Synthesis of TC007

neamine

BzCl, DCM, Et3N →

1) DMSO, (COCl)2, DCM, Et3N
2) NaBH4, MeOH →

1) Tf2O, pyr. DCM
2) NaN3 →

diacetone D-glucose

1) HOAc, TFA, H2O
2) Ac2O →

1) H2NNH2-HOAc
2) Cl3CN, DBU, Bf3-OEt2, DCM →

K2CO3, MeOH →

PMe3, THF →

TC007
Do not follow the exact amounts of reagents and chemicals in the procedures. Discuss with instructor before setting up the reaction.

Preparation of Compound 1.

A solution of NaN₃ (50.0 g, 768.9 mmol) in 46.2 mL H₂O was cooled in an ice-water bath and treated with 77.0 mL CH₂Cl₂. The mixture was stirred vigorously and Tf₂O (26.0 mL, 153.8 mmol) was slowly added over a 5 min period. The system was stirred for 2 hours. After an hour and a half, neamine (9.0 g, 19.2 mmol) was dissolved with 122.6 mL H₂O in another 1L round bottom flask. 245.2 mL MeOH was then added, followed by CuSO₄.5H₂O (0.50 g, 1.92 mmol) and K₂CO₃ (21.2 g, 153.8 mmol). The mixture was heavily stirred at room temperature. 30 minutes later, the first reaction was stopped and transferred to a 1L separatory funnel. Then NaHCO₃ (sat’d, aq) was added and the funnel was heavily shaken to release the gas. The organic phase was separated and the aqueous phase was extracted twice with CH₂Cl₂. The combined organic phases were washed once with NaHCO₃ solution and used without further purification.

The freshly prepared methylene chloride solution of triflic azide was added slowly to the second reaction mixture (neamine). The mixture was stirred overnight at room temperature. If TLC analysis (Hexane/EtOAc=50/50) showed incomplete reaction, an additional portion of triflic azide (50ml) was added, and the mixture was stirred overnight.

The solvent was then removed with compressed air and the residue was redissolved in 600 mL ethyl acetate. This was filtered through a Buchner funnel packed with celite and the residue was washed with an additional 200 mL ethyl acetate. The filtrate was transferred to a 1L separatory funnel and washed with 1N HCl, NaHCO₃ (satd, aq), water and brine. The organic phase was dried over Na₂SO₄, concentrated and purified by column (recycled hexane/ethyl acetate to pure ethyl acetate).

General Procedure for the Preparation of Compound 2.

To the solution of 1 and TEA (4 equiv.) in anhydrous CH₂Cl₂ (20 mL), BzCl (3.3 equiv.) were added at -50°C. The reaction mixture was stirred allowing the reaction temperature to warm up to 0°C and quenched with NaHCO₃(s) after completion of the reaction (Rf =
0.4, monitored by TLC, EtOAc: Hexane = 25:75). After removal of the solvent, the reaction mixture was diluted with EtOAc. The organic layers were washed with 1N HCl(aq), saturated NaHCO$_3$(aq), and brine, then dried over Na$_2$SO$_4$(s). Removal of the solvent followed by purification with gradient column chromatography (Hexanes:EtOAc = 100:0 to 60:40) afforded the product.

Preparation of Compound 3.

To a solution of (COCl)$_2$ (13.5mL, 153.68mmol) in 300 mL dried CH$_2$Cl$_2$ at $-78 \, ^\circ$C, dried DMSO (21.8mL, 307.36mmol) was added and the resulting solution was stirred for 30 minutes, allowing the temperature to warm to $-65 \, ^\circ$C. To the reaction flask, a solution of starting material (20g, 76.84mmol) in 50 mL CH$_2$Cl$_2$ was added. The reaction mixture was stirred for 1 hour, allowing the temperature to warm to $-45 \, ^\circ$C. To this solution, dried DIMEA (107.36mL, 614.72mmol) was added, and the reaction was allowed to warm to 0 $^\circ$C over a 1 hour period. After completion of the reaction, the reaction mixture was quenched with 1 N HCl and diluted with ethyl acetate. The organic layer was washed with pH 7 buffer (three times) and dried over Na$_2$SO$_4$. After removal of the solvent, the crude was used for further reaction without purification.

The crude keto sugar from the previous step was dissolved in MeOH(100mL) and cooled to 0 $^\circ$C. Solid NaBH$_4$ (8.72g, 230.5mmol) was added and the reaction was allowed to stir overnight. Upon completion of the reaction (monitored by TLC, hexanes/ethyl acetate 1/1), the reaction was quenched by addition of concentrated HCl, which was added drop wise until pH ~ 8. MeOH was removed and the mixture was diluted with ethyl acetate and washed with NaHCO$_3$, water, brine, and dried over sodium sulfate. The reaction was concentrated and purified via column chromatography, and the pure compound (6.3g, 31.5%) was obtained as viscous oil.

Preparation of Compound 4.

To a cooled solution (0 $^\circ$C) of starting material (6.3g, 24.23mmol) dissolved in 150mL CH$_2$Cl$_2$, pyridine (3.1mL, 38.77mmol) was added, followed by the slow addition of Tf$_2$O (5.7mL, 33.09mmol), and the resulting mixture was allowed to stir for 0.5 hour at 0 $^\circ$C. After completion of the reaction (monitored by hexanes/ethyl acetate 1/1), the solution was diluted with CH$_2$Cl$_2$ and washed with H$_2$O, saturated NaHCO$_3$, and brine, dried with NaSO$_4$. The solvent was removed via vacuum while maintaining the temperature below 20 $^\circ$C. The crude product was used for further reaction without purification.

Add 10g NaN$_3$, 30mL of DMF to flask, filter above solution to the reaction mixture, and stirred overnight, letting the temperature rise to R.T. If the reaction is uncompleted (monitored by TLC, Hexane/EtOAc=1/1), add more 20mL of DMF and stir overnight. After completion of the reaction, the solvent was removed at 40$^\circ$C oil bath. The resulting mixture was diluted with ethyl acetate and filtered through Celite. The reaction was then concentrated, subjected to column chromatography (pure hexanes-hexanes/ethyl acetate 65/35), and the product (5.3g, 77%) was obtained as a viscous oil.
Preparation of Compound 5.

The starting material (0.08g, 0.30mmol) was dissolved in AcOH/TfA 80%/1% solution, and the resulting reaction mixture was kept in rotate evaporator (without suction) at 60°C for 1 hr. Diluted with water (about 20mL twice), kept in totate evaporator at 100°C with suction to remove the solution. TLC analysis (hexanes/ethyl acetate 65/35 and ethyl acetate/Methanol=90/10) showed the completion of reaction.

To the crude product from the previous reaction, Ac₂O (15mL) was added followed by the addition of 20 drops H₂SO₄ at 0°C. Upon completion of the reaction (monitored by TLC, hexanes/ethyl acetate 65/35), add this solution into saturated sodium bicarbonate solution, diluted with ethyl acetate and washed with H₂O, saturated sodium bicarbonate, brine, and dried over sodium sulfate. Remove of solvent, purified via column chromatography (Hexanes 100-Recycle/ethyl acetate=50/50). Obtained the mixture.

General Procedure for the Preparation of Compound 6.

To a solution of starting material in DMF, hydrazine acetate (1.2 equivalents) was added. The solution was stirred at room temperature till the complete consumption of the starting material. The reaction mixture was filtered through a short column packed with layers of silica gel and Celite. The column was eluted thoroughly with EtOAc. After removal of the solvents, the crude product can be used directly for the next step, or can be further purified with column chromatography providing light yellowish oil. We do, however, recommend a purification of the crude product with a flash column chromatography. To a solution of glycosyl hydroxide and trichloroacetonitrile (12 equivalents) in anhydrous CH₂Cl₂, catalytic amount of DBU was added drop wise. The solution was stirred at room temperature till the complete consumption of the starting material. The reaction mixture was added with charcoal, and filtered through a short column packed with layers of silica gel and Celite. The column was eluted thoroughly with ether. After removal of the solvents, the crude product was purified with column chromatography providing glycosyl trichloroacetimidate as light yellowish oil. The glycosyl trichloroacetimidate may undergo slow decomposition at room temperature. Therefore, these compounds have been characterized only by ¹H and ¹³C NMR.

General procedure for glycosylation (Compound 7): A solution of glycosyl trichloroacetimidate, neamine derivative (1.2 equivalence), and activated powder 4Å molecular sieve was stirred in anhydrous CH₂Cl₂ (ca. 5 mL) at room temperature for 1 h then cooled to -50°C. To this cloudy solution, BF₃·OEt₂ (0.05 mL) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl trichloroacetimidate (ca. 40 minutes). The reaction mixture was quenched by the addition of powder NaHCO₃. After being stirred for 15 minutes, the reaction mixture was filtered through Celite. The residue was washed thoroughly with CH₂Cl₂ and ethyl acetate. After removal of the solvents, the crude product was purified with column chromatography.

General Procedure for Deacylation (Compound 8): A solution of 7 and K₂CO₃ (10 equiv.) was stirred in MeOH (10 mL) at r.t. until the completion of the reaction.
Removed solvent and the residue was re-dissolved in MeOH and filtered through Celite. Removed solvent again and the crude product was used for next step without further purification.

**General procedure for Staudinger reaction (TC007):** To a starting material/THF solution in a reaction vial equipped with a reflux condenser, 0.1 M NaOH$_{aq}$ (0.5 mL) and PMe$_3$ (1M in THF, 5 - 7 equivalents) were added. The reaction mixture was stirred at 50°C for 2 hrs. The product has a R$_f$ of 0 when eluted with EtOAc/MeOH (9/1) solution and a R$_f$ of 0.6 when eluted with iPrOH/1M NH$_4$OAc (2/1) solution. After completion of the reaction, the solvents were removed, and the crude product was purified with Amberlite CG50(NH$_4$$^+$) eluted with a gradient of NH$_4$OH solution (0% – 20%). The final product with Cl$^-$ salt can be prepared with an ion-exchange column packed with Dowex 1X8-200 (Cl$^-$ form) and eluting with water. After collection of the desired fractions and removal of solvent, the final products are subjected to bioassay directly.