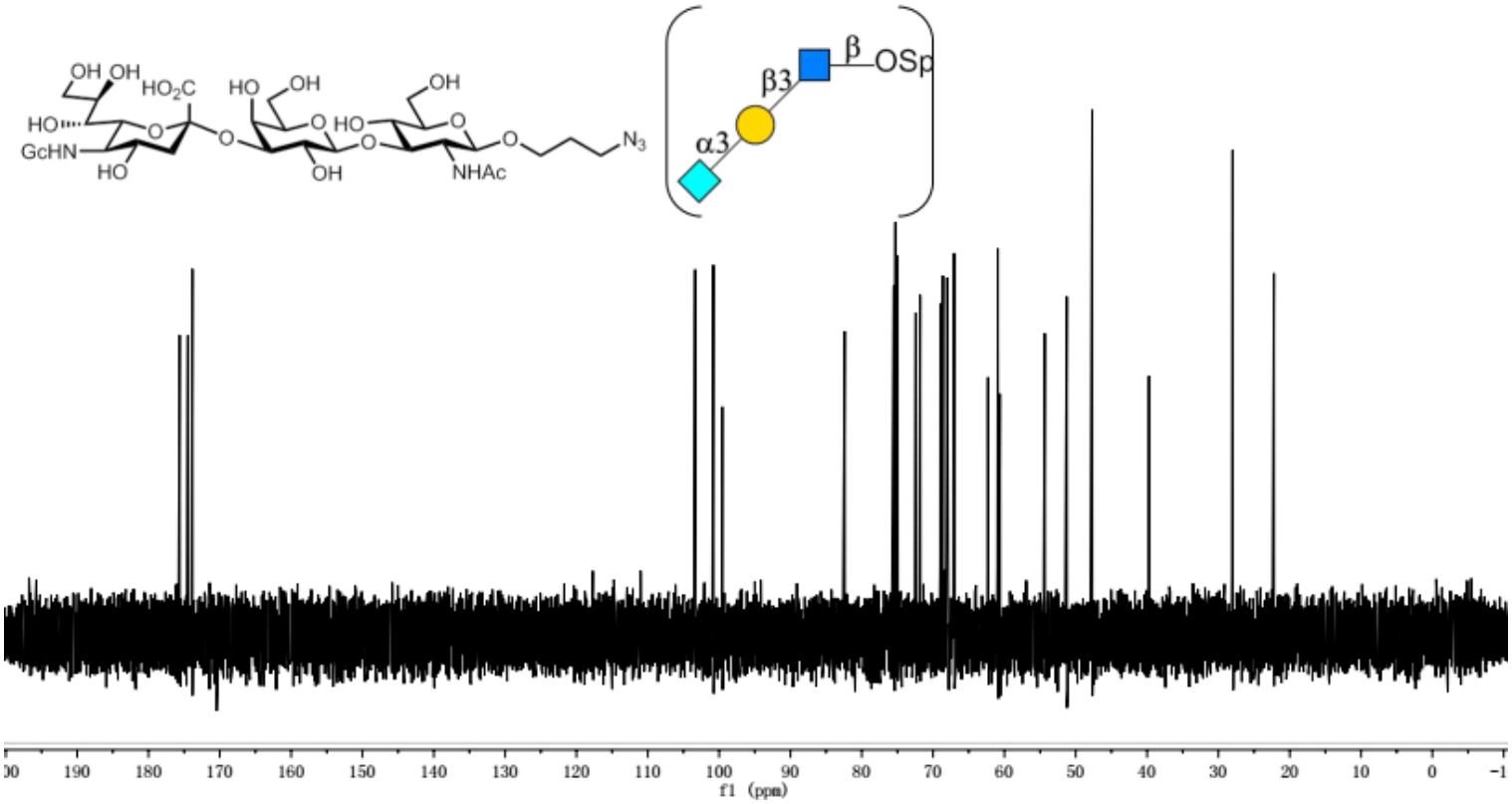


^{13}C NMR of 41

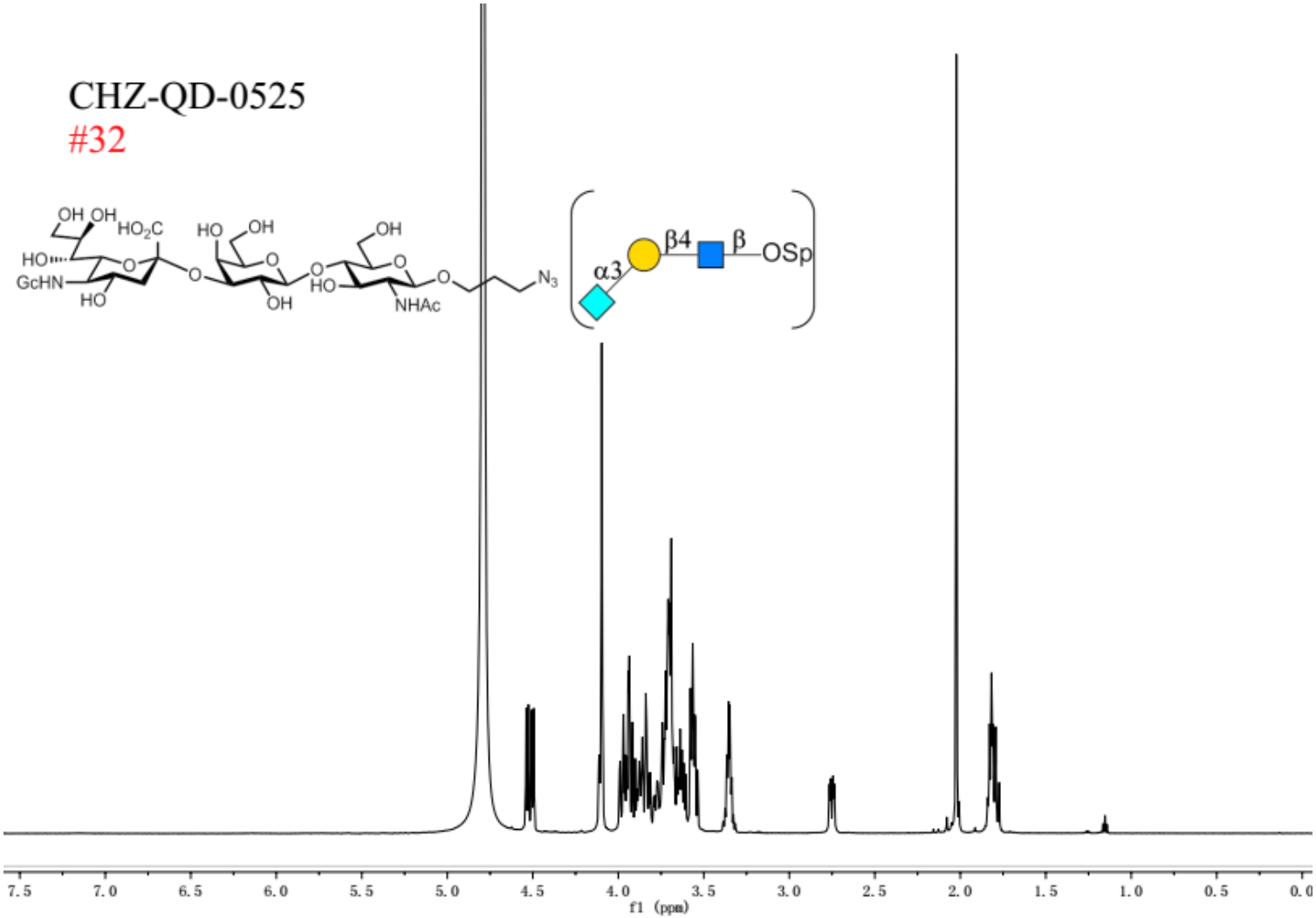

CHZ-QD-0549
#29
OH OH
HO2C
HO
OH
OH
HO
HO
O
O
HO
O
O
O
N3
GcHN
HO
OH
NHAc
β
OSp
β3
α3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{1}H NMR of 29

CHZ-QD-0042
#29



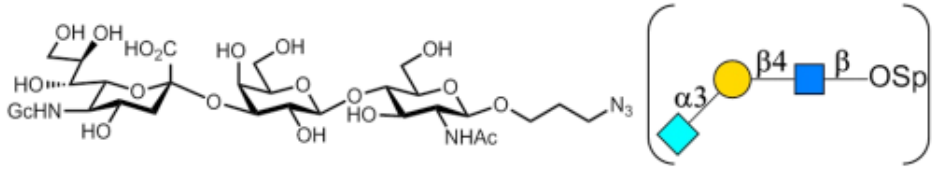
^{13}C NMR of 29

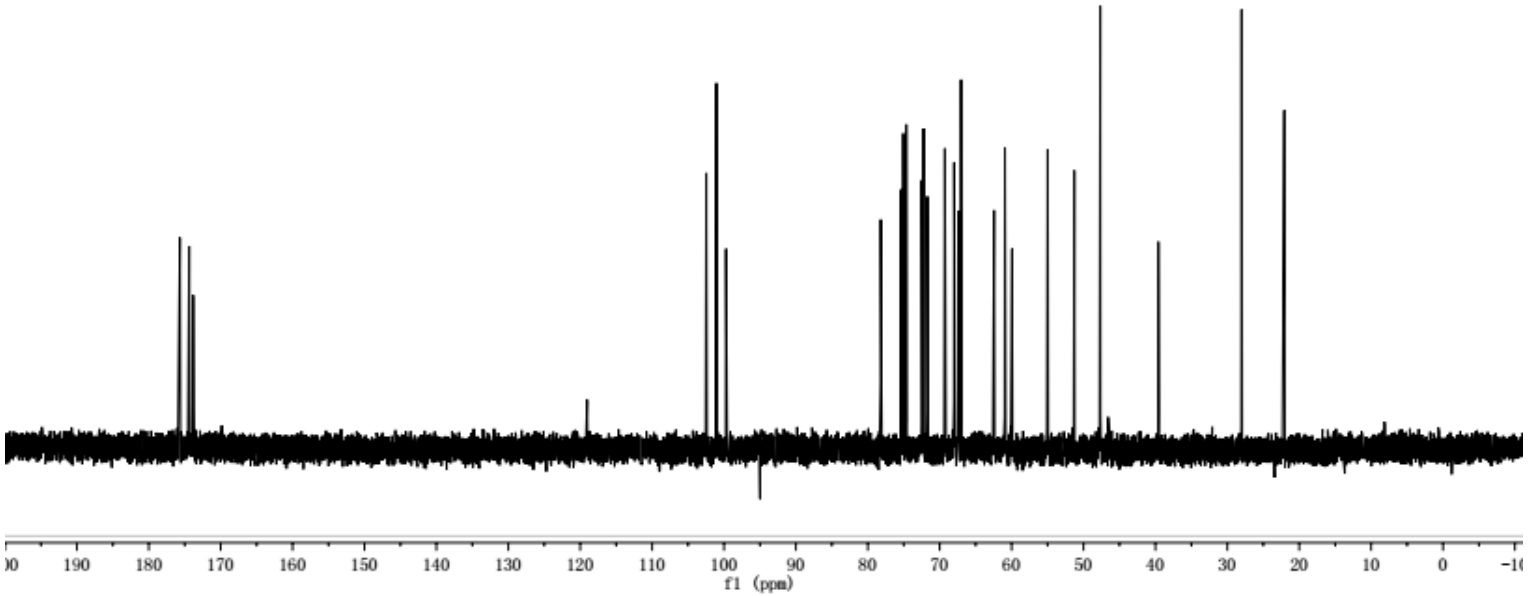

CHZ-QD-0525
#32
OH OH
HO2C
HO
OH
HO
GcHN
HO
O
O
O
O
O
HO
OH
OH
NHAc
N3
β4
β
OSp
α3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{1}H NMR of 32

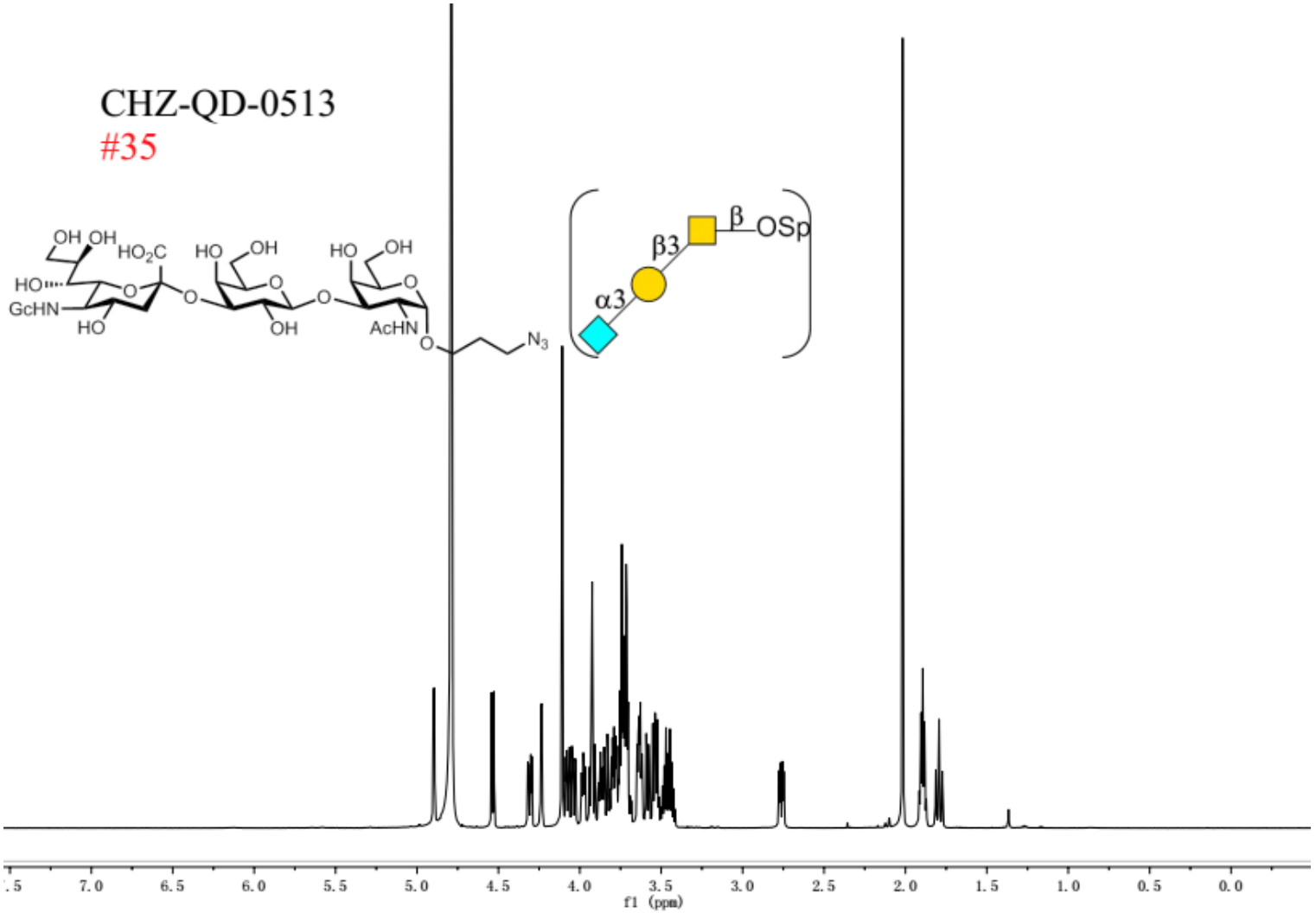
CHZ-QD-0569

#32





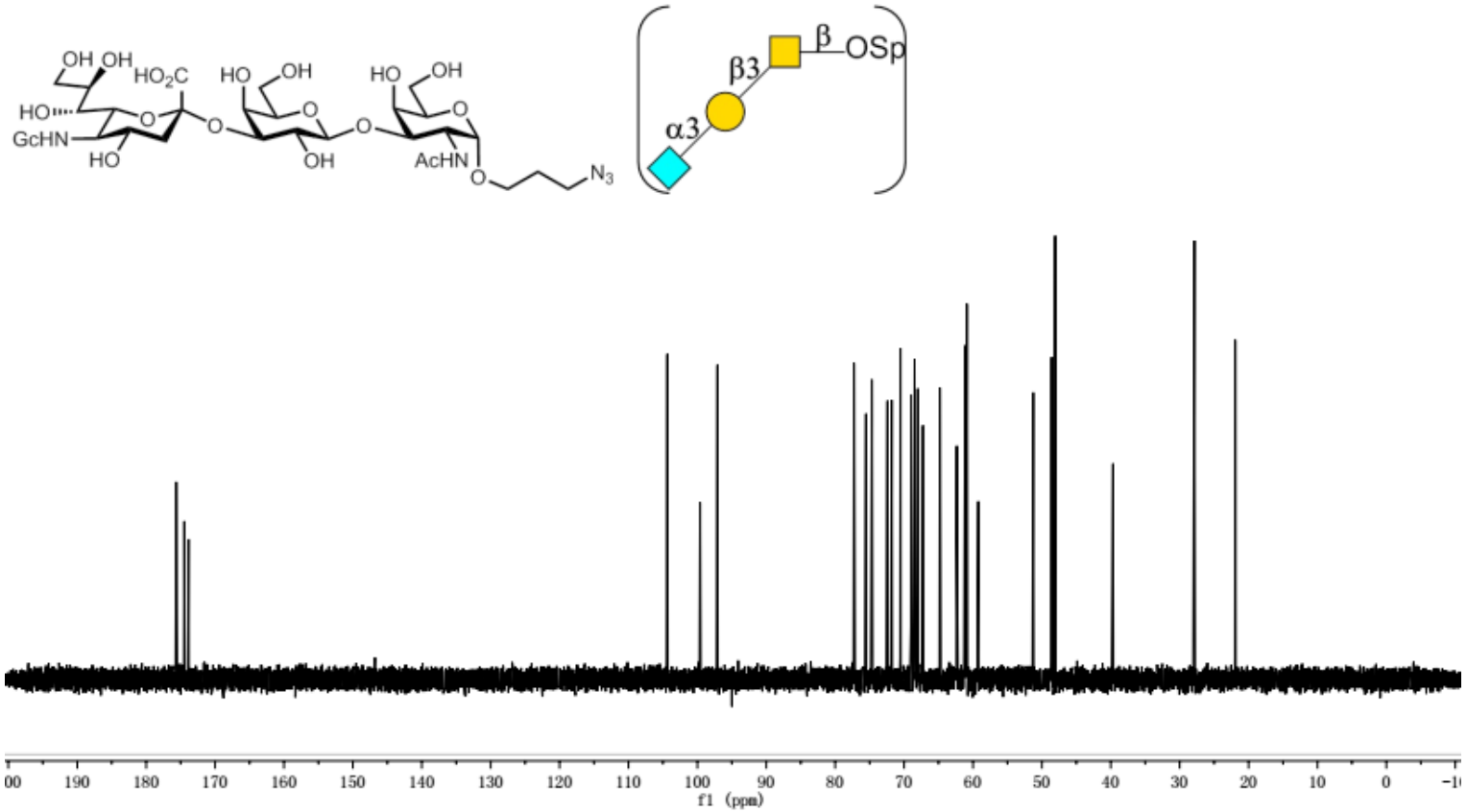
^{13}C NMR of 32


CHZ-QD-0513
#35
OH OH
HO2C
HO
OH
HO
OH
HO
GcHN
HO
OH
AcHN
N3
β
OSp
β3
α3
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
0.0
f1 (ppm)

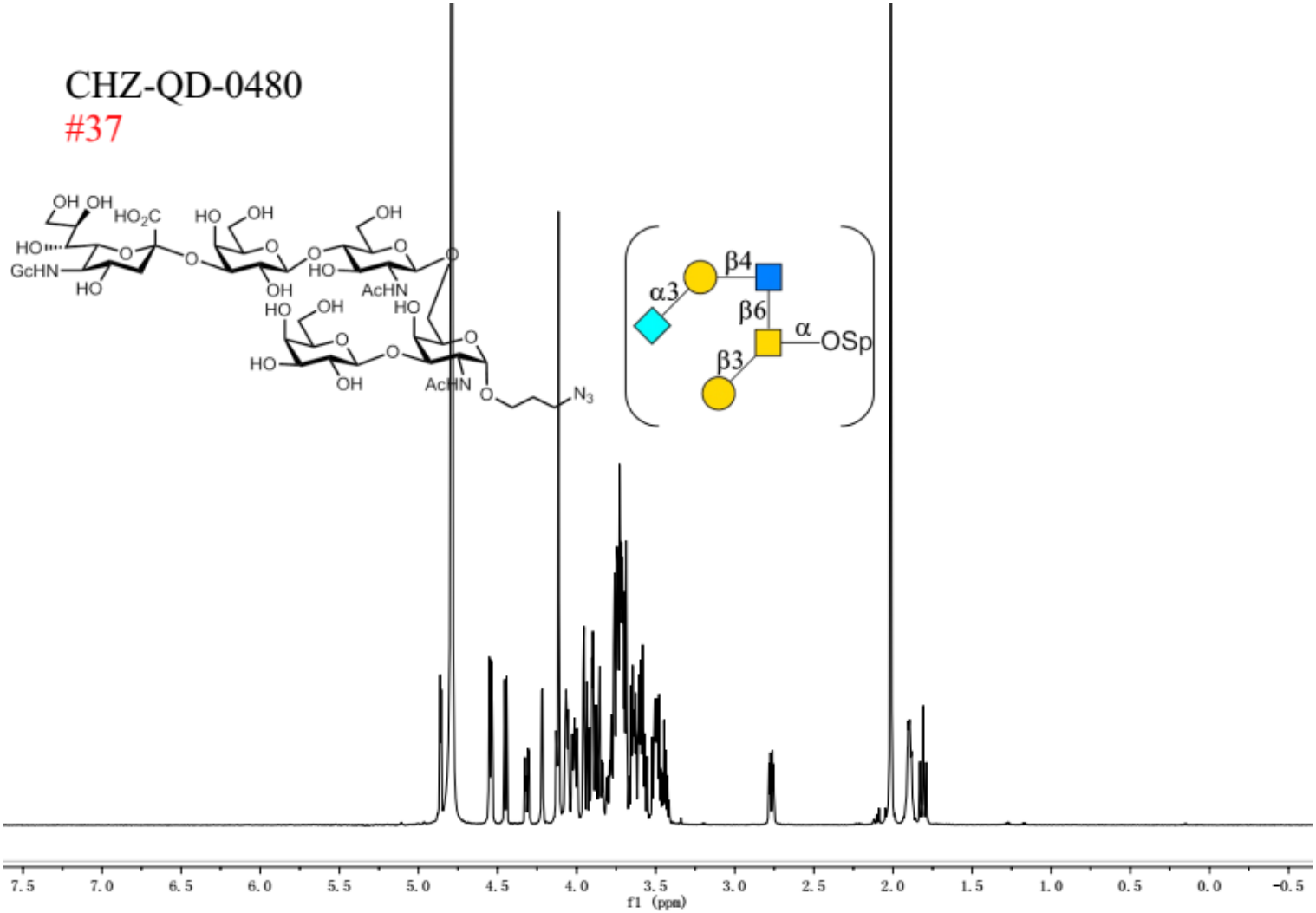
^{1}H NMR of 35

CHZ-QD-0041

#35



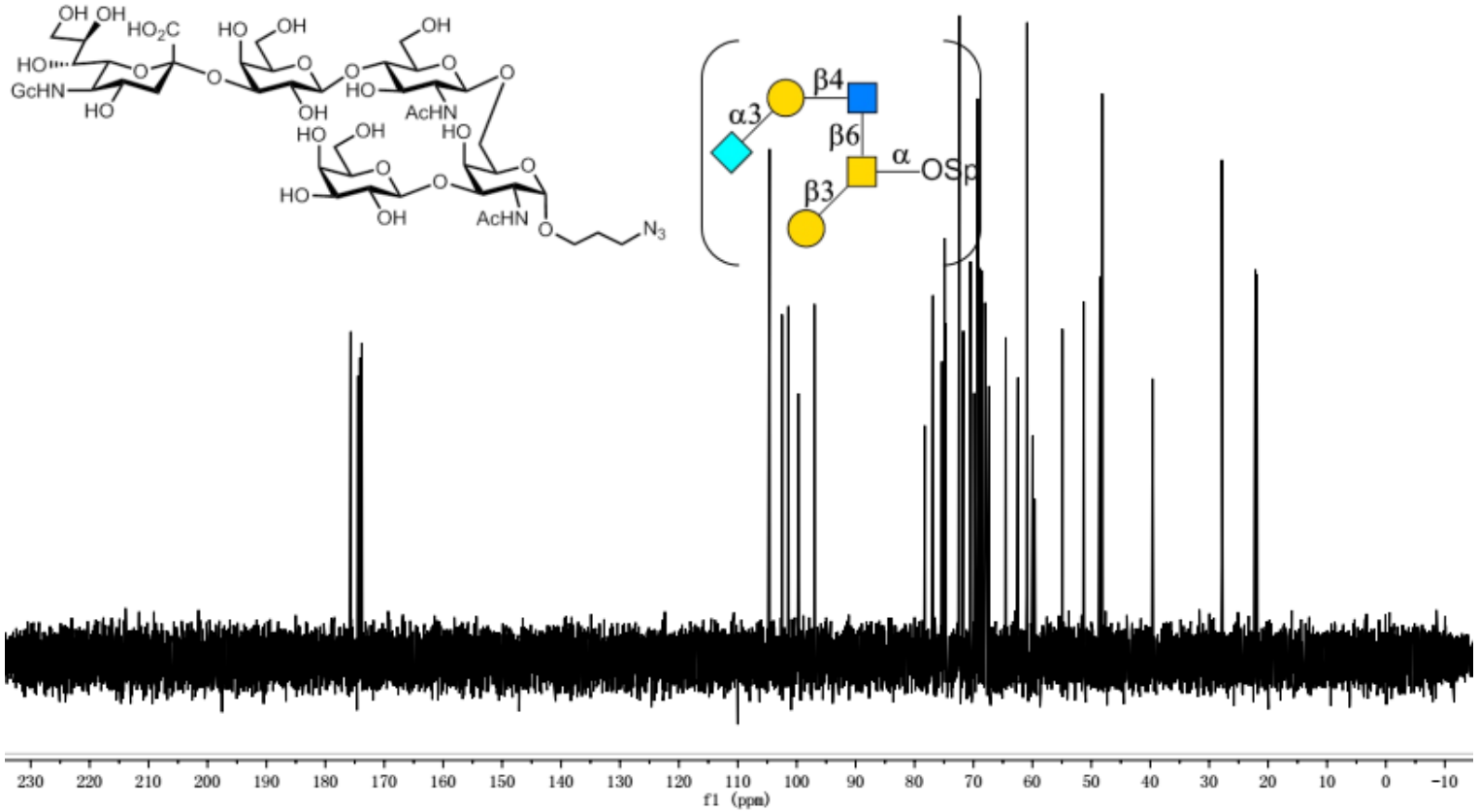
^{13}C NMR of 35


CHZ-QD-0480
#37
OH OH
HO2C
HO
OH
HO
GcHN
HO
O
O
O
O
HO
OH
AcHN
HO
OH
HO
O
HO
OH
O
AcHN
O
N3
β4
α3
β6
α
OSp
β3
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
0.0
-0.5
f1 (ppm)

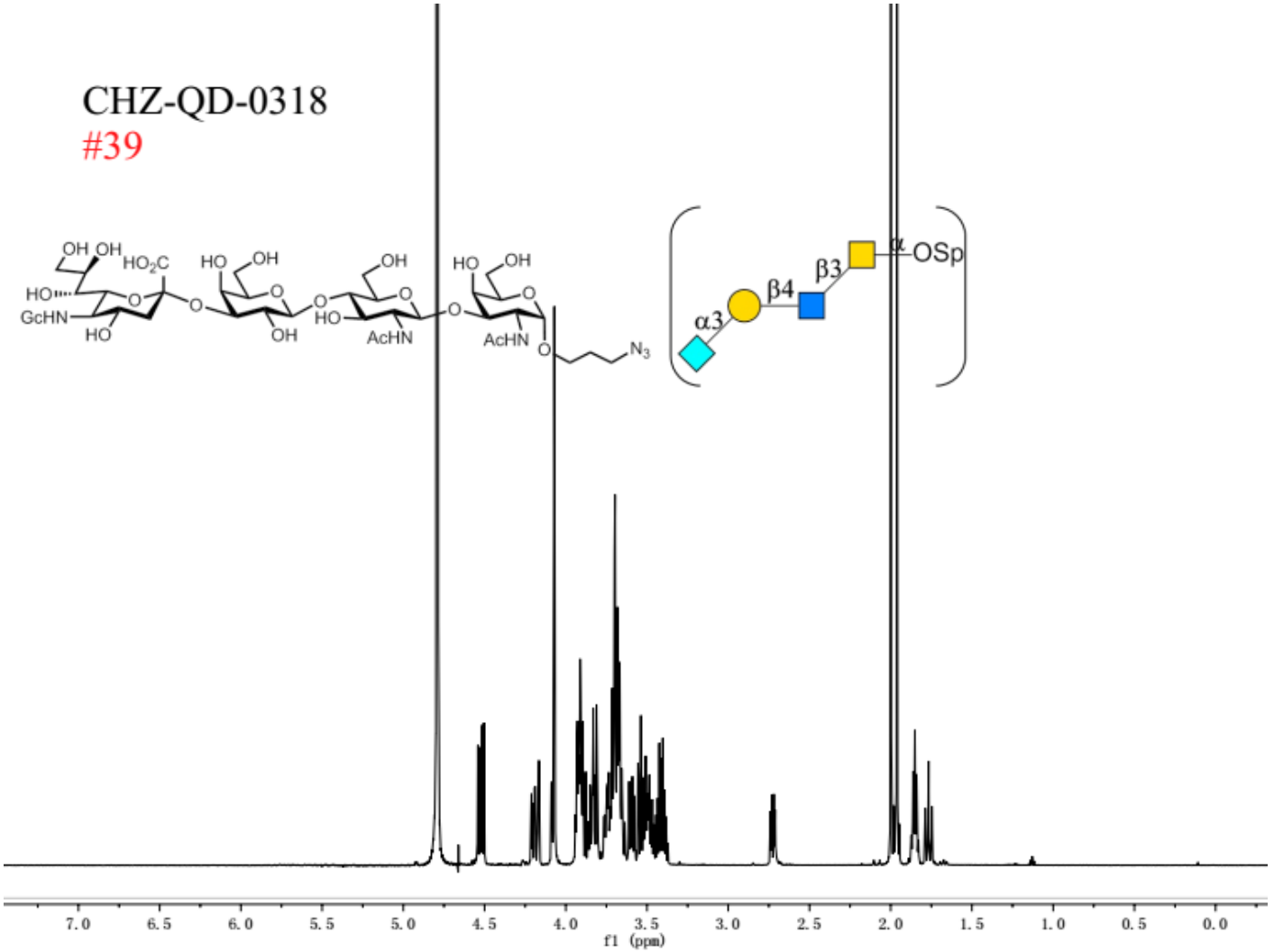
^{1}H NMR of 37

CHZ-QD-0506

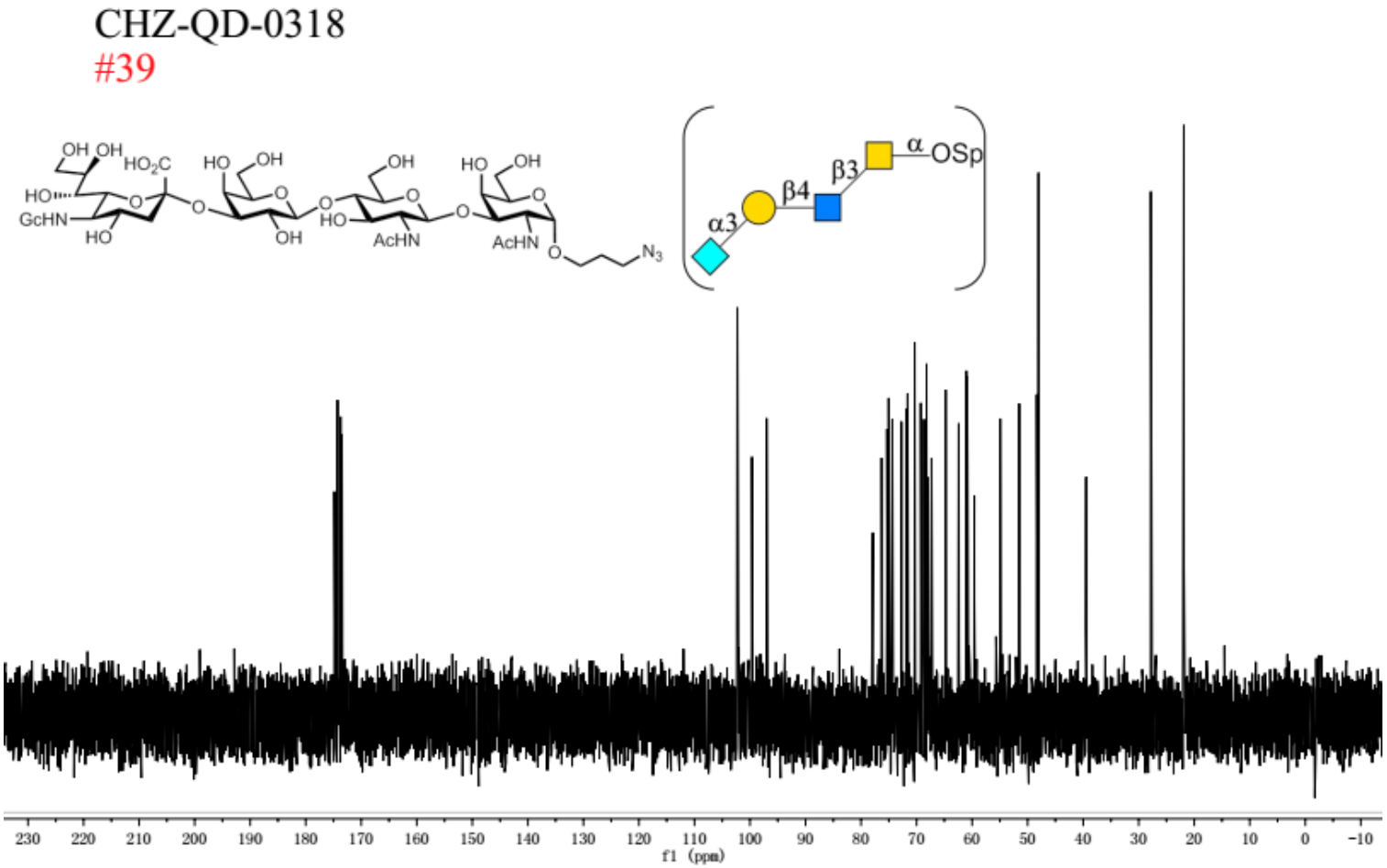
#37



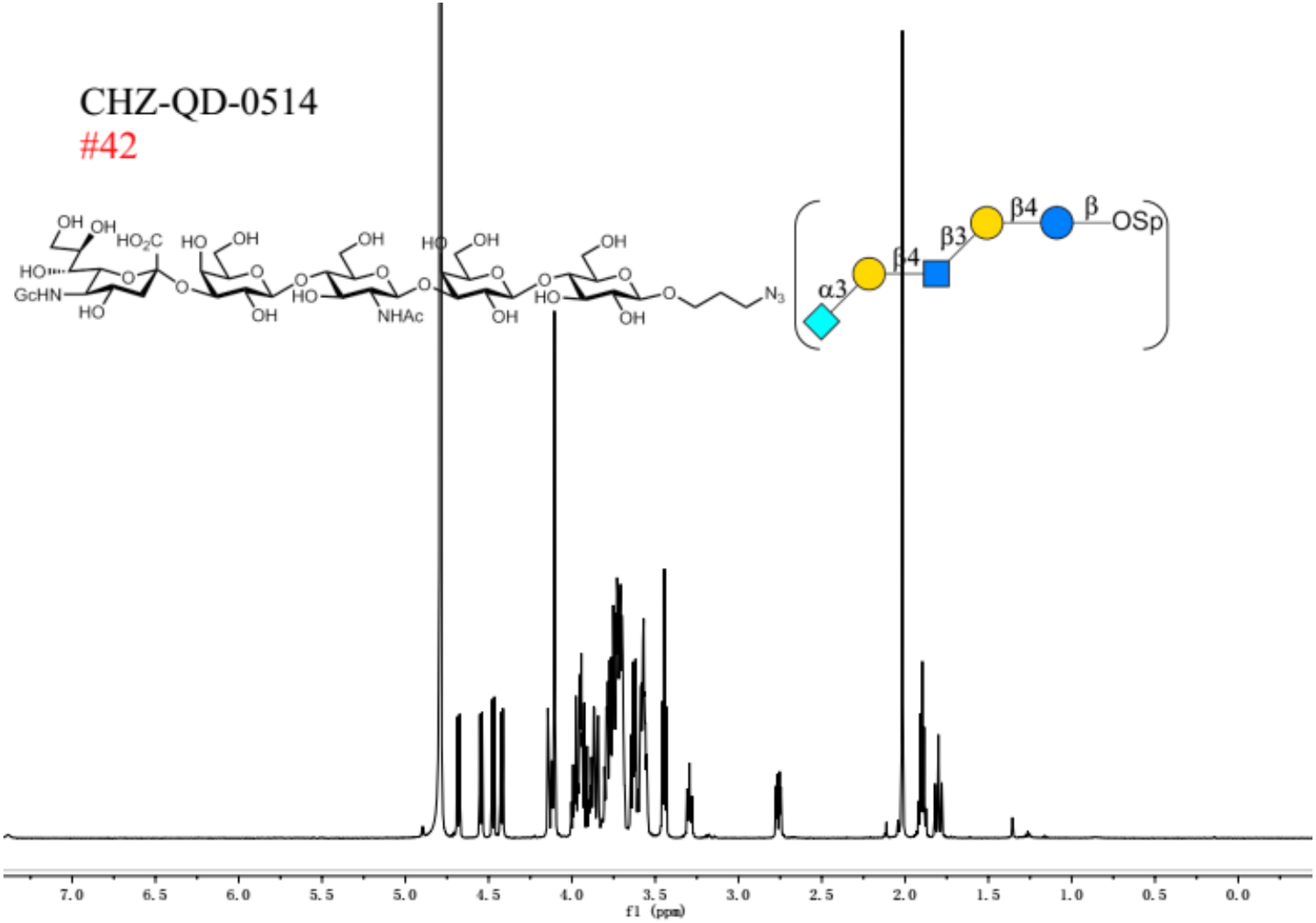
^{13}C NMR of 37


CHZ-QD-0318
#39
OH OH
HO2C
HO
HO
GcHN
HO
OH
O
HO
OH
AcHN
HO
OH
AcHN
N3
α3
β4
β3
α
OSp
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
0.0
f1 (ppm)

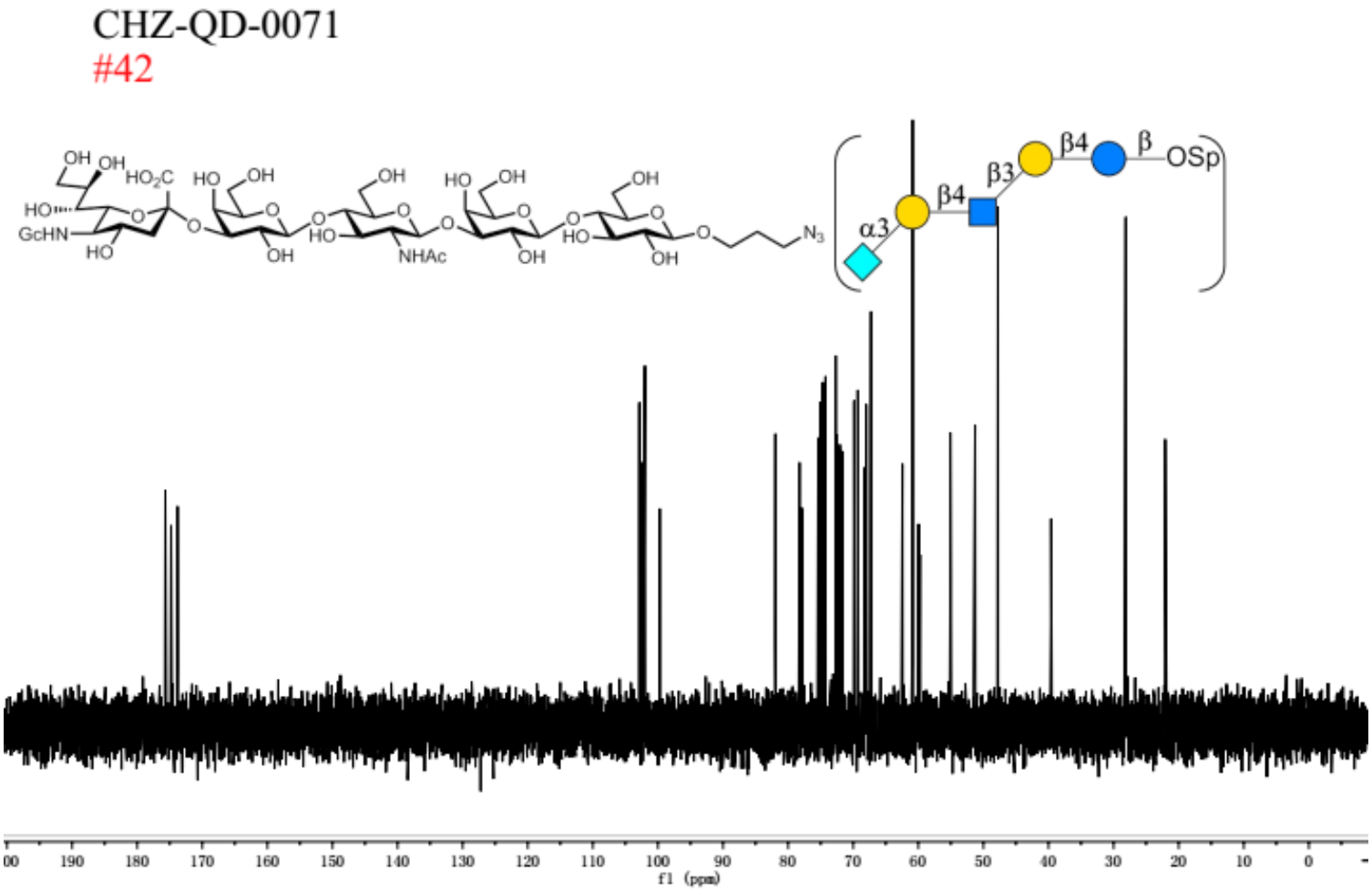
^{1}H NMR of 39


CHZ-QD-0318
#39
OH OH
HO2C
HO
OH
HO
GcHN
HO
O
O
HO
OH
OH
HO
AcHN
OH
HO
AcHN
O
N3
α
OSp
β3
β4
α3
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)

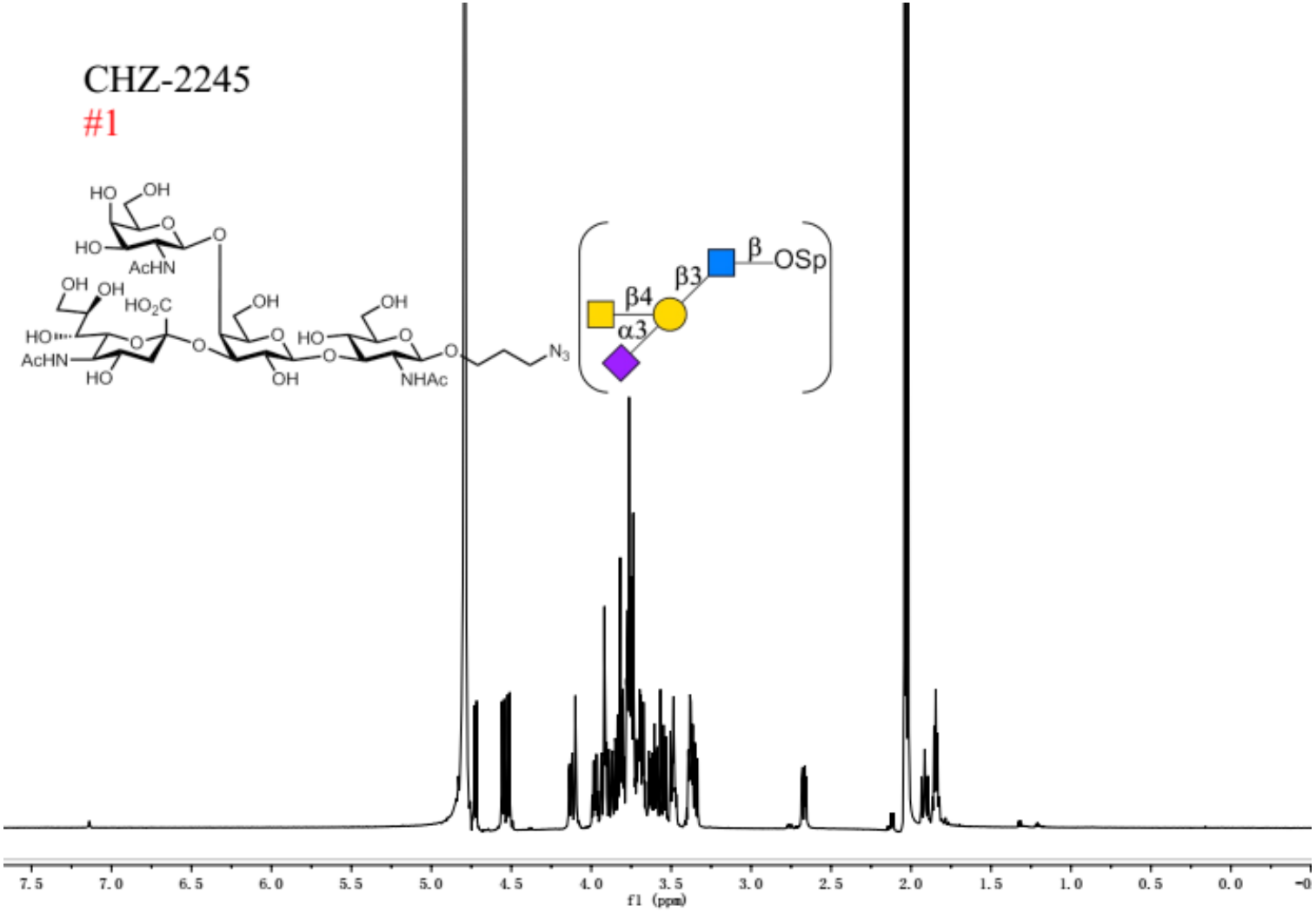
^{13}C NMR of 39


CHZ-QD-0514
#42
OH
OH
HO2C
HO
OH
HO
O
GcHN
HO
O
O
HO
OH
O
O
HO
NHAc
HO
OH
O
O
HO
OH
O
O
N3
α3
β4
β3
β4
β
OSp
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
0.0
f1 (ppm)

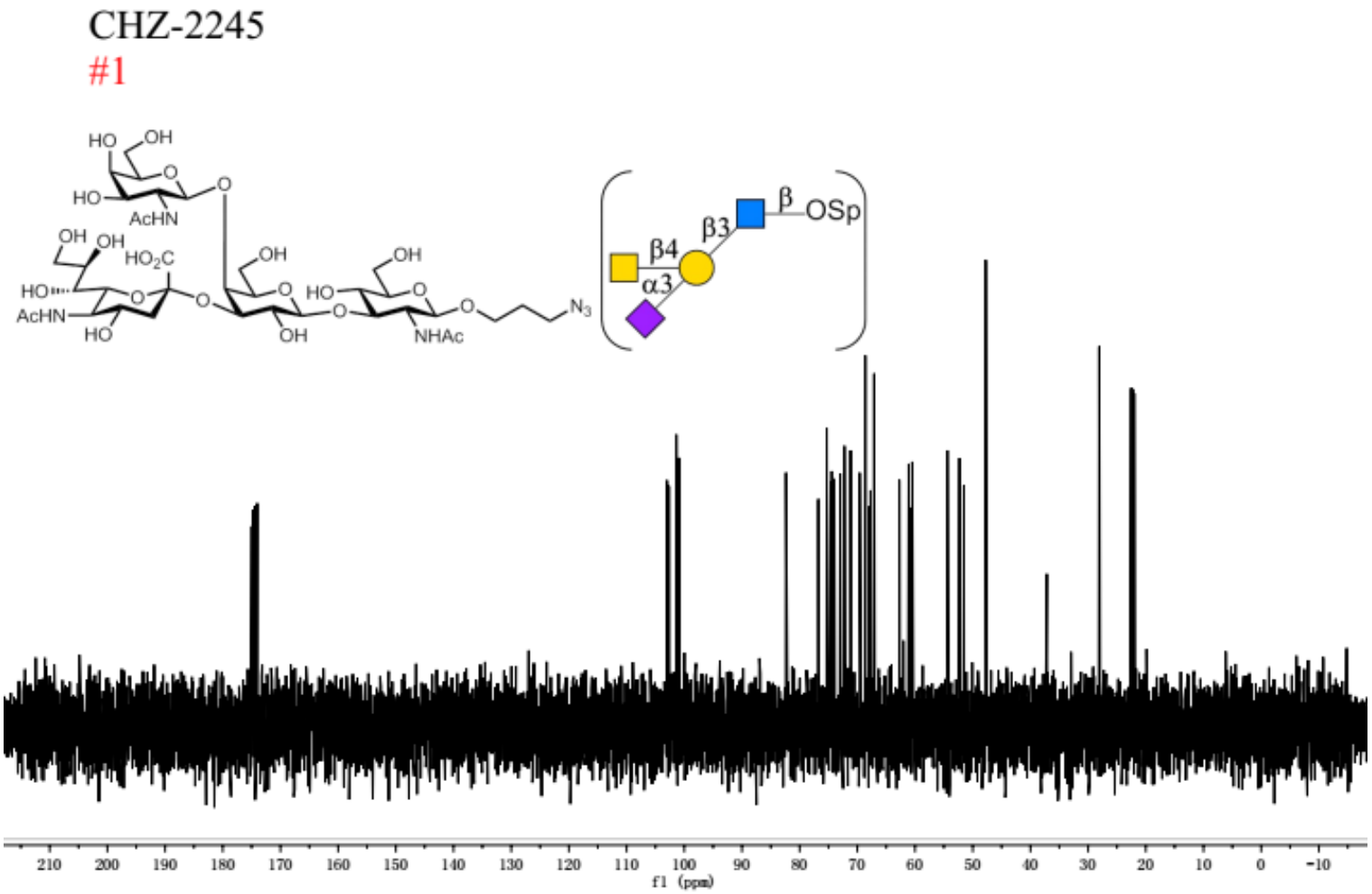
^{1}H NMR of 42



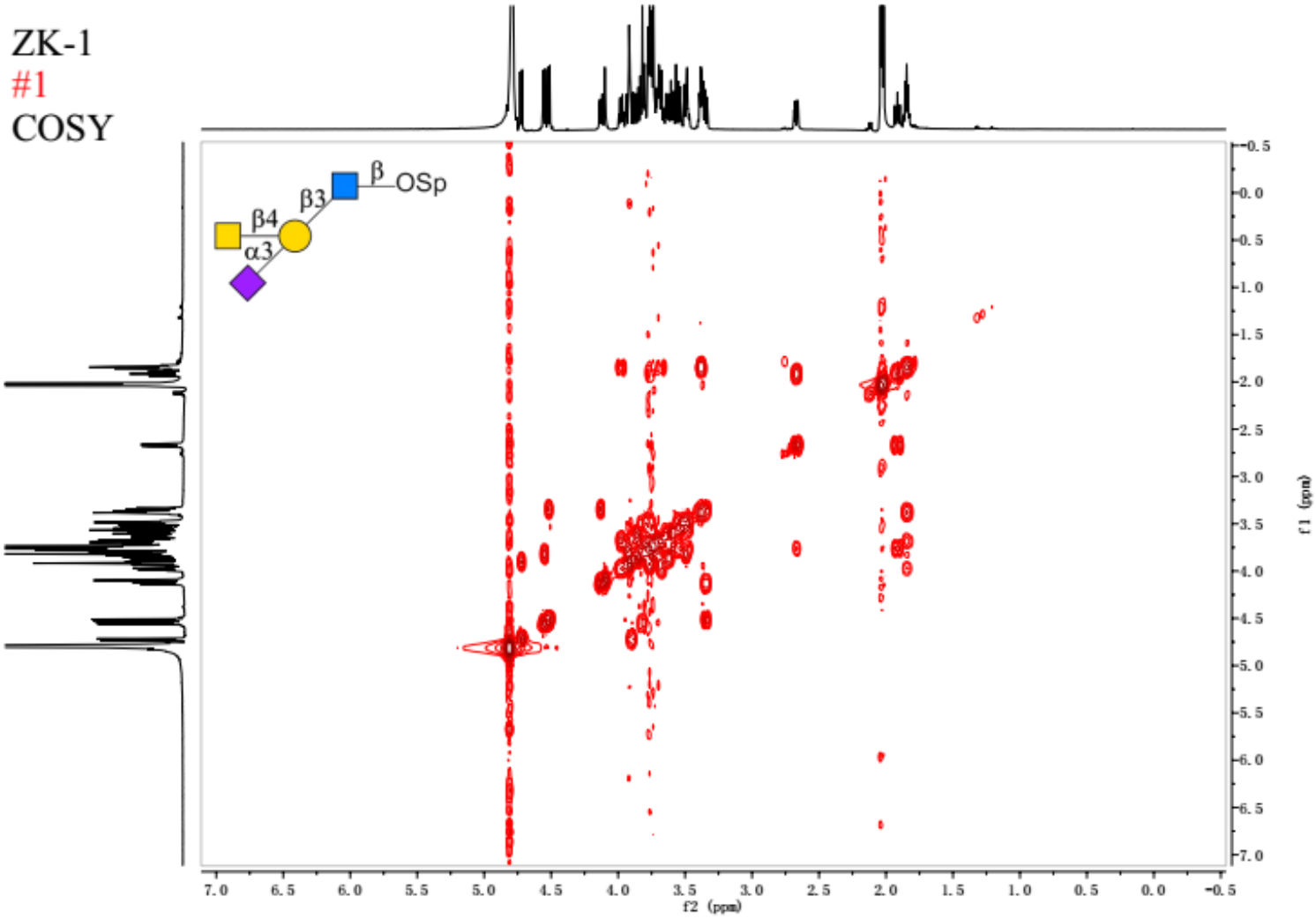
^{13}C NMR of 42


CHZ-2245
#1
HO
OH
HO
AcHN
OH
OH
HO2C
HO
AcHN
HO
OH
OH
HO
NHAc
N3
β
OSp
β3
β4
α3
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
0.0
f1 (ppm)

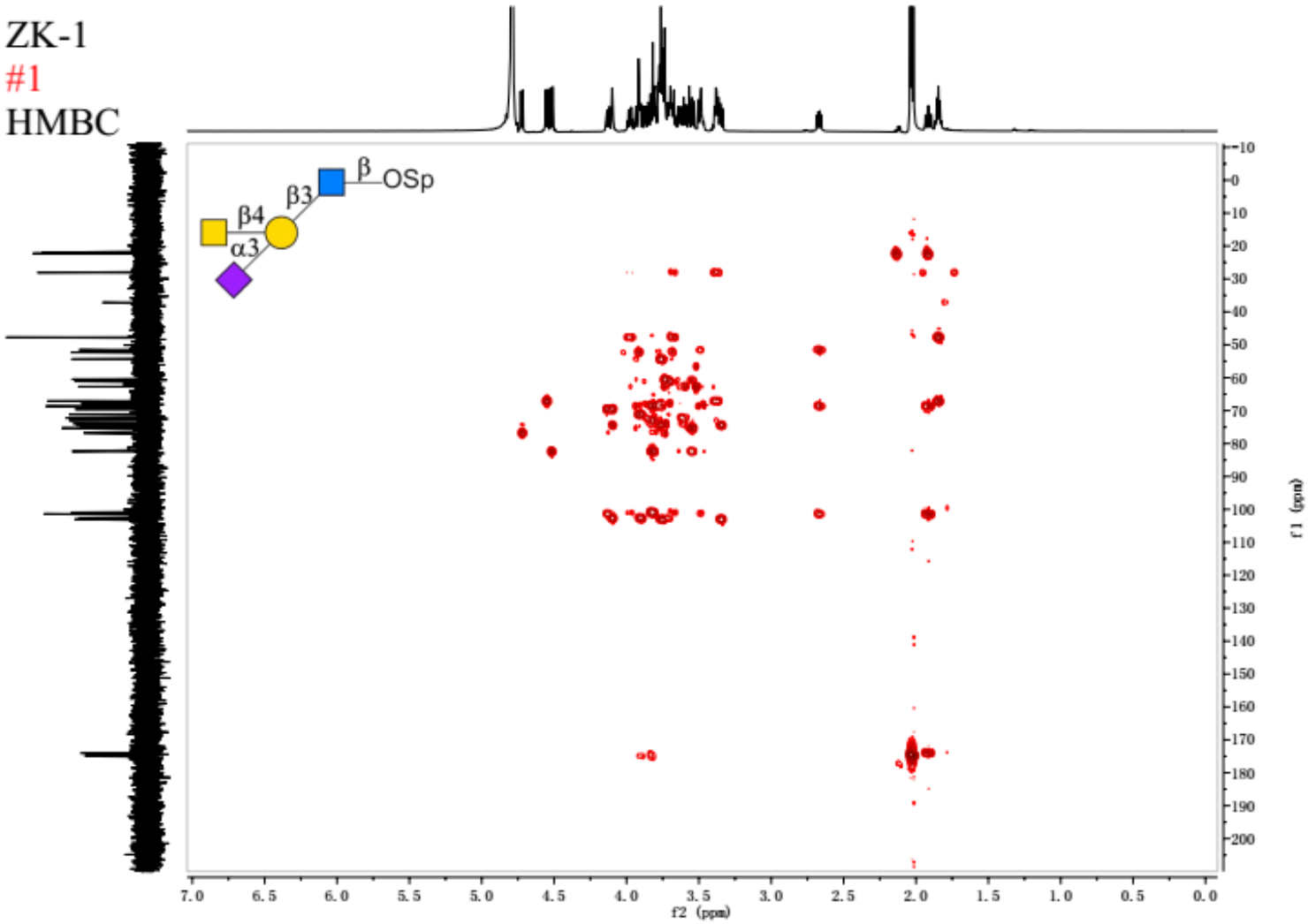
^{1}H NMR of 1


CHZ-2245
#1
HO
OH
HO
AcHN
OH
OH
HO2C
HO
AcHN
HO
OH
OH
HO
NHAc
N3
β
OSp
β3
β4
α3
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)

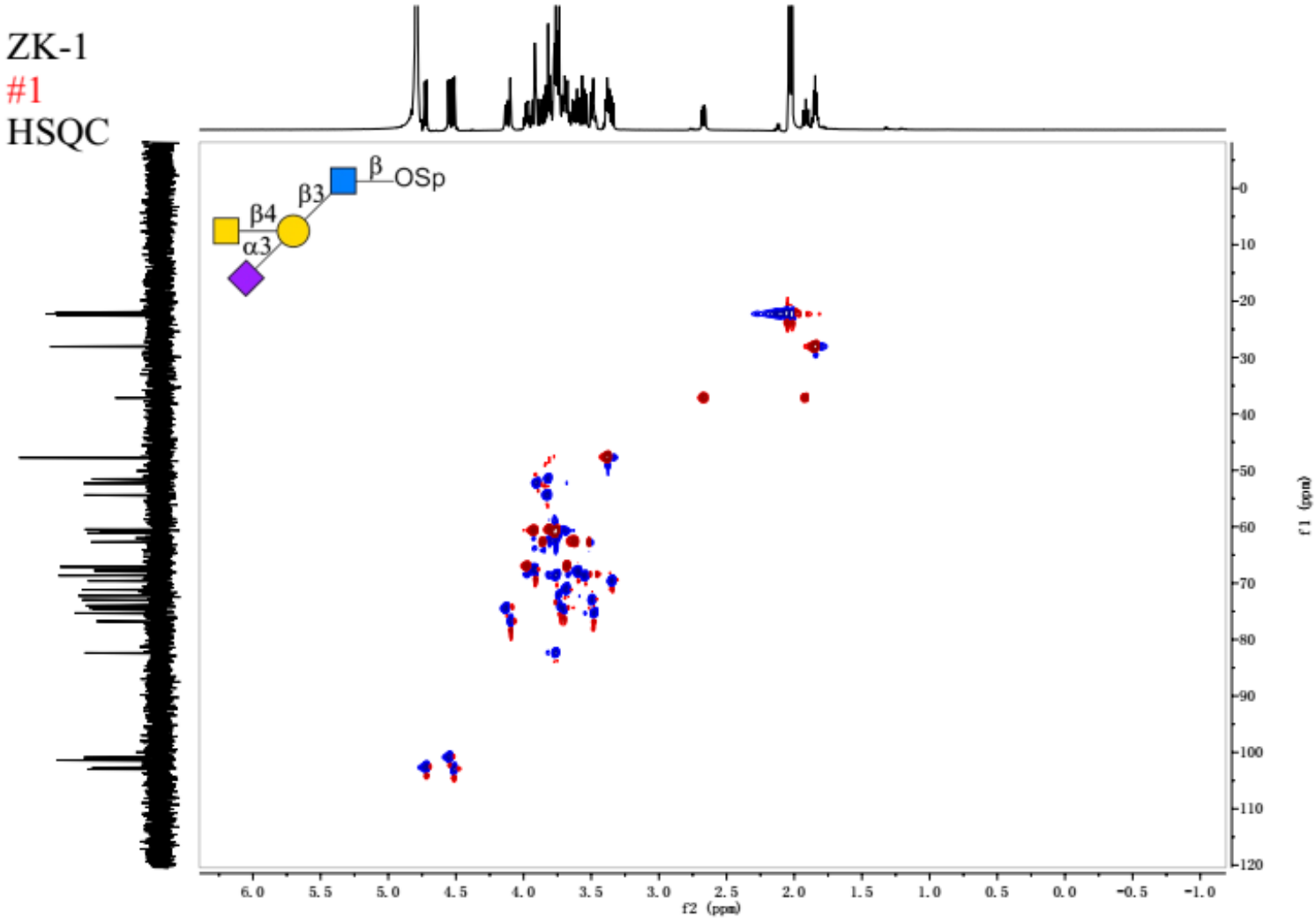
^{13}C NMR of 1



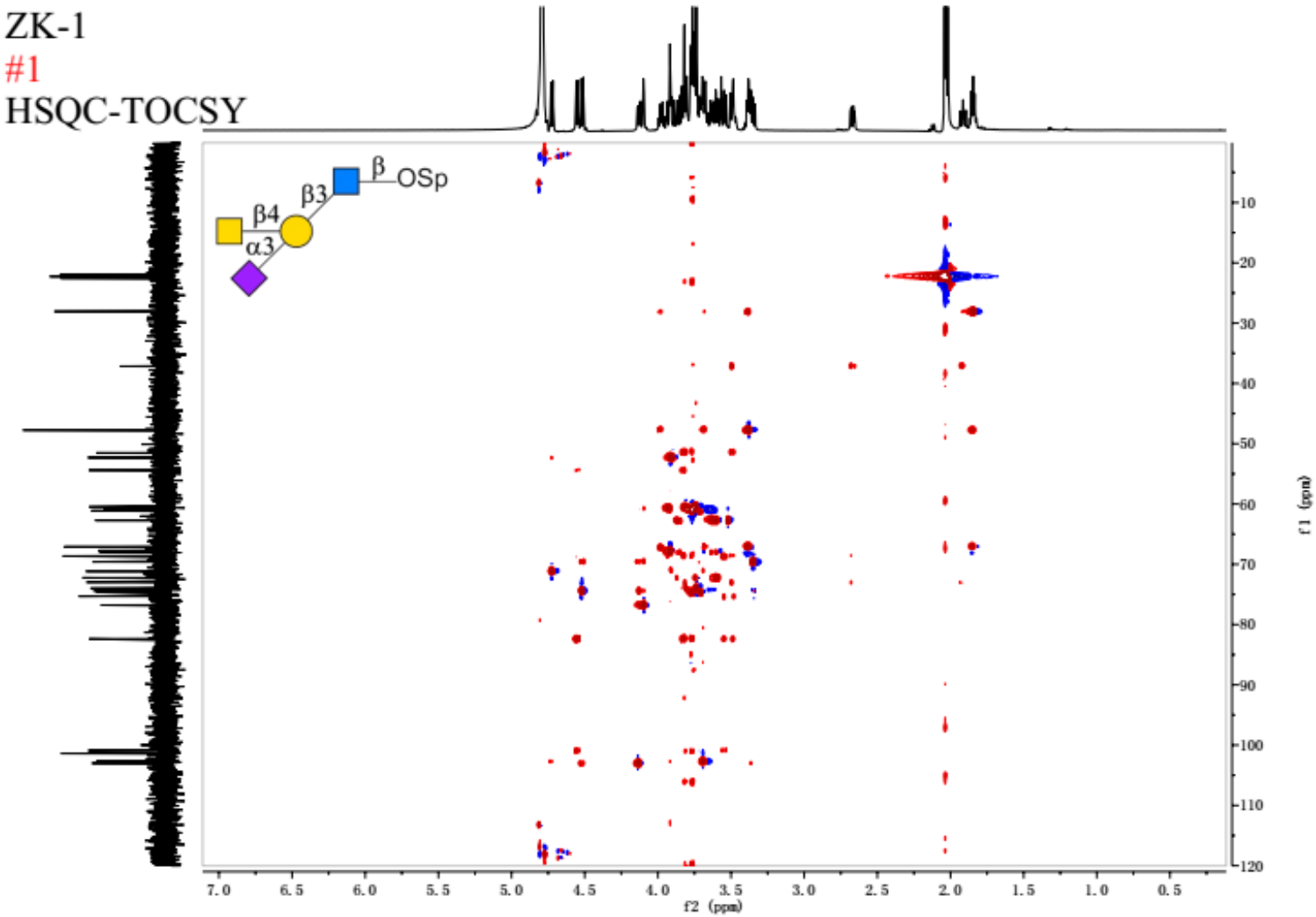
COSY spectra of 1



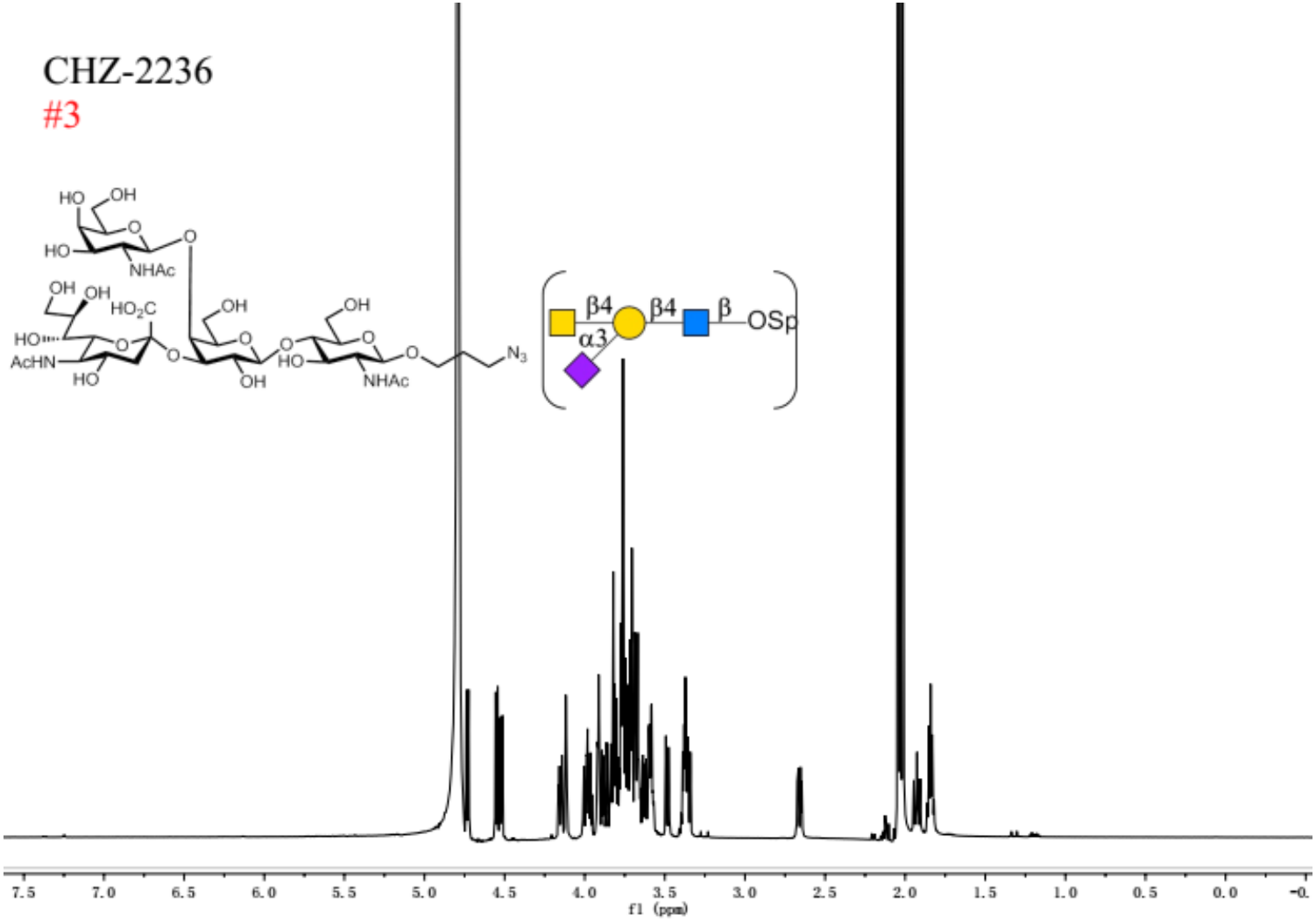
HMBC spectra of 1



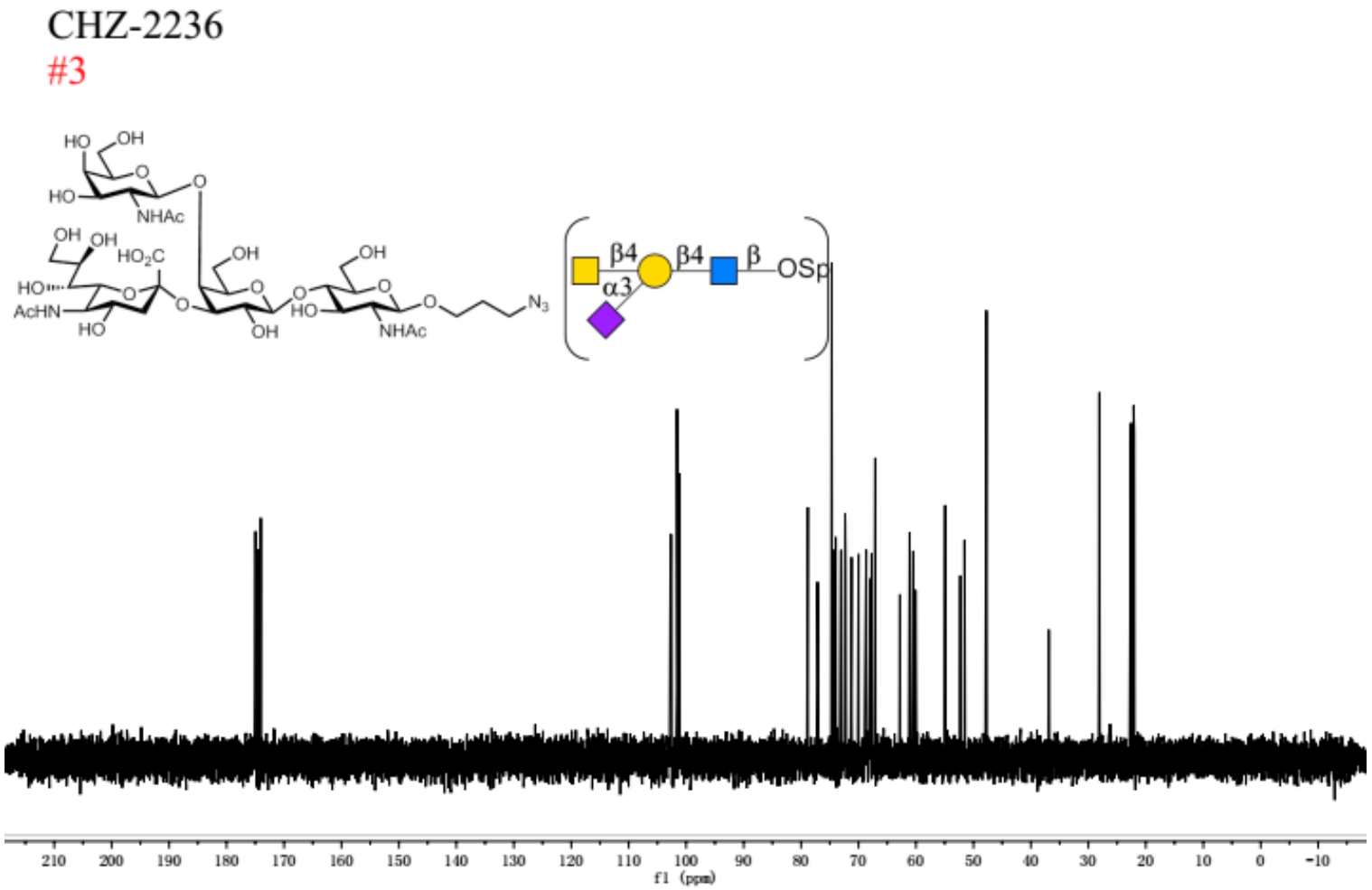
HSQC spectra of 1



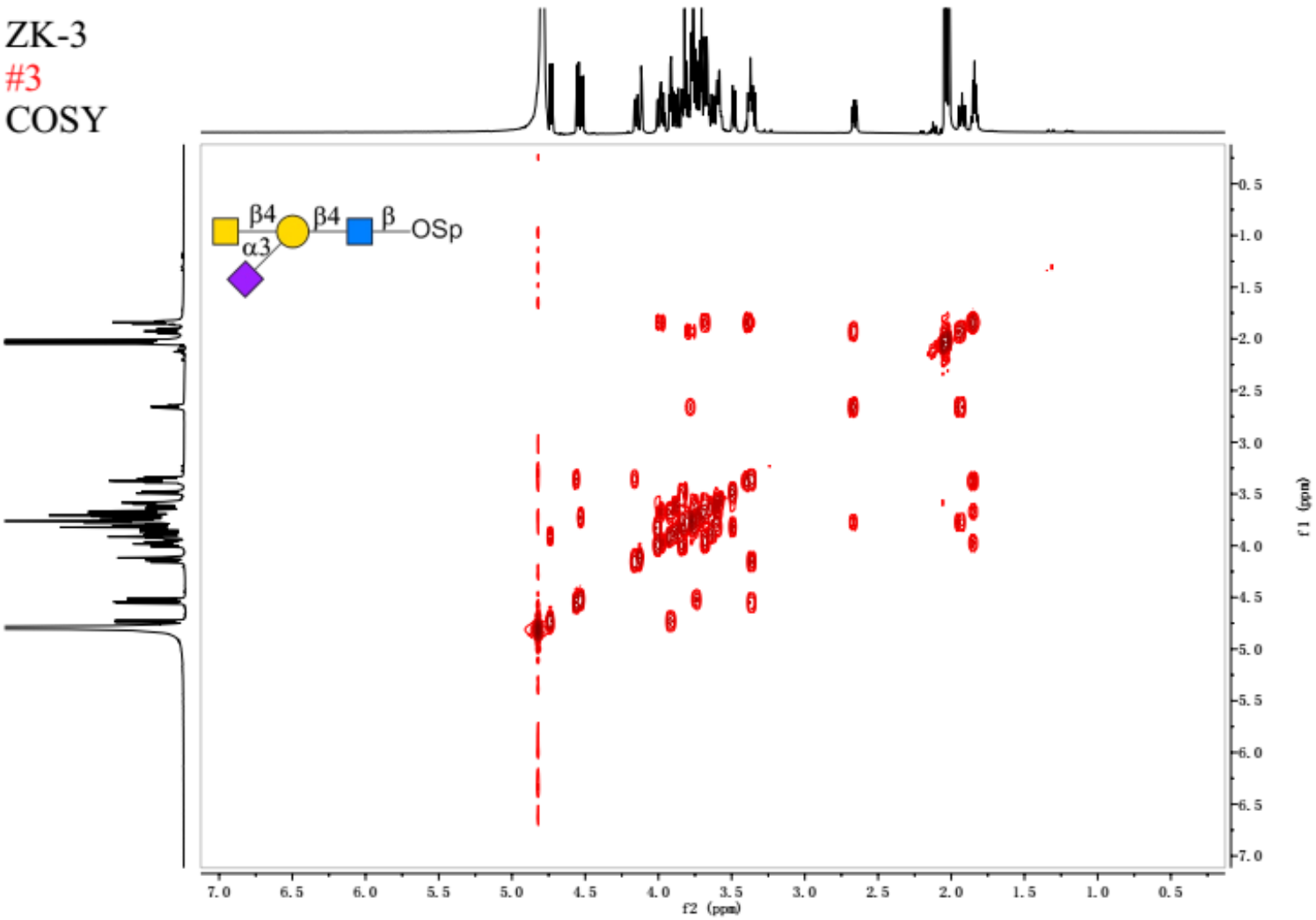
HSQC-TOCSY spectra of 1


CHZ-2236
#3
HO
OH
HO
NHAc
OH
OH
HO2C
HO
AcHN
HO
OH
OH
HO
NHAc
N3
β4
β4
β
OSp
α3
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
0.0
f1 (ppm)

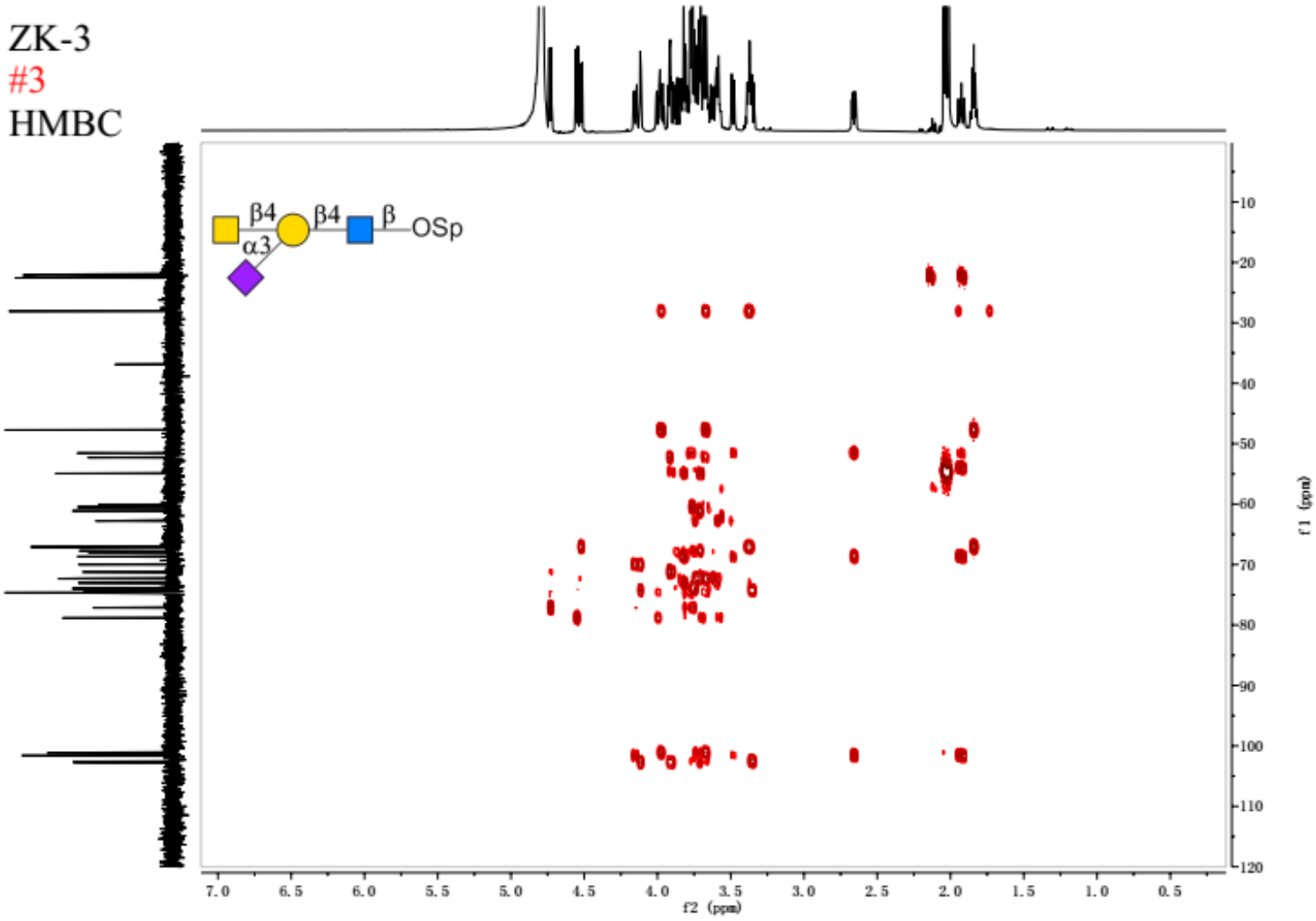
^{1}H NMR of 3


CHZ-2236
#3
HO
OH
HO
NHAc
OH
OH
HO2C
HO
AcHN
HO
OH
OH
HO
NHAc
N3
β4
β4
β
OSp
α3
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)

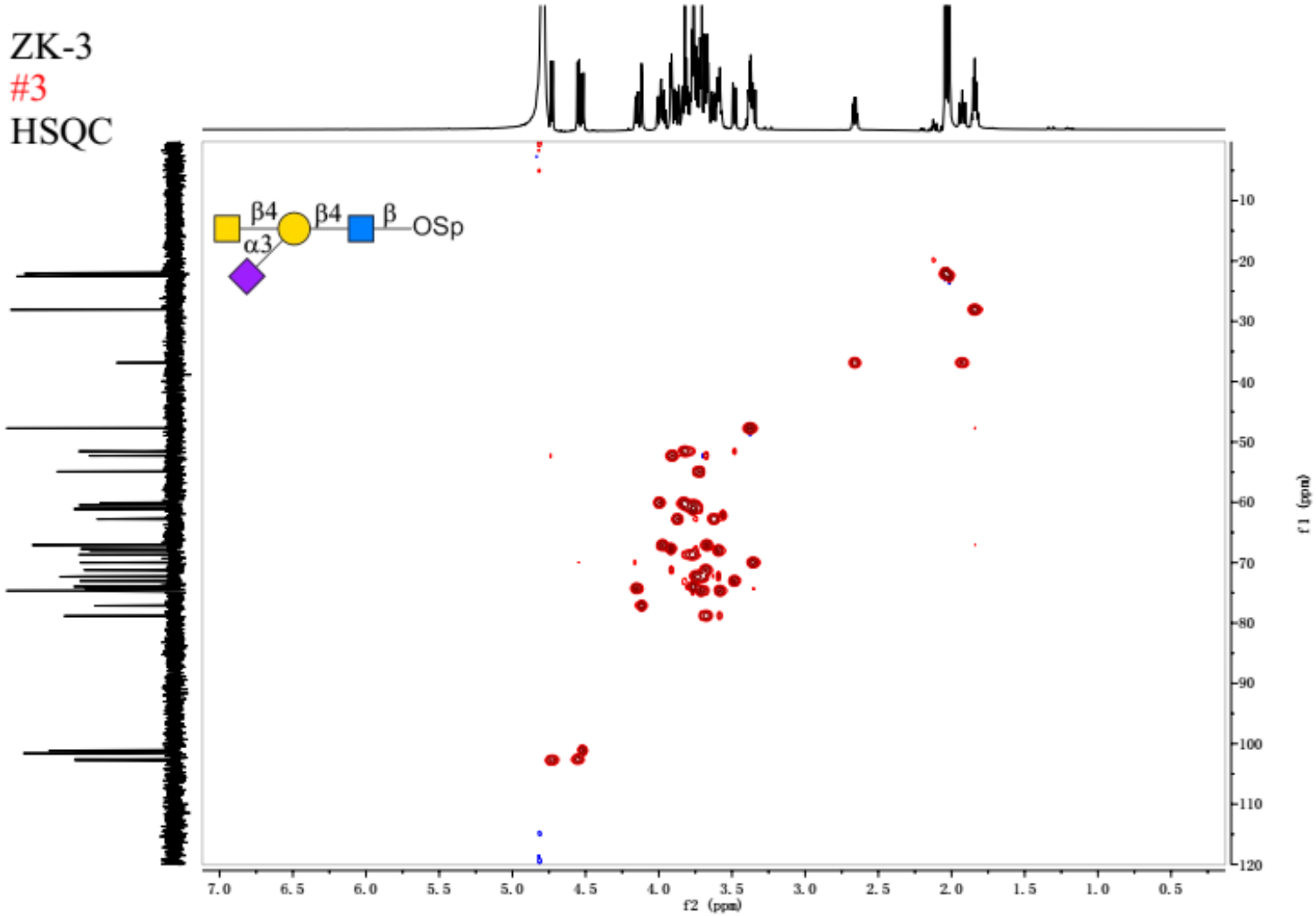
^{13}C NMR of 3



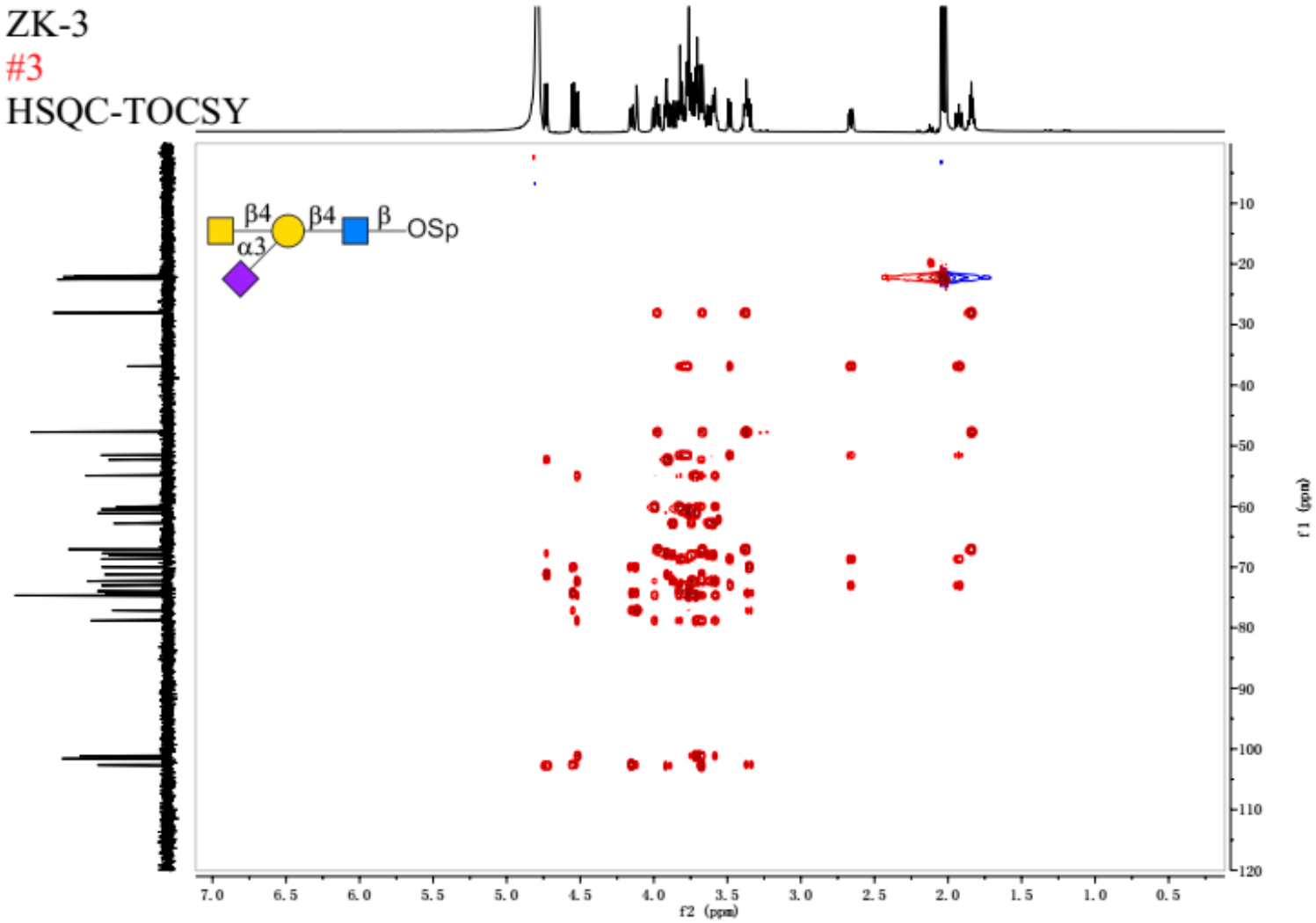
COSY spectra of 3



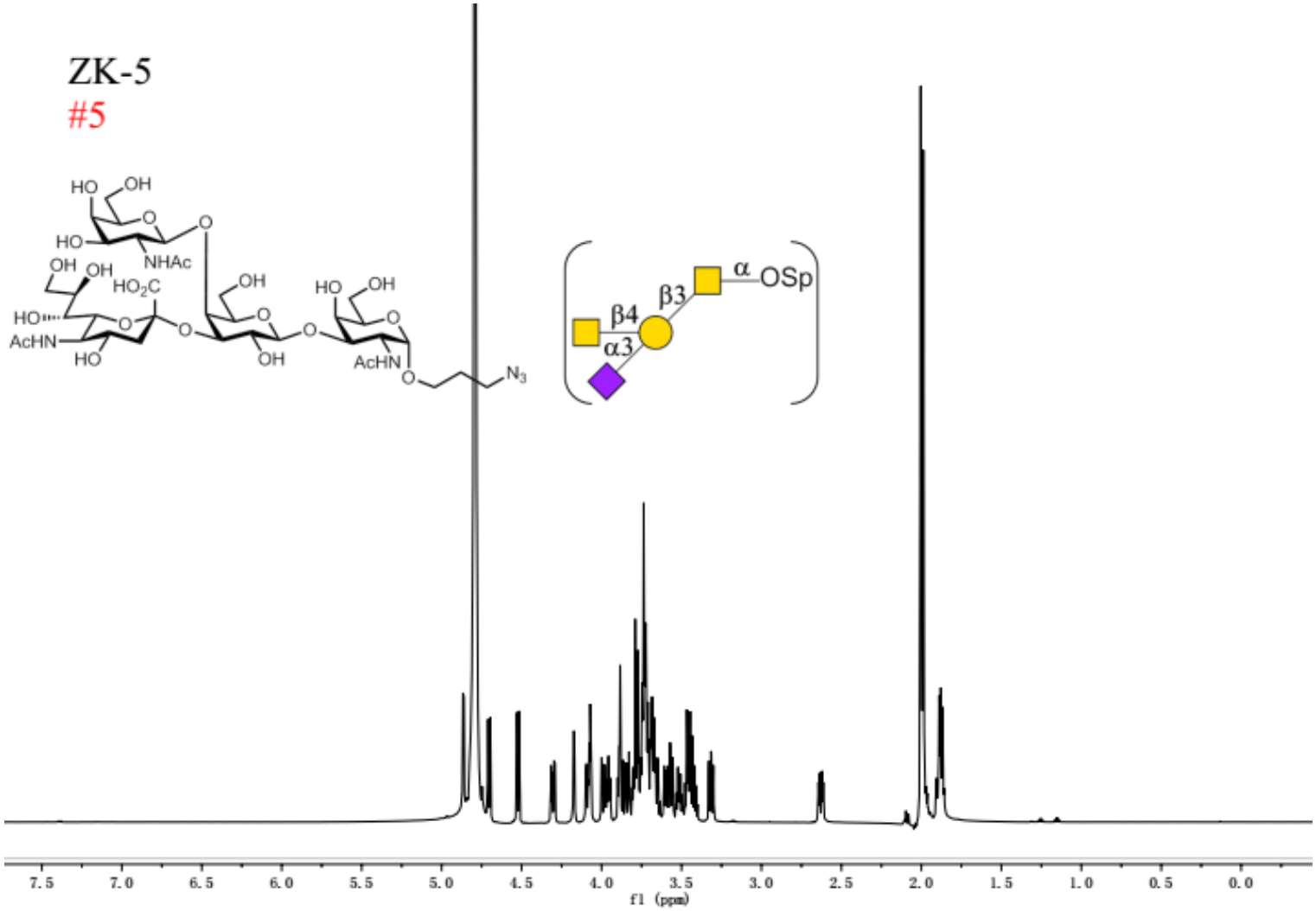
HMBC spectra of 3



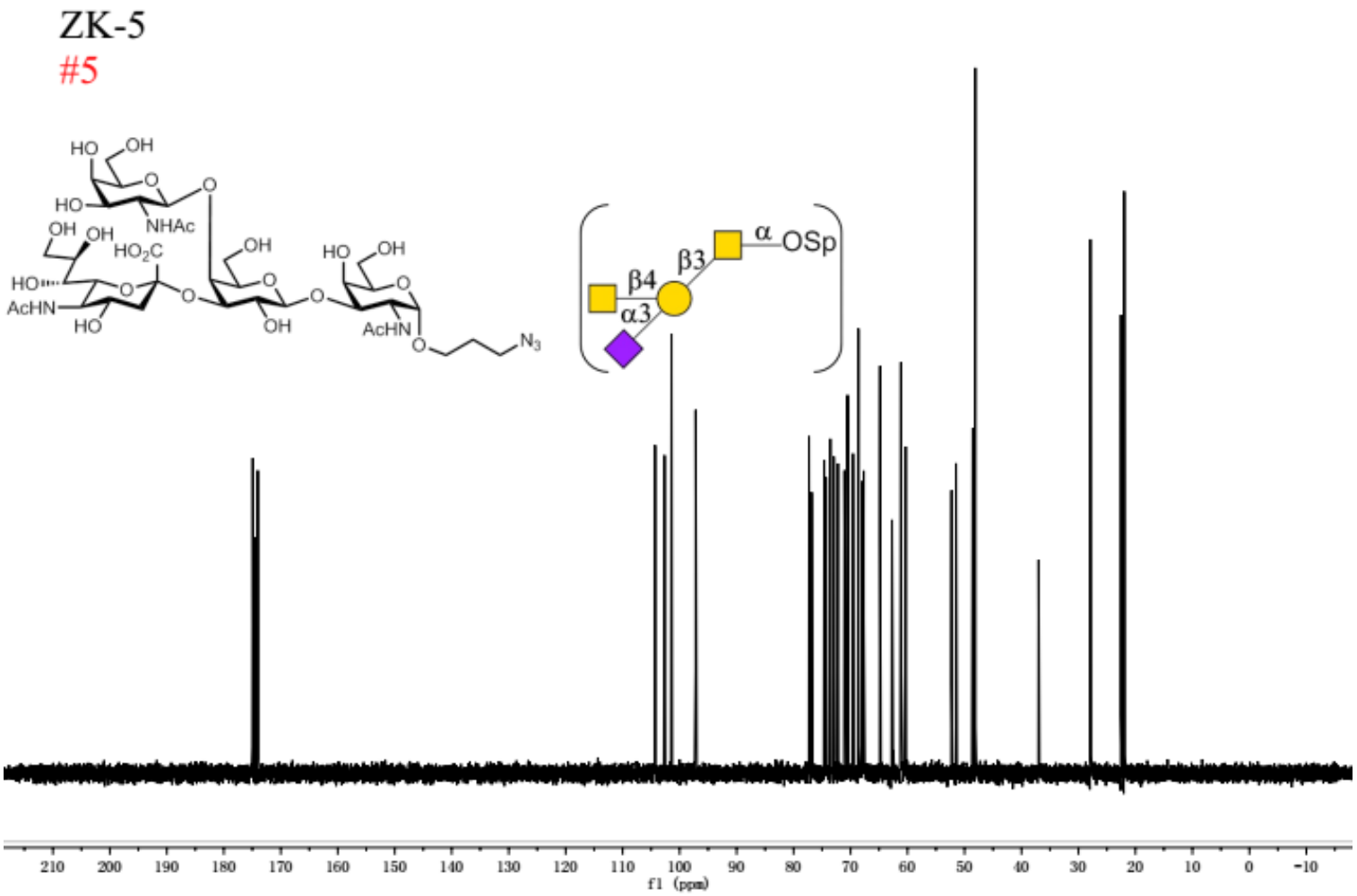
HSQC spectra of 3



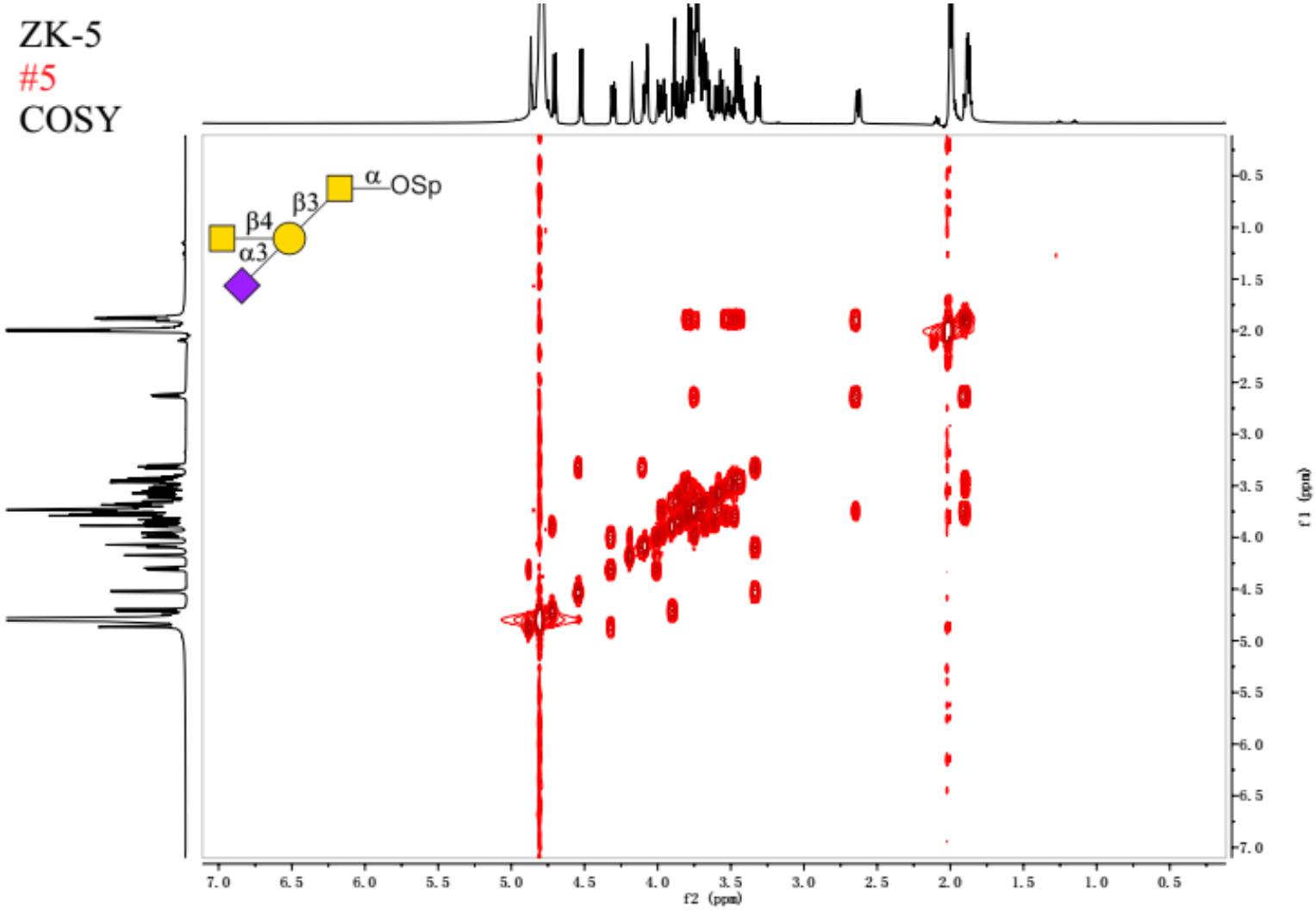
HSQC-TOCSY spectra of 3


ZK-5
#5
HO
OH
O
HO
NHAc
OH
OH
HO2C
HO
AcHN
HO
OH
HO
OH
AcHN
N3
α
OSp
β3
β4
α3
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
0.0
f1 (ppm)

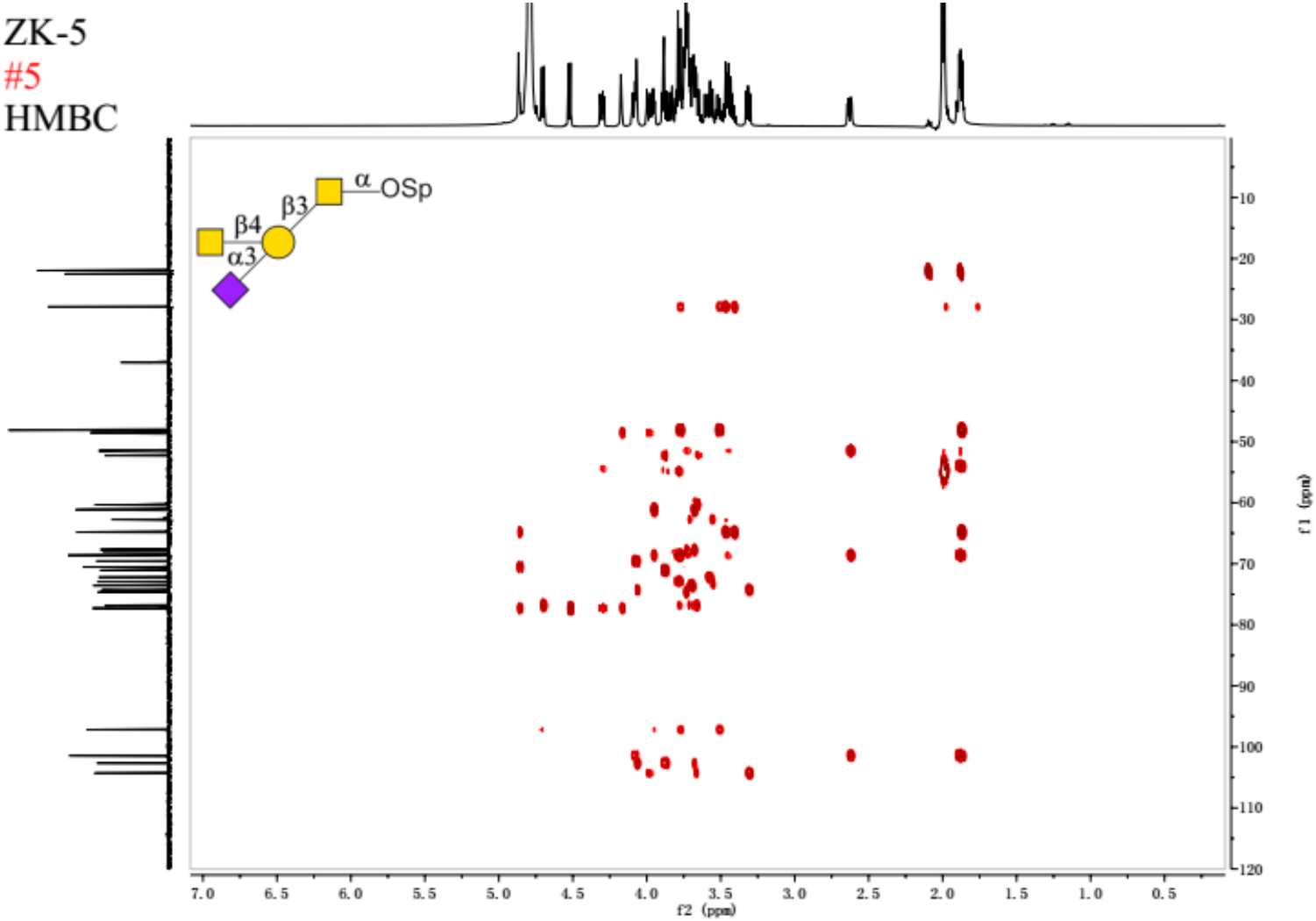
^{1}H NMR of 5


ZK-5
#5
HO
OH
HO
NHAc
OH
OH
HO2C
HO
AcHN
HO
OH
HO
OH
AcHN
N3
α
OSp
β3
β4
α3
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)

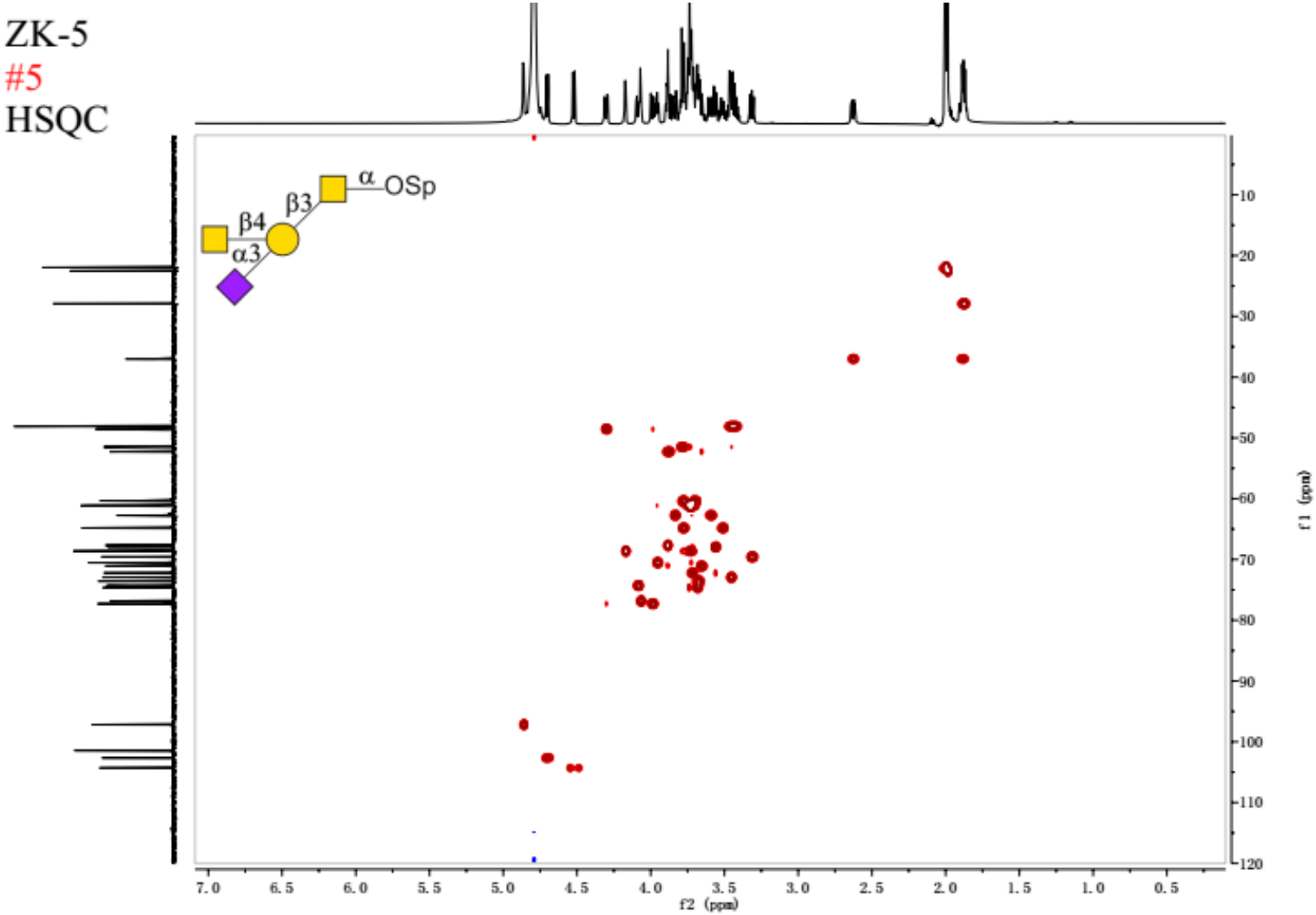
^{13}C NMR of 5



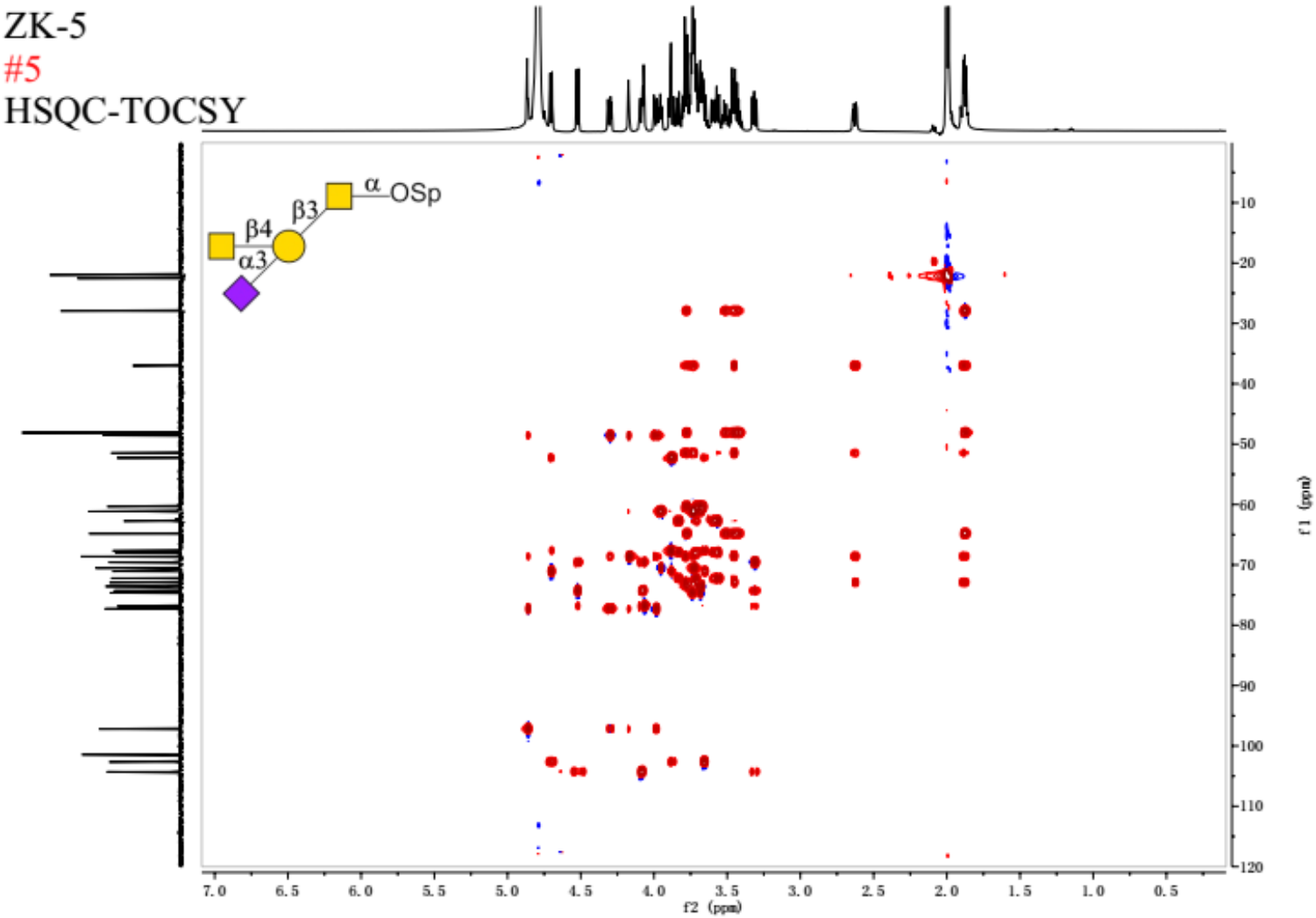
COSY spectra of 5



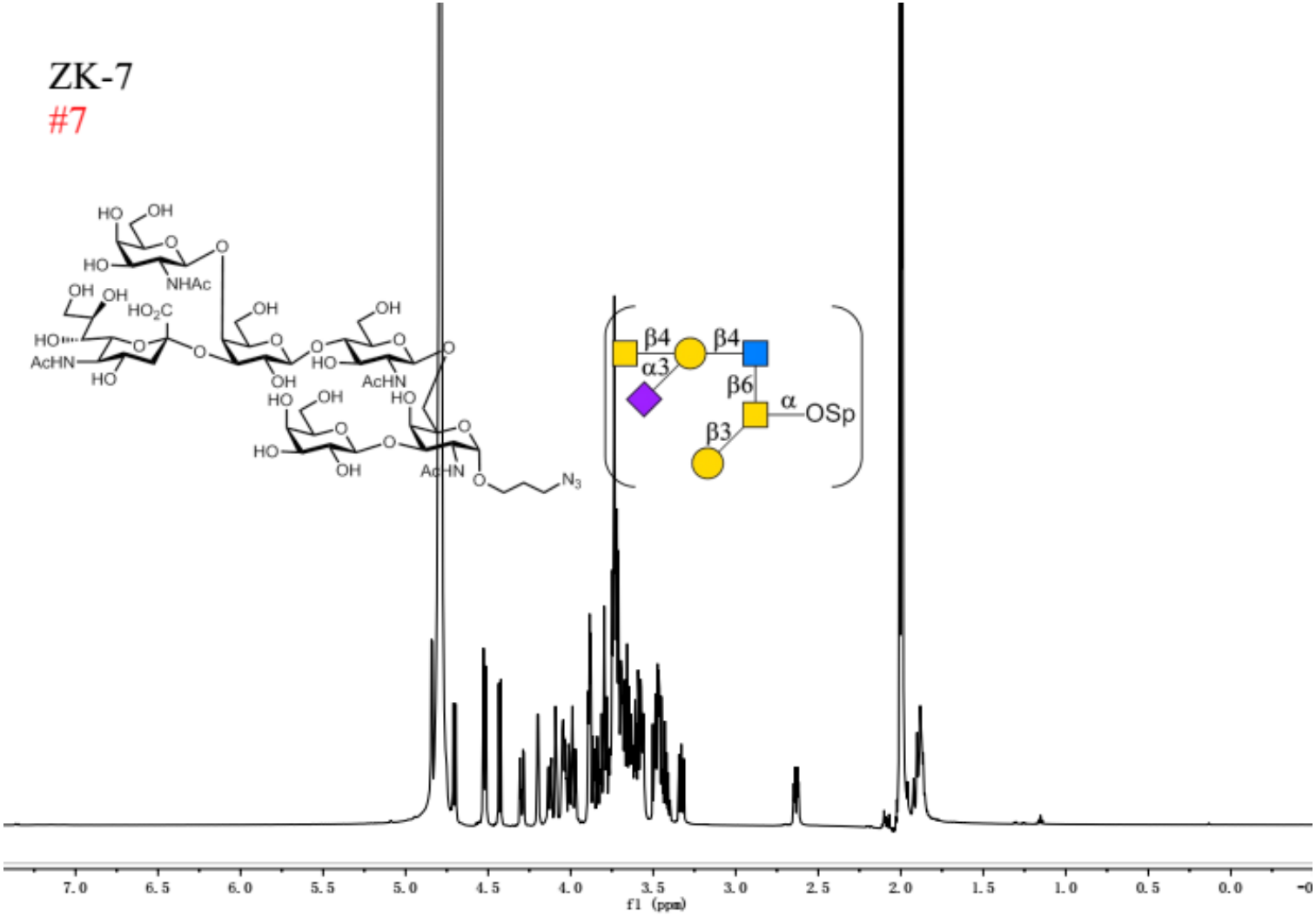
HMBC spectra of 5



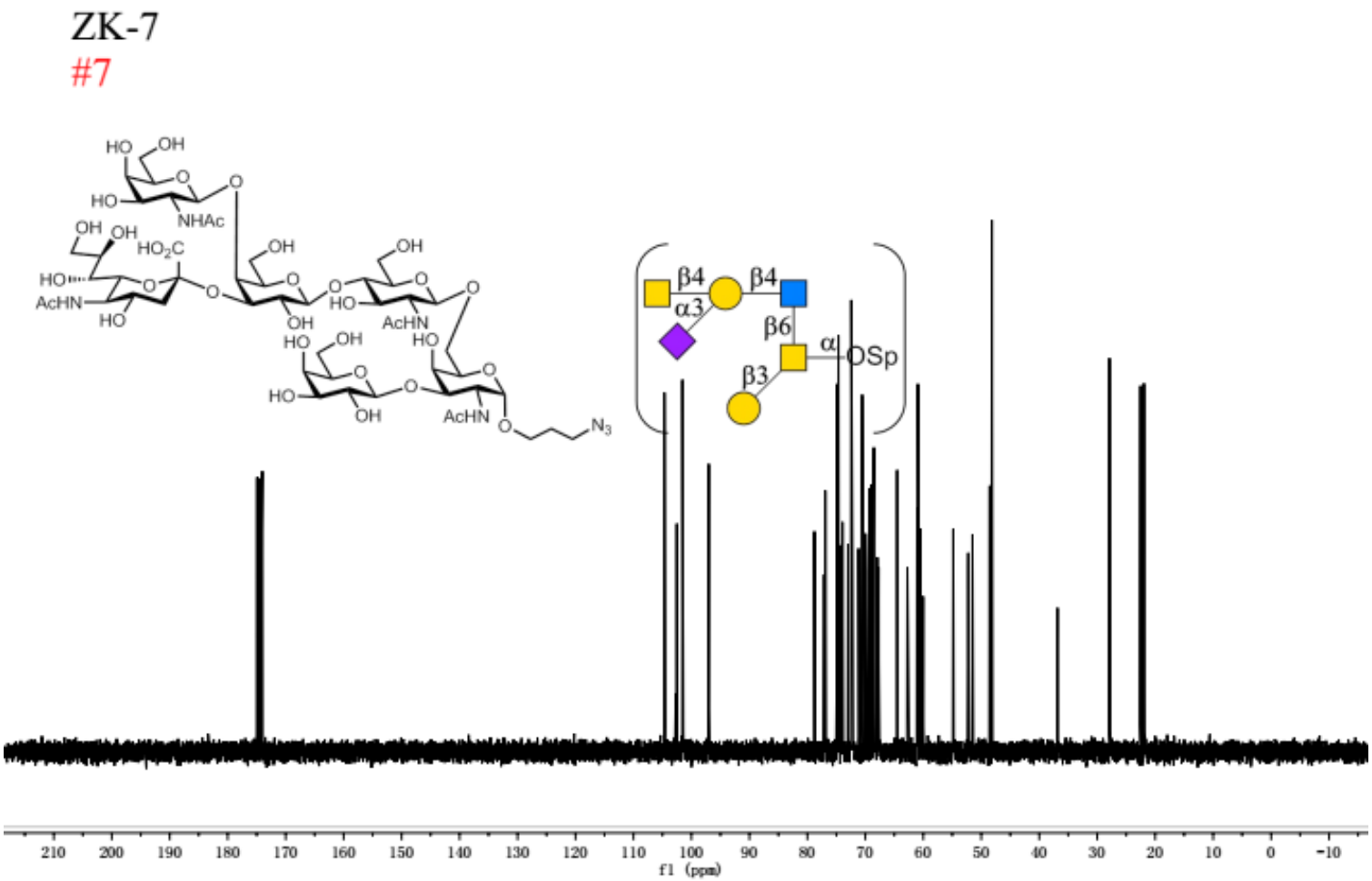
HSQC spectra of 5



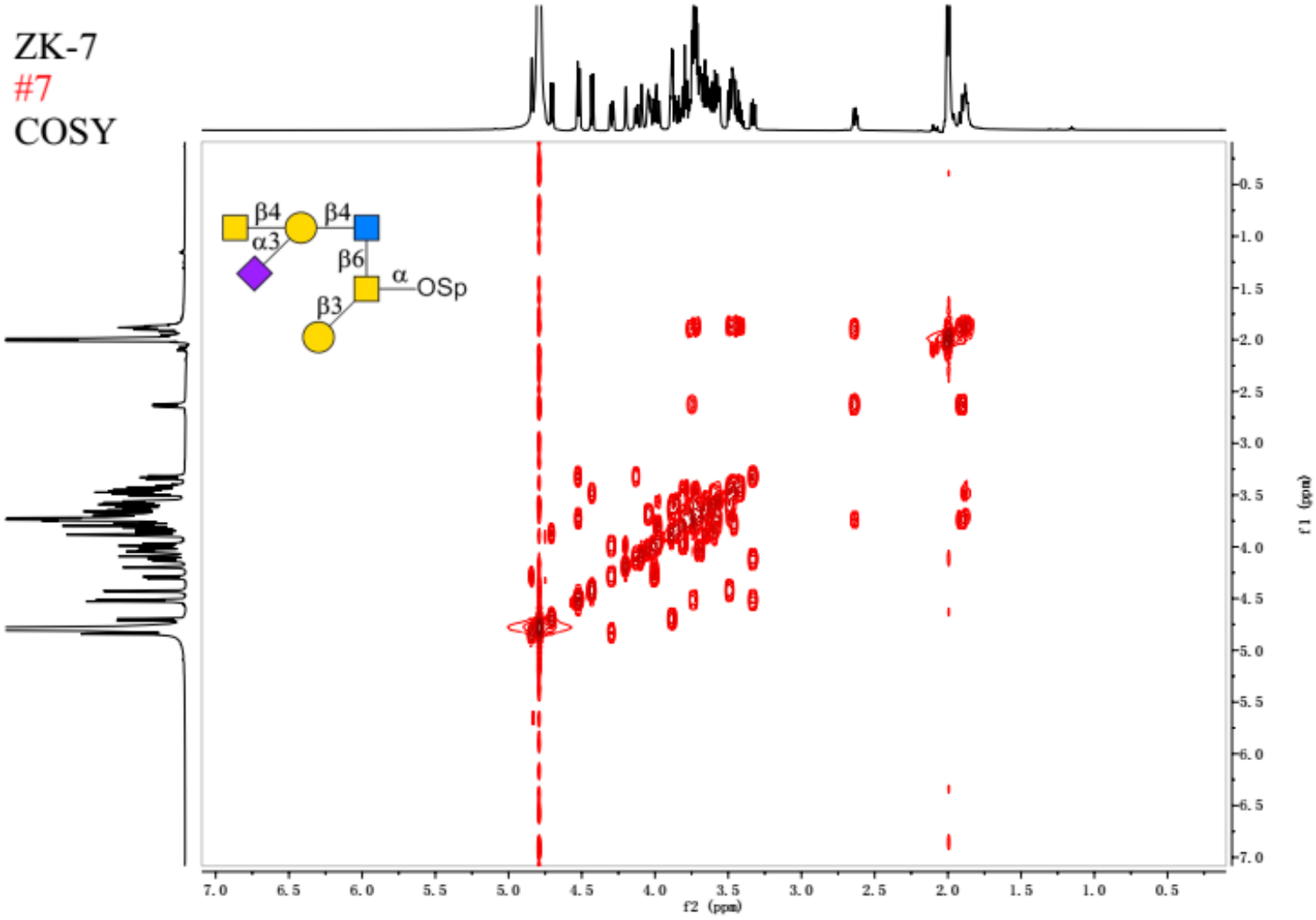
HSQC-TOCSY spectra of 5


ZK-7
#7
β4
β4
α3
β6
α
OSp
β3
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
0.0
f1 (ppm)

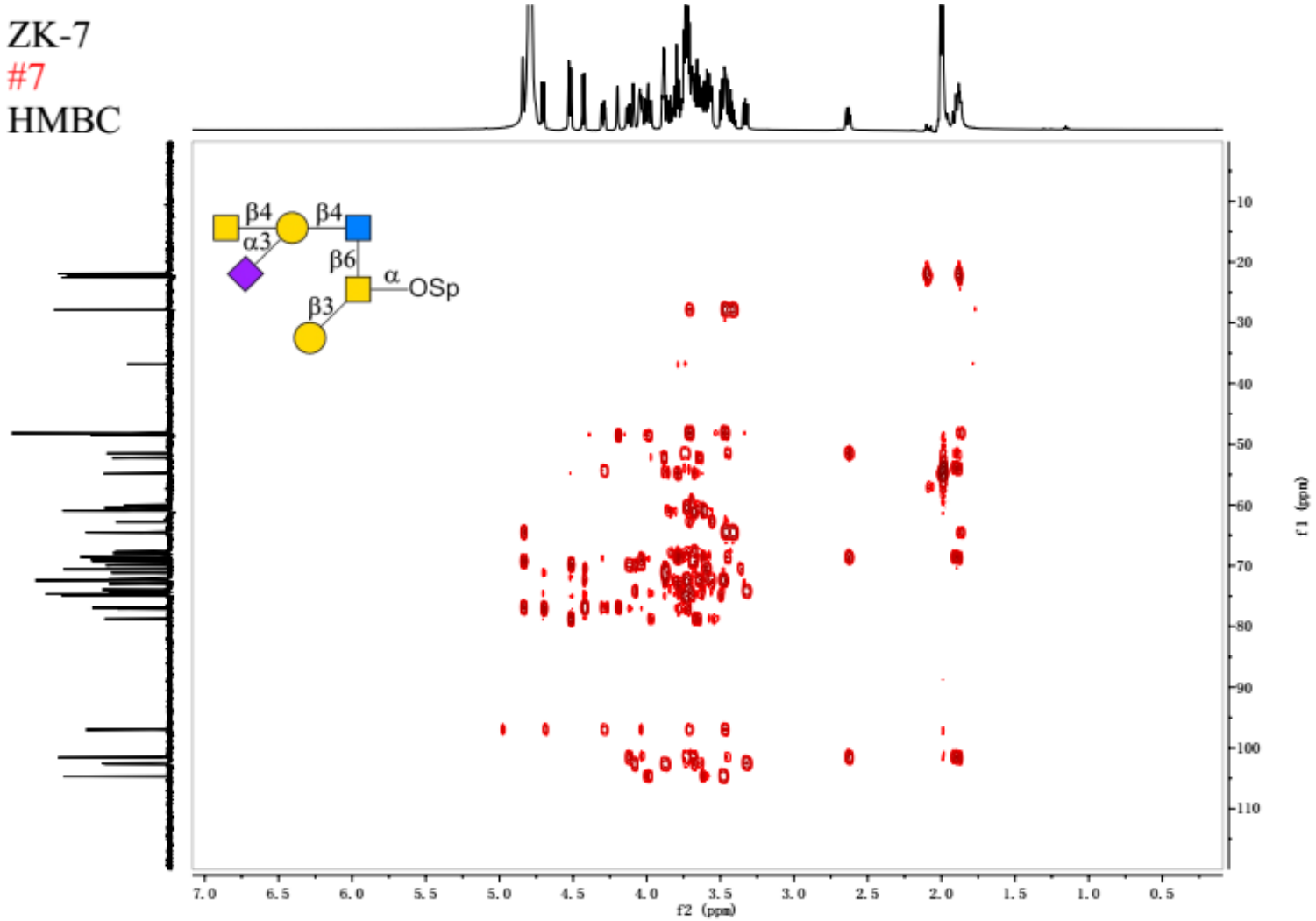
^{1}H NMR of 7


ZK-7
#7
HO
OH
NHAc
AcHN
HO2C
N3
β4
α3
β6
β3
α
OSp
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)

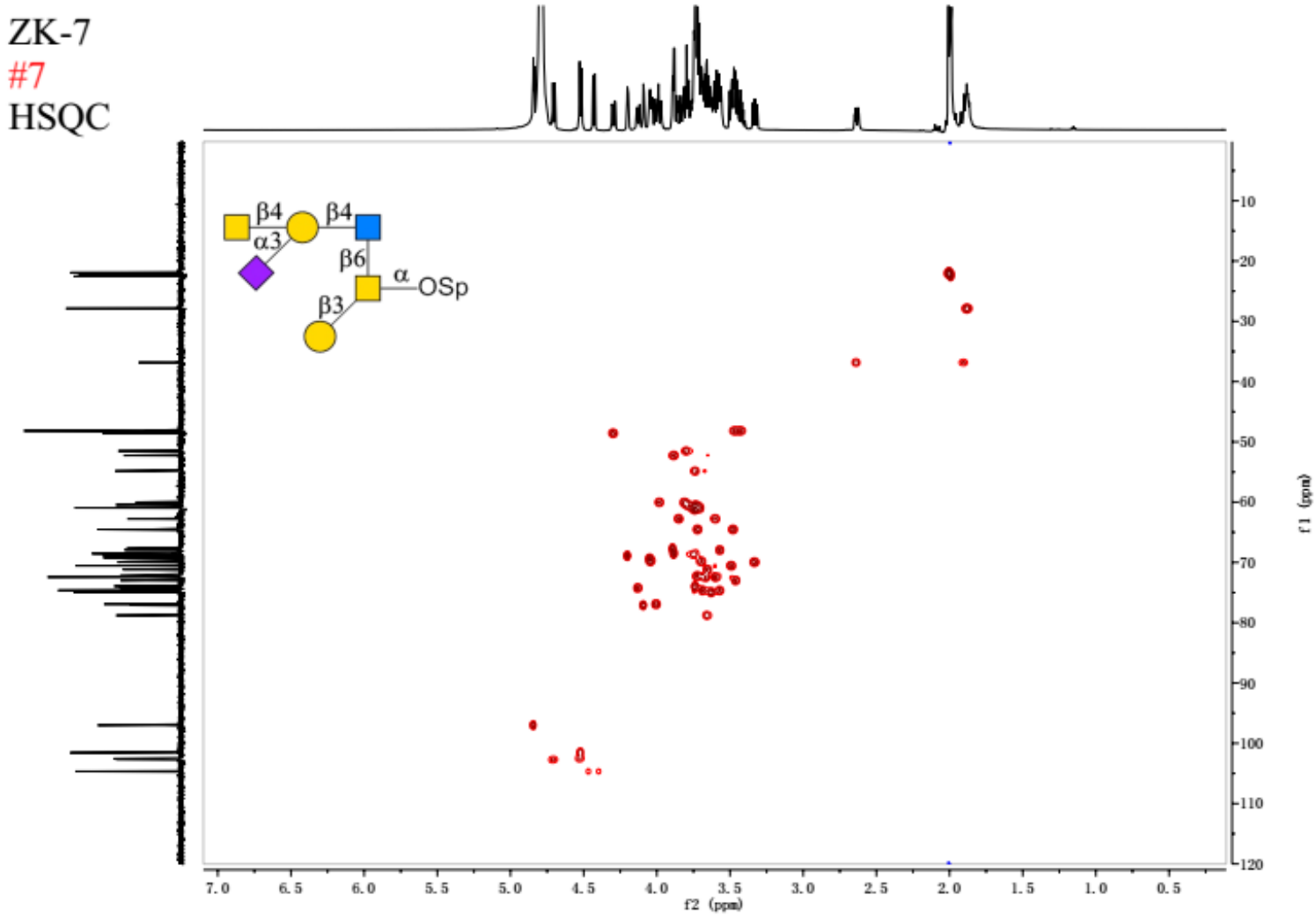
^{13}C NMR of 7



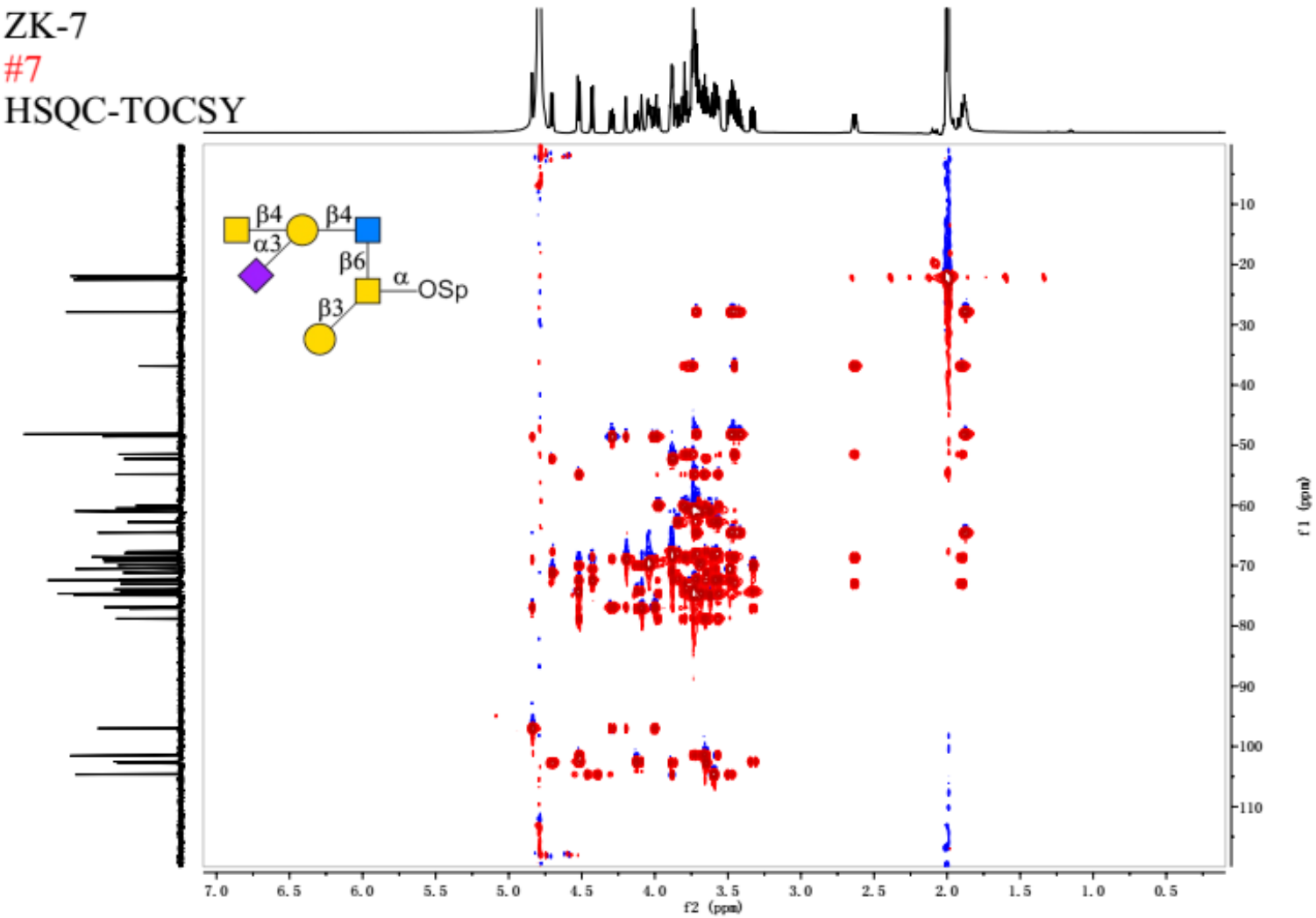
COSY spectra of 7



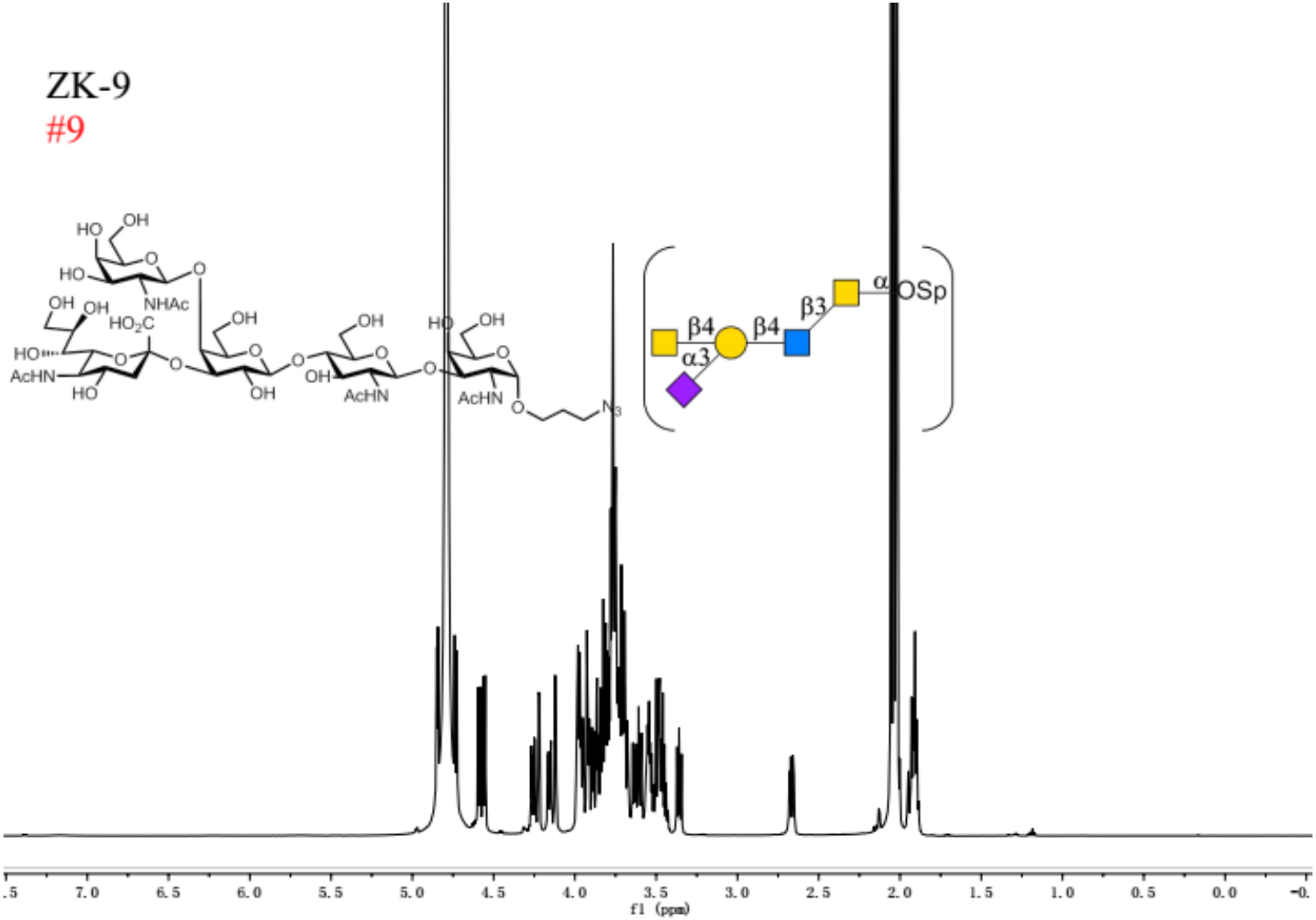
HMBC spectra of 7



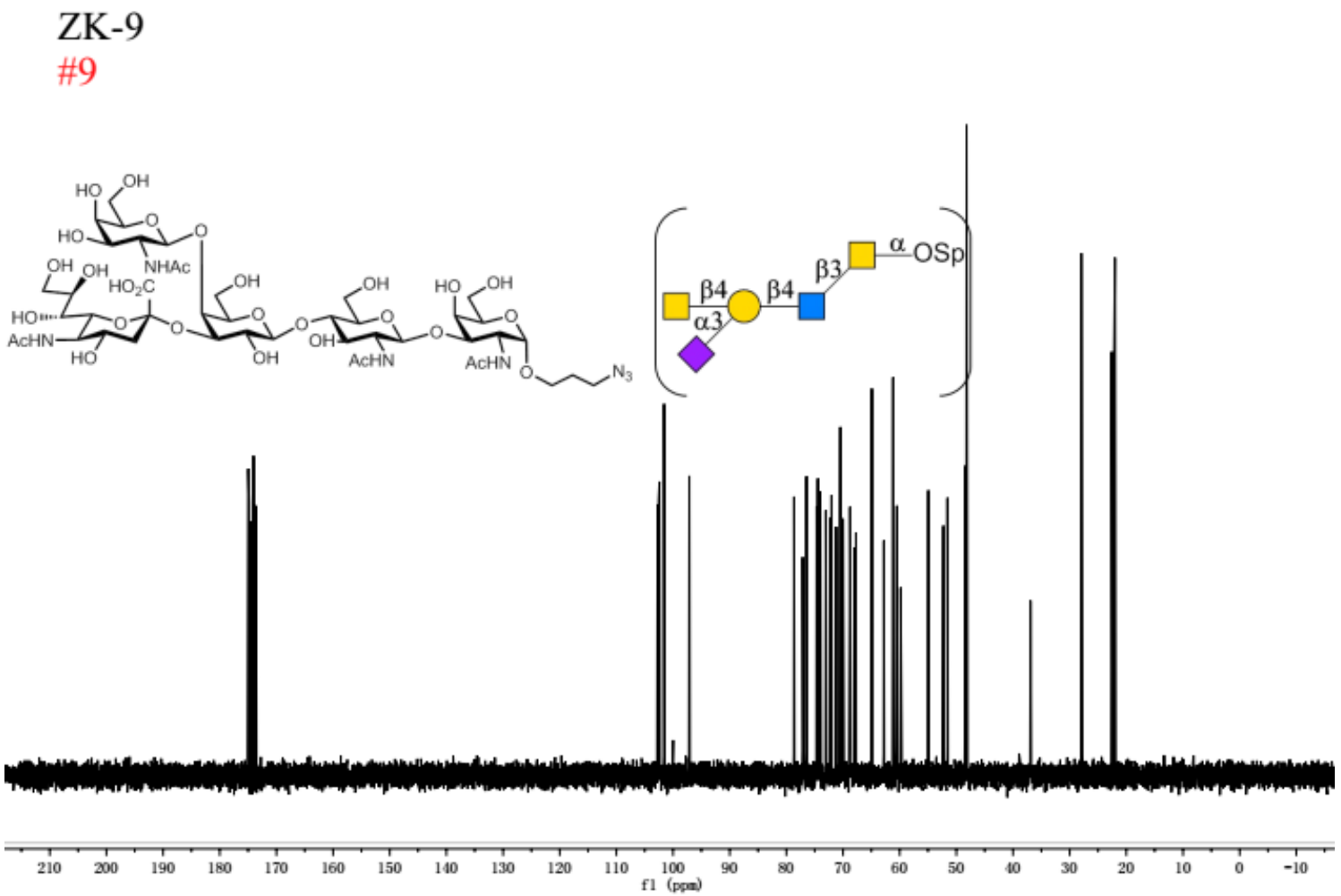
HSQC spectra of 7



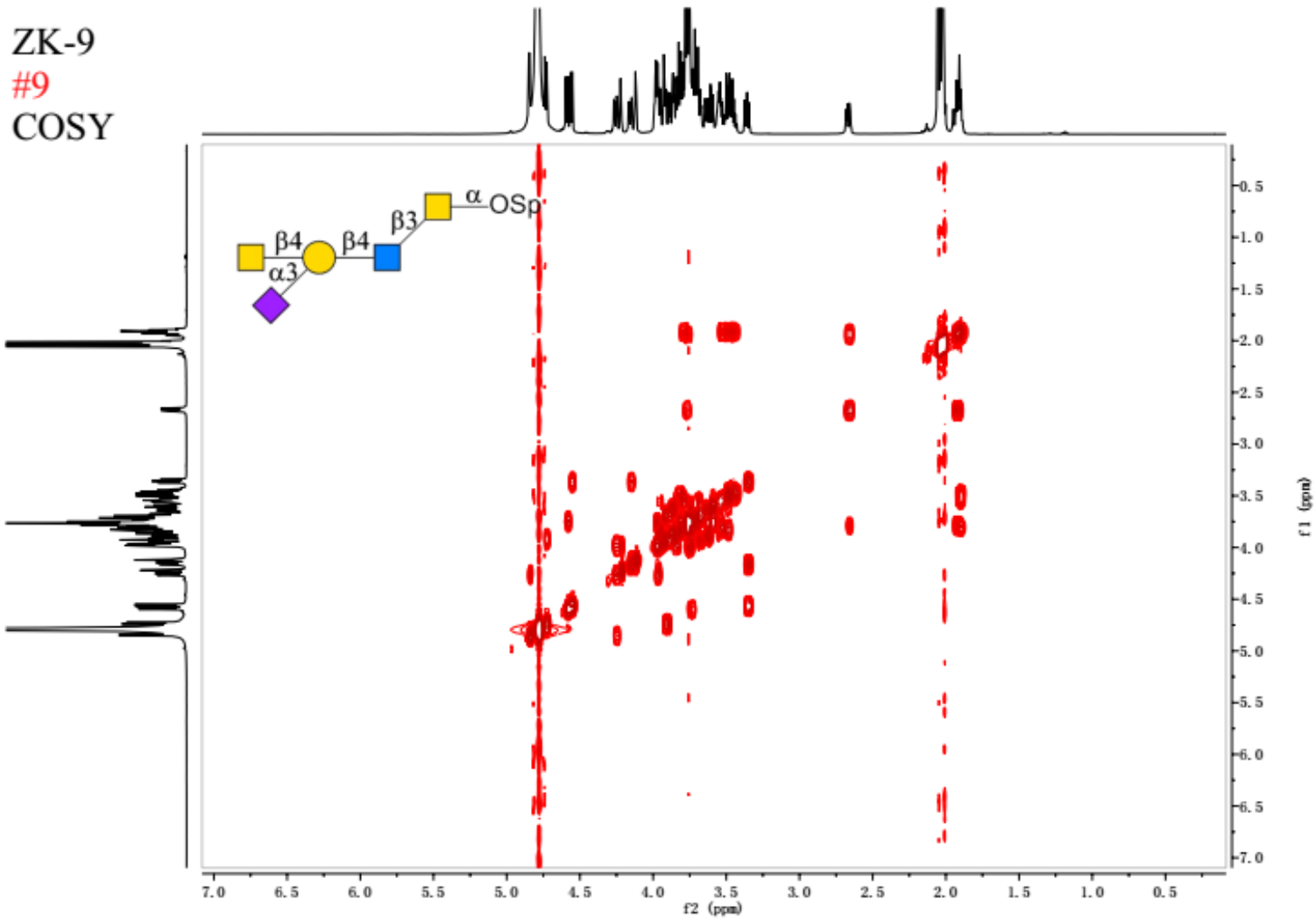
HSQC-TOCSY spectra of 7


ZK-9
#9
HO
OH
HO
O
NHAc
OH
OH
HO2C
HO
AcHN
HO
OH
OH
OH
AcHN
HO
OH
AcHN
O
N3
β4
β4
β3
α
OSp
α3
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
0.0
f1 (ppm)

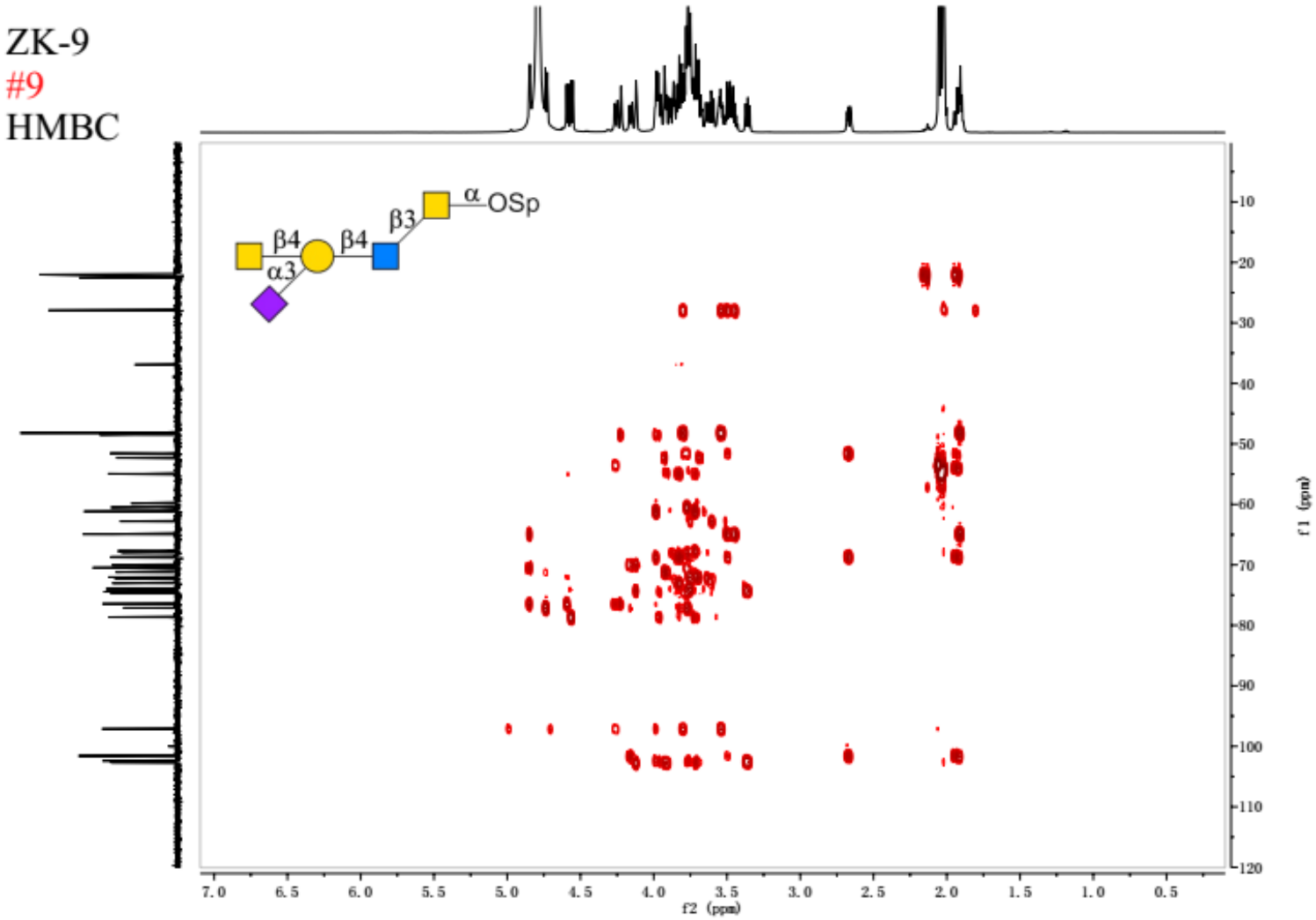
^{1}H NMR of 9


ZK-9
#9
HO
OH
HO
NHAc
OH
OH
HO2C
HO
AcHN
HO
OH
OH
OH
AcHN
HO
OH
AcHN
O
N3
α
OSp
β3
β4
β4
α3
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)

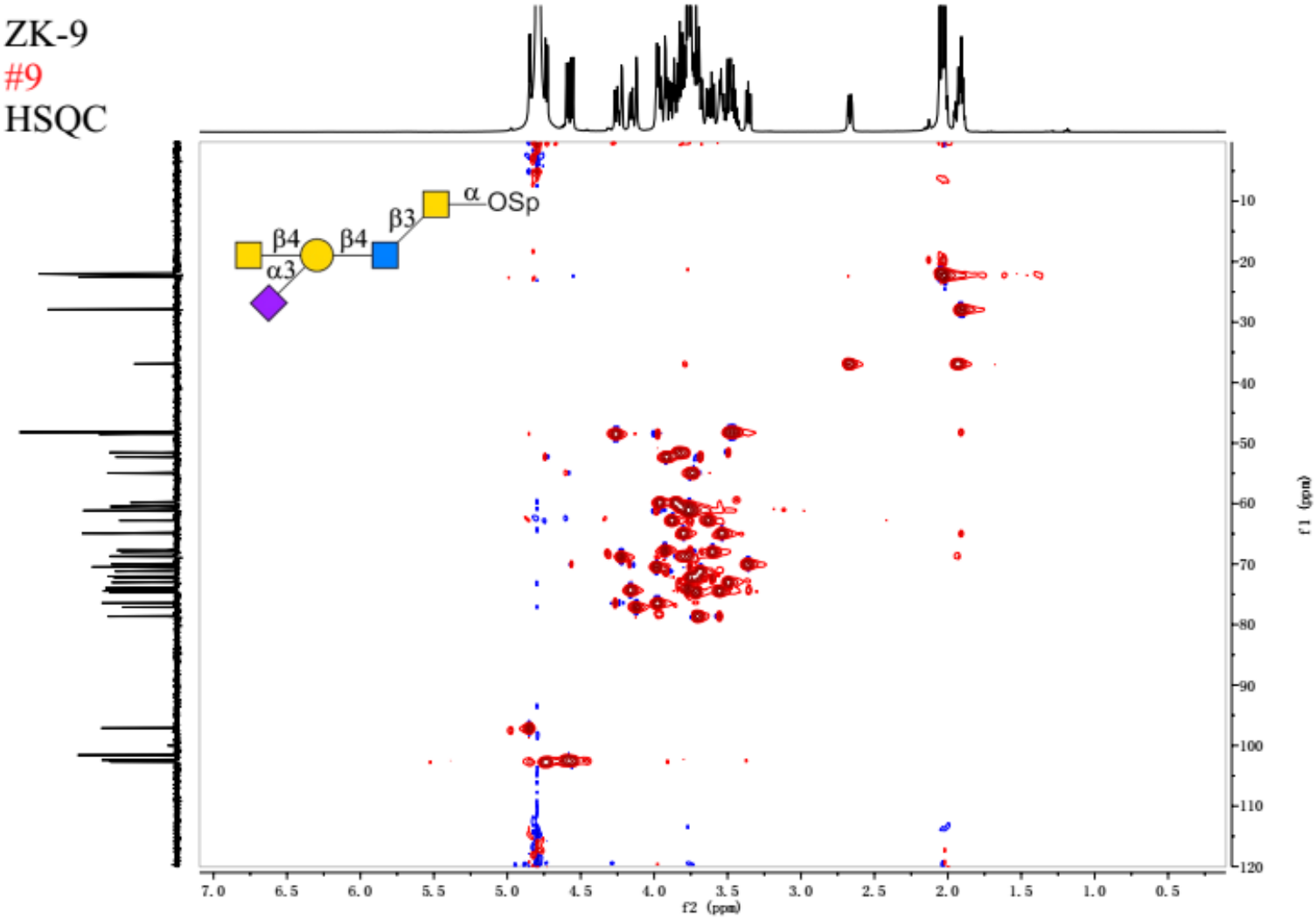
^{13}C NMR of 9



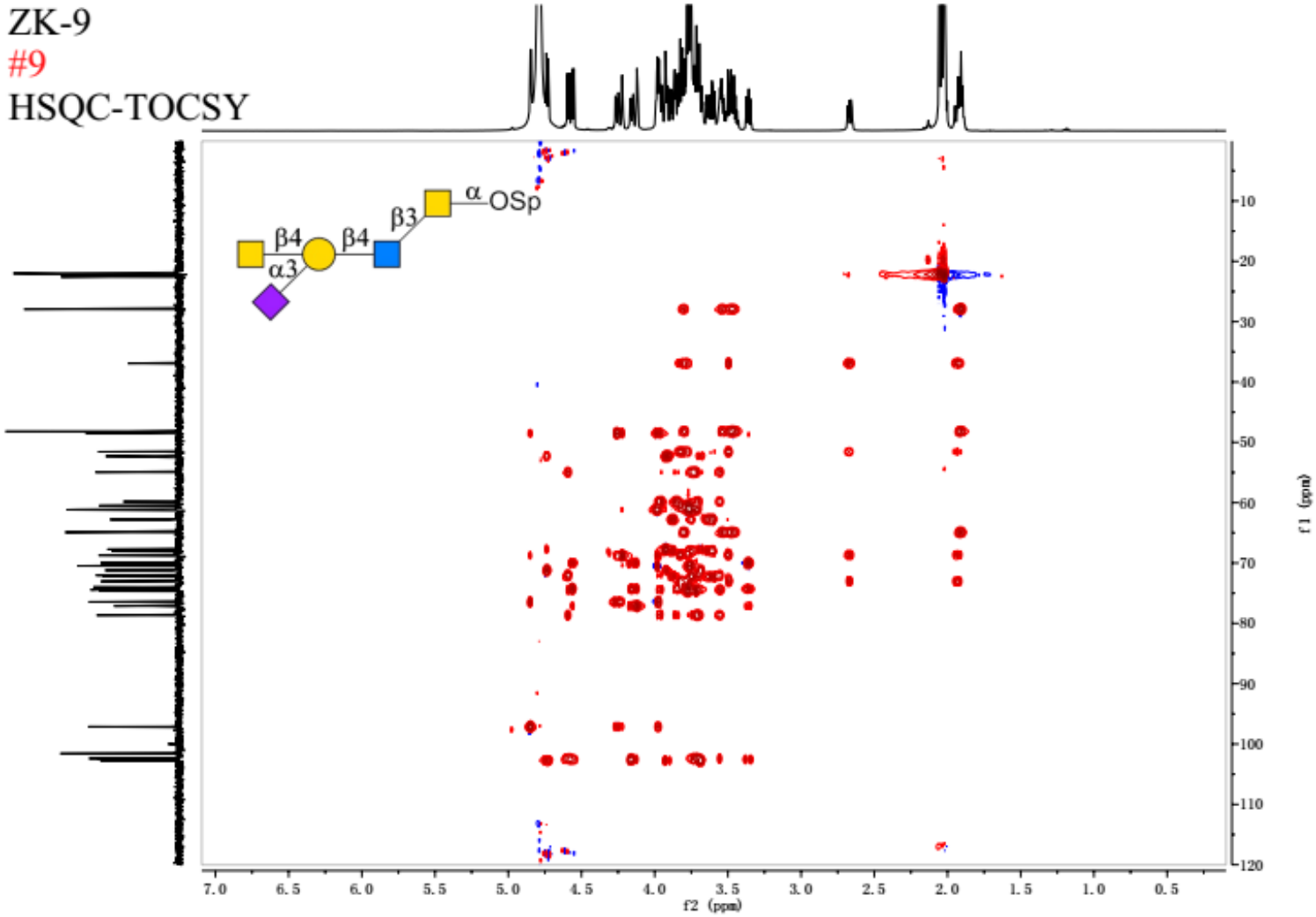
COSY spectra of 9



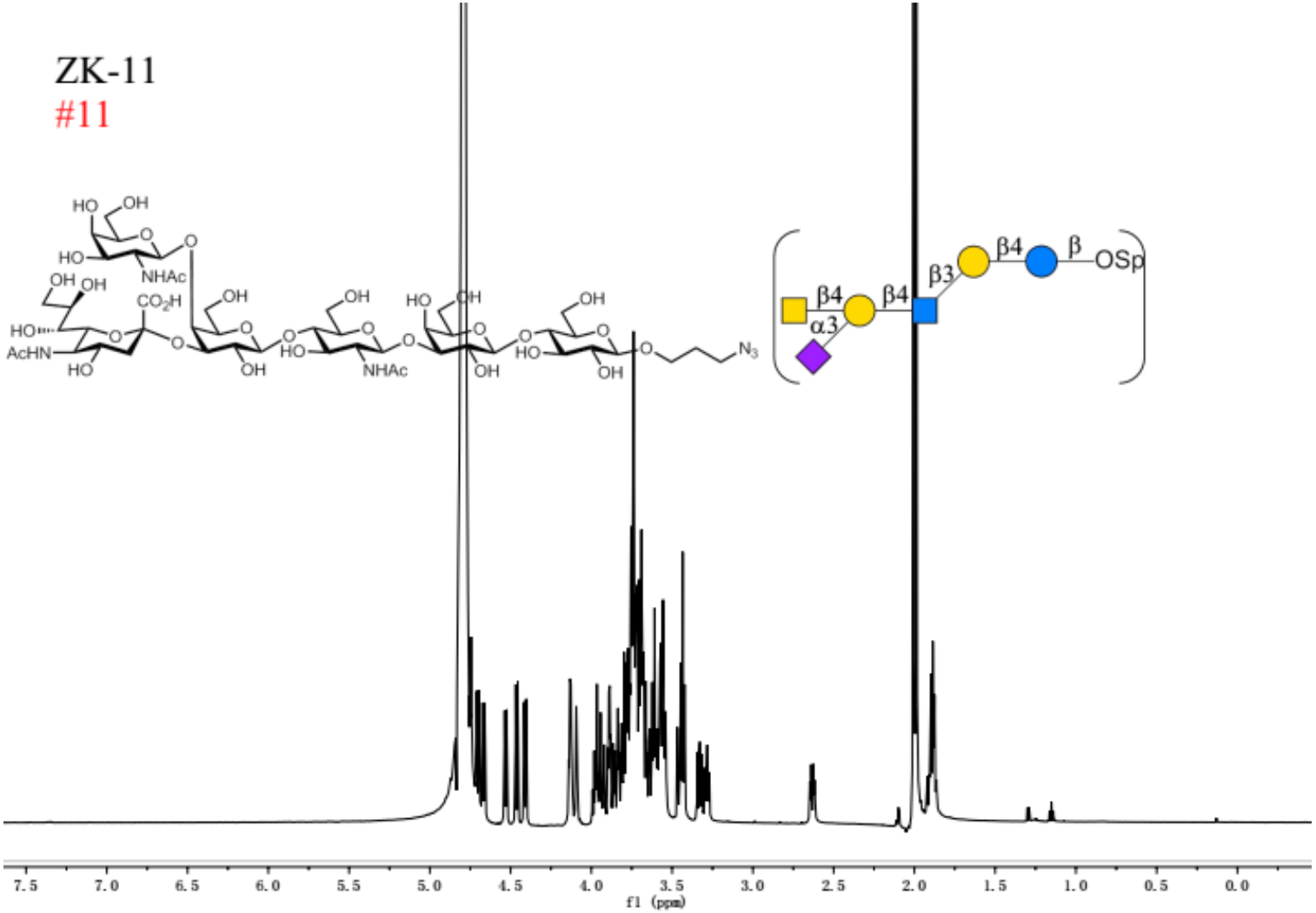
HMBC spectra of 9



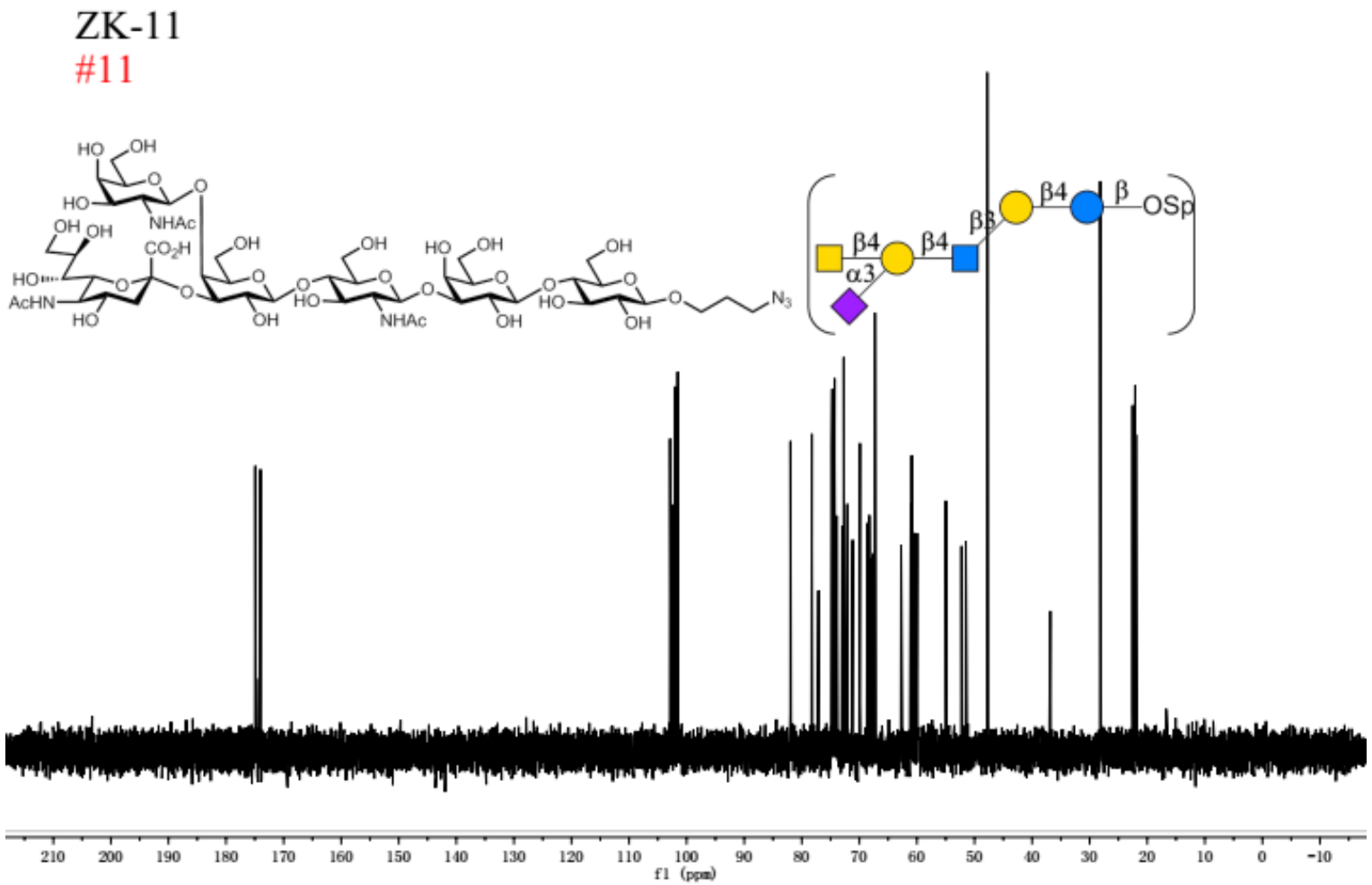
HSQC spectra of 9



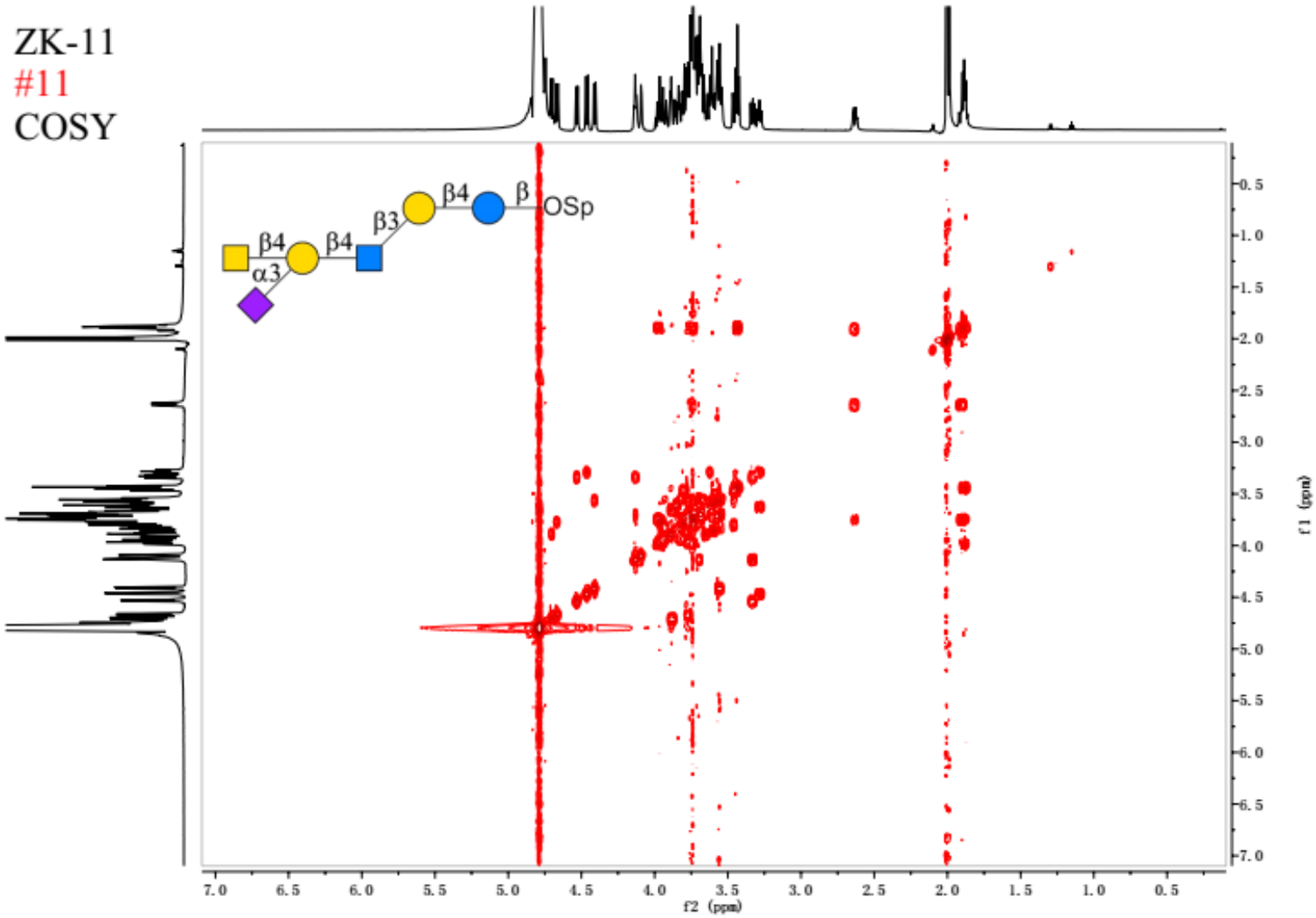
HSQC-TOCSY spectra of 9


ZK-11
#11
HO
OH
HO
NHAc
OH OH
CO2H
HO
AcHN
HO
OH
OH
HO
NHAc
OH
HO
OH
HO
OH
N3
β4
β4
α3
β3
β4
β
OSp
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
0.0
f1 (ppm)

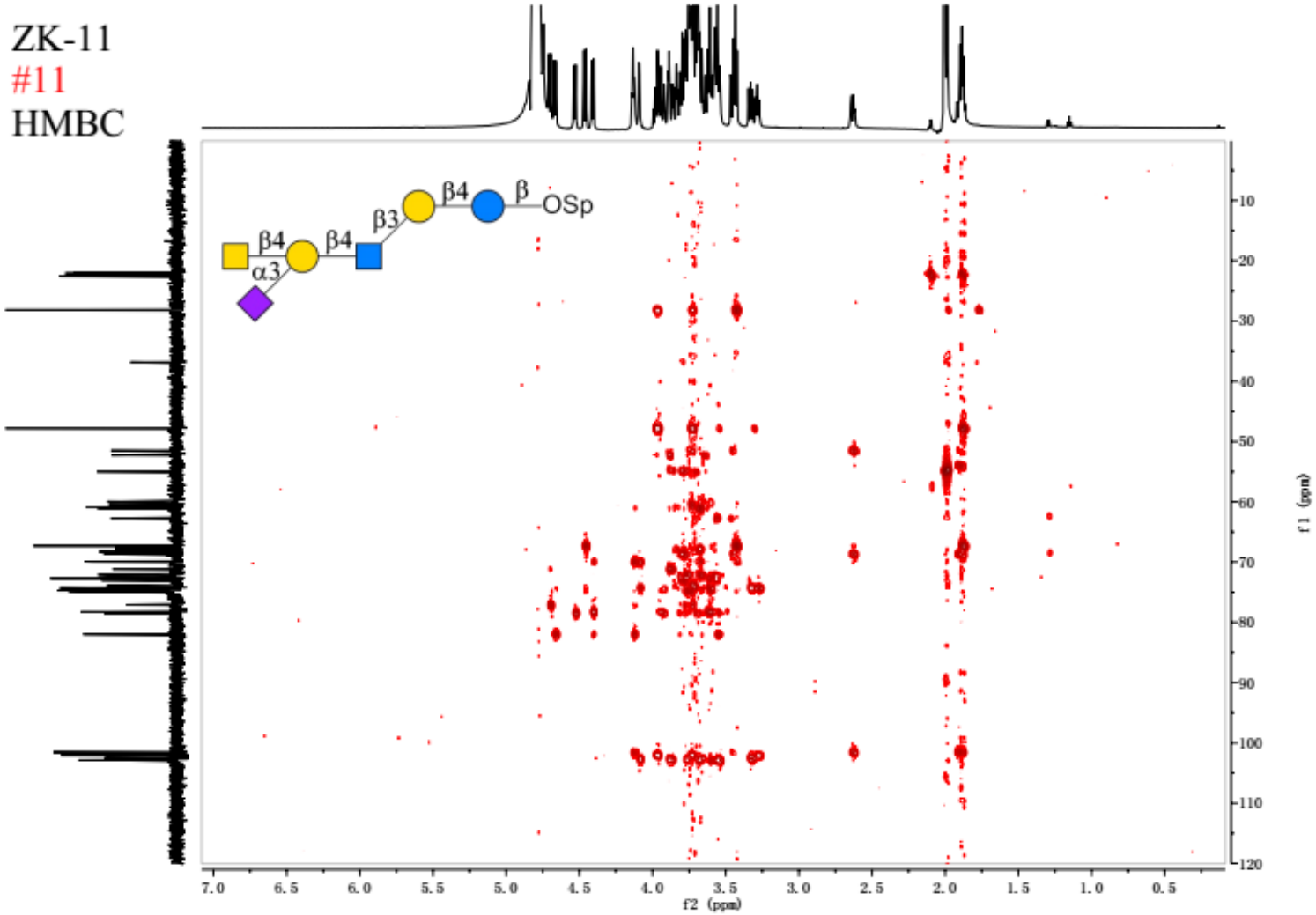
^{1}H NMR of 11


ZK-11
#11
β4
β4
β3
β4
β
OSp
α3
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)

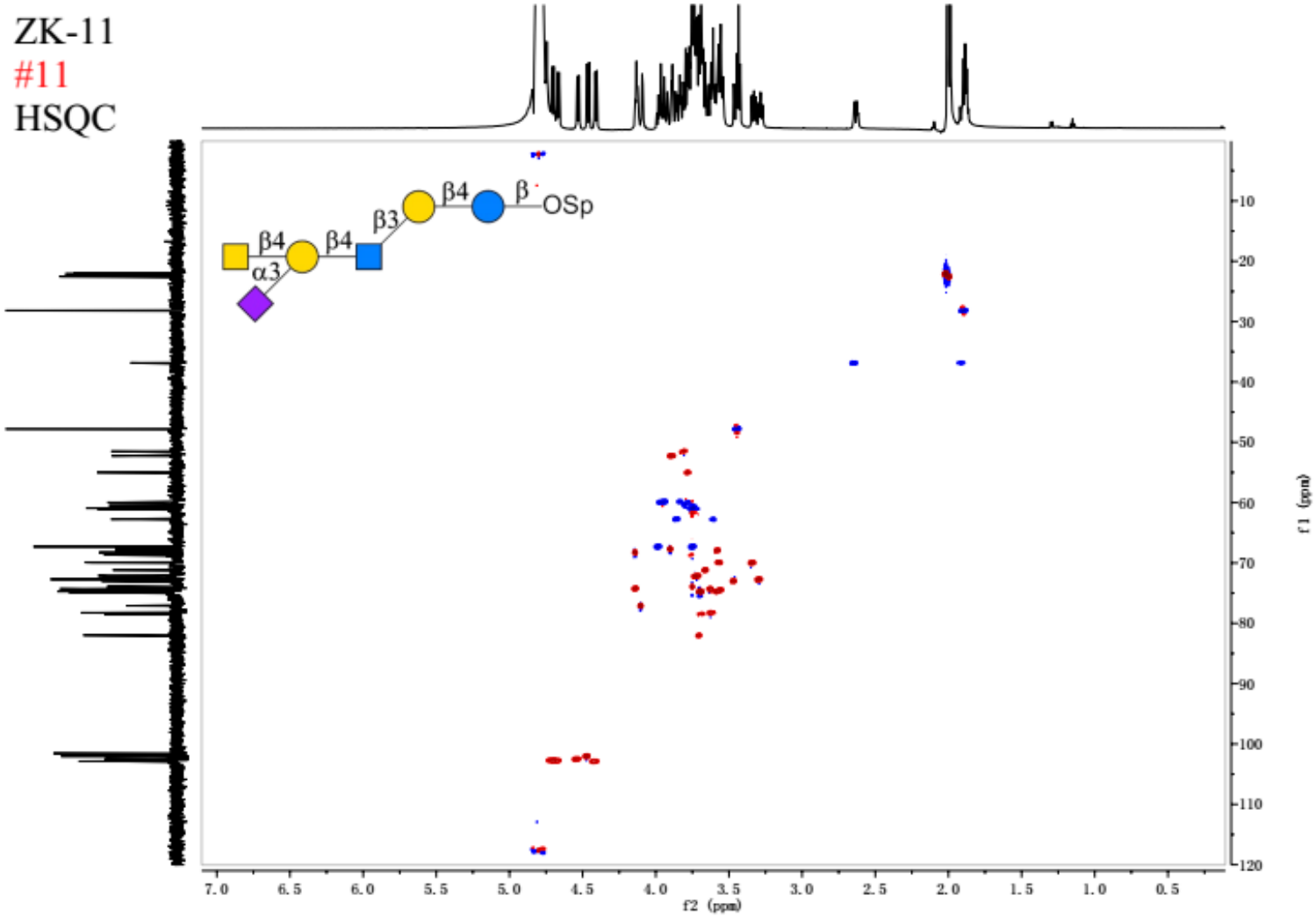
^{13}C NMR of 11



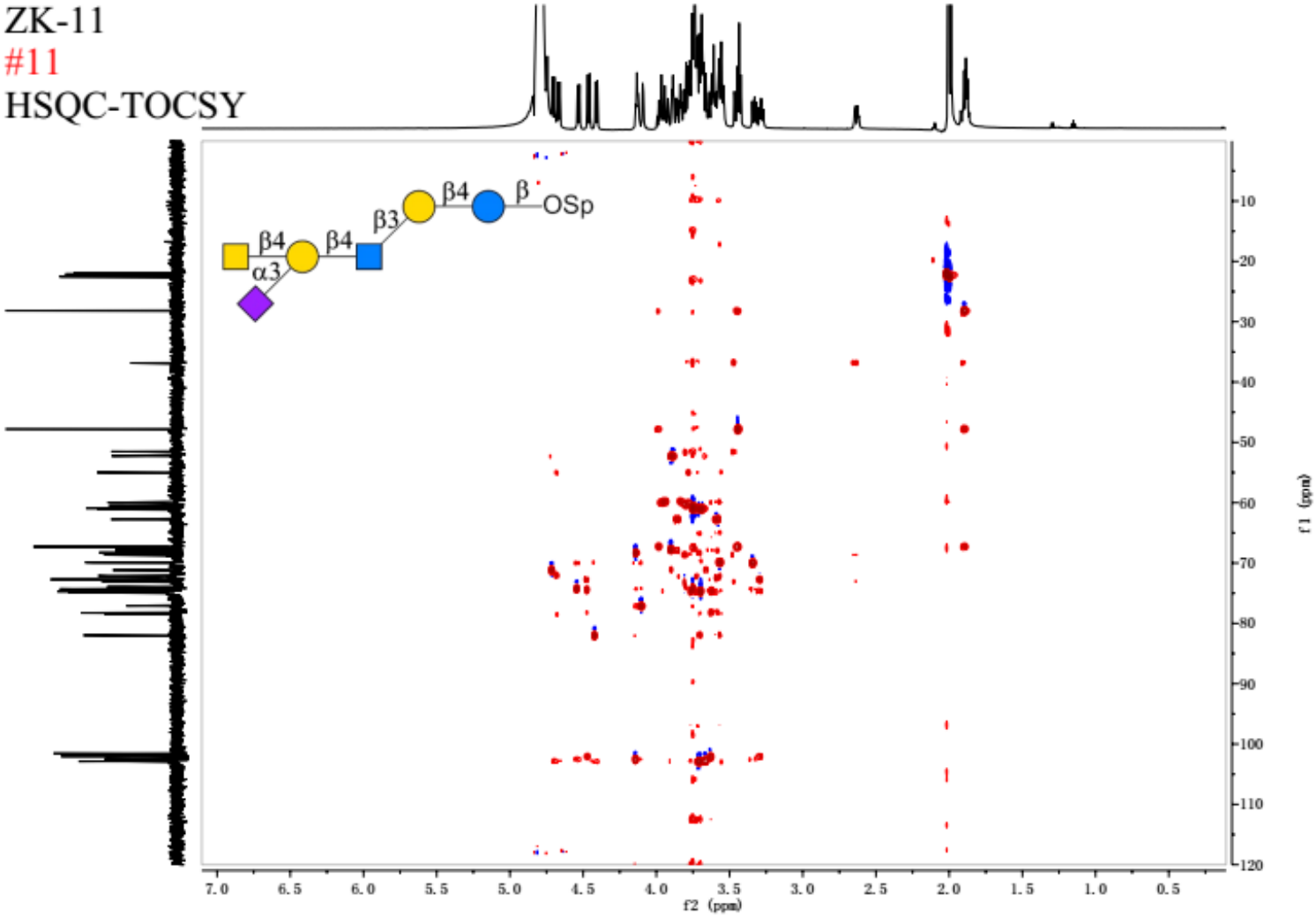
COSY spectra of 11



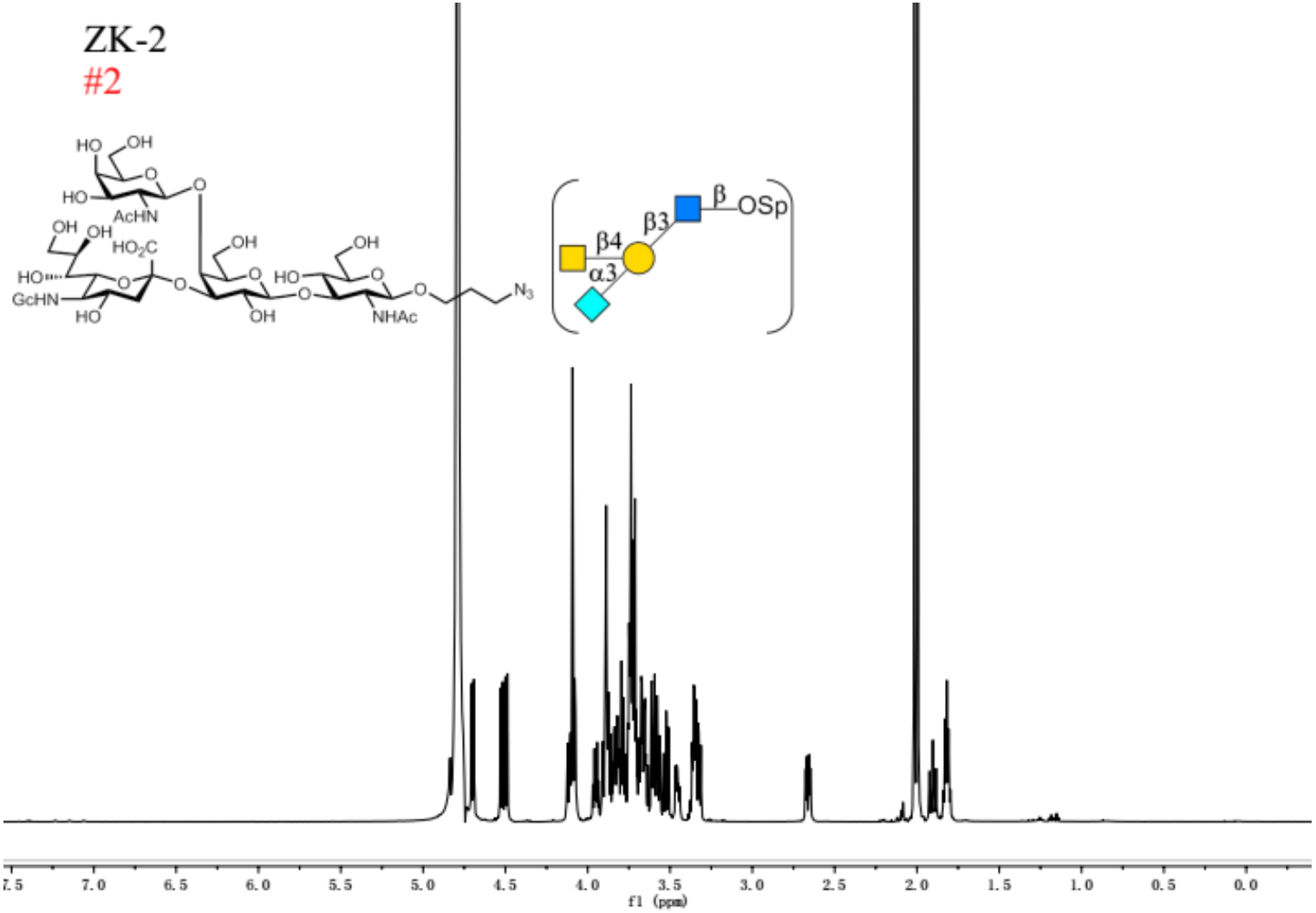
HMBC spectra of 11



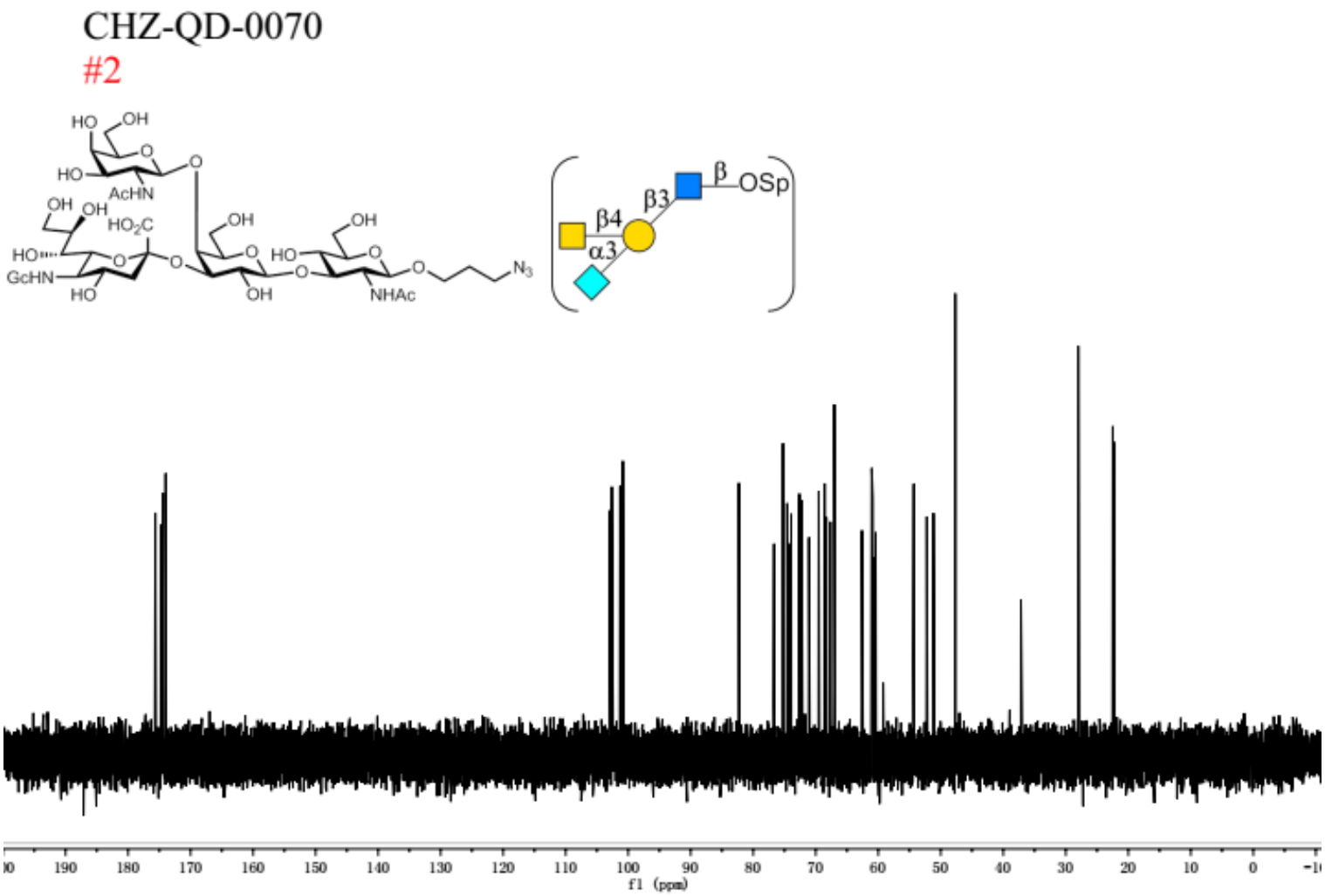
HSQC spectra of 11



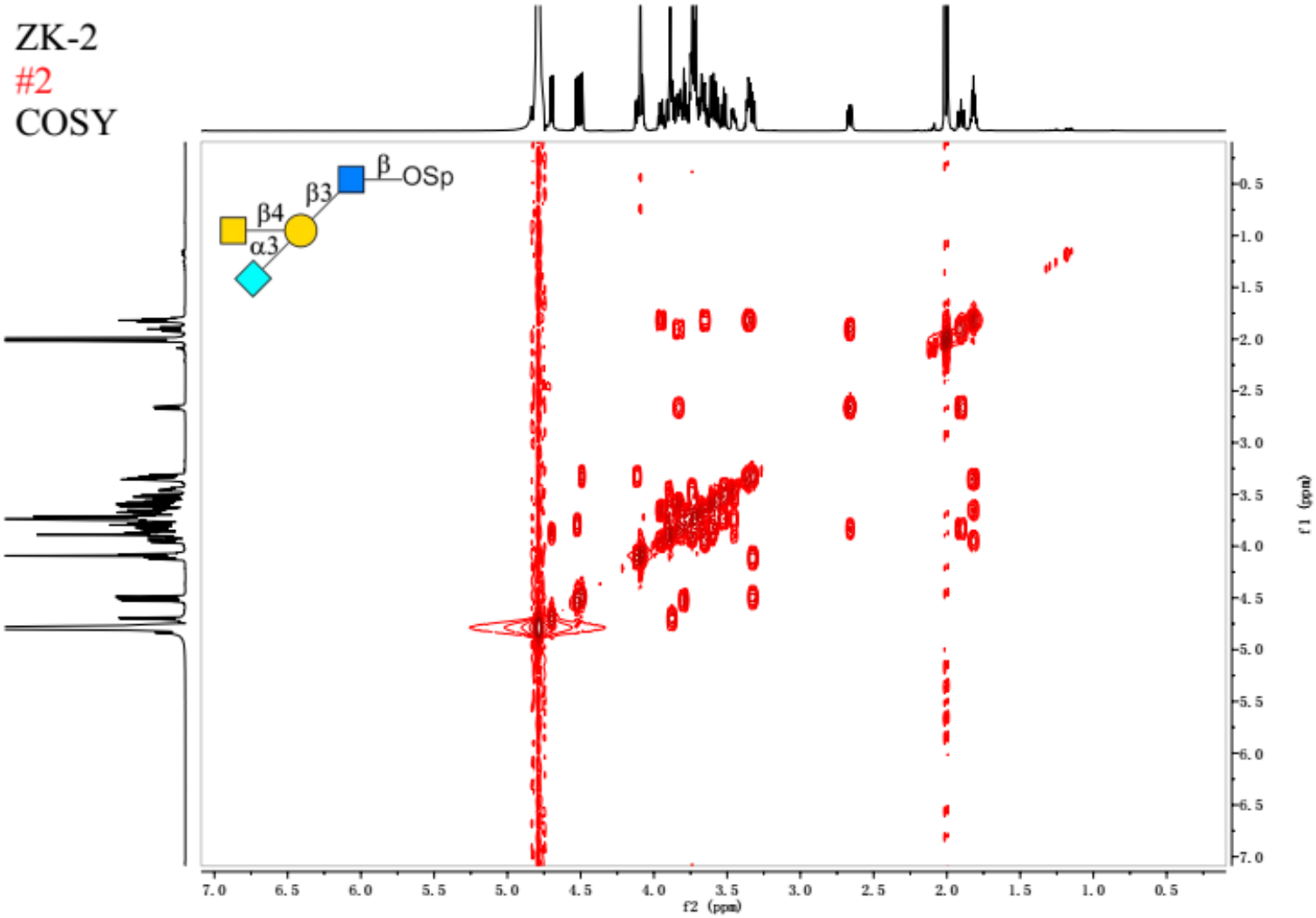
HSQC-TOCSY spectra of 11


ZK-2
#2
HO
OH
HO
AcHN
OH
OH
HO2C
HO
GcHN
HO
OH
OH
HO
OH
NHAc
N3
β
OSp
β3
β4
α3
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
0.0
f1 (ppm)

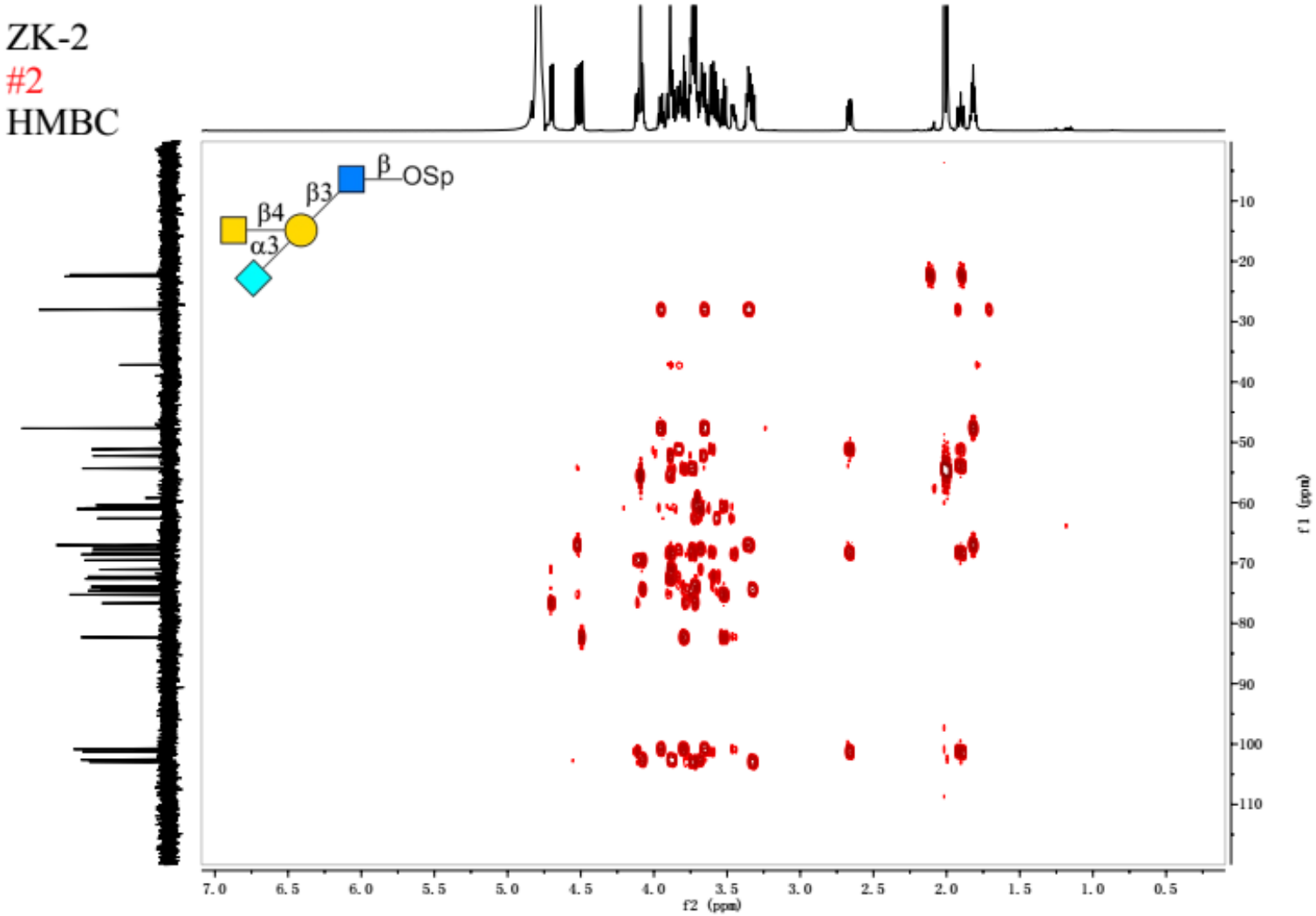
^{1}H NMR of 2


CHZ-QD-0070
#2
HO
OH
HO
AcHN
OH
OH
HO2C
HO
GcHN
HO
OH
HO
OH
HO
NHAc
N3
β
OSp
β3
β4
α3
f1 (ppm)

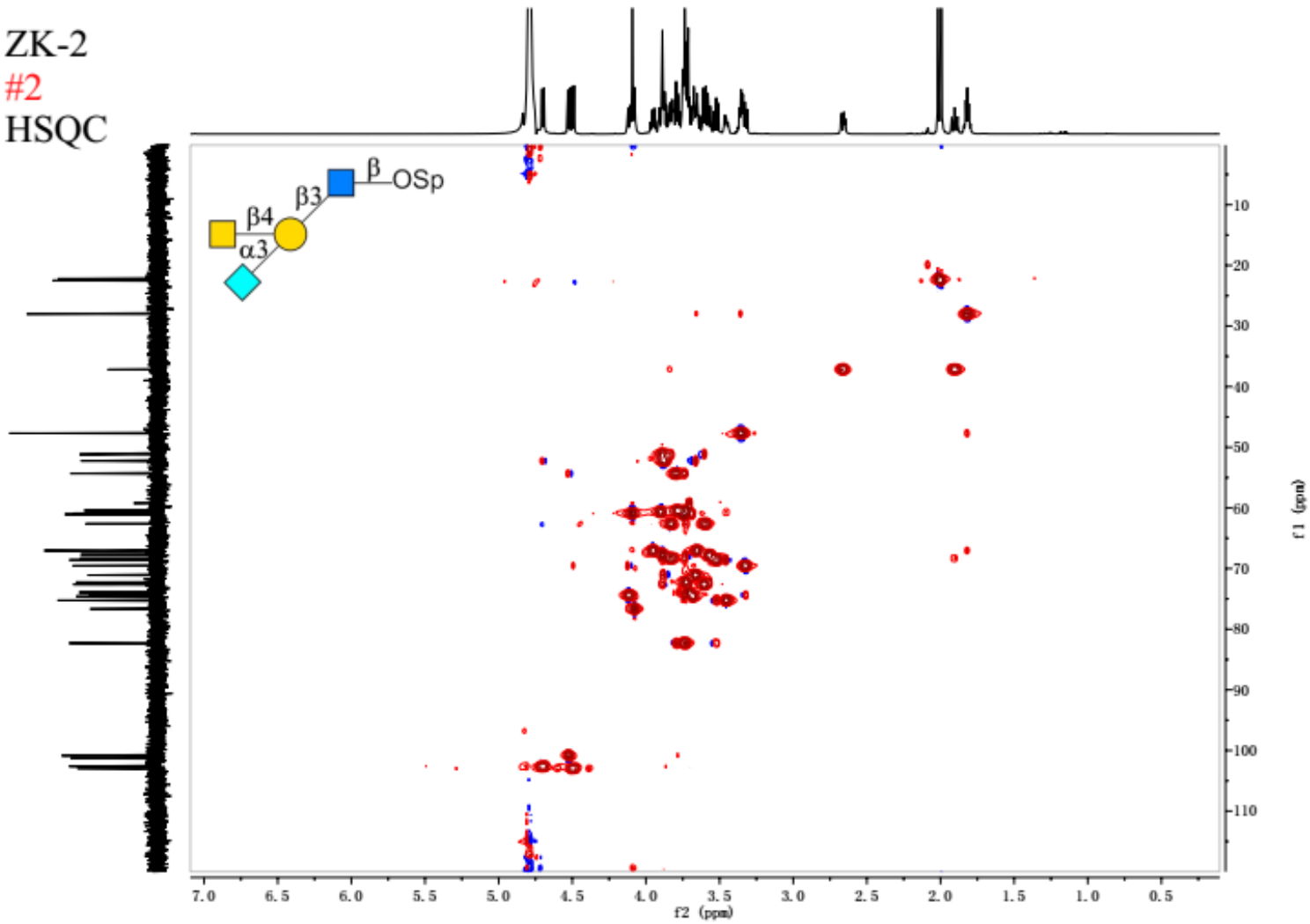
^{13}C NMR of 2



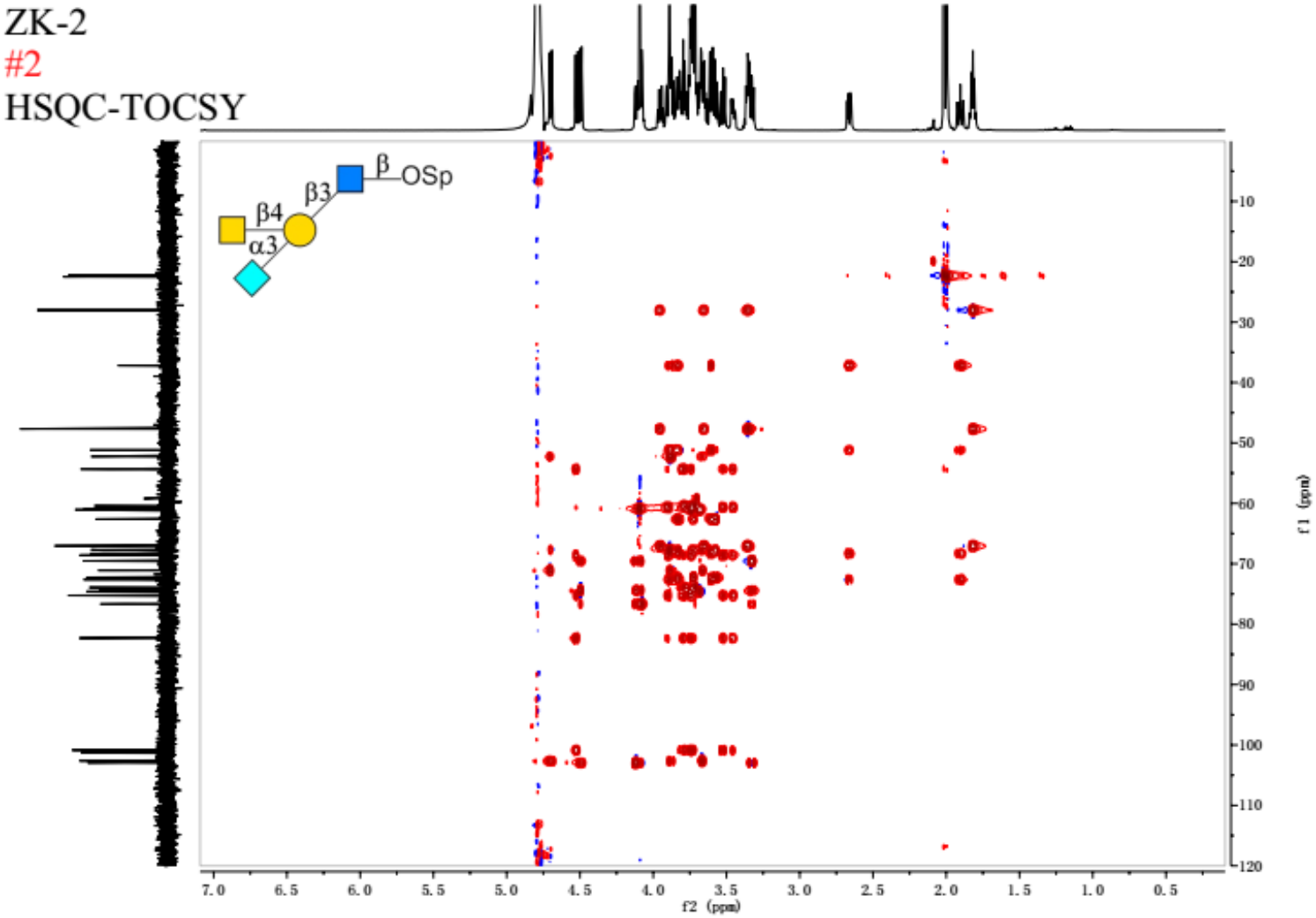
COSY spectra of 2



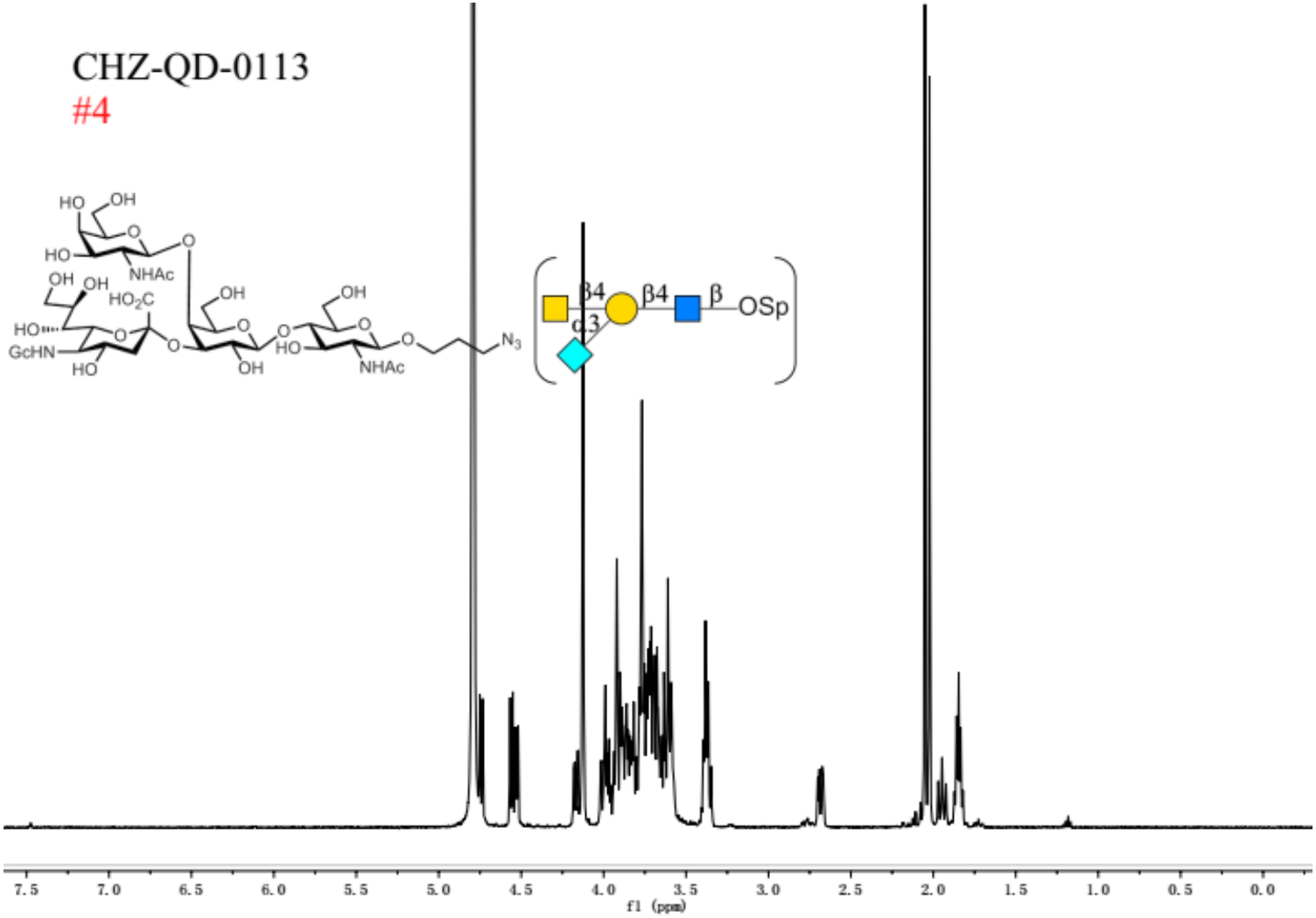
HMBC spectra of 2



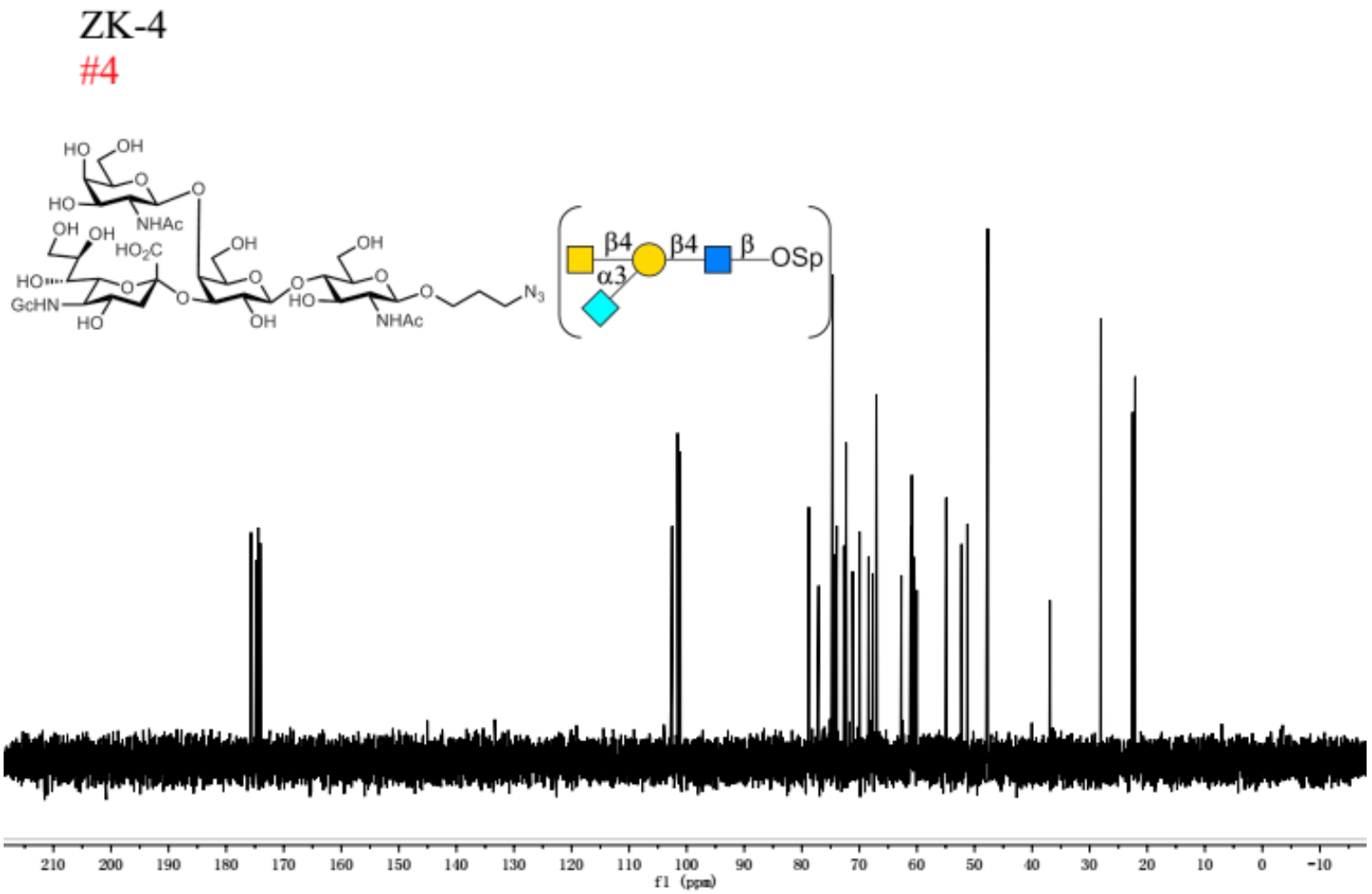
HSQC spectra of 2



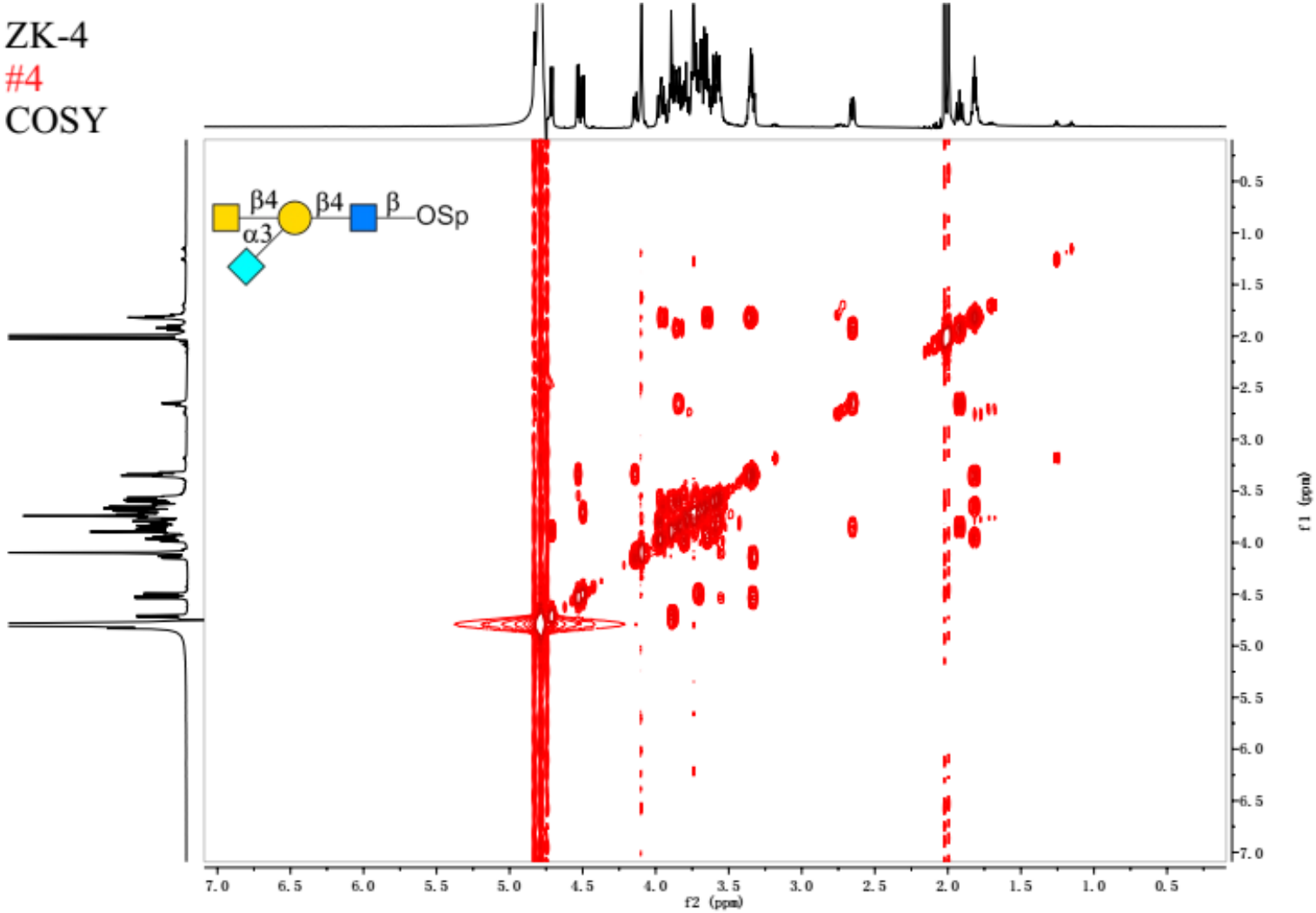
HSQC-TOCSY spectra of 2


CHZ-QD-0113
#4
HO
OH
HO
NHAc
OH
OH
HO2C
HO
GcHN
HO
OH
OH
HO
NHAc
N3
β4
β4
β
OSp
α3
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
0.0
f1 (ppm)

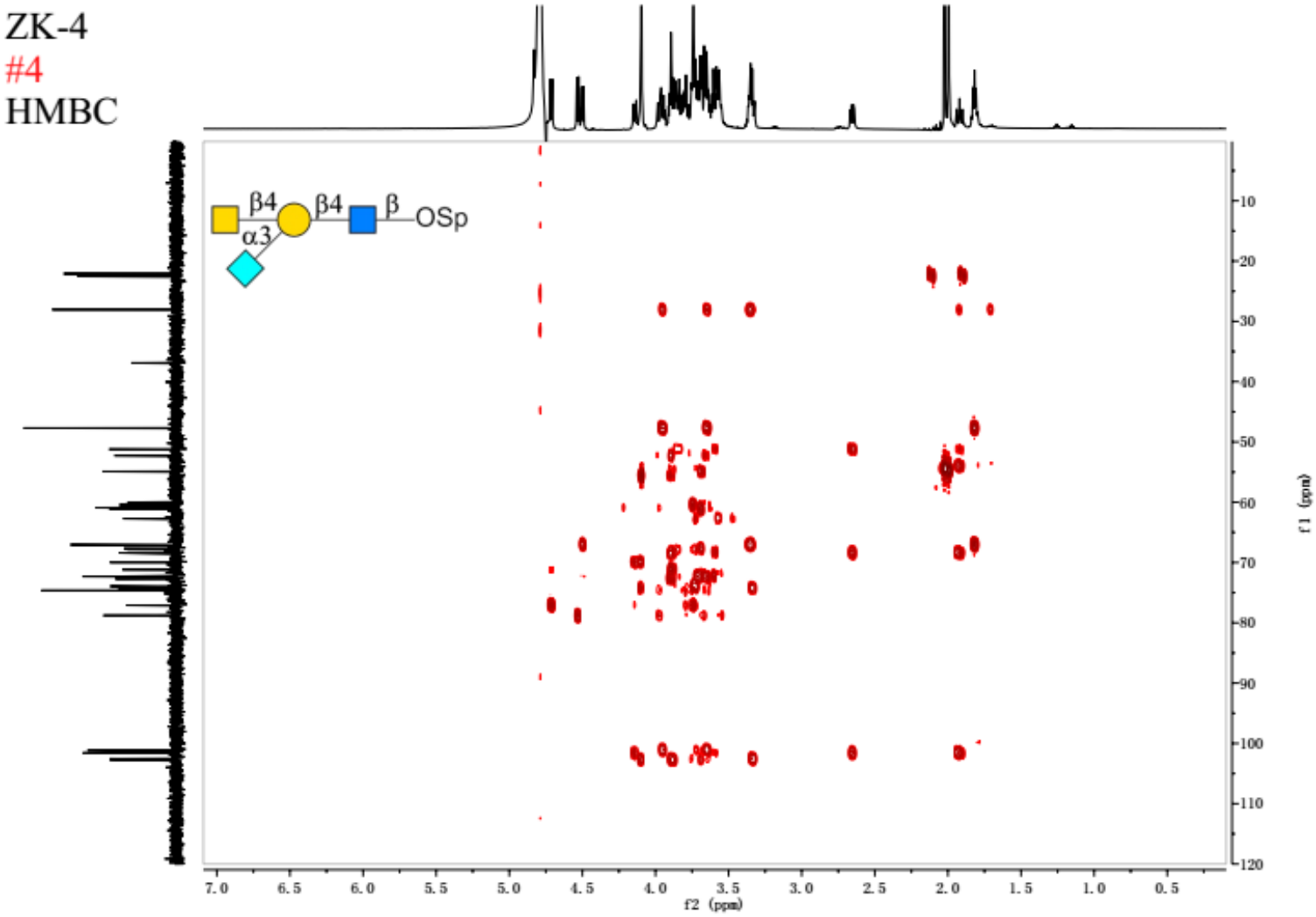
^{1}H NMR of 4


ZK-4
#4
HO
OH
NHAc
HO2C
GcHN
N3
β4
β4
β
α3
OSp
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)

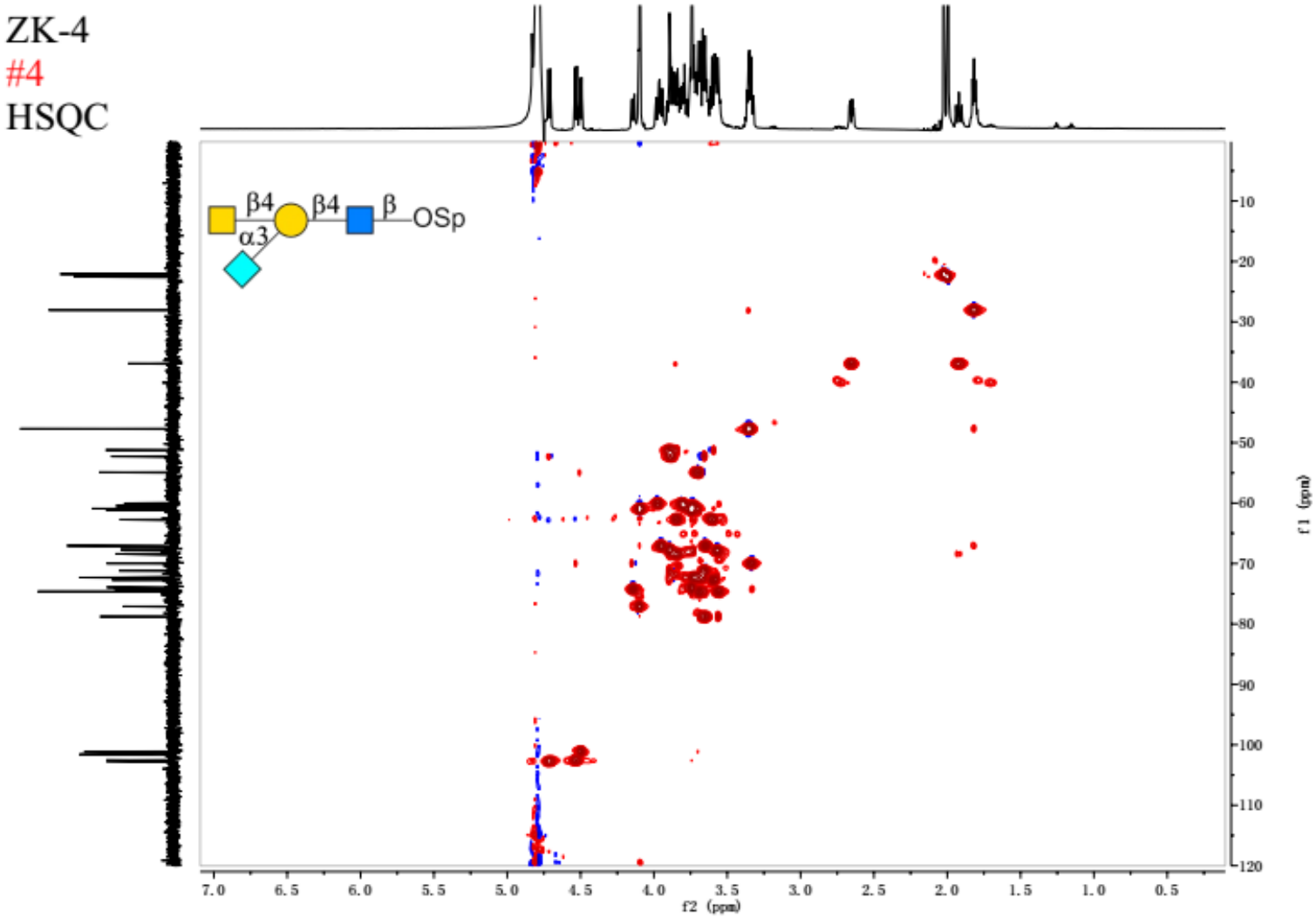
^{13}C NMR of 4



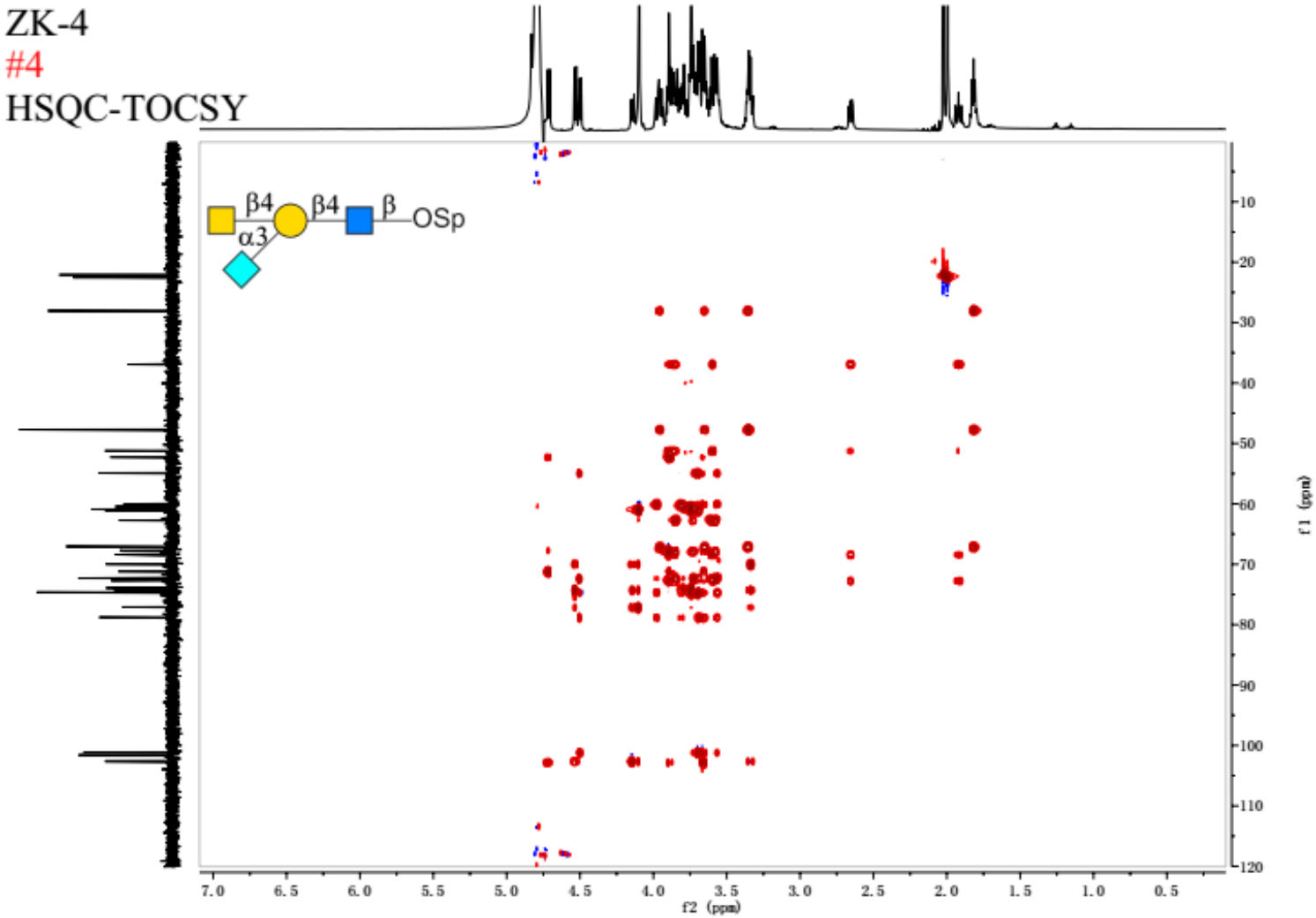
COSY spectra of 4



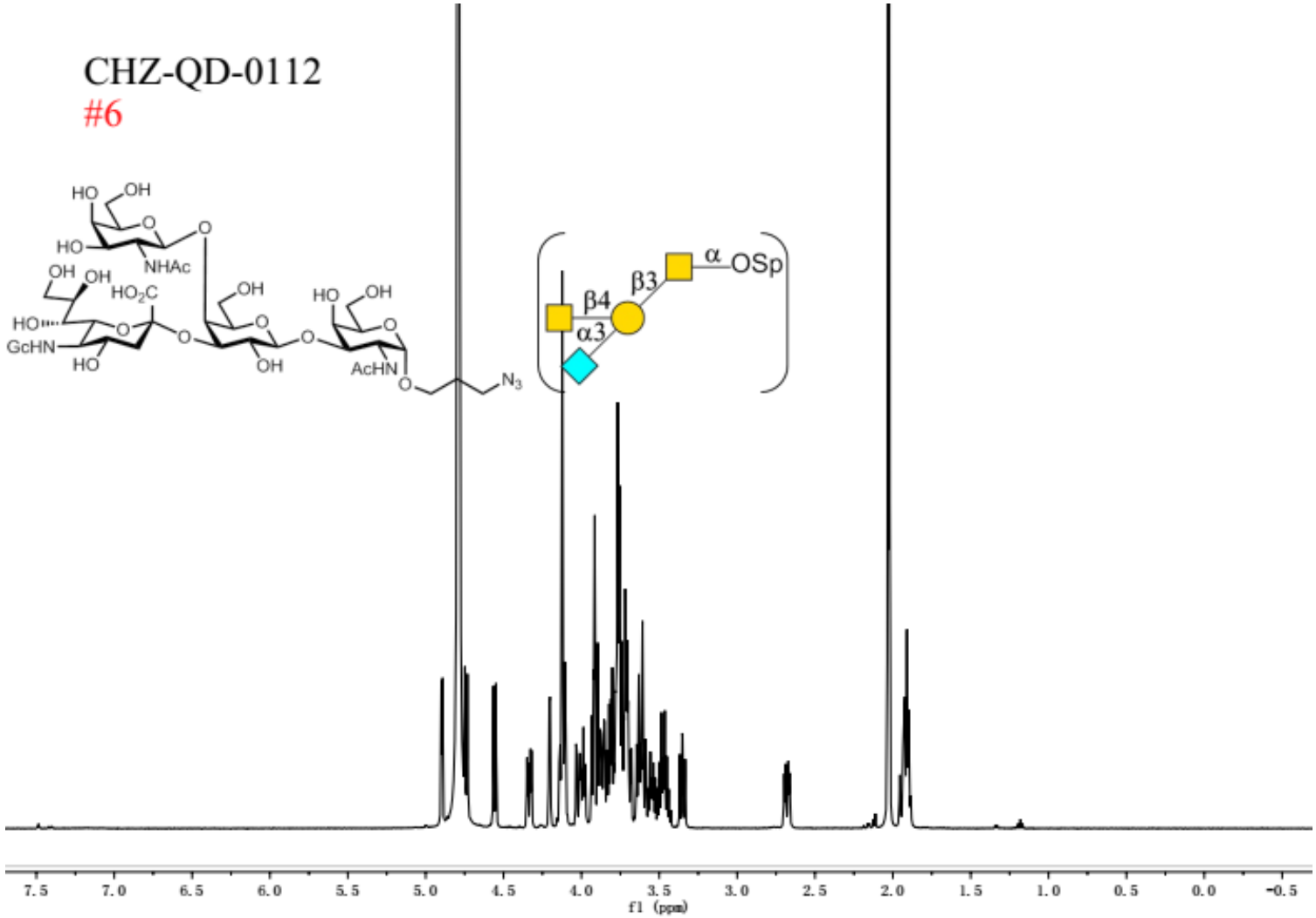
HMBC spectra of 4



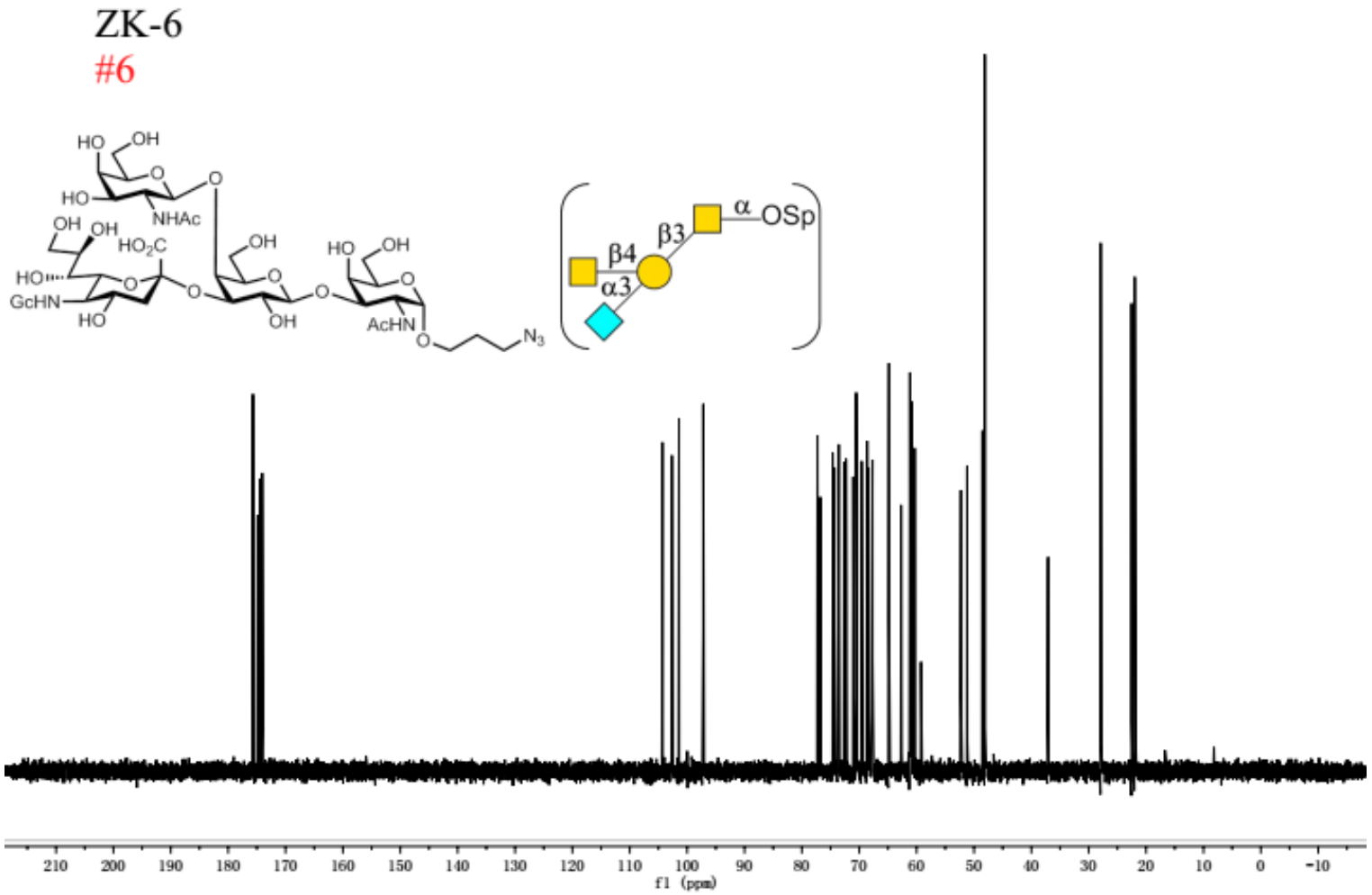
HSQC spectra of 4



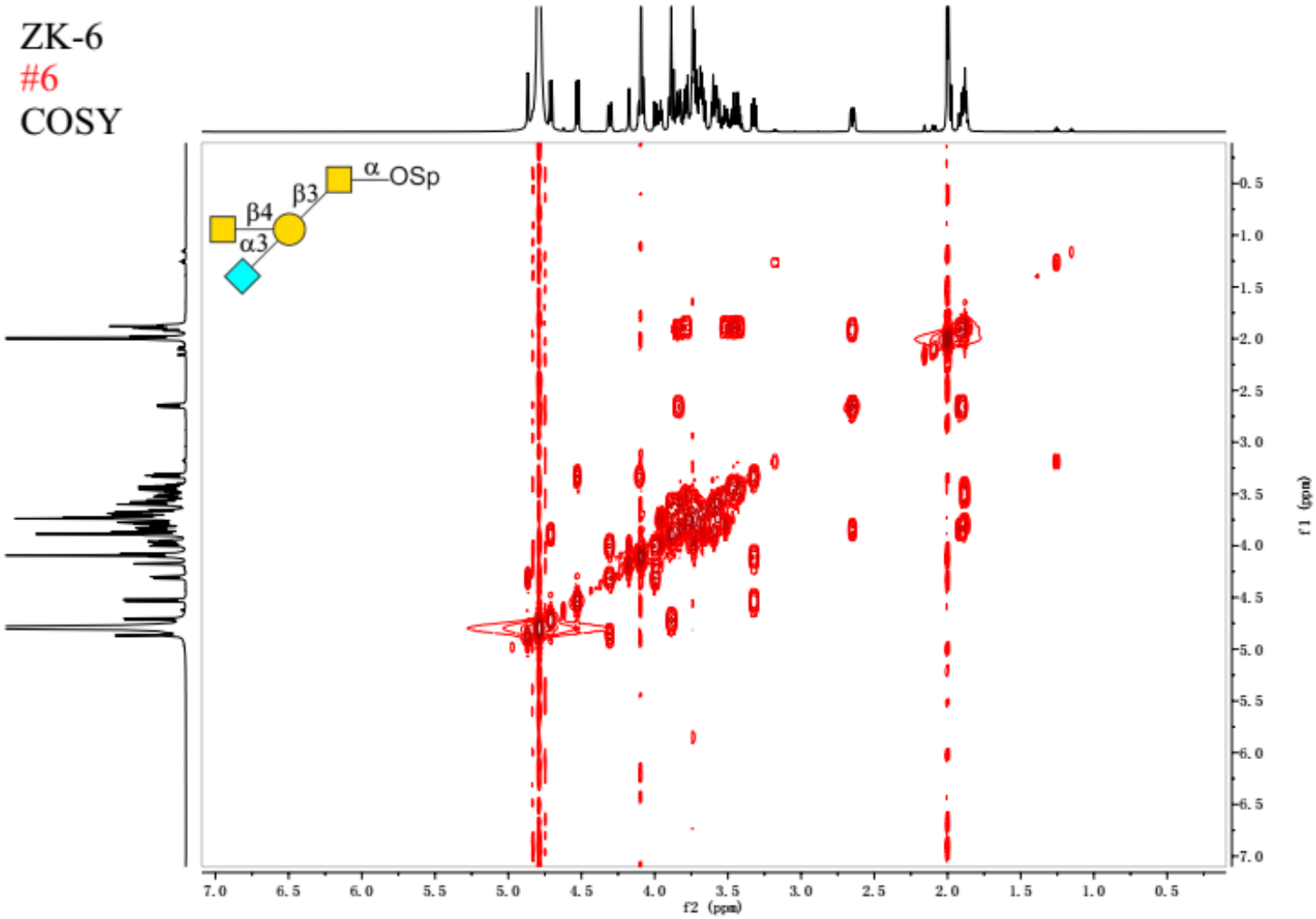
HSQC-TOCSY spectra of 4


CHZ-QD-0112
#6
HO
OH
HO
NHAc
OH
OH
HO2C
HO
GcHN
HO
OH
HO
OH
AcHN
N3
α
OSp
β3
β4
α3
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
0.0
-0.5
f1 (ppm)

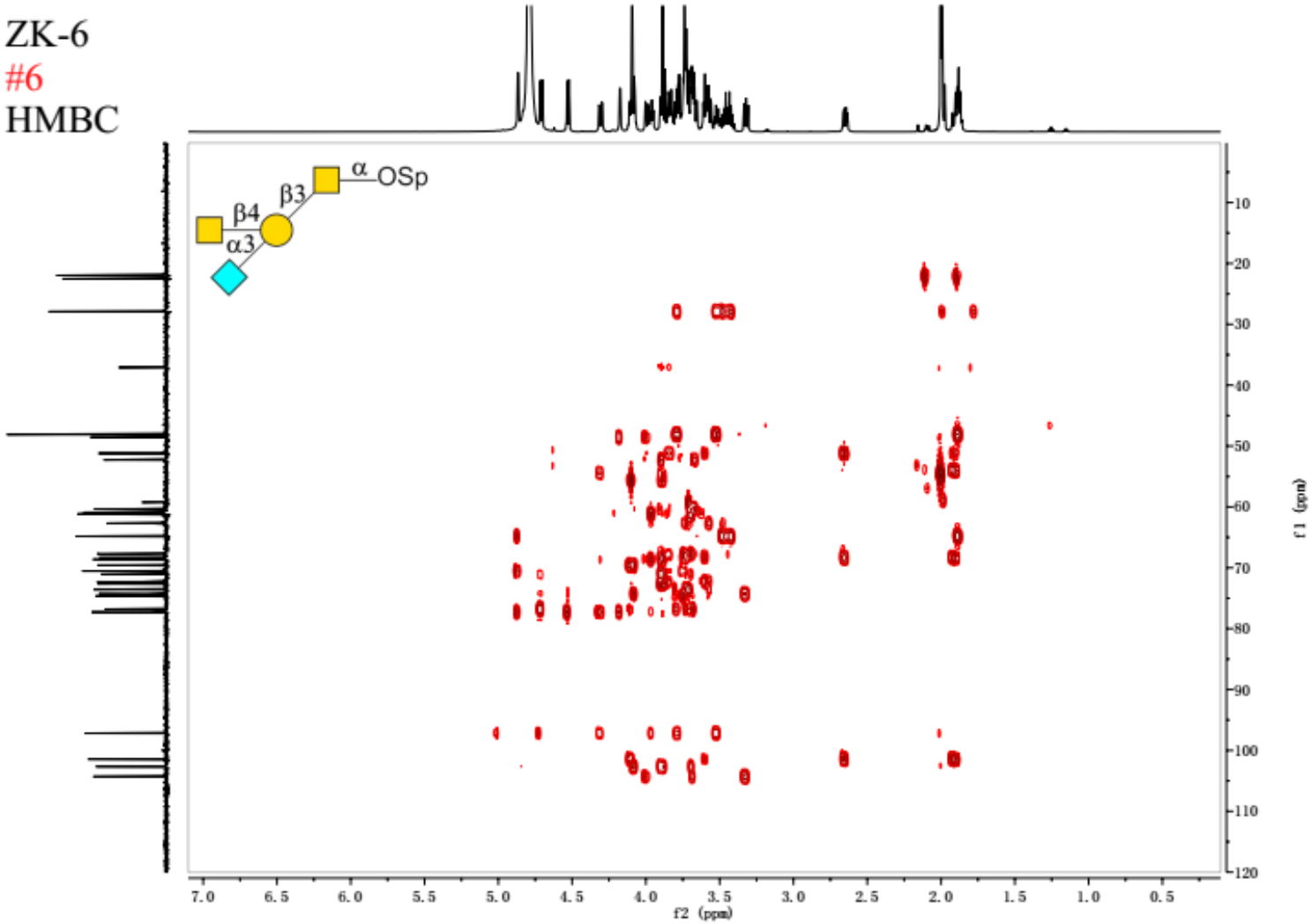
^{1}H NMR of 6


ZK-6
#6
HO
OH
HO
NHAc
OH
OH
HO2C
HO
GcHN
HO
OH
HO
OH
AcHN
N3
α
OSp
β3
β4
α3
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)

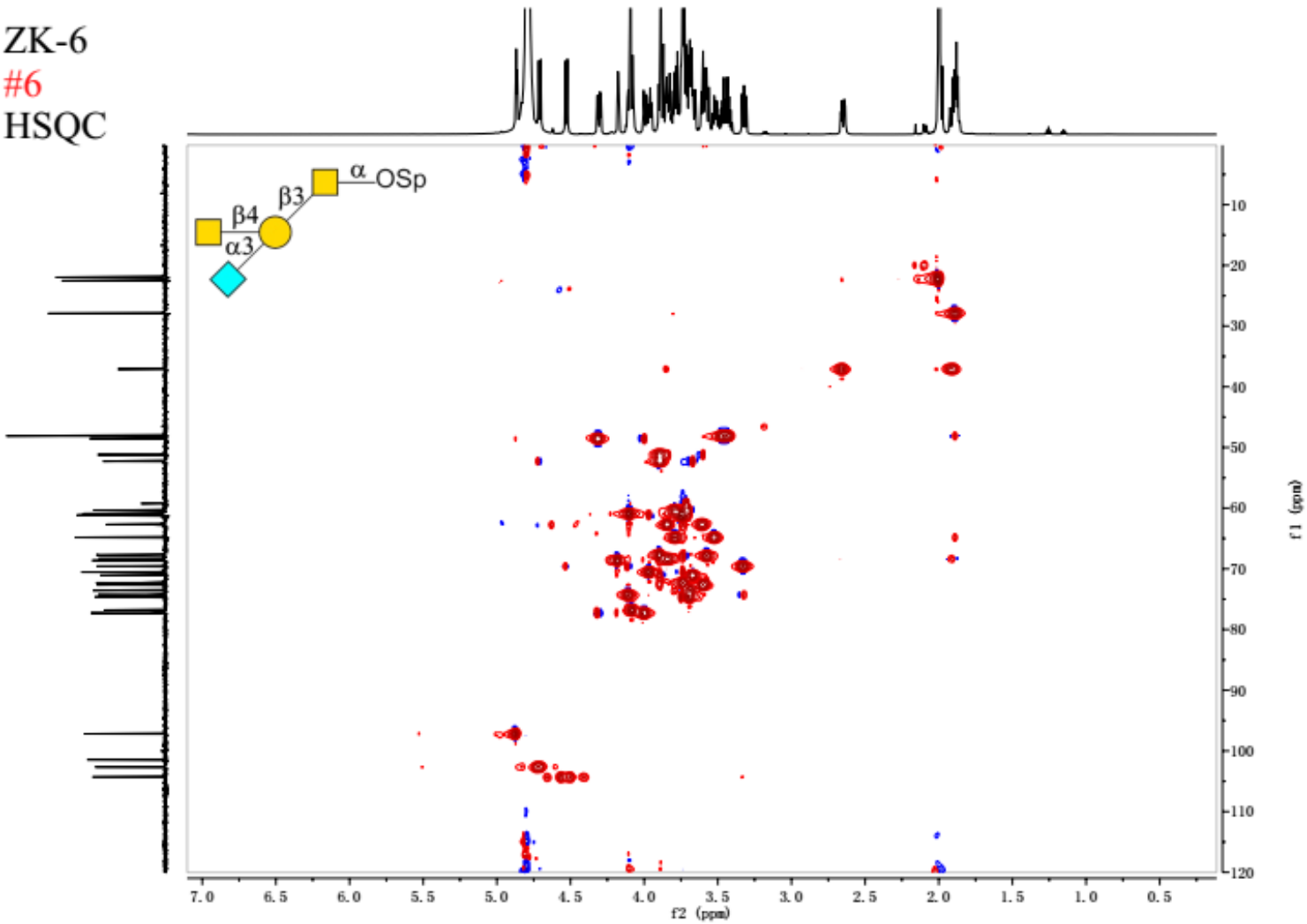
^{13}C NMR of 6



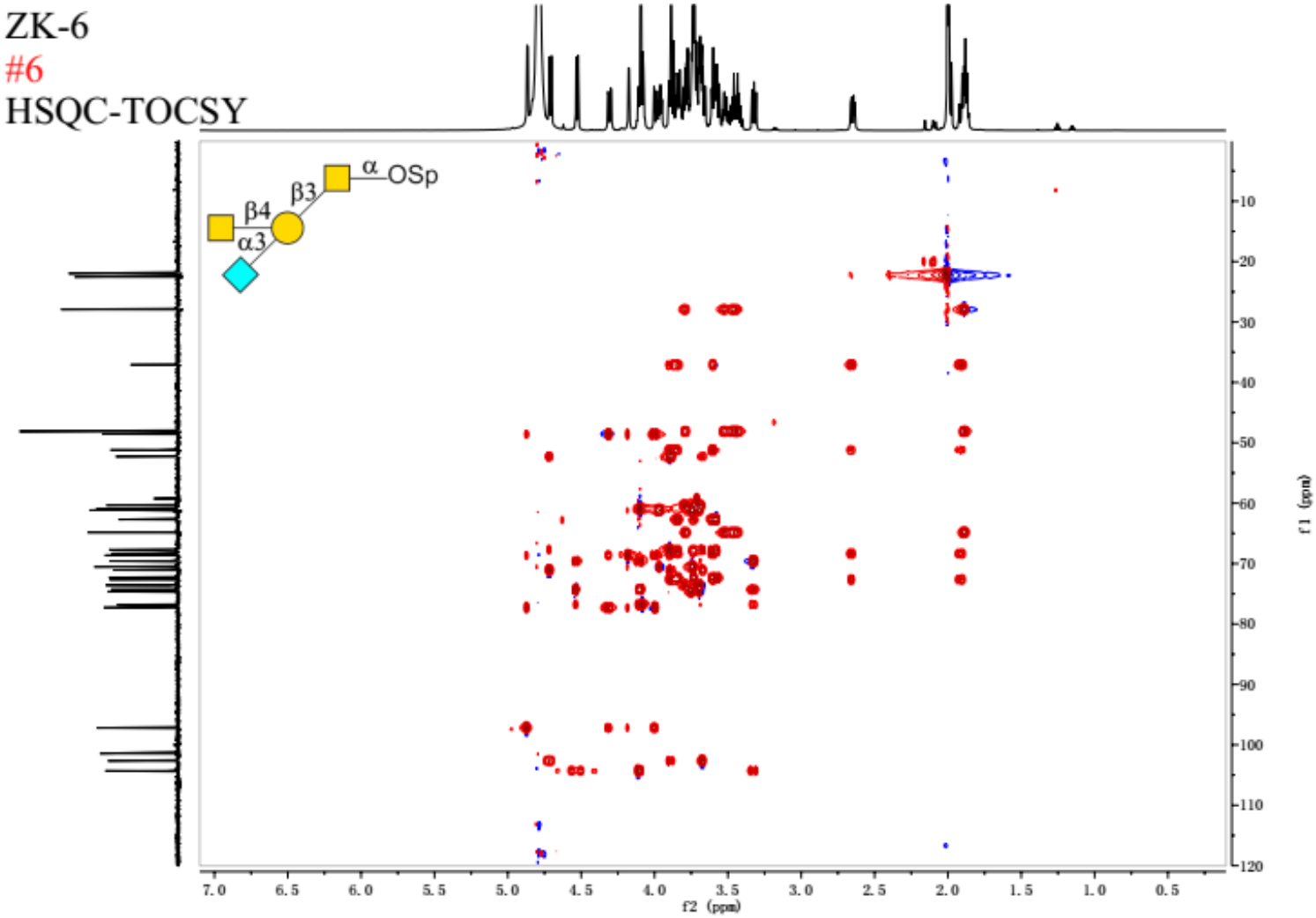
COSY spectra of 6



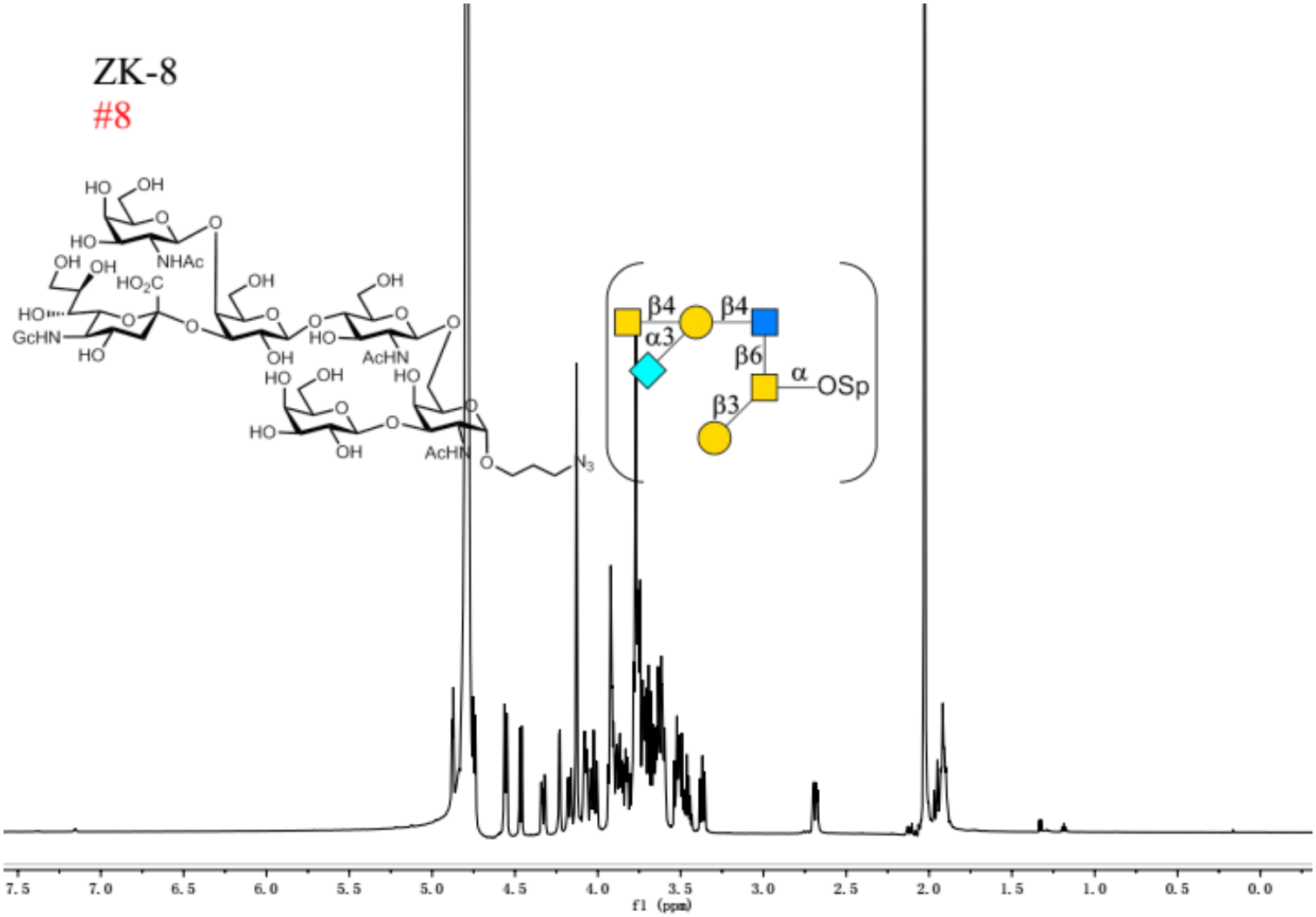
HMBC spectra of 6



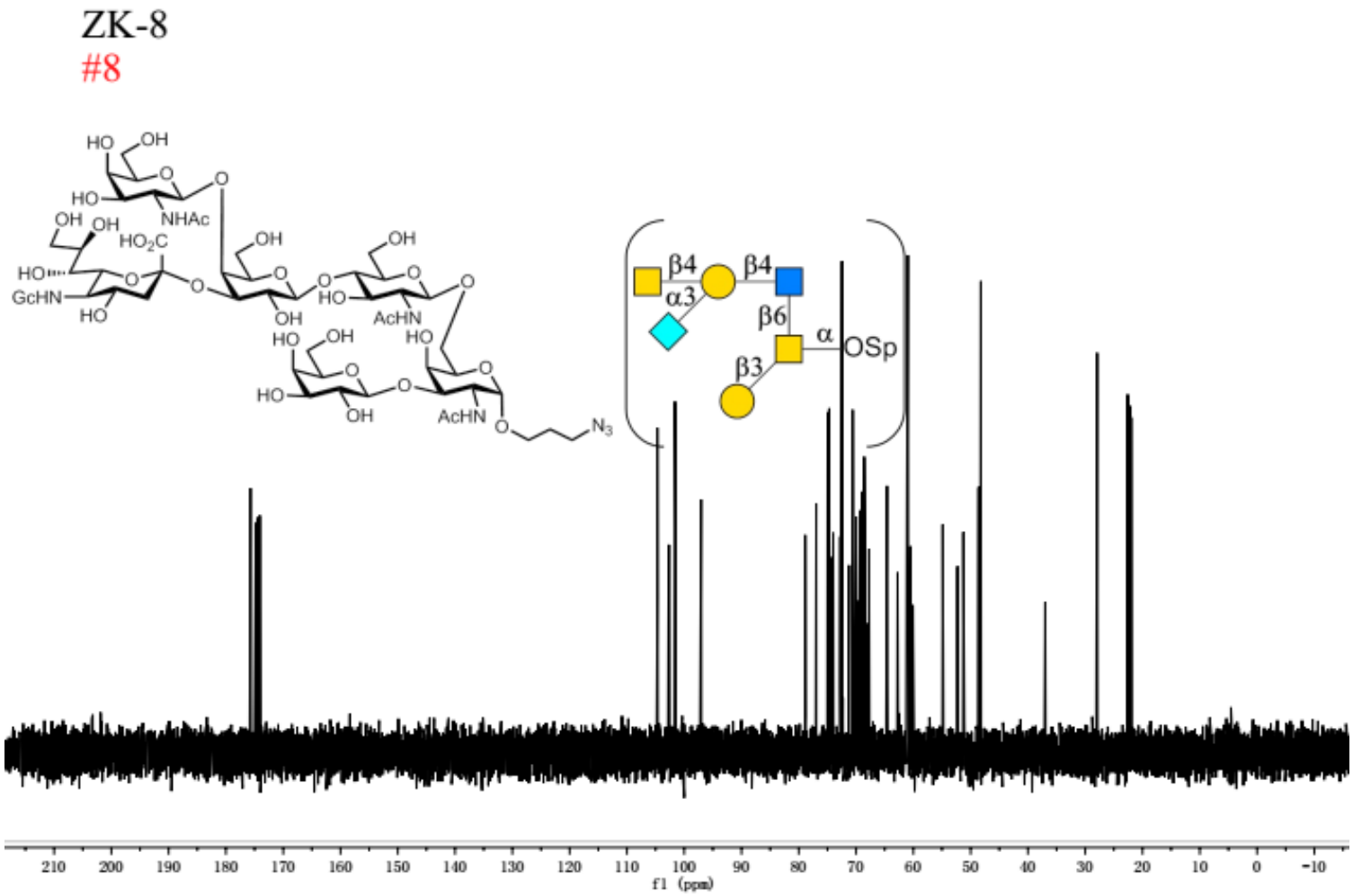
HSQC spectra of 6



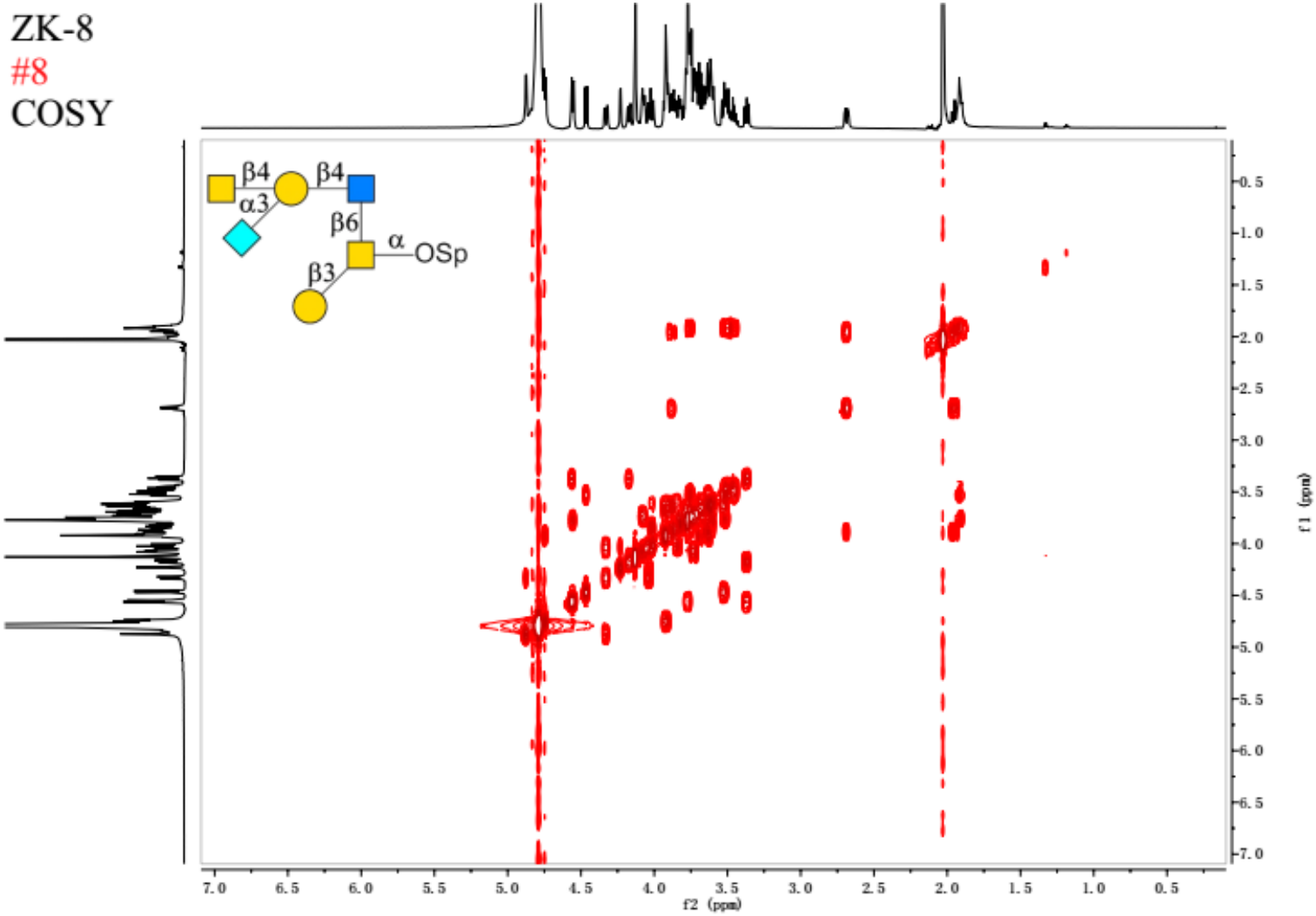
HSQC-TOCSY spectra of 6


ZK-8
#8
HO
OH
NHAc
HO2C
GcHN
AcHN
N3
β4
β4
α3
β6
α
OSp
β3
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
0.0
f1 (ppm)

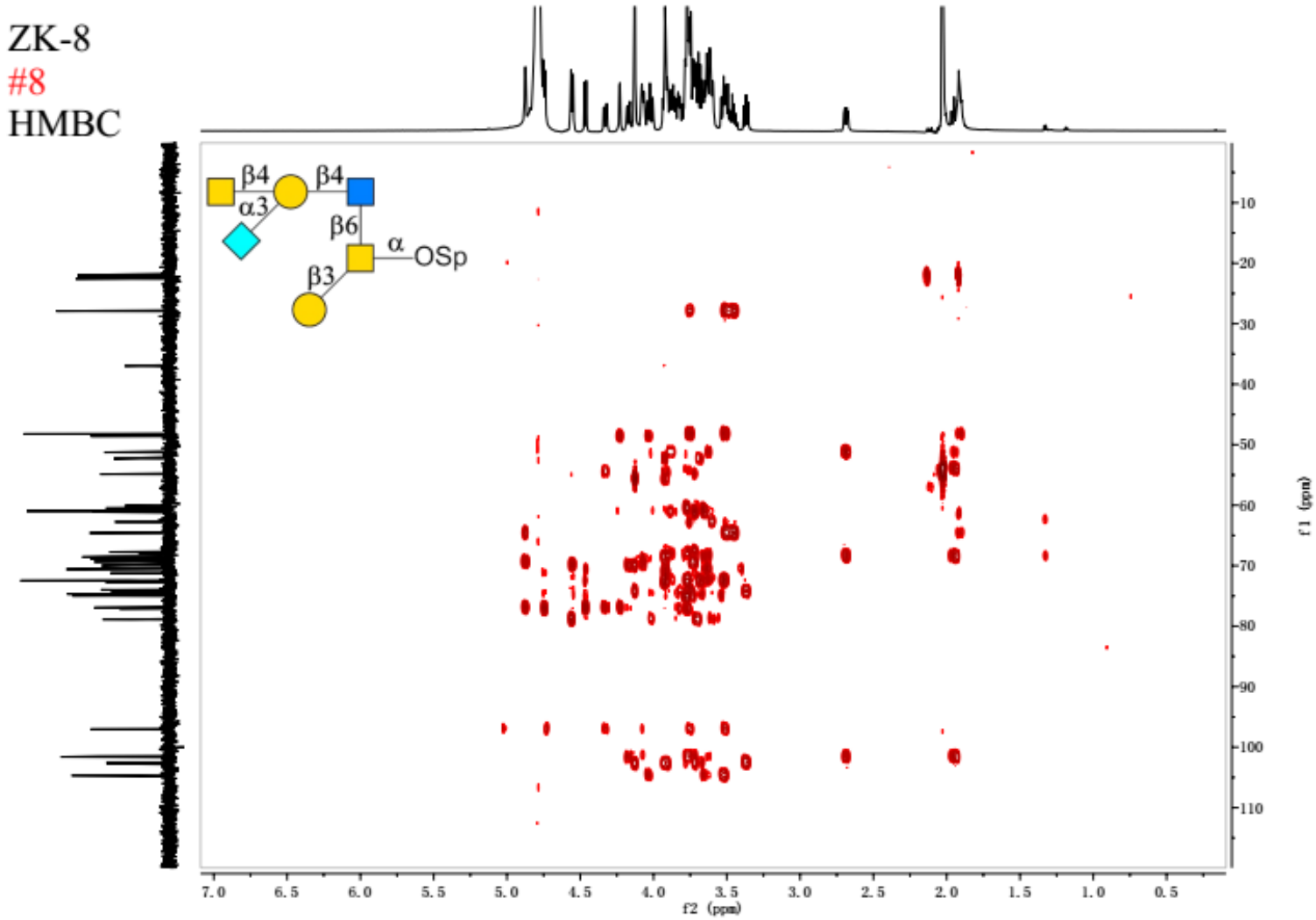
^{1}H NMR of 8


ZK-8
#8
HO
OH
NHAc
HO
OH
OH
HO2C
HO
GcHN
HO
O
OH
HO
AcHN
HO
OH
HO
OH
HO
AcHN
O
N3
β4
β4
α3
β6
α
OSp
β3
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)

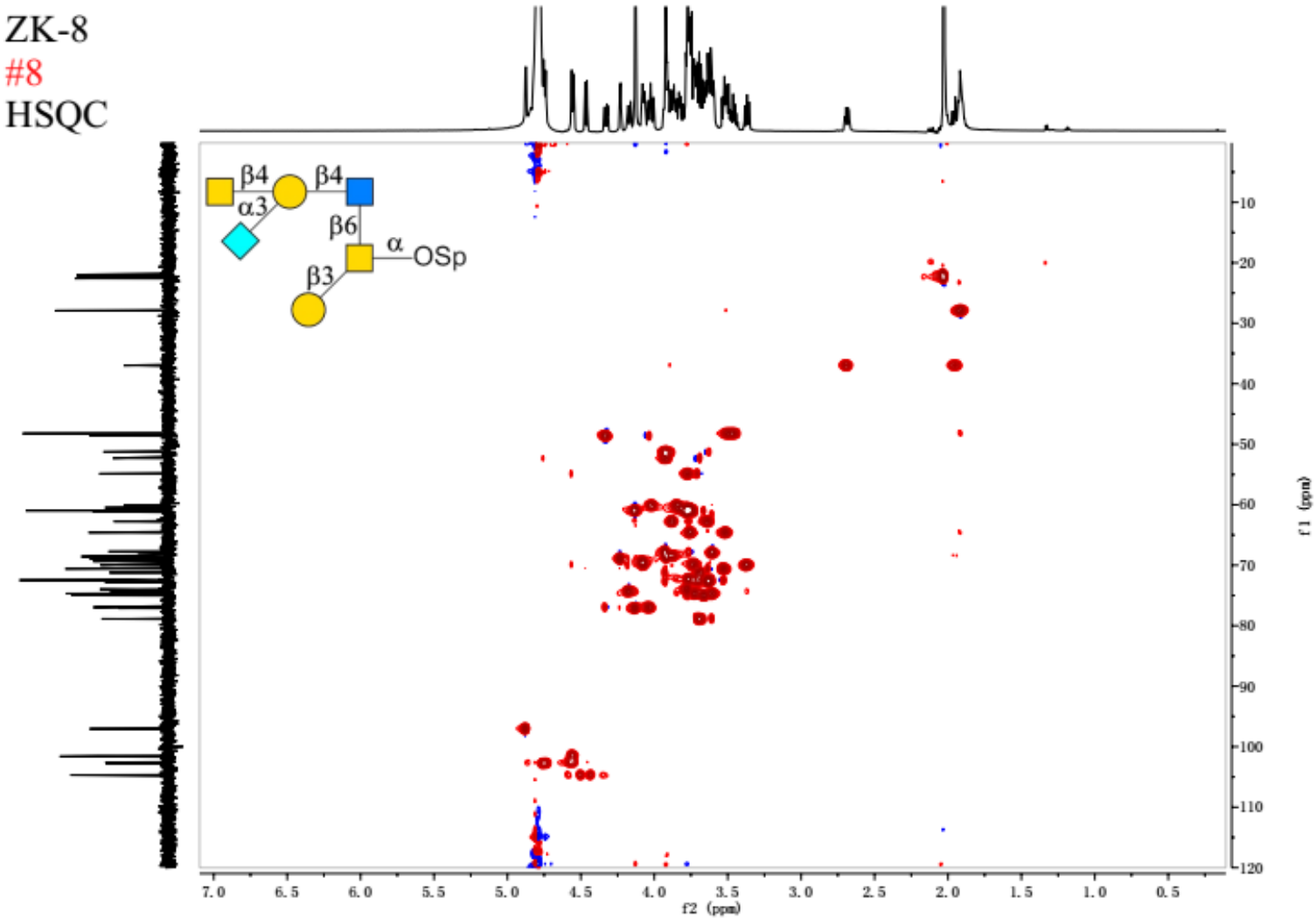
^{13}C NMR of 8



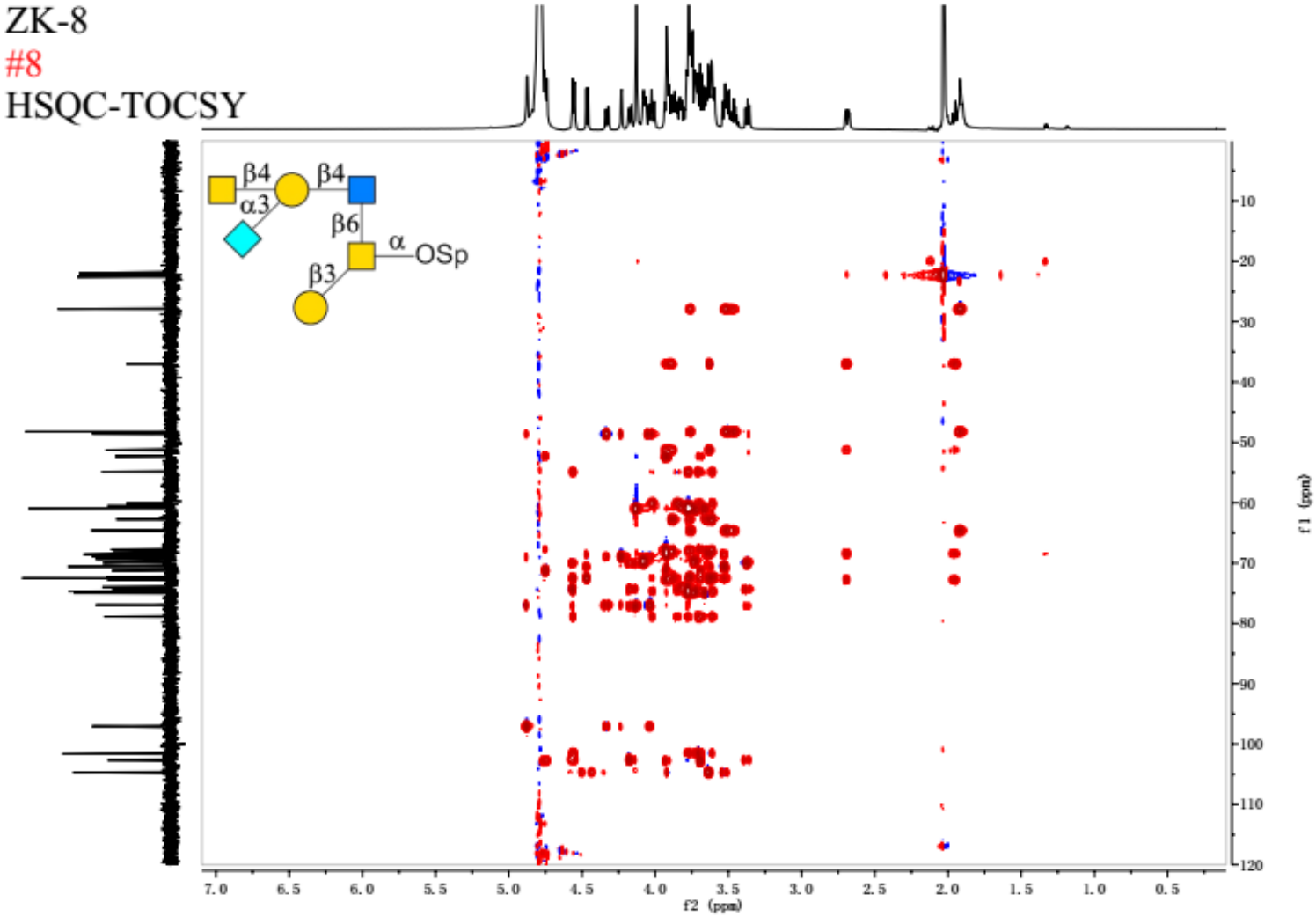
COSY spectra of 8



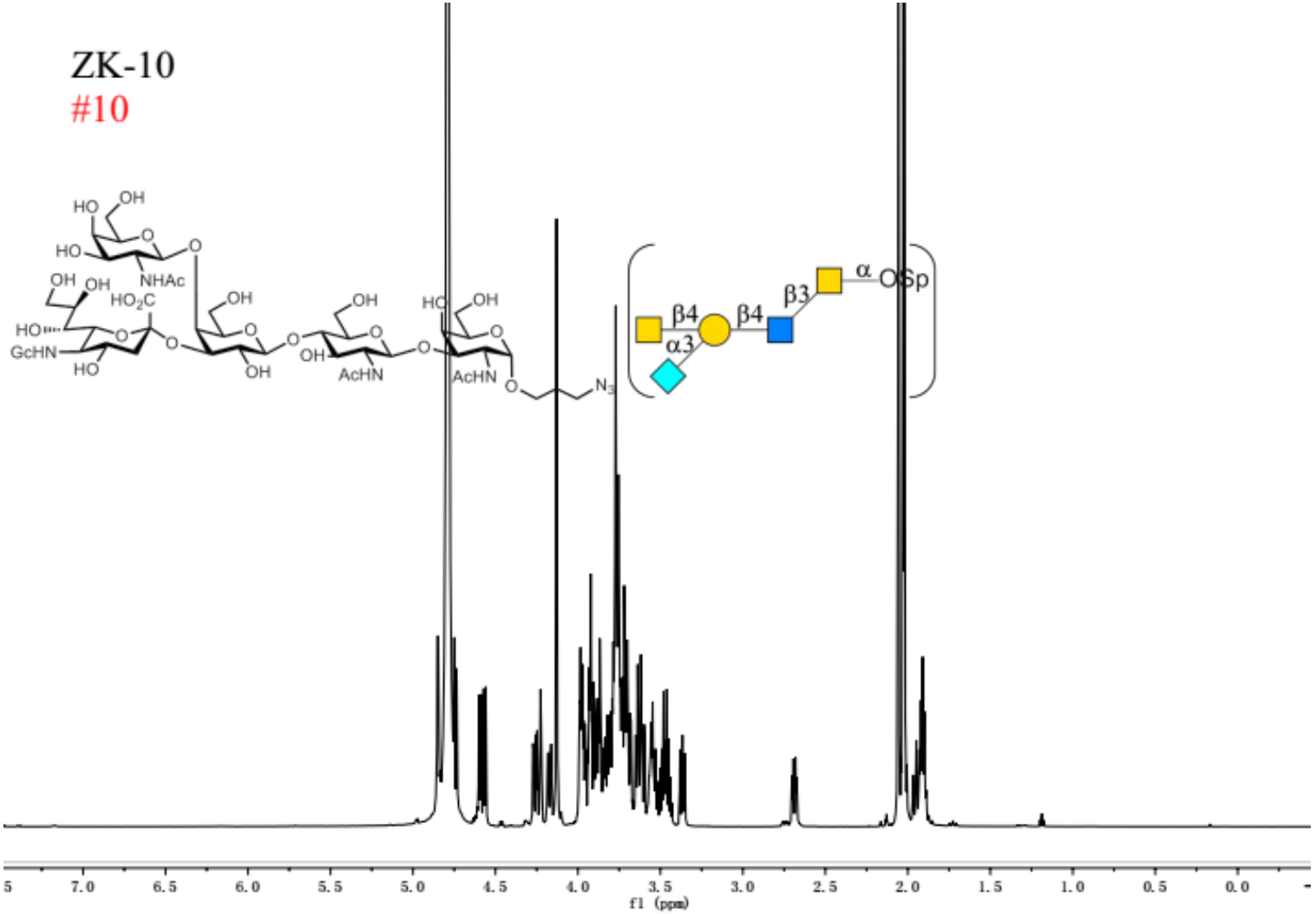
HMBC spectra of 8



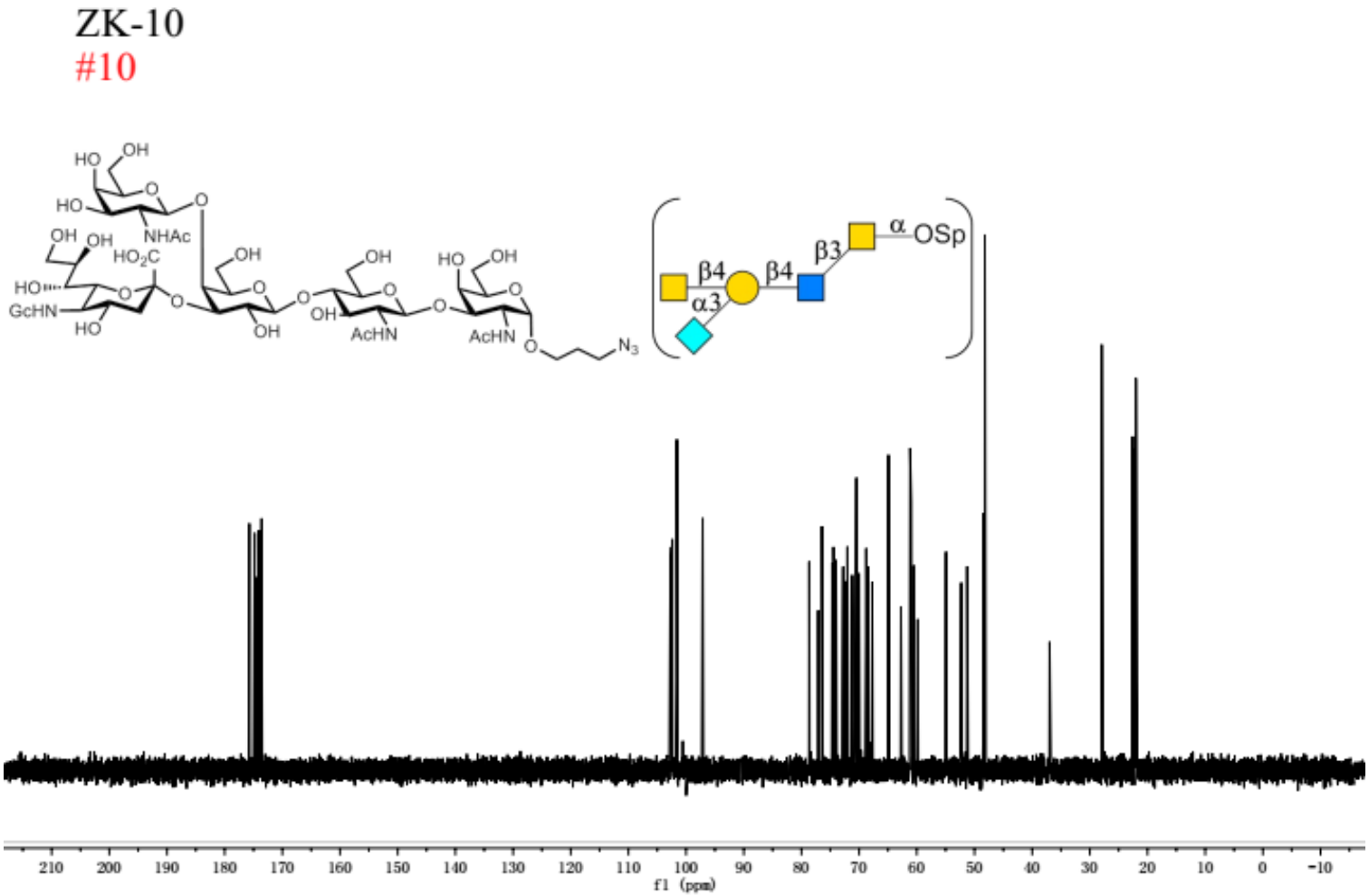
HSQC spectra of 8



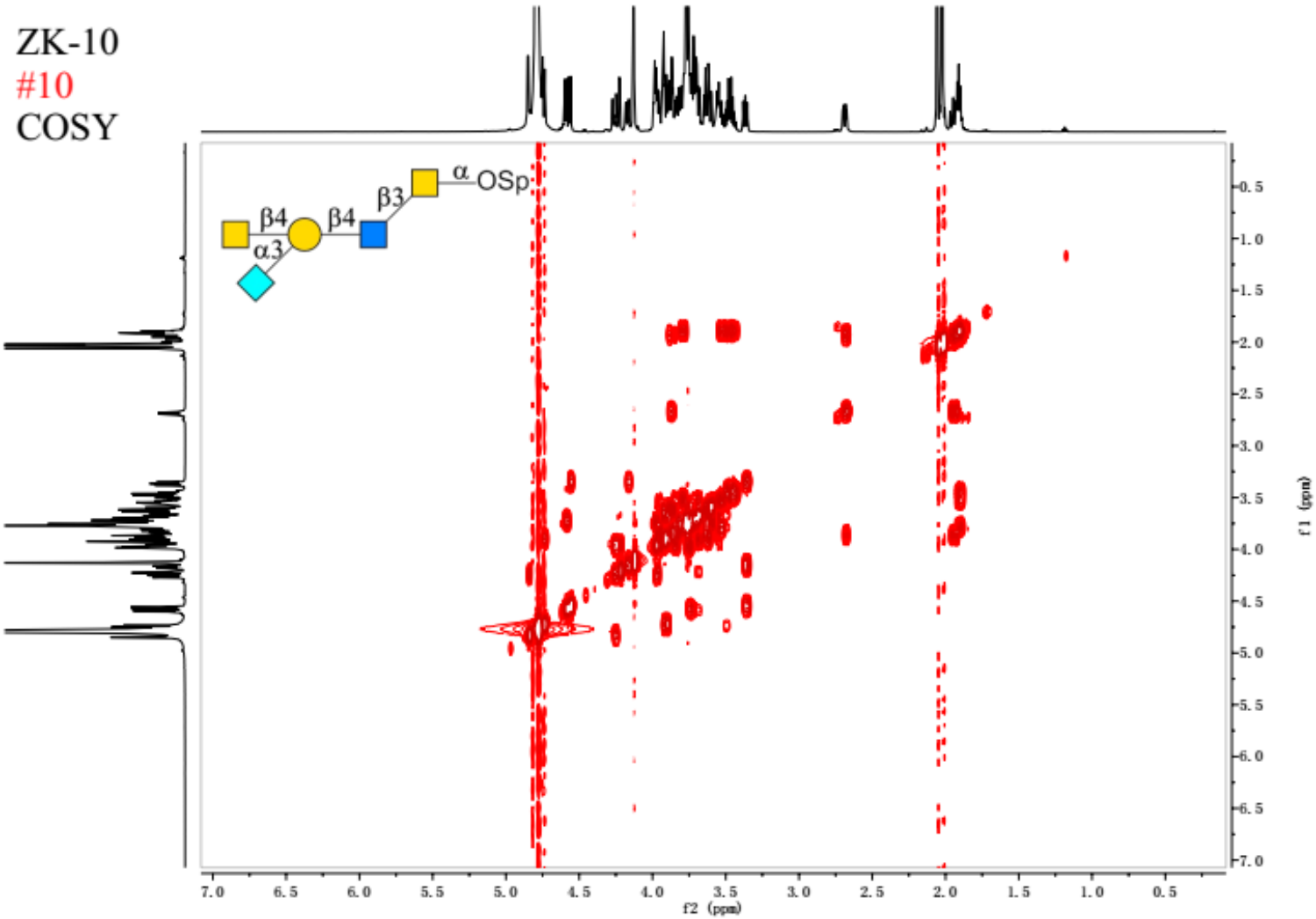
HSQC-TOCSY spectra of 8


ZK-10
#10
HO
OH
NHAc
HO2C
GcHN
AcHN
N3
β4
β3
α3
α
OSp
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
0.0
f1 (ppm)

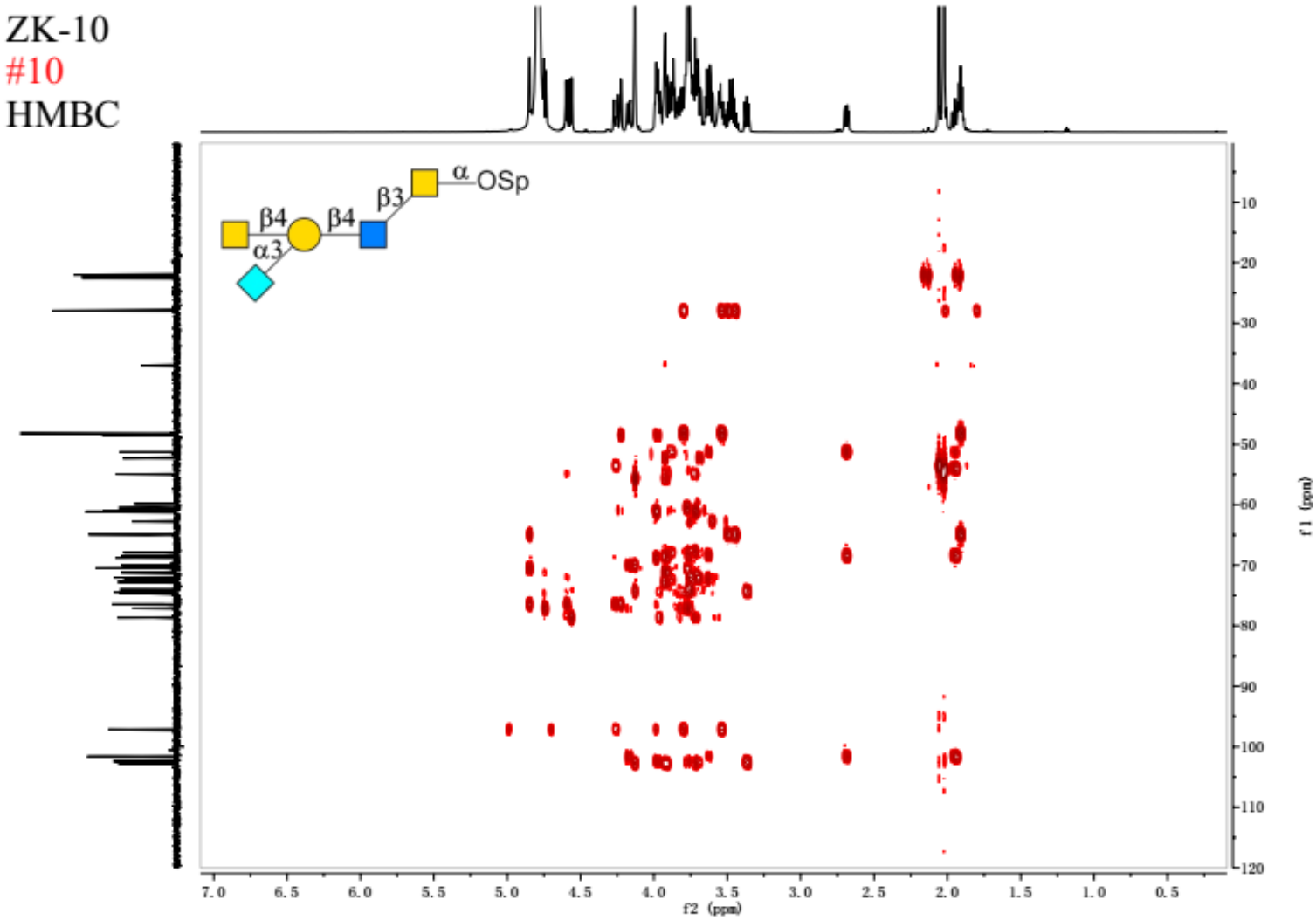
^{1}H NMR of 10


ZK-10
#10
HO
OH
HO
NHAc
OH
OH
HO2C
HO
GcHN
HO
OH
OH
OH
AcHN
HO
OH
AcHN
N3
β4
β4
β3
α
OSp
α3
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)

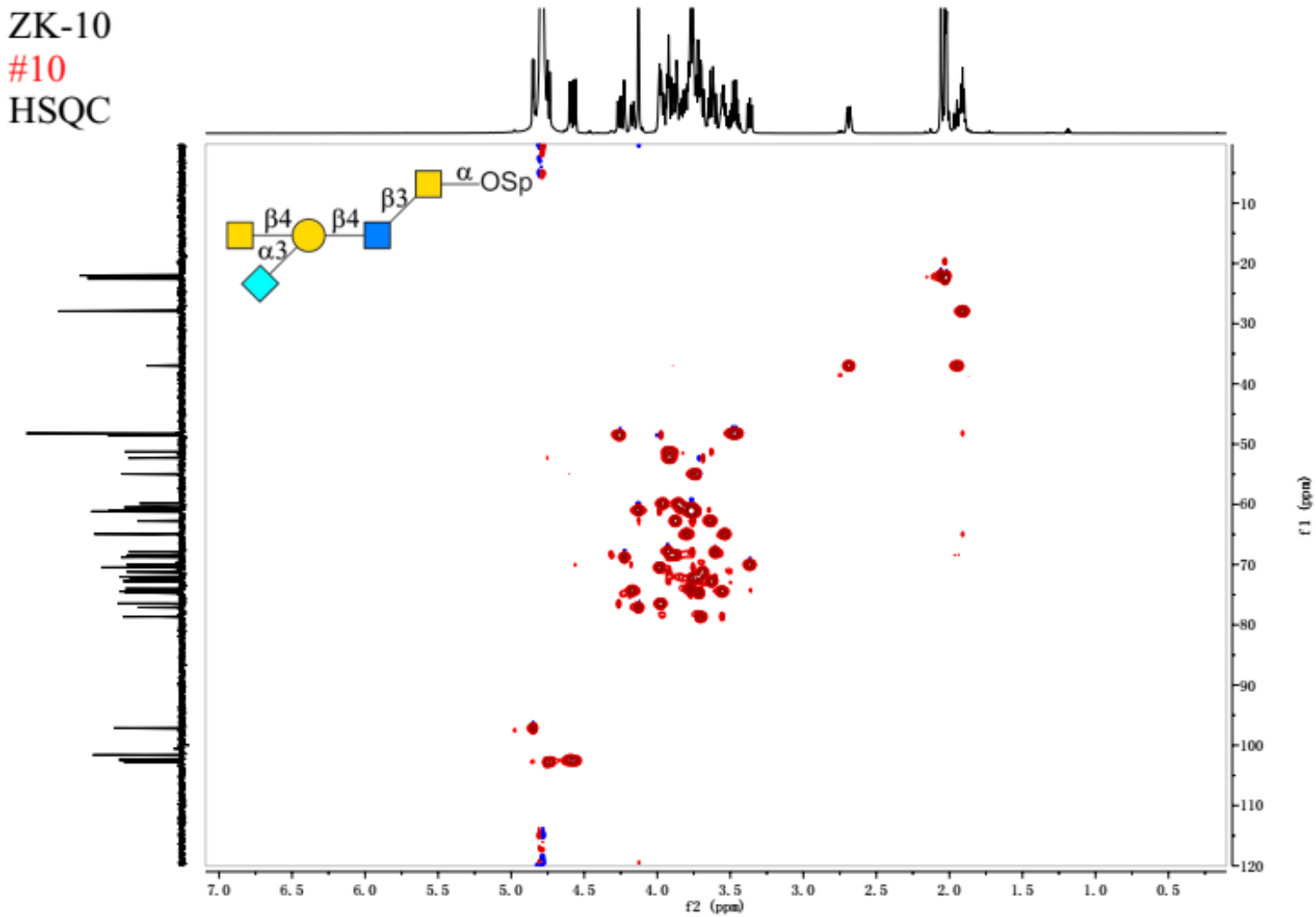
^{13}C NMR of 10



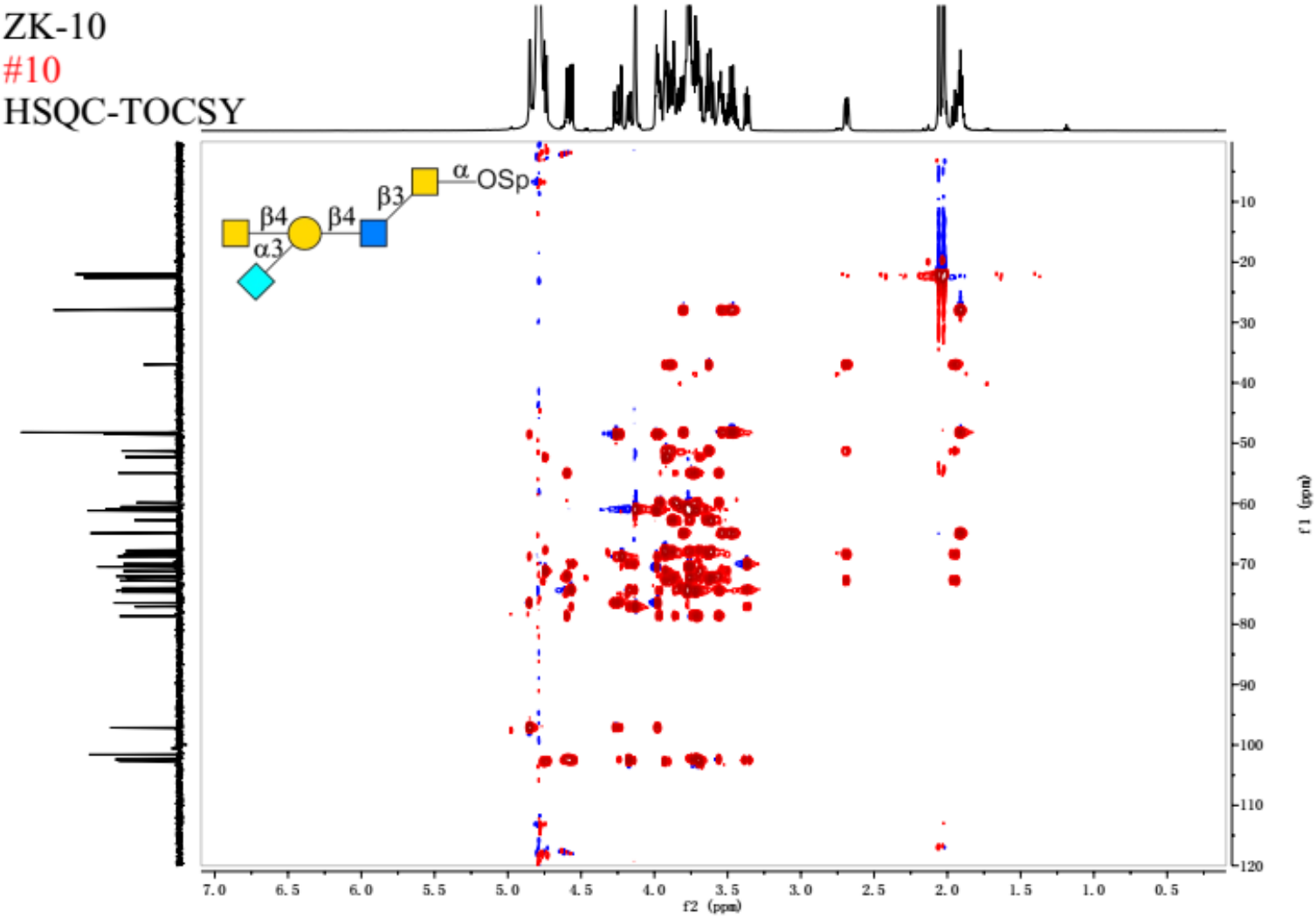
COSY spectra of 10



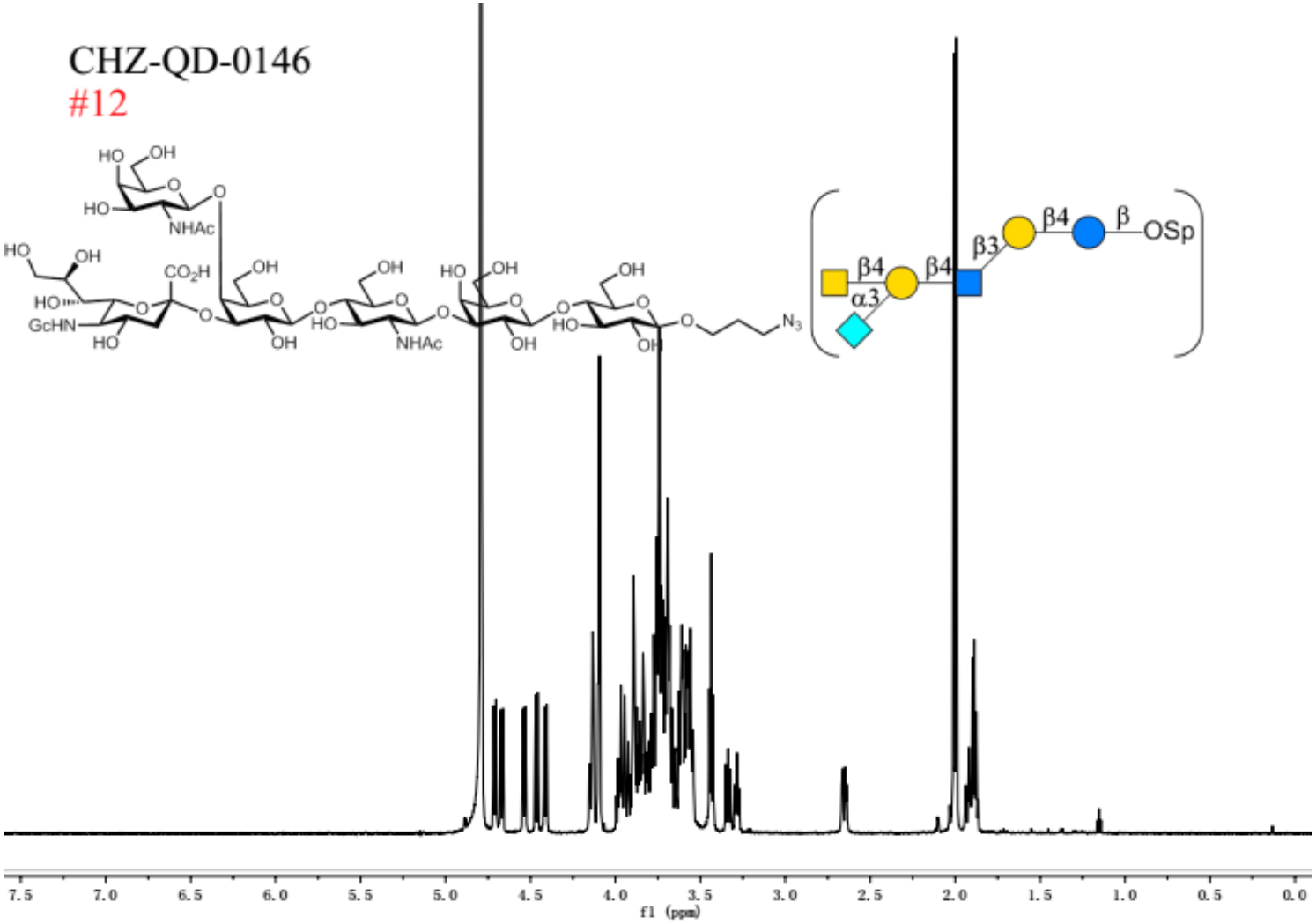
HMBC spectra of 10



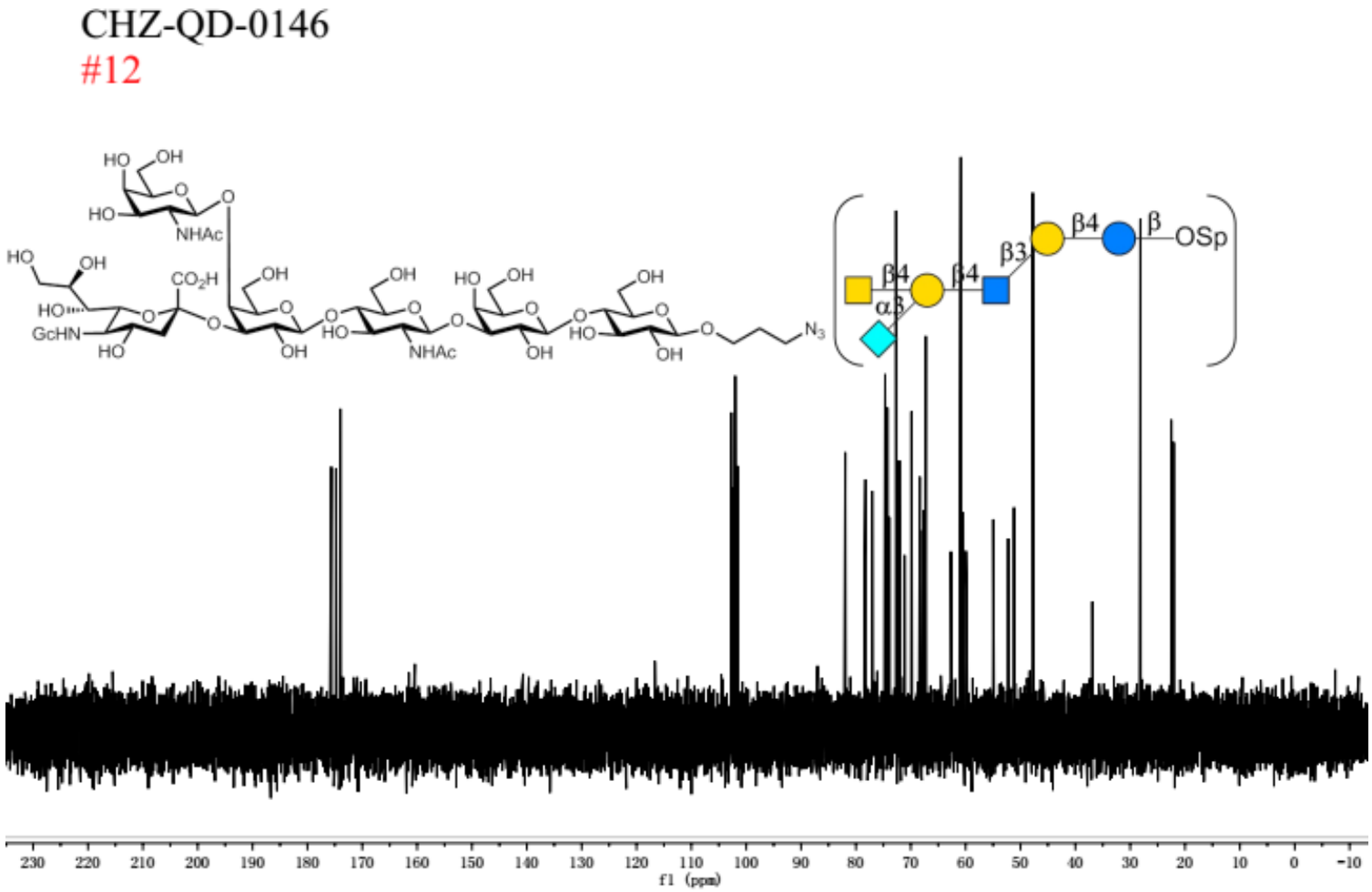
HSQC spectra of 10



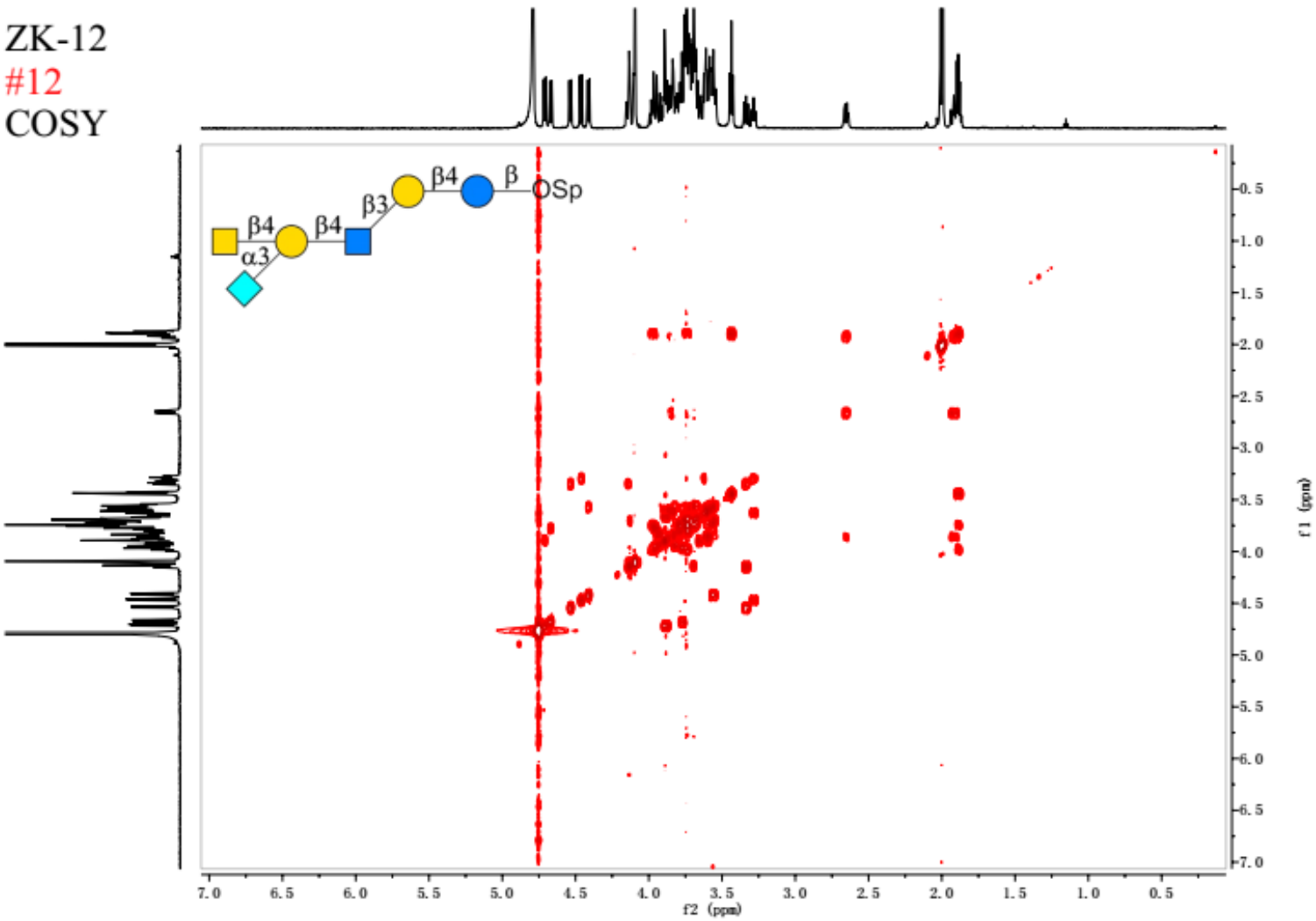
HSQC-TOCSY spectra of 10


CHZ-QD-0146
#12
HO
OH
HO
NHAc
HO
OH
CO2H
HO
GcHN
HO
OH
HO
NHAc
HO
OH
HO
OH
N3
β4
β4
α3
β3
β4
β
OSp
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
0.0
f1 (ppm)

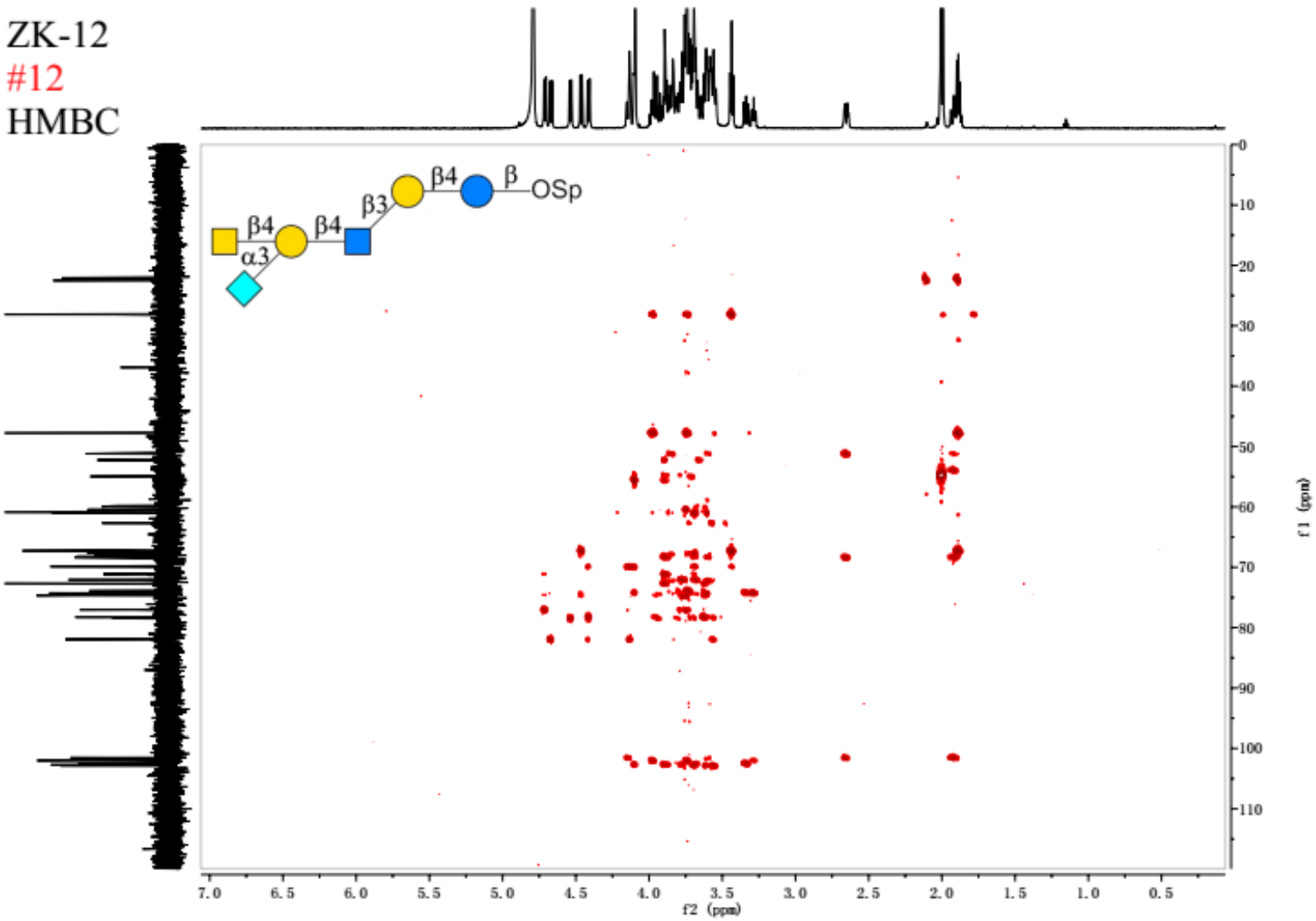
^{1}H NMR of 12


CHZ-QD-0146
#12
HO
OH
O
HO
NHAc
HO
OH
CO2H
OH
HO
GcHN
HO
O
O
OH
HO
NHAc
OH
HO
OH
HO
OH
O
N3
β4
β
OSp
β3
β4
β4
α3
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)

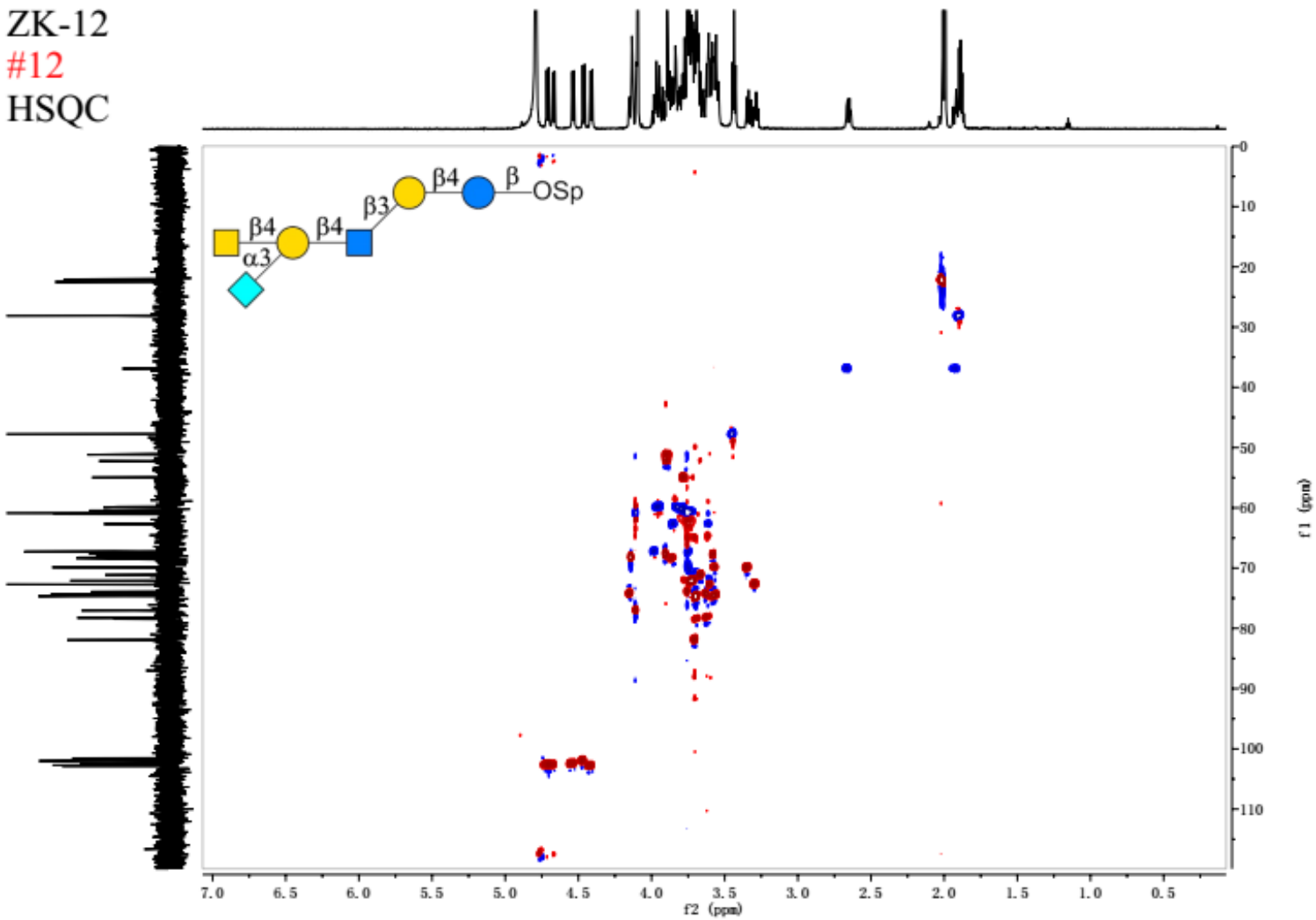
^{13}C NMR of 12



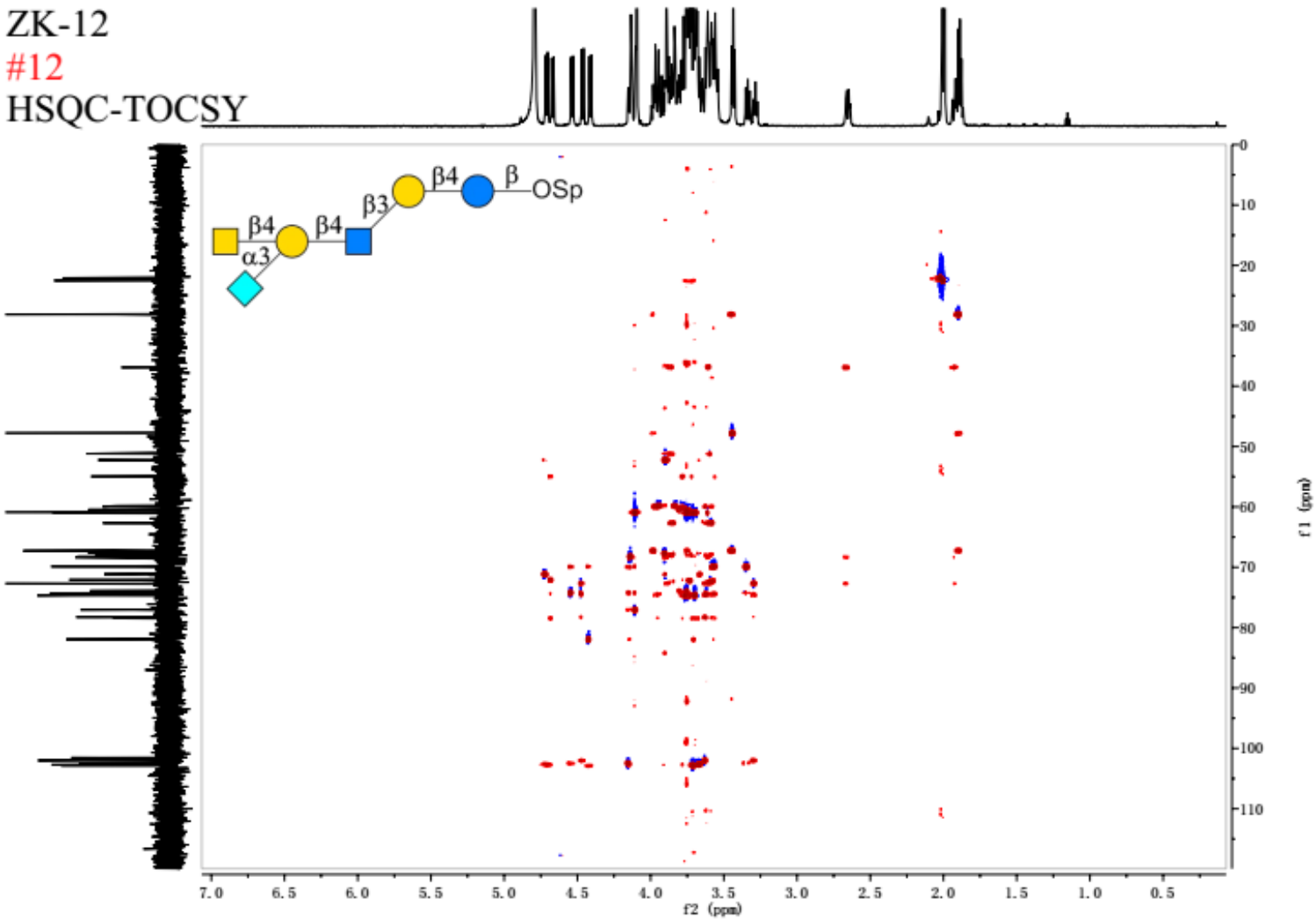
COSY spectra of 12



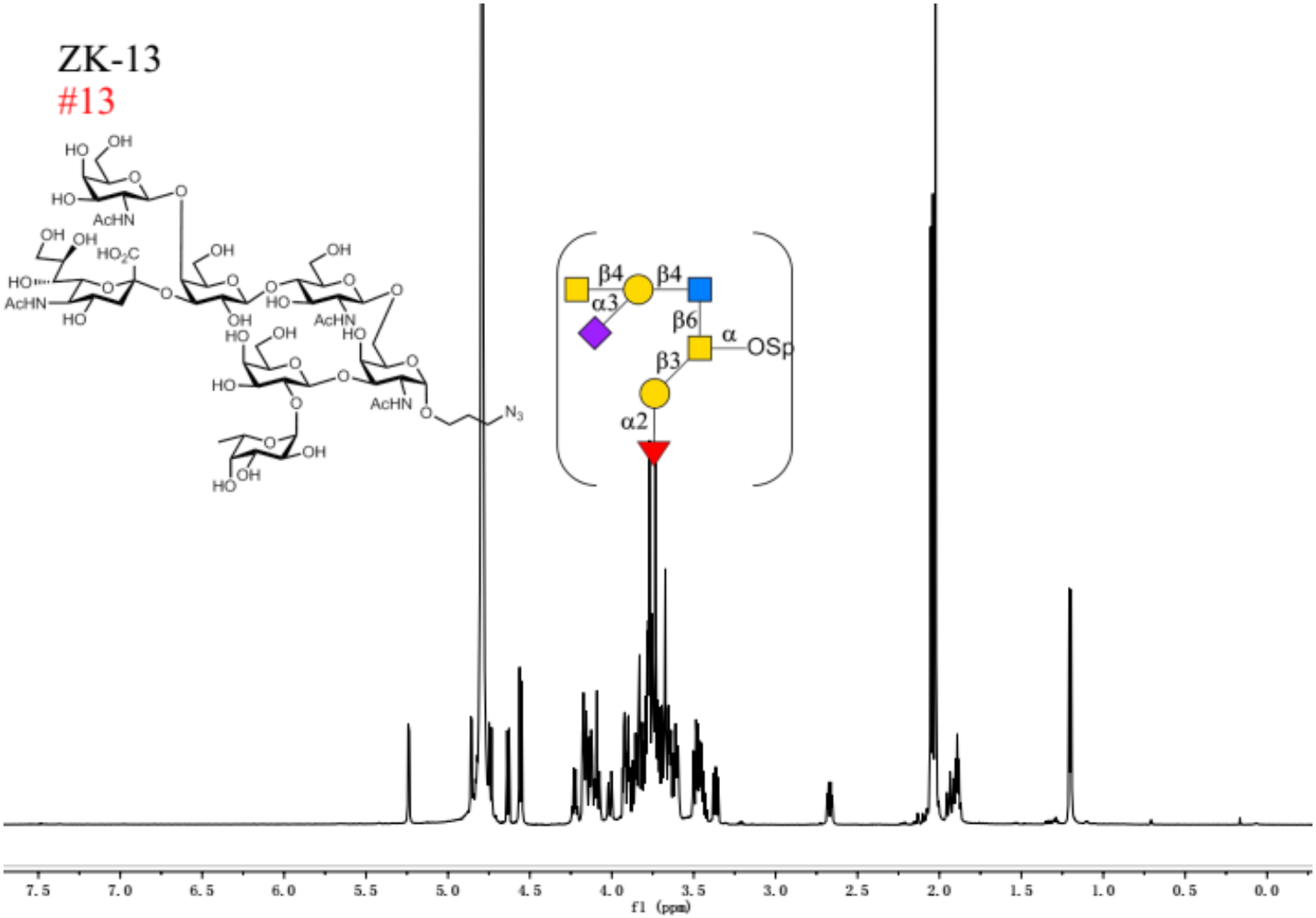
HMBC spectra of 12



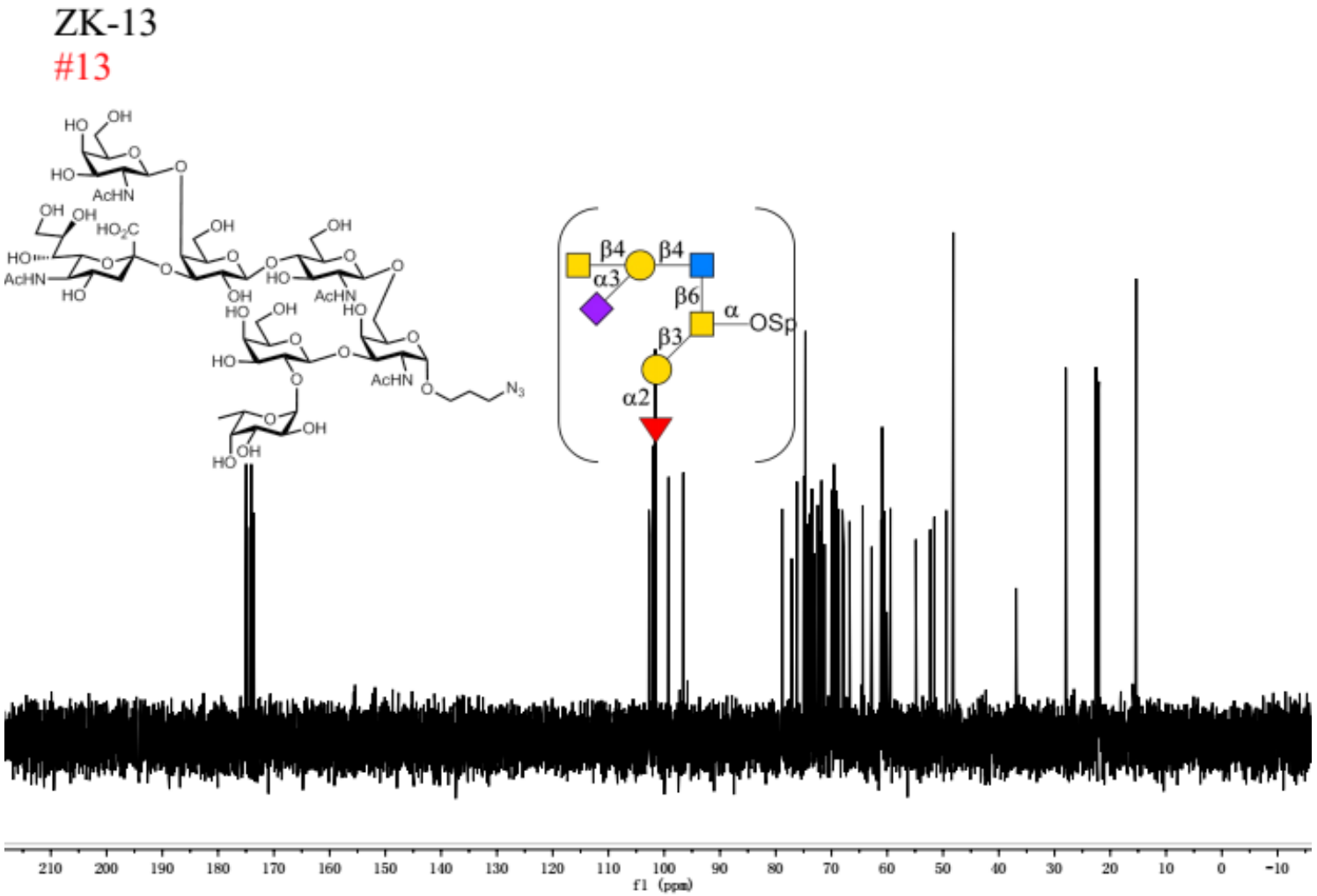
HSQC spectra of 12



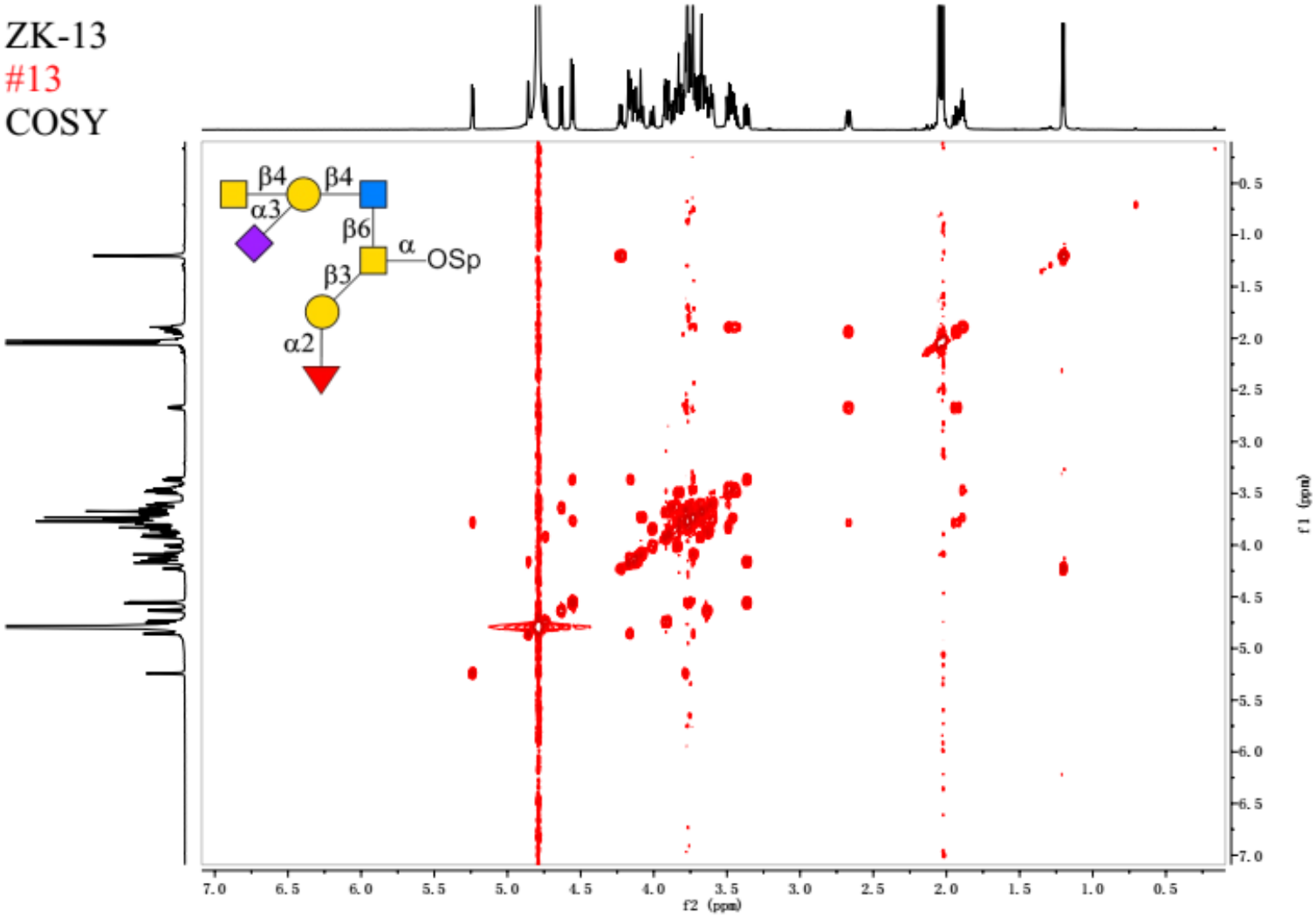
HSQC-TOCSY spectra of 12


ZK-13
#13
HO
OH
AcHN
HO2C
β4
β4
α3
β6
α
OSp
β3
α2
N3
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
0.0
f1 (ppm)

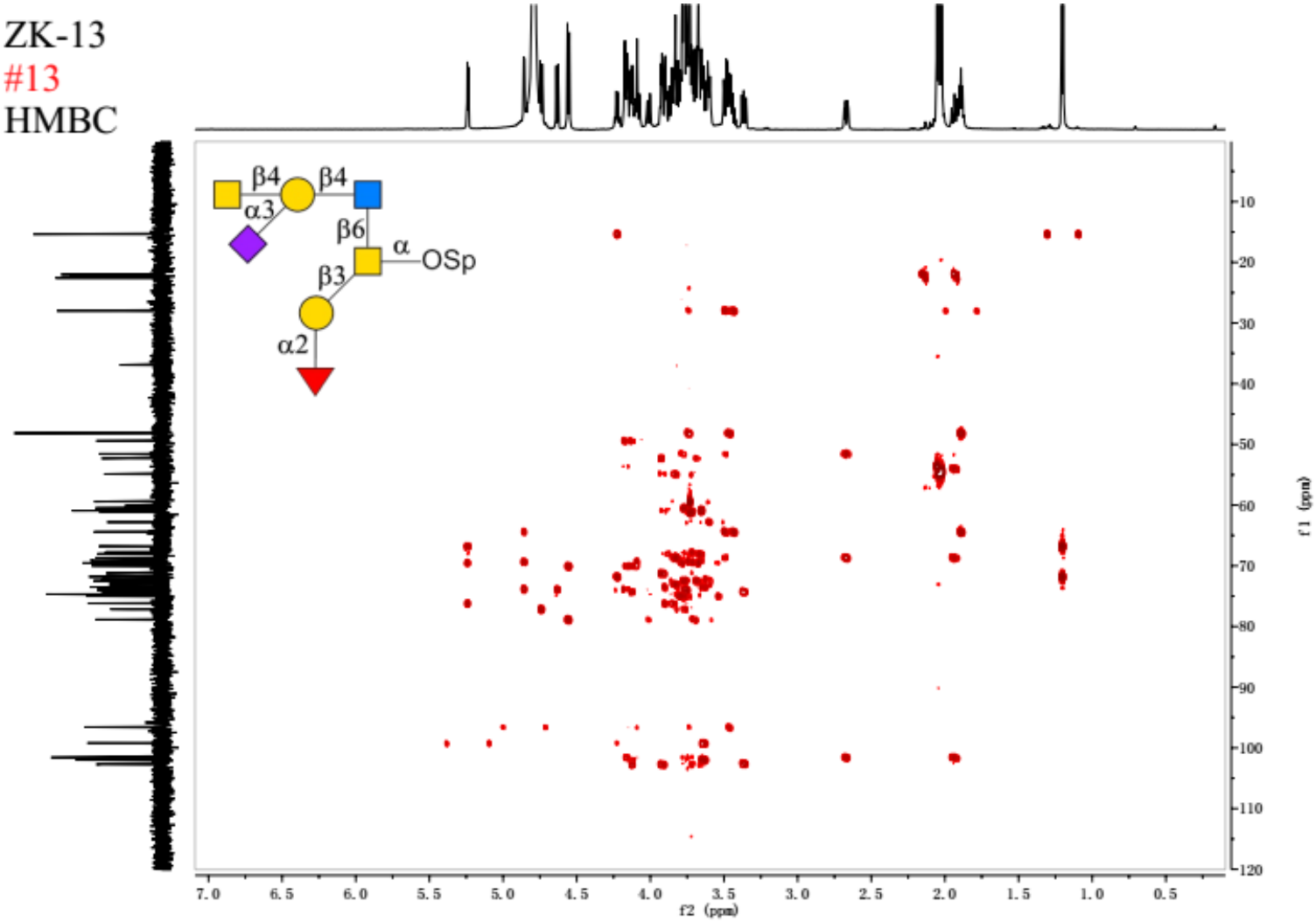
^{1}H NMR of 13


ZK-13
#13
β4
β4
α3
β6
α
OSp
β3
α2
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)

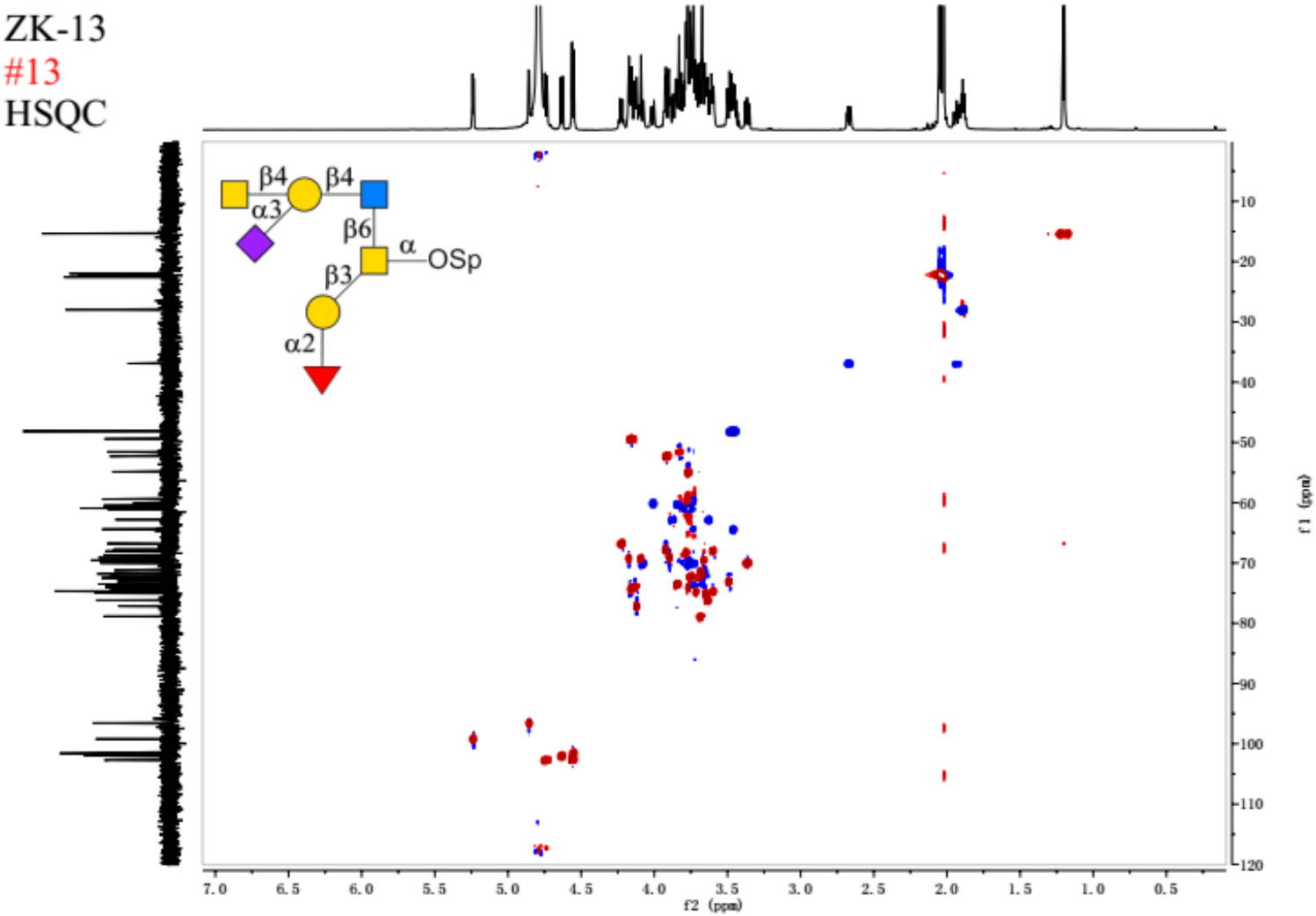
^{13}C NMR of 13



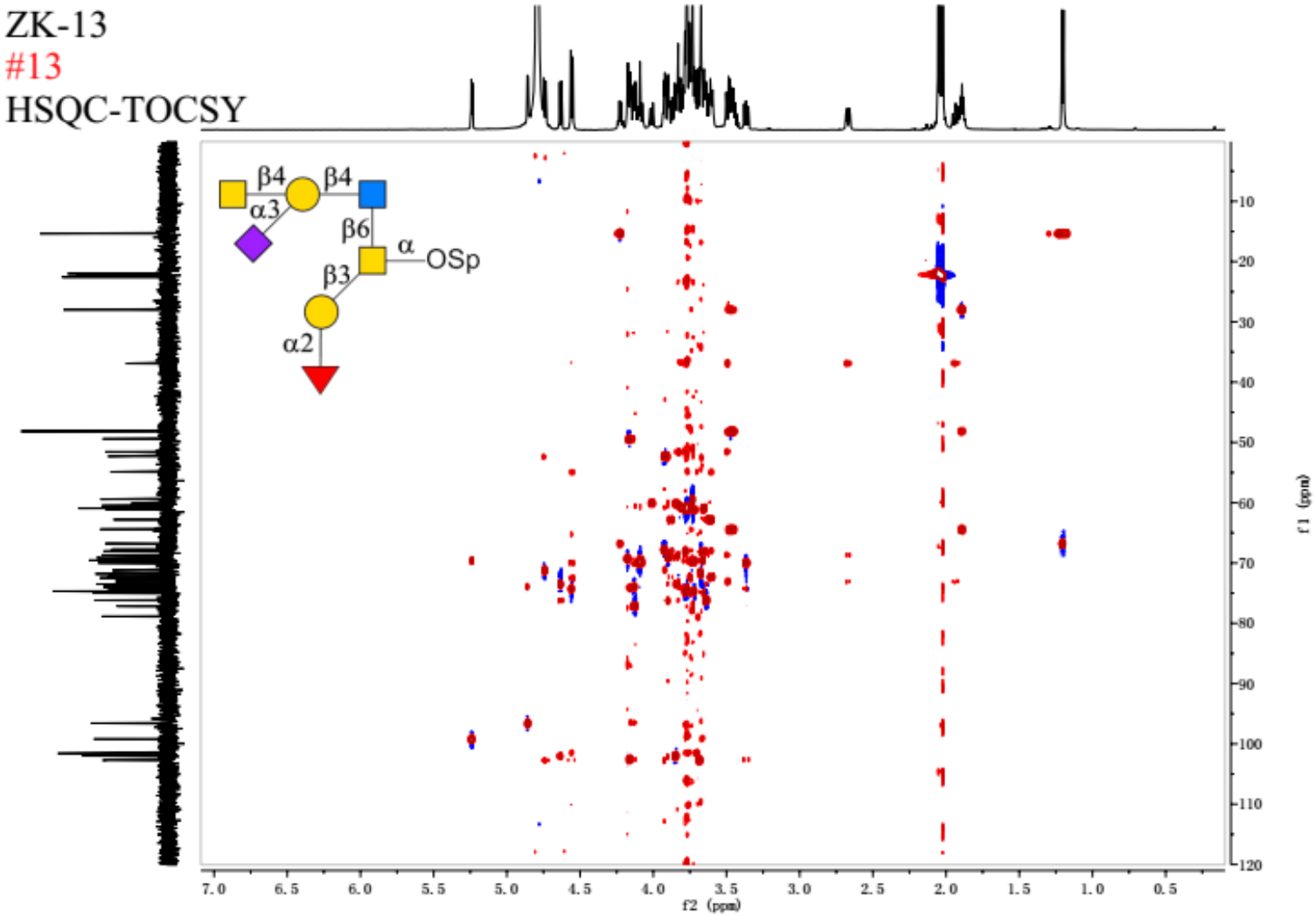
COSY spectra of 13



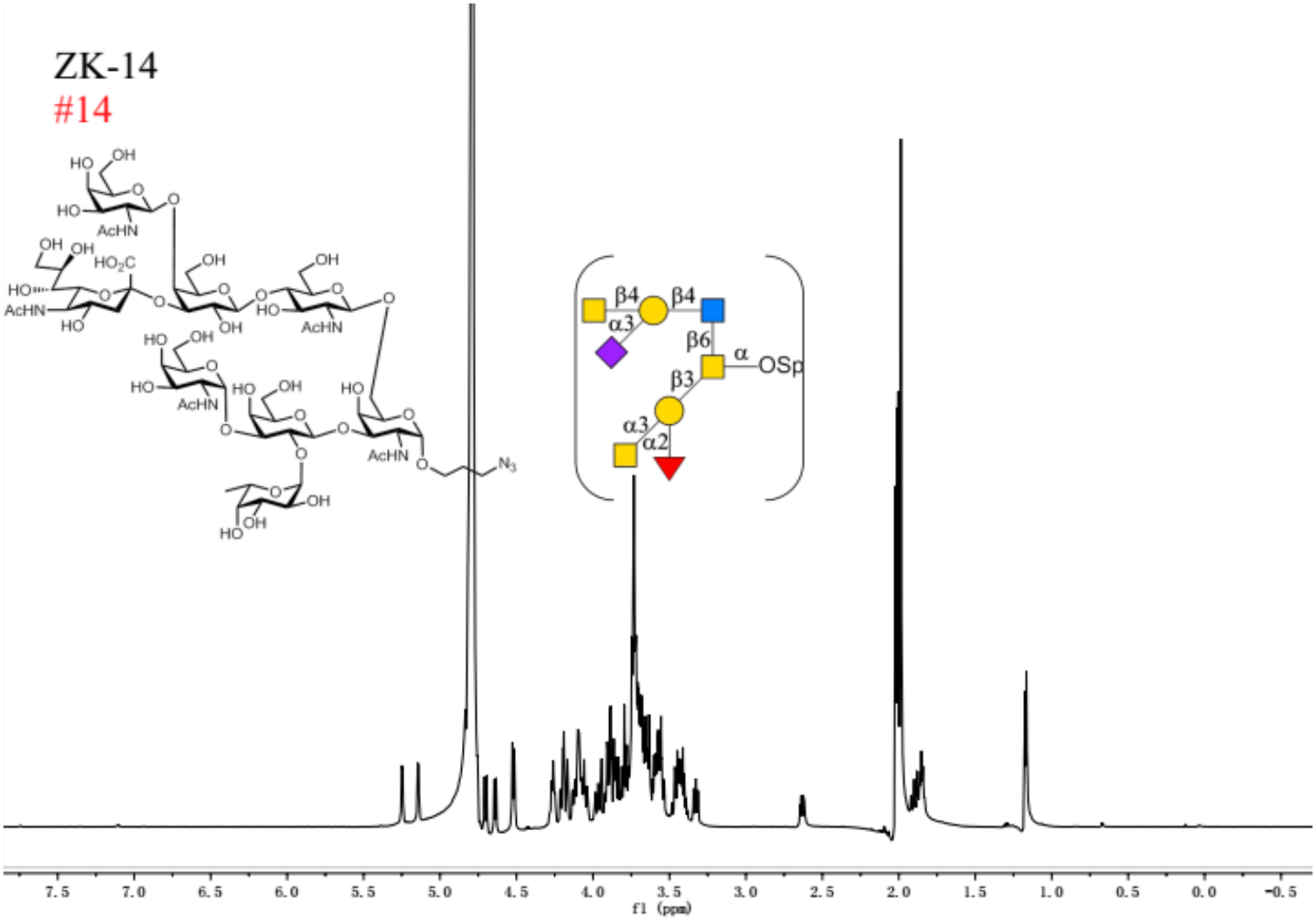
HMBC spectra of 13



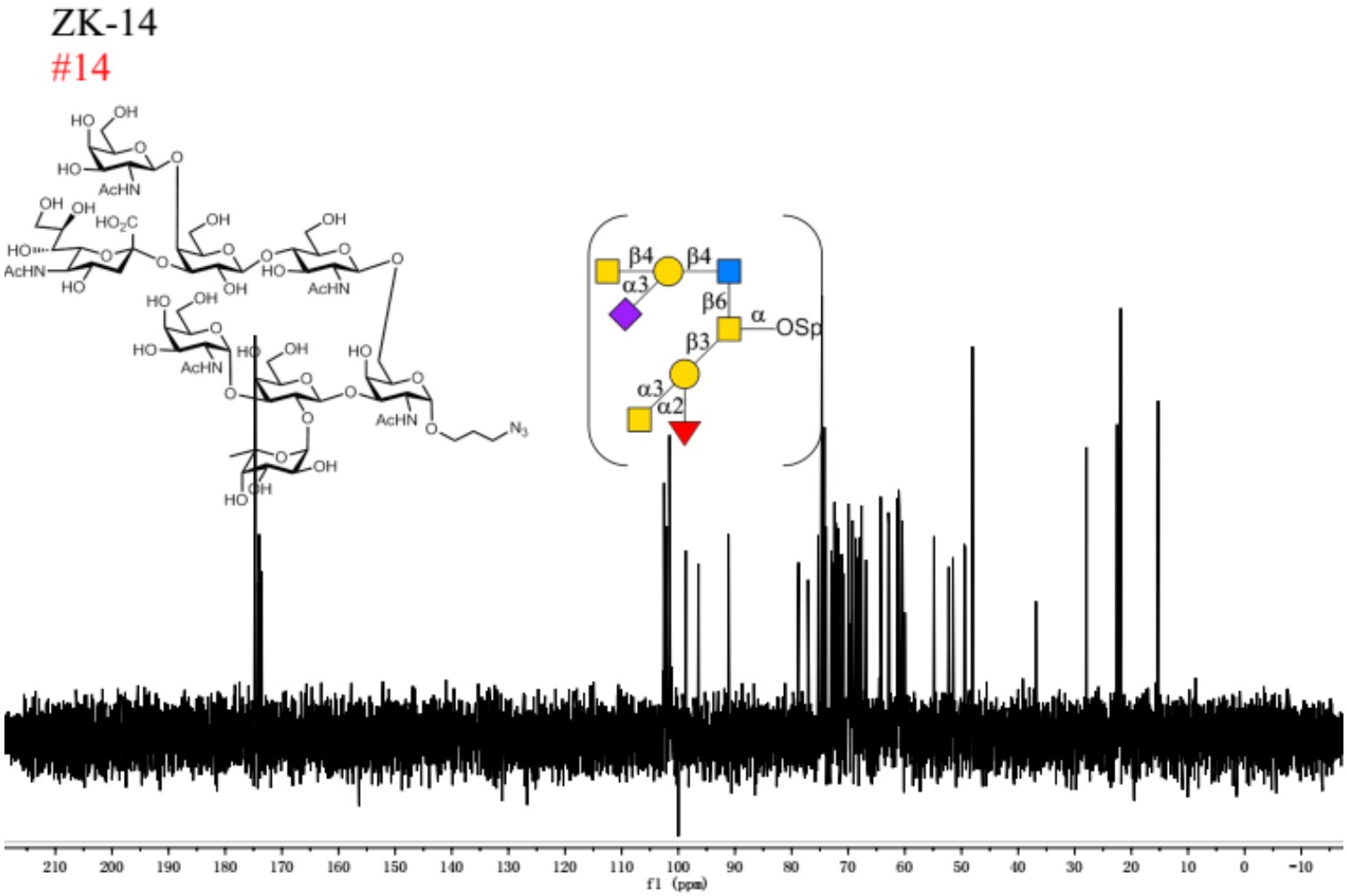
HSQC spectra of 13



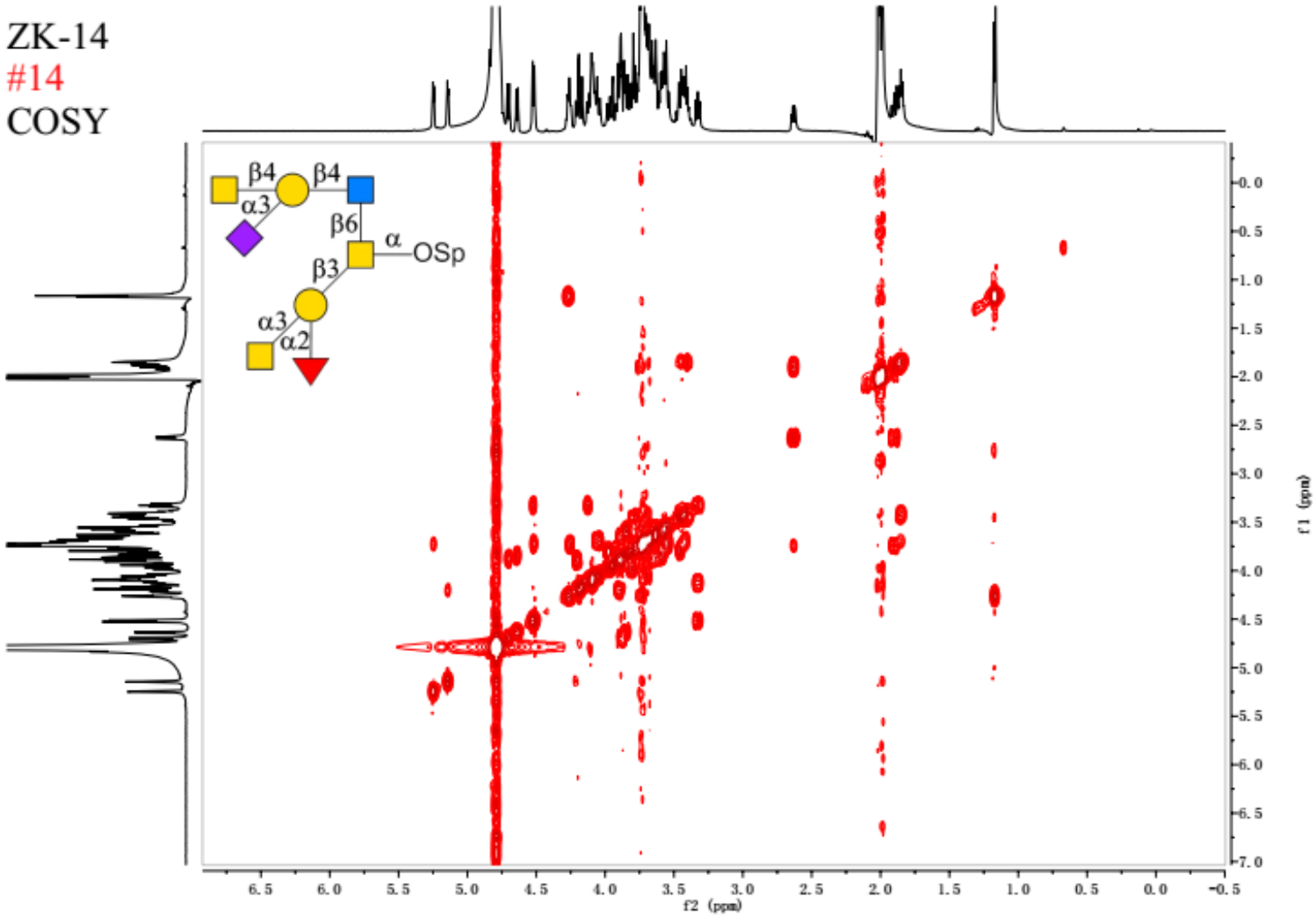
HSQC-TOCSY spectra of 13



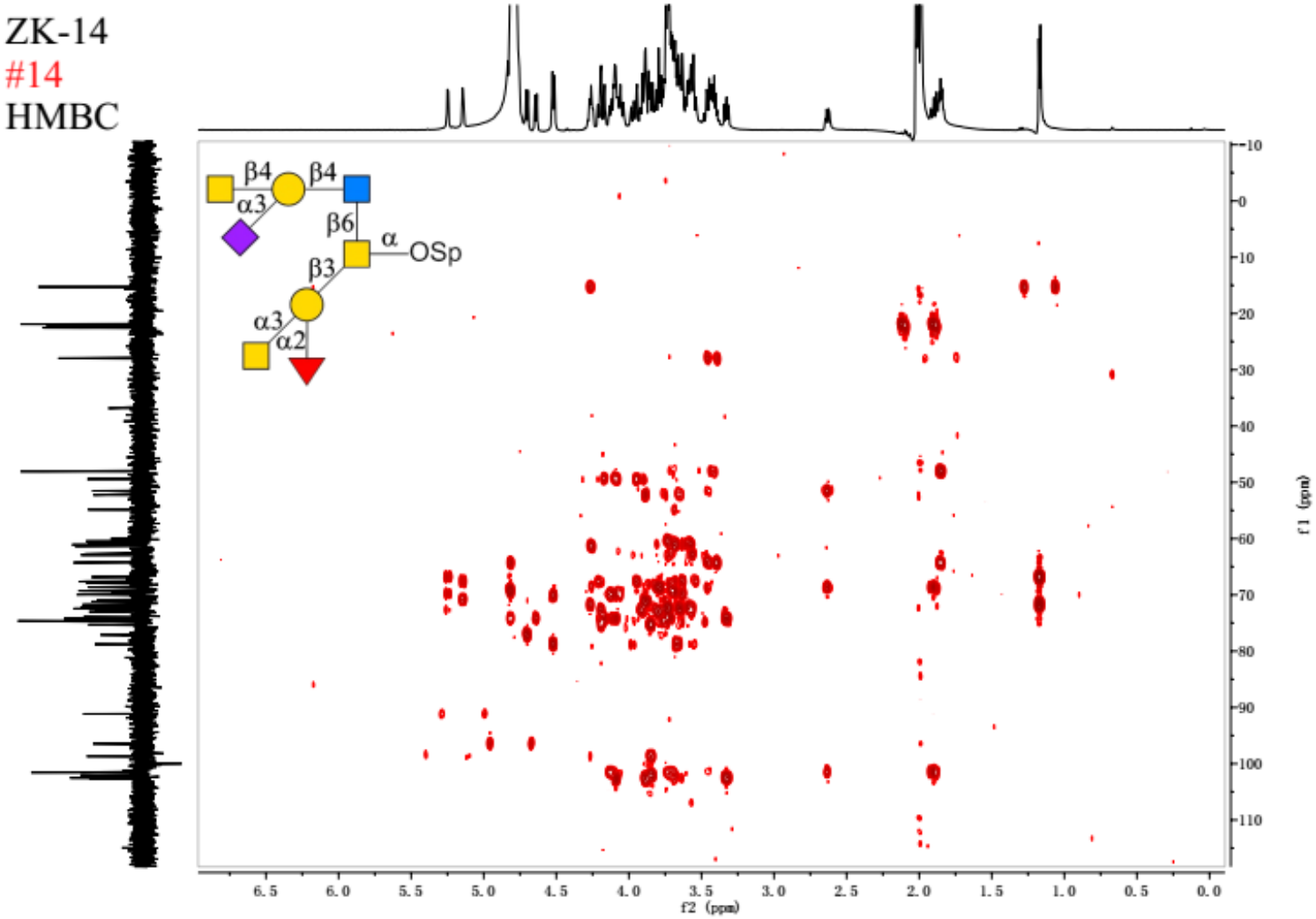
^{1}H NMR of 14


ZK-14
#14
β4
β4
α3
β6
α
OSp
β3
α3
α2
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)

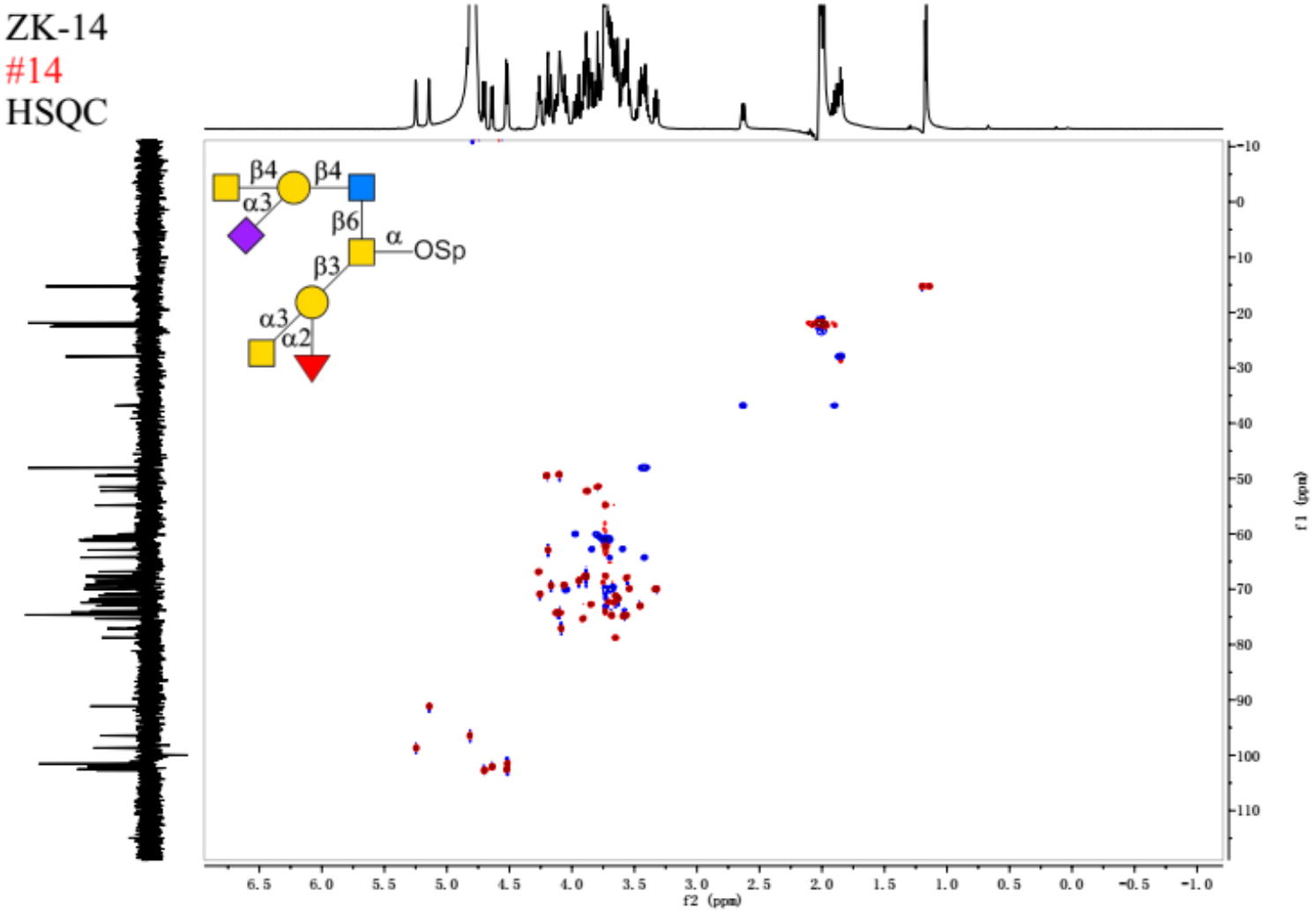
^{13}C NMR of 14



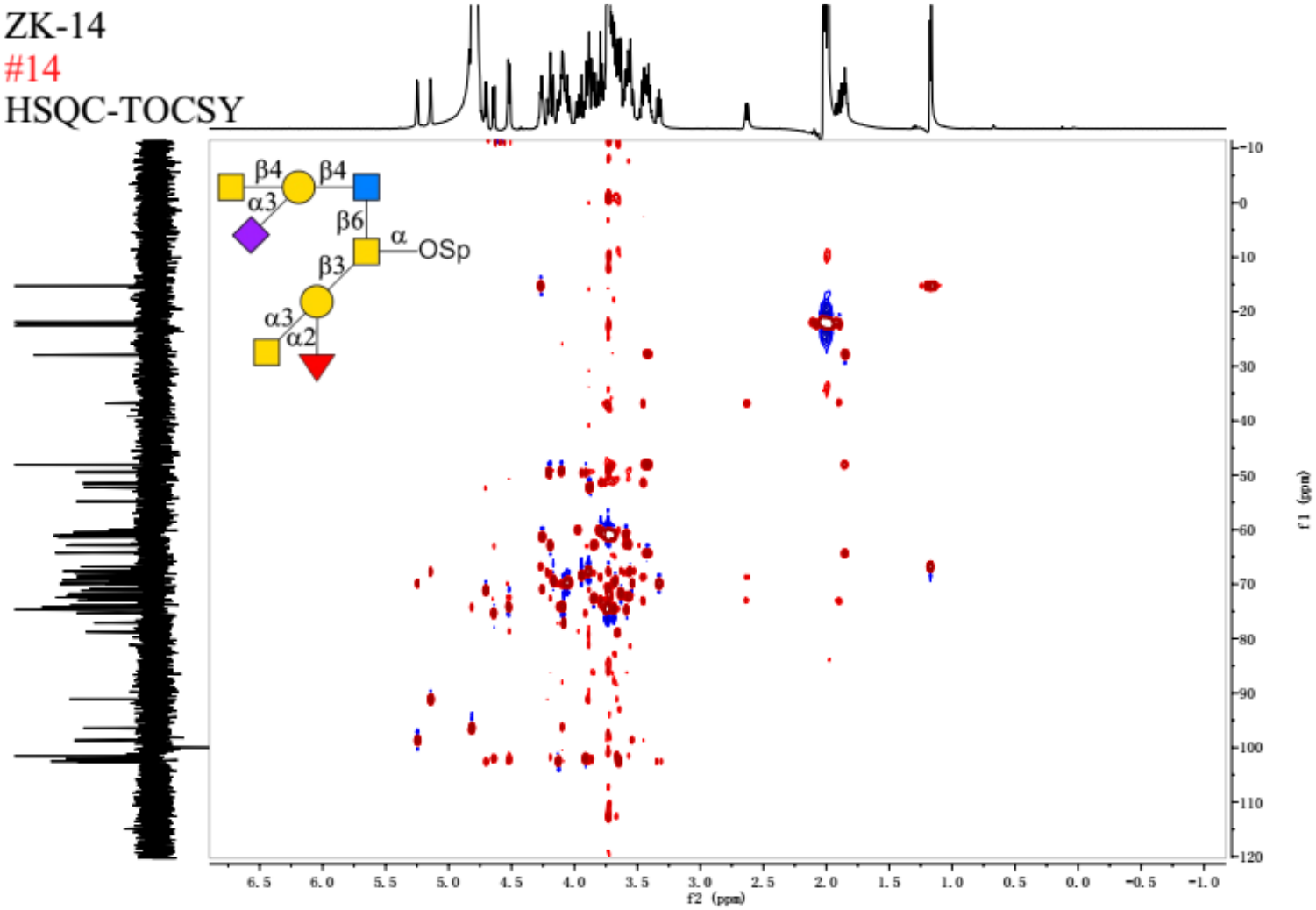
COSY spectra of 14



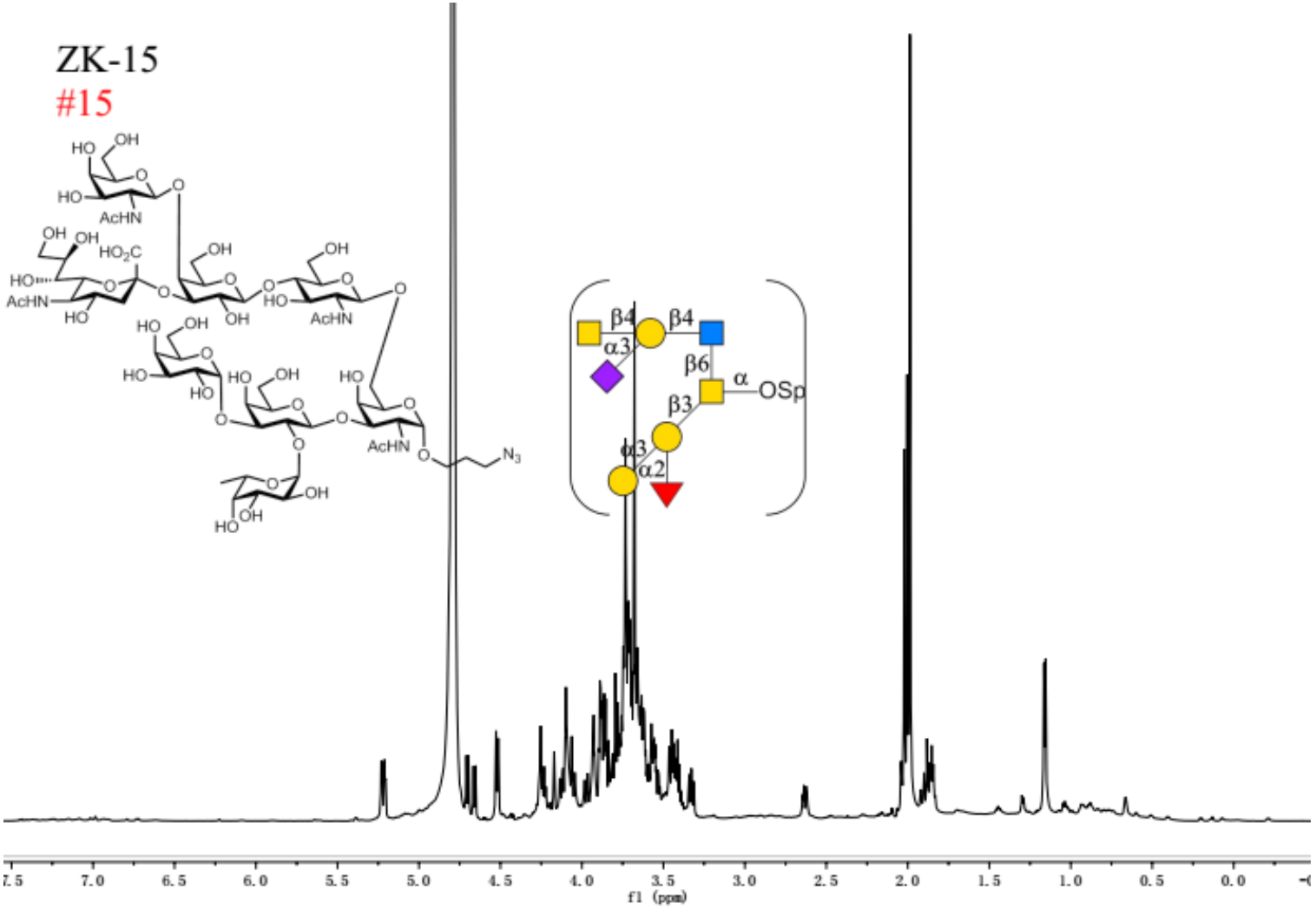
HMBC spectra of 14



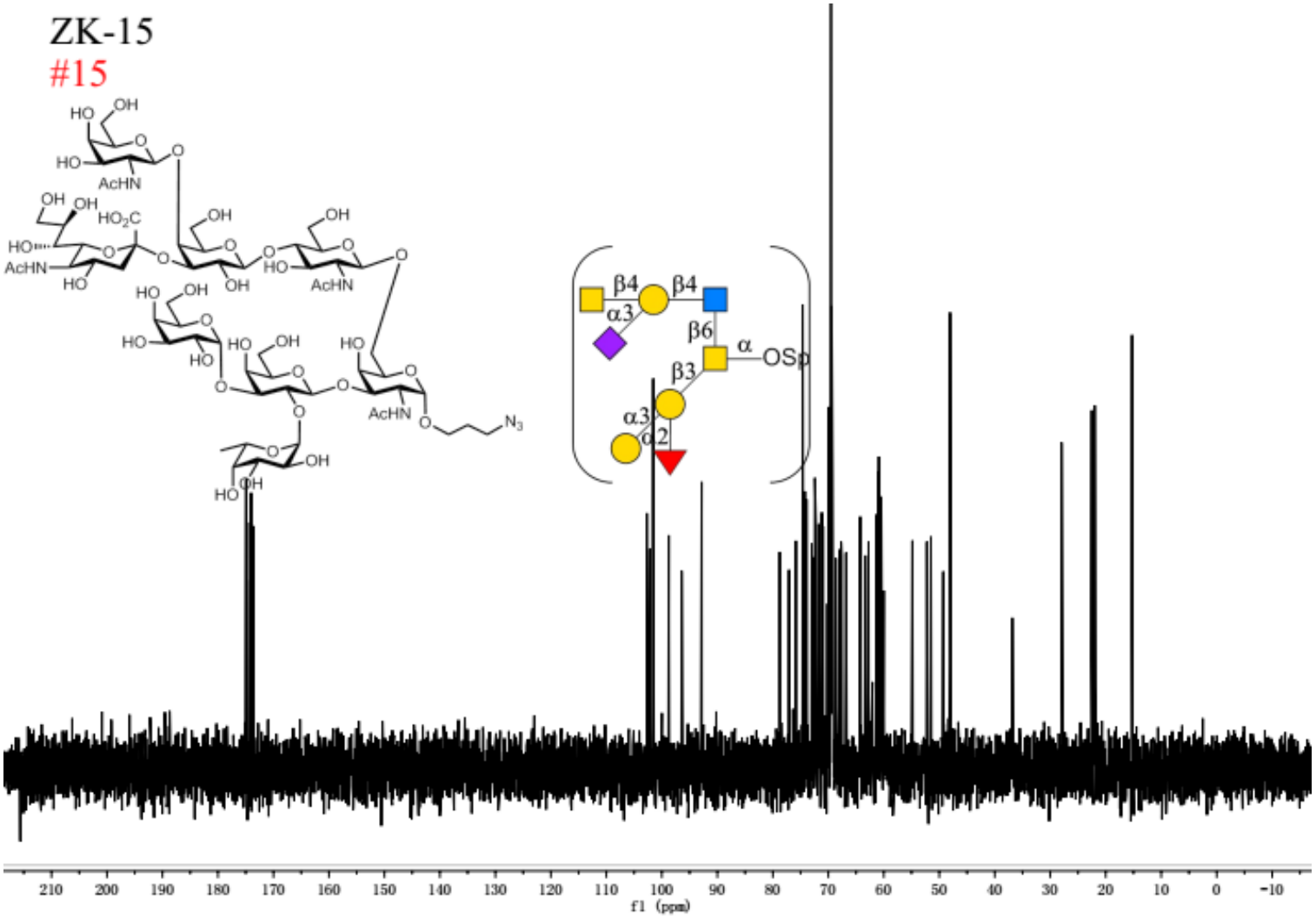
HSQC spectra of 14



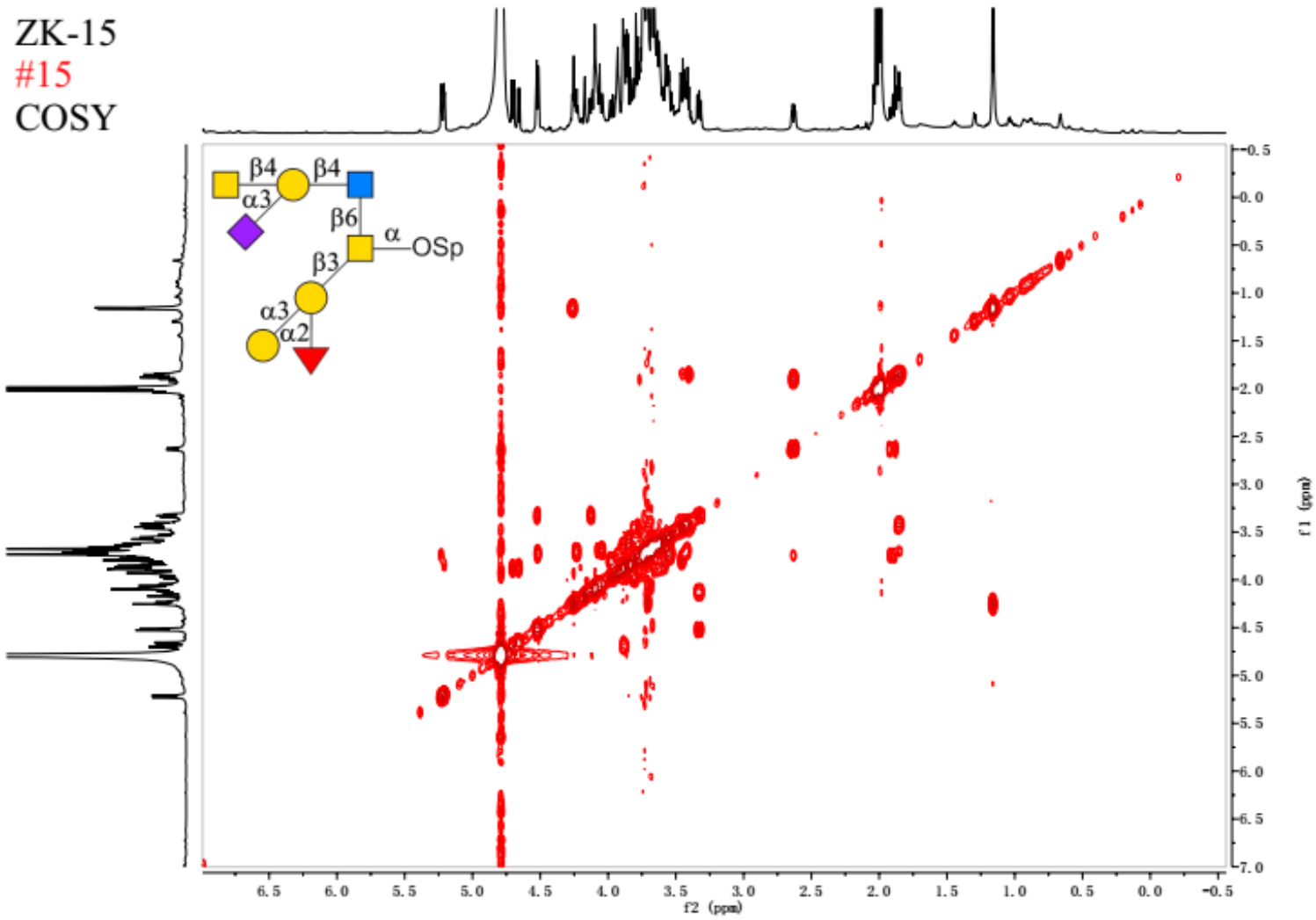
HSQC-TOCSY spectra of 14


ZK-15
#15
AcHN
HO2C
β4
α3
β4
β6
α
OSp
β3
α3
α2
N3
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
0.0
f1 (ppm)

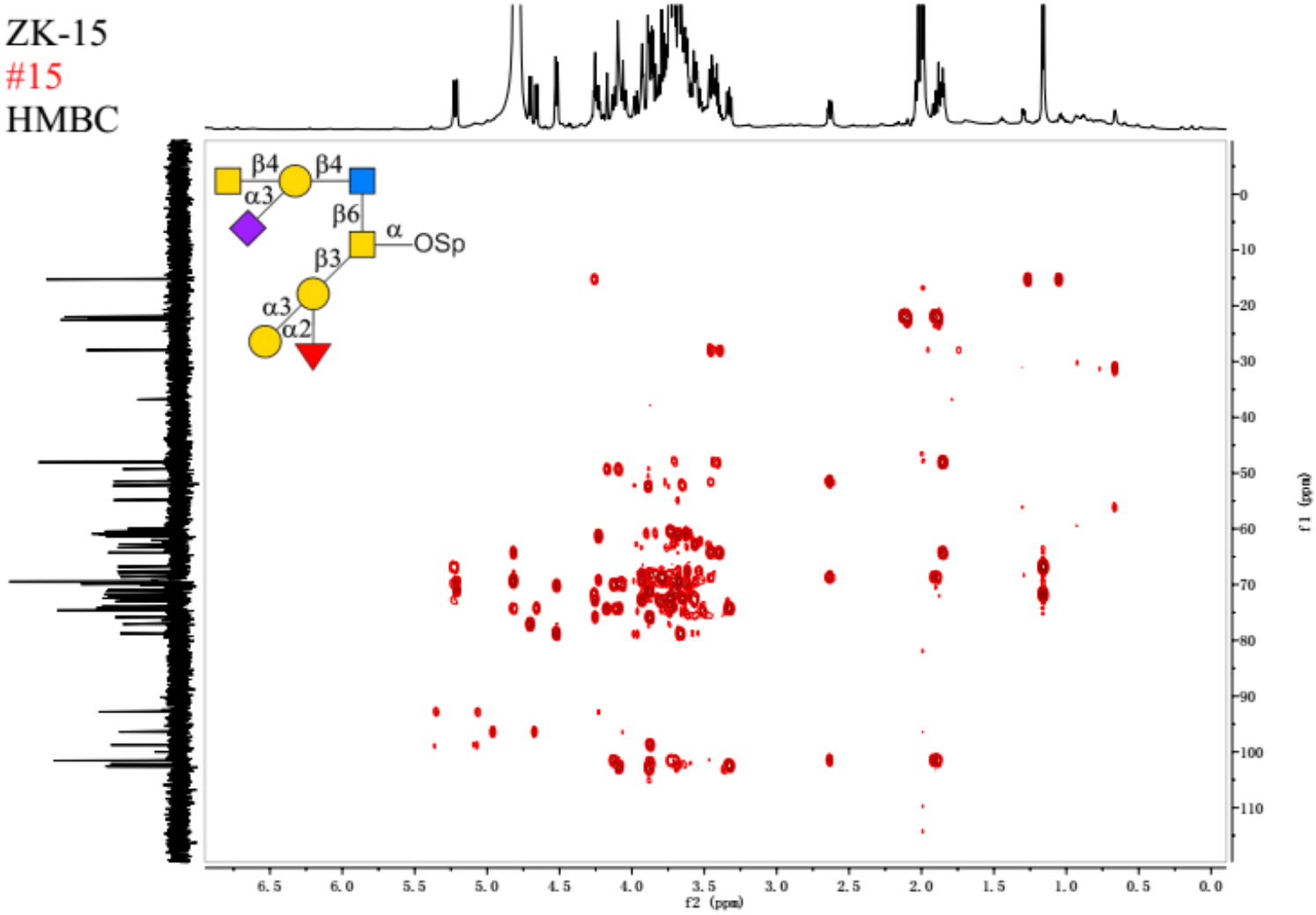
^{1}H NMR of 15


ZK-15
#15
β4
β4
α3
β6
α
OSp
β3
α3
α2
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)

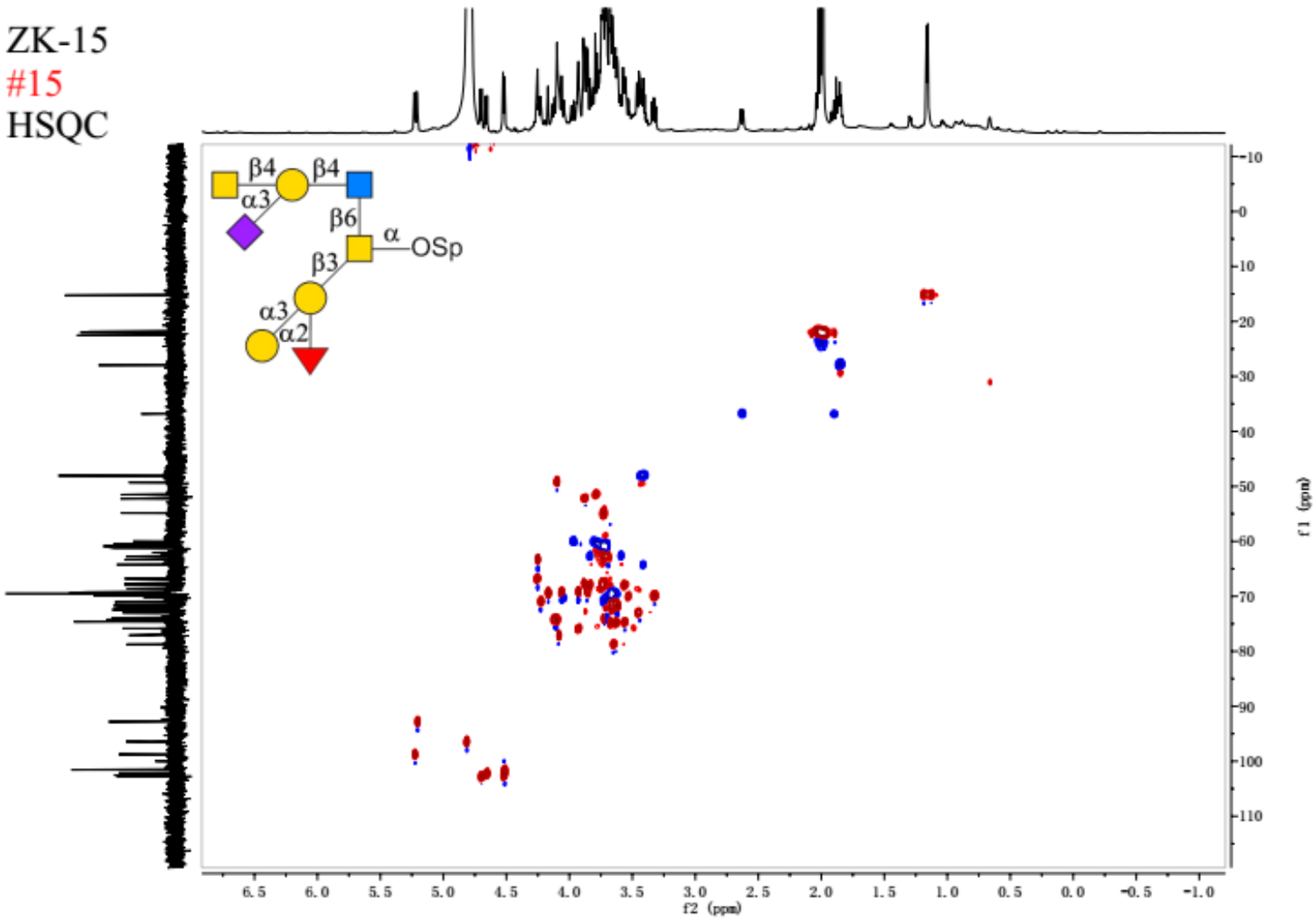
^{13}C NMR of 15



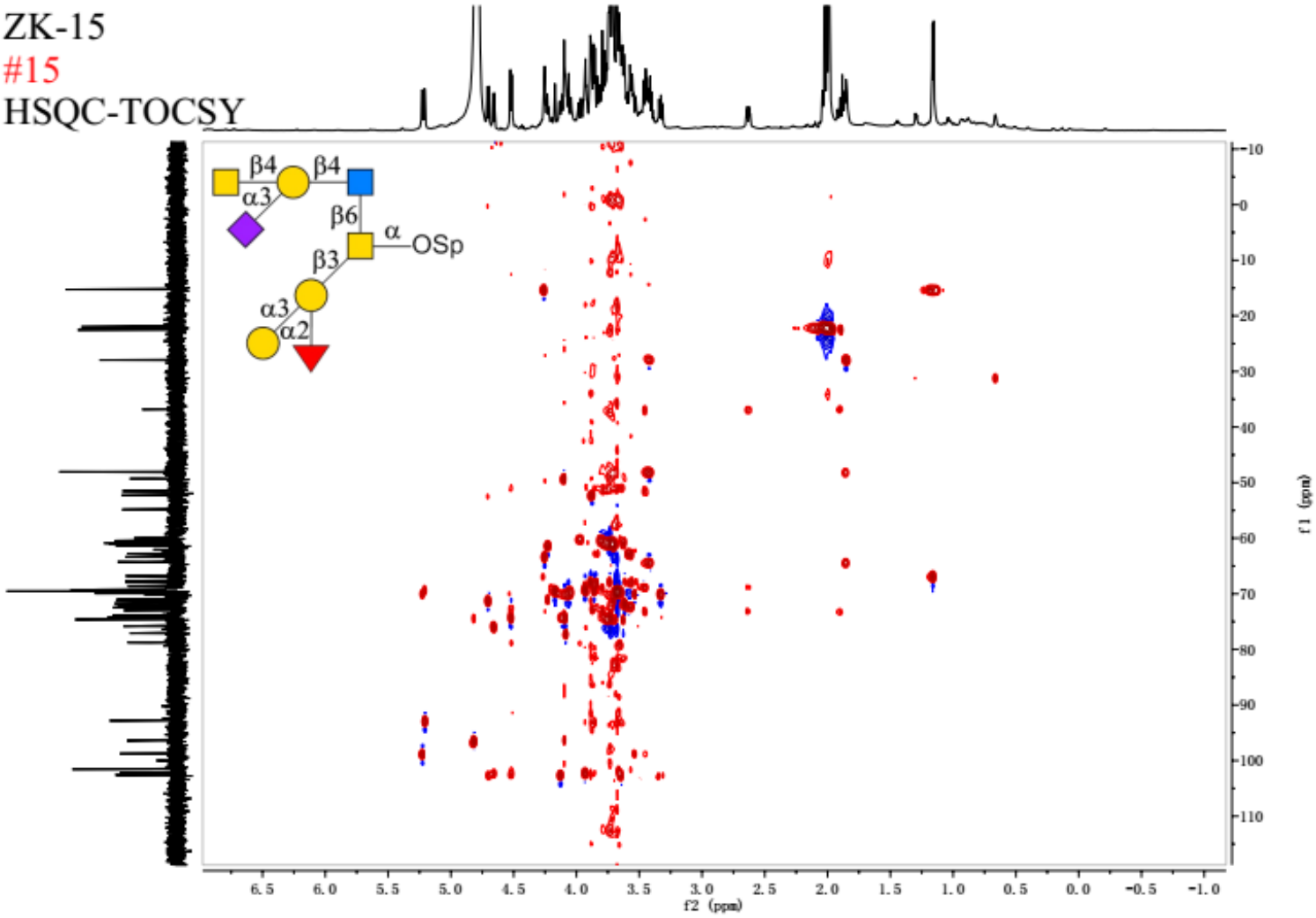
COSY spectra of 15



HMBC spectra of 15



HSQC spectra of 15



HSQC-TOCSY spectra of 15