CHEMISTRY
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Supporting Information

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Bacterial surface engineering utilizing glucosamine phosphate derivatives as cell wall precursor surrogates

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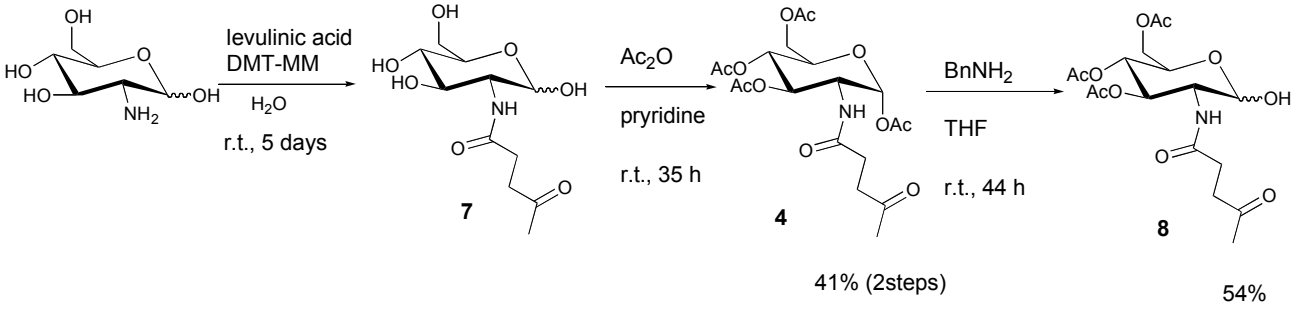
Synthesis

3,4,6-tri-O-acetyl-2-*N*-acetyl-α-D-glucosamine

To a solution of per acetyl GlcNAc (1 g, 2.57 mmol) in THF (20 mL) was added benzylamine (700 μL, 6.43 mmol), and the mixture was stirred for 15 h at 20 °C. under nitrogen. The reaction mixture was then diluted with toluene and concentrated in vacuo. The residue was purified by silica-gel chromatography (EtOAc) to give the target compound (463 mg, 52%).

Dibenzyl-3,4,6-tri-O-acetyl-2-*N*-acetyl-α-D-glucosamine phosphate

3,4,6-tri-O-acetyl-2-*N*-acetyl-α-D-glucosamine (107 mg, 0.308 mmol) was dissolved in toluene and dried by azeotropy. To a solution of the residue in anhydrous CH_2Cl_2 (8 mL) was added 1*H*-tetrazole (80.8 mg, 1.15 mmol) under nitrogen. The mixture was cooled at -30 °C, and then dibenzyl-*N,N*-diethyl phosphamide (203 μL, 0.576 mmol) was added. After stirring for 30 min at -30 °C and for 1 h at 20 °C, the mixture was cooled at -40 °C. m-CPBA (324 mg, 1.44 mmol) was then added and the reaction mixture was stirred for 30 min at 0 °C and for 30 min at 20 °C. The reaction mixture was diluted with $CHCl_3$ and washed successively with 10% aqueous Na_2SO_3, saturated aqueous $NaHCO_3$ and water. The $CHCl_3$ layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel chromatography (EtOAc) to give the target compound (128 mg, 68%).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Ac_2O
pyridine
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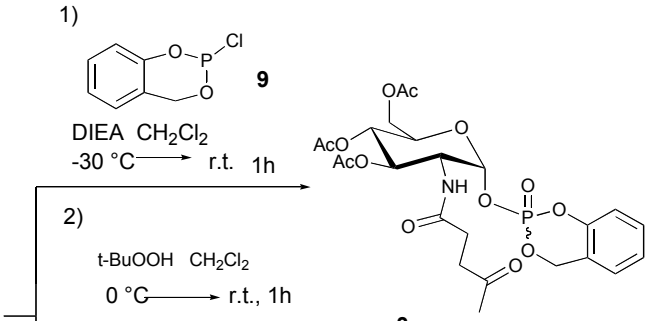
THF
r.t., 44 h
8
54%


1)
9
DIEA CH_2Cl_2
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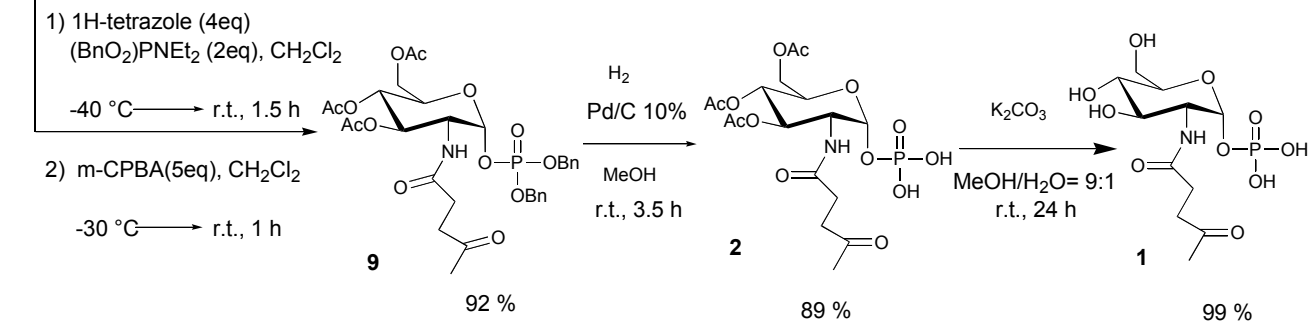
2)
t-BuOOH CH_2Cl_2
0 °C → r.t., 1h
3
54 %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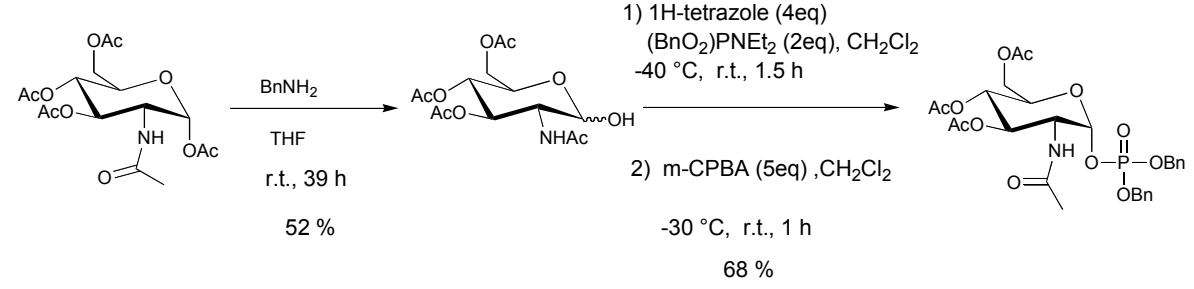
2) m-CPBA(5eq), CH_2Cl_2
-30 °C → r.t., 1 h
9
92 %
H_2
Pd/C 10%
MeOH
r.t., 3.5 h
2
89 %
K_2CO_3
MeOH/H_2O= 9:1
r.t., 24 h
1
99 %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THF
r.t., 39 h
52 %
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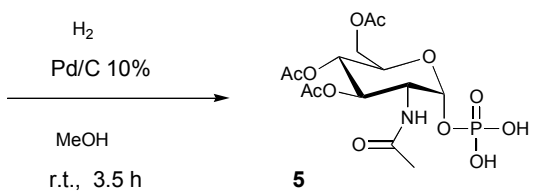
$(BnO_2)PNEt_2$ (2eq), CH_2Cl_2
-40 °C, r.t., 1.5 h
2) m-CPBA (5eq) ,CH_2Cl_2
-30 °C, r.t., 1 h
68 %


H_2
Pd/C 10%
MeOH
r.t., 3.5 h
5
39 %

3,4,6-tri-O-acetyl-2-*N*-acetyl-α-D-glucosamine phosphate (5)

To a solution of dibenzyl-3,4,6-tri-O-acetyl-2-*N*-acetyl-α-D-glucosamine phosphate (128 mg, 0.211 mmol) in MeOH (10 mL) was added 10% Pd/C (catalytic amount). After stirring for 4 h at 20 °C. under hydrogen, the reaction mixture was filtered with celite. The filtrate was concentrated in vacuo, and the residue was dried under reduced pressure. The residue was purified by silica-gel chromatography (EtOAc/EtOH/water = 3:2:1) to give **5** (35.4 mg, 39%).

2-*N*-levulinoyl-D-glucosamine (7)

To a solution of glucosamine hydrochloride (4 g, 18.6 mmol) in H_2O (20 mL) was added levulinic acid (1.90 mL, 18.6 mmol), and the mixture was neutralized with 1 M NaOH. DMT-MM (6.14 g, 22.32 mmol) was then added and the mixture was stirred for 30 h at 20 °C. The reaction mixture was then filtered and the filtrate was concentrated in vacuo. After drying under reduced pressure, the residue was subjected without further purification to the following reaction.

1, 3, 4, 6-tetra-*O*-acetyl-2-*N*-levulinoyl-α-D-glucosamine (4)

The crude **7** was dissolved in pyridine (100 mL) and cooled to 0 °C, and acetic anhydride (53.0 mL, 560 mmol) was then added to the solution. After stirring for 22 h at 20 °C, the reaction mixture was evaporated and the residue was purified by silica-gel chromatography ($CHCl_3$:MeOH = 50:1) to yield **4** (5.67 g, 27%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ=6.14 (d, *J*=3.7 Hz, 1H) (H-1), 5.96 (d, *J*=9.1 Hz, 1H) (NH), 5.27 (t, *J*=10.2 Hz, 1H) (H-3), 5.18 (t, *J*=9.8 Hz, 1H) (H-4), 4.45-4.41 (m, 1H) (H-2), 4.25 (dd,

J=4.1, 12.5 Hz, 1H) (H-6), 4.06 (dd, *J*=2.2, 12.5 Hz, 1H) (H-6), 4.02-3.99 (m, 1H) (H-5), 2.83-2.68 (m, 2H) (levulinic-CH_2-), 2.34 (t, *J*=6.0 Hz, 2H) (levulinic-CH_2-), 2.22 (s, 3H) ($COCH_3$), 2.15 (s, 3H) ($COCH_3$), 2.08 (m, 6H) ($COCH_3$), 2.04 (s, 3H) ($COCH_3$).

3, 4, 6-tri-*O*-acetyl-2-*N*-levulinoyl-α-D-glucosamine (8)

Benzylamine (51.4 μL, 4.71 mmol) was added to a solution of (**4**) (1.40 g, 3.14 mmol) in THF (30 mL) and the mixture was stirred for 24 h at 20 °C. Then, the mixture was neutralized with ice-cooled 1N HCl and extracted with $CHCl_3$. After washing with water, the organic layer was dried with Na_2SO_4, filtered and concentrated by rotary evaporator. The residue was purified by silica-gel chromatography (EtOAc) to yield **8** (703 mg, 56%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ=5.97 (d, *J*=9.0 Hz, 1H) (NH), 5.32 (t, *J*=10.2 Hz, 1H) (H-3), 5.25 (t, *J*=3.6 Hz, 1H) (H-1), 5.11 (t, *J*=9.9 Hz, 1H) (H-4), 4.29-4.24 (m, 1H) (H-2), 4.24-4.19 (m, 2H) (H-5), 4.15-4.11 (m, 1H) (H-6), 3.46 (d, *J*=3.6 Hz, 1H) (OH), 2.81-2.72 (m, 2H) (levulinic-CH_2-), 2.38 (t, *J*=6.2 Hz, 2H) (levulinic-CH_2-), 2.71 (s, 3H) ($COCH_3$), 2.09 (s, 3H) ($COCH_3$), 2.04 (m, 3H) ($COCH_3$), 2.03 (s, 3H) ($COCH_3$).

Dibenzyl-3, 4, 6-tri-*O*-acetyl-2-*N*-levulinoyl-α-D-glucosamine phosphate (9)

To a solution of **8** (700 mg, 1.73 mmol) in CH_2Cl_2 (40 mL), 1*H*-tetrazole (484 mg, 6.92 mmol) was added, and the mixture was cooled to -30 °C. Then, dibenzyl-*N, N*-diethyl phosphamide (1.04 mL, 3.46 mmol) was added, stirred for 30 min at -30 °C and for an additional 1 h at r.t. After cooling the reaction mixture to -40 °C, *m*-CPBA (1.49 g, 8.65 mmol) was added and stirred for 30 min at 0 °C and for an additional 30 min at 20 °C. After

dilution with $CHCl_3$, the solution was washed successively with 10% aqueous sodium sulfite solution, sodium hydrogen carbonate, and water. The organic layer was dried with Na_2SO_4, filtered, and then evaporated. The residue was purified with silica-gel chromatography (EtOAc) to yield **9** (876 mg, 76%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ=7.39-7.34 (m, 10H) (aromatic), 5.81 (d, *J*=9.1 Hz, 1H) (NH), 5.67 (dd, *J*=3.4, 5.7 Hz, 1H) (H-1), 5.20-5.04 (m, 6H) (H-3, H-4, Ph-CH_2-), 4.39-4.33 (m, 1H) (H-2), 4.13 (dd, *J*=4.0, 12.5 Hz, 1H) (H-6), 4.03-3.97 (m, 1H) (H-5), 3.91 (dd, *J*=2.0, 12.5 Hz, 1H) (H-6), 2.73 (ddd, *J*=5.3, 8.5, 18.5 Hz, 1H) (levulinic-CH_2-), 2.52 (dt, *J*=5.8, 18.5 Hz, 1H) (levulinic-CH_2-), 2.27-2.19 (m, 1H) (levulinic-CH_2-), 2.19 (s, 3H) ($COCH_3$), 2.12-2.02 (m, 1H) (levulinic-CH_2-), 2.05 (s, 3H) ($COCH_3$), 2.02 (s, 3H) ($COCH_3$), 2.00 (s, 3H) ($COCH_3$).

3, 4, 6-tri-*O*-acetyl-2-*N*-levulinoyl-α-D-glucosamine phosphate (2)

To a solution of **9** (860 mg, 1.30 mmol) in MeOH (10 mL), 10% Pd / C was added under a nitrogen atmosphere, and the mixture was stirred under a hydrogen atmosphere for 5 h. The reaction mixture was then filtered with celite and evaporated. The residue was purified using silica-gel chromatography to yield **2** (503 mg, 80%).

^{1}H-NMR (MeOD-d_4, 500 MHz): δ=5.48 (m, 1H) (H-1), 5.32 (t, *J*=10.2 Hz, 1H) (H-3), 5.07 (t, *J*=9.8 Hz, 1H) (H-4), 4.34 (d, *J*=10.3 Hz, 1H) (5-H), 4.28 (dd, *J*=2.8, 12.4 Hz, 1H) (H-6), 4.21 (d, *J*=10.8 Hz, 1H) (H-2), 4.14 (d, *J*=11.8 Hz, 1H) (H-6), 2.78 (dt, *J*=7.2, 18.2 Hz, 1H) (levulinic-CH_2-), 2.65 (dt, *J*=6.1, 18.2 Hz, 1H) (levulinic-CH_2-), 2.52-2.37 (m, 2H) (levulinic-CH_2-), 2.13 (s, 3H) ($COCH_3$), 2.03 (s, 3H) ($COCH_3$), 1.99 (s, 3H) ($COCH_3$), 1.96 (s, 3H) ($COCH_3$); FAB MS: *m/z*: calcd for $C_{17}H_{25}NO_{13}P$: 482.1069; found: 482.1064 [*M* - H]$^-$.

2-*N*-levulinoyl-α-D-glucosamine phosphate (1)

To a solution of **2** (30 mg, 0.062 mmol) in MeOH : H_2O = 9 : 1 (3 mL), K_2CO_3 (17 mg, 0.124 mmol) was added and stirred for 12 h at 20 °C. Purification was carried out with P-2 Bio-Gel column chromatography to yield **1** (19 mg, 90%).

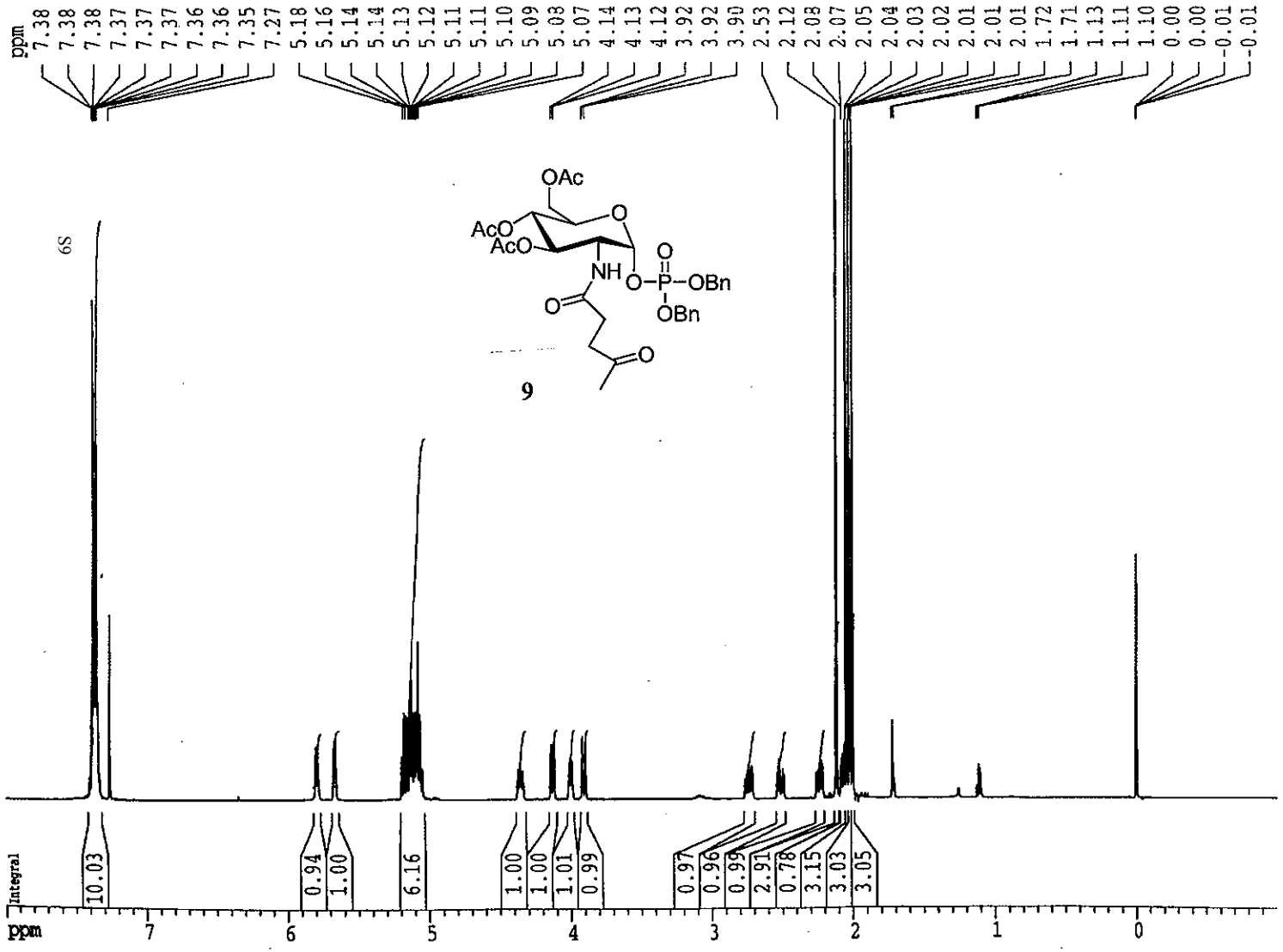
^{1}H-NMR (D_2O, 500 MHz): δ=5.34 (dd, *J*=3.1, 7.1 Hz, 1H) (H-1), 3.90-3.77 (m, 3H) (H-2, H-5, H-6), 3.76-3.70 (m, 2H) (H-3, H-6), 3.46 (t, *J*=9.6 Hz, 1H) (H-4), 2.80 (m, 2H) (levulinic-CH_2-), 2.52 (m, 2H) (levulinic-CH_2-), 2.17 (m, 3H) ($COCH_3$).

***cyclo*Saligenyl-3, 4, 6-tri-*O*-Acetyl-2-*N*-levulinoyl-α-D-glucosamine phosphate (3)**

To a solution of **8** (88 mg, 0.218 mmol) in CH_2Cl_2 (5 mL), DIEA (113 mL, 0.873 mmol) was added. The solution was then cooled to -30 °C, and salicylchlorophosphane (81.9 mg, 0.436 mmol) was added and stirred for 30 min at -30 °C and for an additional 30 min at 20 °C. The mixture was then cooled to -40 °C and *tert*-BuOOH 5-6 M solution in decane (80 mL) was added to the reaction mixture before stirring for 30 min at 0 °C. The mixture was then purified with silica-gel chromatography ($CHCl_3$:MeOH = 50:1) to yield **3** (68 mg, 55%, diastereomeric ratio 1.0:0.6).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ=7.40-7.34 (m, 2H) (aromatic), 7.23-7.08 (m, 6H) (aromatic), 6.07 (d, *J*=8.9 Hz, 1H) (NH), 6.01 (d, *J*=8.8 Hz, 1H) (NH) 5.85 (dd, *J*=3.2, 5.9 Hz, 1H) (H-1), 5.78 (dd, *J*=3.2, 5.7 Hz, 1H) (H-1), 5.50-5.41 (m, 4H) (Ph-CH_2), 5.26-5.31 (m, 4H) (H-3, H-4), 4.46-4.37 (m, 2H) (H-2), 4.24-4.15 (m, 2H) (H-6), 4.13-4.09 (m, 1H) (H-5), 4.07-4.03 (m, 1H) (H-6), 3.95-3.90 (m, 2H) (H-5, H-6), 2.90-2.81 (m, 2H) (levulinic-CH_2-), 2.72-2.62 (m, 2H) (levulinic-CH_2-), 2.47-2.30 (m, 4H) (levulinic-CH_2-), 2.17 (s, 3H) ($COCH_3$), 2.15 (s,

3H) ($COCH_3$), 2.07-1.98 (m, 18H) ($COCH_3$); ESI MS: *m/z:* calcd for $C_{24}H_{30}NO_{13}PNa$: 594.1352; found: 594.1365 $[M + Na]^+$.

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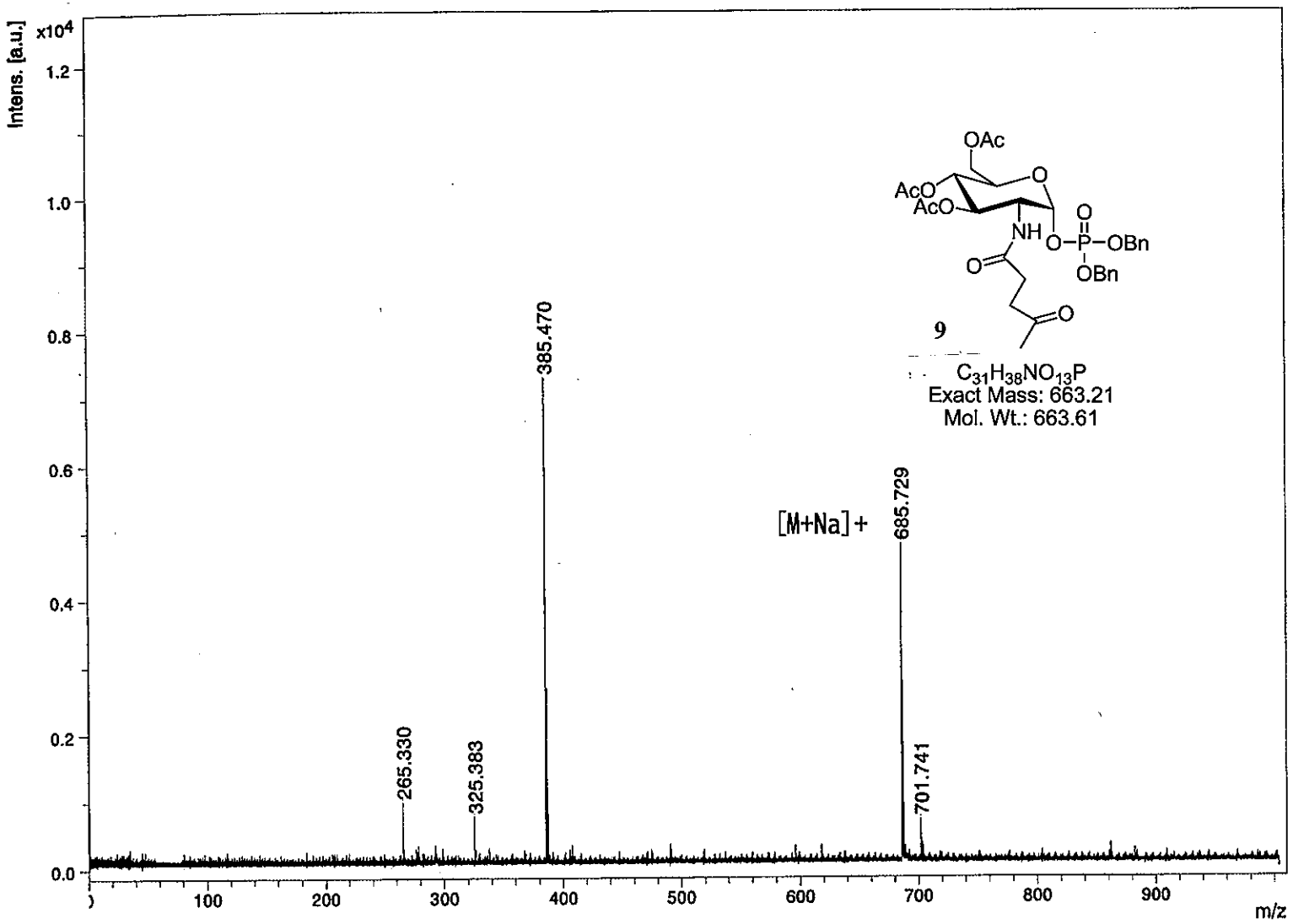

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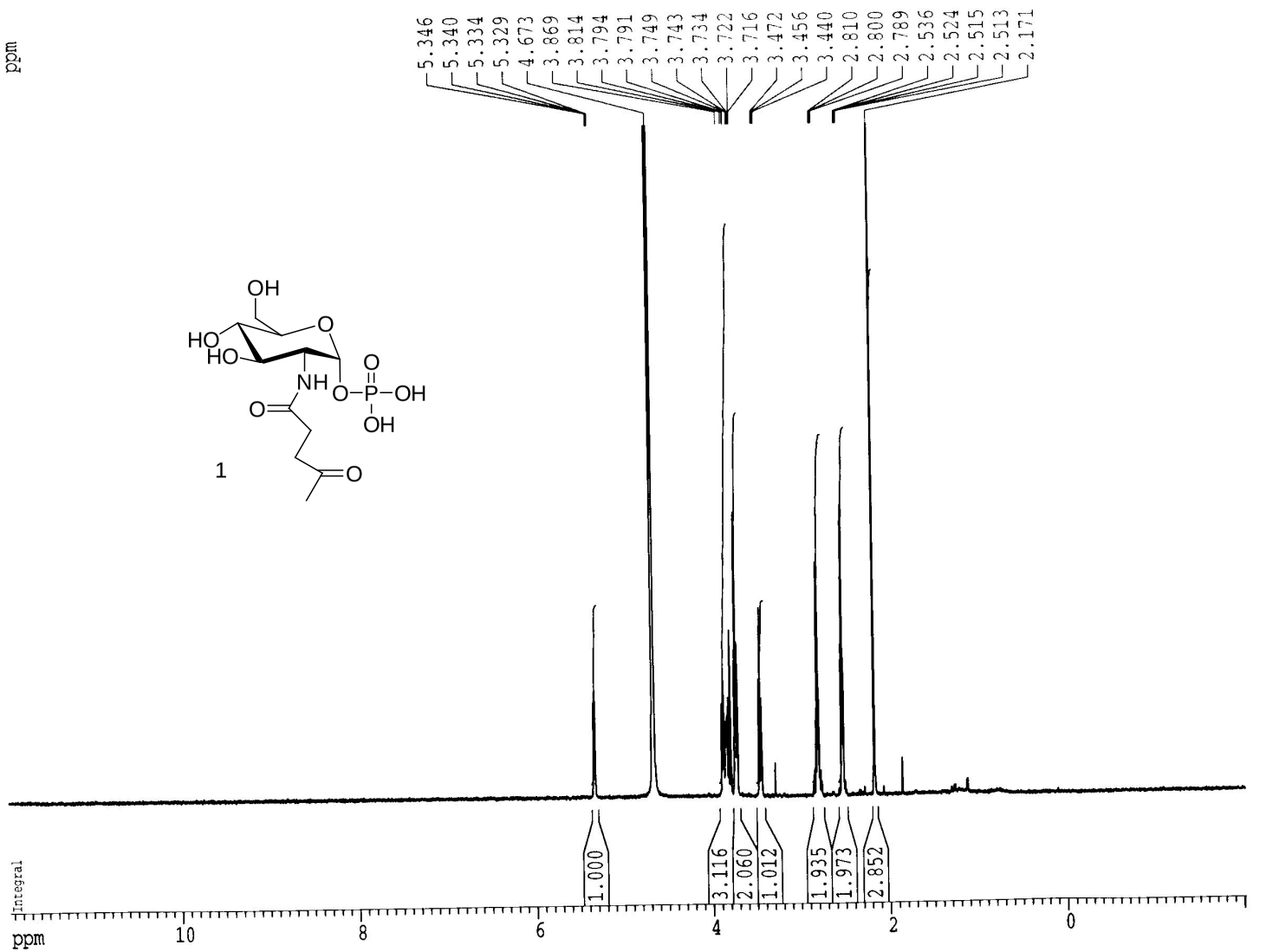

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1D NMR plot parameters

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Output m/z range : 293.1751 to 459.3472 Cut Level : 0.00 %

ESI <JMS-700TZ>
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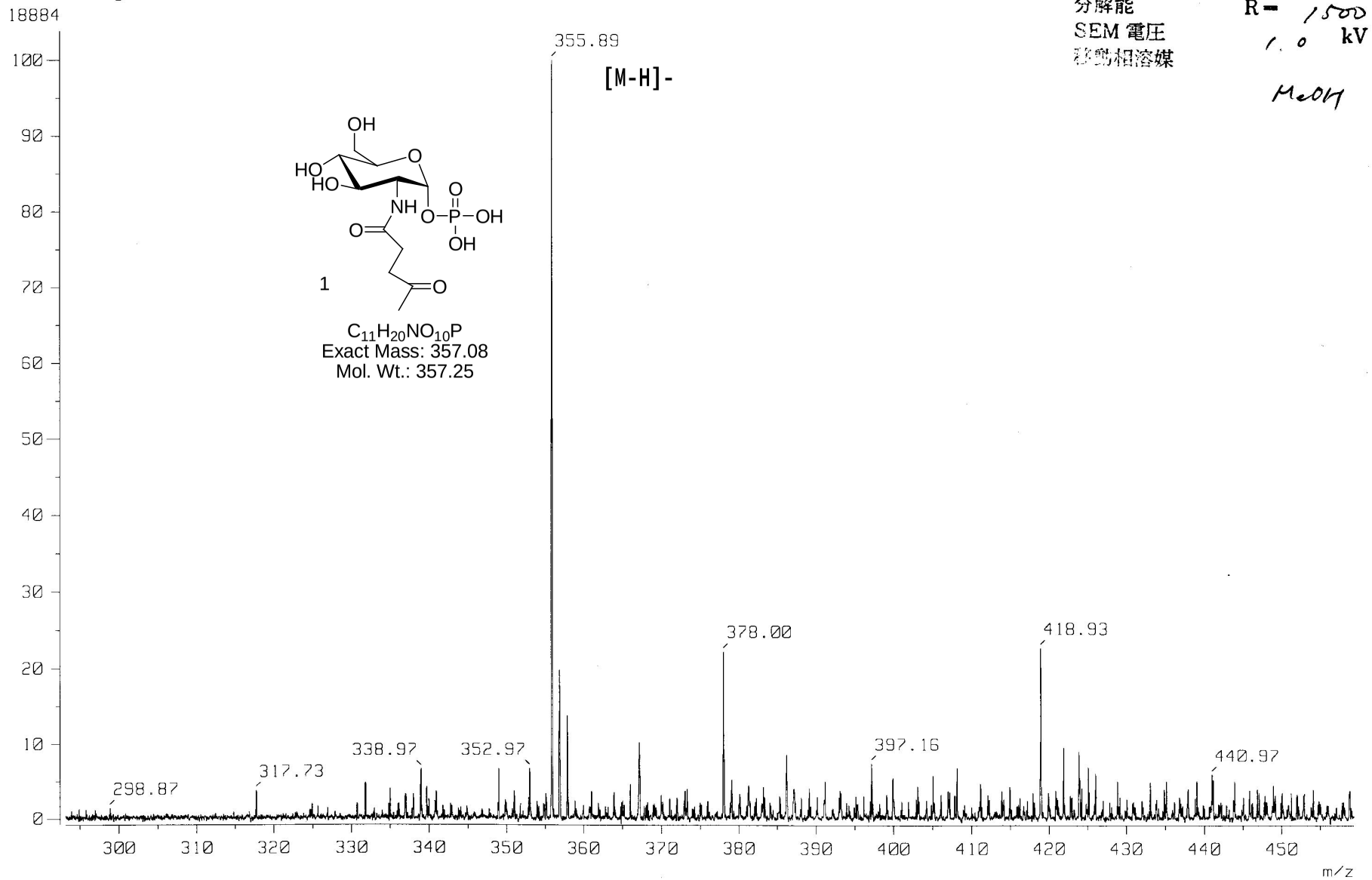

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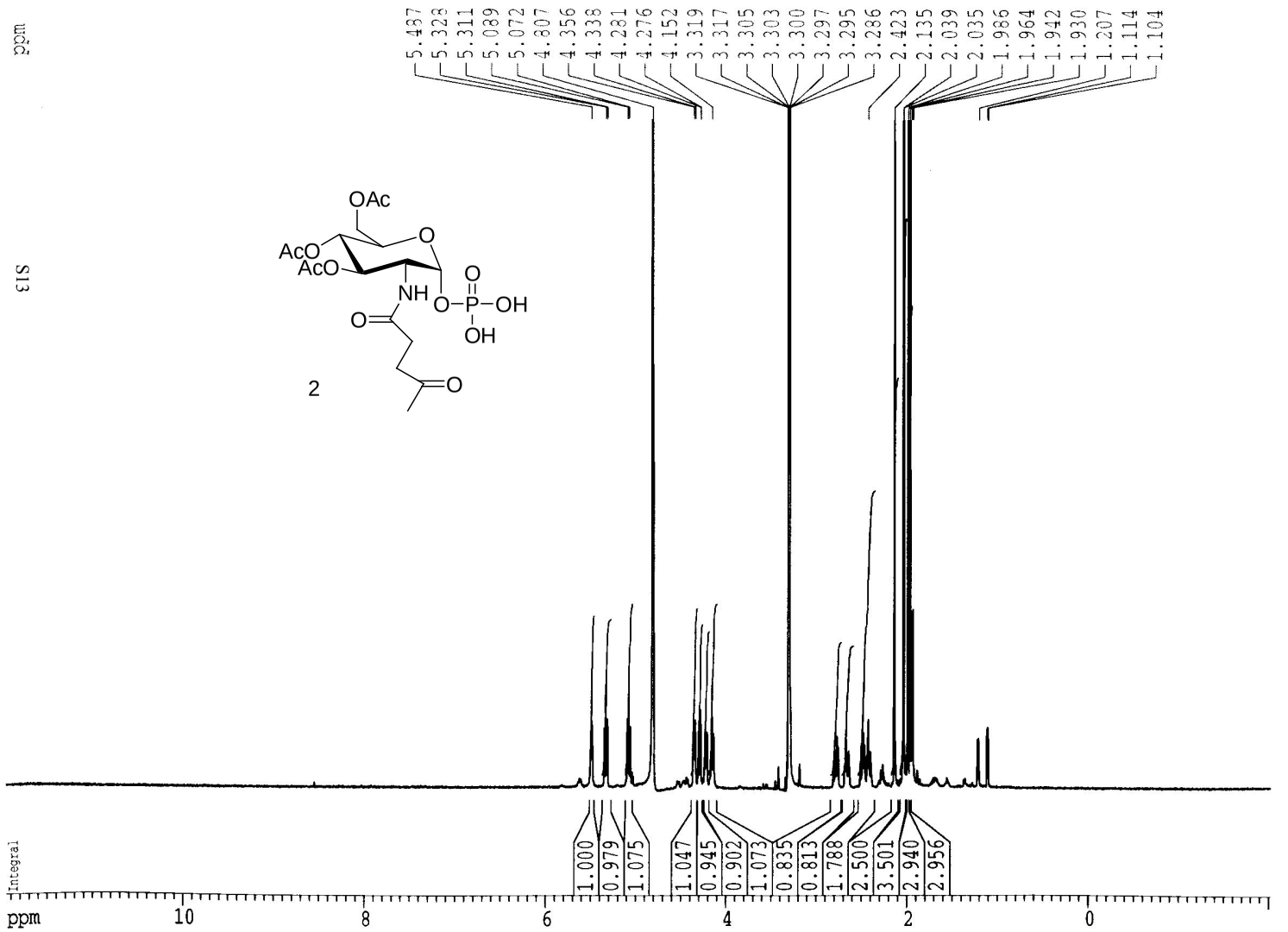

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1D NMR plot parameters

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Output m/z range : 434.0639 to 529.4772 Cut Level : 0.00 %

ESI <JMS-700TZ>
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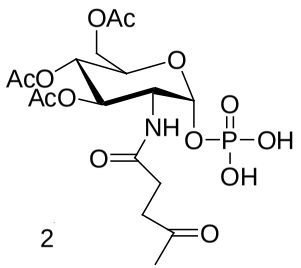
2272281



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S14

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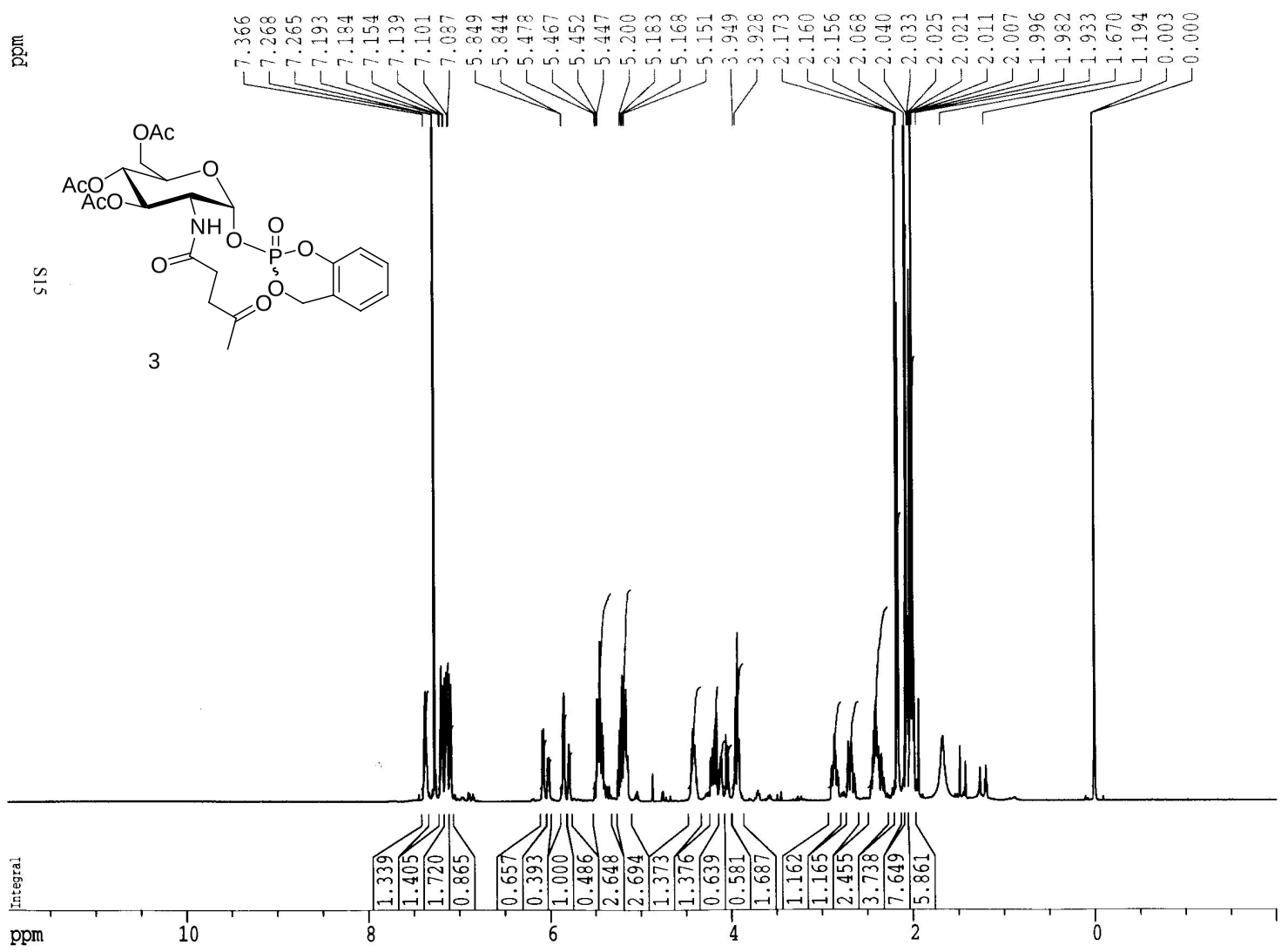


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D1                 2.50000000 sec

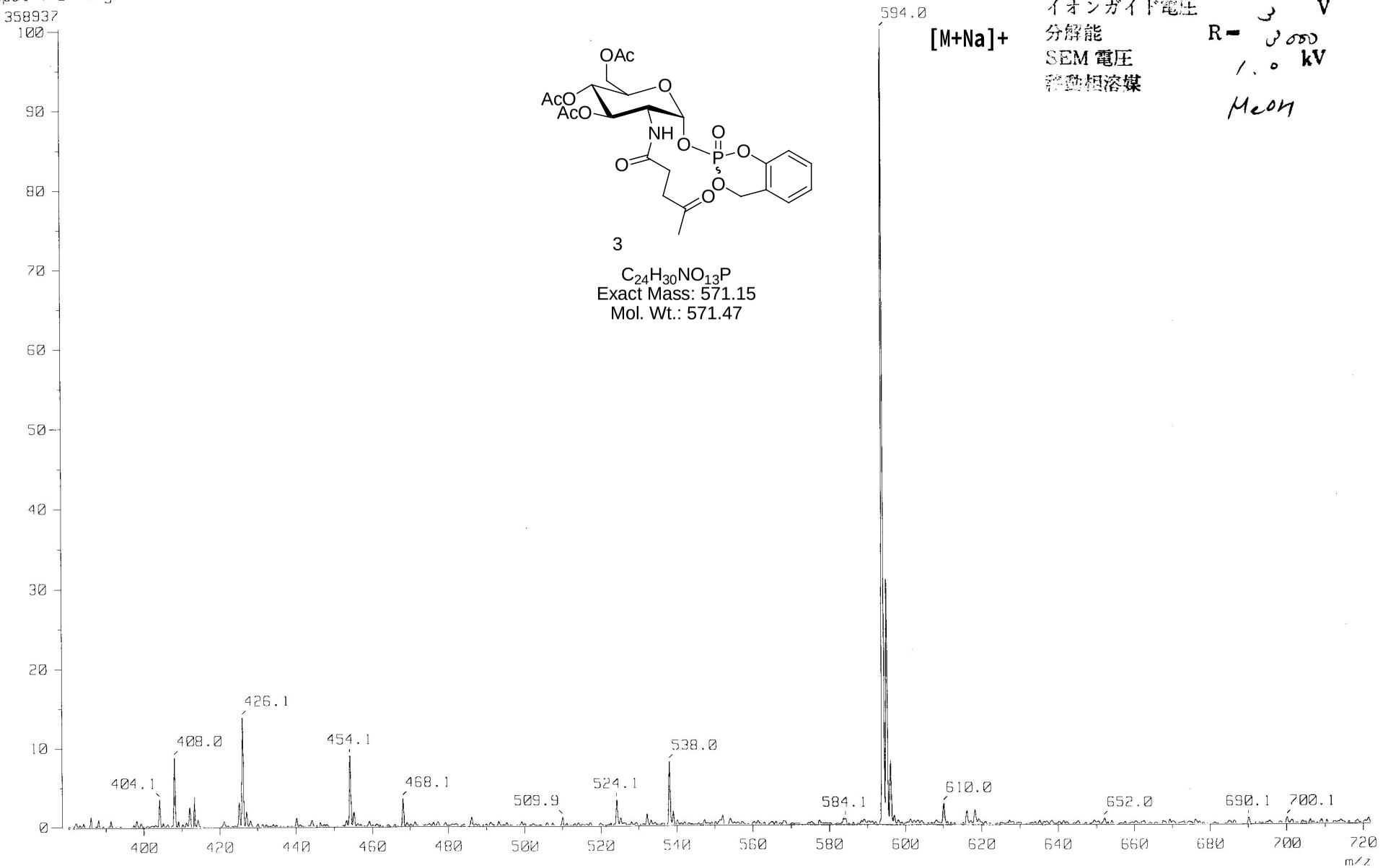
============ CHANNEL f1 =============
NUC1                      1H
P1                     8.10 usec
PL1                    0.00 dB
SFO1             600.1330006 MHz

F2 - Processing parameters
SI                    32768
SF               600.1300132 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00

1D NMR plot parameters
CX                    20.00 cm
F1P                   11.967 ppm
F1                  7182.06 Hz
F2P                   -2.012 ppm
F2                 -1207.21 Hz
PPMCM                 0.69895 ppm/cm
HZCM                419.46307 Hz/cm
```

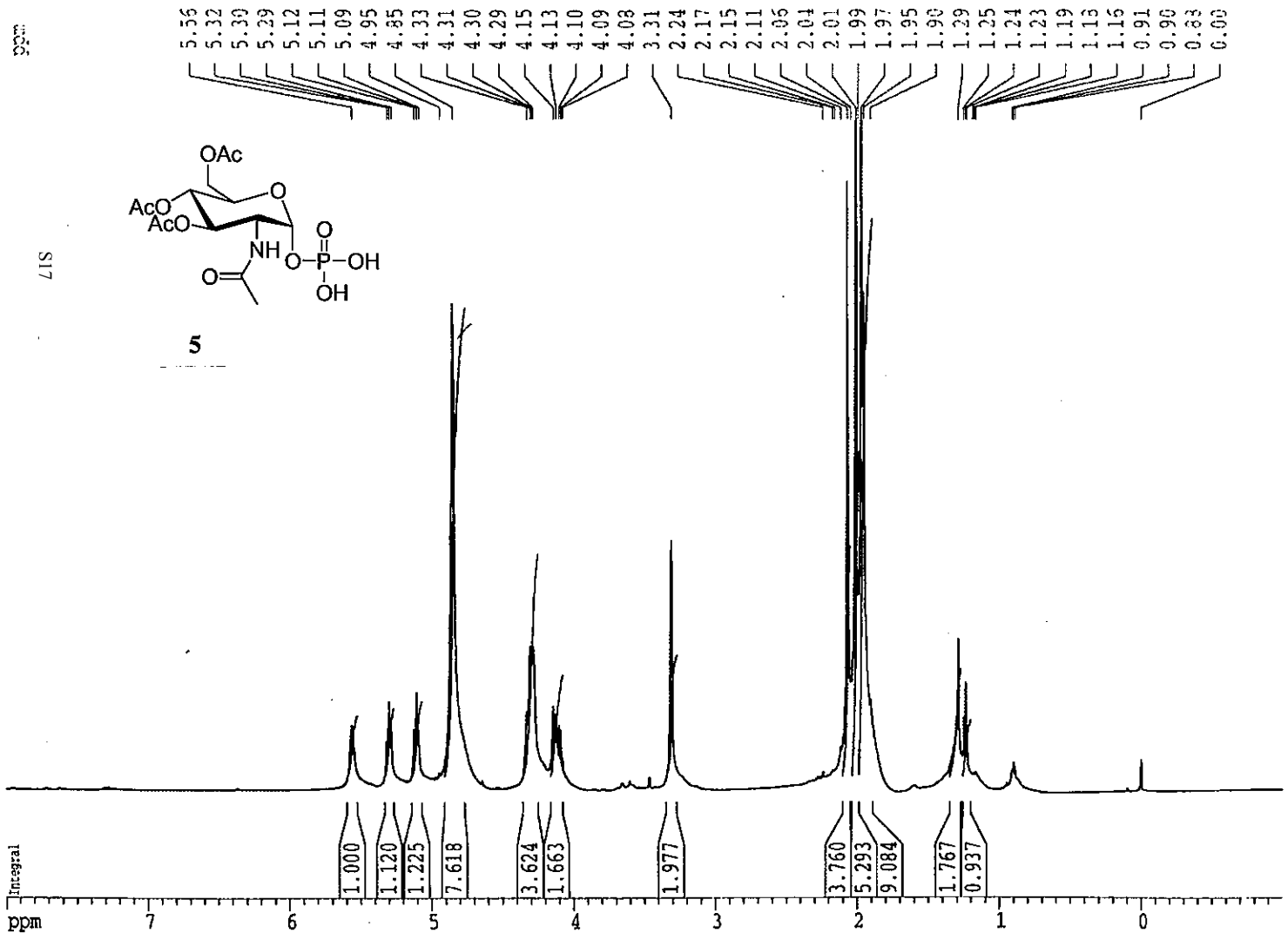
[Mass Spectrum]
Data : 050106-161916-451-001 Date : 06-Jan-2005 09:04
Sample: -
Note : Operator name : S.Oka , Instrument : JMS-700TZ
Inlet : LC Ion Mode : ESI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.65 min Scan# : (10,11)-5
BP : m/z 594.0236 Int. : 17.06
Output m/z range : 380.0297 to 721.9585 Cut Level : 0.00 %

ESI <JMS-700TZ>
ニードル 電圧 2 kV
オリフィス 電圧 0 V
リングレンズ電圧 70 V
イオンガイド電圧 3 V
分解能 R= 3000
SEM 電圧 1.0 kV
移動相溶媒 MeOH



3

$C_{24}H_{30}NO_{13}P$
Exact Mass: 571.15
Mol. Wt.: 571.47




```
Current Data Parameters
NAME      shimizu060926
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20060926
Time              15.42
INSTRUM          drx600
PROBHD     5 mm TXI 1H-
PULPROG            zg30
TD                32768
SOLVENT            MeOH
NS                   16
DS                    1
SWH            8389.300 Hz
FIDRES         0.256021 Hz
AQ           1.9530139 sec
RG                 90.5
DW              59.600 usec
DE               6.00 usec
TE              300.0 K
D1           2.50000000 sec

============ CHANNEL f1 =============
NUC1                  1H
P1                 7.50 usec
PL1               -1.00 dB
SFO1        600.1327291 MHz

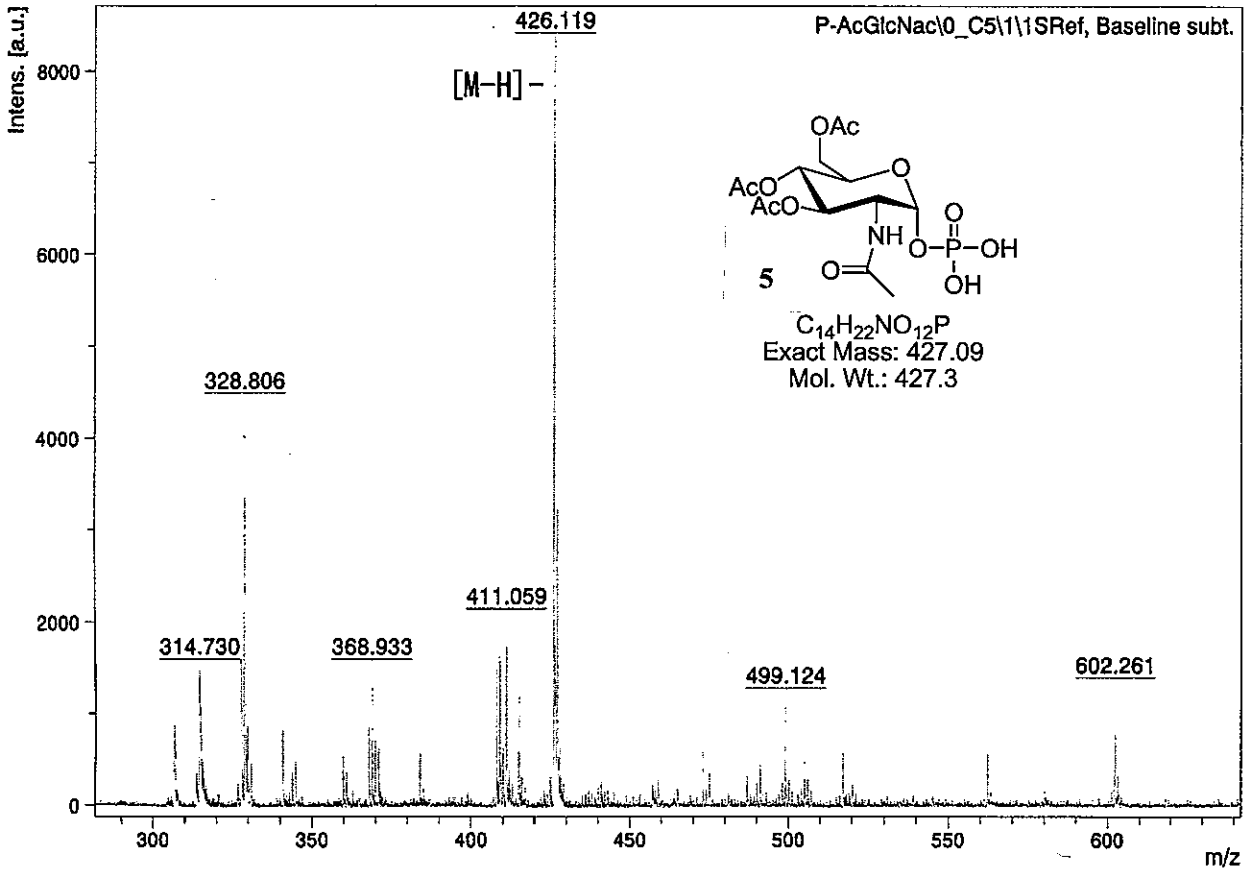
F2 - Processing parameters
SI                32768
SF          600.1300137 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00

1D NMR plot parameters
CX                20.00 cm
F1P                8.000 ppm
F1              4801.04 Hz
F2P               -1.000 ppm
F2              -600.13 Hz
PPMCM          0.45000 ppm/cm
HZCM         270.05850 Hz/cm
```

D:\ooyanagi\shimizu\P-AcGlcNac\0_C5\1

Comment 1

Comment 2



m/z	SN	Quality Fac.	Res.	Intens.	Area
314.730	171.0	238540	968	1562.63	718
328.806	487.2	569954	1185	4451.80	1536
368.933	170.9	96191	2747	1561.38	253
411.059	230.7	319813	3601	2107.50	401
426.119	917.2	1124375	2512	8380.62	2168
499.124	136.3	129038	3895	1245.27	218
602.261	149.1	121955	4199	1362.02	265