Supporting Information

Chemoenzymatic synthesis and lectin array characterization of a class of N-glycan clusters

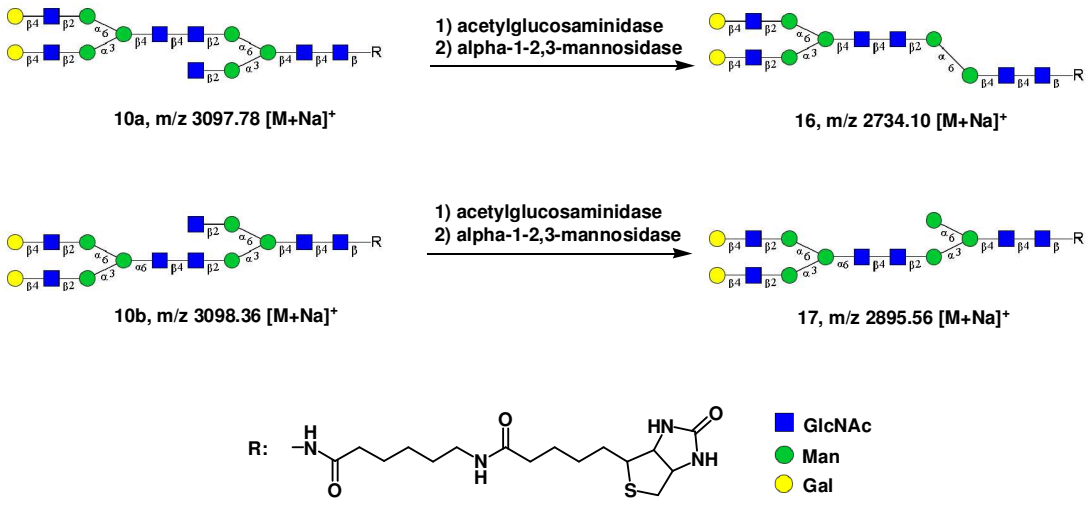
Wei Huang[1], Denong Wang[2], Masao Yamada[3] and Lai-Xi Wang[1*]

[1]Institute of Human Virology and Department of Biochemistry & Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA
[2] Stanford Tumor Glycome Laboratory, Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA
[3]GP Biosciences Ltd, 1-3-3 Azamino-minami, Aoba-ku, Yokohama 225-0012, JAPAN

Complete Ref. 4a:

Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C. H.; Paulson, J. C. *Proc. Natl. Acad. Sci. USA* **2004,** *101*, 17033-17038.

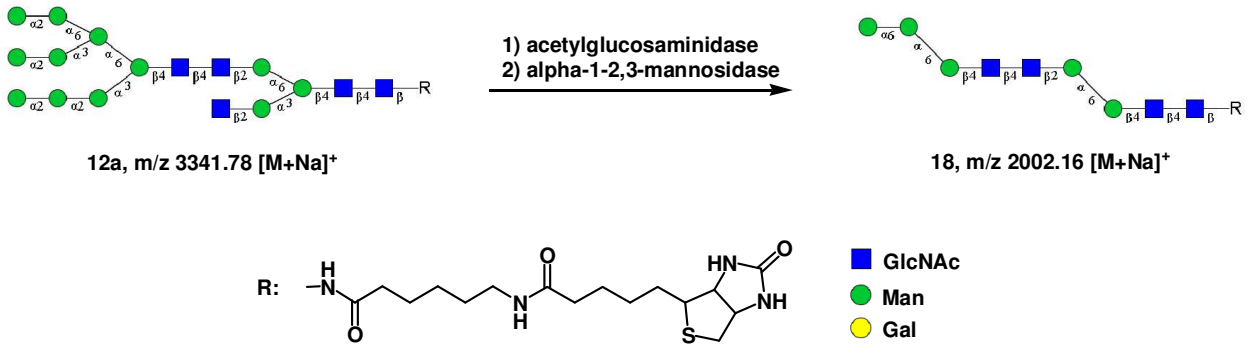
Figure S1: Assignment of the two mono-glycosylated products (**10a** and **10b**) by specific enzymatic transformation coupled with MS analysis.



The attachment of the new glycan at the 6-arm (in **10a**) or at the 3-arm (in **10b**) of the GlcNAc2Man3GlcNAc2-core was confirmed by the following enzymatic transformations coupled with MS analysis. Briefly, compound **10a** or **10b** (20 μg) and BSA (3 μg) was dissolved in a sodium citrate buffer (50 mM, pH 6.0, 30 μL) and the solution was incubated with β-N-acetylglucosaminidase (6 U) from *Xanthomonas manihotis* at 37°C for 2h. The reaction was monitored by MALDI-TOF MS until complete removal of the terminal GlcNAc residue at the non-reducing end. Then the reaction medium was adjusted to pH 5.5 and was incubated with an α-1,2/α-1,3-mannosidase (50 U) from *Xanthomonas manihotis* at 37°C overnight. This enzymatic treatment of **10a** gave product **16**: Analytical HPLC: t_R = 10.9 min; MALDI-TOF-MS: found, 2734.10 $[M + Na]^+$, calculated for $C_{106}H_{176}N_{10}O_{68}S$, M = 2710.62 Da. These results indicated that the GlcNAc residue at the α-1,3-Man arm in the core should be un-substituted,

resulting in sequential removal of the GlcNAc by the β-N-acetylglucosaminidase and then the exposed α-1,3-linked Man residue by the α-1,2/α-1,3-mannosidase to give **16**. On the other hand, the same treatment on compound **10b** gave product **17**: Analytical HPLC: t_R = 11.1 min; MALDI-TOF-MS: calculated for $C_{112}H_{186}N_{10}O_{73}S$, M = 2872.76 Da, found, 2895.56 $[M + Na]^+$. The MS analysis indicated that only a GlcNAc residue was removed from 10b by sequential enzymatic treatment, confirming that the α-1,3-arm was blocked by the attachment of newly introduced glycan. The α-1,6-Man residue exposed would not be hydrolyzed by the α-1,2/α-1,3-mannosidase.

Figure S2. Characterization of mono-glycosylated product **12a** generated from the reaction of Man9GlcNAc-oxazoline (**11**) and acceptor **7**.

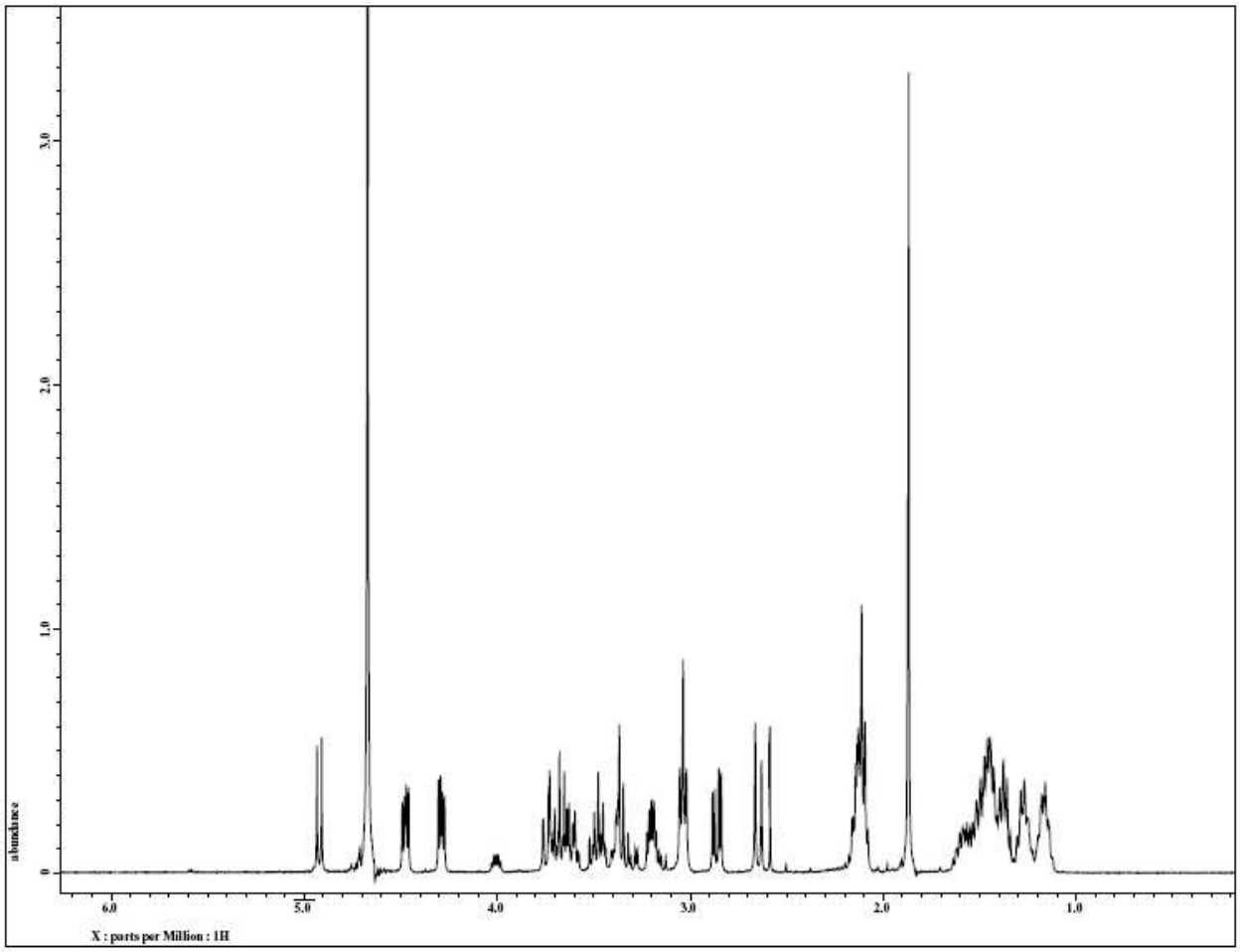


The sequential treatment of **12a** by the β-N-acetylglucosaminidase and the α-1,2/α-1,3-mannosidase was performed in the same way as the characterization of 10a and 10b. The enzymatic transformation gave compound 18: Analytical HPLC: t_R = 10.8 min; MALDI-TOF-MS: calculated for $C_{78}H_{130}N_8O_{48}S$, M = 1978.77 Da, found, 2002.16 $[M + Na]^+$. The MS data of 18 indicated the removal of a GlcNAc and a Man residue from **12a**. These results confirm that the α-1,3-arm was open and the α-1,6-arm in 12a was blocked by the newly introduced N-glycan.

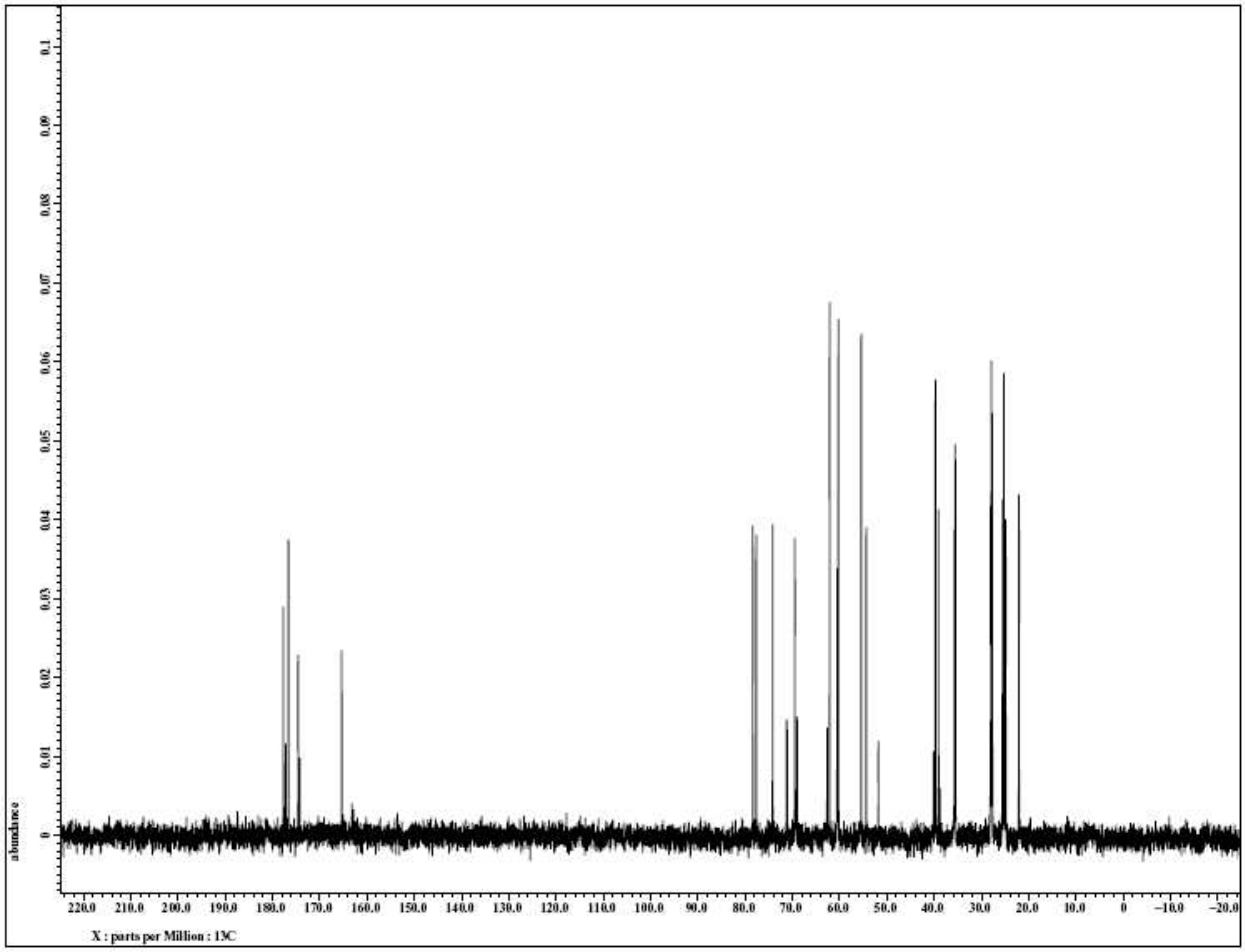
Figure S3. Lectin specificity

Lectin No.	Lectin	Reported specificity
1	LTL	Fuc 1-3 Gal 1-4)GlcNAc, Fuc 1-2Gal 1-4GlcNAc
2	PSA	Fuc 1-6GlcNAc, -D-Glc, -D-Man
3	LCA	Fuc 1-6GlcNAc, -D-Glc, -D-Man
4	UEA-I	Fuc 1-2Gal 1-4GlcNAc
5	AOL	Fuc 1-6GlcNAc (core fucose)
6	AAL	Fuc 1-6GlcNAc, Fuc 1-3(Gal 1-4)GlcNAc
7	MAL	Sia 2-3Gal 1-4GlcNAc
8	SNA	Sia 2-6Gal/GalNAc
9	SSA	Sia 2-6Gal/GalNAc
10	TJA-I	Sia 2-6Gal/GalNAc
11	PHAL	tri/tetra-antennary complex-type N-glycan
12	ECA	Gal 1-4GlcNAc
13	RCA120	Gal 1-4GlcNAc
14	PHAE	bi-antennary complex-type N-glycan with outer Gal and bisecting GlcNAc
15	DSA	(GlcNAc 1-4)n, Gal 1-4GlcNAc
16	GSL-II	agalactosylated tri/tetra antennary glycans, GlcNAc
17	NPA	High-Mannose, Man 1-6Man
18	ConA	High-Mannose, Man 1-6(Man 1-3)Man
19	GNA	High-Mannose, Man 1-3Man
20	HHL	High-Mannose, Man 1-3Man, Man 1-6Man
21	ACG	Sia 2-3Gal 1-4GlcNAc
22	TxLCI	Man 1-3(Man 1-6)Man, bi- and tri-antennary complex-type N-glycan, GalNAc
23	BPL	Gal 1-3GalNAc, GalNAc
24	TJA-II	Fuc 1-2Gal 1-> or GalNAc 1-> groups at their nonreducing terminals
25	EEL	blood group B antigen, Gal 1-3Gal
26	ABA	Gal 1-3GalNAc
27	LEL	GlcNAc trimers/tetramers, chitin
28	STL	GlcNAc oligomers, oligosaccharide containing GlcNAc and MurNAc
29	UDA	b-1,4-linked GlcNAc oligomers
30	PWM	(GlcNAc 1-4)n
31	Jacalin	Gal 1-3GalNAc, GalNAc
32	PNA	Gal 1-3GalNAc
33	WFA	GalNAc 1-4GlcNAc, Gal 1-3(-6)GalNAc
34	ACA	Gal 1-3GalNAc
35	MPA	Gal 1-3GalNAc, GalNAc
36	HPA	a-linked terminal GalNAc
37	VVA	a-linked terminal GalNAc, GalNAc 1-3Gal
38	DBA	blood group A antigen, GalNAc 1-3GalNAc
39	SBA	a- or -linked terminal GalNAc, GalNAc 1-3Gal
40	Calsepa	Mannose, Maltose
41	PTL-I	a-linked terminal GalNAc
42	MAH	Sia 2-3Gal 1-3(Sia 2-6)GalNAc
43	WGA	chitin oligomers, Sia
44	GSL-IA4	a-linked GalNAc
45	GSL-IB4	a-linked Gal

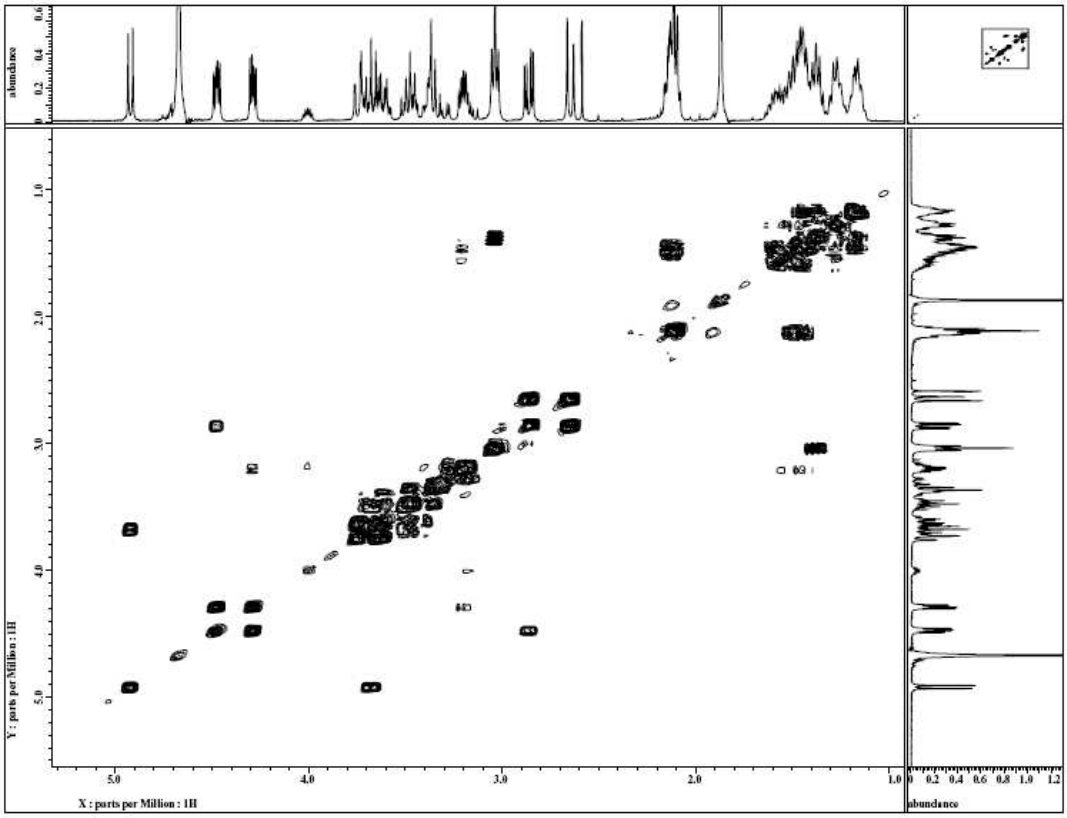
^{1}H NMR of GlcNAc-LC-Biotin (4)



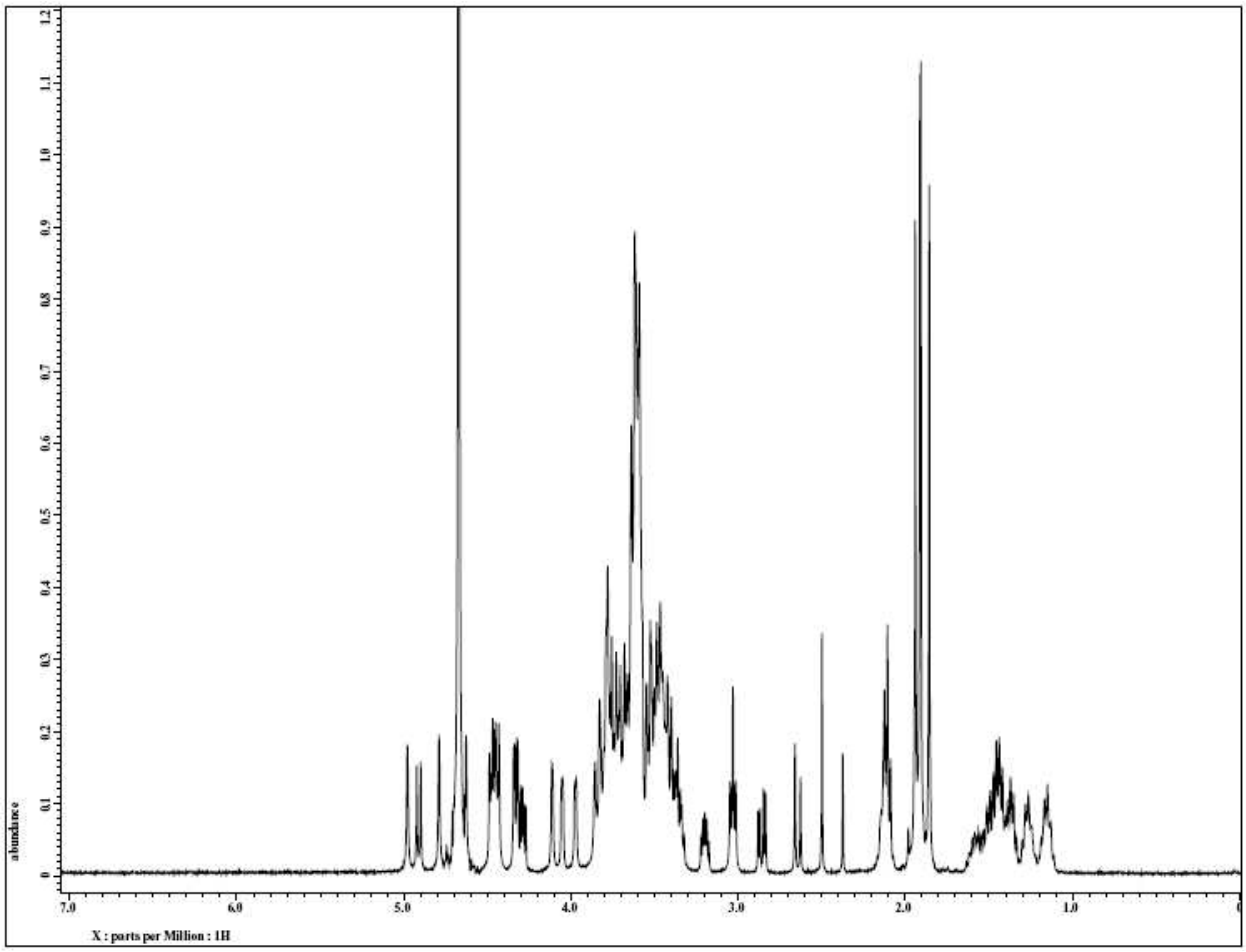
^{13}C NMR of GlcNAc-LC-biotin (4)



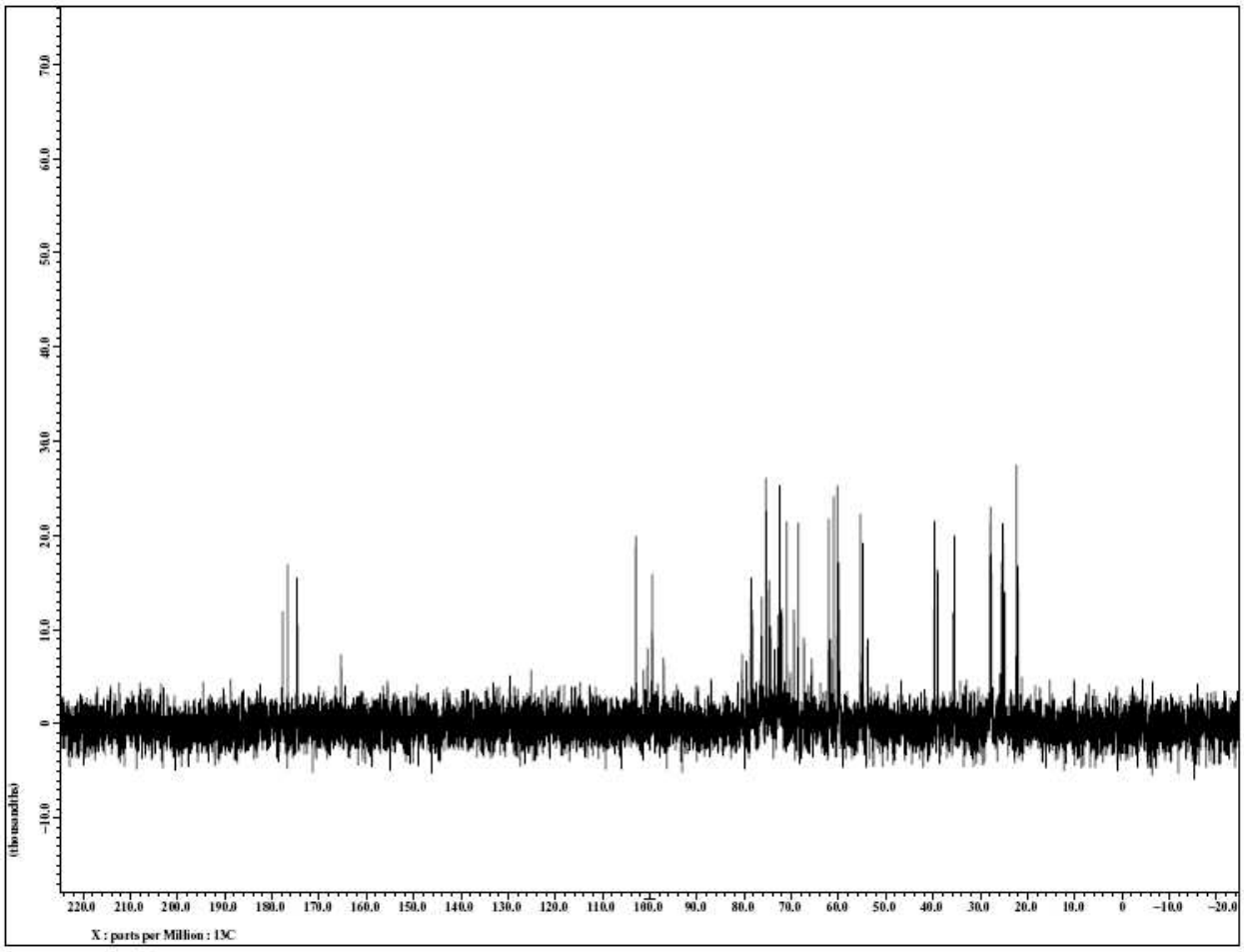
^{1}H-^{1}H COSY NMR of GlcNAc-LC-biotin (4)



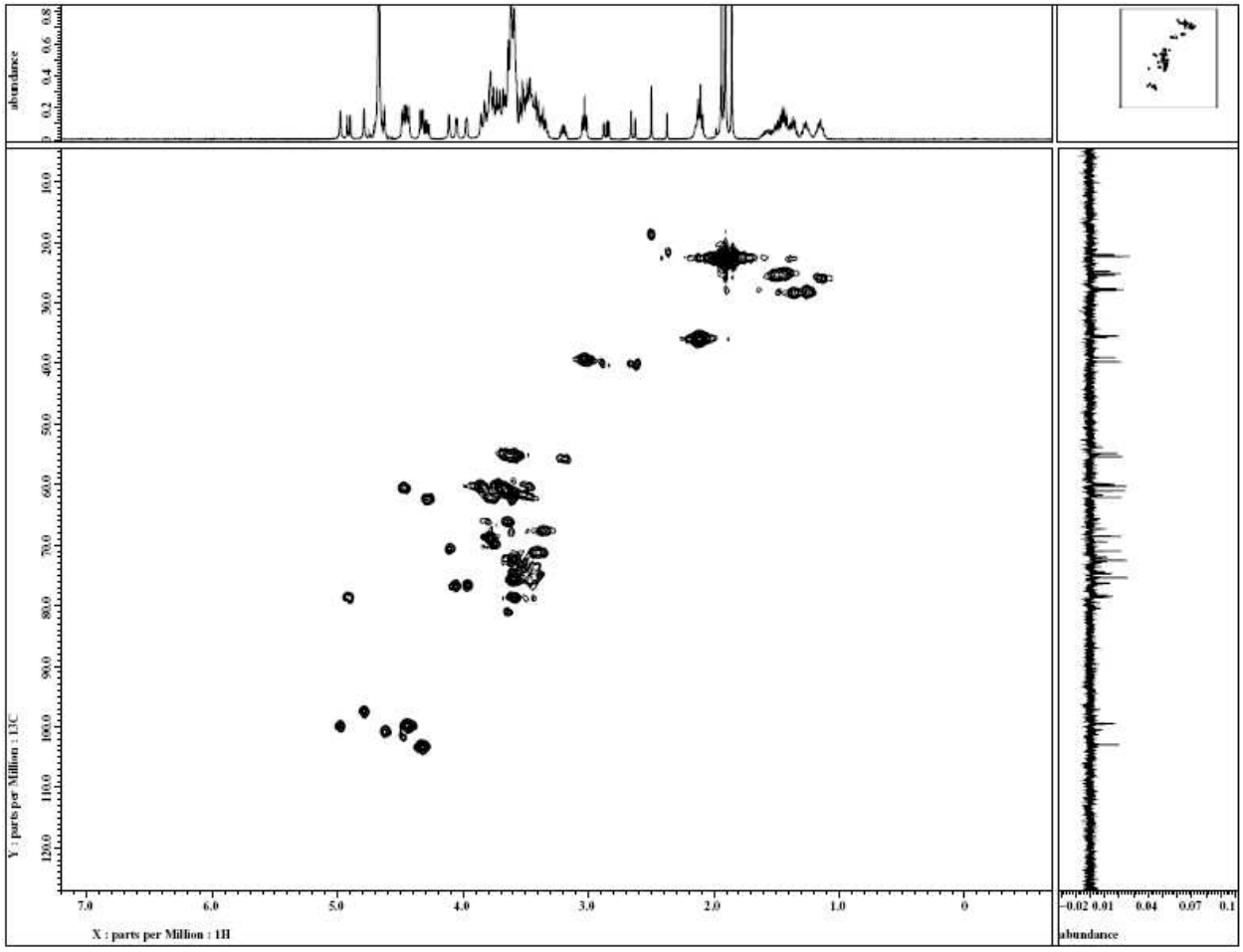
^{1}H NMR of CT-GlcNAc$_2$-LC-biotin (6)



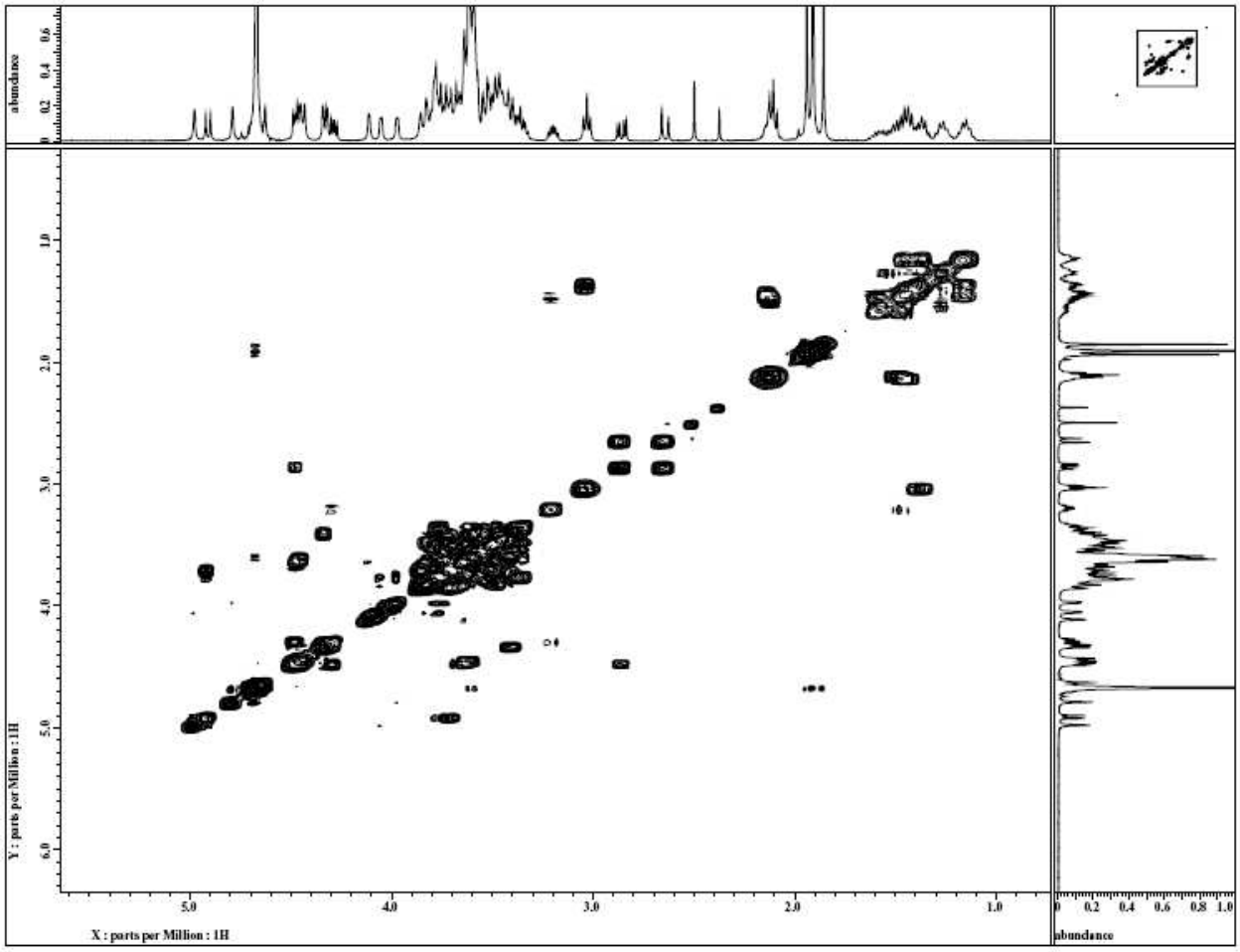
^{13}C NMR of CT-GlcNAc$_2$-LC-biotin (6)



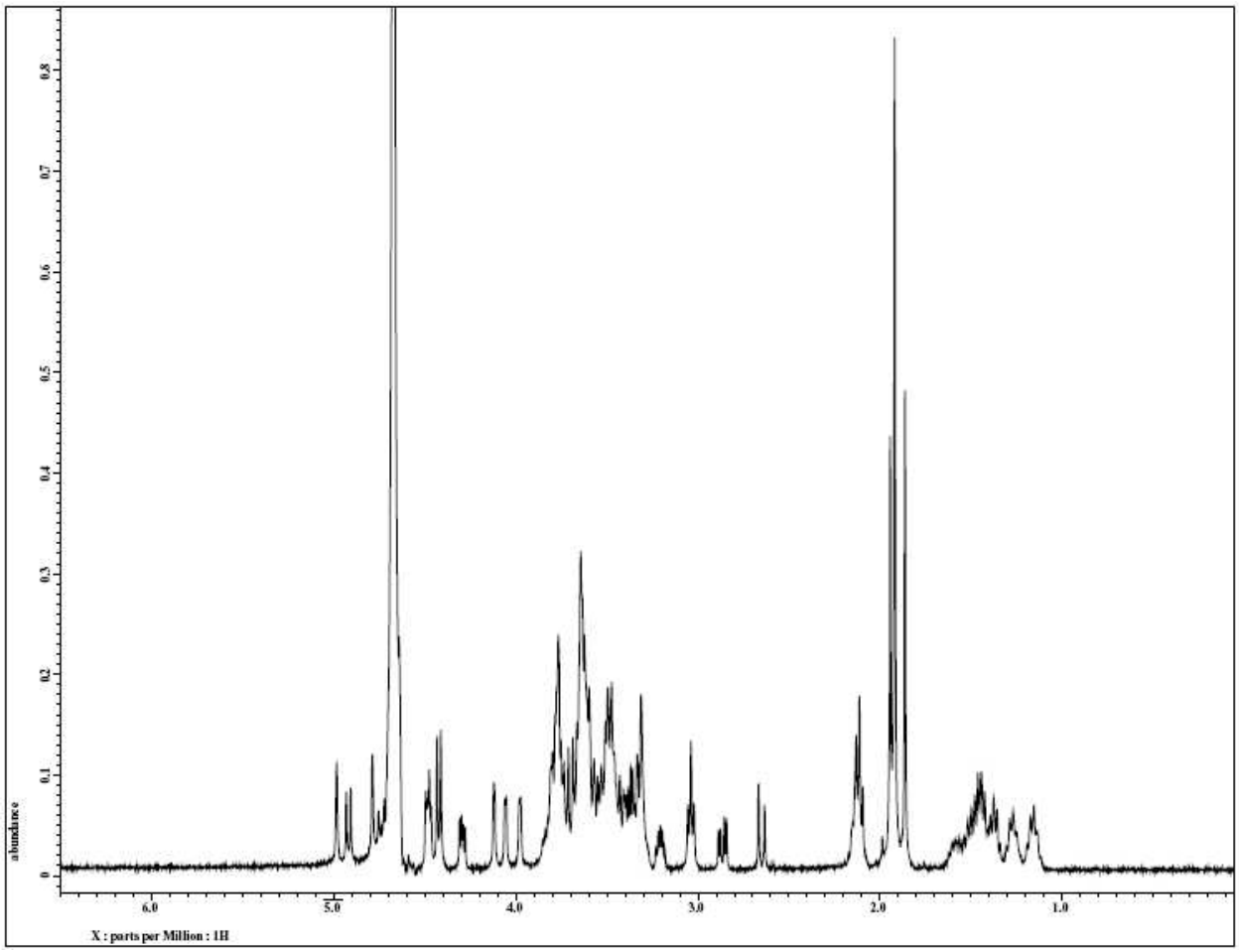
^{1}H-^{13}C HSQC NMR of CT-GlcNAc$_2$-LC-biotin (6)



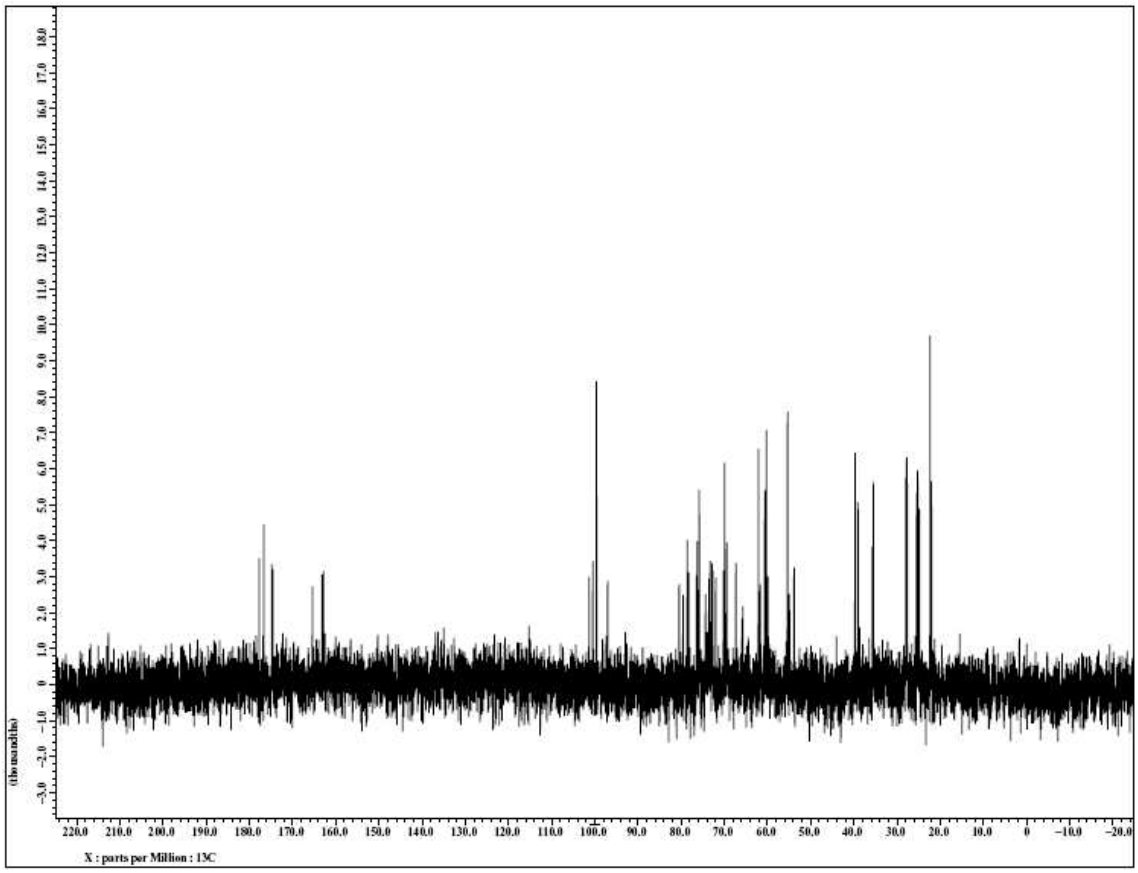
^{1}H-^{1}H COSY NMR of CT-GlcNAc$_2$-LC-biotin (6)



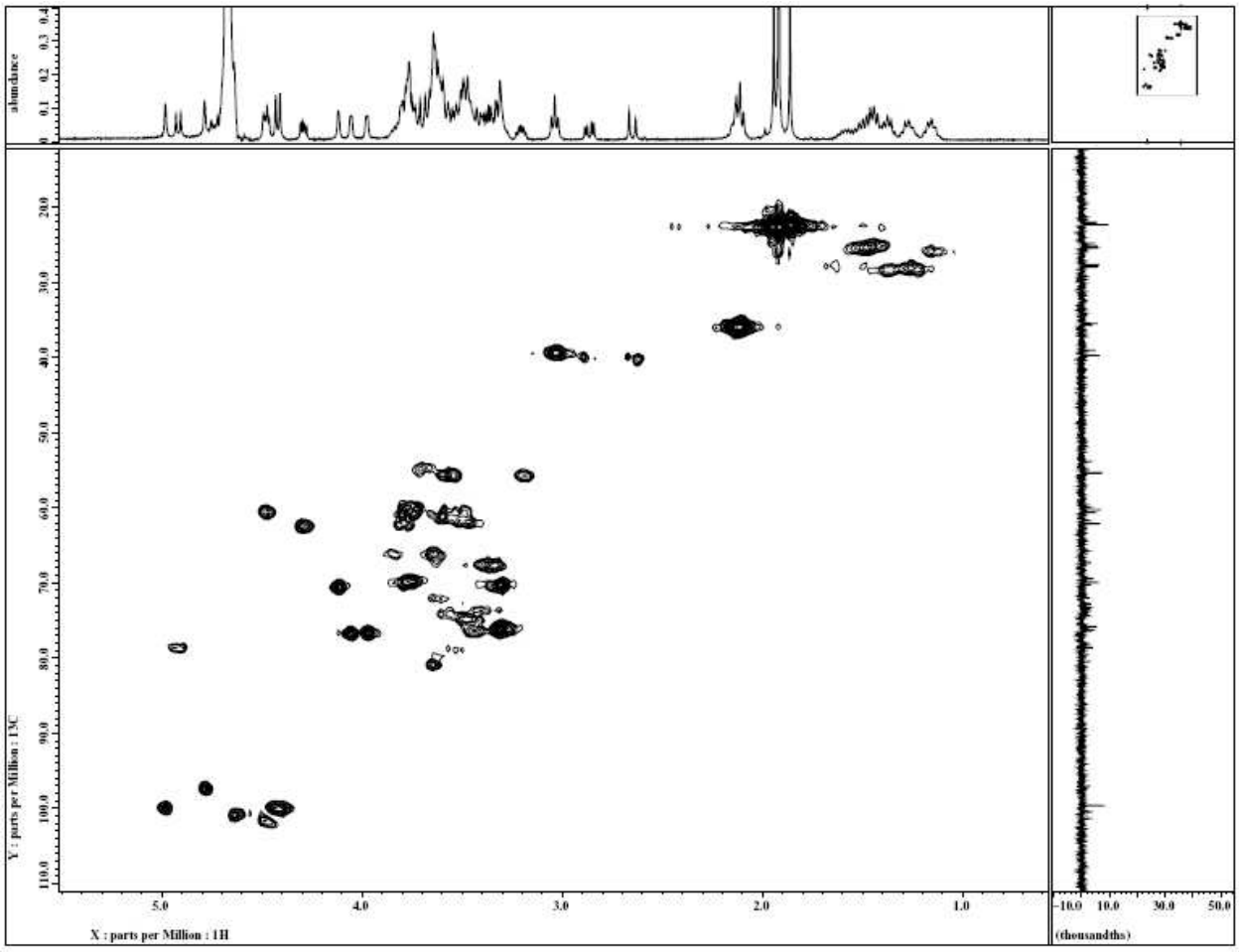
^{1}H NMR of $GlcNAc_2Man_3GlcNAc_2$-LC-biotin (7)



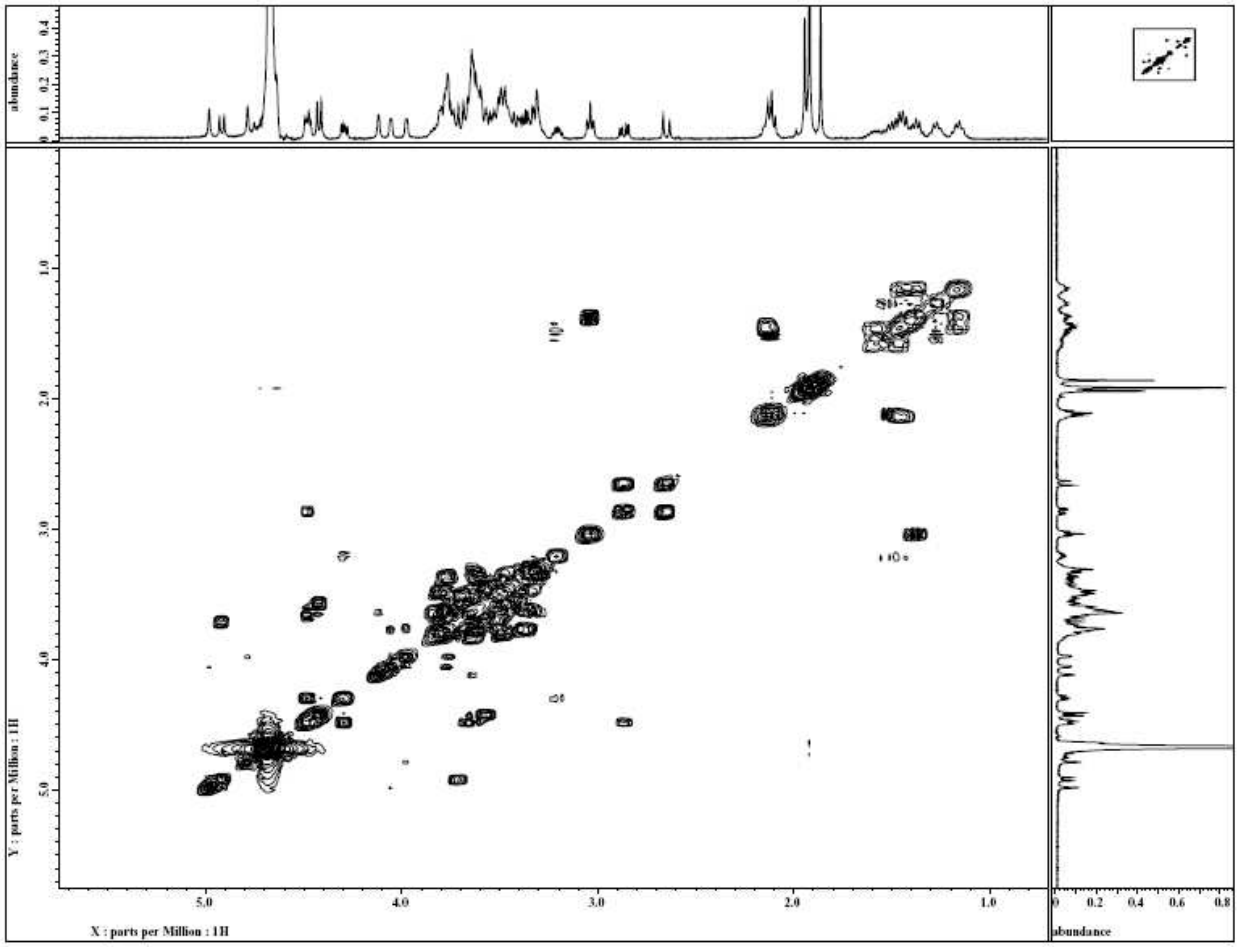
^{13}C NMR of $GlcNAc_2Man_3GlcNAc_2$-LC-biotin (7)



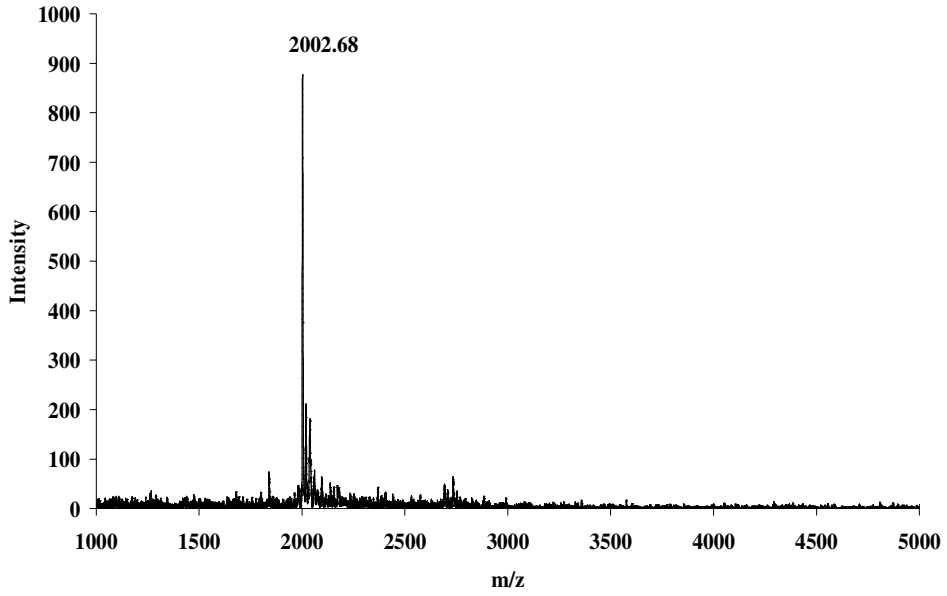
^{1}H-^{13}C HSQC NMR of $GlcNAc_2Man_3GlcNAc_2$-LC-biotin (7)



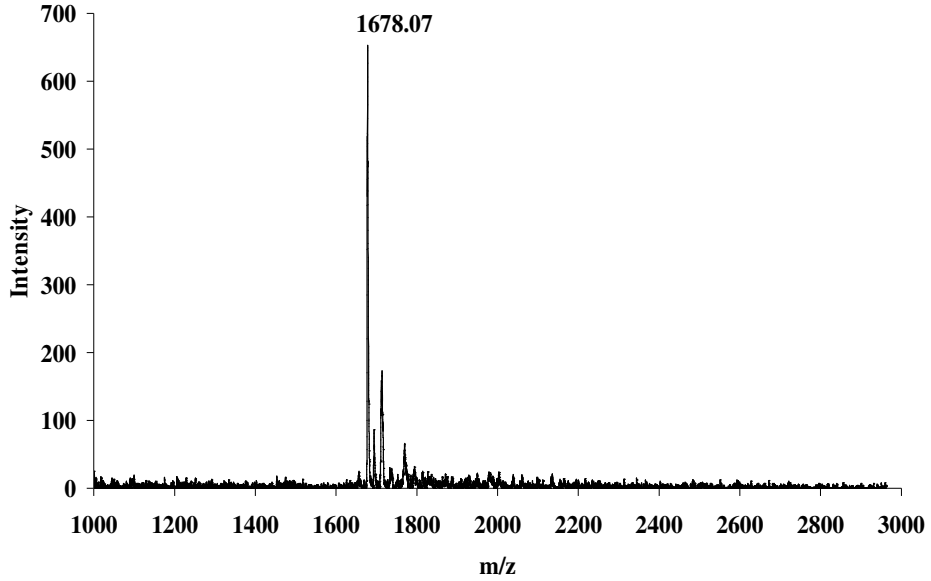
^{1}H-^{1}H COSY NMR of $GlcNAc_2Man_3GlcNAc_2$-LC-biotin (7)



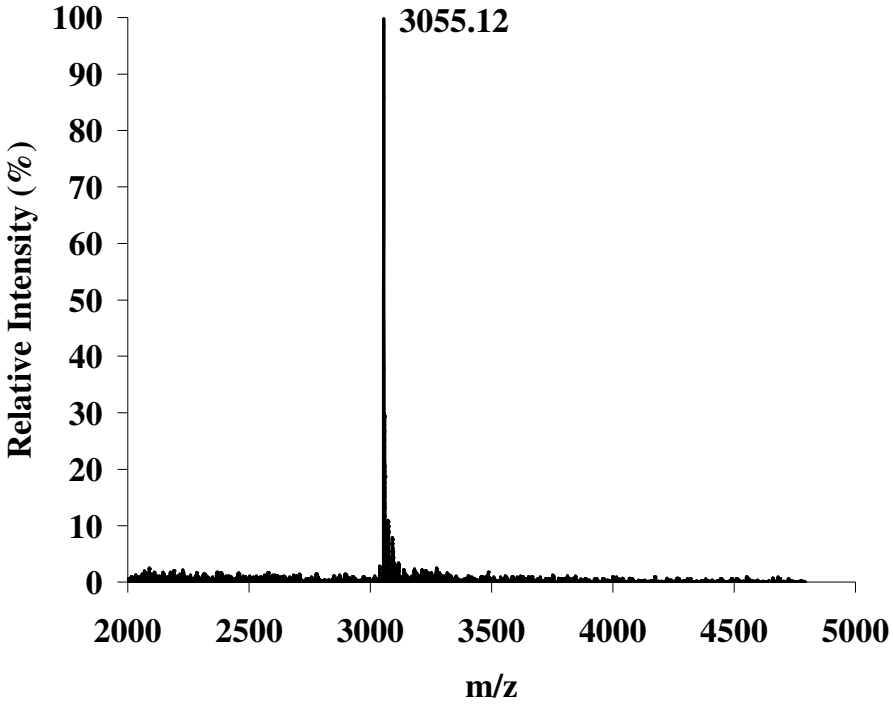
MALDI-TOF MS spectrum of CT-GlcNAc$_2$-LC-biotin (6)



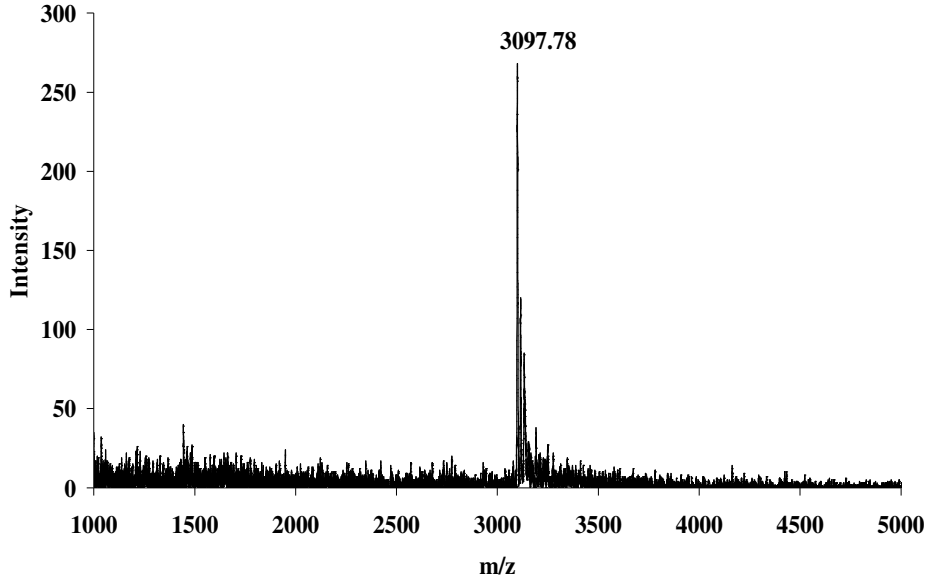
MALDI-TOF MS spectrum of $GlcNAc_2Man_3GlcNAc_2$-LC-biotin (7)



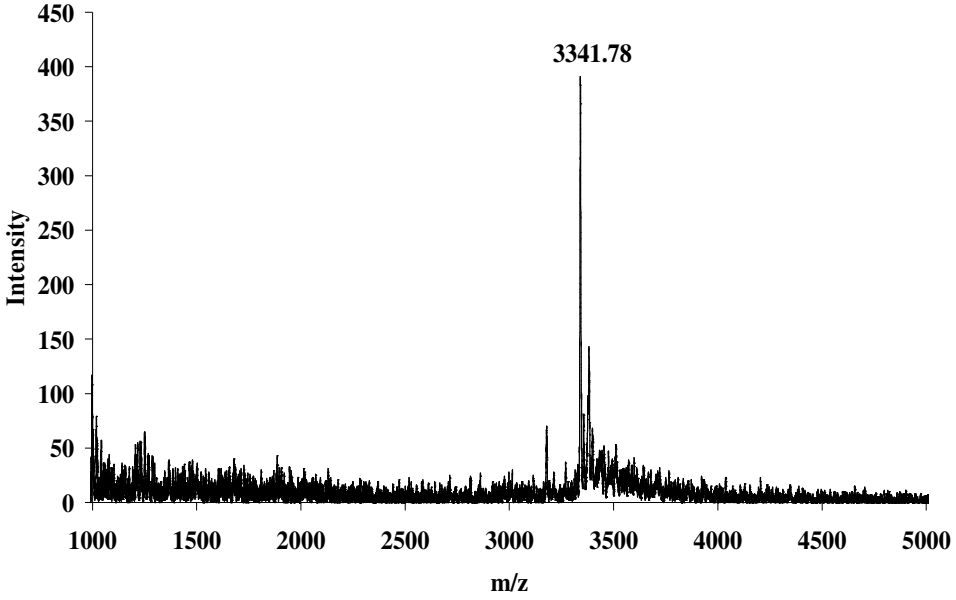
MALDI-TOF MS spectrum of $Man_3GlcNAc_2Man(1,6)$-[$Man_3GlcNAc_2Man(1,3)$]-$ManGlcNAc_2$-LC-biotin (9)



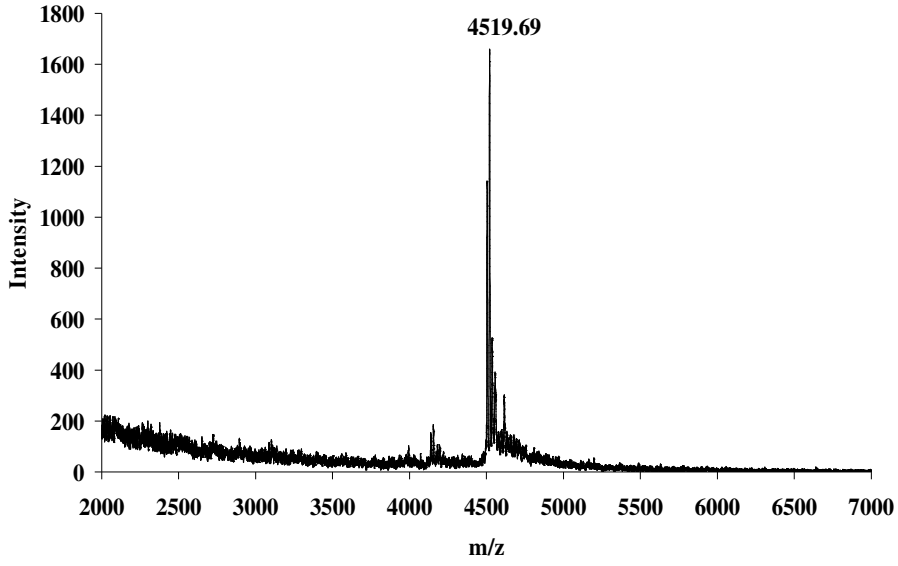
MALDI-TOF MS spectrum of CT-GlcNAc$_2$Man(1,6)-[GlcNAcMan(1,3)]-ManGlcNAc$_2$-LC-biotin (10a)



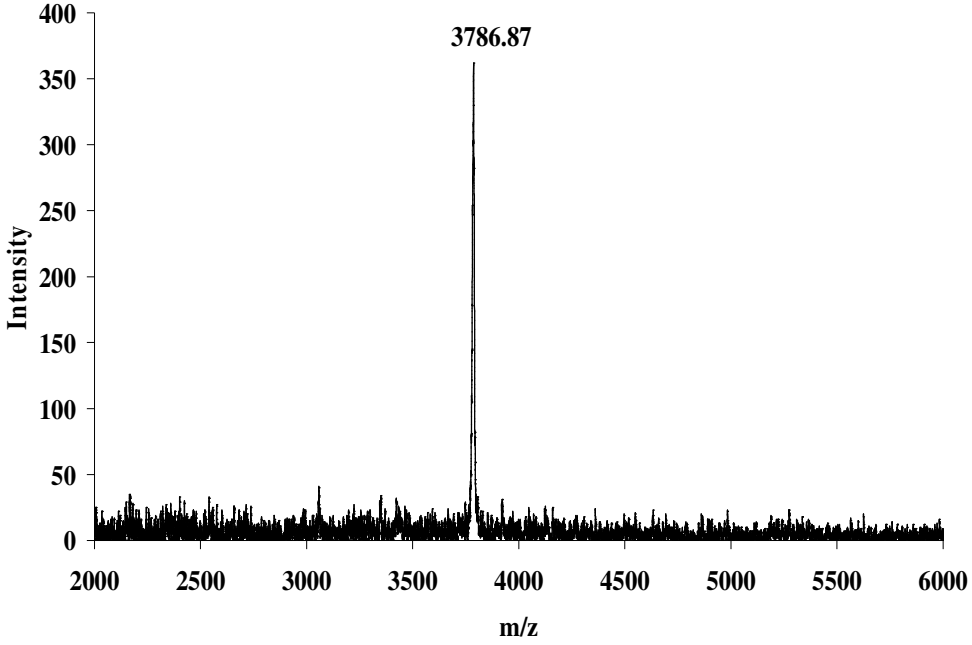
MALDI-TOF MS spectrum of $Man_9GlcNAc_2Man(1,6)$-[GlcNAcMan(1,3)]-$ManGlcNAc_2$-LC-biotin (12a)



MALDI-TOF MS spectrum of CT-GlcNAc$_2$Man(1,6)-[CT-GlcNAc$_2$Man(1,3)]-ManGlcNAc$_2$-LC-biotin (10c)

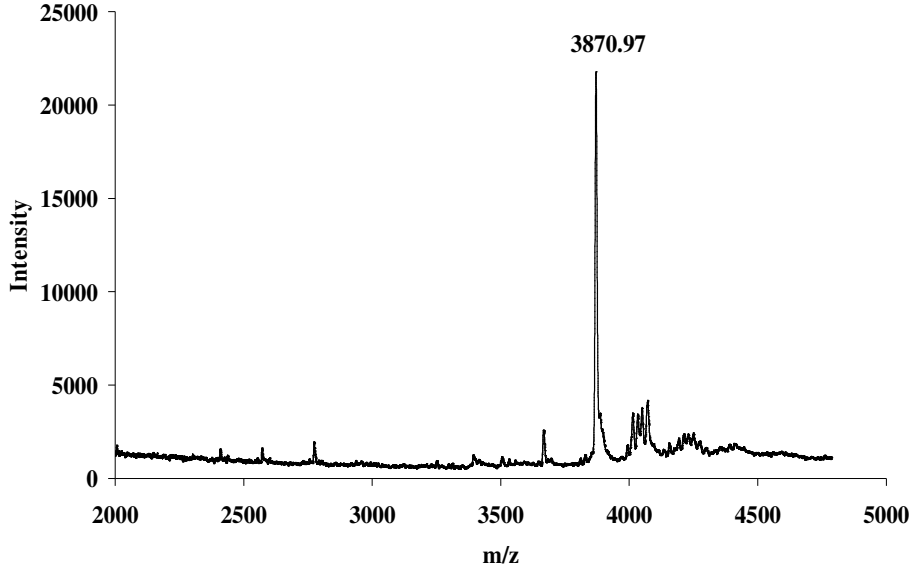


MALDI-TOF MS spectrum of CT-GlcNAc$_2$Man(1,6)-[Man$_3$GlcNAc$_2$Man(1,3)]-ManGlcNAc$_2$-LC-biotin (13)



MALDI-TOF MS spectrum of $GlcNAc_2Man_3GlcNAc_2Man(1,6)$-

$[GlcNAc_2Man_3GlcNAc_2Man(1,3)]$-$ManGlcNAc_2$-LC-biotin (14)



HPLC profiles of (a) glycan **6**; (b) glycan **7**; (c) glycan **9**; (d) glycan **10c**; (e) glycan **13**; (f) glycan **14**.