

Electronic Supplementary Information

Synthetic and Immunological Studies of *N*-Acyl Modified *S*-Linked STn Derivatives as Anticancer Vaccine Candidates

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Supplementary Table 1. Carbohydrate loading levels of the conjugates.

KLH conjugates	Loading (%)	BSA conjugates	Loading (%)
natural STn-KLH	7.48	natural STn-BSA	13.62
13a	10.52	13b	19.52
14a	14.71	14b	29.17
15a	13.91	15b	24.72
16a	10.29	16b	16.83
31a	12.91	31b	16.81
32a	9.59	32b	21.04
33a	8.31	33b	15.97
34a	10.71	34b	14.69
48a	18.29	48b	23.01
49a	11.86	49b	17.88
50a	6.82	50b	15.35
51a	7.04	51b	12.32
53a	8.01	53b	20.20

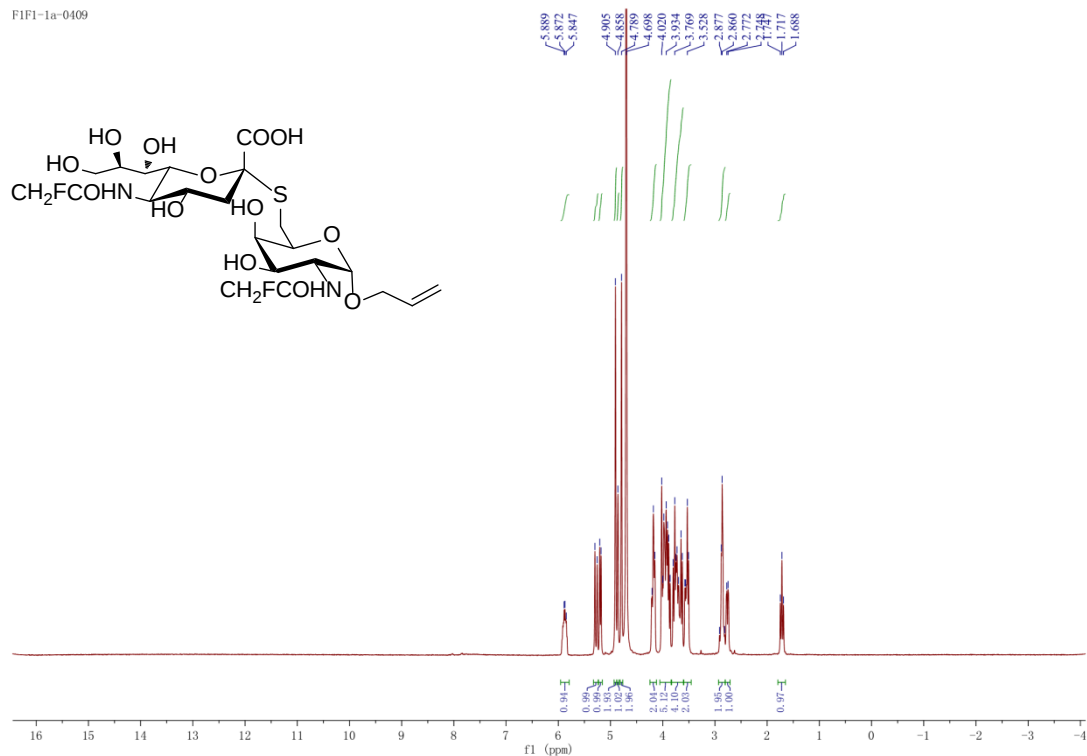
Supplementary Table 2. Immunological results after the 2nd immunization with the carbohydrate conjugates.

	anti-natural STn IgG titer	anti-natural STn IgM titer
natural STn-KLH	137,980	4685
13a	18,621	1321
14a	4006	1925
15a	3787	1856
16a	2830	1541
31a	13,897	1439
32a	18,257	3099
33a	1533	4364
34a	2730	1349
48a	<1000	1593
49a	<1000	2046
50a	9030	1566
51a	1996	<1000
53a	4101	1045

Supplementary Table 3. Immunological results after the 3rd immunization with the carbohydrate conjugates.

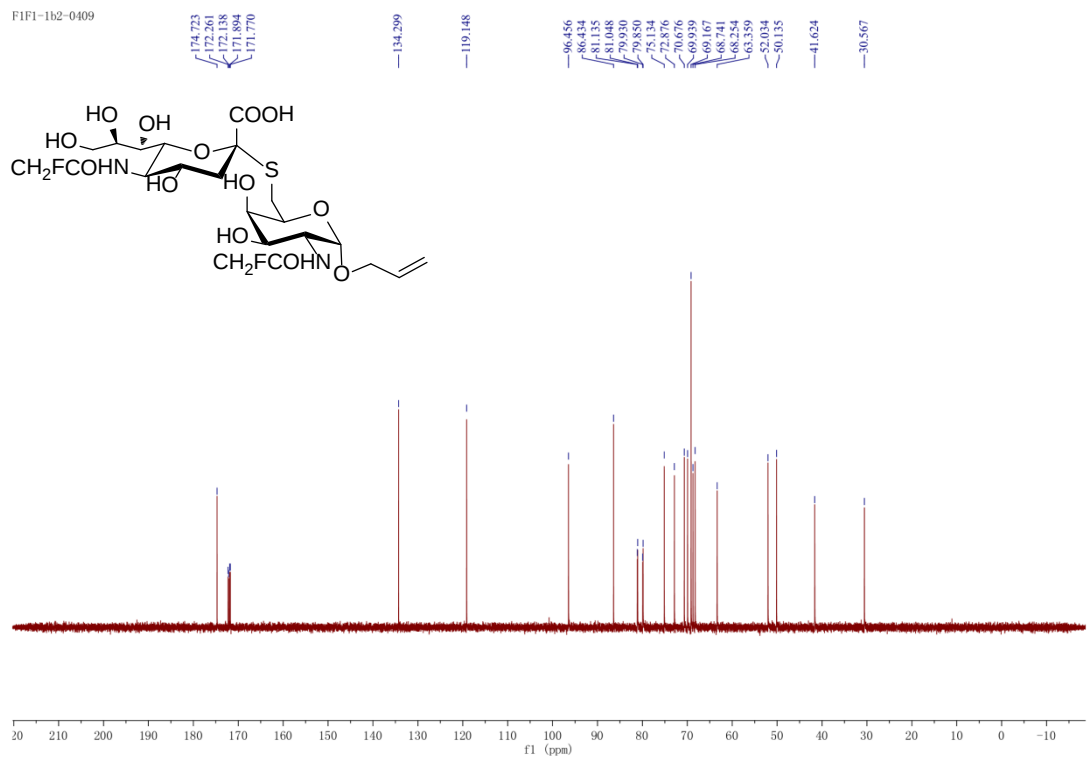
	anti-natural STn IgG titer	anti-natural STn IgM titer
natural STn-KLH	1,930,847	18,717
13a	54,600	4000
14a	24,600	2183
15a	18,862	1675
16a	27,261	3017
31a	43,191	1559
32a	52,320	4075
33a	19,695	15,144
34a	8747	3674
48a	27,819	5131
49a	27,028	1870
50a	33,456	4298
51a	19,500	2146
53a	7894	5465

FIF1-1a-0409

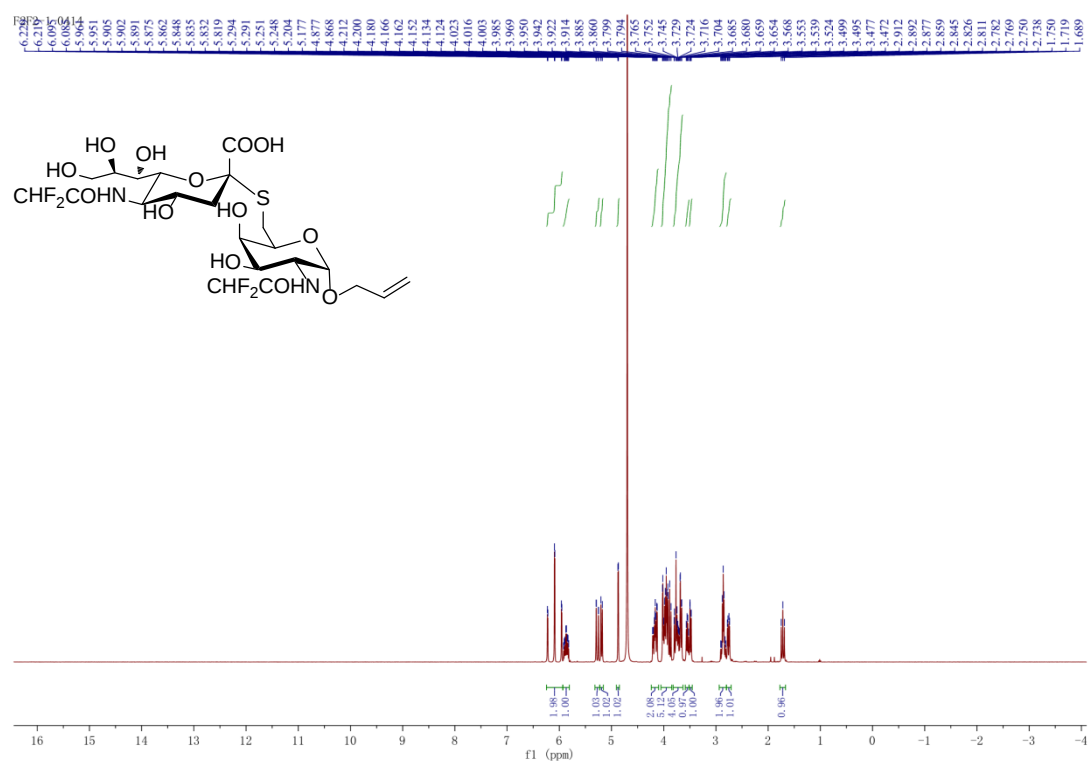


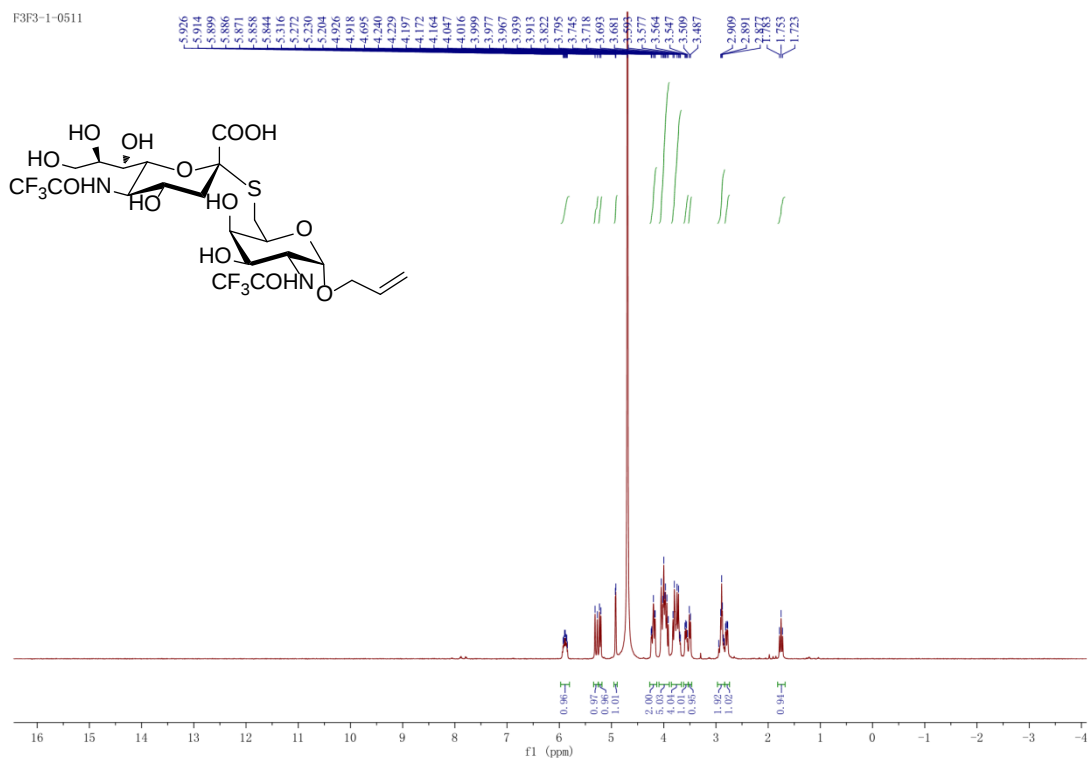
¹H NMR spectrum of compound 4 (400 MHz, D₂O)

FIF1-1b2-0409

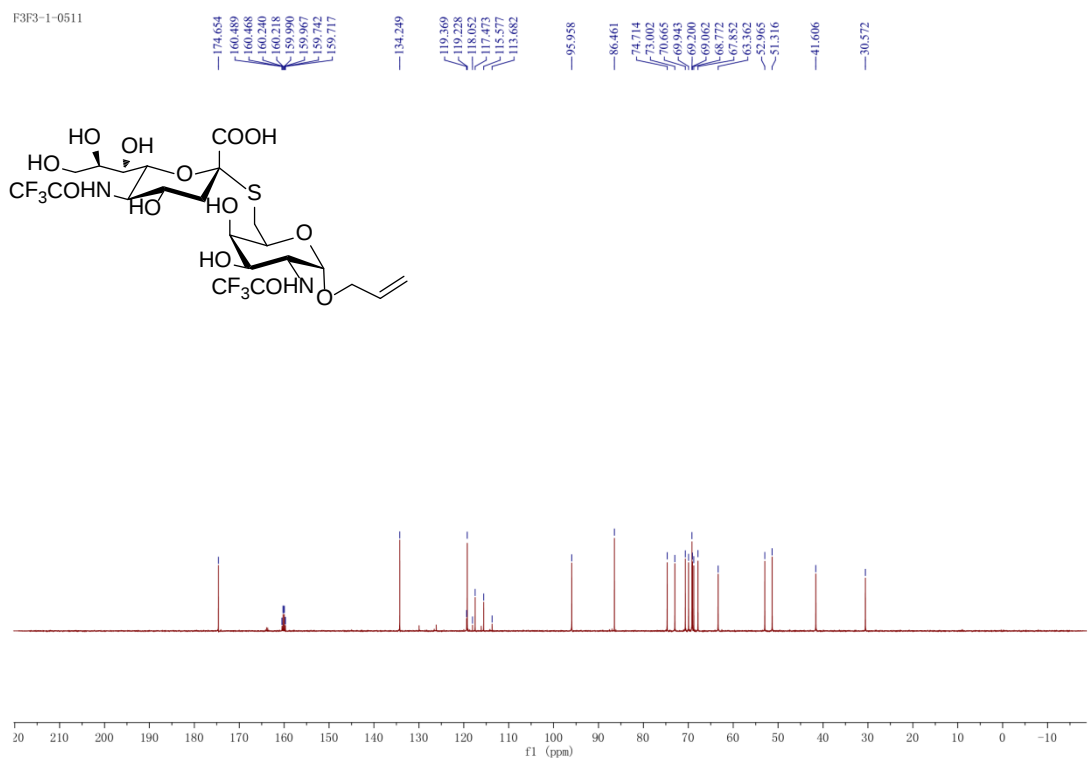


¹³C NMR spectrum of compound 4 (150 MHz, D₂O)





^1H NMR spectrum of compound 6 (400 MHz, D_2O)



^{13}C NMR spectrum of compound 6 (150 MHz, D_2O)

PP-1-0508

179.121
178.631
174.010

133.707

118.367

95.894

85.777

74.895
72.020
69.962
69.261
68.488
68.453
67.696
67.143
49.684

41.048

29.866
29.266
29.164

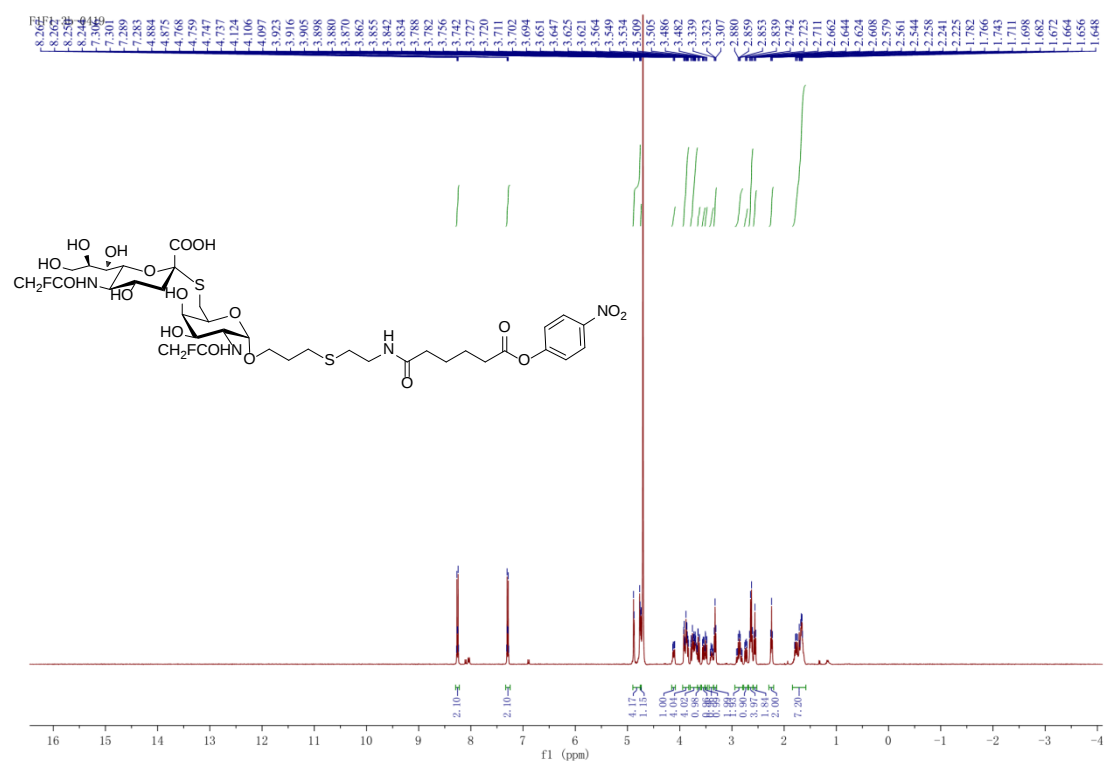
9.604
9.539

Chemical structure of compound 10b: CCCC(=O)N[C@@H]1[C@H](O)[C@@H](O)[C@H](O)[C@@H](O)[C@H]1S[C@@H]2[C@H](O)[C@@H](O)[C@H](O)[C@@H](O)[C@H]2OCC=C

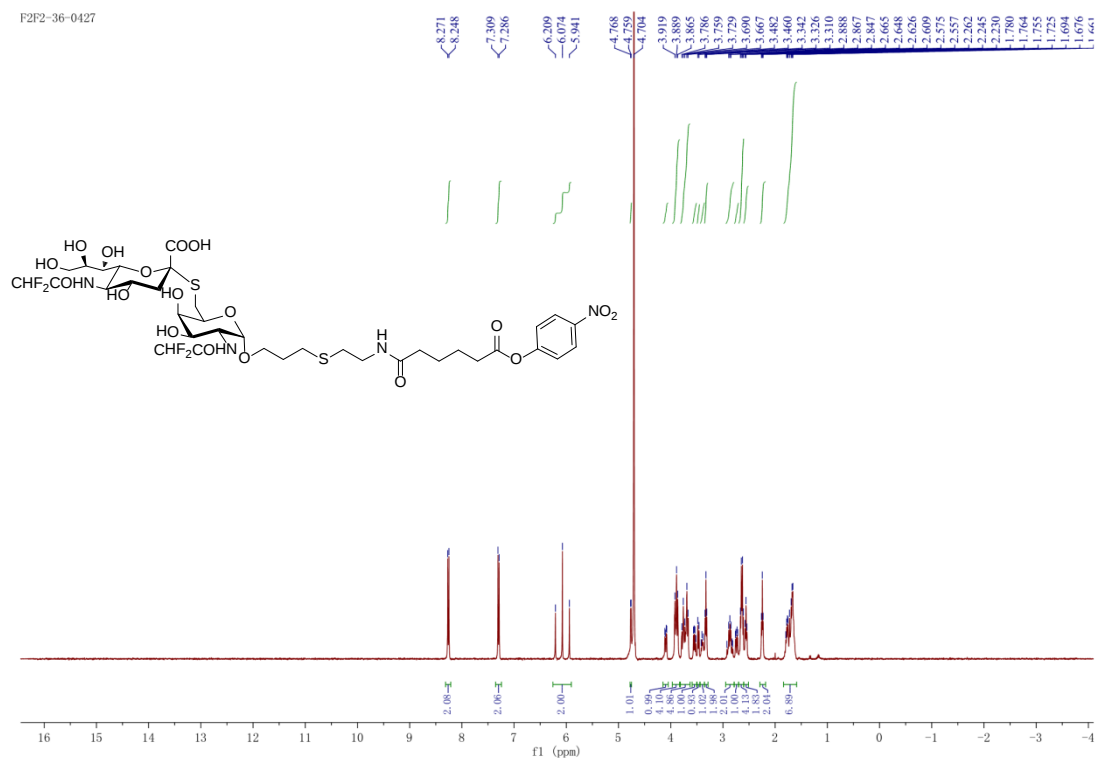
¹³C NMR spectrum (ppm):

- 179.121
- 178.631
- 174.010
- 133.707
- 118.367
- 95.894
- 85.777
- 74.895
- 72.020
- 69.962
- 69.261
- 68.488
- 68.453
- 67.696
- 67.143
- 49.684
- 41.048
- 29.866
- 29.266
- 29.164
- 9.604
- 9.539

S8

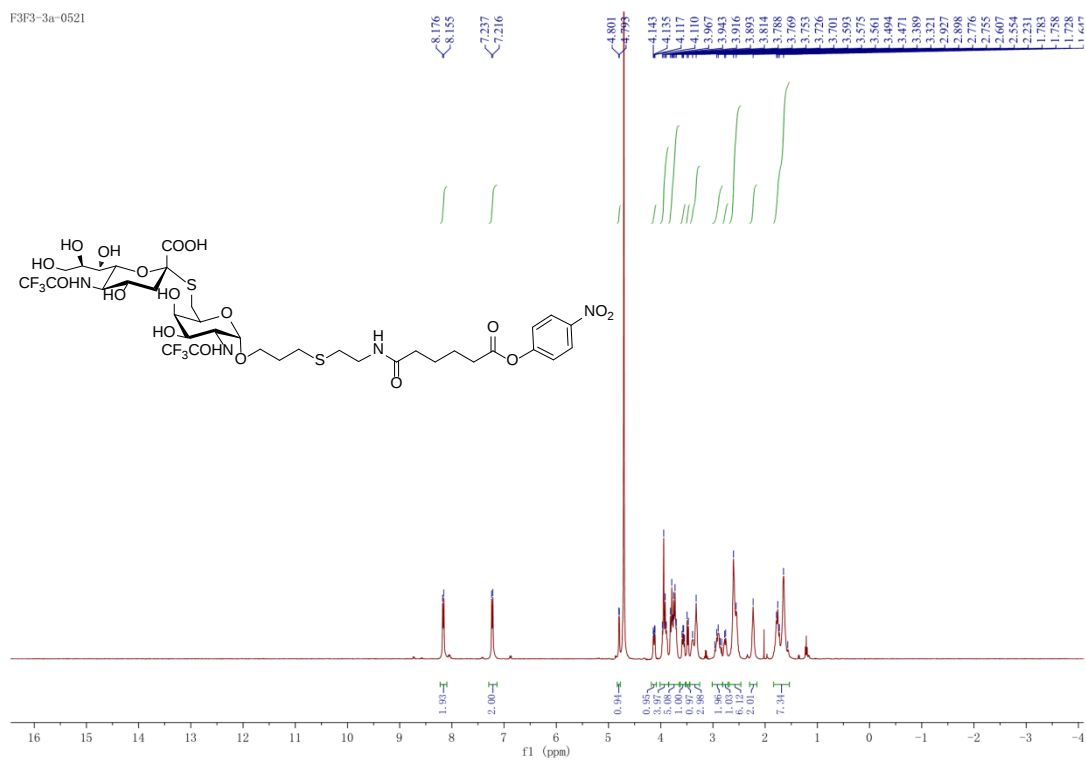


¹H NMR spectrum of compound 9 (400 MHz, D₂O)



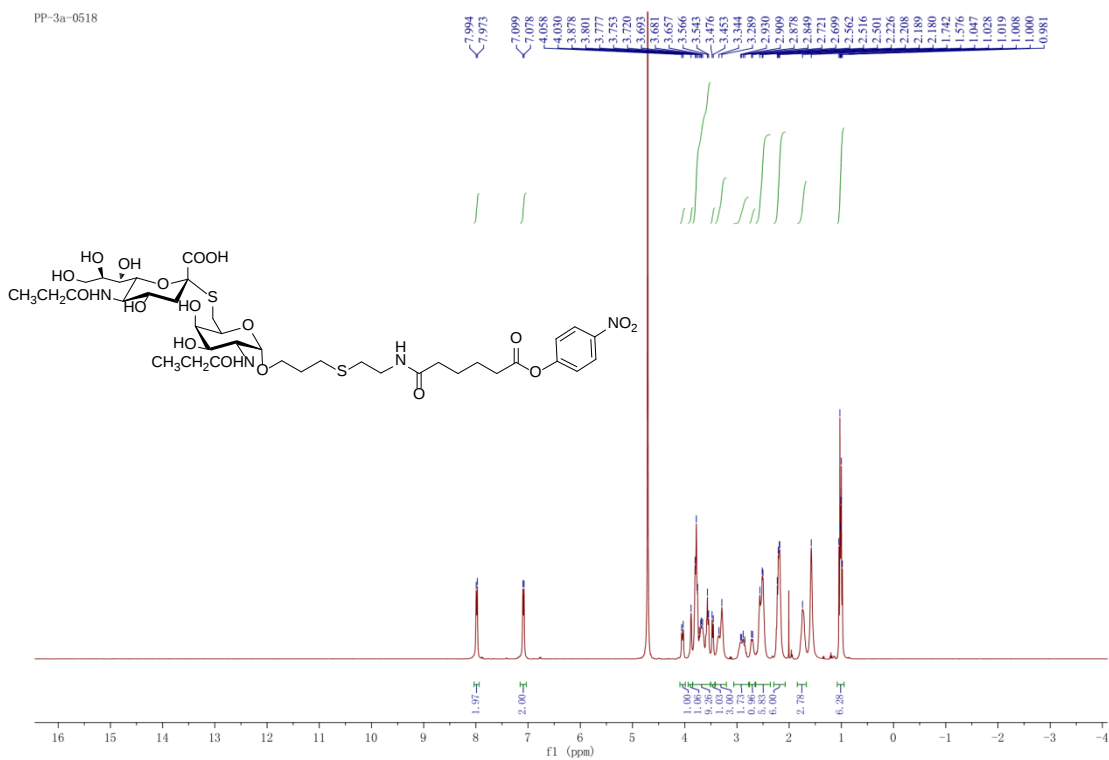
¹H NMR spectrum of compound 10 (400 MHz, D₂O)

F3F3-3a-0521



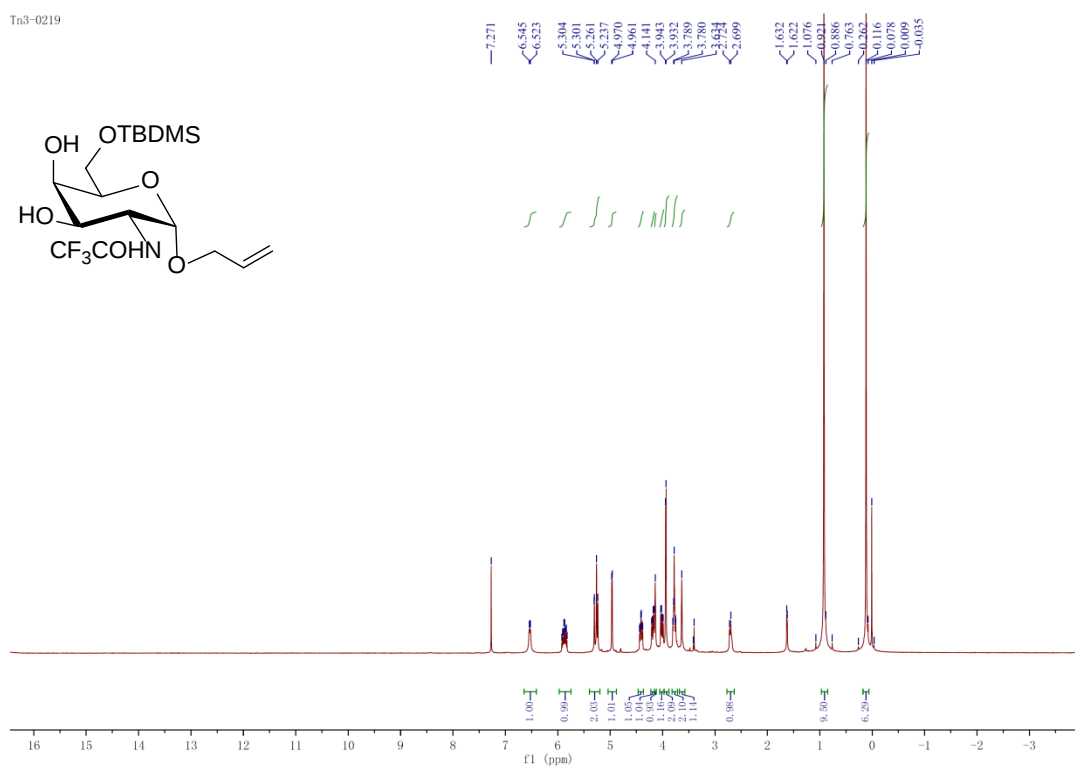
¹H NMR spectrum of compound **11** (400 MHz, D₂O)

PP-3a-0518



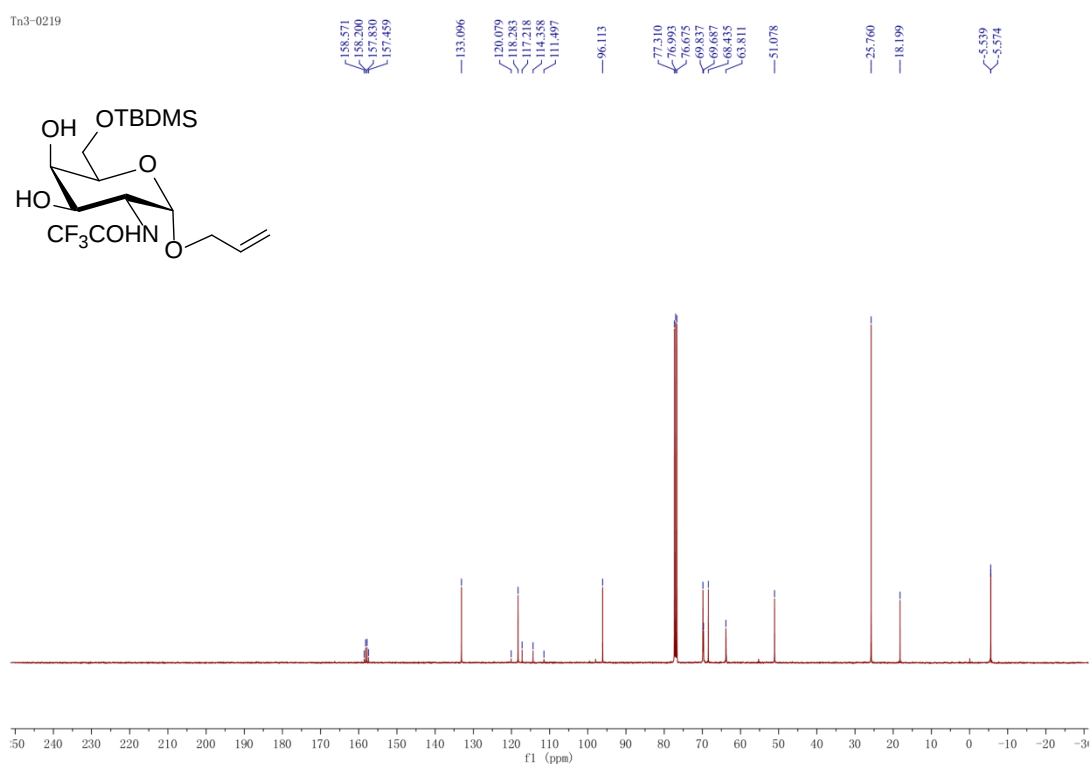
¹H NMR spectrum of compound **12** (400 MHz, D₂O)

Tn3-0219



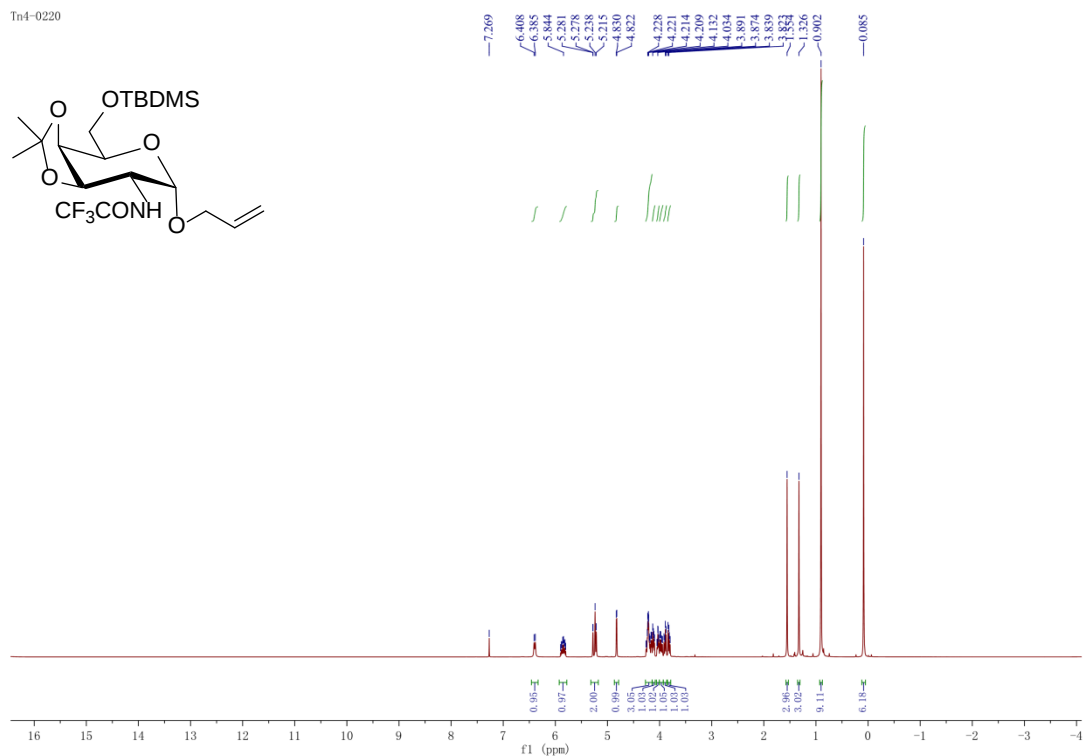
¹H NMR spectrum of compound **18** (400 MHz, CDCl₃)

Tn3-0219



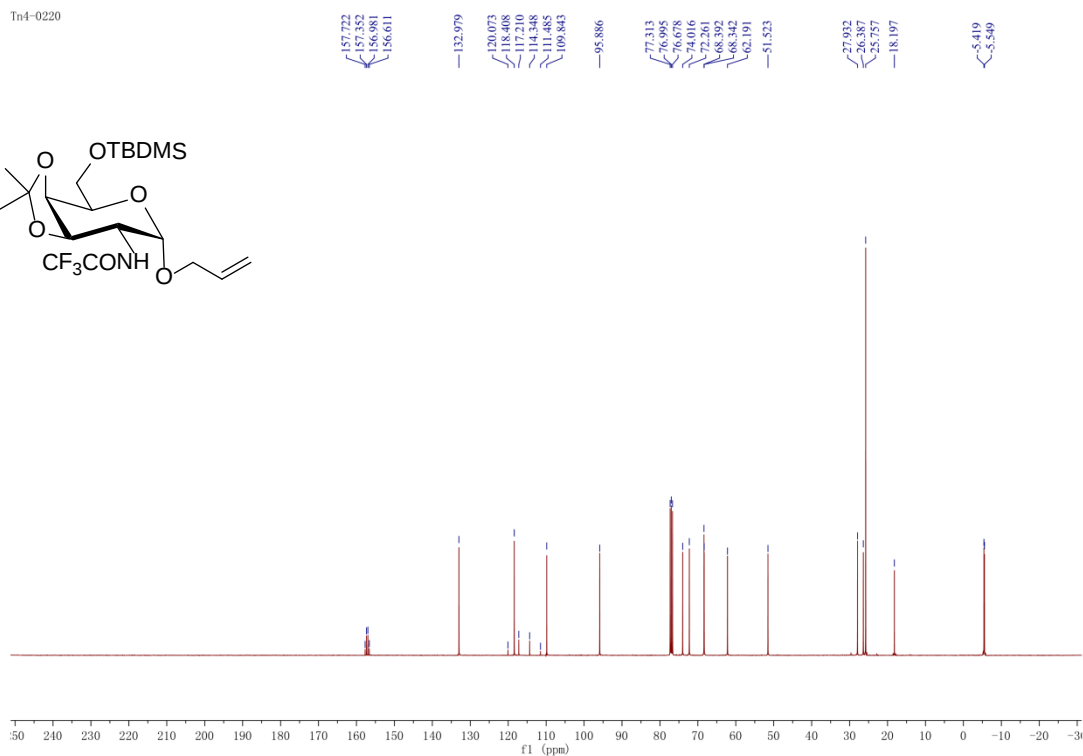
¹³C NMR spectrum of compound **18** (100 MHz, CDCl₃)

Tn4-0220



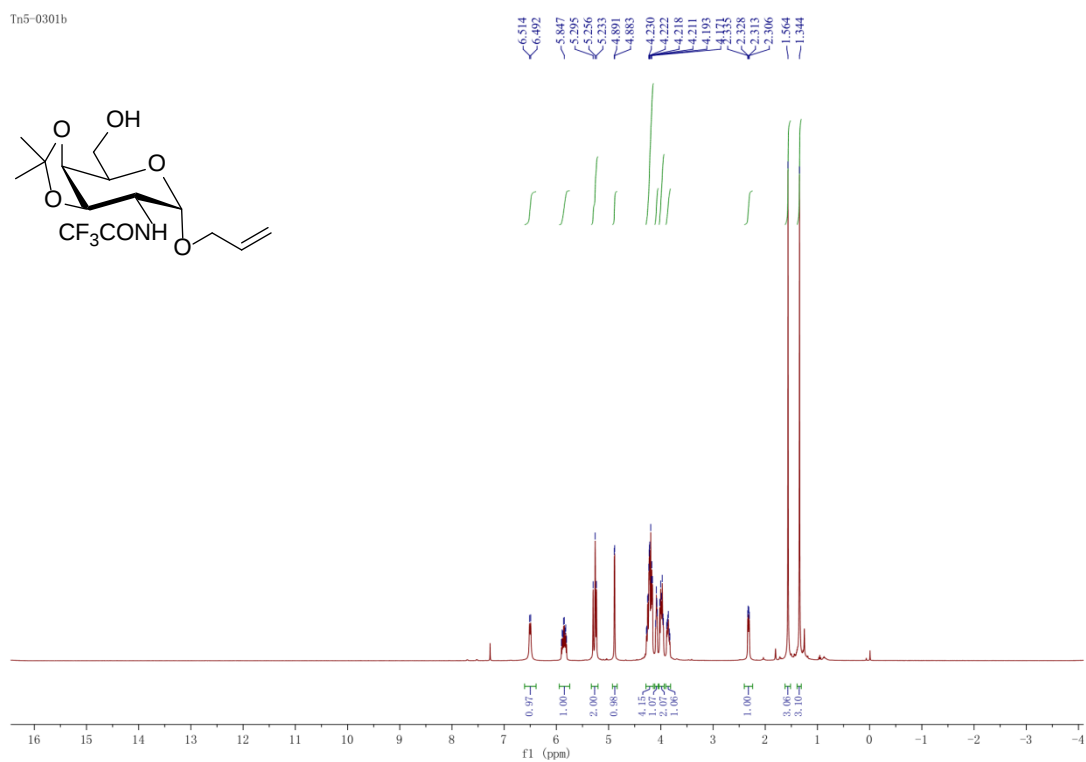
¹H NMR spectrum of compound **19** (400 MHz, CDCl₃)

Tn4-0220



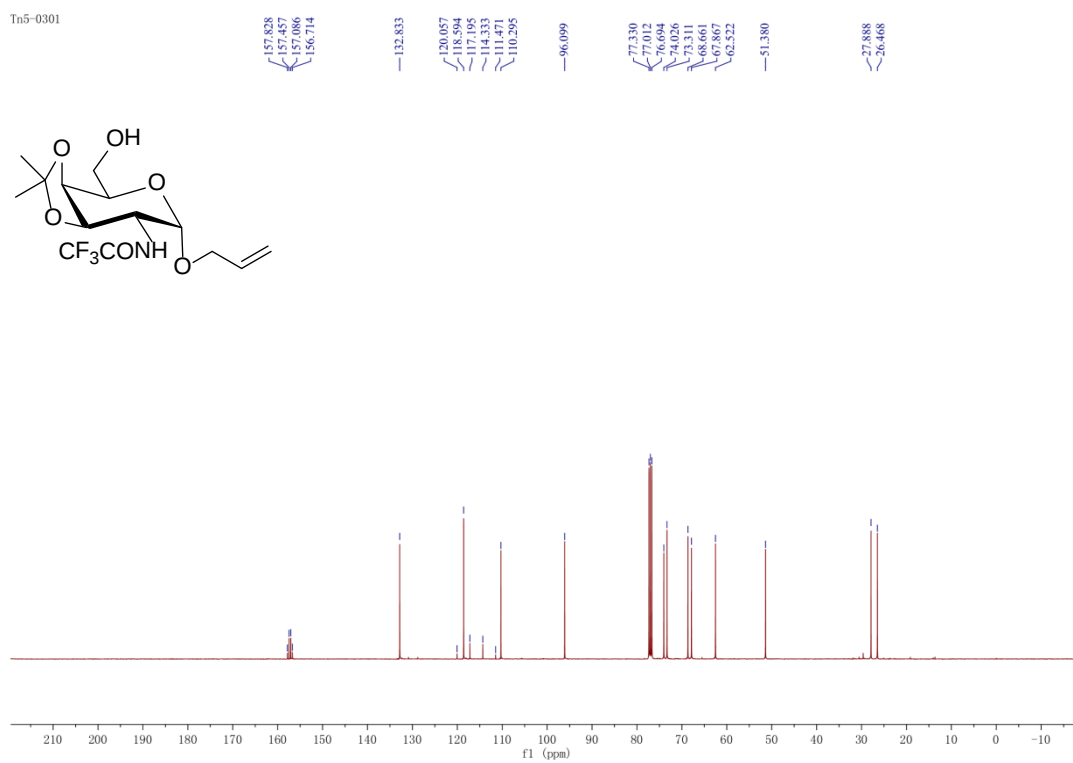
¹³C NMR spectrum of compound **19** (100 MHz, CDCl₃)

Tn5-0301b

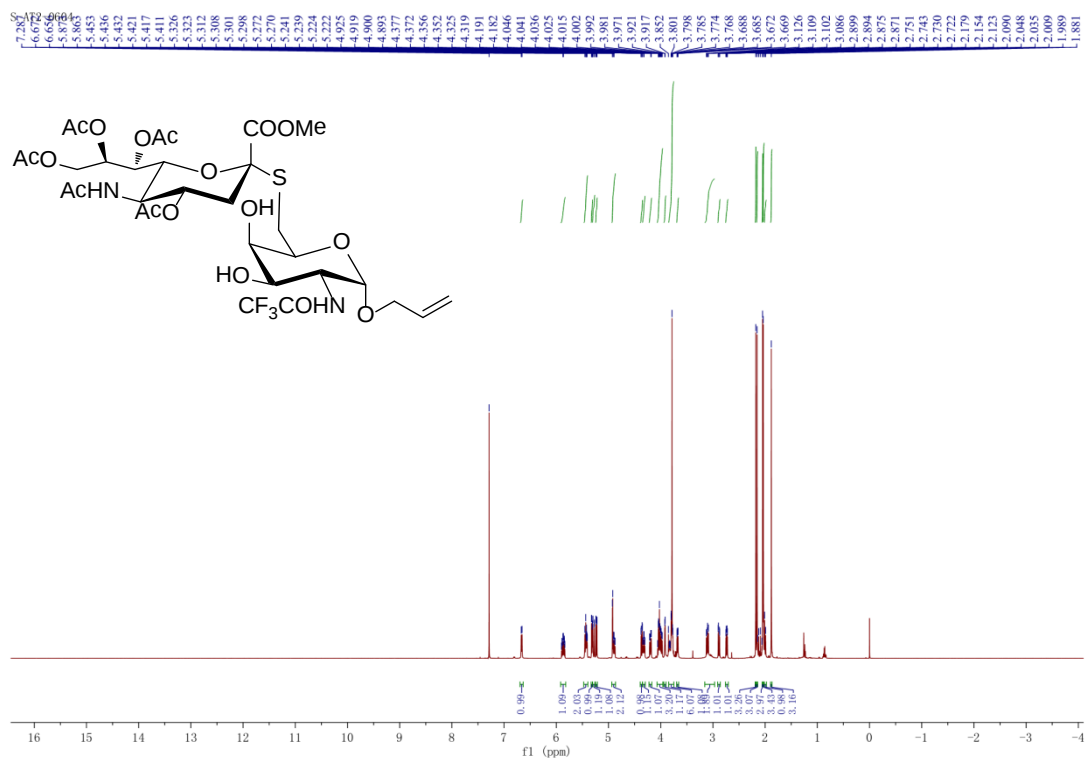


¹H NMR spectrum of compound **20** (400 MHz, CDCl₃)

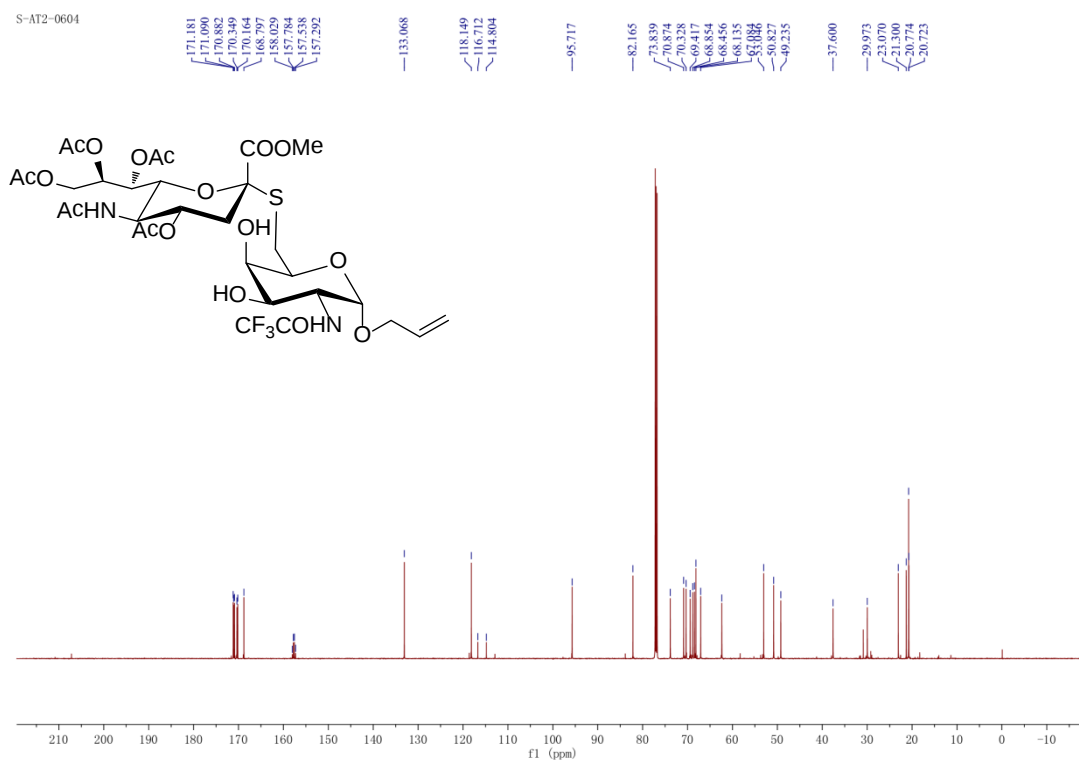
Tn5-0301



¹³C NMR spectrum of compound **20** (100 MHz, CDCl₃)

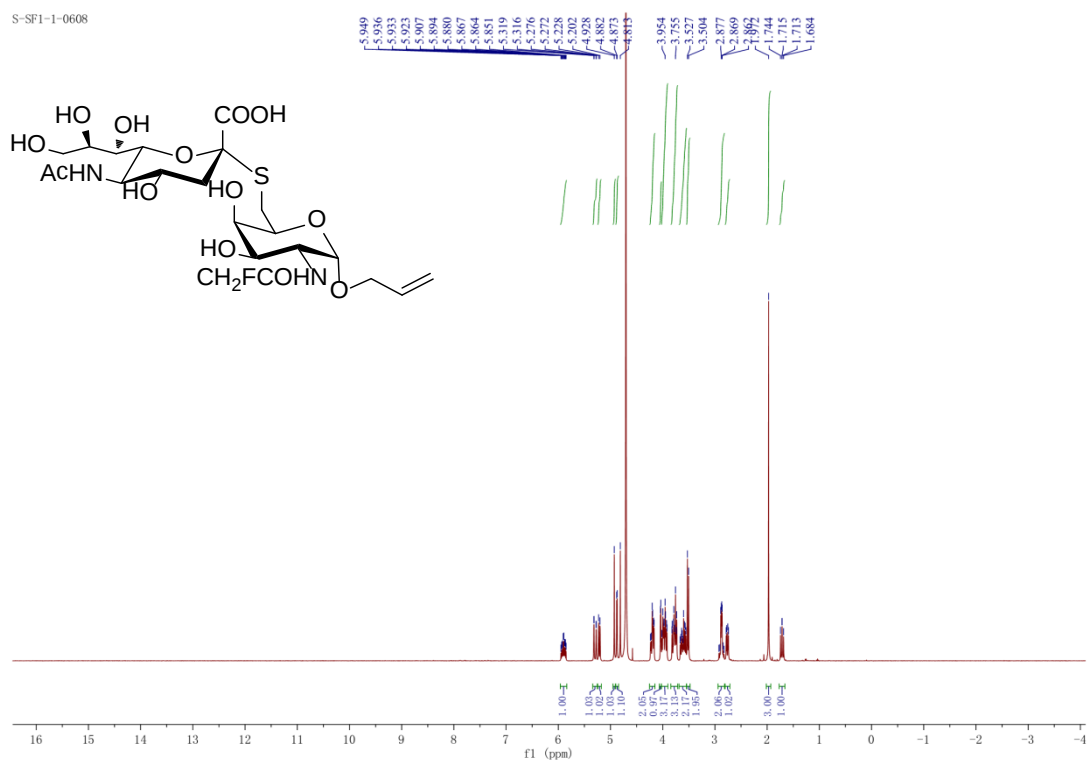


¹H NMR spectrum of compound 22 (600 MHz, CDCl₃)



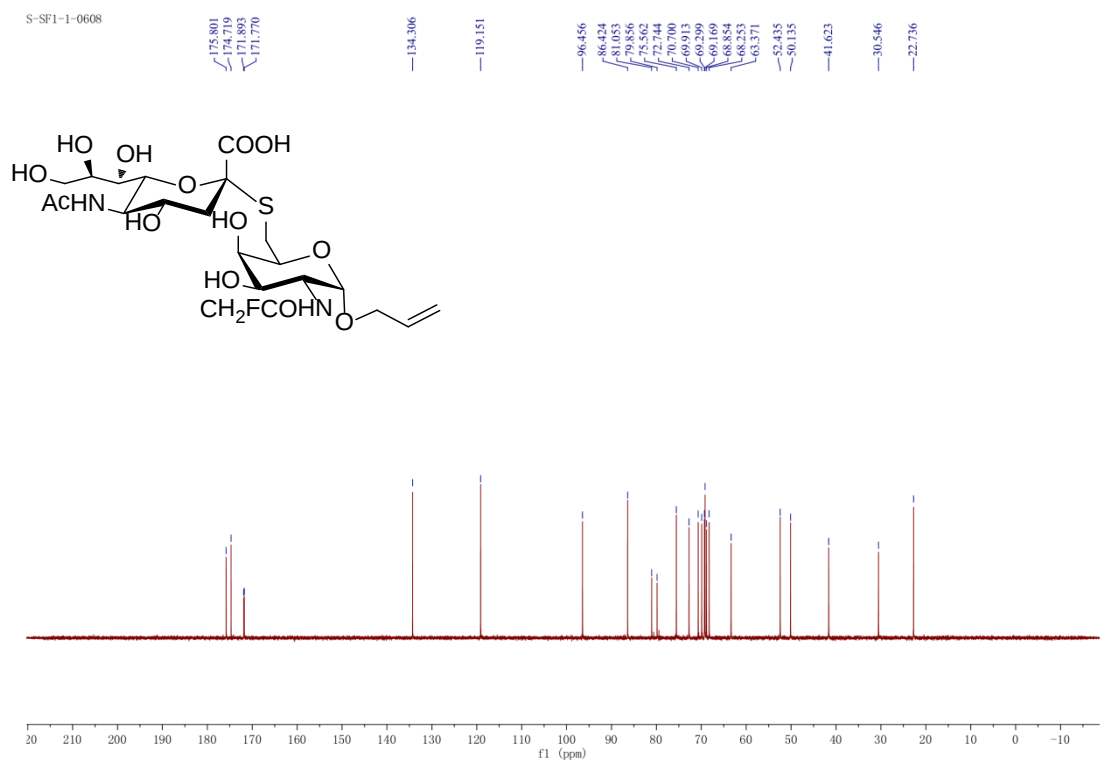
¹³C NMR spectrum of compound 22 (150 MHz, CDCl₃)

S-SF1-1-0608



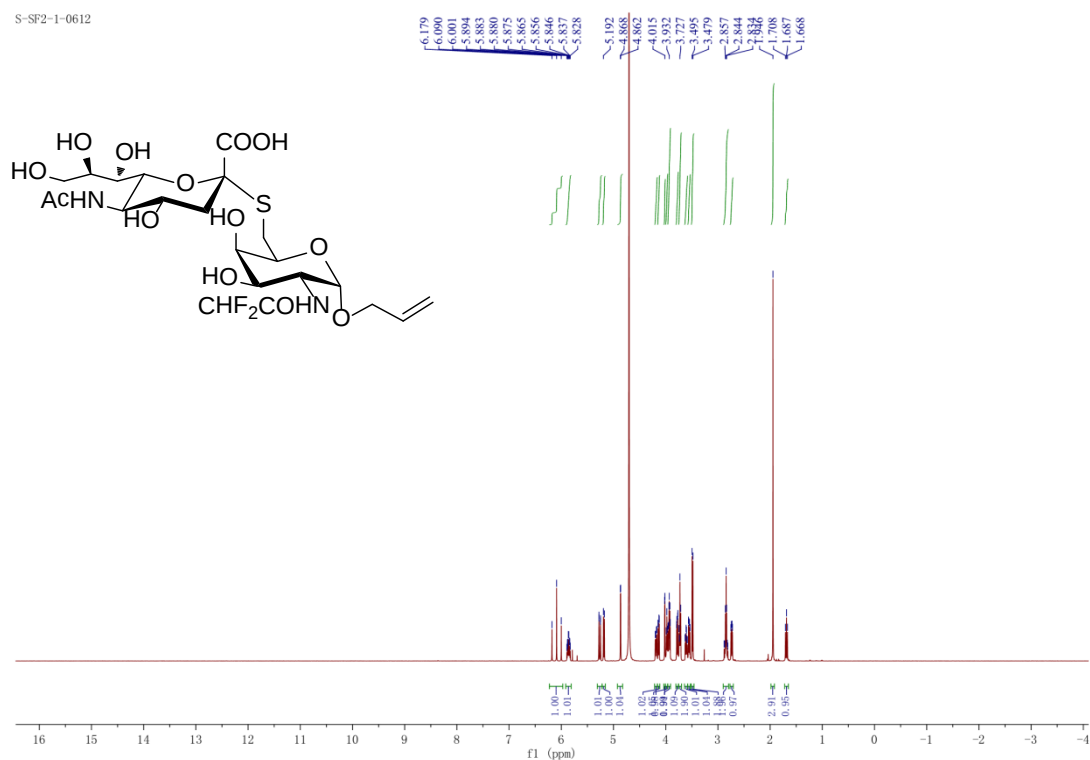
¹H NMR spectrum of compound **23** (400 MHz, D₂O)

S-SF1-1-0608



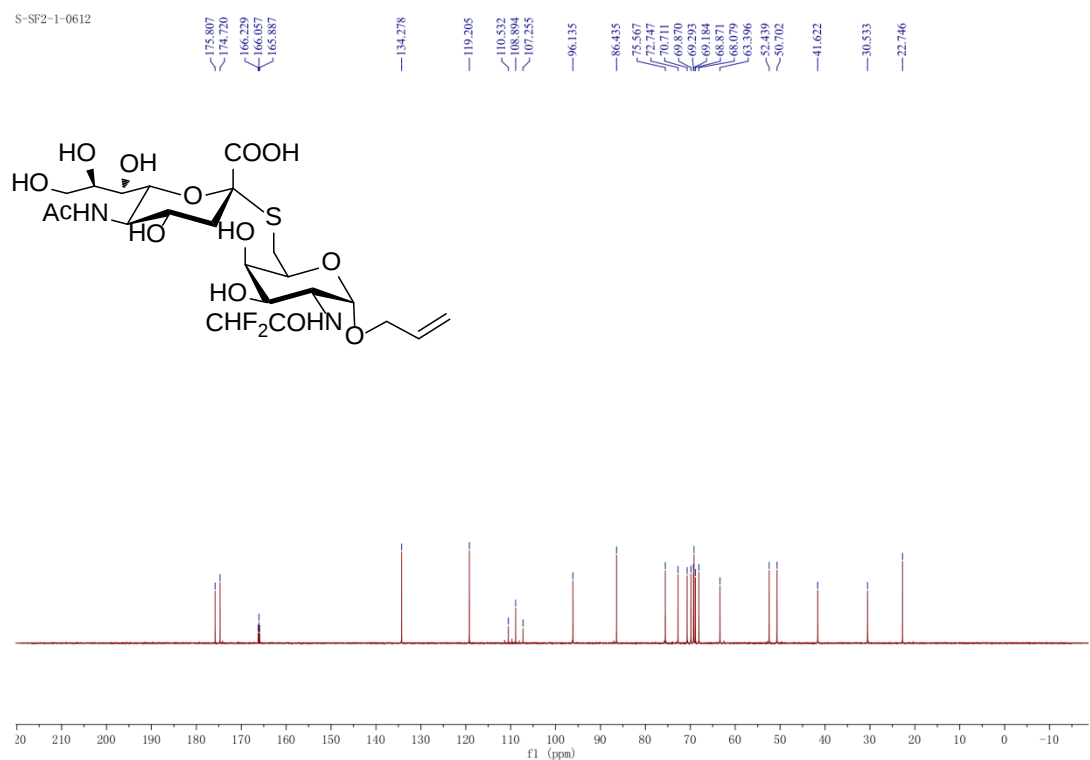
¹³C NMR spectrum of compound **23** (150 MHz, D₂O)

S-SF2-1-0612

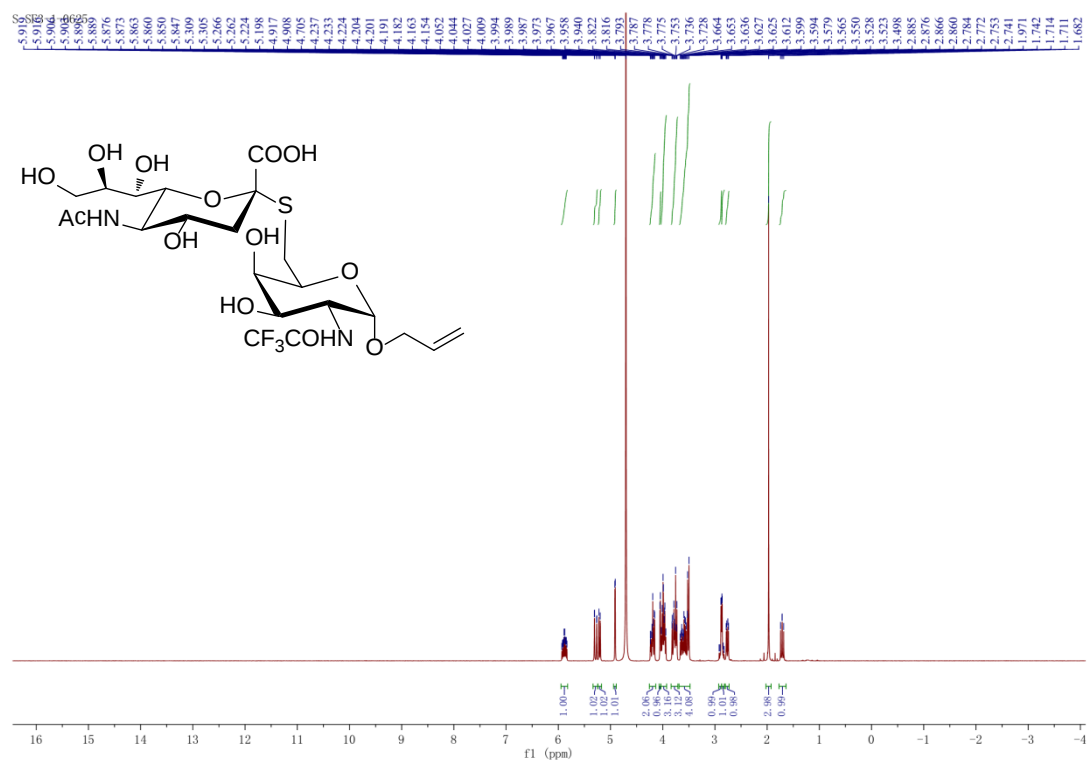


¹H NMR spectrum of compound **24** (600 MHz, D₂O)

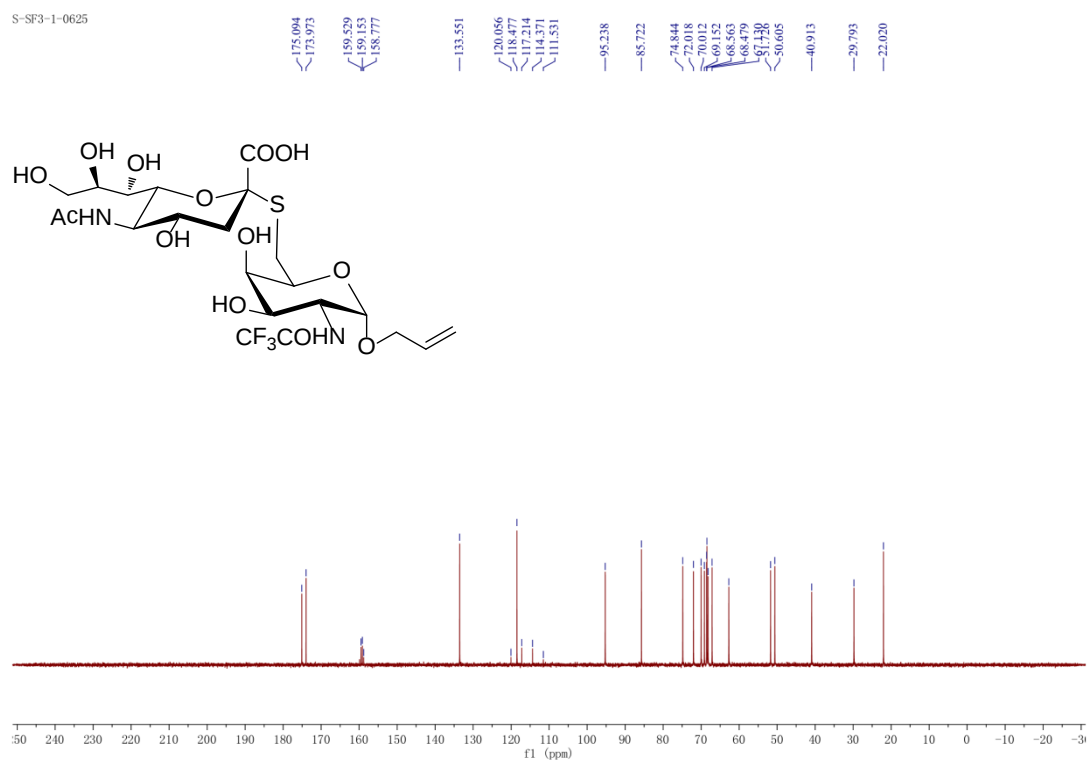
S-SF2-1-0612



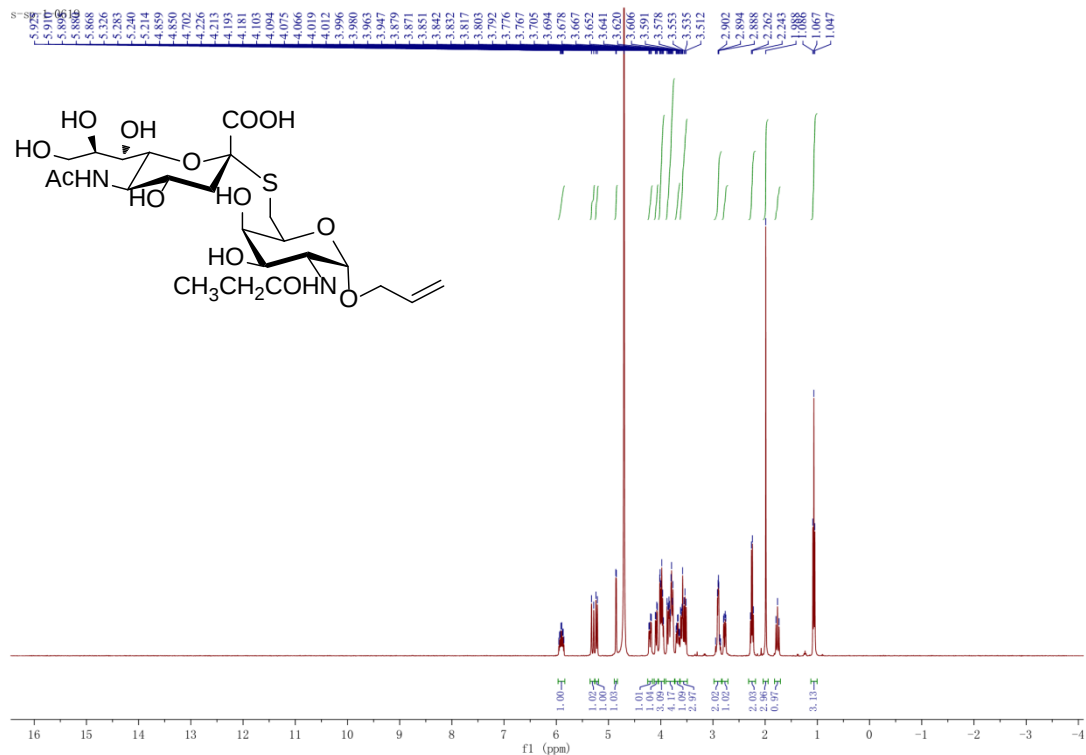
¹³C NMR spectrum of compound **24** (150 MHz, D₂O)



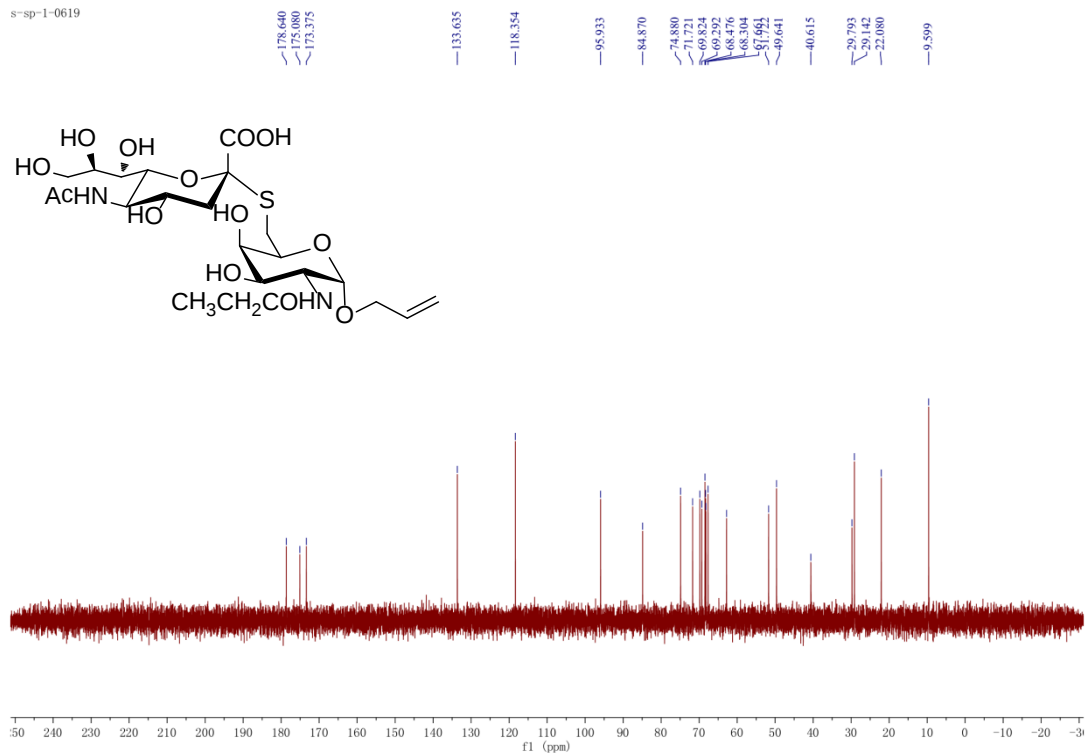
¹H NMR spectrum of compound 25 (400 MHz, D₂O)



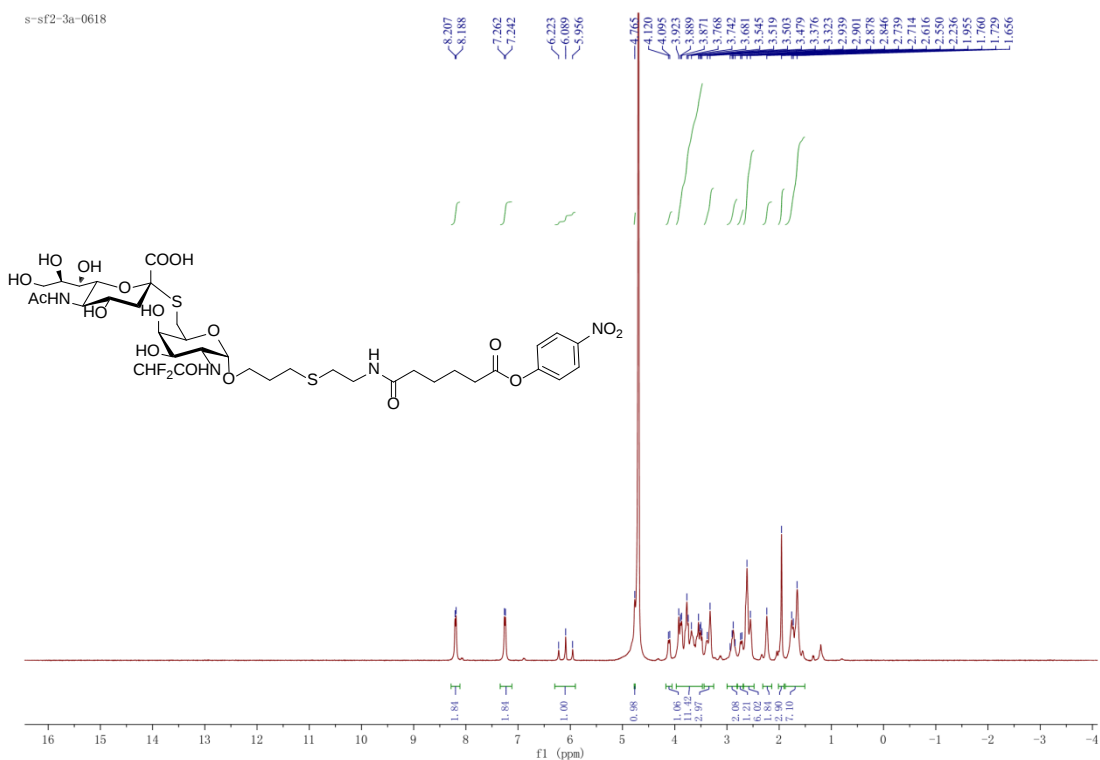
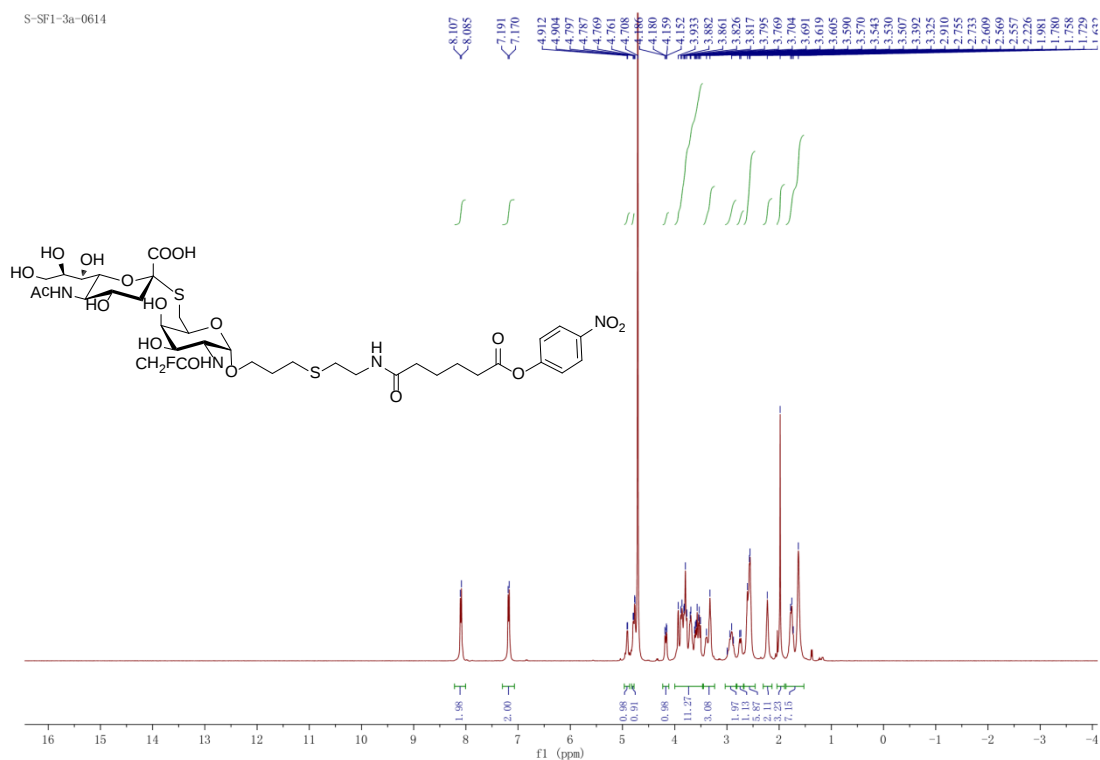
¹³C NMR spectrum of compound 25 (100 MHz, D₂O)



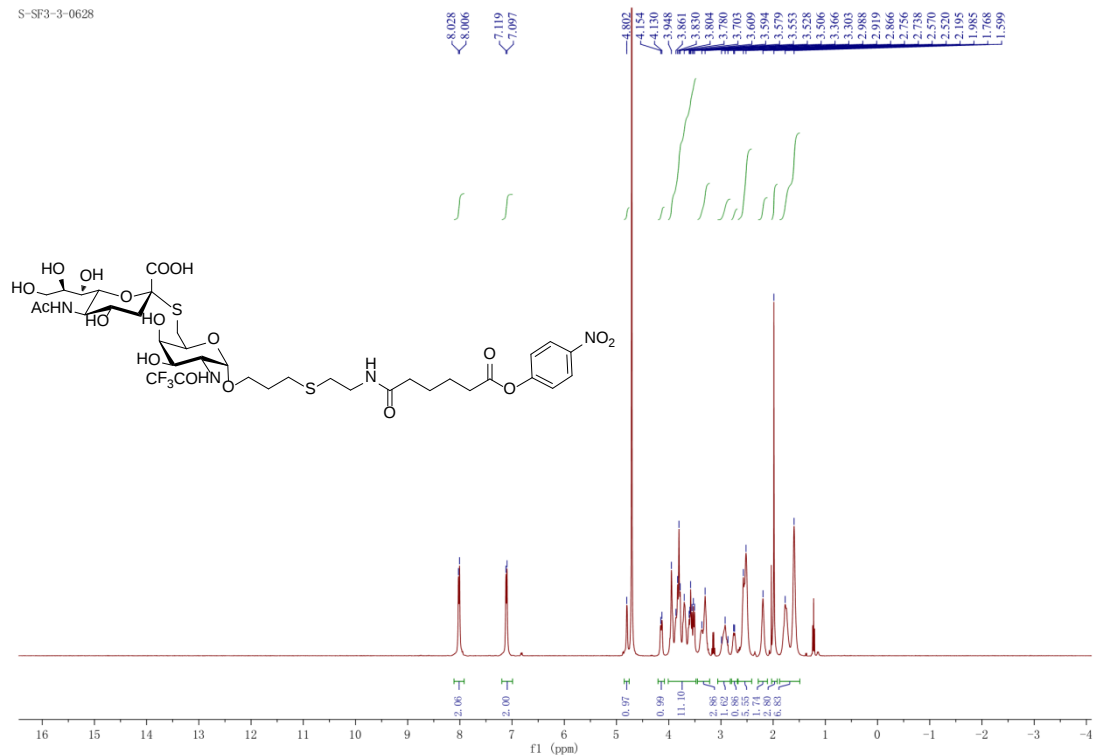
¹H NMR spectrum of compound **26** (400 MHz, D₂O)



¹³C NMR spectrum of compound **26** (100 MHz, D₂O)

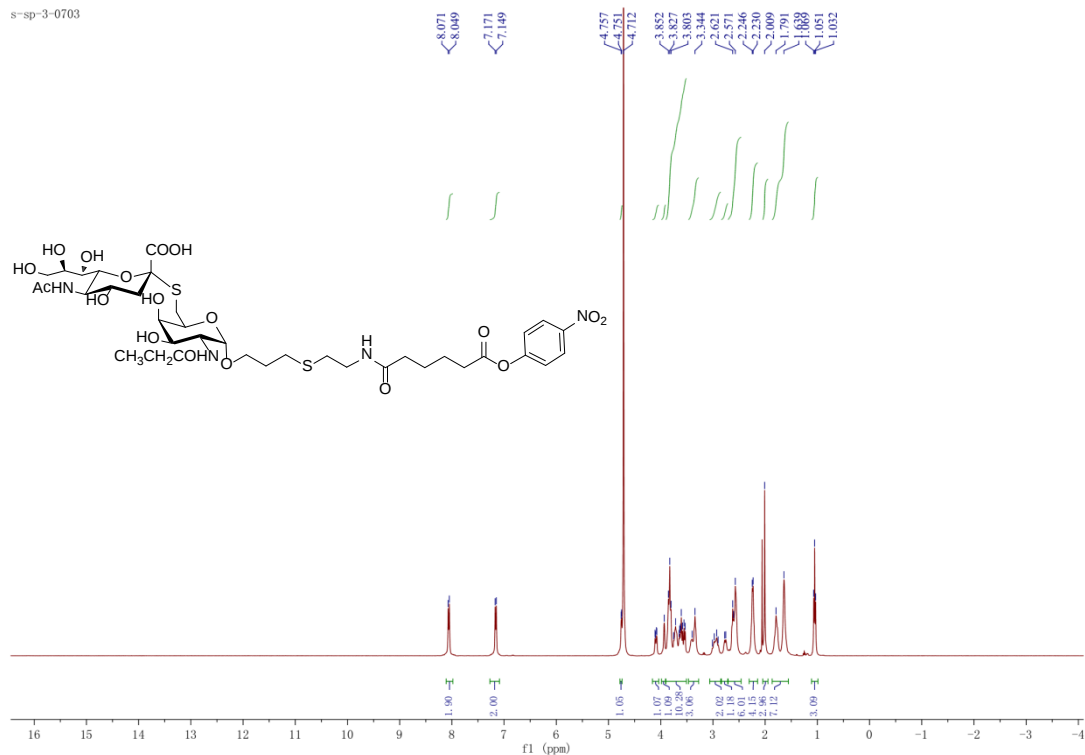


S-SF3-3-0628

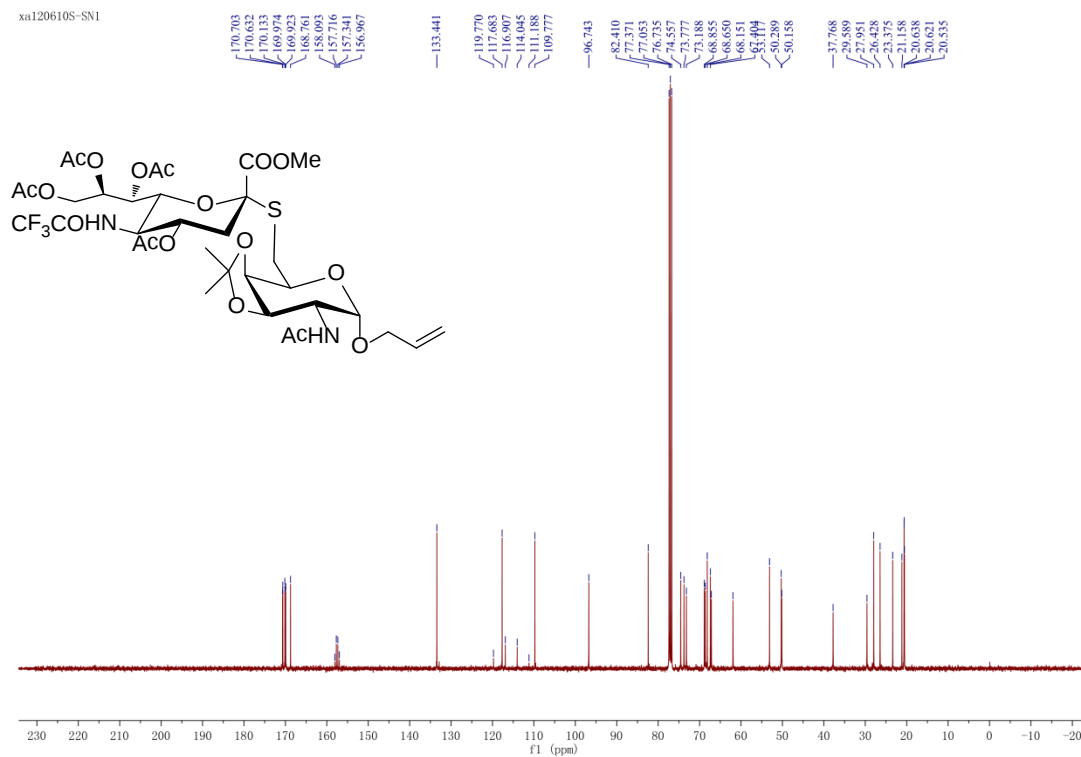
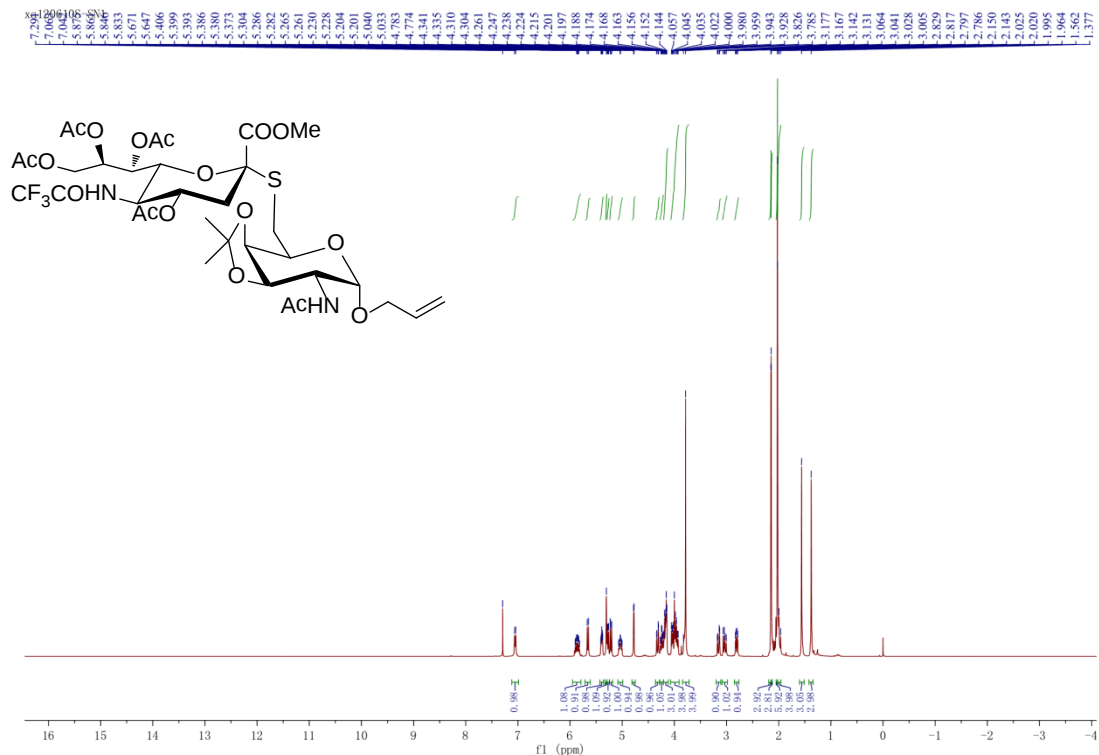


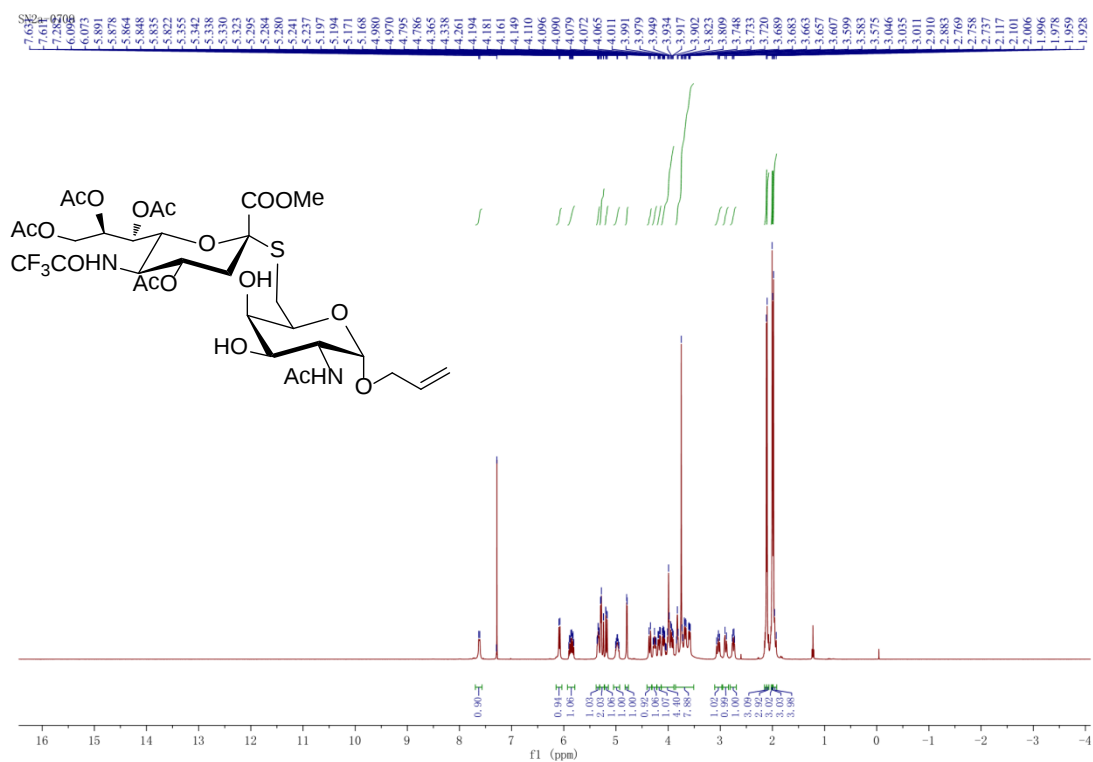
¹H NMR spectrum of compound **29** (400 MHz, D₂O)

s-sp-3-0703

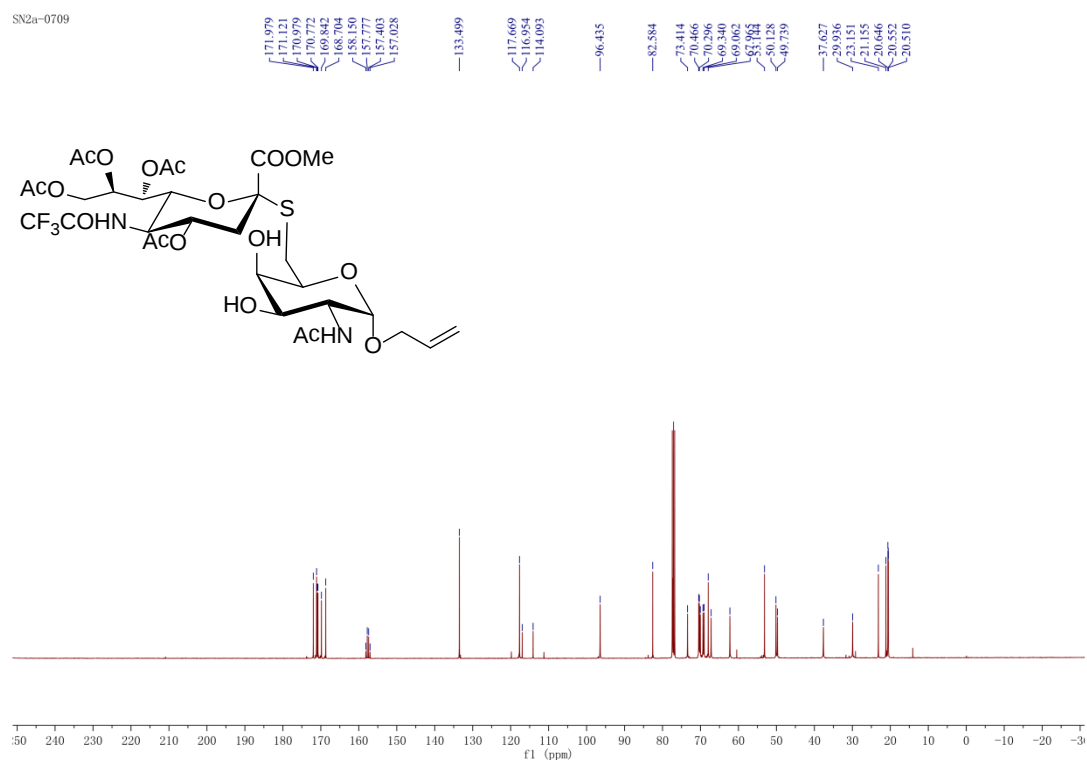


¹H NMR spectrum of compound **30** (400 MHz, D₂O)

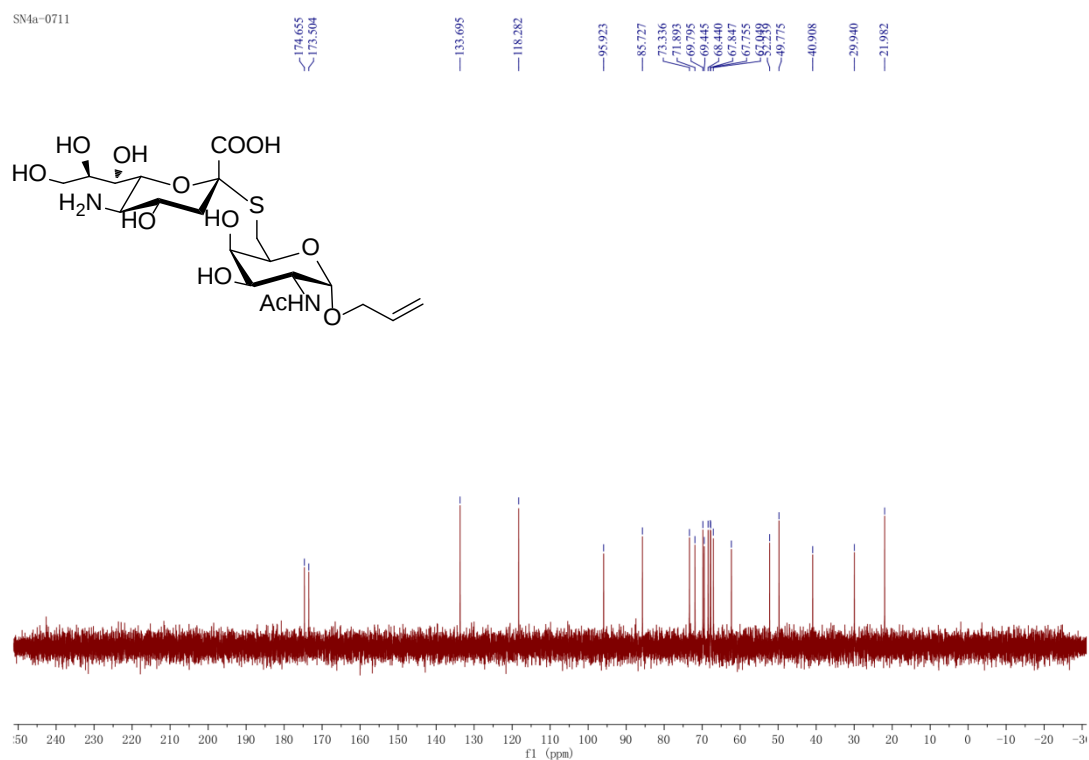
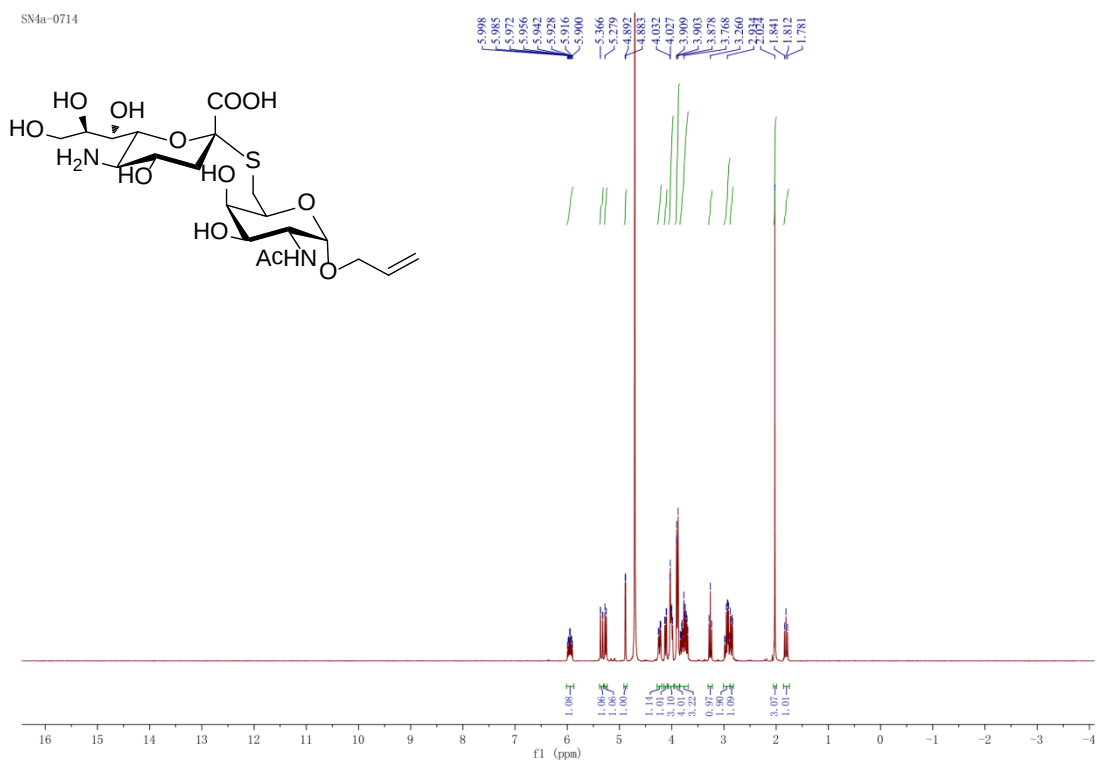




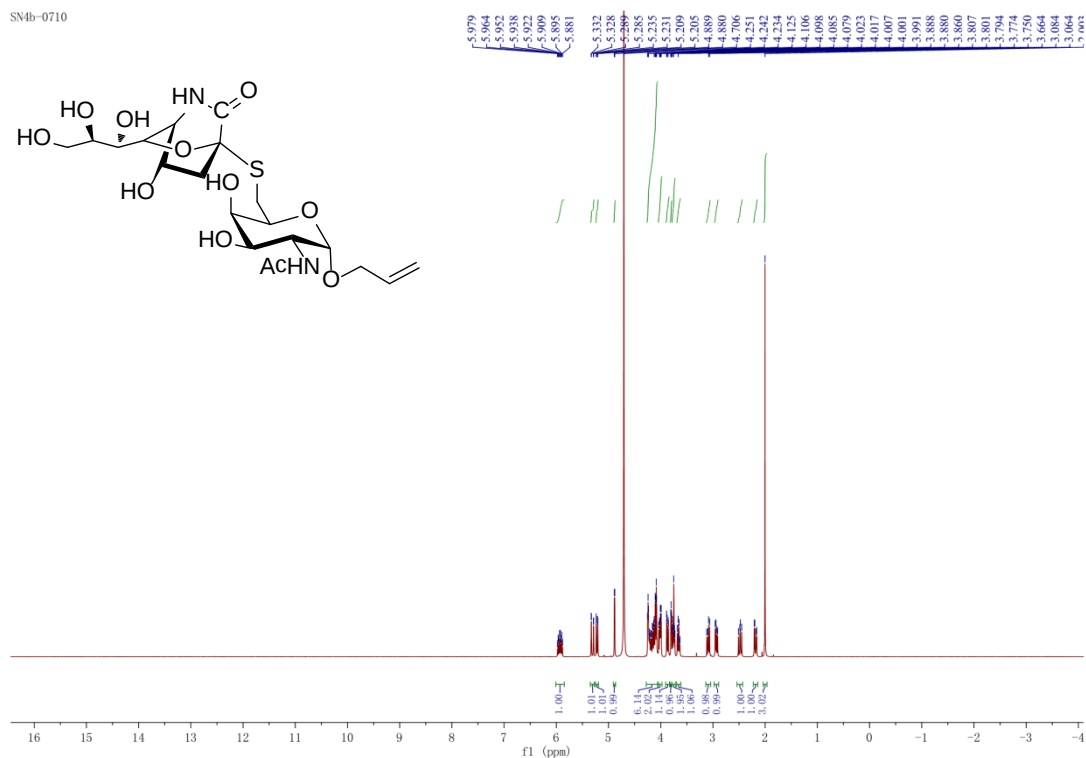
^1H NMR spectrum of compound 37 (400 MHz, CDCl_3)



^{13}C NMR spectrum of compound 37 (100 MHz, CDCl_3)

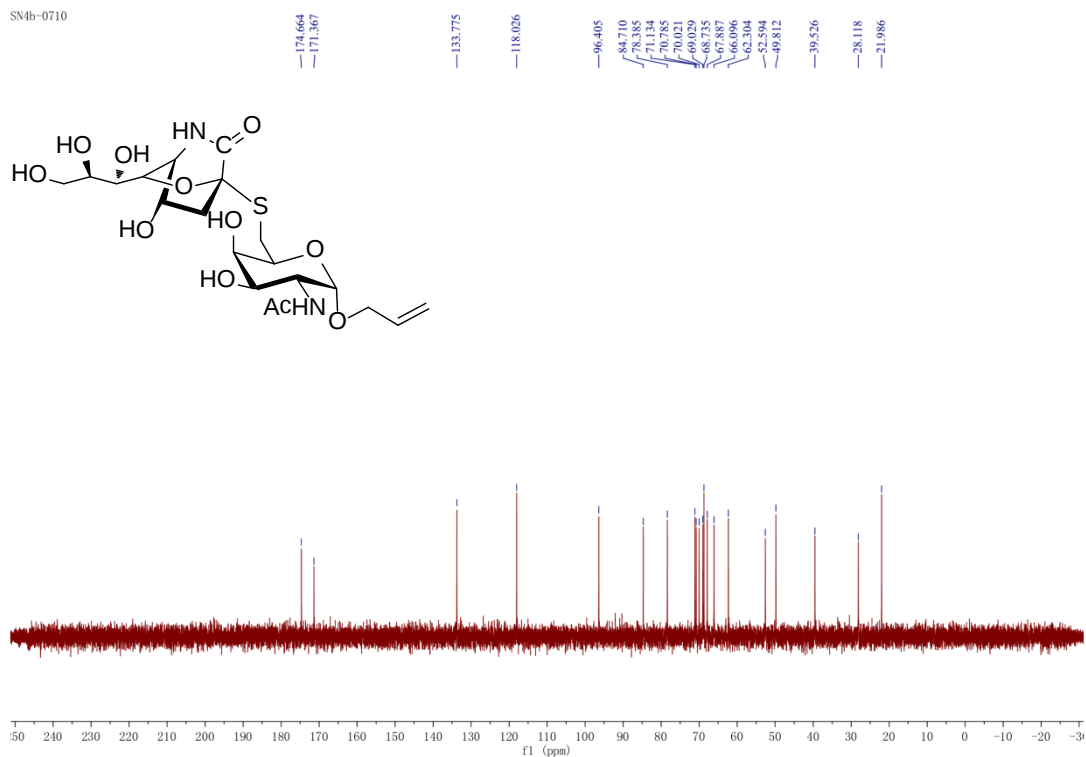


SM4b-0710

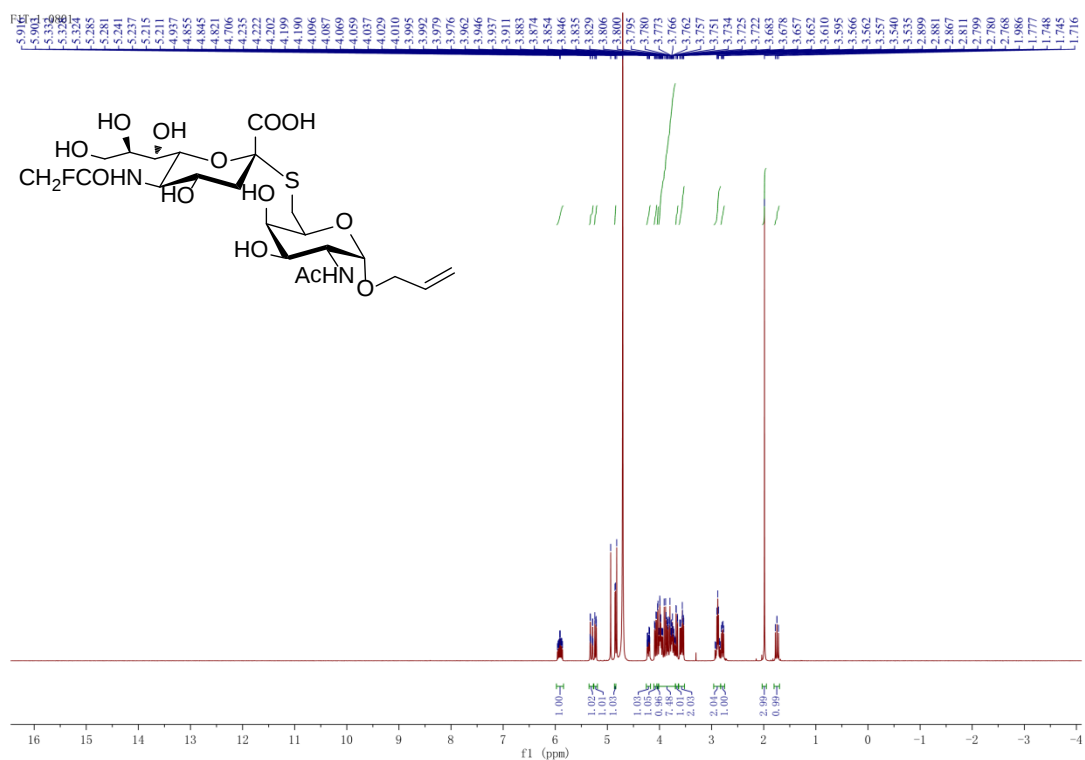


¹H NMR spectrum of compound 39 (400 MHz, D₂O)

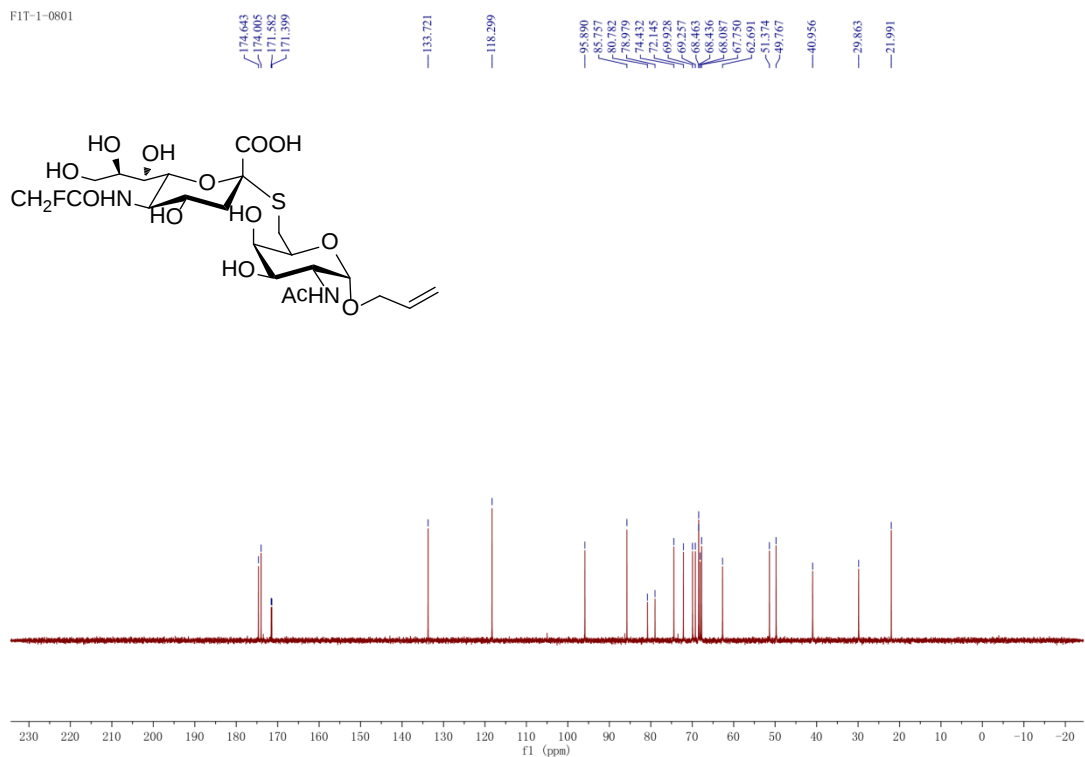
SM4b-0710



¹³C NMR spectrum of compound 39 (100 MHz, D₂O)

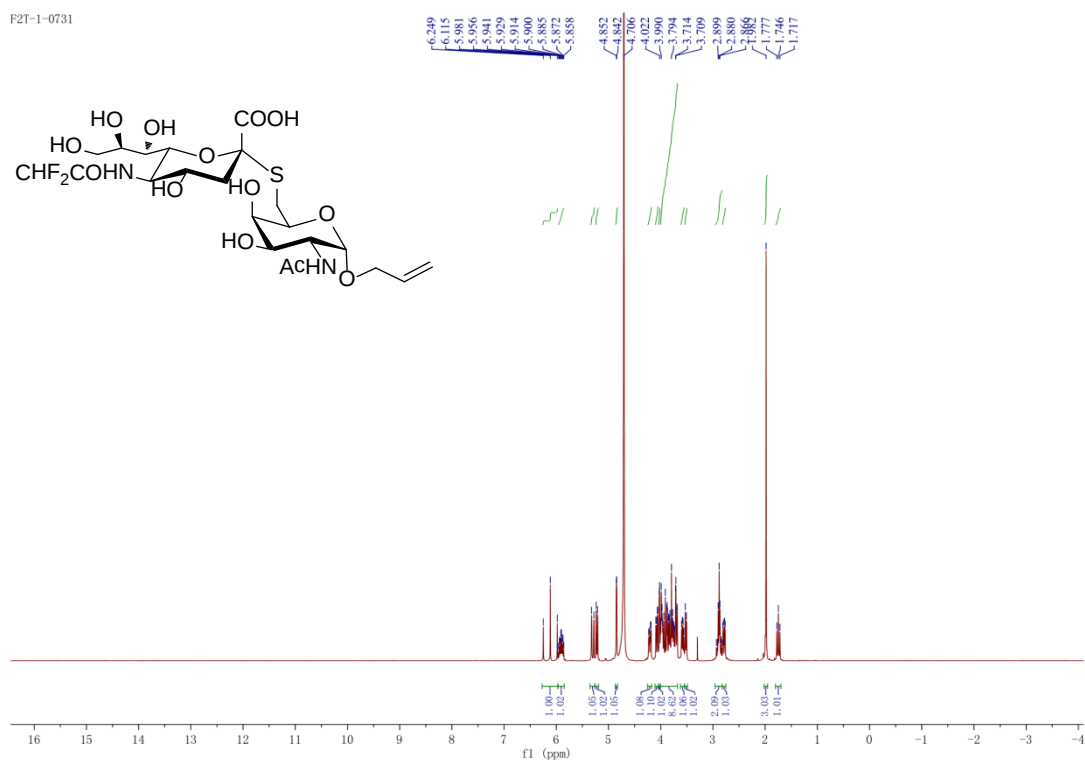


¹H NMR spectrum of compound **40** (400 MHz, D₂O)



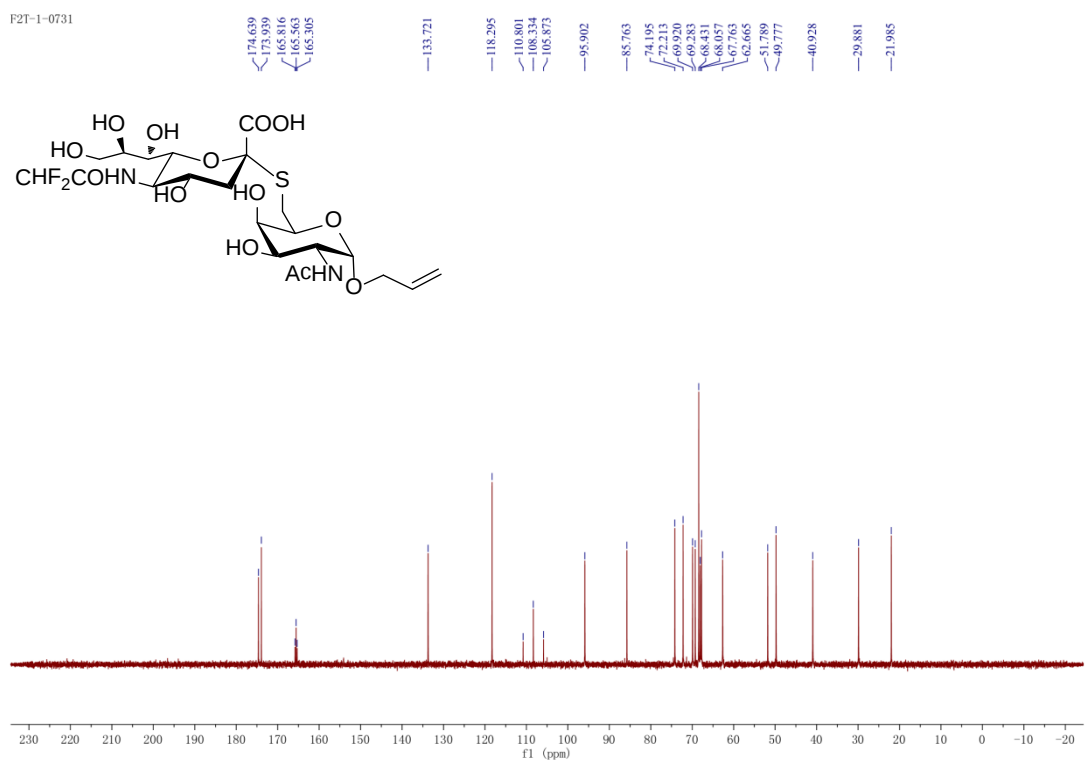
¹³C NMR spectrum of compound **40** (100 MHz, D₂O)

F2T-1-0731



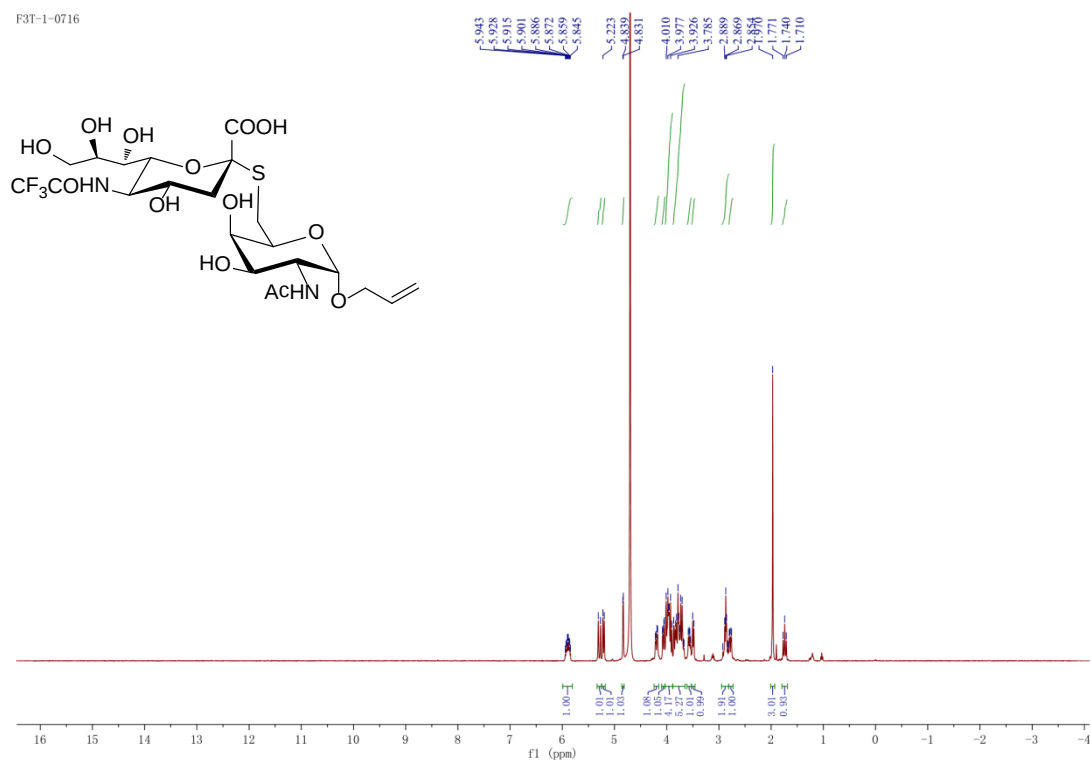
^1H NMR spectrum of compound **41** (400 MHz, D_2O)

F2T-1-0731



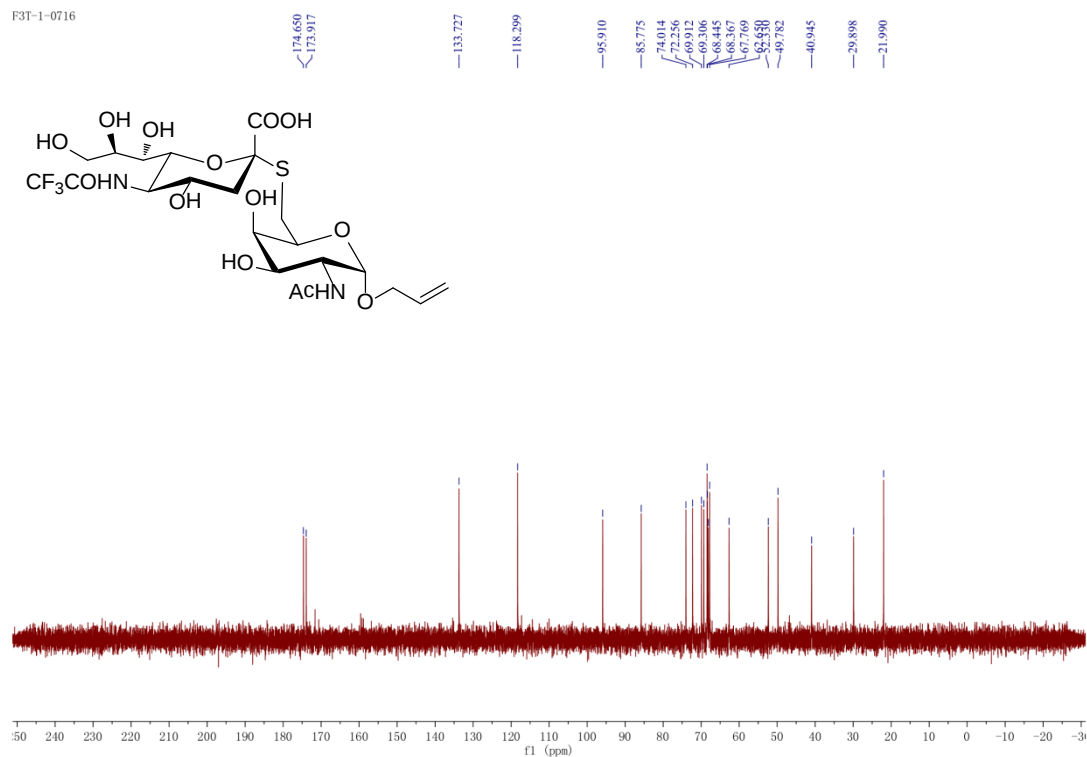
^{13}C NMR spectrum of compound **41** (100 MHz, D_2O)

F3T-1-0716



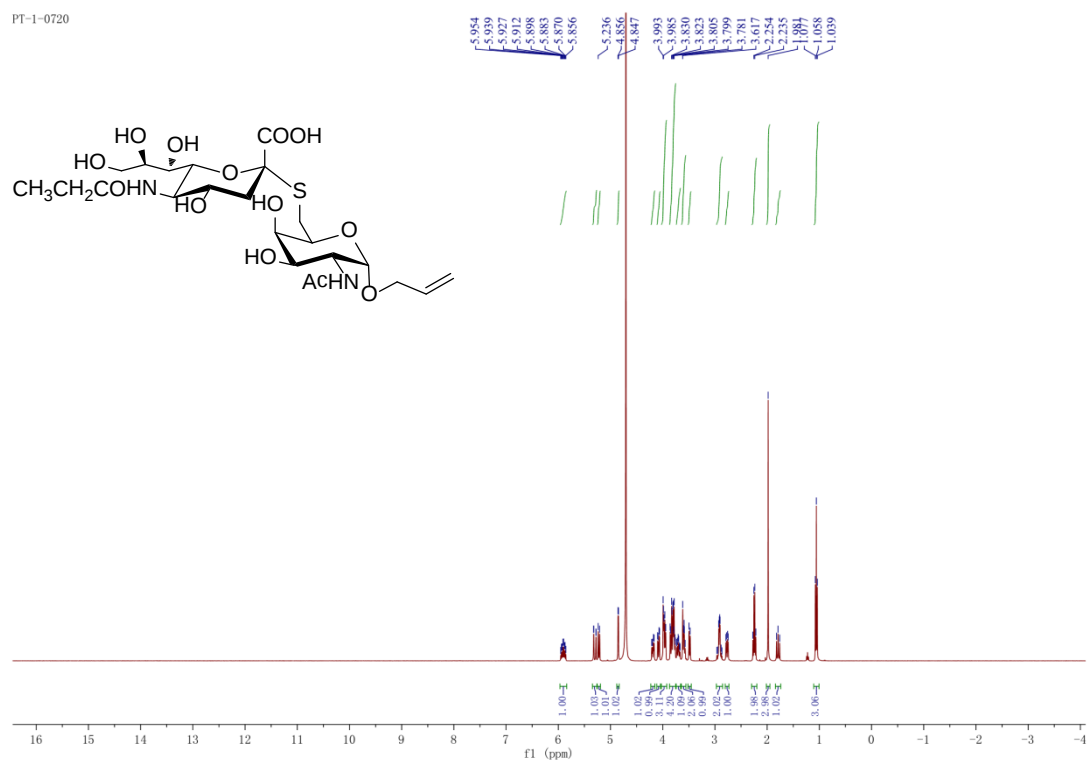
¹H NMR spectrum of compound 42 (400 MHz, D₂O)

F3T-1-0716



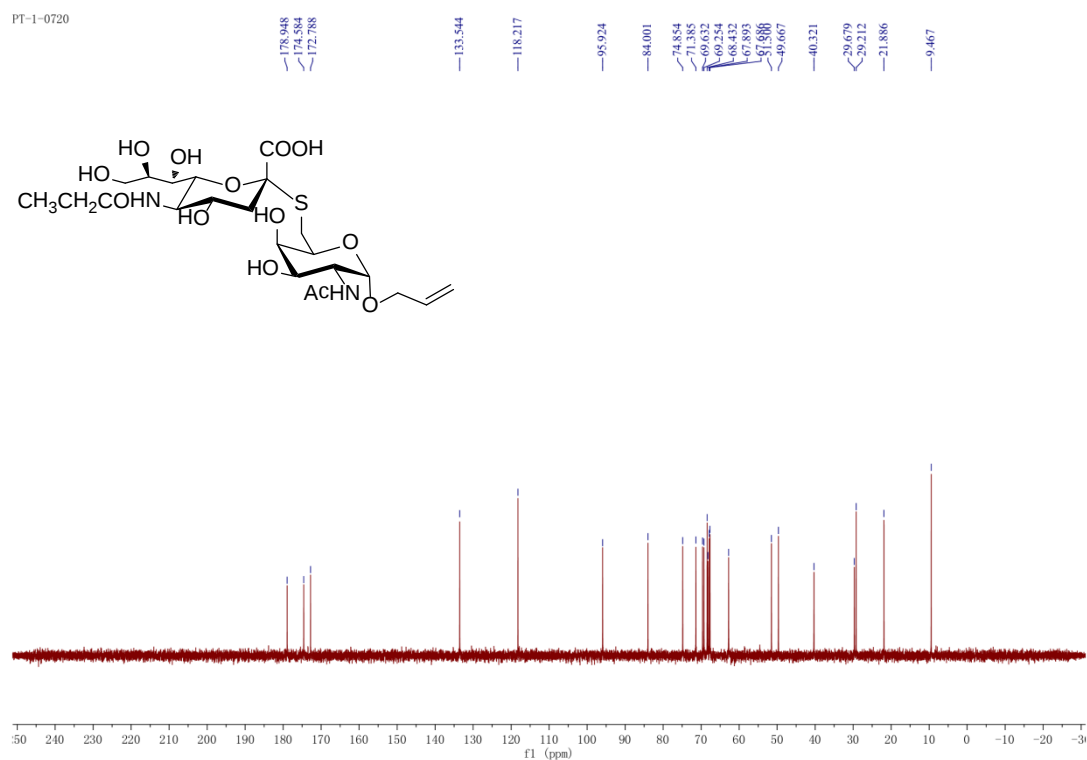
¹³C NMR spectrum of compound 42 (100 MHz, D₂O)

PT-1-0720

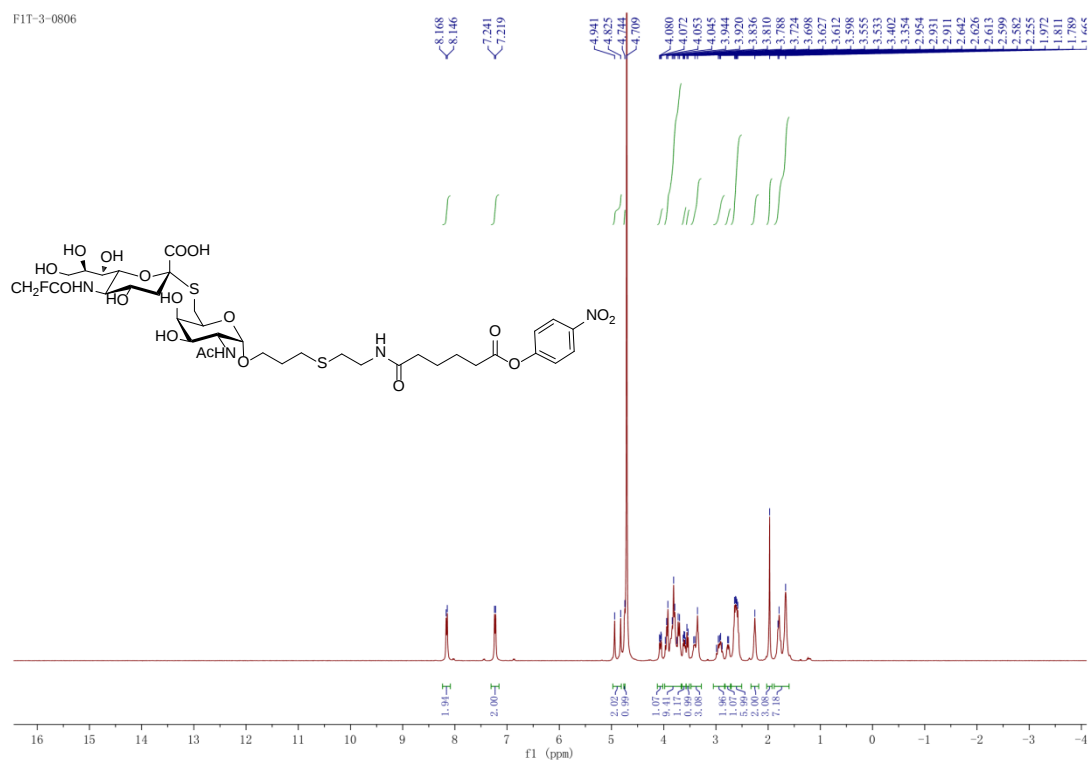


¹H NMR spectrum of compound **43** (400 MHz, D₂O)

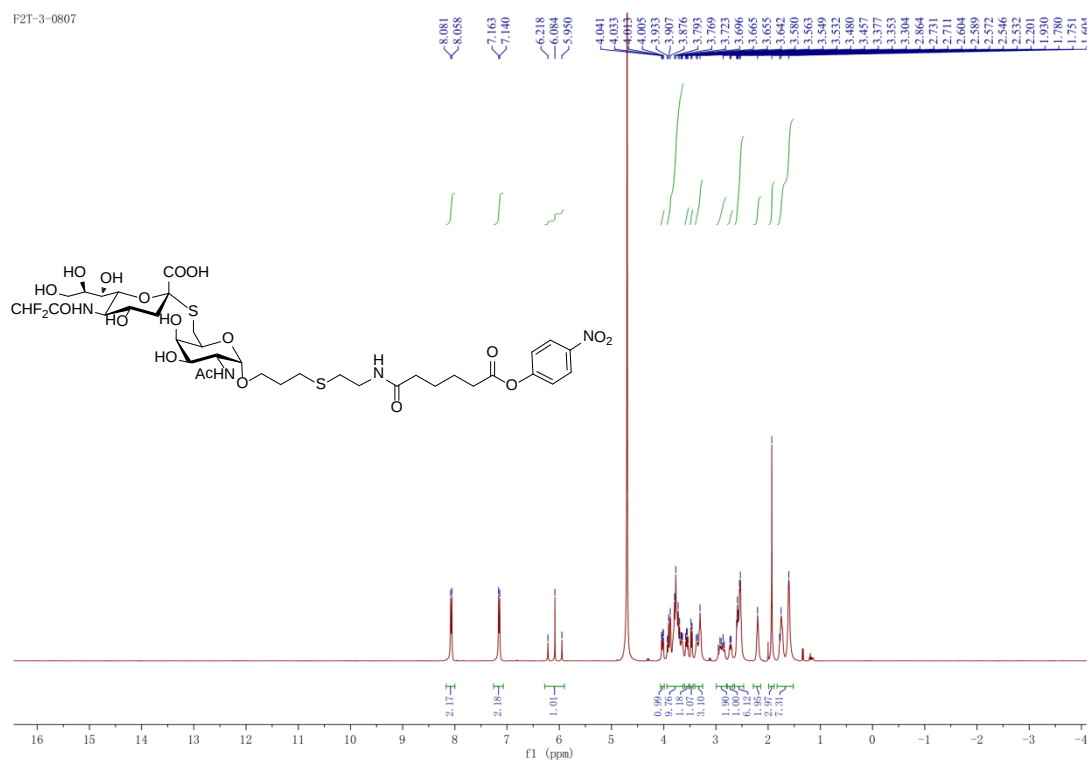
PT-1-0720



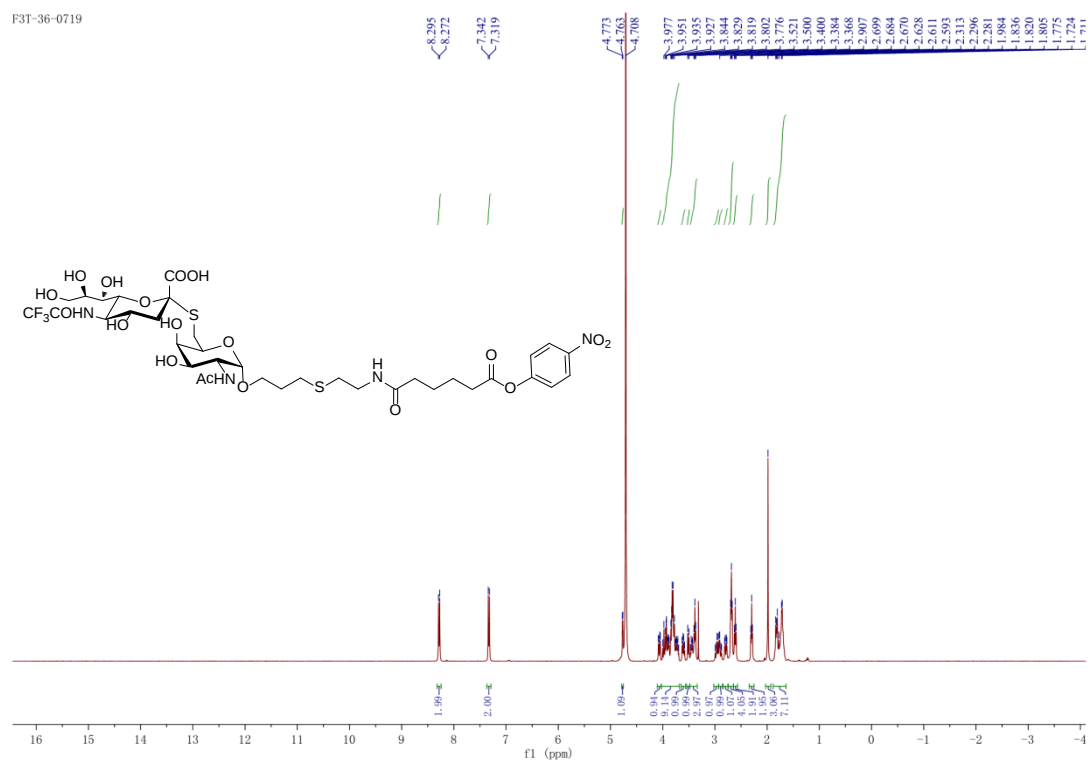
¹³C NMR spectrum of compound **43** (100 MHz, D₂O)



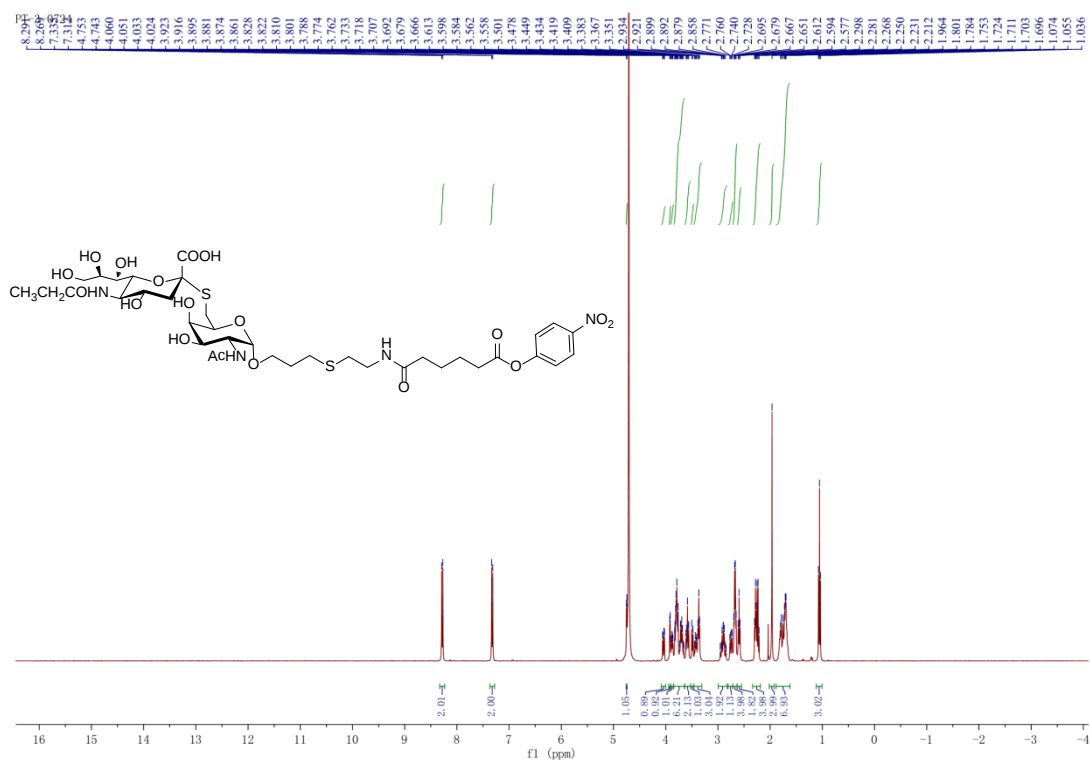
¹H NMR spectrum of compound 44 (400 MHz, D₂O)



¹H NMR spectrum of compound 45 (400 MHz, D₂O)



¹H NMR spectrum of compound **46** (400 MHz, D₂O)



¹H NMR spectrum of compound **47** (400 MHz, D₂O)


$$\begin{array}{r} < 8.244 \\ < 8.221 \end{array}$$