Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information (SI)

Spectroscopic diagnostic for the ring-size of carbohydrates in the gas phase: furanose and pyranose forms of GalNAc

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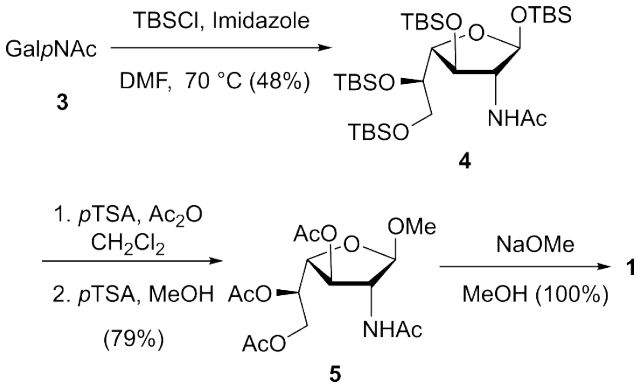
Chemical synthesis

A one-step procedure for the *N*-acetyl galactosylation of methanol was described previously but led to a mixture of methyl pyranoside and furanoside.[1] We have previously described the selective synthesis of per-*O*-silylated Gal*f*NAc starting from *N*-acetyl galactosamine.[2] Such compound could be a useful intermediate of compound **1**. Following this strategy, persilylation of *N*-acetyl galactosamine **3** in the presence of imidazole and an excess of *t*-butyldiphenylsilyl (TBS) chloride allowed setting the carbohydrate ring in the required furanose form to give the corresponding intermediate **4** in 48% yield (Scheme 2). Subequent acido-catalyzed acetolysis followed by glycosylation of methanol resulted in the formation of methyl Gal*f*NAc **5** in 79% yield and without detectable ring expansion. The protecting groups were removed by acetolysis to give the desired furanoside **1** isolated as a white powder in quantitative yield.

All reactions were carried out in oven-dried glassware. All reagents and dry solvents were purchased from commercial sources and were used without further purification unless noted. Unless otherwise stated, all reactions were carried out at room temperature under a positive pressure of nitrogen and were monitored by TLC on Silica Gel 60 F_{254}. TLC spots were detected under 254 nm light and/or by staining with cerium ammonium molybdate solution. Column chromatography was performed on Silica Gel (40-63 µm). Optical rotations were measured at 20 °C. NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are given in δ units (ppm) and referenced to $CDCl_3$ (7.26 ppm) or CD_3OD (3.31 ppm). Coupling constants *J* were calculated in Hertz (Hz). Proton and carbon NMR peaks were unambiguously assigned by COSY (double quantum filtered with gradient pulse for selection), HSQC (gradient echo-anti echo selection and shape pulse) and HMBC (echo-anti echo gradient selection, magnitude mode) correlation experiments. High Resolution Mass was measured by electrospray with a MS/MS ZabSpec TOF Micromass using *m*-nitrobenzylic alcohol as a matrix and accelerated caesium ions for ionization (Centre Regional des Mesures Physiques de l'Ouest, Université de Rennes 1).



Scheme S1: Synthesis of β-D-Gal*f*NAc-OMe 1.

[1] A. P. Rauter, T. Almeida, N. M. Xavier, F. Siopa, A. I. Vicente, S. D. Lucas, J. P. Marques, F. Ramôa Ribeiro, M. Guisnet and M. J. Ferreira, Acid zeolites as efficient catalysts for O- and S-glycosylation, *Journal of Molecular Catalysis A: Chemical*, 2007, **275**, 206–213.

[2] R. Dureau, L. Legentil, R. Daniellou and V. Ferrières, Two-Step Synthesis of Per- *O* -acetylfuranoses: Optimization and Rationalization, *The Journal of Organic Chemistry*, 2012, **77**, 1301–1307.

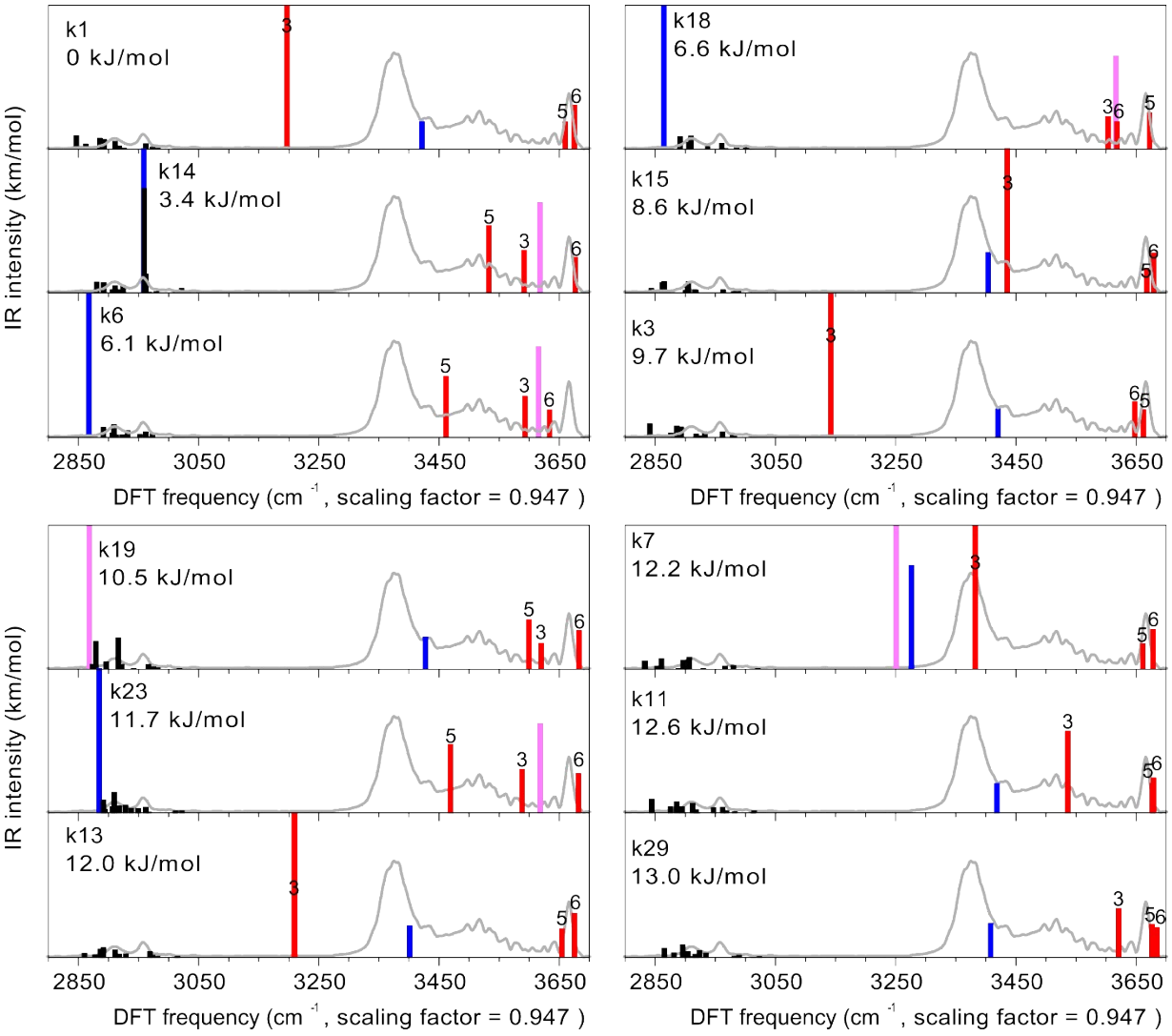


Fig. S1: Experimental spectrum (grey line) compared with IR spectra of conformers of β-D-Gal*f*NAc-OMe **1**. A color code (color version only) is used to identify the vibrational modes: red (ring OH), blue (NH), black (CH), pink (Acetyl OH). The labels indicate the ring OH position.

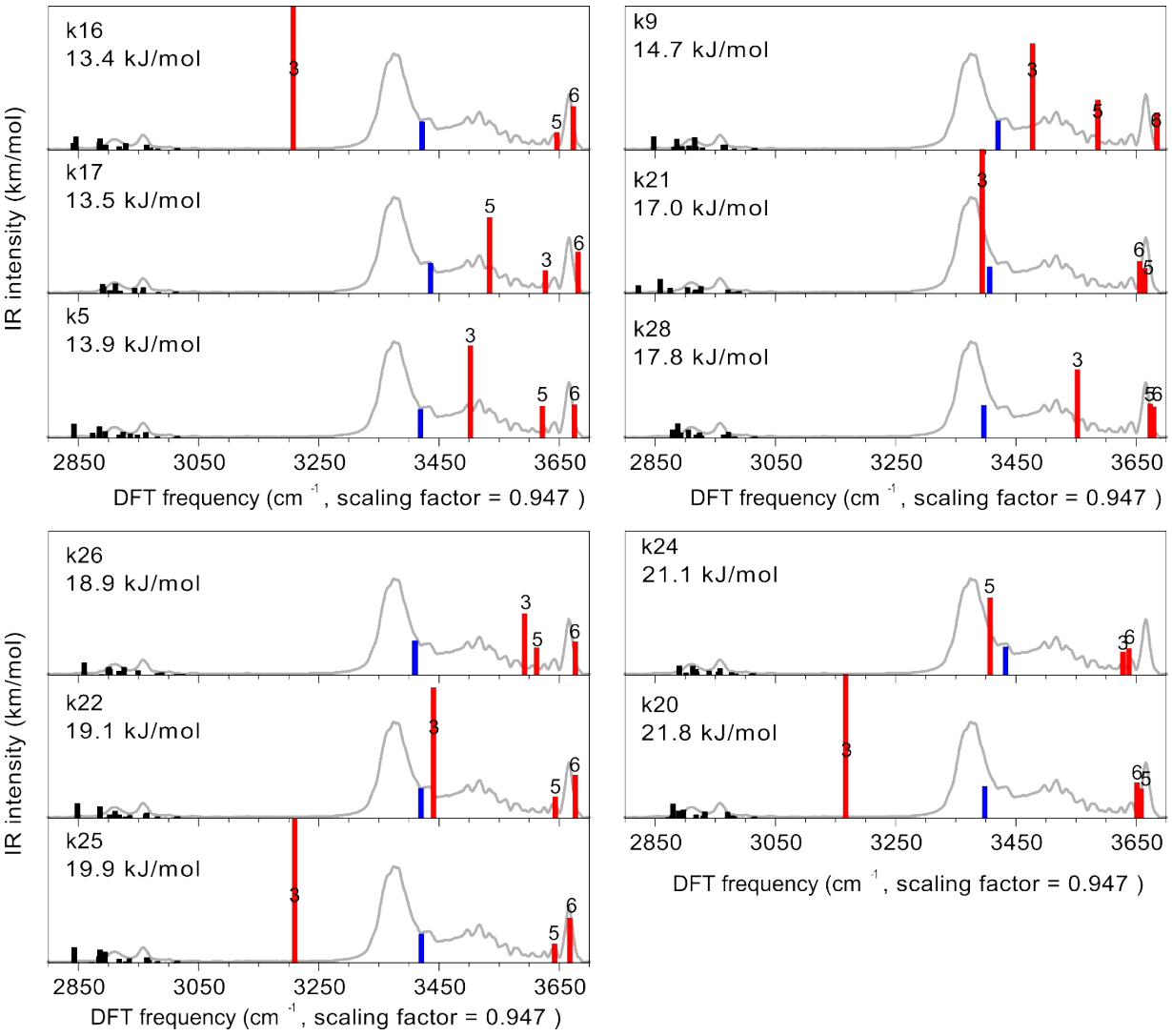


Fig. S2: Experimental spectrum (grey line) compared with IR spectra of conformers of β-D-Gal*f*NAc-OMe **1**. A color code (color version only) is used to identify the vibrational modes: red (ring OH), blue (NH), black (CH), pink (Acetyl OH). The labels indicate the ring OH position.

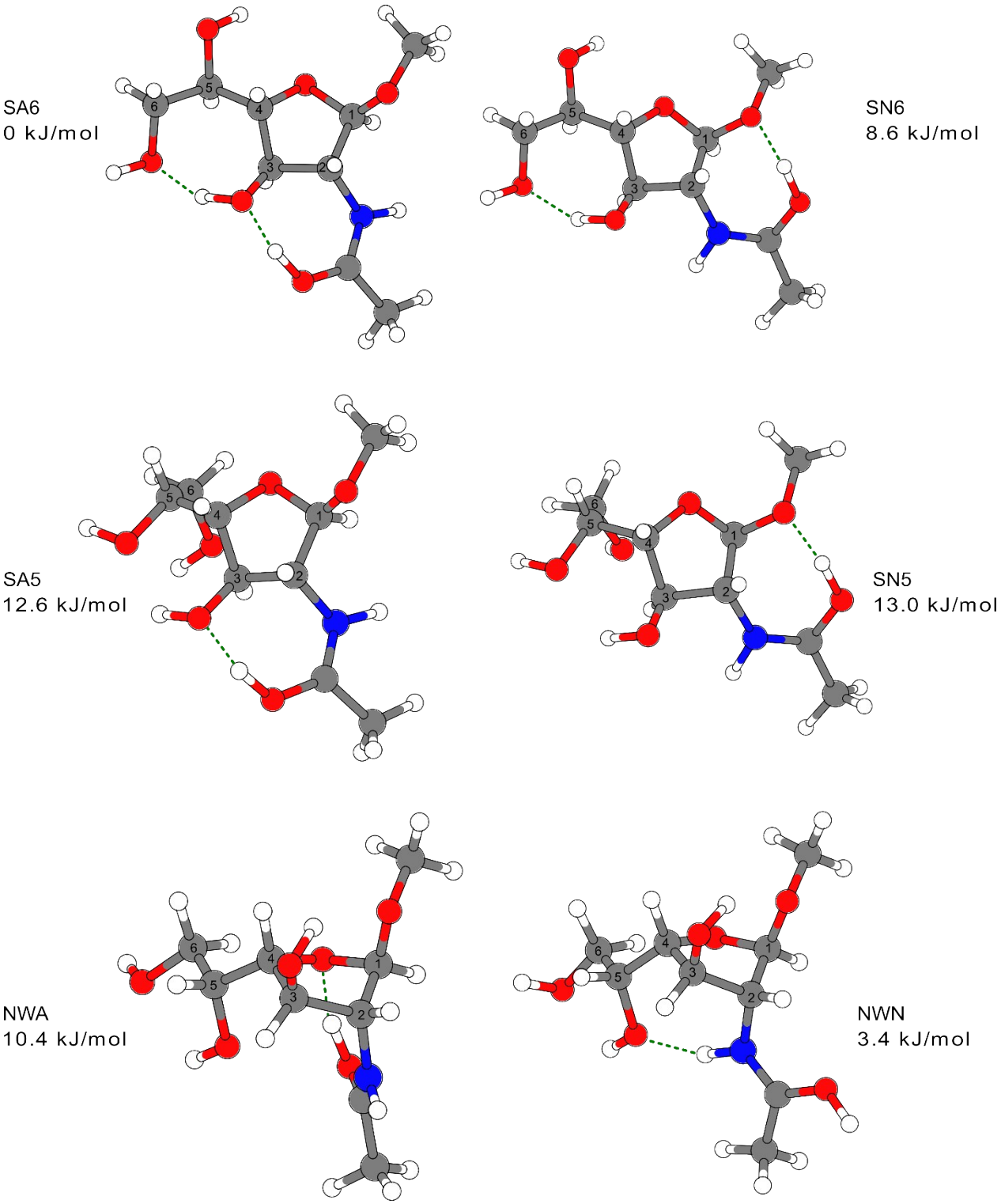


Fig. S3: Molecular drawings of the 6 selected conformers. Hydrogen bonds are shown in green dot line.

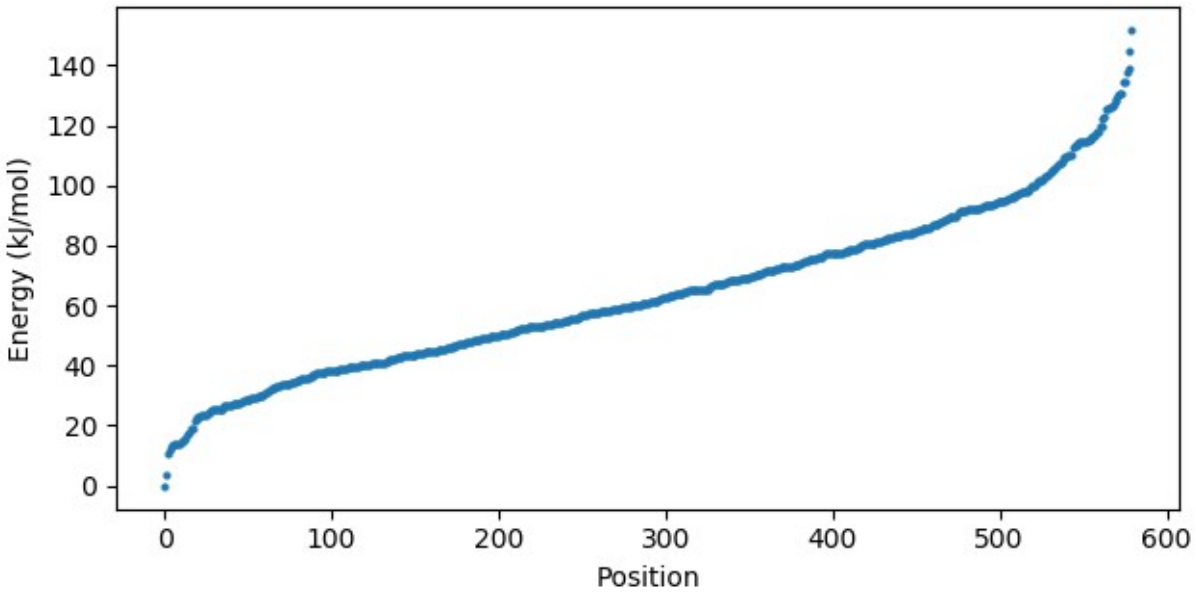


Fig. S4: Sorted energy values of the 608 structures calculated at the CAM-B3LYP/6-31G* level of theory

Preparation of methyl 2-acetamido-2-deoxy-β-D-galactofuranoside 1

2-Acetamido-2-deoxy-1,3,5,6-tetra-*O*-tert-butyldimethylsilyl-D-galactofuranose 4. A solution of *N*-acetyl galactosamine (1 g, 4.5 mmol) in dry DMF (20 mL) was stirred at room temperature for 10 min. before the successive addition of imidazole (2.46 g, 36 mmol) and TBSCl (5.47 g, 36 mmol). The resulting mixture was stirred 10 min at room temperature, then 2 h at 70 °C before placing the mixture at 4 °C overnight. The resulting precipitate was filtered off, then dried under high vacuum to yield 4 as a white solid (1.48 g, 47 %), R_f = 0.5 (cyclohexane/EtOAc, 9:1), mp 144-148 °C, $[\alpha]_D$ -3.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S5 ESI†) δ 5.75 (d, 1H, $J_{2\text{-NH}}$ 9.2 Hz, NH), 5.13 (s, 1H, H-1), 4.23 (dd, 1H, $J_{3,4}$ 3.6, $J_{4,5}$ 2.0 Hz, H-4), 4.21 (d, 1H, H-2), 4.01 (d, 1H, H-3), 3.83 (ddd, 1H, $J_{5,6a}$ 7.6, $J_{5,6b}$ 5.6 Hz, H-5), 3.68 (dd, 1H, $J_{6a,6b}$ 9.6 Hz, H-6a), 3.62 (dd, 1H, H-6b), 1.94 (s, 3H, $COCH_3$), 0.93, 0.89, 0.88, 0.87 [4s, 36H, $C(CH_3)_3$], 0.16, 0.13, 0.10, 0.09, 0.07, 0.05 [7s, 24H, $Si(CH_3)_2$]. ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S6 ESI†) δ 169.7 (CO), 102.0 (C-1), 85.8 (C-4), 77.9 (C-3), 72.9 (C-5), 63.9 (C-6), 63.4 (C-2), 26.1, 25.9, 25.6 [$C(CH_3)_3$], 23.4 ($COCH_3$), 18.4, 17.8 [$C(CH_3)_3$], -4.0, -4.4, -4.5, -4.8, -5.3, -5.4 [$Si(CH_3)_2$]. HRMS calcd. for $C_{32}H_{71}NO_6Si_4$ $[M+Na]^+$ 700.4256, found 700.4251.

Methyl 2-acetamido-2-deoxy-3,5,6-tri-*O*-acetyl-D-galactofuranoside 5. To a solution of **4** (1 g, 1.5 mmol) and acetic anhydride (5.5 mL, 59 mmol) in CH_2Cl_2 (25 mL) was added, at 0 °C, *p*-TsOH (5.6 g, 29.5 mmol). The mixture was stirred overnight at room temperature. Then the solution was washed with saturated aq $NaHCO_3$ solution (3 ×) and brine. The combined organic layers were concentrated, and the resulting oxazoline intermediate was obtained as a colorless oil (440 mg, 91 %). To a solution of this oxazoline (430 mg, 1.3 mmol) in MeOH (15 mL) was added *p*-TsOH (25 mg, 0.13 mmol). The resulting mixture was stirred for 3 h at room temperature. Then the reaction was stopped by addition of few drops of triethylamine and the solvent was evaporated. Purification by flash chromatography (CH_2Cl_2/AcOEt) afforded 5 as a white solid (410 mg, 79% over two steps). mp 125-127 °C, $[\alpha]_D$ -0.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S7 ESI†) δ 6.01 (d, 1H, $J_{2,NH}$ 7.9 Hz, NH), 5.36 (ddd, 1H, $J_{5,6a}$ 8.4, $J_{5,6b}$ 7.0, $J_{5,4}$ 4.2 Hz, H-5), 4.83 (s, 1H, H-1), 4.75 (dd, 1H, $J_{3,4}$ 5.2, $J_{2,3}$ 2.4 Hz, H-3), 4.39-4.33 (m, 2H, H-2, H-6a), 4.24 (dd, 1H, $J_{6b,6a}$ 11.9 Hz, H-6b), 4.19 (dd, 1H, H-4), 3.37 (s, 3H, OCH_3), 2.15, 2.09, 2.06 (3s, 9H, CH_3CO), 2.00 (s, 3H, NHAc). ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S8 ESI†) δ 170.7, 170.6, 170.1, 169.7 (CO), 107.8 (C-1), 79.8 (C-4), 77.8 (C-3), 69.9 (C-5), 62. 6 (C-2), 60.4 (C-6), 55.1 (OCH_3), 22.7, 20.9, 20.7 (CH_3CO). HRMS calcd. for $C_{15}H_{23}NO_9Na$ $[M+Na]^+$ 384.1271, found 384.1267.

Methyl 2-acetamido-2-deoxy-β-D-galactofuranoside 1. To a solution of **5** (400 mg, 1.1 mmol) in MeOH (15 mL) was added a 30% wt. solution of sodium methoxide in MeOH (44 µL, 0.22 mmol). The reaction mixture was stirred at room temperature overnight. Then it was neutralized by addition of Amberlyte IR-120-H^+ form before removing the solvent under reduced pressure. The desired product 1 (258 mg, 100%) was obtained as a light brown solid. $[\alpha]_D^{20}$= +52 (*c* 1, MeOH). ^{1}H NMR (400 MHz, CD_3OD, Fig. S9 ESI†) δ 4.74 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 4.13 (dd, 1H, $J_{2,3}$ 4.2 Hz, H-2), 4.04 (dd, 1H, $J_{3,4}$ 6.3 Hz, H-3), 3.95 (dd, 1H, $J_{4,5}$ 2.8 Hz, H-4), 3.74 (ddd, 1H, $J_{5,6b}$ 7.1, $J_{5,6a}$ 5.6 Hz H-5), 3.65 (dd, 1H, $J_{6b,6a}$ 11.1 Hz, H-6a), 3.61 (dd, 1H, H-6b), 3.35 (s, 3H, OCH_3), 1.96 (s, 3H, NHAc). ^{13}C NMR (100 MHz, CD_3OD, Fig. S10 ESI†) δ 173.0 (CO), 109.0 (C-1), 84.4 (C-4), 77.6 (C-3), 72.2 (C-5), 64.5 (C-6), 64.3 (C-2), 55.1 (OCH_3), 22.6 ($COCH_3$). HRMS calcd. for $C_9H_{17}NNaO_6$ $[M+Na]^+$ 258.0954, found 258.1011.

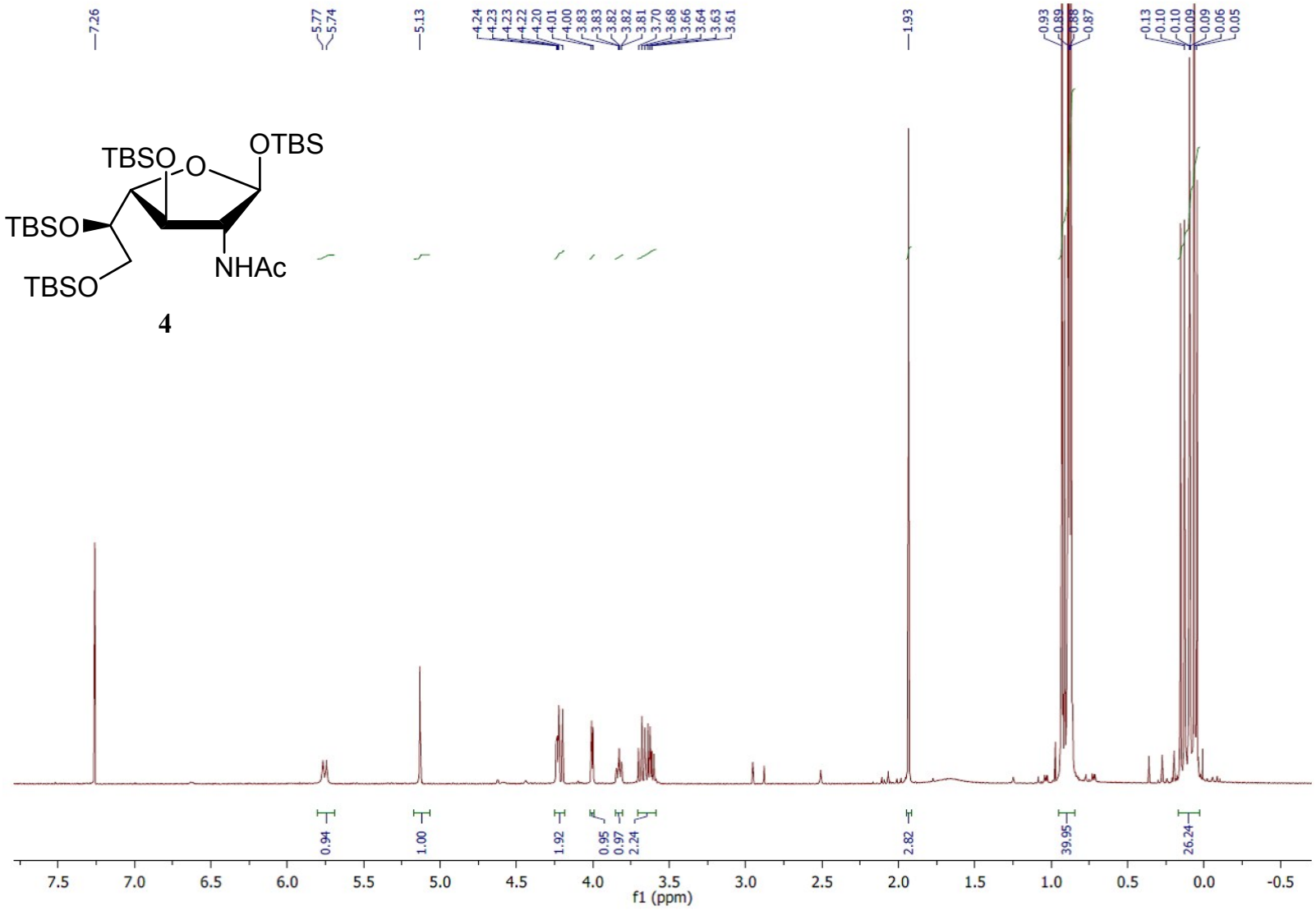
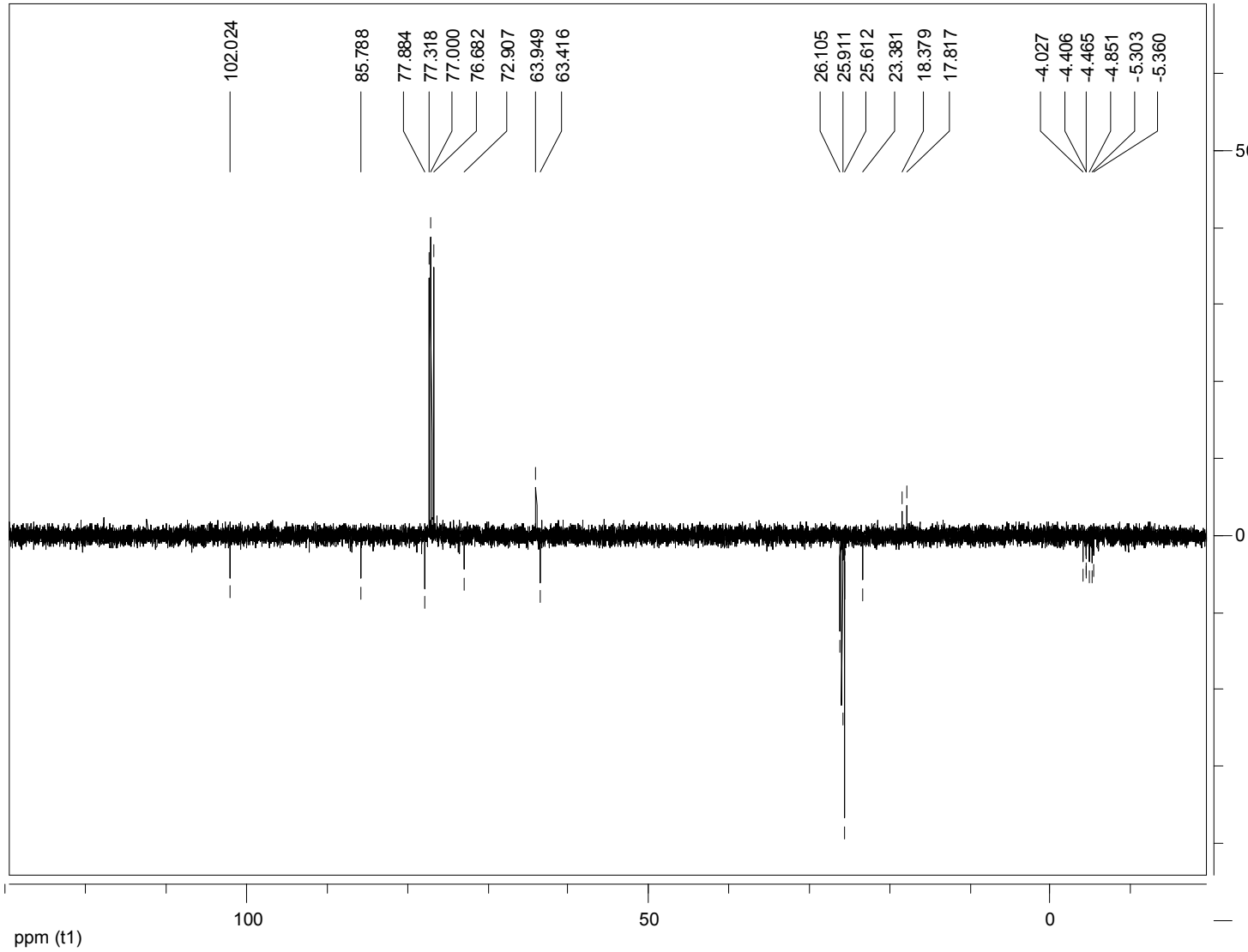


Fig. S5: ^{1}H NMR spectrum of isolated compound 4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77.884
77.318
77.000
76.682
72.907
63.949
63.416
26.105
25.911
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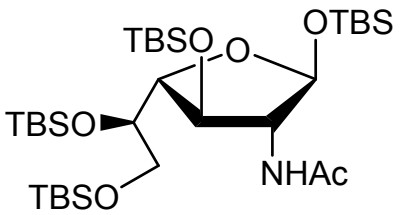
23.381
18.379
17.817
-4.027
-4.406
-4.465
-4.851
-5.303
-5.360
50000
0
100
50
0
ppm (t1)


TBSO
O
OTBS
TBSO
NHAc
TBSO

4

Fig. S6: ^{13}C NMR spectrum of isolated compound 4

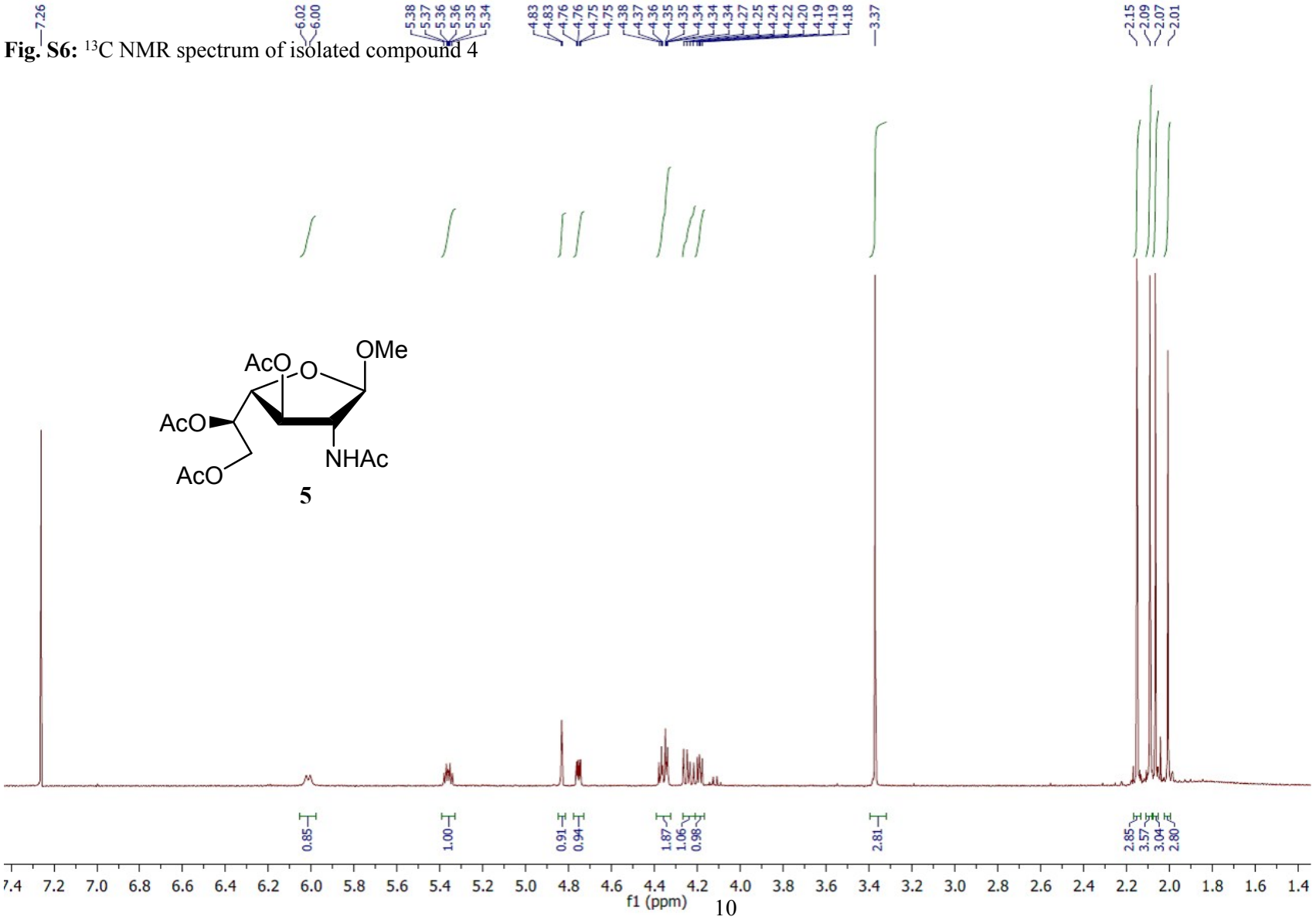


Fig. S7: [1]H NMR spectrum of isolated compound 5

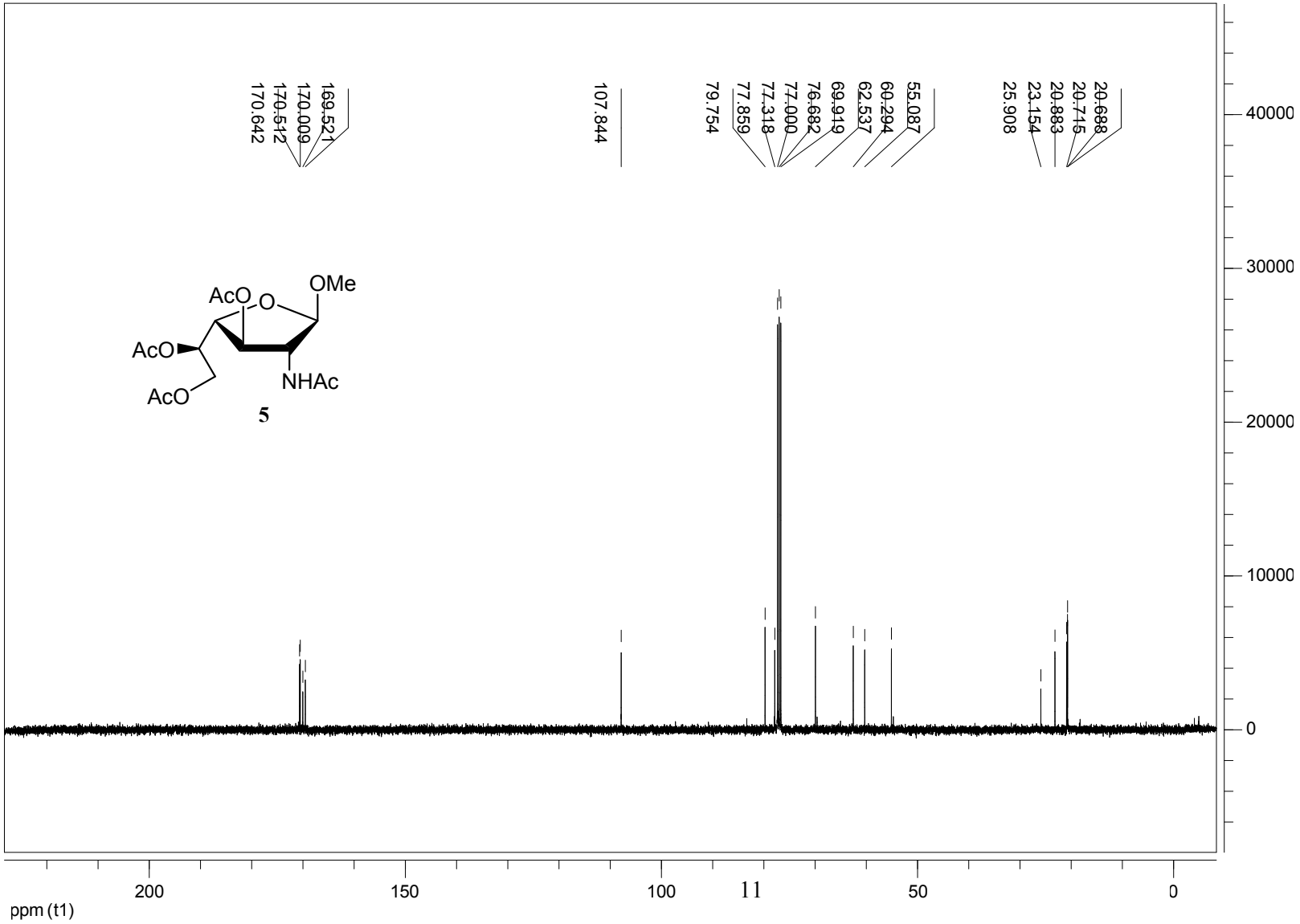
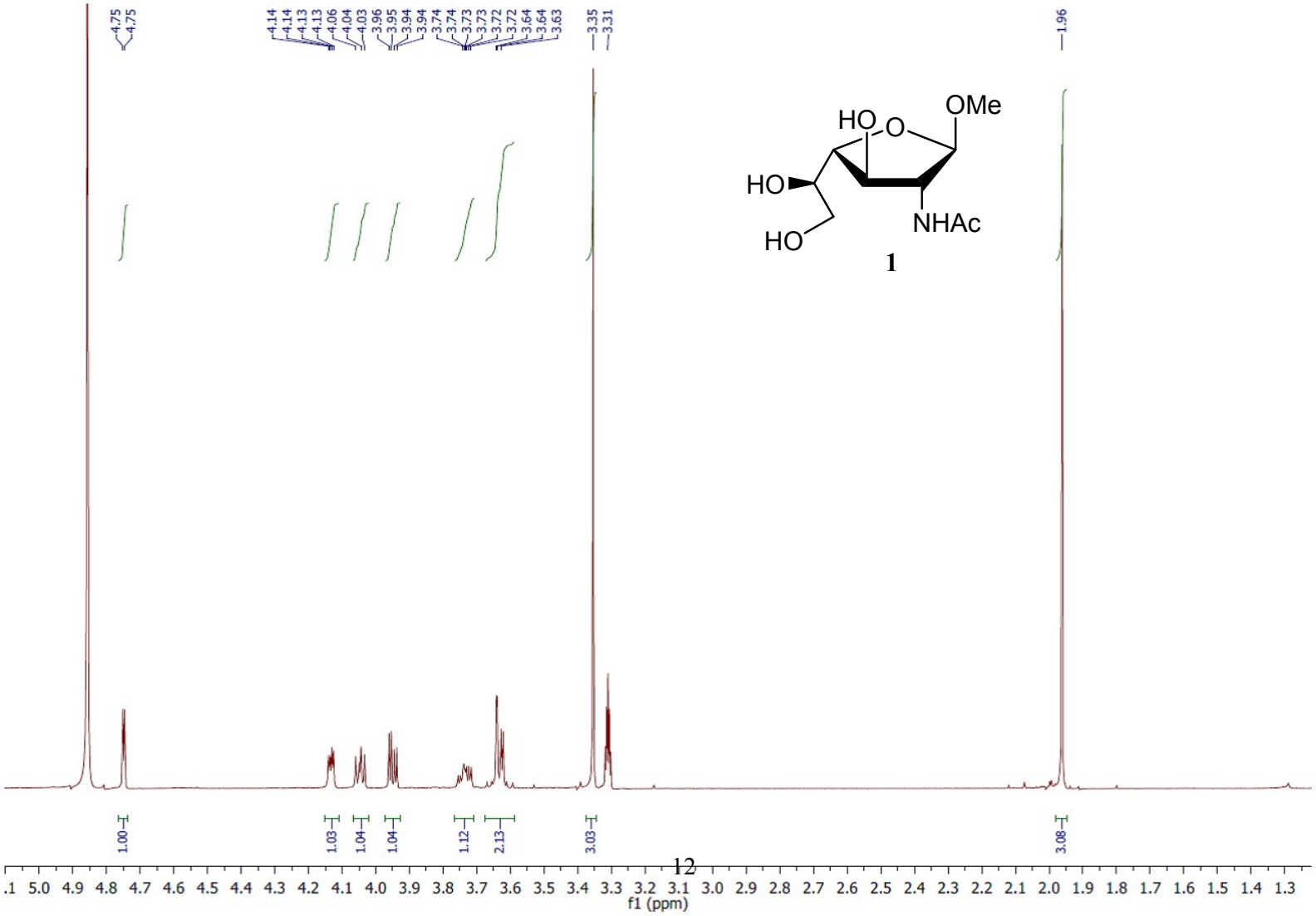
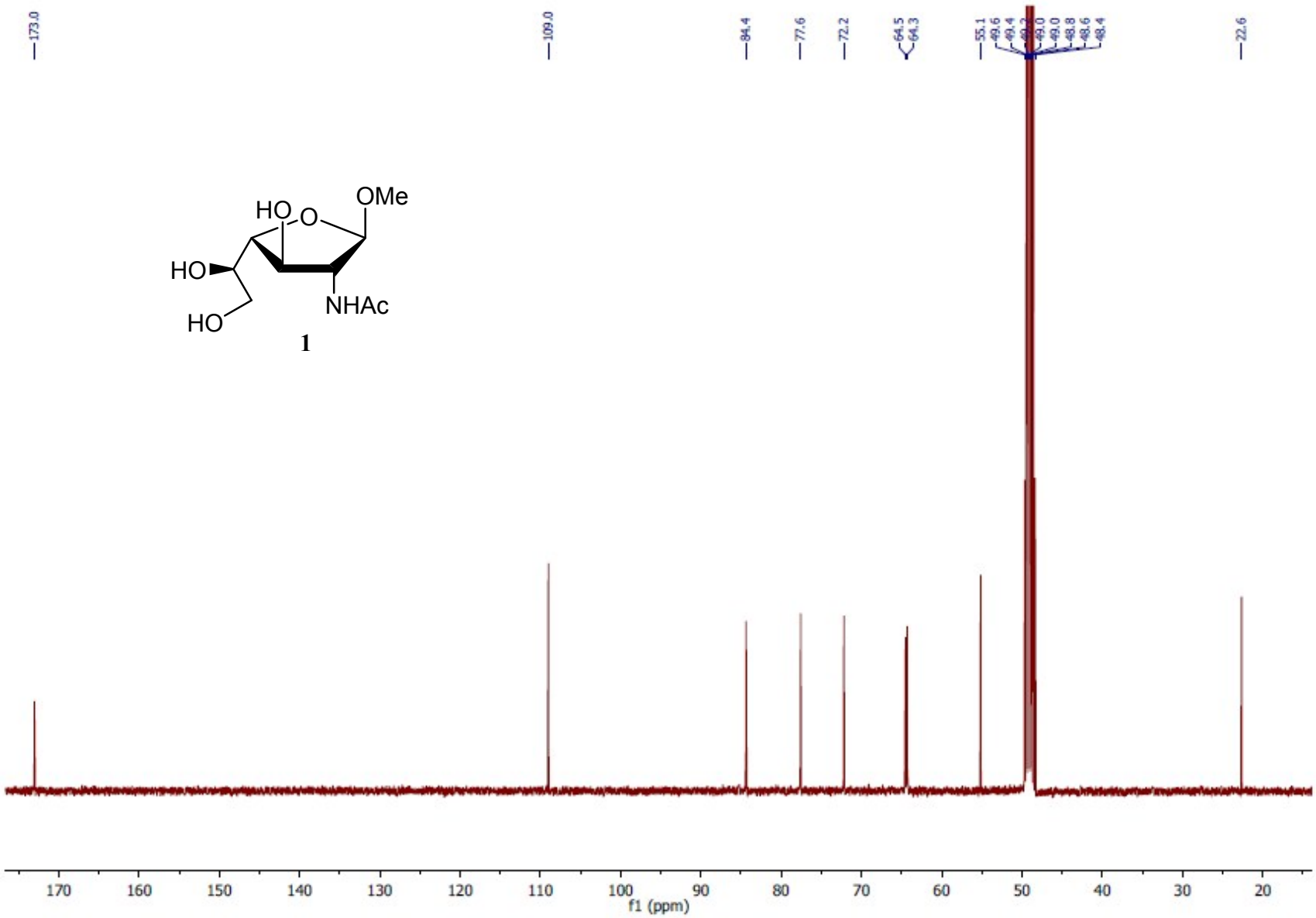


Fig. S8: ^{13}C NMR spectrum of isolated compound 5

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109.0
84.4
77.6
72.2
64.5
64.3
55.1
49.6
49.4
49.2
49.0
49.0
48.8
48.6
48.4
22.6
HO
O
OMe
HO
NHAc
HO
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

1

Fig. S10: ^{13}C NMR spectrum of isolated compound 1

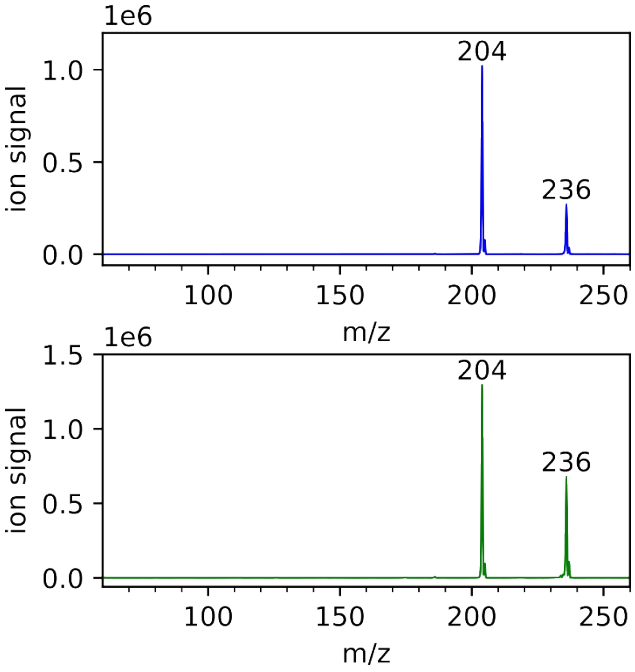

Fig S11. MS/MS spectra of Gal*p*NAc (blue) and Gal*f*NAc(green)