

Supplementary Information

Synthesis and Immunological Properties of *N*-Modified GM3 Antigens as Therapeutic Cancer Vaccines

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Additional Experimental Procedures

General Methods. NMR spectra were recorded on a 300 or 600 MHz NMR spectrometer with chemical shifts reported in ppm (δ) relative to Me₄Si (¹H), CDCl₃ (¹³C) and CFCl₃ (¹⁹F), respectively. Coupling constants (*J*) are reported in hertz (Hz). Thin layer chromatography (TLC) was performed on silica gel 60 GF₂₅₆ with the detection by charring with phosphomolibdic acid in EtOH or 5% H₂SO₄ in EtOH. Commercial solvents and reagents were directly used without further purification.

***O*-(2,6-di-*O*-Acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl Azide (5).** It was prepared as a white solid from lactose (4) in six steps and a 56% overall yield according a reported procedure [Xue, J., Pan, Y., and Guo, Z. (2002). *Tetrahedron Lett.* 43, 1599-1602]. **5:** R_f 0.46 (DCM and MeOH, 16:1); [α]_D -14 (*c* 1.0, CHCl₃); ¹H NMR (CDCl₃, 600 MHz): δ 5.18 (dd, 1 H, *J* 9.0, 9.0 Hz, H-3), 4.87 (dd, 1 H, *J* 9.6, 8.4 Hz, H-2'), 4.86 (dd, 1 H, *J* 9.6, 7.8 Hz, H-2), 4.61 (d, 1 H, *J* 9.0 Hz, H-1'), 4.50 (dd, 1 H, *J* 12.0, 1.8 Hz, H-6'), 4.34 (d, 1 H, *J* 7.8 Hz, H-1), 4.35 (dd, 1 H, *J* 11.4, 6.0 Hz, H-6), 4.22 (dd, *J* 11.4, 6.6 Hz, H-6), 4.17 (dd, 1 H, *J* 12.0, 3.6 Hz, H-6'), 3.85 (bs, 1 H, H-4'), 3.76 (dd, 1 H, *J* 9.0, 9.6 Hz, H-4), 3.72 (m, 1 H, H-5), 3.62 (dd, 1 H, *J* 6.6, 6.6 Hz, H-5'), 3.59

(m, 1 H, H-3'), 3.42 (d, 1 H, J 7.4 Hz, OH), 3.32 (d, 1 H, J 4.8 Hz, OH), 2.12, 2.11, 2.10, 2.07, 2.04 (5 s, $5 \times 3\text{H}$, Ac). ^{13}C NMR (CDCl_3 , 50M Hz): δ 171.4, 171.0, 170.5, 170.4, 169.5, 100.9, 87.8, 75.9, 75.0, 73.5, 72.7, 72.5, 72.4, 70.8, 68.5, 62.4, 62.0, 20.9, 20.8, 20.8, 20.8, 20.6; FABMS: calcd for $\text{C}_{26}\text{H}_{43}\text{N}_4\text{O}_{17}$ $[\text{M} + \text{DEA} + \text{H}]^+$ (DEA: diethanolamine, the matrix) 683.2623, found 683.2613.

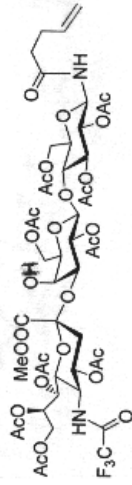
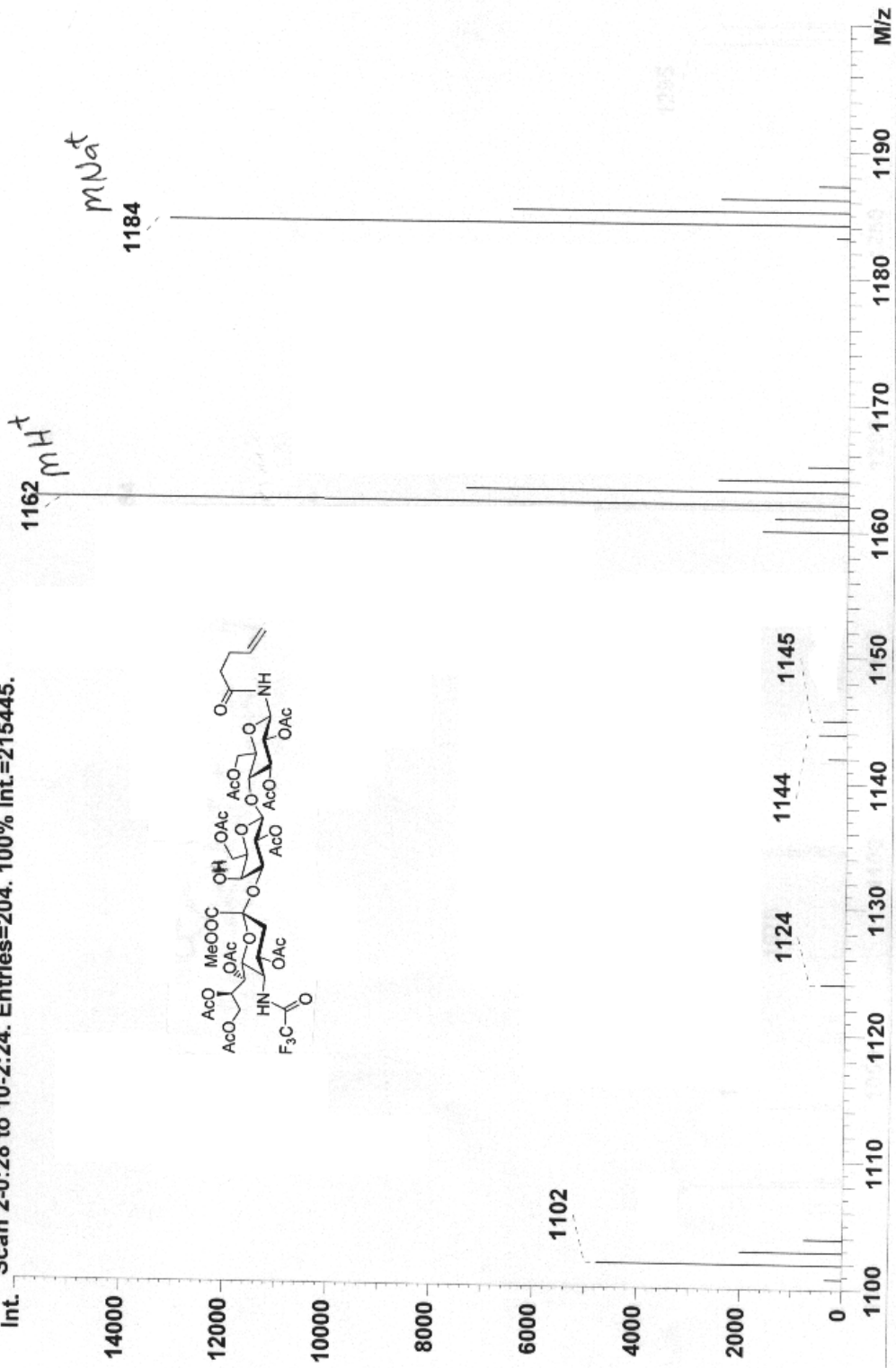
Methyl (Ethyl 4,7,8,9-Tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-*D*-glycero- α -*D*-galacto-non-2-ulopyranosid)onate (8). After a solution of **7** (7.4g, 13.6 mmol) and MsOH (5 mL) in anhydrous MeOH (50 mL) was refluxed for 24 h, Et_3N was added to neutralize the reaction to pH 8. It was concentrated under reduced pressure. The residue was redissolved in methanol (30 mL), and to the solution was added methyl trifluoroacetate (3.0 mL) at 0 °C. The solution was stirred at rt overnight and concentrated. The residue was dissolved in pyridine (30 mL) and acetic anhydride (15 mL) and stirred at rt overnight. After concentration in a vacuum, the product was purified by column chromatography to afford **8** (6.8 g, 84%; $\alpha:\beta = 1:5$) as a white crystalline product. A part of the β -isomer could be isolated in the pure form. **8 β** : $[\alpha]_{\text{D}} -57.1$ (c 1.2, CHCl_3); ^1H NMR (CDCl_3 , 300MHz): δ 7.54 (d, 1 H, J 10.0 Hz, NH), 5.39 (m, 2 H, J 2.8, 6.8, 4.9 Hz, H-4,7), 5.07 (m, 1 H, J 2.5, 2.7, 8.4 Hz, H-8), 4.80 (dd, 1 H, J 12.4, 2.2 Hz, H-9a), 4.55 (dd, 1H, J 10.4, 2.3 Hz, H-6), 4.17 (dd, 1 H, J 12.4, 8.2 Hz, H-9b), 4.05 (dd, 1H, J 11.5, 10.4 Hz, H-5), 3.78 (s, 3 H, OCH_3), 2.45-2.61 (m, 3 H, H-3e, SCH_2CH_3), 2.11, 2.07, 2.02, 2.00 (4s, 4×3 H), 1.17 (t, 3 H, J 7.5 Hz, SCH_2CH_3); ^{19}F NMR (CDCl_3): δ -76.8 (s); ^{13}C NMR (CDCl_3 , 50M Hz): δ 171.5, 171.2, 170.6, 169.9, 168.3, 158.1, 115.6, 85.0, 72.9, 71.2, 68.8, 68.4, 62.4, 52.9, 50.2, 37.3, 22.8, 21.0, 20.7, 20.6, 20.5, 14.0; HR-FABMS: calcd for $\text{C}_{22}\text{H}_{31}\text{F}_3\text{NO}_{12}\text{S}$ $[\text{M} + \text{H}]^+$ 590.1519, found 590.1541.

Selected NMR and MS Spectra

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 Creation Date/Time : 10/29/02 at 12:29:59
 File Type : Lo-Res Mass Data (Centroid)
 File Title : 0460 Yanbin Pan #1 LR FAB mag (1200) 10/29/02
 Description 1 : 2ul sample (in 20ul acetone) & 3ul NBA. Both slits on
 Description 2 : position #3, filter = 100 Hz, set R = 2000.

SCAN GRAPH. Flagging=M/z.

Int. Scan 2-0:28 to 10-2:24. Entries=204. 100% Int.=215445.



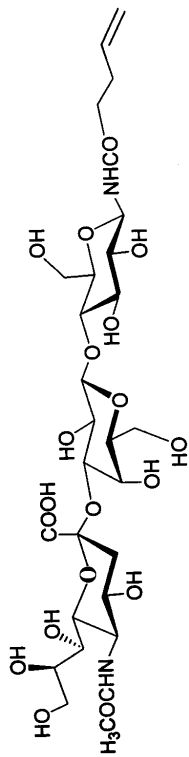
Compound 11a:

Archive directory: /support/home/guo/vnmrsys/data
 Sample directory: AGRM3_05NOV2002
 File: PROTON

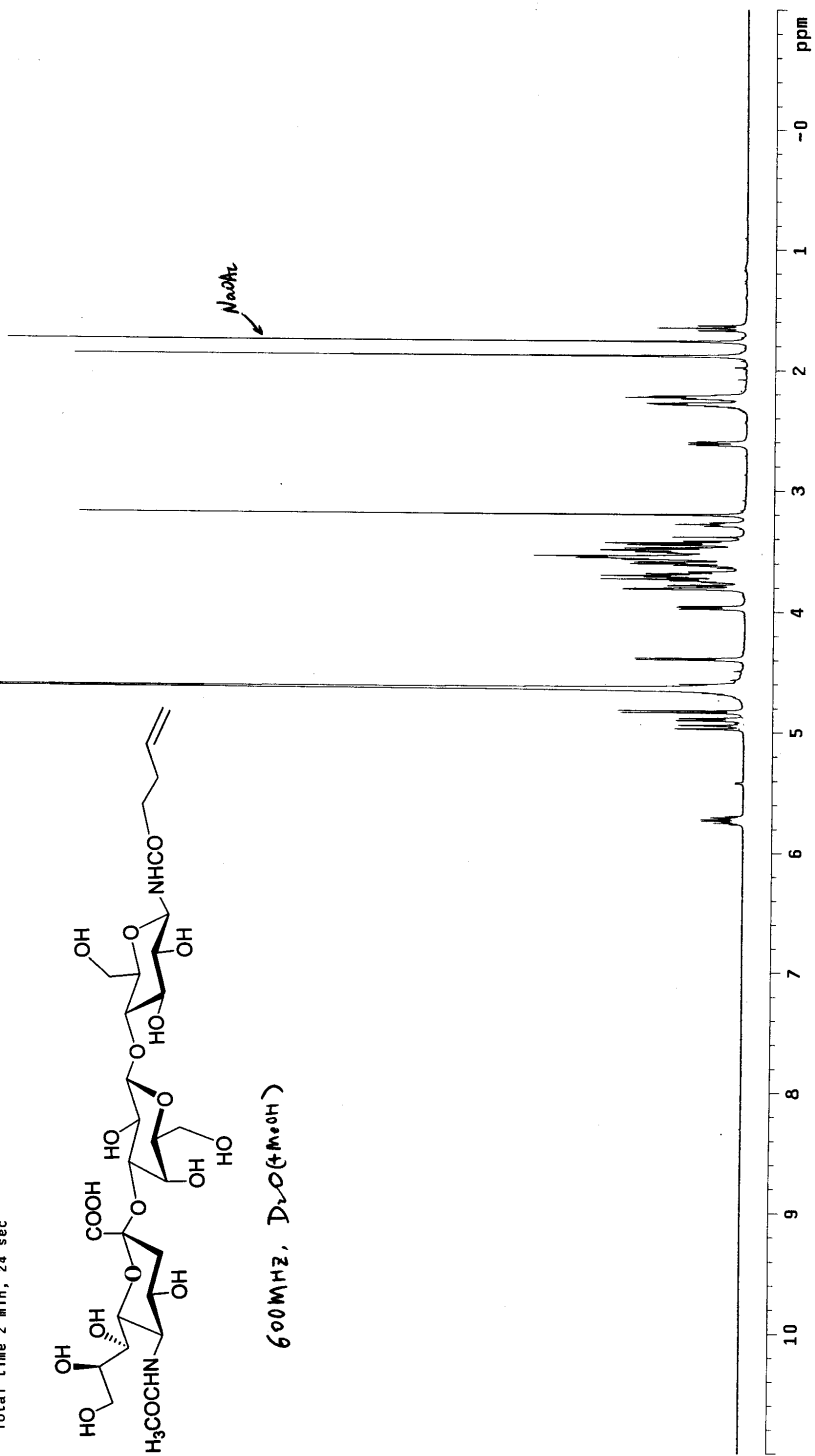
Pulse Sequence: s2pu1
 Solvent: D2O
 Temp.: 25.0 C / 298.1 K
 INOVA-600 "Jarry"

Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 3.495 sec
 Turn/200.7 Hz
 32

OBSERVE F1 500.130796 MHz
 DATA PROCESSING
 FT size 65536
 Total time 2 min, 24 sec

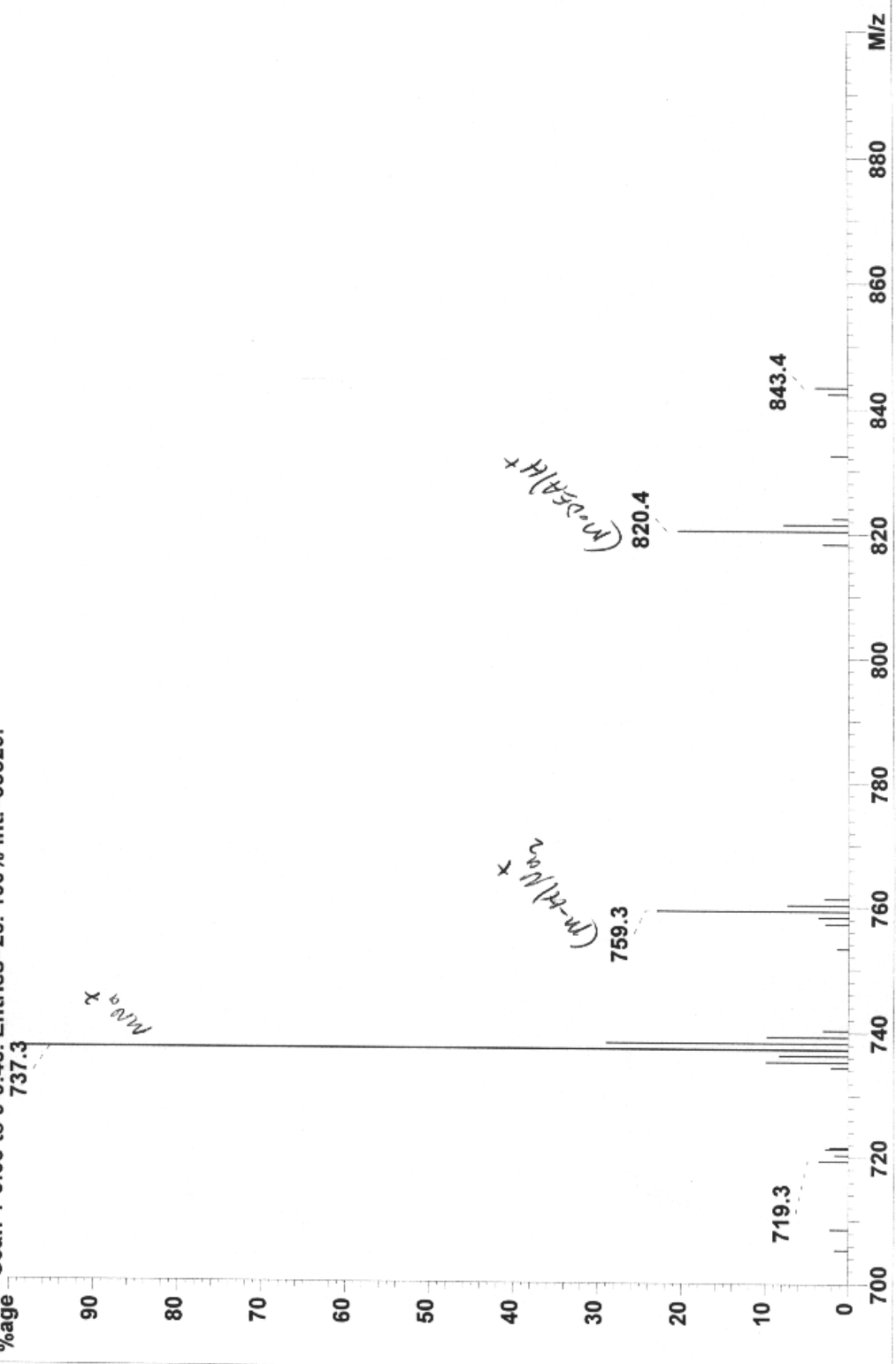


600MHz, D₂O (4MeOH)

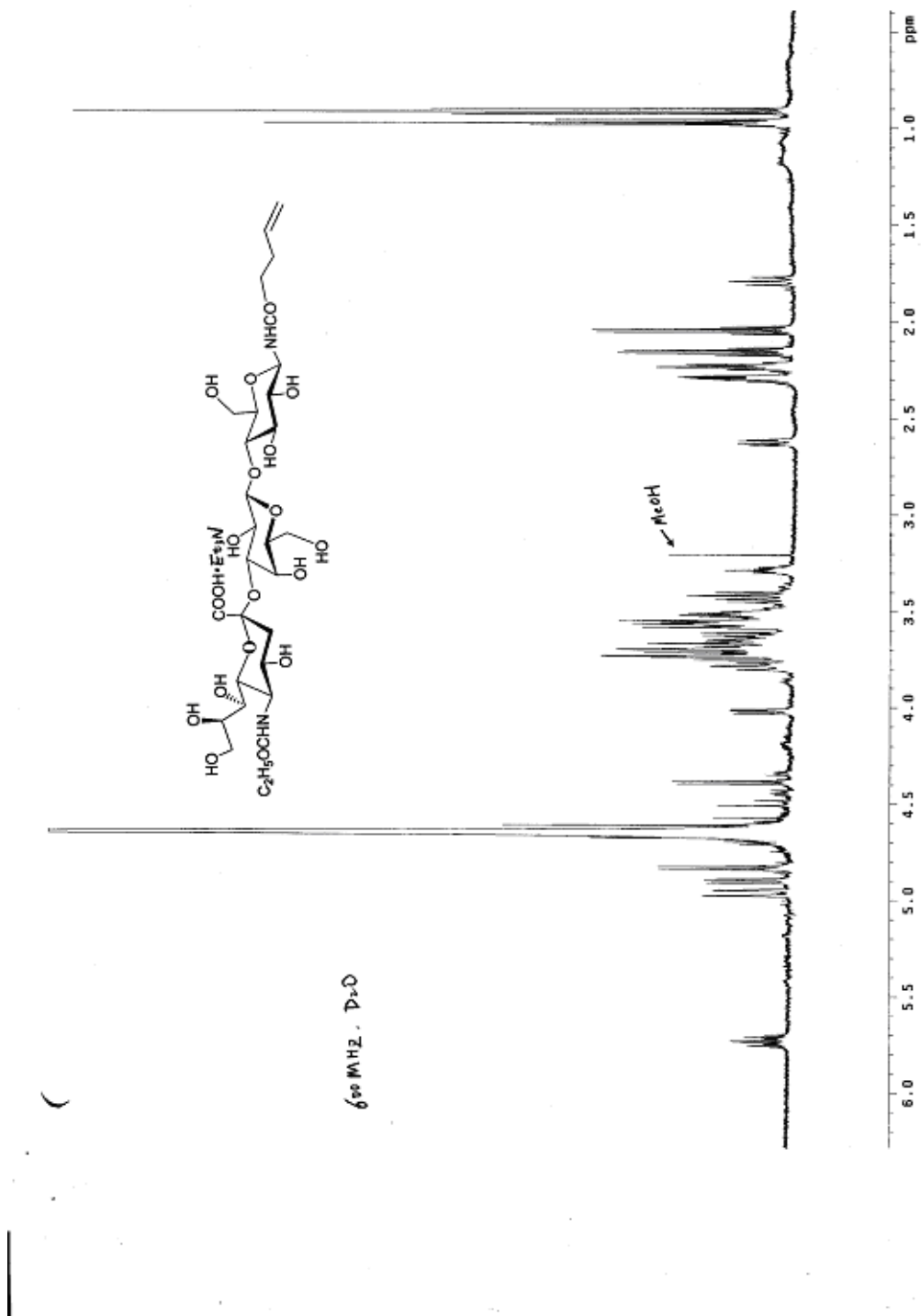


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File Type : Lo-Res V/E Scan Data
File Title : MR FAB v/e (1200) 12/5/02 YANBIN PAN
Description 1 : 4ul sample (in 10ul MeOH) & 3ul DEA

SCAN GRAPH. Flagging=M/Z.
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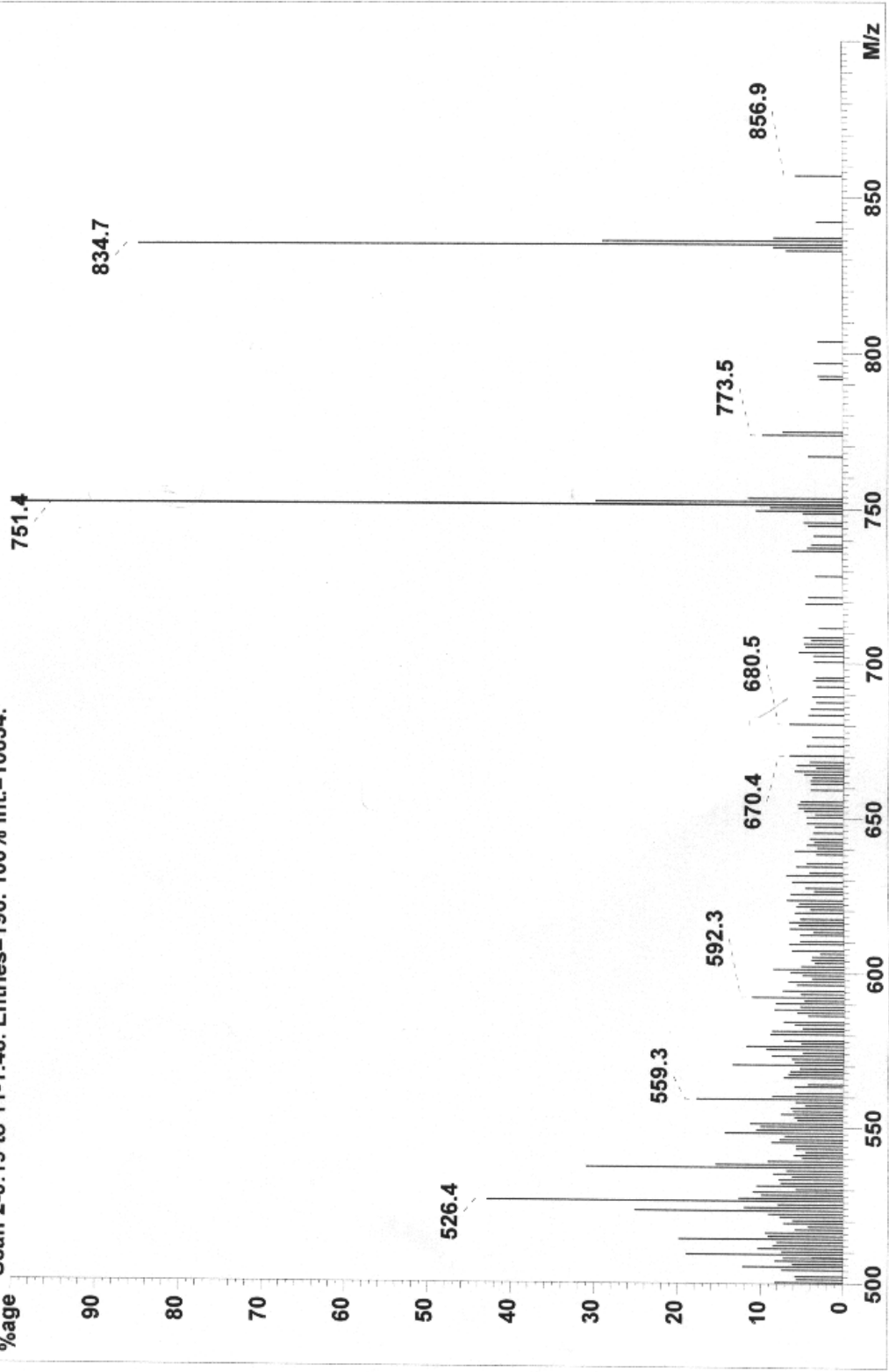
Compound 11b:



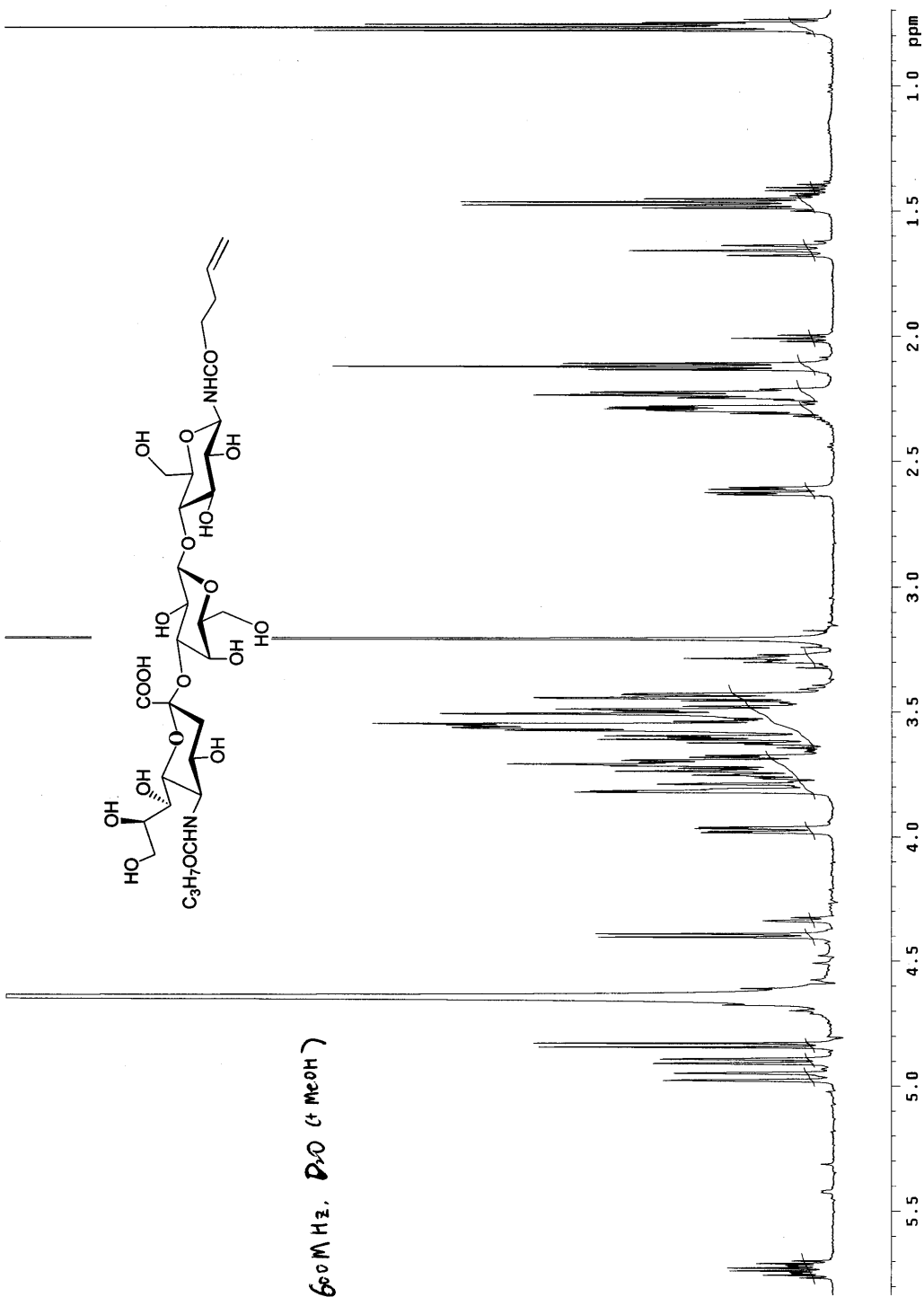
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Creation Date/Time : 12/5/02 at 14:00:02
File Type : Lo-Res Mass Data (Centroid)
File Title : PPrGM3 Yanbin Pan #1 LR FAB mag (1200) 12/5/02
Description 1 : 3ul sample (in 10ul MeOH) & 3ul DEA

SCAN GRAPH. Flagging=M/z.

%age Scan 2-0:19 to 11-1:48. Entries=196. 100% Int.=10654.



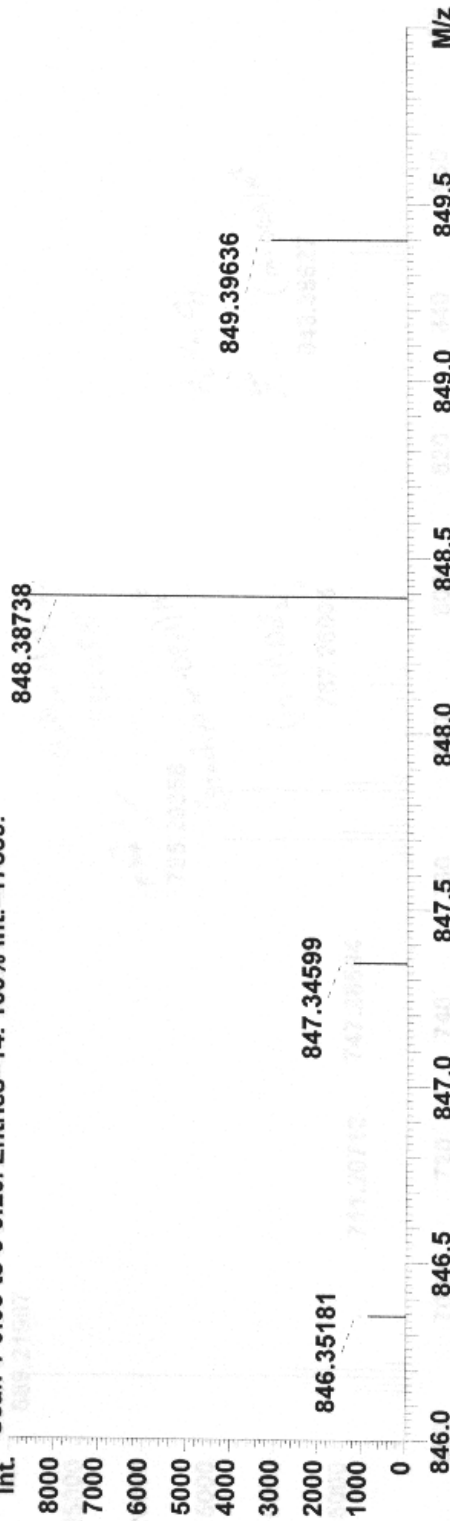
Compound 11c:



File Name : c:\mswin\data\inbugm302.mss
 Creation Date/Time : 12/6/02 at 11:54:10
 File Type : Hi-Res V/E Scan Data
 File Title : MBuGM3 Yanbin Pan #2 HR FAB v/e 12/6/02
 ESA calib range : 206-266 Da using CsI/NaI/Glycerol
 ESA m/z range : 770-900 Da. Locked on 772.3164 Da of Stachyose-DEAH+
 Matrix : 3ul DEA & 1ul Stachyose (52ug/ul water)

SCAN GRAPH. Flaggings=M/z.

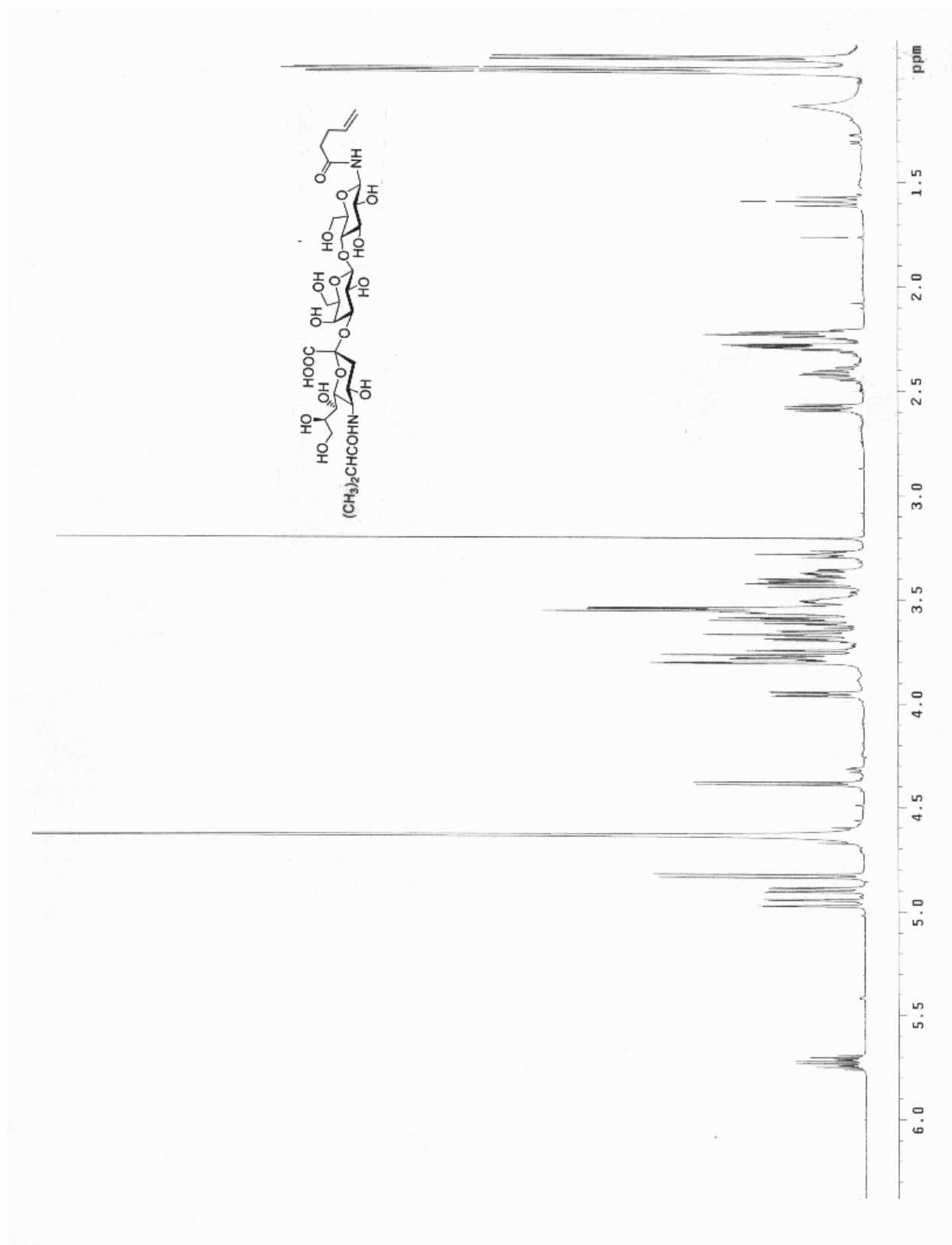
Scan 1-0:03 to 6-0:20. Entries=14. 100% Int.=17559.



SCAN TEXT. Filter=[Range:846-850. Defect:0-0.5. Excl: Ref/Ex.].
Scan 1-0:03 to 6-0:20. Sorted on M/z (ascending). Entries=4.

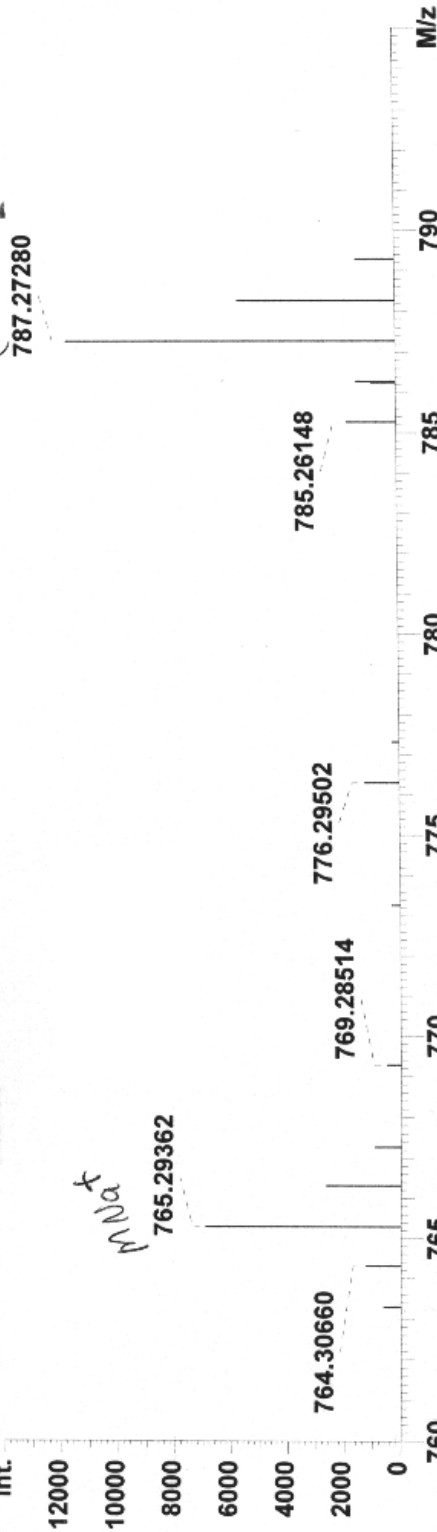
M/z	Int.	Width	Age
846.35181	846	14	4.82
847.34599	1210	17	6.89
848.38738	8569	22	48.80
849.39636	3083	18	17.56

Compound **11d**:



File Name. : c:\mswin\data\libugm02.mss
 Creation Date/Time : 12/6/02 at 13:58:20
 File Type : Hi-Res V/E Scan Data
 File Title : IBuGM Yanbin Pan #2 HR FAB v/e 12/6/02
 ESA calib range : 206-266 Da using CsI/NaI/Glycerol
 ESA m/z range : 687-800 Da. Locked on 689.2197 Da of StachyoseNa+
 Matrix : 3ul DEA & 2ul Stachyose (52ug/ul water)

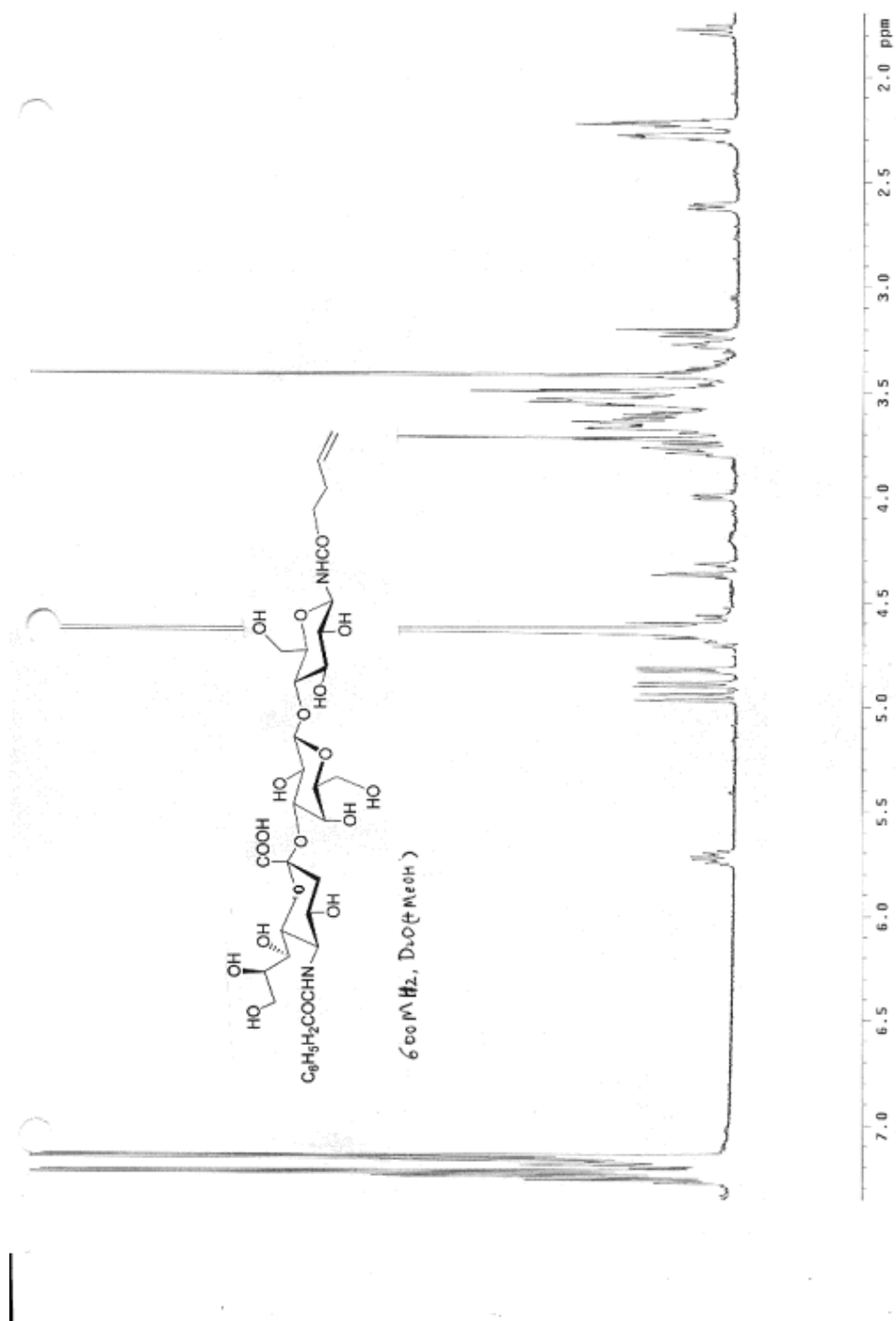
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SCAN TEXT. Filter=[Range:760-795. Defect:0.25-0.32. Excl: Ref/Ex.].
 Scan 1-0:03 to 4-0:13. Sorted on M/z (ascending). Entries=15.

M/z	Int.	M/z	Width	%age
763.29212	622	770	15	m
764.30660	1232	775	12	m
765.29362	6920	780	26	m
766.31066	2645	785	21	m
767.27483	934	790	12	m
769.28514	482	795	12	m
773.26358	284		11	m
776.29502	1208		20	m
777.31408	241		10	m
785.26148	1750		23	m
786.25194	862		18	m
787.27280	11657		18	m
788.27489	5584		24	m
789.28932	1369		22	m

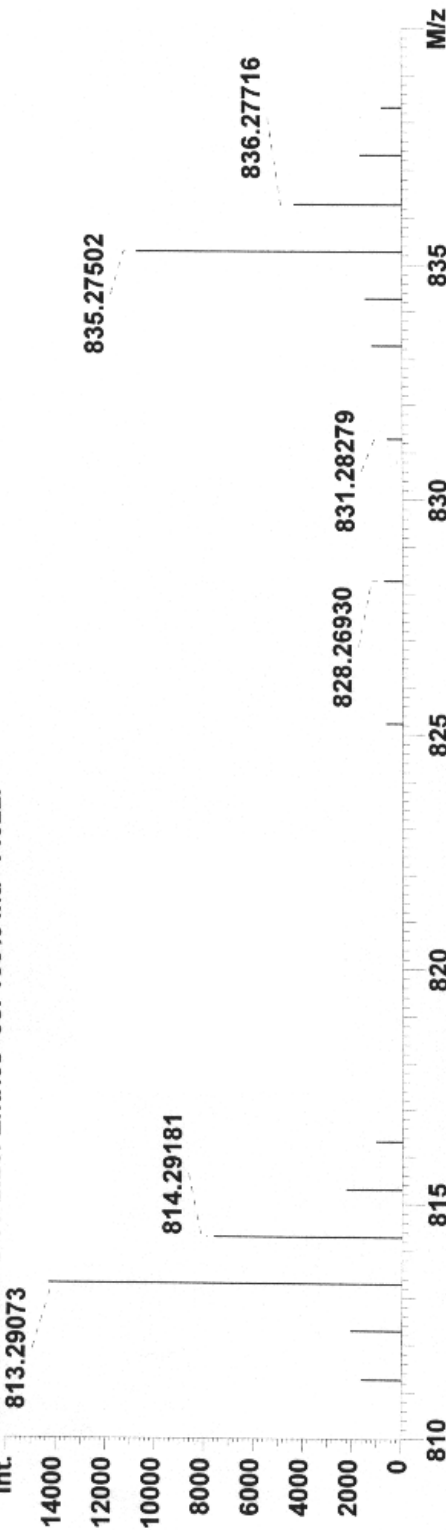
Compound 11e:



File Name : c:\mswin\data\phacgm02.mss
 Creation Date/Time : 12/6/02 at 13:19:31
 File Type : Hi-Res V/E Scan Data
 File Title : PhAcGM Yanbin Pan #2 HR FAB v/e 12/6/02
 ESA calib range : 206-266 Da using CsI/NaI/Glycerol
 ESA m/z range : 770-920 Da. Locked on 772.3164 Da of (Stachyose-DEA)H+
 Matrix : 3ul DEA & 4ul Stachyose (52ul/ug water)

SCAN GRAPH. Flagging=M/z.

Scan 31-1:55 to 39-2:25. Entries=38. 100% Int.=14322.



SCAN TEXT.Filter=[Range:810-840. Defect:0-0.5.]

Scan 31-1:55 to 39-2:25. Sorted on M/z (ascending). Entries=15.

M/z	Int.	Width	Age
811.26403	1608	17	m
812.28529	2052	19	14.33
813.29073	14322	24	100.00
814.29181	7607	25	53.11
815.29580	2256	18	m
816.31687	1062	17	7.42
825.22976	657	14	m
828.26930	714	12	m
831.28279	593	12	m
833.25946	1228	17	m
834.27296	1482	21	10.35
835.27502	10732	24	74.93
836.27716	4364	20	m
837.30560	1681	16	11.74
838.31209	788	15	m

File Name : c:\mswin\data\phacgm01.mss
Creation Date/Time : 12/6/02 at 12:59:44
File Type : Lo-Res Mass Data (Centroid)
File Title : PhAcGM Yanbin Pan #1 LR FAB mag (1200) 12/6/02
Description 1 : 3ul sample (in 20ul MeOH) & 3ul DEA

SCAN GRAPH. Flagging=M/z.
Scan 2-0:16 to 6-0:50. Entries=669. 100% Int.=32826.

