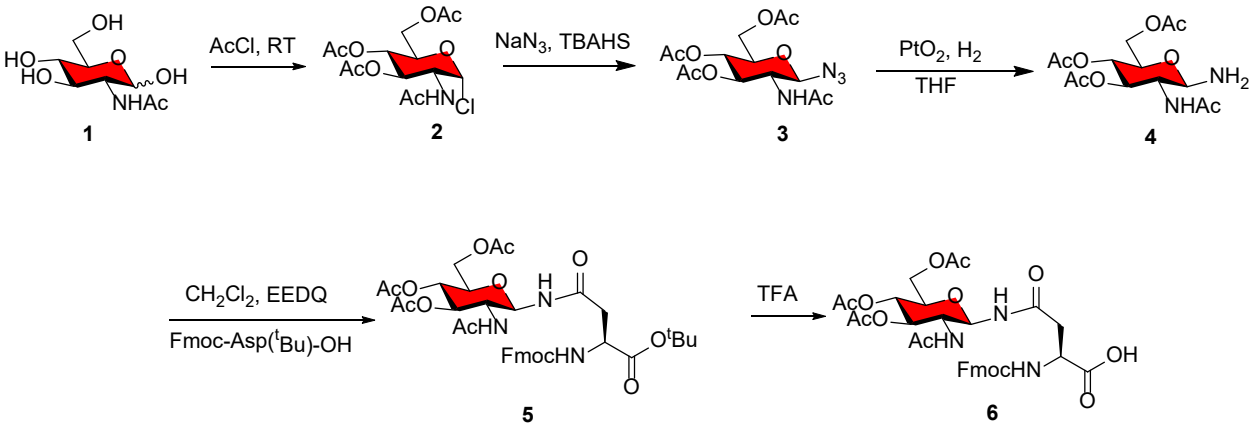


Scheme-1: Chemical Synthesis of GlcNAc-Asn

Protected GlcNAc-Asn can be synthesized from the commercially available GlcNAc in five steps without involving a column chromatography.

Additional Materials:

Acetyl chloride (Sigma Aldrich,Cat No. 320129) **! CAUTION** This compound is corrosive to the respiratory tract., Reacts violently with water.

Sodium Azide (Sigma Aldrich,Cat No. 8.22335) **! CAUTION** This compound on contact with acids liberates very toxic gas

PtO_2 (Acros, Cat. No. AC195320010) **! CAUTION** May cause fire and strong oxidizer.

Fmoc-Asp(^{t}Bu)-OH (Chem-Impex International, Inc, Cat. No. 50-491-711).

EEDQ (2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) (Sigma Aldrich,Cat No. 149837) **! CAUTION** Dust mask must be worn.

TFA (Trifluoroacetic Acid) (Sigma Aldrich,Cat No. T6508) **! CAUTION** Highly Corrosive.

Procedure:

Synthesis of compound 6

1. 80 mL of Acetyl Chloride was taken in a 2 L round bottom flask equipped with a Teflon-coated magnetic bar and guard/drying tube filled with a desiccant bead. The flask was cooled to 0 ^oC using ice-water mixture.

Δ Critical Step The desiccant must be loosely filled for the free gas passage and must be constantly checked for blockage during the reaction.

2. Weigh 40g of compound **1** (0.181 mol, 1 equivalent) and added to the 2L flask from step 1 in one portion. The reaction mixture was slowly allowed to reach room temperature and stirred for 18h. The solution turns to amber colour over the time.

Δ Critical Step A sudden evolution of HCl gas will be observed over the time and drying tube from step 1 must be checked for blockage or replaced with a new one when necessary.

3. 320 mL of Chloroform was added to the mixture from step 2. The resulting solution was poured into 320 g of ice and 80 mL of water mixture with stirring. The organic layer was separated and slowly added into 320 mL of saturate aq. $NaHCO_3$ solution. The mixture was taken into a 2 L separating funnel and shaken until no further evolution of CO_2 gas. The organic layer was separated and washed with 320 mL of brine solution and 320 mL of water successively. The organic layer was dried over 50 g of anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain light brown solid.

! CAUTION Evolution of large amount of gas during aq. $NaHCO_3$ wash.

Δ Critical Step Complete neutralization with aq. NaHCO3 and removal of salts with washings is necessary for the recrystallization.

4. Light brown solid from step 3 was dissolved in 100 mL hot EtOH and left a side at room temperature. White solid precipitate appears over the time and the solid was collected by filtration after 12 h. The solid was washed with cold EtOH and dried to obtain 21 g (31.8%) of compound **2** as a white powder.

Δ Critical Step If there is no precipitation observed, cooling to 4 ^{0}C helps in obtaining the precipitate.

5. To a 2 L round bottom flask, 200 mL of saturated aq. $NaHCO_3$, 19.6 g (57.5 mmol) tetrabutylammonium hydrogen sulphate, 11.2 g (0.173 mol) of sodium azide, 200 mL of dichloromethane and 21 g (57.4 mmol) of compound **2** from step 4 were added successively. The resulting mixture was stirred vigorously at room temperature for 5 h.

Δ Critical Step Proper mixing/stirring is must for the reaction progress.

6. To the biphasic mixture from step 5, 500 mL of ethyl acetate was added and the whole mixture was taken into a 2 L separating funnel. The organic layer was separated and washed with 300 mL of saturated aq. $NaHCO_3$, 300 mL of brine and 300 mL of water successively. The organic phase was dried over 50 g of anhydrous sodium sulphate, filtered and concentrated to obtain cure compound **3**.

Δ Critical Step More ethyl acetate can be taken for the better separation of the layers during the work up.

7. The crude from step 6 was crystallized from 100 mL of ethanol, filtered and washed with 50 mL of cold ethanol to obtain 16 g (74.8%) of compound **3** as an off-white powder.

8. In a 1 L two neck round bottom flask, 16 g (43 mmol) of compound **3** from step 7 was dissolved in 250 mL of anhydrous tetrahydrofuran. The flask was sealed with a rubber stopper and a three-way stopcock connected to vacuum and nitrogen balloon. The flask was evacuated under vacuum and purged with nitrogen and the process was repeated 3 times using a three-way stopcock.

9. To the solution from step 8, 800 mg of platinum (IV) oxide was quickly added from the other neck which was sealed with a rubber stopper under slight nitrogen pressure. The flask was sealed with the rubber stopper and evacuated under vacuum and purged with nitrogen.

The nitrogen balloon on the three-way was replaced with hydrogen gas balloon. The flask was evacuated under vacuum and purged with hydrogen and the process was repeated 3 times. The reaction was allowed to stir under positive hydrogen pressure until completion.

Δ Critical Step Platinum (IV) oxide may cause fire and proper handling is recommended. The reaction can also be done using 10% Pd/C. However, high loading and low yield was observed.

10. The reaction mixture from step 9 was filtered through Celite® 545 and concentrated to dryness to obtain 11.2 g (75.2 %) of compound **4** as a grey powder.

11. In a 1 L round bottom flask, 11.2 g (32.4 mmol) of compound **4** from step 10, 13.3 g (32.4 mmol) of Fmoc-Asp(^{t}Bu)-OH and 8 g (32.4 mmol) of N-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline were dissolved in 500 mL of anhydrous dichloromethane. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 5 h.

12. The reaction mixture from step 11 was concentrated under reduced pressure to obtain crude compound **5** as a solid.

13. The crude solid from step 12 was dissolved in a 400 mL mixture (1:1) of hot ethyl acetate and petroleum ether. The mixture was slowly allowed to reach room temperature for the precipitation of compound **5**. The obtained solid was filtered and dried to obtain 16 g (66.9%) of compound **5** as a grey solid.

14. In a 1 L round bottom flask, 16 g of compound **5** from step 13 was dissolved in 250 mL of 1:1 trifluoroacetic acid and anhydrous dichloromethane at room temperature. The reaction was stirred at room temperature for 1 After completion, the solvents were evaporated under reduced pressure. The crude compound **6** was coevaporated with 50 mL of anhydrous toluene twice.

15. The crude compound **6** from step 14 was dissolved in a 300 mL mixture (1:1) of hot ethyl acetate and petroleum ether. The mixture was slowly allowed to reach room temperature for the precipitation of compound **6**. The obtained solid was filtered and dried to obtain 10 g (67.7%) of compound **6** as an off white solid.

Δ Critical Step Compound **6** was also recrystallized from MeOH at -20 ^{O}C to obtain as a white powder.

Analytical data

Compound **2:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.18 (d, *J* = 3.7, H1), 5.83 (d, *J* = 8.5, NH), 5.32 (t, *J* = 10.4, H3), 5.21 (t, *J* = 9.5, H4), 4.51 - 4.55 (m, H2), 4.27 (m, H6a and H5), 4.13 (dd, *J* = 12.5, 2.0, H6b), 2.10 (s, CH3), 2.05 (s, 2 x CH3), 1.98 (s, CH3). ^{13}C NMR (125 MHz, CDCl3) δ 171.6, 170.7, 170.2, 169.2, 93.7, 71.0, 70.2, 67.0, 61.2, 53.6, 23.2, 20.8, 20.6, 20.5. FAB+ MS (*m/z*) calculated for $C_{14}H_{20}NO_8Cl$ 365.09, found $[M+Na]^+$ 388.08.

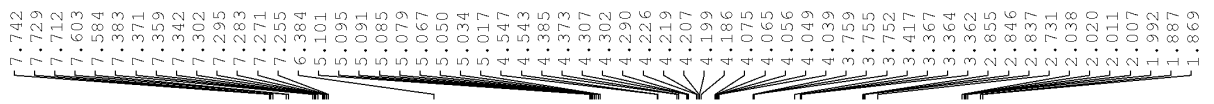
Compound **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (d, *J* = 8.8, NH), 5.24 (t, *J* = 9.6, H3), 5.09 (t, *J* = 9.6, H4), 4.75 (d, *J* = 9.3, H1), 4.26 (dd, *J* = 12.4, 4.8, H6a), 4.16 (dd, *J* = 12.4, 2.0, H6b), 3.92 (q, *J* = 9.2, H2), 3.78 (m, H5), 2.10 (s, CH3), 2.04 (s, CH3), 2.03 (s, CH3), 1.97

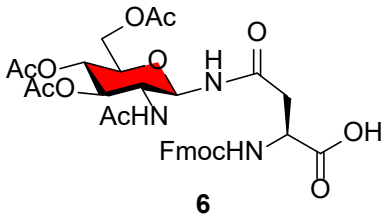
(s, CH3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 170.8, 170.5, 169.4, 88.5, 74.1, 72.2, 68.1, 62.0, 54.2, 23.3, 20.8, 20.7, 20.6. ESI+ MS (*m/z*) calculated for C14H20N4O8 372.13, found $[M+Na]^+$ 395.12.

Compound **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (d, *J* = 8.8, NH), 5.01 - 5.13 (m, H3, H4), 4.21 (dd, *J* = 12.3, 4.9, H6a), 4.09 - 4.12 (m, H1, H6b), 4.02 (q, *J* = 9.4, H2), 3.63 (m, H5), 2.09 (s, CH3), 2.04 (s, CH3), 2.03 (s, CH3), 1.99 (s, CH3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 170.9, 170.8, 169.4, 86.5, 73.5, 72.9, 68.5, 62.5, 55.0, 23.4, 20.9, 20.8, 20.7. ESI+ MS (*m/z*) calculated for C14H22N2O8 346.14 found, $[M+Na]^+$ 369.13.

Compound **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5, 2 x CH), 7.59 (d, *J* = 7.4, 2 x CH), 7.39 (t, *J* = 7.3, 2 x CH), 7.30 (m, 2 x CH, NH), 6.21 (d, *J* = 8.3, NH), 5.96 (d, *J* = 8.6, NH), 5.03 - 5.11 (m, H1, H3, H4), 4.49 - 4.55 (m, CHα), 4.41 (m, CH), 4.17 - 4.30 (m, 2 x CH, H6), 4.12 - 4.14 (m, CH, H2), 3.74 (dd, *J* = 9.7, 2.0, H5), 2.84 (dd, *J* = 16.4, 4.2, Hβ), 2.70 (dd, *J* = 16.3, 3.9, Hβ), 2.06 (s, CH3), 2.05 (s, CH3), 2.04 (s, CH3), 1.94 (s, CH3), 1.44 (s, C(CH3)3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 172.0, 171.2, 170.8, 170.1, 169.4, 156.2, 144.0, 141.3, 127.8, 127.2, 125.2, 120.0, 82.3, 80.2, 73.6, 73.0, 67.7, 67.2, 61.8, 60.5, 53.4, 51.1, 47.2, 38.1, 28.0, 23.1, 20.8, 20.7, 20.6, 14.3. FAB+ MS (*m/z*) calculated for C37H45N3O13 739.30 found $[M+Na]^+$ 762.29.

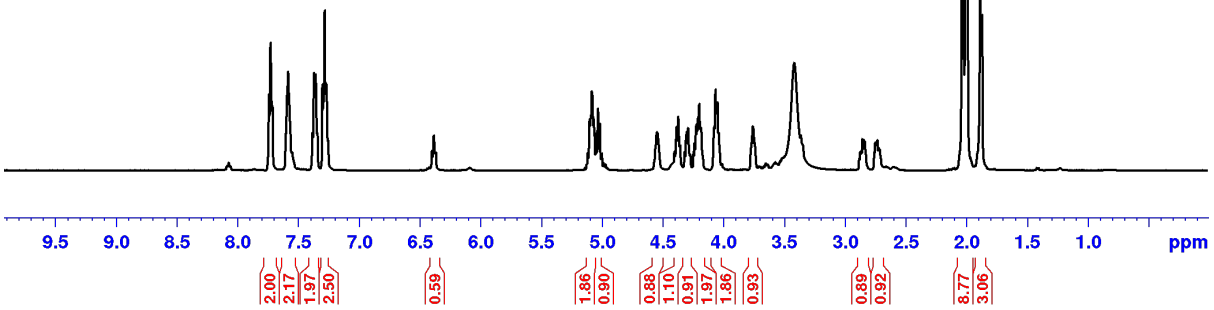
Compound **6**: ^{1}H NMR (500 MHz, DMSO-d6) δ 8.57 (d, *J* = 9.2, NH), 7.87 (d, *J* = 8.0, 2 x CH, NH), 7.69 (d, *J* = 7.4, 2 x CH), 7.45 (d, *J* = 11.5, NH), 7.40 (t, *J* = 7.4, 2 x CH), 7.31 (t, *J* = 7.4, 2 x CH), 5.17 (t, *J* = 9.9, H1), 5.08 (t, *J* = 9.9, H3), 4.80 (t, *J* = 9.8, H4), 4.38 (q, *J* = 8.7, CHα), 4.15 - 4.30 (m, H6a, CH, CH2), 3.93 (dd, *J* = 10.7, 1.6, H6b), 3.86 (q, *J* = 9.7, H2), 3.80 (dd, *J* = 9.9, 1.5, H5), 2.64 (dd, *J* = 16.3, 5.4, CHβ), 2.48 (dd, overlapped by DMSO signal CHβ), 1.98 (s, CH3), 1.95 (s, CH3), 1.89 (s, CH3), 1.70 (s, CH3). ^{13}C NMR (125 MHz, DMSO-d6) δ 173.0, 171.5, 170.0, 169.8, 169.5, 169.3, 155.8, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 78.1, 73.4, 72.3, 68.4, 65.7, 52.1, 50.0, 46.6, 36.8, 22.6, 20.5, 20.4, 20.3. ESI+ MS (*m/z*) calculated for C33H37N3O13 683.23 found $[M+Na]^+$ 706.22.

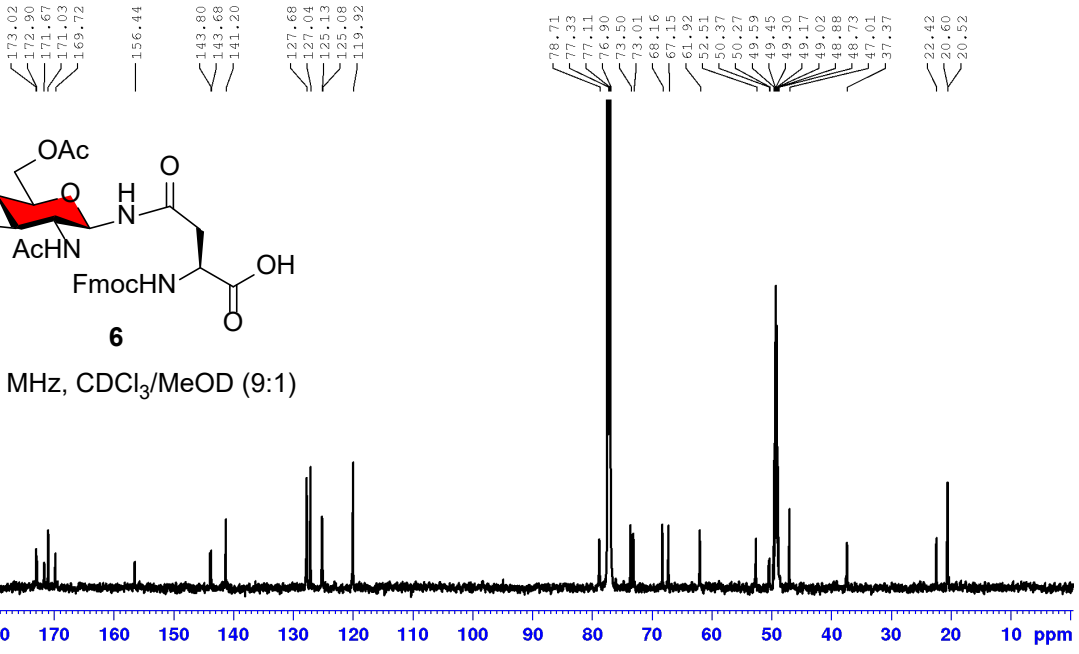




6

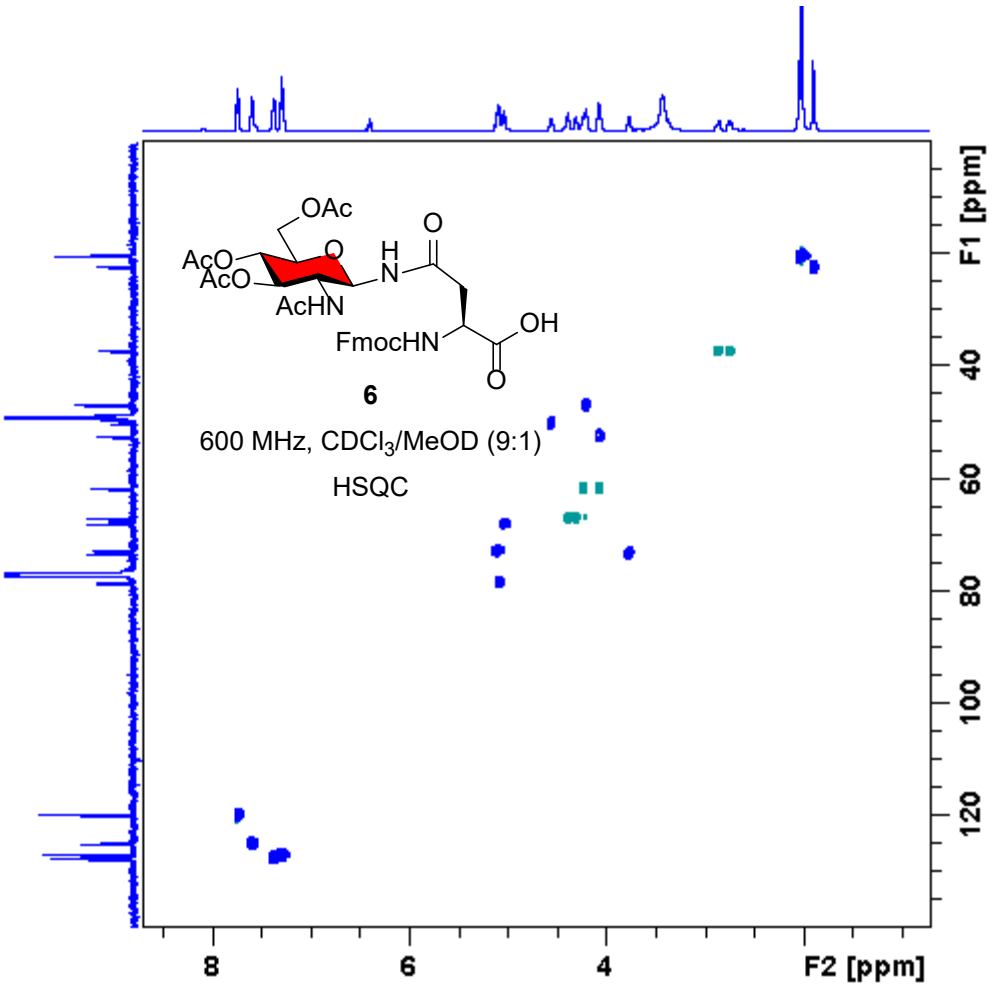
600 MHz, $CDCl_3$/MeOD (9:1)





6

150 MHz, $CDCl_3$/MeOD (9:1)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AcO
AcO
O
H
N
O
AcHN
FmocHN
OH
O
6
600 MHz, CDCl3/MeOD (9:1)
HSQC
F1 [ppm]
40
60
80
100
120
8
6
4
F2 [ppm]